Progress in the Chemistry of Organic Natural Products

A. Douglas Kinghorn · Heinz Falk
Simon Gibbons · Jun'ichi Kobayashi
Yoshinori Asakawa · Ji-Kai Liu *Editors*

108

Progress in the Chemistry of Organic Natural Products

Springer

Progress in the Chemistry of Organic Natural Products

The volumes of this classic series, now referred to simply as "Zechmeister" after its founder, Laszlo Zechmeister, have appeared under the Springer Imprint ever since the series' inauguration in 1938. It is therefore not really surprising to find out that the list of contributing authors, who were awarded a Nobel Prize, is quite long: Kurt Alder, Derek H.R. Barton, George Wells Beadle, Dorothy Crowfoot-Hodgkin, Otto Diels, Hans von Euler-Chelpin, Paul Karrer, Luis Federico Leloir, Linus Pauling, Vladimir Prelog, with Walter Norman Haworth and Adolf F.J. Butenandt serving as members of the editorial board. The volumes contain contributions on various topics related to the origin, distribution, chemistry, synthesis, biochemistry, function or use of various classes of naturally occurring substances ranging from small molecules to biopolymers. Each contribution is written by a recognized authority in the field and provides a comprehensive and up-to-date review of the topic in question. Addressed to biologists, technologists, and chemists alike, the series can be used by the expert as a source of information and literature citations and by the non-expert as a means of orientation in a rapidly developing discipline. Listed in Medline. All contributions are listed in PubMed.

More information about this series at http://www.springer.com/series/10169

A. Douglas Kinghorn • Heinz Falk •
Simon Gibbons • Jun'ichi Kobayashi •
Yoshinori Asakawa • Ji-Kai Liu
Editors

Progress in the Chemistry of Organic Natural Products

Volume 108

With contributions by

R. Mata · M. Figueroa · A. Navarrete · I. Rivero-Cruz

S. Fiorito · F. Epifano · F. Preziuso · V. A. Taddeo · S. Genovese

D. I. Bernardi · F. O. das Chagas · A. F. Monteiro · G. F. dos Santos · R. G. de Souza Berlinck

Springer

Editors
A. Douglas Kinghorn
College of Pharmacy
The Ohio State University
Columbus, OH, USA

Simon Gibbons
UCL School of Pharmacy
University College London, Research
London, UK

Yoshinori Asakawa
Faculty of Pharmaceutical Sciences
Tokushima Bunri University
Tokushima, Japan

Heinz Falk
Institute of Organic Chemistry
Johannes Kepler University
Linz, Austria

Jun'ichi Kobayashi
Grad. School of Pharmaceutical Science
Hokkaido University
Fukuoka, Japan

Ji-Kai Liu
School of Pharmaceutical Sciences
South-Central Univ. for Nationalities
Wuhan, China

ISSN 2191-7043 ISSN 2192-4309 (electronic)
Progress in the Chemistry of Organic Natural Products
ISBN 978-3-030-01098-0 ISBN 978-3-030-01099-7 (eBook)
https://doi.org/10.1007/978-3-030-01099-7

Library of Congress Control Number: 2018965903

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Chemistry and Biology of Selected Mexican Medicinal Plants

Rachel Mata, Mario Figueroa, Andrés Navarrete, and Isabel Rivero-Cruz

Contents

R. Mata (✉) · M. Figueroa (✉) · A. Navarrete · I. Rivero-Cruz
Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad de México, México
e-mail: rachel@unam.mx; mafiguer@unam.mx; anavarrt@unam.mx; riveroic@unam.mx

A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.),
Progress in the Chemistry of Organic Natural Products, Vol. 108,
https://doi.org/10.1007/978-3-030-01099-7_1

1 Introduction

Mexico is a multifaceted and heterogeneous country with high cultural richness and 10–12% of the world's biodiversity. This country ranks 4th in the variety of vascular plants with about 31,000 different species; of this stock more than 3350 form part of the medicinal flora. When the Spanish conquerors arrived to ancient Mexico, they found existing civilizations with a holistic view of illnesses and healing. These early Mesoamericans inhabitants used religious, magic rituals and a variety of plant-based remedies to improve health. The abundance and variety of Mexican medicinal flora can be traced from published work written from the sixteenth century to modern times. Crucial and most important sources of information about traditional Mexican medicine were recently reviewed [1].

The use of herbal medicines survives to this day in modern Mexico; the original Aztec beliefs and practices are interlaced with strands of the European medicine introduced by the Spaniards in the sixteenth century. They are an integral element of alternative medical care and the best testimony of their efficacy and cultural value is the persistence of medicinal plants in present-day Mexican markets, where the highest percentage of medicinal and aromatic plants is sold.

For more than 100 years, researchers have explored Mexican medicinal flora from the ethnobotanical, anthropological, chemical, pharmacological, and biotechnological points of view; in a few cases some clinical investigations have been pursued. The most important investigations have been carried out at the Instituto Nacional de Antropología, Instituto Mexicano del Seguro Social, Universidad Autónoma de Nuevo León, Universidad Autónoma del Estado de Morelos, Instituto Tecnológico y de Estudios Superiores de Monterrey, Universidad Autónoma Metropolitana, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, and Universidad Nacional Autónoma de México. The no-longer existing Instituto Médico Nacional and Instituto Mexicano de Plantas Medicinales deserve special mention since they were devoted to the study of Mexican medicinal plants in different periods of the twentieth century. Both are good examples of important institutions dedicated to the comprehensive analysis of the national Materia Medica, and were pioneering institutions in bioprospecting matters.

In the twenty-first century, the commerce of medicinal plants in Mexico has grown due to a global resurgence of herbal-based remedies. Furthermore, according to a recent survey, 54% of health professionals and 49% of physicians have used medicinal plants as an alternative therapy for several diseases. Twenty-eight percent of health professionals and 26% of physicians, have recommended or prescribed medicinal plants to their patients, in particular for digestive and respiratory ailments; finally, 73%

of health professionals would agree to receiving academic information regarding the use and prescribing of medicinal plants [2].

Concomitantly, a loss of biodiversity, over-exploitation, biopiracy, and weak regulations on the use of medicinal plants are the major impediments to the growth of herbal medicine as an important national industry [3]. Therefore, current research on medicinal plants should also involve conservation issues and the sustainable search for bioactive natural products based on traditional knowledge, regulation, and quality control of the most important species; these are essential issues for the growth of a rational herbal medicine usage.

In the following sections, some work from the authors' laboratories will be highlighted. The most relevant phytochemical and pharmacological profiles of a selected group of plants widely used for treating major national health problems will be discussed.

2 Mexican Medicinal Plants Employed for Treating Major National Health Problems

2.1 Diabetes

The global prevalence of diabetes in adults has been increasing over recent decades, making this disease a major public health threat in countries all over the world. The International Diabetes Federation estimated the global prevalence to be 425 million in 2017, which implied a health expenditure of 673 billion USD [4]. The prevalence of diabetes in adults aged 20–79 years is predicted to rise to 10.4% in 2040. Of the total diabetics, about 95% have type 2 diabetes mellitus (T2DM). Mexico is one of the countries most affected by this metabolic disease, in particular indigenous people owing to changes in their traditional lifestyle and the effects of industrialization on both environmental and sociocultural norms. In 2017, there were more than 12 million people affected by diabetes, representing a prime cause of mortality. In Mexico as in other regions of the world, people use plants to treat the symptoms of diabetes. More than 300 different plants have been described as reputedly beneficial for the diabetic patient [5–7], but most claims are subjective and few have received any suitable scientific evaluation. So far, about 200 plants have been investigated scientifically in Mexico in order to establish their antidiabetic potential. Most studies have been limited to the preclinical evaluation of extracts prepared with selected solvents using different pharmacological models [6]; the depth of their analysis is variable since some authors have reported in detail the mode of action of the extracts while others just measured their hypoglycemic activity. Other studies have determined both the active principles and the preclinical efficacy of the traditional preparations. Finally, only a very few studies have pursued in-depth clinical observations. Most of the work of the present author group falls into the second category, involving detailed phytochemical work coupled with substantial preclinical biological observations.

Some examples of our work on antidiabetic plants are described in the following sections. In addition, other investigations, from other authors and ourselves, carried out

after a survey on diabetic plants was published in 2005 [6], are summarized in the Appendix Table.

2.1.1 *Swietenia humilis*

Swietenia humilis Zuccarini (Meliaceae), locally known as "zopilote", "cobano", "flor de venadillo" and "caoba", is a medium-sized deciduous tree (Fig. 1). The species is regarded as one of the three true American mahogany species. It grows in a very wide ecological range within its native Pacific watershed of Central America and Mexico. The seeds are wind dispersed and highly valued for medicinal purposes. The plant is also a much appreciated hardwood species in the neotropics and is seriously threatened owing to overexploitation and habitat destruction. Therefore, a multilateral treaty called the Convention on International Trade in Endangered Species of Wild Fauna and Flora lists *S. humilis* in Appendix II (all parts and derivatives except the seeds) [8]. Also, it is categorized in the International Union for Conservation of Nature Red List of Threatened Species as "vulnerable" [9].

The medicinal use of the seeds of *S. humilis* can be traced to the sixteenth century; the Spanish royal physician Francisco Hernández, in his magnificent manuscript "Four Books on the Nature and Virtues of Plants and Animals for Medicinal Purposes in New Spain", described the antiulcer, astringent, antitussive, and emollient properties of these seeds. In the middle of the twentieth century, their astringent effects were also described [10]. In the present day, decoctions of the seeds of *S. humilis* (SHD), alone or in combination with other plants, are valued for treating indigestion, stomachache, amebic dysentery, and diarrhea. The ground raw seeds or their decoctions are also ingested as a blood depurative and antidiabetic agent [5, 6].

In general, for conducting our studies focused on the determination of any pharmacological properties of traditional extracts, first acute preclinical toxicity using the Lorke procedure is assessed [11]. This method measures acute toxicity for 14 days in mice using a range of doses between 10 and 5000 mg/kg, in two phases. The dried seeds and SHD (10–5000 mg/kg) showed no acute toxic effects when assessed by the Lorke procedure. The calculated LD_{50} values of the preparation and crude drug were higher than 5000 mg/kg.

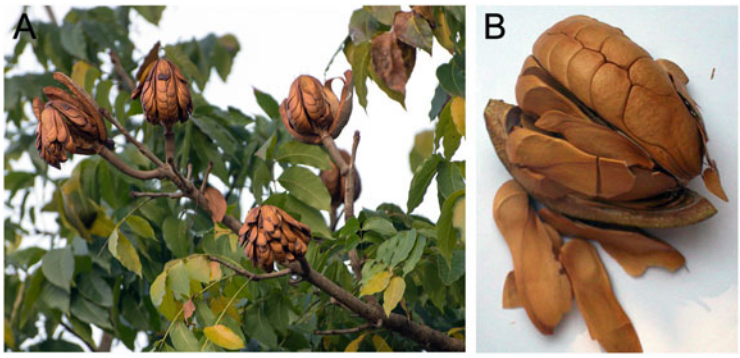


Fig. 1 Leaves, stems (**A**), and seeds (**A** and **B**) of *Swietenia humilis*

Since the plant preparation lacked acute toxic effects, it was next tested for antidiabetic action in vivo by means of animal models using a standard protocol. By means of this protocol, initially the acute hypoglycemic activity in normoglycemic and hyperglycemic animals (ICR mice or Wistar rats) is assessed. If feasible, subchronic (14 days) or chronic (30 days) experiments are also performed. Then, the antihyperglycemic action of the extracts or purified compounds after a glucose (1 g/kg; oral glucose tolerance test, OGTT), sucrose (2 g/kg; oral sucrose tolerance test, OSTT) or starch (2 g/kg; oral starch tolerance test, OStTT) challenge is assessed using normal and hyperglycemic animals. These tests provide relevant information regarding peripheral utilization or absorption of glucose. In all tests, the animals are made hyperglycemic with streptozotocin (STZ, 130 mg/kg for mice; and 50 mg/kg for rats), after previous protection with nicotinamide (NAA, 40 mg/kg for mice; and 65 mg/kg for rats). After 7 days of NAA-STZ administration, the animals are generally hyperglycemic and can be included in the studies conducted subsequently. The NAA-STZ model affords a similar biochemical blood profile and pathogenesis to T2DM in humans. Glibenclamide (15 mg/kg), metformin (200 mg/kg) or acarbose (5 mg/kg) are used as positive controls, depending of the type of experiment. The percentage variation of glycemia for each group of animals is calculated with respect to the initial values at different periods of time. The results are plotted indicating blood glucose values or percentage of variation versus time at several doses [12].

In a series of experiments conducted in NAA-STZ hyperglycemic mice, SHD (100–316 mg/kg) caused a significant reduction in blood glucose levels and inhibited the postprandial peak provoked by a glucose load during an OGTT. On the other hand, SHD (100–316 mg/kg) did not inhibit the postprandial peak at any of the doses tested during an OSTT in normoglycemic mice, ruling out an inhibition of α-glucosidases at the intestinal level [13].

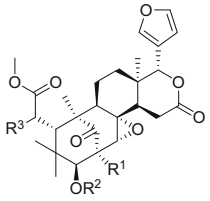
The antihyperglycemic, hypoglycemic, and hypolipidemic effects of *S. humilis* seeds were corroborated in rats with fructose-fed metabolic syndrome. SHD (100 and 316 mg/kg) caused a significant inhibition of the postprandial peak during an OGTT when compared with a vehicle-treated group. Moreover, daily administration of SHD (100 mg/kg) for a week provoked a significant hypoglycemic effect, and reductions in both serum triglycerides and uric acid, without any significant changes in fasting insulin levels or body weight. In addition, a reduction in the abdominal fat of the test animals, and an increment in hepatic glycogen, were observed. Altogether, the results suggested that the traditional preparation of *S. humilis* induced modifications in peripheral glucose uptake, rather than by inhibition of the intestinal α-glucosidases. The reduction of the postprandial peak observed during the OGTT, and the increment of hepatic glycogen in rats with fructose-fed metabolic syndrome indicated that the hypoglycemic effect of SHD involves an insulin-sensitizing mechanism. The reduction in blood triglycerides is compatible with an increment in glucose uptake in adipose tissue, where energy is stored as triglycerides. These effects are also consistent with the use of this species as blood depurative (purifying) agent [13].

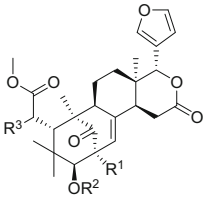
In order to identify the compounds responsible for these pharmacological effects, both the active aqueous and an organic extracts of *S. humulis* seeds were fractionated extensively by chromatographic procedures. These processes led to the isolation of

eight new limonoids of the mexicanolide type, namely, humilinolides A–H (**1**–**8**) along with humulin B (**9**), methyl-2-hydroxy-3β-isobutyroxy-1-oxomeliac-8(30)-enate (**10**), methyl-2-hydroxy-3β-tigloyloxy-1-oxomeliac-8(30)-enate (**11**), swietenin C (**12**), swietemahonin C (**13**) and 2-hydroxy-destigloyl-6-deoxyswietenine acetate (**14**) (Fig. 2) [13]. These mexicanolides can be categorized into two structural subclasses by considering the degree of oxidation at C-8/C-30 of the basic methyl-1-oxomeliacate nucleus. The first one comprises limonoids with an 8,30 double bond, while the second includes those with an 8,30 epoxide function. The compounds in each group differ in the number and position of oxygenated substituents. The acid residues esterifying the hydroxy group at C-3 could be either isobutyric, tiglic or acetic acid. All structures were elucidated using one- and two-dimensional NMR spectroscopic techniques, and with that of humulinolide G (**5**) confirmed by X-ray diffraction analysis [13].

Chromatographic analysis of SHD revealed that compounds **9**, **11**, and **14** are its major components, although the remaining limonoids isolated were also identified. These limonoids were isolated in adequate amounts to perform in vivo assays. As expected, the three major compounds (3.16–31.6 mg/kg) showed hypoglycemic and antihyperglycemic actions when tested in the NAA-STZ mice model using the acute hypoglycemic assay and the OGTT, respectively (Fig. 3). Although limonoids **9**, **11**, and **14** were found as the major hypoglycemic and antihyperglycemic limonoids of the decoction, the remaining compounds could also contribute to the pharmacological action displayed by SHD. Furthermore, they could be acting synergistically on different molecular targets to produce antidiabetic and hypolipidemic effects. Likewise, the mixture of components in SHD might enhance the bioavailability of one or several compounds of the extract, thus improving their pharmacological actions. It is worth mentioning that none of the isolates inhibited α-glucosidases.

The antihyperalgesic effects of SHD and compound **14** were assessed in NAA-STZ hyperglycemic mice using the formalin method. The formalin test in mice is a valid and reliable model of nociception and is sensitive to various classes of analgesic drugs. The noxious stimulus is an injection of dilute formalin (1% in





1 (humilinolide A) R^1 = OH, R^2 = A, R^3 = OH
2 (humilinolide B) R^1 = OH, R^2 = A, R^3 = B
6 (humilinolide F) R^1 = B, R^2 = C, R^3 = B
8 (humilinolide H) R^1 = B, R^2 = A, R^3 = H
9 (humulin B) R^1 = OH, R^2 = A, R^3 = H
13 (swietemahonin C) R^1 = H, R^2 = A, R^3 = B

3 (humilinolide C) R^1 = B, R^2 = C, R^3 = H
4 (humilinolide D) R^1 = OH, R^2 = D, R^3 = B
5 (humilinolide E) R^1 = OH, R^2 = C, R^3 = B
7 (humilinolide G) R^1 = B, R^2 = A, R^3 = H
10 R^1 = OH, R^2 = A, R^3 = H
11 R^1 = OH, R^2 = C, R^3 = H
12 (swietenin C) R^1 = H, R^2 = A, R^3 = OH
14 R^1 = OH, R^2 = D, R^3 = H

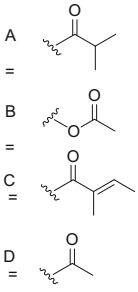


Fig. 2 Limonoids isolated from *Swietenia humilis*

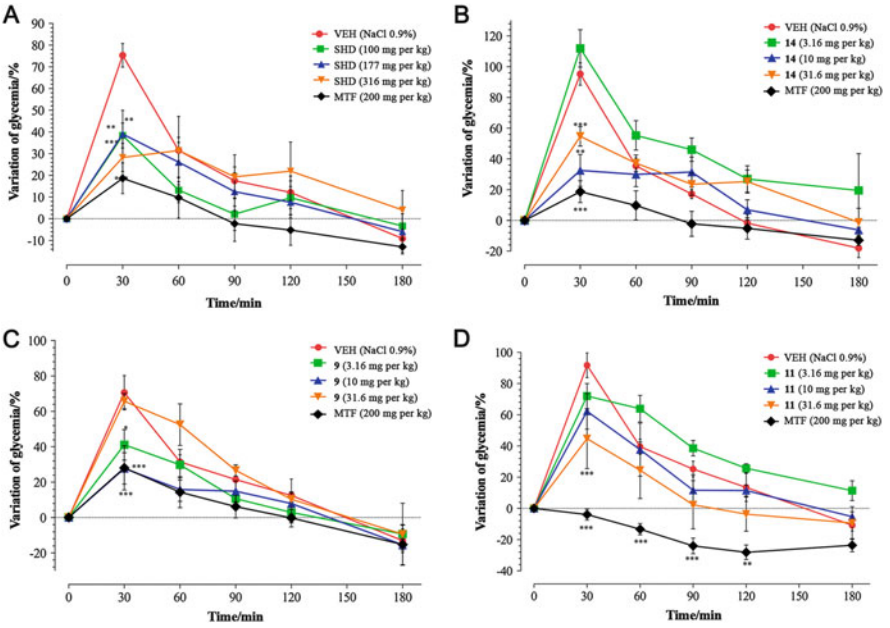


Fig. 3 Effect of SHD (**A**), mexicanolide **14** (**B**), humulin B (**9**) (**C**), and methyl-2-hydroxy-3β-tigloyloxy-1-oxomeliac-8(30)-enate (**11**) (**D**) on blood glucose levels in NAA-STZ-hyperglycemic mice during an OGTT. VEH: vehicle; MTF: metformin. Values are expressed as the means from six data points ±SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Adapted from [13]

saline), placed under the skin of the dorsal surface of the right hind paw. The response observed is the amount of time the animals spend licking the injected paw. Two distinct periods of high licking activity can be identified, an early phase lasting for the first 5 min and a late phase lasting from 20 to 30 min after the injection of formalin. The two phases in the formalin test may have different nociceptive mechanisms. The early phase seems to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord; this pain can be inhibited by anti-inflammatory drugs [14]. Thus, local injection of SHD (10–177 μg) and mexicanolide **14** (0.5–3.5 μg) provoked a concentration-dependent antihyperalgesic action in NAA-STZ hyperglycemic mice (Fig. 4). Ketanserin (6 μg), a 5-$HT_{2A/C}$ receptor antagonist, and flumazenil (6 μg), a $GABA_A$ receptor antagonist, abolished the antihyperalgesic effect of mexicanolide **14** (3 μg) (Fig. 5). On the other hand, naloxone (3 μg), L-arginine (50 μg), and N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME; 150 μg) diminished the antihyperalgesic effect of mexicanolide **14** (Fig. 6). The aqueous extract of the seeds possesses significant antihyperalgesic action [15]. Thus, *S. humilis* seeds have shown also promising results for managing secondary complications (neuropathic pain) of diabetes.

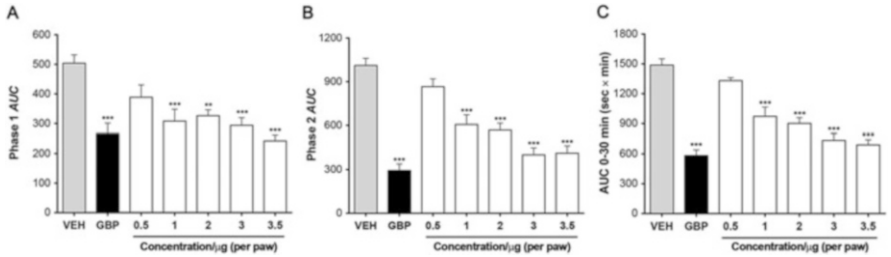


Fig. 4 Antihyperalgesic effect of mexicanolide **14** in NAA-STZ hyperglycemic mice during phases 1 (**A**), 2 (**B**), and the total area under the curve (**C**) in the formalin test. VEH: vehicle; GBP: gabapentin (30 μg per paw) was used as positive control. Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.01$ and $**p < 0.001$. Adapted from [15]

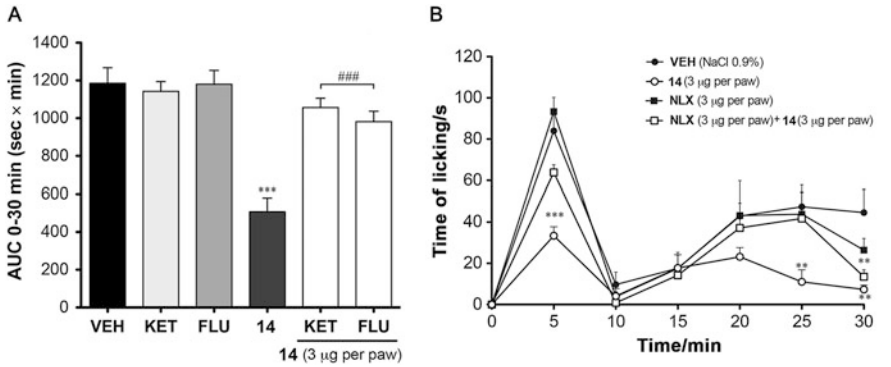


Fig. 5 Possible antihyperalgesic mechanism of mexicanolide **14** (3 μg per paw) in NAA-STZ hyperglycemic mice during the formalin test: serotoninergic, GABAergic (**A**), and opioid modulation (**B**). VEH: vehicle, ketanserin (KET, 6 μg per paw), flumazenil (FLU, 6 μg per paw), and naloxone (NLX, 3 μg per paw). (**A**) Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. (**B**) Each point represents the mean of the time of licking (sec) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Adapted from [15]

Swietenia humilis and/or its limonoids represent promising alternatives for development as safer and cheaper phytotherapeutic agents. The overall results described in the paragraphs above support the use of seeds of this tropical species for treating diabetes in contemporary Mexico. Finally, it is worth mentioning that the potential antiamebiasis effects of the limonoids and extracts from this plant were tested, with negative results being obtained. Moreover, most of the limonoid constituents and an organic extract from *S. humilis* were active against the European corn borer, *Ostrinia nubilalis*, affecting important life cycle parameters such as reduction of the % pupation and the % of adult emergence, in a similar way to the positive control toosendanin [16, 17]. They also inhibited radical growth of a several weed species

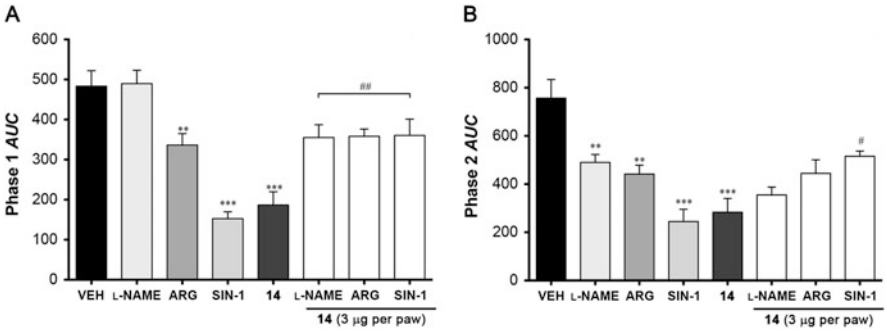


Fig. 6 Possible antihyperalgesic mechanism of mexicanolide **14** (3 µg per paw) in NAA-STZ hyperglycemic mice during phases 1 (**A**) and 2 (**B**) on the formalin test: nitrergic modulation. VEH: vehicle, L-NAME (150 µg per paw), L-arginine (ARG, 50 µg per paw), and 3-morpholinosydnonimine hydrochloride (SIN-1, 200 µg per paw). Each bar represents the mean area under the curve (AUC, time of licking against time, sec × min) from six data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Adapted from [15]

when tested in vitro [18]. In consequence, this species seems valuable not only as a medicinal agent but also as a pesticide.

2.1.2 Mexican "Copalchis": *Hintonia latiflora*, *Hintonia standleyana*, and *Exostema caribaeum*

Hintonia latiflora (Sessé & Moc. ex DC.) Bullock (Rubiaceae) is a species endemic to Mexico, while *H. standleyana* Bullock has a wider distribution area up to Northern Central America. *Hintonia standleyana* was considered to be synonym of *H. latiflora*, which is still widely accepted by some authors, however, recent molecular evidence has revealed that these two species are significantly different [19–21]. Both species are known commonly as "copalquin" and "copalchi", among other colloquial names. The plants are shrubs or trees up to 8 m tall, with gray stems; the leaves are bright green and covered with hairs on the back (Fig. 7). The main area

Fig. 7 Mexican "Copalchis": *Hintonia latiflora* (**A**), *Hintonia standleyana* (**B**) and *Exostema caribaeum* (**C**)

supplying the commercial "copalchi" is the northern state of Guerrero, Mexico. Teas from the bark of these species are used in modern Mexico for a variety of health problems, including malaria, stomach ulcers, diabetes, obesity, infections and fevers. In addition, the Tarahumaras have used *H. latiflora* on body sores [22].

Exostema caribaeum (Jacq.) Schult. (Rubiaceae), the Caribbean prince wood, is an evergreen slender shrub or small tree up to 12 m height (Fig. 7). The plant occurs on all islands within the Bahamian Archipelago, as well as the rest of the Caribbean region, Florida, Mexico, and Central America. In Mexico, the plant is gathered from the wild for local use as a medicine to treat fevers, especially those related to malaria, and also a source of lighting and timber. This species is also regarded as "copalchi", and in some local markets its stem bark is mixed with those of *H. latiflora* or *H. standleyana* [23].

The hypoglycemic and diuretic properties of *H. latiflora* were discovered clinically by researchers at the Instituto Médico Nacional in Mexico City at the beginning of twentieth century (Fig. 8). They also discovered some chemical compounds present that were later on rediscovered by German, French, and Mexican researchers. It is notable that in 1913, when the Instituto Médico Nacional closed, "copalchi" was reintroduced in Europe for the treatment of diabetes. Later on, researchers in Germany and France corroborated the earlier work of the Mexican scientists. Recently, the most relevant historical aspects about this species as well as the research carried out by other scientists were reviewed [12]. Perhaps the most relevant aspect of these historical events was that, after the Royal Botanical Expedition to New Spain (1787–1803), led by Martín Sessé and José Mociño, *H. latiflora*,

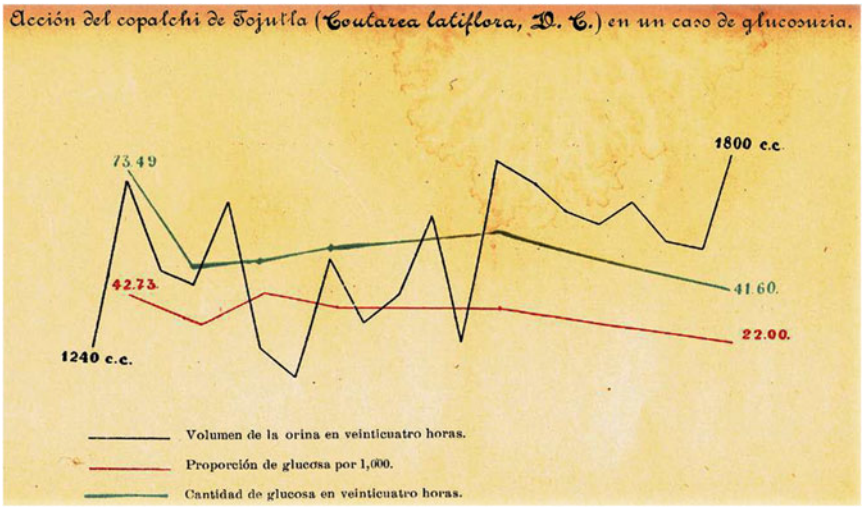
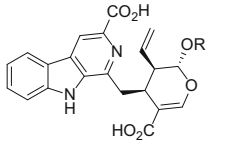


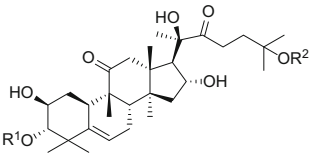
Fig. 8 Hypoglycemic and diuretic effects exerted by a *Hintonia latiflora* hydroalcoholic extract in a clinical trial conducted at IMN, Mexico City. Urine volume during a 24-h period (black line); amount of glucose in urine during a 24-h period (green line); proportion of glucose per liter (red line). Adapted from [12]

under its synonym *Coutarea latiflora* Sessé & Moc. ex DC., and *E. caribaeum* appeared in the list of the most important "Medicinal Plants of New Spain". They were also included in the well-known "Torner Collection" of Sessé and Mociño biological illustrations. Thus, in the following paragraphs, we will review mostly the work carried out by our group.

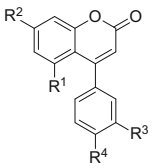
Phytochemical analysis of the stem bark of these three plants allowed the discovery of cucurbitacins in the Rubiaceae family, as well as the characterization of several 4-phenylcoumarins, with most being new chemical entities, and the indole alkaloid desoxycordifolinic acid (**15**) [23–31]. The basic core of the cucurbitacins **16**–**19** is dihydrocucurbitacin F (**16**) (Fig. 9). The 4-phenylcoumarins **20**–**36** of the three species are 5,7,3′,4′- or 5,7,4′-substituted with oxygenated functionalities, with the former having the most common pattern; the sugar unit is usually a monosaccharide (*β*-D-galactose, *β*-D-glucose, 6″-acetyl-*β*-D-glucose or 6″-acetyl-*β*-D-galactose), although some disaccharides have been found (*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranose or *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranose) (Fig. 9). In all cases, the saccharide unit is attached to the hydroxy group at C-5. During the course of our investigations it was demonstrated that 4-phenylcoumarins undergo oxidative cyclization under aerobic alkaline conditions to give oxido-4-phenylcoumarins.

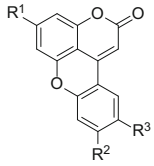


15 (desoxycordifolinic acid) R = *β*-D-glucopyranosyl



16 (dihydrocucurbitacin F) R^1 = H, R^2 = H
17 R^1 = H, R^2 = Ac
18 R^1 = *β*-D-glucopyranosyl, R^2 = H
19 R^1 = *β*-D-glucopyranosyl, R^2 = Ac





20 R^1 = R^2 = R^4 = OCH_3, R^3 = H
21 R^1 = R^2 = R^4 = OCH_3, R^3 = OH
22 R^1 = *O*-*β*-D-galactopyranosyl, R^2 = OCH_3, R^3 = R^4 = OH
23 R^1 = R^3 = R^4 = OH, R^2 = OCH_3
24 R^1 = *O*-*β*-D-glucopyranosyl, R^2 = OCH_3, R^3 = R^4 = OH
25 R^1 = *O*-*β*-D-glucopyranosyl, R^2 = R^3 = R^4 = OH
26 R^1 = R^2 = R^3 = R^4 = OH
27 R^1 = 6″-*O*-acetyl-*O*-*β*-D-glucopyranosyl, R^2 = R^3 = R^4 = OH
28 R^1 = *O*-*β*-D-galactopyranosyl, R^2 = OCH_3, R^3 = H, R^4 = OH
29 R^1 = *O*-*β*-D-glucopyranosyl, R^2 = R^4 = OCH_3, R^3 = OH
30 R^1 = 6″-*O*-acetyl-*O*-*β*-D-galactopyranosyl, R^2 = R^4 = OH, R^3 = H
31 R^1 = 6″-*O*-acetyl-*O*-*β*-D-galactopyranosyl, R^2 = R^3 = R^4 = OH
32 R^1 = *O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyl, R^2 = OCH_3, R^3 = R^4 = OH
33 R^1 = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, R^2 = OCH_3, R^3 = R^4 = OH
34 R^1 = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, R^2 = R^4 = OCH_3, R^3 = H

35 R^1 = OCH_3, R^2 = R^3 = OH
36 R^1 = R^2 = OCH_3, R^3 = OH

Fig. 9 Compounds isolated from Mexican "Copalchis"

Thus, 7-methoxy-5,3′,4′-trihydroxy-4-phenylcoumarin (**23**) was converted to 7-methoxy-4′,5′-dihydroxy-4-phenyl-5,2′-oxido-coumarin (**35**) by treatment with potassium hydroxide in methanol. Since the reaction took place only in basic conditions and in the presence of air, it might proceed via an oxidative phenol coupling process.

Preclinical toxicity studies have revealed that none of the aqueous (traditional preparations) extracts from the stem bark of the two above-mentioned *Hintonia* species and *E. caribaeum* were toxic to mice ($LD_{50} > 5$ g/kg). These results thus suggest the preclinical safety of the traditional preparations of these three plants [32, 33]. The organic extracts of *H. latiflora* and *E. caribaeum*, however, showed LD_{50} values of 2900 and 700 mg/kg, respectively; the extract of *E. caribaeum* generated tremors, respiratory distress as well as decreases in motor activity and in body weight, by 27.1% with respect to the vehicle-treated animals. The organic extract of *H. standleyana* had an LD_{50} of > 5 g/kg. Moreover, none of the extracts induced mutagenic effects when assayed by the Ames test [33]. Rivera et al. [34] reported that a methanol extract from *H. latiflora* induced genotoxic effects, piloerection, excitability, dyspnea, anoxia, mydriasis, tachycardia, overcrowding, decreased muscle tone, burying behavior, and ambulatory movements in a dose-dependent manner in mice. These effects were only observed during the first 24 h of the experiment. At the end of the study (15 days after treatment), all surviving mice showed a normal behavior [34]. Our group has worked extensively with *Hintonia* species and never observed such effects, even in long-term experiments. Unfortunately, the authors did not provide chromatographic profiles of their extract nor a voucher number to compare the plant material they analyzed [34].

The long-term hypoglycemic effect of the organic extracts (CH_2Cl_2:MeOH = 1:1) of the three species (*H. latiflora, H. standleyana*, and *E. caribaeum*) and a commercial mixture of "copalchi" (composed by *H. standleyana* and *E. caribaeum*), and compounds **18**, **22**, **24**, **25**, and **32** (15 mg/kg each time) was established (Fig. 10) [31]. The extract of *H. latiflora* and compound **25** restored blood glucose levels to normal values, with the effect being comparable to that of glibenclamide. Compounds **22** and **24** also restored blood glucose levels to near normal values by the end of the experiment. During this study, it was also demonstrated that the extract of *H. latiflora* regulated both hepatic glycogen and plasma insulin levels ($p < 0.05$) (Fig. 11). These data suggested that its hypoglycemic effect is due in part to stimulation of insulin secretion and regulation of hepatic glycogen metabolism. Comparison of the hypoglycemic activity of the 4-phenylcoumarins tested established that the most active compounds possess a free hydroxy group at C-7 in the 4-phenylcoumarin core. On the other hand, comparison of the activity of all glycosides tested indicated that the nature of the sugar moiety (glucose, galactose, *β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranose or *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranose) had little or no influence on the resultant biological activity [31]. The hypoglycemic activity of such cucurbitacin-type compounds was demonstrated for the first time in these studies.

Infusions of the stem bark of both *Hintonia* species and *E. caribaeum* also showed hypoglycemic and antihyperglycemic effects. The later was demonstrated

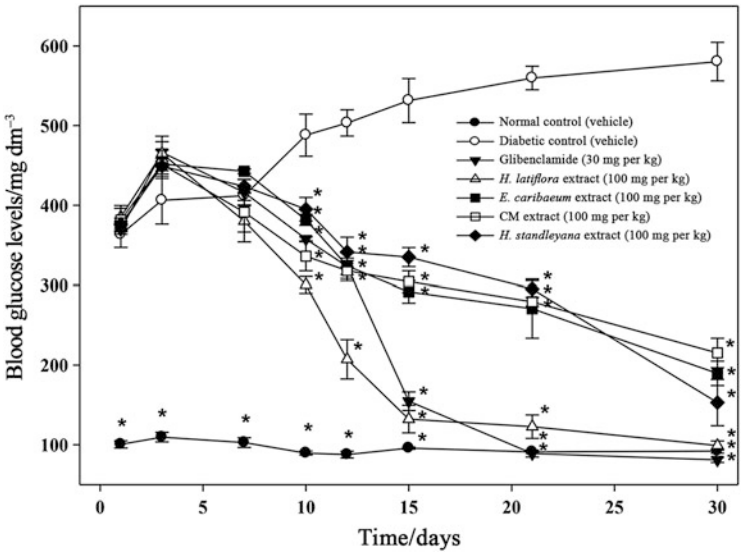


Fig. 10 Long-term effect of extracts of *Hintonia latiflora*, *Hintonia standleyana*, *Exostema caribaeum*, and CM (commercial mixture of "copalchi") on blood glucose levels in NAA-STZ-diabetic rats. Each value is the mean from six data points ±SEM. *$p < 0.05$. Adapted from [31]

during an OSTT in mice suggesting that the plants contained inhibitors of α-glucosidases [12]. In addition, the major components of the infusions were 4-phenylcoumarin glycosides: in the case of *H. latiflora*, the most abundant compound was **33**, for *H. standleyana*, the most relevant was **32**, while for *E. caribaeum* this was **22**. The α-glucosidase inhibitory activities of several glycosides present in the infusions and the aglycones **23** and **26** were demonstrated in vitro using different enzymes. This assay was carried out using a well-known spectrophotocolorimetric procedure that measures the ability of any α-glucosidases (baker's yeast, *Ruminococcus obeum* or mammalian) to hydrolyze a suitable substrate (*p*-nitrophenyl-α-D-glucopyranoside) in the presence of the potential inhibitor; acarbose was also used as positive control. Docking studies, using AutoDock software, predicted that the aglycone **23** ($IC_{50} = 3.0$ μ*M* vs. 0.41 m*M* for acarbose), which turned out to be the most active inhibitor, binds to the yeast α-glucosidases in the same pocket as acarbose. Coumarin **23** and glycoside **32** were also very active in an OStTT, thus indicating that both compounds possess also α-amylase inhibitory activity [12].

For the crude drug (stem bark) of the three species, reliable, reproducible, and accurate high-performance liquid chromatography (HPLC)-UV methods were developed for the quantitative determination of the active compounds (Fig. 12). These methods were included in the second edition of the "Mexican Herbal Pharmacopoeia" [23, 35]. The development of the composition and identity tests for the three "copalchi" species have been very useful to detect the adulteration of

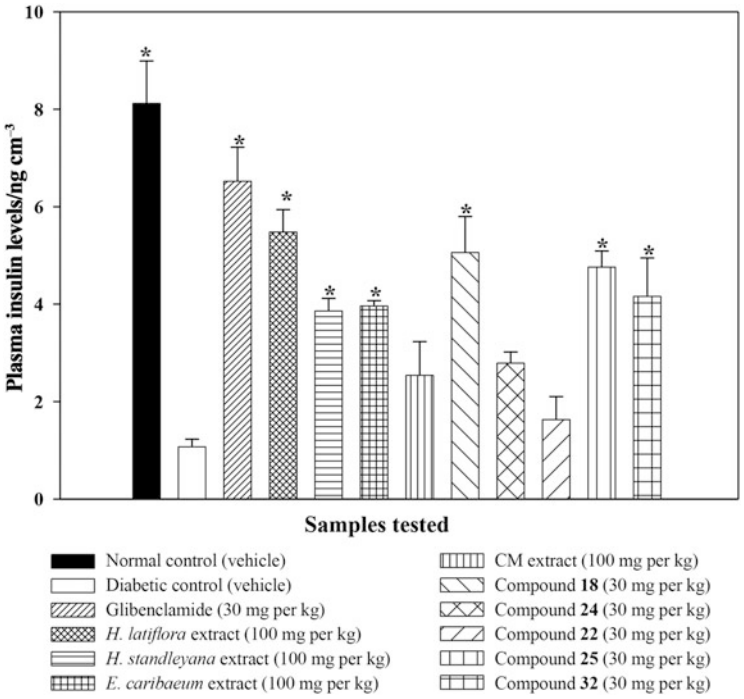


Fig. 11 Effect of extracts of *Hintonia latiflora*, *Hintonia standleyana*, *Exostema caribaeum*, and CM (commercial mixture of "copalchi"), cucurbitacin **18**, and 4-phenylcoumarins **22**, **24**, **25**, and **32** on plasma insulin levels in STZ-diabetic rats. Each value is the mean from six data points ±SEM. $^*p < 0.05$. Adapted from [31]

H. latiflora with *E. caribaeum*. Indeed, the analysis of the commercial crude drugs or preparations made up with "copalchi" samples, Mexican or from abroad, have revealed clearly that most preparations contain a mixture of the two "copalchi" components, with *E. caribeum* almost always the more abundant in the mixture.

The organic extracts [CH_2Cl_2-MeOH (1:1)] and infusions from the leaves of *H. standleyana* and *H. latiflora* were also hypoglycemic and antihyperglycemic, in both normal and hyperglycemic rats. These extracts did not provoke death or damage, behavioral alterations, lesions or bleeding of the internal tissues to the animals, throughout the experiments conducted [32]. Therefore, the leaves of *Hintonia* species represent a therapeutic alternative to the stem bark in terms of conservation, since these species have been extensively exploited and commercialized locally and outside of Mexico from harvesting wild plants. In consequence, the populations of the plants are now scarce and in danger of extinction. From the active leaf extracts, three additional 4-phenylcoumarins (**37**–**39**) were obtained (Fig. 13) [32]. In addition, HPLC profiles of the leaf extracts of both plants revealed the presence of several hypoglycemic 4-phenylcoumarins isolated from the stem bark as well as ursolic (**40**) and chlorogenic (**41**) acids (Fig. 13). The overall results indicated

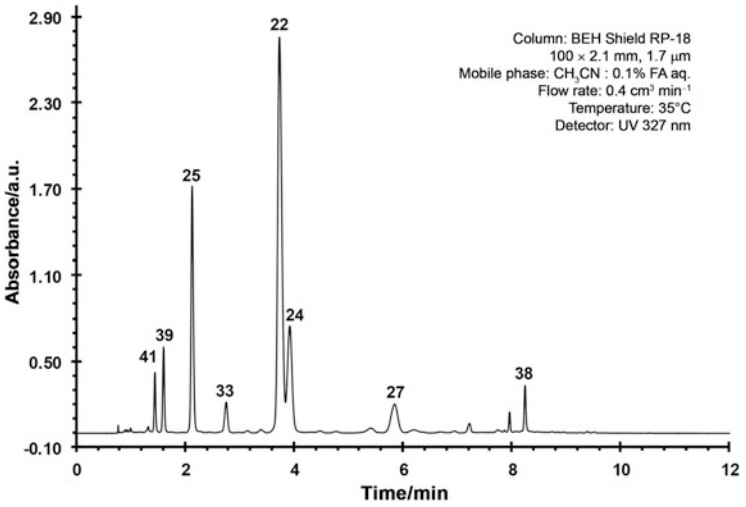
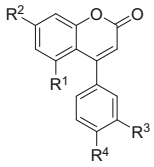
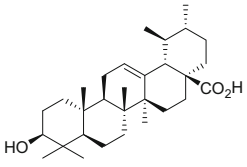


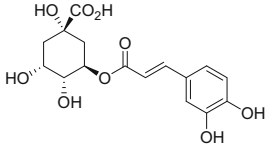
Fig. 12 UHPLC-PDA chromatogram of *Exostema caribaeum* stem bark aqueous extract under optimized conditions; detection wavelength 327 nm. Adapted from [23]



37 R^1 = 6"-*O*-acetyl-5-*O*-*β*-D-galactopyranosyl, R^2 = R^4 = OH, R^3 =H
38 R^1 = 6"-*O*-acetyl-5-*O*-*β*-D-galactopyranosyl, R^2 = R^3 = R^4 = OH
39 R^1 = *O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranosyl, R^2 = R^4 = OCH_3, R^3 = H



40 (ursolic acid)



41 (chlorogenic acid)

Fig. 13 Structures of 4-phenylcoumarins **37**–**39**, and ursolic (**40**) and chlorogenic (**41**) acids

that the leaves of both species possess similar antidiabetic actions to their stem bark. Phenological and geographical analysis of the leaves from two different regions of Mexico (Chihuahua and Michoacán), using a validated UPLC method, revealed that the best harvesting season for the leaves of *H. latiflora* is between the leaf renovation and senescence stages, avoiding the flowering period [32]. In addition, no significant differences were found among the two different geographical populations analyzed.

Recently, a dry concentrated extract from the stem bark of *H. latiflora* in capsule form was tested in an open prospective clinical study in 41 dietetically stabilized subjects with T2DM for 6 months [36]. The results revealed that fasting and postprandial glucose levels and HbA_{1c} values all declined significantly. Moreover, the tolerance was excellent, and liver and lipid values tended to be positively affected. In particular, no side effects and no hypoglycemic episodes or worsening of diabetic symptoms occurred. These results were in agreement with earlier studies [36]. Thus, the use of *Hintonia* dry extract for treating mild to moderate T2DM can be regarded as safe and useful in cases where dietary measures alone cannot provide adequate control of the disease.

Vierling and collaborators showed also that an extract of *H. latiflora* exerted a vasodilatory effect in vitro in aortic rings of the guinea pig and in vivo in rabbits [37]. Aortic rings pre-contracted with noradrenaline (NA) could be relaxed completely by the extract (EC_{50} = 51.98 mg/dm^3). The aglycone also inhibited NA-induced contractions of aortic rings (EC_{50} = 32.55 mg/dm^3) and in aortic cells suppressed calcium transients evoked by vasopressin at a concentration of 60 mg/dm^3, suggesting a possible inhibition of G-protein-induced intracellular calcium release. Ultrasound measurements in conscious rabbits showed that the extract induced vasodilation and lowering of blood flow velocity in the abdominal aorta and the carotid artery. The combination of a blood glucose-lowering with a vasodilating effect may be helpful for reducing vascular long-term complications in patients with T2DM.

During our earlier work on *H. latiflora*, the in vitro anti-*Plasmodium falciparum* activity of the extracts or isolates was not demonstrated. The same type of results were obtained by Noster and Kraus in Germany [38], who found only a moderate effect in vitro with *E. caribaeum* extracts. However, in a paper by Argotte-Ramos et al. [39], it was reported that an ethyl acetate extract of the stem bark of *H. latiflora* provoked suppression of induced parasitemia with *P. berghei* in mice. Bioassay-directed fractionation of the active extract using in vitro and in vivo assays indicated that **23** is the active principle. Also, Rivera and coworkers [34] found that a methanolic extract of *H. latiflora* had an excellent chemosuppression of *P. yoelii* total parasitemia and schizonts number in CD1 male mice in a 4-day test protocol. As indicated above, in this work no voucher nor chemical composition of the plant was provided. Hence, there is an uncertainty about the precise identity of the plant material that was actually analyzed.

The organic extract of *H. standleyana* showed a potent antinociceptive effect when tested in hot-plate and writhing tests [29]. The hot-plate test is a suitable method to evaluate central antinociceptive activity. In addition, this model measures animal behavior and has good sensitivity and specificity. It is based on the principle that when rodents are placed onto a hot surface they will initially demonstrate aversive behaviors to the thermal stimulus by licking their paws and jumping. Compounds that alter the nociceptive threshold increase the latency period to the observed licking/jumping. On the other hand, the acetic acid-induced writhing test, is a classical visceral pain model useful to detect painful symptoms associated with inflammatory disorders of the internal organs such as the stomach or intestines.

The metabolite responsible for this antinociceptive activity of the extract was found to be compound **18**, which significantly reduced the acetic acid-induced abdominal constrictions in mice. In addition, this compound produced a significant increase in thermal latency in the hot-plate test (Fig. 14) [29]. The effect of **18** was

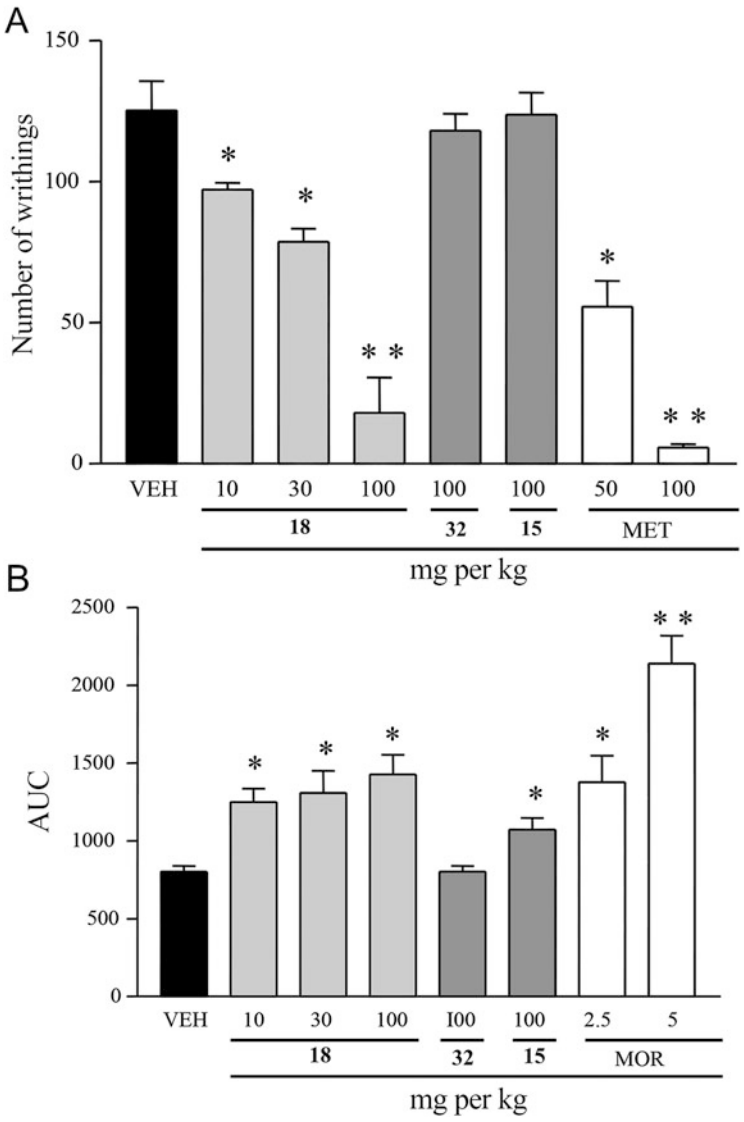


Fig. 14 Antinociceptive effects of desoxycordifolinic acid (**15**), cucurbitacin **18** and 4-phenylcoumarin **32** in mice submitted to the writhing (**A**) and hot-plate (**B**) tests. Metamizol (MET) and morphine (MOR) were used as positive controls in the writhing and hot-plate tests. In both cases, bars are the means from six data points ±SEM. *$p < 0.05$ and **$p < 0.01$. Adapted from [29]

mediated by the nitric oxide-cyclic GMP pathway at the peripheral and/or central levels, opening of ATP-sensitive K^+ channels, and activation of the opioid receptors or increasing the levels of endogenous opioids. Altogether, these results suggested that the extract of *H. standleyana* investigated is able to reduce inflammatory and central pain in mice [29].

The aqueous extracts from the stem bark and leaves of *H. latiflora* and *H. standleyana* (80.5%, 80.2%, 75.1%, and 76.9% of gastroprotection, respectively), as well as compounds **32** ($ED_{50} = 15$ mg/kg) and **38** ($ED_{50} = 26$ mg/kg), were able to inhibit ethanol-induced gastric lesions in rats [40]. Intragastric application of absolute ethanol to produce gastric lesions in experimental animals is a well-known and reproducible method to investigate cytoprotective agents. The mode of action of **32** and **38** involved non-protein sulfhydryl endogenous compounds, because when the rats were pretreated with *N*-ethylmaleimide (NEM), a sulfhydryl alkylator, their gastroprotective action was inhibited [40].

Both *Hintonia* species contain more than one active constituents that act on different targets. Accordingly, these plants exhibit a wide range of biological properties, which collectively could be useful for treating a multifactorial disease such as diabetes.

2.1.3 *Salvia circinata*

Salvia circinata Cav. (syn. *S. amarissima* Ortega) (Lamiaceae) is a perennial shrub native to Mexico (Fig. 15). It grows up to 1.5 m tall. The whitish green oval leaves are rough or wrinkled and usually downy. The flowers, usually pale blue, feature tubular two-lipped corollas and produce nutlet fruits [41]. *Salvia circinata* was also listed as a medicinal in the catalog of plants from the Royal Botanical Expeditions to New Spain. A tea brewed from the aerial parts of the plant is used in Mexican folk medicine for treating ulcers, helminthiasis, and diabetes. According to the Lorke criterion, this tea did not provoke behavior alterations, macroscopic tissue injury, or weight loss, when tested during 14-day in mice; the estimated LD_{50} was higher than 5 g/kg [41].

Single oral administration of the traditional preparation (100–570 mg/kg) to normal and NAA-STZ hyperglycemic mice induced a perceptible decrease of blood glucose level. During an OSTT, the infusion (31.6, 100, and 316 mg/kg) also significantly reduced the postprandial peak when compared with a vehicle-treated group; the effect observed was comparable to that of the positive control, acarbose. However, the preparation (100–570 mg/kg) did not induce a significant drop in the postprandial peak after a glucose challenge in normal and hyperglycemic mice. These results strongly suggested that the antihyperglycemic effect of *S. circinata* infusion could be due to the presence of α-glucosidase inhibitors, which may be able to prevent postprandial hypersecretion of insulin and reactive hypoglycemia [41]. The active compounds included a few new glucosides of

Fig. 15 *Salvia circinata*

tricyclic neo-clerodane type diterpenoids with the six-membered rings *trans*-fused, and the side chain fragment forming ethylbutenolides, ethylfuran or linear substructures. These terpenoids were given the trivial names amarisolides A–E (**42**–**46**) (Fig. 16). Several flavonoids (**47**–**50**) were also among the active principles (Fig. 16). All compounds were characterized on the basis of their spectroscopic properties. The absolute configurations at the stereocenters of diterpenoids **42**–**46** were established by comparison of the experimentally obtained and calculated electronic circular dichroism spectra [41].

Compounds **42**–**50** were tested in vitro against rat small intestine α-glucosidase. The more active compounds were the flavonoids, in particular, compound **47**, which showed an IC_{50} value of 39 μM and was 2.5 times more active than acarbose ($IC_{50} = 100\ \mu M$). Flavonoids **48**–**50** exhibited IC_{50} values of 810, 200, and 1800 μM, respectively. Regarding the diterpenoids, the most active compound was **42**, with an IC_{50} value of 500 μM. Compounds **42** and **48** were also tested against a recombinant α-glucosidase with maltase-glucoamylase activity from *Ruminococcus obeum*, a bacterium found in the human intestine that is involved in carbohydrate metabolism. This enzyme is phylogenetically closer to human *N*-maltase-glucoamylase than those of rats. The results of the assays showed that **42** and **48** inhibited the activity of the pure enzyme with IC_{50} values of 400 and 60 μM,

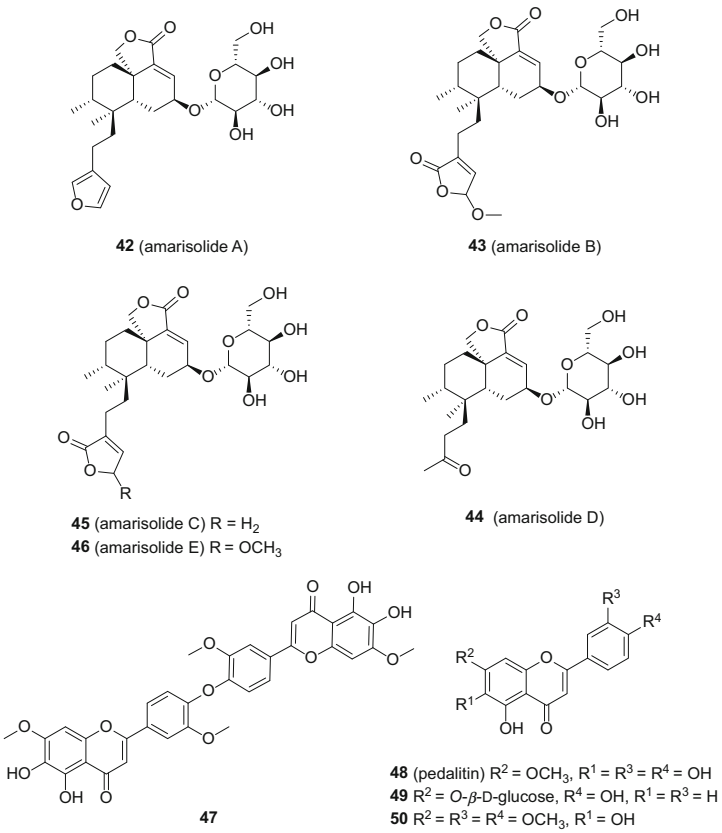


Fig. 16 Compounds isolated from *Salvia circinata*

respectively ($IC_{50} = 1030\ \mu M$ for acarbose). In vivo evaluation of these compounds reduced significantly the postprandial peak in a dose-dependent manner during an OSTT in mice (Fig. 17). In both cases, the effect was comparable to that of the positive control, thus revealing their antihyperglycemic potential. Finally, docking and molecular dynamics analyses of the *R. obeum* α-glucosidase complexes with **42**, **48**, and acarbose, predicted that the three products bind to the catalytic site of the enzyme (Fig. 18). The molecular dynamics studies indicated that the binding of the compounds was stable during the period of analysis (20 ns) [41].

Salvia circinata biosynthesizes also several *seco*-clerodane diterpenoids, some of which are moderately cytotoxic against human cancer cell lines and exhibited modulatory activity in a breast cancer cell line resistant to vinblastine [42, 43].

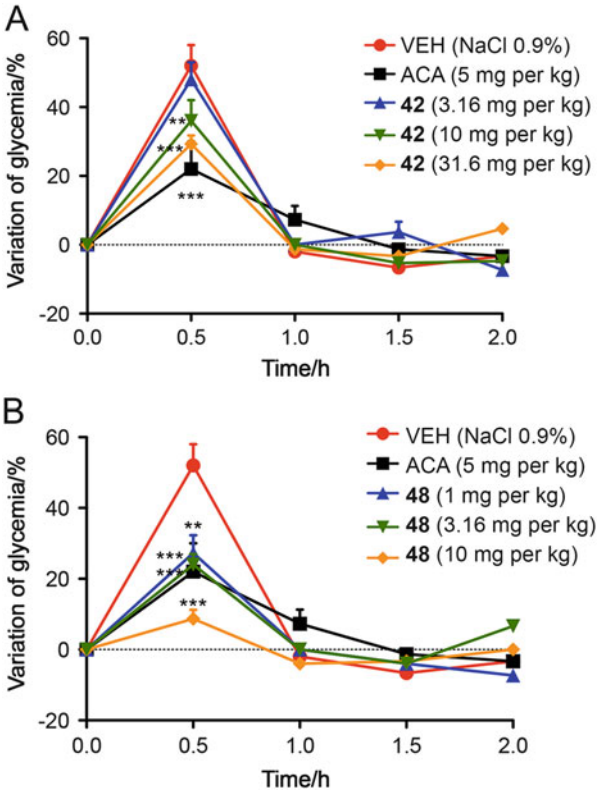


Fig. 17 Antihyperglycemic action of amarisolide A (**42**) (**A**) and pedalitin (**48**) (**B**) in normoglycemic mice during an OSTT. VEH: vehicle; ACA: acarbose. Each point represents the mean from six data points ±SEM. $^{**}p < 0.01$ and $^{***}p < 0.001$. Adapted from [41]

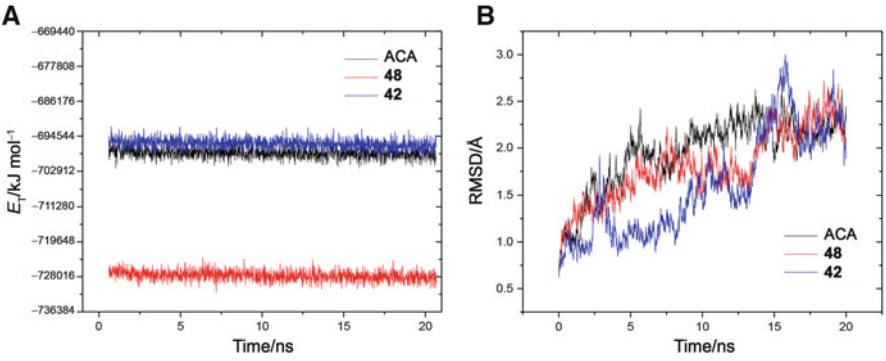


Fig. 18 Molecular dynamics trajectory analysis: Total energy of the system as a function of the time of acarbose (ACA), amarisolide A (**42**) and pedalitin (**48**) (**A**), and RMSD as a function of time (**B**). Adapted from [41]

2.2 *Smooth Muscle-Relaxant Agents for Gastrointestinal and Cardiovascular Illnesses*

Ethnobotanical reports refer to the use of several herbs for the treatment of stomachache (with or without diarrhea), pains of hepatic and splenic origin, or related functional disorders (irritable bowel syndrome, abdominal bloating, and dyspepsia). More than 400 plant extracts have been examined for their spasmolytic action [44–49], but only a few studies have resulted in the isolation and identification of the active principles concerned.

Hypertension affects around 48 million of adults in Mexico and is expected to increase with rising rates of obesity. Hypertension greatly increases the risk of cardiovascular diseases such as ischemic heart disease (the second leading cause of death in Mexico) and stroke (the third leading cause of death in Mexico). It is also a co-morbidity very common in diabetic patients [50]. Different plants with antihypertensive properties are used in Mexican folk medicine [51]. A preliminary pharmacological survey of plants used in Mexico for the treatment of hypertension indicated that 186 plant species belonging to 163 genera and 76 families are used for treating hypertension. The authors pointed out that 47% of the total had been studied at least once from the phytochemical point of view, with 74% subjected to investigations using mostly in vitro pharmacological assays.

On the basis of the above considerations, we investigated three orchid species and the results are summarized in the next few paragraphs. It is worth mentioning that two papers summarizing some of the work carried out in Mexico regarding spasmolytic plants and their active principles were published recently [52, 53]. In addition, the Appendix Table summarizes selected medicinal plants with important smooth muscle-relaxant properties.

2.2.1 *Scaphyglottis livida*, *Maxillaria densa*, and *Nidema boothii*

Mexico produces about 1260 species of orchids with many have been used medicinally since prehispanic times, but, nevertheless, only 9 have been subjected to pharmacological and chemical studies [54]. *Scaphyglottis livida* (Lindley) Schltr. (Orchidaceae) (Fig. 19) is an epiphytic orchid found from Mexico to tropical South America. It is widely distributed along the tropical forests of the state of Veracruz, where the plant colonizes coffee farms and contributes to the shade conditions required for coffee growth. Important characteristics of the plant are the presence of cylindrical pseudobulbs, 5–6 cm long, 0.3 cm wide, unifoliated, arising from each rhizome; the leaves up to 14 cm long and 0.1 cm wide and are linear and grass-like. The inflorescence is fasciculate, with a single flower opening in time. The flower is small with bracts 5 mm long. In Los Tuxtlas in the state of Veracruz, the ground herb is applied topically to the body of humans to eliminate ectoparasites, and for treating wounds; the decoction is employed for treating stomachache and to prevent miscarriage [55, 56].

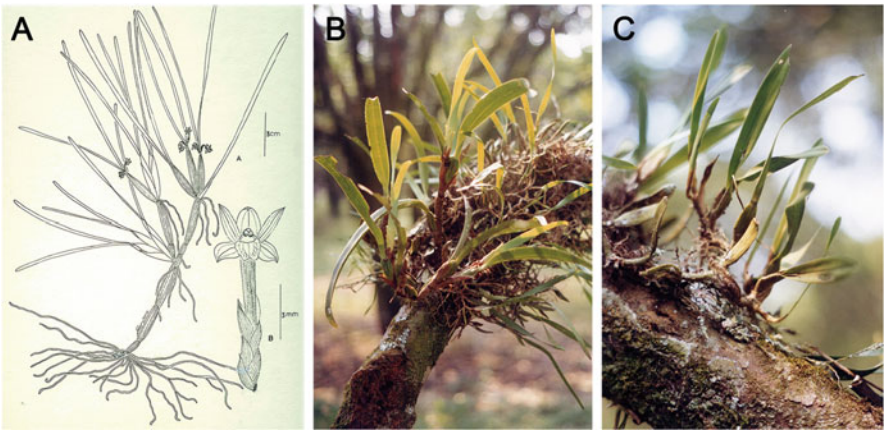


Fig. 19 *Scaphyglottis livida* (**A**), *Maxillaria densa* (**B**), and *Nidema boothii* (**C**)

For assessing the potential smooth muscle relaxant-effect, the plant extracts and compounds were analyzed by means of classical isolated tissue bath assays [14, 55, 57]. These tests are useful for recording responses elicited by increasing concentrations of the test materials on the isometric contractions of guinea-pig or rat ilea and rat thoracic aortic rings (with and without the endothelium), among other contractile tissues. The power of these techniques is in their simplicity and versatility; by recording responses elicited by increasing concentrations of the test material, in the presence or absence of antagonists, a myriad of information can be derived about the pharmacological characteristics of each compound from the extracts and the receptor to which they might bind.

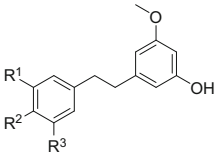
The application of the classical assays mentioned above showed that an organic extract of the whole plant of *S. livida* inhibited the spontaneous contractions of the rat ileum in a concentration-dependent manner ($IC_{50} = 6.0$ μg/cm^3) (Table 1) [58]. Bioassay-guided fractionation of the active extract led to the isolation of a series of stilbenoids including the bibenzyls gigantol (**51**) and batatasin III (**52**) as well as the phenanthrene derivatives coelonin (**53**), 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**), and denthyrsinin (**55**) (Fig. 20). These compounds showed also noted spasmolytic action, better than or comparable with papaverine. Different pharmacological experiments demonstrated that the smooth muscle-relaxant effect of these stilbenoids was mediated by neuronal release of nitric oxide.

Gigantol (**51**) produced the generation of NO/cGMP in the rat whole ileum as detected by a radioimmunoassay [55]. Compound **51** also inhibited the complex Ca^{2+}-calmodulin (CaM)-CaM-sensitive phosphodiesterase 1 (PDE1) and potentiated the antispasmodic action of chlorpromazine, a classical CaM inhibitor [57]. In addition, it quenched the extrinsic fluorescence of the human CaM biosensor, *h*CaMeM124C-mBBr (Fig. 21) [59]. Altogether, these results suggested that gigantol (**51**) is a CaM inhibitor and that its smooth muscle-relaxant activity is

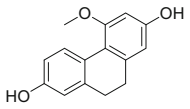
Table 1 Inhibition of spontaneous contractions of the isolated rat ileum induced by an extract and compounds from *Scaphyglottis livida*

Compound	$E_{max}/\%^{a,b}$	$IC_{50}/\mu M^{c}$	Potency[c]
Papaverine	96.70 ± 5.02	1.55 ± 0.12	1.00
Extract	82.10 ± 6.02	6.06 ± 1.02[d]	–
Gigantol (**51**)	93.50 ± 3.02	5.83 ± 0.55	0.27
Batatasin III (**52**)	85.20 ± 2.08	0.74 ± 0.07	2.10
Lusianthridin (**53**)	80.00 ± 1.98	0.95 ± 0.03	1.63
3,7-Dihydroxy-2,4-dimethoxyphenanthrene (**54**)	83.59 ± 1.30	0.66 ± 0.01	2.33
Denthyrsinin (**55**)	88.05 ± 1.80	7.13 ± 0.42	0.22

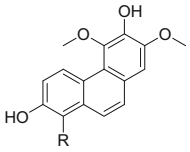
[a]Indicates the percentage of maximum inhibition
[b]Means ±SEM; $n = 6$; $p < 0.05$
[c]Potency was obtained by the formula: $IC_{50}/\mu M_{papaverine}/IC_{50}/\mu M_{compound}$, assuming a value of 1.00 for papaverine
[d]The IC_{50} of the extract is expressed in μg/cm^3



51 (gigantol) $R^1 = OCH_3$, $R^2 = OH$, $R^3 = H$
52 (batatasin III) $R^1 = R^2 = H$, $R^3 = OH$



53 (coelonin)



54 R = H
55 (denthyrsinin) R = OCH_3

Fig. 20 Stilbenoids and phenanthrenes of *Scaphyglottis livida*

also mediated by CaM. More recently, the presence of gigantol (**51**) was reported as the spasmolytic agent of the medicinal Mexican orchid *Encyclia michuacana* [60].

Subsequently, we have screened several Mexican orchid extracts for their ability to relax the spontaneous rat ileum contraction and selected *Maxillaria densa* Lindley and *Nidema boothii* (Lindl.) Schltr. (Fig. 19) for fractionation. Following a similar strategy as described for *S. livida*, *M. densa* yielded six phenanthrene derivatives,

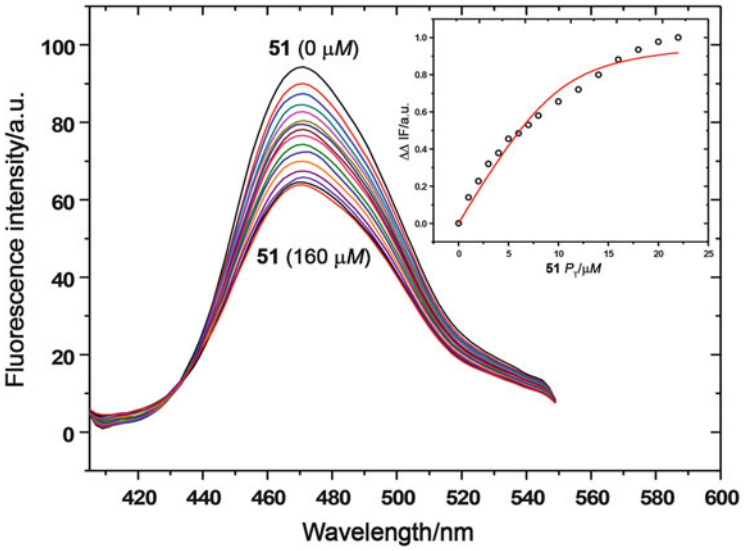
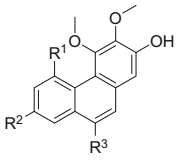
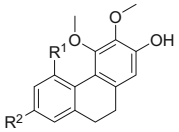


Fig. 21 Titration by fluorescence of *h*CaM M124C-mBBr engineered *h*CaM with gigantol (**51**). Adapted from [59]



56 R^1 = OH, R^2 = H, R^3 = H
58 (nudol) R^1 = H, R^2 = OH, R^3 = H
60 (fimbriol A) R^1 = OH, R^2 = H, R^3 = OCH_3
61 (gymnopusin) R^1 = H, R^2 = OH, R^3 = OCH_3



57 R^1 = OH, R^2 = H
59 (erianthridin) R^1 = H, R^2 = OH

Fig. 22 Phenanthrene derivatives of *Maxillaria densa*

namely, 2,5-dihydroxy-3,4-dimethoxyphenanthrene (**56**), 9,10-dihydro-2,5-dihydroxy-3,4-dimethoxyphenanthrene (**57**), nudol (**58**), erianthridin (**59**), fimbriol A (**60**), and gymnopusin (**61**) (Fig. 22) [61, 62]. Phenanthrenes **56**–**61** provoked also a concentration-dependent inhibition of the spontaneous contractions of the rat ileum with potencies comparable to papaverine. As an example, the smooth muscle-

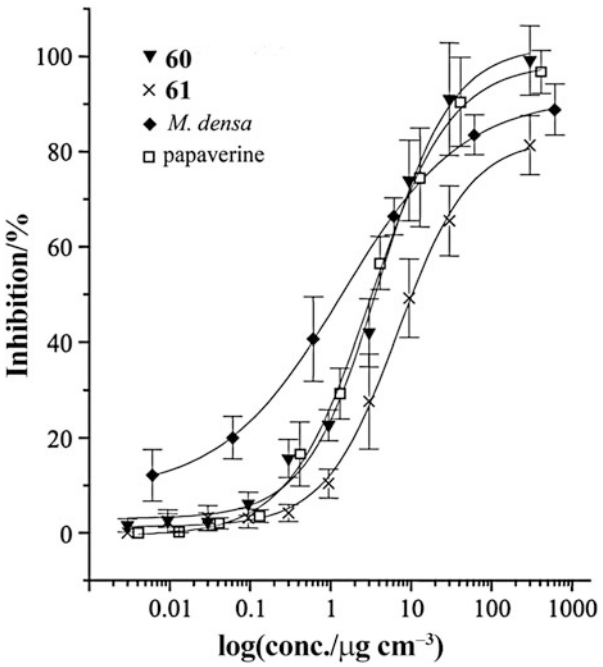


Fig. 23 Effect of fimbriol A (**60**) and gymnopusin (**61**) from *Maxillaria densa* on spontaneous contractions of the isolated rat ileum. Results are presented as means from eight data points ±SEM. $p < 0.05$. Adapted from [62]

relaxant effect of compounds **60** and **61** is shown in Fig. 23. The relaxant activity of these compounds did not involve a direct nitrergic or antihistaminergic mode of action or an interference with calcium influx into the smooth muscle cells.

Gigantol (**51**) and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**) induced also a significant concentration-dependent relaxation of the contractions evoked by NA in endothelium-intact and -denuded rat aorta rings. Incubation with L-NAME (1×10^{-5} *M*) or methylene blue (1×10^{-7} *M*) significantly reduced the relaxation induced by gigantol (**51**) and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**) (Fig. 24). The results suggested that two or more mechanisms are involved in the vasorelaxant effects of both compounds.

Erianthridin (**59**), fimbriol A (**60**), and gymnopusin (**61**) from *M. densa*, induced significant relaxant effects in a concentration-dependent and endothelium-independent manner on aortic rings pre-contracted with norepinephrine (0.1 μ*M*) and KCl (80 mM). Compound **61** was the most active and also inhibited the cumulative concentration-response contraction of norepinephrine or $CaCl_2$. Contractions induced by FPL 64176, an agonist of L-type voltage-dependent calcium channels, were blocked by gymnopusin (**61**). The potassium channel blockers, glibenclamide and tetraethylammonium reduced the relaxations induced by **61**. Nevertheless, its effect was not modified by 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one, a specific soluble guanylate cyclase inhibitor. The functional results obtained suggested that gymnopusin (**61**) induced relaxation through an endothelium-independent pathway

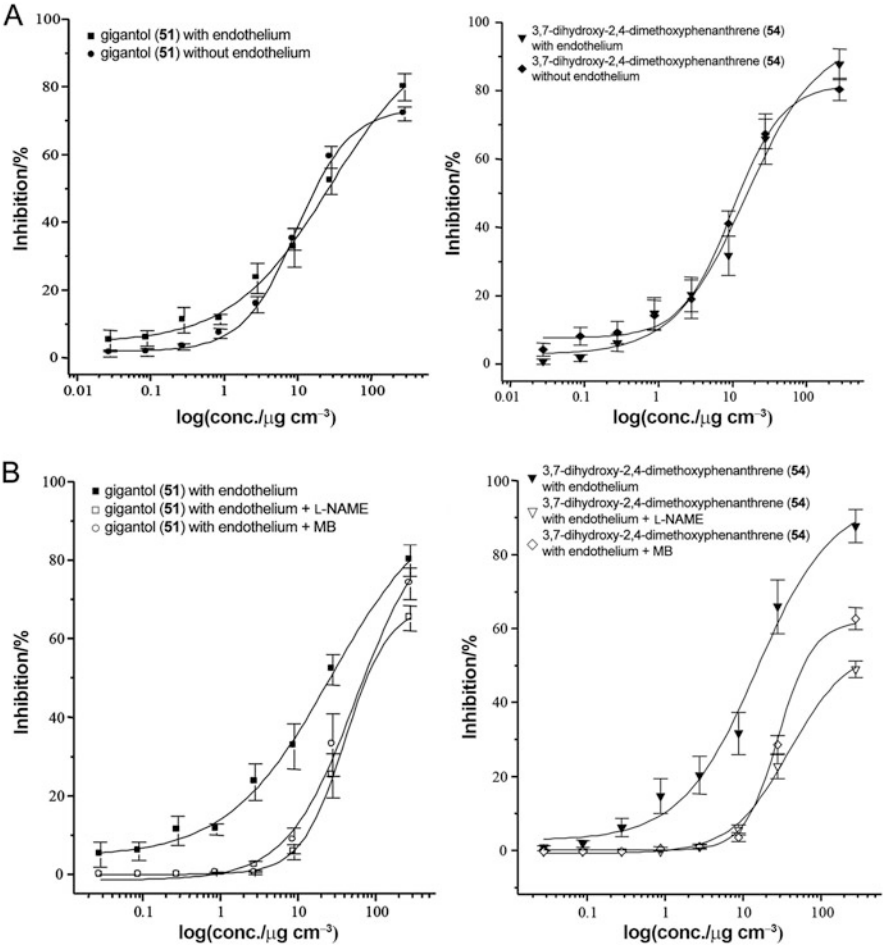


Fig. 24 Concentration-response curves for gigantol (**51**) and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**)-induced relaxation of endothelium-intact and endothelium-removed aortic segments pretreated with NA (**A**). Concentration-response curves for gigantol (**51**) and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**54**)-induced relaxation of NA constricted rat aorta recorded in endothelium intact and denuded rings pretreated for 10 min with L-NAME (1×10^{-5} M) or MB (1×10^{-7} M) (**B**). Adapted from [58]

by the control of cationic channels (calcium channel blockade and potassium channel opening) in the myogenic response of rat aortic rings [63].

From the spasmolytic CH_2Cl_2-MeOH (1:1) extract of the whole plant of *N. boothii* (IC_{50} = 6.26 μg/cm^3), the compounds nidemone (**62**), 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol (**63**), aloifol II (**64**), 1,5,7-trimethoxyphenanthrene-2,6-diol (**65**), ephemeranthoquinone (**66**), ephemeranthol B (**67**), and vitexin (**68**) were characterized (Fig. 25) [57]. Recently, nidemone (**62**), which possesses a unique skeleton, was synthesized (Scheme 1) and the structure corrected [64]. The

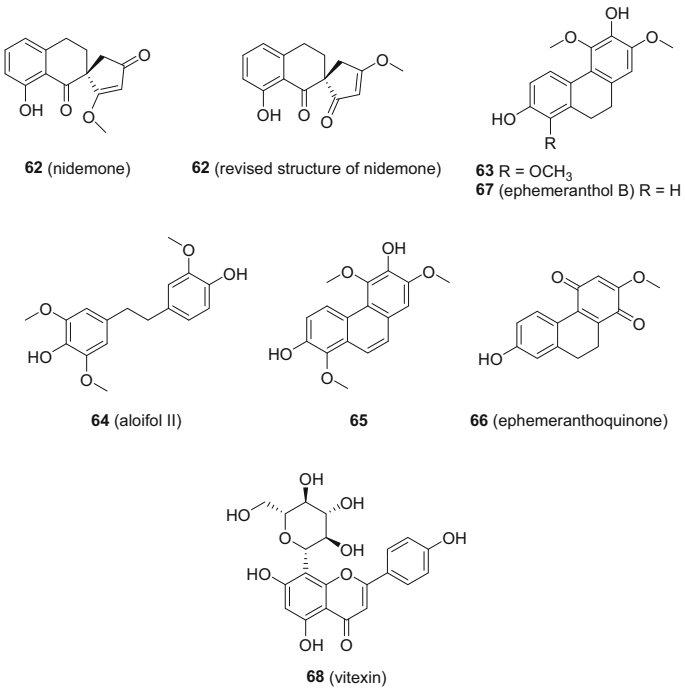
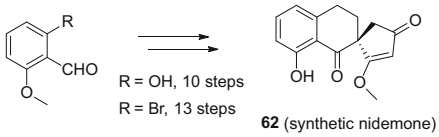


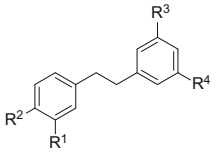
Fig. 25 Compounds isolated from *Nidema boothi*



Scheme 1 Synthesis of nidemone (**62**)

main differences between the natural and the synthetic product were the chemical shifts of three signals of the β-methoxycyclopentanone moiety in the ^{1}H and ^{13}C NMR spectra.

To establish the influence of the nature (phenolic vs. phenolic methyl ether) and location of the oxygenated substituents along the bibenzyl core on the pharmacological effects, gigantol (**51**), batatasin III (**52**), and compounds **69**–**81** were synthesized and tested pharmacologically (Fig. 26) [57]. All compounds but **74** and **79** were obtained using the Wittig reaction, a method widely employed for the synthesis of bibenzyls. Analogues **74** and **79** were synthesized by catalytic reduction of resveratrol and piceatannol, respectively. Most of these compounds are natural products except for **72** and **78**. All analogues were found to be potent inhibitors of spontaneous contractions of the guinea-pig ileum, with IC_{50} values ranging between



69 $R^1 = R^3 = R^4 = OCH_3$, $R^2 = OH$
70 $R^1 = R^2 = R^3 = R^4 = OCH_3$
71 $R^1 = R^3 = OH$, $R^2 = R^4 = OCH_3$
72 $R^2 = OCH_3$, $R^1 = R^3 = R^4 = OH$
73 $R^1 = R^2 = R^4 = OCH_3$, $R^3 = OH$
74 $R^1 = R^2 = R^3 = R^4 = OH$
75 $R^1 = H$, $R^2 = R^3 = OH$, $R^4 = OCH_3$
76 $R^1 = R^4 = OCH_3$, $R^2 = H$, $R^3 = OH$
77 $R^1 = R^3 = R^4 = OH$, $R^2 = H$
78 $R^1 = R^3 = R^4 = OCH_3$, $R^2 = H$
79 $R^1 = H$, $R^2 = R^3 = R^4 = OH$
80 $R^1 = R^2 = H$, $R^3 = R^4 = OH$
81 $R^1 = OH$, $R^2 = OCH_3$, $R^3 = R^4 = H$

Fig. 26 Synthetic analogues of gigantol (**51**)

0.14 and 2.36 μ*M*. However, structural differences influenced the potency of this type of inhibition. Thus, methylation of one or more of the free hydroxy groups and the presence of additional oxygenated groups in relation to the lead compounds decreased the smooth muscle-relaxant activity. Compound **75**, possessing a free hydroxy group at C-4, and **71**, in which the locations of the hydroxy and methoxy groups in ring B are the opposite of gigantol (**51**), were the most potent. Furthermore, the absence of oxygenated substituents in one of the aromatic rings of the stilbenoid moiety, as in the case of compounds **80** and **81**, induced the highest decrease in activity. Hence, it could be inferred that the presence of oxygenated substituents in both aromatic rings is essential for activity within this compound class [57].

Scaphyglottis livida possesses also antinociceptive and anti-inflammatory effects when tested in mice and rats using the hot-plate (150–600 mg/kg) and carrageenan-induced inflammation (150–600 mg/kg) models, respectively. Morphine (1.5–6 mg/kg, p.o.) and indomethacin (10–40 mg/kg, p.o.) were used, in turn, as positive controls. Gigantol (**51**) (25–100 mg/kg, p.o.) significantly increased the hot-plate latency in comparison to vehicle-treated mice and decreased carrageenan-induced inflammation in rats. The antinociception provoked by gigantol (**51**) was partially blocked by naloxone (1 mg/kg, i.p.). However, pretreatment with L-NAME (100 mg/kg, i.p.) and glibenclamide (10 mg/kg, i.p.) did not affect the antinociceptive response induced by gigantol (**51**), suggesting that its pharmacological effect could be partially due to activation of opioid receptors [65]. In summary, the investigation of these orchids has led to the discovery of new leads for the development of smooth muscle-relaxant, antinociceptive, and anti-inflammatory agents; altogether these results have provided also laboratory support for the use of *S. livida* for treating colic in the region of Los Tuxtlas.

The volatile components profile of *S. livida* was assembled via GC-MS analysis of the headspace solid-phase microextraction (HS-SPME)-adsorbed compounds. For

the HS-SPME analysis, a DVB/CAR/PDMS-coated fiber was used. Overall, nine components were characterized including linalool (11.6%), (*E*)-geranyl ketone (25%), benzaldehyde (25.5%), and *p*-menth-1-en-8-ol (12.5%). Interestingly, linalool and oils containing it are used by pest professionals as a flea, fruit fly, and cockroach insecticide. This finding might account for the use of this orchid to eliminate ectoparasites.

During a program conducted to discover new herbicidal agents, erianthridin (**59**) and gymnopusin (**61**) were also highly phytotoxic to axenic cultures of duckweed (*Lemna pausicostata*), which is one of the best characterized models for assessing phytotoxic activity [66]. The duckweed assay system makes it possible to study toxic effects throughout the plant life cycle, as well as plant-specific toxic effects that target photosynthesis. Erianthridin (**59**) provoked cellular leakage, complete growth inhibition, and significant chlorophyll reduction in duckweed. Gymnopusin (**61**) also caused a significant decrease in chlorophyll content at concentrations ranging from 10 to 200 μ*M* (Fig. 27). In addition, both compounds inhibited radicle elongation of *Amaranthus hypochondriacus* seedlings with IC_{50} values of 330 and 58.2 μ*M*, respectively [66]. Also, foliar application of gigantol (**51**) at 1 μ*M* to 4-week old seedlings of *A. hypochondriacus* reduced shoot elongation (69%) and fresh weight accumulation (54%). At 0.1 μ*M*, the treatments inhibited only fresh weight accumulation (20%); at two concentrations, this bibenzyl induced necrosis, desiccation, and leaf abscission. Paraquat (0.1 μ*M*) was used as a positive control; the potency of the biological activity of this commercial herbicide was comparable to that of gigantol (**51**). The studies on phytotoxicity of gigantol (**51**) were extended to axenic

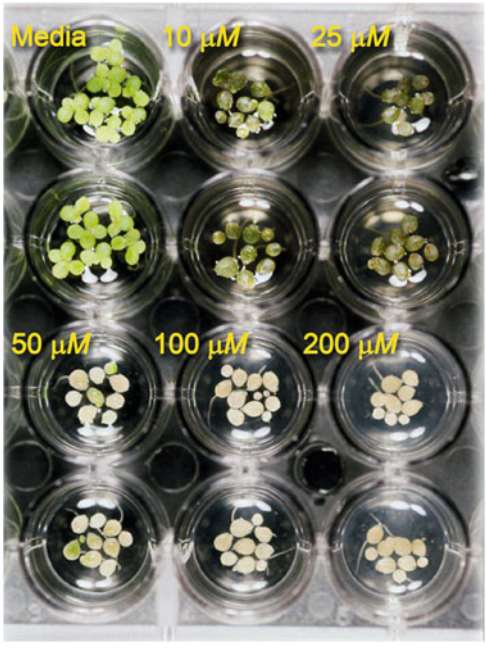


Fig. 27 Effect of gymnopusin (**61**) from *Maxillaria densa* on *Lemna pausicostata* cultures at 72 h. Adapted from [66]

L. pausicostata, and the results revealed also significant growth inhibition ($IC_{50} = 165\ \mu M$) and cellular leakage ($IC_{50} = 180\ \mu M$). Thus, the orchid stilbenoids and phenanthrenes exhibited good phytotoxicity on both amaranth and duckweed and seem good lead compounds for the development of a novel class of herbicidal agents [67].

2.3 Antiulcer Agents

According to the National Social Security Institute, about 70% of all Mexicans suffer from some form of gastric disorder each year. Peptic ulcer is the 10th-most common reason for visiting a physician in Mexico, and this tends to affect women nearly twice as much as men. According to the latest WHO data published in May 2014 peptic ulcer-related fatalities in Mexico reached 2294 or 0.44% of total deaths. The death rate is 2.40 per 100,000 of the population [68]. Thus, peptic ulcer symptoms and their alleviation have a major impact on the public health system.

Although gastric ulcers can be treated with a simple over-the-counter antacids or other drugs, many patients use herbal drugs. Therefore, Navarrete and coworkers initiated a systematic research program using several models of gastric injury, to validate the indigenous use of some species. These models use absolute ethanol [69], non-steroidal anti-inflammatory drugs [70], cold-restrain stress, pyloric ligation [71], and other erosive compounds to induce ulcers in the mouse or rat stomach. It is worth mentioning that in 2009 a list of Mexican medicinal plants used for treating gastrointestinal disorders related to gastritis and peptic ulcer was published. Some of them showed notable anti-*Helicobacter pylori* activity [72].

In the following paragraphs some of our work on antiulcer agents is summarized, and the Appendix Table includes a survey on other medicinal plants with important antiulcer properties.

2.3.1 *Amphipterygium adstringens*

Amphipterygium adstringens Schiede ex Schltdl. (Anacardiaceae) is an indigenous tree up to 8.5 m high found in the deciduous and sub-deciduous tropical forests of the Pacific Slope Regions, from Nayarit to Oaxaca, and Estado de México, Puebla, Morelos and Michoacán (Fig. 28). The grayish wrinkled stem bark of *A. adstringens* has been used medicinally since pre-hispanic times as pointed out by Francisco Hernández in the sixteenth century [73]. The plant was included in the first edition of the "Mexican Pharmacopoeia" in 1846 and remained until the 1930 version [74], and more recently, it was included in the first and second editions of the "Mexican Herbal Pharmacopoeia" [75, 76].

Nowadays, *A. adstringens* is used for treating over 25 different diseases, especially gastritis, peptic ulcer, and wound scarring. Thus, it is considered to be one of the most relevant species of the modern Mexican "herbolaria" [77]. The most common

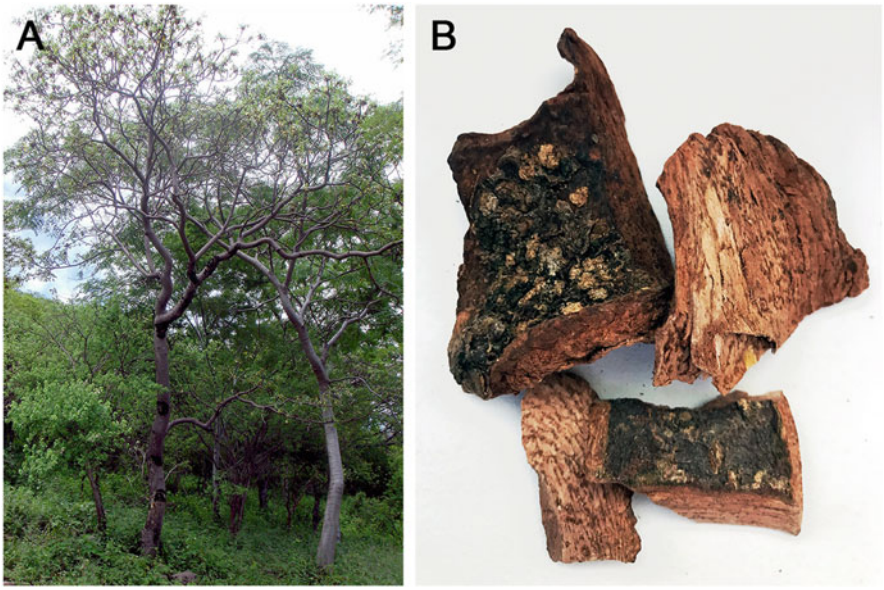


Fig. 28 *Amphipterygium adstringens* tree (**A**) and stem bark (**B**)

vernacular names are "cuachalalate", "cuachalala", "matixerán", "pacueco", "palo santo", and "cuachinala".

Three regions are the most important supplier of "cuachalalate" stem bark in Mexico. These are the southern part of the state of Morelos, the Mixteca Poblana, and the northern area of the Balsas river basin. It is estimated that around 57 tons of the stem bark are collected each year from these areas, mostly for export purposes. In the states of Colima, Jalisco, Michoacán, and Oaxaca, the product is also collected for local use [77].

González and collaborators conducted an initial general phytochemical screening of the stem bark of *A. adstringens* on the basis of its promising anticancer activity, demonstrating the presence of sarsapogenin by means of IR spectrometry and thin-layer chromatography [78, 79]. Later on, different phytochemical studies reported the presence of triterpenoids of the tirucallane, oleanane and dammarane types as well as alkylphenols [80–89]. The tirucallane triterpenoids included instipolinacic acid (**82**), cuachalalic acid (**83**), masticadienonic acid (**84**), 3α-hydroxymasticadienonic acid (**85**), *iso*-masticadienonic acid (**86**), 3β-hydroxymasticadienonic acid (**87**), 3α-hydroxy-6-oxo-7,(24*Z*)-tirucalladien-26-oic acid (**88**), 3,7-dioxo-8,(24*Z*)-tirucalladien-26-oic acid (**89**), 3α-hydroxy-7-oxo-8,(24*Z*)-tirucalladien-26-oic acid (**90**), 7,11-dioxo-3α-hydroxy-8,(24*Z*)-tirucalladien-26-oic acid (**91**), and 3,8-dioxo-7β-hydroxy-7,9-cyclo-7,8-*seco*-(24*Z*)-tirucalladien-26-oic acid (**92**) (Fig. 29); the oleananes comprised oleanolic (**93**) and 3-*epi*-oleanolic acids (**94**), 3α-hydroxy-11α,12α-epoxy-oleanane-28,13β-olide (**95**), and 3β-hydroxy-11α,12α-epoxy-oleanane-28,13β-olide (**96**) (Fig. 30);

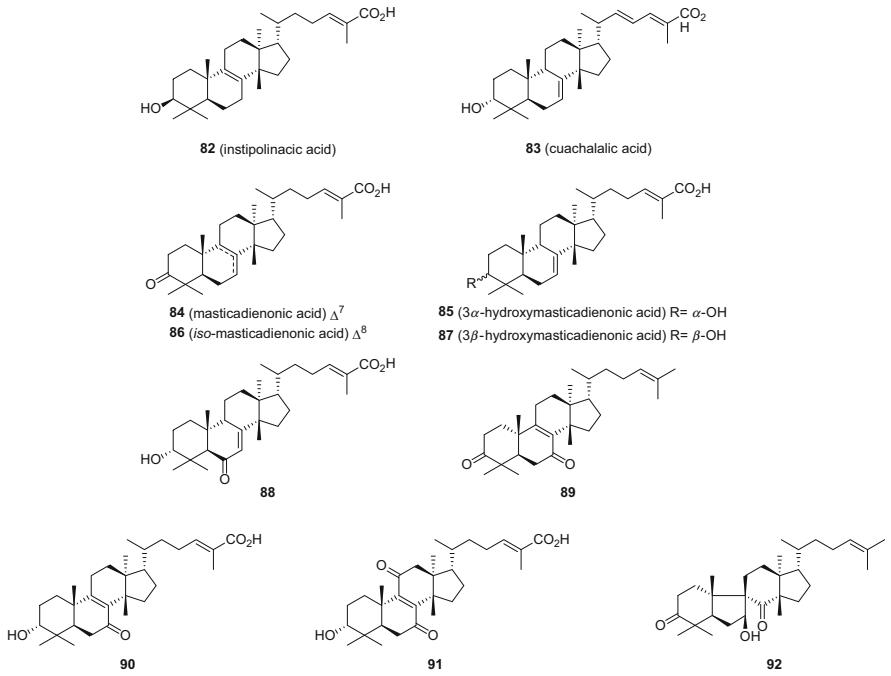


Fig. 29 Tirucallane triterpenoids isolated from *Amphipterygium adstringens*

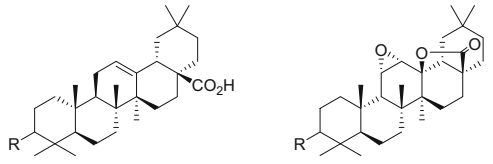
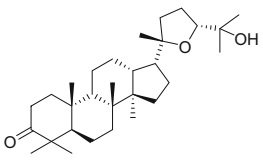


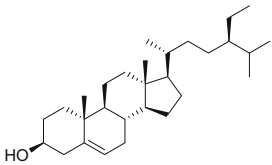
Fig. 30 Oleanane triterpenoid derivatives isolated from *Amphipterygium adstringens*

and the only dammarane was identified as ocotillone (**97**) (Fig. 31). The long-chain phenols encompassed anacardic acids (**98**–**104**), anacardic aldehydes (**105**–**107**) and 3-dodecyl-1,8-dihydroxy-2-naphthoic acid (**108**) (Fig. 32). Finally, β-sitosterol (**109**) was also isolated (Fig. 31).

The first account of the antiulcer activity of a decoction of "cuachalalate" using several experimental models of gastric and duodenal ulcer was published in 1990 [90]. Bioassay-guided analysis of an active methanol extract of the stem bark showed that the gastroprotective action was due to 3-*epi*-oleanolic acid (**94**), which protected

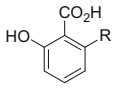


97 (ocotillone)

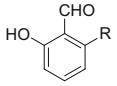


109 (β-sitosterol)

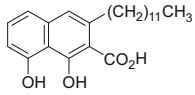
Fig. 31 Structures of ocotillone (**97**) and β-sitosterol (**109**) from *Amphipterygium adstringens*



98 R = $(CH_2)_{14}CH_3$
99 R = $(CH_2)_{15}CH_3$
100 R = $(CH_2)_{16}CH_3$
101 R = $(CH_2)_{18}CH_3$
102 R = $(CH_2)_{14}CH{=}CH(CH_2)_2CH_3$
103 R = $(CH_2)_{15}CH{=}CHCH_2CH_3$
104 R = $(CH_2)_7CH{=}CH(CH_2)_5CH_3$



105 R = $(CH_2)_{17}CH_3$
106 R = $(CH_2)_{19}CH_3$
107 R = $(CH_2)_{21}CH_3$



108

Fig. 32 Anacardic acids (**98**–**104**), anacardic aldehydes (**105**–**107**), and 3-dodecyl-1,8-dihydroxy-2-naphthoic acid (**108**) isolated from *Amphipterygium adstringens*

damaged mucosa by 88.8%, followed by 3α-hydroxymasticadienonic acid (**85**) and β-sitosterol (**109**), with 69.8% and 42.5% of gastroprotection, respectively [84]. The gastroprotection of the methanol extract was completely inhibited by pretreatment with L-NAME and attenuated by pretreatment with indomethacin and NEM. These results suggest that endogenous nitric oxide, prostaglandins and endogenous sulfhydryl groups play an important role in the gastroprotection exerted by the extract on ethanol-induced gastric mucosal lesions. The effect of 3α-hydroxymasticadienonic acid (**85**) was attenuated only by pretreatment with NEM, indicating that endogenous sulfhydryls (thiols) participate in its gastroprotective mechanism. However, capsaicin-sensitive neurons did not participate in the antiulcer activity of *A. adstringens* or its active compounds [84]. On the other hand, the methanol extract of the stem bark of "cuachalalate" inhibited the gastric lesions induced by high doses of sodium diclofenac, without affecting the anti-inflammatory activity and pharmacokinetics of this drug [70].

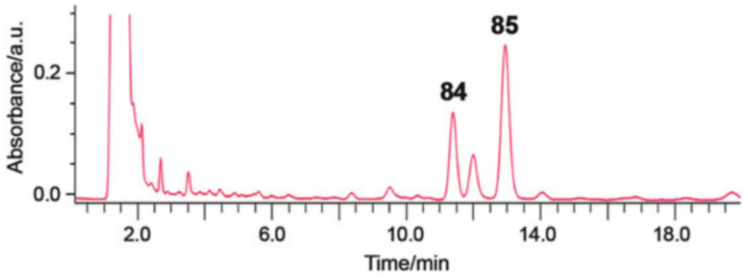


Fig. 33 HPLC-PDA chromatogram of *Amphipterygium adstringens* crude drug: masticadienonic (**84**) and 3α-hydroxymasticadienonic (**85**) acids

Recently, the efficacy of an alcoholic extract of *A. adstringens* in mice with dextran sulphate sodium-induced ulcerative colitis was demonstrated [91]. The protective effect of the extract was determined at 200 mg/kg by oral gavage for 10 days. The treatment increased the survival rate and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in the colonic mucosa, and reduced body weight loss, macroscopic and microscopic changes of the colonic mucosa, and the production of proinflammatory cytokines (TNF-α, IFN-γ, and IL-1β) [91].

Other studies on "cuachalalate" confirmed its anti-*H. pylori* activity, which was attributed to the anacardic acid constituents of the plant [92], and the anti-inflammatory action using the 12-*O*-tetradecanoylphorbol-13-acetate-induced ear edema and carrageenan-induced paw edema models. Masticadienonic (**84**) and 3α-hydroxymasticadienonic acids (**85**) were the active principles, which also induced the production of nitric oxide in resting and LPS-treated macrophages [93]. The cytotoxic activity of the two triterpenoids and the extract was weak [94]. Finally, the anacardic acid mixture isolated from *A. adstringens* inhibited quorum sensing-controlled virulence factors of *Chromobacterium violaceum* and *Pseudomonas aeruginosa* [95].

Regarding the quality control procedures for the crude drug of "cuachalalate", HPLC-UV and high-performance thin-layer chromatography-densitometry methods were developed and validated to quantify masticadienonic (**84**) and 3-α-hydroxymasticadienonic acids (**85**) (Fig. 33) [96]. The former appears as the official composition test for the crude drug in the second edition of the "Mexican Herbal Pharmacopoeia" [76]. Finally, a microscopy-based botanical identity test was also developed.

2.3.2 *Ligusticum porteri*

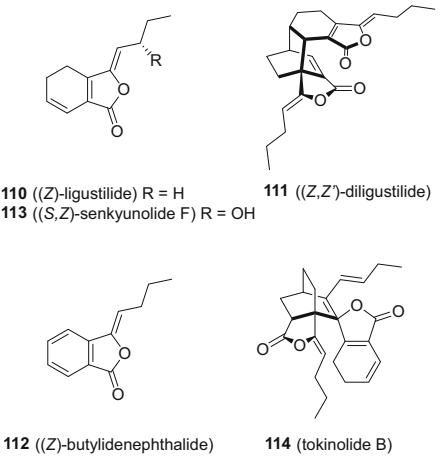
Ligusticum porteri J.M. Coult. & Rose (Apiaceae) is a perennial herb growing from 50 to 100 cm tall or more (Fig. 34). In winter, the above-ground parts die back to a thick, woody and very aromatic rootstock. The plant has deeply incised, elliptic leaf segments that are 5–40 mm in width with larger basal leaves. The flowers are white,

Fig. 34 Roots and rhizomes of *Ligusticum porteri*

approximately 2–5 mm in diameter with five petals. They are grouped in flat-topped, compound umbels and are followed by reddish, oblong, ribbed fruits 5–8 mm in length. The plant is native to the mountains of western North America (in Arizona, Colorado, Idaho, New Mexico, Nevada, Utah, and Wyoming) to the states of Sonora and Chihuahua, Mexico. In Mexico, the plant has several colloquial names including "chuchupate", "raíz de Angélica", "yerba del cochino", "washi", and "Angélica del país", among others [97]. In the USA, the most popular common names are "osha", "Porter's licorice" and "bear root". The last-mentioned name is associated with the practice of Kodiak bears in masticating the roots of *L. porteri* and rubbing the salivated contents over their bodies, possibly to medicate infected topical wounds, while the second is due to its pungent mixture of flavors reminiscent of celery and licorice. In Mexico, Bye and Linares observed the Tarahumaras using infusions of *L. porteri* during ritual curing ceremonies or for protecting individuals against witches and rattlesnakes [97].

The use of "osha" to treat sickness has played an important role in Mexican traditional medicine. According to several reports, preparations (e.g. an extract, infusion, decoction or oil) made with "osha" roots and rhizomes are ingested commonly for treating a broad array of medical ailments, particularly those related to the lungs (bronchitis, pneumonia, tuberculosis, colds, and coughs), and heart. Fevers, gastrointestinal disorders (stomachache, colic, ulcers, and diarrhea), sore throats, and rheumatism are also treated with this herb; externally, the roots and rhizomes may be used to make a dressing, paste or liniment to treat pain, scorpion stings, and skin infections; the leaves, seeds, and roots are used to season meat, beans, and peppers [22, 97, 98].

The chemical composition of the roots and rhizomes of *L. porteri* includes mainly coumarins and phthalides. Most of these studies have been carried out by Delgado and collaborators in Mexico City and its chemistry was recently reviewed [99]. Although numerous compounds have been identified, (*Z*)-ligustilide (**110**), (*Z*,*Z*′)-diligustilide (**111**), and (*Z*)-butylidenephthalide (**112**) (Fig. 35) are most commonly purported to



110 ((*Z*)-ligustilide) R = H
113 ((*S*,*Z*)-senkyunolide F) R = OH

111 ((*Z*,*Z*′)-diligustilide)

112 ((*Z*)-butylidenephthalide)

114 (tokinolide B)

Fig. 35 Phthalides from *Ligusticum porteri*

facilitate the therapeutic effects of the plant. The essential oil also has been analyzed and the major components are mono- and sesquiterpenoids [99].

The gastroprotective effect of several organic solvent crude extracts of *L. porter*i was demonstrated in the ethanol-induced gastric lesion model in male Wistar rats [100]. Thus, oral administration of the organic extracts (30 mg/kg) of *L. porteri* showed gastroprotective effects on ethanol-induced gastric lesions, with the *n*-hexane and dichloromethane extracts being the most active. (*Z*,*Z*′)-Diligustilide (**111**) or levistolide A was the active compound (Fig. 36); at a dose of 10 mg/kg this compound prevented significantly the gastric injuries induced by ethanol via

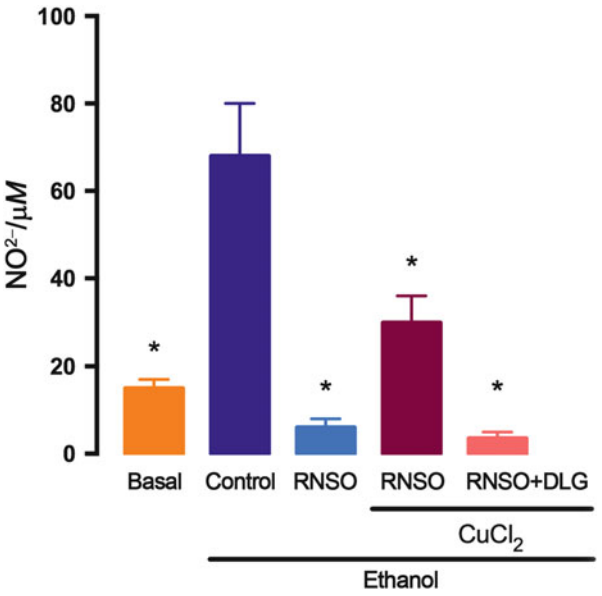


Fig. 36 Effect of (*Z*,*Z*′)-diligustilide (**111**) (DLG) on nitrite levels due the nitrosothiols (RNSO) degradation in the presence of $CuCl_2$. Data represent the means ± SEM of five experiments. $^*p < 0.05$

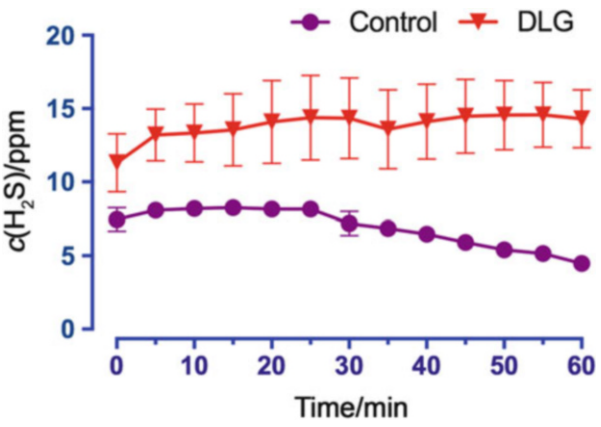


Fig. 37 Time-course of H_2S released in vivo by the stomach in fasted animals treated with saline solution (control) and (*Z*,*Z*′)-diligustilide (**111**) (DLG). Ethanol (0.2 cm^3) was injected directly after 30 min showing a decrease of the total amount of H_2S due to its necrotoxic damage. Adapted from [101]

endogenous non-protein sulfhydryl groups and prostaglandins, without inhibition of gastric secretion. This conclusion was based on the fact that NEM, a sulfhydryl alkylator, and indomethacin, a cyclooxygenase inhibitor, neutralized totally or partially, respectively, the gastroprotective effect of (*Z*,*Z*′)-diligustilide (**111**) [100]. Finally, compound **111** has a significant gastroprotective effect in vivo, regulating two of the most important gastrotransmitters, $NO^{\cdot}$ and H_2S, increasing the total concentration of H_2S and by stabilizing *S*-nitrosothiols (Fig. 37) [101].

