A THESIS ENTITLED

"STUDIES IN NATURAL PRODUCTS"

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Selma Dagli

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SUMMARY

This thesis consists of eleven chapters and deals with phytochemical investigations of several liverwort species together with *Piper chaba* and *Inula khongii*. The first chapter gives a general introduction dealing with the nature of secondary metabolites and the skeletal types found in the Hepaticae. This is followed by an examination of the chemical constituents found in the liverwort *Scapania undulata*. Three known compounds were isolated from this liverwort and these are: (-)-*ent*-longipinanol, (-)-longiborneol, and an amorphane/muurolane(cadinane) type sesquiterpenoid, which probably came from the liverwort *Marsupella aquatica* mixed with *S. undulata* in their growing habitat.

The third chapter consists of an investigation of the extract of a flowering plant called *Piper chaba*, sent to us by a Chinese colleague. Four known compounds, piperine, pipernonaline, guineensine and the isobutylamide of 13-(3,4-methylenedioxyphenyl)undeca-2,4,12-trienoic acid were isolated. The following chapter examines the chemical constituents of the Scottish liverwort *Conocephalum conicum*, which gave 3,4'-dihydroxybibenzyl and 3,4-dimethoxystyrene.

The metabolites of *Nardia scalaris* form the subject matter of Chapter 5. Three known *ent*-kaurane diterpenoids, (15S)-*ent*-kaur-16-en-15-yl hydrogen malonate, (15R)-*ent*-kaur-16-en-15-ol and *ent*-kaur-16-en-15α,3β-diol have been found in this liverwort. The chemical constituents of the liverwort *Trichocolea tomentella* are revealed in the next chapter. Four known compounds, deoxytomentellin, trichocolein, tomentellin, isotomentellin and two new compounds Methyl 4-[7-hydroxy-3,7-dimethyl-2,5-octadienyloxy]-3-methoxybenzoate, and Methyl 4-[5-hydroxy-3,7-dimethyl-2,6-octadienyloxy]-3-methoxybenzoate were isolated.

Chapter 7 deals with the constituents of the Taiwanese liverwort *Scapania robusta* which afforded *ent*-spathulenol, found in many liverworts. The following chapter examines the metabolites of the extract of the flowering plant *Inula khongii*. Two new sesquiterpene lactones, 8β-angeloyloxy-14-acetyl-1(10),4,11(13)-germacratrien-12,6α-olide, and 8β-angeloyloxy-14-hydroxy-1(10),4,11(13)-germacratrien-12,6α-olide as well as the known lactones metampolide and ovatifolin were isolated.

Chapter 9 deals with the constituents of *Marsupella aquatica*, which gave the known sesquiterpenoids marsupellone, acetoxymarsupellone, 9,11α,14-triacetoxymarsupellone, 9,11β,14-triacetoxymarsupellone, and marsupellol. Chapter 10 examines the metabolites of Scottish liverworts *Herbertus dicranus* and *H. stramineus*. Two sesquiterpenoids, α- and β- herbertenols, were present in both liverworts, however a new herbertane-type sesquiterpenoid has been isolated from *Herbertus stramineus*. The next chapter considers the chemical constituents of Scottish *Porella platyphylla*, which afforded pinguisanin and perrottetianal A, and two French *Porella* species, one of which afforded a new sesquiterpenoid, a derivative of pinguisanin. The structures were determined mainly using one and two dimensional NMR experiments.

The final chapter consists of an investigation of the extract of *Miliusa velutina* from the Annonaceae family. Four acetogenin-related compounds, three of which are new, have been identified in the extract of this plant by spectroscopic and GCMS analysis.

CONTENTS

Chapter-1

General Introduction

NATURAL PRODUCT CHEMISTRY

Humans have always been fascinated by Nature. This great interest in Nature drove them to examine it in every aspect. In particular the natural products extracted from plants, animals and lesser organisms, attracted the greatest interest since they were used as treatments for diseases, as poisons, as euphoriants and as stimulants. Natural product chemistry is one of the oldest branches of Chemistry. For more than two centuries chemists have been examining the extracts obtained from natural sources. This interest has increased over the years with the development of separation and purification methods, such as column chromatography, g.c., t.l.c., h.p.l.c., paper chromatography, ion exchange, etc. Following the separation and purification of compounds, different spectroscopic techniques are used for structure elucidation, such as NMR (including two dimensional NMR spectroscopy : COSY , HMBC , HSQC) I.R. , UV and mass spectroscopy. The structural elucidation of complex molecules often available in small amount, is greatly facilitated by these modern spectroscopic techniques. X-ray analysis and molecular force-field calculations are also readily available. The most important factor which makes Natural Product Chemistry an outstanding science is the biological activities that some of these natural products possess. Many of the medicinal, pharmacological and other biological agents used in the world are either natural products themselves, are derivatives of them or are modified templates of natural products. Thus there is an enormous potential for the future development of natural products from many sources (marine and terrestrial environments) to produce key biological agents for the future benefit of mankind.

Traditionally, natural products are considered in two categories: primary and secondary metabolites. However the border between the two classes is somewhat vague in places. The former are the organic compounds which are characteristic of all living systems, such as carbohydrates, lipids, amino acids, peptides, proteins, nucleic acids, nucleosides and nucleotides whereas the secondary metabolites include phenols, quinones, terpenes, alkaloids, aromatics, etc. There is a considerable interest in plant secondary metabolites and many plants have been and are being investigated by chemists in the search for new structural types with useful biological activity. Another interest that captivated chemists was to learn the pathways followed within an organism to these diverse secondary metabolites. The remarkable advances which have been made in the understanding of biosynthesis have depended on specialised techniques drawn from a wide range of disciplines, such as the use of mutants, isolated enzyme systems, radioactive tracer techniques, and the application of N.M.R. methods to metabolites enriched with precursors containing stable isotopes. Natural products play an important role in chemotaxonomy, which is the description and classification of plants, enzyme studies and chemical classification of plants. The biosynthetic pathways to the metabolites that play important roles in our lives are shown in **Fig.1**.

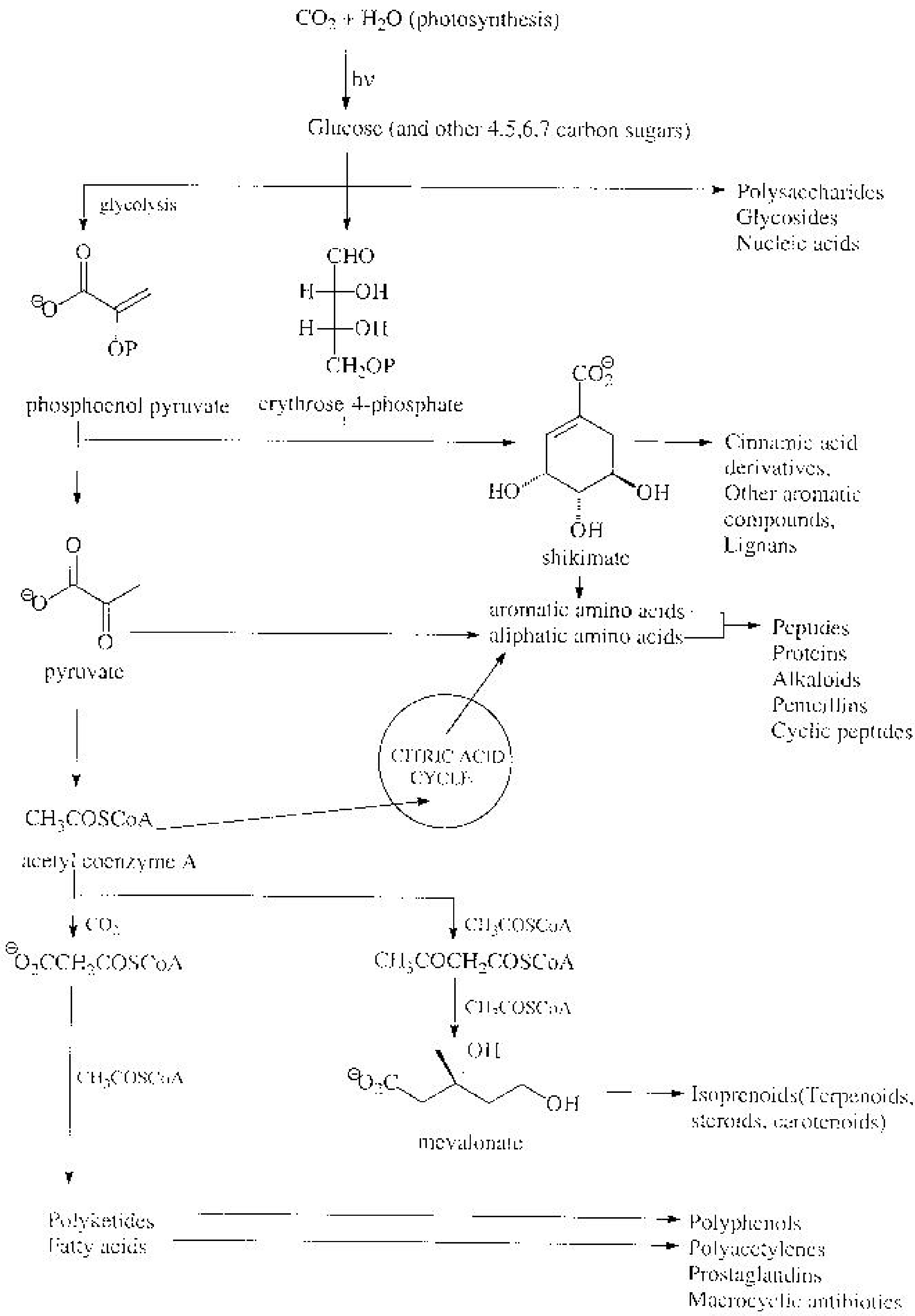

CO2 + H2O (photosynthesis)
hv
Glucose (and other 4,5,6,7 carbon sugars)
glycolysis
Polysaccharides
Glycosides
Nucleic acids
phosphoenol pyruvate
erythrose 4-phosphate
Cinnamic acid derivatives,
Other aromatic compounds,
Lignans
shikimate
aromatic amino acids
aliphatic amino acids
Peptides
Proteins
Alkaloids
Penicillins
Cyclic peptides
pyruvate
CITRIC ACID CYCLE
CH3COSCoA
acetyl coenzyme A
CO2
CH3COSCoA
CH3COCH2COSCoA
CH3COSCoA
mevalonate
Isoprenoids(Terpenoids, steroids, carotenoids)
Polyketides
Fatty acids
Polyphenols
Polyacetylenes
Prostaglandins
Macrocyclic antibiotics

Fig.1

HEPATICAE

On the evolutionary scale, bryophytes are placed in the lower plants and are taxonomically between the algae and the pteridophytes (ferns). Approximately 20,000 species of them are known[1]. They are divided into three classes : Bryopsida or mosses (bryales), Hepaticae or liverworts and Anthocerotae or hornworts. While the hornworts comprise some 300 species the liverworts comprise 6,000 species worldwide. The mosses form the largest bryophyte class with 14,000 species. The liverworts are chemically the most interesting plants because, unlike mosses and hornworts, they have oil bodies in their cells, which contain large quantities of secondary metabolites . The liverworts and mosses form two distinct classes within the Bryophyta. They differ from each other in rhizoid structure, in the manner of development of sex organs and in the prevailing mode of growth and cell structure of their leaves .

The Hepaticae consists of plants with leaves (leafy liverworts) or without leaves ('thalloid liverworts') with a unique life cycle[2] . The family is divided into two sub-classes and six orders by most authors[2] :

1. Jungermanniidae

- Calobryales
- Jungermanniales
- Metzgeriales

2. Marchantiidae

- Monocleales
- Marchantiales
- Sphaerocarpales

Although the Anthocerotales or hornworts and the Takakiales are sometimes classified within the Hepaticae, arguments have been put forward to place them in separate classes. Some features of the Anthocerotales seem to justify the placing of this order in a different subclass. The unusual character of this group had already been recognised by Leitgeb[3] and Cavers[4] and, as early as 1899, Howe[5] put it in a different class under the name of Anthocerotae.

The general difference between the two sub-classes of the Hepaticae is the kind of habitat in which they grow. While the sub class Jungermanniidae typically represent the liverworts adapted to moist climates, the Marchantiidae, on the other hand, are those which grow in dry climatic regions.

As we mentioned above, the characteristic feature of the Hepaticae is their ability to elaborate large quantities of terpenoids and other metabolites which are stored in special cell organelles, called the oil bodies. Under the light microscope, the oil bodies appear as colourless, rarely brownish or bluish, organelles in the cytoplasm, varying from 2 to 20 μm in length. In dried specimens they disintegrate. Several oil body types can be recognised, the most important ones are the homogenous oil body and the segmented oil body. The difference between them is that while homogenous oil bodies are made up of one lipid droplet surrounded by a membrane, the segmented oil bodies have several to numerous droplets bounded within the membrane. The distribution of the oil bodies varies. For example, within the subclass Jungermannidae, oil bodies occur in green, photosynthetic cells whereas in the subclass Marchantiidae, they are restricted to special 'oil cells' lacking chlorophyll.

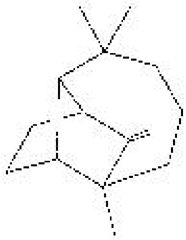
Some characteristic features found in the secondary metabolites also led to a taxonomic classification. For instance, plant species which produce similar chemical constituents might have similar biosynthetic pathways and thus similar genes which encode enzymes. On the other hand, secondary metabolites may also depend on the stage of development of different plants and on environmental factors. Such factors complicate the issue of chemotaxonomy.

Many liverworts prefer wet, humus-rich habitats, such as damp rocks, the forest floor, swamps or marshes, or beside streams and pools and it is often troublesome to collect them in sufficient amounts for chemical research, due to their small size and their tendency to intermingle with other plants. Muller was the first person to investigate liverworts and to report the presence of sesquiterpenoids in the oil bodies in 1905[6]. No further work was carried out until 1956 when Fujita *et al*[7] reported that the essential oil of *Bazzania pompeana* was composed of sesquiterpene hydrocarbons. Finally in 1967, Huneck and Klein[1] isolated two sesquiterpenes (-)-longifolene **(1)** and (-)-longiborneol **(2)** from *Scapania undulata*. This was the first isolation of pure terpenoids from the Hepaticae[1]. These compounds are the enantiomers of those normally found in higher plants.

MONOTERPENOIDS

Monoterpenoids are important in the perfumery and flavour industries and are responsible for the characteristic smell of many liverworts. They are mainly hydrocarbons and generally occur as complex mixtures with sesquiterpene hydrocarbons. Monoterpenoids found in liverworts have been reported on the basis of detection with GC or GCMS[1] and have the same general structure as those found in spermatophytes. However, their optical activity has yet to be measured in the majority of taxa and only this can help to determine their absolute stereochemistry[7].

For the first time in 1974 Svensson[8] reported the composition of the monoterpene hydrocarbons from the essential oils of *Jungermania cordifolia* and *Jungermania obovata*. He found that camphene (**3**) was the main component in *J. cordifolia*, while terpinolene (**4**) and limonene (**5**) were dominant in *J. obovata*. He also found that myrcene (**6**), α-pinene (**7**) were present only in *J. obovata*, which has a characteristic carrot aroma, and α terpinene (**8**) was a minor component in the same liverwort[8]. It was Suire and Asakawa[9,10] who isolated pure monoterpenoids from a liverwort. Both enantiomers of monoterpenoids occur in higher plants; they are often produced as racemic mixture but some species synthesise only one of the two enantiomers[9]. Suire *et al*[9] investigated the Japanese thalloid liverwort *Conocephalum conicum* and examined the chirality of the compounds produced. Two monoterpenes, (-)-limonene (**5**) and (-)-β-sabinene (**9**), were isolated from *C. conicum*[9], while (+)-limonene (***ent*-5**), (+)-α-pinene (**7**) and (+)-camphene (**3**) were previously isolated from a leafy liverwort *Jungermannia exsertifolia*[10]. Thus, the two optical antipodes of limonene occur in liverworts as in higher plants but *C. conicum* and *J. exsertifolia* produce only one of the two antipodes[9]. The absolute configuration of many liverwort monoterpenes still remains to be determined. The highly sensitive two-dimensional GC technique, however, can be used to determine the exact enantiomeric composition of most of the monoterpene hydrocarbons in a crude mixture, even the ones present in very low quantities[11]. Valterová *et al*[1] used two-dimensional GC technique to study the chirality of the monoterpene hydrocarbons present in the liverwort *Conocephalum conicum*.



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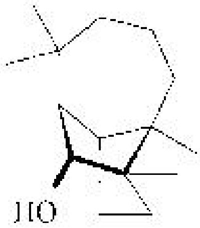

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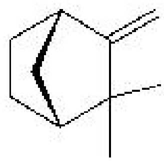

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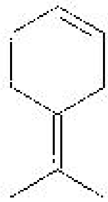

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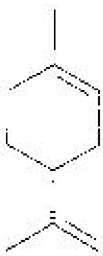

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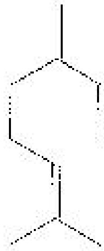

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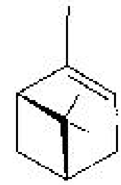

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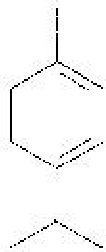

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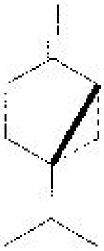

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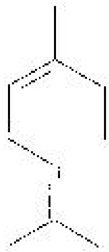

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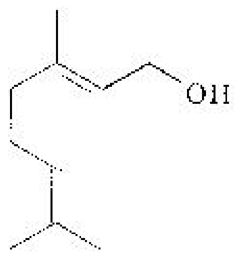

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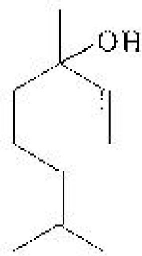

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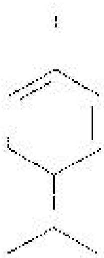

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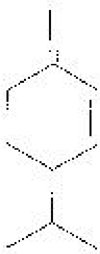

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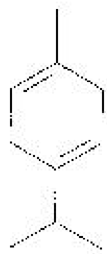

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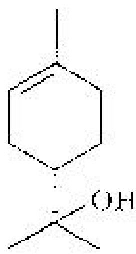

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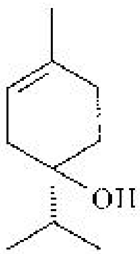
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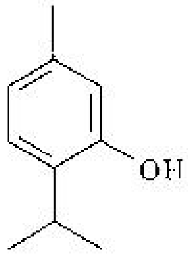
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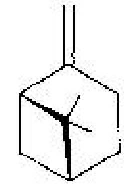
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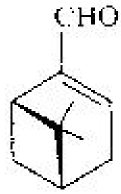
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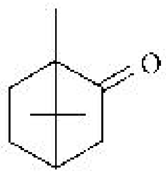
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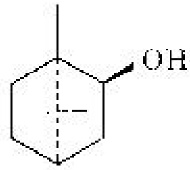
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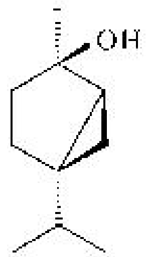
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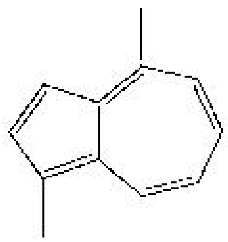
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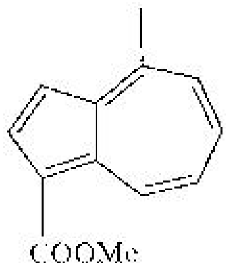
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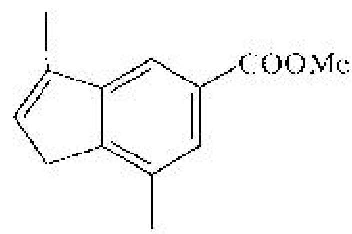
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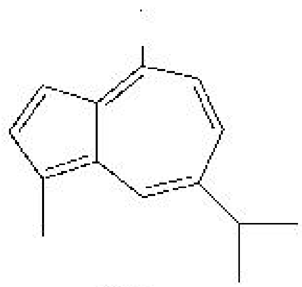
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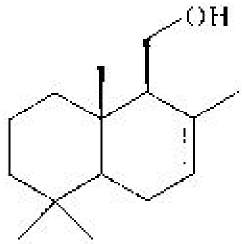
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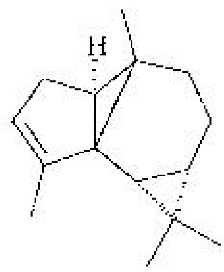
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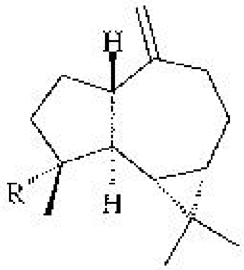
Some monoterpenes found among the Hepaticae[1] are ocimene (**10**), geraniol (**11**), linalool (**12**), α-phellandrene (**13**), β-phellandrene (**14**), γ-terpinene (**15**), α-terpineol (**16**), terpenen-4-ol (**17**), thymol (**18**), β-pinene (**19**), myrtenal (**20**), camphor (**21**), borneol (**22**), thujanol (**23**).

SESQUITERPENOIDS

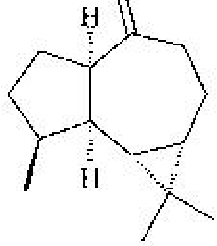
Sesquiterpenoids are defined as a group of C_{15} compounds and they are found in many forms of living systems of which higher plants are the principal member[12]. Although the study of sesquiterpenoids was begun in the early years of the nineteenth century, it was only after 1920 that serious progress was made. For many years it was known that certain essential oils developed a blue colour on distillation. Many sesquiterpenoids gave similar blue substances when dehydrogenated and the blue colour was attributed to the presence of a small group of hydrocarbons. The name "azulene" was given to the oils by Piesse in 1804[13]. Those azulenes which were first obtained from natural sources were given trival names. However as their identity with other hydrocarbons was established the superfluous names were discarded.

Some *Calypogeia* species contain characteristic blue oil bodies[1]. Two azulene derivatives, 1,4-dimethylazulene (**24**) and 4-methyl-1-methoxycarbonylazulene (**25**), have been isolated from *Calypogeia trichomanis*. Further investigation of the essential oil of the same liverwort gave 3,7-dimethyl-5-methoxycarbonylindene (**26**). While azulenes are often obtained as artefacts during isolation procedures, the azulenes in *Calypogeia* species occur naturally since the oil bodies in intact gametophytes show a blue colour and the solvents immediately become blue when the cultured cells are immersed in suitable solvents[14]. Guaiazulene (**27**) was isolated from *Pellia* species. It was suggested that guaiazulene might be an artefact since azulenes were not detected in the ether extract of the same species, even by GC-MS analysis[1].

The first positive identification of a sesquiterpenoid in a liverwort was the isolation of (-)-drimenol (**28**) from *Bazzania trilobata*[15], and (-)-longifolene (**1**) and (-)-longiborneol (**2**) from *Scapania undulata*[16]. Most of the sesquiterpenoids isolated from the Hepaticae are enantiomeric to those found in higher plants and this is the most important endogenous character of the Hepaticae[1].



(30) R = OH
(31) R = H



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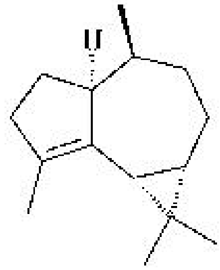

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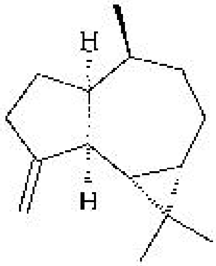

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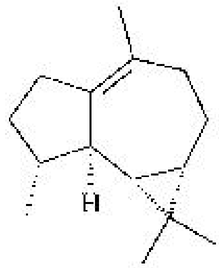

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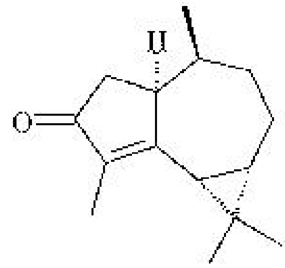

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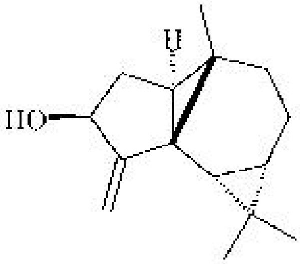

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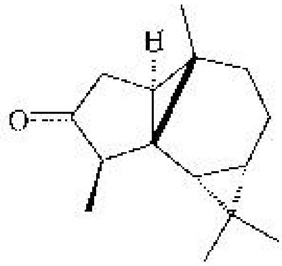

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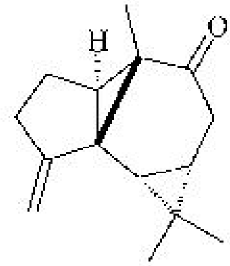

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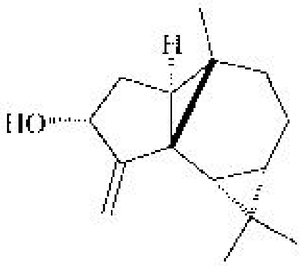

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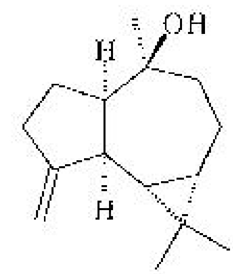

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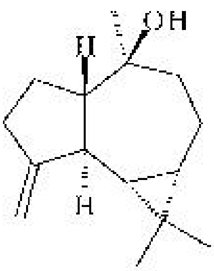

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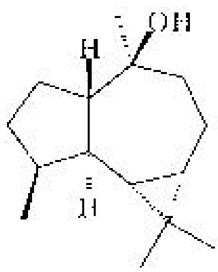

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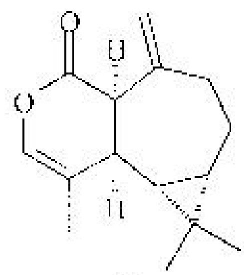


(44)

Aromadendranes, Secoaromadendranes and Norsecoaromadendranes

While *ent*-aromadendrane-type sesquiterpenoids are widespread in Jungermanniales, secoaromadendrane-type sesquiterpenoids are mainly distributed in *Plagiochila* species[17]. Anastreptene (**29**) and *ent* spathulenol (**30**) are the most frequently encountered aromadendrane-type sesquiterpenoids[17]. The previously known aromadendrene (**31**), alloaromadendrene (**32**), α- (**33**) and β- gurjunene (**34**), (-)-ledene (**35**), (+)-cyclocolorenone (**36**), myliol (**37**), and dihydromylione (**38**) have been detected in Jungermanniales[17]. *Mylia taylorii* is a rich source of aromadendrane- and secoaromadendrane-type sesquiterpenoids[1,17]. New aromadendranes, (+)-myli-4(15)-en-9-one (**39**), (-)-3-*epi*-myliol (**40**), (+)-4(15)-dehydroledol (**41**) and (+)-4(15)-dehydroglobulol (**42**), together with (+)-*ent*-globulol (**43**) were isolated from *Mylia taylorii*[17].

2,3-Secoaromadendrane-type sesquiterpenoids, plagiochilide (**44**), plagiochiline A (**45**), B (**46**), C (**47**), D (**48**), E (**49**), H (**50**), hanegokedial (plagiochilal A) (**51**), ovalifolienal (**52**) and 9α-acetoxyovalifoliene (**53**) have been isolated from several South American and Asiatic *Plagiochila* species [17]. Plagiochiline A (**45**), when chewed, is transformed to furanoplagiochilal (**54**) and plagiochilal B (**55**) leaving a persistent pungent taste in the mouth. This transformation is done by amylase and saliva enzymes. The treatment of (**45**) with $KHCO_3$ in aqueous MeOH afforded the same aldehydes, (**54**) and (**55**) as shown in **Fig.2**.

A novel nor 1,10 secoaromadendrane-type sesquiterpenoid (**56**) was isolated from European *Mylia taylorii*, together with previously known *ent*-aromadendranes, myliol (**37**), dihydromylione (**38**) and taylorione (**57**)[18].

Barbatanes (Gymnomitranes)

Barbatane-type sesquiterpenoids are common constituents of the Jungermanniidae and Marchantiidae[17]. The skeleton of the gymnomitranes (barbatanes) was first reported by Connolly *et al*[10] from the liverwort *Gymnomitrion obtusum*, a rich source of gymnomitranes, e.g. gymnomitrol (**58**). This type of skeleton is not found outwith the Hepaticae. Although the corresponding

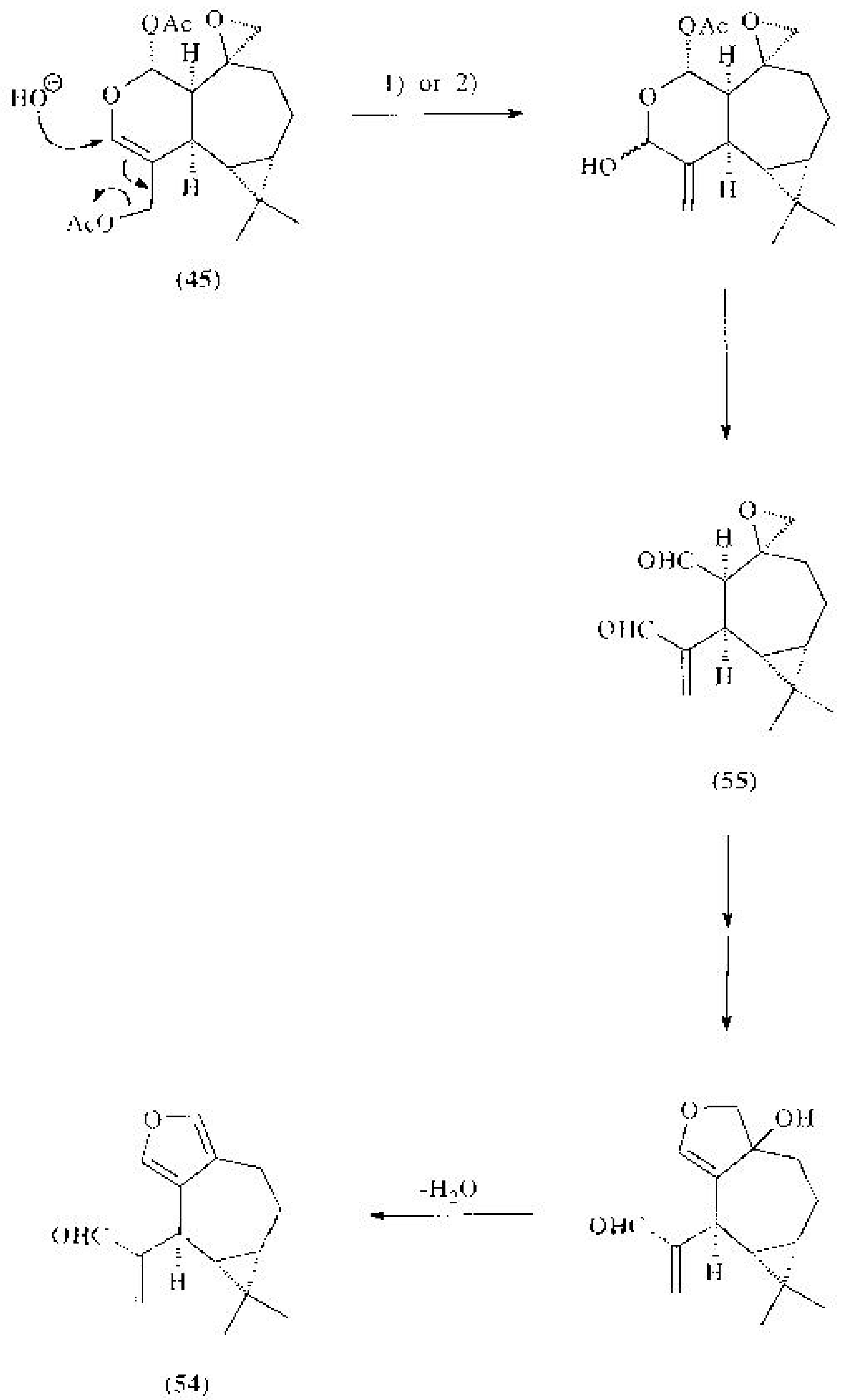
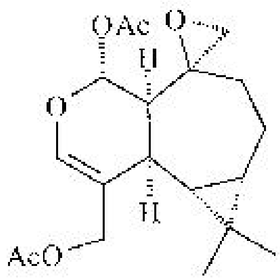


Fig.2 1) Amylase or Saliva 2) $KHCO_3$/MeOH$_{(aq)}$



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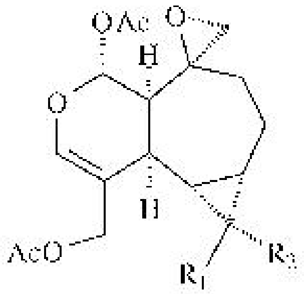
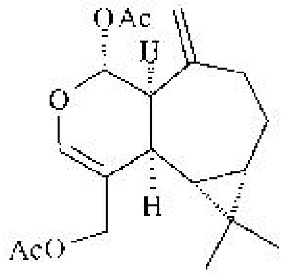


(46) $R_1 = CH_3$ $R_2 = CH_2OAc$

(48) $R_1 = R_2 = CH_2OAc$



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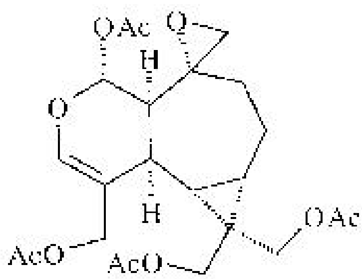

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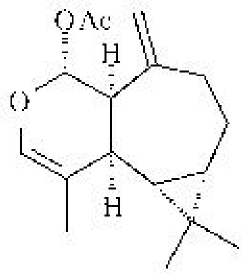

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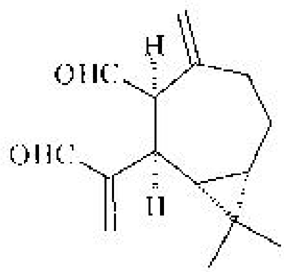

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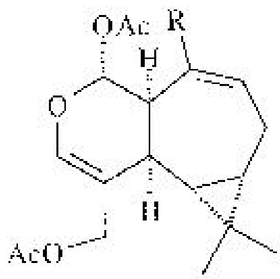
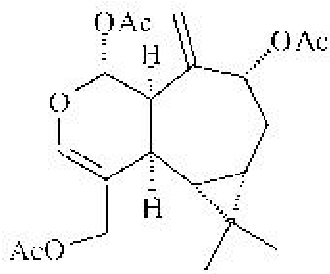


(52) R= CHO



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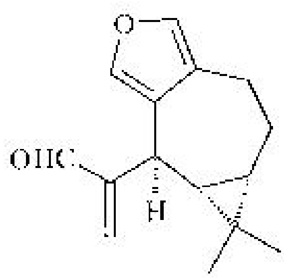

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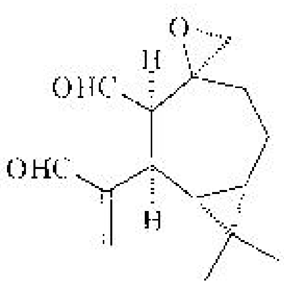

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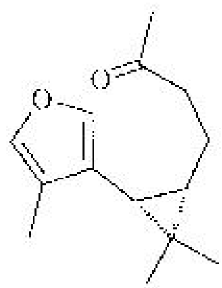

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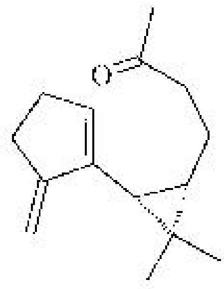

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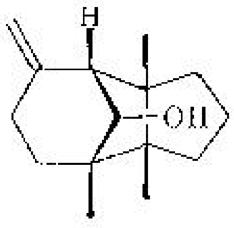

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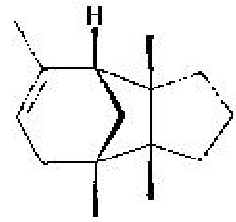

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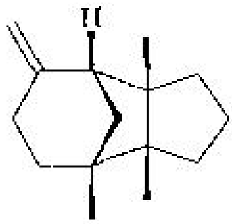

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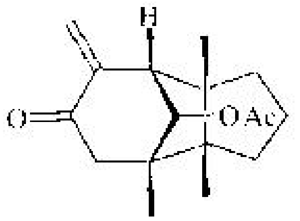

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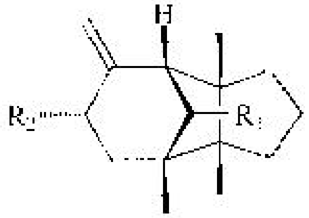
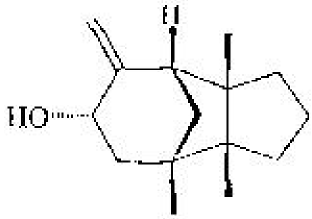


(62) R_1 = OAc, R_2 = OH
(63) R_1 = $OCOCHCHC_6H_5$, R_2 = OH



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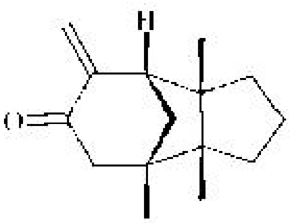

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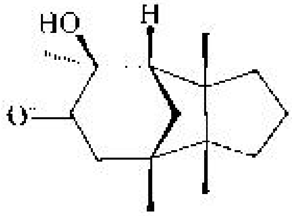

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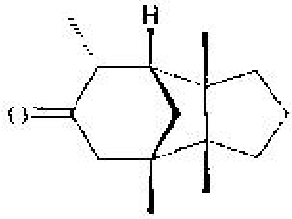

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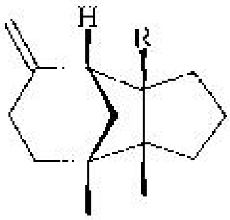
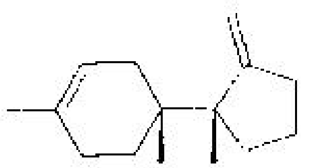


(68) R= CH_2OH
(69) R= CHO
(70) R= CO_2H



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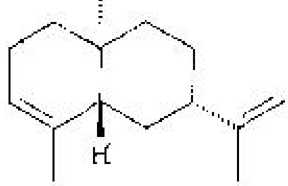

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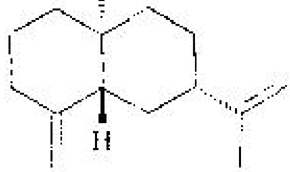

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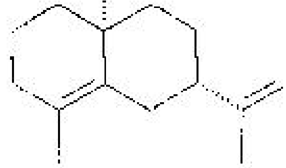

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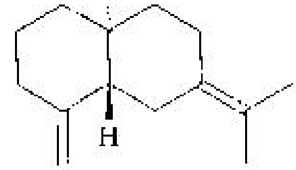


(75)

hydrocarbons α- (**59**) and β-gymnomitrene (**60**) are widespread, oxygenated derivatives are much less common.

Among the barbatanes (gymnomitranes) found in Hepaticae[17] are 9-oxogymnomitryl acetate (**61**), 9α-hydroxygymnomitryl acetate (**62**), 9α-hydroxygymnomitryl cinnamate (**63**), (+)-gymnomitr-8(12)-en-9α-ol (**64**), (+)-gymnomitr-8(12)-en-9α-one (**65**), (+)-8β-hydroxy-gymnomitran-9-one (**66**), (8R)-(+)-gymnomitran-9-one (**67**), (-)-gymnomitr-8(12)-en-15-ol (**68**), (-)-gymnomitr-8(12)-en-15-al (**69**), and (-)-gymnomitr-8(12)-en-15-oic acid (**70**).

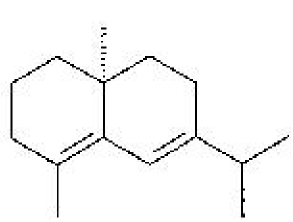
Connolly *et al*[20] suggested that a plausible biogenetic precursor of gymnomitrane-type sesquiterpenes was β-bazzanene (**71**), whose acid catalysed rearrangement has been investigated by Wu and Liu[21].

Eudesmanes and Eremophilanes

Eudesmane sesquiterpenoids are very widely distributed in the Hepaticae, particularly in the Jungermanniales[1, 17]. *Ent*-α-selinene (**72**), *ent*-β-selinene (**73**), selina-4,11-diene (**74**), *ent*-γ-selinene (**75**), δ-selinene (**76**), α-eudesmol (**77**), β-eudesmol (**78**), selin-11-en-4-ol (**79**), neointermediol (**80**), eudesm-3-en-7α-ol (**81**), 4β-methoxyeudesmanal (**82**), eudesmanal (**83**), (+)-5α,7β(H)-eudesmane-4α,6α-diol (**84**), and ajanol (**85**), are some of the eudesmanes found in the Hepaticae[17]. Connolly and his colleagues isolated eudesm-4(15)-ene-6β,7β-diol (**86**) from *Chiloscyphus pallescens*[22] and (+)-eudesm-3-ene-6β,7α-diol (**87**) from *Lepidozia reptans*[23]. The absolute configurations of both diols are yet to be determined.

Frullania species are rich sources of 12,6-eudesmanolides[1,17]. (-)-Frullanolide (**88**) and dihydrofrullanolide (**89**) have been found in many *Frullania* species[24,25]. (-)-Frullanolide (**88**) has also been isolated from *Grangea maderaspatana*(Compositae)[26]. The absolute stereostructeres of two eudesmanolides, (−)-β-frullanolide (**90**) and (+)-brothenolide (**91**) has been publised by Takeda *et al*[27].

Connolly and Thornton[28] isolated (-)-frullanolide (**88**), γ-cyclocostunolide (**92**), better known as arbusculin B[29], α-cyclocostunolide (**93**), and costunolide (**94**) from the Scottish liverwort *Frullania tamarisci*. The isolation of costunolide with two of its cyclised isomers, (**92**) and (**93**), is of special biogenetic interest as costunolide may be cyclised [30] to a mixture of α-, β- and γ-cyclocostunolides.



