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Etude phytochimique de Launaea arborescens et Halophila stipulacea

JURY

Yassine BOUZAHER	**Professeur Univ-Batna**	**Président**
Ammar DIBI	**Professeur Univ-Batna**	**Rapporteur**
Margherita GAVAGNIN	**Docteur (Directrice de recherche) CNR-Italy**	**Co-rapporteur**
Maria Litezia CIAVATTA	**Docteur CNR-Italy**	**Examinateur**
Yassine El Hilou MALEK RASOUL	**Professeur C.U-Oum El-Bouaghi**	**Examinateur**
Mohammed CHERIF ABERKANE	**Maître de conferences Univ-Batna**	**Examinateur**

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My brothers Mohamed, abdelhak,
and my sister Meriem whose moral support and encouragement made this work possible.

Abbreviations and symbols

EtOAc	Ethyl acetate
MeOH	Methanol
Ep	Petroleum ether
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
$CDCl_3$	Deuterated chloroform
CD_2Cl_2	Deuterated dichloromethane
MeOD	Deuterated methanol
DMSO-d6	Deuterated dimethylsulfoxyde
D_2O	Deuterated water
SO_3Na	Sodium-sulphate
SO_3K	Potassium-sulphate
TLC	Thin layer chromatography
CC	Column chromatography
SiO_2	Silica gel
RP-C18	Reversed phase chromatography
HPLC	High pressure liquid chromatography
R_f	Retention factor
R_t	Retention time
TMS	Tetramethylsilane
δ	Chemical shift in ppm
J	Coupling constant in Hertz (Hz)
s	Singlet
br s	Broad singulet
d	Doublet
dd	Doublet doublet
ddd	Doublet doublet doublet
t	Triplet
q	Quartet
dq	Doublet quartet
q	Quintet
m	Multiplet
^{1}H NMR	Proton nuclear magnetique resonance
^{13}C NMR	Carbone nuclear magnetique resonance
COSY	Correlated spectroscopy
HSQC	Heteronuclear single quantum correlation
HMBC	Heteronuclear multiple bond correlation
DEPT	Distortionless enhancement polarisation transfer
NOESY	Nuclear overhauser effect spectroscopy
MS	Mass spectroscopy
EI	Electronic impact

ESIMS	Electrospray ionisation mass spectroscopy
HRESIMS	High resolution electrospray ionisation mass spectroscopy
MALDI	Matrix-assisted laser desorption ionisation
m/z	masse/charge of ion
IR	Infrared
UV	Ultraviolet
$[\alpha]_D$	Optical activity referred to Sodium ray
ATP	Adenosine triphosphate
IPP	Isopentyl pyrophosphate
GPP	Geranyl pyrophosphate
FPP	Farnesyl pyrophosphate
CoA	Coenzyme A as part of thioester
CTP	Cytidine triphosphate
CDP	Cytidine diphosphate
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (Reduced)
DMAPP	Dimethylallyl pyrophosphate
MVA	Mevalonic acid
GFPP	Geranylfarnesyl pyrophosphate
PPPP	Polyprenyl pyrophosphate
MAC	Methacryloyl
ANG	Angeloyl
HMB	2-hydroxy-3-methylbutyryl
HMV	α-hydroxy-β-methylvaleroyl
HMPS	3-hydroxy-2-methylpropanoyl-3-sulphate
PPA	para-hydroxyphenylacetyl
HPP	*para*-hydroxyphenylpropanoyl
PMP	para-methylphenylacetic acid
HPL	*p*-Hydroxy-phenyllactyl
HPLHMB	*p*-Hydroxy-phenyllactyl-α-hydroxymethylbutyryl
PIC	Picriosyl
DDC	1,2-didehydro-3-oxo-costoyl
Clc	Glucopyranosyl
Allo	Allosyl

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INTRODUCTION

Throughout the ages, humans have relied on nature for their basic needs: food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours and fragrances as not the least, medicines.

Plants which have been the basis of traditional medicine systems for thousands of years in countries such as China (Chang et al., 1986) and India (Kapoor, 1990**)**, continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is still based on the empirical findings of hundreds and thousands of years.

Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world and in which higher plants contribute to no less than 25% (Ameenah, 2006). During the last 40 years, at least a dozen potent drugs have been derived from flowering plants including: *Dioscorea* spp. derived diosgenin from which all anovulatory contraceptive agents have been derived; reserpine and other anti-hypertensive and tranquilizing alkaloids from *Rauwolfia* species; pilocarpine to treat glaucoma and dry mouth, derived from a group of south American trees (*Pilocarpus* spp.) in the Citrus family; two powerful anti-cancer agents from the *Rosy periwinkle* (*Catharanthus roseus*); laxative agents from *Cassia* sp. and as a cardiotonic agent to treat heart failure from *Digitalis* species.

Natural products are not restricted to constituents of higher plants, but they include also metabolites from fungi, bacteria, algae, and marine organisms. The variety of structures obtained and the different therapeutic activities shown for the natural products make that the isolation, identification, synthesis and biosynthesis of new natural compounds continue to be a field of enormous interest. Development of spectroscopic techniques in the last decades allows in most of the cases the elucidation of the complete structures.

The terrestrial and marine angiosperm flora seem inexhaustible since only a small part of the 400'000 vegetable species known were investigated from phytochemical and pharmacological aspects, and that each species can contain up to several thousands of different components (Hostettmann et al, 1998).

In this context, and within the framework of the search for molecules with new biological activities of vegetable origin, it is thus preferable not to choose the plants to be studied randomly, but to circumscribe them according to their use in traditional or popular medicine that depends specifically on the empirical knowledge of indigenous peoples concerning medicinal substances.

Algeria with its large area and diversified climate has a varied flora, which is a source of rich and abundant medical matter and, in particular, Sahara part constitutes an important reservoir of many plants which have not been investigated until today. Among this flora, some endemic Algerian plants have been used in the traditional medicine (Cheriti et al., 2005). The ethnopharmacological survey was conducted among people, herbalists and practitioners living in the south west of Algeria.

The plant *Launaea arborescens* (Batt) (local name ‘‘Oum Lbina”) commonly used in the north African popular medicine against diarrhoea and abdominal spasms, is one of the nine endemic plant of north Africa mainly distributed in the south west part of Algeria (Quezel et al., 1963), (Ozenda, 2004). Very interesting antifungal, antibacterial and insecticidal activities have been reported for the methanol extract of this plant (Belboukhari et al., 2006), (Jbilou et al., 2008). However, to the best of our knowledge, very few chemical studies of this species are reported in the literature.

The main objective of this work was to define the chemical constituents of *L. arborescens* and to evaluate the antibacterial properties of the pure isolated compounds with the aim of finding a possible relationship between these molecules and the biological activities reported for the plant.

The second part of this work deals with the phytochemical investigation of the marine plant *Halophila stipulacea*. This topic has been developed in the frame of a research program on marine chemistry conducted by the team working at the Institute of Biomolecular Chemistry (ICB) of National Research Council (CNR), in Italy. The group, one of the most active in Europe in the field of Natural Products Chemistry, is interested in the isolation and structural elucidation of bioactive secondary metabolites from marine organisms as well as in the definition of the chemo-ecological interaction between marine invertebrates and plants.
The plant studied in this work belongs to the family of phanerogams (sea grasses), the only angiosperms that successfully grow in tidal and subtidal marine environment (Kannan et al., 1999).

Due to their ability to produce secondary metabolites with original chemical and biological characteristics, marine phanerogams occupy a particular position between terrestrial and aquatic environment, and play an ecological and evolutionary role in the conservation and the protection of the marine flora. These plants have been recently regarded with great interest in the estimation of the ecological and economic values of the various marine ecosystems.

Halophila stipulacea is an Indo-Pacific plant which reached the Mediterranean Sea through Suez Canal with other exotic species including plants and animals (Lipikin, 1975). It is one of the nine macrophyte species that are considered as invasive playing a significant role in the recipient ecosystems, taking the place of keystone species and being economically harmful (Boudouresque and Verlaque, 2002). Chemo-ecological implications of the establishment of this plant and its predator, the mollusc *Syphonota geographica*, in Mediterranean Sea have been recently discussed by Mollo et al.

As far as we know, few chemical studies on sea grasses have been reported. Metabolites of various classes such as fatty acids, sterols, lignans, neolignanes, phenylpropanes, and phenanthrenes, with algicide effect (Aliotta et al, 1996; Dellagreca et al, 1997, 1998) have been described in the literature. This kind of activity is most likely responsible of the allelopathy interactions that were shown for nearly 100 species of aquatic plants, although in the majority of the cases the active ingredients were not identified (Elakovich et al, 1995).

In this light, the second objective of this work was to study the chemical composition of *Halophila stipulacea* with particular regards to the flavonoid fraction, which has never been analysed. This investigation was planned to get information useful for either chemo-taxonomic or ecological studies of sea grasses.

This thesis is organised into five chapters as following described.

The first chapter is divided in two parts. The first is devoted to the Asteraceae family, the Cichorieae (Lactuceae) tribe and the *Launaea* genus. The previous phytochemical studies reported in the literature for this family are described by referring to the different groups of secondary metabolites. The second part deals with the marine plants, especially those named "sea grasses", the Hydrocharitaceae family, and the *Halophila* genus. Secondary metabolites known in the sea plants as well as the natural compounds isolated from the Hydrocharitaceae family and, in particular, from the *Halophila* genus have been quoted.

In the second chapter, the different groups of the secondary metabolites described in the first chapter, their biogenesis and the metabolic sequences leading to these selected classes of natural compounds and their biological interest are presented in a general way. In particular, the class of terpenes which comprises triterpenes and sesquiterpenes and the class of phenolic compounds which includes flavonoids have been considered in this work.

The third and the fourth chapters describe the chemical results of the phytochemical investigation of the two plants: *L. arborescens* and *H. stipulacea*. The isolation and the structural elucidation of all pure compounds obtained are reported in details including the spectroscopic analysis conducted by using NMR and mass techniques.

The fifth chapter reports the experimental part describing all the steps of separation and purification used in this work, and the physicochemical data of all identified compounds.

The manuscript includes a conclusive part that summarizes the contribution given by this thesis to the phytochemistry of terrestrial and marine plants. A possible future research direction in the study of other plants is also proposed.

Chapter 1
Literature data

1. ASTERACEAE FAMILY

1.1 General introduction

The family of Asteraceae or, alternatively, Compositae, known as the aster, daisy or sunflower family, is one of the largest angiosperm families of a taxon of dicotyledenous flowering plants. It comprises about 1400 genera and more than 25000 species of herbaceous plants, shrubs, and trees, spread throughout the world, and classified over three subfamilies and 17 tribes (Bremer, 1994). The composite nature of the inflorescences of these plants led early taxonomists to call this family Compositae. Though diverse habits and habitats, composites tend to grow in sunlit places, in temperate and subtropical regions. Some Asteraceae plants can share these following characters:

- ✓ Various members of the aster family are familiar species in natural habitats, while others are cultivated plants in gardens and some are grown as food (*Lactuca sativa*) (Lettuce), and *Cichorium* (Chicory).
- ✓ Many members of Asteraceae are pollinated by insects, which explain their value in attracting beneficial insects. Many members of Asteraceae are copious nectar producers and are useful for evaluating pollinator populations during their bloom. *Centuarea cyanus*, *Helianthus annuus* and some species of solidago are major honey plants.
- ✓ Some species in the aster family have anatomical mechanisms of attaching their seeds to the fur of mammals, for the purposes of dispersal.
- ✓ Phytochemically, species of Asteraceae biosynthesize many several metabolites such polyfructanes (especially inulin) as storage carbohydrates as opposed to polysaccharides, in the perennial taxa. In some taxa, some segments of the family accumulate polyacetylenes, flavonoids, various alkaloids and terpenoids essential oils. But iridoids have never been found (Harborne et al., 1994).
- ✓ Plants in Asteraceae are medically important; sesquiterpenes compounds obtained from them are responsible for allergic contact dermatitis. More than 4000 structures with around 30 different skeletal types have so far been reported from several tribes of this family (Seaman, 1982). Due to their chemo-diversity, the sesquiterpene lactones are the most suitable class of naturals products for chemo-systematic studies within the family (Seaman, 1982, Zdero et al., 1990). It is interesting to note that parthenolides derivatives are important sesquiterpene lactones responsible for the pharmacological activity of many botanical drugs e.g. Fever few (*Chrysanthemum parthenium*) and Arnica (*Arnica montana*).

1.2 Morphological and characteristics of Asteraceae

All plants belonging to the Asteraceae family can share the following characteristics according to Judd (Judd et al., 1990).

✓ The inflorescence is an involucrate capitulum, technically called a calathid or calathidium, but generally referred to as flower head, which is a contracted raceme composed of numerous individual sessile flowers, called the florets, all sharing the same receptacle.

✓ The flowers are of two basic types: tubular actinomorphic corollas and those with strap shaped or radiate zygomorphic corollas, often with the same head. Either type may be bisexual or unisexual.

✓ The calyxes (sepals) of the florets are modified to form a pappus, a tuft of hairs, which often appears on the mature fruit.

✓ Anthers are syngenesious with the stamens fused together

✓ Leaves and stems very often contain secretory canals with resin or latex (particularly common among the Cichorioideae). The leaves can be alternate, opposite, or whorled. They may be simple, but are often deeply lobed or otherwise incised, and conduplicated or revoluted. The margins can be entire or dentate.

✓ The fruit of Asteraceae is a specialized type of achene sometimes called cypsela. One seed per fruit is formed. Its morphology is often used to help determining plant relationships at the genus and species level. The seeds usually have little or lack endosperm.

1.3 Importance of Asteraceae family

The biodiversity of metabolites products isolated from Asteraceae makes this family have an important commercial source. Some Asteraceae plants are used as herbs and herbal tea such as a) chamomile, which comes from two different species, the annual *Matricaria recutila* and the perennial *Chamaemelum nobile*, also called Roman chamomile; b) calendula (*Calendula arvensis)*, also called the pot marigold, grown commercially for herbal teas and the potpourri industry, c) echinacea (*Echinacea purpurea*) used as a medicinal tea.

The industrial use of Compositae is also known. Common in all commercial poultry feed, marigold (*Tagetes patula*) is grown primarily in Mexico. Marigold oil, extracted from *Tagetes minuta* is used in the metric ton in the cola and cigarette industry.

Several species of this family are used as natural remedies; such as:

Anthemis arvensis L. the whole plant is used like anti-inflammatory, emetic, and sedative.

Artemisia arborescens L. The flower is used as digestive, stimulant, expectorant.

Calendula arvensis L. The flower and leaf are used as antispasmodic, burns, diuretic, disinfectant and vulnerary.

Cichorium intybus L. The leaf and root are used in blood purification, as arteriosclerosis, anti-arthritis, anti-spasmodic, digestive, hypotensive, aperitif, and laxative.

Helychrysum microphyllum Willd. The flower and leaf were used as an expectorant.

1.4 The tribe Cichorieae (synonym: Lactuceae)

The Lactuceae is a tribe of closely related genera of the Asteraceae family that is easily recognized because the flowering heads are composed of wholly ligulate florets that are usually 5-lobed. Another very distinguishing feature is the milk sap. Although not apparent without magnification, the pollen is distinctive in that the spines are more or less restricted to discrete ridges or flanges on the surface of the grain. In the other members of family the spines are distributed more or less evenly over the surface of the pollen grain. The pappus usually consists of scales or stiff hairs.

According to Bremer, this tribe encompasses approximately 100 genera and 1500 species. These genera are used as vegetables or for salads (e.g. *Cichorium*, *Lactuca*, *Taraxacum*, *Cicerbita*, *Scorzonera*, and *Tragopognon)* as well as a number of genera used in folk medicine (eg. *Crepidiastrum, Ixeris*, *Lactuca*, *Pilosella*, *Taraxacum*, and *Youngia*).

1.5 Metabolites isolated from Lactuceae.

Several phytochemical studies of some genera of Lactuceae tribe (Zidorn, 2006a, 2008)[23,24] revealed to be reach in secondary metabolites, specifically sesquiterpene lactones exhibiting the eudesmane, germacrane and guaiane carbon framework.

As sesquiterpenoids exhibit a wide range of bioactivities which include toxicity for certain cancer cell lines and induction of detoxifying enzymes, the sesquiterpene content of salads and vegetables from the Cichorieae might contribute to the health promoting properties of these groceries.

Some phenolic compounds, such as flavonoids, coumarins were also isolated (Miyase et al., 1985, Kisiel et al., 2000a, 2000b, 2006, Michalska et al 2007, Mulinacci et al., 2001).

In addition, triterpenes have been also detected (Schultz et al., 2006, Shiojima et al., 1995a, Takasaki, 1999).

The extraction of the aerial parts and roots are usually carried out with different solvents, the separation of the constituents are based on the combination of normal liquid chromatography and high performance liquid chromatography (HPLC).

The elucidation of the structures and the stereochemistry of these compounds are determined by the classical spectroscopic techniques such as ^{1}H and ^{13}C NMR (1D and 2D experiments), infrared spectroscopy (IR), mass spectrometry (MS) and X-ray analysis.

1.6 Distribution of sesquiterpenoids subtypes in the genera of the Cichorieae

The literature data on sesquiterpenoids from the Cichorieae tribe until the end of 2007, indicate that a total of 360 sesquiterpene lactones and related compounds have been isolated from 139 taxa belonging to 31 different genera of the Cichorieae (Zidorn, 2008).

The study realized by Zidorn (Zidorn., 2008) for these genera revealed that most sesquiterpenoids within the Cichorieae belong to the guaianolide class and in particular: 92 representatives of costus lactone type, 75 compounds of lactucin type, and 29 representatives of hieracin type.

According to Zidorn, the 31 genera are grouped into seven main clusters, based on the similarity of their sesquiterpenes profiles:

Group 1: comprises the genera *Andryala*, *Chondrilla*, *Soroseris*, *Taeckholmia*, *Willemetica*, *Crepis*, *Nabalus*, *Ixeris, Mycelis*, *Picris*, *Youngia*, *Scorzonera*, and *Lapsana*. This group is characterized by the prevalence of guaianolides, mainly of the costus lactone type.

Group 2: comprises the genera *Scorzoneroides*, *Notoseris*, *Lactuca*, *Cichorium*, *Launaea*, *Crepidiastrum*, *Reichardia*, *Cicerbita*, *Taraxacum*, *Helminthotheca*, and *Hypochaeris*. The cluster sub-divided into four sub-groups: a) *Scorzoneroides*; b) *Notoseris, Lactuca*, and *Cichorium*; c) *Launaea*, *Crepidiastrum*, *Reichardia,* and *Cicerbita*; and d) *Taraxacum*, *Helminthotheca*, and *Hypochaeris*. This group is also characterized by the prevalence of guaianolides. In contrast to group 1 most genera contain predominantly lactucin type compounds.

Group 3: only comprises the genus *Warionia*. This is the only genus in *Cichorieae* to contain epoxyguaian12,5-olides.

Group 4: comprises the genera *Dendroseris* and *Sonchus*. These two genera are characterized by the presence of reynosin type eudesmanolides.

Group 5: comprises only the genus *Hieracium*. It is characterized by the dominance of the tuberiferine type and their non-lactonized precursor acids.

Group 6: comprises the genera *Hedypnois* and *Leontodon* s.str. These are characterized by the presence of guaianolides of the hypocretonolides type.

Group 7: only comprises the genus *Urospermum*, which is characterized by the presence of melampolides type germacranolides.

The relative contribution of different compound classes to the total number of sesquiterpenoids for each genus in the Cichorieae tribe is summarized in the following **Table 1.1.**

Genus	N°	Eudesmanolides	Germacranolides	Guaianolides
Andrayala	11	0.0	0.0	100.0
Chondrilla	1	0.0	0.0	100.0
Cecerbita	6	0.0	16.7	83.3
Cichorium	38	23.7	2.6	73.7
Crepidiastrum	16	0.0	0.0	100.0
Cripis	95	3.2	4.2	92.6
Dendroseris	2	50.0	0.0	50.0
Hedypnois	4	0.0	0.0	100.0
Helminthotheca	12	33.3	16.7	50.0
Hieracium	11	81.8	9.1	9.1
Hypochaeris	41	14.6	9.8	75.6
Ixeris	106	12.3	15.1	75.6
Lactuca	105	1.9	20.0	78.1
Lapsana	5	0.0	0.0	100.0
Launaea	8	0.0	0.0	100.0
Leontodon	12	8.3	0.0	91.7
Mycelis	9	0.0	11.1	88.9
Nabalus	7	0.0	0.0	100.0
Notoseris	49	6.1	8.2	85.7
Picris	58	3.4	6.9	89.7
Reichardia	9	0.0	0.0	100.0
Scorzoneroides	48	0.0	2.1	97.9
Sonchus	48	64.6	18.8	16.7
Soroseris	6	0.0	0.0	100.0
Taeckholmia	11	0.0	0.0	100.0
Taraxacum	57	12.3	35.1	52.6
Urospermum	13	0.0	92.3	7.7
Warionia	15	13.3	0.0	86.7
Willemetica	1	0.0	0.0	100.0
Youngia	30	3.3	3.3	93.3
Total	**838**	**11.2**	**12.2**	**76.6**

Table 1.1 Relative contribution (%) of different compound classes of sesquiterpenoids in each genus of Cichorieae tribe

1.7 MAJOR SESQUITERPENOIDS ISOLATED FROM SOME GENERA OF CICHORIEAE

1.7.1 *Crepidiastrum* (15 species)

The chemical investigation of the aerial parts of the Japanese plant *Crepidiastrum lanceolatum* yielded 11 guaianolides, 10 of the lactucin type guaianolides **1**, **2**, **3**, **4**, **5**, **6**, **8**, **11**, **19**, and **23**, and one the hieracin type **80** (Takeda et al, 2005), while the chemical studied of another Japanese plant *Crepidiastrum keiskeanum* Nakai. afforded lactucin type guaianolides crepidiaside E **7**, crepidiaside C **9**, crepidiaside D **10**, and crepidiaside A **18** (Adegawa et al, 1985).

1.7.2 *Cichorium* (6 species)

A series of guaiane derivatives, 8-deoxylactucin **16**, jacquinelin **21**, crepidiaside B **22**, lactucin **25**, lactucopicrin **27**, 11β, 13-dihydrolactucin **33**, 11β,13-dihydrolactucopicrin **35**, and the eudesmane derivatives epiartesin **101** and santamarin **102** (Elmassry et al, 1984; Kisiel et al, 2003) were reported from the roots of the Poland cultivated plants *Cichorium pimulum*. Sesquiterpenoids reported from *Cichorium intybus L* (Chicory) prior to 2000 have been revised and summarized by Kisiel (Kisiel et al., 2001 a). These authors verified the presence of eight of the lactucin type **16**, **21**, **22**, **25**, **27**, **33**, **35**, and **36**, one costus lactone type **43**, one picridin type guaianolide **81**, and four eudesmane derivatives magnolialide **97**, artesin **98**, and their β-D-glycosides **99**, and **100** respectively. Additionally, Sessa and his collaborators (Sessa et al. 2000) reported from the latex of the Great Britain cultivated plants the 15-oxalates of 8-deoxylactucin **20**, lactucin **26** and lactucopicrin **29**.

Moreover, two lactucin type aldehyde **40** and **41** were reported from USA *Cichorium intybus* (Deng et al., 2001). The chemical study of the aerial parts of the Poland cultivated plants *Cichorium spinosum L.* yielded lactucin type guaianolides **25** and **33** and the eudesmanolide tanacetin **89** (Mickalska et al., 2007).

1.7.3 *Crepis* (200 species)

The chemical study of the aerial plants of the Egyptian plant *Crepis aspera* yielded costus lactone type guaianolide **16** (Ahmed et al, 2000).

The investigation of the Poland cultivated plants of *Crepis mollis* Ashers afforded guaianolides **49**, **58**, **60**, **67**, **68**, **71**, **72**, **74**, and **75** and germacranolide picriside B **112** (Kisiel et al., 2000).

The roots of the Polish plant *Crepis pulchra L* were found to contain the costus lactone type guaianolides **47** and **55** (Kisiel et al., 1994).

Moreover, the roots of the Poland cultivated plants *Crepis rhoeadifolia* M.Bieb yielded four costus lactone type guaianolides **50**, **58**, **69**, and **73** (Kisiel et al., 1996).

The flowers of the Italian plants *Crepis capillaris* yielded costus lactone guaianolide **75** (Barbetti et al., 1979). A more detailed investigation of the aerial parts and roots of the Poland plant yielded costus lactone type guaianolides **60**, **71**, and **72** (Kisiel, 1983a, 1983b 1984).

Similarly, Kisiel reported the isolation of costus lactone type guaianolides **50**, **58**, **60**, **61**, **69**, **72**, and **73** and the germacranolide picriside B **112** from the roots of *Crepis zacintha* L, a Poland cultivated plant (Kisiel et al., 2002a).

1.7.4 *Lactuca* (75 species)

The chemical investigation of the roots of the Polish plant *Lactuca serriola* yielded lactucin derivatives **16**, **21**, **25** and **27** (Marco et al., 1992a). A detailed study of the Poland plants *Lactuca tatarica*, yielded lactucin type guaianolides **21**, **22**, **25**, and **36** and costus guaianolides **50**, **55** and **58**, and germacranolides **112**, **115**, **117**, and **121** (Kisiel et al., 1997a), (Kisiel et al., 1998). In another study, the Chinese plants yielded **28** and **118** (Wang et al., 2006).

Aerial parts of *Lactuca sativa* from Egypt gave three lactucin derivatives **25**, **27**, and **33** and germacranolide **119**, melampolide **113** (Mahmoud et al, 1986). Japanese plants yielded lactucin derivative **12** and costus lactone type guaianolide **55** (Ishihara et al., 1987).

In addition Sessa et al (Sessa et al., 2000) found a number of sesquiterpenoids oxalates **20**, **26**, and **29** and sulfates **13** by direct HPLC–MS of latex obtained from *Lactuca sativa* cultivated in Great Britain. These compounds are the main constituents in the latex of the living plant but decompose rapidly when subjected to standard phytochemical purification techniques.

The whole plants of the Poland plants *Lactuca virosa* L. yielded lactucin derivatives **16**, **21**, **25**, **27**, and **33** (Gromek, 1989). Additionally, a detailed investigation of minor constituents of the roots of *Lactuca virosa* L, yielded four lactucin type **22**, **35**, **36**, and **38**, five costus lactone derivatives **50**, **54**, **55**, **56**, and **58** and two germacranolides **112** and **120** (Kisiel et al, 1997b). The chemical study of the roots of the Japanese plants *Lactuca laciniata* Roth yielded lactucin type **32** and **33**, costus lactone type guaianolides **47**, **50**, **54**, **55**, and **56**, eudesmanolide **103**, melampolides **132**, **133**, and **134** (Nishimura et al., 1986). Aerial parts of Egyptian plants *Lactuca saligna,* yielded **25**, **27**, and **35** (Khalil et al., 1991). Moreover, cultivated plants from Poland yielded lactucin type guaianolides **16**, **21**, **22**, **25**, **27** and **35**, costus lactone type guaianolides **55**, **58** and melampolide **134** (Kisiel et al., 1993).

1.7.5 *Ixeris* (20 species)

The Japanese whole plants *Ixeris debillis* A.Gray yielded costus lactone type guaianolides **47**, **52**, **55**, **60**, **61**, **62**, **63**, **64** and **65** (Warashina et al., 1990), while the chemical investigation of the Japanese whole plants *Ixeris rapens* yielded germacranolides **43**, **47**, **51**, **55**, **55**, **60**, **61**, **63**, and **70** and eudesmane derivatives **90**, **91**, **92**, **93**, **104**, **105**, **106**, **107**, **108** and **109** (Warashina et al., 1990).

The study of the *Ixeris stolonifera* A. Gray afforded costus lactone type guaianolides **55**, **60**, **61**, and **65** as well as hiearacin type **76** (Nishimura et al., 1985).

Taiwanese plant *Ixeris chinensis* Nakai yielded **52**, **60**, and **64** (Lee et al., 1994). In addition, the study of material from Mainland China gave **85** and **86** (Zhang et al., 2002). The whole plants of the Japanese plants *Ixeris tamagawaensis* Kitam yielded costus lactone type guaianolides **52**, **53**, and **58** and germacranolides **113** and **114**, melampolides **124**, **125**, **126**, **127**, **128**, **129**, **130**, and **131** (Assada et al., 1984).

1.7.6 *Andryala* (20 species)

The chemical investigation of aerial parts of *Andryala ragusina* from Spain yielded integrifolin-3β-D-glucopyranoside **60** lactone type guaianolide (Marco et al., 1994).

1.7.7 *Chondrilla* (25 species)

The subaerial parts of Italian plants *Chondrilla juncea* L. was found to contain ixerin F **58** (Zidorn et al., 2006).

1.7.8 *Cicerbita* (35 species)

The chemical investigation of the roots and leaves of *Cicerbita alpina* Wallr from Italy yielded lactucin derivative **33** (Appendino et al., 1991). Additionally, the roots of the Montenegro plants *Cicerbita alpina* afforded **25** and **34** (Djordjevic et al., 2004).

1.7.9 *Helminthothetica* (6 species)

The aerial parts of *Helminthothetica echioides* L(syn: *Picris echioides*) were extracted by diethyl ether in petroleum ether (1:2) to afford 8-deoxy lactucin **16**, jacquinelin **21** and 11-epi-jacquinelin **24** (Bohlmann et al 1981). Furthermore, Marco (Marco et al 1992b) reported two guaianolides **78** and **79**, and new germacranolide **123** from the aerial parts of the Spanish plant *Helminthothetica echioide.*

1.7.10 *Hypochaeris* (60 species)

The extraction of the roots and aerial parts of Venezuelan plants *Hypochaeris setosus* in soxhlet yielded to the isolation after purification of the guaianolides **21** and **84** as well as the eudesmanolide **99** (Gonzaléz et al., 1976).

1.7.11 *Notoseris* (12 species)

The chemical investigation of the aerial parts of Chinese plants *Notoseris psilolepis* C afforded lactucin derivatives **14**, **15**, **18**, **21** and **37**, costus type guaianolide **45** and melampolide **132** (Wang et al., 2000; Ye et al., 2001).

1.7.12 *Picris* (40 species)

The chemical investigation of the aerial parts of the Poland cultivated plant *Picris evaea* Luck yielded lactucin guaianolide **21** and costus lactone type guaianolides **42**, **43**, **44**, **45**, **56**, **58**, and **69** (Kisiel et al., 2001b).

In addition, the aerial parts of the Poland plant *Picris hieracioides* L yielded lactucin type guaianolide **21** and costus lactone guaianolide **50** (Kisiel, 1992) while the Japanese whole plants afforded five lactucin derivatives **25**, **30**, **31**, **33**, and **39**, two costus guaianolides **48** and **58** and two germacranolides **112** and **115** (Nishimura et al., 1986).

In the same way, the study of the Poland cultivated plants (Kisiel et al., 2002b) reported the isolation of two lactucin derivatives **16**, and **21** from the aerial parts of *Picris kamtschatica* Ledeb whereas the roots contained the unusual guaianolide **87**, and eight costus lactone derivatives **42**, **44**, **45**, **49**, **50**, **56**, **57**, and **58**. Recently, the Poland cultivated plants *Picris koreana* (Kitam) was found to possess lactucin derivatives **14**, **33** and **42**, **50**, **56**, and **58** and the germacranolide **117**, in addition to the two eudesmanolides **97**, and **102** (Michalska et al., 2007).

1.7.13 *Reichardia* (8 species)

One new lactucin derivative sulfate **17** was isolated recently from the roots of Spanish plants *Reichardia gaditana* (Willk) (Zidorn et al., 2007).

1.7.14 *Sonchus* (60 species)

The chemical investigation of Japanese whole plants *Sonchus oleraceus* yielded lactucin derivative **18** and costus lactone derivatives **47** and **55**, in addition to the eudesmanolides **88**, and **96** and the germacranolides **112**, **115**, **117**, and **122** (Miyase et al., 1987).

1.7.15 *Taeckholmia* (8 species)

The chemical study of the aerial parts of the species *Taekholmia pinanta* (L.fil.) Boulos, from Canary Islands reported costus lactone guaianolide **59** (Gonzalez et al., 1985).

1.7.16 *Taraxacum* (more than 500 species)

Some species of the genus *Taraxacum* cultivated in Poland have been investigated for the occurrence of the three types of the sesquiterpenoids, as reported by the authors Kisiel and Michalska.

Taraxacum becorne Dahlst yielded germacranolides **110**, and **111** and the costus guaianolide **47** (Michalska et al., 2001). Additionally, *Taraxacum obavatum* DC, yielded lactucin derivatives **14**, and **15** and the germacranolides **110**, **111**, and **117** (Michalska et al., 2003). Lactucin derivatives **14**, and **15**, and costus guaianolides **47**, and **50**, and the germacranolides **110**, **111**, **112**, and **117** were isolated from *Taraxacum hondoense* Nakai ex Koidz (Kisiel et al., 2005). The germacranolides **110** and **111** were also isolated from the roots of the France species *Taraxacum rubicundum* (Dahlst) (Michalska et al., 2005).

The first study of commercial species *Taraxaum officinale* (currently named *Taraxacum* sectio Ruderelia) yielded eudesmanolides **94**, and **95** and the germacranolides **110**, and **111** (Hansel et al., 1980). Moreover, the recent study reported by Kisiel and Barszcz revealed the presence of the lactucin derivative **33**, and the costus lactone **52** (Kisiel et al., 2000), in addition to the germacranolides **110**, and **111**, which were previously reported from *Taraxacum laevigatum* and *Taraxacum disseminatum* **(**Zelinska and Kisiel, 2000).

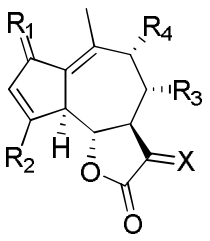
1.7.17 *Warionia* (monotypic)

The dichloromethane extract of the leaves of the Morocco plant *Warionia saharae* Benth. Coss afforded hypochaerin type guaianolides **82** and **83** (Hilmi et al, 2002).

1.7.18 *Youngia* (40 species)

The chemical investigation of the Japanese plant *Youngia japonica* (syn: *Cripis japonica*) (L) Benth. led to the isolation of costus lactone type guaianolides **46**, **47**, **48**, and **59** (Miyase et al, 1983, 1985).

All the structures cited above, sesquiterpenoids **1-134**, are indicated in the following figures with the common name.

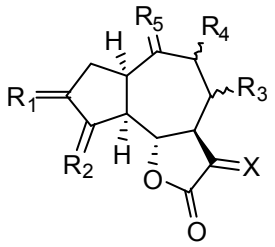


Nr.	R_1	R_2	R_3	R_4	X	Common name

1	H,H	CH_2OGlc	H	OH	CH_2	youngiaside A
2	H, H	CH_2OGlc-6'-*O*-PPA	H	OH	CH_2	youngiaside D
3	H, H	CH_2OGlc	H	OH	αCH_3, βH	lanceocrepidiaside A
4	H, H	CH_2OGlc-6'-*O*-PPA	H	H	αCH_3, βH	lanceocrepidiaside B
5	H, H	CH_2OGlc	αOH	H	CH_2	Ixerin Y
6	H, H	CH_2OGlc	αO-PPA	H	CH_2	Lanceocrepidiaside C
7	H, H	CH_2OGlc-2'-*O*-PPA	αOH	H	CH_2	Crepidiaside E
8	H, H	CH_2OGlc-6'-*O*-PPA	αOH	H	CH_2	Crepidialanceoside A
9	H, H	CH_2OGlc	αOH	H	αCH_3, βH	Crepidiaside C
10	H, H	CH_2OGlc-2'-*O*-PPA	αOH	H	αCH_3, βH	Crepidiaside D
11	H, H	CH_2OGlc-6'-*O*-PPA	αOH	H	αCH_3, βH	Crepidialanceoside B
12	O	CH_3	H	αOGlc	αCH_3, βH	Lactuside C
13	O	CH_3	αOSO_3H	H	CH_2	15-deoxylactucin-8-sulfate
14	O	CH_3	αOH	H	αCH_3, βH	desacetylmatricarin
15	O	CH_3	αOGlc	H	αCH_3, βH	notoserolide A
16	O	CH_2OH	H	H	CH_2	8-deoxylactucin
17	O	CH_2O-HMPS	H	H	CH_2	8-deoxy-15-(3'-hydroxy-2'-metylpropanoyl)-lactucin-3'-sulfate
18	O	CH_2OGlc	H	H	CH_2	Crepidiaside A
19	O	CH_2OGlc-6'-*O*-PPA	H	H	CH_2	lanceocrepidiaside D
20	O	CH_2O-C_2O_3H	H	H	CH_2	15-deoxylactucin-15-oxalate
21	O	CH_2OH	H	H	αCH_3, βH	Jacquinelin
22	O	CH_2OGlc	H	H	αCH_3, βH	Crepidiaside B
23	O	CH_2OGlc-6'-*O*-PPA	H	H	αCH_3, βH	lanceocrepidiaside F
24	O	CH_2OAc	H	H	βCH_3, αH	11-epi-jaquinelin
25	O	CH_2OH	αOH	H	CH_2	lactucin
26	O	CH_2O-C_2O_3H	αOH	H	CH_2	lactucin-15-oxalate
27	O	CH_2OH	αO-PPA	H	CH_2	lactucopicrin
28	O	CH_2OH	αO-PMP	H	CH_2	Lactucin-8-*O*-*p*-methoxyphenylacetate
29	O	CH_2O-C_2O_3H	αO-PPA	H	CH_2	lactucopicrin-15-oxalate
30	O	CH_2OGlc	αOH	H	CH_2	Picriside A
31	O	CH_2OGlc	αO-PIC	H	CH_2	Picrioside A
32	O	CH_2OGlc	αO-PPA	H	CH_2	Lactucopicriside
33	O	CH_2OH	αOH	H	αCH_3, βH	11β,13-dihydrolactucin
34	O	CH_2OH	αO-OAc	H	αCH_3, βH	8-acetyl-11β,13-dihydrolactucin
35	O	CH_2OH	αO-PPA	H	αCH_3, βH	11β,13-dihydrolactucopicrin
36	O	CH_2OGlc	αOH	H	αCH_3, βH	Cichorioside B
37	O	CH_2OGlc	αO-ANG	H	αCH_3, βH	notoserolide
38	O	CH_2OH	αO-MAC	H	αCH_3, βH	11β,13-dihydrolactucopicrin-8-*O*-methylcrylate

39	O	CH_2OGlc	αO-PIC	H	αCH_3, βH	Hypochoreoside B
40	O	CHO	αO-PPA	H	CH_2	15-dehydrolactucopicrin
41	O	CHO	αO-PPA	H	αCH_3, βH	15-dehydro-11β,13-dihydrolactucopicrin

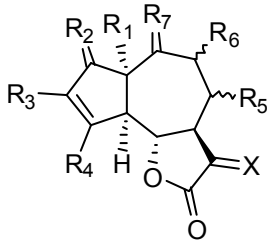
Figure 1.1: Selected lactucin type guaianolides



Nr.	R_1		R_3	R_4	R_5	X	Common name
42	H, H	CH_2	H	αOH	CH_2	CH_2	9α-hydroxy-3-deoxyzaluzanin
43	H, H	CH_2	H	αOGlc	CH_2	CH_2	Ixerisoside D
44	H, H	CH_2	H	αOH	CH_2	αCH_3, βH	Annuolide D
45	H, H	CH_2	H	αOGlc	CH_2	αCH_3, βH	Scorsoside
46	αH, βOH	CH_2	H	H	CH_2	CH_2	Zaluzanin
47	αH, βOGlc	CH_2	H	H	CH_2	CH_2	Glucozaluzanin C
48	αH,βOGlc-6'-	CH_2	H	H	CH_2	CH_2	Crepiside A
49		CH_2	H	H	CH_2	αCH_3, βH	11β, 13-dihydrozaluzanin C
50	αH, βOH	CH_2	H	H	CH_2	αCH_3, βH	11β, 13-dihydrogluco
	αH, βOGlc						zaluzanin C
51		CH_2	H	H	CH_2	αCH_3, βOH	Ixerisoside C
52	αH, βOGlc	CH_2	H	H	αOH,	CH_2	Ixerin D
53	αH, βOGlc	CH_2	H	H		αCH_3, βOH	Ixerin E
54	αH, βOGlc	CH_2	H	αOH	αOH,	CH_2	9α-hydroxyzaluzanin C
55	αH, βOH	CH_2	H	αOH		CH_2	Macrocliniside A
56	αH, βOGlc	CH_2	H	αOH	CH_2	αCH_3, βH	9α-hydroxy-11β,13-dihydro
	αH, βOH				CH_2		zaluzanin C
57		CH_2	H	αOH	CH_2	αCH_3, βH	9α-hydroxy-11β,13-dihydro
	αH, βOAllo						zaluzaninC-3-*O*-β-allopyranoside
58		CH_2	H	αOH	CH_2	αCH_3, βH	Ixerin F
59	αH, βOGlc	CH_2	αOH	H		CH_2	8-desacylcynapicrin
60	αH, βOH	CH_2	βOH	H	CH_2	CH_2	Integrifolin-3β-D-gluco-
	αH, βOGlc				CH_2		pyranoside

61		CH_2	βO-HPL	H	CH_2	CH_2	Ixerin M
62	αH, βOGlc	CH_2	βO-HMV	H		CH_2	Ixerin N
63	αH, βOGlc	CH_2	βO-HPL	H	CH_2	CH_2	tectoroside
64	αH, βOGlc	CH_2	βO-PPA	H	CH_2	CH_2	ixerisoside B
65	αH, βOGlc	CH_2	βO-HMB	H	CH_2	CH_2	Ixerin P
66	αH,βOGlc-	CH_2	βOH	H	CH_2	αCH$_3$, βH	11βH-11, 13-hydrointegrifolin
67		CH_2	βOH	H	CH_2	αCH$_3$, βH	11βH-11,13-hydrointegrifolin D-
68	αH, βOGlc	CH_2	βOH	H	CH_2	αCH$_2$OCH$_3$,βH	-
69	αH, βOH	αCH$_3$,βH	H	αOH	CH_2	αCH$_3$, βH	9α,hydroxy-4β,11β,13,15-tetrahydro zaluzanin C
70	αH, βOGlc	αCH$_3$,βH	βO-HPL	H	CH_2	CH_2	Ixerisoside B
71	αH,βOH	αCH$_3$,βH	βOH	H	CH_2	αCH$_3$, βH	8-epiisolipidiol
72	αH, βOGlc	αCH$_3$,βH	βOH	H	CH_2	αCH$_3$, βH	8-epiisolipidiol-3-*O*-β-D-glucopyranoside
73	αH, βOH	αH,βCH$_3$	H	αOH	CH_2	αCH$_3$, βH	9α,hydroxy-4α,11β,13,15-tetrahydrozaluzanin C
74	O	αCH$_3$,βH	αOH	H	CH_2	αCH$_3$, βH	9α,hydroxy-4β,11β,13,15-tetrahydro-dehydrozaluzanin C
75	O	αCH$_3$,βH	βOH	H	CH_2	CH_2	8-epigrosheimin

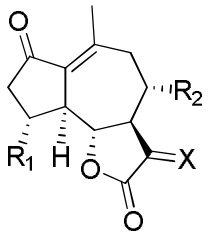
Figure 1.2: Selected costus type guaianolides



Nr.	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**	**X**	**Common name**
76	αH	H, H	OGlc	CH_3	βO-HMB	H	CH_2	CH_2	Ixerin S
77	αH	O	H	CH_3	αO-Ac	H	αH, βCH$_3$	CH_2	montanone
78	αH	O	H	CH_2OH	H	H	αH, βCH$_3$	CH_2	Heriacin II
79	αH	O	H	CH_2OH	H	H	αH, βCH$_3$	αCH$_3$, βH	Heriacin I
80	α/β OH	O	H	CH_2OGlc-6'-*O*-PPA	H	H	αH, βCH$_3$	CH_2	Lanceo-cripidiaside F

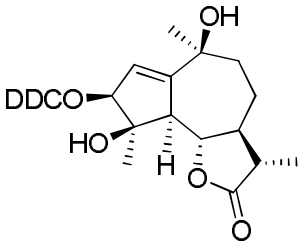
Figure 1.3: Selected heriacin type guaianolides

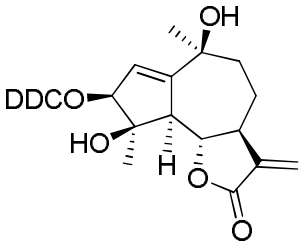
Other picridin, hypocharein, and guai-8-enolides type guaianolides are illustrated.

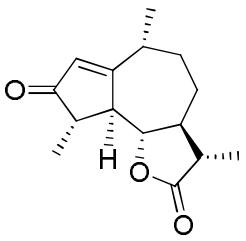


81 R_1 = CHO R_2 = O-PPA X = CH_2

Picridin

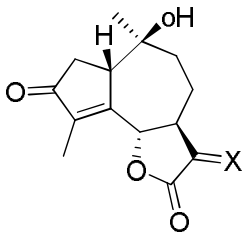


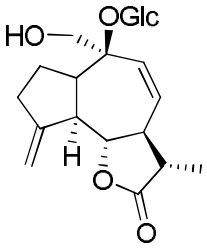




82 **83** **84**

Hypocharein





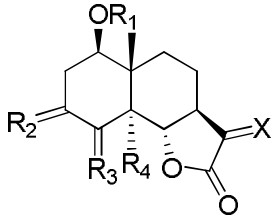
85 X = CH_2; Chinensiolide

86 X = αCH_3, βH

87 Guai-8-enolide

Chinensiolide

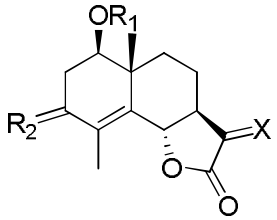
Figure 1.4: Selected additional guaianolide types



Nr.	R_1	R_2	R_3	R_4	X	Common name
88	Glc	H, H	CH_2	H	CH_2	Sonchuside D
89	H	H,H	CH_2	OH	CH_2	Tanacetin
90	HPL	H, H	CHOGlc*	H	CH_2	Ixerisoside M
91	HPL	H, H	αCH_2OGlc, βH	H	CH_2	Ixerisoide G
92	HPLHMB	H, H	αCH_2OGlc, βH	H	CH_2	Ixerisoide H
93	H	H, H	αCH_2OGlc, βH	H	CH_2	Ixerisoide I
94	H	H, βOH	αH, βCH_3	H	αCH_3, βH	4α,15,11β,13-tetrahydroridentin B
95	H	O	αCH_3, βH	H	αCH_3, βH	Taraxacolide-*O*-β-D-glucopyranoside

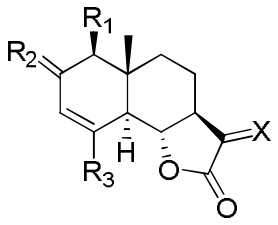
*Double bond from C-4 to C-15

Figure 1.5: Reynosin type 12,6-eudesmanolides.



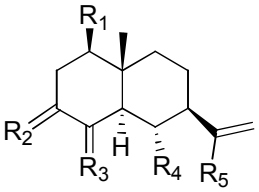
Nr.	R_1	R_2	X	Common name
96	H	αH, βOGlc	αCH_3, βH	Sonchuside C
97	OH	H, H	CH_2	Magnolialide
98	OH	H, H	αCH_3, βH	Artisan
99	OGlc	H, H	CH_2	-
100	OGlc	H, H	αCH_3, βH	-
101	OH	H, H	αCH_3, βH	11-epicartesin

Figure 1.6: Magnolialide type 12,6-eudesmnolides



Nr.	R_1	R_2	R_3	X	Common name
102	OH	H, H	CH_3	CH_2	Santamarin
103	OH	H, H	CH_3	αCH_3, βH	Dihydrosantamarin
104	OH	H, H	CH_2OGlc	CH_2	Ixerisoside E

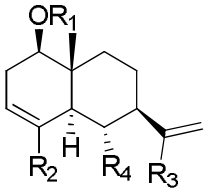
Figure 1.7: Santamarin type 12,6-eudesmanolides



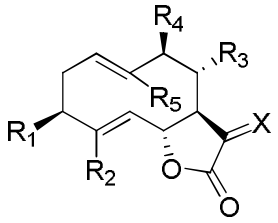
Nr.	R1	R2	R3	R4	R5	Common name
105	O-HMB	H, H	CHOGlc*	OH	$COOCH_3$	Ixerisoside N
106	O-HMB	H, H	αCH_2OGlc, βOH	OH	$COOCH_3$	Ixerisoside I
107	O-HPL	H, H	αCH_2OGlc, βOH	OH	$COOCH_3$	Ixerisoside K
108	O-HPL-HMB	H, H	αCH_2OGlc, βOH	OH	$COOCH_3$	Ixerisoside L

*Double bond from C-4 to C-15

Figure 1.8: Non-lactonized eudesmane derivatives with exocyclic double bonds

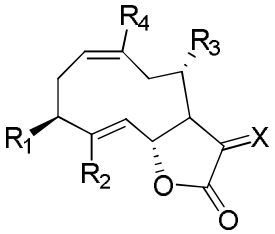


109 R_1 = HPL $R_2 = CH_2OGlc$ $R_3 = COOCH_3$ Ixerisoside F



Nr.	R_1	R_2	R_3	R_4	R_5	X	Common name
110	H	CH_3	H	H	COOGlc	CH_2	Taraxinic acid -1'-*O*-β-D-glycopyranoside
111	H	CH_3	H	H	COOGlc	αCH_3, βH	11β,13-dihydrotaraxinic acid-1'-*O*-β-D-glycopyranoside
112	H	CH_2OGlc	H	H	CH_3	CH_2	Picriside B
113	H	CH_2OGlc	H	H	CH_3	αCH_3, βH	Ixerin H
114	H	CH_2OGlc	H	H	CH_2O-PPA	CH_2	Ixerin I
115	OGlc	CH_3	H	H	CH_3	CH_2	Picriside C
116	OH	CH_3	H	H	CH_3	αCH_3, βH	11β, 13-dihydrohanphillin
117	OGlc	CH_3	H	H	CH_3	αCH_3, βH	Sonchuside A
118	OH	CH_3	H	H	CH_3	αCH_3, βOH	3β,11 β -dihydroxy-11,13-dihydrocostunolide
119	OH	CH_3	H	H	CH_2OH	αCH_3, βH	3β,14-dihydroxy-11β,13-dihydrocostunolide
120	OGlc	CH_3	H	H	CH_2OH	αCH_3, βH	3β,14-dihydroxy-11β,13-dihydrocostunolide-3-*O*-*β*-glucopyranoside.
121	OGlc	CH_3	H	H	CH_2OH	αCH_3, βOH	Tataroside
122	OGlc	CH_3	H	O-PMP	CH_3	CH_2	Sonchuside B
123	OH	CH_3	OH	H	CH_3	αCH_3, βOH	3β,8α,11β-trihydroxy-11,13-dihydrocostunolide

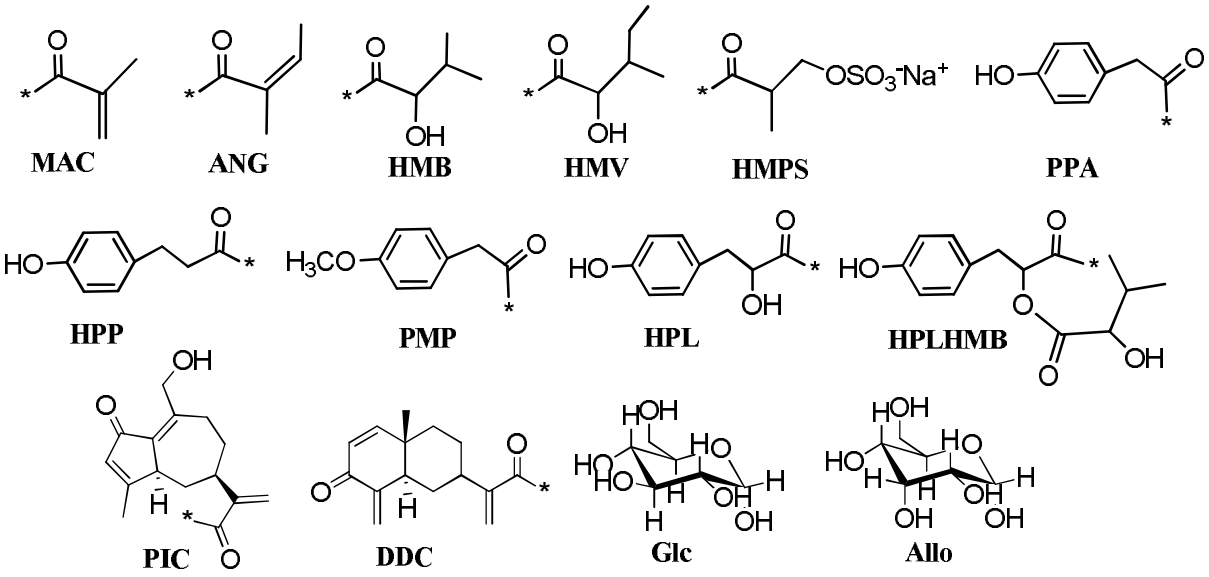
Figure 1.9: Costunolide type 12,6-germacranolides



Nr.	R_1	R_2	R_3	R_4	X	Common name
124	H	CH_2OH	H	CH_2OH	αCH_3, βH	Ixerin K
125	H	CH_2OH	H	CH_2OH	αH, β CH_3	Ixerin L
126	H	CH_2OH	H	CHO	CH_2	8-deoxyurospermal A
127	H	CH_2OGlc	H	CHO	CH_2	Ixerin B
128	H	CH_2OGlc-2'-*O*-PPA	H	CHO	CH_2	Ixerin G

129	H	CH_2OGlc-6'-*O*-PPA	H	CHO	CH_2	Ixerin A
130	H	CH_2OH	H	CHO	αCH_3, βH	Ixerin C
131	H	CH_2OGlc	H	CHO	αCH_3, βH	Ixerin J
132	OGlc	CH_3	H	CH_2OH	αCH_3, βH	Lactuside B
133	OH	CH_3	H	CHO	αCH_3, βH	Lactilide A
134	OGlc	CH_3	H	CHO	αCH_3, βH	Lactuside A

Figure 1.10: Melampolide type 12,6-germacranolides



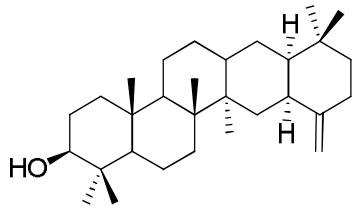
1.8 MAJOR TERPENOIDS ISOLATED FROM SOME GENERA OF CICHORIEAE (LACTUCEAE)

Plants of the tribe Cichorieae have been reported to contain also triterpenoids. The majority of these triterpenes are pentacyclic and belong to lupane, oleanane, migrated oleanane, gammacerane, migrated gammacerane, ursane, and migrated ursane groups, with some tetracyclic compounds.

The fresh roots of the Japanese specie *Picris hieracioides* subsp. *japonica* yielded about forty-five triterpenoids (Shiojima et al., 1995a, 1995b, 1989), whereas the phytochemical study of the dried aerial parts and roots of *Ixeris chinensis* led to the isolation of thirty-nine triterpenoids (Shiojima et al., 1996) with similar structural type skeleton as *Picris hieracioides*.

Eleven triterpenoids: β-amyrin **139**, β-amyrin acetate **141**, taraxerol **142**, taraxeryl acetate **143**, taraxasterol **146**, taraxasteryl acetate **148**, ψ-taraxasteryl acetate **151**, α-amyrin acetate **154**, lupeol **186**, lupenone **187**, and lupenyl acetate **188** (Takasaki et al., 1999) were isolated from the roots of the Japanese specie *Taraxacum japonicum*. These compounds were tested for their anti-carcinogenic activity and compound **146** showed a remarkable inhibitory effect on mouse spontaneous mammary tumour. These results suggested that taraxasterol **146** could be a valuable chemo-preventive agent.

All the structures **135-199** isolated from both plants are described in figure 1.11.



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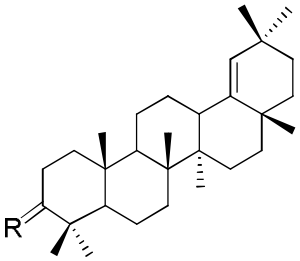
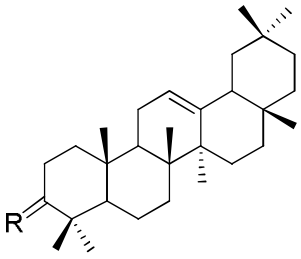
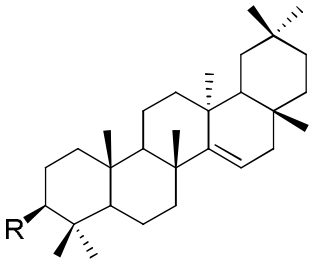


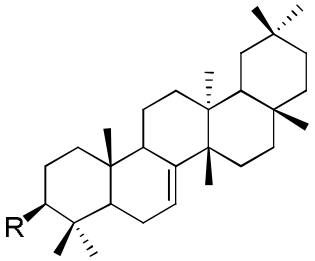
136 R = α H,βOH
137 R = O
138 R = α H, βOAc



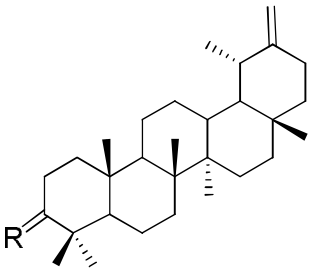
139 R = α H, βOH
140 R = O
141 R = α H, βOAc



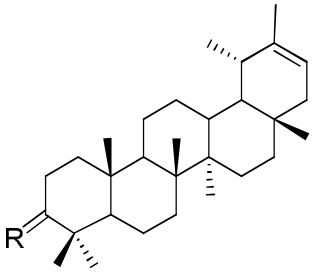
142 R = OH
143 R = OAc



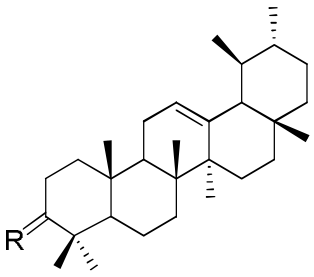
144 R = OH
145 R = OAc



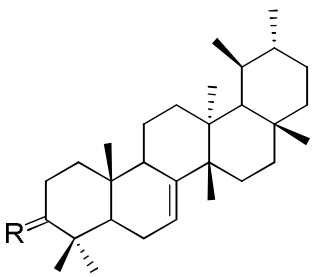
146 R = α H, βOH
147 R = O
148 R = α H, βOAc



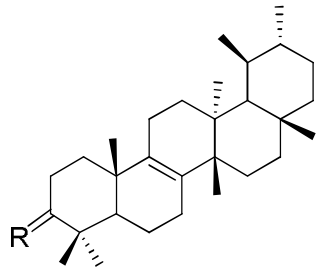
149 R = α H, βOH
150 R = O
151 R = α H, βOAc



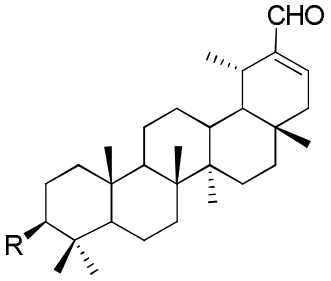
152 R = α H, βOH
153 R = O
154 R = α H, βOAc



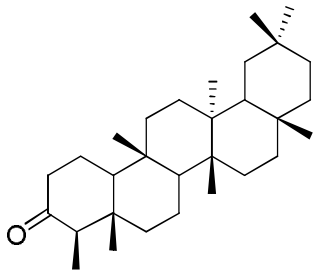
155 R = α H, βOH
156 R = O
157 R = α H, βOAc



158 R = α H, βOH
159 R = O
160 R = α H, βOAc



161 R = OH
162 R = OAc



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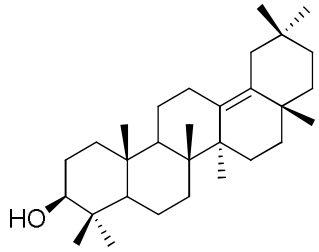

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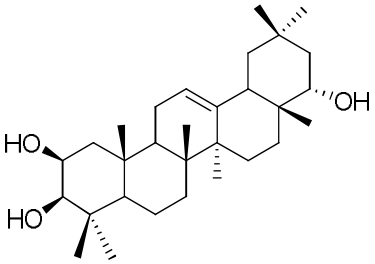

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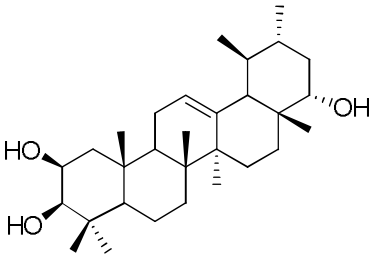

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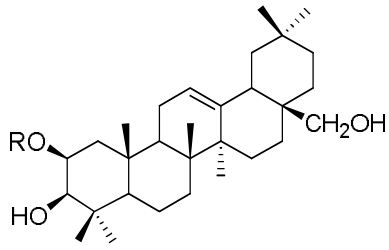
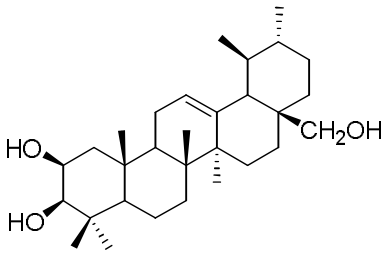


167 R = H
168 R = OH



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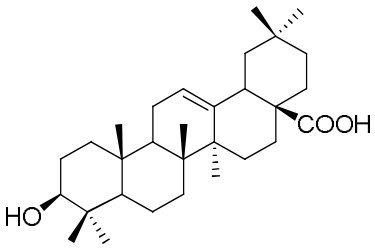

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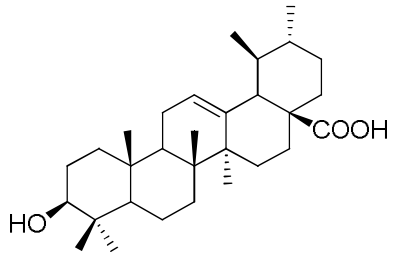

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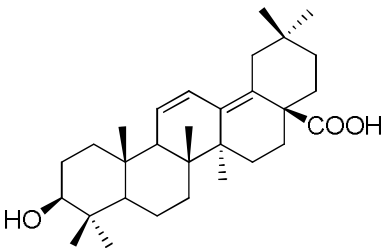

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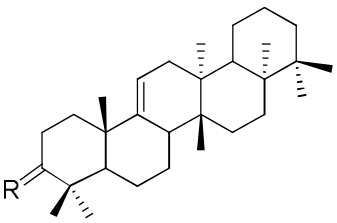
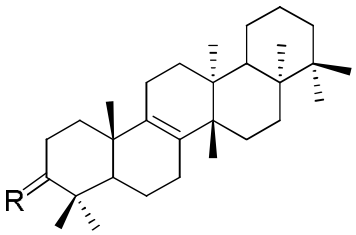
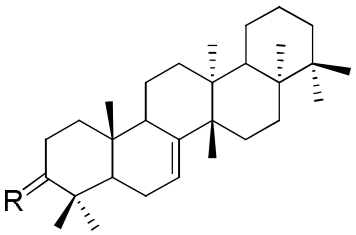


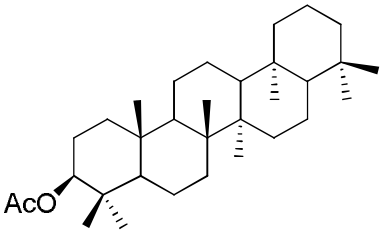
173 R = α H, βOH
174 R = O
175 R = α H, βOAc



176 R = OH
177 R = OAc



178 R = OH
179 R = OAc



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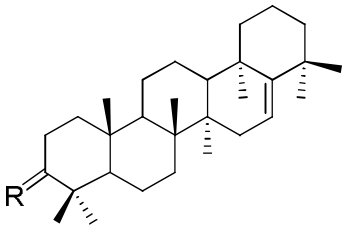
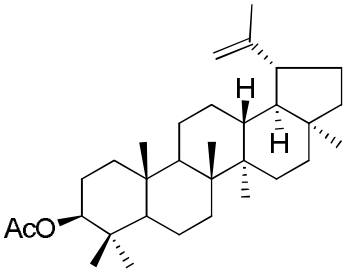


181 R = a H, βOH
182 R = αOH, β H
183 R = O
184 R = α H, βOAc



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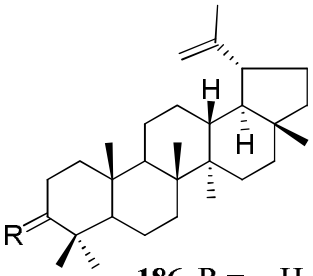
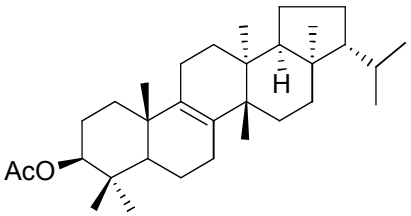


186 R = α H, βOH
187 R = O
188 R = α H, βOAc



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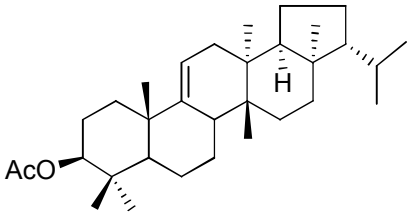

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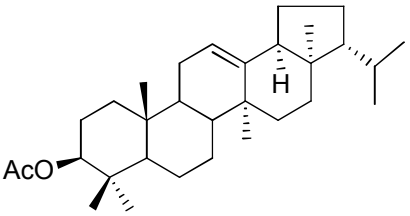

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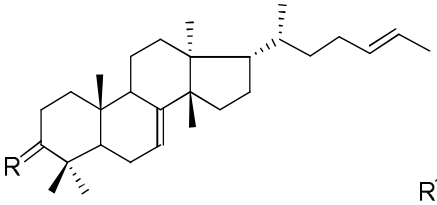
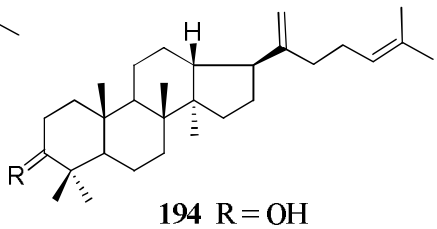
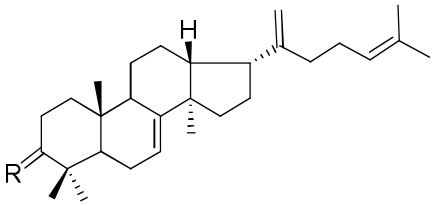


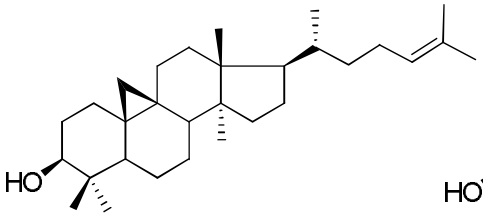
192 R = OH
193 R = OAc



194 R = OH
195 R = OAc



196 R = OH
197 R = OAc



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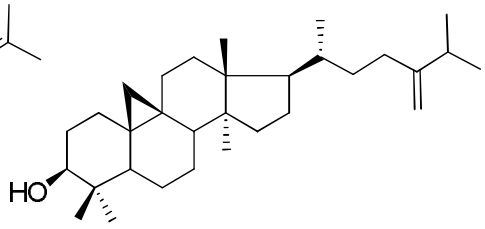

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Figure 1.11: Selected pentacyclic and tetracyclic triterpenoids isolated from Cichorieae tribe

1.9 MAJOR PHENOLIC COMPOUNDS ISOLATED FROM SOME GENERA OF CICHORIEAE (LACTUCEAE)

Several phenolic compounds such as chicoric acid and its isomer, monocaffeoyltartaric, 4-caffeoylquinic, chlorogenic, caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and *p*-hydroxyphenylacetic acids as well as various flavonoids glycosides such as luteolin-7-*O*-glucoside, luteolin-7-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, quercetin-7-*O*-glucoside and apigenin-7-*O*-glucoside were identified in the aerial parts and roots of some species of Cichorieae tribe (**Figure 1.12**), in addition to coumarins, umbelliferone, esculetin, scopoletin and chicoriin.

Crepis capillaris, *Hieracium pilosella*, and *Hypochaeris radicata* were investigated for the influence of the altitude of the collection site on the content of phenolics within the flowering heads. Flowering heads collected from different altitudes ranging from 180 m to 1060 m (*C. capillaris*), from 190 to 1290 m (*H. pilosella*), and from 20 m to 1290 m (*H. radicata)*, respectively, were extracted and analyzed by high performance liquid chromatography. The results showed a positive correlation between the altitude of the growing site and the contents of flavonoids and phenolic acids for all investigated taxa (Zidorn, 2005).

The chemical investigation of roots of *Taraxacum officinale* (Dandelion) yielded dihydroconiferin **200**, a mixture of syringin **201**, and dihydrosyringin **202** (Kisiel et al., 2000b). In addition, *p*-coumaric and caffeic acids **203-204** were previously isolated (Clifford et al, 1987). Moreover, the leaves and flowers of *Taraxacum officinale* afforded flavonoids such as quercetin-7-*O*-glucoside **212**, apigenin-7-*O*-glucoside **215**, luteolin-7-*O*-glucoside **216**, luteolin-4'-*O*-glucoside **217**, and luteolin-7-*O*-rutinoside **218**, and three coumarins, umbelliferone **219**, scopoletin **220**, and esculetin **221** (Williams et al, 1996).

The ethanol extract of the roots of *Crepis mollis* yielded some phenolic compounds such as 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone **209** and 5-methoxyeugenyl-4-*O*-β-glucopyranoside **210** (Kisiel et al, 2000a).

The presence of phenolic compounds has been evidenced also in some species of the genus *Cichorium*.

The roots of *Cichorium endivia* yielded ethyl *trans*-caffeate **205**, methyl and ethyl *p*-hydroxyphenylacetates **207** and **208 (**Kisiel et al, 2006). In addition, the aerial parts of *Cichorium spinosum* afforded many secondary metabolites, including the coumarins umbelliferone **219**, scopoletin **220**, esculetin **221** and cichoriin **222** (Michalska et al., 2007).

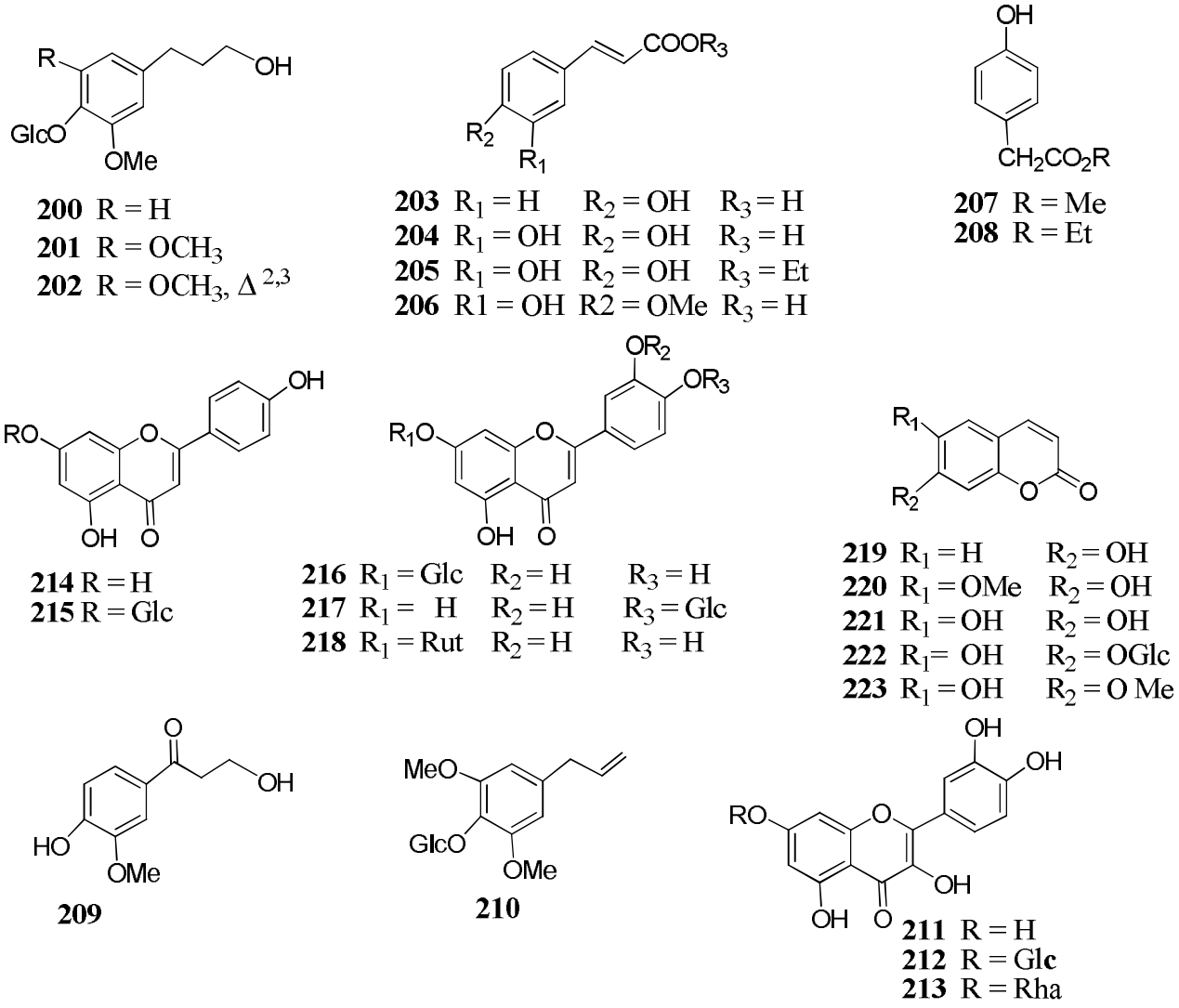


Figure 1.12: Selected phenolic compounds isolated from the tribe Cichorieae

1.10 DESCRIPTION OF THE GENUS *LAUNAEA* CASSINI (ZOLLICOFERIA DC)

The genus *Launaea* Cass. belongs to the tribe Lactuceae of the Asteraceae family and contains about 40 species, most of which are adapted to dry, saline and sandy habits (Ozenda, 2004). Plants of this genus have very row stems, hairless leaves incised into lobes that are themselves lined with white teeth, membranous scales on the edges, yellow ligules, and elongated chain, prismatic or slightly flattened.

The genus *Launaea* is represented in the flora of Algeria by nine species including five endemics of north Africa: *L. angustifolia*, *L. quercifolia*, and *L. cassiniana* are the endemic plants of the north Africa, with limited distribution (Quezel et al., 1963), (Ozenda, 2004), whereas *L. acanthoclada* and *L. arborescens* are two endemic plants of the north-west of Africa. *L. acanthoclada* also grows in the arid regions of the south-east of Spain.

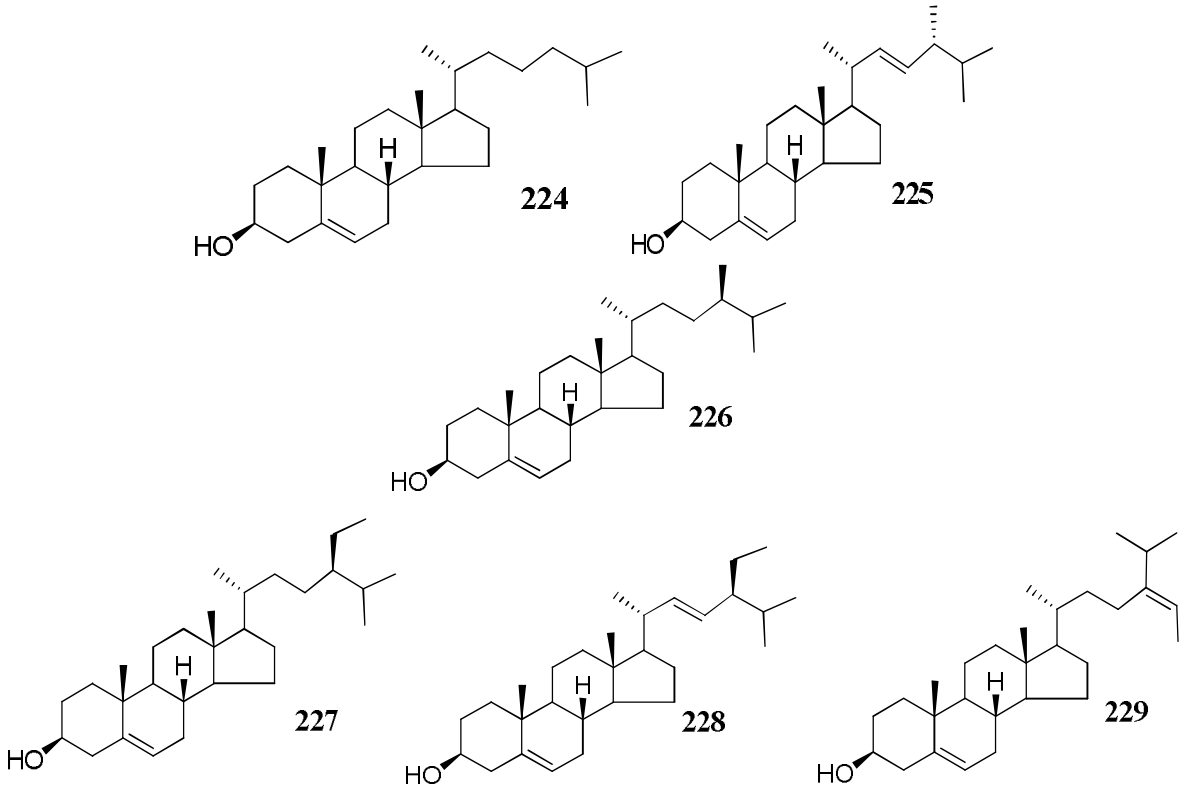
The other four species cover all the Sahara and have a large distribution at the northern, westerner, and central part of the Sahara. *L. nudicaulis* and *L. residifolia* sprout in Algeria and Tunisia Mediterranean Sea. *L. residifolia* can reach even the Sicily, whereas *L. glomerata* and *L. mucronata* grow in the Saharan Atlas (Quezel et al., 1963).

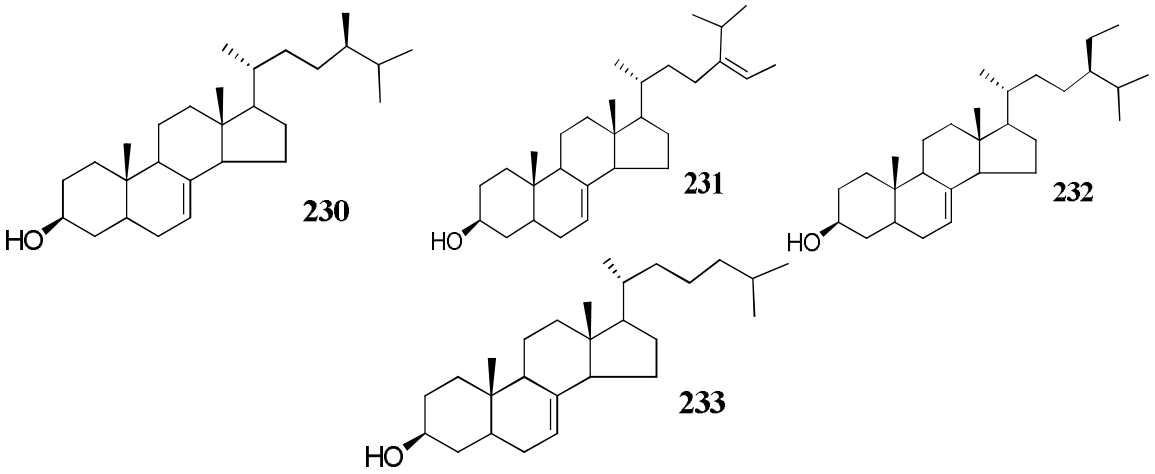
1.10.1 Secondary metabolites isolated from the genus *Launaea*

Different secondary metabolites including terpenoids, steroids and phenolic compounds have been identified from the genus *Launaea*. In addition, few sesquiterpene lactones have been reported from various species of this genus. The occurrence of flavones glycosides is remarkable.

1.10.2 *Launaea nudicaulis Hook.* F

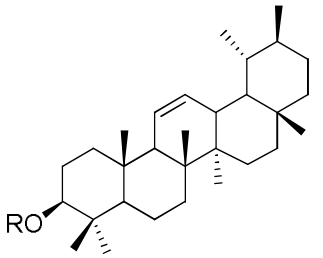
Chemical investigation of the light petroleum extract of *Launaea nudicaulis* leads to the characterization of ten Δ^7 and Δ^5 sterols: cholesterol **224**, brassicasterol **225**, campesterol **226**, β-sitosterol **227**, stigmasterol **228**, fucosterol **229**, 24β-Δ^7-ergosten-3β-ol **230**, stigmasta-7,24(28)-dien-3–ol, **231**, Δ^7-stigmastenol **232**, and Δ^7-cholesterol **233** (Behari et al., 1980).



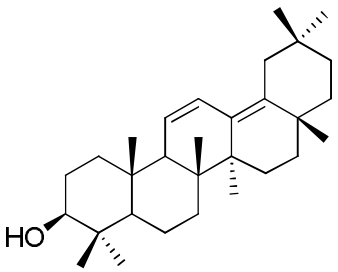


A more detailed investigation of *Launaea nudicaulis* yielded some triterpenes such as taraxasterol **146**, and **149** (Majumder et al., 1982), germanicol **136**, β-amyrin **139**, 3β-taraxerol **142**, ψ-taraxasterol **149**, α-amyrin **152**, and lupeol **186** (Hook et al., 1984).

Recently, two new ursene type triterpenes, nudicauline A **234**, and nudicauline B **235** have been isolated from the aerials parts of *Launaea nudicaulis* collected in Pakistan, along with olean-11,13(18)-diene **240**, 3β-hydroxy-13(28)-epoxy-urs-11-ene **237** and 3-keto-13(28)-epoxy-urs-11-ene **238** (Zaheer et al., 2006).



234 R = OH
235 R = Ac



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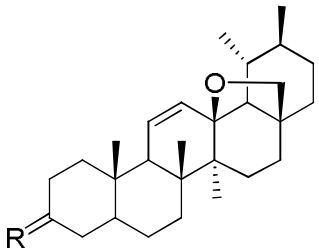
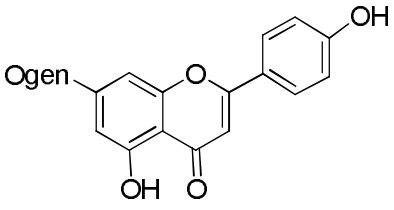


237 R = α H, βOH
238 R = O

Additionally, eight flavone glycosides were reported from the fresh sample of *Launaea nudicaulis* collected in Egypt. These compounds were identified as apigenin-7-*O*-glucoside **215**, luteolin-7-*O*-glucoside **216**, luteolin-7-*O*-rutinoside **218**, which are common metabolites

within the Lactuceae, apigenin-7-*O*-gentiobioside **239**, luteolin-7-*O*-gentiobioside **240**, and three uncommon glycosides luteolin-7,3'-diglucoside **241**, luteolin-7',4'-diglucoside **242** and luteolin-7-*O*-gentiobioside-4'-*O*-glucoside **243** (Ragaa et al., 1983).



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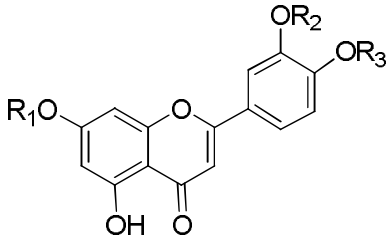
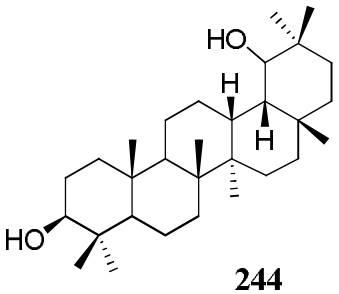


240 R_1= Gent R_2 = H R_3 = H
241 R_1 = Glc R_2 = Glc R_3 = H
242 R_1 = Glc R_2 = H R_3 = Glc
243 R_1 = Gent R_2 = H R_3 = Glc

Moreover, two common coumarins, esculetin **221**, and cichoriin **222**, were also described (Sarg et al., 1986).

1.10.3 *Launaea spinosa (*Forssk.)

The chemical investigation of an Egyptian sample of the plant yielded the isolation of one guaianolide type sesquiterpene lactone crepidiaside A (**18**), in addition to β-sitosterol **222**, stigmasterol **223**, ψ-taraxasterol 30-aldehyde **161**, friedelin **163**, lupeol and its acetate **186** and **188**. Moreover, three other triterpenes, oleanane-3,19-diol **244**, moretene-3,28-diol **245**, and moretene-3,19–diol **246** were isolated from the same sample (Sokkar et al., 1993).



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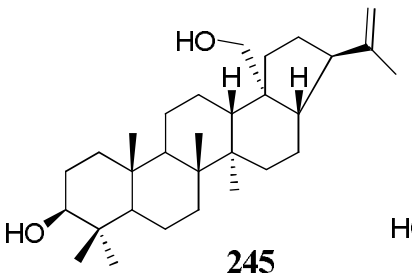

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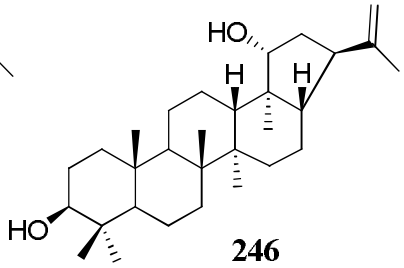
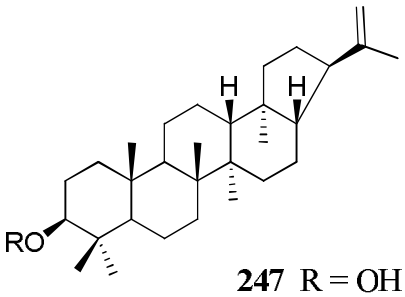


246

A second study reported the isolation of luteolin **211**, luteolin-7-*O*-glucoside **216**, and of coumarins esculetin **221**, and cichoriin **222**, in addition to the 3,4-dihydroscopoletin (isoscopoletin) **223**, which was isolated for the first time from this plant (Sarg et al., 1987).

1.10.4 *Launaea residifolia* (L.) Kunteze

The triterpenes α-amyrin **152**, lupeol **186** and its acetate **188**, moretenol **247** and its acetate **248** have been isolated from an Egyptian sample of the plant. The study led also to the isolation of Δ^7-stigmasterol **232** (Abdel-Fattah et al., 1990).

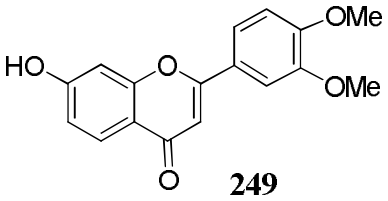


247 R = OH
248 R = Ac

Recently, four coumarin compounds, scopoletin **220**, esculetin **221**, cichoriin **222**, and isoscopoletin **223**, were isolated from the aerials parts of an Algerian sample (Gherraf et al., 2006).

1.10.5 *Launaea asplenifolia* Hook

The presence of triterpenes was also evidenced in *Launaea asplenifolia* that contains taraxasterol **146** taraxasterone **147**, taraxasteryl acetate **148** and lupeol **186** (Gupta et al., 1989). Moreover, the studies on phenolic compounds of this plant revealed the abundance of flavonoids: apigenin-7-*O*-glucoside **215**, luteolin **211**, luteolin-7-glucoside **216**, 7-hydroxy-3',4'-dimethoxyflavone **249**, delphinidin **250**, vitexin **251** (Gupta et al., 1985a), in addition to asplenetin **252** and its glucoside **253** (Gupta et al., 1985b) have been isolated and characterized.



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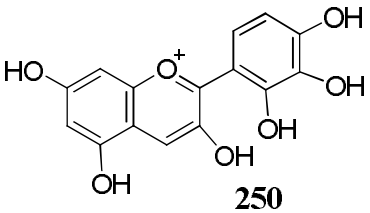

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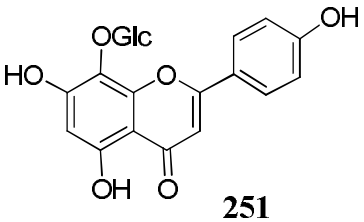

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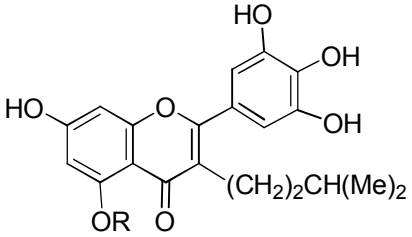


252 R = H
253 R = Neohesperidosyl

1.10.6 *Launaea mucronata* (Forssk)

The light petroleum extract of the aerial parts of *Launaea mucronata* grown in Egypt afforded lupeol **186**, its acetate **188** and β-sitosterol **227** (Abdel-Salem et al., 1982). Additionally, the roots of the same sample yielded the sesquiterpene lactone guaianolide lactucin **25**, the corresponding dihydroderivative 11β-13-dihydrolactucin **33**, and 8-acetyl-11β,13-dihydrolactucin **34** (Sarg et al., 1982).

1.10.7 *Launaea tenuiloba* (Boiss)

Phytochemical study of *L. tennuiloba* collected in Egypt resulted in the isolation of three sesquitepene lactones of guaianolide type, 8-deoxylactucin **16**, jaquinelin (11β-13-dihydrolactucin) **21** and lactucin **25**, two coumarins, esculetin **221** and cichoriin **222**, and the flavone glycosides, apigenin-7-*O*-glucoside **215** and luteolin-7-*O*-glucoside **216** (Abdel-Salem 1986).

1.10.8 Biological activities in plants of the genus *Launaea*

Some species of the genus *Launaea* are used as medicinal plants. Many of them are used in folk medicine as bitter stomachic, anti-tumour, insecticides and against skin diseases. Therefore, these species have been subjected to many pharmacological studies.

Launaea residifolia is a medicinal plant used in the Libyan folkloric medicine mainly for the treatment of hepatic pains.

Launaea nudicaulis is endemic in north Africa and used in the traditional medicine in the West South of Algeria. It is used to treat gastric burns, pain of chest, and pain of stomach. In India and in Pakistan, the milk material from *L. nudicaulis* is taken during the constipation. Leaves are used to relieve fever in children, in the treatment of itches of skin, ulcers, swellings, eczema eruption whereas the roots are used in toothaches.

Antibacterial, antifungal and allelopathic potential activities have been proven for many species of *Launaea*. In an antibacterial assay against *Bacillus subtilis* the extracts of *L. nudicaulis* and *L. residifolia* showed 18.5 and 20.5 mm zones of inhibition, respectively, as determined by the disc diffusion method. The antifungal activity against *Aspergillus* spp. was determined by measuring the linear growth in slants on 4^{th} day of incubation.

Methanol extracts of *L. nudicaulis* and *L. residifolia* were active at 0.209 mg/ml levels exhibiting 45±6 mm and 37±6 mm linear growth which decreased to 22±5 mm and 28±4 mm, respectively, at 0.838 mg/ml concentration (Rashid et al., 2000).

In the same way, the methanol extract of the aerial part of *L. nudicaulis* showed high activities against *C. albicans, E. coli*, *S. aureus*, and *P. aeruginosa*. The highest inhibition

observed in *S. aureus*, a human pathogen, explains the use of this plant against a number of infections for generations (Belboukhari et al., 2008).

Coumarins isolated from *L. resedifolia* (Gherraf et al., 2006) were tested for their antibacterial activity. They showed inhibition against *Bacillus cereus* and *Staphylococcus aureus* in minimum inhibitory concentrations of 200 and 400 µg/ml (El-Bassuony et al., 2006).

Recently, the ethanol extract of *L. resedifolia* showed neuropharmacological properties in animal models. The extract exhibited an inhibitory effect on the locomotor activity of mice in the open field test, an anti-nociceptive effect by increasing the hot plate reaction time in the hot plate test, and an anti-inflammatory activity in the carrageen-induced paw oedema.

Furthermore, a sedative effect was evident from the decrease in the onset of pentobarbitone sleeping time and increase in the duration of pentobarbitone sleeping time in rats. The extract demonstrated also a significant decrease in the mortality rate induced by picrotoxin by about 66%, and a considerable reduction in the body weight of mice compared to the control group (Auzi et al., 2007).

The aqueous extract of *Launaea procumbens* (Roxb.) of three test species: spinach, mustard and corn inhibited germination at different concentrations (25, 50, 75, and 100%).

Roots and shoot growth of all species was substantially reduced by *Launaea procumbens*, and growth reduction was greater at higher concentrations. This allelopathic potential effect was also confirmed when different modes of extract application were tested, it has been found that only the soil application of the aqueous extract has a significant retarding effect on wheat growth while shoot spray or root dip treatment had no such effect (Shaukat et al., 2003).

The methanol extract of *Launaea arborescens* showed antifungal activity against *Candida albicans* and *Saccharomyces cerevisae* and antibacterial activity against gram + *Staphylococcus aureus* and gram – *Escherichia coli*, *P. aeruginosa* and *Klebsiella entrecocus* (Belboukhari et al., 2006). Recently, an insecticidal effect against *Tribolium castaneum* was performed (Jbilou et al., 2008).

1.11 MARINE PHANEROGAMS (SEAGRASSES)

1.11.1 Taxonomy and distribution

Seagrasses comprise <0.02% of the angiosperm flora representing a surprisingly small number of species. These unusual flowering plants are called seagrasses because the leaves are long and narrow and are very often green, and because the plants grow often in large meadows which look like grassland. In other words, many of the species of seagrasses superficially resemble terrestrial grasses of the family Poaceae.

Seagrasses are generally assigned to two families, Potamogetonaceae and Hydrocharitaceae, encompassing twelve genera of angiosperms containing about 50 species.

Three of the twelve genera, *Halophila*, *Zostera* and *Posidonia*, which may have evolved from lineages that appeared relatively early in seagrass evolution, comprise most (55%) of the species, whereas *Enhalus*, the most recently described genus, is represented by a single species (*Enhalus acoroides*).

Table 1.2 groups the distribution of seagrass species and their membership to the different seagrass floras (Hemminga et al., 2000**)**, (Gullstrom et al., 2002)**.** Most seagrass meadows are monospecific, but may develop multispecies, with up to 12 species, meadows in subtropical and tropical waters creating one of the most productive aquatic ecosystems on earth.

Species	Biogeographic membership
Amphibolis antartica	S. Australian flora
Amphibolis griffithii	S. Australian flora
Cymodocea angustata	Indo-Pacific flora
Cymodocea nodosa	Mediterranean flora
Cymodocea rotundata	Indo-Pacific flora
Cymodocea serrulata	Indo-Pacific flora
Enhalus acoroides	Indo-Pacific flora
Halodule pinifolia	Indo-Pacific flora
Haludule uninervis	Indo-Pacific flora
Haludule wrightii	Caribbean flora
Halophila baillonis	Caribbean flora
Halophila beccarii	Indo-Pacific flora
Halophila capricornii	Indo-Pacific flora
Halophila decipiens	Caribbean flora and Indo-Pacific flora
Halophila engelmannii	Caribbean flora
Halophila hawaiiana	Indo-Pacific flora
Halophila ovalis	Indo-Pacific flora
Halophila ovata	Indo-Pacific flora
Halophila spinulosa	Indo-Pacific flora
Halophila stipulacea	Indo-Pacific flora
Heterozostera tasmanica	S. Australian flora
Phyllospadix iwatensis	Temperate w. Pacific flora
Phyllospadix japonicus	Temperate w. Pacific flora

Phyllospadix scouleri	Temperate E. Pacific flora
Phyllospadix serrulatus	Temperate E. Pacific flora
Phyllospadix torreyi	Temperate E. Pacific flora
Posidonia angustifolia	S. Australian flora
Posidonia australis	S. Australian flora
Posidonia coriacea	S. Australian flora
Posidonia denhartogii	S. Australian flora
Posidonia kirkmanii	S. Australian flora
Posidonia oceanica	Mediterranean flora
Posidonia ostenfeldii	S. Australian flora
Posidonia robertsoniae	S. Australian flora
Posidonia sinuosa	S. Australian flora
Syringodium filiforme	Caribbean flora
Syringodium isoetifolium	Indo-Pacific flora
Thalassia hemprichii	Indo-Pacific flora
Thalassia testudinum	Caribbean flora
Thalassodendrom ciliatum	Indo-Pacific flora
Thalassodendrom pachyrhizum	S. Australian flora
Zostera asiatica	Temperate w. Pacific flora
Zostera capensis	S. Atlantic
Zostera capricorni	S. Australian flora
Zostera caulescens	Temperate w. Pacific flora
Zostera japonica	Temperate w. Pacific flora
Zostera marina	N. Atlantic, Mediterranean, W. and E. Pacific floras
Zostera mucronata	S. Australian flora
Zostera mulleri	S. Australian flora
Zostera noltii	N. Atlantic and Mediterranean floras
Zostera novazelandika	New Zealand flora

Table 1.2: List of seagrass species and their membership to the different seagrass floras

Seagrasses or marine phanerogams are widespread in various seas, where they form large meadowlands representing dynamic substrates exceeding the area of the sediment surface several times over and allowing settlement of epiphytic organisms.

Seagrasses are found in waters with salinity greater than 10‰ in estuaries to salinities of about 45‰, in hyper-saline coastal environments. Seagrasses grow from the intertidal, where they are exposed to full sunlight during the emersion periods to depths receiving, on average, 11% of the irradiance incident just below the water surface, allowing seagrasses to grow deeper than 40 m in the clearest ocean waters.

1.11.2 Importance of seagrasses

The true importance of seagrass meadows to the coastal marine ecosystem is not fully understood and generally under estimated. The rapidly expanding scientific knowledge on seagrasses has led to a growing awareness that seagrasses are valuable coastal resources. Where seagrasses abound, humans benefit directly and indirectly from the presence of this marine vegetation (Hemminga et al., 2000), (Gullstrom et al., 2002).

✓ Seagrass meadows enhance the biodiversity and habitat diversity of coastal waters. It has been estimated that over 153 species of microalgae (mostly diatoms), 359 species of macroalgae and 178 species of invertebrates are found on the seagrass blades as epiphytes and epizooties (Phillips et al., 1980).

✓ A seagrass meadow also acts as nursery and foraging area for a number of commercially and recreationally important fish and shellfish and other organisms. There are about 340 animals including green turtles which directly feed on the seagrasses and their epiphytes. Besides, the marine mammal Dugong solely feeds on seagrasses.

✓ Seagrasses improve water quality by acting as roughness elements that deflect currents and dissipate the kinetic energy of the water and thereby creating a relatively quiet environment favourable for sediment deposition and retention. With the help of their well developed root system, they bind the sediments and stabilize them.

✓ Seagrasses play an important role in carbon and nutrient cycling in the marine environment. The large biomass produced by the seagrasses and their epiphytes act as sink for carbon in the oceans. Seagrass meadows are also involved in nitrogen cycling through nitrogen fixation (eg. *Posidonia oceanica* meadows account for an annual input of $57x10^{10}$ gN in the Mediterranean Sea) (Kannan et al., 1990).

✓ Seeds of *Enhalus acoroides* are used as food by the coastal populations as the nutritional value of the flour derived from the seeds is comparable to that of wheat and rice in terms of carbohydrate and protein content and in energetic value and even it surpasses these types of flour in calcium, iron and phosphorous content (Montano et al., 1999). Coastal people use rhizomes of *Cymodocea* sp. (nicknamed as sea sugarcane) as food, for the preparation of salad.

✓ Seagrasses are also used as raw materials in paper industry and in the production of fertilizer, fodder and feed. Most of the seagrasses are used extensively as soil fertilizer for coconut and other plantations.

- ✓ A variety of medicines and chemicals are also prepared from them. The agar-like zosterin is extracted from *Zostera* sp. Many seagrasses play an important role in the protection of the nature by production of many secondary metabolites with ecological activity.
- ✓ Some studies have been carried out on the bioactivity of seagrasses and showed that some species of Hydrocharitaceae such as *Thalassia tistudinum*, *Posidonia oceanica*, *Zostera marina,* and *Enhalus acoroides* had antibacterial, antialgal, antifungal, antiviral, anti-inflammatory, antifouling and feeding-deterrence activities (Harrison et al., 1980), (Jensen et al., 1998), (Bhosale et al., 2002), (Hu et al., 2006), and (Qi et al., 2008).

1.11.3 Chemical investigations of seagrasses

Few chemical studies on seagrasses have been so far published. They report the occurrence of sterols, phenolic acids, and fatty acids as main compounds. The sterol composition of various Mediterranean marine algae and phanerogams has been also described.

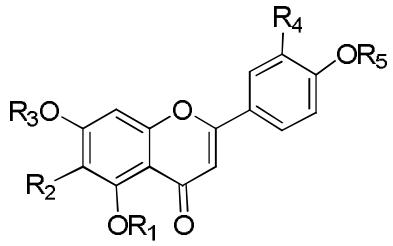
The sterols of *Posidonia oceanica* and *Cymodocea nodosa* were essentially β-sitosterol and stigmasterol; minor sterols including cholesterol, campesterol, and Δ^5-avenasterol were also found (Iatrides et al., (1983), (Sica et al., 1984). Moreover, 3-keto-steroids were isolated from *Dendrophyllia cornigera*, *Cymodocea nodosa*, and *Enhalis acoroids* (Kontiza et al., 2006).

The phenolic acids that widely predominate in land plants also occur in seagrasses. Six phenolic acids including *p*-hydroxybenzoic, *p*-coumaric, caffeic, ferulic, vanillic, and protocatechuic acids were found in leaves of >50% of the sea grasses surveyed. *p*-Hydroxybenzoic acid was ubiquitous and was recorded in each of the 12 genera and in each of the 25 species collected in Caribbean Sea and Indo-Pacific Ocean. These compounds were detected in rhizomes and roots as well as in leaves of four Texas sea grasses, *Thalassia testudinum*, *Halodule wrightii*, *Syringodium filiforme*, and *Halophila engelmannii* (Zapata et al., 1979).

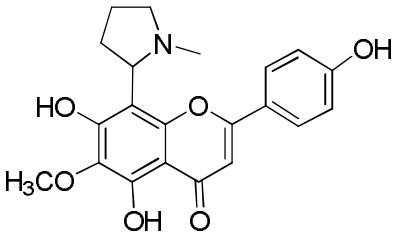
Flavonoids were found to be the most abundant secondary metabolites produced by some Taxes of seagrasses and showed very significant activities, such as feeding-deterrence, antibacterial and antilarval activities (Qi et al., 2008). In all the cases studied, the flavonoid moiety isolated was either a flavone or a flavonol.

Flavonoids are widely present in seagrasses of the family Hydrocharitaceae. Luteolin-5-methyl ether **254**, luteolin-5,4'-dimethylene **255**, acacetin-5-methyl ether **256**, pectolinarigenin **257**, hispidulin **258**, jaceosidin, 5,7,3'-trihydroxy-6,4'-dimethoxyflavone **259**, and 6-hydroxy luteolin **260** were isolated from *Phyllospadix japonica* (Takagi et al., 1979a).

Three flavone acetates were isolated from *Phyllospadix iwatensis* and identified as luteolin acetate **261**, hispidulin acetate **262**, and a flavonoidal alkaloid, 5,7,4'-trihydroxy-6-methoxy-8-(1-methyl-2-pyrrolidinyl)flavone **263 (**Takagi et al., 1979b).

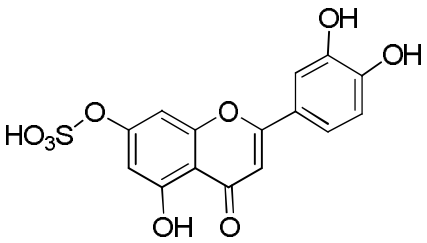


254	$R_1 = CH_3$	$R_2 = H$	$R_3 = H$	$R_4 = OH$	$R_5 = H$
255	$R_1 = CH_3$	$R_2 = H$	$R_3 = H$	$R_4 = OH$	$R_5 = CH_3$
256	$R_1 = CH_3$	$R_2 = H$	$R_3 = H$	$R_4 = H$	$R_5 = CH_3$
257	$R_1 = H$	$R_2 = OCH_3$	$R_3 = H$	$R_4 = H$	$R_5 = CH_3$
258	$R_1 = H$	$R_2 = OCH_3$	$R_3 = H$	$R_4 = H$	$R_5 = H$
259	$R_1 = H$	$R_2 = OCH_3$	$R_3 = H$	$R_4 = OH$	$R_5 = OCH_3$
260	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = OH$	$R_5 = OH$
261	$R_1 = Ac$	$R_2 = H$	$R_3 = Ac$	$R_4 = OAc$	$R_5 = Ac$
262	$R_1 = Ac$	$R_2 = OCH_3$	$R_3 = Ac$	$R_4 = H$	$R_5 = Ac$



263

The chemical study of *Zostera marina* L. and *Zostera nana* led to the isolation of luteolin-7-*O*-glucoside **216**, diosmetin **264**, and diosmetin-7-*O*-glucoside **265** (Milkova et al., 1995). Additionally, *Zostera marina* yielded luteolin-7-sulphate **266**, and luteolin-7,3'-disulphate **267** (Buchsbaum et al., 1990).



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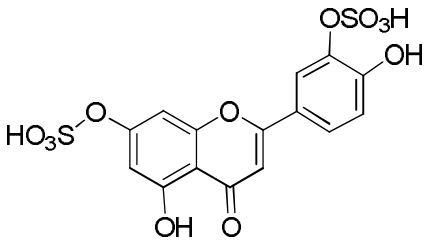
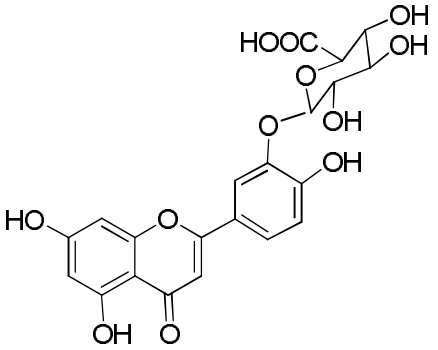


267

Investigation of the chemical composition of the methanolic extract of the leaves of *Zostera noltii* Hornem showed the presence of luteolin-7-*O*-glucoside **216**, caffeic acid, chlorogenic acid and rosmarinic acid (Males et al., 2000).

The EtOH extract of the air-dried *Enhalus acoroides* from South China Sea yielded four flavones which were identified as luteolin **211**, apigenin **214**, luteolin-3'-glucoronide **268** and luteolin-4'-*O*-glucoronide **269** (Qi et al., 2008).



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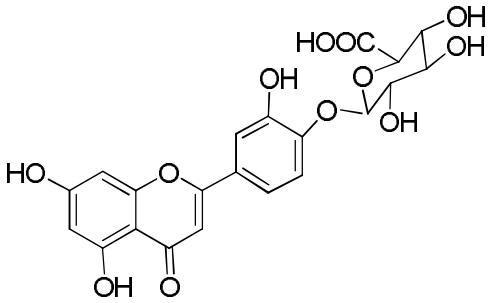
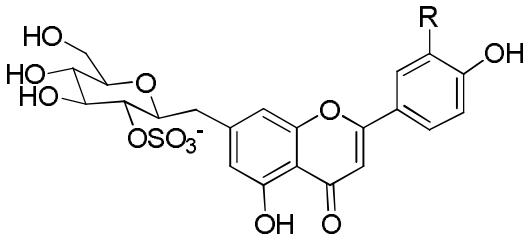


269

Flavonoid sulphates were also present in some sea grasses of Hydrocharitaceae family such as *Thalassia testudinum* and *Halophila ovalis* (McMillan et al., 1980), and (Rowly et al., 2002).

The lyophilized aqueous extract of *Thalassia testudinum* yielded three sulphonated flavones, thalassiolin A (luteolin-7-*O*-β-D-glucopyranosyl-2''-sulphate) **270**, thalassiolin B (3'-methoxyluteolin-7-β-D-glucopyranosyl-2''-sulphate) **271** and thalassiolin C (apigenin-7-*O*-β-D-glucopyranosyl-2''-sulphate) **272** (Rowly et al., 2002)[136].



270 R = OH
271 R = OMe
272 R = H

1.12 FAMILY HYDROCHARITACEAE

Hydrocharitaceae is a plant family that includes a number of aquatic species, broadly called the Tape-grasses, such as the well known *Canadian Waterweed* and *Frog's Bit*. This family includes both fresh and marine aquatic plants. They are distributed throughout the world in a wide variety of habitats, but are primarily tropical.

The family contains 17 genera, three of which, *Thalassia*, *Halophila*, and *Enhalus,* are marine (Larcum et al., 2006). They can share the following properties:

✓ The species are annual or perennial, with a creeping monopdial rhizome with the leaves arranged in two vertical rows, or an erect main shoot with roots at the base and spirally arranged or whorled leaves.

✓ The leaves are simple and usually found submerged, though they may be found floating or partially immerse. As many aquatic plants, they can be very variable in shape - from linear to orbicular, with or without a petiole, and with or without a sheathing base.

✓ The flowers are arranged in a forked, spathe-like bract or between two opposite bracts. They are usually irregular, though in some case they may be slightly irregular, and either bisexual or unisexual. The perianth segments are in 1 or 2 series of (2)3 free segments; the inner series when present are usually showy and petal-like.

✓ Fruits are globular to linear, dry or pulpy, dehiscent or more usually indehiscent and opening by decay of the pericarp. Seeds are normally numerous with straight embryos and no endosperm.

1.12.1 The genus *Halophila*

Halophila is a genus belonging to the family Hydrocharitaceae. It is widespread in tropical waters, and the distribution ranges to subtropical and temperate waters.

1.12.2 Scientific classification

Kingdom:	Plantae
(Unranked):	Angiosperms
(Unranked):	Monocots
Order:	Alismatales
Family:	Hydrocharitaceae
Genus:	***Halophila***

There are approximately 36 species of the genus *Halophila*, widely distributed in north America, south America, Europe, Asia, Africa and Australia, that exhibit the following botanical characters:

✓ Leaf bearing branches arising from the thin rhizome at each node.

✓ Leaves petiolate, in pairs, in pseudo whorls or distichously arranged with a pinnate nervation. Spathal leaves free. Style 3-6, not divided.

✓ Fruit dehiscent by decay of pericarp. Tannins cells absent.

1.12.3 Selected species of *Halophila*

H. aschersonii	*H. decipiens*	*H. jagorii*	*H. linearis*	*H. okinawensis*	*H. tricostata*
H. australis	*H. decipiens*	*H. japonica*	*H. madagascariensis*	*H. ovalis*	-
H. baillonii	*H. engelmannii*	*H. johnsonii*	*H. major*	*H. ovata*	-
H. balfouri	*H. euphlebia*	*H. johnstonii*	*H. mikii*	*H. spinulosa*	-
H. beccarii	*H. gaudichaudii*	*H. kotschyana*	*H. nipponica*	*H. stipulacea*	-
H. capricorni	*H. hawaiiana*	*H. lemnopsis*	*H. minor*	*H. sulawesii*	-

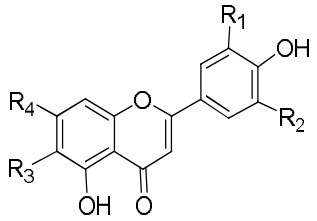
1.12.4 Secondary metabolites isolated from the genus *Halophila*

A survey of literature data revealed that few chemical studies have been conducted on the genus *Halophila*.

