CHEMICAL, BIOLOGICAL AND ANALYTICAL STUDIES ON NATURAL PRODUCTS FROM CENTRAL NERVOUS SYSTEM-(CNS) ACTIVE PLANTS

by

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Abstract

CHEMICAL, BIOLOGICAL AND ANALYTICAL STUDIES ON NATURAL PRODUCTS FROM CENTRAL NERVOUS SYSTEM- (CNS) ACTIVE PLANTS

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Adviser: Professor Wayne W. Harding

There are several CNS activities, including analgesic, antidepressant, sedative, euphoric, stimulant, anxiolytic, psychedelic and oneirogenic activity. Investigation of CNS-active plants has the promise to identify new compounds that may be therapeutically useful or that may be useful as probes for CNS receptors. This thesis deals with three plants that have been reported to possess CNS activity: *Leonotis leonurus, Silene capensis*, and *Ipomoea indica.*

Leonotis leonurus when smoked or consumed as a tea is reported to cause mild marijuana-like sedative effects. Our investigation has uncovered three new labdane diterpenes from an aqueous extract of a commercial source of the plant. The labdane diterpenes tested did not show any CNS receptor affinity at the sites we tested. These compounds are not likely (by themselves) to be responsible for the activity.

Silene capensis known as African Dream Root has oneirogenic activity. Phytochemical isolation of our plant material resulted in the isolation of ten known compounds. Six of these ten compounds are withanolides or withanolide glycosides.

No phytochemical investigations of *Ipomoea indica* have been reported to date. Eight known compounds were isolated during our investigation.

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Abbreviations

CNS	central nervous system
DCM	dichloromethane
DCC	N,N'-Dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarization transfer
FCC	flash column chromatography
HMBC	heteronuclear multiple bond correlation
HMPT	hexamethylphosphorous triamide
HPLC	high-performance liquid chromatography
HRESIMS	high resolution electrospray ionization mass spectroscopy
HSQC	heteronuclear single quantum coherence
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy

Chapter 1. Introduction

Psychoactive agents, especially those plants which cause marked changes in patterns of mood, perception and behavior as a hallucinogenic drug, have always fascinated human beings. Surrounded by superstitions and magic or religious thoughts, they have always occupied man's attention. Those plants which are able to alter the conscience and the sensorium have drawn special consideration. In fact, due to their amazing effects, the psychodysleptic drugs [1] have occupied most of the researchers' time. The researchers tried their best to understand their mechanism of action, and, hence, to understand human behavior, thoughts, humor, sensations, etc.[1]

However, there were so many difficulties and challenges of trying to understand the mechanisms of action on mood, humor, cognition, sensorium, etc., that a lot of people ignored the fact that plants could also have beneficial properties to treat mental disease and some psychic ailments. [2] Furthermore, most of the plants' occasional use by the White occidental culture was relegated to a second plan, being considered as sorcerer's therapeutics because those plants were first used by the so-called primitive cultures. A bad result of such posture was a neglect and a disdain for all kinds of therapeutics based on plants.[2]

Thus, until recently, very little attention was given by the scientific

community to the benefits, as accepted by folk medicine, of the therapeutic usefulness of plants endowed with psycholeptic and psychoanaleptic properties.[1]

Fortunately, this bad tide has recently turned due to several reasons, among them the wrong belief that plants, by originating directly from nature, must be less toxic than synthetic drugs. [3]Another important aspect for this turning point was the realization by the pharmaceutical industry that plants, after all, could be a good business as more and more people were prone to look for this unconventional form of therapy. For example, Eisenberg et al. found that among American citizens, between 20% and 28% used alternative treatments for central nervous system (CNS) symptoms such as insomnia, headache, anxiety and depression; 3% of those patients had used herbal medicines.[3]

1.1 Central nervous system (CNS) - active plants

It is estimated that nearly 25% of modern drugs directly or indirectly originated from plants. [4] Several examples concern the CNS, and include caffeine, ephedrine, cannabinoids, opioids and reserpine. [4] However, for the majority of CNS active plants, the active principles are not yet known. The rational treatment of CNS disorders by plant extract derived drugs is in its infancy due to the complex chemistry and organization of the CNS and also the complex chemistry and pharmacology of a plant extract. [5]

The search for new molecules that act on the CNS and that can be used for therapeutic purposes started with several studies in the 19th century. In fact, the first drugs used to treat pathologic conditions of the CNS were based on natural resources, specifically on plants. However, studies targeting plants with this type of bioactivity represent only a very small percentage of those investigations. In a review of the existing literature, it appears that plants with molecules that produce this kind of activity are increasingly attractive targets for the development of new drugs. [4] It is found that the studies demonstrating either stimulating or depressive activity on the CNS have been in certain classes of compounds, namely, alkaloids, [5, 6, 7] amino acids, flavonoids, [8, 9] and phenolic and polyphenolic compounds. [10, 11, 12, 13]

There are a couple of CNS activities, such as antidepressant, anxiolytic, stimulant, analgesic, psychedelic, oneirogenic and sedative activity. [4, 5, 10] The three plants investigated in this thesis are each associated with psychedelic, oneirogenic or sedative activity.

1.2 Psychedelic plants

Hallucinogens, psychotomimetics, psychometamorphics, entactogens psychotogens, phantastica, psychodysleptics, etc., are all synonymous with the word psychedelics.

A brief description of the effects of these drugs is as follows:

On cognition: interference with memory, attention, reasoning and orientation, all important cognitive functions;

On sensorium: illusion, delusion, depersonalization, lack of contact with reality and sensorial alterations such as loss of sensitivity to corporal movements and posture, and loss of temporal and space discriminations. [14, 15, 16]

Hallucinogenic active principles do not vary much. With the exception of the cannabinoids from *Cannabis. sativa*, all other known active principles have nitrogen and possess one of three chemical moieties: phenylethylamine, indole or the anticholinergic tropane esters [Phenylethylamine (Fig. 1) [17] [typical example: norepinephrine (**1**), mescaline (**2**)], indole (Fig. 2) [17] [tryptamines (**6**), β-carbolines, psilocybin, dimethyltryptamine (**7**), ergolines (**8**), harmaline (**9**)], the anticholinergic tropane esters (atropine, escopolamine)], although not "true hallucinogens" as LSD-25, psilocybin, etc., still are able to produce psychedelic experiences. [17, 18, 19]

Fig. 1 Phenylethylamine-type psychedelic compounds

Fig. 2 Indole-type psychedelic compounds

1.3 Oneirogenic plants

Oneirogenic is used to describe a plant that induces lucid or vivid dreaming when people sleep. Plants have been used for thousands of years to induce states of lucid dreaming by shamans throughout the world. These plants are often classified as oneirogens.[20, 21]

The most famous oneirogenic plants are *Silene capensis, Calea*

zacatechichi and *Entada rheedii*. [21, 22, 23]

Silene capensis, also known as African Dream Root, is a plant native to the Eastern Cape of South Africa, where it is regarded by the Xhosa people as a sacred plant. The root is traditionally used to induce vivid lucid dreams during the initiation process of shaman. It is regarded as the most powerful dream inducing plant, more powerful than *Calea zacatechichi* or *Entada rheedii*.[21]

Calea zacatechichi, also known as Dream Herb, is a plant used by the indigenous Chontal of the Mexican state of Oaxaca. The plant naturally occurs from southern Mexico to northern Costa Rica. Its oneirogenic activity lies within its leaves which are usually boiled with water to make a tea and then drank directly before going to sleep. The leaves are also often smoked, sometimes even at the same time as the tea is drank.[22]

Entada rheedii, also known as African Dream Herb, is a large woody liana or climber. These very large seeds are often washed up onto beaches. Because of this, they also have gained the name Sea Beans. The seeds, which are usually ground up and smoked, are associated with the plants oneirogenic activity. It is traditionally used by shamans in South Africa to remember dreams. [23]

1.4 Sedative plants

Insomnia is a frequent problem that affects people of all the ages

around the world. It is a prevalent and potentially serious condition that adversely affects the diurnal functioning, health status and life quality of people of all of age. Insomnia is often defined by sleeping problems. People who suffer from insomnia may encounter difficulty of getting to sleep or staying asleep, or having non-refreshing sleep, to some degree. Stress, anxiety and depression could trigger insomnia. As well as cough, chronic pain, apnea, circadian rhythm disorders and neural diseases are conditions also associated with insomnia. [24]

There are a lot of medicinal herbs used for treatment of insomnia, such as *Coriandrum sativum, Rosa damascene, Crocus sativus, Salvia leriifolia, Salvia reuterana* and *Stachys lavandulifolia*. [24]

A wide variety of chemical compounds have been employed in clinical therapy as the sedative-hypnoytic drugs, such as zopiclone (**10**), nordiazepam (**11**), flurazepam (**12**), nitrazepam (**13**), diphenhydramine (**14**), glutethimide (**15**), fenadiazole (**16**), clomethiazole (**17**). (Fig. 3) [25-33]

zopiclone (**10**) nordiazepam (**11**) flurazepam (**12**) nitrazepam (**13**)

diphenhydramine (**14**) glutethimide (**15**) fenadiazole (**16**) clomethiazole (**17**)

Fig. 3 Sedative-hypnoytic compounds

This thesis deals with three plants that have been reported to process CNS activity: *Leonotis leonurus*, [34] *Silene capensis*, [35] and *Ipomoea indica*. [36]

Chapter 2. A review of the genus *Leonotis* and *Leonotis leonurus*

2.1 The genus *Leonotis*

Leonotis, family Lamiaceae (Labiatae), tribe Stachyoideae, is a small genus, including about forty species. [34] During the last sixty years, several species were investigated for the search of diterpenoids. The genus *Leonotis* grows in the tropical belt: Caribbean Islands, South America, India, Africa (specifically South Africa). [34]

Leonotis species are used in folk medicine. For example, in Southern Africa, *Leonotis leonurus*is called "wild dagga" or "wild hemp".[34, 50] It is used as anticonvulsant, [34, 51] antinociceptive, [54] antiinflammatory, [34, 53] antidiabetic, antiarthritic, [55]antidote for snake bite, antibacterial.[34, 50-59] Aqueous or ethanolic extracts are usually prepared. [53-55]

Probably the most used species is *Leonotis nepetaefolia*. In Africa, India, Caribbean countries and South America, it has been attributed with a variety of salutary physiological effects. [34] Smoking of dried leaves is a common practice in many countries as a narcotic drug.[34] Folk medicine used extracts as antitumour,[34] antifungal, antimalarial, hypotensive, tonic, laxative, [34] sedative, insecticide, antiviral, antibacteric, [34] to cure coughs, fever, [34] stomachache, headache, [34] kidneydiseases, rheumatism,

dysmenorrhea, asthma, burns. [34] It was ascertained that in Central Mexico *Leonotis nepetaefolia* is a reservoir for several viruses.[36]

Leonotis dubia is used in Africa against whooping cough. [37] *Leonotis ocymifolia*, in Eastern and Southern Africa, is quoted to have ascaricide and anticancer activity and to cure ulcers and wounds.[38] It is also reported to be a narcotic and habit-forming drug. [38]

The most well-known species in the genus *Leonotis* are *Leonotis nepetaefolia* and *Leonotis leonurus.*

The first results on the chemistry of the diterpenes from the genus *Leonotis* appeared in 1962: two products were isolated from the aerial parts of *Leonotis leonurus* R, Br. collected in South Africa and provisionally indicated as compound X (**18**) and compound Y (**19**) (Fig. 4a). [34, 50] Two years later the extraction of the same species yielded the well known marrubiin (**20**) (Fig. 4a), [34, 50-53] previously isolated from *Marrubium vulgare* L.[34, 52]

The first result from *Leonotis nepetaefolia* appeared in 1969: a new natural product, leonotin (**21**), was isolated. The same substance was found in *Leonotis leonitis* R. Br. and in *Leonotis dysophylla* Benth. [34]

Subsequently, a lot of diterpenes and other natural products were isolated from the genus *Leonotis,* as shown in Table 1. [34, 37, 50-53]

Table 1. Compounds isolated from *Leonotis* [34, 37, 50-53]

Species	Compounds
Leonotis leonurus	compound X (**18**), compound Y (**19**), marrubiin (**20**), leonurun (**21**), 13*R*-premarrubiin (**22**), 13*S*-premarrubiin (**23**), apigenin 6-C-α-arabinoside -8-C-β-glucoside (**24**), apigenin 8-C-β-glucoside (**25**), apigenin 7-*O*-β-glucoside (**26**), luteolin 7-*O*-β-glucoside (**27**), luteolin 7-*O*-β-glucoside-3'-methyl ether (**28**), apigenin 7-*O*-(6"-*O*-p-coumaroyl)-β-glucoside (**29**), 6-Methoxyluteolin-4'-methyl ether (**30**), luteolin 3'-methyl ether (**31**), luteolin (**32**), geniposidic acid (**33**)
Leonotis nepetaefolia	leonotin, nepetaefolin, nepetaefuran, nepetaefuranol, methoxynepetaefolin, nepetaefolinol, leonotinin, dilactone, dehydronepetaefolinol, tetrol, leonotinic acid
Leonotis dysophylla	leonotin
Leonotis dubia	dubiin
Leonotis ocymifolia raineriana	leonotin, compound X (**18**), dilactone, lactone, leonotin, leonotinin, nepetaefolin
Leonotis leonitis	leonitin, leonotin
Leonotis leonitis hirtiflora	diolide

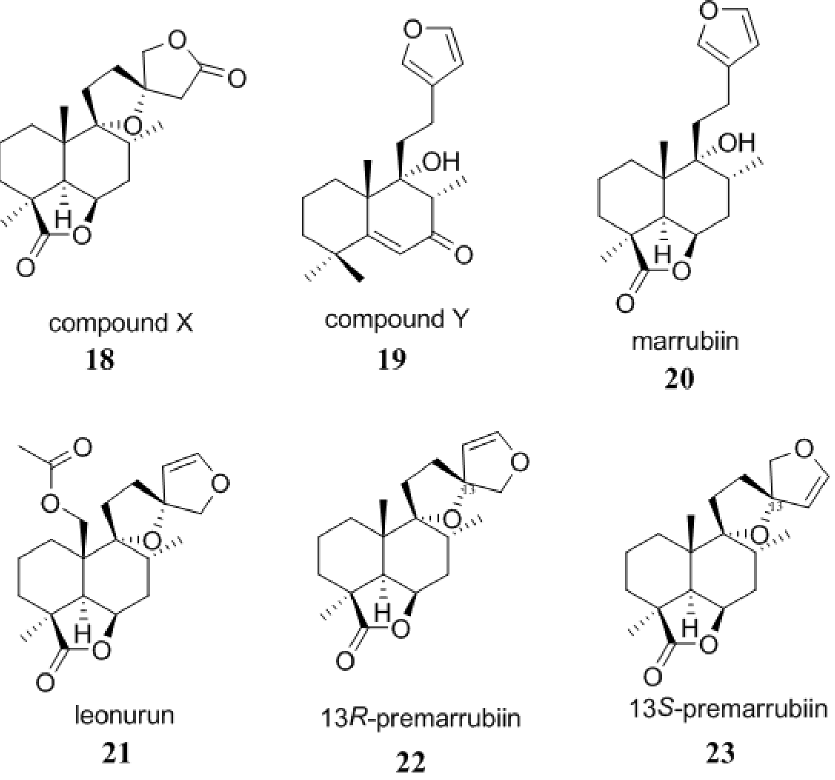


Fig. 4 (a) Diterpenoid compounds isolated from *Leonotis leonurus*

apigenin 6-C-α-arabinoside-8-C-β-glucoside

24

apigenin 8-C-β-glucoside

25

26 apigenin 7-*O*-β-glucoside: R=H
27 luteolin 7-*O*-β-glucoside: R=OH
28 luteolin 7-*O*-β-glucoside-3'-methyl ether: R=OCH_3

apigenin 7-*O*-(6"-*O*-p-coumaroyl)-β-glucoside

29

6-Methoxyluteolin-4'-methyl ether

30

31 luteolin 3'-methyl ether: R=OCH_3
32 luteolin: R=OH

Fig. 4 (b) Phenolic compounds isolated from *Leonotis leonurus*

2.2 *Leonotis leonurus*

2.2.1 *Leonotis leonurus* - general information and biological activity

Leonotis leonurus R. Br. (Lamiaceae), also known as Lion's Tail and Wild Dagga, a plant species in the genus *Leonotis,* is a shrub 2-5 m in height that is native to South Africa. [34]

The leaves of *L. leonurus* have traditionally been smoked for the relief of epilepsy. [39-46] An infusion and a decoction of the leaf and stem have been used internally for coughs, colds, influenza, bronchitis, high blood pressure and headaches. [34, 39-47] A tincture of the flower has also been used for the same purpose.[48, 49] These orange colored flowers are reported to be hallucinogenic. [49] Externally, decoctions have been applied to treat boils, eczema, skin disease, itching and muscular cramps. [48]

The most common use for *Leonotis leonurus* utilizes the picked and dried leaves brewed as a tea. [34] *L. leonurus* has been reported to possess a number of biological activities such as anticonvulsant, antinociceptive, antiinflammatory, and hypoglycemic properties. [34, 50-59] Additionally, the herb when smoked or consumed as a tea is reported to cause mild marijuana-like sedative effects. [34, 60, 61]

The aqueous leaf extract of *Leonotis leonurus* possesses antinociceptive and hypoglycemic properties; thus lending

pharmacological credence to folk usage of the herb in the management and/or control of painful, arthritic, and other inflammatory conditions, as well as for adult-onset, type-2 diabetes mellitus in some communities of South Africa. [69]

Leonotis leonurus aqueous leaf extract also possesses hypotensive activity. [70] Previous scientific studies have shown that extractives from certain African medicinal plants are useful remedies for the control and/or management of high blood pressure. *Leonotis leonurus* is one of the several African medicinal plants commonly used by some South African traditional healers to manage or control essential hypertension. [67]

2.2.2 Compounds isolated from *Leonotis leonurus*

Marrubiin (**18**) (Fig. 4a) has been isolated as the main diterpenoid lactone from *L. leonurus*.[34, 56, 63-65] Two labdane terpenoids, compounds X (**19**) and Y (**20**) (Fig. 4a) also occur in *L. leonurus*. [34, 48] Two stereoisomeric premarrubiins (13*R*) (**21**) and (13*S*) (**22**) (Fig. 4a) were also isolated from *L. leonurus* (Fig. 4a) on a sample collected in Italy. [34] Premarrubiins are probably chiral natural products and marrubiin is not chiral. [34] Another new labdane diterpenoid, leonurun (**23**) (Fig. 4a) was isolated from the same species in South Africa. [34]

Recently, ten flavonoid compounds (Fig. 4b) were also isolated from the same species. Among these are six flavone glycosides, two

methylated flavones, and two flavone aglycons. [65] One iridoid glycoside [Geniposidic acid (**33**)] was also isolated from *L. leonurus* (Fig. 4c). [82]

Geniposidic acid (**33**)

Fig. 4 (c) Geniposidic acid isolated from *Leonotis leonurus*

Chapter 3. Phytochemical and Biological Investigation of *Leonotis leonurus*

3.1 Rationale for phytochemical and pharmacological analysis of *Leonotis leonurus*

In traditional medicine, *Leonotis leonurus* decoctions have been used externally for dermatological problems (rashes, boils and eczema) and internally to treat coughs, fever, headaches and hypertension. [71] Leaves of the plant are smoked for its anti-epileptic effects. [72] Aqueous leaf preparations have been reported to possess anticonvulsant, antinociceptive, anti-inflammatory and antidiabetic properties in rodents. [72, 73, 74] Additionally, crude aqueous extracts have been shown to possess antihelminthic activity. [75, 76]

In addition to folkloric uses mentioned above, *L. leonurus* reportedly produces marijuana-like effects. [77] The plant is commercially available and is marketed largely for its psychoactive effects. Some internet websites claim that this activity is attributable to an alkaloid, leonurine. There is some doubt about the validity of this claim since leonurine has never been reported to occur in the plant (although its presence in related species is documented). [78, 79, 80, 81] Thus the component(s) responsible for the reputed psychoactive effects are scientifically unverified at this time. Prior phytochemical investigations of various extracts of the plant have

uncovered a number of labdane diterpenes, an iridoid glycoside and phenolic compounds, primarily of the flavonoid class. [82-91]

As part of our program to identify natural products with CNS activity from *Leonotis* plants, an investigation of a commercially available source of *L. leonurus* was carried out. Verification of the taxonomic identity of the plant was achieved via molecular methods. Leonurine was synthesized to evaluate the bioactivity of this alkaloid. Details of the taxonomic identification as well as our chemical, spectroscopic and biological studies are discussed herein.

3.2 Results and discussion

3.2.1 Genetic identification of *Leonotis leonurus*

Since the plant material procured was not of sufficient quality to permit positive morphological identification (i.e. as distinct from other similar *Leonotis* species), identification of the plant using genetic methods was done.

Molecular markers have proved a powerful tool in diagnostics of species and varieties of various commercial products in order to identify and delimiting closely related species and to ensure quality control. Both DNA fingerprinting techniques and DNA sequence information have been employed, even from degraded and unrecognizable plant material. [92, 93] The application, sometimes referred to as "DNA barcoding", can assist in the process of identifying unknown plant specimens to known species. [94] For example, this method has been suggested as useful for an accurate and rapid authentication of medicinal plant products and their adulterants. [95]

Recent molecular work in the Lamiaceae subfamily Lamioideae, to which *Leonotis* belongs, has produced a large database of chloroplast DNA sequences. [96] A diagnostic assay was thus employed based on two chloroplast intron gene sequences (trnL intron and rps16 intron) that provide sufficient sequence variation to distinguish members of the group

Leonotis belongs to, including closely related *Leonotis* and *Leucas* R.Br. species. After sequencing these gene regions from the procured *Leonotis* plant material several approaches were taken to ensure a correct identification. First, the sequences were analyzed using the BLAST search tool against sequences in the public database, GenBank, held at the National Center for Biotechnology Information (NCBI). Secondly, the DNA sequences were compared to the large data set of several hundred lamioid sequences, which clearly confirmed a large diagnostic "gap" in the rps16 sequence, which was shared with the *L. leonurus* sample in the data set. Finally, phylogenetic reconstruction was performed verifying that the obtained plant material grouped with the other *L. leonurus* in the data set. Together, these results clearly confirm the obtained material to belong to *Leonotis leonurus*.

3.2.2 Isolation of *Leonotis leonurus* secondary metabolites

The aqueous extracts of the aerial parts of the plant have not been subjected to phytochemical study and given the pharmacological activity attributed to this extract, it was considered worthy of such an investigation. [34, 56]

Repeated purification procedures (High-Performance Liquid Chromatography (HPLC) and flash column chromatography) on an aqueous extract of aerial parts of *L. leonurus* gave compounds Leonurenones A (**34**), [51] Leonurenones B (**35**), [51] Luteolin 7-*O*-β-glucoside (**39**), [103] Luteolin (**40**) [103] and Uracil (**42**). [105] Similarly, repeated chromatography of an acetone extract afforded compounds Leonurenones C (**36**), [51] 9,13:15,16-Diepoxy-6,16-labdanediol (**37**), [88] Nepetaefolin (**38**) [99-102] and β-Sitosterol (**41**). [104] Compounds **34-36** are new compounds and compounds **37-42** are known compounds.

3.2.3 Structure elucidation of compound 34 [51]

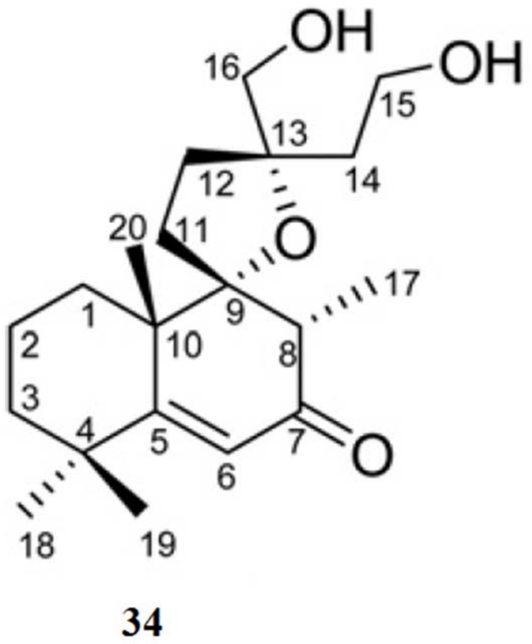


Fig. 5 Structure of compound **34**

The initial diterpenoid nature of **34** came from its Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) spectrum where 20 carbons were observed. The High Resolution Electrospray Ionization Mass Spectroscopy (HRESIMS) spectrum showed a molecular ion peak at *m/z* 336.2380. This implied a molecular formula of $C_{20}H_{32}O_4$ in agreement with the ^{13}C NMR spectroscopic data. (see Table 2.)

Table 2. ^{13}C NMR and ^{1}H NMR Spectroscopic Data for Compound **34**

position	^{13}C		^{1}H	(J in Hz)	HMBC
1a	29.4	CH_2	1.92	m	
1b			2.18	m	
2a	17.9	CH_2	1.72	m	
2b			1.85	m	
3a	39.2	CH_2	1.44	m	
3b			1.60	m	
4	37.1	C			
5	172.4	C			
6	123.1	CH	6.06	s	4, 5, 8, 10
7	200.0	C			
8	47.7	CH	3.01	q (6.8)	7, 9, 10,17
9	95.9	C			
10	45.2	C			
11a	30.8	CH_2	1.67	d (4.0)	
11b			1.71	s	
12a	35.4	CH_2	1.87	m	9, 13, 16
12b			1.88	m	
13	85.7	C			
14a	40.8	CH_2	1.94	m	12, 13, 15, 16
14b			1.95	m	
15a	59.2	CH_2	3.70	m	13, 14, 16,
15b			3.74	m	
16a	66.9	CH_2	3.50	d (11.2)	12, 13, 14
16b			3.56	d (11.2)	
17	9.9	CH_3	1.21	d (6.8)	6, 7, 9
18	31.7	CH_3	1.20	s	3, 5, 19
19	30.6	CH_3	1.21	s	3, 5, 18
20	24.0	CH_3	1.38	s	5, 9, 10

In the ^{13}C NMR spectrum, signals typical of the quaternary C9 and C13 positions of a spiroether labdane framework were observed (δ_C 95.9 and 85.7 ppm, respectively). Other low field quaternary signals were observed at δ_C 200.0 and 172.4 ppm. The former resonance was placed at position 7 of the labdane core based on Heteronuclear Multiple Bond

Correlation (HMBC) correlations with the C17 methyl protons (δ_H 1.21, d). The resonance at δ_C 172.4 showed HMBC cross-peaks to the C18 and C19 methyl protons as well as the C20 methyl protons. This signal was thus attributed to C5 of the diterpene skeleton. A vinylic system appeared to be present based on the signal at δ_C 123.1 (CH). The proton attached to this vinylic carbon (δ_H 6.06) showed HMBC correlations to C8 (δ_C 47.7) and C-10 (δ_C 45.2). On the basis of the preceding, it became evident that the resonances at δ_C 200.0, 172.4 and 123.1 were due to the presence of an α, β-unsaturated enone system in ring B. The presence of two oxymethylene carbons was inferred from Distortionless Enhancement by Polarization Transfer - 135 (DEPT-135) and Heteronuclear Single Quantum Coherence (HSQC) data (δ_C 66.9 and 59.2). That at δ_C 66.9 was attached to mutually coupled doublets at δ_H 3.50 and 3.56, thereby placing this carbon at position 16. Further HMBC correlations from the proton at δ_H 3.56 to C12, C13 and the C14 methylene carbon supported this assignment. The oxymethylene carbon at δ_C 59.2 was assigned to position 15. HMBC cross-peaks were observed for the H15 protons to C13 and C14. The above established the gross structure of compound **34**.

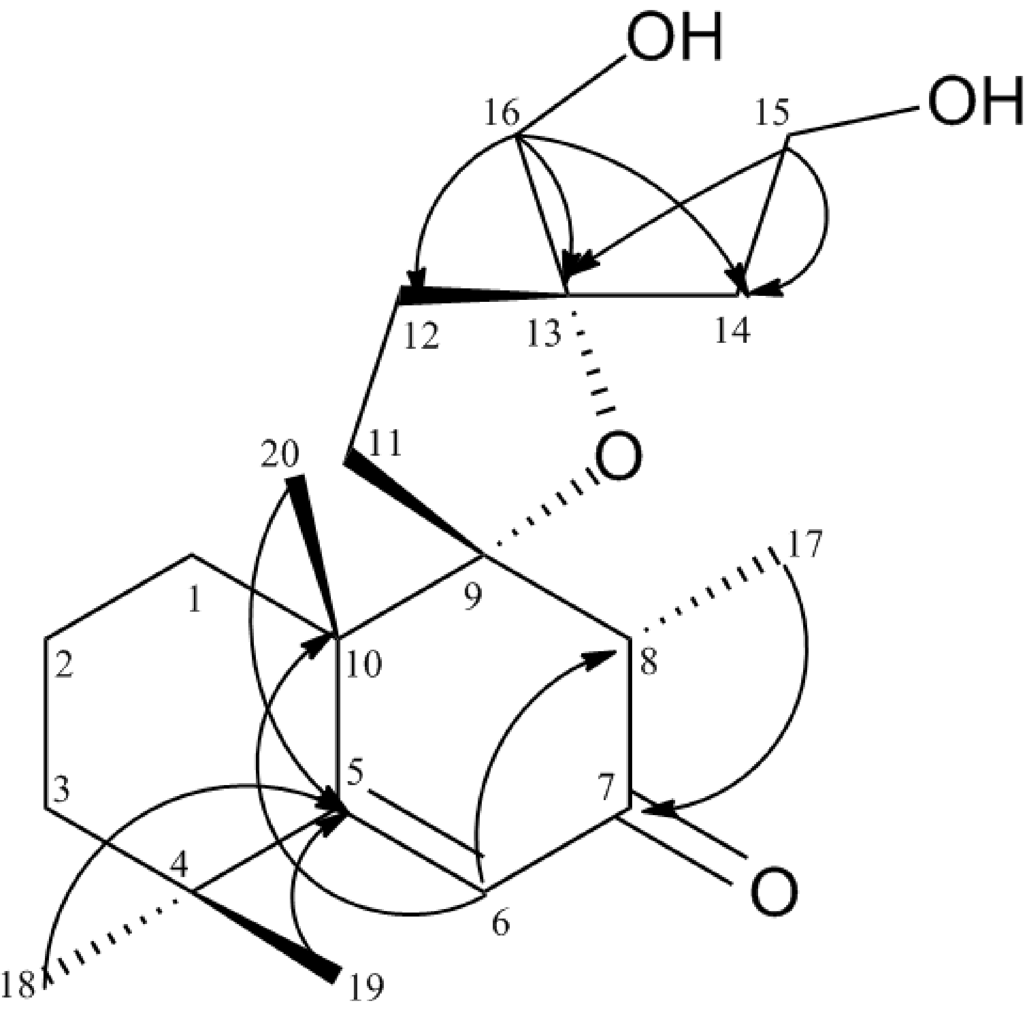


Fig. 6 (a) Key HMBC correlations for compound **34**

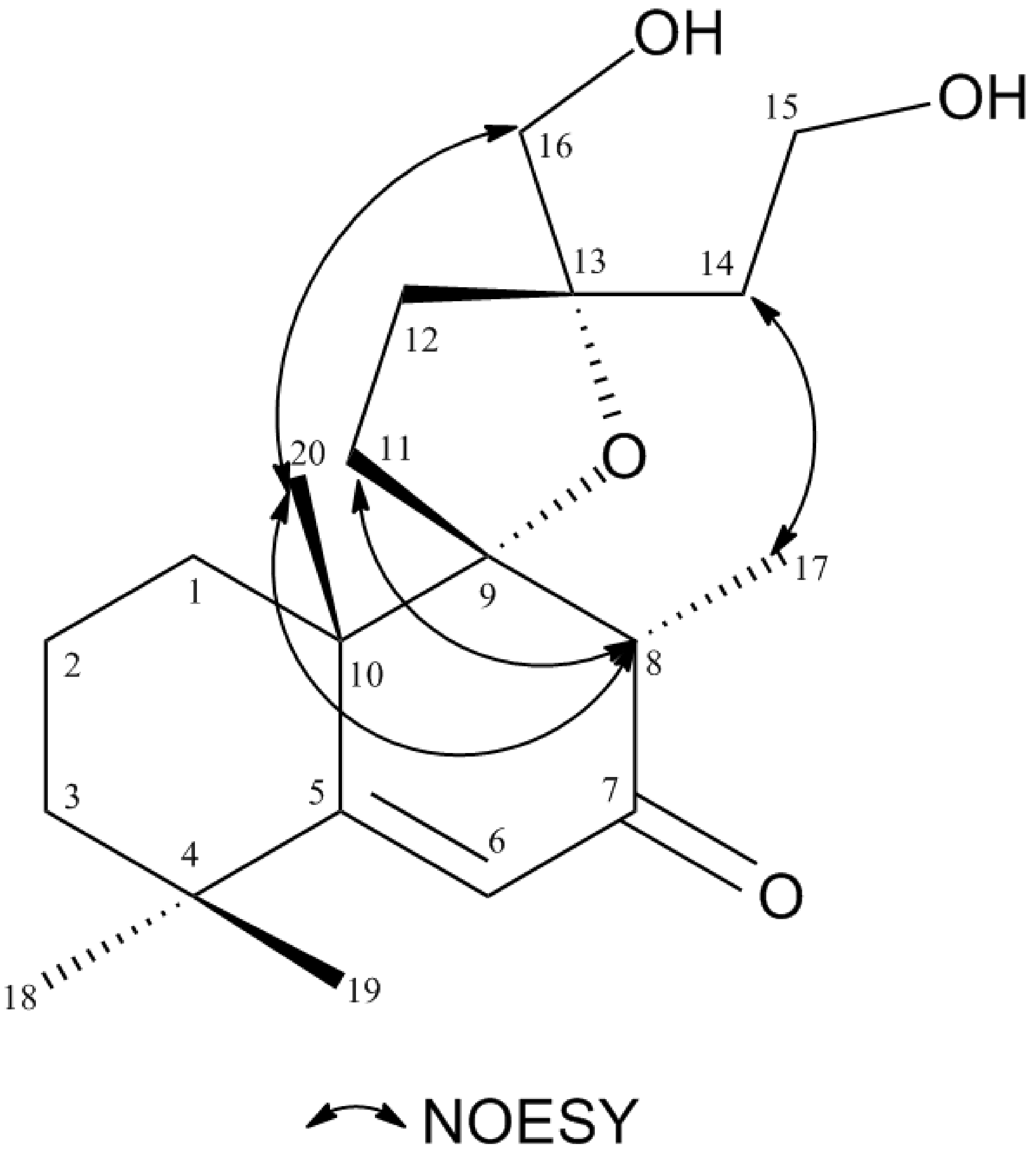


Fig. 6 (b) Key NOESY correlations for compound **34**

The relative stereochemical assignments for **34** were made on the basis of Nuclear Overhauser Effect Spectroscopy (NOESY) spectroscopic data. Here cross-peaks were seen for H8 to the C20 and C11 protons, thereby establishing that these groups were on the same side of the labdane structure. H20 in turn showed NOESY cross-peaks to H16 while H17 showed cross-peaks to H14. The α, β-unsaturated ketone system in ring B seen in **34** is very rare in the *Leonotis* genus having only been reported once in the literature. [84] Compound **34** has been denoted with the trivial name Leonurenone A. [51]

3.2.4 Structure elucidation of compound 35 [51]

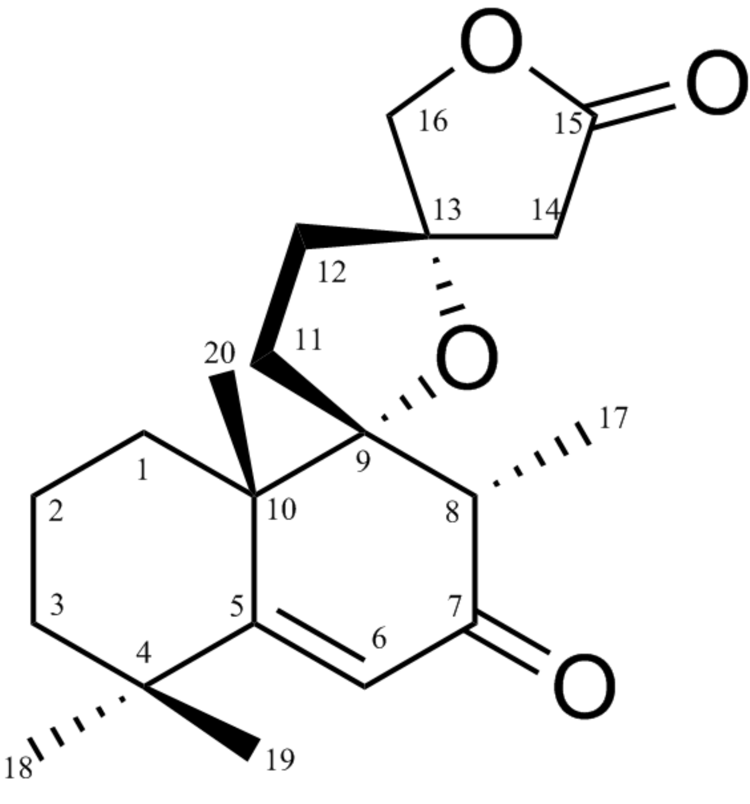


Fig. 7 Structure of compound **35**

Compound **35** was isolated as a colorless oil and showed a molecular ion peak in its HRESIMS spectrum (*m/z* 332.1992) corresponding to the molecular formula $C_{20}H_{28}O_4$. The structural similarity to compound **34** was evident upon inspection of the ^{13}C NMR and ^{1}H NMR spectroscopic data. (see Table 3.)

Table 3. ^{13}C NMR and ^{1}H NMR Spectroscopic Data for Compound **35**

position	^{13}C		^{1}H	(J in Hz)	HMBC
1a	30.4	CH_2	1.73	m	3, 5, 10
1b			1.62	m	2, 5, 9, 10, 20
2a	17.2	CH_2	1.93	m	1
2b			1.72	m	1, 2, 4, 10,
3a	38.8	CH_2	1.61	m	1, 2, 5, 19
3b			1.47	m	1, 2, 4, 19
4	36.6	C			
5	174.6	C			
6	122.3	CH	6.00	s	4, 5, 10
7	201.1	C			
8	47.1	CH	3.05	q (6.8)	7, 9, 17
9	96.5	C			
10	44.9	C			
11a	29.3	CH_2	2.40	m	9, 10, 12, 13
11b			2.10	m	9, 10, 12, 13
12	36.0	CH_2	2.21	m	9, 11, 13, 14, 16
13	86.6	C			
14a	40.9	CH_2	2.80	d (17.2)	12, 13, 15, 16
14b			2.63	d (17.2)	
15	175.8	C			
16a	77.9	CH_2	4.23	d (9.0)	12, 13, 14, 15,
16b			4.30	d (9.0)	
17	8.6	CH_3	1.17	d (7.8)	7, 9,
18	30.6	CH_3	1.19	s	3, 4, 5, 19,
19	29.8	CH_3	1.26	s	3, 5, 18
20	23.0	CH_3	1.43	s	1, 5, 9, 10,

The ^{1}H NMR spectrum showed 3 methyl singlets and a methyl doublet as seen in **34**. Typical ^{13}C NMR signals for the α, β-unsaturated enone in ring B (δ_C 174.6, C5; 122.3, C6; 201.1, C7) and the quaternary C9 and C13 positions (δ_C 96.5 and 86.6, respectively) were also observed. The main difference in the ^{1}H and ^{13}C NMR spectra of the **35**, as compared to **34**, was the absence of additional signals corresponding to an

oxymethylene group. This oxymethylene group was apparently replaced by an ester-like carbonyl functionality (δ_C 175.8). A pair of coupled oxymethylene doublets (δ_H 4.30 and 4.23; J = 9.0 Hz) was reminiscent of similar signals for C16 of **34**. The resonance at δ_H 4.23 correlated to C13 as well as δ_C 175.8. These data support the presence of a γ-butyrolactone moiety comprising C13 to C16.

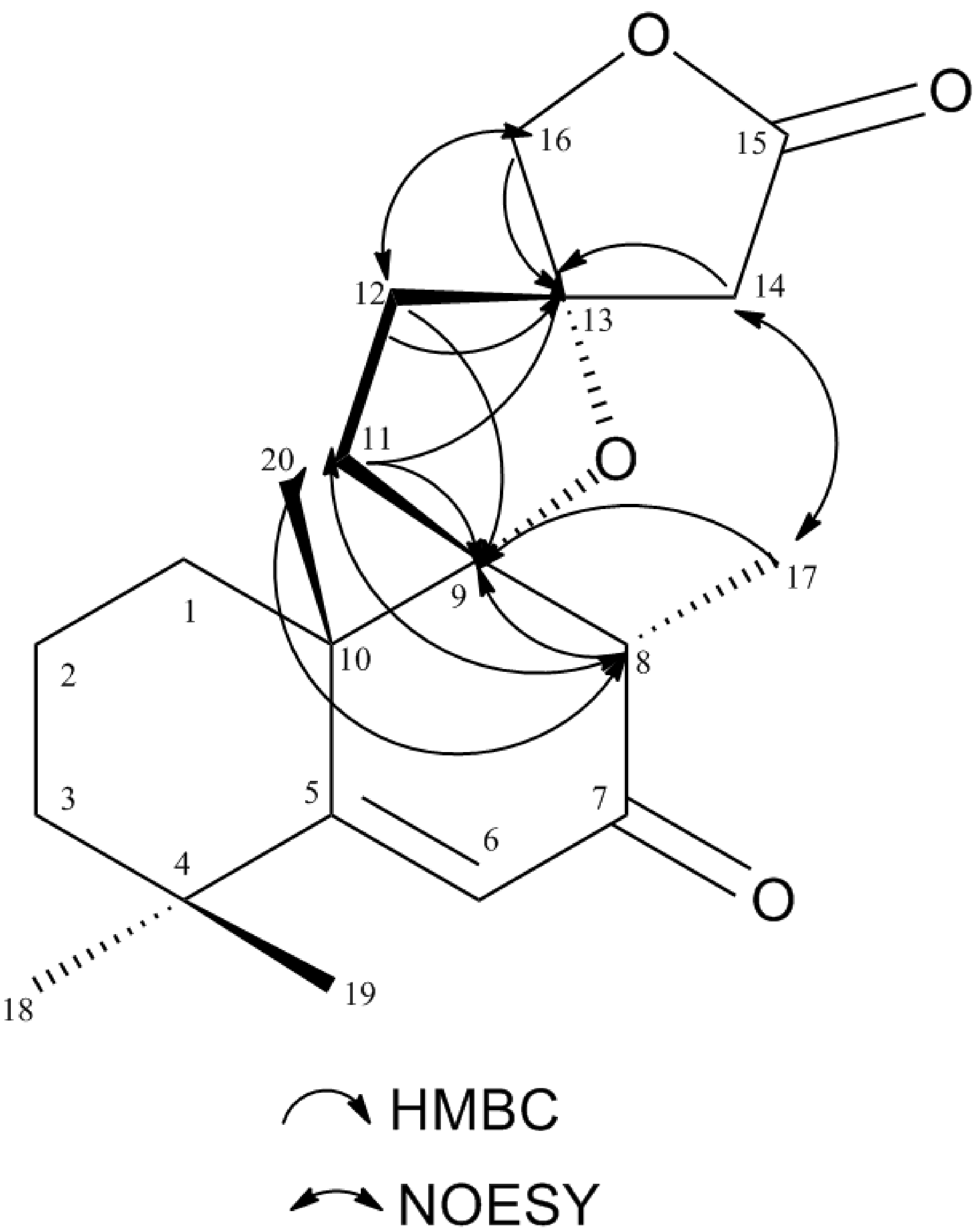


Fig. 8 Key HMBC and NOESY correlations of compound **35**

In the NOESY spectrum, correlations from H8 to H20 and H11 were seen, placing these groups on the same face of the diterpene scaffold. Further NOESY cross-peaks between H14 and H17 and H16 and H12 established

the 13*S* relative stereochemistry depicted. It is interesting that **35** being relatively non-polar was isolated from the aqueous extract; we cannot exclude the possibility that **35** is an artifact being formed via lactonization of a more polar C15 hydroxyl, C16 carboxylate intermediate during the isolation process.