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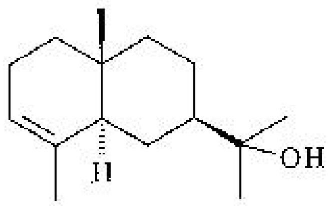

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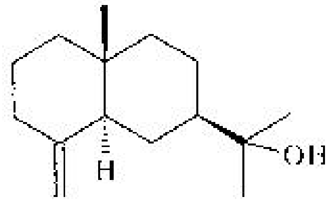

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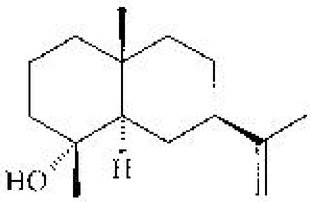

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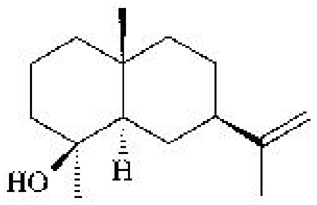

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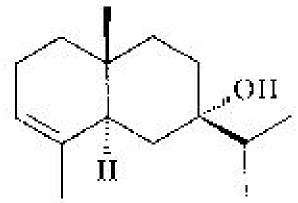

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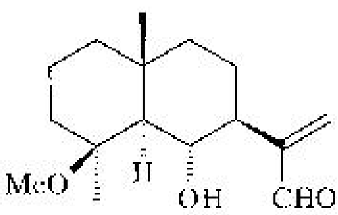

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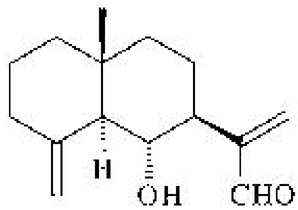

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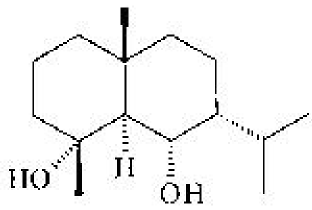

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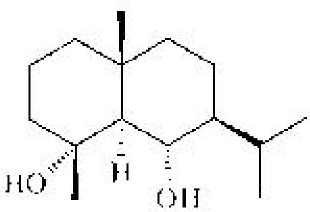

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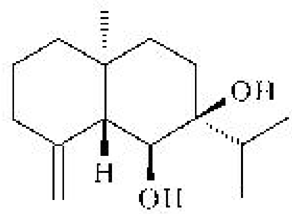

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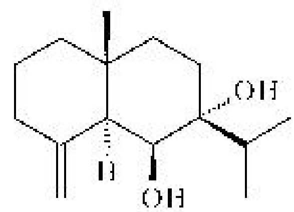

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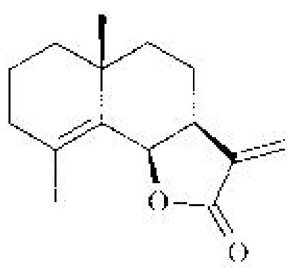

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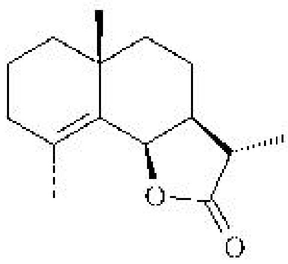

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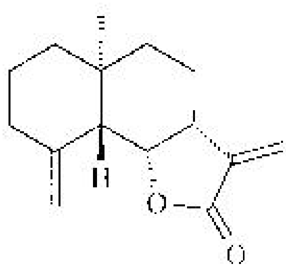

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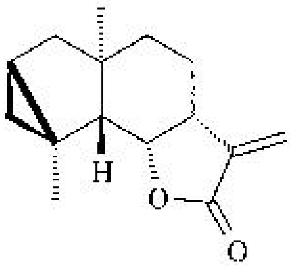

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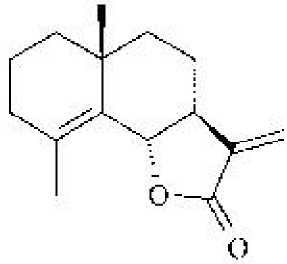

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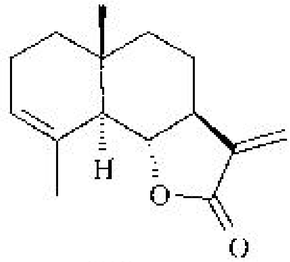

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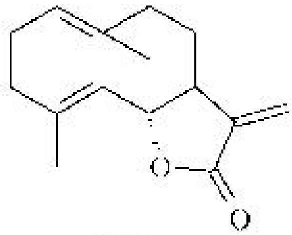

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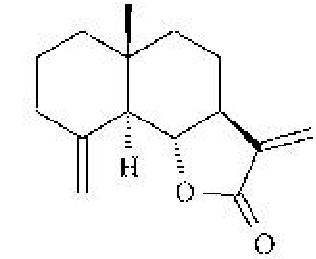

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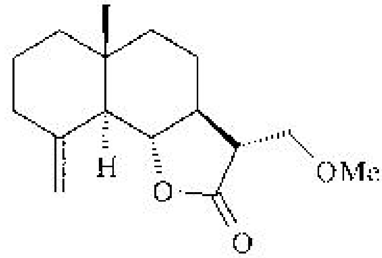

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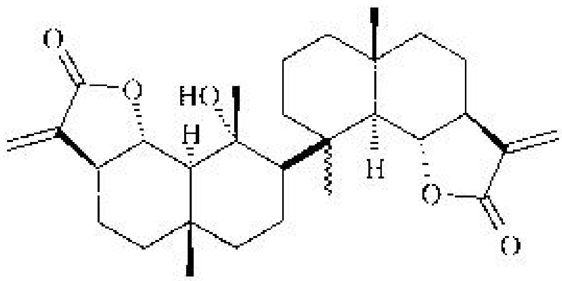

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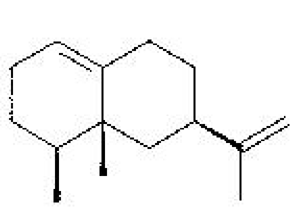

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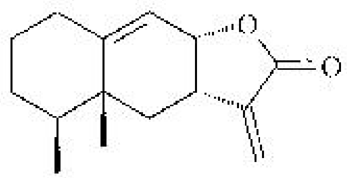

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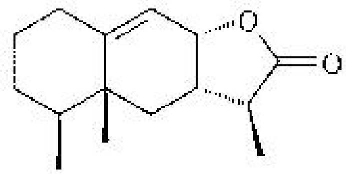

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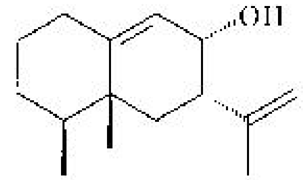

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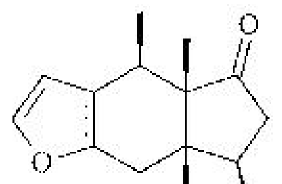

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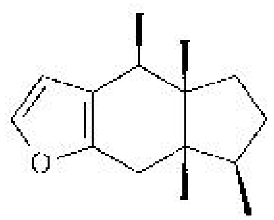

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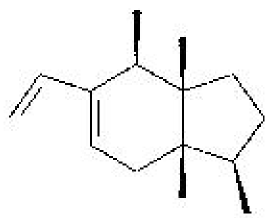

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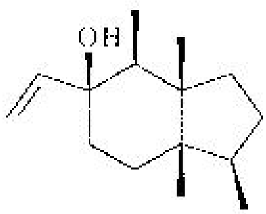

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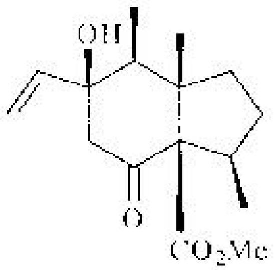

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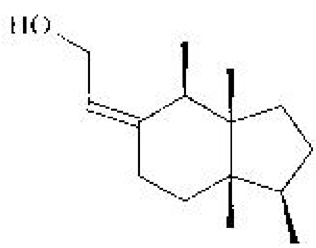


(107)

However, β-cyclocostunolide (**95**) could not be detected in the extract of *F. tamarisci*. This liverwort has been further examined[17] and methoxyfrullanolide (**96**) and the unique eudesmane-type lactone dimer (**97**) were isolated and their structures were deduced by ^{1}H and ^{13}C NMR spectrometry[3].

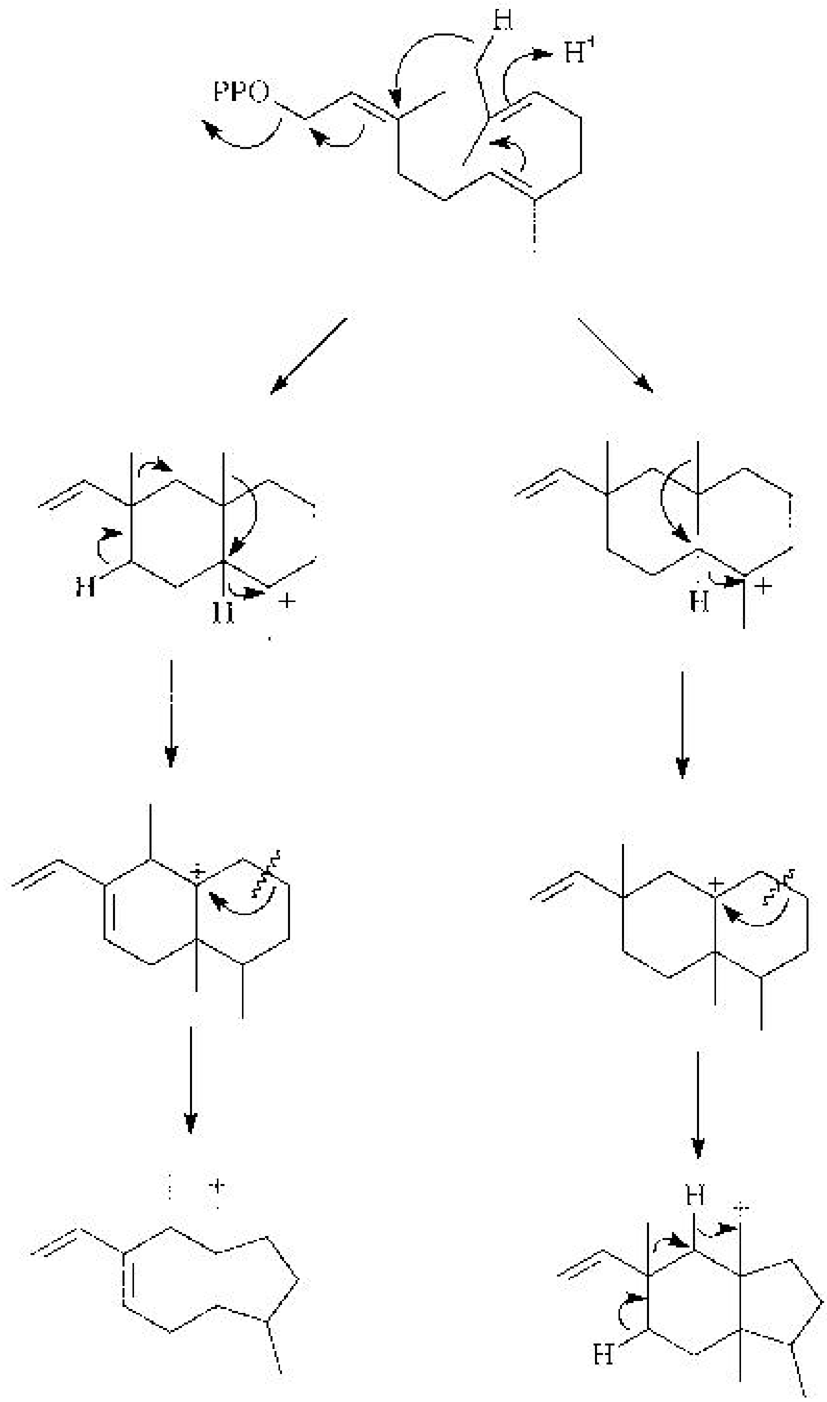
Eremophilane-type sesquiterpenes are quite rare in the Hepaticae. Previously only three eremophilane-type compounds, eremophilene (**98**), eremofrullanolide (**99**) and its dihydro derivative (**100**), had been found in Jungermanniales[1]. In 1990, Asakawa *et al*[32] found eremophilene (**98**) in the extract of *Frullania serrata*. Katoh and Takeda[33,34] showed that the essential oils from cultured cells of *Calypogeia* species also contain eremophilene (**98**). Later in 1992, Harrison *et al*[35] isolated a novel eremophilane-type sesquiterpene, (+)-(4S*,5R*,7S*,8R*)-eremophila-9,11-dien-8α-ol (**101**).

Pinguisanes and Norpinguisanes

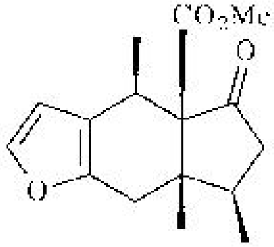
The first representative of this group, the ketone pinguisone (**102**), was isolated[36] from *Aneura pinguis*. It was soon followed by deoxopinguisone (**103**) from *Ptilidium ciliare*[37]. Two possible biogenetic pathways for pinguisane-type sesquiterpenoids found in liverworts have been proposed by Tazaki *et al*[94] as shown in the next page.

Pinguisane-type sesquiterpenoids have so far not been found in higher plants but are limited to liverworts. Various pinguisane-type sesquiterpenes and norpinguisane type sesquiterpenoids have been isolated from the Hepaticae. Some of these sesquiterpenoids are α-pinguisene (**104**), pinguisenol (**105**), 7-keto-8-carbomethoxy-pinguisenol (**106**), naviculol (**107**), pinguisone methyl ester (**108**), porellapinguisenone (**109**), dehydropinguisone (**110**), pinguisenene (**111**), furanopinguisanol (**112**), dehydropinguisenol (**113**), and dehydropinguisenol methyl ether (**114**).

Connolly[31] has showed that pinguisanin (**115**) in $CDCl_3$ solution is slowly transformed into isopinguisanin (**116**). This appears to be due to acid catalysis as the transformation occurs also on treatment with acid while isopinguisanin seems to be stable under these conditions. The allylic cation (**117**) appears to be an intermediate in this transformation[31].



Possible Biogenetic Pathway for the Pinguisane Skeleton



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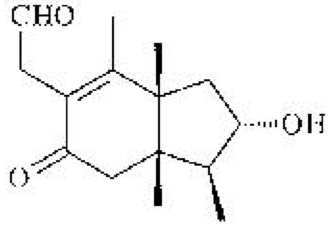

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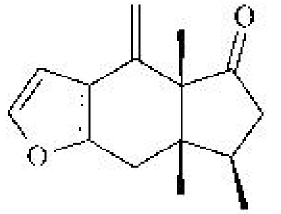

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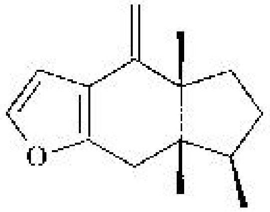
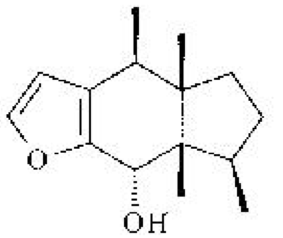
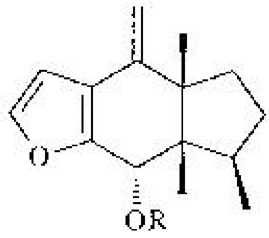


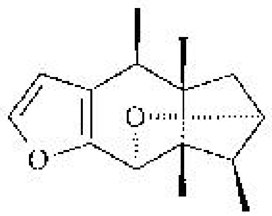
(111)



(112)



(113) R= H
(114) R= Me



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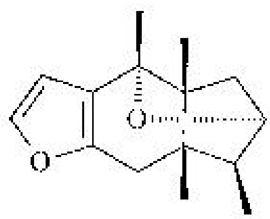

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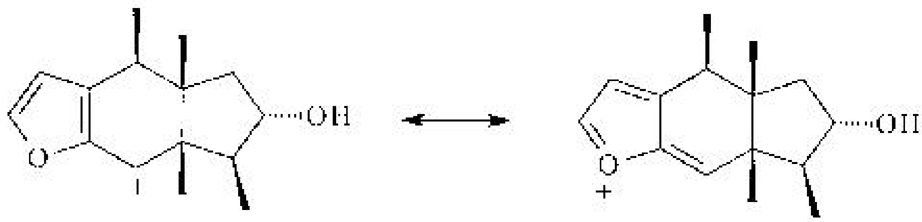

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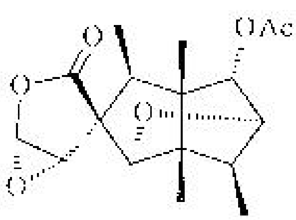

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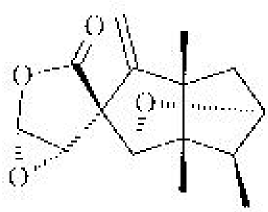

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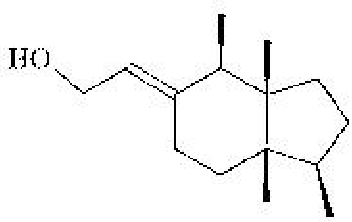


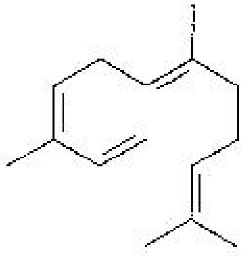
(120)

Two liverworts, *Frullanoides densifolia* and *Trocholejeunea sandvicensis*, were examined by Tori *et al*[38] and new pinguisane derivatives were isolated. *F. densifolia* produced two new rearranged pinguisane sesquiterpenoids, spirodensifolin A **(118)** and spirodensifolin B **(119)** together with the pinguisane-type alcohol isonaviculol **(120)**. The stereochemistry of the epoxide ring in compound **(118)** was established by X-ray crystallographic analysis. *T. sandvicensis* , however, gave furanopinguisanol **(112)**

Farnesanes and Germacranes

Farnesanes are not widely distributed among liverworts but germacranes are common. The first farnesane to be reported is α-farnesene **(121)** from *Scapania ornithopodioides* by Wu *et al*[39]. *Trans*-β-farnesene **(122)** has been found in fourteen liverworts collected in South America[17], and it has been detected in *Gymnocolea, Lophocolea, Monoclea, Radula,* and *Scapania* species as well. *Trans*-farnesol **(123)** has been found in the essential oil of *Plagiochila ovatifolia* and (+) nerolidol **(124)** was isolated from *Lophocolea heterophylla, Plagiochila ovatifolia,* and *Wiesnerella denudata*[17]. 4,5-Dehydronerolidol **(125)** was reported by Bohlmann *et al*[40] from *Brickellia californica* (Compositae).

Germacrene-B **(126)** has been detected [17] in the essential oil of *Conocephalum japonicum* and *Plagiochila ovatifolia* and germacrene-D **(127)** was isolated[17] from *Conocephalum conicum, Bazzania praerupta,* and *Marchantia foliacea*[41]. Nagashima *et al*[42] have reported two new germacrane-type sesquiterpene alcohols, *ent*-germacra-4(15),5,10(14)-trien-1β-ol **(128)** and *ent*-germacra-4(15),5,10(14)-trien-1α-ol **(129)** from *Jackiella javanica*(Adelanthaceae). *Ent*-1(10)E,5E germacradien-11-ol **(130)** has been isolated from a large thalloid liverwort, *Dumortiera hirsuta* and its enantiomer has been obtained earlier from a higher plant, *Ferula communis* (Umbelliferae)[17]. Two new ketogermacrenes, **(131)** and **(132)**, have been isolated from *Porella swartziana* and *Conocephalum conicum*[17]. Four new germacranolides, **(133)**, **(134)**, **(135)**, **(136)**, have been found in the essential oil of *Porella acutifolia* by Loyola *et al*[43].



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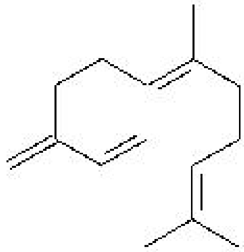

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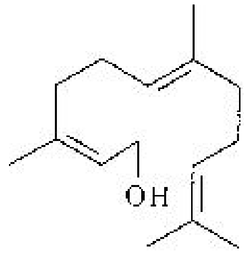

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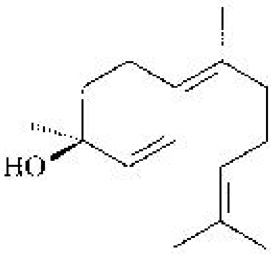

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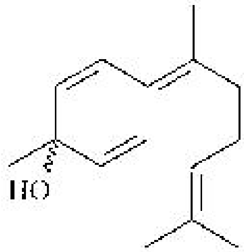

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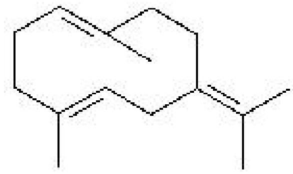

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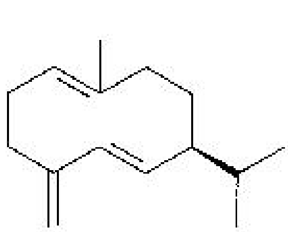

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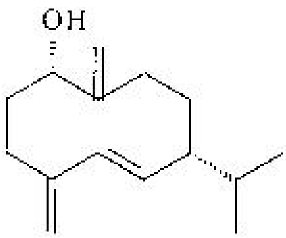

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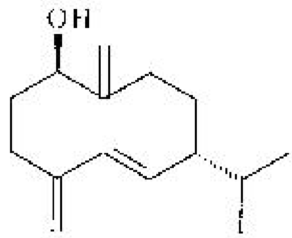

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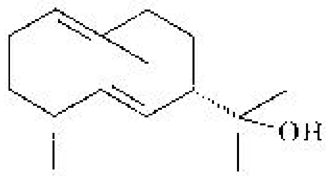

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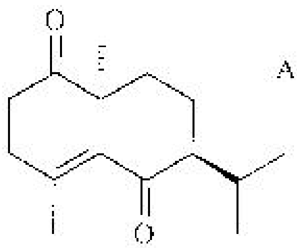

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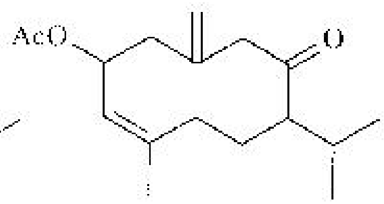

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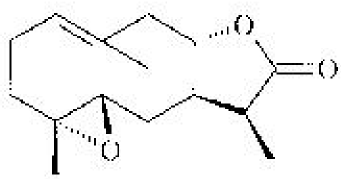

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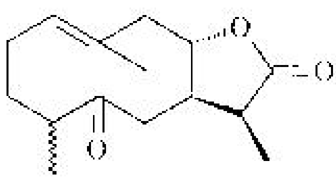

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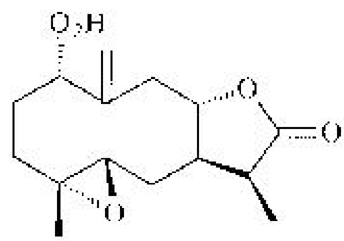


(135)

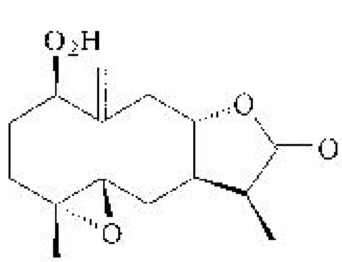
DITERPENOIDS

Only certain genera of liverworts produce diterpenoids. Most of the diterpenoids found in liverworts have representatives from both the 'normal' and *ent* series as in higher plants and only the kauranes belong exclusively to the *ent*-series. The first diterpenoid isolated from liverworts was *ent*-16β-hydroxykaurane (**137**) from *Anthelia julacea* and *A. juratzkana* by Huneck and Velve in 1970[44]

Labdanes

A labdane-type alcohol jungermanool[45] (**138**) has been isolated from *Jungermannia torticalyx*, together with *ent*-manool[46] (**139**) by Matsuo and his colleagues. The structure of the former was determined by spectroscopic methods. The essential oil of *Porella perrottetiana* yielded[47] the novel labdane *ent*-labda-12(E),14-diene-7α,8β-diol (**140**), while *Ptychanthus striatus* gave[48,49] highly oxygenated labdane-type diterpenes, ptychantins A (**141**), B (**142**), C (**143**) and D (**144**). The relative and absolute stereochemistry of (**142**) was established by a combination of chemical reactions, X-ray crystallography, and NOE difference spectrometry[48,49]. The structures and stereochemistry of (**141**), (**143**) and (**144**) were established by a combination of NOE spectrometry and chemical correlations with (**142**). Another highly oxygenated labdane from a liverwort is scapanin (**145**), also called scapanin A, which was isolated from *Scapania undulata* by Huneck and Overton[50].The structure was determined[51] by Connolly and Huneck using spectroscopic and chemical methods.

Taiwanese *Pleurozia acinosa* yielded[52] a new labdane-type diterpene, 8-*epi*-sclareol (**146**). The structure was established by comparison of its ^{13}C NMR spectrum with the spectra of sclareol (**147**) and 13-*epi*-sclareol (**148**). Asakawa *et al.*[53,54] isolated (+)-labda-7,14-dien-13-ol (**149**) and labda-12,14-dien-8α-ol (**150**), first isolated from *Nicotiana tabacum*[55], along with the previously known *ent*-labdane-diol (**140**) from *Porella perrottetiana*. *Marchantia polymorpha* produces[56,57] not only *ent*-



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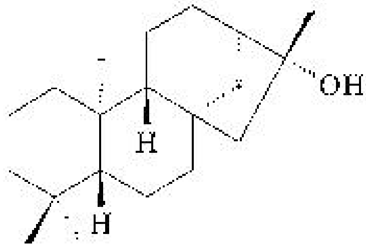

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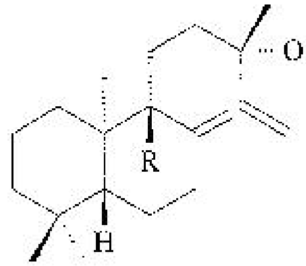
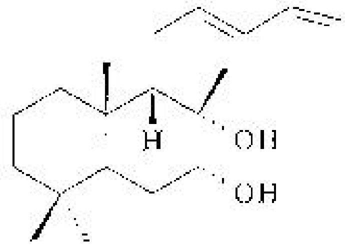


(138) R= H
(139) R= OH



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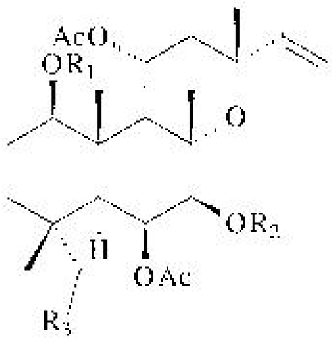
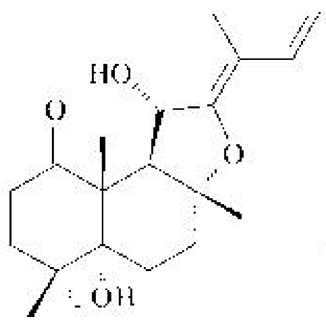


(141) R_1=R_2= Ac, R_3= H
(142) R_1= Ac, R_2=R_3= H
(143) R_1=R_2=R_3= H
(144) R_1= Ac, R_2= H, R_3= OH



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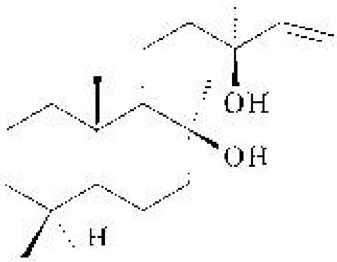

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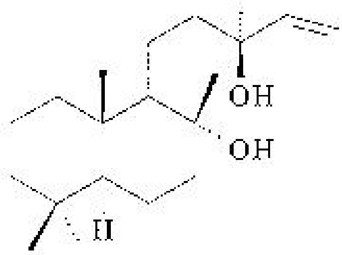

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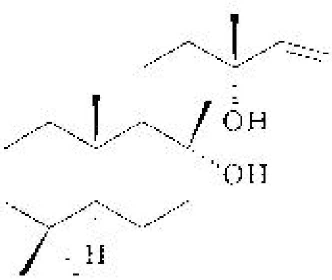

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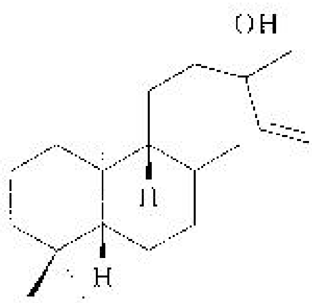

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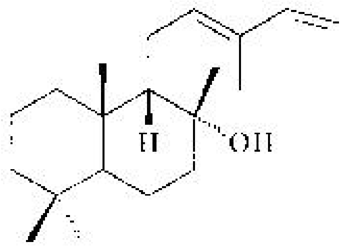


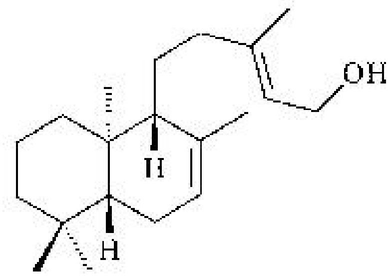
(150)

sesquiterpenoids but also an *ent*-diterpenoid, *ent*-labda-7,13E-dien-15 ol (**151**). The same compound occurs[57] in *Targiona hypophylla* while its enantiomer has been isolated[58] from *Nicotiana setchelli*.

Three labdane-type diterpenoids, haplomitrenolides A (**152**), B (**153**) and C (**154**) have been isolated from the fresh cultured primitive liverwort, *Haplomitrium mnioides* by Asakawa *et al*[59]. The stereochemistry of (**152**) was established by NOE difference. A very similar lactone (**155**), nidorella lactone, has been isolated[60] from *Nidorella hottentotica* (Compositae). Huneck *et al*[61] isolated the dihydro derivative scapanin B (**156**), the rearranged labdane (**157**), labda-12E,14-dien-8α,11ζ-diol (**158**) together with previously known scapanin A (**145**) from the liverwort *Scapania undulata*. Five highly oxygenated new labdane-type diterpenoids, hamatilobenes A (**159**), B (**160**), C (**161**), D (**162**), and E (**163**) have been isolated[63] from *Frullania hamatiloba* and a new chettaphanin-type(= rearranged labdane) diol, pleurodiol (**164**) was reported by Asakawa *et al*[64]. Another diterpenoid of this type (**165**) has been isolated[65] from *Pallavicinia levieri*. Rearranged labdane-type diterpenoids are rare in nature[66] and these are the first reports of the isolation of chettaphanin-type diterpenoids.

Sacculatanes

The first report of this skeletal type was published[67] by Asakawa and Takemoto, who isolated sacculatal (**166**) and the non-pungent isosacculatal (**167**) from the liverwort *Trichocoleopsis sacculata*. *Pellia endiviifolia* is a rich source of sacculatane-type diterpenoids, and six sacculatanes (**168**, **169**, **170**, **171**, **172**, **173**) have been isolated[68] from this liverwort together with sacculatal (**166**) and its C-9 epimer (**167**). The two sacculatanes, perrottetianal A (**174**) and perrottetianal B (**175**) have been isolated[69] from *Porella perrottetiana*. Perrottetianal A (**174**), a common component of non-pungent *Porella* species, occurs both in Jungermanniales and in Metzgeriales. Since then the same dialdehyde has also been isolated from *Fossombronia pusilla*[70], *Porella acutifolia*[71], *P. caespitans*[71], *P. cordaeana*[72], *P. elegantula*[73,74], and *P. navicularis*[75]. Surface and liquid cultures of *Fossombronia pusilla* furnished[70,76] a new saccalatane-type diterpene, 8-hydroxy-9-hydroperrottetianal (**176**) together with perrottetianal A (**174**) and B (**175**). The



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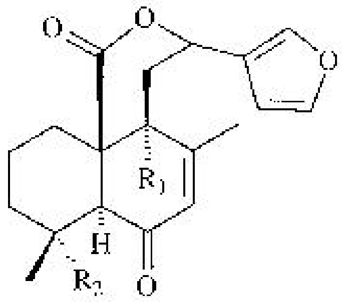
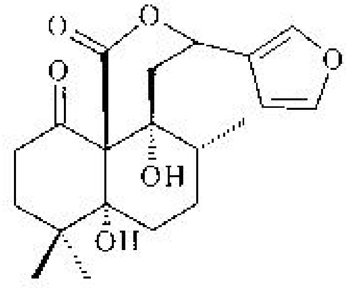


(152) R_1 = H, R_2 = Me
(153) R_1 = OH, R_2 = Me
(154) R_1 = H, R_2 = COOMe



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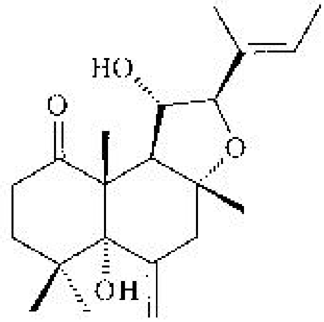

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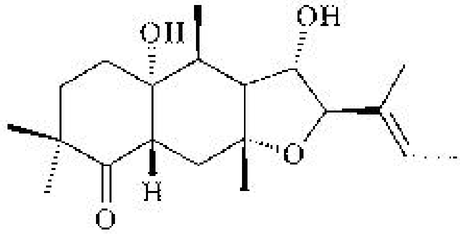

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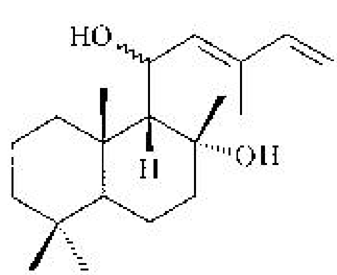

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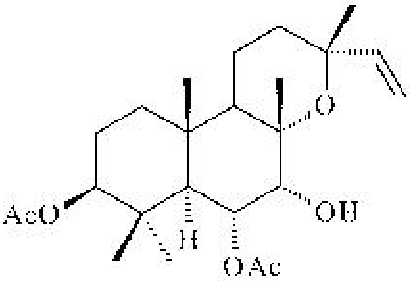

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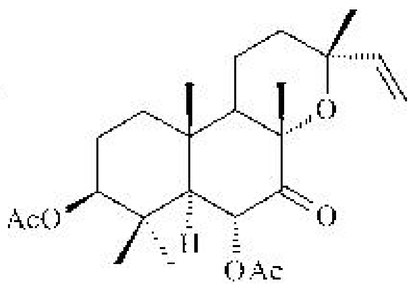

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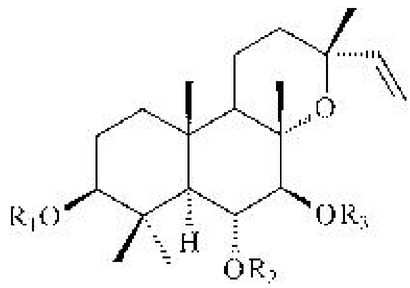
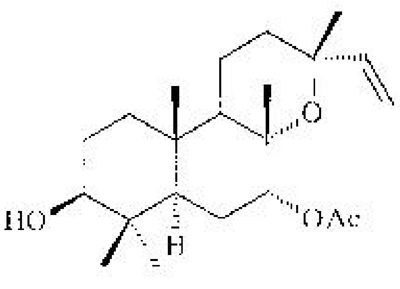


(161) R_1=R_2= Ac, R_3= H
(162) R_1=R_3= Ac, R_2= H



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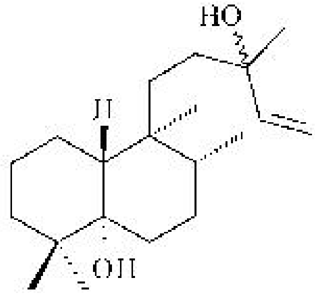

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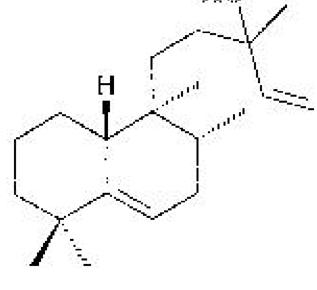

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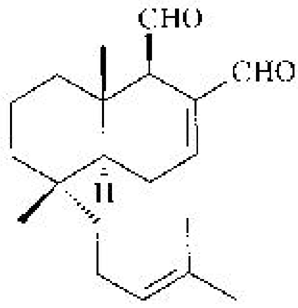

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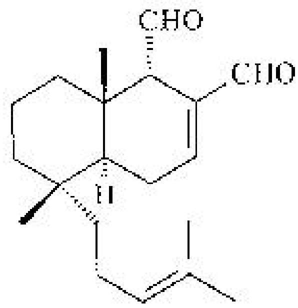

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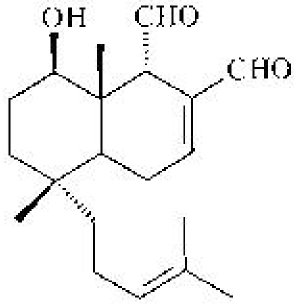

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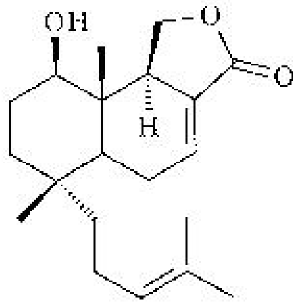

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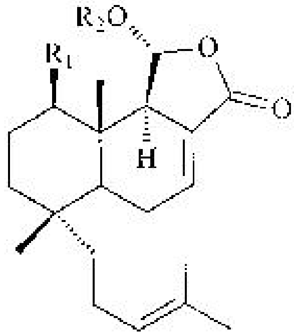
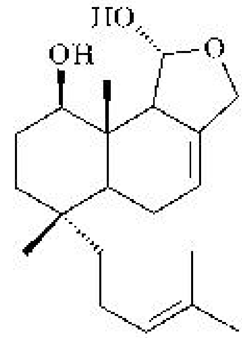


(170) $R_1=R_2=H$

(171) $R_1=OH$, $R_2=H$



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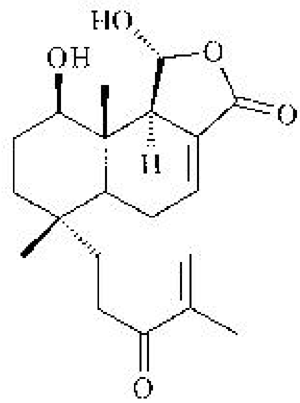

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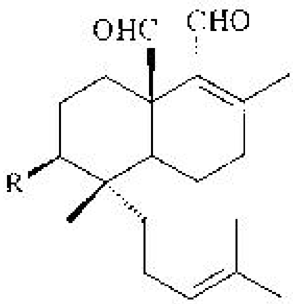
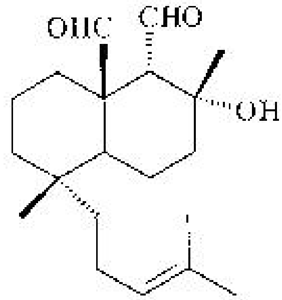


(174) R= H

(175) R= OH



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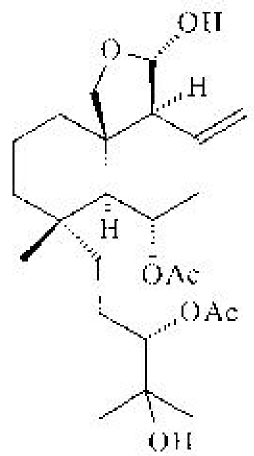

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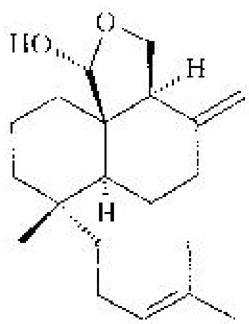
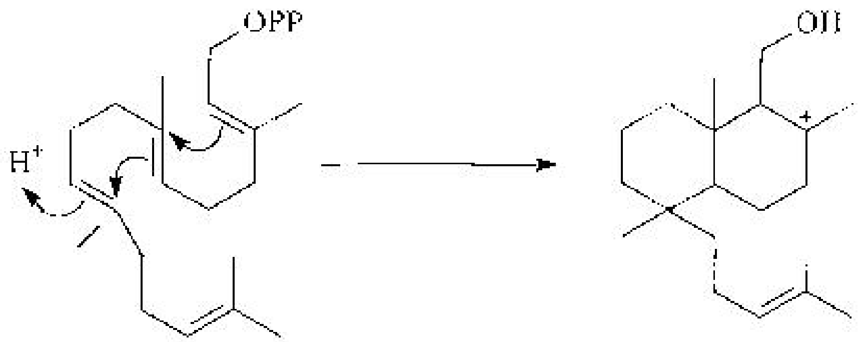


(178)

structure of (**176**) was deduced by comparing the NMR spectral data with those of (**174**) and (**175**) and the relative stereochemistry of (**176**) is based on NOE spectroscopy.

A new highly oxygenated sacculatane-type diterpene hemiacetal, sacculaplagin (**177**) has been isolated[77] from *P. sciophila* (= *P. acanthophylla*). *Porella perrottetiana* elaborates[54] perrottetianal A (**174**) as well as a new sacculatane-type diterpene hemiacetal, sacculaporellin (**178**).

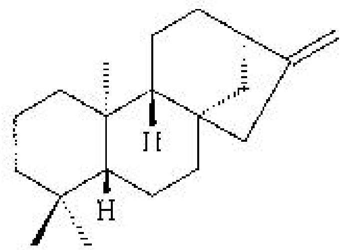
The ring system of sacculatane-type diterpenoids is the same as that of the drimanes with an additional isoprene unit attached to C-13. They may be formed from geranylgeranyl pyrophosphate by a cyclisation analogous to that of the drimanes, as shown below.



Kauranes

The kaurane skeleton is commonly found in Nature and most kauranoids belong to the *ent* series. *Ent*-kaurene (**179**) is the most commonly encountered kaurane-type diterpenoid and has been found in several species. Huneck and Vevle[74] showed that the main component of the waxy coating of the leafy liverworts *Anthelia juratzkana* and *A. julacea* was *ent*-16β-hydroxykaurane (**137**).

Connolly and Thornton[78] isolated four *ent*-kaurane derivatives, (**180-183**) from *Jungermannia atrovirens* (*Solenostoma triste*). Matsuo *et al*[79] isolated *ent*-18-hydroxykaur-16-en-15-one (**184**) and *ent*-18-hydroxy-(16S)-kauran-15-one (**185**) from *Porella densifolia* together with *ent*-kaur-16-en-19-oic acid (**186**). The essential oil of Czechoslovakian *Nardia scalaris* yielded[illegible] *ent*-15α-hydroxykaura-9(11),16-dien-6-one (**187**).