Extracts and compounds from this same plant showed also antinociceptive [102], anti-inflammatory [103], spasmolytic and sedative [104], and antioxidant [105] effects, which together might contribute to the alleviation of the typical discomfort of gastric ulcers. Thus, a traditional preparation and the essential oil (10–316 mg/kg) from *L. porteri* both showed an antinociceptive effect when tested in the acetic acid-induced writhing and hot plate tests in ICR mice. In addition, (*Z*)-ligustilide (**110**) provoked an increment in the latency period to thermal stimuli in the hot-plate test at a dose of 31.6 mg/kg, and a decrease in the number of abdominal writhes at 10 mg/kg. In turn, (*Z*)-butylidenephthalide (**112**) induced a dose-dependent antinociceptive action in the hot-plate assay, and this compound was also effective for controlling the pain provoked by chemical irritation at doses of 10 and 31.6 mg/kg. Also, (*Z*,*Z*′)-diligustilide (**111**) inhibited the number of writhing responses at all doses tested but was inactive in the hot-plate model (Fig. 38) [102].

The anti-inflammatory activity of natural phthalides from *L. porteri* and a few semi-synthetic analogues was demonstrated using the mouse model of ear edema induced by 12-*O*-tetradecanoylphorbol-13-acetate, and the rat paw edema model caused by carrageenan. The anti-inflammatory activity was explained by the reduction of myeloperoxidase activity and the infiltration of leukocytes [103]. (*Z*)-Ligustilide (**110**) and senkyunolide F (**113**) (Fig. 35) showed spasmolytic activity when tested in a guinea pig ileum model. These compounds (50 μg/cm^3) were able to inhibit the induced electrical contractions of the ileum by 41.5% and 54.6%, respectively. Finally, regarding the sedative properties, it was found that the

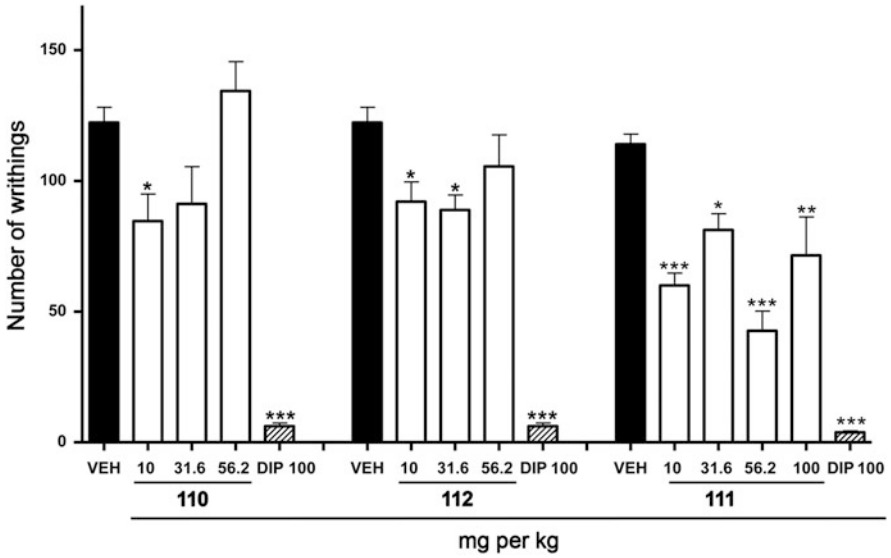


Fig. 38 Antinociceptive activity of (*Z*)-ligustilide (**110**), (*Z*,*Z*′)-diligustilide (**111**), and (*Z*)-butylidenephthalide (**112**) in the acetic acid-induced writhing test. Dipirone (DIP, 100 mg/kg). Data are presented as the means from eight data points ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. Adapted from [102]

hypnotic time induced by sodium pentobarbital in mice was increased significantly by pre-treatment with 50 mg/kg of **110**, **111**, **113**, and tokinolide B (**114**) (Fig. 35) by 46.3%, 24.6%, 34.6%, and 70.8%, respectively [104].

An organic solvent-soluble extract and the essential oil (31.6, 52.6, and 100 mg/kg) from the roots and rhizomes of *L. porteri* were hypoglycemic and decreased the postprandial peak during an OGTT in diabetic mice. In the OSTT, the organic extract, an infusion, and the essential oil lowered glucose levels in both hyperglycemic and normal mice revealing that the inhibition of α-glucosidase may mediate the observed antihyperglycemic effect [12, 106]. These last results were confirmed in vitro by testing the preparations against yeast α-glucosidase. In order to purify the α-glucosidase inhibitors of *L. porteri*, the active organic extract was subjected to assay-guided fractionation to yield (*Z*)-butylidenephthalide (**112**) as the major active component [106]. Compound **112** inhibited also the activity of yeast α-glucosidase ($IC_{50} = 2.35$ m*M* vs. acarbose 0.42 m*M*) in a concentration-dependent manner. A kinetic analysis revealed that **112** is a mixed-type inhibitor. An in vivo OSTT corroborated the above results, since **112** (10.0 and 56.2 mg/kg) was also able to lower the postprandial peak. Phthalides **110**–**112** were tested also in the OGTT and only (*Z*)-ligustilide (**110**) and butylidenephthalide (**112**) (10, 31.2, and 56.2 mg/kg) were able to lower blood glucose levels in NAA-STZ-diabetic mice, suggesting that their mode of action involved also other mechanisms [106].

Since the essential oil was the most active in the OSTT, this preparation was also analyzed by different chromatographic techniques [107]. GC-MS analysis allowed

the identification of 30 constituents, representing about 99% by weight of the total content; the most important feature of the oil was the presence of a high percentage of the phthalides (20.2% of **110** and 24.4% of **112**), among other components [107]. The results of the chemical analysis were consistent with the pharmacological action of the oil. A method for quantifying the major active compounds from the traditional preparation was also developed and validated [107].

In more recent work, the viability of HL-60 cells challenged with H_2O_2 was twofold higher than the control after 7 days of incubation with 200 or 400 μg/cm^3 of a *L. porteri* root extract [105]. Stressed HL-60 cells treated with 100, 200, and 400 μg/cm^3 of the *L. porteri* extract reduced lipid peroxidation by 12–13%. An increase in GPx levels and SOD and CAT activities in stressed HL-60 cells supplemented with 400 μg/cm^3 of this extract was also observed. Treatment with 400 μg/cm^3 of the extract of *L. porteri* increased IFN-γ and IL-2 in H_2O_2-challenged cells. These data suggest a protective function of *L. porteri* root extract against oxidative stress [105].

To complete this subsection on this species, it is worth pointing out that "osha" is not available as a cultivated crop; the seeds are difficult to germinate, so therefore it is an ecologically sensitive plant that has been ignored in terms of conservation. The entire plant is removed when the roots are harvested, thus, this destructive collection of "osha" from slow-growing populations could lead to unsustainable harvesting. With this in mind, Goldhaber-Pasillas and co-workers [108] attempted its in vitro propagation and analyzed the morphogenetic response of *L. porteri*; the leaf and stem calli extracts both proved to contain (*Z*)-butylidenephthalide (**112**). These results demonstrated, therefore, that in vitro cultures of *L. porteri* possess the biosynthetic machinery for the generation of this potentially valuable biologically active metabolite.

2.3.3 *Hippocratea excelsa*

Hippocratea excelsa Kunth (Hippocrataceae) (syn. *Hemiangium excelsum* (Kunth) A.C. Sm.) is a climbing liana up to 8 m tall with glabrous or puberulent opposite leaves. Its flowers are 10 mm in diameter with greenish sepals and green-yellow petals (Fig. 39). The crude drug is the root cortex that possesses a characteristic reddish-brown color with yellow stripes and is somewhat elastic, making it easy to recognize. *Hippocratea excelsa* is commonly known as "cancerina", "ixcate", "izcate rojo", "hierba del piojo", and "mata piojo". The last two names allude to its use to kill lice and as a remedy against mite skin parasites. Throughout Mexico, "cancerina" is collected in the wild and sold in markets and medicinal herbstores. This drug is valued for treating skin ailments, gastric ulcers, kidney disease, and female disorders [109].

The root bark of *H. excelsa* is an important source of (*E*)-polyisoprene [110], but the triterpenoids and sesquiterpene evoinate alkaloids are the most well studied components of the plant [111]. The triterpenoids isolated belong to four major groups: friedelanes, oleananes, ursanes, and taraxanes. The friedelanes included the quinone-methides tingenone (**115**), celastrol (**116**), pristimerin (**117**), excelsine

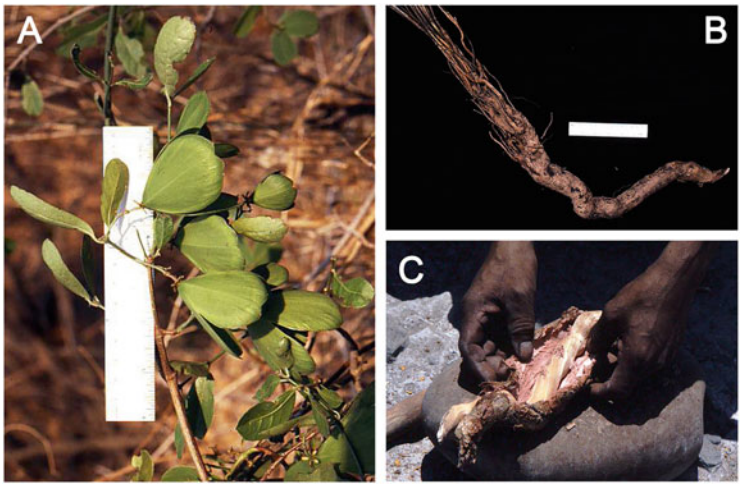
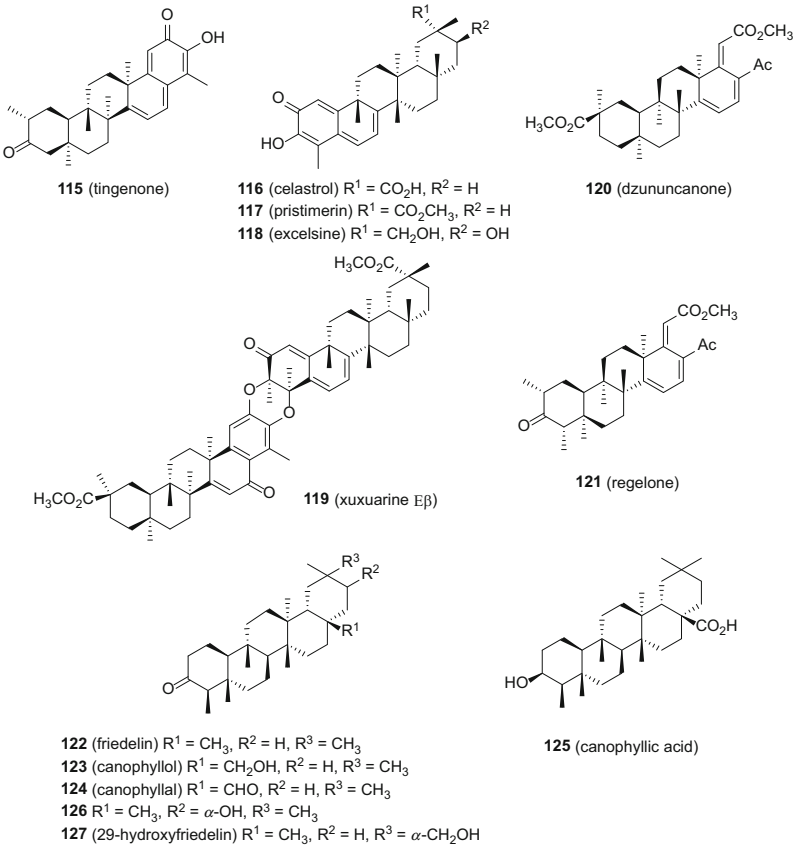


Fig. 39 Stems and leaves (**A**) and root cortex (**B** and **C**) of *Hippocratea excelsa*

(**118**), xuxuarine Eβ (**119**), dzununcanone (**120**), and regelone (**121**), and the simple friedelane derivatives friedelin (**122**), canophyllol (**123**), canophyllal (**124**), canophyllic acid (**125**), 21α-hydroxy-3-oxofriedelane (**126**), and 29-hydroxyfriedelin (**127**) (Fig. 40) [112, 113]. The oleananes included β-amyrin (**128**) and its ferulate (**129**), oleanoic acid (**93**), 21β-hydroxy-β-amyrenone (**130**), and five 21β-dihydroxyoleanane derivatives (**131–135**) (Fig. 41). The ursanes included a 21β-dihydroxyursane derivative (**136**), α-amyrin (**137**) (Fig. 42) and ursolic acid (**40**). Finally, among the taraxanes were 29-hydroxyglutinol (**138**) and 29-hydroxytaraxerol (**139**) (Fig. 42) [114]. The sesquiterpene evoinate alkaloids biosynthesized in *H. excelsa* belonged to two classes: one possessing a 2,3-substituted nicotinic acid as in hippocrateines I (**140**) and II (**141**), emarginatine A (**142**), mayteine (**143**), and the derivatives **144–152**; the second class was represented by hippocrateine III (**153**) and derivatives **154–160**, with a 3,4-substituted nicotinic acid at the diester moiety (Fig. 43) [111, 115, 116]. Other minor compounds reported for this plant are galactitol (**161**) [109], catechin (**162**), (–)-*epi*-catechin (**163**), and the ubiquitous β-sitosterol (**109**) and its 3-*O*-β-glucoside (**164**), as well as 6-hydroxystigmast-4-en-3-one (**165**) (Fig. 44) [71].

The antiulcer effects of the methanol and aqueous extracts of root bark of *H. excelsa* were assessed in various ulcer models induced experimentally with ethanol, acetylsalicylic acid, indomethacin-histamine, a glucose diet, or pyloric ligation in rats [71]. Gastric protection was evaluated by measuring the ulcer index, gastric secretion, and the size of the mucosal lesions. In pyloric ligation, the digestive effect of the accumulated gastric juice and interference with gastric blood circulation are responsible for the induction of ulceration. The extracts (300 mg/kg, p.o.) of the root bark failed to inhibit the gastric secretion in the pyloric-ligated rat model, therefore, the extracts did not inhibit the acid output (Fig. 45) [71]. In the gastroprotective study,



115 (tingenone)

116 (celastrol) $R^1 = CO_2H$, $R^2 = H$
117 (pristimerin) $R^1 = CO_2CH_3$, $R^2 = H$
118 (excelsine) $R^1 = CH_2OH$, $R^2 = OH$

120 (dzununcanone)

119 (xuxuarine Eβ)

121 (regelone)

122 (friedelin) $R^1 = CH_3$, $R^2 = H$, $R^3 = CH_3$
123 (canophyllol) $R^1 = CH_2OH$, $R^2 = H$, $R^3 = CH_3$
124 (canophyllal) $R^1 = CHO$, $R^2 = H$, $R^3 = CH_3$
126 $R^1 = CH_3$, $R^2 = \alpha$-OH, $R^3 = CH_3$
127 (29-hydroxyfriedelin) $R^1 = CH_3$, $R^2 = H$, $R^3 = \alpha$-CH_2OH

125 (canophyllic acid)

Fig. 40 Friedelane triterpenoids isolated from *Hippocratea excelsa*

both extracts at 300 mg/kg produced a significant reduction of the ulcer index of the lesions induced by all ulcerogenic compounds. The aqueous extract (4% *w/v*) completely inhibited the longitudinal fondus lesions induced by a diet based exclusively on a 10% glucose solution ad libitum during a period of eight consecutive days [71]. Altogether, these results were consistent with a gastroprotective activity of the traditional preparation made from the root bark.

Bioassay-guided fractionation of the extract indicated the active gastroprotective principles to be a mixture of α-amyrin (**137**) and β-amyrin (**128**), β-sitosterol (**109**), sitosterol-3-*O*-*β*-glucoside (**164**) and (−)-*epi*-catechin (**163**), which exerted gastroprotective effects at a 100 mg/kg dose of 50.7%, 85.0%, 93.4%, and 72.1%, respectively [71]. The gastroprotective effect of these compounds was compared with that of bismuth subsalicylate (90 mg/kg, 46.2%), used as a positive control. Friedelin (**122**), canophyllol (**123**) and canophyllal (**124**) were inactive.

Further investigation on the gastroprotective activity of stigmasterol (**166**) and β-sitosterol (**109**) was evaluated [117]. Gastric mucosal damage was induced in rats

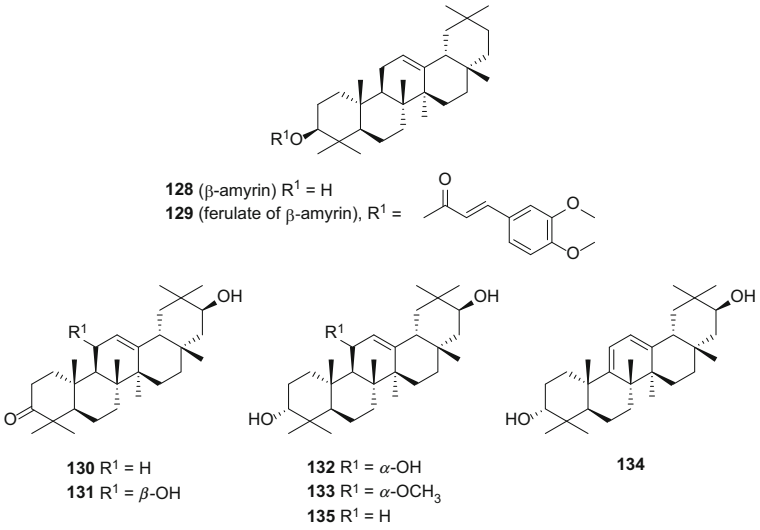


Fig. 41 Oleanane triterpenoids isolated from *Hippocratea excelsa*

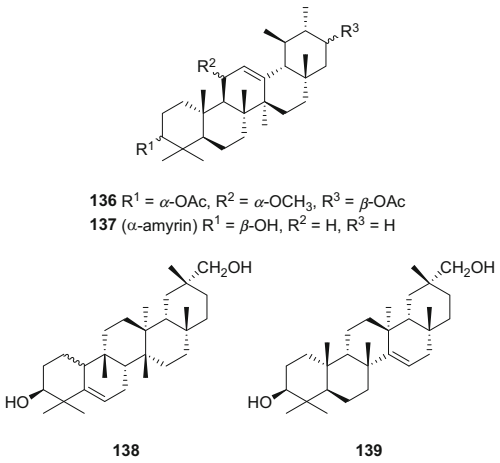
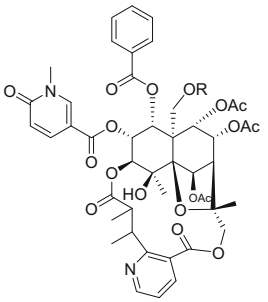
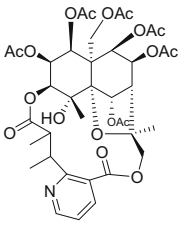


Fig. 42 Ursane and taraxane triterpenoids isolated from *Hippocratea excelsa*

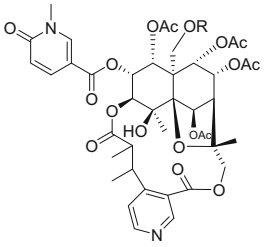
by intragastric ethanol (1 cm^3/rat). Rats were treated orally with stigmasterol (**166**) suspended in Tween 80 at 10, 30, 100, and 300 mg/kg, and showed 26.2, 39.6, 58.3, and 70.7% of gastroprotection (Fig. 46). β-Sitosterol (**109**) at 10, 30, 100, and 300 mg/kg exhibited, in turn, 21.6, 42.5, 48.5, and 71.2% of gastroprotection (Fig. 46) [117]. The gastroprotection observed at 30 mg/kg for both phytosterols was attenuated in rats pretreated with indomethacin (10 mg/kg, s.c.), L-NAME (70 mg/kg, i.p.), and capsaicin (125 mg/kg, s.c.), suggesting that their



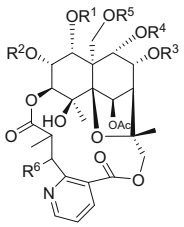
140 (hippocrateine I) R = Ac
141 (hippocrateine II) R = $COCH(CH_3)CH_2CH_3$

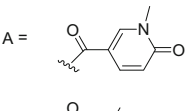


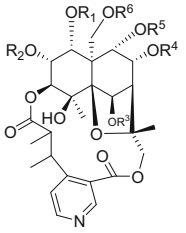
143 (mayteine)



153 (hippocrateine III) R = $COCH(CH_3)CH_2CH_3$



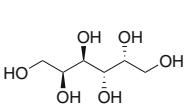




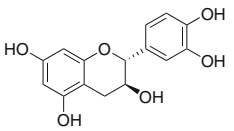
142 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = Ac, R^5 = Ac, R^6 = CH_3 (emarginatine A)
144 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = Ac, R^5 = B, R^6 = CH_3
145 R^1 = Ac, R^2 = A, R^3 =B, R^4 = Ac, R^5 =Ac, R^6 = CH_3
146 R^1 = B, R^2 = A, R^3 = Ac, R^4 = B, R^5 = Ac, R^6 = CH_3
147 R^1 = H, R^2 = A, R^3 = Ac, R^4 = B, R^5 = Ac, R^6 = CH_3
148 R^1 = A, R^2 = Ac, R^3 = Ac, R^4 = Ac, R^5 = Ac, R^6 = CH_3
149 R^1 = A, R^2 = H, R^3 = Ac, R^4 = Ac, R^5 = Ac, R^6 = CH_3
150 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = Ac, R^5 = Ac, R^6 = H
151 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = Ac, R^5 = B, R^6 = H
152 R^1 = Ac, R^2 = A, R^3 = B, R^4 = Ac, R^5 = B, R^6 = H

154 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = B, R^5 = Ac, R^6 = Ac
155 R^1 =Ac, R^2 = A, R^3 = Ac, R^4 = B, R^5 = Ac, R^6 = B
156 R^1 = Ac, R^2 = A, R^3 = Ac, R^4 = B, R^5 = B, R^6 = B
157 R^1 = Ac, R^2 = A, R^3 = H, R^4 = B, R^5 = Ac, R^6 = B
158 R^1 = H, R^2 = A, R^3 = Ac, R^4 = B, R^5 = B, R^6 = B
159 R^1 = Ac, R^2 = Ac, R^3 = Ac, R^4 = B, R^5 = Ac, R^6 = Ac
160 R^1 = B, R^2 = A, R^3 = Ac, R^4 = Ac, R^5 = B, R^6 = B

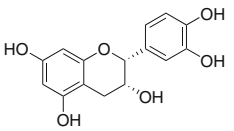
Fig. 43 Sesquiterpene evoinate alkaloids isolated from *Hippocratea excelsa*



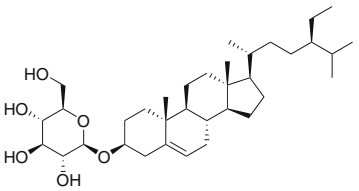
161 (galactitol)



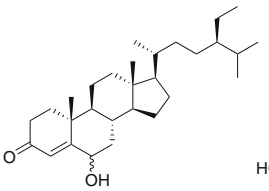
162 ((+)-catechin)



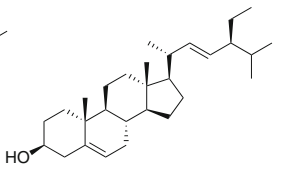
163 ((–)-*epi*-catechin)



164 (β-sitosterol-3-*O*-*β*-D-glucoside)



165



166 (stigmasterol)

Fig. 44 Minor compounds isolated from *Hippocratea excelsa*

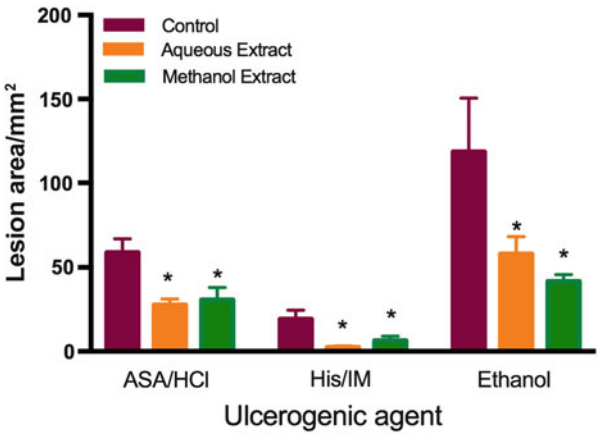


Fig. 45 Gastroprotective effects of methanol (300 mg/kg) and aqueous (300 mg/kg) extracts from the root bark of *Hippocratea excelsa*, in lesions induced by acetylsalicylic acid (200 mg/kg) plus 150 m*M* HCl (ASA/HCl), histamine (40 mg/kg) plus indomethacin (10 mg/kg) (His/IM), and absolute ethanol (1 cm^3). $*p < 0.05$ ($n = 10$)

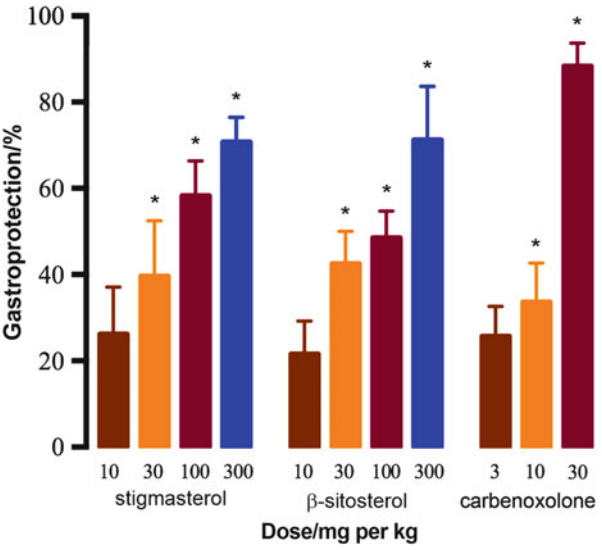


Fig. 46 Gastroprotective effects of stigmasterol (**166**), β-sitosterol (**109**), and carbenoxolone (positive control) on ethanol-induced ulceration in rats. Data represent the means from six experiments ±SEM. $*p < 0.05$

gastroprotective mechanism involved the participation of prostaglandins, nitric oxide and capsaicin-sensitive sensory neurons. The gastroprotection of β-sitosterol (**109**) was also attenuated by pretreatment with NEM (10 mg/kg, s.c.), indicating that endogenous sulfhydryls may be involved in the gastroprotection of this compound. Carbenoxolone was used as a gastroprotective model drug and showed a dose-dependent gastroprotective effect (25.7%, 33.6%, and 88.3% of gastroprotection, at 3, 10, and 30 mg/kg, respectively) [117].

The gastroprotective activities of four esters of β-sitosterol (**109**), namely, the succinate, phthalate, benzoate, and acetate, were also evaluated in the same model [118]. When the rats were treated orally with these compounds (30 mg/kg) suspended

in Tween 80, significant gastroprotective effects (38–75% gastroprotection) were evident. The gastroprotection observed at 30 mg/kg for these phytosterol esters was attenuated in rats pretreated with indomethacin (10 mg/kg), suggesting that the gastroprotective mechanism of these esters involved also the participation of prostaglandins. Overall, these results indicated that the gastroprotective activity of β-sitosterol (**109**) is not modified by the introduction of any of the ester groups at C-3 that were investigated [118].

Other biological studies carried out with this species have included antiprotozoal activity against *Giardia intestinalis* of the extract and isolated compounds. Pristimerin (**117**) and tingenone (**115**) were the most active antigiardial compounds, with IC_{50} values of 0.11 and 0.74 μM, respectively, compared with metronidazole, the current drug of choice ($IC_{50} = 1.23\ \mu M$); however, 21β-hydroxy-β-amyrenone (**130**) showed only weak activity [113]. The aqueous and methanol extracts of the root bark displayed antimicrobial effects against *Escherichia coli*, *Shigella sonnei*, *S. flexneri*, and *Salmonella* sp. [119]; they also revealed antisecretory activity on cholera toxin-induced intestinal secretion in a rat jejunal loop model [120].

Regarding quality control methods for this plant, a colorimetric method to quantify the content of catechin (**162**) in the roots of *H. excelsa* is described in the "Mexican Herbal Pharmacopoeia" [76]. More recently, a method to quantify the quinone-methide triterpenes in the crude drug by HPLC was developed [121].

2.4 Analgesic and Anti-inflammatory Agents

As in other countries, pain is a common cause of medical consultation in Mexico. The number of plant species for treating painful complaints is enormous. For testing antinociceptive action, a few animal models (rats or mice) have been used. According to a recent study that analyzed the use of analgesic plants in the state of Morelos, most of species were native to Mexico but a considerable number have been introduced [122]. Scrutiny of the scientific literature shows that the use of these species by rural populations has been validated pharmacologically. These studies used different nociceptive models in the rat or mouse such as the hot plate, formalin, capsaicin and acetic acid-induced writhing tests, which use different nociceptive stimuli (chemical, thermal, or mechanical) [14].

In the next few paragraphs some of the work of our group on analgesic and anti-inflammatory agents is summarized. In addition, the Appendix Table shows a recent survey on other medicinal plants from Mexico with these important properties.

2.4.1 *Hofmeisteria schaffneri*

Hofmeisteria schaffneri (A. Gray) R. M. King & H. Robinson (Asteraceae) is a rare evergreen aromatic medicinal herb known as "ambar" (Fig. 47). It grows naturally in the oak and pine-oak forested mountains of the central Mexican states of Jalisco and

Fig. 47 *Hofmeisteria schaffneri* shrubs (**A**) and fresh aerial parts (**B**)

San Luis Potosi, and it is cultivated in home gardens in the valley of Mexico. The fresh or dried aerial parts of the plant are available in Mexican markets and its infusion is used for treating skin wounds, vomiting, and stomachache. The species is cultivated commonly in family gardens throughout Mexico for medicinal purposes [123].

The antinociceptive [124], antimicrobial [125], and spasmolytic [126] potential of the essential oil, decoction, and organic extracts from the aerial parts of *H. schaffneri* were analyzed on the basis of its extensive use for treating stomachache and infectious diseases. These preparations were not acutely toxic when tested at a dose range of 10–5000 mg/kg by the Lorke method [33]. After 14 days, treated mice did not present any visible toxic effects, nor lesions in their internal organs.

The potential antinociceptive effect of the above-mentioned preparations was assessed using the writhing and hot plate tests. Graded doses of the organic extract, essential oil, or aqueous extract (10–562 mg/kg, p.o.) did not decrease acetic acid induced contortions in mice [124]. On the other hand, the organic extract significantly increased the latency to thermal stimuli when tested at the doses of 177 and 562 mg/kg; the effect was dose-dependent. The essential oil and aqueous extract were also very active in the hot plate test at doses ranging from 1 to 316 mg/kg, but the effect was not dose dependent [124].

From the active organic CH_2Cl_2-MeOH (1:1) and aqueous extracts a few thymol derivatives were obtained. These compounds were classified based on the level of oxidation and the number of carbons of the thymol core: the first group were the northymol derivatives, hofmeisterin (**167**) and hofmeisterin II (**168**), which are rare in Nature (Fig. 48) [123]. The second group comprised derivatives without oxygenated units in the isopropyl moiety, such as hofmeisterin III (**169**) and thymol (**170**) (Fig. 48). In turn, the third group comprised compounds with two or three oxygen functions in the isopropyl moiety, such as 8,9-epoxy-10-acetoxythymol angelate (**171**), 8,9-epoxy-10-hydroxythymol angelate (**172**), hofmeisterin IV (**173**), and the derivatives **174** and **175** (Fig. 48) [127]. The last of these groups included the dimeric compound, 3′,4′,4a′,9a′-tetrahydro-6,7′-dimethylspiro[benzofuran-3(2*H*),2′-pyrano[2,3-*b*]benzofuran]-2,4a′-diol (**176**), which is an unusual structure (Fig. 48) [127].

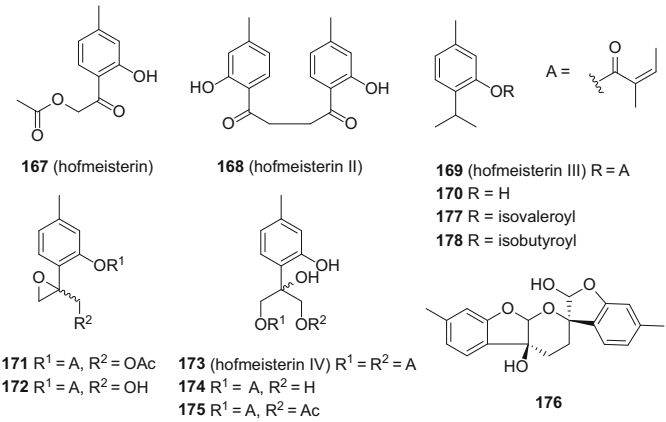


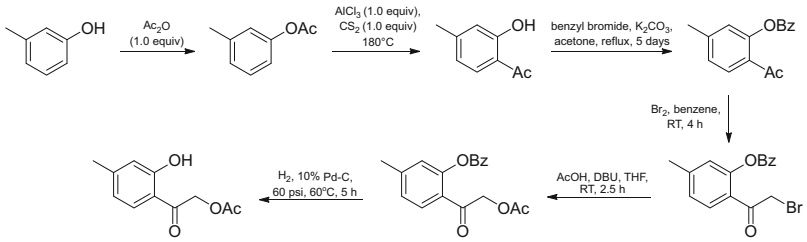
Fig. 48 Thymol derivatives from *Hofmeisteria schaffneri*

The structure of hofmeisterin (**167**) was established unequivocally by synthesis [123], with that of hofmeisterin II (**168**) determined by X-ray analysis [127]. The synthesis path to hofmeisterin (**167**) is outlined in Scheme 2. The general strategy was based on a Fries rearrangement of a suitable acetyl derivative of *m*-cresol. The overall yield obtained of hofmeisterin (**167**) was 15% [123].

Thymol (**170**) and the northymol derivatives **167**–**169** and **171**–**176** can be interrelated biogenetically starting from a suitable intermediate such as 2-[2-(hydroxymethyl)oxiran-2-yl]-5-methylphenol [124]. This compound could undergo an oxidative cleavage to afford the northymol derivative 2-hydroxy-1-(2-hydroxy-4-methylphenyl)ethanone, which upon appropriate "tailoring" reactions would yield hofmeisterin (**167**). 2-Hydroxy-1-(2-hydroxy-4-methylphenyl)ethanone could dimerize through an oxidative coupling reaction to generate hofmeisterin II (**168**) [124]. Alternatively, this compound could arise from 3′,4′,4a′,9a′-tetrahydro-6,7′-dimethylspiro[benzofuran-3(2*H*),2′-pyrano[2,3-*b*]benzofuran]-2,4a′-diol (**176**), by an oxidative degradation reaction. Finally, nucleophilic epoxide opening of 2-[2-(hydroxymethyl)oxiran-2-yl]-5-methylphenol would favor the biosynthesis of hofmeisterin IV (**173**) and analogues [127].

Analysis of the active essential oil of *H. schaffneri* by GC and GC-MS after the plant was collected during the flowering and non-flowering stages revealed the presence of 44 components representing ~90% of the total amount occurring [125]. The more abundant compounds in the active oil were oxygen-containing monoterpenes (72%) including hofmeisterin III (**169**) (30.2%), 8,9-epoxy-10-acetoxythymol angelate (**171**) (15%), thymol isovalerate (**177**) (20.2%), and thymol isobutyrate (**178**) (2.5%). Thus, hofmeisterin III (**169**) is the common metabolite found in the three preparations, but it was more abundant in the essential oil and the organic extract [125].

The major components hofmeisterin III (**169**), 8,9-epoxy-10-acetoxythymol angelate (**171**), thymol isovalerate (**177**), and thymol isobutyrate (**178**) were tested



Scheme 2 Synthesis of hofmeisterin derivatives

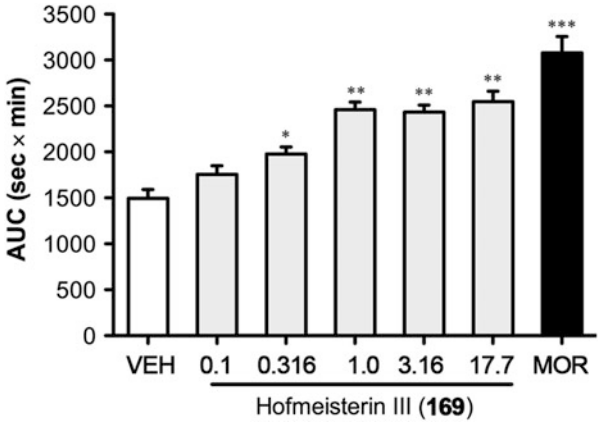


Fig. 49 Antinociceptive effect of hofmeisterin III (**169**) in mice submitted to the hot-plate tests

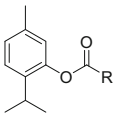
in the hot plate assay and their activity compared with thymol (**169**) and morphine (3.16 mg/kg) [124]. Hofmeisterin III (**169**) (0.1–17 mg/kg) was the most active, increasing significantly the latency to a thermal stimulus in a dose-dependent manner up to a dose of 1 mg/kg (Fig. 49). At higher doses (3.16 and 17.7 mg/kg), the effect remained constant. The pharmacological effect of hofmeisterin III (**169**) was also assessed after pretreatment of mice with either naloxone (1 mg/kg), a non-selective opiate receptor antagonist, glibenclamide (10 mg/kg), a ATP-K channel blocker, or L-NAME (30 mg/kg), an inhibitor of nitric oxide synthase. According to the results obtained, L-NAME and glibenclamide were not able to decrease the antinociceptive activity of hofmeisterin III (**169**). However, the effect of the compound was reversed by treatment with naloxone suggesting that its mode of action involved opioid receptors and/or an increment of endogenous opioids. Thymol isovalerate (**177**) and thymol isobutyrate (**178**) (0.1–17 mg/kg) showed similar effects to hofmeisterin III (**169**), but thymol (**170**) was only active at a dose of 100 mg/kg, suggesting that the esterification of the free phenolic group of thymol improved the resultant activity. 8,9-Epoxy-10-acetoxythymol angelate (**171**) (0.316–17.7 mg/kg) was less

active, suggesting that the presence of the epoxide ring decreased the antinociceptive action. Altogether, this information revealed that the thymol esters of *H. schaffneri* are responsible for the antinociceptive activity of the different preparations tested in this study [124].

Considering that structural modification of a lead molecule may improve its efficacy, 13 analogues (**179**–**191**) (Fig. 50) of hofmeisterin III (**169**) were prepared by condensing thymol with the corresponding acid chlorides in a single-step reaction [124]. The resulting esters were then evaluated in the same pharmacological model at three different doses in the range of 1–17.7 mg/kg. However, they were not better than the lead compound; therefore, the nature of the ester chain did not have a clear impact on the antinociceptive activity. The superior activity of hofmeisterin III (**169**) over its analogs could be due to its better stability and lesser susceptibility to enzymatic degradation, according to in vitro studies in the presence of pig liver esterase in two buffers (pH 7.4 and 5.0), as well as in mouse plasma at 37°C for a period of 24 h [124].

The antimicrobial properties of the oil and extracts of *H. schaffneri* were also assessed using the broth dilution method in 96-microplate wells [125]. The infusion exhibited antibacterial effects against *Staphylococcus aureus* and *Bacillus subtilis*, with a *MIC* value of 64 μg/cm^3 in each case. The essential oil was active against *S. aureus*, having a *MIC* value of 48 μg/cm^3. These were, however, inactive against Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi* (*MIC* $>$ 1024 μg/cm^3). On the other hand, the infusion and the oil displayed moderate anti-*Candida albicans* activity, with *MIC* values of 128 and 192 μg/cm^3, respectively. Finally, the organic extract did not display significant activity against the tested microorganisms (*MIC* $>$ 1024 μg/cm^3). Some of the compounds isolated from the plant were also evaluated including 8,9-epoxy-10-acetoxythymol angelate (**171**) and thymol isovalerate (**177**), present in the essential oil, which displayed the best antibacterial effects against Gram-positive bacteria (*MIC* values ranging between 32 and 64 μg/cm^3) [125].

The last efficacy test performed with the infusion and essential oil of *H. schaffneri* was that for spasmolytic action using an in vivo gastrointestinal transit test in mice [126]. The activity was compared with those of loperamide and atropine. Both preparations inhibited gastrointestinal transit in a dose-dependent manner when tested in a range of doses between 10 and 316 mg/kg; at 316 mg/kg, they inhibited



179 R = $CH_2CH(CH_3)_2$
180 R = $CH(CH_3)_2$
181 R = $C(CH)_3$=$CHCH_3$
182 R = $CH(CH_3)CH_2CH_3$
183 R = CH_2CH_3
184 R = $CH(CH_3)CH_2CH_2CH_3$
185 R = $CH_2CH(CH_3)CH_2CH_3$
186 R = $CH_2CH_2CH(CH_3)_2$
187 R = $CH_2CH_2CH_3$
188 R = $CH_2CH_2CH_2CH_2CH_3$
189 R = C_6H_6
190 R = $CH_2CH_2CH_2CH_3$
191 R = CH=$C(CH_3)_2$

Fig. 50 Synthetic analogues of hofmeisterin III (**169**)

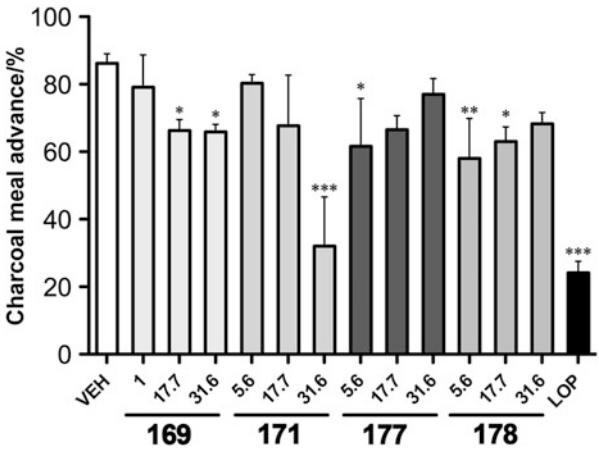


Fig. 51 Spasmolytic effect of extracts and hofmeisterin III (**169**), 8,9-epoxy-10-acetoxythymol angelate (**171**), thymol isovalerate (**177**), and thymol isobutyrate (**178**) from *Hofmeisteria schaffneri*, on gastrointestinal transit in mice

gastrointestinal transit by 47.5% and 52.1%, respectively. The major thymol derivatives of the plant were also evaluated, but only 8,9-epoxy-10-acetoxythymol angelate (**171**) inhibited gastrointestinal transit by 53.4% at a dose of 31.6 mg/kg, in comparison with atropine (3 mg/kg), which caused an inhibition of 50.2% (Fig. 51) [126].

A fast and reliable HPLC-UV method for the quality control of the crude drug of *H. schaffneri* to quantify 8,9-epoxy-10-acetoxythymol angelate (**171**) was developed in order to assure the standardization of the crude drug composition [126]. This method is included in the Second Edition of the "Mexican Herbal Pharmacopoeia" [76].

Finally, it is of interest to note that compounds **167**–**169** and **171**–**176** are of agrochemical interest, since they were phytotoxic against seedlings of *Amaranthus hypochondriacus* and *Echinochloa crus-galli* when tested in a petri dish assay [123, 127]. All compounds inhibited radicle growth of seedlings of both species with IC_{50} values of $\leq 10^{-4}$ *M*. In summary, *H. schaffneri* possesses compounds with antinociceptive, spasmolytic, and antimicrobial properties. Altogether, these effects support the use of this species for treating painful gastrointestinal complaints in Mexican folk medicine.

2.4.2 *Artemisia ludoviciana*

Artemisia ludoviciana Nutt. (Asteraceae) is a rhizomatous white-woolly, perennial herb up to 1 m tall, with a strong odor of sagebrush. The stems are erect and often clustered from creeping rhizomes. The leaves are alternate, entire to irregularly toothed or lobed, 3–11 cm long, and up to 1.5 cm wide (Fig. 52). The flower heads are small tight greenish clusters among the leaves near the ends of the stems, and the plant flowers from August through September. The fruits are dry, smooth, broadly cylindrical achenes. This species is native throughout North

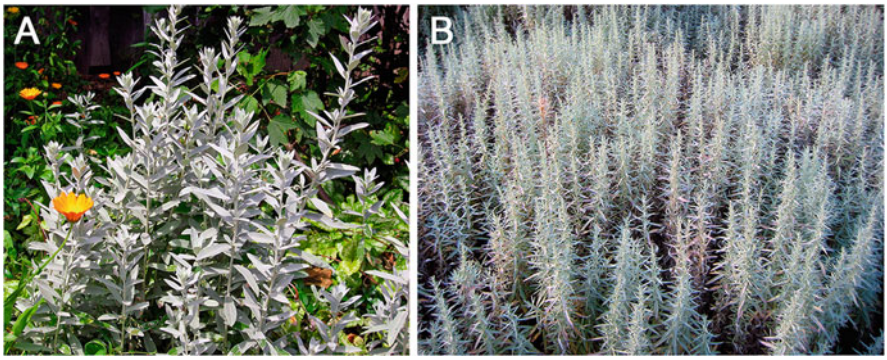


Fig. 52 Wild (**A**) and cultivated (**B**) *Artemisia ludoviciana*

America from Canada to Mexico [128]. The specific epithet "*ludoviciana*" is the Latinized version of the word Louisiana. Thomas Nuttall named the plant in the early nineteenth century during a botanical exploration to the Louisiana Territory. Several subspecies of *A. ludoviciana* are recognized, and probably the most important in Mexico is *A. ludoviciana* Nutt. subsp. *mexicana* (Willd. ex Spreng.) D.D. Keck. Throughout Mexico, this herb is cultivated commonly in home gardens and sold in popular markets, fresh or dry, for medicinal purposes [128].

Artemisia ludoviciana is known with many popular names, some of them of clear indigenous origin. For example, "estafiate" and "istafiate", which derive from the Nahualt term "iztauhyatl", meaning the plant that produces a whitish bitter water. Also, "ambfe" is the Otomi name for the plant; among the Popoloca and the Raramuri, the plant is known as "kamaistra" and "ros'sabl'l", respectively. Other Spanish names include "ajenjo del país" (absinthe of the country), "ajenjo", "artemisia", "hierba maestro", among many others [129]. In the United States, *A. ludoviciana* is termed "silver king", "Louisiana sagewort", and "silver wormwood", just to mention a few [130].

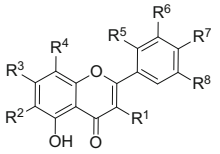
Artemisia ludoviciana has been used medicinally in Mexico since pre-hispanic times, having at that time a divine character. Many authors have reviewed the history of the plant throughout the centuries, and perhaps the most important and complete account appears in the "Atlas of Traditional Medicine of Mexico" [129]. According to the information in this book, "The Libellus de Medicinalibus Indorum Herbis" ("The Badiano Codex, XVI Century"), the recorded uses of "iztauhyatl" were for treating weak hands, injured feet, hemorrhoids, madness, and head lice. In the "Florentine Codex", written between 1555 and 1580, and published between 1829 and 1830, the species was described as valuable for treating pain, fever, and cough. Francisco Hernández in the "Historia Natural de Nueva España" (1571–1577) documented the use of "iztauhyatl" for hemorrhoids, upset stomach, colic, bronchitis, indigestion, flatulence, dandruff, and inflammation. Fray Francisco Ximénez (1615) indicated in "Cuatro Libros de la Naturaleza y Virtudes de las Plantas y Animales en la Nueva España" that "iztauhyatl" has similar medicinal properties to

the European *Artemisia absinthium* [131]. In 1711, Juan de Esteyneffer, a Moravian German lay Jesuit missionary, in his "Florilegio Medicinal" recorded the efficacy of *A. ludoviciana* to treat gastrointestinal disorders, including worms. In the nineteenth century, the plant was described in "Ensayo para la Materia Medica Mexicana" as one of the most important medicinal plants of the country. From 1890 to 1913 at the IMN, several investigations on "estafiate" took place; the researchers concluded that the plant was useful as an antiparasitic, eupectic, antipaludic, analgesic, and cathartic agent. During the twentieth century, many publications attributed a large number of medicinal uses to this species, but consistently its applications for alleviating parasitic diseases, colic, and pain were prominent. Some of the attributed properties have persisted to the present day, although new uses have emerged. Thus, the infusion prepared from the aerial parts of *A. ludoviciana* plays an important role in modern traditional healthcare practices for treating worms, upset stomach, colic, diarrhea, dysentery, many kinds of painful complaints, and diabetes. Rubbing with the whole plant is a remedy for pain and inflammation. In addition, inhalation of the plant is regarded as useful for treating colds and bronchitis as well as sore throats and head sores [6, 7, 10, 122, 132–134]. The oil made up with *A. ludoviciana*, *Persea americana*, *Tagetes erecta*, *Zingiber officinale*, and *Ocimum basilicum*, along with camphor, is utilized as a rub for treating rheumatic pain [129].

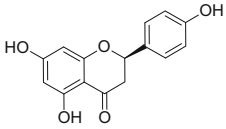
The aerial parts of *A. ludoviciana* subsp. *mexicana* have been thoroughly investigated in terms of phytochemistry and pharmacology. The most relevant compounds found during these investigations were monoterpenoids, sesquiterpenes lactones, and flavonoids [135–146]. The flavonoids belong mostly to the flavone (**192**–**206**), flavonol (**207**–**210**) and flavanone (**211**) types (Fig. 53). On the other hand, the sesquiterpene lactones comprise eudesmanolides (**212**–**234**) (Fig. 54), guaianolides (**235**–**255**) (Fig. 55), and germacranolides (**256**–**264**) (Fig. 56). In addition, a number of thienyl-substituted spiroacetal enol ethers have been isolated from the roots of the plant [147].

The chemical composition of the essential oils from a wide range of *Artemisia* species as well as their pharmacological activities have been recently reviewed [148]. In general, the volatile oils of these species, concentrated in the leaves and flowers, are biologically active. The major active constituents in most species analyzed are camphor, 1,8-cineole, borneol, β-thujone and γ-terpineol [148, 149]. The volatile oil of samples of *A. ludoviciana* from western Canada, Mexico and the United States have been analyzed. In all cases, 1,8-cineole and (±)-camphor were the most relevant components [150–152]. Manjarrez and Medina identified also (−)-α-phellandrene, (+)-limonene, (−)-β-phellandrene, and borneol, from the oil of the leaves of this species, collected in Mexico City [153]. Zavala-Sánchez and co-workers found also the presence of *p*-α-dimethyl benzyl alcohol, linalol, and linalal in the oil of *A. ludoviciana* collected in Huixquilucan, Estado de Mexico, Mexico [154].

We recently analyzed the volatile constituents obtained by HS-SPME and by hydrodistillation of the aerial parts of the plant using GC-MS analysis [155]. The oil contained oxygenated monoterpenes (ten components accounting for 77% of the oil). The most representative were (±)-camphor (21%), γ-terpineol (18%), borneol (18%), terpinen-4-ol (3.5%), 1,8-cineole (3.4%), lavender lactone (3.4%), isoborneol



192 (eupatilin) $R^1 = R^4 = R^5 = R^8 = H$, $R^2 = R^6 = R^7 = OCH_3$, $R^3 = OH$
193 (jaceosidin) $R^1 = R^4 = R^5 = R^8 = H$, $R^2 = R^6 = OCH_3$, $R^3 = R^7 = OH$
194 $R^1 = R^4 = R^6 = H$, $R^2 = CH_3$, $R^3 = R^5 = R^7 = OH$, $R^8 = OCH_3$
195 $R^1 = R^4 = R^5 = H$, $R^2 = R^6 = R^8 = OCH_3$, $R^3 = R^7 = OH$
196 $R^1 = R^4 = R^5 = H$, $R^2 = R^7 = R^8 = OCH_3$, $R^3 = R^6 = OH$
197 $R^1 = R^4 = R^5 = H$, $R^2 = R^8 = OCH_3$, $R^3 = R^6 = R^7 = OH$
198 (selagin) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = R^6 = R^7 = OH$, $R^8 = OCH_3$
199 (luteolin-3',4'-dimethyl ether) $R^1 = R^2 = R^4 = R^5 = R^8 = H$, $R^3 = OH$, $R^6 = R^7 = OCH_3$
200 (tricin) $R^1 = R^2 = R^4 = R^5 = H$, $R^3 = R^7 = OH$, $R^6 = R^8 = OCH_3$
201 (hispidulin) $R^1 = R^4 = R^5 = R^6 = R^8 = H$, $R^2 = OCH_3$, $R^3 = R^7 = OH$
202 (chrysoeriol) $R^1 = R^2 = R^4 = R^5 = R^8 = H$, $R^3 = R^7 = OH$, $R^6 = OCH_3$
203 (apigenin) $R^1 = R^2 = R^4 = R^5 = R^6 = R^8 = H$, $R^3 = R^7 = OH$
204 (luteolin) $R^1 = R^2 = R^4 = R^5 = R^8 = H$, $R^3 = R^6 = R^7 = OH$
205 $R^1 = R^4 = R^5 = R^8 = H$, $R^2 = R^3 = R^6 = R^7 = OCH_3$
206 (eupafolin) $R^1 = R^4 = R^5 = R^6 = H$, $R^2 = OCH_3$, $R^3 = R^7 = R^8 = OH$
207 $R^1 = OCH_3$, $R^2 = R^7 = OCH_3$, $R^3 = OH$, $R^4 = CH_3$, $R^5 = R^6 = R^8 = H$,
208 $R^1 = OCH_3$, $R^2 = OCH_3$, $R^3 = R^7 = OH$, $R^4 = R^5 = R^6 = R^8 = H$
209 (axillarin) $R^1 = OCH_3$, $R^2 = OCH_3$, $R^3 = R^6 =$ $R^7 = OH$, $R^4 = R^5 = R^8 = H$
210 (isokaempferide) $R^1 = OCH_3$, $R^2 = OCH_3$, $R^3 = R^7 = OH$, $R^4 = R^5 = R^6 =$ $R^8 = H$

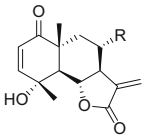


211 (naringenin)

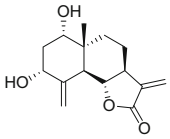
Fig. 53 Flavonoids from *Artemisia ludoviciana*

(2.4%), 6-camphenol (2.3%), *trans*-sabinyl acetate (2.2%), and bornyl acetate (2.2%). HS-SPME using differently coated fibers (carboxen/polydimethylsiloxane, carboxen/divinylbenzene/polydimethylsiloxane, and polydimethylsiloxane) allowed the detection of *trans*-pinocarveol, 1,8-cineole, camphen-6-ol, (±)-camphor, artemiseol, artemisia ketone, borneol, myrtenyl acetate, and bornyl acetate [155].

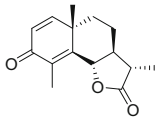
A significant number of publications exist concerning the biological activities of *A. ludoviciana*; these studies have documented in vitro cytotoxic [156], anti-*Mycobacterium tuberculosis* [157], anti-*H. pylori* [72], and anti-*Plasmodium yoelli* properties [158]. A few studies related with the use of "estafiate" preparations for treating gastrointestinal complaints have been described. Thus, Zavala and coworkers [154] described the antidiarrheal activity of the essential oil obtained from the aerial parts of *A. ludoviciana*. Nonanal was identified as the active principle, since it inhibited castor oil-, magnesium sulfate-, and arachidonic acid-induced diarrhea in CD1 mice. Nonanal delayed also castor oil-stimulated intestinal transit in Wistar rats [154]. Velázquez et al. [120] found that the antisecretory activity induced by aqueous or methanol extracts on cholera toxin-induced intestinal secretion using the rat jejunal loop model was rather poor (15% and 5%, respectively). In another



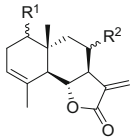
212 (arglanin) R = H
213 (artemexifolin) R = α-OAc



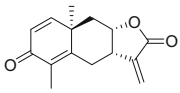
225 (ludovicin B)



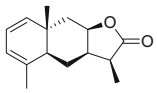
226 (santonin)



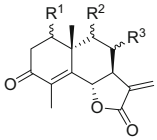
214 (douglanine) R^1 = α-OH, R^2 = H
215 (ludalbin) R^1 = α-OH, R^2 = α-OAc
216 (santamarine) R^1 = β-OH; R^2= H



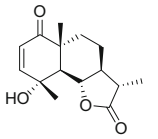
227 (yomogin)



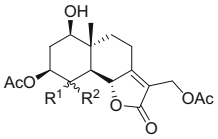
228 (anthemidin)



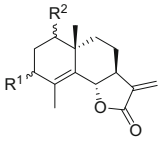
217 (armexifolin) R^1 = β-OH, R^2 = R^3 = H
218 (ludovicin C) R^1 = α-OH, R^2 = R^3 = H
219 (8α-acetoxyarmexifolin) R^1 = α-OH, R^2 = H, R^3 = α-OAc
220 R^1 = R^3 = H, R^2 = OH



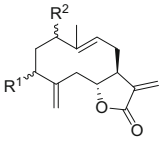
229 (vulgarin)



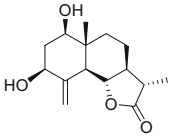
230 R^1 = R^2 = H
231 R^1 = α-OH, R^2 = β-CH_3



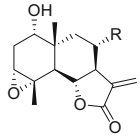
221 (armexin) R^1 = α-OAc, R^2 = β-OAc
222 (armefolin) R^1 = α-OH, R^2 = β-OH



232 (ridentin) R^1 = R^2 = α-OH
233 (ridentin B) R^1 = R^2 = β-OH



234



223 (ludovicin A) R = H
224 (α-epoxyludalbin) R = α-OAc

Fig. 54 Eudesmanolides from *Artemisia ludoviciana*

study, it was found that a methanol extract of *A. ludoviciana* inhibited the adhesion of *Vibrio cholerae* to Chinese hamster ovary cells [159]. Aqueous and methanol extracts of the aerial parts showed weak antiprotozoal activities against *Entamoeba histolytica* and *Giardia lamblia* [160, 161], antibacterial properties against *E. coli* and *S. flexneri* [119], and an antifungal effect, mainly against *Trichoderma viridae* [162]. In turn, the essential oil had inhibitory effects on the growth of bacteria (*E. coli*, *S. aureus*, and *S. epidermidis*), yeasts (*C. albicans* and *C. neoformans*),

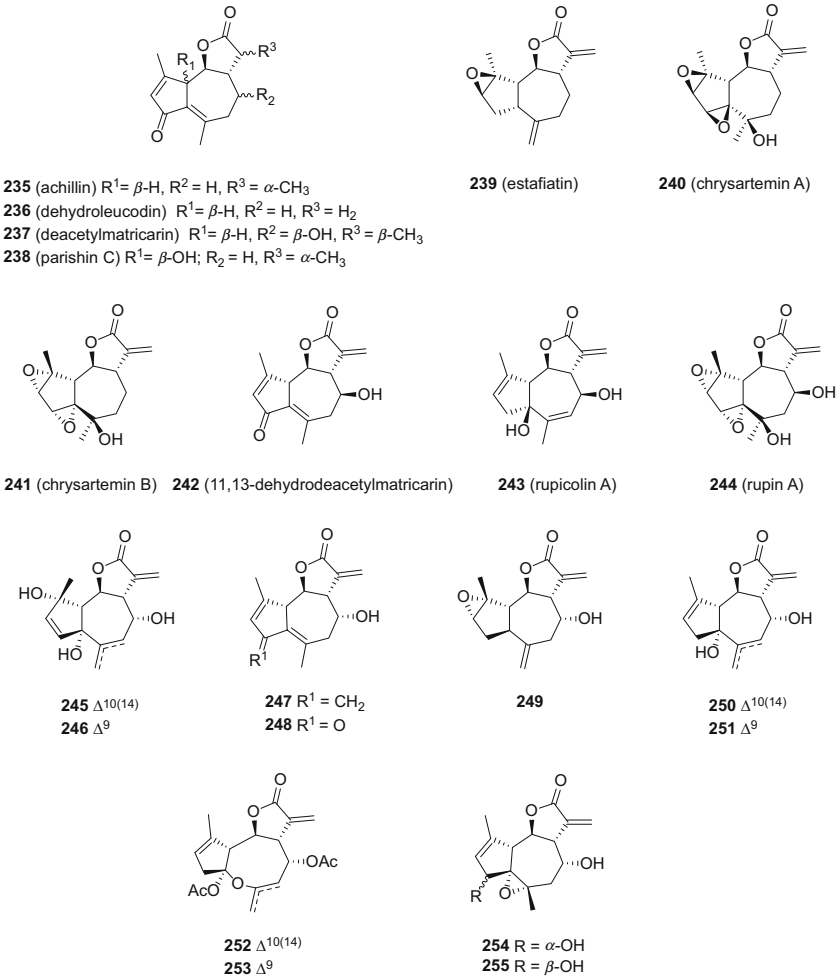


Fig. 55 Guaianolides from *Artemisia ludoviciana*

dermatophytes (*Trichophyton rubrum*, *Microsporum canis*, and *M. gypseum*), *Fonsecaea pedrosoi* and *Aspergillus niger* [152]. *Artemisia ludoviciana* extracts showed also a moderate inhibitory effect (31%) on charcoal-gum acacia-induced hyperperistalsis in rats [163], which was consistent with the same levels of spasmolytic action exerted by extracts of different polarity (methanol, dichloromethane, and *n*-hexane) prepared from the aerial parts of the plant. The best effect was observed by the dichloromethane extract, which induced a concentration-dependent inhibition of the spontaneous contraction of rat ileum strips of not greater than 50% [164]. Finally, the in vitro anti-inflammatory action of the plant has been reported [165].

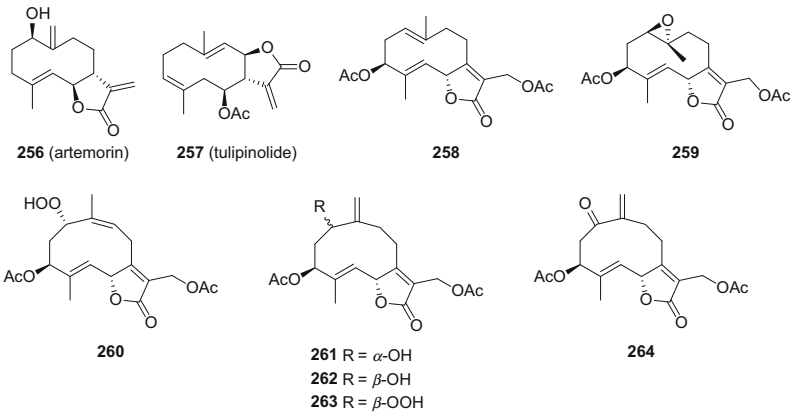


Fig. 56 Germacranolides from *Artemisia ludoviciana*

The use of different preparations of *A. ludoviciana* for treating painful complaints prompted us to evaluate the antinociceptive activity of the essential oil and infusion obtained from the aerial parts of the plant using the hot plate and formalin tests [155, 166]. Thus, injection of 2% formalin solution subcutaneously in the hind paw of mice pretreated with vehicle resulted in intense spontaneous licking of the injected paw with a classic biphasic response. However, pretreatment of the mice with the essential oil of "estafiate" (1, 10, 31.6, 100, and 316 mg/kg, i.p.) decreased both the first and second phases of formalin-induced nociception; in the first stage, the effect was only observed at the highest dose (316 mg/kg) tested, but in the second, the animal responses were attenuated in a dose-dependent manner (Fig. 57). The effectiveness of the oil (1, 10, 17.7, 31.6, and 100 mg/kg) for attenuating neurogenic pain was corroborated using the hot plate test (Fig. 58). The mean ED_{50} value calculated was 25.9 mg/kg. The antinociceptive action of the essential oil was blocked by naloxone, suggesting that its mode of action involved an opioid-related mechanism. It is important to mention that the volatile oil of *A. ludoviciana* (316 mg/kg) did not affect animal motor and coordination functions when tested by the rotarod and open field tests, indicating that the pharmacological effects exerted during the hot plate and formalin tests are truly antinociceptive [155, 166].

In addition, the infusion of the plant when tested with the formalin test at the dose of 316 mg/kg displayed a significant antinociceptive effect during the inflammatory phase, indicating that the traditional preparation contains compounds that might modify the production or release of some inflammatory mediators involved in the second phase. Furthermore, oral administration of the infusion (31.6–316 mg/kg) significantly inhibited the formation of carrageenan-induced edema (Fig. 59). The effect was comparable to that of the positive control group (diclofenac; 31.6 mg/kg, p.o.) [166].

Seven major compounds detected in the infusion were eupatilin (**192**), jaceosidin (**193**), arglanin (**212**), achillin (**235**), dehydroleucodin (**236**), salvinine (**265**), and

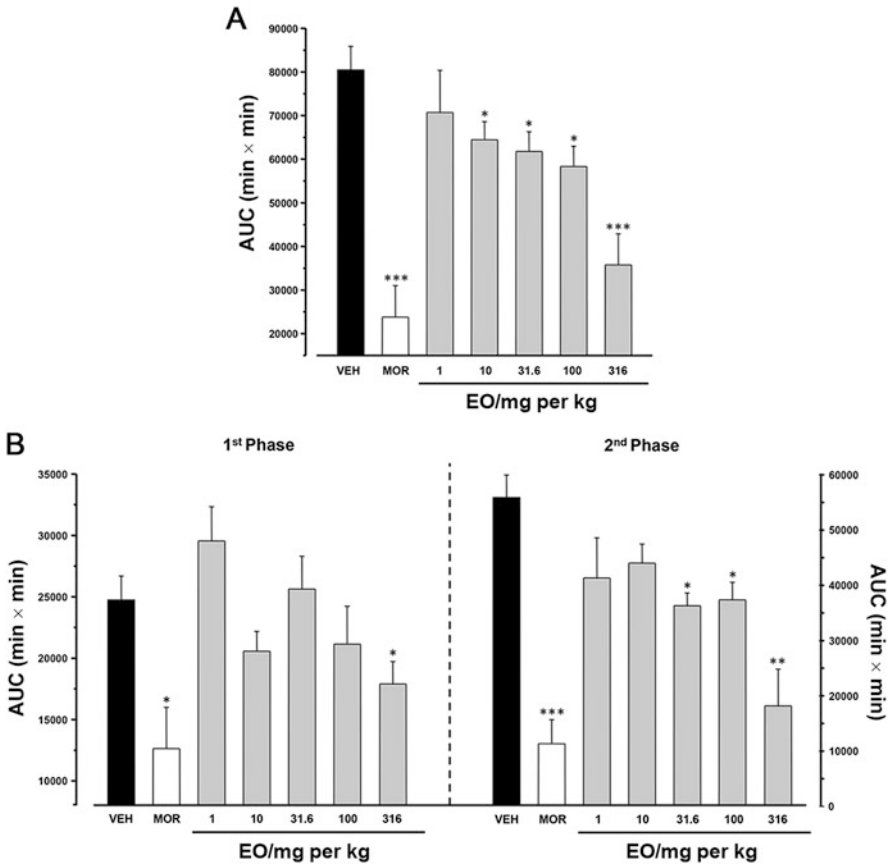


Fig. 57 Antinociceptive effect of the essential oil (EO) from the aerial parts of *Artemisia ludoviciana* in the formalin test (**A**). Time of licking during phases 1 and 2 (**B**). Data represent the means from eight experiments ±SEM of area under the curve (AUC) of the time of licking. VEH: vehicle; MOR: morphine (5 mg/kg). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

3,5-dicaffeoylquinic acid (**266**) (Fig. 60) [166]. Achillin (**235**) and dehydroleucodin (**236**) showed also antinociceptive and anti-inflammatory effects in the formalin (10, 17.7, 31.6 mg/kg) (Fig. 61) and in the carrageenan-induced edema (17.7 mg/kg) (Fig. 62) tests in mice. The best effects were also observed in the inflammatory phase. The action of dehydroleucodin (**236**) was attributed to its inhibitory effect of the transcription factor NF-κB [167]. These results are consistent with the anti-inflammatory and antinociceptive action that have been demonstrated for a number of sesquiterpene lactones.

According to our results and the different studies summarized above, the efficacy of *A. ludoviciana* for treating gastrointestinal diseases are related to its antibacterial, analgesic and anti-inflammatory actions.

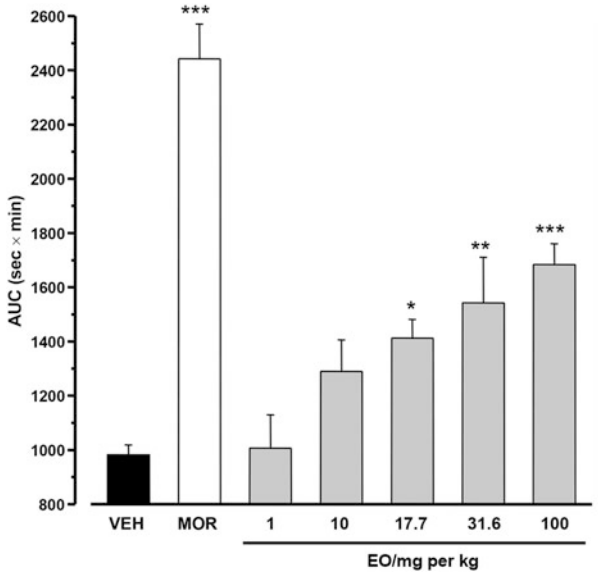


Fig. 58 Antinociceptive effect of the essential oil (EO) from aerial parts of *Artemisia ludoviciana* in the hot plate test. Data represent the means from eight experiments ±SEM of area under the curve (AUC) of latency. VEH: vehicle; MOR: morphine (5 mg/kg). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$

Regarding the antidiabetic properties of *A. ludoviciana*, several ethnobotanical reports in the last two decades have documented this new application for the plant [6, 7, 132]. In the course of our investigation of antidiabetic plants from Mexico, we found that a few preparations (infusion, organic extract and essential oil) made up from the aerial parts of *A. ludoviciana* were very active in both an acute hypoglycemic assay and an OGTT in both normal and NAA-STZ hyperglycemic mice [146]. Treatment of mice with an aqueous extract reduced significantly, in a non-dependent dose manner, blood sugar levels in hyperglycemic mice; the best effect was observed at the dose of 316 mg/kg. In normal mice, no significant changes in blood glucose concentrations were observed at all doses tested, although a lowering trend was observed. In the OGTT, the infusion at doses of 100 and 316 mg/kg provoked a considerable decrease of the postprandial peak in both normal and hyperglycemic mice. Finally, the aqueous extract inhibited by 45% the activity of yeast α-glucosidase. The results of an OSTT were consistent with this effect, and the essential oil did not induce hypoglycemic action in both normal and diabetic mice. However, it induced an important lowering of the postprandial peak during an OGTT in normal and hyperglycemic mice [146].

Biological testing of eupatilin (**192**) and arglanin (**212**), the main components of the infusion of the species, revealed their hypoglycemic effects (Fig. 63). In the case

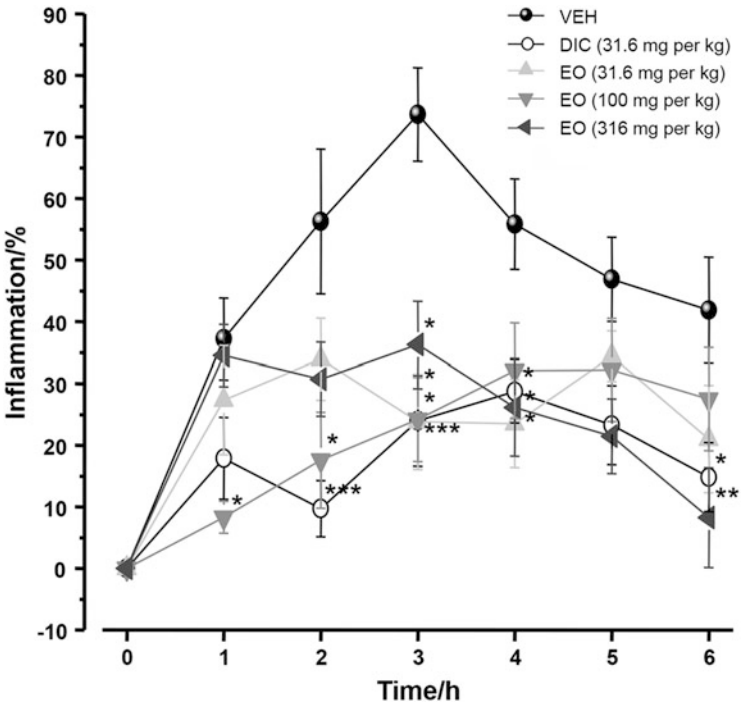
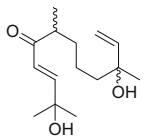
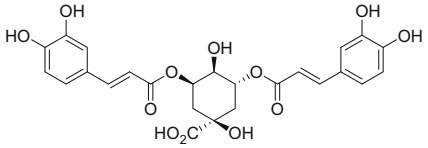


Fig. 59 Anti-inflammatory effect of the essential oil (EO) from *Artemisia ludoviciana* on carrageenan-induced edema formation in the mouse paw. Data represent the means from five experiments ±SEM. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$



265 (salvinine)



266 (3,5-dicaffeoylquinic acid)

Fig. 60 Structures of salvinine (**265**) and 3,5-dicaffeoylquinic acid (**266**) from *Artemisia ludoviciana*

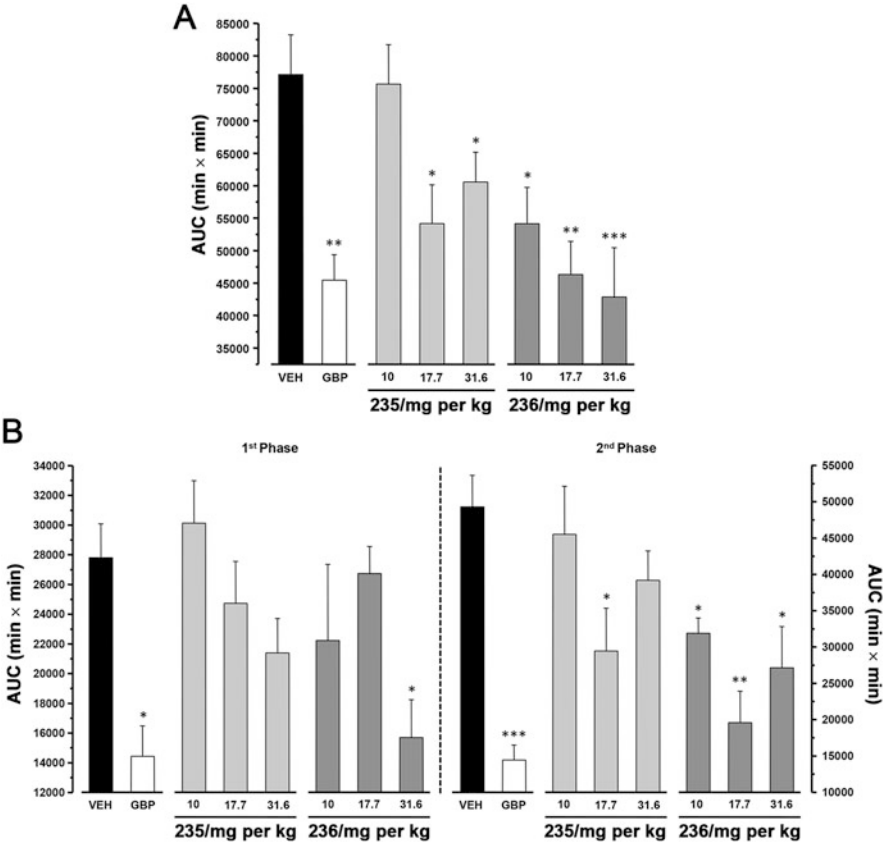


Fig. 61 Effect of achillin (**235**) and dehydroleucodin (**236**) during the formalin test in mice (**A**). Time of licking during phases 1 and 2 (**B**). Data represent the means from six experiments ±SEM of area under the curve (AUC) of the time of licking. VEH: vehicle; GBP: gabapentin (31.6 mg/kg). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$

of arglanin (**212**), the hypoglycemic effect was attenuated in the presence of nicorandil, with this compound thus behaving as an ATP-K^+-channel blocker like glybenclamide (Fig. 64). Salvinine (**265**) turned out to be a mixed α-glucosidase inhibitor. It is noteworthy that there are several papers and patents that refer to eupatilin (**192**) and jaceosidin (**193**) for preventing and treating diabetes mellitus [168]. Altogether, these pharmacological results revealed the potential of *A. ludoviciana* as a hypoglycemic and antihyperglycemic agent.

Other species of the genus *Artemisia* have also demonstrated potential for the development of new preparations for treating diabetes, in particular, *A. herba alba* [169], *A. dracunculus* [170–172], *A. princeps* [173, 174], *A. capillaris* [175, 176],

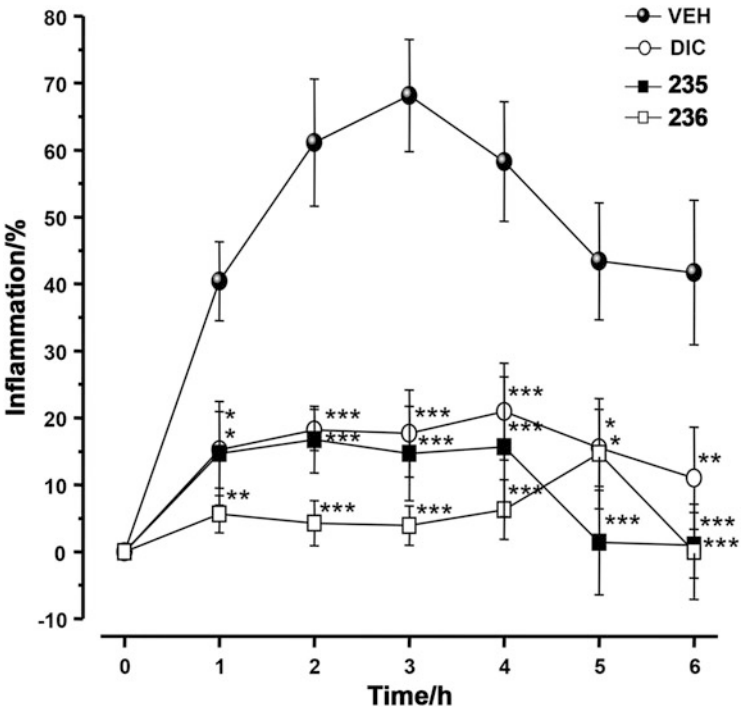


Fig. 62 Effect of achillin (**235**) (17.7 mg/kg) and dehydroleucodin (**236**) (17.7 mg/kg) on carrageenan-induced edema in mice. Data represent the means from six experiments ±SEM of % of inflammation. VEH: vehicle; DIC: diclofenac (31.6 mg/kg). $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$

and *A. judaica* [177]. Furthermore, from *A. dracunculus*, an ethanol preparation, named "Tarralin", has been developed and patented [171, 178]; and from *A. judaica*, another patent was developed concerning a novel methodology for the preparation of clinically useful extracts possessing insulin-like activity [179]. From *A. capillaris*, a tablet dosage form and a rice cracker containing the aqueous extract were also formulated for treating diabetes [180]. Using *A. argyi* and *Prunellae spica*, an insulin mimetic preparation was developed that inhibited also the activity of protein tyrosine phosphatase 1B [181]. From *A. rupestris*, another preparation was developed [182]. Finally, *A. annua* can be combined with drugs and health foods for controlling postprandial blood sugar, alleviating insulin resistance, and for preventing and treating diabetes [183]. These studies support also the potential of *A. ludoviciana* in Mexico for treating diabetes.

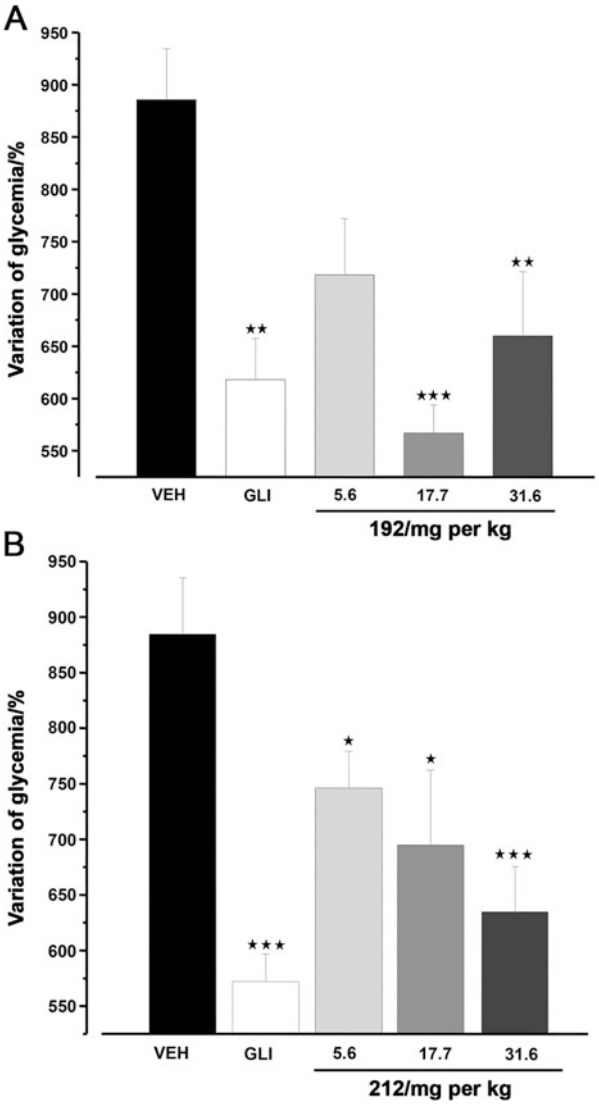


Fig. 63 Hypoglycemic activity of eupatilin (**192**) (**A**) and arglanin (**212**) (**B**) isolated from *Artemisia ludoviciana* in both normal and NAA-STZ-treated mice. Data represent the means from six experiments ±SEM. $*p < 0.05$

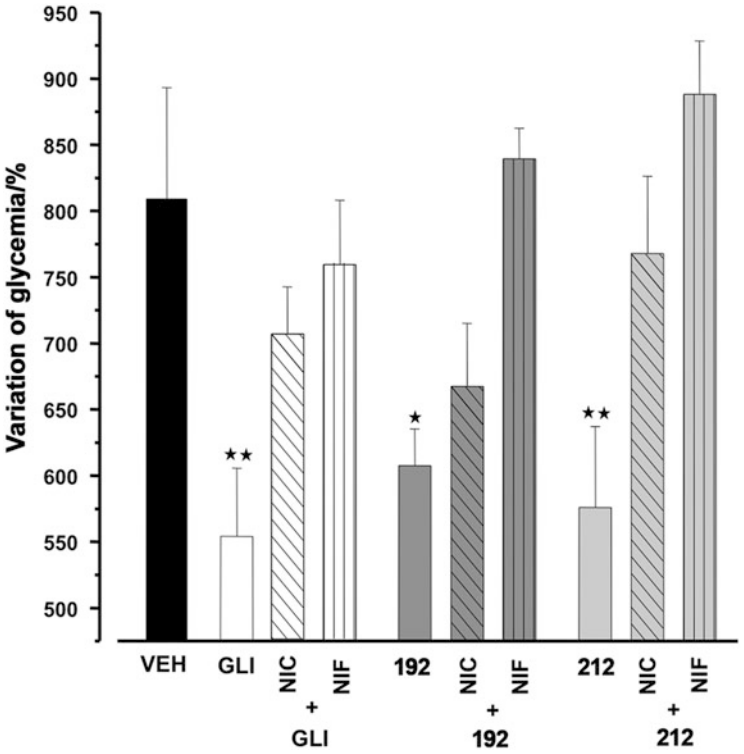


Fig. 64 Effect of nicorandil (NIC, 6.8 mg/kg) and nifedipine (NIF, 13.6 mg/kg), potassium and calcium ion channel regulators, on the hypoglycemic activity of the glibenclamide (GLI, 15 mg/kg), eupatilin (**192**) (17.7 mg/kg), and arglanin (**212**) (17.7 mg/kg) in NAA-STZ-treated mice. Data represent the means from six experiments ±SEM. $*p < 0.05$

2.5 Antiparasitics

Parasitic diseases are among the most prevalent infections worldwide. In Mexico, an important segment of the population is also affected with gastrointestinal parasitic diseases. These ailments are in two principal groups, those caused by protozoa and those caused by helminths, which are probably the most widespread of human parasites [184–187]. In the last decades, certain diseases such as intestinal amebiasis and ascariasis are among the 20 most common causes of morbidity in Mexico. In 2015, the overall mortality associated with these diseases was 2.5%. Intestinal infectious diseases and helminthiasis have also been positioned within the ten leading causes of death in the southern and central part of the country with cysticercosis and ascariasis claiming the most lives, with 2575 and 448 cases, respectively. Other medically relevant protozoa include *G. lamblia*, for which the seroprevalence in Mexico has been determined to be as high as 55.3%. In many

rural areas of the country, these parasitic diseases are treated with medicinal plants, whether under medical supervision or not. Recent ethnopharmacological research in Mexico has brought to light many plants used to treat the most lethal parasitic diseases produced by blood and tissue protozoa, namely, malaria, trypanosomiasis and leishmaniasis [184–187].

2.5.1 *Dysphania graveolens*

Dysphania graveolens (Willd.) Mosyakin & Clemants (syn. *Chenopodium graveolens* Willd. and *Teloxys graveolens* (Willd.) W.A. Weber) is a bright green annual herb, turning red in the autumn, with pinnately lobed leaves and yellow glands, especially on the calyx (Fig. 65). The plant gives off a strong scent reminiscent of the smell of skunks, so the plant is commonly known as "yerba del zorrillo" (skunk herb). A tea prepared from the aerial parts of the plant is ingested (often after the main meal) to alleviate gastrointestinal ailments, to act as a vermifuge, and to relieve headaches and fevers. "Yerba del zorrillo" was noted as an additive to beans and as a medicinal at San Buenaventura mission in 1776 [22].

The anthelmintic properties of *Dysphania ambrosioides* (L.) Mosyakin & Clemants and *D. graveolens* have long been recognized in Mexico and worldwide. Furthermore, the essential oil of the former species was highly valued for many years to treat worms in humans and animals [22, 188]; in the case of *D. ambrosioides*, ascaridol (**267**) is considered the most relevant active constituent (Fig. 66) [189]. It is worth mentioning that archaeological evidence from coprolites suggests its similar use in prehistoric times. Several constituents have been isolated from *D. graveolens*, including: sterols, stigmasterol (**166**), stigmast-22-en-3-ol (**268**), and sitosterol 3-*O*-α-glucoside (**269**) (Fig. 66); flavonoids, pinostrobin (**270**), pinocembrin (**271**), chrysin (**272**), rutin (**273**), narcissin (**274**), and negletein (**275**) (Fig. 66); sesquiterpenoids, (+)-8α-hydroxyelemol (**276**), (+)-8α-acetoxycryptomeridiol

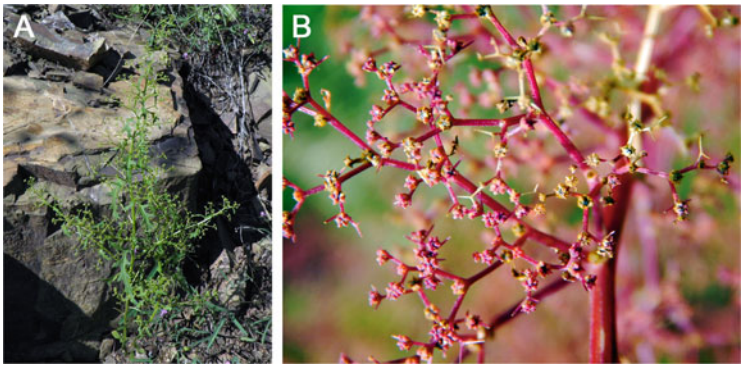


Fig. 65 Stem and leaves (**A**) and flowers (**B**) of *Dysphania graveolens*

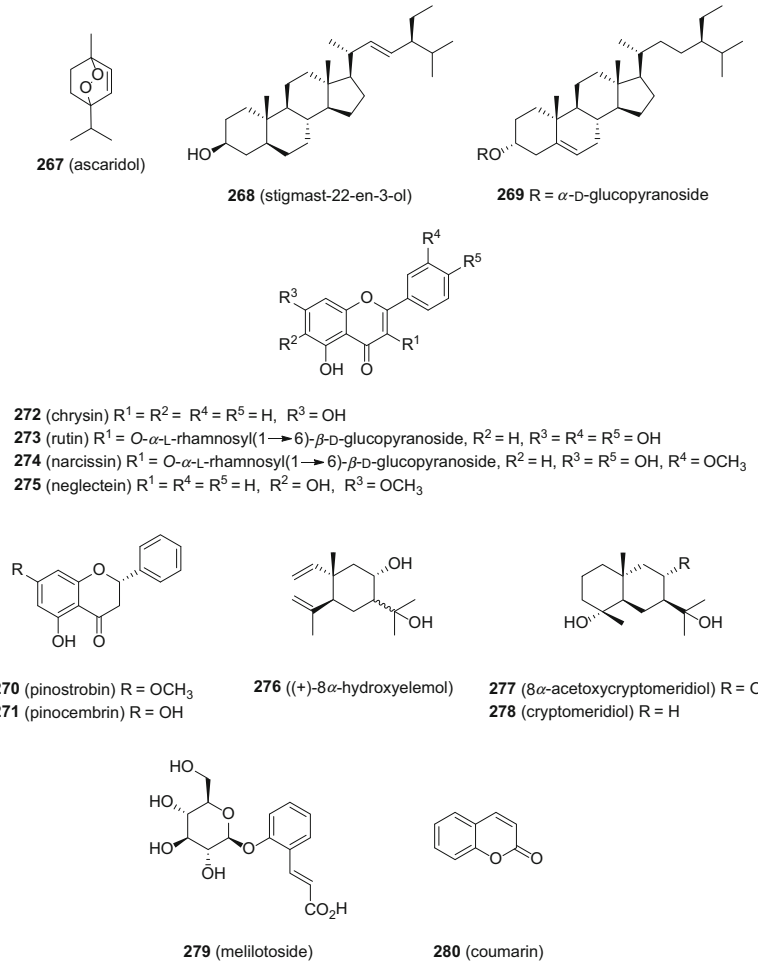


Fig. 66 Compounds isolated from *Dysphania graveolens*

(**277**) and cryptomeridiol (**278**) (Fig. 66); a phenylpropanoid, melilotoside (**279**), and coumarin (**280**) (Fig. 66) [190, 191].

The profile of the volatile components of *D. graveolens* was assembled via GC-MS analyses of both HS-SPME-adsorbed compounds, and those of the essential oils obtained by hydrodistillation of the aerial parts of the plant [192]. The results were compared with those obtained for *D. ambrosioides.* For the HS-SPME analysis, a polydimethylsiloxane-coated fiber was used and 13 components were characterized for *D. graveolens*, with *p*-cymene (84.8%) and eucalyptol (11.3%) as the most relevant components. α-Terpinene, *p*-cymene, (+)-limonene, and ascaridol (**267**) were the most abundant components of *D. ambrosioides*. The essential oil of *D. graveolens* contained 21 chemical constituents, representing about 99% of the total content. The most important components were eucalyptol (42.9%) and *p*-cymene (16.5%) and it did

not contain any ascaridol (**267**). *Dysphania ambrosioides,* on the other hand, proved to contain large amounts of *trans-p*-mentha-1(7),8-dien-2-ol, carvacrol, phytol, carvone, and ascaridol (**267**), thus revealing important differences between the essential oils of these two species [192].

The LD_{50} values for a CH_2Cl_2-MeOH (1:1) extract, an infusion, and an essential oil prepared from the aerial parts of the plant were higher to 5000, 3807, and 3807 mg/kg, respectively, when tested by the Lorke method [193]. Thus, all preparations of *D. graveolens* were deemed safe for mice, when tested in this manner. No adverse effects nor macroscopically detected injuries to the vital organs were observed in any case.

The pharmacological studies conducted on extracts and compounds from *D. graveolens* have shown anthelmintic [194], antiseptic [195], antiprotozoal [191], spasmolytic [196], and antinociceptive [193] actions. Regarding the anthelmintic properties, bioassay-guided fractionation of an organic extract of the plant using a brine shrimp lethality test yielded pinocembrin (**271**); this flavanone showed a fasciolicide effect against newly excysted *Fasciola hepatica* and ovicidal action against the infective eggs of *Ascaridia galli* [194]. The fasciolicide action was assessed at 0.1, 1, 5, and 10 μg/cm^3. The effect at the highest concentration tested was better than that of diamphenetide (10 μg/cm^3), an effective fasciolicidal agent used as the positive control. The ovicidal activity was determined on infective eggs of *A. galli*, a parasitic roundworm that causes ascaridiasis in chickens and turkeys. More recently, extracts from *D. graveolens* and pinocembrin (**271**) showed in vivo and in vitro cysticidal activity against *Taenia crassiceps*, a canid parasitic tapeworm related to *T. solium*, the pork tapeworm, and *T. saginata*, the beef tapeworm [197]. All extracts caused 100% mortality of the cysts at 200 μg/cm^3 of *T. crassiceps* with EC_{50} values ranging from 44.8 to 67.1 μg/cm^3. The in vivo studies were carried out in male BALB/c mice infected by intraperitoneal injection with 30 cysts of *T. crassiceps* ORF strain. The methanol extract, in particular, exhibited good activity in vitro and in vivo with *T. crassiceps* cysts, accompanied by low cytotoxicity. The extract caused significant alterations on the germinal layer of the cysts, with a high accumulation of granules of glycogen and vacuoles; the effect was similar to that of albendazole, used as the positive control. Pinocembrin (**271**) had an EC_{50} value of 46.76 μg/cm^3 and caused an inhibition of the motility of the parasites after 3 days of the treatment and the maximum effect was obtained on day 5 [197].

A methanol extract of *D. graveolens* had amebicidal and giardicidal activities when tested in vitro against axenically grown trophozoites of *Giardia lamblia* and *Entamoeba histolytica* [191]. The effect of the extract was attributed to melilotoside (**279**) (IC_{50} values of 12.5 and 16.8 μg/cm^3 for *E. histolytica* and *G. lamblia*, respectively) and narcissin (**274**), which showed selective activity against *E. histolytica* (IC_{50} = 17.2 μg/cm^3). This extract, pinostrobin (**270**), and chrysin (**272**) inhibited intestinal smooth muscle contractions of guinea-pig ileum preparations by a calcium-mediated mechanism [196].

Three preparations (oil, organic extract and infusion) from the aerial parts of the plant showed substantial antinociceptive properties when tested by the hot plate and writhing models in mice at three different doses (31, 100, and 316 mg/kg). The effects were observed after an acute treatment with the preparations. Morphine and

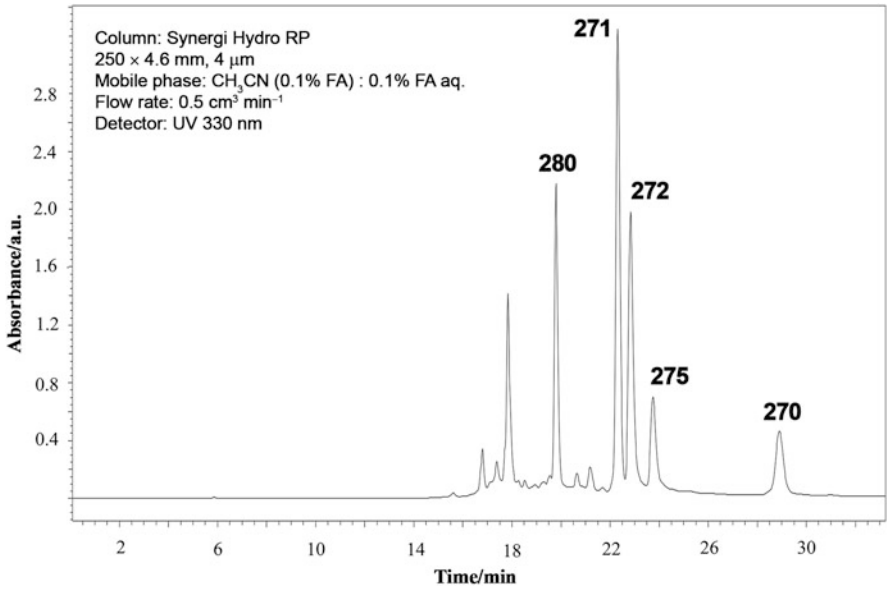


Fig. 67 HPLC chromatogram of the infusion of *Dysphania graveolens*

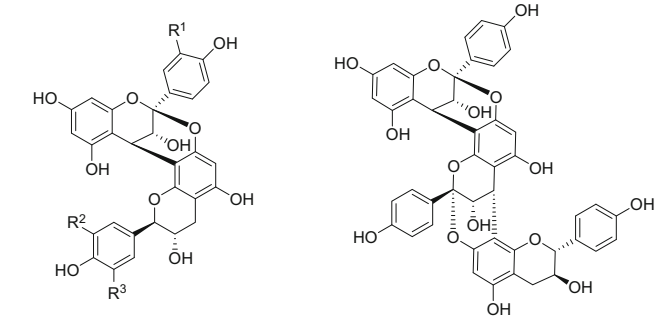
metamizol were used as a positive controls, respectively. The essential oil was the most active in all tests but the traditional preparation was more active in the writhing test. Three major components of the infusion, pinostrobin (**270**), pinocembrin (**271**), and chrysin (**272**) (Fig. 67), were tested also in both models. The results showed that pinocembrin (**271**) exhibited a better antinociceptive effect in the hot plate test and pinostrobin (**270**) in the writhing test. Chrysin (**272**) was active in both assays. These results for the three compounds were confirmed by means of the formalin test. Since **272** was the most active compound, it was selected along with the traditional preparation for the analysis of its mode of action using the formalin test. Thus, peripheral administration of **272** or the infusion reduced the time of licking in either the first or second phase of the formalin test in mice, in a concentration-dependent manner. Pretreatment of mice with ketanserin, a 5-$HT_{2A/2C}$ receptor antagonist, did not reverse the antinociceptive action of **272** in the formalin test at the doses tested, demonstrating that these receptors are not implicated in the effect. In contrast, pretreatment with naltrexone, an opioid antagonist, altered the antinociceptive response produced by chrysin (**272**). On the other hand, pretreatment with flumazenil and bicuculline (antagonists at the $GABA_A$ receptor) before the administration of **272** reversed the antinociceptive effect. These results suggested that the antinociceptive effect of **272** was due to interaction with the $GABA_A$ receptor. Finally, the traditional preparation, pinostrobin (**270**), and chrysin (**272**) all showed notable spasmolytic action by a calcium-mediated mechanism when tested in the guinea-pig ileum model [196]. These last-mentioned set of pharmacological experiments are very pertinent when considering that nowadays the main use of this species is for treating stomachache.

2.5.2 *Geranium niveum*

Geranium niveum S. Watson (Geraniaceae) is a silvery canescent-leaved herb, which grows along the dry stream banks and grassy meadows of pine-oak forests in the high mountains of western Chihuahua, Mexico (Fig. 68). The Tarahumara Indians call this perennial herb "makiki" and employ a decoction of the roots to treat dysentery. They also claim that longevity and stamina emanate from their intake of this local plant [10].

A $CHCl_3$-MeOH (1:1) extract prepared from the roots of *G. niveum* showed evident antiprotozoal activity against axenically grown trophozoites of *G. lamblia* and *E. histolytica* [198]. From the active extract, four new antiprotozoal A-type proanthocyanidins were obtained, namely, geranins A–D (**281**–**284**) (Fig. 69) [199, 200]. Their structural elucidation was accomplished primarily by spectroscopic means. In the case of geranin A (**281**), the molecular formula was

Fig. 68 *Geranium niveum*



281 (geranin A) R^1 = H, R^2 = R^3 = H
282 (geranin B) R^1 = OH, R^2 = R^3 = H
283 (geranin C) R^1 = H, R^2 = R^3 = OH

284 (geranin D)

Fig. 69 Structures of geranins A–D (**281**–**284**) from *Geranium niveum*

determined as $C_{30}H_{24}O_{10}$ based on HRFABMS. The A-type proanthocyanidin nature of geranin A (**281**) was evident from the ^{1}H-, ^{13}C-, and COSY-NMR spectra. Thus, the ^{1}H-NMR spectrum contained the typical signals for the dihydropyran rings of the upper (U) and terminal (T) flavan-3-ol units (Fig. 70). The resonances for the U dihydropyran ring appeared as an AB system at δ_H 4.08 (d, J = 3.5 Hz, H-3U) and 4.26 (d, J = 3.5 Hz, H-4U) ppm. Those of the T unit were observed as an A_2XY system at δ_H 4.80 (d, J = 8.0 Hz, H-2T), 4.17 (ddd, J = 8.5, 8.0, 5.5 Hz, H-3T), 2.93 (dd, J = 16.5, 5.5 Hz, H-4Ta), and 2.58 (dd, J = 16.5, 8.5 Hz, H-4Tb) ppm. The J (8 Hz) value between H-2T and H-3T revealed the *trans* relationship of these protons [199]. In addition, the aromatic region of this spectrum indicated the presence of four aromatic rings in the molecule. The corresponding resonances appeared as a singlet at δ_H 6.10 ppm (H-6T), two A_2B_2 systems and one AB system, consistent with a penta-, two *p*-di-, and one tetra-substituted benzene ring substituent, respectively. The ^{13}C NMR (Fig. 70) data and the HMQC correlations supported the above-mentioned ^{1}H NMR assignments [199].

The linkage between the two flavonoid moieties of geranin A (**281**) was defined through the analysis of a NOESY experiment; thus, the correlations H-4U/H-2T, H-3U and H-6U/H-2′T and H-6′T defined the (4→8, 2→O→7) linkage [199]. The absolute configuration at the chiral centers was established by combining CD

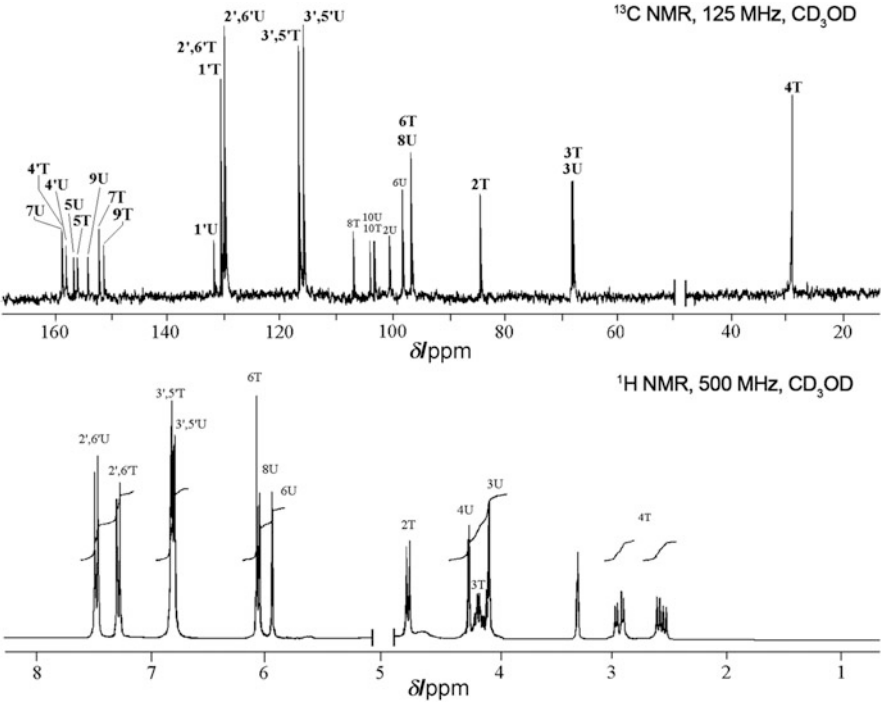


Fig. 70 ^{1}H- and ^{13}C-NMR spectra of geranin A (**281**)

measurements and Mosher ester methodology. The CD spectrum of geranin A (**281**) displayed a strong positive Cotton effect at 220 nm ($[\theta] = 3.26 \times 10^3$ cm^2/mol), so, consequently the configuration at C-4U was (*R*). Accordingly, the configuration at C-2U could be assigned as (*R*). On the other hand, the absolute configurations at C-3U and C-3T were established from the analysis of the $\Delta\delta_{R\text{-}S}$ data of the (+)-(*R*)- and (−)-(*S*)-MTPA esters of a methyl derivative of geranin A (**281**). Thereafter, the absolute configuration at C-2T was determined as (*S*), because of the *trans* relationship between H-3T and H-2T [199].

Geranins A–D (**281**–**284**) exhibited moderate antiprotozoal properties, compared with a standard used, against both *G. lamblia* and *E. histolytica*. *Giardia lamblia* was more sensitive to geranins A (**281**) (IC_{50} = 24 μg/cm^3) and B (**282**) (IC_{50} = 2.6 μg/cm^3); but geranin D (**284**) was more active against *E. histolytica* (IC_{50} = 28.6 μg/cm^3) [199, 200]. None of the geranins displayed discernible cytotoxic activity against three different cell lines (MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, and A-549 lung carcinoma) in a 7-day MTT test.

The in vitro antioxidant activity of geranins A (**281**) and D (**284**), and the extract, using seven different assay systems, namely, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$), showed that they were able to scavenge ABTS, DPPH, $O_2^{\bullet -}$, $OH^{\bullet}$, and HOCl (Fig. 71) [201]. The scavenging ability of these

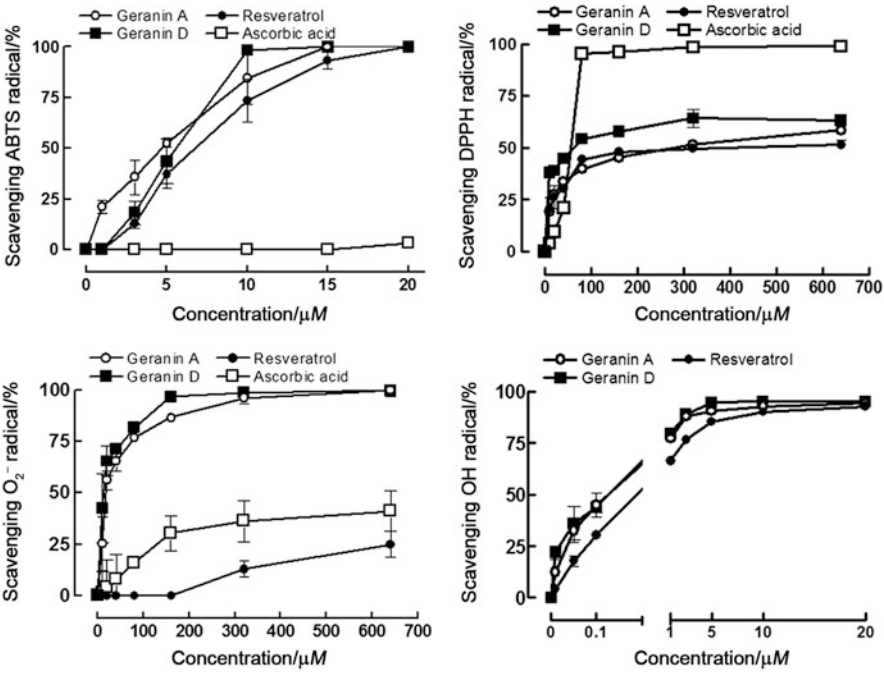


Fig. 71 Antioxidant activities of geranins A (**281**) and D (**284**)

geranins was similar to that of resveratrol and ascorbic acid in the ABTS, $O_2^{\bullet -}$, and HOCl assays. The scavenging capacity of ascorbic acid for DPPH was more potent than that of both geranins and resveratrol. On the other hand, the $OH^{\bullet}$ scavenging action of both geranins and resveratrol was similar. Neither of geranins A (**281**) and D (**284**) was able to scavenge H_2O_2 and $^1O_2$ (Fig. 71) [201]. This potent radical-scavenging in vitro effects of geranins A (**281**) and D (**284**) partially may explain the wide use of this plant in the Tarahumara indigenous system of medicine for the treatment of gastrointestinal illnesses (other than spasms), pain, and fevers associated with oxidative stress.

2.6 Cytotoxic Activity

Cancer is one of the most feared of all diseases. It affects persons in both developed and developing countries alike. According to Mohar et al. [202] in Mexico, cancer is the third leading cause of death, with 45.3% of deaths occurring among members of the economically active population. Approximately half of all cancer deaths were as a result of lung, gastric, liver, prostate, breast, or cervical tumors. Information about risk factors, including the expected aging of the population, points to possibly a substantial increase in the occurrence of new cancer diagnoses in the coming decades [202].

As with other diseases, a significant part of the population in Mexico uses herbs to treat conditions consistent with cancer symptomatology, as alternative or complementary therapy. According to recent reviews, about 300 plant species belonging to 90 botanical families with cytotoxic and antineoplastic properties are used currently for treating cancer in Mexico [203–205]. From these, over 250 compounds have been isolated and deemed to display cytotoxic effects for cancer cell lines. Thus, in the Appendix Table, previous reviews have been updated to include relevant phytochemical studies on Mexican plants from over the last few years.

Two of the most important plants used in Mexico to treat cancer are *Annona mucosa* Jacq. (syn. *Rollinia mucosa* (Jacquin) Baillon) and *A. purpurea* Moc. & Sessé ex Dunal (Annonaceae) (Fig. 72), both of the Annonaceae family, which is distributed abundantly in Mexico, mainly in the tropical lowlands, and produce potent antitumor acetogenins and a variety of isoquinoline alkaloids. Generally speaking, Annonaceous acetogenins possess a broad spectrum of biological effects, such as antineoplastic, antiparasitic, insecticidal, and immunosuppressive activities. These compounds exhibit their potent bioactivities through depletion of ATP levels via inhibiting complex I of the mitochondria and inhibiting the NADH oxidase of plasma membranes of tumor cells. They also cause cell death through the induction of the apoptotic mitochondrial pathway. Recent reviews summarizing chemical and biological work on the acetogenins [206, 207] and alkaloids [208] of plants in the Annonaceae have been published.