3.2.5 Structure elucidation of compound 36 [51]

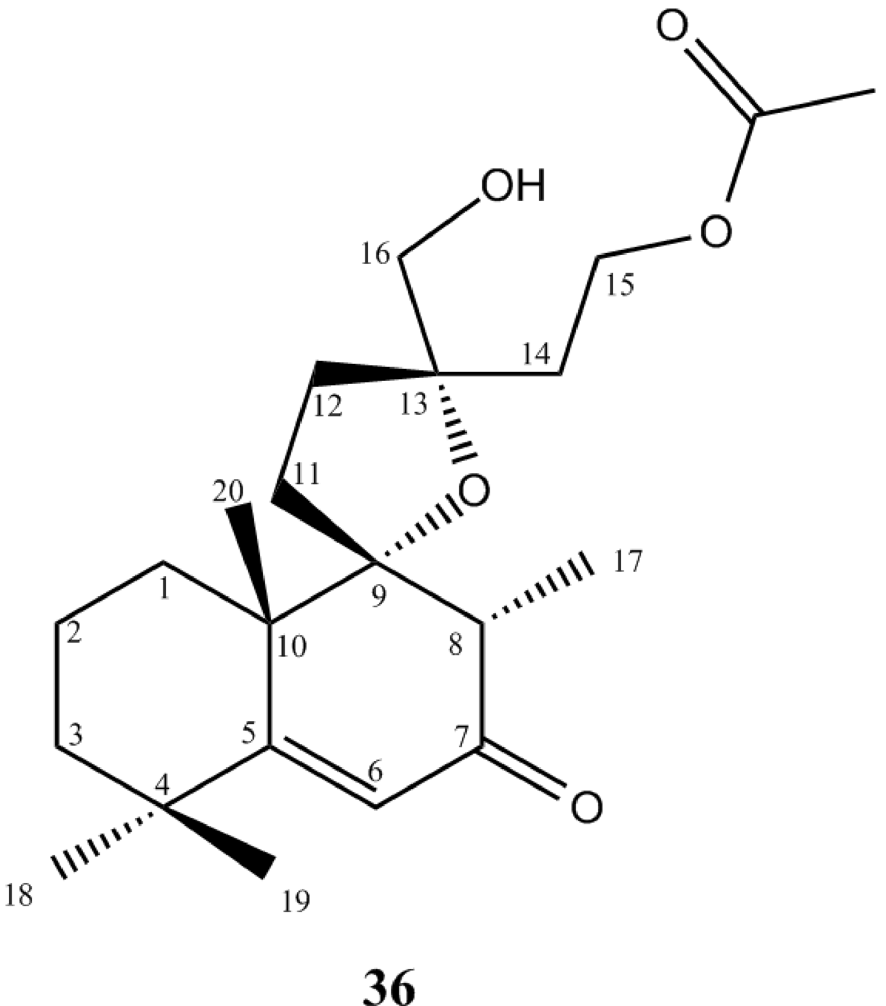


Fig. 9 Structure of compound **36**

The HRESIMS of compound **36** indicated a molecular formula of $C_{22}H_{34}O_5$ (*m/z* 378.2407). The previously described compounds **34** and **35** were obviously similar to **36** based on the NMR spectroscopic data accumulated. (see Table 4.)

Table 4. ^{13}C NMR and ^{1}H NMR Spectroscopic Data for Compound **36**

position	^{13}C		^{1}H	(J in Hz)	HMBC
1	30.7	CH_2	1.66	t (3.6)	5, 9, 10, 20
2a	17.4	CH_2	1.74	m	1, 4, 10
2b			1.87	m	
3	39.3	CH_2	1.58	m	1, 2, 4, 5, 18, 19
4	37.1	C			
5	172.0	C			
6	123.2	CH	6.05	s	4, 5, 8, 10
7	199.8	C			
8	47.7	CH	2.98	q (6.9)	7, 9, 10,17
9	95.7	C			
10	45.0	C			
11a	29.7	CH_2	1.91	m	13
11b			2.18	m	9, 10, 13
12a	34.3	CH_2	1.78	m	9, 14
12b			1.91	m	13, 15
13	85.2	C			
14a	35.7	CH_2	1.93	m	15, 16,
14b			2.15	m	12, 13, 15, 16
15a	61.5	CH_2	4.06	m	13, 14, $CH_3\underline{C}OO$
15b			4.16	m	13, 14, $CH_3\underline{C}OO$
16a	65.4	CH_2	3.40	d (11.3)	13, 16
16b			3.52	d (11.3)	13, 16
17	9.9	CH_3	1.24	d (6.8)	7, 8, 9
18	31.7	CH_3	1.20	s	3, 4, 5, 19
19	30.6	CH_3	1.21	s	3, 4, 5, 18
20	24.1	CH_3	1.38	s	1, 5, 9, 10
$\underline{C}H_3COO$	21.0	CH_3	2.05	s	$CH_3\underline{C}OO$
$CH_3\underline{C}OO$	171.1	C			

Assignments for C1 to C13 (particularly the now familiar α,β-unsaturated enone moiety) could readily be made by comparison of their ^{1}H NMR and ^{13}C NMR spectra in tandem with HMBC analysis. Protons at δ_H 3.52 and 3.40 (both doublets, J = 11.3 Hz) were correlated to C13 (δ_C 85.2) and C12 in the HMBC spectrum. These protons were assigned to

the oxymethylene group at C16. HMBC cross-peaks were observed from protons at δ_H 1.91, 2.15, 4.06 and 4.16 to C13. Protons at δ_H 4.06 and 4.16 were attached to δ_C 61.5. Their downfield chemical shift suggested attachment to an acyloxy group. Indeed, presence of an acetoxyl group was established via resonances at δ_H 2.05 (3H, s); δ_C 21.0 (CH_3) and δ_C 171.1 (C). Thus the carbon at δ_C 61.5 was assigned to C15 enabling complete assignments for the molecule and elucidation of the structure proposed.

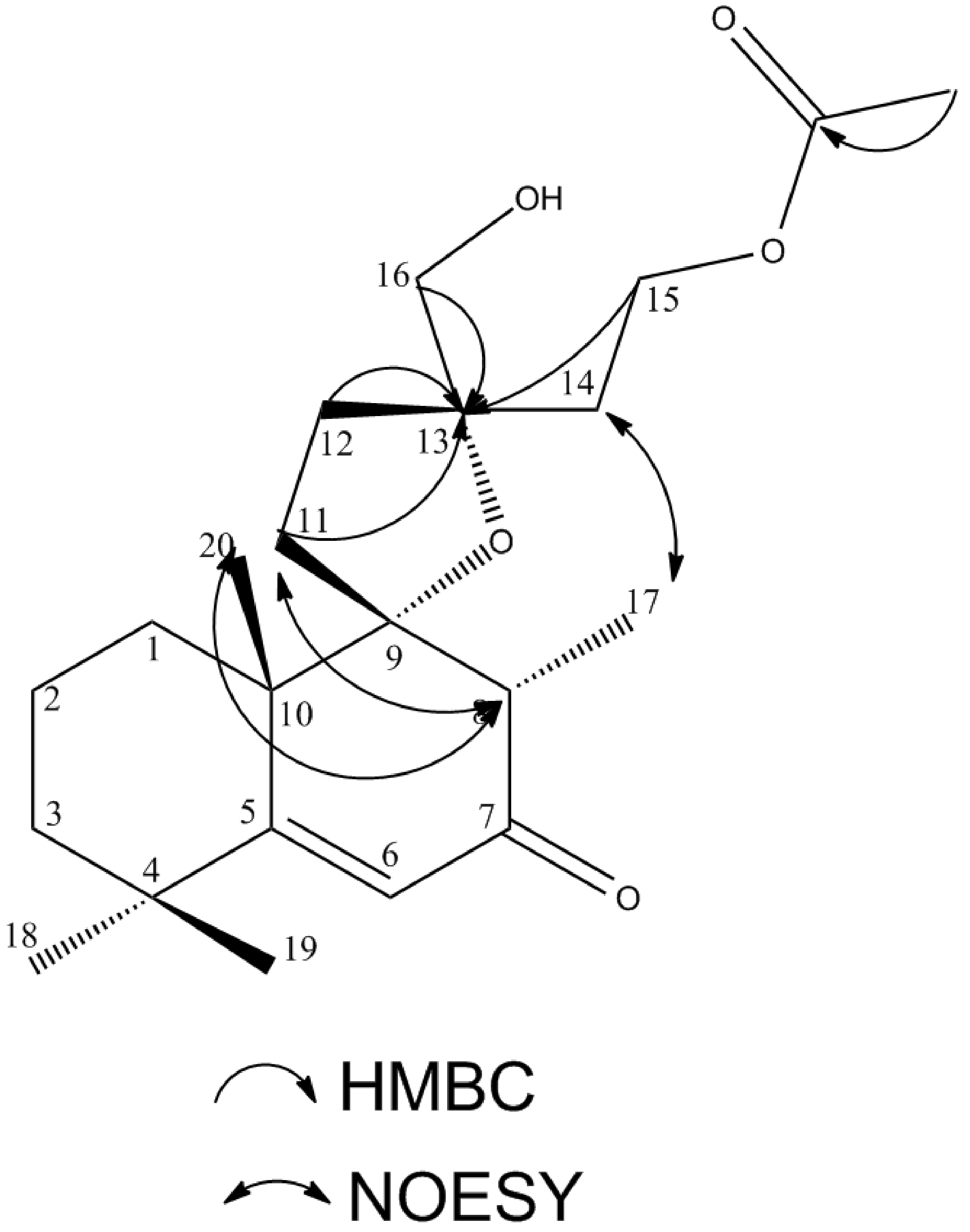


Fig. 10 Key HMBC and NOESY correlations of compound **36**

The NOESY data acquired suggested that **36** had the same relative configuration as seen in **34** and **35**. In that regard, key NOESY correlations were seen for H8 to H20 and H11 and for H17 to H14.

3.2.6 Structure elucidation of compound 37

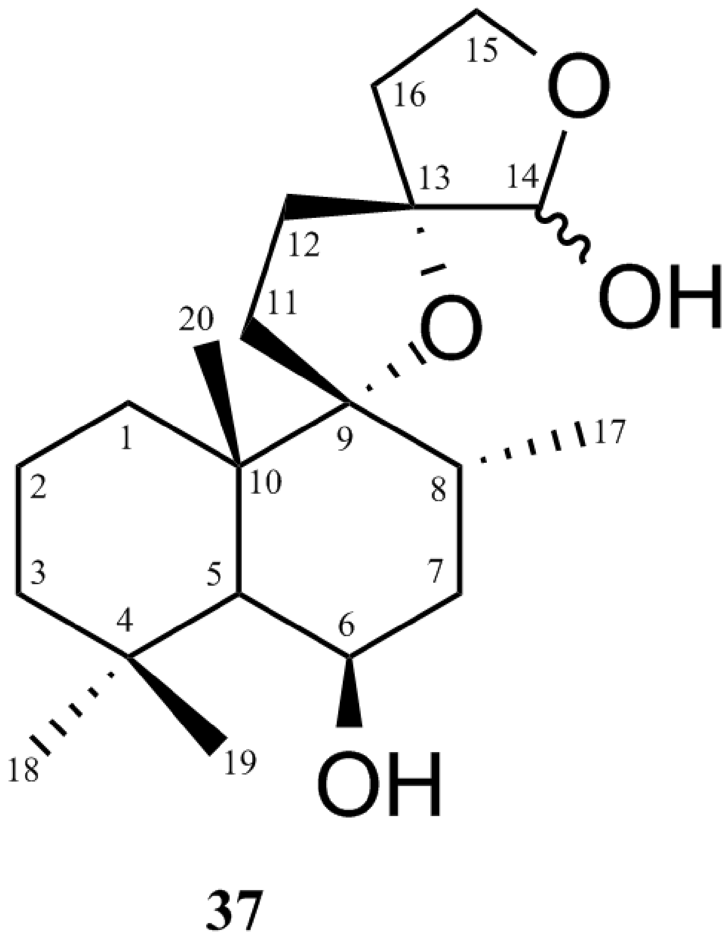


Fig. 11 Structure of compound **37**

Compound **37** was assigned the molecular formula $C_{20}H_{34}O_4$, determined by HRESIMS. The IR spectrum had absorption characteristic of hydroxy group (3427 cm^{-1}). According to the formula$C_{20}H_{34}O_4$ and the other reported compounds isolated from the same or the similar species, this compound **37** also looked like a labdane type lactone with some hydroxy groups. The ^{13}C NMR spectrum showed resonances at δ 100.5 (C16), which appeared to be due to a carbon connected to two oxygens.

In the DEPT 135 spectrum, this peak was positive, which meant it is due to either CH or CH_3. Considering the chemical shift, it was deduced to be a CH. According to the HSQC, one proton δ 5.23 (1 H, s, H16) was directly connected to this carbon. Also considering the carbon at δ 63.6 (C15) and the proton at δ 4.01 (2 H, m, H15), there was possibly a 2-hydroxyl, tetrahydrofuran group in the molecule. Neither of the carbon at δ 89.7 (C13) and the carbon at δ 93.8 (C9) showed up in DEPT 135, which meant both of these two carbons were quaternary carbon. According to the chemical shift, both of these two carbons were possibly connected to an oxygen. Therefore, we figured out the two five-member rings structure (OC9C11C12C13C14C15OC16OH). According to the chemical shift of the carbon at δ 66.9 (C6), the carbon was possibly attached to a hydroxy group. On the other hand, the ^{1}H NMR spectrum showed resonances due to four methyl groups at δ 1.29 (3 H, s, $H_3$20), 1.25 (3 H, d, J=4.4 Hz, $H_3$17), 1.02 (3 H, s, $H_3$19), 0.94 (3 H, s, $H_3$18). Considering all of the data, we determined the structure as in compound **37**. The compound **37** was isolated as an epimeric mixture.The epimeric ratio of **37** was approximately 3:1 based on integration of the H16 signals of the major and minor isomers - δ_H 4.75 and 5.32, respectively.

3.2.7 Structure elucidation of compound 38

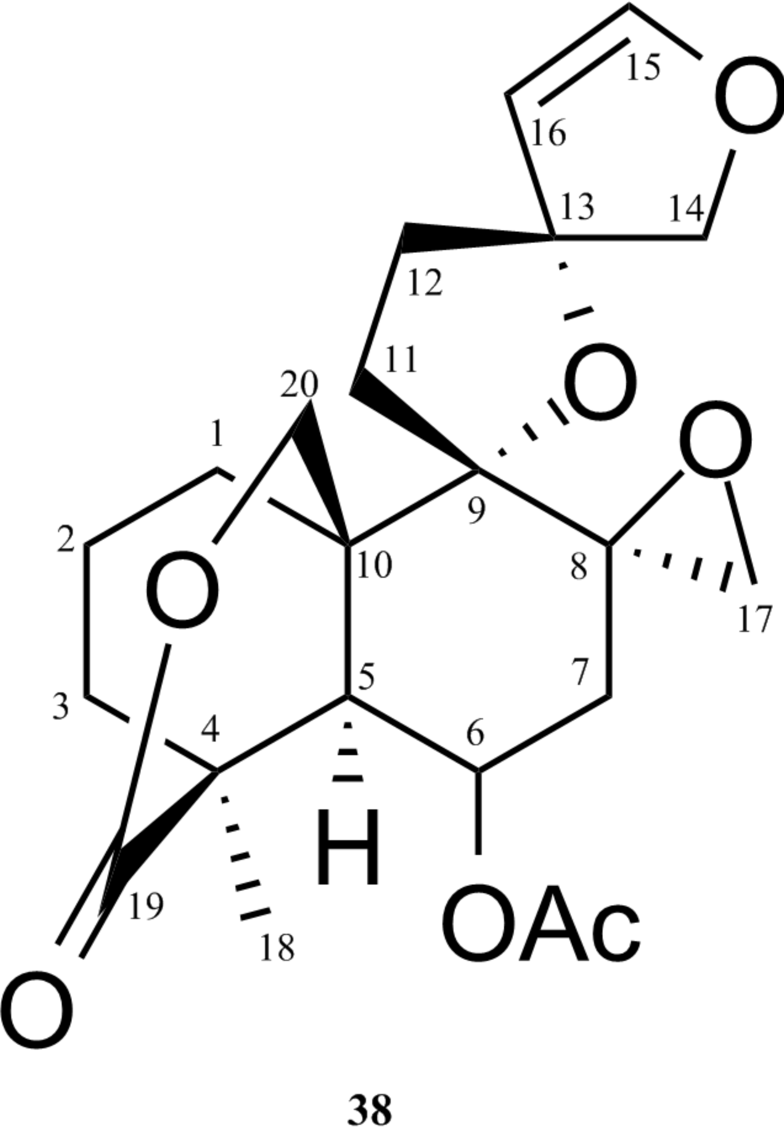


Fig. 12 Structure of compound **38**

Compound **38** had the molecular formula $C_{22}H_{28}O_7$ determined by HRESIMS $[M]^+$ *m/z*: 404.1836, indicating nine degrees of unsaturation. Its molecular formula and the consideration of the compounds previously isolated from the genus *Leonotis* suggested the initial diterpenoid nature

of **38**.

Its ^{13}C NMR spectrum displayed two ester carbonyls resonances at δ 175.9 and 170.4. The ^{1}H NMR and ^{13}C NMR spectral data of **38** showed the presence of one tertiary methyl [δ 1.14 (3H, s); δ 22.2], one acetyl [δ 2.02; δ 21.0, 170.4] and an exocyclic epoxide ring [δ 2.36 (1H, d, *J*=3.9 Hz), 2.58 (1H, d, *J*=3.9 Hz); δ 47.2, 56.2].

By comparison of spectral data with those reported in the literature,[99-102] the structure of compound **38** was elucidated as the previously know nepetaefolin.

3.2.8 Structure elucidation of compound 39

Fig. 13 Structure of compound **39**

Compound **39** had the molecular formula $C_{21}H_{20}O_{11}$ determined by HRESIMS $[M]^+$ *m/z*: 448.1006, indicating twelve degrees of unsaturation. The subsequent scrutiny of its ^1H NMR and ^{13}C NMR spectral data highlighted the presence of a 5, 7, 3', 4'-quasubstituted flavone framework and a β-glucoside group. Considering its molecular formula and the compounds previously isolated from this species *Leonotis leonurus*, it approved that the molecular formula $C_{21}H_{20}O_{11}$ matched exactly with Luteolin 7-*O*-β-glucoside which was previously isolated from this species. [103]

In the end, the structure of compound **39** was confirmed as Luteolin

7-*O*-β-glucoside by comparison of the spectral data with those reported in the literature. [103]

3.2.9 Structure elucidation of compound 40

40

Fig. 14 Structure of compound **40**

Compound **40** had the molecular formula $C_{15}H_{10}O_6$ determined by HRESIMS $[M]^+$ *m/z*: 286.0481, indicating eleven degrees of unsaturation. The ^1H NMR and ^{13}C NMR spectral data of **40** also showed the presence of a 5, 7, 3', 4'-quasubstituted flavone framework, which were very similar to compound **39**. Comparing the spectral data and the molecular formula of **40** with those of **39**, we realized that the only difference between **40** and **39** was the disappearance of the β-glucoside group in **40**.

Then **40** was confirmed as Luteolin, which was also previously isolated from this species *Leonotis leonurus*,[103] by direct comparison with the reported data. [103]

3.2.10 Structure elucidation of compound 41

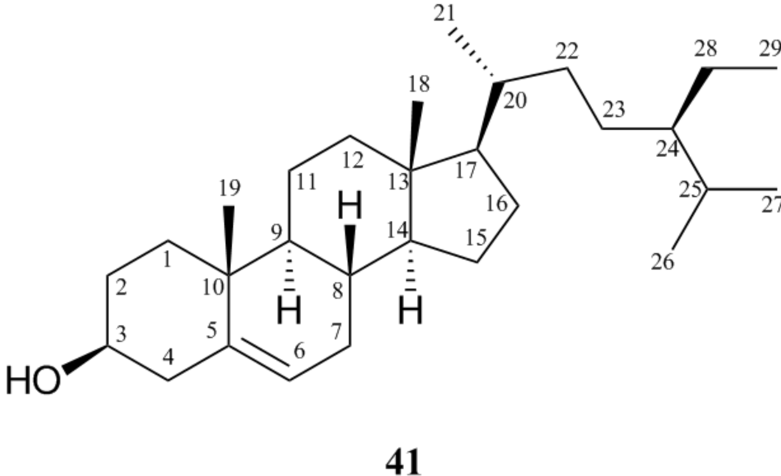


Fig. 15 Structure of compound **41**

Compound **41** had the molecular formula $C_{29}H_{50}O$ determined by HRESIMS $[M]^{+}$ *m/z*: 414.3866, indicating five degrees of unsaturation. The consideration of the typical natural product compounds and its molecular formula suggested the steroid nature of **41**.

The ^{1}H NMR and ^{13}C NMR spectral data of **41** showed the presence of one double bond (C=C) [δ 5.37 (1 H, m); δ 140.8, 121.7] and one carbon which was connected to a hydroxide group [δ 3.55 (1 H, m); δ 71.8].

By comparison of spectral data with those reported in the literature, [104] the structure of compound **41** was elucidated as β-Sitosterol, which is a very common natural product.

3.2.11 Structure elucidation of compound 42

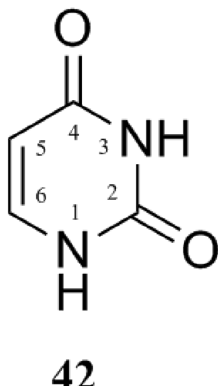


Fig. 16 Structure of compound **42**

Compound **42** had the molecular formula $C_4H_4N_2O_2$ determined by high-resolution MS HRESIMS $[M]^+$ *m/z*: 112.0274, indicating four degrees of unsaturation. The ^{1}H NMR and ^{13}C NMR spectral data of **42** showed the presence of one double bond (C=C) [δ 7.40 (1 H, t, J = 6.0 Hz), 5.46 (1 H, d, J = 8.0 Hz); δ 142.7, 100.7] and two carbonyl groups[δ 164.8, 152.0].According to the chemical shifts of these functional groups and the molecular formula, the structure of **42** should have a ring with nitrogen. The consideration of the typical natural product compounds suggested the nucleobase nature of **42**.

By direct comparison with the reported data, [105] the structure of compound **42** was elucidated as Uracil, which is one of the four nucleobases in the nucleic acid of RNA. [105]

NMR and physicochemical data for the known compounds are in close agreement with those previously reported. [98, 99, 103] The presence of **38**, **39**, **40**, **41** and **42** in this species is known. [98, 103] However, this is the first report of the presence of Nepetaefolin (**38**) in *L. leonurus*. It is proposed that the absolute configurations of the new compounds **34-37** are in agreement with the absolute configurations of other known labdanes given the co-occurrence of **34-37** and **38** (for which the crystal structure has been reported). [101]

3.2.12 Biological evaluations on *Leonotis leonurus* and isolated compounds

Because the most common use for *Leonotis leonurus* utilizes the picked and dried leaves brewed as a tea, the aqueous extract presumably contains components which cause the biological activities of *Leonotis leonurus*.

The aqueous extract was thus submitted for receptor binding assays, which was designed to identify a subset of potential receptors, transporters, ion channels, etc. for which the extract displayed affinity. We selected several neurotransmitter receptors: Adrenergic Alpha 1 Non-selective, Adrenergic Alpha 2 Non-selective, Adrenergic Beta Non-selective, Cannabinoid CBl (h), Cannabinoid CB2 (h), Dopamine Transporter, Dopamine D1, Dopamine D2, GABA A Agonist Site, GABA A BDZ alpha 1 site, GABA-B, Glutamate AMPA Site (Ionotropic), Glutamate NMDA Agonist Site (Ionotropic), Histamine H1, Histamine H2, Muscarinic Non-selective Central, Nicotinic Neuronal (a-BnTx sensitive) rat, Norepinephrine Transporter, Opioid Non-selective, Opioid Orphanin ORL1 (h), Serotonin Transporter, Serotonin Non-selective and sigma Non-selective because these receptors are all involved in mediating CNS' effects. [111] The results of these neurotransmitter receptor assay are showed in Table 5.

Table 5. The results of neurotransmitter receptor assay for *Leonotis leonurus* aqueous extract

Receptor	% inhibition (1.0E2 μg/mL)
Adrenergic Alpha 1 Non-selective	-84.20%
Adrenergic Alpha 2 Non-selective	0.66%
Adrenergic Beta Non-selective	-0.81%
Cannabinoid CBl (h)	-4.38%
Cannabinoid CB2 (h)	10.46%
Dopamine Transporter	13.54%
Dopamine D1	13.32%
Dopamine D2	21.13%
GABA A Agonist Site	-1.30%
GABA A BDZ alpha 1 site	26.54%
GABA-B	11.06%
Glutamate AMPA Site (Ionotropic)	-1.20%
Glutamate NMDA Agonist Site (Ionotropic)	-10.14%
Histamine H1	19.04%
Histamine H2	-20.86%
Muscarinic Non-selective Central	-13.52%
Nicotinic Neuronal (a-BnTx sensitive) rat	3.71%
Norepinephrine Transporter	14.85%
Opioid Non-selective	-3.24%
Opioid Orphanin ORL1 (h)	-244.5%
Serotonin Transporter	16.65%
Serotonin Non-selective	4.27%
sigma Non-selective	10.04%

We got two large negative values for the receptors Adrenergic Alpha 1 Non-selective and Opioid Orphanin ORL1 (h), which meant that the *Leonotis leonurus* aqueous extract had stimulation effect on these two receptors, instead of inhibition.

We also choose the receptors Dopamine D2, GABA A BDZ alpha 1 site and Serotonin Transporter, which showed relatively high positive

values, to do the receptor binding assays for the pure compounds Leonurenone A **(34)** and Leonurenone B (**35**). In the receptor binding assays for the Leonurenone A **(34)** and Leonurenone B (**35**), we used low concentration (3.4 μg/mL), to compare with the high concentration (1.0E2 μg/mL) in the receptor binding assays for the *Leonotis leonurus* aqueous extract.

Nepetaefolin (**38**) was also submitted for receptor binding assays. We selected several neurotransmitter receptors: Cannabinoid CBl (h), Cannabinoid CB2 (h), GABA Non-Selective, GABA-B, Histamine H1, Histamine H2, Histamine H3, Opioid Delta 2 (h), Opioid Kappa 1, Opioid Mu (h), and Serotonin Non-selective, because these receptors may contribute to the diversity of inhibitory synapses in the central nervous system. [110]

The results of the receptor binding assays for the Leonurenone A **(34)**, Leonurenone B (**35**) and Nepetaefolin (**38**) are shown in Table 6.

Table 6. The results of the receptor binding assays for Leonurenone A **(34)**, Leonurenone B (**35**) and Nepetaefolin (**38**)

Receptor	Percent inhibition		
	34	**35**	**38**
Dopamine, D2	-9.15%	-10.12%	N/A
GABA A, BDZ, alpha 1 site	11.38%	10.66%	N/A
Serotonin Transporter	-15.21%	6.08%	N/A
Cannabinoid CBl (h)	N/A	N/A	1.69%
Cannabinoid CB2 (h)	N/A	N/A	10.11%
GABA Non-Selective	N/A	N/A	4.79%
GABA-B	N/A	N/A	1.09%
Histamine H1	N/A	N/A	8.74%
Histamine H2	N/A	N/A	10.47%
Histamine H3	N/A	N/A	6.25%
Opioid Delta 2 (h)	N/A	N/A	4.33%
Opioid Kappa 1	N/A	N/A	32.26%
Opioid Mu (h)	N/A	N/A	31.44%
Serotonin Non-selective	N/A	N/A	-7.51%

Unfortunately none of these three compounds showed good activity (≥ 50% inhibition binding) in the assays requested.

Luteolin (**40**) is one of the major compounds (see Fig. 17) in the aqueous extract of *Leonotis Leonurus* leaves.

It was reported that Luteolin (**40**) apparently has CNS activity with anxiolytic-like effects. [112, 113] Some herbs which also contain Luteolin (**40**), such as lemon catnip herb (*Nepeta cataria L. var. citriodora*), were reported to have mild sedative effects. [102, 114] Affinity of Luteolin (**40**) for the benzodiazepine receptor was detected (K_i>100μM). [115] Therefore Luteolin (**40**) is possibly responsible for the anxiolytic-like effects and the sedative effects of *Leonotis leonurus*.

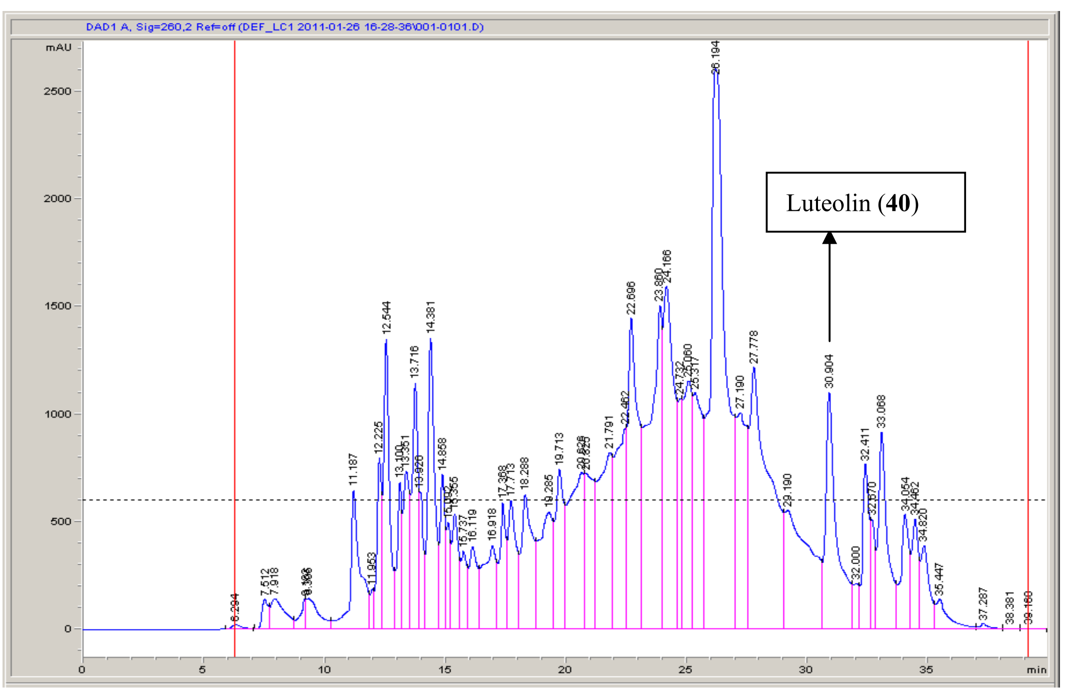


Fig. 17 HPLC chromatogram for *L. leonurus* aqueous extract

3.2.13 Synthesis and biological evaluation of leonurine

Leonurine (**49**)

Fig. 18 (a) Structure of leonurine

It is claimed that the compound responsible for the marijuana-like effects of *Leonotis leonurus* is an alkaloid, leonurine. However, this has not been substantiated scientifically and is questionable since leonurine has never been isolated from the plant and in a recent publication, it was reported that leonurine was not detectable in *Leonotis leonurus* using HPLC. [116] We wish to determine whether leonurine has sedative activity or not. Therefore we need to synthesize leonurine.

The synthesis of leonurine was executed following Fig. 18 (b), using the esterification of syringic acid and 1-(4-hydroxybutyl)guanidine. In each step of the whole synthesis process, the NMR data of the products were in agreement with the literatures.

43

TBDMSCl
Imidazole

room temperature,
12h, 55%

44

EDCI
Et_3N
DMAP

room temperature,
12h, 62%

45

N,*N*'-Di-Boc-1H-pyrazole-1-carboxamidine

room temperature,
12h, 40%

46

47

room temperature,
2h, 79%

TBAF

48

room temperature,
12h, 80%

HCl

49

Fig. 18 (b) Synthetic scheme for leonurine

Leonurine had been prepared previously by Cheng et al. [62] Leonurine was prepared in 80% yield. The synthesis involved the condensation of syringic acid and 4-guanidino-1-butanol hydrochloride in the presence of *N*,*N'*-Dicyclohexylcarbodiimide (DCC) using 1:1 hexamethylphosphorous triamide (HMPT)-ether as solvent. [62]

Although Cheng et al. reported a reasonable yield for the synthesis of leonurine, the known carcinogenic solvent HMPT was used in their method [36]. Comparing with their method, our route for the synthesis of leonurine was more practical and less hazardous.

We successfully finished the synthesis of leonurine and then submitted leonurine for receptor binding assays. We selected several neurotransmitter receptors based on their known involvement in sedation: [120] Adenosine Transporter (h); GABA A, Agonist Site; Glutamate, mGluR5 (Metabotropic); Calcium Channel, Type N. The results of neurotransmitter receptor assay are showed in Table 7.

Table 7. The results of neurotransmitter receptor assay for leonurine

Receptor	K_i values (nM)	Receptor	K_i values (nM)
5-HT_{1A}	N/A	D2	N/A
5-HT_{1B}	N/A	D3	N/A
5-HT_{1D}	N/A	D4	N/A
5-ht_{1e}	N/A	D5	N/A
5-HT_{2A}	1655	DAT	N/A
5-HT_{2B}	721	DOR	N/A
5-HT_{2C}	1916	GABAA	N/A
5-HT_3	N/A	H1	N/A
5-ht_{5a}	N/A	H3	N/A
5-HT_6	N/A	KOR	N/A
5-HT_7	N/A	M1	N/A
$Alpha_{1A}$	N/A	M2	N/A
$Alpha_{1B}$	N/A	M3	N/A
$Alpha_{1D}$	N/A	M4	N/A
$Alpha_{2A}$	N/A	M5	N/A
$Alpha_{2B}$	N/A	MOR	N/A
$Alpha_{2C}$	3558	NET	N/A
Beta1 adrenergic	N/A	PBR	N/A
Beta2 adrenergic	N/A	SERT	N/A
Beta3 adrenergic	N/A	Sigma 1	N/A
BZP Rat Brain Site	N/A	Sigma 2	551
D1	N/A		

* N/A: Activity was not detected. (1° Assay <50%)

Unfortunately leonurine did not show high affinity in the assays requested, which means leonurine is most likely not exerting sedative activity through these receptors.

3.3 Conclusions

There have been a number of phytochemical investigations on *L. leonurus* and interest in this plant continues to be high because of the reported biological activities. Previous phytochemical work on the leaves of *L. leonurus* have concentrated on organic extracts. However, the biological activities ascribed to the plant are attributable to consumption of aqueous preparations. An investigation of the aqueous extract was thus prudent at this time.

Our investigation has uncovered three new labdane diterpenes from an aqueous extract of a commercial source of the plant. Thus this work highlights the utility of commercially available plant sources as a viable option for phytochemical work. Clearly however, as carried out in this thesis, the appropriate validation of the plant material must accompany any such investigation.

Results from our study, as well as a previous investigation on the anticonvulsant effects of *L. leonurus*, [72] suggest that the aqueous extract has $GABA_A$ activity. However, compounds **34**, **35** and **38** isolated herein did not show any activity at this receptor, indicating that these compounds are not by themselves responsible for the activity.

Although leonurine was claimed to be responsible for the marijuana-like effects of *Leonotis leonurus*, it has never been isolated

from the plant and may not have sedative activity through those receptors we tested.

3.4 Experimental

3.4.1 General experimental procedures

Optical rotations were performed on a Rudolph Autopol IV polarimeter with the Na 589 line. IR spectra were recorded on a Thermo Nicolet IR 100 spectrophotometer as thin films. UV spectra were recorded on a Varian Cary 1 Bio UV–Visible spectrophotometer in MeOH solution. NMR spectroscopic data were obtained on a Bruker 500 MHz spectrometer in $CDCl_3$ as solvent unless otherwise stated and with TMS as internal standard. Chemical shift (δ) values are reported in ppm and coupling constants in Hertz (Hz). High Resolution Electrospray Ionization Mass Spectra (HRESIMS) were obtained using an Agilent 6520 Q-TOF instrument. TLC analysis was performed with Analtech Uniplate silica gel G/UV 254 pre-coated plates (0.2 mm). TLC plates were visualized by UV (254 and 360 nm), and by staining with 10% sulfuric acid/vanillin reagent followed by heating. Flash column chromatography (FCC) was performed with Silica gel 60 (EMD Chemicals, 230–400 mesh, 0.04–0.063 μm particle size) and Diaion HP-20 (Supelco/1-3607/176255E). HPLC purifications were performed on an Agilent 1200 system equipped with variable wavelength detector and fraction collector with Partisil 10 (Whatman) (10.0 × 500 mm, 10 μm) or ZORBAX SB-C18, 21.2 × 150 mm, 7 μm (Agilent). Lyophilization was performed with a Labconco Freezone 2.5 system.

3.4.2 Plant material

Plant material (crushed leaves) was purchased from Bouncing Bear Botanicals in September 2009. A voucher specimen (voucher number BKL00104130) is deposited in the Herbarium at the Brooklyn Botanic Gardens, Brooklyn NY 11225. Authentication of the material was performed via genetic analysis as described below. Genetic sequencing data is accessible athttp://www.ncbi.nlm.nih.gov/(GenBank accessions JX073278 and JX073279).

3.4.3 Genetic Identification of *L. leonurus*

Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer ' s instructions. Amplification of the chloroplast trnL and rps16 introns was performed following the method outlined in. [117] Since the DNA was potentially of low quality, the internal primers rpsLR and rpsLF were used to amplify the rps16 intron as two separate, shorter fragments. [117] The DNA was sequenced at the University of Washington High-Throughput Genomics Unit. The sequences were assembled and edited using Sequencher 4.1.4 (Gene Codes) before being aligned manually to an existing database of lamioid mint DNA sequences using BioEdit. [118] Phylogenetic reconstruction was performed using the programs TNT and SplitsTree4. [119] Accession

numbers for GenBank accessions are JX073278 and JX073279.

3.4.4 Extraction and isolation

Ground leaves of *L. leonurus* (50.2 g) were extracted three times with H_2O (3 × 1 L) by percolating in distilled H_2O at room temperature overnight. The extracts were combined and dried by lyophilization to yield a dried extract (4.5 g). A portion of the aqueous extract (3.3 g) was applied to a Diaion HP-20 (Supelco/1－3607/176255E) column (19.0 × 4.5 cm) and fractionated using a H_2O:MeOH gradient solvent system (100:0, 30:70, 0:100). Fractions were collected and pooled by TLC analysis to afford 5 combined fractions. From these combined fractions, the fraction eluted with 100% MeOH (0.12 g) was subjected to a silica gel CC (15.0 × 1.2 cm) using an EtOAc:hexanes gradient solvent elution (20:80, 40:60, 60:40, 80:20, 100:0) to give 12 combined fractions. The fraction eluted in EtOAc:hexanes (80:20) was purified by HPLC [Partisil 10 column; flow rate: 2.00 mL/min; injection volume: 15 μL; UV detector wavelength: 254 nm] to provide **34** (4.1 mg).

Compound **35** (3.0 mg) was isolated following HPLC of the fraction eluted in EtOAc:hexanes (40:60) using the following chromatographic parameters. Column: Partisil 10; flow rate: 2.00 mL/min; injection volume: 20 μL; UV detector wavelength: 254 nm.

Ground *L. leonurus* leaves (180.1 g) were defatted with hexanes (3

× 1 L) by percolation at room temperature overnight. Extraction of the marc of the hexanes extract with acetone provided an acetone extract (2.1 g) after removal of solvent. A portion (1.1 g) of the acetone extract was subjected to repeated HPLC purification in MeOH:CH_2Cl_2 (1:99, v/v) to afford compound **36** (1.3 mg) (see Fig. 19). The following parameters were used. Column: Partisil 10; flow rate: 5.00 mL/min; injection volume: 10 μL; UV detector wavelength: 254 nm.

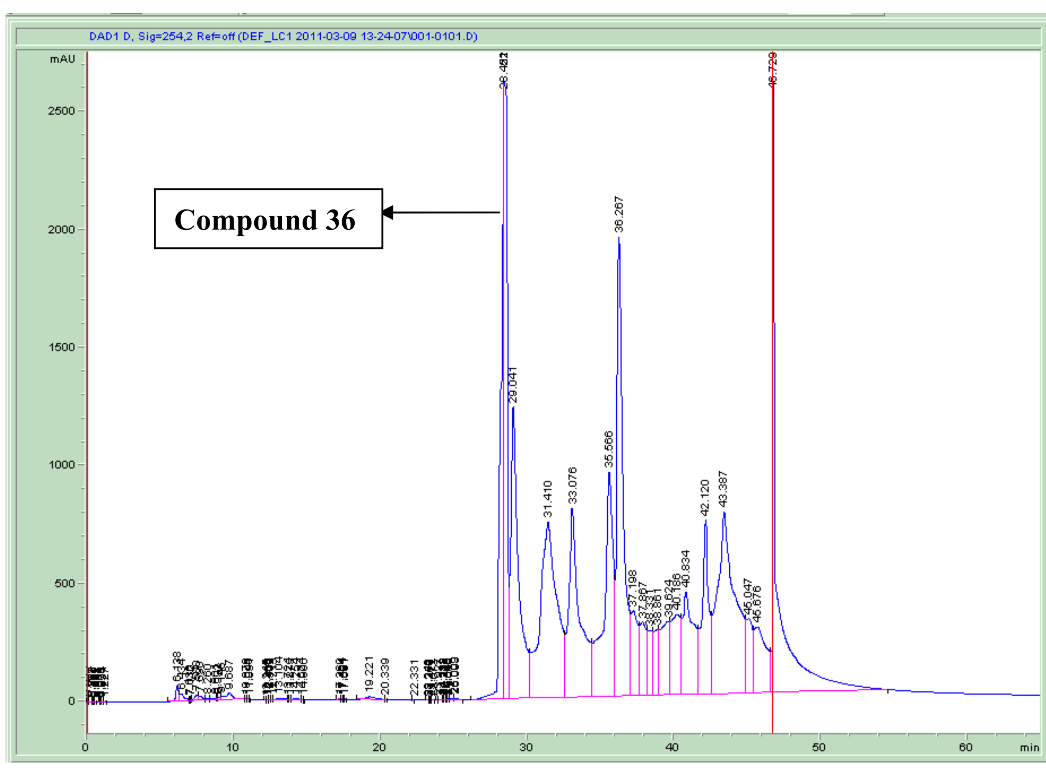


Fig. 19 HPLC chromatogram for *L. leonurus* acetone extract

The fraction eluted in EtOAc:hexanes (20:80, v/v) was applied to a silica gel column using an EtOAc:hexanes gradient solvent system (15:85,

20:80), to give compound **37** (1.2 mg).