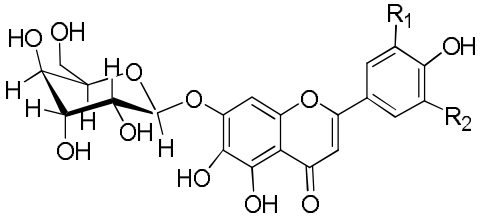
As above cited, *p*-hydroxybenzoic, *p*-coumaric, caffeic, ferulic, vanillic, and protocatechuic acids were detected in rhizomes, leaves and roots of *Halophila engelmannii* (Zapata et al., 1979).

Halophila johnsonii is a threatened marine angiosperm endemic to south eastern Florida coastal lagoons. Recently, Meng (Meng et al., 2008) reported the phytochemical study of the methanol extract of the whole plant of *Halophila jonhsonii* that resulted in the isolation and identification of fifteen flavonoids. Five of them were previously known flavones: luteolin **211**, apigenin **214**, pedalitin **273**, ladanetin **274**, and myricetin **275**; three were known flavonoid glycosides: 6-hydroxyluteolin-7-*O*-β-glucopyranoside **276**, scutellarein-7-*O*-β-glucopyranoside **278**, and spicoside **279**, and finally seven flavonoid glycosides were new: 5,6,7,3',4',5'-hexahydroflavone-7-*O*-β-glucopyranoside **277**, 5,6,7,3',4',5'-hexa-hydro flavone-7-*O*-(6''-*O*-acetyl)-β-glucopyranoside **280**, 6-hydroxyluteolin-7-*O*-(6''-*O*-acetyl) β-

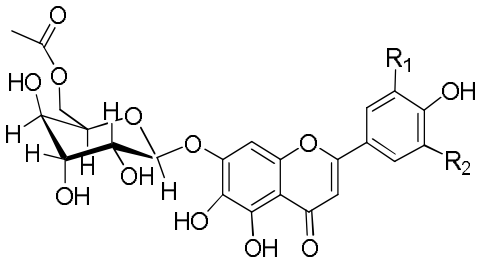
glucopyranoside **281**, 6-hydroxyapigenin-7-*O*-(6''-*O*-acetyl)-β-glucopyranoside **282**, 6-hydroxyapigenin-7-*O*-(6''-*O*-(E)-coumaroyl)-β-glucopyranoside **283**, 6-hydroxy apigenin-7-*O*-(6''-*O*-(E)-caffeoyl)-β-glucopyranoside **284**, and 6-hydroxyluteolin-7-*O*-(6''-*O*-(E)-coumaroyl) β-glucopyranoside **285**.



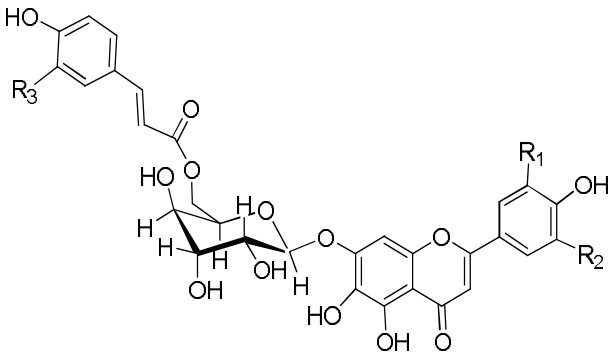
273 $R_1 = R_3 = OH$ $R_2 = H$ $R_4 = OCH_3$
274 $R_1 = R_2 = H$ $R_3 = OH$ $R_4 = OCH_3$
275 $R_1 = R_2 = R_3 = R_4 = OH$ $R_2 = H$ $R_4 = OCH_3$



276 $R_1 = OH$ $R_2 = H$
277 $R_1 = R_2 = OH$
278 $R_1 = R_2 = H$

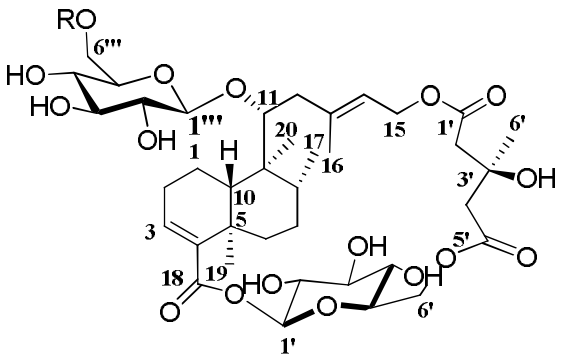


280 $R_1 = R_2 = OH$
281 $R_1 = OH$ $R_2 = H$
282 $R_1 = R_2 = H$



279 $R_1 = R_3 = OH$ $R_2 = H$
283 $R_1 = R_2 = R_3 = H$
284 $R_1 = R_2 = H$ $R_3 = OH$
285 $R_1 = OH$ $R_2 = R_3 = H$

Finally, unusual glycoterpenoids, syphonoside **286** (Gavagnin et al., 2007) and the corresponding acetyl derivative **287** (Carbone et al., 2008), have been recently reported from a sample of *Halophila stipulacea* collected in the Mediterranean Sea.



286 R = H
287 R = Ac

Chapter 2
Study of terpenoids and flavonoids

2. Terpenoids

2.1 Introduction

Isoprenoids or polyprenoids, as they are sometimes called, comprise large and important groups of natural products that can be formally derived from isoprene units.

This group consists of two major classes: terpenoids and steroids. The chemistry of the two classes has developed fairly independently and, usually, they are treated separately.

The term "terpen" is attributed to Kekule who coined it to describe $C_{10}H_{18}$ hydrocarbons occurring in turpentine oil. This term has, over the years, been used to designate isoprene-based secondary metabolites. At present, several groups of terpenoids, classified in terms of C-10 units are recognized.

Classification	**No. of carbon atoms**	**No. of isoprene units**
Hemiterpenoids	5	1
Monoterpenoids	10	2
Sesquiterpenoids	15	3
Diterpenoids	20	4
Sesterterpenoids	25	5
Triterpenoids	30	6
Tetraterpenoids	40	8
Polyterpenoids	>40	>8

Terpenoids are widely distributed in higher plants. Some compounds, such as chlorophyll pigments that have a diterpene side chain or gibberellins, are essential for the growth and well-being of plants. Terpenoids constitute important components of many wood extractives, and are often the major constituents of extracts obtained with non-polar solvents. Essential oils, latexes and resinous exudates from plants are often composed mainly of terpenoids.

Mono and sesquiterpenes are the usual components of essential oils, whereas diterpene, being less steam-volatile, are only rarely found in essential oils. Co-occurrence of diterpenoids or triterpenoids with lower terpenoids is common, though the two seldom occur together in the same tissue.

2.2 Biosynthesis

According to the biogenetic isoprene rule, which was formally enunciated by Ruzica in 1953, terpene structures may be rationalized, or preliminary structures deduced, by accepted reaction mechanisms from hypothesized acyclic precursors such as geraniol, farnesol, geranylgeraniol, etc. It was found that the active C5 unit from mevalonic acid (3-methyl-3,5-

dihydroxypentanoic acid) was isopentyl pyrophosphate (IPP) which recognized as the long sought biological isoprene unit. **Figure 2.1** gives a simplified version of the pathway from acetyl coenzyme **A** to mevalonic acid, to IPP, and subsequently to geranyl pyrophosphate (GPP) in a stereospecific condensation. GPP is then channeled into monoterpene biosynthesis, and serves as a substrate for prenylation by IPP the sesquiterpenoid precursor farnesyl pyrophosphate (FPP) (Rowe, 1989). **Figure 2.2** gives the second version of the pathway from 1-deoxy-D-xylulose 5-phosphate to 2-*C*-methyl-D-erythritol 4-phosphate, to 2-*C*-methyl-D-erythritol 2,4-cyclophosphate which is transformed by steps to IPP.

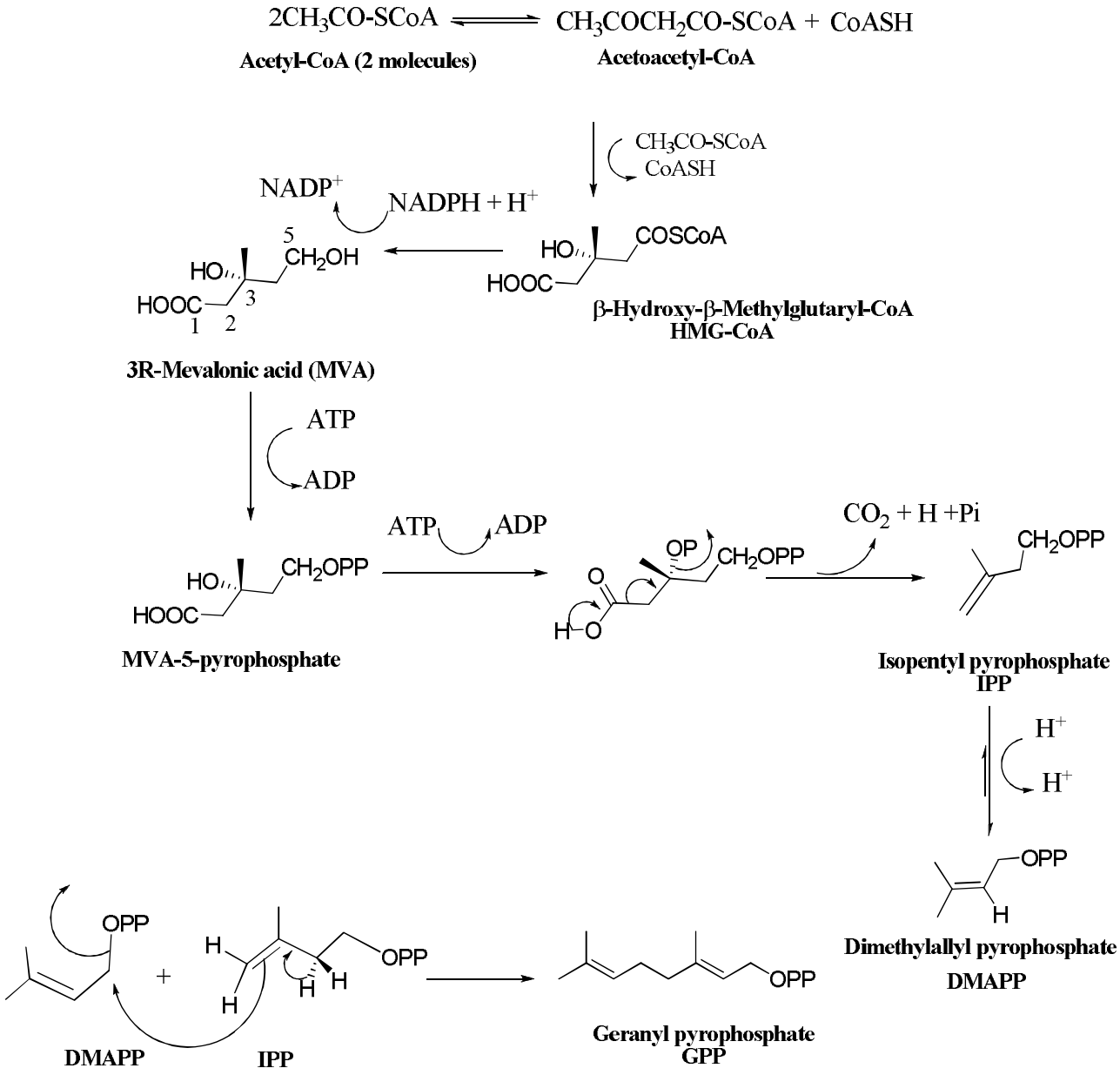


Figure 2.1: Biosynthesis of geranyl pyrophosphate via mevalonate pathway

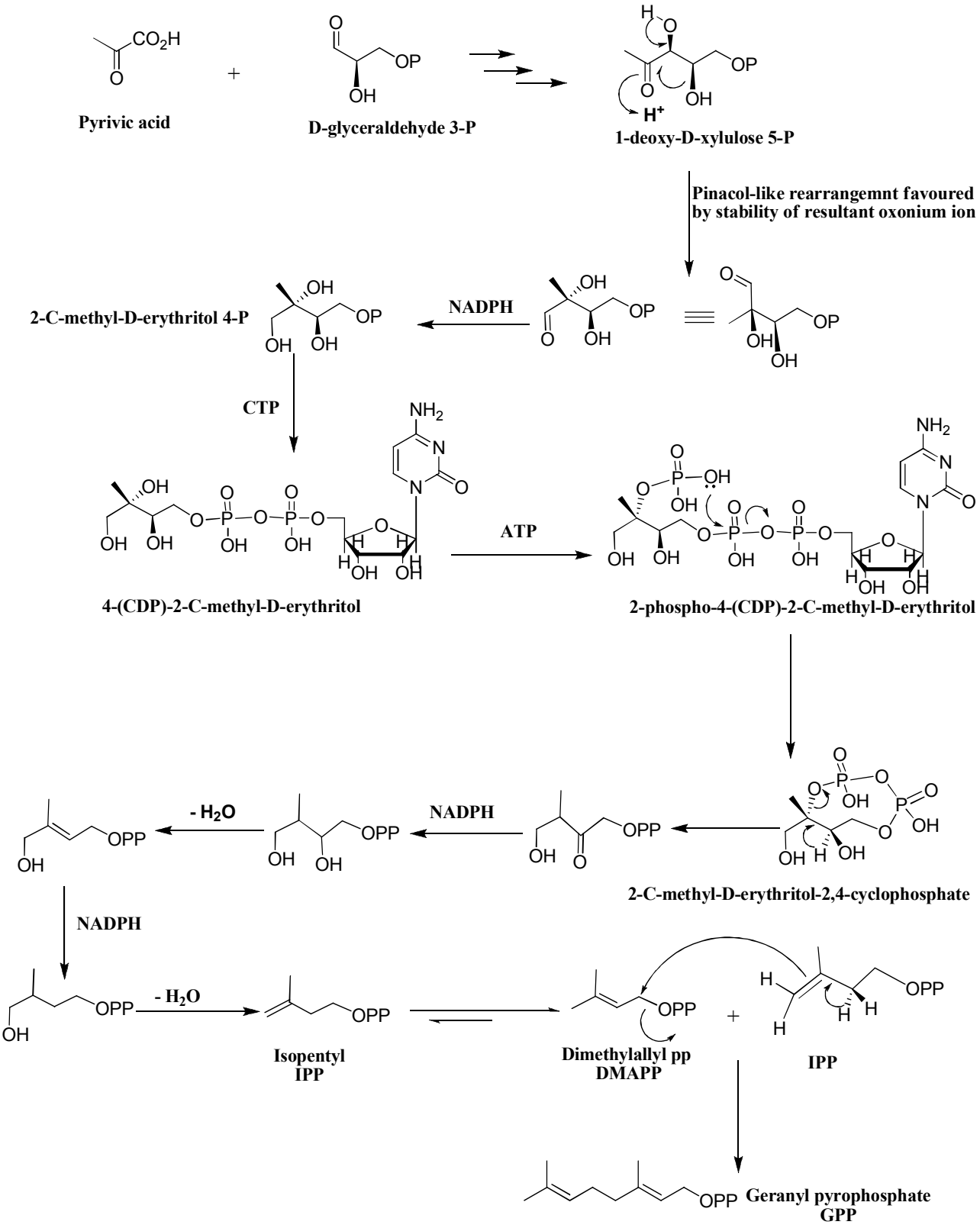


Figure 2.2: Biosynthesis of geranyl pyrophosphate via deoxyxylulose phosphate pathway

Two other metabolic pathways are available to FPP:

1- Tail to tail dimerization by the action of an enzyme (presqualenesynthase) to yield squalene, the precursor of all triterpenoids and steroids.

2- Chain extension to geranylgeranyl pyrophosphate (GGPP).

The same three options are available to GGPP: generating diterpenoids, prenylation to geranylfarnesyl pyrophosphate (GFPP), the sesterterpenoids precursor, and dimerization to phytoene and thence to carotenoids. Finally, chain elongation of GFPP to polyprenyl pyrophosphate (PPPP) yields the formation of polyprenols (**Figure 2.3)**.

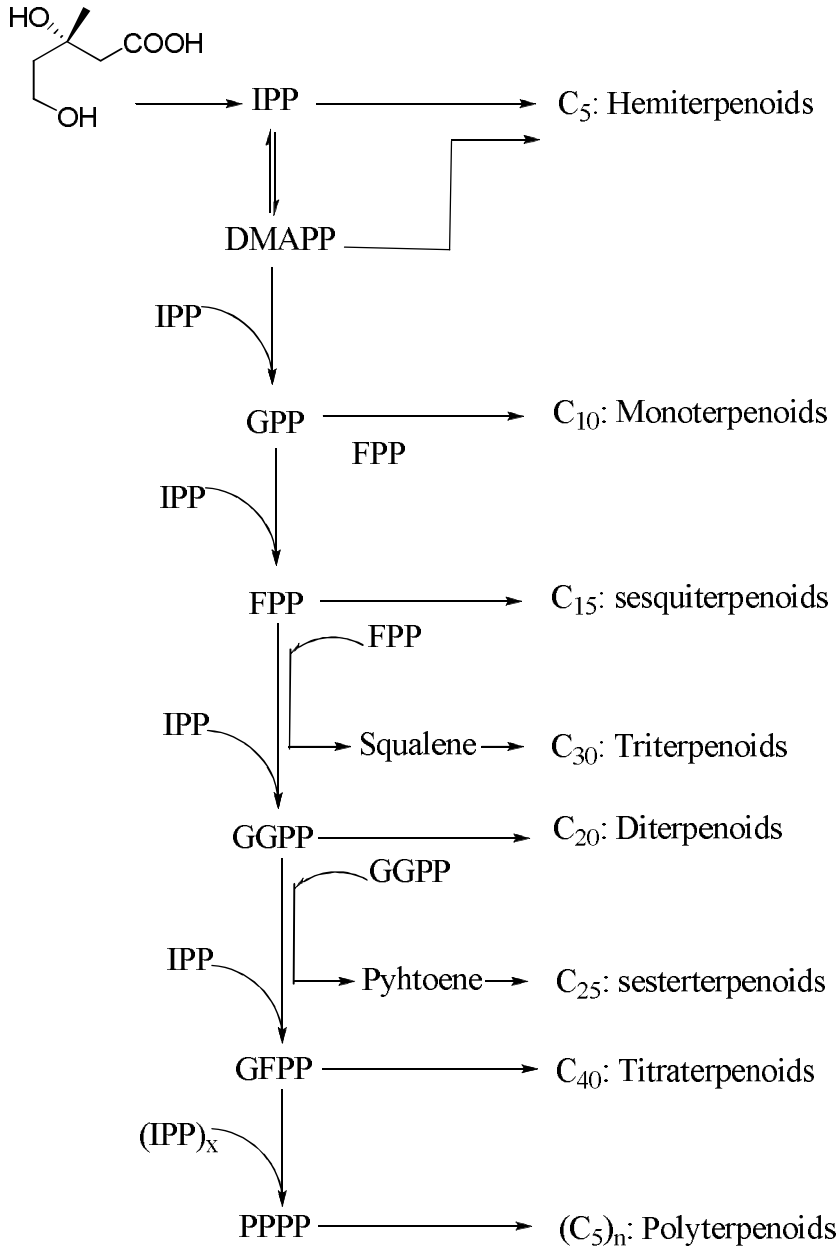


Figure 2.3: General scheme of isoprenoid biosynthesis

2.3 SESQUITERPENOIDS

Sesquiterpenoids, which constitute the largest single class of naturally occurring terpenoids, are widely distributed in nature and often encountered as constituents of wood extractives. According to the biogenetic isoprene rule, sesquiterpenes arise from a linear precursor such as farnesol, and farnesyl pyrophosphate is considered as the immediate biosynthetic precursor of almost all sesquiterpenoids (Manitto, 1981).

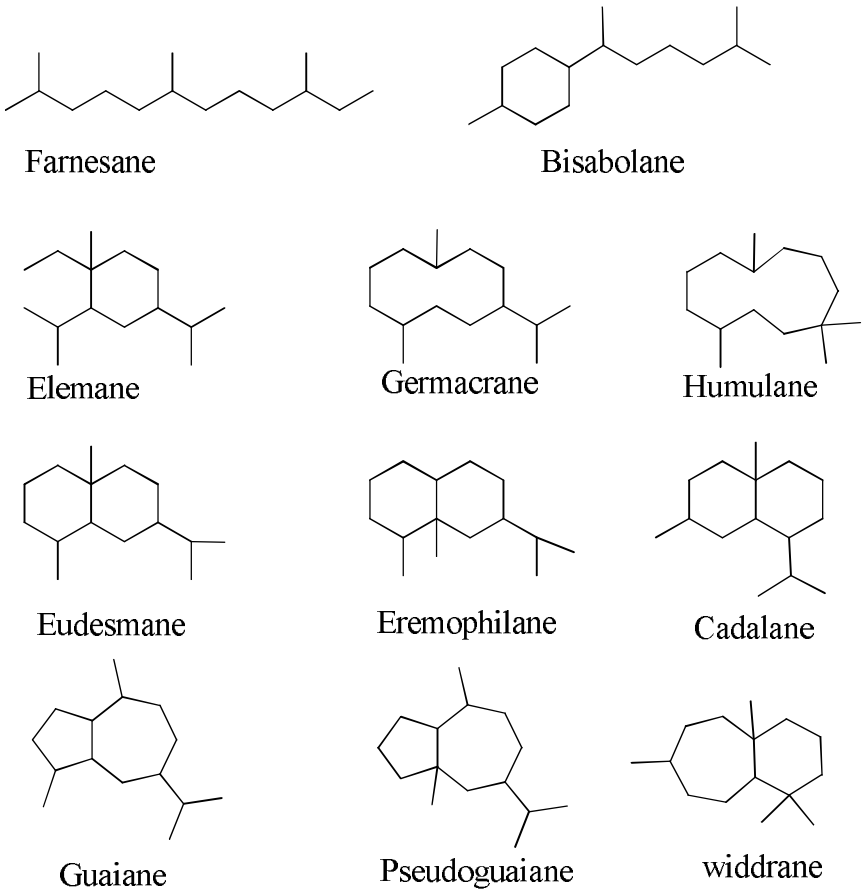


Figure 2.4: Important sesquiterpene skeletal types

Conceptually, cyclization of farnesyl pyrophosphate can proceed from either the tail end (by ionization of the pyrophosphate moiety) or the head (by an H^+ or OH^+ attack on Δ^{10}). **Figure 2.5** depicts possible monocyclic ions that would result from such a cyclization, depending on the nature of the substrate and the involved cyclase.

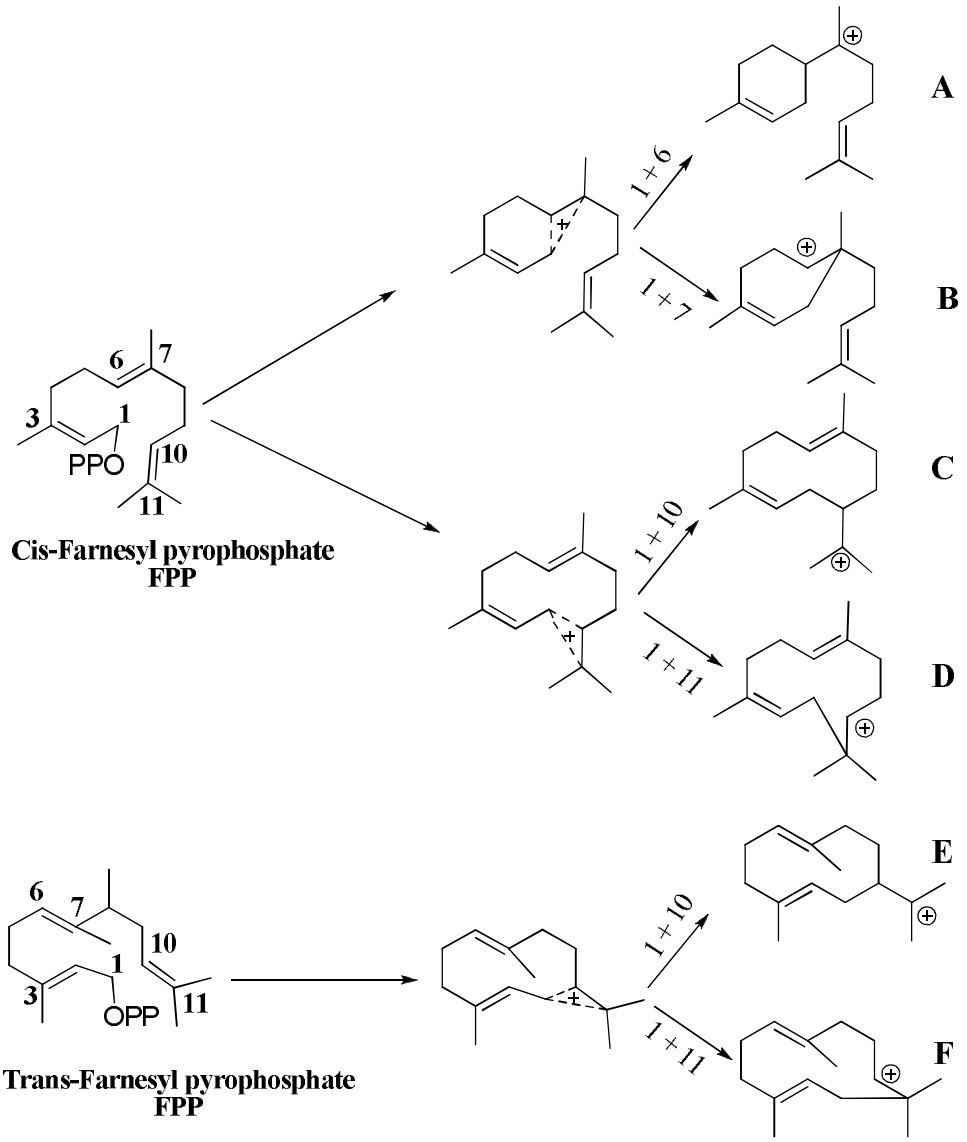


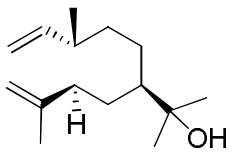
Figure 2.5: Postulated initial cyclization of farnesyl pyrophosphate

2.3.1 Germacranes and elemanes

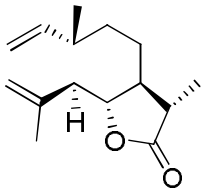
These compounds have been chiefly obtained from higher plants, especially those of the family Compositae. However, the vast majority of known germacranes are oxidatively modified to carry a lactone ring (germacranolides), often along with a variety of other oxygen functionalities. These products are the result of the 1,10-cyclization forming the cation **E** **(Figure 2.5)**.

In the majority of germacranes both olefinic bonds display a *trans* geometry even though an increasing number of molecules having one or both *cis* double bonds have being isolated from nature. This has led to the re-classification of germacranolides into four configurationally isomeric subgroups: germacranolides, melampolides, heliangolides and *cis*-germacranolides.

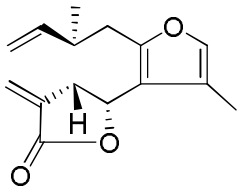
Germacrane with *trans-trans* 1,5-diene system are thermally labile and undergo Cope rearrangement to give products with elemane skeleton. Accordingly, compounds such as elemol saussurea lactone, and isolinderalactone have been isolated from natural sources.



Elemol

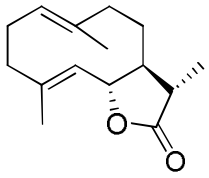


Saussurea lactone



Isolinderalactone

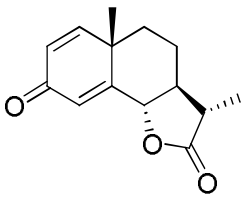
It has been suggested that elemanes arise in nature from suitable germacranes by a biological equivalent of Cope rearrangement, and that many natural elemanes may, in fact, be artifacts. Thus, 11,12-dihydrocustunolide on heating gives saussurea lactone, albeit, in low yield. This lactone had been isolated from costus root oil, but it was found later that it is only an artifact (Newman, 1972).



11,12-dihydrocustunolide

2.3.2 Eudesmanes (selinanes)

Eudesmanes are an important family of sesquiterpenoids. (-)-α-Santonin, the first pure sesquiterpene to be isolated from nature in 1830 belongs to this group. This skeleton arises by a Markownikoff cyclization of a suitable germacrane, such as **G**, deriving from the germacrane cation **E**. This later generates the bicycle **H**, which in principle, is implicated in the genesis of a number of eudesmane, vitispirane, eremophilane, and valerane type (**Figure 2.6**).



(-)-α-santonin

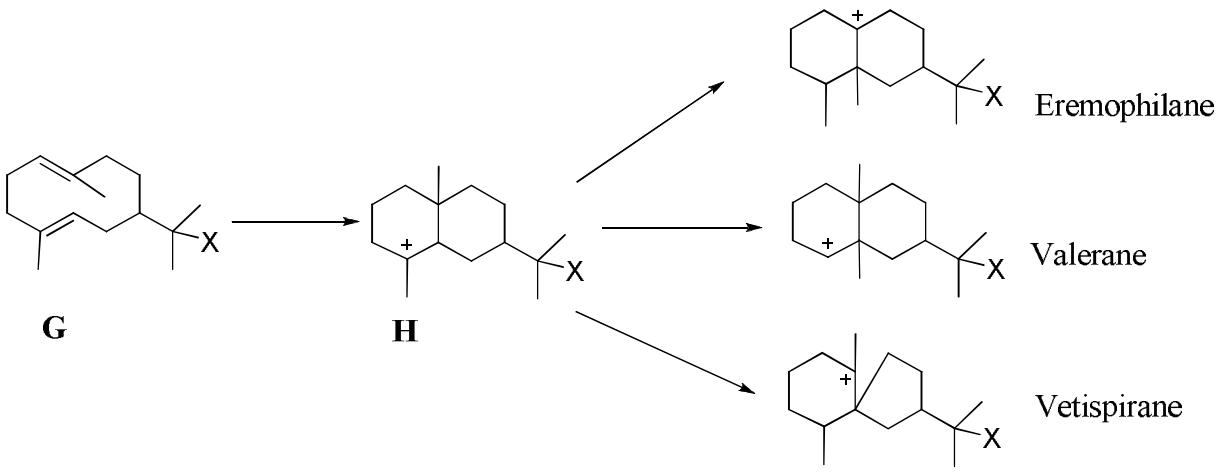
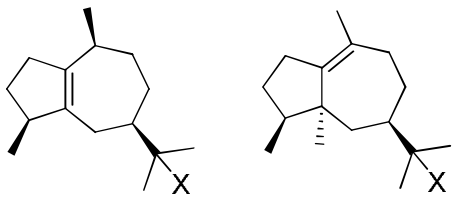


Figure 2.6: Selected important sesquiterpene types based on eudesmane cation

Compounds with the relative stereochemistry depicted in **H** are the most common molecules (*trans* decaline system). As many other major sesquiterpene types, this class exhibits a variety of structures, the majority of them have a γ-lactone ring (eudesmanolides).

2.3.3 Guaianes

They are presently the second largest group of natural sesquiterpenoids, they are most widely distributed in the Compositae (Asteraceae). The formation of guaiane skeleton is due to an anti-Markownikoff cyclization of germacrane **G** leading to the bicyclic ions **I** and **J** with the hydroazulene skeleton. **Figure 2.7** showed the possible rearrangement-cyclization ways for the cation derived from **G**. The simpler members of this group include guaiol, isolated from *Bulnesia sarmienti,* and bulnesol, isolated from *Gurjum balsum*.



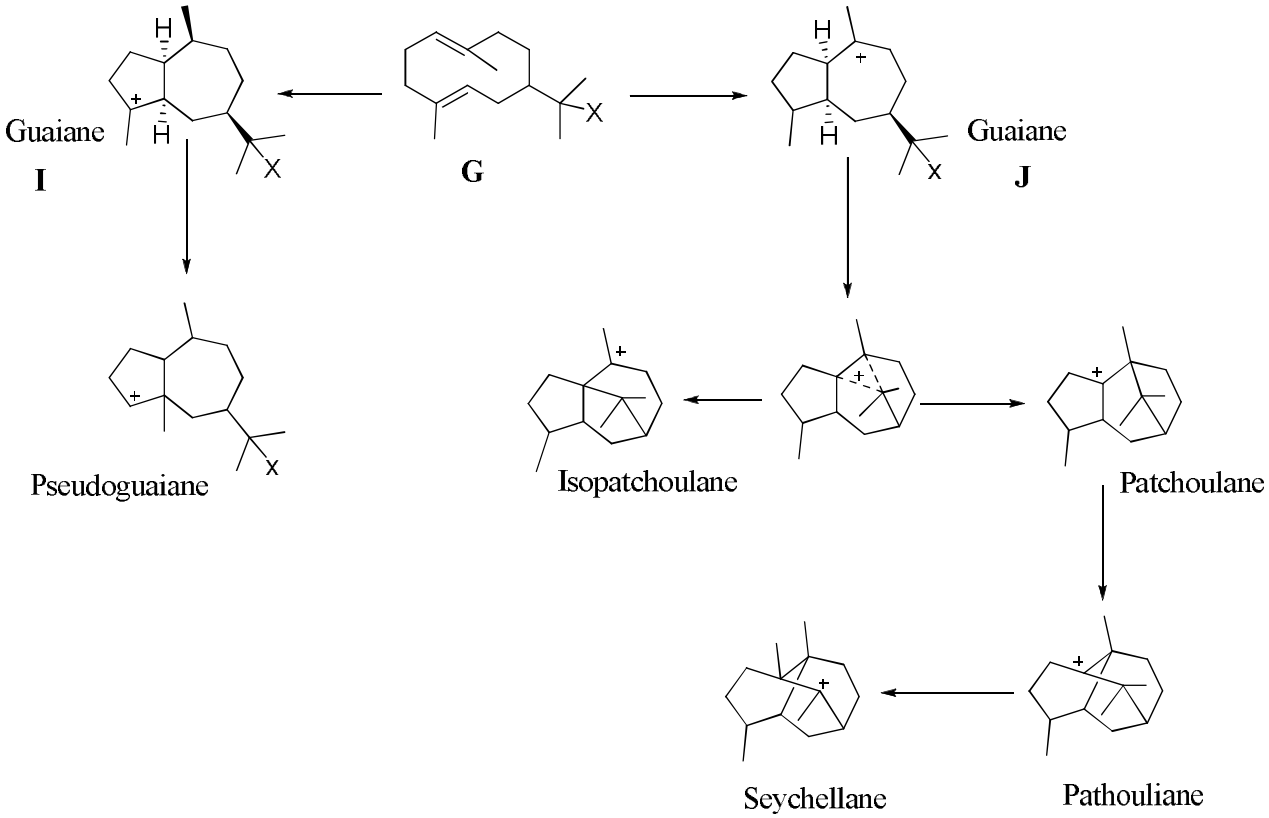


Figure 2.7: Selected important sesquiterpene types based on guaiane cation.

2.4 Sesquiterpene lactones

Sesquiterpene lactones, which have been isolated from fungi, liverworts and members of angiosperm families, mainly the Asteraceae, represent one of the largest class of plant products with over 4.000 reported naturally occurring compounds (Fischer, 1991)[142]. The majority of them are guaianolides or near relatives. Biogenetic proposals for the various skeletal types of sesquiterpene lactones suggest that they are derived from germacradiene precursors which, in turn, are formed via the mevalonic acid-farnesyl (nerolidyl) pyrophosphate pathway (**Figure 2.4)**.

The guaianolide lactones identified so far exhibit three of the four possible lactones fusions: 6α-12-olide, 8α-12-olide, and 8β-12-olide. The same junction stereochemistries are present in pseudoguaianolides.

Usually, guaianolides have the 1α,5α *cis* ring fusion while pseudoguianolides have the 1α ,5β-Me stereochemistry.

A biosynthetic mechanism of 8-deoxylactucin of the tribe Lactuceae of the Asteraceae family was proposed by Song and collaborators (Song et al., 1995).

The enzymatic oxidation at C-6 and C-12 of cation **E** is most likely followed by lactonization to give the germacranolide costunolide, and upon bio-epoxidation, parthenolide. *Trans*-annular cyclization of the hypothetical intermediate parthenolide would provide the

guaianolide skeleton **L** via cation **K**. Further oxidative bio-modifications of **K** would lead to the two lactones (**Figure 2.8**). The most probable initial lactone would be 8-desoxylactucin which must be further bio-modified to give lactone 11βH,13-dihydrolactucin-8-*O*-acetate.

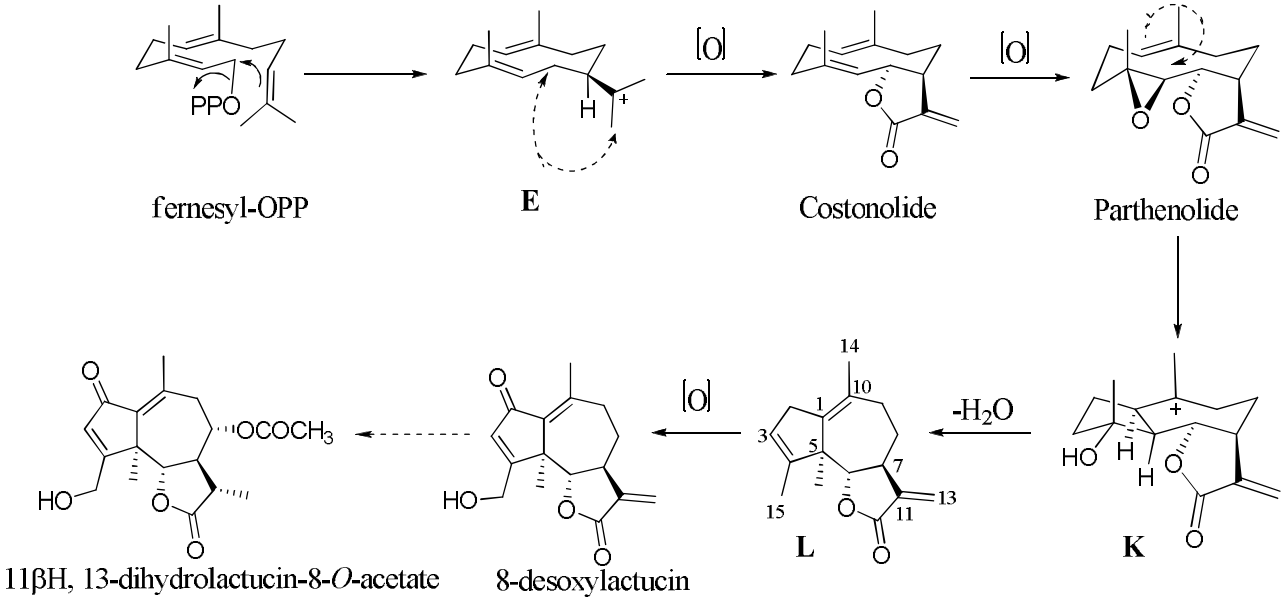
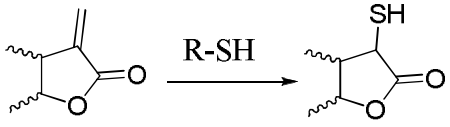


Figure 2.8: Proposed biosynthesis of 8-deoxylactucin

2.5 Biological activities of sesquiterpene lactones

Sesquiterpene lactones (STL) possess a broad variety of conspicuous biological activities directed towards all types of predating organisms. The majority of these compounds appear to be of exceptional ecological value for the plants that synthesize them. They include antineoplastic agents, insect feeding-deterrents, plant growth regulators, antimicrobial agents, schistosomicidal agents, vertebrate poisons and contact human dermatitis promoters. Several structure–activity studies have led to the conclusion that plant growth regulation requires the presence of an exocyclic α, β-unsaturated lactone moiety which can combine with sulphydryl groups in key enzymes that control cell division (Kupchan et al., 1970).



2.5.1 Anti-tumour and cytotoxic activity

Most of the active sesquiterpene lactones are found in species of the Asteraceae although some of them originate in the Magnoliaceae, Apiaceae and even fungi. The majority of known sesquiterpene lactones show cytotoxic activity (KB and P388 leukemia in vitro) and tumour inhibition activity (P388 leukemia in vivo). All listed active sesquiterpene lactones posses an

α,β-unsaturated lactone moiety, and the changes such as saturation or addition to the methylene group result in the loss of cytotoxicity and tumor inhibition. It has been also demonstrated that an additional conjugated ester, cyclopentenone, an epoxy group, or a second α, β-unsaturated lactone moiety appear to enhance cytotoxicity (Picman, 1986). The chemical studies by Kupchan (Kupchan et al., 1970) showed that various cytotoxic sesquiterpene lactones react with thiols such as cysteine by rapid Michael-type addition, and a loss of sulphydryl enzymes, phosphofructokinase and glycogen synthase was observed after reaction with sesquiterpene lactones known to act as tumour inhibitors. Kupchan suggested that these tumour inhibitors may have a triple selectivity: for thiols over other nucleophiles such as amines, for particular sulphydryl enzymes and sulphydryl groups within those enzymes. It has also demonstrated (Kupchan et al., 1970) that sesquiterpene lactones significantly inhibit nuclear DNA synthesis, especially DNA polymerase and thymidylate synthetase enzymatic activity, in tumour cells.

2.5.2 Anti- bacterial activity

The work which has been done by Lee (Lee et al., 1977) showed the positive results of anti-bacterial activity of sesquiterpene lactones. Thirty-six sesquiterpene lactones were tested against six bacterial strains and they were exclusively active against gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. Lee et al. suggested that the anti-bacterial activity of sesquiterpene lactones is strongly influenced by the β-unsubstituted cyclopentenone ring moiety and, in particular, enhanced by esterification of the hydroxyl group or by epoxidation whereas the presence of the α,β-unsaturated lactone moiety seems to be not relevant. In addition, other researchers (Picman, 1986) confirmed that the activity may also be determined by the availability and accessibility of vitally important thiol compounds present in bacteria and by their chemical affinity for the sesquiterpene lactones.

2.5.3 Anti-fungal activity

Some sesquiterpene lactones have been reported to inhibit the growth of several species of fungi, such as *Candida albicans*, *Trichophyton mentagrophytes*, *Saccharomyces cerevisiae Microscoporum cookei* and *Eremothecium ashbyi*. An example of these antifungal sesquiterpene lactones is represented by parthenolide, a germacranolide epoxide which inhibits the growth of several filamentous fungi and yeasts in vitro, and can play a role in the defence of the plant against diseases. Picman and coworkers (Picman et al., 1986) have studied the structure-activity relationship of forty-five sesquiterpene lactones and have suggested that the presence of the α, β-unsaturated lactone moiety may be necessary for the

fungal activity of some lactones and that the presence of other functionalities, their position and/or configuration may also enhance or reduce the activity of sesquiterpene lactones.

2.5.4 Others biological activities

There are numerous reports on the poisonous action of secondary metabolites of several species of the Asteraceae to livestock grazing on them. In many cases, the toxic constituents have been subsequently identified as sesquiterpene lactones. *Parthenium hysterophurus*, a tropical weed causing agricultural problems in some parts of the world, when fed to cattle and buffaloes in India, was found to cause illness or death of the animals, most likely because of the presence of parthenin, a guaianolide sesquiterpene.

Horticultural plants or vegetables from the Asteraceae such as *Artemisia*, *Cichorium* and *Lactuca* (Lactuceae tribe), *Cosmos*, *Matricaria*, *Solidago* and many others have been reported to cause allergic contact dermatitis. It was also postulated that the β-unsaturated lactone moiety is an essential chemical prerequisite for the immunologic reactivity of these compounds.

A number of sesquiterpene lactones have been tested for their insecticidal activity, the results suggest that these compounds play an important role in plant defence against herbivorous insects. Interestingly, the herbivores caused an increase in the concentration of sesquiterpene lactones in the plant. As example, germacranolide glaucolide A, when ingested, significantly reduces the rate of growth, increases the number of days pupation, reduces pupal weight and the survival of three species of *Lepidoptera*.

Therapeutic uses of sesquiterpene lactones have been also reported. Inulicin, a guaianolide sesquiterpene lactone from *Inula japonica*, is used as a drug due to the wide spectrum of pharmacological activities possessed. It acts as a stimulant of the central nervous system and smooth muscles of the intestine, and possess anti-ulcer and capillary-strengthening diuretic properties. Helenin a sesquiterpene isolated from *Inula helenium,* is also well known for its pharmacological activities. It is used therapeutically as expectorant, cholagogue and as drug stimulating intestinal secretion. Finally, enydrin, a highly oxygenated germacranolide isolated from *Enhydra fluctuans,* is known for the blood pressure lowering activity.

2.6 TRITERPENOIDS

Triterpenoids are widely distributed in nature and, in particular, ubiquitous among the dicotyledons. Ursolic acid, β-amyrin and friedelin are the triterpenoids most commonly encountered. Triterpenoids constitute the largest family of terpenoids with over 4.000 compounds embracing about forty skeletal types. **Figure 2.9** lists triterpene skeletal types of frequent occurrence in nature.

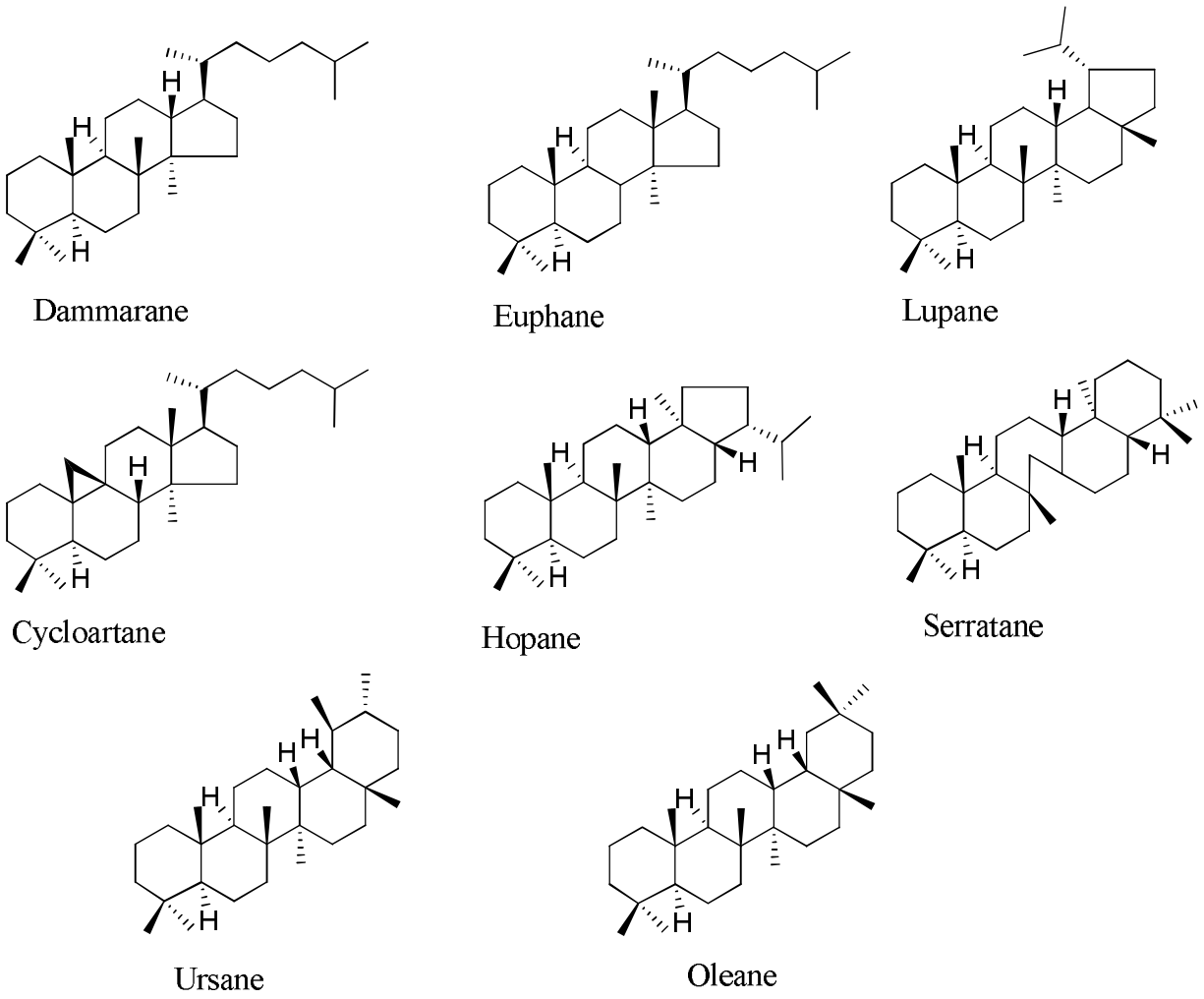


Figure 2.9: Selected triterpene skeletal types

2.6.1 Biogenesis of triterpenes

According to the biogenetic rule, *trans*-squalene is the immediate precursor of all cyclic triterpenoids. In **Figure 2.10** is depicted the pathway of the biosynthesis of squalene:

- ✓ The coupling of two **FPP** units is catalyzed by a membrane-bound enzyme and occurs by way of an SN_2 reaction to generate the species **M**, which undergoes 1,3-deprotonation to furnish presqualene pyrophosphate **N**.

✓ Ionization of presqualene pyrophosphate triggers a rearrangement to cyclopropylcarbinol cation **O** which, by the intervention of NADPH, furnishes squalene **P**.

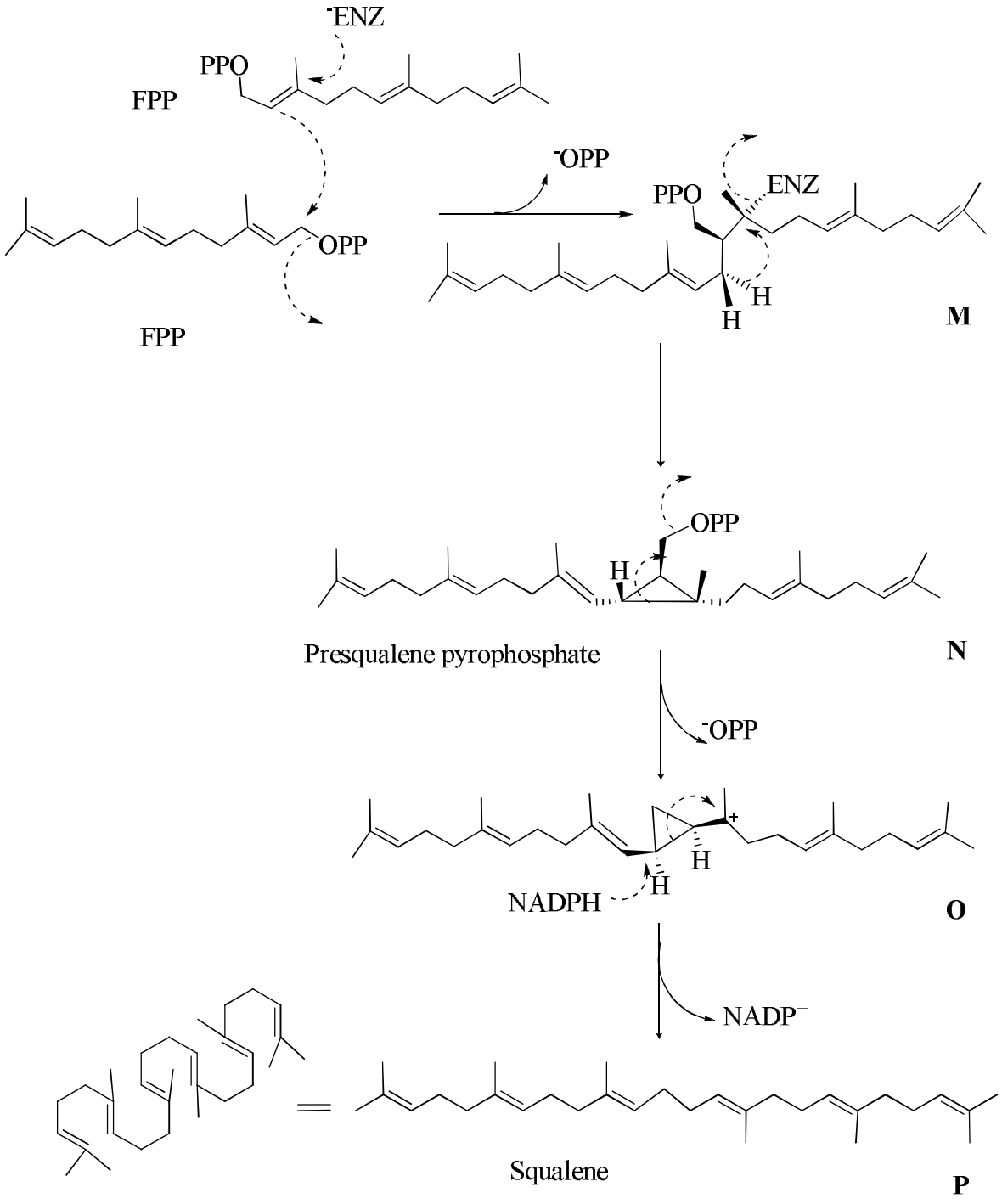


Figure 2.10: Biosynthesis of squalene

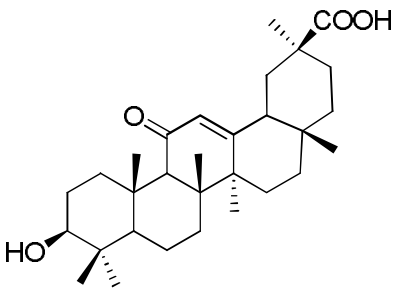
The cyclization of squalene, in the vast majority of cases, proceeds by its oxidation first to 2,3-epoxide, in which the chirality at C-3 is usually *S*. Cyclic triterpenes fall into two main categories, tetracyclic and pentacyclic. It was confirmed (Rowe, 1989), (Manitto, 1981) (Newman, 1972), that the number and the conformation of the formed rings are probably dependent on the folding of the squalene chain into a chair, or boat, or a part remaining unfolded. This possibility is determined by the attachment to the surface of the enzyme catalyzing this conversion. Two main cyclization ways may be distinguished:

✓ If the squalene is in the chair-boat-chair-boat conformation, the cyclization leads to tetracyclic triterpene types like lanostane, protostane, and cycloartane group. These comprise the so-called steroidal triterpenes.

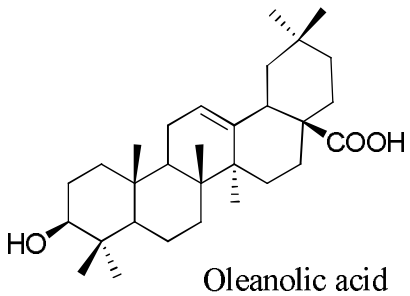
✓ If the squalene is in the chair-chair-chair-boat conformation, this generates the tetracyclic triterpenes like dammarane, euphane, tirucallanes type and pentacyclic triterpenes like lupane, oleanane, ursane, germanicane, taraxastanes and related compounds (**Figure 2.11**).

Lupeol has a wide distribution and has been isolated from many plants, in particular from several genera of families Apocynacae and Leguminosea. It has been also shown that lupeol, betulin (28-CH_2OH) and betulinic acid (28-COOH) are active against walker carcinoma tumour system. Germanicane and taraxastane are minor groups and several of these occur in woody parts of plants.

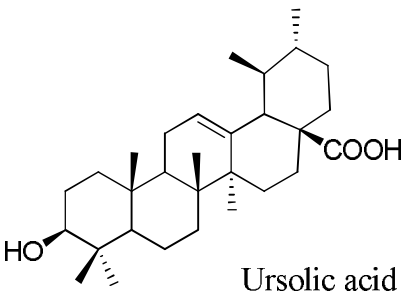
Oleananes constitute the largest class of triterpenoids, and the most widely occurring compounds of this class are represented by α- amyrin (oleanane type), oleanolic acid, and β-amyrin (ursane type). Several oleananes have been obtained from licorice, the root of *Glycyrrhiza glabra*, glycyrrhizin being the most important among these. This compound consists of the calcium and potassium salts of glycyrrhizic acid, the diglucopyranosiduronic acid of the aglycone glycyrrhitic acid.



Glycyrrhetic acid



Oleanolic acid



Ursolic acid

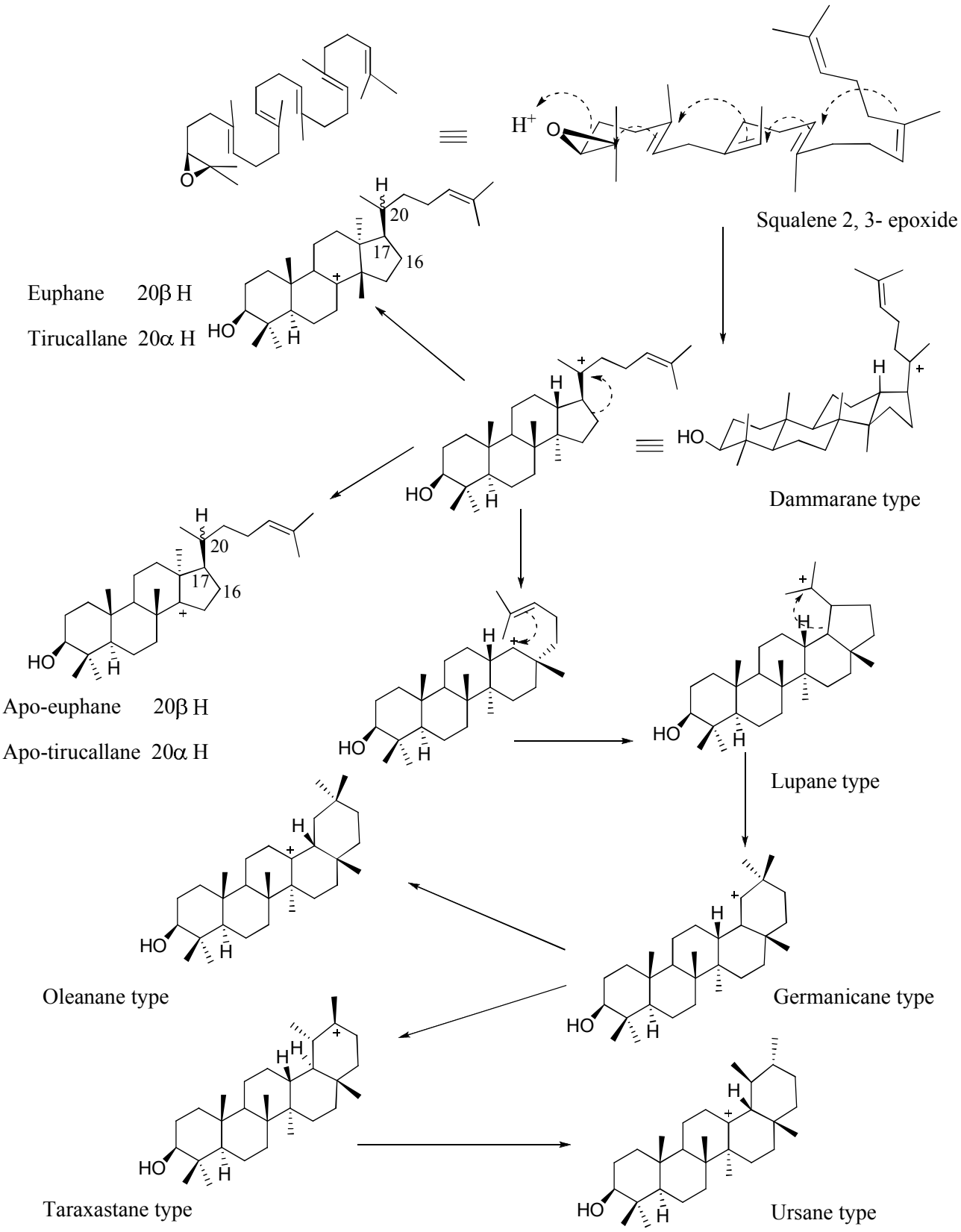


Figure 2.11: Biogenesis of lupane, germanicane, oleanane, taraxastane, and ursane types

Another common oleanane compound is 11β-hydroxy–α-amyrin, which was isolated from black dammar, the resinous exudates of *Canarium strictum*. Several ursanes have been also isolated from nature so far. The most important among them is ursolic acid, which was isolated first from leaves of *Arctostaphylos uva-ursi*.

Naturally-occurring triterpenoids show oxygen functions attached to all expected carbon positions (mainly C-7 and C-11) including the angular methyls with the exception of the methyl at C-8, which has never been reported to be oxidised.

2.6.2 Toxic activities of triterpenoids

Triterpenoids have been reported to have anti-inflammatory activities. α-Amyrin, and β-amyrin showed inhibition of 43.1%, and 26.9% respectively, at the concentration of 40mg/kg on carrageen-induced rat paw oedema, whereas oleanolic acid showed 36.5% inhibition. Several friedelin and dammarane triterpenoids were found to be also active.

Oleanolic, and ursolic acid are relatively non toxic and have been used in cosmetics and health products. Oleanolic acid has been marketed in China as an oral drug for human liver disorders. Several pharmacological studies of oleanolic and ursolic acids indicate that these two triterpenes have many beneficial effects, notably hepatoprotection , anti-inflammation, antitumour-promotion and anti-hyperlipidemia (Lie et al., 1995). Studies on derivatives of these two compounds are also desired to elucidate the structure-activity relationships and to guide the development of novel therapeutic agents.

2.7 POLYPHENOLIC COMPOUNDS

2.7.1 Flavonoids

Flavonoids are a class of polyphenolic compounds that are synthesized solely in plants. They are able to exert numerous biological and pharmacological activities in animal cells.

Flavonoids with low molecule weight are found in all vascular plants and in some of the monocotyledonous families such as seagrasses. They are most concentrated in seeds, citrus fruits, olive oil, tea, etc, and have potent antioxidant, cytoprotective, and anti-inflammatory activities. This family includes five major classes different by their specific chemical structure: flavones, flavonols, flavanones, flavanols and anthocyanidins. They contribute colours to plants, yellows from chalcones and flavonols, and reds, blues, and violets from anthocyanidins. Flavonoids absorb strongly in the UV and the most frequently cited as being UV-protective are flavone and flavonol glycosides having hydroxyl cinnamyl acylation linked through sugar.

2.7.2 Structure variation in flavonoids

Chemically, flavonoids consist of a benzopyran-4-one (rings A and C) carrying a phenyl unit (ring B) at C-2 position as substituent. Flavonoids are distinguished in different classes depending on the degree of oxidation at C-3 in ring C. The major classes of flavonoids are shown in **figure 2.12**.

In flavones and flavonols, the location of the hydroxyl/methoxyl group is usually at C-5, C-7 (ring A) and at C-3', C-4' (ring B) whereas it is rarely at C-2' and C-6' (ring B). The identification of complex mixtures of flavonoids is reported in the literature. This has been achieved by combination of standard techniques including spectroscopy (^{1}H and ^{13}C NMR), hydrolysis (acid, alkali and enzymic) and product analysis, TLC, colour change reaction and comparison with authentic standards.

Some flavonoids contain alkyl groups (mainly methyl group) linked to C-5, C-6, C-7, and C-8 in the ring A; C-2', C-3', C-4' in the ring B; and C-3 in the ring C.

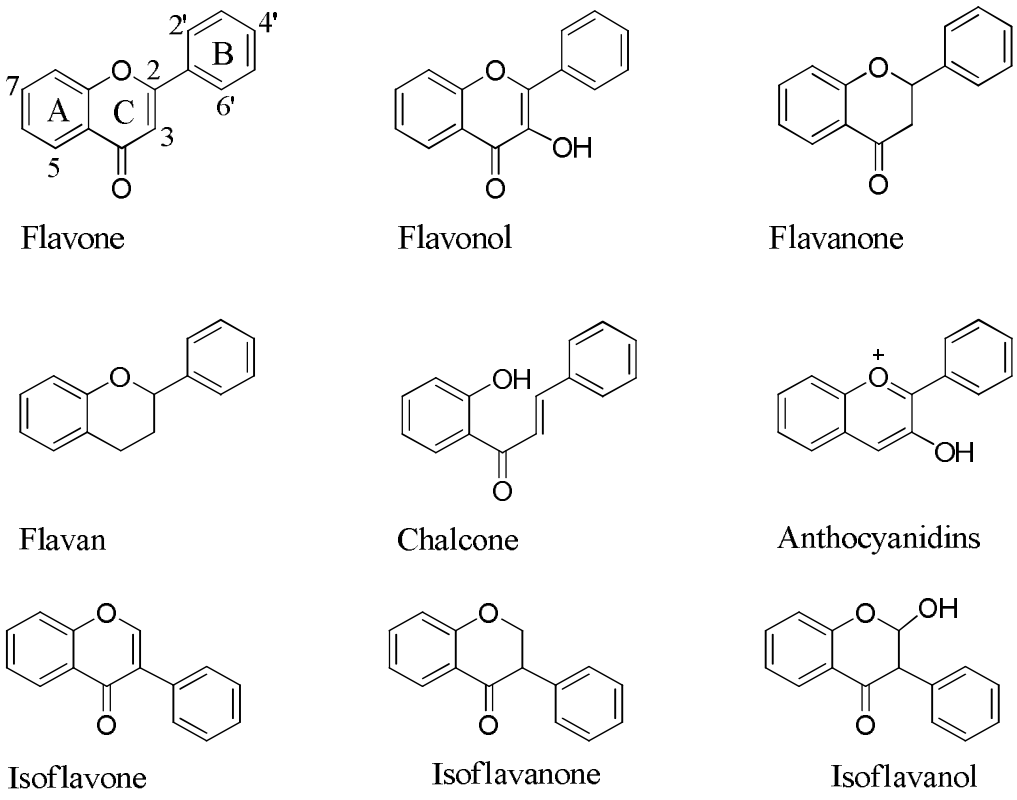


Figure 2.12: Major classes of flavonoids

Modifications to the hydroxylation patterns in the two aromatic rings may occur, generally at flavanone or dihydroflavonol stage, and methylation, glycosylation, and dimethylallylation are also possible, increasing enormously the range of compounds. Thus, a large number of flavonoids occur as *O*-glycosides (mainly glucose derivatives) in which one or more of the hydroxyl groups of the flavonoid are linked to one sugar or more via acid labile hemiacetal bonds. The effect of glycosylation is that flavonoids are less reactive and more soluble in water.

Several flavones and flavonols occur in a conjugated form, covalently linked to inorganic bisulphate (or sulphate). The ^{13}C NMR spectroscopy has been found to be an important tool for the structure elucidation of these compounds, because α, β related carbon resonances with respect to the site of sulphation are mainly affected otherwise remainder of ^{13}C spectrum shows a close resemblance with the non-sulphated hydroxyl compounds.

Such compounds contain one or more sulphate residues attached to a hydroxyl on the flavonoid or sugar moiety. Sulphate flavonoids were found in some seagrasses such as Thalassioideae (*Thalassia testunidum*) and Zosteraceae (*Zostera marina*) (Buchsbaum et al., 1990), and (Rowly et al., 2002).

Acetyl and malonyl are the most common residues among the aliphatic acyl groups and have been reported in various naturally occurring *O*- and *C*-glycosyl flavonoids including flavones, isoflavones, flavonols and anthocyanins.

2.7.3 Biosynthesis of flavonoids

Flavonoids are products from a cinnamoyl-CoA starter unit derived from L-tyrosine *via* the enzyme phenylalanine ammonialyase (PAL), that undergoes a chain extension by adding three molecules of malonyl-CoA. This gives a polyketide, which, according to the nature of the responsible enzyme, can be folded in two different ways generating aromatic rings by either aldol or Claisen–like reaction. Enzymes stilbene synthase and chalcone synthase couple a cinnamoyl-CoA unit with three malonyl-CoA units giving stilbenes, such as resveratrol or chalcones, such as naringenin–chalcone, respectively (Dewick, 2001).

Chalcones act as precursors for a vast range of flavonoid derivatives found throughout the plant kingdom. Most contain a six membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group on the unsaturated ketone giving flavanones such as naringenin (**Figure 2.13**).

Flavanones can then give rise to many variants on this basic skeleton, such as flavones, flavonols, anthocyanidins, and catechins **(Figure 2.14)**.

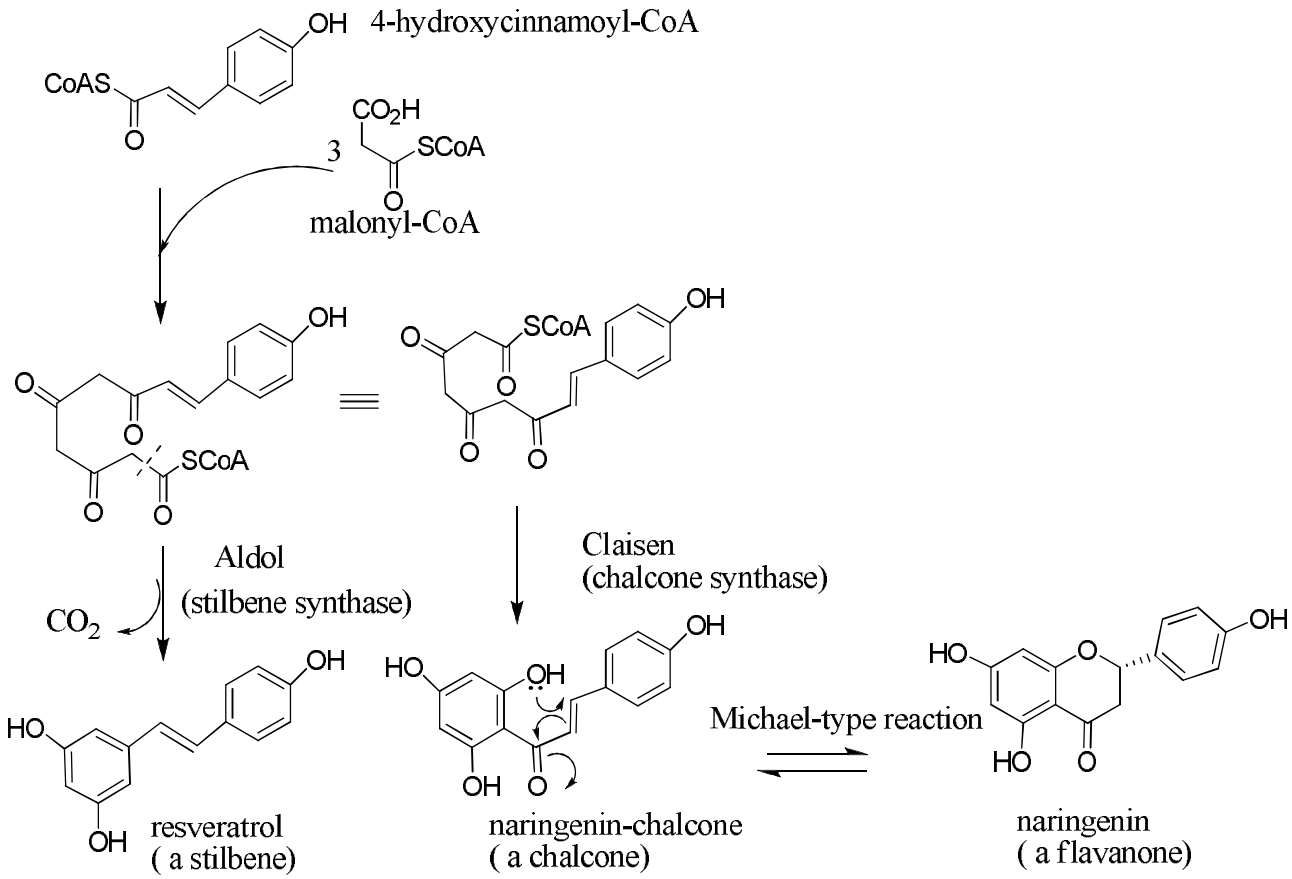


Figure 2.13: Biosynthesis of flavanone: example naringenin

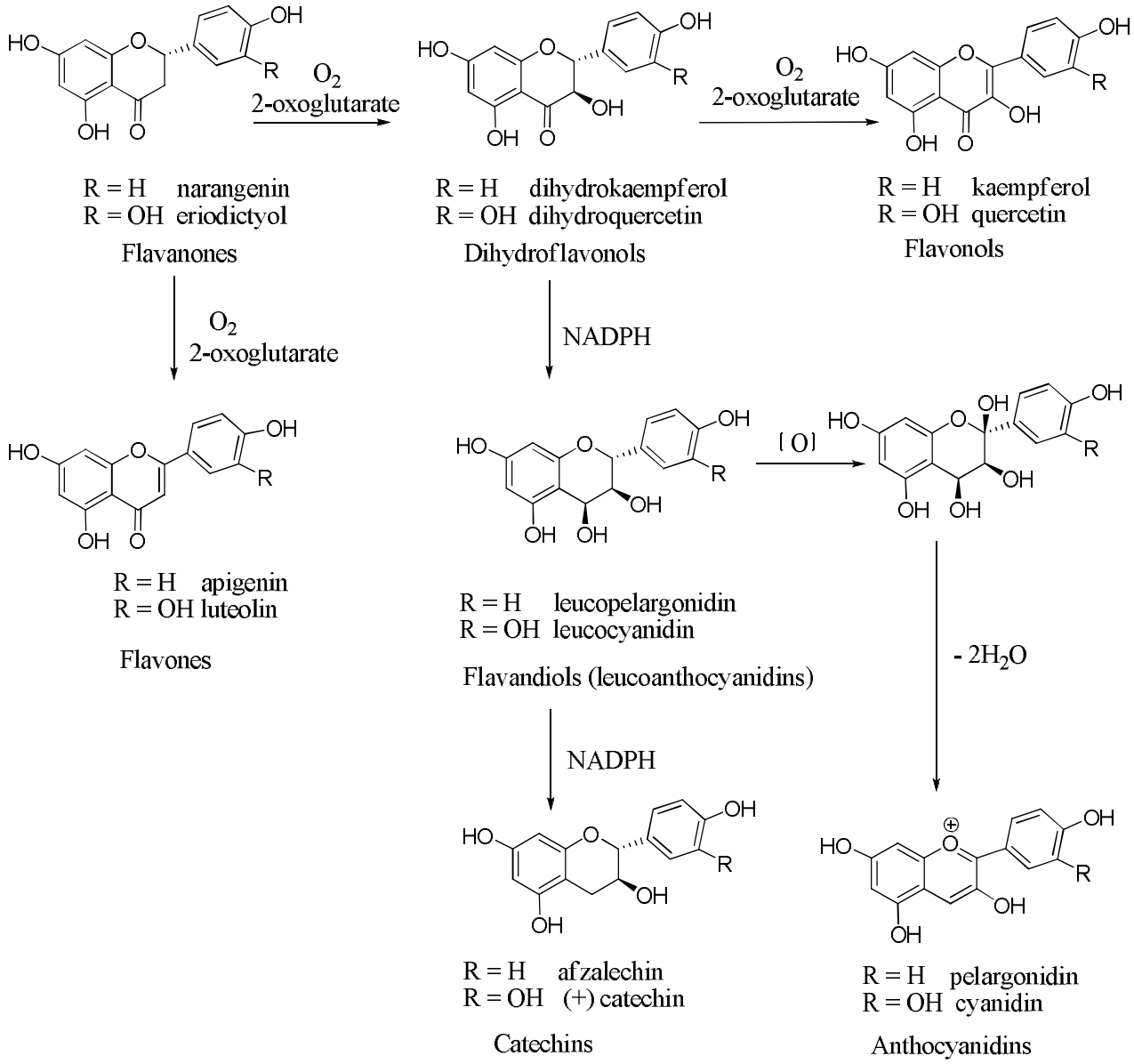


Figure 2.14: Biosynthesis of flavones, flavonols, catechins, and anthocynadins from naringenin

2.7.4 Biological activities of flavonoids

Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, and certain forms of cancer. Their polyphenolic nature enables them to scavenge injurious free radicals such as superoxide and hydroxyl radicals. In particular, quercetin, which is commonly present in substantial amounts in plants tissues, is a powerful antioxidant, chelating metals, scavenging free radicals, and preventing oxidation of low density lipoprotein. Kaempferol, anthocyanidins, and catechins are also demonstrated to be effective antioxidants.

Flavonoids have been previously shown to exhibit anti-inflammatory activity. Apigenin showed significant inhibition of fibroblast growth at all concentration from 0.01 to 100 μg/ml.

Kaempferol is reported to inhibit granulation tissue formation induced by croton oil and to protect against gastric ulcers induced by pyloric ligation and restraint stress in rats.

Anthocyanins and their aglycone, cyanidin, have been also shown to have anti-inflammatory properties. Anthocyanins were tested for their ability to inhibit prostaglandin endoperoxide hydrogen synthase-1 and 2 (PGHS-1 and 2). The glycosides showed little or no activity whereas the aglycone cyanidin displayed significant inhibitory activity against both enzymes with IC50 values of 90 and 60 μM, respectively compared with 1050 μM for aspirin in both tests.

Thalassia testudinum is one of the marine species that contains flavone sulphates: thalassiolin A (luteolin-7-*O*-β-D-glucopyranosyl-2''-sulphate) **270**, thalassiolin B (3'-methoxyluteolin-7-*O*-β-D-glucopyranosyl-2''-sulphate) **271**, and thalassiolin C (apigenin-7-*O*-β-D-glucopyranosyl-2''-sulphate) **272** (Rowly et al., 2002).

Compound **270** has been implicated in the chemical defence of the seagrass against pathogenic marine microorganisms, and showed a good antifungal activity against zoosporic fungi *Schizochytrium aggregatum* (Harborne, 2000). Recently, the three flavones (**270-272)** were tested for their inhibition against HIV virus. Compound **270** was the most active and displayed in vitro inhibition of the integrase catalysed strand transfer reaction (IC_{50}= 0.4 μM) and an antiviral IC_{50} of 30 μM. Molecular modelling studies indicate a favourable binding mode probable at the catalytic core domain of HIV integrase.

Chapter 3
Phytochemical study of *Launaea arborescens* (Butt.) Murb

3. PHYTOCHEMICAL STUDY OF *LAUNAEA ARBORESCENS* (BUTT.) MURB

3.1 Botanical systematic and description

The genus *Launaea* belongs to the tribe Lactuceae of the Asteraceae family and contains about 40 species. In the flora of Algeria, five of the nine *Launaea* species present are endemic of north Africa and include *Launaea arborescens* (Batt.) Murb, synonym *Zollikoferia spinosa* DC (Quezel et al., 1963; Ozenda, 2004).

Launaea arborescens is an almost leafless, xerophilous, perennial shrub reaching a height of 50-150 cm. It occurs in matorrals, on the banks bottoms of temporary streams and often grows on extreme soils. The species has a semi-ruderal character and often rapidly recolonizes stream beds and abandoned fields. The flowers are yellow, and abundant flowering occurs from May to August, but flowers and achenes are produced throughout the year. Seed production is substantial although the percentage of filled fruits varies from 60-90%. Seeds are black, 3-4 mm long and have an average weight of 1.1 mg. They have a well developed pappus that is readily dispersed by wind.

The leaves are in the form of spine and the roots are very deep. The plant emits a very strong smell when we cut branches or roots. It produces a whitish liquid (sap), which is similar in appearance to milk (thus the local name “Oum loubina”).

It is a Saharo-Canarian phytogeographical element, but is also present in the northern and western Sahara. In Algeria, it grows in the area from the South to Beni-Abbés and Tademait **(**Ozenda, 2004).

The plant is appreciated by livestock, mainly by camel. It’s known as a medicinal plant being used as an antidiarrhoic and antispasmodic.

Its milk is used in the treatment of the skin diseases.

Figure 3.1: Aerial parts and roots of Launaea arborescens

Literature chemical data on this species are scarce and few published papers only describe phenolic components of the plant. In the frame of a study by a Spanish group of three species of the genus *Launaea,* including *L. arborescens*, the aerial parts of this species was found to contain eight common phenolic compounds including three flavonoids, luteolin **220**, luteolin-7-*O*-glucoside **225**, luteolin-7-*O*-rhamnoside **222**, two coumarins, aesculetin **230** and its glycoside cichoriin **231**, and ethyl-caffeoate **214** and ferulic acid **215**. It was also found that the other two species *L. acanthoclada* and *L. residifolia* were characterized by the same phenolic content and that cichoriin was the most abundant compound in all samples (Giner et al., 1992).

Recently, Belboukhari and Cheriti ((Belboukhari et al., 2006) reported the phytochemical study of the aerial parts of an Algerian sample of *L. arborescens* collected from Bechar. The authors described the isolation of four compounds from the methanol extract, two flavonoids, 3-acetyl-5-methoxy-7,3',4'-trihydroxyflavan-3-ol-8-*O*-glycoside **288** and 5,7,4'-trihydroxy-3'-methoxyflavone **289**, one lignan, 4,4'-dihydroxy-3,3'-dimethoxy-7,9':7,9'-diepoxylignan **290**, and one diterpene, methyl-15,16-epoxy-12-oxo-8(17),13(16),14-ent-labdatrien-19-oate **291**.

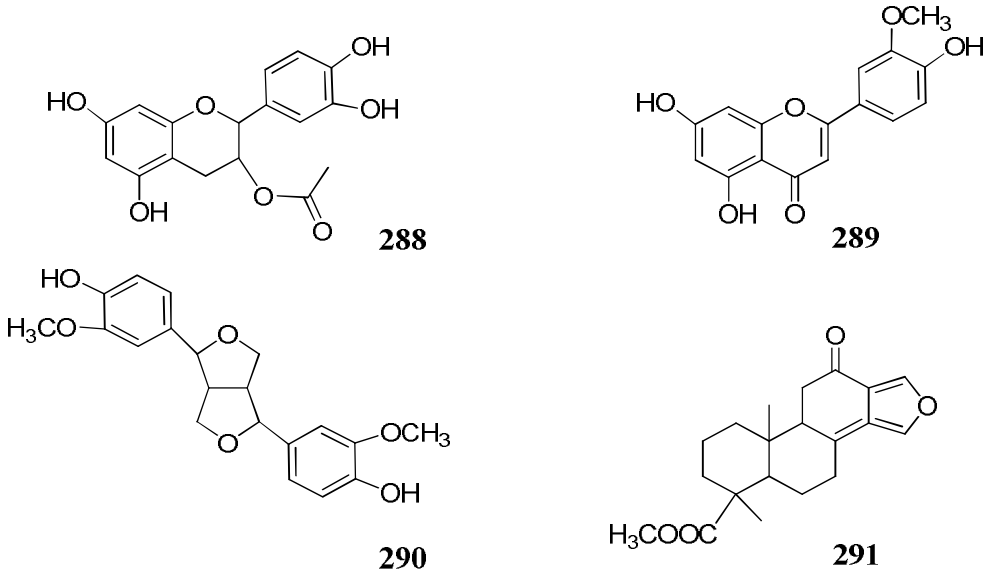


Figure 3.2: Compounds isolated from Launaea arborescens

3.2 Collection of the biological material

The aerial parts of *Launaea arborescens* were collected in 2004 from Bechar (Laabadla), South of Algeria, while the roots were collected in April 2006 from the same place. The plant was identified by the Pr. Oudjehih Bachir of the Agronomy Department of Batna University.

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3.3 CHEMICAL INVESTIGATION OF *LAUNAEA ARBORESCENS*: EXTRACTION AND PURIFICATION

3.3.1 Aerial parts

Dried and powdered aerial parts (900 g) of *Launaea arborescens* were treated with light petroleum ether (3 L, 3 times) to afford, after evaporation of the solvent under reduced pressure, 12 g of a crude extract. The extract was analysed by TLC chromatography, using different elution system (light petroleum ether/ethyl acetate) and (dichloromethane/methanol), and showed a very complex secondary metabolite pattern. Thus, this extract was subjected to a series of purification steps as indicated in the protocol summarised in **Figure 3.3**.

First, the extract was submitted to silica gel column chromatography using a gradient of ethyl acetate in light petroleum ether. The fractions recovered were subsequently subjected to different purification steps including silica gel column chromatography and reverse-phase HPLC

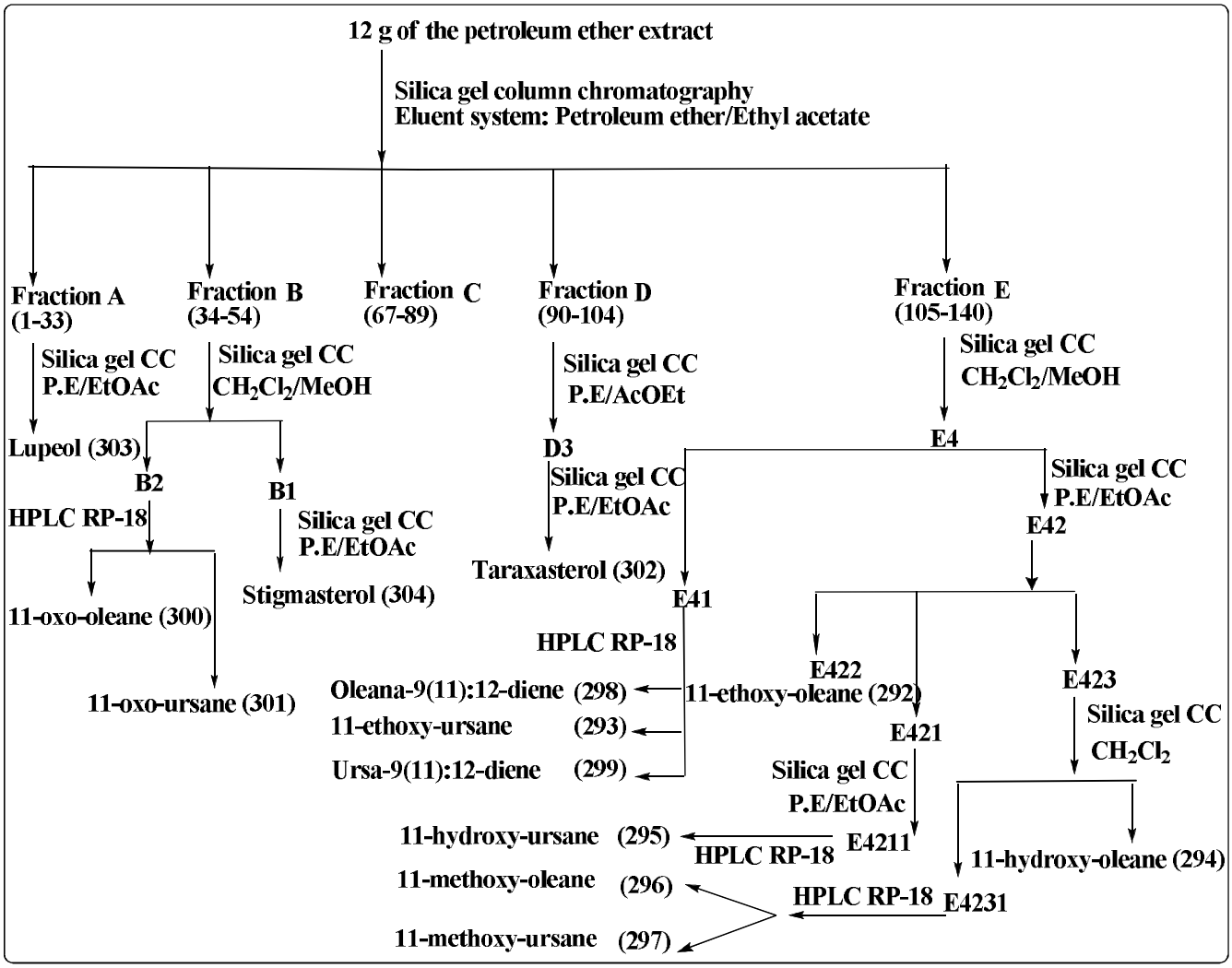


Figure 3.3: Scheme of the purification of the light petroleum ether extract of the aerial parts of Launaea arborescens

3.3.2 Roots

Dried and powdered roots (1 kg) were macerated with methanol (7 L) to give 27 g of a crude extract which was partitioned between water and ethyl acetate. The organic phase yielded 3.7 g of a crude extract after removal of ethyl acetate.

The thin layer chromatography (TLC) of the ethyl acetate extract showed the presence of several spots including some with a strong UV visible absorbance at 254 nm.

An aliquot of this extract (2 g) was subjected to Sephadex LH-20 column chromatography, using $CHCl_3$/MeOH (1:1) as isocratic elution system, to obtain 9 fractions from **A** to **I**. A preliminary spectroscopic analysis of these fractions revealed that fractions **C** and **G** contained terpene components.

Further purification of these fractions has been done by High Liquid Performance Chromatography in reversed phase (HPLC-RP 18) to get compounds **305 - 318**.

The following **Figure 3.4** summarises the chromatographic steps used to purify the extract.

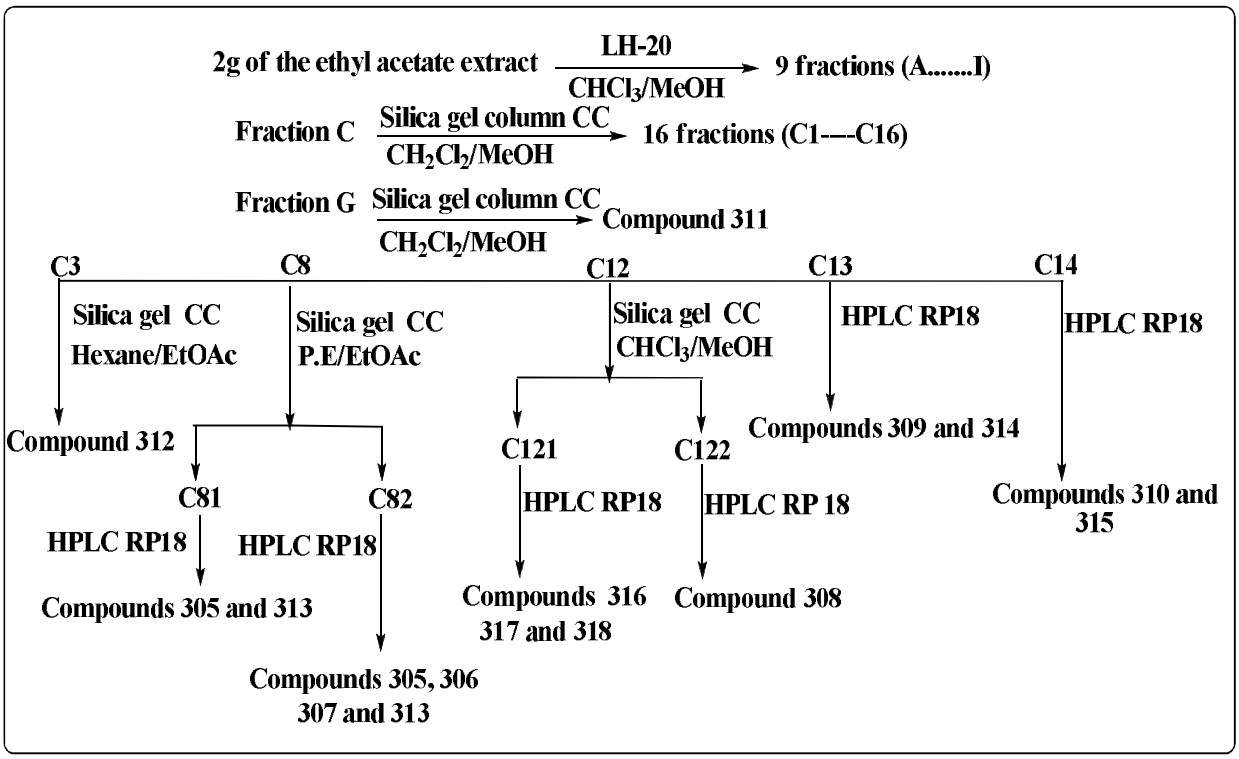
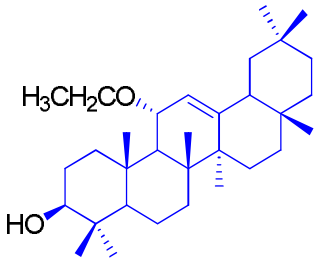
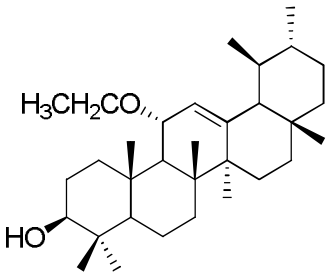


Figure 3.4: Scheme of the purification of the ethyl acetate extract of the roots

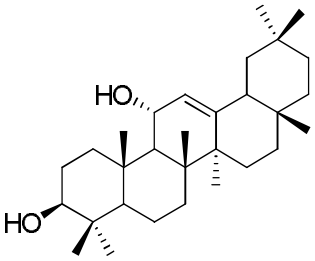
All the structures of the isolated terpenoids (**292-318**) from both aerial parts and roots are illustrated in the **figure 3.5**.



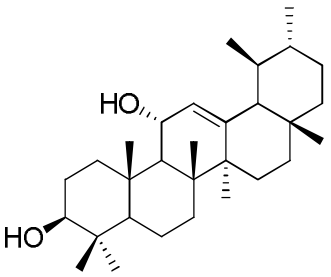
3β-hydroxy-11α-ethoxy-olean-12-ene **(292)**



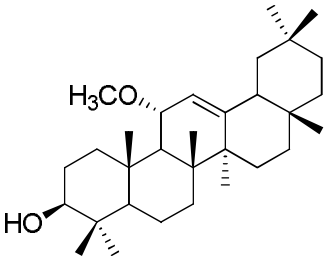
3β-hydroxy-11α-ethoxy-urs-12-ene **(293)**



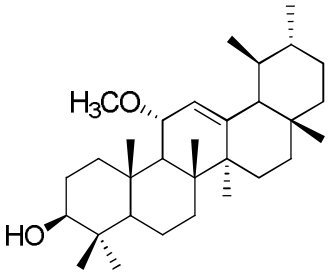
3β-11α-dihydroxy-olean-12-ene **(294)**



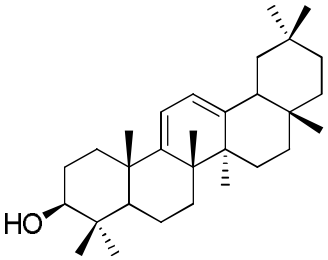
3β-11α-dihydroxy-urs-12-ene **(295)**



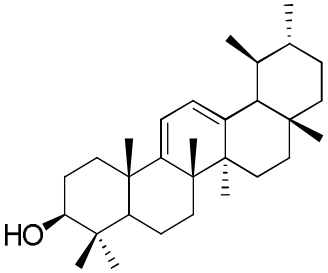
3β-hydroxy-11α-methoxy-olean-12-ene **(296)**



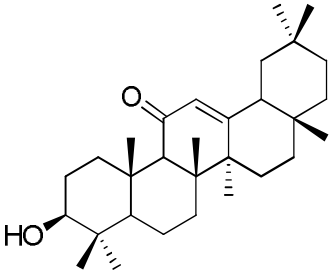
3β-hydroxy-11α-methoxy-urs-12-ene **(297)**



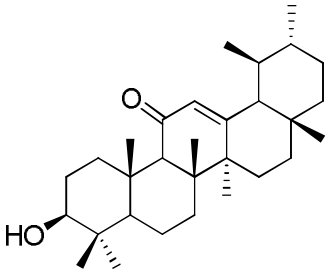
3β-hydroxy-oleana-9(11):12-diene **(298)**



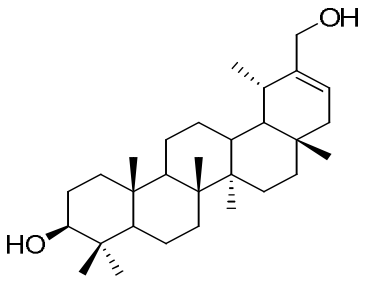
3β-hydroxy-ursa-9(11):12-diene **(299)**



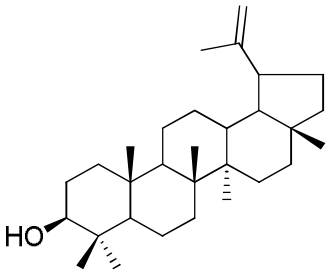
3β- hydroxy-11-oxo-olean-12-ene **(300)**



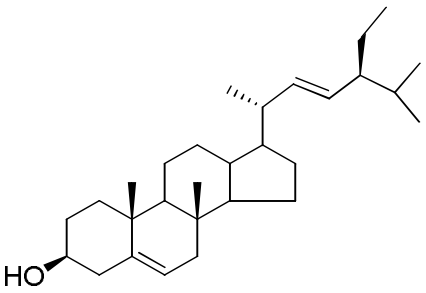
3β-hydroxy-11-oxo-ursa-12-ene **(301)**



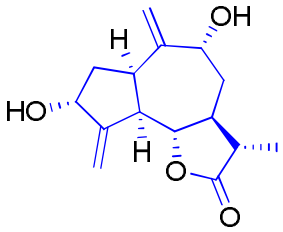
Taraxast-20-ene-3β,30-diol **(302)**



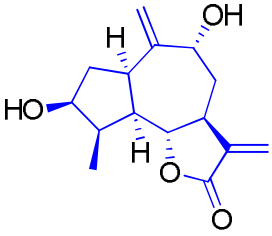
Lup-20(29)-en-3-ol **(303)** (Lupeol)



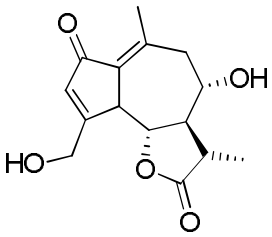
24-ethyl-5,22-cholestadien-3-ol **(304)** (Stigmasterol)



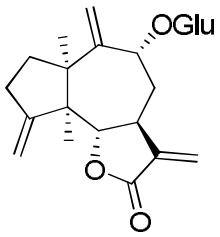
9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C**(305)**



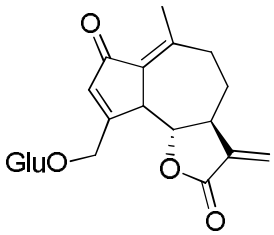
9α-hydroxy-4α,15-dihydrozaluzanin C **306)**



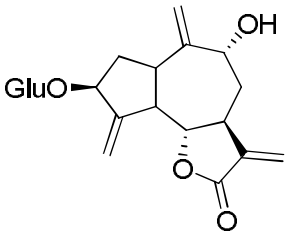
11β,13-dihydrolactucin **(307)**



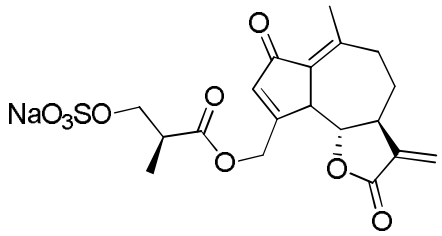
3α-hydroxy- guaia-4(15), 10(14),11(13)-triene-12,6αolide-3-*O*-β-glucopyranoside **(308)** (Ixerisoside D)



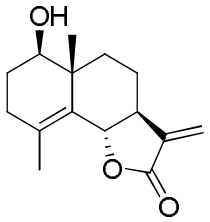
Guaia-2-oxo-1(10),2(3),11(13)-triene-12.6α-olide-15-*O*-β-glycopyranoside Crepidiaside A **(309)**



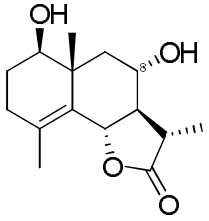
9α-hydroxy zaluzanin-3-*O*-β- glucopyranoside **(310)**



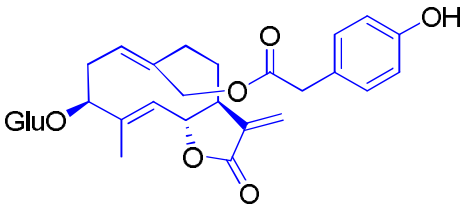
8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl) lactucin-3'-sulfate **(311)**



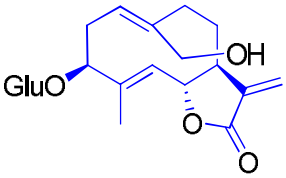
1β-hydroxyeudesm-4(5)-ene-12,6α-olide **(312)**



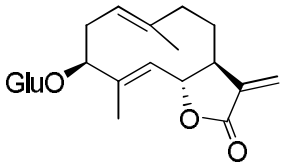
1β,8α-dihydroxy-eudesm-4-en-6β-7α, 11βH-6- olide **(313)**



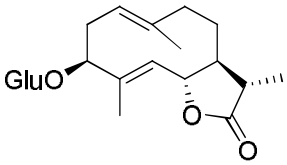
3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate **(314)**



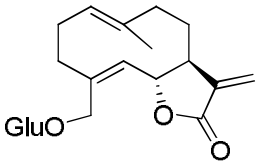
3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside **(315)**



3β-hydroxycostunolide-3-*O*-β-glycopyranoside **(316)** (Picriside C)



3β-hydroxy-11β,13-dihydrocostunolide-3-*O*-β-glucopyranoside **(317)** (Sonchuside A)



Germacra-1(10)E,4E,11(13)-triene-12,6α-olide-15-*O*-β- glycopyranoside **(318)** (Picriside B)

Figure 3.5: Structures of the molecules isolated from both aerial parts and roots of Launaea arborescens

3.4 STRUCTURAL ELUCIDATION OF ISOLATED COMPOUNDS

Among the plethora of terpenoids isolated from both aerial parts and roots, five of them, compounds **292**, **305**, **306**, **314**, and **315**, resulted to be new. Their structures were defined by spectroscopic methods, mainly NMR techniques. Complete carbon and proton assignments were also made for selected known molecules that have been only partially characterised in the literature. In the course of this study, the stereochemical analysis of a lactucin sulphate derivative, compound **311**, which was the main terpenoid of *L. arborescens*, was also conducted for assigning the absolute configuration of the chiral carbon in the side chain.

3.4.1 TRITERPENOIDS STRUCTURES

3.4.1.1 Structure elucidation of compound 292

Compound **292** was obtained as a powder and had the molecular formula $C_{32}H_{54}O_2$ as deduced by both the mass and the ^{13}C NMR spectra. In the mass spectrum (**Figure 3.6**), a very intense peak at *m/z* 425, corresponding to the fragment $[M - 46 + H]^+$ and indicating the loss of an ethanol unit, and a peak at *m/z* 407 corresponding to the loss of both water and ethanol, were observed.

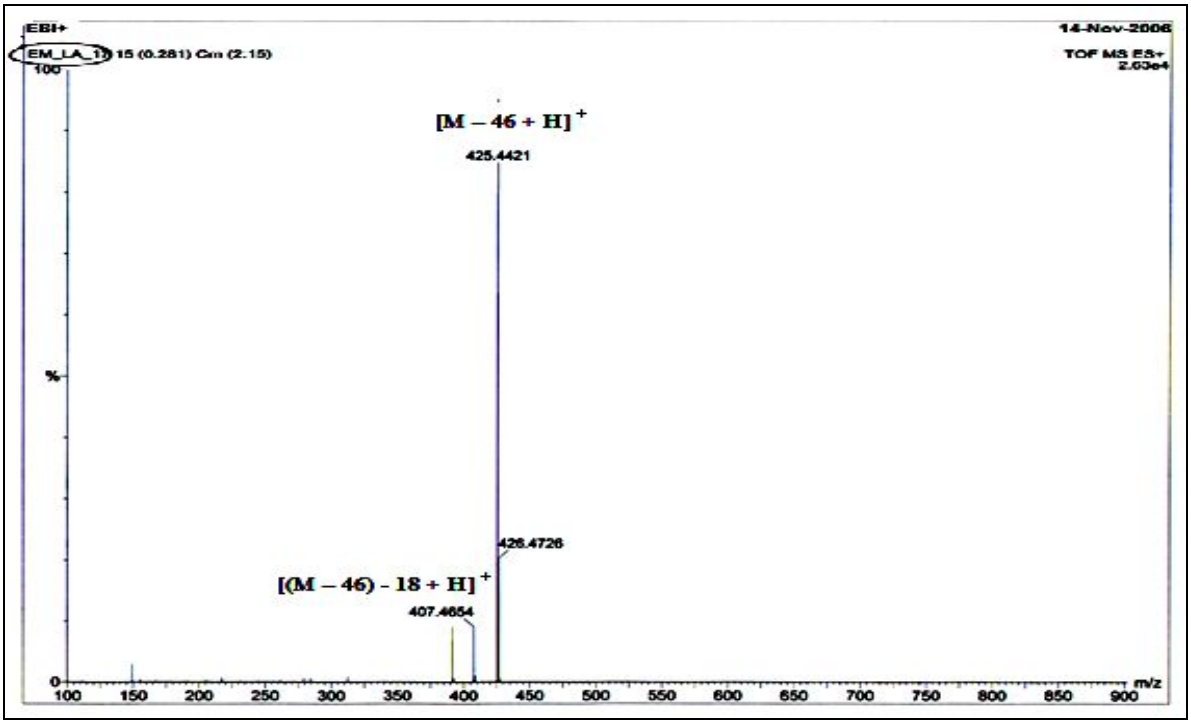


Figure 3.6: ESIMS spectrum of compound 292

The presence of the triterpene skeleton was confirmed by analyzing both ^{1}H and ^{13}C NMR spectra (**Figures 3.7**, **3.8**). In fact, the ^{1}H NMR of compound **292** revealed the presence of eight methyl singlets [δ 0.80 (H_3-24), 0.83 (H_3-28), 0.88 (H_3-30), 0.89 (H_3-29), 0.99 (6H, H_3-23 and H_3-26), 1.04 (H_3-25), and 1.20 (H_3-27)], two methine signals attached to an oxygen function at δ 3.23 (m, H-3) and δ 3.94 (dd, J = 9.4 and 3.3 Hz, H-11) and one olefinic proton at δ 5.31 (1H, d, J = 3.3 Hz, H-12).

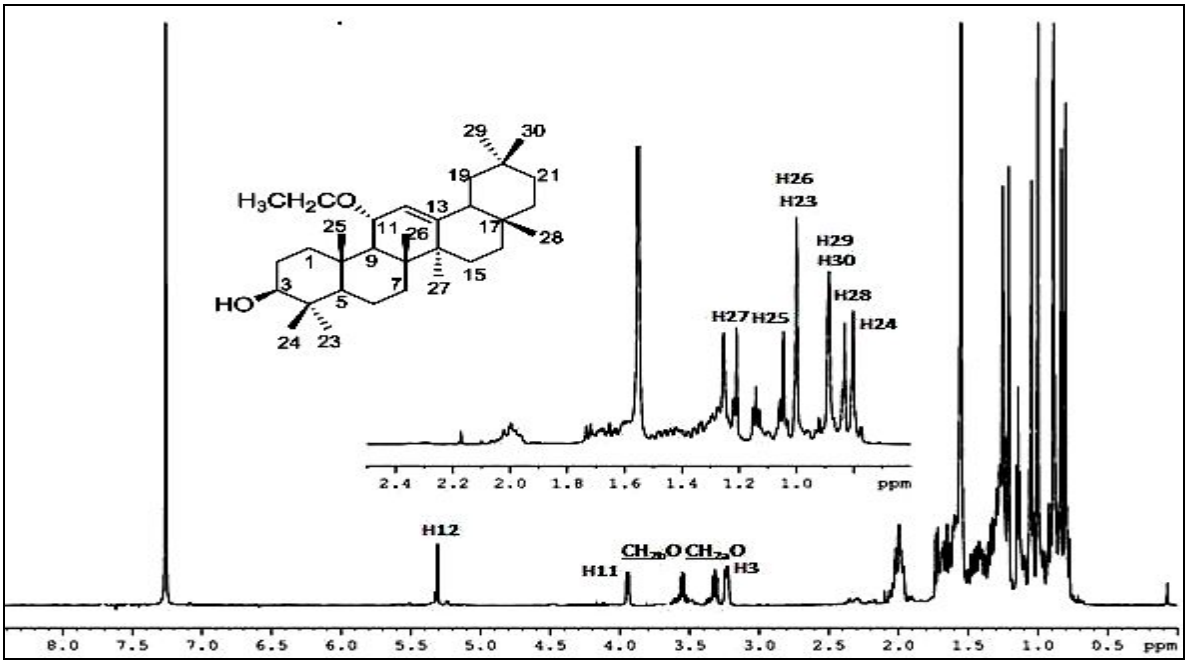


Figure 3.7: ^{1}H NMR spectrum of compound 292 in $CDCl_3$

The ^{13}C NMR spectrum of **292** contained 32 signals which were attributed to the carbons of a double bond, two methines attached to an oxygen function, three methines, nine methylenes, eight methyls, six quaternary carbons and an ethoxy group.

The comparison of the spectroscopic data of **292** with those of different triterpenoid frameworks indicated that compound **292** had a functionalised oleanane skeleton.

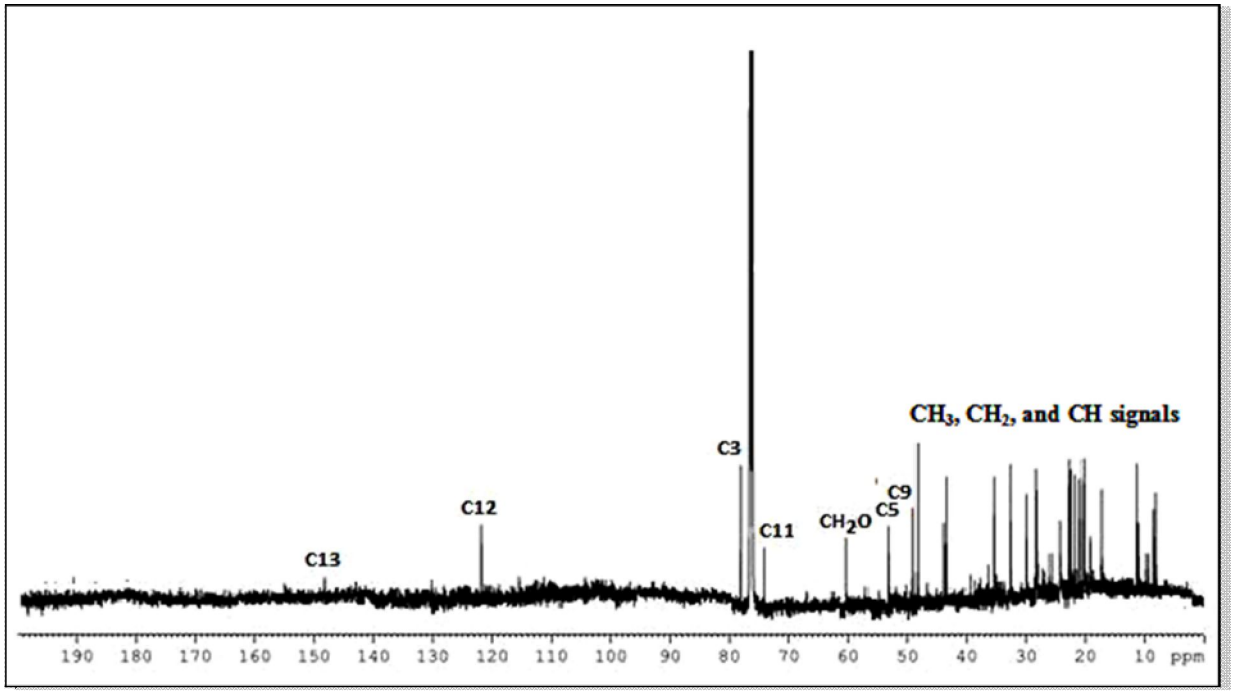


Figure 3.8: ^{13}C NMR spectrum of compound 292

The presence of an ethoxy group was indicated by both two double quartet at δ 3.31 and 3.55 (each 1H, dq, J = 8.7 and 7.2 Hz, CH_3CH_2O–) and the 3H triplet at δ 1.14 (J = 7.2 Hz, CH_3CH_2O–). According to the proposed structure, the ^{13}C NMR spectrum of compound **292** displayed two additional signals due to the ethoxy moiety at δ 61.4 (CH_3CH_2O–) and δ 16.8 (CH_3CH_2O–) along with the typical values of the oleanane skeleton (**Table 3.1**). A detailed 2D NMR analysis (^1H-^1H COSY, HSQC and HMBC) (**Figure 3.9**, **3.10**, and **3.11**) allowed the assignment of all carbon and proton values.

