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An ethnopharmacological survey conducted in the Bolivian Amazon, and identification of *N*-alkylamides and lignans from *Lepidium meyenii* and *Heliopsis helianthoides* var. *scabra* with effects on the central nervous system

Ph.D. Thesis

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LIST OF PUBLICATIONS RELATED TO THE THESIS

I. **Hajdu Z**, Hohmann J.
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II. **Hajdu Z**, Nicolussi S, Rau M, Lorántfy L, Forgo P, Hohmann J, Csupor D, Gertsch J.
Identification of endocannabinoid system-modulating *N*-alkylamides from *Heliopsis helianthoides* var. *scabra* and *Lepidium meyenii*.
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III. **Hajdu Z**, Haskó J, Krizbai I, Wilhelm I, Jedlinszki N, Fazakas, C, Molnár J, Forgo P, Hohmann J, Csupor D.
Evaluation of lignans from *Heliopsis helianthoides* var. *scabra* for their potential antimetastatic effects in the brain.
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IV. **Hajdu Z**, Lorántfy L, Jedlinszki N, Boros K, Hohmann J, Csupor D.
Quality control of Maca-containing (*Lepidium meyenii* Walp) dietary supplements.
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TABLE OF CONTENTS

ABBREVIATIONS AND SYMBOLS

1D	one-dimensional
2D	two-dimensional
AEA	anandamide
APCIMS	atmospheric pressure chemical ionization mass spectrometry
BBB	blood-brain barrier
CB	cannabinoid
CNS	central nervous system
COSY	correlated spectroscopy
CPC	centrifugal partition chromatography
DAD	diode array detector
ESIMS	electronspray ionization mass spectrometry
ECS	endocannabinoid system
FAAH	fatty acid amide hydrolase
HMBC	heteronuclear multiple-bond correlation spectroscopy
HPLC	high-performance liquid chromatography
HRE(S)IMS	high-resolution electron(spray) ionization mass spectrometry
HSQC	heteronuclear single-quantum coherence spectroscopy
JMOD	*J*-modulated spin-echo experiment
MPLC	medium pressure liquid chromatography
MS	mass spectrometry
MTCA	1*R*,3*S*-1-Methyltetrahydro-β-carboline-3-carboxylic acid
NAA	*N*-alkylamides
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
PLC	preparative thin-layer chromatography
RBEC	rat brain endothelial cell
RP	reversed-phase
RPC	rotational planar chromatography
SAR	structure-activity relationship
syn.	synonym
CTO	Communal Territory of Origin
TEER	transendothelial electrical resistance
TLC	thin layer chromatography
VLC	vacuum liquid chromatography
δ	chemical shift

1. INTRODUCTION

The use of medicinal plants for treating illnesses is a worldwide phenomenon that is seen even nowadays from the native people of developing countries to the citizens of the largest metropolises. The traditional knowledge of medicinal plants has led to the development of various medicaments, and is still the basis of many scientific studies.

The South-American rainforests are rich sources of traditional medicines based on a large variety of plants, and the aim of my work was therefore to conduct ethnopharmacological fieldwork in the Amazon. Porvenir is a Bolivian indigenous community in the Bajo Paraguá Communal Territory of Origin (CTO), home to the Chiquitano mestizos and the Guarasug'we indigenous nation, which is currently close to extinction. This region provided a good research area, because no ethnomedicinal fieldwork had previously been conducted in Porvenir.

Numerous South-American plant species are traditionally used for central nervous system (CNS) disturbances, which are at the main focus of neuroscientific research, and therefore we aimed a phytochemical and pharmacological investigation of traditionally used plants with possible effects on the brain. From ethnopharmacological considerations, *Lepidium meyenii* and *Heliopsis helianthoides* var. *scabra* were chosen for detailed analysis. The hypocotyls of *L. meyenii* (Maca, Brassicaceae) are widely consumed as a common vegetable and have a multiplicity of other uses in the Peruvian and Bolivian highlands, among them fertility enhancement being the most popular. Maca has been found to contain certain metabolites characteristic of the species, such as the *N*-alkylamide (NAA) macamides. Some species of the *Heliopsis* genus (Asteraceae) are also used in North-American traditional medicines, and have been reported to contain NAAs and lignans. Among these species, *H. helianthoides* var. *scabra* has not been studied in detail. With the discovery of the functional interaction of plant NAAs with the endocannabinoid system (ECS), these compounds have become important as lead compounds of drug development. The promising anti-metastatic potential of several lignans underlines the significance of these compounds as potential tools in cancer treatment. Our studies on *L. meyenii* and *H. helianthoides* var. *scabra* focused on the phytochemical analysis of these species to isolate and identify NAAs and lignans and to carry out detailed pharmacological analyses to reveal their role in the folk medicinal applications of the plants and as potential tools in modern medicine.

The traditional knowledge of medicinal plants is also utilized in the food industry. Certain South-American plants, such as Maca are marketed in Europe, but their utilization partially differs from the traditional way. This difference raises the suspicion that the quality of certain products cannot meet the requirements, and an analytical study of Maca containing preparations was therefore also proposed.

2. AIMS OF THE STUDY

The aims of our study were

- to describe the medicinal plants applied in the traditional medicine of Porvenir, Bolivia, and to find plant species which may be worthy of further phytochemical and pharmacological investigations on the CNS, by comparison of the folk-medicinal use with the available scientific literature data;
- the isolation and structure elucidation of alkamides from *L. meyenii* and *H. helianthoides* var. *scabra* and, in the frame of cooperation, to test these compounds on different targets within the ECS;
- the isolation and structure elucidation of lignans from *H. helianthoides* var. *scabra* and, in the frame of cooperation, to evaluate their potential antimetastatic activity in the brain;
- to develop an analytical protocol for the qualitative and quantitative analysis of *L. meyenii*-containing food supplements and to screen selected products for the presence of synthetic adulterants (phosphodiesterase inhibitors).

3. LITERATURE OVERVIEW

3.1. ETHNOPHARMACOLOGICAL BACKGROUND

Ethnopharmacological studies in South America comprise part of the age of the discovery of the continent. Adventurers in the New World, missionaries, and later anthropologists and botanists recorded the utilization of the plants, the scientific background of which started to be investigated decades or centuries later. Thanks to the researches and expeditions in the last two centuries, a good number of pharmacons have been developed from the plants and animals of the South-American rainforests.

In Bolivia, there are 37 indigenous groups[1] and numerous mestizo communities living in different natural areas of the country, e.g. Altiplano, Yungas, Chaco or the tropical rainforest lowlands. Some general studies have been carried out on useful plants, including the medicinal species, especially from the Andes.[2,3] In the highlands, there are mainly two indigenous cultures, the Aymara and the Quechua, representing 55% of the Bolivian population,[1] both with great background knowledge on medicinal plants.[4] Medicinal plants are still widely used in the countryside, but also in the main Andean cities, since a considerable proportion of their populations arrived from Aymara and Quechua peasant communities and maintain much of their own cultures.[4] The Kallawaya medicine is the richest and best-known traditional medicine of Bolivia. More than a thousand medicinal plants and animal species and minerals used by Kallawaya healers were described by *Girault,*[5] who provided a broad picture of the traditional healing capacities in the Andean area.

Several ethnobotanical studies have been conducted in the Bolivian tropical rainforest lowlands,[6–9] the subtropical Yungas,[10] the dry region of Chaco,[11] and the semi-deciduous forests of Chiquitanía.[12] There have been numerous unpublished studies, and theses and dissertations accessible only at the local universities, on the ethnobotany of the indigenous nations and mestizo communities of Bolivia (e.g. theses from the Universidad Autónoma Gabriel René Moreno[13–18]).

The ethnopharmacological study presented in this thesis was conducted in the Amazonian Porvenir, which is one of the five indigenous communities in the Territorio Comunitario de Origen Bajo Paraguá (Bajo Paraguá Communal Territory of Origin (CTO)) in Velasco Province, County of Santa Cruz, Bolivia (Figure 1). The flora of the CTO has not been studied thoroughly; there are data only on the flora of the Noel Kempff Mercado National Park, which borders on the CTO, and which is one of the most extensive ecological reservations in the world, with a surface of 1 523 446 hectares. 2705 plant species have been described in the national park, but a total of around 4000 species are estimated to be native to the area.[19] One ethnobotanical work has been published on the region,[9] with information concerning 36 medicinal plants used in 4 of the communities, with the exception of Porvenir. Moreover, ethnobotanical thesis work conducted in one of the communities (Bella Vista) noted 111 medicinal plant species, without discussing their indications.[15]

The 378 163 ha of Bajo Paraguá hauses a population of about 1224 people, living in 4 widely separated settlements (Piso Firme, Cachuela, Porvenir and Florida); the 519 of them

who live in Porvenir are mostly Chiquitano mestizos and some Guarasug'wes.[20] All of them speak Spanish, and have a passive linguistic knowledge of Guarasug'we or Chiquitano. The Guarasug'wes (or Pausernas) are currently close to extinction: there are altogether only 31 Guarasug'we inhabitants in 2 communities, Porvenir and Bella Vista.

Porvenir is a semi-isolated community, the inhabitants of which partly live according to their traditional roots,[21] but also benefit from the external world. The nearest relatively large settlement, San Ignacio de Velasco, with about 23 000 inhabitants, is roughly 12 h away by coach. The CTO is located on the border of the tropical rainforests and the cerrado biome, the vegetation therefore being typical of the south-west Amazonian forest and the inundated savanna. Porvenir is situated on the border of the rainforest, which is secondary woodland, and the savanna, close to the Paraguá River, and thus the locals can easily benefit from the natural resources provided by these ecosystems.

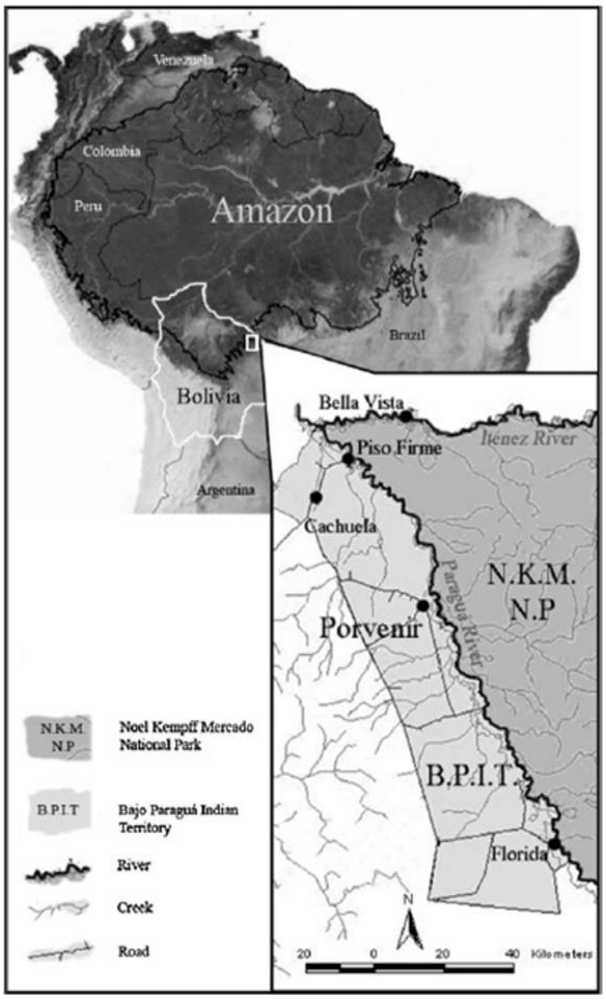


Figure 1. Location of the study*.

Although the traditional medicinal system of the community of Porvenir was previously described in detail by me,[22] in the thesis I only focus on the plants used for CNS disturbances. As the phytochemical and pharmacological investigations of traditionally applied medicinal plants is usually more efficient, I chose the Maca plant from the Bolivian traditions, which has as well a modern widespread application in Europe. The second plant, a *Heliopsis* species selected for my Ph.D. work belongs to the North-American traditional medicine, and available in Hungary too. Its similar phytochemical profile, which presents NAAs with possible action to the CNS, links it to the first species. My aim was to scientifically analyse

* Geographical coordinates of Porvenir: 13°59'13" S, 61°32'30" W. The map of the Amazon was made with the use of the map downloaded from the web page of the Amazon Tour, and the map of Bajo Paraguá was made with the use of the map from the book [9].

plant species with possible CNS effects, starting with an evaluation of traditional applications, followed by phytochemical analysis and pharmacological investigations, and problems of modern consumption.

3.2. BOTANY OF THE *LEPIDIUM* AND *HELIOPSIS* GENERA AND THE INVESTIGATED SPECIES

3.2.1. Botany of the *Lepidium* genus and *L. meyenii* Walp.

The *Lepidium* genus belongs to the Brassicaceae family, order of Brassicales, superorder of Rosanae, subclass of Magnoliidae, Equisetopsida class, Angiospermatophyta subdivision, and Spermatophyta division.[23]

The Brassicaceae family, which contains very important crop plants, comprises approximately 3000 species. The genus *Lepidium* is the largest genus of the family, with approximately 175 species, which are widely distributed throughout the world in all continents except Antarctica. There are 4 cultivated *Lepidium* species: the worldwide known *L. sativum* L. (or garden cress); *L. latifolium* L. (dittander) and *L. virginicum* L. (poor man's pepper), whose leaves are used as salad components; and *L. meyenii* Walp., known as Maca or Peruvian ginseng, the only species in the entire genus that produces fleshy roots.[24,25] Maca was probably domesticated in the Department Junín, Peru, between 1300 and 2000 years ago, but little is known about its origin. Today, the species is mainly cultivated in the Andes of Peru and Bolivia at elevations of 3500-4450 m above sea level. Based on morphological observations and comparative analyses *G. Chacón* attempted to separate the species from its wild type, renaming the cultivated species *L. peruvianum* G. Chacón as a new species. [24] However, this change was not accepted, and nowadays the name *L. peruvianum* is a synonym for *L. meyenii*[26] Furthermore, a search for the wild type of the species identified 3 wild plants (*L. bipinnatifidum* Desvaux, *L. kalenbornii* C. L. Hitchcock and *L. chichicara* Desvaux) in the Andes, none of them closely related to Maca.[24]

The Maca plant is a rosette of 12-20 frilly leaves with an enlarged fleshy underground storage organ formed by the taproot and the lower part of the hypocotyl, resembling a turnip. This is the economic product of Maca; for simplicity, it is called 'hypocotyl'.[24] It is about 10–14 cm in length and 3–5 cm in width.[25] 13 hypocotyl color types are known, the black, yellow and red types being the most frequently used.[27,28] The foliage forms a mat, growing in close contact with the ground. The leaves exhibit dimorphism, being larger in the vegetative phase and reduced in the reproductive cycle. The plant grows in a habitat of intense cold, extremely

intense sunlight, and strong winds. It is an annual crop completing its life cycle within a year when climatic conditions are favorable. The seeds have no dormancy, and germinate in 5–7 days at 25 °C under good moisture conditions. A single plant of Maca produces approximately 14 g of seeds.[24]

3.2.2. Botany of the *Heliopsis* genus and *H. helianthoides* var. *scabra* 'Asahi'

The *Heliopsis* genus belongs to the Asteraceae (Compositae) family, order Asterales, superorder Asteranae, subclass Magnoliidae, Equisetopsida class, Angiospermatophyta subdivision and Spermatophyta division.[29]

The Compositae family has more than 23000 species spread across 1620 genera; among them, the *Heliopsis* genus is represented by only 13 species, all of which are restricted to North, Central and South America. The plants in the genus are similar in appearance and closely related to those in the genus *Helianthus*, the sunflowers. *Heliopsis* is commonly called false sunflower.[30]

H. helianthoides (L.) Sweet, a species commonly called smooth oxeye or false sunflower, is an upright, clump-forming, nearly glabrous, sunflower-like, short-lived perennial that is native to eastern and central North America. The subspecies *H. helianthoides* var. *scabra* (Dunal) Fernald differs from the species by having hairy and rough-textured (scabrous) leaves and stems with the leaves being thicker. The upper leaves may be entire, with the basal leaves toothed. Cultivars of var. *scabra* are more commonly grown in gardens than the species itself.[30] *H. scabra* is a synonym for *H. helianthoides* var. *scabra*.[29] Several cultivars are available with different flower colors and shades; one of them is 'Asahi', with bright-yellow double daisy-like flowers on sturdy stems.[31]

3.3. TRADITIONAL AND MODERN USES OF THE INVESTIGATED SPECIES

The dried and cooked hypocotyl of *L. meyenii* is consumed as a common vegetable and also used traditionally for medicinal purposes, as a general invigorator and as a fertility enhancer for human use and for domesticated animals[24,27,28] Although Maca is not used traditionally for the improvement of CNS symptomes, Peruvian natives have observed more recently that the ingestion of the plant helps children in their school work.[27]

Sexual performance and fertility enhancement are the most popular contemporary applications of Maca in Europe. However, there are several preparations that are claimed to increase mental and physical performances too. The number and variety of industrial products

(predominantly dietary supplements, mono- and multicomponent Maca preparations) on the European herbal market (mainly sold via the internet) are increasing.[24,32]

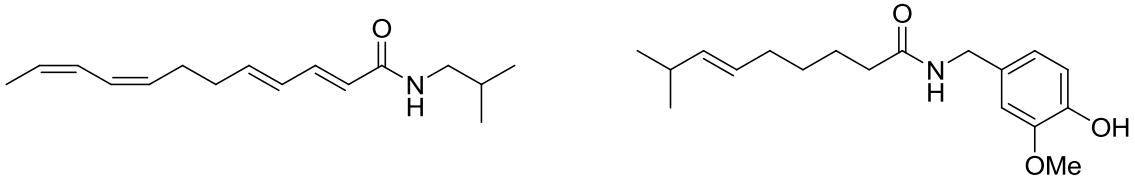
Some species of the *Heliopsis* genus have been used by North-American Indians as medicinal plants. One of the most widely applied species, *H. longipes* (A. Gray) Blake, has been used to relieve toothache, and for the treatment of inflammations and ulcers. The root of *H. helianthoides* has been applied by the Meskwaki Indians to relieve lung troubles, and by the Chippewa Indians to strengthen limbs.[33] Members of the *Heliopsis* genus are cultivated almost worldwide as ornamental plants.