A portion (1.0 g) of the acetone extract prepared as described above, was purified by FCC on a silica gel column using an EtOAc:hexanes gradient solvent system (10:90, 20:80, 30:70, 40:60, 50:50). Repeated FCC of the fraction eluted in EtOAc:hexanes (50:50, v/v) gave compound **38** (3.1 mg).

The fraction eluted in EtOAc:hexanes (20:80, v/v) was applied to on a silica gel column using an MeOH:CH_2Cl_2 gradient solvent system (0:100, 0.5:99.5, 1:99, 1.5:98.5, 2:98), to give compound **41** (4.1 mg).

A separate portion of *L. leonurus* aqueous extract (1.0 g) was subjected to HPLC with a ZORBAX SB-C18 column (eluted in H_2O and MeOH, respectively) yielding three fractions. Compounds **39** (1.1 mg), **40** (1.3 mg)and compound **42** (1.0 mg) were obtained by repeated HPLC of each of these there fractions (eluted in 100% MeOH). The following parameters were used. Column: ZORBAX SB-C18, 21.2 × 150 mm, 7 μm; flow rate: 4.00 mL/min; injection volume: 55 μL; UV detector wavelength: 254 nm.

The isolation scheme for compound **34**, **35** and **37** is as follows:

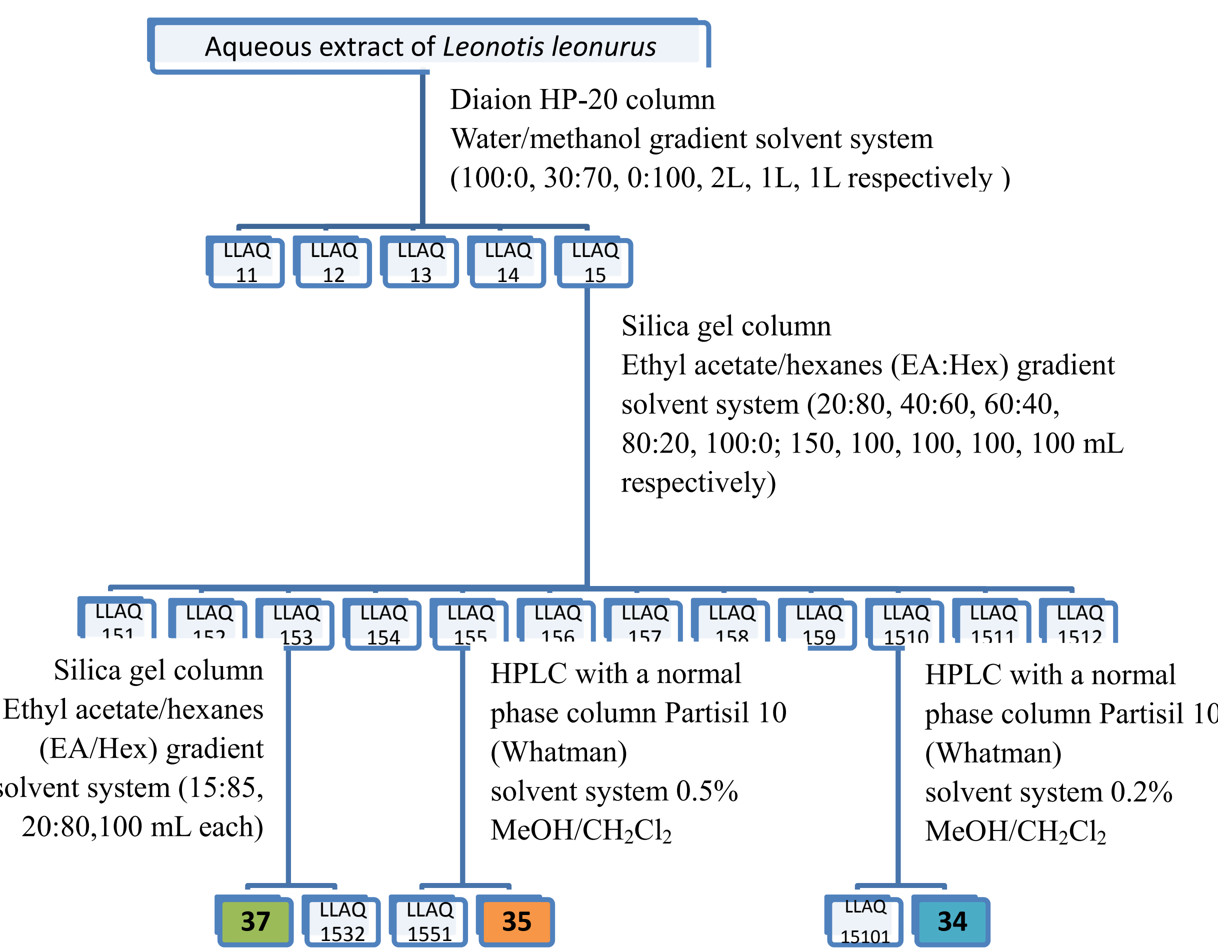


Fig. 20 The isolation scheme for compound **34**, **35** and **37**

The isolation scheme for compound **39, 40** and **42** is as follows:

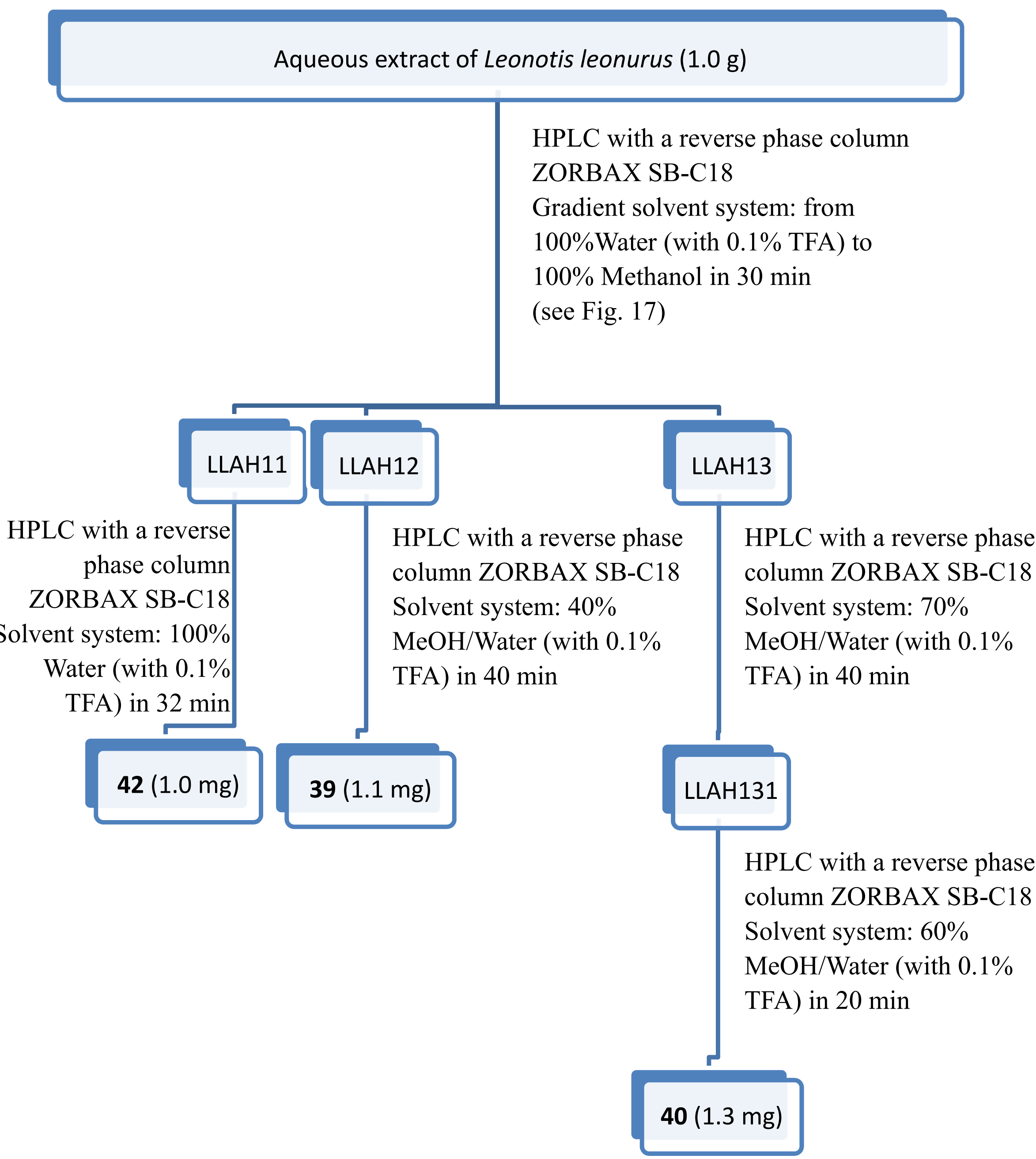


Fig. 21 The isolation scheme for compound **39, 40** and **42**

The isolation scheme for compound **38** and **41** is as follows:

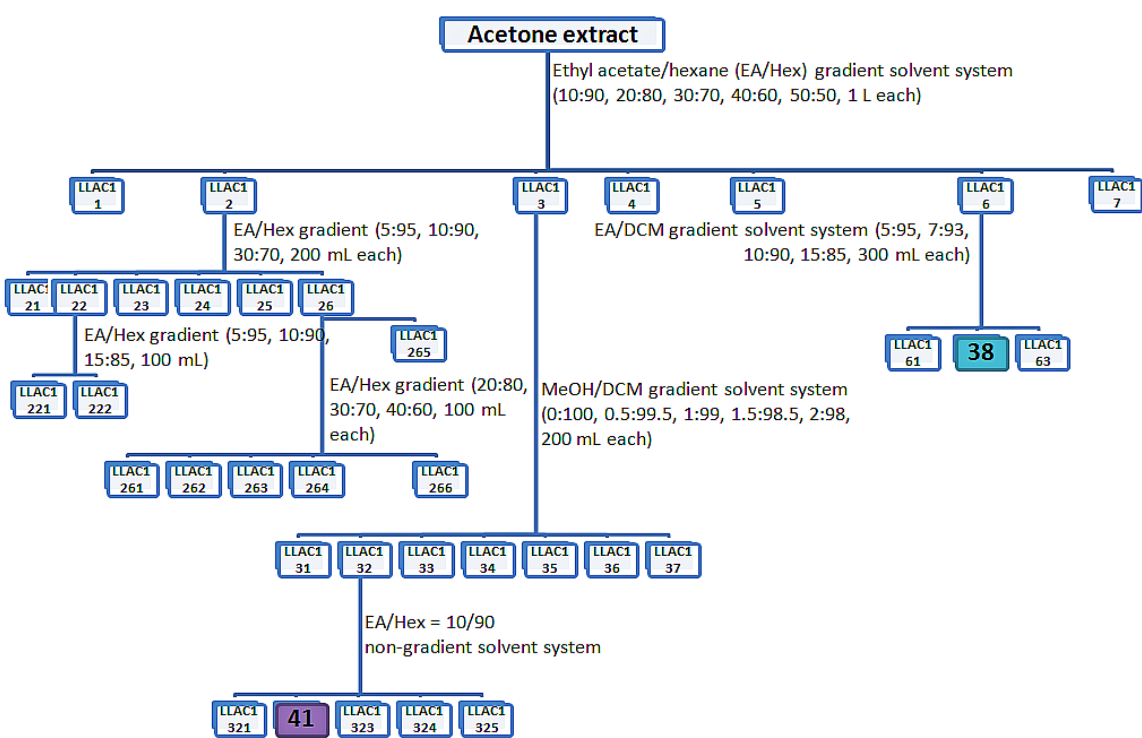


Fig. 22 The isolation scheme for compound **38** and **41**

3.4.4.1 Leonurenone A (34) [51]

Colorless oil; $[\alpha]^{20}_{D}$ − 26.1 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 236 (3.11), 269 (2.52) nm; IR (film) ν_{max} 2933, 1664, 1464, 1360, 1170, 1043 cm^{-1}; for ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, see Table 1. HRESIMS $[M]^{+}$ *m/z*: 336.2308 (calculated for $C_{20}H_{32}O_4$ 336.2380).

3.4.4.2 Leonurenone B (35) [51]

Colorless oil; $[\alpha]^{20}_{D}$ − 8.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 243 (2.90) nm; IR (film) ν_{max} 2932, 1781, 1662, 1464, 1372, 1171 cm^{-1}; for ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data, see Table 1.HRESIMS $[M]^{+}$ *m/z*: 332.1988 (calculated for $C_{20}H_{28}O_4$ 332.1976).

3.4.4.3 Leonurenone C (36) [51]

Colorless oil; $[\alpha]^{20}_{D}$ − 24.9° (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 231 (2.51) nm; IR (film) ν_{max} 3449, 2929, 1737, 1663, 1466, 1365, 1242, 1042 cm^{-1}; for ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz), see Table 1.HRESIMS $[M]^{+}$ *m/z*: 378.2407, (calculated for $C_{22}H_{34}O_5$ 378.2406).

NMR spectra of Compounds **34-36** are in the Appendix.

3.4.4.4 9,13:15,16-Diepoxy-6,16-labdanediol (37) [88]

Colorless oil; $[\alpha]^{20}_{D}$ − 49.2° (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 237 (2.96) nm; IR (film) ν_{max} 3427, 2926, 1722, 1465, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.32 (1 H, s), 4.75 (3 H, s), 4.39 (3 H, s), 4.35 (1 H, s), 4.13 (3 H, t , J = 10 Hz), 4.01 (2 H, m), 3.83 (3 H, q, J = 10 Hz), 3.31 (3 H, s), 1.29 (3 H, s), 1.25 (3 H, d, J=4.4 Hz), 1.02 (3 H, s), 0.94 (3 H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 127.9, 120.3, 101.0, 98.9, 94.4, 90.7, 67.9, 65.1, 50.1, 44.1, 40.8, 36.7, 35.6, 33.3, 30.9, 29.3, 24.7, 20.5, 18.7, 17.1; HRESIMS $[M]^+$ *m/z*: 338.2346 (calculated for $C_{20}H_{34}O_4$ 338.2457)

3.4.4.5 Nepetaefolin (38) [99-102]

White solid; $[\alpha]^{20}_{D}$ − 16.9 (c 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 234 (2.76), 259 (2.73) nm; IR (film) ν_{max} 2933, 2359, 1729, 1235, 1148, 1035, 938 cm^{-1}; m.p. 231.0℃/233.5℃ (literature value is m.p.250℃ /255℃[121]); ^1H NMR ($CDCl_3$, 500 MHz) δ 6.52 (1 H, d, 2.6 Hz), 5.20 (1 H, m), 5.06 (1 H, m), 5.05 (1 H, m), 4.22 (1 H, d, 10.5), 4.01 (1 H, m), 3.97 (1 H, m), 2.64 (1 H, dd, 15.3, 3.4 Hz), 2.58 (1 H, d, 3.9), 2.36 (1 H, d, 3.9 Hz), 2.17 (1 H, m), 2.05 (1 H, m), 2.02 (3 H, s), 1.96 (1 H, m), 1.87 (1 H, m), 1.84 (2 H, m), 1.80 (2 H, m), 1.76 (1 H, m), 1.60 (1 H, dd, 15.3, 2.8 Hz), 1.57 (1 H, m), 1.46 (1 H, m), 1.14 (3 H, s); ^{13}C NMR ($CDCl_3$,

125 MHz) δ 175.9, 170.4, 149.5, 105.5, 92.8, 86.0, 81.1, 73.8, 67.9, 56.2, 47.2, 46.9, 41.1, 40.8, 39.6, 37.6, 33.3, 32.5, 23.6, 22.2, 21.0, 20.4; HRESIMS $[M]^+$ *m/z*: 404.1836 (calculated for $C_{22}H_{28}O_7$ 404.1835).

3.4.4.6 Luteolin 7-*O*-β-glucoside (39) [103]

Colorless oil; UV (MeOH) λ_{max} (log ε) 207 (1.71), 238 (2.19), 244 (3.58), 253 (4.11) nm; IR (film) ν_{max} 3424, 1637, 446, 427, 415 cm^{-1}; ^1H NMR (MeOD, 500 MHz) δ 7.46 (1 H, d, J = 5 Hz), 7.43 (1 H, s), 6.94 (1 H, d, J = 8 Hz), 6.81 (1 H, s), 6.64 (1 H, s), 6.52 (1 H, s), 5.19 (1 H, d, J = 6 Hz), 4.13 (1 H, d, J = 10 Hz), 3.66 (1 H, m), 3.56 (2 H, m); ^{13}C NMR (MeOD, 125 MHz)δ 182.7, 170.6, 165.5, 163.1, 161.6, 157.5, 149.8, 145.7, 122.1, 119.1, 115.4, 112.9, 105.8, 102.8, 100.0, 99.7, 94.6, 75.7, 75.2, 73.0, 71.4; HRESIMS $[M]^+$ *m/z*: 448.1006, (calculated for $C_{21}H_{20}O_{11}$ 448.1006).

3.4.4.7 Luteolin (40) [103]

Colorless oil; UV (MeOH) λ_{max} (log ε) 238 (2.89), 241 (3.51), 253 (4.06) nm; IR (film) ν_{max} 3517, 2981, 1644, 1055, 1033, 1013, 566, 431, 420, 408 cm^{-1}; ^1H NMR (MeOD, 500 MHz) δ 7.42 (1 H, d, J = 8 Hz), 7.41 (2 H, s), 6.94 (2 H, d, J = 8 Hz), 6.57 (2 H, s), 6.47 (2 H, s), 6.23 (1 H, s); ^{13}C NMR (MeOD, 125 MHz) δ 165.0, 164.6, 161.8, 158.0, 122.3, 118.9, 115.4, 112.7, 102.5, 98.7, 93.6; HRESIMS $[M]^+$ *m/z*: 286.0481,

(calculated for $C_{15}H_{10}O_6$ 286.0477).

3.4.4.8 **β-Sitosterol (41)** [104]

Colorless oil; UV (MeOH) λ_{max} (log ε) 190 (1.52) nm; IR (film) ν_{max} 3566, 1033, 668, 450, 431, 427, 420 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.37 (1 H, m), 3.55 (1 H, m), 2.31 (1 H, m), 2.26 (1 H, m), 2.02 (1 H, m), 1.98 (1 H, m), 1.88 (1 H, m), 1.86 (1 H, m), 1.84 (1 H, m), 1.68 (1 H, m), 1.61 (1 H, m), 1.54 (1 H, m), 1.53 (1 H, m), 1.51 (1 H, m), 1.49 (1 H, m), 1.46 (1 H, m), 1.36 (1 H, m), 1.32 (1 H, m), 1.28 (2 H, m), 1.26 (1 H, m), 1.20 (1 H, m), 1.18 (1 H, m), 1.16 (1 H, m), 1.10 (1 H, m), 1.08 (1 H, m), 1.05 (1 H, m), 1.04 (1 H, m), 1.03 (3 H, s), 1.02 (1 H, m), 0.97 (1 H, m), 0.95 (3 H, d, 6.5), 0.94 (1 H, m), 0.90 (1 H, m), 0.86 (3 H, m), 0.84 (3 H, m), 0.83 (3 H, s), 0.70 (3 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.8, 121.7, 71.8, 56.8, 56.0, 50.1, 45.8, 42.3, 42.2, 39.8, 37.3, 36.5, 36.2, 33.9, 31.92, 31.90, 31.7, 29.1, 28.3, 26.0, 24.3, 23.1, 21.1, 19.8, 19.4, 19.0, 18.8, 12.0, 11.9; HRESIMS $[M]^{+}$ *m/z*: 414.3866, (calculated for $C_{29}H_{50}O$ 414.3862)

3.4.4.9 **Uracil (42)** [105]

Colorless oil; UV (MeOH) λ_{max} (log ε) 251 (3.99) nm; IR (film) ν_{max} 3090, 1640, 1206, 538, 431 cm^{-1}; ^{1}H NMR (DMSO, 500 MHz) δ 11.02 (1 H, s), 10.83 (1 H, s), 7.40 (1 H, t, J = 6 Hz), 5.46 (1 H, d, J = 8

Hz);^{13}C NMR(DMSO, 125 MHz) δ 164.8, 152.0, 142.7, 100.7; HRESIMS $[M]^+$ *m/z*: 112.0274, (calculated for $C_4H_4N_2O_2$ 112.0273).

3.4.5 Synthesis of leonurine

3.4.5.1 4-((tert-butyldimethylsilyl)oxy)-3,5- dimethoxybenzoic acid (44) [121]

Syringic acid (**43**) (2.1 g, 10.1 mmol) and imidazole (2.1g, 30.3 mmol) were added into THF (30 mL) and cooled to 0 ºC. *tert*-Butyldimethylsilyl chloride (TBDMSCl) (5.1 g, 30.3 mmol) in THF (10 mL) was added dropwise. Once TBDMSCl was added, the reaction mixture was warmed up to room temperature and allowed to stir for 12h. After the reaction, there was some white deposit formed. The solvent was evaporated. The product was dissolved in a mixture of DCM (20 mL) and EtOAc (20 mL). Then the white solid was filtered. The DCM and EtOAc solution was collected. Then the organic solution was washed with brine solution twice (50 mL brine solution each time). The organic layer was collected. After the solvent was evaporated, 4-((tert-butyldimethylsilyl)oxy)-3,5-dimeth-oxybenzoic acid (**44)** was obtained as a white solid (1.7g, 5.5 mmol, yield 55%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32 (2 H, s), 3.85 (6 H, s), 1.03 (9 H, s), 0.17 (6 H, s); ^{13}C NMR($CDCl_3$, 125 MHz) δ 166.6, 151.2, 139.1, 132.6, 107.3, 55.6, 25.7, 18.8, 4.6.

3.4.5.2 N,N'-Di-Boc-1-(4-hydroxybutyl)guanidine (46) [121]

4-aminobutan-1-ol (**45**) (0.9 g, 9.9 mmol) and *N*,*N*'-Di-Boc-1H-pyrazole-1-carboxamidine (1.0g, 3.0 mmol) were dissolved in MeCN (15 mL). After 12h at rt, the solvent was evaporated. The crude residue was purified by column chromatography (Si gel, EtOAc:hexanes 1:3) to yield **46** as a white solid (0.4 g, 1.2 mmol, yield 40%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.25 (1 H, s), 6.14 (1 H, s), 3.48 (2 H, m), 3.26 (2 H, m), 1.50 (2 H, m), 1.43 (2 H, m), 1.33 (18 H, m); ^{13}C NMR($CDCl_3$, 125 MHz) δ 163.2, 156.1, 153.0, 82.9, 79.1, 61.3, 40.5, 29.4, 28.1, 27.9, 25.4.

3.4.5.3 (E)-4-(2,3-bis(tert-butoxycarbonyl)guanidino)butyl4-((tert-butyl dimethylsilyl)oxy)-3,5-dimethoxybenzoate (47) [121]

4-((tert-butyldimethylsilyl)oxy)-3,5-dimethoxybenzoic acid (**44)** (284.0 mg, 0.9 mmol), compound **46** (103.6 mg, 0.3 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (143.0 mg, 0.9 mmol), triethylamine (Et_3N) (103.1 mg, 1.0 mmol) and 4-Dimethylaminopyridine (DMAP) (25.0 mg, 0.2 mmol) were dissolved in CH_2Cl_2 (20 mL). After 12h at rt, the crude residue was washed with HCl (2 M) twice (20 mL each time), washed with saturated $NaHCO_3$ solution three times (20 mL each time), washed with brine solution twice

(20 mL each time). Then the residue was purified by column chromatography (Si gel, EtOAc:hexanes 1:4) to yield (*E*)-4-(2,3-bis(tert-butoxycarbonyl)guanidino)butyl4-((tert-butyldimethylsilyl)oxy)-3,5-dimethoxybenzoate (**47**) as a white solid (116.7 mg, 0.2 mmol, yield 62%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23 (2 H, s), 4.29 (2 H, m), 3.81 (6 H, s), 3.47 (2 H, m), 1.81 (2 H, m), 1.70 (2 H, m), 1.44 (18 H, m), 0.97 (9 H, s), 0.10 (6 H, s); ^{13}C NMR($CDCl_3$, 125 MHz) δ 166.6, 165.6, 157.2, 152.5, 147.5, 140.6, 120.1, 106.7, 84.1, 83.3, 63.9, 55.5, 40.8, 26.8, 25.7, 25.6, 25.5, 25.3, 19.6, 12.6.

3.4.5.4 (E)-4-(2,3-bis(tert-butoxycarbonyl)guanidino)butyl4-hydroxy-3,5-dimethoxybenzoate (48) [121]

(*E*)-4-(2,3-bis(tert-butoxycarbonyl)guanidino)butyl4-((tert-butyldimethylsilyl)oxy)-3,5-dimethoxybenzoate (**47**) (1.1g, 1.8 mmol) was dissolved in THF (6 mL). 6 mL tetra-n-butylammonium fluoride (TBAF) (1M TBAF in THF) was added. The mixture was stirred for 3 h. The starting material was consumed at this time. The solvent was evaporated. The crude residue was purified by column chromatography (Si gel, EtOAc:hexanes 1:1) to yield (*E*)-4-(2,3-bis(tert-butoxycarbonyl) guanidino)butyl-4-hydroxy-3,5-dimethoxybenzoate (**48**) as a white solid (0.7 g, 1.4 mmol, yield 79%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.21 (2 H, s), 4.24 (2 H, m), 3.82 (6 H, s), 3.43 (2 H, m), 1.77 (2 H, m), 1.64 (2 H, m),

1.38 (18 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 166.6, 165.1, 157.2, 152.4, 147.4, 140.4, 120.0, 106.6, 81.3, 78.1, 64.2, 55.8, 41.0, 29.6, 28.9, 25.8, 25.4.

3.4.5.5 Leonurine **(49)** [121]

(*E*)-4-(2,3-bis(tert-butoxycarbonyl)guanidino)butyl4-hydroxy-3,5-dimethoxybenzoate (**48**) (340.5 mg, 0.67 mmol) and 2 mL HCl (1 M) dissolved in THF (10 mL) were stirred together for 12h. The solvent was evaporated and the product was washed with ether for three times (10 mL each time) to yield leonurine **(49)** as white solid (185.0 mg, 0.53 mmol, yield 80%): ^{1}H NMR (MeOD, 500 MHz) δ 7.32 (2 H, s, H-2, 6), 4.35 (2 H, m, H-1'), 1.86 (2 H, m, H-2'), 1.75 (2 H, m, H-3'), 3.28 (2 H, m, H-4'), 3.89 (6 H, s, OCH_3); ^{13}C NMR (MeOD, 125 MHz) δ 119.8 (C-1), 107.2 (C-2, 6), 148.0 (C-3, 5), 141.2 (C-4), 166.1 (C-7), 64.4 (C-1'), 26.0 (C-2'), 25.7 (C-3'), 40.9 (C-4'), 157.6 (C-5'), 56.6 (OCH_3). HRESIMS $[M]^+$ *m/z*: 311.1484, (calculated for $C_{14}H_{21}N_3O_5$311.1481).

Chapter 4. A literature review on the genus *Silene* and *Silene capensis*

4.1 The genus *Silene*

4.1.1 The genus *Silene* - general information

Silene (Caryophyllaceae) is a genus of flowering plants, [133] which are mainly distributed in Northern Hemisphere, but also in Africa, Asia and South American. [133] The *Silene* genus includes more than 700 species (allocated to 39 sections) of annuals, biennials, and perennials with a worldwide distribution and its taxonomy appears very complex. [133] Red campion (*Silene dioica*), white campion (*Silene latifolia*) and bladder campion (*Silene vulgaris*) are common wildflowers throughout Europe. [133]

4.1.2 The genus *Silene* - biological activities

The plant of *Silene acaulis* has been used in the treatment of children with colic. [135] Some of members of this genus are used in folk medicine, mainly as an emollient and used as fumigant. [136] Juice of *Silene cucubalis* is prescribed in ophthalmia. [137] The flowers of *Silene nigrescens* used in Tibetan medicine in the treatment for hearing loss, blocking otic canal and volvulus. [138] The seeds of *Silene dioica* have also

been used to cure snakebites. [139] *Silene szechuensis* has been used in Chinese medicine as antipyretic, analgesic, diuretic. [138] *Silene vulgaris* also widely used in medicine as antianemic, [134] sedative, anti-inflammatory and antitoxic agent. [134]The plant *Silene undulata* is used as a medicine in treating many diseases particularly fevers and delirium. [140] The root bark of *Silene undulata* [141] is used by the Zulu and Xhosa of Africa people as an oneirogenic agent. [142] Other *Silene* species such as *Silene bellidioides* and *Silene pilosellifolia* used by the Zulus and taken as a love charm emetic, treatment of scrofula, to combat sleepiness, in tonic baths after severe illness, to produce dreams relating to the ancestral spirits in South Africa. [143]

Some plants of genus *Silene* such as *Silene acaulis, Silene multifida* and *Silene regia* as ornamental plants and have beautiful flowers and have been cultured as garden plants in many countries. [134]

4.1.3 Secondary metabolites produced by *Silene* plants

Chemical investigations of the genus *Silene* have led to the isolation of phytoecdysteroids, triterpene glycosides, [140] terpenoids, benzenoids, flavonoids, [143] anthocynanins, N-containing compounds, [141] fatty acids, [142] amino acids, [145] polysaccharides, [144] sugars, [143] sterols, vitamins, organic acids and microelements. [138]

Many species in the genus *Silene* have previously been shown to

contain phytoecdysteroids [134] and this genus is recognised as a good source of novel phytoecdysteroid analogues. [133, 134] Phytoecdysteroids are the predominant constituents within the genus *Silene* (Table 8).

Phytoecdysteroids are plant-derived ecdysteroids. The structures of phytoecdysteroids are shown in Fig. 26.

Phytoecdysteroids are contentedly widespread in the plant world. They are isolated from the main types of higher plants - ferns, gymnosperms and angiosperms, but their function in plants are yet studied insufficiently. One can conclude that the role of phytoecdysteroids in various plants and plant families may differ. [133, 134]

Phytoecdysteroids are analogues of insect steroid hormones occurring in plants.[133] They are believed to contribute to insect deterrence by acting as antifeedants or by interfering in ecdysteroid metabolism or mode of action on ingestion by phytophagous insects. [133]

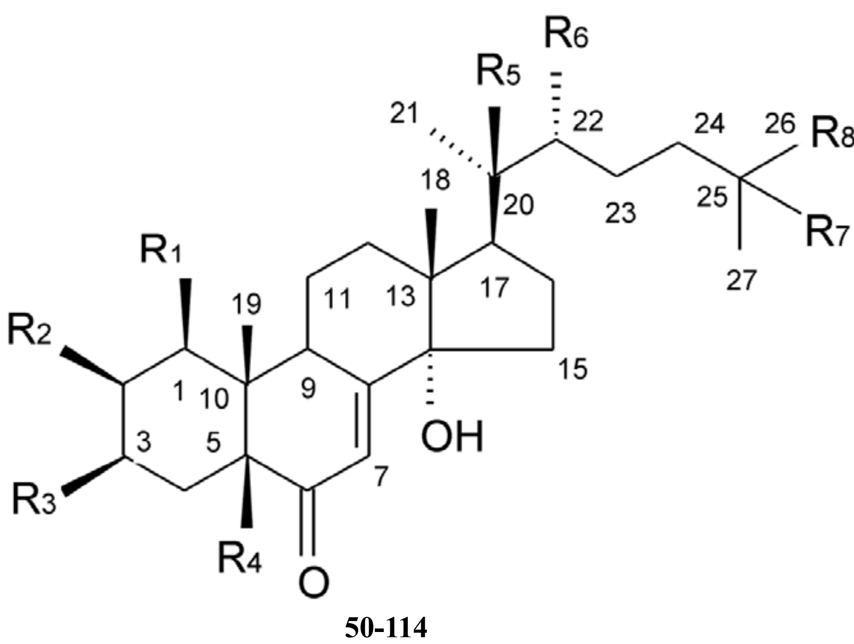


Fig. 26 Chemical structures of phytoecdysteroids [133, 134]

Table 8. Chemical structures of phytoecdysteroids (**50-114**) isolated from *Silene* plants [133, 134]

	Name	R1	R2	R3	R4	R5	R6	R7	R8
50	Brahuisterone	H	H	OH	OH	H	OH	OH	CH_3
51	2-Deoxy-20,26-dihydroxyecdysone	H	H	OH	H	OH	OH	OH	CH_2 OH
52	22-Deoxy-20,26-dihydroxyecdysone	H	OH	OH	H	OH	H	OH	CH_2 OH
53	2-Deoxyecdysone	H	H	OH	H	H	OH	OH	CH_3
54	2-Deoxyecdysone-3-acetate	H	H	OAc	H	H	OH	OH	CH_3
55	2-Deoxyecdysone-22 - acetate	H	H	OH	H	H	OAc	OH	CH_3
56	2-Deoxyecdysone-22 - benzoate	H	H	OH	H	H	OBz	OH	CH_3
57	2-Deoxyecdysone-22 - glucoside	H	H	OH	H	H	OGlu	OH	CH_3

58	2-Deoxy-20-hydroxyecdysone	H	H	OH	H	OH	OH	OH	CH_3
59	2-Deoxy-20-hydroxyecdysone-3-acetate	H	H	OAc	H	OH	OH	OH	CH_3
60	5α-2-Deoxy-20-hydroxyecdysone-3-acetate	H	H	OAc	H (α)	OH	OH	OH	CH_3
61	2-Deoxy-20-hydroxyecdysone-22-acetate	H	H	OH	H	OH	OAc	OH	CH_3
62	2-Deoxy-20-hydroxyecdysone-25-acetate	H	H	OH	H	OH	OH	OAc	CH_3
63	2-Deoxy-20-hydroxyecdysone-3-benzoate	H	H	OBz	H	OH	OH	OH	CH_3
64	2-Deoxy-20-hydroxyecdysone-22-benzoate	H	H	OH	H	OH	OBz	OH	CH_3
65	2-Deoxy-20-hydroxyecdysone-3-crotonate	H	H	$OCOC_2H_2CH_3$	H	OH	OH	OH	CH_3
66	2-Deoxy-20-hydroxyecdysone-3,22-diacetate	H	H	OAc	H	OH	OAc	OH	CH_3
67	2-Deoxy-20-hydroxyecdysone-22-glucoside	H	H	OH	H	OH	O-β-D-Glu	OH	CH_3
68	2-Deoxy-20-hydroxyecdysone-25-glucoside	H	H	OH	H	OH	OH	O-β-D-Glu	CH_3
69	2-Deoxyintegristerone A	OH	OH	OH	H	OH	OH	OH	CH_3
70	5α-2-Deoxyintegristerone A	OH	OH	OH	H (α)	OH	OH	OH	CH_3
71	22-Deoxyintegristerone A	OH	OH	OH	H	OH	H	OH	CH_3
72	5α-22-Deoxyintegristerone A	OH	OH	OH	H (α)	OH	H	OH	CH_3
73	2-Deoxypolypodine B-3-glucoside	H	H	O-β-D-Glu	OH	OH	OH	OH	CH_3
74	2-Deoxy-5,20,26-trihydroxyecdysone	H	H	OH	OH	OH	OH	OH	CH_2OH

75	20,26-Dihydroxyecdysone (Podecdysone C)	H	OH	OH	H	OH	OH	OH	CH_2OH
76	20,26-Dihydroxyecdysone-2,22-diacetate	H	OAc	OH	H	OH	OAc	OH	CH_2OH
77	20,26-Dihydroxyecdysone-3,22-diacetate	H	OH	OAc	H	OH	OAc	OH	CH_2OH
78	Ecdysone	H	OH	OH	H	H	OH	OH	CH_3
79	Ecdysone-22-sulfate	H	OH	OH	H	H	OSO_3H	OH	CH_3
80	Ecdysteroside	H	OH	O-α-D-Gal(1→6)α-D-Gal	H	OH	OH	OH	CH_3
81	5α-20-Hydroxyecdysone	H	OH	OH	H (α)	OH	OH	OH	CH_3
82	5α-20-Hydroxyecdysone-22-benzoate	H	OH	OH	H (α)	OH	OBz	OH	CH_3
83	20-Hydroxyecdysone	H	OH	OH	H	OH	OH	OH	CH_3
84	20-Hydroxyecdysone-2-acetate	H	OAc	OH	H	OH	OH	OH	CH_3
85	20-Hydroxyecdysone-3-acetate	H	OH	OAc	H	OH	OH	OH	CH_3
86	20-Hydroxyecdysone-22-acetate	H	OH	OH	H	OH	OAc	OH	CH_3
87	20-Hydroxyecdysone-20-benzoate	H	OH	OH	H	OBz	OH	OH	CH_3
88	20-Hydroxyecdysone-22-benzoate	H	OH	OH	H	OH	OBz	OH	CH_3
89	20-Hydroxyecdysone-22-benzoate-25-glucoside	H	OH	OH	H	OH	OBz	O-β-D-Glu	CH_3
90	20-Hydroxyecdysone-2,3-diacetate-22-benzoate	H	OAc	OAc	H	OH	OBz	OH	CH_3
91	20-Hydroxyecdysone-22,25-dibenzoate	H	OH	OH	H	OH	OBz	OBz	CH_3
92	20-Hydroxyecdysone-3-glucoside	H	OH	O-β-D-Glu	H	OH	OH	OH	CH_3
93	20-Hydroxyecdysone-25- glucoside	H	OH	OH	H	OH	OH	O-β-D-Glu	CH_3

94	26-Hydroxyintegristerone A	OH	OH	OH	H	OH	OH	OH	CH_2 OH
95	26-Hydroxypolypodine B	H	OH	OH	OH	OH	OH	OH	CH_2 OH
96	Inokosterone	H	OH	OH	H	OH	OH	H	CH_2 OH
97	Integristerone A	OH	OH	OH	H	OH	OH	OH	CH_3
98	Integristerone A-25-acetate	OH	OH	OH	H	OH	OH	OAc	CH_3
99	Polypodine B	H	OH	OH	OH	OH	OH	OH	CH_3
100	Ponasterone A	H	OH	OH	H	OH	OH	H	CH_3
101	Sileneoside A	H	OH	OH	H	OH	O-α-D-Gal	OH	CH_3
102	Sileneoside B	H	OH	O-β-D-Gal	H	OH	O-β-D-Gal	OH	CH_3
103	Sileneoside C	OH	OH	OH	H	OH	O-α-D-Gal	OH	CH_3
104	Sileneoside D	H	OH	O-β-D-Gal	H	OH	OH	OH	CH_3
105	Silenoside E (Blechnoside A)	H	H	O-β-D-Glu	H	H	OH	OH	CH_3
106	5α-Silenoside E	H	H	O-β-D-Glu	H (α)	H	OH	OH	CH_3
107	Sileneoside F	H	H	O-β-D-Glu	OH	H	OH	OH	CH_3
108	Sileneoside G	H	OH	O-α-D-Glu	H	OH	O-α-D-Gal	OH	CH_3
109	Sileneoside H	OH	OH	OH	H	OH	O-α -D-Gal	OAc	CH_3
110	Taxisterone	H	OH	OH	H	OH	H	OH	CH_3
111	Tomentesterone A	H	H	OH	H (α)	H	OAc	OBz	CH_3
112	Tomentesterone B	H	H	OH	H (α)	H	OH	OBz	CH_3
113	Viticosterone E	H	OH	OH	H	OH	OH	OAc	CH_3
114	Viticosterone E-22-benzoate	H	OH	OH	H	OH	OAc	OAc	CH_3

4.2 *Silene capensis*

4.2.1 *Silene capensis* - general information

Silene capensis known as African Dream Root or Ubulawu, in cultivation, *S. capensis* is an easily grown, but moisture hungry herb. [122] It is tolerant of extreme heat (>40 °C) and moderate cold (-5 °C). A moisture retentive seedbed is essential. The flowers open at night and close in the day. They are very fragrant and smell something like cloves, jasmine and bananas. It acts a biennial to short lived perennial and the root can be harvested after the second year. [122]

4.2.2 *Silene capensis* - biological activities

S. capensis is reportedly more oneirogenic than the better known *Calea zacatechichi*, often referred to as the Dream Herb. [122] It has been used for countless years by a culture who believes ancestors are contacted through dreams, so they cultivate and seek out plants that enhance dreaming. African Dream Root is one of the best-known in Africa. [123,124]

4.2.3 *Silene capensis* - our isolation work

Up to now, there are no scientific reports about the natural product isolation work on *S. capensis*. Our primary goal is to isolate and identify components of the extract of the plant and determine what compound(s)

is(are) responsible for the reputed bioactivity of *S. capensis*.

Ten compounds have been isolated from the roots of *S. capensis*. Details of the isolation and structure elucidation of these compounds are described herein.

Chapter 5. Phytochemical investigation of *Silene capensis*

5.1 Introduction

Ten compounds (**41**,**115-123**) were obtained by repeated chromatography of the extracts of *S. capensis* roots.

The details for the isolation are presented in the experimental section. These compounds were identified by MS and NMR data.

5.2 Results and discussion

5.2.1 Structure elucidation of compound 115

115

Fig. 27 Structure of compound **115**

Compound **115** was assigned the molecular formula $C_{18}H_{32}O_2$ (*m/z*:280.2398), determined by HRESIMS, indicating three degrees of unsaturation. According to the molecular formula, it appeared to be a 18-carbon fatty acid.