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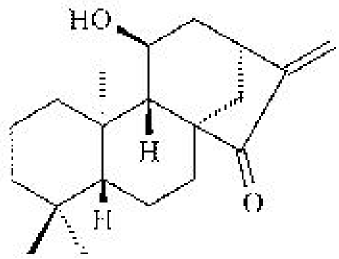

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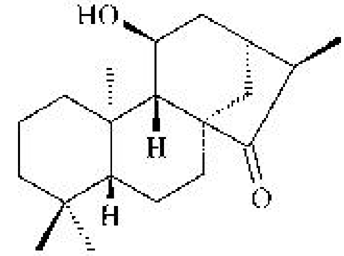
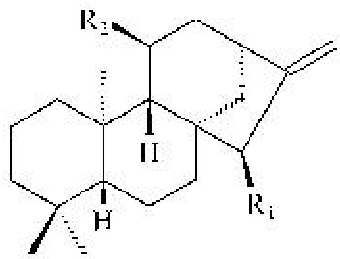
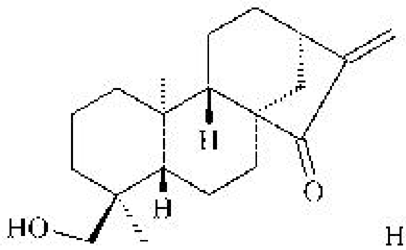


(181)



(182) $R_1 = R_2 = OH$
(183) $R_1 = OAc$, $R_2 = OH$



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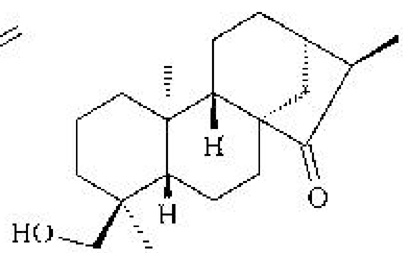

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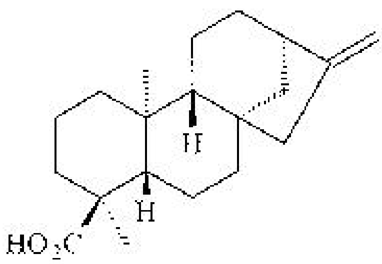

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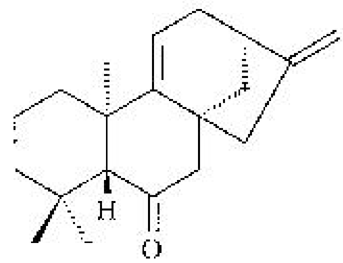

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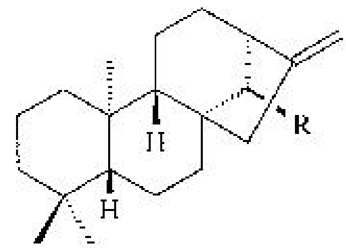
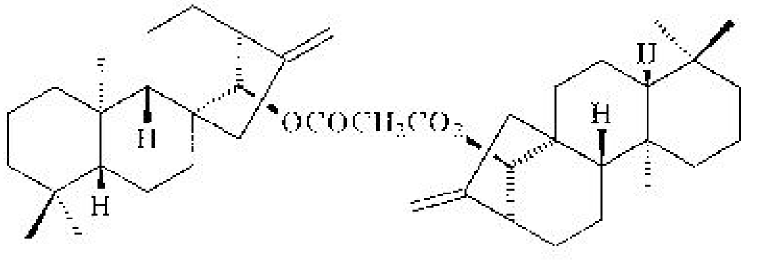
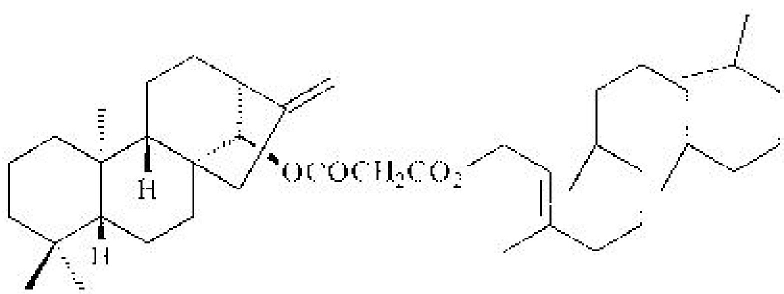


(188) $R = OCOCH_2CO_2CH_3$
(191) $R = OH$



(189)

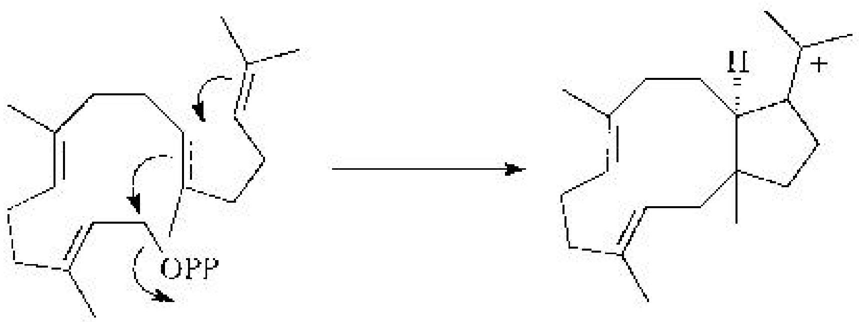


(190)

Connolly *et al*[81] have further investigated the ether extract of *N. scalaris* and isolated the *ent*-kaurane malonate in the form of its methyl ester **(188)**. C-14 oxygenated kaurane-type diterpenoids are rare in Nature[82,83]. Two more complex *ent* kaurane malonates **(189)** and **(190)** together with the parent *ent*-14α-kaurenol **(191)** have been isolated[84] from Japanese *Nardia subclavata*.

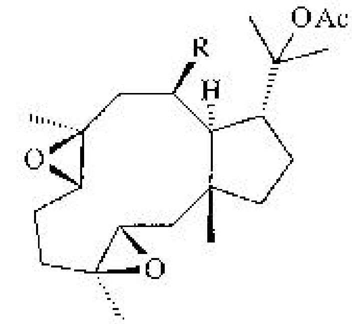
Dolabellanes

In his review of new terpenoids from the Hepaticae, Connolly has reported[51] the occurrence of the dolabellane barbilycopodin **(192)** in the extract of *Barbilophozia floerkei*. This is the first report of a dolabellane from plants, this class having previously been found[85] only in marine organisms. The biogenesis of dolabellane-type diterpenoids can be represented by cyclisation of all-*trans*-geranylgeranyl pyrophosphate, as shown below.

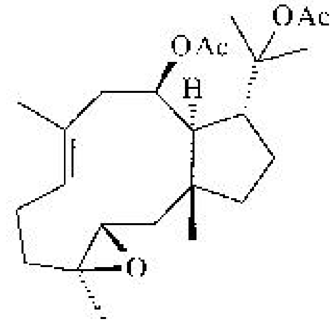


The ether extract of the dried and powdered *B. attenuata*, *B. floerkei*, and *B. lycopodioides*, was chromatographed on silica gel to give barbilycopodin **(192)**, while *B. floerkei* produced three additional dolabellane-type diterpenoids **(193-195)**[86]. Barbilycopodin **(192)** has also been isolated from *Barbilophozia hatcheri*[87], and *Chandonanthus setiformis* (= *Tetralophozia setiformis*)[88], which belongs to the Lophoziaceae. *Odontoschisma denudatum* of the Lophoziaceae produces[89,90] five dolabellane-type diterpenoids, (+)-acetoxyodontoschismenol **(196)** and the related compounds, **(197-200)**.

Asakawa *et al*[52] have isolated a new dolabellane-type diterpenoid, 18-hydroxy-4,8-dolabelladiene **(201)** from the liverwort *Pleurozia gigantea*. The first



(192) R= OAc
(193) R= H



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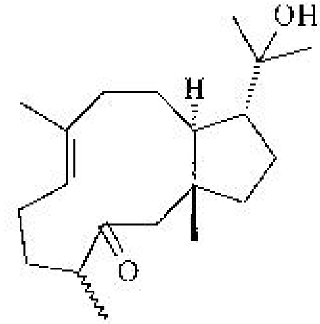
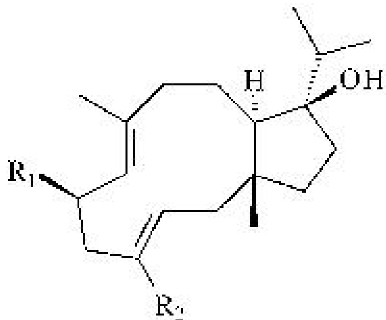
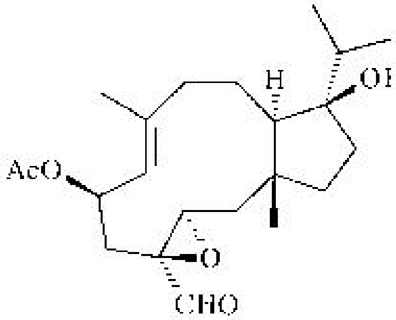


(195)



(196) R_1 = OAc, R_2 = CH_3
(197) R_1 = OH, R_2 = CH_3
(198) R_1 = OAc, R_2 = CH_2OH
(199) R_1 = OAc, R_2 = CH_2OAc



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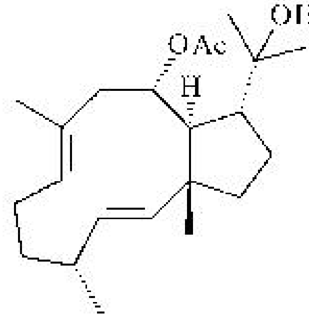

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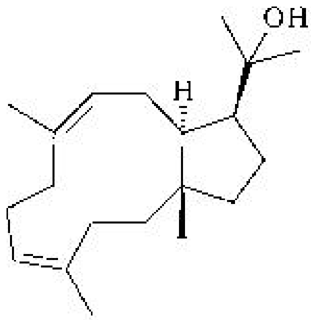

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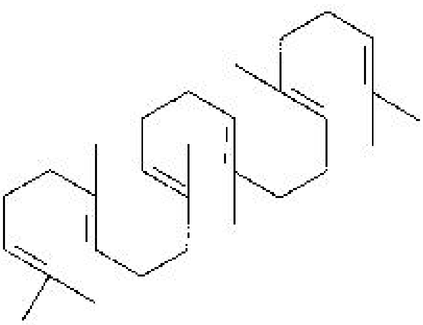

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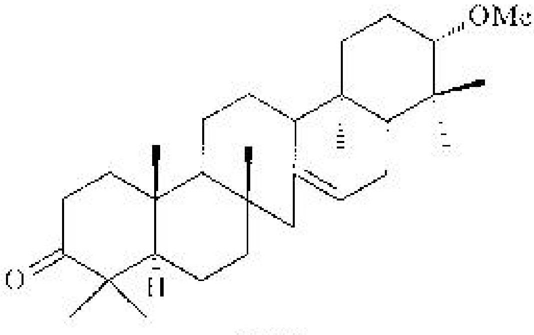

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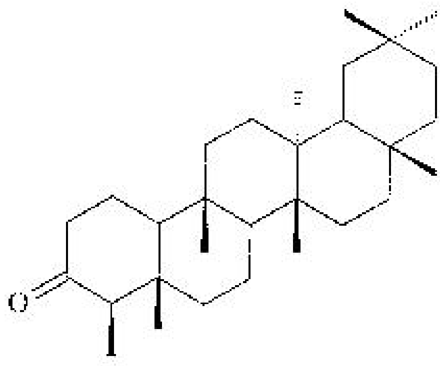


(205)

dolabellane-type diterpenoid **(202)** was isolated[91] from the sea hare *Dolabella californica*.

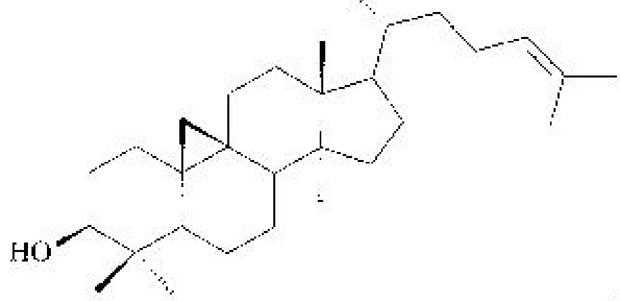
TRITERPENOIDS

Reports of triterpenoids in the Hepaticae are comparatively rare. Recently about 700 species of Hepaticae have been investigated[17] by GC-MS. Almost all species contain squalene **(203)**. Benes *et al*[92] reported the presence of (+)-21α-methoxyserrat-14-en-3-one **(204)** in *Nardia scalaris*. From *Bazzania japonica*, friedelin **(205)** has been obtained[93] in the pure state. *Mastigophora diclados* produced[94] cycloartenol **(206)**. *Mylia taylorii* also produces[95] cycloartenol. Later, Wu *et al*[96] isolated cycloart-23-en-3β,25-diol **(207)** from *Plagiochila peculiaris*. Diploptene **(208)**, hop-22,23-diol **(209)** and α-zeorin **(210)**, which was previously isolated by Harrison *et al*[98] from *Plagiochasma rupestre*, have been found in *Conocephalum japonicum*[97,84].

AROMATICS

The main types of aromatic compounds found in liverworts are bibenzyls and bisbibenzyls. The bibenzyls are the most characteristic and many of them are biologically active. Lunularic acid **(211)**, a growth inhibitor, isolated from the liverwort *Lunularia cruciata* in 1969[100], is the first representative of the bibenzyl type. It occurs widely and has been reported in 76 species of the Hepaticae[101]. Many variations of the basic bibenzyl unit[1,102] have been published, e.g. the interesting cyclohexenone, 4-(p-methoxyphenethyl)-cyclohex-2-en-1-one **(212)** from *Plagiochila longispina*[103] and a new bibenzyl cannabinoid derivative **(213)** from *Radula perrottetii*[104]. Another variation is the cyclopropanochroman type e.g. radulanin I **(214)** from the liverwort *Radula javanica*[105].

Bisbibenzyls are two bibenzyl units joined by ether and biphenyl linkages. These compounds are unique to the liverworts and are exemplified by perrottetin E-11 methyl ether **(215)** from *Pellia endiviifolia*[106], and isoplagin A **(216)** from *Plagiochila fruticosa*.



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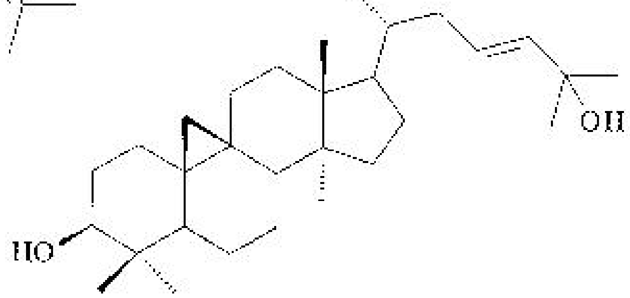

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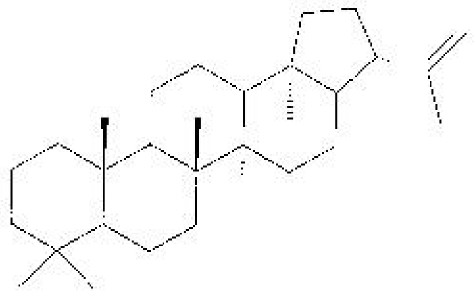

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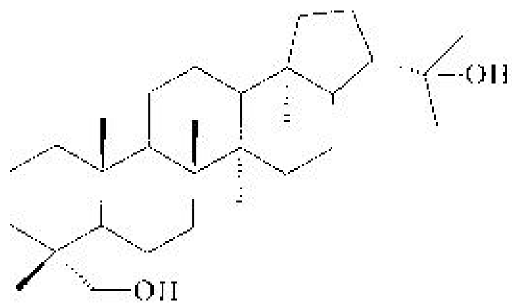

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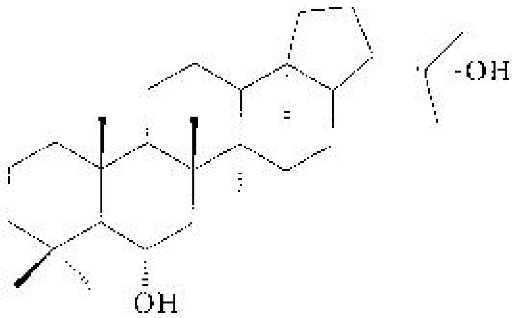

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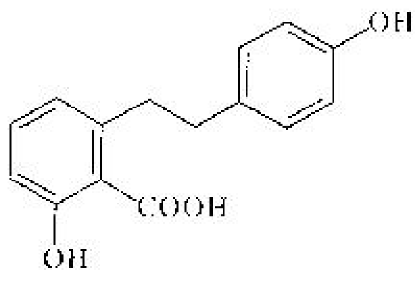

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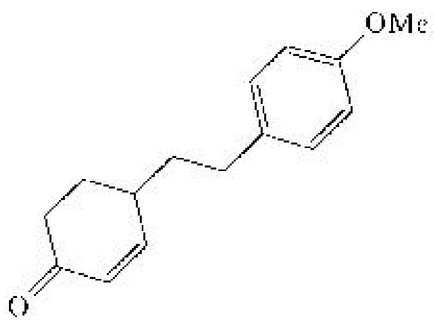

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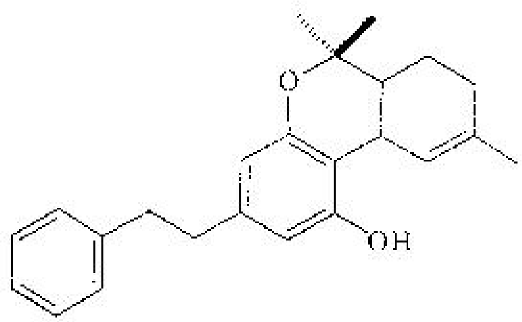
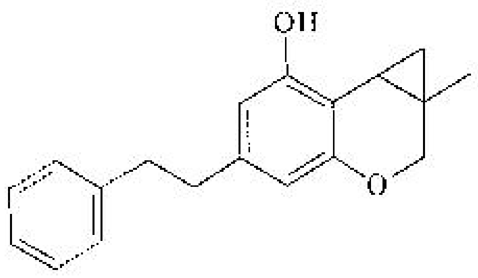


(213)

Flavonoids are widely distributed in the Hepaticae, particularly in the Marchantiales[1]. Most of the flavonoids found in the Hepaticae are flavonoid glycosides, except for a few flavonoid aglycones found in *Corsinia*[107] and *Frullania* species[108, 109, 110]. Apigenin (**217**) and luteolin (**218**) glycosides are the most common flavonoids.



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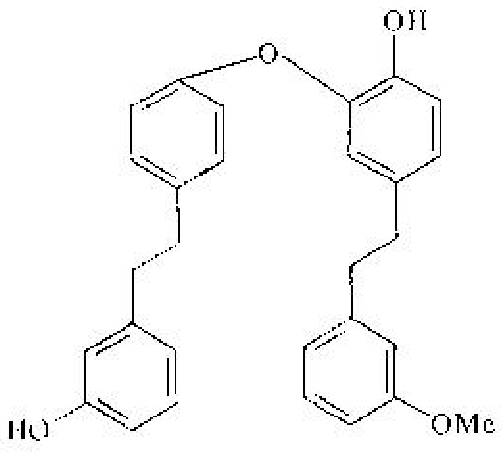

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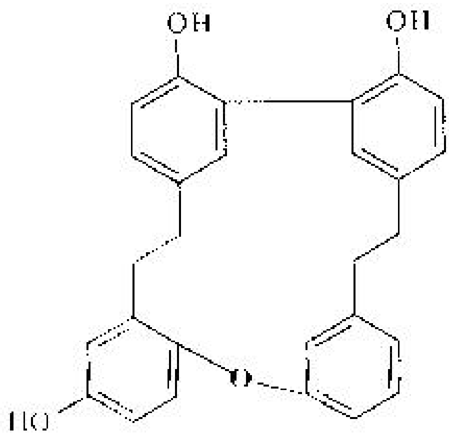

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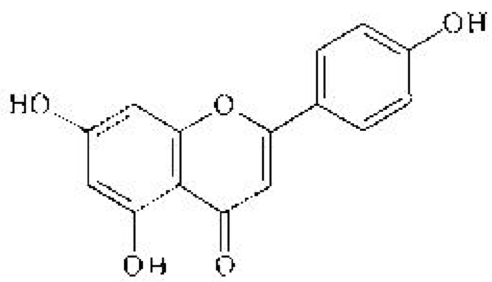

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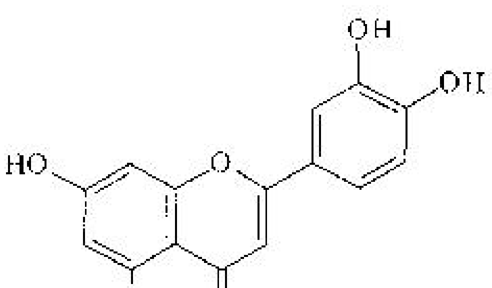

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Chapter-2

Scapania undulata

INTRODUCTION

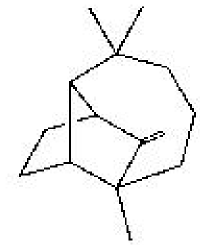
The *Scapania* species belonging to the Scapaniaceae have already been shown to contain sesquiterpenoids. No monoterpenoids have been reported which is suprising since *Scapania* species are extremely rich in metabolites. Among the first terpenoids to be isolated from *Scapania undulata* and indeed from the Hepaticae were (-)-*ent*-longifolene **(1)** and (-)-*ent*-longiborneol **(2)** which were shown to be enantiomeric with those compounds isolated from higher plants[1].

Many sesquiterpenoids[2,3] and labdane-type diterpenoids[4] have been isolated from *Scapania undulata*. The sesquiterpenes reported from this species are: (-)-longifolene **(1)**, (-)-longiborneol **(2)**, (-)-α-longipinene **(3)**, (+)-α-himachalene **(4)**, γ-himachalene **(5)**, (-)-α-ylangene **(6)**, (-)-β-farnesene **(7)**, (-)-longicyclene **(8)**, sativene **(9)**, (-)-α-helminscapene **(10)**, α-bisabolene **(11)**, β-gymnomitrene **(12)**, (+)-α-chamigrene **(13)**, β-chamigrene **(14)**, γ-cadinene **(15)**, (-)- longipinanol **(16)**, (+)-*ent*-epicubenol **(17)** and the labdane diterpenoids are *inter alia* 5α, 8α, 9α-trihydroxy-13E-labden-12-one **(18)**, 1α, 5α, 8α-trihydroxy-13E-labden-12-one **(19)**, 5α, 8α-dihydroxy-13E-labden-12-one **(20)**, scapaundulin A **(21)** and B **(22)**.

RESULTS AND DISCUSSION

The plant material (247 g.) collected near Aberfoyle, was air-dried, ground and extracted with diethyl ether. It yielded 2 g. crude extract, which showed several spots on analytical TLC (UV) and was subjected to flash chromatography on silica gel, eluting with light petroleum and increasing amounts of ether in light petroleum. Preparative TLC was used to isolate and purify the compounds. Three compounds, which are previously known, were isolated from this species : (-)-*ent*-longipinanol **(16)**, (-)-longiborneol **(2)** and an amorphane/muurolane(cadinane) type sesquiterpenoid **(23)**.

(-)-*ent*-Longipinanol **(16)** was present in the fraction eluted with 40 % ether in light petroleum. It was obtained following purification by preparative TLC (eluent 25 % ether in light petroleum). The molecular formula [$C_{15}H_{26}O$, 204 m/z (M^+-H_2O)] was readily apparent from the ^{13}C NMR spectrum which revealed the presence of four methyl groups, five methylene groups, three methine carbons and three tertiary



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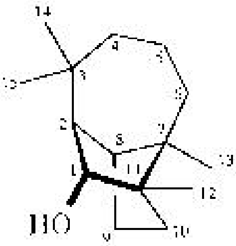

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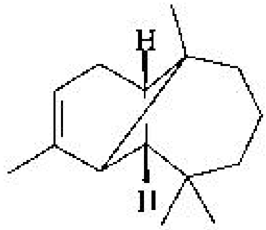

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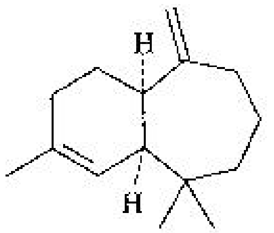

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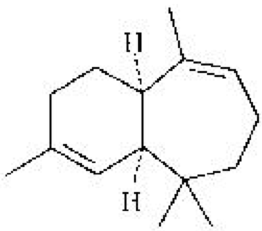

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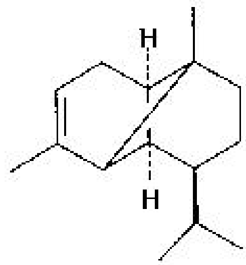

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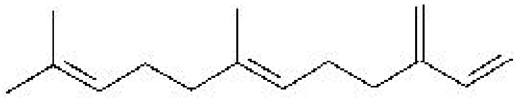

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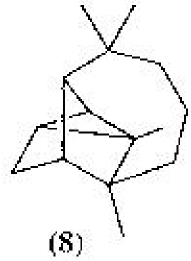

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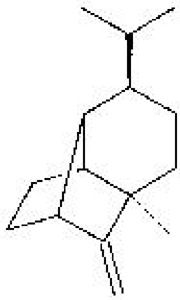

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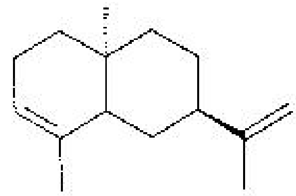

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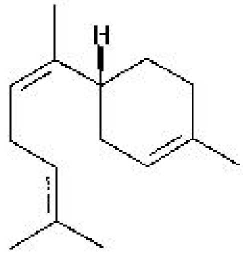

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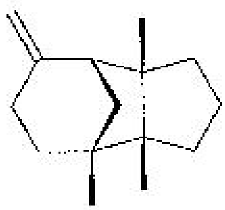

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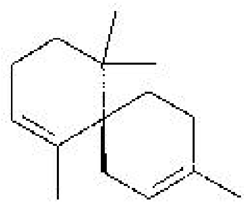

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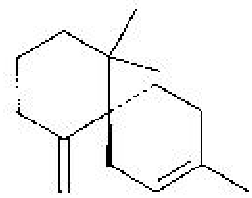

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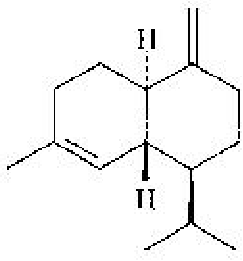

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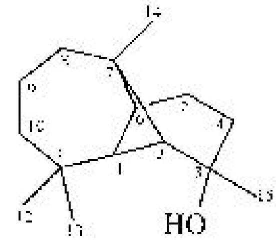

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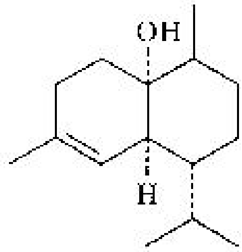

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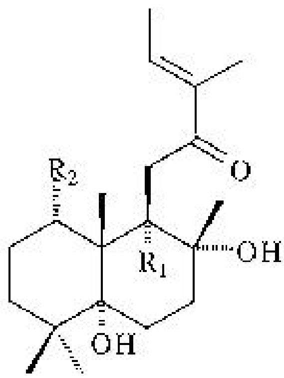
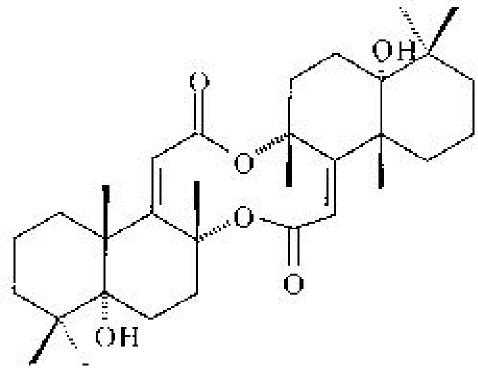


(18) R_1= OH, R_2= H
(19) R_1= H, R_2= OH
(20) R_1= H, R_2= H



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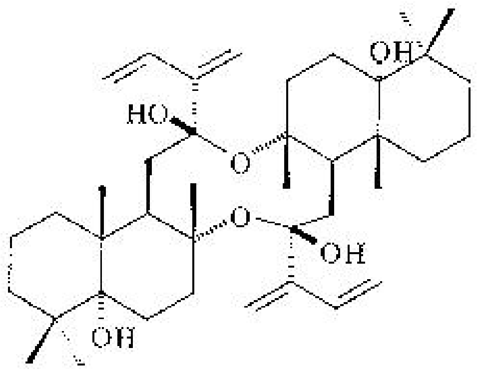

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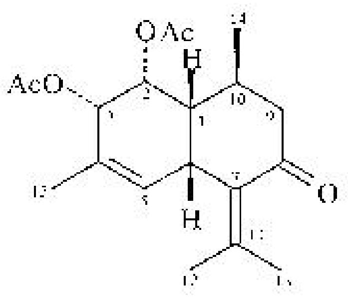
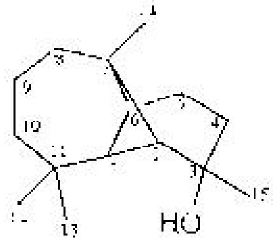


(23)

carbons, one of which is oxygenated. The molecule is thus bicarbocyclic. Although the ^1H NMR spectrum showed the four methyl groups clearly, the other methylene and methine protons appeared as multiplets between 1.4 and 1.96 ppm. The structure of the compound was finally deduced from proton and carbon correlations in HMBC and HMQC spectra. Its ^1H and ^{13}C NMR shifts are given below.



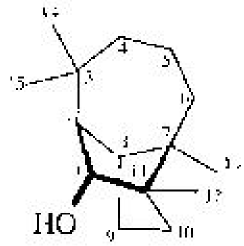
(16)

^1H and ^{13}C NMR Spectrum of Compound (16)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	52.6	1.64-1.50	m (overlapping with 2H-10, 2H-9)
2	53.0	1.90-1.75	m (overlapping with 2H-8, 2H-5)
3	77.1		
4	39.3	1.38-1.30	m
5	27.9	1.90-1.75	m
6	40.0	1.97-1.90	m
7	39.9		
8	32.6	1.90-1.75	m
9	21.7	1.64-1.50	m
10	42.6	1.64-1.40	m
11	32.2		
12*	27.2	0.88	s
13*	28.6	0.89	s
14	25.7	0.90	s
15	31.1	1.32	s

* 12 and 13 maybe interchanged.

The second compound, (-)-longiborneol (2), was obtained from the fraction eluted with 30 % ether in light petroleum. It is a known compound and its ^{13}C chemical shifts are identical with those of the literature data[5]. Its ^{13}C NMR spectrum showed four methyl groups, five methylene groups, three methine carbons and three tertiary carbons and the structure of the compound was supported by HMBC and HMQC spectra.



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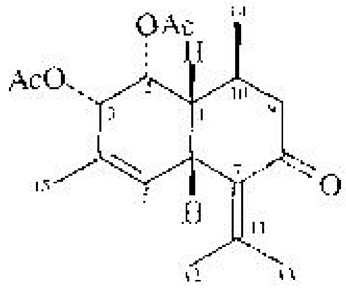
^1H and ^{13}C NMR Spectrum of Compound (2)

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	79.7	3.75	dd (J= 5.0 Hz & 2.0 Hz)
2	64.6	0.95	brd (J= 5.0 Hz)
3	33.4		
4	41.0	1.50-1.05	m
5	22.5	1.50-1.05	m
6	35.1	1.50-1.05	m
7	50.0		
8	44.0	1.52	brd (J= 5.0 Hz)
9	30.3	1.69	m
		1.16	m

10	26.3	1.87	m
		1.17	m
11	51.3		
12	13.0	0.85	s
13	22.7	0.82	s
14	29.2	0.92	s
15	28.9	0.92	s

The third compound, on the other hand, was present in the fraction eluted with 50 % ether with light petroleum. Its ^{13}C NMR spectrum clearly showed that it was an amorphane/muurolane(cadinane) type sesquiterpenoid **(23)** and its molecular formula was $C_{19}H_{26}O_5$. The ^{13}C NMR spectrum revealed the presence of six methyl groups (including two acetates), one methylene group, six methine carbons (including two secondary oxygen bearing carbons and a vinyl carbon), a trisubstituted and a tetrasubstituted double bond, two acetate carbonyls and a ketonic carbonyl. The molecule is thus bicarbocyclic. The ^{1}H NMR spectrum showed *inter alia* a secondary methyl group, three deshielded methines, two of which overlapped, arising from the two secondary acetates and the vinyl proton, and four sharp methyl signals between 2.1 and 1.8 ppm. Two of these methyl signals must belong to the acetates while the other two must arise from vinyl methyls with little or no coupling. The data are consistent with a cadinane type structure with vicinal acetates attached to C-2 and C-3. The sharp deshielded methyl signals can be accommodated by the presence of an isopropylidene group at C-7 in conjugation with a ketone at C-6 as in structure **(23)**. Support for the structure was obtained from its COSY spectrum in which H-2 correlates with H-3 ; H-5 correlates with H-15 and H-6 ; H-6 correlates with H-5, H-15, and a little with H-3 , H-1 correlates with 2H-9. This compound was previously isolated by us[5].



(23)

^1H and ^{13}C NMR Spectrum of Compound (23)

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	28.3	2.55	m
2	72.8	5.23	overlapping with H-5
3	68.0	5.47	brd (J= 3.0 Hz)
4	131.3		
5	129.4	5.23	overlapping with H-2
6	42.3	3.71	m
7	133.6		
8	203.3		
9	50.4	2.42	dd (J= 15.0 Hz & 4.0 Hz)
		2.0	under the acetate signals
10	41.8	2.0	under the acetate signals
11	143.7		
12	23.2	2.04	s
13	22.0	1.83	s
14	19.8	1.04	d (J= 6.0 Hz)
15	20.9	1.63	brs
2(OAc)	21.9	2.1	s
	21.1	2.01	s
	170.3		
	170.1		

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Chapter-3

Piper chaba

INTRODUCTION

The Piperaceae is a family of flowering plants. It is a tropical family of five genera and ca. 3000 species. *Peperomia* and *Piper* especially are richly represented in tropical forests, particularly in South Asia and tropical America. The family, Piperaceae, ranges from shrubs, lianas, often spindly trees to vigorous climbers and terrestrial or epiphytic herbs[1]. Their leaves vary in size from 2 mm to up to 70 cm in length, and can be round, ovate, oblong, elliptic, cordate or sagittate. The fruits are drupaceous or berry-like, often fleshly at maturity. These fleshly mature fruits of some species are eaten by bats.

The secondary metabolism of the Piperaceae is highly diversified. Ethereal oil cells contain monoterpenes, sesquiterpenes and phenylpropanes in more or less equal proportions[1]. The more characteristic compounds include simple neolignans, a furfuran derivate of a common lignan, cinnamoyl amides, α-pyrones, and highly oxygenated and hence rare aporphine derivatives[1]. Methylenedioxy substitution of aromatic rings is frequent. Chemically, the Piperaceae are typical members of the Ranalean complex[1]. Recently, the structures of lignans and neolignans have been summarized by Jensen *et al*[2].

The genus *Piper* in the Piperaceae family is composed of approximately 2000 species distributed primarily in tropical regions[3]. A number of *Piper* species are noted for their ethnomedical properties, of which the reputed stimulant, carminative, diuretic and diaphoretic activities of *Piper nigrum* are probably the best known. *P. nigrum* is also known as the most economically important species, and many varieties are cultivated for the production of peppercorns, used for flavouring[1]. *Piper longum*, the long pepper from India, is mainly used locally, but the commercially grown culinary pepper is taken only from *P. nigrum*[1]. *Piper darienense* from Central America has fruits with a clove-like flavour and sometimes it is used as an effective painkiller for toothache[1]. In Thailand the plant *Piper sarmentosum* Roxb., also known as 'Cha-plu', and its fruit are used as an expectorant[5]. In the Malay and Indonesian Archipelago, the leaves and the roots of this species have been reported to provide an effective remedy for toothache, fungoid dermatitis on the feet, coughing asthma and pleurisy. The species *P. sarmentosum* has exhibited *in vitro* activity in the reduction of blood sugar in alloxan diabetic rabbits[1]. Other species of *Piper* have been used locally for medicinal purposes, for brewing stimulating drinks or as bait for fishing[1].

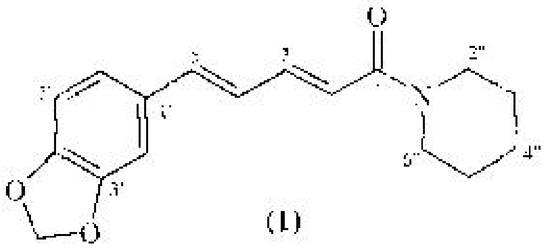
The *Piper* species examined by us is called *Piper chaba*. It is a climbing glabrous creeper, cultivated in various parts of India and Malaya Islands[4]. Its roots and fruits find numerous applications in medicine, and are particularly useful in asthma, bronchitis, fever, and pain in abdomen, as stimulant and in haemorrhoidal effections[4]. It is used as an insecticide. Because *Piper* species are capable of producing physiologically active compounds, they have attracted considerable interest[5]. Piperine (1) is the characteristic compound of the species and it was first isolated by Oersted in 1819 from the fruits of *P. nigrum*[6].

Earlier investigations[7] of *Piper* species led to the isolation of several alkaloids, the structures of which contain a piperidine moiety. These include piperettine[8] (2) from *P. nigrum*, piperovatine[9] (3) from *P. ovatum*. The physiological action of piperovatine has been investigated by Cash, and he found it to act as a temporary depressant of both motor and sensory nerve fibres, and also of sensory nerve terminations, producing some anaesthesia[9]. The recent work done on *P. nigrum* by Siddiqui *et al*[10] resulted in the isolation of a new amide, pipericine, the *N*-isobutyl amide of octadeca-2E,4Z-dienoic acid (4), along with piperine (1).

Two alkaloids, piperlongumine (5) and piperlonguminine (6) have been isolated by Chatterjee *et al*[7] from the root of *Piper longum* L. Previous work on the same plant gave an alkaloid amide, piplartine[11] (7). Recently, examination of the fruits of this plant by Tabuneng *et al*[12] afforded two piperidine alkaloids, pipernonaline (8) and piperundecalidine (9). The crude drug "Piperis Longi Fructus" is widely used as an anodyne and a treatment for stomach disease in China[12].

The amide $\Delta^{\alpha,\beta}$-dihydropiperine (10) has been isolated from the wood of *Piper novae-hollandiae* by Loder *et al*[13]. The species is a rain-forest vine, found in New South Wales and Queensland. It is reputed to contain a medicinally active principle, which was used in the treatment of gonorrhoea[13].

From the seeds of *Piper sylvaticum*, Banerji *et al*[14] isolated an alkamide, sylvatine (11), in addition to 4′, 7-dimethoxy-5 hydroxyflavone (12). The roots of *P. sylvaticum* Roxb. are widely used as an effective antidote to snake poison in the indigenous system of Indian medicine[15]. Some flavones and chalcones like flavokawain[14], N-pyrrolidinyl eicosa-2E,4E-dienamide[15] and lignans such as cubebin[16] (14) and sesamin[17] (15) were previously isolated from different *Piper* species.



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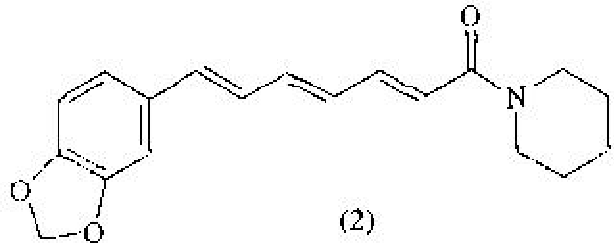

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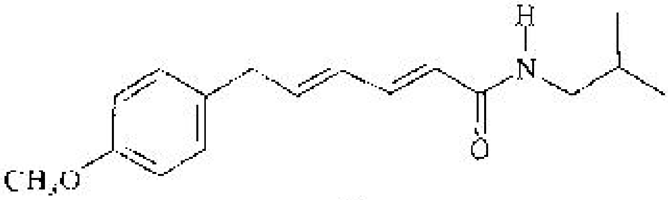

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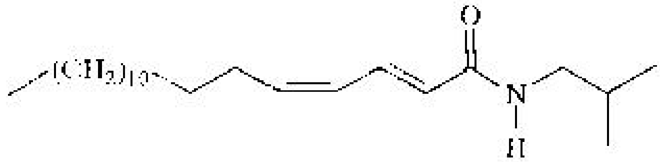

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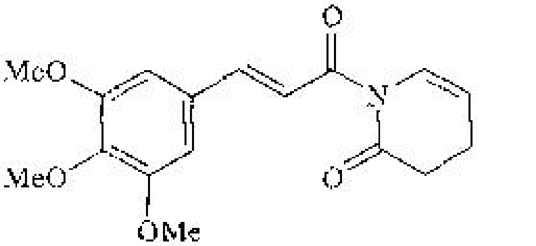

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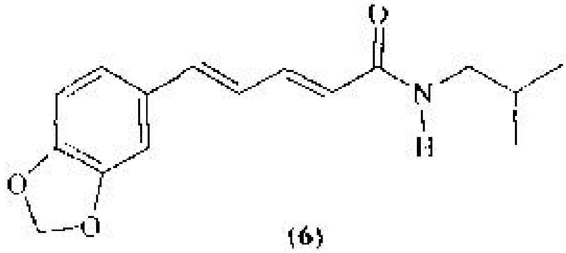

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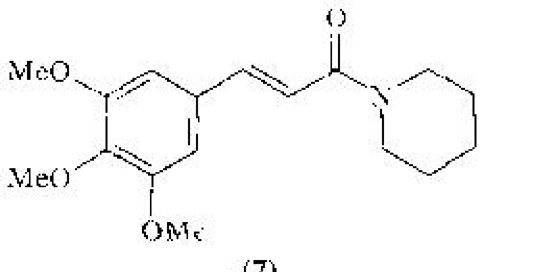

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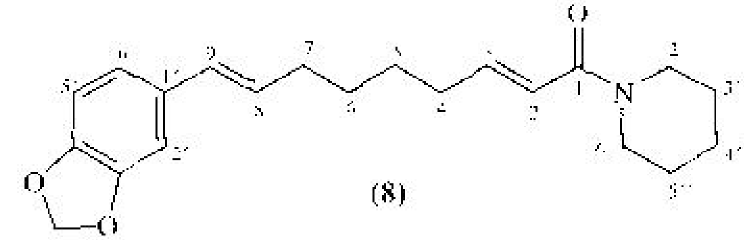

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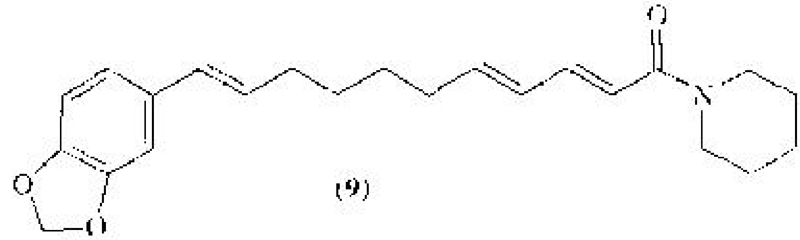

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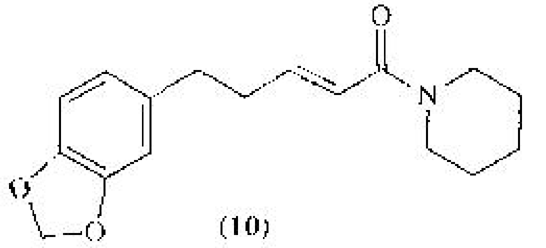

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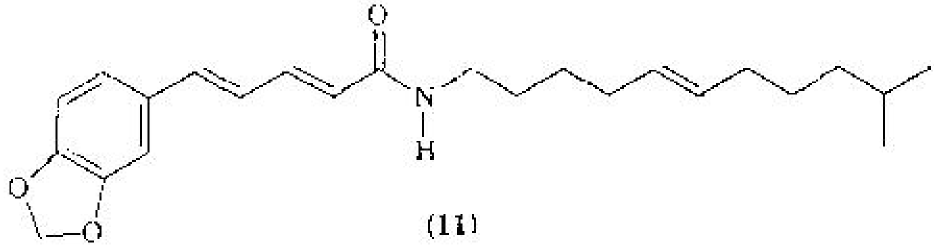

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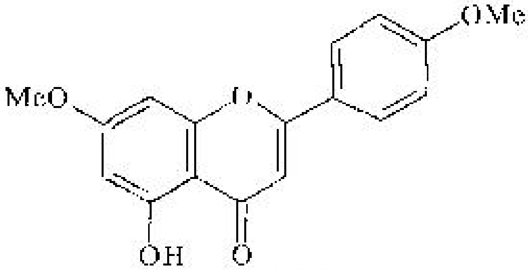

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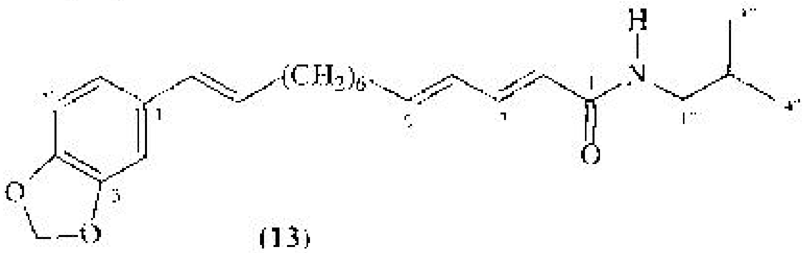

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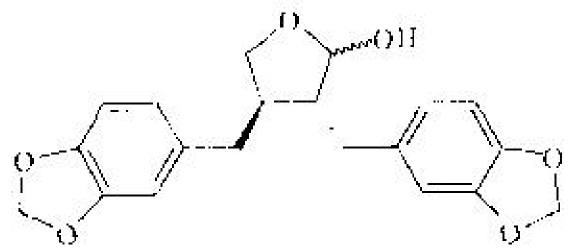

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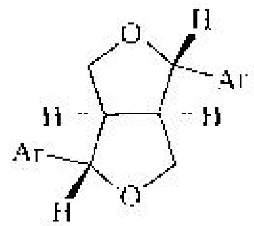

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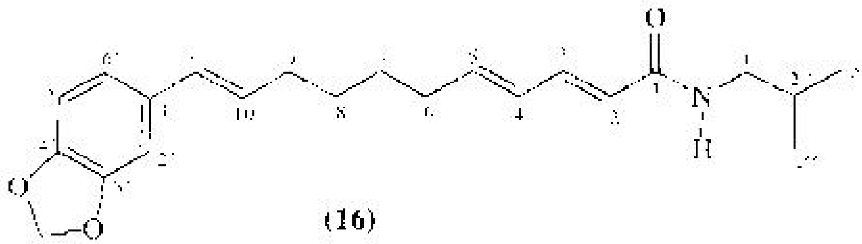

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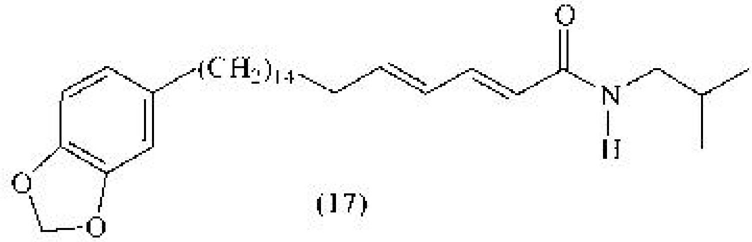
The occurrence of sylvatine (**11**) and piperlonguminine (**6**) in the species of *Piper chaba* Hunter was first proved by Patra *et al*[18] in 1974.