3.4. CHEMISTRY OF THE *HELIOPSIS* AND *LEPIDIUM* GENERA AND THE INVESTIGATED SPECIES

3.4.1. *N*-Alkylamides

Plant-derived NAAs or alkamides are lipophilic substances, a heterogeneous class of structurally different molecules. These compounds mostly contain a polyunsaturated aliphatic fatty acid chain and a shorter substituent on the amine side. Both substituents may include cyclic systems and/or heteroatoms (nitrogen, sulfur or oxygen).

NAAs have been identified in 26 plant families, and are characteristic of certain Asteraceae species, especially in the genera *Achillea*, *Acmella*, *Echinacea*, *Heliopsis* and *Spilanthes*, and in some species of the plant families Brassicaceae, Piperaceae, Rutaceae and Solanaceae.[34] To date, around 400 NAAs have been identified, more than 70 of them in the Heliantheae tribe (Asteraceae).[35,36] Typical examples of an aliphatic NAA from *Echinacea purpurea* (L.) Moench[37] and the aromatic ring-containing capsaicin from *Capsicum annuum* L. can be seen in Figure 2.



dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide capsaicin

Figure 2. Two main types of plant *N*-alkylamides

3.4.1.1. *N*-Alkylamides from *Lepidium meyenii*

In the Brassicaceae family, some NAAs have been described from *Arabidopsis thaliana* and *Brassica oleracea*, and a large variety have been found in *L. meyenii*.[35] The unsaturated fatty

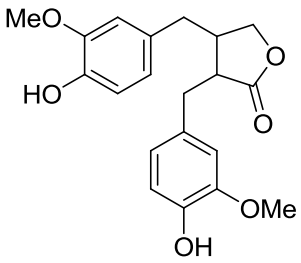
acid amides, the alkylamide macamides are typical markers of the Maca plant. To date, 19 macamides have been described[38–41] (see in Annex 1).

3.4.1.2. *N*-Alkylamides from the *Heliopsis* genus and *H. helianthoides* var. *scabra*

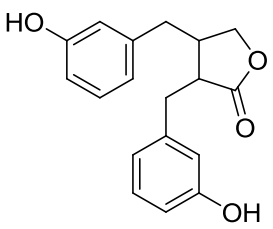
Spilanthol (deca-2*E*,6*Z*,8*E*-trienoic acid isobutylamide, *syn*. affinin) is the best-known NAA in several *Spilanthes* species and, together with homospilanthol (deca-2*E*,6*Z*,8*E*-trienoic acid 2-methylbutylamide)[42], can be found in *H. longipes* (A. Gray) Blake. Undeca-2*E*,4*E*-diene-8,10-diynoic acid isobutylamide, undeca-2*E*-en-8,10-diynoic acid isobutylamide and *N*-isobutyl-2*E*-decenamide[43] have also been reported from *H. longipes*. As compared with the alkamides of *H. longipes* bearing 1-3 double bonds, *H. buphthalmoides* (Jacq.) Dunal and *H. helianthoides* (L.) Sweet contain C_{18} NAAs with the rarely occurring pentaene acids. Octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide, octadeca-2*E*,4*E*,8*E*,10*Z*-tetraen-12-ynoic acid isobutylamide, tetradeca-2*E*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide and octadeca-2*E*,4*E*,8*E*,10*E*,14*Z*-pentaen-12-ynoic acid isobutylamide have been isolated from *H. buphthalmoides*, and the latter compound also from *H. helianthoides*.[44] Two NAAs, scabrin (octadeca-2,4,8,10,14-pentaenoic acid isobutylamide) and heliopsin (octadeca-2,4,8,10,12,16-hexaenoic acid isobutylamide or octadeca-2,4,8,12,14,16-hexaenoic acid isobutylamide) have also been identified from *H. scabra*.[45,46] The structures of the compounds are given in Annex 2.

3.4.2. Lignans

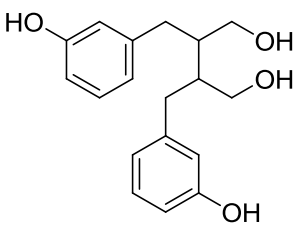
The lignans are a large group of phenolic compounds: nearly 500 lignan structures have been identified from plants so far.[47] These compounds are present in many edible plants, such as oil seeds, seaweed, whole grains, fruits and vegetables.[48] Some of the edible plant lignans, e.g. secoisolariciresinol and matairesinol isolated from flaxseed[48] or hydroxymatairesinol from *Picea abies*,[49] are converted by the intestinal bacteria to enterolignans, enterodiol and enterolactone, the latter of which is thought to be one of the major biologically active lignans in the human organism (Figure 3).



matairesinol



enterolactone



enterodiol

Figure 3. Structures of the plant lignan matairesinol and 2 enterolignans derived from it

3.4.2.1. Lignans from the *Heliopsis* genus and the investigated species

In the *Heliopsis* genus, a wide variety of lignans have been identified: the dibenzylbutane derivatives heliobuphthalmin, 8-hydroxyheliobuphthalmin, 7*Z*-7,8-dehydroheliobuphthalmin and 2-[(2H-1,3-benzodioxo-5-yl)methyl]-3-[(3,4-dimethoxyphenyl)methyl]succinic acid dimethyl ester; the dibenzylbutyrolactones heliobuphthalmin lactone and 5,7'-dehydroheliobuphthalmin lactone in the aerial parts of *H. buphthalmoides* (Jacq.) Dunal;[44,50,51] the dibenzylbutyrolactones hinokinin and 2′-hydroxyhinokinin in the stem of *H. longipes* (A. Gray) Blake.[43] Two lignan derivatives, the dibenzylbutyrolactone helianthoidin and the arylnaphthalene helioxanthin, were isolated previously from the root of *H. helianthoides* var. *scabra* (Dunal) Fernald. [52,53] Lignans isolated from the genus are to be seen in Annex 3.

3.4.3. Other compounds

3.4.3.1. Other compounds from *Lepidium meyenii*

The hypocotyls of *L. meyenii* contains alkaloids. 1*R*,3*S*-1-Methyltetrahydro-β-carboline-3-carboxylic acid (MTCA) is found in several species,[54] but the majority of the alkaloids found in Maca (macaridin, lepidilin A and B) have been identified only in this species.[28] Other important secondary metabolites of *L. meyenii* are glucosinolates and their derivatives,[54,55] most of them containing an aromatic ring, e.g. glucotropaeolin, *m*-methoxybenzylglucosinolate and benzylisothiocyanate..

Depending on the origin and color of the hypocotyl, the composition and quantity of the secondary metabolites of Maca may be very different,[27] macaenes, macamides and glucosinolates differ substantially. Black and lead-colored Maca contain the largest quantity of glucosinolates, while yellow Maca is the richest source of macaenes, macamides and phenols.[56]

3.4.3.2. Other compounds from *Heliopsis helianthoides*

Besides the alkamides and lignans, 13 guaianolides, 1 germacranolide, 3 derivatives of homogeranylnerol, 1 daucane and 1 tremetone derivative have been reported from *H. helianthoides* previously.[44,57]

3.5. PHARMACOLOGY OF THE INVESTIGATED SPECIES AND THE RELATED COMPOUNDS

3.5.1. Pharmacology of the *N*-alkylamides

NAAs are present in some plants, where these compounds exert growth regulatory functions, similarly to *N*-acylethanolamines.[58] Various bioactivities of plant-derived NAAs have been described in mammalian tissues, such as antimicrobial and related activities, effects on anti-inflammatory and immunomodulatory processes, and activity on the ECS.[35]

3.5.1.1. Antimicrobial activities

Numerous studies have dealt with the antibacterial and antifungal, and also the antiparasitic, molluscicidal and insecticidal activities of NAAs.[35] The antibacterial effects of several NAAs against *Escherichia coli*, *Pseudomonas solanacearum*, *Erwinia carotovora*, and *Bacillus subtilis* were studied by *Molina-Torres et al*.[59–61]

The minimal inhibitory concentrations of the investigated NAAs for *E. coli*, *P. solanacearum* and *E. carotovora* range between 5 and 300 μg/mL.[59] Those of 4 phenylethylamides for the Gram-positive bacterium *B. subtilis* were 10-150 μg/mL; all of them reached more than 90% of inhibition.[61] The degree of unsaturation and the chain length of the acid moiety influences the growth inhibition of *B. subtilis*. The amide moiety impacts the inhibitory effect, phenylethylamides generally exhibiting higher activities, than those of isobutylamides.[35] Affinin isolated from *H. longipes* inhibited the growth of *E. coli* and *S. cerevisiae* at concentrations as low as 25 mg/mL. Higher concentrations of affinin were necessary to inhibit the growth of *P. solanacearum* and *B. subtilis*.[59]

Antifungal effects of several NAAs have been studied against *Sclerotium rolfsii*, *Cladosporium sphaerospermum* and *C. cladosporioides*.[60,62] Penta-2*E*-ene-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidide and penta-2*E*,4*E*-ene-5-(benzo[1,3]dioxol-5-yl) acid pyrrolidine were the most active, with minimal inhibitory concentrations of 10 μg/mL. A 2*E* unsaturation in the acid or the amine side-chain was generally favorable for fungal growth inhibition. NAAs possessing a sulfur atom in their acid or amine moieties displayed increased

antifungal effects. In straight-chain acid isobutylamides such as affinin, the 2*E*,6*Z*,8*Z* unsaturation was necessary for fungal inhibition.[35] The 2*E* and fully unsaturated affinin derivatives pellitorine and fagaramide, isolated from *Zanthoxylum gilletii* (De Wild.) P.G. Waterman, were ineffective against fungal growth.[63]

The antiplasmodial activities of some NAAs have been evaluated. Lemairamide and zanthomamide isolated from *Z. rubescens* Planch. ex Hook. f. (Rutaceae) exerted weak activity, with average IC_{50} values ranging from 45.6 μM to 149.9 μM.[64] Pellitorin and fagaramide were more active, with IC_{50} values of 24.1 μM and 50.0 μM, respectively.[63] It was suggested that the α,β-unsaturated carbonyl function rather than the *N*-isobutyl substituent is the responsible active site. [63,64].

3.5.1.2. Anti-inflammatory and immunomodulatory activities

The anti-inflammatory and immunomodulatory properties of *Echinacea* species have been well investigated, confirmed and patented.[35,65–68] Further, the immunomodulatory effects of *Anacyclus pyrethrum* and the anti-inflammatory effects of *Spilanthes*, *Heliopsis*, *Piper* and *Achillea* species have also been established.[36] Affinin inactivates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), shows significant topical anti-inflammatory effects in the mouse ear edema test and is the only NAA that has a proved influence on the transcription and translation of the cyclooxygenase enzymes, which play roles in inflammatory processes.[36]

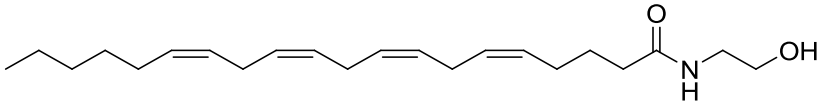
3.5.1.3. Analgesic and anticonvulsant effects

The dichloromethane extract of *H. longipes* has been shown to exert an analgesic effect, which may be attributed to the alkylamide content of the root and especially to affinin.[43] Affinin also demonstrates an antinociceptive effect,[69] modifies anxiety behaviour, prolongs the duration of sodium pentobarbital-induced hypnosis, and decreases the time of clonic and tonic pentylenetetrazol-induced seizures in rats.[70]

3.5.1.4. Activity on the endogenous cannabinoid system

With the discovery of the functional interaction of NAAs with the ECS,[66–68,71–73] a new pharmacological mode of action of medicinal and food plants containing *N*-acylethanolamines was revealed. In mammalian tissues, different NAAs from medicinal plants exert analgesic, anti-inflammatory and immunomodulatory effects, potentially also via the ECS.[65,74] For instance, NAAs seem to play a major role in the bioactivity of the widely used medicinal plants of the *Echinacea* genus.[35,65–68] The immunomodulatory *Echinacea* alkylamides dodeca-

2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (Figure 2) and dodeca-2*E*,4*E*-dienoic acid isobutylamide bind to the cannabinoid-2 (CB_2) receptor more strongly than the endogenous cannabinoids.[68] While the interactions of *Echinacea* NAAs with CB receptors have been studied in more detail, ECS-interacting NAAs from other plants have been described only recently.[72,73,75,76] The assumption that NAAs may interfere with the ECS due to their structural similarity to endocannabinoids such as anandamide (*N*-arachidonoylethanolamide, AEA) and palmitoylethanolamide[35] is intuitive, but the confirmation of this assumption is hampered by the lack of structure – activity relationship (SAR) data.



anandamide (AEA)

Chemically diverse plant NAAs showing different saturation patterns and double bond configurations therefore provide a source for further analysis of the ECS-binding interactions of this class of natural products. Moreover, insights from such studies may inspire the generation of scaffolds for the development of novel chemical probes or may explain the traditional use of certain plants.

3.5.2. Pharmacology of lignans

Several bioactivities of lignans have been described, such as antimicrobial and antiviral activities, anti-inflammatory and immunosuppressive, hepatoprotective effects, roles in osteoporosis and cancer prevention, and antitumor activities.[77] Only the antitumor and the related antimetastatic activities are discussed here.

3.5.2.1. Antitumor and antimetastatic activities

Experimental evidence has demonstrated clear anticarcinogenic effects of enterolignans,[78] lignan-containing flaxseed extracts[78] or pure lignans such as hydroxymatairesinol[49] and honokiol[79,80] in animals. A number of investigations have revealed the cytostatic effects of numerous lignans on different types of cancer cells.[81–89] Certain compounds, including schizandrin B,[84] picropodophyllin,[85] podophyllotoxin and their derivatives,[90] display marked anticancer and antimetastatic activities.

One of the possible strategies to prevent tumor angiogenesis is to inhibit endothelial cell migration into the tumor. Interestingly, the angiogenic effects of secoisolariciresinol

diglucoside, isolated from the flaxseed,[91] and sesamin, naturally present in sesame oil,[92] have been proved. Nevertheless, another study indicated the antiangiogenic property of honokiol because of the pronounced downregulation of vascular endothelial growth factor (VEGF) and intercellular adhesion molecule 1 (ICAM-1) in H1299 human lung adenocarcinoma cells treated with this lignan.[93] Various lignans have been demonstrated to downregulate different matrix metalloproteinases[80,84,94] and cellular adhesion molecules,[95–98] such as vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1, which are considered to be important in the extravasation of tumor cells. Moreover, treatment with different lignans reduces the migratory potential of highly metastatic tumor cells.[94,99] However, the effects of lignans on the transmigration of metastatic cells through endothelial barriers (a critical step in metastasis formation) have not been investigated so far.

3.5.3. Pharmacology of *Lepidium meyenii*

Numerous studies have been carried out with the hypocotyl of Maca, such as investigations on its sexual activity and fertility enhancement effects, antioxidant and antiproliferative functions, adaptogenic effects, and activities on postmenopausal osteoporosis and the CNS.[25,27,28] Only the studies conducted on its activities on the CNS, and its sexual activity and fertility enhancement effects are discussed here.

In vivo experiments on rats and mice have shown that a boiled aqueous or hydroalcoholic extract of the black variety of Maca helps studying, improves memory and has a positive effect in scopolamine-induced memory impairment.[100–102] Black Maca inhibited acetylcholinesterase in ovariectomized mice, and it is therefore proposed that it improves experimental memory impairment.[27] MTCA isolated from Maca acts as an inhibitor of the enzyme monoamine oxidase.[54] However, *Gonzales et al.* have proven that MTCA as a component of Maca extracts does not inhibit monoamine oxidase.[103]

It has been shown that the macamide *N*-3-methoxybenzyl linoleamide weakly inhibits the major AEA degrading enzyme fatty acid amide hydrolase (FAAH, which is a membrane bound mammalian enzyme responsible for the destruction of a number of endogenous signaling amides by hydrolysis), and may thus exert indirect cannabimimetic neuroprotective effects.[75] This was recently confirmed in a study in which synthesized macamides and analogs were shown to act as FAAH inhibitors, although no further ECS targets were tested.[76]

In some pharmacological experiments, the sexual behaviour of male rats and the erectile function of castrated rats were enhanced.[104,105] In a similar experiment, Maca was inactive, but the reason for this could be the much lower dosage.[106]

Fertility-enhancing effects were observed in both male and female rats. The weights of the testicles and epididymis of healthy rats increased after the consumption of an aqueous extract of the plant, and the weight increase of the epididymis was considered to be related to an increase of the sperm count.[107–109] The rate of spermatogenesis and the sperm motility also improved in healthy[110,111] and pathological[107–109,112] conditions. These parameters changed similarly in healthy bulls[113] and in mice following spermatogenic damage induced by malathion.[114]

In vitro[115] and *in vivo* experiments suggest that Maca may have an estrogen-like effect, which may be related to the fertility improvement observed in female rats and mice. The uterus weight was increased in healthy mice fed with Maca,[100,116] and the rate of osteoporosis in ovariectomized rats decreased, but an increase in uterus weight related to an estrogen effect was not observed in this case.[117] The explanation for the contradictory estrogen-like effects may be the different compositions of the applied plant materials and the presence of selective estrogen receptor modulators in the plant, which behave as agonists or antagonists depending on the tissue.

Maca consumption affects neither the estradiol level[109,111,117–120] nor the plasma concentrations of testosterone, luteinizing hormone, follicle stimulating hormone and prolactin.[118] Several reviews discuss whether the effects of Maca on the reproductive system may be due to the estrogen-like phytosterols of the hypocotyl,[28,115] but this has been refuted by other studies.[121,122]

Comprehensive phytochemical and pharmacological comparisons of hypocotyls from the different color types have not been performed to date in detail. The application of different hypocotyl color types could explain the contradictions in the pharmacological results. Red Maca decreases the prostate size, and provides prostatic hyperplasia even in the presence of testosterone, but this is not characteristic for yellow and black Maca. The latter increases the number of spermatides in the testes and the sperm count in the epididymis, but red Maca does not. The best reproductive character is attributed to the black type, followed by yellow and red Maca. The black one is also considered the best energy source and invigorator.[27,28]

The mechanisms involved in the reproductive system of Maca have not been revealed in detail so far. Examinations of different Maca extracts did not confirm androgen receptor binding,[123] or changes in testosterone level,[111,120,124] – with the exception of one study where the testosterone level of mice increased after Maca consumption.[119] According to *Gonzales*, the potency-enhancing effect of Maca is not related to changes in depression or anxiety,[125] and is independent of its nutritive values.[105] The current knowledge on the chemistry of *L. meyenii* indicates that macamides, alkaloids, glucosinolates and their derivatives, polyphenols and flavonoids may also take part in the pharmacological activities of the species,[56] though their exact roles have not been elucidated so far.