In the ^{13}C NMR spectrum, one singlet at δ(C) 180.3 was attributed to carboxyl group. Four singlets at δ(C) 130.2, 130.0, 128.1 and 127.9 were attributed to vinylic carbons. Then it was confirmed as linoleic acid by direct comparison with reported data. [122]

5.2.2 Structure elucidation of compound 41

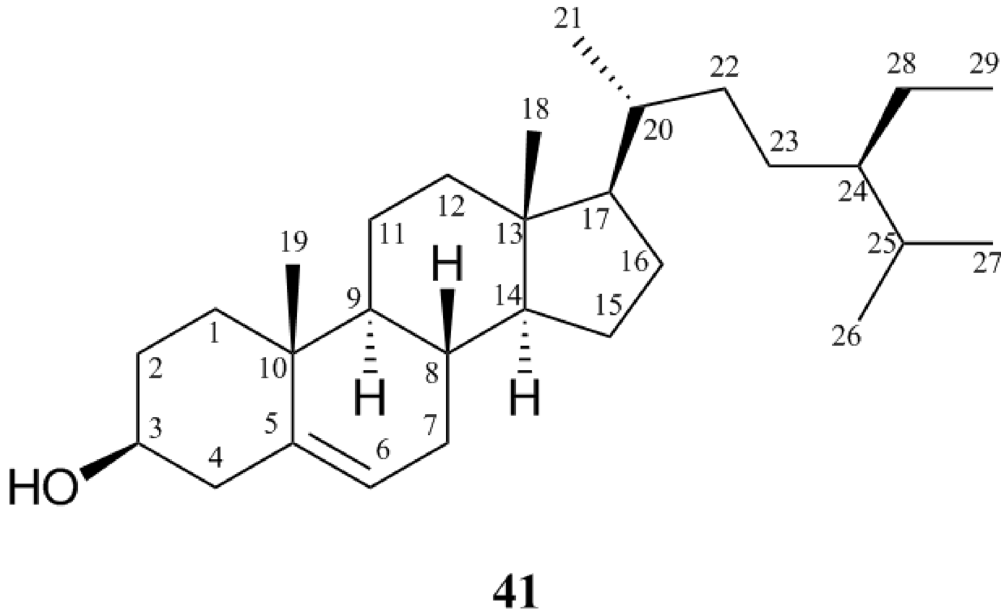


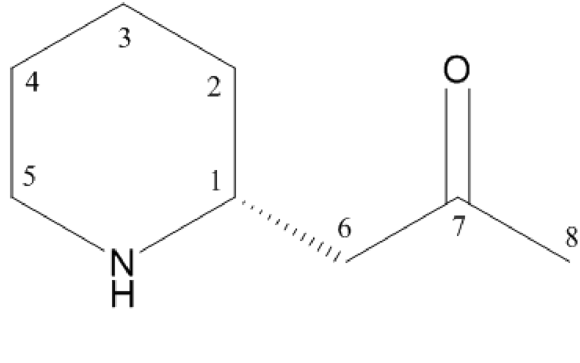
Fig. 28 Structure of compound **41**

Compound **41** had the molecular formula $C_{29}H_{50}O$ determined by HRESIMS $[M]^+$ *m/z*: 414.3863, indicating five degrees of unsaturation. The consideration of the typical natural product compounds and its molecular formula suggested the steroid nature of **41**.

The ^1H NMR and ^{13}C NMR spectral data of **41** showed the presence of one pair of vinylic carbons [δ 5.37 (1 H, m); δ 140.7, 121.8] and one carbon which was connected to a hydroxyl group [δ3.52 (1 H, m); δ 71.8].

By comparison of spectral data with those reported in the literature, [126] the structure of compound **41** was elucidated as β-Sitosterol.

5.2.3 Structure elucidation of compound 116



116

Fig. 29 Structure of compound **116**

Compound **116** had the molecular formula $C_8H_{15}NO$ determined by HRESIMS $[M]^+$ *m/z*: 141.1163, indicating two degrees of unsaturation.

The ^{13}C NMR spectrum of **116** presented a ketone group at δ 205.5 and the ^{1}H NMR spectrum of **116** presented a methyl group (CH_3) at δ 1.99 (3 H, s), which was connected to the ketone group. Therefore there was only one degree of unsaturation left unidentified. According to the ^{13}C NMR spectrum, there seemed to be no vinylic carbon in **116**. Therefore there seemed to be a ring in **116**.

Considering the molecular formula of **116**, **116** was proposed to be pelletierine and it was confirmed by the direct comparison with the reported data. [128]

5.2.4 Structure elucidation of compound 117

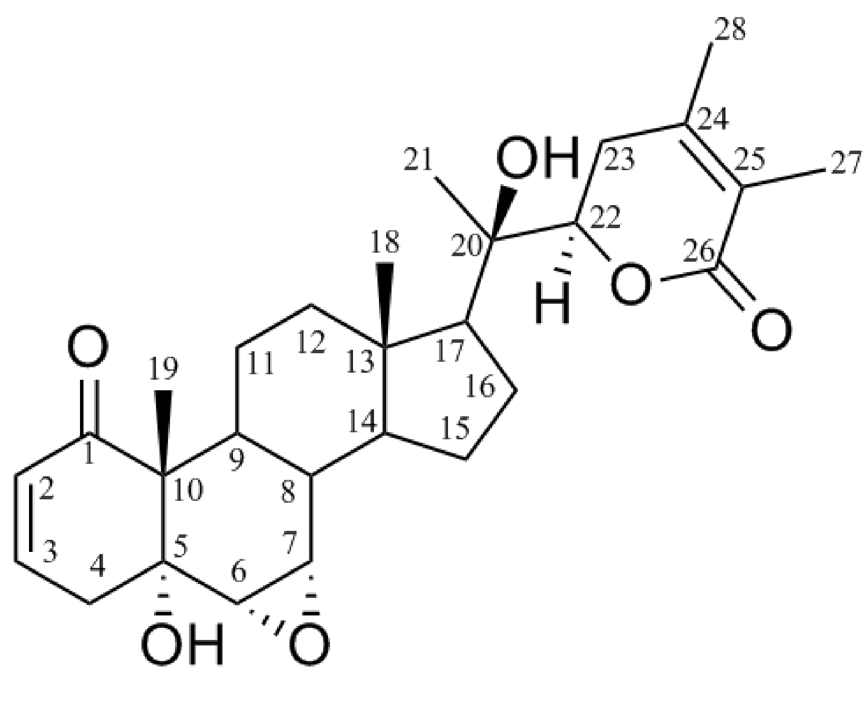


Fig. 30 Structure of compound **117**

Compound **117** showed a molecular ion peak in its HRESIMS spectrum (*m/z* 470.2715) corresponding to the molecular formula $C_{28}H_{38}O_6$.

The hydrogen signals (H2 and H3) of the conjugated enone system at the A ring were observed at δ 5.89 (dd, *J* = 10.2 and 2.4 Hz) and 6.62 (ddd, *J* = 10.2, 5.2 and 2.2 Hz), respectively. The C4 was connected to two hygrogens (δ 2.69 and 2.57), which indicated that C4 was unsubstituted. The chemical shift of the deshielded H6 (δ 3.07) and H7 (δ

3.35) indicated that there was an epoxide group between C6 and C7.

The ^{1}H NMR spectrum of **117** showed three singlets at δ(H) 0.99, 1.21 and 1.35 which were assigned to C18, C19, and C21 methyl group, respectively. The chemical shift of the deshielded C21 methyl singlet and the appearance of the H22 methine signal (δ 4.22) as a doublet of doublets (J = 13.4 and 3.5 Hz) indicated the presence of an OH group at the C20 position. The two vinylic methyl singlets at δ 1.92 and 1.99 were assigned to the methyl groups attached to the conjugated lactone group in the side chain. It was confirmed as withanolide A by direct comparison with the reported data.[122, 123]

Table 9. NMR spectral data for compound **117**

Position	^{13}C NMR ($CDCl_3$) δ		^{13}C NMR ($CDCl_3$) δ reported data [123]
1	203.2	C	203.1
2	129.0	CH	129.1
3	139.6	CH	139.6
4a	36.7	CH_2	36.8
4b			
5	73.3	C	73.3
6	56.3	CH	56.4
7	57.3	CH	57.3
8	35.1	CH	35.2
9	35.5	CH	35.7
10	51.0	C	51.1
11a	21.7	CH_2	21.9
11b			
12a	40.4	CH_2	40.5
12b			
13	43.9	C	43.9
14	51.9	CH	50.2
15a	23.2	CH_2	23.3
15b			
16a	21.8	CH_2	21.9
16b			
17	54.5	CH	54.6
18	13.8	CH_3	13.8
19	14.7	CH_3	14.8
20	75.1	C	75.1
21	21.1	CH_3	21.2
22	81.0	CH	81.0
23a	31.7	CH_2	31.7
23b			
24	148.8	C	148.8
25	122.1	C	122.1
26	166.0	C	166.0
27	12.5	CH_3	12.5
28	20.6	CH_3	20.5

(The assigment was made acording to ^{1}H NMR, ^{13}C NMR, DEPT, HMBC, HSQC, and COSY)

5.2.5 Structure elucidation of compound 118

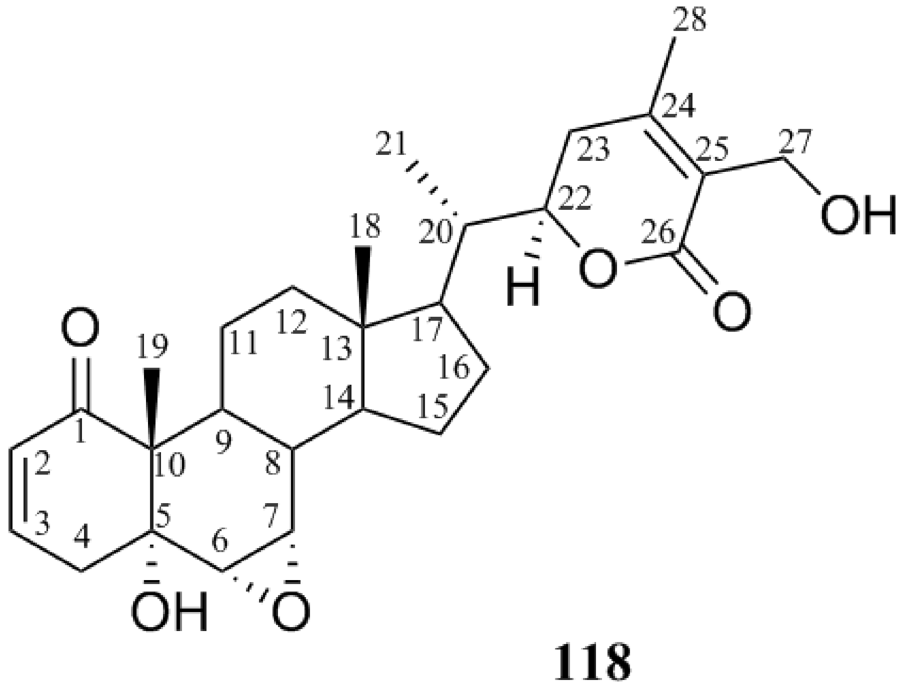


Fig. 31 Structure of compound **118**

Compound **118** showed a molecular ion peak in its HRESIMS spectrum (*m/z* 470.2677) corresponding to the molecular formula $C_{28}H_{38}O_6$. According to the HRESIMS, compound **118** and Compound **117** had the same molecular formula $C_{28}H_{38}O_6$.

^{1}H NMR and ^{13}C NMR of Compound **118** were very similar to those of Compound **117**. As compared to **117**, the ^{1}H NMR spectrum of **118** had only one vinylic methyl singlet at δ 2.08. In addition, in compound **118** there was a deshielded methylene doublet (J=12.6 Hz) at δ 4.42 (2H), while compound **117** had a singlet at δ 1.92 (3H), indicating the presence of a hydroxyl group at the C27 position. The presence of a H at C20

position (δ 2.06) indicating that there was no hydroxyl group at the C20 position. It was confirmed as 12-deoxywithastramonolide by direct comparison with the reported data. [124]

Table 10. NMR spectral data for compound **118**

position	^{13}C NMR ($CDCl_3$) δ		^{13}C NMR ($CDCl_3$) δ reported data [124]
1	202.2	C	202.2
2	128.0	CH	128.9
3	138.6	CH	138.6
4a	35.7	CH_2	36.8
4b			
5	71.3	C	72.2
6	55.3	CH	55.2
7	56.3	CH	56.2
8	34.7	CH	34.5
9	34.5	CH	34.7
10	50.0	C	49.9
11a	20.9	CH_2	20.8
11b			
12a	38.8	CH_2	38.7
12b			
13	42.5	C	42.4
14	50.4	CH	50.7
15a	22.6	CH_2	23.1
15b			
16a	26.3	CH_2	26.6
16b			
17	50.8	CH	50.4
18	11.1	CH_3	11.0
19	13.7	CH_3	13.2
20	37.9	CH	37.8
21	12.3	CH_3	12.0
22	77.7	CH	77.7
23a	28.8	CH_2	28.7
23b			
24	151.9	C	152.0
25	124.7	C	124.0
26	166.0	C	165.9
27	56.5	CH_2	56.4
28	19.0	CH_3	18.9

(The assigment was made acording to ^{1}H NMR, ^{13}C NMR, DEPT, HMBC, HSQC, and COSY)

5.2.6 Structure elucidation of compound 119

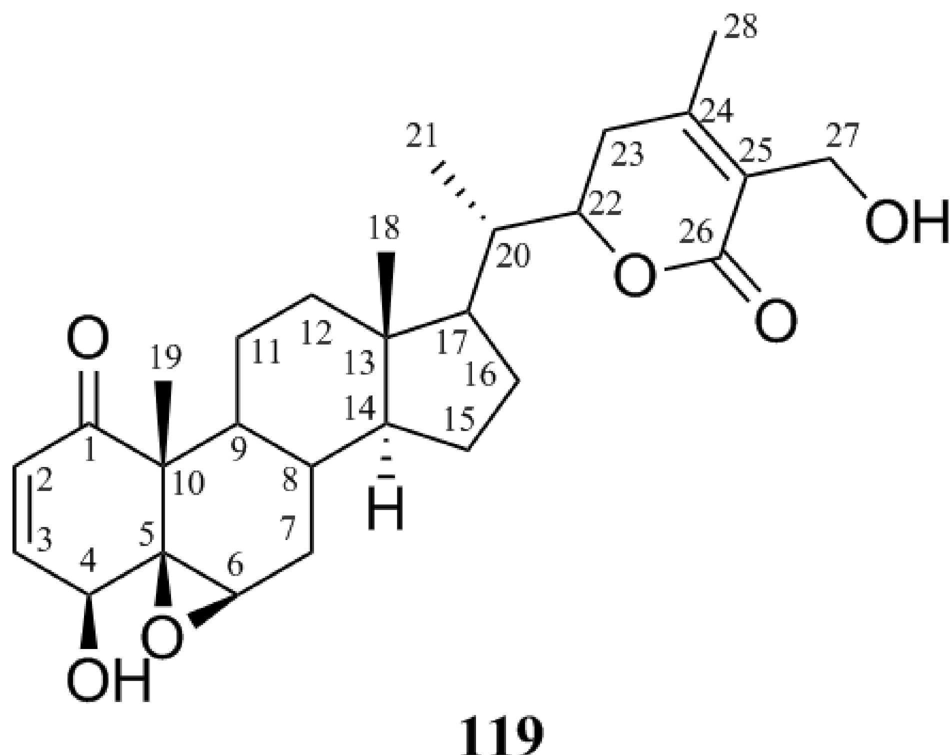


Fig. 32 Structure of compound **119**

According to the HRESIMS, Compound **119** and Compound **118** had the same molecular formula $C_{28}H_{38}O_6$. Comparing ^{1}H NMR and ^{13}C NMR of Compound **119** with those of Compound **118,** the major differences were on C4, C5, C6 and C7. Compound **118** had chemical shift δ 36.7 (C4), 73.3 (C5), 56.3 (C6), 57.3 (C7) while Compound **119** had chemical shift δ 69.9 (C4), 63.8 (C5), 62.7 (C6), 31.2 (C7). According to the DEPT, Compound **118** had CH2 (C4) and CH (C7) while Compound **119** had CH (C4) and CH2 (C7). Therefore it was very obvious that Compound **119** had C4, C5 and C6 connected to oxygen.

Considering that Compound **119** and Compound **118** had the same molecular formula, Compound **119** was identified as withaferin A, which was confirmed by direct comparison with the reported data. [125, 127]

Table 11. NMR spectral data for compound **119**

position	^{13}C NMR ($CDCl_3$) δ		^{13}C NMR ($CDCl_3$) δ reported data [35]
1	202.3	C	202.3
2	132.3	CH	132.3
3	141.8	CH	142.5
4	69.9	CH	69.8
5	63.8	C	63.9
6	62.7	CH	61.7
7a	31.2	CH_2	31.1
7b			
8	29.79	CH	29.8
9	44.1	CH	44.0
10	47.7	C	47.8
11a	22.2	CH_2	21.8
11b			
12a	27.3	CH_2	27.2
12b			
13	42.6	C	42.5
14	56.1	CH	56.0
15a	24.3	CH_2	24.2
15b			
16a	39.4	CH_2	39.2
16b			
17	52.0	CH	51.8
18	11.6	CH_3	11.6
19	17.5	CH_3	17.0
20	38.8	CH	38.7
21	13.3	CH_3	13.3
22	78.7	CH	78.7
23a	29.83	CH_2	29.8
23b			
24	152.7	C	153.5
25	125.7	C	125.6
26	167.0	C	167.0
27	57.5	CH_2	57.0
28	20.0	CH_3	20.0

(The assigment was made according to ^{1}H NMR, ^{13}C NMR, DEPT, HMBC, HSQC, and COSY)

5.2.7 Structure elucidation of compound 120

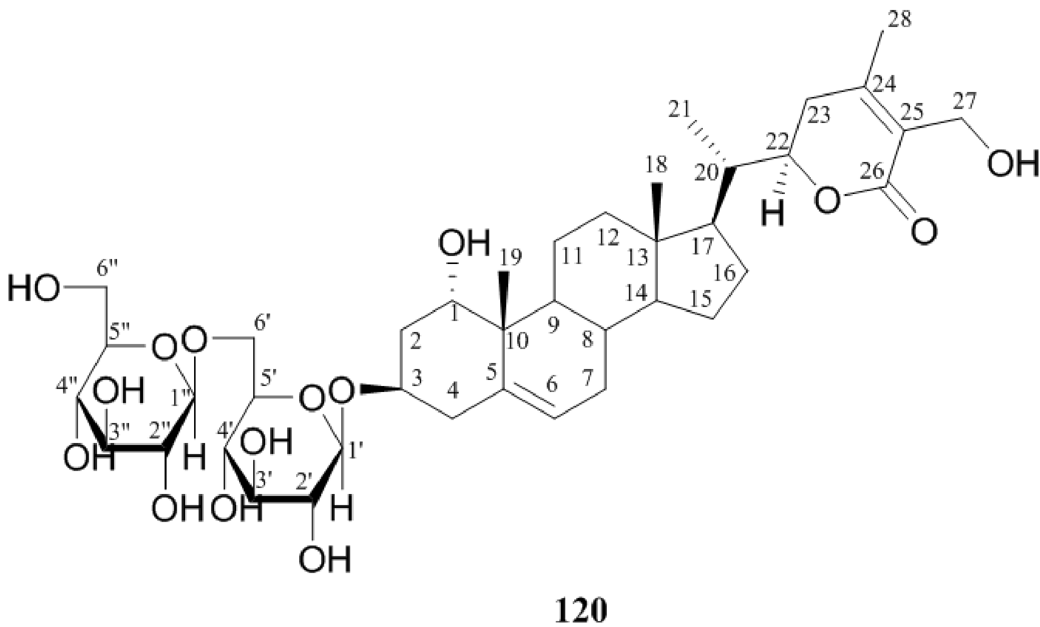


Fig. 33 Structure of compound **120**

Compound **120** had the molecular formula $C_{40}H_{62}O_{15}$ determined by HRESIMS $[M]^+$ *m/z*: 782.4100, indicating ten degrees of unsaturation.

^1H NMR and ^{13}C NMR of **120** were very similar to those of **119**. The most obvious difference was that the ^{13}C NMR spectrum of **120** presented two more glycosyl groups than that of **119** at the chemical shifts δ 103.4, 75.2, 78.5, 71.5, 77.1, 69.9, 105.6, 75.3, 78.4, 71.7, 78.5, 62.7. According to the ^{13}C NMR spectrum δ 72.4 (C1), 38.0 (C2), 74.5 (C3), it seemed that there was no double bond on C1, C2 and C3 and C1 and C3 were connected to oxygen. According to the ^{13}C NMR spectrum δ139.4 (C5) and 124.1 (C6), it was obvious that C5 and C6 were a pair of vinylic carbons.

Considering the molecular formula and NMR of **120**, **120** was proposed to be withanoside IV and it was confirmed by the direct comparison with the reported data. [130]

Table 12. NMR spectral data for compound **120**

position	**120** ^{13}C NMR (pyridine-d_5) δ	**120** reported data [130] (pyridine-d_5) δ
1	72.4	72.4
2	38.0	37.8
3	74.5	74.5
4	39.3	39.2
5	139.4	139.3
6	124.1	124.0
7	32.3	32.2
8	32.2	32.2
9	41.5	41.5
10	42.1	42.0
11	20.6	20.6
12	39.8	39.8
13	42.9	42.9
14	56.5	56.4
15	27.3	27.3
16	24.7	24.6
17	52.2	52.2
18	11.8	11.8
19	19.6	19.6
20	39.2	39.2
21	13.6	13.5
22	78.6	78.5
23	29.9	30.0
24	154.2	154.2
25	127.4	127.2
26	166.5	166.5
27	56.2	56.1
28	20.2	20.1
Glc-1'	103.4	103.2
2'	75.2	75.0
3'	78.5	78.2
4'	71.5	71.4
5'	77.1	76.9
6'	69.9	69.8
Glc-1"	105.6	105.2
2"	75.3	75.0
3"	78.4	78.1
4"	71.7	71.6
5"	78.5	78.2
6"	62.7	62.6

5.2.8 Structure elucidation of compound 121

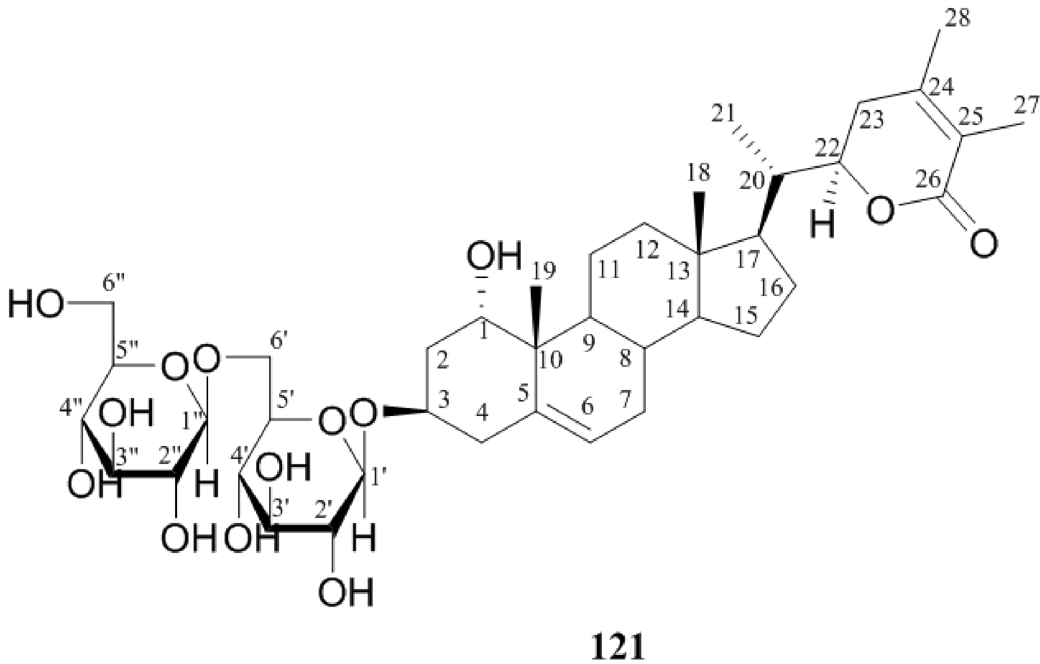


Fig. 34 Structure of compound **121**

Compound **121** had the molecular formula $C_{40}H_{62}O_{14}$ determined by HRESIMS $[M]^{+}$ *m/z*: 766.4157, indicating ten degrees of unsaturation.

^{1}H NMR and ^{13}C NMR of **121** were very similar to those of **120**. The major difference was that the ^{13}C NMR and DEPT spectrum of **121** presented a methyl (CH_3) at the chemical shifts δ 12.6 (C27) while, in **120**, C27 was a CH_2 connected to the oxygenat the chemical shifts δ 56.2 (C27).

Considering the molecular formula and NMR of **121**, **121** was proposed to be withanoside V and it was confirmed by the direct comparison with the reported data. [130]

Table 13. NMR spectral data for compound **121**

position	**121** ^{13}C NMR (pyridine-d_5) δ	**121** reported data [130] (pyridine-d_5) δ
1	72.3	72.4
2	37.8	37.8
3	74.3	74.5
4	39.0	39.1
5	139.2	139.2
6	123.9	124.0
7	32.1	32.2
8	32.1	32.2
9	41.3	41.5
10	41.9	42.0
11	20.4	20.5
12	39.6	39.7
13	42.7	42.9
14	56.3	56.4
15	27.2	27.3
16	24.5	24.6
17	52.0	52.2
18	11.6	11.8
19	19.4	19.6
20	39.1	39.2
21	13.5	13.5
22	78.4	78.5
23	29.4	29.6
24	149.3	149.8
25	121.7	121.8
26	166.6	166.9
27	12.6	12.7
28	20.0	20.2
Glc-1'	103.2	103.2
2'	75.0	74.9
3'	78.3	78.1
4'	71.3	71.3
5'	76.9	76.8
6'	69.7	69.7
Glc-1"	105.4	105.1
2"	75.1	75.0
3"	78.2	78.0
4"	71.5	71.5
5"	78.3	78.2
6"	62.5	62.6

5.2.9 Structure elucidation of compound 122

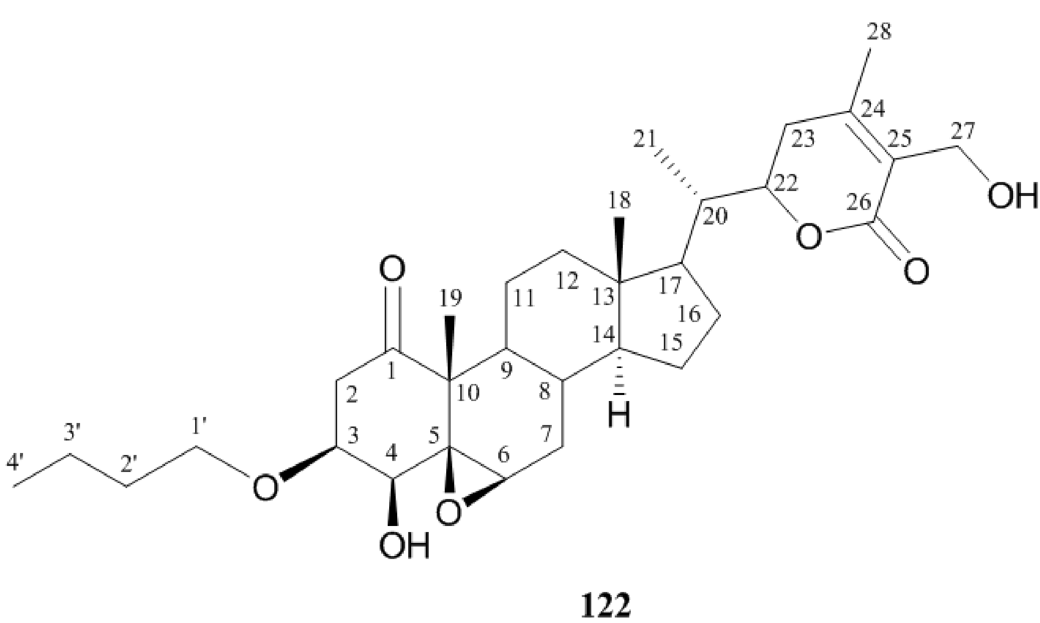


Fig. 35 Structure of compound **122**

Compound **122** had the molecular formula $C_{32}H_{48}O_7$ determined by HRESIMS $[M]^+$*m/z*: 544.3383, indicating nine degrees of unsaturation.

^{1}H NMR and ^{13}C NMR of **122** were very similar to those of **119**. But according to the ^{13}C NMR and DEPT spectrum of **122** [δ 41.8 (C2), 75.5 (C3)], it seemed that there was no double bond between C2 and C3 and that C3 was connected to oxygen. According to the molecular formula of **122**, **122** had four more carbons and one more oxygen than **119**, which was also proved by ^{13}C NMR of **122**: δ69.3 (C1'), 32.4 (C2'), 22.1 (C3'), 16.0 (C4').

Considering the molecular formula and NMR of **122** and compound **119**, **122** was proposed to be 3β-*O*-Butyl-2,3-dihydrowithaferin A and it was confirmed by the direct comparison with the reported data. [131]

Compound **122** is presumed to be an artifact formed in the isolation process as *n*-butanol was used as extraction solvent during the preparation of the crude extract. In the reference, [131] it was also presumed to be an artifact.

Table 14. NMR spectral data for compound **122**

position	**122** ^{13}C NMR ($CDCl_3$) δ	**122** reported data [131] ($CDCl_3$) δ
1	210.1	209.9 (s)
2	40.0	40.0 (t)
3	77.6	77.6 (d)
4	77.7	77.7 (d)
5	64.9	64.9 (s)
6	60.5	60.4 (d)
7	31.2	31.3 (t)
8	29.4	29.4 (d)
9	42.8	42.9 (d)
10	50.4	50.4 (s)
11	21.7	21.7 (t)
12	27.3	27.3 (t)
13	42.7	42.7 (s)
14	56.1	56.2 (d)
15	24.3	24.3 (t)
16	39.1	39.2 (t)
17	52.0	52.0 (d)
18	11.6	11.6 (q)
19	15.8	15.8 (q)
20	38.8	38.8 (d)
21	13.4	13.4 (q)
22	78.8	78.7 (d)
23	29.8	29.9 (t)
24	152.8	152.7 (s)
25	125.7	125.7 (s)
26	167.0	166.9 (s)
27	57.5	57.5 (t)
28	20.0	20.0 (q)
1'	68.8	68.8 (t)
2'	31.9	32.0 (t)
3'	19.3	19.4 (t)
4'	13.9	13.9 (q)

5.2.10 Structure elucidation of compound 123

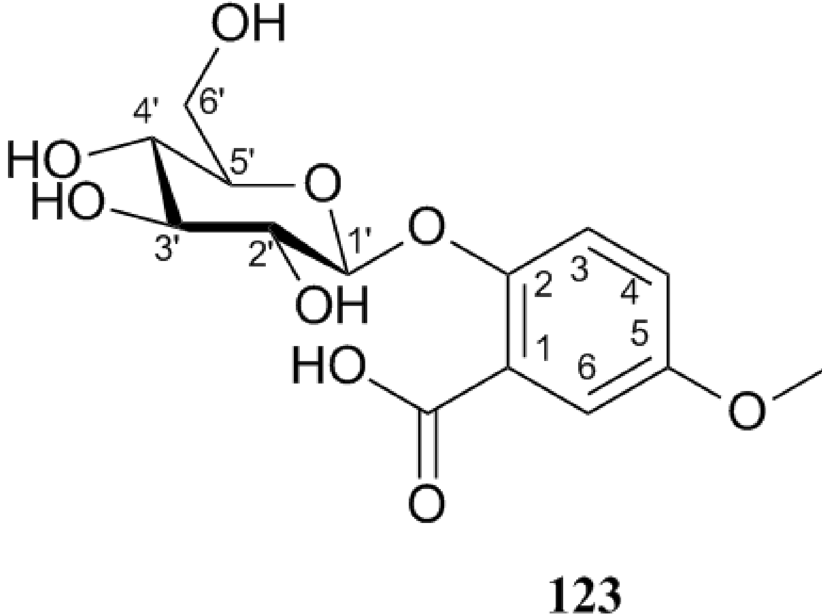


Fig. 36 Structure of compound **123**

Compound **123** had the molecular formula $C_{14}H_{18}O_9$ determined by HRESIMS $[M]^+$ *m/z*: 330.0952, indicating six degrees of unsaturation.

In the ^{13}C NMR spectrum, one singlet at δ(C) 170.4 was attributed to a carboxyl group. Six singlets at δ(C) 127.3, 151.7, 116.4, 124.6, 150.2 and 114.4 were attributed to aromatic carbons. This aromatic group was also proved by ^{1}H NMR spectrum δ(H) 7.21 (d, J=8.3 Hz) (H3), 7.66 (dd, 1.2, 8.3) (H4), 7.64 (d, J=1.2 Hz) (H6), which meant this aromatic ring was trisubstituted. The large coupling constant 8.3 Hz indicated that H4 was ortho-couple to H3, while the small coupling constant 1.2 Hz indicated that H4 was meta-couple to H6. In the ^{1}H NMR and ^{13}C NMR spectrum, δ(C) 56.7 and δ(H) 3.92 (3H, s) were attributed to a methoxy

group (OCH_3). δ(C) 102.0, 77.9, 74.8, 71.3, 78.3, 62.4 and δ(H) 5.04 (d, J=7.5 Hz), 3.47 (m), 3.55 (m), 3.43 (m), 3.50 (m), 3.89 (m), 3.72 (m) were attributed to a glucose residue.

Considering the molecular formula and NMR of **123**, **123** was proposed to be 2-β-*D*-glucopyranosyloxy-5-methoxy benzoic acid and it was confirmed by the direct comparison with the reported data. [132]

Table 15. NMR spectral data for compound **123**

position	^{13}C NMR (MeOD) δ	^{1}H NMR (MeOD) δ	(J in Hz)	^{13}C NMR (MeOD) reported data [132] δ	^{1}H NMR (MeOD) reported data [132] δ	(J in Hz)
1	127.3			126.1		
2	151.7			152.0		
3	116.4	7.21	(d, 8.3)	116.5	7.21	(d, 7.8)
4	124.6	7.66	(dd, 1.2, 8.3)	124.3	7.66	(dd,1.6, 7.8)
5	150.2			150.4		
6	114.4	7.64	(d, 1.2)	114.4	7.62	(d, 1.6)
1'	102.0	5.04	(d, 7.5)	102.1	5.06	(d, 7.2)
2'	77.9	3.47	(m)	77.9	3.45	(m)
3'	74.8	3.55	(m)	74.8	3.53	(m)
4'	71.3	3.43	(m)	71.3	3.42	(m)
5'	78.3	3.50	(m)	78.3	3.50	(m)
6'a	62.4	3.89	(m)	62.5	3.87	(d, 7.0 and 12.1)
6'b		3.72	(m)		3.69	(d, 2.4 and 12.1)
OCH_3	56.7	3.92	(3H, s)	56.7	3.87	(3H, s)
C=O	170.4			169.6		

5.3 Conclusions

There have been no phytochemical investigation on *S. capensis*. Our phytochemical isolation of the plant gave ten known compounds. Six of these ten compounds are withanolides (compound **117**, **118**, **119** and **122**) or withanolide glycosides (compound **120** and **121**).

Our plant material was purchased from Bouncing Bear Botanicals in 2011. During the purchase, they were claimed to be the roots of *Silene capensis*. Very unfortunately, due to the physical state of the plant material, identification based on morphological characteristics was not possible. The lack of appropriate genetic sequences have also precluded the identification by genetic means. Based on our phytochemical investigations on the plant material, it is possible that our plant material are the roots of *Withania somnifera* because *Withania somnifera* is very good source of withanolides and our isolated withanolides (compound **117**, **118**, **119** and **122**) were found in *Withania somnifera* too. [122, 123, 124, 125, 127, 131] Additionally, similar to *Silene capensis*, *Withania somnifera* also has oneirogenic effect. [122, 123, 124, 125, 127, 131]

5.4 Experimental section

5.4.1 General Experimental Procedures

IR spectra were recorded on a Thermo Nicolet IR 100 spectrophotometer as thin films. UV spectra were recorded on a Varian Cary 1 Bio UV‒Visible spectrophotometer in MeOH solution. Nuclear magnetic resonance spectra (NMR) spectra were recorded using a Bruker Avance DRX 500MHz instrument. Chemical shifts are given in parts per million (ppm) and were referenced to solvent signals. High-resolution mass spectra were determined on a Agilent 6520 Q-TOF instrument, followed by deconvolution of the data using Agilent's Bioconfirmation software. Optical rotations were performed on a Rudolph Autopol IV polarimeter with the Na 589 line. HPLC was performed by Agilent 1200 HPLC system, with normal phase column Partisil 10 (Whatman) and reverse phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). TLC was performed on commercially precoated plates (Whatman, aluminum backing, UV254 fluorescence, silica gel coating, 250 μm layer,CAT NO: 4420 222). Column chromatography was carried out on Silica Gel 60 [particle size: 0.040-0.063 mm, 230-400 mesh ASTM] (EMD). Visualization was by examination under visible light, UV (254 nm), and UV (365 nm) or by spraying with 10% sulfuric acid/vanillin reagent followed by heating.

5.4.2 Plant Material

Roots of *Silene capensis* were purchased from Bouncing Bear Botanicals in 2011. Due to the physical state of the plant material, identification based on morphological characteristics was not possible. The lack of appropriate genetic sequences have also precluded the identification by genetic means.

5.4.3 Extraction of the plant material

The roots of *S. capensis* (410.6 g) were ground and then extracted three times with 90% ethanol/water (1 L each time) by percolating the root powder in solvent overnight. The extracts were combined and dried by rotary evaporator *in vacuo* and lyophilization to yield 31.4 g of solvent-free extract. The extract (31.4g) was dissolved in water and extracted using hexanes, ethyl acetate and n-butanol sequentially (0.5 L for each solvent). Each extraction procedure was repeated three times. In the end, hexane extract (2.3g), ethyl acetate extract (0.8g), n-butanol (3.9g) extract and aqueous extract (14.3g) were obtained respectively. Each extract was separated further by chromatography.

5.4.4 Extraction and Isolation of compound 41, 115 and 117

The isolation scheme for compound **41, 115** and **117** is as follows:

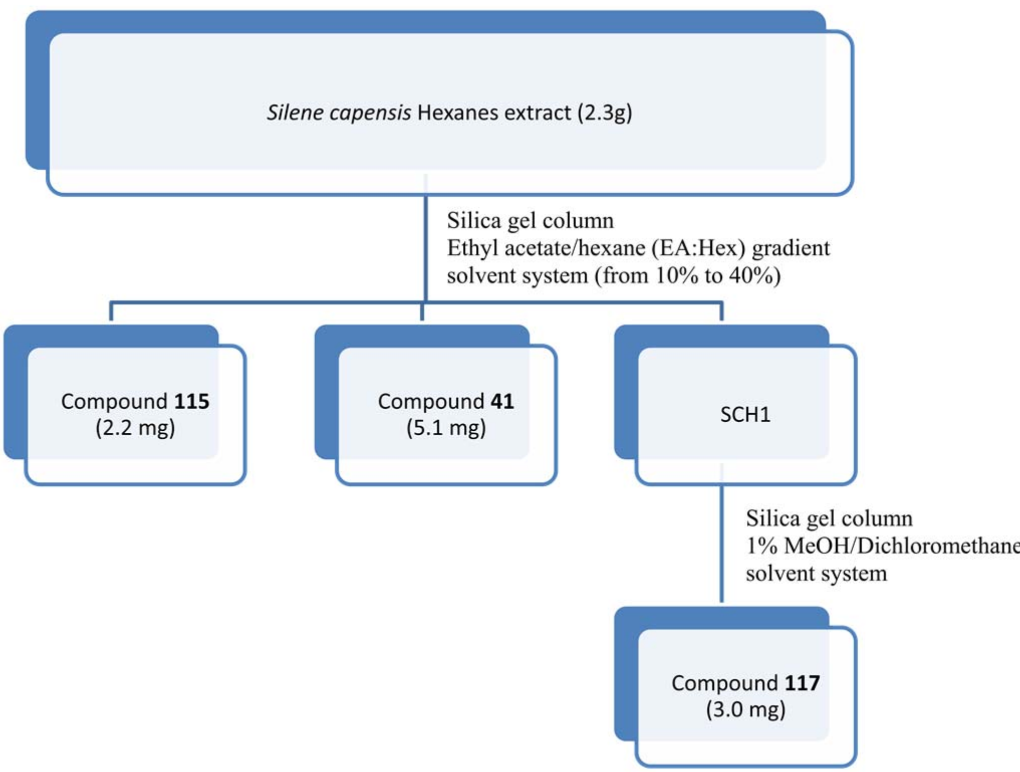


Fig. 37 The isolation scheme for compound **41, 115** and **117**

S. capensis hexanes extract was chromatographed on a silica gel column (7.0×2.0 cm) and fractionated (5 mL for each fraction) using an ethyl acetate/hexanes (EA:Hex) gradient solvent system (10:90, 20:80, 30:70, 40:60, 50:50, 60:40;100, 50, 50, 50, 50, 50 mL respectively). Fractions were collected and pooled by TLC analysis to afford 10 combined fractions.

Fraction 2 was Compound **115** (linoleic acid) [122] [2.2 mg; eluted with EA/Hex (10:90)]: UV (MeOH) λ_{max} (log ε) 211 (1.02) nm; IR (film) ν_{max} 3455, 2844, 2077, 1645, 1455, 1424, 1338, 1235, 1117, 1033, 1015, 581, 442, 430 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500MHz) δ 5.38 (4 H, m), 2.79 (2 H, m), 2.37 (2 H, m), 2.07 (2 H, m), 1.65 (4 H, m), 1.27 (12 H, m), 0.91 (6 H, m); ^{13}C NMR ($CDCl_3$, 125MHz) δ 180.3, 130.2, 130.0, 128.1, 127.9, 34.1, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 24.7, 22.7, 14.2. HRESIMS $[M]^+$ *m/z*: 280.2405, (calculated for $C_{18}H_{32}O_2$ 280.2402).

Fraction 3 was Compound **41** (β-Sitosterol) [126] [5.1 mg; eluted with EA/Hex (20:80)]: UV (MeOH) λ_{max} (log ε) 190 (1.54) nm; IR (film) ν_{max} 3567, 1033, 668, 450, 431, 420 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500MHz) δ 5.37 (1 H, m), 3.52 (1 H, m), 0.70 (3 H, s), 1.01 (3 H, s), 0.93 (3 H, m), 0.85 (3 H, m), 0.81 (3 H, m), 2.26 (2 H, m); ^{13}C NMR ($CDCl_3$, 125MHz) δ 37.2, 31.9, 71.8, 42.3, 140.7, 121.8, 31.7, 31.9, 50.1, 36.5, 21.1, 39.8, 42.3, 56.7, 24.3, 28.2, 56.1, 12.0, 19.0, 36.1, 19.4, 33.9, 26.1, 45.8, 29.1, 19.0, 18.8, 23.1, 11.9. HRESIMS $[M]^+$ *m/z*: 414.3863, (calculated for

$C_{29}H_{50}O$ 414.3862).