2.6.1 *Annona mucosa*

Annona mucosa is an evergreen tree growing from 4 to 15 m in height with brown, hairy twigs and alternate, oblong-elliptic glossy green leaves from 10 to 25 cm long, with prominent veins, sunken on the upper surface. The flowers are perfect, whitish, and have a three-lobed triangular form, and occur singly or in pairs in the leaf axils. The fruit is heart-shaped, yellow and 15 cm in diameter, and with very conspicuous protuberances (Fig. 72). The fruit pulp is whitish, juicy, aromatic and flavorful, with

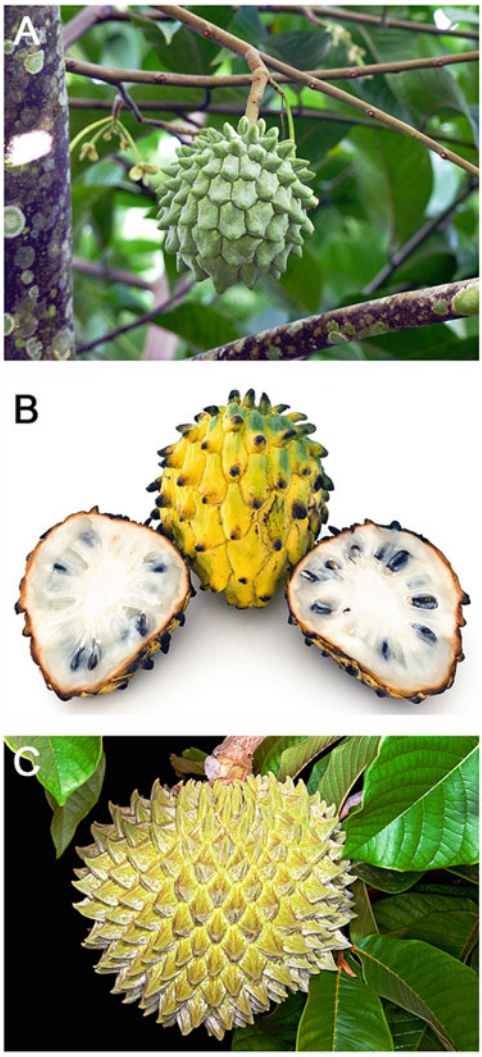


Fig. 72 Fruits of *Annona mucosa* (**A** and **B**) and *Annona purpurea* (**C**)

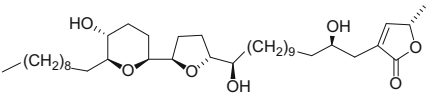
a creamy texture and a delicious sweet-sour balance. The fruits are edible and contain shiny, brown and elliptical seeds, about 1.6 cm long [209].

This species has an extensive natural range, from northern Argentina to southern Mexico, the Lesser Antilles, Puerto Rico, and Hispaniola. The plant, known in Mexico as "anona babosa", "anonita del monte", and "zambo", is employed in folk medicine for the treatment of cancer and gastrointestinal disorders. The powdered seeds are considered an effective medicine for enterocolitis. In Mexico, *A. mucosa* is mainly distributed in the states of Oaxaca, Tabasco, Veracruz, Chiapas, and Yucatán, where this species is cultivated for commercial purposes [210].

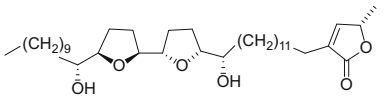
Chemical investigations of the fruit, leaves, bark, and seeds of *A. mucosa* worldwide have led to the isolation and identification of a number of alkaloids, mainly aphorphinoids [208], furofuran lignans [211], a pyrromethenone derivative [212], and aliphatic acetogenins with one or two terminal γ-lactone rings, a terminal aliphatic side chain, connected with some oxygen-bearing moieties, and several hydroxy groups along the basic core [207]. From *A. mucosa*, all structural sub-types of acetogenins known so far have been isolated. The essential oil of *A. mucosa* from Brazil was analyzed by GC/MS; 42 compounds were identified of which the major volatiles were α-pinene, β-pinene and β-caryophyllene [213].

In a study of the seeds of *A. mucosa* collected in Los Tuxtlas, Veracruz, in Mexico, a novel cytotoxic acetogenin, jimenezin (**285**), was found along with membranacin (**286**), desacetyluvaricin (**287**), rolliniastatin 1 (**288**), bullatacin (**289**), squamocin (**290**), and motrilin (**291**) (Fig. 73). Jimenezin (**285**) is a rare example of an acetogenin possessing a tetrahydropyran (THP) ring along with an adjacent tetrahydrofuran (THF) ring. Jimenezin (**285**) is structurally related to the antitumor acetogenin muconin, differing, however, in the stereo-relationship of the THP and THF rings (*erythro* versus *threo*) and in bearing a hydroxy group on the THP ring [214, 215].

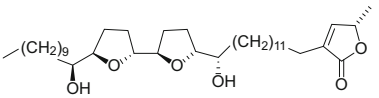
The structure elucidation of jimenezin (**285**) was pursued by spectroscopic, chiroptical, and chemical methods [214]. The molecular formula was established as $C_{37}H_{66}O_7$ by HRFABMS. Sequential losses of three molecules of H_2O from the MH^+ in the FABMS as well as the formation of a tri-TMSi derivative confirmed the presence of three hydroxy groups in jimenezin (**285**). The basic structure was established from its ^{1}H NMR and ^{13}C NMR spectra. The position of the adjacent THP-THF moiety along the aliphatic chain was determined by the analysis of the fragmentation pattern displayed by the tri-TMSi derivative. The relative configuration at C-15/C-16 was assigned as *threo* based on the chemical shift values of both C-15 (δ_C 73.9 ppm) and H-15 (δ_H 3.35 ppm) following Born's rule. On the other hand, the relationship between H-19 and H-20 was initially determined as *erythro* by comparing the NMR information with that of other acetogenins. However, when Takashi and coworkers [216] performed the first total synthesis of jimenezin (**285**), using carbohydrates as chiral building blocks, they found a discrepancy between the $J_{H\text{-}19\text{-}H\text{-}20}$ values for the natural and the synthetic products ($J = 6.3$ Hz of synthetic vs. $J = 2.3$ Hz of natural). In addition, the natural product exhibited two multiplets at δ_H 3.90 (H-16) and 3.94 (H-19) ppm, which were observed at δ_H 3.77 and 3.82 ppm, respectively, in the ^{1}H NMR spectrum of the synthetic product. These results



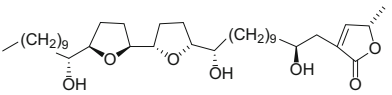
285 (jimenezin)



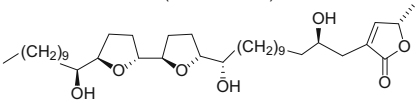
286 (membranacin)



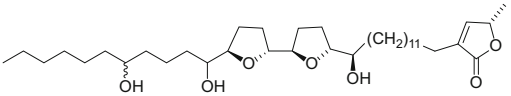
287 (desacetyluvaricin)



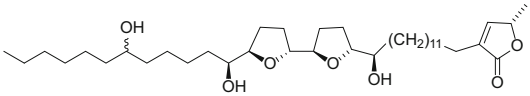
288 (rolliniastatin 1)



289 (bullatacin)



290 (squamocin)



291 (motrilin)

suggested a difference in the stereochemistry at C-19. Thus, the relationship between H-19 and H-20 of natural jimenezin (**285**) should be *threo*, rather than *erythro*. The synthesis of the compound possessing the *threo* relationship between H-19 and H-20 confirmed this proposal since it turned out to be identical to the natural product [216].

The *cis* stereochemistry of the THP ring in jimenezin (**285**) was established by interpreting the NOESY NMR spectrum, which exhibited an intense cross-peak between H-20 (δ_H 3.24 ppm) and H-24 (δ_H 3.00 ppm). On the other hand, the *trans*-diaxial relationship of H-23 and H-24 was determined by a homo-decoupling NMR experiment. Thus, irradiation of the resonance at δ_H 1.42 ppm (H-25) simplified the signal assignable to H-24 to a doublet ($J = 9.2$ Hz). The magnitude of the coupling constant observed for this doublet was consistent with the *trans* relationship between H-23 and H-24, and therefore with the *equatorial* orientation of the hydroxy group at C-23. The absolute configuration of the stereogenic carbinol

Fig. 73 Acetogenins isolated from the seeds of *Annona mucosa*

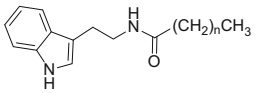
centers was established using Mosher ester methodology. Finally, the (*S*) configuration at C-36 was established according to Hoye's Mosher ester method. The negative Cotton effect at 238 nm in the CD spectrum of jimenezin (**285**) provided further evidence for the (*S*) configuration at C-36 [214].

Two more efficient total syntheses of jimenezin (**285**) were completed. Thus, in the synthesis of Hwang et al. [217], the oxane and oxolane moieties were introduced in high stereoselectivity via a samarium iodide-mediated radical cyclization of β-alkoxyacrylate and β-alkoxyvinyl sulfoxide intermediates. A ring-closing olefin metathesis reaction was employed for the butenolide side-chain elongation. One year later, Bandur et al. [218] achieved the enantioselective synthesis of (−)-jimenezin (**285**) in 3.8% yield, having the longest linear sequence of 24 steps starting from commercially available (*S*)-glycidol. Their strategy highlighted intramolecular allylboration as a highly stereocontrolled access to the THP part and an intramolecular Williamson reaction to close the THF ring of jimenezin (**285**) [218].

The seeds of *A. mucosa* from Los Tuxtlas contained also a series of tryptamine amides characterized as *N*-palmitoyltryptamine (**292**), *N*-stearoyltryptamine (**293**), *N*-arachidoyltryptamine (**294**), *N*-behenoyltryptamine (**295**), *N*-tricosanoyltryptamine (**296**), *N*-lignoceroyltryptamine (**297**), *N*-pentacosanoyltryptamine (**298**), and *N*-cerotoyltryptamine (**299**) (Fig. 74) [215]. The structural identification of these amides was accomplished by a combination of chemical, spectrometric and spectroscopic methods. It is relevant to indicate that this is the first report of tryptamine-derived alkaloids from a member of the Annonaceae, a family well known as a source of a variety of isoquinoline alkaloids [215].

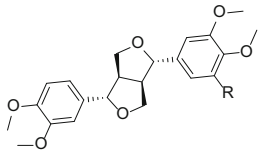
Jimenezin (**285**) was cytotoxic for six human solid tumor cell lines in a 7-day MTT test using adriamycin as the positive control: human lung carcinoma ($ED_{50} = 1.64 \times 10^{-2}$ μg/cm^3), human breast carcinoma ($ED_{50} > 10^{-1}$ μg/cm^3), human colon adenocarcinoma ($ED_{50} = 4.25 \times 10^{-3}$ μg/cm^3), human kidney carcinoma ($ED_{50} = 4.94 \times 10^{-2}$ μg/cm^3), human prostate adenocarcinoma ($ED_{50} = 2.77 \times 10^{-4}$ μg/cm^3) and human pancreatic carcinoma ($ED_{50} = 1.69 \times 10^{-4}$ μg/cm^3) [214].

A *n*-hexane extract of *A. mucosa* collected in Chiapas, Mexico, induced anxiolytic-like actions similar to those induced by diazepam using the avoidance exploratory behavior paradigm. Its significant activity was shown at doses from 1.62 to 6.25 mg/kg [219]. It also enhanced the pentobarbital-induced hypnosis time, and at high doses produced motor coordination impairment. The benzodiazepine receptor binding was evaluated by in vitro autoradiography following a single administration of

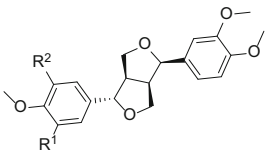


292 (*N*-palmitoyltryptamine) n = 14
293 (*N*-stearoyltryptamine) n = 16
294 (*N*-arachidoyltryptamine) n = 18
295 (*N*-behenoyltryptamine) n = 20
296 (*N*-tricosanoyltryptamine) n = 21
297 (*N*-lignoceroyltryptamine) n = 22
298 (*N*-pentacosanoyltryptamine) n = 23
299 (*N*-cerotoyltryptamine) n = 24

Fig. 74 Tryptamine amide derivatives isolated from *Annona mucosa*



300 (pinoresinol dimethyl ether) R = H
301 (magnolin) R = OCH_3



302 ((+)-*epi*-membrine) $R^1 = R^2 = H$
303 ((+)-*epi*-eudesmine) $R^1 = H$, $R^2 = OCH_3$
304 ((+)-*epi*-magnolin A) $R^1 = R^2 = OCH_3$

Fig. 75 Lignans isolated from *Annona mucosa*

A. mucosa, and revealed that this plant extract reduced benzodiazepine receptor binding in the hippocampus (29%), amygdala (26%), and temporal cortex (36%) of mice [219]. Finally, the species when collected in Mexico either in Veracruz or Chiapas also contained lignans of the furofuran type including pinoresinol dimethyl ether (**300**), magnolin (**301**), *epi*-membrine (**302**), *epi*-eudesmine (**303**), and *epi*-magnolin A (**304**) (Fig. 75) [214, 220].

2.6.2 *Annona purpurea*

Annona purpurea Moc. & Sessé ex Dunal (Anonnaceae) is a tree up to 7 m high with large and coarse round fruits, covered with sharp conical protuberances, with the seeds dark brown or black, and frequently broken open (Fig. 72). The flesh of the fruit is orange colored, and resembles in flavor the North American pawpaw (*Asimina triloba* Dunal). It is unquestionably indigenous to the southern part of Mexico, being found abundantly along water courses. In contrast to *A. muricata*, *A. squamosa*, and *A. cherimola*, *A. purpurea* is not cultivated in Mexico for commercial purposes. In Mexico, the plant is designated with the common names of "chincuya", "cabeza negra" (black head), and as "frio y caliente", with this last name meaning cold and hot or chills and fever. This is due perhaps to the effect experienced on the single consumption of the fruit, so hence it is not popular and rarely seen in marketplaces [133, 221].

The leaves and fruits of *A. purpurea* are used in folk medicine as a remedy for fevers, diabetes, and colds, while the stem bark is used for dysentery. In the Mixe lowland community of San Juan Guichicovi, Oaxaca, Mexico, a bath with the leaves of the plant is used for fevers and body pain. As with many other species of the same genus its use as a potential anticancer agent is now widespread in Mexico [133].

The first chemical study on *A. purpurea* was carried out by Sonnet and Jacobson [222], who isolated several aporphine and oxoaporphine alkaloids, some of which showed limited growth inhibitory effects against the 9KB cell line. A few years later, Cepleanu and co-workers [221] obtained besides cherimoline (**305**) five acetogenins, namely, rolliniastatin 1 (**288**), bullatacin (**289**), sylvaticin (**306**), purpureacin 1 (**307**), and purpureacin 2 (**308**) (Fig. 76) from the leaves of this species collected in Panama; these compounds showed activity against larvae of the yellow-fever mosquito *Aedes aegypti* and showed antifungal activity against *Candida albicans* [221]. Chang et al. [223–225] isolated from the methanol extract of the leaves and stems of *A. purpurea*, when collected in Taiwan, three oxoaporphines, six aporphines, two proaporphines, one phenanthrene, four dehydroaporphines, and one lactam amide alkaloid, together with four flavonols. Some of these compounds inhibited the aggregation of rabbit platelets induced by thrombin, arachidonic acid, collagen, and platelet-activating factor [223–225].

From the seeds of *A. purpurea* collected in Los Tuxtlas, Mexico, three novel adjacent bis-THF acetogenins were obtained, purpurediolin (**309**), purpurenin (**310**), and purpuracenin (**311**) (Fig. 77) [226, 227]. In addition, bullatacin (**289**), squamocin (**290**), motrilin (**291**), annoglaucin (**312**), xylomatenin (**313**), and annonacin A (**314**) were also isolated (Fig. 77) [226, 227]. The new acetogenins varied in the location of the hydroxy groups along the aliphatic chain and in the configuration around the bis-THF rings.

The structure elucidation of these new compounds from *A. purpurea* was accomplished following standard strategies for identifying this type of acetogenin. The

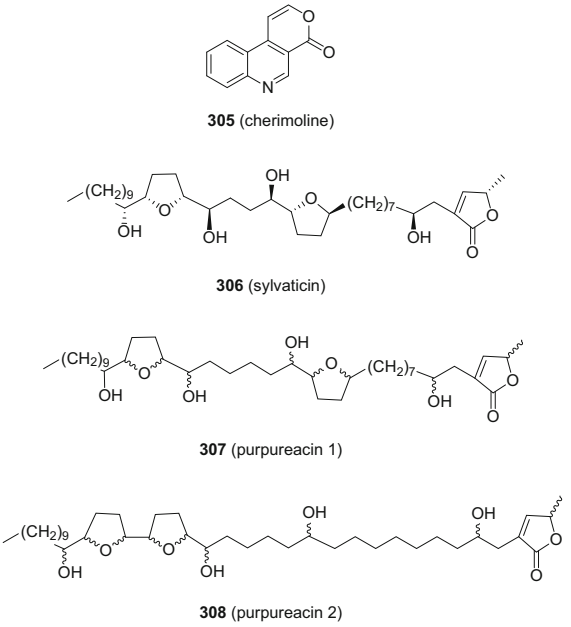


Fig. 76 Acetogenins isolated from *Annona purpurea*

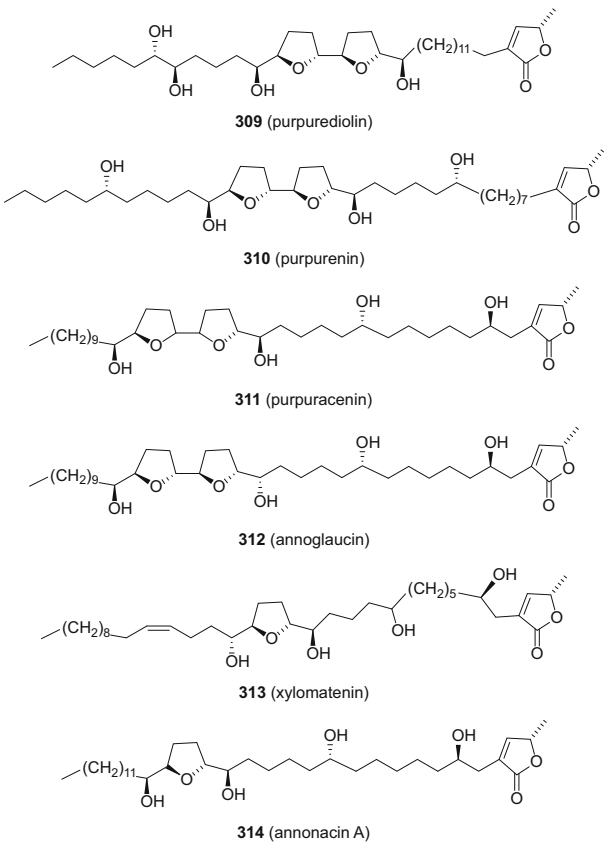


Fig. 77 Bis-THF acetogenins isolated from *Annona purpurea*

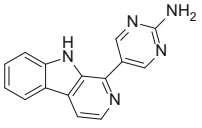
application of the Mosher ester method and CD measurements were used to establish the absolute stereochemistry of the stereogenic centers in compounds **311**–**314** [226, 227]. The absolute configuration at C-10 in the acetogenins purpuracenin (**311**) and annoglaucin (**312**) was not able to be resolved by spectrometric analysis of the (*S*)- and (*R*)-per-Mosher ester derivatives of the natural products, because the chemical shifts of the protons at C-9 and C-11 were indistinguishable. Therefore, both compounds were converted into their respective ketolactones. The translactonization was accomplished by treating purpuracenin (**311**) and annoglaucin (**312**) with diethylamine. Analysis of the $\Delta\delta_{S\text{-}R}$ values of the (*S*)- and (*R*)-per-Mosher ester derivatives of the resulting C-2/C-4 *cis* and *trans* translactonized isomers showed positive differences for H-4. Accordingly, the configuration at C-10 was assigned as (*R*) [227].

The acetogenins jimenezin (**285**), purpurediolin (**309**), purpurenin (**310**), purpuracenin (**311**), and annoglaucin (**312**) were significantly active in a brine shrimp lethality test and were also cytotoxic for six human solid tumor cell lines

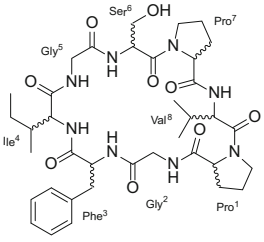
in a 7-day MTT test using adriamycin as the positive control [227]. Purpuracenin (**311**) and annoglaucin (**312**) showed selectivity for the HT-29 human colon cancer cell line. The stereochemistry around the adjacent bis-THF made a notable difference in the resultant cytotoxic activities of purpuracenin (**311**) and annoglaucin (**312**). Purpuracenin (**311**) with the *threo*/*cis*/*threo*/*trans*/*erythro* configuration showed potency against the A-549, A-498 and PC-3 cell lines, while annoglaucin (**312**) with *threo*/*trans*/*threo*/*trans*/*erythro* configuration displaying selectivity against HT-29 cells, as mentioned above.

Annomontine (**315**) (Fig. 78), a pyrimidine-β-carboline alkaloid, isolated from the roots of *A. purpurea* collected in Hermenegildo Galeana, in the state of Chiapas, Mexico, showed an anxiolytic-like effect by a mechanism similar to that of the benzodiazepines, which bind allosterically to the $GABA_A$ receptor [228]. The alkaloid was tested in mice using the elevated plus-maze, the pentobarbital-induced hypnosis, for locomotor activity in an open field, and for motor coordination in the rotarod models. The intraperitoneal injection of annomontine (**315**) (1–30 mg/kg) increased, in a dose-dependent way, the number of visits to and the time spent in the open arms of the elevated plus-maze test in comparison to the control animals. Such effects were blocked by the prior application of flumazenil (3 mg/kg; i.p.), a specific antagonist for the binding of benzodiazepines on the $GABA_A$ receptor. Under the same experimental conditions, annomontine (**315**) failed to affect the behavior of the animals in the pentobarbital-induced hypnosis test and had no effects on locomotion and motor coordination [228].

More recently, from a hydroalcoholic extract from the seeds of *A. purpurea* from the Las Salinas community of Chicomuselo, also in the state of Chiapas, a novel cyclooctapeptide named cyclopurpuracin (**316**) (Fig. 78) was isolated [229]. The peptide was characterized as cyclo(-Pro^1-Gly^2-Phe^3-Ile^4-Gly^5-Ser^6-Pro^7-Val^8-), by



315 (annomontine)



316 (cyclopurpuracin)

Fig. 78 Structures of annomontine (**315**) and cyclopurpuracin (**316**)

de novo sequencing based on it MS/MS spectra and using 1D and 2D NMR techniques [229].

The effects of bullatacin (**289**), squamocin (**290**), and motrilin (**291**) also were investigated on several photosynthetic activities in spinach thylakoids. The results indicated that compounds **289**–**291** significantly inhibited both ATP synthesis and uncoupled electron transport [230]. In addition, they enhanced light-activated Mg^{2+}-ATPase, and basal electron flow. Therefore, acetogenins **289**–**291** behaved as uncouplers and Hill reaction inhibitors, and while they did not affect photosystem I (PSI) activity, they inhibited photosystem II (PSII) electron flow. A study of the partial PSII reactions from H_2O to $DCPIP_{ox}$, H_2O to SiMo and diphenylcarbazide to DCPIP established that the site of inhibition was at the oxygen-evolving complex (OEC). Chlorophyll a fluorescence measurements confirmed the behavior of the acetogenins of the Annonaceae as water-splitting enzyme inhibitors. Since OEC is unique to plant chloroplasts and cyanobacteria, these acetogenins represent good candidates for the development of new specific, biodegradable, and possibly environmentally safe herbicides [230].

2.7 Anxiolytic and Sleep-Aid Agents

The prevalence of mental disorders (depression, anxiety, and insomnia) in Mexico has increased notably in the last two decades, affecting both the physical and professional development of individuals. Statistics from the National Institute of Statistics and Geography have shown that the number of deaths due to mental and behavioral disorders increased by 33% between 2008 and 2014 [231]. In addition, the mortality associated with mental health disorders has increased during the same period for both men and women, to 17 and 18%. It is possible that even more people suffer from mental disorders in Mexico than have been accounted for because prevalence studies do not usually reach indigenous communities but rather urban populations only. In a recent study, 3021 Mexicans from metropolitan and rural areas showing a frequency of 62.3% insomnia were analyzed. Difficulty initiating sleep was evident in 1135 patients (82.4%), and difficulty maintaining sleep in 1108 patients (80.4%), while early morning awakenings and restless sleep occurred in 983 (71.3%) and 1144 patients (83%), respectively. Their social, emotional, and professional functioning was affected in 75% of patients and was in 25% these incidences severe [231]. Furthermore, according to recent information from the World Health Organization, in Mexico the prevalence of depressive and anxiety disorders was 4.2% and 3.6%, respectively [232–234].

Many synthetic psychoactive molecules such as neuroleptics, antidepressants, and anxiolytics are used in modern medicine to treat these pathologies and other mental disorders. However, as with many other maladies, a substantial proportion of the population prefers to use alternative or complementary therapies, including herbs for their treatment. Some of the plants concerned have been summarized in recent reviews with emphasis on those useful for anxiety, depression, and sleep disorders

[235–237]. In some cases, the efficacy of these treatments has been assessed using several animal models including distress- (forced swimming test), reward- (sucrose preference test), and anxiety- (open-field, light and dark, and elevated plus tests) based tests [14, 235–237]. Perhaps Mexican valerian and *Galphimia glauca* have been the most studied Mexican medicinal plants that are used as mild sedative, tranquilizer and sleep-aid agents. In addition, the Appendix Table summarizes selected medicinal plants used as anxiolytic and sleep-aid agents.

2.7.1 *Valeriana procera*

Valerian ("valeriana" in Spanish) is the common name for over 200 plant species of the genus *Valeriana* (Valerianaceae) [238]. The most important commercial valerian species are *V. officinalis* L. (European valerian), *V. jatamansi* Jhones (syn. *V. wallichii* DC., Indian or Pakistani valerian), *V. fauriei* Briq. (Japanese valerian), and *V. procera* Kunth (syn. *V. edulis* subsp. *procera* (Kunth) Meyer, Mexican valerian).

The name "valerian", derived from the Latin term, *valere*, refers to health or well-being. The roots of *V. officinalis* have been used as a remedy for insomnia at least for 2000 years. It was first applied as a treatment for brain disorders in the late sixteenth century. The English physician John Hill was the first to use valerian therapeutically as a sedative agent in the middle of the eighteenth century [239]. Towards the end of the nineteenth century, the pharmacy text "Dispensatory of the United States of America", published in 1883, described the use of valerian in the form in which it is used today [240]. During World War I, the tincture of valerian was used as a tranquilizer for some soldiers. However, during World War II, there was a shortage of *V. officinalis*, and in the "Martindale" of 1941, it was established that it could be replaced by the valerian of India (*V. wallichii* or *V. jatamansi*) as a sedative agent to treat cases of hysteria and neurosis [240, 241]. There is no record of the date from when the Mexican valerian began to be used as a substitute for the European valerian. However, a book by Maximino Martínez cited work from 1871 concerning the use of the Mexican valerian [133].

Valeriana edulis Nutt. ex Torr. & A. Gray is believed to be a polyphyletic assemblage of three geographically distinct subspecies, namely, *V. edulis* ssp. *edulis* Nutt. ex Torr. & A. Gray, distributed in the western United States and northern Mexico; *V. edulis* ssp. *ciliata*, found in the north-central United States and adjoining Canada; and *V. procera* [syn. *V. edulis* ssp. *procera* (Kunth) F.G. Meyer], which is exclusively distributed in Mexico [239]. Thus, in a true sense, *V. procera*, endemic to the central and northern part of Mexico, should be regarded as Mexican valerian. The correct scientific name for Mexican valerian, frequently designated as *V. edulis* ssp. *procera* (Kunth) Meyer or *V. edulis* Nutt ex Torr. & A. Gray, should be *V. procera* Kunth [242].

Valeriana procera grows in open rocky slopes, in pine-oak forest, mainly on sandy soil, from about 1800 to nearly 3200 m above sea level. It is a perennial herb 30–100 cm in height, commonly with a glabrous stem and having conical rhizomes

with ramifications. The rhizome is about 30 cm long and up to 5 cm wide, fusiform and tapering into a small point, being pale yellowish-brown, with longitudinal ridges (Fig. 79). At the crown, one or more stem scars are seen, and several rootlets appear halfway to the root. Roots are tasteless at first; later, they taste slightly bitter and have a foul odor [242].

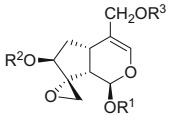
According to Lorke's method, a $CHCl_3$-MeOH (1:1) extract of the roots of *V. procera* showed an LD_{50} value of 3807 mg/kg [33]. This extract showed a mutagenic effect in the Ames test. However, a tincture of the same sample plant did not show a mutagenic effect [33]. The mutagenic effect of this plant was previously reported by Bos and co-workers and attributed to its valepotriate content [243]. The content of valepotriates is higher in the $CHCl_3$-MeOH extract than in the tincture. Also, it is known that valepotriates are unstable when in the form of a tincture and as a result of heating [238]. The cytotoxic potential of the valerian tinctures of commercially important *Valeriana* species, including *V. procera*, was established [243]. In this report, the authors showed a correlation between the cytotoxic effect and the valepotriate content; they also reported that a fresh tincture is more highly cytotoxic than when stored. Therefore, it is highly preferred to use an aged tincture than a fresh one.

Valepotriates and valerenic acids are representative compounds of *Valeriana* species. While there has been no rigorous phytochemical study of *V. procera*, a report indicated that it is devoid of valerenic acid, but has the highest content of

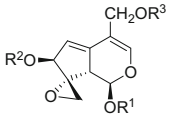
Fig. 79 *Valeriana procera*

valpotriates among all *Valeriana* species [243]. Herrera-Arellano and colleagues [244] reported that a hydroalcoholic extract of *V. procera* contained 0.26% of dihydroisovaltrate (**317**) as the main valepotriate, and also that this extract did not contain valerenic acid (**318**) (Fig. 80). Subsequently, an analysis by HPLC of the valepotriate levels in wild and in vitro-regenerated plants of *V. procera* revealed the presence of valtrate (**319**), isovaltrate (**320**), dihydrovaltrate (**321**), and dihydroisovaltrate (**317**), and the absence of valerenic acid (**318**) was confirmed (Fig. 80) [245, 246]. In 2006, Navarrete et al. [238], reported the presence in the plant of chlorogenic acid (**41**), and the lignans massoniresinol-4′-*O*-*β*-D-glucoside (**322**), berchemol-4′-*O*-*β*-D-glucoside (**323**), pinoresinol-4,4′-di-*O*-*β*-D-glucoside (**324**), pinoresinol-4-*O*-*β*-D-glucoside (**325**), 8-hydroxypinoresinol-4′-*O*-*β*-D-glucoside (**326**), and 8-hydroxypinoresinol-4-*O*-*β*-D-glucoside (**327**) (Fig. 81), in addition to the flavonoids hesperidin (**328**), and linarin (**329**) (Fig. 81). Also, we recently reported the root volatile composition of *V. procera* using HS-SPME-GC/MS [247]. The major components identified were isobutyl isovalerate (46.3%), methyl isovalerate (13.4%), isopropyl isovalerate (9.1%), and ethyl isovalerate (8.3%). These compounds are present in *V. officinalis* in much lower proportions [247].

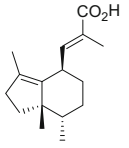
The first preclinical study of the roots of *V. procera* was performed by Oliva and coworkers, who determined the neuropharmacological profile in mice of a hydroalcoholic extract (100, 300, and 1000 mg/kg, i.p.) of the roots and rhizomes of the plant [248]. The extract showed a significant dose-dependent anticonvulsant effect on pentylenetetrazole-induced seizures; the latency of the onset of the clonic convulsions was increased from 0.8 min to 1.85 min, and the beginning of the tonic convulsions was prolonged from 2.5 min to 11.3 min. This extract also showed a



317 (dihydroisovaltrate) $R^1 = R^3 = COCH_2CH(CH_3)_2$, $R^2 = COCH_3$
321 (dihydrovaltrate) $R^1 = R^2 = COCH_2CH(CH_3)_2$, $R^3 = COCH_3$



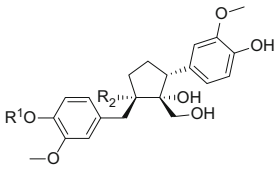
319 (valtrate) $R^1 = R^2 = COCH_2CH(CH_3)_2$, $R^3 = COCH_3$
320 (isovaltrate) $R^1 = R^3 = COCH_2CH(CH_3)_2$, $R^2 = COCH_3$



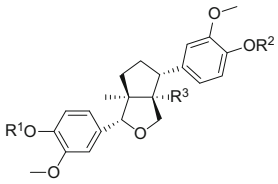
318 (valerenic acid)

Fig. 80 Compounds isolated from *Valeriana procera*

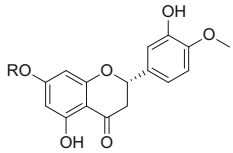
Fig. 81 Lignans and flavonoids isolated from *Valeriana procera*



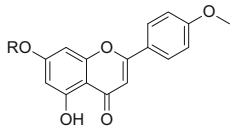
322 (massoniresinol-4´-*O*-*β*-D-glucoside) R^1 = *β*-D-glucose, R^2 = OH
323 (berchemol-4´-*O*-*β*-D-glucoside) R^1 = *β*-D-glucose, R^2 = OH



324 (pinoresinol-4,4´-di-*O*-*β*-D-glucoside) R^1 = R^2 = *β*-D-glucose, R^3 = H
325 (pinoresinol-4-*O*-*β*-D-glucoside) R^1 = H, R^2 = *β*-D-glucose, R^3 = H
326 (8-hydroxypinoresinol-4´-*O*-*β*-D-glucoside) R^1 = *β*-D-glucose, R^2 = R^3 = OH
327 (8-hydroxypinoresinol-4-*O*-*β*-D-glucoside) R^1 = H, R^2 = *β*-D-glucose, R^3 = OH



328 (hesperidin) R = *O*-rutinoside



329 (linarin) R = *O*-*α*-L-rhamnosyl(1→6)-*β*-D-glucopyranoside

significant dose-dependent sedative response in the exploratory cylinder model (Fig. 82) and impaired motor coordination during the rotarod test (Fig. 82) for more than 5 h. Finally, the preparation induced a myo-relaxant effect in the traction test and significantly prolonged the sodium pentobarbital-induced sleep time [248].

Through an isobolographic analysis, it was reported that a hydroalcoholic extract of *V. procera* did not show a pharmacodynamic interaction with any of diazepam, haloperidol, diphenhydramine, sodium pentobarbital, ethanol, and buspirone, all central nervous system-depressant drugs [249].

Valeriana procera has been the subject of two clinical studies. One of them was performed in children with intellectual deficits and with difficulties in initiating and maintaining sleep. In this study, which was double-blinded, placebo-controlled and randomized designed, tablets were prepared each with 500 mg of the dried and

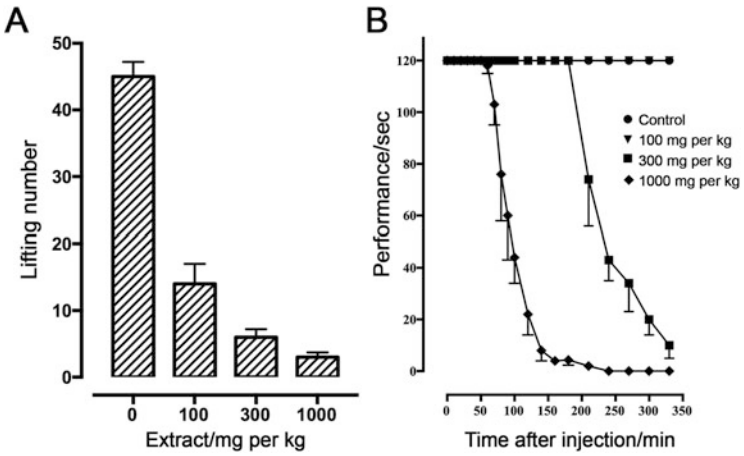


Fig. 82 Sedative effect of a hydroalcoholic extract of *Valeriana procera* in the exploratory cylinder model (**A**) and in the rotarod test (**B**) in mice. Taken from [248]

crushed whole roots of *V. procera*, containing 5.52 mg of valtrate (**319**)/isovaltrate (**320**), and was administered for 8 weeks at 20 mg/kg body weight [250]. Valerian treatment led to significant reductions in sleep latencies and the nocturnal time awake, and also lengthened total sleep time and improved sleep quality. These authors pointed out that the treatment was most effective in children with deficits that involved hyperactivity [250].

In the second clinical study, the effects were determined of 450 mg (three capsules of 150 mg each) of a *V. procera* standardized hydroalcoholic extract (0.54% of valepotriate content), given to adult patients (29–55 years old) with insomnia, in a double-blind, cross-over, controlled study [244]. An extract of *V. officinalis* was used as positive control at the same doses. The study was performed in a sleep laboratory, and a polysomnographic recording was used to analyze the quality and architecture of the sleep, with morning sleepiness, memory quotient, and side effects also evaluated. The results showed that the *V. procera* extract reduced the number of waking episodes, increased the sleep efficiency index, and produced beneficial effects on sleep architecture, diminishing the time of stages I and II in the non-rapid eye movement sleep, with increased delta sleep. Also, rapid eye movement sleep was augmented; this last parameter was better for an extract of *V. officinalis*, but there were no significant differences between these two valerian species in the other polysomnographic parameters evaluated. These results provide documented support of the hypnotic effect of Mexican valerian on patients suffering insomnia [244].

Several organic extracts (0.15–50 μg/cm^3) from the rhizomes of this species induced a significant concentration-dependent and endothelium independent relaxation on the isolated rat aorta pre-contracted with NA (0.1 μ*M*) [251]. Of these, a *n*-hexane extract was the most active (IC_{50} = 34.61 μg/cm^3; E_{max} = 85.0%). Pretreatment of aortic rings with the *n*-hexane extract (30 μg/cm^3) also inhibited

the contractile response to $CaCl_2$, reduced the NA-induced transient contractions in Ca^{2+}-free solution, and inhibited contractions induced by KCl (80 mM). Several experiments with endothelium-denuded rings revealed that the relaxation effect followed an endothelium-independent pathway, involving blockade of Ca^{2+} channels. The active principles responsible for these effects were not determined [251].

Currently, the raw material of Mexican valerian used commercially originates from controlled crops, although there is still overuse of the wild material. Fortunately, propagation studies have been described to reduce overexploitation of wild material of this species [252]. Also, a procedure for the regeneration of *V. procera* via indirect organogenesis and somatic embryogenesis from leaf-derived callus and suspension culture to massive propagation of this plant was proposed as an alternative method to obtain the plant rhizomes [245]. The levels of valepotriates were the same in these in vitro cultures as in wild plants of *V. procera* [246].

A HPLC-PDA method was developed for quality control of raw material of *V. procera*; linarin (**329**) was used as marker compound because *V. procera* does not accumulate valerenic acid (**318**) as does *V. officinalis* [238]. This compound seems to be the main chemical difference between the two valerian types. This composition test has been included in the Second Edition of the "Mexican Herbal Pharmacopoeia" [76].

2.8 Antiasthmatic Agents

Asthma is a complex inflammatory disease associated with changes in the levels of eosinophils, mast cells, lymphocytes, cytokines and other inflammatory cell products. It is a chronic lung disease that inflames and narrows the airways causing recurring periods of wheezing, chest tightness, shortness of breath, and coughing. The causes of the asthma are not clear but are probably due to a combination of environmental and genetic factors. Most people who have asthma need to take long-term control medicines daily to help prevent their symptoms. The most effective long-term medicines include inhaled corticosteroids, leukotriene modifiers, long-acting beta agonists, combination inhalers, and theophylline. There are also quick-relief medications comprising short-acting beta agonists and ipratropium, as well as oral or intravenous corticosteroids. Finally, some allergy drugs (immunotherapy and omalizumab) are used only if the cause is triggered or worsened by allergies [253].

Asthma most commonly develops in early childhood, and more than three-quarters of children who develop asthma symptoms before the age of 7 no longer have symptoms by the age of 16. However, asthma can develop at any stage in life, including adulthood [253].

Asthma is a public health problem worldwide, not just in high-income countries. This illness occurs in all countries regardless of their level of development, being generally under-diagnosed and undertreated, and most asthma-related deaths occur in low-income and lower-middle-income countries. According to the latest WHO data published in 2017, asthma deaths in Mexico reached 1555 or 0.29% of the total

deaths. The age adjusted death rate was 1.42 per 100,000 of the population, ranking Mexico as number 131 in the world [68].

According to a recent review, 100 herbs belonging to 43 botanical families are used in Mexico to treat asthma [254]. The information provided, according to their common (vernacular) and scientific names, plant part used, the forms of administration, and preparations, as well as data on its chemical composition, was described in detail. These species are rich in essential oils, organic acids, alkaloids, carotenes, phenols, phytosterols, flavonoids, glycosides, lignans, mucilage, pectin, saponins, terpenes and tannins, with expectorant, antitussive, anti-inflammatory or antiasthmatic properties. In the following paragraphs, some of our studies with selected species are reviewed. The pharmacological model used to test these plants has involved mostly tracheal smooth muscle relaxation [14]. In addition, the Appendix Table summarizes other recent work on medicinal plants with potential therapeutic value in the treatment of asthma.

2.8.1 *Pseudognaphalium liebmannii*

The genus *Gnaphalium* (Asteraceae) comprises more than 200 species, annual or perennial herbs, distributed worldwide [255]. The name *Gnaphalium* comes from the Greek word "gnaphalon", which means "tuft of wool" in connection to the shaggy appearance of these plants. In a broad sense, *Gnaphalium* L. is a heterogeneous and polyphyletic genus. *Pseudognaphalium* Kirp. is one of the many segregated genera from *Gnaphalium* that have been proposed to produce subgroups that are better defined and presumably monophyletic. Anderberg [256] moved about 70 additional species from *Gnaphalium* to *Pseudognaphalium*, including the majority of the Mexican species of *Gnaphalium*; later on phylogenetic analysis with molecular data supported these changes [257]. Apparently, the ancient Aztecs knew the medicinal uses of some species of *Gnaphalium*, since Francisco Hernández in his "Natural History of New Spain" book [73] describes two plants, one with the name "popohtli", for which the description corresponded to a *Gnaphalium* sp., and the second, "tzonpotonic" (fetid hairs in Nahuatl language), which according to an illustration included in the book matched *Gnaphalium conoideum*, and this was indicated for respiratory and gastric affections [258].

A recent review by Zheng et al. [255] indicated that phytochemical investigations of *Gnaphalium* and related genera have led to the identification of around 125 metabolites, including flavonoids, terpenes, phytosterols, anthraquinones, and caffeoylquinic acid derivatives, with antioxidant, antimicrobial, antitussive, expectorant, cytotoxic, anti-inflammatory, and antidiabetic properties, to mention those most relevant. Most of *Gnaphalium* and *Pseudognaphalium* species are designated nowadays with the trivial name "gordolobo" and used medicinally for the treatment of several respiratory diseases (bronchitis, asthma cough, and cold) as well as fever [259].

Pseudognaphalium liebmannii (Sch. Bip. ex Klatt) Anderb. var. *monticola* (McVaugh) Hinojosa & Villaseñor, is an annual to perennial herb or subshrub; its stems are usually erect, and the leaves basal and cauline or mostly cauline; the heads

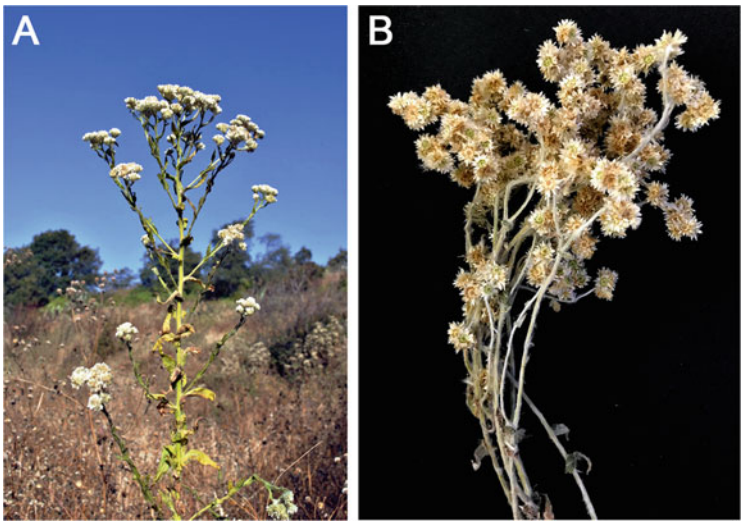
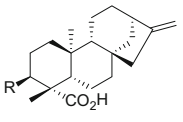


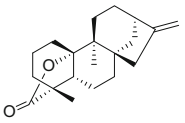
Fig. 83 *Pseudognaphalium liebmannii*

are disciform, usually in corymbiform or paniculiform arrays; the involucres are mostly campanulate to cylindric, and the stereomes, usually green, divided or fenestrated (Fig. 83) [260].

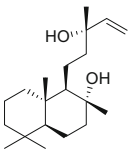
The first phytochemical and biological investigation of *P. liebmannii* was carried out by Villagomez-Ibarra and co-workers who reported the presence of *ent*-kaur-16-en-19-oic acid (**330**), *ent*-3β-hydroxykaur-16-en-19-oic (**331**), zoapatlin (**332**), 13-*epi*-sclareol (**333**), 13-*epi*-cyclosclareol (**334**), luteolin (**205**), 3-methoxyquercetin (**335**), 5-hydroxy-3,7-dimethoxyflavone (**336**) (Fig. 84), and a mixture of sterols [260]. The *n*-hexane extract of the leaves and flowers showed noted antimicrobial activity against *S. aureus* and *Bacillus cereus* [260]. Then, Sanchez-Mendoza et al. [261] demonstrated the smooth muscle-relaxant effect in guinea-pig tracheal segments of a few extracts of the inflorescences of the plant. The less polar *n*-hexane extract was the most active ($IC_{30} = 54.23$ μg/cm^3 with 99.5% of relaxation). This extract produced a parallel rightward shift of the concentration-response curve of carbachol ($IC_{50} = 0.04$ μ*M*) in a competitive manner at concentrations of 237 μg/cm^3 ($IC_{50} = 0.20$ μ*M*) and 316 μg/cm^3 ($IC_{50} = 0.19$ μ*M*), but did not inhibit the cumulative concentration-response curve of histamine. In the presence of propranolol (3 μ*M*), the effect of the extract was not affected. Also, glibenclamide (10 μ*M*) did not change the log concentration-response curves of the extract [261]. Bioassay-guided fractionation of the active extract, following the relaxant effect on guinea pig tracheal smooth muscle, led to the isolation of 5,7-dihydroxy-3,8-dimethoxyflavone (**337**) and 3,5-dihydroxy-7,8-dimethoxyflavone (**338**) (Fig. 85) as the active relaxant compounds. These compounds showed more potent relaxant properties than aminophylline in the guinea-pig tracheal model. 3,5-Dihydroxy-7,8-dimethoxyflavone (**338**) ($EC_{50} = 134.04$ μ*M*) showed a better relaxant activity than 5,7-dihydroxy-3,8-dimethoxyflavone (**337**) ($EC_{50} = 195.04$ μ*M*). The activity was compared with that



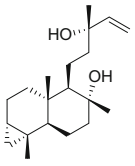
330 (*ent*-kaur-16-en-19-oic acid) R = H
331 (*ent*-3β-hydroxykaur-16-en-19-oic acid) R = β-OH



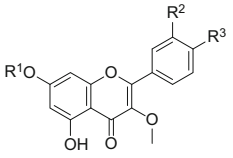
332 (zoapatlin)



333 (13-*epi*-sclareol)

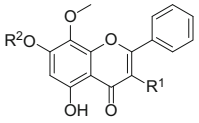


334 (13-*epi*-cyclosclareol)



335 (3-methoxyquercetin) R^1 = H, R^2 = R^3 = OH
336 R^1 = CH_3, R^2 = R^3 = H

Fig. 84 Compounds isolated from *Pseudognaphalium liebmannii*



337 (gnaphaliin A) R^1 = OCH_3, R^2 = H
338 (gnaphaliin B) R^1 = OH, R^2 = CH_3

Fig. 85 Structures of gnaphaliins A (**337**) and B (**338**) from *Pseudognaphalium liebmannii*

of aminophylline (EC_{50} = 534.04 μM). Surprisingly, compounds **337** and **338** were described as the flavonoid gnaphaliin in the past, however, based on NMR experiments and X-ray diffraction analysis, these compounds were renamed as gnaphaliins A (**337**) and B (**338**) [255, 262, 263].

Gnaphaliins A (**337**) and B (**338**) relaxed carbachol pre-contracted (3 μM) guinea-pig tracheal rings (EC_{50} = 181.58 and 128.36 μM) and NA pre-contracted (0.1 μM) rat aortic rings, in a concentration-dependent manner (EC_{50} = 1.53 and 5.36 μM, respectively) [262]. The rat aorta tissue was then more sensitive than guinea-pig trachea in its response to the relaxant effect induced by gnaphaliins A (**337**) and B (**338**). The relaxant effect of both flavones in the guinea-pig trachea was potentiated by nitroprusside and forskolin, and blocked by 1*H*-[1,2,4]-oxadiazolo[4,3-*a*] quinoxalin-1-one but not by 2′,5′-dideoxyadenosine. L-NAME did not modify the relaxant effect of either flavone [262]. On the other hand, gnaphaliins A (**337**) and B (**338**) were more potent relaxant agents of the guinea-pig trachea than enoximone (PDE-3 inhibitor), rolipram (PDE-4 inhibitor), and aminophylline (unspecific PDE (phosphodiesterase) inhibitor), but they were less potent than sildenafil (PDE-5 inhibitor) [262]. In the rat aorta model, both flavones were slightly more potent than aminophylline, and showed similar potencies to enoximone and rolipram, but

Table 2 $IC_{50}/\mu M$ values of gnaphaliins A (**337**) and B (**338**) and standard inhibitory drugs on phosphodiesterase (PDE) enzymes

Compound	cAMP	cGMP
Gnaphaliin A (**337**)	120.798 ± 26.599 ($r^2 = 0.980$)	2.963 ± 0.350 ($r^2 = 0.985$)
Gnaphaliin B (**338**)	72.153 ± 11.858 ($r^2 = 0.993$)	6.454 ± 2.293 ($r^2 = 0.933$)
Sildenafil	0.116 ± 0.016 ($r^2 = 0.997$)	0.0037 ± 0.0008 ($r^2 = 0.991$)
Aminophylline	>3000	182.770 ± 20.386 ($r^2 = 0.989$)
Rolipram	74.877 ± 9.190 ($r^2 = 0.995$)	2.866 ± 0.739 ($r^2 = 0.986$)
IBMX	21.347 ± 4.411 ($r^2 = 0.899$)	>300
Enoximone	>3000	>3000

Values are presented as the means ±SEM, $n = 3$

were less potent than sildenafil. 1*H*-[1,2,4]-Oxadiazolo[4,3-*a*]quinoxalin-1-one (10 μ*M*), a soluble guanylate cyclase inhibitor, significantly increased the EC_{50} values of **337** and **338** in guinea-pig tracheal rings. 2′,5′-Dideoxyadenosine, an adenylate cyclase inhibitor, did not modify the EC_{50} values of either gnaphaliins. However, sodium nitroprusside (10 μ*M*), a guanylate cyclase activator and forskolin (10 μ*M*), an adenylate cyclase activator, potentiated the relaxant effect of both gnaphaliins [262].

Gnaphaliins A (**337**) and B (**338**) and the other PDE inhibitors evaluated were more potent in this regard when cGMP was used as substrate (Table 2) [262]. For cAMP as substrate, the PDE inhibitory activity order was: sildenafil > IBMX > gnaphaliin B (**338**) > rolipram > gnaphaliin A (**337**) > aminophylline, and enoximone. For cGMP as substrate, the activity order was: sildenafil > rolipram > gnaphaliin A (**337**) > gnaphaliin B (**338**) > aminophylline > IBMX, and enoximone [262]. Thus, gnaphaliins A (**337**) and B (**338**) showed relaxant effects in the guinea-pig trachea and the rat aorta through the inhibition of PDE with an ability to inhibit the degradation of cGMP. In addition to PDE inhibition as a relaxant mechanism of gnaphaliins, this was due to inhibition of calcium influx in the guinea-pig trachea, although the inhibition was not significant in the rat aorta.

A HPLC method was developed for the simultaneous determination of gnaphaliins A (**337**) and B (**338**). The method was validated for linearity, repeatability, LOD, and LOQ. The LOD and LOQ for gnaphaliin A and B were found to be in the range of 0.4–0.5 and 1.0–1.4 μg/cm^3, respectively. This analytical method would be useful for the analysis of raw material and commercial products based on *P. liebmannii* [264].

Another "gordolobo" is *G. conoideum* H.B.K., a species distributed in pine and evergreen oak forests. It is an herbaceous annual plant of 6–60 cm height with glandular and wooly leaves, a paniculated-corymbous inflorescence, and odorous flowers [265, 266]. Among the Tarahumaras, this plant is known as "rasó" and "rasábori", and the tea prepared from the leaves is used for diarrhea, stomach problems, colds, and as an anti-inflammatory [258]. A methanol extract of *G. conoideum* (20 and 100 μg/cm^3) significantly reduced the contractile responses to histamine and KCl but not to carbachol in the guinea-pig trachea; the effect was mediated by L-type Ca^{2+} channel blockers, since D-600, a compound with this type of activity, abolished

the intracellular Ca^{2+} changes and the contractile response to histamine [267]. Furthermore, the extract inhibited the KCl-induced intracellular Ca^{2+} rise in isolated tracheal smooth muscle cells. Although a higher concentration of this extract (3.1 mg/cm^3) prevented this KCl-induced response, it also caused a decrease in the response to caffeine, suggesting a possible additional effect on the ryanodine receptor. Therefore, it seems that the *G. conoideum* methanol extract might be considered as a partial blocker of L-type Ca^{2+} channels. Unfortunately, the active principles involved were not isolated in this study [267].

3 Concluding Remarks

From this contribution, it is evident that in the last five decades a significant amount of medicinal plant research has been conducted by Mexican scientists. Such efforts have led to the publication of many research papers in noted peer-reviewed journals and technical books, which are included in the most important scientific databases. A large percentage of this research has been published mostly in the "Phytochemistry", the "Journal of Natural Products", "Planta Medica", the "Journal of Pharmacy and Pharmacology", the "Journal of Ethnopharmacology", "Phytomedicine", and "Phytotherapy Research". The isolation and characterization of hundreds of bioactive secondary metabolites have been accomplished, and most importantly, these studies have supported the ethnomedical uses of the different species investigated. Some of the most relevant activities determined are antimicrobial, antitumor, antiprotozoal, antihelmintic, antidiabetic, antioxidant, anti-inflammatory, antidepressant, anxiolytic, and antihypertensive effects. Conventional methodology, focusing on the isolation and structure elucidation of individual compounds or just the pharmacological evaluation of extracts, still prevail among many research groups. However, a multidisciplinary approach for analyzing medicinal plants has shown an increased focus on areas such as phytopharmacology, phytotoxicology, quality control, regulation, and conservation issues for these valuable resources. The medicinal plants analyzed so far have a very broad chemical diversity of their constituents, which have a high potential for exhibiting of novel mechanistic effects biologically. Some weaknesses are evident; for example, there is need to develop the area of clinical studies of herbal drugs, in particular because the longstanding traditional evidence for their safety is not always sufficient to assure their rational use. There is also need to move to "omics" approaches for investigating the holistic effect and the influence of groups of phytochemicals on the whole organism. Mexican scientists are expected to have bright prospects in this regard, which will imbue medicinal plant research with a new dynamism in the future.

Acknowledgments This work has been conducted under the auspices of several project grants funded by Consejo Nacional de Ciencia y Tecnología (CONACyT, CB219765 and INFRA268233) and the Dirección General de Asuntos del Personal Académico UNAM (DGAPA, IN217516 and IN213418). R. Mata is very grateful to all her colleagues, postdoctoral fellows, and B.S., M.S., and Ph.D. students, whose collaboration has contributed to the results described in this chapter.

Appendix

Selected medicinal plants employed for treating major Mexican health problems

Scientific name and family	Pharmacological studies	Active principles	Ref.
Diabetes			
Achillea millefolium L. (Asteraceae)	A hydromethanolic extract (100 mg/kg) from the aerial parts possessed in vivo antidiabetic effects in NAA-STZ mice, involving antihyperglycemic (α-glucosidases inhibition), hypoglycemic (insulin secretion), and potential insulin sensitizer (PPARγ/GLUT4 overexpression) actions	Not determined, but the plant is rich in bioactive flavonoids and sesquiterpene lactones	[268]
Acosmium panamense (Benth.) Yacolev (Fabaceae)	Oral administration of aqueous (20 and 200 mg/kg) and butanol (20 and 100 mg/kg) extracts from the bark induced hypoglycemic action in STZ-rats within 3 h	Not determined but the authors isolated 4′β-D-*O*-glucopyranosyl-desmethylyangonin and 4′β-D-*O*-di(1→6) glucopyranosyl-desmethylyangonin	[269]
Acourtia thurberi (A. Gray) Reveal & R.M. King (Asteraceae)	Acute oral administration of a decoction from the roots did not produce toxic effects in mice according to the Lorke procedure; it (31.6–316.2 mg/kg, p.o.) decreased blood glucose levels during acute hypoglycemic testing, an OGTT, and an OSTT in normoglycemic and hyperglycemic animals. Pharmacological evaluation in vivo of the active principles (3.2–31.6 mg/kg) using the same assays revealed their hypoglycemic and antihyperglycemic actions	Perezone, α-pipitzol and β-pipitzol, and 8β-D-glucopyranosyloxy-4D-methoxy-5-methyl-coumarin	[270, 271]
Ageratina petiolaris (Moc. & Sessé ex DC.) R.M. King & H. Robinson (Asteraceae)	Aqueous (40 and 160 mg/kg) and methanol (67 and 268 mg/kg) extracts obtained from the aerial parts showed hypoglycemic action in NAA-STZ-induced diabetic rats. The authors attributed the activity to chlorogenic acid (**41**) and L-chiro-inositol	Not determined but the plant contains chlorogenic acid (**41**), L-chiro-inositol, 2α-iso-valeroyloxyeperuic acid, benzyl 2-hydroxy-6-methoxybenzoate, benzyl 2,6-dimethoxybenzoate, 3-methoxybenzyl 2,6-dimethoxybenzoate, benzyl 2-hydroxy-3,6-dimethoxybenzoate, and 2α-tigloyloxyeperuic acid	[272]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Annona cherimola Miller (Annonaceae)	The ethanol extract (300 mg/kg) and rutin (**273**) (30 mg/kg) attenuated postprandial hyperglycemia in alloxan-induced DM during an OGTT and an OSTT	Rutin (**273**)	[273]
Anoda cristata (L.) Schltdl. (Malvaceae)	The traditional extract, the mucilage and the traditional extract free of mucilage (FMAE) from the plant were effective for reducing blood glucose levels in healthy and NAA-STZ-hyperglycemic mice during an acute hypoglycemic test, an OGTT, and an OSTT. The FMAE also induced important metabolic changes in rats with metabolic syndrome induced by fructose. The rats fed with FMAE (316 mg/kg) had a higher fat depot and a lower Lee index. The high levels of serum glucose, triglycerides and uric acid observed in fructose-fed rats also decreased significantly in the groups treated with FMAE; moreover they exhibited significantly higher insulin levels than those in control groups. Acacetin and diosmetin (3, 10, and 31.6 mg/kg) also caused significant hypoglycemic effects in healthy and NAA-STZ hyperglycemic mice	Acacetin and diosmetin	[274]
Bromelia plumieri (E. Morren) L.B. Sm. (Bromeliaceae)	Aqueous (35 and 350 mg/kg) and ethanol-water (30 and 300 mg/kg) leaf extracts both produced a significant hypoglycemic effect in NAA-STZ rats	Not determined but flavonoids were the main components according to TLC analysis	[275]
Calea zacatechichi Schltdl. (Asteraceae)	An aqueous extract made up from aerial parts of the plant was effective for controlling fasting and postprandial blood glucose levels in normoglycemic and NAA-STZ hyperglycemic mice and inhibited the activity of α-glucosidases from different sources. The essential oil (31.6–316.2 mg/kg) provoked also a significant decrease of blood glucose levels during an OSTT. Demethylisoencecalin and caleins A and C (3.16–31.6 mg/kg) controlled postprandial glucose levels during an OSTT in normal and NAA-STZ, hyperglycemic mice. 6-Hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene inhibited the activity of α-glucosidases	Demethylisoencecalin, caleins A and C, 6-acetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene, and 6-hydroxyacetyl-5-hydroxy-2,2-dimethyl-2*H*-chromene	[276, 277]

Cecropia obtusifolia Bertol. (Cecropiaceae)	The hypoglycemic action in different animal models and DM patients of different preparations from the leaves has been demonstrated. The infusion and chlorogenic acid (**41**) stimulate glucose uptake in both insulin-sensitive and insulin-resistant adipocytes without appreciable pro-adipogenic effects. The aqueous and butanol extracts inhibited the glucose-6-phosphatase activity with IC_{50} values of 224 and 160 mg/cm^3, respectively, suggesting that glycemic control could also block hepatic glucose output. Clinical trials with the infusion prepared with the leaves (containing 2.99 ± 0.14 mg of chlorogenic acid (**41**)/g of dried plant) produced beneficial effects on carbohydrate and lipid metabolism when it was administered as an adjunct on patients with T2DM with poor responses to conventional medical treatment	Chlorogenic acid (**41**) and the presence of *iso*-orientin was established	[278–282]
Cochlospermum vitifolium (Willd.) Sprenge (Bixaceae)	A *n*-hexane extract (120 mg/kg) from the whole plant showed hypoglycemic effect on normoglycemic rats. The active naringenin (**211**) exerted an antidiabetic effect via a PPARγ/GLUT4 dual agonistic action, among other mechanisms	Naringenin (**211**)	[283–285]
Coriandrum sativum L. (Apiaceae)	An aqueous extract (100, 300, and 500 mg/kg) from the leaves inhibited postprandial peak in a dose-dependent manner during an OSTT in rats. In vitro experiments with yeast α-glucosidase revealed a competitive-type inhibition of this enzyme	Not determined, but rutin (**273**) was identified in the aqueous extract	[286]
Equisetum myriochaetum L. (Equisetaceae)	A single oral administration of the water (7 and 13 mg/kg) and butanol (8 and 16 mg/kg) extracts from the aerial parts significantly lowered plasma glucose levels in STZ-diabetic rats after 3 h of administration	Not determined, but the authors isolated kaempferol-3-*O*-sophoroside-4′-*O*-β-D-glucoside	[287, 288]
Guazuma ulmifolia Lam. (Sterculiaceae)	An aqueous extract from the stem bark of the plant lowered plasma glucose levels by 22% in healthy rabbits and exerted stimulated glucose uptake in both insulin-sensitive and insulin-resistant adipocytes without inducing adipogenesis in vitro	Not determined	[289, 290]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Ibervillea sonorae (S. Watson) Greene (Cucurbitaceae)	The decoction and the juice from the roots significantly reduced hyperglycemia in mice when administered (600 mg/kg) to healthy and alloxan-diabetic animals. A dichloromethane extract (300 mg/kg) produced also hypoglycemic action. Daily administration of the decoction to rats (300 mg/kg/day) produced reductions of the glycemia and triglycerides levels, as well as an improvement in body weight. A decoction stimulated glucose uptake in insulin-sensitive and insulin-resistant adipocytes. In human adipocytes, the glucose uptake proceeded via a PI3K-(phosphatidylinositol-3-kinase) independent pathway	Not determined, but the authors identified phenolic compounds including gallic acid (decoction), and a mixture (1:1) of 11 monoglycerides and 5 fatty acids (dichloromethane extract)	[291–293]
Justicia spicigera Schltdl. (Acanthaceae)	A traditional preparation obtained from the leaves of the plant exerted glucose-lowering effects in normoglycemic and STZ-induced diabetic rats. The antidiabetic effects from the administration of an aqueous extract of the plant were related to the stimulation of glucose uptake in both insulin-sensitive and insulin-resistant murine and human adipocytes	Not determined	[294]
Larrea tridentata J.M. Coult. (Zygophyllaceae)	An ethanol extract (0.2%) from the leaves and twigs reduced plasma levels of triglycerides, total cholesterol, and glucose, leptin. and improved insulin sensitivity in hamsters fed with a high fat and cholesterol diet	Not determined	[295]
Malmea depressa (Baill.) R.E. Fries (Annonaceae)	The aqueous (40 and 80 mg/kg), butanol (80 mg/kg), and ethanol (113 mg/kg) root extracts showed acute hypoglycemic action on STZ-diabetic rats. The butanol extract (10 mg/kg) decreased glucose and HbA_{1c} after 30 or 45 days of daily administration to n5-STZ rats. The ethanol (60 and 80 mg/kg) extract of the root bark reduced blood glucose levels after a pyruvate tolerance test in n5-STZ-diabetic rats. The gluconeogenesis inhibition was confirmed in vitro	Not determined, but the authors isolated 2-hydroxy-3,4,5-trimethoxy-1-(2′,4′-hydroxy-3′-dihydroxy)butyl-benzene, 2-hydroxy-3,4,5-trimethoxy-1-(2′,3′,4′-hydroxy)butyl-benzene, and 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol	[296, 297]

Opuntia ficus-indica (L.) Mill. and *O. streptacantha* Lem. (Cactaceae)	A recent study conducted on a murine model suggested that a lyophilized aqueous extract of *O. ficus-indica* stimulated glucose transport in L6 myoblasts through GLUT4 translocation to the plasma membrane by activation of AMPK and its downstream target p38MAPK; this extract (1 and 10 μg/cm^3) inhibited *α*-glucosidase activity in a concentration-dependent manner and suppressed (200 and 500 μg/cm^3) Na^+-dependent glucose uptake into intestinal brush border membrane vesicles. The dietary intake of *O. ficus-indica* improved the postprandial response of glucose, insulin, glucose-dependent insulinotropic peptide index, and the glucagon-like peptide 1 index on T2DM patients after consumption of a high-carbohydrate or high-soy protein breakfast. The juice had antihyperglycemic effects. The liquefied extract (135 mg/kg) of *O. streptacantha* did not reduce glycemia in STZ-treated rats when compared to the control, but exhibited an antihyperglycemic effect when administered before a glucose challenge. Some polysaccharides isolated from both *Opuntia* species had hypoglycemic activity in diabetic mice	Not determined, but the plant contains fibers, pectin polysaccharides, phenolic acids, quercetin 3-*O*-rhamnosyl-(1→2)-[rhamnosyl-(1→6)]-glucoside, kaempferol, isorhamnetin, and kaempferol 3-*O*-arabinofuranoside	[298–305]
Rhizophora mangle L. (Rhizophoraceae)	An ethanol-aqueous extract (90 mg/kg) from the bark exerted acute and chronic (42 days of treatment) hypoglycemic as well as hypolipidemic effects in NAA-STZ-induced diabetic rats	Not determined, but the plant contains (−)-*epi*-catechin (**163**), catechin-3-*O*-rhamnopyranoside, lyoniside, and nudiposide	[306, 307]
Smilax moranensis Mart. & Gal. (Smilacaceae)	Aqueous and ethanol-aqueous extracts from the roots produced a dose-dependent hypoglycemic effect in n5-STZ diabetic rats	Not determined, but flavonoids were the main components according to TLC analysis	[308]
Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae)	In healthy and hyperglycemic STZ-induced male Sprague-Dawley rats, a leaf aqueous extract decreased hyperglycemic peak values during an oral corn-starch tolerance test. The preparation showed also *α*-glucosidase inhibitory activity in vitro. Moreover, sub-chronic administration of the preparation (500 mg/kg) for 21 days significantly reduced cholesterol and triglyceride levels in rats but did not affected fasting glucose	Chlorogenic acid (**41**)	[309–311]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Tournefortia hartwegiana Steud. (Boraginaceae)	A methanol extract from the aerial parts had important α-glucosidase inhibitory activity in vitro; in addition, the extract (310 mg/kg) exerted hypoglycemic and antihyperglycemic action during different carbohydrate tolerance tests in normoglycemic and alloxan-diabetic rats	Not determined, but the plant contains β-sitosterol (**109**), stigmasterol (**166**), lupeol, and ursolic (**40**) and oleanolic (**93**) acids	[312, 313]
Tournefortia hirsutissima L. (Boraginaceae)	The stem aqueous (20 and 80 mg/kg) and butanol (8 and 80 mg/kg) extracts showed hypoglycemic effects when tested in neonatal STZ-diabetic rats	Not determined	[314]
Gastrointestinal and cardiovascular illnesses			
Acalypha phleoides Cav. (Euphorbiaceae)	A $CHCl_3$-MeOH (1:1) extract from the aerial parts of the plant showed an inhibitory effect on the gastrointestinal propulsion of a charcoal meal in mice. In the isolated guinea-pig ileum, this extract produced a concentration-dependent inhibition of the contractions induced by 5-HT. The extract produced also a concentration-dependent inhibition of the spontaneous pendular movement of the isolated rabbit jejunum. The essential oil was more potent than the extract in inhibiting the spontaneous pendular movement of the rabbit jejunum. Thymol (**170**), camphor, and γ-terpinene showed also antispasmodic activity in the rabbit jejunum preparation; thymol (**170**) was the most active compound, followed by camphor and γ-terpinene	Thymol (**170**), camphor, and γ-terpinene	[315]
Agastache mexicana (Kunth) Lint & Epling (Lamiaceae)	The dichloromethane and methanol extracts induced a significant vasorelaxant effect in a concentration-dependent and endothelium-independent manner. The principal active compound tilianin induced significant relaxation in a concentration- and endothelium-dependent and -independent manner in aortic rings pre-contracted with NA and serotonin. Also, acacetin, ursolic acid (**40**), and acacetin-oleanolic acid (**93**) and acacetin-ursolic acid (**40**) mixtures, showed significant vasodilation. Finally, the essential oil relaxed carbachol and histamine-induced contractions, and antagonized calcium chloride-induced contractions	Tilianin, acacetin, ursolic (**40**) and oleanolic (**93**) acids, and the essential oil	[316–318]

Aloysia citrodora Paláu (syn. *Aloysia triphylla* (L'Hér.) Britton) (Verbenaceae)	A *n*-hexane extract of the leaves showed an inhibitory effect on the spontaneous phasic contractions of myometrial smooth muscle in a concentration-dependent manner (3–56 μg/cm^3). The extract also inhibited the maximal contraction induced by the high-K^+ depolarizing solution, oxytocin, carbachol, and PGF2α in a concentration-dependent manner. Citral (3–230 μg/cm^3) inhibited the contractile response induced by PGF2α. The extract was also anti-inflammatory	Citral	[52]
Arracacia tolucensis var. *multifida* Hemsley (S. Wats.) Mathias & Constance (Umbelliferae)	The essential oil (IC_{50} = 116.4 μg/cm^3) and the extract (IC_{50} = 1153.1 μg/cm^3) prepared from the aerial parts of the plant provoked concentration-dependent inhibition of the tone and amplitude of the guinea-pig ileum spontaneous contractions. The crude extract as well as the essential oil antagonized the contractions exerted by acetylcholine, histamine, 5-HT and $BaCl_2$. Altogether, this information revealed that the smooth muscle-relaxant activity displayed by the extract and essential oil involved an unspecific inhibition of the calcium influx into the smooth muscle cells	Osthol, suberosin, isoimperatorin, 8-methoxypsoralen, herniarin, scoparone, umbelliferone, and dihydroxypeucedanin, 5-methoxypsoralen, isoscopoletin, and scopoletin	[319]
Brickellia cavanillesii (Cass.) Gray (Asteraceae)	A methanol extract produced a vasorelaxant effect on the contraction induced by norepinephrine in endothelium-intact (E_{max} = 98.2%) and denuded (E_{max} = 100.0%) aortal rings	Not determined	[320]
Brickellia paniculata (Mill.) B.L. Rob (Asteraceae)	A methanol extract from the leaves provoked relaxation of K^+-induced contractions in the guinea-pig ileum. Xanthomicrol and 3α-angeloyloxy-2α-hydroxy-13,(14*Z*)-dehydrocativic acid inhibited the tonic contraction (IC_{50} = 4.6 and 6.6 μg/cm^3, respectively); the effect was potentiated in a mixture 3:1 (IC_{50} = 2.4 μg/cm^3) of both compounds. Xanthomicrol reduced the spasms induced with acetylcholine, histamine, and $BaCl_2$, and blocked voltage-operated calcium channels	Xanthomicrol and 3α-angeloyloxy-2-α-hydroxy-13,(14*Z*)-dehydrocativic acid	[321, 322]
Casimiroa edulis Llave et Lex. and *C. pubescens* Ramirez (Rutaceae)	The methanol seed extract induced vasorelaxation in arterial rat tissues by M3 receptor through the activation of cGMP-dependent NO signaling	Not determined	[323]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Chirantodendron pentadactylon Larreat (Sterculiaceae)	The aqueous extract from the flowers induced a concentration-dependent relaxation on rat aortic segments with E_{max} of 66.5% ($IC_{50} = 30.6$ μg/cm^3)	Not determined	[324]
Cochlospermum vitifolium (Willd.) Sprengel (Bixaceae)	A *n*-hexane extract showed a significant endothelium-independent relaxation on rat aorta rings ($E_{max} = 100\%$ and 78.7% in intact and denuded endothelium, respectively), and the methanol extract produced an endothelium-dependent relaxation ($E_{max} = 79.1\%$). Naringenin (**211**) exhibited a concentration-dependent and partially endothelium-dependent vasorelaxant effect. Also, the methanol extract (120 mg/kg) and the active principle (50 and 160 mg/kg) showed acute antihypertensive effects on spontaneously hypertensive rats when the systolic and diastolic pressure were decreased at 1 and 24 h after administration, respectively	Naringenin (**211**)	[283, 325]
Conyza filaginoides (DC.) Hieron (Asteraceae)	A $CHCl_3$-MeOH (1:1) extract induced an inhibition of the spontaneous contraction of the rat ileum ($IC_{50} = 43$ μg/cm^3). Activity-guided fractionation of the smooth muscle-relaxant extract led to the isolation of several active principles that induced a concentration-dependent inhibition of the spontaneous contractions of rat ileum	Quercetin 3-glucoside, rutin (**273**), pinostrobin (**270**), α-spinasterol, β-caryophyllene 4,5-*α*-oxide, erythrodiol, and *β*-tridecanoyloxy-28-hydroxyolean-12-ene	[326]
Dodonaea viscosa (L.) Jacq. (Sapindaceae)	The CH_2Cl_2-MeOH (1:1) extract of the aerial parts of the plant triggered a concentration-dependent inhibition of the tone and the amplitude of the spontaneous contraction of the guinea-pig ileum. Bioassay-guided fractionation resulted in the isolation of four spasmolytic agents. These compounds inhibited also the spontaneous contractions of the guinea-pig ileum; the mechanism of action involved the interference with calcium metabolism in smooth muscle cells	Sakuranetin, *ent*-15,16-epoxy-9*αH*-labda-13(16)14-diene-3*β*,8*α*-diol, hautriwaic acid, and 6-hydroxykaempferyl 3,7-dimethyl ether	[327, 328]
Dracocephalum moldavica L. (Lamiaceae)	The aqueous extract from the whole plant including flowers, leaves, and stems induced a concentration-dependent relaxation of rat aortic segments with E_{max} of 76.4% ($IC_{50} = 39.1$ μg/cm^3)	Not determined	[324]

Galphimia glauca Cav. (Malpighiaceae)	The aqueous extracts from the leaves (IC_{50} = 2.3 μg/cm^3) and flowers (IC_{50} = 3.3 μg/cm^3) significantly inhibited in a concentration-dependent manner, the maximal contractile response induced by NA in isolated rat thoracic aorta. A methanol extract of the leaves (135 μg/cm^3) inhibited the electrically induced contraction of the isolated guinea pig ileum by 80.4%. The active principles galphimines A–F also inhibited the electrically induced contractions of the guinea-pig ileum (EC_{50} values ranged from 5.43 to 200 μg/cm^3)	Galphimines A–F	[329, 330]
Juglans regia L. (Juglandaceae)	The aqueous extracts from the leaves (IC_{50} = 3.2 μg/cm^3) significantly inhibited in a concentration-dependent manner, the maximal contractile response induced by NA in isolated rat thoracic aorta	Not determined	[329]
Laelia anceps Lindl. (Orchidaceae)	A methanol extract from the roots induced relaxation in aortic rings precontracted with norepinephrine, 5-HT and KCl. It also reduced norepinephrine-induced transient contraction in Ca^{2+}-free solution and inhibited contraction induced by increasing external calcium. Oral administration of the extract (100 mg/kg) exhibited a significant decrease in systolic and diastolic blood pressures in SHR rats. The active principle induced a concentration-dependent and endothelium-independent relaxant activity on norepinephrine precontracted aortic rings with E_{max} of 90.0% (with endothelium) and 96.5% (without endothelium)	2,7-Dihydroxy-3,4,9-trimethoxyphenantrene	[331, 332]
Laelia autumnalis (Lex.) Lindley (Orchidaceae)	A methanol extract produced vasorelaxant effect on the contraction induced by norepinephrine and KCl in endothelium-intact (E_{max} = 84.6% and 85.0%, respectively) and denuded (E_{max} = 54.7% and 80.0%, respectively) aortal rings. The mechanisms of action involved blockade of Ca^{2+} channels, inhibition of Ca^{2+} mobilization from intracellular stores, and probably by enhanced cGMP levels	Not determined	[320, 333]
Laelia speciosa (Kunth) Schltr. (Orchidaceae)	The *n*-hexane and methanol extracts obtained from the roots and pseudobulbs showed a concentration-dependent and endothelium-independent vasorelaxant effect	Gigantol (**51**)	[332]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Lepechinia caulescens (Ortega) Epling (Lamiaceae)	A methanol extract produced a vasorelaxant effect ($E_{max} = 90.2\%$) on the contraction induced by norepinephrine in endothelium-intact aorta rings. It also induced a concentration-dependent relaxation (0.001–100 μg/cm^3) of the rat ileum ($IC_{50} = 11.2$ μg/cm^3). The spasmolytic effect was mediated by a blockade of Ca^{2+} influx and NO release. In a different study, bioassay-guided fractionation using the rat uterus model of a *n*-hexane extract of the leaves led to the isolation of four active terpenoids. The spasmolytic activity of spathulenol involved a blocking action on voltage-operated calcium channels	Ursolic (**40**) and oleanolic (**93**) acids, spathulenol, 9*α*,13α-epidioxyabiet-8(14)-en-18-oate, 9*α*-hydroxydehydroabietyl alcohol, and dehydroabietic acid	[48, 320, 334]
Lippia graveolens Kunth (syn: *L. berlandieri* Schauer) (Verbenaceae)	The essential oil provoked a concentration-dependent inhibition of the carbachol- and histamine-induced contractions ($IC_{50} = 19.9$ and 1.6 μg/cm^3, respectively) when tested in the isolated guinea-pig ileum. It also caused a dose-dependent inhibition of the gastrointestinal transit; at a dose of 200 mg/kg the inhibition was 46.6%	Thymol (**170**), carvacrol, and *p*-cymene were the most abundant components of the oil, and were tested	[335]
Lonchocarpus xuul Lundell (Fabaceae)	The active principles each induced a significant relaxant effect in a concentration-dependent manner on aortic rat rings pre-contracted with NA. Oral administration of 50 mg/kg of dihydrospinochalcone A decreased indiastolic and systolic blood pressure in SHR rats	Dihydrospinochalcone A and isocordoin	[336]
Ipomoea stans Cav. (Convolvulaceae)	An aqueous extract from the roots ($IC_{50} = 8.2$ μg/cm^3) significantly inhibited in a concentration-dependent manner, the maximal contractile response induced by NA in isolated rat thoracic aorta	Not determined	[336]
Phoradendron reichenbachianum (Seem.) Oliv. (Viscaceae)	The active principles isolated from the acetone extract of leaves and stems showed a significant relaxant effect in a concentration- and endothelium-dependent manner in rat aorta rings after contraction with NA	Ursolic (**40**), moronic, morolic, betulinic, and 3,4-*seco*-olean-18-ene-3,28-dioic acids	[337]
Poliomintha longiflora A. Gray (Lamiaceae)	The essential oil induced a concentration-dependent inhibition of carbachol- and histamine-induced contractions ($IC_{50} = 45.7$ and 48.1 μg/cm^3, respectively), using the isolated guinea-pig ileum model	Carvacrol and *p*-cymene were the most abundant components of the oil, and were tested	[335]

Prunus serotina ssp. *capuli* (Cav. ex Spreng) McVaugh (Rosaceae)	An aqueous extract from the aerial parts induced a concentration-dependent relaxation of rat aortic segments with E_{max} of 70.2% ($IC_{50} = 190.4$ μg/cm^3)	Not determined	[324]
Psittacanthus calyculatus G. Don (Loranthaceae)	An aqueous extract from the aerial parts induced a concentration-dependent relaxation of rat aortic segments with E_{max} of 97.2% ($IC_{50} = 531.8$ μg/cm^3)	Not determined	[324]
Sechium edule (Jacq.) Sw. (Cucurbitaceae)	The hydroalcoholic extract obtained from the roots inhibited angiotensin II-induced hypertension in vagotomized rats and acute hypertension induced in mice with angiotensin II. The extract also showed a vasorelaxant effect in an in vitro aortal ring assay. Also, the acetone fraction of the hydroalcoholic extract at a dose of 10 mg/kg was able to control hypertension, as well as the prooxidative and proinflammatory status in the kidney as efficiently as losartan, returning mice to normotensive levels	Not determined	[338, 339]
Taxodium mucronatum Ten. (Taxodiaceae)	An aqueous extract from the aerial parts ($IC_{50} = 3.4$ μg/cm^3) significantly inhibited in a concentration-dependent manner, the maximal contractile response induced by NA in the isolated rat thoracic aorta	Not determined	[329]
Valeriana edulis ssp. *procera* (Valerianaceae)	A *n*-hexane extract from the rhizomes induced relaxation in aortic rings precontracted with KCl ($E_{max} = 85.0\%$), NA, and $CaCl_2$	Not determined, but the plant contains valepotriates	[251]
Gastroprotective agents			
Ageratina pichinchensis (Kunth) R.M. King & H. Robinson (Asteraceae)	3,5-Diprenyl-4-hydroxyacetophenone showed a gastroprotective effect against an ethanol-induced ulcer model in rats at 100 mg/kg. The results suggested that NO, prostaglandins, and SH groups are involved in their mechanism of action	3,5-Diprenyl-4-hydroxyacetophenone	[340]
Astragalus zahlbruckner Hand.-Mazz. (Fabaceae)	Astragaloside IV showed gastroprotective effects when tested at 3, 10 and 30 mg/kg in a model of ethanol-induced gastric damage in rats. This study suggested the participation of NO in the mechanism of action	Astragaloside IV	[341]
Calea urticifolia (Mill.) DC. (Asteraceae)	The calealactone B and 2,3-epoxyjuanislamin isolated revealed gastroprotective effects in an ethanol-induced injury model in rats. The modulation of NO, prostaglandins, and SH groups was not involved in their mechanism of action	Calealactone B and 2,3-epoxyjuanislamin	[342, 343]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Croton reflexifolius Kunth (Euphorbiaceae)	The main gastroprotective compound isolated from the plant, polyalthic acid, was tested in an ethanol-induced injury model in rats and showed a similar effectiveness to carbenoxolone. NO and SH groups were involved in its mechanism of action	Polyalthic acid	[344]
Eupatorium aschenbornianum S. Schauer (Asteraceae)	Encecanescin was tested in ethanol-induced gastric lesions in rats. The participation of NO, prostaglandins and SH groups in gastroprotection was observed	Encecanescin	[345]
Hyptis suaveolens (L.) Poit. (Lamiaceae)	Suaveolol was tested in an ethanol-induced gastric injury model in rats at 3, 10, 30, and 100 mg/kg. The results suggested that NO, prostaglandins and SH groups are involved in its gastroprotective mechanism of action	Suaveolol	[346]
Peperomia hispidula (Sw.) A. Dietr. (Piperaceae)	Methyleugenol (100 mg/kg) showed a gastroprotective effect when tested in an ethanol-induced gastric lesion model in rats. Modulation of NO, SH groups or prostaglandins did not seem to be involved in its mechanism of action	Methyleugenol	[347]
Peperomia pellucida (L.) Kunth (Piperaceae)	The gastroprotective effect of an extract of the plant was tested against ethanol-induced ulceration in rats. Dillapiole was tested at 3, 10, 30, and 100 mg/kg and showed gastroprotective effects. The results suggested that its mechanism of action did not involve prostaglandin, NO or SH groups	Dillapiole	[348]
Pseudobombax ellipticum (Kunth) Dugand (Malvaceae)	The active principle, β-lupeol, was tested in an ethanol-induced ulcer model in rats. The results suggested the participation of NO and non-protein SH groups in the mechanism of action	β-Lupeol	[349]
Pain and inflammatory disorders			
Acourtia thurberi (A. Gray) Reveal & R.M. King (Asteraceae)	A decoction from the roots and the active principles produced significant attenuation of the licking time in both the neurogenic and inflammatory phases in normal and hyperglycemic-hyperalgesic mice, using the formalin test	Perezone, α-pipitzol, β-pipitzol, and 8-*β*-D-glucopyranosyloxy-4-methoxy-5-methyl-coumarin	[271]

Agastache mexicana (Kunth) Lint & Epling (Lamiaceae)	The methanol and ethyl acetate extracts and the active principles produced antinociception in the writhing and formalin tests and capsaicin model in mice. Ursolic acid (**40**) exhibited its activity thorough the participation of TRPV1 and 5-HT_{1A} receptors	Ursolic acid (**40**) and acacetin	[350, 351]
Alnus acuminata ssp. *arguta* (Schltdl.) Furlow (Betulaceae)	The stem bark methanol extract showed anti-inflammatory activity in the carrageenan-induced hind paw edema model. The active principle possessed anti-inflammatory activity against TPA-induced inflammation in mice and on NO production in lipopolysaccharide-activated macrophages	Centrolobol	[352]
Brickellia veronicifollia (Kunth) Gray (Asteraceae)	The CH_2Cl_2-MeOH extract and the active principles showed significant analgesic effects in both the writhing and hot-plate tests in mice	6-Methoxysalicylic acid, 2-methoxybenzoic acid and taraxasteryl acetate	[353, 354]
Buddleia cordata H.B.K. (Loganiaceae)	An aqueous extract of the leaves and the active principle exhibited significant and dose-dependent analgesic and anti-inflammatory activities in both the writhing and hot-plate tests in mice	Linarin (**329**)	[355, 356]
Calea zacatechichi Schltdl. (Asteraceae)	The dichloromethane extract displayed a reduction of abdominal pain using the behavioral model elicited by mustard oil and the writhing test. The ethanol extract contained biologically active sesquiterpene lactones, which were shown in vitro to inhibit activation of NF-κB, a transcriptional factor and one of the major mediators of inflammatory pathways	Sesquiterpene lactones mixture	[357, 358]
Choisya ternata Kunth (Rutaceae)	The essential oil, the ethanol leaf extract, and the active principle showed antinociception in the acetic acid-induced writhing and hot-plate tests	Isopropyl *N*-methylanthranilate	[359]
Cirsium subcoriaceum (Less.) Schultz-Bip. (Asteraceae)	An aqueous extract of the aerial parts and the active principle exerted significant and dose-dependent analgesic and anti-inflammatory activities in mice and rats	Pectolinarin	[356]
Clinopodium mexicanum (Benth.) Govaerts (Lamiaceae)	The antinociceptive effect of the aqueous extract and the active principle were studied in the hot-plate model. The pure compound did not produce changes in either the latency sedation or the sleeping time induced by sodium pentobarbital	(2*S*)-Neoponcirin	[360]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Conyza filaginoides (DC.) Hieron (Asteraceae)	The CH_2Cl_2-MeOH extract and the main active compound of the plant showed antinociceptive and antihyperalgesic effects in the writhing, hot-plate, and formalin tests in mice	Rutin (**273**)	[361]
Cyrtopodium macrobulbon Lindley (Orchidaceae)	The peripheral antinociceptive activity of the dichloromethane and aqueous extracts were established by acetic acid-induced writhing and the hot-plate tests	Ephemeranthol B (**67**)	[54]
Decatropis bicolor (Zucc.) Radlk. (Rutaceae)	The active principles showed a dose-dependent anti-inflammatory activity in the TPA mouse ear model	Heraclenin and heraclenol	[362]
Geranium bellum Rose (Geraniaceae)	The acetone-water extract of the aerial parts and of some isolated compounds, showed antinociception activity in the hot-plate test and after acetic acid injection in CD1 female albino mice. Their anti-inflammatory properties were demonstrated by systemic administration in the carrageenan-induced paw edema model	Geraniin, corilagin, ellagic acid, and quercetin	[363]
Heliopsis longipes (A. Gray) S.F. Blake (Asteraceae)	The dichloromethane and ethanol extracts both showed analgesic activity as determined by $GABA_A$ release in mice brain slices, and in the writhing and hot-plate tests, respectively. The extract and active principle produced significant antinociceptive effects against chemical nociception in mice induced by intraperitoneal acetic acid and subplantar capsaicin. The anti-inflammatory properties of the ethanol extract and the active principle were evaluated through the mouse ear edema test using arachidonic acid and TPA as irritating agents	Affinin (*N*-isobutyldeca-2,6,8-trienamide)	[364–366]
Heterotheca inuloides Cass. (Asteraceae)	The antinociceptive activity of the *n*-hexane extract was demonstrated using the formalin test. The active principle reduced the number of flinches in the formalin test and increased the pain threshold to mechanical stimulation in the Randall-Selitto model in a dose-dependent manner. It also reduced the carrageenan-induced mechanical hyperalgesia and inflammation in a dose-dependent manner. The aqueous extract produced inhibition in the carrageenan- and dextran-induced paw edema models in rats. In the TPA-induced mouse ear edema model, the dichloromethane, acetone and methanol	7-Hydroxycadalene, 7-hydroxy-3,4-dihydrocadalene, dicadalenol, cadalen-15-oic acid, caryolan-1,9β-diol, and quercetin	[367, 368]

	extracts reduced inflammation. The active principles also exhibited anti-inflammatory effects in the same experimental models		
Justicia spicigera Schltdl. (Acanthaceae)	The ethanol extract showed central and peripheral antinociceptive effects in mice without inducing sedation. The anti-inflammatory effects were demonstrated in the carrageenan-induced paw edema model in rats	Kaempferitrin	[369]
Lippia graveolens H.B.K. (Verbenaceae)	The methanol and ethyl acetate extracts, and the active principle revealed moderate anti-inflammatory activities in the TPA ear mice model	(−)-(2*S*)-5,6,7,3′,5′-Pentahydroxyflavanone-7-*O*-*β*-D-glucopyranoside	[370]
Mimosa albida Humb. & Bonpl. ex Willd. (Fabaceae)	An aqueous extract from the roots exhibited antinociceptive activity in the acetic acid-induced writhing and hot plate tests. No effects were, however, observed either in the elevated plus-maze and hole board tests	Not determined	[371]
Myrtillocactus geometrizans (Mart. ex Pfeiff.) Con. (Cactaceae)	The active principles showed anti-inflammatory activity in both the TPA-induced ear edema in mice and the carrageenan-induced rat paw edema models	Chichipegenin, peniocerol and macdougallin	[372]
Parthenium argentatum (Gray) (Asteraceae)	The active principles exhibited a dose-related anti-inflammatory activity in the TPA-induced edema model. These compounds also inhibited COX-2 activity	Argentatins A, B, and D	[373]
Rosmarinus officinalis L. (Lamiaceae)	The essential oil produced a dose-dependent antinociceptive effect manifested as a significant reduction in the dysfunction in the pain-induced functional impairment model in the rat (arthritic pain). The serotonergic system via 5-HT_{1A} receptors and endogenous opioids in the antinociceptive effect were involved	Not determined	[374]
Ternstroemia sylvatica Schltdl. & Cham. (Theaceae)	The anti-inflammatory and antinociceptive effects of the leaf extracts were evaluated using the in vivo croton oil- and TPA-induced ear edema, carrageenan-induced paw edema, acetic acid-induced writhing, and formalin murine models. The chloroform and ethanol extracts showed strong activities	Not determined	[375]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Antiparasitic agents			
Annona cherimola Miller (Annonaceae)	Bioassay-guided fractionation of the ethanol extract of the leaves of the plant afforded a series of phenolic compounds. Using an in vitro antiprotozoal assay, kaempferol was the most potent antiamoebic and antigiardial compound, with IC_{50} values of 7.9 and 8.7 μg/cm^3 for *E. histolytica* and *G. lamblia*, respectively	Kaempferol	[376]
Aristolochia elegans Mast. (Aristolochiaceae)	The active principles isolated from a *n*-hexane extract from the rhizomes of the plant displayed antiprotozoal activity against *E. histolytica* and *G. lamblia.* Eupomatenoid-1 ($IC_{50} = 0.624$ and 0.545 μg/cm^3, respectively) was the most active compound, while fargesin and (8*R*,8′*R*,9*R*)-cubebin were moderately active ($IC_{50} < 100$ μg/cm^3)	Eupomatenoid-1, fargesin, and (8*R*,8′*R*,9*R*)-cubebin	[377]
Bursera fagaroides (H.B. K.) Engl. (Burseraceae)	The podophyllotoxin-type-lignans isolated from the stem bark of the plant were tested against *G. lamblia* trophozoites. The compounds displayed IC_{50} values ranging from 2.12 to 42.2 μg/cm^3. In addition, the effects of these lignans were investigated using *Giardia* adhesion electron microscopic images and revealed also morphological alterations in the caudal region, ventral disk, membrane, and flagella, to different extents	Burseranin, 5′-demethoxy-β-peltatin-A-methyl ether, acetylpodophyllotoxin, and podophyllotoxin	[378]
Citrus aurantifolia (Christm.) Swingle (Rutaceae)	The *n*-hexane extract obtained from fresh fruit peels of the plant showed antiprotozoal activity against trophozoites of *G. lamblia* strain 0989:IMSS. The IC_{50} values of the active principles ranged from 34.7 to 229.5 μg/cm^3	Citral, geraniol and 4-hexen-3-one	[379]
Clerodendron mexicanum M. Brand (Verbenaceae)	Bioactivity-directed fractionation of the $CHCl_3$-MeOH (1:1) extract yielded the active principles, 3-oxotirucalla-7,(24*Z*)-dien-26-oic and *epi*-oleanolic (**94**) acids. Both compounds showed antiprotozoal activity against *Leishmania donovani* promastigotes with IC_{50} values of 13.7 and 18.8 μg/cm^3, respectively. The tirucallane-type triterpene was also active against *Tripanosoma bruceri* bloodstream forms with an IC_{50} value of 16.8 μg/cm^3	3-Oxotirucalla-7,(24*Z*)-dien-26-oic and *epi*-oleanolic (**94**) acids	[380]

Cnidoscolus chayamansa (McVaugh) (Euphorbiaceae)	The $CHCl_3$-MeOH (1:1) leaf extract and isolated compounds from the plant showed significant antimycobacterial and antiprotozoal activities. Moretenol and moretenyl acetate exhibited moderate effects against *E. histolytica* and *G. lamblia* ($IC_{50} < 71.7$ μg/cm^3), while kaempferol-3,7-dimethyl ether and 5-hydroxy-7 -3′,4′-trimethoxyflavanone were more active than the extract ($IC_{50} < 27.4$ μg/cm^3)	Moretenol, moretenyl acetate, kaempferol-3,7-dimethyl ether, and 5-hydroxy-7-3′,4′-trimethoxyflavanone	[381]
Colubrina greggii S. Watson var. *yucatanensis* M.C. Johnst. (Rhamnaceae)	The ethanol roots extract of the plant showed antiprotozoal activity against *L. amazonensis* ($IC_{50} = 32.4$ μg/cm^3) and *P. falciparum* ($IC_{50} = 8.0$ μg/m^3). The active principles displayed activity against *L. amazonensis*, *T. cruzi tulahuen* and *P. falciparum* in the μg/cm^3 range	3-*O*-Acetyl-ceanothic, ceanothic, ceanothenic, and betulinic acids, discarine B, and chrysophanein	[382]
Conyza filaginoides (DC.) Hieron (Asteraceae)	Bioassay-guided fractionation of a $CHCl_3$-MeOH (1:1) extract from the aerial parts of the plant yielded a series of compounds with moderate antiprotozoal activity against the trophozoites of *E. histolytica* and *G. lamblia*	3-*O*-(6″-*O*-(*E*)-Caffeoyl)-*β*-D-galactopyranoside, isorhamnetin 3-*O*-(6-″-*O*-(*E*)-caffeoyl)-*β*-D-galactopyranoside, quercetin 3-*O*-(6″-*O*-(*E*)-caffeoyl)-*β*-D-galactopyranoside, erythrodiol, β-caryophyllene-4,5-*α*-oxide, astragalin, isoquercitrin, nicotiflorin, rutin (**273**), and narcissin (**274**)	[383]
Cuphea pinetorum Benth. (Lythraceae)	A methanol extract from the roots was active in vitro against trophozoites of *E. histolytica* and *G. lamblia* ($IC_{50} = 73.2$ and 74.7 μg/cm^3). Kaempferol ($IC_{50} = 7.9$ and 8.7 μg/cm^3) and quercetin ($IC_{50} = 114.3$ and 26.6 μg/cm^3) showed antiprotozoal activity against *E. histolytica* or *G. lamblia*, respectively. Kaempferol also showed in vivo anti-*G. lamblia* activity in suckling female CD-1 mice with an ED_{50} of 2.06 mg/kg	Kaempferol and quercetin	[384, 385]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Helianthemum glomeratum Lag. (Cistaceae)	A methanol extract from the leaves and stems, and roots of the plant showed activity against the trophozoites of *E. histolytica* and *G. lamblia*. From the first extract, the flavonol glycosides tiliroside, kaempferol-3-*O*-(3″,6″di-*O*-(*E*)-*p*-coumaroyl)-*β*-D-glucopyranoside, astragalin, quercitrin, and isoquercitrin were isolated. Tiliroside was the most potent antiamoebic and antigiardial compound with IC_{50} values of 17.5 μg/cm^3 against both parasites. Also, isoquercitrin showed selectivity against *E. histolytica* (IC_{50} = 14.7 μg/cm^3) and quercitrin towards *G. lamblia* (IC_{50} = 24.3 μg/cm^3). From the root extract, (−)-*epi*-gallocatechin and (−)-*epi*-gallocatechin gallate were isolated. The first was active against *E. histolytica* and *G. lamblia*, with IC_{50} values of 6.9 and 8.1 μg/cm^3, respectively. Finally, a crude methanol extract of the whole plant and tiliroside showed in vivo anti-*G. lamblia* activity in suckling female CD-1 mice with ED_{50} values of 0.125 and 1.429 mg/kg, respectively	Tiliroside, kaempferol-3-*O*-(3″,6″-di-*O*-(*E*)-*p*-coumaroyl)-*β*-D-glucopyranoside, astragalin, quercitrin, isoquercitrin, (−)-*epi*-gallocatechin, and (−)-*epi*-gallocatechin gallate	[385–388]
Heterotheca inuloides Cass. (Compositae)	From the acetone extract of the dried flowers of the plant, a series of sesquiterpenes and flavonoids was isolated. The active principles, 7-hydroxy-3,4-dihydrocadalene and 7-hydroxycadalene, displayed anti-*G. intestinalis* trophozoite activity with IC_{50} values of 15.3 and 22.9 μg/cm^3, respectively	7-Hydroxy-3,4-dihydrocadalene and 7-hydroxycadalene	[389]
Lepidium virginicum L. (Brassicaceae)	The ethanol-water partitioning fraction obtained from the methanol extract of the roots of the plant showed antiprotozoal activity against *E. histolytica* trophozoites (IC_{50} = 100.1 μg/cm^3). Bioassay-guided fractionation resulted in the isolation of benzyl glucosinolate, which revealed in vitro activity against *E. histolytica* strain HM1-IMSS (IC_{50} = 20.4 μg/cm^3)	Benzyl glucosinolate	[390]
Lippia graveolens Kunth (Verbenaceae)	A methanol extract of the plant displayed antiprotozoal activity against *E. histolytica* (IC_{50} = 59.1 μg/cm^3). The active principle, carvacrol, inhibited the parasite with an IC_{50} value of 44.3 μg/cm^3	Carvacrol	[391]

Lonchocarpus xuul Lundell and *L. yucatanensis* Pittier (Leguminosae)	From the leaves, stem bark and root extracts of the plant, a series of flavans, flavones, chromenes and chalcones was isolated. Most of the compounds were inactive against *Leishmania* and *Tripanosoma* parasites, but 5,4′-dimethoxy-(6:7)-2,2-dimethylpyrano-flavone, found in both species, and 2′,4′-dihydroxy-3′-(3-methylbut-2-enyl) chalcone and 2′,4′-dihydroxy-3′-(γ,γ-dimethylallyl)-dihydrochalcone isolated only from *L. xuul*, were the active principles	5,4′-Dimethoxy-(6:7)-2,2-dimethylpyrano-flavone, isocordoin, and 2′,4′-Dihydroxy-3′-(γ,γ-dimethylallyl)-dihydrochalcone	[392–394]
Pentalinon andrieuxii Muell.-Arg. (Apocynaceae)	Several cholesterol derivatives, coumarins and triterpenes were isolated from an *n*-hexane partition of a methanol extract of the roots of the plant. Among these compounds, 6,7-dihydroneridienone, pentalinonsterol, 24-methylcholest-4,24(28)-dien-3-one, and neridienone exhibited significant activity against promastigotes and amastigotes of *L. mexicana*	6,7-Dihydroneridienone, pentalinonsterol, 24-methylcholest-4,24 (28)-dien-3-one, and neridienone	[395]
Prunus serotina subsp. *capuli* (Cav.) McVaugh (Rosaceae)	Bioassay-guided fractionation of the methanol extract of the bark of the plant yielded the active principle naringenin (**211**). This compound exhibited cysticidal activity against *Taenia crassiceps* in a time-concentration manner ($EC_{50} = 89.3\ \mu M$), and also caused a high level of damage in all parasite tissue	Naringenin (**211**)	[197]
Rubus coriifolius Focke (Rosaceae)	A CH_2Cl_2-MeOH (1:1) extract of the aerial parts of the plant showed activity against the trophozoites of *E. histolytica* and *G. lamblia* ($IC_{50} = 11.6$ and 55.6 μg/cm^3, respectively). Chemical analysis of the active fractions led to the isolation of several active compounds against of *E. histolytica* and *G. lamblia*. (−)-*epi*-Catechin (**163**) was the most active with IC_{50} values of 1.9 and 1.6 μg/cm^3, respectively, and also showed in vivo anti-*G. lamblia* activity in suckling female CD-1 mice with an ED_{50} of 0.072 mg/kg	(−)-*epi*-Catechin (**163**), (+)-catechin (**162**), nigaichigoside F1, *β*-sitosterol (**109**), β-sitosterol-3-*O*-*β*-D-galactopyranoside, hyperin, and gallic and ellagic acids	[385, 387, 396]
Ruta chalepensis Pers. (Rutaceae)	A methanol extract of the plant displayed antiprotozoal activity against *E. histolytica* ($IC_{50} = 60.0$ μg/cm^3). The active principle inhibited the parasite with an IC_{50} value of 45.95 μg/cm^3	Chalepensin	[391]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Salvia polystachya Ort. (Lamiaceae)	An acetone extract from the aerial parts displayed activities against *E. histolytica* ($IC_{50} = 29.5$ μg/cm^3) and *G. lamblia* ($IC_{50} = 29.5$ μg/cm^3) trophozoites. The main neo-clerodane diterpenoids isolated, polystachynes A, B, and D, and linearolactone, showed antiprotozoal activity in vitro. Linearolactone was the most potent antiamoebic and antigiardial compound with IC_{50} values of 22.9 μ*M* for *E. histolytica* and 28.2 μ*M* for *G. lamblia*	Polystachynes A, B, and D, and linearolactone	[397, 398]
Serjania yucatanensis Standl. (Sapindaceae)	A *n*-hexane extract of the leaves of the plant showed activity against trypomastigotes of *T. cruzi* ($IC_{50} = 78.0$ μg/cm^3). The mixture of lup-20(29)-en-3-one and β-caryophyllene oxide displayed anti-*T. cruzi* activity ($IC_{50} = 80.3$ μg/cm^3)	Lup-20(29)-en-3-one and β-caryophyllene oxide	[399]
Sphaeralcea angustifolia (Cav.) G. Don (Malvaceae)	From a crude ethanol extract of the aerial parts of the plant, a series of compounds was isolated. All compounds showed antiprotozoal activity against *E. histolytica* and *G. lamblia* but tiliroside was the most active against both parasites with IC_{50} values of 17.5 and 17.4 μg/cm^3, respectively	β-Sitosterol (**109**), tiliroside, caffeic and protocatechuic acids, and apigenin (**203**)	[400]
Zanthoxylum liebmannianum (Engelm.) P. Wilson (Rutaceae)	The decoction of the stem bark decreased the count of intestinal nematode eggs in naturally infected sheep. The chloroform extract was toxic to *Ascaris suum* and bioassay-guided fractionation led to the isolation of α-sanshool ($LC_{50} = 83.4 \times 10^{-5}$ *M*) as the only active compound. Also, the crude ethanol extract from the leaves exhibited inhibitory effects on the reproduction of trophozoites of *E. histolytica* and *G. lamblia* ($IC_{50} = 3.8$ and 58.0 μg/cm^3, respectively). From this extract, asarinin, hyperin, β-sitosterol (**109**), and β-sitosterol glucoside (**164**) were isolated. The most active compound was asarinin ($IC_{50} = 18.9$ and 35.4 μg/cm^3, respectively). The remaining compounds each showed moderate activity against both parasites	α-Sanshool, asarinin, hyperin, β-sitosterol (**109**), and β-sitosterol glucoside (**164**)	[401, 402]

Cytotoxic activity			
Bursera fagaroides (Kunth) Engl. var. *fagaroides* (Burseraceae)	All compounds showed in vitro antiproliferative activity against lung (A549) and ovarian (A2780) human carcinoma cell lines with IC_{50} values ranging from 15 to 587 n*M*. Also, the isolated lignans disrupted microtubule networks in cells and caused cell cycle arrest in the G2/M phase in the A549 cell line. Finally, these compounds were potent tubulin assembly inhibitors, displaying binding to the colchicine site with K_b values ranging from 11.8 to 185.0 $\times$ 10^5 M^{-1}. In addition, in vivo studies of the effect of these natural lignans on the cell cycle, cell migration and microtubule cytoskeleton of developing zebrafish embryos, demonstrated their antimitotic molecular activity by disturbing tubulin	Podophyllotoxin, acetyl podophyllotoxin, 5′-desmethoxy-β-peltatin A methyl ether, and 7′,8′-dehydro acetyl podophyllotoxin	[403, 404]
Hyptis brevipes Poit. (Lamiaceae)	From the chloroform extract of the aerial parts of the plant, a series of 6-heptyl-5,6-dihydro-2*H*-pyran-2-ones was isolated. These compounds displayed cytotoxicity against a variety of tumor cell lines. The most significant values were obtained against nasopharyngeal (KB) and cervix (HeLa) cancer cells with IC_{50} values of 1.7-10 μ*M*	Brevipolides G–O	[405, 406]
Ipomea alba L. (Convolvulaceae)	The active principles from the seed of the plant showed potential as mammalian multidrug-resistance-modifying agents. The potentiation of vinblastine susceptibility in multidrug-resistant human breast carcinoma cells of albinosides IV–XI was evaluated by modulation assays. The non-cytotoxic albinosides VII–IX (25 μg/cm^3) exerted the most effective potentiation of vinblastine susceptibility, with reversal factors ($RF_{MCF\text{-}7/Vin+}$) of 201- and >2517-fold, respectively	Albinosides I–XI	[407, 408]
Ipomea purga (Wender) Hayne (Convolvulaceae)	The glycolipids from the resin of the plant significantly lowered the efflux rate of rhodamine 123, a fluorescent efflux pump substrate used to determine its accumulation in efflux assays with MDR MCF-7/Vin cells. The reversal fold ($RF_{MCF\text{-}7/Vin+} > 1906$) value indicated these natural products to be vinblastine chemosensitizers at 25 μg/cm^3 in modulation assays	Jalapinosides I and II	[409, 410]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Ipomoea wolcottiana Rose var. *wolcottiana* (Convolvulaceae)	The resin glycosides isolated from the $CHCl_3$-soluble extract prepared using flowers of the plant showed cytotoxic ($IC_{50} > 8.68$ μ*M*) activity and modulated vinblastine susceptibility at 25 μg/cm^3 in MFC-7/Vin$^+$ cells, with reversal factor ($RF_{MCF\text{-}7/Vin+}$) of 2- to 130-fold	Wolcottinosides I–IV and intrapilosin VII	[411]
Schinus molle L. (Anacardiaceae)	The antilymphoma properties of an ethanol extract of the leaves of the plant and the active principle rutin (**273**) were established on athymic CD-1 nu/nu ($EC_{50} = 52.2$ and 9.5 mg/kg, respectively) and CD-1 ($EC_{50} = 99.4$ and 6.8 mg/kg, respectively) mice inoculated with U-937 cell line (human leukemic monocyte lymphoma, HLML). The antiproliferative effects of the extract and the active principle ($IC_{50} = 172.0$ and 9.6 μg/cm^3, respectively) were evaluated on the U-937 cell line using the MTT assay	Rutin (**273**)	[412]
Stillingia sanguinolenta Muell.-Arg. (Euphorbiaceae)	The active principle showed antiproliferative activities for the cell lines used with GI_{50} values ranging from 5 to 50 μ*M*. When PtK2 potoroo kidney cells were incubated for 18 h with 21.5 μ*M* of tonantzitlolone A dissolved in methanol, around 20% of mitotic cells were arrested in mitosis and showed a non-physiological monoastral half spindle instead of a normal bipolar spindle apparatus. This compound also reduced the attachment of kinesin-5 molecules to microtubules with a half maximal inhibitory effect at $IC_{50} = 147$ μ*M*	Tonantzitlolone A	[413]
Thevetia peruviana (Pers.) K. Schum (Apocynaceae)	The methanol extract of the plant exhibited cytotoxic activity against four human cancer cell lines: prostate adenocarcinoma (HTB-81) ($IC_{50} = 1.9$ μg/cm^3), breast adenocarcinoma (HTB-22) ($IC_{50} = 5.8$ μg/cm^3), colorectal adenocarcinoma (HTB-38) ($IC_{50} = 6.3$ μg/cm^3), and lung carcinoma (HTB-177) ($IC_{50} = 12.0$ μg/cm^3). The extract caused also a significant reduction of cell motility and colony formation, cell size reduction, membrane blebbing, and detachment of cells on all evaluated cancer cell lines. Finally, the extract induced apoptotic cell death, which was confirmed by DNA, and morphological examination displayed fragmentation and AO/EB double staining	Not determined, but the active fractions contains thevetiaflavone and cardiac glycosides	[414]

Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae)	The essential oil of the plant displayed significant cytotoxic effects on A375, MDA-MB 231, HCT 116, and T98 G tumor cells with IC_{50} values of 3.02, 3.79, 3.46, and 12.82 μg/cm^3, respectively	α-Pinene, limonene, and spathulenol	[415]
Anxiolytic and sleep-aid agents			
Agastache mexicana (Kunth) Lint & Epling (Lamiaceae)	Anxiolytic-like and sedative effects were measured in the avoidance exploratory behavior, burying behavior and the hole-board tests. The aqueous extracts of *A. mexicana* ssp. *mexicana* and *A. mexicana* ssp. *xolocotziana* at low doses (0.1–10.0 mg/kg) induced anxiolytic-like actions, and at higher doses (over 100 mg/kg) they induced sedative actions that affected general activity and motor coordination. In another study, tilianin (30–300 mg/kg) and a crude methanol extract (10–300 mg/kg) from *A. mexicana* were evaluated for potential sedative and anxiolytic-like responses using open-field, hole-board, cylinder of exploration, plus-maze, and sodium pentobarbital-induced hypnosis mouse methods. The results indicated that tilianin is one of the bioactive metabolites for the anxiolytic-like activity of *A. mexicana*, where $GABA_A$/BZD, but not 5-HT_{1A}, receptors are partially involved	Tilianin	[235, 416]
Galphimia glauca Cav. (Malpighiaceae)	A ethanol extract of *G. glauca* showed a sedative effect in several experimental models in mice. Galphimine B was identified as the active depressant principle in this extract. In a clinical study, a standardized aqueous extract (310 mg twice a day for 4 weeks; $n = 72$) showed anxiolytic effectiveness similar to lorazepam (1 mg) using the same schedule	Galphimine B	[417–419]
Citrus sinensis (L.) Osbeck (Rutaceae)	Methanol and dichloromethane extracts from the flowers of *C. sinensis* both showed a sedative effect in the exploratory cylinder model in mice. Hesperidin (**328**) was identified as the sedative active principle of the methanol extract with an $ED_{50} = 11.34$ mg/kg	Hesperidin (**328**)	[420]

(continued)

Scientific name and family	Pharmacological studies	Active principles	Ref.
Annona diversifolia Saff. (Annonaceae)	Palmitone (16-hentriacontanone), isolated from *A. diversifolia* leaves, at 0.3, 1, 3, 10, and 30 mg/kg, showed anxiolytic-like effects in the plus-maze, hole-board, and exploratory cylinder models in mice	Palmitone	[421]
Ternstroemia sylvatica Schltdl. and Cham. (Theaceae)	Methanol and aqueous extracts of the fruits of the *T. pringleii* showed CNS-depressant effects in the exploratory cylinder and hole-board models. From the aqueous extract, a triterpene glycoside with toxic rather than a CNS-depressant effect was isolated	28-*O*-[β-L-6-rhamnopyranosyl]-R1-barrigenol	[422]
Antiasthmatic agents			
Acalypha phleoides Cav. (Euphorbiaceae)	The $CHCl_3$-MeOH (1:1) extract and essential oil of the aerial parts inhibited the contractions elicited by serotonin (1×10^{-5} *M*) in the guinea pig ileum and pendular spontaneous contractions in the rabbit jejunum. Thymol (**170**) and camphor, identified as major components of the essential oil, showed relaxant effects in the guinea pig trachea	Thymol (**170**) and camphor	[315]
Croton reflexifolius H.B.K. (Euphorbiaceae)	The dichloromethane extract of *C. reflexifolius* was the most active relaxant extract ($EC_{50} = 118.98 \pm 5.927$ µg/cm^3) on the guinea pig trachea. Polyalthic acid was identified as the main active relaxing agent. The authors suggested an antimuscarinic effect as the mechanism of action of this compound	Polyalthic acid	[423]
Lippia dulcis Trev. (Verbenaceae)	The essential oil showed anti-histaminergic and anti-cholinergic activities at 100 µg/cm^3 in porcine bronchial rings	Not determined	[424]
Bryophyllum pinnatum Lam. (Crassulaceae)	An aqueous leaf extract (0.25–1.0 mg/cm^3) showed anti-histaminergic and anti-cholinergic activities in guinea pig trachea	Not determined	[425]

Artemisia ludoviciana spp. *mexicana* (Willd. ex Spring) D.D. Keck (Asteraceae),	The *n*-hexane, dichloromethane and methanol extracts showed significant relaxant effects on the pre-contracted rat tracheal tissue in a concentration-dependent manner. The dichloromethane extract shifted to the right, in a parallel manner, the concentration-response curves induced by carbachol. Also, this extract showed relaxant effects of the rat trachea. Pre-incubation with 1*H*-[1,2,4]-oxadiazolo [4,3-*a*]quinoxalin-1-one (10 μ*M*), indomethacin (10 μ*M*), L-NAME (10 μ*M*), glibenclamide (10 μ*M*), and 2-aminopyridine (100 μ*M*) did not modify the dichloromethane extract relaxant curves. This extract contains some sesquiterpene lactones and flavonoids	Not determined	[164]
Taxodium mucronatum Ten. (Cupressaceae)	The *n*-hexane leaf extract inhibited both 1 μ*M* carbachol ($EC_{50} = 33.9 \pm 2.5$ μg/cm^3) and 60 m*M* K^+ ($EC_{50} = 20.6 \pm 1.1$ μg/cm^3)-induced contractions. In rat tracheal rings it also caused the concentration-response to Ca^{2+} curves to shift to the right in a noncompetitive manner	Not determined	[426]

References

1. Bye R, Linares E (2016) Ethnobotany and ethnohistorical sources of Mesoamerica. In: Lira R, Casas A, Blancas J (eds) Ethnobotany of Mexico: interactions of people and plants in Mesoamerica. Springer, New York, p 41
2. Alonso-Castro AJ, Dominguez F, Maldonado-Miranda JJ, Castillo-Perez LJ, Carranza-Alvarez C, Solano E, Isiordia-Espinoza MA, Juarez-Vazquez MC, Zapata-Morales JR, Argueta-Fuertes MA, Ruiz-Padilla AJ, Solorio-Alvarado CR, Rangel-Velazquez JE, Ortiz-Andrade R, Gonzalez-Sanchez I, Cruz-Jimenez G, Orozco-Castellanos LM (2017) Use of medicinal plants by health professionals in Mexico. J Ethnopharmacol 198:81
3. Soria-Lopez M, Fuentes-Paramo I (2016) The identification of biopiracy in patents. World Pat Inf 47:67
4. International Diabetes Federation (2017) IDF Diabetes Atlas, 2017. http://www.diabetesatlas.org
5. Alarcon-Aguilar F, Roman-Ramos R (2006) Antidiabetic plants in Mexico and Central America) In: Soumyanath A (ed) Traditional herbal medicines for modern times: antidiabetic plants. CRC Press, Boca Raton, FL, p 179
6. Andrade-Cetto A, Heinrich M (2005) Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 99:325
7. Romero-Cerecero O, Reyes-Morales H, Aguilar-Santamaria L, Huerta-Reyes M, Tortoriello-Garcia J (2009) Use of medicinal plants among patients with diabetes mellitus type 2 in Morelos, Mexico. Bol Latinoam Caribe Plant Med Aromat 8:380
8. Convention on International Trade in Endangered Species of Wild Fauna and Flora (2017) Apendix II, 2017. https://www.cites.org/
9. The IUCN Red List of Threatened Species (2017) Version 2017-3. https://www.iucnredlist.org
10. Biblioteca Digital de la Medicina Tradicional (2009) Atlas de las plantas de la medicina tradicional Mexicana, 2009. http://www.medicinatradicionalmexicana.unam.mx
11. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54:275
12. Mata R, Cristians S, Escandon-Rivera S, Juarez-Reyes K, Rivero-Cruz I (2013) Mexican antidiabetic herbs: valuable sources of inhibitors of α-glucosidases. J Nat Prod 76:468
13. Ovalle-Magallanes B, Medina-Campos ON, Pedraza-Chaverri J, Mata R (2015) Hypoglycemic and antihyperglycemic effects of phytopreparations and limonoids from *Swietenia humilis*. Phytochemistry 110:111
14. Vogel HG (2008) Drug discovery and evaluation: pharmacological assays, vol 1, 3rd edn. Springer, Berlin
15. Ovalle-Magallanes B, Deciga-Campos M, Mata R (2017) Antihyperalgesic activity of a mexicanolide isolated from *Swietenia humilis* extract in nicotinamide-streptozotocin hyperglycemic mice. Biomed Pharmacother 92:324
16. Jimenez A, Mata R, Pereda-Miranda R, Calderon J, Isman MB, Nicol R, Arnason JT (1997) Insecticidal limonoids from *Swietenia humilis* and *Cedrela salvadorensis*. J Chem Ecol 23:1225
17. Jimenez A, Villarreal C, Toscano RA, Cook M, Arnason JT, Bye R, Mata R (1998) Limonoids from *Swietenia humilis* and *Guarea grandiflora* (Meliaceae). Phytochemistry 49:1981
18. Segura-Correa R, Mata R, Anaya AL, Hernandez-Bautista B, Villena R, Soriano-Garcia M, Bye R, Linares E (1993) New tetranortriterpenoids from *Swietenia humilis*. J Nat Prod 56:1567
19. Stranczinger S, Szentpéteri JL, Borhidi A (2006) Sequence differentiation between some DNA regions of *Hintonia latiflora* and *Hintonia standleyana*. Acta Bot Hung 48:435
20. Motley TJ, Wurdack KJ, Delprete PG (2005) Molecular systematics of the Catesbaeeae-Chiococceae complex (Rubiaceae): flower and fruit evolution and biogeographic implications. Am J Bot 92:316
21. Borhidi A (2012) Rubíaceas de México, 2nd edn. Akadémiai Kiadó, Budapest

22. Bye RA (1986) Medicinal plants of the Sierra-Madre: comparative study of Tarahumara and Mexican market plants. Econ Bot 40:103
23. Perez-Vasquez A, Castillejos-Ramirez E, Cristians S, Mata R (2014) Development of a UHPLC-PDA method for the simultaneous quantification of 4-phenylcoumarins and chlorogenic acid in *Exostema caribaeum* stem bark. J Nat Prod 77:516
24. Reguero MT, Mata R, Bye R, Linares E, Delgado G (1987) Chemical studies on Mexican plants used in traditional medicine II. Cucurbitacins from *Hintonia latiflora*. J Nat Prod 50:315
25. Mata R, Calzada F, Garcia MR, Reguero MT (1987) Chemical studies on Mexican plants used in traditional medicine III. New 4-phenylcoumarins from *Exostema caribaeum*. J Nat Prod 50:866
26. Mata R, Calzada F, Garcia MD (1988) Chemical studies on Mexican plants used in traditional medicine VI. Additional new 4-phenylcoumarins from *Exostema caribaeum*. J Nat Prod 51:851
27. Mata R, Camacho MDR, Cervera E, Bye R, Linares E (1990) Secondary metabolites from *Hintonia latiflora*. Phytochemistry 29:2037
28. Mata R, Camacho MDR, Mendoza S, Cruz MD (1992) A phenylstyrene from *Hintonia latiflora*. Phytochemistry 31:3199
29. Deciga-Campos M, Guerrero-Analco JA, Quijano L, Mata R (2006) Antinociceptive activity of 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F from *Hintonia standleyana* (Rubiaceae). Pharmacol Biochem Behav 83:342
30. Guerrero-Analco JA, Hersch-Martinez P, Pedraza-Chaverri J, Navarrete A, Mata R (2005) Antihyperglycemic effect of constituents from *Hintonia standleyana* in streptozotocin-induced diabetic rats. Planta Med 71:1099
31. Guerrero-Analco J, Medina-Campos O, Brindis F, Bye R, Pedraza-Chaverri J, Navarrete A, Mata R (2007) Antidiabetic properties of selected Mexican copalchis of the Rubiaceae family. Phytochemistry 68:2087
32. Cristians S, Guerrero-Analco JA, Perez-Vasquez A, Palacios-Espinosa F, Ciangherotti C, Bye R, Mata R (2009) Hypoglycemic activity of extracts and compounds from the leaves of *Hintonia standleyana* and *H. latiflora*: potential alternatives to the use of the stem bark of these species. J Nat Prod 72:408
33. Deciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castaneda-Corral G, Angeles-Lopez GE, Navarrete A, Mata R (2007) Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. J Ethnopharmacol 110:334
34. Rivera N, Lopez PY, Rojas M, Fortoul TI, Reynada D, Reyes AJ, Rivera E, Beltran HI, Malagon F (2014) Antimalarial efficacy, cytotoxicity, and genotoxicity of methanolic stem bark extract from *Hintonia latiflora* in a *Plasmodium yoelii yoelii* lethal murine malaria model. Parasitol Res 113:1529
35. Mata R, Acevedo L, Mendez-Bautista DI, Guerrero-Analco JA, Rivero BE, Rodriguez JM (2008) Development and validation of liquid chromatography method for quantification of the active markers of *Hintonia standleyana* and *Hintonia latiflora* crude drugs. Pharm Biol 46:105
36. Korecova M, Hladikova M (2014) Treatment of mild and moderate type-2 diabetes: open prospective trial with *Hintonia latiflora* extract. Eur J Med Res 19:16
37. Vierling C (2005) Zum vasodilatierenden Effekt des antidiabetisch wirkenden *Hintonia-latiflora*-Extraktes: in-vitro- und in-vivo-Messungen. PhD Thesis, Ludwig-Maximilians-Universität, p 154
38. Noster S, Kraus L (1990) In vitro antimalarial activity of *Coutarea latiflora* and *Exostema caribaeum* extracts on *Plasmodium falciparum*. Planta Med 56:63
39. Argotte-Ramos R, Ramirez-Avila G, Rodriguez-Gutierrez MDC, Ovilla-Munoz M, Lanz-Mendoza H, Rodriguez MH, Gonzalez-Cortazar M, Alvarez L (2006) Antimalarial 4-phenylcoumarins from the stem bark of *Hintonia latiflora*. J Nat Prod 69:1442
40. Cristians S, Bye R, Navarrete A, Mata R (2013) Gastroprotective effect of *Hintonia latiflora* and *Hintonia standleyana* aqueous extracts and compounds. J Ethnopharmacol 145:530

41. Flores-Bocanegra L, Gonzalez-Andrade M, Bye R, Linares E, Mata R (2017) α-Glucosidase inhibitors from *Salvia circinata*. J Nat Prod 80:1584
42. Bautista E, Fragoso-Serrano M, Ortiz-Pastrana N, Toscano RA, Ortega A (2016) Structural elucidation and evaluation of multidrug-resistance modulatory capability of amarissinins A-C, diterpenes derived from *Salvia amarissima*. Fitoterapia 114:1
43. Bautista E, Fragoso-Serrano M, Toscano RA, Garcia-Pena MR, Ortega A (2015) Teotihuacanin, a diterpene with an unusual spiro-10/6 system from *Salvia amarissima* with potent modulatory activity of multidrug resistance in cancer cells. Org Lett 17:3280
44. Tortoriello J, Meckes-Fischer M, Villarreal ML, Berlin B, Berlin E (1995) Spasmolytic activity of medicinal plants used to treat gastrointestinal and respiratory diseases in the Highland of Chiapas. Phytomedicine 2:57
45. Rojas A, Cruz S, Rauch V, Bye R, Linares E, Mata R (1995) Spasmolytic potential of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. Phytomedicine 2:51
46. Rojas A, Bah M, Rojas JI, Serrano V, Pacheco S (1999) Spasmolytic activity of some plants used by the Otomi Indians of Queretaro (Mexico) for the treatment of gastrointestinal disorders. Phytomedicine 6:367
47. Rodriguez-Lopez V, Salazar L, Estrada S (2003) Spasmolytic activity of several extracts obtained from some Mexican medicinal plants. Fitoterapia 74:725
48. Estrada-Soto S, Rodriguez-Avilez A, Castaneda-Avila C, Castillo-Espana P, Navarrete-Vazquez G, Hernandez L, Aguirre-Crespo F (2007) Spasmolytic action of *Lepechinia caulescens* is through calcium channel blockade and NO release. J Ethnopharmacol 114:364
49. Osuna L, Tapia-Perez ME, Jimenez-Ferrer JE, Carrillo-Quiroz BA, Silva-Sanchez J (2005) Screening of *Alternanthera repens*, *Boerhavia coccinea*, *Flaveria trinervia*, *Tournefortia densiflora*, and *Vitex mollis* extracts to evaluate their antibacterial activity and effect on smooth muscle. Pharm Biol 43:749
50. Rosas-Peralta M, Borrayo-Sánchez G, Santiago-López J, Ramirez Árias E, García Méndez R, Borja-Aburto VH, Sepúlveda-Vildósola AC, Arriaga-Dávila JJ (2017) What is new in hypertension of Mexico 2018? Impact of the new classification of high blood pressure in adults from American College of Cardiology/American Heart Association (ACC/AHA). Ann Clin Hypertens 2:24
51. Castillo-España P, Cisneros-Estrada A, Garduno-Ramirez ML, Hernandez-Abreu O, Rodríguez R, Estrada-Soto S (2009) Preliminary ethnopharmacological survey of plants used in Mexico for the treatment of hypertension. Pharmacogn Rev 3:41
52. Ponce-Monter H, Fernandez M, Eduardo OMI, Ramirez-Montiel ML, Cruz-Elizalde D, Perez-Hernandez N, Carino-Cortez R (2010) Spasmolytic and anti-inflammatory effects of *Aloysia triphylla* and citral, in vitro and in vivo studies. J Smooth Muscle Res 46:309
53. Perez-Gutierrez RM (2011) Spasmolytic effect of constituents from ethnomedicinal plants on smooth muscle. In: Rai M, Acharya D, Rios JL (eds) Ethnomedicinal plants: revitalizing of traditional knowledge of herbs. CRC Press, Boca Raton, FL, p 25
54. Morales-Sanchez V, Rivero-Cruz I, Laguna-Hernandez G, Salazar-Chavez G, Mata R (2014) Chemical composition, potential toxicity, and quality control procedures of the crude drug of *Cyrtopodium macrobulbon*. J Ethnopharmacol 154:790
55. Estrada S, Rojas A, Mathison Y, Israel A, Mata R (1999) Nitric oxide/cGMP mediates the spasmolytic action of 3,4′-dihydroxy-5,5′-dimethoxybibenzyl from *Scaphyglottis livida*. Planta Med 65:109
56. Meisel JE, Kaufmann RS, Pupulin F (2014) Orchids of tropical America: an introduction and guide, 1st edn. Cornell University Press, Ithaca, NY
57. Hernandez-Romero Y, Rojas JI, Castillo R, Rojas A, Mata R (2004) Spasmolytic effects, mode of action, and structure-activity relationships of stilbenoids from *Nidema boothii*. J Nat Prod 67:160

58. Estrada-Soto S, López-Guerrero JJ, Villalobos-Molina R, Mata R (2006) Endothelium-independent relaxation of aorta rings by two stilbenoids from the orchids *Scaphyglottis livida*. Fitoterapia 77:236
59. Reyes-Ramirez A, Leyte-Lugo M, Figueroa M, Serrano-Alba T, Gonzalez-Andrade M, Mata R (2011) Synthesis, biological evaluation, and docking studies of gigantol analogs as calmodulin inhibitors. Eur J Med Chem 46:2699
60. Perez-Gutierrez RM, Vargas-Solis R (2009) Relaxant and antispasmodic effects of extracts of the orchid *Encyclia michuacana* on isolated guinea pig ileum. J Nat Med 63:65
61. Estrada S, Toscano RA, Mata R (1999) New phenanthrene derivatives from *Maxillaria densa*. J Nat Prod 62:1175
62. Estrada S, Lopez-Guerrero JJ, Villalobos-Molina R, Mata R (2004) Spasmolytic stilbenoids from *Maxillaria densa*. Fitoterapia 75:690
63. Rendon-Vallejo P, Hernandez-Abreu O, Vergara-Galicia J, Millan-Pacheco C, Mejia A, Ibarra-Barajas M, Estrada-Soto S (2012) Ex vivo study of the vasorelaxant activity induced by phenanthrene derivatives isolated from *Maxillaria densa*. J Nat Prod 75:2241
64. Hsu D-S, Yeh J-Y, Cheng C-Y (2017) Total synthesis of the proposed structure of (±)-nidemone. Org Lett 19:5549
65. Deciga-Campos M, Palacios-Espinosa JF, Reyes-Ramirez A, Mata R (2007) Antinociceptive and anti-inflammatory effects of compounds isolated from *Scaphyglottis livida* and *Maxillaria densa*. J Ethnopharmacol 114:161
66. Valencia-Islas NA, Paul RN, Shier WT, Mata R, Abbas HK (2002) Phytotoxicity and ultrastructural effects of gymnopusin from the orchid *Maxillaria densa* on duckweed (*Lemna pausicostata*) frond and root tissues. Phytochemistry 61:141
67. Hernandez-Romero Y, Acevedo L, Sanchez MD, Shier WT, Abbas HK, Mata R (2005) Phytotoxic activity of bibenzyl derivatives from the orchid *Epidendrum rigidum*. J Agric Food Chem 53:6276
68. World Life Expectancy (2018) World Health Rankings, 2018. http://www.worldlifeexpectancy.com/country-health-profile/Mexico
69. Navarrete A, Martínez-Uribe LS, Reyes B (1998) Gastroprotective activity of the stem bark of *Amphipterygium adstringens* in rats. Phytother Res 12:1
70. Navarrete A, Oliva I, Sanchez-Mendoza ME, Arrieta J, Cruz-Antonio L, Castaneda-Hernandez G (2005) Gastroprotection and effect of the simultaneous administration of cuachalalate (*Amphipterygium adstringens*) on the pharmacokinetics and anti-inflammatory activity of diclofenac in rats. J Pharm Pharmacol 57:1629
71. Navarrete A, Trejo-Miranda JL, Reyes-Trejo L (2002) Principles of root bark of *Hippocratea excelsa* (Hippocrataceae) with gastroprotective activity. J Ethnopharmacol 79:383–388
72. Castillo-Juarez I, Gonzalez V, Jaime-Aguilar H, Martinez G, Linares E, Bye R, Romero I (2009) Anti-*Helicobacter pylori* activity of plants used in Mexican traditional medicine for gastrointestinal disorders. J Ethnopharmacol 122:402
73. Hernández F (1943) Historia de las plantas de la Nueva España. Instituto de Biología, Universidad Nacional Autónoma de México. Imprenta Universitaria. Mexico
74. Navarrete A, Mata R (2009) Monografías científicas de plantas medicinales de México: Cuachalalate *Amphipterygium adstringens*. Sentido Giratorio Ediciones, Mexico City
75. Comisión Permanente de la Farmacopea (2001) Farmacopea Herbolaria de los Estados Unidos Mexicanos, 1st edn. Secretaría de Salud de Mexico, Mexico City
76. Comisión Permanente de la Farmacopea (2013) Farmacopea Herbolaria de los Estados Unidos Mexicanos, 2nd edn. Secretaría de Salud de Mexico, Mexico City
77. Solares Arenas F, Vázquez Alvarado JMP, Gálvez Cortés MC (2012) Canales de comercialización de la corteza de cuachalalate (*Amphipterigium adstringens* Schiede ex Schlecht.) en México. Rev Mex Cienc Forestales 3:29
78. Gonzalez EE, Delgado JN, McKenna GF (1962) Anticancer evaluation of *Amphipterygium adstringens*. J Pharm Sci 51:901

79. Gonzalez EE, Delgado JN (1962) Phytochemical investigation of *Amphiterygium adstringens*. J Pharm Sci 51:786
80. Soriano-Garcia M, Toscano RA, Ortiz B, Navarrete A, Sanchez-Obregon R, Barrios H, Yuste F (1987) Structure and stereochemistry of the methyl-ester of (5α,13α,14β,17α,20*S*,24*Z*)-3-oxolanosta-7,24-dien-26-oic acid (masticadienonic acid). Acta Crystallogr Sect C Cryst Struct Commun 43:990
81. Navarrete A, Mata R, Delgado G (1989) Alkylanacardic acids from *Amphypterygium adstringens*. Planta Med 55:579
82. Mata R, Calzada F, Navarrete A, Del Rio F, Delgado G (1991) Long-chain phenols from the bark of *Amphypterygium adstringens*. J Ethnopharmacol 34:147
83. Makino M, Motegi T, Fujimoto Y (2004) Tirucallane-type triterpenes from *Juliania adstringens*. Phytochemistry 65:891
84. Arrieta J, Benitez J, Flores E, Castillo C, Navarrete A (2003) Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*. Role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. Planta Med 69:905
85. Dominguez XA, Franco R, Garcia S, Porras ME, Vazquez G, Amescua B (1983) Plantas medicinales mexicanas. XLVIII: estructura del ácido instipolinácico separado de la corteza del cuachalalate (*Amphipterygium adstringens*). Rev Latinoam Quim 14:99
86. Mata R (1993) Chemical studies and biological aspects of some Mexican plants used in traditional medicine. In: Downum KR, Romeo JT, Stafford HA (eds) Phytochemical potential of tropical plants. Springer, New York, p 41
87. Rivero-Cruz BE, Esturau N, Sánchez-Nieto S, Romero I, Castillo-Juárez I, Rivero-Cruz JF (2011) Isolation of the new anacardic acid 6-[(16′*Z*)-nonadecenyl]-salicylic acid and evaluation of its antimicrobial activity against *Streptococcus mutans* and *Porphyromonas gingivalis*. Nat Prod Res 25:1282
88. Rivero-Cruz I, Acevedo L, Guerrero JA, Martinez S, Bye R, Pereda-Miranda R, Franzblau S, Timmermann BN, Mata R (2005) Antimycobacterial agents from selected Mexican medicinal plants. J Pharm Pharmacol 57:1117
89. Watson WH, Dominguez XA, Vazquez G, Garcia S (1987) Cuachalalic acid, a new triterpene from *Amphipterygium adstringens*. Rev Latinoam Quim 18:89
90. Navarrete A, Reyes B, Silva A, Sixtos C, Islas P, Estrada E (1990) Evaluación farmacológica de la actividad antiulcerosa de *Amphipterygium adstringens* (cuachalalate). Rev Mex Cienc Farm 21:28
91. Rodriguez-Canales M, Jimenez-Rivas R, Canales-Martinez MM, Garcia-Lopez AJ, Rivera-Yanez N, Nieto-Yanez O, Ledesma-Soto Y, Sanchez-Torres LE, Rodriguez-Sosa M, Terrazas LI, Rodriguez-Monroy MA (2016) Protective effect of *Amphipterygium adstringens* extract on dextran sulphate sodium-induced ulcerative colitis in mice. Mediators Inflamm 2016:ID 8543561
92. Castillo-Juarez I, Rivero-Cruz F, Celis H, Romero I (2007) Anti-*Helicobacter pylori* activity of anacardic acids from *Amphipterygium adstringens*. J Ethnopharmacol 114:72
93. Oviedo-Chavez I, Ramirez-Apan T, Soto-Hernandez M, Martinez-Vazquez M (2004) Principles of the bark of *Amphipterygium adstringens* (Julianaceae) with anti-inflammatory activity. Phytomedicine 11:436
94. Oviedo-Chavez I, Ramirez-Apan T, Martinez-Vazquez M (2005) Cytotoxic activity and effect on nitric oxide production of tirucallane-type triterpenes. J Pharm Pharmacol 57:1087
95. Castillo-Juarez I, Garcia-Contreras R, Velazquez-Guadarrarma N, Soto-Hernandez M, Martinez-Vazquez M (2013) *Amphypterygium adstringens* anacardic acid mixture inhibits quorum sensing-controlled virulence factors of *Chromobacteriurn violaceum* and *Pseudomonas aeruginosa*. Arch Med Res 44:488
96. Navarrete A, Avula B, Joshi VC, Ji XH, Hersh P, Khan IA (2006) Quantitative determination of triterpenes from *Amphiptherygium* adstringens by liquid chromatography and morphological analysis of cuachalalate preparations. J AOAC Int 89:1

97. Linares E, Bye RA (1987) A study of four medicinal plant complexes of Mexico and adjacent United States. J Ethnopharmacol 19:153
98. Appelt GD (1985) Pharmacological aspects of selected herbs employed in hispanic folk medicine in the San Luis Valley of Colorado, USA. I. *Ligusticum porteri* (Osha) and *Matricaria chamomilla* (Manzanilla). J Ethnopharmacol 13:51
99. Leon A, Del Angel M, Avila JL, Delgado G (2017) Phthalides: distribution in nature, chemical reactivity, synthesis, and biological activity. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J (eds) Progress in the chemistry of organic natural products, vol 104. Springer International Publishing AG, Cham, Switzerland, p 127
100. Velazquez-Moyado JA, Martinez-Gonzalez A, Linares E, Bye R, Mata R, Navarrete A (2015) Gastroprotective effect of diligustilide isolated from roots of *Ligusticum porteri* Coulter & Rose (Apiaceae) on ethanol-induced lesions in rats. J Ethnopharmacol 174:403
101. Velazquez-Moyado J, Balderas-Lopez JL, Pineda-Pena EA, Sanchez-Ortiz BL, Tavares-Carvalho JC, Navarrete A (2018) Diligustilide releases H_2S and stabilizes S-nitrosothiols in ethanol-induced lesions on rat gastric mucosa. Inflammopharmacology 26:611
102. Juarez-Reyes K, Angeles-Lopez GE, Rivero-Cruz I, Bye R, Mata R (2014) Antinociceptive activity of *Ligusticum porteri* preparations and compounds. Pharm Biol 52:14
103. Del Angel M, Nieto A, Ramirez-Apan T, Delgado G (2015) Anti-inflammatory effect of natural and semi-synthetic phthalides. Eur J Pharmacol 752:40
104. Leon A, Toscano RA, Tortoriello J, Delgado G (2011) Phthalides and other constituents from *Ligusticum porteri*: sedative and spasmolytic activities of some natural products and derivatives. Nat Prod Res 25:1234
105. Nguyen K, Sparks J, Omoruyi F (2017) Effects of *Ligusticum porteri* (Osha) root extract on human promyelocytic leukemia cells. Pharmacogn Res 9:156
106. Brindis F, Rodriguez R, Bye R, Gonzalez-Andrade M, Mata R (2011) (*Z*)-3-Butylidenephthalide from *Ligusticum porteri*, an α-glucosidase inhibitor. J Nat Prod 74:314
107. Rivero I, Juarez K, Zuluaga M, Bye R, Mata R (2012) Quantitative HPLC method for determining two of the major active phthalides from *Ligusticum porteri* roots. J AOAC Int 95:84
108. Goldhaber-Pasillas D, Bye R, Chávez-Ávila VM, Mata R (2012) In vitro morphogenetic responses and comparative analysis of phthalides in the highly valued medicinal plant *Ligusticum porteri* Coulter & Rose. Plant Growth Regul 67:107
109. Reyes-Chilpa R, Jimenez-Estrada M, Cristobal-Telesforo E, Torres-Colin L, Villavicencio MA, Perez-Escandon BE, Mercado-Gonzalez R (2003) Natural insecticides from *Hippocratea excelsa* and *Hippocratea celastroides*. Econ Bot 57:54
110. Palacios J, Mata R, Lopez R, Linares E, Bye R (1989) Notes on economic plants. *Hippocratea excelsa* (Hippocrateaeeae), a new source of *trans* polyisoprene. Econ Bot 43:508
111. Mata R, Calzada F, Diaz E, Toscano RA (1990) Chemical studies on Mexican plants used in traditional medicine. XV. Sesquiterpene evoninoate alkaloids from *Hippocratea excelsa*. J Nat Prod 53:1212
112. Calzada F, Mata R, Lopez R, Linares E, Bye R, Barreto VM, Delrio F (1991) Chemical studies on Mexican plants used in traditional medicine. XI. Friedelanes and triterpenoid quinone methides from *Hippocratea excelsa*. Planta Med 57:194
113. Mena-Rejon GJ, Perez-Espadas AR, Moo-Puc RE, Cedillo-Rivera R, Bazzocchi IL, Jimenez-Diaz IA, Quijano L (2007) Antigiardial activity of triterpenoids from root bark of *Hippocratea excelsa*. J Nat Prod 70:863
114. Aguilar-Gonzalez AR, Mena-Rejon GJ, Padilla-Montano N, Toscano A, Quijano L (2005) Triterpenoids from *Hippocratea excelsa*. The crystal structure of 29-hydroxytaraxerol. Z Naturforsch B 60:577
115. Calzada F, Mata R (1995) Hippocrateine III, a sesquiterpene alkaloid from *Hippocratea excelsa*. Phytochemistry 40:583
116. Furukawa M, Makino M, Uchiyama T, Ishimi K, Ichinohe Y, Fujimoto Y (2002) Sesquiterpene pyridine alkaloids from *Hippocratea excelsa*. Phytochemistry 59:767

117. Sanchez-Mendoza ME, Arrieta J, Navarrete A (2008) Role of prostaglandins, nitric oxide, sulfhydryls and capsaicin-sensitive neurons in gastroprotection of stigmasterol and β-sitosterol. Nat Prod Commun 3:505
118. Arrieta J, Sánchez-Mendoza ME, Castillo-Henkel C, Navarrete A (2006) Role of endogenous prostaglandins in gastroprotection of β-sitosterol and four derivative esters on ethanol-induced gastric mucosal lesions in rats. Proc West Pharmacol Soc 49:130
119. Alanís AD, Calzada F, Cervantes JA, Torres J, Ceballos GM (2005) Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 100:153
120. Velázquez C, Calzada F, Torres J, González F, Ceballos G (2006) Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico. J Ethnopharmacol 103:66
121. Araujo Leon JA, Ruiz Ciau DV, Coral Martinez TI, Cantillo Ciau ZO (2015) Comparative fingerprint analyses of extracts from the root bark of wild *Hippocratea excelsa* and "Cancerina" by high-performance liquid chromatography. J Sep Sci 38:3870
122. Castillo-Espana P, Monroy-Ortiz C, Arellano Garcia J, Estrada-Soto SE, Perea-Arango I (2015) Traditional medicinal plants used for the treatment pain and inflammation in Morelos (Mexico): an ethno-botanical survey. Nat Prod Chem Res 3:216
123. Perez-Vasquez A, Reyes A, Linares E, Bye R, Mata R (2005) Phytotoxins from *Hofmeisteria schaffneri*: isolation and synthesis of 2′-(2″-hydroxy-4″-methylphenyl)-2′-oxoethyl acetate. J Nat Prod 68:959
124. Angeles-Lopez G, Perez-Vasquez A, Hernandez-Luis F, Deciga-Campos M, Bye R, Linares E, Mata R (2010) Antinociceptive effect of extracts and compounds from *Hofmeisteria schaffneri*. J Ethnopharmacol 131:425
125. Perez-Vasquez A, Capella S, Linares E, Bye R, Angeles-Lopez G, Mata R (2011) Antimicrobial activity and chemical composition of the essential oil of *Hofmeisteria schaffneri*. J Pharm Pharmacol 63:579
126. Pérez-Vásquez A, Ángeles-López G, Rivero-Cruz I, Flores-Bocanegra L, Linares E, Bye R, Mata R (2017) Spasmolytic action of preparations and compounds from *Hofmeisteria schaffneri*. Nat Prod Commun 12:475
127. Perez-Vasquez A, Linares E, Bye R, Cerda-Garcia-Rojas CM, Mata R (2008) Phytotoxic activity and conformational analysis of thymol analogs from *Hofmeisteria schaffneri*. Phytochemistry 69:1339
128. Villaseñor Ríos JL, Espinosa García FJ (1998) Catálogo de malezas de México, 1st edn. Universidad Nacional Autónoma de México, Consejo Nacional Consultivo Fitosanitario, Fondo de Cultura Económica, Mexico City
129. Argueta A, Cano Asseleih LM, Rodarte ME (1994) Atlas de las plantas de la medicina tradicional mexicana, tomo I-III, 1st edn. Instituto Nacional Indigenista, Mexico City
130. Missouri Botanical Garden (2018) http://www.missouribotanicalgarden.org/
131. Ximénez F (1615) Cuatro libros de la naturaleza y virtudes de las plantas y animales de uso medicinal en la Nueva España. Mexico City
132. Aguilar Contreras A, Xolalpa Molina S (2002) La herbolaria mexicana en el tratamiento de la diabetes. Ciencia 2002:24
133. Martínez M (1989) Las plantas medicinales de México, 6th edn. Botas, Mexico City
134. Osuna Torres L, Tapia-Pérez ME, Aguilar Contreras A (2005) Plantas medicinales de la medicina tradicional mexicana para tratar afecciones gastrointestinales. Estudio etnobotánico, fitoquímico y farmacológico. Edicions, Universitat de Barcelona, Spain
135. Sanchez-Viesca F, Romo J (1963) Estafiatin, a new sesquiterpene lactone isolated from *Artemisia mexicana* (Willd). Tetrahedron 19:1285
136. Viehoever A, Capen RG (1923) New sources of santonin. J Am Chem Soc 45:1941–1944
137. Lee KH, Geissman TA (1970) Sesquiterpene lactones of *Artemisia* constituents of *A. ludoviciana* ssp. *mexicana*. Phytochemistry 9:403
138. Geissman TA, Saitoh T (1972) Ludalbin, a new lactone from *Artemisia ludoviciana*. Phytochemistry 11:1157

139. Domínguez XA, Enrique Cárdenas G (1975) Achillin and deacetylmatricarin from two *Artemisia* species. Phytochemistry 14:2511
140. Epstein WW, Ubben Jenkins EE (1979) Anthemidin, a new sesquiterpene lactone from *Artemisia ludoviciana*. J Nat Prod 42:279
141. Ruiz-Cancino A, Cano AE, Delgado G (1993) Sesquiterpene lactones and flavonoids from *Artemisia ludoviciana* ssp. *mexicana*. Phytochemistry 33:1113
142. Liu YL, Mabry TJ (1982) Flavonoids from *Artemisia ludoviciana* var. *ludoviciana*. Phytochemistry 21:209
143. Mata R, Delgado G, Devivar AR (1984) Sesquiterpene lactones of *Artemisia mexicana* var. *angustifolia*. Phytochemistry 23:1665
144. Jakupovic J, Tan RX, Bohlmann F, Boldt PE, Jia ZJ (1991) Sesquiterpene lactones from *Artemisia ludoviciana*. Phytochemistry 30:1573
145. Bora KS, Sharma A (2011) The genus *Artemisia*: a comprehensive review. Pharm Biol 49:101
146. Anaya-Eugenio GD, Rivero-Cruz I, Rivera-Chavez J, Mata R (2014) Hypoglycemic properties of some preparations and compounds from *Artemisia ludoviciana* Nutt. J Ethnopharmacol 155:416
147. Hofer O, Wallnöfer B, Widhalm M, Greger H (1988) Naturally occurring thienyl-substituted spiroacetal enol ethers from *Artemisia ludoviciana*. Liebigs Ann Chem 1988:525
148. Abad MJ, Bedoya LM, Apaza L, Bermejo P (2012) The *Artemisia* L. genus: a review of bioactive essential oils. Molecules 17:2542
149. Bakkali F, Averbeck S, Averbeck D, Waomar M (2008) Biological effects of essential oils – a review. Food Chem Toxicol 46:446
150. Blust MH, Hopkins TL (1987) Gustatory responses of a specialist and a generalist grasshopper to terpenoids of *Artemisia ludoviciana*. Entomol Exp Appl 45:37
151. Blust MH, Hopkins TL (1987) Olfactory responses of a specialist and a generalist grasshopper to volatiles of *Artemisia ludoviciana* Nutt. (Asteraceae). J Chem Ecol 13:1893
152. Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP (2008) Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochemistry 69:1732
153. Manjarrez A, Medina F (1964) Analysis of volatile oils of leaves of *Artemisia mexicana* and *Artemisia klotzchiana*. Can J Chem 42:2085
154. Zavala-Sanchez MA, Perez-Gutierrez S, Perez-Gonzalez C, Sanchez-Saldivar D, Arias-Garcia L (2002) Antidiarrhoeal activity of nonanal, an aldehyde isolated from *Artemisia ludoviciana*. Pharm Biol 40:263
155. Anaya-Eugenio GD, Rivero-Cruz I, Bye R, Linares E, Mata R (2016) Antinociceptive activity of the essential oil from *Artemisia ludoviciana*. J Ethnopharmacol 179:403
156. Vahdati-Mashhadian N, Emami SA, Oghazian MB, Vosough R (2009) The cytotoxicity evaluation of seven species of *Artemisia* on human tumor cell lines. Pharmacologyonline 1:229
157. Jimenez-Arellanes A, Meckes M, Ramirez R, Torres J, Luna-Herrera J (2003) Activity against multidrug-resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory diseases. Phytother Res 17:903
158. Malagón F, Vázquez J, Delgado G, Ruiz A (1997) Antimalaric effect of an alcoholic extract of *Artemisia ludoviciana mexicana* in a rodent malaria model. Parasitologia 39:3
159. Garcia S, Alarcon G, Rodriguez C, Heredia N (2006) Extracts of *Acacia farnesiana* and *Artemisia ludoviciana* inhibit growth, enterotoxin production and adhesion of *Vibrio cholerae*. World J Microbiol Biotechnol 22:669
160. Calzada F, Yepez-Mulia L, Aguilar A (2006) In vitro susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 108:367
161. Fernandez SS, Guerra MCR, Cardenas BDM, Villarreal JV, Trevino LV (2005) In vitro antiprotozoal activity of the leaves of *Artemisia ludoviciana*. Fitoterapia 76:466

162. McCutcheon AR, Ellis SM, Hancock RE, Towers GH (1995) Antifungal screening of medicinal plants of British Columbian native peoples. J Ethnopharmacol 44:157
163. Calzada F, Arista R, Perez H (2010) Effect of plants used in Mexico to treat gastrointestinal disorders on charcoal-gum acacia-induced hyperperistalsis in rats. J Ethnopharmacol 128:49
164. Estrada-Soto S, Sanchez-Recillas A, Navarrete-Vazqueza G, Castillo-Espana P, Villalobos-Molina R, Ibarra-Barajas M (2012) Relaxant effects of *Artemisia ludoviciana* on isolated rat smooth muscle tissues. J Ethnopharmacol 139:513
165. Bork Peter M, Schmitz ML, Kuhnt M, Escher C, Heinrich M (1998) Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-κB. FEBS Lett 402:8
166. Rivero-Cruz I, Anaya-Eugenio G, Perez-Vasquez A, Martinez AL, Mata R (2017) Quantitative analysis and pharmacological effects of *Artemisia ludoviciana* aqueous extract and compounds. Nat Prod Commun 12:1531
167. Castro V, Murillo R, Klaas CA, Meunier C, Mora G, Pahl HL, Merfort I (2000) Inhibition of the transcription factor NF-κB by sesquiterpene lactones from *Podachaenium eminens*. Planta Med 66:591
168. Myeong SC, Yeong JK, Seon MJ (2009) Eupatilin and jaceosidin for preventing and treating diabetes mellitus. Korean Patent, Kongkae Taeho Kongbo, KR 2009066858, A 20090624
169. Al-Waili NSD (1986) Treatment of diabetes mellitus by *Artemisia herba alba* extract – preliminary study. Clin Exp Pharmacol Physiol 13:569
170. Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR (1989) Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice. Acta Diabetol Lat 26:51
171. Ribnicky DM, Poulev A, Watford M, Cefalu WT, Raskin I (2006) Antihyperglycemic activity of Tarralin, an ethanolic extract of *Artemisia dracunculus* L. Phytomedicine 13:550
172. Obanda DN, Hernandez A, Ribnicky D, Yu YM, Zhang XH, Wang ZQ, Cefalu WT (2012) Bioactives of *Artemisia dracunculus* L. mitigate the role of ceramides in attenuating insulin signaling in rat skeletal muscle cells. Diabetes 61:597
173. Jung UJ, Baek NI, Chung HG, Bang MH, Yoo JS, Jeong TS, Lee KT, Kang YJ, Lee MK, Kim HJ, Yeo JY, Choi MS (2007) The anti-diabetic effects of ethanol extract from two variants of *Artemisia princeps* Pampanini in C57BL/KsJ-db/db mice. Food Chem Toxicol 45:2022
174. Cho YY, Baek NI, Chung HG, Jeong TS, Lee KT, Jeon SM, Kim HJ, McGregor RA, Choi MS (2012) Randomized controlled trial of Sajabalssuk (*Artemisia princeps* Pampanini) to treat pre-diabetes. Eur J Integr Med 4:E299–E308
175. Pan J, Liu G, Liu H, Qiu Z, Chen L (1998) Effects of *Artemisia capillaris* on blood glucose and lipid in mice. Zhong Yao Cai 21:408
176. Nurul Islam M, Jung HA, Sohn HS, Kim HM, Choi JS (2013) Potent α-glucosidase and protein tyrosine phosphatase 1B inhibitors from *Artemisia capillaris*. Arch Pharmacal Res 36:542
177. Nofal SM, Mahmoud SS, Ramadan A, Soliman G, Abdel-Rahman R (2009) Anti-diabetic effect of *Artemisia judaica* extracts. Res J Med Med Sci 4:42
178. Ribnicky DM, Logendra S, Poulev A, Cefalu WT, Raskin I (2007) Compounds from an extract of *Artemisia* and methods for treating type II diabetes. US Patent, PCT Int. Appl. WO 2007109217 A2 20070927
179. Whittle BA, Skett PG (2002) *Artemisia judaica* fractionation method. US Patent, US6350478B1
180. Masao T, Yoshihiko N, Takashi T, Hiroshi H, Atsuko Y (2013) Adiponectin production promoting compositions containing aqueous extract of *Artemisia capillaris* and cosmetics, health food, and pharmaceuticals containing the compositions. Japanese Patent, Kokai Tokkyo Koho. JP 2013237658A 20131128
181. Gyeong MY, Sang OK, Bok NK, Jae HJ, Chung GN, Seon PN (2013) *Prunellae spica* and *Artemisia argyi* extracts for inhibiting protein tyrosine phosphatase. Korean Patent, Kongkae Taeho Kongbo. KR 2013055395A 20130528

182. Chao Q, Fang M, Liu X, Fan Y, Wang Y, Xu X (2011) Application of *Artemisia rupestris* preparation in medicine for treating diabetes. Chinese Patent, Faming Zhuanli Shenqing CN 102166243
183. Han S, Wang Z, Wang J, Chu J, Yu H, Wang L (2011) Process for manufacturing extract of *Artemisia annua* for controlling postprandial blood sugar. Chinese Patent, Faming Zhuanli Shenqing CN 101933965
184. Ximénez-García C (2002) Las parasitosis intestinales en México, vol 36. Cuadernos Funsalud, Fundación Mexicana para la Salud, A.C., Mexico City
185. Rodríguez Guzmán LM (2003) Sistema Nacional de Vigilancia Epidemiológica, Sistema Único Información. Mexico City
186. Quihui-Cota L, Valencia ME, Crompton DWT, Phillips S, Hagan P, Diaz-Camacho SP, Tejas AT (2004) Prevalence and intensity of intestinal parasitic infections in relation to nutritional status in Mexican schoolchildren. Trans R Soc Trop Med Hyg 98:653
187. Instituto Nacional de Estadística y Geografía (INEGI) (2017) Estadísticas a propósito del día de muertos. http://www.inegi.org.mx/
188. Githiori JB, Athanasiadou S, Thamsborg SM (2006) Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. Vet Parasitol 139:308
189. Monzote L, Nance MR, Garcia M, Scull R, Setzer WN (2011) Comparative chemical, cytotoxicity and antileishmanial properties of essential oils from *Chenopodium ambrosioides*. Nat Prod Commun 6:281
190. Mata R, Navarrete A, Alvarez L, Pereda Miranda R, Delgado G, De Vivar AR (1987) Chemical studies on Mexican plants used in traditional medicine I. Flavonoids and terpenoids of *Chenopodium graveolens*. Phytochemistry 26:191
191. Calzada F, Velazquez C, Cedillo-Rivera R, Esquivel B (2003) Antiprotozoal activity of the constituents of *Teloxys graveolens*. Phytother Res 17:731
192. Álvarez-Ospina H, Rivero Cruz I, Duarte G, Bye R, Mata R (2012) HPLC determination of the major active flavonoids and GC-MS analysis of volatile components of *Dysphania graveolens* (Amaranthaceae). Phytochem Anal 24:248
193. Deciga-Campos M, Mata R, Rivero-Cruz I (2017) Antinociceptive pharmacological profile of *Dysphania graveolens* in mouse. Biomed Pharmacother 89:933
194. Del Rayo Camacho M, Sanchez B, Quiroz H, Contreras JL, Mata R (1991) Pinocembrine: a bioactive flavanone from *Teloxys graveolens*. J Ethnopharmacol 31:383
195. Rojas A, Hernandez L, Pereda Miranda R, Mata R (1992) Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. J Ethnopharmacol 35:275
196. Meckes M, Paz D, Acosta J, Mata R (1998) The effects of chrysin and pinostrobin, two flavonoids isolated from *Teloxys graveolens* leaves, on isolated guinea-pig ileum. Phytomedicine 5:459
197. Palomares-Alonso F, Rojas-Tome IS, Rocha VJ, Hernandez GP, Gonzalez-Maciel A, Ramos-Morales A, Santiago-Reyes R, Gonzalez-Hernandez IE, Jung-Cook H (2015) Cysticidal activity of extracts and isolated compounds from *Teloxys graveolens*: in vitro and in vivo studies. Exp Parasitol 156:79
198. Calzada F, Meckes M, Cedillo-Rivera R, Tapia-Contreras A, Mata R (1998) Screening of Mexican medicinal plants for antiprotozoal activity. Pharm Biol 36:305
199. Calzada F, Cerda-Garcia-Rojas CM, Meckes M, Cedillo-Rivera R, Bye R, Mata R (1999) Geranins A and B, new antiprotozoal A-type proanthocyanidins from *Geranium niveum*. J Nat Prod 62:705
200. Calzada F, Cedillo-Rivera R, Bye R, Mata R (2001) Geranins C and D, additional new antiprotozoal A-type proanthocyanidins from *Geranium niveum*. Planta Med 67:677
201. Maldonado PD, Rivero-Cruz I, Mata R, Pedraza-Chaverrí J (2005) Antioxidant activity of A-type proanthocyanidins from *Geranium niveum* (Geraniaceae). J Agric Food Chem 53:1996

202. Mohar-Betancourt A, Reynoso-Noverón N, Armas-Texta D, Gutiérrez-Delgado C, Torres-Domínguez JA (2017) Cancer trends in Mexico: essential data for the creation and follow-up of public policies. J Glob Oncol 3:740
203. Alonso-Castro AJ, Villarreal ML, Salazar-Olivo LA, Gomez-Sanchez M, Dominguez F, Garcia-Carranca A (2011) Mexican medicinal plants used for cancer treatment: pharmacological, phytochemical and ethnobotanical studies. J Ethnopharmacol 133:945
204. Alonso-Castro AJ, Juarez-Vazquez MC, Campos-Xolalpa N (2016) Medicinal plants from Mexico, Central America, and the Caribbean used as immunostimulants. Evid-Based Complement Altern Med 2016:Article ID 4017676
205. Jacobo-Herrera NJ, Jacobo-Herrera FE, Zentella-Dehesa A, Andrade-Cetto A, Heinrich M, Pérez-Plasencia C (2016) Medicinal plants used in Mexican traditional medicine for the treatment of colorectal cancer. J Ethnopharmacol 179:391
206. Liaw CC, Wu TY, Chang FR, Wu YC (2010) Historic perspectives on Annonaceous acetogenins from the chemical bench to preclinical trials. Planta Med 76:1390
207. Liaw C-C, Liou J-R, Wu T-Y, Chang F-R, Wu Y-C (2016) Acetogenins from Annonaceae. In: Kinghorn AD, Falk H, Gibbons S, Kobayashi J (eds) Progress in the chemistry of organic natural products, vol 101. Springer International Publishing, Cham, Switzerland, p 113
208. Lúcio ASSC, Almeida JRGS, da-Cunha EVL, Tavares JF, Barbosa Filho JM (2015) Alkaloids of the Annonaceae: occurrence and a compilation of their biological activities. In: Knölker H-J (ed) The alkaloids: chemistry and biology, vol 74. Academic Press, Cambridge, MA, p 233
209. Mata R, Rivero-Cruz JF, Chávez D (2000) Bioactive secondary metabolites from selected Mexican medicinal plants: recent progress. In: Corrado T (ed) Bioactive compounds from natural sources: isolation, characterization and biological properties, 1st edn. CRC Press, Boca Raton, FL, p 129
210. Romero-Soler K, Cetzal-Ix W (2015) Las especies del género *Annona* (Annonaceae) cultivadas de la Península de Yucatán, México. Herbario CICY 7:147
211. Chen YY, Chang FR, Wu YC (1996) Isoquinoline alkaloids and lignans from *Rollinia mucosa*. J Nat Prod 59:904
212. Kuo RY, Chang FR, Wu YC (2001) A new propentdyopent derivative, rollipyrrole, from *Rollinia mucosa* Baill. Tetrahedron Lett 42:7907
213. Pino JA (2000) Volatile components of *Rollinia mucosa* (Jacq.) Baill. J Essent Oil Res 12:97
214. Chavez D, Acevedo LA, Mata R (1998) Jimenezin, a novel annonaceous acetogenin from the seeds of *Rollinia mucosa* containing adjacent tetrahydrofuran-tetrahydropyran ring systems. J Nat Prod 61:419
215. Chavez D, Acevedo LA, Mata R (1999) Tryptamine derived amides and acetogenins from the seeds of *Rollinia mucosa*. J Nat Prod 62:1119
216. Takahashi S, Maeda K, Hirota S, Nakata T (1999) Total synthesis of a new cytotoxic acetogenin, jimenezin, and the revised structure. Org Lett 1:2025
217. Hwang CH, Keum G, Sohn KI, Lee DH, Lee E (2005) Stereoselective synthesis of (-)-jimenezin. Tetrahedron Lett 46:6621
218. Bandur NG, Brueckner D, Hoffmann RW, Koert U (2006) Total synthesis of jimenezin via an intramolecular allylboration. Org Lett 8:3829
219. Estrada-Reyes R, Lopez-Rubalcava C, Rocha L, Heinze G, Esquinca ARG, Martinez-Vazquez M (2010) Anxiolytic-like and sedative actions of *Rollinia mucosa*: possible involvement of the GABA/benzodiazepine receptor complex. Pharm Biol 48:70
220. Estrada-Reyes R, Alvarez AL, Lopez-Rubalcava C, Rocha L, Heinze G, Moreno J, Martinez-Vazquez M (2002) Lignans from leaves of *Rollinia mucosa*. Z Naturforsch C 57:29
221. Cepleanu F, Ohtani K, Hamburger M, Hostettmann K, Gupta MP, Solis P (1993) Novel acetogenins from the leaves of *Annona purpurea*. Helv Chim Acta 76:1379
222. Sonnet PE, Jacobson M (1971) Tumor inhibitors II: cytotoxic alkaloids from *Annona purpurea*. J Pharm Sci 60:1254
223. Chang F-R, Chen C-Y, Wu P-H, Kuo R-Y, Chang Y-C, Wu Y-C (2000) New alkaloids from *Annona purpurea*. J Nat Prod 63:746

224. Chang F-R, Wei J-L, Teng C-M, Wu Y-C (1998) Two new 7-dehydroaporphine alkaloids and antiplatelet action aporphines from the leaves of *Annona purpurea*. Phytochemistry 49:2015
225. Chang F-R, Wei J-L, Teng C-M, Wu Y-C (1998) Antiplatelet aggregation constituents from *Annona purpurea*. J Nat Prod 61:1457
226. Chavez D, Mata R (1998) Purpurediolin and purpurenin, two new cytotoxic adjacent bis-tetrahydrofuran annonaceous acetogenins from the seeds of *Annona purpurea*. J Nat Prod 61:580
227. Chavez D, Mata R (1999) Purpuracenin: a new cytotoxic adjacent bis-tetrahydrofuran annonaceous acetogenin from the seeds of *Annona purpurea*. Phytochemistry 50:823–828
228. Rejón-Orantes JC, González-Esquinca AR, Pérez de la Mora M, Roldan Roldan G, Cortes D (2011) Annomontine, an alkaloid isolated from *Annona purpurea*, has anxiolytic-like effects in the elevated plus-maze. Planta Med 77:322
229. González-Tepale MR, Reyes L, Mayorga-Flores M, Reyes-Trejo B, Gómez-Zepeda D, del Rio Portilla F, Ordaz-Ortiz JJ, Herbert-Pucheta JE (2018) Cyclopurpuracin, a cyclopeptide from *Annona purpurea* seeds. Phytochem Lett 23:164
230. Chávez D, Mata R, Iglesias-Prieto R, Lotina-Hennsen B (2001) Annonaceous acetogenins: naturally occurring inhibitors of ATP synthesis and photosystem II in spinach chloroplasts. Physiol Plant 111:262
231. Márquez-Romero JM, Chiquete E (2013) Frequency of insomnia and daytime consequences in Mexican patients: insights from the EQUINOX study. Rev Mex Neuroci 14:314–320
232. Jimenez-Genchi A, Caraveo-Anduaga J (2017) Crude and adjusted prevalence of sleep complaints in Mexico City. Sleep Sci 10:113
233. Milian MJ, Goodwin GM, Meyer-Lindenberg A, Ogren SO (2015) Learning from the past and looking to the future: emerging perspectives for improving the treatment of psychiatric disorders. Eur Neuropsychopharmacol 25:599
234. World-Health-Organization (2017) Depression and other common mental disorders: global health estimates. World Health Organization, Geneva
235. González-Trujano ME, Lopez-Meraz ML, Navarrete A, Martinez A (2015) Preclinical studies of three Mexican plants used in folk medicine to treat epilepsy: a short review. Rev Latinoam Quim 43:31
236. Gutierrez SLG, Chilpa RR, Jaime HB (2014) Medicinal plants for the treatment of "nervios", anxiety, and depression in Mexican traditional medicine. Rev Bras Farmacogn 24:591
237. Lopez-Rubalcava C, Estrada-Camarena E (2016) Mexican medicinal plants with anxiolytic or antidepressant activity: focus on preclinical research. J Ethnopharmacol 186:377
238. Navarrete A, Avula B, Choi YW, Khan IA (2006) Chemical fingerprinting of *Valeriana* species: simultaneous determination of valerenic acids, flavonoids, and phenylpropanoids using liquid chromatography with ultraviolet detection. J AOAC Int 89:8
239. Meyer FG (1951) *Valeriana* in North America and the West Indies (Valerianaceae). Ann Mo Bot Gard 38:377
240. Dweck AC (1997) An introduction to Valerian (*Valeriana officinalis*) and related species. In: Houghton P (ed) Valerian: the genus *Valeriana*. Harwood Academic Publishers, Amsterdam, p 1
241. Nandhini S, Narayanan KB, Ilango K (2018) *Valeriana officinalis*: a review of its traditional uses, phytochemistry and pharmacology. Asian J Pharm Clin Res 11:36
242. Joshi VC, Navarrete A, Khan IA (2005) Authentication of *Valeriana procera* Kunth and comparative account of five *Valeriana* species. J AOAC Int 88:1621
243. Bos R, Hendriks H, Scheffer JJC, Woerdenbag HJ (1998) Cytotoxic potential of valerian constituents and valerian tinctures. Phytomedicine 5:219
244. Herrera-Arellano A, Luna-Villegas G, Cuevas-Uriostegui ML, Alvarez L, Vargas-Pineda G, Zamilpa-Alvarez A, Tortoriello J (2001) Polysomnographic evaluation of the hypnotic effect of *Valeriana edulis* standardized extract in patients suffering from insomnia. Planta Med 67:695

245. Castillo P, Marquez J, Rubluo A, Hernandez G, Lara M (2000) Plant regeneration from callus and suspension cultures of *Valeriana edulis* ssp. *procera* via simultaneous organogenesis and somatic embryogenesis. Plant Sci 151:115
246. Castillo P, Zamilpa A, Marquez J, Hernandez G, Lara M, Alvarez L (2002) Comparative study of differentiation levels and valepotriate content of in vitro cultures and regenerated and wild plants of *Valeriana edulis* ssp. *procera*. J Nat Prod 65:573
247. Alfaro-Romero A, Balderas-López JL, Duarte-Lisci G, Navarrete A (2016) Root scent composition in *Valeriana officinalis* and *Valeriana edulis* ssp. *procera* analyzed by HS-SPME-GC-MS. J Essent Oil-Bear Plants 19:1821
248. Oliva I, González-Trujano ME, Arrieta J, Enciso-Rodríguez R, Navarrete A (2004) Neuropharmacological profile of hydroalcohol extract of *Valeriana edulis* ssp. *procera* roots in mice. Phytother Res 18:290
249. Ugalde M, Reza V, Gonzalez-Trujano ME, Avula B, Khan IA, Navarrete A (2005) Isobolographic analysis of the sedative interaction between six central nervous system depressant drugs and *Valeriana edulis* hydroalcoholic extract in mice. J Pharm Pharmacol 57:631
250. Francis AJP, Dempster RJW (2002) Effect of valerian, *Valeriana edulis*, on sleep difficulties in children with intellectual deficits: randomised trial. Phytomedicine 9:273–279
251. Estrada-Soto S, Rivera-Leyva J, Ramirez-Espinosa JJ, Castillo-Espana P, Aguirre-Crespo F, Hernandez-Abreu O (2010) Vasorelaxant effect of *Valeriana edulis* ssp. *procera* (Valerianaceae) and its mode of action as calcium channel blocker. J Pharm Pharmacol 62:1167
252. Enciso Rodriguez R (1997) Micropropagation of *Valeriana edulis* ssp. *procera*. Planta Med 63:274
253. Global Asthma Report (2014) Global Asthma Network, 2014. http://www.globalasthmareport.org/
254. Waizel Haiat S, Waizel Bucay J (2009) Algunas plantas utilizadas en México para el tratamiento del asma. An Orl Mex 54:145
255. Zheng X, Wang W, Piao HS, Xu WQ, Shi HB, Zhao CG (2013) The genus *Gnaphalium* L. (Compositae): phytochemical and pharmacological characteristics. Molecules 18:8298
256. Anderberg A (1991) Taxonomy and phylogeny of the tribe Gnaphalieae (Asteraceae). Opera Bot 104:1
257. Hinojosa-Espinosa O, Villaseñor JL (2014) New combinations in *Pseudognaphalium* (Gnaphalieae-Asteraceae) of Mexico. Bot Sci 92:489
258. Espinosa-García FJ (1987) Nota sobre la identidad del segundo Tzonpotónic mencionado por Francisco Hernández en su *Historia Natural de la Nueva España*. Bol Soc Bot Mex 47:87
259. Rodriguez-Ramos F, Andrade-Moreno MA, Alfaro-Romero A, Balderas-Lopez JL, Navarrete A (2016) Gnaphaliin A and gnaphaliin B synergize the relaxant effect of salbutamol but not of ipratropium in guinea pig trachea. J Pharm Pharmacol 68:533
260. Villagomez-Ibarra JR, Sanchez M, Espejo O, Zuniga-Estrada A, Torres-Valencia JM, Joseph-Nathan P (2001) Antimicrobial activity of three Mexican *Gnaphalium* species. Fitoterapia 72:692
261. Sanchez-Mendoza ME, Torres G, Arrieta J, Aguilar A, Castillo-Henkel C, Navarrete A (2007) Mechanisms of relaxant action of a crude hexane extract of *Gnaphalium liebmannii* in guinea pig tracheal smooth muscle. J Ethnopharmacol 111:142
262. Rodriguez-Ramos F, Gonzalez-Andrade M, Navarrete A (2011) Gnaphaliin A and B relax smooth muscle of guinea-pig trachea and rat aorta via phosphodiesterase inhibition. J Pharm Pharmacol 63:926
263. Rodriguez-Ramos F, Navarrete A (2009) Solving the confusion of gnaphaliin structure: gnaphaliin A and gnaphaliin B identified as active principles of *Gnaphalium liebmannii* with tracheal smooth muscle relaxant properties. J Nat Prod 72:1061
264. Rodriguez-Ramos F, Sánchez-Estrada V, Alfaro-Romero A, Tapia-Alvarez G, Navarrete A (2011) Development and validation of a column high-performance liquid chromatography

method for quantification of ganaphaliin A and B in inflorescences of *Gnaphalium liebmannii* Sch. Bip. ex Klatt. J AOAC Int 9:1076
265. Macia MJ, Garcia E, Vidaurre PJ (2005) An ethnobotanical survey of medicinal plants commercialized in the markets of La Paz and El Alto, Bolivia. J Ethnopharmacol 97:337
266. Thomas E, Vandebroek I, Sanca S, Van Damme P (2009) Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. J Ethnopharmacol 122:60
267. Campos-Bedolla P, Montano LM, Flores-Soto E, Aguilar A, Puebla AM, Lozoya X, Vargas MH (2005) Effect of *Gnaphalium conoideum* H.B.K. on guinea pig airway smooth muscle: role of L-type Ca^{2+} channels. J Ethnopharmacol 97:267
268. Chavez-Silva F, Ceron-Romero L, Arias-Duran L, Navarrete-Vazquez G, Almanza-Perez J, Roman-Ramos R, Ramirez-Avila G, Perea-Arango I, Villalobos-Molina R, Estrada-Soto S (2018) Antidiabetic effect of *Achillea millefollium* through multitarget interactions: α-glucosidases inhibition, insulin sensitization and insulin secretagogue activities. J Ethnopharmacol 212:1
269. Andrade-Cetto A, Wiedenfeld H (2004) Hypoglycemic effect of *Acosmium panamense* bark on streptozotocin diabetic rats. J Ethnopharmacol 90:217
270. Alarcon-Aguilar FJ, Roman-Ramos R, Jimenez-Estrada M, Reyes-Chilpa R, Gonzalez-Paredes B, Flores-Saenz JL (1997) Effects of three Mexican medicinal plants (Asteraceae) on blood glucose levels in healthy mice and rabbits. J Ethnopharmacol 55:171
271. Martinez AL, Madariaga-Mazon A, Rivero-Cruz I, Bye R, Mata R (2017) Antidiabetic and antihyperalgesic effects of a decoction and compounds from *Acourtia thurberi*. Planta Med 83:534
272. Bustos-Brito C, Andrade-Cetto A, Giraldo-Aguirre JD, Moreno-Vargas AD, Quijano L (2016) Acute hypoglycemic effect and phytochemical composition of *Ageratina petiolaris*. J Ethnopharmacol 185:341
273. Calzada F, Solares-Pascasio JI, Ordonez-Razo RM, Velazquez C, Barbosa E, Garcia-Hernandez N, Mendez-Luna D, Correa-Basurto J (2017) Antihyperglycemic activity of the leaves from *Annona cherimola* Miller and rutin on alloxan-induced diabetic rats. Pharmacogn Res 9:1
274. Juarez-Reyes K, Brindis F, Medina-Campos ON, Pedraza-Chaverri J, Bye R, Linares E, Mata R (2015) Hypoglycemic, antihyperglycemic, and antioxidant effects of the edible plant *Anoda cristata*. J Ethnopharmacol 161:36
275. Andrade-Cetto A, Medina-Hernandez AE (2013) Hypoglycemic effect of *Bromelia plumieri* (E. Morren) L.B. Sm. leaves in STZ-NA-induced diabetic rats. Front Pharmacol 4:36
276. Escandon-Rivera S, Gonzalez-Andrade M, Bye R, Linares E, Navarrete A, Mata R (2012) α-Glucosidase inhibitors from *Brickellia cavanillesii*. J Nat Prod 75:968
277. Escandon-Rivera S, Perez-Vasquez A, Navarrete A, Hernandez M, Linares E, Bye R, Mata R (2017) Anti-hyperglycemic activity of major compounds from *Calea ternifolia*. Molecules 22: E289
278. Andrade-Cetto A, Wiedenfeld H (2001) Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. J Ethnopharmacol 78:145
279. Revilla-Monsalve MC, Andrade-Cetto A, Palomino-Garibay MA, Wiedenfeld H, Islas-Andrade S (2007) Hypoglycemic effect of *Cecropia obtusifolia* Bertol. aqueous extracts on type 2 diabetic patients. J Ethnopharmacol 111:636
280. Herrera-Arellano A, Aguilar-Santamaria L, Garcia-Hernandez B, Nicasio-Torres P, Tortoriello J (2004) Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. Phytomedicine 11:561
281. Andrade-Cetto A, Vazquez RC (2010) Gluconeogenesis inhibition and phytochemical composition of two *Cecropia* species. J Ethnopharmacol 130:93
282. Alonso-Castro AJ, Miranda-Torres AC, Gonzalez-Chavez MM, Salazar-Olivo LA (2008) *Cecropia obtusifolia* Bertol. and its active compound, chlorogenic acid, stimulate 2-NBDglucose uptake in both insulin-sensitive and insulin-resistant 3T3 adipocytes. J Ethnopharmacol 120:458

283. Sanchez-Salgado JC, Ortiz-Andrade RR, Aguirre-Crespo F, Vergara-Galicia J, Leon-Rivera I, Montes S, Villalobos-Molina R, Estrada-Soto S (2007) Hypoglycemic, vasorelaxant and hepatoprotective effects of *Cochlospermum vitifolium* (Willd.) Sprengel: a potential agent for the treatment of metabolic syndrome. J Ethnopharmacol 109:400
284. Singh AK, Raj V, Keshari AK, Rai A, Kumar P, Rawat A, Maity B, Kumar D, Prakash A, De A, Samanta A, Bhattacharya B, Saha S (2018) Isolated mangiferin and naringenin exert antidiabetic effect via PPAR(gamma)/GLUT4 dual agonistic action with strong metabolic regulation. Chem-Biol Interact 280:33
285. Hossain MK, Dayem AA, Han J, Yin Y, Kim K, Saha SK, Yang GM, Choi HY, Cho SG (2016) Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. Int J Mol Sci 17:569
286. Brindis F, Gonzalez-Andrade M, Gonzalez-Trujano ME, Estrada-Soto S, Villalobos-Molina R (2014) Postprandial glycaemia and inhibition of α-glucosidase activity by aqueous extract from *Coriandrum sativum*. Nat Prod Res 28:2021
287. Andrade-Cetto AA, Wiedenfeld H, Revilla MC, Sergio IA (2000) Hypoglycemic effect of *Equisetum myriochaetum* aerial parts on streptozotocin diabetic rats. J Ethnopharmacol 72:129
288. Wiedenfeld H, Andrade-Cetto AA, Amador CP (2000) Flavonol glycosides from *Equisetum myriochaetum*. Biochem Syst Ecol 28:395
289. Alarcon-Aguilar FJ, Roman-Ramos R, Perez-Gutierrez S, Aguilar-Contreras A, Contreras-Weber CC, Flores-Saenz JL (1998) Study of the anti-hyperglycemic effect of plants used as antidiabetics. J Ethnopharmacol 61:101
290. Alonso-Castro AJ, Salazar-Olivo LA (2008) The anti-diabetic properties of *Guazuma ulmifolia* Lam. are mediated by the stimulation of glucose uptake in normal and diabetic adipocytes without inducing adipogenesis. J Ethnopharmacol 118:252
291. Alarcon-Aguilar FJ, Calzada-Bermejo R, Hernandez-Galicia E, Ruiz-Angeles C, Roman-Ramos R (2005) Acute and chronic hypoglycernic effect of *Ibervillea sonorae* root extracts-II. J Ethnopharmacol 97:447
292. Zapata-Bustos R, Alonso-Castro AJ, Gomez-Sanchez M, Salazar-Olivo LA (2014) *Ibervillea sonorae* (Cucurbitaceae) induces the glucose uptake in human adipocytes by activating a PI3K-independent pathway. J Ethnopharmacol 152:546
293. Hernandez-Galicia E, Calzada F, Roman-Ramos R, Alarcon-Aguilar FJ (2007) Monoglycerides and fatty acids from *Ibervillea sonorae* root: isolation and hypoglycemic activity. Planta Med 73:236
294. Ortiz-Andrade R, Cabanas-Wuan A, Arana-Argaez VE, Alonso-Castro AJ, Zapata-Bustos R, Salazar-Olivo LA, Dominguez F, Chavez M, Carranza-Alvarez C, Garcia-Carranca A (2012) Antidiabetic effects of *Justicia spicigera* Schltdl. (Acanthaceae). J Ethnopharmacol 143:455
295. Del Vecchyo-Tenorio G, Rodriguez-Cruz M, Andrade-Cetto A, Cardenas-Vazquez R (2016) Creosote bush (*Larrea tridentata*) improves insulin sensitivity and reduces plasma and hepatic lipids in hamsters fed a high fat and cholesterol diet. Front Pharmacol 7:194
296. Andrade-Cetto A (2011) Inhibition of gluconeogenesis by *Malmea depressa* root. J Ethnopharmacol 137:930
297. Andrade-Cetto A (2013) Hypoglycemic effect of *Malmea depressa*, a plant used in the treatment of type 2 diabetes in Yucatan, Mexico. Planta Med 79:1140
298. Diaz MDS, Barba de la Rosa AP, Helies-Toussaint C, Gueraud F, Negre-Salvayre A (2017) *Opuntia* spp.: characterization and benefits in chronic diseases. Oxid Med Cell Longev 2017: Article ID 8634249
299. Lopez-Romero P, Pichardo-Ontiveros E, Avila-Nava A, Vazquez-Manjarrez N, Tovar AR, Pedraza-Chaverri J, Torres N (2014) The effect of nopal (*Opuntia ficus-indica*) on postprandial blood glucose, incretins, and antioxidant activity in Mexican patients with type 2 diabetes after consumption of two different composition breakfasts. J Acad Nutr Diet 114:1811
300. Butterweck V, Semlin L, Feistel B, Pischel I, Bauer K, Verspohl EJ (2011) Comparative evaluation of two different *Opuntia ficus-indica* extracts for blood sugar lowering effects in rats. Phytother Res 25:370

301. Leem KH, Kim MG, Hahm YT, Kim HK (2016) Hypoglycemic effect of *Opuntia ficus-indica* var. *saboten* is due to enhanced peripheral glucose uptake through activation of aMPK/p38 MAPK pathway. Nutrients 8:800
302. Frati-Munari AC, Gordillo BE, Altamirano P, Ariza CR (1988) Hypoglycemic effect of *Opuntia streptacantha* Lemaire in NIDDM. Diabetes Care 11:63
303. Andrade-Cetto A, Wiedenfeld H (2011) Anti-hyperglycemic effect of *Opuntia streptacantha* Lem. J Ethnopharmacol 133:940
304. Alarcon-Aguilar FJ, Valdes-Arzate A, Xolalpa-Molina S, Banderas-Dorantes T, Jimenez-Estrada M, Hernandez-Galicia E, Roman-Ramos R (2003) Hypoglycemic activity of two polysaccharides isolated from *Opuntia ficus-indica* and *O. streptacantha*. Proc West Pharmacol Soc 46:139
305. Becerra-Jimenez J, Andrade-Cetto A (2012) Effect of *Opuntia streptacantha* Lem. on α-glucosidase activity. J Ethnopharmacol 139:493
306. Andrade-Cetto A, Mares MLR (2012) Hypoglycemic effect of the *Rhizophora mangle* cortex on STZ-NA-induced diabetic rats. Pharmacologyonline 3:1
307. Andrade-Cetto A, Escandon-Rivera SM, Torres-Valle GM, Quijano L (2017) Phytochemical composition and chronic hypoglycemic effect of *Rhizophora mangle* cortex on STZ-NA-induced diabetic rats. Rev Bras Farmacogn 27:744–750
308. Andrade-Cetto A (2011) Hypoglycemic effect of *Smilax moranensis* root on N5-STZ diabetic rats. Pharmacologyonline 1:111
309. Perez RM, Ocegueda A, Munoz JL, Avila JG, Morrow WW (1984) A study of the hypoglycemic effect of some Mexican plants. J Ethnopharmacol 12:253
310. Aguilar-Santamaria L, Ramirez G, Nicasio P, Alegria-Reyes C, Herrera-Arellano A (2009) Antidiabetic activities of *Tecoma stans* (L.) Juss. ex Kunth. J Ethnopharmacol 124:284
311. Aguilar LC, Masias S, Chagoya A, Cardenas A, Diaz P, Cantu JM (1993) Antidiabetic activity of *Tecoma stans* in rats. Fitoterapia 64:304
312. Ortiz-Andrade RR, Rodriguez-Lopez V, Garduno-Ramirez ML, Castillo-Espana P, Estrada-Soto S (2005) Anti-diabetic effect on alloxanized and normoglycemic rats and some pharmacological evaluations of *Tournefortia hartwegiana*. J Ethnopharmacol 101:37
313. Ortiz-Andrade RR, Garcia-Jimenez S, Castillo-Espana P, Ramirez-Avila G, Villalobos-Molina R, Estrada-Soto S (2007) α-Glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an anti-hyperglycemic agent. J Ethnopharmacol 109:48
314. Andrade-Cetto A, Revilla-Monsalve C, Wiedenfeld H (2007) Hypoglycemic effect of *Tournefortia hirsutissima* L., on n-streptozotocin diabetic rats. J Ethnopharmacol 112:96
315. Astudillo A, Hong E, Bye R, Navarrete A (2004) Antispasmodic activity of extracts and compounds of *Acalypha phleoides* Cav. Phytother Res 18:102
316. Hernández-Abreu O, Castillo-España P, León-Rivera I, Ibarra-Barajas M, Villalobos-Molina-R, González-Christen J, Vergara-Galicia J, Estrada-Soto S (2009) Antihypertensive and vasorelaxant effects of tilianin isolated from *Agastache mexicana* are mediated by NO/cGMP pathway and potassium channel opening. Biochem Pharmacol 78:54
317. Flores-Flores A, Hernandez-Abreu O, Rios MY, Leon-Rivera I, Aguilar-Guadarrama B, Castillo-Espana P, Perea-Arango I, Estrada-Soto S (2016) Vasorelaxant mode of action of dichloromethane-soluble extract from *Agastache mexicana* and its main bioactive compounds. Pharm Biol 54:2807
318. Navarrete A, Avila-Rosas N, Majin-Leon M, Balderas-Lopez JL, Alfaro-Romero A, Tavares-Carvalho JC (2017) Mechanism of action of relaxant effect of *Agastache mexicana* ssp. *mexicana* essential oil in guinea-pig trachea smooth muscle. Pharm Biol 55:96
319. Figueroa M, Rivero-Cruz I, Rivero-Cruz B, Bye R, Navarrete A, Mata R (2007) Constituents, biological activities and quality control parameters of the crude extract and essential oil from *Arracacia tolucensis* var. *multifida*. J Ethnopharmacol 113:125
320. Aguirre-Crespo F, Castillo-Espana P, Villalobos-Molina R, Lopez-Guerrero JJ, Estrada-Soto S (2005) Vasorelaxant effect of Mexican medicinal plants on isolated rat aorta. Pharm Biol 43:540

321. Ponce-Monter H, Perez S, Zavala MA, Perez C, Meckes M, Macias A, Campos M (2006) Relaxant effect of xanthomicrol and 3α-angeloyloxy-2α-hydroxy-13,14*Z*-dehydrocativic acid from *Brickellia paniculata* on rat uterus. Biol Pharm Bull 29:1501
322. Meckes M, Calzada F, Paz D, Rodriguez J, Ponce-Monter H (2002) Inhibitory effect of xanthomicrol and 3α-angeloyloxy-2α-hydroxy-13,(14*Z*)-dehydrocativic acid from *Brickellia paniculata* on the contractility of guinea-pig ileum. Planta Med 68:467
323. Froldi G, Bertin R, Secchi E, Zagotto G, Martinez-Vazquez M, Garcia-Argaez A (2011) Vasorelaxation by extracts of *Casimiroa* spp. in rat resistance vessels and pharmacological study of cellular mechanisms. J Ethnopharmacol 134:637
324. Ibarra-Alvarado C, Rojas A, Mendoza S, Bah M, Gutierrez DM, Hernandez-Sandoval L, Martinez M (2010) Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. Pharm Biol 48:732
325. Sanchez-Salgado JC, Castillo-Espana P, Ibarra-Barajas M, Villalobos-Molina R, Estrada-Soto S (2010) *Cochlospermum vitifolium* induces vasorelaxant and antihypertensive effects mainly by activation of NO/cGMP signaling pathway. J Ethnopharmacol 130:477
326. Mata R, Rojas A, Acevedo L, Estrada S, Calzada F, Rojas I, Bye R, Linares E (1997) Smooth muscle relaxing flavonoids and terpenoids from *Conyza filaginoides*. Planta Med 63:31
327. Rojas A, Cruz S, Ponce-Monter H, Mata R (1996) Smooth muscle relaxing compounds from *Dodonaea viscosa*. Planta Med 62:154
328. Mata R, Contreras JL, Crisanto D, Peredmiranda R, Castaneda P, Delrio F (1991) Chemical studies on Mexican plants used in traditional medicine, XVIII. New secondary metabolites from *Dodonaea viscosa*. J Nat Prod 54:913
329. Perusquia M, Mendoza S, Bye R, Linares E, Mata R (1995) Vasoactive effects of aqueous extracts from five Mexican medicinal plants on isolated rat aorta. J Ethnopharmacol 46:63
330. Gonzalez-Cortazar M, Tortoriello J, Alvarez L (2005) Norsecofriedelanes as spasmolytics, advances of structure-activity relationships. Planta Med 71:711
331. Vergara-Galicia J, Ortiz-Andrade R, Rivera-Leyva J, Castillo-España P, Villalobos-Molina R, Ibarra-Barajas M, Gallardo-Ortiz I, Estrada-Soto S (2010) Vasorelaxant and antihypertensive effects of methanolic extract from roots of *Laelia anceps* are mediated by calcium-channel antagonism. Fitoterapia 81:350
332. Vergara-Galicia J, Castillo-España P, Villalobos-Molina R, Estrada-Soto S (2013) Vasorelaxant effect of *Laelia speciosa* and *Laelia anceps*: two orchids as potential sources for the isolation of bioactive molecules. J Appl Pharm Sci 3:34
333. Vergara-Galicia J, Ortiz-Andrade R, Castillo-Espana P, Ibarra-Barajas M, Gallardo-Ortiz I, Villalobos-Molina R, Estrada-Soto S (2008) Antihypertensive and vasorelaxant activities of *Laelia autumnalis* are mainly through calcium channel blockade. Vasc Pharmacol 49:26
334. Perez-Hernandez N, Ponce-Monter H, Medina JA, Joseph-Nathan P (2008) Spasmolytic effect of constituents from *Lepechinia caulescens* on rat uterus. J Ethnopharmacol 115:30
335. Rivero-Cruz I, Duarte G, Navarrete A, Bye R, Linares E, Mata R (2011) Chemical composition and antimicrobial and spasmolytic properties of *Poliomintha longiflora* and *Lippia graveolens* essential oils. J Food Sci 76:C309
336. Avila-Villarreal G, Hernandez-Abreu O, Hidalgo-Figueroa S, Navarrete-Vazquez G, Escalante-Erosa F, Pena-Rodriguez LM, Villalobos-Molina R, Estrada-Soto S (2013) Antihypertensive and vasorelaxant effects of dihydrospinochalcone-A isolated from *Lonchocarpus xuul* Lundell by NO production: computational and ex vivo approaches. Phytomedicine 20:1241
337. Rios MY, Lopez-Martinez S, Lopez-Vallejo F, Medina-Franco JL, Villalobos-Molina R, Ibarra-Barajas M, Navarrete-Vazquez G, Hidalgo-Figueroa S, Hernandez-Abreu O, Estrada-Soto S (2012) Vasorelaxant activity of some structurally related triterpenic acids from *Phoradendron reichenbachianum* (Viscaceae) mainly by NO production: ex vivo and in silico studies. Fitoterapia 83:1023
338. Lombardo-Earl G, Roman-Ramos R, Zamilpa A, Herrera-Ruiz M, Rosas-Salgado G, Tortoriello J, Jimenez-Ferrer E (2014) Extracts and fractions from edible roots of *Sechium*

edule (Jacq.) Sw. with antihypertensive activity. Evid-Based Complement Alternat Med 2014: Article ID 594326
339. Trejo-Moreno C, Castro-Martínez G, Méndez-Martínez M, Jiménez-Ferrer JE, Pedraza-Chaverri J, Arrellín G, Zamilpa A, Medina-Campos ON, Lombardo-Earl G, Barrita-Cruz GJ, Hernández B, Ramírez CC, Santana MA, Fragoso G, Rosas G (2018) Acetone fraction from *Sechium edule* (Jacq.) Sw. edible roots exhibits anti-endothelial dysfunction activity. J Ethnopharmacol 220:75
340. Sanchez-Mendoza ME, Rodriguez-Silverio J, Rivero-Cruz JF, Rocha-Gonzalez HI, Pineda-Farias JB, Arrieta J (2013) Antinociceptive effect and gastroprotective mechanisms of 3,5-diprenyl-4-hydroxyacetophenone from *Ageratina pichinchensis*. Fitoterapia 87:11
341. Navarrete A, Arrieta J, Terrones L, Abou-Gazar H, Calis I (2005) Gastroprotective effect of astragaloside IV: role of prostaglandins, sulfhydryls and nitric oxide. J Pharm Pharmacol 57:1059
342. Sanchez-Mendoza ME, Lopez-Lorenzo Y, Matus-Meza AS, Arrieta J (2018) Gastroprotective effect of calealactone B: lack of involvement of prostaglandins, nitric oxide and sulfhydryls. Drug Dev Res 79:11
343. Garcia-Martinez LE, Sanchez-Mendoza ME, Arrieta-Baez D, Cruz-Antonio L, Mejia-Barradas CM, Soto-Perulero C, Arrieta J (2016) Gastroprotection of 2,3-epoxyjuanislamin, isolated from *Calea urticifolia,* against ethanol-induced gastric lesions in Wistar rats. Int J Pharmacol 12:893
344. Reyes-Trejo B, Sanchez-Mendoza ME, Becerra-Garcia AA, Cedillo-Portugal E, Castillo-Henkel C, Arrieta J (2008) Bioassay-guided isolation of an anti-ulcer diterpenoid from *Croton reflexifolius*: role of nitric oxide, prostaglandins and sulfhydryls. J Pharm Pharmacol 60:931
345. Sanchez-Mendoza ME, Reyes-Trejo B, Sanchez-Gomez P, Rodriguez-Silverio J, Castillo-Henkel C, Cervantes-Cuevas H, Arrieta J (2010) Bioassay-guided isolation of an anti-ulcer chromene from *Eupatorium aschenbornianum*: role of nitric oxide, prostaglandins and sulfydryls. Fitoterapia 81:66
346. Vera-Arzave C, Antonio LC, Arrieta J, Cruz-Hernandez G, Velazquez-Mendez AM, Reyes-Ramirez A, Sanchez-Mendoza ME (2012) Gastroprotection of suaveolol, isolated from *Hyptis suaveolens*, against ethanol-induced gastric lesions in wistar rats: role of prostaglandins, nitric oxide and sulfhydryls. Molecules 17:8917
347. Sanchez-Mendoza ME, Cruz-Antonio L, Arrieta-Baez D, Olivares-Corichi IM, Rojas-Martinez R, Martinez-Cabrera D, Arrieta J (2015) Gastroprotective activity of methyleugenol from *Peperomia hispidula* on ethanol-induced gastric lesions in rats. Int J Pharmacol 11:697
348. Rojas-Martinez R, Arrieta J, Cruz-Antonio L, Arrieta-Baez D, Velazquez-Mendez AM, Sanchez-Mendoza ME (2013) Dillapiole, isolated from *Peperomia pellucida*, shows gastroprotector activity against ethanol-induced gastric lesions in Wistar rats. Molecules 18:11327
349. Chávez-Piña AE, Navarrete A (2009) Gastroprotective triterpenoids: pharmacological mechanism. In: Varela A, Ibañez J (eds) Medicinal plants: classification, biosynthesis and pharmacology. Nova Science, Hauppauge, NY, p 97
350. Gonzalez-Trujano ME, Ventura-Martinez R, Chavez M, Diaz-Reval I, Pellicer F (2012) Spasmolytic and antinociceptive activities of ursolic acid and acacetin identified in *Agastache mexicana*. Planta Med 78:793
351. Verano J, Gonzalez-Trujano ME, Deciga-Campos M, Ventura-Martinez R, Pellicer F (2013) Ursolic acid from *Agastache mexicana* aerial parts produces antinociceptive activity involving TRPV1 receptors, cGMP and a serotonergic synergism. Pharmacol Biochem Behav 110:255
352. Aguilar MI, Rovelo R, Verjan JG, Illescas O, Baeza AE, De La Fuente M, Avila I, Navarrete A (2011) Anti-inflammatory activities, triterpenoids, and diarylheptanoids of *Alnus acuminata* ssp. *arguta*. Pharm Biol 49:1052
353. Deciga-Campos M, Gonzalez-Trujano E, Navarrete A, Mata R (2005) Antinociceptive effect of selected Mexican traditional medicinal species. Proc West Pharmacol Soc 48:70

354. Palacios-Espinosa F, Deciga-Campos M, Mata R (2008) Antinociceptive, hypoglycemic and spasmolytic effects of *Brickellia veronicifolia*. J Ethnopharmacol 118:448
355. Martinez-Vazquez M, Apan TOR, Aguilar H, Bye R (1996) Analgesic and antipyretic activities of an aqueous extract and of the flavone linarin of *Buddleia cordata*. Planta Med 62:137
356. Martinez-Vazquez M, Apan TOR, Lastra AL, Bye R (1998) A comparative study of the analgesic and anti-inflammatory activities of pectolinarin isolated from *Cirsium subcoriaceum* and linarin isolated from *Buddleia cordata*. Planta Med 64:134
357. Sałaga M, Kowalczuk A, Zielinska M, Błażewicz A, Fichna J (2015) *Calea zacatechichi* dichloromethane extract exhibits antidiarrheal and antinociceptive effects in mouse models mimicking irritable bowel syndrome. Naunyn-Schmiedeberg's Arch Pharmacol 388:1069
358. Hehner SP, Heinrich M, Bork PM, Vogt M, Ratter F, Lehmann V, Schulze-Osthoff K, Droge W, Schmitz ML (1998) Sesquiterpene lactones specifically inhibit activation of NF-kappa B by preventing the degradation of I kappa B-alpha and I kappa B-beta. J Biol Chem 273:1288
359. Radulovic NS, Miltojevic AB, McDermott M, Waldren S, Parnell JA, Pinheiro MMG, Fernandes PD, Menezes FD (2011) Identification of a new antinociceptive alkaloid isopropyl *N*-methylanthranilate from the essential oil of *Choisya ternata* Kunth. J Ethnopharmacol 135:610
360. Cassani J, Araujo AGE, Martinez-Vazquez M, Manjarrez N, Moreno J, Estrada-Reyes R (2013) Anxiolytic-like and antinociceptive effects of (2*S*)-neoponcirin in mice. Molecules 18:7584
361. Ovalle-Magallanes B, Deciga-Campos M, Mata R (2015) Antinociceptive and hypoglycaemic evaluation of *Conyza filaginoides* (DC.) Hieron Asteraceae. J Pharm Pharmacol 67:1733
362. Garcia-Argaez AN, Apan TOR, Delgado HP, Velazquez G, Martinez-Vazquez M (2000) Anti-inflammatory activity of coumarins from *Decatropis bicolor* on TPA ear mice model. Planta Med 66:279
363. Velazquez-Gonzalez C, Carino-Cortes R, de Lucio JAG, Ortiz MI, Arciniega MD, Altamirano-Baez DA, Jimenez-Angeles L, Bautista-Avila M (2014) Antinociceptive and anti-inflammatory activities of *Geranium bellum* and its isolated compounds. BMC Complement Altern Med 14:506
364. Hernandez I, Marquez L, Martinez I, Dieguez R, Delporte C, Prieto S, Molina-Torres J, Garrido G (2009) Anti-inflammatory effects of ethanolic extract and alkamides derived from *Heliopsis longipes* roots. J Ethnopharmacol 124:649
365. Carino-Cortes R, Gayosso-De-Lucio JA, Ortiz MI, Sanchez-Gutierrez M, Garcia-Reyna PB, Cilia-Lopez VG, Perez-Hernandez N, Moreno E, Ponce-Monter H (2010) Antinociceptive, genotoxic and histopathological study of *Heliopsis longipes* S.F. Blake in mice. J Ethnopharmacol 130:216
366. Deciga-Campos M, Rios MY, Aguilar-Guadarrama AB (2010) Antinociceptive effect of *Heliopsis longipes* extract and affinin in mice. Planta Med 76:665
367. Rocha-Gonzalez HI, Blaisdell-Lopez E, Granados-Soto V, Navarrete A (2010) Antinociceptive effect of 7-hydroxy-3,4-dihydrocadalin isolated from *Heterotheca inuloides:* role of peripheral 5-HT1 serotonergic receptors. Eur J Pharmacol 649:154–160
368. Rodriguez-Chavez JL, Egas V, Linares E, Bye R, Hernandez T, Espinosa-Garcia FJ, Delgado G (2017) Mexican Arnica (*Heterotheca inuloides* Cass. Asteraceae: Astereae): ethnomedical uses, chemical constituents and biological properties. J Ethnopharmacol 195:39
369. Zapata-Morales JR, Alonso-Castro AJ, Dominguez F, Carranza-Alvarez C, Castellanos LMO, Martinez-Medina RM, Perez-Urizar J (2016) Antinociceptive activity of an ethanol extract of *Justicia spicigera*. Drug Devel Res 77:180
370. González-Guëreca MC, Soto-Hernández M, Martínez-Vázquez M (2010) Isolation of (−)-(2*S*)-5,6,7,3′,5′-pentahydroxyflavanone-7-*O*-*β*-D-glucopyranoside, from *Lippia graveolens* H.B.K. var. *berlandieri* Schauer, a new anti-inflammatory and cytotoxic flavanone. Nat Prod Res 24:1528

371. Rejon-Orantes JC, Suarez DPP, Rejon-Rodriguez A, Hernandez SH, Lievano OEG, Rodriguez DL, de la Mora MP (2013) Aqueous root extracts from *Mimosa albida* Humb. & Bonpl. ex Willd. display antinociceptive activity in mice. J Ethnopharmacol 149:522
372. Salazar JR, Martinez-Vazquez M, Cespedes CL, Ramirez-Apan T, Nieto-Camacho A, Rodriguez-Silverio J, Flores-Murrieta F (2011) Anti-inflammatory and cytotoxic activities of chichipegenin, peniocerol, and macdougallin isolated from *Myrtillocactus geometrizans* (Mart. ex Pfeiff.) Con. Z Naturforsch C 66:24
373. Romero JC, Martinez-Vazquez A, Herrera MP, Martinez-Mayorga K, Parra-Delgado H, Perez-Flores FJ, Martinez-Vazquez M (2014) Synthesis, anti-inflammatory activity and modeling studies of cycloartane-type terpenes derivatives isolated from *Parthenium argentatum*. Bioorg Med Chem 22:6893
374. Martinez AL, Gonzalez-Trujano ME, Pellicer F, Lopez-Munoz FJ, Navarrete A (2009) Antinociceptive effect and GC/MS analysis of *Rosmarinus officinalis* L. essential oil from its aerial parts. Planta Med 75:508
375. Moreno-Quirós CV, Sánchez-Medina A, Vázquez-Hernández M, Hernández Reyes AG, García-Rodríguez RV (2017) Antioxidant, anti-inflammatory and antinociceptive potential of *Ternstroemia sylvatica* Schltdl. & Cham. Asian Pac J Trop Med 10:1047
376. Calzada F, Correa-Basurto J, Barbosa E, Mendez-Luna D, Yepez-Mulia L (2017) Antiprotozoal constituents from *Annona cherimola* Miller, a plant used in Mexican traditional medicine for the treatment of diarrhea and dysentery. Pharmacogn Mag 13:148
377. Jimenez-Arellanes A, Leon-Diaz R, Meckes M, Tapia A, Molina-Salinas GM, Luna-Herrera J, Yepez-Mulia L (2012) Antiprotozoal and antimycobacterial activities of pure compounds from *Aristolochia elegans* rhizomes. Evid-Based Complement Altern Med 2012:ID 593403
378. Gutierrez-Gutierrez F, Puebla-Perez AM, Gonzalez-Pozos S, Hernandez-Hernandez JM, Perez-Rangel A, Alvarez LP, Tapia-Pastrana G, Castillo-Romero A (2017) Antigiardial activity of podophyllotoxin-type lignans from *Bursera fagaroides* var. *fagaroides*. Molecules 22
379. Dominguez-Vigil IG, Camacho-Corona MD, Heredia-Rojas JA, Vargas-Villarreal J, Rodriguez-De la Fuente AO, Heredia-Rodriguez O, Mata-Cardenas BD (2015) Anti-giardia activity of hexane extract of *Citrus aurantifolia* (Christm.) Swingle and some of its constituents. Afr J Tradit Complem 12:55
380. Camacho MD, Mata R, Castaneda P, Kirby GC, Warhurst DC, Croft SL, Phillipson JD (2000) Bioactive compounds from *Celaenodendron mexicanum*. Planta Med 66:463
381. Perez-Gonzalez MZ, Gutierrez-Rebolledo GA, Yepez-Mulia L, Rojas-Tome IS, Luna-Herrera J, Jimenez-Arellanes MA (2017) Antiprotozoal, antimycobacterial, and anti-inflammatory evaluation of *Cnidoscolus chayamansa* (McVaugh) extract and the isolated compounds. Biomed Pharmacother 89:89
382. Dominguez-Carmona DB, Escalante-Erosa F, Garcia-Sosa K, Ruiz-Pinell G, Gutierrez-Yapu D, Chan-Bacab MJ, Moo-Puc RE, Veitch NC, Gimenez-Turba A, Pena-Rodriguez LM (2011) Metabolites from roots of *Colubrina greggii* var. *yucatanensis* and evaluation of their antiprotozoan, cytotoxic and antiproliferative activities. J Brazil Chem Soc 22:1279
383. Calzada F, Cedillo-Rivera R, Mata R (2001) Antiprotozoal activity of the constituents of *Conyza filaginoides*. J Nat Prod 64:671
384. Calzada F, Alanis AD, Meckes M, Tapia-Contreras A, Cedillo-Rivera R (1998) In vitro susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to some medicinal plants used by the people of Southern Mexico. Phytother Res 12:70
385. Barbosa E, Calzada F, Campos R (2007) In vivo antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. J Ethnopharmacol 109:552
386. Meckes M, Calzada F, Tapia-Contreras A, Cedillo-Rivera R (1999) Antiprotozoal properties of *Helianthemum glomeratum*. Phytother Res 13:102

387. Barbosa E, Calzada F, Campos R (2006) Antigiardial activity of methanolic extracts from *Helianthemum glomeratum* Lag. and *Rubus coriifolius* Focke in suckling mice CD-1. J Ethnopharmacol 108:395
388. Calzada F, Alanis AD (2007) Additional antiprotozoal flavonol glycosides of the aerial parts of *Helianthemum glomeratum*. Phytother Res 21:78
389. Rodriguez-Chavez JL, Rufino-Gonzalez Y, Ponce-Macotela M, Delgado G (2015) In vitro activity of "Mexican Arnica" *Heterotheca inuloides* Cass. natural products and some derivatives against *Giardia intestinalis*. Parasitology 142:576
390. Calzada F, Barbosa E, Cedillo-Rivera R (2003) Antiamoebic activity of benzyl glucosinolate from *Lepidium virginicum*. Phytother Res 17:618
391. Quintanilla-Licea R, Mata-Cardenas BD, Vargas-Villarreal J, Bazaldua-Rodriguez AF, Angeles-Hernandez IK, Garza-Gonzalez JN, Hernandez-Garcia ME (2014) Antiprotozoal activity against *Entamoeba histolytica* of plants used in Northeast Mexican traditional medicine. Bioactive compounds from *Lippia graveolens* and *Ruta chalepensis*. Molecules 19:21044
392. Borges-Argaez R, Pena-Rodriguez LM, Waterman PG (2002) Flavonoids from two *Lonchocarpus* species of the Yucatan Peninsula. Phytochemistry 60:533
393. Borges-Argaez R, Balnbury L, Flowers A, Gimenez-Turba A, Ruiz G, Waterman PG, Pena-Rodriguez LM (2007) Cytotoxic and antiprotozoal activity of flavonoids from *Lonchocarpus* spp. Phytomedicine 14:530
394. Borges-Argaez R, Vela-Catzin T, Yam-Puc A, Chan-Bacab MJ, Moo-Puc RE, Caceres-Farfan M (2009) Antiprotozoal and cytotoxic studies on some isocordoin derivatives. Planta Med 75:1336
395. Pan L, Lezama-Davila CM, Isaac-Marquez AP, Calomeni EP, Fuchs JR, Satoskar AR, Kinghorn AD (2012) Sterols with antileishmanial activity isolated from the roots of *Pentalinon andrieuxii*. Phytochemistry 82:128
396. Alanis AD, Calzada F, Cedillo-Rivera R, Meckes M (2003) Antiprotozoal activity of the constituents of *Rubus coriifolius*. Phytother Res 17:681
397. Calzada F, Yepez-Mulia L, Tapia-Contreras A, Bautista E, Maldonado E, Ortega A (2010) Evaluation of the antiprotozoal activity of neo-clerodane type diterpenes from *Salvia polystachya* against *Entamoeba histolytica* and *Giardia lamblia*. Phytother Res 24:662
398. Calzada F, Bautista E, Yepez-Mulia L, Garcia-Hernandez N, Ortega A (2015) Antiamoebic and antigiardial activity of clerodane diterpenes from Mexican *Salvia* species used for the treatment of diarrhea. Phytother Res 29:1600
399. Polanco-Hernandez G, Escalante-Erosa F, Garcia-Sosa K, Chan-Bacab MJ, Sagua-Franco H, Gonzalez J, Osorio-Rodriguez L, Pena-Rodriguez LM (2012) Metabolites from the leaf extract of *Serjania yucatanensis* with trypanocidal activity against *Trypanosoma cruzi*. Parasitol Res 111:451
400. Calzada F, Basurto JC, Barbosa E, Velazquez C, Hernandez NG, Razo RMO, Luna DM, Mulia LY (2017) Antiprotozoal activities of tiliroside and other compounds from *Sphaeralcea angustifolia* (Cav.) G. Don. Pharmacogn Res 9:133
401. Navarrete A, Hong E (1996) Anthelmintic properties of alpha-sanshool from *Zanthoxylum liebmannianum*. Planta Med 62:250
402. Arrieta J, Reyes B, Calzada F, Cedillo-Rivera R, Navarrete A (2001) Amoebicidal and giardicidal compounds from the leaves of *Zanthoxylum liebmannianun*. Fitoterapia 72:295
403. Antunez-Mojica M, Rodriguez-Salarichs J, Redondo-Horcajo M, Leon A, Barasoain I, Canales A, Canada FJ, Jimenez-Barbero J, Alvarez L, Diaz JF (2016) Structural and biochemical characterization of the interaction of tubulin with potent natural analogues of podophyllotoxin. J Nat Prod 79:2113
404. Mojica MA, Leon A, Rojas-Sepulveda AM, Marquina S, Mendieta-Serrano MA, Salas-Vidal-E, Villarreal ML, Alvarez L (2016) Aryldihydronaphthalene-type lignans from *Bursera fagaroides* var. *fagaroides* and their antimitotic mechanism of action. RSC Adv 6:4950

405. Mendoza-Espinoza JA, Lopez-Vallejo F, Fragoso-Serrano M, Pereda-Miranda R, Cerda-Garcia-Rojas CM (2009) Structural reassignment, absolute configuration, and conformation of hypurticin, a highly flexible polyacyloxy-6-heptenyl-5,6-dihydro-2*H*-pyran-2-one. J Nat Prod 72:700
406. Suarez-Ortiz GA, Cerda-Garcia-Rojas CM, Fragoso-Serrano M, Pereda-Miranda R (2017) Complementarity of DFT calculations, NMR anisotropy, and ECD for the configurational analysis of brevipolides K-O from *Hyptis brevipes*. J Nat Prod 80:181
407. Cruz-Morales S, Castaneda-Gomez J, Rosas-Ramirez D, Fragoso-Serrano M, Figueroa-Gonzalez G, Lorence A, Pereda-Miranda R (2016) Resin glycosides from *Ipomoea alba* seeds as potential chemosensitizers in breast carcinoma cells. J Nat Prod 79:3093
408. Castaneda-Gomez J, Rosas-Ramirez D, Cruz-Morales S, Fragoso-Serrano M, Pereda-Miranda R (2017) HPLC-MS profiling of the multidrug-resistance modifying resin glycoside content of *Ipomoea alba* seeds. Rev Bras Farmacogn 27:434
409. Bautista E, Fragoso-Serrano M, Pereda-Miranda R (2016) Jalapinoside II, a bisdesmoside resin glycoside, and related glycosidic acids from the officinal jalap root (*Ipomoea purga*). Phytochem Lett 17:85
410. Bautista E, Fragoso-Serrano M, Pereda-Miranda R (2015) Jalapinoside, a macrocyclic bisdesmoside from the resin glycosides of *Ipomea purga*, as a modulator of multidrug resistance in human cancer cells. J Nat Prod 78:168
411. Corona-Castaneda B, Rosas-Ramirez D, Castaneda-Gomez J, Aparicio-Cuevas MA, Fragoso-Serrano M, Figueroa-Gonzalez G, Pereda-Miranda R (2016) Resin glycosides from *Ipomoea wolcottiana* as modulators of the multidrug resistance phenotype in vitro. Phytochemistry 123:48
412. Calzada F, Solares-Pascasio JI, Valdes M, Garcia-Hernandez N, Velazquez C, Ordonez-Razo RM, Barbosa E (2018) Antilymphoma potential of the ethanol extract and rutin obtained of the leaves from *Schinus molle* Linn. Pharmacogn Res 10:119
413. Pfeffer TJ, Sasse F, Schmidt CF, Lakamper S, Kirschning A, Scholz T (2016) The natural diterpene tonantzitlolone A and its synthetic enantiomer inhibit cell proliferation and kinesin-5 function. Eur J Med Chem 112:164
414. Ramos-Silva A, Tavares-Carreon F, Figueroa M, De la Torre-Zavala S, Gastelum-Arellanez A, Rodriguez-Garcia A, Galan-Wong LJ, Aviles-Arnaut H (2017) Anticancer potential of *Thevetia peruviana* fruit methanolic extract. BMC Complement Altern Med 17:241
415. Orsomando G, Agostinelli S, Bramucci M, Cappellacci L, Damiano S, Lupidi G, Maggi F, Kamte SLN, Nya PCB, Papa F, Petrelli D, Quassinti L, Sorci L, Vitali LA, Petrelli R (2016) Mexican sunflower (*Tithonia diversifolia*, Asteraceae) volatile oil as a selective inhibitor of *Staphylococcus aureus* nicotinate mononucleotide adenylyltransferase (NadD). Ind Crop Prod 85:181
416. Estrada-Reyes R, Lopez-Rubalcava C, Ferreyra-Cruz OA, Dorantes-Barron AM, Heinze G, Aguilar JM, Martinez-Vazquez M (2014) Central nervous system effects and chemical composition of two subspecies of *Agastache mexicana*; an ethnomedicine of Mexico. J Ethnopharmacol 153:98
417. Tortoriello J, Lozoya X (1992) Effect of *Galphimia glauca* methanolic extract on neuropharmacological tests. Planta Med 58:234
418. Tortoriello J, Ortega A (1993) Sedative effect of galphimine B, a *nor-seco*-triterpenoid from *Galphimia glauca*. Planta Med 59:398
419. Herrera-Arellano A, Jimenez-Ferrer E, Zamilpa A, Morales-Valdez M, Garcia-Valencia CE, Tortoriello J (2007) Efficacy and tolerability of a standardized herbal product from *Galphimia glauca* on generalized anxiety disorder. A randomized, double-blind clinical trial controlled with lorazepam. Planta Med 73:713
420. Guzman-Gutierrez SL, Navarrete A (2009) Pharmacological exploration of the sedative mechanism of hesperidin identified as the active principle of *Citrus sinensis* flowers. Planta Med 75:295

421. Gonzalez-Trujano ME, Tapia E, Lopez-Merez L, Navarrete A, Reyes-Ramirez A, Martinez A (2006) Anticonvulsant effect of *Annona diversifolia* Saff. and palmitone on penicillin-induced convulsive activity. A behavioral and EEG study in rats. Epilepsia 47:1810
422. Balderas-Lopez JL, Alfaro-Romero A, Monroy A, Lopez-Villafranco ME, Rivero-Cruz JF, Navarrete A (2013) Toxic rather than neuropharmacological effect of *Ternstroemia sylvatica* fruits and identification of 28-*O*-[*β*-L-6-rhamnopyranosyl]-*R*-1-barrigenol as a new compound with toxic effects in mice. Pharm Biol 51:1451
423. Sanchez-Mendoza ME, Reyes-Trejo B, de la Rosa L, Rodriguez-Silverio J, Castillo-Henkel C, Arrieta J (2008) Polyalthic acid isolated from *Croton reflexifolius* has relaxing effect in guinea pig tracheal smooth muscle. Pharm Biol 46:800
424. Gornemann T, Nayal R, Pertz HH, Melzig MF (2008) Antispasmodic activity of essential oil from *Lippia dulcis* Trev. J Ethnopharmacol 117:166
425. Ozoluaa RI, Ebokab CJ, Durua CN, Uwaya DO (2010) Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. Nig J Physiol Sci 25:149
426. Cortes-Arroyo AR, Dominguez-Ramirez AM, Gomez-Hernandez M, Lopez JRM, de la Pena MHY, Lopez-Munoz FJ (2011) Antispasmodic and bronchodilator activities of *Taxodium mucronatum* Ten. leaf extract. Afr J Biotechnol 10:54

Rachel Mata is an Emeritus Professor at the National Autonomous University of Mexico (UNAM). She obtained her Ph.D. degree from Purdue University, W. Lafayette, IN, USA, in 1979. Following a postdoctoral stay at the Institute of Chemistry at UNAM, she became an Assistant Professor of the School of Chemistry at the same university in 1985, and was promoted to Full Professor in 1992. Prof. Mata served as Head of the Department of Pharmacy from 2003 to 2012, and since 2013 has been a member of the Advisory Board of the School of Chemistry. Moreover, she has served on several National and International Advisory Boards, including the National Center for Natural Products Research at the University of Mississippi, Oxford, MS, USA, from 2003 to 2007. Prof. Mata is a Past-President of the Phytochemical Society of North America (1996–1997) and was named as a Fellow of the American Society of Pharmacognosy in 2014. Her work has contributed significantly to knowledge of Mexican traditional medicine by establishing the chemical composition and biological properties of many widely used herbal drugs. She has published over 200 original papers as well as several book chapters and books on medicinal plants from Mexico. She was a Visiting Professor at the University of Rhode Island, Kingston, RI, USA, and has collaborated with many research groups from Mexico, Canada, the UK, and the USA. These contributions have earned her both national and international recognition, including being named as an Emeritus National Researcher from CONACyT-Mexico in 2015, and in receiving the Distinguished Alumni Award of the School of Pharmacy and Pharmaceutical Sciences, Purdue University in 1998, the National Chemistry Research Award from the Mexican Chemical Society in 2013, and the Norman Farnsworth Research Achievement Award from the American Society of Pharmacognosy in 2014. She has trained numerous Ph.D. students, of whom many are now gainfully employed in academia.