3.5.4. Pharmacology of *Heliopsis helianthoides* var. *scabra*

Pharmacological investigations with extracts of this species have not been published, but several studies have been conducted with pure compounds isolated from it.

The insecticidal activity of the NAAs scabrin and heliopsin has been demonstrated on house flies.[45,46] The lignan helioxanthin inhibits hepatitis B virus gene expression and viral particle production *in vitro*,[126] and also has some effect on the presumed molecular mechanisms of hepatic inflammatory disease.[127] Its synthetic derivatives exhibit significant *in vitro* antiviral activity against various viruses, including hepatitis B and C and herpes simplex, and moderate activity against HIV.[128] The compound displays cytotoxicity on an HeLa cell line.[89]

Heliobuphthalmin exhibits high antineoplastic activities against the classical multidrug resistant subline derived from gastric carcinoma;[129] and reduces the viability of hepatocellular carcinoma cells (HuH-7 cells) as a strong inducer of apoptosis.[130] In contrast, heliobuphthalmin has been shown not to have cytotoxic activity on the human lung cancer cell line A549 and an inhibitory effect on human cytomegalovirus IE gene expression in A549 cells,[131] and does not show significant cytotoxic effects *in vitro* on human hepatoma and cervical carcinoma cell lines.[132] It exerts weak *in vitro* activity against the 3D7 *Plasmodium falciparum* strain.[133] Dehydroheliobuphthalmin has significant neuroprotective activity against glutamate-induced neurotoxicity.[134]

3.7. TOXICOLOGY OF THE INVESTIGATED SPECIES

3.7.1. Toxicology of *Lepidium meyenii*

Review data on *in vivo* and *in vitro* studies with Maca indicate that its use is safe.[27] According to a study based on questionnaires and laboratory investigations (liver and kidney function and blood profile) with 600 Peruvian participants, Maca consumption is safe and the health status of Maca-consuming people is high.[135]

3.7.2. Toxicology of *Heliopsis helianthoides* var. *scabra*

No studies are available on the toxicity of the species. The related *H. longipes* has been shown in an acute toxicity study on mice to be non-toxic (LD_{50}=288 mg ethanol extract/kg).[136]

3.8. CLINICAL STUDIES OF *LEPIDIUM MEYENII*

The effects of the species on sexual function and fertility in humans have been studied by many groups. A systematic review[137] of 7 studies on sexual function found that only 4 randomized trials met all their inclusion criteria, in which 1.5-3.5 g dry Maca powder was given orally daily to 8-57 participants for 4-6 weeks. Consumption of the hypocotyl has been found effective in mild erectile dysfunction,[138] and to improve sexual desire in healthy man,[125] and positive psychological changes have been observed in menopausal women.[120] A study including the examination of the physical performance-enhancing effect of the plant (performed with only 9 participants) showed slightly enhanced sexual activity, but changes in sport achievements were not observed.[139] In another study with 9 participants (but without a control group), the number and motility of spermatocytes was approximately doubled when the dry powder was consumed in daily dosages of 1.5-3 g during 4 months.[107] Overall, the numbers of participants were too low, and the studies were too heterogeneous to allow appropriate conclusions.

3.9. FOOD SUPPLEMENTS

The term food supplement* was defined in 1994, and practically covered preparations of minerals and vitamins of good quality at the beginning, but increased commerce in preparations containing materials of herbal or animal origin has become a worldwide phenomenon in the last decade.[140] The public opinion that these products are natural and

*"Food supplements means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients (vitamins, minerals) or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities"[176]

hence safe plays an important role in their increased consumption. Due to the lack of proper quality control in some cases, however, the declared and the real compositions of the products may differ, and certain products contain non-labelled substances, including synthetic compounds, to enhance the effect. Adulteration is particularly frequently in the case of products marked as potency enhancers.[141] Since the male sexual performance-enhancing effect of *L. meyenii* and its metabolites has not been confirmed convincingly either preclinically or clinically, Maca products marketed for this purpose are candidates for quality control, focusing on activity-potentiating synthetic compounds. In the frame of the research, the quality control of 14 namely Maca-containing products has been carried out.[32,142]

4. MATERIALS AND METHODS

4.1. ETHNOPHARMACOLOGICAL METHODS

4.1.1. Fieldwork

My fieldwork was conducted for 5 months between September 2004 and June 2005, in two phases, partly in the dry and partly in the humid season. The first month involved familiarization with the field, and participant observation and informal interviews with the inhabitants, which was important in order to establish the basis of cooperation. A total of 16 adults from 16 different families were selected to participate in semi-structured interviews with open questionnaires. The information recorded included personal data, the vernacular names of the medicinal plants used in Porvenir, the parts used, the plant habits, the modes of preparation and application, the formulas, the doses and the therapeutic purposes. Two samples of each plant were collected with the participation of the interviewed subjects. The specimens were dried in Porvenir with the aid of a hand-made drying box, utilizing the heat of the fireside in a kitchen.

4.1.2. Identification of plants

Voucher specimens were deposited at the National Herbarium of the Noel Kempff Mercado Natural History Museum in Santa Cruz, Bolivia, where they were botanically identified, using handbooks and by comparison with the specimens in the Herbarium, and with the help of the taxonomists of the Museum.

4.1.3. Ethnobotanical data analysis

The knowledge of the inhabitants on medicinal plants was analyzed by means of the modified method of *Gentry* and *Phillips*,[143,144] which assesses the frequency and the variety of use of plants. The frequency of use was determined from the number of people interviewed who used the given species, while the variety of use involves the number of different diseases that can be treated with the given species.

4.1.4. Scientific data collection and data analysis

Ethnobotanical, chemical and pharmacological data were collected from the following databases: SciFinder Scholar, Web of Knowledge, Science Direct, PubMed and Scopus up to 2012. Additionally, ethnobotanical data were gathered from Bolivian university dissertations, ethnobotanical books, journals and relevant web pages. The data found in the literature were compared with the application in Porvenir. Mainly the following points were taken into consideration: (1) whether the given species was used in a similar way in other regions, countries or traditional communities, (2) whether chemical components were described, and (3) whether the described pharmacological effects support the application in Porvenir.

4.2. PLANT MATERIAL

The roots of *H. helianthoides* var. *scabra* (Dunal) Fernald 'Asahi' were obtained from a nursery (Hegede Flower Nursery Ltd., Kecskemét, Hungary) in the flowering period in September 2009. A voucher specimen (No. 819) has been preserved in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary. The plant material was washed, cleaned and processed in a fresh form.

The yellow dry hypocotyl powder of *L. meyenii* Walp originated from Peru and was purchased from Raw Organic Maca Powder, EverTrust Ltd, UK (batch number M-010177-11-220312). The material was stored at room temperature until preparation. A representative sample (No. 823) is available at the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

4.3. PURIFICATION AND ISOLATION OF COMPOUNDS

For **vacuum liquid chromatography (VLC)**, SiO_2 (silica gel 60 GF_{254}, 15 μm, Merck) and reversed-phase (RP) SiO_2 (LiChroprep RP-C_{18}, 40-63 μm, Merck) were applied. Separations were monitored by **thin layer chromatography (TLC)** (aluminum sheets coated with silica gel 60 F_{254}, 0.25 mm, Merck 5554 and silica gel 60 RP-C_{18} F_{254}s, Merck). The chromatograms

were visualized at 254 and 366 nm, and by spraying with concentrated H_2SO_4, followed by heating at 110 °C.

Medium pressure liquid chromatography (MPLC) was performed with a Büchi apparatus (Büchi Labortechnik AG, Flawil, Switzerland), using a 40 × 75 mm RP18ec column (Büchi, 40-63 μm).

Preparative thin-layer chromatography (PLC) was carried out on silica gel 60 F_{254} (0.25 mm, Merck).

Rotational planar chromatography (RPC) was performed with a Chromatotron instrument (model 8924, Harrison Research, Palo Alto, CA, USA) on manually coated SiO_2 plates (silica gel 60 GF_{254}, Merck 7730).

High pressure liquid chromatography (HPLC) experiments were carried out on a Young-Lin 9100 series HPLC system (Young-Lin, Korea), equipped with a UV detector and on-line degasser using an RP-C_{18} column (YMC-Pack ODS-A 250 × 4.6 mm, 5 μm, 120 Å, YMC, Germany) at 30 °C and on a Waters 600 system (Waters Corporation, Milford, USA), equipped with an UV detector and on-line degasser using a RP C_{18} column (LiChroCART 5 μm, 100 Å, 250 × 4 mm column, Merck KGaA, Darmstadt, Germany) at 25 °C.

Centrifugal partition chromatography (CPC) was carried out on Armen SCPC apparatus (Armen Instrument Sas, Saint-Avé, France) equipped with a gradient pump, a 10 mL sample loop, an ASC/DSC valve, a 250 mL column, a UV detector, and an automatic fraction collector. The system was controlled by Armen Glider software.

4.4. CHARACTERIZATION AND STRUCTURE ELUCIDATION

Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) or 125 MHz (^{13}C). Two-dimensional (2D) data were acquired and processed with standard Bruker software. For ^{1}H–^{1}H COSY, HSQC and HMBC experiments, gradient-enhanced versions were used.

Mass spectrometry (MS) was carried out in positive ionization mode with an API 2000 MS/MS equipped with an atmospheric pressure chemical ionization (APCI) interface. The source temperature was 400 °C (*H. helianthoides* var. *scabra* samples) or 450 °C (*L. meyenii* samples). Data acquisition and evaluation were performed with Analyst 1.5.2 software.

Optical rotation values were determined in $CHCl_3$ at room temperature by using a Perkin-Elmer 341 polarimeter.

4.5. PHARMACOLOGICAL TESTS WITH THE ISOLATED COMPOUNDS

4.5.1. Evaluation of the isolated *N*-alkylamides in the endocannabinoid system

Evaluation of the NAAs on the endocannabinoid system was carried out by *Jürg Gertsch* and his research group (Institute of Biochemistry and Molecular Medicine, NCCR TransCure, University of Bern, Bern, Switzerland).

The isolated compounds were investigated on different targets of the ECS, including CB_1 and CB_2 receptor binding, inhibition of FAAH, AEA transport, and monoacyl glycerol lipase (MAGL). The methods are described in detail in [145].

4.5.2. Evaluation of antimetastatic effects of lignans in the brain

Investigation of the pharmacological effects of the isolated lignans were performed by *János Haskó* and his colleagues (Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary).

In the first set of experiments, the isolated lignans were tested on the viability of cerebral endothelial cells and melanoma cells. The morphological changes and the effects on the migration of melanoma cells, phalloidin staining of the actin-cytoskeleton, the attachment of melanoma cells to brain endothelial cells, and improvement of the barrier function of the endothelial cells were then evaluated. Measurement of transendothelial electrical resistance (TEER) and a wound healing assay were also applied. The methods can be seen in detail in [146].

4.6. QUANTIFICATION OF MACAMIDE CONTENT OF DIETARY SUPPLEMENTS

4.6.1. Chemicals and reagents

The macamide *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide that we isolated was used as chemical marker. Peak purity analysis carried out with the Empower Pro software confirmed that the chromatographic peak of the compound was spectrally pure. Millipore Direct-Q UV3 clarifier (Millipore Corporation, Billerica, MA, USA) was used to obtain purified water for HPLC measurements. Synthetic phosphodiesterase inhibitors were isolated previously from adulterated dietary supplements (sildenafil (Viagra), tadalafil (Cialis) and vardenafil (Levitra); and their derivatives (pseudovardenafil, nor-acetildenafil, thiosildenafil, dimethyl-

thiosildenafil, and aminotadalafil). Ten Maca-containing dietary supplements were purchased in Hungarian shops, 3 Maca powder samples were provided by Ashaninka Pharma Ltd. (Hungary), and 1 Maca powder was purchased in a webshop (UK).

4.6.2. Sample preparation

Dry Maca hypocotyl powder (Raw Organic Maca Powder, EverTrust Ltd, New Malden, UK, originating from Peru) was used for validation of the analytical method. 500 mg of the product was extracted with 10 mL *n*-hexane (10 min extraction in an ultrasonic bath at room temperature), then diluted with *n*-hexane to 25.0 mL in a volumetric flask and centrifuged (10 min, 2500 rpm). 20.0 mL of the supernatant was pipetted into a round-bottomed flask and evaporated under vacuo. Extracts were redissolved in 2 mL of *n*-hexane, and filtered through a filter membrane (Acrodisc® GHP 13 mm, 0.45 μm, Waters, USA). The first 0.5 mL was rejected; the other 1.5 mL was analyzed by HPLC-DAD. Three extracts were prepared and analyzed in triplicate.

In the case of product quality analysis, 500 mg of the powder products and 380–1000 mg of the multicomponent preparations (depending on the tablet or capsule sizes) were processed as described above.

4.6.3. Calibration and linearity

As calibration standard, *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide, a major alkamide of *L. meyenii*, was applied. In order to obtain the linear range of quantification, stock standard solutions (1.2 mg/mL) were prepared with *n*-hexane. 1.0 mL of the stock standard solution was transferred to a 10.0 mL volumetric flask and diluted with *n*-hexane. Different amounts of this solution were diluted with *n*-hexane to obtain 6 different concentrations in the range 0.0048–0.06 mg/mL. The calibration range of *N*-benzyl-(9Z,12Z)-octadecadienamide was 0.048–12.0 μg/injection. 10-μL of the standards were injected in triplicate. The slope, intercept and correlation coefficient were determined. For calculation of the calibration curve, peak areas were plotted against the injected mass of the macamide standard.

4.6.4. HPLC apparatus and measurement conditions

HPLC analysis was carried out on a Waters 600 system (Waters Corporation, Milford, USA), equipped with a 2998 photodiode array detector, an on-line degasser, a column thermostat and autosampler using a RP Kinetex XBC_{18} (2.6 μm, 100 Å, 100 × 4.6 mm column (Phenomenex, Torrance, USA) at 25 °C). Chromatographic elution of the samples was accomplished with an

isocratic solvent system consisting of MeCN–H_2O 85:15 at a flow rate of 0.7 mL/min. For analysis, 10.0 μL of extract was injected. The samples were monitored at 210 nm. Data acquisition and evaluation were performed with Empower Pro software.

4.6.5. Method validation

Repeatability was evaluated via experiments with Raw Organic Maca Powder extracts. Intraday precisions were calculated from data acquired during a 3-day validation. Precision was expressed as relative standard deviation (RSD%). The signal to noise ratio was used to express the limit of detection (3 times the noise) and quantitation (10 times the noise). Recovery analysis was carried out by adding known amounts of macamide (64, 128 and 256% of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide) to the Maca extract (n=3 at each concentration). To assess the stability of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide in the *n*-hexane extract, the extracts were stored at room temperature and analyzed after 3 and 4 days.

4.6.6. Detection of phosphodiesterase inhibitors

For the presence of phosphodiesterase inhibitors, multicomponent preparations were analyzed by TLC and HPLC-DAD according to a protocol developed in our laboratory.[147] Briefly, 1 capsule or tablet was extracted with 10 mL of MeOH for 10 min in an ultrasonic bath at room temperature, and then filtered through a membrane filter. 10 μL of the extract was applied to a silica gel stationary phase (Merck 105553 60, 20 × 20) and developed in toluene–Me_2CO–EtOH–25% NH_3 (70:50:10:3) in a developing chamber saturated with solvent vapor. The development distance was 20 cm. The detection was performed under UV light (254 nm) and by spraying the plate with Dragendorff's reagent or vanillin–sulfuric acid and heating at 120 °C. This method allows the identification of phosphodiesterase inhibitors by comparing their retention factors and the colors of the spots with those of analytical standards. Selective detection with Dragendorff's reagent gives a possibility for the detection of so far unidentified synthetic derivatives.

For HPLC-DAD analysis, a Gemini-NX 5u C_{18} 100A, 100 × 4.6 mm column was used. The solvent system was NH_4HCO_3 (10 mM, pH = 10.00±0.2) – MeCN (7:3) at a flow rate of 1 mL/min. 20 μL of extract was injected. The samples were monitored in the whole UV range (200–400 nm) and at 220, 254, 282 and 292 nm. Data acquisition and evaluation were performed with Empower Pro software. Synthetic phosphodiesterase inhibitors can be identified via their retention times and characteristic UV spectra.

5. RESULTS

5.1. ETHNOPHARMACOLOGICAL FIELDWORK

Accounts of the lifestyle and beliefs in Porvenir, botanical data regarding the plants used, the diseases that occur and their possible treatment, methods of plant application, frequency and variety of use, and some species and formulas are discussed in detail in [22]. The plants used for illnesses of the CNS, pain or fever are discussed here. A total of 145 medicinal plant species were registered in Porvenir, which are applied for 107 known complaints or symptoms. The latter are grouped in main categories in Table 1.

Table 1. The use of the plants by illness categories

Illness categories	No. of indications	No. of species
Diseases of the alimentary tract and the metabolism	18	60
Diseases of the CNS, pain and fever	11	37
Diseases of the genitourinary tract	10	35
Dermatological diseases	12	34
Diseases of the respiratory system	7	32
Infectious and parasitic diseases	9	30
Diseases of the musculoskeletal system, traumatic injuries	9	28
Pediatric diseases	6	10
Diseases of sense organs	3	9
Cardiovascular diseases	2	9
Intoxications by animals	3	8
Tumorous diseases	1	5
Hematological diseases	2	4
Other diseases and symptoms	8	17
Total	**101**	

The CNS, pain and fever therapeutic area can be divided into 11 subcategories, in which a total of 37 species were used (Annex 4). Among these subcategories, headache (12 species) and fever, including Dengue-fever (12), were the most frequent. To alleviate toothache, 8 plants were noted; 8 species were used against *arrebato* (probably stroke); "cold exudation" (2) probably appears in febrile stages; 4 species were used to relieve nervousness, 3 against sadness (melancholy), 2 for weight control, 2 for weakness, and 2 species for alcoholism. The

fieldwork disclosed 1 species for “keeping ghosts away”. The species and their indications can be found in Annex 4.