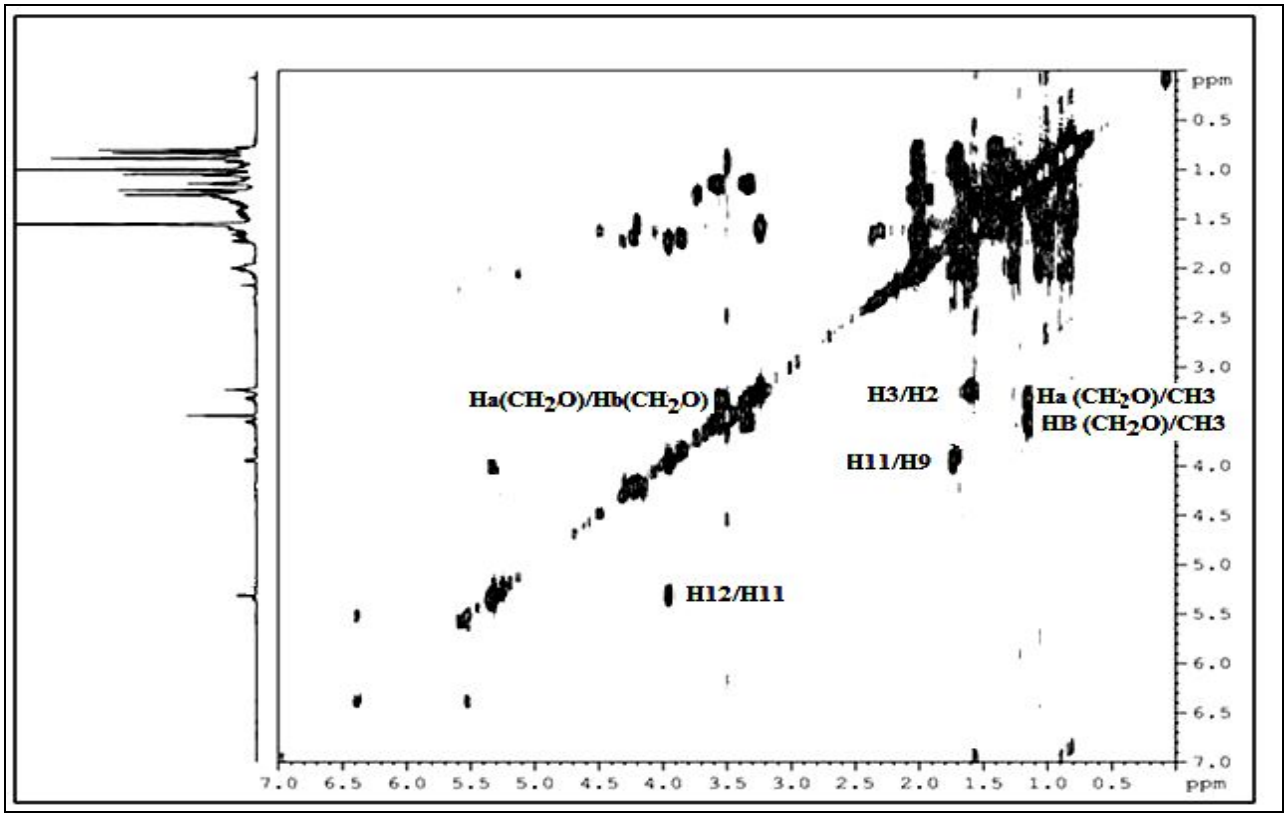


Figure 3.9: ^{1}H-^{1}H COSY spectrum of compound 292

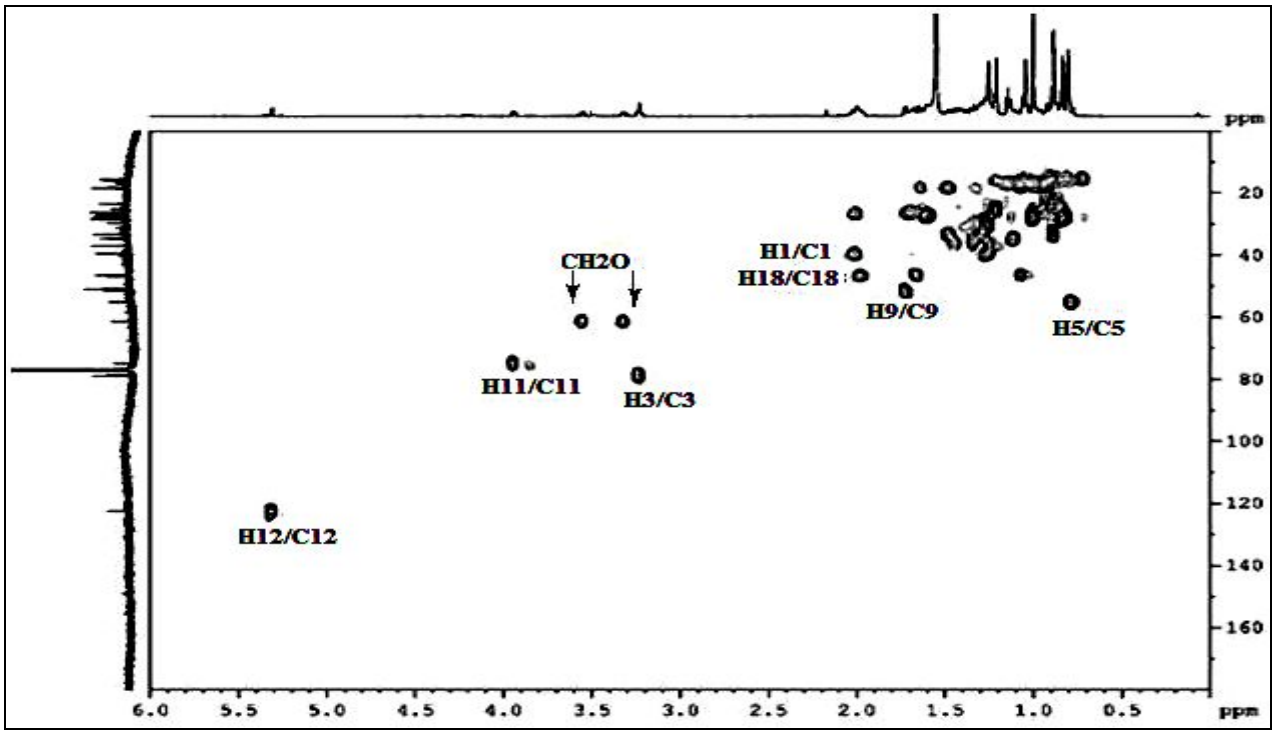


Figure 3.10: HSQC spectrum of compound 292

In the HMBC spectrum, the proton signal at δ 3.94 (dd, *J* = 9.4 and 3.3 Hz, H-11) showed correlations with carbon resonances at δ 122.7 (C-12) and δ 61.4 ($CH_3\underline{CH_2O}$–), clearly indicating that the ethoxy group was attached to the carbon C-11. Other correlations are depicted in the **Figure 3.11**.

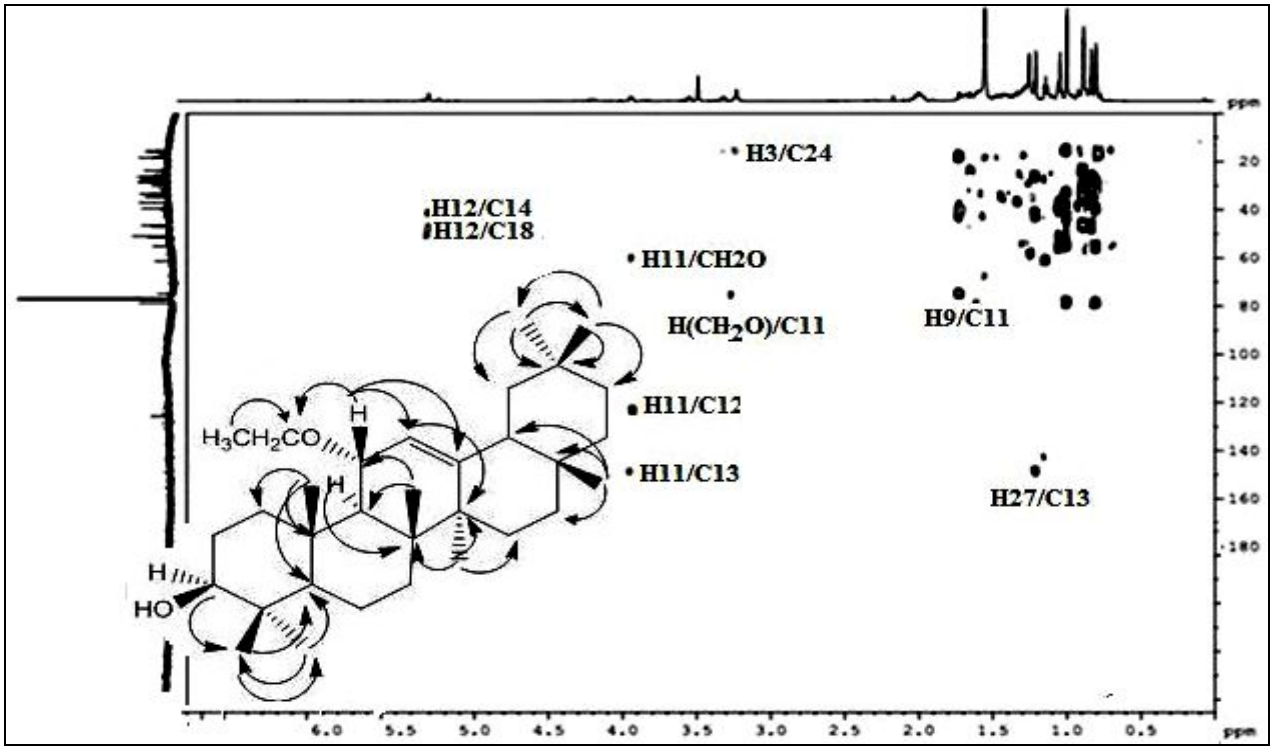


Figure 3.11: HMBC spectrum of compound 292

The relative stereochemistry at C-11 was suggested by analysis of the coupling constant value of H-11 resonating as a double doublet (*J* = 9.4 and 3.3 Hz) in agreement with a β-orientation, analogously with the data reported for the corresponding hydroxyl (Bohlmann et al., 1984), (Xiao et al., 1994) and methoxyl (Fujita et al., 2000) derivatives.

Table 3.1: ^{1}H and ^{13}C NMR data of conpound 292 in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	39.4	1.75	m	H-25
		2.02	m	-
2	27.5	1.62	m	-
3	78.6	3.23	-	H-23, H-24
4	38.7	-	-	H-23, H-24
5	55.2	0.77	m	H-23, H-24, H-25
6	18.4	1.37	m	-
		1.42	m	-
7	33.3	1.57	m	-
		1.73	m	-
8	42.6	-	-	H-9, H-27
9	51.5	1.72	d (9.0)	H-25, H-26
10	38.3	-	-	H-9, H-25
11	74.8	3.94	dd (9.4, 3.3)	H-9
12	122.7	5.31	d (3.3)	H-11
13	148.9	-	-	H-11, H-27
14	41.8	-	-	H-9, H-12, H-27
15	26.3	1.70	m	H-27
		2.02	m	-
16	26.8	1.61	m	H-28
17	32.3	-	-	H-28
18	46.9	1.99	-	H-12, H-28
19	46.5	1.05	m	H-29, H-30
		1.64	m	-
20	31.1	-	-	H-29, H-30
21	34.7	1.11	m	H-29, H-30
22	37.0	2.02	m	-
23	28.2	0.99	s	H-3, H-24
24	15.6	0.80	s	H-3, H-23
25	15.9	1.04	s	H-9
26	18.2	0.99	s	H-7
27	25.3	1.20	s	-
28	28.5	0.83	s	-
29	33.6	0.89	s	H-30
30	23.7	0.88	s	H-29
Ethoxy group				
OCH_2	61.4	3.31	dq (8.7, 7.2)	CH_3, H-11
		3.55	dq (8.7, 7.2)	-
CH_3	16.8	1.14	t (7.2)	-

Compound **292** underwent rapidly to an elimination reaction of the ethanol residue at C-11 to give the corresponding conjugated diene (Tanaka et al., 1988), which was more stable than the starting compound. This elimination was favoured by the presence of acid impurities in the solvent as evidenced by recording the ^{1}H NMR spectrum on a sample allowed to stand at room temperature for some time. In this spectrum two olefinic signals at δ 5.50 (1H, d, *J* = 6.0, H-11) and δ 5.59 (d, *J* = 6.0, H-12) were present rather then the signal at δ 3.94 (1H, dd, *J* = 9.4 and 3.3 Hz, H-11) according to the described transformation.

All these data led us to propose without ambiguity the structure 3β-hydroxy-11α-ethoxy-olean-12-ene for compound **292**.

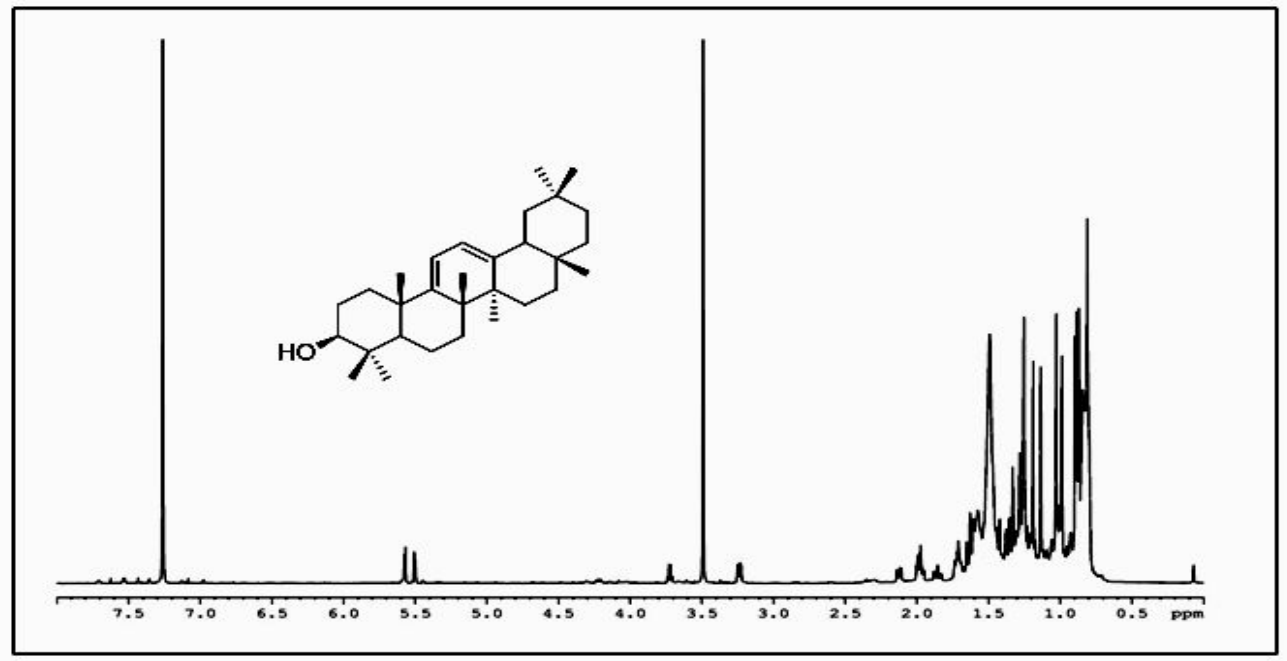


Figure 3.12: ^{1}H NMR spectrum of compound 292 in $CDCl_3$ after dehydration

3.4.1.2 Structure elucidation of compound 293

Careful analysis of the mass spectrum of compound **293** (see **Figure 3.13**) showed that it has the same mass as compound **292**. The pseudo molecular peak at *m/z* 493 $[M + Na]^+$ corresponds to the formula $C_{32}H_{54}O_2$.

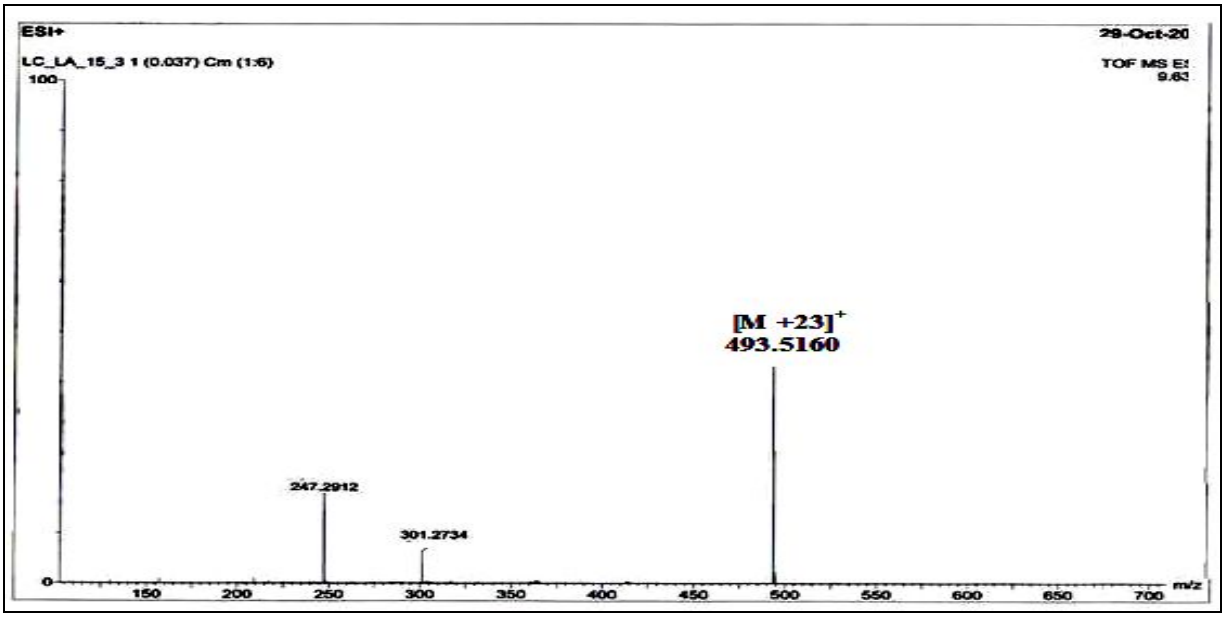


Figure 3.13: ESIMS spectrum of compound 293

The NMR spectra showed strong similarities with those of compound **292**. The main difference was evidenced by the ^{1}H NMR spectrum (**Figure 3.14**) revealing the presence of six methyl singlets [δ 0.80 (H_3-24), 0.81 (H_3-28), 1.01 (H_3-23), 1.04 (H_3-26), 1.07 (H_3- 25), and 1.16 (H_3-27)], and two methyl doublets [δ 0.88 (3H, d, J = 6.0 Hz, H_3-29), and 0.91 (3 H, br d, J = 6.0 Hz, H_3-30)] instead of eight tertiary methyl groups as in compound **292**. This indicated that compound **290** had a different triterpenoid carbon skeleton and, in particular, an ursane-type framework.

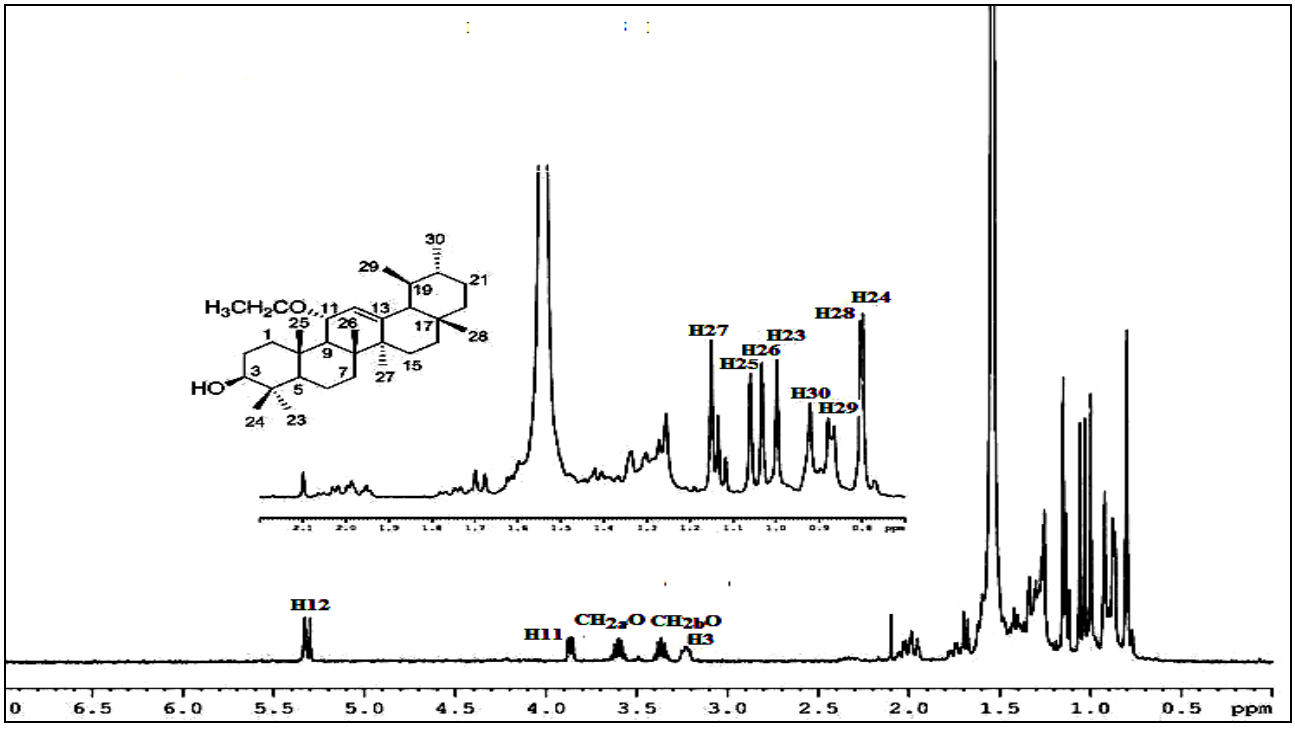
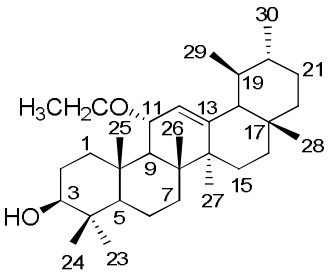


Figure 3.14: ^{1}H NMR spectrum of compound 293 in $CDCl_3$

Compound **293** showed the same functionalisation pattern as compound **1** with an hydroxyl group at C-3 [δ 3.24 (1H, m, H-3)], a double bond at C-12 [δ 5.33 (1H, d, J = 2.6 Hz, H-12)], and an ethoxyl residue at C-11 [δ 3.87 (1H, dd, J = 8.9 and 3.0 Hz, H-11), δ 3.37 (1H, dq, J = 8.5 and 6.9 Hz, CH_3CH_2O), and δ 3.60 (1H, dq, J = 8.5 and 6.9 Hz, CH_3CH_2O)].

The structure was finally confirmed by comparing the NMR assignments with those reported in the literature for the compound isolated from the roots bark of *Tripterigyum hypoglaucum,* belonging to the Celastraceae family (Fujita et al., 2000). Thus, compound **293** was identified as 3β-hydroxy-11α-ethoxy-urs-12-ene.



3.4.1.3 Structure elucidation of compound 294

Compound **294** was identified as 3β,11α-dihydroxyolean-12-ene by spectroscopic data that were collected by recording the ^{1}H NMR, HSQC, HMBC and the mass spectra. The ^{1}H NMR spectrum (**Figure 3.15**) showed the presence of eight methyl singlets [δ 0.79 (H_3-24), 0.84 (H_3-28), 0.88 (H_3-29 and H_3-30), 0.98 (H_3-23), 1.00 (H_3-26), 1.05 (H_3-25) and 1.21 (H_3-27)] according to an oleanane-type skeleton. The spectrum contained also signals at δ 5.20 (1H, d, J = 3.73 Hz, H-12), δ 4.25 (1H, dd, J = 8.0 and 3.8 Hz, H-11), and δ 3.22 (1H, dd, J = 10.3 and 6.9 Hz, H-3) that were attributed to a vinyl proton and two oxygenated methine protons, respectively.

Analysis of the COSY experiment allowed the positioning of the two oxygenated functions at C-3 and C-11 and of the double bond at C-12, analogously with above described closely related compound **292**.

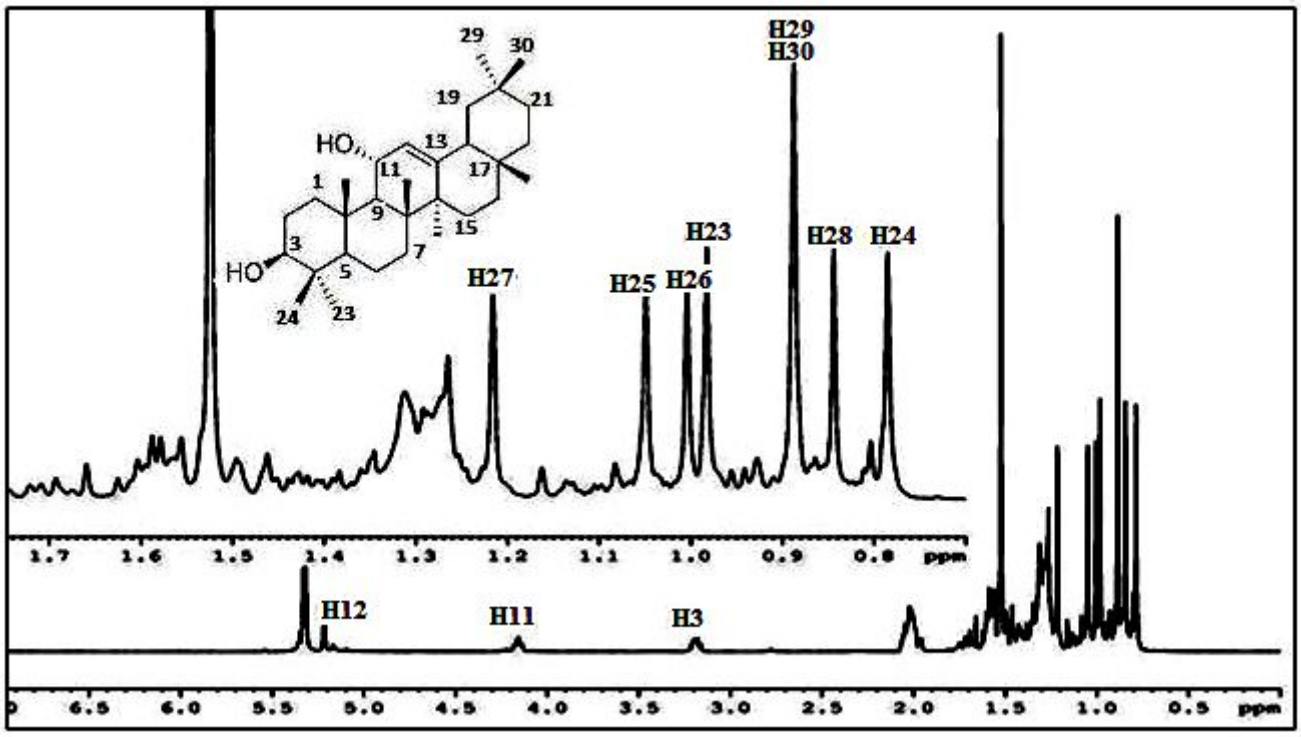


Figure 3.15: ^{1}H NMR spectrum of compound 294 in CD_2Cl_2

The relative stereochemistry at C-11 was deduced by the analysis of coupling constant value of the proton H-11. The high value of 8.0 Hz supported a β orientation, which is very common in these triterpenoids. All assignments of proton and carbon values were made by 2D NMR experiments (^{1}H-^{1}H COSY, HSQC and HMBC) (**Table 3.2**).

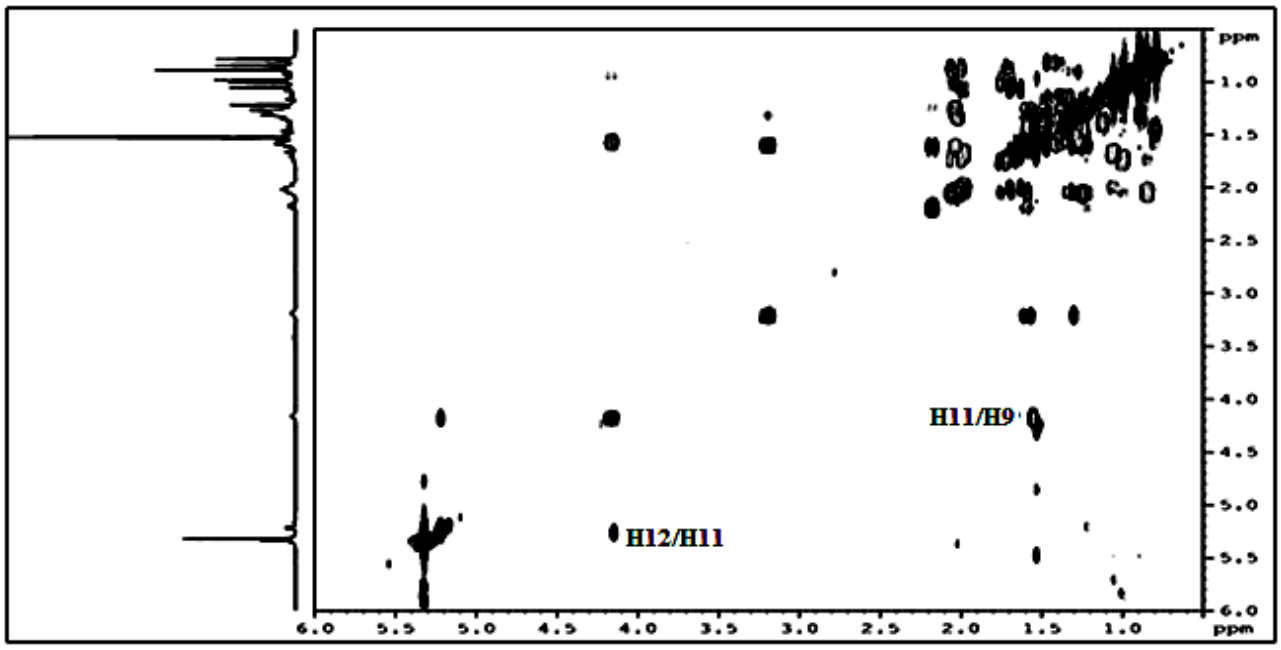


Figure 3.16: ^{1}H-^{1}H COSY spectrum of compound 294 in CD_2Cl_2

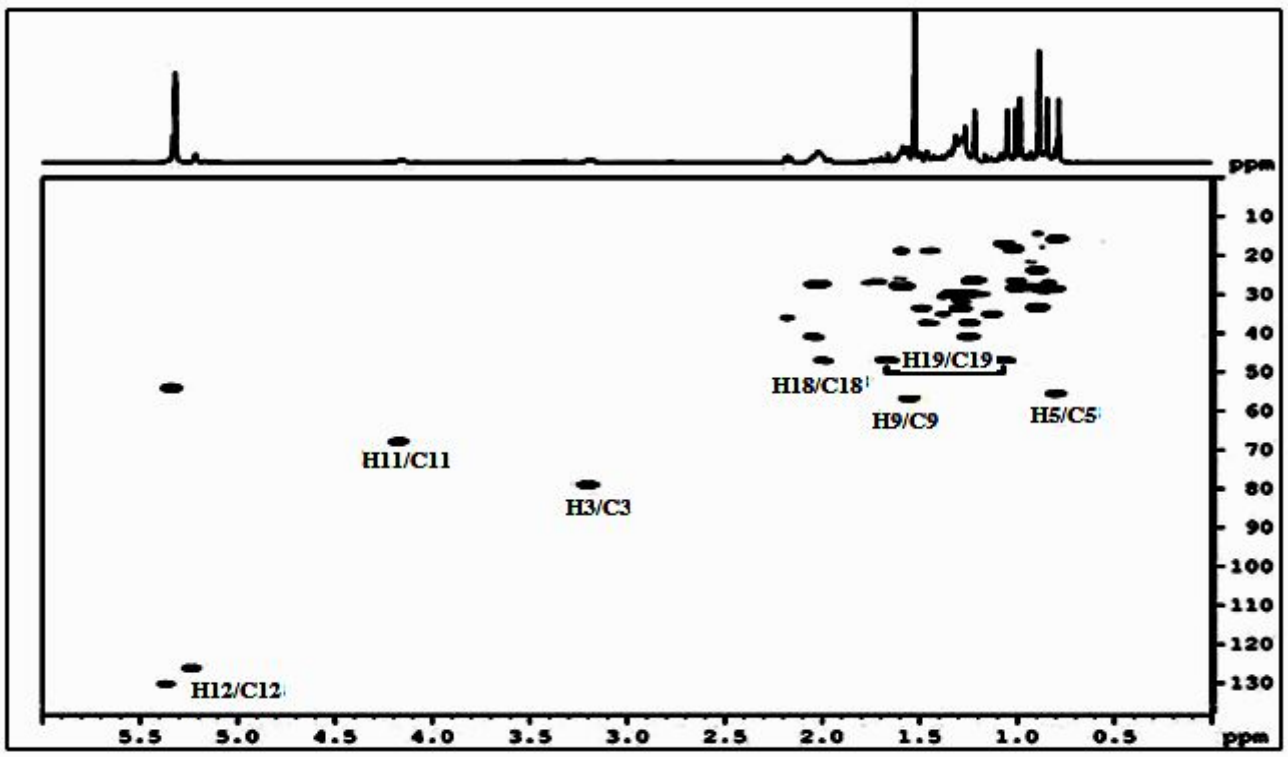


Figure 3.17: HSQC spectrum of compound 294 in CD_2Cl_2

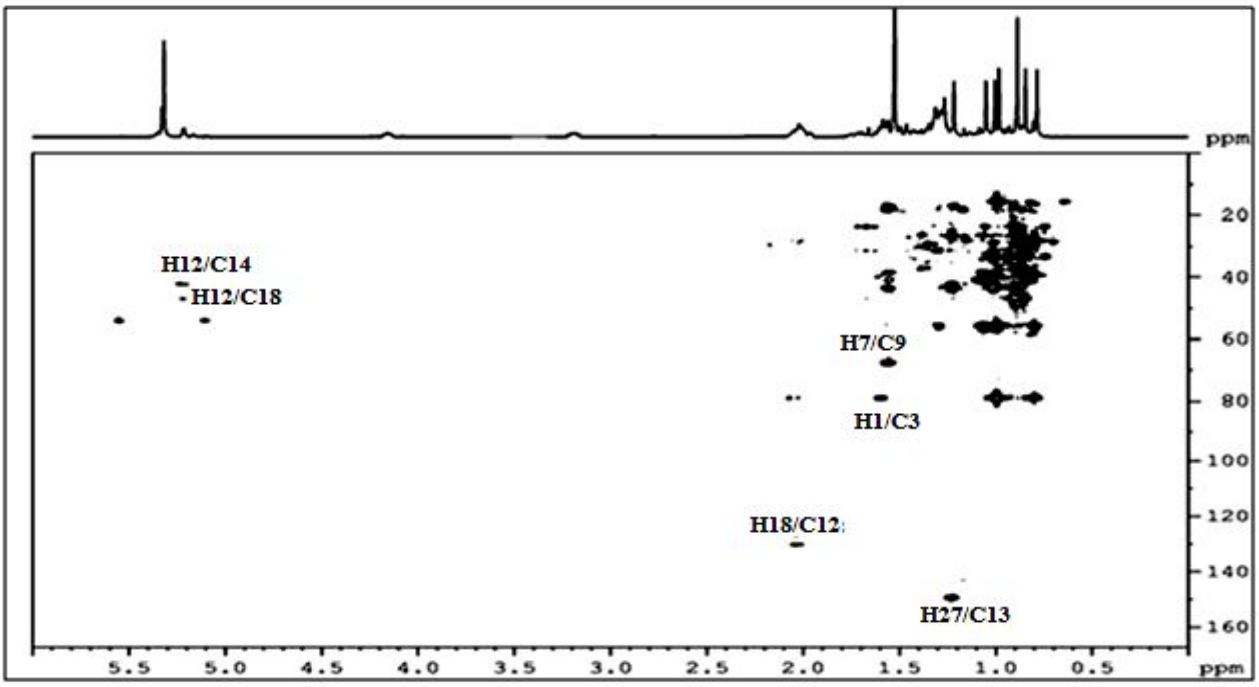


Figure 3.18: HMBC spectrum of compound 294 in CD_2Cl_2

Table 3.2: ^1H and ^{13}C NMR data of conpound 294 in CD_2Cl_2

Position	$\delta^{13}C$	δ^1H	m	*J* (Hz)	HMBC (C to H)
1	40.3	2.01	m		H-25
		2.02	m		-
2	27.3	1.30	m		-
		1.60	m		
3	78.3	3.23	dd	(10.3, 6.9)	H-23, H-24
4	38.9	-	-		H-23, H-24
5	55.1	0.82	m		H-23, H-24, H-25
6	18.3	1.38	m		-
		1.40	m		-
7	32.9	-	m		-
		-	m		-
8	42.0	-	-		H-9, H-27
9	56.3	1.56	m		H-25, H-26
10	38.0	-	-		H-9, H-25
11	67.1	4.25	dd	(8.0, 3.8)	H-9
12	127.1	5.20	d	(3.7)	H-11
13	150.1	-	-		H-11, H-27
14	43.2	-	-		H-9, H-12, H-27
15	26.6	-	-		H-27
		-	-		-
16	26.8	1.61	m		H-28
17	32.3	-	-		H-28
18	46.3	2.00	-		H-12, H-28
19	46.3	1.05	m		H-29, H-30
		1.64	m		-
20	31.1	-	-		H-29, H-30
21	33.1	1.30	m		H-29, H-30
22	37.0	-	-		-
23	28.3	0.98	s		H-3, H-24
24	15.5	0.79	s		H-3, H-23
25	16.8	1.05	s		H-9
26	18.2	1.00	s		H-7
27	26.1	1.21	s		-
28	28.2	0.84	s		-
29	33.2	0.88	s		H-30
30	23.7	0.88	s		H-29

Analogously with compound **292**, a rapid conversion of compound **294** to the corresponding diene derivative by elimination of a water molecule was observed to occur in chloroform solution. In the ^{1}H NMR spectrum recorded after some time (**Figure 3.20**), two vinyl signals of a conjugated diene system at δ 5.50 and δ 5.58 appeared in the place of the carbinol and the olefinic signals at δ 4.25 (H-11) and 5.20 (H-12), respectively.

The ESIMS spectrum of this compound (**Figure 3.19**) showed the peak at *m/z* 425 confirming the loss of the water molecule.

A survey of the literature on triterpenoids showed that compound **294** was first isolated from the roots of *Sabia schumanniana* Diels (Xiao et al., 1994) and subsequently from the aerial parts of *Pseudobrickellia brasiliensis* (Bohlmann et al., 1984) along with the corresponding 3-acetyl derivative.

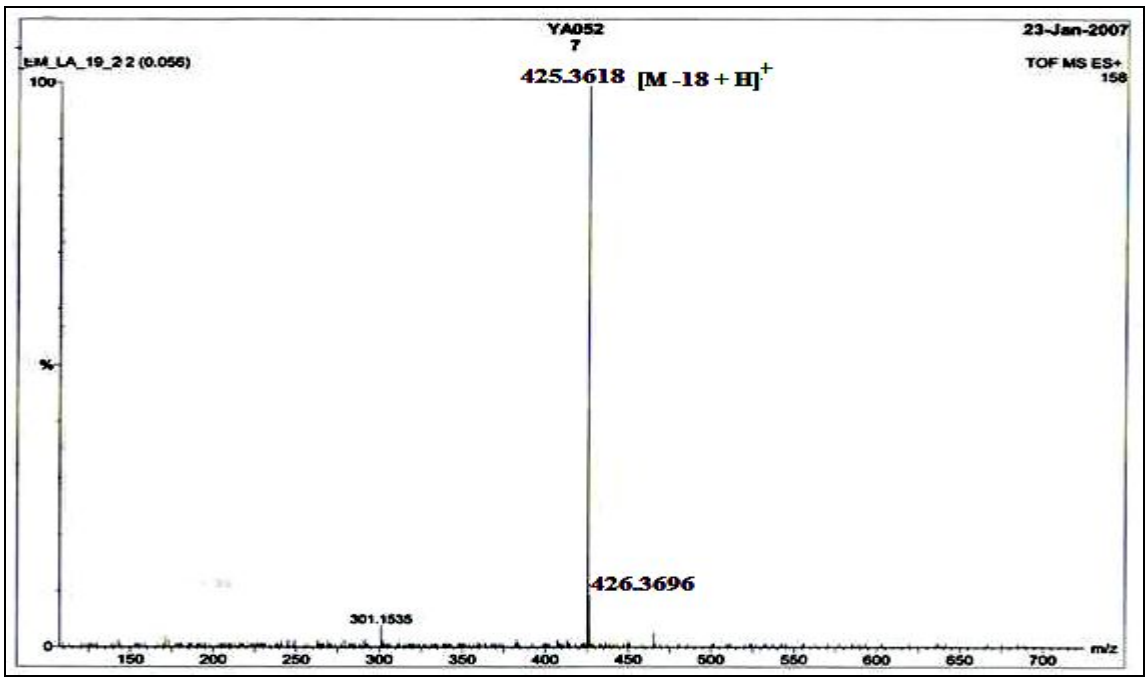


Figure 3.19: ESIMS dehydration spectrum of compound 294

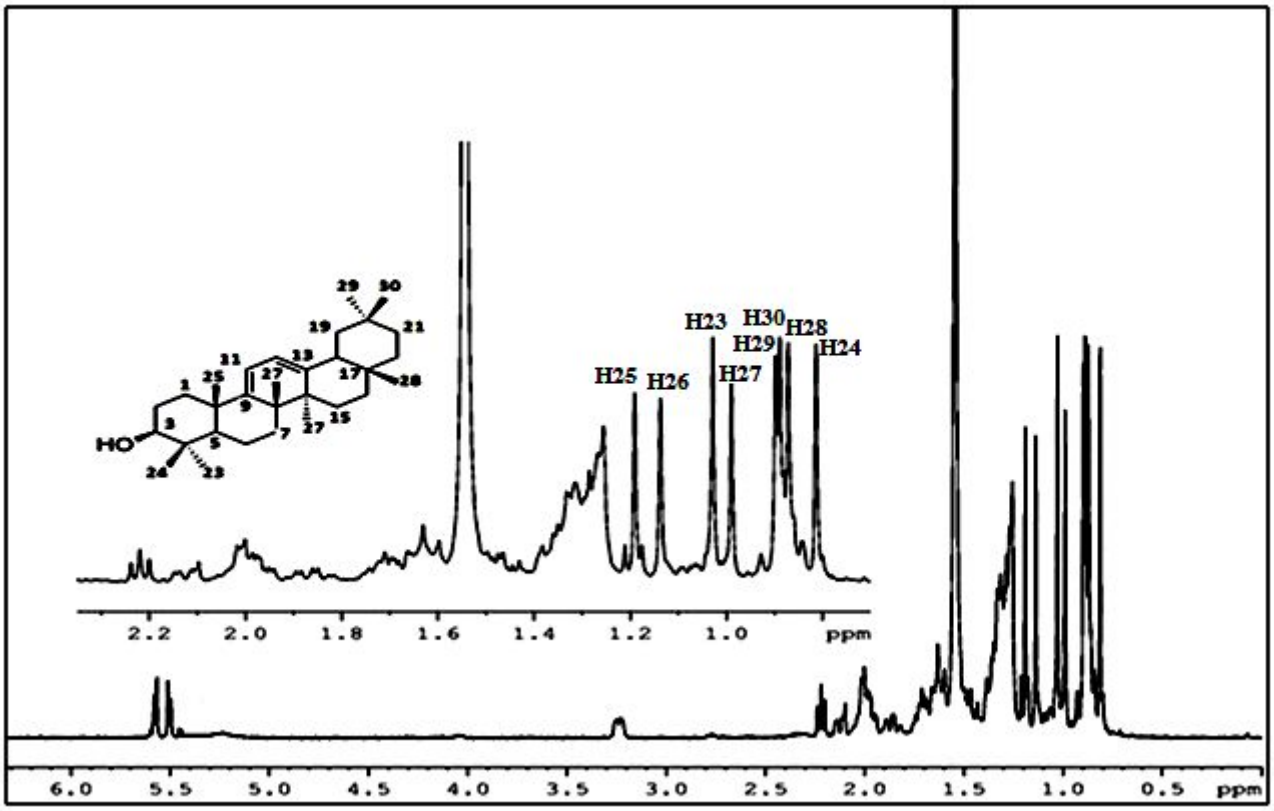


Figure 3.20: ^{1}H NMR spectrum of compound 294 after dehydration

3.4.1.4 Structure elucidation of compound 295

Analysis of the ^{1}H NMR spectrum of compound **295** (**Figure 3.21**) immediately revealed an oxygenated ursane-type triterpenoid closely related to compound **2**. In fact, in the spectrum were present six methyl singlets [δ 0.79 (H_3-24), 0.81 (H_3-28), 1.01 (3H, H_3-23), 1.04 (H_3-26), 1.07 (H_3-25), and 1.16 (H_3-27)], and two methyl doublets [δ 0.87 (3H, d, J = 6.0, H_3-29), and δ 0.93 (3H, br s, J = 6.0 Hz, H_3-30)] along with signals due to two oxygenated methines [δ 3.22 (H-3) and δ 4.25 (H-11)] and an olefinic proton [δ 5.18 (H-12)].

Compound **295** underwent the same dehydration reaction as **293**. The presence in the mass spectrum of an intense fragmentation peak at *m/z* 425 $[M-18]^{+}$ corresponding to the loss of a molecule of water was in agreement with the observed reactivity. By analogy with compound **293**, compound **295** was thus identified as 3β-11α-dihydroxy-urs-12-ene. This compound has been previously isolated from *Pseudobrickellia brasiliensis* and chemically characterised as 3β-11α-diacetyl derivative (Bohlmann et al., 1984). The related 3α-hydroxyl epimer exhibiting equatorial H-3 (δ 3.41 Hz, t, $J_{e,a} = J_{e,e}$ = 2.7 Hz) has been reported from the aerial parts of *Salvia willeana* (Dela Torre et al., 1990). However, the rapid dehydration reaction of **295** into the corresponding diene prevented the recording of 2D NMR experiments and the full

characterisation.

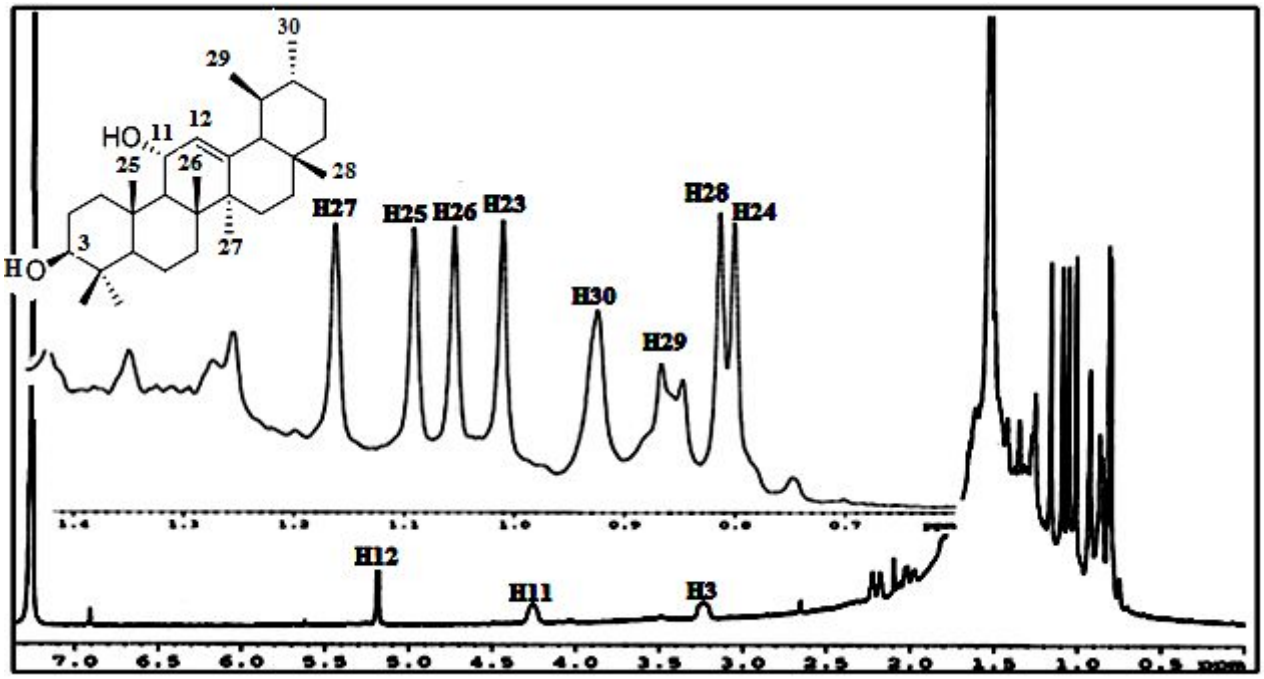


Figure 3.21: ^{1}H NMR spectrum of compound 295 in $CDCl_3$

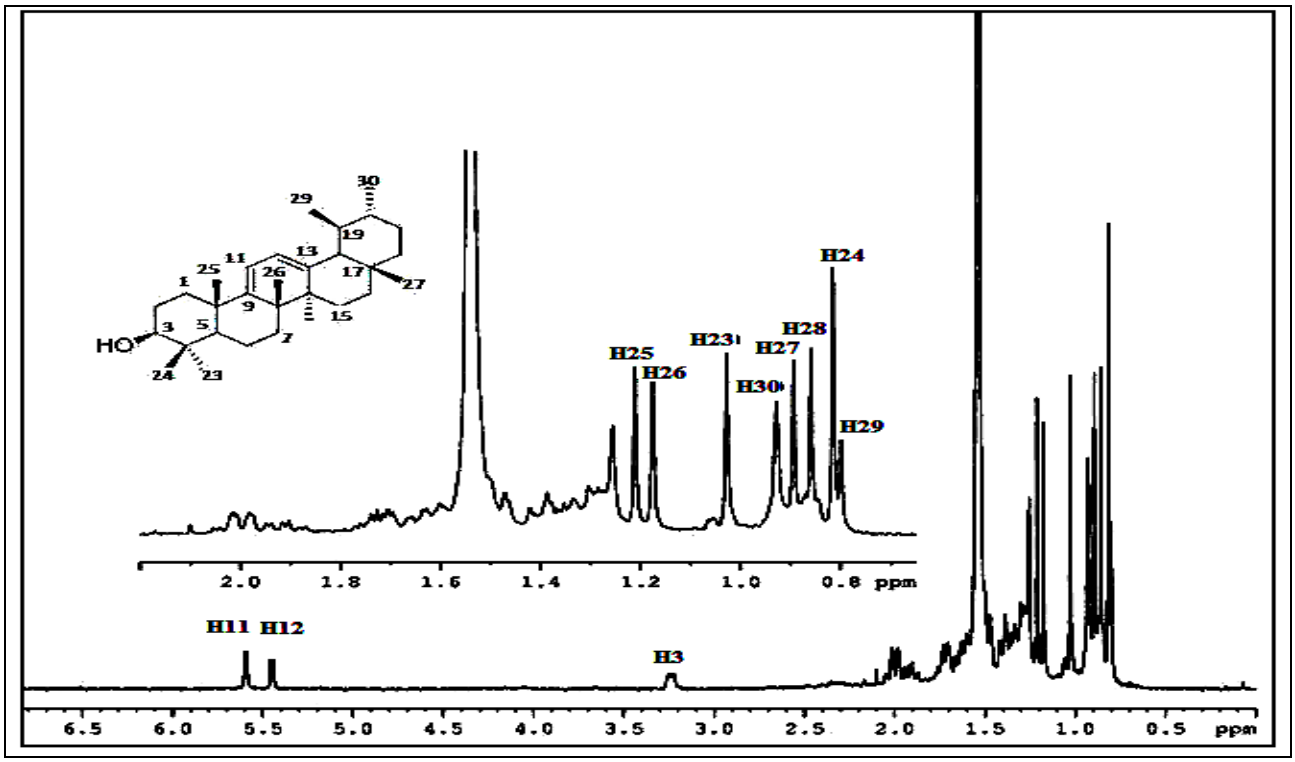


Figure 3.22: ^{1}H NMR spectrum of compound 295 in $CDCl_3$ after dehydration

3.4.1.5 Structure elucidation of compound 296

The ESIMS spectrum of compound **296** showed two sodiated molecular peak at *m/z* 479 [M + Na]$^+$, and *m/z* 935 [2M +Na]$^+$, indicating the molecular formula $C_{31}H_{52}O_2$ (*m/z* 456).

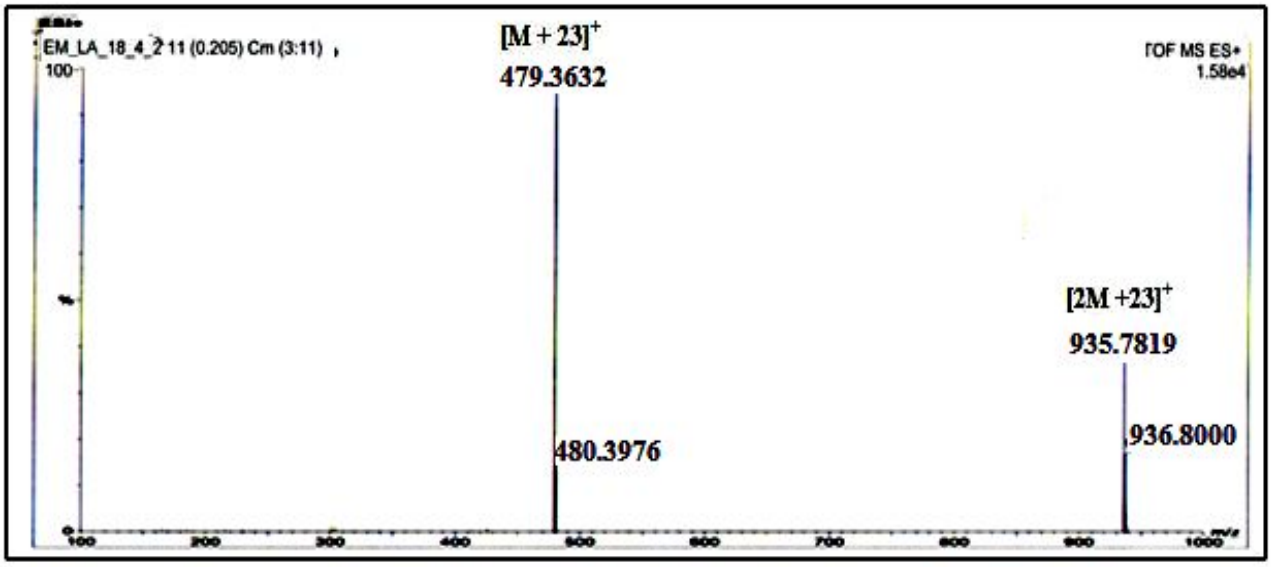


Figure 3.23: ESIMS spectrum of compound 296

The ^{1}H NMR spectrum (**Figure 3.24**) revealed the presence of eight methyl groups [δ 0.82 (H_3-24), 0.84(H_3-28), 0.89 (H_3-30), 0.90 (H_3-29), 1.01 (6H, H_3-23 and H_3-26), 1.05 (H_3-25), and 1.22 (H_3-27)], two methines attached to oxygen functions at δ 3.84 (1H, dd, *J* = 8.4 and 3.4 Hz, H-11) and at δ 3.24 (1H, m, H-3), an olefinic proton at δ 5.35 (1H, d, *J* = 3.4) and a methoxy group at δ 3.24. These data indicated a very close structural analogy with compound **292** and **294** due to the presence of the same functionalised oleanane-type framework. Compound **296** was characterised by a methoxy group at C-11 rather than the ethoxyl moiety as in **292** or the hydroxyl group as in **294**. Compound **296** was therefore identified as 3β-hydroxy-11α-methoxy-olean-12-ene.

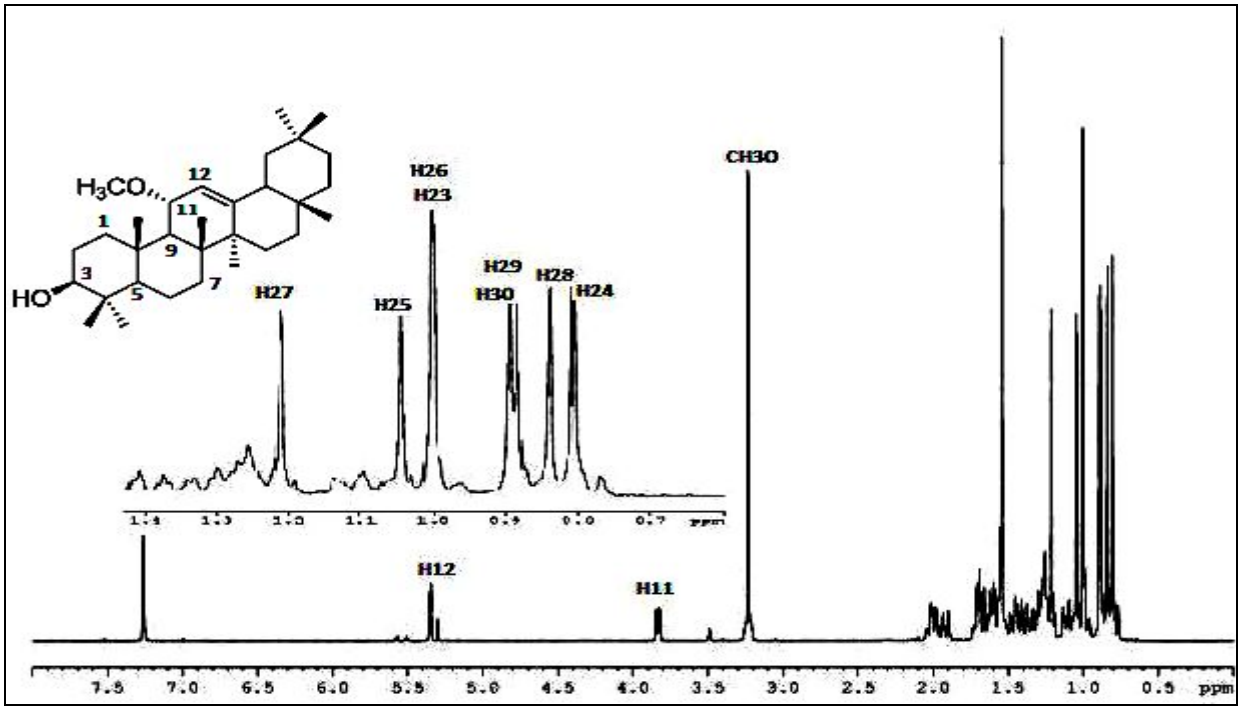


Figure 3.24: ^{1}H NMR spectrum of the compound 296 in $CDCl_3$

Analogously with related **292** and **294**, compound **296** underwent the elimination reaction of methanol to give the same diene derivative as **292** and **294** further confirming the structural assignment.

This compound was isolated for the first time from the root bark of *Tripterygium hypoglaucum* of the Celastraceae family (Fujita et al., 2000).

3.4.1.6 Structure elucidation of compound 297

Compound **297** had the same molecular formula ($C_{31}H_{52}O_2$) as compound **296**. The ^{1}H NMR spectrum of **297** (**Figure 3.25**) revealed the presence of six tertiary methyl groups [singles at δ 0.81 (H_3-24), 0.82 (H_3-28), 1.01 (H_3-23), 1.04 (H_3-26), 1.07 (H_3- 25), and 1.15 (H_3-27)], and two secondary methyl groups [δ 0.87 (3H, d, J = 6.0, H_3-29), and δ 0.93 (3H, br d, J = 6.0 Hz, H_3-30)] according to the ursane-type skeleton.

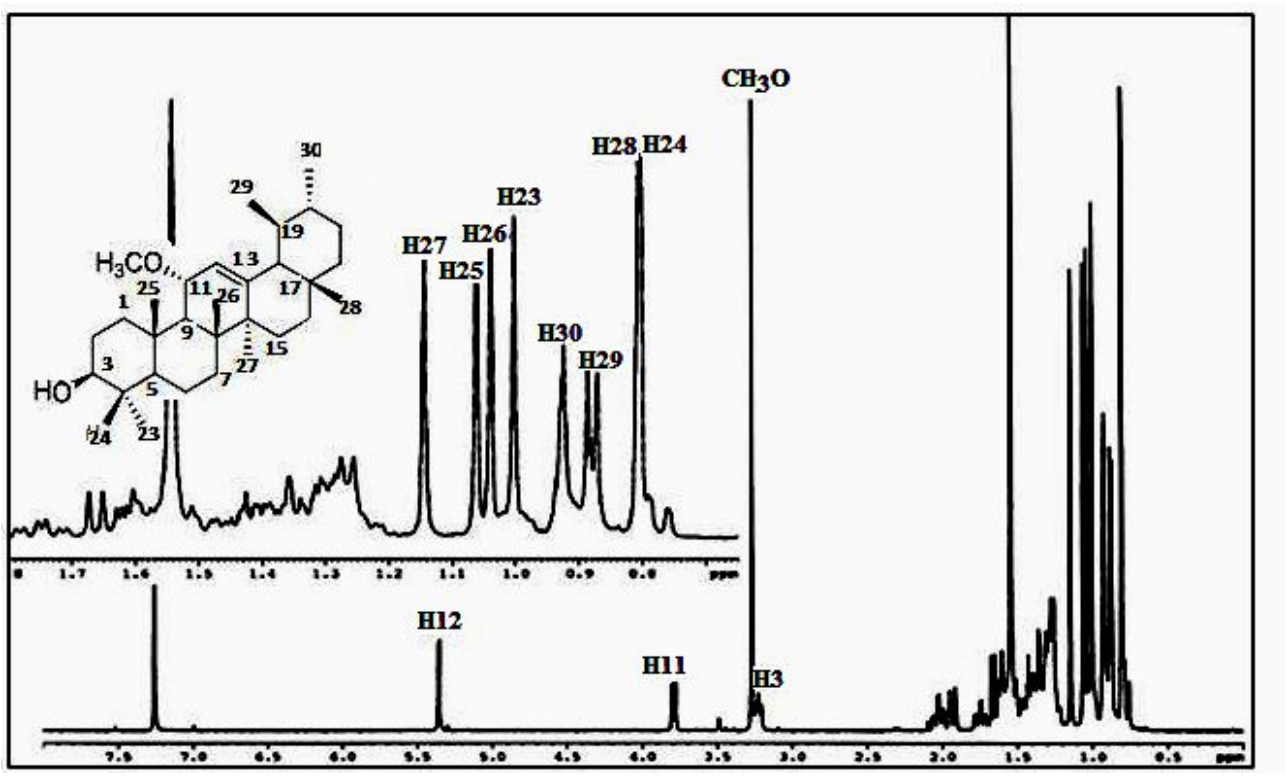


Figure 3.25: ^{1}H NMR spectrum of compound 297 in $CDCl_3$

The proton resonances of **297** resembled those of compounds **293** and **295**. Their spectra differed only in the signals due to the substituent at C-11, which was a methoxyl group (δ 3.38, 3H, s, -OMe) in compound **297** rather then the ethoxyl residue in compound **293** and the hydroxyl group in compound **295**. Thus, compound **297** was 3β-hydroxy-11α-methoxy-urs-12-ene, previously reported in the literature as triterpene metabolite of the plant *Tripterygium hypoglaucum* (Fujita et al., 2000) in which it occurs along with the corresponding compound **296** of the oleanane series.

The elimination reaction of methanol residue and the formation of the diene derivative was observed to occur partially for compound **294** (**Figure 3.26**).

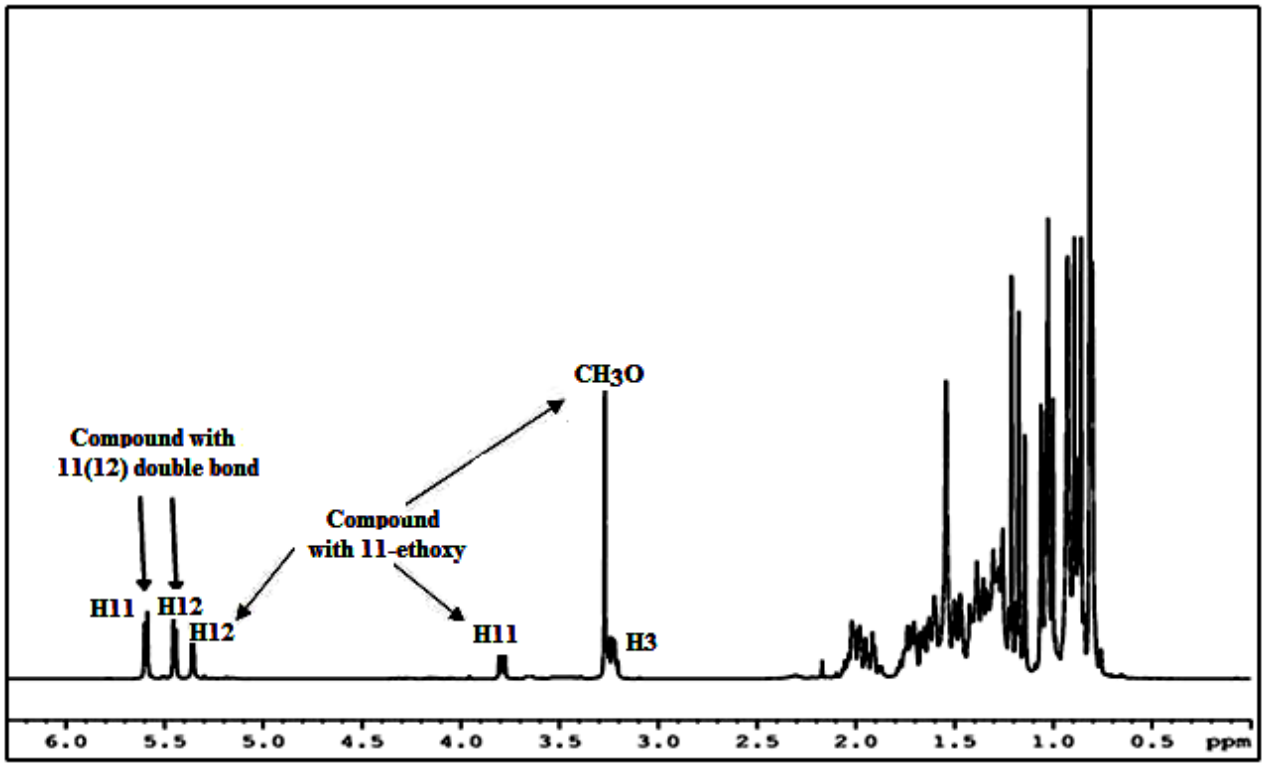


Figure 3.26: ^{1}H NMR spectrum of compound 297 in $CDCl_3$ after dehydration

3.4.1.7 Structure elucidation of compound 298

Compound **298** showed a molecular peak at *m/z* 425 $[M + H]^+$ that was identical to that observed in the ESIMS spectra of dehydration derivatives from compounds **292-297**.

The ^{1}H NMR spectrum of compound **298 (Figure 3.27)** exhibited the typical signals of the oleanane-type framework due to the eight methyl singlets [δ 0.81 (H_3-24), 0.87 (H_3-28), 0.89 (H_3-29), 0.90 (H_3-30), 1.01 (H_3-27), 1.03 (H_3-23), 1.14 (H_3-26), and 1.19 (H_3-25)] and the carbinol proton at δ 3.24 (1H, dd, J = 11.5 and 6.0 Hz, H-3).

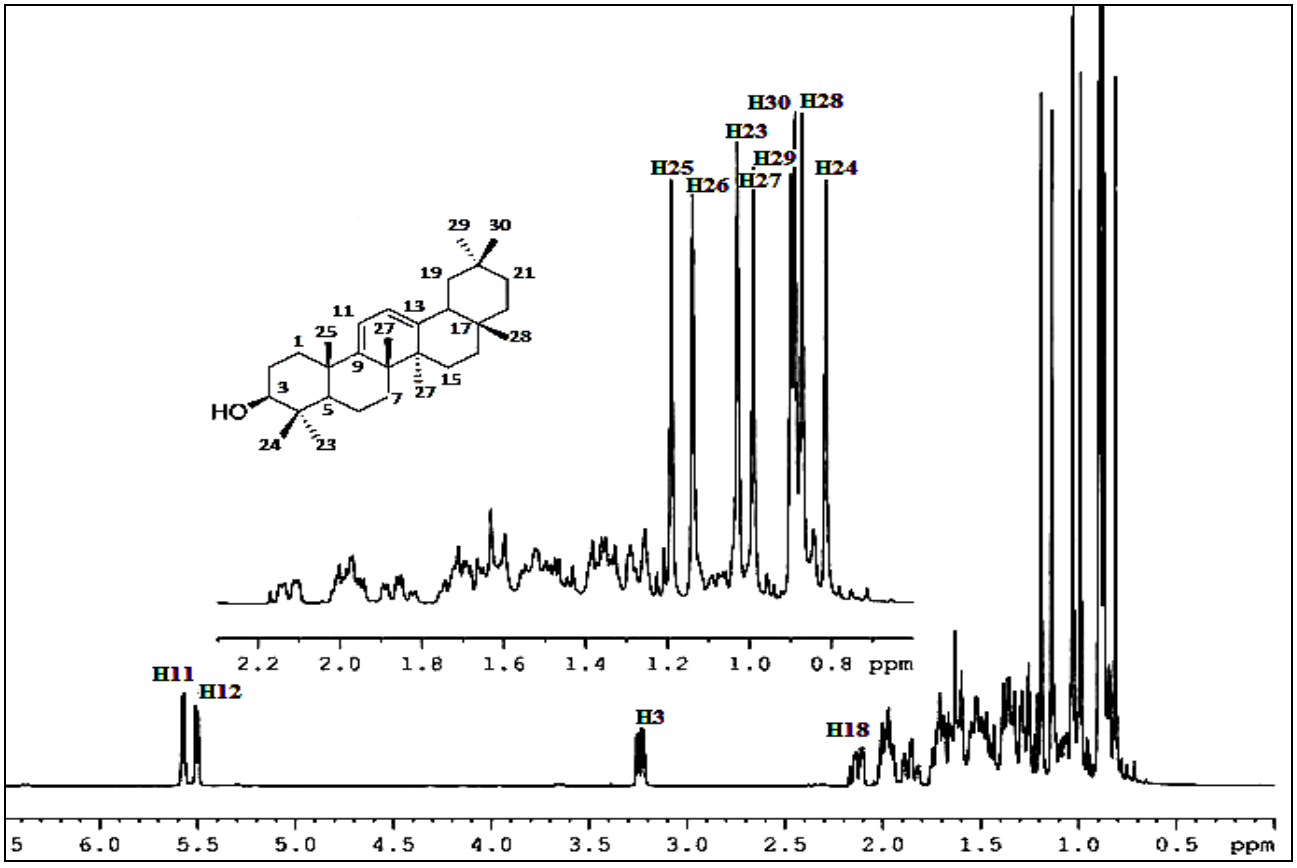


Figure 3.27: ^{1}H NMR spectrum of compound 298 in $CDCl_3$

In addition, the ^{1}H NMR spectrum showed signals attributed to two olefinic protons at δ 5.50 (1H, d, *J* = 6.0 Hz, H-12), and δ 5.58 (1H, d, *J* = 6.0 Hz, H-11). The analysis of the COSY experiment (**Figure 3.28**) revealed cross-peaks between these two protons, indicating a cisoid diene system. Accordingly, carbons C-11 and C-12 resonated at δ 115.8 and 120.7, respectively, as deduced from the HSQC experiment (**Figure 3.29**).

The position of the diene system was confirmed by diagnostic HMBC correlations (**Figure 3.30**) between the quaternary sp^2 carbon at δ 154.2 (C-9) and H-12, H_3-25, and H_3-26 and between the second quaternary sp^2 carbon at δ 147.1 (C-13) and both H-11 and H_3-27, consistently with the proposed 3β-hydroxy-oleana-9(11):12-diene arrangement.

Proton and carbon assignment was made by analysis of ^{1}H-^{1}H COSY, HSQC and HMBC spectra **(Table 3.3)**.

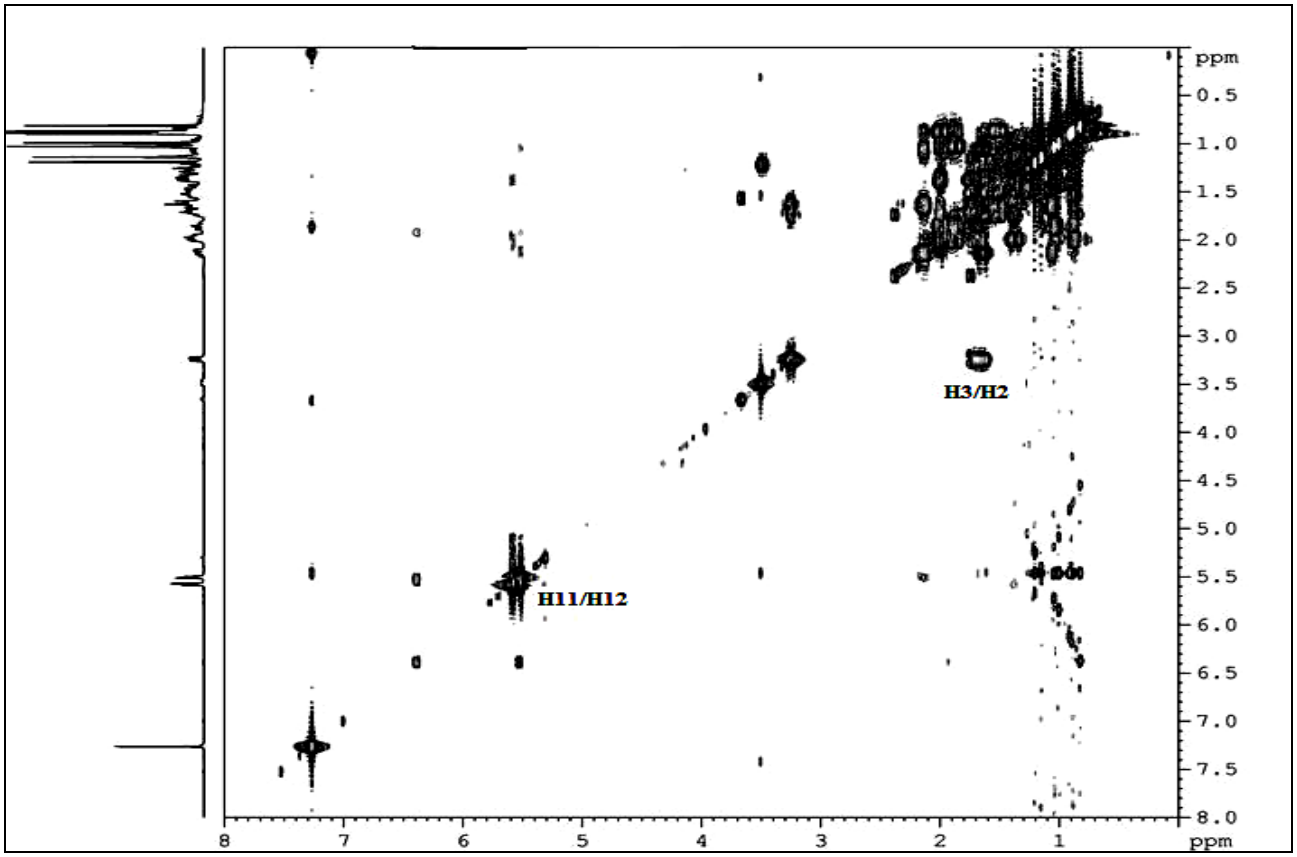


Figure 3.28: ^{1}H-^{1}H COSY spectrum of compound 298

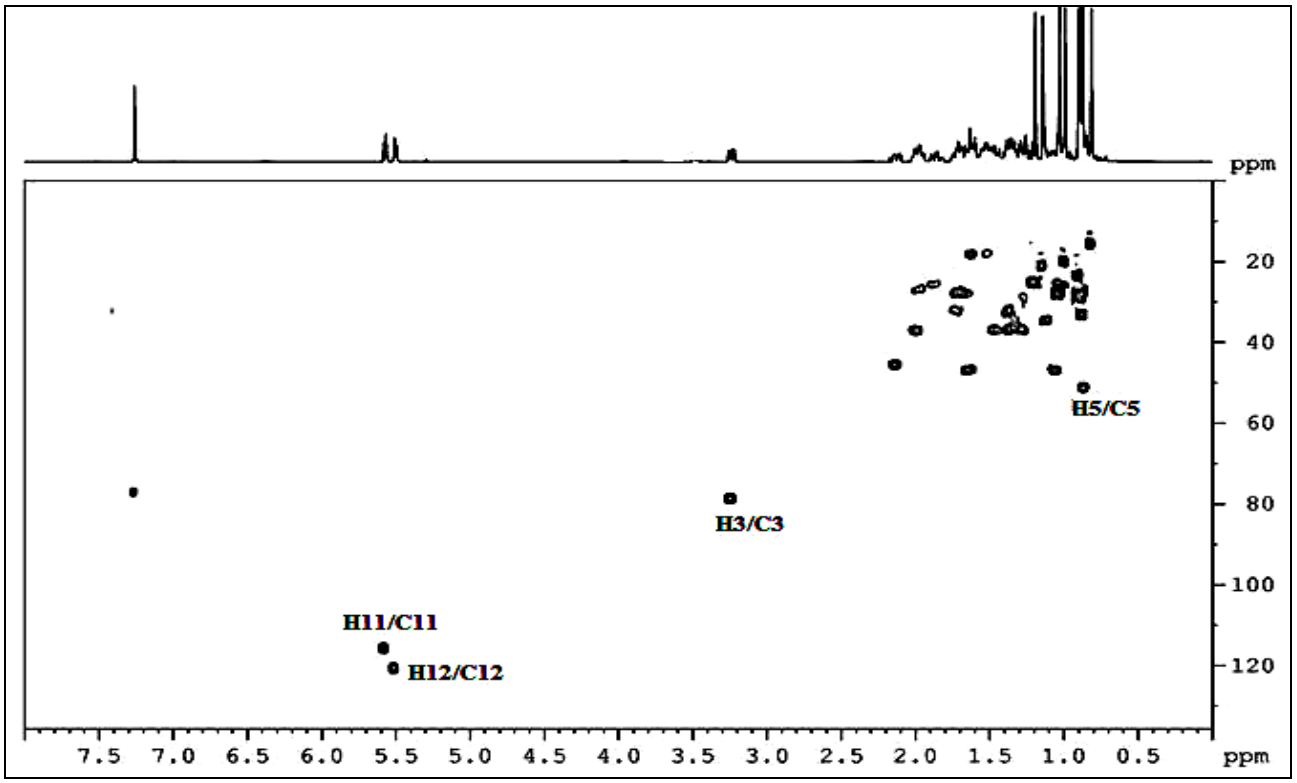


Figure 3.29: HSQC spectrum of compound 298

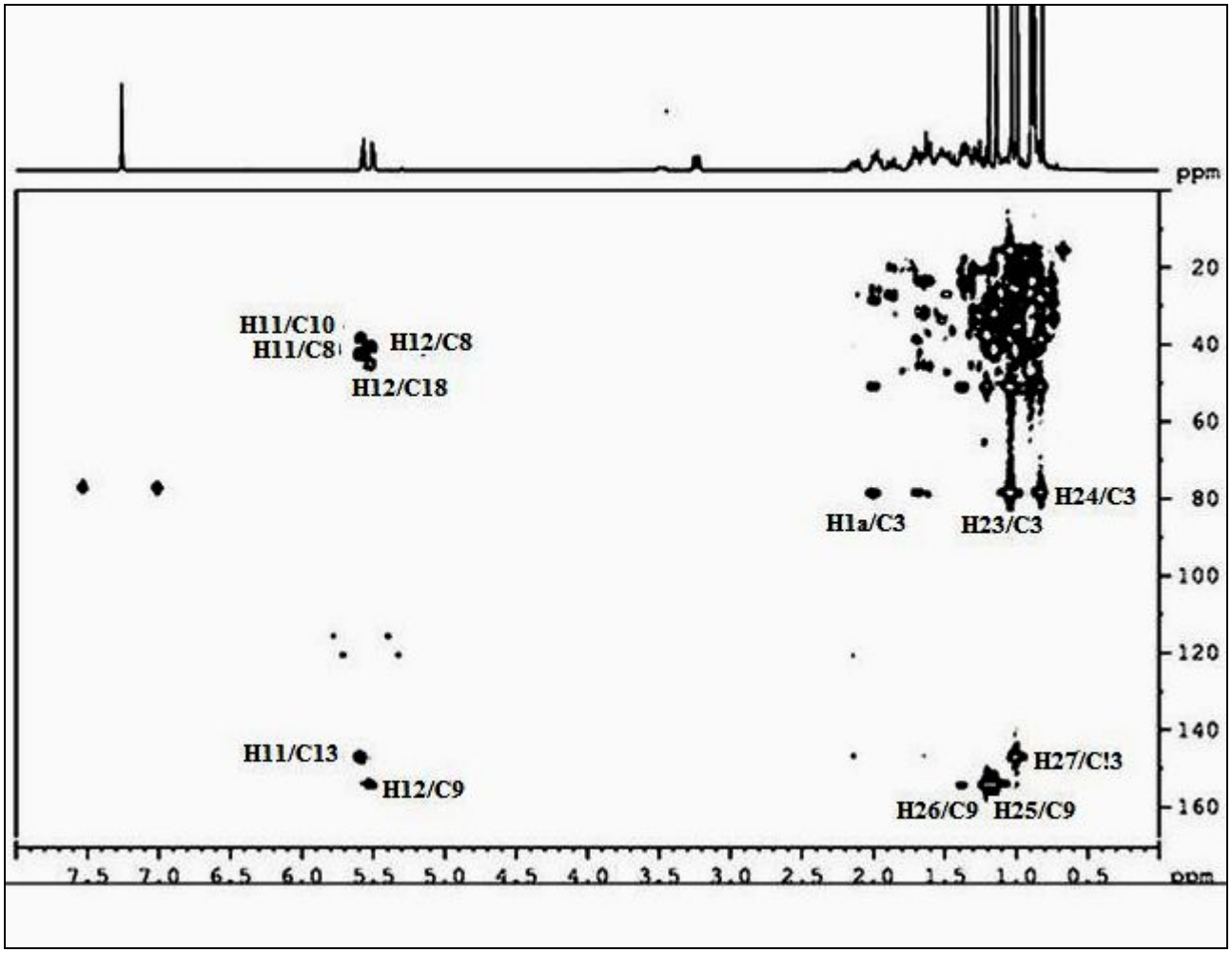


Figure 3.30: HMBC spectrum of compound 298

Compound **298** was first found in the stem bark of *Phyllanthus flexuosus* of Euphorbiaceae family (Tanaka et al., 1988).

Table 3.3: ^{1}H and ^{13}C NMR data of conpound 298 in $CDCl_3$

Position	$\delta^{13}C$	$\delta^{1}H$	m *J* (Hz)	HMBC (C to H)
1	36.5	1.90	m	H-25
		2.02	m	-
2	27.9	1.30	m	-
		1.65	m	
3	78.6	3.23	dd (10.3, 6.9)	H-23, H-24, H-1
4	41.2	-	-	H-23, H-24
5	50.8	0.84	m	H-1, H-7, H-23, H-24, H-25
6	17.9	1.55	m	-
		1.62	m	-
7	32.7	1.38	m	H-6, H-11, H-26
		1.73	m	-
8	37.0	-	-	H-11, H-25, H-26
9	154.2	-	m	H-7, H-12, H-25, H-26
10	40.7	-	-	H-2, H-11, H-25
11	115.8	5.58	d (6.0)	H-12
12	120.8	5.50	d (6.0)	H-11, H-27
13	147.0	-	-	H-9, H-12, H-27
14	42.1	-	-	H-27
15	35.2	1.2-1.4	-	-
			-	-
16	27.1	1.61	m	H-28
17	32.1	-	-	H-28
18	45.6	2.20	m	H-28
19	46.9	1.09	m	H-29, H-30
		1.68	m	-
20	31.2	-	-	H-29, H-30
21	34.6	1.17	m	H-29, H-30
		1.35	m	-
22	37.8	1.27	m	-
		1.48	m	-
23	28.1	1.03	s	H-24
24	15.1	0.81	s	H-23
25	24.9	1.19	s	-
26	21.0	1.14	s	H-7
27	20.1	1.01	s	H-15
28	28.7	0.87	s	-
29	33.2	0.89	s	H-30
30	23.7	0.90	s	H-29

3.4.1.8 Structure elucidation of compound 299

Compound **299** was isomeric with **298** as it was evidenced by the same molecular peak at *m/z* 425 $[M + H]^+$ in the ESIMS spectrum.

Comparison of the ^{1}H NMR spectrum (**Figure 3.31**) with that of compound **298** revealed strong similarities in particular with regards to the presence of the same diene system with two olefinic protons resonating δ 5.45 (1H, d, *J* = 6 Hz, H-12) and δ 5.60 (1H, d, *J* = 6.0 Hz, H-11).

In contrast, the methyl pattern was constituted by six tertiary methyl groups [δ 0.82 (H_3-24), 0.86 (H_3-28), 0.89 (H_3-27), 1.03 (H_3-23), 1.18 (H_3-26), and 1.21 (H_3-25)] and two secondary methyl groups [δ 0.81 (H_3-29, d, *J* = 6.0 Hz), and 0.93 (H_3 - 30, br s)] in accordance with the presence of the ursane-type skeleton.

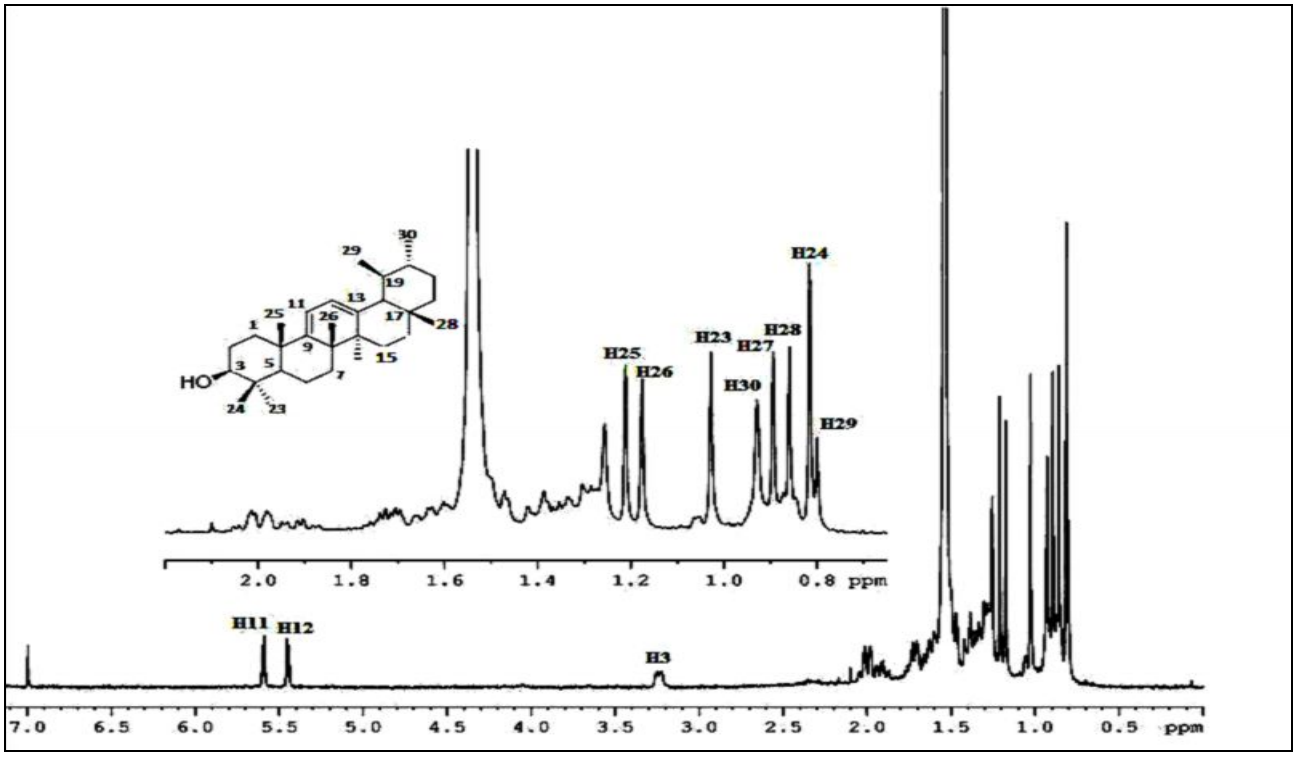


Figure 3.31: ^{1}H NMR spectrum of compound 299 in $CDCl_3$

Thus, compound **299** was identified as ursa-9(11):12-dien-3β-ol, previously isolated from the whole herb of *Euphorbia maculata* belonging to Euphorbiaceae family (Matsunaga et al., 1988). All ^{1}H and ^{13}C resonances were attributed by 2D NMR experiments (^{1}H-^{1}HCOSY, HSQC and HMBC).

The assignments made for compounds **298** and **299** were compared with those reported in the literature (Tanaka et al., 1988), (Matsunaga et al., 1988) suggesting a re-assignment of methyls H_3-25 and H_3-27 for both compounds. However, due to the easy transformation observed for triterpenoids **292-297** of either oleanane or ursane type into the corresponding diene derivatives **298** or **299**, it is probable that these compounds are non-natural metabolites but work-up derivatives.

3.4.1.9 Structure elucidation of compounds 300 and 301

These two compounds showed the same ESIMS sodiated molecular peak at *m/z* 463 $[M+23]^{+}$ (**Figure 3.32**), consistent with the molecular formula $C_{30}H_{48}O_2$.

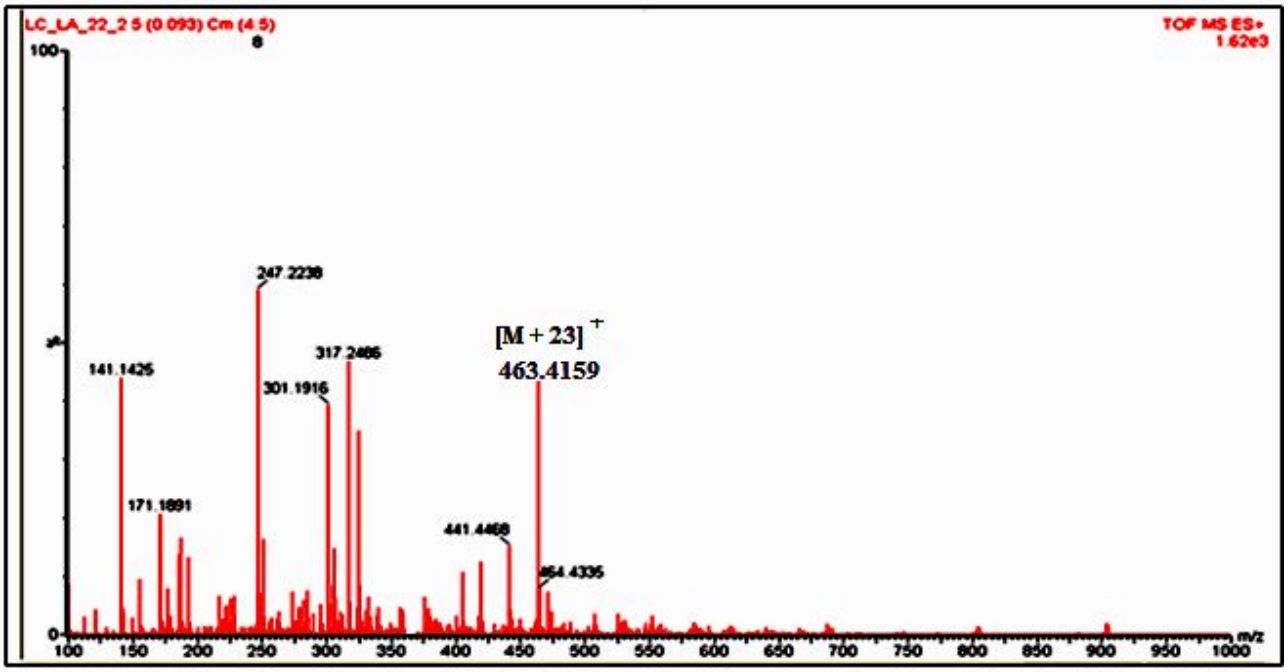


Figure 3.32: ESIMS spectrum of compound 300

The ^{1}H NMR spectra of both compounds (**Figures 3.33**, **3.34**) were similar indicating identical structural features in **300** and **301**. In particular, the presence of an α,β-unsaturated ketone system at C-11/C-12 position in both molecules was suggested by the down-field shifted olefinic proton H-12 resonating at δ 5.59 in **300** and δ 5.52 in **301**, with respect to the corresponding typical value (δ 5.35) observed in α- or β-amyrin, as well as by the multiplicity of H-9 signal resonating at δ 2.32 as a singlet in both **300** and **301**. Accordingly, these molecules were UV-visible (λ 254 nm).

The ^{1}H NMR spectrum of **300** exhibited eight methyl singlets [δ 0.8 (H_3-24), 0.85 (H_3-28), 0.88 (H_3-30), 0.90 (H_3-29), 1.00 (H_3-23), 1.13 (H_6-25 and 26), and 1.35 (H_3-27)] according to the oleanane framework (β-amyrin) whereas the ^{1}H NMR spectrum of **301** contained six methyl singlets [δ (0.81 (H_3-24), 0.82 (H_3-28), 1.00 (H_3-23), 1.17 (H_6-25 and 26), and 1.30 (H_3-27)], and two methyl doublets [δ 0.80 (H_3-29, J = 6.0 Hz), and δ 0.94 (H_3-30, br s)] according to the ursane skeleton (α-amyrin).

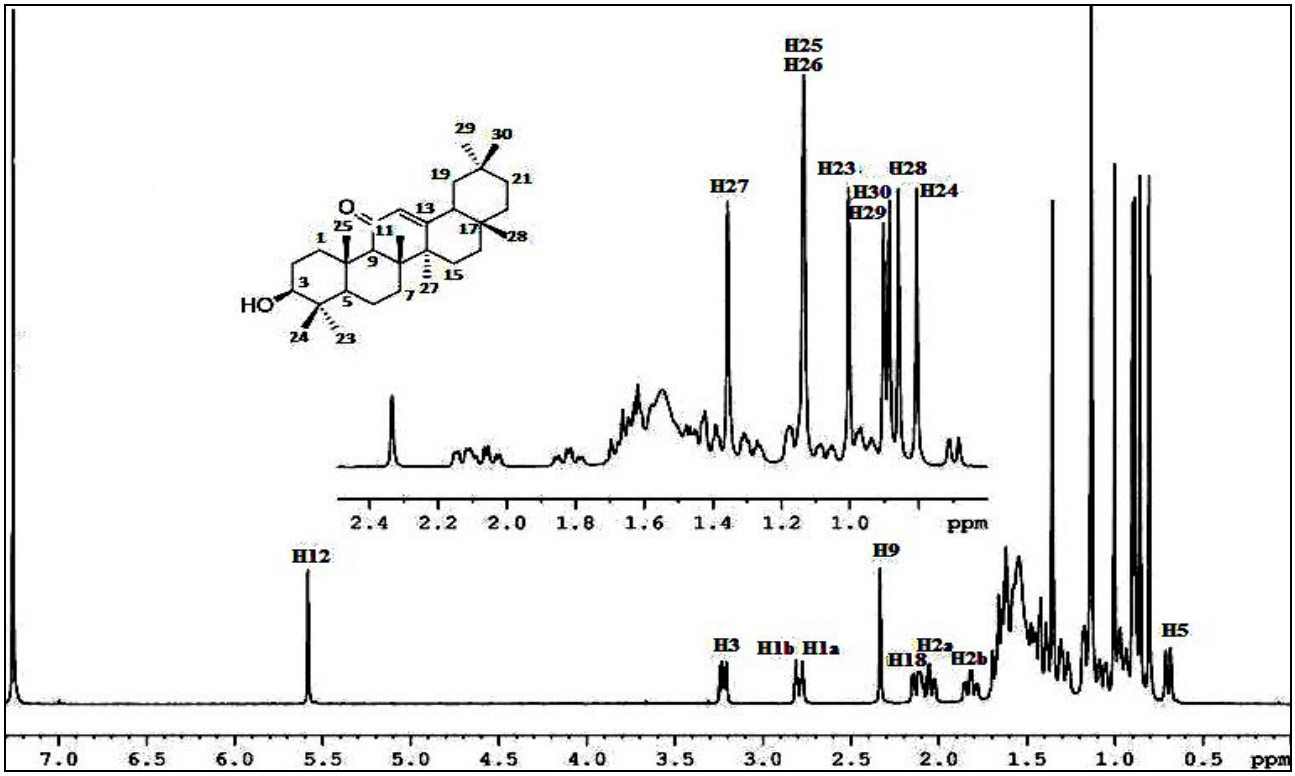


Figure 3.33: ^{1}H NMR spectrum of compound 300 in $CDCl_3$

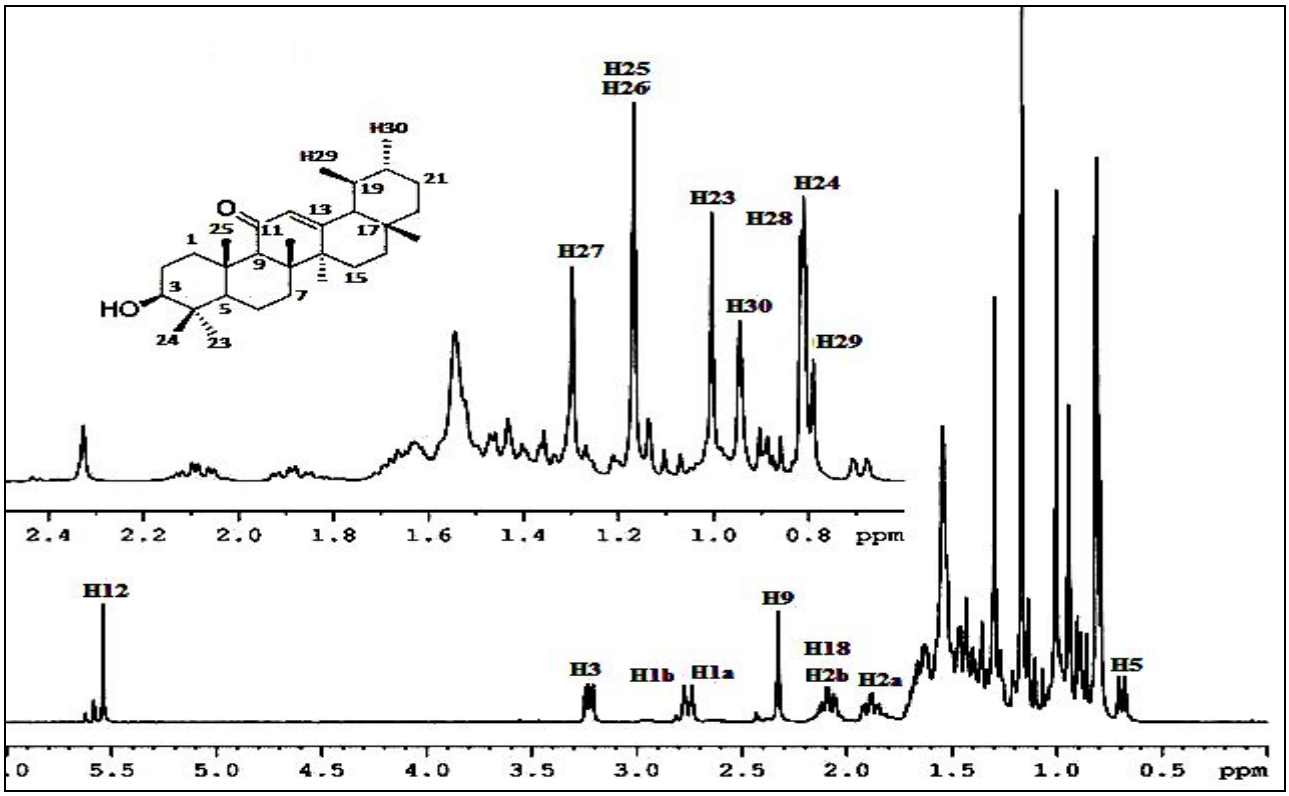


Figure 3.34: ^{1}H NMR spectrum of the compound 301 in $CDCl_3$

By these data, compounds **300** and **301** were identified as 3β-hydroxy-11-oxo-olean-12-ene and 3β-hydroxy-11-oxo-ursa-12-ene, respectively.

These two terpenoids were previously isolated and identified from oleoresin, bark and timber of *Canarium zeylanicum* from Burseraceae family (Bandaranayake et al., 1980).

3.4.1.10 Structure elucidation of compound 302

The molecular formula of compound **302** was deduced to be $C_{30}H_{50}O_2$ by both the EIMS (electronic impact mass spectrum) (**Figure 3.35**) displaying a molecular ion peak at *m/z* 442 and the ^{13}C NMR spectrum exhibiting 30 signals (seven methyls, ten methylenes, seven methines, and six quaternary carbons).

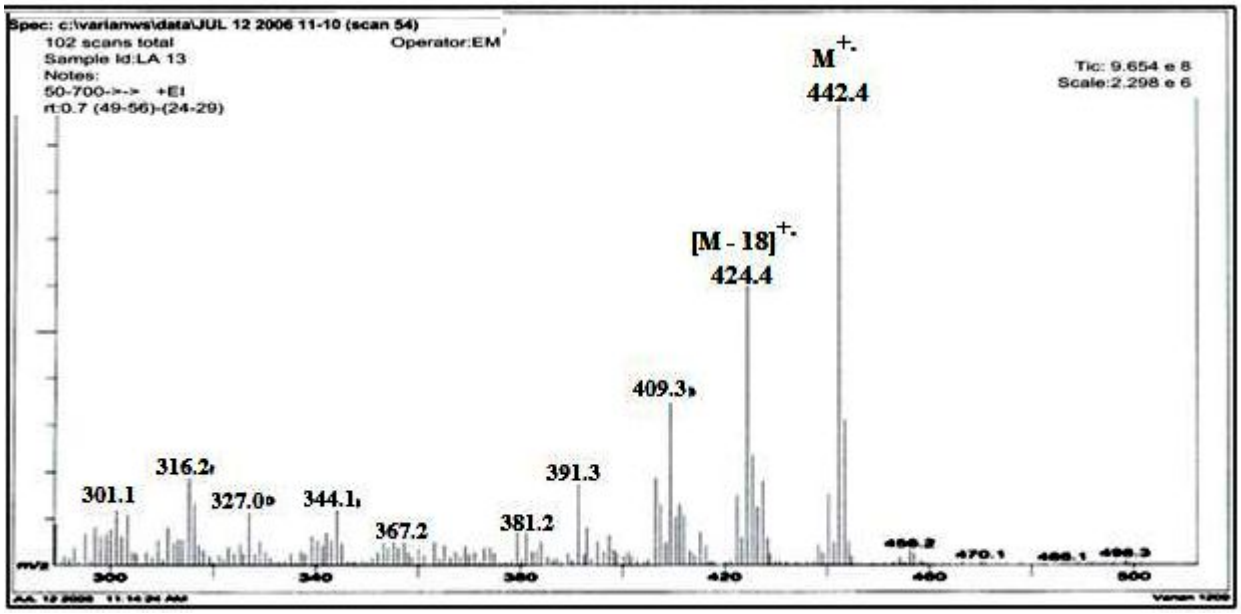


Figure 3.35: EI spectrum of compound 302

The ^1H NMR spectrum of **302** (**Figure 3.36**) disclosed the presence of six tertiary methyl groups [δ 0.75 (3H, s, H_3-28), 0.76 (3H, s, H_3-24), 0.85 (3H, s, H_3-25), 0.95 (3H, s, H_3-27), 0.96 (3H, s, H_3-23), and 1.04 (3H, s, H_3-26)], a secondary methyl [δ 1.01 (3H, d, J = 6.5 Hz, H-29), and a vinyl proton at δ 5.58 (1H, dd, J = 6.5 and 1.8 Hz, H-21). The ^1H NMR spectrum contained also an AB system δ 4.13 (1H, d, J = 12.3 Hz, H-30a) and δ 4.02 (1H, d, J = 12.3 Hz, H-30b), which was attributed to an isolated hydroxyl methylene group, along with the typical carbinol signal at δ 3.21 (1H, dd, J = 11.2 and 4.9 Hz, H-3).

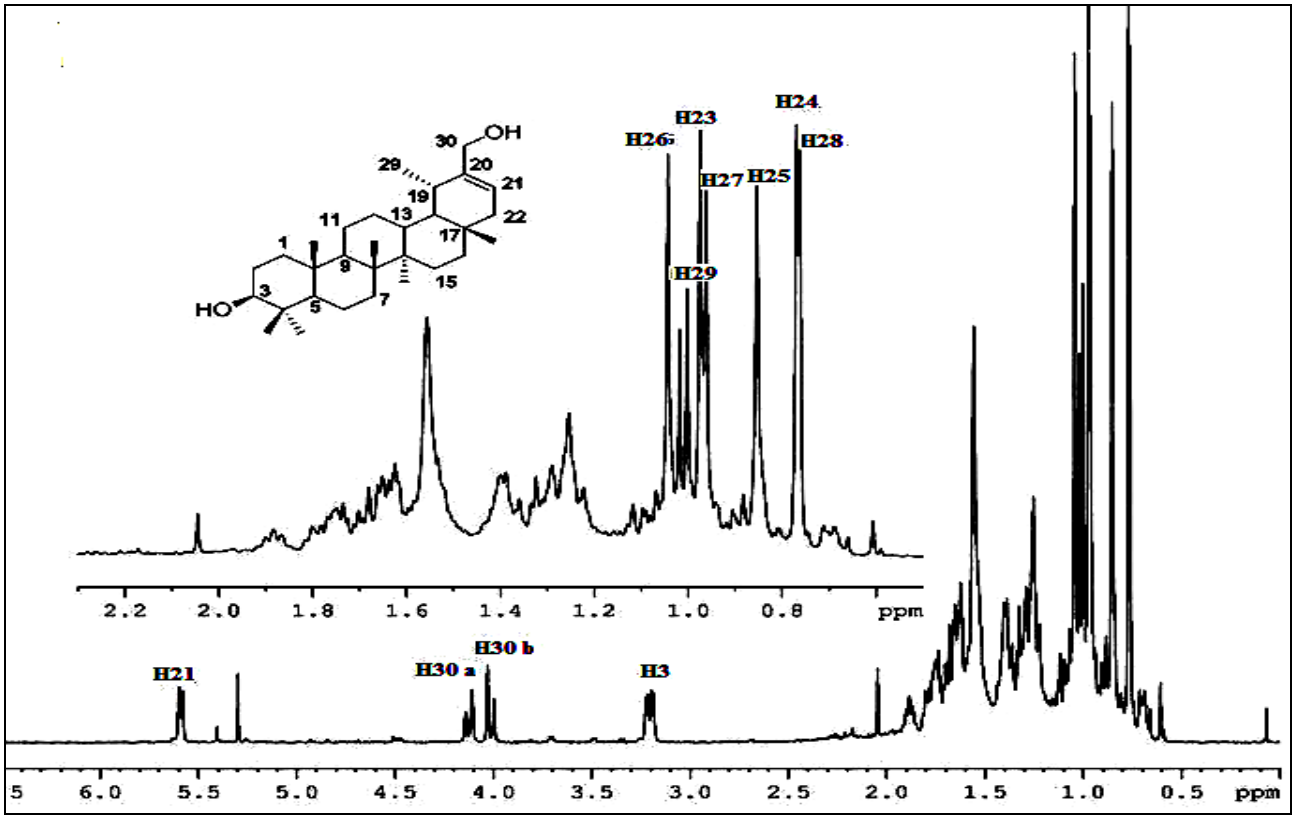
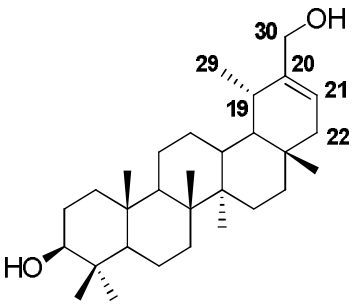


Figure 3.36: ^{1}H NMR spectrum of compound 302 in $CDCl_3$

These spectral data suggested that compound **302** was an unsaturated pentacyclic triterpene with two hydroxyl groups. A comparison of ^{1}H NMR data with those recorded for the series of oleanane and ursane terpenoids discussed above strongly suggested that in **299** the double bond was not positioned at C-12 and one of the methyl groups was functionalised.

The comparison of the chemical data with those of pseudotaraxasterol (Reynolds et al., 1986) showed that the chemical shift values were almost identical strongly supporting the location of the double bond and the hydroxymethylene group in the ring E, the same as pseudotaraxasterol. Thus, compound **302** should be a derivative of ψ-taraxasterol.

In the ^{1}H-^{1}H COSY spectrum of **302** (**Figure 3.37**), the vinyl signal H-21 showed cross-peak correlations with two non-equivalent methylene proton signals at δ 1.64 (1H, m, H-22a) and δ 1.78 (1H, m, H-22b), and with the hydroxyl methylene H_2-30 by allylic coupling. The methyl doublet at δ 1.01 (H_3-29) was correlated with a methine proton signal at δ 1.88 (H-18), which in turn showed a W-coupling with the hydroxyl methylene H_2-30. Significant HMBC correlationswere observed between the quaternary sp^2 carbon at δ 143.7 (C-20) and the isolated hydroxyl methylene H_2-30 thus confirming the presence of the fragment -$CH(CH_3)$-$C(CH_2OH)$=CH-CH_2- in the ring E. These data led us to identify compound **302** as taraxast-20-ene-3β,30-diol.



The interpretation of ^{1}H-^{1}H COSY, HSQC and HMBC experiments (**Figures 3.37**, **3.38**, **3.39**) allowed all proton and carbon assignments **(Table 3.4)**.