Fraction 10 [10.0 mg; eluted with EA/Hex (60:40)] was chromatographed on a silica gel column (7.0×2.0 cm) again and fractionated (5mL for each fraction) using a 1% MeOH/Dichloromethane solvent system to give compound **117** (withanolide A) [122, 123] [3.0 mg]: UV (MeOH) λ_{max} (log ε) 214 (3.98) nm; IR (film) ν_{max} 3474, 2948, 2836, 1652, 1412, 1113, 1027, 667, 440 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500MHz) δ 5.89 (1 H, dd, 10.2, 2.4 Hz, H2), 6.62 (1 H, ddd, 10.2, 5.2, 2.2 Hz, H3), 2.69 (1 H, m, H4a), 2.57 (1 H, m, H4b), 3.07 (1 H, d, 3.9 Hz, H6), 3.35 (1 H, m, H7), 1.80 (1 H, m, H8), 1.58 (1 H, m, H9), 2.76 (1 H, m, H11a), 2.05 (1 H, m, H11b), 2.12(1 H, m, H12a), 1.37 (1 H, m, H12b), 1.43 (1 H, m, H14), 1.86 (1 H, m, H15a), 1.39 (1 H, m, H15b), 1.60 (1 H, m, H16a), 1.40 (1 H, m, H16b), 1.51 (1 H, m, H17), 0.99 (3 H, s, H18), 1.21 (3 H, s, H19), 1.35 (3 H, s, H21), 4.22 (1 H, dd, 13.4, 3.5 Hz, H22) 2.41 (1 H, m, H23a), 2.13 (1 H, m, H23b), 1.92 (3 H, s, H27), 1.99 (3 H, s, H28). ^{13}C NMR spectroscopic data, see Table. 6. HRESIMS $[M]^+$ *m/z*: 470.2715, (calculated for $C_{28}H_{38}O_6$ 470.2668).

5.4.5 Extraction and Isolation of compound 118 and 119

The isolation scheme for compound **118** and **119** is as follows:

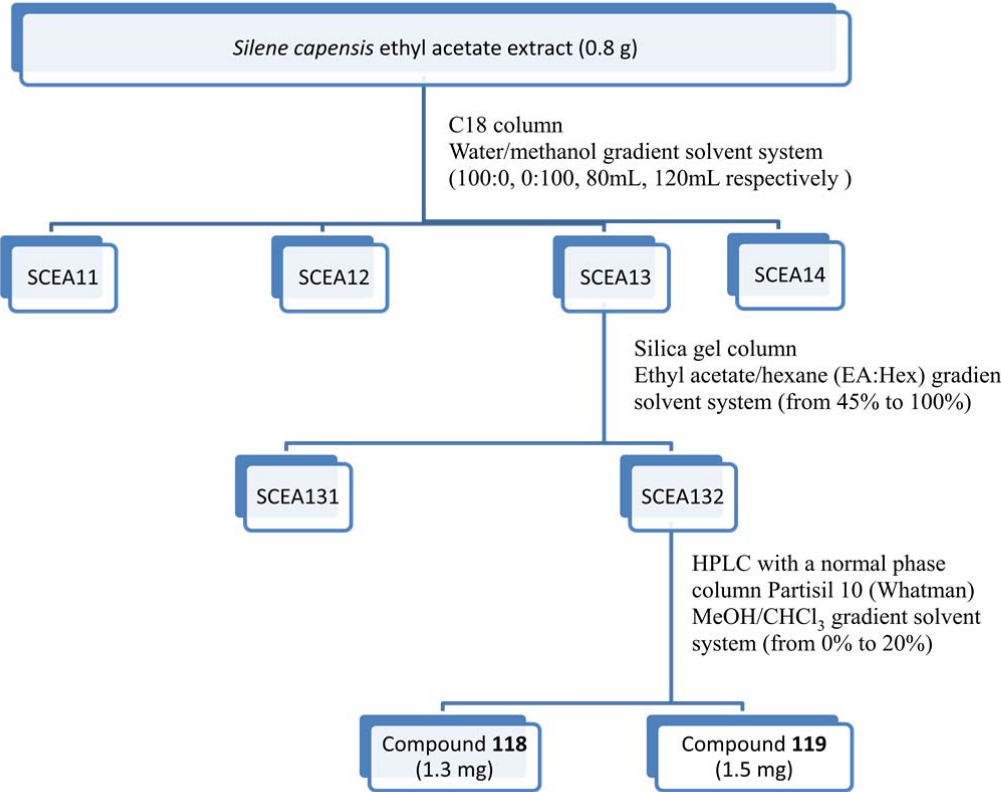


Fig. 38 The isolation scheme for compound **118** and **119**

S. capensis ethyl acetate extract was chromatographed on a C18 column (3.0×3.0 cm) and fractionated (20mL for each fraction) using 80 mL pure water and then 120 mL pure methanol. Fractions were collected and pooled by TLC analysis to afford 4 combined fractions.

Fraction 3 (SCEA13) [20.0mg; eluted with pure methanol] was chromatographed on a silica gel column (7.0×2.0 cm) and fractionated (5mL for each fraction) using an ethyl acetate/hexanes (EA:Hex) gradient solvent system (45:55, 55:45, 65:35, 80:20, 100:0; 30, 50, 30, 30, 30 mL respectively). Fractions were collected and pooled by TLC analysis to afford 2 combined fractions.

Fraction 2 (SCEA22) [5.0 mg; eluted with EA/Hex (80:20)] was purified by HPLC with a normal phase column Partisil 10 (Whatman). Flow rate: 3.00 mL/min; Injection volume: 40μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 20% MeOH/$CHCl_3$ (from 0 to 30 min), 100% MeOH (from 30 to 55 min). Compound **118** (12-deoxywithastramonolide) [124] (1.3 mg) was collected at 16.968 min and Compound **119** (withaferin A) [125, 127] (1.5 mg) was collected at 18.356 min.

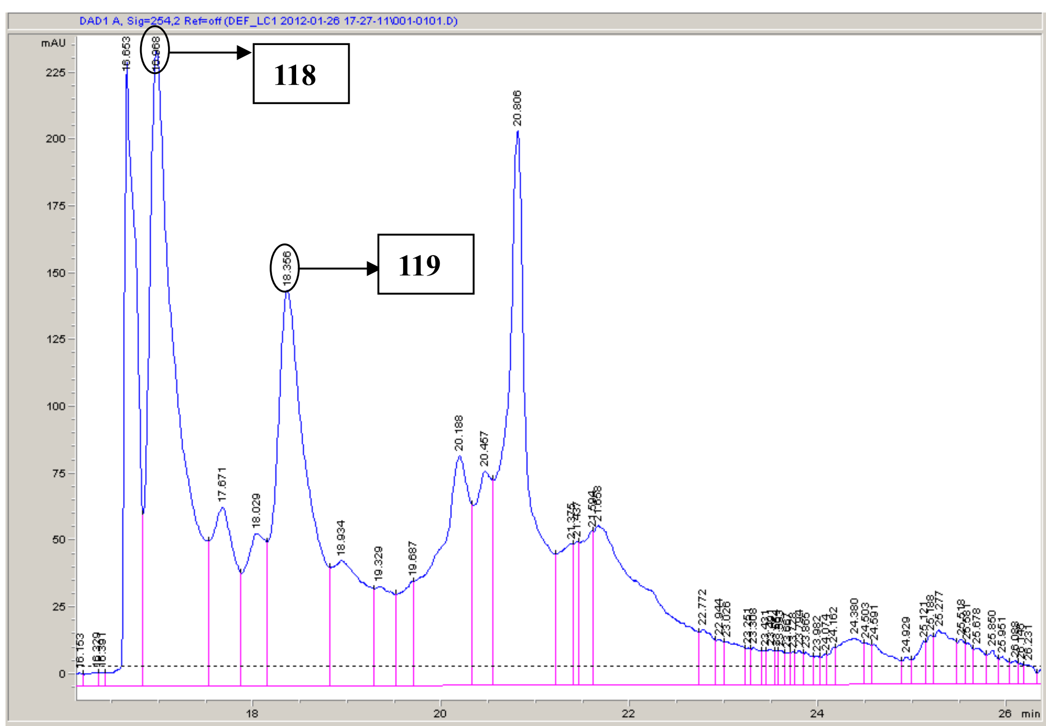


Fig. 39 HPLC chromatogram for *S. capensis* ethyl acetate extract

Compound **118** (12-deoxywithastramonolide) [124]: UV (MeOH) λ_{max} (log ε) 227 (3.11) nm; IR (film) ν_{max} 3489, 2947, 2835, 1656, 1413, 1113, 1028, 667, 428 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500MHz) δ 5.87 (1 H, dd, 10.2, 2.8 Hz, H2), 6.63 (1 H, m, H3), 2.70 (1 H, m, H4a), 2.58 (1 H, m, H4b), 3.07 (1 H, d, 3.7 Hz, H6), 3.34 (1 H, m, H7), 1.78(1 H, m, H8), 1.59 (1 H, m, H9), 2.78 (1 H, m, H11a), 1.36 (1 H, m, H11b), 2.02 (1 H, m, H12a), 1.34 (1 H, m, H12b), 1.45 (1 H, m, H14), 1.87 (1 H, m, H15a), 1.37 (1 H, m, H15b), 1.80 (1 H, m, H16a), 1.43 (1 H, m, H16b), 1.23 (1 H, m, H17), 0.80 (3 H, s, H18), 1.21 (3 H, s, H19), 2.06 (1 H, m, H20), 1.05 (3 H, d, 6.6 Hz, H21), 4.49 (1 H, dt, 13.1, 3.1 Hz, H22), 2.54 (1 H, m, H23a), 2.04 (1 H, m, H23b), 4.42 (2 H, d, 12.6 Hz, H27), 2.08 (3 H, s, H28). ^{13}C NMR spectroscopic data, see Table.7. HRESIMS $[M]^+$ *m/z*: 470.2677, (calculated for $C_{28}H_{38}O_6$ 470.2668).

Compound **119** (withaferin A) [125, 127]: UV (MeOH) λ_{max} (log ε) 239 (3.01) nm; IR (film) ν_{max} 3467, 2948, 2836, 1653, 1450, 1412, 1113, 1025, 668 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500MHz) δ 6.24 (1 H, d, 10.1 Hz, H2), 6.96 (1 H, dd, 9.95, 5.80 Hz, H3), 3.79 (1 H, d, 5.8 Hz, H4), 3.27 (1 H, m, H6), 2.16 (1 H, m, H7a), 1.30 (1 H, m, H7b), 2.52 (1 H, m, H8), 1.05 (1 H, m, H9), 1.88 (1 H, m, H11a), 1.48 (1 H, m, H11b), 1.67 (1 H, m, H12a), 1.39 (1 H, m, H12b), 0.97 (1 H, m, H14), 1.64 (1 H, m, H15a),

1.18 (1 H, m, H15b), 1.97 (1 H, m, H16a), 1.15 (1 H, m, H16b), 1.10 (1 H, m, H17), 0.73 (3 H, s, H18), 1.44 (3 H, s, H19), 2.02 (1 H, m, H20), 1.04 (3 H, d, 6.6 Hz, H21), 4.43 (1 H, dt, 13.1, 3.1 Hz, H22), 1.99 (1 H, m, H23a), 1.54 (1 H, m, H23b), 4.38 (2 H, m, H27), 2.06 (3 H, s, H28). ^{13}C NMR spectroscopic data, see Table.8. HRESIMS $[M]^+$ *m/z*: 470.2776, (calculated for $C_{28}H_{38}O_6$ 470.2668).

5.4.6 Extraction and isolation of compound 116

S. capensis aqueous extract was chromatographed by HPLC with a reversed phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). Flow rate: 5.00 mL/min; Injection: 60μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 100% MeOH/Water (with 0.1% TFA) (from 0 to 30 min), 100% MeOH (from 30 to 55 min). Compound **116** (pelletierine) [128] (2.0 mg) was collected at 13.019 min. UV (MeOH) λ_{max} (log ε) 206 (1.03) nm; IR (film) ν_{max} 1654, 1345, 1033, 1013, 668, 440 cm^{-1}; ^{1}H NMR (pyr-D, 500MHz) δ 1.56 (1 H, m), 1.72 (1 H, m), 1.81 (4 H, m), 1.99 (3 H, s), 2.88 (1 H, m), 2.91 (1 H, m), 3.37 (1 H, m), 3.48 (1 H, m); ^{13}C NMR (pyr-D, 125MHz)δ 22.4, 22.5, 28.8, 29.6, 44.6, 46.2, 52.3, 205.5. HRESIMS $[M]^{+}$ *m/z*: 141.1163, (calculated for $C_8H_{15}NO$ 141.1154).

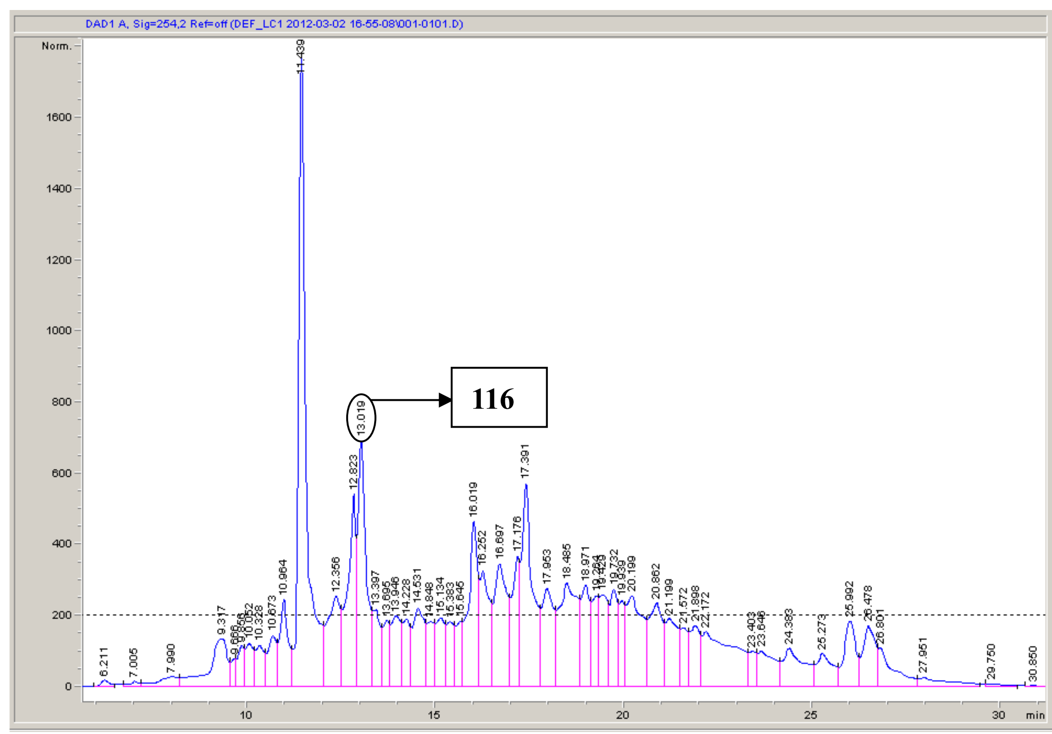


Fig. 40 HPLC chromatogram for *S. capensis* aqueous extract

5.4.7 Extraction and isolation of compound 120-123

S. capensis n-BuOH extract was chromatographed by HPLC with a reversed phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). Flow rate: 5.00 mL/min; Injection: 30μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 100% MeOH/Water (with 0.1% TFA) (from 0 to 30 min), 100% MeOH (from 30 to 50 min). Compound **120** (withanoside IV) [130] (1.1 mg) was collected at 29.948 min. Compound **121** (withanoside V) [130] (2.2 mg) was collected at 33.097 min. Compound **122** (3β-*O*-Butyl-2,3-dihydrowithaferin A) [131] (2.1 mg) was collected at 33.911 min. Compound **123** (2-β-*D*-glucopyranosyloxy-5-methoxy benzoic acid) [132] (1.0 mg) was collected at 16.179 min.

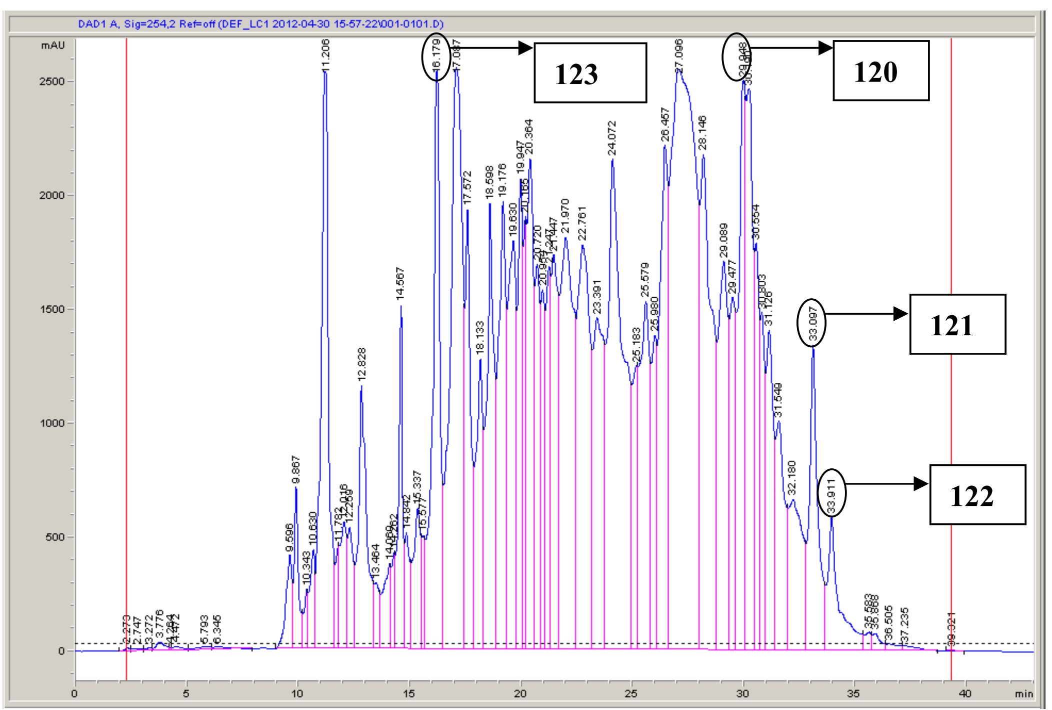


Fig. 41 HPLC chromatogram for *S. capensis* n-BuOH extract

Compound **120** (withanoside IV) [130] (1.1 mg) was collected at 29.948 min. UV (MeOH) λ_{max} (log ε) 218 (2.12), 228 (1.99) nm; IR (film) ν_{max} 3517, 2844, 1644, 1033, 1015, 573, 427 cm^{-1}; ^{1}H NMR (pyr-D, 500MHz) δ 0.62(3 H, s, H18), 0.98 (3 H, d, 5.1 Hz, H21), 1.01 (3 H, s, H19), 2.14 (3 H, s, H28), 4.08 (1 H, m, H1), 4.39 (1 H, m, H22), 4.73 (1 H, m, H3), 4.76 (1 H, m, H27a), 4.87 (1 H, m, H27b), 4.93 (1 H, d, 7.4 Hz, H1'), 5.14 (1 H, d, 7.4 Hz, H1"). ^{13}C NMR spectroscopic data, see Table. 9. HRESIMS $[M]^{+}$ *m/z*:782.4100, (calculated for $C_{40}H_{62}O_{15}$ 782.4089).

Compound **121** (withanoside V) [130] (2.2 mg) was collected at 33.097 min. UV (MeOH) λ_{max} (log ε) 233 (1.97) nm; IR (film) ν_{max} 3467, 2950, 2843, 1648, 1453, 1412, 1112, 1016, 631, 428 cm^{-1}; ^{1}H NMR (pyr-D, 500MHz) δ 0.63 (3 H, s, H18), 1.00 (3 H, m, H21), 1.01 (3 H, s, H19), 1.83 (3 H, s, H28), 1.95 (3 H, s, H27), 4.07 (1 H, m, H1), 4.08 (1 H, m, H22), 4.71 (1 H, m, H3), 4.93 (1 H, d, 7.8 Hz, H1'), 5.60 (1 H, m, H6). ^{13}C NMR spectroscopic data, see Table. 9. HRESIMS $[M]^{+}$ *m/z*: 766.4157, (calculated for$C_{40}H_{62}O_{14}$ 766.4140).

Compound **122** (3β-*O*-Butyl-2,3-dihydrowithaferin A) [131] (2.1 mg) was collected at 33.911 min. UV (MeOH) λ_{max} (log ε) 231 (2.39) nm;

IR (film) ν_{max} 3497, 2951, 2843, 1647, 1398, 1016, 627, 429, 421 cm^{-1}; ^{1}H NMR (pyr-D, 500MHz) δ 0.52 (3 H, s, H18), 0.88 (3 H, t, 7.4 Hz, H4'), 0.94 (3 H, d, 6.6 Hz, H21), 2.10 (3 H, s, H28), 2.95 (1 H, dd, 3.2, 15.6 Hz, H7), 3.20 (1 H, m, H6), 3.42 (1 H, m, H4), 3.45 (1 H, m, H2'), 3.91 (1 H, m, H3), 4.35 (2 H, m, H27), 4.40 (1 H, m, H22). ^{13}C NMR spectroscopic data, see Table. 10. HRESIMS $[M]^+$ *m/z*: 544.3383, (calculated for $C_{32}H_{48}O_7$ 544.3400).

Compound **123** (2-β-*D*-glucopyranosyloxy-5-methoxy benzoic acid) [132] (1.0 mg) was collected at 16.179 min. UV (MeOH) λ_{max} (log ε) 247 (3.74), 250 (4.13) nm; IR (film) ν_{max} 3424, 2981, 2844, 1641, 1055, 1033, 1014, 573, 429 cm^{-1}; ^{1}H NMR, ^{13}C NMR spectroscopic data, see Table. 11. HRESIMS $[M]^+$ *m/z*: 330.0952, (calculated for $C_{14}H_{18}O_9$ 330.0951).

Chapter 6. A literature review of the genus *Ipomoea* and *Ipomoea indica*

6.1 The genus *Ipomoea*

The genus *Ipomoea* (family: Convolvulaceae) occurs in the tropics of the world although some species also reach temperate zones. [146, 147] The species of this genus are mainly distributed throughout the South and Central America countries, and Tropical African territories. [146, 147] One of the most noticeable anatomical characteristics of the Convolvulaceae is the existence of cells, which secrete resin glycosides in the foliar tissues and in the roots of the plants. These glycoresins constitute one important chemotaxonomic marker of this family [147, 148] and are responsible for the purgative properties of some species of the Convolvulaceae. [147, 149]

The genus *Ipomoea* since time immemorial have been in continuous use for different purposes, such as, nutritional, medicinal, ritual and agricultural. [149] The knowledge constitutes a rich source of ethnomedical information for effective selection of plants to be evaluated by chemical studies. [149] With regard to these nutritional purposes, it is necessary to highlight the importance of the *I. batatas* (L.) Lam. This species originated from Central America, was widely cultivated and consumed almost throughout the world. [151] *I. aquática* Forsk is consumed as food

in Sri Lanka, Hong Kong, Taiwan and China.[150] *I aquatica* is one of the richest sources of carotenoids and chlorophylls.[153] The leaves of *I. aquatica* contain adequate quantities of most of the essential amino acids and are comparable to conventional food stuffs such as soybean or whole egg, indicating the potential of *I. aquatica* for utilisation as a food supplement. Moreover, the leaves of *I. aquatica* are an excellent source of bioelements such as calcium, magnesium, iron, zinc, and copper.[154]

Some species of *Ipomoea* showed antimicrobial, analgesic,[152, 150] spasmolitic, spasmogenic,[153, 154] hypotensive,[155] psychotomimetic[156] and anticancer[157] activities. The most common biologically active constituents from these plants are alkaloids (Fig. 42 and Table 16),[158, 159, 161] mainly including ergoline alkaloids (**124-135**),[158] indolizidine alkaloids (**136-138**),[159] nortropane alkaloids (**139-142**).[161] Phenolic compounds,[160] coumarins,[162] norisoprenoids,[163] diterpene, isocoumarin[163, 164, 166] and benzenoids flavonoids[163] and antocianosides, glycolipids,[161] lignan and triterpenes[161, 165, 166] are also important constituents from the *Ipomoea* genus.

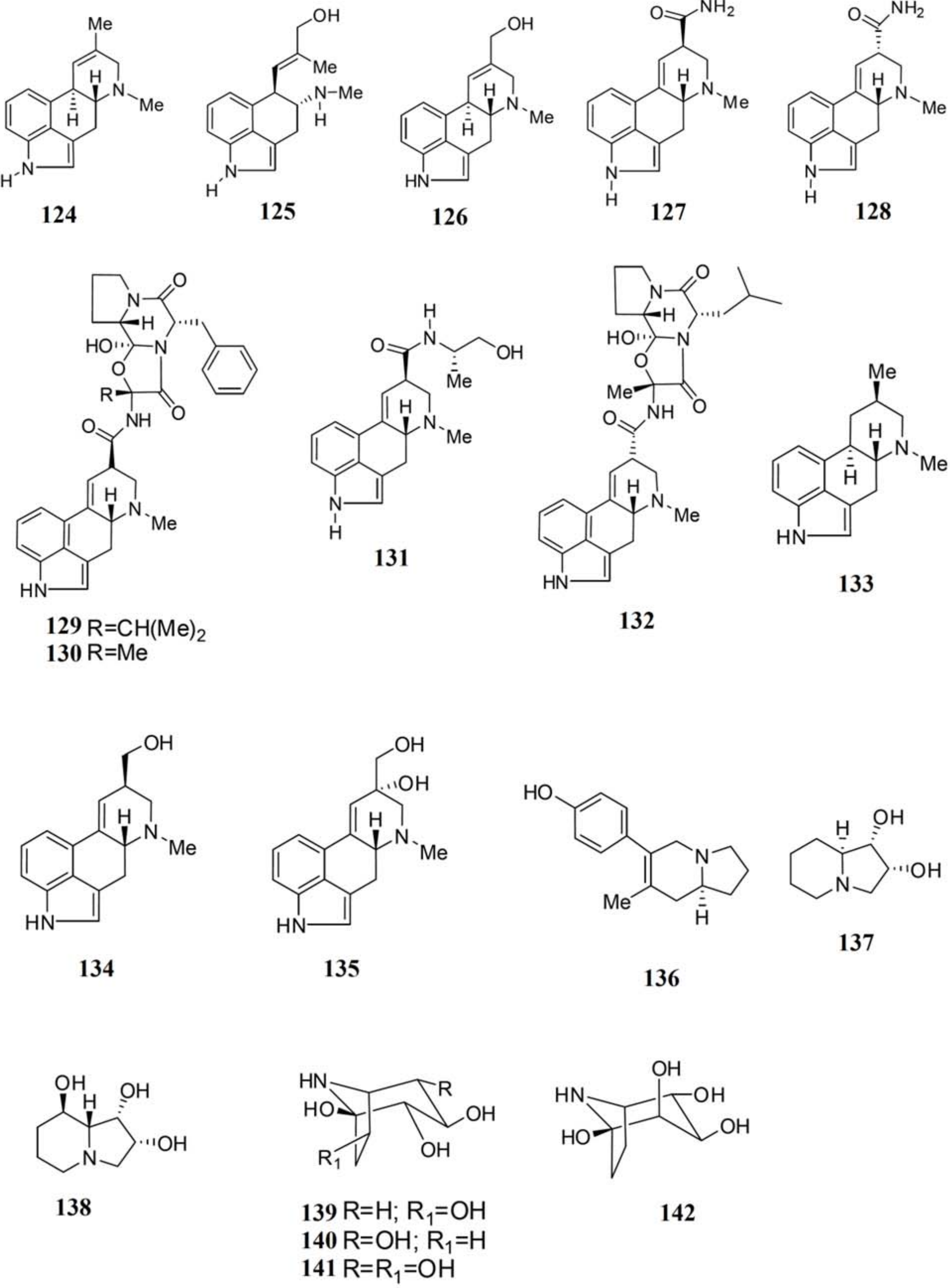


Fig. 42 Structures of alkaloids from the *Ipomoea* genus [158, 159, 161]

Table 16. Alkaloids from the *Ipomoea* genus [158, 159, 161]

Substances	Species	Activities
Ergoline alkaloids		
agroclavine (**124**)	*I. fistulosa* *I. mueller* *I. tricolor*	Antimicrobial Cytostatic
hanoclavine I (**125**)	*I. asarifolia* *I. hederacea* *I. muelleri* *I. corymbosa* *I. tricolor* *I. violacea*	Psychotropic Psychotomimetic
elymoclavine (**126**)	*I. hederacea* *I. muelleri* *I. corymbosa* *I. parasitica* *I. violacea*	Psychotropic Psychotomimetic
ergine (LSA) (**127**)	*I. asarifolia* *I. muelleri* *I. corymbosa* *I. tricolor* *I. violacea*	Psychotropic Psychotomimetic
erginine (**128**)	*I. muelleri* *I. corymbosa* *I. tricolor* *I. violacea*	Psychotropic Psychotomimetic
ergocristine (**129**) ergotamine (**130**)	*I. tricolor*	Psychotropic Psychotomimetic
ergometrine or ergonovine (**131**)	*I. muelleri* *I. corymbosa* *I. tricolor* *I. violacea*	Psychotropic Psychotomimetic Vasoconstrictor Hemostatic Uterotonic
ergosinine (**132**)	*I. palmata*	Uterotonic
festuclavine (**133**)	*I. muelleri*	Antimicrobial
lysergol (**134**)	*I. hederacea* *I. muelleri* *I. parasitica* *I. petaloidea* *I. corymbosa* *I. violacea*	Psychotropic Psychotomimetic

Table 16. continued

penniclavine (**135**)	*I. hederacea* *I. muelleri* *I. corymbosa* *I. violacea*	Psychotropic
Indolizidine alkaloids		
ipalbidine (**136**)	*I. alba* *I. muricata* *I. hardwickki*	Analgesic Antioxidant
2-*epi*-lentiginosine (**137**)	*I. carnea*	Potent inhibitory activity toward rat α-mannosidase
swainsonine (**138**)	*I. carnea*	Immunomodulatory Antimetastatic Potent inhibitory activity toward rat α-mannosidase
Nortropane alkaloids		
calystegine B1 (**139**) calystegine B2 (**140**) calystegine C1 (**141**)	*I. alba* *I. aquatica* *I. batatas* *I. carnea* *I. hederifolia* *I. eremnobrocha* *I. obscura* *I. pes-caprae* *I. setifera* *I. violacea*	Potent inhibitory activity toward rat lysosomal β-glucosidase.
calystegine B3 (**142**)	*I. alba* *I. aquatica* *I. batatas* *I. carnea* *I. hederifolia* *I. eremnobrocha* *I. obscura* *I. pes-caprae* *I. setifera* *I. violacea*	Moderate inhibitory activity toward rat α- and β-mannosidases

6.2 *Ipomoea indica*

Ipomoea indica is widely grown ornamentally in the Mediterranean basin, where it is frequently naturalized. [164] The putative role of *I. indica* and other species in the genus as a reservoir for sweet potato pathogens has not been explored. [164] Methanolic extract from the seeds of *I. indica* presented biological activity against Herpes Simplex-1. [154] Methanolic and aqueous extracts from the seeds of this species were also investigated for anti-bacterial activity against *Streptococus pyogenes, Staphylococus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* However, they did not present activity. [151] Acetonitrile extract from the seeds of *I. indica* was evaluated for its ability to inhibit the growth of three species of fungi, *Microsporum canis, Epidermophyton floccosum* and *Trichophyton rubrum. I. indica* showed activity against *Microsporum canis* and *Epidermophyton floccosum* at a concentration of 1000 μg/mL but no growth inhibition was observed against *Trichophyton rubrum*. [151] The glycoside called ipolearoside, with significant activity against Walker carcinosarcoma 256 in rats, has been isolated from ethanol extracts of the whole plants of *I. leari* Paxt. [149]

Up to now, there are no scientific report about the natural product isolation work of *I. indica*. Our primary goal is to isolate and identify components of the extract of *I. indica.*

Eight compounds have been isolated from the stems of *I. indica*. Details of the isolation and structure elucidation of these compounds are described herein.

Chapter 7. Phytochemical investigation of *Ipomoea indica*

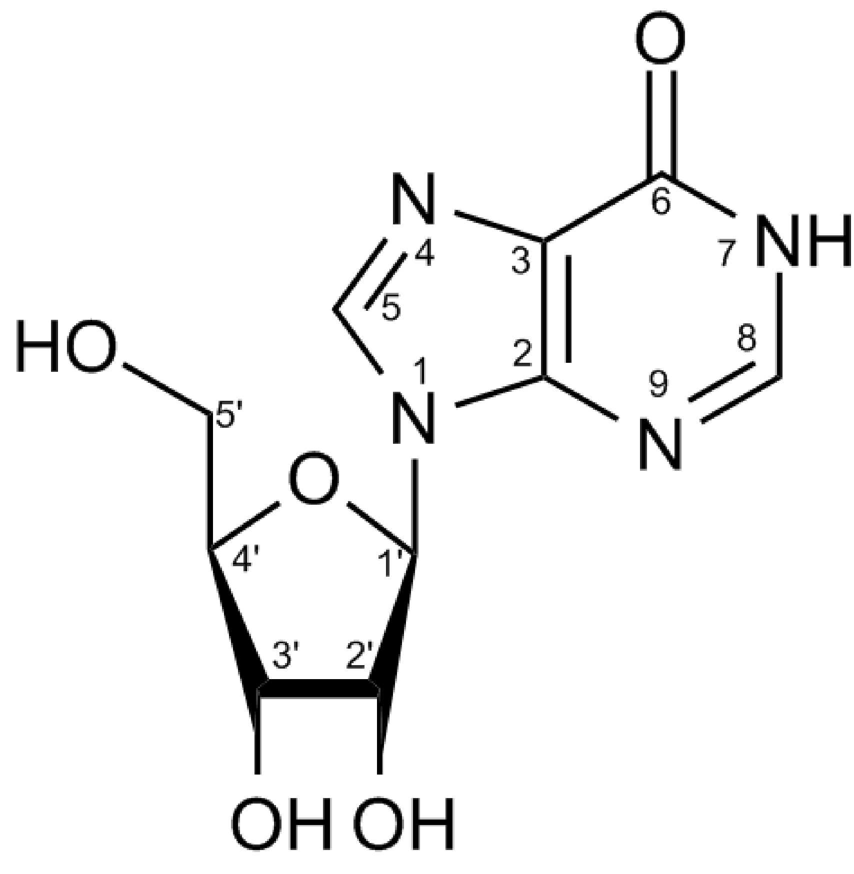
7.1 Introduction

Eight compounds (**41**, **42**, **115**, **143-147**) were obtained by repeated chromatography of the extracts of the stems of *I. indica*.

The details for the isolation are presented in the experimental section. These compounds were identified by MS and NMR data.

7.2 Results and discussion

7.2.1 Structure elucidation of compound 143



143

Fig. 43 Structure of compound **143**

Compound **143** showed a molecular ion peak in its HRESIMS spectrum (*m/z*268.1042) corresponding to the molecular formula $C_{10}H_{12}N_4O_5$. indicating seven degrees of unsaturation. According to the molecular formula, it appeared to be an alkaloid, possibly with aromatic character.

According to the ^{1}H NMR,^{13}C NMR, DEPT, HSQC and HMBC spectrum and molecular formula, there should be a sugar group in the molecule: ^{1}H NMR δ 3.65 (1 H, dd, 12.4 Hz, 2.8 Hz, H 5'), 3.78 (1 H, dd, 12.4 Hz, 2.6 Hz, H 5'), 4.07 (1 H, dd, 5.6 Hz, 2.8 Hz, H 4'), 4.23 (1 H, dd, 4.9 Hz, 3.0 Hz, H 3'), 4.59 (1 H, t, 5.7 Hz, H 2'), 5.92 (1 H, d, 6.0 Hz, H1'), ^{13}C NMR δ 60.2, 69.4, 72.9, 85.0, 88.0. The carbon at δ 60.2 (C 5') was a CH_2, according to DEPT. δ 88.0 (C 1') was connected to δ 5.92 (1 H, d, 6.0 Hz, H 1'), according to HSQC. All these data were indicative of a sugar group.

Table 17. NMR spectral data for compound **143**

position	^{13}C NMR (MeOD) δ	^{1}H NMR (MeOD) δ	(J in Hz)	^{13}C NMR (MeOD) reported data [167, 168] δ	^{1}H NMR (MeOD) reported data [167, 168] δ	(J in Hz)
2	146.9			146.8		
3	117.9			118.1		
5	147.6	8.17	(s)	147.3	8.15	(s)
6	159.6			159.4		
8	139.8	8.35	(s)	140.2	8.38	(s)
1'	88.0	5.92	(d, 6.0)	88.5	5.93	(d)
2'	72.9	4.59	(t, 5.7)	73.1	4.59	(dd)
3'	69.4	4.23	(dd, 4.9, 3.0)	69.4	4.26	(dd)
4'	85.0	4.07	(dd, 5.6, 2.8)	85.6	4.11	(ddd)
5'a	60.2	3.78	(dd, 12.4, 2.6)	60.3	3.72	(m)
5'b		3.65	(dd, 12.4, 2.8)		3.62	(m)

According to HSQC, ^{13}C NMR δ 139.8 was connected to ^{1}H NMR δ 8.35 (1 H, s, H8) and ^{13}C NMR δ 147.6 was connected to ^{1}H NMR δ 8.17 (1 H, s, H5). Based on the low field chemical shift, these two carbons

should be aromatic carbons and there should be some nitrogens next to these two carbons.

In the ^{13}C NMR spectrum, one singlet at δ(C) 159.6 (C6) was attributed to a carbonyl group. Based on this data and molecular formula, we proposed that there should be a hypoxanthine unit in the molecule. This proposal was corroborated by the HMBC data. δ 8.17 (1 H, s, H5) showed cross peak to the δ 117.9 (C3) and δ 146.9 (C2) respectively and δ 8.35 (1 H, s, H8) also showed cross peak to the δ 146.9 (C2) in HMBC.

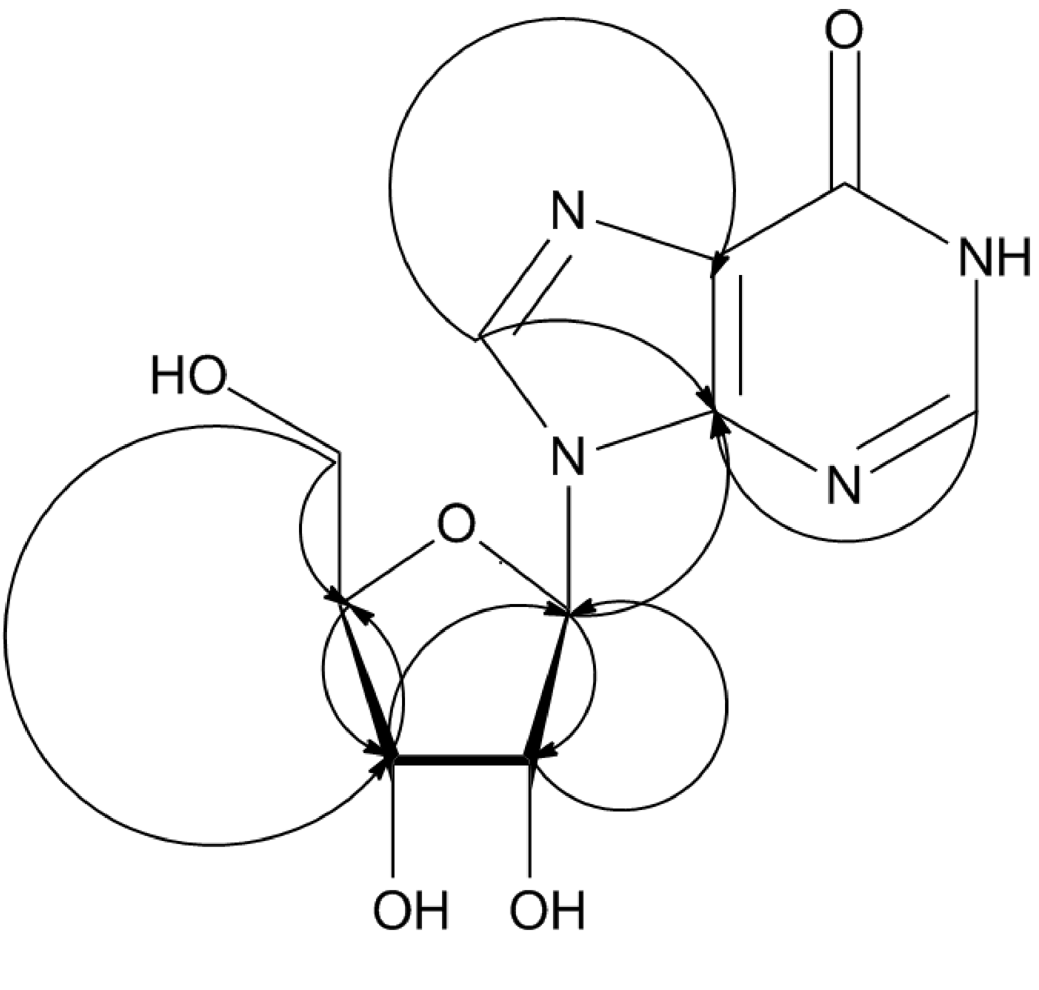
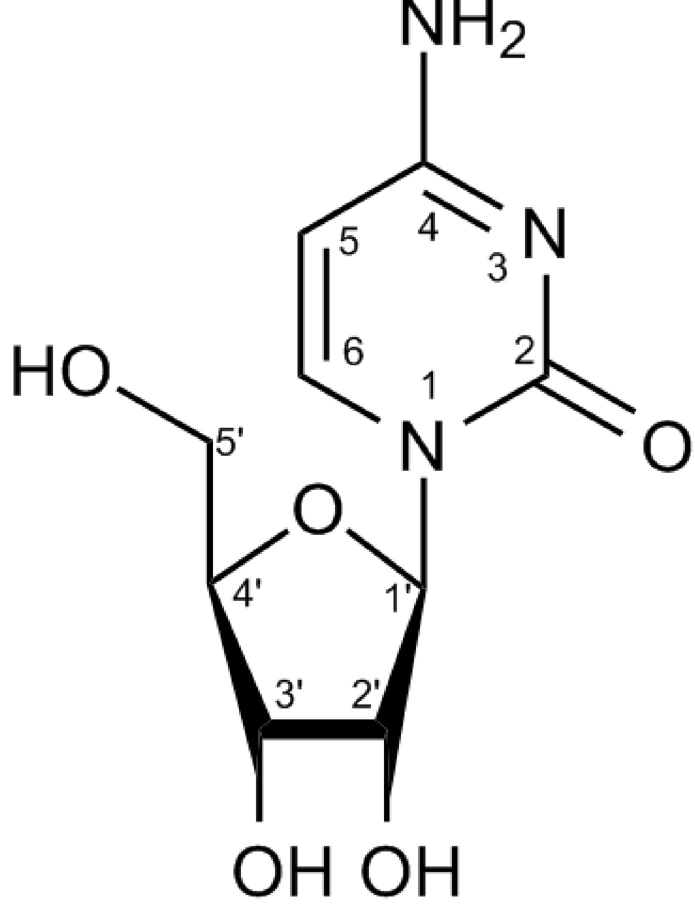


Fig. 44 Key HMBC correlations of compound **143**

According to the HMBC, δ 5.92 (1 H, d, 6.0 Hz, H1') showed cross peak to the δ 146.9 (C2). Therefore the sugar group should be connected to N 1.

In the end, Compound **143** was identified as inosine, which is a very common natural product. [168] It was also confirmed by the reported data. [167, 168] Inosine has never been identified in any other *Ipomoea* species. [158, 159, 161]

7.2.2 Structure elucidation of compound 144



144

Fig. 45 Structure of compound **144**

Compound **144** showed a molecular ion peak in its HRESIMS spectrum (*m/z* 243.0632) corresponding to the molecular formula $C_9H_{13}N_3O_5$ indicating five degrees of unsaturation.