The examination of *Piper guineense* by Okogun[19] yielded the isobutylamides of 13-(3,4-methylenedioxyphenyl)-2,4,12-tridecatrienoic (**13**), 13-(3,4-methylenedioxyphenyl)undeca-2,4,12-trienoic (**16**), eicosa-2,4-dienoic (**17**), octadeca-2,4-dienoic (**18**), and hexadeca-2,4-dienoic (**19**) acids as well as the known amides piperine (**1**), trichostachine (**20**), piperlonguminine (**6**). *P. guineense*, West African Black Pepper or Ashanti Pepper, is widely distributed throughout West Africa. The fruits have been used as a flavorant while preparations of leaves, roots and seeds have been used internally as medicinal agents for the treatment of bronchitis, gastrointestinal disease and upset, venereal disease and rheumatism. It is also used as a spice in foods[20,19]. Some other work carried on *P. guineense* resulted in isolation of an *iso*butylamide, N-*iso*butyloctadeca-*trans*-2-*trans*-4 dienamide (**21**)[20] from the seeds of this plant, and two novel amide alkaloids , wisanine[21] (**22**) and wisanidine[21] (**23**), from its roots.

The roots of the shrub *P. callosum* have been examined by Pring[22], resulting in the isolation of piperovatine (**3**) [N-isobutyl-6-(p-methoxyphenyl) sorbamide] and two amides, the isobutylamides of (2E,4E)-9-(3,4-methylenedioxypheny)-nona-2,4-dienoic acid and (E)-7-(3,4-methylenedioxyphenyl) hept-2-enoic acid, named pipercallosine[22] (**24**) and pipercallosidine[22] (**25**) respectively.

Piper officinarum is found in Indonesia and used in the Ayuverdic system of medicine. The first examination of this species was carried out by Gupta *et al*[23] and methyl piperate (**26**) was isolated for the first time from the fruits of this plant. Later, Gupta and his colleagues reported N-isobutyl-trideca-13-(3,4-methylenedioxyphenyl)-2,4,12-trienamide[24] (**13**), and filfiline[25] (**27**) from the same source. In 1985, Banerji *et al*[26] examined the total above-ground parts of *Piper retrofractum* Vahl. (syn. *P. chaba* Hunter and *P. officinarum* PC) and found two unsaturated amides : retrofractamides A (**28**) and C (**29**). Later work on the same plant resulted in isolation of two new piperidine alkaloids, piperoctadecalidine[27] (**30**) and pipereicosalidine[27] (**31**).

Extraction of the stems and leaves of *Piper ridleyi* afforded ridleyamide[28] (N-isobutyl-15-(3',4'-methylenedioxyphenyl)-2E,4E,12E-pentadecatrienamide) (**32**). The examination of another species, *P. tuberculatum* resulted in a piperidine alkaloid piperdardine[29] (**33**). The plant *P. tuberculatum* is used in the Brazilian state of



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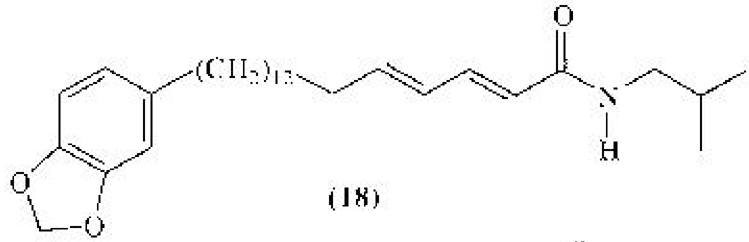

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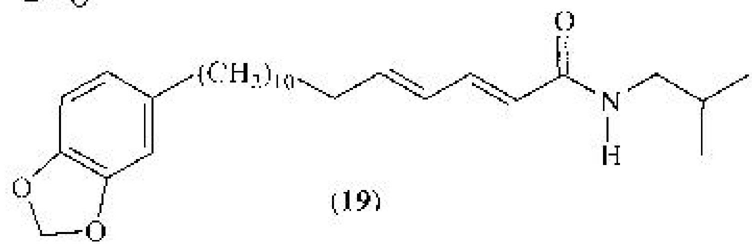

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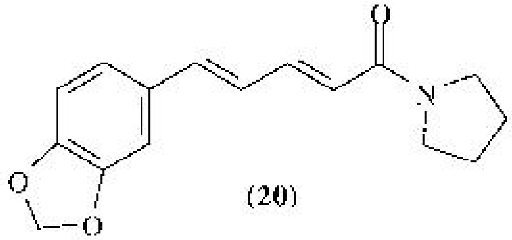

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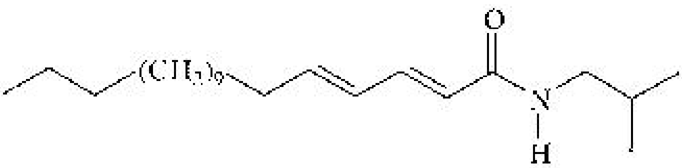

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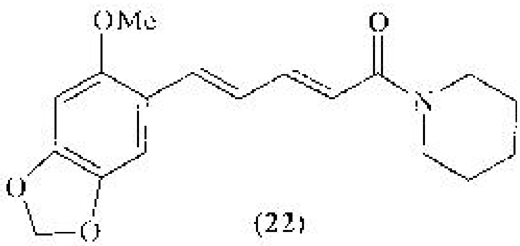

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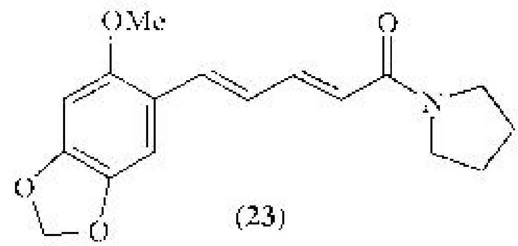

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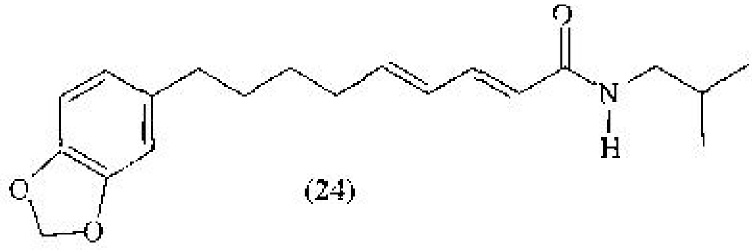

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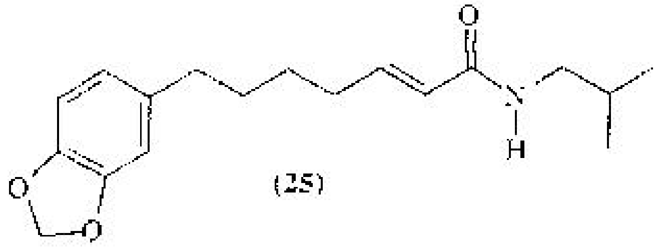

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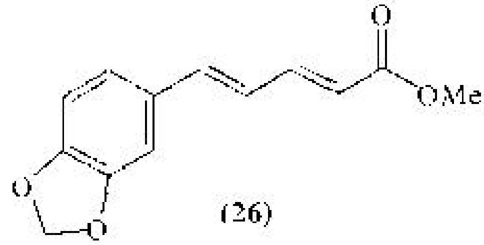

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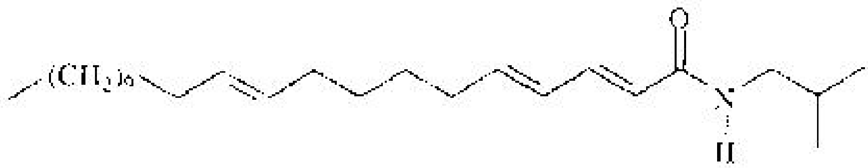

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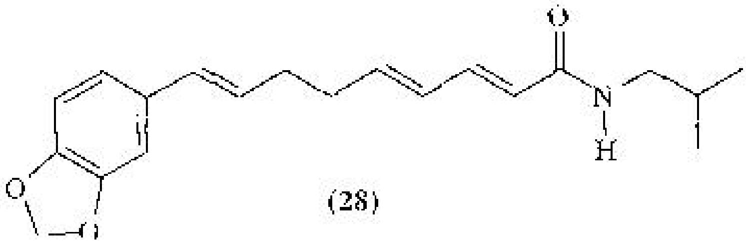

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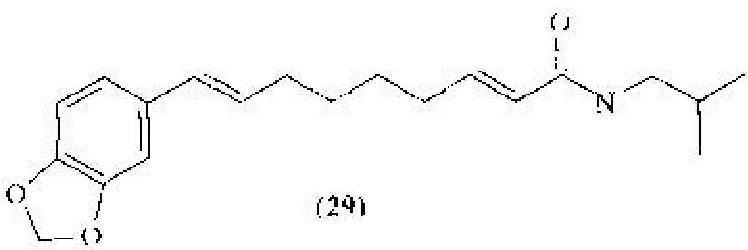


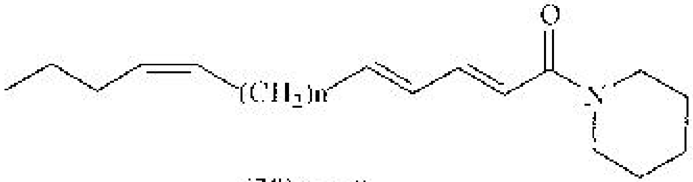
(29)

Paraiba as a sedative and as an antidote for snake-bite and is locally known as " pimenta d'arda"[29].

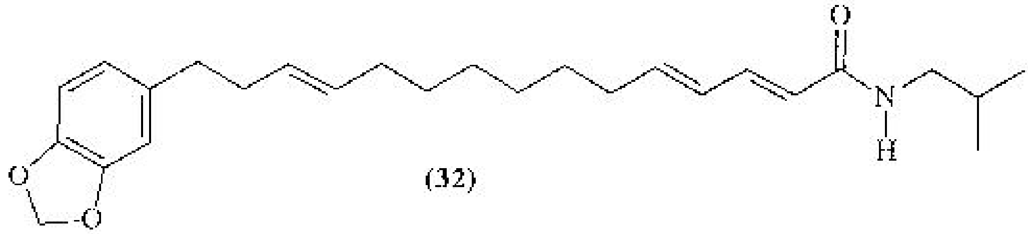
Two new alkaloids named piperlactam S[30] (**34**), piperine S[30] (**35**) and a new natural product pubenillumine[30] (**36**) have been isolated from the stem and leaves of *P. puberulum*. Previous workers on *P. puberulum* reported the presence of lignans[30]. This plant is widely used in Chinese herbal medicine for the treatment of arthritic conditions[30].

Parmar *et al*[31] have examined twelve *Piper* species (*P. khasiana, P. manii, P. pedicellosum, P. thomsoni, P. acutisleginum, P. aduncum, P. attenuatum, P. betle, P. brachystachyum, P. falconeri, P. longum and P. peepuloides*) and isolated thirty eight compounds, of which 2,6-dimethoxy-4-(2-propenyl) benzene (**37**), β-sitosteryl palmitate (**38**) and turacridone (**39**) were reported for the first time from the genus *Piper*, and 2-acetoxy-1,3-dimethoxy-5-(2-propenyl)-benzene (**40**), 14-benzo[1,3]dioxol-5-yl-tetradecan-2-ol (**41**) and 3-(3,4-dimethoxyphenyl)-propanoylpyrrole (**42**) were new compounds.

Another investigation has been carried out by Maxwell *et al*[32] on the aerial parts of *Piper aequale*, and they have isolated ten benzofuranoid neolignans, three [(**43**), (**44**), (**45**)] of which are new natural products.



(30) n = 8
(31) n = 10



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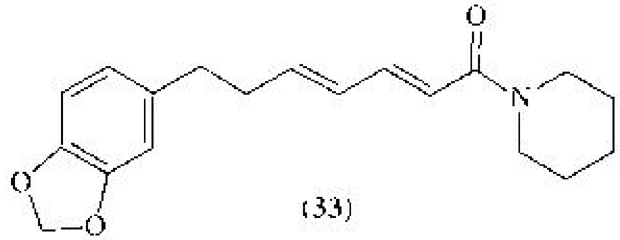

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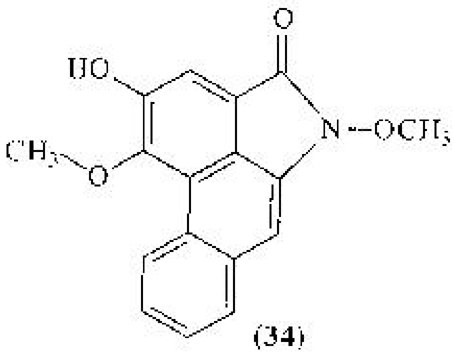

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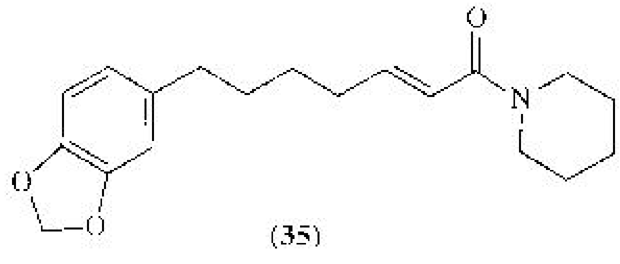

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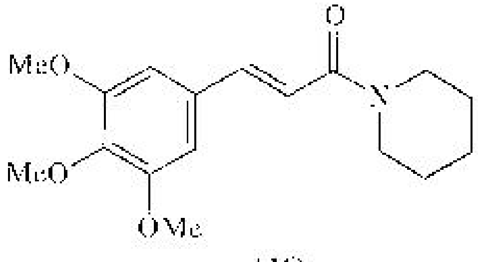

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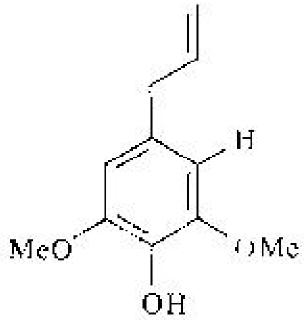

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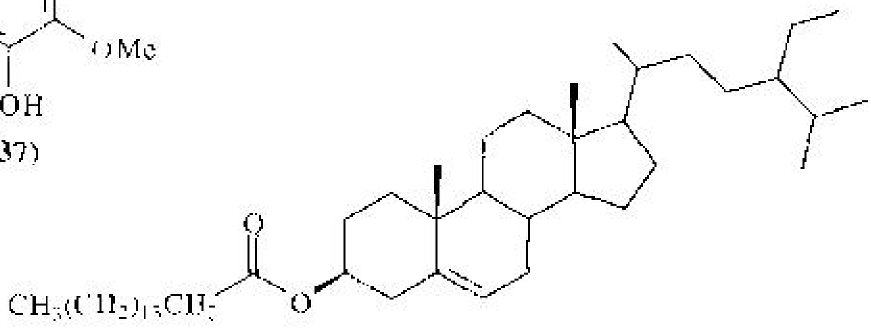

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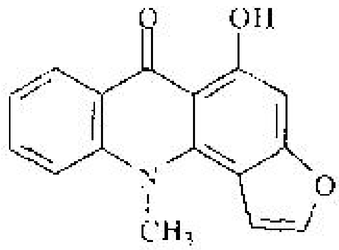

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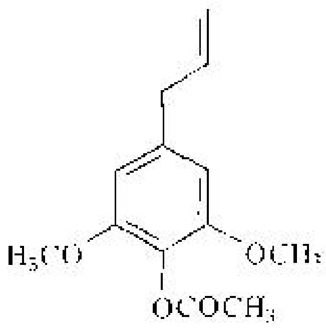

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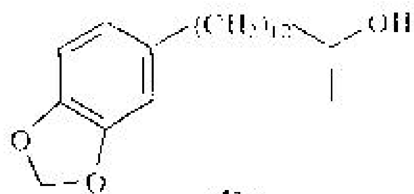

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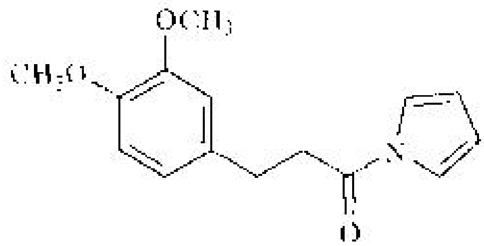

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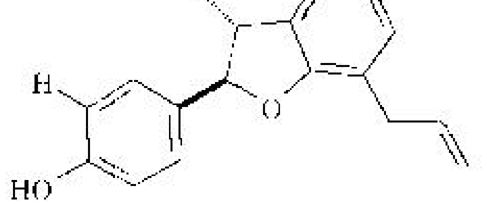

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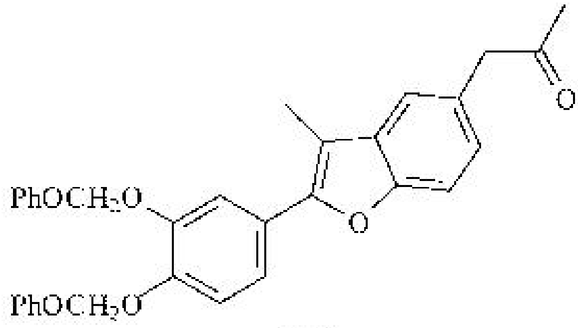

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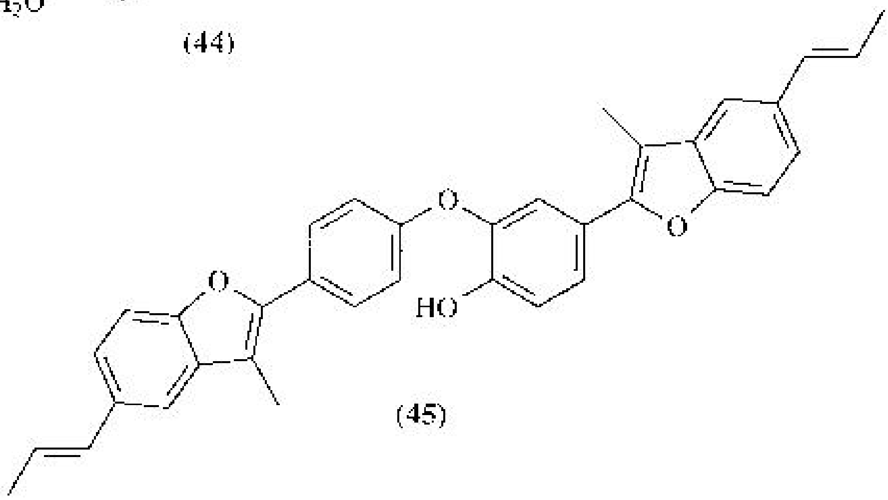
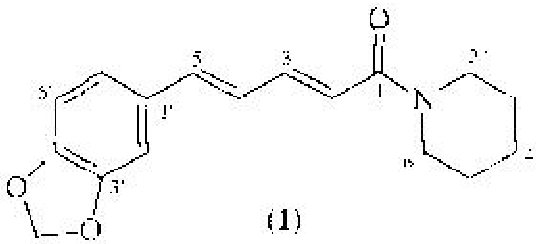


(45)

RESULTS AND DISCUSSION

Several fractions of the crude extract of *Piper chaba* were sent to us by a Chinese colleague. One fraction (3.32 g.) was examined by analytical TLC. Under U.V. light it showed a distinct spot, which seemed to be almost pure. The examination of the sample by spectroscopic methods led us to assign the structure as that of piperine (1). The formula, $C_{17}H_{19}NO_3$, was readily apparent from its ^{13}C NMR spectrum, which was identical with literature data[6,10,11,12]. The ^{13}C NMR spectrum showed five methylene groups which belong to the piperidine ring, two double bonds, one aromatic ring, a methylenedioxy group and a carbonyl group. In its ^{1}H NMR spectrum, the hydrogens [pip-H(2) and pip-H(6)] of the piperidine ring, which are next to the nitrogen, were clearly visible at around 3.48 and 3.57 ppm. The other methylene protons in the piperidine ring appeared at 1.54 as a broad singlet. The two double bond hydrogens, overlapping multiplets, were at 6.38 and 7.36 ppm, while the aromatic ring hydrogens appeared between 6.5 ppm and 7.0 ppm. The other two vinyl hydrogens (γ- and δ-) were overlapping with one of the aromatic ring protons, Ar-H(5), at δ_H 6.73. In the ^{1}H NMR spectrum, apart from these signals, there were some others (appearing as multiplets), at 0.78 ppm and between 7.1 and 7.3 ppm, which clearly belong to minor impurities.



(1)

δ_H ; 6.93 ppm [Ar-H(2), d, J= 1.5 Hz] ; 6.84 ppm [Ar-H(6), dd, J= 6.0 and 1.5 Hz] ; 6.38 ppm (α-H, d, J= 14.7 Hz) , 7.36 ppm (β-H, ddd J= 14,7, 8.25 and 1.89 Hz.) ; 3.48 ppm [pip-H(2), s, br] ; 1.54 ppm [pip-H(3), (4), (5), br] ; 3.57 ppm [pip-H(6), s, br] ; 5.9 ppm [methlenedioxy].

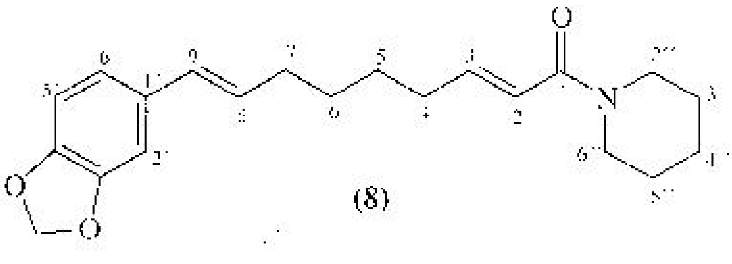
δ_C ; 130.9 ppm [Ar-C(1)] ; 105.6 ppm [Ar-C(2)] ; 148.06 ppm [Ar-C(3)] ; 148.09 [Ar-C(4)] ; 108.4 ppm [Ar-C(5)] , 122.5 ppm [Ar-C(6)] , 119.7 ppm [α-C] ; 142.8 ppm [β-C] ; 125.2 ppm [γ-C] ; 138.4 ppm [δ-C] ; 165.6 ppm [carbonyl] ; 43.3 ppm [pip-C(2)] ; 26.6 ppm [pip-C(3)] ; 24.5 ppm [pip-C(4)] ; 25.5 ppm [pip-C(5)] ; 46.9 ppm [pip-C(6)] ; 101.2 ppm [methylenedioxy]

Most of the remaining fractions showed the same TLC profiles. They were combined and subjected to flash chromatography. The flash chromatography gave us many fractions and the further purification of these fractions by preparative TLC afforded these compounds in pure form.

The fraction eluted with 60% ethyl acetate and petroleum ether (40-60^0) was subjected to preparative TLC with the eluent 35 % ethyl acetate and light petroleum. It showed two bands under U.V. light. Examination of the spectral data showed these two compounds to be the piperidine alkaloid pipernonaline (**8**) and the isobutylamide of 13-(3,4-methylenedioxyphenyl) undeca-2,4,12-trienoic acid (**16**).

The molecular formula of compound (**8**), $C_{21}H_{27}NO_3$, was readily apparent from the ^{13}C N.M.R. spectrum (Table 1). The mass spectrum supported the molecular formula, with a parent ion peak at 341 m/z (M^+) and the fragment at m/z 84 arising from the piperidine ring. The ^{13}C N.M.R. spectrum revealed the presence of a carbonyl group (amide carbonyl) at 165.5 ppm, which is shielded by the electron releasing effect of the nitrogen on the piperidine ring, and a methylenedioxy group at 100.9 ppm. The methylene carbons on the piperidine ring adjacent to nitrogen were seen at 43.0 and 46.8 ppm. The signal of the C-4′′methylene carbon was a sharp singlet at 24.6 ppm while the C-3′′ and C-5′′ methylenes appeared at 26.6 and at 25.5 ppm. The quaternary carbon (C-1′) of the aromatic ring was at 132.3 ppm and the other two (C-3′ and C-4′) adjacent to the oxygens of the methylenedioxy group were seen at 146.5 and 147.9 ppm. There were seven methine carbons, three of which belong to the aromatic ring and the other four must be the olefinic protons. Therefore, apart from an aromatic ring, two double bonds must be present in the compound. The ^{13}C N.M.R. shifts were identical with those reported in the literature[72].

The ^{1}H N.M.R. spectrum (Table 1) clearly showed the two broad signals belonging to the two methylene protons on the piperidine ring next to nitrogen at 3.46 and 3.58 ppm. A clear sharp singlet at 5.91 ppm was assigned to the protons of the methylenedioxy group, which are deshielded by the electron withdrawing effect of the two oxygen atoms. The aromatic proton 2′ was seen at 6.87 ppm as a broad doublet due to a slight splitting (J= 1.1 Hz) with the aromatic proton 6′. Because the coupling constant between H-2′ and H-5′ is too small, we cannot see the splitting pattern of this coupling. Instead a slight broadening of the signal was observed. The aromatic protons H-5′ and H-6′ on the other hand appeared at 6.72 ppm. The coupling between H-5′ and H-6′ resulted in the aromatic H-5′ appearing as a doublet (J=8.0 Hz) at 6.71 ppm. H-6′ on the other hand was a doublet of doublets at around 6.74 ppm. The couplings of H-6′ with both aromatic protons H-5′ and H-2′ were clearly observed (J= 8.0 Hz and 1.5 Hz). Thus the aromatic ring was a 1,3,4 substituted benzene ring. In the ^{1}H N.M.R. spectrum two allylic methylene protons (H-4 and H-7) appeared as a multiplet at around 2.18 ppm. Thus the two double bonds in our structure are well separated, one of which is in conjugation with the carbonyl group and the other is in conjugation with the aromatic ring. The carbonyl is a π acceptor group and hence it withdraws the electrons of the double bond, causing a deshielding effect on both the α and β protons. However the effect at β position is larger. Therefore the β proton was seen at 6.81 ppm as a dt (J= 15.0 and 7.0 Hz) and the α proton at 6.22 ppm, dt [J= 15.0 and 1.5 Hz (allylic coupling with H-4)]. The coupling constant (15.0 Hz) shows that the α and β protons are trans to each other. The other double bond protons next to the aromatic ring were seen at 6.27 ppm [H-9, dt (J= 15.4 Hz and a slight broadening arising from the effect of allylic protons H-7)] and at 6.10 ppm [H-8, dt (J= 15.7 and 7.0 Hz)]. The large vicinal coupling constants showed that these protons are also trans to each other. Finally the 2D spectra, COSY (Table 2) and HMQC (Table 3), supported the structure as that of pipernonaline (8). The same compound, pipernonaline, was also present in the fraction eluted with 70% ethyl acetate and light petroleum.



(8)

Table 1, ^{13}C and ^{1}H NMR Spectra of Compound (8)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	165.5		
2	120.5	6.22	dt (J= 15.0 Hz, 1.5 Hz)
3	145.5	6.81	dt (J= 15.0 Hz, 7.0 Hz)
4*	32.6	2.18	m, overlapping with H-7
5**	27.9	1.48	m, overlapping with H-6
6**	28.9	1.48	m, overlapping with H-5
7*	32.3	2.18	m, overlapping with H-4
8	128.8	6.01	dt (J= 15.7 Hz, 7.0 Hz)
9	129.5	6.27	dt (J= 15.4 and a slight broadening)
1′	132.3		
2′	105.3	6.87	d (J= 1.1 Hz)
3′	146.5		
4′	147.9		
5′	108.2	6.71	d (J= 8.0 Hz)
6′	120.2	6.74	dd (J= 8.0 Hz, 1.5 Hz)
-OCH$_2$O-	100.9	5.91	s
2″	43.0	3.58	brs
3″	26.6	1.55	m, overlapping with H-5″
4″	24.6	1.63	m
5″	25.5	1.55	m, overlapping with H-3″
6″	46.8	3.46	brs

* Maybe interchanged

**Maybe interchanged

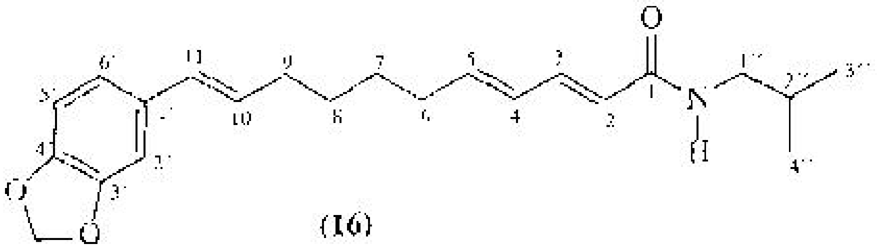
Table 2, COSY Correlations of Compound **(8)**

H-5 and H-6	H-4, H-7
H-3″ and H-5″	H-2″ and H-6″
H-4″	H-3″ and H-5″
H-7 and H-4	H-5, H-6, H-8, H2 and H-3
H-6″	H3″, H5″ and H-2″
H-2′	H-5′ and H-6′
H-9	H-8, H-7

Table 3, HMQC Correlations of Compound **(8)**

δ_C	C	δ_H	No.
120.5	2	6.22	1H
145.5	3	6.81	1H
32.6	4	2.18	2H
27.9	5	1.48	2H
28.9	6	1.48	2H
32.3	7	2.18	2H
128.8	8	6.10	1H
129.5	9	6.27	1H
105.3	2′	6.87	1H
108.2	5′	6.71	1H
120.2	6′	6.74	1H
43.0	2″	3.58	2H
26.6	3″	1.55	2H
24.6	4″	1.63	2H
25.5	5″	1.55	2H
46.8	6″	3.46	2H

The other compound obtained from fraction 60 was the isobutylamide of 1,3-(3,4-methylenedioxyphenyl)undeca-2,4,12-trienoic acid (**16**). Its empirical formula, $C_{22}H_{29}NO_3$, was apparent from the ^{13}C NMR spectrum (Table 4). The mass spectrum showed a parent ion peak at 355 m/z (M^+) and another peak at 57 m/z for the isobutyl fragment. This time no piperidine ring moiety was present in the ^1H NMR spectrum (Table 4). Instead, an isobutyl moiety was observed, showing one methylene (2H-1″) at 3.15 ppm, dd (J= 6.6 and 6.3 Hz), one methine (1H-2″) at 1.78 ppm, m, and two methyl protons (3H-3″ and 3H-4″) at 0.91 ppm, d (J= 6.7 Hz). The ^1H NMR spectrum (Table 4) was quite similar to that of Guineensine (**13**)[19]. The olefinic proton, α to the carbonyl, was seen at 5.74 ppm as a doublet (J= 15.0 Hz), only coupling to the proton in the β position. The β proton was shifted downfield to around 7.18 ppm and observed as a doublet of doublets (J= 15.0 and 10.2 Hz). The proton H-11 was observed at 6.27 ppm as a doublet (J= 16.0 Hz). The other olefinic protons H-10, H-4, and H-5 were overlapping and seen as multiplets around 6.07 ppm. The olefinic protons showed a coupling constant of around 15.0 Hz, thus they must be trans to each other. The aromatic proton H-5′ was at 6.72 ppm [d (J= 8.0 Hz)], H-6′ at 6.75 ppm [dd (J= 8.0 and 1.5 Hz)], and H-2′ at 6.87 ppm [d (J= 1.1 Hz)]. The two allylic methylene protons H-9 and H-6 were observed at 2.17 ppm, as a multiplet. Another multiplet seen at 1.45 ppm belonged to the methylene protons in the chain (H-7 and H-8). The ^{13}C NMR spectrum revealed the presence of an amide carbonyl at 166.3 ppm, and six methylene carbons, one of which is the methylenedioxy at 100.9 ppm and another one is the methylene carbon (1″) at 46.9 ppm. The other four methylenes were in the region between 25 ppm and 35 ppm. Nine methine carbons were observed, three of which belong to the aromatic ring, and thus six olefinic protons were present in the structure. Because they are both chemically and magnetically equal, the two isobutyl methyl carbons appeared as one signal at 20.1 ppm. This is a characteristic chemical shift for the isobutyl methyl groups. Finally the HMQC spectrum (Table 5) supported the structure.



(16)

Table 4, ^{13}C and ^{1}H NMR Spectra of Compound **(16)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	166.3		
2	121.8	5.74	d (J= 15.0 Hz)
3	141.2	7.18	dd (J= 15.0 Hz, 10.1 Hz)
4	128.4	6.07	m (overlapping with H-5 and H-10)
5	129.0	6.07	m (overlapping with H-4 and H-10)
6**	32.7	2.17	m (overlapping with H-9)
7*	29.0	1.45	m (overlapping with H-8)
8*	28.3	1.45	m (overlapping with H-7)
9**	32.8	2.17	m (overlapping with H-6)
10	142.8	6.07	m (overlapping with H-5 and H-4)
11	129.5	6.27	d (J= 16.0 Hz)
1′	132.3		
2′	105.4	6.87	d (J= 1.1 Hz)
3′	147.7		
4′	146.5		
5′	108.2	6.72	d (J= 8.0 Hz)
6′	120.2	6.75	dd (J= 8.0 Hz, 1.5 Hz)
-OCH$_2$O-	100.9	5.91	s
1″	46.9	3.15	dd (J= 6.6 Hz, 6.3 Hz)
2″	28.6	1.78	m
3″, 4″	20.1	0.91	d (J= 6.7 Hz)

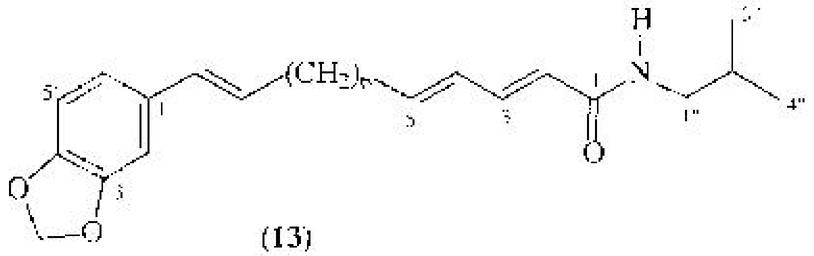
Table 5, HMQC Correlations of Compound **(16)**

δ_C	C	δ_H	No.
121.8	**2**	5.74	1H
141.2	**3**	7.18	1H
128.4	**4**	6.07	1H
129.0	**5**	6.07	1H
32.7	**6**	2.17	2H
29.0	**7**	1.45	2H
28.3	**8**	1.45	2H
32.8	**9**	2.17	2H
142.8	**10**	6.07	1H
129.5	**11**	6.27	1H
105.4	**2′**	6.87	1H
108.2	**5′**	6.72	1H
120.2	**6′**	6.75	1H
100.9	**-OCH$_2$O-**	5.91	2H
46.9	**1″**	3.15	2H
28.6	**2″**	1.78	1H
20.1	**3″, 4″**	0.91	6H

The analytical t.l.c. of fraction 50 (eluted with 50 % ethyl acetate/light petroleum) showed a few spots, and it was subjected to preparative TLC for purification with 20 % ethyl acetate and petroleum ether (40-60^0) as eluent. Under UV light, a main band was observed. It was extracted and its structure assigned on the basis of its spectroscopic properties as the isobutylamide of 13-(3,4-methylenedioxyphenyl)-2,4,12-tridecatrienoic acid **(13)**.

Compound **(13)** was previously isolated[19] and named as guineensine. The molecular formula, $C_{24}H_{33}NO_3$, was supported by a parent ion peak at 383 *m/z* (M^+)

in the mass spectrum. Its spectroscopic properties were very similar to those of **(16)**. The only difference, apart from the slight change in the chemical shifts, was the presence of two additional methylene groups in the chain, appearing at around 1.31 ppm. The ^1H and ^{13}C NMR shifts are given in Table 6. and the 2D spectra, COSY and HMQC are in Table 7, and Table 8.



(13)

Table 6. ^{13}C and ^1H NMR Spectra of Compound **(13)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	166.4		
2	121.7	5.73	d (J= 15.0 Hz)
3	141.3	7.18	dd (J= 15.0 Hz, 10.0 Hz)
4	128.2	6.06	m
5	129.3	6.06	m (overlapping with H-4 and H-12)
6**	32.8	2.14	m (overlapping with H-11)
7*	28.9	1.42	m (overlapping with H-10)
8+	28.7	1.31	m (overlapping with H-9)
9+	29.2	1.31	m (overlapping with H-8)
10*	29.0	1.42	m (overlapping with H-7)
11**	32.9	2.14	m (overlapping with H-6)
12	143.1	6.06	m (overlapping with H-4 and H-5)
13	129.3	6.27	d (J= 16.0 Hz)
1'	132.5		
2'	105.4	6.88	d (J= 1.2 Hz)
3'	147.9		
4'	146.6		

5′	108.2	6.73	d (J= 8.0 Hz)
6′	120.2	6.75	dd (J= 8.0 Hz, 1.4 Hz)
-OCH$_2$O-	100.9	5.92	s
1″	46.9	3.15	dd (J= 6.6 Hz, 6.4 Hz)
2″	28.6	1.79	m
3″, 4″	20.1	0.91	d (J= 6.7 Hz)

Table 7. COSY Correlations of Compound **(13)**

H-2	H-3
H-3	H-2
H-12	H-11
H-13	H-12
H-11 and H-6	H-7, H-10, H-12, H-5
H-7 and H-10	H-11, H-6, H-9, H-8
H-9 and H-8	H-7, H-10
H-1″	H-2″, and NH
H-2″	H-3″, H-4″, H-1″
H-3″, H-4″	H-2″
H-2′	H-6′, H-5′
H-6′ and H-5′	H-2′

Table 8. HMQC Correlations of Compound (**13**)

C	δ_H	No.
2	5.73	1H
3	7.18	1H
4	6.06	1H
5	6.06	1H
6	2.14	2H
7	1.42	2H
8	1.31	2H
9	1.31	2H
10	1.42	2H
11	2.14	2H
12	6.06	1H
13	6.27	1H
2′	6.88	1H
5′	6.73	1H
6′	6.75	1H
-OCH$_2$O-	5.92	2H
1″	3.15	2H
2″	1.79	1H
3″, 4″	0.91	6H

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Chapter-4

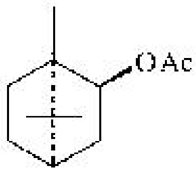
Conocephalum conicum

INTRODUCTION

The large thalloid liverwort *Conocephalum conicum*, belonging to the Marchantiales, produces an intense mushroom-like odour[1]. It has been used as a diuretic drug and against gallstones[1]. Among the monoterpenoids found in this liverwort (+)-bornyl acetate **(1)** and (-)-β-sabinene **(2)** are the major constituents. Other monoterpenoids are[1] ; myrcene **(3)**, linalyl acetate **(4)**, β-phellandrene **(5)**, α-terpinene **(6)**, γ-terpinene **(7)**, terpinolene **(8)**, α-terpineol **(9)**, terpinene-4-ol **(10)**, *p*-cymene **(11)**, α-pinene **(12)**, β-pinene **(13)**, and camphene **(14)**.

The sesquiterpenoids found in *C. conicum* are[1] : bicyclogermacrene **(15)**, δ-cadinene **(16)**, 10-epi-zonarene **(17)**, calamenene **(18)**, α-calacorene **(19)**, β-caryophyllene **(20)**, β-chamigrene **(21)**, α-elemene **(22)**, β-elemene **(23)**, α-selinene **(24)**, β-eudesmol **(25)**, tulipinolide **(26)**, zaluzanin C **(27)**, zaluzanin D **(28)**, 8α-acetoxyzaluzanin C **(29)**, 8α-acetoxyzaluzanin D **(30)**. The triterpenoid friedelin **(31)** has also been reported.

Recently, Suire[2] and his colleagues examined a Japanese sample of *C. conicum* and found a new monoterpene ester, bornyl 2-methoxy-4-hydroxycinnamate **(32)**, (+)-bornyl ferulate **(33)**, (-)-limonene **(34)**, (-)-β-sabinene **(35)**, (+)-bornyl acetate **(1)**, *ent*-sesquiterpenes (+)-bicycloelemene **(36)**, (+)-β-elemene **(23)**, (-)-bicyclogermacrene **(15)**, and lunularin (3,4'-dihydroxybibenzyl) **(37)**. Bicyclogermacrene-13-al **(38)**, a new sesquiterpene aldehyde, was found in *C. conicum* by Toyota *et al*[3]. A sesquiterpene alcohol, conocephalenol **(39)**, was isolated from Scottish *C. conicum* by Tori *et al*[4]. Valterova *et al*[5] found some monoterpene hydrocarbons in the essential oil of this liverwort. These were; α-thujene **(40)**, α-pinene **(12)**, camphene **(14)**, β-pinene **(13)**, myrcene **(3)**, α-terpinene **(6)**, β-phellandrene **(5)**, *p*-cymene **(11)**, γ-terpinene **(7)** and terpinolene **(8)**. Among these α-thujene **(40)**, has not been previously reported. A phenethyl glycoside, 2-(3,4-dihydroxyphenyl)ethyl-β–allopyranoside **(41)** was isolated from a Japanese collection of *C. conicum* by Toyota *et al*[6]. Two new monoterpene esters; bornyl *cis*-4-hydroxycinnamate **(42)** and (+)-bornyl *cis*-4-hydroxy-3-methoxycinnamate **(43)** were also found in this liverwort by Toyota *et al*[7]. Another sample of *C. conicum*, collected in Southern Germany, was examined by Melching *et al*[8]. The GC-mass spectra of a hydrodistillate of the liverwort revealed the presence of (+)-cadina-3,5-diene **(44)** as a natural constituent.