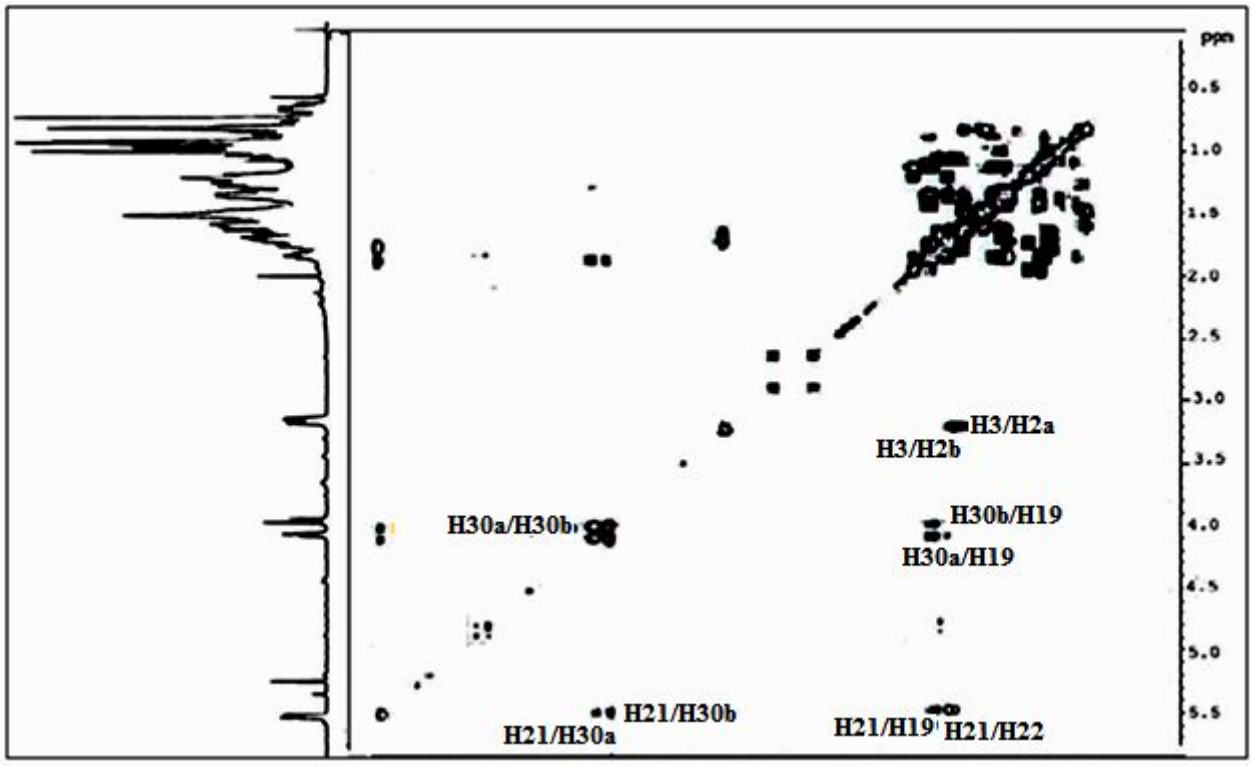


Figure 3.37: ^{1}H-^{1}H COSY spectrum of compound 302 in $CDCl_3$

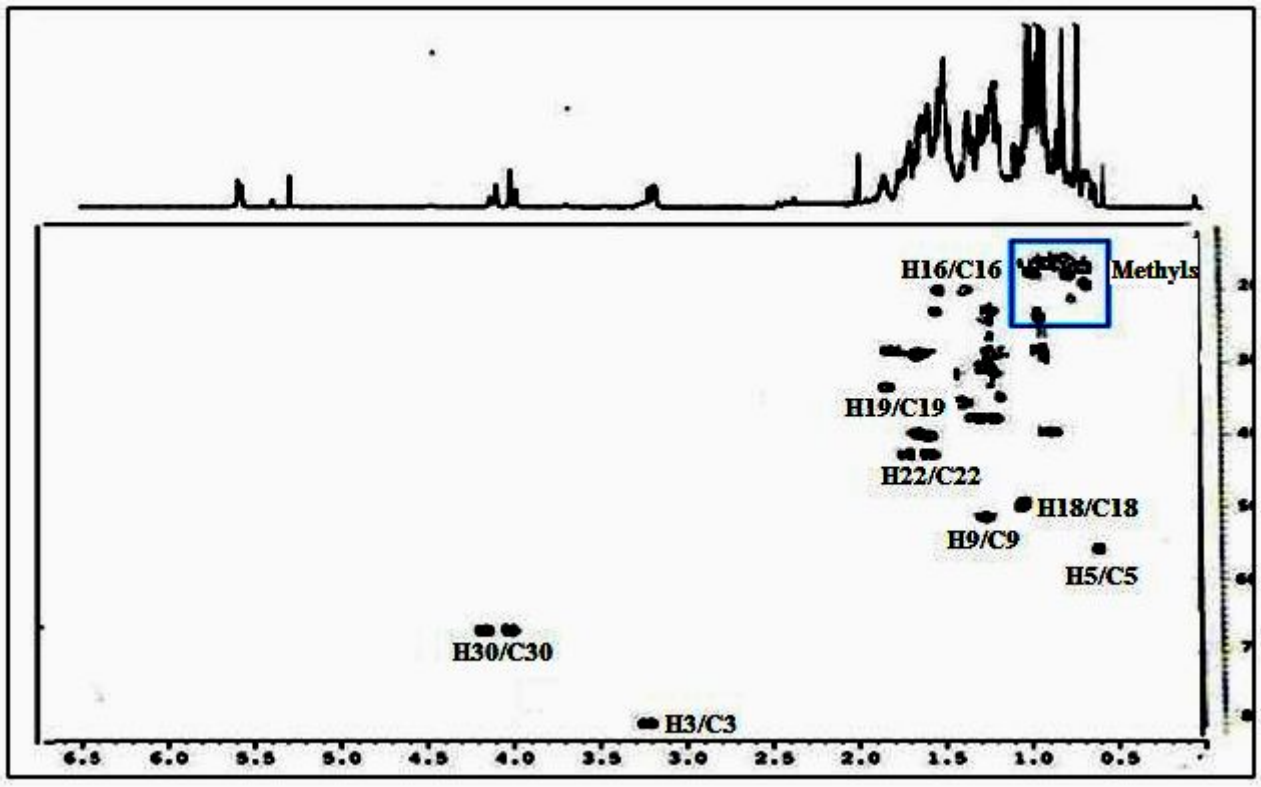


Figure 3.38: HSQC spectrum of the compound 302 in $CDCl_3$

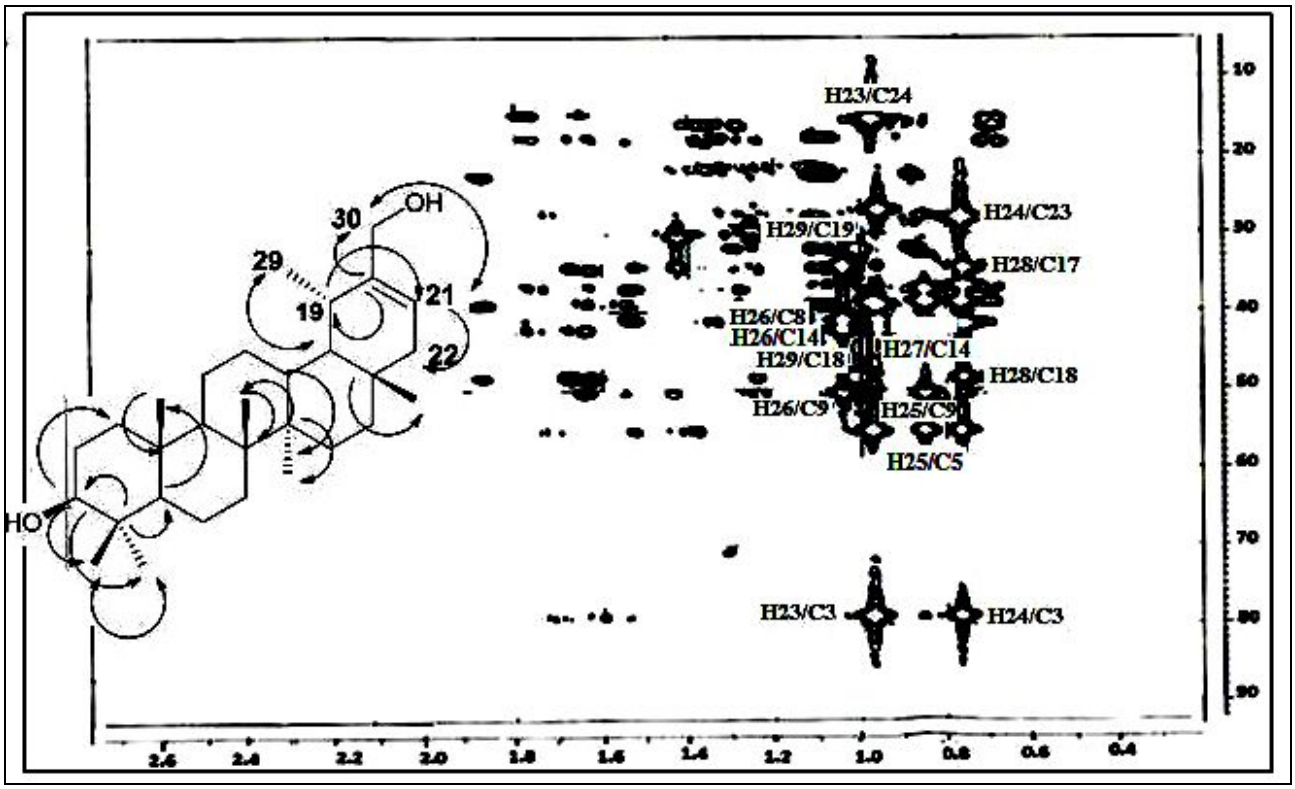


Figure 3.39: HMBC spectrum of compound 302 in $CDCl_3$

Derivatives of ψ-taraxasterol oxygenated at C-30 are rare in nature. To date, only three compounds including **302** have been reported from the Cichorieae (Shiojima et al., 1996). In particular, taraxast-20-ene-3β,30-diol (**302**) was isolated from *Ixeris chinensis* from the Lactuceae tribe (Shiojima et al., 1996), from the whole plant of *Saussurea petrovii* belonging to the Asteraceae family (Dai et al., 2001), and from the aerial parts of *Picris evae* of the Lactuceae tribe (Kisiel et al., 2001a).

Our findings confirmed the typical occurrence of this compound in the Lactuceae tribe.

Table 3.4: ^{1}H and ^{13}C NMR data of compound 302 in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	38.7	0.91	m	H-2
		1.73	m	-
2	27.6	1.54	m	-
		1.55	m	
3	79.1	3.23	dd (11.2, 4.9)	H-23, H-24, H-1
4	38.9	-	-	H-3, H-5, H-23, H-24
5	55.3	0.76	m	H-1, H-7, H-23, H-24, H-25
6	18.3	1.54	m	-
		1.45	-	-
7	34.2	1.38	m	H-6, H-11, H-26
		-	-	-
8	41.1	-	-	H-7, H-9, H-26
9	50.4	1.36	dd (11.7, 4.7)	H-7, H-12, H-25, H-26
10	37.1	-	-	H-1, H-5, H-9, H-25
11	21.6	1.27	m	H-11
		1.59	m	
12	27.1	0.92	-	H-11, H-27
13	39.2	1.65	m	H-9, H-12, H-27
14	42.4	-	-	H-13, H15, H-27
15	27.4	1.11	m	-
		1.68	m	-
16	36.7	1.30	m	H-28
17	34.5	1.35	-	H-16, H-18, H-22, H-28
18	48.5	1.25	m	H-28
19	32.1	1.88	m	H-21
		-	m	-
20	143.7	-	-	H-19 ,H-21, H-29, H-30
21	120.7	5.58	br d (6.2)	H-30
22	41.7	1.64	m	H-21
		1.78	m	-
23	15.4	0.96	s	H-24
24	27.9	0.76	s	H-23
25	16.3	0.85	s	-
26	16.0	1.04	s	H-7
27	14.8	0.95	s	H-15
28	17.7	0.75	s	H-18
29	22.5	1.01	d (6.5)	H-18
30a	65.5	4.02	d (12.3)	-
30b	-	4.13	d (12.3)	H-21

3.4.1.11 Structure elucidation of compound 303

This compound was isolated in large amount; representing the main component of the light petroleum ether extract of the plant. The analysis of ^{1}H NMR spectrum of compound **303** revealed the presence of six methyl groups attached to quaternary sp^3 carbons [δ 0.77 (3H, s, H_3-24), 0.79 (3H, s, H_3-28), 0.82 (3H, s, H_3-25), 0.95 (3H, s, H_3-27), 0.97 (3H, s, H_3-23), 1.03 (3H, s, H_3-26)] and a vinyl methyl at δ 1.68 (3H, br s, H_3-30). The proton spectrum also contained proton signals at δ 4.56 (1H, br s, H-29a) and 4.69 (1H, br s, H-29b), that were due to an exomethylene group, and at δ 3.20 (1H, dd, *J* = 11.3 and 5.2 Hz, H-3), which was assigned to a hydroxyl methine.

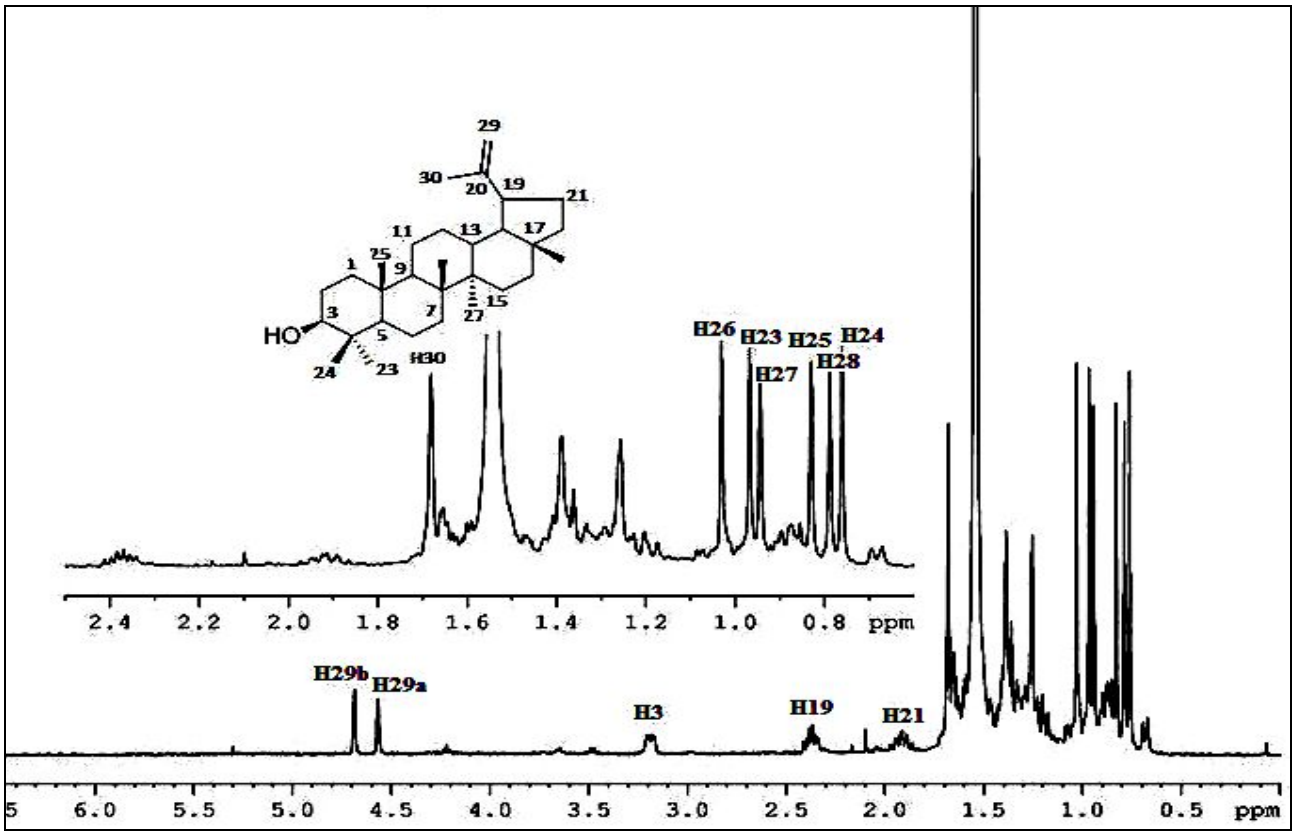
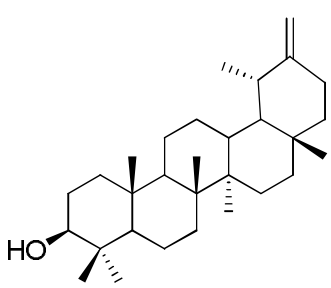


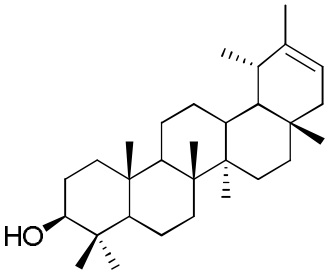
Figure 3.40: ^{1}H NMR spectrum of compound 303 in $CDCl_3$

The comparison of the chemical shift values with those reported in the literature for triterpenoid alcohols such as ψ-taraxasterol (18α,19α-urs-20(30)-en-3β-ol), pseudotaraxasterol (18α,19α-urs-20-en-3β-ol) and lupeol (lup-20(29)-en-3β-ol), immediately revealed that compound **303** was lupeol (Reynolds et al., 1986).

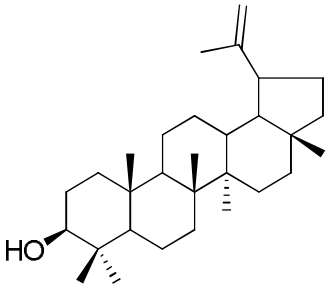
The mass spectrum of **303** displayed the molecular peak at *m/z* 426 consistently with the molecular formula $C_{30}H_{50}$ confirming the identity of triterpenoid.



ψ-taraxasterol



Pseudotaraxasterol



Lupeol

3.4.1.12 Structure elucidation of compound 304

Compound **304** was easily identified as stigmasterol by comparison of the ESIMS (**Figure 3.41**) and the ^{1}H NMR (**Figure 3.42**) data with the literature (Kojima et al., 1990). The ^{1}H NMR spectrum was recorded in $CDCl_3$ and indicated the characteristic signals of a C29 sterol due to six methyl groups [δ 0.69 (H_3-18), 1.01 (H_3-19), 1.02 (H_3-21, d, J = 6.5 Hz),0.83 (H_3-26, d, J = 6.5 Hz), and 0.81 (H_3-27, d, J = 6.5 Hz), 0.84 (H_3-29, t, J = 7.5 Hz)]; a trisubstituted double bond in ring B [δ 5.35 (H-6, m); a disubstituted double bond in the side chain [δ 5.01 (H-23, dd, J = 15.0 and 9.1 Hz), and 5.15 (H-22, dd, J = 15.0 and 9.1 Hz); and the 3-OH function [δ 3.52 (H-3, m)].

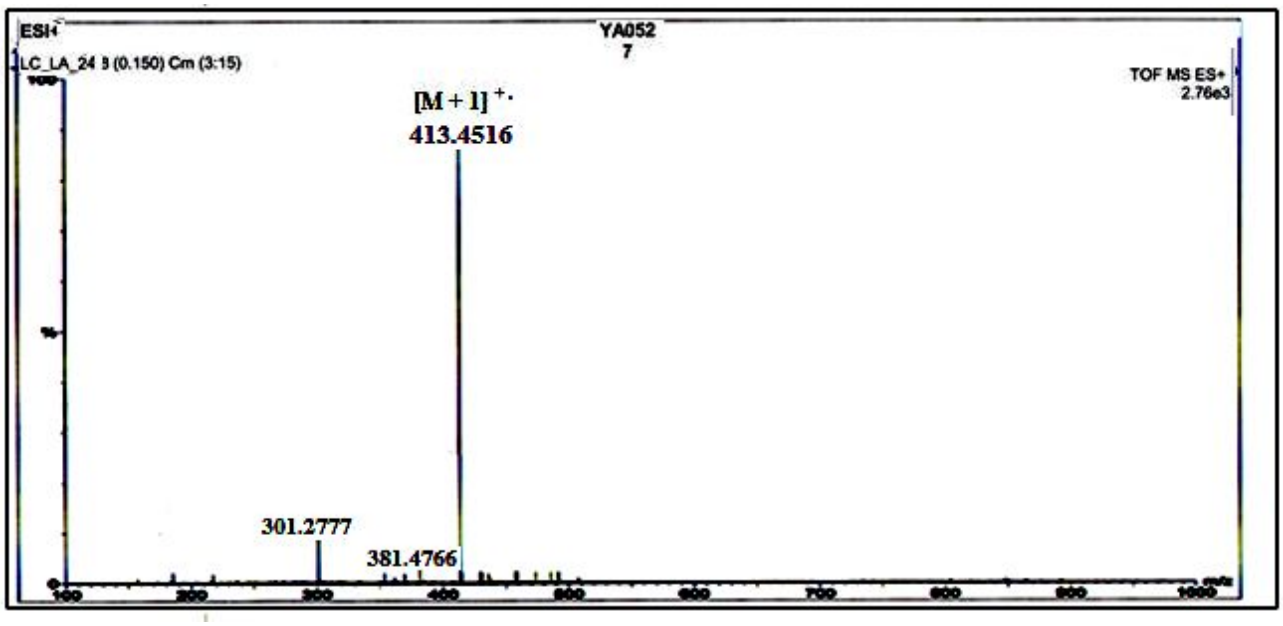


Figure 3.41: ESIMS spectrum of compound 304

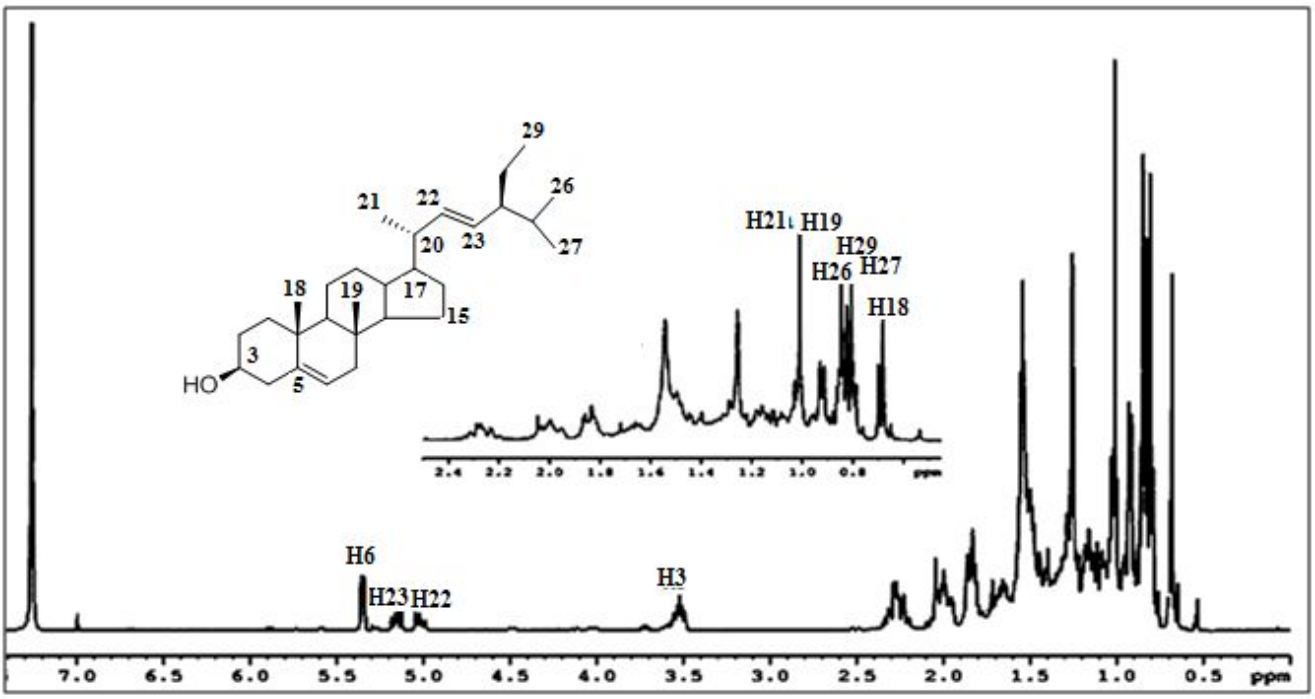


Figure 3.42: ^{1}H NMR spectrum of compound 304 in $CDCl_3$

3.4.2 SESQUITERPENOIDS STRUCTURES

3.4.2.1 STRUCTURE ELUCIDATION OF GUAIANOLIDE COMPOUNDS

3.4.2.1.1 Compound 305

Compound **305** had the molecular formula $C_{15}H_{20}O_4$ as deduced by both ESIMS and ^{13}C NMR spectra (**Figures 3.43**, **3.45**) suggesting a sesquiterpenoid structure.

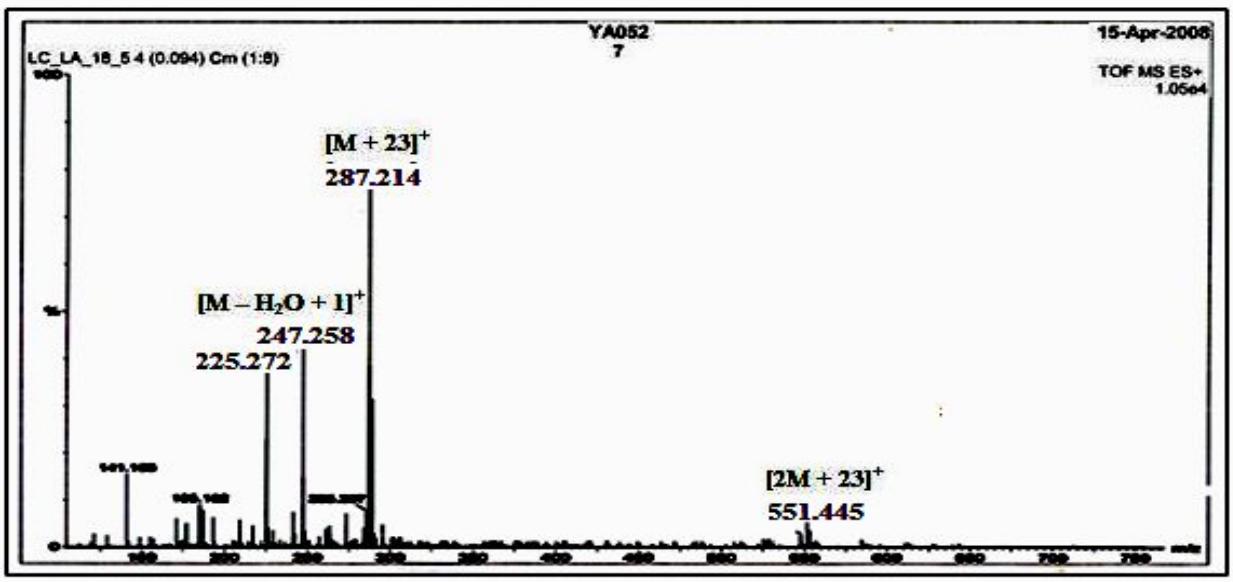


Figure 3.43: ESIMS spectrum of compound 305

The comparison of ^{1}H and ^{13}C NMR data (**Figure 3.44** and **Figure 3.45**) with those reported in the literature for different cyclic sesquiterpenoids indicated the presence of a guaianolide sesquiterpene framework. Accordingly, the ^{1}H NMR spectrum showed the presence of two exocyclic methylene protons signals at δ 4.81 (1H, br s, H-14a), 5.08 (1H, br s, H-14b), 5.38 (1H, br s, H-15a), and 5.46 (1H, br s, H-15b); three oxymethine protons at δ 4.71 (1H, m, H-3), δ 4.58 (1H, sharp m, H-9), and δ 3.81 (1H, t, J = 9.7, H-6) and a secondary methyl at δ 1.26 (3H, d, J = 7.2 Hz).

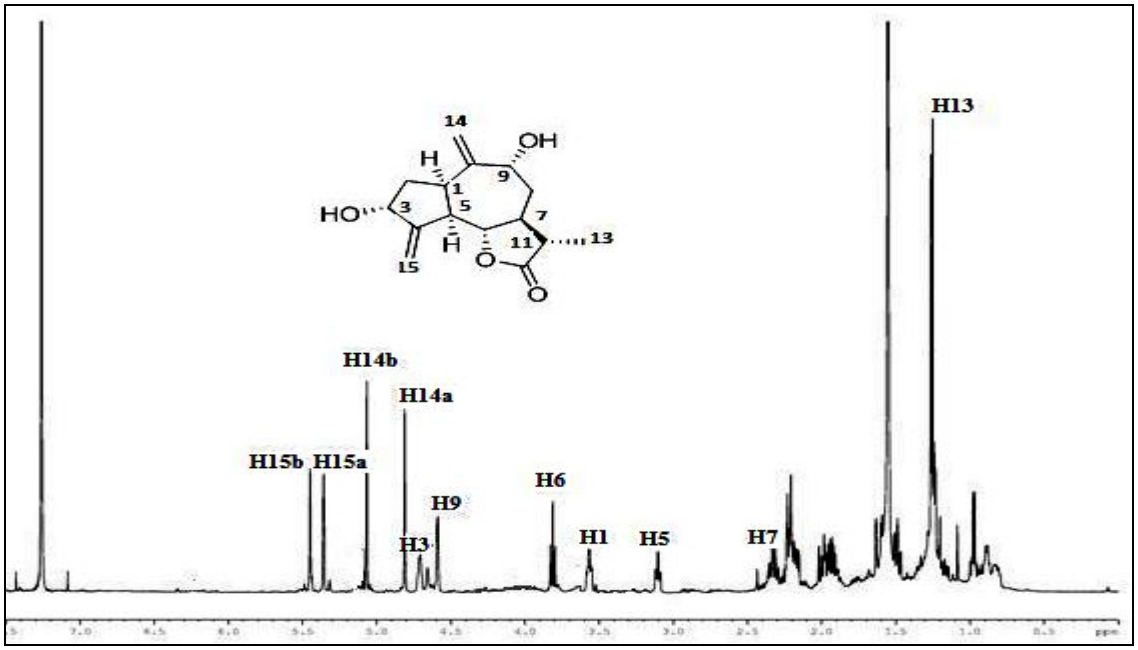


Figure 3.44: ^{1}H NMR spectrum of compound 305 in $CDCl_3$

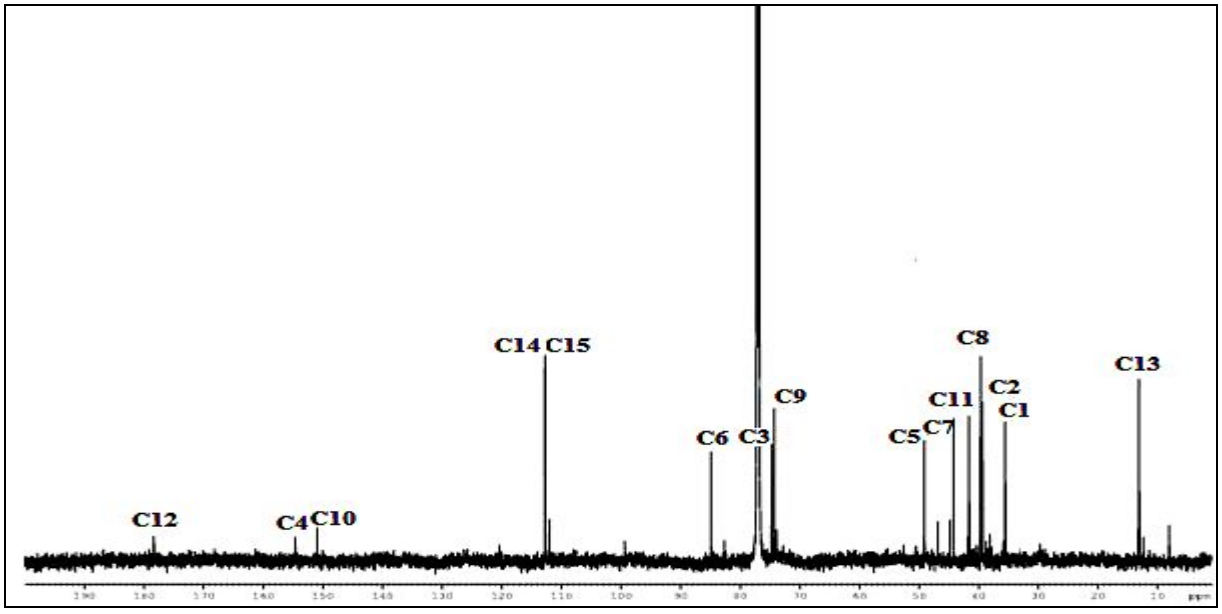


Figure 3.45: ^{13}C NMR spectrum of compound 305 in $CDCl_3$

Analysis of the ^{1}H-^{1}H COSY spectrum (**Figure 3.46**) allowed the definition of the single spin system of the tricyclic skeleton. Particularly diagnostic were the correlations between the proton H-6 (δ 3.81) and the two protons H-5 (δ 3.10) and H-7 (δ 2.32), and between the proton H-1 (δ 3.58) and the protons H-5 (δ 3.10), H-2 (δ 2.20) and H-14 (δ 4.81).

The presence of a lactone functionality was suggested by the carbonyl signal at δ 178.4 (C-12) in the ^{13}C NMR spectrum. A significant HMBC correlation (**Figure 3.48**) between C-12 and the methyl doublet at δ 1.26 (H-13) supported the arrangement of the lactone ring as depicted in structure **305** with the C-6/C-7 junction.

The interpretation of ^{1}H-^{1}H COSY, HSQC, and HMBC experiments (**Figures 3.46**, **3.48**, **3.49**) allowed the assignment of all protons and carbons values (**Table 3.5)**.

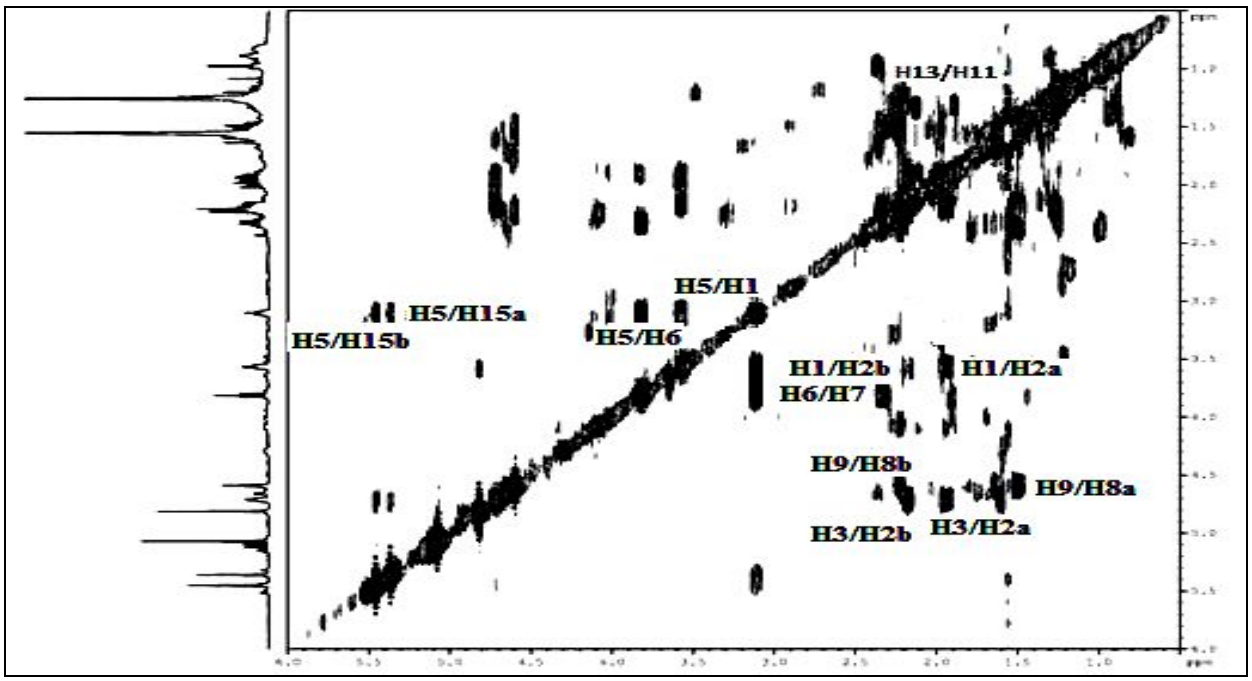


Figure 3.46: ^{1}H-^{1}H COSY spectrum of compound 305 in $CDCl_3$

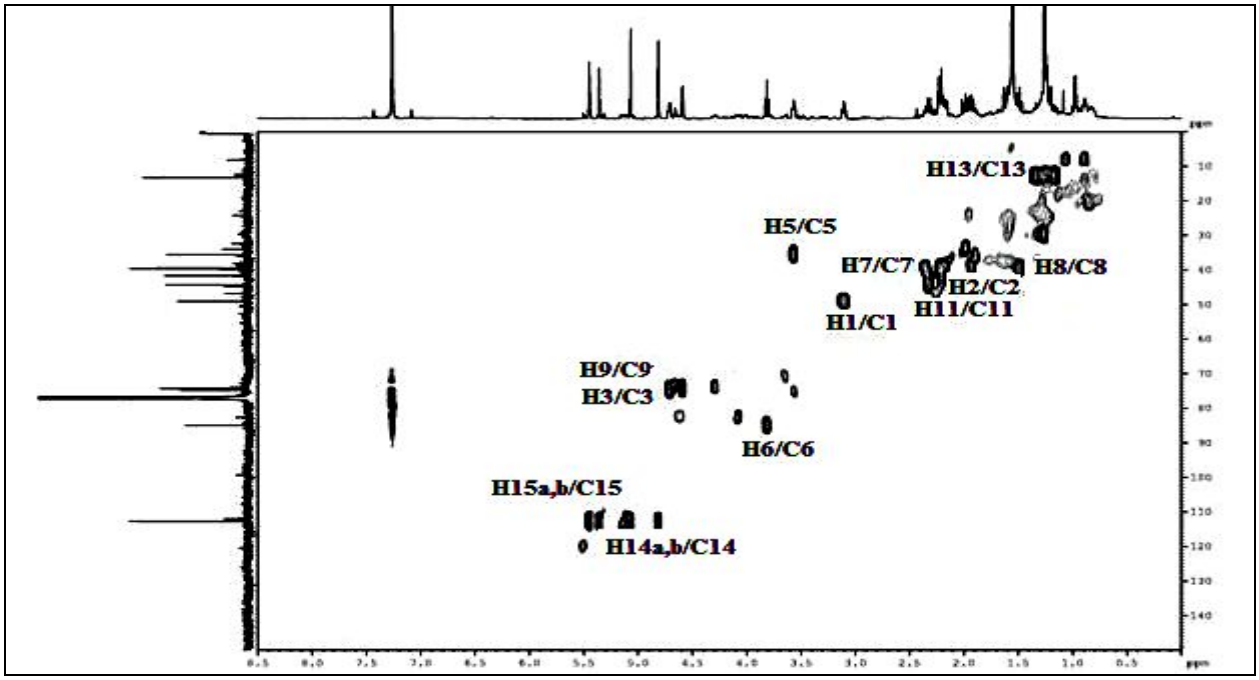


Figure 3.47: HSQC spectrum of compound 305 in $CDCl_3$

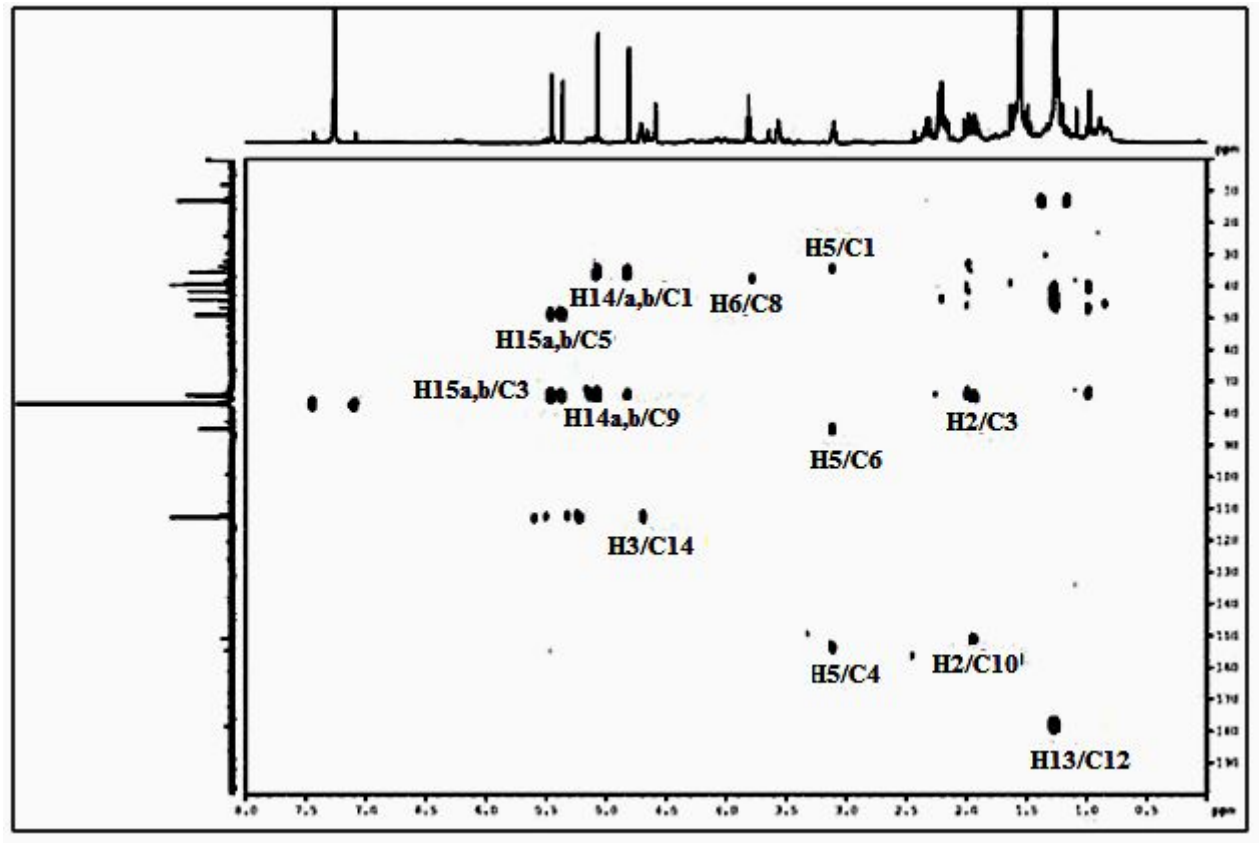


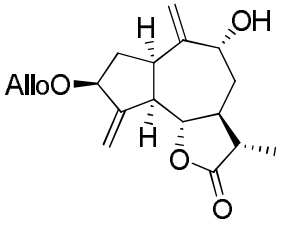
Figure 3.48: HMBC spectrum of compound 305 in $CDCl_3$

Table 3.5 : ^{1}H and ^{13}C NMR of compound 305 in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	35.6	3.58	ddd (12.3, 8.7, 4.1)	H-5, H-14a, H-14b
2	39.4	2.20	m	-
		1.95	m	-
3	74.7	4.71	m	H-15a, H-15b, H-2a
4	154.0	-		H-5, H-6
5	49.9	3.10	t (9.7)	H-15a, H-15b
6	84.9	3.81	t (9.7)	H-5
7	44.3	2.32	m	H-11, H-13
8	39.7	1.50	m	H-6, H-11
		2.23	m	-
9	74.3	4.58	m	H-8a, H-14a, H-14b
		-		-
10	150.9	-		H-1, H-2a
11	41.6	2.18	m	H-6, H-13
12	178.4	-		H-13
13	13.6	1.26	d (7.2)	-
				-
14	112.7	4.81	br s	-
		5.08	br s	-
15	112.7	5.38	br s	H-3b
		5.46	br s	-

Compound **305** was structurally related to zaluzanin C (Romo et al., 1967), (Spring et al., 1994) differing from this in the presence of both a secondary methyl [δ 1.26 (3H, d, *J* = 7.2 Hz, H_3-13) replacing the exomethylene moiety at C-11 and an additional hydroxyl group at C-9 [δ 4.58 (1H, sharp m, H-9)].

In particular, comparison of the NMR data of compound **305** in pyridine-d_5 (**Figure 3.49**) with those reported in the literature for related guaianolides (Asada et al., 1984), (Kisiel et al., 2002b), showed a close structural similarity of compound **305** with 9α-hydroxy-11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside (Kisiel et al., 2002b), which is the aglycone of the glycosides guaianolide previously isolated from different genera of Asteraceae family.



9α-hydroxy-11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside

In particular, the spin-system sequence from C-1 to C-9 in **305** was the same as 9α-hydroxy-

11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside, whereas differences were observed in the carbons and protons chemical shifts (**Figures 3.49**, **3.50**) of the 5-membered ring strongly suggesting the opposite stereochemistry at C-3. According to this suggestion, H-1 and H-5 values were downfield shifted [δ 4.01 (H-1), δ 3.25 (H-5) in **305**; δ 3.65 (H-1), δ 2.91 (H-5 in 9α-hydroxy-11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside (values in pyridine)] due to the α-oriented 3-OH group.

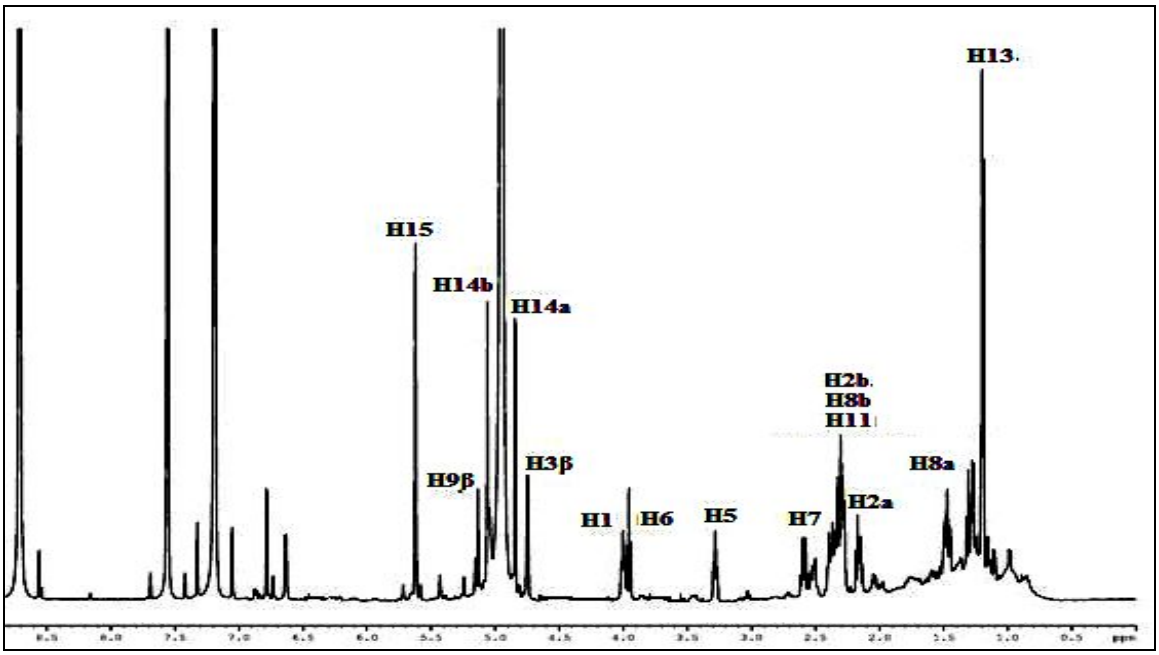


Figure 3.49: ^{1}H NMR spectrum of compound 305 in pyridine-d_5

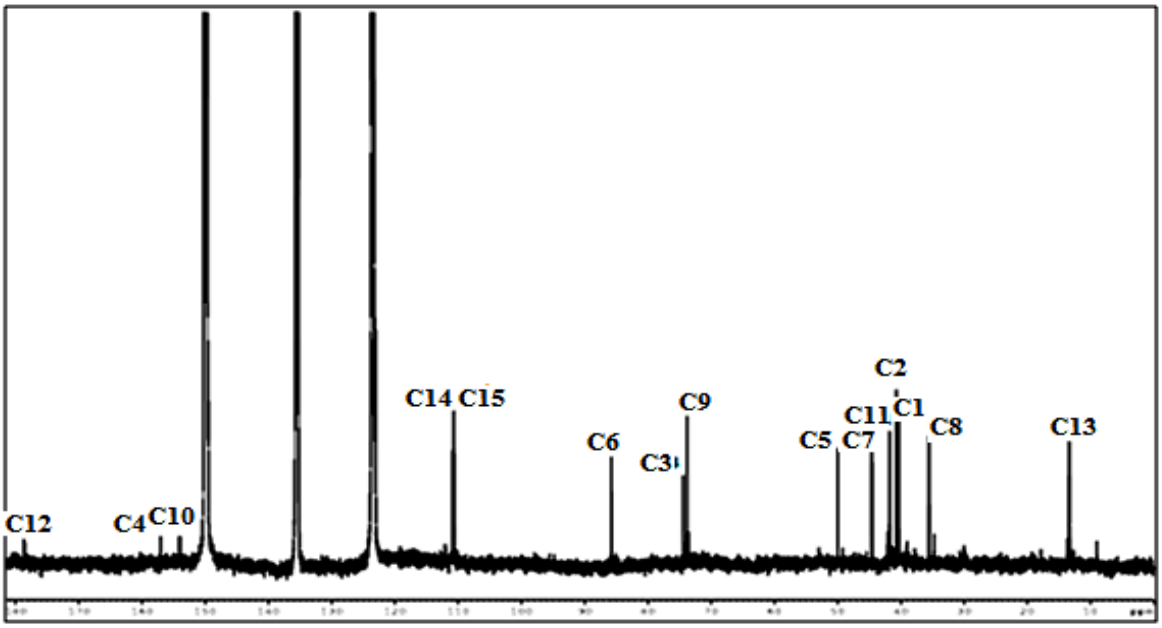


Figure 3.50: ^{13}C NMR spectrum of compound 305 in pyridine-d_5

The proposed relative stereochemical arrangement was further supported by a series of steric effects observed in the NOESY spectrum of compound **305 (Figures 3.51, 3.52)**. Along with the expected correlations between α oriented H-1 and H-5, diagnostic cross-peaks were observed between H-2α and H-1, and between H-2β and both H-14 and H-3 according to the β-orientation of H-3. Analogously, H-9 showed correlations only with H-14b and both protons H_2-8 thus supporting a β-orientation.

All these data confirmed the structure proposed and compound **305** was 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C.

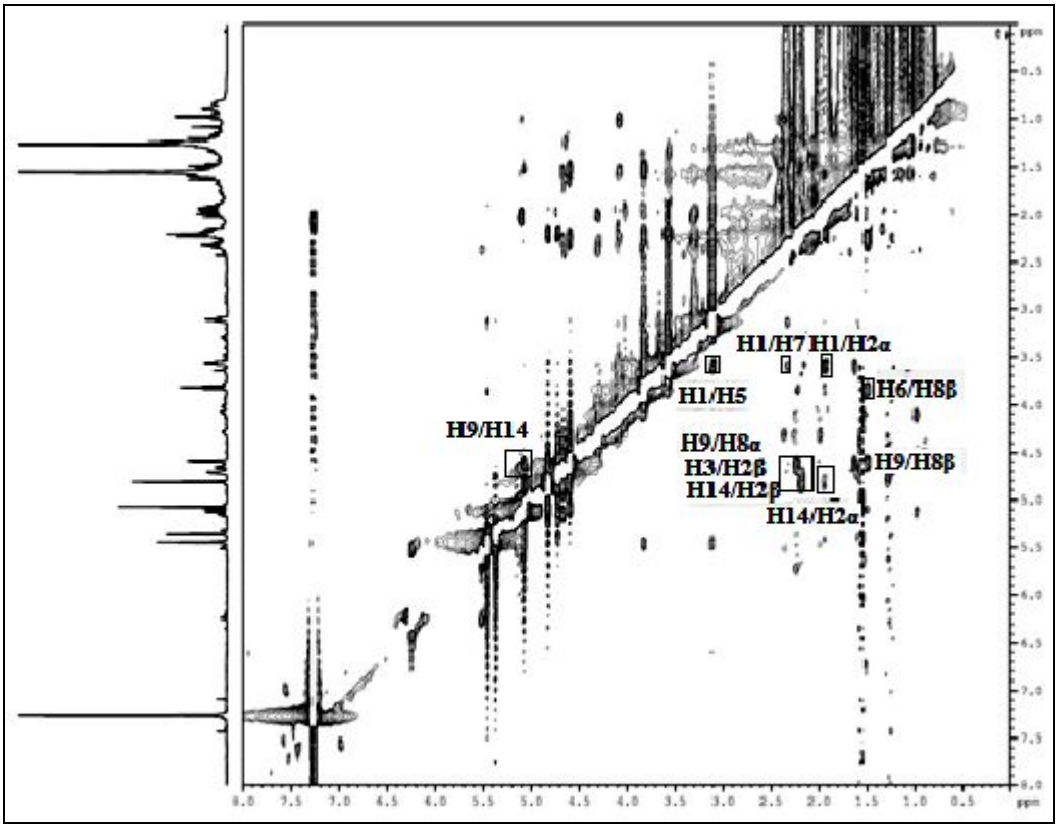


Figure 3.51: NOESY spectrum of compound 305 in $CDCl_3$

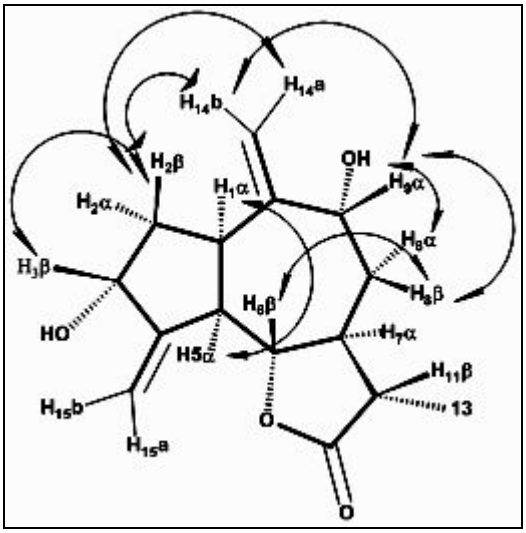


Figure 3.52: NOESY correlations observed in compound 305

3.4.2.1.2 Compound 306

The high resolution electron spray mass spectrum (HRESIMS) of compound **303** (**Figure 3.53**), showed the sodiated molecular peak at *m/z* 264.1259 consistent with the molecular formula $C_{25}H_{20}O_4$, the same as compound **305**.

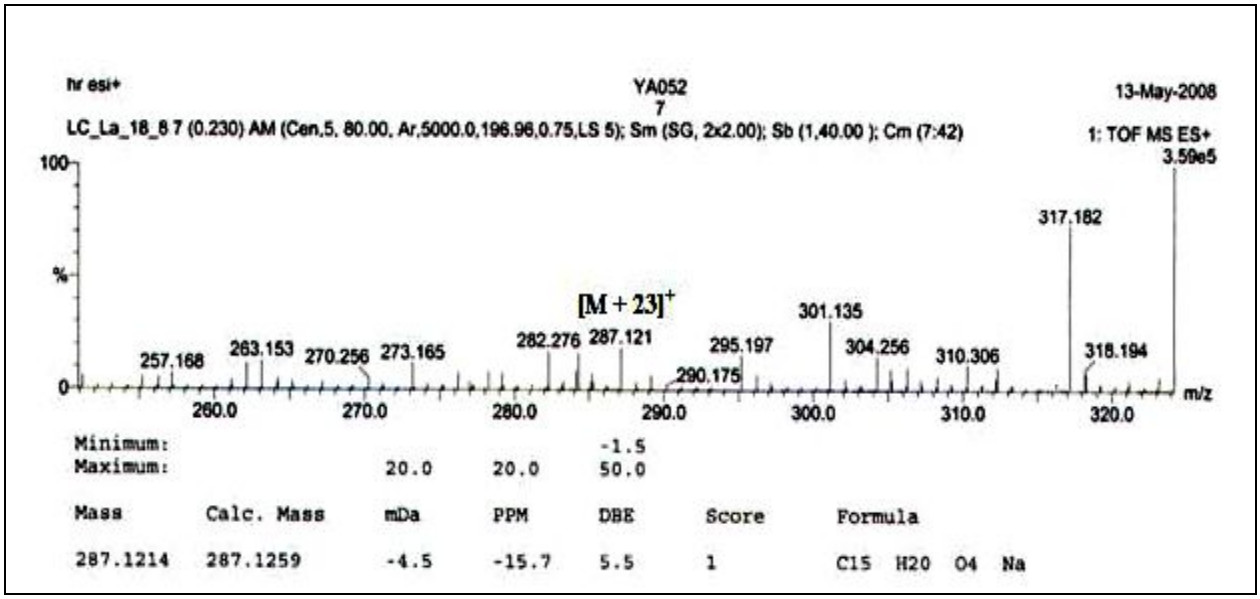


Figure 3.53: HRESIMS spectrum of compound 306

The analysis of the proton and carbon spectra (**Figures 3.54**, **3.55**) revealed the same guaianolide framework as compound **305** exhibiting two exomethylene groups [δ_H 5.12 (1H, br s, H-14a), 5.16 (1H, br s, H-14b), 5.50 (1H, d, *J* = 3.5, H-13a) and 6.21 (1H, d, *J* = 3.5, H-13b); δ_C 112.7 (C-14), 120.1 (C-13), 139.3 (C-11), and 149.8 (C-10)], a secondary methyl [δ_H 0.98

(3H, d, J = 7.1 Hz, H_3-15); δ_C 8.1 (C-15)], and two secondary hydroxyl functions [δ_H 4.30 (1H, m, H-3) and 4.72 (1H, br s, H-9); δ_C 74.1 (2C, C-3 and C-9)].

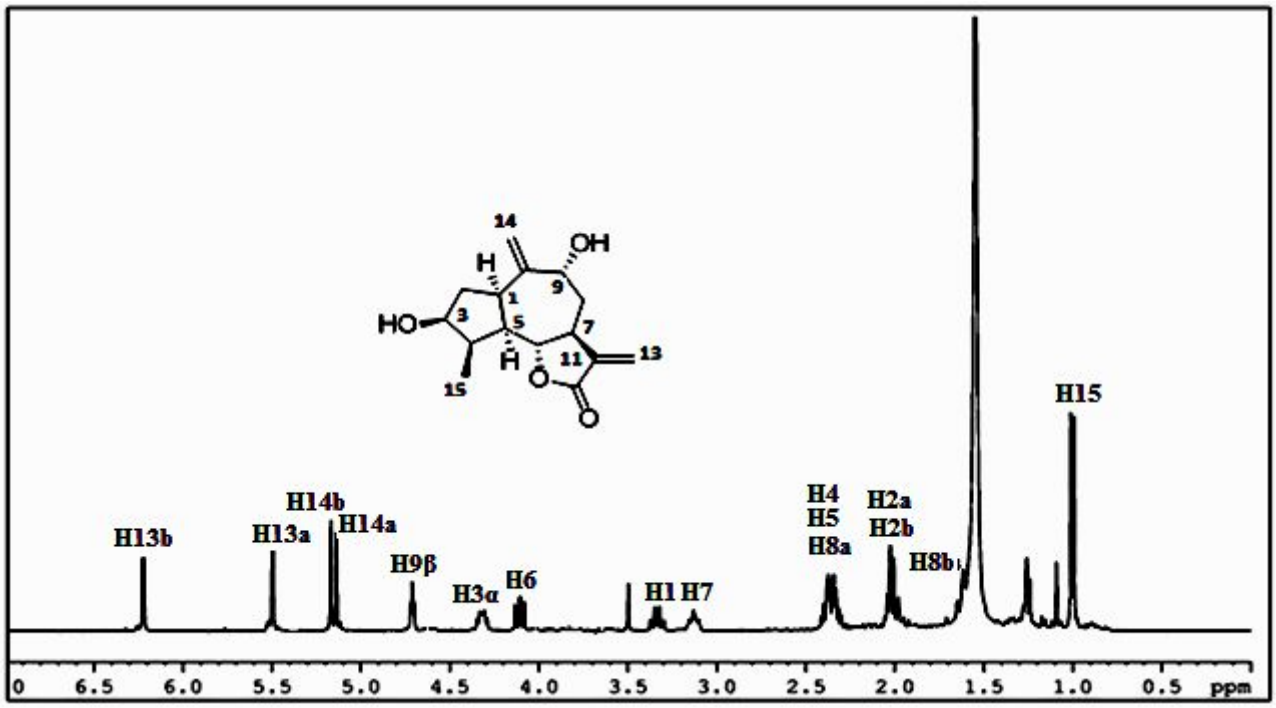


Figure 3.54: ^{1}H NMR spectrum of the compound 306 in $CDCl_3$

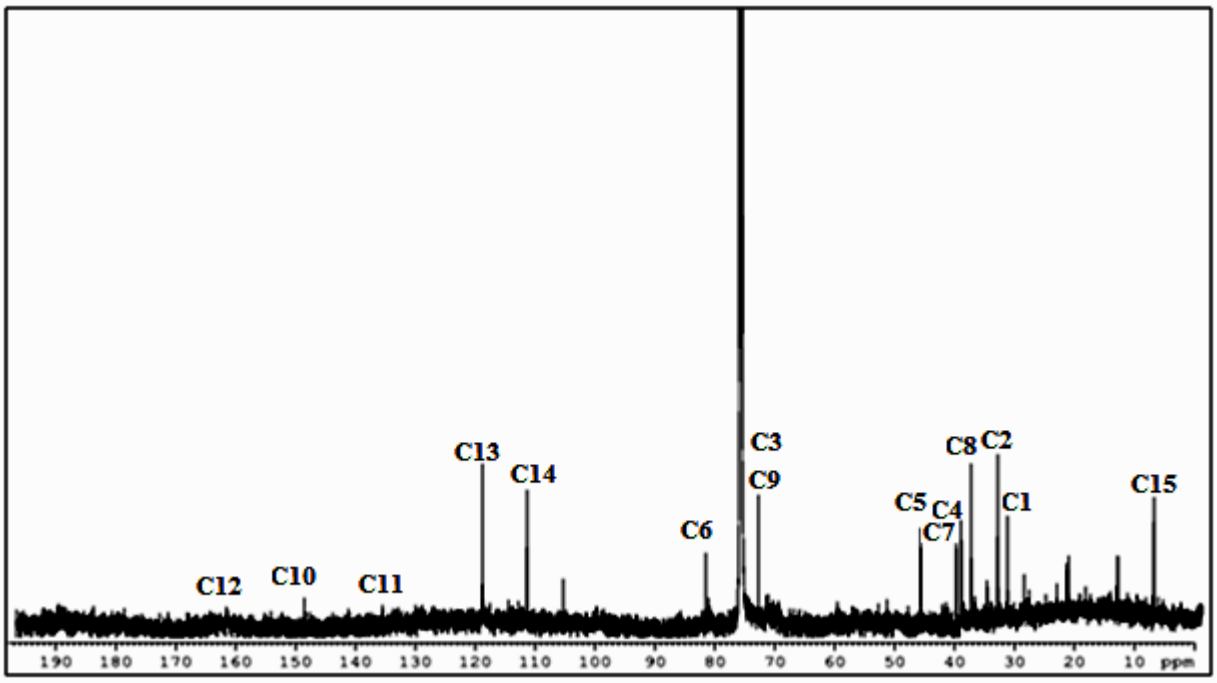


Figure 3.55: ^{13}C NMR Spectrum of f the compound 306 in $CDCl_3$

Careful analysis of the $^{1}H–^{1}H$ COSY experiment (**Figure 3.56**) led us to define all the proton sequence from H-1 to H-9 indicating that the two hydroxyl groups were located at C-3 and C-9 analogously with compound **305**, whereas the secondary methyl was at C-4, and the exocyclic double bonds were located at C-10 and C-11. The interpretation of the HSQC (**Figure 3.57**) and HMBC (**Figure 3.58**) spectra of **306** allowed the assignment of all proton and their corresponding carbon values as well as the attribution of the resonances of all quaternary carbons, as it is reported in **Table 3.6**.

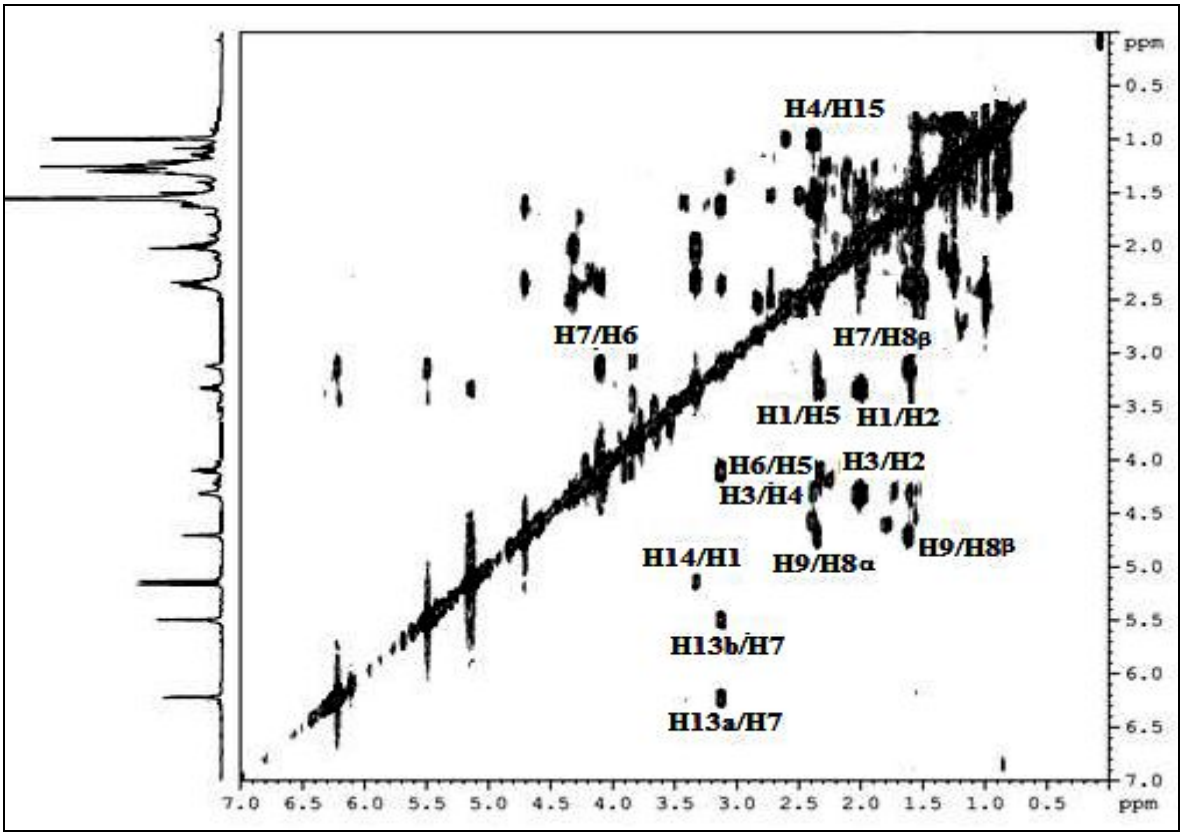


Figure 3.56: ^{1}H-^{1}H COSY spectrum of compound 306 in $CDCl_3$

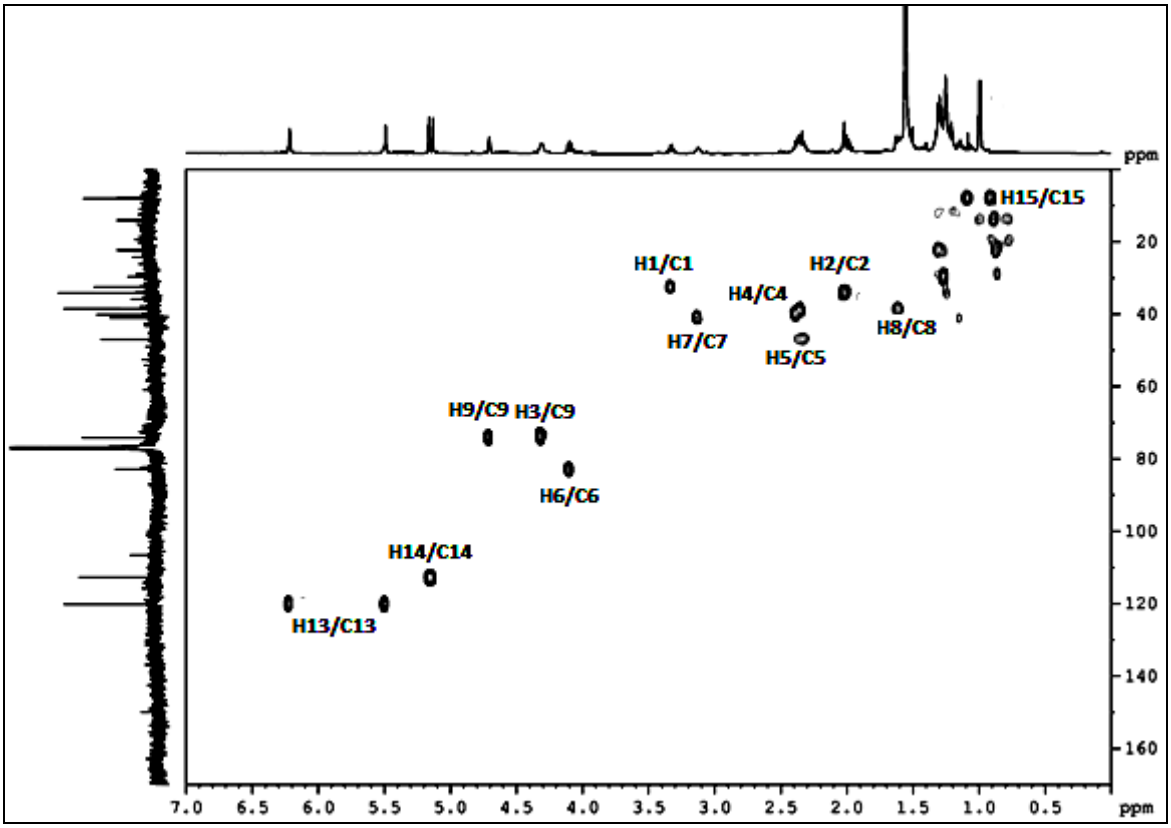


Figure 3.57: HSQC spectrum of compound 306 in $CDCl_3$

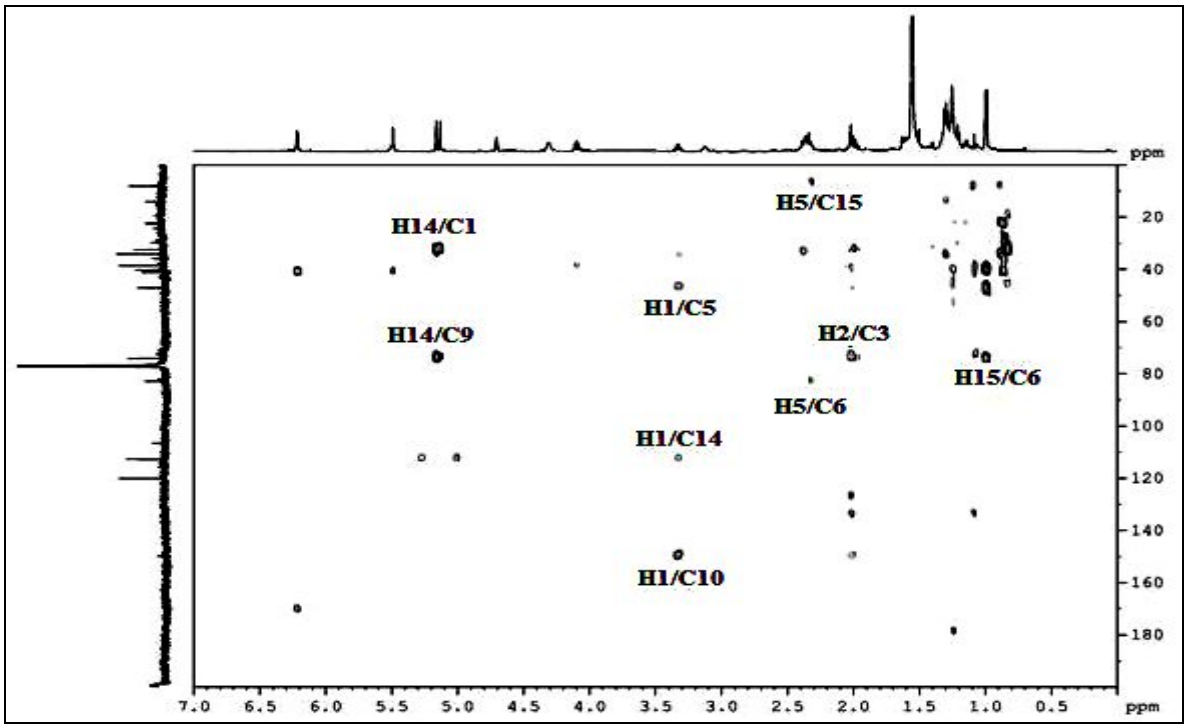
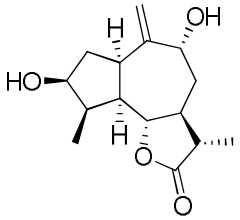


Figure 3.58: HMBC spectrum of compound 306 in $CDCl_3$

A survey of the literature on the phytochemistry of *Crepis* species indicates that compound **306** had strong similarities with 9α-hydroxy-4α,11β,13,15-tetrahydrozaluzanin, which was isolated from *Crepis rhoeadifolia* (Kisiel et al., 1996), belonging to the Lactuceae tribe.



9α-hydroxy-4α,11β,13,15-tetrahydrozaluzanin

The only difference was the presence of an additional double bond in the lactone ring at C-11 (C-13) in compound **306** The analogies with 9α-hydroxy-4α,11β,13,15-tetrahydrozaluzanin regarded the perhydroazulene moiety indicating for this part the same substitution pattern including the relative stereochemistry. The relative orientation of the substituents was supported by analysis of the coupling constant values in the proton spectrum of compound **306** as well as by comparison of the chemical shift values with those of related compounds with opposite configuration at either C-4 or C-9 (Kisiel et al., 1996).

Table 3.6 : ^{1}H and ^{13}C NMR of compound 306 in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	32.5	3.32	q (10.1)	H-2a, H-14a, H-14b
2	34.1	2.00	m	H-1, H-4, H-14a
		2.00	m	-
3	74.1	4.30	m	H-2a, H-15a, H-15b
4	40.2	2.35	m	H-15
5	46.9	2.35	m	H-1, H-2, H-13
6	82.9	4.10	t (8.1)	H-4 , H-5
7	41.0	3.15	m	H-13a, H-13b
8	38.6	1.6o	m	H-6
		2.35	m	-
9	74.1	4.72	br s	H-14a, H-14b,
		-		
10	149.8	-		H-1, H-2a, H-2b, H-14
11	139.3	-		H-13
12	169.8			H-13
13	120.1	5.50	d (3.5)	-
		6.21	d (3.5)	-
14	112.7	5.12	br s	H-1
		5.16	br s	-
15	8.1	0.98	d (7.1)	H-4, H-5

Finally, the proposed stereochemistry was confirmed by interpretation of the NOESY experiment (**Figure 3.59**) that showed diagnostic correlations between the α-oriented protons H-1 and H-3, and between the β-oriented H-6 and H_3-15. Expected NOE interactions between H-5 and both H-1 and H-7 according to the guaianolide skeleton with 1,5-*cis* and 6,7-*trans* junctions were also detected.

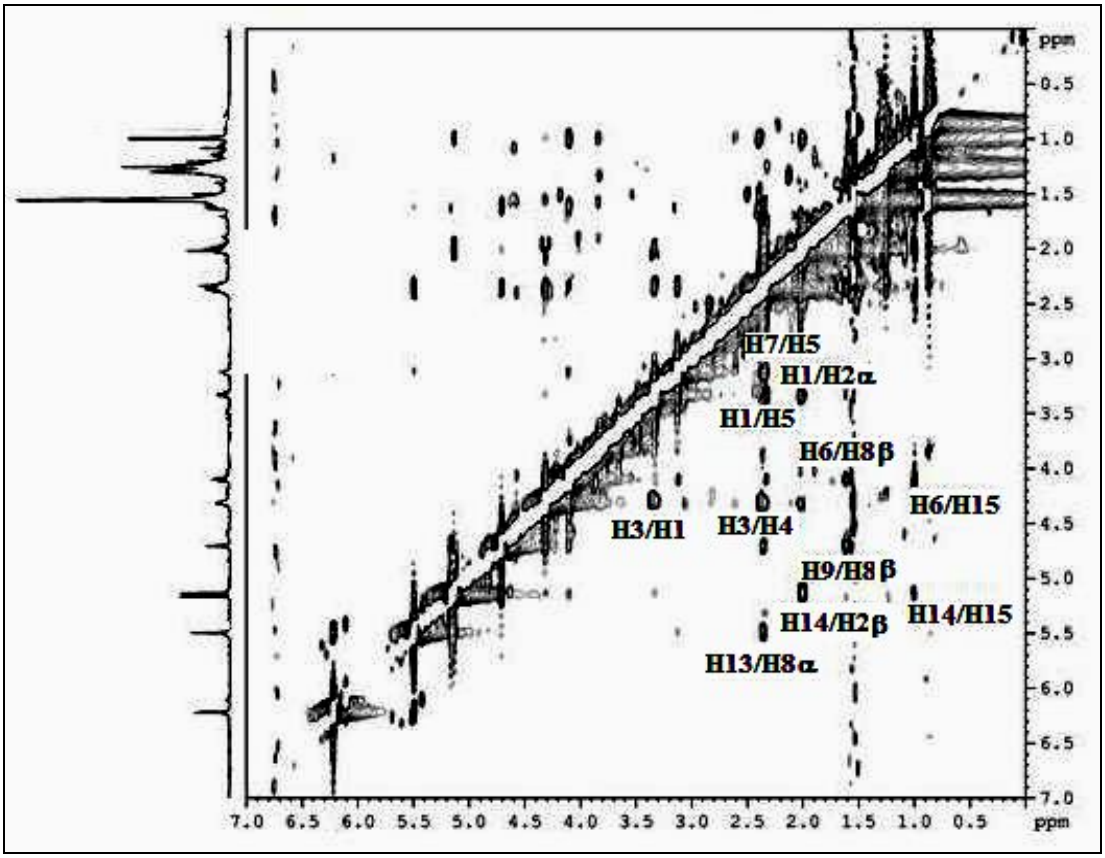


Figure 3.59: NOESY spectrum of compound 306 in $CDCl_3$

3.4.2.2 RELATED GUAIANOLIDES STRUCTURES: 307, 308, 309, 310 AND 311

3.4.2.2.1 Compound 307

The ESIMS spectrum (positive ions) (**Figure 3.60**) of compound **307** showed a sodiated molecular peak at *m/z* 301 $[M + Na]^+$ consistent with the molecular formula $C_{15}H_{18}O_5$ (molecular mass 278).

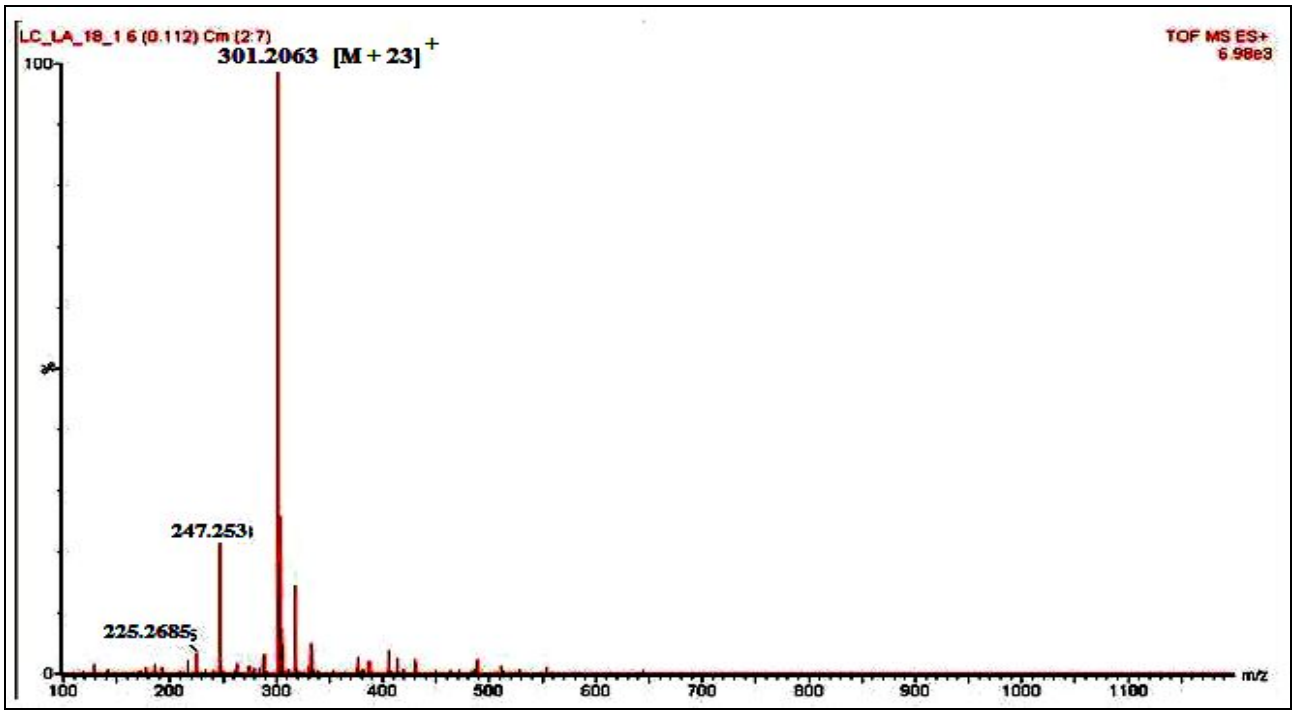


Figure 3.60: ESIMS spectrum of compound 307

The ^{1}H NMR spectrum (**Figure 3.61**) of **307** revealed a guaianolide framework exhibiting a conjugated trisubstituted double bond [δ 6.43 (1H, bs, H-3)], a vinyl methyl [δ 2.44 (3H, br s, H_3-14)], and a secondary methyl linked to an sp^3 carbon [δ 1.44 (3H, d, J = 6.9 Hz, H_3-13)].
In addition to the oxygenated proton H-6 of the lactone *trans* junction, that resonated as a triplet at δ 3.65 (J = 8.2 Hz), the ^{1}H NMR spectrum also indicated the presence of a hydroxyl methylene attached to an sp^2 carbon [δ 4.53 (1H, d, J = 18.0 Hz, H-15a) and 4.86 (1H, d, J = 18.0 Hz, H-15b)], and a hydroxyl group in the ring B [δ 3.75 (1H, m, H-8)].

A careful check of the literature data on guaianolides allowed the identification of **307** as 11β,13-dihydroxylactucin, which was isolated for the first time from *Launaea micronata* (Sarg et al., 1982).

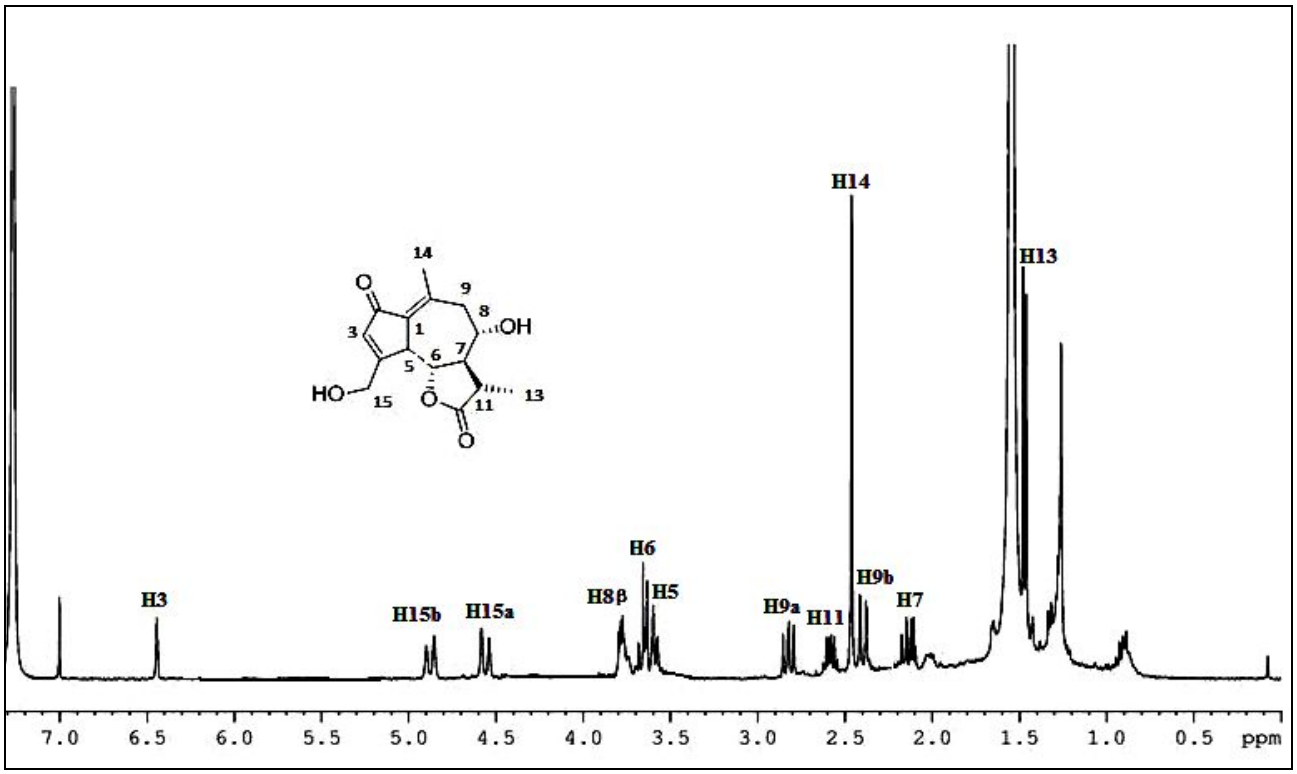


Figure 3.61: ^{1}H NMR spectrum of compound 307 in $CDCl_3$

3.4.2.2.2 Compound 308

The mass spectrum of compound **308 (Figure 3.62)**, showed a sodiated molecular peak at *m/z* 431 corresponding to the molecular formula $C_{21}H_{28}O_8$ (molecular weight 408).

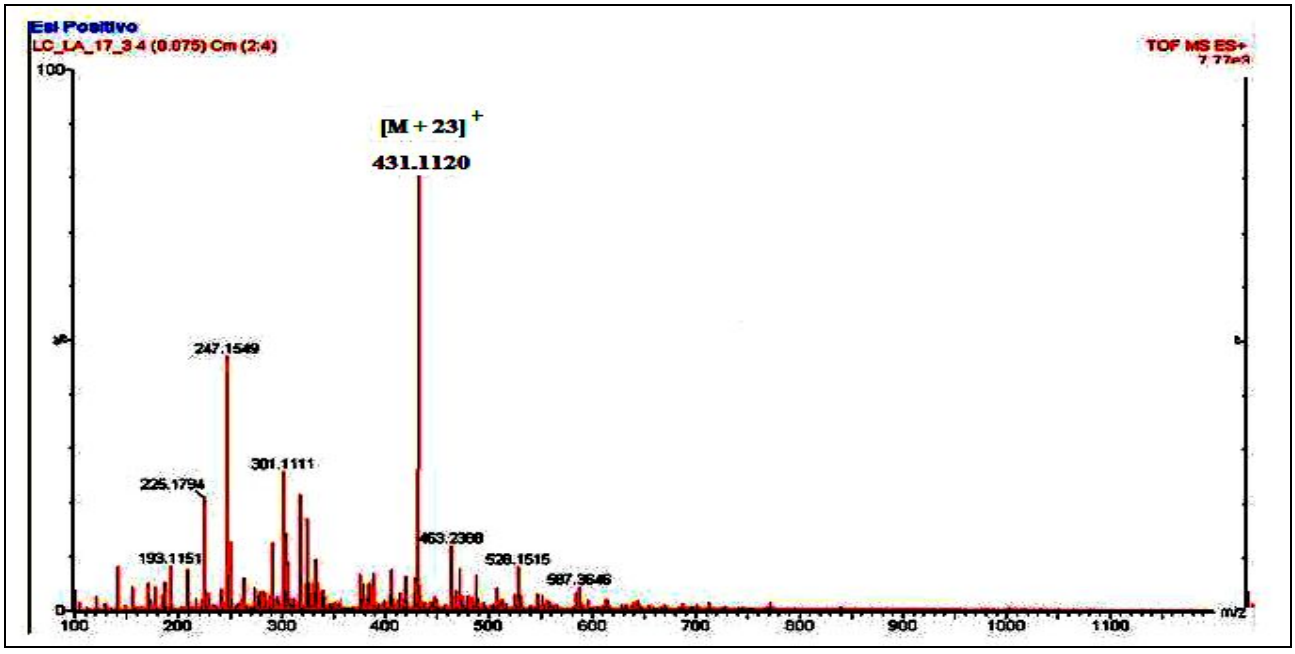


Figure 3.62: ESIMS spectrum of compound 308

Compound **308** was isolated as a glucoside as it was evidenced by the ^{1}H NMR spectrum (**Figure 3.63**) exhibiting typical signals between 3.70 and 4.50 ppm due to a glucose moiety. The spectrum also contained signals attributed to the protons of exocyclic double bonds resonating at δ 6.25 (1H, br s, H-13a), 5.35 (2H, br s, H-13b and H-15a), 5.09 (1H, br s, H-15b), δ 5.01 (1H, br s, H-14a), and 4.80 (1H, br s, H-14b). These data suggested that compound **308** had a guaianolide-type skeleton with three exomethylene groups and a glucose residue. A comparison of the ^{1}H NMR spectrum with that of compound **305** recorded in pyridine-d_5 revealed that compound **308** was closely related to **305**. Analysis of both ^{1}H-^{1}H COSY and HSQC experiments allowed the definition of the structure exhibiting an α-glucosidic linkage at C-9.

Accordingly, the chemical shift value of C-9 was downfield shifted (δ 82.3) compared with that of free aglycone (δ 73.3) and the coupling constant value of H-9 (br t) was J = 2.5 Hz).

Compound **308** was thus identified as ixerisoside D, first isolated from *Ixeris debilis* of the Lactuceae tribe (Warashina et al., 1990).

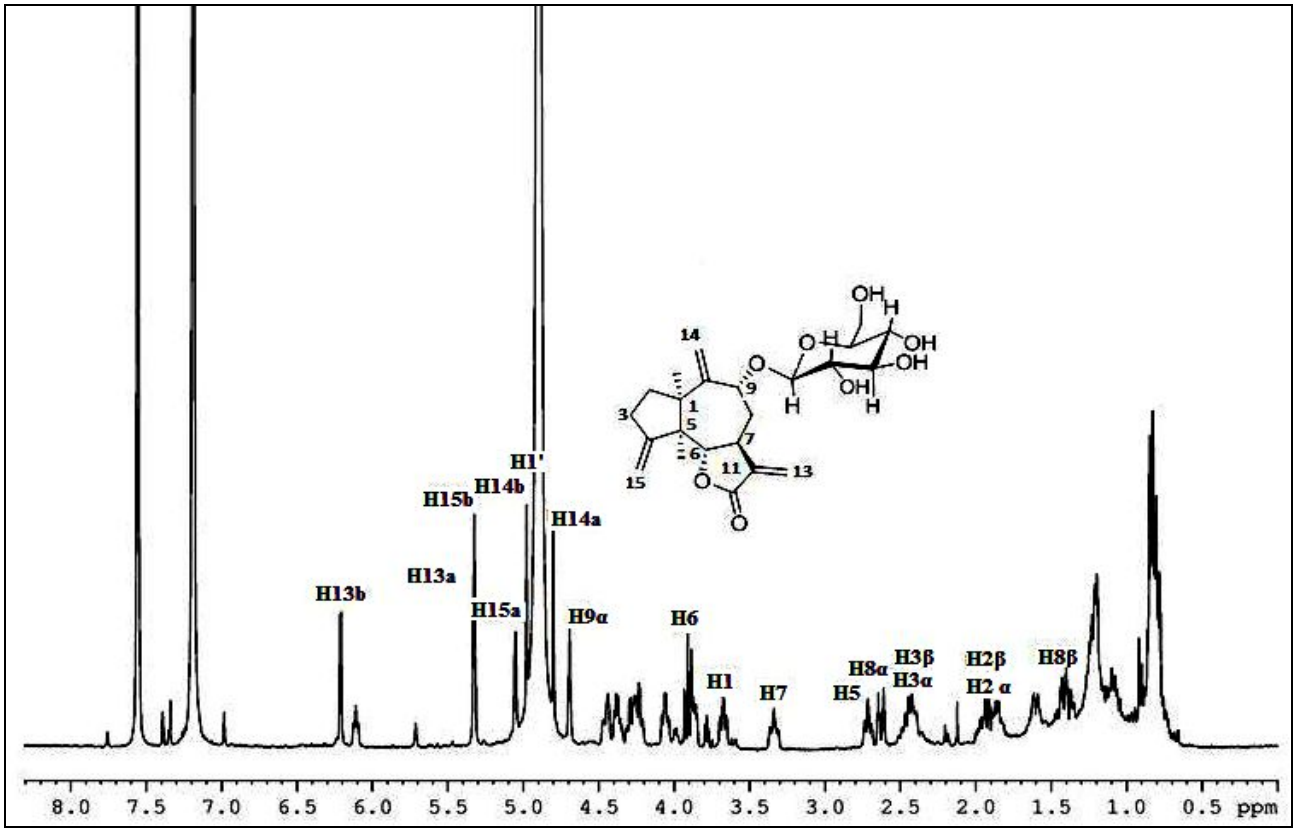


Figure 3.63: ^{1}H NMR spectrum of compound 308 in pyridine-d_5

3.4.2.2.3 Compound 309

The ESIMS spectrum of compound **309** showed the sodiated molecular peak at *m/z* 445 (**Figure 3.64**) along with a sodiated fragmentation peak at *m/z* 283 $[M - 162 + Na]^+$, which was due to the loss of a glucosyl moiety from the molecular ion.

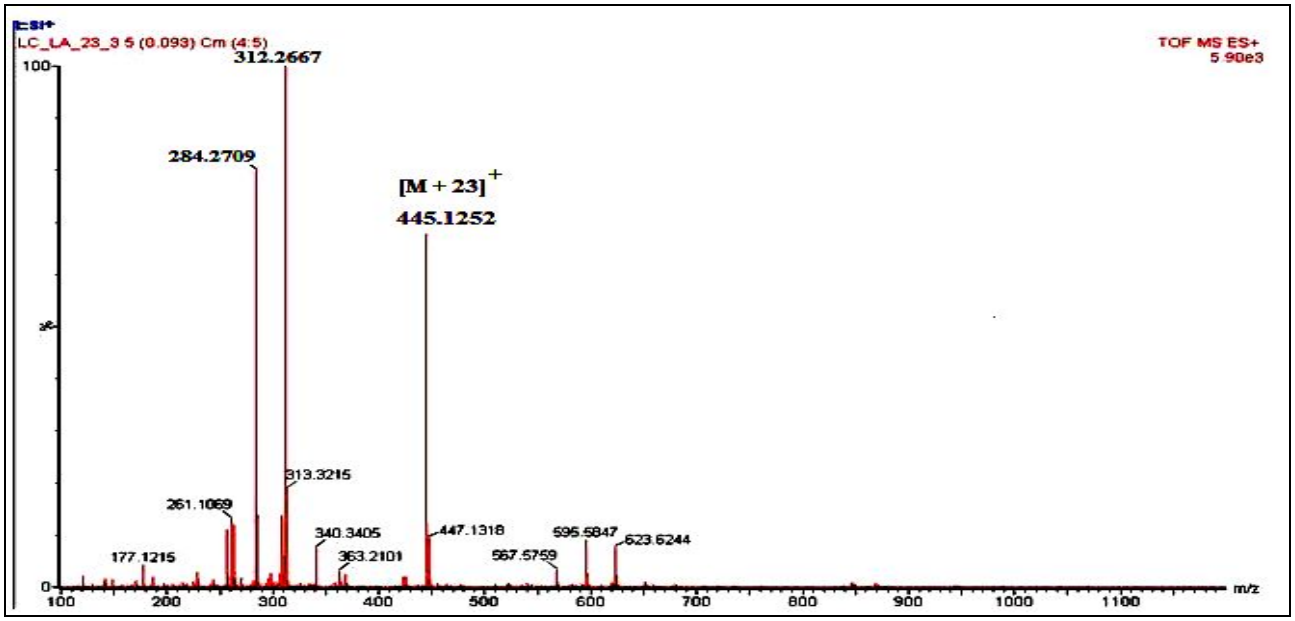


Figure 3.64: ESIMS spectrum of compound 309

The ^{1}H NMR spectrum of compound **309** (**Figure 3.65**) revealed a glucoside structure with the aglycone moiety resembling compound **307**. In fact, a series of signals at δ 3.8-4.6 due to the sugar moiety of a β-D-glucopyranoside were observed in the spectrum. Analogously with **307**, the spectrum contained a vinyl methyl signal at δ 2.45 (3H, br s, H_3-14) and an olefinic proton signal at δ 6.90 (1H, s, H-3), according to the presence of the α,β-unsaturated ketone system in rings A/B. In addition, signals at δ 5.35 (1H, br d, J = 3.1 Hz) and δ 6.15 (1H, br d, J = 3.1 Hz) attributed to the exomethylene protons H-13a and H-13b, respectively, were present in the spectrum.

Comparison of these data with those reported in the literature for 8-deoxylactucin (Kisiel et al., 2000a, 2000b), (Kisiel et al., 2006), (Michalska et al., 2007) clearly confirmed the presence of this aglycone in compound **309**. A survey on guaianolide glycosides led to identify compound **309** as crepidiaside A, previously isolated from *Crepidiastrum Keiskheanum* (syn. *Lactuca keiskeana*) **(**Adegawa et al., 1985), and later from other species of the Lactuceae tribe.

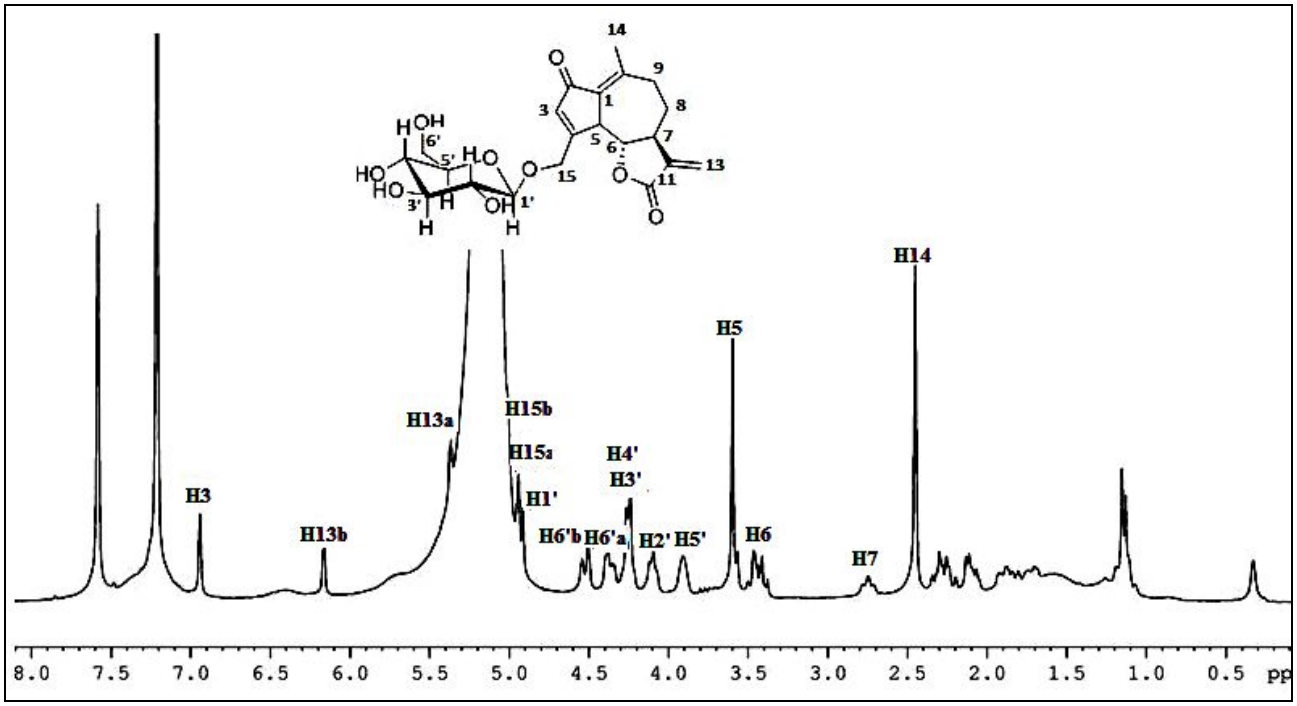


Figure 3.65: ^{1}H NMR spectrum of compound 309 in pyridine-d_5

3.4.2.2.4 Compound 310

The ion molecular observed for this compound at *m/z* 447 $[M + 23]^+$ as shown in the **figure 3.66**, was attributed to the molecular ion *m/z* 424 which corresponds to the molecular formula $C_{21}H_{28}O_9$.

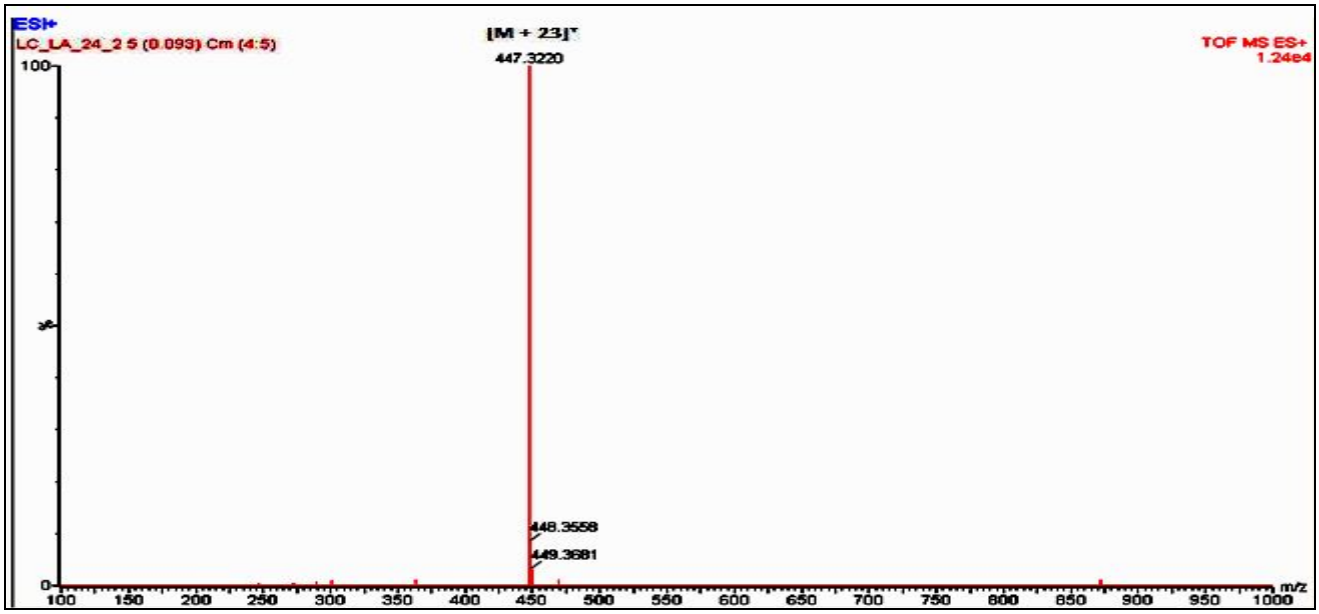


Figure 3.66: ESIMS spectrum of compound 310

The ^{1}H NMR spectrum of compound **310** (**Figure 3.67**) exhibited signals similar to those of compound **308**. In particular, the presence of three exomethylene groups [δ 5.15 (2H, br s), δ 5.56 (1H, br s), δ 5.96 (1H, br s), δ 5.42 (1H, d, *J* = 3.1 Hz) and δ 6.25 (1H, d, *J* = 3.1)] suggested that compound **310** had also a guaianolide-type skeleton as compound **308**. In addition, the ^{1}H NMR spectrum showed the presence of two signals at δ 4.81 (1H, br t, *J* = 3.5 Hz) and δ 4.88 (1H, t, *J* = 7.63 Hz) attributed to two carbinol methine protons. The set of signals due to a glucopyranosyl residue was also observed. A search of literature related metabolites allowed to identify compound **310** as macrocliniside A isolated from *Macroclinidium trilobum* Makino of the Asteraceae family (Kisiel et al., 2000a), and also from some *Lactuca* species of the Lactuceae tribe, such as *Lactuca virosa* (Gromek, 1989), *Lactuca laciniata* (Nishimura et al., 1986) and *Lactuca saligna* (Kisiel et al., 1993).

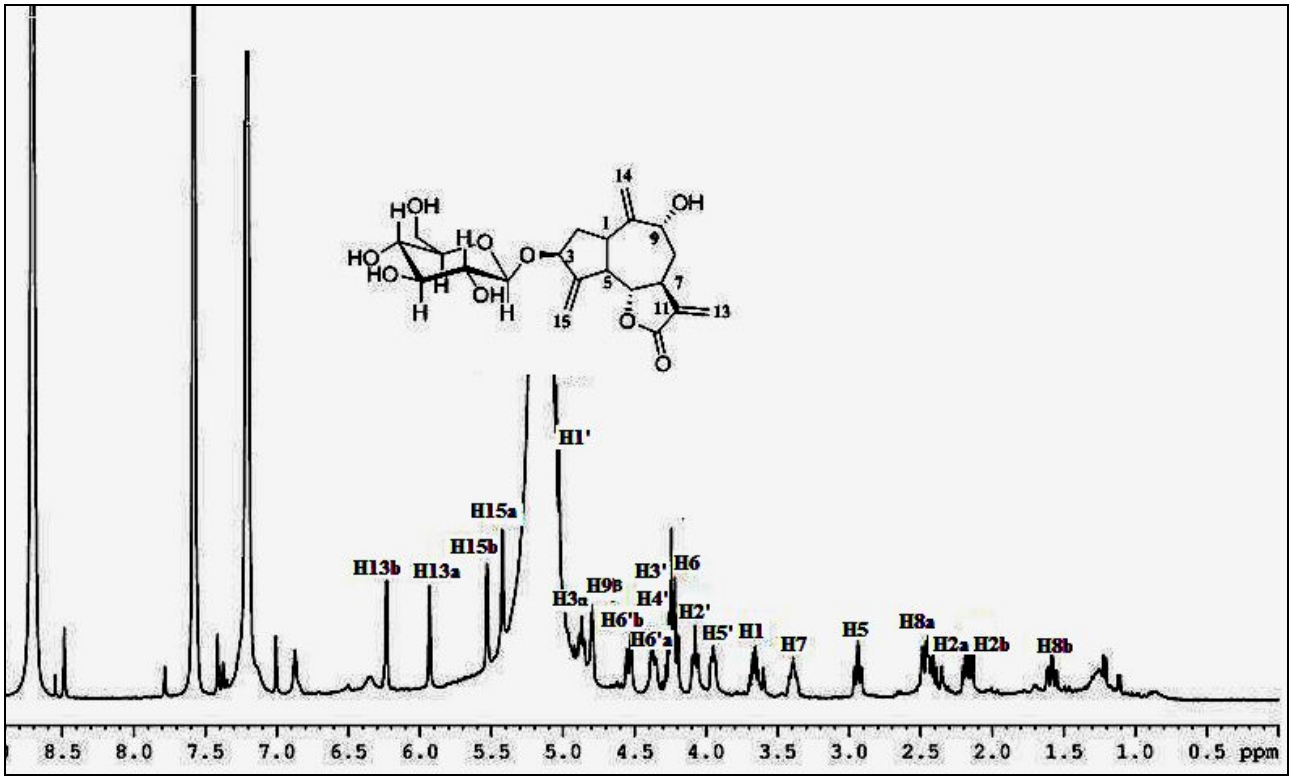


Figure 3.67: ^{1}H NMR spectrum of compound 310 in pyridine-d_5

3.3.2.2.5 Compound 311

Compound **311** was the main component and most polar of the sesquiterpene fraction. The strong absorption bands observed in the UV spectrum of **311** at 250, 212, and 234 nm suggested the presence of carbonyl groups (γ–lactone, carbonyl, and carbonyl ester). Additionally, in the IR spectrum, the absorption band at 3427 cm^{-1} was attributed to the hydroxyl group, while the

absorption bonds at 1776, 1747, and 1689 cm^{-1} confirmed the presence of both an α,β-unsaturated carbonyl group and an ester function. These indicated the presence of an highly functionalised carbon skeleton.

The ^{1}H and ^{13}C NMR spectra **(Figures 3.68**, **3.69)** of compound **311** showed typical signals that well fit a guaianolide skeleton. In particular, compound **311** appeared structurally related to the aglycone of above described compound **309**. The *J*-modulated ^{13}C NMR spectrum displayed 21 carbon signals that were assigned to seven quaternary carbons, five methines, five methylenes and two methyls by HSQC and HMBC experiments **(Figures 3.71**, **3.72**). The presence of the α-methylene-γ–lactone motif was confirmed by signals at δ 169.2 (C-12), 138.5 (C-11) and 119.0 (C-13).