5.2. ISOLATION OF THE COMPOUNDS FROM *LEPIDIUM MEYENII*

Dried *L. meyenii* hypocotyl powder (1.2 kg) was extracted twice with *n*-hexane (30 °C, ultrasonic bath, 2 × 15 min, 2 × 3000 mL), filtered and then centrifuged. The supernatant was concentrated in vacuo, yielding 9 g of material, which was subjected in 4 parts to CPC, using a two-phase solvent system consisting of *n*-hexane–EtOAc–MeOH–H_2O 9:1:9:1 (2200 rpm, 12 mL/min flow rate, 60 min) in the ascending mode (CPC I). Nine main fractions (I–IX) were obtained, from which V (370 mg) and VI (235 mg) were purified again with CPC (MeCN–H_2O, 1:1, descending mode, 2200 rpm, 12 mL/min flow rate, 60 min program, CPC II) and the alkylamide-containing fractions and main fraction VII (80 mg) were then subjected to HPLC (Young-Lin, semi-preparative RP-C_{18} column, MeCN–H_2O, 9:1): three almost pure compounds were obtained, and the final purification was carried out by RP-HPLC (Waters, MeCN–H_2O, 95:5), which resulted in the isolation of compounds **1** (1 mg), **2** (8 mg) and **3** (92 mg).

5.3. ISOLATION FROM *HELIOPSIS HELIANTHOIDES* VAR. *SCABRA*

The fresh roots of *H. helianthoides* var. *scabra* ‘Asahi’ (9 kg) were extracted with MeOH (90 L) at room temperature (Figure 4). After evaporation, the MeOH extract (after dilution with H_2O) was subjected to solvent-solvent partitioning to obtain $CHCl_3$- and H_2O-soluble fractions. The alkylamide-containing $CHCl_3$ phase was concentrated in vacuo, yielding 80 g of material, which was subjected to silica gel VLC (2 × 250 g), with a gradient system of *n*-hexane–EtOAc (10:0, 9:1, 8:2, 7:3, 1:1, 0:10). In total, 78 fractions were collected, and combined into 12 main fractions (I–XII) on the basis of TLC monitoring.

Alkylamide- and lignan-containing fractions (VIII–IX, 9 g) were selected for further purification, which was carried out by RP-VLC, using MeOH–H_2O (8:2, 85:15, 9:1, 10:0) and then MeOH–EtOAc (9:1, 6:4, 10:0) gradient systems. From the total of 75 fractions, selected ones (fractions 17–22, 470 mg; 23-34, 2.4 g; 35–40, 1.15 g; and 41–46, 668 mg) were further processed.

Fraction 17–22 was chromatographed on a 1-mm silica gel RPC plate with a gradient system of *n*-hexane–EtOAc (9:1, 8:2, 7:3, 0:1) at a flow rate of 3 mL/min (RPC I). Finally, the fractions obtained were purified by preparative TLC (benzene–CH_2Cl_2–Et_2O, 1:1:1), which resulted in pure compound **6** (4 mg).

Fractions 23–34, 35–40 and 41–46 were purified by MPLC with a gradient system of *n*-hexane–EtOAc (1:0, 9:1, 8:2, 7:3, 6:4, 1:1, 0:1) at a flow rate of 60 mL/min (MPLC I).

Through the above-mentioned MPLC purification fraction 23–34 was separated into 5 main subfractions, 1 of which contained the pure compound **3** (5 mg). Subfraction 1 was further chromatographed with MPLC I, yielding 60 fractions, among which fraction 25–42 was subjected to RPC (4-mm RPC plate, 8 mL/min flow rate, gradient system of *n*-hexane–EtOAc (9:1, 8:2, 7:3, 0:1) (RPC II), which resulted in the isolation of compound **5** (6 mg).

Fraction 35–40 was separated into 45 subfractions, among which 25–29 was subjected to multiple RPC (2-mm silica gel RPC plate, gradient system of benzene–CH_2Cl_2–Et_2O 9:3:0, 6:3:0, 6:3:0,5; 5 mL/min flow rate) (RPC III). Final purification was carried out by preparative TLC (benzene–CH_2Cl_2–Et_2O 6:3:1) (PLC II), which yielded compounds **4** (13 mg) and **5** (8 mg). Subfraction 30–40 was chromatographed on a 2-mm silica gel RPC plate (*n*-hexane–Me_2CO 9:1, 8:2, 7:3, 0:1; 5 mL/min flow rate), and then purified twice on a 1-mm RPC plate at a 3 mL/min flow rate (RPC IV), affording compound **4** (15 mg). Fraction 41–45 was purified by RPC (1 mm plate, benzene–CH_2Cl_2–Et_2O, 1:1:1; 3 mL/min flow rate) (RPC V), and then by PLC, using 2 solvent systems: PLC I and then *n*-hexane–Me_2CO, 8:2 (PLC III), resulting in compound **6** (3 mg).

Fraction 41–46 was separated into 78 fractions, of which fractions 7–19 and 20–36 were selected for further separations. Fraction 7–19 was subjected to PLC (*n*-hexane–Et_2O, 6:4, PLC IV), and the almost pure fractions were then injected for RP-HPLC (MeCN–H_2O, 8:2), which resulted in the isolation of compounds **1** (6 mg) and **2** (2 mg). Fraction 20–36 was further chromatographed by RPC with a gradient system of *n*-hexane–Me_2CO (9:1, 8:2, 1:0). The material was subjected to a 1-mm silica gel RPC plate, at a flow rate of 3 mL/min (RPC VI). Three main fractions were collected (1–3), two of which (1 and 3) were further purified by PLC I, affording the pure compounds **5** (4 mg) and **7** (3 mg). The third fraction was finally purified by HPLC (*n*-hexane–EtOAc, 8:2), resulting in compound **3** (2 mg).

Fraction 47–55 was subjected to RPC with a gradient system of *n*-hexane–EtOAc (9:1, 8:2, 0:1). The material was chromatographed on a 1-mm silica gel RPC plate at a 3 mL/min flow rate (RPC VII). Some of the yielded fractions were subjected to HPLC (*n*-hexane–EtOAc, 8:2), affording compound **1** (5 mg).

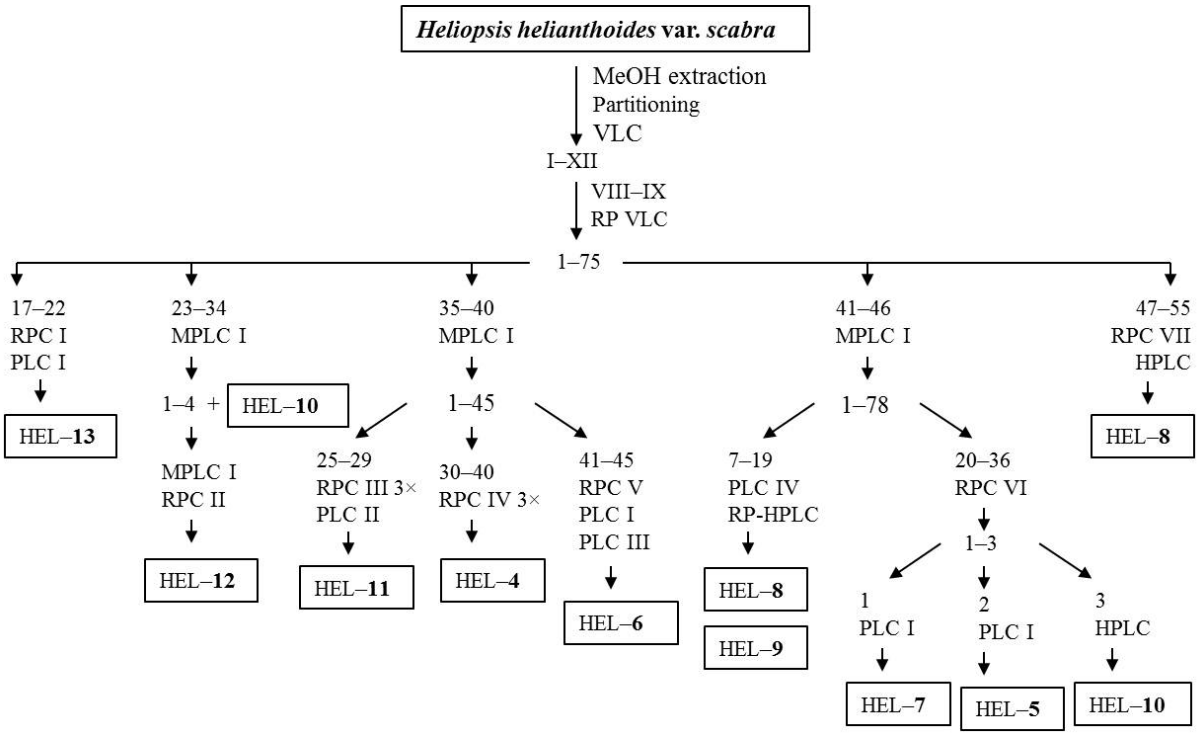


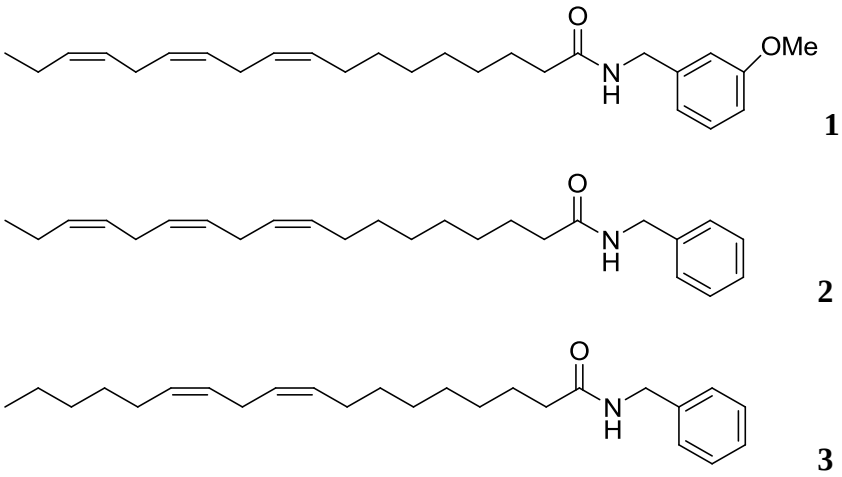
Figure 4. Isolation of compounds from *H. helianthoides* var. *scabra*

5.4. STRUCTURE ELUCIDATION

The structures of the isolated compounds were determined by means of spectroscopic methods and by comparison of the spectral data with literature data. HRMS measurements allowed the determination of the exact molecular weights and molecular compositions of the compounds. Fragmentation of the molecules was studied by APCIMS. The most informative methods in the structure elucidation were 1D and 2D NMR methods, including ^{1}H NMR, JMOD, ^{1}H-^{1}H-COSY, NOESY, HMQC and HMBC techniques.

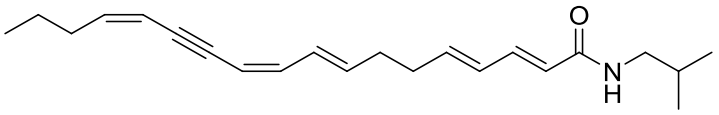
5.4.1. Alkamides from *Lepidium meyenii*

Three macamides were identified as *N*-(3-methoxybenzyl)-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**1**), *N*-benzyl-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**2**) and *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**) by comparison of their ^{1}H NMR spectral data with those reported in the literature.[41]



5.4.2. Alkamides from *Heliopsis helianthoides* var. *scabra*

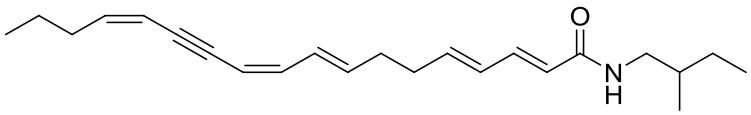
Compound 4 was isolated as a colorless oil with a strong tingling effect characteristic of numerous NAAs.[35] It was shown by APCIMS to have a quasimolecular ion peak at *m/z* 326 $[M+H]^+$, which resulted in a fragment ion at *m/z* 253 in the MS^2 due to the loss of isobutylamine. In accordance with this, the ^1H NMR spectrum of **4** confirmed an *N*-isobutylamide part of the molecule [δ_H 3.15 t, 1.80 sept, 0.91 d (6H)], and additionally contained signals proving the **octadeca-2*E*,4E,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide** structure.[44] This compound was isolated earlier from another species of the genus (*H. buphthalmoides*),[44,51] but its ^{13}C NMR data are reported here for the first time.



(4): ^1H NMR ($CDCl_3$, 500 MHz) δ 5.85 (1H, d, *J* = 15.0 Hz, H-2), 7.18 (1H, dd, *J* = 15.0, 10.8 Hz, H-3), 6.15 (1H, dd, *J* = 15.1, 10.8 Hz, H.4), 6.07 (1H, m, H-5), 2.29 (4H, m, H-6, H-7), 5.93 (1H, dt, *J* = 10.8, 3.2 Hz, H-8), 6.63 (1H, dd, *J* = 15.1, 10.8, H-9), 6.33 (1H, t, *J* = 10.8, H-10), 5.50 (1H, dd, *J* = 10.6, 1.6, H-11), 5.66 (1H, d, *J* = 10.5, H-14), 6.08 (1H, m, H-15), 2.33 (2H, q, *J* = 7.4 Hz, H-16), 1.47 (2H, m, H-17), 0.95 (3H, t, *J* = 6.5 Hz, H-18), 3.15 (1H, t, *J* = 6.5, H-2'), 1.80 (1H, sept., *J* = 6.7 Hz, H-3'), 0.91 (6H, d, *J* = 6.7 Hz, H-4', H-5'); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.4 (C-1), 143.5, 141.1, 140.6, 139.0, 136.7, 128.8, 128.4, 122.4, 109.3, 107.7 (C-2 – C-5, C-8 – C-11, C-14, C-15), 92.4, 90.8 (C-12, C-13), 46.9 (C-1'), 32.4, 32.3, 32.2, 32.1 (C-6, C-7, C-16, C-17), 28.5 (C-1'), 22.0 (C-2'), 20.0 (C-3', C-4'), 13.8 (C-18); APCIMS (positive) *m/z* 326 $[M+H]^+$, 253 $[(M+H)–C_4H_{11}N]^+$, 225 $[(M+H)–C_4H_{11}N–CO]^+$, 211 $[(M+H)–C_4H_{11}N–CH_2CO]^+$, 185, 181, 171, 158, 143, 131, 117.

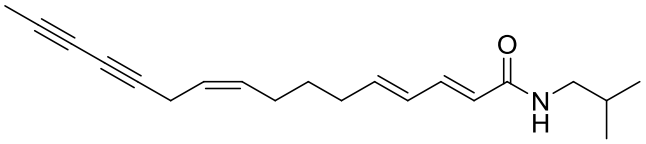
Compound 5 was obtained as a colorless oil with a light tingling effect. Its APCIMS spectrum contained a quasimolecular peak at *m/z* 340 $[M+H]^+$, which afforded the fragment ions *m/z* 253 $[(M+H)–C_5H_{13}N]^+$, 235 $[(M+H)–C_5H_{13}N–H_2O]^+$, 225 $[(M+H)–C_5H_{13}N–CO]^+$, 211 $[(M+H)–C_5H_{13}N–CH_2CO]^+$, revealing a methylbutylamide structural part. Analysis of the

^{1}H NMR spectrum confirmed that compounds **4** and **5** are homologues since they exhibited the same spectroscopic features as regards the acid part of the molecule. The ^{1}H NMR spectra were different only in the signals of a 2-methylbutylamide [δ_H 3.28 m (H-2'a), 3.15 m (H-2'b), 1.57 m (H-3'), 1.40 m (H-4'a), 1.17 m (H-4'b) and 0.91 (H-5', H-6')] in compound **5** instead of isobutylamide. The new compound **5** was therefore assigned as **octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid 2'-methylbutylamide**.



(5): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.78 (1H, d, *J* = 15.0 Hz, H-2), 7.19 (1H, dd, *J* = 15.0, 10.9 Hz, H-3), 6.15 (1H, dd, *J* = 15.0, 10.4 Hz, H.4), 6.06 (1H, m, H-5), 2.27 (4H, m, H-6, H-7), 5.93 (1H, m, H-8), 6.64 (1H, dd, *J* = 15.0, 10.9, H-9), 6.33 (1H, t, *J* = 10.9, H-10), 5.51 (1H, brd, *J* = 10.4, H-11), 5.66 (1H, d, *J* = 10.4, H-14), 6.06 (1H, m, H-15), 2.33 (2H, q, *J* = 7.3 Hz, H-16), 1.47 (2H, m, H-17), 0.96 (3H, t, *J* = 7.4 Hz, H-18), 3.28 (1H, m, H-2'a), 3.15 (1H, m, H-2'b), 1.57 (1H, m, H-3'), 1.40 (1H, m, H-4'a), 1.17 (1H, m, H-4'b), 0.91 (3H, t, *J* = 7.2 Hz, H-5'), 0.91 (3H, d, *J* = 6.8 Hz, H-6'); APCIMS (positive) *m/z* 340 $[M+H]^+$, 253 $[(M+H)–C_5H_{13}N]^+$, 235 $[(M+H)–C_5H_{13}N–H_2O]^+$, 225 $[(M+H)–C_5H_{13}N–CO]^+$, 211 $[(M+H)–C_5H_{13}N–CH_2CO]^+$, 185, 181, 159; HRESIMS *m/z* 340.2559 $[M+H]^+$ (calcd for $C_{23}H_{34}NO$, 340.2562).