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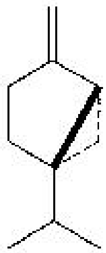

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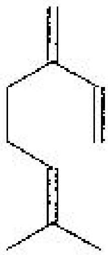

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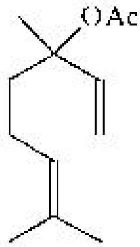

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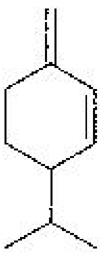

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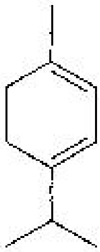

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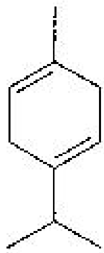

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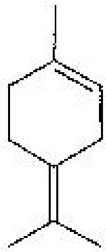

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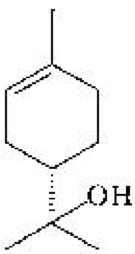

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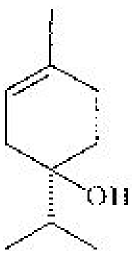

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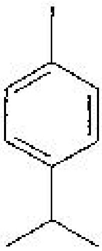

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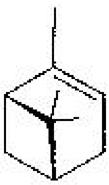

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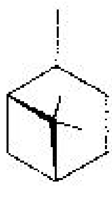

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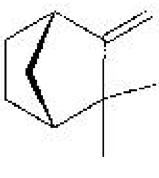

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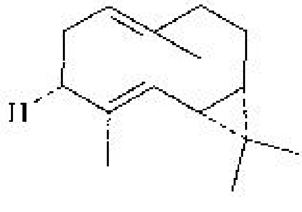

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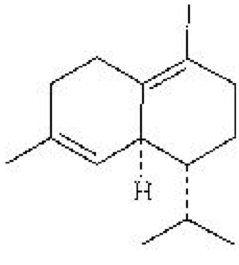

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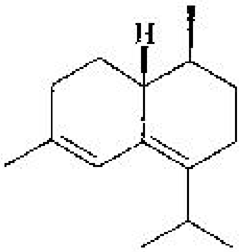

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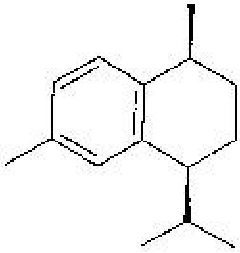

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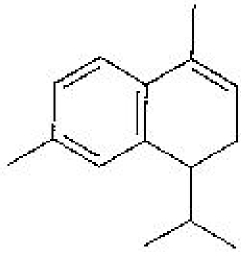

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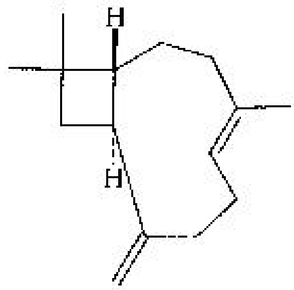

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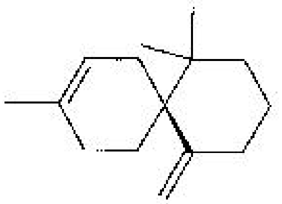

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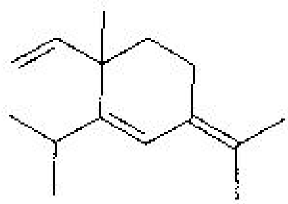

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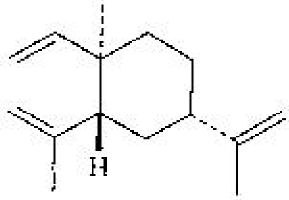

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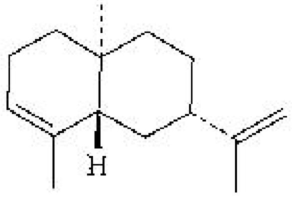

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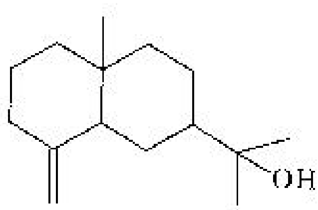

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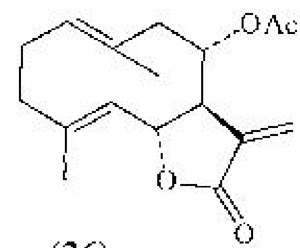

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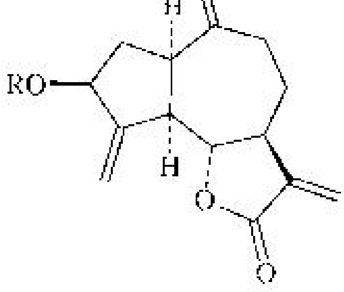
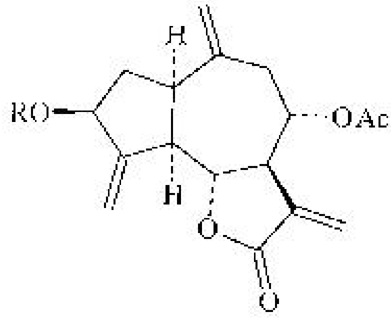
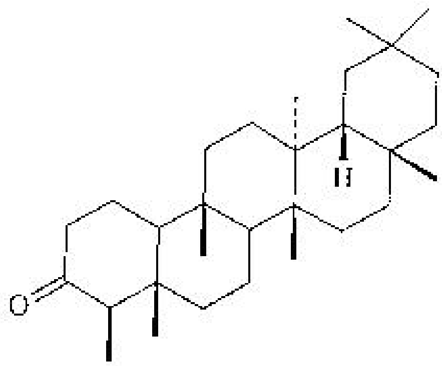


(27) R = H
(28) R = Ac



(29) R = H
(30) R = Ac



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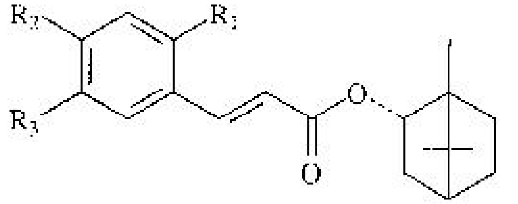
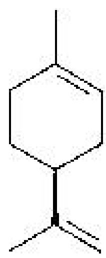


(32) R_1 = OMe, R_2 = OH, R_3 = H
(33) R_1 = H, R_2 = OH, R_3 = OMe



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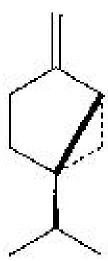

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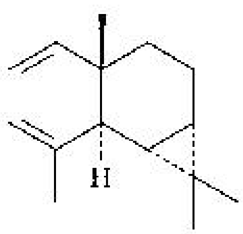

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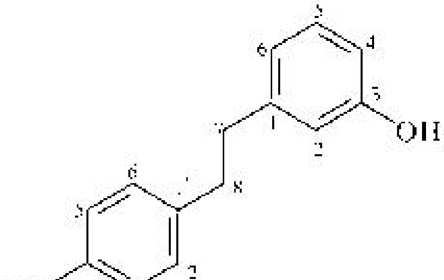

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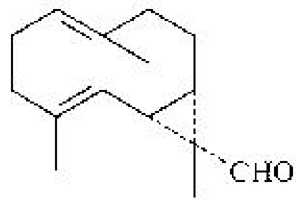

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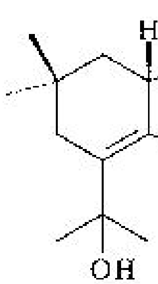

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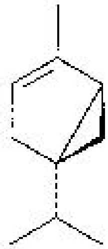

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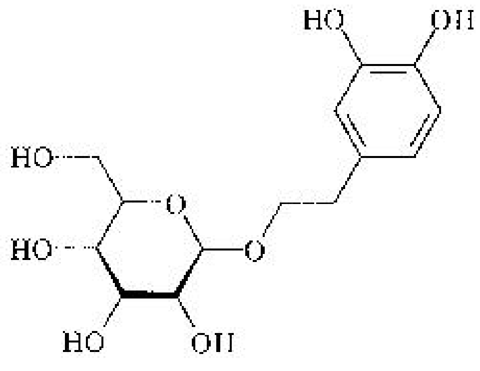

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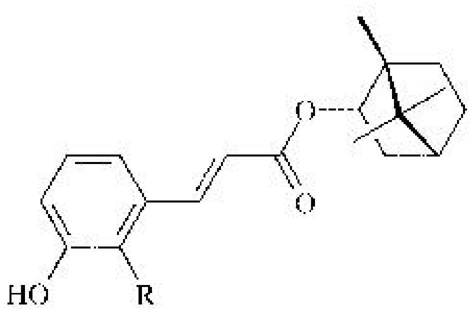
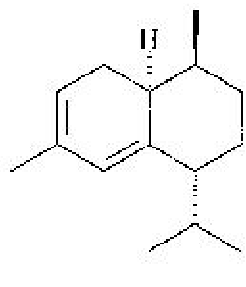


(42) R = H
(43) R = OMe



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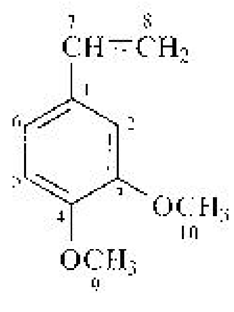


(45)

RESULTS AND DISCUSSION

Conocephalum conicum was collected in Ayrshire, near Mauchline. It was dried and powdered, and extracted with ethyl acetate by percolation over several hours. The crude extract (25g) was subjected to flash chromatography followed by preparative TLC and gel chromatography. The eluent for flash chromatography was light petroleum ether with increasing percentages of ethyl acetate. Unfortunately the analytical plates of the fractions did not show many UV or non-UV active spots, and because chlorophyll was the main compound in these fractions it was hard to see any clear spots.

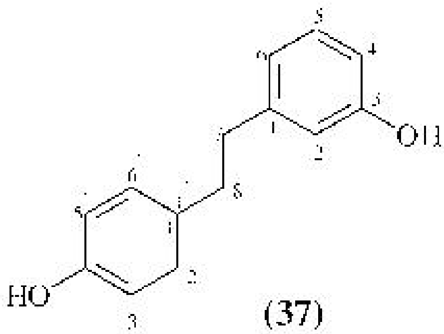
Fraction 60, eluted with 60% ethyl acetate and petroleum ether, was chromatographed on LH20, eluting with methanol and chloroform (1:1). The analytical plates of the several fractions gave a distinct spot under the UV light. Further purification was carried by preparative TLC with eluent 20% ethyl acetate and petroleum ether. Spectroscopic analysis of the compound showed it to be 3,4′-dihydroxybibenzyl (Lunularin) **(37)**, which was previously isolated by Suire *et al*[2]. It has also been found in the liverworts *Marchantia polymorpha*[7] and *Plagiochila stephensoniana*[?] (a New Zealand liverwort).

In its ^{13}C NMR spectrum (Table-1), four quaternary carbons [C-1(143.7 ppm), C-3(155.5 ppm), C-1′(133.9 ppm), C-4′(153.7 ppm)], two of which bear oxygen, two methylene carbons attached to the two aromatic rings[C-7[§](38.0 ppm), C-8 (36.8 ppm)] and six aromatic methine carbons [C-2(115.4 ppm), C-4(112.8 ppm), C-5′(129.5 ppm), C-6(121.0 ppm), C-2′′(129.5 ppm), C-3′(115.1 ppm)] were observed. One of the aromatic rings is 1,4 disubstituted, [C-2′&C-6′(129.5 ppm) and C-3′&C-5′(115.1 ppm)].

In the ^{1}H NMR spectrum (Table-1), the aromatic protons appeared between 6.6 ppm and 7.2 ppm. The triplet seen further downfield was ascribed to H-5(7.13 ppm, t, J= 7.7 Hz). The AA′BB′ system associated with H-2′,6′ and H-3′,5′ appeared at 7.02 ppm, d, J= 8.5 Hz and at 6.71 ppm, d, J= 8.5 Hz, and H-6 also appeared at 6.74 ppm, d, J= 8.5 Hz, overlapping with H-3′. The two other aromatic protons were seen at around 6.65 ppm: H-4(6.65 ppm, d, J= 7.9 Hz) and H-2(6.65 ppm, t, J= 1.5 Hz). Two methylene protons (2H-7 and 2H-8) were seen as a singlet at 2.82 ppm and the hydroxyl protons gave a singlet at 4.80 ppm. The ^{1}H NMR data are identical with those given in the literature[7,9].

According to its HMBC spectrum, the two methylene protons (2H-7 and 2H-8) had correlations with C-1, C-2, C-6, C-1', and C-2'. The other correlations in the HMBC spectrum of **(37)** are given in (Table-2). The correlations in the HSQC and COSY spectra of compound **(37)** are given in (Table-3) and (Table-4).

Finally the molecular formula $C_{14}H_{14}O_2$ was supported by the mass spectrum with a parent ion(M^+) peak at 214. Approximately 10mg lunularin **(37)** was obtained.



(37)

Table-1. ^{13}C and ^{1}H NMR Spectra of Compound **(37)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	143.7		
2	115.4	6.63	t (J = 1.8)
3	155.5		
4	112.8	6.65	d (J = 7.9, 1.5)
5	129.5	7.13	t (J = 7.7)
6	121.0	6.74	d (J = 8.5)
7	38.0	2.82	s
8	36.8	2.82	s
1'	133.9		
2', 6'	129.5	7.02	d (J = 8.5)
3', 5'	115.1	6.74	d (J = 8.5)
4'	153.7		
OH(2H)		4.8	s

Table-2. HMBC Correlations Of Compound (37)

δ_C	C	Type	H
143.7	**1**	quaternary	H-5, 2H-7, 2H-8
115.4	**2**	methine	H-4, H-6, 2H-7
155.5	**3**	quaternary	H-2, H-5
112.8	**4**	methine	H-2, H-5,H-6
129.5	**5**	methine	H-6
121.0	**6**	methine	H-2, H-4, 2H-7
38.0	**7**	methylene	H-6, H-2
36.8	**8**	methylene	H-3′, H-5′
133.9	**1′**	quaternary	H-3′, H-5′, 2H-8, 2H-7
129.5	**2′,6′**	methine	H-3′, 2H-8
115.1	**3′,5′**	methine	H-2′
153.7	**4′**	quaternary	H-2′, H-6′, H-3′

Table-3. HSQC Correlations of Compound (37)

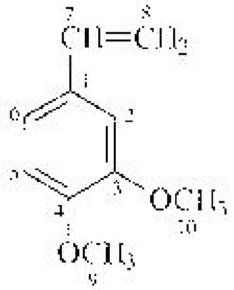
δ_C	C	δ_H	No
115.4	**2**	6.65	1H
112.8	**4**	6.65	1H
129.5	**5**	7.13	1H
121.0	**6**	6.74	1H
38.0	**7**	2.82	2H
36.8	**8**	2.82	2H
129.5	**2′,6′**	7.02	2H
115.1	**3′,5′**	6.74	2H

Table-4. COSY Correlations of Compound **(37)**

δ_H	H	TYPE	H
6.63	2	methine	H-4, H-5
6.65	4	methine	H-2, H-5, H-6
7.13	5	methine	H-6, H-4, H-2
6.74	6	methine	H-5, H-2, 2H-7, H-4
2.82	7	methylene	H-2, H-6, 2H-8
2.82	8	methylene	2H-7, H-2′
7.02	2′,6′	methine	2H-8, H-3′, H-5′
6.74	3′,5′	methine	H-2′

The other fraction 30, eluted with 30% ethyl acetate and petroleum ether, gave a UV active spot on an analytical plate. After purification by preparative TLC, its spectroscopic analysis showed that it was still a mixture of two compounds. We were able to recognize one of them as 3,4-dimethoxystyrene **(45)** from its ^{13}C and ^{1}H NMR spectra. The molecular formula, $C_{10}H_{12}O_2$, was readily apparent from its ^{13}C NMR spectrum (**Table-13**), which showed three aromatic quaternary carbons [C-1(130.7 ppm), C-3**(149.0 ppm), C-4**(149.0 ppm)], two of which bear oxygen, three aromatic methine carbons [C-2(108.5 ppm), C-5(111.0 ppm), C-6(119.4 ppm)], two methoxy carbons [C-9†(55.9 ppm), C-10†(55.8 ppm)] and a vinyl group [C-7(136.5 ppm), C-8(111.8 ppm)], attached to the aromatic ring. The ^{1}H NMR spectrum (Table-13) of **(45)**, on the other hand, showed two methoxyl groups (3H-9 and 3H-10) at 3.89 ppm and 3.87ppm as singlets. H-7 was seen at 6.64 ppm as dd(J= 10.9, 17.6 Hz), and the other two protons of the vinyl group (2H-8) were at 5.61 ppm as dd(J= 18.3, 0.7 Hz) and at 5.13 ppm as dd(J= 11.6, 0.7 Hz). Aromatic methine protons were observed between 6.77 ppm and 7.0 ppm, H-2 was observed at 6.96 ppm as a doublet(J= 2.0 Hz), which had a long-range coupling(^{4}J) to H-6. H-6 gave doublet of doublets (J= 8.2, 2.0 Hz) at 6.93, and finally the doublet(J= 7.2 Hz) at 6.85 ppm belonged to H-5.

The HMQC experiment of compound **(45)** also supported the structure showing the direct correlations between the carbons and the protons (Table-6).



(45)

Table-5. ^{13}C and ^{1}H NMR Spectra of Compound **(45)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	130.7		
2	108.5	6.96	d (J= 2.0)
3**	149.0		
4**	149.9		
5	111.0	6.85	d (J= 7.2)
6	119.4	6.93	dd (J= 8.2, 2.0)
7	136.4	6.64	dd (J= 10.9, 17.6)
8	111.8	5.61	dd (J= 18.3, 0.7)
		5.13	dd (J= 11.6, 0.7)
9*	55.9	3.89	s
10*	55.8	3.87	s

** maybe interchanged.

* maybe interchanged.

Table-6, HSQC Correlations of Compound **(45)**

δ_c	**C**	δ_H	**No**
108.5	**2**	6.96	1H
111.0	**5**	6.85	1H
119.4	**6**	6.93	1H
136.5	**7**	6.64	1H
111.8	**8**	5.61	2H
		5.13	
55.9	**9'**	3.89	3H
55.8	**10'**	3.87	3H

maybe interchanged

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Chapter-5

Nardia scalaris

INTRODUCTION

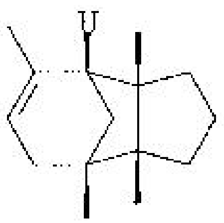
The liverwort *Nardia scalaris* is typically low-growing and nearly prostrate[1]. The almost unbranched stems of this species are short (1-3 cm). They form rather extensive patches, of a yellowish-green to red-brown colour, and grow on gravelly banks, ledges, moors and in the mountains. It is the commonest entire-leafed liverwort found in Britain[1]. The distribution of the species of the genus *Nardia* (six species known world-wide)[2], which is in the group of the Jungermanniidae, has been reported in the Atlas of the Bryophytes[3].

Early investigations[4] of *Nardia scalaris* revealed some sesquiterpenoids, α-barbatene **(1)**, β-barbatene **(2)**, β-bisabolene **(3)**, cuparene **(4)**, and some aromatic compounds, e.g. lunularic acid **(5)**. In addition, Benes *et al*[4,5] isolated an *ent*-kaurane diterpenoid **(6)** and (+)-21α-methoxyserrat-14-en-3-one **(7)** from the same plant.

In a later study Connolly *et al*[6] isolated an acidic diterpenoid. It was characterised as its methyl ester, methyl *ent*-kaur-16-en-14-yl malonate **(8)**. They have also found the related ketone **(9)**. The same diterpenoid has been reported by Langenbahn *et al*[2] together with compound **(10)**.

The related species *N. succulenta* contains, in addition to the simple kaurene derivatives *ent*-kaur-16-en-15β-ol[7], *ent*-(16R)-kauran 15 one and *ent*-kaur-16-en-15-one[8], a wide range of terpenoid malonates and half-malonates. Langenbahn *et al*[2] isolated compounds **(11)** and **(12)** from the acidic fraction from *N. succulenta*. They also reported compounds **(13)**, **(14)**, **(15)** and **(16)** from neutral fractions of the same liverwort.

Similar terpenoid esters were found in *N. scalaris* by the same authors[2] and were recognised as derivatives of *ent*-kaurene alcohols with the functional group predominantly at C-14, though derivatives with functionalization at C-15 were also observed[2]. Compounds **(17)**, **(18)**, **(19)**, **(20)** and **(21)** have been reported from the acidic fraction of *N. scalaris*[2] while the neutral fractions afforded the compounds **(22)**, and **(23)**.



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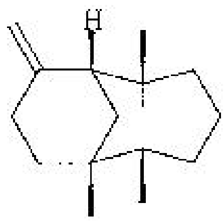

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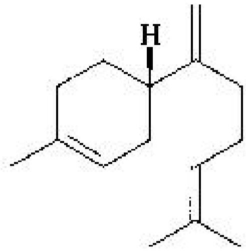

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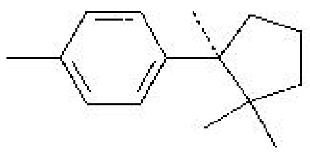

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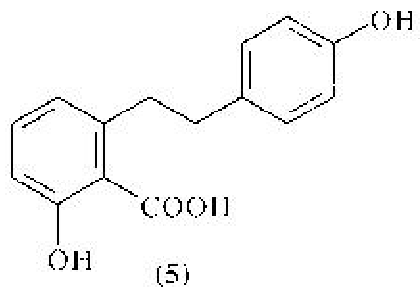

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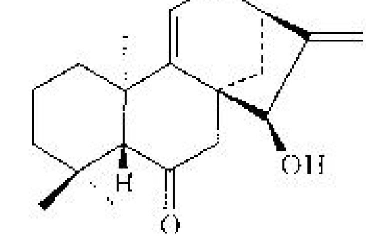

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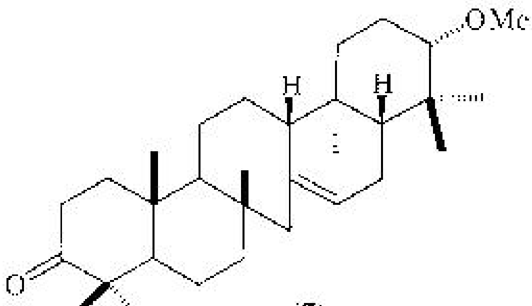

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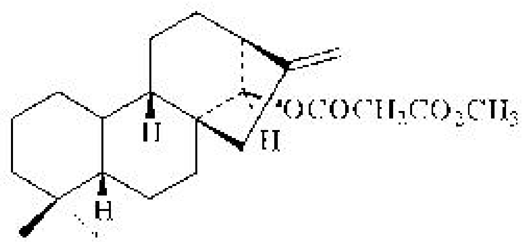

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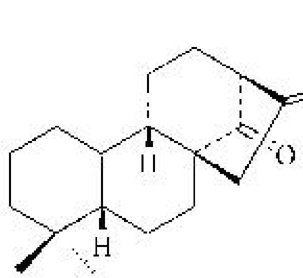

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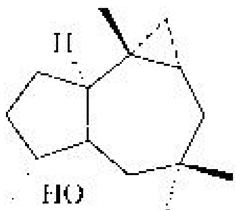

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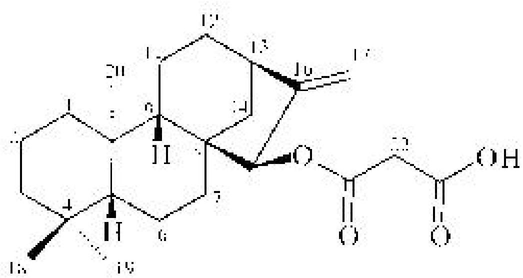

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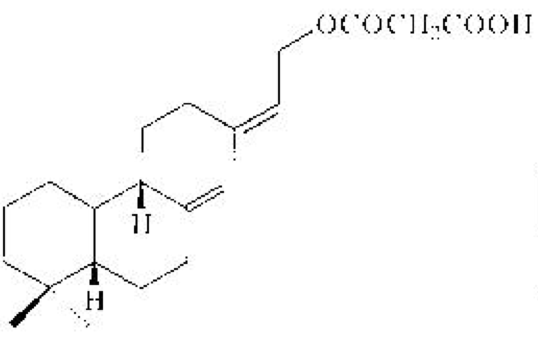

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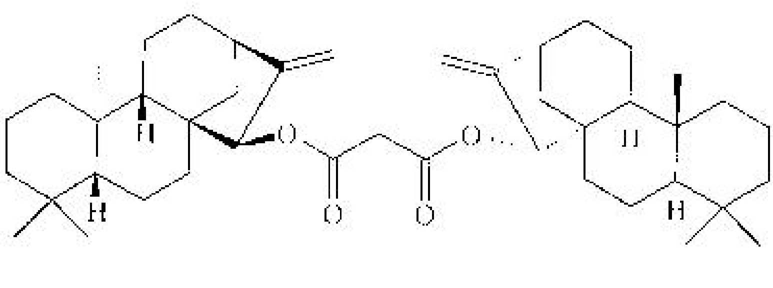

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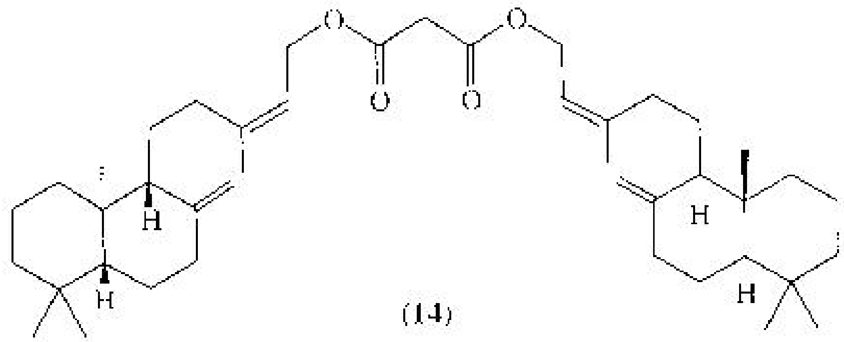

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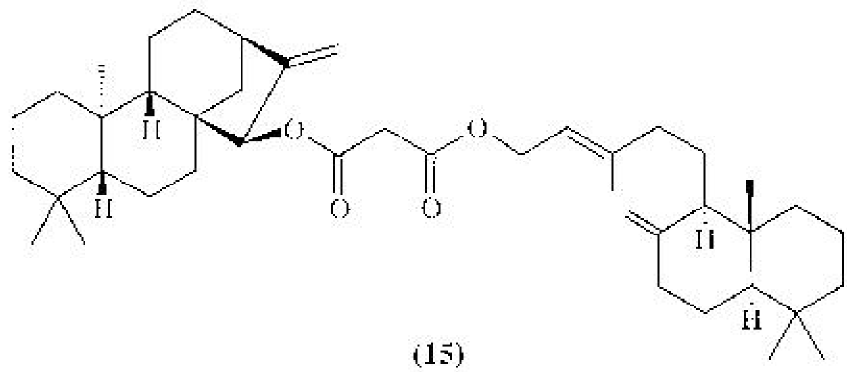

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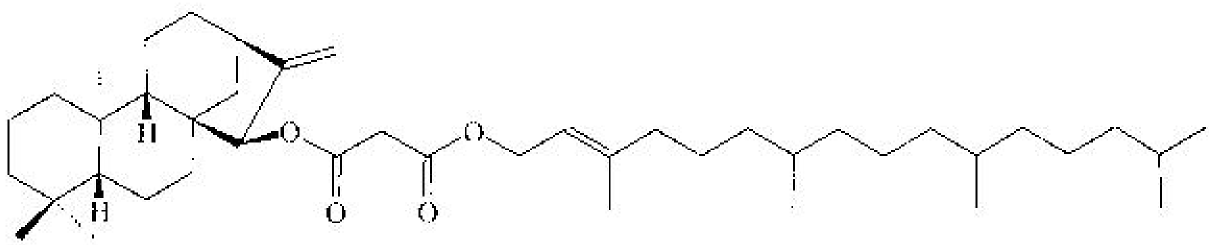

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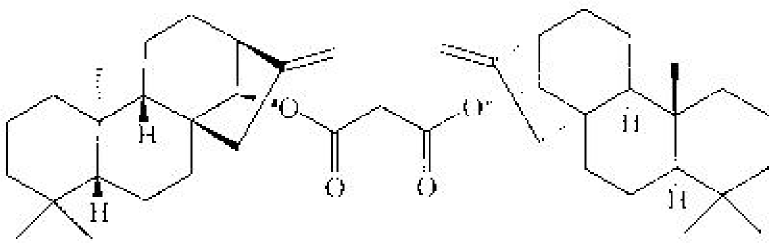

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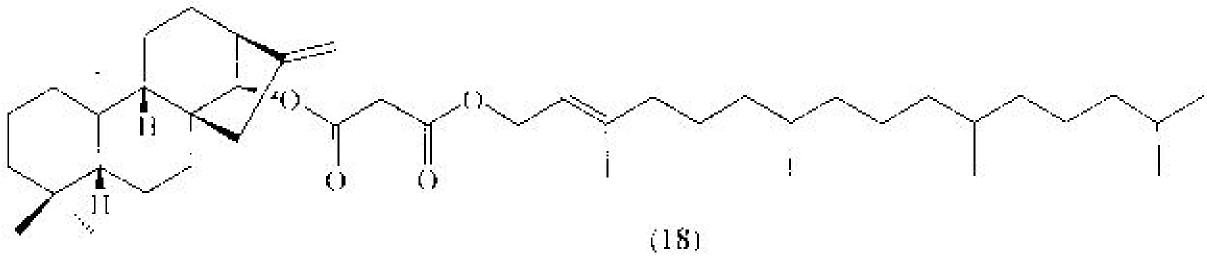

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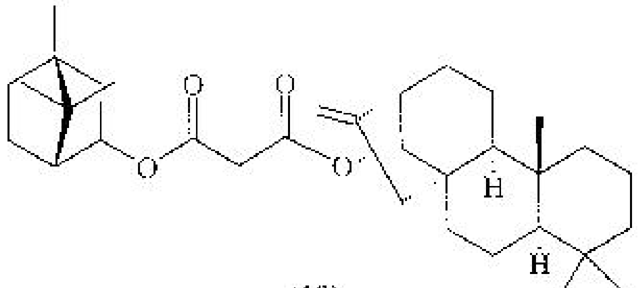

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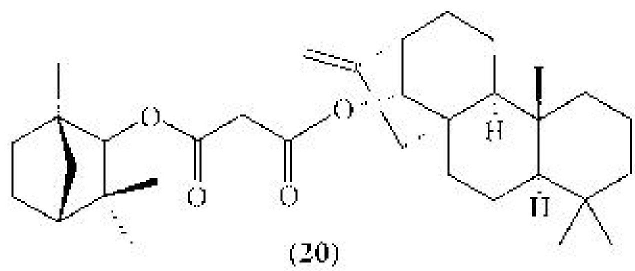

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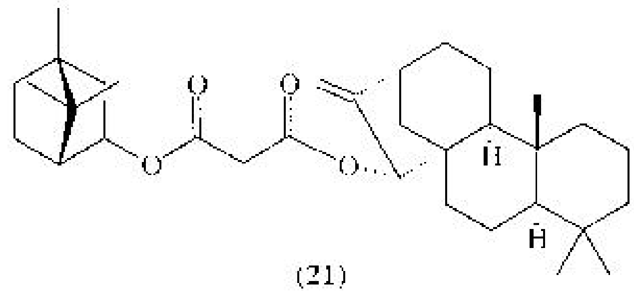

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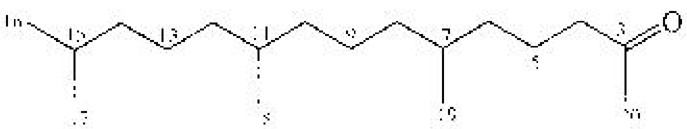

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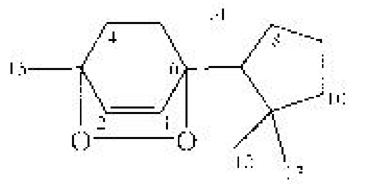

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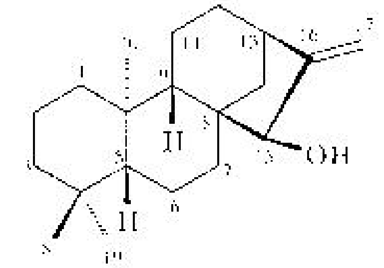

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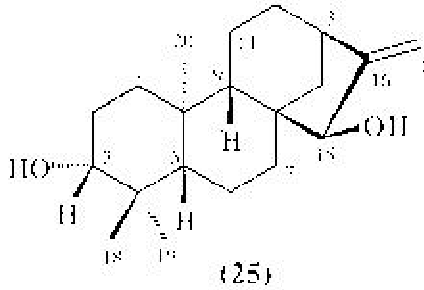

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RESULTS AND DISCUSSION

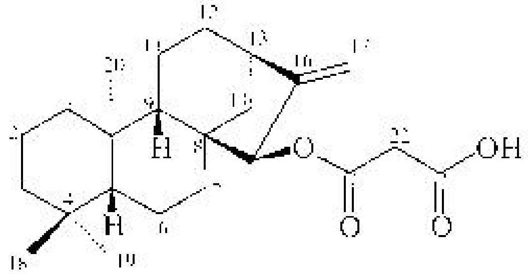
The plant material (203 g) was air dried, ground and extracted with diethyl ether. It yielded a crude extract (3.95 g), which showed several spots on analytical TLC (UV), and was subjected to flash chromatography on silica gel, eluting with light petroleum and increasing percentages of ethyl acetate. Several fractions were obtained.

Three main compounds were present in the fractions : *ent*-kaur-(15R)-16-en-15-yl hydrogen malonate **(11)**, *ent*-kaur-(15R)-16-en-15-ol **(24)** and *ent*-kaur-16-en-15α,3β-diol **(25)**. All of them are known compounds[2]. Compounds **(11)** and **(24)** were previously reported by Langenbahn *et al*[2] from the related species *Nardia succulenta*, and compound **(25)** has been isolated by Nagashima *et al*[1] from the liverwort *Jungermannia vulcanicola*. However this is the first time that it has been isolated from the liverwort *Nardia scalaris*.

ent-Kaur-(15R)-16-en-15-yl hydrogen malonate **(11)** was present in the fraction eluted with 100% ethyl acetate. The analytical plate showed a distinct spot, which was purified by preparative TLC. It showed a carbonyl bond at 1768 cm^{-1} in the IR spectrum and a parent ion at m/z 374 in the mass spectrum. The molecular formula ($C_{23}H_{34}O_4$) was deduced from the ^{13}C NMR spectrum (Table 1), which showed an exomethylene group at δ_C 157.6 (C-16) and δ_C 106.7 (C-17), and a malonyl residue at δ_C 41.0 (C-22), δ_C 167.3 and δ_C 170.8. In addition, three tertiary methyl carbons [δ_C 33.5 (C-18), δ_C 21.7 (C-19), δ_C 17.7 (C-20)], ten methylene groups, two of which were the exomethylene group(C-17) and the malonyl residue(C-22),and four methine carbons, one of which belonged to C-15 (δ_C 83.0), were observed. The malonyl group was attached to C-15, the secondary oxygen bearing carbon. In the HMBC spectrum a correlation was observed between C-15 and one of the hydrogens of C-14, and between C-15 and one of the hydrogens of C-17.

The exomethylene group (2H-17) was clearly observed in the ^{1}H NMR spectrum (Table 1) at δ_H 4.92 (brd, J=2.4 Hz) and δ_H 4.89 (brs). The malonyl residue (2H-22) was seen at δ_H 3.50 (s). The methine H-13 appeared at δ_H 2.63 (brs) and revealed correlations with the exomethylene protons (2H-17) in the COSY spectrum. Three methyl groups (3H-18, 3H-19, 3H-20) resonated at δ_H 0.81, δ_H 0.76 and at δ_H 1.01 as singlets. The spectral data were identical with those given in literature[2,6].

Unfortunately due to the massive overlap of signals it was not easy to interpret the entire proton spectrum.



(11)

Table-1: ^{13}C and ^{1}H NMR Data of Compound (11)

	δ_C	δ_H	J (Hz)
1	40.4	1.81 (H_β-1)	brd (12.9 Hz)
2	18.6		
3	41.8		
4	33.2		
5	55.5		
6	19.7		
7	38.6		
8	46.2		
9	47.7		
10	39.0		
11	17.7		
12	33.4		
13	40.7	2.63	brs
14	36.4	2.04 (H_α-14)	d (12.0 Hz)
15	83.0	5.17	t (2.3 Hz)
16	152.8		
17	106.7	4.92 4.89	brd (2.4 Hz) brs

18	33.5	0.81	s
19	21.7	0.76	s
20	17.7	1.01	s

[**] The signals of the malonyl residue appeared at δ_H 3.50 (2H-22) in the ^1H NMR spectrum, and at δ_C 41.0, 167.3, 170.8 in the ^{13}C NMR spectrum.

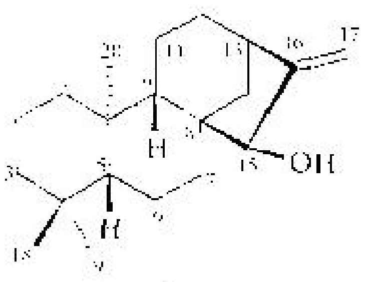
Table-2. HMQC Correlations of Compound (**11**)

δ_C	C	δ_H	**No**
40.4	**1**	1.81	1H(H$_a$-1)
40.7	**13**	2.63	1H
36.4	**14**	2.04	1H(H$_a$-14)
83.0	**15**	5.17	1H
106.7	**17**	4.92 & 4.89	2H
33.5	**18**	0.81	3H
21.7	**19**	0.76	3H
17.7	**20**	1.01	3H

Table-3. HMBC Correlations of Compound (**11**)

δ_C	C	**TYPE**	**H**
40.4	**1**	methylene	3H-20
33.2	**4**	quaternary	3H-19, 3H-18
55.5	**5**	methine	3H-20, 3H-19, 3H-18
40.7	**13**	methine	2H-17, 2H-14
83.0	**15**	methine	H$_a$-14, 2H-17
152.8	**16**	exomethylene	H-15, H$_a$-14, 2H-17
21.7	**19**	methyl	H-5, 3H-18

ent Kaur-(15R)-16-en 15-ol **(24)** was present in fraction 30, which was subjected to preparative TLC, following Sephadex (LH-20), for further purification. Its spectral data were similar to those of compound **(11)**. The only difference was the absence of the malonic acid residue. Instead, the compound had a hydroxyl group attached to C-15. Its ^{13}C NMR spectrum revealed the emprical formula to be $C_{20}H_{32}O$, which is supported by the mass spectrum showing a parent ion peak (M^+) at 288 m/z. Three tertiary methyl groups [δ_C 17.6 (C-20), δ_C 21.7 (C-19), δ_C 33.6 (C-18)], four methine carbons [δ_C 82.6 (C-15), δ_C 55.5 (C-5), δ_C 46.[illegible] (C-9), δ_C 40.1 (C-13)], nine methylene carbons [δ_C 104.7 (C-17), δ_C 36.5 (C-14), δ_C 33.3 (C-12), δ_C 18.1 (C-11), δ_C 38.8 (C-7), δ_C 19.9 (C-6), δ_C 42.0 (C-3), δ_C 18.7 (C-2), δ_C 40.4 (C-1)] and three quaternary carbons [δ_C 33.3 (C-4), δ_C 38.9 (C-10), δ_C 45.8 (C-8)] were observed in its ^{13}C NMR spectrum (Table-4). Its ^{1}H NMR spectrum revealed the exomethylene group (2H-17) at δ_H 5.08 (s) and δ_H 4.91 (d, J=2.5 Hz). The three tertiary methyl groups (3H-18, 3H-19, 3H-20) appeared at δ_H 0.85, δ_H 0.80 and δ_H 1.0 as singlets and the two methine protons (H-15 and H-13) at δ_H 3.72 and at δ_H 2.64 as broad singlets. The rest of the protons that were observable are given in Table-4. In the COSY spectrum, the correlations between 2H-17 and H-15, and between 2H-17 and H-13 are observable. Its ^{13}C and ^{1}H NMR spectra are in agreement with those in literature[2]. The other spectral data are given in Tables 5-6.



(24)

Table-4, ^{13}C and ^{1}H NMR Spectra of Compound **(24)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	40.4	1.83 (H, 1)	d (12.9 Hz)
2	18.7		
3	42.0	1.13 (H, 3)	td (13.2 Hz & 4.4 Hz)

4	35.3		
5	55.5		
6	19.9		
7	38.8		
8	45.8		
9	46.5		
10	38.9		
11	18.1		
12	33.3		
13	40.1	2.64	s
14	36.5	1.99 (H_a-14)	d (11.9 Hz)
15	82.6	3.72	s
16	158.5		
17	104.7	5.08	s
		4.94	d (2.8 Hz)
18	33.6	0.85	s
19	21.7	0.80	s
20	17.6	1.0	s

Table-5. HMQC Correlations of Compound **(24)**

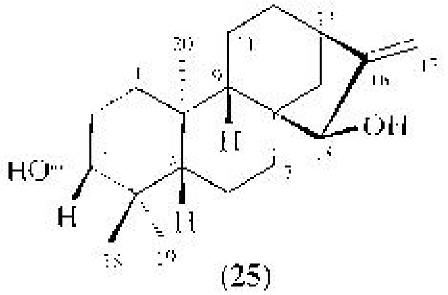
δ_C	**C**	δ_H	**No**
40.4	**1**	1.83	1H(H_a-1)
42.0	**3**	1.13	1H(H_a-3)
40.1	**13**	2.64	1H
36.5	**14**	1.99	1H(H_a-14)
82.6	**15**	3.72	1H(H_a-15)
104.7	**17**	5.08 & 4.94	2H
33.6	**18**	0.85	3H

21.7	**19**	0.80	3H
17.6	**20**	1.0	3H

Table-6. HMBC Correlations of Compound **(24)**

δ_C	C	TYPE	H
40.4	**1**	methylene	3H-20
42.0	**3**	methylene	3H-18, 3H-19
33.3	**4**	quaternary	3H-19, 3H-18, H_2-3
55.5	**5**	methine	3H-18, 3H-19, 3H-20
46.5	**9**	methine	3H-20
38.9	**10**	quaternary	3H-20
33.3	**12**	methylene	3H-19, 3H-18
36.5	**14**	methylene	2H-17
82.6	**15**	methine	H_2-14, 2H-17
158.5	**16**	quaternary	H_2-14
33.6	**18**	methyl	3H-19
21.7	**19**	methyl	3H-18, H_2-3

The third compound, *ent*-kaur-16-en-15α, 3β-diol **(25)**, was present in fraction 40 eluted with 40% ethyl acetate and light petroleum ether. Most of its spectral data were identical with those of **(24)**. However, compound **(25)**, contained an additional hydroxyl group attached to C-3. The mass spectrum showed a parent ion (M^+) peak at 304.3 m/z. Its ^{13}C NMR spectrum showed that there were two oxygenated methine carbons [δ_C 79.0 (C-3) and δ_C 82.3 (C-15)], and the HMBC spectrum showed distinct correlations between C-3 and 3H-18 and 3H-19. The COSY spectrum was difficult to interpret but a correlation between the exomethylene protons (2H-17) and the methine proton (H-15) was observed. The spectral data, which are identical with those given *in literature*[11-13] can be seen in Tables 7-9.