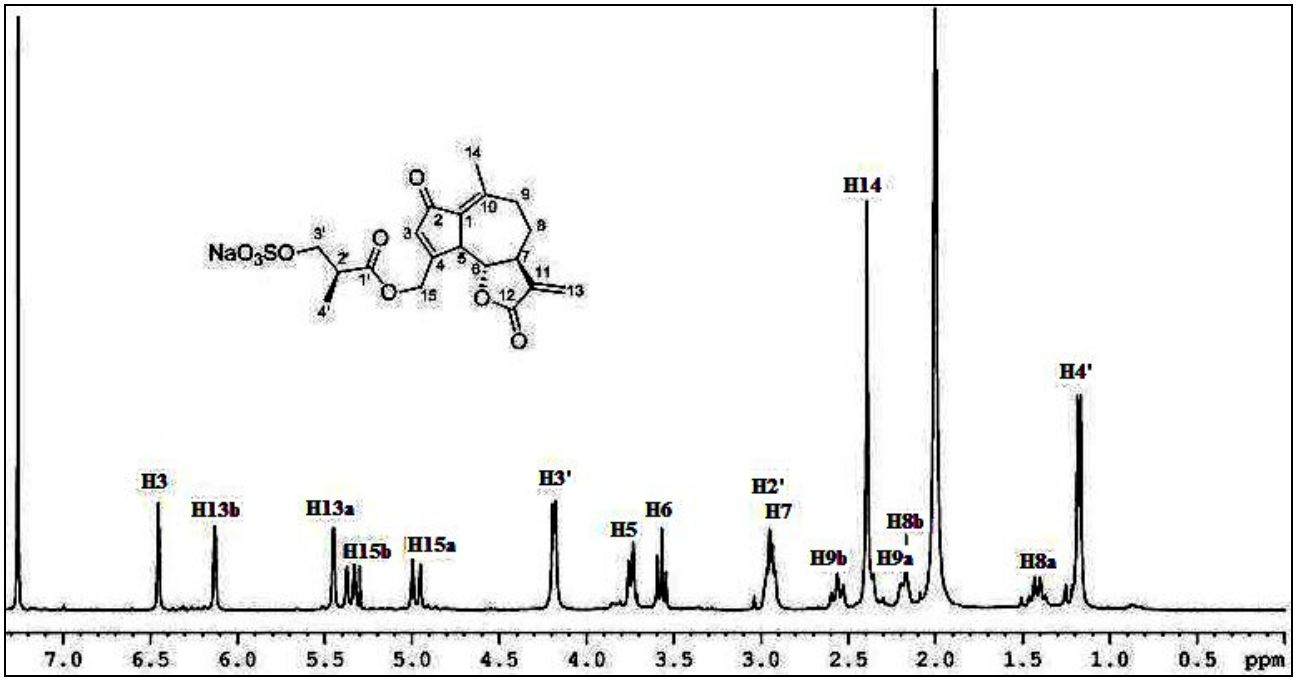


Figure 3.68: ^{1}H NMR spectrum of the compound 311 in $CDCl_3$

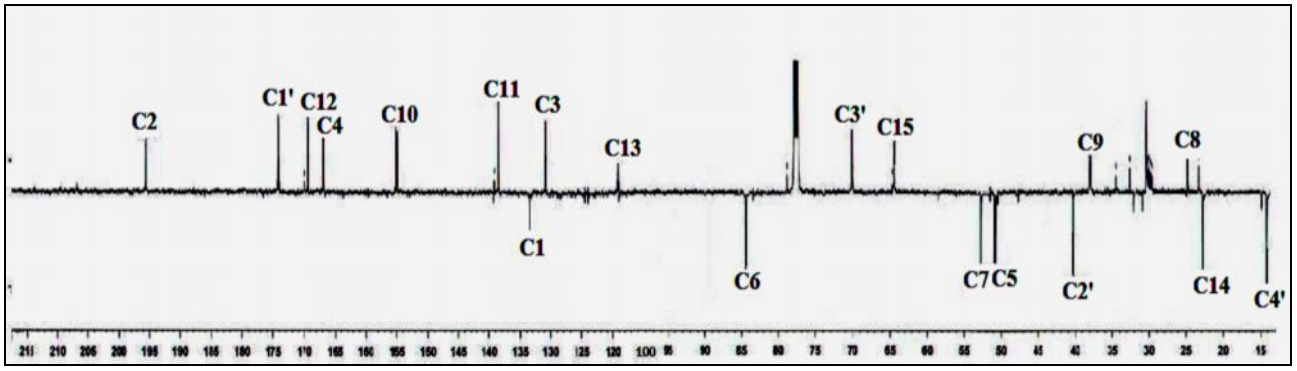


Figure 3.69: ^{13}C J-MOD spectrum of the compound 311 in $CDCl_3$

The ^{1}H-^{1}H COSY spectrum (**Figure 3.70**) contained all cross-peak correlations defining the spin system from H-5 to H_2-9 of the guaianolide skeleton. The coupling constant between H-5 and H-6 as well as between H-6 and H-7 (J = 10.0 Hz) confirmed the *trans* stereochemistry of the ring junction at C-5/C-6.

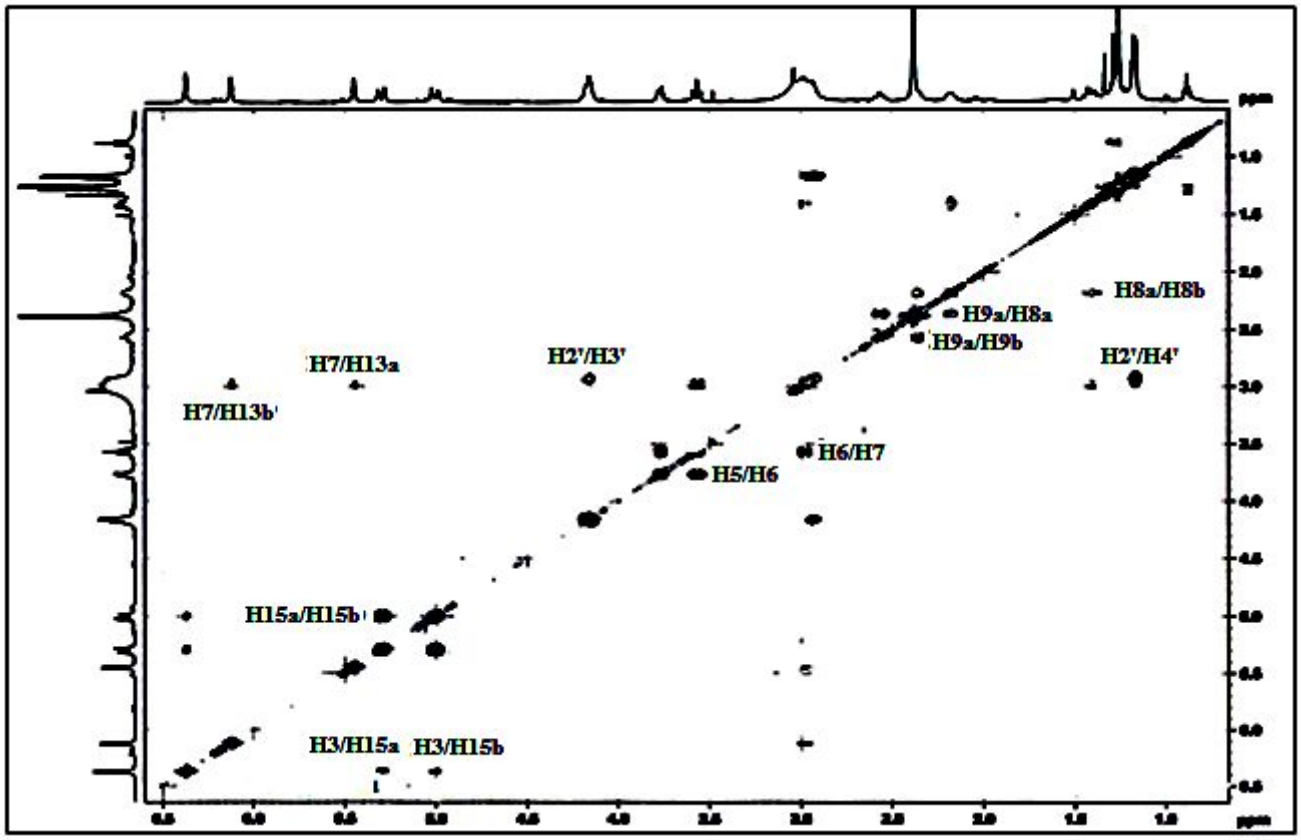


Figure 3.70: ^{1}H-^{1}H COSY spectrum of compound 311 in $CDCl_3$

The proton and carbon spectra of compound **311** showed also the presence of the cyclopentenone moiety bearing the hydroxymethyl residue the same as compounds **307** and **309** and, in addition, an acyl alkyl moiety [δc 174.1(C-1'), 39.5 (C-2 '), 69.3 (C-3'), and 13.5 (C-4 '); δ_H 2.9 (H-2 '), 4.18 (H-3'), 1.17 (Me-4 ')]. This residue had to be linked to the primary hydroxyl group 15-O.

Accordingly, the chemical shift values of hydroxymethyl protons were observed at δ 5.00 and 5.35 (H_2-15), downfield shifted when comparing with the same protons in compound **307**. This structural assumption was also supported by the diagnostic long-range correlations in the HMBC spectrum (**Figure 3.72**) between the ester carboxyl C-1' (δ 174.1) and H_2-15.

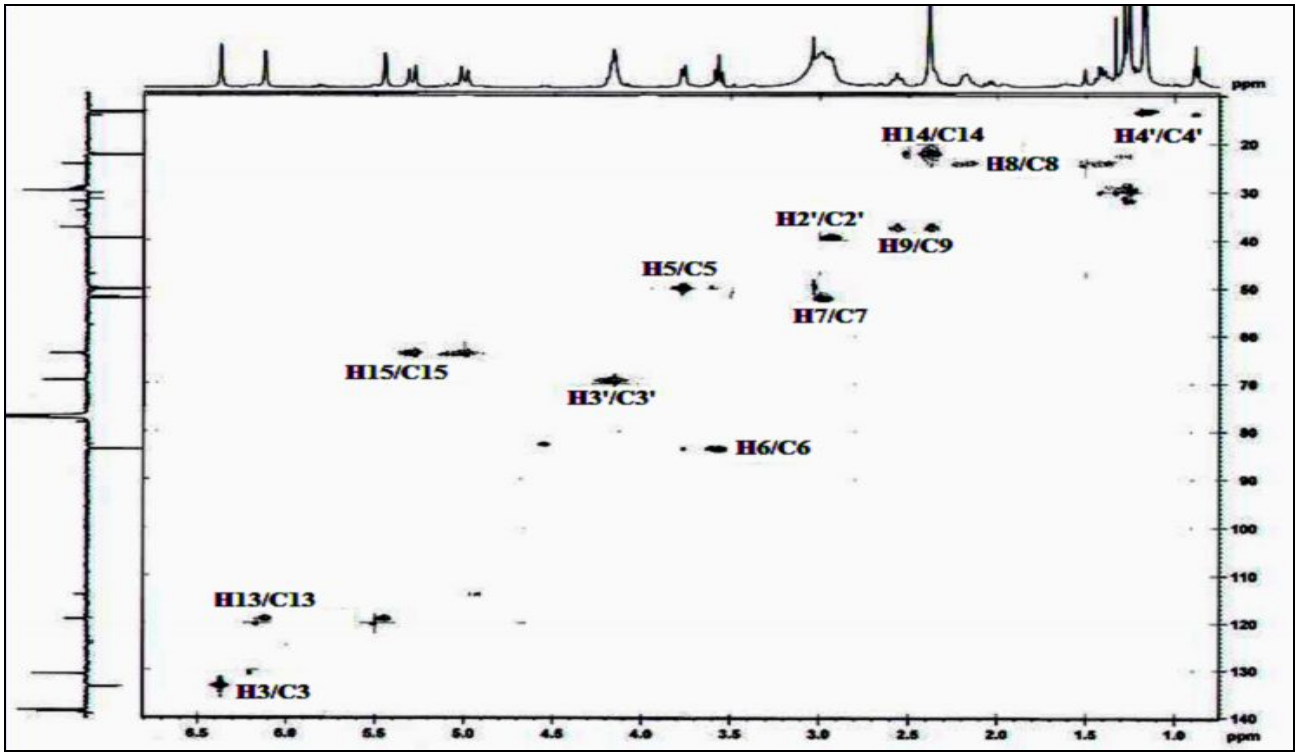


Figure 3.71: HSQC spectrum of compound 311 in $CDCl_3$

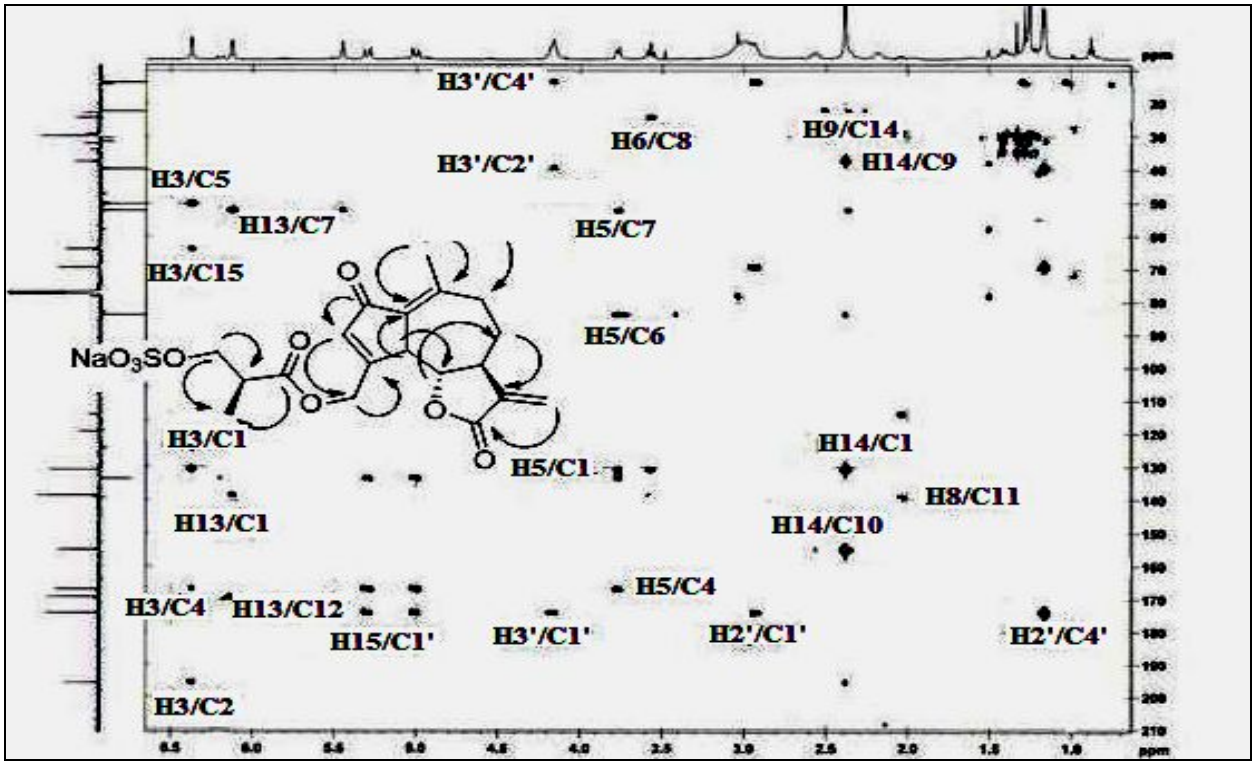


Figure 3.72: HMBC spectrum of compound 311 in $CDCl_3$, and selected correlations

The high polarity of compound **311** and the downfield shifted resonance of C-3' (δc 69.3) compared with the hydroxyl group (δc = ca. 62) in model compounds strongly suggested the presence of a sulphate group which was attached to C-3'.

The ESIMS mass spectrum (recorded in both positive and negative mode) (**Figure 3.73**) contained peaks which were consistent with the molecular formula $C_{19}H_{21}SO_9Na$ (molecular mass 448).

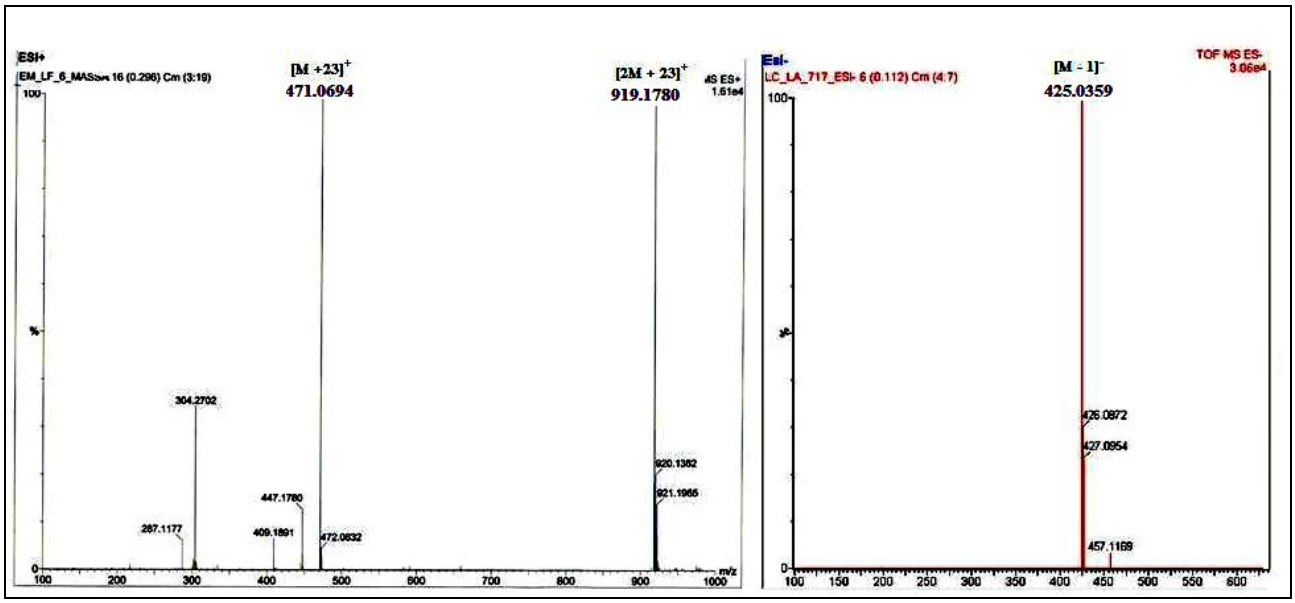


Figure 3.73: ESIMS spectrum of compound 311 in positive and negative mode

These spectral data lead us to identify compound **311** as 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin-3'-sulfate, which has been recently reported (Zidorn et al., 2007). In this paper, the absolute configuration of the chiral centre C-2' of 3'-hydroxy-2'-methyl-propanoyl fragment was not defined, thus we undertook a stereochemical study with the aim of clarifying this structural aspect.

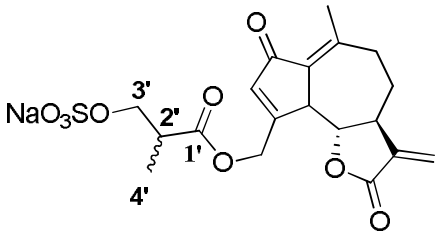
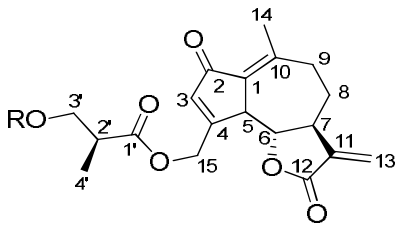


Table 3.7: ^{1}H and ^{13}C NMR data of conpound 311 in $CDCl_3$

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC[c] (C to H)
1	130.8	-		H-3, H-5, H-14
2	195.5	-		H-3
3	133.5	6.37	s	H-15
4	166.9	-		H-3, H-5, H-15
5	50.0	3.77	d (9.95)	H-3
6	83.8	3.58	t (10.0)	H-5
7	52.0	2.90	m	H-5, H-9, H-13
8	24.1	1.40	m	H-6, H-9
		2.15	m	-
9	37.3	2.35	m	H-8
		2.56	m	
10	154.9	-		H-9, H-14
11	138.6	-		H-7, H-13
12	169.3	-		H-13
13	119.0	5.45	s	-
		6.12	s	
14	22.2	2.44	s	-
15	63.8	5.00	d (17.6)	H-3
		5.35	d (17.6)	
Ester moiety				
1'	174.2	-		H2', H-3', H-4', H-15
2'	39.5	2.90	m	H-3'
3'	69.3	4.18	m	H-2'
4'	13.5	1.17	d (7.0)	H-2', H-3'

3.4.2.2.6 Determination of the absolute stereochemistry of compound 311

The stereochemistry at the side chain of the compound **311** was assigned by comparison of the ^{1}H NMR spectra of Mosher derivatives with those of the corresponding MTPA esters of model compounds (Seco et al., 2004).



311a R = H
311b R = Ac
311c R = *S*-MTPA
311d R = *R*-MTPA

The first step was the methanolysis of compound **311** under acid conditions to give the corresponding alcohol **311a**, the HRESIMS and ^{1}H NMR spectra of which are depicted in **Figures 3.74 and 3.75**.

The HRESIMS spectrum (**Figure 3.74**) displayed the sodiated molecular peak at *m/z* 369.1298 consistently with the molecular formula $C_{19}H_{22}O_6$. The ^{1}H NMR spectrum (**Figure 3.75**) showed the signals attributed to the hydroxyl methylene H_2-3' at δ 3.78, which were up-field shifted with respect to the corresponding signals in compound **311** due the hydrolysis of sulphate ester group.

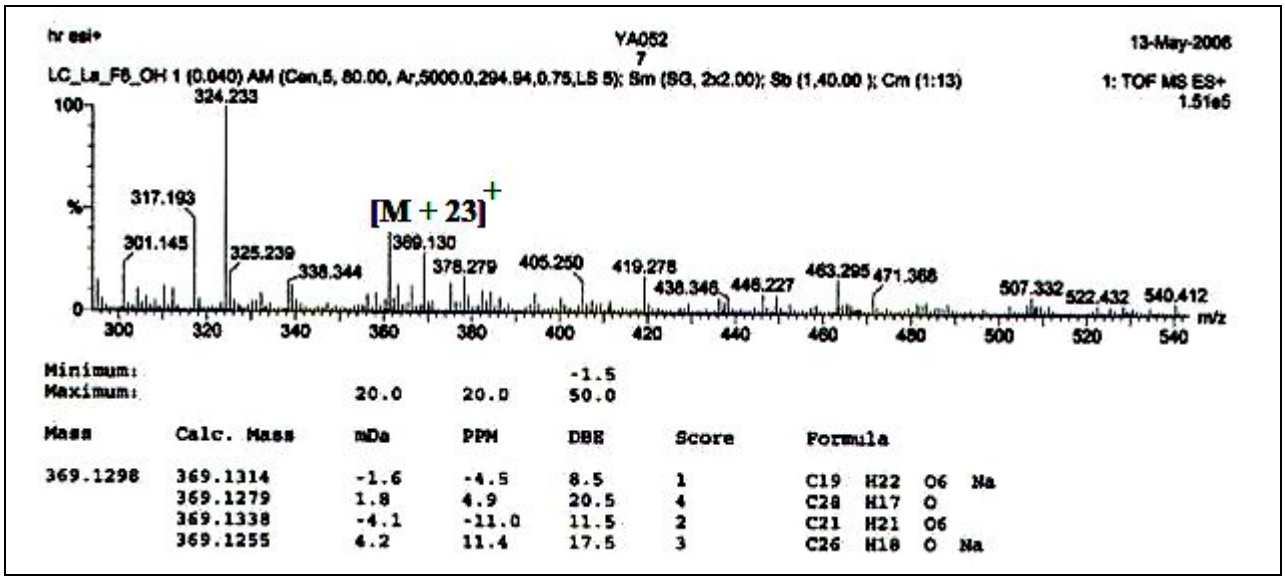


Figure 3.74: HRESIMS spectrum of compound 311a

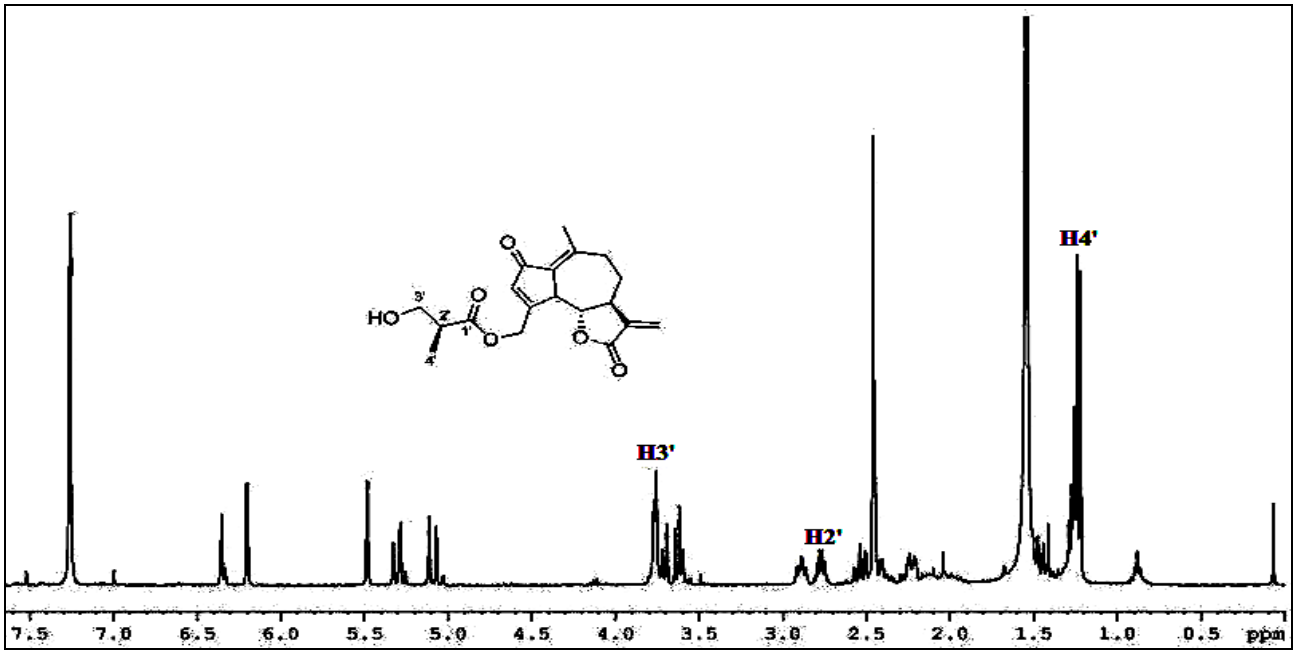


Figure 3.75: ^{1}H NMR spectrum of compound 311a in $CDCl_3$

The acetyl derivative **311b** was fully characterized by ESIMS and 2D NMR experiments. The ESIMS spectrum (**Figure 3.76**) showed the sodiated molecular peak at *m/z* 411, indicating the molecular formula $C_{21}H_{24}O_7$.

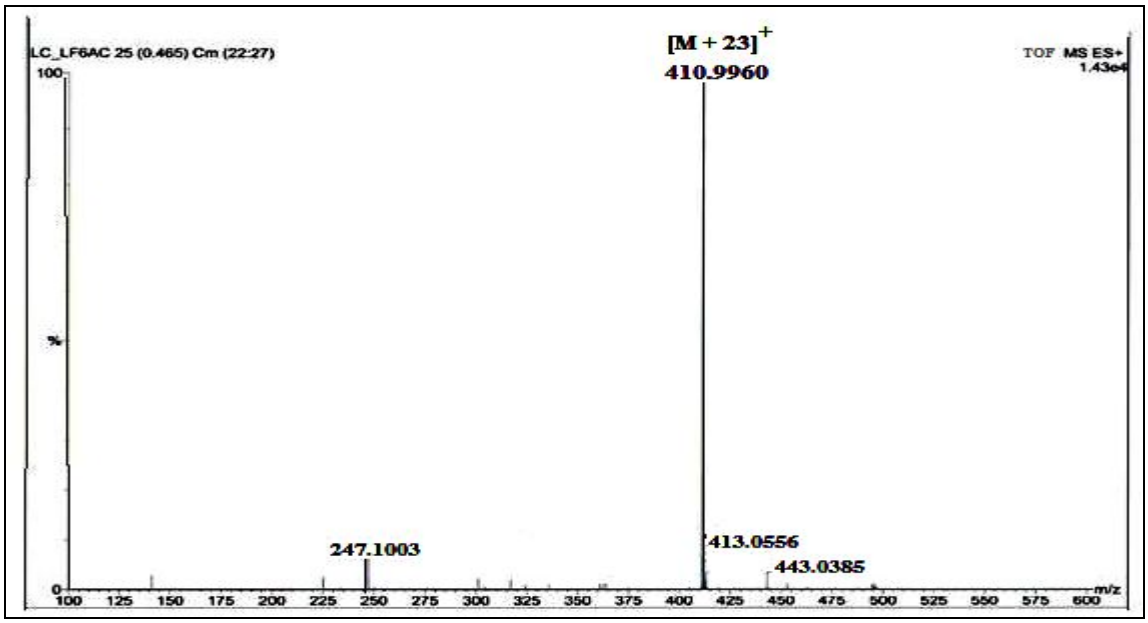


Figure 3.76: ESIMS spectrum of compound 311b

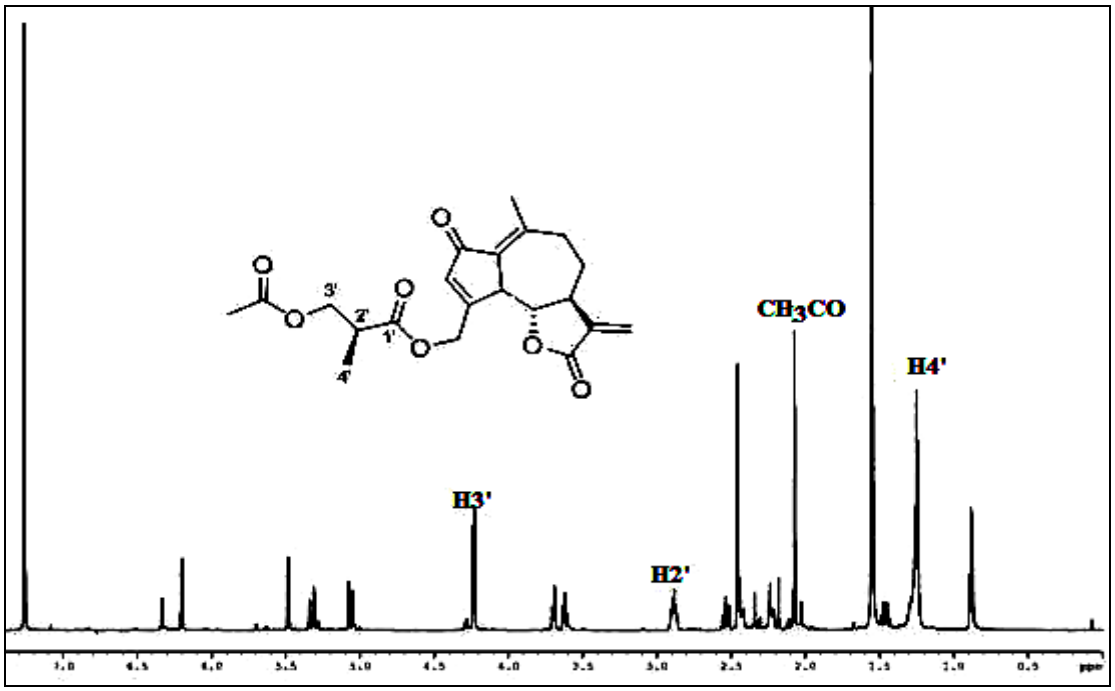


Figure 3.77: ^{1}H NMR spectrum of compound 311b in $CDCl_3$

All NMR assignment of compound **311b** are summarized in **Table 3.8**.

Table 3.8: ^{1}H and ^{13}C NMR data of conpound 311b in $CDCl_3$

Position	$\delta^{13}C$	$\delta\ ^{1}H$	m *J* (Hz)	HMBC (C to H)
1	130.8	-		H-3, H-5, H-14
2	195.5	-		H-3
3	133.5	6.32	s	H-15
4	166.9	-		H-3, H-5, H-15
5	50.0	3.70	d (10.0)	H-3
6	83.8	3.58	t (10.0)	H-5
7	52.0	2.85	m	H-5, H-9, H-13
8	24.1	1.45	m	H-6, H-9
		2.22	m	-
9	37.3	2.24	m	H-8
		2.56	t (13.0)	
10	154.9	-		H-9, H-14
11	138.6	-		H-7, H-13
12	169.3	-		H-13
13	119.0	5.48	s	-
		6.20	s	
14	22.2	2.44	s	-
15	63.8	5.05	d (17.5)	H-3
		5.35	d (17.5)	
Ester moiety				
1'	174.2	-		H2', H-3', H-4', H-15
2'	39.5	2.90	m	H-3'
3'	69.3	4.18	m	H-2'
4'	13.5	1.17	d (7.0)	H-2', H-3'
Acetyl moiety				
	21.5	2.07	s	H-3', CH_3CO
	171.0			

Compound **311a** was allowed to react with *R*-(-) and *S*-(+)-α-methoxy-α-trifluoromethyl-phenylacetic acid chlorides to obtain the *S*- and *R*-MTPA ester derivatives **311c** and **311d**, respectively. In **Figure 3.78** is depicted the ESIMS spectrum of **311c** with the sodiated molecular peak at *m/z* 585 $[M + 23]^+$ that was in agreement with the molecular formula $C_{29}H_{29}O_8$.

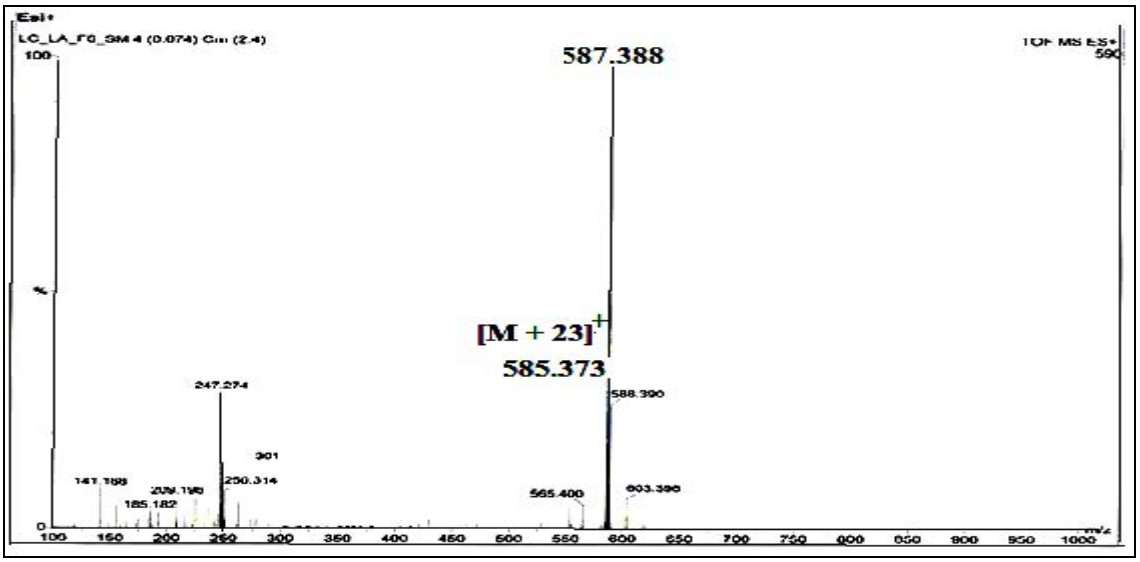


Figure 3.78 ESIMS spectrum of compound 311c

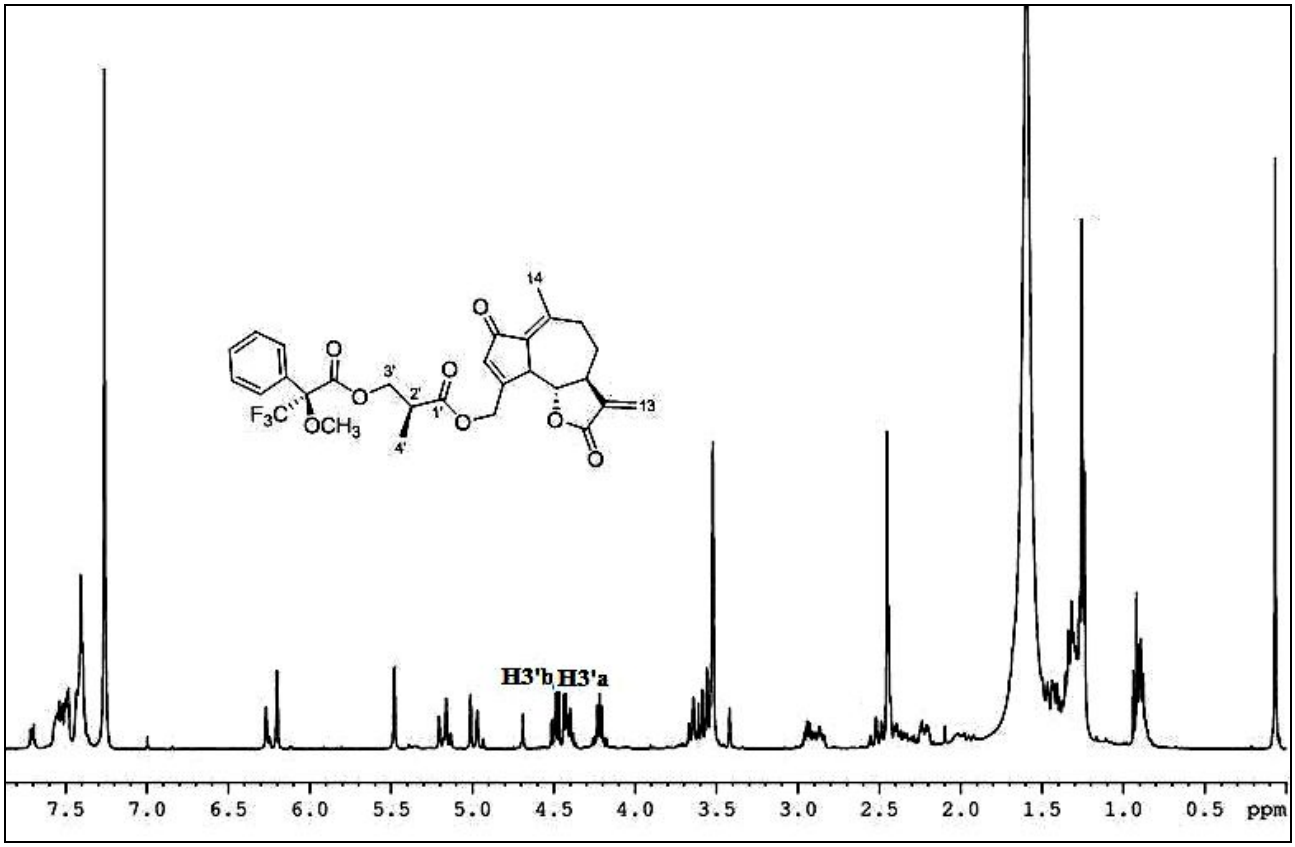


Figure 3.79: ^{1}H NMR spectrum of compound 311c (S-MTPA ester) in $CDCl_3$

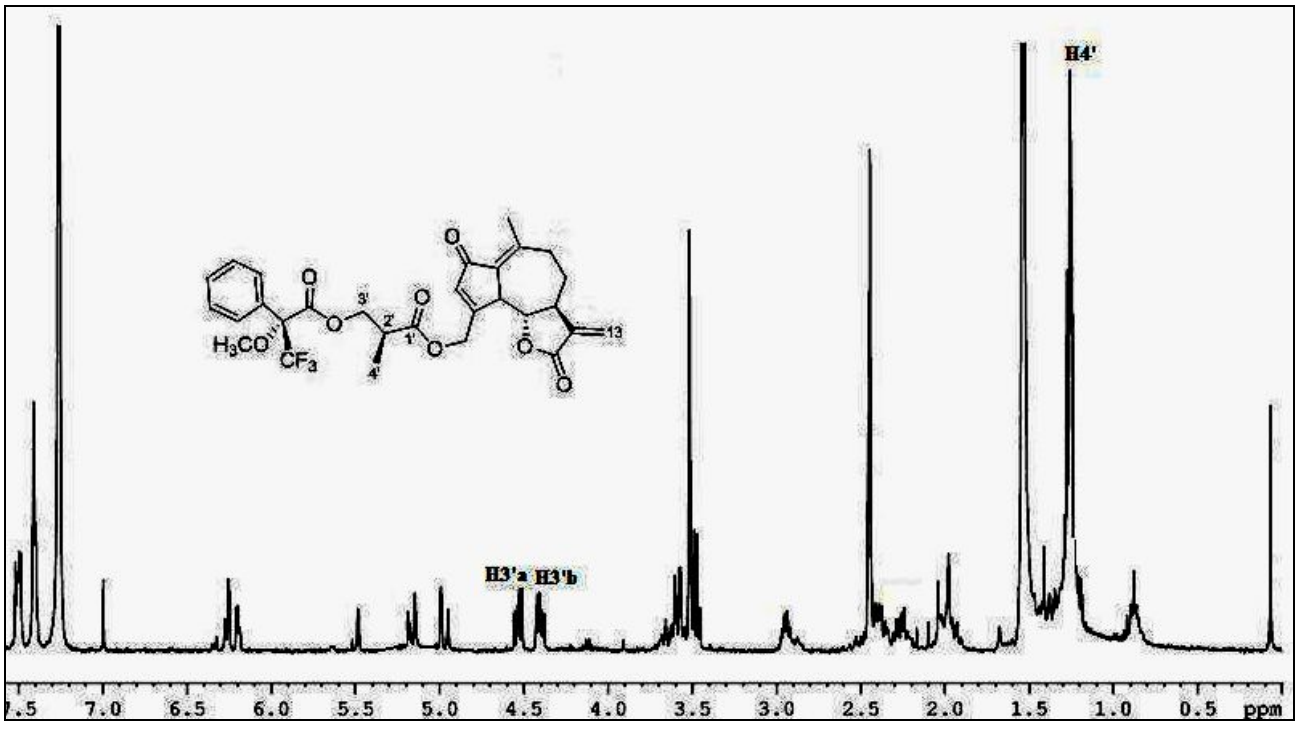


Figure 3.80: ^{1}H NMR spectrum of compound 311d (R-MTPA ester) in $CDCl_3$

The ^{1}H NMR spectra (**Figures 3.79** and **3.80**) of the two Mosher derivatives were slightly different with regards to the multiplet pattern due to H_2-3' methylene being this latter influenced by the chirality of the Mosher acyl residue attached.

The second step was to repeat the same Mosher reaction on both commercial methyl-(*S*)-(+)-3-hydroxy-2-methyl-propionate **(I)** and methyl-(*R*)-(-)-3-hydroxy-2-methylpropionate **(II).** These two reactions afforded two pairs of MTPA derivatives, (S)/(S)-MTPA ester **(Ia)**, (*S*)/(*R*)-MTPA ester **(Ib)**, (*R*)/(*S*)-MTPA ester **(IIa)** and (*R*)/(*R*)-MTPA ester (**IIb) (Figures 3.82**, **3.83**, **3.84**, **3.85)**.

All ESIMS spectra (in **Figure 3.81** is depicted one of them) of Mosher derivatives of **I** or **II** showed the sodiated molecular peak at *m/z* 357 $[M + 23]^+$, consistent with the molecular formula $C_{15}H_{17}O_5F_3$.

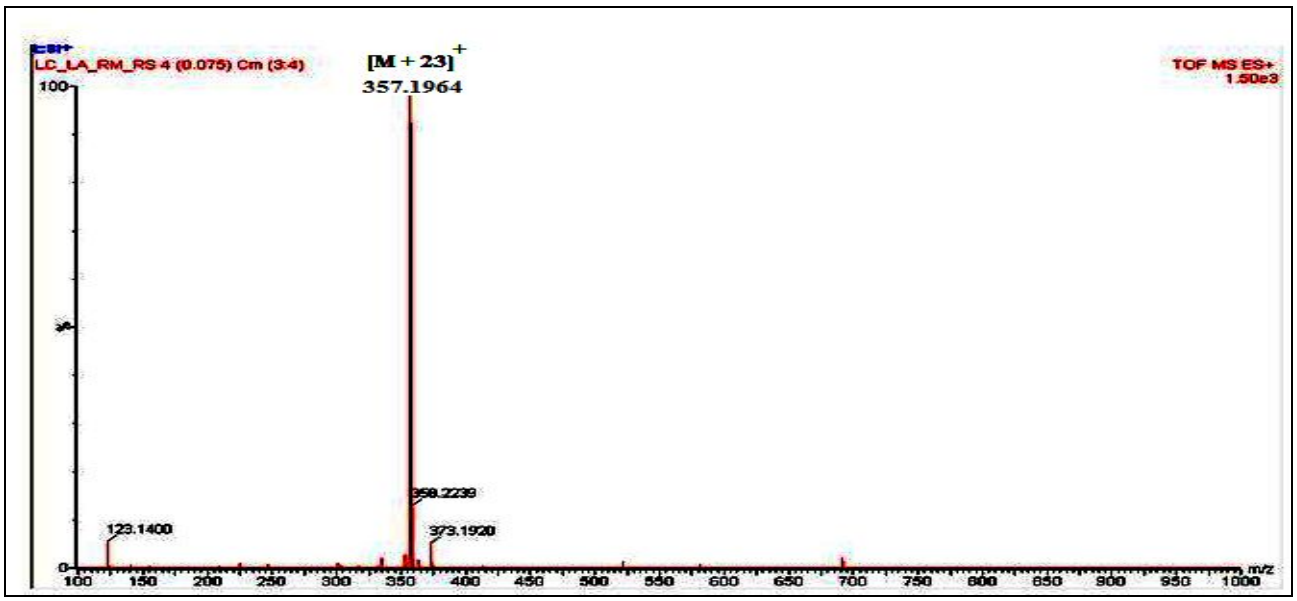


Figure 3.81: ESIMS mass spectrum of a Mosher ester of compound I (or II)

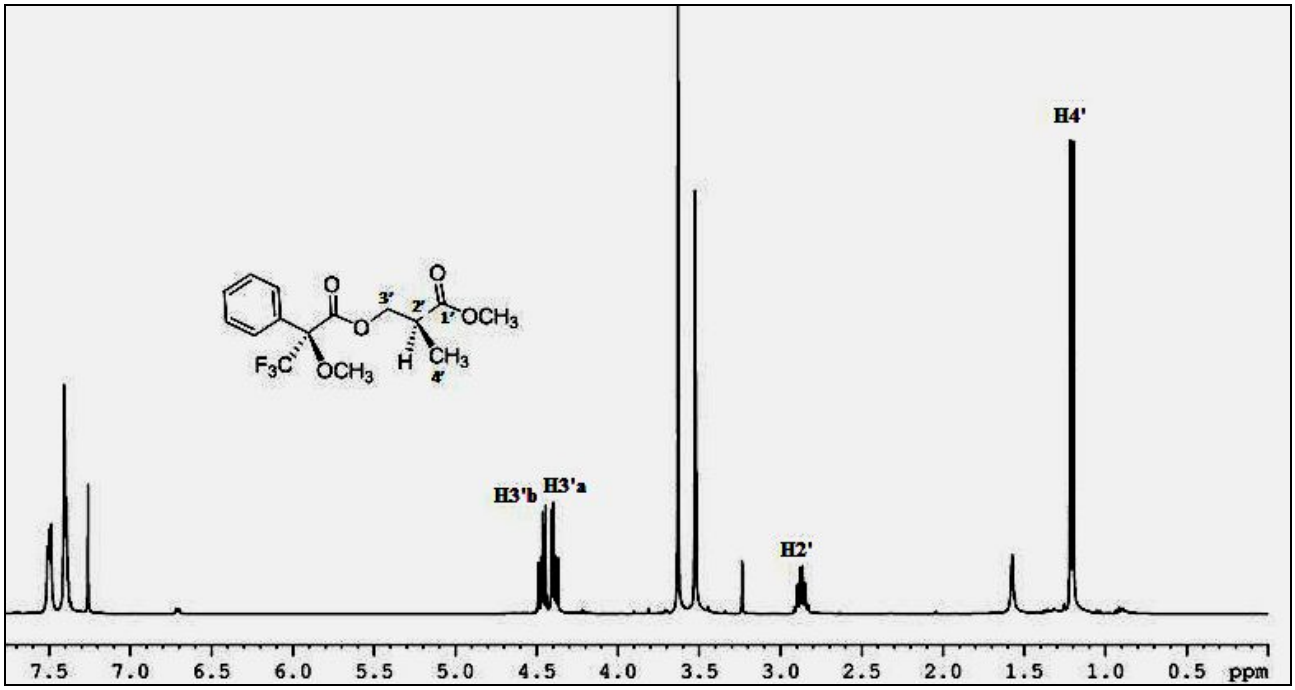


Figure 3.82: ^{1}H NMR spectrum of the compound Ia (S/S-MTPA ester) in $CDCl_3$

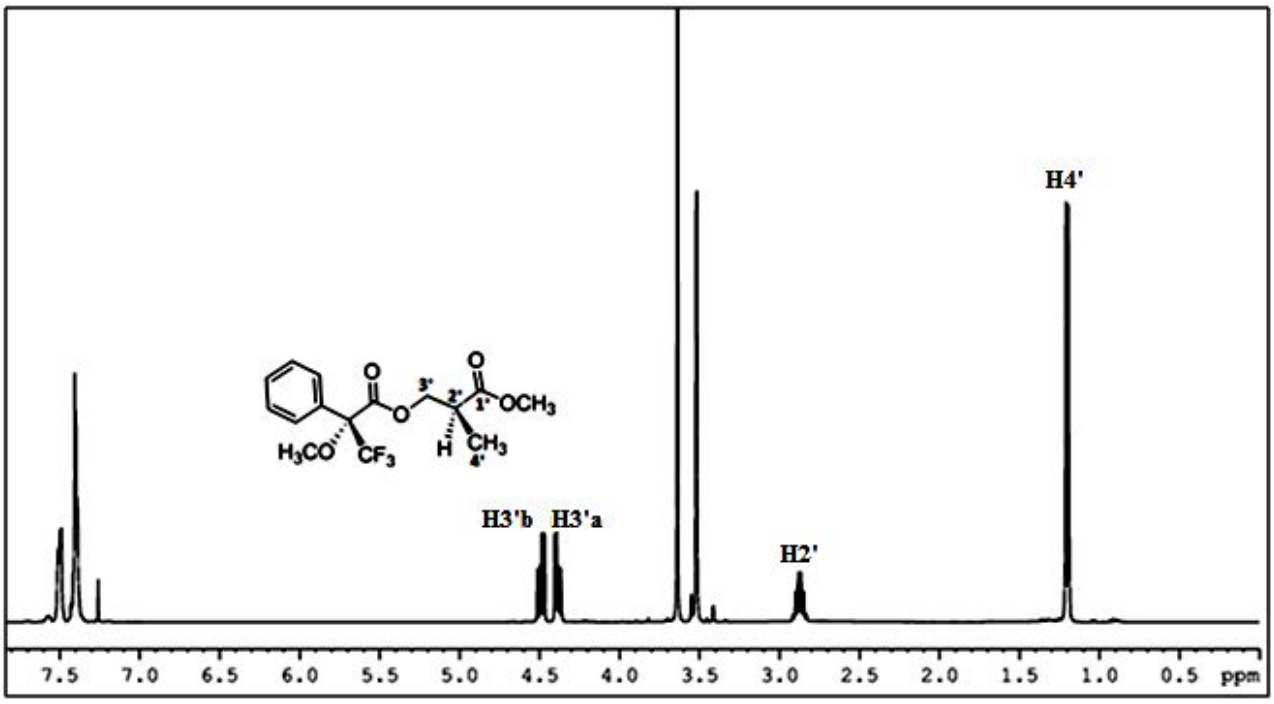


Figure 3.83: ^{1}H NMR spectrum of compound Ib (S/R-MTPA ester) in $CDCl_3$

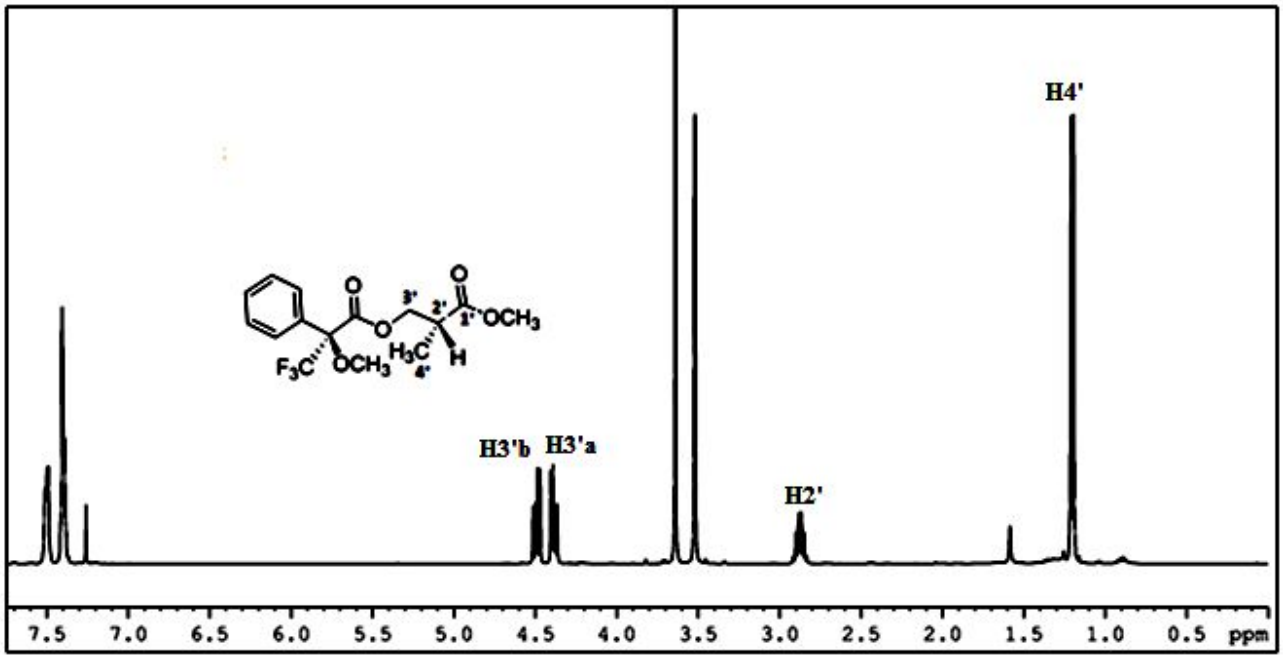


Figure 3.84: ^{1}H NMR spectrum of compound IIa (R/S-MTPA ester) in $CDCl_3$

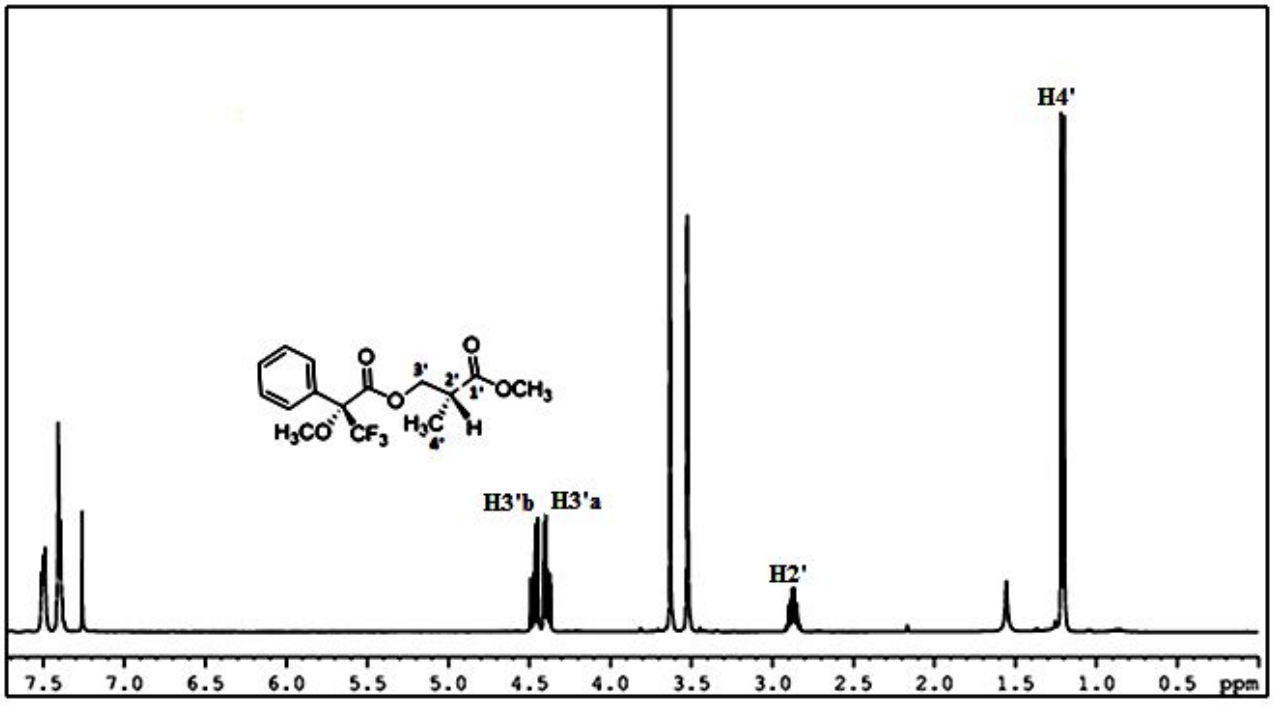


Figure 3.85: ^{1}H NMR spectrum of compound IIb (R/R-MTPA ester) in $CDCl_3$

Analysis of the ^{1}H NMR spectra of the two esters showed significant differences in the multiplet patterns of H_2-3' (**Figure 3.86**).

Comparison of the ^{1}H NMR spectra of each pair with those of *S*- and *R*-MTPA esters **311c** and **311d,** clearly showed that H_2-3' multiplet patterns of **311c** and **311d** were the same as the pair **Ia** and **Ib**, thus inferring the *S* absolute stereochemistry at C-2' of compound **308** (**Figure 3.86**).

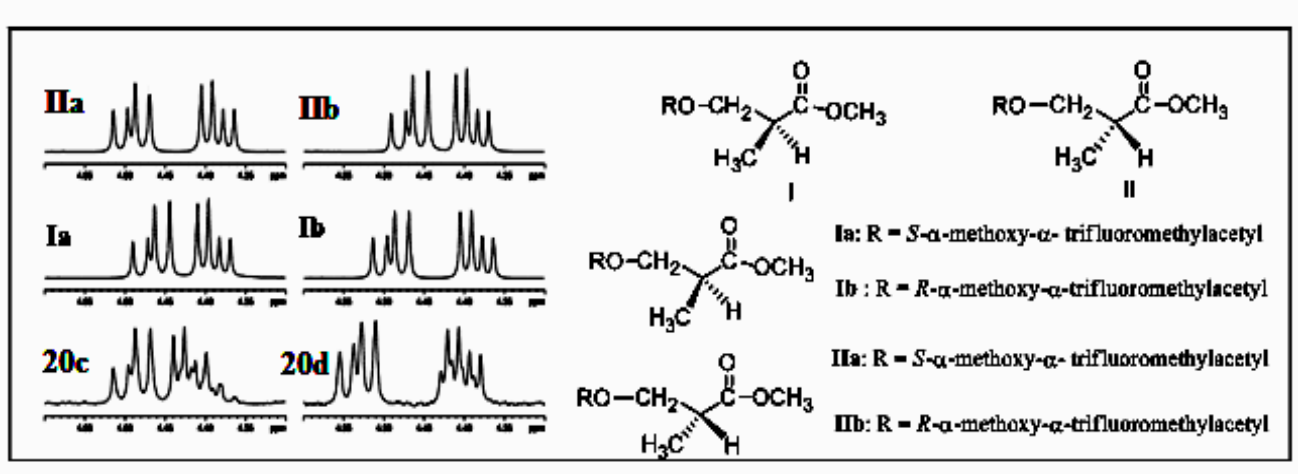


Figure 3.86: ^{1}H NMR signals (400 MHz; $CDCl_3$) due to the methylene protons at C-3 for the compounds Ia, IIa, Ib and IIb

3.4.2.3 STRUCTURE ELUCIDATION OF EUDESMANOLIDE COMPOUNDS

3.4.2.3.1 Compound 312

Compound **312** was obtained as colourless prisms. The ESIMS spectrum showed the sodiated molecular peak at *m/z* 271 $[M + Na]^+$, which indicated the molecular formula $C_{15}H_{20}O_3$.

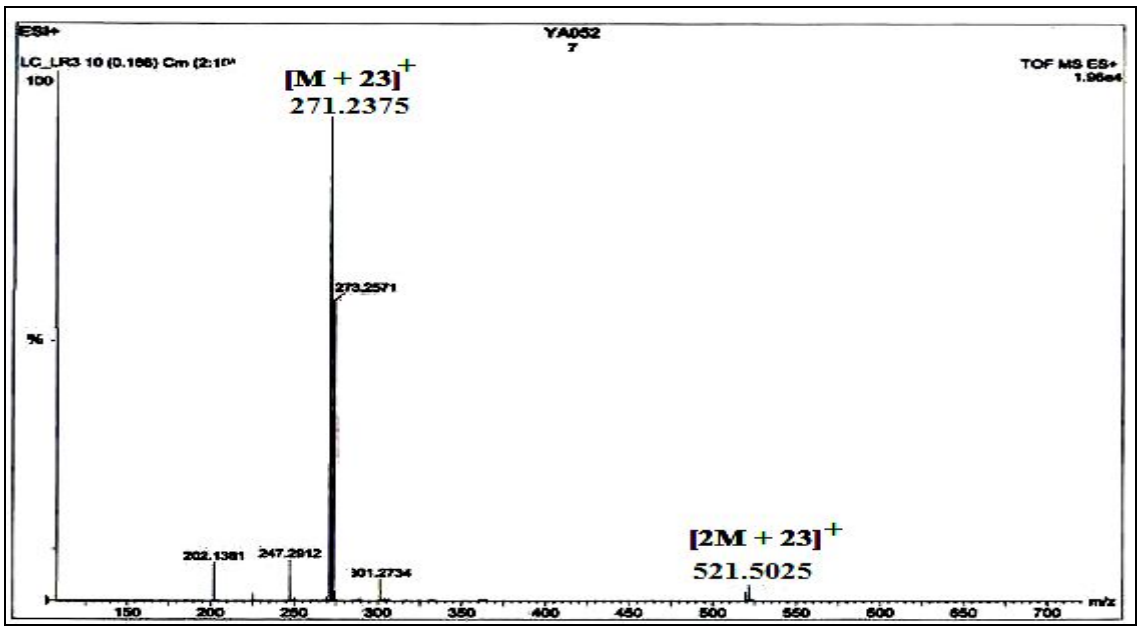


Figure 3.87: ESIMS spectrum of compound 312

The ^{1}H NMR spectrum of compound **312 (Figure 3.88)** revealed characteristic signals of an eudesman-12,6-olide with Δ^{4}-double bond. The spectrum showed a sharp singlet for the angular methyl group at δ 1.10 (H_3-14), a broad singlet for a vinyl methyl group at δ 1.86 (H_3-15), a broad doublet at δ 4.56 (*J* = 11.6 Hz, H-6) attributed to an oxygenated proton, the exocyclic methylene proton doublets at δ 5.47 (*J* = 3.0 Hz, H-13a) and δ 6.15 (*J* = 3.2 Hz, H-13b) and a methine proton multiplet at δ 3.55, attributed to proton H-1. The ^{13}C NMR spectrum carried out in $CDCl_3$ (**Figure 3.89**) was consistent with the assigned structure showing two methyl signals at δ 18.4 (C-14) and δ 19.6 (C-15), four methylene signals at δ 23.1 (C-8), 27.1 (C-2), 33.2 (C-3) and 38.1 (C-9), three methine signals at δ 49.6 (C-7), 77.7 (C-1), 83.1 (C-6), a quaternary carbon at δ 41.1 (C-10), three quaternary sp^2 carbons at δ 126.3 (C-4), 128.9 (C-5) and 139.1 (C-11), an exocyclic methylene signal at δ 118.5 (C-13) and a carbonyl signal at δ 170.3 (C-12).

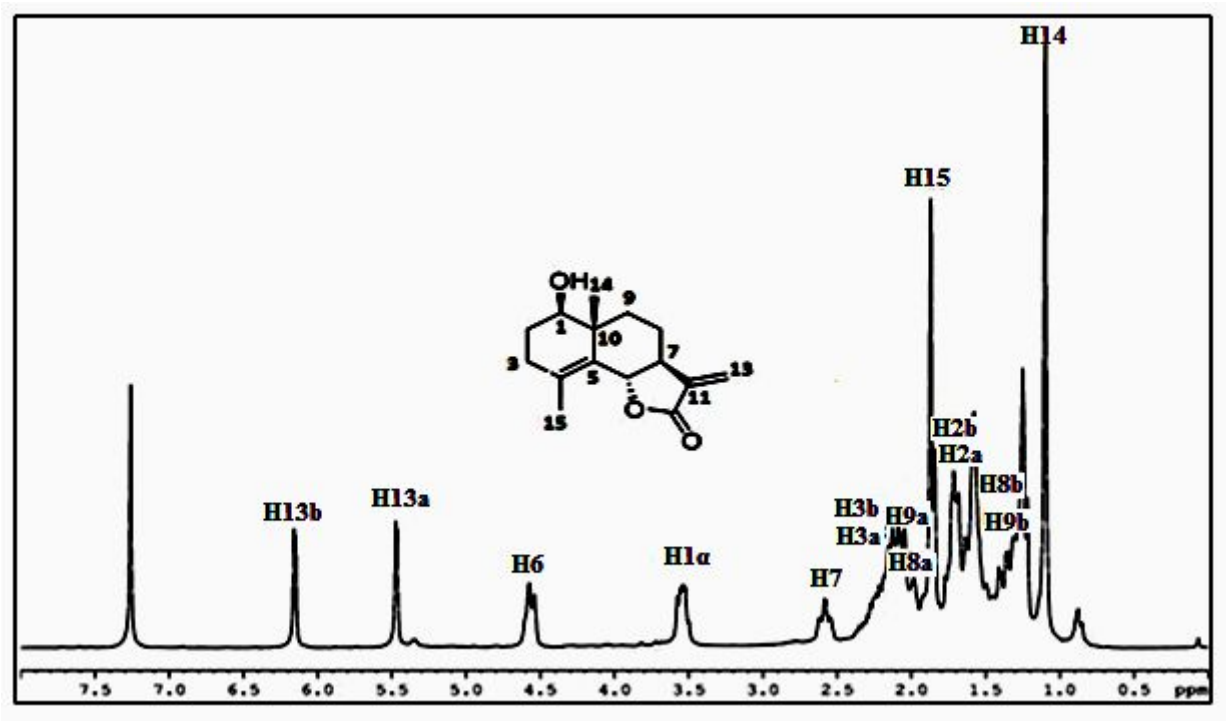


Figure 3.88: ^{1}H NMR spectrum of compound 312 in $CDCl_3$

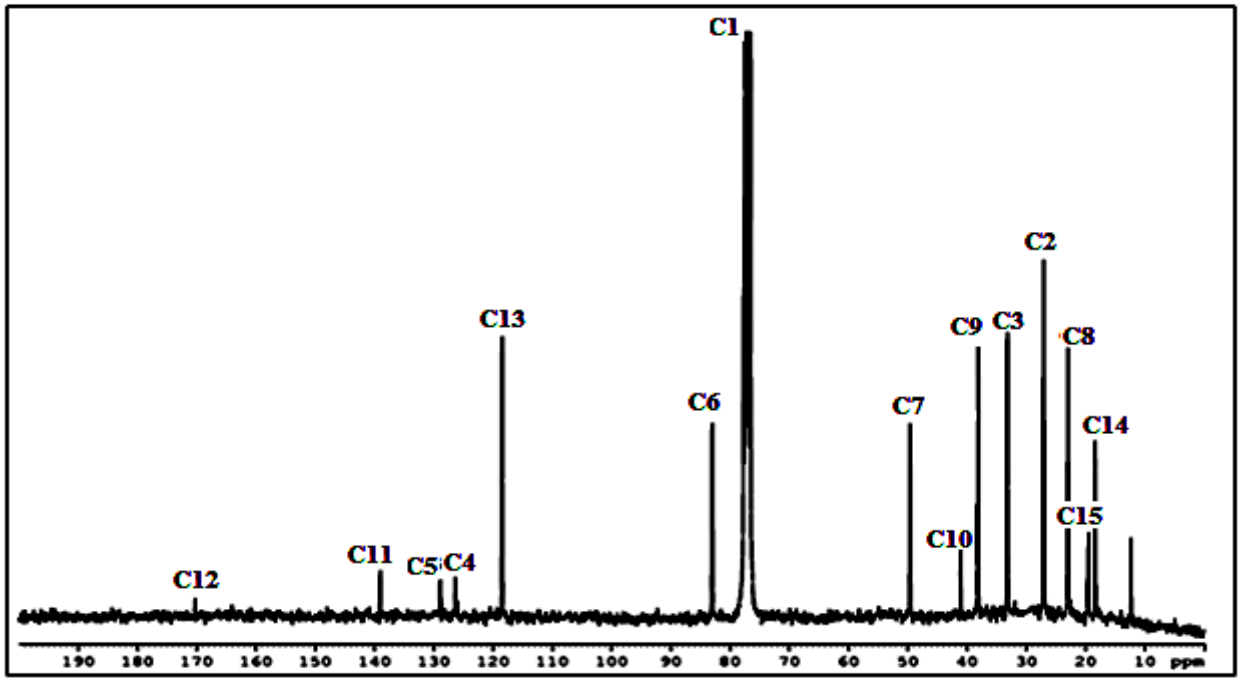


Figure 3.89: ^{13}C NMR spectrum of compound 312 in $CDCl_3$

The proton and carbon assignment of compound **312** was made by interpretation of ^{1}H-^{13}C correlations (HSQC) (**Figure 3.90**) and confirmed by comparison of the spectral data with those reported in literature (Kisiel et al., 2001a), (Deng et al., 2001).

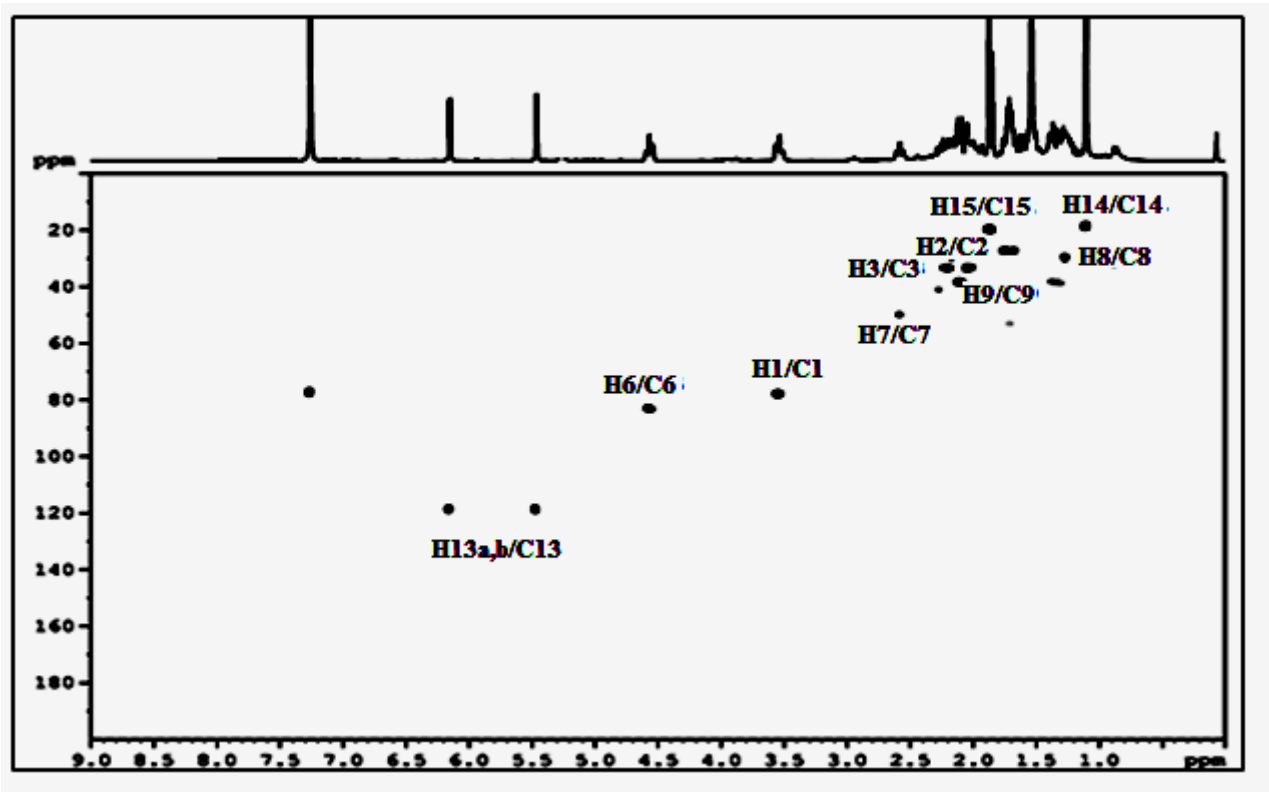


Figure 3.90: HSQC spectrum of compound 312 in $CDCl_3$

The coupling constant of protons H-6 and H-7 ($J_{6,7}$ = 11.5 Hz) was in good agreement with the *trans* junction as reported in literature for most of natural eudesmanolides. The ^{13}C NMR values of carbons C-1, C-2 and C-3 were consistent with the β-orientation of the OH group attached at C-1. Finally, the positive sign of the optical rotation value of compound **312** ($[\alpha]_D$ = + 67° (c 0.35, $CHCl_3$) in comparison with the literature value ($[\alpha]_D$ = + 74° (C = 0.23, $CHCl_3$) indicated the same configuration as that reported (El-Feraly et al., 1979).

From the above data, compound **312** was identified as magnolialide (1β-hydroxyeudesm-4(5)-ene-12, 6α-olide), which was isolated from *Magnolia grandiflora* 30 years ago. The structure proposed was confirmed by comparison with the synthetic product obtained by cyclization of costunolide-1,10 epoxide (El-Feraly et al., 1979).

Compound **312** was also isolated from the roots of *Chicorium intybus* (Chicory) of the Lactuceae tribe of Asteraceae family (Kisiel et al., 2001a), (Deng et al., 2001).

3.4.2.3.2 Compound 313

The molecular formula $C_{15}H_{22}O_4$ of compound **313** was deduced by the ESIMS spectrum (**Figure 3.91**) containing the sodiated molecular peak at *m/z* 289 $[M + Na]^+$, 18 mass units more than magnolialide **312**.

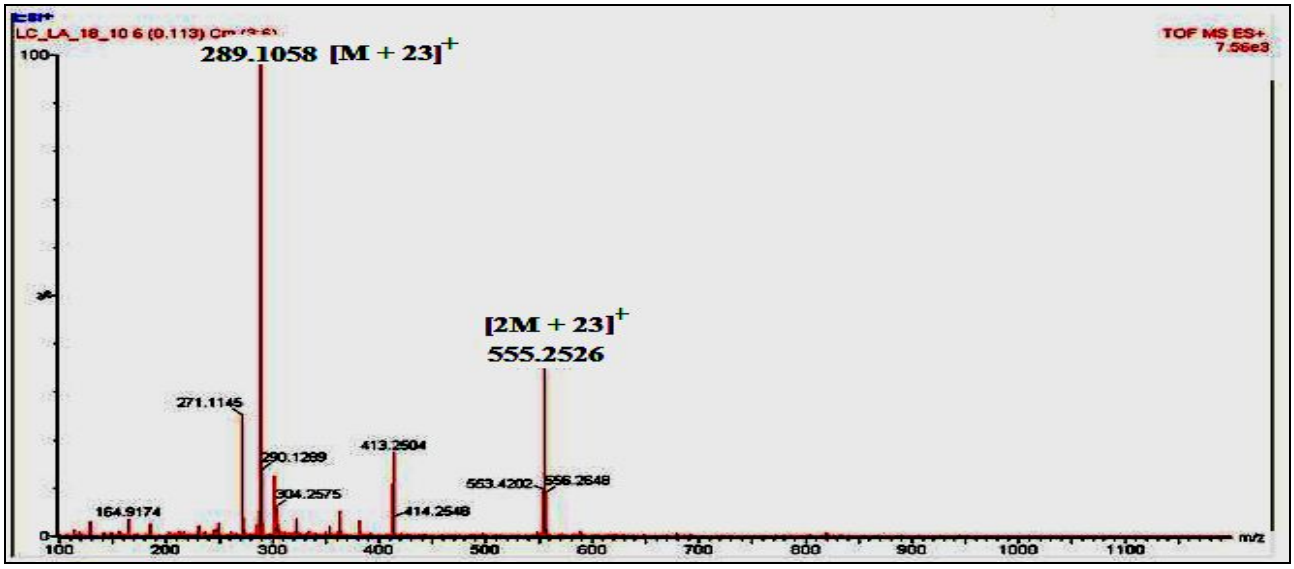


Figure 3.91: ESIMS spectrum of compound 313

The analysis of the ^1H NMR spectrum of compound **313** (**Figure 3.92**) showed the presence of structural features similar to compound **312** according to the same Δ^4-eudesmanolide skeleton. The differences consisted in an additional secondary hydroxyl function in the ring B [δ 3.88 (1H, dt, *J* = 10.5 and 4.5 Hz, H-8)] and a secondary methyl group in the lactone cycle [δ 1.33 (3H, d, *J*

= 6.7 Hz, H_3-13)] replacing the exomethylene group. The coupling constant value of H-8 suggested the equatorial orientation of the hydroxyl group.

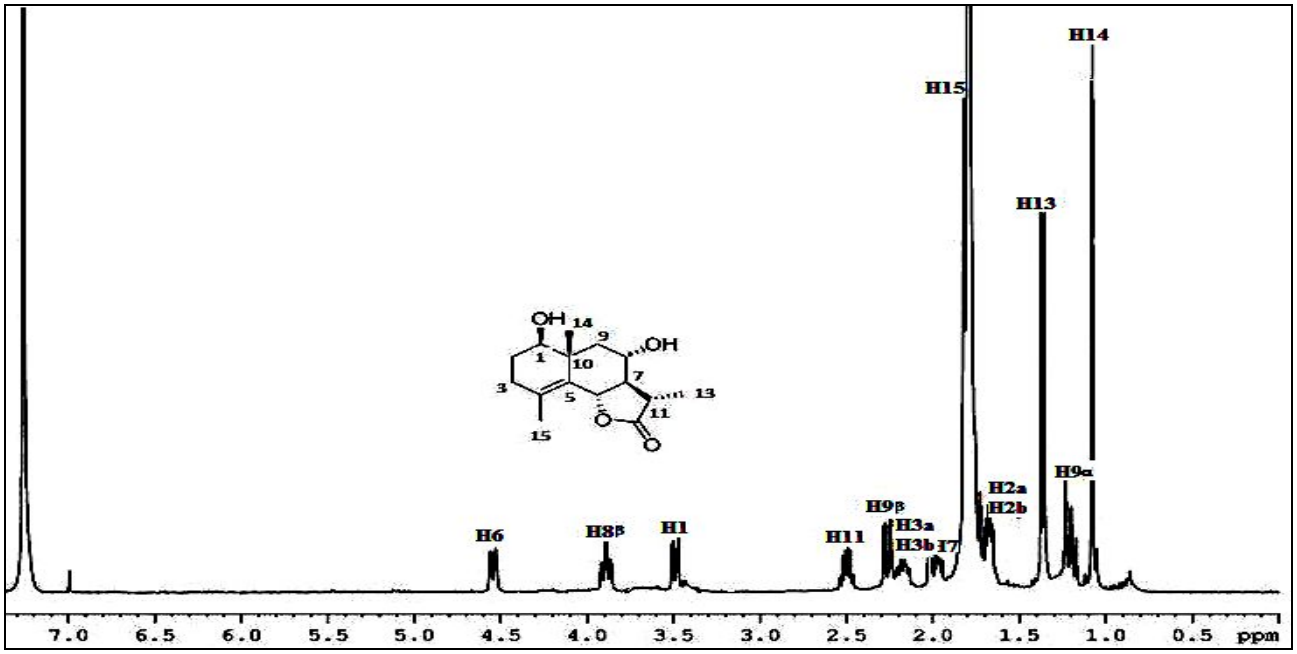


Figure 3.92: ^{1}H NMR spectrum of the compound 313 in $CDCl_3$

A search in the literature data led us to identify compound **313** as 1β,8α-dihydroxyeudesm-4-en-6β-7α,11βH-6-olide, which was previously reported from the aerial parts of *Artimisia herba–alba* from Asteraceae family (Marco, 1989).

3.4.2.4 STRUCTURE ELUCIDATION OF GERMACRANOLIDE COMPOUNDS

3.4.2.4.1 Compound 314

Compound **314** is a new natural compound. The molecular formula $C_{29}H_{36}O_{11}$ was deduced by the HRESIMS spectrum (**Figure 3.93**) that showed the sodiated molecular peak at *m/z* 560.2155.

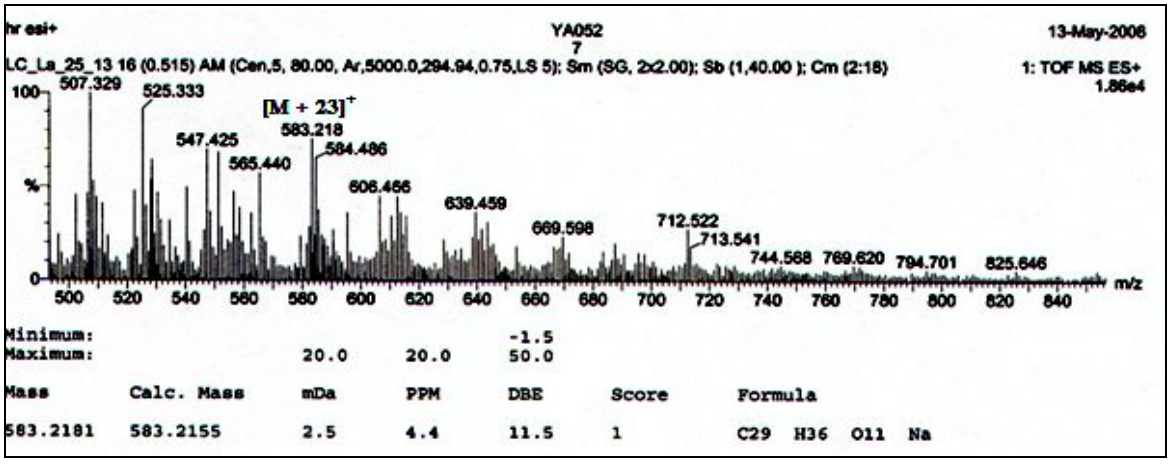


Figure 3.93: HRESIMS spectrum of compound 314

The IR spectrum suggested the presence of hydroxyl groups (3393 cm^{-1}), ester groups (1738 cm^{-1}) and double bonds (1616, 1516 cm^{-1}).

Analysis of the ^{1}H and ^{13}C NMR data of compound **314** and comparison with literature data of cyclic sesquiterpenoids strongly suggested a glucosyl functionalised germacrane framework. In particular, the NMR spectra (**Figures 3.94**, **3.95**, **3.96**) indicated the following structural features: two trisubstituted double bonds [δ_C 131.3 (C-1), 127.4 (C-5), 140.8 (C-4), 135.9 (C-10); δ 5.05 (1H, overlapped, H-1), 5.02 (1H, overlapped, H-5)], a vinyl methyl [δ 1.85 (3H, s, H_3-15)], and a secondary hydroxyl group connected to a glucopyranose moiety by a glycosyl linkage [δ 3.90 (1H, m, H-5''), δ 4.09 (1H, m, H-2''), δ 4.23 (1H, m, H-3''), δ 4.23 (1H, m, H-4''), δ 4.40 (1H, dd, *J* = 12.1, 5.3 Hz, H-6''a), δ 4.60 (1H, dd, *J* = 12.1, 4.8 Hz, H-6''b), δ H 4.82 (1H, d, *J* = 7.6 Hz, H-1'')]. The presence of an unsaturated γ-lactone ring bearing an exomethylene group was also evidenced [δ_H 5.49 (1H, d, *J* = 3.5 Hz, H-13a), and 6.35 (1H, d, *J* = 3.5 Hz, H-13b); δ_C 119.8 (t, C-13), 142.2 (s, C-11), 172.0 (s, C12)].

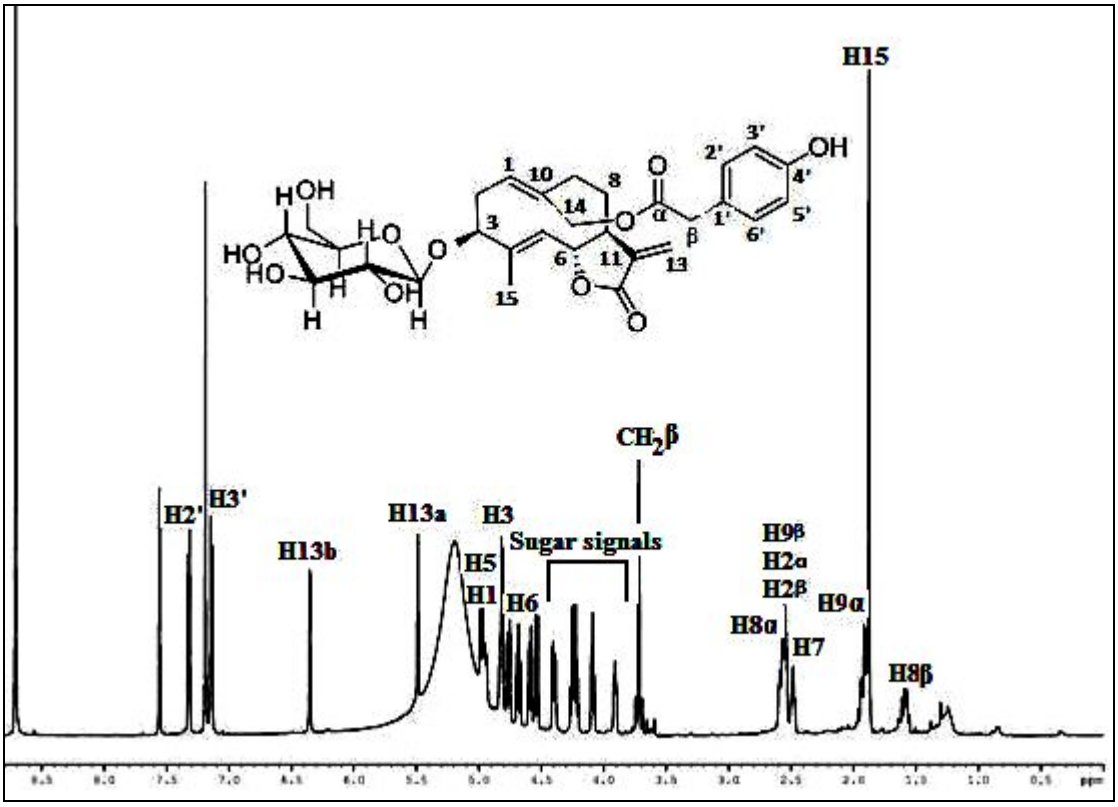


Figure 3.94: ^{1}H NMR spectrum of compound 314 in pyridine-d5

The interpretation of ^{1}H-^{1}H COSY and HSQC experiments (**Figures 3.97**, **3.98**) delineated both the -C=CH-CH_2-CH(O)- (through H-1) and the –CH(O)-CH-CH_2-CH_2- (through H-6) spin systems of the germacranolide skeleton.

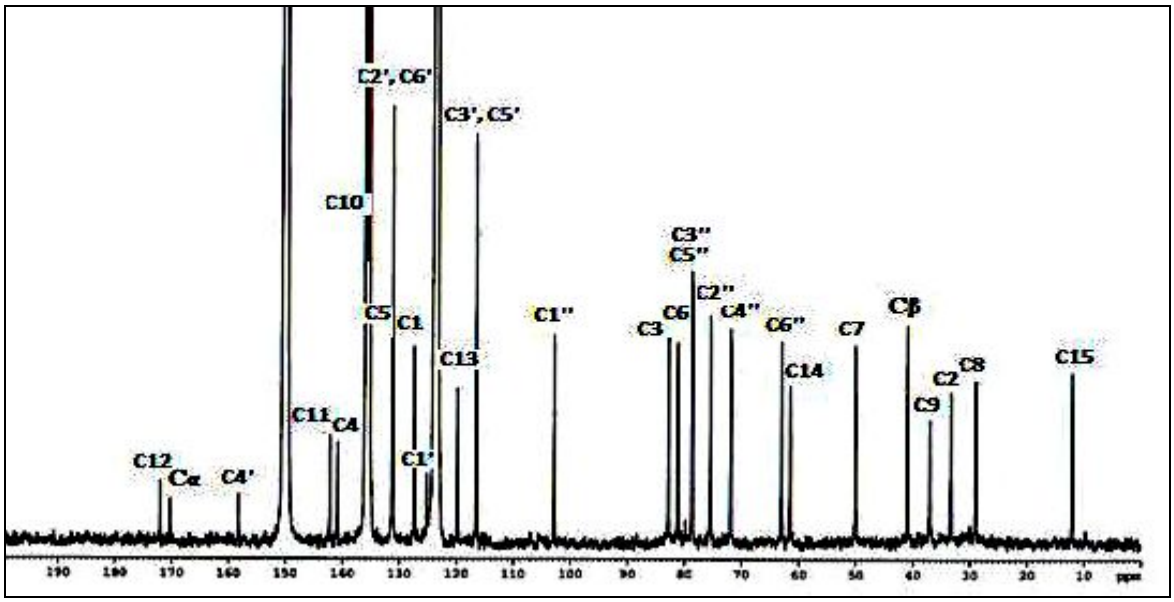


Figure 3.95: ^{13}C spectrum of compound 314 in pyridine- d_5

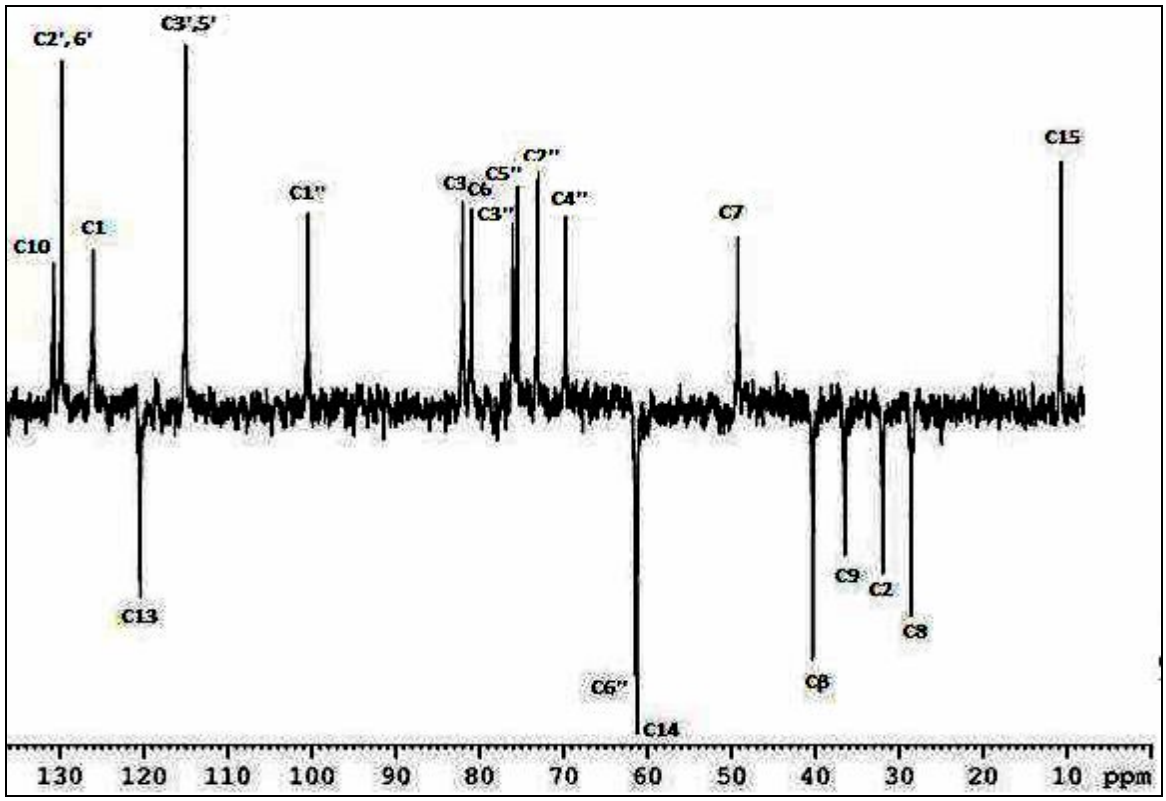


Figure 3.96: DEPT ^{13}C NMR spectrum of compound 314 in ($CDCl_3$ + MeOD)

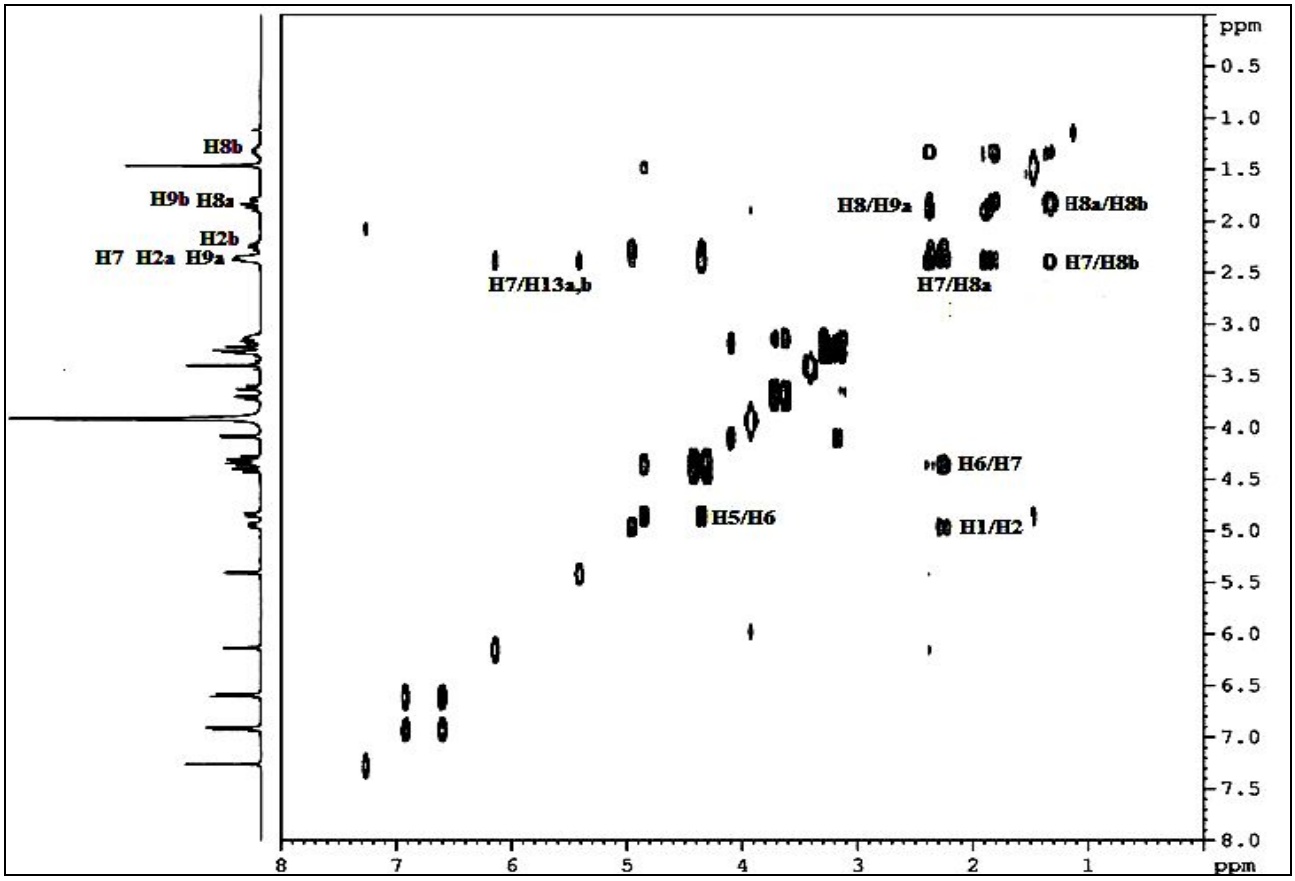


Figure 3.97: H-H COSY spectrum of compound 314 in ($CDCl_3$ + MeOD)

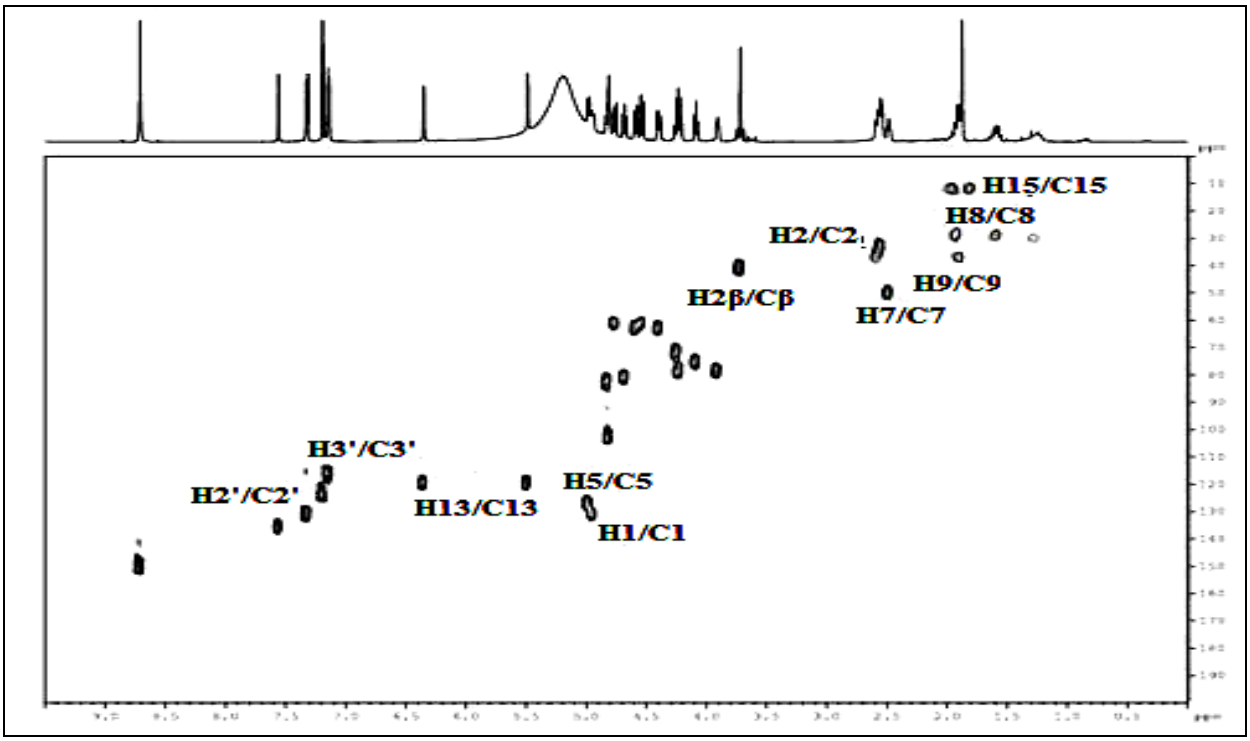
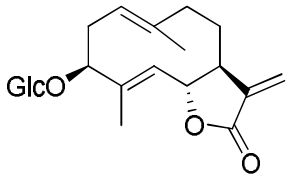


Figure 3.98: HSQC spectrum of compound 314 in pyridine-d5

By these data compound **314** was deduced to be a germacranolide analogue of costunolide-3-β-glucopyranoside (picriside C) (Nishimura et al., 1986), that had been previously reported along with related metabolites from *Lactuca* (Kisiel et al., 1997a), *Picris* (Nishimura et al., 1986), *Youngia* (Adegawa et al., 1986) and *Cichorium* (Seto et al., 1986), all belonging to the Lactuceae tribe.



picriside C

The ^{1}H NMR spectrum of compound **314** was completed by signals at δ 4.57 (1H, d, *J* = 12.3 Hz) and 4.76 (1H, d, *J* = 12.3 Hz) due to the presence of an isolated -CH_2O- moiety, and by three signals at δ 3.72 (2H, d, *J* = 3.5 Hz, CH_2β), 7.14 (2H, d, *J* = 8.2 Hz, H-3' and H-5'), and 7.32 (2H, d, *J* = 8.2 Hz, H-2' and H-6') which were attributed to a *p*-hydroxy-phenyl acetic residue. In the ^{13}C NMR spectrum, the corresponding signals at δ 170.4 (CO, Cα), 41.0 (CH_2, Cβ), 125.2 (s, C-10), 131.2 (2CH, d, C-2' and C-6'), 116.5 (2CH, d, C-3' and C-5'), and 158.3 (s, C-4') were observed. The absence in the ^{1}H NMR spectrum of the vinyl broad singlet due to H_3-14 when compared to picriside C led us to suppose that in compound **314** the hydroxymethylene function replaced the vinyl methyl C-14 and that it was esterified by a *p*-hydroxy-phenyl acetic residue.

A series of HMBC correlations (**Figure 3.99**) aided us to link the defined partial structures. In particular, the location of the acyl residue at the position C-14 was confirmed by the diagnostic HMBC correlation observed between the carbonyl signal at δ 170.4 (Cα) and the two methylene protons at δ 4.57 and δ 4.76. Furthermore, carbon C-3 was correlated with the anomeric proton at δ 4.82 (d, *J* = 7.6 Hz), confirming the linkage of the sugar moiety at this position.

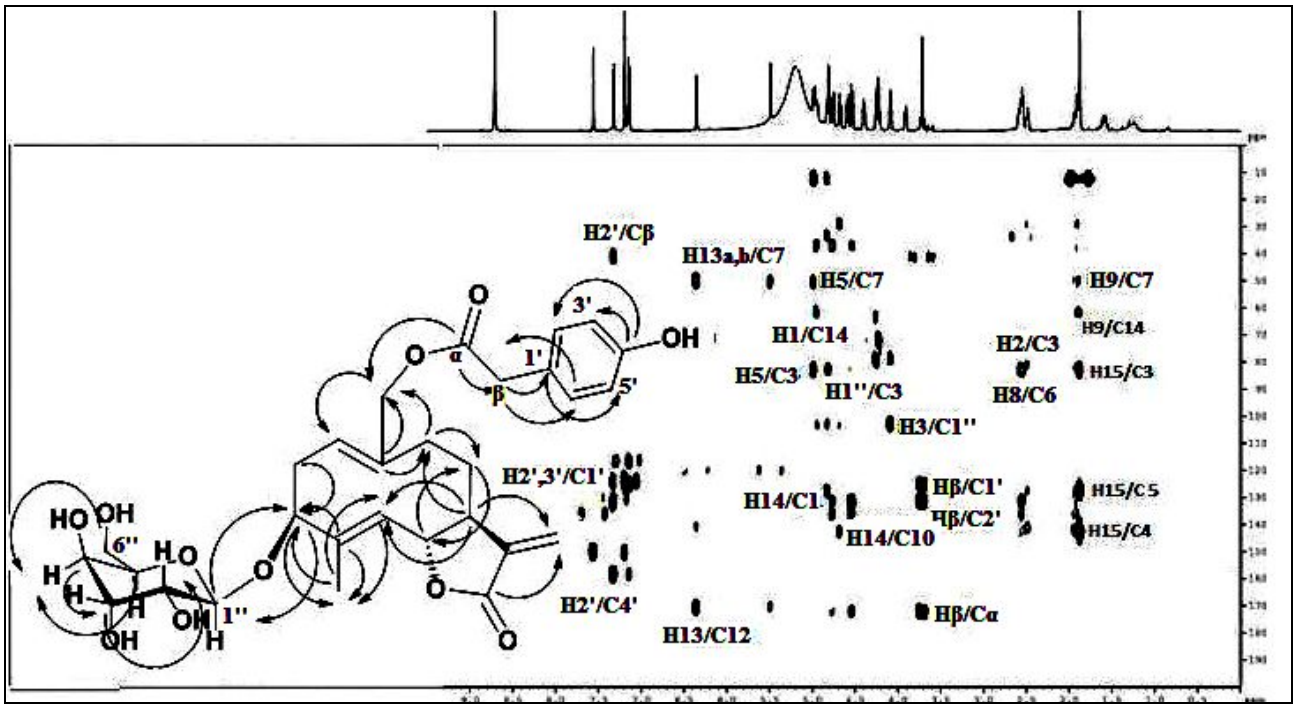
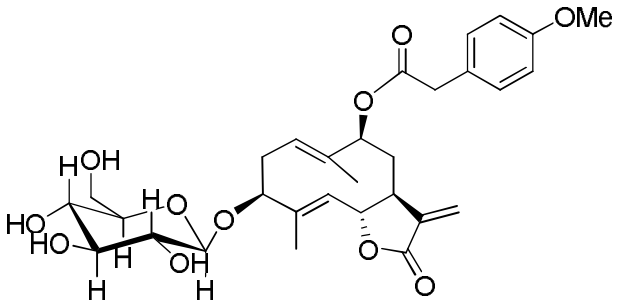


Figure 3.99: HMBC spectrum and the significant correlations observed for compound 314 in pyridine-d_5

Careful comparison of proton and carbon NMR data of compound **314** with those of sonchuside B (Miyase et al., 1987) showed a substantial similarity supporting the structural hypothesis**.**



Sonchuside B

The above data allowed the formulation of **314** as 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate.

All NMR resonances were assigned as reported in **table 3.9** by 2D experiments.

Table 3.9 : ^{1}H and ^{13}C NMR data of conpound 314 in C_5D_5N

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	131.3	4.95	dd (10.6, 5.9)	H-9
2	33.3	2.50	m	H-3
		2.58	q (11.8)	-
3	82.8	4.83	m	H-1, H-1", H-2, H-15
4	140.8	-		H-3, H-6, H-15
5	127.4	4.98	d (10.2)	H-3, H-15
6	81.2	4.68	t (8.8)	H-7
7	50.0	2.46	m	H-5, H-9, H-13a, H-13b
8	29.0	1.58	m	H-6, H-7
		1.93	m	-
9	37.0	1.90	m	H-1, H-8, H-14
		2.58	m	-
10	135.9	-		H-9, H-14
11	142.2	-		H-6, H-7, H-13a, H-13b
12	172.0	-		H-13a, H-13b
13	119.8	5.49	d (3.5)	-
		6.35	d (3.5)	-
14	61.5	4.57	d (12.3)	H-1, H-9
		4.76	d (12.3)	-
15	12.0	1.85	s	H-3, H-5
		-		-
Ester moiety				
C α	170.4	-		CH_2β, H-14
CH_2 β	41.0	3.72	d (3.5)	H-2', H-6'
1'	125.2	-		CH_2β, H-5'
2', 6'	131.2	7.32	d (8.2)	CH_2β
3', 5'	116.5	7.14	d (8.2)	-
4'	158.3	-		H-2', H-3'
Sugar moiety				
1"	102.8	4.82	d (7.6)	H-3, H-2"
2"	75.4	4.09	t (8.2)	-
3"	78.6	4.23	m	H-2"
4"	71.9	4.23	m	H-3"
5"	78.7	3.90	m	H4", H-6"
6"a	63.0	4.40	dd (11.7, 5.3)	H-4"
6"b	-	4.60	dd (11.7, 2.3)	-

The stereochemistry of compound **314** was determined by analysis of the NOESY spectrum (**Figure 3.100**). The correlations observed between the proton H-14 and H-2β (δ 2.50) as well as between H-1 and H-9β (δ 2.58) suggested a *Z* geometry for the double bond C-1(10), while the cross-peak observed between H_3-15 and H-6 indicated an *E* geometry for the double bond C-4(5).

The *trans* fusion at C-6/C-7 was indicated by the absence of the correlation between the two protons H-6 and H-7 as well as by the coupling constant value ($J_{6,7}$ = 8.8 Hz). The hydroxyl group at C-3 was suggested to be β-oriented from a NOESY correlation observed between the

proton H-3 and H-2α, and from the ^{13}C NMR values which were compared with model germacranolide compounds bearing a β-hydroxyl group at the position C-3 (Miyase et al., 1987). All NOESY correlations observed are showed in the following **Figure 3.100**.

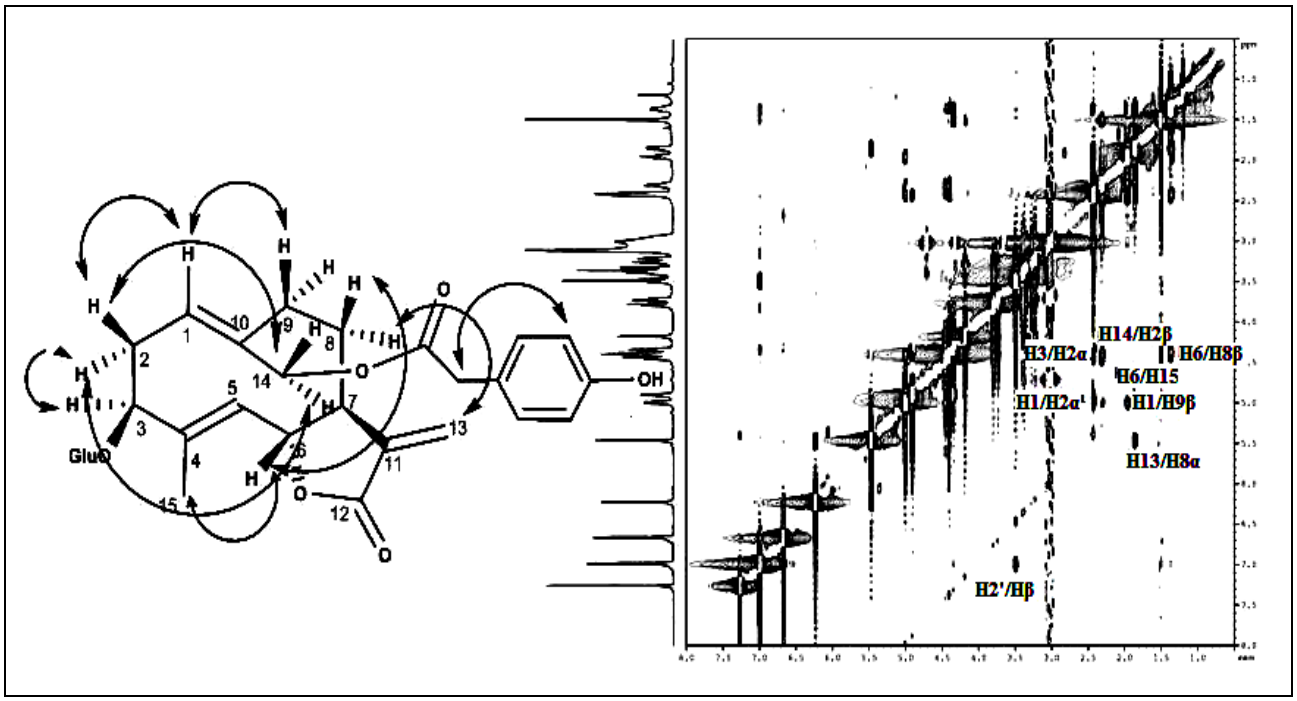


Figure 3.100: NOESY spectrum and the important correlations observed in the compound 314 in ($CDCl_3$ + MeOD)

3.4.2.4.2 Compound 315

Compound **315** is a new natural compound. The molecular formula $C_{21}H_{30}O_9$ was indicated by the HRESIMS spectrum **(Figure 3.101)** that showed peaks at *m/z* 449.1776 $[M + Na]^+$ and at *m/z* 875.364 $[2M + 23]^+$.

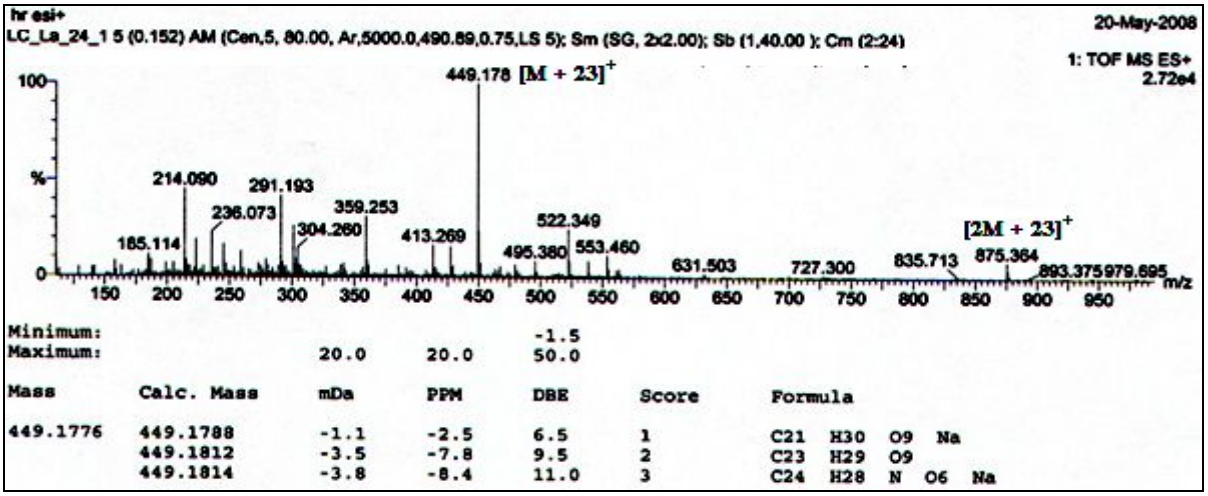


Figure 3.101: HRESIMS spectrum of compound 315

The ^{1}H NMR spectrum of compound **315** in pyridine-d_5 (**Figure 3.102**) resembled that of compound **314**, according to the presence of the same germacranolide skeleton.