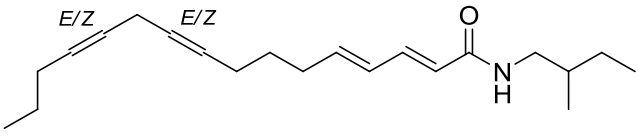
Compound 6 was isolated as colorless oil, and its HRESIMS displayed a quasimolecular ion peak at *m/z* 298.2094 $[M+H]^+$, indicating the molecular formula $C_{20}H_{27}NO$. The ^{1}H and ^{13}C NMR spectra exhibited resonances for an isobutylamide [δ_H 3.17 t (H-2'), 1.81 sept. (H-3'), 0.93 d (H-4', 5'); δ_C 46.9 (C-2'), 28.6 (C-3') and 20.1 (C-4',5')] and a polyunsaturated C_{16} acid part. From the HSQC spectrum, the chemical shifts of the protonated carbons were assigned, and the proton-proton connectivities were then studied. The ^{1}H-^{1}H COSY spectrum defined the following structural fragment with correlated protons: -CH=CH-CH=CH-CH_2-CH_2-CH_2-CH=CH-CH_2- [δ_H 5.78 d, 7.19 dd, 6.15 dd, 6.03 dt, 2.16, 1.52 dq, 2.04 q, 5.48 dt, 5.40 dt and 3.01 d (H-2 – H-11)]. This structural unit, a tertiary methyl (δ_H 1.95 s), the carbonyl carbon (δ_C 166.3) and acetylene carbons (δ_C 75.2, 64.9, 60.9 and 59.7), were connected by analysis of the long-range C–H correlations observed in the HMBC spectrum. The two- and three-bond correlations between the carbonyl carbon (δ_C 166.3), and the proton signals at δ_H 5.78 and 7.19 (H-2 and H-3), between the methyl group (δ_H 1.95 s) and δ_C 64.9 and 60.9 (C-14 and C-15) demonstrated the presence of a hexadeca-2,4,9-trien-12,14-diynoic acid isobutylamide structure. The geometry of the C-2/C-3 and C-4/C-5 double bonds was concluded to be *E* from the coupling constants (*J* = 15.5 and 15.1 Hz, respectively), and *Z* for the C-9/C-10 olefinic unit with regard to the *J* = 10.3 Hz coupling constant. Based on these data, the



structure **hexadeca-2*E*,4*E*,9*Z*-trien-12,14-diynoic acid isobutylamide** (**6)** was elucidated for this compound.

(6): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.78 (1H, d, *J* = 15.5 Hz, H-2), 7.19 (1H, dd, *J* = 15.5, 10.8 Hz, H-3), 6.15 (1H, dd, *J* = 15.1, 10.8 Hz, H-4), 6.03 (1H, dt, *J* = 15.1, 7.0, H-5), 2.16 (2H, *J* = 7.0 Hz, H-6), 1.52 (2H, dq, *J* = 7.3, 7.3 Hz, H-7), 2.04 (2H, q, *J* = 7.1 Hz, H-8), 5.48 (1H, dt, *J* = 10.3, 7.2 Hz, H-9), 5.40 (1H, dt, *J* =10.3, 6.8 Hz, H-10), 3.01 (2H, d, *J* = 7.3 Hz, H-11), 1.95 (3H, s, H-16), 5.53 (1H, brs, NH), 3.17 (2H, t, *J* = 6.5 Hz, H-2'), 1.81 (1H, sept, *J* = 6.7 Hz, H-3'), 0.93 (6H, d, *J* = 6.7 Hz, H-4', 5'); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.3 (C-1), 122.1 (C-2), 141.0 (C-3), 128.9 (C-4), 142.1 (C-5), 32.1 (C-6), 28.0 (C-7), 26.4 (C-8), 132.3 (C-9), 122.4 (C-10), 17.8 (C-11), 75.2, 64.9, 60.9, 59.7 (C-12 – C-15), 4.5 (C-16), 46.9 (C-2'), 28.6 (C-3'), 20.1 (C-4', C-5'); HRESIMS *m/z* 298.2094 $[M+H]^+$ (calcd for $C_{20}H_{28}NO$, 298.2092).

Compound 7, a colorless oil with a strong tingling effect, was found to have the molecular formula $C_{21}H_{35}NO$ as confirmed by the quasimolecular ion peak at *m/z* 317.2717 in the HRESIMS. In accordance with this,

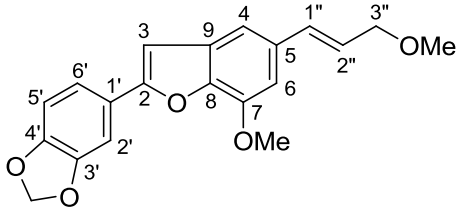


the ^{1}H and ^{13}C NMR spectra showed signals for a 2-methylbutylamide [δ_H 3.28 m (H-2'a), 3.15 m (H-2'b), 1.57 m (H-3'), 1.40 m (H-4'a), 1.17 m (H-4'b) and 0.93 t (H-5', H-6'); δ_C 45.2 (C-2'), 35.0 (C-3'), 27.0 (C-4'), 11.3 (C-5') and 17.2 (C-6')]. Additionally, the NMR spectra exhibited resonances for a C_{16} unsaturated fatty acid residue containing 1 methyl, 6 methylene, 8 methine and 1 carbonyl group. Analysis of the proton-proton connectivities in the ^{1}H-^{1}H COSY spectrum led to the identification of the hexadeca-2,4,9,12-tetraenoate chain. The stereochemistry of the C-2/C-3 and C-4/C-5 olefins was established as *E* on the basis of the coupling constants $J_{2,3}$ 15.0 and $J_{4,5}$ 15.3 Hz. Unfortunately, the *E* or *Z* geometry of the C-9/C-10 and C-12/C-13 double bond could not be determined because of the overlapping proton signals. The HMBC spectrum provided information on the connection of the acyl and amine parts of the molecule by long-range correlation between C-1 (δ_C 166.1) and H-2' (δ_H 3.28 m and 3.15 m), and corroborated the hexadeca-2,4,9,12-tetraenoate structure. The above evidence confirmed the structure of compound **7** as **hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2'-methylbutylamide**.

(7): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.75 (1H, d, *J* = 15.0 Hz, H-2), 7.19 (1H, dd, *J* = 15.0, 10.6 Hz, H-3), 6.14 (1H, dd, *J* = 15.3, 10.6 Hz, H-4), 6.05 (1H, dt, *J* = 15.3, 6.8, H-5), 2.17 (2H, *J* = 6.8 Hz, H-6), 1.50 (2H, m, H-7), 2.06 (2H, m, H-8), 5.46 (4H, m, H-9, H-10, H-12, H-13), 3.02 (1H, brd, *J* = 5.1 Hz, H-11a), 3.09 (1H, d, *J* = 5.7 Hz, H-11b), 2.06 (2H, m, H-14), 1.41 (2H, m, H-15), 0.92 (3H, t, *J* = 7.0 Hz, H-16), 3.28 (1H, m, H-2'a), 3.15 (1H, m, H-2'b), 1.57 (1H, m, H-3'), 1.41 (1H, m, H-4'a), 1.17 (1H, m, H-4'b), 0.93 (3H, t, *J* = 7.2 Hz, H-5'), 0.93 (3H, d, *J* = 6.8 Hz, H-6'); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.1 (C-1), 122.0 (C-2), 141.0 (C-3), 128.6 (C-4), 142.4 (C-5), 32.3 (C-6), 28.4 (C-7), 26.5 (C-8), 2 × 124.9 and 2 × 130.9 (C-9, C-10, C-12, C-13), 17.8 (C-11), 26.5 (C-14), 22.5 (C-15), 13.6 (C-16), 45.2 (C-2'), 35.0 (C-3'), 27.0 (C-4'), 11.3 (C-5'), 17.2 (C-6'); HRESIMS *m/z* 318.2717 $[M+H]^+$ (calcd for $C_{21}H_{36}NO$, 318.2718).

5.4.3. Lignans from *Heliopsis helianthoides* var. *scabra*

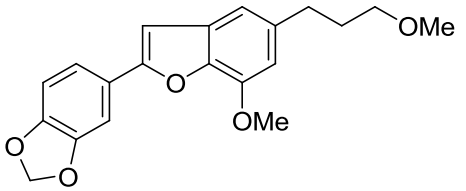
Compound 8 was isolated as an amorphous solid. HRESIMS and detailed 1D and 2D NMR spectroscopic studies indicated the 2-arylbenzofuran-type norneolignan structure. The positive-ion HRESIMS spectrum revealed the quasimolecular ion peak at *m/z* 361.1045 $[M+Na]^+$ (calcd for 361.1052 $C_{20}H_{18}O_5Na$), indicative of the molecular formula $C_{20}H_{18}O_5$. From the ^1H NMR and JMOD spectra, 2 methoxy (δ_H 4.05 s, 3.41 s, δ_C 56.1, 56.9) and 1 methylenedioxy groups (δ_H 6.01 s, δ_C 101.3) were clearly identified, in addition to a 17 carbon-containing skeleton involving 8 quaternary carbons, 8 methine groups and 1 methylene groups. The ^1H-^1H COSY spectrum revealed 2 aromatic rings, 1 tetrasubstituted ring (ring A) according to the correlation of the protons at δ_H 7.14 (H-4) and 6.87 (H-6), and 1 trisubstituted ring (ring B), as demonstrated by the correlative signals of an ABX spin system [δ_H 7.32 d (0.7) (H-2'), 6.87 d (8.1) (H-5') and 7.40 brd (8.1) (H-6')]. Further, the ^1H-^1H COSY spectrum showed the presence of an unsaturated C_3 unit with correlated protons at δ_H 6.67, 6.26 and 4.12 ppm. These structural parts, together with a quaternary carbon (δ_C 156.3) and an isolated methine (δ_H 6.82 s, δ_C 100.5), were connected by inspection of the long-range C–H correlations observed in the HMBC spectrum. The 2- and 3-bond correlations between the quaternary carbon C-2 and H-3 and H-2', and between the methine proton H-3 and C-9 and C-8 signals revealed that rings A and B are connected through a furan ring, altogether forming a norneolignan structure. The correlations of the methoxy signal at δ_H 4.05 with the carbon signal δ_C 145.2 (C-7) indicated the presence of the methoxy group on C-7. Similarly, the HMBC cross-peak of the methoxy signal at δ_H 3.41 with the carbon signals at δ_C 73.2 (C-3") demonstrated the connection of the methoxy group to the C_3 unit. The position of this 3"-methoxypropenyl group on C-5 was proved by the long-range correlation between C-1" and H-4 and H-6. The methylenedioxy group on ring B was indicated by the 3-bond HMBC correlations between the proton signal at δ_H 6.01 and C-3' and C-4'. The substitution pattern was also corroborated by the NOESY cross-peaks between H-3/H-2', 7-OCH_3/H-6, H-6/H-1", H-1"/H-4, H-4/H-3 and 3"-OCH_3/H-3". The *E* geometry of the C-1"/C-2" olefin was deduced from the coupling constant of 15.8 Hz. All of the above evidence confirmed the structure of this compound as **1"-dehydroegonol 3"-methyl ether** (**8**).



(**8**): ^1H NMR ($CDCl_3$, 500 MHz) δ 6.82 (1H, s, H-3), 7.14 (1H, brs, H-4), 6.87 (1H, brs, H-6), 7.32 (1H, d, *J* = 0.7 Hz, H-2'), 6.87 (1H, d, *J* = 8.1 Hz, H-5'), 7.40 (1H, brd, *J* = 8.1 Hz, H-6'), 6.67 (1H, d, *J* = 15.8 Hz, H-1"), 6.26 (1H, dt, *J* = 15.8, 6.1 Hz, H-2"), 4.12 (2H, d, *J* = 6.0 Hz, H-3"), 4.05 (3H, s, 7-OCH_3), 3.41 (3H, s, 3"-OCH_3), 6.01 (2H, s, 3"-OCH_2O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.7 (C-2), 100.5 (C-3), 111.8 (C-4), 137.8

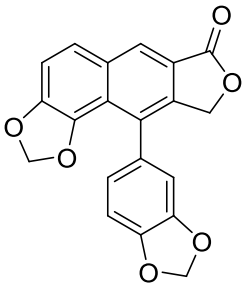
(C-5), 104.7 (C-6), 144.8 (C-7), 142.8 (C-8), 131.0 (C-9), 124.8 (C-1'), 105.5 (C-2'), 148.2 (C-3'), 148.2 (C-4'), 108.6 (C-5'), 119.3 (C-6'), 133.1 (C-1"), 124.8 (C-2"), 73.2 (C-3"), 56.1 (7-OCH_3), 56.9 (3"-OCH_3), 101.3 (-OCH_2O-). HRESIMS *m/z* 361.1045 $[M+Na]^+$ (calcd for $C_{20}H_{18}O_5Na$, 361.1052); APCIMS positive *m/z* 339 $[M+H]^+$, 307 $[(M+H)–CH_3OH]^+$, 279 $[(M+H)–CH_3OH–CO]^+$, 249, 221, 149.

Compound 9 was obtained as an amorphous solid. Its HRESIMS spectrum displayed a quasimolecular ion peak at *m/z* 363.1202 $[M+Na]^+$, indicating the molecular formula $C_{20}H_{20}O_5$. Similarly as in the case of compound **8**, the ^1H NMR and JMOD spectra showed the presence of 2 methoxy (δ_H 4.03 s, 3.36 s, δ_C 56.1, 58.6) and 1 methylenedioxy (δ_H 6.01 s, δ_C 101.3) substituents in **9**. After the ^1H and ^{13}C NMR data on **9** had been assigned by analysis of its ^1H-^1H COSY, HSQC and HMBC spectra, it was obvious that compounds **8** and **9** are based on the same parent system and differ only in the C_3 side-chain. The absence of signals of a disubstituted olefin group (C-1", C-2") and the appearance of signals of a saturated C_3 side-chain (δ_H 2.75 t, 1.94 dq, 3.41 t, δ_C 32.6, 31.8, 71.9) indicated the presence of a 3"-methoxypropyl group in **9**, in contrast with the 3"-methoxypropenyl residue in **8**. All of the above data were compatible with the **egonol 3"-methyl ether** structure of compound **9**.



(**9**): ^1H NMR ($CDCl_3$, 500 MHz) δ 6.79 (1H, s, H-3), 6.96 (1H, s, H-4), 6.63 (1H, s, H-6), 7.32 (1H, s, H-2'), 6.87 (1H, d, *J* = 8.1 Hz, H-5'), 7.40 (1H, d, *J* = 8.0 Hz, H-6'), 2.75 (2H, t, *J* = 7.7 Hz, H-1"), 1.94 (2H, dq, *J* = 7.1, 6.5 Hz, H-2"), 3.41 (2H, t, *J* = 6.4 Hz, H-3"), 4.03 (3H, s, 7-OCH_3), 3.36 (3H, s, 3"-OCH_3), 6.01 (2H, s, -OCH_2O-); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.3 (C-2), 100.4 (C-3), 112.3 (C-4), 107.5 (C-6), 145.2 (C-7), 143.8 (C-8), 131.1 (C-9), 124.8 (C-1'), 105.5 (C-2'), 148.1 (C-3'), 147.9 (C-4'), 108.6 (C-5'), 119.2 (C-6'), 32.6 (C-1"), 31.8 (C-2"), 71.9 (C-3"), 56.1 (7-OCH_3), 58.6 (3"-OCH_3), 101.3 (-OCH_2O-). HRESIMS *m/z* 363.1202 $[M+Na]^+$ (calcd for $C_{20}H_{20}O_5Na$, 363.1208); APCIMS positive *m/z* 341 $[M+H]^+$, 309 $[(M+H)–CH_3OH]^+$, 281 $[(M+H)–CH_3OH–CO]^+$, 251, 223, 149.

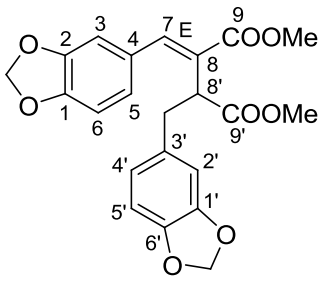
Compound **10** was obtained as a yellow substance with a strong blue fluorescence at 360 nm. It was identified as **helioxanthin** by comparison of its spectroscopic data with those published in the literature.[148] Helioxanthin has been isolated from several genera, and it is the most widely investigated lignan from the *Heliopsis* genus.



(**10**): ^1H and ^{13}C NMR data identical with those published in [148]. APCIMS (positive mode) *m/z* 349 $[M+H]^+$, 319 $[(M+H)–CH_2O]^+$, 305, 301, 291, 275.

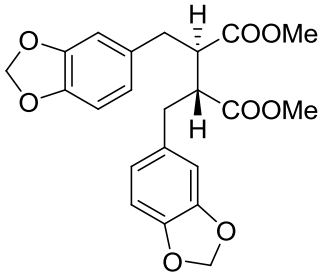
Compound **11**, a colorless oil, was identified on the basis of ESIMS and 1D and 2D NMR spectroscopy as **(7*E*)-7,8-dehydroheliobuphthalmin**. This compound has earlier been reported from *H. bupthalmoides*, without ^{13}C NMR spectroscopic data.[51] In our 2D NMR

experiments, the chemical shifts assignment of all carbons were assigned as listed below. It has also been published from *Biota orientalis* leaves.[134]



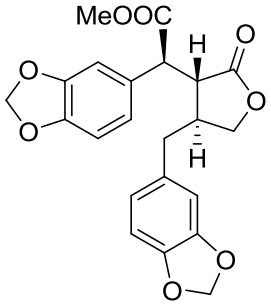
(**11**): $[\alpha]^{24}_{D}$ = –175 (*c* 0.1, $CHCl_3$); ^{1}H NMR data: identical with published data;[51] ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.5 (C-1, C-1'), 147.7 (C-2, C-2'), 108.4 (C-3), 128.8 (C-4), 122.5 (C-5), 108.2 (C-6), 142.5 (C-7), 129.5 (C-8), 167.1 (C-9), 109.4 (C-3'), 133.2 (C-4'), 122.1 (C-5'), 107.8 (C-6'), 35.7 (C-7'), 45.5 (C-8'), 173.1 (C-9'), 101.2 (1,2- -OCH_2O-), 100.7 (1',2'- -OCH_2O-), 52.0 (9-OCH_3), 52.2 (9'-OCH_3).

By means of APCIMS and ^{1}H and ^{13}C NMR spectroscopy and comparison of the measured data with published values,[133] the amorphous solid compound **12** was determined to be **heliobuphthalmi**n with the *threo* configuration at C-8 and C-8'. This compound has also been identified from the whole plant of *Justicia ciliata*[149] and the stem bark of *Pycnanthus angolensis*.[132]



(**12**): $[\alpha]_D^{24}$ = –3 (*c* 0.2, $CHCl_3$); ^{1}H and ^{13}C NMR data: identical with those published in.[133] APCIMS (positive mode) *m/z* 415 $[M+H]^+$, 383 $[(M+H)–CH_3OH]^+$, 351, 333, 135.

From a consideration of its APCIMS spectrum and ^{13}C NMR chemical shift values,[150] compound **13** was found to be identical with **7-acetoxyhinokinin**. Our 2D NMR analysis led to the complete assignment of the ^{1}H NMR data as listed below.