(25)

Table-7: ^{13}C and ^{1}H NMR Spectra of Compound (25)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	38.6	1.87(H, 1)	dt(13.2 Hz & 3.5 Hz)
2	27.3		
3	79.0	3.20	dd(11.2 Hz & 5.3 Hz)
4	38.7		
5	54.5		
6	19.6		
7[b]	38.8		
8	45.6		
9	46.3		
10[b]	38.8		
11	18.2		
12	33.2		
13	40.07	2.65	brs
14	36.4	1.97(H,-14)	d(12.0 Hz)
15	82.3	3.75	t(2.6 Hz)
16	158.3		
17	101.8	5.08	s
		4.94	d (2.8 Hz)
18	28.3	0.97	s
19	15.5	0.77	s
20	17.6	1.02	s

[b] May be interchanged.

Table-8, HMQC Correlations of Compound **(25)**

δ_C	C	δ_H	No
38.6	1	1.87	1H(H$_a$-1)
79.0	**3**	3.20	1H
40.07	**13**	2.65	1H
36.4	**14**	1.97	1H(H$_a$-14)
82.3	**15**	3.75	1H
104.8	**17**	5.08 & 4.94	2H
28.3	**18**	0.97	3H
15.5	**19**	0.77	3H
17.6	**20**	1.02	3H

Table-9, HMBC Correlations of Compound **(25)**

δ_C	C	**TYPE**	**H**
38.6	1	methylene	3H-20
27.3	2	methylene	H-3
79.0	**3**	methine	3H-18, 3H-19
54.5	**5**	methine	3H-18, 3H-19, 3H-20
46.3	**9**	methine	3H-20
38.2	**12**	methylene	H$_a$-14
40.07	**13**	methine	2H-17
82.3	**15**	methine	H$_a$-14
158.3	**16**	exomethylene	H$_a$-14
28.3	**18**	methyl	3H-19
15.5	**19**	methyl	3H-18, H-3

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Chapter-6

Trichocolea tomentella

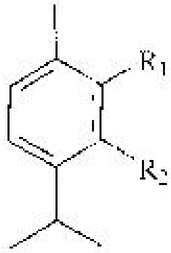
INTRODUCTION

The liverwort *Trichocolea tomentella* of the family of the Trichocoleaceae belongs to Jungermanniales[1]. It is found in Japan, North America and Europe[1]. Carvacrol (**1**), thymol (**2**), α-pinene (**3**) are the monoterpenoids[2], and α-gurjunene (**4**), β-barbatene (**5**), calamenene (**6**), drimenol (**7**), α-himachalene (**8**) are the sesquiterpenoids[2] found in this liverwort. Among these, compounds (**5**) and (**6**) were in *Trichocoleopsis sacculata*[2], and compound (**7**) was found in *Neotrichocolea bissetii*[2], belonging to the same family, Trichocoleaceae, which is also known to produce flavanoid glycosides[2]. The aromatic compounds[2] found in *T. tomentella* are 3,4 dihydroxybenzoic acid (**9**), lunularic acid (**10**), and lunularin (**11**). *T. tomentella* also produces some compounds with a rare combination of both terpenoid and aromatic portions[3,4]. Among these compounds are trichocolein (**12**), tomentellin (**13**), isotomentellin (**14**), demethoxytomentellin (**15**), and deoxytomentellin (**16**), which were isolated by Asakawa Y. *et al*[1]. Although the structures of the compounds were reported wrongly at first, they have been corrected in their later work[3]. Among these compounds, (**12**), (**13**) and (**14**) were also discovered in *Trichocolea pluma* by Asakawa Y. *et al*[5]. A sample of *Trichocolea tomentella* was collected in Scotland by Dr. David Rycroft and forms the subject of this investigation.

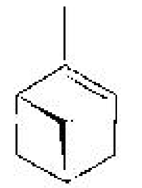
RESULTS AND DISCUSSION

Plant material was air-dried, ground and extracted with diethyl ether a few times. The crude extract (1.41 g) was subjected to flash chromatography with increasing percentages of ethyl acetate and petroleum ether. Further purification was done by preparative TLC. Four known compounds, deoxytomentellin (**16**), trichocolein (**12**), tomentellin (**13**), isotomentellin (**14**) and two new compounds, methyl 4-[7-hydroxy-3,7-dimethyl-2,5-octadienyloxy]-3-methoxybenzoate (**17**), and methyl 4-[5-hydroxy-3,7-dimethyl-2,6-octadienyloxy]-3-methoxybenzoate (**18**) have been isolated from this liverwort.

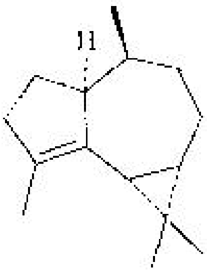
Compounds (**16**) and (**12**) were present in fraction 30, eluted with ethyl acetate and petroleum ether (3:7). The ^{1}H NMR spectrum (Table 1) of compound (**16**)



(1) R_1 = OH, R_2 = H
(2) R_1 = H, R_2 = OH



(3)



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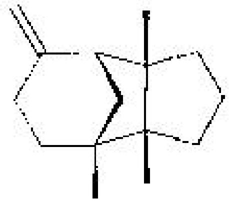

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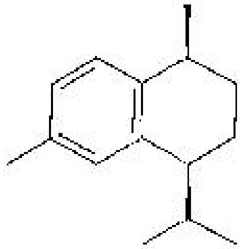

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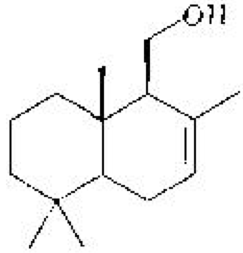

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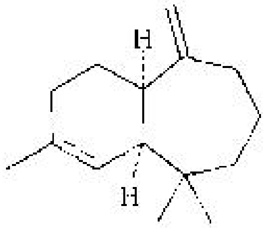

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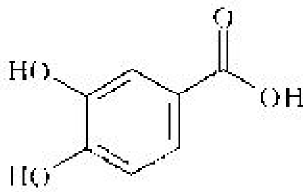

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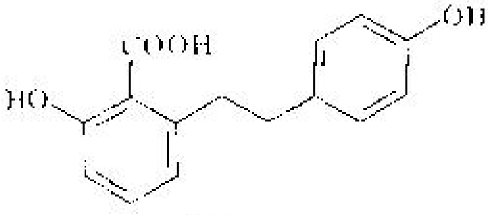

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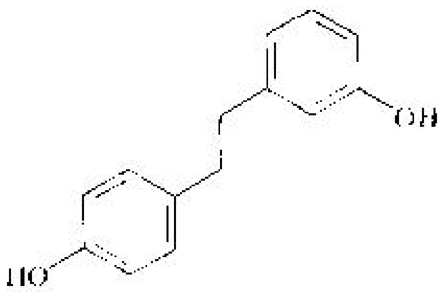

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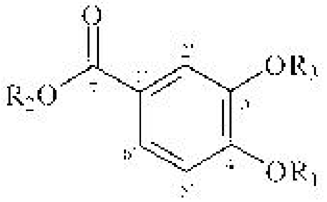
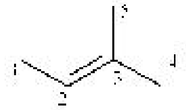
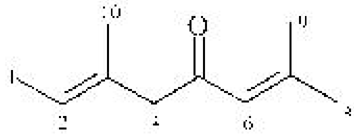


(12) R_1=



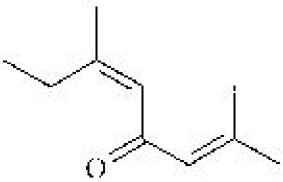
$R_2=R_3= CH_3$

(13) R_1=



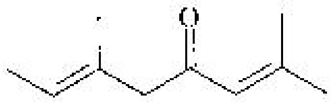
$R_2=R_3= CH_3$

(14) R_1=



$R_2=R_3= CH_3$

(15) R_1=



$R_2= CH_3$, $R_3= H$

(16) R_1=

$R_2=R_3= CH_3$

(17) R_1=

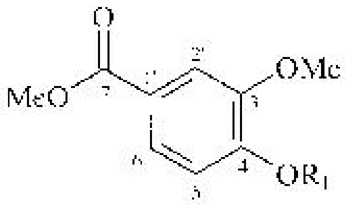
$R_2=R_3= CH_3$

(18) R_1=

$R_2=R_3= CH_3$

revealed that a trisubstituted benzene ring δ 7.63 [dd, J= 8.4, 2.0 Hz, H-6'] ; δ 7.53 [d, J= 2.0 Hz, H-2']; δ 6.86 [d, J= 8.4 Hz, H-5'] was present in the structure along with two methylene groups δ 2.07 [m, (2H-4 and 2H-5)], an oxygenated methylene δ 4.67 [d, J= 6.5 Hz, 2H-1] and five methyl groups, two of which were methoxyls δ 3.88 [s, 7'-OMe], δ 3.90 [s, 3'-OMe]. *The other three methyl groups appeared between* δ 1.76 and δ 1.57 and were hence vinylic. The molecular formula, $C_{19}H_{26}O_4$, was readily apparent from the ^{13}C NMR spectrum (Table 1), which showed an ester carbonyl (C-7') at δ 167.0, five quaternary carbons, *three of which belonged to the aromatic ring* [δ 122.5 (C-1') , δ 148.9 (C-3') ; δ 152.3 (C-4')] and the other two to the two trisubstituted double bonds present in the structure [δ 141.3 (C-3) ; δ 131.8 (C-7)]. Typical chemical shifts were observed for a carbomethoxy group (7'-OMe) at δ 51.9 and for a methoxy (3'-OMe) at δ 56.0. The oxygenated methylene carbon (C-1) resonated at δ 65.9. The other two methylene carbons were seen at δ 39.5 (C-4) and at δ 26.2 (C-5). The latter is shielded by the neighbouring methyl group (3H-9) attached to the adjacent carbon (C-7). The three methyl groups (3H-8, 3H-9, 3H-10) were at δ 25.7, δ 17.7 and δ 16.7. The mass spectrum supported its molecular formula by showing a parent ion peak at 318 (M+1). The UV spectrum (ethanol) had bands at λ_{max} (ε) : 206 nm (8955), 210 nm (6782), 262 nm (3370), 292 nm (1852), 328 nm (73), 334 nm (68). NMR data can be seen in Tables 1-4.

The other compound present in this fraction was trichocolein (**12**), which was previously reported by Perry *et al*[3]. The spectroscopic properties of compounds (**16**) and (**12**) were quite similar. The main difference was the presence of a simple isoprenyloxy side chain in place of the more complex side chain of (**16**). Its mass spectrum showed a parent ion peak at 251 $(M+H)^+$, supporting the molecular formula, $C_{14}H_{18}O_4$. The UV spectrum (ethanol) showed bands at λ_{max} (ε) : 206 nm (8955), 210 nm (6782), 262 nm (3370), 292 nm (1852), 328 nm (73), 334 nm (68) and the IR spectrum gave carbonyl absorption at 1706 cm^{-1}. The spectral data of compound (**12**) can be seen in Tables 5-8.



(16) R =

(12) R_1 =

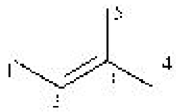


Table-1. ^{13}C and ^{1}H NMR Data of Compound **(16)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	65.9	4.67	d (6.5 Hz)
2	119.1	5.49	brt (6.5 Hz)
3	141.3		
4	39.5	2.07	m (overlapping with 2H-5)
5	26.2	2.07	m (overlapping with 2H-4)
6	123.7	5.06	brt (6.8 Hz)
7	131.8		
8	25.7	1.65	s
9	17.7	1.58	s
10	16.7	1.73	s
1'	122.5		
2'	112.1	7.53	d (2.0 Hz)
3'	148.9		
4'	152.3		
5'	111.7	6.86	d (8.4 Hz)
6'	123.4	7.63	dd (8.4 Hz, 2.0 Hz)
7'	167.0		
7'-OMe	51.9	3.88	s
3'-OMe	56.0	3.90	s

Table-2, HMQC Correlations of Compound **(16)**

δ_C	C	δ_H	No
65.9	**1**	4.67	2H
119.1	**2**	5.49	1H
39.5	**4**	2.07	2H
26.2	**5**	2.07	2H
123.7	**6**	5.06	1H
25.7	**8**	1.65	3H
17.7	**9**	1.58	3H
16.7	**10**	1.73	3H
112.1	**2'**	7.53	1H
111.7	**5'**	6.86	1H
123.4	**6'**	7.63	1H
51.9	**7'-OMe**	3.88	3H
56.0	**3'-OMe**	3.90	3H

Table-3, HMBC Correlations of Compound **(16)**

δ_C	C	TYPE	H
119.1	**2**	methine	2H-1, 3H-10
141.3	**3**	quaternary	2H-1, 3H-10
39.5	**4**	methylene	3H-10, 2H-5
26.2	**5**	methylene	2H-4
123.7	**6**	methine	3H-8, 3H-9
131.8	**7**	quaternary	3H-8, 3H-9
25.7	**8**	methyl	3H-9

17.7	**9**	methyl	3H-8
16.7	**10**	methyl	H-2
122.5	**1'**	quaternary	H-5'
112.1	**2'**	methine	H-6'
148.9	**3'**	quaternary	H-5', 3' OMe
152.3	**4'**	quaternary	H-6', H-2', H-5', 2H-1
111.7	**5'**	methine	H-6'
123.4	**6'**	methine	H-2'
167.0	**7'**	carbonyl	H-6', H-2', 7'-OMe

Table-4. COSY Correlations of Compound (**16**)

δ_H	**H**	**TYPE**	**H**
7.63	**6'**	methine	H-5', H-2'
7.53	**2'**	methine	H-6'
6.86	**5'**	methine	H-6'
5.49	**2**	methine	2H-1, 3H-10
5.06	**6**	methine	3H-8, 3H-9, 2H-5
4.67	**1**	methylene	3H-10, H-2

Table-5. ^{13}C and ^{1}H NMR Data of Compound (**12**)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	65.7	4.62	d (6.7 Hz)
2	119.2	5.49	brt (6.7 Hz)
3	138.3		

4	25.8	1.76	s
5	18.2	1.73	s
1'	122.4		
2'	112.0	7.52	d (2.0 Hz)
3'	148.9		
4'	152.3		
5'	111.6	6.86	d (8.4 Hz)
6'	123.4	7.63	dd (8.4, 2.0 Hz)
7'	166.9		
7'-OMe	51.9	3.87	s
3'-OMe	56.0	3.90	s

Table-6. HMQC Correlations of Compound **(12)**

δ_C	C	δ_H	No
65.7	**1**	4.62	2H
119.2	**2**	5.49	1H
25.8	**4**	1.76	3H
18.2	**5**	1.73	3H
112.0	**2'**	7.52	1H
111.6	**5'**	6.86	1H
123.4	**6'**	7.63	1H
51.9	**7'-OMe**	3.87	3H
56.0	**3'-OMe**	3.90	3H

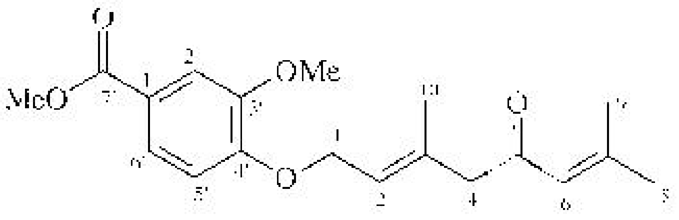
Table-7. HMBC Correlations of Compound **(12)**

δ_C	C	TYPE	H
119.2	2	methine	2H-1, 3H-4, 3H-5
138.3	3	quaternary	2H-1, 3H-4, 3H-5
25.8	4	methyl	H-2, 3H-5
18.2	5	methyl	H-2, 3H-4
122.4	1'	quaternary	H-5'
112.0	2'	methine	H-6'
148.9	3'	quaternary	3'-OMe, H-5'
152.3	4'	quaternary	2H-1, H-6', H-2', H-5'
123.4	6'	methine	H-2'
166.9	7'	carbonyl	7'-OMe, H-6', H-2'

Table-8. COSY Correlations of Compound **(12)**

δ_H	H	TYPE	H
4.62	1	methylene	H-2
5.49	2	methine	2H-1
7.52	2'	methine	H-6'
6.86	5'	methine	H-6'
7.63	6'	methine	H-5', H-2'

Compounds **(13)** and **(14)** were present in fraction 50 eluted with ethyl acetate and petroleum ether (1:1). Compound **(13)** is known as tomentellin, and was previously isolated by Perry *et al*[3]. Due to the similarity of its spectral data to the compound **(16)**, it was easy to recognise the typical chemical shifts in both its proton and carbon NMR spectra. The only difference was the oxygenation of the geranyl chain at C-5, which is one of the methylenes in compound **(16)**, deoxytomentellin. Some differences in the chemical shifts were observed due to the newly introduced carbonyl group. The three methyls, δ 1.84 [s, 3H-8], δ 2.12 [s, 3H-9], δ 1.75 [s, 3H-10] and one of the two double bond protons δ 6.07 [m, H-6] appeared slightly more downfield than those in the ^1H NMR spectrum of deoxytomentellin **(16)**. The other two methylene protons of the geranyl chain were at δ 4.71 [d, J= 6 Hz, 2H-1] and at δ 3.12 [s, 2H-4]. The spectral data are given in Tables 9-12. The mass spectrum supported the molecular formula, $C_{19}H_{24}O_5$, with a parent ion peak (M^+) at 332 m/z and a fragment, arising from the geranyl group, at 151 m/z. The UV spectrum (ethanol) showed bands at λ_{max} (ε) : 202 nm (12016), 240 nm (13268) and the IR spectrum showed a carbonyl absorption at 1711 cm^{-1}



(13)

Table-9. ^{13}C and ^1H NMR Data of Compound **(13)**

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	65.6	4.71	d (6.0 Hz)
2	123.8	5.60	td (6.0 Hz, 1.0 Hz)
3	135.5		
4	55.0	3.12	s
5	198.0		
6	122.7	6.07	m
7	156.7		
8	27.7	1.84	s
9	20.8	2.12	s

10	17.0	1.75	s
1'	122.6		
2'	112.2	7.53	d (2.0 Hz)
3'	148.9		
4'	152.0		
5'	111.8	6.86	d (8.5 Hz)
6'	123.4	7.63	dd (8.5, 2.0 Hz)
7'	166.9		
7'-OMe	51.9	3.87	s
3'-OMe	56.0	3.90	s

Table-10. HMQC Correlations of Compound (**13**)

δ_C	C	δ_H	No
65.6	**1**	4.71	2H
123.8	**2**	5.60	1H
55.0	**4**	3.12	2H
122.7	**6**	6.07	1H
27.7	**8**	1.84	3H
20.8	**9**	2.12	3H
17.0	**10**	1.75	3H
112.2	**2'**	7.53	1H
111.8	**5'**	6.86	1H
123.4	**6'**	7.63	1H
51.9	**7'-OMe**	3.87	3H
56.0	**3'-OMe**	3.90	3H

Table-11. HMBC Correlations of Compound (13)

δ_C	C	TYPE	H
123.8	**2**	methine	2H-1, 2H-4, 3H-10
135.5	**3**	quaternary	2H-1, 2H-4, 3H-10
55.0	**4**	methylene	3H-10, H-2
198.0	**5**	carbonyl	2H-4, H-6
122.7	**6**	methine	3H-8, 3H-9
156.7	**7**	quaternary	3H-8, 3H-9
27.7	**8**	methyl	3H-9, H-6
20.8	**9**	methyl	3H-8, H-6
17.0	**10**	methyl	2H-4, H-2
122.6	**1'**	quaternary	H-5'
112.2	**2'**	methine	H-6'
148.9	**3'**	quaternary	3'-OMe, H-5', H-2'
152.0	**4'**	quaternary	H-2', H-5', H-6', 2H-1
111.8	**5'**	quaternary	H-6'
123.4	**6'**	methine	H-2'
166.9	**7'**	carbonyl	7'-OMe, H-2', H-6'

Table-12. COSY Correlations of Compound (13)

δ_H	H	TYPE	H
1.71	1	methylene	3H-10, H-2, 2H-4
1.60	**2**	methine	2H-1, 3H-10
3.12	**4**	methylene	3H-10, H-2, 2H-1
6.07	**6**	methine	3H-8, 3H-9
1.84	**8**	methyl	3H-9, H-6
2.12	**9**	methyl	3H-8, H-6

1.75	**10**	methyl	2H-4, 2H-1, H-2
7.53	**2'**	methine	H-6'
6.86	**5'**	methine	H-6'
7.63	**6'**	methine	H-2', H-5'

Compound **(14)** has been previously discovered in the liverworts *T. tomentellia* and *T. mollissima*[5]. Its spectral data were very similar to those of compound **(13)**. The empirical formula of the two compounds, $C_{19}H_{32}O_5$, were deduced from their ^{13}C NMR spectra. The mass spectrum of **(14)**, which gave a parent ion (M^+) peak at 332 m/z, proved that the two compounds were isomeric. The only difference between the two compounds was the different position of one of the two double bonds. The 2,3 double bond in tomentellin **(13)** was now a 3,4 double bond in isotomentellin **(14)**. In the ^1H NMR spectrum of **(14)**, the two methylene protons [δ 4.29 (2H-1), t (6.6 Hz) ; δ 3.09 (2H-2), t (6.6 Hz)], and the two methine protons [δ 6.14 (H-4), brs ; δ 6.06 (H-6), brs] in the geranyl chain were clearly observed. The rest of the spectral data are given in Tables 13-16.

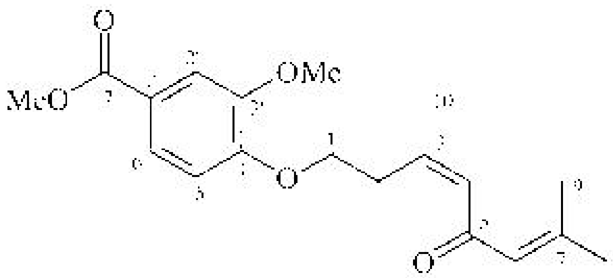


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Table-13. ^{13}C and ^1H NMR Data of Compound **(14)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	67.9	4.29	t (6.6 Hz)
2	33.6	3.09	t (6.6 Hz)

3	151.5		
4	127.6	6.14	brs
5	190.7		
6	125.9	6.05	brs
7	155.3		
8	27.8	1.88	s
9	20.6	2.16	s
10	27.0	2.02	s
1'	122.4		
2'	112.2	7.51	brs
3'	148.8		
4'	152.4		
5'	111.6	7.03	d (8.4 Hz)
6'	123.6	7.64	d (8.4 Hz)
7'	167.0		
7'-OMe	51.9	3.87	s
3'-OMe	56.0	3.88	s

Table-14. HMQC Correlations of Compound (**14**)

δ_C	C	δ_H	No
67.9	**1**	4.29	2H
33.6	**2**	3.09	2H
127.6	**4**	6.14	1H
125.9	**6**	6.05	1H
27.8	**8**	1.88	3H
20.6	**9**	2.16	3H
27.0	**10**	2.02	3H
112.2	**2'**	7.51	1H

111.6	5'	7.03	1H
123.6	6'	7.64	1H
51.9	7'-OMe	3.87	3H
56.0	3'-OMe	3.88	3H

Table-15. HMBC Correlations of Compound (14)

δ_C	C	TYPE	H
67.9	1	methylene	2H-2
33.6	2	methylene	2H-1, 3H-10, H-4
154.5	3	quaternary	2H-1, 2H-2, 3H-10
127.6	4	methine	2H-2, 3H-10
190.7	5	carbonyl	H-4, H-6
125.9	6	methine	3H-8, 3H-9
155.3	7	quaternary	3H-8, 3H-9
27.8	8	methyl	3H-9, H-6
20.6	9	methyl	3H-8, H-6
27.0	10	methyl	2H-2, H-4
122.4	1'	quaternary	H-5'
112.2	2'	methine	H-6'
148.8	3'	quaternary	H-5', 3'-OMe
152.4	4'	quaternary	H-2', H-6', H-5'
123.6	6'	methine	H-2'
167.0	7'	carbonyl	7'-OMe, H-6', H-2'

Table-16. COSY Correlations of Compound (**14**)

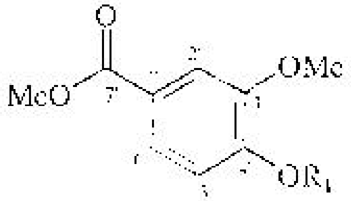
δ_H	H	TYPE	H
4.29	1	methylene	2H-2
3.09	2	methylene	2H-1
6.14	4	methine	3H-10
6.05	6	methine	3H-8, 3H-9
7.51	2'	methine	H-6'
7.03	5'	methine	H-6'
7.64	6'	methine	H-2', H-5'

Compounds (**17**) and (**18**) were present in equimolar amounts in fraction 60, **whose analytical plate showed a UV active spot containing two compounds.** Purification of the two compounds by preparative TLC failed due to their close polarity. Two-dimensional NMR experiments carried out on the mixture enabled us to interpret the correlations of the two compounds. The proton NMR spectrum of the mixture showed some signals, which are present in both structures. These were the three aromatic protons between δ_H 6.70 and δ_H 7.70, the methoxy and carbomethoxy groups at δ_H 3.90 and δ_H 3.80 and the oxygenated methylene signal, which was at δ_H 4.67 as a triplet. The other signals observed in the ^1H NMR spectrum were six methyl groups between δ_H 1.20 and δ_H 1.90, two methylene groups at δ_H 2.20 and δ_H 2.74, and six methine protons, one of which is oxygenated and seen at δ_H 4.50 as a multiplet. The other five belonged to the double bonds present in the structures. One of them was at δ_H 5.14 as a doublet (J= 8.4 Hz) and the other four were massively overlapped between δ_H 5.49 and δ_H 5.70.

The ^{13}C NMR spectrum of the mixture showed eight methyl carbons, two of which belonging to methoxy (δ_C 56.0) and carbomethoxy (δ_C 52.0) groups. Eleven methine signals, one oxygenated at δ_C 66.4, and the others consisting of aromatic and double bond carbons between region δ_C 110 and δ_C 154 were observed. Four methylene carbons were apparent, two of which oxygenated at δ_C 65.6, and δ_C 65.8 and the others at δ_C 42.2 and δ_C 47.8.

Although the purification by preparative TLC failed to give clean products, the ^{1}H NMR spectra of the two bands still gave some information about the compounds. We were able to assign the different methyl signals and the methylene signals of each compound, which enabled us to build the structures of the two compounds with the help of their two dimensional spectra. The HMBC spectrum showed that the oxygenated methylenes correlate with a trisubstituted double bond next to them. The methylene signal at δ_H 2.74 [d, J= 5.8 Hz] showed correlations with three methine carbons, a trisubstituted and a disubstituted double bonds. The methyl signals at δ_H 1.71 [s, 3H-10] and at δ_H 1.30 [s, 3H-8, and, s 3H-9] and the methylene signal at 2.74 [d, J= 5.8 Hz, 2H-4] belong to one of the two unknowns. The methyl signals at δ_H 1.30 correlated with each other, with a tertiary oxygenated carbon and with a methine carbon, belonging to a trans double bond (J= 15.6 Hz). These results helped us elucidate the structure **(17)**, as one of the unknowns. A literature search showed a similar compound whose NMR data were quite similar to those of compound **(17)**. The other compound, **(18)**, was easily deduced because of its similar chemical shifts to those of compound **(16)**. The COSY spectrum of the mixture was not very clear due to the overlap of the signals. However the methylene protons (2H-4), δ_H 2.74 in compound **(17)** gave a homoallylic coupling to one of the oxygenated methylenes at δ_H 4.67 and another coupling to the methine signals overlapped between δ_H 5.49 and δ_H 5.70. The two methine signals δ_H 5.58 [m, H-5] and δ_H 5.15 [brd, J= 8.5 Hz, H-6] that belong to compound **(18)** also coupled in the COSY spectrum of the mixture. The empirical formula, $C_{16}H_{28}O_5$, was the same for both compound **(17)** and **(18)**, and the mass spectrum showed a peak, (M^+-H_2O) at 316 m/z.

The spectral data of compounds **(17)** and **(18)** can be seen in Tables 17-22.



(17) R_1=

(18) R_1=

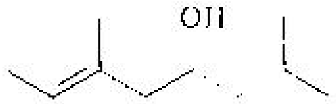


Table-17. ^{13}C and ^{1}H NMR Data of Compound **(17)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	65.6	4.66	d (J= 6.7 Hz)
2	119.9	5.53	dt (J= 6.3 Hz, 1.3 Hz)
3	140.1		
4	42.2	2.74	d (J= 5.8 Hz)
5	124.0	5.60	dt (J= 15.6 Hz, 5.8 Hz)
6	140.3	5.62	d (J= 15.6 Hz)
7	70.7		
8	29.8	1.30	s
9	29.5	1.30	s
10	16.7	1.71	s
1′	122.6		
2′	112.1	7.53	d (2.0 Hz)
3′	149.0		
4′	152.2		
5′	111.7	6.87	d (J= 8.5 Hz)
6′	123.4	7.64	dd (J= 8.5 Hz, 2.0 Hz)
7′	166.9		

7′-OMe	52.0	3.88	s
3′-OMe	56.0	3.91	s

Table-18. ^{13}C and ^{1}H NMR Data of Compound **(18)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1*	65.8	4.68	d (J= 6.7 Hz)
2	122.5	5.62	overlapped
3	138.0		
4	47.8	2.20	dd (J= 13.4 Hz, 8.3 Hz)
		2.27	dd (J= 13.4 Hz, 5.1 Hz)
5	66.4	4.50	m
6	127.3	5.15	brd (J= 8.5 Hz)
7	135.4		
8	25.7	1.70	s
9	18.2	1.67	s
10	17.0	1.80	s
1′	122.6		
2′[illegible]	112.2	7.53	d (J= 2.0 Hz)
3′[illegible]	148.9		
4′[illegible]	152.1		
5′[illegible]	111.8	6.86	d (J= 8.4 Hz)
6′	123.4	7.61	dd (J= 8.4 Hz, 2.0 Hz)
7′	167.0		
7′-OMe	52.0	3.88	s
3′-OMe	56.0	3.90	s

- The carbons that are marked with the same character in Table-17 and Table 18 can be exchangeable within the δ_C column.

Table-19. HMQC Correlations of Compound (**17**)

δ_C	C	δ_H	No
65.6	**1′**	4.66	2H
119.9	**2**	5.55	1H
42.2	**4**	2.75	2H
124.0	**5**	5.60	1H
140.3	**6**	5.62	1H
29.8	**8**	1.30	3H
29.8	**9**	1.30	3H
16.7	**10**	1.71	3H
112.1	**2′′**	7.53	1H
111.7	**5′′**	6.87	1H
123.4	**6′**	7.64	1H
52.0	7′-OMe	3.88	3H
56.0	3′-OMe	3.91	3H

Table-20. HMQC Correlations of Compound (**18**)

δ_C	C	δ_H	No
65.8	**1′**	4.68	2H
122.5	**2**	5.62	1H
47.8	**4**	2.20 & 2.27	2H
66.4	**5**	4.50	1H
127.3	**6**	5.45	1H
25.7	**8**	1.70	3H

18.2	**9**	1.67	3H
17.0	**10**	1.80	3H
112.2	**2′***	7.53	1H
111.8	**5′****	6.86	1H
123.4	**6′**	7.64	1H
52.0	7′-OMe	3.88	3H
56.0	3′-OMe	3.90	3H

Table-21. HMBC Correlations of Compound (17)

δ_C	C	TYPE	H
119.9	**2**	methine	3H-10, 2H-4, 2H-1
140.1	**3**	quaternary	2H-4
42.2	**4**	methylene	3H-10
124.0	**5**	methine	2H-4
140.3	**6**	methine	3H-8, 3H-9, 2H-4
70.7	**7**	quaternary	3H-8, 3H-9
29.8	**8**	methyl	3H-9
29.8	**9**	methyl	3H-8
122.6	**1′**	quaternary	H-5′
112.1	**2′***	methine	H-6′
149.0	**3′****	quaternary	H-5′, 3′-OMe
152.2	**4′***	quaternary	2H-1, H-6′, H-2′
123.4	**6′**	methine	H-2′
166.9	7′	carbonyl	7′-OMe, H-2′, H-6′

Table-22. HMBC Correlations of Compound (18)

δ_C	C	TYPE	H
122.5	**2**	methine	3H-10, 2H-1
138.0	**3**	quaternary	3H-10, 2H-4, 2H-1
47.8	**4**	methylene	3H-10
66.4	**5**	methine	2H-4
127.3	**6**	methine	3H-8, 3H-9
135.4	**7**	quaternary	3H-8, 3H-9
25.7	**8**	methyl	3H-9
18.2	**9**	methyl	3H-8
122.6	**1′**	quaternary	H-5′
112.2	**2′**†	methine	H-6′
148.9	**3′**†	quaternary	H-5′, 3′-OMe
152.1	**4′**†	quaternary	2H-1, H-6′, H-2′
123.4	**6′**	methine	H-2′
167.0	**7′**	carbonyl	7′-OMe, H-2′, H-6′

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Chapter-7

Scapania robusta

INTRODUCTION

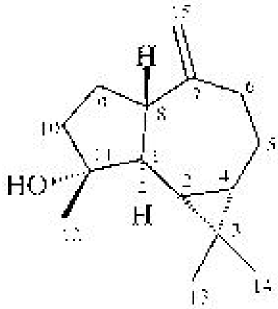
Scapania robusta of the family Scapaniaceae belongs to Jungermanniales. No monoterpenes have been reported in this liverwort. However some trisnorsesquiterpenoids and sesquiterpenoids discovered in this liverwort[1] are: aromadendrene (1), β-gurjunene (2), anastreptene (3), β-barbatene (4), α-amorphene (5), 1S,4S-calamenene (6), cuparene (7), 2-hydroxycuparene (8), (-)-δ-cuparene (9), *ent*-α-selinene (10), *ent*-β-selinene (11), selina-4,11-diene (12), α-humulene (13), longifolene (14), and α-spirovetivene (15).

Scapania undulata is another liverwort belonging to the same family. It has already been shown to contain sesquiterpenoids and labdane type diterpenoids[2,3] and is highly evolved in terms of chemical criteria since its sesquiterpenoid features are very complex[4]. European *Scapania undulata* comprises three chemical races : an (+)-*ent*-epicubenol (16) type, a longifolene (14) type and a longiborneol (17) type[4]. *Ent*-longifolene (14), *ent*-longiborneol (17), *ent*-α-longipinene (18), *ent*-β-longipinene (19), and longipinanol (20) were earlier reported from *Scapania undulata* together with *ent*-longicyclene (21). Among these, longifolene (14) has also been detected in *Scapania robusta*[5].

RESULTS AND DISCUSSION

The plant material (160 g) was collected in Taiwan, ground and extracted with diethyl ether for several days. The crude extract (1.8 g) was subjected to flash chromatography with increasing percentages of ethyl acetate and petroleum ether. The analytical plates of the fractions showed a main spot, which was non-uv active and present in most of the fractions. Further purification was carried out by preparative TLC, and the NMR spectra of this main compound showed that it was *ent*-spathulenol (1), which is a constituent of many liverworts[6].

The molecular formula, $C_{15}H_{24}O$, was readily apparent from the ^{13}C NMR spectrum which showed an exomethylene group [δ 153.4 (C-7) ; δ 106.2 (C-15)], a tertiary alcohol [δ 81.0 (C-11)], three methyls [δ 26.0 (C-12) ; δ 28.6 (C-13) ; δ 16.4 (C-14)], four methylenes [δ 24.8 (C-5) ; δ 38.8 (C-6) ; 26.7 (C-9) ; δ 41.7 (C-10)] and



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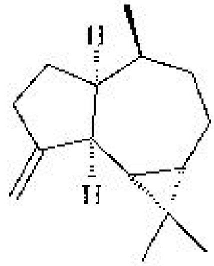

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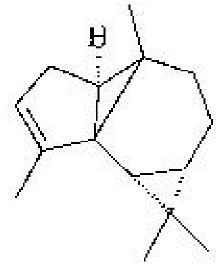

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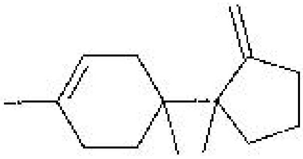

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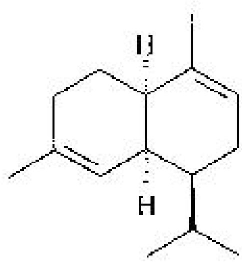

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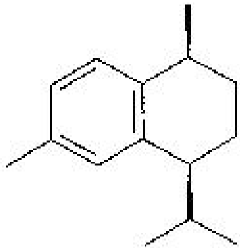

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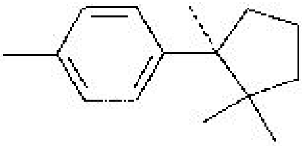

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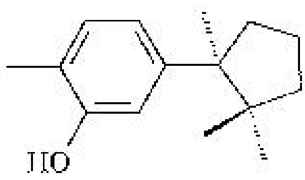

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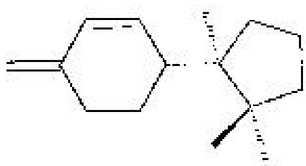

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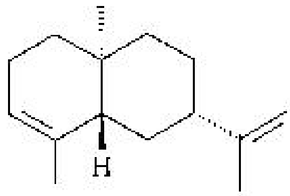

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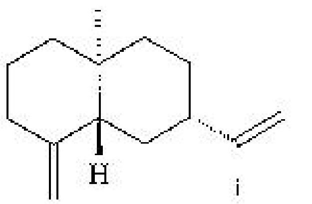

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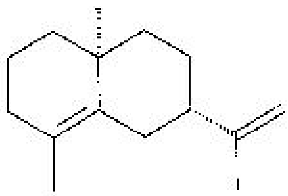

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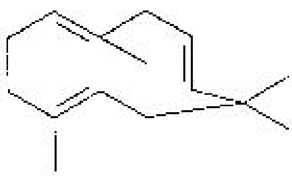

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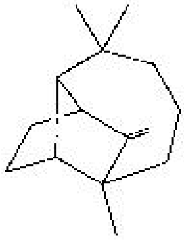

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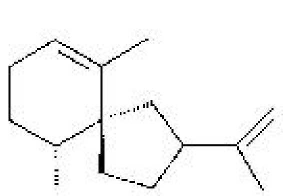

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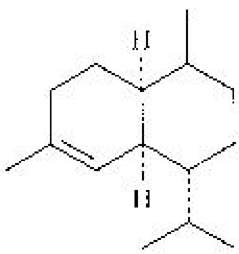

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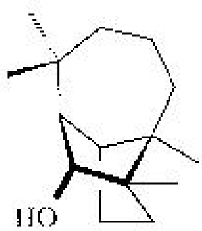

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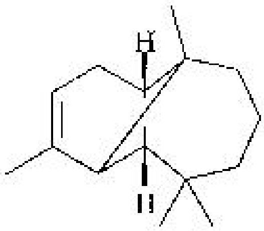

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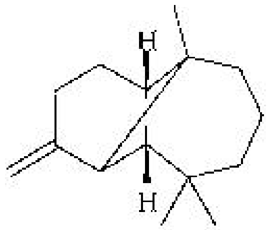

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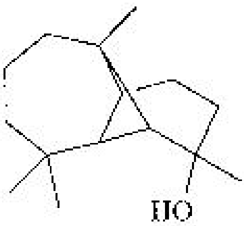

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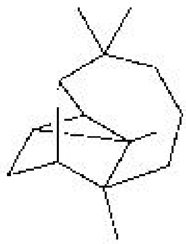
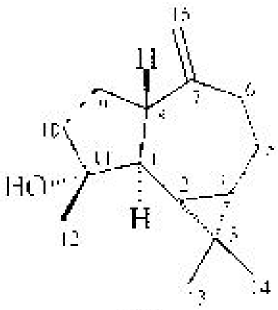


(21)

four methines [δ 54.3 (C-1) ; δ53.4 (C-8) ; δ 29.9 (C-2) ; δ 27.1 (C-4)]. Hence it was a tricyclic compound containing an exomethylene group, a tertiary alcohol and a cyclopropane. The presence of the cyclopropane group δ 0.46 [t, J= 9.7 Hz, H-2], δ 0.70 [m (H-4)] and the exomethylene group, δ 4.65 [brs, H_a-15], δ 4.68 [brs, H_b-15], was readily observed in the ^{1}H-NMR spectrum, which also revealed three tertiary methyl groups δ 1.27 [s, 3H-12], δ 1.05 [s, 3H-13], δ 1.03 [s, 3H-14], two of which are the geminal methyl groups. Apart from these a methine group was observed at δ 2.20 [m, H-8] and one of the protons of C-6 was at δ 2.41 [dd, J= 13.3 6.2 Hz, H_a-6]. The NMR data are given in Tables 1-4.



(1)

Table-1. ^{13}C and ^{1}H NMR Data of Compound (1)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	54.3	1.28	Overlapping with 3H-12
2	29.9	0.46	t (9.7 Hz)
3	20.3		
4	27.5	0.70	m
5	24.8	0.99 (H_a-5)	Overlapping with 3H-13 and 3H-14
6	38.8	2.41 (H_a-6)	dd (13.3 Hz, 6.2 Hz)
7	153.4		
8	53.4	2.20	m
9	26.7		
10	41.7	1.76 (H_a-10)	m
11	81.0		

12	26.0	1.27	s
13	28.6	1.05	s
14	16.3	1.03	s
15	106.2	4.65 (H_a-15) 4.68 (H_b-15)	brs brs

Table-2. HMQC Correlations of Compound (**1**)

δ_C	C	δ_H	No
54.3	**1**	1.28	1H
29.9	**2**	0.46	1H
27.5	**4**	0.70	1H
24.8	**5**	0.99 (H_a-5)	2H
38.8	**6**	2.41 (H_a-6)	2H
53.4	**8**	2.20	1H
26.7	**9**		2H
41.7	**10**	1.76 (H_a-10)	2H
26.0	**12**	1.27	3H
28.6	**13**	1.05	3H
16.3	**14**	1.03	3H
106.2	**15**	4.65 (H_a-15) 4.68 (H_b-15)	2H

Table-3. HMBC Correlations of Compound (**1**)

δ_C	C	**TYPE**	**H**
54.3	**1**	methine	3H-12
29.9	**2**	methine	3H-13, 3H-14, H-1
20.3	**3**	quaternary	3H-13, 3H-14
27.5	**4**	methine	3H-14, 3H-13, 2H-6

24.8	**5**	methylene	2H-6
38.8	**6**	methylene	2H-15
153.4	7	quaternary	2H-6, H-1
53.4	**8**	methine	2H-6, 2H-15
41.7	**10**	methylene	3H-12
28.6	**13**	methyl	H-2, H-4
106.2	**15**	exomethylene	2H-6

Table-4. NOEDIFF Correlations of Compound (**I**)

IRRADIATION	**ENHANCEMENT**
H_b-15	H_a-15, H_a-9
H_a-6	H_c-6, H_b-15
H-1	3H-13
3H-12	H-2, 3H-13
3H-14	H-2, H-4
3H-13	H-1
H-2	H-4
H-4	H-2

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Chapter-8

Inula klengii

INTRODUCTION

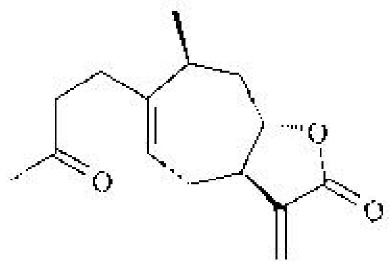
The leaves of the Compositae (*Asteraceae* Dumort.) family are usually alternate or opposite, toothed, lobed or variously dissected, exstipulate[1]. The flowers are generally small and called florets, and the fruits are small, dry, seed like nuts called achenes[1]. The Compositae is one of the largest families of flowering plants comprising about 950 genera with probably some 20,000 species[1]. The wide medicinal use of many composites inspired the early organic chemists to explore their chemistry in order to identify the active constituents[2]. Many substances elaborated by the family are toxic or show other significant physiological activity[2].