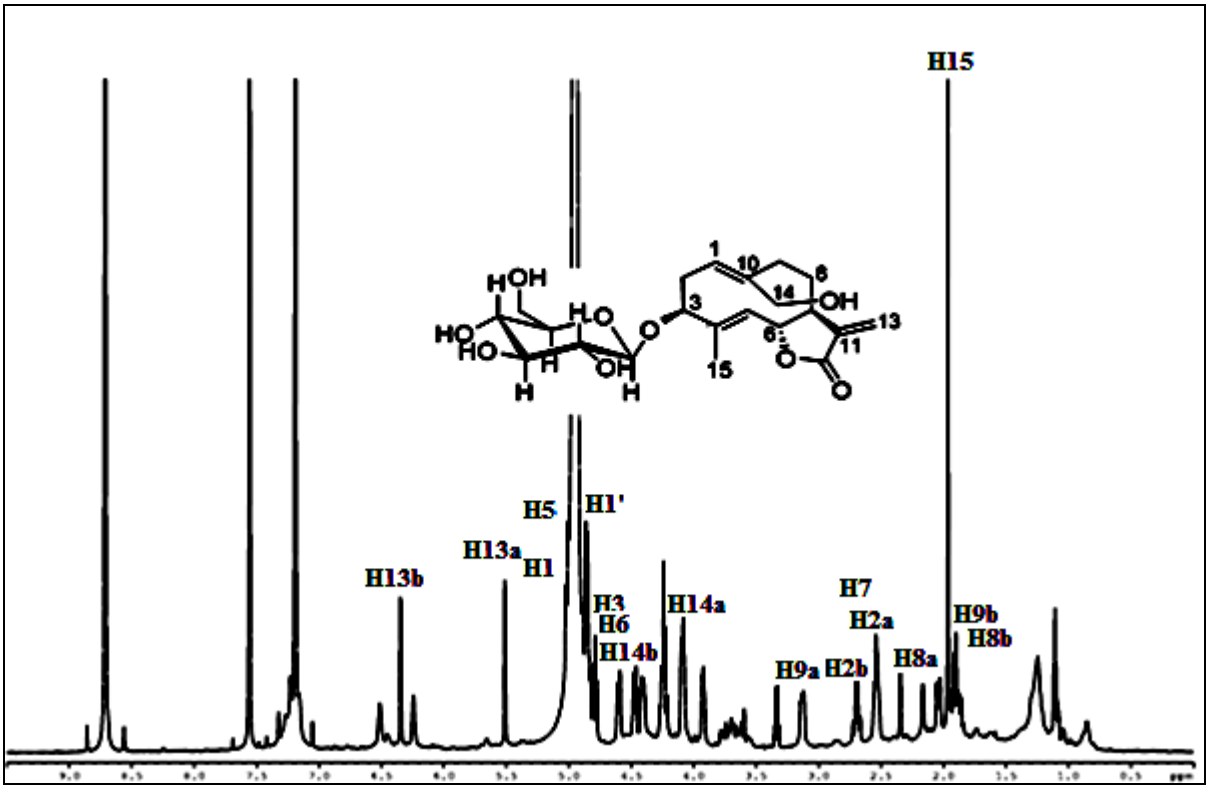


Figure 3.102: ^{1}H NMR spectrum of compound 315 in pyridine-d5

The spectrum was lacking of the signals due to the *p*-hydroxyphenyl acetic moiety and also displayed the hydroxymethylene AB system H_2-14 at δ 4.10 and 4.47 upfield shifted with respect to compound **314** This implied the 14-OH group had to be free. This was confirmed by the ^{13}C NMR value of C-14 (**Figure 3.103**) resonating at δ 58.5, which was upfield shifted with respect to the corresponding value in compound **311**.

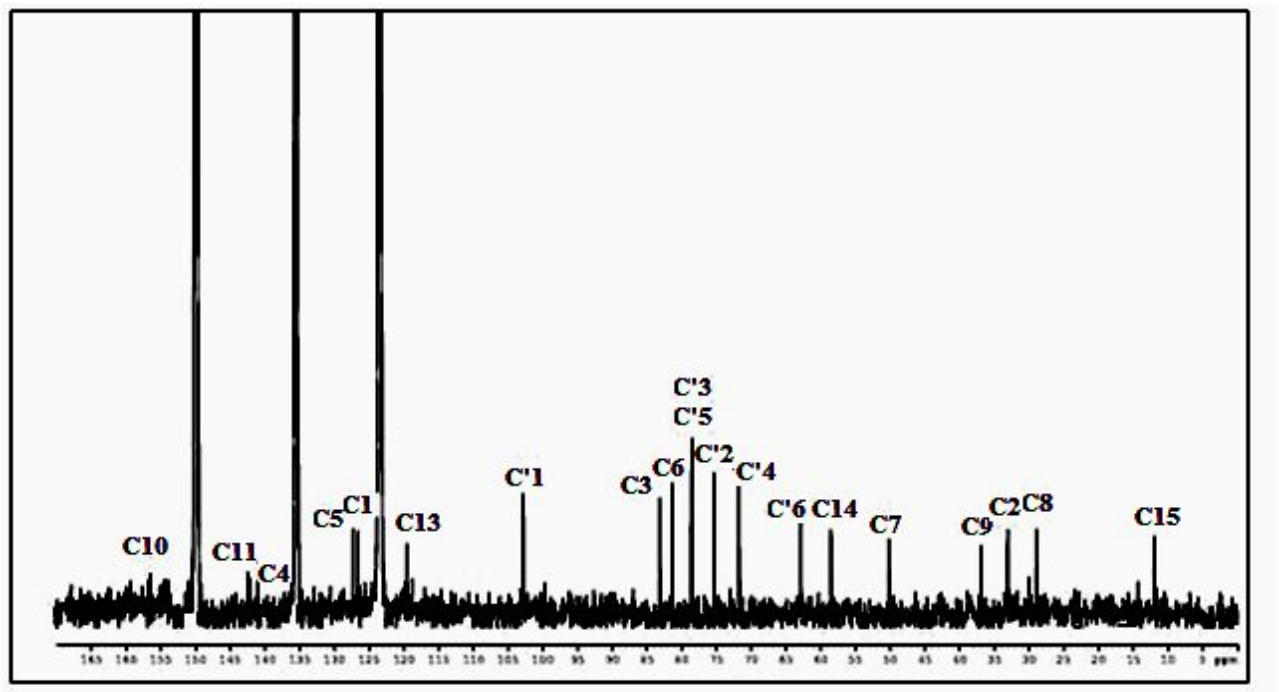


Figure 3.103: ^{13}C NMR spectrum of compound 315 in pyridine-d5

The ^{1}H-^{1}H COSY spectrum of **315 (Figure 3.104)** revealed the presence of the same spin systems as compound **314**. The HSQC and HMBC experiments (**Figures 3.105**, **3.106**) allowed the assignment of carbon and proton resonances as reported in **Table 3.9**.

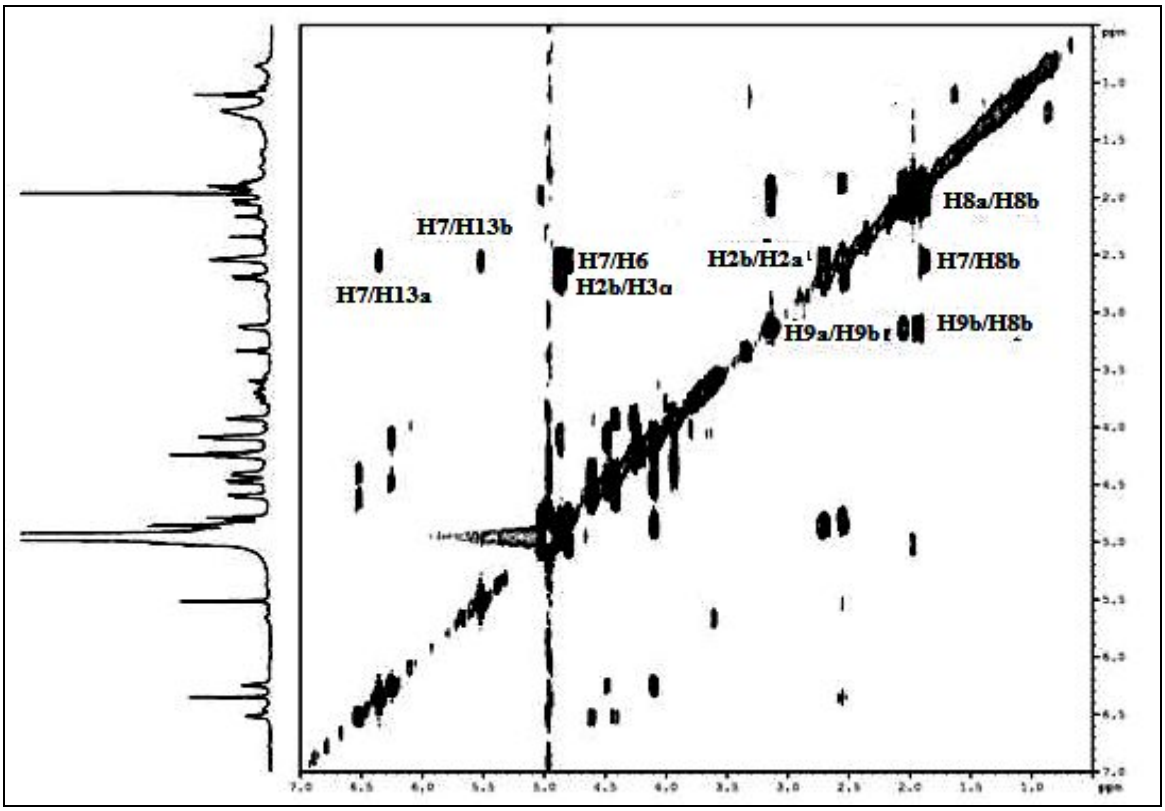


Figure 3.104: ^{1}H-^{1}H COSY spectrum of compound 315 in pyridine-d_5

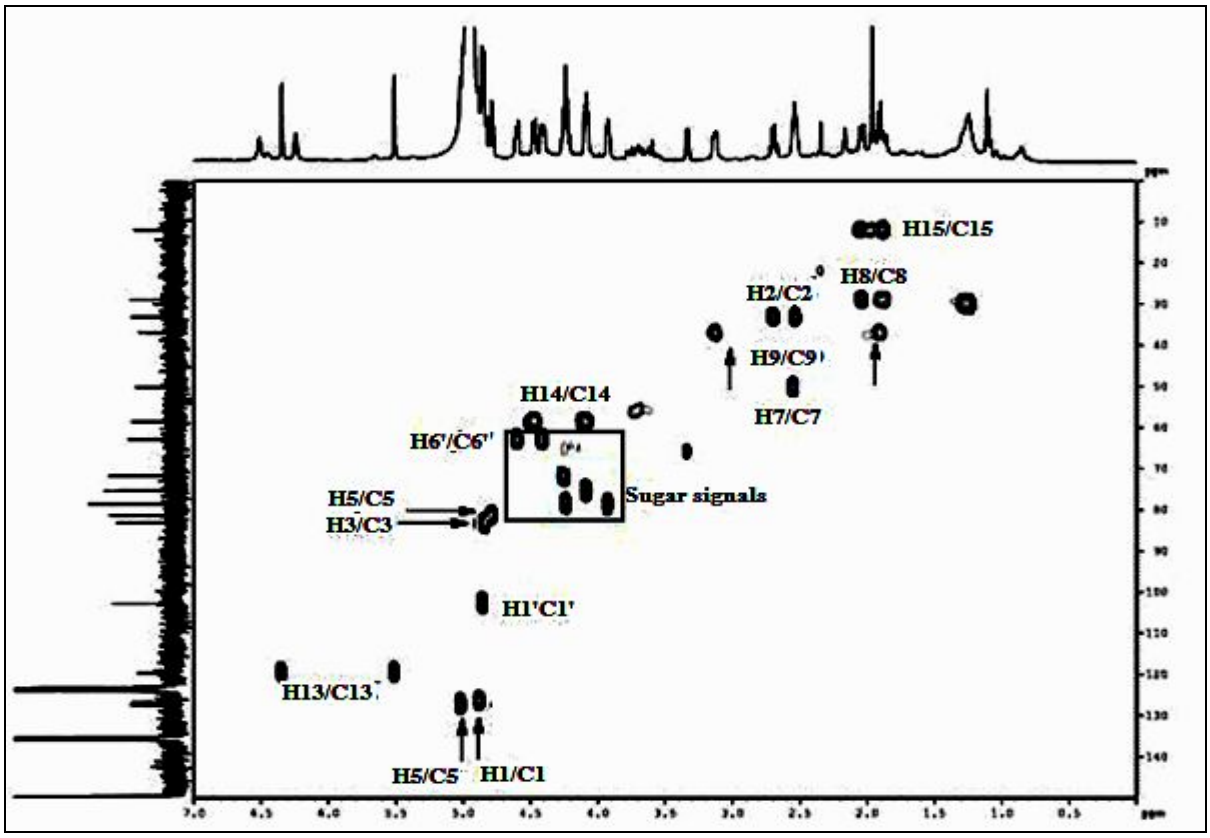


Figure 3.105: HSQC spectrum of compound 315 in pyridine-d_5

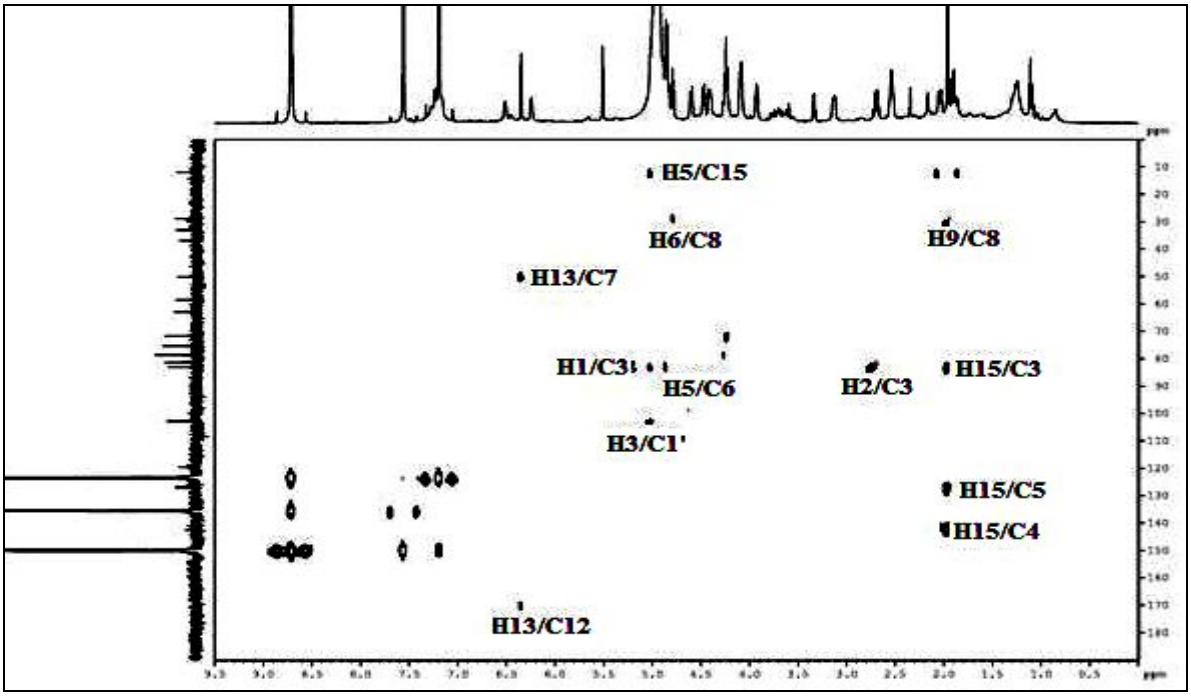


Figure 3.106: HMBC spectrum of compound 315 in pyridine-d_5

Table 3.10 : ^{1}H and ^{13}C NMR data of conpound 315 in C_5D_5N

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	HMBC (C to H)
1	126.6	5.05		-
2	33.0	2.68	q (11.8)	-
		2.55	m	-
3	83.2	4.90	m	H-2, H-5, H-1',H-15
4	141.5	-		H-5, H-15
5	127.3	5.02	m	H-7, H-15
6	81.4	4.80	t (4.8)	-
7	50.2	2.52	m	H-13
8	28.9	2.15	m	H-6, H-9
		1.90	m	-
9	36.9	1.90	m	-
		3.15	m	-
10	135.2	-		H-2, H-9
11	139.0	-		-
12	168.8	-		H-13
13	119.6	5.50	d (2.8)	-
		6.32	d (2.8)	-
14	58.5	4.10	d (2.3)	-
		4.47	d (12.3)	-
15	12.0	1.95	s	H-5
		-		
Sugar moiety				
1"	102.8	4.83	d (8.0)	H-3
2"	75.3	4.10	m	-
3"	78.6	4.25	m	H-5"
4"	71.7	4.28	m	H-3"
5"	78.6	3.90	m	-
6"a	62.9	4.42	dd (12.1, 5.3)	-
6"b	-	4.61	dd (12.1, 4.8)	-

The relative stereochemistry of compound **315** including the geometry of the two endocyclic double bonds C-1 (10) and C-4 (5) was determined by analysis of the NOESY spectrum (**Figure 3.107**). Analogously with **314**, the cross-peak correlations between protons H-14 and H-2a , and between H_3-15 and H-6 were in good agreement with the *Z* and *E* geometry for the two double bonds C-1(10), and C-4(5), respectively.

The orientation of 3-OH was suggested to be β by comparing the proton and carbon chemical shift values (δ_H 4.80 and δ_C 83.2) with those reported in the literature for related germacranolides exhibiting two hydroxyl groups at both C-3 and C-14 positions (Kisiel et al., 1997a, 1998), (Nishimura et al., 1986). Thus, compound **315** was defined to be 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside.

The chemical correlation between compounds **314** and **315** was finally confirmed by hydrolysis of compound **314** that afforded *p*-hydroxyphenyl acetic acid and a glucosyl alcohol identical to 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**315**).

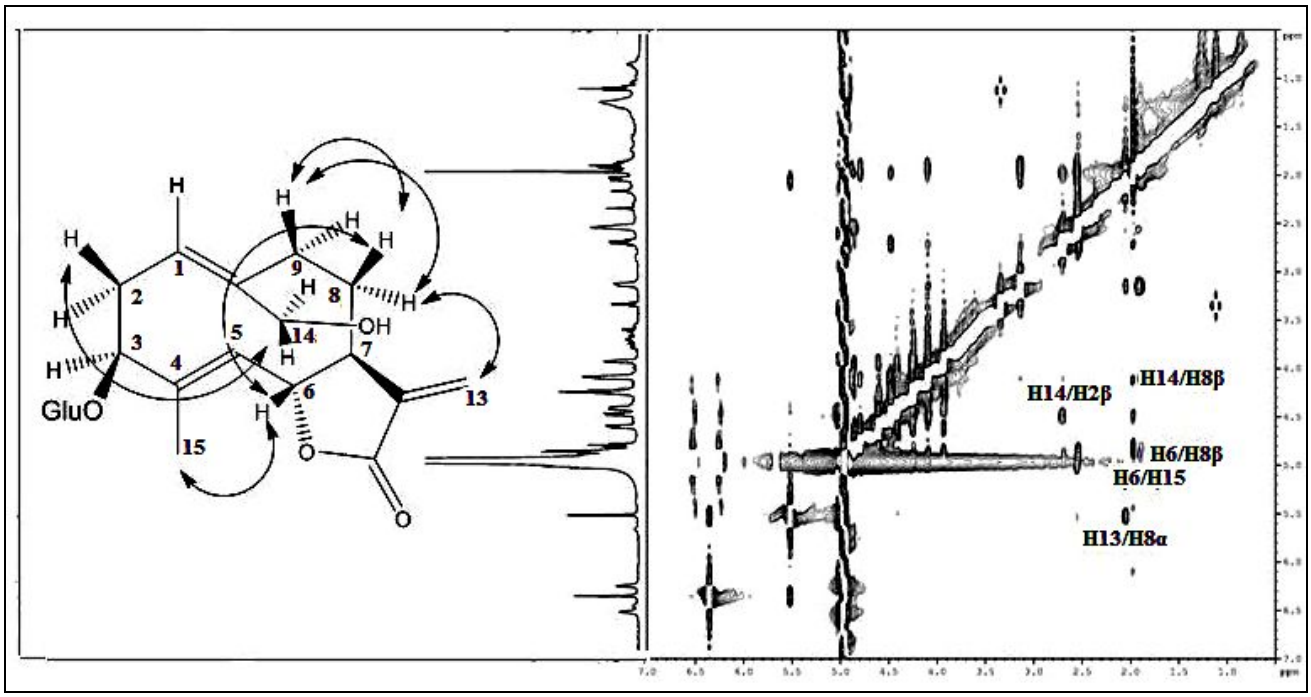


Figure 3.107: spectrum and selected correlations observed for compound 315 in pyridine-d_7

3.4.2.5 RELATED GERMACRANOLIDE STRUCTURES: 316, 317 and 318

3.4.2.5.1 Compound 316

Compound **316** had a molecular formula $C_{21}H_{30}O_8$ as deduced from the ESIMS mass spectrum that showed a peak at *m/z* 433 $[M + Na]^+$ (**Figure 3.108**).

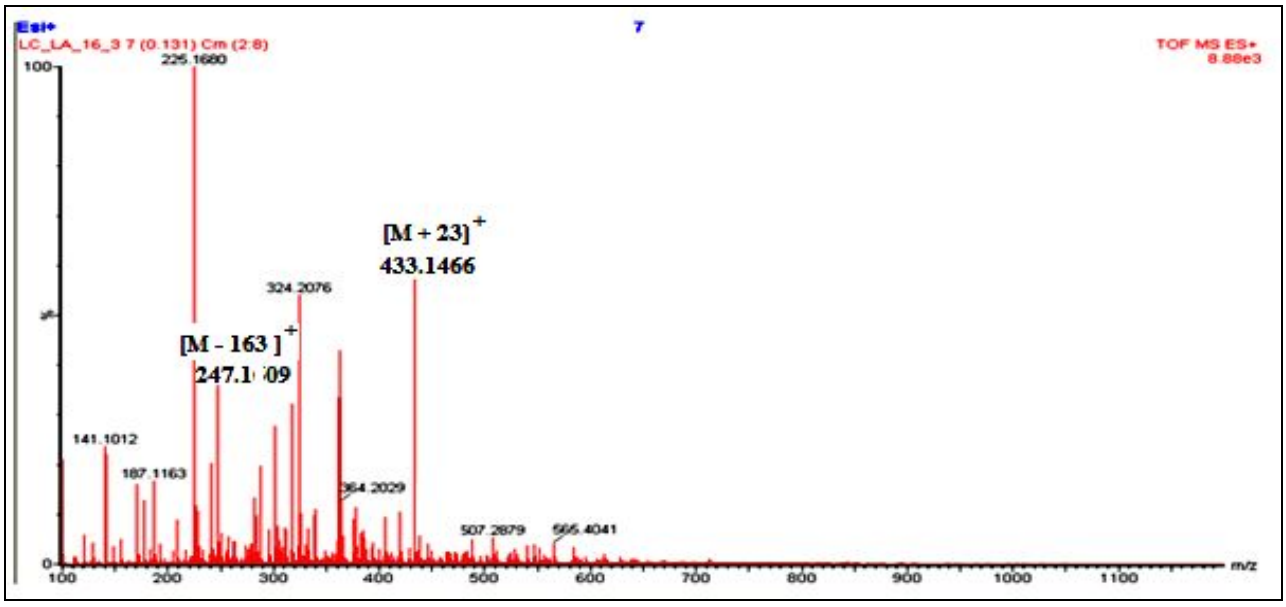


Figure 3.108: ESIMS spectrum of compound 316

The ^{1}H NMR spectrum (**Figure 3.109**) exhibited signals comparable with those of both compounds **314** and **315** strongly suggesting the presence of the same germacranolide skeleton. Compound **316** exhibited a glucosyl residue at C-3, analogously with **314** and **315**, whereas the hydroxymethylene group linked to C-5 was substituted by a methyl group. In fact, a 3H broad singlet at δ 1.39 was present in the proton spectrum of **316** due to the vinyl methyl C-14.

All signals were attributed by ^{1}H NMR and ^{1}H-^{1}H COSY spectra (**Figure 3.110** and **Figure 3.111**). Compound **316** was identified as picriside C (3β-hydroxycostunolide-3-*O*-β-glycopyranoside), that was isolated from some species of *Picris* (Bohlmann at al., 1981), (Nishimura et al., 1986).

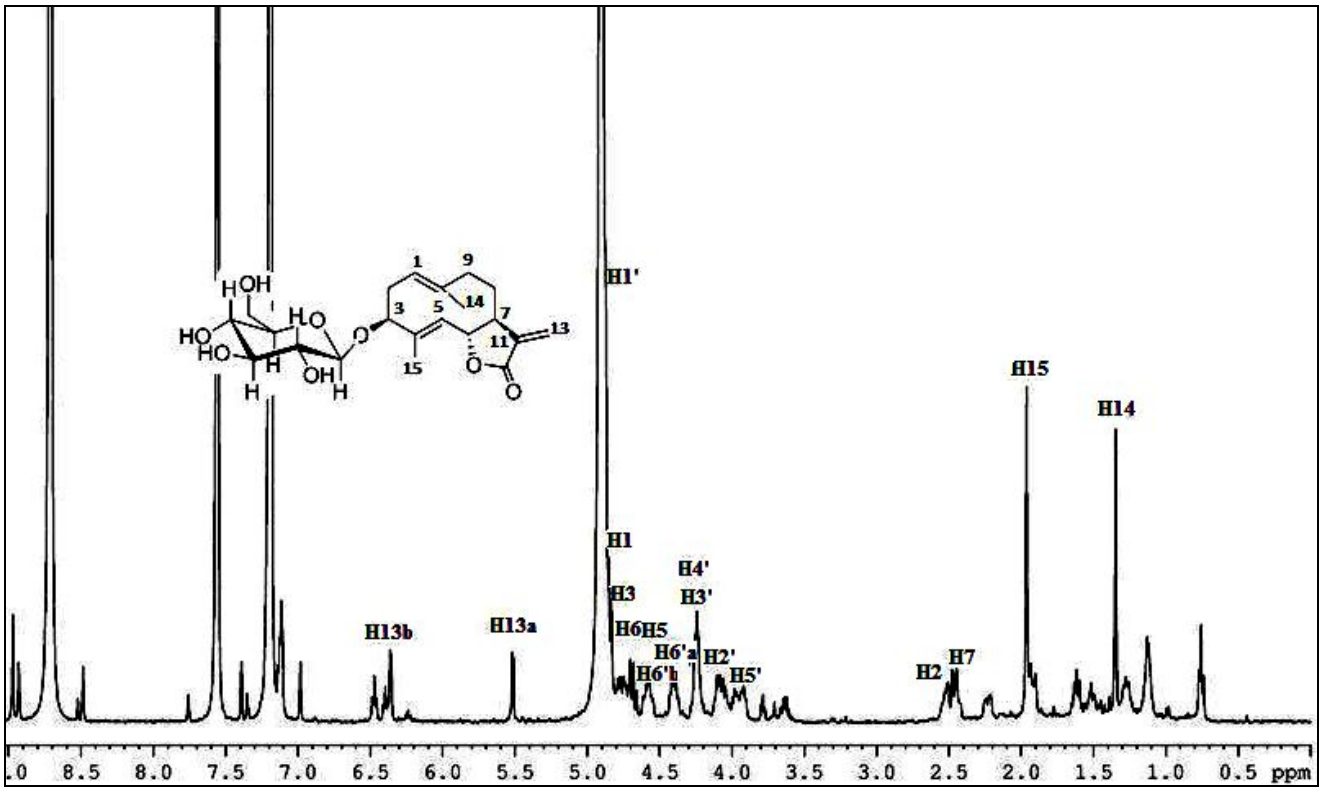


Figure 3.109: ^{1}H NMR spectrum of compound 316 in pyridine-d5

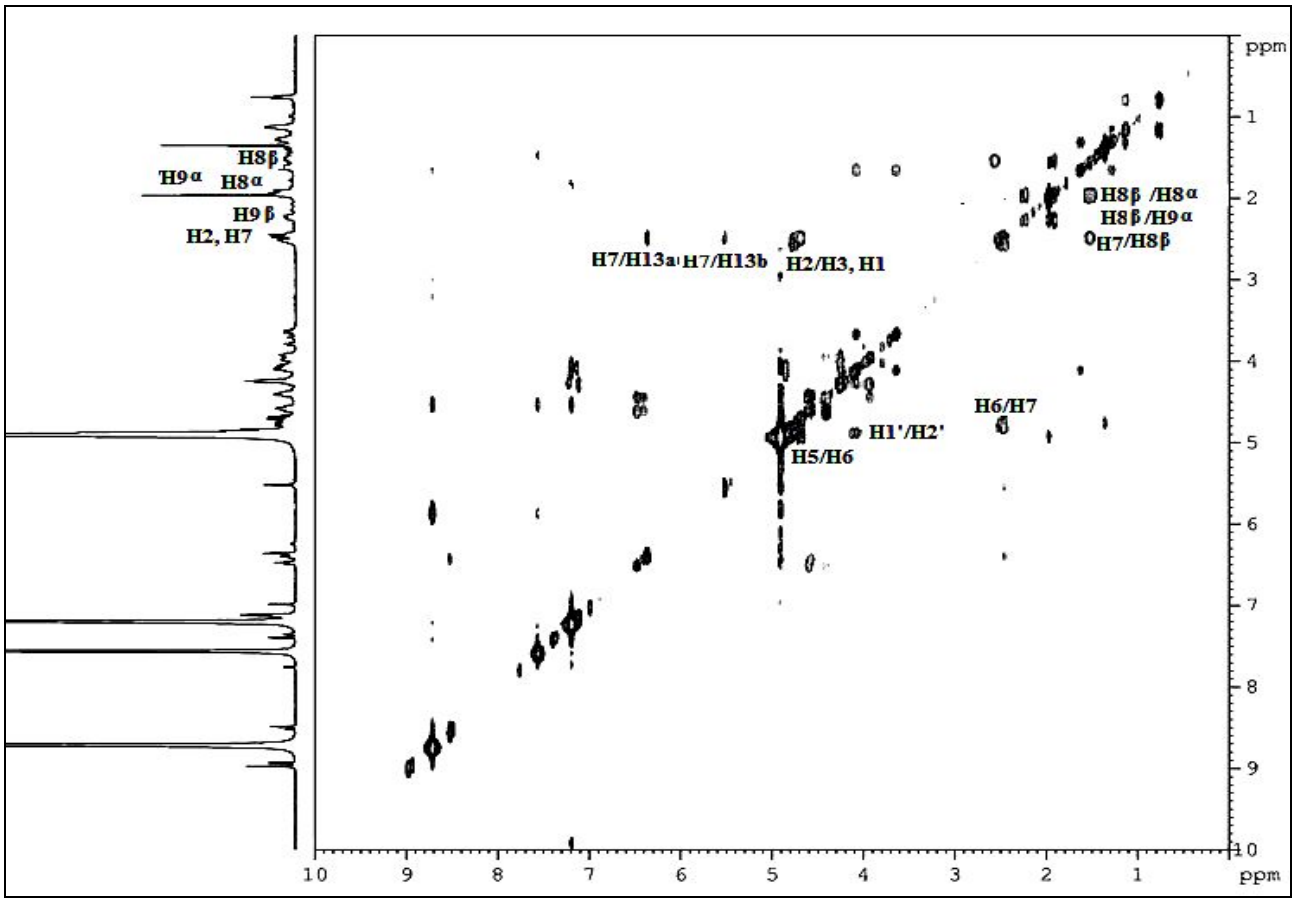


Figure 3.110: ^{1}H-^{1}H COSY spectrum of compound 316 in pyridine-d5

3.4.2.5.2 Compound 317

The ESIMS spectrum of the compound **317** (**Figure 3.111**) showed a peak at *m/z* 435 $[M + Na]^+$ consistent with the molecular formula $C_{21}H_{32}O_8$, that containing two hydrogen atoms more than compound **316**.

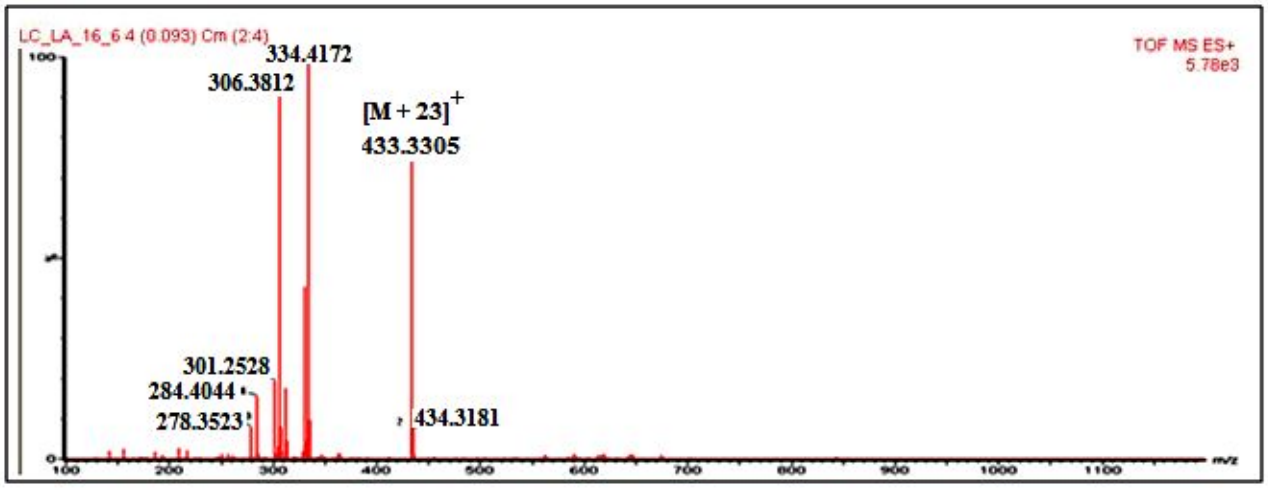


Figure 3.111: ESIMS spectrum of the compound 317

The ^{1}H NMR spectrum of compound **317 (Figure 3.112)** was closely related to that of compound **316**. The only difference was the presence of 3H doublet at δ 1.27 (J = 7.3 Hz) due to the secondary methyl H_3-13 replacing the corresponding exomethylene signals. This indicated that **317** was the 11,13-dihydroderivative of compound **316**.

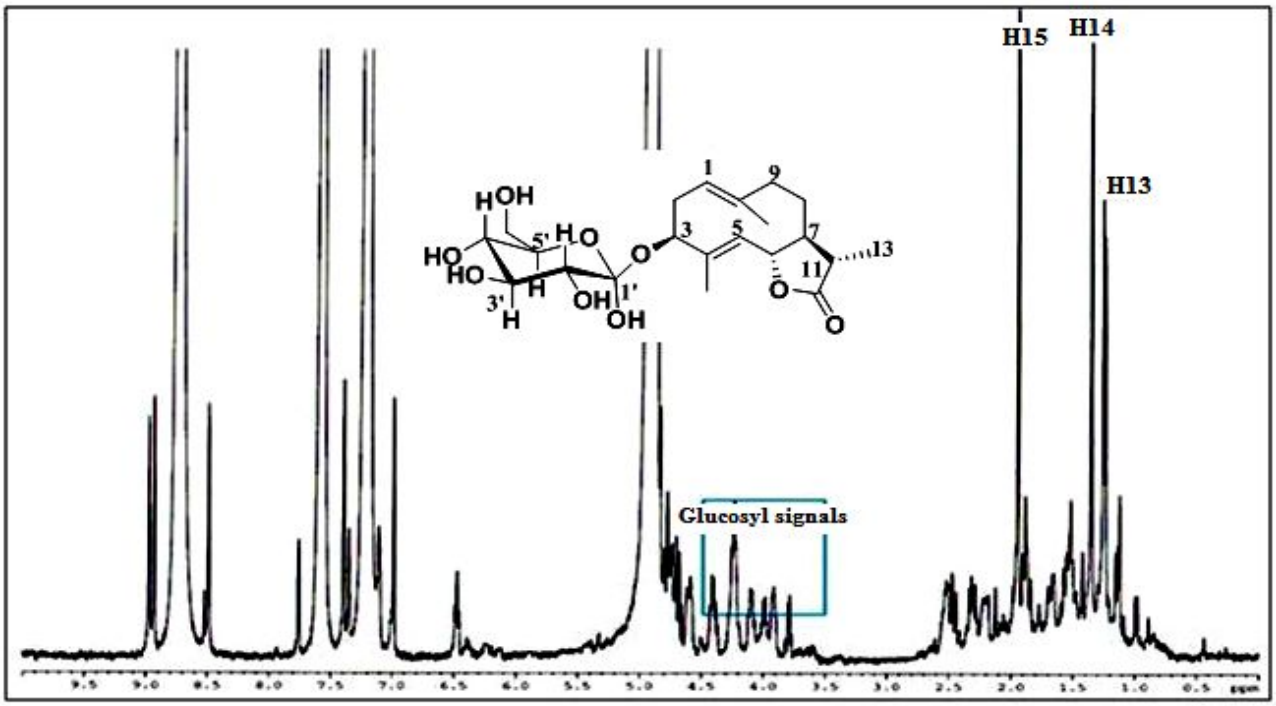


Figure 3.112: ^{1}H NMR spectrum of compound 317 in pyridine-d_5

These data led us to identify compound **317** as sonchuside A (3β-hydroxy-11β,13-dihydrocostunolide-3-*O*-β-glycopyranoside), previously isolated from *Sonchus oleracens* (Miyase et al., 1987).

3.4.2.5.3 Compound 318

Compound **315** had the same molecular formula as compound **317**.

The ^{1}H NMR spectrum of compound **318** (**Figure 3.113**) exhibited similar signals as those of compound **317** including the set of signals due to the glucosyl moiety. The difference between the two molecules was in the position of the glucosylated hydroxyl group (C-3 in **317**, C-15 in **318**). In the proton spectrum of compound **318**, the methyl signal H_3-15 was replaced by a hydroxymethylene attached to the glucosyl moiety. Thus, this compound was identified as picriside B, previously reported from *Picris hieracioides L*. (Nishimura et al., 1986).

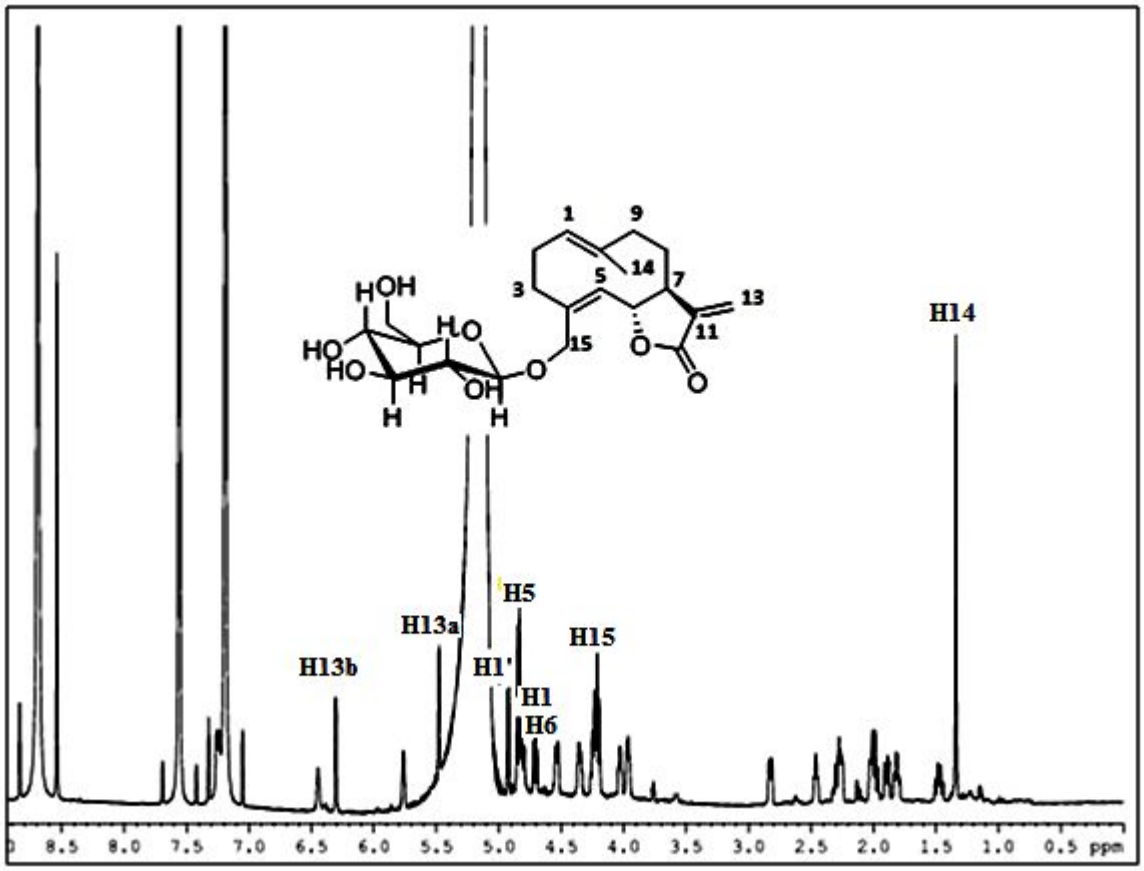


Figure 3.113: ^{1}H NMR spectrum of compound 318 in pyridine-d_5

3.5 BIOLOGICAL ACTIVITIES OF SELECTED ISOLATED COMPOUNDS

Selected sesquiterpenes lactones **305**, **306**, **311**, **314** and **315** were tested for their antifungal activity against *Candida albicans* and antibacterial activity against gram positive *Staphylococcus aureus* bacteria and gram negative *Escherichia coli* bacteria using a broth macrodilution method (Rodriguez-Tudela et al., 1996) at a concentration of 5µg/ml. The minimum inhibitory concentrations (MICs, µg/ml) were determined after 48h of incubation at 35° for the antifungal activity, and after 24h at 37° for the anti bacterial activity.

The results obtained showed that there was no growth inhibition at tested concentrations.

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Chapter 4
Phytochemical study of *Halophila stipulacea* (Forssk.) Aschers

4. PHYTOCHEMICAL STUDY OF *HALOPHILA STIPULACEA* (FORSSKÁL) ASCHERS

4.1 Botanical and systematic description

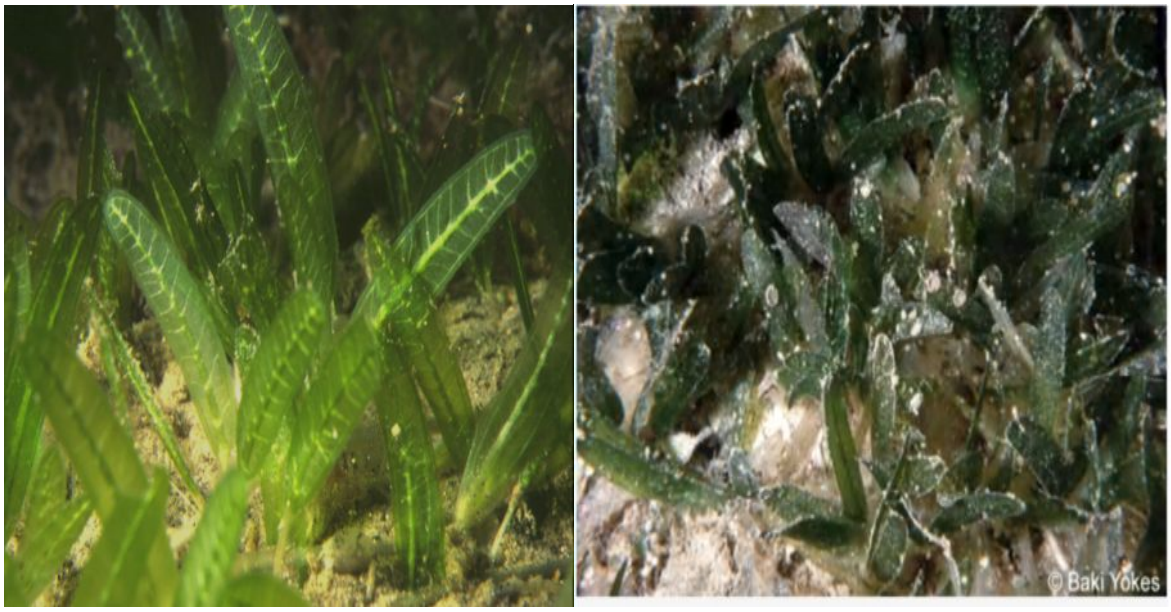
The seagrass *Halophila stipulacea* is one of the marine species which have entered in the Mediterranean from the Red Sea since the opening of the Suez Canal in 1869. The first record of *Halophila stipulacea* in the Mediterranean Sea was in Greece in 1894. *H. stipulacea* developed extensive meadows in the Eastern Mediterranean basin and now is extending its distribution into the western basin, in ports and near ports, in Levantine Sea, southern Aegean, Greece, Malta, Sicily, and Tunisia. It occupied sublittoral sediments, sandy and muddy bottoms, intertidal 65 m, but mainly at depth of 30-45 m, mostly in harbours, or in their vicinity.

Halophila stipulacea is known by its grazing for invertebrates and fishes.

Taxon : *Halophila stipulacea* (Forsskal, 1775) Aschers .

Family/Order/Class/Phylum: Hydrocharitaceae/Hydrocharitales/Liliopsida/Magniliophyta (Angiosperm).

Plants are dioecious with male and female flowers produced at each leaf node. Rhizomes are creeping, branched and fleshy, and roots appear solitary at each node of the rhizome, unbranched and thick with dense soft root hairs. Pairs of leaves are distributed on petioles along a rhizome, rooted in the sand. Leaves from 3-8 mm wide, ovate, not narrowing at the base, thin and hairy, margin spinulose. Petioles are from 3-15 mm long.

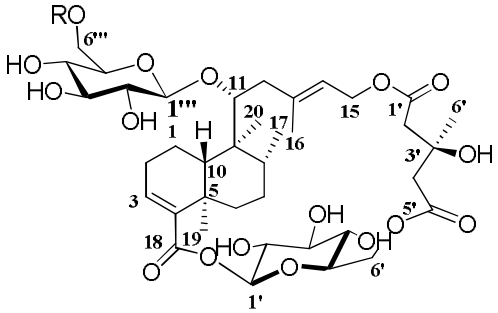


Halophila stipulacea

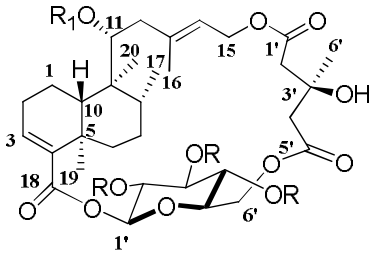
A previous study on a sample of this plant, which was collected in the Gulf of Corinth, Greece, and analysed together with its prey, the anaspidean mollusc *Syphonota geographica*, resulted in the isolation and the characterization of an unusual macrocyclic glycoterpenoid, syphonoside (**286**) (Gavagnin et al., 2007). In a subsequent work, the acetyl derivative of syphonoside, compound **287**, was also isolated from the phanerogam (Carbone et al., 2008), whereas the diethyl extract of the mollusc afforded the three minor derivatives **319**, **320** and **321**. It was suggested that these latter compounds could be obtained by a biotransformation of the main metabolite syphonoside (**286**) in the mollusc and that they could be involved in the defensive mechanisms of the animal (Carbone et al., 2008). The finding of syphonoside (**286**) in both the mollusc and the plant chemically demonstrated the trophic relationship between the two organisms, that had been previously suggested by the detection of fragments of *H. stipulacea* in the stomach of *S. geographica*.

Furthermore, chemo-ecological implications of the introduction of two exotic species, the mollusc and the sea-grass, in the Mediterranean Sea have been discussed by Mollo et al. (2008).

Syphonata geographica feeding on Halophila



286 R = H
287 R = Ac



319 R = R_1 = H
320 R = H R_1 = $CH_3(CH_2)_{14}CO$- or $CH_3(CH_2)_{16}CO$-
321 R = R_1 = Ac

In a preliminary cytotoxicity evaluation, syphonoside **286** inhibited high density induced apoptosis in a selected human and murine cancer cell lines, suggesting a possible involvement in the regulation of the cell survival and death under specific conditions (Gavagnin et al., 2007).

4.2 Collection of the material, extraction and purification

The seagrass *Halophila stipulacea* was collected in 2007 off Porto Germeno (Gulf of Corinth, Greece) at 5-10 m depth. Specimens of the plant were frozen at -20°C until chemical analysis. The fresh sample was extracted with acetone several times, and the aqueous residue after evaporation of the organic solvent was partitioned between diethyl ether and *n*-BuOH to get 1.31g of a butanolic crude extract. Some UV polar components which give a yellow coloration by reaction with cerium sulfate were detected at R*f* 0.15 and R*f* 0.70-0.60 (chloroform/methanol, 7:3) together with previously reported glycoterpenoids syphonoside **286** (Gavagnin et al, 2007) and its acetyl derivative **287** (Carbone et al., 2008).

An aliquot of the butanolic extract (380 mg) was subjected to Sephadex LH-20 chromatography eluting with a mixture of chloroform/methanol in 1:1 ratio. The collected fractions were analyzed by both TLC chromatography and NMR, and then combined to obtain three main flavonoid glycosides-containing fractions **A**, **B** and **C**. The results of the purification protocol are summarised in the following scheme (**Figure 4.1**).

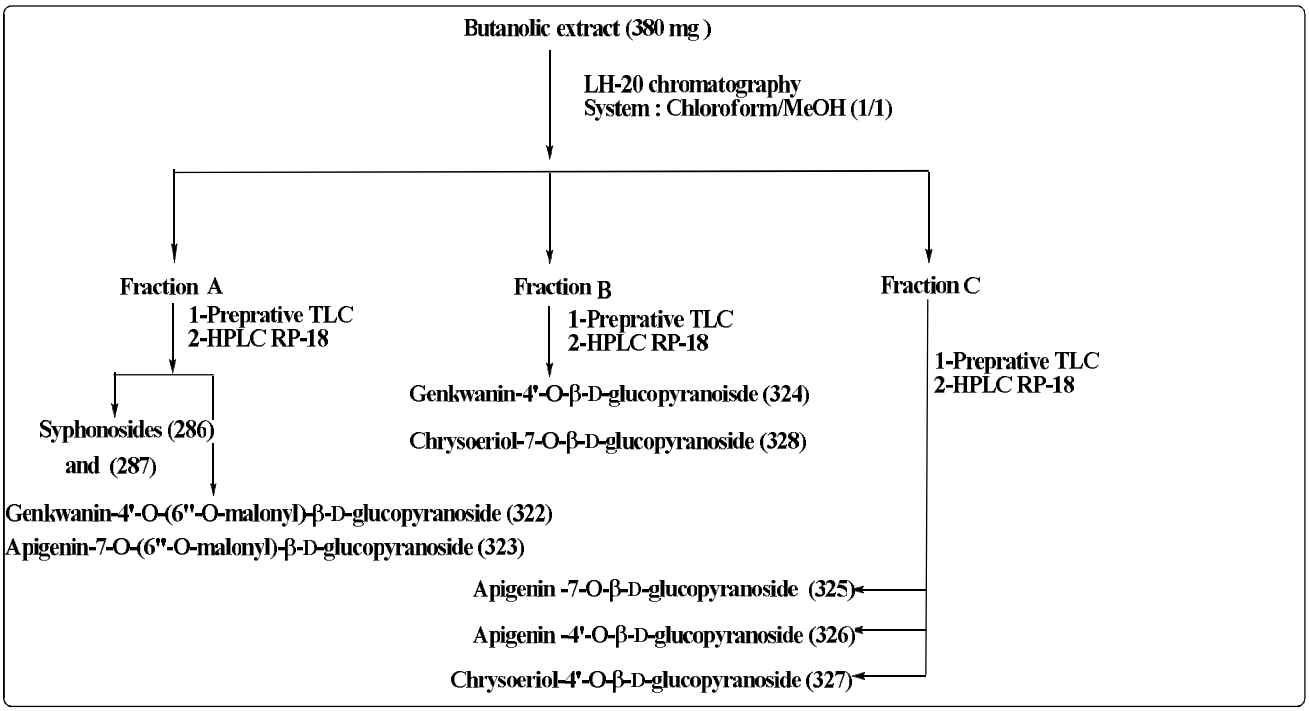
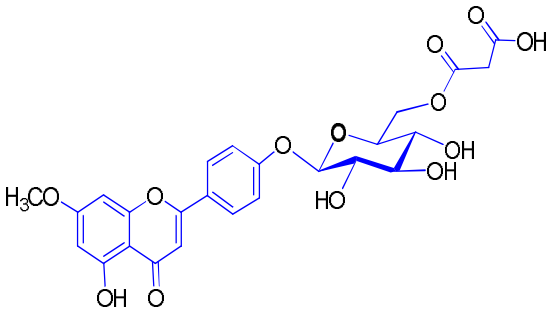
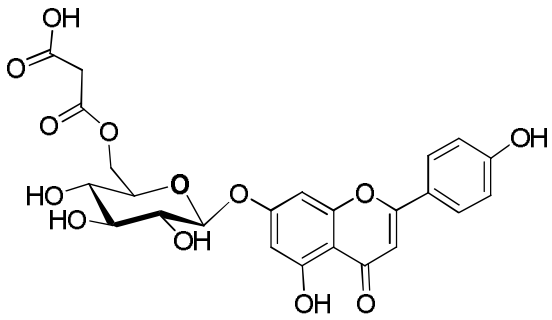


Figure 4.1 Scheme of the purification of the butanol extract of the whole plant of Halophila stipulacea

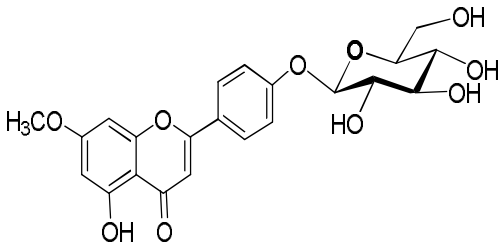
The structures of all metabolites (**286-287**, **322-328)**, isolated from the butanol extract of *H. stipulacea* are depicted in **Figure 4.2**. Compound **322** was an unprecedented compound whereas the other co-occurring flavonoids **323-328** have been reported so far only from terrestrial sources. Compounds **286** and **287** have been previously isolated from the same plant.



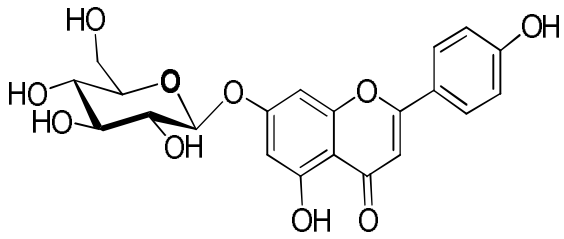
Genkwanin-4'-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside (**322**)



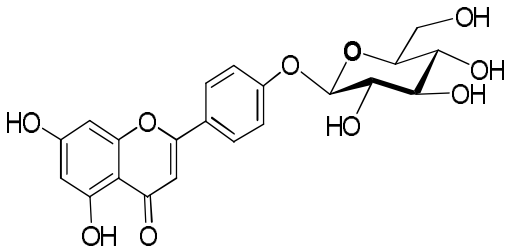
Apigenin-7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside **(323)**



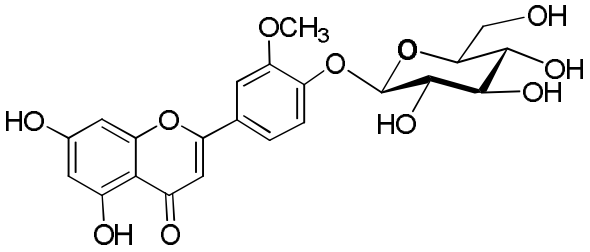
Genkwanin-4'-*O*-β-D-glucopyranoside (**324)**



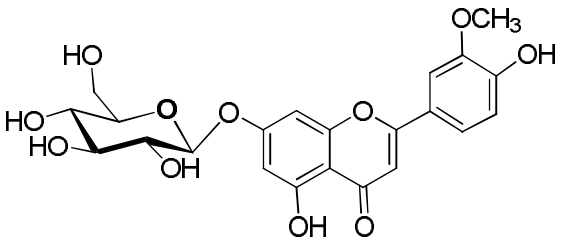
Apigenin-7-*O*-β-D-glucopyranoisde (**325**)



Apigenin-4'-*O*-β-D-glucopyranoisde (**326)**



Chrysoeriol-4'-*O*-β-D-glucopyranoisde (**327**)



Chrysoeriol-7-*O*-β-D-glucopyranoisde (**328)**

Figure 4.2: Compounds isolated from Halophila stipulacea

4.3 STRUCTURAL ELUCIDATION OF ISOLATED FLAVONOID COMPOUNDS

4.3.1 Compound 322

Compound **322** was obtained as pale yellow powder. It showed a strong UV absorbance at 254 nm and a typical yellow reaction by spraying with cerium sulphate suggesting the presence of a flavonoid framework. The NMR and mass spectra supported this assumption.

The ^{1}H NMR spectrum (**Figure 4.3**) showed the typical aromatic pattern of a disubstituted flavone that was constituted by signals at δ 6.39 (1H, d, *J* = 2.8 Hz, H-6), 6.71 (1H, d, *J* = 2.8 Hz, H-8) , 6.75 (1H, s, H-3), 7.26 (2H, d, *J* = 8.8 Hz, H-3'and H-5'), and 8.05 (2H, d, *J* = 8.8 Hz, H-2' and H-6'). Accordingly, in the ^{13}C NMR spectrum, the corresponding carbon atoms resonated at δ 98.9 (C-6), 93.6 (C-8), 105.2 (C-3), 118.1 (2C, C-3' and C-5'), and 129.6 (2C, C-2' and C-6')], respectively, as deduced from the HSQC spectrum (**Figure 4.4)**.

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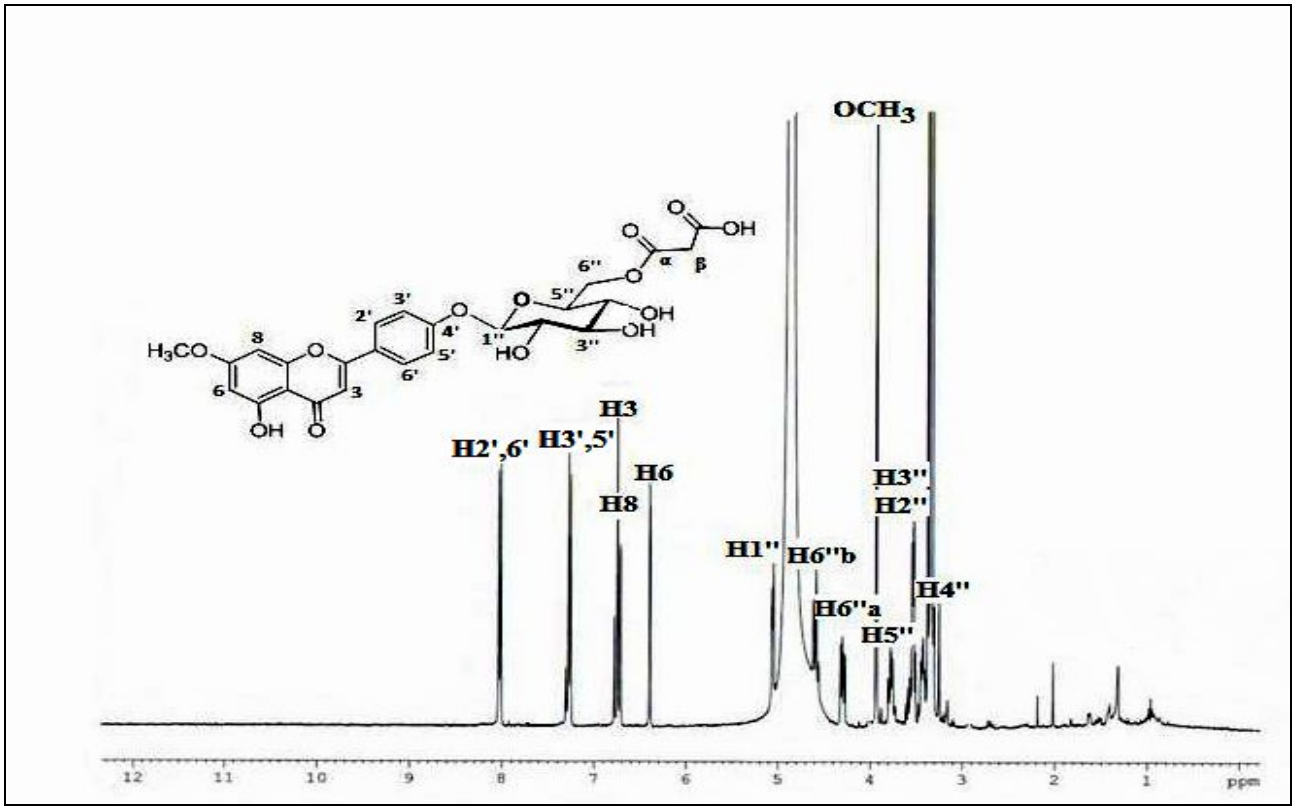


Figure 4.3: ^{1}H NMR spectrum of compound 322 in MeOD

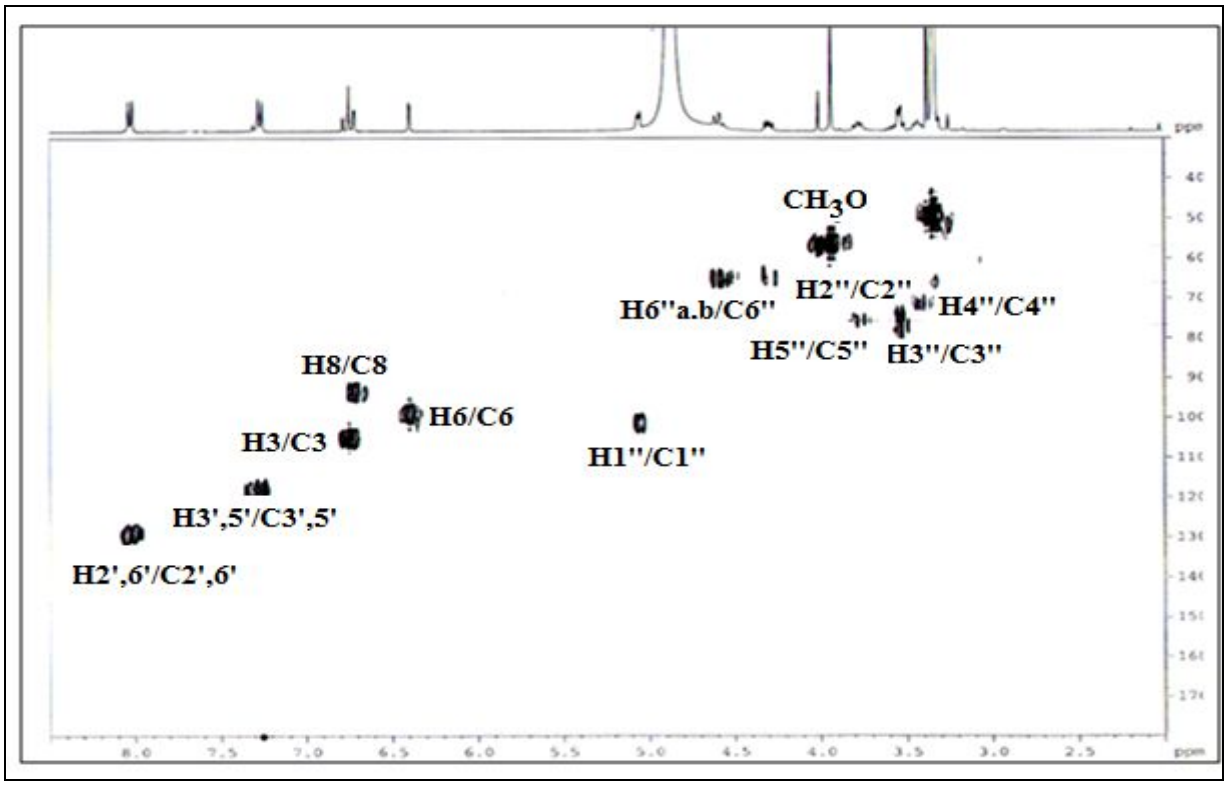


Figure 4.4: HSQC spectrum of compound 322 in MeOD

These data well fit a flavone skeleton exhibiting a *p*-substituted ring C. In addition, the ^{1}H NMR spectrum displayed a set of oxymethine signals in the range of δ 3.40–4.60, suggesting the presence of a sugar moiety in compound **322**. A signal integrating for three protons and resonating at δ 3.93 was also observed in the spectrum and assigned to a methoxyl group.

The interpretation of the ^{1}H-^{1}H COSY spectrum (**Figure 4.5**) led us to define the sugar spin system and assign all protons: δ 3.54 (1H, m, H-2’’), δ 3.52 (1H, m, H3’’), δ 3.43 (1H, m, H-4’’), δ 3.78 (1H, m, H-5’’), δ 4.33 (1H, dd, *J* = 11.8, 6.1 Hz, H-6’’a), and δ 4.54 (1H, d , *J* = 11.8 Hz, H-6’’b). The corresponding carbon values at δ 74.6 (C-2’’), 77.8 (C-3’’), 71.5 (C-4’’), 75.7 (C-5’’), and 64.7 (C-6’’) were easily attributed from the HSQC spectrum (**Figure 4.4**).

The sugar moiety was readily determined as a glucopyranosyl residue based on the previously published data (Ragaa et al., 1983), (Abdel-salem et al., 1986), Takagi et al., 1979a, 1979b), (Meng et al., 2008).

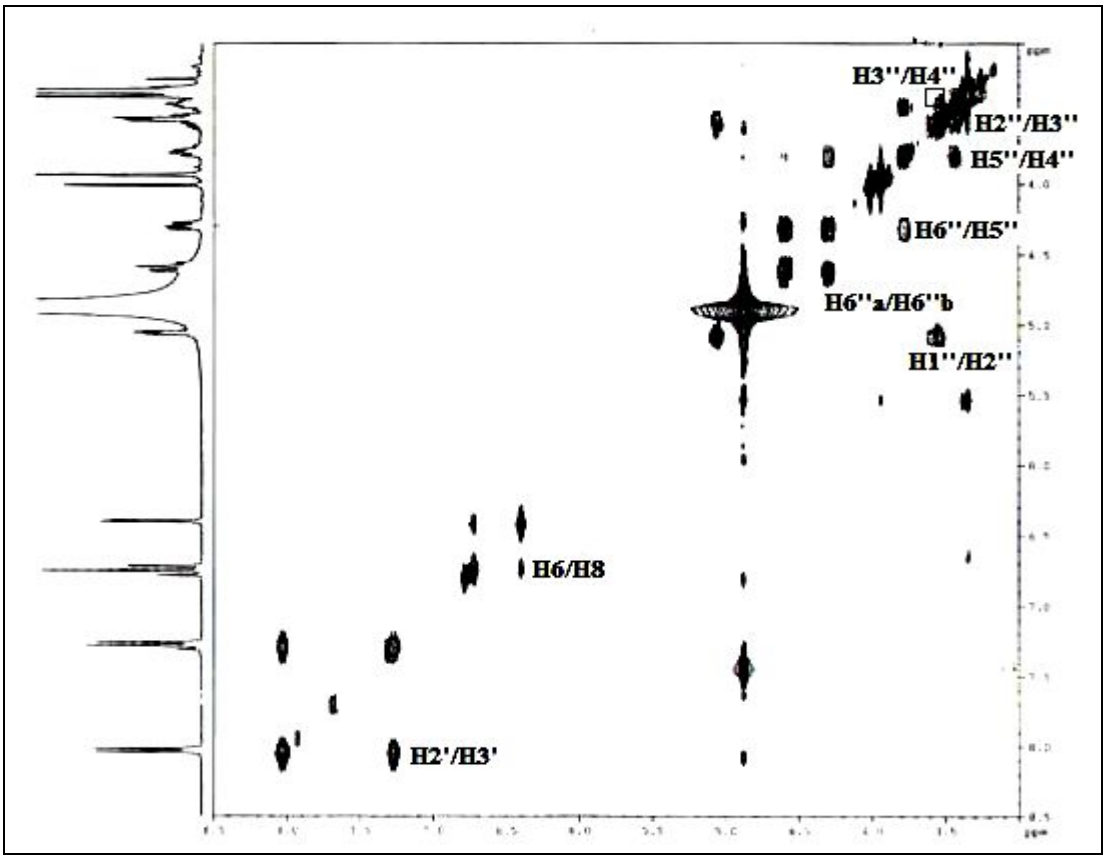


Figure 4.5: ^{1}H-^{1}H COSY spectrum of compound 322 in MeOD

The coupling constant of the anomeric proton H-1'' (J = 7.2 Hz), indicated the β-configuration of the glucopyranosyl moiety.

The comparison of the above data with the literature led us to identify the flavone framework of compound **322** as genkwanin bearing a methoxyl group at C-7 (ring B) and also connected to the glucopyranosyl residue through an ether linkage at C-4' (ring C). However, the MALDI mass spectrum of **319** (**Figure 4.6**) displayed a molecular peak at *m/z* 533 $[M + H]^+$ consistent with the molecular formula $C_{25}H_{24}O_{13}$. The assessed genkwanin-4'-*O*-β-D-glucopyranosyl moiety accounted for $C_{22}H_{22}O_{10}$ thus implying that a further fragment $C_3O_3H_2$ remained to be arranged in the structure of **322**. This residue was suggested to be an acyl moiety esterifying an hydroxyl group of the glucospyranose portion of the molecule.

Comparison of the proton and carbon chemical shifts values of compound **322** with those of genkwanin-4'-*O*-β-D-glucopyranoside revealed differences in the resonances of H_2-6''protons of the sugar moiety. In fact, they resonated at δ_H 4.08 and 4.25, which were downfield shifted with respect to the corresponding values (δ_H 3.11-3.79) observed for the

non-esterified compound (Veit et al., 1990). According to the expected acylation chemical shift difference, C-6 resonated at δ_C 64.5 in compound **322**, compared with δ_C 60.7 observed in genkwanin-4'-*O*-β-D-glucopyranoside (**324**). Thus the remaining fragment was established to be a malonyl residue linked to 6''-OH of the glucosyl portion. A fragmentation peak at *m/z* 447 corresponding to the loss of the malonyl acid unit (*m/z* 86) was observed in the MALDI mass spectrum (**Figure 4.6**) of **28**, further supporting this assumption.

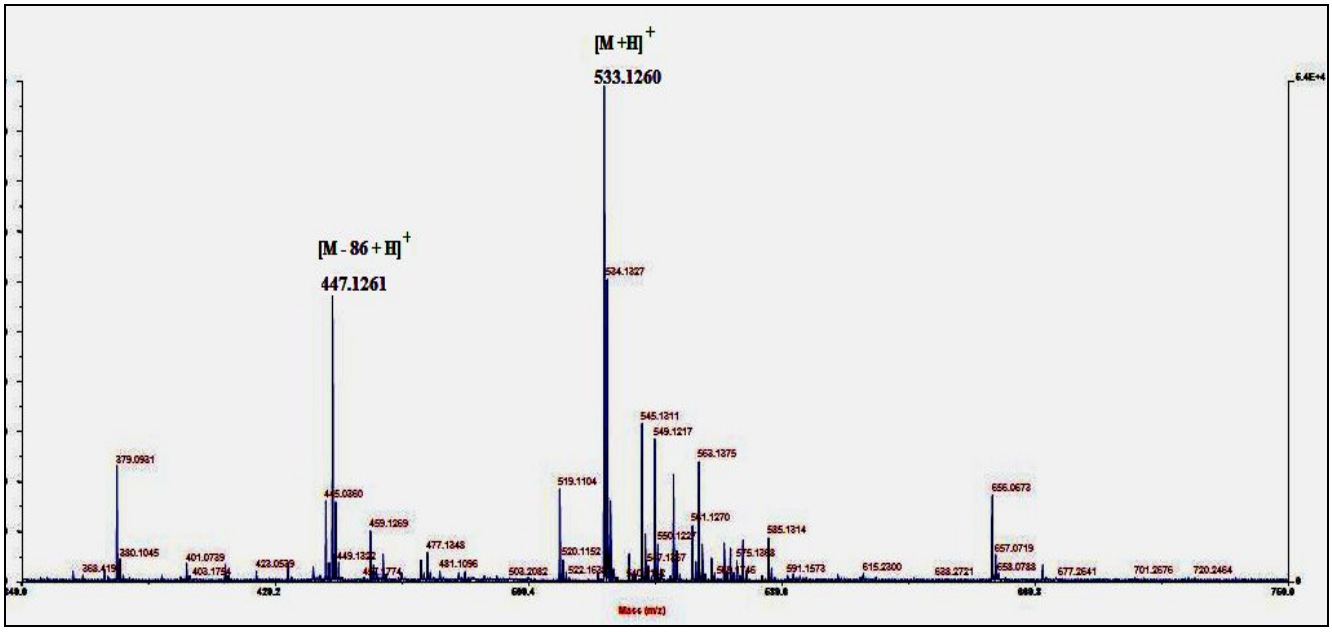


Figure 4.6: MALDI mass spectrum of compound 322

The chromatographic behaviour of compound **322** was consistent with the proposed structure. In fact, it showed higher R_f value in the solvent system $CHCl_3$/MeOH (70:30) in comparison with non-acylated glycosyl flavone. Compound **322** was also observed to be very unstable in aqueous or methanolic solution, readily transforming into the corresponding genkwanin-4'-*O*-β-D-glucopyranoside **(324)**. Furthermore, the signal due to methylene protons of the malonyl group rapidly disappeared in the ^{1}H NMR spectrum of **322** when it was recorded in a protic solvent (chloroform, methanol, water) due to the exchange of the acid methylene protons with the deuterated solvent.

Decarboxylation reactions have been reported in the literature for the malonyl esters of some previously isolated flavonoid glycosides (Markham et al., 1988), (Horowitz et al., 1989), (Takeda et al., 1993). The acidity of malonyl methylene suggested the use of an aprotic solvent (non-containing exchangeable deuterium) such as DMSO. The ^{1}H NMR and HSQC spectra (**Figures 4.7**, **4.8**) carried out in DMSO-d_6 showed the expected signals of the malonyl methylene at δ_H 2.95 and δ_C 45.0.

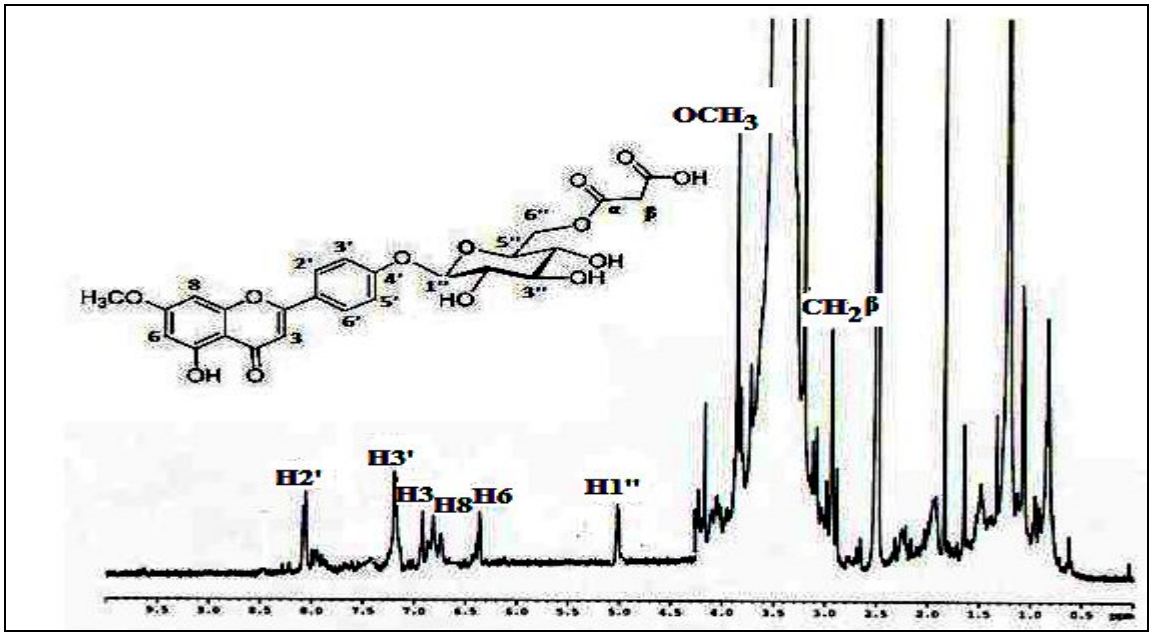


Figure 4.7: ^{1}H NMR spectrum of compound 322 in DMSO

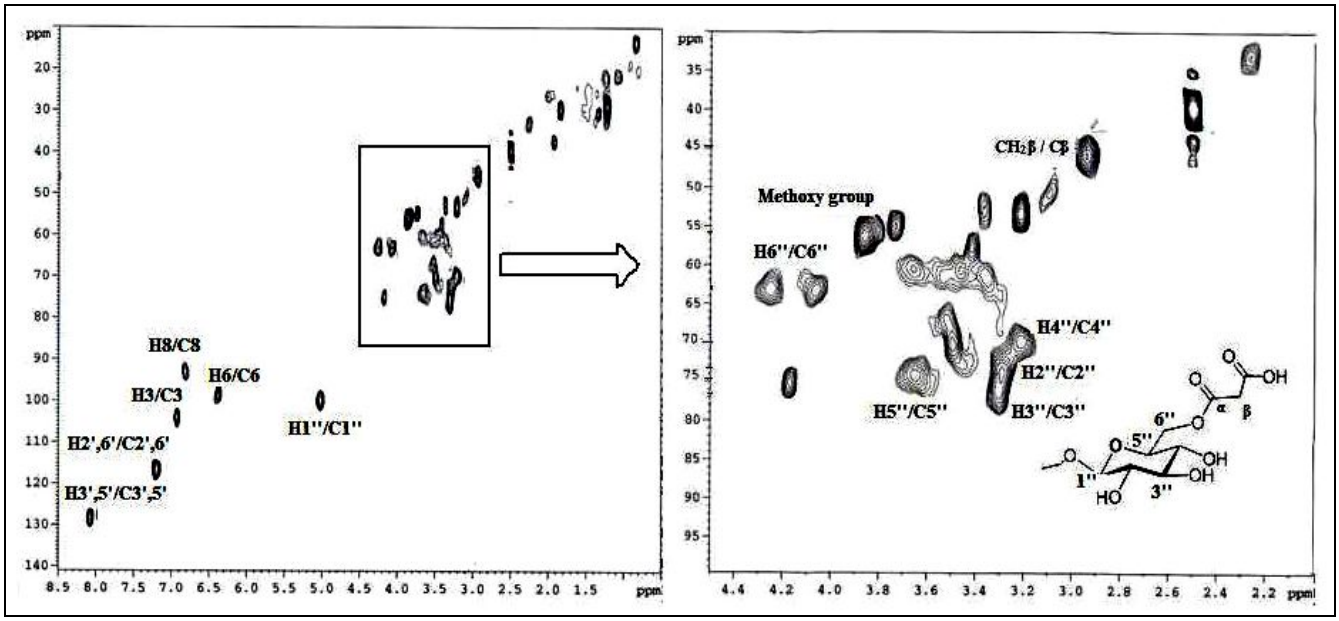


Figure 4.8: HSQC spectrum of compound 322 in DMSO-d_6

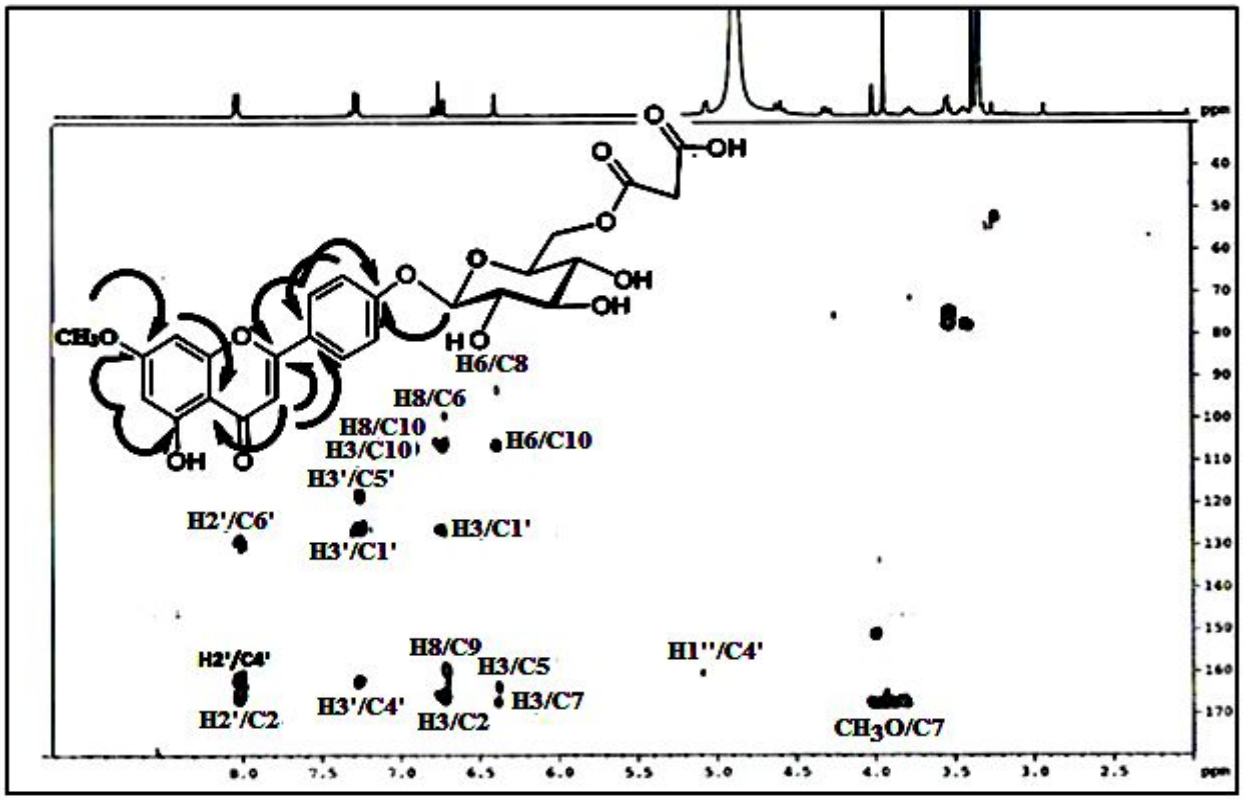


Figure 4.9: HMBC spectrum of compound 322 in MeOD

The HMBC spectrum recorded in DMSO-d_6 (**Figure 4.9**) revealed diagnostic correlations between the β-methylene singlet at δ_H 2.95 and the two ester carbonyl carbons at δ_C 168.2 and δ_C 169.5 of the malonyl moiety further confirming the structural hypothesis.

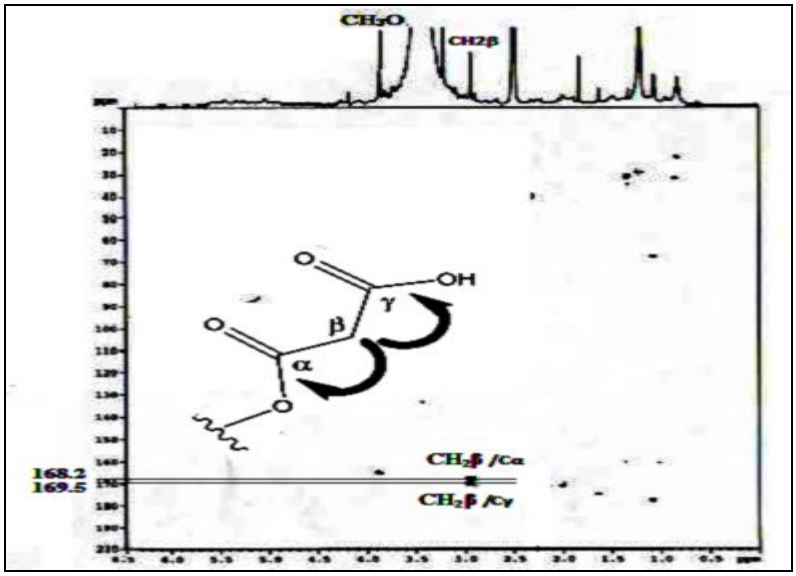


Figure 4.10: Correlations observed for the malonyl group in the HMBC spectrum (DMSO)

Table 4.1 : ^{1}H and ^{13}C NMR data of conpound 322

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)	δ ^{13}C	δ ^{1}H	m *J* (Hz)
	in MeOD			in DMSO		
2	165.6	-		163.4	-	
3	105.2	6.75	s	105.2	6.96	s
4	184.6	-		182.4	-	
5	164.2	-		160.3	-	
6	98.6	6.39	d (2.4)	98.6	6.4	d (2.4)
7	167.4	-		165.2	-	
8	93.6	6.71	d (2.4)	92.8	6.82	d (2.4)
9	159.3	-		157.3	-	
10	106.6	-		104.8	-	
1'	126.0	-		-	-	
2'.6'	129.6	8.05	d (8.9)	128.2	8.05	d (8.9)
3'.5'	118.2	7.26	d (8.9)	116.5	7.26	d (8.9)
4'	161.8	-		160.3	-	
OCH_3	56.4	3.95	s	56.5	3.82	s
Sugar moiety						
1"	101.5	5.05	d (7.2)	99.8	5.05	d (7.2)
2"	74.6	3.54	m	73.6	3.28	m
3"	77.8	3.52	m	75.9	3.30	m
4"	71.5	3.43	m	69.6	3.20	m
5"	75.7	3.87	m	73.8	3.64	m
6"a	64.7	4.33	dd (6.1, 11.8)	64.5	4.08	dd (11.8, 6.1)
6"b	-	4.54	d (11.8)		4.25	d (11.8)
Malonyl moiety						
α				168.2	-	
β	-			45.7	2.94	
γ	-			169.5	-	
	-					

In conclusion, the structure of new compound **322** was established to be genkwanin-4'-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside. All proton and carbons resonances were assigned by HSQC and HMBC experiments (**Figures 4.4**, **4.9**) as reported in **Table 4.1**.

4.3.2 Compound 323

Compound **323** displayed similar spectral data as compound **322**, suggesting that they exhibited the same flavone aglycone. The molecular formula $C_{24}H_{22}O_{13}$ of compound **323**, that was established by MALDI mass spectrum (**Figure 4.11**) containing the molecular peak at *m/z* 519, was lacking of a CH_2 moiety (14 mass units) with respect to compound **322**.

Accordingly, in the ^{1}H NMR spectrum (**Figure 4.12**) of **323** the methyl singlet due to the methoxyl group, which was present in the proton spectrum of **322,** disappeared.

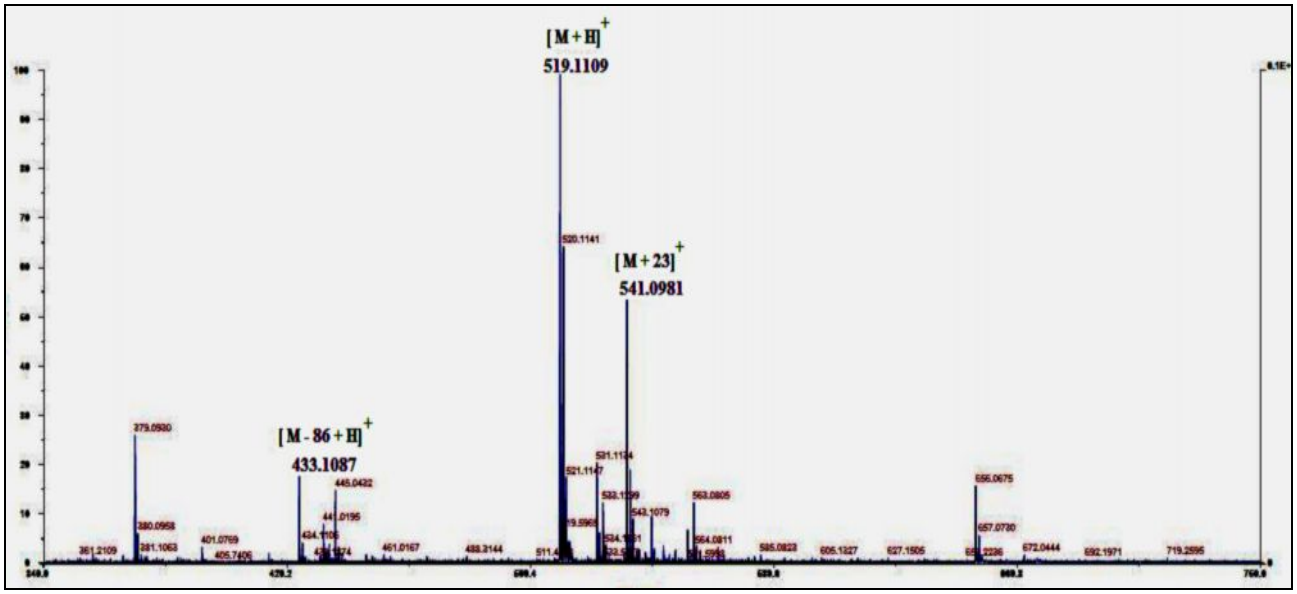


Figure 4.11: MALDI mass spectrum of compound 323

The ^{1}H NMR spectrum (**Figure 4.12**) showed a singlet at δ 6.68 (1H, s), and two doublets at δ 6.51 (1H, d, *J* = 2.4 Hz) and δ 6.84 (1H, d, *J* = 2.4 Hz), which were assigned to protons H-3, H-6, and H-8. The presence of two pairs of ortho-coupled aromatic protons was revealed by signals at δ 7.92 (2H, d, *J* = 8.9 Hz, H-2',6') and δ 6.98 (2H, d, *J* = 8.9 Hz, H-3',5') and indicated a 1,4 substituted benzene ring. Thus, the flavonoid aglycone was identified as 6-hydroxyapigenin (Redaelli et al., 1980), (Stockmal et al., 2001).

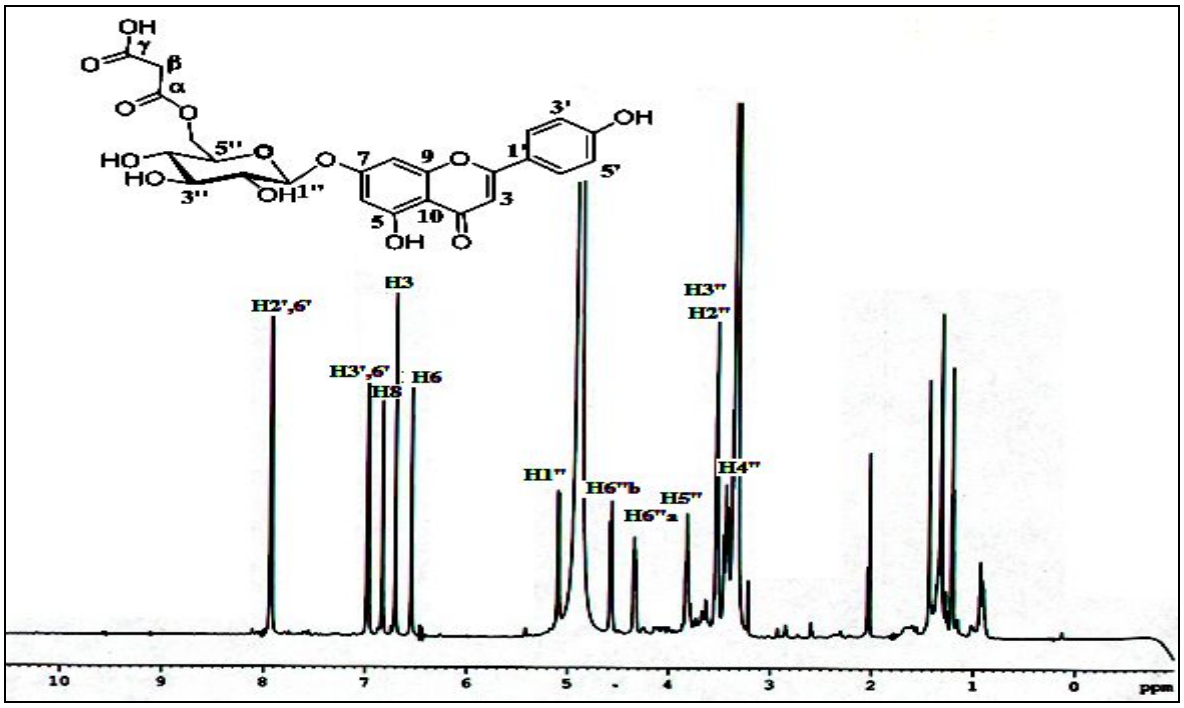


Figure 4.12: ^{1}H NMR spectrum of compound 323 in MeOD

A malonyl glucose moiety accounted for the remaining signals in the ^{1}H NMR spectrum including the anomeric signal at δ 5.08 (1H, d, *J* = 7.2 Hz, H-1') and six oxymethine multiplets in the range δ 3.40 – 4.60. The H_2-6''methylene resonated at δ 4.33 (1H, dd, *J* = 11.8, 6.1 Hz) and 4.50 (1H, d, *J* = 11.8 Hz), downfield shifted with respect to non-esterified corresponding derivative, that was due to acylation influence by the malonyl residue. This inferred that the malonyl group was attached at the carbon C-6''. The stability of compound **323** was very low. In fact, it was observed that **323** underwent to a rapid deacylation reaction during work-up to give the corresponding non-esterified derivative apigenin-7-*O*-β-D-glucopyranoside (**326**).

Thus, compound **323** was identified as apigenin-7-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside. This compound was first isolated from parsley cell cultures in 1973, but the location of the malonic acyl residue was not confirmed (Kreuzaler et al., 1973). Later, it was isolated from the blue flowers of the garden lupin *Russell hybrids*, derived from *Lupinus polyphyllus* and *Lupinus arboreus* (Takeda et al., 1993) and in some chamomile species, *Chamomilla recutita* and *Matricaria chamomilla* (Švenhlíková et al., 2004, 2006).

The NMR data are summarised in **Table 4.2**. The assignment was made on the basis of ^{1}H and ^{13}C NMR and HSQC spectra (**Figures 4.12**, **4.13**, **4.14**) as well as by comparing the spectroscopic data with those of compound **322**.

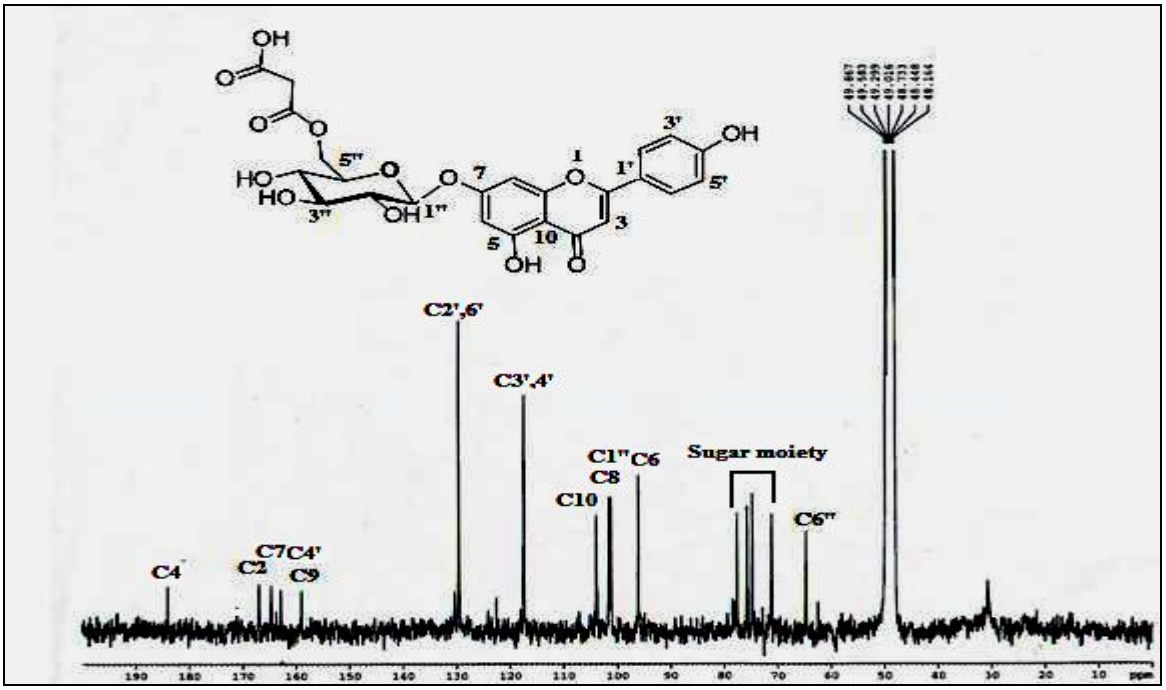


Figure 4.13: ^{13}C spectrum of compound 323 in MeOD

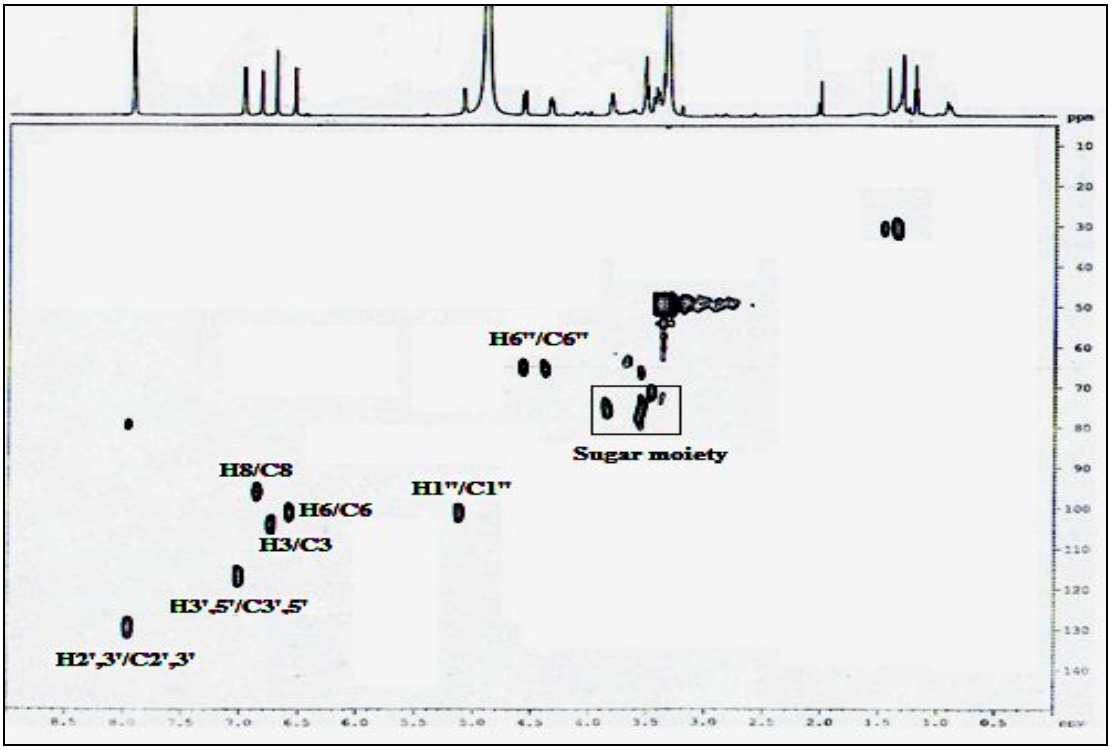


Figure 4.14: HSQC spectrum of compound 323 in MeOD

Table 4.2 : ^{1}H and ^{13}C NMR data of conpound 323 in MeOD

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)
2	165.7	-	
3	103.9	6.70	s
4	184.5	-	
5	160.3	-	
6	101.2	6.51	d (2.4)
7	163.2	-	
8	96.1	6.82	d (2.4)
9	159.4	-	
10	107.2	-	
1'	126.0	-	
2',6'	129.6	8.05	d (8.9)
3',5'	117.4	7.26	d (8.9)
4'	161.8	-	
Sugar moiety			
1"	101.6	5.08	d (7.2)
2"	74.8	3.55	m
3"	77.6	3.55	m
4"	71.2	3.43	m
5"	75.7	3.81	m
6"a	64.7	4.35	dd (11.8, 6.1)
6"b	-	4.50	d (11.8)
Malonyl moiety			
α	-		
β	-		
γ	-		

It is interesting to note that this is the first finding of malonylated flavonoids in marine organisms whereas apigenin and luteolin malonyl glycosides have been reported from some terrestrial plants.

4.3.3 Compounds 324 and 325

Compounds **324** and **325** were also isolated as pale yellow powders. Their ESIMS spectra (**Figures 4.15**, **4.16**) showed sodiated molecular peaks at *m/z* 469 [M + 23], and *m/z* 455 [M + 23] which were consistent with the molecular formulas $C_{22}H_{22}O_{10}$ and $C_{21}H_{20}O_{10}$, assigned to compounds **324** and **325**, respectively. The difference of 14 units between the two mass values suggested the presence of a methoxy group in compound **324**.

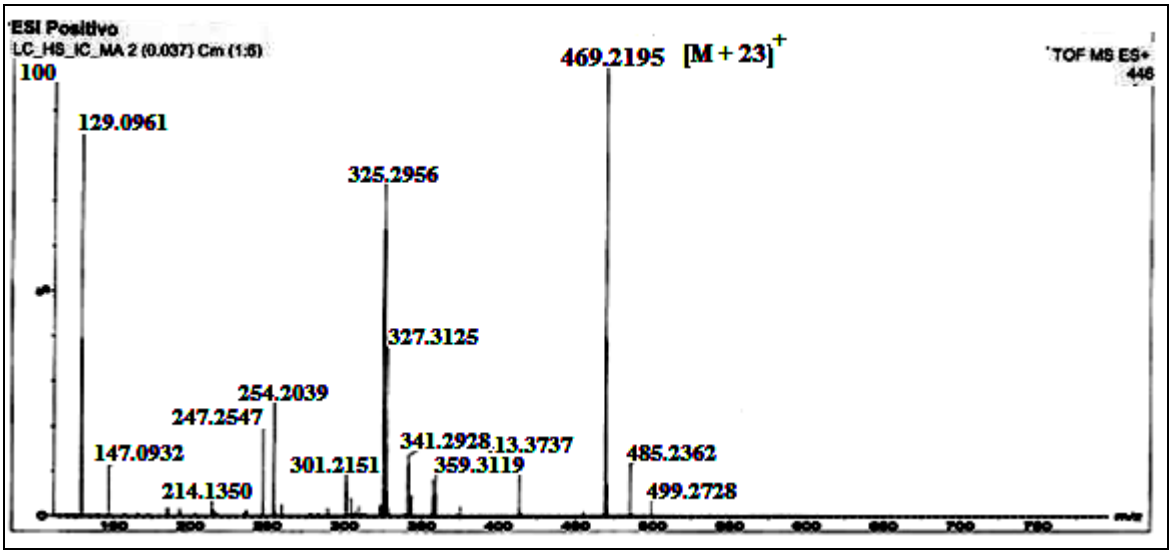


Figure 4.15: ESIMS spectrum of compound 324

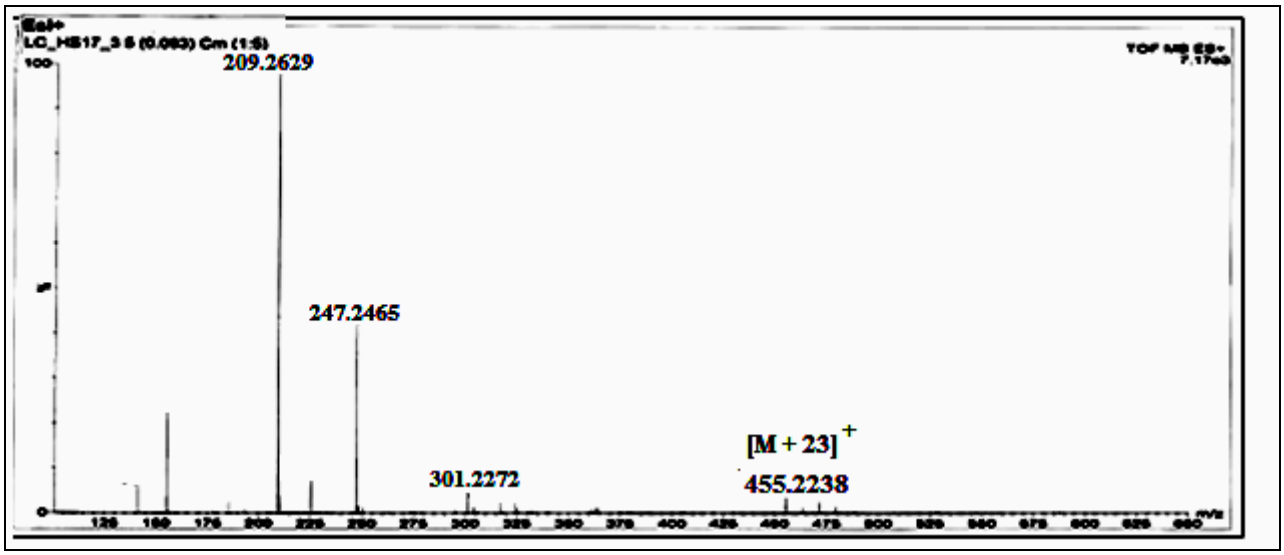


Figure 4.16: ESIMS spectrum of compound 325

The ^{1}H NMR spectra of both compounds (**Figure 4.17** and **Figure 4.19**) revealed similar signals as compounds **322** and **323**, respectively. The only difference was the chemical shift values of H_2-6'' protons of the glycopyranoside moiety that resonated in both **324** and **325** at δ 3.74 (1H, dd, *J* = 11.7, 5.3 Hz, H-6''a) and δ 3.95 (1H, d, *J* = 11.7, H-6''b), upfield shifted with respect to the corresponding protons (δ 4.33 and 4.54 in **322**, 4.35 and 4.50 in **323**) in related compounds. This clearly indicated that in compounds **324** and **325** the 6-OH group was free and therefore they were the non-esterified derivatives of **322** and **323**, respectively.

Accordingly, the carbon value of C-6 was observed at δ 62.5 in both compounds (**Figures 4.18, 4.20**). These molecules were finally identified as genkwanin-4'-*O*-β-D-glucopyranoside (**324)** and apigenin-7-*O*-β-D-glucopyranoside (**325)**.