Table 18. NMR spectral data for compound **144**

position	^{13}C NMR (MeOD) δ	^{1}H NMR (MeOD) δ	(J in Hz)	^{13}C NMR (MeOD) reported data [169] δ	^{1}H NMR (MeOD) reported data [169] δ
2	152.5			152.6	
4	166.2			166.7	
5	102.6	5.71	(d, 8.1)	102.0	5.74
6	142.7	8.00	(d, 8.1)	142.8	8.03
1'	90.7	5.91	(d, 4.6)	90.1	5.99
2'	71.3	4.19	(m)	71.6	4.18
3'	75.7	4.16	(m)	75.1	4.15
4'	86.4	4.02	(m)	86.3	4.08
5'a	62.3	3.86	(dd, 12.2, 2.7)	61.9	3.88
5'b		3.75	(dd, 12.2, 3.1)		3.76

In the ^{1}H NMR and ^{13}C NMR, signals for Compound **144** were very similar to that of Compound **143** [^{1}H NMR δ 3.75 (1 H, dd, 12.2 Hz, 3.1 Hz, H 5'), 3.86 (1 H, dd, 12.2 Hz, 2.7 Hz, H 5'), 4.02 (1 H, m, H 4'), 4.16 (1 H, m, 3.0 Hz, H 3'), 4.19 (1 H, m, H 2'), 5.91 (1 H, d, 4.6 Hz, H1'),^{13}C NMR δ 62.3, 71.3, 75.7, 86.4, 90.7]. This suggested that Compound **144** also had a sugar group in the molecule. This supposition was supported by a cross-peak between δ(H) 4.19 (1 H, m, H 2') and δ(H) 5.91 (1 H, d, 4.6 Hz, H 1') in the COSY, which indicated a furanose sugar moiety. According to DEPT, δ 62.3 (C 5') was a CH_2. All these information matched up with a sugar group.

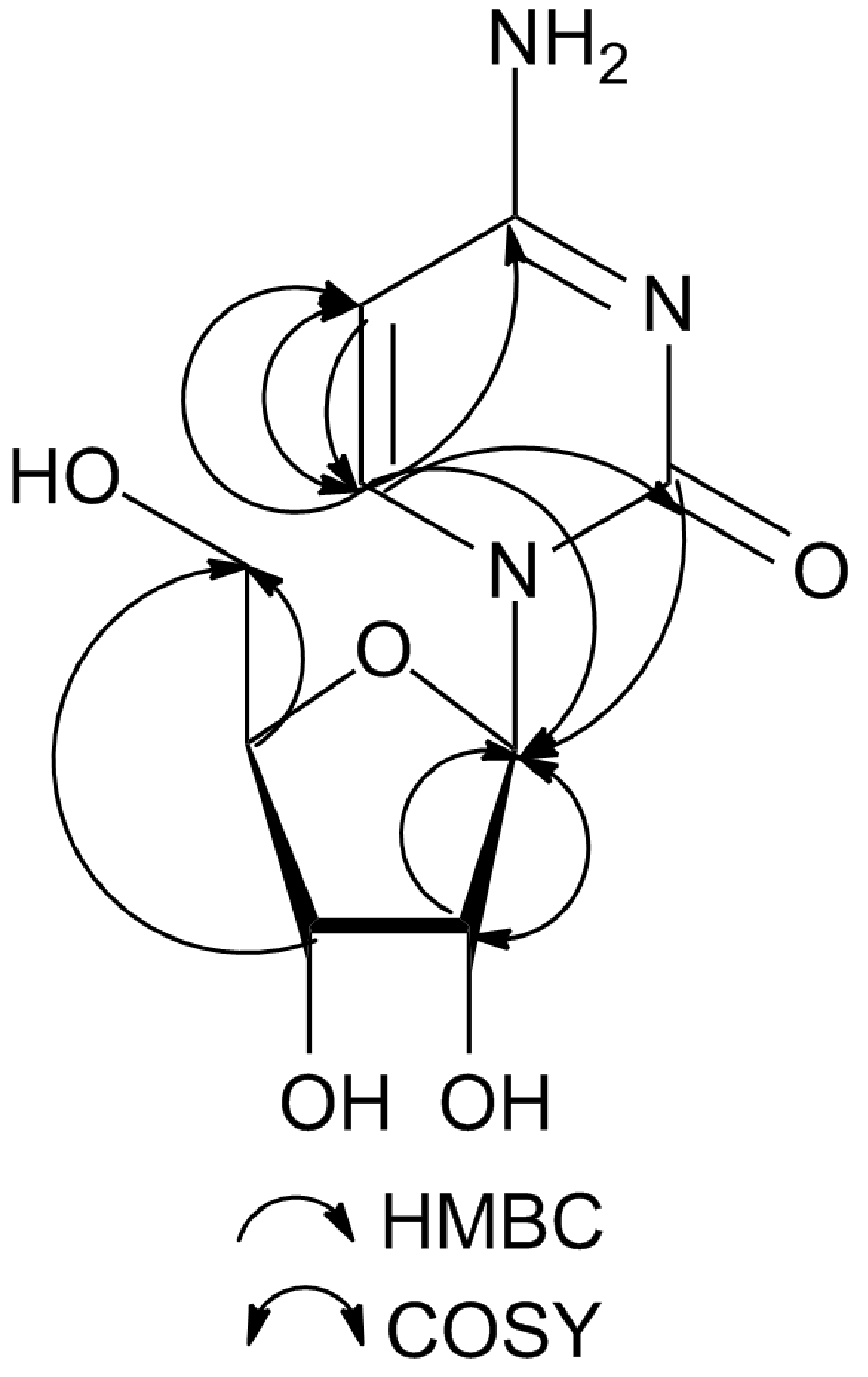


Fig. 46 Key HMBC and COSY correlations of compound **144**

The carbon at δ 142.7 was connected to the proton at δ 8.00 (1 H, d, 8.1 Hz, H6). Based on the low field chemical shift, this carbon should be an aromatic carbon possibly adjacent to a nitrogen atom.

The carbon at δ 102.6 (C 5)and the doublet at δ_H 5.71 (1 H, d,8.1 Hz, H 5) were attributed to a carbon in pair with C 6, forming a double bond, because 5-H and 6-H were in couple, having the same coupling constant. It was also confirmed by the cross peak between δ (H) 5.71 (1 H, d, 8.1 Hz, H 5) and δ (H) 8.00 (1 H, d, 8.1 Hz, H 6) in the COSY, the cross peak between δ(C) 142.7 (C 6) and δ (H) 5.71 (1 H, d,8.1 Hz, H 5) in the HMBC and the cross peak between δ(C) 102.6 (C 5) and δ(H) 8.00 (1 H, d, 8.1 Hz, H 6) in the HMBC.

Based on all these information and molecular formula, we proposed that there should be a cytosine unit in the molecule. This proposal was confirmed by the cross peak between δ(C) 152.5 (C 2) and δ(H) 8.00 (1 H, d, 8.1 Hz, H 6) and the cross peak between δ(C) 166.2 (C 4) and δ(H) 8.00 (1 H, d, 8.1 Hz, H 6) in the HMBC.

According to the HMBC, δ 5.91 (1 H, d, 4.6 Hz, H 1') showed cross peak to the δ 142.7 (C 6) and δ 152.5 (C 2) respectively. Therefore the sugar group should connected to N 1.

Based on all of these data and molecular formula and the similarity between Compound **144** and Compound **143**, Compound **144** was identified as cytidine. Data were in agreement with those previously

reported. [169] Cytidine has never been identified in any other *Ipomoea* species. [158, 159, 161]

7.2.3 Structure elucidation of compound 115

115

Fig. 47 Structure of compound **115**

Compound **115** was assigned the molecular formula $C_{18}H_{32}O_2$ (*m/z*: 280.2625), determined by HRESIMS, indicating three degrees of unsaturation. According to the molecular formula, it looked like a 18-carbon fatty acid.

The ^{1}H NMR and ^{13}C NMR spectra of Compound **115** from *I. indica* were almost same as those of Compound **115** from *S. capensis*.

In the ^{13}C NMR spectrum, one singlet at δ(C) 178.7 was attributed to carboxyl group. Four singlets at δ(C) 130.2, 130.0, 128.1 and 127.9 were attributed to vinylic carbons.

According to the NMR and molecular formula, also considering common 18-carbon fatty acid, Compound **115** was identified as linoleic acid, which was a very common natural product compound. It was also confirmed by the comparison with reported data. [122]

7.2.4 Structure elucidation of compound 41

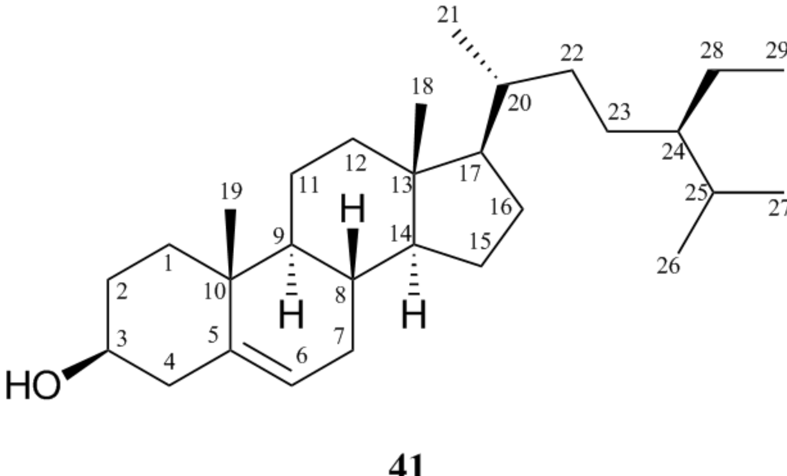


Fig. 48 Structure of compound **41**

Compound **41** had the molecular formula $C_{29}H_{50}O$ determined by HRESIMS $[M]^{+}$ *m/z*: 414.3869, indicating five degrees of unsaturation.

The ^{1}H NMR and ^{13}C NMR spectra of Compound **41** from *I. indica* were almost identical with those of Compound **41** from *S. capensis* and *L. leonurus*.

The ^{1}H NMR and ^{13}C NMR spectral data of **41** showed the presence of one pair of vinylic carbons [δ 5.38 (1 H, m); δ 140.7, 121.7] and one carbon which was connected to a hydroxyl group [δ 3.55 (1 H, m); δ 71.8].

The consideration of the typical natural product compounds and its molecular formula suggested the steroid nature of **41**.

By comparison of spectral data with those reported in the literature, [126] compound **41** was confirmed as β-Sitosterol.

7.2.5 Structure elucidation of compound 42

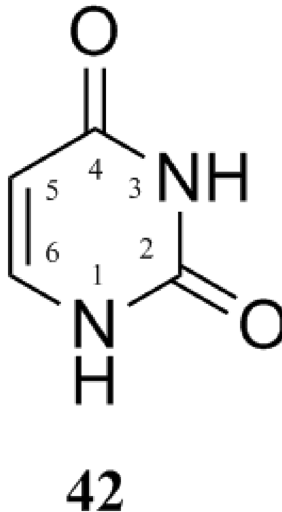


Fig. 49 Structure of compound **42**

Compound **42** had the molecular formula $C_4H_4N_2O_2$ determined by high-resolution MS HRESIMS $[M]^+$ *m/z*: 112.0596, indicating four degrees of unsaturation.

The ^{1}H NMR and ^{13}C NMR spectra of Compound **42** from *I. indica* were almost identical with those of Compound **42** from *L. leonurus*.

The ^{1}H NMR and ^{13}C NMR spectral data of **42** showed the presence of one pair of vinylic carbons [δ 7.41 (1 H, t, J = 5.8 Hz), 5.44 (1 H, d, J = 7.6 Hz); δ 142.7, 100.7] and two carbonyl groups [δ 164.8, 152.0]. According to the chemical shifts of these functional groups and the molecular formula, the structure of **42** should have a ring with nitrogen. The consideration of the typical natural product compounds suggested the nucleobase nature of **42**.

Compound **42** was identified as uracil, which was one of the four nucleobases in the nucleic acid of RNA. [105] It was also confirmed by the reported data. [105]

7.2.6 Structure elucidation of compound 145

Fig. 50 Structure of compound **145**

Compound **145** had the molecular formula $C_6H_6O_3$ determined by HRESIMS $[M]^+$ *m/z*: 126.0761, indicating four degrees of unsaturation.

The ^1H NMR and ^{13}C NMR spectral data of **145** showed the presence of one trisubstituted aromatic ring [δ (H) 7.02 (2 H, m), 6.40 (1 H, d, J = 7.9 Hz); δ (C) 150.2, 145.7, 137.5, 113.9, 107.7, 105.8]. According to the chemical shift, δ (C) 150.2, 145.7, 137.5 should connected to the oxygen and δ (C) 113.9, 107.7, 105.8 should connected to the hydrogen. It was also confirmed by the molecular formula.

According to the large coupling constant of δ (H) 6.40 (1 H, d, J = 7.9 Hz), this hydrogen should be coupled to a hydrogen, which was next to it. The peak of the second hydrogen overlapped with that of the third

hydrogen, showing a multiplet peak at δ 7.02 (2 H, m). Because δ (H) 6.40 (1 H, d, J = 7.9 Hz) showed up as doublet, this hydrogen should be only coupled to one hydrogen, which meant the third hydrogen should be on the opposite side of this hydrogen.

Therefore Compound **145** was identified as 1,2,4-benzenetriol, which was also confirmed by the reported data. [170]

7.2.7 Structure elucidation of compound 146

146

Fig. 51 Structure of compound **146**

Compound **146** had the molecular formula $C_8H_8O_4$ determined by HRESIMS $[M]^+$ *m/z*: 168.0030, indicating five degrees of unsaturation.

In the ^1H NMR and ^{13}C NMR, the trisubstituted aromatic ring of Compound **146** was very similar to that of Compound **145** [^1H NMR δ 7.45 (2 H, m), 6.73 (1 H, d, J = 8.7 Hz), ^{13}C NMR δ 160.9, 160.6, 131.7, 123.0, 113.6, 111.5]. Therefore Compound **146** should also have a trisubstituted aromatic ring.

According to the large coupling constant of δ (H) 6.73 (1 H, d, J = 8.7 Hz), this hydrogen should be coupled to a hydrogen, which was next to it. The peak of the second hydrogen overlapped with that of the third

hydrogen, showing a multiplet peak at δ 7.45 (2 H, m). Because δ (H) 6.73 (1 H, d, J = 8.7 Hz) showed up as doublet, this hydrogen should be only coupled to one hydrogen, which meant the third hydrogen should be on the opposite side of this hydrogen. The ^{1}H NMR and ^{13}C NMR spectral data of **146** showed the presence of one methoxy group [δ (H) 3.80 (3 H, s); δ (C) 54.1] and one carbonyl group [δ (C) 170.5]. That was also confirmed by the molecular formula.

Based on all of these information, Compound **146** was identified as methyl 2,4-dihydroxybenzoate, which was also confirmed by the reported data. [171]

7.2.8 Structure elucidation of compound 147

147

Fig. 52 Structure of compound **147**

Compound **147** had the molecular formula $C_{15}H_{10}O_7$ determined by HRESIMS $[M]^+$ *m/z*: 302.0646, indicating eleven degrees of unsaturation.

The ^1H NMR and ^{13}C NMR of Compound **147** was very similar to those of Compound **40** (Luteolin). Therefore Compound **147** could also be a flavone. This was also substantiated by the molecular formula.

According to the molecular formula, Compound **147** should have one more hydroxyl group than Compound **40** (Luteolin). According to the large coupling constant of δ (H) 6.86 (1 H, d, J = 7.9 Hz) and 6.67 (1 H, d, J = 8.2 Hz), these two hydrogens were in an ortho relationship. Because

both of these two hydrogen showed up as doublet peaks, there should be no more hydrogen next to these two hydrogen.

Based on this data and the similarity between Compound **147** and Luteolin (**40**), Compound **147** was identified as 2',3',4',5,7-pentahydroxyflavone, which was also confirmed by the reported data.[172]

7.3 Conclusions

There have been very few phytochemical investigations on *I. indica*. There was no scientific report about any compound isolated from *I. indica*. Therefore any compound we got from *I. indica* would be the first time of the isolation from *I. indica.*

Our phytochemical isolation of *I. indica* provided eight known compounds. Inosine (**143**) and cytidine (**144**) have never been identified in any other *Ipomoea* species. [158, 159, 161]

7.4 Experimental section

7.4.1 General Experimental Procedures

IR spectra were recorded on a Thermo Nicolet IR 100 spectrophotometer as thin films. UV spectra were recorded on a Varian Cary 1 Bio UV – Visible spectrophotometer in MeOH solution. Nuclear magnetic resonance spectra (NMR) spectra were recorded using a Bruker Avance DRX 500MHz instrument. Chemical shifts are given in parts per million (ppm) and were referenced to solvent signals. High-resolution mass spectra were determined on a Agilent 6520 Q-TOF instrument, followed by deconvolution of the data using Agilent's Bioconfirmation software. Optical rotations were performed on a Rudolph Autopol IV polarimeter with the Na 589 line. HPLC was performed by Agilent 1200 HPLC system, with normal phase column Partisil 10(Whatman) and reverse phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). TLC was performed on commercially precoated plates (Whatman, aluminum backing, UV254 fluorescence, silica gel coating, 250 μm layer, CAT NO: 4420 222). Column chromatography was carried out on Silica Gel 60 [particle size: 0.040-0.063 mm, 230-400 mesh ASTM] (EMD) and Diaion HP-20 (Supelco/1-3607/176255E). Visualization was by examination under visible light, UV (254 nm), and UV (365 nm) or using iodine or by spraying with 10% sulfuric acid/vanillin reagent followed by heating.

7.4.2 Extraction of the plant material

The stems of *I. indica* (882.2 g) were ground and then they were extracted with hexanes, ethyl acetate and n-Butanol (n-BuOH) in sequence by percolating the plant material in the solvent at room temperature overnight, three times for each solvent (3 × 1 L). The extracts were combined and dried to yield hexanes extract (5.4 g), ethyl acetate extract (6.3 g) and n-BuOH extract (5.9 g). Each extract was separated further by chromatography.

7.4.3 Extraction and Isolation of compound 42, 143, 144, 146 and 147

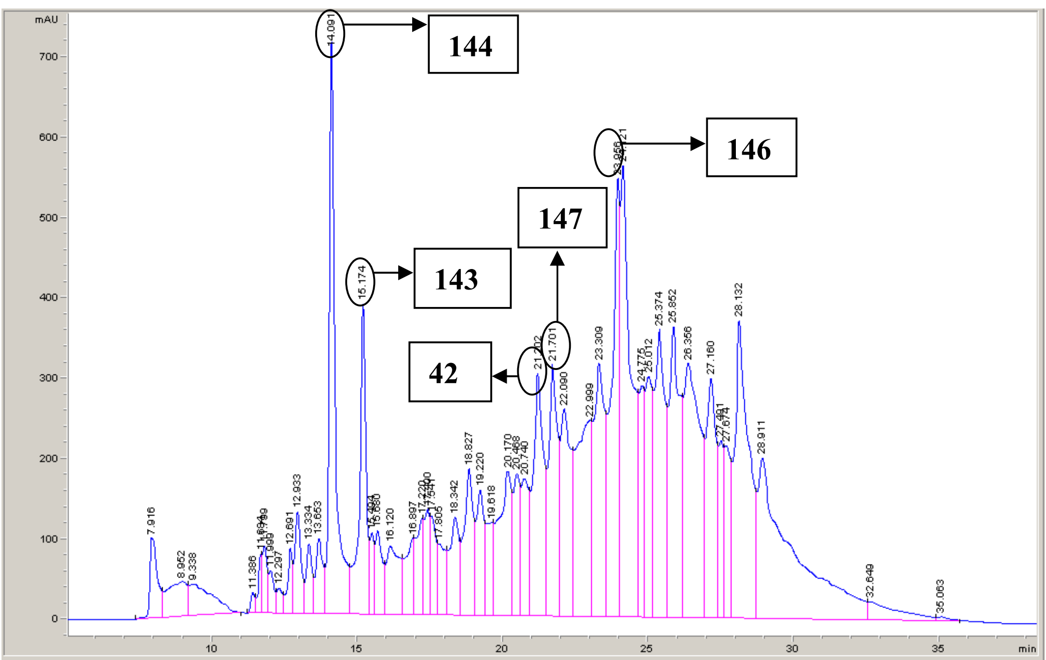


Fig. 53 HPLC chromatogram for *I. indica* n-BuOH extract

I. indica n-BuOH extract was chromatographed by HPLC with a reverse phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). Flow rate: 5.00 mL/min; Injection: 100μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 100% MeOH/Water (with 0.1% TFA) (from 0 to 30 min), 100% MeOH (from 30 to 45min). Five fractions were collected. Compounds **143** (2.0 mg), **144** (1.7 mg), **42** (1.0 mg), **146** (1.2 mg), **147** (0.9 mg) were obtained by repeated HPLC of each of these five fractions.

The isolation scheme for compound **42, 143, 144, 146 and 147** is as follows:

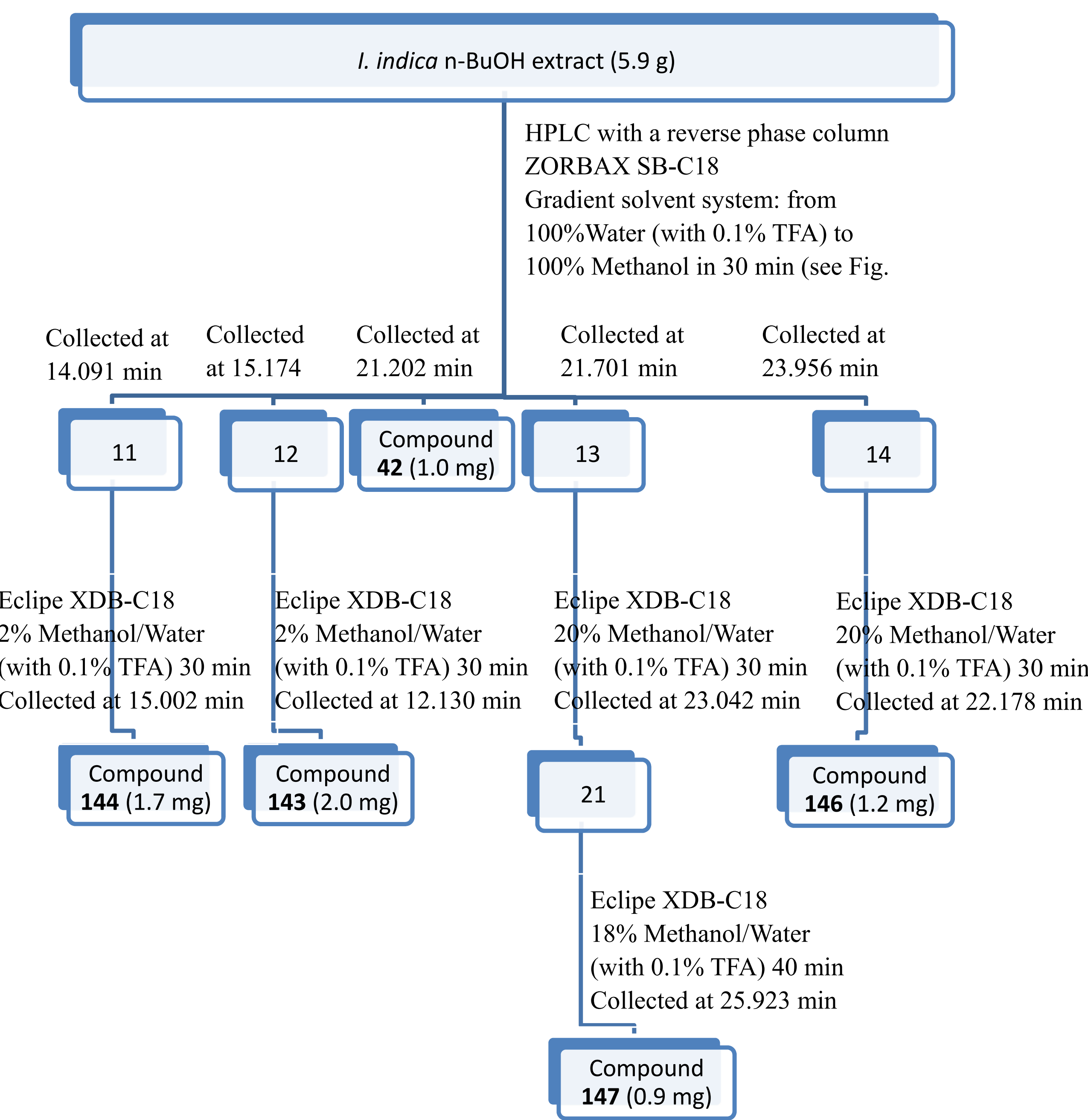


Fig. 54 The isolation scheme for compound **42, 143, 144, 146 and 147**

Compound **143** (inosine) [167, 168] (2.0 mg): UV (MeOH) λ_{max} (log ε) 259 (3.32) nm; IR (film) ν_{max} 3416, 1641, 1054, 1033, 1013, 569 cm^{-1}; ^{1}H NMR (MeOD, 500MHz) δ 3.65 (1 H, dd, 12.4, 2.8 Hz, H 5'), 3.78 (1 H, dd, 12.4, 2.6 Hz, H 5'), 4.07 (1 H, dd, 5.6, 2.8 Hz, H 4'), 4.23 (1 H, dd, 4.9 Hz, 3.0 Hz, H 3'), 4.59 (1 H, t, 5.7 Hz, H 2'), 5.92 (1 H, d, 6.0 Hz, H 1'),8.17 (1 H, s, H 5), 8.35 (1 H, s, H 8). The ^{13}C NMR (MeOD, 125MHz) δ 60.2, 69.4, 72.9, 85.0, 88.0, 117.9, 139.8, 146.9, 147.6, 159.6. HRESIMS $[M]^+$ *m/z*: 268.1042, (calculated for $C_{10}H_{12}N_4O_5$ 268.0808).

Compound **144** (cytidine) [169] (1.7 mg): UV (MeOH) λ_{max} (log ε) 258 (3.10) nm; IR (film) ν_{max} 3423, 2089, 1645, 550, 435, 422 cm^{-1}; ^{1}H NMR (MeOD, 500MHz) δ 3.75 (1 H, dd, 12.2, 3.1 Hz, H 5'), 3.86 (1 H, dd, 12.2, 2.7 Hz, H 5'), 4.02 (1 H, m, H 4'), 4.16 (1 H, m, 3.0 Hz, H 3'), 4.19 (1 H, m, H 2'), 5.91 (1 H, d, 4.6 Hz, H 1'),5.71 (1 H, d,8.1 Hz, H 5), 8.00 (1 H, d, 8.1 Hz, H 6). The ^{13}C NMR (MeOD, 125MHz) δ 62.3, 71.3, 75.7, 86.4, 90.7, 102.6, 142.7, 152.5, 166.2. HRESIMS $[M]^+$ *m/z*: 243.0632, (calculated for $C_9H_{13}N_3O_5$ 243.0855).

Compound **42** (uracil) [105] (1.0 mg): UV (MeOH) λ_{max} (log ε) 252 (3.78) nm; IR (film) ν_{max} 3098, 1644, 1201, 521, 505, 496, 489, 480, 458 cm^{-1}; ^{1}H NMR (DMSO, 500 MHz) δ 7.41 (1 H, t, J = 5.8 Hz), 5.44 (1 H, d, J = 7.6 Hz); ^{13}C NMR (DMSO, 125 MHz) δ 164.8, 152.0, 142.7,

100.7; HRESIMS $[M]^+$ *m/z*: 112.0596, (calculated for $C_4H_4N_2O_2$ 112.0273).

Compound **146 (**methyl 2,4-dihydroxybenzoate) [171] (1.2 mg): UV (MeOH) λ_{max} (log ε) 256 (4.05) nm; IR (film) ν_{max} 3474, 2981, 2865, 1641, 1346, 1055, 1033, 1014, 573 cm^{-1}; ^{1}H NMR (MeOD, 500 MHz) δ 7.45 (2 H, m), 6.73 (1 H, d, J = 8.7 Hz), 3.80 (3 H, s); ^{13}C NMR (MeOD, 125 MHz) δ 170.5, 160.9, 160.6, 131.7, 123.0, 113.6, 111.5, 54.1; HRESIMS $[M]^+$ *m/z*: 168.0030, (calculated for $C_8H_8O_4$ 168.0423).

Compound **147 (**2',3',4',5,7-pentahydroxyflavone) [172] (0.9 mg): UV (MeOH) λ_{max} (log ε) 260 (4.21) nm; IR (film) ν_{max} 3423, 2981, 2865, 1643, 1454, 1346, 1055, 1033, 1014, 573, 423 cm^{-1}; ^{1}H NMR (MeOD, 500 MHz) δ 7.51 (1 H, m), 6.97 (1 H, s), 6.86 (1 H, d, J = 7.9 Hz), 6.67 (1 H, d, J = 8.2 Hz), 6.29 (1 H, m); ^{13}C NMR (MeOD, 125 MHz) δ 181.6, 165.0, 164.5, 161.8, 160.1, 147.0, 146.9, 135.0, 123.0, 118.6, 115.1, 112.7, 102.8, 98.9, 93.9; HRESIMS $[M]^+$ *m/z*: 302.0646, (calculated for $C_{15}H_{10}O_7$ 302.0427).

7.4.4 Extraction and Isolation of compound 115 and 145

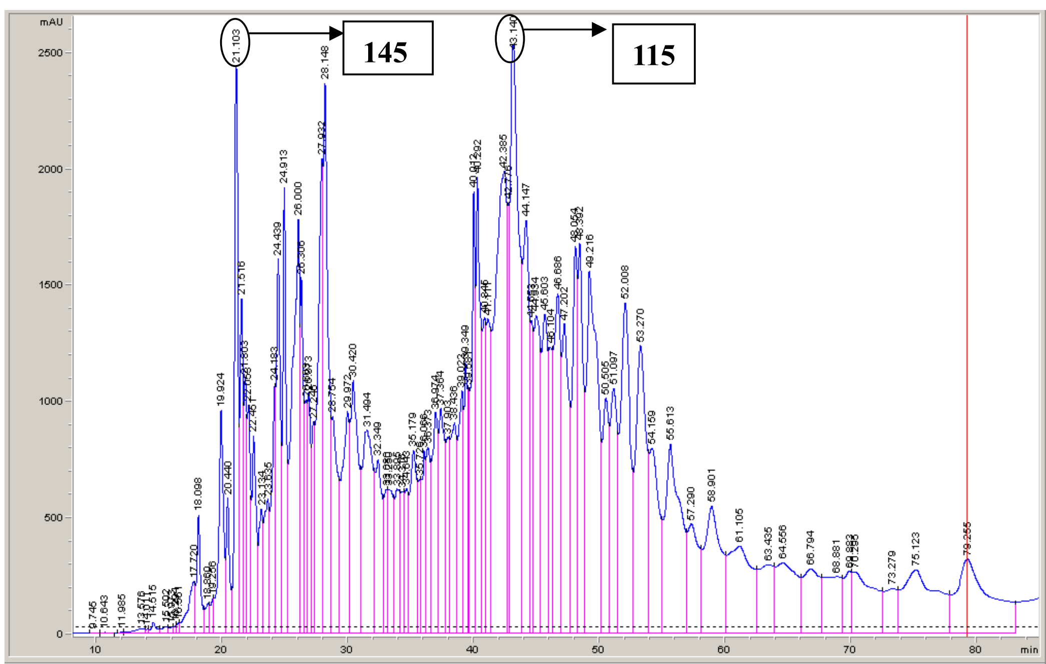


Fig. 55 HPLC chromatogram for *I. indica* ethyl acetate extract

I. indica ethyl acetate extract was chromatographed by HPLC with a reverse phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). Flow rate: 5.00 mL/min; Injection: 100μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 100% MeOH/Water (with 0.1% TFA) (from 0 to 30 min), 100% MeOH (from 30 to 85 min). Compound **115** (1.6 mg) was collected at 43.140 min. Compound **145** (1.1 mg) was collected at 21.103 min.

Compound **115** (linoleic acid) [122] (1.6 mg): UV (MeOH) λ_{max} (log ε) 210 (1.04) nm; IR (film) ν_{max} 3423, 2837, 2041, 1652, 1450, 1410, 1112, 1024, 658 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.38 (4 H, m), 2.76 (2 H, m), 2.33 (2 H, m), 2.05 (2 H, m), 1.62 (4 H, m), 1.27 (12 H, m), 0.91 (6 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 178.7, 130.2, 130.0, 128.1, 127.9, 33.8, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 24.7, 22.7, 14.1. HRESIMS $[M]^+$ *m/z*: 280.2625, (calculated for $C_{18}H_{32}O_2$ 280.2402).

Compound **145** (1,2,4-benzenetriol) [170] (1.1 mg): UV (MeOH) λ_{max} (log ε) 254 (3.92) nm; IR (film) ν_{max} 3566, 2084, 1054, 1033, 1014, 428 cm^{-1}; ^{1}H NMR (MeOD, 500 MHz) δ 7.02 (2 H, m), 6.40 (1 H, d, J = 7.9 Hz); ^{13}C NMR (MeOD, 125 MHz) δ 150.2, 145.7, 137.5, 113.9, 107.7, 105.8; HRESIMS $[M]^+$ *m/z*: 126.0761, (calculated for $C_6H_6O_3$ 126.0317).

7.4.5 Extraction and Isolation of compound 41

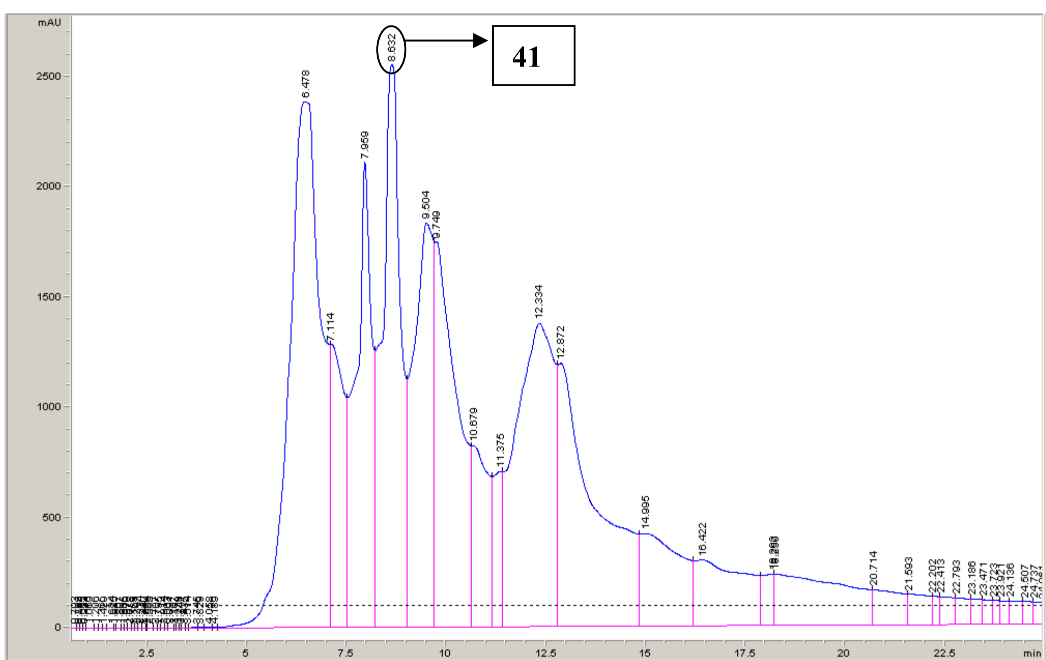


Fig. 56 HPLC chromatogram for *I. indica* hexanes extract

I. indica hexanes extract was chromatographed by HPLC with a reverse phase column ZORBAX SB-C18, 21.2*150 mm, 7μm (Agilent). Flow rate: 5.00 mL/min; Injection: 100μL; UV detector wavelength: 254 nm; Solvent system: from 0% to 100% MeOH/Water (with 0.1% TFA) (from 0 to 30 min), 100% MeOH (from 30 to 60 min). Compound **41** (1.3 mg) was collected at 8.632 min.

Compound **41** (β-Sitosterol) [126] (1.3 mg): UV (MeOH) λ_{max} (log ε) 190 (1.52) nm; IR (film) ν_{max} 3417, 515, 439, 421, 402 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.38 (1 H, m), 3.55 (1 H, m), 2.31 (1 H, m), 2.26 (1 H, m), 2.02 (1 H, m), 1.97 (1 H, m), 1.88 (1 H, m), 1.86 (1 H, m), 1.85 (1 H, m), 1.65 (1 H, m), 1.63 (1 H, m), 1.54 (1 H, m), 1.52 (1 H, m), 1.51 (1 H, m), 1.49 (1 H, m), 1.46 (1 H, m), 1.36 (1 H, m), 1.32 (1 H, m), 1.27 (2 H, m), 1.26 (1 H, m), 1.20 (1 H, m), 1.18 (1 H, m), 1.16 (1 H, m), 1.10 (1 H, m), 1.08 (1 H, m), 1.05 (1 H, m), 1.04 (1 H, m), 1.03 (3 H, s), 1.02 (1 H, m), 0.97 (1 H, m), 0.95 (3 H, m), 0.93 (1 H, m), 0.90 (1 H, m), 0.86 (3 H, m), 0.84 (3 H, m), 0.82 (3 H, s), 0.70 (3 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.7, 121.7, 71.8, 56.7, 56.0, 50.1, 45.8, 42.3, 42.2, 39.8, 37.2, 36.5, 36.2, 33.9, 31.92, 31.90, 31.6, 29.1, 28.3, 26.0, 24.3, 23.1, 21.1, 19.8, 19.4, 19.0, 18.8, 12.1, 11.9. HRESIMS $[M]^+$ *m/z*: 414.3869, (calculated for $C_{29}H_{50}O$ 414.3862).

Appendix I.

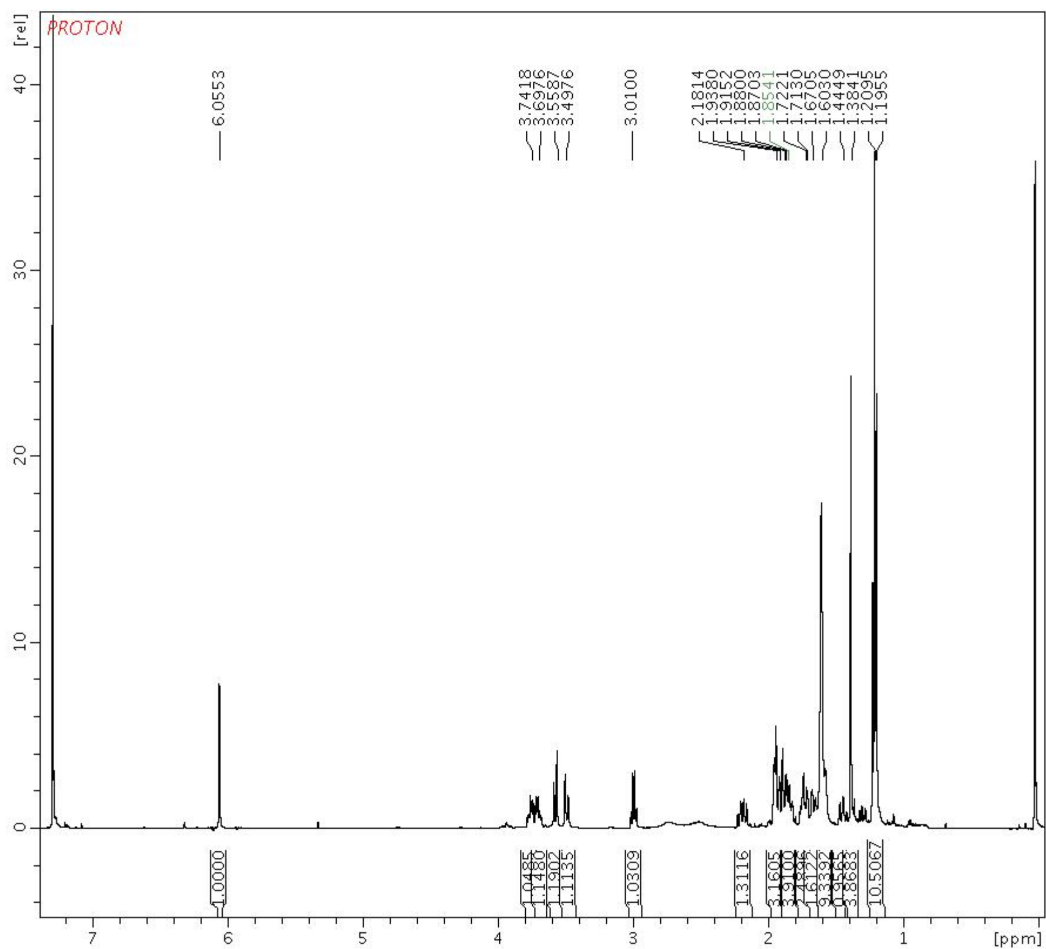
Compounds isolated from each plant

Plant	Compound
L. leonurus	34, 35, 36, 37, 38, 39, 40, 41, 42
S. capensis	115, 41, 116, 117, 118

	119 120 121 122 123
I. indica	143 144 115 41 42 145 146 147

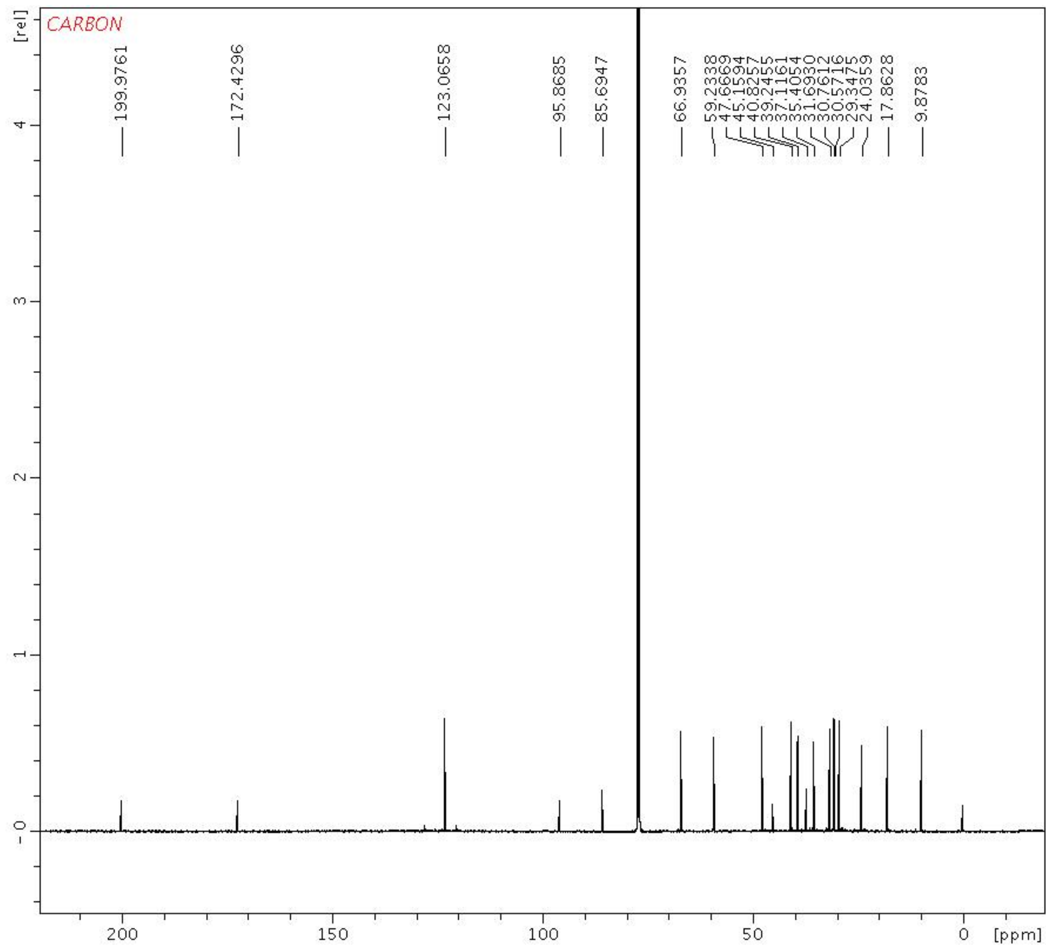
Appendix II.