Sesquiterpene lactones are the most characteristic single group of chemicals known in the Compositae[2]. They are colourless, often bitter-tasting, lipophilic constituents mainly found in leaf tissues[2]. Sesquiterpene lactones are known to have feeding deterrent effects on rabbits, deer and other browsing animals. Some show insecticidal activity[2]. The lactones are not only the feeding toxins in the case of mammals but they also cause allergic contact dermatitis. For a lactone to possess dermatitic activity, it should contain a γ-lactone grouping with an exocyclic α-methylene function. This was deduced from several experiments carried out on man, and over eighty sesquiterpene lactones have been used[2]. This grouping is present in practically all the common lactones[2].

One of the most distinctive features in the biochemistry of the Compositae is the production of storage polysaccharides based on fructose instead of glucose. These unusual polysaccharides are known as fructans[2]. The tribe *Inuleae* is known to have 180-200 genera with some 2100 species seen generally in South Africa, Australia and to some extent in South America and the Mediterranean[2]. The *Inuleae* include a selection of plants which have useful properties, e.g. elecampane, *Inula helenium*, was used for treating chest diseases and its roots have been used as sweetmeats[2].

Commonly occurring mono- and sesquiterpenes have been identified in several plants of the *Inuleae*. Pinene, limonene, caryophyllene have been found in *Achyrocline satureioides*, borneol and camphor in *Blumea balsamifera*, and α-ionone and cadinene were found in *Sphaeranthus indicus*[2]. Some other compounds that are found in the *Inuleae* species are summarised below.

One of the species of the *Inuleae*, *Inula viscosa*, was examined by Bohlmann *et al*[3] several years ago. They found two sesquiterpene lactones (1), (2), and a new



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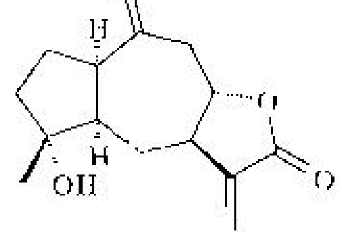

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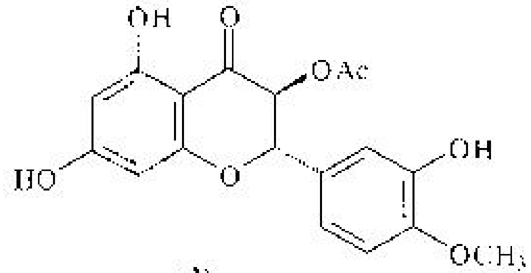

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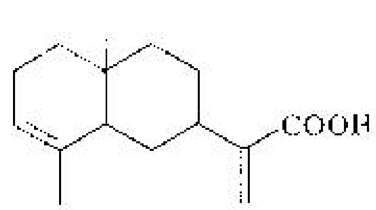

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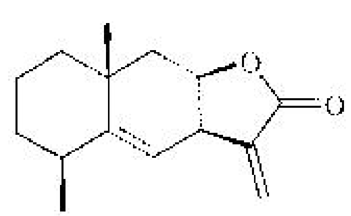

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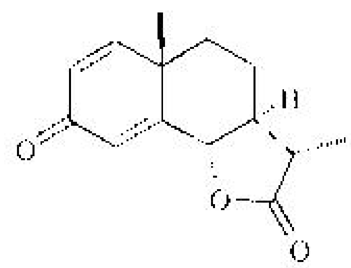

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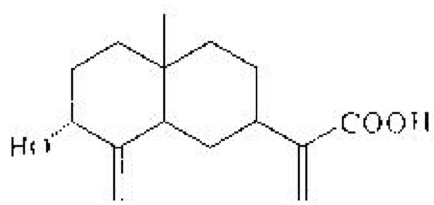

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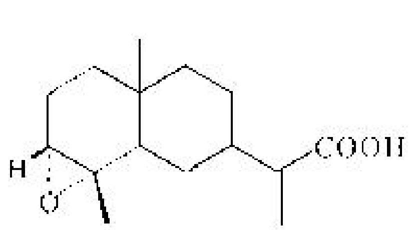

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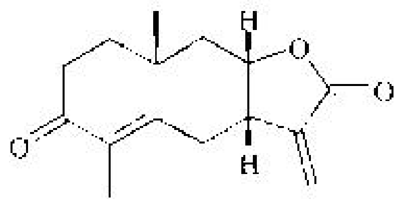

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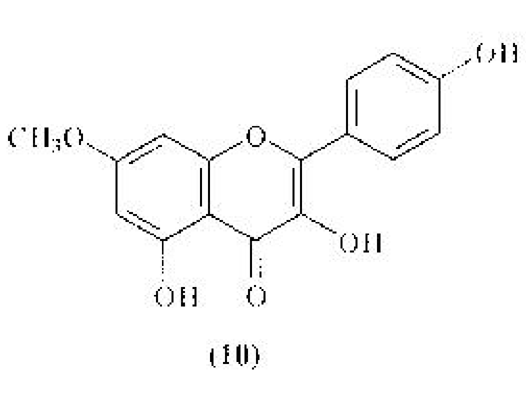

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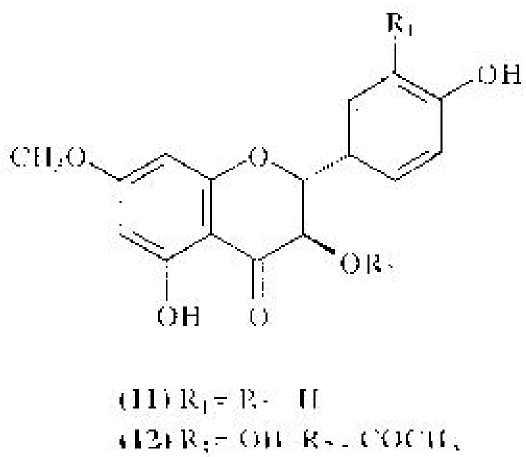
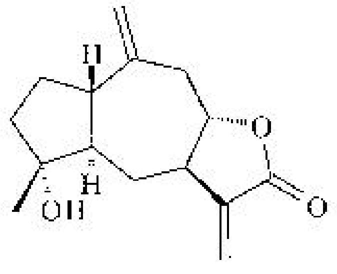


(11) $R_1 = R_2 = H$

(12) $R_1 = OH$, $R_2 = COCH_3$



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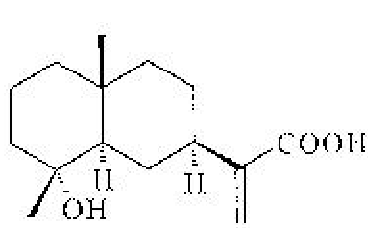

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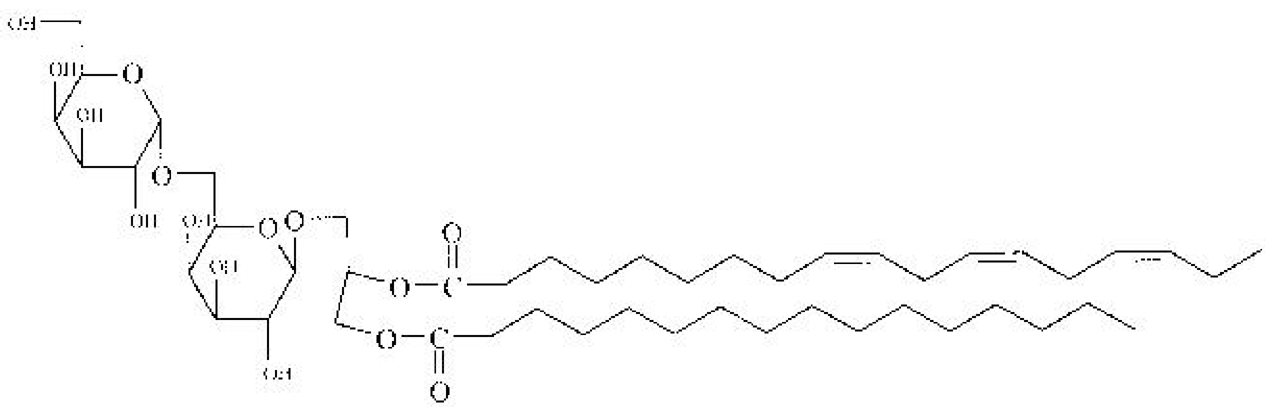

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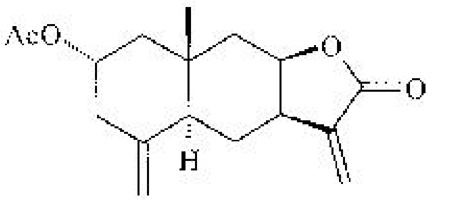

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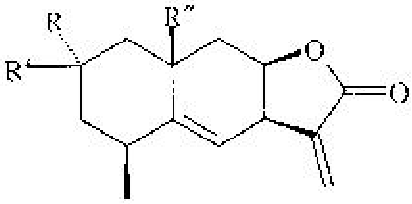
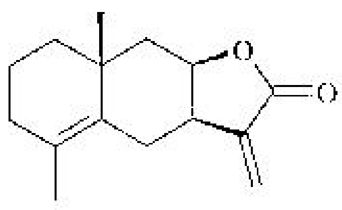


(**17**) R′= R″= H, R= OH
(**18**) R= R′= H, R″= OH



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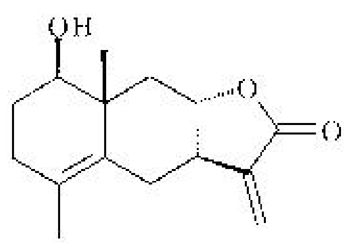

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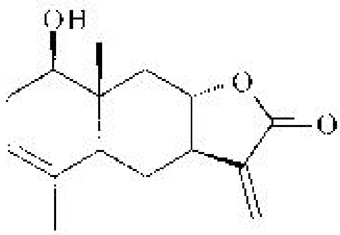

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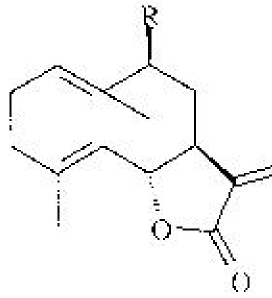
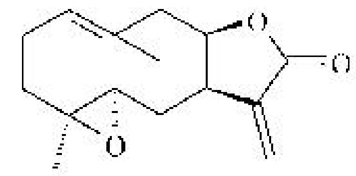


(22) R= OH
(23) R= OCOEt
(24) R= OCOCH(Me)Et
(25) R= $COCH_2CHMe_2$
(26) R= $COCHMe_2$



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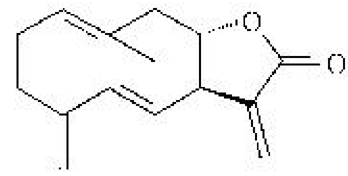

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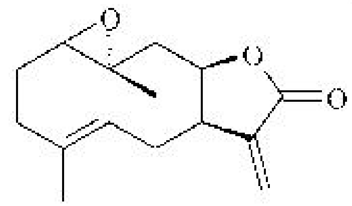

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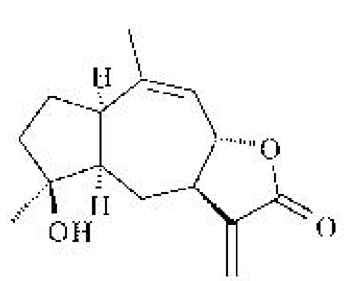

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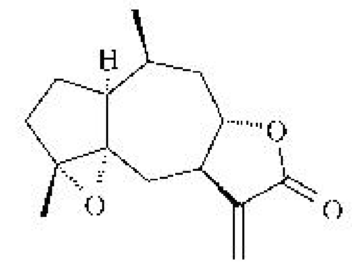

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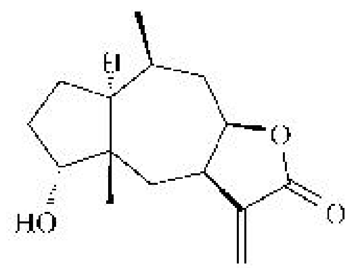

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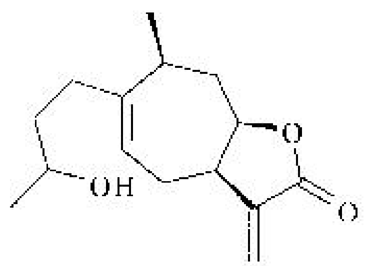

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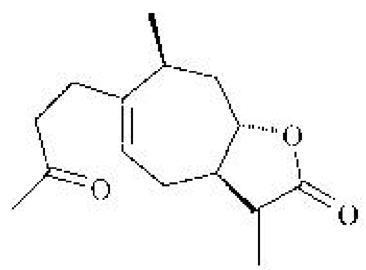

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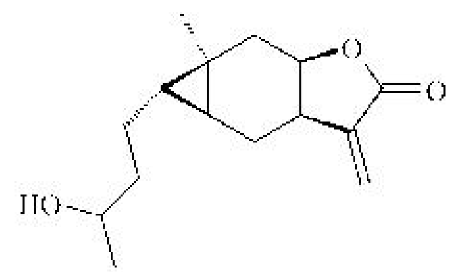

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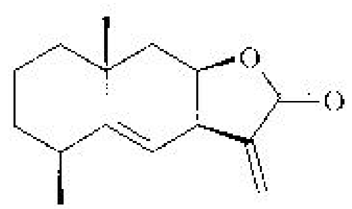

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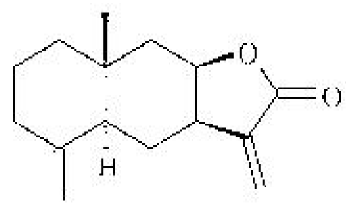

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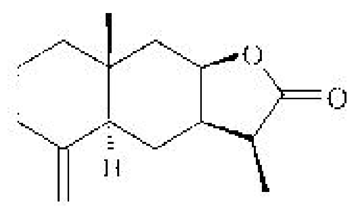

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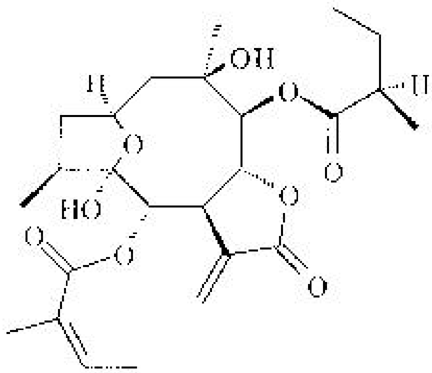

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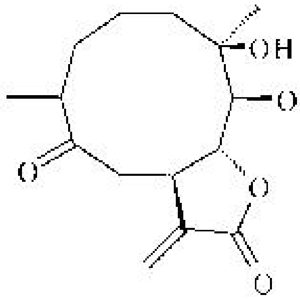
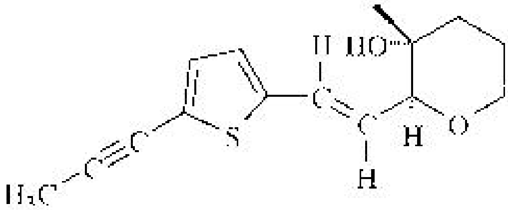


(40) R= MeBu
(41) R= Ang



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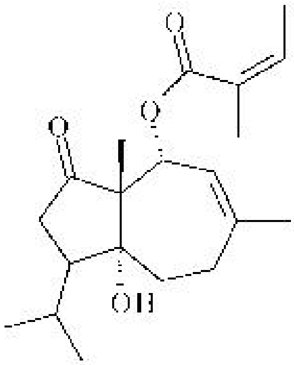

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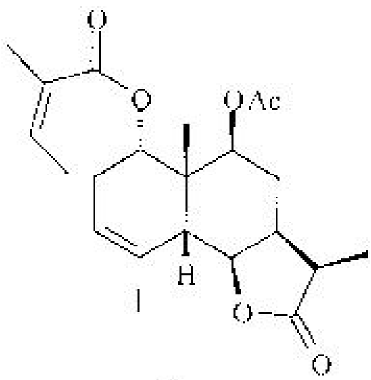

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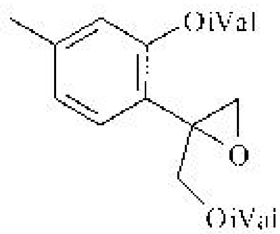

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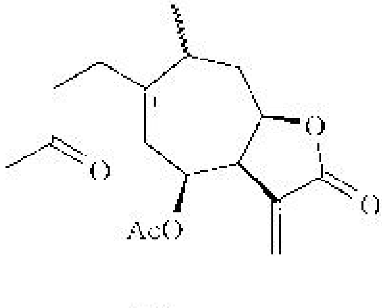

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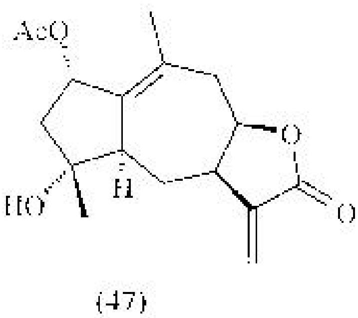

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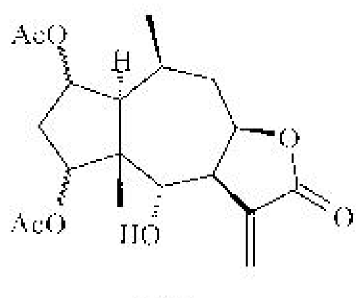


(48)

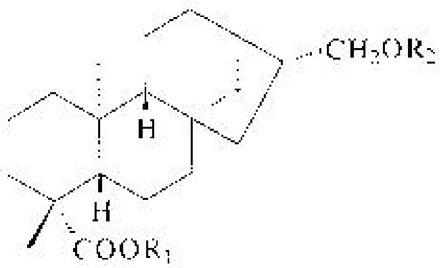
flavanone (3). Another examination of the above-ground portions of *Inula viscosa* by Azoulay *et al*[4] gave 5,11(13)-eudesmadien-12-oic acid (4), helenin (5) and santonin (6). Two new sesquiterpene acids, viscic acid (7) and viscosic acid (8) have been isolated from the aerial parts of *Inula viscosa* by Ulubelen *et al*[5]. Recently, Maoz *et al*[6] have isolated a new sesquiterpene, tayunin (9), from the leaves of *I. viscosa* while Máñez *et al*[7] have found three flavanoids, rhamnocitrin (10), 7-O-methylaromadendrin (11) and 3-O-acetylpadmatin (12), a sesquiterpene lactone, inuviscolide (13), a sesquiterpene acid, ilicic acid (14), and a digalactosyl-diacylglycerol, inugalactolipid A (15), in the extracts of *Inula viscosa*.

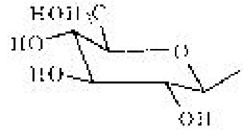
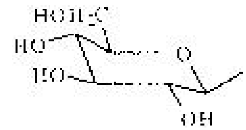
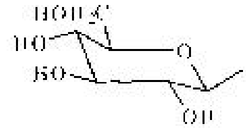
Examination of some *Inula* species, *I. helenium*, *I. royleana*, *I. salicina* and *I. bifrons* by Bohlmann *et al*[8] gave some new sesquiterpene lactones; the eudesmanolides, (16)-(21), the germacranolides, (22)-(26) and (27)-(29), the guaianolides (30) and (31), the pseudoguaianolide, (32), the xanthanolides, (33) and (34) and the cyclopropane analogue (35). In a recent investigation, some antimycobacterial eudesmanolides, (36), (37), (38), have been found in one of these species, *I. helenium*, by Cantrell *et al*[9].

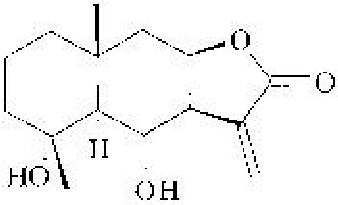
Three new germacranolides, ineupatolide (39), ineupatorolide A (40) and B (41) have been isolated from the chloroform extract of *I. eupatorioides* by Baruah *et al*[10] and the acetylenic 3-hydroxy-tetrahydropyran, ineupatoriol (42) was found in the non polar part of the extract[11].

The aerial parts of *Inula crithmoides*, examined by Mahmoud *et al*[12], afforded a new carotene derivative (43) and a eudesmanolide, inucrithmolide (44). A later study on the same plant by Metwally *et al*[13] gave a new epoxythymol isovalerate (45).

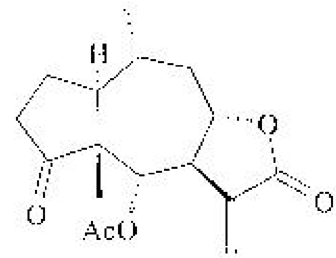
Another species, *Inula britannica*, was investigated by Ito *et al*[14] and three new lactones, in addition to four known sesquiterpene lactones, have been isolated. These new compounds are inuchinenolides A (46), B (47) and C (48). Later, Shao *et al*[15] isolated two new diterpene glycosides, (49) and (50). *Inula britannica* has been used as a traditional medicine in Eastern Asia for the treatment of digestive disorder, bronchitis and inflammation[16]. Four sesquiterpene lactones, (51), (52), (53), (54), that showed cytotoxicity against human tumor cell lines, were isolated by Park *et al*[16] from *I. britannica*. In a recent study on the same plant by Park *et al*[17] revealed three new acylated flavonol glycosides, (55), (56) and (57).



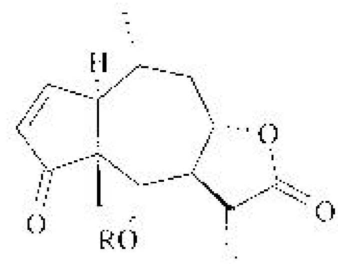
	R_1	R_2
(49)	H	
(50)		



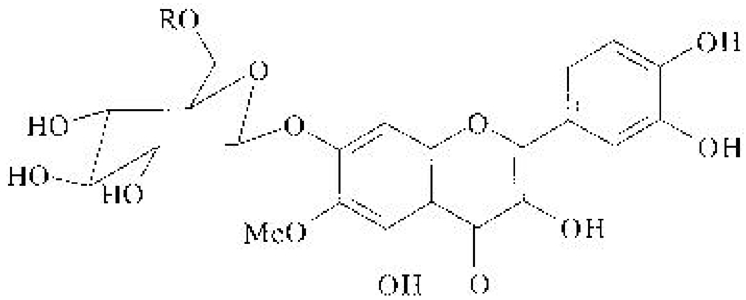
(51)



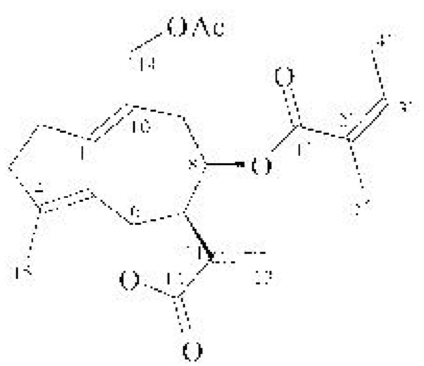
(52)



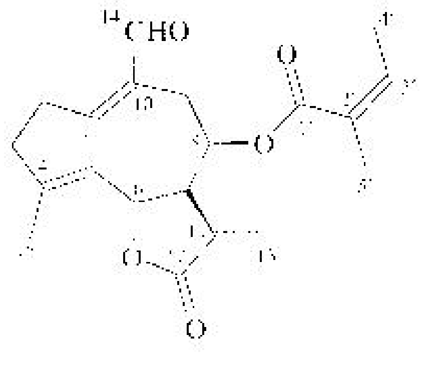
(53) R = H
(54) R = Ac



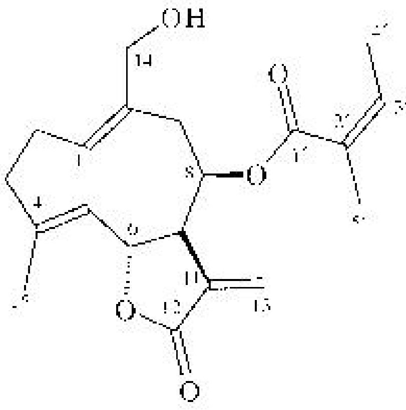
(55) R = $COCH(CH_3)_2$
(56) R = $COCH(CH_3)CH_2CH_3$
(57) R = $COCH_2CH(CH_3)_2$



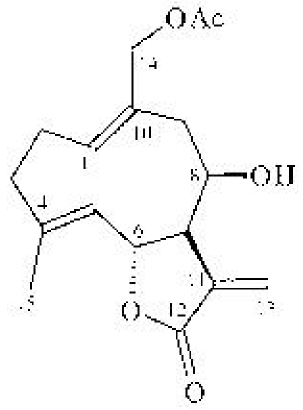
(58)



(59)



(60)



(61)

Apart from the species mentioned above, many others have also been investigated: e.g. *I. cappa*[18], *I. cuspidata*[19], *I. heterolepis*[20], *I. indica*[21], *I. oculus christi*[22], *I. racemosa*[23].

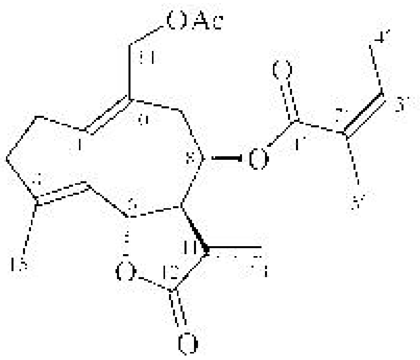
RESULTS AND DISCUSSION

The plant extract of *Inula klengii* was sent to us by a colleague in Cameroon. The extract consisted largely of wax and fat, which made it difficult for us to run a decent analytical plate. Around 58.7 g of the extract was taken and evaporated until all the remaining solvent [methanol and dichloromethane (1:1)] had been removed. Then some *n*-hexane was added in order to get a clean extract. By shaking the flask vigorously and decanting the hexane extract from the rest we successfully managed to remove the wax. This procedure was done several times. The crude hexane extract obtained was 15 g and 7 g of it was subjected to flash chromatography with increasing percentages of ethyl acetate with petroleum ether. The first fractions of the flash chromatography consisted of fats as expected. The crude ^{1}H NMR spectra of the other fractions showed that the compounds present in these fractions were sesquiterpene lactones. Most of the fractions were subjected to a sephadex column for purification followed by preparative TLC.

Four sesquiterpene lactones, 8β-angeloyloxy-14-acetyl-1(10),4,11(13)-germacratrien-12,6α-olide **(58)**, melampolide **(59)**, 8β-angeloyloxy-14-hydroxy-1(10),4,11(13)-germacratrien-12,6α-olide **(60)**, and ovatifolin **(61)**, were isolated from these fractions. However, this is the first time that these four sesquiterpene lactones have been isolated from the species, *Inula klengii* and the two compounds **(58)** and **(60)** are new.

Compound **(58)** was present in fraction 40, which was eluted with 40% ethyl acetate and petroleum ether. The molecular formula, $C_{22}H_{28}O_6$, was readily apparent from the ^{13}C NMR spectrum, which showed three vinyl methyl carbons [δ 17.0 (C-15), δ 15.9 (C-4'), δ 20.5 (C-5')] and one acetyl methyl at δ 21.0. There are five methylene carbons, one of which is the exomethylene carbon at δ 120.5 (C-13) and the other at δ 68.3 (C-14) bears the acetoxy group, and six methine carbons, three of which belong to the three trisubstituted double bonds at δ 131.0 (C-1), δ 125.6 (C-5), δ 139.2 (C-3'), two are oxygenated at δ 76.0 (C-6) and δ 66.4 (C-8) and the other one appears at δ 49.5 (C-7). Three ester carbonyls appear at δ 170.5 (OAc), δ 166.6 (C-1′) and at δ 169.6 (C-12). In the ^{1}H NMR spectrum there are well separated exomethylene protons, α to the γ-lactone group, at δ 6.23 [d, J= 3.5 Hz, (H_a-13)] and at δ 5.48 [d, J= 3.2 Hz, (H_b-13)]. One of the trisubstituted double bond protons is at δ 6.08 [qq, J= 8.7, 1.4 Hz, (H-3′)], and belongs to the angelate moiety. The other two double bond protons are at δ 5.58 [brt, J= 7.0 Hz, (H-1)] and at δ 5.11 [brd, J= 10.0 Hz, (H-5)], the latter was overlapping with a methine proton, H-6, that is at δ 5.06 [brt, J= 10.0, 9.0 Hz]. One of the oxygenated methine protons is quite deshielded, at δ 5.85 [ddd, J= 11.0, 7.0, 2.0 Hz, (H-8)]. Another methine proton is at δ 2.96 [dq, J= 9.0, 2.0 Hz, (H-7)]. The two methylene protons that are next to the acetyl group appear as doublets at δ 4.64 [d, J= 12.5 Hz, (H_a-14)] and at δ 4.46 [d, J= 12.5 Hz, H_b-14)]. Other methylene protons are observed at δ 2.51 [brdd, J= 14.0, 7.0 Hz, (H_a-9)] and at δ 2.11 [brdd, J= 14.0, 2.5 Hz, (H_b-9)]. The two vinyl methyl groups of the angelate moiety are at δ 1.95 [dq, J= 7.2, 1.5 Hz, (3H-4′)] and at δ 1.82 [quintet, J= 1.5 Hz, (3H-5′)]. The other vinyl methyl, 3H-15, is observed at δ 1.88 as singlet. The IR spectrum showed αβ-unsaturated γ-lactone absorption at 1760.

The stereochemistry of these lactones was revealed by NOE DIFFERENCE experiments, carried out on compound **(61)** (see Table 15). The ring junction between C-6 and C-7 is trans since that the coupling constant between H-6 and H-7 is around 9.0 Hz. Irradiation of H-6 gave NOE only to 3H-15 which means H-6 and H-7 are not cis. However H-7 and H-8 are cis to each other (see Table 15). Compound **(58)** is a new sesquiterpene lactone. The NMR data can be seen in Tables 1-4.



(58)

Table 1- ^{13}C and ^{1}H NMR Data of Compound **(58)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	131.0	5.58	brt (7.0 Hz)
2	25.0	2.15-1.90	m, (overlapping with 2H-3)
3	38.0	2.15-1.90	m, (overlapping with 2H-2)
4	138.6		
5	125.6	5.11	brd (10.0 Hz)
6	76.0	5.06	brt (10.0 Hz, 9.0 Hz)
7	49.5	2.96	dq (9.0 Hz, 2.0 Hz)
8	66.4	5.85	ddd (11.0 Hz, 7.0 Hz, 2.0 Hz)
9	31.6	2.51	brdd (14.0 Hz, 7.0 Hz)
		2.11	brdd (14.0 Hz, 2.5 Hz)
10	133.0		
11	135.6		
12	169.6		
13	120.2	6.23	d (3.5 Hz)
		5.48	d (3.2 Hz)
14	68.3	4.64	d (12.5 Hz)
		4.46	d (12.5 Hz)
15	17.0	1.88	s
1′	166.6		
2′	127.1		
3′	139.2	6.08	qq (8.7 Hz, 1.4 Hz)
4′	15.9	1.95	dq (7.2 Hz, 1.5 Hz)
5′	20.5	1.82	quintet (1.5 Hz)

OAc	21.0 170.5	2.06	s

Table 2-HMQC Correlations of compound (58)

δ_C	C	δ_H	No
131.0	**1**	5.58	1H
25.6	**2**	2.15-1.90	2H
38.0	**3**	2.15-1.90	2H
125.6	**5**	5.11	1H
76.0	**6**	5.06	1H
49.5	**7**	2.96	1H
66.4	**8**	5.85	1H
31.6	**9**	2.51, 2.11	2H
120.2	**13**	6.23, 5.48	2H
68.3	**14**	4.64, 4.46	2H
17.0	**15**	1.88	3H
139.2	**3'**	6.08	1H
15.9	**4'**	1.95	3H
20.5	**5'**	1.82	3H

Table 3-HMBC Correlations of Compound (**58**)

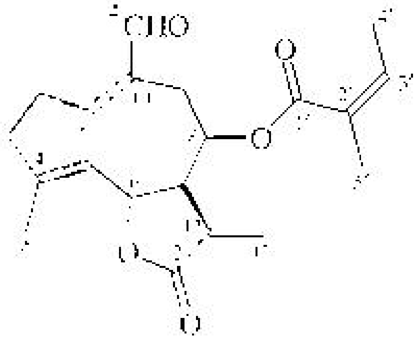
δ_C	C	TYPE	H
131.0	**1**	methine	2H-14, 2H-9
25.0	**2**	methylene	H-1, 2H-3
38.0	**3**	methylene	H-5, 3H-15
138.6	**4**	quaternary	H-6, 3H-15
125.6	**5**	methine	3H-15, 2H-3
76.0	**6**	methine	H-8, H-7
49.5	**7**	methine	2H-13, 2H-9, H-5
66.4	**8**	methine	H-6, 2H-9
31.6	**9**	methylene	H-8, H-1, 2H-14
133.0	**10**	quaternary	2H-9, 2H-14
135.6	**11**	quaternary	2H-13, H-7
169.6	**12**	carbonyl	2H-13
68.3	**14**	methylene	H-1, 2H-9
17.0	**15**	methyl	H-5
166.6	**1′**	carbonyl	H-8, 3H-5′
127.1	**2′**	quaternary	3H-5′, 3H-4′
139.2	**3′**	methine	3H-5′, 3H-4′
20.5	**5′**	methyl	H-3′
170.5	**OAc**	carbonyl	2H-14

Table 4-COSY Correlations of Compound (**58**)

δ_H	**H**	**TYPE**	**H**
5.11	5	methine	3H-15
5.06	6	methine	H-7
2.96	7	methine	H-6, 2H-13
5.82	8	methine	2H-9

2.51, 2.11	**9**	methylene	H-8
6.23, 5.48	**13**	methylene	H-7
6.08	**3'**	methine	3H-4'
1.95	**4'**	methyl	H-3'

Compound **(59)** was isolated from fraction 60. It has the molecular formula $C_{20}H_{24}O_5$ deduced from the ^{13}C NMR spectrum. Its ^1H NMR spectrum revealed the presence of α,β-unsaturated γ-lactone group, which was previously observed in compound **(58)**. Among the distinct features of its ^1H NMR spectrum is an aldehyde signal seen at δ 9.14 [d, J= 1.5 Hz, (H-14)], which has caused deshielding effects especially on the nearby atoms (see Table 5). The primary acetate function of compound **(58)**, was not seen in the ^1H NMR spectrum of compound **(59)**. We could still see the angelate moiety at δ 6.09 [qq, J= 7.3, 1.5 Hz (H-3')], and the three vinyl methyls at δ 1.91 [s, (3H-15)], δ 1.97 [dq, J= 7.2, 1.5 Hz, (3H-4')], δ 1.83 [quintet, J= 1.5 Hz, (3H-5')]. The NMR data of **(59)** are listed in Tables 5-7. This compound was previously isolated by Bohlmann *et al*[24] from the aerial parts of *Gracielia intermedia* but this is the first time that this compound has been isolated from, *Inula klengii*. The NMR data are in agreement with those given in literature[23,25].



(59)

Table 5: ^{13}C and ^1H NMR Data of Compound (59)

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	153.9	6.60	m
2	26.2	2.53, 2.30	m, (overlapping with 2H-3)

3	37.2	2.39, 2.09	m, (overlapping with 2H-2)
4	143.0		
5	126.4	5.08	m, (overlapping with H-6)
6	75.6	5.04	m, (overlapping with H-5)
7	49.5	2.49	m
8	65.6	6.47	ddd (10.0 Hz, 7.5 Hz, 2.0 Hz)
9	29.0	2.82	brdd (14.0 Hz, 7.0 Hz)
		1.95	overlapping with 3H-4′
10	135.0		
11	137.7		
12	169.5		
13	121.0	6.23	d (3.5 Hz)
		5.59	d (3.1 Hz)
14	195.3	9.44	d (1.5 Hz)
15	17.0	1.91	s
1′	166.4		
2′	127.1		
3′	139.4	6.09	qq (7.3 Hz, 1.5 Hz)
4′	15.9	1.97	dq (7.2 Hz, 1.5 Hz)
5′	20.6	1.83	quintet (1.5 Hz)

Table 6-HMQC Correlations of Compound **(59)**

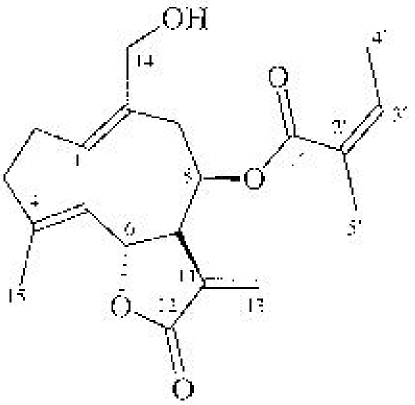
δ_C		δ_H	**No**
153.9	**1**	6.66	1H
26.2	**2**	2.53, 2.30	2H

37.2	**3**	2.39, 2.09	2H
126.4	**5**	5.08	1H
75.6	**6**	5.04	1H
49.5	**7**	2.49	1H
65.6	**8**	6.47	1H
29.0	**9**	2.82, 1.95	2H
121.0	**13**	6.23, 5.59	2H
195.3	**14**	9.44	1H
17.0	**15**	1.91	3H
139.4	**3′**	6.09	1H
15.9	**4′**	1.97	3H
20.6	**5′**	1.83	3H

Table 7-HMBC Correlations of Compound **(59)**

δ_C		**TYPE**	**H**
37.2	3	methylene	3H-15
49.5	**7**	methine	2H-9, 2H-13
65.6	**8**	methine	2H-9
29.0	**9**	methylene	H-14, H-1
169.5	**12**	carbonyl	2H-13
195.3	**14**	carbonyl	H-1
166.4	**1′**	carbonyl	3H-5′
127.1	**2′**	quaternary	3H-4′
139.4	**3′**	methine	3H-4′

The other two compounds **(60)** and **(61)** were present in fraction 70. The NMR data of compound **(60)** are almost identical with those of compound **(58)**. The only difference is the replacement of the acetoxy group, which is attached to C-14 in **(58)**, by a hydroxyl group. The methylene protons 2H-14 are observed at higher field, δ 4.14 [d, J= 12.5 Hz, (H_a-14)] and δ 4.10 [d, J= 12.3 Hz, (H_b-14)], overlapping with each other (see Tables 8-10). A sesquiterpene lactone with a tiglate moiety on C-8 has been isolated from *Stevia maimarensis* by Hernández *et al*[26]. However this is the first time that a lactone with an angelate moiety on C-8 has been found. The other compound, **(61)**, is known as ovatifolin and has been isolated previously by Gnecco *et al*[27] from *Podanthus ovatifolius* and, in a later study, by Hoeneisen *et al*[28] in *Podanthus mitiqui*. The NMR data are consistent with those given in literature[28, 27] (see Tables 11-15).