Mario Figueroa received his B.Sc., M.S. and Ph.D. degrees in Chemistry from the National Autonomous University of Mexico (UNAM). Then, from 2009 to 2013, he worked as a Postdoctoral Fellow at the Lehman College, City University of New York, Bronx, NY, USA, and The University of North Carolina at Greensboro, NC, USA, in the laboratories of Professors Edward J. Kennelly and Nicholas H. Oberlies, respectively. During this period, Dr. Figueroa discovered numerous new natural compounds with potential anticancer and anti-MRSA properties from plants and fungi. He is currently an Associate Professor at the School of Chemistry at UNAM, where his research focuses in the chemistry and biology of fungi and bacteria from unexplored sources of Mexico. He has published over 45 papers in leading internationally recognized peer-reviewed journals and 2 book chapters, and has been named as co-inventor on three patents, and he is member of the National Council of Science and Technology (2018–2021) Level II.

Andrés Navarrete obtained his Ph.D. degree in Pharmacology from the Instituto Politécnico Nacional of Mexico in 1996. He worked at the University of Chapingo, Estado de Mexico, from 1983 to 1997, where he undertook a pharmacological study on Mexican medicinal plants. He then moved to the School of Chemistry at the National University Autonomous of Mexico, where he continued his research on the pharmacology of medicinal plants and developed analytical methods for the quality control of autochthonous medicinal plant species from Mexico. His primary research area focuses on the identification of gastroprotective compounds from Mexican medicinal plants and their mechanism of action. Other research areas of interest include the study of antiasthmatic, analgesic, and sedative herbal drugs. He has proposed several methods to investigate the pharmacological action of natural products, such as the real-time and in situ determination of the changes of hydrogen sulfide in the gastroprotective process and the use of the exploratory cylinder to obtain quantitative measurements of sedative effects. Currently, he is interested in alternative methods for the investigation of the pharmacology of natural products with the limited use of laboratory animals. He spent a sabbatical period at the National Center for Natural Products Research of the University of Mississippi, Oxford, MS, USA. In 2001, he received the National Award in Health "Martín de la Cruz."

Isabel Rivero-Cruz holds a Ph.D. degree in Pharmacognosy from the National Autonomous University of Mexico; her Ph.D. research dealt with Mexican oregano specimens. She joined the laboratory of Professor Rachel Mata as an Associate Researcher in 2001. The main research interests of Dr. Rivero-Cruz involve the isolation and characterization of bioactive compounds from different natural sources as well as the development and validation of analytical methods for quality control of herbal drugs. These methods are included in the Second Edition of the "Mexican Herbal Pharmacopoeia". She has extensive knowledge in the application of different contemporary chromatographic methods. She has published a number of technical contributions including original research papers and book chapters. Dr. Rivero-Cruz is a member of the Mexican National Research Council.

Biomolecular Targets of Oxyprenylated Phenylpropanoids and Polyketides

Serena Fiorito, Francesco Epifano, Francesca Preziuso, Vito Alessandro Taddeo, and Salvatore Genovese

Contents

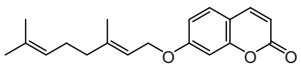
S. Fiorito · F. Epifano (✉) · F. Preziuso · V. A. Taddeo · S. Genovese
Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Chieti Scalo, Italy
e-mail: serena.fiorito@unich.it; fepifano@unich.it; francesca.preziuso@unich.it; vito.taddeo@unich.it; s.genovese@unich.it

A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.),
Progress in the Chemistry of Organic Natural Products, Vol. 108,
https://doi.org/10.1007/978-3-030-01099-7_2

1 Introduction

Oxyprenylated secondary metabolites from plants and fungi are a class of rarely occurring natural products. In the case of plants, these are examples of compounds of mixed biosynthetic origin resulting from a convergence of two of the main metabolic pathways represented in the plant kingdom: one starting from phenylalanine leading to the phenylpropanoids and from the decarboxylative condensation of malonyl-CoA generating polyketides, and the other being the mevalonate and 1-DOXP pathways leading to the isopentenoids. The most important structural feature consequently is the linkage of one or more 3,3-dimethylallyl, geranyl, and farnesyl chains to oxygen atoms to produce this mixed biosynthesis group of natural products. Such skeletons can contain either only carbon and hydrogen atoms, or be functionalized with oxygen atoms, as alcohols, ethers, epoxides, ketones, aldehydes, carboxylic acids and esters, and in some cases with halogens, especially in metabolites coming from marine organisms [1]. Furthermore, another peculiarity is that oxyprenylated secondary metabolites are found in a very restricted number of plant families (Rutaceae, Apiaceae, Asteraceae, Fabaceae, and a few others) and, in the most cases, occur only in very low concentration levels.

Over the past two decades, the interest in oxyprenylated phenylpropanoids and polyketides has largely increased due to two major reasons: the refinement and the higher sensitivity of analytical techniques (e.g. HPLC and UHPLC) available to allow the detection of such phytochemicals in a wider range of plants, despite their very low amounts present, and the development of greener and high yielding synthesis methodologies, allowing the production of hundreds of milligrams to grams of products so that more complete pharmacological profiles may be obtained, as well as yielding pure chemical standards for analysis and structural characterization. From the discovery of the first example of the characterization of an oxyprenylated compound reported in the literature at the beginning of 1930s by Karyione and Matsuno [2], namely, the isolation and purification of auraptene (**1**) from the fruits of *Citrus aurantium* L. (Rutaceae) (Plate 1), to date approximately 400 such chemical entities have been described.



1 (auraptene)

While between the years 1990 and 2000, studies on oxyprenylated secondary metabolites were focused mainly on qualitative and quantitative analysis as well as structural characterization, from 2000 onwards an increasing number of pharmacological studies have appeared in the literature and these have highlighted how such phytochemicals are able to act in vitro and in vivo as effective anticarcinogenic, neuroprotective, and anti-inflammatory agents. These effects have been focused not only in the context of potential drug therapy, but also for chemoprevention and have

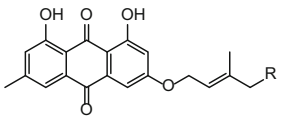
Plate 1 *Citrus aurantium*. Photograph: A. Barra, Wikimedia Commons

been supported by laboratory experiments involving oral administration to test animals. With a plethora of pharmacological data having been accumulated, some groups have begun to study during the last decade mechanisms of the biomolecular actions underlying the observed effects of test compounds both in vitro and in vivo. Thus, it was demonstrated that oxyprenylated secondary metabolites are able to interact with several biological targets inside and outside cells leading to different biological responses and yielding benefits for human welfare. The aim of this comprehensive chapter is to provide an up-to-date detailed survey of the literature data on the capacity of *O*-prenylphenylpropanoids and *O*-prenylpolyketides to trigger selected cell receptors, enzymes, and related substructures, thus accounting for their ability to positively modulate key metabolic processes and biological functions. The literature period covered is from 2006 to 2018. Bibliographic searches in web-based databases such as Scifinder, Medline, Scopus, ISI Web of Science, and Google Scholar have indicated that to date more than 150 articles and/or patents on this topic have appeared or been issued. These concern three main subclasses of oxyprenylated secondary metabolites, anthraquinones, coumarins, and ferulic acid derivatives, for which the most detailed descriptions of their interactions with biological targets have been provided and validated. The chapter is divided into sections having subsections that each deal with an individual compound, in turn. The major perspectives in the field with suggestions on possible ways to address future research approaches are also presented.

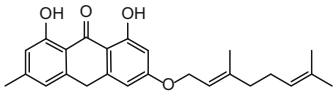
2 Oxyprenylated Anthraquinones

Despite the large amount of phytochemical and pharmacological data currently in the literature on naturally occurring anthraquinones, including detailed studies about their mechanisms of action at a biomolecular level, very few investigations have been reported on this topic for oxyprenylated anthraquinones. The activities described refer mainly to the in vitro growth inhibition of selected cancer cell

lines, and antiprotozoal and antibacterial effects, in particular against *Leishmania, Plasmodium*, and *Trypanosoma* spp., in addition to their potential neuroprotective capacities [3]. In this context, however, only three reports on the specific targets of oxyprenylated anthraquinones have been cited. Compounds for which such data are available are 3-geranyloxyemodin (**2**), 3-geranyloxyemodin anthrone (**3**), and madagascin (**4**).



2 R = CH_2–CH=$C(CH_3)_2$ (3-geranyloxyemodin)
4 R = H (madagascin)



3 (3-geranyloxyemodin anthrone)

3-Geranyloxyemodin (**2**) has been isolated from *Vismia guineensis* (L.) Choisy (Clusiaceae) (Plate 2) [4], *Cratoxylum glaucum* Korth., and *C. arborescens* (Vahl) Blume (Clusiaceae) [5]. The first and up to now only example of biological activity reported is the description of in vitro growth inhibitory effects against the HepG2 (human liver cancer), PC-3 (human prostate cancer), DU-145 (human prostate cancer), MCF-7 (human breast adenocarcinoma), and HEK-293 (human embryonic kidney) cell lines, with IC_{50} values ranging from 10 to 17 μM [6]. The mechanism of action seems to be linked to the capacity of 3-geranyloxyemodin to effectively intercalate into the DNA double helix. Using a specific assay, namely, the displacement capacity of ethidium bromide from the binding to calf thymus DNA evaluated by fluorescence detection, it was found that **2** displayed an effect comparable to that evoked by doxorubicin when used as a reference compound. This is a more than plausible hypothesis considering that 3-geranyloxyemodin (**3**) shares with doxorubicin a large planar moiety, but also has a markedly higher lipophilicity. The latter feature may be favorable in rendering **2** as being more able to permeate cell and nuclear membranes and to interact with DNA with greater ease. These data highlight the value of this compound structural class as a template for the design of novel anthraquinone-based DNA intercalating agents.

3-Geranyloxyemodin anthrone (**3**) has been obtained from root extracts of *Psorospermum febrifugum* Spach var. *ferrugineum* (Hook.) Keay & Milne-Redh. (Plate 3) and *P. glaberrimum* Hochr. (Clusiaceae) [7, 8]. However, only one citation concerned with a potential biological target for this anthraquinone has been reported. Both plant sources are used in the traditional medicine systems of several African countries to treat epilepsy and related disorders. Thus, the in vitro modulatory properties of 3-geranyloxyemodin anthrone (**3**) on acetyl- and butyryl-cholinesterases (AChE and BChE) have been investigated [8]. While displaying a

Plate 2 *Vismia guineensis*. Photograph: C. Mas, (CC) BY-NC-ND

Plate 3 *Psorospermum febrifugum*, Mount Ribaue, N. Mozambique. Photograph: Rulkens Ton, Wikimedia Commons

very poor inhibitory activity against AChE (5.4% inhibition), **3** exerted a more potent effect on BChE with an IC_{50} of 11.6 μ*M*. Unfortunately, these data are too weak to hypothesize that **3** can be considered an effective agent against neurological syndromes involving a deficit of acetylcholine (e.g. Alzheimer's disease) and to correlate the folkloric usage of the source plant, *P. glaberrimum*, as a neuroprotective remedy. There is, however, the potential to conduct additional studies to better investigate the interaction of **3** with other components and targets of the central and peripheral nervous systems both in vitro and in vivo, and to consider this compound further as a lead for the design and synthesis of more potent anti-AChE and anti-BChE agents.

Madagascin (**4**) is the most widespread prenyloxyanthraquinone found in Nature. Its name is derived from that of the African plant *Harungana madascariensis* Poir. (Clusiaceae) (Plate 4), a species from which this compound was obtained for the first time in 1964 [9]. Subsequently, **4** has been isolated from several other source plants: *Vismia guaramiranga* Huber [10], *V. magnoliifolia Cham.* & Schltdl. [11], *V. martiana* Reichardt [12], *V. guineensis* Choisy [13], and *V. parviflora* Cham. & Schltdl. [14], *Psorosporum tenuifolium* Hook. f. [15], *P. glaberrimum* [16], *P. febrifugum* Spach, and *P. adamauense* Engl. [17], *Cratoxylum formosum* (Jack) Benth. & Hook.f. ex Dyer [18], and finally from *Rhamnus* spp. (Rhamnaceae) [19–22]. Although reported to have multiple effects as an antioxidant, antimicrobial, and cytotoxic agent [3, 4, 8], the only report concerning the interaction of **4** with biological targets was published in 2016 by Chen and coworkers [23]. These authors investigated the mechanism underlying the capacity of **4** to reduce K^+-induced constrictions in isolated rat mesenteric resistance arteries and provide relaxation in the same pharmacological model pre-treated with methoxamine and endothelin-1. The finding that these effects are antagonized by the endothelial NO synthase inhibitor L-NAME and the AMPK inhibitor compound C served to corroborate that **4** can interfere with the AMP-activated protein kinase and nitric oxide (NO) synthase (eNOS) systems. This anthraquinone **4** was able to induce the phosphorylation of AMPK that in turn provided the same metabolic reaction on

Plate 4 *Harungana madascariensis*, in Bioko. Photograph: D. Barthel, Wikimedia Commons

Table 1 Reported biological targets of oxyprenylated anthraquinones

Target		Refs.
DNA-intercalation	↑	[6]
AChE, BChE	↓	[8]
AMPK	↑	[23]

↑ = activation; ↓ = inactivation

eNOS and led to a large decrease in the phosphorylation of myosin phosphatase target subunit 1 (MYPT1), resulting in nitrite-mediated blood vessel relaxation. The highlighted effect of activation of AMPK is noteworthy as it may also account for other reported activities of **4**, such as its cytotoxic effects. It is now well understood that activation of AMPK by phosphorylation gives rise to a potent tumor suppressor able to positively modulate the activity of p53 and to regulate overall cell growth and metabolism [24]. As for the other two anthraquinones, the so far reported pharmacological data have confirmed the considerable potential of **4**, the profile of which deserves to be further and more deeply studied in the future. Table 1 gives a summary of the so far described mechanisms of action of oxyprenylated anthraquinones.

3 Oxyprenylated Coumarins

3.1 Auraptene

Auraptene (**1**) represents one of the most abundant oxyprenylated coumarins occurring in Nature, in particular in the fruits of plants of the Rutaceae belonging to the genus *Citrus* (as an example, see *C. aurantium* L., Plate 1) and one for which a well-detailed description of its pharmacological effects and mechanisms of action have been described during the last decade [25]. In particular, **1** was shown to exert potentially beneficial chemopreventive and therapeutic effects in laboratory systems representative of several syndromes affecting humans, like cancer, inflammation, neurological diseases, and metabolic disorders.

The first report on the interaction of **1** with a biological target well known to be deeply involved in cancer progression was reported by Kawabata and co-workers in 2006 [26]. These authors investigated the relationship between **1** and the matrix metalloproteinase (MMP)-7 enzyme, also known as matrylisin. This enzyme is overexpressed in several types of cancer, such as those affecting the gastrointestinal system, and contributes to tumor progression, dysregulated cell proliferation, tumor invasion, and metastasis. A previous finding that auraptene (**1**) can act as an effective dietary colon cancer chemopreventive agent indicates that the mechanism of action underlying such an effect could be attributed to an inhibition of MMP-7. In the HT-29 cell line (human colorectal adenocarcinoma), Kawabata and coworkers determined that auraptene decreased the extent of both intracellular and extracellular proMMP-7 production, with IC_{50} values of 2.8 and 3 μM. As **1** was not able to

inhibit MMP-7 mRNA expression, it was hypothesized that this coumarin may act on proMMP-7 production at a post-translational level. Interestingly, and to underline the role of the geranyloxy side chain, the parent unprenylated coumarin, umbelliferone, was found to be totally ineffective at this same experiment. The authors also examined the effects of **1** on the m-TOR mediated signaling pathway. Compound **1** at a concentration of 25 μ*M* led to the dephosphorylation at Thr37, Thr46, and Thr70 of the 4E binding protein (4EBP1), in a way similar to that evoked by rapamycin, while having opposite effects with respect to rapamycin on the phosphorylation status of eukaryotic initiation factor (eIF4B), for which **1** produced dephosphorylation at Ser 422. Compound **1** was also able to suppress, again by dephosphorylation, the activity of ERK1/2 when applied at the same dose over a period of 6 h. This effect, in turn, would lead to the suppression of proMMP-7 biosynthesis. The interaction of auraptene (**1**) with mTOR was also investigated by Moon and coworkers in 2015 [27]. These authors confirmed the experimental evidence previously provided by Kawabata and coworkers and found that **1** behaves like rapamycin in SNU-1 cancer cells (human gastric carcinoma) in dephosphorylating mTOR and thus leading to down-regulation of phospho-p70S6K and downstream production of mTOR proteins.

Mitochondria and the endoplasmic reticulum (EndR) represent other targets of auraptene (**1**). Jun and coworkers in 2007 demonstrated that the induction of apoptosis in leukemia Jurkat T cells is due to the influence of **1** on the structure and functionality of the EndR [28]. Using a series of experiments, these authors provided clear evidence that **1** dose-dependently induced ER-stress-mediated activation of caspase-8, representing the initial event that would then lead to apoptosis. Such events comprise in sequence c-Jun N-terminal kinase (JNK) activation, cleavage of the FLICE inhibitory protein and Bid, cytochrome c release at the mitochondrial level, activation of caspases-9 and -3, degradation of poly (ADP-ribose) polymerase, and finally apoptotic DNA fragmentation. Interestingly non-tumor T-cells are much less sensitive to auraptene action than Jurkat T-cells, in which their viability was only affected at high doses. Induction of apoptosis through caspase activation by **1** (applied at the concentration range of 30–60 μ*M*) was also recorded in PC3 and DU145 human prostate cancer cells [29]. Auraptene (**1**) has also a direct effect on mitochondrial respiration, notably without affecting cell growth and viability. Assayed in human renal carcinoma cells, **1** inhibited mitochondrial complex-I and reduced by 30–60% the expression of mRNA encoding enzymes of the glycolytic enzymes glucose transporter 1 (GLUT1), hexokinase 2 (HK2), phosphofructokinase (PFK) and lactate dehydrogenase A (LDHA), thus depriving cancer cells of the glycolytic ATP necessary to maintain a high proliferation rate [30]. The recorded large reduction in energy metabolism resulted in a diminished cell motility and inhibition of vascular endothelial growth factor (VEGF)-promoted neovascularization (also demonstrated in vivo). These latter effects were also determined by another effect exerted by auraptene in the same cancer cell line, namely, the dose-dependent degradation of hypoxia-inducible factor 1a (HIF-1a) at a translation initiation level.

Auraptene (**1**) might also contribute to cancer chemoprevention and cancer chemotherapy by modulating the expression of several genes encoding proteins that play a key role in cell proliferation. By binding as a partial agonist to the α- and β-estrogen receptors (ERs) with equal affinity values (7.8 and 7.9 μ*M*, respectively), this coumarin, assayed at a concentration of 20 μ*M* in MCF-7 (human breast cancer) cells, was able to modulate the expression of endogenous genes regulated by 17*β*-estradiol and reported genes, such as the progesterone receptor (PR), trefoil factor 1 (TFF1), and transforming growth factor-α (TGF-α) [31]. Auraptene (**1**) slightly increased (by 1.1-fold) the transcription of TGF-α and inhibited (by 0.5-fold) the expression of TFF1. In addition, it antagonized the stimulation by 17*β*-estradiol of TFF1 and TGF-α expression, with IC_{50} values of 4.50 and 3.38 μ*M*. This resulted in a marked reduction in the invasiveness capacities of four human breast cancer cell lines: MCF-7, MDA-MB-231, SW-620, and E151A. Similar observations were also made by Khrishnan and Kleiner-Hancock in 2012 concerning the regulation of genes controlling the overall cell cycle in MCF-7 cells pre-treated with insulin growth factor (IGF)-1 [32]. In a time period between 8 and 24 h after application of **1** in cultured cancer cells, the transcription of more than 20 genes was modulated and these included the down-regulation of E2F1 (E2F transcription factor 1, –7.88-fold), CDC45L (cell division cycle 45 homolog, –6.51-fold), E2F2 (E2F transcription factor 2, –9.81-fold), MCM3 (minichromosome maintenance complex component 3, –6.08-fold), MCM6 (minichromosome maintenance complex component 6, –3.71-fold), UHRF1 (ubiquitin-like with PHD and ring finger domains 1, –17.01-fold), CDC2 (cyclin dependent kinase 1, –38.29-fold), CCNA2 (cyclin A2, –20.25-fold), KIF20B (kinesin family member 20B, –32.48-fold), CHEK1 (CHK1 checkpoint homolog, –9.01-fold), CDKN2C (cyclin-dependent kinase inhibitor 2C, –8.98-fold), CHEK2 (CHK2 checkpoint homolog, –10.83-fold), and CCNB2 (cyclin B2, –6.81-fold), and up-regulation of DDIT3 (DNA-damage-inducible transcript 3, 9.36-fold), CDKN2B (cyclin-dependent kinase inhibitor 2B, 5.83-fold), GADD45A (growth arrest and DNA-damage-inducible, alpha, 10.16-fold), and DUSP1 (dual specificity phosphatase 1, 7.51-fold).

Auraptene (**1**) also attenuated resistance to chemotherapeutic agents acting on factors that confer such characteristics to several cancer cell lines. Nabekura and coworkers in 2008 showed that **1** provided an increase in accumulation of daunorubicin in multidrug-resistant KB-C2 (human papillomavirus-related endocervical adenocarcinoma) cells by inhibiting P-glycoprotein (P-gp) when assayed at a concentration of 50 μ*M* in a manner very similar to the known P-gp substrate verapamil [33]. Furthermore, **1** afforded a significant increase in liver glutathione *S*-transferase (GST) when administered orally in Nrf2 heterozygous mice, thus leading to an enhancement of cell antioxidant resources [34].

Although not yet fully understood, it is worth highlighting recent results suggesting that auraptene (**1**) also can act on cancer stem cells. The first evidence of this was provided by Epifano and co-workers in 2013 [35]. These authors demonstrated that coumarin **1** at a concentration of 10 μ*M* was able to inhibit

(40%) of the growth and formation of colonospheres of FOLFOX-resistant colon cancer HT-29 cells (highly enriched in cancer stem cells). The basis of this effect seems to depend on a decrease (50%) of phospho-epidermal growth factor receptor (PEGFR). Similar results have been obtained more recently by Saboor-Maleki and coworkers using KYSE30 (human esophageal carcinoma) cells [36]. These authors also demonstrated that **1** is a very good adjuvant agent for some known cancer chemotherapeutic agents (e.g. cisplatin, paclitaxel, and 5-fluorouracil), in being able to increase greatly the sensitivity of cancer stem cells towards their action. Among the data collected so far on the modulatory properties of **1** on selected key biomolecular targets, those dealing with their interactions with cancer stem cells deserve to be investigated in much greater more detail. For cancer chemoprevention, the approach of combating the growth of cancer-triggering stem cells is perhaps one of the greatest challenges to be met in the coming years. Very few test compounds have shown similar properties to those exhibited by **1** in this regard, and this encourages the investigation of analogous effects of structurally related natural products.

Inflammation is well recognized as a physiological response closely related to cancer development and progression. Studies on the potential anticarcinogenic properties of auraptene (**1**) have been accompanied by investigations on its putative anti-inflammatory properties. The first contribution in this area was provided in 2005 by Murakami and co-workers, who assessed that auraptene is able to inhibit to a large extent the activity of cyclooxygenase (COX)-2 but did not prevent its expression [37]. One year later, Kawabata and co-workers provided evidence on the effectiveness of **1** in the regression of ulcerative colitis induced by dextran sodium sulfate in ICR mice in targeting three MMPs (2, 7, and 9), for which their expression commonly is enhanced in patients suffering from inflammatory bowel diseases [38]. In animals fed auraptene 0.1% *w/w* for 2 weeks, the expression and/or activity of these MMPs were drastically reduced. In particular, the gelatinolytic activity of MMP-7 was totally abolished while the expression of the three proMMPs underwent a decrease in the range from 82 to 100%. Additional experimental data by Kawabata and co-workers indicated that the effective mechanism of action for these effects may be not related to a direct action on MMPs, but on the inhibition of ERK1/2, in turn leading to the abolition of genomic expression of proMMP-2, -7, and -9. In co-cultures of 3T3-L1 adipocytes and RAW 264.7 macrophages, auraptene decreased the release of NO and tumor necrosis factor (TNF)-α [39]. This effect is related to the capacity of **1** to inhibit mitogen-activated protein kinases (MAPK), and, in particular, the activation of p38 in suppressing the secretion of inflammatory mediators. A large decrease in interleukin [IL] and chemokine levels, in particular IL-6, IL-8, and chemokine (C–C motif) ligand-5, were also observed in *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS)-stimulated oral epithelial cells [40]. This, together with an increase in collagen I biosynthesis [41] and MMP-2 inhibition, contributed to observed marked wound-healing effects by **1**. Despite the importance of inflammation as a pathological process, the anti-

inflammatory properties of **1** still remain described merely in terms of final effect (e.g. effects on NO and/or prostaglandins [PGs] release, effects on pro-inflammatory ILs, and similar activities) while, as indicated above, few investigations about its mechanism of action at a biomolecular level have been reported. Many more detailed studies are needed in this regard. Knowledge of the exact target of the observed and effective anti-inflammatory action by **1** would be of help for the design and synthesis of a new generation of hopefully more selective drug lead compounds. It can be hypothesized that earlier cancer-focused studies could also be of relevance for investigating further the potential anti-inflammatory activity of **1**.

Another group of biological activity reports on auraptene **1** concern its observed neuroprotective effects. The first documentation of this was in 2012 by Furukawa and coworkers. These authors studied the activity of **1** to promote neuronal proliferation and differentiation in rat cortical neurons and in the rat pheochromocytoma cell line (PC12 cells) [42]. Auraptene (**1**), at a dose of 100 μ*M*, was able to promote phosphorylation of ERK1/2 in cultured neurons in a time-dependent manner, with the highest activity being recorded between 10 and 30 min from administration of this coumarin to cultured cells. The same effect was recorded in PC12 cells. As a consequence of ERK1/2 activation, **1** showed the ability to induce neurite outgrowth in a dose-dependent manner (10–50 μ*M*) in PC12 cells and also induced oligodendrocyte lineage precursor cells to prevent demyelination [43]. The anti-inflammatory-related properties of **1**, in particular its capacity to modulate the expression and activity of COX-2 in selected areas of the brain, have been investigated in an animal model of brain inflammation. Okuyama and coworkers in 2015 demonstrated that **1** could reduce the extent of the inflammatory process upon local administration 5 days before and 3 days after ischemic surgery in mice at doses of 10 and 25 mg/kg [44]. Auraptene (**1**) largely diminished, especially when assayed at 25 mg/kg, the COX-2 translation process and expression in the hippocampus (notably this effect was demonstrated during this investigation for the first time), as well as providing a decrease in microglial activation during inflammatory processes stimulated by bacterial LPS. It is noteworthy that, in the course of this study, Okuyama and coworkers determined for the first time that **1** is able to entirely permeate the blood-brain barrier. The capacity to inhibit astro- and microglial-hyperactivation induced by LPS in the substantia nigra was also exploited by the same research group one year later to provide evidence as to how **1** can exert beneficial in vivo effects in a mouse model of Parkinson's disease. As a result, a substantial decrease in dopaminergic neuronal cell loss was observed [45]. Finally, auraptene (**1**) has been shown to have a good affinity ($K_i = 1.9$ μ*M*) for the melatonin receptor-1 (MT-1). This effect can help to explain its observed neuroprotective effects [46]. The findings to date on the mechanism of action of **1** that show some the beneficial properties for the central and peripheral nervous systems support the potential of this molecule to serve as a lead compound for further studies for its effects on neurological syndromes. They also suggest the potential of auraptene-containing foods (e.g. *Citrus* fruits) to act as chemopreventives for the dietary prevention of such disorders.

Auraptene (**1**) has pronounced effects on lipid and sugar metabolism and therefore has the capacity to positively modulate metabolic syndrome in humans [47, 48]. The first biomolecular targets identified in this area were the peroxisome proliferator-activated receptors (PPARs). In two independent studies published in 2008, Kawada and coworkers described in detail the interaction of **1** with PPARγ in 3T3-L1 adipocytes [49] and PPARα in HepG2 (human hepatoma) cells [50]. In the first of these cell lines, these authors provided evidence that **1** is able to selectively activate in a dose-dependent manner the isoforms PPARα and PPARγ, notably without affecting PPARδ. The agonist activity recorded in this experiment led in turn to an up-regulation of the gene and protein expression of adinopectin, and to the induction of the formation of high-molecular-weight multimers of adiponectin, and finally to the inhibition of the gene expression and secretion of monocyte chemoattractant protein (MCP)-1. The interaction of **1** with PPARα was studied in greater detail by Kawada and coworkers in the HepG2 line. In these cells, auraptene (**1**) stimulated the receptors in a dose-dependent manner. Consequently, **1** was found to be able to modulate the expression of several genes encoding proteins directly involved in lipid metabolism and homeostasis, among which were acyl-CoA oxidase (ACO), carnitine palmitoyl-CoA transferase-1A (CPT1A), and acyl-CoA synthetase (ACS), and therefore to provide an enhancement in fatty acid uptake in HepG2 cells. The same research group in 2011 performed in vivo studies using high-fat-diet (HFD)-fed KK-Ay diabetic obese mice [51]. Animals were fed a HFD containing 0.1 and 0.2% *w/w* of **1** for 4 weeks. This treatment led to an induction of the mRNA expression of PPAR target genes in the liver and in the skeletal muscle, and to a reduction of the average size of adipocytes and an increase of the number of smaller adipocytes in comparison to that of the control HFD-fed mice, and finally to a suppression of hyperglycemia development and triglyceride accumulation. Another receptor-regulating lipid and glucose metabolism-related factor is the farnesoid X receptor (FXR), towards which **1** exhibited dose-dependent activation in a manner very similar to the natural ligand chenodeoxycholic acid [52]. Such an interaction is important in determining the positive modulation of bile acid metabolism and in ameliorating liver injury [53, 54].

Finally, auraptene (**1**) has the capacity to dose-dependently increase glucose uptake in L6 myotubes (thereby mimicking skeletal muscle cells) and to efficiently promote the translocation of GLUT4 to the plasma membrane. The effect recorded was equal to that evoked by insulin at a concentration of 0.1 μ*M* when used as control [48]. Considered together, these data indicate that the consumption of *Citrus* fruits that contain **1** as normal components of the diet, may contribute to the prevention and improvement of symptoms associated with metabolic syndrome such as obesity and diabetes. Table 2 provides a summary of the mechanisms of action of auraptene (**1**) thus far documented.

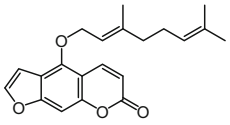
Table 2 Reported biological targets of auraptene (**1**)

Target		Refs.
MMPs	↓	[26, 38]
4EBP1	↓	[26, 27, 38]
eIF4B	↓	[26, 27, 38]
mTOR	↓	[26, 27, 38]
ERK1/2	↓	[26, 27, 38]
Caspases 3, 8, 9	↑	[28]
PARP	↑	[28]
Mitochondrial complex 1	↓	[30]
Glycolysis	↓	[30]
VEGF	↓	[30]
HIF-1α	↓	[30]
ERs	↑	[31]
GlP	↓	[33]
GST	↑	[34]
PEGFR	↓	[35]
COX-2	↓	[37]
MAPK	↓	[41]
Neuronal proliferation/differentiation	↑	[46]
MT-1	↑	[46]
PPAR α/γ	↑	[49, 50]
FXR	↑	[52, 54]
GLUT-4	↑	[48]

↑ = activation; ↓ = inactivation

3.2 *Bergamottin*

Bergamottin (**5**) is a linear furanocoumarin mainly found in grapefruit (*Citrus paradisi* Macfad. (Plate 5)) juice, but also extracted from the oils of the bergamot orange (*Citrus bergamia* Risso & Poit.) and other *Citrus* fruits [55].



5 (bergamottin)

The best characterized mechanism of action of coumarin **5** presently is its interaction with liver cytochromes. This effect was shown for the first time in 1998 by He and coworkers [56]. This investigation resulted from epidemiological data for which a high consumption of grapefruit juice is often associated with marked increases of the oral bioavailability of several drugs that are metabolized by cytochrome (CY) P450 3A4, through the inhibition of this enzymatic activity and a decrease in the intestinal content of these drugs. The bioassay-guided fractionation

Plate 5 *Citrus paradisi*. Photograph: F. and K. Starr, Wikimedia Commons

of an ethyl acetate of grapefruit juice led to the isolation of bergamottin (**5**) as the most effective inhibitor of CYP450 3A4. In a reconstituted in vitro system consisting of purified P450 3A4, NADPH-cytochrome P450 reductase, cytochrome *b*5, and phospholipids and by monitoring erythromycin N-demethylation as a reference reaction, furanocoumarin **5** demonstrated a 70% inhibition in a period of 15 min and a 40% decrease in the content of CYP450 3A4. The inhibitory activity recorded was time- and dose-dependent, following pseudo-first-order kinetics, and provided $k_{\text{inactivation}}$ and K_{i} values of 0.3 min^{-1} and 7.7 μ*M*. The interaction of bergamottin (**5**) with CYP450 3A4 occurs by structural modification of the active site of the corresponding apoenzyme without affecting the heme group. A similar pattern of reactivity was also recorded in a preliminary manner towards CYPs 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in human liver microsomes. In 2000, Tassaneeyakul and co-workers studied in more detail the inhibitory properties of **5** on CYPs using nifedipine oxidation as the reference reaction [57]. The results from this study indicated that **5** exerted much higher inhibitory properties (approximately sixfold) on the CYP isoforms 1A2, 2C9, 2C19, and 2D6 than on 3A4 [58, 59]. Using the oxidation of testosterone as a reference reaction in cultured human and monkey hepatocytes, **5**, when applied at the concentration range 1–5 μ*M*, showed an inhibition of CYP450 3A4 of 75–100%, while complete inhibition was recorded in monkey hepatocytes treated with **5** at a dose of 1 μ*M* against CYP450 3A8 [60]. Furthermore, **5** (5 μ*M*) completely inhibited α-naphthoflavone-induced ethoxyresorufin-*O*-dealkylase (EROD) and methoxyresorufin-*O*-dealkylase (MROD) in human hepatocytes and EROD activities in monkey hepatocytes. On the other hand, **5** did not affect phase-II conjugating enzymes primarily in both experimental models. Notably **5**, at a dose of 5 μ*M* and after a 48 h treatment in human hepatocytes, increased the mRNA of CYPs, providing the following values with respect to the untreated controls used: 8-fold for 3A4, 53-fold for 1A1 [61], and 12-fold for 1A2. Subsequent studies demonstrated that **5** is able to inhibit other CYP

isoforms. These included CYP2B6, CYP3A5 (60% reduction of the catalytic activity and also showing heme degradation and a covalent binding to the apoCYP [62, 63]), and CYP1B1 [64]. Such a great capacity of **5** to interact with a wide array of phase-I metabolizing enzymes explains how this phytochemical and food and plant-based preparations containing it (e.g. grapefruit and lime juices) can decrease the liver metabolism of a large number of drugs, prolonging their half-life and enhancing their pharmacological activities and side effects [65–68]. Notably, in some cases, such as CYP1A1, the action of **5** was not only directed at the enzyme but also effected by the inhibition of the expression and activity of the aryl hydrocarbon receptor (AhR), which, in turn, is the main trigger responsible for CYP expression in response to stimulation by several xenobiotics [69]. Based on the hypothesis that activation of the oxidative metabolic pathways of CYPs may generate an increase of reactive oxygen species (ROS), some authors have suggested employing **5** as a possible anti-inflammatory agent [70]. Indeed, this coumarin showed an appreciable reduction of the expression of the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in response to TNF-α-stimulation in a concentration-dependent manner, in the range of 20–50 μ*M*, and with a large decrease in lymphocyte adhesion. However, the poorly defined role of CYPs on inflammatory processes has discouraged further studies on **5** as an anti-inflammatory agent.

On the other hand, compound **5** has been investigated primarily as a potential anticarcinogenic agent [71]. Some of the effects documented are mediated by the direct action by **5** of CYPs. As reported by Cai and coworkers in 1997, this furocoumarin is able to modulate the formation of DNA mouse epidermal adducts and skin tumors when induced by benzo[*a*]pyrene (B[*a*]P) and 7,12-dimethylbenz[*a*] anthracene (DMBA). In particular, bergamottin (**5**), when applied topically at a dose of 400 n*M* 5 min prior to polycyclic aromatic hydrocarbon administration (B[*a*]P 200 nm and DMBA 10 n*M*), selectively inhibited the formation of covalent binding between B[*a*]P and epidermal DNA in a dose-dependent manner, while having no appreciable effect on the same binding induced by DMBA [72]. A similar pattern of inhibition has been observed for the binding of B[*a*]P metabolic derivatives, namely (+) *anti*-B[*a*]P–diol epoxide [(+) antiBPDE], with selected bases such as guanosine (80% inhibition at 400 n*M*). This was also consistent with the recorded in vivo effects for which **5**, when topically administered to mice, exhibited a dose-dependent decrease in papilloma formation (75% inhibition at the maximum dose applied of 800 n*M*). The high selectivity of **5** with respect to other coumarins in the interaction with adducts between DNA—B[*a*]P rather than DNA—DMBA was been also confirmed some years later by the same research group [73, 74].

Other cancer-related inhibitory effects of **5** may include also an influence on P-glycoprotein (P-gp), now well known to be the most important determinant for resistance to chemotherapeutic agents for several cancer types [75]. However, contrasting results have been obtained as reported by a series of studies published in the literature between 2000 and 2008. Bergamottin (**5**) was first shown to increase to a large extent the uptake of [^{3}H]vincristine in adriamycin-resistant human myelogenous leukemia (K562/ADM) cells but not to have the capacity to directly interact with P-gp [76]. With a different cancer cell line, namely, Caco-2 (human

colon carcinoma), **5** showed a similar pattern, yielding an increase of drug influx into cells (in this case also saquinavir rather than vincristine) but with little interaction with the membrane carrier [77]. Additionally, in 2008, an in vivo study demonstrated that **5** when orally administered to Sprague-Dawley rats at a dose of 0.22 mg/kg led to a 2.4-fold increase of the intestinal carrier-mediated transport of talinolol [78]. The limited data obtained so far about a putative interaction between **5** and P-gp and other transporters cannot allow a definitive picture as to whether or not an effective direct interaction occurs. Consequently, more focused studies to evaluate this rather than the accumulation and influx of marker drugs into cells are needed, hopefully with a greater variety of pharmacological models and reference drugs. The current state of knowledge leads the present authors to hypothesize that the observed increases of drug accumulation may be mediated by the effects of bergamottin on CYPs [79].

Bergamottin (**5**) is able to exert its cancer-related effects by means of an additional mechanism. This is the case of gastric cancer, for which a definitive role of the pathogen *Helicobacter pylori* has been ascertained [80]. In this context, Sekiguchi and coworkers first determined how a crucial adhesion factor for the urease of this microorganism, namely, CD74 from gastric epithelial cells, is expressed abundantly in a constitutive manner using the NCI-N87 cancer cell line (human gastric carcinoma), and found that bergamottin, when evaluated at a concentration of 20 μM, inhibited adhesion by 91%, notably without exerting any appreciable cytotoxic effect [80]. Other targets of bergamottin are represented by MMPs. In 2010, Hwang and coworkers investigated the effects of **5** on the growth of the HT-1080 cancer line (human fibrosarcoma) [81]. While having no effects on cell viability in the concentration range of 1–50 μM, bergamottin was able to suppress migration and phorbol 12-myristate-13-acetate (PMA) stimulated invasion of cancer cells by 50% at 50 μM. Other actions induced by PMA were blocked by this coumarin and these included a dose-dependent inhibition of MMP-2 and MMP-9 at concentration levels of >5 μM, an effect also reached by inhibition at the same doses of TNF-α-induced MMP-9 and -2 production. The mechanism underlying this last set of activities relies on a marked decrease of the transcriptional activity of the PMA-induced MMP-9 and MMP-2 expression and on PMA-induced nuclear translocation of NF-κB, both in a dose-dependent manner. Furthermore, bergamottin was shown to suppress PMA-mediated MMP-9 gene activation through the PKC, p38 MAPK, and JNK signaling pathways.

Bergamottin (**5**) also has shown a major influence on the apoptotic process in several cancer cell lines. At an initial concentration of 100 μM after 6 h, this coumarin was able to inhibit the phosphorylation at Tyr 705 of the signal transducer and activator of transcription 3 (STAT3) in the U266 cancer cell line (human, peripheral blood, multiple myeloma) [82]. In the course of this same investigation, it was also found that bergamottin evoked a decrease in STAT3 DNA-binding activity in a dose- and time-dependent manner, inhibiting its translocation to the nucleus. Such an effect could be related to the capacity of **5** to abolish the constitutive phosphorylation of JAK1, JAK2, and Src kinases in a time-dependent manner, which, in turn, suppressed STAT3 activation. U266 cell survival was also affected

by **5** through an inhibitory effect on the phosphorylation of ERK 1/2 and AKT. The negative modulation of STAT3 observed in the U266 cell line resulted in a marked decrease in the genomic expression of pro-inflammatory and pro-carcinogenic stimuli such as COX-2, VEGF, and cyclin D1, and a negative regulation of the antiapoptotic gene products Bcl-2, Bcl-x1, IAP-1, and survivin, and finally the activation of caspase and PARP. As a result of these different and multiple effects, a potent induction of apoptosis was observed for this cancer cell line. Similar activities have also been recorded using other cancer cell lines. In A549 cells, **5** inhibited mitochondrial membrane potential in the range –32.8% to –52.8% at 25 and 50 μ*M* and caspase enzymes and resulted in apoptosis [83]. Induction of apoptosis by bergamottin can also be achieved effectively in combination with other drugs such as simvastatin. As observed by Kim and coworkers in 2016, **5** in combination with this antilipidemic drug led to a marked potentiation of apoptosis evoked by TNF-α in KBM-5 (human chronic myeloid leukemia) cells and suppression of the NF-κB signaling pathway [84].

From the data reported above, it is evident that **5** has been the subject of intensive research investigations. These have allowed a better understanding of its role as an inhibitor of CYPs and, in the case of grapefruit and other citrus fruit juices, why potentially dangerous side effects may occur in patients undergoing chronic drug therapy as a result of the high consumption of such fruits and related food preparations (e.g. juices, jams, etc.). Furthermore, **5** has exhibited some potential as a cytotoxic agent, and is able to negatively modulate key factors of carcinogenesis. Its consumption as a food component for the promotion of food health, should be more intensively investigated, taking into account its potent inhibitory properties on CYPs. Such a side effect might be able to be attenuated by making semi-synthetic derivatives of **5** with enhanced cancer related inhibitory effects (e.g. as P-gp inhibitors, or as negative modulators of pro-inflammatory factors, and as potent inducers

Table 3 Reported biological targets of bergamottin (**5**)

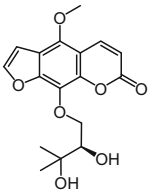
Target		Refs.
CYPs	↓	[56, 69]
MMPs	↓	[82]
STAT3	↓	[83]
ERK1/2	↓	[83]
Akt	↓	[83]
Caspases 3, 8, 9	↑	[83]
PARP	↑	[83]
COX-2	↓	[83]
VEGF	↓	[83]
Cyclin D1	↓	[83]
Bcl-2	↓	[83]
Bcl-x1	↓	[83]
IAP-1	↓	[83]
Survivin	↓	[83]

↑ = activation; ↓ = inactivation

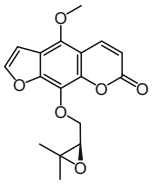
of apoptosis in tumor cells, etc.) and with less affinity for CYPs. Finally, limited information is available in the literature on the potential anti-inflammatory properties of **5**. It is necessary for knowledge of the interaction of this coumarin to be enhanced with pro-inflammatory enzymes such as COX-2 and NOS and their mRNA expression, as has been performed with several other structurally related phenylpropanoids. Indeed, the limited data available, as mentioned above, are concerned with the effects of bergamottin with TNF-α and NF-κB and their mediated signaling pathways. Table 3 summarizes the thus far described mechanisms of action of bergamottin.

3.3 Byakangelicin and Byakangelicol

Byakangelicin (**6**) and byakangelicol (**7**) are two structurally related furanocoumarins that have been mainly isolated from the genus *Angelica* (as an example, see *A. sylvestris* L., Plate 6), comprising several species with well-established use in the traditional Chinese and Ayurvedic systems of medicine [85].



6 ((+)-(*R*)-byakangelicin)



7 ((+)-(*R*)-byakangelicol)

Both compounds show some potential as antidiabetic, anti-inflammatory, and anticarcinogenic agents although much more data on their individual mechanisms of action related to these effects are still required. As a potential antidiabetic agent, **6** has been shown to inhibit aldose reductase in vitro with an IC_{50} value of 6.2 μ*M* [86] and demonstrated in vivo to act as a modulator of Na^+/K^+ ATPase in streptozotocin-induced diabetic rats [87]. More recently, the interaction of byakangelicin with the "classic" target of naturally occurring furanocoumarins, namely CYPs, has been investigated [88]. Coumarin **6**, at concentrations of 5 and 10 μ*M*, induced the genomic expression of CYP3A4 at both the mRNA level (around fivefold) and the protein level (around threefold) in primary cultured human hepatocytes by activation of its promoter in a concentration-dependent manner ($EC_{50} = 5$ μ*M*). Such an activation is increased by co-transfection with human pregnane X receptor (PXR) and effectively achieved by the transactivation of PXR rather than its expression. As a consequence, through this mechanism, byakangelicin is in principle able to augment the expression of all PXR target genes and lead to a wide range of drug–drug interactions.

Plate 6 *Angelica sylvestris*, The Hague, Netherlands. Photograph: TeunSpaans, Wikimedia Commons

Byakangelicol (**7**) has been the subject during recent years of some attention with respect to its mechanism of action at the biomolecular level, in particular for its cytotoxic activity. This coumarin was first seen to be an effective and selective inhibitory agent of COX-2 in A549 cancer cells acting on IL-1β stimulated expression with an IC_{50} value of 20.5 μ*M* and able to suppress NF-κB activity [89]. In Caco-2 cells at a concentration of 10 μ*M*, **7** suppressed P-gp activity as measured by a recorded large decrease in the efflux of quinidine and digoxin [90]. This effect was also confirmed by an in vivo study [91]. Byakangelicol (**7**), when assayed at the same concentration was able to greatly enhance the uptake of calcein acetoxymethyl ester (a substrate of P-gp that is metabolized to calcein by the carrier) into bovine brain microvascular endothelial cells (391%). A similar pattern, with an increase of 1.33-fold of rat blood brain barrier permeation, was recorded using verapamil as the model compound. Moreover, byakangelicol was shown to inhibit β-secretase (BACE-1) ($IC_{50} = 104.9$ μ*M*) [92].

Data reported so far on the mechanisms of action of byakangelicin (**6**) and byakalingelicol (**7**) are still inadequate to allow a proper understanding of their exact role as biologically active constituents of widely used medicinal plants belonging to the genus *Angelica*. Thus, it is hoped that further studies will be performed on these two furocoumarins. In particular, investigations on the

Table 4 Reported biological targets of byakangelicin (**6**) and byakangelicol (**7**)

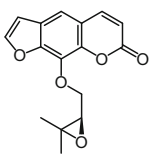
Target		Refs.
Aldose reductase	↓	[87, 88]
PXR	↑	[89]
COX-2	↓	[90]
NF-κB	↓	[90]
GlP	↓	[91, 92]
BACE-1	↓	[93]

↑ = activation; ↓ = inactivation

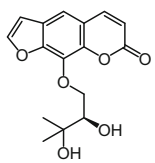
diabetes-related properties of byakangelicin (**6**) as an aldose-reductase inhibitor should be confirmed in more in vivo pharmacological models of diabetes and information about its interaction with PXR should be studied in more detail. Similar considerations for byakangelicol (**7**) are required, and, in particular, how its potential anticarcinogenic activity is related to the inhibition of P-glycoprotein and BACE-1. Importantly, this latter activity is now also well recognized as a key determinant for the pathology of severe neurological syndromes such as Alzheimer's disease [94]. However, the only study reported is too limited in scope to be able to ascribe a potential role of **7** as a neuroprotective agent or as a lead compound for the design of novel alternative BACE-1 inhibitors. Table 4 summarizes the mechanisms of action described to date for both byakangelicin (**6**) and byakangelicol (**7**).

3.4 Heraclenin and Heraclenol

Heraclenin (**8**) and heraclenol (**9**) are two furanocoumarins possessing a functionalized isopentenyloxy side chain and have been isolated primarily from species in the genera *Angelica* (see Plate 6) and *Opopanax* (for example, *O. chironium* Guss. (Apiaceae), Plate 7), of which a number of these plants are used in Chinese traditional medicine [85, 94].



8 ((*R*)-heraclenin)



9 ((*R*)-heraclenol)

Despite their relative abundance in Nature and their well-described possible beneficial effects for human welfare in terms of potential anticancer and anti-inflammatory activities [96], very few reports have appeared in the literature about the mechanisms of action of the coumarins **8** and **9**.

Plate 7 *Opopanax chironium*, Monte Fasce, Genova, Italy. Photograph: Hectonichus, Wikimedia Commons

The cancer-inhibitory properties claimed for heraclenin (**8**) may be due to its strong capacity to interact with DNA. Such an effect was described for the first time in 1986 by Abel and Schimmer [97]. These authors observed that **8** is able to induce sister chromatid exchange and lead to an almost complete block of the metaphase in cultured human lymphocytes when assayed at 20 μ*M*. This may be the result of the presence of the epoxide moiety as other compounds without this functional group were not active. A more complete investigation was provided two decades later by Appendino and coworkers [95]. These authors confirmed the importance of the presence of the epoxide ring for the observed activity. In particular, they established that **8**, applied at concentrations of 10 and 25 μ*M*, arrested mitosis in Jurkat T cells in the G2/M phase without recording appreciable interactions with tubulin depolymerization and/or polymerization. Instead, a large breakdown of DNA structure was observed.

For heraclenol (**9**), the only report published in the literature refers to the anticholinesterase activity of its angelate ester [98]. As observed by Wszelaki and co-workers in 2011 in the course of a bioassay-guided fractionation of extracts from roots and fruits of *Angelica archangelica*, heraclenol-2′-*O*-angelate exhibited

Table 5 Reported biological targets of heraclenin (**8**) and heraclenol (**9**)

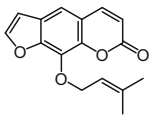
Target		Refs.
DNA breakdown	↑	[96]
BChE	↓	[99]

↑ = activation; ↓ = inactivation

butyryl cholinesterase (BChE) inhibitory activity with an IC_{50} value of 7.5 μ*M*, but the authors did not provide any follow-up biological information. Thus, it is evident that in the case of both **8** and **9** the available data to corroborate their potential to mediate cancer symptoms and eventually serve as a neuroprotective agents are so far not sufficient. More studies are needed to properly validate their mechanisms of action and additional targets must be checked for having an affinity with these compounds, especially when compared with the large amount of data currently known for other oxyprenylated furanocoumarin analogs. Table 5 provides a summary of the mechanisms of action information available for heraclenin (**8**) and heraclenol (**9**) to date.

3.5 Imperatorin

Imperatorin (**10**) is an *O*-isopentenylfuranocoumarin that is widespread in several plant genera belonging to the families Rutaceae and Apiaceae. This phytochemical has attributed to it a plethora of well-documented pharmacological properties. Together with auraptene (**1**) and bergamottin (**5**), imperatorin (**10**) is a candidate for drug development as a member of new generation of agents with potential anticancer, anti-inflammatory, antiviral, and neuroprotective effects [99].



10 (imperatorin)

The first study on the enzymatic inhibitory properties of this coumarin was carried out in 1993, when Cai and co-workers demonstrated that **10** is a potent negative modulator of EROD and pentoxyresorufin-*O*-dealkylase (PROD) [100]. In particular, it was shown that **10** is a highly active inhibitor of this latter enzyme with an IC_{50} value of 54 n*M* (IC_{50} = 2.32 μ*M* for EROD). In the same year, Maenpaa and co-workers revealed that **10** is a moderate inhibitor of CYP2A5 activity (IC_{50} = 40 μ*M*) [101]. From these preliminary observations, the attention of researchers turned to the consequences of the inhibition of CYPs by **10**, namely, by preventing the metabolic activation of known carcinogens. Consequently, in 2001 Kleiner and co-workers confirmed the above-mentioned inhibitory in vitro data obtained for **10** against EROD and PROD, but extended this work by

performing in vivo studies on the oral administration of **10** to SENCAR mice at a dose of 70 mg/kg daily for 4 consecutive days. They also measured the activities of these enzymes in several tissues [102]. After 1 h from the time of compound administration, EROD activity decreased by 40% in the lung and this percentage was maintained after 24 h. A similar pattern was recorded in the epidermis (–45%), and forestomach (–21%). Values were higher in the case of PROD inhibition: –72%, –52%, and –48% in the lung, epidermis, and forestomach, respectively. The liver content of CYP450 increased to 49% as did GSH activity (1.6-fold) in the same organ. As a consequence, **10** inhibited the linkage of DMBA to DNA, although only to an appreciable extent in the forestomach (–49%), while it had no discernible effect on the formation of B[*a*]P-DNA adducts. A similar pattern of effects was recorded in MCF-7 cells by the same research group [73]. In particular, these authors also observed that **10** is able to inhibit a wide panel of CYPs comprising the 1A1 (IC_{50} = 2.76 μ*M*), 1B1 (IC_{50} = 0.71 μ*M*), 1A2 (IC_{50} = 0.38 μ*M*), 2A6 (IC_{50} = 11.7 μ*M*), 2B6 (IC_{50} = 0.32 μ*M*), and 3A4 (IC_{50} = 0.53 μ*M*) isoforms. Subsequently, it was revealed that, due to these properties, **10** at a concentration of 10 μ*M* blocked DMBA-DNA adduct formation by 63%, but higher concentrations were needed (20 and 40 μ*M*) to also inhibit B[*a*]P DNA adduct formation (37% and 70%). A similar range of inhibitory capacities towards DNA adduct formation was observed also in skin tissues from SENCAR mice (43% and 81% inhibition of DMBA-DNA adducts at concentrations of 100 and 1600 n*M*) [73]. Some of the above-reported results have been confirmed and enforced by subsequent studies [103, 104], which also revealed inhibitory capacities of imperatorin (**10**) on other isoforms such as CYP2C9 (IC_{50} = 7.0 μ*M*) [105], and in addition the basis for in vivo studies on the protective effects by the diet with procarcinogen activation by CYPs by **10** and imperatorin-enriched plant extracts. In 2006, Prince and co-workers investigated the inhibitory capacities on the formation of DMBA-DNA adducts promoted by CYPs in female SENCAR mice mammary glands [106]. These authors administered **10** at a dose of 150 mg/kg prior to the administration of DMBA (50 μg as a single dose or 20 μg daily for 3 and 6 weeks). Upon application of the single dose of DMBA, **10** blocked the formation of the corresponding adduct with DNA by 41%, while this percentage increased upon application of multiple doses to 55%. The CYP isoforms mainly inhibited by the dietary administration of **10** were CYP1A1 and 1B1. A concomitant induction of liver GST was also observed, probably via the Nrf2/ARE mechanism [34].

Imperatorin (**10**) can act as an antiproliferative agent by several other mechanisms. Among these, there is the induction of apoptosis in the G1/S transition phases of the cell cycle [95] and this was assessed in several human cancer cell lines. In 2011, Luo and coworkers determined that **10** is able to induce apoptosis in a time- and dose-dependent manner acting on death-receptor and mitochondria-mediated pathways [107]. Hep-G2 cells were the most sensitive to this phenomenon among a wide panel of human cancer cell lines and were therefore selected for further studies. When administered to cells at concentrations of 30 and 60 μ*M*, **10** induced apoptosis accompanied by extensive DNA fragmentation, activation of caspases 3, 8, and 9, inhibition of MMP, a decline of the expression of the antiapoptotic

protein Bcl-2, release of cytochrome c, and interference with mitochondrial membrane functionality. Similar results were obtained in T98G glioma cells in which imperatorin induced approximately 20% of apoptosis at concentrations from 25 to 50 μ*M* [108]. Furthermore, using the same cancer cell line, a more potent effect was revealed when **10** was added into the culture medium with quercetin at the same doses. This combination led to the significant induction of each of caspases 3, 8, and 9, and to a large decrease of the expression of heat shock proteins (HSP) 27 and 72. In vitro and in vivo, imperatonin (**10**) triggered Mcl-1 in human resistant hepatoma cells, producing proteosomal degradation, and evoking the release of the proapoptotic proteins Bak and Bax [109]. The action on Mcl-1 also reverses the resistance to several chemotherapeutics such as cisplatin in hepatocellular carcinoma and enhances their toxicity in other cancer cell lines [110]. Promotion of caspase activity has also been observed in the colon cancer cell line HT-29 [111]. In lung cancer cells (H292 and A549 lines), imperatorin, at a concentration of 1 μg/cm^3, produced an increase of p53 protein levels and exhibited a strong inhibitory effect on the anchorage-independent growth of cancer cells, diminishing their metastatic potential [112].

Another factor related to imperatorin-promoted cancer cell growth inhibition is that the dose-dependent abolishment of HIF-1α was observed in HCT116, HeLa, and Hep3B cells [113]. This effect was deemed as being due to an impairment of protein synthesis, in turn linked to a largely diminished target gene expression. This effect on HIF-1α led to a down-regulation of the mTOR/p70S6K/4E-BP1 and MAPK signaling pathways and abolition of angiogenesis. Another target accounting for the observed antiproliferative activity by **10** is P-gp, for which a marked inhibition was observed in MDCK-MDR1 Madin-Darby canine kidney cells. The strong affinity of coumarin **10** for the carrier was also confirmed by docking studies [114].

Closely related to the above reported cancer-related effects and mechanisms are the anti-inflammatory effects exerted by **10**. The first recognized target of this coumarin was COX-2. In a paper published in 2003 by Ban and coworkers, evidence on how **10** was able to abolish prostaglandin (PG)E2 in LPS-stimulated rat peritoneal macrophages was presented [115]. When assayed at a concentration of 30 μ*M*, **10** performed slightly better than the well-known and widely used non-steroidal anti-inflammatory drug (NSAID) indomethacin (assayed at a concentration of 0.1 μ*M*), which was used as a control substance. The effect by **10** was ascribed as being due to inhibition of the LPS-induced expression of COX-2 and microsomal PGE synthase. The expression of another enzyme that plays a crucial role in the progression of inflammatory processes, namely, iNOS, is also inhibited by imperatorin, using RAW 264.7 murine macrophages as the laboratory model [116]. In the course of a study to demonstrate such activity, the in vivo anti-inflammatory properties of **10** were investigated. Employing the widely used carrageenan-evoked paw edema model in mice, **10** effectively counteracted the progression of the inflammatory process and completely prevented edema formation when administered topically to animals at a dose of 10 mg/kg 5 h prior to the application of a promoter. Notably, the effect was of greater magnitude than that evoked by indomethacin used as control at the same

concentration. The inhibition of genomic expression of both iNOS and COX-2 was also observed in vivo with percentages of 79.8 and 70.3%. Histopathological examination revealed that **10** largely decreased the number of inflammatory cells with no infiltration into the intercellular space. Its antiedema effect was accompanied by a marked decrease in malondialdehyde (MDA) content, and the release of TNF-α, in addition to the enhancement of activity of antioxidant enzymes inclusive of superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPx). A similar effect of a NO-mediated signaling pathway was observed using another pharmacological model [117]. A contribution to the inhibitory properties by **10** on the expression of genes encoding iNOS and COX-2 has come from its capacity to modulate other components of the inflammatory machinery. In RAW 264.7 cells, imperatonin (**10**) resulted in a decrease of interleukin (IL) release, in particular of IL-1β, IL-6, and IL-10 as well as of TNF-α, when assayed at the concentration range 25 to 40 μg/cm^3. Surprisingly, at an expression level, only TNF-α and IL-6 were affected. Moreover, using the same model, **10** was able to suppress activation of NF-κB and the phosphorylation of MAPK. This effect was also confirmed in a number of in vivo experiments, namely, the dimethylbenzene-induced ear edema, the acetic acid-induced vascular permeability, and the formation of cotton pellet granuloma in mice [119] models, and also in cultured cancer (HeLa) cells [120]. In addition, **10** proved to be an efficient IL-2 gene transcription inhibitor, with this effect caused by its capacity to inhibit the binding of nuclear factor of activated T-cells (NFAT) to DNA and related transcriptional events [121].

Moreover, imperatorin (**10**) also possesses an ability to modulate the immune response to inflammatory processes and in general to stress stimuli. Oh and co-workers in 2011 demonstrated that this coumarin, when orally administered at dose levels of 0.1 and 1 mg/kg, in an ovalbumin (OVA)-induced allergic rhinitis (AR) model in mice, led to a decrease of IgE, IL-1β, IL-4, and histamine release, and showed a marked reduction of spleen weight. It also diminished the content of pro-inflammatory chemotactic factors such as macrophage-inflammatory protein 2 (MIP-2) and the expression of intercellular adhesion molecule-1 (ICAM-1), and slowed down the infiltration of eosinophils, mast cells, macrophages, and T-cells, and finally, partially accounted for all the previously listed effects, by substantially decreasing the activity of caspase-1 in mice nasal mucosa tissue [122]. As a confirmation of the immunomodulatory properties of **10**, in 2015 Jeong and coworkers assessed the biomolecular basis by which this phytochemical is able to suppress degranulation and biosynthesis of leukotriene (LT) (in particular LTC4) and eicosanoids (PGD2) in IgE-activated bone marrow-derived mast cells (BMMC) [123]. Imperatorin (**10**) largely diminished the degranulation and production of 5-lipoxygenase (LOX)-dependent LTC4 and COX-2-dependent PGD2, abolishing intracellular Ca^{2+} influx, and in turn stimulating the activity of phospholipase Cγ1, and inhibiting cytosolic phospholipase A2 (PLA-2)/mitogen-activated protein kinases and the NF-κB pathways in this cell line. As a further contribution to the immunomodulatory mechanisms of **10**, in 2016 Lin and co-workers, in a murine model of OVA-induced asthma, observed that this coumarin is able to effectively counteract symptoms such as airway hyper-responsiveness and inflammation

through reduction of pro-inflammatory chemokines (among which were IL-1β, IL-6, IL-12 and TNF-α), and to produce an increase of IL-10-producing regulatory T cells [124].

Notable are the potential neuroprotective properties displayed by imperatorin (**10**). The first report of this was provided by Deng and co-workers in 2006 [125]. These authors asserted that, in the course of a bioactivity-guided fractionation procedure of a root extract from a Chinese *Angelica* species, this coumarin, at a concentration of 10 μ*M*, exhibited selective agonistic properties on 5-HT_7 receptors, in being able to displace by 57% of the binding of [^{3}H]LSD. The interaction of **10** with the serotoninergic system was further investigated in 2017 by Cao and co-workers [126]. They used three behavioral tests (i.e. the sucrose-preference test, the forced-swimming test, and the open field test) in prenatally stressed offspring rats followed by serotonin (5-HT) measurements in key areas of the central nervous system (CNS), such as the hippocampus and frontal cortex. These authors determined that the intragastric administration of **10** at dose levels of 15 and 30 mg/kg/day for 4 weeks led to major increases of 5-HT in these two regions as well as an enhancement of 5-HT1AR mRNA expression, accompanied by a decrease in the concentration of 5-HT membrane transporters. This can account for the observed antidepressant effects of **10** in rats. Another system modulated by **10** is metabotropic glutamate (mGlu) receptor one. In 2008, Wang and coworkers observed that **10** in depolarized rat hippocampal nerve terminals evoked Glu release promoted by 4-aminopyridine in a dose-dependent manner and with an IC_{50} value of 4.7 μ*M* [127]. This observation was linked to an enhancement of vesicular exocytosis rather than by an increase via Glu transporter-mediated Ca^{2+} influx. Furthermore, imperatorin activated N-and P/Q-type Ca^{2+} channels, thus providing the observed activation of Glu release. The same research group confirmed these observations by investigations performed subsequently, and suggested a possible role in the facilitation of Glu release by Ca^{2+}/calmodulin dependent kinase II (CaMKII) and synapsin I [128].

Imperatorin (**10**) has been seen to exert efficient anticonvulsive effects [129]. The underlying mechanism of action seems to rely on its capacity to interact with $GABA_A$ receptors [130] and to potentiate GABA activity by inhibiting GABA-transaminase [112]. Imperatorin can also increase melatonin activity in vivo indirectly by inhibiting its oxidative catabolism by CYP 1A1, 1A2, and 1B1 [132]. By its significant permeability to the blood brain barrier by passive and carrier-mediated transport [133], **10** may be able to effectively contribute to prevent neuronal loss and death. This may occur during cerebral ischemia, during which cells of the CNS are exposed to a period of hypoxia followed by re-oxygenation. Imperatorin treatment in the oxygen glucose deprived/reperfusion (OGD-R) SH-SY5Y cell line led to an inhibition of apoptosis by its capacity to dose-dependently decrease levels of the pre-apoptosis factor Bax and its downstream factor caspase-3 and upregulate the Bcl-2 protein levels [134]. The antiapoptotic effects by imperatorin rely also on its capacity to negatively interfere with ERK signaling, in turn leading to an inhibition of NMDA receptor/intracellular calcium influx [135, 136]. Such effects might also be useful to enhance the potential of **10** as a neuroprotective agent against severe

neurological syndromes such as Alzheimer's disease (AD). As shown by Budzynska and coworkers in 2013, **10** was able to protect male Swiss mice from scopolamine-invoked memory deficit measured using a passive avoidance (PA) paradigm employed in an animal model of AD [137]. When administered acutely at doses of 5 and 10 mg/kg prior to the injection of scopolamine (1 mg/kg), **10** improved memory acquisition and consolidation as disrupted by this tropane alkaloid. Chronic (7 days, twice daily) administration of the highest dose (10 mg/kg) significantly ameliorated the effects of scopolamine on memory acquisition. Furthermore, imperatorin increased the activities of SOD and GPx in the cortex and hippocampus and decreased also the level of NMDA. Beneficial effects against AD may also be due to the capacity of **10** to inhibit the activity of key enzymes like BACE-1 [92] and/or to promote one of the cholinesterases [138]. In particular, this interaction has been studied in detail and it was shown that imperatorin inhibits AChE in a non-competitive way with an IC_{50} value of 63.7 μ*M* [140] and BChE with an IC_{50} value of 14.4 μ*M* [98]. This large difference of activity against these two forms of cholinesterase may be due to the different structures of the active site of the two enzymes, in which the bulkier 3,3-dimethylallyloxy side chain of **10** does not fit optimally into the smaller pocket of AChE when compared to BChE.

Other than interacting with specific receptors, **10** was also found to block the functionality of ion channels, in particular those conducting cations. The first observation of this type came from investigations carried out by Zhang and coworkers [140]. These authors used spontaneously hypertensive rats (SHRs) as the pharmacological model in which the chronic administration of **10** at concentrations ranging from 6.25 to 25 mg/kg led to a decrease of the systolic blood pressure by approximately 40 mm Hg. In isolated aortic rings, this coumarin exerted a pronounced vasodilatory effect. The basis for these observed activities relies on the ability of imperatorin to trigger L-type Ca^{2+} channels, providing a substantial decrease in currents mediated by these channels and a large depletion of cytosolic Ca^{2+} content. Two years later, Wu and co-workers studied the interaction of **10** with voltage-gated Na^{+} channels (VGSC) in differentiated neuronal NG108-15 cells [141]. Imperatorin blocked VGSC in a dose-dependent manner in its closed state at a dose range of 10 to 100 μ*M*. Furthermore, this coumarin reduced neuronal excitability in CA1 hippocampal cells from neonatal rats, suppressing Na^{+} currents when applied at a concentration of 30 μ*M*. The same research group also studied the interaction of imperatorin with voltage-gated K^{+} channels (K_v) and ATP-sensitive K^{+} channels (K_{ATP}) in differentiated neuronal NG108-15 cells [142]. Coumarin **10** exerted an inhibitory effect on K_v in a similar way to that observed for VGSC without affecting the activation gating. The block of K_{ATP} occurred only at the highest concentration tested (100 μ*M*).

The inactivation of cationic channels may also contribute to the analgesic properties of imperatorin (**10**) reported, together with its interaction with transient receptor potential vanilloid type 1 receptors (TRPV1) [143]. Compound **10** is an agonist of such receptors with an IC_{50} of 12.6 μ*M*, acting on a site different from that binding capsaicin. Furthermore, imperatorin delayed the TRPV1 recovery from desensitization, but sensitized the same to acid activation without affecting current

amplitudes. Moreover, the effects on cationic channels represent the basis of the observed vasodilatory and anti-hypertensive activities of imperatorin. These properties of **10** have been seen both in vitro, using isolated pre-contracted rat arterial rings, and in rat isolated jejunum strips [144–146] and in vivo in hypertensive rats. However, other mechanisms may contribute to the vasodilatory properties of **10**. First, this coumarin is able to negatively modulate the release of NO, inhibiting NOS in the denuded endothelium from the rat aorta [147]. Second, in vivo studies have revealed that imperatorin is an efficient antihypertensive agent acting on mRNA expression and the protein levels of NADPH oxidase [148], inhibiting the MAPK pathway, and exhibiting antioxidant properties (enhancement of CAT, SOD, and GSH levels and decreases in the NMDA concentration and the release of PGs) [149].

A last set of data on the pharmacological properties of imperatorin (**10**) and the related mechanisms of action, concerns its activities on the metabolism of sugars and lipids. While investigations of the effects of this coumarin on mitochondrial function date back to more than 50 years, indicating an inhibition towards an enzyme that is part of the Krebs cycle [150, 151], other investigations are more recent. Imperatorin (**10**) has modulating properties on lipid biosynthesis acting on squalene production and inhibiting squalene synthase promoter activity [152]. Such an activity is the result of the inhibition of the phosphorylation of Akt by insulin growth factor-1 (IGF-1), and down-regulation of PPAR-γ and sterol regulatory element-binding transcription factor 1 (SREBP-1), the main transcriptional factors for lipid biosynthesis. In Chinese hamster ovarian cells (CHO), **10** stimulated glucose uptake. This effect was confirmed in in vivo studies using streptozocin type 1-induced like diabetic rats, in which a significant decrease of glucose concentration in blood was observed [153]. The basis of this effect consisted of the activation of glucagon-like peptide 1 (GLP-1), in turn evoked by activation of G-protein-coupled bile acid receptor 1 (GPBAR1) and G-protein-coupled receptor 119 (GPR119).

Other reported activities of **10**, although the description of which is limited to only one report in each case, include anti-HIV effects, related to the capacity of **10** to inhibit the Sp1 viral transcription factor [154], and promotion of osteogenesis in vivo via the p38 and ERK-dependent pathway in osteoblasts [155]. However, neither of these pharmacological properties has been the topic of any further investigations during the last decade.

Imperatorin (**10**) currently is the most extensively investigated prenyl-oxy-coumarin and phenylpropanoid in general, from both a pharmacological point of view and for its composite mechanisms of action. More than 45 different biological targets, mainly receptors and enzymes, have been identified for this furocoumarin thus far. Accordingly, it can be stated that **10** exerts a pleiotropic activity. Ultimately, this may limit its future development for effective therapeutic use, however. Imperatorin (**10**), with its multiple effects, can be considered as a template molecule for a host of potential cancer inhibitory, anti-inflammatory, neuroprotective, lipid blood lowering, and antidiabetic drugs, which could be developed to possess more selective and more highly targeted modes of action. Such studies have been carried out in several laboratories [138, 156] and it can be predicted that the number of studies on semi-synthetic imperatorin derivatives will increase greatly in the coming

Table 6 Reported biological targets of imperatorin (**10**)

Target		Refs.
CYPs	↓	[37, 74, 101–107, 133]
DNA breakdown	↑	[108, 109]
Caspases 3, 8, 9	↑	[108, 109, 112]
MMPs	↓	[108, 109]
Bcl-2	↓	[108, 109]
Mcl-1	↓	[110, 111]
HIF-1α	↓	[114]
GlP	↓	[115]
COX-2	↓	[116]
NOS	↓	[117, 148]
TNF-α	↓	[118, 125]
SOD	↑	[118, 150]
CAT	↑	[118, 150]
GPx	↑	[118, 150]
NF-κB	↓	[118]
MAPK	↓	[118, 150]
MIP-2	↓	[123]
ICAM-1	↓	[123]
Caspase 1	↓	[123]
LOXs	↓	[124]
COX-2	↓	[124]
PLA-2	↓	[124]
ILs 1β, 6, 12	↓	[125]
IL-10	↑	[125]
$5HT_7$ receptor	↑	[126, 127]
mGlu	↑	[128]
Ca^{+2} channels	↑	[128]
$GABA_A$ receptor	↑	[131]
GABA-T	↓	[132]
Caspases 3, 8, 9	↑	[135]
ERK1/2	↓	[136, 137]
SOD	↑	[138]
GPx	↑	[138]
BACE-1	↓	[93]
AChE, BChE	↑	[99, 139, 140]
Na^+ channels	↑	[142]
K^+ channels	↑	[143]
TRPV-1	↑	[144]
NADPH oxidase	↓	[149]
Squalene synthase	↓	[153]
Akt	↓	[153]
IGF-1	↓	[153]

(continued)

Table 6 (continued)

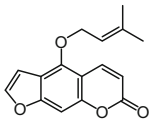
Target		Refs.
PPAR-γ	↓	[153]
SREBP-1	↓	[153]
GLP-1	↑	[154]
GPBAR-1	↑	[154]
GPR119	↑	[154]

↑ = activation; ↓ = inactivation

years. Table 6 summarizes the mechanisms of action described to date for imperatorin (**10**).

3.6 Isoimperatorin

Isoimperatorin (**11**), the 5-substituted isomer of imperatorin (**10**), is particularly abundant in Nature, in plants belonging to the genera *Citrus* (like lemon and lime), *Prangos*, and *Ferulago* (for example, see *F. campestris* Grec. (Apiaceae), Plate 8), and several others [157].



11 (isoimperatorin)

Although few studies concerned with the mechanisms of action of this compound have been so far reported in the literature, those that have appeared reveal the potential of **11**. When considering its anticarcinogenic properties, the first observation was reported in 1997, when Cai and co-workers assessed that **11** is able to reduce the formation of water-soluble metabolites of the carcinogen B[*a*]P by 59% [100]. After this preliminary result, 20 years later Tong and co-workers reported that the basis of the growth inhibitory effects of **11** are consistent with enhancements of the expression levels of the proapoptotic proteins Bax, caspase 3, and caspase 9 and a reduction of the antiapoptotic factors survivin and Bcl-2, when this coumarin was administered to cultured SGC-7901 (human gastric carcinoma) cancer cells in doses ranging from 5 to 20 μg/cm^3 [156]. Such activity was also observed in vivo. The anticarcinogenic properties of **11** may also contribute to its capacity to inhibit CYP2B6 in a time-, concentration-, and NADPH-dependent manner (60% at 25 μ*M*) [159] as well as CYP1A1, although in this latter case a completely different mechanism of action was observed [160]. Studying the potential cancer chemopreventive effect of coumarin (**11**) against aflatoxin B1 (AFB1)-induced cytotoxicity in H4IIE (*Rattus norvegicus* liver hepatoma) cells, it was found that isoimperatorin,

Plate 8 *Ferulago campestris*, Monte Fasce, Genova, Italy. Photograph: Hectonichus, Wikimedia Commons

when administered to cultured cells in the concentration range 0.3–30 μ*M*, increased GST activity in a concentration-dependent manner, via activation of Nrf2/ARE, with a concomitant inactivation of CYP1A1. Moreover, **11** was found to be an effective inhibitor of EROD and PROD, with IC_{50} values of 1.4 and 1.3 μ*M* [100]. Furthermore, **11** possesses potential anti-inflammatory activity in being able to reduce PGE2 and PGD2 production by COX-2 ($IC_{50} = 10.7$ μ*M*) [114] and LTC4 biosynthesis by 5-LOX ($IC_{50} = 5.7$ μ*M*) [161], thus revealing its dual action on the main factors responsible of the progress of inflammatory processes. Isoimperatorin is also able to reduce ROS production in the human umbilical endothelial cell line, EA.hy926 [162]. In particular, **11** effectively inhibited TNF-α-activated VCAM-1, activated the expression of PPAR-γ, and also abolished phosphorylation of ERK1/2 and Akt.

Isoimperatorin has shown promising neuroprotective effects and has potential as a therapeutic agent against chronic neurological syndromes like AD. This compound exerted weak inhibitory activities on the key enzymes ACh-E with an IC_{50} value of 63.7 μ*M* [139], BACE-1 ($IC_{50} = 24.4$ μ*M*) [92], and GABA-T (IC_{50} not reported) [131].

Considering its wide distribution in Nature [157], studies on the biological effects of isoimperatorin (**11**) are still quite sparse, in particular with respect to its mechanism of activation and the identification of its cellular targets. Data for the potential of this compound as an anticarcinogenic agent are limited and such effects still remain to be properly established and validated. Thus, it is hoped that in future further studies on the interaction of **11** with a wider panel of biological targets will be carried out. It would be also interesting to compare the activities displayed by imperatorin (**10**) and isoimperatorin (**11**) and to assess the exact role played by the position of the geranyloxy side-chain with respect to the observed effects by these two coumarins. The need for more studies is even more urgent in terms of the putative activity of **11** on the central and peripheral nervous systems. Indeed, only

Table 7 Reported biological targets of isoimperatorin (**11**)

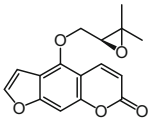
Target		Refs.
Bax	↑	[159]
Caspases 3, 8, 9	↑	[159]
Survivin	↓	[159]
Bcl-2	↓	[159]
CYP2B6	↓	[160]
CYP1A1	↓	[101, 161]
GST	↑	[101]
COX-2	↓	[162]
LOXs	↓	[162]
TNF-α	↓	[163]
PPAR-γ	↑	[163]
ERK-1/2	↓	[163]
Akt	↓	[163]

↑ = activation; ↓ = inactivation

three reports are currently available and these have employed only enzymatic assays. A deeper knowledge of the potential of **11** as a biologically active agent is needed to probe further the nutraceutical value of the many edible fruits and vegetables in which this coumarin is a component. Table 7 summarizes the described mechanisms of action of isoimperatorin (**11**) established to date.

3.7 Oxypeucedanin

Oxypeucedanin (**12**) is a linear furanocoumarin having an epoxide ring in the oxygenated-side chain. It can be found commonly in plants belonging to the genus *Angelica* genus (for example, see Plate 6) [157].



12 ((+)-(*S*)-oxypeucedanin)

As for some of the previously mentioned compounds, there are few literature reports on the mechanisms of action underlying the observed biological effects of oxypeucedanin (**12**). Growth-inhibitory properties on different human cancer cell lines by prevention of the incorporation of inorganic phosphate into relevant biomolecules have been claimed [163], and **12** was also found to exert anti-inflammatory properties that may contribute to its potential anticarcinogenic activity. These comprise inhibition of PGE2 biosynthesis by COX-2 (IC_{50} not reported) [115], inhibition of NO production in IFN-γ and LPS-stimulated RAW 264.7 macrophages [164], and modulation of gene expression of proteins of the MAPK

signaling pathway [165]. In particular, this latter effect was studied by Choi and co-workers in mouse neuroblastoma neuro-2A cells, in which some proteins were downregulated by the action of oxypeucedanin administered to cells at a concentration of 10 μ*M*. These included many ribosomal subunit proteins that were upregulated and among these several are responsible for the phosphorylation of target proteins, namely, Mapk1 (Erk2), Mapk14 (p38 MAPK-α), dual specificity phosphatase 4 (Dusp4), thymoma viral proto-oncogene 1 (Akt1), and Akt interacting protein (Fts), and those for intracellular protein trafficking (e.g. protein transport protein Sec23B (Sec23b), protein transport protein Sec61 subunit α isoform 1 (Sec61a1), and protein transport protein Sec61 subunit γ (Sec61g) and for mitochondrial protein transport (e.g. translocase of outer mitochondrial membrane 40 homolog (Tomm40), translocase of inner mitochondrial membrane 10 homolog (Timm10), and Timm16). Furthermore, and notably, genes encoding proteins regulating the mitochondrial electron transport chain were all downregulated and these included NADH dehydrogenase (ubiquinone) 1α subcomplex subunit 2 (Ndufa2), ubiquinol-cytochrome c reductase complex, subunit X (Ucrc), and ubiquinolcytochrome c reductase.

As with several other furanocoumarins, oxypeucedanin (**12**) also has exhibited potential as a neuroprotective agent, in exerting a mild inhibitory activity on AChE with an IC_{50} value of 89.7 μ*M* [139], and in having a moderate affinity for $GABA_A$ receptors expressed in *Xenopus laevis* oocytes ($EC_{50} = 25.0$ μ*M*) [130]. In particular, for this latter effect. a potentiation of the receptor affinity of 550% with respect to the control was observed. It is also noteworthy that **12** binds to $GABA_A$ receptors at a site different from that of the benzodiazepines, and its action is not antagonized by flumazenil. Moreover, the epoxidized *O*-side chain is a key structural element for the potent affinity observed (cf, the longer sidechain in imperatorin (**10**) did not lead to any effects of this type).

Finally, antiarrhythmic activity reported for **12** is due to a direct action of this coumarin on human Kv1.5 (hKv1.5) channels and to a prolongation of the cardiac action potential duration (APD) [168]. In particular, oxypeucedanin (**12**) decreased the hKv1.5 current in a concentration-dependent manner ($IC_{50} = 76$ n*M*). Furthermore, **12** induced an initial fast decline of the hKv1.5 current during depolarization. The observed inhibition of the hKv1.5 channel was voltage-dependent, especially with depolarizing pulses between –40 and 0 mV, which corresponded to the voltage range of the opening of the channel. Moreover, **12** prolonged the APD of rat atrial and ventricular myocytes in a dose-dependent manner.

From these data reported above, it is clear that oxypeucedanin (**12**) has been investigated more for its effects on the central and peripheral nervous system and much less for its possible anticarcinogenic and putative anti-inflammatory activities. In particular, the well-characterized and affinity for the $GABA_A$ receptor led to the hypothesis that **12** could serve effectively as a template for the design and synthesis of a new generation of anxiolytic drugs to act as alternatives to benzodiazepines, as well as having possible general use as neuroprotective agents. However, this must first be corroborated by further in vitro and in vivo studies. When compared to reported data on other prenyloxyfuranocoumarins, further evidence for the

Table 8 Reported biological targets of oxypeucedanin (**12**)

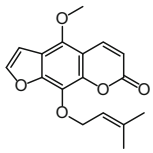
Target		Refs.
COX-2	↓	[116]
NOS	↓	[165]
MAPK	↓	[166]
AChE	↓	[140]
$GABA_A$ receptor	↑	[131]
K^+ channels	↑	[167]

↑ = activation; ↓ = inactivation

interaction of **12** with biological targets that play a crucial role in cancer pathogenesis and inflammation is required. It should be noted that the presence of an epoxide ring may be a fundamental structural element for the efficacy of compound **12**. Together with the planar moiety and overall high lipophilicity, this feature may enable oxypeucedanin (**12**) to interact irreversibly with several cellular targets. Table 8 summarizes prior studies on the mechanisms of action of oxypeucedanin (**12**).

3.8 Phellopterin

Phellopterin (**13**) is a methoxy-substituted furanocoumarin found widely in plants of the genus *Angelica* (for example, see Plate 6) as well as in many other species of the family Apiaceae [167].



13 (phellopterin)

The best described mechanism of action of this coumarin relates to its documented anti-inflammatory activity. A first report of this was the stated capacity of **13** to inhibit the production of prostaglandin E2 by COX-2 (IC_{50} not reported) [115]. Some years later, it was demonstrated that **13** possesses inhibitory properties on VCAM-1 expression in a dose-dependent manner leading to the total abolishment of activity on administration to cultured cells at a dose of 50 μ*M* [169]. Through this effect, **13** was able also to reduce (2.5-fold) the TNF-α-induced adhesion of human monocyte U937 cells to HUVEC cells. Furthermore, this coumarin also showed activity against NO production by iNOS ($IC_{50} = 38.3$ μ*M*), due to a dose-dependent inhibition of mRNA expression of this enzyme in the concentration range of 15–60 μ*M* in RAW 264.7 macrophages [169, 170].

The interaction of **13** with CYPs has not been characterized in much depth when compared to many other furanocoumarins. In the only report published so far using melatonin as the substrate, this coumarin was found to inhibit the activity of CYP 1A2, 1A1, and 1B1 with a K_i of 6.34 n*M* in human liver microsomes from healthy human subjects [132]. Other effects on human metabolism included evidence for an effective antidiabetic activity. Thus, **13** was found to decrease glucose blood concentrations in streptozocin type-1-induced diabetic like rats [153]. Coumarin **13** activated GLP-1, GPBAR1, GPR119, and, more notably, insulin secretion in vitro. Such results were then confirmed by in vivo experiments for which **13** improved glucose tolerance in diabetic db/db mice.

Phellopterin (**13**) exhibits a high affinity towards the $GABA_A$ receptor and behaves as a partial agonist [171, 172]. This coumarin was able to inhibit the binding of [^{3}H]diazepam at the benzodiazepine receptor binding site, with an IC_{50} value of 360 n*M*. Phellopterin (**13**) also exhibited a mild inhibitory effect on GABA-T [132].

Phellopterin (**13**) may effect several organs and tissues in order to exert potential cancer inhibitory, anti-inflammatory, antilipidemic, antidiabetic, and neuroprotective effects. However, data on the mechanisms of action underlying such biological activities are too scarce to allow any insight on the therapeutic potential of this coumarin as a useful pharmacologically active laboratory probe or lead compound. Thus, many more studies are needed on phellopterin using a broad range of in vitro and in vivo experimental models. Table 9 summarizes literature reports on the mechanisms of action described for phellopterin (**13**).

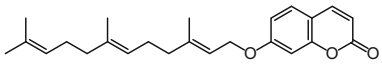
Table 9 Reported biological targets of phellopterin (**13**)

Target		Refs.
COX-2	↓	[116]
VCAM-1	↓	[169]
TNF-α	↓	[169]
NOS	↓	[169]
CYPs	↓	[170, 171]
GLP-1	↑	[133]
GPBAR-1	↑	[133]
GPR119	↑	[133]

↑ = activation; ↓ = inactivation

3.9 Umbelliprenin

Umbelliprenin (**14**) is a rare example of a coumarin bearing a farnesyloxy side chain and it is found particularly in plants belonging to the genus *Ferula* (for example, see *Ferula communis* L. (Apiaceae), Plate 9) [173].



14 (umbelliprenin)

The so far reported data on the mode of actions of coumarin **14** mainly concern its possible anticarcinogenic activity. In 2006 Shahverdi and co-workers, further to the bioassay-guided fractionation of a chloroform extract of *Ferula persica* var. *persica*, determined that **14** at a concentration of 51 μg/cm^3 is able to block fibrosarcoma Wehi-164 cell invasion, and this effect was ascribed as being due to inhibition of MMP-2 [174]. Two years later, the same research group observed that **14** caused the

Plate 9 *Ferula communis*, near Zavitan river, Golan Heights, Israel. Photograph: Iorsh, Wikimedia Commons

arrest of the cell cycle in the G1 phase in the M4Beu (metastatic pigmented malignant melanoma) cell line when applied at a concentration of 25 μ*M* and that this was due to an induction of apoptosis by enhancement of caspase activities [175]. Other results related to the antineoplastic activity of this coumarin have emanated from in vivo studies. In 2014, Khaghanzadeh and coworkers used the Lewis lung cancer mouse model to investigate the effects of **14** administered i.p. at a dose of 2.5 mg/kg and measured the levels of mRNA expressions of pro-inflammatory and pro-carcinogenic factors such as Foxp3, TNF-α and TGF-β as well as the release extent of IL-10, IFN-γ and IL-4 [176]. The expression of TNF-α in tumor tissues was 35-fold higher than in the untreated group of animals while that of Foxp3 decreased by 20-fold and that of TGF-β by 2.2-fold. The effect on chemokine production on the other hand was only slightly pronounced with respect to the control. The same research group investigated subsequently the effects of administration of **14** (at a concentration of 31 μ*M*) for two types of human lung cancer cell lines, namely, QU-DB (large-cell lung carcinoma) and A549 (adenocarcinoma), specifically on the expression of immune-associated proteins using two-dimensional electrophoresis coupled to mass spectrometry [176]. In QU-DB cells, a downregulation of HSP90, HSP27, endoplasmin, vimentin, heterogeneous nuclear ribonucleoproteins (hnRNP) C1/C2, transitional endoplasmic reticulum ATPase (p97/VCP), NADH dehydrogenase [ubiquinone] iron-sulfur protein-3 (NDUFS3), importin-α2, importin-β1, tubulin α-1B, FK506-binding protein (FKBP4), and splicing factor 3A subunit-3 (SF3a3), and an upregulation of Nipsnap1 and glycine-tRNA ligase (GRS), were observed. In turn, in A549 cells, a downregulation of cyclophilin B, adenine phosphoribosyl transferase (APRT), dimethylarginine dimethylaminohydrolase-2 (DDAH-2), dual specificity protein phosphatase-3 (VHR), annexin A4, prohibitin, proteasome α-1, MST, and keratin-1, and the upregulation of glucose-regulated protein (GRP) 78 kDa, aortic and skeletal muscle α-actins, activator of HSP90, ATPase homolog-1 (AHA1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), stathmin, and calreticulin, were recorded. The inactivation of P-gp has been observed also to contribute to the cytotoxic activity by umbelliprenin [177]. An intriguing hypothesis, namely, induction of apoptosis by hormesis, has been postulated very recently by Gholami for umbelliprenin (**14**) [179]. Finally, **14** was seen to inhibit melanogenesis acting on different components of the melanogenic machinery [180].

As in the case of auraptene (**1**), the linear oxyprenylated coumarin (**14**) that has shown extensive evidence of being a biologically active agent. As a demonstration of this potential, the number of studies on coumarin (**14**) has dramatically increased

Table 10 Reported biological targets of umbelliprenin (**14**)

Target		Refs.
MMP-2	↓	[116]
Caspases 3, 8, 9	↑	[176]
GlP	↓	[178]
Hormesis	↑	[179]

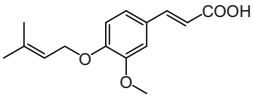
↑ = activation; ↓ = inactivation

over the past 5 years. Table 10 summarizes studies that have described the mechanisms of action of umbelliprenin (**14**) thus far.

4 Oxyprenylated Ferulic Acid Derivatives

4.1 Boropinic Acid

Boropinic acid (**15**) is a ferulic acid derivative having an *O*-isopentenyl side chain, which was isolated originally from the Australian shrub *Boronia pinnata* Sm. (Rutaceae) (Plate 10) and later also found in *Citrus* fruits [180].



15 (boropinic acid)

Boropinic acid (**15**) is claimed to exert an anti-inflammatory activity both in vitro and in vivo. Such an activity results from selective inhibition of LOX thus preventing the biosynthesis of leucotrienes (LTs). The interaction of **15** with soybean 5-LOX has been studied in detail by molecular modeling techniques and revealed a key role of the isopentenyloxy side chain that is oriented towards a specific cluster of amino acids of the active site [181]. This mode of action has been confirmed by in vivo studies (using the croton oil-induced mouse ear edema model) in which **15** showed inhibitory effects similar to the known LOX inhibitor, nordihydroguaiaretic acid [182].

Boropinic acid (**15**) is a molecule that was characterized structurally for the first time only in 2000. Hence it may be expected that relatively few studies on its properties have been reported in the literature to date. This compound has great

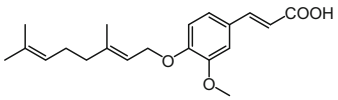
Plate 10 *Boronia pinnata*, Muogamarra Reserve, Kuring-ai National Park, N. of Sidney, Australia. Photograph: Moonlight0551, Wikimedia Commons

potential as an anti-inflammatory drug lead compound with the advantage of being selective toward LOXs. Thus, **15** may be used as a template to obtain more potent agents in this area, as has been done by Epifano and co-workers in 2011 [183]. Second, **15** and its derivatives have shown promising activities in vitro and in vivo against *Helicobacter pylori* [184, 185]. Although having demonstrated encouraging experimental data in this regard, there is a lack of explanatory mechanistic knowledge, apart from an effect that seems to be consistent with the disruption of the bacterial cell wall.

4.2 4′-Geranyloxyferulic Acid

4′-Geranyloxyferulic acid (**16**) is a ferulic acid derivative possessing an *O*-geranyl side chain. It was first isolated from the Australian plant *Acronychia baueri* Schott (Rutaceae) (Plate 11) and later also found in *Citrus* fruits and bee products, including raw propolis [186].

Plate 11 *Acronychia baueri* = *Sarcomelicope simplicifolia*, West Pennant Hills, Australia. Photograph: P. Woodard, Wikimedia Commons



16 (4'-geranyloxyferulic acid)

The main pharmacological activities of **16** are associated with dietary feeding, and cancer chemopreventive and anti-inflammatory effects [186, 187]. Compound **16** has shown the capacity to dose-dependently inhibit the proinflammatory enzymes COX-2 in LPS-stimulated human monocytes with an IC_{50} value of 54.2 μ*M* [188] and *i*NOS, especially in conjunction with the known iNOS inhibitor L-NAME (IC_{50} = 64.2 μ*M*), in RAW 264.7 macrophages [189]. Furthermore, when applied at a concentration of 100 μ*M*, **16** selectively inhibited geranylgeranyl transferase I (GGTase I) (72.4%, IC_{50} = 39.0 μ*M*), while it exhibited only a slight effect on farnesyl transferase (FTase) (12.7%) [188]. Considering the interaction of this compound with COX-2 and the links between peroxisome proliferator-activated receptors (PPARs) and the expression of COX-2 encoded genes, in 2010, Genovese and co-workers investigated its efficacy as an activator of PPARs [189]. In these experiments, it was revealed that **16** had a greater affinity towards PPAR β/δ. These authors also used wild-type mouse keratinocytes, in which the activation of PPARs β/δ by selective ligands is known to cause a marked increase in the expression of mRNA encoding the Angptl4 gene, but in PPARs β/δ-null cells, this effect was not recorded. At a concentration of 100 μ*M*, **16** caused a marked increase in the expression of Angptl4 mRNA, comparable to that observed with GW0742, used as the reference compound, and this change was not observed in PPARs β/δ-null keratinocytes. To underline its selectivity on PPARs β/δ, culturing wild-type keratinocytes in the presence of **16** provided no changes in the expression of the PPARα target gene Aco or the PPARγ target gene Klf4. Proliferation effects under the influence of **16** were also investigated in the A431 human epithelial carcinoma cell line. A growth inhibitory effect was observed after 72 h following treatment with 100 μ*M* of **16**, similar to that recorded with the positive control, GW0742. To assess whether the inhibition of cell proliferation by **16** was mediated by PPARs β/δ, this parameter was examined in wild-type and PPARs β/δ-null mouse primary keratinocytes. Ligand activation of PPARs β/δ with GW0742 applied at a concentration of 1 μ*M* led to the inhibition of cellular proliferation in wild-type keratinocytes, and this effect was not observed in similarly treated PPARs β/δ-null keratinocytes. No changes in cellular proliferation were recorded in keratinocytes following treatment with 25 μ*M* of **16**. On the other hand, inhibition of cell growth was revealed in wild-type keratinocytes treated with 100 μ*M* of **16** and this effect was not observed in similarly treated PPARs β/δ-null keratinocytes. Furthermore, inhibition of cell proliferation was found in primary keratinocytes lacking expression of PPARs β/δ. Thus, these authors came to the conclusion that the inhibitory effect of **16** was influenced by other PPARs β/δ-independent mechanisms.

The same research group investigated the influence of **16** on the catalytic efficiency and expression of human CYPs as well as the effects on the activity of the

Table 11 Reported biological targets of 4′-geranyloxyferulic acid (**16**)

Target		Refs.
COX-2	↓	[186, 187]
NOS	↓	[186, 187]
GGT-ase I	↓	[188]
PPAR βδ	↑	[189]
CYPs	↓	[190]

↑ = activation; ↓ = inactivation

major drug efflux transporter P-gp [190]. On the three different CYP isoforms studied, namely, CYP3A4, CYP2D6, and CYP2C19, compound **16** was found to moderately inhibit CYP2D6 by 25% at a concentration of 100 μ*M*. Furthermore, **16** weakly induced CYP3A4 and CYP2C9 at the mRNA level in LS180 cells at concentrations of 50 and 10 μ*M*. Finally, compound **16** significantly inhibited GlP with an IC_{50} value of 34.2 μ*M*.

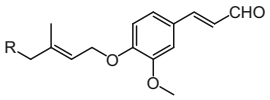
4′-Geranyloxyferulic acid (**16**) has been the subject of quite intensive research activities during the last 15 years. This natural product has demonstrated promising and interesting effects as a dietary cancer chemopreventive agent when orally administered as such and in the form of a prodrug [191], and has been linked to peptides hydrolyzable in the distal part of the small intestine, as an inclusion compound [192], and finally included as a co-drug with NOS inhibitors [193]. The results obtained have been extremely encouraging, but at the moment the exact mechanism of action underlying the observed in vivo anticarcinogenic effects remains poorly defined. The only data available are values resulting from histopathological examinations that have revealed an anti-inflammatory-based cancer chemopreventive activity in mice [194, 195]. Thus, studies are needed to better define the mode of action of **16**, hopefully using on a wider panel of cancer types either in vitro or in vivo, since up to now the collected data have emanated from investigations carried out on gastrointestinal-related cancer models (tongue, oesophageal, gastric, and colon). On the other hand, the first encouraging results related to neuroprotective effects of **16** have been reported, indicating a second future research line of investigation for this natural product. Table 11 provides a summary of the mechanisms of action described for 4′-geranyloxyferulic acid (**16**).

5 Miscellaneous Compounds

In this section, several oxyprenylated phenylpropanoids for which only very preliminary data on their individual mechanism of action have been reported in the recent literature, are grouped together.

Boropinal (**17**) has been identified so far only in extracts of the aerial parts of *Boronia pinnata* Sm. (Rutaceae) (Plate 10) [196]. Two reports have been published on its molecular pharmacological properties leading to indications of its mode of action and cellular targeting. In 2016, Hill and coworkers observed **17** to be an

effective agonist of the transient receptor potential ankyrin subtype 1 channel (TRPA1) in HEK 293 cells, overexpressing this receptor and exhibiting an IC_{50} value of 9.8 μ*M*, similar to that registered for the known inhibitor, allyl isothiocyanate [197]. More recently, **17** was found to be a potent ligand of MT1 receptors with a K_i value of 786 n*M* [46].



17 R = H (boropinal)
18 R = isopentenyl (nelumal A)

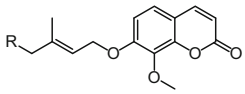
Nelumal A (**18**) also has been identified from only one natural source, namely, the Chinese medicinal plant *Ligularia nelumbifolia* Hand.-Mazz. (Asteraceae). Two relevant biological properties and related mechanisms of action for this compound have recently been identified by Epifano and coworkers [52, 198]. Using the FXR reporter assay in transfected HepG2 cells, **18** exhibited a high affinity as an agonist of this receptor. Notably, the potency recorded for **18** was higher than for the endogenous FXR ligand, chenodeoxycholic acid [52]. Another notable biological target identified for **18** is aromatase. In HEK293 cells transfected with aromatase cDNA, and using anastrazole as the reference compound, **18** showed a dose-dependent inhibition of this enzyme, displaying an activity that, at the concentration of 10 μ*M*, was slightly higher to that of anastrazole assayed at a dose of 1 μ*M* [198]. Furthermore, when tested in KGN (human ovary granulosa-like carcinoma) cells, well known to present aromatase activity constitutively, nelumal A (**18**), after 48 h of pretreatment in the concentration range 10–30 μ*M*, revealed a more potent effect than a positive control drug. Surprisingly, compound **18** did not decrease the activity of aromatase under these experimental conditions, leading to the hypothesis that the observed inhibitory activity was not due to the competitive inhibition of aromatase and may be related to the abolishment of aromatase mRNA expression.

Lacinartin (**19**) is an isopentenyloxycoumarin isolated from *Zanthoxylum schinifolium* Siebold & Zucc. (Rutaceae) (Plate 12) [199]. This compound has been characterized as a potent and selective non-competitive inhibitor of MAO-A from the mouse brain, with an IC_{50} value of 5.7 μ*M* (IC_{50} for MAO-B 28.6 μ*M*). Subsequently, Marquis and coworkers found that **19** exhibited antibacterial effects against the periodontopathogen *Porphyromonas gingivalis* and evoked significant inhibitory activities on IL-8 and TNF-α secretion (95 and 99% inhibition), when applied in the concentration range of 12.5–50 μg/cm^3. Furthermore, at the highest concentration tested, **19** nearly abolished MMP-8 and MMP-9 secretion (89 and 82%), and inhibited bacterial collagenase by 64%, at 100 μg/cm^3 [200].

Collinin (**20**), as with lacinartin (**19**), has been isolated from *Z. schinifolium* (Plate 12) [201, 202]. This compound also exhibited promising inhibitory effects against *P. gingivalis* at different concentration levels [203]. In particular, collinin dose-dependently diminished reducing type-I collagen degradation in a *P. gingivalis* culture supernatant by 46–82% when applied at the concentration range 5–25 μg/cm^3,

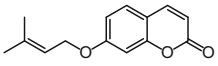
Plate 12 *Zanthoxylum schinifolium*, Bupyeong, Korea. Photograph: Dalgial, Wikimedia Commons

and decreased the release of pro-inflammatory cytokines and mediators such as IL-6, CCL-5, and PGE2, even at low concentrations (<0.5 μg/cm^3), and moreover reduced MMP-9 secretion by osteoclasts (60% at 5 μg/cm^3). Although having documented dietary colon cancer chemopreventive effects in a murine colon carcinogenesis model [204], the only data relevant to this observation have been a report of **20** to reduce NO production by iNOS in LPS-stimulated RAW 264.7 macrophages ($IC_{50} = 5.9$ μM) [205]. In the course of the same study, it was also revealed that collinin (**20**) possesses a slight inhibitory activity for α-glucosidase ($IC_{50} = 92.1$ μM).



19 R = H (lacinartin)
20 R = isopentenyl (collinin)

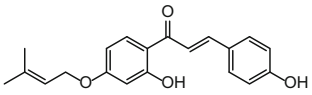
7-Isopentenyloxycoumarin (**21**) is widespread in plants belonging to the Rutaceae family, particularly in those of the genus *Citrus* [158].



21 (7-isopentenyoxyxcoumarin)

Very recently, **21** has been found to interact with the aryl hydrocarbon receptor (ArH), a transcription factor acting as a sensor of small synthetic xenobiotic molecules [206]. In particular, using mouse hepatoma (H1L1.1c2) cells, containing the stably integrated AhR xenobiotic responsive element driven by a firefly luciferase reporter plasmid, pGudLuc6.1, **21** activated AhR in a dose-dependent manner at the highest concentration tested (100 μM). Furthermore, **21** showed an affinity for MT1 receptors with a K_i of 5.2 μM [46]. Both observations may in part account for the reported neuroprotective effects of **21** [45, 207].

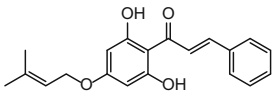
4′-Hydroxycordoin (**22**) is an isopentenyloxychalcone isolated from *Lonchocarpus neuroscapha* Benth. (Fabaceae) [208].



22 (4'-hydroxycordoin)

The only report available refers to the marked anti-inflammatory-related properties of compound **22** [208]. In LPS-stimulated monocyte-derived macrophages pre-exposed (2 h) to (**22**) applied at a dose of 5 μg/cm^3, this chalcone was able to inhibit the secretion of IL-8 (38%), IL-1β/29%), CCL-5 (67%), and TNF-α (23%), while decreasing the activity of MMP-9, and also to induce NF-κB p65 and AP-1.

2,6-Dihydroxy-4-isopentenyloxychalcone (**23**) has been isolated from several species of the genera *Helychrysum* [209], *Pleiotaxis* [210], and *Metalasia* (for example, see *M. muricata* R. Br., Plate 13) [211].



23 (2,6-dihydroxy-4-isopentenyloxychalcone)

In LPS-stimulated oral epithelial cells, **23** dose-dependently inhibited the release of IL-8 (33% at 25 μg/cm^3), and CCL-5 (86% at 25 μg/cm^3), and attenuated the secretion of MMP-2 by gingival fibroblasts [212].

Plate 13 *Metalasia muricata*, Nature's Valley, South Africa. Photograph: P. Venter, Wikimedia Commons

6 Synthesis Aspects

As stated in previous sections of this chapter, most of the oxyprenylated phenylpropanoids and polyketides may only be extracted from their respective plant natural sources in very low amounts. This is the main reason why over the last two decades chemical synthesis methods have been developed and carried out in order to generate such natural products in quantities of hundreds of milligrams to grams for pharmacological assay purposes and therefore to depict their biological activity profiles in greater detail.

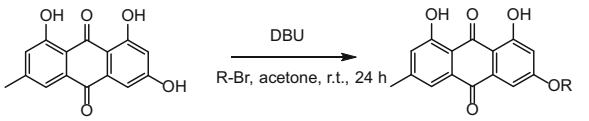
3-Geranyloxyemodin (**2**), 3-geranyloxyemodin anthrone (**3**), and madagascin (**4**) have been produced with a single-step reaction from commercially available emodin or emodinanthrone by a 1,8-diazabiciclo[5.4.0]undec-7-ene (DBU)-promoted selective alkyation of the OH group in position 3, as outlined in Scheme 1.

The use of a sterically hindered base such as DBU coupled to the lesser nucleophilicity of OH groups in positions 1 and 9, gave only one adduct, in 74, 67, and 88% yields for 3-geranyloxyemodin (**2**), 3-geranyloxyemodin anthrone (**3**), and madagascin (**4**) [213].

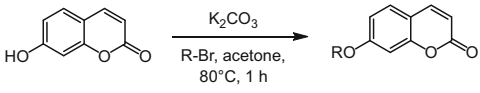
Coumarins having an oxygenated side chain containing only carbon and hydrogen atoms, such as auraptene (**1**), bergamottin (**5**), imperatorin (**10**), isoimperatorin (**11**), phellopterin (**13**), and umbelliprenin (**14**), have been synthesized in a single step from the respective commercially available hydroxylated parent samples by a Williamson reaction, using 3,3-dimethylallyl, geranyl, or (*E*,*E*)-farnesyl bromide as alkylating agents in acetone at 80°C for 1 h in the presence of dry K_2CO_3 as the base, as depicted in Scheme 2. The desired adducts have been obtained in high yields (>85%) and purity (>97.7%), after crystallization from *n*-hexane [214].

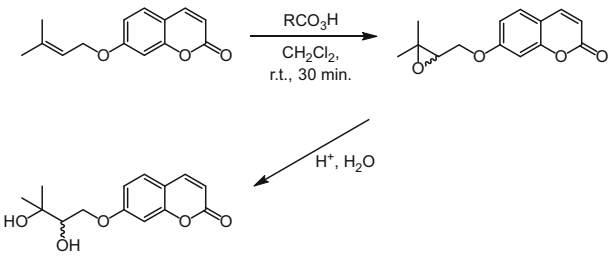
When functionalized side chains are present, as for byakangelicin (**6**), byakangelicol (**7**), heraclenin (**8**), heraclenol (**9**), and oxypeucedanin (**13**), first an epoxidation has been achieved by reaction of oxyprenylated coumarins with peracids (compounds **8**, **9**, and **13**), followed by aqueous acid-catalyzed ring opening to provide the corresponding diols (compounds **6** and **7**), as outlined in Scheme 3 [215]. Yields were in the range 45–66%.

Scheme 1 Chemical synthesis of oxyprenylated anthraquinones



Scheme 2 Chemical synthesis of oxyprenylated coumarins





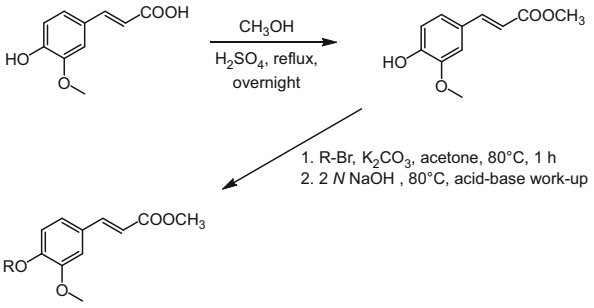
Scheme 3 Chemical synthesis of functionalized oxyprenylated coumarins

Boropinic acid (**15**) and 4′-geranyloxyferulic acid (**16**) have been synthesized by a two-step sequence consisting of the protection of the carboxylic acid group as the methyl ester by reaction in refluxing MeOH catalyzed by concentrated H_2SO_4 for 12 h, followed by a one-pot alkylation under the same experimental conditions as above, leading to alkaline hydrolysis and acid-base work-up, to get these pure acids in high yields and in purities of >96%, as illustrated in Scheme 4 [213].