(**13**): $[\alpha]_D^{26}$ = –46 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.65 (1H, s, H-2), 6.36 (1H, d, *J* = 7.9 Hz, H-5), 6.64 (1H, d, *J* = 7.9 Hz, H-6), 6.14 (1H, d, *J* = 3.4, H-7), 2.70 (1H, dd, *J* = 6.1, 3.4 Hz, H-8), 6.34 (1H, s, H-2'), 6.73 (1H, d, *J* = 7.9 Hz, H-5'), 6.65 (1H, d, *J* = 7.9 Hz, H-6'), 2.46 (1H, dd, *J* = 13.8, 8.2 Hz, H-7'a), 2.35 (1H, dd, *J* = 13.8, 7.4 Hz, H-7'b), 2.81 (1H, m, H-8'), 4.29 (1H, t, *J* = 8.7 Hz, H-9'a), 3.97 (1H, dd, *J* = 8.7, 5.6 Hz, H-9'b), 5.97, 5.96 (each 1H, s, -OC*H*$_2$O-), 5.93, 5.92 (each 1H, d, *J* = 1.0 Hz, -OC*H*$_2$O-), 2.13 (3H, s, OAc); APCIMS (positive mode) *m/z* 413 $[M+H]^+$, 353 $[(M+H)–CH_3COOH]^+$.

5.5. PHARMACOLOGICAL INVESTIGATIONS

5.5.1. Pharmacological effects of *N*-Alkylamides

The isolated NAAs were examined for their possible activities on different targets within the ECS by *Jürg Gertsch et al* (Institute of Biochemistry and Molecular Medicine, NCCR TransCure, University of Bern, Bern, Switzerland).[145]

From the studied compounds (**1**, **3**-**7**) no NAA had any significant effect on MAGL (which is the major 2-arachidonoylglycerol-degrading enzyme). Only macamide **3** showed a

low micromolar inhibition of FAAH (IC_{50} = 4.07 μM), as expected.[75] Compound **3** caused a significant submicromolar inhibition of AEA uptake (IC_{50} = 0.67 μM), which was even more potent than the inhibition measured with the reference inhibitors OMDM-2 (IC_{50} = 4.12 μM) and UCM707 (IC_{50} = 1.46 μM. Because the AEA cellular uptake inhibition by **3** was more potent than the FAAH inhibition, this compound shows certain selectivity toward the first process. Only **3** also showed a significant binding affinity toward CB receptors, with an unexpected tenfold selectivity towards CB_1 (K_i = 0.48) over CB_2 receptors (K_i = 4.11). The NAAs from *H. helianthoides* var. *scabra* were not active on FAAH or AEA uptake, but **7** showed a potent binding interaction with the CB_1 receptor (K_i = 0.31 μM).

Table 2. Summary of the effects of the isolated NAAs on ECS targets.[a]

Cpd.	**AEA cell uptake** IC_{50} (μM) (95% CI)	Effi-cacy (%)	**FAAH** IC_{50} (μM) (95% CI)	Effi-cacy (%)	**MAGL** IC_{50} (μM) -	**CB_1** K_i (μM) (95% CI)	**CB_2** K_i (μM) (95% CI)
1	>100	44	19.05 (13.8–28.18)	85	>100	8.67 (4.66–13.75)	>50
2	84.36 (72.44–>100)	53	11.48 (6.81–18.78)	85	>100	8.88 (3.79–10.19)	43.96 (24.16–>50)
3	0.67 (0.47–0.97)	73	4.07 (2.95–5.62)	83	>100	0.48 (0.33–0.67)	4.11 (3.11–5.66)
4	2.45 (1.28–4.70)	75	17.78 (13.18–25.70)	84	> 100	8.60 (2.39–10.92)	9.18 (5.41–9.84)
6	4.33 (2.69–6.97)	75	12.30	79	>100	>20 (6.28–>20)	22.55 (12.68–25.30)
7	2.15 (0.87–5.31)	51	19.95 (12.59–33.11)	80	>100	0.31 (0.18–0.59)	1.21 (0.90–1.71)
OMDM-2	4.12 (2.01 – 12.19)	72	23.29 (10.72 – 48.98)	82	n.d.	n.d.	n.d.
UCM707	1.46 (1.18 – 1.80)	67	7.24 (6.03 – 13.18)	79	n.d.	n.d.	n.d.

[a] Data are mean values from at least three independent experiments, and the 95% confidence interval (CI) is shown; OMDM-2 and UCM-707 were used as positive controls for AEA uptake. n.d., not determined.

5.5.2. Pharmacological effects of lignans

All of the isolated lignans (**8-13**) were examined for their possible activities on melanomas brain metastases formation by *János Haskó et al.* (Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary). The results are discussed in detail in [146] and in the Ph.D. thesis of *János Haskó* (under preparation).

Briefly, the tested lignans had no cytotoxic effect on the brain endothelial cells and A2058 melanoma cells in the range of concentrations used during the experiments (1-10 μM). Only **10** and **11** (each 5 μM) induced morphological changes and both lignans effectively reduced the rate of migration of melanoma cells. Compound **10** (2 μM) decreased the number of melanoma cells attached to the endothelial layer compared to the untreated control, however the other compounds had no observed effect on the melanoma cell – endothelial cell interactions.

Since only **10** and **11** were effective in the above-mentioned experiments, subsequent experiments were performed with **10** (5 μM) and **11** (5 μM in rat, 2 and 5 μM in human brain endothelial cells). A significant improvement of the barrier function of cerebral endothelial cells was observed in the presence of both compounds.

Treatment of endothelial cells with **10** or **11** resulted in a more pronounced immunostaining of ZO-1 (an important protein component of tight junctions) on the edge of endothelial cells. Each of these compounds led to a significant concentration-dependent reduction (2-10 μM) in endothelial cell migration.

5.6. QUALITY CONTROL OF MACA-CONTAINING DIETARY SUPPLEMENTS

5.6.1. Method validation

A linear calibration plot (y = 1 871 710.20x + 3 015.43) was established with a correlation coefficient (R^2) of 1.00, which allowed reliable quantification. The *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**) (a major alkamide of Maca) content of 6 preparations was below the limit of detection and above the limit of quantification in the case of 8 products. The intraday precision ranged between 0.80 and 1.11%. These results underline the good repeatability of our method. The recovery was 97% (RSD% 0.80) for the first concentration, and 99.6% (RSD% 2.90) and 98.8% (RSD%) for the second and third concentrations, respectively. These results are in accordance with recovery values of previously published methods[41,151] and confirm the good accuracy of our method. The stability revealed resulted that the *N*-benzyl-

(9*Z*,12*Z*)-octadecadienamide contents of *n*-hexane extracts stored for 3 and 4 days at room temperature were 96.53 and 103.40% of those of the original samples. These results indicated that the decomposition of this macamide is not significant and analysis can therefore be carried out reliably on fresh samples.

5.6.2. Product analysis

A total of 14 Maca containing mono- (5) and multicomponent (9) preparations (distributed by 11 companies) were randomly selected and purchased. The presence of Maca was confirmed in 8 products (Table 3). The HPLC-UV chromatograms (210 nm) of the Maca-containing products were similar to that of Raw Organic Maca Powder (Figure 5).

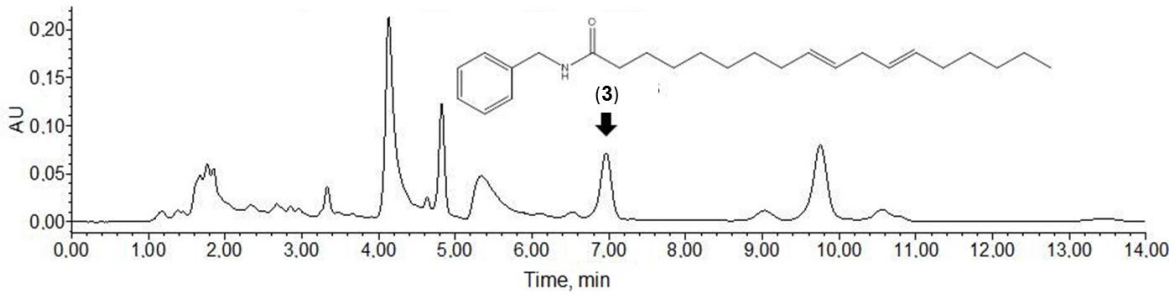


Figure 5. HPLC-UV chromatogram of a Maca extract (210 nm)

The Maca powders contained 28.0-225.8 μg *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**) /g. In 6 preparations, the concentration of the marker macamide was below the detection limit and 1 preparation was adulterated with a synthetic phosphodiesterase inhibitor. Thiosildenafil, a derivative of sildenafil, was detected by TLC and HPLC-DAD (retention time 7.2 min, maximal UV absorption at 282 nm) in this product.

Table 3. Analysis of 14 Maca-containing dietary supplements

Product (origin)	Pharmaceutical form	Recommended daily dosage	MACA-3 (μg/g product)	MACA-3 in the recommended daily dosage (μg)
1. (Peru) Raw Organic Maca Powder (yellow)	powder[1]	3-5 g	193.1	579.3–955.5
2. (Peru) Red Maca Powder	powder[1]	no data	225.8	
3. (Peru) Black Maca Powder	powder[1]	no data	63.0	
4. (Peru) Gelatinized Maca Powder	powder[1]	no data	28.0	
5. (Peru)	tablet[1]	1500 mg	45.2	67.8
6. (EU)	capsule[2]	1 capsule	30.9	15.45
7. (UK)	capsule[2]	1 capsule per day for 5 days, then 2 days break	2.6	1.3
8. (China)	tablet[2]	1-2 tablets per day, for 4-6 weeks	13.9	13.9–27.8
9. (China)	capsule[2]	maximum 1 capsule in every 3 days	n.d. **Presence of thiosildenafil**	
10. (China)	capsule[2]	1-2 capsules 30 min before sexual activity	n.d.	
11. (EU)	capsule[2]	2 capsules before sexual activity	n.d.	
12. (China)	capsule[2]	1-2 capsules 1 hour before sexual activity	n.d.	
13. (China)	ampole[2]	1 ampole	n.d.	
14. (UK)	spray[2]	3-5 puffs locally	n.d.	

[1,] monocomponent, [2] multicomponent preparations

6. DISCUSSION

6.1. TREATMENT OF CNS DISTURBANCES WITH PLANTS FROM THE MEDICINE OF PORVENIR

The Bajo Paraguá CTO has one physician, who lives in the largest community, Piso Firme, and visits the other villages about once a month or in the event of emergency. There is a nurse too, who lives in Porvenir, but the inhabitants prefer their own, traditional medicine and contact the nurse merely secondarily, or the physician only in grave cases. There is one

traditional healer in the community, but he is known as a "black healer", and is therefore not too popular. The majority of the inhabitants who are advanced in years tend to make use of self-therapy. The medicine in the community reflects the local traditions and their changes very well. The low number of rituals and plants used for them, and the ever more frequent communication with other communities or towns demonstrate how the community is coming under the influence of conventional medicine, and converting to a modern Bolivian rural community.

In the most recent review article on this topic, more than 300 plant species are listed that are used for the treatment of CNS ailments by 26 native ethnic groups in Brazil.[152] The study reviews 67 different diseases or effects possibly related to the CNS, where the main categories are analgesics, antifebriles, tonics and/or adaptogens, hallucinogens, anxiolytics, anticonvulsants, hypnotics, stimulants, weight control agents, memory enhancers, and others. The high proportion of plant species used as analgesics or to counteract fever is similar in Porvenir. The reason for the high number of antifebriles is the common occurrence of malaria accompanied by fever in Bajo Paraguá. Traditional ceremonies have barely been observed; the utilization of psychoactive plants is not characteristic for the community. Three of the four plants used for nervousness are not endemic in the region; the species, and probably their application too arrived in Porvenir from the medicinal knowledge of the urban areas of Bolivia. It is believed by Bolivian people that if someone starts to work hard after eating a lot, or goes out into a strong "bad wind", or bathes in a cold stream with a heated body, his face or other parts of the body may become paralysed for a time or forever. This is the *arrebato*, the symptoms of which suggest that it may be a stroke. Less aggressive forms of the symptoms are vertigo, nausea and pain in the bones. Utilization of weight-controlling species occurs in Porvenir, but in comparison with some Amazonian native traditions, where a slim appearance is not preferable and weight-gaining plants are used, one species used in Porvenir is indicated for gaining weight, and another is for losing weight, as in modern societies. The bath prepared with one species, *Cedrela fissilis*, is applied to "keep ghosts away". It seems to be only a belief, but taking into consideration that this species contains a sedative volatile oil,[153] the calming effect during the bath may also serve as a background for its application.

The ethnobotanical data that do exist in the literature correspond closely with the utilization of the plants in Porvenir, but the great majority of the species used have not been widely investigated from phytochemical or pharmacological points of view. For example, *Maclura tinctoria* (Moraceae), mora, is a tree from the rainforest, the bark of which contains a

resin that was used to treat tooth pain by 5 interviewees in Porvenir, and is also utilized in numerous other communities for similar purposes. It is used by people in other communities of Bajo Paraguá,[9] by the Ese'eja Indians from Bolivia,[154] in Peru[155] and by people in the Brazilian Mato Grosso.[156] Peruvian people treat a sore throat with the plant, and use it as a purgative, diuretic and antiviral remedy, and, similarly as in the Mato Grosso, mora is applied to combat rheumatism.[156]

6.2. ISOLATION OF COMPOUNDS

The dried hypocotyl powder of *L. meyenii* was extracted with *n*-hexane, which was the most adequate among the different solvents tested for the extraction of NAAs. Further purification was carried out with centrifugal partition chromatography (CPC) combined with HPLC. CPC was used by us for the first time to isolate NAAs and, since it has no solid stationary phase, it proved to be very useful in the isolation of highly unstable compounds such as alkamides. From Maca, three compounds (**1**-**3**) were isolated in 5.0–92.0 mg (0.00041–0.0076% of the dry plant material).

The phytochemical work on *H. helianthoides* var. *scabra* started with the TLC analysis of different cultivars of the species and their comparison with the TLC profiles of several NAA-containing plants and NAAs isolated previously in our laboratory. In view of the similar TLC profile and the tingling effect typical for NAAs[35] presented by the fresh root, the cultivar 'Asahi' was selected for further work. The plant material was extracted with MeOH at room temperature by percolation. MeOH, an amphipolar solvent, was suitable for the extraction of both lipophilic and polar compounds. Solvent–solvent extraction with $CHCl_3$ was applied in order to separate the apolar constituents. The purification was continued with more selective methods (VLC, RP-VLC, MPLC, RPC, PLC and HPLC). VLC and MPLC separation of the NAA- and lignan-containing fractions afforded crude fractionation of the main components. For final purification, RPC, PLC and HPLC were applied since these were the most effective and most selective methods. The preparative work was carried out with analytical TLC on silica gel with various solvent systems. TLC analysis was used to model the separation methods, to combine fractions, and to check the purity of the isolated compounds. The detection was carried out in UV light at 254 and 360 nm, followed by spraying with cc. H_2SO_4. Because of the instability of the double or triple bond-containing NAAs, fractions were stored under nitrogen at -10 °C during the isolation process. Ten compounds (**4**-**13**) were

isolated from *H. helianthoides* var. *scabra* yielding 2.0-15.0 mg (0.000022-0.00016% of the fresh plant material).

6.3. STRUCTURE ELUCIDATION OF THE ISOLATED COMPOUNDS

The structure determination of compounds **1-3** isolated from *L. meyenii* led to the identification of previously described[41] polyunsaturated aromatic diene and triene amide macamides, which contain 18 carbon atoms in their aliphatic chain: *N*-(3-methoxybenzyl)-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**1**), *N*-benzyl-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**2**) and *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**).

From *Heliopsis helianthoides* var. *scabra,* three new (**5**, **6**, **7**) and one known (**4**) aliphatic isobutyl- and methylbutylamides with 16 and 18 carbon atoms and three to six double and triple bonds on their fatty acid chain were identified.

Alkylamides are characteristic compounds for the *Heliopsis* genus. Octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide (**4**) was isolated previously from *H. helianthoides*,[44] but its ^{13}C NMR data are reported by us for the first time. Including the latter compound four isobutylamides were described from *H. buphthalmoides.*[44] Two more isobutylamides, scabrin and heliopsin were identified previously from *H. scabra.*[45,46] Compared with the five known alkamides of *H. longipes* (A. Gray) Blake[42,43] bearing 1-3 double bonds, *H. buphthalmoides* (Jacq.) Dunal and *H. helianthoides* (L.) Sweet contain C_{18} NAAs with the rarely occurring pentaene and hexaene acids. In the cases of the three new natural products octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid 2'-methylbutylamide (**5**), hexadeca-2*E*,4*E*,9*Z*-triene-12,14-diynoic acid isobutylamide (**6**), and hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2'-methylbutylamide (**7**), two of them contain acetylene bonds as well.