^{1}H NMR spectrum for Leonurenone A (**34**)



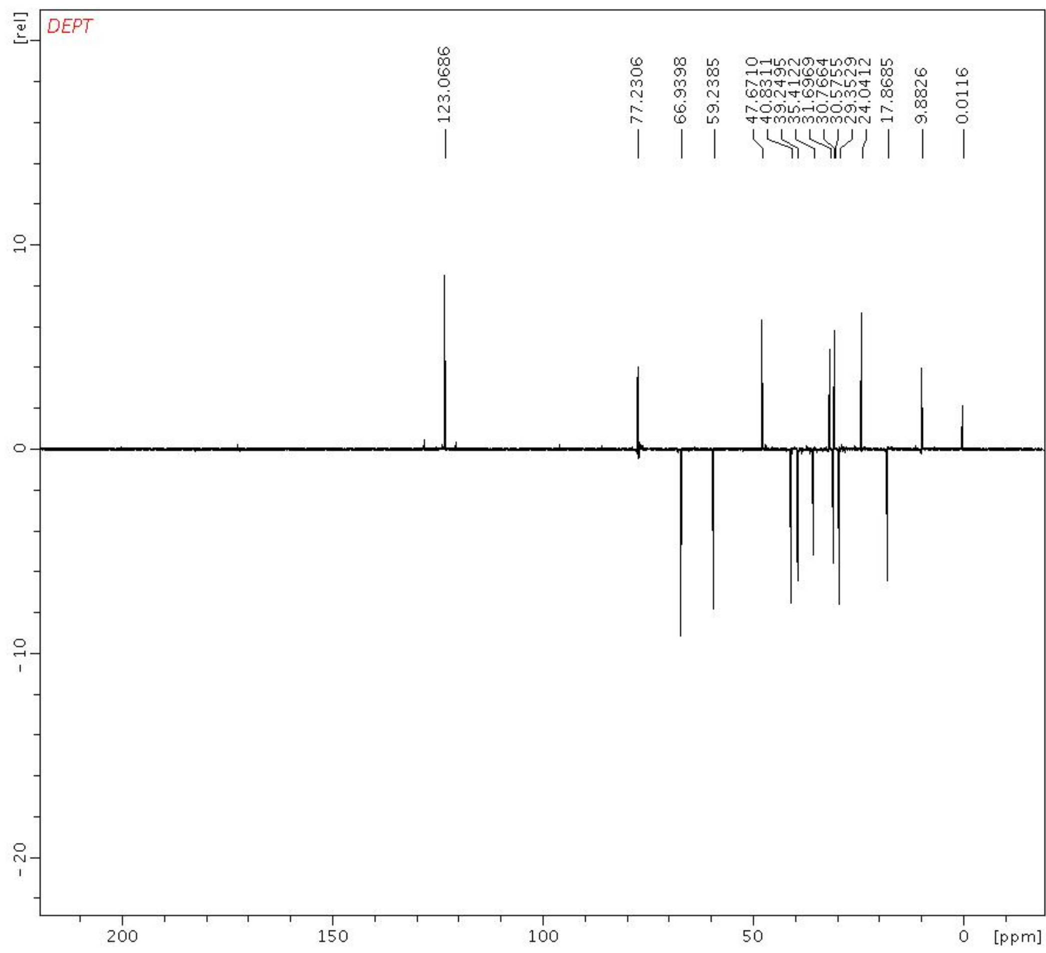
Appendix III.

^{13}C NMR spectrum for Leonurenone A (**34**)



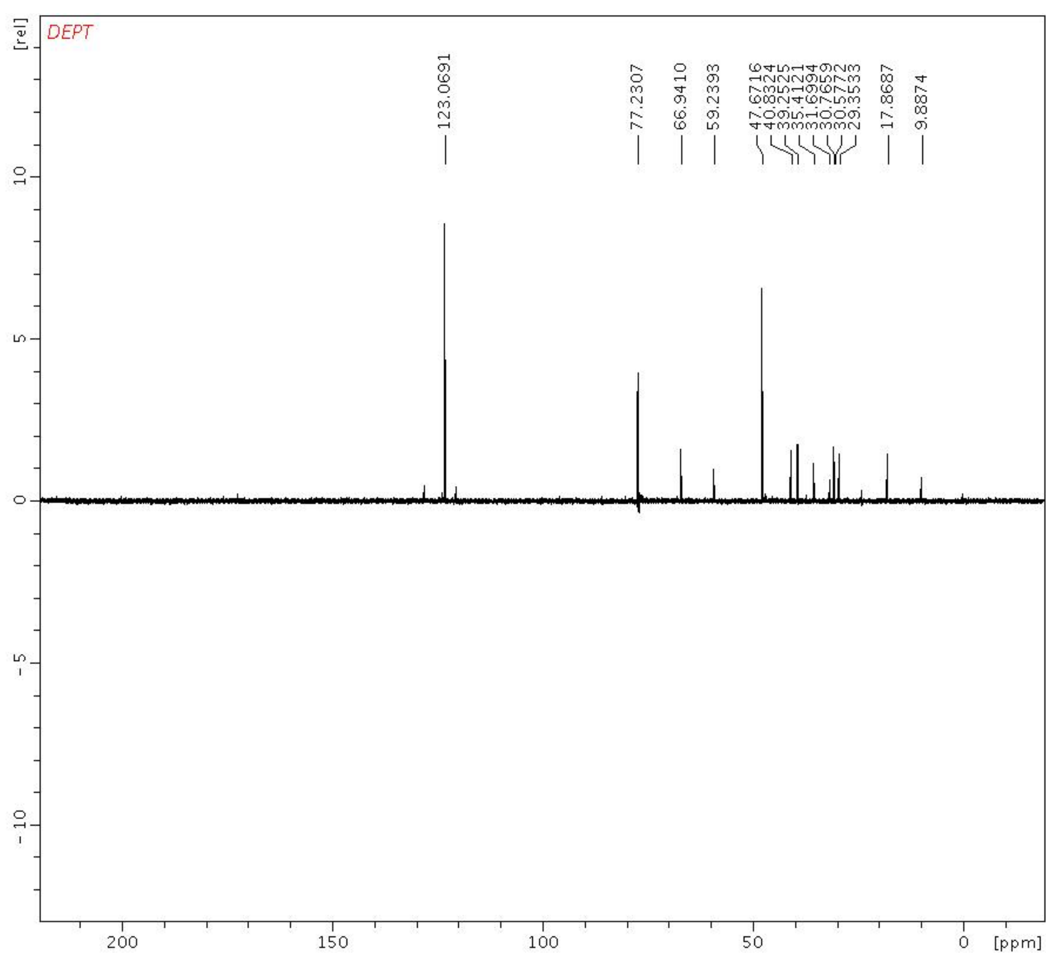
Appendix IV.

DEPT135 spectrum for Leonurenone A (**34**)



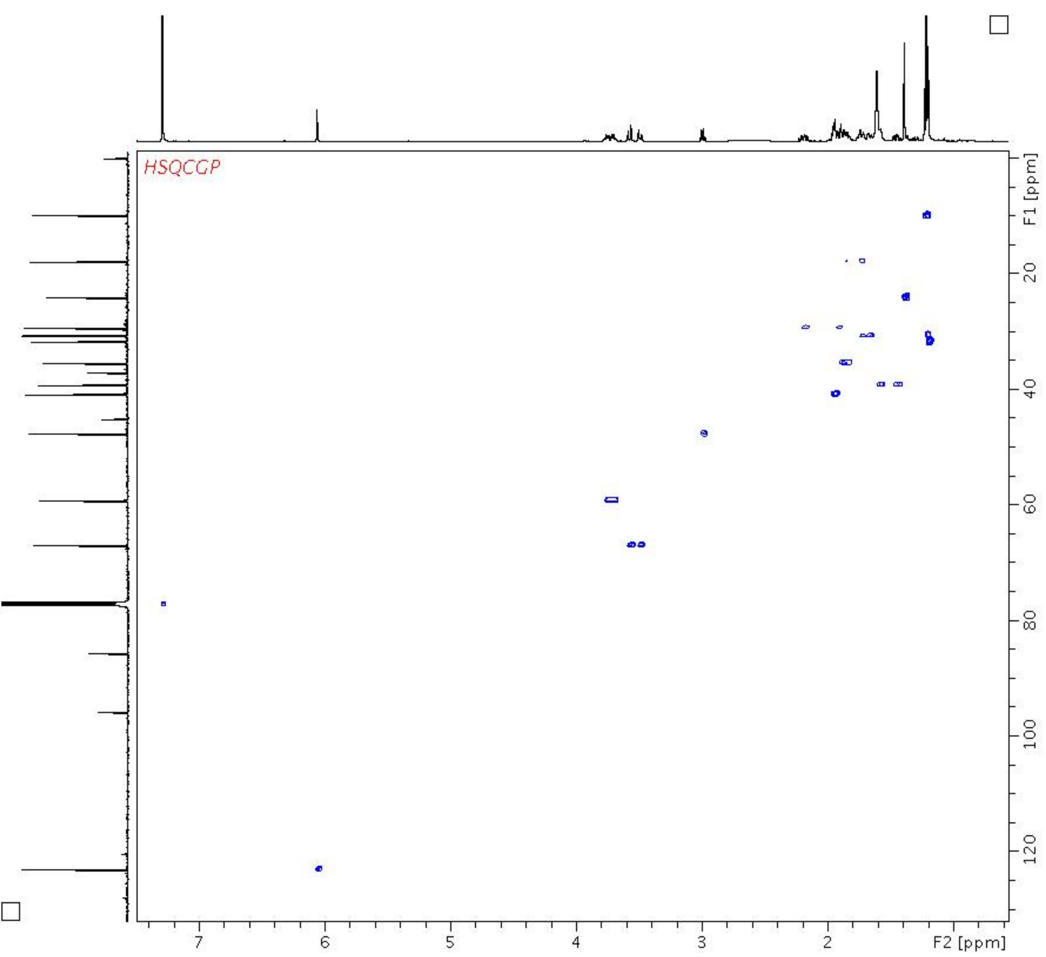
Appendix V.

DEPT90 spectrum for Leonurenone A (**34**)



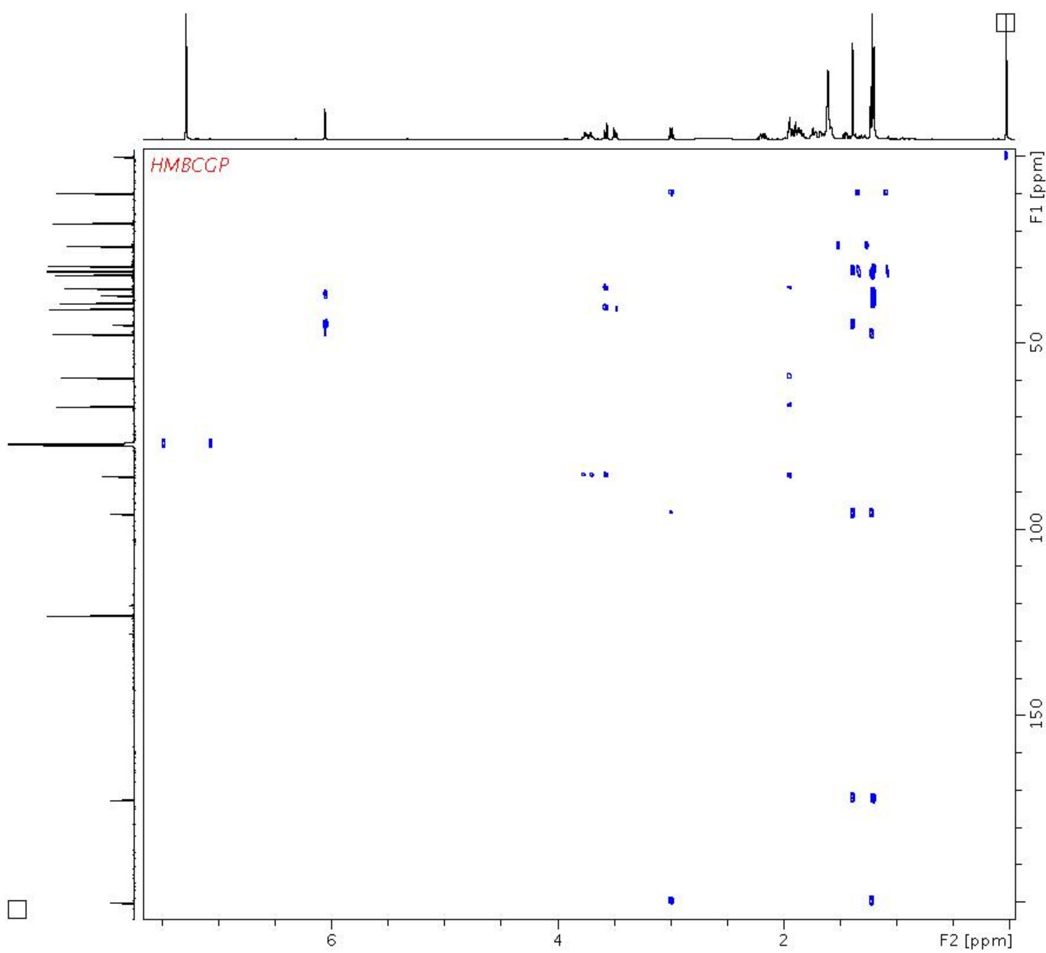
Appendix VI.

HSQC spectrum for Leonurenone A (**34**)



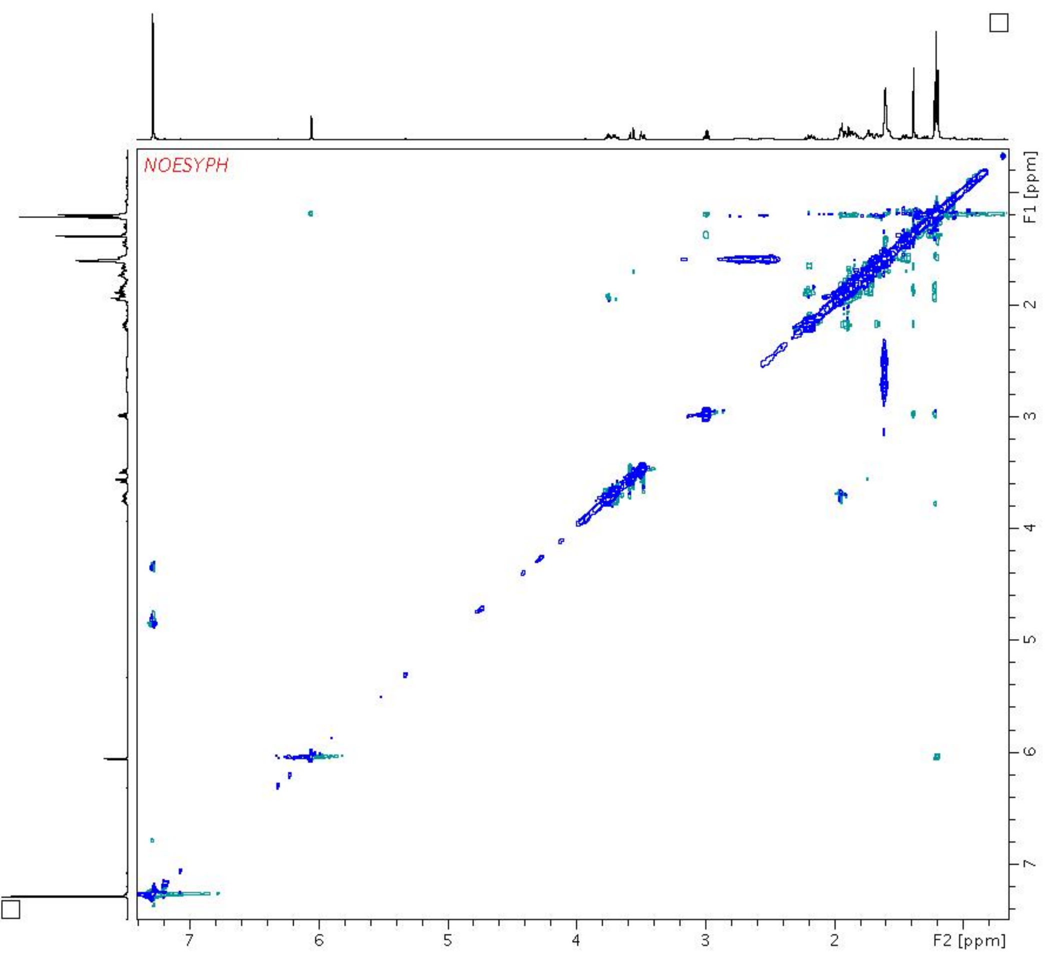
Appendix VII.

HMBC spectrum for Leonurenone A (**34**)



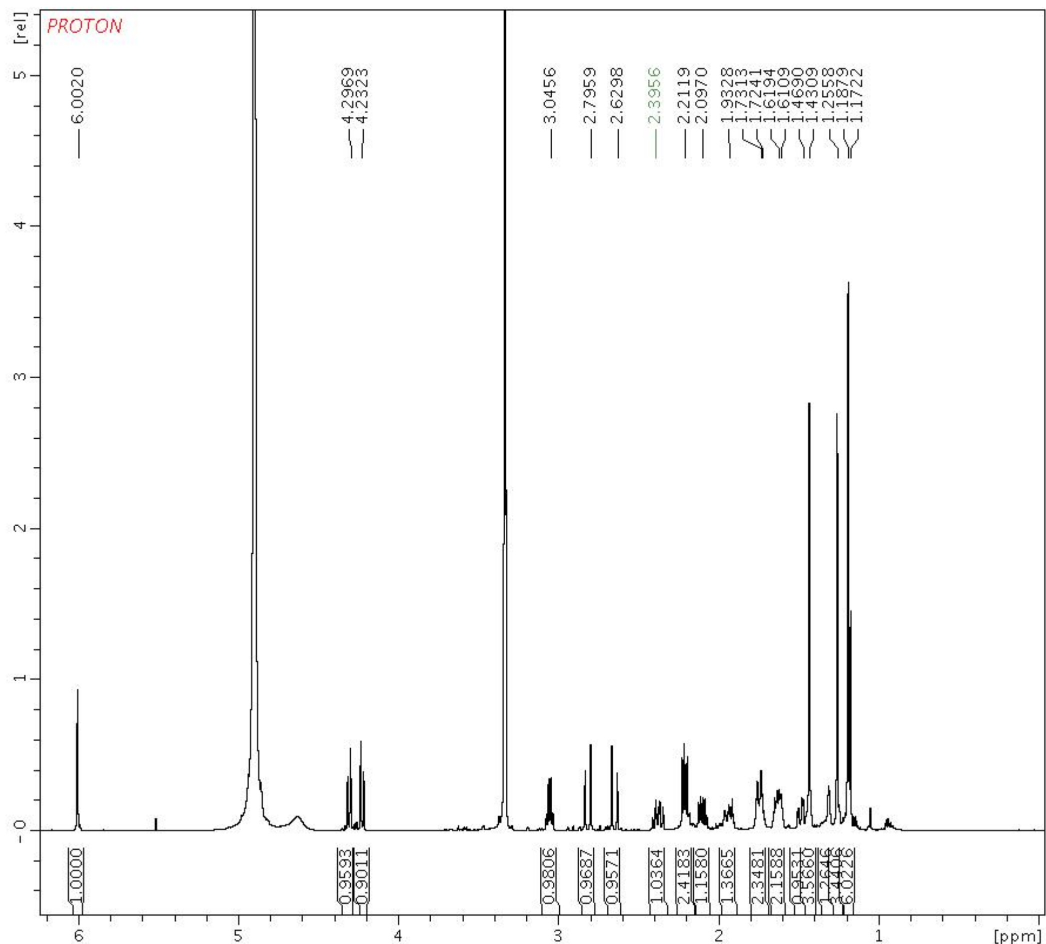
Appendix VIII.

NOESY spectrum for Leonurenone A (**34**)



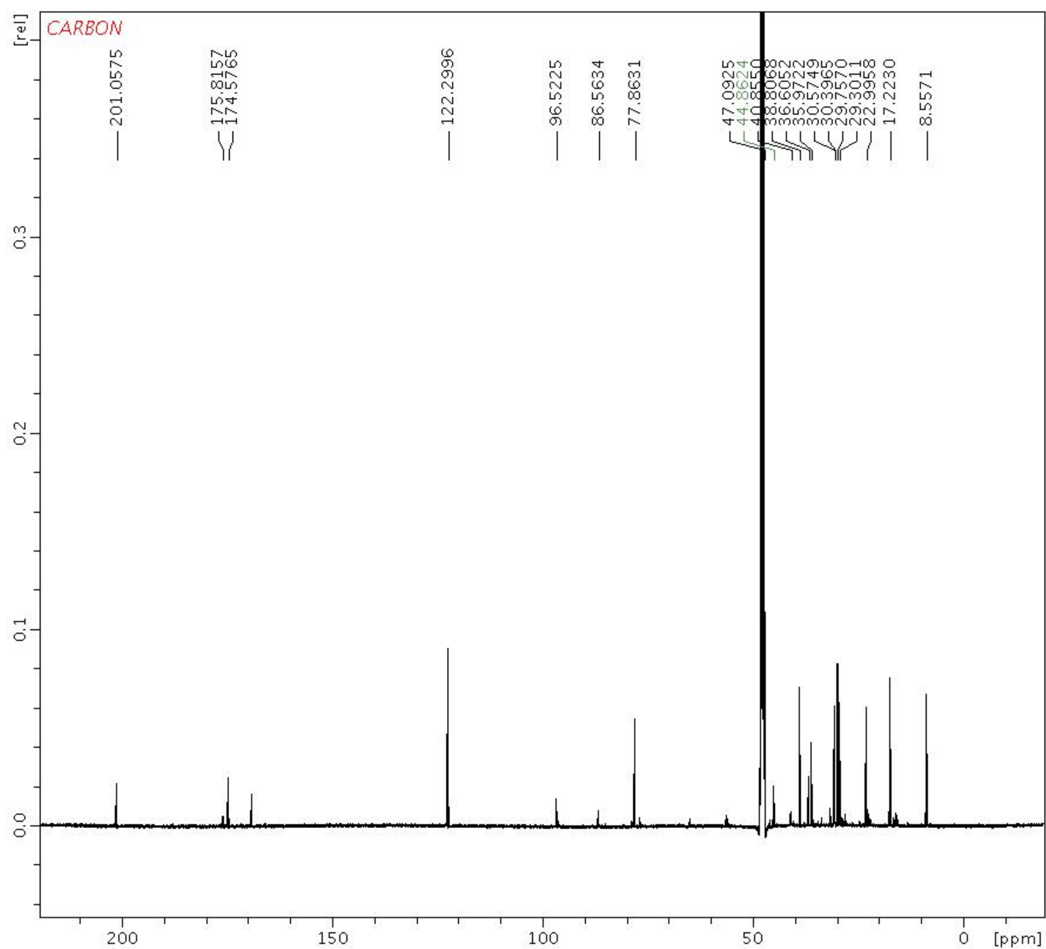
Appendix IX.

^{1}H NMR spectrum for Leonurenone B (**35**)



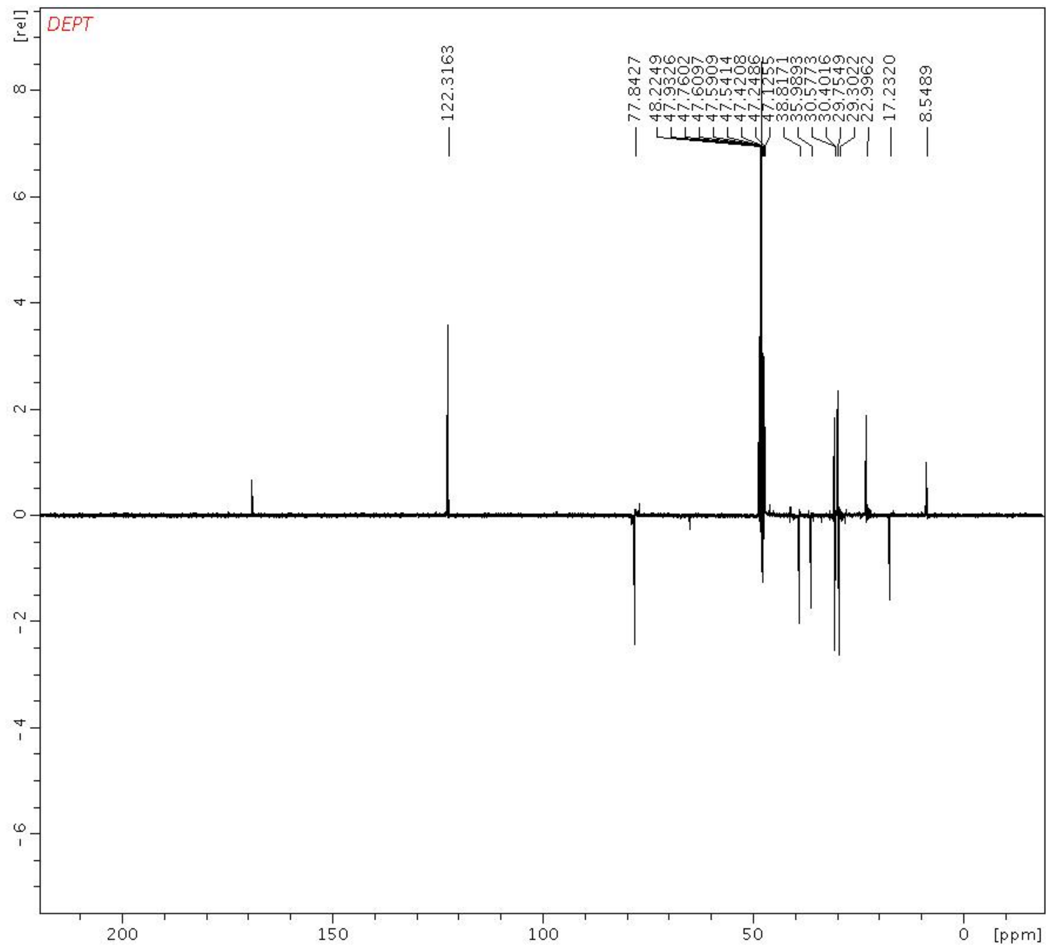
Appendix X.

^{13}C NMR spectrum for Leonurenone B (**35**)



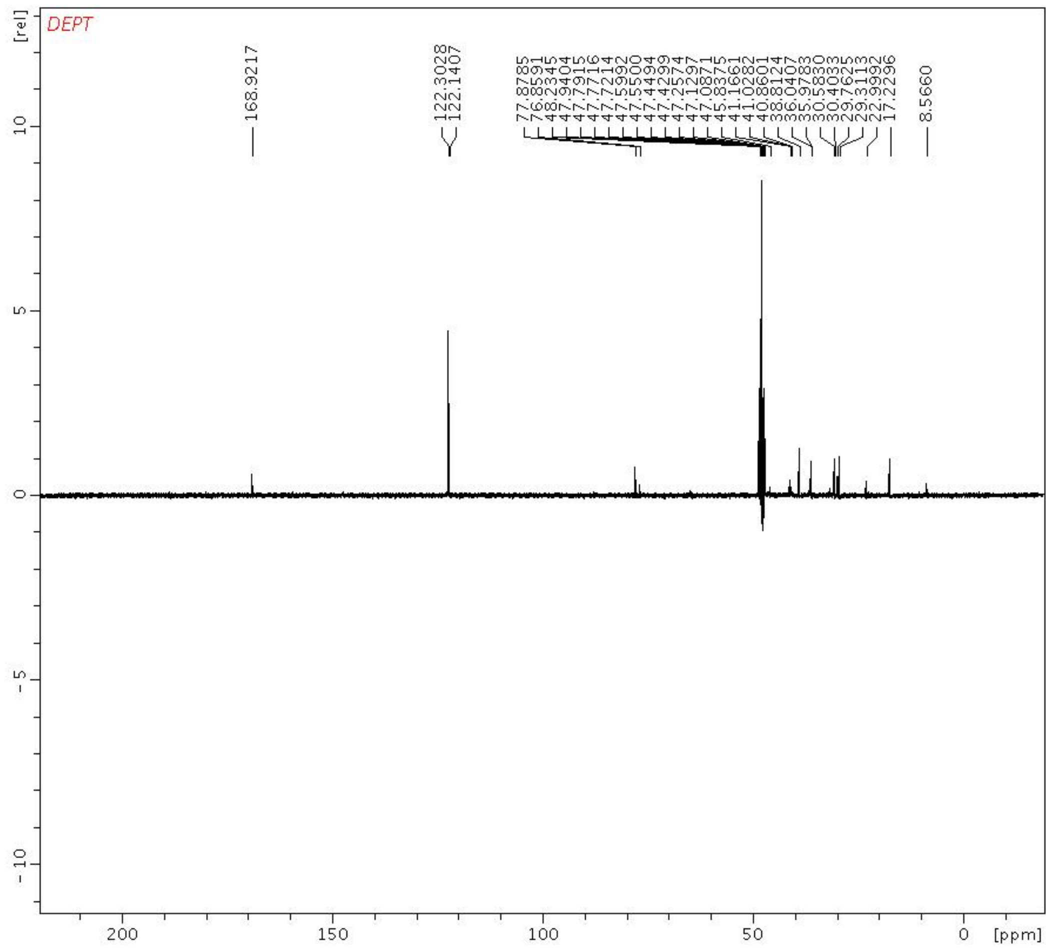
Appendix XI.

DEPT135 spectrum for Leonurenone B (**35**)



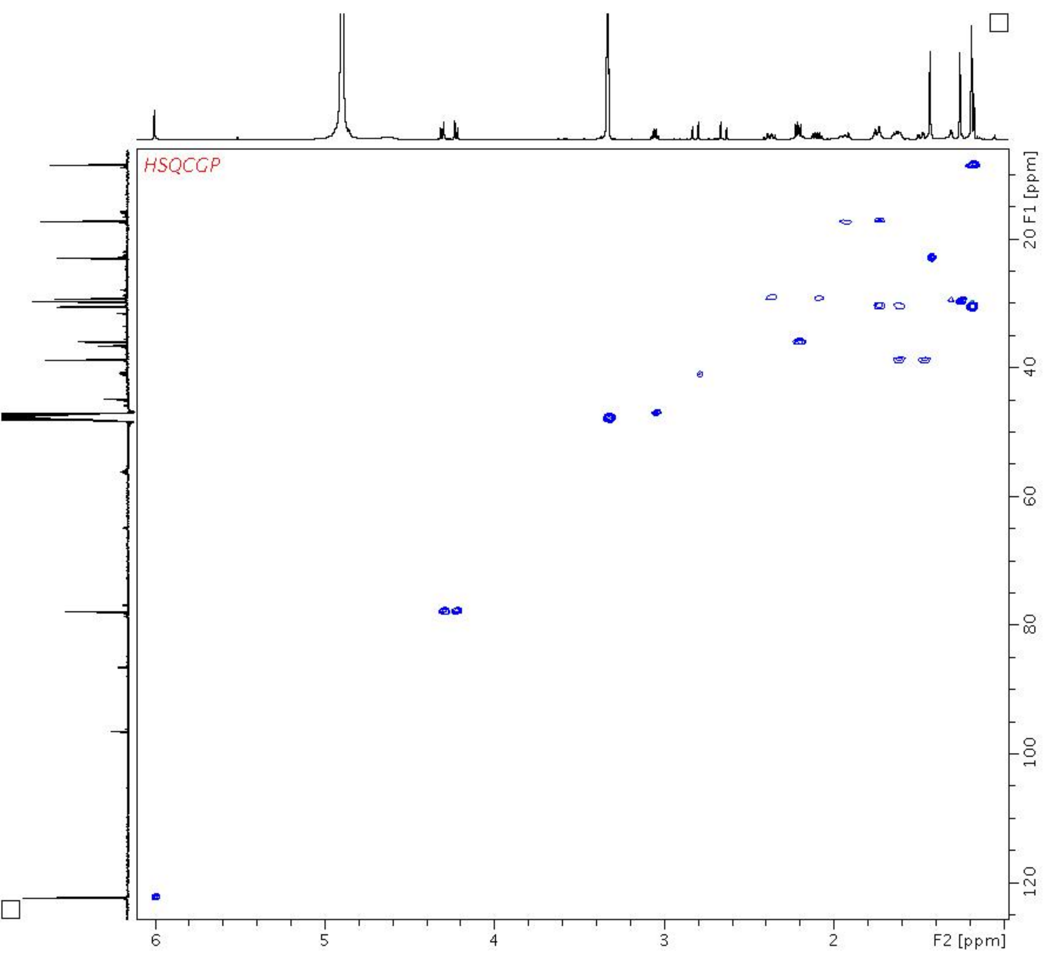
Appendix XII.

DEPT90 spectrum for Leonurenone B (**35**)



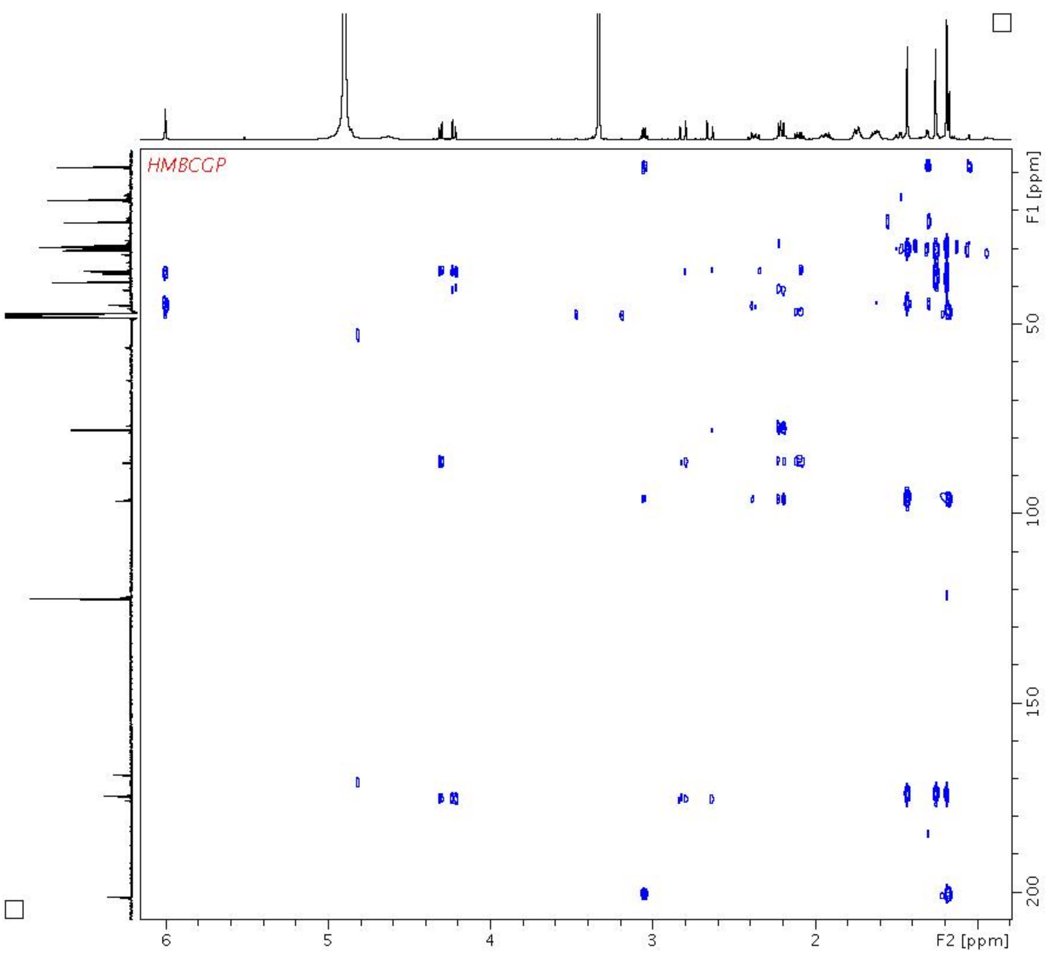
Appendix XIII.

HSQC spectrum for Leonurenone B (**35**)



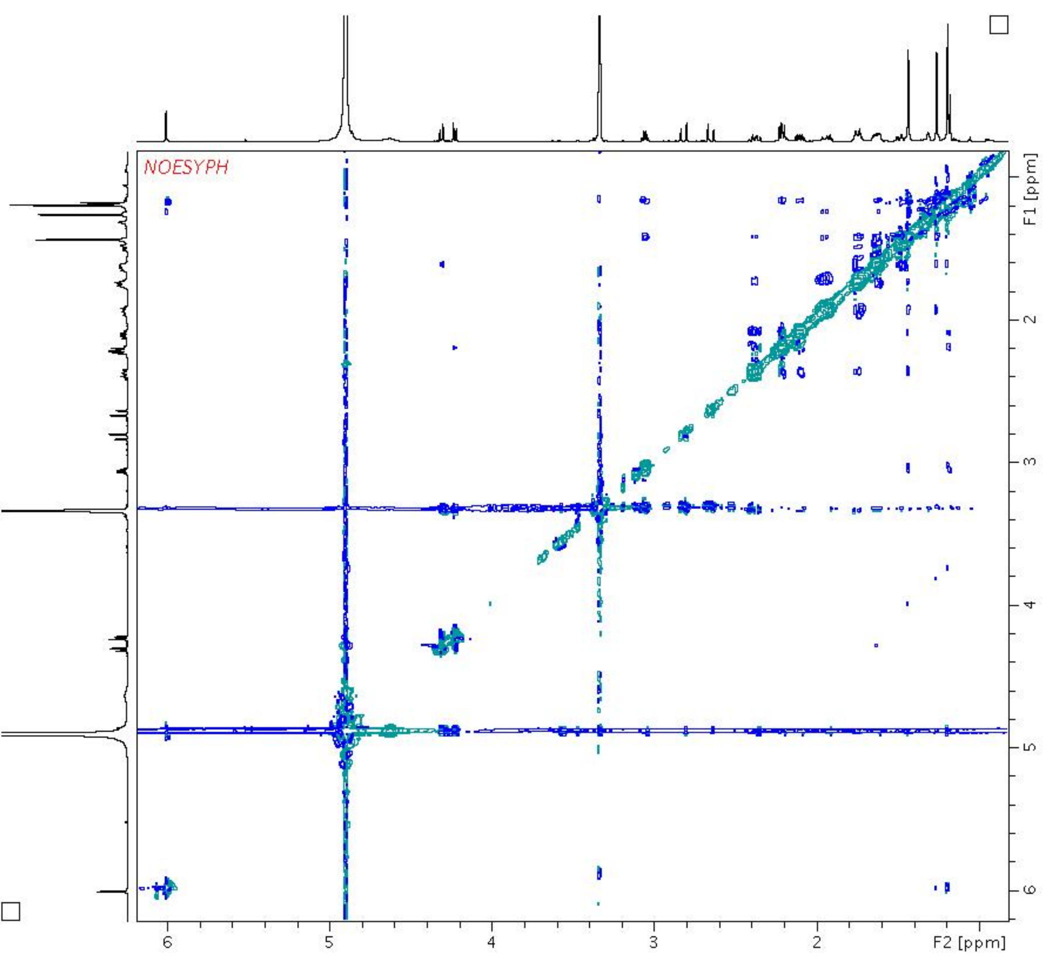
Appendix XIV.

HMBC spectrum for Leonurenone B (**35**)



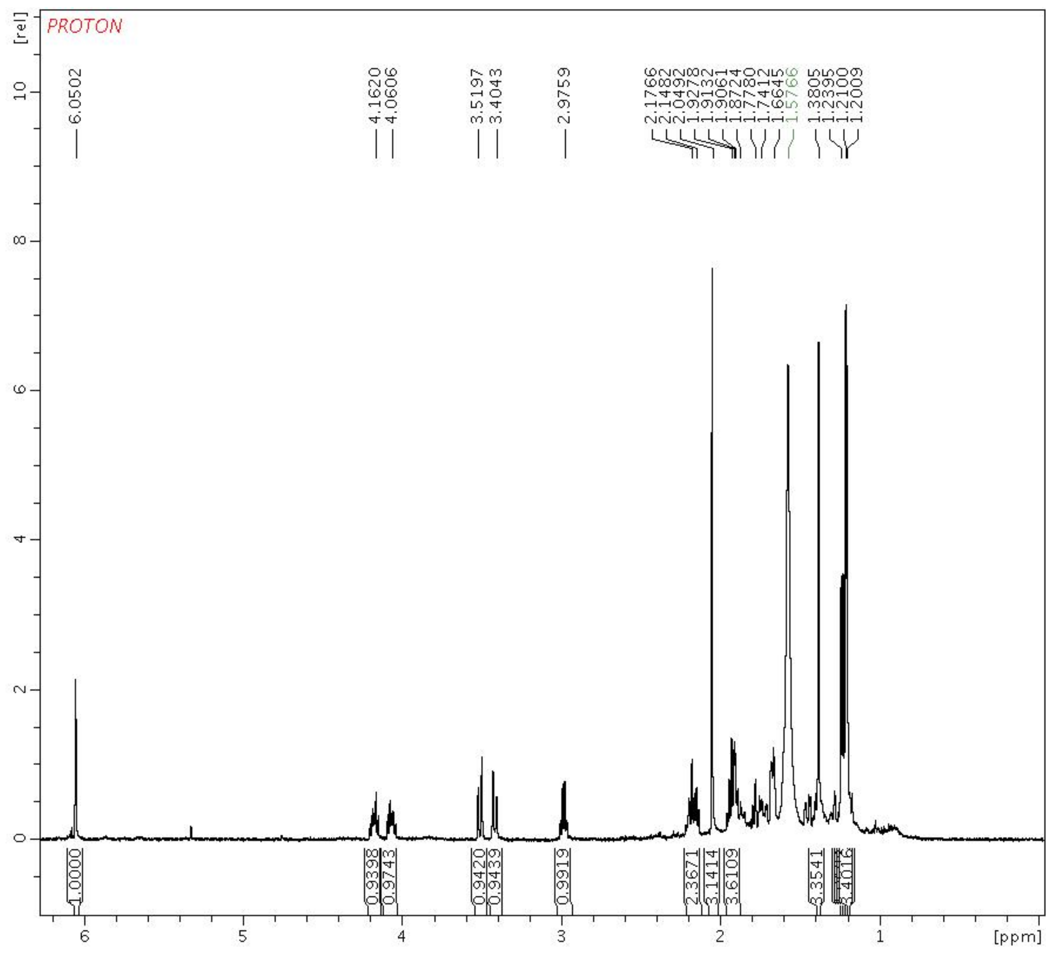
Appendix XV.