Boropinal (**17**), nelumal (**18**), and 7-isopentenyloxycoumarin (**21**) have been synthesized from the respective commercially available OH-free native compound by the same method outlined for auraptene (**1**) [213]. Lacinartin (**19**) and collinin (**20**), on the other hand, required a multi-step procedure, since the corresponding parent products were not commercially available [200, 203]. Thus, the basic coumarin core was obtained by a Pechmann reaction between pyrogallol and propionic acid using a few drops of concentrated H_2SO_4 as the solvent at 120°C for 30 min, followed by purification by crystallization from H_2O (yield 32%). The adduct so obtained was first selectively alkylated by a DBU-promoted reaction, followed by methylation at position C-8 with MeI in DMF in the presence of Et_3N as the base at 80°C for 12 h. The overall process is outlined in Scheme 5 and the desired adducts were provided in yields of >65% and purities of >95.4%.

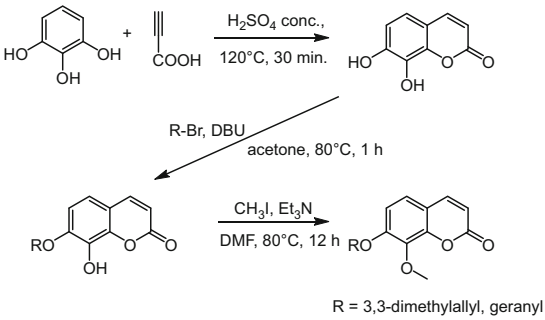
4′-Hydroxycordoin (**22**) has been obtained from commercially available 2,4-dihydroxyacetophenone, which was first selectively alkylated with 3,3-dimethylallyl bromide at position C-4 by the above-described DBU-promoted

Scheme 4 Chemical synthesis of oxyprenylated ferulic acid derivatives

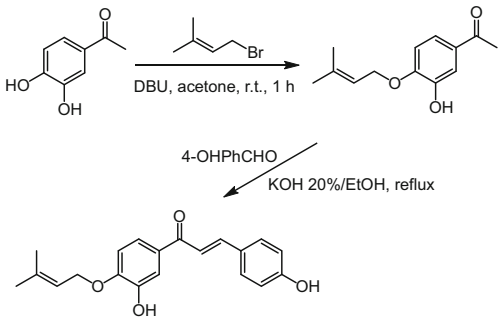


R = 3,3-dimethylallyl, geranyl

Scheme 5 Chemical synthesis of lacinartin (**19**) and collinin (**20**)



Scheme 6 Chemical synthesis of oxyprenylated chalcones



reaction. Subsequently, the chalcone core was built by reaction of this adduct with 4-hydroxybenzaldehyde in a boiling ethanolic solution of KOH (20%), providing compound **22** in 48% overall yield, with a purity of >95%, after crystallization from *n*-hexane, as depicted in Scheme 6 [208]. A similar process was used to achieve the synthesis of 2,6-dihydroxy-4-isopentenyloxychalcone (**23**) [212].

7 Conclusions

This contribution has provided a detailed survey of the so far reported mechanisms of actions and cellular targets of selected biologically active oxyprenylated phenylpropanoids and polyketides from natural sources. Only for a restricted number (around 5%) of such chemical entities out of the more than 400 currently characterized oxyprenylated secondary metabolites belonging to these groups having substantial biological and mechanistic data appeared in the literature. Of these, only for three compounds, namely, auraptene (**1**), bergamottin (**5**), and imperatorin (**10**), can the descriptions of their interactions with biological targets be considered both exhaustive and explicit. Hopefully, the present contribution might serve as a stimulus for further research, since oxyprenylated phenylpropanoids and polyketides

and their semi-synthetic derivatives have great potential not only for possible use in future drug therapy, but also as lead compounds following a medicinal chemistry approach to obtain more selective and more efficient agents. It should also be noted that most of the phytochemicals described herein are common and abundant constituents of edible fruits and vegetables and other food preparations (e.g. *Citrus* fruits, culinary herbs like amaranth [216], dill, anise, wild celery [217], bee products [218, 219], and several others). These latter materials are also claimed to have a great nutraceutical value due to their composition in not only in terms of vitamins, fiber, minerals, and complex phenolic compounds but also because of the of oxyprenylated coumarins and ferulic acid derivatives present, although the last-mentioned compounds are present only in low concentrations in most cases. Being able to clarify the individual biological targets of such natural products, on the one hand enhances the nutraceutical and commercial value of these food plants, and on the other hand renders a better understanding as to the true basis of the claimed and proven beneficial effects and eventually any important side effects (e.g. interference between grapefruit consumption and the efficacy of certain prescription drugs). Finally, it must be pointed out that an overview of the above reported data sheds light on the importance of an oxygenated side chain for the mediation of the observed effects and interactions with biological targets. In most cases, phytochemicals with free hydroxy groups are less potent in terms of their biological potencies when compared with their prenylated counterparts. Their past investigation has augmented knowledge of the importance of prenylation in Nature and the potential practical application of the resulting secondary metabolites to enhance human welfare [220]. Given the rather small amounts of these compounds available from natural sources. their chemical synthesis is also of increasing importance, as discussed herein.

References

1. Epifano F, Genovese S, Menghini L, Curini M (2007) Chemistry and pharmacology of oxyprenylated secondary plant metabolites. Phytochemistry 68:93
2. Karyione T, Matsuno T (1932) Studies on the constituents of orange oil. On the structure of auraptene. Pharm Bull 1:19
3. Epifano F, Fiorito S, Carlucci G, Locatelli M, Genovese S (2013) Phytochemistry and pharmacognosy of naturally occurring prenyloxyanthraquinones. Curr Drug Targets 14:959
4. Mbaveng AT, Kuete V, Nguemeving JR (2008) Antimicrobial activity of the extracts and compounds obtained from *Vismia guineensis* (Guttiferae). Asian J Trad Med 3:211
5. Sim WC, Ee GCL, Lim CJ (2011) *Cratoxylum glaucum* and *Cratoxylum arborescens* (Guttiferae) two potential sources of antioxidant agents. Asian J Chem 23:569
6. Narender T, Sukanya P, Sharma K, Bathula SR (2013) Apoptosis and DNA intercalating activities of novel emodin derivatives. RSC Adv 3:6123
7. Amonkar A, Chang CJ, Cassady JM (1981) 6-Geranyloxy-3-methyl-1,8-dihydroxyanthrone, a novel antileukemic agent from *Psorospermum febrifugum* Sprach var. *ferrugineum* (Hook. fil.). Experientia 37:1138

8. Ndjakou Lenta B, Devkota KP, Ngouela S, Fekam Boyom F, Naz Q, Choudhary MI, Tsamo E, Rosenthal PJ, Sewald N (2008) Anti-plasmodial and cholinesterase inhibiting activities of some constituents of *Psorospermum glaberrimum*. Chem Pharm Bull 56:222
9. Ritchie E, Taylor WG (1964) Constituents of *Harungana* (Haronga) *madagascariensis*. Tetrahedron Lett 23–24:1431
10. Camele G, Delle Monache F, Delle Monache G, Marini Bettolo GB, Alves de Lima R (1982) Chemistry of the *Vismia* genus. Part 8. 2-Isoprenylemodin and 5,5′-dimethoxysesamin from *Vismia guaramirangae*. Phytochemistry 21:417
11. Nagem TJ, Faria TJ (1990) Quinonoid and other constituents from *Vismia martiana*. Phytochemistry 29:3382
12. Nagem TJ, Alves VL (1995) Constituents of *Vismia magnoliifolia*. Fitoterapia 66:278
13. Bilia AR, Yusuf AW, Braca A, Keita M, Morelli I (2000) New prenylated anthraquinones and xanthones from *Vismia guineensis*. J Nat Prod 63:16
14. Nagem TJ, de Oliveira FF (1997) Xanthones and other constituents of *Vismia parviflora*. J Braz Chem Soc 8:505
15. Delle Monache G, Delle Monache F, Di Benedetto R, Ogukawa JU (1987) Chemistry of the *Psorospermum* genus. Part 5. New metabolites from *Psorospermum tenuifolium*. Phytochemistry 26:2611
16. Botta B, Delle Monache F, Delle Monache G, Menichini F (1988) Chemistry of the *Psorospermum* genus. Part 7. Psorolactones and other metabolites from *Psorospermum glaberrimum*. Tetrahedron 44:7193
17. Tsaffack M, Nguemeving JR, Kuete V (2009) Two new antimicrobial dimeric compounds: febriquinone, a vismione-anthraquinone coupled pigment and adamabianthrone, from two *Psorospermum* species. Chem Pharm Bull 57:1113
18. Boonsri S, Karalai C, Ponglimanont C, Kanjana-Opas A, Chantrapromma K (2006) Antibacterial and cytotoxic xanthones from the roots of *Cratoxylum formosum*. Phytochemistry 67:723
19. Epifano F, Genovese S, Kremer M, Randic M, Carlucci G, Locatelli M (2012) Re-investigation of the anthraquinone pool of *Rhamnus* spp.: madagascin from the fruits of *Rhamnus cathartica* and *R. intermedia*. Nat Prod Commun 7:1029
20. Locatelli M, Genovese S, Carlucci G, Kremer D, Randic M, Epifano F (2012) Development and application of high-performance liquid chromatography for the study of two new oxyprenylated anthraquinones produced by *Rhamnus* spp. J Chromatogr A 1225:113
21. Genovese S, Epifano F, Curini M, Kremer D, Carlucci G, Locatelli M (2012) Screening for oxyprenylated anthraquinones in Mediterranean *Rhamnus* species. Biochem Syst Ecol 43:125
22. Kremer D, Kosalec I, Locatelli M, Epifano F, Genovese S, Carlucci G, Zovko Koncic M (2012) Amthraquinone profiles, antioxidant, and antimicrobial properties of *Frangula rupestris* (Scop.) Schur. and *Frangula alnus* Mill. bark. Food Chem 131:1174
23. Chen D, Lv B, Kobayashi S, Xiong Y, Sun P, Lin Y, Genovese S, Epifano F, Hou S, Tang F, Ji Y, Yu D (2016) Madagascine induces vasodilation via activation of AMPK. Front Pharmacol 7:435
24. Luo Z, Zang M, Guo W (2010) AMPK as a metabolic tumor suppressor: control of metabolism and cell growth. Future Oncol 6:457
25. Genovese S, Epifano F (2011) Auraptene: a natural biologically active compound with multiple targets. Curr Drug Targets 12:381
26. Kawabata K, Murakami A, Ohigashi H (2006) Citrus auraptene targets translation of MMP-7 (matrylisin) via ERK 1/2-dependent and mTOR-independent mechasnism. FEBS Lett 580:5288
27. Moon JY, Kim H, Cho SK (2015) Auraptene, a major compound of supercritical fluid extract of Phalsak (*Citrus hassaku* Hort. ex Tanaka), induces apoptosis through the suppression of mTOR pathways in human gastric cancer SNU-1 cells. Evid Based Compl Altern Med 2015: ID 402385

28. Jun DY, Kim JS, Park HS, Han CR, Fang Z, Woo MH, Rhee IK, Kim YH (2007) Apoptogenic activity of auraptene of *Zanthoxylum schinifolium* toward human acute leukemia Jurkat T cells is associated with ER-stress mediated caspase-8 activation that stimulates mithochondria-dependent or independent caspase cascade. Carcinogenesis 28:1303
29. Lee JC, Shin EA, Kim B, Kim BI, Chitsazian-Yasdi M, Iranshahi M, Kim SH (2017) Auraptene induces apoptosis via myeloid cell leukemia 1-mediated activation of caspase in PC3 and DU145 prostate cancer cells. Phytother Res 31:891
30. Jang Y, Han J, Kim SJ, Kim J, Lee MJ, Jeong S, Ryu MJ, Seo KS, Choi SY, Shong M, Lim K, Heo JY, Kweon GR (2015) Suppression of mitochondrial respiration with auraptene inhibits the progression of renal cell carcinoma: involvement of HIF-1a degradation. Oncotarget 6:38127
31. De Medina P, Genovese S, Paillasse MR, Mazaheri M, Caze-Subra S, Bystrcky K, Curini M, Silvente-Poirot S, Epifano F, Poirot S (2010) Auraptene is an inhibitor of cholesterol esterification and a modulator of estrogen receptors. Mol Pharmacol 78:827
32. Khrishnan P, Kleiner-Hancock H (2012) Effects of auraptene on IGF-1 stimulated cell cycle progression in the human breast cancer cell line, MCF-7. Int J Breast Cancer 2012: ID 502092
33. Nabekura T, Yamaki T, Kitagawa S (2008) Effects of chemopreventive citrus phytochemicals on human P-glycoprotein and multidrug resistance protein 1. Eur J Pharmacol 600:45
34. Prince M, Li Y, Childers A, Itoh K, Yamamoto M, Kleiner-Hancock H (2009) Comparison of citrus coumarins on carcinogen-detoxifying enzymes in Nrf2 knockout mice. Toxicol Lett 185:180
35. Epifano F, Genovese S, Miller R, Majumdar APN (2013) Auraptene and its effects on the re-emergence of colon cancer stem cells. Phytother Res 27:784
36. Saboor-Maleki S, Rassouli FB, Matin MM, Iranshahi M (2016) Auraptene attenuates malignant properties of esophageal stem-like cancer cells. Technol Cancer Res Treat 16:519
37. Murakami A, Shigemori T, Ohigashi H (2005) Zingiberaceous and Citrus constituents, 1-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264.7 murine macrophages through different modes of action. J Nutr 135:2987S
38. Kawabata K, Murakami A, Ohigashi H (2006) Auraptene decreases the activity of matrix metalloproteinases in dextran sulfate sodium-induced ulcerative colitis in ICR mice. Biosci Biotechnol Biochem 70:3062
39. Lin S, Hirai S, Goto T, Sakamoto T, Takahashi N, Yano M, Sasaki T, Yu R, Kawada T (2013) Auraptene suppresses inflammatory responses in activated RAW264 macrophages by inhibiting p38 mitogen-activated protein kinase activation. Mol Nutr Food Res 57:1135
40. La VD, Zhao L, Epifano F, Genovese S, Grenier D (2013) Anti-inflammatory and wound healing potential of *Citrus* auraptene. J Med Food 16:961
41. Adams DH, Shou Q, Wohlmuth H, Cowin AJ (2016) Native Australian plant extracts differentially induce Collagen I and Collagen III in vitro and could be important targets for the development of new wound healing therapies. Fitoterapia 109:45
42. Furukawa Y, Watanabe S, Okuyama S, Nakajima M (2012) Neurotrophic effect of *Citrus* auraptene: neuritogenic activity in PC12 cells. Int J Mol Sci 13:5338
43. Nakajima M, Shimizu R, Furuta K, Sugino M, Watanabe T, Aoki R, Okuyama S, Furukawa Y (2016) Auraptene induces oligodendrocyte lineage precursor cells in a cuprizone-induced animal model of demyelination. Brain Res 1639:28
44. Okuyama S, Morita M, Kaji M, Amakura Y, Yoshimura M, Shimamoto K, Ookido Y, Nakajima M, Furukawa Y (2015) Auraptene acts as an anti-inflammatory agent in the mouse brain. Molecules 20:20230
45. Okuyama S, Semba T, Toyoda N, Epifano F, Genovese S, Fiorito S, Taddeo VA, Sawamoto A, Nakajima M, Furukawa Y (2016) Auraptene and other prenyloxyphenylpropanoids suppress microglial activation and dopaminergic neuronal cell death in a lipopolysaccharide-induced model of Parkinson's disease. Int J Mol Sci 17:1716

46. Hasan M, Genovese S, Fiorito S, Epifano F, Witt-Enderby PA (2017) Oxyprenylated phenylpropanoids bind to MT1 melatonin receptors and inhibit breast cancer cell proliferation and migration. J Nat Prod 80:3324
47. Nagao K, Yamano N, Shirouchi B, Inoue N, Murakami S, Sasaki T, Yanagita T (2010) Effects of citrus auraptene (7-geranyloxycoumarin) on hepatic lipid metabolism in vitro and in vivo. J Agric Food Chem 58:9028
48. Genovese S, Ashida H, Yamashita Y, Nakgano T, Ikeda M, Daishi S, Epifano F, Taddeo VA, Fiorito S (2017) The interaction of auraptene and other oxyprenylated phenylpropanoids with glucose transporter type 4. Phytomedicine 32:74
49. Kuroyanagi K, Kang MS, Goto T, Hirai S, Ohyama K, Kusudo T, Yu R, Yano M, Sasaki T, Takahashi N, Kawada T (2008) *Citrus* auraptene acts as an agonist for PPARs and enhances adiponectin production and MCP-1 reduction in 3T3-L1 adipocytes. Biochem Biophys Res Commun 366:219
50. Takahashi N, Kang MS, Kuroyanagi K, Goto T, Hirai S, Lee JY, Yu R, Yano M, Sasaki T, Murakami S, Kawada T (2008) Auraptene, a citrus fruit compound, regulates gene expression as a PPARα agonist in HepG2 hepatocytes. Biofactors 33:25
51. Takahashi N, Senda M, Lin S, Goto T, Yano M, Sasaki T, Murakami S, Kawada T (2011) Auraptene regulates gene expression involved in lipid metabolism through PPARa activation in diabetic obese mice. Mol Nutr Food Res 55:1791
52. Epifano F, Genovese S, Squires JE, Gray MA (2012) Nelumal A, the active principle from *Ligularia nelumbifolia*, is a novel farnesoid X receptor agonist. Bioorg Med Chem Lett 22:3130
53. Gao X, Fu T, Wang C, Ning C, Kong Y, Liu Z, Sun H, Ma X, Liu K, Meng Q (2017) Computational discovery and experimental verification of farnesoid X receptor agonist auraptene to protect against cholestatic liver injury. Biochem Pharmacol 146:127
54. Gao X, Wang C, Ning C, Liu K, Wang X, Liu Z, Sun H, Ma X, Sun P, Meng Q (2018) Hepatoprotection of auraptene from peels of citrus fruits against thioacetamide-induced hepatic fibrosis in mice by activating farnesoid X receptor. Food Funct. https://doi.org/10.1039/c8fo00107c
55. Hung WL, Suh JH, Wang Y (2017) Chemistry and health effects of furanocoumarins in grapefruit. J Food Drug Anal 25:71
56. He K, Iyer KR, Hayes RN, Sinz MW, Woolf TF, Hollenberg PF (1998) Inactivation of cytochrome P450 3A4 by bergamottin, a component of grapefruit juice. Chem Res Toxicol 11:252
57. Tassaneeyakul W, Guo LQ, Fukuda K, Ohta T, Yamazoe Y (2000) Inhibition selectivity of grapefruit juice components on human cytochromes P450. Arch Biochem Biophys 378:356
58. Lim HK, Duczak Jr. N, Brougham L, Elliot M, Patel K, Chan K (2005) Automated screening with confirmation of mechanism-based inactivation of CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP1A2 in pooled human liver microsomes. Drug Metab Dispos 33:1211
59. Girennavar BA, Jayaprakasha GK, Patil BS (2007) Potent inhibition of human cytochrome P450 3A4, 2D6, and 2C9 isoenzymes by grapefruit juice and its furocoumarins. Food Chem Toxicol 72:417
60. Wen YH, Sahi J, Urda E, Kulkarni S, Rose K, Zheng X, Sinclair JF, Cai H, Strom SC, Kostrubsky VE (2002) Effects of bergamottin on human and monkey drug metabolizing enzymes in primary cultured hepatocytes. Drug Metab Dispos 30:977
61. Olguin-Reyes S, Camacho-Carranza R, Hernandez-Ojeda S, Elinos-Baez M, Espinosa-Aguirre JJ (2012) Bergamottin is a competitive inhibitor of CYP1A1 and is antimutagenic in the Ames test. Food Chem Toxicol 50:3094
62. Lin HL, Kent UM, Hollenberg PF (2005) The grapefruit juice effect is not limited to cytochrome P450 (P450) 3A4: evidence for bergamottin-dependent inactivation, heme destruction, and covalent binding to protein in P450s 2B6 and 3A5. J Pharmacol Exp Ther 313:154

63. Kent UM, Lin HL, Noon KR, Harris DL, Hollenberg PF (2006) Metabolism of bergamottin by cytochromes P4502B6 and 3A5. J Pharmacol Exp Ther 318:992
64. Girennavar BA, Poulose SM, Jayaprakasha GK, Bhat NG, Patil BS (2006) Furocoumarins from grapefruit juice and their effect on human CYP 3A4 and CYP 1B1 isoenzymes. Bioorg Med Chem 14:2606
65. Le Goff-Klein N, Koffel JC, Jung L, Ubeaud G (2003) In vitro inhibition of simvastatin metabolism, a HMG-CoA reductase inhibitor in human and rat liver by bergamottin, a component of grapefruit juice. Eur J Pharm Sci 18:31
66. Le Goff-Klein N, Klein L, Herin M, Koffel JC, Ubeaud G (2004) Inhibition of in-vitro simvastatin metabolism in rat liver microsomes by bergamottin, a component of grapefruit juice. J Pharm Pharmacol 56:1007
67. Cesar TB, Manthey JA, Myung K (2009) Minor furanocoumarins and coumarins in grapefruit peel oil as inhibitors of human cytochrome P450 3A4. J Nat Prod 72:1702
68. Baumgart A, Schmidt M, Schmitz HJ, Schrenk D (2005) Natural furocoumarins as inducers and inhibitors of cytochrome P450 1A1 in rat hepatocytes. Biochem Pharmacol 69:657
69. Sasaki M, Elrod JW, Jordan P, Itoh M, Joh T, Minagar A, Alexander JS (2004) CYP450 dietary inhibitors attenuate TNF-α-stimulated endothelial molecule expression and leukocyte adhesion. Am J Physiol Cell Physiol 286:931
70. Hung WL, Suh JH, Wang Y (2017) Chemistry and health effects of furanocoumarins in grapefruit. J Food Drug Anal 25:71
71. Cai Y, Kleiner H, Johnston D, Dubowski A, Bostic S, Ivie W, DiGiovanni J (1997) Effect of naturally occurring coumarins on the formation of epidermal DNA adducts and skin tumors induced by benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene in SENCAR mice. Carcinogenesis 18:1521
72. Kleiner HE, Vulimiri VS, Reed MJ, Uberecken A, DiGiovanni J (2002) Role of cytochrome P450 1a1 and 1b1 in the metabolic activation of 7,12-dimethylbenz[*a*]anthracene and the effects of naturally occurring furanocoumarins on skin tumor initiation. Chem Res Toxicol 15:226
73. Kleiner HE, Reed MJ, DiGiovanni J (2003) Naturally occurring coumarins inhibit human cytochromes P450 and block benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene DNA adduct formation in MCF-7 cells. Chem Res Toxicol 16:415
74. Pan ST, Li ZL, He ZX, Qiu JX, Zhou SF (2016) Molecular mechanisms for tumour resistance to chemotherapy. Clin Exp Pharmacol Physiol 43:723
75. Ikegawa T, Ushigome F, Koyabu N, Morimoto S, Shoyama Y, Naito M, Tsuruo T, Ohtani H, Sawada Y (2000) Inhibition of P-glycoprotein by orange juice components, polymethoxyflavones in adriamycin-resistant human myelogenous leukemia (K562/ADM) cells. Cancer Lett 160:21
76. Honda Y, Ushigome F, Koyabu N, Morimoto S, Shoyama Y, Uchiumi T, Kuwano N, Ohtani H, Sawada Y (2004) Effects of grapefruit juice and orange juice components on P-glycoprotein- and MRP2-mediated drug efflux. Br J Pharmacol 143:856
77. de Castro WV, Mertens-Talcott S, Derendorf H, Butterweck V (2008) Effect of grapefruit juice, naringin, naringenin, and bergamottin on the intestinal carrier-mediated transport of talinolol in rats. J Agric Food Chem 56:4840
78. Hochman JH, Chiba M, Yamazaki M, Tang C, Lin JH (2001) P-glycoprotein-mediated efflux of indinavir metabolites in Caco-2 cells expressing cytochrome P450 3A4. J Pharmacol Exp Ther 298:323
79. Pormohammad A, Ghotaslo R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A (2018) Risk of gastric cancer in association with *Helicobacter pylori* different virulence factors: a systematic review and meta-analysis. Microb Pathogen 118:214
80. Sekiguchi H, Washida K, Murakami A (2008) Suppressive effects of selected food phytochemicals on CD74 expression in NCI-N87 gastric carcinoma cells. J Clin Biochem Nutr 43:109

81. Hwang YP, Yun HJ, Choi JH, Kang KW, Jeong HG (2010) Suppression of phorbol-12-myristate-13-acetate-induced tumor cell invasion by bergamottin via the inhibition of protein kinase Cδ/p38 mitogen-activated protein kinase and JNK/nuclear factor-kB-dependent matrix metalloproteinase-9 expression. Mol Nutr Food Res 54:977
82. Kim SM, Lee JH, Sethi G, Kim C, Baek SH, Nam D, Chung WS, Kim SH, Shim BS, Ahn KS (2014) Bergamottin, a natural furanocoumarin obtained from grapefruit juice induces chemosensitization and apoptosis through the inhibition of STAT3 signaling pathway in tumor cells. Cancer Lett 354:153
83. Wu HJ, Wu HB, Zhiao YQ, Chen LJ, Zou HZ (2016) Bergamottin isolated from *Citrus bergamia* exerts in vitro and in vivo antitumor activity in lung adenocarcinoma through the induction of apoptosis, cell cycle arrest, mitochondrial membrane potential loss and inhibition of cell migration and invasion. Oncol Rep 36:324
84. Kim SM, Lee EJ, Lee JH, Yang WM, Nam D, Lee JH, Lee SG, Um JY, Shim BS, Ahn KS (2016) Simvastatin in combination with bergamottin potentiates TNF-induced apoptosis through modulation of NF-κB signalling pathway in human chronic myelogenous leukaemia. Pharm Biol 54:2050
85. Sowndhararajan K, Deepa P, Kim M, Park SJ, Kim S (2017) A review of the composition of the essential oils and biological activities of *Angelica* species. Sci Pharm 85:33
86. Shin KH, Chung MS, Cho TS (1994) Effect of furanocoumarins from *Angelica dahurica* on aldose reductase and galactosemic cataract formation in rats. Arch Pharmacal Res 17:331
87. Shin KH, Lim SS, Kim DK (1998) Effect of byakangelicin, an aldose reductase inhibitor, on galactosemic cataracts, the polyol contents and Na^+, K^+ATPase activity in sciatic nerves of streptozotocin-induced diabetic rats. Phytomedicine 5:121
88. Yang J, Luan X, Gui H, Yan P, Yang D, Song X, Liu W, Hu G, Yan B (2011) Byakangelicin induces cytochrome P450 3A4 expression via transactivation of pregnane X receptors in human hepatocytes. Br J Pharmacol 162:441
89. Lin CH, Chang CW, Wang CC, Chang MS, Yang LL (2002) Byakangelicol, isolated from *Angelica dahurica*, inhibits both the activity and induction of cyclooxygenase-2 in human pulmonary epithelial cells. J Pharm Pharmacol 54:1271
90. Iwanaga K, Hayashi M, Hamahata Y, Miyazaki M, Shibano M, Taniguchi M, Baba K, Kakemi M (2010) Furanocoumarin derivatives in Kampo extract medicines inhibit cytochrome P450 3A4 and P-glycoprotein. Drug Metab Dispos 38:1286
91. Iwanaga K, Yoneda S, Hamahata Y, Miyazaki M, Shibano M, Taniguchi M, Baba K, Kakemi M (2011) Inhibitory effects of furanocoumarin derivatives in Kampo extract medicines on P-glycoprotein at the blood–brain barrier. Biol Pharm Bull 34:1246
92. Marumoto S, Miyazawa M (2010) Secretase inhibitory effects of furanocoumarins from the root of *Angelica dahurica*. Phytother Res 24:510
93. Kumar D, Ganeshpurkar A, Kumar D, Modi G, Gupta SK, Singh SK (2018) Secretase inhibitors for the treatment of Alzheimer's disease: long road ahead. Eur J Med Chem 48:436
94. Shikov AN, Pozharitskaya ON, Makarov VG, Yang WZ, Guo DA (2014) *Oplopanax elatus* (Nakai) Nakai: chemistry, traditional use and pharmacology. Chin J Nat Med 12:721
95. Appendino G, Bianchi F, Bader A, Campagnuolo C, Fattorusso E, Taglialatela-Scafati O, Blanco-Molina M, Macho A, Fiebich BL, Bremner P, Heinrich M, Ballero M, Muñoz E (2004) Coumarins from *Opopanax chironium*. New dihydrofuranocoumarins and differential induction of apoptosis by imperatorin and heraclenin. J Nat Prod 67:532
96. García-Argáez AN, Ramírez Apan TO, Parra Delgado H, Velázquez G, Martínez-Vázquez M (2000) Anti-inflammatory activity of coumarins from *Decatropis bicolor* on TPA ear mice model. Planta Med 66:279
97. Abel G, Schimmer O (1986) Chromosome damaging effects of heraclenin in human lymphocytes in vitro. Mutat Res 169:51
98. Wszelaki N, Paradowska K, Jamroz MK, Granica S, Kiss AK (2011) Bioactivity-guided fractionation for the butyryl-cholinesterase inhibitory activity of furanocoumarins from *Angelica archangelica* L. roots and fruits. J Agric Food Chem 59:9186

99. Koziol E, Skalicka-Wozniak K (2016) Imperatorin pharmacocological meaning and analytical clues: profound investigation. Phytochem Rev 15:627
100. Cai Y, Bennett D, Nair RV, Ceska O, Ashwood-Smith MJ, Di Giovanni J (1993) Inhibition and inactivation of murine hepatic ethoxy- and pentoxyresorufin-*O*-dealkylase by naturally occurring coumarins. Chem Res Toxicol 6:872
101. Maenpaa J, Sigusch H, Raunio H, Syngelma T, Vuorela P, Vuorela H, Pelkonen O (1993) Differential inhibition of coumarin 7-hydroxylase activity in mouse and human liver microsomes. Biochem Pharmacol 45:1035
102. Kleiner HE, Vulimiri SV, Miller L, Johnson WH Jr, Whitmann CP, Di Giovanni J (2001) Oral administration of naturally occurring coumarins leads to altered phase I and phase II enzyme activities and reduced DNA adducts formation by polycyclic aromatic hydrocarbons in various tissues of SENCAR mice. Carcinogenesis 22:73
103. Kang AY, Young LR, Dingfelder C, Peterson S (2011) Effects of furanocoumarins from Apiaceous vegetables on the catalytic activity of recombinant human cytochrome P-450 1A2. Protein J 30:447
104. Zheng L, Cao J, Lu D, Ji L, Peng Y, Zheng J (2015) Imperatorin is a mechanism-based inactivator of CYP2B6. Drug Metab Dispos 43:82
105. Kimura Y, Ito H, Ohnishi R, Hatano T (2006) Inhibitory effects of polyphenols on human cytochrome P450 3A4 and 2C9 activity. Food Chem Toxicol 48:429
106. Prince M, Campbell CT, Robertson TA, Wells AJ, Kleiner HE (2006) Naturally occurring coumarins inhibit 7,12-dimethylbenz[*a*]anthracene DNA adduct formation in mouse mammary gland. Carcinogenesis 27:1204
107. Luo KW, Sun JG, Wa Chan JY, Yang L, Wu SH, Fung KP, Liu FY (2011) Anticancer effects of imperatorin isolated from *Angelica dahurica*: induction of apoptosis in HepG2 cells through both death-receptor- and mitochondria-mediated pathways. Chemotherapy 57:449
108. Badziul D, Jakubowicz-Gil J, Langner E, Rzeski W, Glowniak K, Gawron A (2014) The effect of quercetin and imperatorin on programmed cell death induction in T98G cells in vitro. Pharmacol Rep 66:292
109. Li X, Zeng X, Sun J, Li H, Wu P, Fung KP, Liu F (2014) Imperatorin induces Mcl-1 degradation to cooperatively trigger Bax translocation and Bak activation to suppress drug-resistant human hepatoma. Cancer Lett 348:146
110. Hu J, Xu C, Cheng B, Jin L, Li J, Gong Y, Lin W, Pan Z, Pan C (2016) Imperatorin acts as a cisplatin sensitizer via downregulating Mcl-1 expression in HCC chemotherapy. Tumor Biol 37:331
111. Zheng YM, Lu AX, Shen JZ, Kwok AHY, Ho WS (2016) Imperatorin exhibits anticancer activities in human colon cancer cells via the caspase cascade. Oncol Rep 35:1995
112. Choochuay K, Chunhacha P, Pongrakhananon V, Luechapudiporn R, Chanvorachote P (2012) Imperatorin sensitizes anoikis and inhibits anchorage independent growth of lung cancer cells. J Nat Med 67:599
113. Mi C, Ma J, Wang KS, Zuo HX, Wang Z, Li MY, Piao LX, Xu GH, Li X, Quan ZS, Jin X (2017) Imperatorin suppresses proliferation and angiogenesis of human colon cancer cell by targeting HIF-1α via the mTOR/p70S6K/4E-BP1 and MAPK pathways. J Ethnopharmacol 203:27
114. Liao ZG, Tang T, Guan XJ, Dong W, Zhang J, Zhao GW, Yang M, Liang XL (2016) Improvement of transmembrane transport mechanism study of imperatorin on P-glycoprotein-mediated drug transport. Molecules 21:1606
115. Ban HS, Lim SS, Suzuki K, Jung SH, Lee S, Lee YS, Shin KH, Ohuchi K (2003) Inhibitory effects of furanocoumarins isolated from the roots of *Angelica dahurica* on prostaglandin E2 production. Planta Med 69:408
116. Huang GJ, Deng JS, Liao JC, Hou WC, Wang SY, Sung PJ, Kuo YH (2011) Inducible nitric oxide synthase and cyclooxygenase-2 participate in anti-inflammatory activity of imperatorin from *Glehnia littoralis*. J Agric Food Chem 60:1673

117. Zhang Y, Cao Y, Duan H, Wang H, He LC (2012) Imperatorin prevents cardiac hypertrophy and the transition to heart failure via NO-dependent mechanisms in mice. Fitoterapia 83:60
118. Guo W, Sun J, Jiang L, Duan L, Huo M, Chen N, Zhong W, Wassy L, Yang Z, Feng H (2012) Imperatorin attenuates LPS-induced inflammation by suppressing NF-κB and MAPKs activation in RAW 264.7 macrophages. Inflammation 35:1764
119. Zhang X, Li W, Abudureheman A, Cheng T, Peng P (2017) Imperatorin possesses notable anti-inflammatory activity in vitro and in vivo through inhibition of the NF-κB pathway. Mol Med Rep 16:8619
120. Wang KS, Lv Y, Wang Z, Ma J, Mi C, Li X, Xu GH, Piao LX, Zheng SZ, Jin X (2017) Imperatorin efficiently blocks TNF-α-mediated activation of ROS/PI3K/Akt/NF-κB pathway. Oncol Rep 37:3397
121. Marquez N, Sancho R, Ballero M, Bremmer P, Appendino G, Fiebich BL, Heinrich M, Munoz E (2004) Imperatorin inhibits T-cell proliferation by targeting the transcription factor NFAT. Planta Med 70:1016
122. Oh HA, Kim HM, Jeong HJ (2011) Distinct effects of imperatorin on allergic rhinitis: imperatorin inhibits caspase-1 activity in vivo and in vitro. J Pharmacol Exp Ther 339:72
123. Jeong KT, Lee E, Park NY, Kim SG, Park HH, Lee J, Lee YJ, Lee E (2016) Imperatorin suppresses degranulation and eicosanoid generation in activated bone marrow-derived mast cells. Biomol Ther 23:421
124. Lin CL, Hsiao G, Wang CC, Lee YL (2016) Imperatorin exerts antiallergic effects in Th2-mediated allergic asthma via induction of IL-10-producing regulatory T cells by modulating the function of dendritic cells. Pharm Res 110:111
125. Deng S, Chen SN, Yao P, Nikolic D, van Breemen RB, Bolton JL, Fong HHS, Farnsworth NR, Pauli GF (2006) Serotonergic activity-guided phytochemical investigation of the roots of *Angelica sinensis*. J Nat Prod 69:536
126. Cao Y, Liu J, Wang Q, Liu M, Cheng Y, Zhang X, Lin T, Zhu Z (2017) Antidepressive-like effect of imperatorin from *Angelica dahurica* in prenatally stressed offspring rats through 5-hydroxytryptamine system. Neuroreport 28:426
127. Wang SJ, Lin TY, Lu CW, Huang WJ (2008) Osthole and imperatorin, the active constituents of *Cnidium monnieri* (L.) Cusson, facilitate glutamate release from rat hippocampal nerve terminals. Neurochem Int 53:416
128. Lin TY, Lu CW, Huang WJ, Wang SJ (2010) Osthole or imperatorin-mediated facilitation of glutamate release is associated with a synaptic vesicle mobilization in rat hippocampal glutamatergic nerve endings. Synapse 64:390
129. Luszczki JJ, Wojda E, Andres-Mach M, Cisowski W, Glensk M, Glowniak K, Czuczwar SJ (2009) Anticonvulsant and acute neurotoxic effects of imperatorin, osthole and valproate in the maximal electroshock seizure and chimney tests in mice: a comparative study. Epilepsy Res 85:293
130. Singhuber J, Baburin I, Ecker GF, Kopp B, Hering S (2011) Insights into structure–activity relationship of GABAA receptor modulating coumarins and furanocoumarins. Eur J Pharmacol 668:57
131. Choi SY, Ahn EM, Song MC, Kim DW, Kang JH, Kwon OS, Kang TC, Baek NI (2005) In vitro GABA-transaminase inhibitory compounds from the root of *Angelica dahurica*. Phytother Res 19:839
132. Wang C, Huo X, Tian X, Xu M, Dong P, Luan Z, Wang X, Zhang B, Zhang B, Huang S, Deng S, Ma X (2016) Inhibition of melatonin metabolism in humans induced by chemical components from herbs and effective prediction of this risk using a computational model. Br J Pharmacol 173:3261
133. Tun T, Kang YS (2017) Imperatorin is transported through blood-brain barrier by carrier-mediated transporters. Biomol Ther 25:441
134. Wang N, Wu L, Cao Y, Whang Y, Zhang Y (2013) The protective activity of imperatorin in cultured neural cells exposed to hypoxia re-oxygenation injury via anti-apoptosis. Fitoterapia 90:38

135. Lee E, Choi SY, Yang JH, Lee YJ (2016) Preventive effects of imperatorin on perfluorohexanesulfonate-induced neuronal apoptosis via inhibition of intracellular calcium-mediated ERK pathway. Kor J Physiol Pharmacol 20:399
136. Liao B, Chen R, Lin F, Mai A, Chen J, Li H, Dong S, Xu Z (2017) Imperatorin protects H9c2 cardiomyoblasts cells from hypoxia/reoxygenation-induced injury through activation of ERK signaling pathway. Saudi Pharm J 25:615
137. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I, Biala G (2015) Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology 232:931
138. Granica S, Kiss AK, Jarończyk M, Maurin JK, Mazurek AP, Czarnocki Z (2009) Synthesis of imperatorin analogs and their evaluation as acetylcholinesterase and butyrylcholinesterase inhibitors. Arch Pharm 346:775
139. Kim DK, Lim JP, Yang JH, Eom DO, Eun JS, Leem KH (2002) Acetylcholinesterase inhibitors from the roots of *Angelica dahurica*. Arch Pharm Res 25:856
140. Zhang Y, Cao Y, Wang QL, Zheng L, Zhang J, He LC (2011) A potential calcium antagonist and its antihypertensive effects. Fitoterapia 82:988
141. Wu KC, Chen YH, Cheng KS, Kuo YH, Yang CT, Wong KL, Tu YK, Chan P, Leung YM (2013) Suppression of voltage-gated Na^+ channels and neuronal excitability by imperatorin. Eur J Pharmacol 721:49
142. Wang YW, Yang CT, Chen YH, Gong CL, Chen YF, Kuo YH, Leung YM (2015) Inhibitory effects of imperatorin on voltage-gated K^+ channels and ATP-sensitive K^+ channels. Pharmacol Rep 67:134
143. Chen X, Sun W, Gianaris NG, Riley AM, Cummins TR, Fehrenbacher JC, Obukhov AG (2014) Furanocoumarins are a novel class of modulators for the transient receptor potential vanilloid type 1 (TRPV1) channels. J Biol Chem 289:9600
144. He JY, Zhang W, He LC, Cao YX (2008) Imperatorin induces vasodilatation possibly inhibiting voltage dependent calcium channel and receptor mediated Ca^{+2} influx and release. Eur J Pharmacol 573:170
145. Zhang Y, Wang QL, Zhan YZ, Duan HJ, Cao YJ, He LC (2010) Role of store-operated calcium in imperatorin-induced vasodilatation of rat small mesenteric artery. Eur J Pharmacol 647:126
146. Mendel M, Skalicka-Wozniak K, Chlopecka M, Dziekan N (2015) Effect of imperatorin on the spontaneous motor activity of rat jejunum strips. Evid Based Compl Altern Med 2015: ID 614849
147. Nie H, Meng LZ, Zhou JY, Fan XF, Luo Y, Zhang GW (2009) Imperatorin is responsible for the vasodilatation of *Angelica dahurica* var. *formosana* regulated by nitric oxide in an endothelium-dependent manner. Chin J Integr Med 15:442
148. Cao YJ, He X, Wang N, He LC (2013) Effects of imperatorin, the active component from *Radix Angelicae* (Baizhi), on the blood pressure and oxidative stress in 2K,1C hypertensive rats. Phytomedicine 20:1048
149. Cao Y, Zhang Y, Wang N, He L (2014) Antioxidant effect of imperatorin from *Angelica dahurica* in hypertension via inhibiting NADPH oxidase activation and MAPK pathway. J Am Soc Hypertens 8:527
150. Kaiser E, Kramar R, Farkouh E (1966) Imperatorin, a respiration inhibitor of succinate oxidation in liver mithocondria. Enzymologia 30:64
151. Kramar R, Kaiser E (1968) Effect of imperatorin, a toxic principle from *Ammi majus*, on energy transfer in mitochrondia. Toxicon 6:145
152. Hwang YL, Im M, Lee MH, Roh SS, Choi BW, Kim SJ, Sohn KC, Lee Y, Seo YJ, Lee JH, Kim CD (2016) Inhibitory effect of imperatorin on insulin-like growth factor-1-induced production in human sebocytes cultured in vitro. Life Sci 144:49
153. Wang LY, Cheng KC, Li Y, Niu CS, Cheng JT, Niu HS (2017) The dietary furocoumarin imperatorin increases plasma GLP-1 levels in type 1-like diabetic rats. Nutrients 9:1192

154. Sancho R, Marquez N, Gomez-Gonzalo M, Calzado MA, Bettoni G, Coiras MT, Alcami J, Lopez-Cabrera M, Appendino G, Munoz E (2004) Imperatorin inhibits HIV-1 replication through an Sp-1 dependent pathway. J Biol Chem 279:37349
155. Tang CH, Yang RS, Chien MY, Chen CC, Fu WM (2008) Enhancement of bone morphogenetic protein-2 expression and bone formation by coumarin derivatives via p38 and ERK-dependent pathway in osteoblasts. Eur J Pharmacol 579:40
156. Tong K, Xin C, Chen W (2017) Isoimperatorin induces apoptosis of the SGC-7901 human gastric cancer cell line via the mitochondria-mediated pathway. Oncol Lett 13:518
157. Hou YJ, Wang C, Wang T, Huang LM, Lin YY, He HZ (2017) Design, synthesis, and evaluation of new series of imperatorin analogs with potential vasodilatory activity. J Asian Nat Prod Res 30:1
158. Curini M, Cravotto G, Epifano F, Giannone G (2006) Chemistry and biological activity of natural and synthetic prenyloxycoumarins. Curr Med Chem 2:199
159. Cao J, Zheng L, Ji L, Lu D, Peng Y, Zheng J (2015) Mechanism-based inactivation of cytochrome P450 2B6 by isoimperatorin. Chem Biol Interact 226:23
160. Pokharel YR, Han EH, Kim JY, Oh SJ, Kim SK, Woo ER, Jeong HG, Kang KW (2006) Potent protective effect of isoimperatorin against aflatoxin B1-inducible cytotoxicity in H4IIE cells: bifunctional effects on glutathione S-transferase and CYP1A. Carcinogenesis 27:2483
161. Moon TC, Jin M, Son JK, Chang HW (2003) The effects of isoimperatorin isolated from *Angelicae dahuricae* on cyclooxygenase-2 and 5-lipoxygenase in mouse bone marrow-derived mast cells. Arch Pharmacal Res 31:210
162. Moon L, Ha YM, Jang HJ, Kim HS, Jun MS, Kim YM, Lee YS, Lee DH, Son KH, Kim HJ, Seo HG, Lee JH, Kim YS, Chang KC (2011) Isoimperatorin, cimiside E and 23-*O*-acetylshengmanol-3-xyloside from Cimicifugae rhizome inhibit TNF-α-induced VCAM-1 expression in human endothelial cells: involvement of PPAR-γ upregulation and PI3K, ERK1/2, and PKC signal pathways. J Ethnopharmacol 133:336
163. Okuyama T, Takata M, Nishino H, Nishino A, Takayasu J, Iwashima A (1990) Studies on the anti-tumor-promoting activity of naturally occurring substances II. Inhibition of tumor-promoter enhanced phospholipid metabolism by umbelliferous material. Chem Pharm Bull 38:1084
164. Murakami A, Gao G, Kim OK, Omura M, Yano M, Ito C, Furukawa H, Jiwajinda S, Koshimizu K, Ohigashi H (1999) Identification of coumarins from the fruit of *Citrus hystrix* DC. as inhibitors of nitric oxide generation in mouse macrophage RAW 264.7 cells. J Agric Food Chem 47:333
165. Choi JS, Shin HY, Kwon KS, Shin S, Choung SY, Kwon YS, Lee JW, Choi BH, Lee CK (2011) Effects of oxypeucedanin on global gene expression and MAPK signaling pathway in mouse neuroblastoma neuro-2A cells. Planta Med 77:1512
166. Eun JS, Park JA, Choi BH, Cho SK, Kim DK, Kwak YG (2005) Effects of oxypeucedanin on hKv1.5 and action potential duration. Biol Pharm Bull 28:657
167. Nizamutdinova IT, Jeong JJ, Xu GH, Lee SH, Kang SS, Kim YS, Chang KC, Kim HJ (2008) Hesperidin, hesperidin methyl chalone and phellopterin from *Poncirus trifoliata* (Rutaceae) differentially regulate the expression of adhesion molecules in tumor necrosis factor-α-stimulated human umbilical vein endothelial cells. Int Immunopharmacol 8:670
168. Azietaku JT, Ma H, Yu XA, Li J, Oppong MB, Cao J, An M, Chang YX (2017) A review of the ethnopharmacology, phytochemistry and pharmacology of *Notopterygium incisum*. J Ethnopharmacol 18:202
169. Zhao AH, Yang XB, Yang XW, Zhang YB, Xu W, Liu JX (2012) Biotransformation products of phellopterin by rat liver microsomes and the inhibition on NO production in LPS-activated RAW264.7 cells. J Asian Nat Prod Res 14:956
170. Deng GG, Wei W, Yang XW, Zhang YB, Xu W, Gong NB, Lu Y, Wang FF (2015) New coumarins from the roots of *Angelica dahurica* var. *formosana* cv. Chuanbaizhi and their inhibition on NO production in LPS-activated RAW264.7 cells. Fitoterapia 101:194

171. Dekermendijian K, Ai J, Nielsen M, Sterner O, Shan R, Witt MR (1996) Characterisaztion of the furanocoumarin phellopterin as a rat brain benzodiazepine receptor partial agonist in vitro. Neurosci Lett 219:151
172. Bergendorff O, Dekermendijian K, Nielsen M, Shan R, Witt MR, Ai J, Sterner O (1997) Furanocoumarins with affinity to brain benzodiazepine receptors in vitro. Phytochemistry 44:1121
173. Shahverdi AR, Saadat F, Khorramizadeh MR, Iranshahi M, Khoshayand MR (2006) Two matrix metalloproteinases inhibitors from *Ferula persica* var. *persica*. Phytomedicine 13:712
174. Shakeri A, Iranshahy M, Iranshahi M (2014) Biological properties and molecular targets of umbelliprenin—a mini-review. J Asian Nat Prod Res 16:884
175. Barthomeuf C, Lim S, Iranshahi M, Chollet P (2008) Umbelliprenin from *Ferula szowitsiana* inhibits the growth of human M4Beu metastatic pigmented malignant melanoma cells through cell-cycle arrest in G1 and induction of caspase-dependent apoptosis. Phytomedicine 15:103
176. Khaghanzadeh N, Samiei A, Ramezani M, Mojtahedi Z, Hosseinzadeh M, Ghaderi A (2014) Umbelliprenin induced production of IFN-γ and TNF-α, and reduced IL-10, IL-4, Foxp3 and TGF-β in a mouse model of lung cancer. Immunopharmacol Immunotoxicol 36:25
177. Khaghanzadeh N, Nakamura K, Kuramitsu Y, Ghaderi A, Mojtahedi Z (2016) Immune-associated proteins with potential in vivo anti-tumor activities are upregulated in lung cancer cells treated with umbelliprenin: a proteomic approach. Oncol Lett 12:5295
178. Kasaian J, Mosaffa F, Behravan J, Masullo M, Piacente S, Ghandadi M, Iranshahi M (2015) Reversal of P-glycoprotein-mediated multidrug resistance in MCF-7/Adr cancer cells by sesquiterpene coumarins. Fitoterapia 103:149
179. Gholami O (2017) Umbelliprenin mediates its apoptotic effect by hormesis: a commentary. Dose-Response 2017:1
180. Fiorito S, Epifano F, Preziuso F, Cacciatore I, Di Stefano A, Taddeo VA, de Medina P, Genovese S (2018) Natural oxyprenylated coumarins are modulators of melanogenesis. Eur J Med Chem 152:274
181. Curini M, Genovese S, Menghini L, Marcotullio MC, Epifano F (2008) Phytochemistry and pharmacology of *Boronia pinnata* Sm. Nat Prod Commun 3:2145
182. Curini M, Epifano F, Genovese S, Menghini L, Ricci D, Fraternale D, Giamperi L, Bucchini A, Bellacchio E (2006) Lipoxygenase inhibitory activity of boropinic acid, active principle of *Boronia pinnata*. Nat Prod Commun 1:1141
183. Epifano F, Sosa S, Tubaro A, Marcotullio MC, Curini M, Genovese S (2011) Topical anti-inflammatory activity of boropinic acid and its natural and semi-synthetic derivatives. Bioorg Med Chem Lett 21:769
184. Epifano F, Menghini L, Pagiotti R, Angelini P, Genovese S, Curini M (2006) In vitro inhibitory activity of boropinic acid against *Helicobacter pylori*. Bioorg Med Chem Lett 16:5523
185. Touati E, Michel V, Correia M, Menghini L, Genovese S, Curini M, Epifano F (2009) Boropinic acid, a novel inhibitor of *Helicobacter pylori* stomach colonization. J Antimicrob Chemother:210
186. Genovese S, Epifano F (2013) Recent developments in the pharmacological properties of 4′-geranyloxyferulic acid, a colon cancer chemopreventive agent of natural origin. Curr Drug Targets 13:1083
187. Curini M, Epifano F, Genovese S, Marcotullio MC, Menghini L (2006) 3-(4′-Geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid: a novel promising cancer chemopreventive agent. Anticancer Agents Med Chem 6:571
188. Genovese S, Curini M, Gresele P, Corazzi T, Epifano F (2011) Inhibition of COX-1 activity and COX-2 expression by 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans*-propenoic acid and its semi-synthetic derivatives. Bioorg Med Chem Lett 21:5995
189. Genovese S, Epifano F, Fiorito S, Curini M, Marrelli M, Menichini F, Conforti F (2013) Conjugation of L-NAME to prenyloxycinnamic acids improves its inhibitory effects on nitric oxide production. Bioorg Med Chem Lett 23:2933

190. Epifano F, Curini M, Genovese S, Blaskovich M, Hamilton A, Sebti SM (2007) Prenyloxyphenylpropanoids as novel lead compounds for the selective inhibition of geranylgeranyl transferase I. Bioorg Med Chem Lett 17:2639
191. Genovese S, Foreman JE, Borland MG (2010) A natural propenoic acid derivative activates peroxisome proliferator-activated receptor-β/δ (PPAR β/δ). Life Sci 86:493
192. Genovese S, Epifano F, Curini M, Menger D, Zembruski NCL, Weiss J (2011) In vitro effects of natural prenyloxycinnamic acids on human cytochrome P450 isozyme activity and expression. Phytomedicine 18:586
193. Miyamoto S, Epifano F, Curini M, Genovese S, Kimi M, Ishigamori-Suzuki R, Yasui Y, Sugie S, Tanaka T (2008) A novel prodrug of 4′-geranyloxyferulic acid suppresses colitis-related colon carcinogenesis in mice. Nutr Cancer 60:675
194. Tanaka T, de Azevedo MB, Durán N, Alderete JB, Epifano F, Genovese S, Tanaka M, Tanaka T, Curini M (2010) Colorectal cancer chemoprevention by 2β-cyclodextrin inclusion compounds of auraptene and 4′-geranyloxyferulic acid. Int J Cancer 126:830
195. Shimizu M, Kochi T, Shirakami Y, Genovese S, Epifano F, Fiorito S, Mori T, Tanaka T, Moriwaki H (2014) A newly synthesized compound, 4′-geranyloxyferulic acid–*N*(ω)-nitro-L-arginine methyl ester suppresses inflammation associated colorectal carcinogenesis in male mice. Int J Cancer 135:774
196. Ito C, Itoigawa M, Otsuka T, Tokuda H, Nishino H, Furukawa H (2000) Constituents of *Boronia pinnata*. J Nat Prod 63:1344
197. Hill K, Fiorito S, Taddeo VA, Schulze A, Leonhardt M, Epifano F, Genovese S (2016) Plumbagin, juglone, and boropinal as novel TRPA1 agonists. J Nat Prod 79:697
198. Epifano F, Genovese S, Fiorito S, Magne Nde C, Clyne C (2014) Nelumal A, the active principle of *Ligularia nelumbifolia*, is a novel aromatase inhibitor. Nat Prod Commun 9:823
199. Jo YS, Huong DT, Bae K, Lee MK, Kim YH (2002) Monoamine oxidase inhibitory coumarin from *Zanthoxylum schinifolium*. Planta Med 68:84
200. Marquis A, Epifano F, Genovese S, Grenier D (2014) The plant coumarins auraptene and lacinartin as potential multifunctional therapeutic agents for treating periodontal disease. BMC Compl Altern Med 12:80
201. Chen IS, Lin YC, Tsai IL, Teng CM, Ko FN, Ishikawa T, Ishii H (1995) Coumarins and antiplatelet aggregation constituents from *Zanthoxylum schinifolium*. Phytochemistry 39:1091
202. Tsai IL, Lin WY, Teng CM, Ishikawa T, Doong SL, Huang MW, Chen YC, Chen IS (2000) Coumarins and antiplatelet constituents from the root bark of *Zanthoxylum schinifolium*. Planta Med 66:618
203. Santos J, Marquis A, Epifano F, Genovese S, Curini M, Grenier D (2013) Collinin reduces *Porphyromonas gingivalis* growth and collagenase activity and inhibits the lipopolysaccharide-induced macrophage inflammatory response and osteoclast differentiation and function. J Periodont 84:704
204. Kohno H, Suzuki R, Curini M, Epifano F, Maltese F, Prieto Gonzales S, Tanaka T (2006) Dietary administration with prenyloxycoumarins, auraptene and collinin, inhibits colitis-related colon carcinogenesis in mice. Int J Cancer 118:2936
205. Nguyen PH, Zhao BT, Kim O, Lee JH, Choi JS, Min BS, Woo MH (2016) Anti-inflammatory terpenylated coumarins from the leaves of *Zanthoxylum schinifolium* with α-glucosidase inhibitory activity. J Nat Med 70:276
206. Gargaro M, Epifano F, Fiorito S, Taddeo VA, Genovese S, Turco A, Puccetti P, Schmidt-Weber CB, Fallarino F (2017) Interaction of 7-alkoxycoumarins with the aryl hydrocarbon receptor. J Nat Prod 80:1939
207. Epifano F, Molinaro G, Genovese S, Teke Ngomba R, Nicoletti F, Curini M (2008) Neuroprotective effect of prenyloxycoumarins from edible vegetables. Neurosci Lett 443:57
208. Feldman M, Tanabe SI, Epifano F, Genovese S, Curini M, Grenier D (2011) Antibacterial and anti-inflammatory activities of 4-hydroxycordoin: potential benefits for periodontal disease. J Nat Prod 74:26

209. Randriaminahy M, Proksch P, Witte L (1992) Lipophilic phenolic constituents from *Helychrisum* species endemic to Madagscar. Nat C J Biosci 47:10
210. Bohlmann F, Zdero C (1982) Naturally occurring terpene derivatives. Part 408. Chemotaxonomy of the genus *Pleiotaxis*. Phytochemistry 21:1434
211. Zdero C, Bohlmann F (1990) Glycerol derivatives and other constituents from *Metalasia* species. Phytochemistry 29:2179
212. Bonifait L, Zhao L, Azelmat J, Genovese S, Epifano F, Grenier D (2014) Synthesis and biological activities of 2,6-dihydroxy-4-isopentenyloxychalcone as an antimicrobial and anti-inflammatory compound. Med Chem 3:300
213. Locatelli M, Genovese S, Carlucci G, Kremer D, Randic M, Epifano F (2012) Development and application of high-performance liquid chromatography for the study of two new oxyprenylated anthraquinones produced by *Rhamnus* species. J Chromatogr A 1225:113
214. Bruyere C, Genovese S, Lallemand B, Ionescu-Motatu A, Curini M, Kiss R, Epifano F (2011) Growth inhibitory activities of oxyprenylated and non-prenylated naturally occurring phenylpropanoids in cancer cell lines. Bioorg Med Chem Lett 21:4173
215. Abu-Mustafa EA, El-Bay FKA, El-Khrisy EAM, Fayez MBE (1973) Natural coumarins. Part XIV. Synthesis of some isoprenyl ethers of psoralene hydroquinone and related products. J Heterocycl Chem 10:443
216. Fiorito S, Epifano F, Palmisano R, Genovese S, Taddeo VA (2017) A re-investigation of the phytochemical composition of the edible herb *Amaranthus retroflexus* L. J Pharm Biomed Anal 143:183
217. Taddeo VA, Genovese S, de Medina P, Palmisano R, Epifano F, Fiorito S (2017) Quantification of biologically active *O*-prenylated and unprenylated phenylpropanoids in dill (*Anethum graveolens*), anise (*Pimpinella anisum*), and wild celery (*Angelica archangelica*). J Pharm Biomed Anal 134:319
218. Taddeo VA, Epifano F, Fiorito S, Genovese S (2017) Comparison of different extraction methods and HPLC quantification of prenylated and unprenylated phenylpropanoids in raw Italian propolis. J Pharm Biomed Anal 129:219
219. Fiorito S, Epifano F, de Medina P, Taddeo VA, Genovese S (2016) Two novel cinnamic acid derivatives in honey and propolis. J Apicult Res 55:228
220. Alhassain AM, Abdullahi MI, Uba A, Umar A (2014) Prenylation of aromatic secondary metabolites: a new frontier for the development of novel drugs. Trop J Pharm Res (2):307

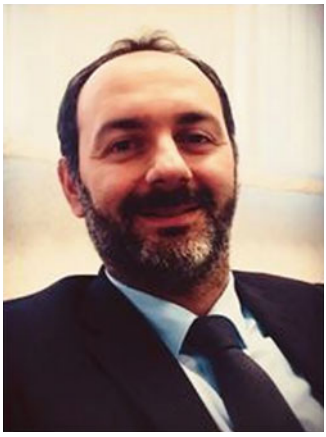
Serena Fiorito obtained her degree in Pharmacy in October 2012 at the Faculty of Pharmacy of the University "Gabriele D'Annunzio" of Chieti-Pescara. She soon after started her academic career in January 2013, having been appointed as a Ph.D. student in "Drug Sciences" at the Department of Pharmacy of the same University under the tutorship of Prof. Francesco Epifano. The research topics chosen for the elaboration of her doctoral thesis concerned: (a) the synthesis and biological investigations of natural and semisynthetic prenylated 1,4-naphthoquinones and their complexes with transition metals, (b) the synthesis and the study of pharmacological effects of natural and semisynthetic prenyloxy- and prenylazo-phenylpropanoids and polyketides, and (c) the development of novel HPLC methodologies for the analysis of prenyloxyphenylpropanoids in edible fruits and vegetables and their preparations. During her Ph.D. studies she spent 7 months at the Faculty of Pharmacy of the Universitè Libre de Bruxelles (Belgium) working in the research group of Prof. Robert Kiss and Prof. Veronique Mathieu. From February 2017 she obtained a 2-year postdoctoral grant at the Department of Drug Sciences of the University of Perugia (Italy) where she is currently working. She is presently author of 57 publications in international peer-reviewed journals. In 2015 she was awarded with the "Young Researcher Award 2015" issued by SIROE (Italian Society for Research on Essential Oils).

Francesco Epifano obtained his degree in Medicinal Chemistry and Pharmaceutical Technology in November 1993 at the University of Perugia. He started his academic career in 1994 at the Institute of Organic Chemistry of the University of Perugia in the research group of Prof. P. Ceccherelli, working in the field of the use of diazo compounds and organoselenium reagents in the synthesis of biologically active natural products. He obtained his Ph. D. in Agricultural Entomology at the Faculty of Agricultural Sciences of the University of Perugia in 1998 under the supervision of Prof. P. Ceccherelli, Prof. M. Curini, and Prof. R. Tiberi (University of Florence). The subject of his thesis was the synthesis, properties, and ecological role of semiochemicals isolated from insects and plants. From 1999 to 2005 he obtained a postdoctoral research grant at the Department of Medicinal Chemistry and Pharmaceutical Technology of the University of Perugia, under the tutorship of Prof. M. Curini working in the field of the use of lanthanide triflates as catalysts of reactions in the synthesis of natural products. After having switched his research interests towards the design and development of novel and eco-friendly synthesis methodologies and the study of pharmacological activities of rarely occurring natural compounds in 2003, in February 2006 he became Research Associate of Pharmaceutical Biology and Phytochemistry at the Faculty of Pharmacy of the University "Gabriele D'Annunzio" of

Chieti-Pescara. From October 2016 he has been Associate Professor of Medicinal Chemistry at the Department of Pharmacy of the same University. His work is currently concerned with the synthesis and pharmacological properties of secondary metabolites from plants, fungi, and bacteria. Among his main research topics are the synthesis and the study of biological effects of natural and semisynthetic prenyloxy- and prenylazo-phenylpropanoids, polyketides, and naphthoquinones. He is currently author of 217 peer-reviewed publications and has presented several invited lectures, keynote, oral, and poster communications at national and international congresses. In 2010 he was awarded the IADR/Glaxo Smith Kline Innovation in Oral Care Award as the co-investigator of the project entitled "Therapeutic potential of *Citrus* auraptene for periodontal disease". He received in 2012 the Apivita Award for Phytochemistry and in 2017 the Pierre Fabre-Phytochemical Society of Europe Award, both from the Phytochemical Society of Europe.

Francesca Preziuso obtained her degree in Pharmacy in March 2017 at the Faculty of Pharmacy of the University "Gabriele D'Annunzio" of Chieti-Pescara. She soon after started her academic career in April 2017 as the recipient of a 1-year postgraduate grant. In November 2017 she was appointed as a Ph.D. student of an Italian-German joint-doctoral course in "Biomolecular and Drug Sciences" at the Department of Pharmacy of the same University and the Department of Pharmaceutical Biology of the University "Christian Albrecht" of Kiel under the tutorship of Prof. Antonio di Stefano and Prof. Christian Zidorn. The research topics chosen for the elaboration of her doctoral thesis concern: (a) the development of novel HPLC, UHPLC, and LC-MS methodologies for the analysis of oxyprenylated secondary metabolites in edible fruits and vegetables, and their preparations and (b) phytochemical investigations on plants belonging to the flora of central and southern Europe. She is currently author of eight publications in international peer-reviewed journals.

Vito Alessandro Taddeo obtained his degree in Medicinal Chemistry and Pharmaceutical Technology in July 2014 at the Faculty of Pharmacy of the University "Gabriele D'Annunzio" of Chieti-Pescara. He soon after started his academic career in September 2014 as the recipient of a 1-year post-graduate grant. In November 2015 he was appointed as a Ph.D. student of an Italian-Spanish joint-doctoral course in "Biomolecular and Drug Sciences" at the Department of Pharmacy of the same University and "Chemistry and Chemical Engineering" at the Instituto Universitario de Bio-Orgánica Antonio González in La Laguna-Tenerife under the tutorship of Prof. Salvatore Genovese and Prof. Isabel López Bazzocchi. The research topics chosen for the elaboration of his doctoral thesis concern: (a) the development of novel HPLC, UHPLC, and LC-MS methodologies for the analysis of oxyprenylated secondary metabolites in edible fruits and vegetables, bee products, and their preparations and (b) phytochemical investigations on plants belonging to the flora of the Canary Islands and South and Central America. He is currently author of 42 publications in international peer-reviewed journals.



Salvatore Genovese obtained his degree in Pharmacy in July 2004 at the Faculty of Pharmacy of the University of Perugia. He started his academic career in September 2004 at the Section of Organic Chemistry of the Department of Medicinal Chemistry and Pharmaceutical Technology of the University of Perugia under the direction of Prof. M. Curini and Dr. F. Epifano, investigating the use of lanthanide triflates as catalysts of reactions in the synthesis of biologically active natural products. He obtained his Ph.D. in "Medicinal Chemistry and Pharmaceutical Technology" in November 2007 at the Faculty of Pharmacy of the same University under the tutorship of Prof. M. Curini, having as the research topic for the elaboration of his doctoral thesis being the synthesis and pharmacological activity of 3-(4′-geranyloxy-3′-methoxyphenyl)-(2*E*) propenoic acid, active principle of *Acronychia baueri*, and its derivatives. After having spent an additional year with a postdoctoral grant in the above-cited Department in the research group of Prof. M. Curini, in November 2008 he became Research Associate of Pharmaceutical Biology and Phytochemistry at the Faculty of Pharmacy at the University "G. D'Annunzio" of Chieti-Pescara. Since July 2017 he has been Associate Professor of Medicinal Chemistry at the Department of Pharmacy of the same University. His work is concerned with the synthesis, pharmacological properties, and HPLC and UHPLC analysis of secondary metabolites from plants and fungi. He is currently author of 145 peer-reviewed publications and has given several invited lectures, keynote, oral, and poster communications at national and international congresses.

Secondary Metabolites of Endophytic Actinomycetes: Isolation, Synthesis, Biosynthesis, and Biological Activities

Darlon Irineu Bernardi, Fernanda Oliveira das Chagas, Afif Felix Monteiro, Gabriel Franco dos Santos, and Roberto Gomes de Souza Berlinck

Contents

D. I. Bernardi · A. F. Monteiro · G. F. dos Santos · R. G. de Souza Berlinck (✉)
Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, São Paulo, Brazil
e-mail: rgsberlinck@iqsc.usp.br

F. O. das Chagas
Instituto de Pesquisa de Produtos Naturais Walter Mors, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.),
Progress in the Chemistry of Organic Natural Products, Vol. 108,
https://doi.org/10.1007/978-3-030-01099-7_3

1 Introduction

Life on Earth is regulated chemically at the adaptative dimension by molecules selected during evolution that are biosynthesized by carefully tuned enzymatic machineries [1]. Selection of secondary metabolites has been driven by interactions with proteins, which ultimately results in a phenotypic expression known as a biological activity. While the actual functions of secondary metabolites are still largely unknown, it is well recognized that natural products exert various biological effects, which have impacted human activities and health since ancient times [2]. Approximately 20–25% of secondary metabolites display a defined bioactivity. Among the ≈300,000 natural products isolated from all biological sources, 10% have been obtained from micro-organisms. Metabolites produced by organisms belonging to the phylum Actinobacteria represent almost 50% of all known microbial natural products [3]. Microbial antibiotics have mainly been isolated from actinobacterial cultures (≈65%), with the remaining portion being of fungal origin [4]. Over 140 actinobacterial genera are known, but only a fraction of those described have been explored as a source of bioactive secondary metabolites [5]. Most bioactive natural products isolated from actinobacterial cultures are produced by *Actinoplanes*, *Amycolatopsis*, *Micromonospora*, *Nocardia*, *Saccharopolyspora*, and *Streptomyces* species [6]. Actinobacterial secondary metabolites are relevant for agriculture and food production [7], but mainly for the potential treatment of human illnesses, in particular as antibiotics [8, 9].

Actinobacteria are considered a remarkable group of microorganisms, not only because of their unique characteristic features, but also as a result of their ability to produce chemically diverse biologically active secondary metabolites [10]. Actinobacteria, also known as Actinomycetes, are Gram-positive bacteria defined by having a high G+C content in the genome and are considered as a major evolutionary bacterial lineage. Many additional specific characteristics of Actinobacteria have been described [11]. These are cosmopolitan, highly diverse bacteria, found in every single terrestrial environment, from extreme conditions, including deep oceans, to every soil sample, as well as associated or symbiont with other organisms [10]. Actinobacteria are well-known antibiotic producers [9, 12], and also of an array of bioactive compounds [5, 13–16]. The biodiversity and taxonomy of Actinobacteria has been the subject of several reviews [12–15, 17–19]. Biosynthesis gene clusters of this bacterial group also have been reviewed several times [19–21], as well as their capacity to produce natural compounds [12, 18, 20, 22].

Since there are few reports in the literature on new natural products isolated from endophytic Actinobacteria, the intent of this contribution is to present a comprehensive overview on endophytic bacteria biology and on secondary metabolites produced by this microbial group, covering the period between 1998 and 2018.

2 The Actinobacteria Phylum

Actinobacteria belong to one of the largest phyla of bacteria [5, 14], with distinct morphologic and physiologic characteristics [14]. The divergence of the Actinobacteria from other bacterial phyla is so ancient that it is currently not possible to identify its most closely related group. The earliest descriptions of the Actinobacteria in 1875 were provided by the German microbiologist Ferdinand Julius Cohn; however, the actinobacterial microbial group was officially recognized only in 1916 [6]. The taxonomy of Actinobacteria evolved significantly over time, including the nomenclature of its subdivisions. The Actinobacteria phylum encompasses the classes Actinobacteria, Acidimicrobiia, Coriobacteriia, Nitriliruptoria, Rubrobacteria, and Thermoleophilia. The Actinobacteria class comprises 16 orders, including Actinomycetales [14, 23].

Once the Actinomycetales order was established by Buchanan in 1917 [14, 24], its members have been designated as Actinomycetes. Thus, the term "Actinomycetes" refers to members of the Actinomycetales order, while the term "Actinobacteria" indistinctly refers to members of the Actinobacteria class and the Actinobacteria phylum. The designation "Actinobacteria" has been used generally in reference to a specific taxonomic class of microorganisms. However, it is important to note that the scientific literature is unclear when referring to these two terms, which usually have been used interchangeably. For example, a common recurrent error is the assignment of the Actinobacteria exclusively within Actinomycetes. The word "Actinomycetes" derives from the Greek "ακτίνα" ("aktina" = ray or radiation) and "μύκητες" ("myketes" = fungi). In the past, Actinomycetes were believed to be transitional forms between fungi and bacteria, because they present cellular characteristics of bacteria but produce mycelia in the same manner as fungi [14].

Actinobacteria are widely distributed in Nature [14, 19, 25, 26] (for examples of typical Actinomycetes cultures, see Plate 1). Actinomycetes are typified by the formation of a substrate and aerial mycelium, which explains their filamentous characteristics, and the presence of many spore forms, or their complete absence. Actinobacteria can also grow as rods, such as observed for *Mycobacterium*, *Corynebacterium*, *Gordona*, *Nocardia*, and *Rhodococcus*. In several instances, it has been reported that Actinobacteria may be able to produce filamentous cells at some stage during their cycle life. For instance, *Mycobacterium tuberculosis* undergoes morphological alterations into filamentous cells when it infects human cells [27]. Detailed taxonomic and morphological aspects of Actinobacteria can be found in specialized reviews [9, 14].

Streptomyces represent the dominant strains of Actinobacteria currently known in Nature [19]. Species belonging to other genera are collectively known as "rare Actinobacteria", since the frequency of the isolation of such strains by conventional methods is much lower [8, 28]. These "rare Actinobacteria" are now of great biotechnological interest, because they are considerably lesser known and represent a repository of novel natural compounds. Therefore, new microbial isolation methods have been developed continuously for the isolation of these strains,

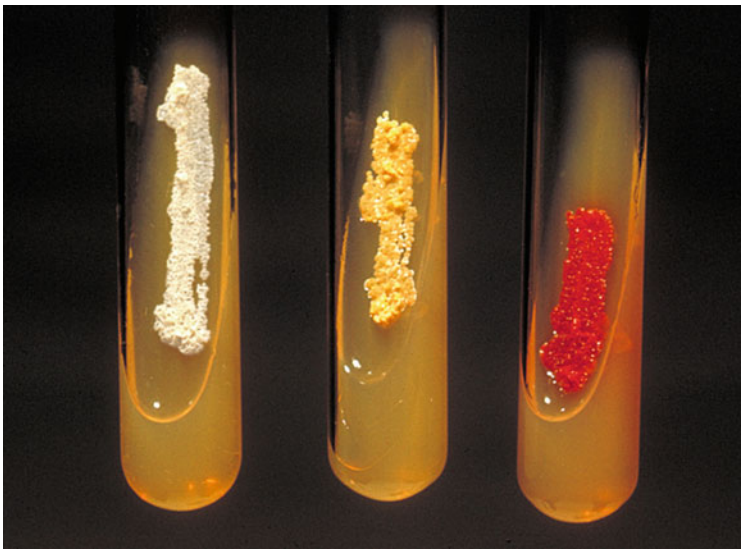


Plate 1 Slant cultures of Actinomycetes spp. Left: *Actinomadura madurae*; middle: *Nocardia asteroides*; right: *Micromonospora* spp. Photograph courtesy of David Berd, 1972, CDC, Public Health Image Library via Wikimedia Commons

including specific or selective isolation techniques [8, 28] as well as metagenomic analyses [18, 29]. A variety of novel actinobacterial species, including the rare ones, have been found as plant endophytes [19, 30].

3 Actinobacterial Life Cycle and Endophytism

Actinobacteria adopt different lifestyles, such as those free-living in both terrestrial and aquatic ecosystems or those as plant and animal symbionts. Once engaged in symbiotic associations, these microorganisms may become pathogens, mutualists, or commensalists [7, 14]. For example, anaerobic Actinobacteria belonging to *Bifidobacterium* spp. are gastrointestinal commensals. *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Corynebacterium diphtheriae*, and *Propionibacterium acnes* are human pathogens, causing leprosy, tuberculosis, diphtheria, and acne, respectively [6].

Plants produce root exudates that act as chemical signals for recruiting surrounding beneficial microbes and pathogens also sense those signals [31]. Microorganisms frequently infect plants injured with root wounds. Alternatively, microbes may also produce hydrolytic enzymes for degrading epidermal cells and penetrating plant tissues [32]. Actinobacteria are very abundant in the soil and include rhizosphere microbes that may be attracted by root exudates and colonize plants. However, the specific mechanisms by which Actinobacteria colonize plants and evade the plant immune system is still poorly understood [9, 32].

During symbiotic associations with plants, Actinobacteria may change their status from pathogens to mutualists. Deleterious interactions among plants and Actinobacteria are less common than those observed for bacteria [14]. Still, some species of Actinobacteria are the causal agents of particular crop diseases [33]. Actinobacterial traits of pathogenicity are not yet well defined, but the production of lytic enzymes and phytotoxins has been associated with severe plant damage [14, 34]. On the other hand, various Actinobacteria establish beneficial relationships with plants. Some species have been described as plant growth-promoting rhizobacteria and nitrogen-fixing Actinobacteria, which comprise mutualistic associations with plants [31]. Consequentially, Actinobacteria have been isolated as endophytes from a variety of plants [19].

Endophytic microorganisms can be considered as mutualists, such as in the case of obligate fungal ryegrass-endophytes, or commensalists, which comprise the most well-studied endophytic microorganisms [31, 35]. However, little is known about actinobacterial endophytic physiology as plant colonizers and habitants [9]. Certain actinobacterial strains improve plant fitness by promoting soil nutrient uptake and growth stimuli, and may also act in the biological control of pathogens by producing bioactive secondary metabolites and eliciting plant defense [32]. Although the exact mechanisms involved in these processes are not yet well known [9, 14], these findings provide evidence that Actinobacteria play key roles in the improvement of plant performance similar to other endophytic microbes [31, 35].

4 Physiological and Morphological Aspects in Actinobacterial Development

Physiological investigations mainly have been performed for the genus *Streptomyces*, the most representative of Actinobacteria. Details of the actinobacterial life cycle will not be addressed here, since they have been well described elsewhere [9, 14, 36].

In general, actinobacterial spores germinate to produce the initial hyphae that grow further and then branch to form vegetative or substrate mycelia. The stage from spore germination to substrate mycelium involves a primary compartmentalized mycelium that partially dies and differentiates to form a second multinucleated mycelium, which is the substrate mycelium itself [36]. Growth occurs through tip

extension, in which cells multiply without subsequent fission, making *Streptomyces* an example of a multicellular bacterium [14, 37]. Under unfavorable conditions, the sporulation process is triggered and involves programmed cell death. Then, the vegetative mycelium is degraded enzymatically, releasing material and nutrients that are consumed during the development of aerial hyphae. Aerial hyphae form the reproductive mycelium that differentiates to produce chains of spores. Thereafter, spores may disseminate and find appropriate conditions for germination [9, 14]. In addition to these well-characterized developmental stages, *Streptomyces* spp. may also exhibit an additional trait that allows them to rapidly traverse solid surfaces, which was recently described and termed as "exploratory growth" [38].

In submerged cultures, *Streptomyces* mycelia grow as pellets, clumps (less condensed than pellets), branched hyphae, or nonbranched hyphae [39, 40]. Most species do not sporulate in liquid cultures, but are able to differentiate morphologically in these conditions [40, 41]. The developmental process in liquid media is comparable to that on solid surfaces. Spores germinate into primary compartmentalized mycelia that form radially growing pellets. At the center of these pellets, the primary mycelia die and give rise to second multinucleated mycelia, which are responsible for producing antibiotics [36, 40]. Differentiation into the second mycelia may not ensure secondary metabolite production, because environmental factors such as culture nutrients and conditions also regulate the biosynthesis of secondary metabolites [36]. Moreover, mycelial differentiation and antibiotic production depends on cell density [40].

With the aim of simulating a "natural environment of growth", spore dilution and autoclaved forest soil is used for culturing *Streptomyces*. Primary compartmentalized mycelia predominate in the developmental cycle under these conditions [42]. The second multinucleated mycelia is a transient feature that arises under unfavorable conditions. It plays a role in sporulation and antibiotic production, diverging from what is observed in ordinary laboratory conditions [36].

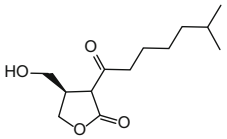
5 Regulation of Secondary Metabolite Production

In submerged cultures, actinobacterial morphogenesis and its role in antibiotic production needs to be further investigated [41]. As mentioned earlier, the second multinucleated mycelium is the structure responsible for the production of secondary metabolites. Compound titers largely depend on mycelia macroscopic morphology [36, 40]. While aggregated mycelia may favor the production and release of some metabolites, a fragmented mycelium improves the biosynthesis of additional metabolites [14].

In surface-grown conditions, Actinobacteria usually produce antibiotics at the onset of the development, which is the moment when these microorganisms spore. Since sporulation is related to resource scarcity, secondary metabolite biosynthesis usually occurs under nutritional depletion [43, 44].

Complex regulatory mechanisms control the transcription of actinobacterial genes, including those involved in morphogenesis and natural product biosynthesis [44]. Activators and repressors are part of a regulatory cascade that involves specific and global regulators [4]. Pathway-specific transcriptional regulators are proteins encoded by genes present in a specific biosynthetic gene cluster and these proteins directly regulate the respective gene cluster. On the other hand, global or pleiotropic regulators act by activating or repressing the expression of other regulatory genes. Thus, pleiotropic regulators are at the top of regulatory cascades and can control the expression of many genes [43–45].

The proteins SARP and LAL are the two major families of pathway-specific activators in *Streptomyces*, whereas regulators belonging to the TetR family act as repressors in the absence of a cognate ligand [43–45]. The γ-butyrolactone **1**, named A-factor, is a cognate ligand of a TetR sub-family and was the first signaling molecule discovered as a regulator of secondary metabolism in the Actinobacteria [46]. Various secondary metabolites acting as ligands in TetR proteins were reported subsequently, including butanolides and analogues, furans, peptides, and bile salts [45–49]. Biosynthesis end products may also act as self-regulators of their own metabolic pathways [50]. Certain TetR regulators have a large cavity that enables interactions of various ligands, while some TetR proteins have no cavity [51], indicating that the mechanism of regulation of these proteins is more complex than expected and needs to be better investigated.



1 (A-factor)

Two main pathways dependent on the nutritional status of Actinobacteria regulate antibiotic production [43, 44]. Under depleted conditions, vegetative mycelia degrade and release *N*-acetylglucosamine, which serves as a signal to control both development and antibiotic production. *N*-Acetylglucosamine is converted into *N*-acetylglucosamine-6-phosphate and glucosamine-6-phosphate, allosteric inhibitors of the global regulator DasR [9]. Without modulation, the protein DasR acts pleiotropically by repressing pathway-specific genes involved in the activation of natural product biosynthesis, which ultimately leads to the inhibition of metabolite production [52]. Therefore, inhibition of DasR results in induction of biosynthesis. On the other hand, under nutritional abundance, accumulation of *N*-acetylglucosamine in culture media promotes an opposite effect, which is known as the feast or famine response [52]. The regulatory signaling pathway mediated by *N*-acetylglucosamine has been discussed in detail [9, 14, 52–54].

In addition to the regulatory pathway mediated by *N*-acetylglucosamine, a stringent control or response has also been reported relying on the accumulation of the alarmones guanosine pentaphosphate or tetraphosphate. Under nutrient depletion,

balanced activities of synthetase RelA and hydrolase SpoT, result in the production of signaling guanosine pentaphosphate or tetraphosphate. RelA is activated when nitrogen and amino acids are lacking, whereas SpoT becomes active in the absence of carbon, phosphate, iron, and fatty acids [55, 56]. Guanosine pentaphosphate or tetraphosphate may affect many actinobacterial physiological processes, including secondary metabolism biosynthesis [56, 57]. During a stringent response, the microbial growth is usually repressed and antibiotic biosynthesis is increased, except for secondary metabolites, which are produced during the exponential growth phase [9, 56, 58].

Many environmental factors may regulate the production of secondary metabolites [43, 44]. Not only a specific nutritional status, but also carbon sources play roles in regulation [59]. Preferential consumption of glucose and other carbohydrates as carbon sources leads to a phenomenon known as carbon catabolite repression, which involves regulatory proteins and other signaling molecules such as cyclic adenosine monophosphate and adenosine triphosphate [59–64]. Actinobacterial production of secondary metabolites is specifically adjusted, a trait that may explain why some screening campaigns aiming for the discovery of further Actinobacteria secondary metabolites have been unsuccessful.

6 Role of Chemical Ecology in Natural Product Biosynthesis

Actinobacterial secondary metabolism is influenced by both biotic and abiotic environmental stimuli. Interactions with other microorganisms and with higher organisms, such as a host plant, may trigger antibiotic production by expressing silent biosynthetic genes [9, 65]. Endophyte microbe-microbe interactions have been investigated. Co-cultures have been applied for culturing endophytes isolated from the same host plant. The results indicated that secondary metabolism is induced under microbial interactions [6, 66–68].

Interaction with higher organisms should also be considered and all ecological aspects involved in such relationships are very complex. Herein, emphasis will be directed to microbe-plant interactions. Not only do microbes promote the alteration of plant physiology [14, 32], but plants may also modify the microbial metabolism. Plant tissues constitute a particular environment where microbes have to thrive. An understanding of the chemical ecology of plants-microorganisms should enable the development of new strategies to elicit the expression of actinobacterial biosynthesis gene clusters under unnatural conditions.

Actinobacteria genomics that regulate secondary metabolism has been extensively investigated. The expression of Actinobacteria gene clusters can be modulated by environmental conditions. The main distinction between endophytic and non-endophytic Actinobacteria relies on the selective activation of specific metabolic pathways, which is controlled for transcriptional taxa.

Evolutionary selection of genetic contents has been reported for obligate symbionts [69, 70]. Evolutionary selection has also been suggested for fungi and bacteria, including Actinobacteria that co-evolved with plants during the early colonization of land by terrestrial plants [35]. The biosynthesis of common secondary metabolites by plants and their endophytic microorganisms should be considered as resulting from co-evolutionary pressures [66]. The biosynthesis of common metabolites by plants and their associated microbiota has been observed for both prokaryotic and eukaryotic endophytes. This is a "hot topic" of research, since many potently active compounds, such as paclitaxel, camptothecin, podophyllotoxin, hypericin, vincristine, vinblastine, and the ansamytocins, have been detected in cultures of endophytic fungi and bacteria [71–80].

It seems that the biosynthesis machinery of endophytic Actinobacteria is different from that of free-living Actinobacteria. For instance, genomic analysis of different *Streptomyces albus* strains has indicated that a common core of secondary metabolites is genetically coded; only a few strains are able to produce additional metabolites. Few of such additional compounds are produced by more than one strain. Other additional natural products are strain-specific, indicating that different strains of a same species may harbor specific gene clusters [81]. Transcriptomic analyses of marine *Salinispora* sp. actinomycete strains have indicated the different expression of genes conserved within this genus. Regulatory silencing mechanisms or the absence of activators for specific gene clusters producing a common metabolite are responsible for an inactive pathway. The presence of silent gene clusters maintained in strains during evolution likely implies the existence of another level of regulation for gene expression [82].

Nevertheless, the diversification between the secondary metabolism of endophytic and non-endophytic Actinobacteria has not yet been investigated, because most studied Actinobacteria have been isolated from the soil and only a small portion of the scientific literature to date has dealt with secondary metabolites from endophytic Actinobacteria. Therefore, it is clear that when referring to endophytic Actinobacteria and their metabolic particularities, there are more questions to be addressed than currently there are answers.

7 Strategies for the Isolation of Endophytic Actinobacteria

General procedures for isolation of endophytic Actinobacteria consist of plant selection, surface sterilization, the choice of culture medium and growth conditions, strain isolation and purification, genetic and morphological identification, as well as taxonomic classification [83–85].

Actinobacteria have been isolated from a variety of plants, most of which are known for traditional medicinal purposes [13, 25, 86]. After collection, plants are cleaned in water to remove soil and organic matter. In order to remove epiphytic microorganisms, surface sterilization is performed by consecutive immersions in 70% ethanol and 2% sodium hypochlorite, followed by washing the tissues with

sterile water [83–85]. Additional procedures may include immersion in an antifungal solution for the inhibition of fungal growth [85].

The effectiveness of surface sterilization can be checked by aliquoting the water of the last washing step onto isolation media plates. An alternative procedure is to imprint surface-sterilized plants onto the plates. If the growth of fungal or bacterial contaminants is not observed, plants are then cut aseptically or crushed into small pieces, plated on selective culture media, and incubated. Selective growth media include supplemented VL70 gellan gum, Bennett's agar, starch casein agar, and humic acid vitamin B, all containing antibacterial and antifungal compounds, such as nalidixic acid and nystatin, to inhibit the growth of Gram-negative bacteria and fungi, respectively [83–85]. Plates are usually incubated at 25–30°C for up to 16 weeks. During this period, plates are checked for emerging microorganisms. Each colony is completely removed as soon as it appears and then transferred to other plates [84]. Large colonies of fast-growing Actinobacteria may overgrow slow-growing strains, which are isolated last or are frequently lost. Most streptomycete strains emerge within 3 weeks; rare Actinobacteria usually appear after 6 weeks [84]. This is the most likely reason why rare Actinobacteria have a low isolation frequency when researchers apply a non-selective methodology and isolate strains for just 1 month. Ideally, several distinct isolation experiments should be performed with the same plant samples if isolation to exhaustion is aimed at, frequently yielding novel and unusual strains. Axenic strains are then grown in diverse culturing media for morphological characterization and DNA extraction for microbial identification, usually by 16S rRNA gene sequencing [83–85].

Alternative methods have been applied for the isolation of rare endophytic Actinobacteria [87]. Also, culture-independent approaches have been used to evaluate the diversity of endophytic Actinobacteria in a host plant [87–89].

A major problem found in culture-independent methods, such as metagenomic analysis, is determining the ecological status of those identified microbes. Resident microbiota may present different interactions with plants that are not easily assessed. Additionally, endophytic isolation protocols do not guarantee that the isolated strains are really mutualistic [35]. Strains may be transient endophytes, or latent pathogens, not expressing signals of pathogenicity at the moment of plant harvesting.

Differentiation of endophytic and pathogenic microbes is not easily accomplished using genetic analyses. General traits related to performance as plant symbionts may be observed in both beneficial and malefic microorganisms [66]. Such traits are not sufficient for a confident classification of their relationship with plants. Virtually all microorganisms present a genetic arsenal for surviving and establishing a population in a particular environment, which makes their metabolic features very similar. The endophytic actinobacterial microbiome is known to promote plant growth, health, and beneficial effects, in many cases mediated by metabolic interactions. Microsymbionts produce a variety of metabolites. These compounds play crucial roles in defense and competition, and may also be needed for specific interaction and communication with the host plant. Bilateral metabolite production is also known, as well as endophyte-modulated plant synthesis [90].

8 Culturing Endophytic Actinobacteria and Their Secondary Metabolism

Despite the impressive number of known Actinobacteria, it has been estimated that these represent less than 1% of their total diversity. Therefore, "yet uncultivated" strains remain largely untapped for the production of structurally unique and bioactive metabolites. Considerable progress has been achieved for the cultivation of Actinobacteria in the last few decades. The main reason why many Actinobacteria still remain uncultivated is probably that growth under laboratory conditions fails to mimic essential aspects of the environment, like the absence of competitors [91].

Successful culturing of Actinobacteria in the laboratory is essentially dependent upon the nutritional composition of the media and incubation conditions. When culturing endophytic Actinobacteria, a suitable approach is using a medium composition that mimics the micro-environments of internal parts of the host plant [92]. For instance, Tap Water Yeast Extract medium is a depleted nutrient medium very effective for the isolation of endophytic Actinobacteria from several plant species [93–95], since high nutrient concentrations propitiates the overgrowth of fast-growing over slow-growing Actinobacteria [92]. Media composed of amino acids such as proline, arginine, and asparagine as nitrogen sources, as well as xylan, sodium propionate, and sodium succinate as carbon sources, provide effective actinobacterial isolation during a large-scale investigation of plants from a tropical rainforest, yielding Actinobacteria of uncommon and rare genera [94]. For many plants, amino acids correspond to the major nitrogen source, while cellulose and xylan are the primary constituents of the plant cell wall [96, 97]. The addition of a plant extract into the growth medium also improves the isolation of rare Actinobacteria [98]. Media supplemented with low concentrations of plant polymers (gellan gum, xylan, and pectin), constituent sugars (glucose, galactose, xylose, arabinose, gluconate, and carboxymethyl cellulose), and 17 amino acids, improved the isolation of 16 rare Actinobacteria genera, including a new genus *Flindersiella*, within the family *Nocardioides* [84].

Understanding the physiological properties of endophytic Actinobacteria is still very challenging due to their complex symbiotic microbial-host relationships. The aforementioned examples emphasize that simulating the micro-environments of inner plant tissues when designing the isolation and growth media is a promising approach for accessing plant-endophytic Actinobacteria [97].

The culturing of Actinobacteria at the laboratory scale is often limited to small amounts of liquid and solid fermentations for accessing their bioactive metabolites. Each strain may require different fermentation conditions, including nutrients, concentration, pH, incubation time, temperature, and aeration in order to achieve an optimal production of compounds. An analysis of the diversity of cultivated endophytic Actinobacteria (Fig. 1) reveals a total of 6 genera comprising 28 isolates, including rare species. Therefore, the associated plant sources (especially medicinal plants) possess a tremendous potential for the discovery of novel actinobacterial species. Not surprisingly, Streptomycetes are the most abundant among the isolates, with probably new strains to be completely identified further due to their low genomic similarity with known species.

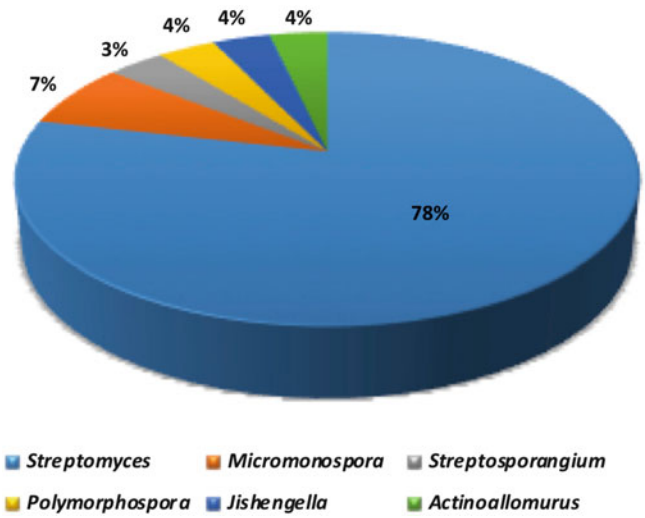


Fig. 1 Diversity of culturable endophytic Actinobacteria

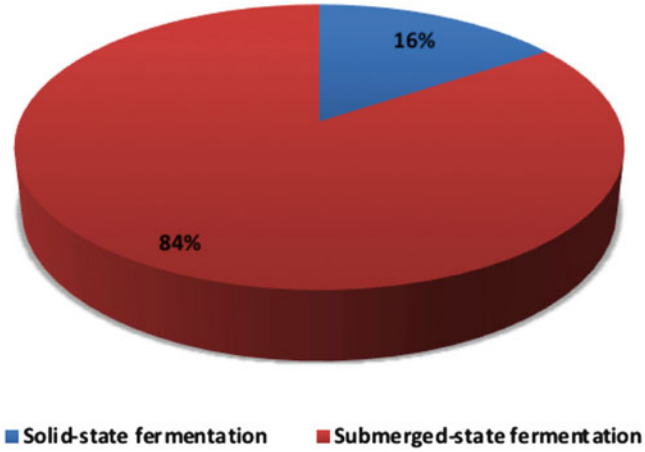


Fig. 2 Overview of solid and liquid culturing for the growth of endophytic Actinobacteria

Submerged fermentation is largely employed for culturing endophytic Actinobacteria for the isolation of secondary metabolites (Fig. 2), probably because liquid media can be easily scaled-up.

Time of incubation for the production of secondary metabolites is varied (Fig. 3) in the fermentation processes since it often depends on the distinct rate of metabolization of the carbon source by actinobacterial strains, which is also influenced by compositional media variations and incubation conditions.

Sequence data have revealed that the majority of secondary metabolites encoded in Actinobacteria genomes is not accessed, probably because biosynthesis genes are not conveniently transcribed under standard laboratory conditions. Efforts to circumvent this drawback have been developed by applying strategies to activate silent

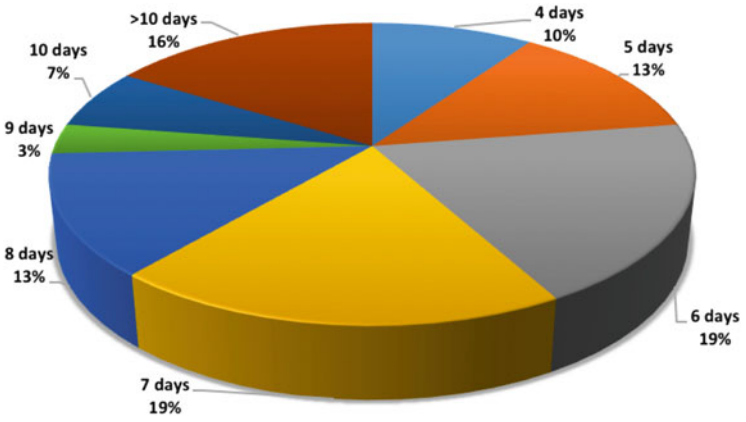


Fig. 3 Incubation time for the growth of endophytic Actinobacteria towards the isolation of secondary metabolites

gene clusters, which include chemical stimulation, biological stimulation (by co-cultivation or mixed fermentation), and molecular elicitation [22]. Such methods have the potential to affect the cellular machinery at distinct levels—in the genome, transcriptome, proteome, or at the metabolome [99].

Co-cultivation and selective variation in fermentation conditions (medium composition, light exposure, pH, temperature, oxygen supply) induce significant alterations in actinomycete metabolic profiles [22]. Systematic variations in fermentation parameters aiming to optimize metabolic production such as OSMAC (one strain, many compounds) [100] are effective in activating silent or under-expressed metabolic pathways [22]. Co-cultivation experiments have been performed between Actinobacteria-Actinobacteria, Actinobacteria-bacteria, and Actinobacteria-fungi. Biological elicitors, such as microbial lysates and microbial cell components, can be recognized by bacterial cells as external signals, triggering responses that promote overproduction of stress-related metabolites. Chemical elicitation by non-biological compounds to induce changes in the metabolome include inorganic compounds, rare Earth elements, and heavy metal ions, among others. Cultivation-independent approaches have also emerged, driven by bioinformatic tools and analytical techniques (LC-MS, MALDI-TOF), such as genome mining combined with genetic manipulation approaches. Such strategies are powerful approaches to access the underexploited potential of endophytic Actinobacteria [22].

9 Secondary Metabolites Produced in Culture by Endophytic Actinobacteria: Isolation, Activities, Biosynthesis, and Synthesis

Endophytic bacteria are unique sources of structurally diverse and bioactive secondary metabolites [101–103]. Investigations on the secondary metabolism of endophytic bacteria have led to the isolation of approximately 100 new compounds. The

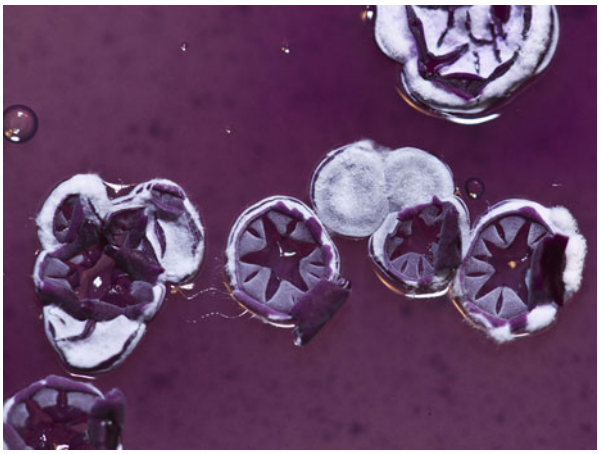
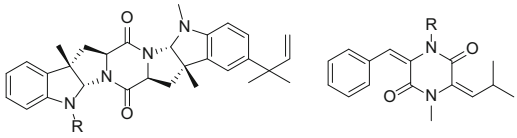


Plate 2 Colony of *Streptomyces olivaceus*. Photograph courtesy of Tetyana Yana-t Petruk, Creative Commons

genus *Streptomyces* (for an example of a *Streptomyces* culture, see Plate 2) is the most studied and yielded 82.8% of all metabolites reported since 1998.

9.1 Alkaloids

Four highly modified diketopiperazines, lansai A–D (**2**–**5**), have been isolated from cultures of the endophytic *Streptomyces* sp. SUC1, obtained from the aerial roots of *Ficus benjamina* [104]. Only the relative configuration has been established for alkaloids **2** and **3**. Alkaloids **2**–**5** were tested for cytotoxic and antifungal activities, but only compound **3** was weakly active against the BC cell line.

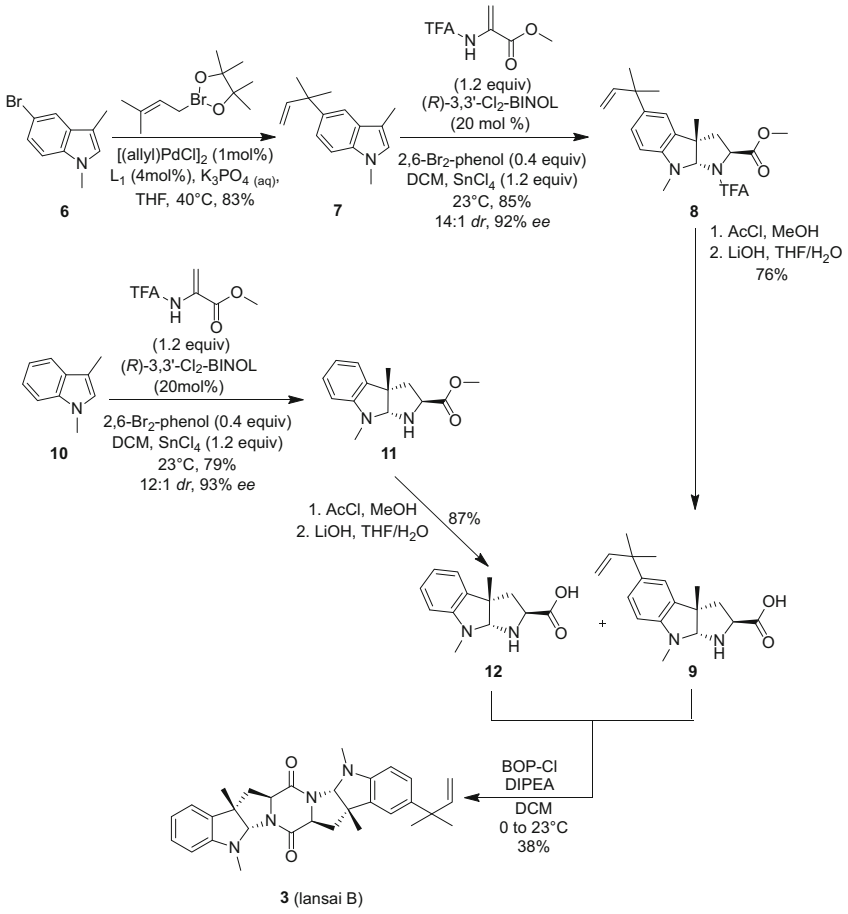


2 R = H (lansai A)
3 R = Me (lansai B)

4 R = OH (lansai C)
5 R = H (lansai D)

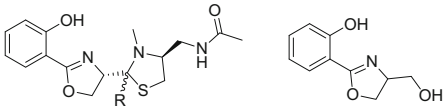
The total synthesis of lansai B (**3**) was achieved via an enantioselective [3+2] cycloaddition. The reversed-prenylated indole **7** was constructed via a Suzuki-Miyaura coupling between 5-bromo-1,3-dimethyl-1*H*-indole (**6**) and phenylboronate. A [3+2] cycloaddition between **7** and 2-trifluoroacetamidoacrylate yielded the pyrroloindoline **8**. Chemoselective transesterification led to the carboxylic acid **9**. The indole **12** was prepared by the same approach from **10**. Coupling of **9** and **12** was performed with BOPCl, to provide lansai B (**3**) in 38% yield. This total synthesis was achieved in six linear steps with 20% overall yield [105] (Scheme 1).

Three dihydrooxazole alkaloids, spoxazomicins A–C (**13**–**15**), have been isolated from cultures of *Streptosporangium oxazolinicum* K07-0460T by silica gel column



Scheme 1 Total enantioselective synthesis of lansai B (**3**) [105]

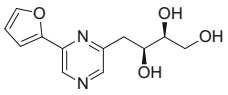
chromatography and purification by HPLC. *S. oxazolinicum* K07-0460T was isolated from an unidentified orchid collected in Okinawa prefecture, Japan. The structures of spoxazomicins A–C (**13**–**15**) were established by NMR spectrometric and X-ray crystal analyses. Spoxazomicin A displayed potent and selective antitrypanosomal activity in vitro with an IC_{50} value of 0.11 μg/cm^3, with weak cytotoxicity against MRC-5 human fetal lung cells (IC_{50} 27.8 μg/cm^3) [106].



13 R = αH (spoxazomicin A)
14 R = βH (spoxazomicin B)

15 (spoxazomicin C)

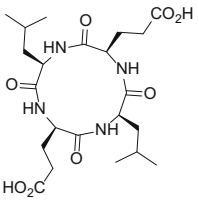
A new pyrazine alkaloid, (2*S*,3*S*)-2-(furan-2-yl)-6-(4-trihydroxybutyl)pyrazine (**16**), was isolated along with 12 known related derivatives from the fermentation broth of *Jishengella endophytica* 161111, isolated from the roots of the mangrove plant *Xylocarpus granatum* (Meliaceae) [107]. While the relative configuration of **16** was established by NMR *J*-based configurational analysis, its absolute configuration was assigned by use of quantum chemical ECD calculations [107].



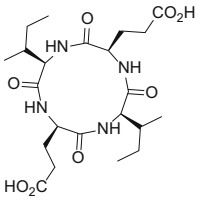
16

9.2 Peptides

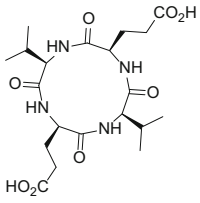
Three cyclotetrapeptides, cyclo-(L-Leu-L-Glu-L-Leu-L-Glu) (**17**), cyclo-(L-Ile-L-Glu-L-Ile-L-Glu) (**18**), and cyclo-(L-Val-L-Glu-L-Val-L-Glu) (**19**), have been isolated from cultures of *Streptomyces* sp. 447 [108], obtained from medicinal plants collected at the Panxi Plateau of southwest China. The absolute configuration of the amino acids was established after hydrolysis and derivatization with trifluoroacetic acid anhydride. Gas chromatography–mass spectrometry analysis by comparison with derivatized amino acid standards provided the absolute configuration of the hydrolyzed amino acids.



17



18

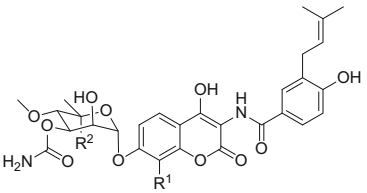


19

9.3 Polyketides

The glycosylated and prenylated antibiotic coumarins TPU-0031-A (**20**) and B (**21**) were isolated from cultures of *Streptomyces* sp. TP-A0556 grown at 32°C for 4 days. This actinobacterium was isolated from the plant *Aucuba japonica* collected in

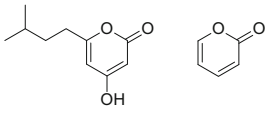
Toyama, Japan. TPU-0031-A (**20**) and B (**21**) displayed antibiotic activity against Gram-positive and Gram-negative bacteria. The antibacterial activities of **20** and **21** were significant against *Staphylococcus aureus* 209P and were 3.1 and 0.79 μg/cm^3, while against *Staphylococcus aureus* F-507 MRSA, they were 12.5 and 3.1 μg/cm^3 [109].



20 R^1 = H R^2 = CH_3 (TPU-0031-A)
21 R^1 = CH_3 R^2 = H (TPU-0031-B)

Some metabolites can exhibit a protective effect to plants against pathogenic fungi. Fistupyrone (**22**) was isolated from the culture broth of *Streptomyces* sp. TP-A0569, obtained from stem of the onion *Allium fistulosum*, also found in Toyama, Japan. Fistupyrone (**22**) inhibited in vivo the infection of the seedlings of Chinese cabbage by *Alternaria brassicicola* [110]. When spores of *A. brassicicola* were suspended in a solution of **22** and then inoculated on host leaves, a single concentration at 0.1 ppm inhibited *Streptomyces* sp. TP-A0569 spore germination, appressorial formation, and hypha formation [111].

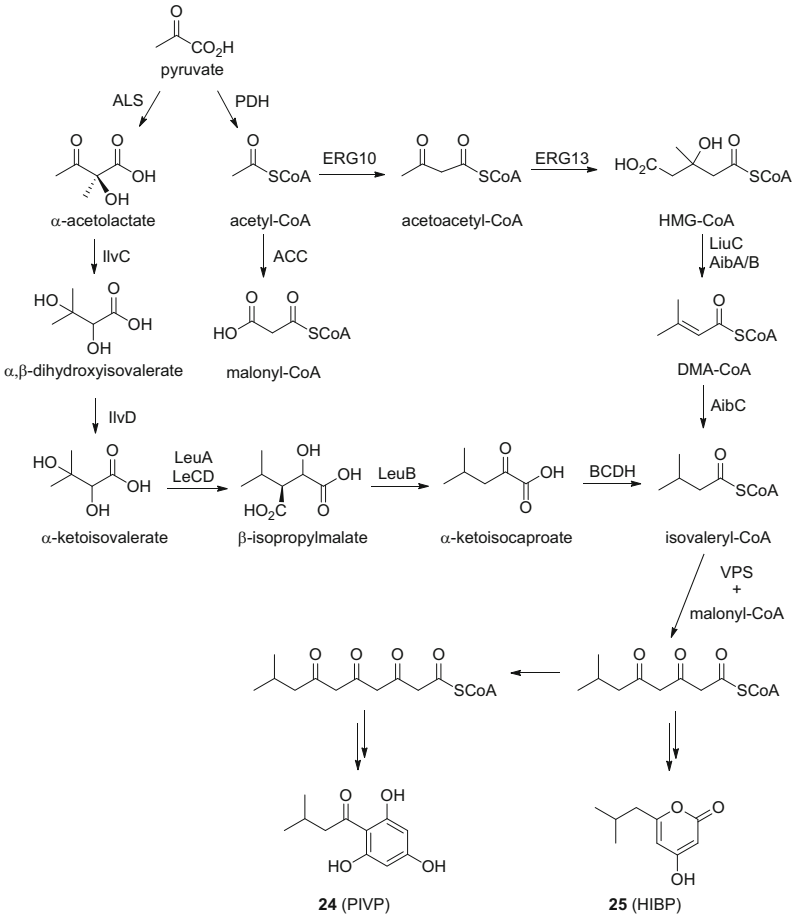
Fistupyrone (**22**) is a derivative of 2-pyrone (**23**), which is widespread in bacteria, fungi, plants, insects, and animals, influencing different biological processes, acting as a chemical defense against other organisms and as a biosynthesis intermediate of different secondary metabolites [112].