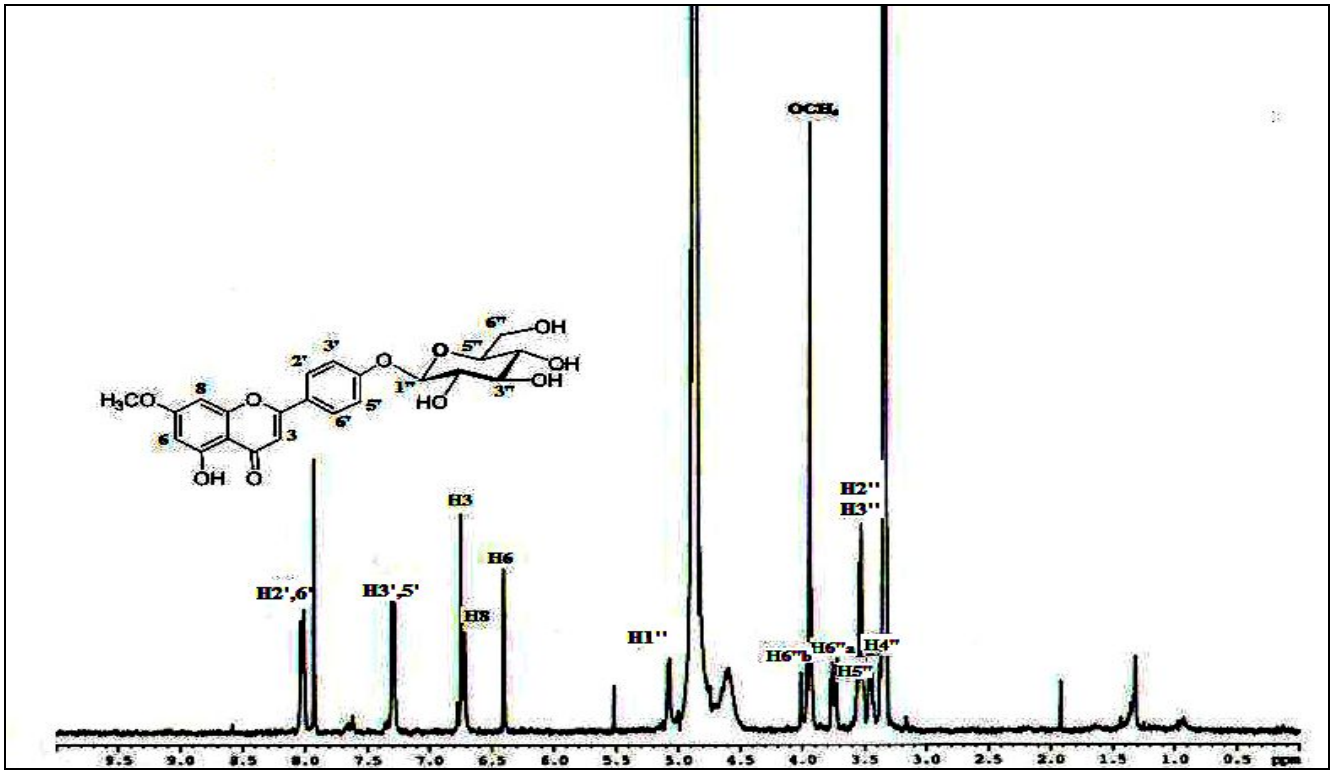


Figure 4.17: [1]H NMR spectrum of compound 324 in MeOD

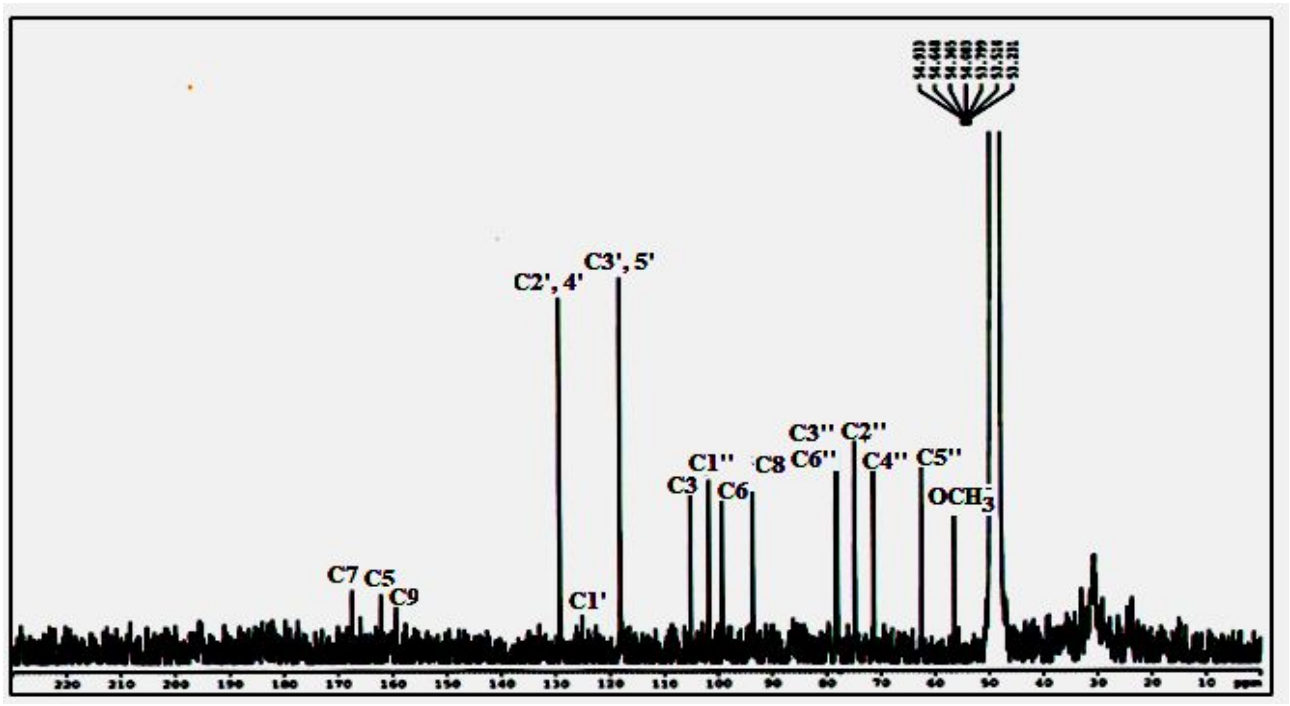


Figure 4.18: [13]C NMR spectrum of compound 324 in MeOD

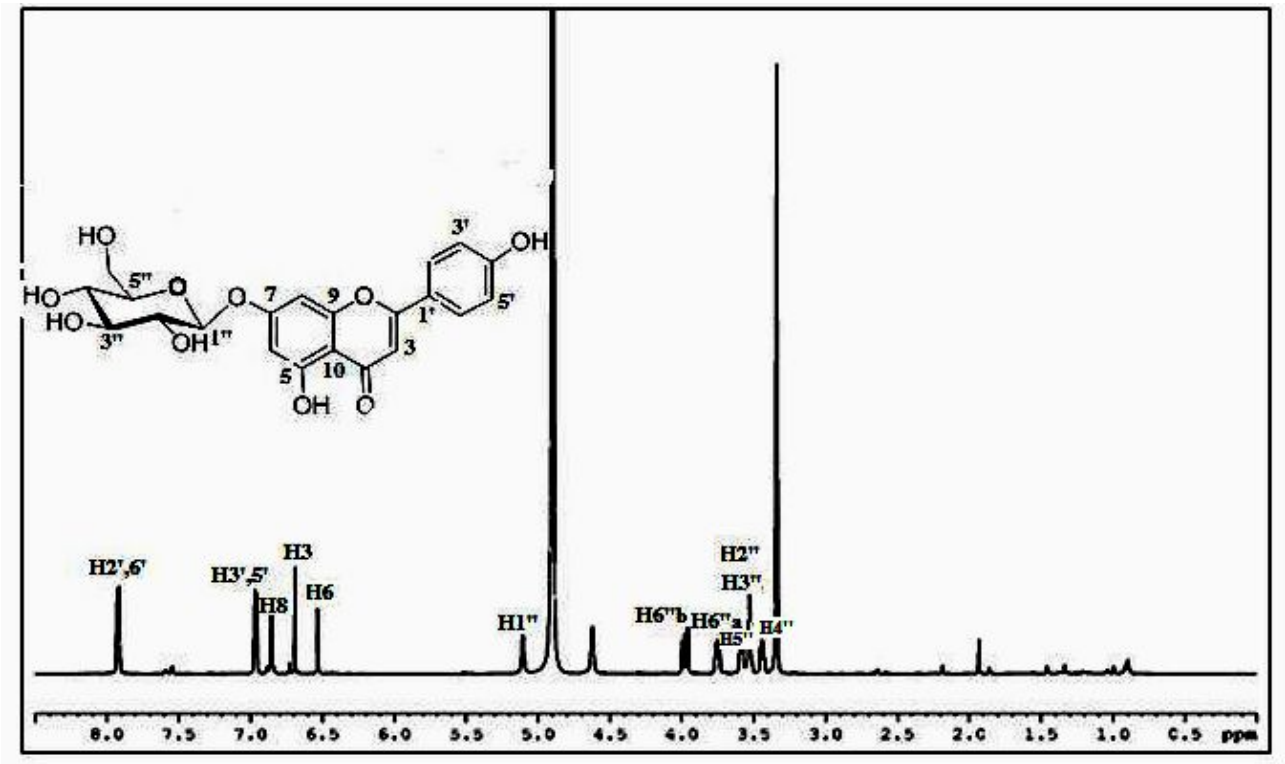


Figure 4.19: ^{1}H NMR spectrum of compound 325 in MeOD

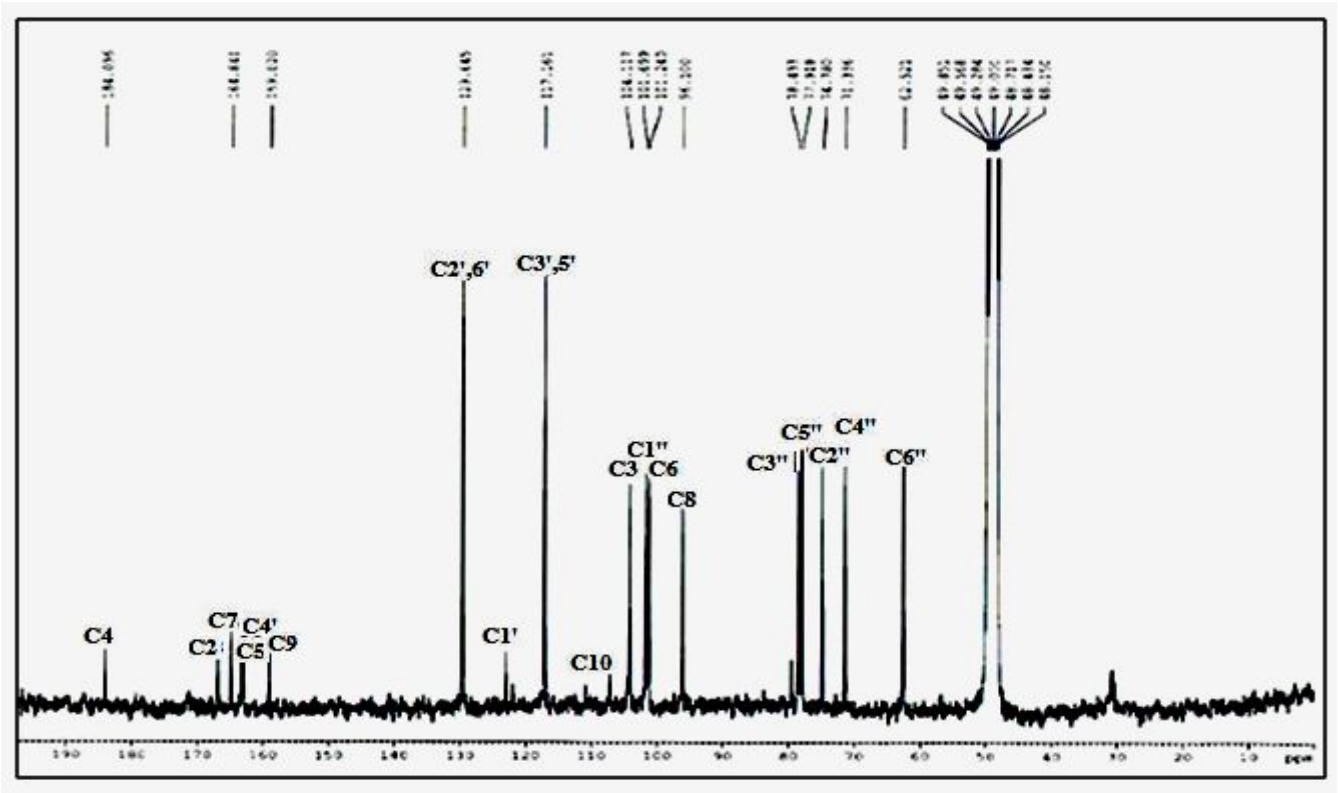


Figure 4.20: ^{13}C NMR spectrum of compound 325 in MeOD

All carbon and proton assignments are reported in **Table 4.3**.

Table 4.3 : ^{1}H and ^{13}C NMR data of compounds 324 and 325 in MeOD

Position	Compond 324			Compound 325		
	δ ^{13}C	δ ^{1}H	m *J* (Hz)	δ ^{13}C	δ ^{1}H	m *J* (Hz)
2	164.8	-		167.2	-	
3	104.5	6.75	s	104.1	6.70	s
4	182.9	-		184.1	-	
5	162.2	-		162.8	-	
6	98.2	6.40	d (2.4)	101.2	6.50	d (2.4)
7	166.3	-		164.8	-	
8	92.2	6.71	d (2.4)	96.1	6.82	d (2.4)
9	157.9	-		159.1	-	
10	105.1	-		107.8	-	
1'	124.9	-		124.8	-	
2'.6'	129.3	8.05	d (8.9)	129.6	7.92	d (8.9)
3'.5'	118.1	7.30	d (8.9)	117.2	6.98	d (8.9)
4'	160.9	-		162.4	-	
Sugar moiety						
1"	102.1	5.08	d (7.2)	101.7	5.09	d (7.2)
2"	74.8	3.52	m	74.8	3.52	m
3"	77.9	3.52	m	78.4	3.52	m
4"	71.3	3.42	m	71.3	3.43	m
5"	78.4	3.54	m	77.9	3.57	m
6"a	62.5	3.74	dd (11.8, 6.1)	62.5	3.74	dd (11.8, 6.1)
6"b	-	3.95	d (11.8)	-	3.95	d (11.8)

Apigenin and glycoside derivatives have been identified from many plant sources, such as alfalfa (*Medigaco sativa)* (Stockmal et al., 2001), *Chamomilla recutita* and *Matricaria chamomilla* (Švenhl□ková et al., 2004, 2006). Genkwanin and glycoside derivatives have been reported for the first time from the barren sprouts of *Equisetum arvense* (Horowitz et al., 1989). However, to the best of our knowledge, compounds **324** and **325** have never been reported from the marine organisms whereas apigenin was found in *Halophila jonhsonii* (Meng et al., 2008).

4.3.4 Compound 326

Compound **326** was isolated in small amount. The ^{1}H NMR spectrum (**Figure 4.21**) showed practically similar signals as compound **324** with the exception of the methoxyl singlet which was absent. Thus, the compound was identified as apigenin-4'-*O*-β-D-glucopyranoside.

The structure was finally confirmed by comparison with literature NMR data (Nawwar et al., 1994).

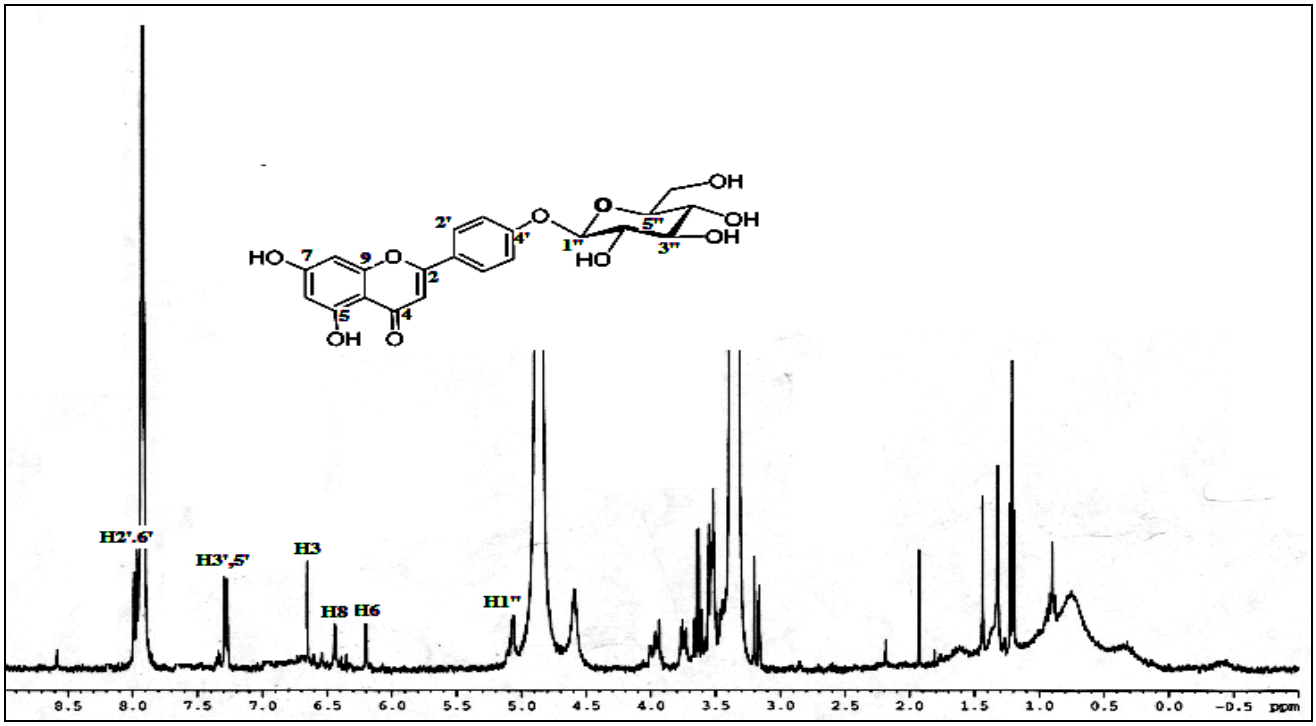


Figure 4.21: ^{1}H NMR spectrum of compound 326 in MeOD

4.3.5 Compound 327

Comparison of the ^{1}H NMR spectrum of compound **327** (**Figure 4.22**) with literature data (Hartwing et al., 1990), (Kellam et al., 1994), indicated the presence of the luteolin skeleton. In the spectrum, which was similar to that of co-occurring compound **326**, were present signals at δ 6.20 (1H, s, H-6), δ 6.40 (1H, d, J = 1.8 Hz, H-8), and δ 6.70 (1H, d, J = 1.8 Hz, H-3), according to a susbstitution pattern of rings A and B the same as **323**. In contrast, ring C exhibited a different substitution. In particular, the presence of a *meta*-coupled proton doublet at δ 7.57 (1H, d, J = 1.9 Hz), and the double doublet at δ 7.61 (1H, dd, J = 8.9, 1.9 Hz) suggested an additional substituent in ring C'.

The comparison of ^{1}H NMR data with those of luteolin-4'-*O*-β-D-glucopyranoside (Kellam et al., 1993), revealed immediately that the methoxyl group resonating at δ 4.0 was attached at the position C-3', and thus this compound was identified as chrysoeriol-4'-*O*-β-D-glucopyranoside.

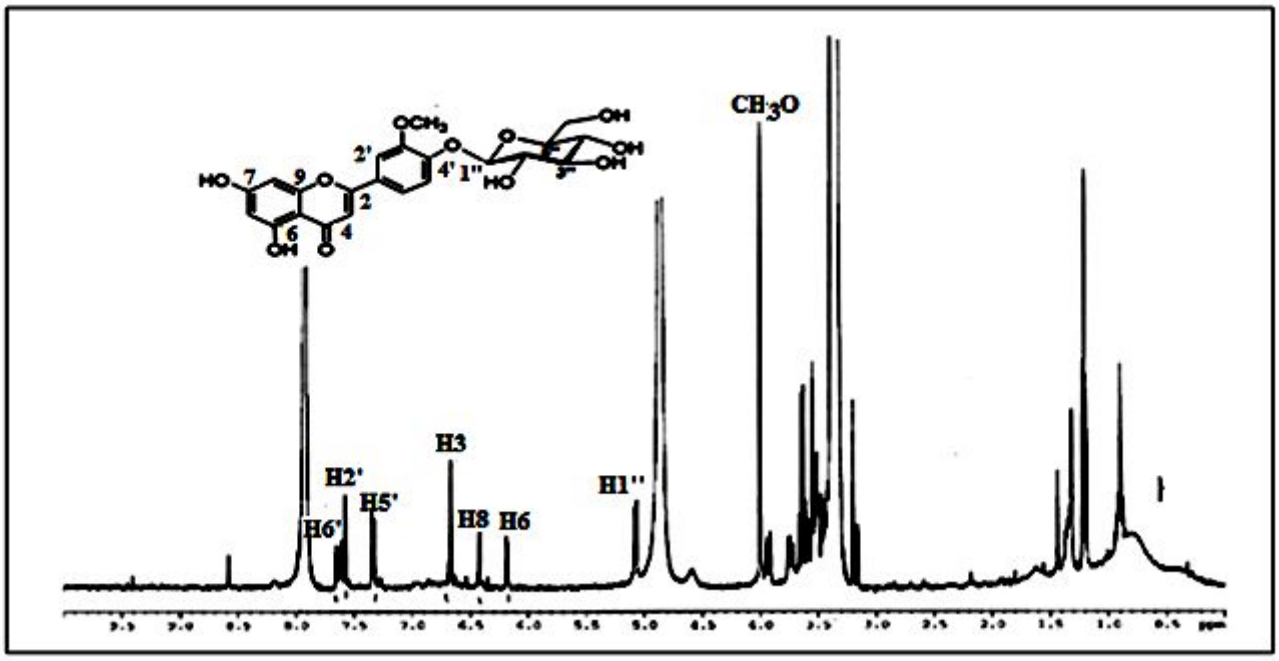


Figure 4.22: ^{1}H NMR spectrum of compound 327 in MeOD

4.3.6 Compound 328

The analysis of the ^{1}H NMR spectrum of compound **328** (**Figure 4.23**) revealed that it had the same skeleton as compound **327**. The two compounds differed in the linkage position of the glucopyranosyl residue. The protons H-6, H-3, and H-8 resonated at chemical shift values slightly different from those of compound **327** [δ 6.51, δ 6.73, and δ 6.90, respectively] indicating that the sugar moiety was attached in ring A.

Compound **328** was finally confirmed to be chrysoeriol-7-*O*-β-D-glucopyranoside, by comparing the NMR values with literature data (Harput et al., 2006).

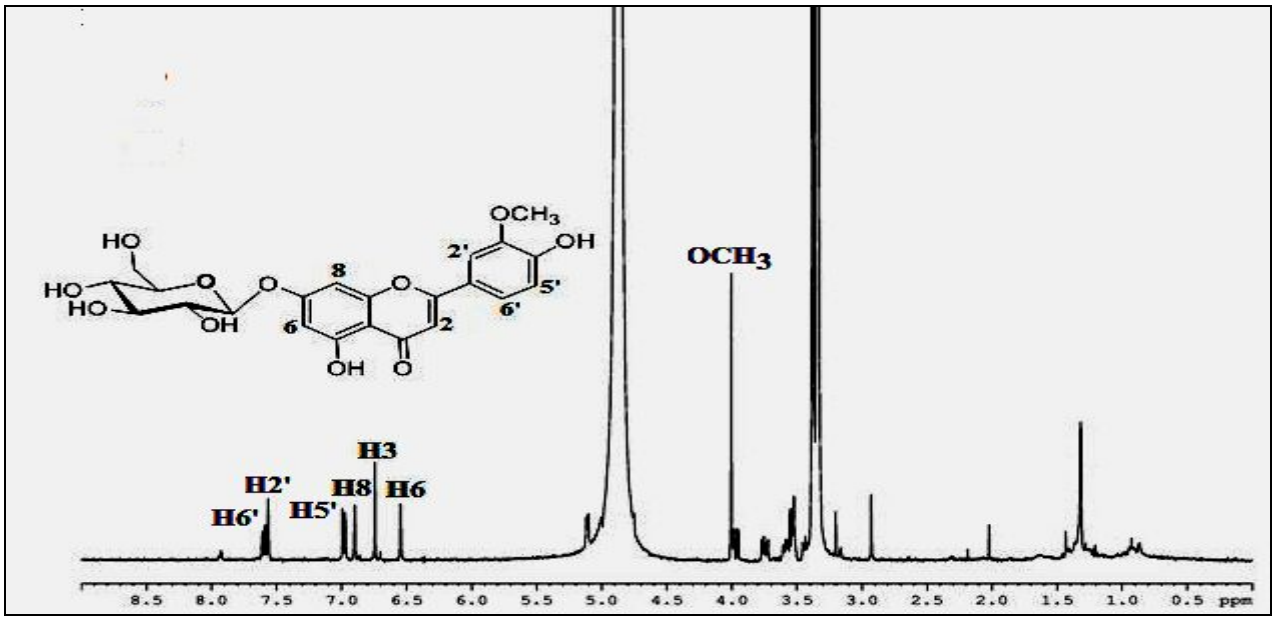
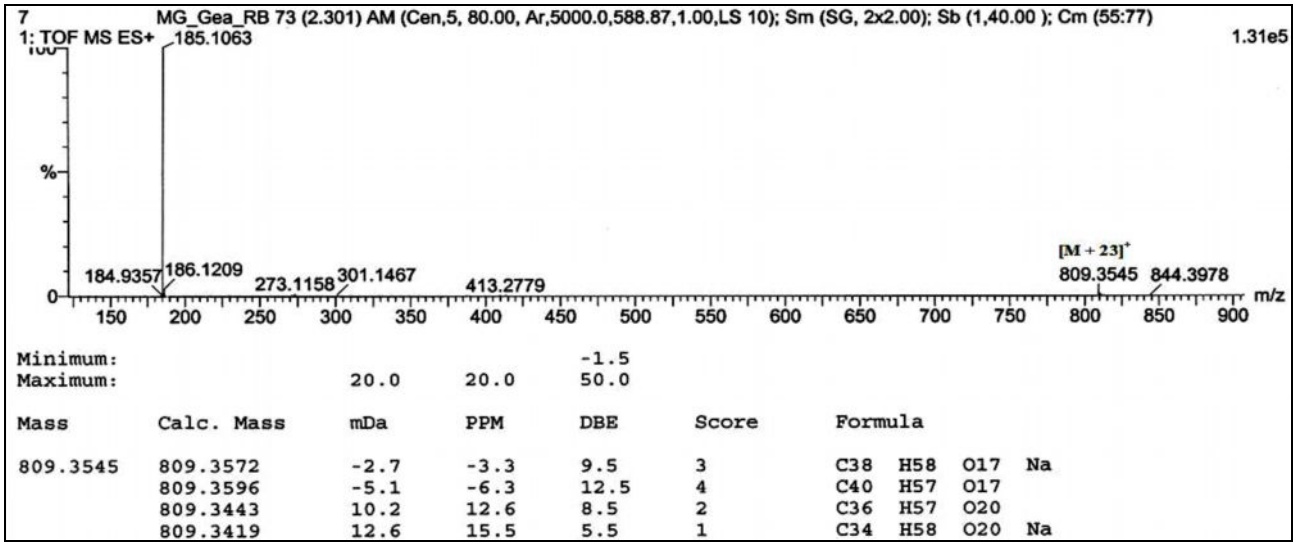


Figure 4.23: ^{1}H NMR spectrum of compound 328

4.3.7 Compounds 286 and 287

Compound **286** was the main compound of the polar fraction whereas compound **287** was isolated in traces.

The HRESIMS spectrum (**Figure 4.24**) displayed a pseudo-sodiated molecular peak at *m/z* 809.3545 which indicated the molecular formula $C_{38}H_{58}O_{17}$, implying 10 degrees of unsaturation.



Minimum:				-1.5		
Maximum:		20.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula
809.3545	809.3572	-2.7	-3.3	9.5	3	C38 H58 O17 Na
	809.3596	-5.1	-6.3	12.5	4	C40 H57 O17
	809.3443	10.2	12.6	8.5	2	C36 H57 O20
	809.3419	12.6	15.5	5.5	1	C34 H58 O20 Na

Figure 4.24: HRESIMS mass spectrum of compound 286

A preliminary analysis of both proton and carbon spectra of compound **286** revealed the presence of a complex carbon framework formed by structurally distinct portions.

The ^{1}H NMR spectrum (**Figure 4.25**) contained signals that suggested the presence of a diterpenoid skeleton. In fact, four methyl signals at δ 0.99 (s, H_3-20), 1.03 (d, J = 7.0 Hz, H_3-17), 1.33 (s, H_3-19), and 1.86 (br s, H_3-16) were observed in the spectrum. Two multiplets at δ 5.46 (br t, J = 7.0 Hz, H-14) and δ 6.44 (t, J = 3.0 Hz, H-3) were assigned to vinyl protons of two trisubstituted double bonds one of which α,β,-unsaturated.

In addition, a methine and a methylene both linked to an oxygen atom were also present in the terpenoid portion as it was indicated by the signals at δ 4.12 (1H, dd, J = 8.1 and 1.3 Hz, H-11) and 4.64 (2H, m, H_2-15). A search in the literature on different diterpenoid arrangements revealed that the terpenoid part in compound **286** could be a clerodane exhibiting the methyl C-18 oxidised and two hydroxyl functions at C-11 and C-15. Accordingly, the ^{13}C NMR spectrum (**Figure 4.26**) of **286** showed the typical set of signals due to a clerodane including the resonances due to the suggested functionalisation: the α,β,-unsaturated ester carboxyl at δ 169.2 (C-18), the hydroxylmethylene at δ 62.4 (C-15) and the carbinolic methine at δ 81.1 (C-11).

The ^{1}H NMR spectrum also displayed a series of signals in the region of δ 3.20-4.64 that were attributed to protons linked to oxygen-bearing carbons. Two diagnostic doublets at δ 5.51 (1H, d, J = 8.0 Hz, H-1') and δ 4.30 (1H, d, J = 8.0 Hz, H-1''') were assigned to the anomeric protons of two sugar moieties, the first of which connected through an ester linkage.

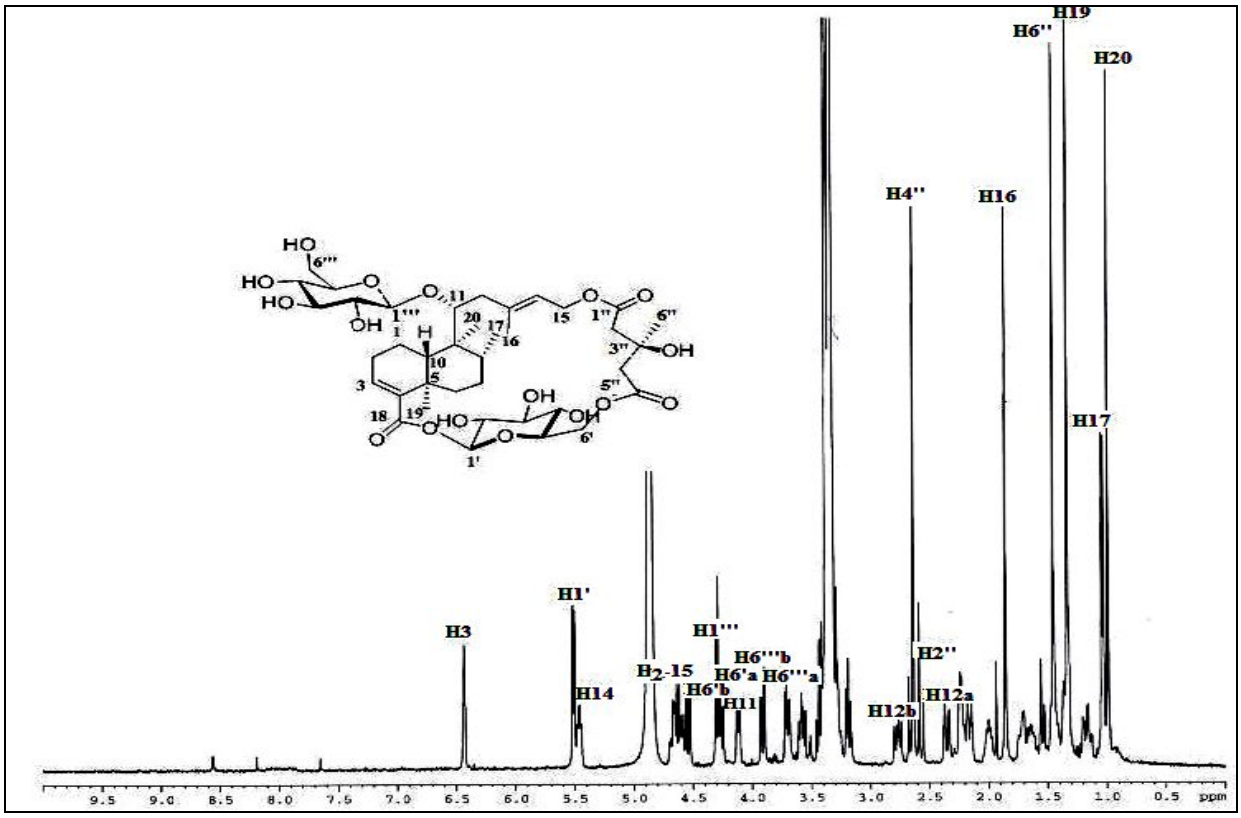


Figure 4.25: ^{1}H NMR spectrum of compound 286 in MeOD

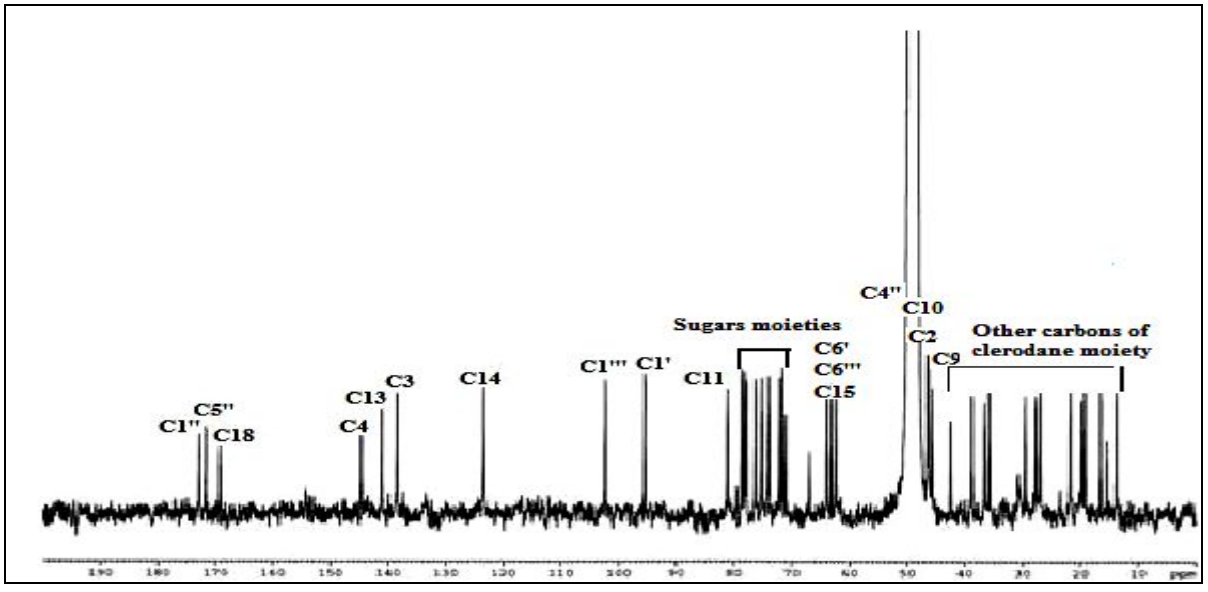


Figure 4.26: ^{13}C NMR spectrum of compound 286 in MeOD

The ^{1}H-^{1}H COSY spectrum (**Figure 4.27**) aided the definition of the spin sequences in the clerodane moiety and in the two glycosyl units in agreement with the above structural suggestions. Furthermore, the HSQC spectrum (**figure 4.28**) allowed to correlate the proton and carbon values for these parts.

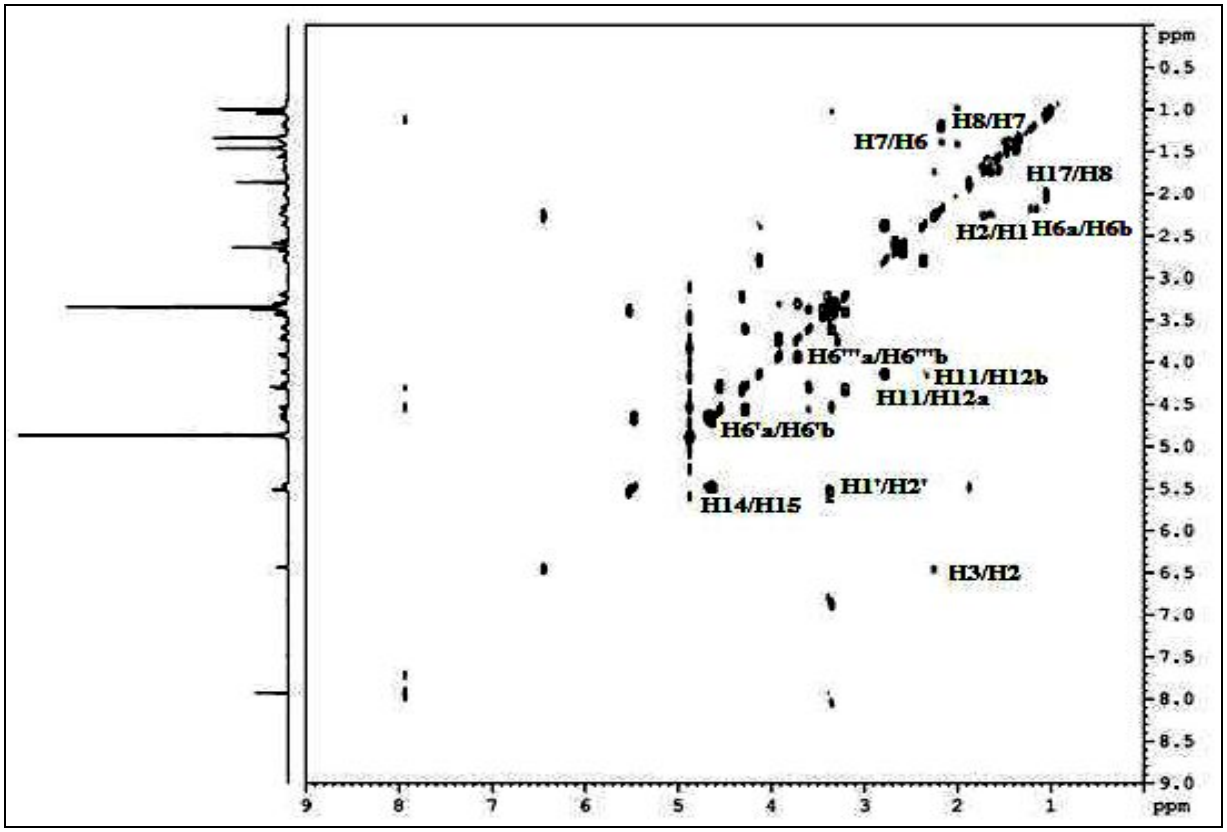
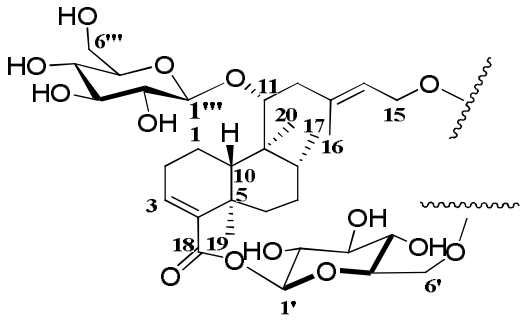


Figure 4.27: ^{1}H-^{1}H COSY spectrum of compound 286 in MeOD

The three above distinct structural moieties were connected by interpretation of the HMBC spectrum (**Figure 4.29**). The anomeric proton H-1''' resonating at δ 4.30 showed long-range correlations with C-11 (δ 81.1) inferring that one of the two sugar units was attached in the side chain of the clerodane framework. The other anomeric proton H-1' resonating at δ 5.51 (lower field with respect to typical glycosyl values) was correlated to the ester carboxyl at δ 169.2 supporting that the second sugar unit was connected to the α, β unsaturated acyl function through an ester linkage. By these data the structure of compound **286** was partially defined as



The downfield shift of the two protons H_2-6' of the second sugar (δ 4.27 and 4.54) proved that the hydroxyl function of the primary alcohol was esterified. The remaining signals in the ^{1}H NMR spectrum, the methyl singlet at δ 1.46 (H_3-6'') and the two signals at δ 2.64 (2H, s) and δ 2.61 (2H, AB q, J = 14.0 Hz) were attributed to the protons of 3-hydroxy-3-methyl-glutaric acid.

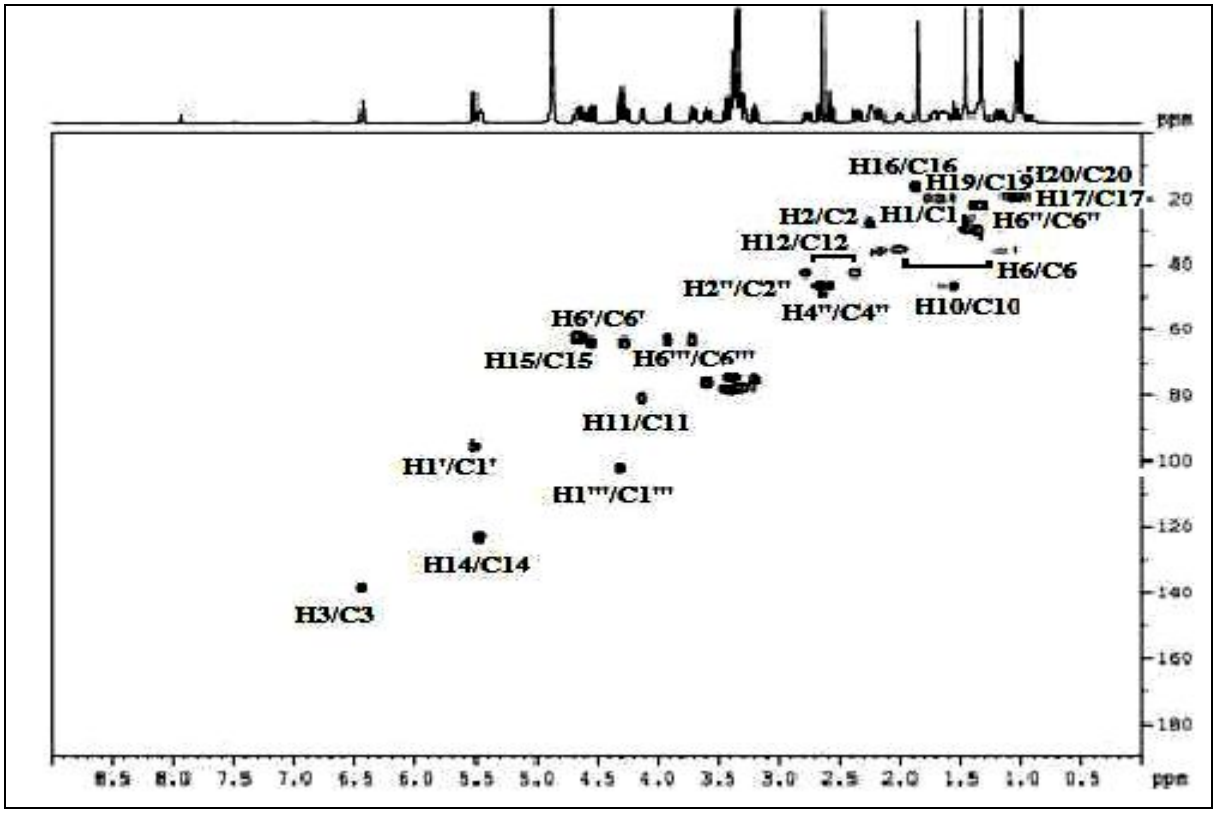


Figure 4.28: HSQC spectrum of compound 286 in MeOD

Long-range correlations (**Figure 4.29**) between the ester carboxyl at δ 171.7 (C-5'') and the protons H_2-6' (δ 4.27 and 4.54), and between the ester carboxyl at δ 172.8 (C-1'') and the protons H_2-15 connected this fragment to the remaining part of the proposed structure.

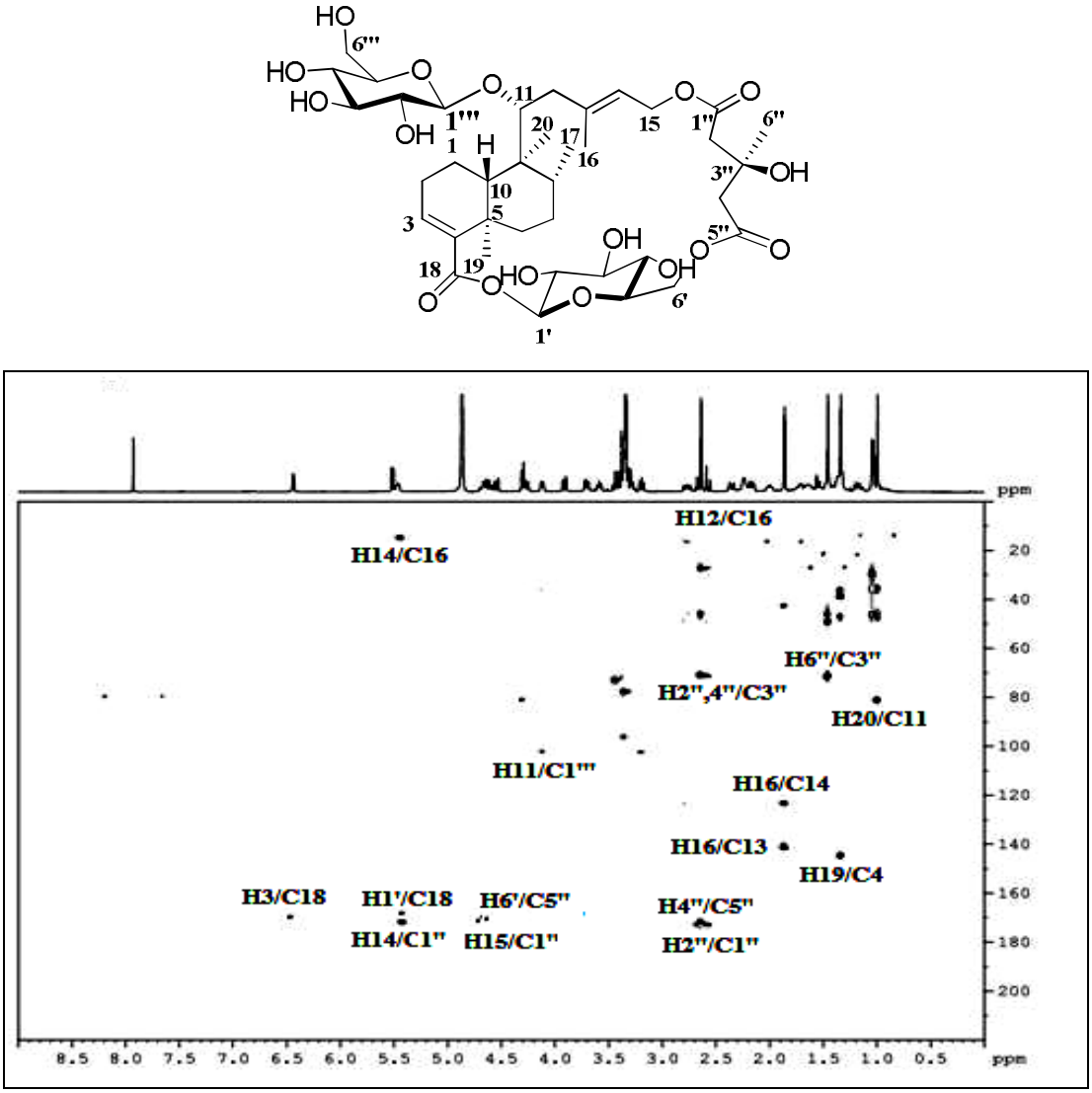


Figure 4.29: HMBC spectrum of compound 286 in MeOD

At this point, compound **286** was identified as syphonoside, previously isolated from the same source (Gavagnin et al., 2007). The spectroscopic data were identical with the literature thus inferring the identity also in the stereochemical aspects as well as in the nature of the sugar moieties that were D-glucose.

Table 4.4 : ^{1}H and ^{13}C NMR data of compound 286 in MeOD

Position	δ ^{13}C	δ ^{1}H	m *J* (Hz)
1	19.8	1.68	m
2	27.7	2.23	m
3	138.5	6.44	t (t)
4	144.6	-	
5	38.7	-	
6	36.5	1.16	ddd (14.2, 13.1, 3.4)
	-	2.16	bd (13.2)
7	29.5	1.36	m
8	35.8	1.98	m
9	45.7	-	-
10	46.6	1.55	m
11	81.1	4.12	dd (8.1, 1.3)
12	42.2	2.35	bd (15.3)
	-	2.76	dd (15.3, 9.2)
13	141.1	-	
14	123.4	5.46	bt (7.3)
15	62.4	4,64	m
16	16.4	1.86	s
17	19.2	1.03	d (7.4)
18	169.2	-	
19	21.7	1.33	s
20	13.8	0.99	s
Sugar moiety 1			
1'	95.5	5.51	d (8.2)
2'	72.0	3.37	m
3'	78.1	3.43	t (9.4)
4'	71.5	3.37	m
5'	76.3	3.59	m
6'	63.9	4.27	d (12.3)
		4.54	dd (12.3, 2.4)
Glutaric ester moiety			-
1"	172.8	-	
2"	46.3	2.61	d (14.3)
3"	71.0	-	
4"	49.6	2,64	s
5"	171.7	-	-
6"	26.9	1.46	s
Sugar moiety 2			
1'"	102.3	4.30	d (8.2)
2'"	75.2	3.20	m
3'"	78.6	3.40	m
4'"	74.0	3.40	m
5'"	77.8	3.28	m
6'"	63.1	3.71	dd (12.3, 2.3)
	ι1ψ–	3.91	dd (12.3, 6.3)

Compound **287** exhibited spectral data very similar with syphonoside **286**. The only difference in the ^{1}H NMR spectrum (**Figure 4.30**) of **287** was an additional methyl signal at δ 2.05, which was attributed to an acetyl group. The HRESIMS (**Figure 4.31**) of **287** showed the sodiated molecular peak at *m/z* 851 $[M + Na]^{+}$ consistent with the molecular formula $C_{40}H_{60}O_{18}$ that indicated the additional presence of an acyl group. Thus, compound **287** was identified as the acetyl derivative of syphonoside **286**, also isolated from the same seagrass (Carbone et al., 2008).

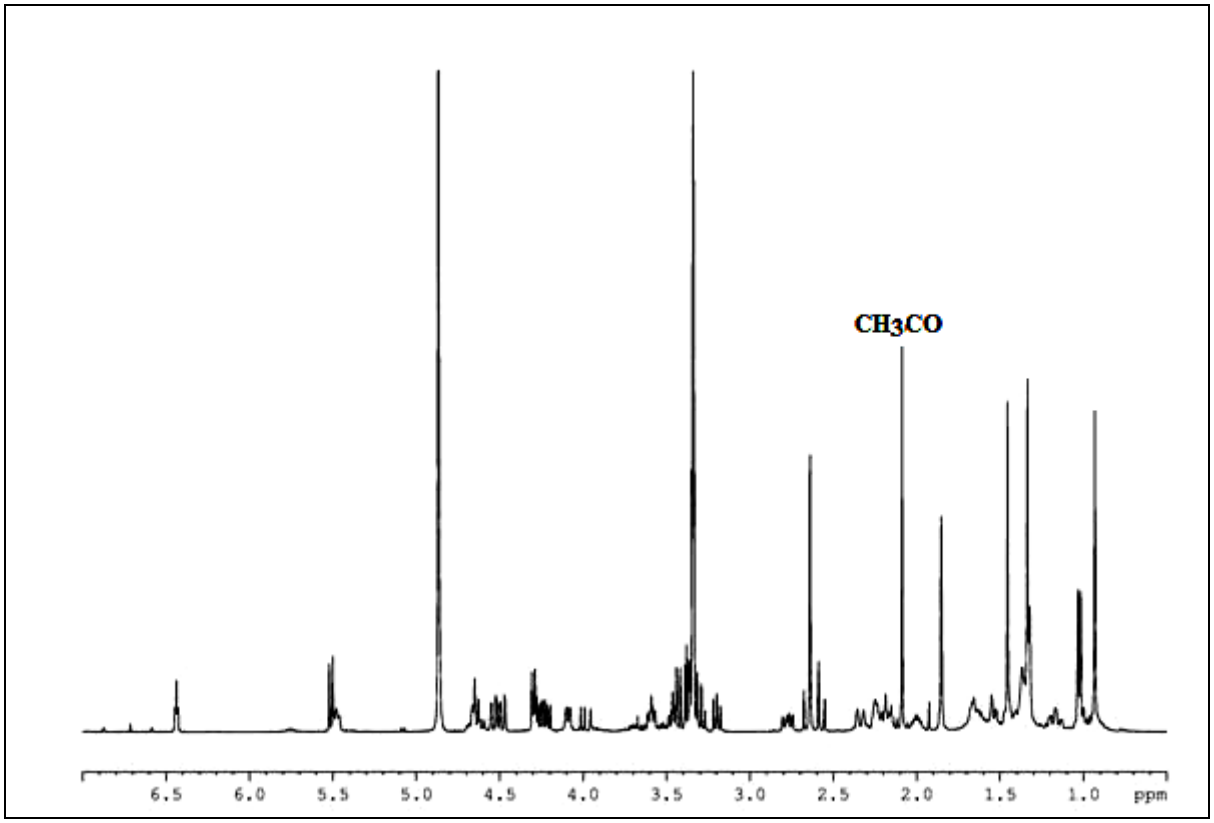
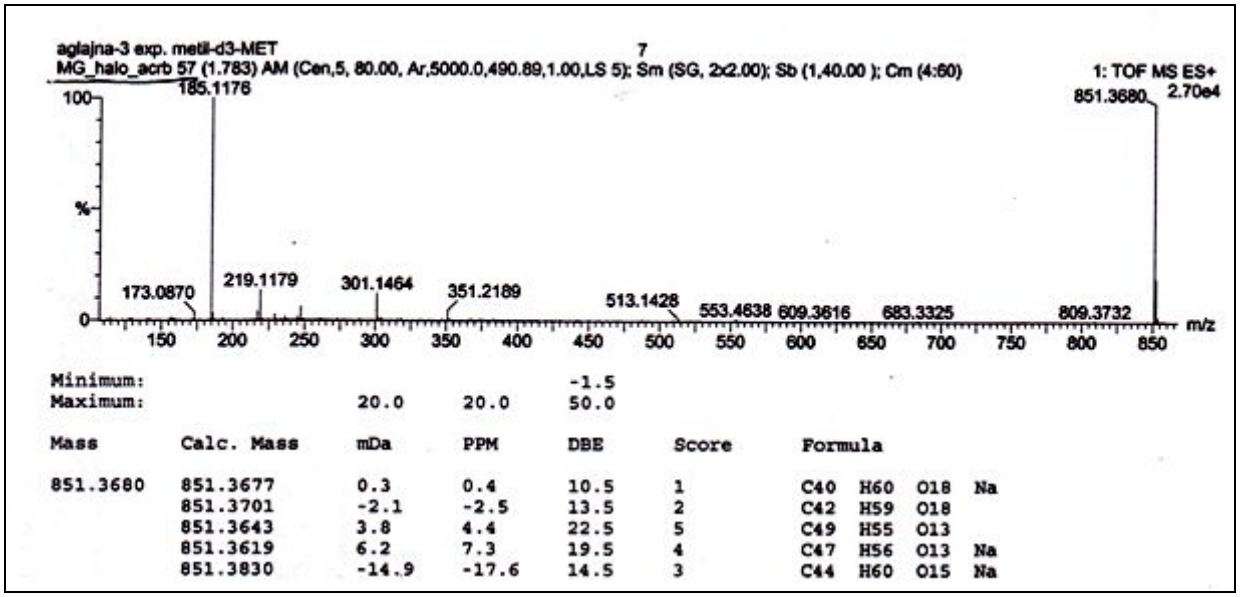


Figure 4.30: ^{1}H NMR spectrum of compound 287 in MeOD



Minimum:				-1.5					
Maximum:		20.0	20.0	50.0					
Mass	Calc. Mass	mDa	PPM	DBE	Score	Formula			
851.3680	851.3677	0.3	0.4	10.5	1	C40	H60	O18	Na
	851.3701	-2.1	-2.5	13.5	2	C42	H59	O18	
	851.3643	3.8	4.4	22.5	5	C49	H55	O13	
	851.3619	6.2	7.3	19.5	4	C47	H56	O13	Na
	851.3830	-14.9	-17.6	14.5	3	C44	H60	O15	Na

Figure 4.31: HRESIMS mass spectrum of compound 287

CONCLUSION

The phytochemical investigations of the terrestrial plant *Launaea arborescens* and marine phanerogam *Halophila stipulacea* resulted in the isolation of a plethora of natural compounds, including terpenoids and flavonoids.

First, the chemical study of the endemic Algerian plant *Launaea arborescens*, belonging to the Asteraceae family, led to the isolation of a series of sesquiterpenes and triterpenes skeletons. Twenty seven pure compounds were isolated from the liposoluble extracts of both aerial parts and roots, five of them are new.

The separation of the petroleum ether extract of the aerial parts, by using different techniques of chromatography, mainly the high pressure liquid chromatography (HPLC), allowed us to purify and identify twelve triterpenes with oleanane and ursane type skeleton, including one novel derivative of β-amyrin: 3β-hydroxy-11β-ethoxy-olean-12-ene (**1**) and the known 3β-hydroxy-11β-ethoxyurs-12-ene (**2**), 3β,11β-dihydroxy-olean-12-ene (**3**), 3β,11β-dihydroxy-urs-12-ene (**4**), 3β-hydroxy-11β-methoxyolean-12-ene (**5**), 3β-hydroxy-11β-methoxyurs-12-ene (**6**), oleana-9(11):12-dien-3β-ol **(7)**, ursa-9(11):12-dien-3β-ol (**8**), 3β-hydroxy-11-oxo-olean-12-ene (**9**) 3β-hydroxy-11-oxo-urs-12-ene (**10**) and taraxast-20-ene-3β,30-diol (**11**). Lupeol **(12)** was found to be the main triterpene of this extract and stigmasterol (**13**) was the major sterol compound of the plant extract. The structure of the new compound was fully characterized by means of spectral methods, including ^{1}H NMR, ^{13}C NMR, and 2D experiments (COSY, HSQC, HMBC), while the structures of known triterpenes were established either by comparing the chemical data with those reported in the literature or by carrying some experimental 2D NMR.

The chemical investigation of the ethyl acetate extract of roots led to the isolation of a series of sesquiterpene lactones including guaianolides, eudesmanolides and germacranolides. Fourteen sesquiterpenes have been isolated leading to seven guaianolides, two eudesmanolides and five germacranolides.

The known structures were elucidated as11β,13-dihydrolactucin **(16)**, 3α-hydroxyguaia-4(15),10(14),11(13)-triene-12,6α-olide-3-*O*-β-glycopyranoside **(17),** guaia-2-oxo-1(10),2(3), ,11(13)-triene-12,6α-olide-15-*O*-β-glycopyranoside **(18)**, 9α-hydroxy zaluzanin-3-*O*-β-glyco pyranoside **(19)**, 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin-3'-sulphate **(20)**, 1β-hydroxyeudesm-4(5)-ene-12,6α-olide **(21)**, 1β,8α-dihydroxyeudesm-4-en-6β-7α,11βH-6-olide

(22), 3β,14-dihydroxy-costunolide-3-*O*-β-glucopyranosyl-14-*O*-p-hydroxyphenyl acetate **(23)**, 3β-hydroxy-11α-metoxy-urs-12-ene **(26)**, and germacra-1(10)E,4E, 11 (13)-triene-12,6α-olide-15-*O*-β-glycopyranoside **(27)**.

Among these sesquiterpene lactones, four unprecedented compounds as 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin **(14)**, 9α-hydroxy-4α,15-dihydrozaluzanin C **(15)**, 3β,14-dihydroxy costunolide-3-*O*-β-glycopyranoside **(24)**, and 3β-hydroxycostunolide-3-*O*-β-glycopyranoside **(25)**, were isolated and fully characterized by spectral methods, mainly NMR techniques, including ^{1}H NMR 1D and 2D dimension,^{13}C NMR, IR , and mass spectrometry.

The main compound of this plethora and of the whole plant was the derivative of the lactucin skeleton 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin-3'-sulphate (**20**) recently reported in literature.

Among different approaches to the problem of determining the absolute configuration based on the NMR spectroscopy, and in order to assign the absolute configuration at the chiral centre of the alkyl chain of compound **20**, we adopted the chemical Moscher's method, which uses MTPA R-(-) and S-(+)-α-methoxy-α-trifluoromethyl-phenylacetic chlorides as a chiral derivatizing agent. The assignment of the configuration was based on the difference between the observed chemical shifts of the two diastereomers derivatives resulted from the reaction of the auxiliary reagent (MTPA) (Cα: known configuration) with the substrate **20** (C'2: unknown configuration). The NMR spectra results were compared with those obtained with the same Moscher's method, that was conducted on commercial substrates methyl-(S)-(+) 3-hydroxy-2-methyl-propionate, and methyl-(R)-(-)-3-hydroxy-2-methylpropionate.

However, the assays to establish the absolute configuration at the 6,7-junction of the lactone using the same method were unsuccessful, due to the rapid dehydration of the secondary alcohol obtained by opening the ring.

The second part of this work was devoted to the study of some phanerogams plants, among them *Halophila stipulacea* was chosen. Regardless of the chosen plant, few chemical studies have been reported on marine plants contrary to the terrestrial counterpart, but recently an increasing interest in these plants has focused on their secondary metabolites because of their ecological and economic roles played in the sea environment. Sterols and phenolic compounds were the major secondary metabolites reported from some species of seagrasses. Our study on the investigation of *Halophila stipulacea* evidenced that the flavonoid profile of *H. stipulacea* was dominated by apigenin-7-*O*-β-D-glucopyranoside **(31)** co-occurring with other minor flavone glucosides including two malonyl derivatives, the new compound

genkwanin-4'-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside **(28)** and apigenin-7-*O*-(6"-*O*- malonyl) β-D-glucopyranoside **(29)**.

Additionally, five known related glucopyranosyl flavones have been also isolated genkwanin-4'-O-β-D-glucopyranoside **(30)**, apigenin-7-*O*-β-D-glucopyranoside **(31)**, apigenin-4'-O-β-D-glucopyranoside **(32)**, chrysoeriol-4'-*O*-β-D-glucopyranoside **(33)**, and chrysoeriol-7-*O*-β-D-glucopyranoside **(34)**. The structure of the new compound was fully established by NMR techniques, mainly ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

It is noteworthy that this is the first finding of malonylated flavones in the marine environment. However, to the best of our knowledge, with the exception of a malonyl amide alkaloid that has been recently found in a marine bacterium associated with a zooanthid, no other malonyl-containing metabolites have been reported from marine organisms.

The presence of the syphonoside (**35)**, a novel macrocyclic in *Halophia stipulacea* previously isolated from the marine mollusc *Syphonata geographica* confirmed well the trophic relationship between this mollusc and its prey *Halophila stipulacea*.

Finally, it is interesting to mention here, that with the exception of a recent investigation on *H. johnsonii* resulting in the chemical characterization of all main components of the flavone pattern of this sea grass, the previous studies on different *Halophila* species including *H. stipulacea* only reported qualitative analysis of flavone profiles and indicated the presence of unidentified flavones and flavone sulphates.

This is the first time of inding of these flavones compounds in this sea grass. Accordingly, and in perspective, *Posidonia oceanica*, a second phanerogam plant will be a subject of our next phytochemical study.

Conclusion

CONCLUSION

The chemical investigation of the secondary metabolism of two plants, the endemic Algerian plant *Launaea arborescens* and the marine phanerogam *Halophila stipulacea*, has resulted in the isolation of a plethora of natural products including five new terpenoids (Bitam et al., 2008) and one new glycosyl flavonoid (Bitam et al., accepted for publication).

This study represented the first chemical characterization of the terpene fraction of *L. arborescens*, belonging to the Asteraceae family, and the first investigation of the flavone components of *H. stipulacea*, a seagrass migrated into Mediterranean Sea from Indo-Pacific Ocean. Both species are particularly interesting under either the pharmacological or the ecological aspect. In fact, *L. arborescens* is commonly used in North African popular medicine against diarrhoea and abdominal spasms whereas *H. stipulacea* is one of nine macrophyte species that are considered as invasive playing an important role in the ecology of Mediterranean Sea.

The structures of new compounds isolated from both plants were determined by means of spectroscopic methods, mainly 1D and 2D NMR techniques (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC experiments). Known molecules were identified by comparing the spectroscopic data with the literature. Additional 2D NMR experiments were conducted in some case with the aim of fully characterising compounds for which a partial NMR assignment had been reported in the literature.

L. arborescens - The liposoluble extracts of both aerial parts and roots of *L. arborescens* were submitted to subsequent purification steps by using different chromatographic techniques, mainly the high pressure liquid chromatography (HPLC), to give twenty-seven pure compounds including sesquiterpenoids and triterpenoids. Five of them were unprecedented molecules. The terpenoid composition of the two parts of the plant was different. In particular, the extract of the aerial parts was found to be dominated by triterpenoids whereas the constituents of the extract of the roots were sesquiterpenoids.

Twelve triterpenes exhibiting either oleanane- or ursane-type skeleton were isolated from aerial parts: 3β-hydroxy-11β-ethoxy-olean-12-ene (**292**), which was a novel derivative of β-amyrin, and the known 3β-hydroxy-11β-ethoxyurs-12-ene (**293**), 3β,11β-dihydroxy-olean-12-ene (**294**), 3β,11β-dihydroxy-urs-12-ene (**295**), 3β-hydroxy-11β-methoxyolean-12-ene (**296**), 3β-hydroxy-11β-methoxyurs-12-ene (**297**), oleana-9(11):12-dien-3β-ol (**298**), ursa-9(11):12-dien-3β-ol (**299**), 3β-hydroxy-11-oxo-olean-12-ene (**300**), 3β-hydroxy-11-oxo-urs-12-ene (**301**) and taraxast-20-ene-3β,30-diol (**301**). Lupeol (**303**) and stigmasterol (**304**) were found to be the main triterpenoid and sterol, respectively, of this extract.

Fourteen sesquiterpenoids displaying eudesmane, guaiane and germacrane skeletons were isolated from the roots. The novel compounds were: 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin (**305**), 9α-hydroxy-4α,15-dihydrozaluzanin C (**306**), 3β,14-dihydroxy costunolide-3-*O*-β-glycopyranoside (**314**), and 3β-hydroxycostunolide-3-*O*-β-glycopyranoside (**315**). The known molecules were identified as 11β,13-dihydrolactucin (**307**), 3α-hydroxyguaia-4(15),10(14),11(13)-triene-12,6α-olide-3-*O*-β-glycopyranoside (**308**)**,** guaia-2-oxo-1(10),2(3), ,11(13)-triene-12,6α-olide-15-*O*-β-glycopyranoside (**309**), 9α-hydroxy zaluzanin-3-*O*-β-glycopyranoside (**310**), 8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin-3'-sulphate (**311**), 1β-hydroxyeudesm-4(5)-ene-12,6α-olide (**312**), 1β,8α-dihydroxyeudesm-4-en-6β-7α,11βH-6-olide (**313**), 3β-hydroxycostunolide-3-*O*-β-glycopyranoside (**316**), 3β-hydroxy-11β,13-dihydrocostunolide-3-*O*-β- glucopyranoside (**317**), and germacra-1(10)E,4E,11(13)-triene-12,6α-olide-15-*O*-β- glycopyranoside (**318**).

Among the novel molecules, sesquiterpenoids **305** and **306** exhibited a dihydroxylated guaianolide skeleton and were both related to zaluzanin C whereas sesquiterpenoids **314** and **315** were characterized by a glucosylated germacranolide framework structurally related to that of the co-occurring picriside C (**316**).

A stereochemical analysis was also conducted on known 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin-3'-sulphate (**311**), which was the main component of the sesquiterpene pool of *L. arborescens*. This compound had been very recently described from *Reichardia gaditana* (Zidorn et al., 2007) but the absolute configuration was not assigned.

Two stereochemical questions were taken into consideration. The first concerned the absolute stereochemistry at the 6,7-junction of the guaianolide framework whereas the second regarded the absolute configuration of the chiral centre in 3'-hydroxy-2'-methyl-propanoyl fragment.

The application of the Mosher method was planned in both cases: in the first, on the secondary alcohol function of the derivative obtained by opening the lactone ring of **311**; in the second, on the primary alcohol derived by hydrolysis of sulphate ester linkage. Unfortunately, due to the rapid dehydration reaction that was observed to occur under different methanolysis conditions, every attempt to obtain the free secondary alcohol by opening of the lactone ring was unsuccessful.

Thus, the absolute stereochemistry of the junction was suggested the same as most literature natural guaianolides. Conversely, the *S* absolute configuration of C-2' in the propanoyl side chain was successfully determined by synthesising the Mosher ester derivatives of the alcohol obtained by hydrolysis of sulphate **308** and, subsequently, by comparing the ^{1}H NMR spectra of such derivatives with those of Mosher esters of model compounds, which were opportunely prepared.

All new terpenoids **292**, **305**, **306**, **314** and **315** were tested for both antifungal and antibacterial activity at a concentration of 5 μg/ml. No growth inhibition was exhibited on *C. albicans* as well as on gram – *E. coli* and gram + *S. aureus* by the studied metabolites.

Finally, it is noteworthy that the terpenoid pattern of *L. arborescens*, described here for the first time, shows striking similarities with those reported in the literature for different species of the same genus (Zaheer et al., 2006; Sokkar et al., 1993; Abdel-Fattah et al., 1990; Gupta et al., 1989; Abdel Salam et al., 1986 and 1982; Behari et al., 1984; Majumder et al., 1982; Prabhu et al., 1969).

H. stipulacea - The flavonoid profile of *H. stipulacea* was found to be dominated by apigenin-7-*O*-β-D-glucopyranoside (**325**) co-occurring with other minor flavone glucosides including two uncommon malonyl derivatives, the new compound genkwanin-4'-*O*-(6"-*O*-malonyl)-β-D-glucopyranoside (**322**) and apigenin-7-*O*-(6"-*O*- malonyl) β-D-glucopyranoside (**323**). The other known related glucopyranosyl flavones were: genkwanin-4'-O-β-D-glucopyranoside (**324**), apigenin-4'-O-β-D-glucopyranoside (**323**), chrysoeriol-4'-*O*-β-D-glucopyranoside (**324**), and chrysoeriol-7-*O*-β-D-glucopyranoside (**325**).

This represents the first finding of malonylated flavones in the marine environment. With the exception of a malonyl amide alkaloid that has been recently found in a marine bacterium associated with a zooanthid (Kita, 2007), no other malonyl-containing metabolites have been reported from marine organisms to date.

Malonyl flavones **322** and **323** were observed to be quite instable undergoing a degradation reaction to form the corresponding derivatives **324** and **325**. This observation was in agreement with that reported in the literature on the reactivity of natural malonyl esters.

Different acetyl-malonyl derivatives of apigenin glucoside have been reported to undergo rapidly to decarboxylation and, subsequently, under different solvent conditions, to complete deacetylation (Švehlíková et al., 2004). Thus, the deacylation reaction of compounds **322** and **323** occurring during the work-up suggested that malonyl-flavones were most likely more abundant in the plant than it was detected by chemical analysis and, consequently, the corresponding non-esterified derivatives **324** and **325** should be considered artefacts.

With the exception of a recent investigation on *H. johnsonii* resulting in the chemical characterization of all main components of the flavone pattern of this sea grass (Meng et al., 2008), previous studies on different *Halophila* species including *H. stipulacea* only reported qualitative analysis of flavone profiles and indicated the presence of unidentified flavones and flavone sulphates (McMillan et al., 1980; McMillan et al., 1986).

The present study on *H. stipulacea* revealed the presence of a flavone glycoside pattern less complex and characterized by different aglycone moieties with respect to that reported for *H. johnsonii* (Meng et al., 2008). Unfortunately, chemical data on the identity of flavonoid metabolites of other *Halophila* species are lacking in the literature. Additional studies in this field should be necessary to correlate the flavone composition of seagrasses of genus *Halophila* to their taxonomy. In general, further investigations on seagrasses are required to complete the chemical scenario of marine phanerogams.

Accordingly, and in perspective, the Mediterranean phanerogam *Posidonia oceanica*, that is extremely important for the ecological role played in the Mediterranean ecosystem, has been planned to be investigated in the next future.

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Chapter 5
Experimental part

5.1 PLANTS MATERIAL

The aerial parts and the roots of *Launaea arborescens* were collected during the period of flowering in April 2004 and 2006 from the area of Laabadla of the Bechar city in the south of Algeria. The plant was identified by Prof. Oudjhih Bachir of the Agronomy department, Sciences Faculty of Batna University. A voucher specimen was deposited under the code number 423/HIAB. The aerial parts and the roots were separated and dried by air and then powdered. The dry weights were 900 g for the aerial parts and 1 kg for the roots.

Specimens of *Halophila stipulacea* were collected from the Greek coast of the Mediterranean Sea by SCUBA diving during December 2003. The sample was frozen at -20°C and stored until the use. A voucher specimen (Halo-71) has been deposited at the Institute of Biomolecular Chemistry (ICB), National Council of Research, Italy.

5.2 CHROMATOGRAPHIC METHODS OF ANALYSIS (ANALYTICAL AND PREPARATIVE)

5.2.1 Thin Layer Chromatography (TLC)

The chromatographic analysis was carried out on glass or aluminium plates, but in most cases on glass plates in normal phase or reversed phase.

For normal phase, we usually used plates on glass Kieselgel 60 F_{254} (Merck) 0.25 mm (5 cm x 10cm), or Kieselgel 60 F_{254} (10 cm x 20 cm), and $RP_{18}F_{254}$ 0.25 mm (5 cm X 10 cm) plates were used in reversed phase. The compound mixture was put in the preparative or analytical plates at around 2 cm from the bottom and let migrate in a tank containing the proper solvent.

The migration let the metabolite mixture to be separated according to their polarity. After that, the plates were observed under UV lamp at 254 nm, and then sprayed by an aqueous solution of cerium sulphate. Finally, they were heated until the appearance of various coloured spots

5.2.2 Column Chromatography (CC)

5.2.2.1 Adsorption chromatography

Silica gel chromatography columns Kieselgel 60 (70-230 mesh ASTM 0.036-0.200 mm) were used in the first or second stages of the extract fractionation. The size of the columns, the granulometry of the solid phase, and the flow of the mobile phase were adapted to the quantity, the nature of the extract, and the fractions of the samples to be separated.

The choice of the elution conditions, the follow-up of separation, and the final combination of the fractions were carried out on the basis of TLC analysis.

The samples were introduced through the columns in liquid form after their dissolution with the minimum of the appropriate solvent.

Gradients of solvents such as ethyl acetate in petroleum ether or hexane, and methanol in chloroform or dichloromethane were used as elution systems, and the fractions were collected manually or by an automatic collector.

5.2.2.2 Size Exclusion Chromatography (SEC)

Size exclusion chromatography or gel filtration was usually carried out as the first stage of the fractionation of the crude extract, with the aim to get a good separation. This is a non-destructive method for the recovery of a high quantity of extract. The separation was carried out on Sephadex LH-20 with an isocratic eluent system: $CHCl_3$/MeOH (1:1) as mobile phase and with a variable flow according to the manner of separation.

The samples to be separated were introduced in liquid form after dissolution of the extract into small volume of the same eluent solvent system. The progression of the separation and the final combination of the fractions were monitored by TLC and combined according to their homogeneity. The fractions were collected manually, or by an automatic collector.

5.2.2.3 High Pressure Liquid Chromatography (HPLC)

The separation by analytical or semi-preparative high pressure liquid chromatography has been widely used for the analysis and separation of the majority of compounds. It was performed on a Shimadzu LC-10AD equipped with an UV-SPD-10A wave length detector (at 210 and 254 nm) and controlled by Class-VP software. The system was equiped with two pumps (Pump A for aqueous solvent and pump B for organic solvent). The columns used were in different in types and sizes. In particular Kromasil 5micron 100A C18 (250 x 4.60) Phenomenex, Kromasil 5 micron 100A C18 (250 x 10 mm), Kromasil 5 micron 100A Silica (250 x 4.6mm), Kromasil 5 micron 100A Silica (250 x 10 mm) and Synergi 4 micron Hydro-RP 80A (250 x 4.60) Phenomenex columns were used.

The injection was made by syringe and the samples were injected through the loop after their dissolution in the proper solvent and elimination of the insoluble particles by filtering or centrifuge.

All solvents used were HPLC grade and the water was ultra-pure. Systems of solvents used for the reverse phase chromatography were MeOH/ H_2O and MeCN/H_2O, either in isocratic mode or in gradient mode as eluent solvent. The flow was 1ml/min in analytical and 2 ml/min in semi-preparative separation.

5.3 PHYSICO-CHEMICAL METHODS

5.3.1 Optical Activity ($[\alpha]_D$)

Optical rotations of the compounds, containing one or more asymmetric centers, were measured on a Jasco DIP 370 digital polarimeter. The compounds were dissolved in chloroform or methanol and the rotation α of the polarized light was measured in a cell of 1 ml of volume and 10 cm of length at room temperature. The line D (589 nm) of a sodium lamp was used as source of incidental light.

5.3.2 Infrared Spectrometry (IR)

IR spectra were recorded on a BIORAD FTS 155 FT-IR spectrophotometer controlled by BIORAD Win IR software. The samples were dissolved in chloroform and then deposed as film on KBr pastille.

5.3.3 Mass Spectroscopy (MS)

Low and high resolution mass spectra of the isolated products were acquired by electrospray (ESI) in positive or negative mode on a spectrometer Micromass Q-TOF MicroTM coupled with a HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm RMS in the presence of a known lock mass). The samples must be soluble in low boiling solvents (acetonitrile, Methanol……) and stable at very low concentrations.

The MALDI (Matrix Assisted Laser Desorption Ionization) spectra were acquired using a mass spectrometer Voyager-DE Pro (Applied Biosystem, Foster City, CA, USA), a source of laser ionization equipped with a time of flight analyzer which operates in a "reflectron mode.

The sample was irradiated by a pulsed laser beam (337 nm) and each spectrum was obtained by summing the intensities from different ion irradiations. The range of mass analysis was calibrated using two internal standards with an appropriate value of the mass. The sample was dispersed in a solid matrix such as α-cyano-4-hydrosuccinic acid (solution 10 mg/ml in acetonitrile/trifluoroacetic acid 0.2% in ratio 70/30).

5.3.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were measured on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the Z-axis and a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the Z-axis.

^{13}C NMR spectra were recorded on a Bruker DPX-300 operating at 300 MHz using a dual probe. Two-dimensional (NOESY, COSY, HSQC, and HMBC) spectra were recorded using specific impulse sequences.

The samples were dissolved in deuterated $CDCl_3$, CD_3OD, and C_5D_5N-d_5. The chemical shifts were given as δ values (ppm) with tetramethylsilane (TMS) as an internal standard.

5.4 CHEMICAL METHODS

5.4.1 Hydrolysis of compound 311

Compound **311** (8.0 mg) was dissolved in H_2SO_4/MeOH (3 drops in 1 ml) and stirred for 10 minutes. The reaction mixture was chromatographed by silica gel column (light petroleum ether /EtOAc, 50:50) to afford 4.8 mg of pure compound **311a:** 8-Deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin (^{1}H NMR and HRESIMS spectra in page 130).

5.4.2 Preparation of the ester derivative of compound 311a

Acetyl derivative **311b** was prepared by treating 2.0 mg of compound **311a** with acetic anhydride (2 drops) in pyridine (1 ml) at room temperature. The product was purified by Pasteur-pipette silica gel chromatography using light petroleum ether-ethyl acetate (60:40) to give 1.7 mg of pure compound **311b**. 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin-3'-acetate (^{1}H NMR data in page 132).

5.4.3 Preparation of the Mosher esters derivatives of compound 311a

5.4.3.1 Compound 311c

Compound **311c** was prepared by treating 1 mg of **311a** in pyridine (1 ml) with *R*-(-)-MTPA chloride (0.07 ml) at room temperature overnight. The residue was purified by Pasteur-pipette silica gel chromatography using light petroleum ether/ethyl acetate (60:40) to give 0.4 mg of pure ester **311c**. 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin-3'-(S)-MTPA-ester (ESIMS and ^{1}H NMR spectra in pages 133, 134).

5.4.3.2 Compound 311d

Compound **311d** was prepared by treating 1 mg of **311a** in pyridine (1 ml) with *S*-(-)-MTPA chloride (0.07 ml) at room temperature overnight. The residue was purified by Pasteur-pipette silica gel chromatography using light petroleum ether/ethyl acetate (60:40) to give 0.4 mg of pure ester **311d**. 8-deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin-3'-(*R*)-MTPA-ester (^{1}H NMR spectrum in page 134).

5.4.4 Preparation of the model Moscher esters

5.4.4.1 Compound Ia

Compound **Ia** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature.

The reaction mixture was purified by silica gel chromatography using light petroleum ether/ethyl acetate (90:10) to get pure **Ia** (9.0 mg).

Methyl-(*S*)-(+)-3-hydroxy-2-methyl propionate-*S*-MTPA-ester (^{1}H NMR spectrum in page 136).

5.4.4.2 Compound Ib

Compound **Ib** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of S-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After usual work up, the reaction mixture was purified by silica gel chromatography using light petroleum ether/ethyl acetate (90:10) to get pure compound **Ib** (30 mg). Methyl-(*S*)-(+)-3-hydroxy-2-methyl propionate-*R*-MTPA-ester (^{1}H NMR spectrum in page 136).

5.4.4.3 Compound IIa

Compound **IIa** was prepared by treating 0.1 ml of methyl (*R*)-(-)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound IIa (23.0 mg) was obtained. Methyl-(*R*)-(-)-3-hydroxy-2-methyl propionate-*S*-MTPA-ester (^{1}H NMR spectrum in page 137).

5.4.4.4 Compound IIb

Compound **IIb** was prepared by treating 0.1 ml of methyl (*R*)-(+)-3-methyl propionate with 0.1 ml of *S*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound **IIb** (8.8 mg) was obtained. Methyl-(*R*)-(-)-3-hydroxy-2-methylpropionate-*R*-MTPA-ester (^{1}H NMR spectrum in page 137).

5.4.5 Hydrolysis of compound 314

Compound **311** (3.0 mg) was treated with NaOH/H_2O (1% solution) under stirring at room temperature for 3 h. After neutralisation with acidic solution (2% H_2SO_4), the residue was extracted with *n*-butanol for 3 times. The organic phase was concentrated to give after usual work up a product (1.7 mg), which was identical (^{1}H NMR, mass and $[\alpha]_D$) to compound **315**.

5.5 BIOLOGICAL ASSAYS

Antifungal assay was performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P. The medium used to prepare the 10 x drug dilutions and the inoculum suspension as liquid RPMI 1640 with L-Glutamine (Sigma–Aldrich), 0.165 M Morpholinopropansulfonic

acid (MOPS) and 2% Glucose (pH 7.0). The yeast suspension was adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland (2 x10^8 CFU/ml) standard at 530 nm and diluted 1:4000 (50,000 CFU/ml) in RPMI 1640 broth medium. The yeast inoculum (0.9 ml) was added to each test tube that contained 0.1 ml of 10^{-2} fold dilutions (256–0.05 µg/ml final) of each compound. Broth macrodilution MICs were determined after 48 h of incubation at 35°C. The MIC was defined as the lowest concentration of the compound that completely inhibited the growth of the test organism. The antibacterial assay was performed by using the same method as the antifungal test, only differing in the assay medium (Luria Bertani medium: 10 g/l Bactotryptone, 5 g/l Bactoyeast and 10 g/l NaCl, pH 7.5) and in the incubation temperature (37 °C for 24 h).

5.6 EXTRACTION AND PURIFICATION OF *LAUNAEA ARBORESCENS.*

5.6.1 Aerial parts

The aerial parts of *Launaea arborescens* were dried and crushed to give 900 g of dry weight that was macerated in light petroleum ether (3l) for 3 times. The evaporation of the solvent under reduced pressure gave 12 g of a crude extract. First analysis by TLC revealed many spots, confirming the richness of this extract.

The first column realized on this extract to separate its constituents was in silica gel, by eluting with a gradient of petroleum ether and ethyl acetate.

The results of this fractionation are summarised in the following figure.

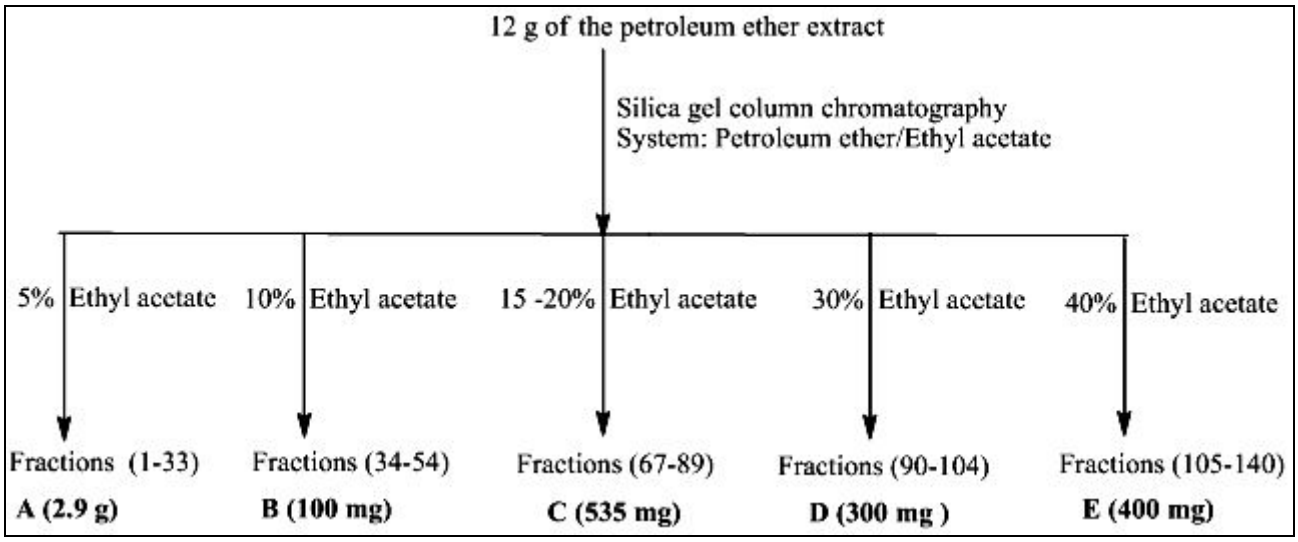


Figure 5.1: First fractionation of the petroleum ether extract of the aerial part of L. arborescens

✓ The fraction **A** (2.9 g) was further subjected to silica gel column chromatography eluting with petroleum ether/ethyl acetate (98:2) to get the pure lupeol **303** as the main compound (1.35 g).

✓ Fraction **B** (100 g) was subjected to silica gel column chromatography eluting with CH_2Cl_2/ MeOH (98:2) to yield stigmasterol **304** (1.8 mg) and a mixture of triterpenes that were further separated by RP18-HPLC using isocratic elution system MeOH/H_2O (9:1) to give compounds **300** (1.2 mg, R_t = 8.8 min), and **301** (1 mg, R_t = 9.2 min).

✓ Fraction **D** (300 g) was further purified on a silica gel column using the system solvent CH_2Cl_2/ MeOH (98:2) to afford the main fraction **D3** (42-60), which was filtrated again through silica gel column using the same system solvent CH_2Cl_2/MeOH (98:2) to afford the compound **302** (13 mg).

✓ Fraction **E** (400 g) was chromatographed by a silica gel column using the light petroleum ether/ethyl acetate to afford two main fractions **E41** (100 mg), and **E42** (70 mg). However, the attempts to purify the fraction **E41** in silica gel column did not allow us to isolate pure compounds; it showed only one spot in TLC analysis. The analytical RP18-HPLC using pure MeOH as eluent led us to isolate, in few amounts, the compounds **293** (0.4 mg, R_t = 22.3 min), **298** (0.6 mg, R_t = 23 min), and **299** (0.6 mg, R_t = 19.2 min)**.**

✓ Fraction **E42** was purified by silica gel column with light petroleum ether-ethyl acetate eluent containing increasing amounts of ethyl acetate. Three main fractions **E421** (18-25)**,** **E422** (22-26), and **E423** (29-36) were collected, and showed R_f value around 0.2 (silica gel TLC, solvent: CH_2Cl_2)

✓ The fraction **E422** contained compound **292** as a pure white powder (1 mg), while the fractions **E421** and **E423** showed a complex mixture in reverse phase TLC (RP-18 TLC, solvent: MeOH). Analytical RP18-HPLC was used to purify these two fractions using MeOH as elution system. The fraction **E4211** from the fraction **E421** yielded the pure compound **295** (1.2 mg, R_t = 20 min), while the fraction **E4231** from the fraction **E423** gave two compounds **296** (1.5 mg, R_t = 14.9 min), and **297**(1.2 mg, R_t = 20 min).

5.6.2 Roots

The dried and powdered roots were exhaustively extracted with methanol for 3 times at room temperature. After evaporation of the solvent under vacuum, the crude extract was partitioned between water and ethyl acetate and between water in a separatory funnel, and then between water and n-butanol. The ethyl acetate fraction was evaporated under vacuum to give 3.7 g of brown oil.

Two grams of this extract was prefractionated on LH-20 column chromatography using $CHCl_3$/MeOH (1:1) as an isocratic eluent system to obtain 54 fractions of 20 ml each. These fractions were combined into nine fractions from **A** to **I**. The weights of the fractions obtained were 332 mg, 384 mg, 260 mg, 257.8 mg, 94.6 mg, 183.9 mg, 69.3 mg, 399.4 mg and 35.9 mg, respectively.

The summary TLC of the nine fractions showed very similar compounds. The fraction **C** was the richest one in terms of constituents, thus this fraction was further purified. The amount of 260 mg was subjected to column chromatography on silica gel (20 g) eluting with CH_2Cl_2/MeOH (up to 50% MeOH) gradient solvent system. 16 fractions from **C1-C16** were collected as shown in the table below:

Eluent (CH_2Cl_2/MeOH)	Fraction	Mass (mg)	Eluent (CH_2Cl_2/MeOH)	Fractions	Mass (mg)
CH_2Cl_2	Fr: 1-4 **C1**	12	5% MeOH	Fr: 36-39 **C9**	4.8
CH_2Cl_2	Fr: 5-6 **C2**	9	5% MeOH	Fr: 40-45 **C10**	3.8
CH_2Cl_2	Fr: 7-10 **C3**	21.4	10% MeOH	Fr: 46-50 **C11**	5.1
CH_2Cl_2	Fr: 11-14 **C4**	1.8	10% MeOH	Fr: 51-55 **C12**	4.6
CH_2Cl_2	Fr: 15-27 **C5**	1.6	10% MeOH	Fr: 56-58 **C13**	22.7
5% MeOH	Fr: 28 **C6**	0.4	15% MeOH	Fr: 59-62 **C14**	17.4
5% MeOH	Fr: 29-30 **C7**	36.7	15% MeOH	Fr: 63-74 **C15**	30.5
5% MeOH	Fr: 31-35 **C8**	25.9	20% MeOH	Fr: 75-83 **C16**	39.4

✓ The fraction C**3** (21.4 mg) was subsequently chromatographed on silica gel column (2g) using a gradient elution system starting from hexane with increasing amounts of ethyl acetate to give eight fractions from **C31**-**C38**. The fractions **C37** and **C38** eluted with 10% ethyl acetate showed the same spots by TLC analysis. These two combined fractions yielded the compound **312** (6 mg).

✓ The fraction **C8** (25.9 mg) was further subjected to silica gel column chromatography eluting with the petroleum ether and a gradient ethyl acetate/petroleum ether (30:70, 40:60, 50:50, and 70:50). The fractions eluted with ethyl acetate 40% were combined into three subfractions **C81** (24-30), **C82** (34-40), and **C83** (41-51). The TLC analysis in reverse phase showed many spots. Consequently, these three fractions were purified by analytical RP18-HPLC using the same gradient MeOH/H_2O [flow rate was 1ml/min, Solvent A: ultra-pure water, Solvent B: 100% MeOH, gradient program: 0 min (65% A, 35%B), 10 min (55% A, 45% B), 20 min (40% A, 60% B), 30 min (30%A, 70% B), 40 min(10%, 90%), 50 min (100% B)]. The fraction **C81** (2.5 mg) afforded compounds **306** (0.6 mg, R_t = 17 min) and **313** (1.0 mg, R_t = 24 min). On the other hand, the fraction **C82** (4 mg) gave compounds **305** (1.6 mg, R_t = 12.6 min), **307** (0.8 mg, R_t = 8.2 min), in addition to the compounds **306** and **313** that were isolated from the fraction **C81**. Fraction **C83** yielded a mixture of many compounds in few amounts which were not sufficient for NMR analyses.

✓ The fraction **C12** (4.6 mg) was first subjected to a silica gel column chromatography (40-63 µm) using the system $CHCl_3$/MeOH (95:5) to yield two fractions **C121** (2.4 mg) and **C122** (1.4 mg). Fraction **C121** was purified by analytical RP18-HPLC eluting with a gradient of MeOH/H_2O starting by 35% MeOH. The chromatogram showed three main peaks that correspond to compounds **316** (0.2 mg, R_t = 33.3 min), **317** (0.3 mg, R_t = 35.6 min), and **318** (0.2 mg, R_t = 38.4 min), respectively. The fraction **C122** was purified in the same condition as fraction **C121** to give at the end the main compound **308** (0.6 mg, R_t = 29.5).

✓ The purification of the fraction **C13** (22.7 mg) was carried out on RP-HPLC column (gradient MeOH/H_2O) starting from 30 % of MeOH to 100% MeOH; the chromatogram revealed many peaks in which two were very intense and corresponded to the compounds **309** (1.2 mg, *Rt* = 30 min) and **314** (7.1 mg, R*t* = 41 min).

✓ The fraction **C14** (17.0 mg) was directly subjected to RP-HPLC chromatography (gradient: MeOH/H_2O) starting from 38 % of MeOH to 100% MeOH to afford compounds **315** (2.1 mg, R_t = 7.5 min) and **310** (2.2 mg, R_t = 15 min).

✓ Finally, fraction **G** was processed through silica gel column chromatography eluting with CH_2Cl_2/MeOH (90:10) to give the pure compound **311** (60 mg).

5.7 EXTRACTION AND PURIFICATION OF *HALOPHILA STIPULACEA*

The frozen specimen of *Halophila stipulacea* (103g dry weight) was extracted by acetone several times at room temperature by using ultrasound vibrations. The remaining aqueous solution was filtrated and concentrated at reduced pressure. The aqueous residue was extracted with diethyl ether and subsequently with n-butanol. The two extracts (197.3 mg and 1.13 g) were, firstly, analyzed by TLC using $CHCl_3$/MeOH as mobile phase in different ratio. The butanolic extract showed some UV-absorbing compounds in the system $CHCl_3$/MeOH (9:1, and 7:3), which they gave yellow coloration when they were sprayed with cerium sulphate.

In order to separate the constituents of the butanolic extract, an aliquot of butanolic extract (380 mg) was fractionated by Sephadex LH-20 chromatography eluted with $CHCl_3$/MeOH in the ration 1:1 ratio to give 3 main fractions from **A-C** with the following weights 100 mg, 14.5 mg, and 71.5 mg, respectively.

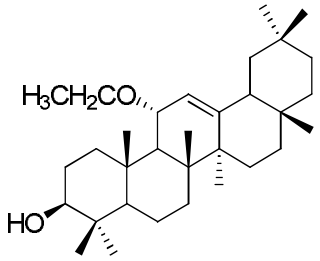
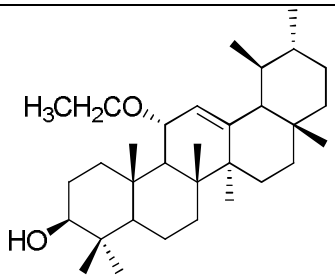
✓ A portion of fraction **A** (33.7 mg), which was also characterized by the presence of syphonosides **286** and **287**, was first subjected to preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a very polar flavonoid glycoside mixture (9.4 mg) corresponding to the UV components at R*f* 0.15 (chloroform/methanol, 7:3). This latter fraction was further purified by RP-HPLC (Synergi-Hydro RP C18, 250x 4.6 mm; 40 min gradient from 50% to 100% CH_3OH in H_2O, flow 1 mL/min, UV detector) to give the new genkwanin-4'-*O*-β-D-(6''-*O*-malonyl)-glucopyranoside **(322)** (1.2 mg, R_t = 28.5 min) and the known apigenin-7-*O*-β-D-(6''-*O*-malonyl)-glucopyranoside) **(323)** (4.0 mg, R_t = 17.3 min).

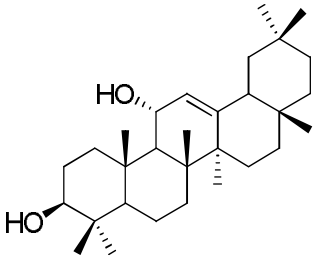
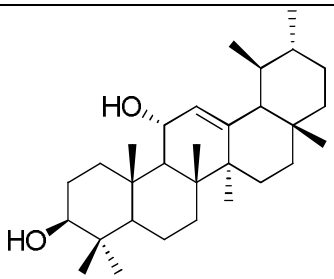
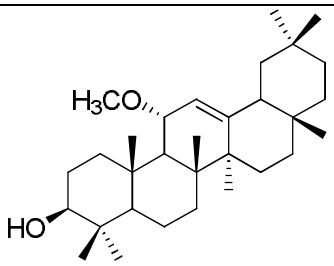
✓ Purification of the fraction **B** on preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) afforded a mixture (4.1 mg) of less polar flavonoid glycosides (R*f* 0.70, chloroform/methanol, 7:3), which was further purified by RP-HPLC (Kromasil 5µ C18, 250x10 mm; CH_3OH/H_2O, 60:40; flow 2 mL/min, UV detector) to obtain the known genkwanin-4'-*O*-β-D-glucopyranoside **(324)** (1.7 mg, R_t = 15 min) and chrysoeriol-7-*O*-β-D-glucopyranoside **(328)** (0.9 mg, R_t = 17 min).

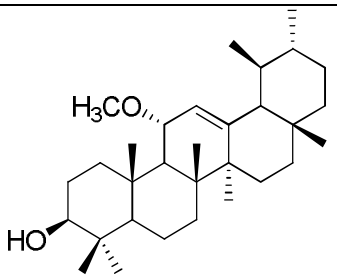
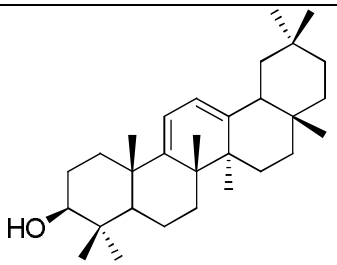
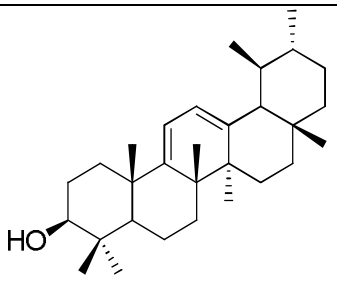
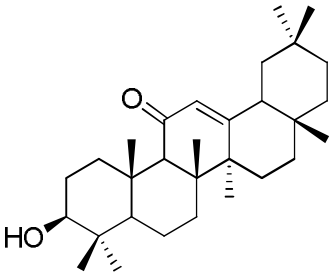
✓ An aliquot of fraction **C** (19 mg) was subjected to a preparative TLC purification (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a mixture of the flavonoid glycosides at Rf 0.65-0.60 (chloroform/methanol, 7:3). The subsequent purification of this fraction by RP-HPLC (Phenomenex: Kromasil 5µ C18, 250x10 mm; CH_3OH/H_2O, 1:1; flow 2 mL/min, UV detector) yielded the known apigenin-7-*O*-β-D-glucopyranoside **(325)** (6.9 mg, R_t = 31 min), apigenin-4'-*O*-β-D-glucopyranoside **(326)** (2.2 mg, R_t = 32.5 min), and chrysoeriol-4'-*O*-β-D-glucopyranoside **(327)** (1.8 mg, R_t = 33 min).

5.8 PHYSICAL CONSTANTS AND SPECTRAL DATA OF THE ISOLATED COMPOUNDS

5.8.1 TRITERPENOIDS

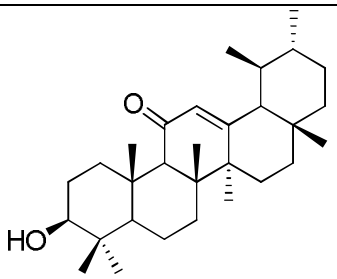
Compound 292	3β-hydroxy-11α-ethoxy-olean-12-ene
$C_{32}H_{54}O_2$ MW 470 White powder $[\alpha]_D$: -10° (c 0.10, $CHCl_3$)	
IR γ_{max}^{film} cm^{-1}: 3852, 2956, 2919, 1457, 1379, 1163, 1080, 974. ESIMS positive mode: *m/z* 425 $[M - EtOH + 1]^+$, *m/z* 407 $[M - EtOH - H_2O + 1]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 76 ^{13}C NMR (150.92 MHz, $CDCl_3$): see page 77 NMR data: see **Table 3.1** in page 80 ^1H NMR of the dehydration of **292** (400 MHz, $CDCl_3$): see page 81	
Compound 293	3β-hydroxy-11α-ethoxy-urs-12-ene
$C_{32}H_{54}O_2$ MW 470 White powder $[\alpha]_D$: -17.6°(c 0.04, $CHCl_3$)	
ESIMS positive mode: *m/z* 493 $[M + 23]^+$	

^{1}H NMR (400 MHz, $CDCl_3$): see page 82	
Compound 294	3β-11α-dihydroxy-olean-12-ene
$C_{30}H_{50}O_2$ MW 442 White powder	
^{1}H NMR (400 MHz, CD_2Cl_2): see page 83 NMR data: see **Table 3.2** in page 85 ^{1}H NMR of the dehydration of **294** (400 MHz, $CDCl_3$): see page 87	
Compound 295	3β-11α-dihydroxy-urs-12-ene
$C_{30}H_{50}O_2$ MW 442 White powder	
ESIMS positive mode : *m/z* 425 $[M - H_2O + 1]^+$ ^{1}H NMR (400 MHz, $CDCl_3$): see page 88 ^{1}H NMR of the dehydration of **295** (400 MHz, $CDCl_3$): see page 88	
Compound 296	3β-hydroxy-11α-metoxy-olean-12-ene
$C_{31}H_{52}O_2$ MW 456 White powder	
ESIMS positive mode: *m/z* 479 $[M + 23]^+$, *m/z* 935 $[2M + 23]^+$ ^{1}H NMR (400 MHz, $CDCl_3$): see page 90	
Compound 297	3β-hydroxy-11α-metoxy-urs-12-ene

$C_{31}H_{52}O_2$ MW 456 White powder	
ESIMS positive mode: *m/z* 479 $[M + 23]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 91 ^1H NMR of the dehydration of **297** (400 MHz, $CDCl_3$): see page 92	
Compound 298	3β- hydroxy-oleana-9(11):12-diene
$C_{30}H_{48}O$ MW 424 White powder $[\alpha]_D$: -6.9° (c 0.06, $CHCl_3$)	
ESIMS positive mode: *m/z* 425 $[M + 1]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 93 NMR data: see **Table 3.3** in page 96	
Compound 299	3β- hydroxy-ursa-9(11):12-diene
$C_{30}H_{48}O$ MW 424 White powder $[\alpha]_D$: +15.9° (c 0.06, $CHCl_3$)	
ESIMS positive mode: *m/z* 425 $[M + 1]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 97	
Compound 300	3β-hydroxy-11-oxo-olean-12-ene
$C_{30}H_{48}O_2$ MW 440 White powder $[\alpha]_D$: +351.3° (c 0.12, $CHCl_3$)	

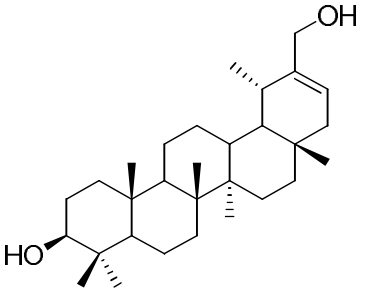
ESIMS positive mode: *m/z* 463 $[M + 23]^+$

^{1}H NMR (400 MHz, $CDCl_3$): see page 99

Compound 301	3β-hydroxy-11-oxo-ursa-12-ene
$C_{30}H_{48}O_2$ MW 440 White powder $[\alpha]_D$: +219.2°(c 0.1, $CHCl_3$)	

ESIMS positive mode: *m/z* 463 $[M + 23]^+$

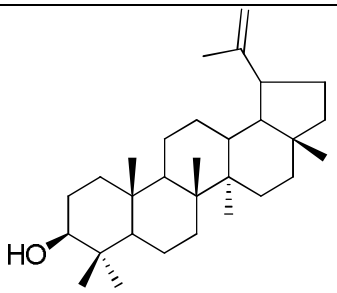
^{1}H NMR (400 MHz, $CDCl_3$): see page 99

Compound 302	Taraxast-20-ene-3β,30-diol
$C_{30}H_{50}O_2$ MW 442 White powder $[\alpha]_D$: +18.1°(c 0.13, $CHCl_3$)	

IE positive mode (70 eV) : 442.2 $[M]^{+\cdot}$, 424 $[M -18]^{+\cdot}$

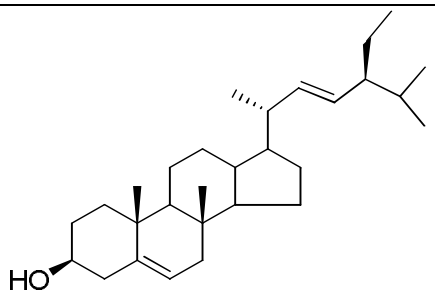
^{1}H NMR (400 MHz, $CDCl_3$): see page 101

NMR data: see **table 3.4** in page 104

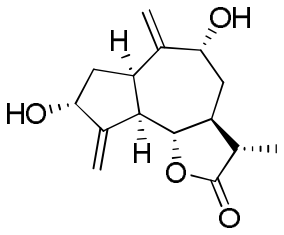
Compound 303	Lup-20(29)-en-3-ol (Lupeol)
$C_{30}H_{50}0$ MW 426 White powder $[\alpha]_D$: +18° (c 0.12, $CHCl_3$)	

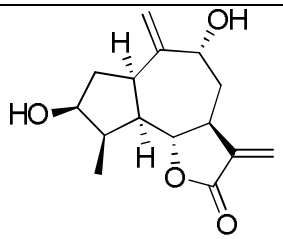
ESIMS positive mode: *m/z* 449 $[M + 23]^+$

^{1}H NMR (400 MHz, $CDCl_3$): see page 105

Compound 304	24-ethyl-5,22-cholestadien-3-ol (Stigmasterol)
$C_{29}H_{48}O$ MW 412 White powder $[\alpha]_D$: -7.8°(c 0.18, $CHCl_3$)	
ESIMS positive mode: *m/z* 413 $[M + 1]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 107	

5.8.2 SESQUITERPENOIDS

Compound 305	9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin
$C_{15}H_{20}O_4$ MW 264 Colourless oil $[\alpha]_D$: -3°(c 0.06, $CHCl_3$)	
IR γ_{max}^{film} cm^{-1}: 3407, 1759, 1456, 1327, 1186, 1064, 991, 910. ESIMS positive mode: *m/z* 287 $[M + 23]^+$, and *m/z* 551 $[2M + 23]$ ^1H NMR (600 MHz, $CDCl_3$): see page 108 ^{13}C NMR (150.92 MHz, $CDCl_3$): see page 108 NMR data: see **Table 3.5** in page 111 ^{1} H NMR (600 MHz, pyridine-d_5): see page 112 ^{13}C NMR (150.92 MHz, pyridine-d_5): see page 112 **NMR data in pyridine-d_5**	

Position	**δ H (m, J (Hz) (600 MHz)**	**δ C (150.92 MHz)**
1	4.01 br t (8.2)	34.7
2a	2.17 m	40.3
2b	2.30 m	-
3β	4.71 br s	73.8
4	-	157.1
5	3.25 t (9.7)	49.9
6	3.95 t (9.7)	85.7
7	2.61 q (11.8)	44.5
8a	1.48 br t (11.8)	40.7
8b	2.30 m	-
9β	5.13 m	74.8
10	-	154.0
11	2.30 m	41.7
12	-	178.6
13	1.20 d (6.7)	13.3
14	4.88 s	110.6
	5.05 s	-
15	5.60 s	110.8
	5.60 s	-
Compound 306		9α-hydroxy-4α,15-dihydrozaluzanin C
$C_{15}H_{20}O_4$ MW 264 Colourless oil $[\alpha]_D$: -11°(c 0.1, $CHCl_3$)		

IR γ_{max}^{film} cm^{-1}: 3047,2926, 1757, 1267, 1167, 984, 770

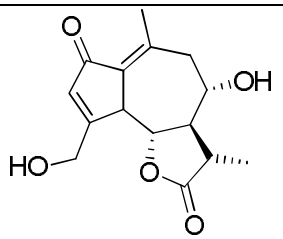
ESIMS positive mode: *m/z* 287 $[M + 23]^+$

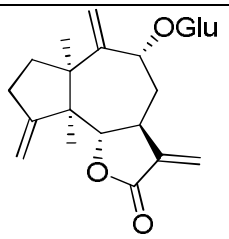
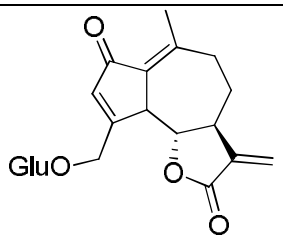
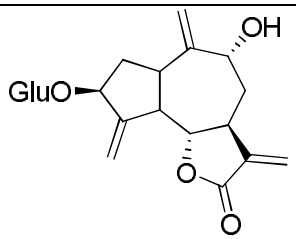
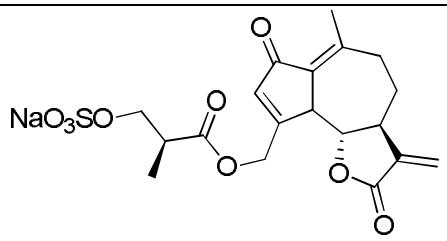
HRESIMS positive mode: *m/z* 287.1214 (calcd for $C_{15}H_{20}O_4Na$, 287.1259)

^1H NMR (600 MHz, $CDCl_3$): see page 115

^{13}C NMR (150.92 MHz, $CDCl_3$): see page 115

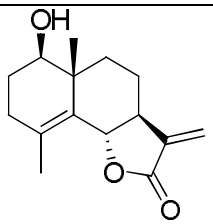
NMR data: see **Table 3.6** in page 118

NMR data in pyridine-d_5		
Position	**δ H (m, J (Hz) (600 MHz)**	**δ C (150.92 MHz)**
1	3.75 br q (10.2)	32.6
2a	2.06 m	34.4
2b	2.34 m	-
3α	4.50 br s	73.5
4	2.54 m	40.4
5	2.43 m	47.3
6	4.22 t (9.7)	83.3
7	3.43 m	41.2
8a	1.57 m	39.4
8b	2.53	-
9β	4.87 m	73.3
10	-	-
11	-	-
12	-	-
13	5.43 d (3.1)	118.7
	6.25 d (3.1)	-
14	5.16 s	110.5
	5.16 s	-
15	1.28 d (7.0)	8.41
Compound 307		11β,13-dihydrolactucin
$C_{15}H_{18}O_5$ MW 278 Colourless crystals $[\alpha]_D$: +7.2° (c 0.08, $CHCl_3$)		
ESIMS positive mode : *m/z* 301 $[M+23]^+$ ^{1}H NMR (400 MHz, $CDCl_3$): see page 121		
Compound 308		3α-hydroxy- guaia-4(15), 10(14),11(13)-triene-12,6α-olide-3-*O*-β-glycopyranoside

$C_{21}H_{28}O_8$ MW 408 Amorphous powder $[\alpha]_D$: - 21° (c 0.06, MeOH)	
ESIMS positive mode : *m/z* 431 $[M +23]^+$ ^1H NMR (400 MHz, pyridine-d_5): see page 122	
Compound 309	Guaia-2-oxo-1(10),2(3),11(13)-triene-12.6α-olide-15-*O*-β-glycopyranoside
$C_{21}H_{26}O_9$ MW 422 Amorphous powder $[\alpha]_D$: -34.5°(c 0.27, MeOH)	
ESIMS positive mode: *m/z* 445$[M + 23]^+$, and *m/z* 283 $[(M-162)+23]^+$ ^1H NMR (400 MHz, $CDCl_3$): see page 124	
Compound 310	9α-hydroxy zaluzanin-3-*O*-β- glycopyranoside
$C_{21}H_{28}O_9$ MW 424 Amorphous powder $[\alpha]_D$: -8.6° (c 0.22, MeOH)	
ESIMS positive mode: *m/z* 447 $[M + 23]^+$ ^1H NMR (400 MHz, pyridine-d_5) : see page 125	
Compound 311	8-deoxy-15-(3'-hydroxy-2'-methylpropanoyl)-lactucin-3'-sulfate
$C_{19}H_{21}NaO_9S$ MW 448 Colourless amorphous solid $[\alpha]_D$: +7.4° (c 0.2, $CHCl_3$)	
ESIMS positive mode: *m/z* 471 $[M + 23]^+$ ESIMS negative mode: *m/z* 425 ($C_{19}H_{21}O_9S^-$)	

^{1}H NMR (600 MHz, $CDCl_3$): see page 126

NMR data: see **Table 3.7** in page 130

Compound 312	1β-hydroxyeudesm-4(5)-ene-12, 6α-olide
$C_{15}H_{20}O_3$ MW 248 colourless prisms $[\alpha]_D$: +67.0° (c 0.35, $CHCl_3$)	

ESIMS positive mode: *m/z* 271 $[M + 23]^+$, *m/z* 521 [2M + 23]

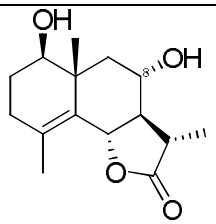
^{1}H NMR (300 MHz, $CHCl_3$): see page 139

^{13}C NMR (75.46 MHz, $CDCl_3$): see page 140

NMR data in $CDCl_3$

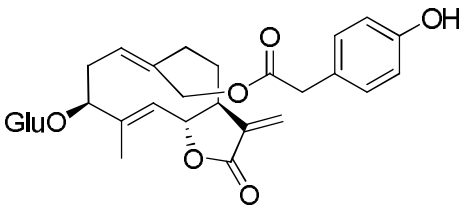
Position	**δ H (m, J (Hz)**	**δ C**
1α	3.55 m	77.7
2	1.66 m	27.1
	1.73 m	-
3	2.02 m	33.2
	2.03 m	-
4	-	126.3
5	-	128.9
6	4.56 d (11.5)	83.1
7	2.58 dt (11.6, 3.2)	49.6
8	1.61 m	23.1
	2.10 m	-
9	1.35 m	38.2
	2.10 m	-
10	-	41.1

11	-	139.3
12	5.50 d (3.2)	118.5
13	6.15 d (3.1)	-
14	1.10 s	18.4
15	1.86 s	19.6

Compound 313	1β,8α-dihydroxy-eudesm-4-en-6β-7α,11βH-6-olide
$C_{15}H_{22}O_4$ MW 266 White needles $[\alpha]_D$: +11.2° (c 0.1, $CHCl_3$)	

ESIMS positive mode: *m/z* 289 $[M + 23]^+$

^1H NMR (400 MHz, $CDCl_3$): see page 142

Compound 314	3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate
$C_{29}H_{36}O_{11}$ MW 560 Amorphous powder $[\alpha]_D$: - 0.4° (c 0.55, MeOH)	

IR γ_{max}^{film} cm^{-1}: 3393, 2922, 1738, 1616, 1516, 1448, 1259, 1145, 1018.