NOESY spectrum for Leonurenone B (**35**)



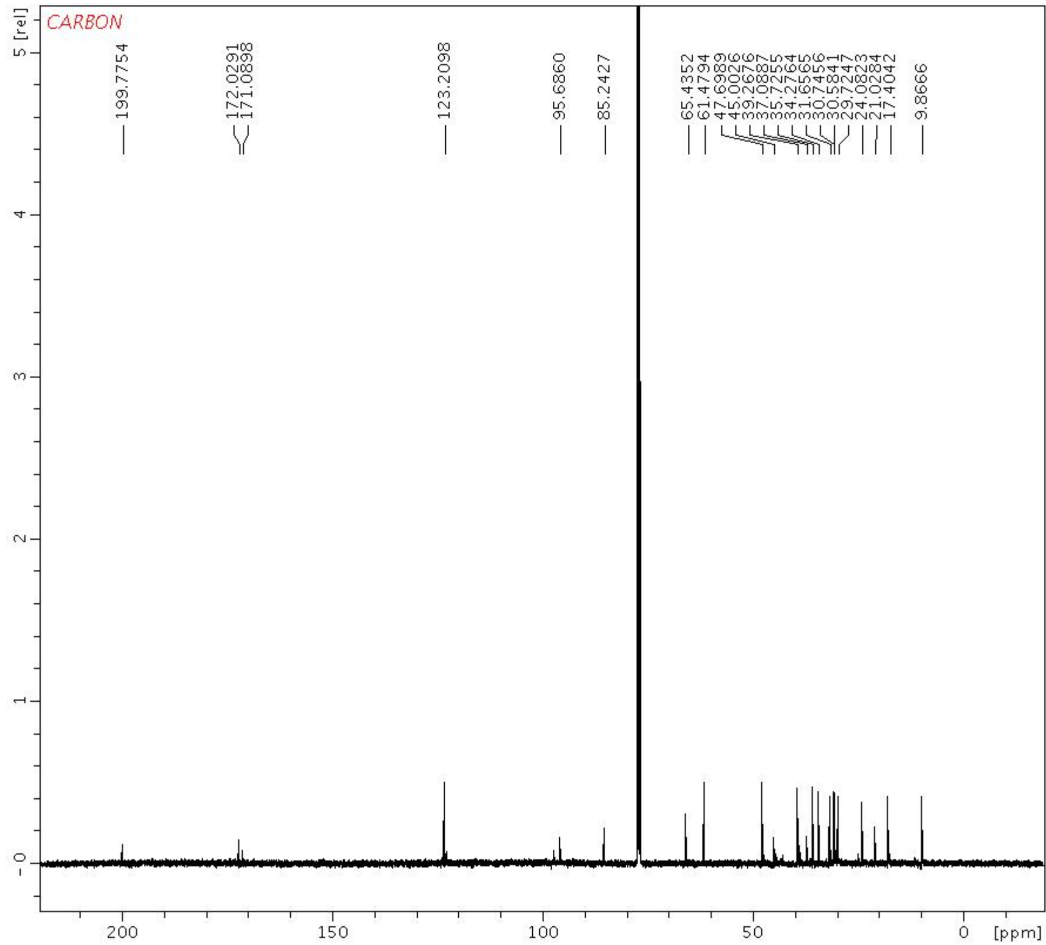
Appendix XVI.

^{1}H NMR spectrum for Leonurenone C (**36**)



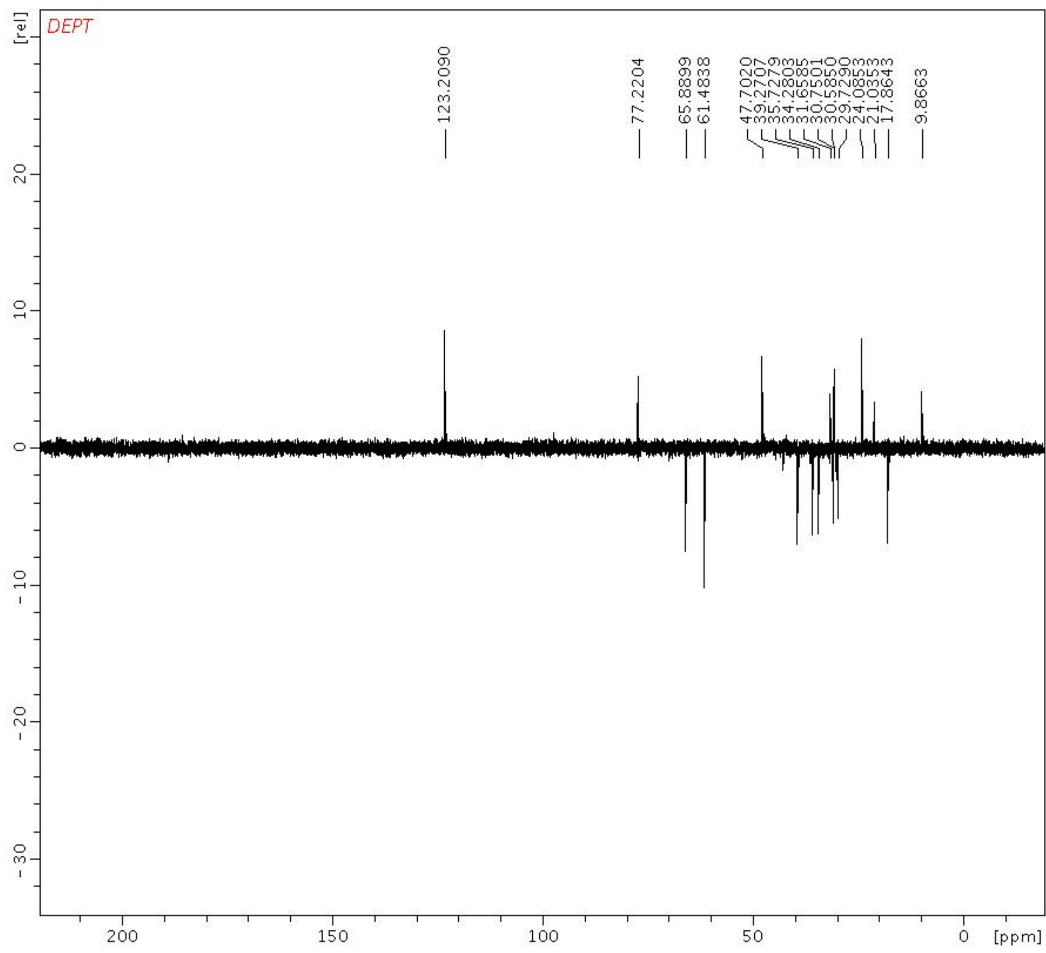
Appendix XVII.

^{13}C NMR spectrum for Leonurenone C (**36**)



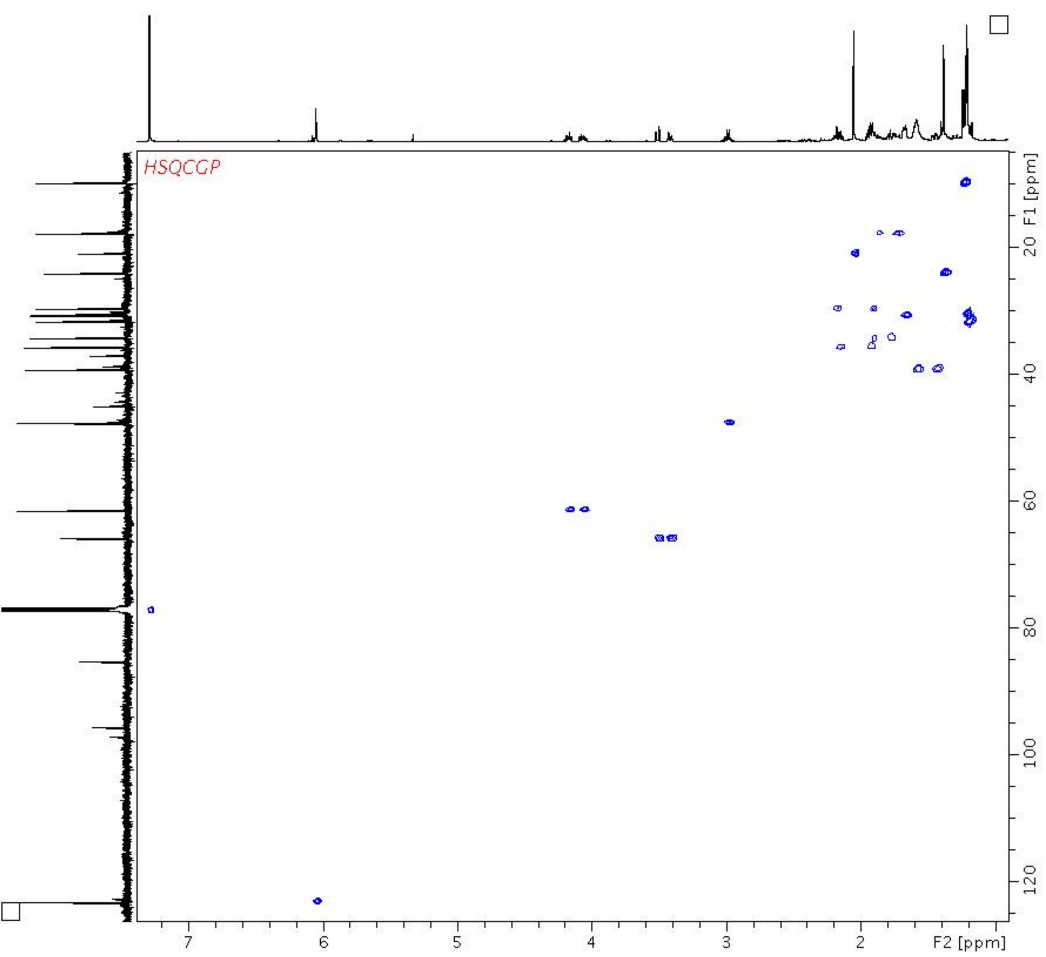
Appendix XVIII.

DEPT135 spectrum for Leonurenone C (**36**)



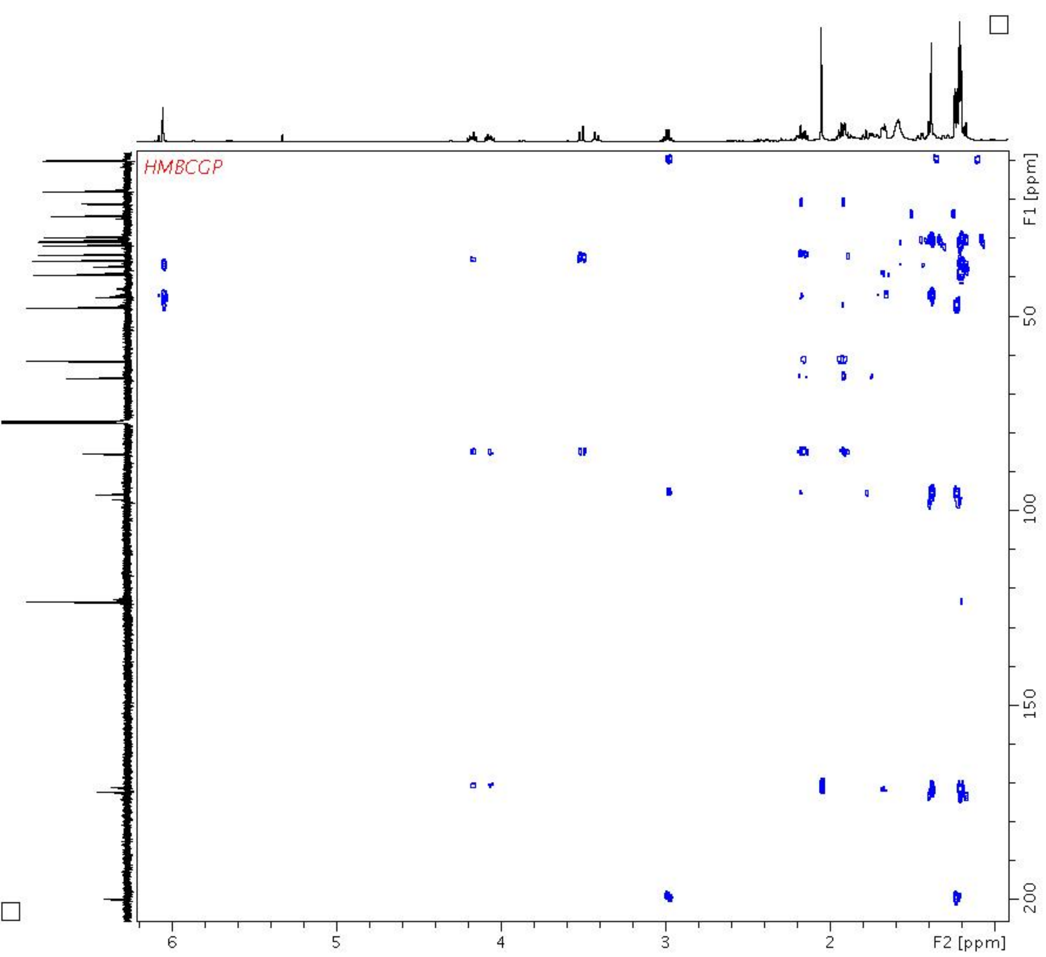
Appendix XIX.

HSQC spectrum for Leonurenone C (**36**)



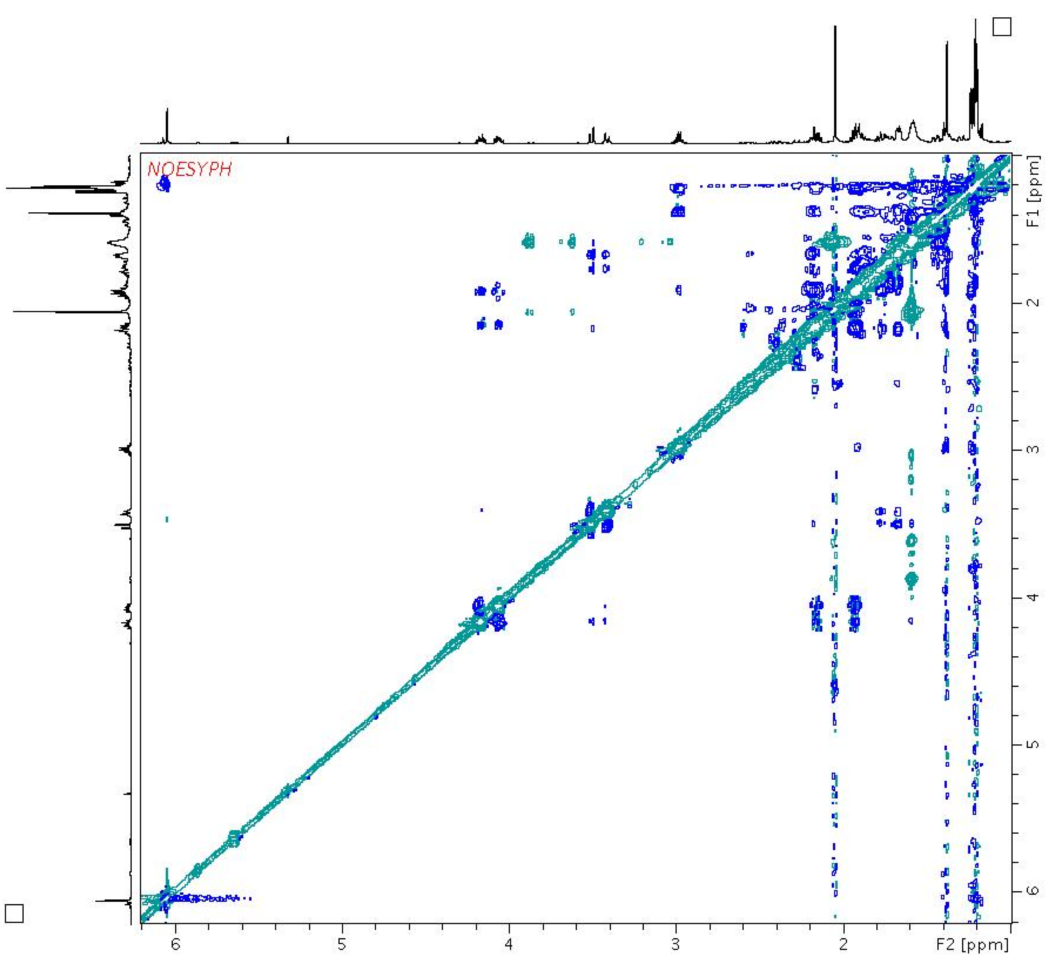
Appendix XX.

HMBC spectrum for Leonurenone C (**36**)



Appendix XXI.

NOESY spectrum for Leonurenone C (**36**)



References

(1) Hofmann, A. *Pharm. Int.*, **1982**, *3*, 216–217

(2) Ozarowski, M.; Mikolajczak, P. L.; Bobkiewicz, K. T.; Kujawski, R.; Mrozikiewicz, P. M. *Herba Polonica,* **2008**, *54,* 108-139

(3) Eisenberg, D. M.; Kessler, R. C.; Foster, C.; Norlock, F. E.; Calkins, D. R.; Delbanco, T. L. *N. Engl. J. Med.,* **1993**, *328*, 246–252

(4) Chung, L. Y.; Goh, S. H.; Imiyabir, Z. *Pharm. Biol.,* **2005**, *43*, 280–288

(5) Taber, W. A.; Vinig, L. C.; Heacock, R. A. *Phytochemistry,* **1963**, *2*, 65–70

(6) Schwarz, M. J.; Houghton, P. J.; Rose, S.; Jenner, P. *Pharmacol. Biochem. Behav.*, **2003**, *75*, 627–633

(7) Tarragó, T.; Kichik, N.; Seguí, J.; Giralt, E. *Chem. Med. Chem.*, **2007**, *2*, 354–359

(8) Tarragó, T.; Kichik, N.; Claasen, B.; Prades, R.; Teixidó, M.; Giralt, E. *Bioorg. Med. Chem.*, **2008**, *16*, 7516–7524

(9) Picq, M.; Cheav, S. L.; Prigent, A. F. *Life Sciences*, **1991**, *49*, 1979-1988

(10) Coleta, M.; Campos, M. G.; Cotrim, M. D.; Cunha, A. P. *Pharmacopsychiatry*, **2001**, *34*, 1–2

(11) Coleta, M.; Batista, M. T.; Campos, M. G.; Carvalho, R.; Cotrim, M. D.; Lima, T. C. *Phytother. Res.*, **2006**, *20*, 1067–1073

(12) Coleta, M.; Campos, M. G.; Cotrim, M. D.; Lima, T. C.; Cunha, A. P. *Behav. Brain. Res.*, **2008**, *189,*75–82

(13) Gomes, N. G.; Campos, M. G.; Órfão, J. M.; Ribeiro, C. A. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, **2009**, *33,* 1372–1389

(14) Abraham, H. D.; Aldridge, A. M.; Gogia, P. *Neuropsychopharmacology*, **1996**, *14*, 285–298

(15) Schultes, R. E.; Hofmann, A. *Plants of the gods: origins of hallucinogenic use*, EMB—Service for Publishers, Lucerne, **1979**

(16) Desmet, P. A. *J. Ethnopharmacol.*, **1996**, *50,* 141–146

(17) Dilsaver, S. C. *J. Clin. Psychopharmacol*., **1988**, *8,* 14–22

(18) Marken, P. A.; Stoner, S. C.; Bunker, M. T. *CNS Drugs*, **1996**, *5*, 190–199

(19) Müller, J. L. *Clin. Toxicol*., **1998**, *36*, 617–627

(20) Mayagoitia, L.; Diaz, J. L.; Contreras, C. M. *Journal of ethnopharmacology*, **1986**, *18*, 229-243

(21) Niu, G.; Starman, T.; Byrne, D. *HortScience*, **2013**, *48*, 756-761

(22) Umezu, T. *Phytotherapy Research*, **2012**, *26*, 884-891

(23) Imanshahidi, M.; Hosseinzadeh, H. *Phytotherapy Research*, **2006**, *20*, 427-437

(24) Andalib, S.; Vaseghi, A.; Vaseghi, G.; Naeini, A. M. *EXCLI. Journal*, **2011**, *10*, 192–197

(25) Nau, S. D.; Mccrae, C. S.; Cook, K. G.; Lichstein, K. L. *Clin. Psychol. Rev.*, **2005**, *25*, 645–672

(26) Roth, T. *J. Clin. Psychiatry.*, **2001**, *62*, 3–4

(27) Kamel, N. S.; Gammack, J. K. *Am. J. Med.*, **2006**, *119*, 463–469

(28) Benca, R. M. *Psychiatr. Serv.*, **2005**, *56*, 332–343

(29) Gottesmann, C. *Neuroscience*, **2002**, *111*, 231–239

(30) Wheatley, D. *J. Psychopharmacol.*, **2005**, *19*, 414–421

(31) Marder, M.; Viola, H.; Wasowski, C.; Fernández, S.; Medina, J. H.; Paladini, A. C. *Pharmacol. Biochem. Behav.*, **2003**, *75*, 537–545

(32) Garg, A.; Garg, S.; Zaneveld, L. J. D.; Sinlge, A. K. *Phytother. Res.*, **2001**, *15*, 655–669

(33) Loscalzo, L. M.; Wasowski, C.; Paladini, A. C.; Marder, M. *Eur. J. Pharmacol.*, **2008**, *580*, 306–313

(34) Piozzi, F.; Bruno, M.; Rosselli, S.; Maggio, A., *Heterocycles,* **2007**, *74,* 31–52.

(35) Willey, N. J.; Wilkins, J. *Environmental Science & Technology* **2008**, *42*, 2162-2167

(36) Lozano, G.; Trenado, H. P.; Valverde, R. A.; Navas, C. J. *Journal of General Virology* **2009**, *90*, 2550-2562

(37) Hall, R.; Kalasz, H. *Principles and Methods of Toxicology*, 4th edn **2001**, 1001-1006

(38) Jager, A.; Lafont, R. *J. Ethnopharmacol.* **1996**, *52*, 95-99

(39) Harborne, J.; Ohmacht R. *The Flavonoids - Advances in Research* **1982**, *19*, 165-169

(40) Jafri, M.; Kalasz, H. *J. Ethnopharmacol.* **1999**, *66*, 355-359

(41) Jollow, D.; Szendrei, K. *Pharmacology* **1974**, *12*, 251-256

(42) Mitchell, J.; Girault, J. P.; Kalasz, H. *J. Pharmacol. Exp. Ther.* **1973**, *187*, 185-189

(43) Watt, J.M.; Breyer, B.N., Ed. *Medicinal and poisonous plants of Southern and Eastern Africa;* 2nd ed., Churchill Livingstone, Edinburgh **1962**, 514–520.

(44) Modnicki, D.; Sharaf, M. *Acta. Pol. Pharm.* **2007**, *64*, 247

(45) Ojewole, J.; Farrag, A. R. *Methods. Find. Exp. Clin. Pharmacol.* **2005**, *27*, 257

(46) Peres, W.; Aboutabl, E. A. *J. Hepatol.* **2000**, *33*, 742

(47) Qiusheng, Z.; Hawas, U. W.; G. M.; Soliman, G. S. *Pharmazie.* **2004**, *59*, 286

(48) Watt, J. M.; Breyer, B. N., Ed. *Medicinal and poisonous plants of Southern and Eastern Africa;* 2nd ed., Churchill Livingstone, Edinburgh **1962**, 514–520.

(49) Agnihotri, V. K.; ElSohly, H. N.; Smillie, T. J.; Khan, I. A.; Walker, L. A., *Phytochemistry Letters* **2009,** *3* 103–105

(50) Rebrin, I; Elgorashi, E. E. *Exp. Gerontol.* **2004**, *39*, 1513-1519

(51) He, F.; Lindqvist, C.; Harding, W. *Phytochemistry,* **2012**, 83, 168–172

(52) Savides, M.; Smillie, T. J. *J. Appl. Toxicol.* **1983**, *3*, 95-101

(53) Kaplan, E.; Naidu, K; Rivett, D; *J. Chem. Soc. (C)*, **1970**, 1656-1660

(54) Stafford, G.; Smillie, T. J.; Khan, I. A. *J. Ethnopharmacol.* **2005**, *97*,107-111

(55) Tolmen, K. *Tietz Book of Clinical Chemistry* **1999**, 1136-1138

(56) Wyk, B.E. V; Oudtshoorn, B.V.; Gericke,N. *Medicinal plants of South Africa* 2nd ed.; Briza, Pretoria, **2000**, 166-171.

(57) Upasani, C.; Elgorashi, E. E. *Indian J. Exp. Biol.* **2001**, *39*, 70-78

(58) van Wyk, B.; Naidu, K; Rivett, D. *Medicinal Plants of South Africa*, 2nd edn **2000**, 166-172

(59) Vermeulen, N.; Tolmen, K. *Drug Metab. Rev.* **1992**, *24*, 367-372

(60) Visen, P.; Lindsey, K. L.; McGaw, L. J.; Sparg, S. G. *J. Ethnopharmacol* **1993**, *40*, 131-139

(61) Watt, J.; Lindsey, K. L. *Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd edn **1962**, 514-519

(62) Cheng, K. F.; Yip, C. S.; Yeung, H. W.; Hong, Y. C. *Experientia* **1979**, *35*, 571-572

(63) Zheng, Q. *Biomed. Environ. Sci.* **2005**, *18*, 65-72

(64) Ansari, M. A.; Aboutabl, E. A.; Farrag, R. H.; Sharaf, M.; Hawas, U. W.; Soliman, G. M.; Elseed, G. S., *Pharm. Biol.*, **2009**, *47*(9), 894–902

(65) Zhang, X.; Geoffroy, P.; Miesch, M.; David, D. J.; Raul, F.; Werner, D. A.; Marchioni, E., *Steroids* **2005,** *70*, 886-895

(66) Sobiecki, J. F., *Transactions of the Royal Society of South Africa* **2002**, *57,* 1–24.

(67) Bienvenu, E.; Amabeoku, G. J.; Eagles, P. K.; Scott, G.; Springfield, E. P., *Phytomedicine* **2002**, *9*217–223.

(68) Fennell, C. W.; Lindsey, K. L.; McGaw, L. J.; Sparg, S. G.; Stafford, G. I.; Elgorashi, E. E.; Grace, O. M.; Staden, J. V., *J. Ethnopharmacology* **2004**, *94,* 205–217.

(69) Ojewole, J. A. *Methods and Findings in Experimental and Clinical Pharmacology* **2005**, *27,* 257–264.

(70) Ojewole, J. A., *American Journal of Hypertension* **2003**, *16*,

40-46.

(71) Scott, G.; Springfield, E. P.; Coldrey, N. *Pharm. Biol. (Lisse, Neth.),* **2004**, *42*, 186–213

(72) Bienvenu, E.; Amabeoku, G. J.; Eagles, P. K.; Scott, G.; Springfield, E. P. *Phytomedicine*, **2002**, *9*, 217–223

(73) Ojewole, J. A. *Methods Find. Exp. Clin. Pharmacol.,* **2005**, *27*, 257–264

(74) Oyedemi, S. O.; Yakubu, M. T.; Afolayan, A. J. *J. Med. Plants Res.,* **2011**, *5*, 119–125

(75) Maphosa, V.; Masika, P. J. *Parasitol. Res.,* **2012**, *110*, 103–108

(76) Maphosa, V.; Masika, P. J.; Bizimenyera, E. S.; Eloff, J. N. *Trop. Anim. Health Prod.,* **2010**, *42*, 301–307

(77) Stafford, G. I.; Pedersen, M. E.; van, S. J.; Jager, A. K. *J. Ethnopharmacol.,* **2008**, *119,* 513–537

(78) Chen, C. X.; Kwan, C.Y. *Life Sci.,* **2001**, *68*, 953–960

(79) Hayashi, Y. *Yakugaku. Zasshi.,* **1962**, *82*, 1025–1027

(80) Kong, Y. C.; Yeung, H. W.; Cheung, Y. M.; Yeung, C. H. *Am. J. Chin. Med. (Gard City, N. Y.),* **1976**, *4*, 373–382

(81) Luo, S. R. *Zhong. Yao. Tong. Bao.*, **1985**, *10*, 32–35

(82) Agnihotri, V. K.; ElSohly, H. N.; Smillie, T. J.; Khan, I. A.; Walker, L. A. *Phytochem. Lett.,* **2009**, *2*, 103–105

(83) ElAnsari, M. A.; Aboutabl, E. A.; Farrag, A. R.; Sharaf, M.;

Hawas, U. W.; Soliman, G. M. *Pharm. Biol. (London, U. K.),* **2009**, *47*, 894–902

(84) Kaplan, E. R.; Rivett, D. E. *J. Chem. Soc.* **1968**, *3*, 262–266

(85) Kruger, G. J.; Rivett, D. E. *S. Afr. J. Chem*., **1988**, *41*, 124–125

(86) Laonigro, G.; Lanzetta, R.; Parrilli, M.; Adinolfi, M.; Mangoni, L. *Gazz. Chim. Ital.*, **1979**, *109*, 145–150

(87) McKenzie, J. M.; Green, I. R.; Mugabo, P. *S. Afr. J. Chem*., **2006**, *59*, 114–116

(88) Naidoo, D.; Maharaj, V.; Crouch, N. R.; Ngwane, A. *Biochem. Syst. Ecol.,* **2011**, *39*, 216–219

(89) Obikeze, K. C.; McKenzie, J. M.; Green, I. R.; Mugabo, P. *S. Afr. J. Chem*., **2008**, *61*, 119–122

(90) Piozzi, F.; Bruno, M.; Rosselli, S.; Maggio, A. *Heterocycles*, **2007**, *74*, 31–52

(91) Rivett, D. E. *J. Chem. Soc*. **1964**, *1,* 1857–1858

(92) Martellossi, C.; Taylor, E. J.; Lee, D.; Graziosi, G.; Donini, P. *J. Agric. Food Chem.*, **2005**, *53*, 8432–8436

(93) Zerega, N. J.; Mori, S.; Lindqvist, C.; Zheng, Q.; Motley, T. J. *Econ. Bot*., **2002**, *56*, 154–164

(94) Hollingsworth, P. M.; Graham, S. W.; Little, D. P. *PLoS. One*, **2011**, *6*, 19254-19258

(95) Chen, S.; Yao, H.; Han, J.; Jia, X.; Lin, Y.; Leon, C. *PLoS.*

One, **2010**, *5*, 8613-8619

(96) Scheen, A. C.; Albert, V. A. *Syst. Bot*., **2009**, *34*, 173–181

(97) Oyedemi, S. O.; Yakubu, M. T.; Afolayan, A. J. *J. Med. Plant. Res.,* **2011**, *5*, 119-125

(98) Oyedeji, O. A.; Afolayan, A. J. *South African Journal of Botany,* **2005**, *71*, 114-116

(99) Hussein, A. A.; Meyer, M. J.; Rodriguez, B. *Magn. Reson. Chem*., **2003**, *41*, 147–151

(100) Ohsaki, A.; Kishimoto, Y.; Isobe, T.; Fukuyama, Y. *Chem. Pharm. Bull. (Tokyo),* **2005**, *53*, 1577–1579

(101) Von Dreele, R. B.; Pettit, G. R.; Ode, R. H.; Perdue Jr., R. E.; White, J. D.; Manchand, P. S. *J. Am. Chem. Soc*., **1975**, *97*, 6236–6240

(102) White, J. D.; Manchand, P. S. *J. Org. Chem*. **1973**, *38*, 720–728

(103) Ansari, M. A.; Aboutabl, E. A.; Farrag, A. R.; Sharaf, M.; Hawas, U. W.; G. M.; Soliman, G. S. *Pharm. Biol. (London, U. K.),* **2009**, *47,* 894–902

(104) Sosińska, E.; Przybylski, R.; Hazendonk, P.; Zhao, Y. Y.; Curtis, J. M. *Food Chemistry*, **2013**, *139*, 464–474

(105) Blicharska, B.; Kupka, T. *Journal of Molecular Structure*, **2002**, *6*, 153–166

(106) Kruk, Z. L.; Pycock, C. J., Ed. *Neurotransmitters and Drugs*, 3rd edn., Chapman and Hall, London, **1990**

(107) Zhu, M.; Bowery, N. G.; Greengrass, P. M.; Phillipson, J. D. *J. Ethnopharmacology* **1996**, *54*, 153-164.

(108) Bennett, J. P.; Yamamura, H. I., *Neurotransmitter hormone, or drug receptor binding methods*. Raven Press, New York, **1985**, 61-89.

(109) Hulme, E. C.; Birdsall, N. J. *Strategy and tacticsin receptor binding studies.* In: Hulme, E.C. (Ed.), *Receptor Ligand Interactions, A Practical Approach,* IRC Press, Oxford, UK, **1992**, 72-75.

(110) Hartmann, K.; Stief, F.; Draguhn, A.; Frahm, C., *Eur. J. pharmacol.* **2004,** *497,*139-146**.**

(111) Chang, L.; Spanjersberg, R.; Drabbe, K.; Mulder-Krieger, T.; van den Hout, G.; Beukers, M., *J. Med. Chem.*, **2004**, *47* (26), 6529–6540.

(112) Raines, T.; Jones, P.; Moe, N.; Duncan, R.; Mccall, S.; Ceremuga, T. E., *AANA J.* **2009**, *77*(1) 33-6.

(113) Modnicki, D.; Tokar, M.; Klimek, B., *Acta. Poloniae. Pharmaceutica.* **2007**, *64*(3) 247-252.

(114) Mohamed, M. H.; Assaf, M. H.; Ahmed, Amany S.; Ahmed, M. S., *Bulletin of the Faculty of Pharmacy (Cairo University)*

2003, *41*(1) 239-252.

(115) Paladini, A. C.; Marder, M.; Viola, H.; Wolfman, C.; Wasowski, C.; Medina, J. H. *J. Pharm. Pharmacol.* **1999,** *51*, 519-526.

(116) Kuchta, K.; Ortwein, J.; Rauwald, H. W. *Pharmazie,* **2012,** *67*, 973-979.

(117) Lindqvist, C.; Scheen, A. C.; Bendiksby, M.; Ryding, O.; Mathiesen, C.; Albert, V. A. *Ann. Mo. Bot. Gard.* **2010**, *97,* 191–217

(118) Hall, T. A. *Nucleic Acids Symp. Ser.* **1999**, *41,* 95–98

(119) Huson, D. H.; Bryant, D. *Mol. Biol. Evol.* **2006**, *23*, 254–267

(120) Chang, L.; Spanjersberg, R.; Drabbe, K.; Mulder-Krieger, T.; van den Hout, G.; Beukers, M., *J. Med. Chem.* **2004**, *47*, 6529–6540.

(121) Liu, C.; Gu, X.; Zhu, Z. Y. *Bioorg. Med. Chem. Lett.***2010,** *20*, 6942-6946.

(122) Vankar, P.; Srivastava, J.; Molčanov, K.; Kojic-Prodić, B. *Phytochem. Lett.*, **2009**, *2*, 67–71

(123) Neogi, P.; Kawai, M.; Butsugan, Y.; Mori, Y.; Suzuki, M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4479-4481

(124) Gupta, M.; Bagchi, A.; Ray, A. B. *J. Nat. Prod.* **1991**, *54*, 599-602

(125) Joshi, D.; Dahake, A. *Der. Pharma. Chemica.* **2009**, *1(2),* 109-112

(126) Zhang, X.; Geoffroy, P.; Miesch, M.; David, D. J.; Raul, F.; Werner, D. A.; Marchioni, E., *Steroids* **2005,** *70*, 886-895

(127) Pelletier, W.; Mody, N.; Nowacki, J.; Bhattaaharyya, J., *J. Nat. Prod.,* **1979**, *42*, 512-521

(128) Takahata, H.; Kubota, M.; Takahashi, S.; Momose, T., *H. Takahata et al.*, **1996**, *7*, 3047-3054

(129) Chang, L.; Spanjersberg, R.; Drabbe, K.; Mulder-Krieger, T.; van den Hout, G.; Beukers, M., *J. Med. Chem.*, **2004**, *47* (26), 6529–6540.

(130) Matsuda, H.; Murakami, T.; Kishi, A.; Yoshikawa, M. *Bioorg. Med. Chem.*, **2001**, *9*, 1499-1507

(131) Xu, Y.; Gao, S.; Bunting, D.; Gunatilaka, L., *Phytochemistry*, **2011**, *72*, 518-522

(132) Khan, M.; Kumar, M.; Jaiswal, N.; Srivastava, A.; Maurya, R., *Trends In Carbohydrate Research*, **2010**, *2*, 29-34

(133) Lafont, R.; Bouthier, A.; Wilson, I. D. *I Hrdy (Ed.), Insect Chemical Ecology, Academia, Prague* **1991**, 197-199

(134) Mamadalieva, N. Z. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* **2012**, *11(6)*, 474-497

(135) Báthori, M.; Girault, J. P.; Kalasz, H.; Mathé, I.; Lafont, R. *J.*

Pharm. Biomed. Anal. **1997**,*16*, 327-332

(136) Girault, J. P.; Báthori, M.; Kalasz, H.; Mathé, I.; Lafont, R. *J. Nat. Prod.* **1996**, *59*, 522-528

(137) Báthori, M.; Szendrei, K.; Kalasz, H.; Ohmacht R. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1986**, *9*, 539-545

(138) Girault, J. P.; Báthori, M.; Varga, E.; Lafont R. *J. Nat. Prod.* **1990**, *53*, 279-284

(139) Báthori, M.; Girault, J. P.; Kalasz, H.; Dinan, L. N.; Lafont R. *Arch. Insect. Biochem. Physiol.* **1999**, *41*, 1-6

(140) Dinan, L. *Phytochem. Anal.* **1992**, 3, 132-136

(141) Clément, C. Y.; Bradbrook, D. A.; Lafont, R.; Dinan, L. *Insect. Biochem. Mol. Biol.* **1993**, *23,* 187-192

(142) Lafont, R.; Wilson I. *The Ecdysone Handbook, Chromatographic Society, Nottingham, 2nd ed.* **1996**, 398-399

(143) Horn, D. H.; Bergamasco, R. *Comprehensive Insect Physiology, Biochemistry and Pharmacology Vol. 7 Pergamon, Oxford* **1985**, 185-186

(144) Saatov, Z.; Gorovits, M. B.; Abubakirov, N. K. *Khim. Prir. Soedin.* **1993**, *21*, 627-632

(145) Báthori, M.; Mathé, I.; Solymosi, P.; Szendrei, K. *Acta. Bot. Hung.* **1987**, *33*, 125-129

(146) Cambie, R. C.; Ferguson, L. R. *Mutat. Res.* **2003**, *52,* 109-117,

(147) Cao, S.; Guzza, R. C.; Wisse, J. H.; Miller, J. S.; Evans, R.; Kingston, D. *J. Nat. Prod.* **2005**, *68,* 487-492.

(148) Austin, D. F.; Huáman, Z. *Taxon.* **1996**, *45*, 3-38.

(149) Baek, N. I.; Ahn, E. M.; Bang, M. H.; Kim, H. Y. *Han'guk. Nonghwa. Hakhoechi.* **1997**, *40*, 583-587.

(150) Luo, J. G.; Kong, L. Y. *Zhongguo. Zhong. Yao. Za. Zhi.* **2005**, *30*, 516-518.

(151) Ma, S. C.; Du, J.; But, P. P.; Deng, X. L.; Zhang, Y. W.; Oive, C.; Xu, H. X.; Lee, S.; Lee, S. F. *J. Ethnopharmacol.* **2002**, *79,* 205-211.

(152) Macleod, J. K.; Ward, A.; Oelrichs, P. B. *J. Nat. Prod.* **1997**, *60,* 467-471.

(153) Prasad, K. N.; Ashok, G.; Raghu, C.; Shivamurthy, G. R.; Vijayan, P.; Aradhya, S. M. *Indian. J. Pharmacol.* **2005**, *37*, 397-398.

(154) Rabah, I. O.; Hou, D. X.; Komine, S.; Fujii, M. *J. Agric. Food. Chem.* **2004**, *52*, 7152-7157.

(155) Sun, Y.L.; Sun, J. M.; Li, Q. P. *Agric. Sci. (China)* **2009**, *8,* 808-820.

(156) Taber, W. A.; Vinig, L. C.; Heacock, R. A. *Phytochemistry* **1963**, *2*, 65-70.

(157) Pereda-Miranda, R.; Bah, M. *Curr. Top. Med. Chem.* **2003**, *3*, 111-131.

(158) Pereda-Miranda, R.; Hernández-Carlos, B. *Tetrahedron* **2002**. *58*, 3145-3154.

(159) Noda, N.; Nishi, M.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1988**, *36*, 1707-1713.

(160) Noda, N.; Kobayashi, H.; Miyahara, K.; Kawasaki, T. *Chem. Pharm. Bull.* **1988**, *36*, 627-633.

(161) Zheng, W.; Clifford, M. N. *Food Chem.* **2008**, *106*, 147-152.

(162) Zhou, J.; Zhao, G.; Jin, W.; Zheng, W.; Chi, Z. *Chin. Acad. Sci. Shanghai* **1988**, *9*, 107-111

(163) Yuan, S. Q.; Zhao, Y. M.; You, Y. *Acta. Pharm. Sin.* **2004**, *39*, 818-20.

(164) Zhao, G.; Kan, J.; Li, Z.; Chen, Z. *Int. Immunopharmacol.* **2005**, *5*, 1436-1445.

(165) Wang, L. M.; Chu, Y.H. *Acta. Pharm. Sinica.* **1996**, *31*, 806-811.

(166) Wasuwat, S. *Nature* **1970**, *225*, 758-59.

(167) Sun, X.-F.; Wang, N.; Wu, Q.; Lin, X.-F. *Biotechnology Letters* **2004**, *26*, 1019–1022.

(168) Kim, S.-H.; Yoo, S.-M.; Park, I.-S.; Kim, Y.-H. *J. Nat. Prod.* **2000**, *63*, 1188-1191.

(169) Wada, T.; Moriguchi, T.; Sekine, M. *J .Am. Chem. SOC.* **1994, *116,*** 9901-9911

(170) Ozaki, Y.; Kim, S. *Chem. Pharm. Bull.* **1989***, 37(2),* 304-307

(171) Ploypradith, P.; Cheryklin, P.; Niyomtham, N.; Bertoni, D. R.; Ruchirawat, S. *Organic Letters* **2007**, *9(14)*, 2637-2640

(172) Lamaty, G.; Moreau, C.; Mouloungui, Z. *Can. J. Chem.* **1983***, 61*, 2643-2650