22 (fistupyrone) **23**

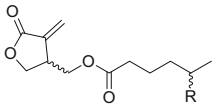
The biosynthesis of phlorisovalerophenone (PIVP) (**24**) and 4-hydroxy-6-isobutyl-2-pyrone (HIBP) (**25**) by *Escherichia coli* has been investigated. Both polyketides are produced from pyruvate, through the acetyl-CoA (via HMG-CoA) or α-acetolactate pathways (via the BCDH complex), as shown in Scheme 2. Both biosynthesis pathways lead to isovaleryl-CoA. The formation of both **24** and **25** arises from isovaleryl-CoA and malonyl-CoA, and is catalyzed by valerophenone synthase (VPS) [113].

Two new butyrolactone antibiotics, cedarmycins A (**26**) and B (**27**), were isolated from cultures of the Actinobacteria *Streptomyces* sp. TP-A045, found in



Scheme 2 Biosynthesis of phlorisovalerophenone (PIVP) (**24**) and 4-hydroxy-6-isobutyl-2-pyrone (HIBP) (**25**). PDH pyruvate dehydrogenase complex, ACC acetyl-CoA carboxylase, ERG10 acetyl-CoA acetyltransferase, ERG13 HMG-CoA synthase, LiuC HMG-CoA dehydratase, AibA/B MG-CoA decarboxylase, AibC DMA-CoA reductase, Als acetolactate synthase, IlvC ketol-acid reductoisomerase, IlvD dihydroxy-acid dehydratase, LeuA α-isopropylmalate synthase, LeCD α-isopropylmalate isomerase complex, LeuB isopropylmalate dehydrogenase, BCDH branched-chain α-keto acid dehydrogenase complex, BCAT branched-chain amino acid transaminase, VPS valerophenone synthase [113]

Cryptomeria japonica collected in Kosugi-Machi, Toyama, Japan [114]. These compounds displayed weak antibiotic activity against Gram-positive and Gram-negative bacteria, and yeasts, as well as weak cytotoxic activity for some cancer cell lines. These results showing a lack of potency are rather surprising, considering the α,β-unsaturated lactone moiety in the structures of both **26** and **27**.

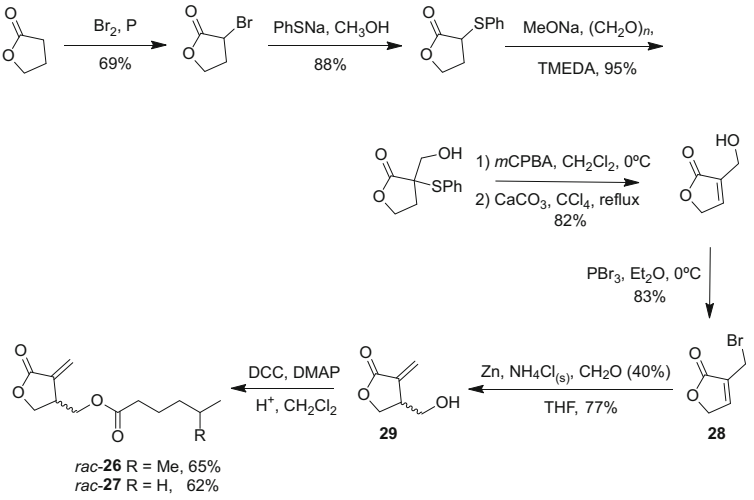


26 R = CH_3 (cedarmycin A)
27 R = H (cedarmycin B)

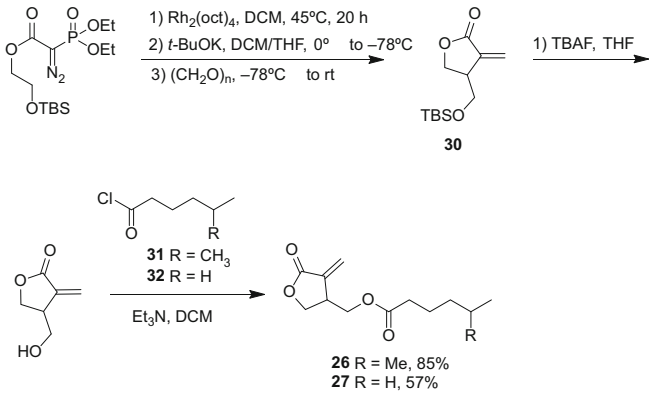
The first synthesis of *racemic* **26** and **27** was achieved in seven steps, starting with the bromination of γ-butyrolactone. The key step was a Barbier reaction between 3-bromomethyl-*5H*-dihydrofuran-2-one (**28**) with formaldehyde in the presence of zinc to provide the α,β-unsaturated **29**, which upon acylation was transformed into **26** and **27** (Scheme 3) [115].

A second synthesis of cedarmycins **26** and **27** has been developed via a one-pot C-H insertion/olefination for the formation of the α-methylene-γ-butyrolactone (**30**) (Scheme 4). The reaction proceeded via a rhodium(II) catalyzed C-H insertion reaction on a diazophosphonate. Following subsequent treatment with TBAF to withdraw the silyl protecting group to give the respective alcohol, the product was acylated with the acid chlorides **31** and **32**. The products **26** and **27** were then obtained in 85 and 57% overall yields (Scheme 4) [116].

Three polyketides, pteridic acids A (**33**) and B (**34**) [117], and pterocidin (**35**) [118], were isolated from cultures of *Streptomyces hygroscopicus* TP-A0451, isolated from the stems of *Pteridium aquilinum*, collected in Toyama, Japan. The structures of pteridic acids A (**33**) and B (**34**) were found to contain a [6,6]-spirocyclic moiety, epimeric at C-11. The complex structures include seven stereogenic centers and an unsaturated side chain with a terminal carboxylic group. Compounds **33–35** exhibited potent plant growth-promoting properties, inducing the formation of roots in kidney beans at 1 n*M*, as effectively as auxin [117]. Pterocidin (**35**) also showed cytotoxicity against the NCI-H522, OVCAR-3,

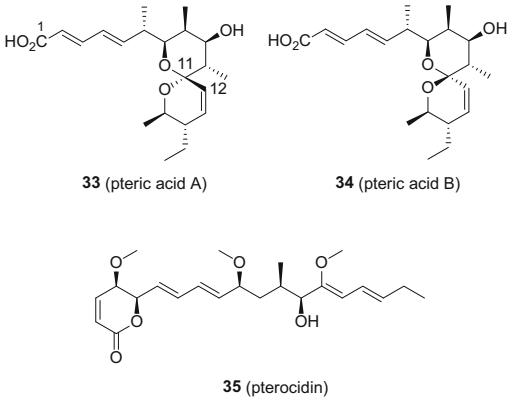


Scheme 3 First synthesis of cedarmycins A (**26**) and B (**27**) [115]



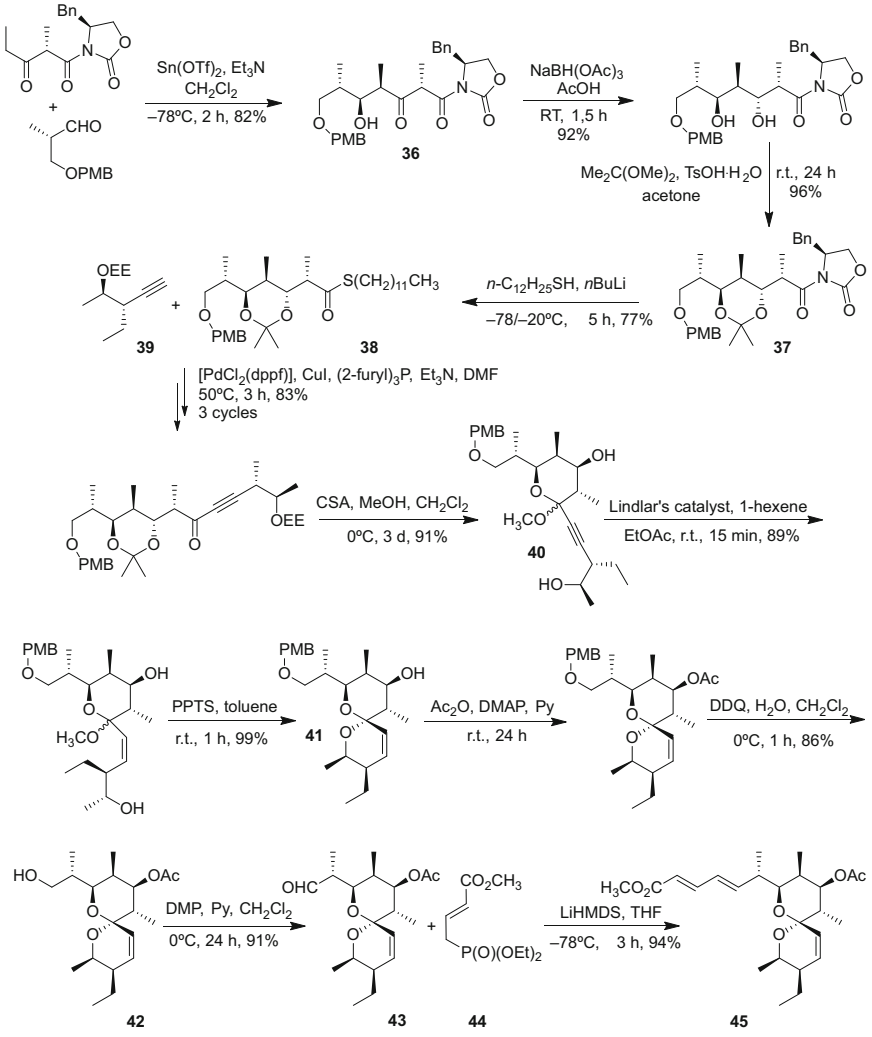
Scheme 4 Second synthesis of cedarmycins A (**26**) and B (**27**) [116]

SF539, and LOX-IMVI cancer cell lines, with IC_{50} values of 2.9, 3.9, 5.0, and 7.1 μ*M* [117, 118].

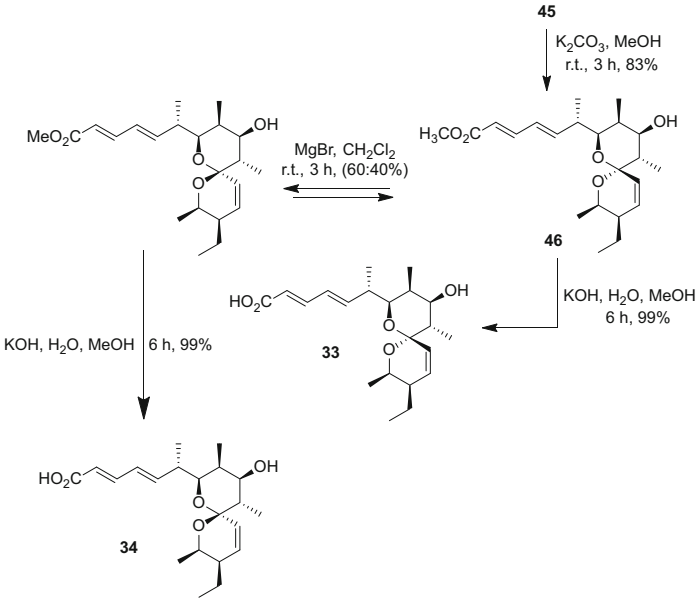


Several total syntheses of pteridic acids A (**33**) and B (**34**) have been developed [119–123]. The first started with the preparation of the main intermediate **36** using an aldol reaction with a β-keto imide derivative and a protected aldehyde to yield an inseparable mixture of the *anti*-aldol **36** and its undesired (7*R*,8*S*)-diastereomer. The mixture was subjected to a diastereoselective reduction with $NaBH(OAc)_3$ to give the 7,9-*anti*-diol with good diastereoselectivity (10:1 7,9-*anti*/7,9-*syn*). The diol was then protected as an acetonide (**37**). The C-11–C-12 fragment was then prepared via the Fukuyama method, in which the oxazolidinone intermediate **37** was esterified into the corresponding dodecanethiol ester **38** in the presence of lithium dodecanethiolate and DMF. The coupling reaction between the thiol ester **38** and the protected acetylene derivative **39** was conducted with a palladium catalyst in three cycles (83%) and with no by-product formation. Removal of the acetonide in the presence of camphorsulfonic acid and MeOH led to the formation of the cyclic ketal **40** as an inseparable diastereomeric mixture (3:1, 91%). Exposure of **40** to reductive conditions to provide the (*Z*)-alkene, followed by spirocyclization in acidic

conditions, provided **41** as a single diastereomer (99%). Alcohol protection of **41** and oxidation of the PMB protecting group with DDQ provided **42**, which was then oxidized with DMP to give the aldehyde **43**. Elongation via a Horner-Wadsworth-Emmons reaction between **43** and **44** phosphonate produced **45** in 94% yield. Completion of the synthesis of pteridic acid A (**33**) was conducted by deprotecting the acetyl group, followed by hydrolysis of the methyl ester. Pteridic acid B (**34**) was obtained by C-11 epimerization of C-11 of **46** in anhydrous $MgBr_2$. The products could be separated by chromatography. The 11-*epi*-7H isomer could be obtained in enhanced amounts by the participation of Mg^{2+} in the formation of **34** after basic hydrolysis [120] (Scheme 5).



Scheme 5 First synthesis of pteridic acids A (**33**) and B (**34**) [119, 120]

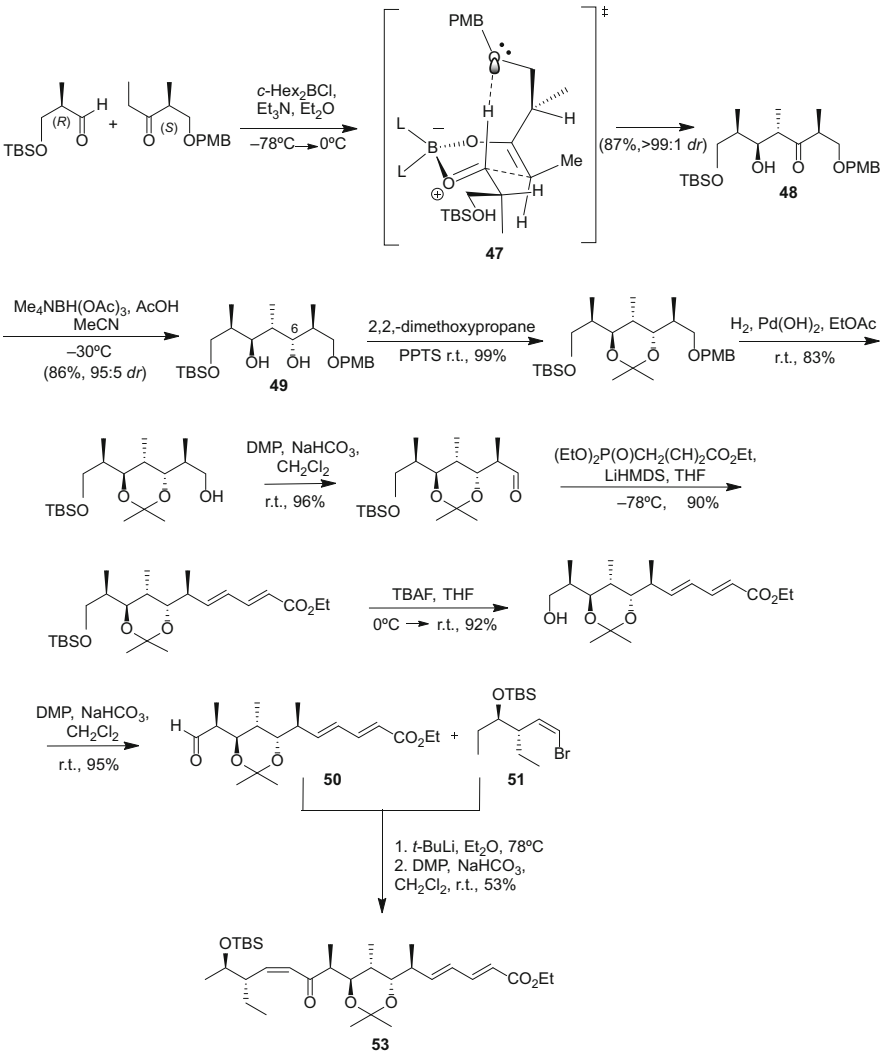


Scheme 5 (continued)

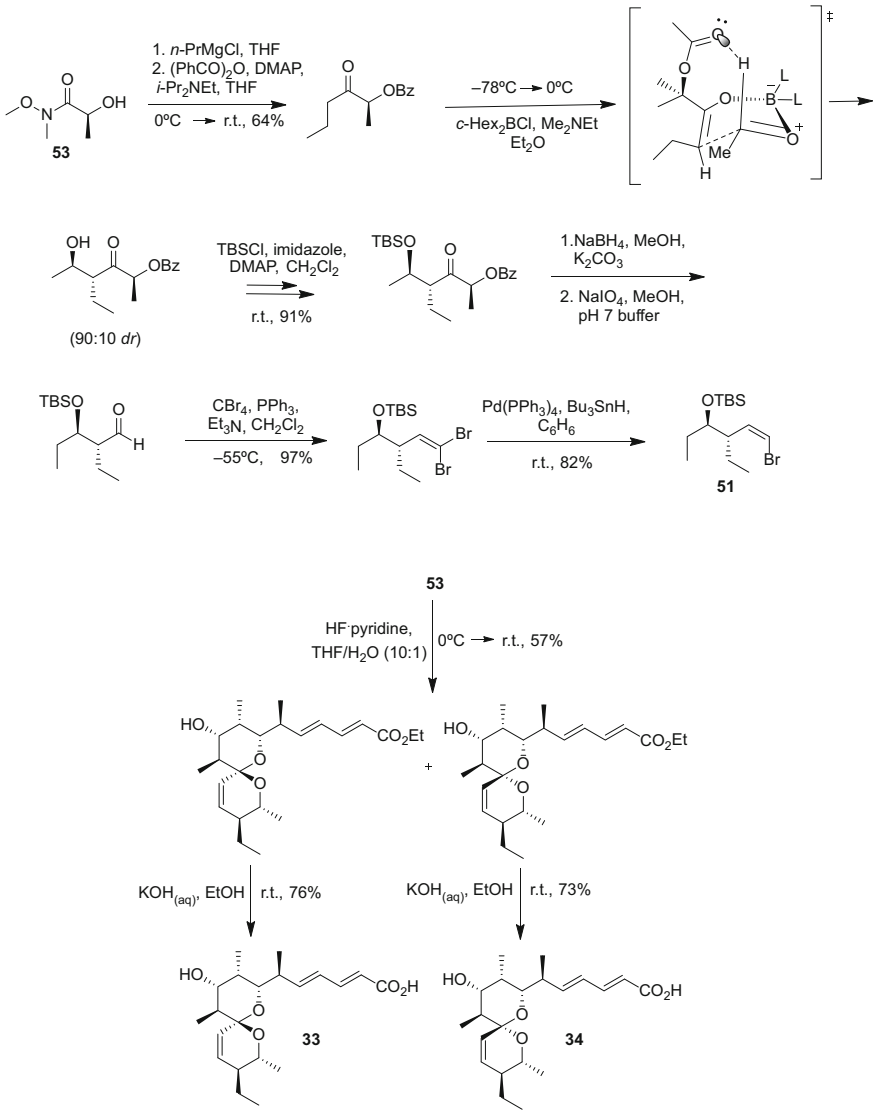
The second synthesis of **33** and **34** was developed by Paterson and co-workers [121], based on the formation of an aldol boronate **47**, in order to establish the stereocenters present in **33** and **34**. The selectivity towards the formation of **47** was driven by the formation of a highly stabilized formyl hydrogen bond with the oxygen of the *p*-methoxybenzyl (PMB) and the chiral aldehyde. Evans-Saksena reduction of **48** with $Me_4NBH(OAc)_3$ provided the 1,3-*anti* diol **49** with the correct configuration. Diol **49** was subjected to a series of stepwise transformations towards the formation of aldehyde **50**, which was coupled with the vinyl bromide **51**, itself prepared from a Weinreb amide **53**. Coupling of **50** and **51** with *tert*-butyl lithium gave an epimeric mixture of two alcohols that was quickly oxidized by DMP to give the key aliphatic intermediate **52** in 53% yield. The cleavage of acetonide and deprotection of the TBS ether, followed by in situ spirocyclization, gave both spiroacetal epimers in a 1:1 mixture. The epimers were separated by chromatography. Alkaline hydrolysis gave pteridic acids A (**33**) and B (**34**) (Scheme 6).

The total synthesis of **33** and **34** developed by Dias and Salles [122] had features based on Paterson's [121] and Nakata's [119, 120] approaches. The oxazolidinone **54** was treated with the protected aldehyde **55** to form the aldol adduct **56** with an excellent diastereoselectivity (>95:5 *ds*) and very good yield (75%). The oxazolidinone protecting group was replaced by a Weinreb amide in 85% yield. To complete the preparation of fragment **57**, the Weinreb amide was protected with TBS, followed by reduction to the corresponding aldehyde with diisobutylaluminum hydride in 90% yield. The C-10–C-16 fragment to be coupled with C-5–C-9 was constructed via a Frater alkylation of a commercially available hydroxy ester,

providing the 1,2-*anti* ester in 85% yield and 95:5 *ds*. Protection of the alcohol as its TBS ether, followed by ester reduction provided the corresponding primary alcohol, which was subjected to a Swern oxidation giving the necessary aldehyde to form the (*Z*)-α,β-unsaturated ester **58** under Ando's protocols in 95:5 *d*s (*Z*)/(*E*) excess. Conversion of **58** into its corresponding Weinreb amide was followed by treatment with EtMgBr to give the corresponding (*Z*)-α,β-unsaturated ketone **59**, in two steps. An aldol coupling reaction between fragments **57** and **59** was performed in 70% yield and an 80:20 diastereoselectivity ratio gave the 1,2-*syn anti*-Felkin adduct,



Scheme 6 Second synthesis of pteridic acids A (**33**) and B (**34**) [121]

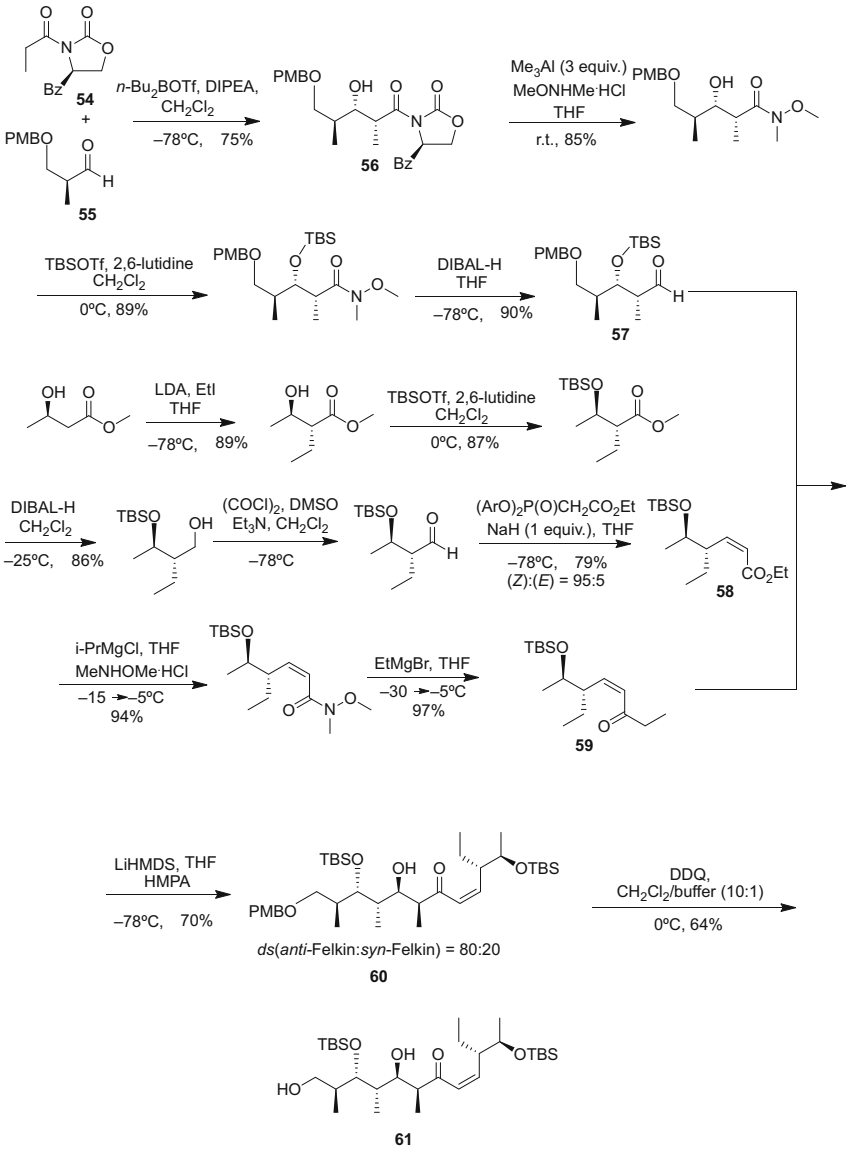


Scheme 6 (continued)

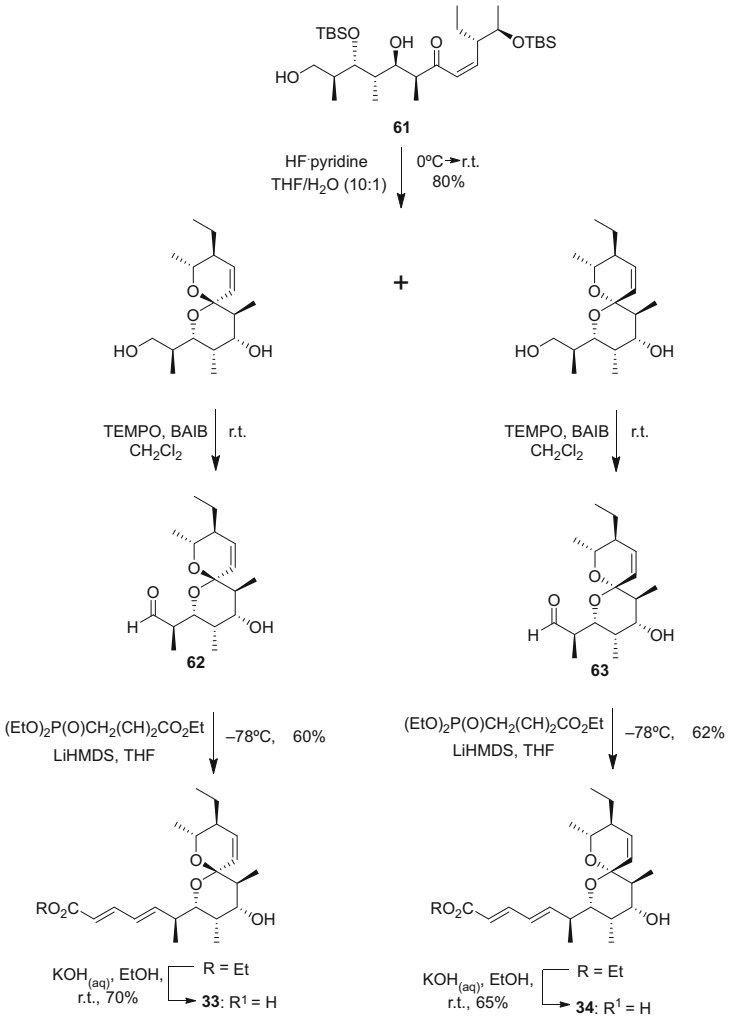
purified by flash column chromatography to give 56% of the pure 1,2-*syn* product **60**. The alcohol **61** was obtained by oxidation of **60** with DDQ. TBS deprotection and simultaneous spirocyclization with HF·pyridine in THF/H_2O (10:1) gave a 1:1 mixture of the spiroketal precursors for **33** and **34**, separated by silica gel column chromatography. After alcohol oxidation followed by a Horner-Wadsworth-Emmons homologation of aldehydes **62** and **63** to the corresponding α,β-unsaturated esters, the products were hydrolyzed with aqueous KOH in EtOH to give pteridic

acids A (**33**) (70% yield) and B (**34**) (65% yield). Thirteen steps were required for the Dias and Salles total synthesis of **33** and **34** in about a 3.0% overall yield (Scheme 7).

Yadav and collaborators [123] elaborated a completely distinct total synthesis of pteridic acid A (**33**). It featured a desymmetrization methodology to give the polyketide scaffold. Construction of the C-5–C-1 fragment was achieved in a few



Scheme 7 Third synthesis of pteridic acids A (**33**) and B (**34**) [122]

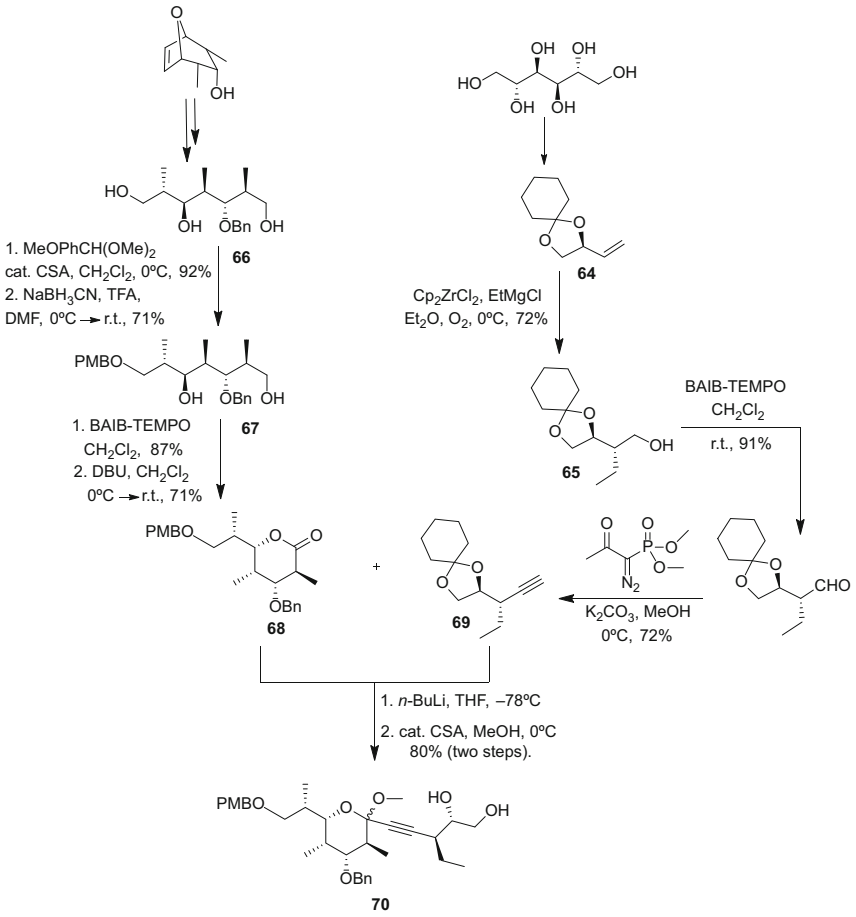


Scheme 7 (continued)

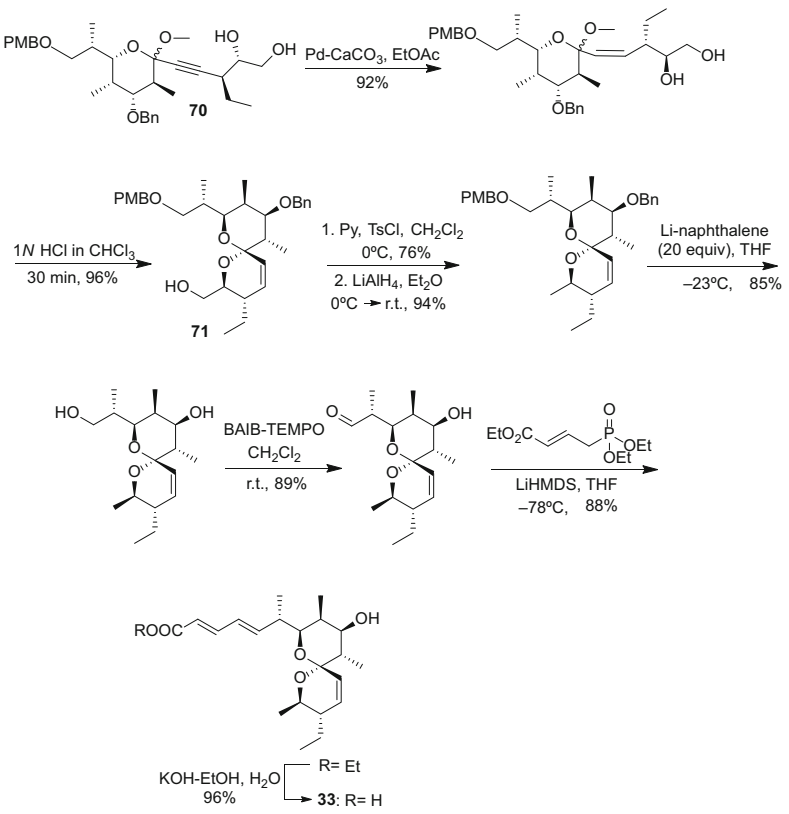
steps, employing a bicyclic olefin **64** via Brown's chiral hydroboration to form a protected triol intermediate **65**. In parallel, a mono-protected triol **66** was generated from (1*R*,3*S*,4*S*,5*S*)-2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-ol. Compound **66** was treated with CSA, followed by reductive acetal opening to engender a 1,5-diol **67**, required for an oxidative lactonization and epimerization with DBU, to provide a Prelog–Djerassi-type lactone **68**. The construction of the C-12–C-15 segment required the installation of an ethyl center at C-14 using Hoveyda's protocol. The alcohol single diastereomer **65** was converted to the corresponding aldehyde, itself transformed into to the alkyne **69** in Ohira-Bestmann conditions. Coupling between **68** and **69** gave the key hemiacetal **70** in 80% yield for two steps. Stereoselective

hydrogenation followed by spirocyclization in mild acidic conditions and high diastereoselectivity (96%) yielded **71**. A series of stepwise interconversions in very good yields led to the formation of pteridic acid A (**33**), which was synthesized in 13 steps and 17.4% overall yield (Scheme 8).

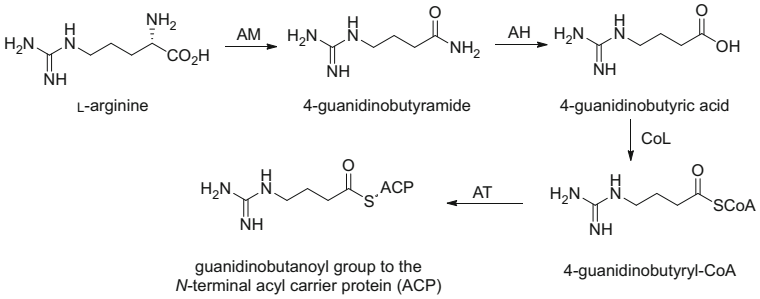
A *Streptomyces hygroscopicus* TP-A0623 strain isolated from the roots of *Clethra barbinervis* collected in Toyama, Japan, produced clethramycin (**72**). Compound **72** showed in vitro antifungal activity with a *MIC* value of ≥ 8 μg/cm^3 against *Candida*, *Cryptococcus*, and *Aspergillus* [124, 125]. The clethramycin biosynthesis gene cluster was identified in *Streptomyces mediocidicus* ATCC23936, which encodes PKS and tailoring enzymes [126]. Clethramycin (**72**) biosynthesis starts with the formation of a guanidinobutanoyl group originating from L-arginine, which is transformed by an arginine monooxygenase (AM—CleD), a



Scheme 8 Fourth synthesis of pteridic acid A (**33**) [123]



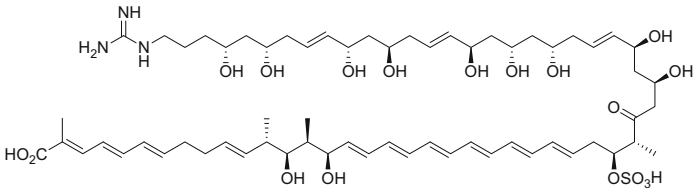
Scheme 8 (continued)



Scheme 9 Biosynthesis of guanidinobutanoyl group of clethramycin (**72**) [127, 128]

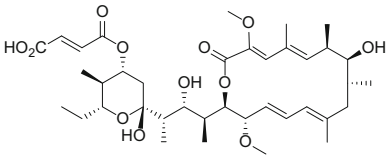
4-guanidinobutyramide hydrolase (AH—CleO), a 4-guanidinobutanoate:CoA ligase (CoL—CleE), and a 4-guanidinobutyryl-CoA:ACP acyltransferase (CleG), respectively [127, 128] (Scheme 9). CleG recruits the γ-aminobutyryl group into the ACP domain, serving as starter unit for the extension of the polyketide chain. After

polyene polyketide formation, the putative sulfotransferase CleB was proposed to be involved in sulfate group inclusion [126].

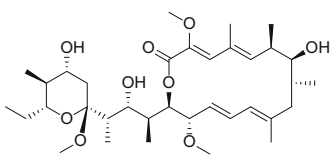


72 (clethramycin)

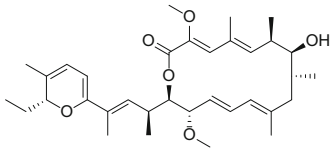
Streptomyces sp. CS, isolated from *Maytenus hookeri*, displayed potent antifungal activity against *Penicillium avellaneum* UC-4376 in a diffusion agar plate assay. Antifungal activity-guided isolation afforded 24-demethyl-bafilomycin C1 (**73**), which exhibited potent antifungal effects and cytotoxic activity against the P388 and A-549 tumor cell lines [129]. The absolute configuration of **73** was inferred as being identical to that reported for bafilomycin C1, by comparison with NMR spectrometric and optical rotation data [130]. The stereochemistry of bafilomycin C1 was established as identical to that of bafilomycin A1, assigned by analysis of X-ray crystallographic data [131]. Two additional 16-membered macrolides, 24-demethylbafilomycin A2 (**74**) and its dehydrated form (**75**), were also isolated from cultures of *Streptomyces* sp. CS [132]. Compounds **74** and **75** were not antifungal but **75** displayed cytotoxic activity similar in potency to that of **73**. Additional bafilomycin analogues (**76**–**80**) were isolated from the same source. The cytotoxic activities of **76**–**80** were assessed against the MDA-MB-435 cell line, and the IC_{50} values observed were 4.2, 4.5, 5.5, 3.8, and 11.4 μ*M*, respectively [133]. Large-scale fermentation of *Streptomyces* sp. YIM56209, isolated from a healthy stem of *Drymaria cordata*, led to the isolation of 9-hydroxybafilomycin D (**81**) and 29-hydroxybafilomycin D (**82**) [134]. The stereochemistry of the asymmetric centers was determined by NMR spectrometric data interpretation. Derivative **77** provided crystals, which by X-ray diffraction enabled the assignment of its absolute configuration.



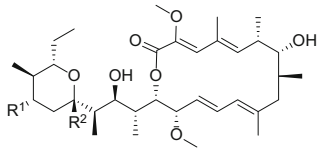
73 (24-demethyl-bafilomycin C1)



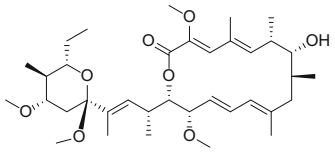
74 (24-demethyl-bafilomycin A2)



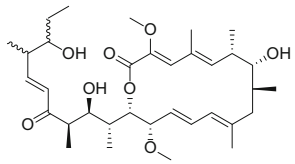
75



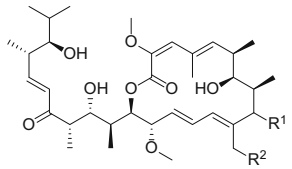
76 $R^1 = R^2 = OH$
77 $R^1 = OMe$, $R^2 = OH$
78 $R^1 = R^2 = OMe$



79

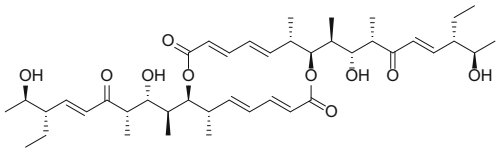


80



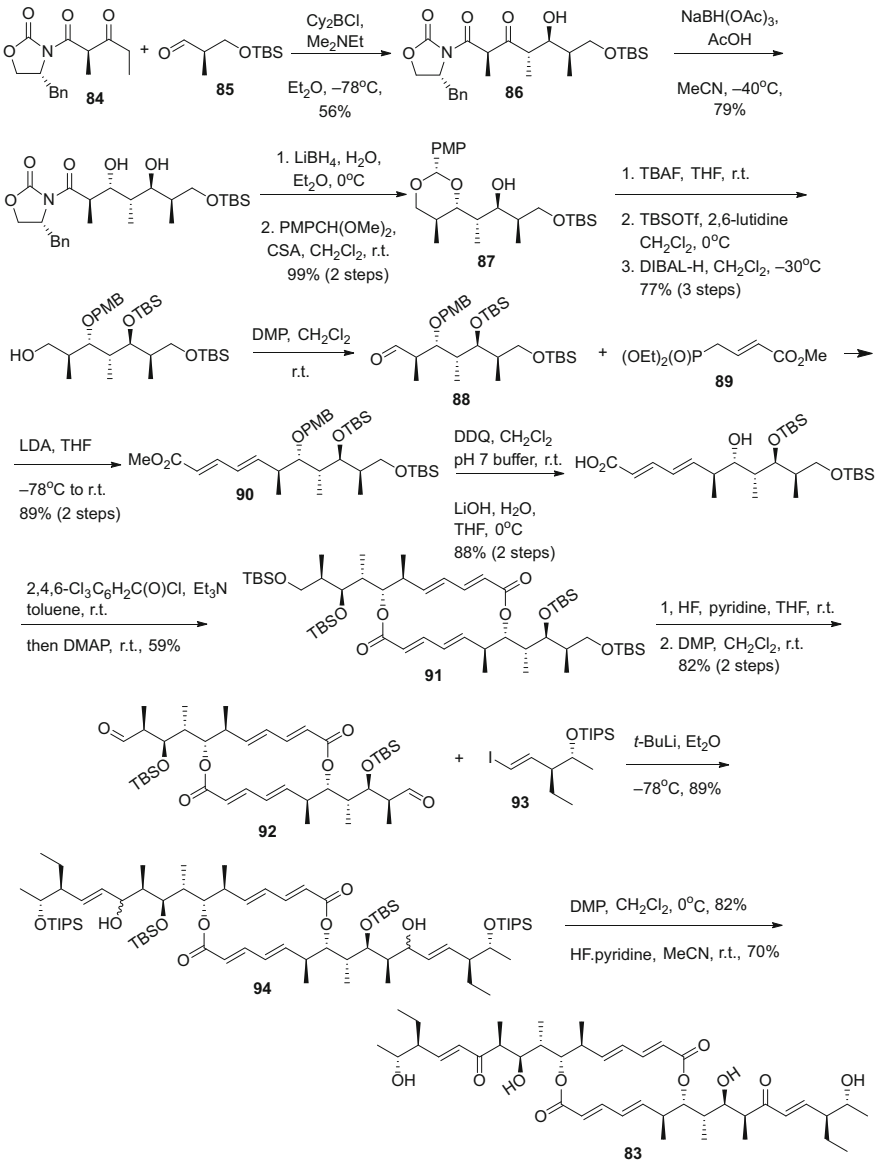
81 $R^1 = OH$, $R^2 = H$ (9-hydroxybafilomycin D)
82 $R^1 = H$, $R^2 = OH$ (29-hydroxybafilomycin D)

A large-scale fermentation extract from cultures of the endophytic actinomycete *Streptomyces* sp. BCC72023, originating from rice (*Oryza sativa* L.), led to the isolation of efomycin M (**83**). Efomycin M displayed antimalarial activity against *Plasmodium falciparum* K-1, a multidrug-resistant strain, with an IC_{50} value of 5.23 μg/cm^3 [135]. The stereochemistry was determined by total synthesis of **83** before its isolation (Scheme 10) [136].



83 (efomycin M)

An anti-selective aldol condensation between β-ketoimide **84** and the aldehyde **85** gave **86** in 56% yield and 95:5 *dr*. Ketone reduction followed by carbamate

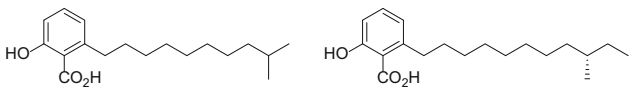


Scheme 10 Total synthesis of efomycin M (**83**) [136]

removal and hemiketal cyclization led to **87**, which was then transformed into the aldehyde **88** by a series of stepwise interconversions. Horner-Wadsworth-Emmons olefination between **88** and phosphonate **89** gave the ester **90** in 89% yield in two steps. Deprotection and dimerization forming the bis-lactone **91** was achieved

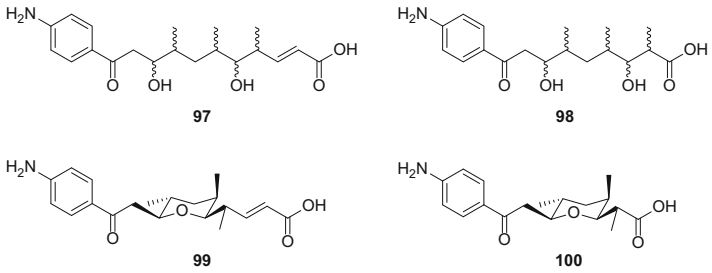
in 59% yield. Transformation of **91** into the aldehyde **92** was followed by a Nozaki-Hiyama-Kishi coupling between **92** and the vinylic iodide **93** and provided the immediate precursor of **83**, which was obtained after a two-step deprotection (Scheme 10).

Two new 6-alkylsalicylic acids related to anacardic acids, salaceyins A (**95**) and B (**96**), have been purified by bioassay-guided fractionation from cultures of endophytic *Streptomyces laceyi* MS53, isolated from a surface-sterilized stem of *Ricinus communis*. Salaceyins A (**95**) and B (**96**) exhibited cytotoxicity against the human breast cancer cell line SKBR3, with IC_{50} values of 3.0 and 5.5 μg/cm^3 [137].

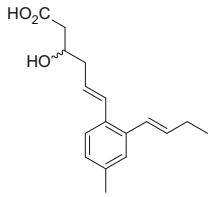


95 (salaceyin A) **96** (salaceyin B)

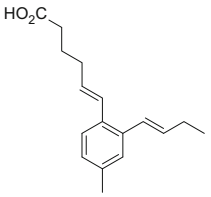
Four new 4-aminoacetophenonic acids, (2*E*)-11-(4′-aminophenyl)-5,9-dihydroxy-4,6,8-trimethyl-11-oxo-undec-2-enoic acid (**97**), 9-(4′-aminophenyl)-3,7-dihydroxy-2,4,6-trimethyl-9-oxo-nonoic acid (**98**), (2*E*)-11-(4′-aminophenyl)-5,9-*O*-cyclo-4,6,8-trimethyl-11-oxo-undec-2-enoic acid (**99**), and 9-(4′-aminophenyl)-3,7-*O*-cyclo-2,4,6-trimethyl-9-oxo-nonoic acid (**100**), were isolated from cultures of the endophyte *Streptomyces* sp. (strain HK10552), obtained from the mangrove plant *Aegiceras corniculatum*. The structures as well as the relative configuration of the stereocenters present were assigned by analysis of spectrometric data [138].



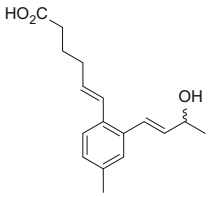
Lorneic acids E–J (**101**–**106**) were isolated from cultures of *Streptomyces* sp. KIB-H1289, a strain isolated from the bark inner tissue of *Betula mandshurica*, a plant used in traditional Chinese medicine. Metabolites **101**–**106** were evaluated for tyrosinase inhibitory activity, but only **102** and **105** displayed weak inhibition [139].



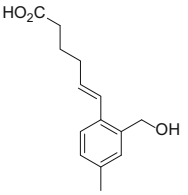
101 (lorneic acid E)



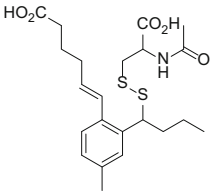
102 (lorneic acid F)



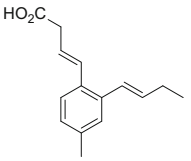
103 (lorneic acid G)



104 (lorneic acid H)

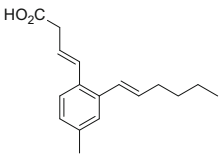


105 (lorneic acid I)



106 (lorneic acid J)

The biosynthesis of lorneic acid A (**107**) was elucidated by feeding experiments with ^{13}C-labeled precursors (Fig. 4) into the growth medium of *Streptomyces* sp. NPS554 [140]. The labeling pattern indicated that the polyketide chain extension begins with an acetate residue, followed by condensation with three malonate residues, one methylmalonate, and again three malonates. The biosynthesis gene cluster of **107** has also been identified. Condensation with PKS affords a polyene intermediate that has all-(*E*) double bonds (Scheme 11). The enoyl-reductase (ER) domain present in the fourth extender domain, which would catalyze the reduction of the C-8/C-9 double bond, is most likely nonfunctional, because no reduction was observed in the lorneic acid analogues. After being released from the PKS, the intermediate would undergo a double bond isomerization at C-6/C-7. The presence of a gene coding for a cytochrome P450 suggests an epoxidation, followed by the cyclization of the polyketide chain retaining the hydroxy group at C-11 [140]. Further dehydration would afford lorneic acid A (**107**) and lorneic acid analogues.



107 (lorneic acid A)

A purple-red pigment has been isolated from cultures of *Streptomyces* sp. KIB-033, an endophyte from the plant *Camellia sinensis*. The purple pigment was identified as rubrolone B (**108**), a cationic tropolone alkaloid that contains an additional benzoic acid unit attached to a pyridinium moiety. The absolute configuration of the rubrolone B (**108**) stereogenic centers at the deoxy sugar fragment were assigned as being identical to those of rubrolone A (**109**), based on their

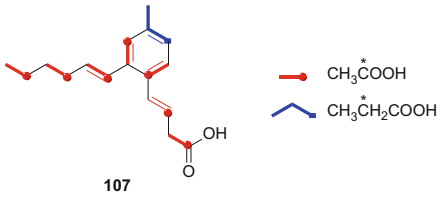
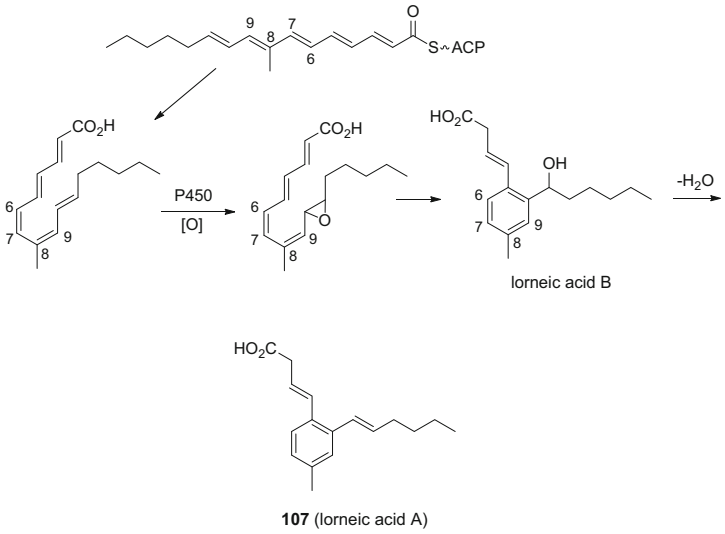
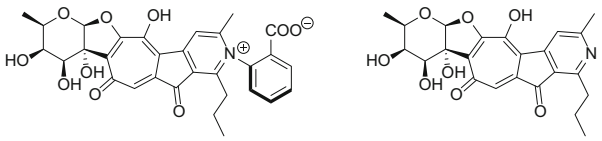


Fig. 4 ^{13}C-labeling pattern observed for the biosynthesis of lorneic acid A (**107**)

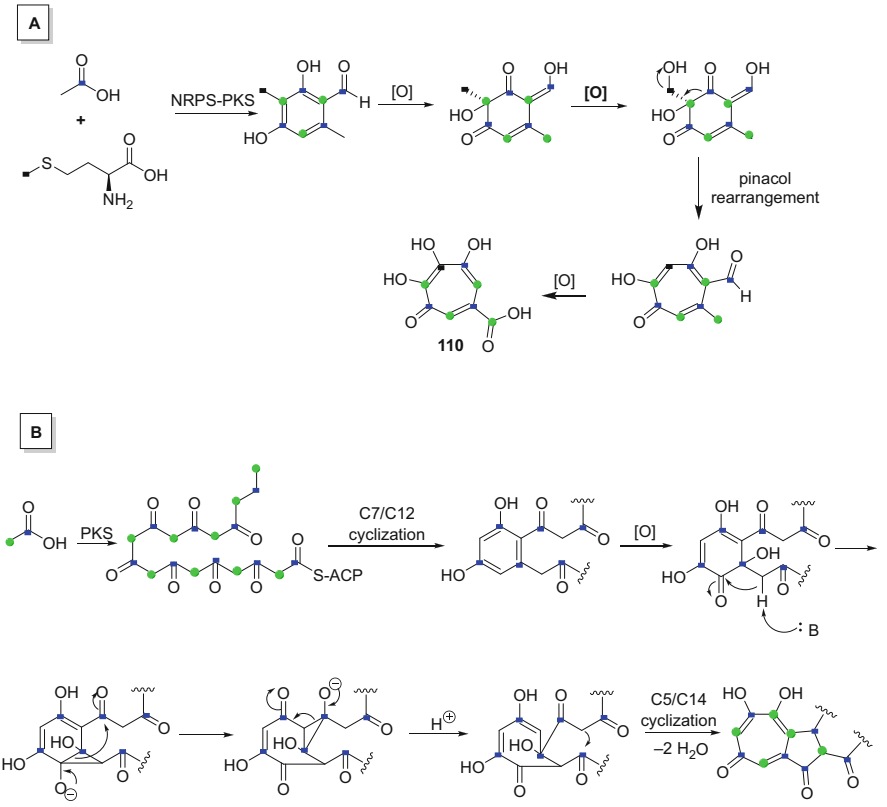


Scheme 11 Biosynthesis of lorneic acid A (**107**) [140]

identical physicochemical properties and by assuming a common biosynthesis origin. The axial configuration of the iminium-aryl axis was established by circular dichroism analysis and quantum mechanics calculations [141].

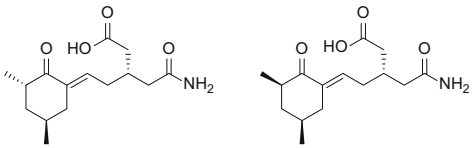


Since the tropolone skeleton in stipitatic acid (**110**) (see Scheme 12) originates from acetate and *S*-adenosyl methionine [142], this suggests a similar biosynthetic origin for the rubrolone aglycone, originating from a polyketide synthase (PKS) and a C-methyltransferase. Feeding experiments were performed with ^{13}C-labeled acetate and [*methyl*-^{13}C]-L-methionine. Addition of [1-^{13}C]acetate to the fermentation medium resulted in ^{13}C enrichment at odd positions (between C-3 and C-17) of the tropolone core. The even positions (C-2 to C-14) were enriched when [2-^{13}C]acetate was fed. No enrichment was noted for the sugar (C-19 to C-24) or benzoic acid (C-1′ to C-7′) moieties in such experiments. No isotopic incorporation was observed with the addition of [methyl-^{13}C]-L-methionine to the culture medium. These labeling experiments indicated the absence of a methyltransferase and, therefore, a distinct bacterial metabolism of tropolone formation when compared to fungi (Scheme 12) [141].



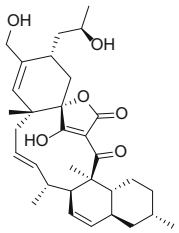
Scheme 12 (**A**) Mechanism of the tropolone ring formation in stipatic acid (**110**) isolated from fungi; (**B**) Proposed mechanism of tropolone ring formation in bacterial rubrolones [141, 142]

Two new cycloheximide congeners, secocycloheximides A (**111**) and B (**112**), have been isolated from *Streptomyces* sp. YIM56132, obtained from *Carex baccaus*, and *Streptomyces* sp. YIM56141, from *Fagopyrum cumosum*. Both plants are used to treat arthritis. The relative configurations of **111** and **112** were determined by analysis of ROESY NMR data [143].



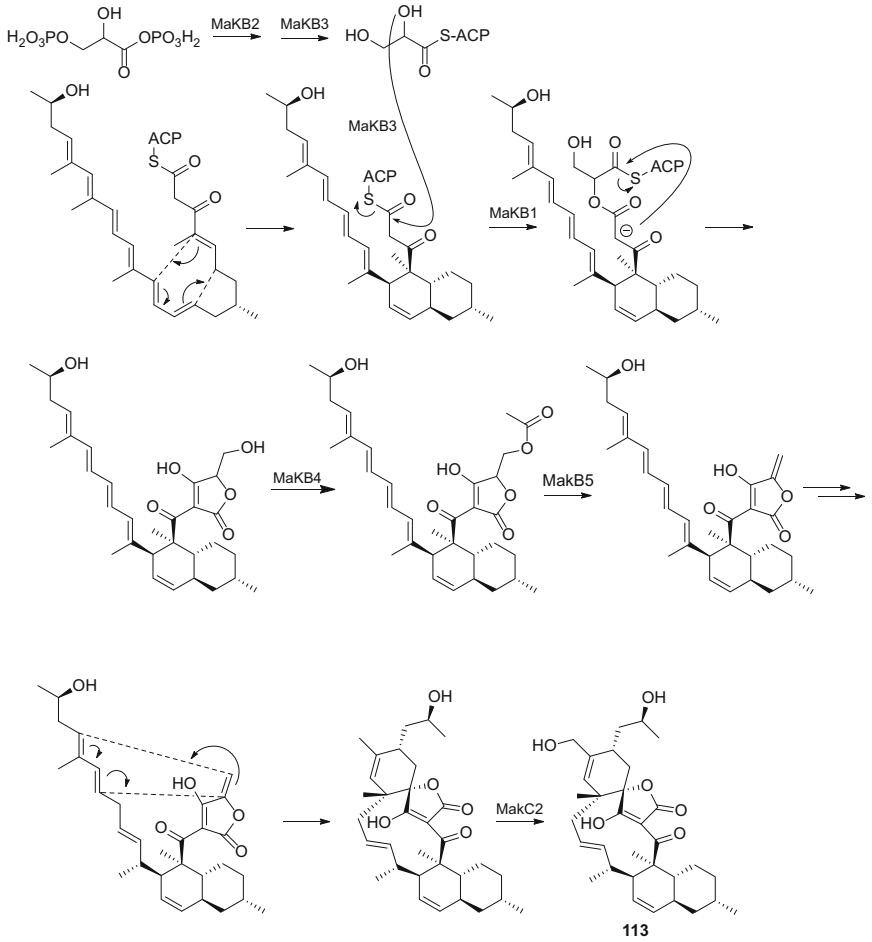
111 (secocycloheximide A) **112** (secocycloheximide B)

A new spirotetronate-class polyketide, maklamicin (**113**), has been isolated from cultures of *Micromonospora* sp. GMKU326, obtained from the roots of *Abrus pulchellus* subsp. *pulchellus*, collected in Thailand [144].



113 (maklamicin)

The genome of the maklamicin (**113**) producer *Micromonospora* sp. NBRC 110955 was sequenced and revealed the gene cluster responsible for the biosynthesis of the polyketide backbone [145]. The biosynthesis of the tetronate moiety in maklamicin involves a glyceryl-*S*-ACP synthase (MakB2) that shows both glyceryltransferase and phosphatase activities, and activates 1,3-bisphosphoglycerate from the glycolytic pathway by loading onto the discrete ACP (MakB3), yielding a glyceryl-*S*-ACP. A ketoacyl-ACP synthase (MakB1) catalyzes the condensation between the glyceryl-*S*-ACP and the polyketide intermediate to form an intermediate, which has the premature tetronate moiety in a *trans*-decalin moiety. Subsequently, the premature tetronate moiety is acetylated by an acyltransferase (MakB4). A dehydratase (MakB5) then generates the exocyclic double bond of the mature tetronate moiety [145]. Prior to the generation of the cyclohexene moiety, the C-14/C-17 conjugated diene structure is reduced and converted to a single double bond between C-15 and C-16. The reduced intermediate is cyclized by a second Diels-Alder reaction, between the exocyclic double bond of the

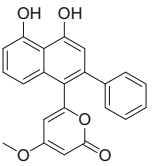


Scheme 13 Biosynthesis of maklamicin (**113**)

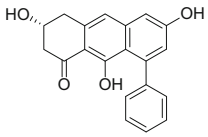
tetronate moiety (dienophile) and a conjugated C-18/C-21 diene structure in the tail region. Two cytochrome P450 monooxygenases, MakC2 and MakC3, are also encoded, responsible for post-modifications of maklamicin biosynthesis. MakC2 is required for the specific hydroxylation at C-29, which is probably the final step in the biosynthesis pathway of maklamicin (**113**) [145] (Scheme 13).

Five phenolic polyketides, 3-*O*-methylwailupemycin G (**114**), wailupemycin J (**115**), (*R*)- and (*S*)-wailupemycin K (**116** and **117**) and wailupemycin L (**118**), as well as the known enterocin and 5-deoxyenterocin, have been isolated from cultures

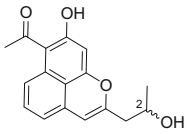
of *Streptomyces* sp. OUCMDZ-3434, associated with the marine green alga *Enteromorpha prolifera* [146].



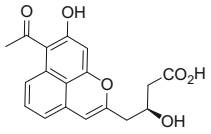
114 (3-*O*-methyl-wailupemycin G)



115 (wailupemycin J)

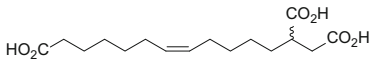


116 ((2*R*)-wailupemycin K)
117 ((2*S*)-wailupemycin K)



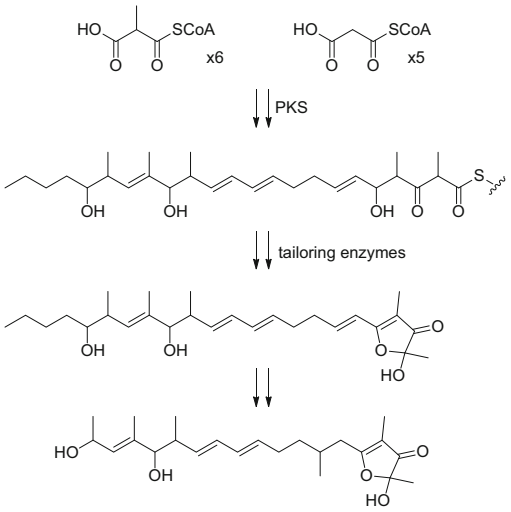
118 (wailupemycin L)

Cultured extracts of *Streptomyces* sp. HUST012, isolated from the stems of the medicinal plant *Dracaena cochinchinensis* showed antimicrobial and cytotoxic activities significantly better than those of "dragon's blood" extracted from *D. cochinchinensis*. The strain produces (*Z*)-tridec-7-ene-1,2,13-tricarboxylic acid (**119**) [147].



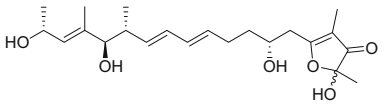
119 ((*Z*)-tridec-7-ene-1,2,13-tricarboxylic acid)

Linfuranones A (**120**), B (**121**), and C (**122**) are furanone-containing polyketides isolated from cultures of *Sphaerimonospora mesophila* GMKU 363, an endophyte isolated from the roots of the Thai medicinal plant *Clinacanthus siamensis*, collected at the Eastern Botanical Garden, Khao Hin Son, Thailand. Linfuranones A (**120**), B (**121**), and C (**122**) did not display antimicrobial and cytotoxic activities. When tested in antidiabetic and antiatherogenic bioassays, 47% of pre-adipocytes were differentiated into their respective matured form, further accumulated in lipid droplets [148, 149]. The absolute configurations of **120–122** were established using chiral anisotropic methods and chemical degradation. The genome sequence of *Sphaerimonospora mesophila* GMKU 363 was investigated in order to unveil the biosynthesis of linfuranones A and B. In silico analysis of the biosynthetic gene cluster, which comprises five PKS genes and tailoring enzymes for the furanone

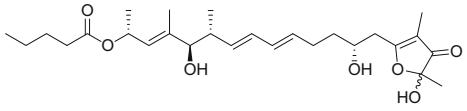


Scheme 14 Linfuranones skeleton predicted by in silico analysis of *Sphaerimonospora mesophila* GMKU 363 biosynthesis genes

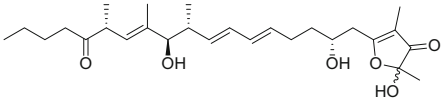
construction, indicated that intact PKS products result from six methylmalonate and five malonate residues (Scheme 14) [149].



120 (linfuranone A)



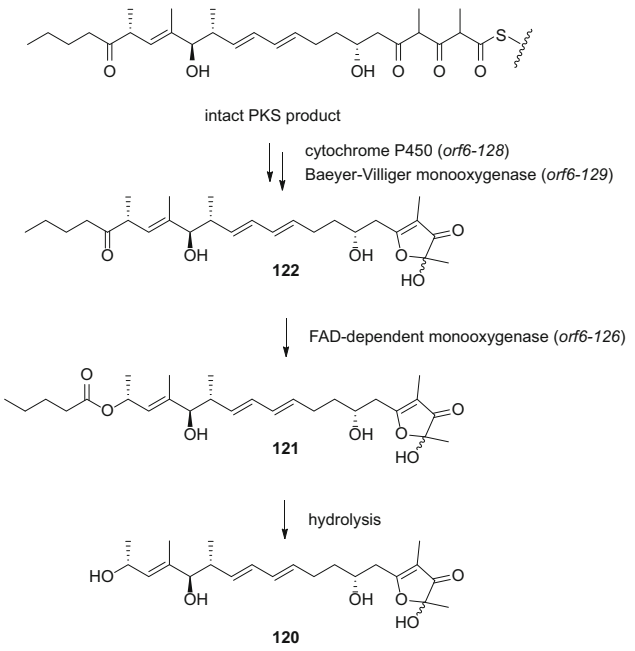
121 (linfuranone B)



122 (linfuranone C)

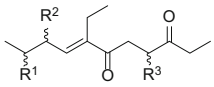
Since the carbon chain of linfuranones A (**120**) and B (**121**) are five carbons shorter than predicted, it was inferred that linfuranone B is generated from linfuranone C (**122**) by oxidative cleavage of the polyketide backbone by a Baeyer-Villigerase enzyme (Scheme 15), possibly a monooxygenase ORF6-129 [149].

The compounds (*7E*)-7-ethyl-9-hydroxyundec-7-ene-3,6-dione (**123**), (*7E*)-7-ethyl-10-hydroxyundec-7-ene-3,6-dione (**124**), (*7E*)-7-ethyl-4-hydroxyundec-7-ene-3,6-dione (**125**), (*7E*)-7-ethyl-4-hydroxyundec-7-ene-3,6-dione (**126**), and (*7E*)-7-ethyl-4,10-dihydroxyundec-7-ene-3,6-dione (**127**) have been isolated from



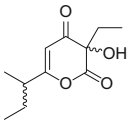
Scheme 15 A plausible biosynthesis relationship for linfuranones A–C (**120**–**122**)

cultures of *Streptomyces* sp. CS, isolated from the callus of *Maytenus hookeri*. No biological activities were described for these compounds [150].

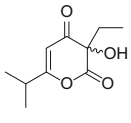


123 $R^1 = R^3 = H$, $R^2 = OH$ (*rac*)
124 $R^1 = OH$, $R^2 = R^3 = H$
125 $R^1 = R^2 = H$, $R^3 = OH$
126 $R^1 = H$, $R^2 = R^3 = OH$
127 $R^1 = R^3 = OH$, $R^2 = H$

Germicidins D (**128**) and E (**129**) are polyketides isolated from the growth medium of *Streptomyces* sp. A00122, present in the roots of *Camptotheca acuminata*, native to mainland China. Antimicrobial activities of **128** and **129** were evaluated against the bacteria *E. coli* (CMCC (B) 44103), *B. subtilis* (CMCC (B) 63501), *B. pumilus* (CMCC (B) 63202), *S. aureus* (CMCC (B) 26003), and the yeast *C. albicans* (AS 2.538). However, no activity was observed at a single concentration of 1 mg/cm^3 [151]. In this report, Li et al. introduced a nomenclatural discrepancy, since germicidin D is a different compound, previously reported from *Streptomyces coelicolor* A3(2) [152].



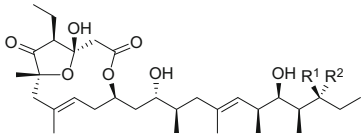
128 (germicidin D)



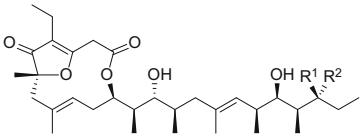
129 (germicidin E)

Bioinformatic analysis of *Streptomyces coelicolor* genome sequence data allowed the identification of germicidin synthases (Gcs) as a type II polyketide synthase [152, 153]. A pathway for germicidins comprises the incorporation of branched acyl-chain starter units [154]. In vitro reconstitution of germicidin biosynthesis was accomplished by coupling Gcs with fatty acid biosynthesis. Two possible parallel paths to germicidins were represented, involving acyl-AcpP and acyl-CoA starter units, respectively (Scheme 16) [154].

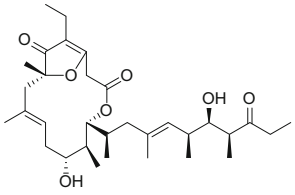
Actinoallolides A–E (**130–134**) were discovered from cultures of *Actinoallomurus fulvus* MK10-036, isolated from the roots of *Capsicum frutescens*, collected in Thailand. Compound **130** displayed the most potent and selective in vitro antitrypanosomal activity against *T. brucei* GUT 3.1 strain, with an IC_{50} value of 0.0049 μg/cm^3, without any discernible cytotoxicity against MRC-5 cells ($IC_{50} > 100$ μg/cm^3). The selectivity index (>20,408) was over fivefold better than that of pentamidine ($IC_{50} = 0.0016$ μg/cm^3) and over 300-fold higher than that of suramin and eflornithine. Moreover, **130** also displayed in vitro antitrypanosomal activity against the *T. b. rhodesiense* STIB900 strain, with an IC_{50} value of 0.086 μg/cm^3 [155].



	R^1	R^2	
130	=O		(actinoallolide A)
131	OH	H	(actinoallolide B)

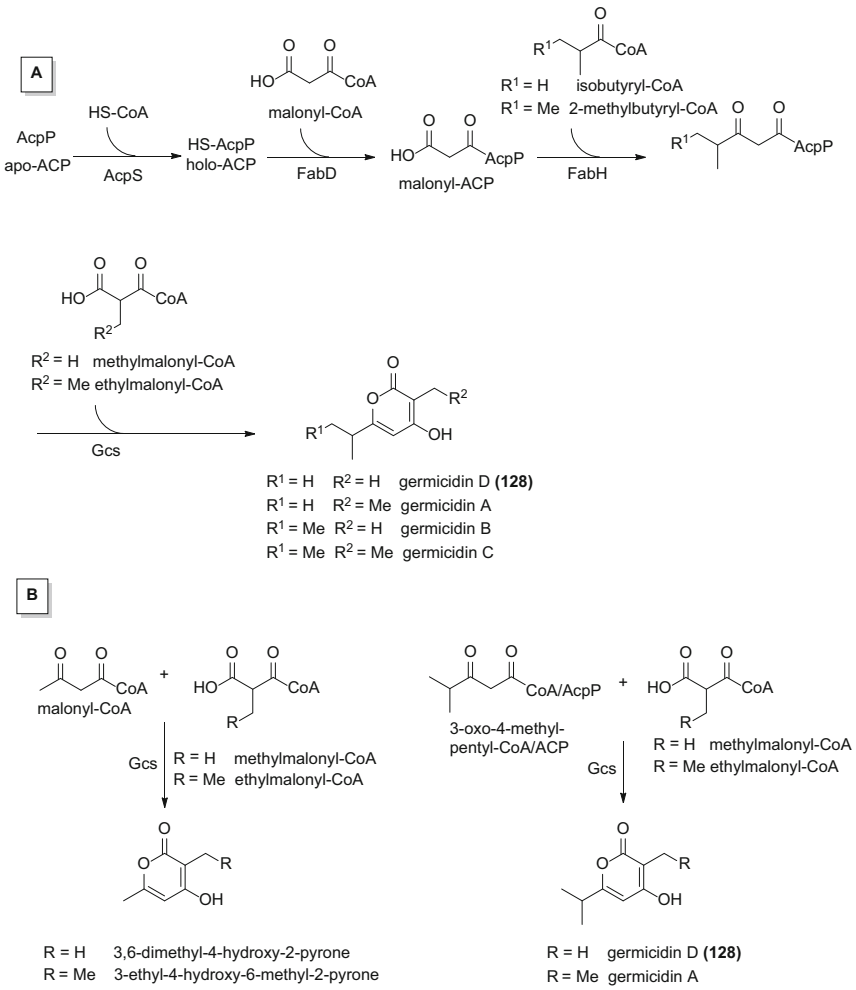


	R^1	R^2	
132	=O		(actinoallolide C)
133	OH	H	(actinoallolide D)



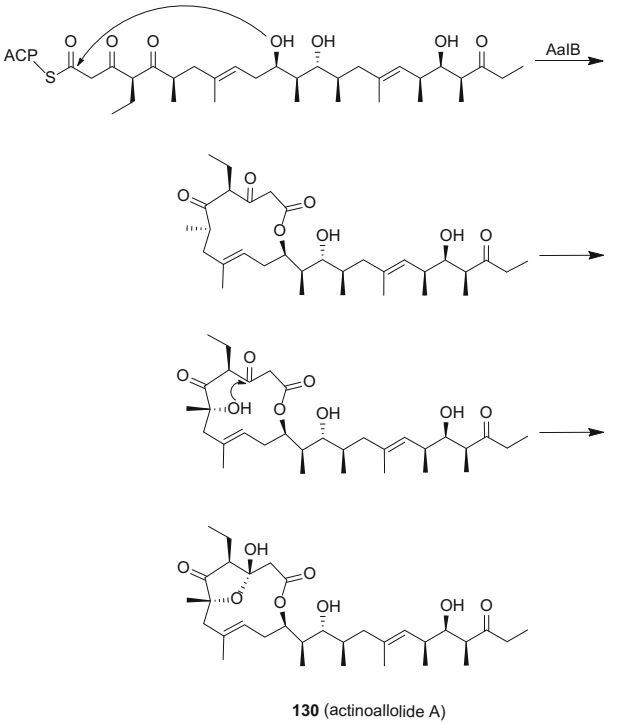
134 (actinoallolide E)

The putative biosynthesis gene cluster of the actinoallolides was predicted from another strain of *A. fulvus* (K09-0307) that produces actinoallolide A (**130**). The



Scheme 16 (**A**) Proposed metabolic pathway for germicidins A–C (**130**–**132**) in *S. coelicolor*; (**B**) In vitro reactions catalyzed by Gcs

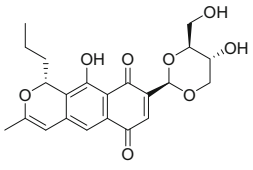
gene cluster comprises three genes for polyketide synthases (PKSs), and individual genes for a cytochrome P450, an acyl-CoA dehydrogenase, a crotonyl-CoA reductase, and a TetR regulator. There is one loading module and ten extension modules, which are responsible for condensing two malonyl-CoA and eight methylmalonyl-CoA residues, and one ethylmalonyl-CoA residue. All structural modifications and stereochemistry in the polyketide backbone were in agreement with domains present in each module (Scheme 17). One ketoreductase (KR) domain seems to be inactive because a ketone at C-21 is present in the structure of **130**. After building block condensations, the macrolactone may be formed by the thioesterase (TE) domain.



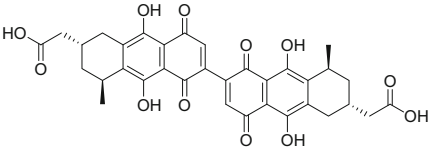
130 (actinoallolide A)

Scheme 17 Final steps in the biosynthesis of actinoallolide A (**130**)

Finally, a cytochrome P450 hydroxylates C-6, followed by a nucleophilic addition to a ketone at C-3 to produce the five-membered hemiacetal of this macrolide [156].



135 (alnumycin)



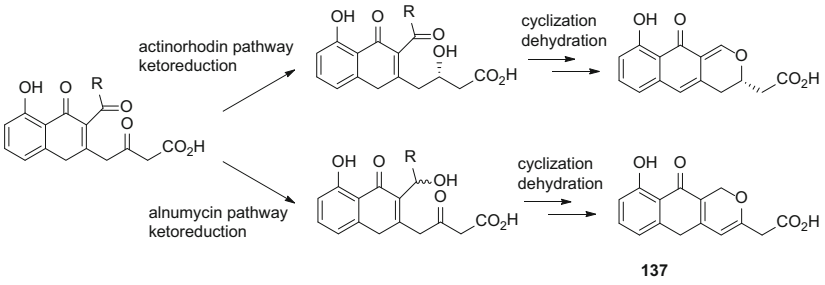
136 (actinorhodin)

Alnumycin (**135**) belongs to the naphthopyranomycin family of polyketides. It was isolated simultaneously by three different research groups. Alnumycin (**135**) was first designed as Be-41956A [157], then as K1115 B_1 [158], and by Bieber et al. as alnumycin [159]. Cultures of *Streptomyces* sp. (DSM 11575), isolated from root nodules of *Alnus glutinosa* collected near Jena (Germany), provided **135**, which has a variety of biological activities, including antibiotic, cytostatic, gyrase inhibitory, and topoisomerase inhibitory actions. The relative stereochemistry of the 1,3-dioxane moiety was determined by NMR NOE analysis [159].

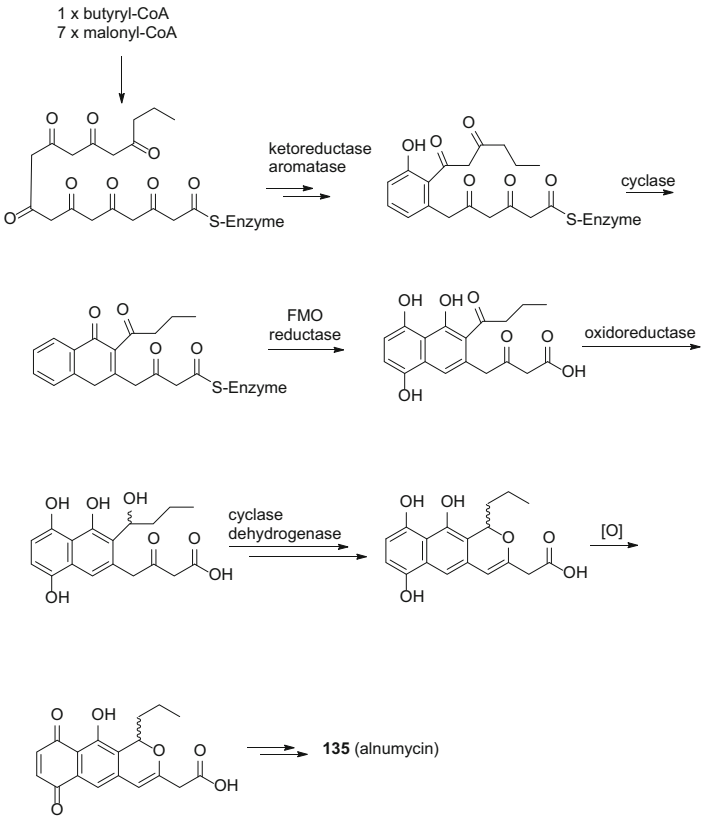
Alnumycin (**135**) possesses an unique chromophore and an aglycone with a 4-hydroxymethyl-5-hydroxy-1,3-dioxane moiety. The alnumycin gene cluster of *Streptomyces* sp. CM has the expected genes for polyketide assembly, with additional atypical genes for tailoring enzymes. It was suggested that a diketide starter unit is first biosynthesized by three gene products coding a ketoacyl synthase III, a malonyl-CoA:ACP transacylase and an additional ACP. The absence of gene products resulting from reduction of the diketide, such as a ketoreductase, a dehydratase, and an enoyl reductase, indicates that primary fatty acid metabolism may exert this catalytic activity [160, 161].

Mutagenesis studies revealed that the mechanism of pyran ring formation differs from other anthraquinones. While actinorhodin (**136**) requires a ketoreductase for reducing C-3, in alnumycin aglycone precursor **137**, the reduction step is catalyzed by an enzyme related to aldo-keto reductases that reduces the C-15 ketone (Scheme 18). Another gene seems to be involved in the cyclization reaction, but only a low similarity was observed (less than 50%) to other sequences, which precluded the prediction of this function. This second gene might produce an accessory protein for the reductase encoded by the first gene. According to the proposed biosynthetic steps, a double bond is formed between C-2 and C-3 after cyclization and dehydration (Scheme 18) [160].

The alnumycin chromophore carries a *p*-quinone arrangement in the lateral ring instead of in the central ring, which is commonly observed for benzoisochromanequinones [160, 162]. A two-component flavin-dependent monooxygenase (FMO) and a flavin reductase (named the FMO system) involved in the biosynthesis of alnumycin have significant homology to FMOs involved in the biosynthesis of other benzoisochromanequinones; however, the enzymes participating in the biosynthesis of alnumycin are monofunctional and catalyze oxygenation only at the lateral ring, leading to the formation of the lateral *p*-quinone. Formation of the bicyclic intermediate was suggested to occur before the action of the FMO system and it is likely to be the substrate for the FMO system, differing from that observed in the biosynthesis of actinorhodin, for which the quinone is generated after pyran ring formation (Scheme 19) [162].



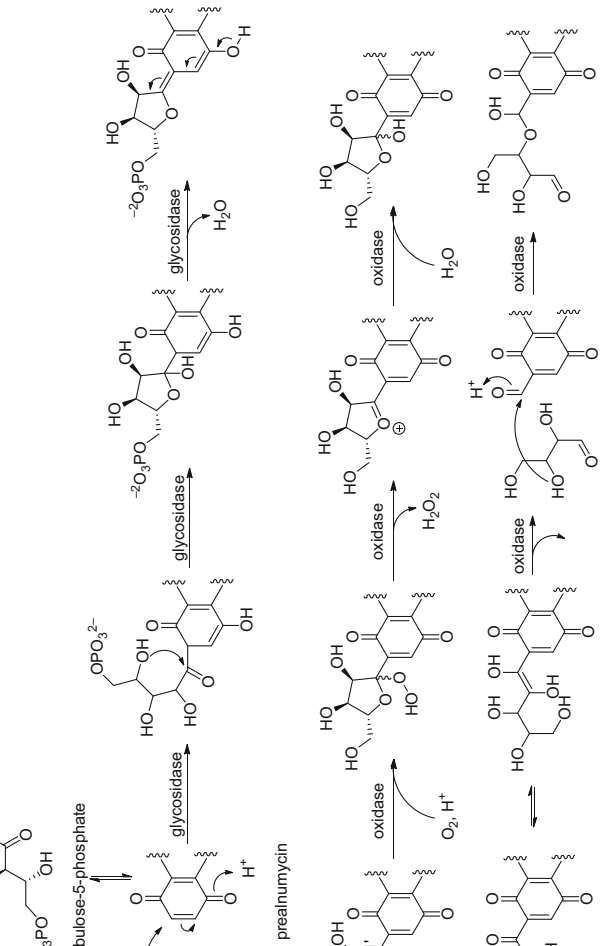
Scheme 18 Comparison of pyran ring formation between alnumycin aglycone precursor **137**



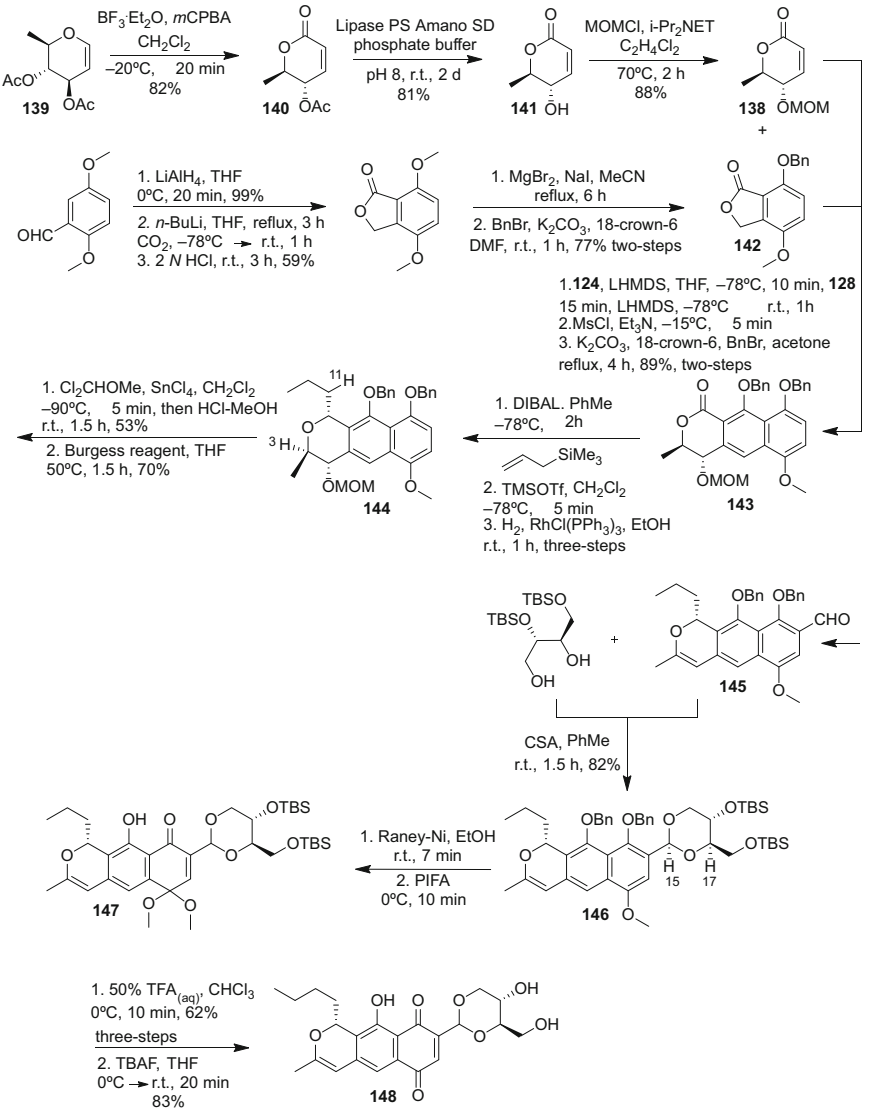
Scheme 19 Proposed biosynthesis pathway of alnumycin (**135**)

Mutagenesis studies have enabled the identification of two genes involved in the biosynthesis and attachment of the dioxane moiety [160]. The entire dioxane moiety might be fully synthesized prior to its attachment to the aglycone. One of the gene products catalyzes acetal formation while the other is responsible for attachment of a complete dioxane moiety to the alnumycin aglycone. However, no gene products homologous to glycosyl transferases were found in the alnumycin gene cluster [160].

More recent investigations have reported that the dioxane moiety of alnumycin is derived from D-ribose-5-phosphate (Scheme 20) [161]. A glycosidase (AlnA) catalyzes the attachment of D-ribose-5-phosphate to the aglycone, via a Michael-type addition, followed by hemiacetal formation and dehydration. A phosphatase (AlnB) catalyzes the dephosphorylation of the attached group [163]. A novel co-factor independent oxidase (Aln6), catalyzes the cleavage of the C-1′–C-2′ bond via a hydroxylation at C-1′ followed by retro-aldol cleavage and acetal formation. Finally, an aldo-ketoreductase (Aln4) performs an aldehyde reduction, followed by its cyclization, to form the dioxane unit [164]. This entire mechanism for the dioxane moiety biosynthesis clarifies the absence of glycosyl transferases in the gene cluster (Scheme 20).



Scheme 20 Biosynthesis of the dioxane unit attached to the alnumycin aglycone

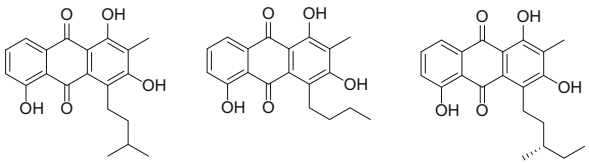


Scheme 21 Total synthesis of (1*R*,17*R*)-alnumycin (**148**)

The first total synthesis of **135** was achieved by Tatsuta et al. [165] (Scheme 21), who synthesized four stereoisomers of **135**. The intermediate **138** was prepared from **139** and converted to its corresponding lactone **140**. A stereoselective lipase hydrolysis yielded (+)-osmundalactone (**141**) in 81% yield, which was O-protected to give **138**. The isobenzofuranone **142** was synthesized from 2,5-dimethoxybenzaldehyde by a series of stepwise interconversions. Construction of **143** was achieved via a Michael-Dieckmann cyclization. Condensation of **138** with **142** was performed with

excess of LHMDS to give the Michael adduct. Additional LHMDS was added to conduct a Dieckmann condensation at room temperature. The product **143** was formed after aromatization and protection as a benzyl ether in 89% yield for two steps. Reduction of the lactone and stereoselective allylation insertion with allyltrimethylsilane gave **144**. The stereochemistry of **144** was determined by NOE NMR analysis. Formylation, de-O-methoxymethylation and dehydration yielded the vinyl ether **145**, which was converted to the 1,3-dioxane **146** in 82% yield. The stereochemistry of **146** also was determined by NMR NOE analysis. Treatment of **146** with Raney-Nickel followed by immediate oxidation with (bis (trifluoroacetoxy)iodo)benzene (PIFA), in order to avoid the degradation of the dihydroquinone, gave **147**. Acid hydrolysis and deprotection provided (1*R*,17*R*)-alnumycin **148** in 83% yield. Additional alnumycin stereoisomers were prepared by changing the diol configuration.

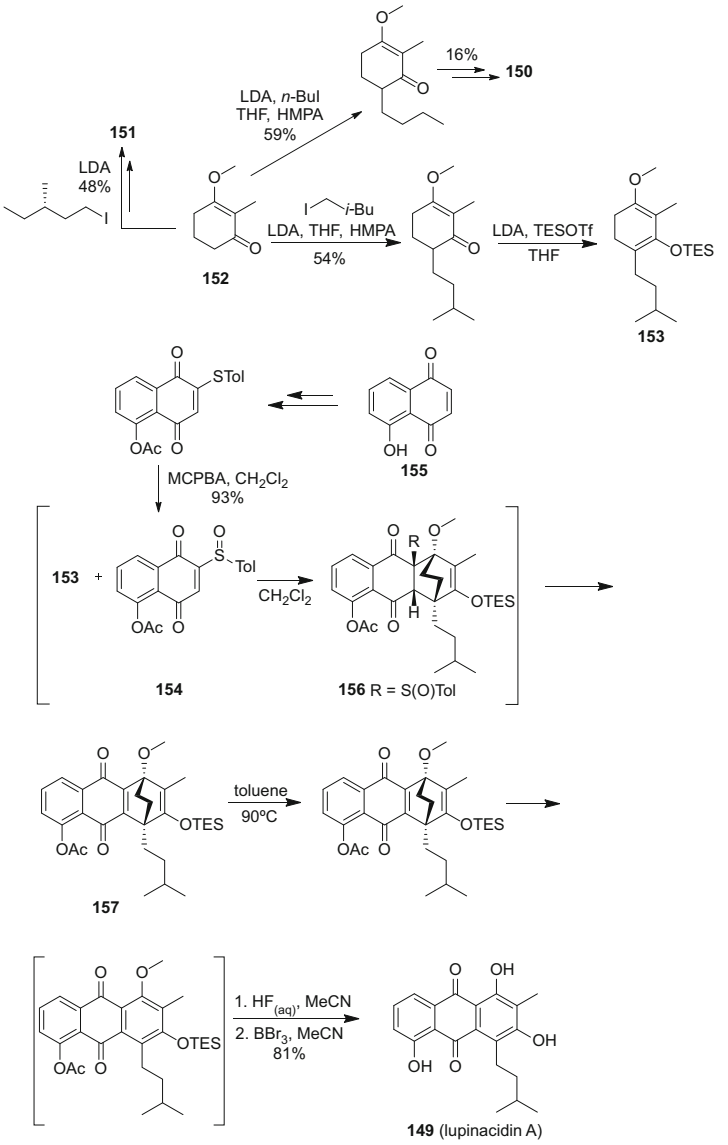
Lupinacidins A (**149**), B (**150**), and C (**151**) have been isolated from *Micromonospora lupini* Lupac 08 isolated from the root nodules of *Lupinus angustifolius*, collected in Saelices el Chico (Salamanca, Spain). Lupinacidins exhibited potent inhibitory effects on the invasion of murine colon carcinoma cells into the reconstituted basement membrane, with IC_{50} values of 0.21, 0.92, and 0.054 μ*M*, respectively, without inhibiting normal cell growth [166, 167].



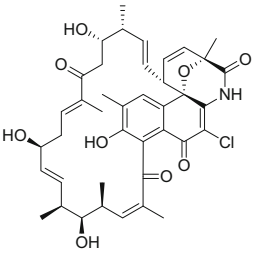
149 (lupinacidin A) **150** (lupinacidin B) **151** (lupinacidin C)

The total synthesis of **149–151** was completed using a Diels-Alder cycloaddition as the key step (Scheme 22). Alkylation of 3-methoxy-2-methylcyclohex-2-enone (**152**) constituted a common step for the preparation of the lupinacidins. In the case of lupinacidin A (**149**), protection of the enol with triethylsilyl ether (TES) provided the unstable **153**, which was reacted in situ in a Diels-Alder reaction with the diene **154** prepared from juglone (**155**). The cycloadduct **156** was subjected to elimination of the 4-toluenesulfonic acid moiety to give **157**, which was heated in toluene to produce the anthraquinone core. Two additional steps gave lupinacidin A (**149**) in 81% yield [167, 168].

Naphthomycin K (**158**), a new ansamycin, was isolated from *Streptomyces* sp. CS obtained from the medicinal plant *Maytenus hookeri*. Naphthomycin K (**158**) displayed cytotoxicity against the P388 and A-549 cell lines, but no inhibitory activities against *Staphylococcus aureus* and *Mycobacterium tuberculosis* were observed [169].

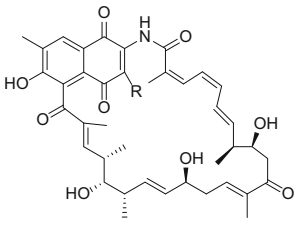


Scheme 22 Total synthesis of lupinacidins A (**149**), B (**150**), and C (**151**)



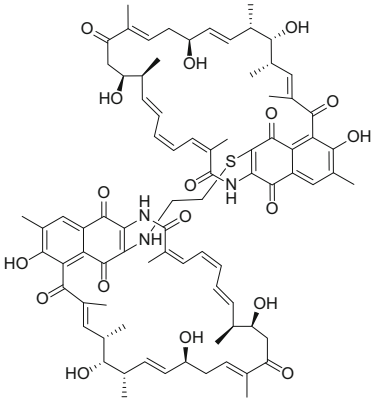
158 (naphthomycin K)

Gene mining of *Streptomyces* sp. CS genome searching for naphthomycin biosynthesis gene clusters indicated that additional naphthomycin derivatives potentially could be produced in culture. A HPLC-MS screening of *Streptomyces* sp. CS products formed under different culture conditions revealed several unknown peaks in extracts derived from oatmeal medium cultures. Subsequently, naphthomycins L (**159**), M (**160**), and N (**161**) were isolated and identified [170]. Only naphthomycin L (**159**) showed very weak antifungal activity against phytopathogenic fungi. The minimal inhibitory concentration of **159** was 300 μg/cm^3 against *Fusarium moniliforme* and 600 μg/cm^3 against several other phytopathogenic fungi (*Fusarium oxysporum*, *Fusarium moniliforme*, *Gaeumannomyces graminis*, *Verticillium cinnabarinum*, and *Pyricularia oryzae*).



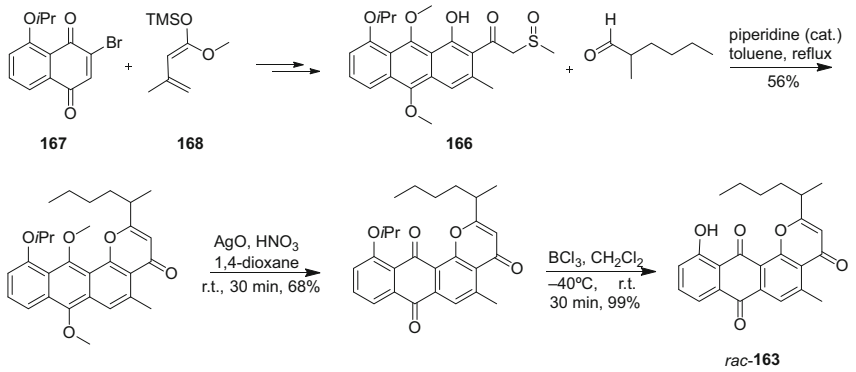
159 R = NH_2 (naphthomycin L)

160 R = SCH_2CO_2H (naphthomycin M)



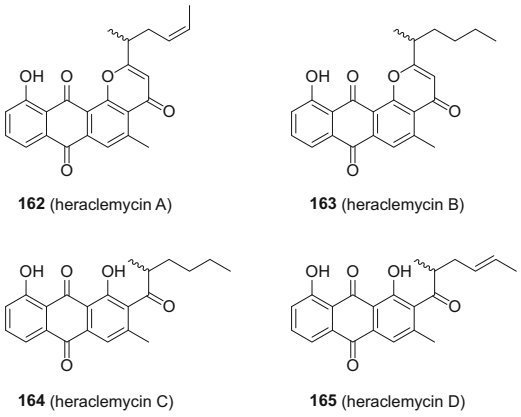
161 (naphthomycin N)

The extract from the growth medium of *Streptomyces* sp. strain Y3111, associated with the stems of *Heracleum souliei*, showed good anti-BCG activity. Bioassay-guided isolation led to the isolation of heraclemycins A–D (**162**–**165**). These are the first examples of *Bacillus* Calmette-Guérin (BCG)-selective inhibitory pluramycins. Heraclemycin C (**164**) displayed inhibitory activity of BCG with an *MIC* value of 6.25 μg/cm^3, with a potential new mode of action [171]. A short synthesis of heraclemycin B (**163**) was developed (Scheme 23). The anthracene

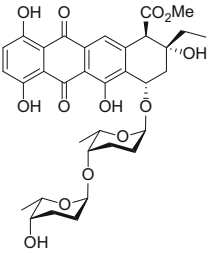


Scheme 23 Total synthesis of heraclemycin B (**163**)

moiety **166** was prepared by a Diels-Alder cycloaddition of 2-bromo-8-isopropoxynaphthalene-1,4-dione **167** and the diene **168**. Condensation of sulfoxide **166** with 2-methylhexanal, followed by deprotection and oxidation steps, provided heraclemycin B **163** [172].



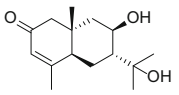
Large-scale fermentation and subsequent fractionation of the broth and mycelium extract of *Streptomyces* sp. YIM66403, isolated from a healthy stem of the traditional Chinese medicinal plant *Isodon eriocalyx* obtained from Xishuangbanna, Yunnan, People's Republic of China, yielded misamycin (**169**). Misamycin (**169**) displayed cytotoxicity and antibacterial activity against *Staphylococcus aureus* [173].



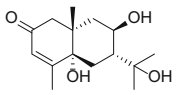
169 (misamycin)

9.4 Terpenoids

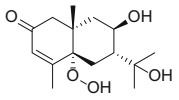
Five eudesmene sesquiterpenes, kandenols A−E (**170**–**174**), have been isolated from the growth medium of *Streptomyces* sp. HKI0595 derived from the mangrove plant *Kandelia candel*. The structures were established by spectrometric analysis, while absolute configurations were established by the Mosher ester method [174].



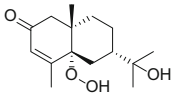
170 (kandenol A)



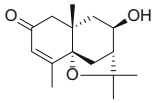
171 (kandenol B)



172 (kandenol C)



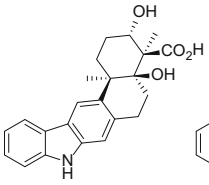
173 (kandenol D)



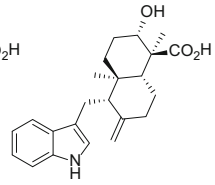
174 (kandenol E)

9.5 Secondary Metabolites of Mixed Biosynthesis

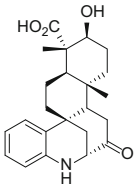
Three indolosesquiterpenes, xiamycin B (**175**), indosespene (**176**), and sespenine (**177**), were isolated from the culture broth of *Streptomyces* sp. HKI0595, a bacterial endophyte of the mangrove tree *Kandelia candel*. Agar diffusion assays revealed moderate to strong antimicrobial activities against several bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*. No cytotoxicity against human tumor cell lines was observed [175]. Indosespene (**176**) is an intermediate in the biosynthesis of xiamycin A and oxiamycin, produced by the marine-derived *Streptomyces* sp. SCSIO 02999 [176, 177].



175 (xiamycin)



176 (indosespene)

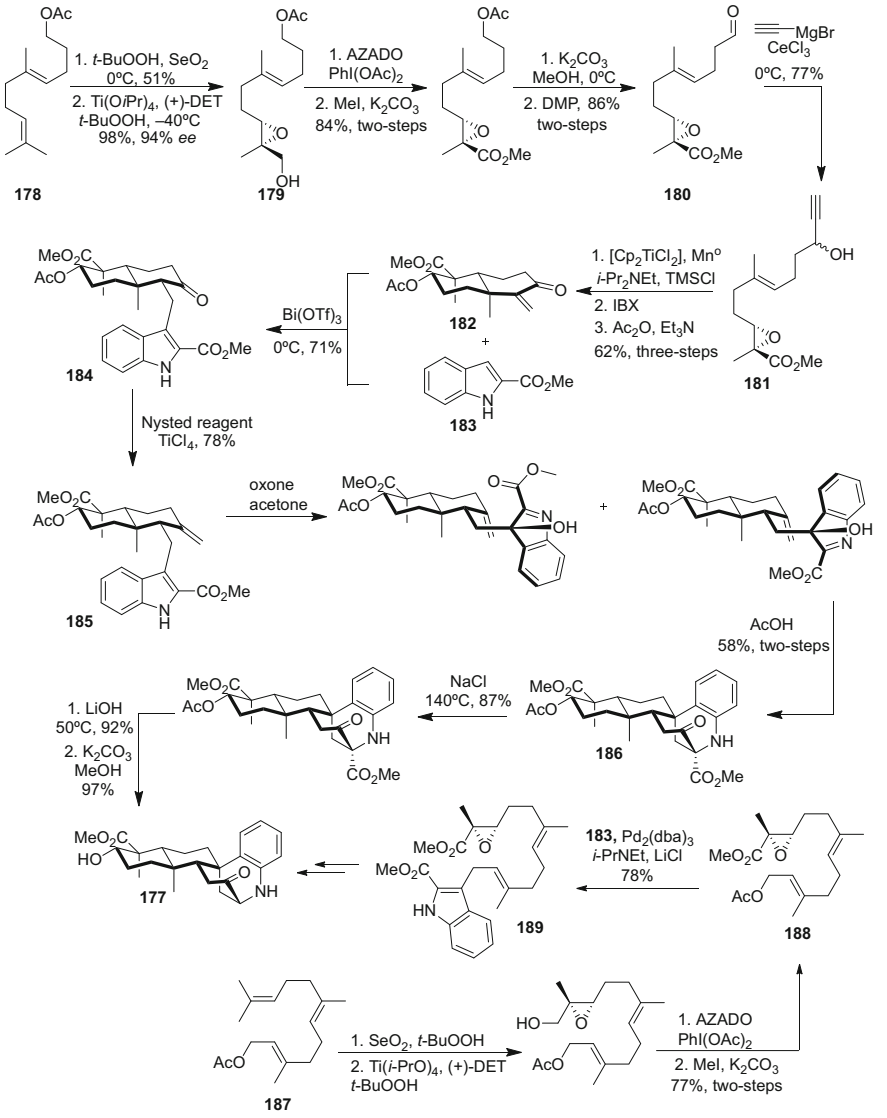


177 (sespenine)

Different approaches towards the total synthesis of indosespene (**176**) and sepenine (**177**) have been developed [178–181]. In the first synthesis of sepenine (**177**) (Scheme 24), conversion of acetylgeraniol (**178**) into **179** involved allylic oxidation and Sharpless epoxidation in 50% overall yield and 94% *ee*. The alcohol **179** was converted into its corresponding carboxylic acid with 2-azaadamantane-*N*-oxyl (AZADO), followed by esterification, transesterification, and oxidation to give the aldehyde **180**. Grignard condensation with $CeCl_3$ mediated using ethynylmagnesium bromide provided the secondary alcohol **181** in 77% yield. Titanium(III)-catalyzed epoxide opening followed by in situ cyclization, oxidation and secondary alcohol acetylation gave the *trans*-decalin-moiety **182** in three steps and 62% yield. Coupling of **182** with methyl 1*H*-indole-2-carboxylate (**183**) enabled the formation of the sespenine scaffold **184**, which reacted with Nysted reagent (cyclo-dibromodi-μ-methylene[μ-(tetrahydrofuran)]trizinc) to transform the ketone into its corresponding olefin **185**. Oxidation of the indole ring led to the formation of an inseparable epimeric mixture, which was subjected to an acid-catalyzed cyclization to give the aniline **186**. The final steps of sespenine (**177**) synthesis included a Krapcho demethoxycarbonylation followed by hydrolysis [178].

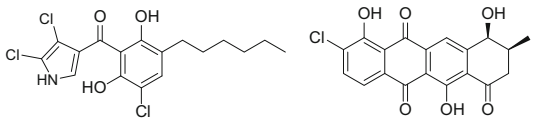
A second sespenine synthesis was reported by Sun et al. [181] (Scheme 24). Farnesyl acetate (**187**) was transformed into its ester by using the same conditions as those previously described. Coupling **188** with methyl 1*H*-indole-2-carboxylate (**183**) led to **189** in 78% yield. The additional steps were quite similar to the first synthesis of sespenine (**177**).

Indosespene (**176**) is a putative precursor of sespenine (**177**). Meng et al. [179] developed a synthesis for **176** (Scheme 25), starting from protected farnesyl acetate, which reacted with *N*-Boc-3-(tributylstannyl)indole via Stille-coupling followed by deprotection to give the intermediate **190**. Ti(III)-catalyzed cyclization give the *trans*-decalin motif as a single diastereomer (60%). After TMSE deprotection, the indosespene (**176**) synthesis was completed.



Scheme 24 First and second syntheses of sespenine (**177**)

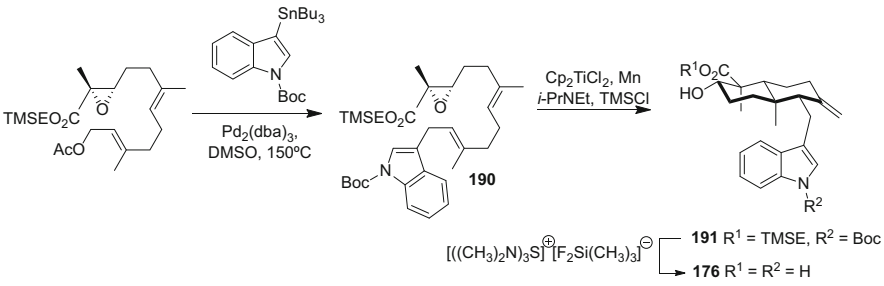
Celastramycins A (**192**) and B (**193**) have been isolated from cultures of *Streptomyces* MaB-QuH-8, isolated from *Maytenus aquifolia*. Celastramycin A (**192**) was active against *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Mycobacterium aurum*, *Mycobacterium vaccae*, *Mycobacterium fortuitum*, and *Bacillus subtilis*, while celastramycin B (**193**) was active only against *M. vaccae* and *B. subtilis* [182].



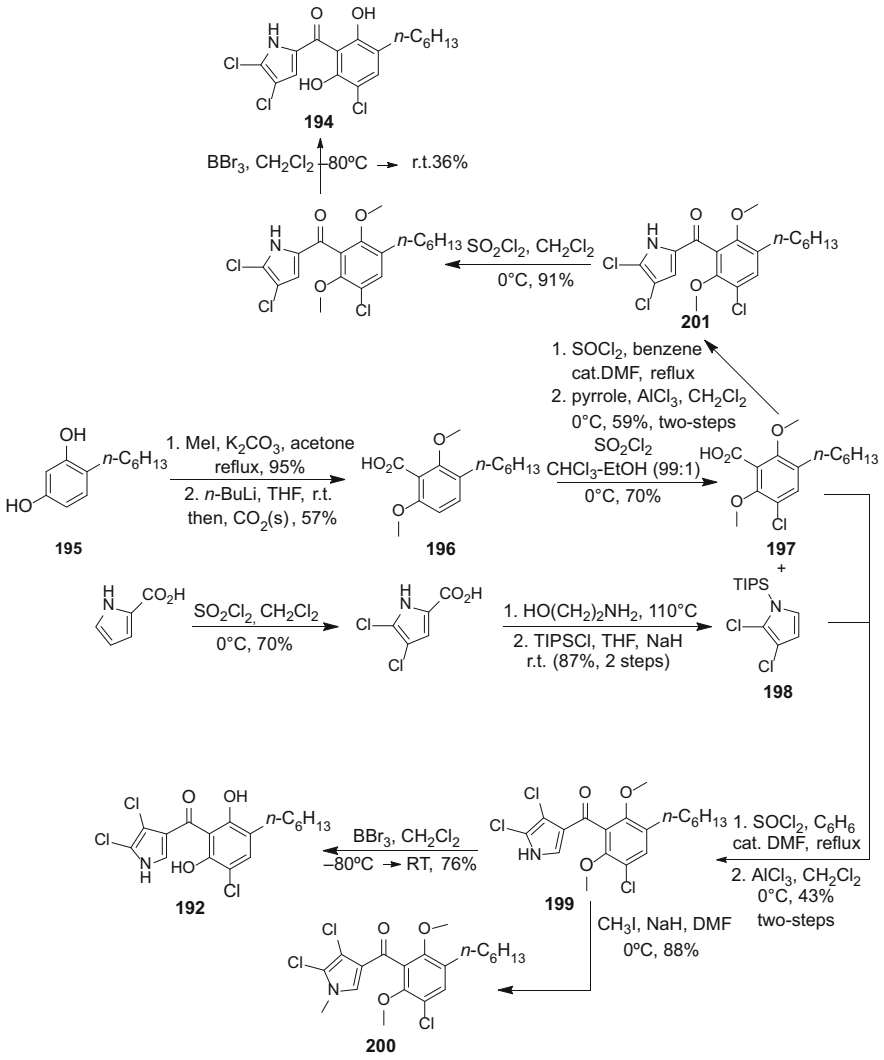
192 (celastramycin A) **193** (celastramycin B)

Kikuchi et al. [183] reported the synthesis of celastramycin A (**192**) and revised the structure previously reported by Pullen et al. [182] (Scheme 26). Curiously, **192** and **194**, reported in a Japanese patent, had the same ^{1}H and ^{13}C NMR spectra as a compound isolated by Kikuchi et al. [183]. To investigate which structure was correct, both **192** and **194** were synthesized. The methodology began from *O*-methylation of 4-*n*-hexylresorcinol (**195**), followed by *ortho*-lithiation and carboxylation to give 3-hexyl-2,6-dimethoxybenzoic acid (**196**). After chlorination of **196**, the chlorinated pyrrole moiety was constructed from pyrrole-2-carboxylic acid via chlorination, decarboxylation and *N*-silyl-protection, to give **198**. Coupling between **197** and **198** was promoted via a Friedel-Crafts β-acylation to yield the β-benzoylpyrrole **199**. Demethylation completed the synthesis of **192**. However, its ^{1}H and ^{13}C NMR spectra were different of those reported previously for celastramycin A and the compound found by Kikuchi et al. [182]. Analysis of the HMBC spectrum of the corresponding *N*-methyl derivative **200** placed the pyrrole motif β-positioned to a carbonyl group. In order to achieve a resolution between the isomeric structures, **194** was synthesized by converting **197** into its acid chloride, which reacted via Friedel-Crafts α-acylation to give **201**. After chlorination and demethylation of **201**, **194** was obtained. Analysis of the ^{1}H and ^{13}C NMR spectra of **194** confirmed that the structure reported by Pullen et al. [182] was incorrect, and that both **192** and the compound isolated by Kikuchi et al. [183] is **194**.