Moreover, six lignans were determined from *H. helianthoides* var. *scabra*: two new arylbenzofuran neolignans, 1''-dehydroegonol 3''-methyl ether (**8**) and egonol 3''-methyl ether (**9**), and four known lignan derivatives (**10**-**13**). From three species of the *Heliopsis* genus, a wide variety of lignans have been identified previously. Helioxanthin (**10),** an arylnaphthalene derivative, was identified earlier from the root of this species[52] and from thirteen other plants.[89,148,157–167] The dibenzylbutyrolactone helianthoidin was isolated previously[53] from the root of *H. helianthoides* var. *scabra*, but was not found by us. The dibenzylbutane (7*E*)-7,8-dehydroheliobuphthalmin (**11)** and heliobuphthalmin (**12)** were isolated for the first time from *H. helianthoides* var. *scabra*, but earlier from *H. buphthalmoides.*[50] Three more dibenzylbutane derivatives and the dibenzylbutyrolactone 7-acetoxyhinokinin (**13**) were

identified for the first time from *H. helianthoides* var. *scabra*, but earlier from *Ruta pinnata* L.[168]

6.4. ACTIVITIES OF *N*-ALKYLAMIDES ON THE ENDOCANNABINOID SYSTEM

Previous studies with NAAs have enabled the generation of some SAR information with respect to CB receptor binding.[169,170] However, the binding interactions of NAAs with the CB_1 or CB_2 receptors do not reflect a straightforward SAR, because head group modifications and variations in alkyl chain length, and double bond configurations all appear to play a role.[67,73,169] The potent new CB_1 receptor ligand **7** comprises a methylbutylamide head group combined with the typical 2*E*,4*E* double bonds in the alkyl chain,[169] with additional double bonds at carbons 9 and 12. This NAA shows an approximately four-fold selectivity for CB_1 receptors over CB_2 receptors. Moderate CB_1 receptor interactions have been described for NAAs, all containing the 2*E* double bond,[67,73] but there is no clear SAR. Based on the findings with the macamides, it is possible to identify further crucial molecular elements for CB_1 binding, FAAH and AEA transport inhibition. Macamide **3** seems to fulfil the criteria for endocannabinoid substrate mimicking, as this is the only NAA known so far to interact with several ECS targets at low micromolar or even submicromolar concentrations. It is tempting to speculate that the 9*Z*,12*Z*-octadecadiene alkyl chain present in **3** mimics the end of the arachidonoyl chain present in endocannabinoids. Thus, it will be interesting to develop this new insight further by studying NAAs with ideal head groups containing the 9*Z*,12*Z*-octadecadiene alkyl chain. In the case of the macamide **3**, the additional 15*Z* double bond present in compounds **1** and **2** is detrimental to potency. Interestingly, the *N*-benzylamide head group present in **3** seems to be ideal as substituted *N*-phenylamides have weaker potency.[171] With respect to the combined significant direct and indirect ECS effects of macamide **3**, future studies will have to establish whether this natural product is a functional agonist or antagonist at CB receptors. Moreover, it should be interesting to test whether the cannabimimetic action expected from this compound could relate to the ethnopharmacological use of Maca. Given the fact that CB_1 receptors are expressed in sperm and that the ECS appears to play a role in fertility and sperm quality,[172] the fertility enhancing effects of Maca that is widely reported in the ethnopharmacological literature[24,27,28] should be studied in the light of the present data. It has already been shown that Maca supplementation improves rat[101–105] and bovine[113] sperm quality and it would be interesting to test the hypothesis that these effects are mediated, at least in part, by ECS-targeting macamides such

as compound **3**. Overall, these results provide additional evidence of the structural and functional similarity between NAAs and endocannabinoids, potentially interlinking NAA chemodiversity, the use of NAA-containing medicinal plants and botanical dietary supplements with the ECS as a potentially major site of action.

6.5. ACTIVITIES OF LIGNANS ON MELANOMAS BRAIN METASTASIS FORMATION

Among various tumors, melanoma has very high tendency (75% of patients) to form brain metastases.[173,174] After intravasation and dissemination of cancer cells, the adhesion and the migration of tumor cells across the wall of the blood vessels formed by endothelial cells, are the key steps in the formation of brain metastases.[175] The successful inhibition of these steps might prevent the formation of these tumors.

The *in vitro* studies show that **10** and **11** exhibit various effects on melanoma cells and brain endothelial cells: impede the migration of them and **10** inhibits the adhesion of melanoma cells to the brain endothelial cells. These observations suggest that these lignans may have the potential to interfere the dissemination and the attachment of melanoma cells to the blood vessels and the angiogenesis of the metastatic tumors. The enhancement of the barrier function of the endothelial cells caused by **10** and **11** was presumably due to the strengthening of tight junctions, as the ZO-1 staining indicated.

The improvement of the barrier function – which seems to be the first confirmation in the case of lignans – may contribute to hinder the transmigration of tumor cells across the blood vessels of the brain, however further investigations are needed to study this effect. These results also draw attention to the possibility that dietary consumed lignans may play a role in the inhibition of brain metastases.

6.6. QUALITY CONTROL OF DIETARY SUPPLEMENTS

The ways and goals of application of Maca are Europe is largely different from the traditional uses. The most preferred recommendations for the products are “potency enhancer”, “for men”, “for the reproductive system”, and “mental and physical capacity enhancer”. Among thirty other herbal (28), animal (2) or fungal (1) ingredients the most preferred ones are *Serenoa repens* (labeled in 7 products), *Panax ginseng* (5), *Ginkgo biloba* (4) *Rhodiola rosea* (3) and *Urtica dioica* (3).[142] The majority of these are widely used either for symptoms of the genitourinary system or as physical or sexual performance enhancers. In cases of

monocomponent preparations, the recommended daily posologies of Maca (1500–5000 mg dry hypocotyl powder) overlap with the effective dosages used in human studies,[24,27,28] but for multicomponent products they are much lower, between 80 and 125 mg for 4 products; 500 and 750 mg in two cases; and not declared in one case. 4 products were claimed to contain "maca extract" (unspecified), and in one case dry hypocotyl powder, while in four cases no further information was given. Since Peruvian Maca consumer natives ingest 65–260 g dry hypocotyl per day,[135] even the relatively higher dosages used in human studies are too low to make a comparison between the traditional and the European applications of the plant.

The HPLC method that we developed allowed the reliable analysis of one of the major macamides of *L. meyenii*. The good separation of *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide from other components of the extracts proved the robustness of the method. HPLC-DAD analysis revealed that significant peaks with higher retention times than that of this compound do not relate to the class of macamides. Marked differences in macamide content could be detected in the cases of the different color types. The presence of Maca was confirmed in only 8 products, but five of them were the only Maca-containing preparations. Thus, according to our method two-thirds of the randomly selected multicomponent products were of inferior quality, without any presence of Maca. Moreover, one of them was adulterated with the synthetic phosphodiesterase inhibitor, thiosildenafil.

7. SUMMARY

The availability of the traditional knowledge about plants may lead to the development of several drugs for modern medicine or dietary supplements. The aims of the present study were to describe the traditional medicine utilized in Porvenir, Bolivia, and to discuss the plant species traditionally applied for CNS disturbances. Further goals were the isolation and structure elucidation of alkamides and lignans from *Lepidium meyenii* (Maca) and *Heliopsis helianthoides* var. *scabra*, both plants used in ethnomedicine, and in the frame of cooperation to test them on different targets of the CNS. Quality control of Maca-containing dietary supplements was also an aim.

Porvenir is an indigenous Bolivian community, which houses the Chiquitano mestizos and the Guarasug'we indigenous nation currently close to extinction. The medicine in Porvenir reflects the local traditional roots and their changes very well, revealing how the population is coming under the influence of conventional medicine. Altogether 107 known complaints or symptoms were described, 11 of them relating for CNS illnesses or symptoms, for which 37 plant species are used.

Phytochemical analysis of *L. meyenii* led to the isolation and structure elucidation of previously described macamides, *N*-(3-methoxybenzyl)-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**1**), *N*-benzyl-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**2**) and *N*-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**). Analysis of the fresh roots of *H. helianthoides* var. *scabra* (Dunal) Fernald led to the isolation and structure elucidation of four *N*-alkylamides, octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide (**4**), octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid 2'-methylbutylamide (**5**), hexadeca-2*E*,4*E*,9*Z*-triene-12,14-diynoic acid isobutylamide (**6**) and hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2'-methylbutylamide (**7**). Compounds **5**-**7** are new natural products, while **4** was isolated for the first time from this species.

The *N*-methylbutylamide **7** and the *N*-benzylamide **3** showed submicromolar and selective binding affinities to the CB_1 receptor (Ki values = 0.31 and 0.48 μM, respectively). Compound **3** also exhibited weak FAAH inhibition (IC_{50} = 4 μM) and was a potent inhibitor of AEA cellular uptake (IC_{50} = 0.67 μM), this inhibition being stronger than that observed with the controls. The pronounced ECS polypharmacology of compound **3** highlights the potential involvement of the arachidonoyl-mimicking 9*Z*,12*Z* double bond system in the linoleoyl group for the overall cannabimimetic action of NAAs. This study provides additional strong evidence for the endocannabinoid substrate mimicking of plant-derived

NAAs and reveals both the direct and indirect cannabimimetic action of the Peruvian Maca root.

Further phytochemical investigation of *H. helianthoides* var. *scabra* resulted in the isolation of six lignans: two new arylbenzofuran neolignans, 1"-dehydroegonol 3"-methyl ether (**8**) and egonol 3"-methyl ether (**9**), and four known lignan derivatives, helioxanthin (**10**), (7*E*)-7,8-dehydroheliobuphthalmin (**11**), heliobuphthalmin (**12**) and 7-acetoxyhinokinin (**13**).

Helioxanthin (**10**) and (7*E*)-7,8-dehydroheliobuphthalmin (**11**) exhibited various effects on melanoma and brain endothelial cells, with the potential to interfere with different steps of metastasis formation. These findings indicate that these compounds impede the migration of melanoma cells, and **11** inhibits the adhesion of melanoma cells to the brain endothelial cells. Both compounds also enhanced the barrier function and decreased the migratory properties of cerebral endothelial cells. These effects might be instrumental in preventing the transendothelial migration of melanoma cells and the vascularization of tumors.

A simple and reliable analytical protocol for the qualitative and quantitative analysis of the Maca content of dietary supplements was developed. In half of the 14 randomly selected Maca products, through the measurement of marker compounds, the Maca content could be determined by our method based on TLC and HPLC-DAD analysis. However, in 6 food supplements the concentration of the marker macamide was below the limit of detection and one of the products was adulterated with a synthetic phosphodiesterase inhibitor. The calculated Maca contents in the recommended daily doses of the majority of products were below the widely accepted posology of the plant. Our results draw attention to the need for proper quality control of Maca-containing dietary supplements.

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Annexes

Annex 1. Macamides isolated previously from *Lepidium meyenii*

Annex 2. *N*-Alkylamides isolated previously from the *Heliopsis* genus

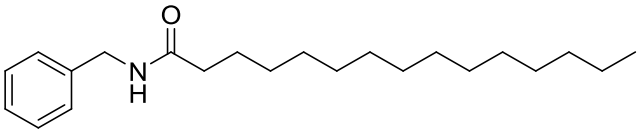
Annex 3. Lignans isolated previously from the *Heliopsis* genus

Annex 4. Plants used in Porvenir for the treatment of central nervous system disturbances

Annex 5. The isolated compounds from *L. meyenii* and *H. helianthoides* var. *scabra*

Publications on which the thesis is based

Annex 1. Macamides isolated previously from *Lepidium meyenii*[38–41]



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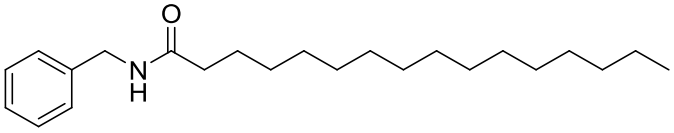

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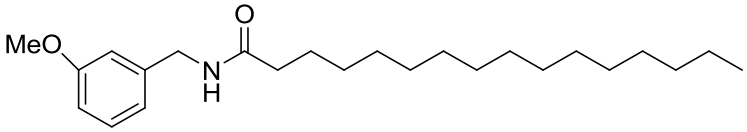

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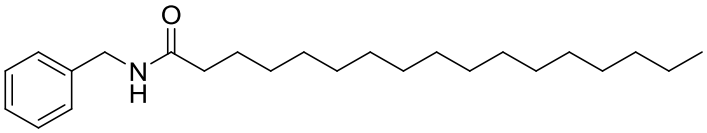

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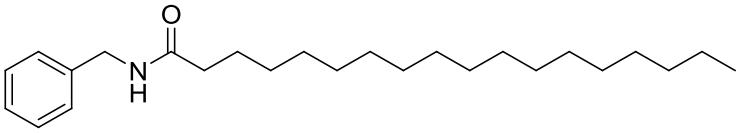

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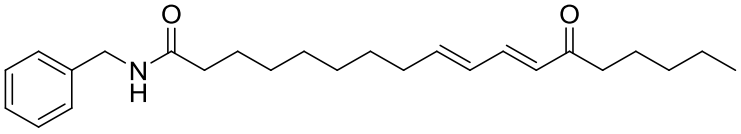

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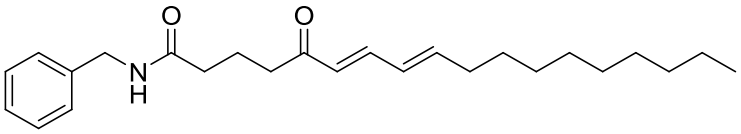

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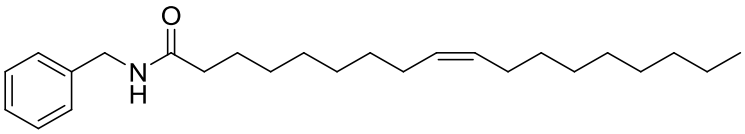

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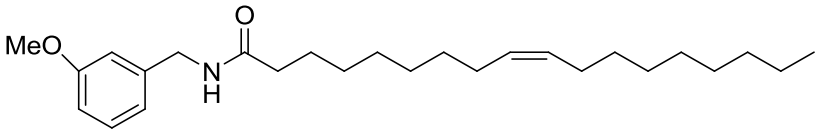

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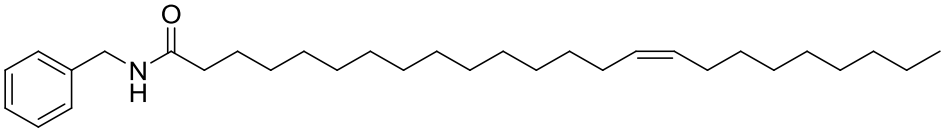

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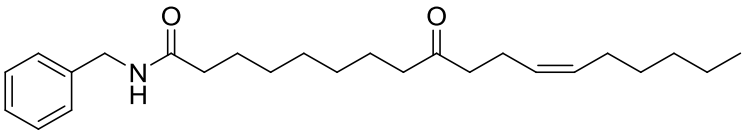

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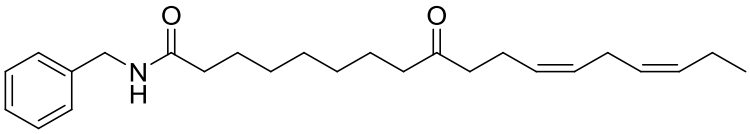

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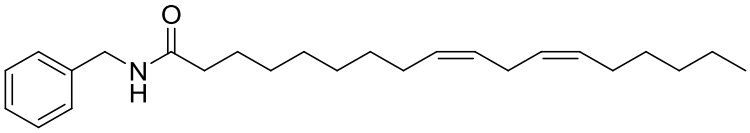

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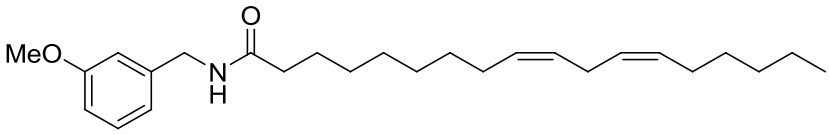

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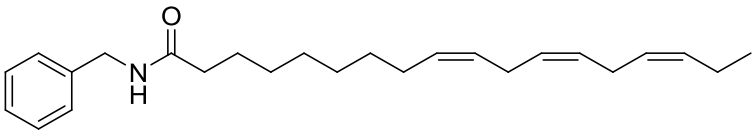

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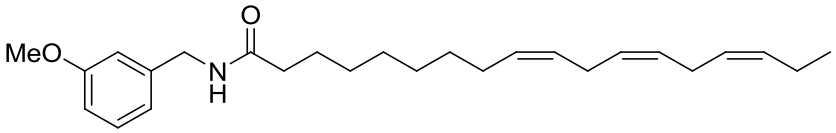

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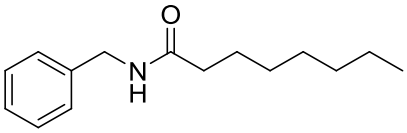

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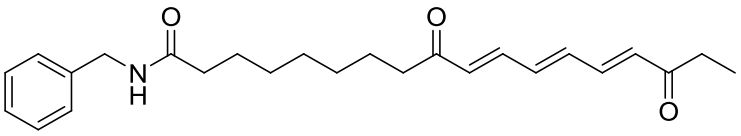
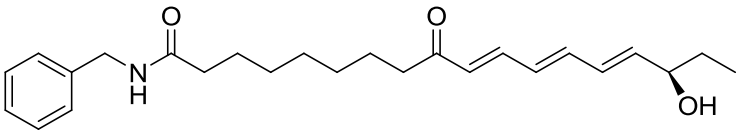
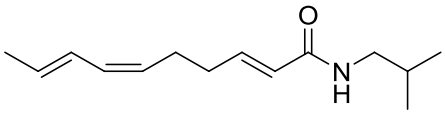


N-benzyl-9,16-dioxo-10*E*,12*E*,14*E*-octadecatriene amide

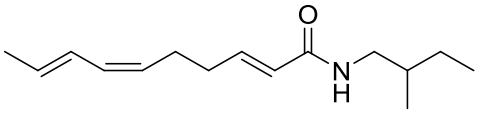


N-benzyl-9-oxo-16-hydroxy-10*E*,12*E*,14*E*-octadecatrienamide

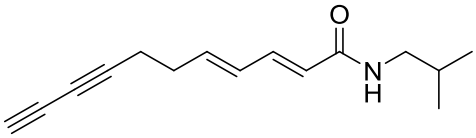
Annex 2. *N*-Alkylamides isolated previously from the *Heliopsis* genus[41–45]



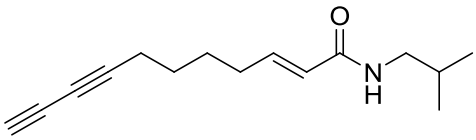
spilanthol or affinin (deca-2*E*,6*Z*,8*E*-trienoic acid isobutylamide), (*H. longipes*)



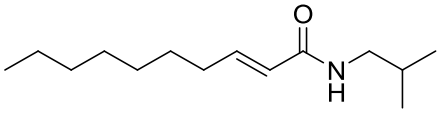
homospilanthol (deca-2*E*,6*Z*,8*E*-trienoic acid 2-methylbutylamide), (*H. longipes*)



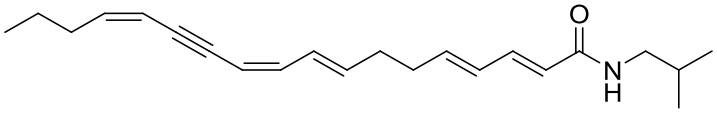
undeca-2*E*,4*E*-diene-8,10-diynoic acid isobutylamide (*H. longipes*)



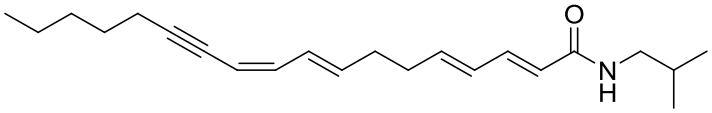
undeca-2*E*-ene-8,10-diynoic acid isobutylamide (*H. longipes*)