ESIMS positive mode: *m/z* 583 $[M + 23]^+$

HRESIMS: *m/z* 583.2181 (calcd for $C_{29}H_{36}O_{11}Na$, 583.2155)

^1H NMR (600 MHz, pyridine-d_5): see page 143

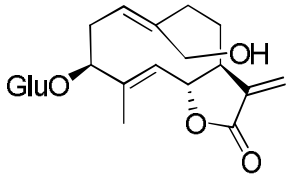
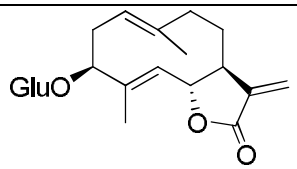
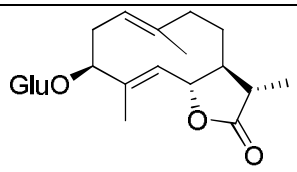
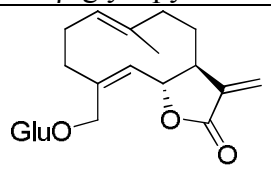
^{13}C NMR, and DEPT ^{13}C (150.92 MHz, pyridine-d_5): see page 144

NMR data: see **Table 3.9** in page 148

NMR data in MeOD

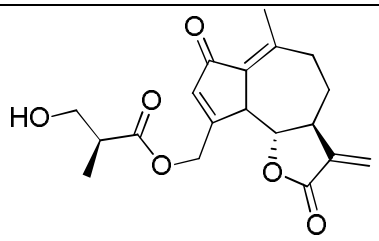
Position	**δ H (m, J (Hz) (600 MHz)**	**δ C (75.46 MHz)**
1	4.95 dd (12.0, 3.5)	126.3

2a	2.25 q (12.0)	32.2
2b	2.38 m	-
3α	4.38 m	81.3
4	-	141.1
5	4.82 d (10.0)	131.0
6	4.35 t (8.8)	81.2
7	2.38 m	49.4
8a	1.80 m	28.8
8b	1.32 m	-
9a	1.88 m	36.7
9b	2.4 m	-
10	-	134.6
11	-	139.0
12	-	170.8
13a	5.50 d (3.8)	120.6
13 b	6.3 d (3.8)	-
14a	4.3 m	61.5
14b	4.4 m	-
15	1.48 s	11.0
Ester moiety		
CH_2β	3.40 s	40.5
C=O (α)	-	172.0
1’	-	124.2
2’, 6’	6.90 d (8.3)	130.0
3’, 5’	6.6 d d (8.3)	115.2
4’	-	155.9
Sugar moiety		
1’’	4.10 d (8.0)	100.7
2’’	3.18 m	73.3
3’’	3.25 m	76.3
4’’	3.27 m	70.0
5’’	3.15 m	75.7
6’’a	3.62 dd (12.0, 4.8)	61.6

6'' b	3.71 dd (12.0, 2.6)	-
Compound 315		3β,14-dihydroxycostunolide-3-O-β-glycopyranoside
$C_{21}H_{30}O_9$ MW 426 Amorphous powder $[\alpha]_D$: +4° (c 0.1, MeOH)		
IR γ_{max}^{film} cm^{-1}: 3420, 2926, 2861, 1757, 1556, 1416; 1286, 1234. ESIMS positive mode: *m/z* 449 $[M + 23]^+$ HRESIMS: *m/z* 449.1776 (calcd for $C_{21}H_{30}O_9Na$, 449.1788). ^1H NMR (600 MHz, pyridine-d_5): see page 150 ^{13}C NMR (150.92 MHz, pyridine-d_5): see page 151 NMR data: see **Table 3.10** in page 153		
Compound 316		3β-hydroxycostunolide-3-*O*-β-glycopyranoside
$C_{21}H_{30}O_8$ MW 410 Amorphous powder $[\alpha]_D$: +43.1° (c 0.02, MeOH)		
ESIMS positive mode: *m/z* 433 $[M + 23]^+$, and *m/z* 247 $[M -163]^+$ ^1H NMR (600 MHz, pyridine-d_5): see page 155		
Compound 317		3β-hydroxy-11α-metoxy-urs-12-ene
$C_{21}H_{32}O_8$ MW 412 Amorphous powder $[\alpha]_D$: +29° (c 0.03, MeOH)		
ESIMS positive mode: *m/z* 435 $[M + 23]^+$ ^1H NMR (600 MHz, pyridine-d_5): see page 157		
Compound 318		Germacra-1(10)E,4E, 11(13)-triene-12,6α-olide-15-*O*-β-glycopyranoside
$C_{21}H_{30}O_8$ MW 410 Amorphous powder		

$[\alpha]_D$: +56° (c 0.02, MeOH)	
ESIMS positive mode: *m/z* 433 $[M +23]^+$ ^1H NMR (600 MHz, pyridine-d_5): see page 158	

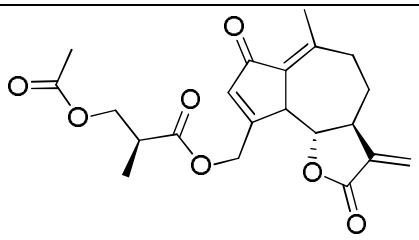
5.8.3 MOSCHER DERIVATIVES

Compound 311a	8-Deoxy-15-(3'-hydroxy-2'-methyl-propanoyl)-lactucin
$C_{19}H_{22}O_6$ MW 346 Colourless oil $[\alpha]_D$: +26.1° (c 0.12, $CHCl_3$)	
ESIMS positive mode: *m/z* 369 $[M + 23]^+$ HRESIMS positive mode: *m/z* 369.1298 (calcd for $C_{19}H_{22}O_6Na$, 369.1314). ^1H NMR spectrum (400 MHz, $CDCl_3$): see page 131	

NMR data in $CHCl_3$

Position	**δ H (m, J (Hz) (400 MHz)**
3	6.35 br s
5	3,71 d (10.2)
6	3.62 t (10.2)
7	2.89 m
8a	1.46 m
8b	2,23 m
9a	2.42 m
9b	2.54 br t (12.7)
13a	5.48 d (3.2)
13b	6.20 d (3.2)
14	2.45 s

15a	5.09 d (16.9)
15b	5.31 d (16.9)
Ester moiety	
2’	2.77 m
3’	3.76 m
4’	1.23 d (7.3)

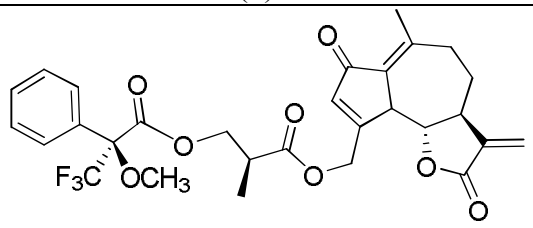
Compound 311b	8-deoxy-15-(3’-hydroxy-2’-methyl-propanoyl)-lactucin-3’- acetate
$C_{21}H_{24}O_7$ MW 388 Colourless oil $[\alpha]_D$: +8° (c 0.17, $CHCl_3$)	

IR γ_{max}^{film} cm^{-1}: 1718, 1688, 1621, 1373, 1251, 1135, 989.

ESIMS positive mode: *m/z* 411 $[M + 23]^+$

^1H NMR (600 MHz, $CDCl_3$): see page 132

NMR data: see **Table 3.8** in page 133

Compound 311c	8-Deoxy-15-(3’-hydroxy-2’-methyl-propanoyl)-lactucin-3’- (*S*)-MTPA- ester
$C_{29}H_{29}O_8F_3$ MW 562 Colourless oil $[\alpha]_D$: +21° (c 0.04, $CHCl_3$)	

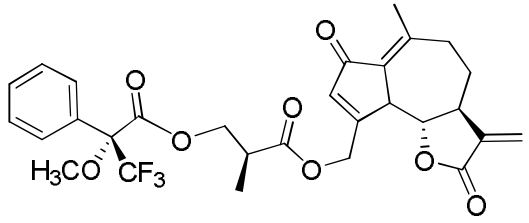
IR γ_{max}^{film} cm^{-1}: 1784,1744, 1684, 1630, 1448, 1381, 1273, 1180, 1113, 970.

ESIMS positive mode: *m/z* 583 $[M + 23]^+$

^1H NMR spectrum (400 MHz, $CDCl_3$): see page 134

NMR data in $CHCl_3$

Position	**δ H (m, J (Hz) (400 MHz)**
3	6.25 br s
5	3.59 d (8.9)
6	3.47 t (7.0)

7	2.94 m
8a	1.44 m
8b	2.03 m
9a	2.39 m
9b	2.56 br t (13.0)
13a	5.48 d (3.2)
13b	6.20 d (3.2)
14	2.45 s
15a	4.97 d (17.5)
15b	5.17 d (17.5)
Ester moiety	
2’	2.27 m
3’a	4.42 dd (10.8, 5.4)
3’b	4.49 dd (10.8, 7.3)
4’	1.23 d (7.3)
Compound 311d	8-Deoxy-15-(3’-hydroxy-2’-methyl-propanoyl)-lactucin-3’- (*R*)-MTPA- ester
$C_{29}H_{29}O_8F_3$ MW 562 Colourless oil $[\alpha]_D$: -40° (c 0.12, $CHCl_3$)	

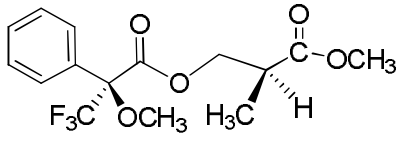
IR γ_{max}^{film} cm^{-1}: 1751, 1684, 1616, 1448, 1381, 1267, 1113, 1032.

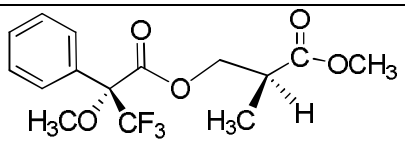
ESIMS positive mode: *m/z* 583 $[M + 23]^+$

^1H NMR spectrum (400 MHz, $CDCl_3$): see page 134

NMR data in $CHCl_3$

Position	**δ H (m, J (Hz) (400 MHz)**
3	6.27 br s
5	3.66 d (10.2)
6	3.59 m
7	2.94 m
8a	1.43 m
8b	2.22 m

9a	2.40 m
9b	2.52 m
13a	5.48 d (3.2)
13b	6.20 d (3.2)
14	2.45 s
15a	5.0 d (17.4)
15b	5.18 d (17.4)
Ester moiety	
2’	2.87 m
3’a	4.40 dd (10.8, 5.7)
3’b	4.53 dd (10.8, 6.7)
4’	1.24 d (6.7)
Compound Ia	Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-*S*-MTPA-ester
$C_{15}H_{17}O_5F_3$ MW 334 Oil $[\alpha]_D$: -25° (c 0.9, $CHCl_3$)	
IR γ_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436, 1272, 1171, 1123, 1082. ESIMS positive mode: *m/z* 357 $[M + 23]^+$ ^1H NMR spectrum (400 MHz, $CDCl_3$): see page 136	
NMR data in $CHCl_3$	
Position	**δ H (m, J (Hz) (400 MHz)**
2’	2.86 m
3’a	4.38 dd (10.8, 5.7)
3’b	4.47 dd (10.8, 7.3)
4’	1.27 d (6.7
Compound Ib	Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-*R*-MTPA-ester

$C_{15}H_{17}O_5F_3$ MW 334 Oil $[\alpha]_D$: +47° (c 3.0, $CHCl_3$)	

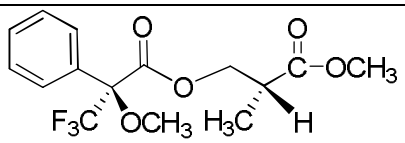
IR γ_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436,1272, 1171, 1123, 1082.

ESIMS positive mode: *m/z* 357 $[M + 23]^+$

^1H NMR spectrum (400 MHz, $CDCl_3$): see page 136

NMR data in $CHCl_3$

Position	**δ H (m, J (Hz) (400 MHz)**
2’	2.86 m
3’a	4.38 dd (10.8, 5.7)
3’b	4.49 dd (10.8, 6.3)
4’	1.27 d (6.7)

Compound IIa	Methyl-(*R*)-(-)-3-hydroxy-2-methyl propionate-*S*-MTPA-ester
$C_{15}H_{17}O_5F_3$ MW 334 Oil $[\alpha]_D$: -46° (c 0.23, $CHCl_3$)	

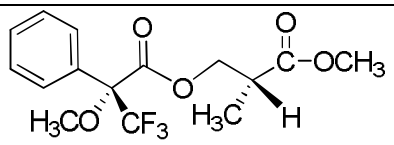
IR γ_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082.

ESIMS positive mode: *m/z* 357 $[M + 23]^+$

^1H NMR spectrum (400 MHz, $CDCl_3$): see page 137

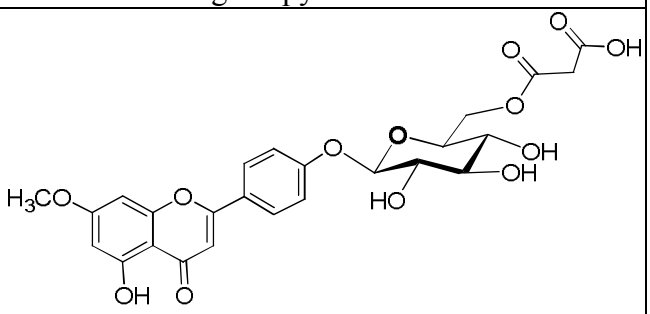
NMR data in $CHCl_3$

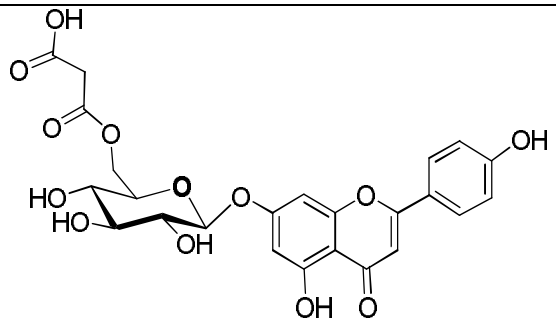
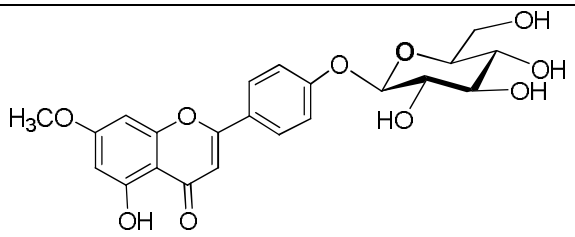
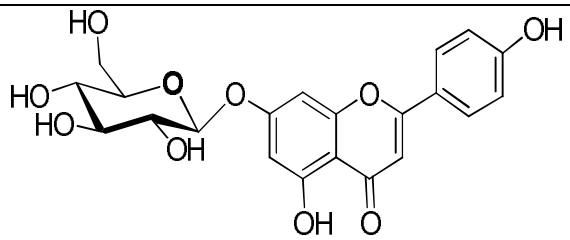
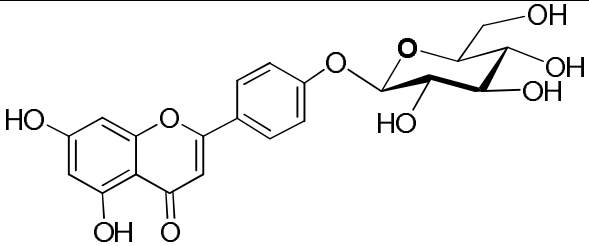
Position	**δ H (m, J (Hz) (400 MHz)**
2’	2.86 m
3’a	4.38 dd (11.1, 5.7)
3’b	4.49 dd (11.1, 6.7)
4’	1.27 d (6.7)

Compound IIb	Methyl-(*R*)-(-)-3-hydroxy-2-methyl propionate-*R*-MTPA-ester
$C_{15}H_{17}O_5F_3$ MW 334 Oil	

$[\alpha]_D$: +26° (c 0.23, $CHCl_3$)	
IR γ_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082. ESIMS positive mode: *m/z* 357 $[M + 23]^+$ ^1H NMR spectrum (400 MHz, $CDCl_3$): see page 137	
NMR data in $CHCl_3$	
Position	**δ H (m, J (Hz) (400 MHz)**
2’	2.86 m
3’a	4.38 dd (10.8, 5.7)
3’b	4.47 dd (10.8, 7.3)
4’	1.27 (6.7)

5.8.4 FLAVONOIDS

Compound 322	Genkwanin-4'-O-(6"-O-malonyl)- β-□-glucopyranoside
$C_{25}H_{24}O_{13}$ MW 532 Yellow powder	
MALDI spectrum: *m/z* 533 $[M + 1]^+$, *m/z* 447 $[M – 86]^+$ ^1H NMR (400 MHz, MeOD): see page 165 NMR data: see **Table 4.1** in page 171	
Compound 323	Apigenin-7-*O*-(6"-*O*-malonyl)- β-□-glucopyranoside

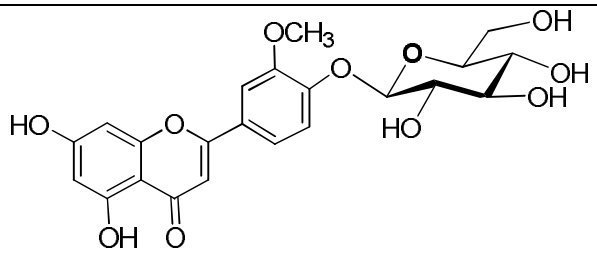
$C_{24}H_{22}O_{13}$ MW 518 Yellow powder	
MALDI spectrum: *m/z* 519 $[M + 1]^+$, *m/z* 541 $[M + 23]^+$, *m/z* 433 $[M - 86 + H]^+$ ^1H NMR (400 MHz, MeOD): see page 173 NMR data: see **Table 4.2** in page 175	
Compound 324	Genkwanin-4'-*O*-β-□-glucopyranoside
$C_{22}H_{22}O_{10}$ MW 446 Yellow powder	
ESIMS spectrum: *m/z* 469 $[M + 23]^+$ ^1H NMR (400 MHz, MeOD): see page 177 NMR data: see **Table 4.3** in page 179	
Compound 325	Apigenin -7-*O*-β-□-glucopyranoisde
$C_{21}H_{20}O_{10}$ MW 432 Yellow powder	
ESIMS spectrum: *m/z* 455$[M + 23]^+$ ^1H NMR (400 MHz, MeOD): see page 177 NMR data: see **Table 4.3** in page 179	
Compound 326	Apigenin -4'-*O*-β-D-glucopyranoisde
$C_{21}H_{20}O_{10}$ MW 432 Yellow powder	

ESIMS spectrum: *m/z* 455[M + 23]$^+$

^{1}H NMR (400 MHz, MeOD): see page 179

NMR data in MeOD

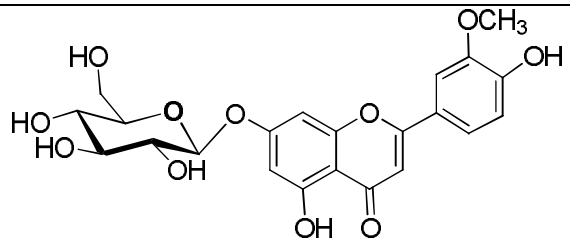
Position	**δ H (m, *J* (Hz) (400 MHz)**
3	6.75 s
6	6.28 s
8	6.42 s
2',6'	7.98 d (8.9)
3',5'	7.28 d (8.9)
1''	5.08 d (7.2)
2''	3.52 m
3''	3.52 m
4''	3.42 m
5''	3.54 m
6''a	3.74 dd(11.8, 6.1)
6''b	3.95 d (11.8)

Compound 327	Chrysoeriol-4'-*O*-β-D-glucopyranoside
$C_{22}H_{22}O_{11}$ MW 462 Yellow powder	

^{1}H NMR (400 MHz, MeOD): see page 181

NMR data in MeOD

Position	**δ H (m, J (Hz) (400 MHz)**
3	6.67 s
6	6.40 s
8	6.18 s
2'	7.57 d (1.9)
5'	7.32 d (8.9)
6'	7.61 dd (8.9, 1.9)

1''	5.08 d (7.2)
2''	3.52 m
3''	3.52 m
4''	3.42 m
5''	3.54 m
6''a	3.74 dd (11.8, 6.1)
6''b	3.95 d (11.8)
CH_3O	4.0 s
Compound 328	Chrysoeriol-7-*O*-β-D-glucopyranoisde
$C_{22}H_{22}O_{11}$ MW 462 Yellow powder	

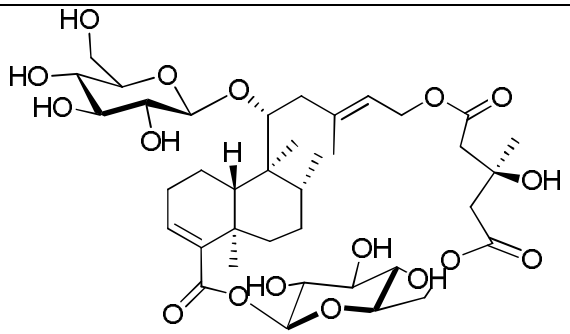
^{1}H NMR (400 MHz, MeOD) : see page 182

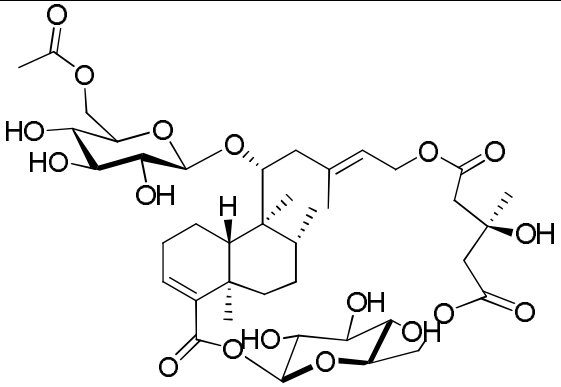
NMR data in MeOD

Position	**δ H (m, J (Hz) (400 MHz)**
3	6.75 s
6	6.40 s
8	6.71 s
2'	7.61 d (2.1)
5'	7.36 d (8.9)
6'	7.65 dd (8.9, 2.1)
1''	5.08 d (7.2)
2''	3.52 m
3''	3.52 m
4''	3.42 m
5''	3.54 m
6''a	3.74 dd (11.8, 6.1)
6''b	3.95 d (11.8)

CH_3O	3.98 s

5.8.5 SHYPHONOSIDES

Compound 286	Syphonoside
$C_{38}H_{58}O_{17}$ MW 786 White powder $[\alpha]_D$: -7° (c 0.54, MeOH)	
IR γ_{max}^{film} cm^{-1}: 3500, 2887, 1714. HRESIMS: *m/z* 809.3545 (calcd for $C_{21}H_{30}O_9Na$, 809.3672). ^{1}H NMR (400 MHz, MeOD): see page 184 ^{13}C NMR (150.92 MHz, $CDCl_3$): see page 184 NMR data: see **Table 4.4** in page 188	

Compound 287	Acetyl syphonoside
$C_{40}H_60_{18}$ MW 828 White powder $[\alpha]_D$: -15.5° (c0.54,MeOH)	
IR γ_{max}^{film} cm^{-1}: 3584, 2922, 1714. HRESIMS: *m/z* 851.3680 (calcd for $C_{21}H_{30}O_9Na$, 851.3677). ^{1}H NMR (400 MHz, MeOD): see page 189	

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Chemical analysis of flavonoid constituents of the seagrass *Halophila stipulacea*: First finding of malonylated derivatives in marine phanerogams

Fatma Bitam [1], Maria Letizia Ciavatta*, Marianna Carbone, Emiliano Manzo, Ernesto Mollo, Margherita Gavagnin

Istituto di Chimica Biomolecolare (CNR),Via Campi Flegrei 34, I-80078 Pozzuoli (Na), Italy

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ABSTRACT

The flavonoid fraction from the butanol extract of a Mediterranean sample of the seagrass *Halophila stipulacea* was chemically analyzed. A new malonylated flavone glucoside, genkwanin-4′-*O*-(6“-malonyl-glucopyranoside) (**3**), was isolated together with known flavone glucosides **4**-**9**, previously reported only from terrestrial sources. The structure of **3** was established by means of spectroscopic techniques, mainly NMR methods.

1. Introduction

The ecological importance of the marine flowering plants, the so-called seagrasses, is not only due to their extraordinarily high rate of primary production, but also to their ability to serve as nurseries, providing a habitat and protection from predators for many diverse benthic organisms. Consequently, several ecological studies on these species have been extensively carried out to determine the environmental “health” of coastal and estuary ecosystems (Dawes, 1998). Despite this, analysis and chemical elucidation of the secondary metabolite products from seagrasses has only recently been undertaken, highlighting antifouling, antibacterial, antiviral, anti-inflammatory and cytotoxic bioactivities (Kong et al., 2008; Kumar et al., 2008; Rowley et al., 2002; Qi et al., 2008).

Seagrasses belonging to the genus *Halophila* are widely distributed along the western coasts of the Indian Ocean, Red Sea and South-eastern Florida coasts. This genus has been the subject of many ecological studies whereas few phytochemical investigations have been conducted to date. The latter reported the presence of unidentified sulphated phenolic compounds from nine different species of *Halophila* including *Halophila stipulacea* (McMillan et al., 1980), unidentified sulphated and non-sulphated flavones from *Halophila ovalis-H. minor* complex (McMillan, 1986), flavones and flavone glycosides from *Halophila johnsonii* (Meng et al., 2008). Antibacterial activity against a series of microorganisms has been described for methanolic and ethyl acetate extracts of *H. ovalis* from the South Indian Sea (Kumar et al., 2008).

H. stipulacea is an Indo-Pacific seagrass introduced through the Suez Canal to the Mediterranean Sea where it was recorded for the first time in 1895 (Lipkin, 1975). It is one of the nine macrophyte species that are considered as invasive playing a significant role in the recipient ecosystems, taking the place of keystone species and being economically harmful

* Corresponding author. Tel.: +39 081 8675243; fax: +39 081 8041770.
E-mail address: lciavatta@icb.cnr.it (M.L. Ciavatta).
[1] On leave from Faculté des Sciences, Département de Chimie, Université de Batna, 05000 Batna, Algeria.

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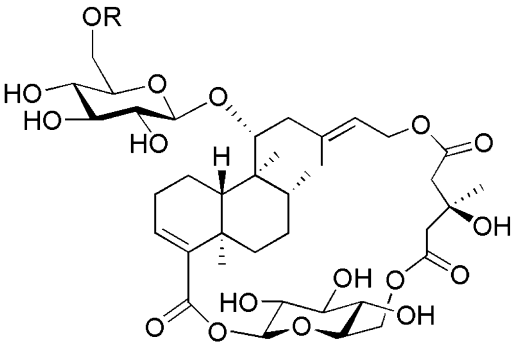
(Boudouresque and Verlaque, 2002). In a previous study on a sample of this plant collected in the Gulf of Corinth, Greece, and analyzed together with the anaspidean mollusc *Syphonota geographica* feeding on the seagrass, we reported the isolation and the characterization of an unusual glycoterpenoid, syphonoside **1** (Gavagnin et al., 2007). In a subsequent work, the acetyl derivative of syphonoside, compound **2**, was also isolated from the phanerogam (Carbone et al., 2008). Furthermore, chemo-ecological implications of the introduction of both exotic species, the mollusc *S. geographica* and the seagrass *H. stipulacea*, in the Mediterranean Sea were discussed by Mollo et al. (2008).



1: R =H
2: R=Ac

Here, we report the investigation of additional chemical constituents of *H. stipulacea* with regard to the flavonoid fraction that led to the characterization of a new malonylated glucopyranosyl flavone (**3**), isolated along with known flavonoids such as malonylated glucopyranosylapigenin (**4**) and five related glucopyranosyl flavones (**5**-**9**).

2. Material and methods

2.1. Plant material

H. stipulacea (Forsskål) Aschers. was collected off Porto Germeno coasts (Gulf of Corinth, Greece) at 5–10 m depth by SCUBA diving during December 2003. The sample was stored at −20 °C until chemical analysis. A voucher specimen (Halo-71) has been deposited at the Institute of Biomolecular Chemistry (ICB), National Council of Research, Italy.

2.2. Chemical procedure

The butanolic soluble portion (1.31 g) of the acetone extract was obtained as already described (Gavagnin et al., 2007) from *H. stipulacea* frozen sample (103 g dry weight), and analyzed by TLC. Some UV polar components which gave a yellow coloration by reaction with cerium sulphate were detected at R*f* 0.15 and R*f* 0.70–0.60 (chloroform/methanol, 7:3) together with the previously reported syphonoside (**1**) (Gavagnin et al., 2007) and its acetyl derivative **2** (Carbone et al., 2008).

An aliquot of butanolic extract (380 mg) was subjected to Sephadex LH-20 chromatography eluting with a mixture of chloroform/methanol in 1:1 ratio. The collected fractions were analyzed by both TLC chromatography and NMR, and then combined to obtain three main flavonoid glycoside-containing fractions: A (100 mg), B (14.1 mg), and C (71.5 mg). A portion of fraction A (33.7 mg), which was also characterized by the presence of syphonosides **1** and **2**, was first submitted to preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a flavonoid glycoside mixture (9.4 mg) corresponding to the UV components at R*f* 0.15 (chloroform/methanol, 7:3). The latter was further purified by RP-HPLC (Synergi-HydroRP C18, 250 × 4.6 mm; 40 min gradient from 50% to 100% CH_3OH in H_2O, flow 1 mL/min, UV detector) to give the new genkwanin-4′-*O*-β-(6″-*O*-malonyl-glucopyranoside) (**3**, 1.2 mg) and the known apigenin-7-*O*-β-(6″-*O*-malonyl-glucopyranoside) (**4**, 4.0 mg). Purification of fraction B on preparative TLC (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) afforded a mixture (4.1 mg) of less polar flavonoid glycosides (R*f* 0.70, chloroform/methanol, 7:3), which was further purified by RP-HPLC (Phenomenex: Kromasil 5 μ C18, 250 × 10 mm; CH_3OH/H_2O, 60:40; flow 2 mL/min, UV detector) to obtain the known genkwanin-4′-*O*-β-glucopyranoside (**5**, 1.7 mg) and chrysoeriol-7-*O*-β-glucopyranoside (**6**, 0.9 mg).

An aliquot of fraction C (19 mg) was subject to a preparative TLC purification (silica gel, 0.5 mm plates; chloroform/methanol, 7:3) to obtain a mixture of the flavonoid glycosides at R*f* 0.65–0.60 (chloroform/methanol, 7:3). The subsequent purification of this fraction by RP-HPLC (Phenomenex: Kromasil 5 μ C18, 250 × 10 mm; CH_3OH/H_2O, 1:1; flow 2 mL/min, UV detector) yielded the known apigenin-7-*O*-β-glucopyranoside (**7,** 6.9 mg), apigenin-4′-*O*-β-glucopyranoside (**8,** 2.2 mg), and chrysoeriol-4′-*O*-β-glucopyranoside (**9,** 1.8 mg).

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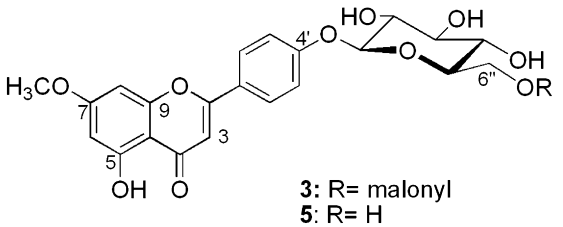
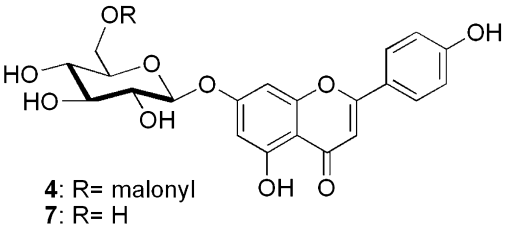
3. Results and discussion

3.1. Flavonoid characterization

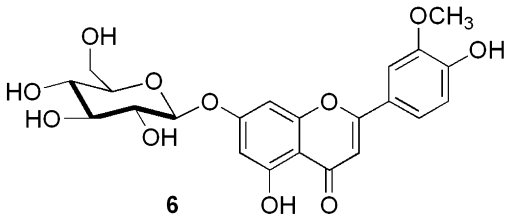
The flavonoid components of the butanol extract of the seagrass *H. stipulacea* were purified as described in Material and Methods to obtain seven pure glucosyl flavones (**3**-**9**). In particular, two malonyl derivatives, genkwanin-4′-*O*-β-(6″-*O*-malonyl)-glucopyranoside (**3**) (1% of the butanol extract) and apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) (Takeda et al., 1993; Švehlíková et al., 2004) (3%), were isolated from the polar fraction of the flavone mixture whereas five non-esterified glucosyl flavones, genkwanin-4′-*O*-β-glucopyranoside (**5**, 0.4% of the butanol extract) (Veit et al., 1990), chrysoeriol-7-*O*-β-glucopyranoside (**6**, 0.2%) (Harput et al., 2006), apigenin-7-*O*-β-glucopyranoside (Takeda et al., 1993) (**7**, 7%), apigenin-4′-*O*-β-glucopyranoside (Nawar et al., 1994) (**8**, 2%), and chrysoeriol-4′-*O*-β-glucopyranoside (**9**, 2%) (Fukunaga et al., 1989) were isolated from the less polar portion of the extract. Malonyl derivatives **3** and **4** were unstable and a rapid degradation was observed to occur under the purification conditions used. This made the isolation of the pure compounds quite difficult. However, we were able to obtain pure **3** in an amount sufficient for a full spectroscopic analysis.



3: R= malonyl
5: R= H



4: R= malonyl
7: R= H



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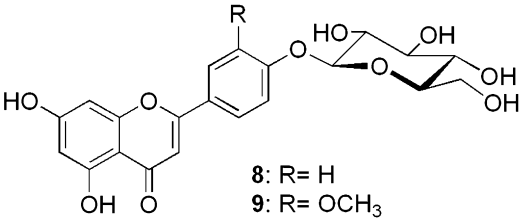

8: R= H
9: R= OCH_3

The main flavonoid metabolites of *H. stipulacea* were apigenin-7-*O*-β-glucopyranoside (**7**), a very common terrestrial flavonoid, and the corresponding malonyl ester **4**, previously described only from the blue flowers of lupin (Takeda et al., 1993) and from chamomile (Švehlíková et al., 2004). The minor co-occurring malonyl derivative of genkwanin-4′-*O*-glucoside (**3**) was an unprecedented compound. The known molecules **4**-**9** were identified by comparing NMR and mass data with those reported in the literature whereas the structure of **3** was defined as follows.

Genkwanin-4′-*O*-β-(6″-*O*-malonyl)-glucopyranoside (**3**): $C_{25}H_{24}O_{13}$, obtained as a yellow amorphous powder; UV (CH_3OH) λ_{max} (log ϵ): 206 (4.32), 269 (4.66) nm; MALDI/MS, *m/z* 533 $[M + H]^+$, 447 $[M - 86$ (malonyl) $+ H]^+$; HR-MALDI/MS *m/z* 533.1269 (533.1295 calculated for $C_{25}H_{24}O_{13} + H$). The molecular formula of **3** was consistent with both the MS and ^{13}C NMR data. Analysis of the ^{13}C NMR and DEPT spectra immediately indicated that compound **3** had the same flavonoid nature as the co-occurring genkwanin-4′-*O*-β-glucopyranoside (**5**) (Veit et al., 1990). In fact, analogously with **5**, the aglycone moiety was identified as genkwanin as well as the sugar residue was determined to be glucose (see Table 1).

The additional mass fragment $C_3H_3O_3$ required by the molecular formula of **3** was attributed to an acyl residue attached to an hydroxyl group of the molecule. Comparison of the ^1H NMR spectrum of **3** recorded in DMSO-d_6 with **5** (Veit et al., 1990) clearly revealed that in **3** the hydroxyl esterified was at C-6 position of the glucose unit. In fact, H_2-6″ protons resonated at δ_H 4.08 and 4.25, which were downfield shifted with respect to the corresponding proton values (δ_H 3.11–3.79) observed for the non-esterified compound (Veit et al., 1990). According to the expected acylation chemical shift difference, C-6″ resonated at δ_C 64.5 in **3**, compared with δ_C 60.7 in **5** (Veit et al., 1990). Finally, the acyl residue was identified as a malonyl unit by the diagnostic signals observed in the ^1H and ^{13}C NMR spectra of **3** (in DMSO-d_6) [δ_C 169.5 (C-3‴), 168.2 (C-1″), 45.7 (C-2‴); δ_H 2.94 (2H, s, H_2-2‴)] (Table 1).

A detailed analysis of 2D-NMR experiments (^1H-^1H COSY, HSQC and HMBC) carried out in CD_3OD and DMSO-d_6 (Table 1) allowed the full assignment of proton and carbon values of **3** and also confirmed the proposed structure including the sites of malonylation and glucosylation at C-6″ and C-4′, respectively. Accordingly, diagnostic long-range correlations were observed in the HMBC spectrum of **3** (in CD_3OD) between C-4′ and H-1a″ as well as between C-1‴ and H-1′ (Table 1).

The stability of compound **3** was very low. In fact it was observed that **3** underwent a rapid deacylation reaction during work-up to give the corresponding non-esterified genkwanin-4′-*O*-β-glucopyranoside (**5**). An analogous chemical behaviour

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Table 1
NMR data of genkwanin-4'-*O*-β-(6"-*O*-malonyl-glucopyranoside) (**3**)

C	DMSO-d_6					CD_3OD				
	δ ^{13}C[a]	m[b]	δ ^{1}H[c]	m *J* (Hz)	HMBC[d]	δ ^{13}C[a]	m[b]	^{1}H[c]	m *J* (Hz)	HMBC[d]
2	163.4	s	–			165.6	s	–		
3	104.1	d	6.96	s	C-2, C-4	105.2	d	6.75	s	C-l′, C-2, C-4
4	182.8	s	–			184.6	s	–		
5	160.3	s	–			164.2	s	–		
6	98.0	d	6.40	d (2.4)	C-5, C-7	98.9	d	6.39	d (2.4)	C-5, C-7, C-8, C-10
7	165.2	s	–			167.4	s	–		
8	92.8	d	6.80	d (2.1)	C-9, C-10	93.6	d	6.71	d (2.1)	C-6, C-9
9	157.3	s	–			159.3	s	–		
10	104.7	s	–			106.6	s	–		
1'	123.8	s	–			126.1	s	–		
2',6'	128.2	d	8.05	d (8.9)	C-2	129.6	d	8.05	d (8.9)	C-2, C-4'
3',5'	116.5	d	7.15	d (8.9)	C-l'	118.2	d	7.26	d (8.9)	C-4', C-2′, C-6'
4'	160.3	s	–	s		161.8	s	–		
1"	99.8	d	5.05	d (6.1)	C-4'	101.5	d	5.05	d (7.2)	C-4'
2"	73.6	d	3.28	m		74.7	d	3.54	m	
3"	75.9	d	3.30	m		77.8	d	3.52	m	
4"	69.6	d	3.20	m		715	d	3.43	m	
5"	73.8	d	3.64	m		75.7	d	3.78	m	
6"a	64.5	t	4.08	d (11.8)		64.7	t	4.33	d (11.8)	C-1""
6"b			4.25	dd (11.8, 6.1)				4.54	dd (11.8, 6.1)	
$-OCH_3$	56.0	q	3.82	s		56.4	q	3.95	s	C-7
1""	168.2	s	–			170.5		–		
2""	45.7	t	2.94	s	C-l"", C-3""	45.4	t	2.95	s	
3""	169.5	s	–			nd		–		

[a] Bruker 300 MHz
[b] Multiplicity deduced by DEPT
[c] Bruker 600 MHz
[d] Long-range coupling constants optimized for $J = 10$ and 6 Hz)

was observed for apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) which degraded under silica–gel chromatographic conditions to form the corresponding non-esterified derivative **7**.

3.2. Previous studies and chemo-ecological significance

Following our previous study on the chemical constituents of the invasive seagrass *H. stipulacea* (Gavagnin et al., 2007; Carbone et al., 2008) that resulted in the isolation of the glucoterpenoid macrocycles, syphonoside (**1**) and its acetyl derivative **2**, we have now investigated the flavonoid content of the plant and identified the components. This study indicated that the flavonoid profile of *H. stipulacea* was dominated by apigenin-7-*O*-β-glucopyranoside (**7**) co-occurring with other minor flavone glucosides including two malonyl derivatives, the new compound **3** and apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**). It is noteworthy that this is the first finding of malonylated flavone glycosides in the marine environment. However, to the best of our knowledge, with the exception of a malonyl amide alkaloid that has been recently found in a marine bacterium associated with a zoanthid (Kita et al., 2007), no other malonyl-containing metabolites have been reported from marine organisms. On the contrary, malonyl flavone glucosides derivatives have been reported from many terrestrial sources (Veit et al., 1990; Takeda et al., 1993; Švehlíková et al., 2004; Montoro et al., 2005; Kim et al., 2009).

The instability of malonylated flavone glycosides that is due to the known reactivity of the malonyl ester linkage *in vitro*, has been studied for different acetyl and malonyl derivatives of apigenin glucosides from chamomile by Švehlíková et al. (2004). In this paper, it was reported that apigenin-7-*O*-β-(6″-*O*-malonyl)-glucopyranoside) (**4**) undergoes rapid decarboxylation at room temperature forming apigenin-7-(6″acetyl-glucopyranoside) and, subsequently, under different solvent conditions, deacetylates completely giving apigenin-7-*O*-β-glucopyranoside. This was in agreement with the observations in the present work. The deacylation reaction of compounds **3** and **4** occurring during the work-up suggested that malonylated flavone glycosides were most likely more abundant in the plant than chemical analysis indicates and, consequently, the corresponding non-esterified derivatives **5** and **7** should be considered as artefacts.

With the exception of a recent investigation on *H. johnsonii* resulting in the chemical characterization of all main components of the flavone profile of this seagrass (Meng et al., 2008), previous studies on different *Halophila* species including *H. stipulacea* only reported qualitative analysis of flavone profiles and indicated the presence of unidentified flavones and flavone sulphates (McMillan et al., 1980; McMillan, 1986). In particular, these studies revealed a high variability in the flavone composition for the different *Halophila* species analyzed with regard to the complexity of the flavone mixture and to the presence of sulphate derivatives thus preventing reliable interspecific taxonomic relationships in *Halophila* seagrasses based on qualitative evaluation of flavone profiles. It is interesting to note that some polar flavonoids exhibiting a chromatographic behaviour similar – but not identical – to that of sulphate flavone derivatives found in other seagrasses were detected in the *H. ovalis-H. minor* complex (McMillan, 1986). By analogy with *H. stipulacea* and considering that flavone

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malonylglycosides show chromatographic properties similar to those of sulphate derivatives, malonyl-containing structures could be tentatively suggested for these flavonoids.

In conclusion, our studies on *H. stipulacea* revealed the presence of a flavone glycoside pattern less complex and characterized by different aglycone moieties with respect to that reported for *H. johnsonii* (Meng et al., 2008). The occurrence of flavone malonylglycosides has not been previously reported in marine phanerogams even though it should be taken into consideration that flavone acetylglycosides isolated from *H. johnsonii* (Meng et al., 2008) could be derived from the corresponding malonyl derivatives by decarboxylation. Unfortunately, chemical data on the identity of flavonoid metabolites of other *Halophila* species are lacking in the literature. Additional studies in this field should be necessary to get useful information for relating flavone chemistry to taxonomy in seagrasses of genus *Halophila*, and to explore the ecological role of malonylated flavone glycosides in marine phanerogams.

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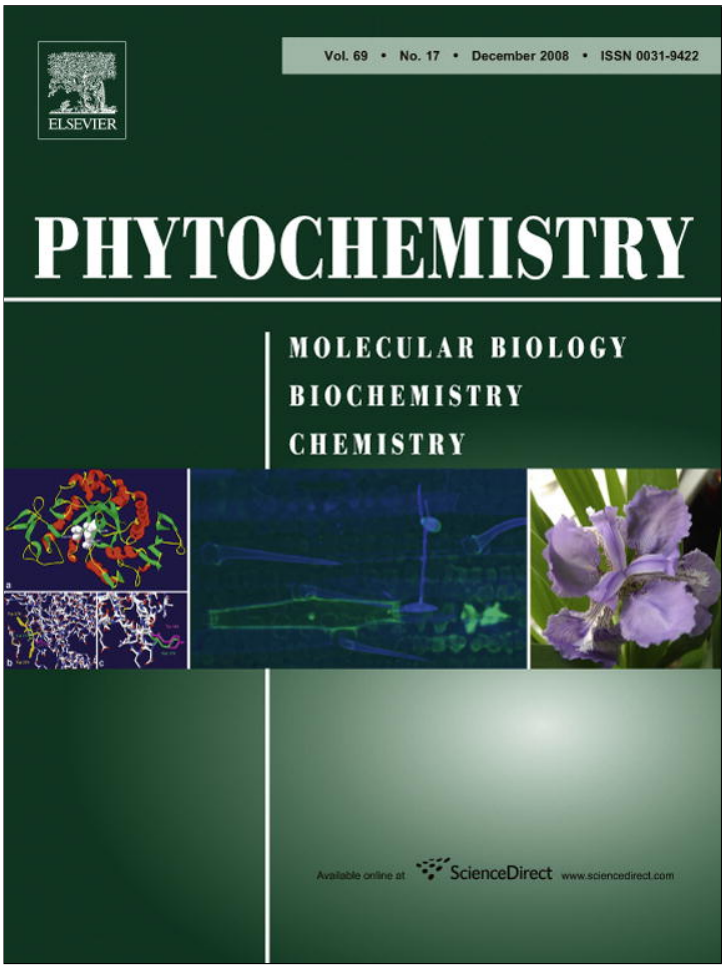

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Chemical characterisation of the terpenoid constituents of the Algerian plant *Launaea arborescens*

Fatma Bitam [a,b], M. Letizia Ciavatta [a], Emiliano Manzo [a], Ammar Dibi [b], Margherita Gavagnin [a,*]

[a] *Istituto di Chimica Biomolecolare, C.N.R., Via Campi Flegrei 34, I-80078 Pozzuoli, Naples, Italy*
[b] *Faculté des Sciences, Département de Chimie, Université de Batna, Batna 05000, Algeria*

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ABSTRACT

Chemical investigation of endemic Algerian plant *Launaea arborescens* resulted in the isolation of a series of triterpenes and sesquiterpenes from both the aerial parts and roots. Five terpenoids have been chemically characterised by means of spectral methods mainly NMR techniques. In addition, the absolute stereochemistry at the chiral carbon in the side chain of 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) has been determined by comparison of the ^{1}H NMR spectra of Mosher derivatives of **6** with those of the corresponding MTPA esters of model compounds.

1. Introduction

The genus *Launaea* belongs to the tribe Lactuceae of the Asteraceae family and contains about 40 species, most of which are adapted to dry, saline and sandy habits (Ozenda, 2004). In the flora of Algeria, five of the nine *Launaea* species present are endemic of North Africa and include *Launaea arborescens* (Batt.) Murb., which is a perennial medicinal shrub mainly distributed in the Southwest part of the country (Quezel and Santa, 1963; Ozenda, 2004). Despite to the pharmacological interest in this plant (local name "Oum Lbina") commonly used in the North African popular medicine against diarrhoea and abdominal spasms, a very few chemical studies on *L. arborescens* have been so far reported. To the best of our knowledge, only flavonoid (Mansour et al., 1983; Belboukhari and Cheriti, 2006), phenolic (Giner et al., 1992) and essential oil (Cheriti et al., 2006) constituents have been described in the literature. In addition, very interesting antifungal, antibacterial and insecticidal activities have been reported for the methanol extract of the plant (Belboukhari and Cheriti, 2006; Jbilou et al., 2008).

We describe here the results of our chemical investigation on the liposoluble extracts of both roots and aerial parts of Algerian specimens of *L. arborescens* that led to the finding of a plethora of sesquiterpenes and triterpenes, which were found to be differently distributed in both parts. Among these compounds, five unprecedented terpenes, 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**), 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C (**2**), 9α-hydroxy-4α,15-dihydro-zaluzanin C (**3**), 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**), and 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate (**5**), were isolated and fully characterised by spectral methods, mainly NMR techniques. Biological evaluation of pure novel compounds **1**–**5** against gram positive and gram negative bacteria was also carried out.

Finally, a stereochemical analysis was conducted on 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) (Zidorn et al., 2007), which was the main component of sesquiterpene fraction, with the aim at assigning the absolute configuration of the chiral centre in the 3′-hydroxy-2′-methyl-propanoyl fragment of the molecule, not previously determined.

2. Results and discussion

The whole plants of *L. arborescens* were collected in Bechar (Algeria) during flowering in 2006. The aerial parts of the plants were separated from the roots and both were allowed to dry before the extraction. The TLC chromatographic analysis of the liposoluble extracts obtained from both parts of the plant showed very complex and substantially distinct terpenoid secondary metabolite patterns even though some analogies were also observed. The extracts were submitted to subsequent chromatographic steps (see experimental) and the terpene-containing fractions were further purified by HPLC to obtain 27 pure metabolites, five of which were novel compounds.

* Corresponding author. Tel.: +39 0818675096; fax: +39 0818041770.
E-mail address: margherita.gavagnin@icb.cnr.it (M. Gavagnin).

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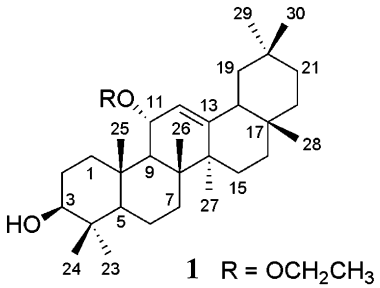
In particular, the terpenoid fraction of the aerial parts extract was found to be dominated by triterpene lupeol (**7**) (Reynolds et al., 1986) (2.9 g, 25% of the extract) co-occurring with a series of oleanane and ursane triterpenes including the novel 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**) and the known: 3-β-hydroxy-11-oxo-urs-12-ene (**8**) and 3-β-hydroxy-11-oxo-olean-12-ene (**9**) (Bandaranayake, 1980), taraxast-20-ene-3β,30-diol (**10**) (Dai et al., 2001; Kisiel and Zielinska, 2001a), oleana-9(11):12-dien-3β-ol **(11)** (Tanaka and Matsunaga, 1988), 3β-hydroxy-11α-ethoxyurs-12-ene (**12**) (Fujita et al., 2000), ursa-9(11):12-dien-3β-ol (**13**) (Matsunaga et al., 1988), 3β-11α-dihydroxy-olean-12-ene (**14**) (Xiao et al., 1994), 3β-hydroxy-11α-methoxyolean-12-ene (**15**) (Fujita et al., 2000), 3β-hydroxy-11α-methoxyurs-12-ene (**16)** and 3β-11α-dihydroxy-urs-12-ene (**17**) (Bohlmann et al., 1984). Stigmasterol (**18**) (Kojima et al., 1990), was also isolated as major sterol component of the plant extract.

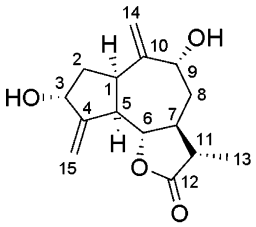
On the other side the terpenoid fraction of the ethyl acetate extract of the roots was characterised by a series of sesquiterpene metabolites exhibiting eudesmane, guaiane and germacrane skeletons, the main of which was 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) (Zidorn et al., 2007). Four unprecedented sesquiterpenes, 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C (**2**), 9α-hydroxy-4α,15-dihydrozaluzanin C (**3**), 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**), and 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate (**5**) were isolated along with known magnolialide (**19**) (El-Feraly et al., 1979; Kisiel and Zielinska, 2001a, b), 1β,8α-dihydroxy-eudesm-4-en-6β,7α,11βH-6-olide (**20**) (Marco, 1989), 11β,13-dihydrolactucin **21** (Sarg et al., 1982), picriside C (**22**) (Nishimura et al., 1986a; Miyase and Fukushima, 1987), sonchuside A **23** (Miyase and Fukushima, 1987), picriside B (**24**) (Nishimura et al., 1986a), ixerisoside D (**25**) (Warashina et al., 1990), crepidiaside A (**26**) (Adegawa et al., 1985) and macrocliniside A (**27**) (Miyase et al., 1984; Kisiel and Gromek, 1993). In addition, few amounts of triterpenes containing lupeol as main component were also detected in the ethyl acetate extract of the roots.

The known metabolites were identified by comparison of their spectral data (^{1}H NMR and MS spectra) with the literature. The structures of triterpene **1** and sesquiterpenes **2**–**5** were determined as described below. In particular, triterpene **1** displayed an oleanane skeleton, sesquiterpenes **2** and **3** exhibited a dihydroxylated guaianolide skeleton and were both related to zaluzanin C (Romo et al., 1967; Spring et al., 1995) whereas sesquiterpenes **4** and **5** were characterised by a glucosylated germacranolide framework structurally related to that of the co-occurring picriside C (**22)** (Nishimura et al., 1986a; Miyase and Fukushima, 1987).

The molecular formula of 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**) was $C_{32}H_{54}O_2$ as deduced by both mass spectrum and ^{13}C NMR spectra. Analysis of the ^{1}H NMR spectrum of compound **1** (Table 1) immediately revealed the close structural relationship with



1 R = OCH_2CH_3
14 R = OH
15 R = OCH_3



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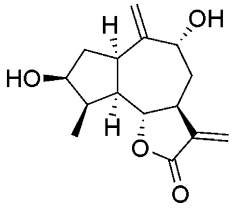

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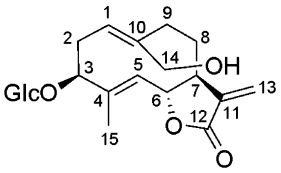

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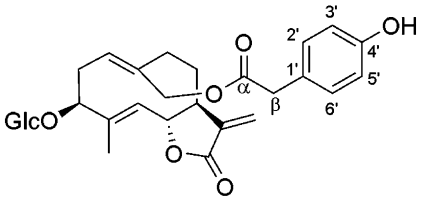

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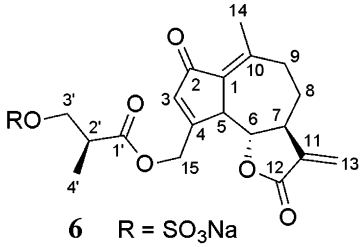

6 R = SO_3Na
6a R = H
6b R = Ac
6c R = *S*-MTPA
6d R = *R*-MTPA

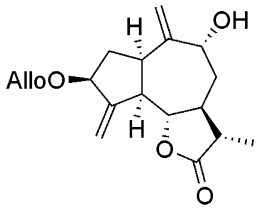

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Table 1
^{1}H and ^{13}C NMR data[a,b] of compound **1** in $CDCl_3$.

Position	δ c (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)
1	39.4 (*t*)	1.75 (*m*)	H-25
		2.02 (*m*)	
2	27.5 (*t*)	1.62 (*m*)	
3	78.6 (*d*)	3.23 (*m*)	H-23, H-24
4	38.7 (*s*)		H-23, H-24
5	55.2 (*d*)	0.77 (*m*)	H-23, H-24, H-25
6	18.4 (*t*)	1.37 (*m*)	
		1.42 (*m*)	
7	33.3 (*t*)	1.57 (*m*)	
		1.73 (*m*)	
8	42.6 (*s*)		H-9, H-27
9	51.5 (*d*)	1.72 (*d*, 9.0)	H-25, H-26
10	38.3 (*s*)		H-9, H-25
11	74.8 (*d*)	3.94 (*dd*, 9.4,3.3)	H-9
12	122.7 (*d*)	5.31 (*d*, 3.3)	H-11
13	148.9 (*s*)		H-11, H-27
14	41.8 (*s*)		H-9, H-12, H-27
15	26.3 (*t*)	1.70 (*m*)	H-27
		2.02 (*m*)	
16	26.8 (*t*)	1.61 (*m*)	H-28
17	32.3 (*s*)		H-28
18	46.9 (*d*)	1.99 (*m*)	H-12, H-28
19	46.5 (*t*)	1.05 (*m*)	H-29, H-30
		1.64 (*m*)	
20	31.1 (*s*)		H-29, H-30
21	34.7 (*t*)	1.11 (*m*)	H-29, H-30
22	37.0 (*t*)	2.02 (*m*)	
23	28.2 (*q*)	0.99 (*s*)	H-3, H-24
24	15.6 (*q*)	0.80 (*s*)	H-3, H-23
25	15.9 (*q*)	1.04 (*s*)	H-9
26	18.2 (*q*)	0.99 (*s*)	H-7
27	25.3 (*q*)	1.20 (*s*)	
28	28.5 (*q*)	0.83 (*s*)	
29	33.6 (*q*)	0.89 (*s*)	H-30
30	23.7 (*q*)	0.88 (*s*)	H-29
Ethoxy group			
OCH_2	61.4 (*t*)	3.31 (*dq*, 8.7,7.2)	CH_3, H-11
		3.55 (*dq*, 8.7,7.2)	
CH_3	16.8 (*q*)	1.14 (*t*, 7.2)	

[a] Bruker DRX 600 spectrometer in $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$(δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

the co-occurring 3β,11α-dihydroxy-olean-12-ene (**14**) and 3β-hydroxy-11α-methoxy-olean-12-ene (**15**), suggesting the presence of the same functionalised triterpenoid oleanane skeleton. In fact, eight methyl singlets [δ 0.80 (H_3-24), 0.83 (H_3-28), 0.88 (H_3-30), 0.89 (H_3-29), 0.99 (6H, H_3-23 and H_3-26), 1.04 (H_3-25), and 1.20 (H_3-27)] were observed in the proton spectrum of **1** that also contained two methine signals at δ 3.23 (*m*, H-3) and 3.94 (*dd*, *J* = 9.4 and 3.3 Hz, H-11) and the olefinic proton at δ 5.31 (1H, *d*, *J* = 3.3 Hz, H-12), similarly to compounds **12** and **14** (Fujita et al., 2000; Xiao et al., 1994), both isolated from the extract of the plant. The presence in compound **1** of an ethoxy group replacing the hydroxyl and the methoxyl function in **14** and in **15**, respectively, was indicated by both two double quartet at δ 3.31 and 3.55 (each 1 H, *dq*, *J* = 8.7 and 7.2 Hz, CH_3CH_2O–) and the 3H triplet at δ 1.14 (*J* = 7.2 Hz, CH_3CH_2O–). According to the proposed structure, in the ^{13}C NMR spectrum of **1** two additional signals due to the ethoxy moiety at δ 61.4 (CH_3CH_2O–) and 16.8 (CH_3CH_2O–) were observed along with the typical values of the oleanane skeleton (Table 1).

The relative stereochemistry at C-11 was suggested by analysis of the coupling constants of H-11 resonating as a double doublet (*J* = 9.4 and 3.3 Hz) in agreement with a β-orientation, analogously with the data reported for the corresponding methoxyl (Fujita et al., 2000) and hydroxyl (Bohlmann et al., 1984; Xiao et al., 1994) derivatives. A detailed 2D NMR analysis (^{1}H–^{1}H COSY, HSQC and HMBC) allowed the assignment of all carbon and proton values as reported in Table 1. Compound **1** underwent rapidly to an elimination reaction of the ethanol residue at C-11 to give the corresponding conjugated diene **11** (Tanaka and Matsunaga, 1988), also isolated from the extract. This conversion was also observed for **14** and **15**, thus suggesting that 3β-hydroxy-olean-9(11):12-diene (**11**) found in the extract is most likely a work-up derivative.

9α-Hydroxy-3-epi-11β,13-dihydrozaluzanin C (**2**) had the molecular formula $C_{15}H_{20}O_4$ as deduced by both ESIMS and ^{13}C NMR spectra. Analysis of the ^{1}H and ^{13}C NMR spectra (Table 2) of **2** indicated the presence of a guaianolide sesquiterpene skeleton the same as the co-occurring known metabolites **21**–**23**, **26** and **27**. Accordingly, two exomethylene groups [δ 4.81 (1H, br *s*, H-14a), 5.08 (1H, br *s*, H-14b), 5.38 (1H, br *s*, H-15a), and 5.46 (1H, br *s*, H-15b)], three oxymethine protons [δ 4.58 (1H, sharp *m*, H-9), 4.71 (1H, *m*, H-3) and δ 3.81 (1H, *t*, *J* = 9.7, H-6)], and a second-

Table 2
^{1}H and ^{13}C NMR data[a,b] of compounds **2** and **3** in $CDCl_3$.

Position	**2**			**3**		
	δ C (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)	δ C (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)
1	35.6 (*d*)	3.58 (*ddd*, 12.3,8.7,4.1)	H-5, H-14a, H-14b	32.5 (*d*)	3.32 (*q*, 10.1)	H-2a, H-14a, H-14b
2	39.4 (*t*)	2.20 (*m*)		3.41 (t)	2.00 (*m*)	H-l, H-4, H-14a
		1.95 (*m*)			2.00 (*m*)	
3	74.7 (*d*)	4.71 (*m*)	H-15a, H-15b, H-2a	74.1 (*d*)	4.30 (*m*)	H-2a, H-15a, H-15b
4	154.0 (s)		H-5, H-6	40.2 (*d*)	2.35 (*m*)	H-15
5	49.9 (*d*)	3.10 (*t*, 9.7)	H-15a, H-15b	46.9 (*d*)	2.35 (*m*)	H-1, H-2, H-13
6	84.9 (*d*)	3.81 (*t*, 9.7)	H-5	82.9 (*d*)	4.10 (*t*, 8.1)	H-4, H-5
7	44.3 (*d*)	2.32 (*m*)	H-11, H-13	41.0 (*d*)	3.15 (*m*)	H-13a, H-13b
8	39.7 (*t*)	1.50 (*m*)	H-6, H-11	38.6 (*t*)	1.60 (*m*)	H-6
		2.23 (*m*)			2.35 (*m*)	
9	74.3 (*d*)	4.58 (*m*)	H-8a, H-14a, H-14b	74.1 (*d*)	4.72 (br *s*)	H-14a, H-14b
10	150.9 (*s*)		H-l, H-2a	149.8 (*s*)		H-l, H-2a, H-2b, H-14
11	41.6 (*d*)	2.18 (*m*)	H-6, H-13	139.3 (s)		H-13
12	178.4 (*s*)		H-13	169.8 (s)		H-13
13	13.6 (*q*)	1.26 (*d*,7.2)		120.1 (*t*)	5.50 (*d*, 3.5)	
					6.21 (*d*, 3.5)	
14	112.7 (*t*)	4.81 (br *s*)		112.7 (*t*)	5.12 (br *s*)	H-l
		5.08 (br *s*)			5.16 (br *s*)	
15	112.7 (*t*)	5.38 (br *s*)	H-3b	8.1 (*q*)	0.98 (*d*,7.1)	H-4, H-5
		5.46 (br *s*)				

[a] Bruker DRX 600 spectrometer in $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

ary methyl [δ 1.26 (3H, *d*, *J* = 7.2, H_3-13)] were easily recognized by analysis of the ^{1}H NMR spectrum whereas the presence of a lactone functionality was suggested by the carbonyl signal at δ 178.4 in the ^{13}C NMR spectrum. Comparison of the NMR data in pyridine of **2** with those reported in the literature for related guaianolides (Asada et al., 1984; Kisiel and Michalska, 2002) showed a close structural similarity of **2** with 9α-hydroxy-11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside (**28**) (Kisiel and Michalska, 2002), which is the aglycone of glucosides previously isolated from different genera of Asteraceae family. In particular, the spin-system sequence from C-1 to C-9 in **2** was the same as **28** whereas differences were observed in the carbon and proton chemical shifts of the 5-membered ring strongly suggesting the opposite stereochemistry at C-3. According to this suggestion, H-1 and H-5 values were downfield shifted [δ H-1 4.01, δ H-5 3.25 in **2**; δ H-1 3.65, δ H-5 2.91 in **28** (values in pyridine)] due to the α-oriented 3-OH group. The proposed relative stereochemical arrangement was further supported by a series of steric effects observed in the NOESY spectrum of **2**. Along with the expected correlations between α oriented H-1 and H-5, diagnostic cross-peaks were observed between H-2α and H-1, and between H-2β and both H-14 and H-3 according to the β-orientation of H-3. Analogously, H-9 showed correlations only with H-14b and both protons H_2-8 thus supporting a β-orientation. All carbon and proton resonances were assigned by 2D-NMR experiments (^{1}H–^{1}H COSY, HSQC and HMBC) as reported in Table 2.

The molecular formula $C_{15}H_{20}O_4$ of 9α-hydroxy-4α,15-dihydro zaluzanin C (**3**) was established by HRESIMS on the sodiated molecular peak at 264.1259 *m/z*. The spectral data of **3** (Table 2) indicated the same guaianolide framework as compound **2** exhibiting two exomethylene groups [δ C 112.7 (C-14), 120.1 (C-13), 139.3 (C-11), and 149.8 (C-10)]; δ H 5.12 (1H, br *s*, H-14a), 5.16 (1H, br *s*, H-14b), 5.50 (1H, *d*, *J* = 3.5, H-13a), and 6.21 (1H, *d*, *J* = 3.5, H-13b)], a secondary methyl [δ C 8.1 (C-15), δ H 0.98 (3H, *d*, *J* = 7.1 Hz, H_3-15)] and two secondary hydroxyl functions [δ C 74.1 (2C, C-3 and C-9), δ H 4.30 (1H, *m*, H-3) and 4.72 (1H, br *s*, H-9)]. Careful analysis of the ^{1}H–^{1}H COSY experiment led us to define all the proton sequence from H-1 to H-9 indicating that the two hydroxyl groups were located at C-3 and C-9 analogously with **2** whereas the secondary methyl was at C-4, and the exocyclic double bonds were at C-10 and C-11. A survey of the literature on guaianolides showed that **3** was structurally related to known 9α-hydroxy-4α,11β,13,15-tetrahydrozaluzanin C (Kisiel and Barszcz, 1996). In particular, strong similarities were observed in the proton spectra of the two molecules with regards to the perhydroazulene bicyclic portion clearly suggesting for this part the same substitution pattern including the relative stereochemistry. The only difference was the presence in **3** of an additional double bond in the lactone ring at C-11(13). The suggested relative stereochemistry was further supported by a NOESY experiment that showed diagnostic correlations between the α-oriented protons H-1 and H-3, and between the β-oriented H_3-15 and H-6. Expected NOE interactions between H-5 and both H-1 and H-7 according to the guaianolide skeleton with 1,5-*cis* and 6,7-*trans* junctions were also detected. Full assignment of proton and carbon values (Table 2) was made by means of detailed analysis of 2D–NMR experiments (^{1}H–^{1}H COSY, HSQC and HMBC).

The HRESIMS spectrum of 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**) showed a sodiated molecular peak at 426.1788 *m/z* according to the molecular formula $C_{21}H_{30}O_9$. The ^{1}H and ^{13}C NMR spectra of **4** indicated the presence of the following structural features: two trisubstituted double bonds [δ C 126.6 (C-1), 127.3 (C-5), 141.5 (C-4), 135.2 (C-10); δ H 5.05 (1H, overlapped, H-1), 5.02 (1H, overlapped, H-5)], an exocyclic double bond conjugated to a lactone carbonyl [δ C 119.6 (*t*, C-13), 139.0 (*s*, C-11), 168.8 (*s*, C-12); δ H 5.50 (1H, *d*, *J* = 2.8 Hz, H-13a) and 6.32 (1H, *d*, *J* = 2.8 Hz, H-13b)], a vinyl methyl [δ H 1.95 (3H, *s*, H_3-15), a vinyl hydroxymethyl [δ H 4.10 (1H, *d*, *J* = 12.3 Hz, H-14a), δ H 4.47 (1H, *d*, *J* = 12.3 Hz, H-14b] and a secondary hydroxyl group connected to a glucopyranose moiety by a glycosyl linkage [δ H 3.90 (1H, *m*, H-5″), δ H 4.10 (1H, *m*, H-2″), δ H 4.25 (1H, *m*, H-3″), δ H 4.28 (1H, *m*, H-4″), δ H 4.42 (1H, *dd*, *J* = 12.1, 5.3 Hz, H-6″a), δ H 4.61 (1H, *dd*, *J* = 12.1, 4.8 Hz, H-6″b), δ H 4.83 (1H, *d*, *J* = 8.0 Hz, H-1″)]. The spectral data were reminiscent with those of the co-occurring picriside C (**22**) (Miyase and Fukushima, 1987) strongly suggesting the presence of the same germacranolide skeleton bearing a glucosylated hydroxyl function at C-3 and containing an additional hydroxyl group at C-14. The geometry of the two endocyclic double bonds at C-1 (10) and C-4 (5) was suggested *Z* and *E*, respectively, as reported in formula **4**, by analogy with **22** and further supported by diagnostic NOE effects observed between H_2-14 and H-2a and between H-6 and H_3-15, respectively. Analogously, the *trans*-junction at C-6/C-7 and the β-orientation of the *O*-glucosyl residue were suggested to be the same as picriside C. A detailed analysis of 2D NMR spectra supported the proposed structure and allowed the attribution of all carbon and proton resonances (Table 3). Our assignment was in agreement with those reported in the literature for related germacranolides exhibiting two hydroxyl groups at both C-3 and C-14 (Nishimura et al., 1986b; Kisiel and Barszcz, 1997; 1998).

Analysis of the spectral data of 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate (**5**) immediately revealed the close relationship with **4**. The molecular formula $C_{29}H_{36}O_{11}$ of **5**, deduced by HRESIMS spectrum on the sodiated molecular peak at *m/z* 560.2155 indicated the presence of an additional $C_8H_6O_2$ moiety with respect to **4**. The ^{1}H and ^{13}C NMR spectra of the two compounds were very similar according to the presence of the same germacranolide skeleton bearing the glucosylated hydroxyl function at C-3 and the hydroxyl group at C-14. Further three signals at δ 3.72 (2H, *d*, *J* = 3.5 Hz, CH_2β), 7.14 (2H, *d*, *J* = 8.2 Hz, H-3′ and H-5′), and 7.32 (2H, *d*, *J* = 8.2 Hz, H-2′ and H-6′) were observed in the proton spectrum of **5** suggesting that the molecule contained a *p*-hydroxy-phenyl acetic residue esterified to an OH group. Accordingly, the carbon spectrum contained additional signals at δ 170.4 (CO, Cα), 41.0 (*t*, Cβ), 125.2 (*s*, C-1′), 131.2 (2C, *d*, C-2′ and C-6′), 116.5 (2C, *d*, C-3′ and C-5′), and 158.3 (*s*, C-4′). Careful comparison of proton and carbon NMR spectra of **5** with those of **4** showed a substantial similarity for δ H and δ C values of the glucosyl moieties of both compounds whereas significant differences were observed for C-14 values strongly supporting the location of the acyl residue at 14-OH. This suggestion was further confirmed by diagnostic HMBC correlations observed between the carbonyl signal at δ 170.4 (Cα) and the methylene protons at δ 4.57 and 4.76 (H_2-14). Thus compound **5** was the 14-*O*-*p*-hydroxy-phenyl acetic ester derivative of **4**. All NMR resonances were assigned as reported in Table 3 by 2D experiments. The chemical correlation between the two molecules was finally confirmed by hydrolysis of **5** that afforded *p*-hydroxy-phenyl acetic acid and a glucosyl alcohol that resulted to be identical to 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**).

Finally, a stereochemical analysis was conducted on the known compound **6** that was the main component of the sesquiterpene pool of *L. arborescens*. First of all, with the aim at confirming the absolute stereochemistry at the 6,7-junction of the guaianolide framework, suggested as reported for the most literature natural guaianolides, we decided to apply the Mosher method on the corresponding alcohol derivative obtained by opening of the lactone ring of **6**. Unfortunately, due to the rapid dehydration reaction that was observed to occur under different methanolysis conditions, every attempt to obtain the free secondary alcohol at C-6 was unsuccessful. Subsequently we decided to determine the absolute configuration of the chiral centre in 3′-hydroxy-2′-methyl-propanoyl fragment as follows. Compound **6** was subjected to

Table 3
^1H and ^{13}C NMR data [a,b] of compounds **4** and **5** in C_5D_5N.

Position	**4**			**5**		
	δ C (*m*)	δ H (*m*,*J* Hz)	HMBC[c] (C to H)	δ C(*m*)	δ H (*m*,*J* Hz)	HMBC[c] (C to H)
1	126.6 (*d*)	5.05 (*m*)		131.3 (*d*)	4.95 (*dd*, 10.6, 5.9)	H-9
2	33.0 (*t*)	2.68 (*q*, 11.8)		33.3 (*t*)	2.50 (*m*)	H-3
		2.55 (*m*)			2.58 (*q*)	
3	83.2 (*d*)	4.90 (*m*)	H-2, H-5, H-1′, H-15	82.8 (*d*)	4.83 (*m*)	H-1, H-1", H-2, H-15
4	141.5 (*s*)		H-5, H-15	140.8 (*s*)		H-3, H-6, H-15
5	127.3 (*d*)	5.02 (*m*)	H-7, H-15	127.4 (*d*)	4.98 (*d*, 10.2)	H-3, H-15
6	81.4 (*d*)	4.80 (*t*, 4.8)		81.2 (*d*)	4.68 (*t*, 8.8)	H-7
7	50.2 (*d*)	2.52 (*m*)	H-13	50.0 (*d*)	2.46 (*m*)	H-5, H-9, H-13a, H-13b
8	28.9 (*t*)	2.15 (*m*) 1.90 (*m*)	H-6, H-9	29.0 (*t*)	1.58 (*m*) 1.93 (*m*)	H-6, H-7
9	36.9 (*t*)	1.90 (*m*) 3.15 (*m*)		37.0 (*t*)	1.90 (*m*) 2.58 (*m*)	H-1, H-8, H-14
10	135.2 (*s*)		H-2, H-9	135.9 (*s*)		H-9, H-14
11	139.0 (*s*)			142.2 (*s*)		H-6, H-7, H-13a, H-13b
12	168.8 (*s*)		H-13	172.0 (*s*)		H-13a, H-13b
13	119.6 (*t*)	5.50 (*d*, 2.8)		119.8 (*t*)	5.49 (*d*, 3.5)	
		6.32 (*d*, 2.8)			6.35 (*d*, 3.5)	
14	58.5 (*t*)	4.10 (*d*, 12.3)		61.5 (*t*)	4.57 (*d*, 12.3)	H-l, H-9
		4.47 (*d*, 12.3)			4.76 (*d*, 12.3)	
15	12.0 (*q*)	1.95 (*s*)	H-5	12.0 (*q*)	1.85 (*s*)	H-3, H-5
Ester moiety						
Cα				170.4 (*s*)		CH_2β, H-14
CH_2β				41.0 (*t*)	3.72 (*d*, 3.5)	H-2′, H-6′
1′				125.2 (*s*)		CH_2β, H-5′
2′, 6′				131.2 (*d*)	7.32 (*d*, 8.2)	CH_2β
3′, 5′				116.5 (*d*)	7.14 (*d*, 8.2)	
4′				158.3 (*s*)		H-2′, H-3′
Sugar moiety						
1"	102.8 (*d*)	4.83 (*d*, 8.0)	H-3	102.8 (*d*)	4.82 (*d*, 7.6)	H-3, H-2"
2"	75.3 (*d*)	4.10 (*m*)		75.4 (*d*)	4.09 (*t*, 8.2)	
3"	78.6 (*d*)	4.25 (*m*)	H-5"	78.6 (*d*)	4.23 (*m*)	H-2"
4"	71.7 (*d*)	4.28 (*m*)	H-3"	*71.9* (*d*)	4.23 (*m*)	H-3"
5"	78.6 (*d*)	3.90 (*m*)		78.7 (*d*)	3.90 (*m*)	H4", H-6"
6"a	62.9 (*t*)	4.42 (*dd*, 12.1,5.3)		63.0 (*t*)	4.40 (*dd*, 11.7,5.3)	H-4"
6"b		4.61 (*dd*, 12.1,4.8)			3.71 (*dd*, 11.7,2.3)	

[a] Bruker DRX 600 spectrometer in C_5D_5N, chemical shifts (ppm) refered to pyridine-d_5 (δ 8.71, 7.56, 7.19) and (δ 149.9, 135.5, 123.5).
[b] Assignmens made by ^1H–^1H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

methanolysis in acid conditions to give the corresponding alcohol **6a**, which was fully characterised as acetyl derivative **6b**. Compound **6a** was allowed to react with *R*-(−) and *S*-(+)-α-methoxy-α-trifluoromethyl-phenylacetic acid chlorides to obtain the *S*- and *R*-MTPA ester derivatives **6c** and **6d**, respectively. Analysis of the ^1H NMR spectra of the two esters showed significant differences in the multiplet patterns of H_2-3′ (see Fig. 1). The same reaction conducted on both commercial methyl-(*S*)-(+)-3-hydroxy-2-methyl-propionate (**I**) and methyl-(*R*)-(−)-3-hydroxy-2-methyl-propionate (**II**) afforded two pairs of MTPA derivatives, (*S*)/(*S*)-MTPA ester (**Ia**), (*S*)/(*R*)-MTPA ester (**Ib**), (*R*)/(*S*)-MTPA ester (**IIa**) and (*R*)/(*R*)-MTPA ester (**IIb**). Comparison of the ^1H NMR spectra of each pair with those of *S*- and *R*-MTPA esters **6c** and **6d** clearly showed that H_2-3′ multiplet patterns of **6c** and **6d** were the same as the pair **Ia** and **Ib**, thus inferring the *S* absolute stereochemistry at C-2′ of **6** (Fig. 1).

All terpenoids **1**-**5** were tested for both antifungal and antibacterial activity at a concentration of 5 μg/ml. No growth inhibition was exhibited on *C. albicans* as well as on gram – *E. coli* and gram + *S. aureus* by the studied metabolites.

The terpenoid pattern of *L. arborescens*, described here for the first time, shows striking similarities with those reported in the lit-

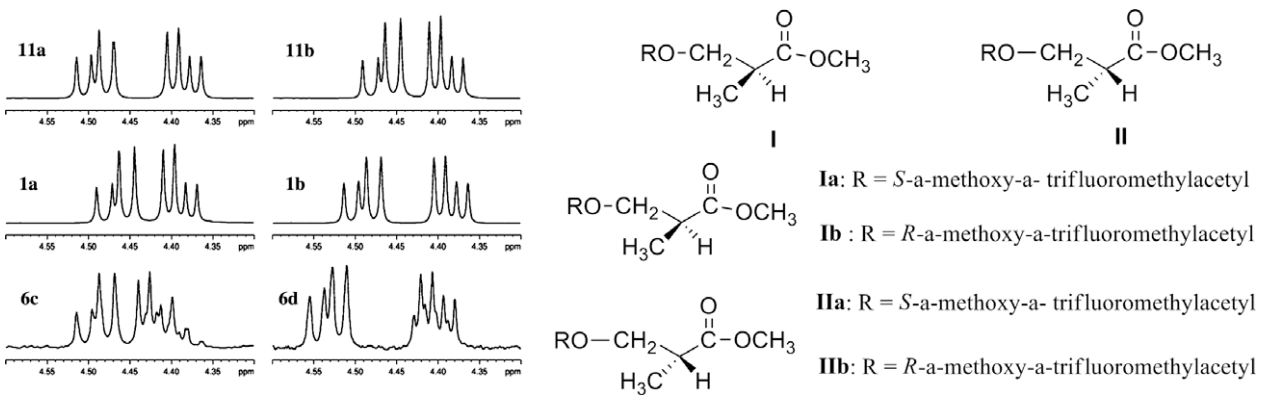

Fig. 1. ^1H NMR signals (400 MHz: $CDCl_3$) due to the methylene protons at C-3 for compounds Ia, IIa, Ib, and IIb.

erature for different species of the same genus (Zaheer et al., 2006; Sokkar et al., 1993; Abdel-Fattah et al., 1990; Gupta et al., 1989; Abdel-Salem et al., 1986; Abdel-Salem et al., 1982; Hook et al., 1984; Majumder and Laha, 1982; Prabhu and Venkateswarlu, 1969).

3. Experimental

3.1. General experimental procedures

Silica-gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were recorded on a Biorad FTS 155 FT-IR spectrophotometer. HPLC separation was performed on a Shimazdu high-performance liquid chromatography using a Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector. NMR experiments were recorded at ICB-NMR Service Centre. 1D and 2D NMR spectra were acquired in $CDCl_3$, CD_3OD and pyridine-d_5 (shifts are referenced to the solvent signal) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis and a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis.^{13}C NMR were recorded on a Bruker DPX-300 operating at 300 MHz using a dual probe. High resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with a HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm RMS in the presence of a known lock mass).

3.2. Plant material

The plant *L. arborescens* was collected in April 2006 in Bechar (Laabadla, South of Algeria) and identified by Prof. Bachir Oudjehih, Institute of Agronomy of University of Batna (Algeria). A voucher specimen is deposited in the herbarium of the department of the same University under the number code 423/HIAB.

3.3. Extraction and isolation

Dried and powdered aerial parts (900 g) of *L. arborescens* were treated with light petroleum ether (3 l, 3 times) to afford, after evaporation of the solvent under reduced pressure, 12 g of crude extract. This extract was subjected to silica gel column using light petroleum ether and increasing amount of ethyl acetate up to 100% ethyl acetate to obtain 140 fractions of 50 ml combined together into five total fractions A (2.9 g), B (100 mg), C (535 mg), D (300 mg) and E (400 mg). The fractions A, B, D and E containing terpenoid molecules were considered for further purification steps. Fraction A was purified by silica gel column chromatography using petroleum ether ethyl acetate (98:2) to afford **7** (1.35 g) as main compound. Fraction B was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 98:2) to yield **18** (1.8 mg) and a mixture of triterpenes that was further separated by RP-HPLC using isocratic elution MeOH/H_2O (9:1) to give **8** (1.2 mg) and **9** (1.0 mg). Fraction D was purified on a silica gel column in the same conditions as fraction B to obtain **10** (13.0 mg). Fraction E was chromatographed by a silica gel column in the same conditions as fraction A to afford two main fractions (E1 and E2), which were passed through analytical RP-18-HPLC (Phenomenex) using pure MeOH as eluent to give **11** (0.6 mg), **12** (0.4 mg) and **13** (0.6 mg) from E1 and **1** (1.5 mg), **14** (1.0 mg), **15** (1.5 mg), **16** (1.2 mg) and **17** (1.2 mg) from E2.

Dried and powdered roots (1 kg) of *L. arborescens* were macerated with methanol (7 l) to give a crude methanolic extract (27.0 g) that was partitioned between water and ethyl acetate. An aliquot (2.0 g) of the ethyl acetate soluble part (3.7 g) was subjected to column chromatography using LH-20, to give 9 fractions from A to I, two of which (C and G) containing terpene components. Fraction C (260 mg) was further purified by silica gel column chromatography using CH_2Cl_2/MeOH from 100% CH_2Cl_2 to 20% MeOH to yield 16 fractions (C1–C16) whereas fraction G afforded pure compound **6**. Fraction C3 (21.4 mg) was subsequently subjected to silica gel column chromatography using *n*-hexane-EtOAc (95:5) as eluent to afford pure compound **19** (6.0 mg). Fraction C8 (25.9 mg) was purified by silica gel column chromatography (gradient light petroleum ether/EtOAc) and following RP-HPLC (gradient MeOH/H_2O) to give **2** (1.6 mg), **20** (1.0 mg), **21** (0.8 mg), and **3** (0.6 mg) respectively. Fraction C12 (4.6 mg) was chromatographed on a silica gel column ($CHCl_3$/MeOH, 95:5) and subsequently RP-HPLC (gradient MeOH/H_2O) to afford pure compounds **22** (0.2 mg), **23** (0.3 mg), **24** (0.2 mg) and **25** (0.6 mg) respectively. Purification of fraction C13 (22.7 mg) on RP-HPLC column (gradient MeOH/H_2O) led to the isolation of compounds **26** (1.2 mg) and **5** (7.1 mg). Finally, fraction C14 (17.0 mg) was directly submitted to RP-HPLC chromatography (gradient MeOH/H_2O) to afford compounds **4** (2.1 mg) and **27** (2.2 mg).

3.3.1. 3β-Hydroxy-11α-ethoxy-olean-12-ene (**1**)

Amorphous powder; $[\alpha]_D^{25}$ −10° (*c* 0.10, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3852, 2956, 2919, 1457, 1379, 1163, 1080, 974; ^{1}H and ^{13}C NMR values ($CDCl_3$) see Table 1; ESIMS positive mode: *m/z* 425 $[M-EtOH+H]^+$.

3.3.2. 9α-Hydroxy-11β,13-dihydro-3-epizaluzanin C (**2**)

Colourless oil; $[\alpha]_D^{25}$ −3° (*c* 0.06, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3407, 1759, 1456, 1327, 1186, 1064, 991, 910; ^{1}H and ^{13}C NMR values ($CDCl_3$) see Table 2; ^{1}H NMR values (600 MHz, pyridine-d_5): δ 1.20 (3H, *d*, *J* = 6.7 Hz, H_3-13), 1.48 (1H, br *t*, *J* = 11.8 Hz, H-8a), 2.17 (1H, *m*, H-2b), 2.30 (1H, *m*, H-2a), 2.30 (1H, *m*, H-8b), 2.30 (1H, *m*, H-11β), 2.61 (1H, *q*, *J* = 10.8 Hz, H-7), 3.25 (1H, *t*, *J* = 9.7 Hz, H-5), 3.95 (1H, *t*, *J* = 9.7 Hz, H-6), 4.01 (1H, br *t*, *J* = 8.2 Hz, H-1), 4.71 (1H, br *s*, H-3β), 5.13 (1H, *m*, H-9β), 4.88 (1H, *s*, H-14a), 5.05 (1H, *s*, H-14b), 5.60 (2H, *s*, H-15); ^{13}C NMR values (150.92 MHz, pyridine-d_5): δ 13.3 (*q*, C-13), 34.7 (*d*, C-1), 40.3 (*t*, C-2), 40.7 (*t*, C-8), 41.7 (*d*, C-11), 44.5 (*d*, C-7), 49.9 (*d*, C-5), 73.8 (*d*, C-3), 74.8 (*d*, C-9), 85.7 (*d*, C-6), 110.6 (*t*, C-14), 110.8 (*t*, C-15), 154.0 (*s*, C-10), 157.1 (*s*, C-4), 178.6 (*s*, C-12); ESIMS positive mode: *m/z* 287 $[M+Na]^+$.

3.3.3. 9α-Hydroxy-4α,15-dihydro zaluzanin C (**3**)

Colourless oil; $[\alpha]_D^{25}$ −11° (*c* 0.10, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3047, 2926, 1757, 1267, 1167, 984, 770; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2; ^{1}H NMR values (600 MHz, pyridine-d_5): δ 1.28 (3H, *d*, *J* = 7.0 Hz, H-15), 1.57 (1H, *m*, H-8a), 2.06 (1H, *m*, H-2a), 2.34 (1H, *m*, H-2b), 2.43 (1H, *m*, H-5), 2.53 (1H, *m*, H-8b), 2.54 (1H, *m*, H-4α), 3.43 (1H, *m*, H-7), 3.75 (1H, br *q*, *J* = 10.2 Hz, H-1), 4.22 (1H, *t*, *J* = 9.7 Hz, H-6), 4.50 (1H, br *s*, H-3), 4.87 (1H, *m*, H-9β), 5.16 (2H, *s*, H-14), 5.43 (1H, *d*, *J* = 3.1 Hz, H-13a), 6.25 (1H, *d*, *J* = 3.1 Hz, H-13b); ^{13}C NMR values (150.92 MHz, pyridine-d_5): δ 8.41 (*q*, C-15), 32.6 (*d*, C-1), 34.4 (*t*, C-2), 39.4 (*t*, C-8), 40.4 (*d*, C-4), 41.2 (*d*, C-7), 47.3 (*d*, C-5), 73.3 (*d*, C-9), 73.5 (*d*, C-3), 83.3 (*d*, C-6), 110.5 (*t*, C-14), 118.7 (*t*, C-13), C-10, C-11 and C-12 were not detected; ESIMS positive mode: *m/z* 287 $[M+Na]^+$, HRESIMS: *m/z* 287.1214 (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

3.3.4. 3β, 14-Dihydroxycostunolide-3-O-β-glycopyranoside (**4**)

Amorphous powder; $[\alpha]_D^{25}$+4° (*c* 0.10, MeOH); IR ν_{max}^{film} cm^{-1}; 3420, 2926, 2861, 1757, 1556, 1416; 1286, 1234; ^{1}H and ^{13}C NMR (pyridine-d_5) see Table 3; HRESIMS positive *m/z* 449.1776 (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*3.3.5. 3β,14-Dihydroxycostunolide-3-O-β-glucopyranosyl-14-O-p-hydroxyphenylacetate (**5**)*

Amorphous powder; $[\alpha]_D^{25}$ −0.4° (*c* 0.55, MeOH); IR ν_{max}^{film} cm^{-1}: 3393, 2922, 1738, 1616, 1516, 1448, 1259, 1145, 1018; ^1H and ^{13}C NMR (pyridine-d_5) see Table 3; ^1H NMR values (600 MHz, $CDCl_3$): δ 1.32 (1H, *m*, H-8a), 1.48 (3H, *s*, H-15), 1.80 (1H, *m*, H-8b), 1.88 (1H, *m*, H-9a), 2.25 (1H, *q*, *J* = 12.0 Hz, H-2a), 2.38 (1H, *m*, H-2b), 2.38 (1H, *m*, H-7), 2.40 (1H, *m*, H-9b), 4.30 (1H, *m*, H-14a), 4.35 (1H, *t*, *J* = 8.8 Hz, H-6), 4.38 (1 H, *m*, H-3), 4.40 (1H, *m*, H-14b), 4.82 (1H, *d*, *J* = 10.0 Hz, H-5), 4.95 (1H, *dd*, *J* = 12.0, 3.5 Hz, H-1), 5.50 (1H, *d*, *J* = 3.8 Hz, H-13a), 6.31 (1H, *d*, *J* = 3.8 Hz, H-13b), ester moiety: δ 3.40 (2H, *s*, H_2-β), 6.60 (2H, *d*, *J* = 8.3 Hz, H-3′ and H-5′), 6.90 (2H, *d*, *J* = 8.3 Hz, H-2′and H-6′), sugar moiety: 3.15 (1H, *m*, H-5″), 3.18 (1H, *m*, H-2″), 3.25 (1H, *m*, H-3″), 3.27 (1H, *m*, H-4″), 3.62 (1H, *dd*, *J* = 12.0, 4.8 Hz, H-6″a), 3.71 (1H, *dd*, *J* = 12.0, 2.6 Hz, H-6″b); 4.10 (1H, *d*, *J* = 8.0 Hz, H-1″); ^{13}C NMR values (75.46 MHz, $CDCl_3$): δ 11.0 (*q*, C-15), 28.8 (*t*, C-8), 32.2 (*t*, C-2), 36.7 (*t*, C-9), 49.4 (*d*, C-7), 61.5 (*t*, C-14), 81.2 (*d*, C-6), 81.3 (*d*, C-3), 120.6 (*t*, C-13), 126.3 (*d*, C-1), 131.0 (*d*, C-5), 134.6 (*s*, C-10), 141.1 (*s*, C-4), 139.0 (*s*, C-11), 170.8 (*s*, C-12), ester moiety: δ 40.6 (*t*, CH_2-α), 115.2 (*d*, C-3′ and C-5′), 124.2 (*s*, C-1′), 130.0 (*d*, C-2′ and C-6′), 155.9 (*s*, C-4′), 172.0 (*s*, C-α), sugar moiety: δ 61.6 (*t*, C″-6), 70.0 (*d*, C″-4), 73.3 (*d*, C″-2), 76.3 (*d*, C″-3), 75.7 (*d*, C″-5) 100.7 (*d*, C″-1), ESIMS positive mode: *m/z* 560 $[M + Na]^+$, HRESIMS: *m/z* 583.2181 (calcd for $C_{29}H_{36}O_{11}Na$, 583.2155).

*3.3.6. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**)*

Colourless amorphous solid; $[\alpha]_D^{25}$+7° (*c* 0.20, $CHCl_3$); ^1H NMR values (600 MHz, $CDCl_3$): δ 1.40 (1H, *m*, H-8a), 2.15 (1H, *m*, H-2a), 2.35 (1H, *m*, H-9a), 2.55 (3H, *s*, H-14), 2.56 (1H, *t*, *J* = 12.8 Hz, H9-b), 2.90 (1H, *m*, H-7), 3.58 (1H, *t*, *J* = 10.0 Hz, H-6), 3.77 (1H, *d*, *J* = 10.0 Hz, H-5), 5.00 (1H, *d*, *J* = 17.6 Hz, H-15 a), 5.35 (1H, *d*, *J* = 17.6 Hz, H-15 b), 5.45 (1H, *s*, H-13a), 6.12 (1H, *s*, H-13b), 6.37 (1H, *s*, H-3), ester moiety: δ 1.17 (3H, *d*, *J* = 7.0 Hz, H-4′), 2.90 (1H, *m*, H-2′), 4.18 (2H, *m*, H-3′); ^{13}C NMR values (75.46 MHz, $CDCl_3$): δ 22.2 (*q*, C-14), 24.1 (*t*, C-8), 37.3 (*t*, C-9), 50.0 (*d*, C-5), 52.0 (*d*, C-7), 63.8 (*t*, C-15), 83.8 (*d*, C-6), 119.0 (*t*, C-13), 130.8 (*s*, C-1), 133.5 (*d*, C-3), 138.6 (*s*, C-11), 154.9 (*s*, C-10), 166.9 (*s*, C-4), 169.3 (*s*, C-12), 195.6 (*s*, C-2), ester moiety: δ 13.5 (*q*, C-4′), 39.5 (*d*, C-2′), 69.3 (*t*, C-3′), 174.2 (*s*, C-1′).

*3.4. Preparation of the ester derivatives of compound **6***

*3.4.1. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin (**6a**)*

Compound **6** (2.0 mg) was dissolved in H_2SO_4/MeOH (3 drops in 1 ml) and stirred for 10 min. After usual work up, the residue was chromatographed by silica gel column (light petroleum ether/EtOAc, 50:50) to afford 1.2 mg of pure compound **6a**.

Colourless oil, $[\alpha]_D^{25}$+26.1° (*c* 0.12, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 3434, 2936, 2836, 2861, 1784, 1738, 1684, 1630, 1549, 1259, 1140, 1051, 978; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.46 (1H, *m*, H8-a), 2.23 (1H, *m*, H-8b), 2.42 (1H, *m*, H-9a), 2.45 (3H, *s*, H-14), 2.54 (1H, br *t*, *J* = 12.7 Hz, H-9b), 2.89 (1H, *m*, H-7), 3.62 (1H, *t*, *J* = 10.2 Hz, H-6), 3.71 (1H, *d*, *J* = 10.2 Hz, H-5), 5.09 (1H, *d*, *J* = 16.9 Hz, H-15a), 5.31 (1H, *d*, *J* = 16.9 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.35 (1H, br *s*, H-3), ester moiety: δ 1.23 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.77 (1H, *m*, H-2′), 3,76 (2H, *m*, H-3′); ESIMS positive mode: *m/z* 369 $[M + Na]^+$; HRESIMS: *m/z* 369.1298 (calcd for $C_{19}H_{22}O_6Na$, 369.1314).

*3.4.2. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-acetate (**6b**)*

Acetyl derivative **6b** was prepared by treating 2.0 mg of compound **6a** with acetic anhydride (2 drops) in pyridine (1 ml) at room temperature. After usual work-up the product was purified by Pasteur-pipette silica gel chromatography using light petroleum ether-ethyl acetate (60:40) to give 1.7 mg of pure **6b**.

Colourless oil; $[\alpha]_D^{25}$+8° (*c* 0.17, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1718, 1688, 1621, 1373, 1251, 1135, 989; ^1H-NMR spectral data (600 MHz, $CDCl_3$): δ 1.45 (1H, *q*, *J* = 12.0 Hz, H8-a), 2.22 (1H, *m*, H-8b), 2.24 (1H, *m*, H-9a), 2.44 (3H, *s*, H-14), 2.56 (1H, br *t*, *J* = 13.0 Hz, H-9b), 2.85 (1H, *m*, H-7), 3.58 (1H, *t*, *J* = 10.0 Hz, H-6), 3.70 (1H, *d*, *J* = 10.0 Hz, H-5), 5.05 (1H, *d*, *J* = 17.1 Hz, H-15a), 5.31 (1H, *d*, *J* = 17.1 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.32 (1H, br *s*, H-3), ester moiety: δ 1.25 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.85 (1H, *m*, H-2′), 4.22 (2H, *d*, *J* = 8.0 Hz, H-3′), 2.07 (3H, *s*, CH_3CO); ^{13}C-NMR values (75.46 MHz, $CDCl_3$): δ 22.2 (*q*, C-14), 24.1 (*t*, C-8), 37.3 (*t*, C-9), 50.0 (*d*, C-5), 52.0 (*d*, C-7), 63.8 (*t*, C-15), 83.7 (*d*, C-6), 119.0 (*t*, C-13), 130.9 (*s*, C-1), 133.5 (*d*, C-3), 138.6 (*s*, C-11), 154.9 (*s*, C-10), 166.8 (*s*, C-4), 169.2 (*s*, C-12), 195.6 (*s*, C-2), ester moiety: δ 13.5 (*q*, C-4′), 21.5 (*q*, CH_3CO), 39.5 (*d*, C-2′), 63.8 (*t*, C-3′), 171.0 (*s*, CH_3COO), 173.2 (*s*, C-1′); ESIMS positive mode: *m/z* 411 $[M + Na]^+$.

*3.4.3. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-(S)-MTPA-ester (**6c**)*

Compound **6c** was prepared by treating 1 mg of **6a** in pyridine (1 ml) with *R*-(−)-MTPA chloride (0.07 ml) at room temperature overnight. After usual work up, the residue was purified by Pasteur-pipette silica gel chromatography using light petroleum ether-ethyl acetate (60:40) to give 0.4 mg of pure ester **6c**.

Colourless oil; $[\alpha]_D^{25}$+21° (*c* 0.04, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1784, 1744, 1684, 1630, 1448, 1381, 1273, 1180, 1113, 970; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.44 (1H, *m*, H8-a), 2.03 (1H, *m*, H-8b), 2.39 (1H, *m*, H-9a), 2.45 (3H, *s*, H-14), 2.56 (1H, br *t*, 13.0 Hz, H-9b), 2.94 (1H, *m*, H-7), 3.47 (1H, *t*, *J* = 7.0 Hz, H-6), 3.59 (1H, *d*, *J* = 8.9 Hz, H-5), 4.97 (1H, *d*, *J* = 17.5 Hz, H-15a), 5.17 (1H, *d*, *J* = 17.5 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2 Hz, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.25 (1H, br *s*, H-3), ester moiety: δ 1.23 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.27 (1H, *m*, H-2′), 4.42 (1H, *dd*, *J* = 5.4, 10.8 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 7.3, 10.8 Hz, H-3′b); ESIMS positive mode: *m/z* 585 $[M + Na]^+$.

*3.4.4. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-(R)-MTPA-ester **(6d)***

Compound **6d** was prepared by treating 1.0 mg of **6a** in pyridine (0.5 ml) of *S*-(−)-MTPA chloride (0.07 ml) at room temperature overnight. The reaction mixture was purified as described for **6c** to obtain pure **6d** (1.2 mg).

Colourless oil; $[\alpha]_D^{25}$−40° (*c* 0.12, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1751, 1684, 1616, 1448, 1381, 1267, 1167, 1113, 1032; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.43 (1H, *m*, H8-a), 2.22 (1H, *m*, H-8b), 2.40 (1H, *m*, H-9b), 2.45 (3H, *s*, H-14), 2.52 (1H, *m*, H-9a), 2.94 (1H, *m*, H-7), 3.59 (1H, *m*, H-6), 3.66 (1H, *d*, *J* = 10.2 Hz, H-5), 5.00 (1H, *d*, *J* = 17.4, H-15a), 5.18 (1H, *d*, *J* = 17.4 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2 Hz, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.27 (1H, br *s*, H-3), ester moiety: δ 1.24 (3H, *d*, *J* = 6.7 Hz, H-4′), 2.87 (1H, *m*, H-2′), 4.40 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.53 (1H, *dd*, *J* = 6.7, 10.8 Hz, H-3′b); ESIMS positive mode: *m/z* 585 $[M + Na]^+$.

3.5. Preparation of model Mosher esters

*3.5.1. Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-S-MTPA-ester (**Ia**)*

Compound **Ia** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After usual work up, the reaction mixture was purified by silica gel chromatography using light petroleum ether-ethyl acetate (90:10) to get pure **Ia** (9.0 mg).

Oil; $[\alpha]_D^{25}$−25° (*c* 0.90, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436, 1272, 1171, 1123, 1082; Selected ^1H NMR values

(400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.47 (1H, *dd*, *J* = 7.3, 10.8 Hz, H-3′b).

3.5.2. Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-R-MTPA-ester (**Ib**)

Compound **Ib** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of *S*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After usual work up the reaction mixture was purified by silica gel chromatography using light petroleum ether-ethyl acetate (90:10) to get pure compound **Ib** (30 mg).

Oil; $[\alpha]_D^{25}$+47° (*c* 3.0, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436, 1272, 1171, 1123, 1082; Selected ^{1}H NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 6.7, 10.8 Hz, H-3′b).

3.5.3. Methyl-(R)-(−)-3-hydroxy-2-methyl propionate-S-MTPA-ester (**IIa**)

Compound **IIa** was prepared by treating 0.1 ml of methyl (*R*)-(−)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound **IIa** (23.0 mg) was obtained.

Oil, $[\alpha]_D^{25}$−46° (*c* 0.23, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082; Selected ^{1}H-NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 11.1 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 6.7, 11.1 Hz, H-3′b).

3.5.4. Methyl-(R)-(−)-3-hydroxy-2-methyl propionate-R-MTPA-ester (**IIb**)

Compound **IIb** was prepared by treating 0.1 ml of methyl (*R*)-(+)-3-methyl propionate with 0.1 ml of *S*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound **IIb** (8.8 mg) was obtained.

Oil; $[\alpha]_D^{25}$+26° (*c* 0.88, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082; Selected ^{1}H-NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.72, 10.8 Hz, H-3′a), 4.47 (1H, *dd*, *J* = 7.3, 10.8 Hz).

3.5.5. Hydrolysis of **5**

Compound **5** (3.0 mg) was treated with NaOH/H_2O (1% solution) under stirring at room temperature for 3 h. After neutralisation with acidic solution (2% H_2SO_4), the residue was extracted with *n*-butanol for 3 times. The organic phase was concentrated to give after usual work up a product (1.7 mg), which was identical (^{1}H NMR, mass and $[\alpha]_D$) with compound **4**.

3.6. Biological assays

Antifungal assay was performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P. The medium used to prepare the 10 x drug dilutions and the inoculum suspension was liquid RPMI 1640 with L-Glutamine (Sigma–Aldrich), 0.165 M Morpholinopropansulfonic acid (MOPS) and 2% Glucose (pH 7.0) (Rodriguez-Tudela et al., 1996; Hong et al., 1998). The yeast suspension was adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland (2×10^8 CFU/ml) standard at 530 nm and diluted 1:4000 (50,000 CFU/ml) in RPMI 1640 broth medium. The yeast inoculum (0.9 ml) was added to each test tube that contained 0.1 ml of 10 2-fold dilutions (256–0.05 μg/ml final) of each compound. Broth macrodilution MICs were determined after 48 h of incubation at 35 °C. The MIC was defined as the lowest concentration of the compound that completely inhibited the growth of the test organism. The antibacterial assay was performed by using the same method as the antifungal test, only differing in the assay medium (Luria Bertani medium: 10 g/l Bactotryptone, 5 g/l Bactoyeast and 10 g/l NaCl, pH 7.5) and in the incubation temperature (37 °C for 24 h).

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RESUME

La nature comme source d'agents potentiels chimio-thérapeutiques suscite un intérêt considérable. Quelle que soit les raisons de la présence de ces composés dans la nature, ils fournissent des ressources de valeur inestimable qui ont été employées pour trouver de nouvelles drogues.

Bien que les plantes terrestres soient les plus étudiés en leur métabolites secondaires, du fait de leur abondance relative dans la nature et par leur accessibilité, la recherche actuelle s'est orientée vers la chimie des métabolites secondaires issus des organismes marins: animaux, bactérie, champignons, algues et plantes.

En faite, l'élaboration des méthodes spectroscopiques couplées à de bonnes techniques d'extraction et de purification, ont contribué au succès phénoménal de la chimie des produits naturels, et ont ouvert des nouvelles voies pour découvrir des structures originales.

Dans ce contexte, notre travail phytochimique est consacré à l'isolement et la caractérisation des nouveaux composés naturels d'origine végétale. Notre choix est ciblé sur deux plantes, l'une terrestre: *Launaea arborescens* et l'autre marine: *Halophila stipulacea*.

Launaea arborescens est une plante saharienne et endémique appartenant à la famille des Composèes et qui est très utilisée dans la médecine traditionnelle. L'extraction des parties aériennes et les racines de cette espèce suivies par la purification, en utilisant les méthodes chromatographiques avancées, nous a permis l'identification de vingt sept terpenoides contenant douze triterpenes de la série oleanane et ursane, et quatorze sesquiterpene lactones de squelette guaiane, eudesmane et germacrane. L'élucidation structurale a été achevée par les méthodes spectrales, notamment les techniques de RMN.

Holophila stipulacea, l'une des plantes aquatiques appartenant à la famille Hydrocharitaceae, qui jouent un rôle primordial dans la protection des écosystèmes aquatiques et la production des métabolites secondaires de caractère original. Notre étude phytochimique sur la plante a révélée la présence des flavonoïdes glycosylés, et particulièrement les flavones. Ces flavones identifiées sont en fait toutes dérivées de l'apigénine, genkwanin et chrysoériol, connues dans les plantes terrestres. On a pu aussi isoler des flavones malonylées détectées pour la première fois dans la flore marine et dont un est une nouvelle rare flavone malonylèe glycosylée.

ABSTRACT

The nature was and is still an interesting source of potential chemotherapeutic agents. Whatever the reasons for the presence of these compounds in nature, they provide invaluable resources that have been used to find new drug molecules.

Although the terrestrial plants are the old origin of the secondary metabolites, by their relative abundance in nature and their accessibility, current research is focused the study of the secondary metabolites resulting from the marine organisms such as: animals, bacteria, fungi, algae and plants.

Actually, the development of the spectroscopic methods coupled with a good extraction and purification techniques, has contributed to the phenomenal success of natural product chemistry, and has given another ways for discovering new structures.

In this context, our phytochemical work has been devoted in the isolation and the characterization of the new natural compounds of vegetable origin. Our choice is targeted on two plants, one terrestrial: *Launaea arborescens* and the other one is marine: *Halophila stipulacea*.

Launaea arborescens is a saharan and endemic plant belonging to the Asteraceae family and which is very common in the traditional medicine. The extraction of the aerial parts and roots of this species, followed by the purification using the advances chromatographic methods, allowed us to identify twenty seven terpenoids, including 12 triterpenes of the series oleanane and ursane, and fourteen sesquiterpene lactones, exhibiting guaiane, eudesmane and germacrane skeleton. The elucidation of the structures was achieved by means of spectral methods mainly NMR techniques.

Holophila stipulacea, is one of the aquatic plants which belongs to the Hydrocharitaceae family that play a principal role in the protection of the ecosystems, and in the production of the secondary metabolites of original nature. Our phytochemical study on the plant revealed the presence of flavonoid glycosides, particularly flavones. These flavones are derivatives of apigenin, genkwanin and chrysoeriol known in the terrestrial plants.

We also isolated two malonylated flavones described here for the first time from the marine flora and including one novel rare malonyl flavone glycoside.

ملخص

تعتبر الطبيعة مصدرا معتبرا للمواد ذات الكمون الكيميائي العلاجي. مهما تكن أسباب وجود هذه المواد في الطبيعة فإنها تشكل موارد مهمة مستعملة لاكتشاف عقارات جديدة.

لوجودها المعتبر في الطبيعة و لسهولة الحصول عليها, عرفت النباتات البرية على أنها الأكثر دراسة لاحتوائها الأيضي الثانوي, إلا أن البحث الكيمائي الحالي توجه إلى دراسة المحتوى الأيض الثانوية لمصادر بحرية منها: حيوانات, بكتيريا, فطريات و نباتات ....

على هذا, فان توفر الطرق المطيافية المكملة لطرق الفصل و التنقية الجيدة ساهم كثيرا في نجاح كيمياء المواد الطبيعية مما سهل سبلا كثيرة لإيجاد مركبات طبيعية جديدة.

في هذا المضمار, اقتصرت دراستنا الفيتوكيميائية على استخلاص وتعريف مواد طبيعية ذات مصادر نباتية بحتة و وقع اختيارنا على نبتتين: *Launaea arborescens* من مصدر بري ونبات *Halophila stipulacea* من مصدر بحري.

نبات *launaea arborescens* هو نبات أصيل من عائلة المركبات Asteraceae لـــه اســـتعمال واسع في مجال الطب التقليدي. استخلاص القسم الهوائي و الأرضي متبوعا بطرق الكروماتوغرفيا الحديثة مكنتنا من عزل 27 مركبا من عائلة التربينات والتي تم تحديد صيغها الكيميائية بواسطة التحاليل المطيافية المختلفة و خاصة مطيافية الرنين النووي المغناطيسي.

12 مركب من عائلة التربينات الثلاثية و المنتمية لهيكل الأوليان و الاورسان تم عزلها من مستخلص الايثر petroleum ether للقسم الهوائي و التي تضمنت مركبا واحدا جديدا.

12 مركب من عائلة الاكتونات السسكوتيربينية و المنتمية لهيكل الغوايان ,الجرماكران و الأودسمان تم عزلها من مستخلص الاثيل أستات للقسم الأرضي و التي تضمنت مركبين جديدين من هيكل الغوايان و مركبين جديدين من هيكل الجرماكران.

Halophila stipulacea إحدى النباتات المائية تنتمي إلى عائلة Hydrocharitacea التي تلعب دورا هاما في حماية الأنظمة المائية و انتاج مركبات ذات أيضي ثانوي جديد.

دراستنا الفيتوكيميائية على هذا النبات سمحت بالكشف على وجود فلافونويدات سكرية تنتمي الى هيكل الفلافون و المشتقة من الأبيجنين, جنكوانين و كريزيول المعروفة لدى النباتات البرية.

تمكنا أيضا من عزل فلافونات مالونيلية تستخلص لأول مرة من البحر والتي تضمنت فلافون ملونيلي نادر.