Three glycosylated esters, trehangelins A–C (**202**–**204**) were isolated from the cultured broth of *Polymorphospora rubra* K07-0510, obtained from the roots of an orchid collected at Iriomote Island, Okinawa, Japan. The trehangelins were found to consist of a trehalose moiety and two angelic acid moieties [184]. Compound **202** (IC_{50} of 0.1 μg/cm^3) and **204** (IC_{50} of 0.4 μg/cm^3), with symmetrical structures, showed potent inhibitory activities on the hemolysis of red blood cells induced by

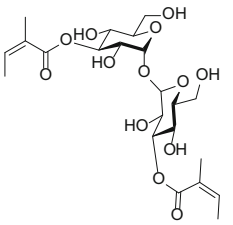


Scheme 25 Synthesis of indosespene (**176**)

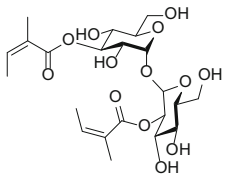


Scheme 26 Total synthesis and structural revision of celastramycin A (**192**) to **194**

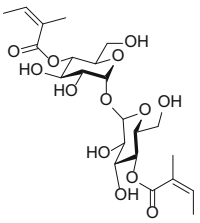
light-activated pheophorbide a. Compound **203**, with an asymmetric structure, displayed only a moderate inhibition (IC_{50} of 1.0 μg/cm^3) [184].



202 (trehangelin A)



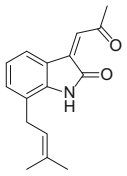
203 (trehangelin B)



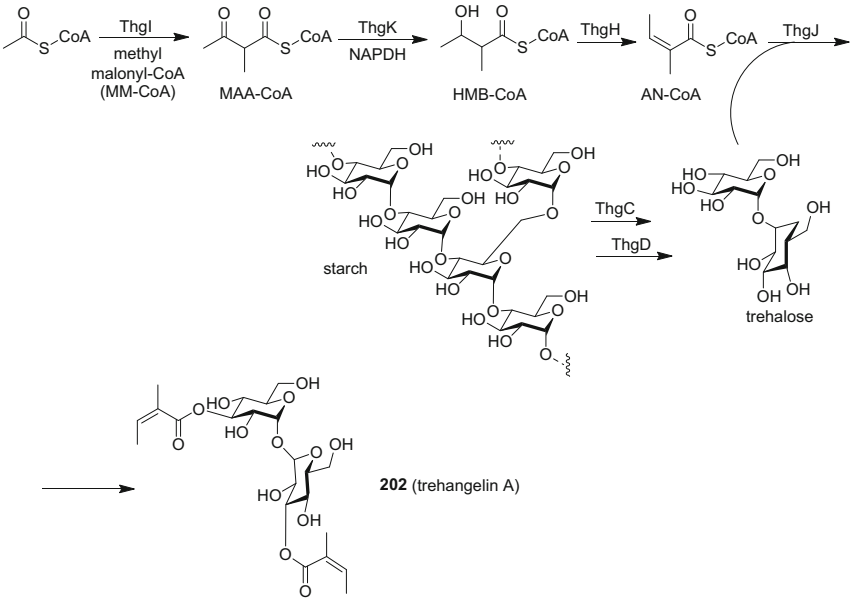
204 (trehangelin C)

The discovery of the trehangelin putative gene cluster was identified as contiguous within a 16 kb DNA region comprising 12 genes. Three genes responsible for formation of the angelyl moiety were determined: ThgI, ThgK, and ThgH. The thiolase ThgI catalyzes a Claisen condensation between Ac-CoA and MM-CoA. It was characterized as a NADPH-dependent reductase, which is involved in the formation of HMB-CoA from MAA-CoA. ThgH catalyzes the dehydration of HMB-CoA to AN-CoA, and could be classified as an enoyl-CoA hydratase. The trehalose formation was only hypothesized from the action of two enzymes, ThgC and ThgD, which would produce trehalose from soluble-starch, followed by ThgJ acyltransferase-mediated esterification (Scheme 27) [185].

The prenylated indole 3-acetonylidene-7-prenylindolin-2-one (**205**) was isolated from cultures of the endophytic actinobacterium *Streptomyces* sp. neau-D50, obtained from a healthy soybean root in Harbin, Heilongjiang Province, China. Compound **205** displayed cytotoxic activity for the A549 human lung adenocarcinoma cell line with an IC_{50} value of 3.3 μg/cm^3, comparable to that of the positive control doxorubicin (4.2 μg/cm^3). Furthermore, compound **205** also exhibited antifungal activity against the phytopathogenic fungi *Colletotrichum orbiculare*, *Phytophthora capsici*, *Corynespora cassiicola*, and *Fusarium oxysporum* [186].

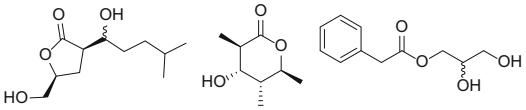


205



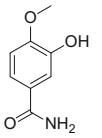
Scheme 27 Biosynthesis of trehangelin A (**188**); ThgC, glycoside hydrolase; ThgD, glycosidase; ThgI, Ketoacyl-ACP synthase III; ThgK, 3-ketoacyl-ACP reductase; ThgH, enoyl-CoA hydratase; ThgJ, acetyl transferase [184]

The endophytic actinobacterium *Streptomyces albospinus* RLe7, isolated from the roots of the Brazilian medicinal plant *Lychnophora ericoides*, produced (2*R**,4*S**)-2-((1′*S**)-hydroxy-4′-methylpentyl)-4-(hydroxymethyl)butanolide (**206**), (3*R**,4*S**,5*R**,6*S**)-tetrahydro-4-hydroxy-3,5,6-trimethyl-2-pyranone (**207**), and 1-*O*-(phenylacetyl)glycerol (**208**) [67].

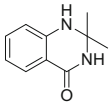


206 **207** **208**

Another *Streptomyces* sp. RLe8 endophytic actinobacterium, isolated from the same Brazilian medicinal plant, *L. ericoides*, furnished 3-hydroxy-4-methoxybenzamide (**209**) and 2,3-dihydro-2,2-dimethyl-4(1*H*)-quinazolinone (**210**). Compound **210** displayed potent cytotoxic activity against the MDA-MB435, HCT-8, SF-95, and HL-60 cancer cell lines [83].

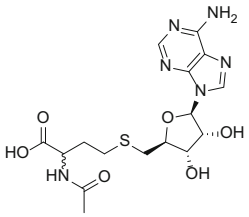


209



210

Investigation of endophytic Actinomycetes associated with the roots of *Pueraria candollei*, a Thai herb with phytoestrogenic-related effects, yielded 32 isolates. Screening by LC-MS-based of medium extracts produced by the endophytic strains led to the isolation of *S*-adenosyl-*N*-acetyl-homocysteine (**211**) from *Micromonospora chalcea* DSM 43026^T [187].



211 (*S*-adenosyl-*N*-acetyl-homocysteine)

10 Perspectives

The fraction of endophytes and of the respective endophyte metabolic capabilities that have been already unveiled is still quite small, but these have an array of features that need to be much better understood [188, 189]. There is a tacit assumption that every single plant host has an average of at least four different endophyte species; however, species-specificity traits that establish such associations have been poorly investigated [102, 190]. Such traits are obviously related to the production and accumulation of secondary metabolites [189, 191], for which successful strain isolation is required prior to manipulation under appropriate conditions and procedures [102]. Fundamental questions concerning endophyte-plant host relationships remain to be addressed, including: how are such relationships established during evolution? Does lateral gene transfer play a significant role in either host or endophyte acquisition of biosynthetic gene clusters? Are endophytes latent pathogens or not? What are the actual roles of endophyte secondary metabolites within the scope of endophyte-plant interactions? These questions add to the challenges of exploiting endophytes as reliable sources of bioactive natural products and require deeper investigation. Such investigations will include the reproduction of the endophyte natural living environment, not only the physical conditions (pH, pCO, pCO_2, nutrient concentration, and sources), but also biological factors that regulate

microbe-plant and microbe-microbe interactions, which may be crucial for the phenotypic expression of endophyte secondary metabolism [190]. The importance in addressing such questions should not be minimized, since the potential of endophytes for the sustainable, long-term and large-scale production of secondary metabolites has yet to be proven and realized [103, 192].

Assessment of endophyte diversity in a single plant species using dilution-to-extinction cultivation techniques has enabled the isolation of additional taxa not observed using traditional plating procedures [193]. The knowledge of tropical plant endophytes is still very preliminary when compared to studies performed in temperate regions, even though species from temperate regions have been little investigated in Asia, Australia, New Zealand, South Africa, and South America [194], regions where a higher level of plant endemism is expected. Investigations dealing with endophyte isolation for bioprospecting purposes usually report a lower number and the occurrence of less diverse species than careful assessments of endophyte diversity performed by microbiology specialists. Therefore, there is a clear need for collaboration between "bioprospectors" and microbiologists, not only to abandon some preconceived conceptions about endophytes [194], but also to properly explore the endophyte diversity towards the discovery of new bioactive compounds. Considerable challenges related to the co-occurrence of both fungal and bacterial endophytes in a single host need to be addressed, aiming to understand the evolution and biology of such systems that allowed life to be successful on Earth.

While fungal endophytes have been much more frequently investigated towards the isolation of bioactive secondary metabolites, endophytic bacteria, including Actinomycetes, have been much less so [195, 196]. This is rather surprising considering that Actinomycetes are considered to be one of the most promising sources of successfully marketed bioactive natural products. Assessments of endophytic Actinobacteria, including analysis of biosynthetic capabilities, has indicated their wide occurrence in wild tropical plant species. Investigation of endophytic Actinobacteria from plants collected in Papua New Guinea and on the Solomon Islands led to the isolation of 137 strains from 37 plant species, among 113 plants surveyed. After culturing, 91 strains showed unique ribotypes and almost half of such strains yielded extracts with antibiotic activity [196]. More than 200 strains of endophytic Actinobacteria have been isolated from the roots, stems, and leaves of *Maytenus austroyunnanensis* occurring in the Xishuangbanna tropical rainforest in mainland China. Actinomycete species distribution was related to the tissue from which strains were isolated. Culture-independent metagenomic analysis showed that the actinomycete taxonomy overlap with culture-dependent analysis was minimal, indicating that suitable methods for the isolation of unusual endophyte Actinomycetes still need to be developed [87]. Nevertheless, concerns on developing long-term biodiscovery programs have been raised [197], suggesting that additional questions need to be debated.

10.1 How Relevant Still Is the Discovery of Microbial Natural Products?

There is a well-established consensus that natural products have been in the past, are presently, and should be considered in the future as the main source of new diversified chemotypes for drug discovery and development [197–200]. This is true even though there are some major difficulties associated with obtaining significant outcomes resulting from the effort invested in the discovery of new drug leads from secondary metabolites, such as these being highly work intensive, costly, and sometimes of low financial return [197]. However, nearly 70% of antiinfective agents and 63% of anticancer drug leads are based on natural products [201]. Although microbial natural products correspond to ca. 11% of all described secondary metabolites, microbially derived pharmaceuticals correspond to ca. 50% of all natural product-based marketed drugs [202]. Microbial strains are likely to be the main source of structurally novel and bioactive secondary metabolites, because these organisms are ancient, as well as taxonomically and metabolically very diverse and account to ca. 60% of the Earth's biomass [203, 204]. It has been estimated that of the ca. 20,000–23,000 natural products isolated from microbial strains, 38% are from fungi, 17% from unicellular bacteria and 45% are produced by Actinomycetes [205]. Another assessment has claimed that about 80% of the microbial secondary metabolites have been isolated from Actinomycetales cultures, of which 50% correspond to Streptomycetes [206]. Microorganisms constitute an essential source of novel, structurally unique, and biologically active secondary metabolites [204], frequently associated with biological activities encompassing new modes-of-action, to which potential beneficial traits can be assigned to the producer organism [204, 207]. Even if the actual origin and functions of microbial natural products remain largely unknown [208, 209], the importance of secondary metabolism for the success of microbial life on Earth is undeniable [204, 208, 209].

One of the unique aspects of microorganism-based drug discovery programs is that microbial strains constitute two faces of the same coin: at the same time microorganisms are the main source of antibiotics and are the main threats associated with antibiotic resistance. Antibiotic resistance is currently of a major concern for public and animal health. Although it is well-known that the anthropogenic production and use of antibiotics has promoted the appearance of antibiotic-resistant pathogenic strains, there is a growing realization that microbial antibiotic resistance is environmentally spread and of common occurrence, since resistance mechanisms are likely to be selected during evolution. Therefore, considerable efforts are underway to understand the phenomenon of antibiotic resistance in order to increase the therapeutic armamentarium against antibiotic-resistant pathogens [210, 211]. Nevertheless, while the largest portion of antimicrobial agents has been isolated from microbial sources [204], it seems that only 1–3% of antimicrobial natural products are known [205]. While the discovery and development of new antibiotics seems to be unrewarding to most pharmaceutical companies, there is an increasing need for new and effective antibiotic agents [204, 212, 213]. The apparent amount of

antibiotic discovery and development activity at pharmaceutical companies is rather worrying. From 2002 to 2007, only two new antibiotics were approved. The number of new antibacterial agents released on the market has diminished from year to year since 1983. Many factors contribute to the lack of interest for the production of new antibiotic agents by companies, including the short patent life and the very high cost of antibiotic research and development, as well as a lack of prospective financial revenues [212]. Although microorganisms are recognized as the most important source of antibiotics, it appears to be necessary to explore a larger number of new microbial strains in screening programs to find new antimicrobial agents [204, 214]. Novel strategies to find strains that produce antimicrobials have been developed, such as genome mining to reveal "hidden" or "silent" biosynthetic gene clusters, to enhance heterologous expression, as well as to remove or inactivate inhibitors of gene expression [212]. New mechanism-based assays have been developed towards finding new antibacterials, such as the inhibition of 4-aminobenzoic acid biosynthesis, the inhibition of FabF enzyme expression, and inhibition of the bacterial translation apparatus [214]. Microbial antitumor antibiotics are considered as some of the most important chemotherapeutic agents as well, including, for example, the anthracyclines, bleomycins, and epothilones [201, 215]. Most of antituberculosis agents have also been isolated from microbial sources, although not during the last 40 years. Streptomycin, discovered during the 1940s, is still used in tuberculosis treatment. Several new approaches have been developed recently in order to discover new antitubercular agents from microorganisms, including new dereplication strategies and target-based assays [205]. Some antibiotics most recently discovered from microorganisms that are currently being used to treat pathogenic infections include daptomycin produced by *Streptomyces roseosporus*, which is an inhibitor of peptidoglycan biosynthesis and a membrane-depolarizing agent [216], as well as the echinocandins, micafungin and related lipopeptides, which are potent antifungal lipopeptides, active at sub-μg/cm^3 concentrations and inhibitors of 1,3-β-glucan synthesis [217].

Anticancer agents from microbial strains have been reviewed in detail by Newman and Cragg [201]. Promising antitumor microbial natural products include the epothilones from the myxobacterium *Sorangium cellulosum*, which are inhibitors of tubulin polymerization. The immunosuppressants cyclosporin A, FK506, and rapamycin have also been isolated from microbial sources [197, 215]. New microbial chemical entities have more recently been discovered as drug leads, such as antibacterials, antifungals, antiparasitic and antiviral agents, as well as to treat neurological, cardiovascular, immunological and cancer-related diseases [198, 201, 204]. Clearly, microorganisms remain one of the major sources of new chemical entities to be included in drug discovery programs.

Even though microorganisms have been continuously investigated as a source of bioactive secondary metabolites for the last 70 years, they are still considered as largely underexplored, because of the enormous microbial diversity and strategies used in drug discovery programs, which have not been diversified until recently [198, 201]. This is rather astonishing, considering that microbial production of bioactive natural products using fermentation is one of the most reliable sources

for a sustainable supply of chemicals in drug development programs. Microbial production processes can be enhanced efficiently and/or diversified, using the compounds produced either as actual drug entities per se or as advanced synthetic intermediates that can be transformed into a commercial product [198].

Screening approaches have been developed during the last few years that may be applied in the discovery of microbially derived natural products, the majority of which include the extensive use of liquid chromatography coupled to diverse hyphenated detection systems. HPLC fractionation and subsequent analysis enables access to the microbial metabolome in a rather straightforward manner [218]. Screening of microbial metabolites using hyphenated strategies includes the use of HPLC-ESI-MS and principal component analysis (PCA) [219], UPLC/HRESI-TOF-MS and PCA [220], and HPLC-UV-MS-ELSD [221]. Exploratory solid-phase extraction (SPE), using stationary phases with orthogonal selectivity, has been employed to improve the separation strategies for the fractionation of microbial natural products. These have included passage over cation-exchange, anion-exchange, mixed-mode polymeric reversed-phase anion-exchange, and Sephadex LH-20 columns. Active and non-active fractions analyzed by HPLC-UV-MS, and by their chemical features coupled with microbial taxonomic analysis, have provided "hits" that indicate strains for further investigation after larger-scale growth. The same approach is suitable for dereplication to trace bioactive fractions and in the optimization and scaling-up of microbial cultures [222]. While the methods mentioned herein are far from being exhaustive, screening systems applied to the discovery of bioactive natural products from other types of source organisms may be adapted for the discovery of microbial metabolites as well.

Dereplication methods have been developed during the last three decades, aiming to avoid redundancy from the isolation of already known compounds and hence to accelerate the discovery of novel, bioactive metabolites [218]. Approaches for the dereplication of microbial metabolites have included the use of HPLC-UV-MS [223, 224], HPLC-ESI-MS-ELSD [225], and direct infusion ESI-MS analysis. Dereplication tools have illustrated the importance of growth media and growth parameter selection to improve the production of secondary metabolites by Actinomycetes. The use of screening and dereplication tools to avoid redundancy and to save time and resources is essential, particularly if traditional methods of strain isolation and culturing are utilized. Dereplication is critical in bioprospecting microbial metabolites because the production of secondary metabolites is not uniformly distributed among microbial strains, while finding and selecting metabolically active strains should be performed along with microbial taxonomic analysis [197, 226].

10.2 Microbial Diversity Is Related to Metabolic Diversity

In dealing with microorganisms, it is difficult to establish the best approaches to fully explore microbial metabolic capabilities for the discovery of novel bioactive natural products. There are several possibilities related to the search for metabolites from

particular microbial taxonomic groups, the location of sample collections, techniques of strain sampling, and conditions for initial cultivation and large-scale culturing. Comprehensive monographs cover many such aspects of microbial prospecting [227–229]. New approaches for strain isolation and cultivation are of vital importance for the discovery of novel microbial secondary metabolites. These include a careful construction of diverse strain libraries using polyphasic approaches for strain identification, as well as the use of unusual and innovative culturing methods to improve the recovery of "difficult to culture" or "yet uncultivated" strains from diverse environmental samples. Exotic or extreme environmental locations are considered of high interest for the finding of novel strains. The use of state-of-the-art culture-independent molecular biology techniques to uncover the metabolic potential of microbial strains has been of increasing interest as well. A summary on such topics has been recently published [230].

Bioprospecting in unknown, pristine, exotic, and extreme geographic locations appears to contribute to the finding of new microbial strains, not only in number but also in taxonomic diversity. This seems to be valid for bacteria as well as for fungi [197, 198, 205, 231, 232]. Even if the prospecting of unusual, rare or novel microbial strains from unique geographic regions or environments seems very attractive, there is a long-time debate as to whether microorganisms are cosmopolitan or if there are truly endemic microbial species [197, 233, 234]. Furthermore, the as-yet underexplored metabolic capabilities of microbes from culture collections should not be overlooked [235].

The paradigm of microbial biogeography proposed by Baas Becking in 1934 that "everything is everywhere, but the environment selects" appears to still be valid to some extent. On the other hand, it is difficult to assess the comprehensive microbial diversity of specific locations or samples, even with the advent of "omics" technologies, which greatly enhance the capability to unveil microbial species distribution. There is evidence of microbial endemism in hot springs, and in plants (endophytes), insects and animals. Strains associated with specific organisms seem less likely to be able to colonize new habitats. Microorganism taxonomic uniqueness can be revealed in most terrestrial and aquatic environments and such microbial communities can be highly diverse [234]. Since there are a number of environmental factors influencing microbial species distribution, the search for microbial strains in unexplored environments is likely to lead to the discovery of potentially completely new metabolic pathways [197]. The isolation of the very unusual sulfur-rich closthioamide from cultures of the anaerobic bacterium *Clostridium cellulolyticum* has initiated the investigation of anaerobic bacterial strain secondary metabolism [235, 236]. Microbial symbionts have been barely investigated as producers of secondary metabolites, partially due to the particular challenge in cultivating such strains. Genomic and metagenomic investigations of several holobionts showed that the microbial diversity associated with specific hosts present astonishing metabolic profiles, which constitute a forum towards the knowledge of symbiotic associations and how guest-host interactions can influence or be influenced by such specific metabolic pathways [237–241].

The importance of considering a careful selection of microbial strains to be included in bioprospecting and drug discovery programs should not be overlooked. The success in finding new and active metabolites produced by microbial strains is increasingly more difficult, because many metabolites produced by microbial strains under usual conditions are widespread in Nature. Therefore, a careful selection of strains is of extreme importance, as well as the strategies used for microbial isolation, growth, and metabolite dereplication, in order to improve the efficiency of microbial bioactive compound discovery.

10.3 Exploring the Unknown and the Unseen, but No Longer the Undetectable

The advent of genomic and molecular biology tools has completely revolutionized access to the microbial world, enabling first the collection of larger amounts of DNA and then the analysis of environmental DNA. Metagenomic analysis [242] became rapidly adopted. Its versatility and power in providing an enormous amount of information was soon realized. The importance of using metagenomic analysis and other cultivation-independent analyses was highlighted by the fact that possibly 99% of all microbial species have not yet been cultivated [243]. Of the 40 known prokaryotic phyla, only half have been cultured [206, 243]. Therefore, culture-dependent methods for microbial taxonomic surveys may bias the view on microbial diversity [243]. Culture-independent methods allow the investigation of unknown microorganisms from exotic and extreme environments, including Antarctica and mineral mines [243]. Metagenomic analysis became by far the most utilized strategy to uncover the microbial diversity of biological and non-biological samples [203, 243]. The use of functional-based screening of clone libraries by metagenomic analysis has been proposed for the discovery and expression of relevant genes for the potential discovery of new natural products and direct industrial applications [203, 233]. Metagenomic analysis has been used to isolate and identify genes of antibiotic resistance from soil samples [244]. The use of metagenomics has been proposed as a powerful tool in bioprospecting for microbial metabolites, since in principle it should provide access to biosynthetic gene clusters bearing large DNA inserts, which can be analyzed using high-throughput screening robots and should provide information on new biosynthetic pathways. Moreover, metagenomics data can be used in combination with secondary metabolite HPLC profiling, providing access to a yet unimagined diversity of metabolic phenotypic expression [245]. Screening of environmental DNA libraries using metagenomics combined with metabolomics, metaproteomics or transcriptomics tools may considerably enhance the capabilities of novel and unusual biosynthetic pathways. However, a major challenge to be addressed is the enormous amount of information generated during such surveys, which may make difficult the finding of gene clusters. A laser-based metagenomics screen technology may provide the analysis of one billion

clones/day. Also, the combination of metagenomics and nanotechnology has been indicated as a completely new approach to investigate and explore the potential of microbial metabolic capabilities [203].

Additional "omics" technologies and approaches have been developed aiming to investigate in detail the expression of microbial metabolism. The use of metaproteomics, for example, enables the characterization of protein content of environmental samples, as well as the description of functional genes, enzymes, and metabolic pathways [243]. The use of single-cell genomics is based on the amplification of DNA from individual cells obtained by either microfluidics, flow cytometry, or micromanipulation, and enables the obtaining of refined information, since the genome to be investigated is related to a unique taxonomic unit [243, 246]. While a detailed review on the use of metagenomics and related "omics" approaches in the investigation of microbial diversity and metabolic capabilities is largely beyond the scope of this chapter, it is clear that the advent of the "omics" technologies will have a major impact on the investigation of microbial diversity, metabolism and functions. It has even been questioned if microbial culturing will still be necessary in the near future.

10.4 Is "Traditional Microbiology" an Outdated Science?

The availability of molecular biology tools from the late 1980s promoted a resurgence in bioprospecting of microorganisms as source of bioactive secondary metabolites. Such tools soon provided high-throughput genome analysis, and revealed that microbes very often present unexpressed secondary metabolite biosynthetic pathways. Genomic analysis of environmental samples has given evidence that cultured microorganisms correspond to only a very small fraction of the whole microbial diversity [247].

Almost at the same time, microbiologists started to invest considerable effort in developing completely new cultivation approaches aimed at isolating and growing unknown microorganism species in artificial media [248]. These accomplishments have been remarkable, since very little is known about the physiology of the "yet uncultured" microbial strains, with it being a challenge to establish the appropriate conditions for such strain isolation and cultivation. In 2008, only 7031 prokaryotic species were described after 120 years of the invention of the Petri dish [249]. A decade ago it was assumed that bioactive secondary metabolites were produced only by strains belonging to 5 of the 53 phyla of bacterial strains—Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria—and these 5 phyla corresponded to 95% of all cultivable bacterial species, overall belonging to 27 of the 53 bacterial phyla. The remaining 26 bacterial phyla corresponded to 5% of all known cultivable species of bacteria [232]. By 2008, it was assumed that of the 100 bacterial phyla that had then been described, only a third gave a cultivable representative [249].

However, the yet uncultivated "missing bacteria" may represent the largest portion of metabolic diversity on Earth [250]. While it requires considerable effort to evaluate if the current knowledge on cultured microbial diversity has changed substantially, novel culturing approaches and methods that have been developed resulted in the discovery of several "yet uncultivated" microbes and promoted a renewal in the development of innovative microbial culturing technologies.

Although the "unculturability" of most microbial strains has been assumed for a long time as a very difficult problem to be overcome, it has been stated that >90% of cells in soil samples are viable, with it being possible to increase their culturability to at least 5%. Nearly a decade ago it was realized that one of the best ways to address the "unculturability" problem of microbial strains was to establish culture conditions similar to the environment where samples of isolated strains have been obtained [232, 251–253]. This is because conventional cultivation seems to be highly selective and biased towards the isolation of a large number of already known or already isolated microbial strains, usually fast growing although not always dominant [206, 232]. Moreover, improper microbial strain manipulation may damage cells by oxidative stress, making them unculturable. Furthermore, cell-to-cell communication chemicals appear to be essential to "awake" microbial strains that are difficult to recover in artificial culturing conditions [197, 254]. Many such uncultivated microorganisms may play essential ecological roles, and a comprehensive understanding of the biology and ecology of microbes can only be achieved using cultivation-dependent techniques. Thus, new isolation and cultivation approaches are needed to uncover the unknown microbial diversity, very often assumed to be "unculturable". Several excellent reviews, as well as a number of books, discuss many such issues in detail and other interesting questions as well, and are recommended to those interested in the fascinating questions related to microbial (un)culturability [206, 229, 231, 232, 248, 249, 252–261].

While the analysis of microorganism cultures is rarely redundant [256], the apparent "unculturability" of the majority of microbial strains is not due to intrinsic features that make such strains difficult to cultivate, but it is rather due to the lack of knowledge as to how to isolate and grow them in artificial media [248–250, 253, 254]. Therefore, the innovative design of novel culturing approaches and methods is of paramount importance to achieve the cultivation of as yet uncultivated microbial strains [248]. Multiple culture conditions appear to be particularly important if one is aiming to explore microbial metabolic capabilities [196]. For example, the isolation of bacteria from freshwater samples was better achieved by testing multiple distinct growth conditions [258]. Patience in waiting for microbial cultures, which may require weeks, even months, or more than a year to appear, seems to be a crucial factor towards the isolation of unusual strains [248, 249].

While the cultivation of as yet uncultured strains may appear a challenging task [253], change of medium formulations is essential to successfully isolate and grow "unculturable" strains in artificial media, since many strains require specific components [249, 256]. Fast-growing microorganisms appear to be resistant to high nutrient concentrations and high microbial cell density [232], while uncultivated microorganisms seem to be inhibited by rich nutrient media and very often require

oligotrophic media to be cultured [196, 217, 256]. For example, high-level nitrogen-containing media likely inhibit most microbial growth, and inorganic phosphate inhibits the production of secondary metabolites, while trace elements are often necessary to uncover as yet uncultured microbial species [196]. A strategy using a diverse set of varying conditions in the isolation of soil bacteria, including nutrient-poor media, more than 30 days of incubation, cell protection from sources of exogenous peroxides, and the inclusion of humic acids and quorum-sensing signaling agents, has led to the isolation of a number of previously uncultured Acidobacteria and Verrucomicrobia [262].

The addition of components of the natural environment, such as a soil, a plant, or a marine source extract, may substantially enhance the isolation of as yet uncultured strains [206]. A diffusion chamber was designed to mimic natural environmental conditions allowing the growth communities of as yet uncultured wild bacteria. Such strains subsequently could be domesticated, even if they belong to unusual taxa. Frequently, a co-culture is necessary in order to achieve a considerable population growth [257]. The soil substrate membrane system (SSMS) efficiently allows the isolation and growth of previously uncultured bacteria in micro-colonies, especially when a diluted medium is used, allowing slow-growing strains to be cultured in stable Petri dish conditions [263]. A standard protocol has been reported for SSMS [264]. The iChip technology functions essentially as microbial traps while allowing single cell isolation in microwells. The use of this technology has provided for the isolation of an impressive number of new bacterial strains from both soil and seawater, belonging not only to new species, but also to new genera, families, orders, or of higher taxonomic taxa. Many of the isolated strains proved to be Actinomycetes belonging to rare genera. The growth of such strains was much more efficiently performed in gellan-gum based Petri dish media than in agar-based media [265]. Specific culturing requirements are very often required for the isolation of rare or not yet cultured microorganisms.

Several factors that may affect the initial stage of cultivation may be determinants in the success of maintaining healthy cultures under artificial conditions [249]. Extended incubation times improve the isolation and the culturability of as yet uncultured strains [196, 206, 249, 254]. The transition from a non-growing condition to a growing state is critical to achieve culture maintenance of strains and very often a slow process, but appears to be yet poorly known [254]. Long incubation times are required for the isolation of rare soil bacterial strains [256]. However, a recent assessment showed no significant increase in diversity richness of unusual bacteria recovered after long-term incubation up to 18 months, when compared to bacteria recovered from short-term incubations. These results indicated that success in the isolation of novel strains is consequential of the degree of cultivation effort rather than of the length of incubation [266].

Traditionally, solid-state fermentation and substrates have been used for a long time in Asian and African countries, but much less frequently elsewhere. In using solid-state fermentation, frequently the substrate constitutes the carbon source of growth, with very low water content. Both advantages and disadvantages in using solid-state fermentation (SSF) over submerged fermentation have been discussed in

detail, but one advantage in using crop-based solid-state fermentation is the much lower medium cost [267]. Different classes of antibiotics have been produced using SSF, such as penicillins, cyclosporin, cephamycin and tetracyclines. Limitations of SSF use are particularly related to the scale-up process [267]. A comparative study of paclitaxel (taxol) production by *Nigrospora* sp. in both SSF and liquid fermentation (LF) media showed the superiority of the former. Paclitaxel was produced more rapidly and in higher amounts in SSF than in LF, except when the medium concentration was higher [268]. Therefore, variation of the medium while screening for secondary metabolite production is very much worthwhile not only to generate chemical novelty, but also to improve yields [221, 269].

It has been observed that once optimal isolation and growth conditions are found, "yet uncultured" microbial strains are able to grow under usual development kinetics, also providing usual yields of biomass [249]. An example of a recent culture of unique bacterial strains is the bacteria of the phylum OP10, originally detected in geothermal springs such as the Obsidian Pool at the Yellowstone National Park. Although these thermophilic strains were first detected in 1998, only in 2011 has culturing been reported. It required a high natural microbial abundance at the collection sites, the use of diluted and low nutrient media, as well as extended incubation times to be observed. Moreover, gellan gum was used instead of agar as the substrate in plating [270].

Although complex to deal with, the culturing of mixed populations appears to have a remarkable influence on the isolation of as yet uncultured strains [249, 254]. The use of culturing mixed populations was essential to cultivate strains that are intrinsically dependent on each other. A culture of two dependent strains, of which one was assumed to be a "helper strain", allowed a subsequential adaptation of the "helper dependent strain" in pure culture. Such interactions have been demonstrated to be mediated chemically by peptides or siderophores, which are essential for the growth of the "helper dependent strain". Enrichment of the medium with a Fe(II) source has been sometimes sufficient to substitute for the helper production of siderophores. Such experiments have allowed for the isolation of a high number of as yet uncultured bacterial strains [250, 257]. Simultaneous cultivation of several strains appears to be an extension of two axenic strain co-cultivation as a strategy to trigger cryptic or not-active biosynthetic gene clusters. Although two-strain co-cultivation has been used successfully during the past few years for production of unusual secondary metabolites by both bacteria and fungi [271, 272], this remains largely underexplored possibly because the particularities involving optimal co-culturing conditions may vary substantially. Co-cultivation of distinct *Sorangium cellulosum* myxobacterial strains presented an average increase in the production of epothilones by a 2.45 factor. Changes in epothilone yield were affected by 50% in 33 of the 45 pairwise experiments, while some co-cultivation experiments resulted in a tenfold increase of epothilone production [273].

As essential as culturing of pure strains is, recently it has been suggested that "pure cultures" frequently considered as axenic may not exactly be so, since they may contain cryptic strain contaminants unless single-cell isolation procedures are employed [274]. Single-cell isolation techniques have been developed for the

isolation of previously uncultured microbes in order to better understand the physiology and function of such strains that may be useful for the discovery of new enzymes and products [255]. Such approaches usually require careful media elaboration, the search for specific microbial groups, the simulation of the natural environment for growth, in situ cultivation, and particular isolation tools. Dilution-to-extinction is now used commonly during the isolation of rare bacterial strains, but requires enrichment of the targeted strains to be isolated. The best-known example of using single-cell isolation of as yet uncultured strains was the isolation of the SAR11 strain *Pelagibacter ubique*. Once it was isolated, new members belonging to this taxon could be isolated and cultured [250]. Several strains of *Proteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Acidobacteria*, *Verrucomicrobia*, and *Gammaproteobacteria* have been isolated and cultured initially using extinction-to-dilution techniques approximately a decade ago [275]. Incubation of diluted samples along with extended incubation times in order to considerably increase the isolation and culture of previously unknown bacteria has also been reported for soil bacteria [276]. The first dilution-to-extinction culturing procedure was used along with the incubation of leaf fragments in order to assess unusual fungal endophytes. Both approaches presented excellent complementarity in species recovery from *Fagus sylvatica*. Fragment plating led to the isolation of 27 fungal taxa from 228 isolates (77% of total species), while dilution-to-extinction culturing yielded 19 recovered fungal taxa from 192 colonies (54% of total species), among which 8 were not detected in plating experiments. Additional improvements have been suggested aiming to ameliorate fungal endophyte diversity assessment [193]. The use of single-cell encapsulation is another approach that has been developed and used very successfully towards the isolation of rare, as yet uncultured bacterial strains [206].

Adhesion to solid surfaces has been used for the enrichment of previously uncultivated bacterial strains, starting from the formation of biofilms. Natural bacteria populations form dense and highly enriched biofilms on artificial polypropylene surfaces. Comparison with previous studies performed with the same natural samples using liquid media with identical composition showed no overlap at all of species grown in solid substrate and submerged cultures [277]. Solid-state substrates include not only each growth medium itself, but also a variety of supports, such as polymers. Different classes of fungal metabolites have been produced on solid-state substrates, using, for example, a moistened polyester-cellulose support. The results obtained showed higher production yields, a wider chemodiversity of the products obtained, and also the presence of high polarity medium contaminants to a lesser extent [278].

Automated high-throughput isolation and cultivation methods allow the recovery of thousands of strains in a relatively short time frame, of which several may be unusual and as yet unknown strains [206]. Miniaturization seems to be mandatory when a large number of environmental samples needs to be collected or culturing experiments are necessary to be performed. The use of nutritional arrays and microarrays for microbial strain isolation and secondary metabolite production is being evaluated currently, along with micromanipulation, flow cytometry, cell

sorting, and microfluidics [255, 256, 279]. Miniaturized systems allow high-throughput screening of strains and growth conditions under much less labor intense efforts, leading to reliable results much faster and enabling better conditions for microbial growth and secondary metabolite production to be established. As an example, fungal cultures have been evaluated using nutritional arrays for the production of antifungals. The results showed little cross-contamination among incubated wells. Chemical profiles of selected extracts collected from 96 wells showed a remarkable similarity with extracts obtained from traditional Erlenmeyer flask cultures. The smaller volume cultures yielded more chemically diverse antifungal extracts since a larger variety of media could be used [280]. The use of miniaturized systems is particularly suitable when a variety of growth conditions is to be explored towards finding suitable parameters for the production of new metabolites by a particular strain. This approach has been named "OSMAC" (one strain, many compounds), and, although it seems quite logical, only a decade ago it received a more detailed attention [100]. The use of the OSMAC approach is currently widely accepted and has led to the successful isolation of a number of unusual metabolites produced in culture by both fungal and bacterial strains [281–289]. The usefulness of the OSMAC approach is undeniable, and also has resulted in finding optimal conditions to perform biosynthesis investigations [290–292]. Experimental design has been used traditionally in industry for the optimization of enzymes and other primary metabolite production by microorganisms, but more recently it has also been employed for the optimal production of minor microbial secondary metabolites [196, 293–306].

Concerning growth technologies, although shaking has been used for over a century in culturing microorganisms, recently new tools have been developed in order to address issues related to high-throughput culturing, screening, and optimization. Large-scale shakers have also been improved for biotechnology culturing while optimizing several factors affecting production yields [250, 307–311].

To conclude this section, it is worth mentioning that it has been estimated that the discovery rate of new antibiotic producing bacteria will require more than 10^7 isolates in order to find the next class of antibiotics. Therefore, new cultivation approaches and high-throughput cultivation equipment are both needed to provide adequate conditions for the discovery of as yet uncultured strains in a reasonable time frame [250]. On the other hand, it has been stated that less culturable strains could not be used in biodiscovery programs [196]. However, if true, this assumption may be only temporary and dependent on the development of new technologies that translate the particularities of as yet uncultured microorganisms from the microbiology research laboratory into biotechnological and biodiscovery programs, in order to give access to the presently underestimated diversity of new microbial strains and their biosynthetic products [257]. Such new microbiology and molecular biology technologies will certainly enhance the capabilities to explore microbial diversity and biotechnology [206]. Currently, bioactive compound discovery research requires truly innovative approaches in order to not only achieve the isolation of uncultured strains, but also to develop reliable and robust conditions for culturing such microorganisms under artificial conditions [254], as well as the use of

technological advances that allow the deep and broad analysis of microbial secondary metabolism. A recent example of how technological advances may strongly impact the advances of microbiological sciences is the first report of in situ real-time analysis of bacterial cultures using fast and very sensitive mass spectrometric analysis [312–314]. Stewart optimistically looks forward to an outstanding development of microorganism cultivation science during the next 30 years, suggesting that its impact on health, ecology and science will surmount its previous development during the last 300 years [250].

11 Conclusions

For no apparently obvious reason, the biology and chemistry of endophytic Actinobacteria has been so far more or less neglected. This fact may be due to the as yet poorly known particular requirements of these endophytes, which are certainly difficult to cultivate and maintain as viable strains. Nevertheless, currently the biology of bacterial endophytes is under a deep scrutiny provided by "omics" technologies, and certainly should reveal unusual traits of plant-associated bacteria. Considering that each plant species potentially has tens to hundreds of endophytic bacteria and fungi, the future will reveal a whole new microbial world of the phytomicrobiota, surely comprising several new species. Bacterial endophytes have a particular metabolism resulting from long-term associations with plants. Consequentially, the discovery of novel biologically active chemical entities produced by endophytic bacteria will enlarge the chemical diversity of the microbial universe. Among these, endophytic Actinobacteria constitute the group of choice for the discovery of bioactive compounds, an assumption based on a strong historical background of the richness of actinobacterial secondary metabolism. This contribution shows that investigations during the last 20 years have not disappointed those searching for novel natural products produced by endophytic Actinobacteria, with there being strong evidence to the contrary. However, the opportunities evident certainly represent the very tip of this immense microbial iceberg.

Acknowledgments The authors thank Professor Heinz Falk (Johannes Kepler Universität Linz) for many suggestions that improved the manuscript. Financial support was provided by Fundação de Amparo à Pesquisa do Estado de São Paulo to RGSB (2013/50228-8), as well as a Ph.D. scholarship awarded to DIB (2016/21341-9). The Brazilian funding agency Conselho Nacional de Desenvolvimento Científico e Tecnológico is also gratefully acknowledged for postdoctoral fellowships awarded to AFM and GFS.

References

1. Walsh CT, Tang Y (2017) Natural product biosynthesis: chemical logic and enzymatic machinery. RSC, London

2. Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629 (and references therein)
3. Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5
4. Aigle B, Corre C (2012) Waking up *Streptomyces* secondary metabolism by constitutive expression of activators or genetic disruption of repressors. Methods Enzymol 517:343
5. Solecka J, Zajko J, Postek M, Rajnisz A (2012) Biologically active secondary metabolites from Actinomycetes. Cent Eur J Biol 7:373
6. Ul-Hassan A, Wellington EM (2009) Actinobacteria. In: Schaechter M (ed) Encyclopedia of microbiology, 3rd edn. Academic, New York
7. Seipke RF, Kaltenpoth M, Hutchings MI (2012) *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol Rev 36:862
8. Tiwari K, Gupta RK (2012) Rare Actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol 32:108
9. van der Meij A, Worsley SF, Hutchings MI, van Wezel GP (2017) Chemical ecology of antibiotic production by Actinomycetes. FEMS Microbiol Rev 41:392
10. Walsh C, Wencewicz TA (2016) Antibiotics: challenges, mechanisms, opportunities. ASM, Washington, DC
11. Ward AC, Bora N (2009) The Actinobacteria. In: Goldman E, Green LH (eds) Practical handbook of microbiology, 2nd edn. CRC Press/Taylor & Francis Group, Boca Raton, FL, p 375
12. Genilloud O (2017) Actinomycetes: still a source of novel antibiotics. Nat Prod Rep 34:1203
13. Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M (2015) Endophytic Actinobacteria of medicinal plants: diversity and bioactivity. Anton Leeuw Int J G 108:267
14. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H-P, Clément C, Ouhdouch Y, van Wezel GP (2016) Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80:1
15. Mohammadipanah F, Wink J (2016) Actinobacteria from arid and desert habitats: diversity and biological activity. Front Microbiol 6:1541
16. Singh M, Kumar A, Singh R, Pandey KD (2017) Endophytic bacteria: a new source of bioactive compounds. Biotech 7:315
17. Tiwari K, Gupta RK (2013) Diversity and isolation of rare Actinomycetes: an overview. Crit Rev Microbiol 39:256
18. Azman A-S, Othman I, Velu SS, Chan K-G, Lee L-H (2015) Mangrove rare Actinobacteria: taxonomy, natural compound, and discovery of bioactivity. Front Microbiol 6:856
19. Dinesh R, Srinivasan V, Sheeja TE, Anandaraj M, Srambikkal H (2017) Endophytic Actinobacteria: diversity, secondary metabolism, and mechanisms to unsilence biosynthetic gene clusters. Crit Rev Microbiol 43:546
20. Choi S-S, Kim H-J, Lee H-S, Kim P, Kim E-S (2015) Genome mining of rare Actinomycetes and cryptic pathway awakening. Process Biochem 50:1184
21. Matsumoto A, Takahashi Y (2017) Endophytic Actinomycetes: promising source of novel bioactive compounds. J Antibiot 70:514
22. Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS, Quinn RJ, Hentschel U (2015) Elicitation of secondary metabolism in Actinomycetes. Biotechnol Adv 33:798
23. Gao B, Gupta RS (2012) Phylogenetic framework and molecular signatures for the main clades of the phylum Actinobacteria. Microbiol Mol Biol Rev 76:66
24. Buchanan RE (1917) Studies in the nomenclature and classification of the bacteria. II. The primary subdivisions of the Schizomycetes. J Bacteriol 2:155
25. Nalini MS, Prakash HS (2017) Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett Appl Microbiol 64:261
26. Silambarasan S, kumar EP, Murugan T, Saravanan D, Balagurunathan R (2012) Antibacterial and antifungal activities of Actinobacteria isolated from Rathnagiri hills. J Appl Pharm Sci 2:99

27. Scherr N, Nguyen L (2009) *Mycobacterium* versus *Streptomyces*—we are different, we are the same. Curr Opin Microbiol 12:699
28. Subramani R, Aalbersberg W (2013) Culturable rare Actinomycetes: diversity, isolation and marine natural product discovery. Appl Microbiol Biotechnol 97:9291
29. Dhakal D, Pokhrel AR, Shrestha B, Sohng JK (2017) Marine rare Actinobacteria: isolation, characterization, and strategies for harnessing bioactive compounds. Front Microbiol 8:1106
30. Masand M, Jose PA, Menghani E, Jebakumar SR (2015) Continuing hunt for endophytic Actinomycetes as a source of novel biologically active metabolites. World J Microbiol Biotechnol 31:1863
31. Chagas FO, Pessoti RC, Caraballo-Rodríguez AM, Pupo MT (2018) Chemical signaling involved in plant–microbe interactions. Chem Soc Rev 47:1652
32. Viaene T, Langendries S, Beirinckx S, Maes M, Goormachtig S (2016) *Streptomyces* as a plant's best friend? FEMS Microbiol Ecol 92:fiw119
33. Loria R, Kers J, Joshi M (2006) Evolution of plant pathogenicity in *Streptomyces*. Annu Rev Phytopathol 44:469
34. Duval I, Brochu V, Simard M, Beaulieu C, Beaudoin N (2005) Thaxtomin A induces programmed cell death in *Arabidopsis thaliana* suspension suspension-cultured cells. Planta 222:820
35. Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293
36. Yague P, López-García MT, Rioseras B, Sánchez J, Manteca A (2013) Pre-sporulation stages of *Streptomyces* differentiation: state-of-the-art and future perspectives. FEMS Microbiol Lett 342:79
37. Claessen D, Rozen DE, Kuipers OP, Sogaard-Andersen L, van Wezel GP (2014) Bacterial solutions to multicellularity: a tale of biofilms, filaments, and fruiting bodies. Nat Rev Microbiol 12:115
38. Jones SE, Ho L, Rees CA, Hill JE, Nodwell JR, Elliot MA (2017) *Streptomyces* exploration is triggered by fungal interactions and volatile signals. Elife 6:e21738
39. Denser CR, Guimaraes LM, Candida M (2002) Applications of image analysis in the characterization of *Streptomyces olindensis* in submerged culture. Braz J Microbiol 33:17
40. Manteca A, Alvarez R, Salazar N, Yagüe P, Sanchez J (2008) Mycelium differentiation and antibiotic production in submerged cultures of *Streptomyces coelicolor*. Appl Environ Microbiol 74:3877
41. van Dissel D, Claessen D, van Wezel GP (2014) Morphogenesis of *Streptomyces* in submerged cultures. Adv Appl Microbiol 89:1
42. Manteca A, Sánchez J (2009) *Streptomyces* development in colonies and soils. Appl Environ Microbiol 75:2920
43. Bibb MJ (2005) Regulation of secondary metabolism in Streptomycetes. Curr Opin Microbiol 8:208
44. vanWezel GP, McDowall KJ (2011) The regulation of the secondary metabolism of *Streptomyces*: new links and experimental advances. Nat Prod Rep 28:1311
45. Willey JM, Gaskell AA (2010) Morphogenetic signaling molecules of the Streptomycetes. Chem Rev 111:174
46. Sidda JD, Corre C (2012) Gamma-butyrolactone and furan signaling systems in *Streptomyces*. Methods Enzymol 517:71
47. Goeke D, Kaspar D, Stoeckle C, Grubmüller S, Berens C, Klotzsche M, Hillen W (2012) Short peptides act as inducers, anti-inducers and corepressors of Tet repressor. J Mol Biol 416:33
48. Corre C, Song L, O'Rourke S, Chater KF, Challis GL (2008) 2-Alkyl-4-hydroxymethylfuran-3-carboxylic acids, antibiotic production inducers discovered by *Streptomyces coelicolor* genome mining. Proc Natl Acad Sci USA 105:17510

49. Kitani S, Miyamoto KT, Takamatsu S, Herawati E, Iguchi H, Nishitomi K, Uchida M, Nagamitsu T, Omura S, Ikeda H (2011) Avenolide, a *Streptomyces* hormone controlling antibiotic production in *Streptomyces avermitilis*. Proc Natl Acad Sci USA 108:16410
50. Wang L, Tian X, Wang J, Yang H, Fan K, Xu G, Yang K, Tan H (2009) Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator. Proc Natl Acad Sci USA 106:8617
51. Deng W, Li C, Xie J (2013) The underlying mechanism of bacterial TetR/AcrR family transcriptional repressors. Cell Signal 25:1608
52. Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA, van Wezel GP (2008) Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*. EMBO Rep 9:670
53. Swiatek-Polatynska MA, Tenconi E, Rigali S, van Wezel GP (2012) Functional analysis of the *N*-acetylglucosamine metabolic genes of *Streptomyces coelicolor* and role in the control of development and antibiotic production. J Bacteriol 194:113
54. Swiatek-Polatynska MA, Bucca G, Laing E, Gubbens J, Titgemeyer F, Smith CP, Rigali S, van Wezel GP (2015) Genome-wide analysis of in vivo binding of the master regulator DasR in *Streptomyces coelicolor* identifies novel non-canonical targets. PLoS One 10:e0122479
55. Battesti A, Bouveret E (2006) Acyl carrier protein/SpoT interaction, the switch linking SpoT-dependent stress response to fatty acid metabolism. Mol Microbiol 62:1048
56. Hesketh A, Chen WJ, Ryding J, Chang S, Bibb M (2007) The global role of ppGpp synthesis in morphological differentiation and antibiotic production in *Streptomyces coelicolor* A3(2). Genome Biol 8:R161
57. Potrykus K, Cashel M (2008) (p)ppGpp: still magical? Annu Rev Microbiol 62:35
58. Gomez-Escribano JP, Martin JF, Hesketh A, Bibb MJ, Liras P (2008) *Streptomyces clavuligerus* relA-null mutants overproduce clavulanic acid and cephamycin C: negative regulation of secondary metabolism by (p)ppGpp. Microbiology 154:744
59. Sanchez S, Chavez A, Forero A, Garcia-Huante Y, Romero A, Sanchez M, Rocha D, Sanchez B, Avalos M, Guzman-Trampe S, Rodriguez-Sanoja R, Langley E, Ruiz B (2010) Carbon source regulation of antibiotic production. J Antibiot 63:442
60. Bruckner R, Titgemeyer F (2002) Carbon catabolite repression in bacteria: choice of the carbon source and autoregulatory limitation of sugar utilization. FEMS Microbiol Lett 209:141
61. Titgemeyer F, Hillen W (2002) Global control of sugar metabolism: a Gram-positive solution. Anton Leeuw Int J G 82:59
62. Susstrunk U, Pidoux J, Taubert S, Ullmann A, Thompson CJ (1998) Pleiotropic effects of cAMP on germination, antibiotic biosynthesis, and morphological development in *Streptomyces coelicolor*. Mol Microbiol 30:33
63. Li M, Kim T, Kwon H, Suh J (2008) Effects of extracellular ATP on the physiology of *Streptomyces coelicolor* A3(2). FEMS Microbiol Lett 286:2
64. Rajkarnikar A, Kwon HJ, Suh JW (2007) Role of adenosine kinase in the control of *Streptomyces* differentiations: loss of adenosine kinase suppresses sporulation and actinorhodin biosynthesis while inducing hyperproduction of undecylprodigiosin in *Streptomyces lividans*. Biochem Biophys Res Commun 363:322
65. Abrudan MI, Smakman F, Grimbergen AJ, Westhoff S, Miller EL, van Wezel GP, Rozen DE (2015) Socially mediated induction and suppression of antibiosis during bacterial coexistence. Proc Natl Acad Sci USA 112:11054
66. Chagas FO, Pupo MT (2018) Chemical interaction of endophytic fungi and actinobacteria from *Lychnophora ericoides* in co-cultures. Microbiol Res 212–213:10
67. Chagas FO, Caraballo-Rodriguez AM, Dorrestein PC, Pupo MT (2017) Expanding the chemical repertoire of the endophyte *Streptomyces albospinus* RLe7 reveals amphotericin B as an inducer of a fungal phenotype. J Nat Prod 80:1302
68. Chagas FO, Dias LG, Pupo MT (2013) A mixed culture of endophytic fungi increases production of antifungal polyketides. J Chem Ecol 39:1335

69. McCutcheon JP, Moran NA (2011) Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol 10:13
70. Lo W-S, Huang Y-Y, Kuo C-H (2016) Winding paths to simplicity: genome evolution in facultative insect symbionts. FEMS Microbiol Rev 40:855
71. Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. J Nat Prod 71:159
72. Heinig U, Scholz S, Jennewein S (2013) Getting to the bottom of taxol biosynthesis by fungi. Fungal Divers 60:161
73. Stierle A, Strobel GA, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214
74. Stierle A, Strobel G, Stierle D, Grothaus P, Bignami G (1995) The search for a taxol-producing microorganism among the endophytic fungi of the Pacific yew, *Taxus brevifolia*. J Nat Prod 58:1315
75. Puri SC, Verma V, Amna T, Qazi GN, Spiteller M (2005) An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. J Nat Prod 68:1717
76. Puri SC, Nazir A, Chawla R, Arora R, Riyaz-ul-Hasan S, Amma T, Ahmed BV, Verma S, Singh R, Sagar A, Sharma R, Kumar RK, Sharma GN, Qazi G (2006) The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol 122:494
77. Eyberger AL, Dondapati R, Porter JR (2006) Endophyte fungal isolates from *Podophyllum peltatum* produce podophyllotoxin. J Nat Prod 69:1121
78. Kour A, Shawl AS, Rehman S, Qazi PH, Sudan P, Khajuria RK, Sultan P, Verma V (2008) Isolation and identification of an endophytic strain of *Fusarium oxysporum* producing podophyllotoxin from *Juniperus recursa*. World J Microbiol Biotechnol 24:1115
79. Cragg GM, Grothaus PG, Newman DJ (2012) Natural products in drug discovery: recent advances. In: Cechinel-Filho V (ed) Plant bioactives and drug discovery: principles, practice, and perspectives. Wiley, New York, p 1
80. Cassady JM, Chan KK, Floss HG, Leistner E (2004) Recent developments in the maytansinoid antitumor agents. Chem Pharm Bull 52:1
81. Seipke RF (2015) Strain-level diversity of secondary metabolism in *Streptomyces albus*. PLoS One 10:e0116457
82. Amos GCA, Awakawa T, Tuttle RN, Letzel AC, Kim MC, Kudo Y, Fenical W, Moore BS, Jensen PR (2017) Comparative transcriptomics as a guide to natural product discovery and biosynthetic gene cluster functionality. Proc Natl Acad Sci USA 114:E11121
83. Conti R, Chagas FO, Caraballo-Rodriguez AM, Melo WG, do Nascimento AM, Cavalcanti BC, de Moraes MO, Pessoa C, Costa-Lotufo LV, Krogh R, Andricopulo AD, Lopes NP, Pupo MT (2016) Endophytic Actinobacteria from the Brazilian medicinal plant *Lychnophora ericoides* Mart. and the biological potential of their secondary metabolites. Chem Biodivers 13:727
84. Kaewkla O, Franco CM (2013) Rational approaches to improving the isolation of endophytic Actinobacteria from Australian native trees. Microb Ecol 65:384
85. Zin NM, Loi CS, Sarmin NM, Rosli AN (2010) Cultivation-dependent characterization of endophytic Actinomycetes. Res J Microbiol 5:717
86. Passari AK, Mishra VK, Saikia R, Gupta VK, Singh BP (2015) Isolation, abundance and phylogenetic affiliation of endophytic Actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. Front Microbiol 6:273
87. Qin S, Chen HH, Zhao GZ, Li J, Zhu WY, Xu LH, Jiang JH, Li WJ (2012) Abundant and diverse endophytic Actinobacteria associated with medicinal plant *Maytenus austroyunnanensis* in Xishuangbanna tropical rainforest revealed by culture-dependent and culture-independent methods. Environ Microbiol Rep 4:522
88. Dos-Santos CM, de Souza DG, Balsanelli E, Cruz LM, de Souza EM, Baldani JI, Schwab S (2017) A culture-independent approach to enrich endophytic bacterial cells from sugarcane stems for community characterization. Microb Ecol 74:453

89. Nimnoi P, Pongsilp N, Lumyong S (2010) Genetic diversity and community of endophytic Actinomycetes within the roots of *Aquilaria crassna* Pierre ex Lec assessed by Actinomycetes-specific PCR and PCR-DGGE of 16S rRNA gene. Biochem Syst Ecol 38:595
90. Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30
91. Hug J, Bader C, Remškar M, Cirnski K, Müller R (2018) Concepts and methods to access novel antibiotics from Actinomycetes. Antibiotics 7:44
92. Govindasamy V, Franco CMM, Gupta VVSR (2013) Endophytic Actinobacteria: diversity and ecology. In: Verma VC, Gange AC (eds) Advances in endophytic research. Springer, Berlin, p 27
93. Coombs JT, Franco CMM (2003) Isolation and identification of Actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603
94. Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, Xu LH, Li WJ (2009) Isolation, diversity, and antimicrobial activity of rare Actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176
95. Li J, Zhao GZ, Huang HY, Qin S, Zhu WY, Li WJ (2009) *Kineosporia mesophila* sp. nov., isolated from the surface sterilized stem of *Tripterygium wilfordii*. Int J Syst Evol Microbiol 59:3150
96. Zhu F, Qin C, Tao L, Liu X, Shi Z, Ma X, Jia J, Tan Y, Cui C, Lin J, Tan C, Jiang Y, Chen Y (2011) Clustered patterns of species origins of nature-derived drugs and clues for future bioprospecting. Proc Natl Acad Sci USA 108:12943
97. Qin S, Xing K, Jiang J-H, Xu L-H, Li W-J (2011) Biodiversity, bioactive natural products, and biotechnological potential of plant-associated endophytic Actinobacteria. Appl Microbiol Biotechnol 89:457
98. Okazaki T (2003) Studies on Actinomycetes isolated from plant leaves. In: Kurtböke DI (ed) Selective isolation of rare Actinomycetes. Queensland Complete Printing Service, Nambour, p 102
99. Bertrand S, Bohni N, Schnee S, Schumpp O, Gindro K, Wolfender J-L (2014) Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery. Biotechnol Adv 32:1180
100. Bode HB, Bethe B, Hofs R, Zeeck A (2002) Big effects from small changes: possible ways to explore Nature's chemical diversity. Chembiochem 3:619
101. Gunatilaka AAL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69:509
102. Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23:753
103. Kusari S, Spiteller M (2011) Are we ready for industrial production of bioactive plant secondary metabolites utilizing endophytes? Nat Prod Rep 28:1203
104. Tuntiwachwuttikul P, Taechowisan T, Wanbanjob A, Thadaniti S, Taylor WC (2008) Lansai A-D, secondary metabolites from *Streptomyces* sp. SUC1. Tetrahedron 64:7583
105. Wang H, Reisman SE (2014) Enantioselective total synthesis of (–)-lansai B and (+)-nocardioazines A and B. Angew Chem Int Ed 53:6206
106. Inahashi Y, Iwatsuki M, Ishiyama A, Namatame M, Nishihara-Tsukashima A, Matsumoto A, Hirose T, Sunazuka T, Yamada H, Otoguro K, Takahashi Y, Omura S, Shiomi K (2011) Spoxazomicins A–C, novel antitrypanosomal alkaloids produced by an endophytic actinomycete, *Streptosporangium oxazolinicum* K07-0460(T). J Antibiot 64:303
107. Wang P, Kong F, Wei J, Wang Y, Wang W, Hong K, Zhu W (2014) Alkaloids from the mangrove-derived actinomycete *Jishengella endophytica* 161111. Mar Drugs 12:477
108. Abdalla MA (2016) Three new cyclotetrapeptides isolated from *Streptomyces* sp. 447. Nat Prod Res 31:1014
109. Sasaki T, Igarashi Y, Saito N, Furumai T (2001) TPU-0031-A and B, new antibiotics of the novobiocin group produced by *Streptomyces* sp. TP-A0556. J Antibiot 54:441

110. Igarashi Y, Ogawa M, Sato Y, Saito N, Yoshida R, Kunoh H, Onaka H, Furumai T (2000) Fistupyrone, a novel inhibitor of the infection of Chinese cabbage by *Alternaria brassicicola*, from *Streptomyces* sp. TP-A0569. J Antibiot 53:1117
111. Aremu EA, Furumai T, Igarashi Y, Sato Y, Akamatsu H, Kodama M, Otani H (2003) Specific inhibition of spore germination of *Alternaria brassicicola* by fistupyrone from *Streptomyces* sp. TP-A0569. J Gen Plant Pathol 69:211
112. McGlackena GP, Fairlamb IJS (2005) 2-Pyrone natural products and mimetics: isolation, characterisation and biological activity. Nat Prod Rep 22:369
113. Zhou W, Zhuang Y, Bai Y, Bi H, Liu T, Ma Y (2016) Biosynthesis of phlorisovalerophenone and 4-hydroxy-6-isobutyl-2-pyrone in *Escherichia coli* from glucose. Microb Cell Fact 15:149
114. Sasaki T, Igarashi Y, Saito N, Furumai T (2001) Cedarmycins A and B, new antimicrobial antibiotics from *Streptomyces* sp. TP-A0456. J Antibiot 54:567
115. Cui Q, Wang J, Yang H-S, Xie L-G, Xu X-H (2010) First synthesis of cedarmycins A and B and their analogues. Chin J Org Chem 30:1705
116. Lloyd MG, D'Acunto M, Taylor RJK, Unsworth WP (2014) α-Alkylidene-γ-butyrolactone synthesis via one-pot C–H insertion/olefination: substrate scope and the total synthesis of (±)-cedarmycins A and B. Tetrahedron 71:7107
117. Igarashi Y, Iida T, Yoshida R, Furumai T (2002) Pteridic acids A and B, novel plant growth promoters with auxin-like activity from *Streptomyces hygroscopicus* TP-A0451. J Antibiot 55:764
118. Igarashi Y, Miura S-S, Fujita T, Furumai T (2006) Pterocidin, a cytotoxic compound from the endophytic *Streptomyces hygroscopicus*. J Antibiot 59:193
119. Nakahata T, Kuwahara S (2005) Enantioselective total synthesis of pteridic acid A. Chem Commun:1028
120. Nakahata T, Fujimura S, Kuwahara S (2006) Total synthesis of pteridic acids A and B. Chem A Eur J 12:4584
121. Paterson I, Anderson EA, Findlay AD, Knappy CS (2008) Total synthesis of pteridic acids A and B. Tetrahedron 64:4768
122. Dias LC, Salles AG Jr (2009) Total synthesis of pteridic acids A and B. J Org Chem 74:5584
123. Yadav JS, Rajender V, Rao YG (2010) Total synthesis of pteridic acid A. Org Lett 12:348
124. Furumai T, Yamakawa T, Yoshida R, Igarashi Y (2003) Clethramycin, a new inhibitor of pollen tube growth with antifungal activity from *Streptomyces hygroscopicus* TP-A0623. I. Screening, taxonomy, fermentation, isolation, and biological properties. J Antibiot 56:700
125. Igarashi Y, Iwashita T, Fujita T, Naoki H, Yamakawa T, Yoshida R, Furumai T (2003) Clethramycin, a new inhibitor of pollen tube growth with antifungal activity from *Streptomyces hygroscopicus* TP-A0623. II. Physico-chemical properties and structure determination. J Antibiot 56:705
126. Sun F, Xu S, Jiang F, Liu W (2018) Genomic-driven discovery of an amidinohydrolase involved in the biosynthesis of mediomycin A. Appl Microbiol Biotechnol 102:2225
127. Hong H, Fill T, Leadlay PF (2013) A common origin for guanidinobutanoate starter units in antifungal natural products. Angew Chem Int Ed 52:13096
128. Berlinck RGS, Romminger S (2016) The chemistry and biology of guanidine natural products. Nat Prod Rep 33:456
129. Lu C, Shen Y (2003) A new macrolide antibiotic with antitumor activity produced by *Streptomyces* sp. CS, a commensal microbe of *Maytenus hookeri*. J Antibiot 56:415
130. Hensens OD, Monaghan RL, Huang L, Albers-Schonberg G (1983) Structure of the sodium and potassium ion activated adenosine triphosphatase inhibitor L-681,110. J Am Chem Soc 105:3672
131. O'Shea MG, Rickards RW, Rothschild JM, Lacey E (1997) Absolute configurations of macrolide antibiotics of the bafilomycin and leucanicidin groups. J Antibiot 50:1073
132. Lu C, Shen Y (2004) Two new macrolides produced by *Streptomyces* sp. CS. J Antibiot 57:597

133. Li J, Lu C, Shen Y (2010) Macrolides of the bafilomycin family produced by *Streptomyces* sp. CS. J Antibiot 63:595
134. Yu Z, Zhao LX, Jiang CL, Duan Y, Wong L, Carver KC, Schuler LA, Shen B (2011) Bafilomycins produced by an endophytic actinomycete *Streptomyces* sp. YIM56209. J Antibiot 64:159
135. Supong K, Thawai C, Choowong W, Kittiwongwattana C, Thanaboripat D, Laosinwattana C, Koohakan P, Parinthawong N, Pittayakhajonwut P (2016) Antimicrobial compounds from endophytic *Streptomyces* sp. BCC72023 isolated from rice (*Oryza sativa* L.). Res Microbiol 167:290
136. Barth R, Mulzer J (2008) Two-directional total synthesis of efomycine M and formal total synthesis of elaiolide. Tetrahedron 64:4718
137. Kim N, Shin JC, Kim W, Hwang BY, Kim BS, Hong Y-S, Lee D (2006) Cytotoxic 6-alkylsalicylic acids from the endophytic *Streptomyces laceyi*. J Antibiot 59:797
138. Wang F, Xu M, Li Q, Sattler I, Lin W (2010) P-aminoacetophenonic acids produced by a mangrove endophyte *Streptomyces* sp. (strain HK10552). Molecules 15:2782
139. Yang R, Yang J, Wang L, Huang J-P, Xiong Z, Luo J, Yu M, Yan Y, Huang S-X (2017) Lorneic acid analogues from an endophytic actinomycete. J Nat Prod 80:2615
140. Zhou T, Komaki H, Ichikawa N, Hosoyama A, Sato S, Igarashi Y (2015) Biosynthesis of akaeolide and lorneic acids and annotation of type I polyketide synthase gene clusters in the genome of *Streptomyces* sp. NPS554. Mar Drugs 13:581
141. Yan Y, Ma Y-T, Yang J, Horsman GP, Luo D, Ji X, Huang S-X (2016) Tropolone ring construction in the biosynthesis of rubrolone B, a cationic tropolone alkaloid from endophytic *Streptomyces*. Org Lett 8:1254
142. Davison J, al Fahad A, Cai M, Song Z, Yehia SY, Lazarus CM, Bailey AM, Simpson TJ, Cox RJ (2012) Genetic, molecular, and biochemical basis of fungal tropolone biosynthesis. Proc Natl Acad Sci USA 109:7642
143. Huang S-X, Yu Z, Robert F, Zhao L-X, Jiang Y, Duan Y, Pelletier J, Shen B (2011) Cycloheximide and congeners as inhibitors of eukaryotic protein synthesis from endophytic Actinomycetes *Streptomyces* spp. YIM56132 and YIM56141. J Antibiot 64:163
144. Igarashi Y, Ogura H, Furihata K, Oku N, Indananda C, Thamchaipenet A (2011) Maklamicin, an antibacterial polyketide from an endophytic *Micromonospora* sp. J Nat Prod 74:670
145. Daduang R, Kitani S, Hashimoto J, Thamchaipenet A, Igarashi Y, Shin-ya K, Ikeda H, Nihira T (2015) Characterization of the biosynthetic gene cluster for maklamicin, a spirotetronate-class antibiotic of the endophytic *Micromonospora* sp. NBRC 110955. Microbiol Res 180:30
146. Liu H, Chen Z, Zhu G, Wang L, Du Y, Wang Y, Zhu W (2017) Phenolic polyketides from the marine alga-derived *Streptomyces* sp. OUCMDZ-3434. Tetrahedron 73:5451
147. Khieu T-N, Liu M-J, Nimaichand S, Quach N-T, Chu-Ky S, Phi Q-T, Vu T-T, Nguyen T-D, Xiong Z, Prabhu DM, Li W-J (2015) Characterization and evaluation of antimicrobial and cytotoxic effects of *Streptomyces* sp. HUST012 isolated from medicinal plant *Dracaena cochinchinensis* Lour. Front Microbiol 6:574
148. Indananda C, Igarashi Y, Ikeda M, Oikawa T, Thamchaipenet A (2013) Linfuranone A, a new polyketide from plant-derived *Microbispora* sp. GMKU 363. J Antibiot 66:675
149. Akiyama H, Indananda C, Thamchaipenet A, Motojima A, Oikawa T, Komaki H, Hosoyama A, Kimura A, Oku N, Igarashi Y (2018) Linfuranones B and C, furanone-containing polyketides from a plant-associated *Sphaerimonospora mesophila*. J Nat Prod 81:1561
150. Li J, Lu C-H, Shen Y-M (2008) Novel polyketides isolated from *Streptomyces* sp. Helv Chim Acta 91:741
151. Li Y, Wu Y, Wang H, Huang Y, Lu C, Shen Y (2013) Two new germicidins from the endophytic *Streptomyces* sp. A00122 of *Camptotheca acuminata*. Rec Nat Prod 7:45
152. Aoki Y, Matsumoto D, Kawaide H, Natsume M (2011) Physiological role of germicidins in spore germination and hyphal elongation in *Streptomyces coelicolor* A3(2). J Antibiot 64:607

153. Song L, Barona-Gomez F, Corre C, Xiang L, Udwary DW, Austin MB, Noel JP, Moore BS, Challis GL (2006) Type III polyketide synthase β-ketoacyl-ACP starter unit and ethylmalonyl-CoA extender unit selectivity discovered by *Streptomyces coelicolor* genome mining. J Am Chem Soc 128:14754
154. Chemler JA, Buchholz TJ, Geders TW, Akey DL, Rath CM, Chlipala GE, Smith JL, Sherman DH (2012) Biochemical and structural characterization of germicidin synthase: analysis of a type III polyketide synthase that employs Acyl-ACP as a starter unit donor. J Am Chem Soc 134:7359
155. Inahashi Y, Iwatsuki M, Ishiyama A, Matsumoto A, Hirose T, Oshita J, Sunazuka T, Panbangred W, Takahashi Y, Kaiser M, Otoguro K, Ōmura S (2015) Actinoallolides A–E, new anti-trypanosomal macrolides, produced by an endophytic actinomycete, *Actinoallomurus fulvus* MK10-036. Org Lett 17:864
156. Inahashi Y, Shiraishi T, Také A, Matsumoto A, Takahashi Y, Ōmura S, Kuzuyama T, Nakashima T (2018) Identification and heterologous expression of the actinoallolide biosynthetic gene cluster. J Antibiot 71:749
157. Tsukamoto M, Hirayama M, Nakajima S, Okabe T, Ojiri K, Suda H (1997) Manufacture of antitumor BE-41956 derivatives by fermentation of *Streptomyces*. Jpn Kokai Tokkyo Koho, JP 09241257
158. Naruse N, Goto M, Watanabe Y, Terasawa T, Dobashi K (1998) K1115 A, a new anthraquinone derivative that inhibits the binding of activator protein-1 (AP-1) to its recognition sites. I. Biological activities. J Antibiot 51:545
159. Bieber B, Nüske J, Ritzau M, Gräfe U (1998) Alnumycin a new naphthoquinone antibiotic produced by an endophytic *Streptomyces* sp. J Antibiot 51:381
160. Oja T, Palmu K, Lehmussola H, Leppäranta O, Hännikäinen K, Niemi J, Mäntsälä P, Metsä-Ketelä M (2008) Characterization of the alnumycin gene cluster reveals unusual gene products for pyran ring formation and dioxan biosynthesis. Chem Biol 15:1046
161. Metsä-Ketelä M, Oja T, Taguchi T, Okamoto S, Ichinose K (2013) Biosynthesis of pyranonaphthoquinone polyketides reveals diverse strategies for enzymatic carbon-carbon bond formation. Curr Opin Chem Biol 17:562
162. Taguchi T, Yabe M, Odaki H, Shinozaki M, Metsä-Ketelä M, Arai T, Okamoto S, Ichinose K (2013) Biosynthetic conclusions from the functional dissection of oxygenases for biosynthesis of actinorhodin and related *Streptomyces* antibiotics. Chem Biol 20:510
163. Oja T, Niiranen L, Sandalova T, Klika KD, Niemi J, Mäntsälä P, Schneider G, Metsä-Ketelä M (2012) Structural basis for C-ribosylation in the alnumycin A biosynthetic pathway. Proc Natl Acad Sci USA 110:1291
164. Oja T, Klika KD, Appassamy L, Sinkkonen J, Mäntsälä P, Niemi J, Metsä-Ketelä M (2012) Biosynthetic pathway toward carbohydrate-like moieties of alnumycins contains unusual steps for C-C bond formation and cleavage. Proc Natl Acad Sci USA 109:6024
165. Tatsuta K, Tokishita S, Fukuda T, Kano T, Komiya T, Hosokawa S (2011) The first total synthesis and structural determination of antibiotics K1115 B1s (alnumycins). Tetrahedron Lett 52:983
166. Igarashi Y, Trujillo ME, Martínez-Molina E, Yanase S, Miyanaga S, Obata T, Sakuraic H, Saiki I, Fujita T, Furumai T (2007) Antitumor anthraquinones from an endophytic actinomycete *Micromonospora lupini* sp. nov. Bioorg Med Chem Lett 17:3702
167. Igarashi Y, Yanase S, Sugimoto K, Enomoto M, Miyanaga S, Trujillo ME, Saiki I, Kuwahara S (2011) Lupinacidin C, an inhibitor of tumor cell invasion from *Micromonospora lupini*. J Nat Prod 74:862
168. Sugimoto K, Enomoto M, Kuwahara S (2010) Synthesis of lupinacidins A and B via sequential cycloaddition–double elimination. Tetrahedron Lett 51:4570
169. Lu C, Shen Y (2007) A novel ansamycin, naphthomycin K from *Streptomyces* sp. J Antibiot 60:649
170. Yang Y-H, Fu X-L, Li L-Q, Zeng Y, Li C-Y, He Y-N, Zhao P-J (2012) Naphthomycins L–N, ansamycin antibiotics from *Streptomyces* sp. CS. J Nat Prod 75:1409

171. Liu M, Abdel-Mageed WM, Ren B, He W, Huang P, Li X, Bolla K, Guo H, Chen C, Song F, Dai H, Quinn RJ, Grkovic T, Zhang X, Liu X, Zhang L (2014) Endophytic *Streptomyces* sp. Y3111 from traditional Chinese medicine produced antitubercular pluramycins. Appl Microbiol Biotechnol 98:1077
172. Jones KD, Rixson JE, Skelton BW, Gericke KM, Stewart SG (2015) The total synthesis of heraclemycin B through β-ketosulfoxide and aldehyde annulation. Asian J Org Chem 4:936
173. Li W, Yang X, Yang Y, Zhao L, Xu L, Ding Z (2015) A new anthracycline from endophytic *Streptomyces* sp. YIM66403. J Antibiot 68:216
174. Ding L, Maier A, Fiebig H-H, Lin W-H, Peschel G, Hertweck C (2012) Kandenols A–E, eudesmenes from an endophytic *Streptomyces* sp. of the mangrove tree *Kandelia candel*. J Nat Prod 75:2223
175. Ding L, Maier A, Fiebig H-H, Lin W-H, Hertweck C (2011) A family of multicyclic indolosesquiterpenes from a bacterial endophyte. Org Biomol Chem 9:4029
176. Li H, Zhang Q, Li S, Zhu Y, Zhang G, Zhang H, Tian X, Zhang S, Ju J, Zhang C (2012) Identification and characterization of xiamycin A and oxiamycin gene cluster reveals an oxidative cyclization strategy tailoring indolosesquiterpene biosynthesis (2012). J Am Chem Soc 134:8996
177. Kugel S, Baunach M, Baer P, Ishida-Ito M, Sundaram S, Xu Z, Groll M, Hertweck C (2017) Cryptic indole hydroxylation by a non-canonical terpenoid cyclase parallels bacterial xenobiotic detoxification. Nat Commun 8:15804
178. Sun Y, Chen P, Zhang D, Baunach M, Hertweck C, Li A (2014) Bioinspired total synthesis of sespenine. Angew Chem Int Ed 126:9158
179. Meng Z, Yu H, Li L, Tao W, Chen H, Wan M, Yang P, Edmonds DJ, Zhong J, Li A (2015) Total synthesis and antiviral activity of indolosesquiterpenoids from the xiamycin and oridamycin families. Nat Commun 4:6096
180. Xiong X, Zhang D, Li J, Sun Y, Zhou S, Yang M, Shao H, Li A (2015) Synthesis of indole terpenoid mimics through a functionality tolerated $Eu(fod)_3$-catalyzed conjugate addition. Chem Asian J 10:869
181. Sun Y, Meng Z, Chen P, Zhang D, Baunach M, Hertweck C Li A (2016) A concise total synthesis of sespenine, a structurally unusual indole terpenoid from *Streptomyces*. Org Chem Front 3:368
182. Pullen C, Schmitz P, Meurer K, Bamberg DD, Lohmann S, França SC, Groth I, Schlegel B, Möllmann U, Gollmick F, Gräfe U, Leistner E (2002) New and bioactive compounds from *Streptomyces* strains residing in the wood of Celastraceae. Planta 216:162
183. Kikuchi H, Sekiya M, Katou Y, Ueda K, Kabeya T, Kurata S, Oshima Y (2009) Revised structure and synthesis of celastramycin A, a potent innate immune suppressor. Org Lett 11:1693
184. Nakashima T, Okuyama R, Kamiya Y, Matsumoto A, Iwatsuki M, Inahashi Y, Yamaji K, Takahashi Y, Ōmura S (2013) Trehangelins A, B and C, novel photo-oxidative hemolysis inhibitors produced by an endophytic actinomycete, *Polymorphospora rubra* K07-0510. J Antibiot 66:311
185. Inahashi Y, Shiraishi T, Kaia P, Takahashi Y, Omura S, Kuzuyama T, Nakashima T (2016) Biosynthesis of trehagelin in *Polymorphospora rubra* K07-0510: identification of metabolic pathway to angelyl-CoA. Chembiochem 17:1442
186. Zhang J, Wang J-D, Liu C-X, Yuan J-H, Wang X-J, Xiang W-S (2014) A new prenylated indole derivative from endophytic Actinobacteria *Streptomyces* sp. neau-D50. Nat Prod Res 28:431
187. Boonsnongcheep P, Nakashima T, Takahashi Y, Prathanturarug S (2017) Diversity of endophytic Actinomycetes isolated from roots and root nodules of *Pueraria candollei* Grah. ex Benth. and the analyses of their secondary metabolites. Chiang Mai J Sci 44:1
188. Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314

189. Kusari S, Pandey SP, Spiteller M (2013) Untapped mutualistic paradigms linking host plant and endophytic fungal production of similar bioactive secondary metabolites. Phytochemistry 91:81
190. Borges WS, Borges KB, Bonato PS, Said S, Pupo MT (2009) Endophytic fungi: natural products, enzymes and biotransformation reactions. Curr Org Chem 13:1137
191. Staniek A, Woerdenbag HJ, Kayser O (2008) Endophytes: exploiting biodiversity for the improvement of natural product-based drug discovery. J Plant Interact 3:75
192. Kusari S, Hertweck C, Spiteller M (2012) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19:792
193. Unterseher M, Schnittler M (2009) Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (*Fagus sylvatica* L.) – Different cultivation techniques influence fungal biodiversity assessment. Mycol Res 113:645
194. Unterseher M (2011) Diversity of fungal endophytes in temperate forest trees. In: Pirttilä AM, Frank AC (eds) Endophytes of forest trees: biology and applications. Springer, Dordrecht, p 31
195. Igarashi Y (2004) Screening of novel bioactive compounds from plant-associated Actinomycetes. Actinomycetologica 18:63
196. Janso JE, Carter GT (2010) Biosynthetic potential of phylogenetically unique endophytic Actinomycetes from tropical plants. Appl Environ Microbiol 76:4377
197. Knight V, Sanglier JJ, DiTullio D, Braccili S, Bonner P, Waters J, Hughes D, Zhang L (2003) Diversifying microbial natural products for drug discovery. Appl Microbiol Biotechnol 62:446
198. Baker DD, Chu M, Oza U, Rajgarhia V (2007) The value of natural products to future pharmaceutical discovery. Nat Prod Rep 24:1225
199. Carter GT (2011) Natural products and Pharma 2011: strategic changes spur new opportunities. Nat Prod Rep 28:1783
200. Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75:311
201. Cragg GM, Newman DJ (2009) Nature: a vital source of leads for anticancer drug development. Phytochem Res 8:313
202. Bérdy J (2012) Thoughts and facts about antibiotics: where we are now and where we are heading. J Antibiot 65:385
203. Singh BK, Macdonald CA (2010) Drug discovery from uncultivable microorganisms. Drug Discov Today 15:792
204. Demain AL (2009) Antibiotics: natural products essential to human health. Med Res Rev 29:821
205. Ashforth EJ, Fu C, Liu X, Dai H, Song F, Guo H, Zhang L (2010) Bioprospecting for antituberculosis leads from microbial metabolites. Nat Prod Rep 27:1709
206. Zengler K, Paradkar A, Keller M (2005) New methods to access microbial diversity for small molecule discovery. In: Zhang L, Demain AL (eds) Natural products: drug discovery and therapeutic medicine. Humana, Totowa, NJ, p 275
207. Vining LC (1990) Functions of secondary metabolites. Annu Rev Microbiol 44:395
208. Davies J, Ryan KS (2012) Introducing the parvome: bioactive compounds in the microbial world. ACS Chem Biol 7:252
209. Davies J (2013) Specialized microbial metabolites: functions and origins. J Antibiot 66:361
210. Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. Science 321:365
211. Mitscher LA (2008) Coevolution: mankind and microbes. J Nat Prod 71:497
212. Scheffler RJ, Colmer S, Tynan H, Demain AL, Gullo VP (2013) Antimicrobials, drug discovery, and genome mining. Appl Microbiol Biotechnol 97:969
213. Singh SB, Barrett JF (2006) Empirical antibacterial drug discovery—foundation in natural products. Biochem Pharmacol 71:1006
214. Donadio S, Brandi L, Monciardini P, Sosio M, Gualerzi CO (2007) Novel assays and novel strains – promising routes to new antibiotics? Expert Opin Drug Discov 2:789

215. Wrigley SK (2004) Pharmacologically active agents of microbial origin. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM, Washington, DC, p 356
216. Baltz RD (2010) Daptomycin. In: Buss AD, Butler MS (eds) Natural product chemistry for drug discovery. RSC, Cambridge, UK, p 395
217. Fujie A, Tawara S, Hashimoto S (2010) Micafubgin. In: Buss AD, Butler MS (eds) Natural product chemistry for drug discovery. RSC, Cambridge, UK, p 410
218. Bugni TS, Harper MK, McCulloch MWB, Whitson EL (2010) Advances in instrumentation, automation, dereplication and prefractionation. In: Buss AD, Butler MS (eds) Natural product chemistry for drug discovery. RSC, Cambridge, UK, p 272
219. Krug D, Zurek G, Schneider B, Garcia R, Müller R (2008) Efficient mining of myxobacterial metabolite profiles enabled by liquid chromatography–electrospray ionisation-time-of-flight mass spectrometry and compound-based principal component analysis. Anal Chim Acta 624:97
220. Hou Y, Braun DR, Michel CR, Klassen JL, Adnani N, Wyche TP, Bugni TS (2012) Microbial strain prioritization using metabolomics tools for the discovery of natural products. Anal Chem 84:4277
221. Wagenaar MM (2008) Pre-fractionated microbial samples – the second generation natural products library at Wyeth. Molecules 13:1406
222. Månsson M, Phipps RK, Gram L, Munro MH, Larsen TO, Nielsen KF (2010) Explorative solid-phase extraction (E-SPE) for accelerated microbial natural product discovery, dereplication, and purification. J Nat Prod 73:1126
223. Nielsen KF, Smedsgaard J (2003) Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography–UV–mass spectrometry methodology. J Chromatogr A 1002:111
224. Nielsen KF, Smedsgaard J, Larsen TO, Lund F, Thrane U, Frisvad JC (2004) Chemical identification of fungi: metabolite profiling and metabolomics. In: Arora DK (ed) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, Basel, p 31
225. Higgs RE, Zahn JA, Gygi JD, Hilton MD (2001) Rapid method to estimate the presence of secondary metabolites in microbial extracts. Appl Environ Microbiol 67:371
226. Larsen TO, Smedsgaard J, Nielsen KF, Hansen ME, Frisvad JC (2005) Phenotypic taxonomy and metabolite profiling in microbial drug discovery. Nat Prod Rep 22:672
227. Baltz RH, Davies JE, Demain AL (2010) Manual of industrial microbiology and biotechnology. ASM, Washington, DC, p 766
228. Bull AT (2004) Microbial diversity and bioprospecting. ASM, Washington, DC, p 496
229. Zengler K (2008) Accessing uncultivated microorganisms: from the environment to organisms and genomes and back. ACM, Washington DC, UK, p 308
230. Pearce C, Eckard P, Gruen-Wollny I, Hansske FG (2010) Microorganisms: their role in the discovery and development of medicines. In: Buss AD, Butler MS (eds) Natural product chemistry for drug discovery. RSC, Cambridge, UK, p 215
231. Hawksworth DL, Rossman AY (1997) Where are all the undescribed fungi. Phytopathology 87:888
232. Keller M, Zengler K (2004) Tapping into microbial diversity. Nat Rev Microbiol 2:141
233. Hedlund BP, Staley JT (2004) Microbial endemism and biogeography. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM, Washington, DC, p 225
234. Fierer N (2008) Microbial biogeography: patterns in microbial diversity across space and time. In: Zengler K (ed) Accessing uncultivated microorganisms: from the environment to organisms and genomes and back. ASM, Washington, DC, p 95
235. Kingston DGI (2010) Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod 74:496
236. Gallagher KA, Fenical W, Jensen PR (2010) Hybrid isoprenoid secondary metabolite production in terrestrial and marine actinomycetes. Curr Opin Biotechnol 21:794

237. Piel J (2006) Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. Curr Med Chem 13:39
238. Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. Nat Rev Microbiol 10:641
239. Lackner G, Peters EE, Helfrich EJ, Piel J (2017) Insights into the lifestyle of uncultured bacterial natural product factories associated with marine sponges. Proc Natl Acad Sci USA 114:E347
240. Mori T, Cahn JK, Wilson MC, Meoded RA, Wiebach V, Martinez AFC, Helfrich EJN, Wibberg D, Dätwyler S, Keren R, Lavy A, Rückert C, Ilan M, Kalinowski J, Matsunaga S, Takeyama H, Piel J (2018) Single-bacterial genomics validates rich and varied specialized metabolism of uncultivated *Entotheonella* sponge symbionts. Proc Natl Acad Sci USA 115:1718
241. Wilson MC, Mori T, Rückert C, Uria AR, Helf MJ, Takada K, Gernert C, Steffens UAE, Heycke N, Schmitt S, Rinke C, Helfrich EJN, Brachmann AO, Gurgui C, Wakimoto T, Kracht M, Crüsemann M, Hentschel U, Abe I, Matsunaga S, Kalinowski J, Takeyama H, Piel J (2014) An environmental bacterial taxon with a large and distinct metabolic repertoire. Nature 506:58
242. Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, Tiong CL, Gilman M, Osburne MS, Clardy J, Handelsman J, Goodman RM, Tiong CL (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl Environ Microbiol 66:2541
243. Su C, Lei L, Duan Y, Zhang KQ, Yang J (2012) Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. Appl Microbiol Biotechnol 93:993
244. Riesenfeld CS, Goodman RM, Handelsman J (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. Environ Microbiol 6:981
245. Lefevre F, Robe P, Jarrin C, Ginolhac A, Zago C, Auriol D, Vogel TM, Simonet P, Nalin R (2008) Drugs from hidden bugs: their discovery via untapped resources. Res Microbiol 159:153
246. Schofield MM, Sherman DH (2013) Meta-omic characterization of prokaryotic gene clusters for natural product biosynthesis. Curr Opin Biotechnol 24:1
247. Sloan WT, Quince C, Curtis TP (2008) The uncountables. In: Zengler K (ed) Accessing uncultivated microorganisms: from the environment to organisms and genomes and back. ASM, Washington, DC, p 52
248. Leadbetter JR (2003) Cultivation of recalcitrant microbes: cells are alive, well and revealing their secrets in the 21st century laboratory. Curr Opin Microbiol 6:274
249. Alain K, Querellou J (2009) Cultivating the uncultured: limits, advances and future challenges. Extremophiles 13:583
250. Stewart EJ (2012) Growing unculturable bacteria. J Bacteriol 194:4151
251. Kaeberlein T, Lewis K, Epstein SS (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296:1127
252. Nichols D (2007) Cultivation gives context to the microbial ecologist. FEMS Microbiol Ecol 60:351
253. Pham VHT, Kim J (2012) Cultivation of unculturable soil bacteria. Trends Biotechnol 30:475
254. Joint J, Mühling M, Querellou J (2010) Culturing marine bacteria—an essential prerequisite for biodiscovery. J Microb Biotechnol 3:564
255. Ishii S, Tago K, Senoo K (2010) Single-cell analysis and isolation for microbiology and biotechnology: methods and applications. Appl Microbiol Biotechnol 86:1281
256. Vartoukian SR, Palmer RM, Wade WG (2010) Strategies for culture of "unculturable" bacteria. FEMS Microbiol Lett 309:1
257. Lewis K, Epstein S, D'Onofrio A, Ling LL (2010) Uncultured microorganisms as a source of secondary metabolites. J Antibiot 63:468

258. Puspita ID, Kamagata Y, Tanaka M, Asano K, Nakatsu CH (2012) Are uncultivated bacteria really uncultivable? Microbes Environ 27:356
259. Hames-Kocabas EE, Uzel A (2012) Isolation strategies of marine-derived Actinomycetes from sponge and sediment samples. J Microbiol Methods 88:342
260. Prakash O, Shouche Y, Jangid K, Kostka JE (2013) Microbial cultivation and the role of microbial resource centers in the omics era. Appl Microbiol Biotechnol 97:51
261. Epstein SS (2009) Uncultivated microorganisms. In: Steinbüchel A (ed) Microbiology monographs, vol 10. Springer, Berlin, p 214
262. Stevenson BS, Eichorst SA, Wertz JT, Schmidt TM, Breznak JA (2004) New strategies for cultivation and detection of previously uncultured microbes. Appl Environ Microbiol 70:4748
263. Ferrari BC, Binnerup SJ, Gillings M (2005) Microcolony cultivation on a soil substrate membrane system selects for previously uncultured soil bacteria. Appl Environ Microbiol 71:8714
264. Ferrari BC, Winsley T, Gillings M, Binnerup S (2008) Cultivating previously uncultured soil bacteria using a soil substrate membrane system. Nat Protoc 3:1261
265. Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, Belanger A, Kanigan T, Lewis K, Epstein SS (2010) Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. Appl Environ Microbiol 76:2445
266. Buerger S, Spoering A, Gavrish E, Leslin C, Ling L, Epstein SS (2012) Microbial scout hypothesis and microbial discovery. Appl Environ Microbiol 78:3229
267. Krishna C (2005) Solid-state fermentation systems-an overview. Crit Rev Biotechnol 25:1
268. Ruiz-Sanchez J, Flores-Bustamante ZR, Dendooven L, Favela-Torres E, Soca-Chafre G, Galindez-Mayer J, Flores-Cotera LB (2010) A comparative study of taxol production in liquid and solid-state fermentation with *Nigrospora* sp. a fungus isolated from *Taxus globosa*. J Appl Microbiol 109:2144
269. Frisvad JC (2013) Media and growth conditions for induction of secondary metabolite production. Methods Mol Biol 944:47
270. Dunfield PF, Tamas I, Lee KC, Morgan XC, McDonald IR, Stott MB (2012) Electing a candidate: a speculative history of the bacterial phylum OP10. Environ Microbiol 14:3069
271. Bode HB (2006) No need to be pure: mix the cultures. Chem Biol 13:1245
272. Pettit RK (2009) Mixed fermentation for natural product drug discovery. Appl Microbiol Biotechnol 83:19
273. Li PF, Li SG, Li ZF, Zhao L, Wang T, Pan HW, Liu H, Wu ZH, Li YZ (2013) Co-cultivation of *Sorangium cellulosum* strains affects cellular growth and biosynthesis of secondary metabolite epothilones. FEMS Microbiol Ecol 85:358
274. Shrestha PM, Nevin KP, Shrestha M, Lovley DR (2013) When is a microbial culture "pure"? Persistent cryptic contaminant escapes detection even with deep genome sequencing. mBio 4: e00591
275. Cho J-C, Giovannoni SJ (2004) Cultivation and growth characteristics of a diverse group of oligotrophic marine gammaproteobacteria. Appl Environ Microbiol 70:432
276. Davis KER, Joseph SJ, Janssen PH (2005) Effects of growth medium, inoculum size, and incubation time on culturability and isolation of soil bacteria. Appl Environ Microbiol 71:826
277. Gich F, Janys MA, König M, Overmann J (2012) Enrichment of previously uncultured bacteria from natural complex communities by adhesion to solid surfaces. Environ Microbiol 14:2984
278. Bigelis R, He H, Yang HY, Chang L-P, Greenstein M (2006) Production of fungal antibiotics using polymeric solid supports in solid-state and liquid fermentation. J Ind Microbiol Biotechnol 33:815
279. Tormo JR, Asensio FJ, Bills GF (2013) Manipulating filamentous fungus chemical phenotypes by growth on nutritional arrays. In: Keller NP, Turner G (eds) Fungal secondary metabolism: methods and protocols, methods in molecular biology, vol 944. Humana/ Springer, Totowa, NJ, p 217
280. Bills GF, Platas G, Fillola A, Jiménez MR, Collado J, Vicente F, Martín J, González A, Bur-Zimmermann J, Tormo JR, Peláez F (2008) Enhancement of antibiotic and secondary

metabolite detection from filamentous fungi by growth on nutritional arrays. J Appl Microbiol 104:1644
281. Shao H-J, Qin X-D, Dong Z-J, Zhang H-B, Liu J-K (2008) Induced daldinin A, B, C with a new skeleton from cultures of the ascomycete *Daldinia concentrica*. J Antibiot 61:115
282. Seibert SF, Krick A, Eguereva E, Kehraus S, König GM (2007) Ascospiroketals A and B, unprecedented cycloethers from the marine-derived fungus *Ascochyta salicorniae*. Org Lett 9:239
283. Du L, Ai J, Li D, Zhu T, Wang Y, Knauer M, Bruhn T, Liu H, Geng M, Gu Q, Bringmann G (2010) Aspergiolides C and D: spirocyclic aromatic polyketides with potent protein kinase c-Met inhibitory effects. Chem A Eur J 17:1319
284. Li D, Chen L, Zhu T, Kurtán T, Mándi A, Zhao Z, Li J, Gu Q (2011) Chloctanspirones A and B, novel chlorinated polyketides with an unprecedented skeleton, from marine sediment derived fungus *Penicillium terrestre*. Tetrahedron 67:7913
285. Nett M, Hertweck C (2011) Farinamycin, a quinazoline from *Streptomyces griseus*. J Nat Prod 74:2265
286. Lösgen S, Magull J, Schulz B, Draeger S, Zeeck A (2008) Isofusidienols: novel chromone-3-oxepines produced by the endophytic fungus *Chalara* sp. Eur J Org Chem 2008:698
287. Wang X-J, Zhang J, Liu C-X, Gong D-L, Zhang H, Wang J-D, Yan Y, Xiang W (2011) A novel macrocyclic lactone with insecticidal bioactivity from *Streptomyces microflavus* neau3. Bioorg Med Chem Lett 21:5145
288. Lin Z, Zhu T, Wei H, Zhang G, Wang H, Gu Q (2009) Spicochalasin A and new aspochalasins from the marine-derived fungus *Spicaria elegans*. Eur J Org Chem 18:3045
289. Loesgen S, Bruhn T, Meindl K, Dix I, Schulz B, Zeeck A, Bringmann G (2011) (+)-Flavipucine, the missing member of the pyridione epoxide family of fungal antibiotics. Eur J Org Chem 26:5156
290. Bender T, Schuhmann T, Magull J, Grond S, von Zezschwitz P (2006) Comprehensive study of okaspirodiol: characterization, total synthesis, and biosynthesis of a new metabolite from *Streptomyces*. J Org Chem 71:7125
291. Surup F, Wagner O, von Frieling J, Schleicher M, Oess S, Müller P, Grond S (2007) The iromycins, a new family of pyridone metabolites from *Streptomyces* sp. I. Structure, NOS inhibitory activity, and biosynthesis. J Org Chem 72:5085
292. Kang M, Jones BD, Mandel AL, Hammons JC, DiPasquale AG, Rheingold AL, La Clair JJ, Burkart MD (2009) Isolation, structure elucidation, and antitumor activity of spirohexenolides A and B. J Org Chem 74:9054
293. Pimenta EF, Vita-Marques AM, Tininis A, Seleghim MHR, Sette LD, Veloso K, Ferreira AG, Williams DE, Patrick BO, Salisay DS, Andersen RJ, Berlinck RGS (2010) Use of experimental design for the optimization of the production of new secondary metabolites by two *Penicillium* species. J Nat Prod 73:1821
294. Ferreira ELF, Williams DE, Ióca LP, Morais-Urano RP, Santos MFC, Patrick BO, Elias LM, Lira SP, Ferreira AG, Passarini MRZ, Sette LD, Andersen RJ, Berlinck RGS (2015) Structure and biogenesis of roussoellatide, a dichlorinated polyketide from the marine-derived fungus *Roussoella* sp. DLM33. J Nat Prod 17:5152
295. Silva GH, Oliveira CM, Teles HL, Pauletti PM, Castro-Gamboa I, Silva DHS, Bolzani VS, Young MCM, Costa-Neto CM, Pfenning LH, Berlinck RGS, Araujo AR (2010) Sesquiterpenes from *Xylaria* sp., an endophytic fungus associated with *Piper aduncum* (Piperaceae). Phytochem Lett 3:164
296. Oliveira CM, Regasini LO, Silva GH, Pfenning LH, Young MCM, Berlinck RGS, Bolzani VS, Araujo AR (2011) Dihydroisocoumarins produced by *Xylaria* sp. and *Penicillium* sp., endophytic fungi associated with *Piper aduncum* and *Alibertia macrophylla*. Phytochem Lett 4:93
297. Oliveira CM, Silva GH, Regasini LO, Flausino O Jr, Lopez SN, Abissi BM, Berlinck RGS, Sette LD, Bonugli-Santos RC, Rodrigues A, Bolzani VS, Araujo AR (2011) Xylarenones C-E from an endophytic fungus isolated from *Alibertia macrophylla*. J Nat Prod 74:1353

298. Romminger S, Pimenta EF, Nascimento ES, Ferreira AG, Berlinck RGS (2012) Biosynthesis of two dihydropyrrole-polyketides from a marine-derived *Penicillium citrinum*. J Braz Chem Soc 23:1783
299. Kossuga MH, Ferreira AG, Sette LD, Berlinck RGS (2013) Two polyketides from a co-culture of two marine-derived fungal strains. Nat Prod Commun 8:721
300. Mercado-Marin EV, Garcia-Reynaga P, Romminger S, Pimenta EF, Romney DK, Lodewyk MW, Williams DE, Andersen RJ, Miller SJ, Tantillo DJ, Berlinck RGS, Sarpong R (2014) Total synthesis and isolation of citrinalin and cyclopiamine congeners. Nature 509:318
301. Rodriguez JP, Williams DE, Sabater ID, Bonugli-Santos RC, Sette LD, Andersen RJ, Berlinck RGS (2015) The marine-derived fungus *Tinctoporellus* sp. CBMAI 1061 degrades the dye Remazol Brilliant Blue R producing anthraquinones and unique tremulane sesquiterpenes. RSC Adv 5:66360
302. Ióca LP, Romminger S, Santos MFC, Bandeira KF, Rodrigues FT, Kossuga MH, Nicacio KJ, Ferreira ELF, Morais-Urano RP, Passos MS, Kohn LK, Arns CW, Sette LD, Berlinck RGS (2016) A strategy for the rapid identification of fungal metabolites and the discovery of the antiviral activity of pyrenocine A and harzianopyridone. Quim Nova 39:720
303. Newmister SA, Gober CM, Romminger S, Yu F, Tripathi A, Parra LLL, Williams RM, Berlinck RGS, Joullié MM, Sherman DH (2016) OxaD: a versatile indolic nitrone synthase from the marine-derived fungus *Penicillium oxalicum* F30. J Am Chem Soc 138:11176
304. Castro MV, Ióca LP, Williams DE, Costa BZ, Mizuno CM, Santos MFC, Jesus K, Ferreira ELF, Seleghim MHR, Sette LD, Pereira-Filho ER, Ferreira AG, Gonçalves NS, Santos RA, Patrick BO, Andersen RJ, Berlinck RGS (2016) Condensation of macrocyclic polyketides produced by *Penicillium* sp. DRF2 with mercaptopyruvate represents a new fungal detoxification pathway. J Nat Prod 79:1668
305. Nicacio KJ, Ióca LP, Fróes AM, Leomil L, Appolinario LR, Thompson CC, Thompson FL, Ferreira AG, Williams DE, Andersen RJ, Eustaquio AS, Berlinck RGS (2017) Cultures of the marine bacterium *Pseudovibrio denitrificans* Ab134 produce bromotyrosine-derived alkaloids previously only isolated from marine sponges. J Nat Prod 80:235
306. Kuroda Y, Nicacio KJ, Silva-Jr IA, Leger PR, Chang S, Gubiani JR, Deflon VM, Nagashima N, Rode A, Blackford K, Ferreira AG, Sette LD, Williams DE, Andersen RJ, Jancar S, Berlinck RGS, Sarpong R (2018) Isolation, synthesis and bioactivity studies of phomactin terpenoids. Nat Chem. 10:938
307. Duetz WA (2007) Microtiter plates as mini-bioreactors: miniaturization of fermentation methods. Trends Microbiol 15:469
308. Siebenberg S, Bapat PM, Lantz AE, Gust B, Heide L (2010) Reducing the variability of antibiotic production in *Streptomyces* by cultivation in 24-square deepwell plates. J Biosci Bioeng 109:230
309. Schäpper D, Stocks SM, Szita N, Lantz AE, Gernaey KV (2010) Development of a single-use microbioreactor for cultivation of microorganisms. Chem Eng J 160:891
310. Klöckner W, Büchs J (2012) Advances in shaking technologies. Trends Biotechnol 30:307
311. Tan J, Chu J, Hao Y, Guo Y, Zhuang Y, Zhang S (2013) High-throughput system for screening of cephalosporin C high-yield strain by 48-deep-well microtiter plates. Appl Biochem Biotechnol 169:1683
312. Watrous JD, Dorrestein PC (2011) Imaging mass spectrometry in microbiology (perspective). Nat Rev Microbiol 9:683
313. Watrous J, Roach P, Alexandrov T, Heath BS, Yang JY, Kersten RD, van der Voort M, Pogliano K, Gross H, Raaijmakers JM, Moore BS, Laskin J, Bandeira N, Dorrestein PC (2012) Mass spectral molecular networking of living microbial colonies. Proc Natl Acad Sci USA 109:E1743
314. Zahn JA, Higgs RE, Hilton MD (2001) Use of direct-infusion electrospray mass spectrometry to guide empirical development of improved conditions for expression of secondary metabolites from Actinomycetes. Appl Environ Microbiol 67:377

Darlon Irineu Bernardi obtained his degree in Chemistry (2016) at the State University of Maringa, where he pursued research on terrestrial plant natural products. He is currently a Ph.D. student at the University of São Paulo, under the supervision of Prof. Roberto G. S. Berlinck. His Ph.D. projects include investigating approaches towards the isolation of water-soluble secondary metabolites from microorganism culture media and marine invertebrates. His main research interests include the discovery of novel natural products, chemotaxonomy, biosynthesis and biological activities.

Fernanda O. Chagas received a M.S. in Sciences (2010) and a B.S. in Pharmacy-Biochemistry (2007) from the University of São Paulo. Her Ph.D. in Sciences (2014) was obtained at the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil, under the supervision of Prof. Mônica T. Pupo. During her Ph.D. studies she spent 1 year (2011–2012) at Prof. Eric W. Schmidt's laboratory, L. S. Skaggs Pharmacy Research Institute, University of Utah, United States. She completed her training as a postdoctoral researcher at Prof. Pupo's laboratory for 2 years (2014–2016), working on the chemistry and genetics of microorganisms. Currently she is Assistant Professor of Natural Products at the Walter Mors Institute of Research on Natural Products, Federal University of Rio de Janeiro, Brazil. Her research interests include microbial interactions and their ecological roles, genetic manipulation, and biosynthesis studies on microorganisms.

Afif Felix Monteiro received his M.Sc. degree in Chemistry (2014) from the Universidade Federal de Goiás and his Ph.D. degree in Chemistry (2018) from the Universidade Estadual Paulista at Araraquara, under the supervision of Prof. Ian Castro-Gamboa. He is currently a postdoctoral researcher under the supervision of Professor Roberto G. S. Berlinck. His current research interests include the discovery of novel microbial bioactive metabolites, as well as biocatalysis.

Gabriel Franco dos Santos received a M.Sc. in Chemistry (2012) at the Universidade Federal de São Carlos, Brazil, under the supervision of Prof. Edson Rodrigues Filho, and a Ph.D. (2017) at the Universidade Federal de Minas Gerais under the supervision of Prof. Jacqueline A. Takahashi, with co-supervision of Prof. Isidro G. Collado, Universidad de Cádiz, Spain. Dr. Santos trained as a postdoctoral researcher in secondary metabolites isolated from microorganisms under the supervision of Prof. Roberto Berlinck (2017–2018). Currently he is a visiting Professor at the Division of Chemistry, Pontificia Universidad Católica del Perú. His research interests focus on microorganism natural products, including their isolation and structural determination by nuclear magnetic resonance and mass spectrometry, biocatalytic transformations, and natural product biosynthesis.

Roberto G. S. Berlinck graduated in Chemistry (1988) at the University of Campinas, Brazil. He obtained his Ph.D. in Sciences (Organic Chemistry) at the Université Libre de Bruxelles (1992). In 1993 he moved to the Universidade de São Paulo as assistant professor. During 1997–1998 was a visiting professor at the University of British Columbia (UBC), Canada, in collaboration with Professor Raymond J. Andersen (Departments of Chemistry and Earth and Ocean Sciences). Since 1993, Dr. Berlinck's research interests have included the discovery of pharmacologically active chemicals from marine invertebrates. During the period between 2001 and 2006 he participated in the National Cooperative Drug Discovery Group program funded by the National Institutes of Health, leaded by Professor Chris M. Ireland (Department of Medicinal Chemistry, University of Utah, USA), working in collaboration with Professors Ireland and Andersen and the pharmaceutical company Wyeth-Ayerst. In 1998 he started the first Brazilian program on the investigation of microbial secondary metabolism, including Streptomycetes and fungi. Dr. Berlinck established collaborations with a number of Brazilian and international investigators, including specialists in cancer, inflammation and immunomodulation, antibiotics, parasitic diseases, in biodiscovery, organic synthesis and natural product biosynthesis. His interest in the discovery of antiparasitic is currently a very active program. More recently, Dr. Berlinck's interests have moved towards the understanding of the biochemical pathways controlling the biosynthesis of biologically active secondary metabolites produced by fungi in culture conditions. These include complex alkaloids and highly modified polyketides, in collaboration with Professors David H. Sherman (University of Michigan, USA), Richmond Sarpong (University of California, Berkeley, USA) and Prof. Alessandra Eustaquio (University of Illinois, Chicago). Dr. Berlinck has supervised over 50 M.Sc. and Ph.D. students and post-doctoral researchers and published over 100 articles in peer-reviewed journals.