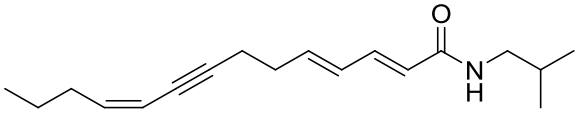
deca-2*E*-enoic acid isobutylamide (*H. longipes*)



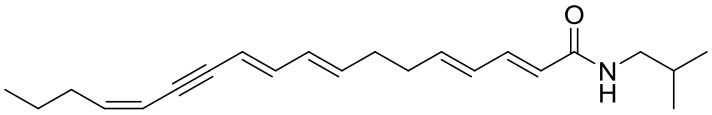
octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide (*H. buphthalmoides*)



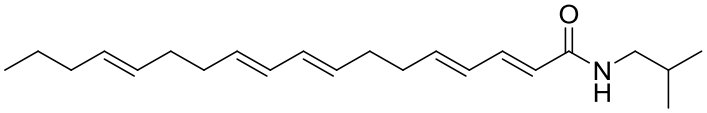
octadeca-2*E*,4*E*,8*E*,10*Z*-tetraen-12-ynoic acid isobutylamide (*H. buphthalmoides*)



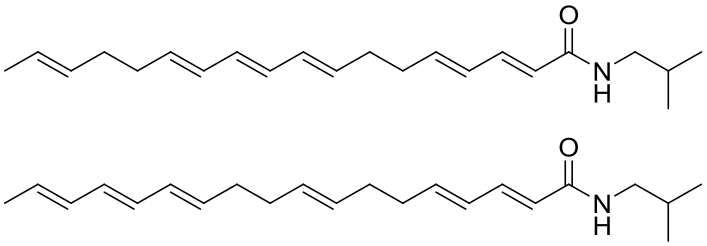
tetradeca-2*E*,4*E*,10*Z*-trien-8-ynoic acid isobutylamide (*H. buphthalmoides*)



octadeca-2*E*,4*E*,8*E*,10*E*,14*Z*-pentaen-12-ynoic acid isobutylamide (*H. buphthalmoides, H. helianthoides*)

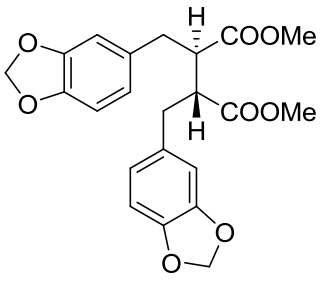


scabrin (octadeca-2,4,8,10,14-pentaenoic acid isobutylamide) (*H. scabra)*

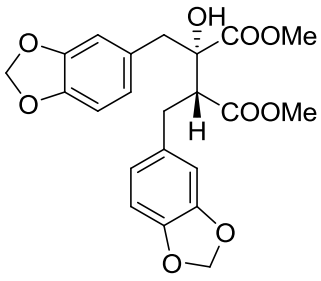


heliopsin (octadeca-2,4,8,10,12,16-hexaenoic acid isobutylamide or octadeca-2,4,8,12,14,16-hexaenoic acid isobutylamide), (*H. scabra)*

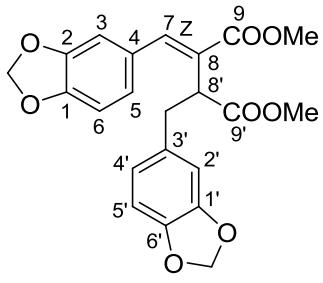
Annex 3. Lignans isolated previously from the *Heliopsis* genus[43,44,49–52]



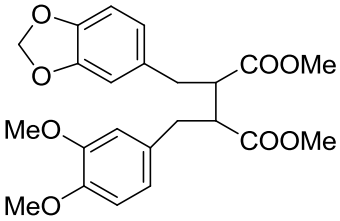
heliobuphthalmin



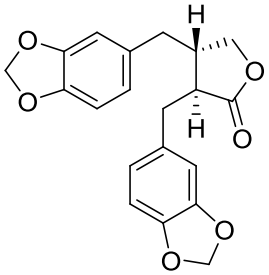
8-hydroxyheliobuphthalmin



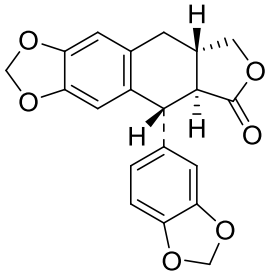
7*Z*-7,8-dehydroheliobuphthalmin



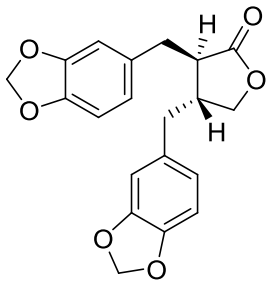
2-[(2H-1,3-benzodioxol-5-yl)methyl]-3-[(3,4-dimethoxyphenyl)methyl] succinic acid dimethylester



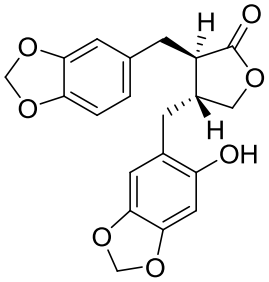
heliobuphthalmin lactone



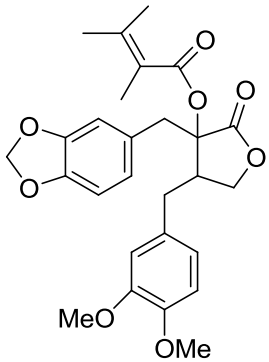
5,7'-dehydroheliobuphthalmin lactone



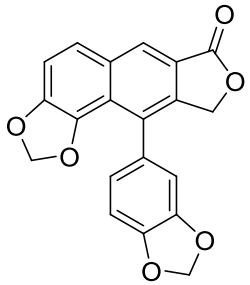
hinokinin



2′-hydroxyhinokinin



helianthoidin



helioxanthin

Annex 4. Plants used in Porvenir for the treatment of central nervous system disturbances

No./Family/Latin name/ Voucher number	Local name	FU[a]	VU[b]	Part used	CNS indication	Preparative method	Route of administration	In formulas with
Chenopodiaceae								
(***1***) *Chenopodium ambrosioides* L. 148	caré	13	10	Leaves	*Arrebato*[c]	Squeeze	Internal and embrocation	The fat of *Ateles paniscus* or cooking oil
Apocynaceae								
(2) *Aspidosperma rigidum* Rusby	gabetillo	7	5	Stem bark	Fever	Decoction	Bath	
Arecaceae								
(3) *Euterpe precatoria* Mart. 83	asaí	7	7	Root	Headache, dorsalgia	Jam	Internal	*Saccharum officinarum*
(4) *Attalea maripa* (Aublet) Mart. 176	cusi macho, casi cusi	1	1	Seed oil	Headache	Crude	Massage	
(5) *Attalea speciosa* Mart.	cusi, cusi hembra	13	8	Seed oil	Fever, headache, A*rrebato*[c]	Crude	Embrocation	
Asteraceae								
(6) *Bidens cynapiifolia* Kunth 4	pega pega espinuda			Root	Sadness	Decoction	Internal	*Imperata brasiliensis* and *Carica papaya*
Bignoniaceae								
(7) *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore 164	alcornoque	13	13	Stem bark	Fever, weakness, alcoholism	Decoction	Internal	*Tabebuia impetiginosa*
Burseraceae								
(8) *Protium spruceanum* (Benth.) Engl. 87	quina	3	3	Stem bark	Dengue-fever	Decoction	Internal	*Citrus limon*
				Resin	Headache	Crude	Massage	
(9) *Trattinnickia* 135	isiga	6	2	Resin	*Arrebato*[c], headache	Crude	Massage of the temporal	The bone of *Chrysocyon brachyurus*

No./Family/Latin name/ Voucher number	Local name	FU[a]	VU[b]	Part used	CNS indication	Preparative method	Route of administration	In formulas with
Caricaceae								
(10) *Carica papaya* L.	papaya	13	11	Flower	Headache, nervousness	Infusion	Internal	Colonia
				Root	Sadness	Infusion	Internal	*Imperata brasiliensis* and *Bidens cynapiifolia*
Cucurbitaceae								
(11) *Citrullus lanatus* (Thunb.) Matsum. & Nakai	sandía	5	2	Seed	*Arrebato*[c]	Milling, decoction	Massage	
Euphorbiaceae								
(12) *Jatropha curcas* L. 48	piñón	13	10	Resin	Alcoholism	Put in an alcoholic drink	Internal	It provokes vomiting
				Seed	Alcoholism	Milling and added to the food	Internal	
				Leaves	To lose weight	Infusion	Washing	
(13) *Ricinus comunis* L. 53	macororó	8	8	Fruit, seed	Dorsalgia	Milling and decoction	Internal (drinking) and massage	
				Leaves, seed oil	Fever, headache	Crude	Massage and/or covering	
Fabaceae								
(14) *Hymenaea courbaril* L. 31	paquió	8	6	Stem bark	To gain weight	Syrup	Internal	
(15) *Senna occidentalis* (L.) Link 38	mamúri 2	11	4	Root, leaves	Fever	Infusion	Bath	
(16) *Senna alata* (L.) Roxb. 52	mamúri 1	11	6	Root, leaves	*Arrebato*[c], fever	Decoction	Bath	
(17) *Mucuna rostrata* Benth 174	ojo de toro	1	1	Seed	Fever	Toasting and maceration	Internal	
(18) *Ormosia coarctata* Jackson 1	sirari	1	1	Endosperm	Tooth ache	Milling	Covering	

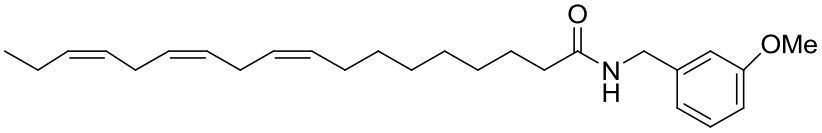
No./Family/Latin name/ Voucher number	Local name	FU[a]	VU[b]	Part used	CNS indication	Preparative method	Route of administration	In formulas with
Lamiaceae								
(19) *Ocimum americanum* L. 121	alba haca	8	3	Whole plant	*Arrebato*[c]	Infusion	Bath	
Malvaceae								
(20) *Gossypium barbadense* L. 40	algodón	7	6	Leaves	Cold exudation[c]	Infusion	Internal	
Meliaceae								
(21) *Cedrela fissilis* Vell. 102	cedro	3	3	Leaves / stem bark	Keeping ghosts away	Decoction	Bath	
(22) *Swietenia macrophylla* King	mara	1	4	Fruit	Tooth ache	Squeeze for juice	Gargle	
Moraceae								
(23) *Maclura tinctoria* (L.) D. Don ex Steud 116	mora	5	1	Resin	Tooth ache	Crude	Dropping	Salt
Piperaceae								
(24) *Piper* 105	matico chico	7	2	Stem / flower	Tooth ache	Crude	Chew	
Poaceae								
(25) *Imperata brasiliensis* Trin. 107	paja sujo	4	3	Root	Sadness	Decoction	Internal	*Carica papaya* and *Bidens cynapiifolia*
(26) *Cymbopogon citratus* (DC.) Stapf 156	paja cedrón	11	3	Leaves	Nervousness	Infusion	Internal	The hoof of *Tapirus baiirdei* / colonia
Rubiaceae								
(27) *Cephaelis ipecacucanha* (Brot.) A. Rich.	poalla	3	2	Root	Tooth ache	Crude	Covering	
				Root	Fever	No data	No data	
Rutaceae								
(28) *Citrus limon* (L.) Burm 100	limon	13	20	Pulp	Tooth ache, weakness	Squeeze for juice	Internal, massage, compress	Honey, salt
				Pulp	Tooth ache	Squeeze for	Gargle	Salt

No./Family/Latin name/ Voucher number	Local name	FU[a]	VU[b]	Part used	CNS indication	Preparative method	Route of administration	In formulas with
						juice		
				Stem with leaves	Tooth ache	Infusion	Gargle and washing of the head	
				Pulp	Headache, fever	Squeeze for juice	Compress, washing of the head	
				Pulp	*Arrebato*[c]	Squeeze for juice	Foot-bath	
				Pulp	Dengue-fever	Squeeze for juice	Internal	*Protium spruceanum*
(29) *Citrus sinensis* (L.) Osbeck 152	naranja dulce	7	3	Seed oil	Headache	Crude	Head massage	*Jatropha curcas* and cinnamon
(30) *Citrus reticulata* Blanco 154	mandarina	2	2	Leaves	Nervousness	Infusion	Internal	
Sapindaceae								
(31) *Scoparia dulcis* L. 36	basuriña	13	5	Whole plant	Tooth ache	Infusion	Inhalation	Salt and ashes
Smilacaceae								
(32) *Nicotiana tabacum* L.	tabaco	13	8	Leaves	Headache,	Heating	Covering	
Indeterminated species								
(***33***)	pesoé	10	9	Seed oil	Headache, tooth ache	Crude	Embrocation	
(***34***)	colonia	8	3	Flower	Nervousness, headache	Infusion	Internal	*Carica papaya*
(***35***)	pateanta	4	2	Seed oil?	*Arrebato*[c], fever	Crude	Massage	
(***36***)	luisiña	1	1	Whole plant	Fever	Infusion	Internal	
(***37***)	cuta	1	2	Young leaves	Cold exudation		Bath	

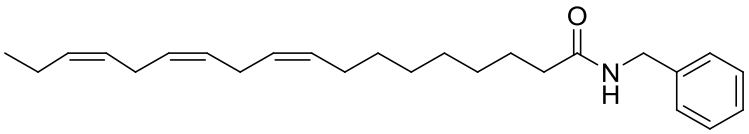
[a] Frequency of use, [b] Variety of use, [c] Explanations of the local words and expresions are to be found in the text.

Annex 5. Isolated compounds

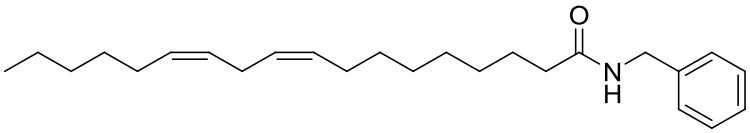
N*-Alkylamides from *Lepidium meyenii



N-(3-methoxybenzyl)-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**1**)

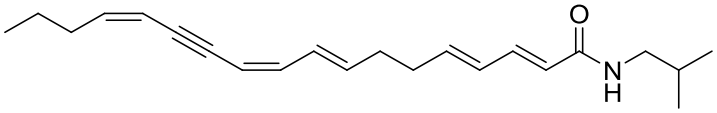


N-benzyl-(9*Z*,12*Z*,15*Z*)-octadecatrienamide (**2**)

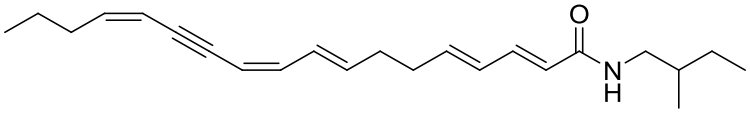


N-benzyl-(9*Z*,12*Z*)-octadecadienamide (**3**)

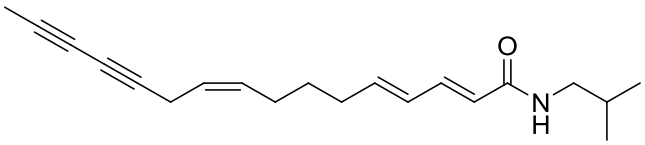
N*-Alkylamides from *Heliopsis helianthoides* var. *scabra



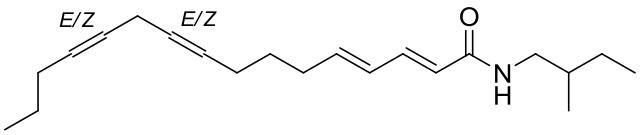
octadeca-2*E*,4E,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid isobutylamide (**4**)



octadeca-2*E*,4*E*,8*E*,10*Z*,14*Z*-pentaen-12-ynoic acid 2'-methylbutylamide (**5**)

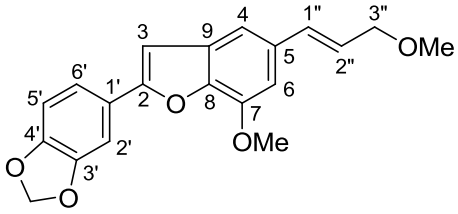


hexadeca-2*E*,4*E*,9*Z*-trien-12,14-diynoic acid isobutylamide (**6**)

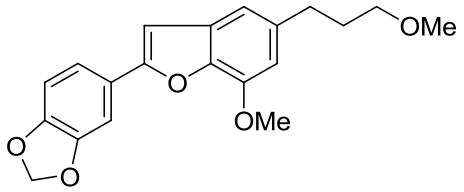


hexadeca-2*E*,4*E*,9,12-tetraenoic acid 2’-methylbutylamide (**7**)

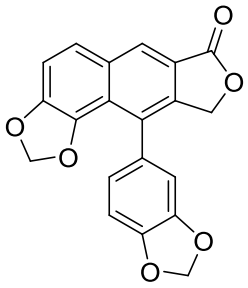
Lignans isolated from *Heliopsis helianthoides* var. *scabra*



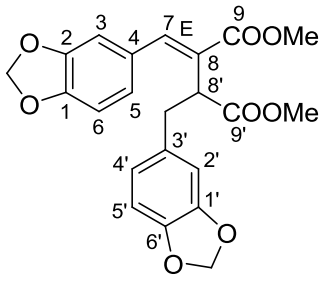
1"-dehydroegonol 3"-methyl ether (**8**)



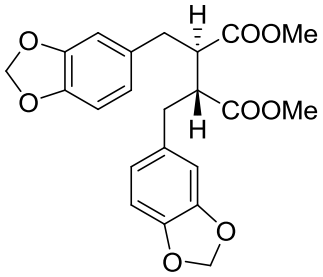
egonol 3"-ethyl ether (**9**)



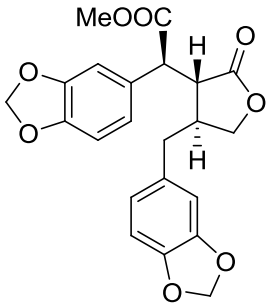
helioxanthin (**10**)



(7*E*)-7,8-dehydroheliobuphthalmin (**11**)



heliobuphthalmin (**12**)



7-acetoxyhinokinin (**13**)