(60)

Table 8 ^{13}C and ^{1}H NMR Data of **(60)**

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	127.8	5.59	brt (7.3 Hz)
2	24.9	2.17-1.91	m, (overlapping with 2H-3)
3	38.2	2.17-1.91	m, (overlapping with 2H-2)
4	138.6		

5	125.5	5.11	brd (10.2 Hz)
6	76.0	5.06	brt (10.2 Hz, 9.0 Hz)
7	49.5	2.98	dq (9.0 Hz, 2.0 Hz)
8	66.8	5.94	ddd (11.0 Hz, 7.0 Hz, 2.0 Hz)
9	31.4	2.56 2.06	dd (13.0 Hz, 7.0 Hz) dd (13.0 Hz, 2.4 Hz)
10	137.5		
11	135.7		
12	169.8		
13	120.2	6.22 5.48	d (3.4 Hz) d (3.1 Hz)
14	67.0	4.14 4.10	d (12.5 Hz) d (12.3 Hz)
15	17.0	1.87	s
1′	166.8		
2′	127.2		
3′	139.2	6.09	qq (8.4 H, 1.1 Hz)
4′	15.9	1.95	dq (8.4 Hz, 1.1 Hz)
5′	20.6	1.83	quintet (1.3 Hz)

Table 9-HMQC Correlations of **(60)**

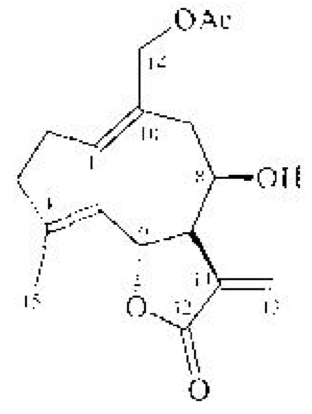
δ_C	**C**	δ_H	**No**
127.6	**1**	5.59	1H
24.9	**2**	2.17-1.91	2H
38.2	**3**	2.17-1.91	2H

125.5	**5**	5.11	1H
76.0	**6**	5.06	1H
49.5	**7**	2.98	1H
66.8	**8**	5.94	1H
31.4	**9**	2.56, 2.06	2H
120.2	**13**	6.22, 5.48	2H
67.0	**14**	4.14, 4.10	2H
17.0	**15**	1.87	3H
139.2	**3′**	6.09	1H
15.9	**4′**	1.95	3H
20.6	**5′**	1.83	3H

Table 10-HMBC Correlations of **(60)**

δ_C	**C**	**TYPE**	**H**
127.8	**1**	methine	2H-14, 2H-9, 2H-3
24.9	**2**	methylene	H-1, 2H-3
38.2	**3**	methylene	H-5, 3H-15
138.6	**4**	quaternary	H-6, 3H-15
125.5	**5**	methine	3H-15, 2H-3
76.0	**6**	methine	H-8
49.5	**7**	methine	2H-13, H-6, 2H-9
66.8	**8**	methine	H-6, 2H-9
31.4	**9**	methylene	H-8, H-1, 2H-14
137.5	**10**	quaternary	2H-14, 2H-9
135.7	**11**	quaternary	2H-13
169.8	**12**	carbonyl	2H-13
67.0	**14**	methylene	2H-9, H-1

17.0	**15**	methyl	H-5, 2H-3
166.8	**1′**	carbonyl	H-8, 3H-5′
127.2	**2′**	quaternary	3H-4′, 3H-5′
130.2	**3′**	methine	3H-4′, 3H-5′
20.6	**5′**	methyl	H-3′



(61)

Table 11: ^{13}C and ^{1}H NMR Data of **(61)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	136.6	5.15	dd (12.0 Hz, 4.0 Hz)
2	25.7	2.25-2.50	m, (overlapping with 2H-3)
3	39.0	2.10-2.50	m, (overlapping with 2H-2)
4	142.0		
5	127.7	4.84	brd (10.0 Hz)
6	74.7	5.30	dd (10.0 Hz, 8.0 Hz)
7	53.6	2.75	m
8	71.2	4.56	m, (overlapping with H_b-14)
9	42.3	2.93	dd (15.0 Hz, 5.4 Hz)
		2.20	brd (15.0 Hz)

10	132.9		
11	138.3		
12	170.0		
13	120.3	6.35 5.56	d (3.0 Hz) d (3.0 Hz)
14	63.0	4.79 4.56	brd (12.0 Hz) brd (12.0 Hz)
15	16.9	1.62	d (1 Hz)
OAc	21.0 171.5	2.07	s

Table 12-HMQC Correlations of (**61**)

δ_C	C	δ_H	No
136.6	**1**	5.13	1H
25.7	**2**	2.25-2.50	2H
39.0	**3**	2.10-2.50	2H
127.7	**5**	4.84	1H
74.7	**6**	5.20	1H
53.6	**7**	2.75	1H
71.2	**8**	4.56	1H
42.3	**9**	2.93, 2.20	2H
120.3	**13**	6.35, 5.56	2H
63.0	**14**	4.79, 4.56	2H
16.9	**15**	1.62	3H

Table 13-HMBC Correlations of (**61**)

δ_C	C	TYPE	H
136.6	**1**	methine	2H-14, 2H-9
25.7	**2**	methylene	2H-3
39.0	**3**	methylene	3H-15, 2H-2, H-5
142.0	**4**	quaternary	3H-15, 2H-2, H-6
121.7	**5**	methine	2H-3, 3H-15
74.7	**6**	methine	H-7, H-8
53.6	**7**	methine	2H-9, H-5, 2H-13, H-6
71.2	**8**	methine	2H-9, H-6
42.3	**9**	methylene	2H-14, H-1
132.9	**10**	quaternary	2H-14, 2H-9, 2H-2
138.3	**11**	quaternary	2H-13, H-7
170.0	**12**	carbonyl	2H-13
120.3	**13**	methylene	H-7
63.0	**14**	methylene	2H-9, H-1
16.9	**15**	methyl	H-5, 2H-3
171.5	**OAc**	carbonyl	2H-14

Table 14-COSY Correlations of (**61**)

δ_H	H	TYPE	H
4.84	H-5	methine	H-6, 3H-15
5.20	H-6	methine	H-7, H-5
2.78	H-7	methine	H-8, H-6, 2H-13
4.56	H-8	methine	2H-9, H-7
2.93, 2.20	2H-9	methylene	H-8
6.35	H_2-13	exomethylene	H-7

5.56	H_b-13	exomethylene	H-7
4.79	H_a-14	methylene	H_b-14
4.56	H_b-14	methylene	H_a-14
1.62	3H-15	methyl	H-5

Table 15 NOEDIFF Correlations of **(61)**

IRRADIATIONS	**ENHANCEMENTS**
H-1	H-5, H_b-9
H-5	H-1, H-7
H-6	3H-15
H-7	H-5, H_b-9, H-8
H-8	H_b-13, 2H-9, H-7
H_a-9	H_b-9, H-8
H_a-13	H_b-13
H_b-13	H_a-13, H-8
H_a-14	H_b-14, H-6, 3H-15
H_b-14	H_a-14
3H-15	H-6, 2H-14

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Chapter-9

Marsupella aquatica

INTRODUCTION

In Britain, the liverwort *Marsupella aquatica* is very common on rocks in hill streams. It differs from the species *M. emarginata* in a number of small points. It grows taller (3-8 cm), is dull blackish green rather than red brown in colour, and has the leaves more channelled in form and less deeply bifid[1]. *Marsupella* species are rich sources of longipinane type sesquiterpenoids which are significant chemical markers of the Marsupellaceae[2].

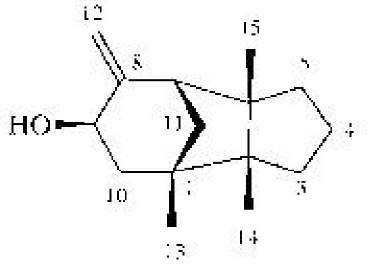
The compounds isolated previously from the species *M. aquatica* include, gymnomitr-8(12)-en-9β-ol (**1**)[3], 9,11α,14-triacetoxymarsupellone (**2**), 9,11β,14-triacetoxymarsupellone (**3**) and 9,14-diacetoxymarsupellone (**4**)[4], (-)-*ent*-12α-acetoxylongipin-2(10)-en-3-one (**5**)[5], lemnalol[6] (**6**), marsupellone[10] (**7**), and acetoxymarsupellone (**8**). The related species *M. emarginata* contains the *ent*-longipinane,9-acetoxymarsupellol (**9**)[7], eremophila-9,11-dien-8α-ol (**11**)[8] and the three gymnomitranes (**12**)-(**14**)[9].

RESULTS AND DISCUSSION

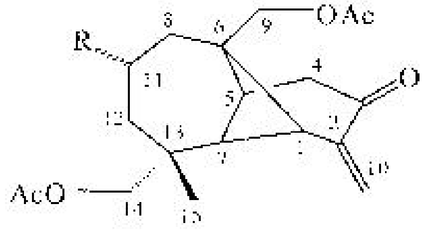
Plant material (250 g) collected on the Cobbler was ground and extracted with diethyl ether. Total crude extract, 2.32 g, was subjected to flash chromatography with increasing percentages of ethyl acetate and petroleum ether. Further purification was done by gel chromatography and preparative TLC.

Compounds (**7**) and (**8**) were present in the fraction eluted with 30 % diethyl ether in light petroleum. They were obtained as oils following purification by preparative TLC, eluent 10% ethyl acetate in petroleum ether.

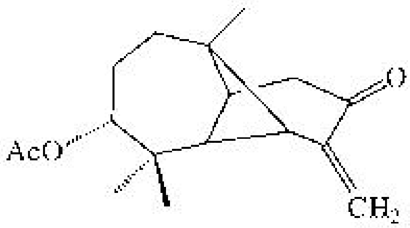
The molecular formula of compound (**7**), $C_{15}H_{22}O$, was readily apparent from its ^{13}C NMR spectrum (Table-1), which showed a carbonyl signal at δ_C 201.0, four methylenes (δ_C 44.6 ; δ_C 41.1 ; δ_C 39.4 ; δ_C 21.3), an exomethylene carbon at δ_C 115.5, three methines (δ_C 59.2 ; δ_C 46.9 ; δ_C 37.2), three methyls (δ_C 27.7 ; δ_C 27.5 ; δ_C 23.1) and three quaternary carbons at δ_C 33.1, δ_C 42.8 and at δ_C 150.5, which is the quaternary carbon of the exomethylene group. Its ^{1}H NMR spectrum (Table-1)



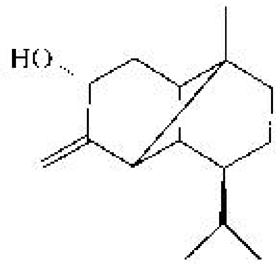
(1)



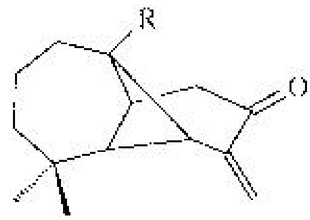
(2) R = α-OAc
(3) R = β-OAc
(4) R = H



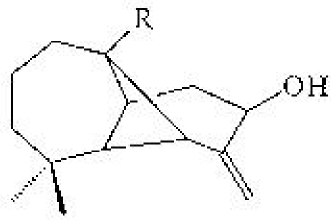
(5)



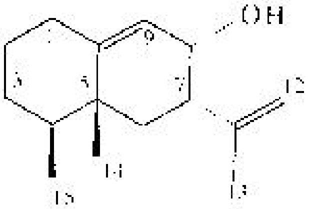
(6)



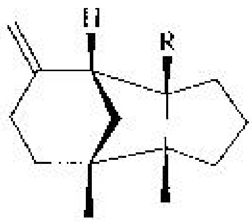
(7) R= CH_3
(8) R= CH_2OAc



(9) R= CH_2OAc
(10) R=CH_3



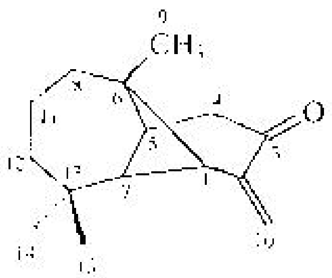
(11)



(12) R = CH_2OH
(13) R = CO_2H
(14) R = CHO

clearly showed the exomethylene protons at δ_H 5.87(d, J= 2.0 Hz) and at δ_H 4.91(d, J= 2.0 Hz) and the three methy groups at δ_H 0.75, δ_H 0.89 and at δ_H 0.90 as singlets, the latter two were very close to each other in the ^{1}H NMR spectrum giving almost one big singlet. Apart from the overlapping signals, the rest of the ^{1}H NMR spectrum was quite clear and the signals were easily assigned. The three methine protons appeared at δ_H 2.81(d, J= 6.8 Hz), δ_H 2.27(m) and at δ_H 1.35(s). The methylene protons next to the carbonyl were seen at δ_H 2.68 (dd, J= 19.0 Hz and 3.0 Hz) and at δ_H 2.47 (dd, J= 19.0 Hz and 3.0 Hz), while the other methylene group appeared at δ_H 1.43 as multiplet.

The molecular formula, $C_{15}H_{22}O$, indicates five double bond equivalents and hence the molecule is tricyclic. However the elucidation of the structure was difficult due to overlapping of the signals in the ^{1}H NMR spectrum in deuteriochloroform. However in deuteriobenzene solution (Table-2), three well resolved tertiary methyl groups were seen at δ_H 0.60 (3H-9) ; δ_H 0.77 (3H-14) ; δ_H 0.71 (3H-15). Changes in chemical shifts were also observed for some of the other resonances: the exomethylene protons [δ_H 6.10(d, J= 2.0 Hz) ; δ_H 4.70(d, J= 2.0 Hz)], the methylene adjacent to the carbonyl group [δ_H 2.53(H_a-4, dd, overlapped with H-1) ; δ_H 2.30(H_b-4, dd, J= 18.8 Hz, 3.2 Hz)] and the methine protons [δ_H 2.55 (H-1, d, J= 6.8 Hz) ; δ_H 1.80 (H-5, m) ; δ_H 1.18 (H-7, s)]. All the protons are shielded in deuteriobenzene relative to deuteriochloroform except one of the exomethylene protons that is seen at around δ_H 6.10 as doublet. From its COSY correlations (in C_6D_6) (Table-3), we were able to get some fragments related to the structure. The most striking feature in the COSY spectrum was the ^{4}J (J= 6.8 Hz) correlation observed between H-1 and H-5. However the HMBC spectrum (Table-4) of the compound in deuteriobenzene provided more information about the structure. The correlations of the three tertiary methyls, especially, made the elucidation of the structure easier. In the HMBC spectrum, the highest field methyl correlates with C-1 (δ_C 47.0), C-5 (δ_C 37.3) and C-8 (δ_C 41.1). Thus these three carbons must be three bonds away from the methyl proton. The other two tertiary methyls [δ_H 0.77 (3H-14) ; δ_H 0.71 (3H-15)] correlate with C-12 (δ_C 39.6) and C-7 (δ_C 59.2), which are also three bonds away from the two methyls. The methine singlet H-7 (δ_H 1.18) correlates with the exomethylene quaternary carbon. This must be C-6, since H-1 is already occupying the allylic position. The NOE experiments (Table-5) provided further evidence about the structure of compound (7). Irradiation of the methyl at highest field gave NOEs at one of the methylene protons [δ_H 2.53(H_a-4)] adjacent to the carbonyl and at two of the methylene protons [δ_H 1.35 (2H-8)]. Irradiation of the methyl at δ_H 0.71 resulted in NOEs at H-7 (δ_H 1.15), H-5 (δ_H 1.80) and at two methylene protons 2H-12 (δ_H 1.23), while the third methyl at δ_H 0.77 resulted in NOEs at two methane protons, H-7 and H-1, and at two methylene protons (δ_H 1.23). The mass spectrum showed a parent ion peak at 218 m/z, which supported the structure (7). It is a compound, known as marsupellone that was isolated by Matsuo *et al*[18] from the liverwort *Marsupella emarginata* in 1979.



(7)

Table-1, ^{13}C and ^1H NMR Spectra of Compound **(7)** in $CDCl_3$

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	46.9	2.81	d (J= 6.8 Hz)
2	150.5		
3	201.0		
4	41.6	2.68	dd (J= 19.0 Hz, 3.0 Hz)
		2.47	dd (J= 19.0 Hz, 3.0 Hz)
5	37.2	2.27	m
6	42.5		
7	59.2	1.35	s
8	41.1	1.63	overlapping with 2H-11
9	23.1	0.75	s
10	115.5	5.87	d (J= 2.0 Hz)
		4.91	d (J= 2.0 Hz)

11	21.3	1.63	overlapping with 2H-8
12	39.4	1.43	m
13	33.1		
14	27.7	0.90	s
15	27.5	0.89	s

Table-2. ^{13}C and ^{1}H NMR Spectra of Compound (7) in C_6D_6

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	47.0	2.55	d (J= 6.8 Hz)
2	151.0		
3	198.8		
4	44.7	2.53 (H_a)	dd (overlapping with H-1)
		2.30 (H_b)	dd (J= 18.8 Hz, 3.2 Hz)
5	37.3	1.80	m
6	42.4		
7	59.2	1.18	s
8	41.1	1.35	m
9	23.0	0.60	s
10	114.9	6.10 (H_a)	d (J= 2.0 Hz)
		4.70 (H_b)	d (J=2.0 Hz)
11	21.6	1.40	m
12	39.6	1.23	m
13	33.0		
14	27.8	0.77	s
15	27.4	0.71	s

Table-3. COSY Correlations of Compound (7) in C_6D_6

δ_H	H	TYPE	H
2.55	**H-1**	methine	H-5
2.53, 2.30	**2H-4**	methylene	H-5
1.80	**H-5**	methine	2H-4, H-1
6.10	**H_a-10**	methylene	H_b-10
4.70	**H_b-10**	methylene	H_a-10

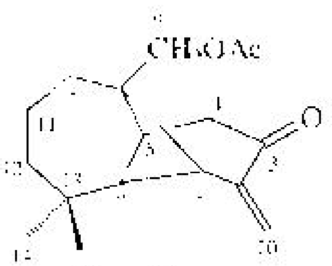
Table-4. HMBC Correlations of Compound (7) in C_6D_6

δ_C	C	TYPE	H
47.0	**1**	methine	2H-10, H-5, 3H-9
151.0	**2**	quaternary	H_a-10, H-1, H-7
198.8	**3**	carbonyl	2H-10, H-1, 2H-4, H-5
44.7	**4**	methylene	H-7
37.3	**5**	methine	3H-9, 2H-4, 2H-8, H-1
42.4	**6**	quaternary	H-7, 2H-11, 2H-4
59.2	**7**	methine	3H-14, 3H-15, 2H-4, 2H-12
11.3	**8**	methylene	3H-9, 2H-12, 2H-11
114.9	**10**	methylene	H-5
21.6	**11**	methylene	2H-8, 2H-12
39.6	**12**	methylene	3H-14, 3H-15, H-7
33.0	**13**	quaternary	3H-14, 3H-15, 2H-12, H-7, H-5
27.8	**14**	methyl	3H-15, H-7, 2H-12
27.4	**15**	methyl	3H-14, H-7, 2H-12

Table-5. NOEDIFF Correlations of Compound (7) in C_6D_6

IRRADIATIONS	ENHANCEMENTS
H-1	H_b-10, H-5, H_b-4
H_a-4	H_b-4, H-5
H_b-4	H_a-4, H-5
H-7	H_a-4, 3H-14, 3H-15
3H-9	H_b-4, 2H-8
H_a-10	H_a-10
H_b-10	H_a-10, H-1
3H-14	H-7, 2H-12, H-1
3H-15	H-7, H-5, 2H-12

The other compound **(8)** obtained from the same fraction, 30% ether in light petroleum, is known as acetoxymarsupellone[16]. Its NMR data are quite similar to those of compound (7). The differences it has in the ^{1}H-NMR spectrum (Table-6) is the acetate methyl which was observed at δ_H 2.0, the methylene protons at δ_H 3.75 (2H-9, d, J= 11.8 Hz) attached to the acetoxy group and some more overlap among the methylene protons. Its ^{13}C NMR spectrum also showed the acetoxy group ($OCOCH_3$) at δ_C 171.0 and at δ_C 20.8. The methylene carbon that is next to the acetoxy group was seen at δ_C 67.8. Its mass spectrum also supported the molecular structure, $C_{17}H_{24}O_3$, by showing a parent ion peak $[M^+]$ at 276 m/z. The NMR data (Table 6) are identical with those given in literature[18].



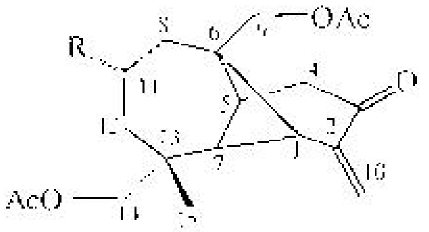
(8)

Table-6. ^{13}C and ^{1}H NMR Spectra of Compound **(8)** in $CDCl_3$

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	45.1	2.94	d(6.8 Hz)
2	149.0		
3	199.5		
4	44.2	2.72	dd(19.0 Hz, 3.0 Hz)
		2.55	dd(19.0 Hz, 3.0 Hz)
5	35.7	2.44	m
6	44.0		
7	59.2	1.40	s
8	35.7	1.79	m
		1.61	overlapping with H-11
9	67.8	3.75	d(11.8 Hz)
10	116.7	5.90	d(1.4 Hz)
		5.01	d(1.4 Hz)
11	20.9	2.0	overlapping with the acetate
		1.67	m
12	39.4	1.46	m
13	33.0		
14	27.8	0.92	s
15	27.3	0.93	s
-OAc	171.0		
	20.8	2.0	s

The other two compounds that we have found in this liverwort have been previously isolated by Nagashima *et al.*[1]. These two isomeric longipinane type sesquiterpenoids are called 9,11α,14-triacetoxy marsupellone **(2)**, and 9,11β,14-triacetoxy marsupellone **(3)**. Their NMR data were similar to those of marsupellone

(7) and acetoxymarsupellone (8) with respect to the signals of exomethylene protons, three methine protons in the four membered ring and the methylene protons next to the carbonyl. The difference lies in the presence of three acetate signals, one tertiary methyl and two oxygenated methylene signals that both isomers have. The ^{1}H NMR spectrum of (2) showed acetate signals at δ_H 2.06, 2.04, 2.02, one tertiary methyl at δ_H 1.10, two oxygenated methylenes at δ_H 3.77 3.80 as singlets, and an oxygenated methine at δ_H 5.22 as a multiplet. In its carbon NMR spectra the carbonyl carbon (δ_C 198.0), the three acetate carbonyls (δ_C 170.9, 170.8, 170.2), the exomethylene carbons (δ_C 147.5, 118.4), two oxygenated methylenes (δ_C 67.2, 71.9) and an oxygenated methine carbon (δ_C 68.6) were clearly observed. The rest of the NMR data for both isomers can be seen between (Table-7) and (Table-10). To understand the stereochemistry of the two isomers, NOEDIFF experiments were carried out. Irradiation of the tertiary methyl in compound (2) gave NOEs at the oxygenated methine (H-11) and the methine next to the exomethylene group (H-1). Thus, these protons must be on the same side of the molecule, which is β. However irradiation of the same methyl in the other compound (3) gave NOE only at H-1, which shows that the oxygenated methine must be α. Their mass spectra of both compounds showed a parent ion peak [M$^+$] at 392 m/z and in the IR spectra the acetoxyl groups (1725 and 1230 cm^{-1}) and a ketone group (1700 cm^{-1}) were observed.



(2) R = α -OAc
(3) R = β -OAc

Table-7 ^{13}C and ^{1}H NMR Spectra of Compound (2)

	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	43.5	3.11	d (J= 7.0 Hz)

2	147.5		
3	198.0		
4	43.5	2.77 (H_a-4) 2.56 (H_b-4)	dd (J= 19.2 Hz, 3.0 Hz) dd (J= 19.2 Hz, 3.0 Hz)
5	37.6	2.47	m
6	42.5		
7	54.1	1.60	s
8	40.7	2.20 (H_a) 1.80 (H_b)	ddd (J= 13.6 Hz, 4.8 Hz, 2.0 Hz)) dd (J= 11.0 Hz, 3.0 Hz)
9	67.2	3.77	s
10	118.4	5.99 (H_a) 5.10 (H_b)	d (J= 1.0 Hz) d (J= 1.0 Hz)
11	68.6	5.22	m
12	40.1	1.85 (H_a) 1.69 (H_b)	d (J= 13.0 Hz) d (J= 13.0 Hz)
13	35.1		
14	71.9	3.30	s
15	20.4	1.10	s
OAc	21.4 20.9 20.7 170.9 170.8 170.2	2.06 2.04 2.02	s s s

Table-8. NOEDIFF Correlations of Compound (**2**)

IRRADIATIONS	**ENHANCEMENTS**
H-1	H-11, 3H-15, H_b-10
H_a-4	H_b-4
H-7	2H-14, H_a-4, H-1, H-5

H_a-8	H-11, H_b-8
2H-9	H-1, H_a-4, H_a-8
H-11	H-1, 3H-15
H_a-12	H_b-12, H-5
2H-14	H-7, H-8, 3H-15
3H-15	H-11, H-1, 2H-14

Table-9. ^{13}C and ^{1}H NMR Spectra of Compound (3)

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	46.0	2.99	d (J= 6.8 Hz)
2	147.8		
3	198.2		
4	43.6	2.70 (H_a)	dd (J= 19.2 Hz, 3.0 Hz)
		2.52 (H_b)	dd (J= 19.2 Hz, 3.0 Hz)
5	34.6	2.60	m
6	42.6		
7	54.7	1.61	s
8	40.3	2.25 (H_a)	ddd (J= 13.6 Hz, 4.8 Hz, 2.0 Hz)
		1.76-1.65 (H_b)	overlapping with H_a-12
9	66.7	3.80 (H_a)	d (J= 12.0 Hz)
		3.73 (H_b)	d (J= 12.0 Hz)
10	117.6	5.97 (H_a)	d (J= 1.0 Hz)
		5.08 (H_b)	d (J= 1.0 Hz)
11	68.5	5.14	m
12	40.5	1.90	d
		1.76-1.65	overlapping with H_b-8
13	35.3		
14	68.6	4.16 (H_a)	d (J= 11.2 Hz)
		3.95 (H_b)	d (J= 11.2 Hz)
15	25.0	0.96	s

OAc	21.3	2.05	s	
	20.8	2.03	s	
	20.7	2.02	s	
	170.8			
	170.0			

Table-10. NOEDIFF Correlations of Compound (**5**)

IRRADIATIONS	**ENHANCEMENTS**
H-1	H_3-10, H-7
H-5	H-11, H-7
H_b-9	H_a-4, H_a-8
H-11	H-5
H_a-14	H_b-14, H-11, H-7
3H-15	H-1, H-7

Another known compound that we have isolated from this liverwort is called marsupellol (**10**), which was isolated by Matsuo *et al*[11] from *Marsupella emarginata*. The carbonyl group that was present in marsupellone is now replaced with a secondary hydroxyl group in marsupellol (**10**), which was seen at δ_H 4.46 as doublet of doublets in its ^1H NMR spectrum. Because of the newly introduced hydroxyl group, some chemical shift changes were observed for the protons compared to marsupellone. The signals of the exomethylene protons, which were quite separated in marsupellone, were now closer, appearing at δ_H 4.96 and 4.80 as broad singlets. The three tertiary methyls were observed at δ_H 0.89, 0.90, 0.60 as singlets. The methylene protons next to the secondary hydroxyl were at δ_H 2.34(ddd, J= 14.4 Hz, 8.2 Hz, 2.3 Hz) and at δ_H 1.82(dd, J= 14.3 Hz, 3.9 Hz). The signals of three methine protons in the four membered ring were also clearly seen. In its ^{13}C NMR spectrum the secondary hydroxyl was present at δ_C 67.5 and the exomethylene carbons were seen at δ_C 158.6 and 109.8. The rest of the ^1H and ^{13}C NMR shifts and multiplicities can be seen in Table-11. Finally, a parent ion peak [M^+] at 220 m/z was observed in its mass spectrum.

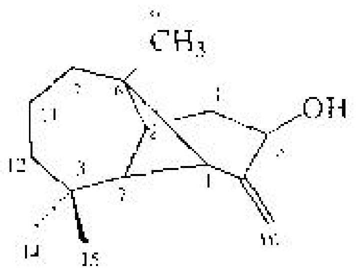


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Table-11. ^{13}C and ^1H NMR Spectra of Compound (10)

	δ_C	δ_H	**MULTIPLICITIES**
1	50.0	2.58	d (J= 5.7 Hz)
2	158.6		
3	67.5	4.46	dd (J= 8.1 Hz, 1.0 Hz)
4	37.5	2.34 (H_a)	ddd (J= 14.4 Hz, 8.2 Hz, 2.3 Hz)
		1.82 (H_b)	dd (J= 14.3 Hz, 3.9 Hz)
5	39.0	2.10	m
6	41.9		
7	55.5	1.76	s
8	41.2	1.70-1.45	m
9	24.0	0.60	s
10	109.8	4.96	brs
		4.80	brs
11	21.7	1.70-1.45	m
12	39.5	1.45-1.30	m
13	32.7		
14	28.2	0.89	s
15	27.7	0.90	s

Table-12, HMBC Correlations of Compound **(10)**

δ_C	C	TYPE	H
50.0	**1**	methine	2H-10, 2H-9, H-7
158.6	**2**	quaternary	H-7
67.5	**3**	methine	2H-10
39.0	**5**	methine	H-1, H-7, 3H-9
41.9	**6**	quaternary	H-7, 3H-9
55.5	**7**	methine	3H-14, 3H-15, H_a-1
41.2	**8**	merhylene	3H-9, 2H-12
39.5	**12**	methylene	3H-14, 3H-15
32.7	**13**	quaternary	3H-14, 3H-15, H-7

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Herbertus dicramus

&

Herbertus stramineus

INTRODUCTION

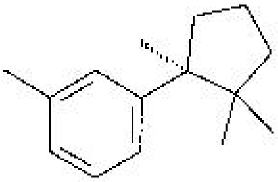
Among liverworts, the Herbertaceae is considered to form an ancient family in the Jungermanniales, based on their morphology and distribution[1]. Liverworts from the genus *Herbertus* contain herbertane-type sesquiterpenoids that can be considered as chemical markers of the genus[2-4].

Among the related species known in the genus *Herbertus*, Matsuo *et al*[4] have examined the liverwort *Herbertus aduncus* and isolated a sesquiterpene which they called (-)-herbertene (**1**). Then, Asakawa *et al*[5] examined *H. aduncus*, *H. sakuraii* and *H. subdentatus* collected in Canada, Colombia and Japan. These three species were chemically quite similar to each other. They all synthesised (-)-α-herbertenol (**2**), (-)-β-herbertenol (**3**) and herbertene (**1**). *H. aduncus* and *H. subdentatus* also synthesized (-)-herbertenediol (**4**), which was named as 2,3-dihydroxycuparene at the time by Asakawa *et al*[5], and they have concluded that herbertane- and cuparane-type sesquiterpenoids are characteristic constituents of *Herbertus* species[5]. However there is no substantiated, published evidence for the occurrence of any cuparenes in *Herbertus* species[1].

Later Matsuo *et al*[6] examined *Herbertus aduncus* and they have isolated (-)-α-herbertenol (**2**), (-)-β-herbertenol (**3**), and a new sesquiterpene phenol (-)-α-formylherbertenol (**5**). Again, from the same liverwort, *Herbertus aduncus*, (-)-herbertenediol (**4**), and a new aromatic sesquiterpenoid, (-)-herbertenolide (**6**), have been isolated by Matsuo *et al*[7].

Investigation of Scottish *H. aduncus* by Buchanan *et al*[1] led to the isolation of two new herbertane sesquiterpenoids, (-)-1,2-dihydroxyherberten-12-al (**7**) and methyl 1,2-dihydroxyherberten-12-oate (**8**) along with previously known (-)-herbertene (**1**), (-)-α-herbertenol (**2**), (-)-β-herbertenol (**3**) and (-)-herbertenediol (**4**).

Asakawa *et al*[8] reported seven new herbertane-type sesquiterpenoids from the liverwort *Herbertus sakuraii*. These were 1,13-dihydroxyherbertene (**9**), 1,14-dihydroxyherbertene (**10**), 1,15-dihydroxyherbertene (**11**), 12-methoxyherbertene-1,2-diol (**12**), herbertenelactol (**13**), herbertenone A (**14**), herbertenone B (**15**). *Herbertus sakuraii* was further investigated by Hashimoto *et al*[9] and three new bis(bibenzyls), 2,12-dichloroisoplagiochin D (**16**), 12,7′-dichloroisoplagiochin D (**17**), and 12,10′-dichloroisoplagiochin C (**18**), were reported.



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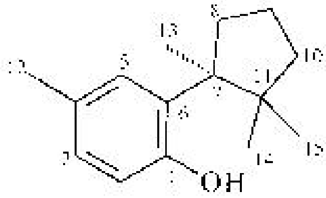

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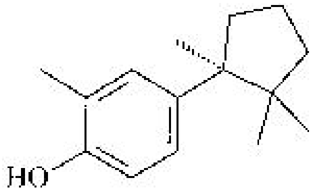

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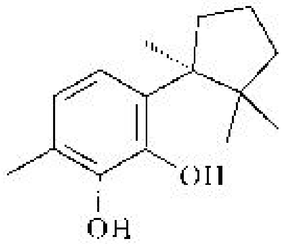

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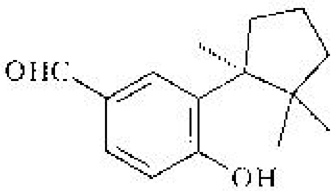

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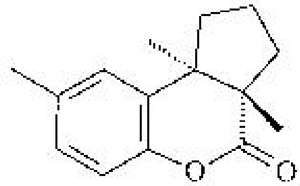

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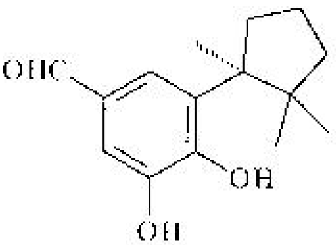

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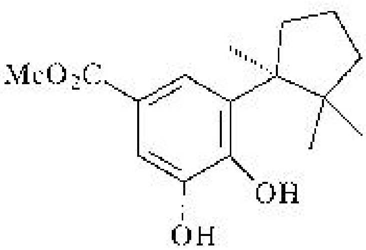

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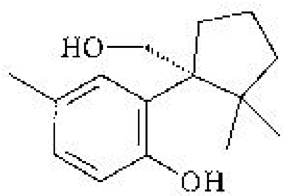

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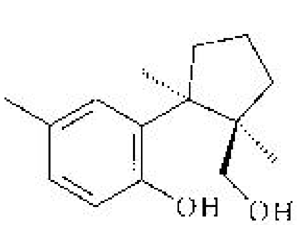

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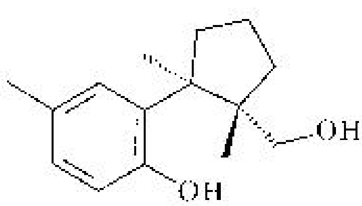

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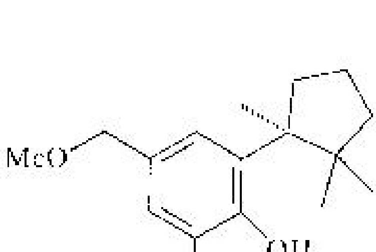

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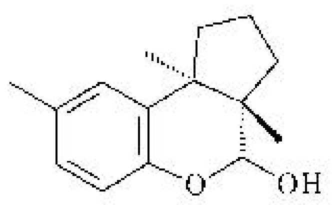

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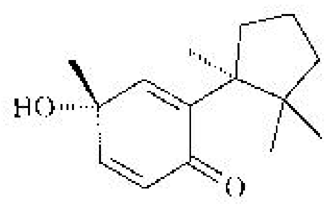

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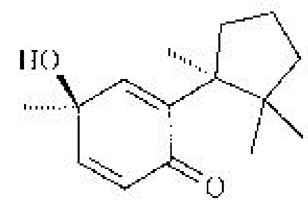

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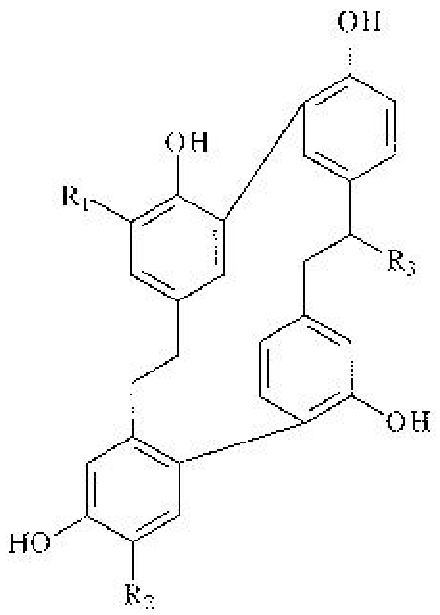
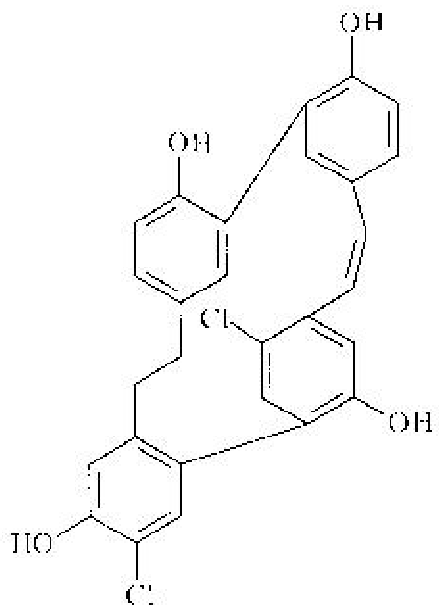


(16) $R_1=R_2=Cl$, $R_3=H$

(17) $R_1=H$, $R_2=R_3=Cl$



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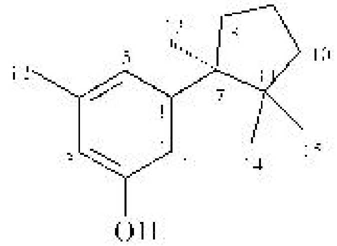

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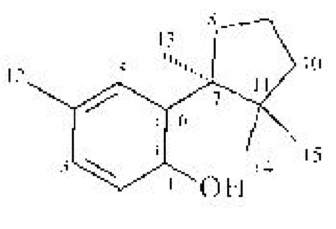
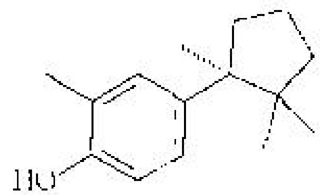
RESULTS AND DISCUSSION

The plants *Herbertus dicranus* and *Herbertus stramineus*, collected in Scotland, have been examined for the first time by us. The plant materials were dried, ground and extracted with diethyl ether. The total crude extract of *H. stramineus* was 2.4 g (from 135 g plant material) and 2.2 g crude extract (from 117 g plant material) was obtained from *H. dicranus*. Both extracts were subjected to flash chromatography and preparative TLC for purification. Two main constituents, (-)-α-herbertenol **(2)** and (-)-β herbertenol **(3)**, were present in both extracts. A new herbertane-type sesquiterpene **(19)** was isolated from *H. stramineus* (see below).

The structures of both α- and β- herbertenols were determined by comparison of their NMR data with those in the literature[6,7]. The NMR data of α- and β herbertenols found in both plants are given below. Because of a lack of material, ^{13}C NMR spectra could not be obtained for some compounds. However the mass spectra of all the samples supported the molecular formula of α- and β- herbertenols, $C_{15}H_{22}O$, by showing parent ion peaks at 218 m/z.





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(-)-α-herbertenol isolated from *H. dicranus* :

δ_H: 7.09 (H-5, d, J=1.7 Hz), 6.84 (H-3, dd, J=8.0 Hz, 2.0 Hz); 6.56 (H-2, d, J=8.0 Hz); 4.64 (OH, s); 2.25 (3H-12, s); 1.40 (3H-13, s); 1.18 (3H-14, s), 0.75 (3H-15, s); 2.58 and 1.77 (2H-9, m); 1.73 (2H-8, m), 1.64 and 1.55 (2H-10, m).

δ_C: 152.3 (C-1); 116.7 (C-2); 127.2 (C-3); 128.9 (C-4); 130.0 (C-5); 133.0 (C-6); 50.9 (C-7); 20.3 (C-8); 39.4 (C-9); 41.2 (C-10); 44.7 (C-11); 20.9 (C-12); 22.9 (C-13); 25.5 (C-14); 27.0 (C-15).

(-)-β-herbertenol isolated from *H. dicranus* :

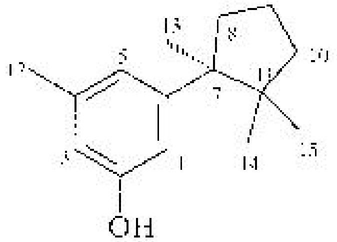
δ_H: 7.07 (H-5, s); 7.04 (H-1, dd, J=8.4 Hz, 2.4 Hz); 6.67 (H-2, d, J=8.3 Hz); 4.53 (OH, brs); 2.23 (3H-12, s); 0.55 (3H-13, s); 1.22 (3H-14, s); 1.03 (3H-15, s).

(-)-α-herbertenol isolated from *H. stramineus* :

δ_H: 7.09 (H-5, s); 6.86 (H-3, dd, J=8.0 Hz, 2.0 Hz); 6.56 (H-2, d, J=8.0 Hz); 4.64 (OH, s); 2.26 (3H-12, s); 1.40 (3H-13, s); 1.18 (3H-14, s); 0.75 (3H-15, s).

(-)-β-herbertenol isolated from *H. stramineus* :

δ_H: 7.07 (H-5, s); 7.04 (H-1, dd, J=8.3 Hz, 2.4 Hz); 6.67 (H-2, d J=8.3 Hz); 4.52 (OH, s); 2.24 (3H-12, s); 0.55 (3H-13, s); 1.22 (3H-14, s), 1.03 (3H-15, s)



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The new sesquiterpene **(19)** had a similar ^{1}H NMR spectrum to those of α- and β- herbertenols. The main difference concerned the proton signals of the benzene ring. The signals of the three benzene protons were now seen as broad singlets, at δ_H 6.73, 6.63 and at 6.47, which showed that the hydroxyl must be attached to C-2 on the ring, leaving a 1,3,5-trisubstituted benzene ring. The rest of the spectrum showed the four methyls, one of which is attached to the benzene ring, at δ_H 2.28, 1.22, 1.06 and at 0.58. The hydroxyl proton appeared at 4.53 as broad singlet. Due to the lack of quantity of the sample, it was not possible to obtain a ^{13}C NMR spectrum of the compound **(19)**. However, its mass spectrum showed a parent ion peak, $[M^+]$, at 218 m/z supporting the molecular formula, $C_{15}H_{22}O$, for this new compound, named as 2-hydroxyherbertene **(19)**.

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Chapter-11

Porella platyphylla

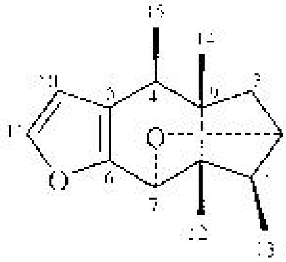
INTRODUCTION

Porella platyphylla is a robust plant which grows in fairly compact, flat, dark, or (more rarely) yellowish green patches[1]. It is mainly a plant of chalk and limestone districts, where it will grow on rock, tree roots and soil. It demands some shade and is sometimes the chief hepatic on the ground in beech woods on chalk[1]. One of the earliest investigations on *Porella platyphylla* was done by Nilsson[2], who found isovitexin, saponarin and another flavone in this liverwort.

The *Porella* species of liverworts produce various sesquiterpenes[3]. Asakawa and his colleagues have reported the isolation and structures of several drimane-, pinguisane-, and aromadendrane-type sesquiterpenes from *P. vernicosa* complex. The pungency of these species is due to a sesquiterpene dialdehyde, polygodial **(19)**[4,5]. Later in 1979, the chemical constituents of *P. platyphylla* were examined by Asakawa *et al*[3] and they found three new pinguisane-type sesquiterpenes, pinguisanin, pinguisanolide **(2)** and β-pinguisenediol **(3)**. They reported the ether linkage in pinguisanin to be between C-3 and C-7 position. However in 1987 Asakawa and his co-workers[6] isolated pinguisanin from British and French samples of *P. platyphylla* and re examined its structure by NMR including NOE difference experiments, which proved the structure of pinguisanin to be **(1)** with the ether linkage at C-2 and C-7. Pinguisanolide was further investigated by Connolly[19], who proved its structure to be **(2)**.

Another *Porella* species, *P. perrottetiana*, was investigated by Asakawa *et al*[7]. They found two diterpene dialdehydes, perrottetianal A **(4)**, perrottetianal B **(5)** and a labdane-type diol **(6)**. Perrottetianal A and B inhibit the germination of rice in the husk at *ca* 500 ppm[7]. In later work, Asakawa and Campbell[13] isolated perrottetianal A **(4)** from a related *Porella* species, *P. elegantula*, collected in New Zealand. The structure of perrottetianal B **(5)** was established by Nagashima *et al*[14] by the detailed analysis of its 2D NMR spectra.

Porella species are considered to be two different types, one containing a sharp pungent substance, polygodial, and one containing no pungent substance[5]. Asakawa and his colleagues examined non-pungent *Porella* species and found a cyclopropanoid, caespitenone **(9)**, whose relative stereochemistry was established by Tori *et al*[10], and the previously known sesquiterpenoids, (+)-aristolone **(7)**, (-)-α-



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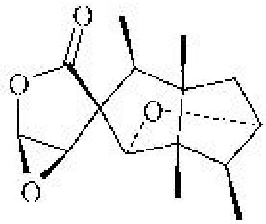

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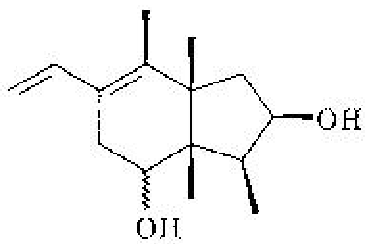

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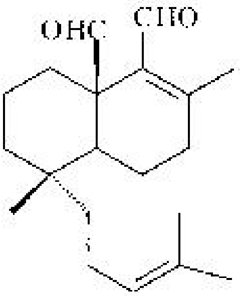

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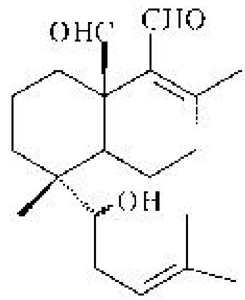

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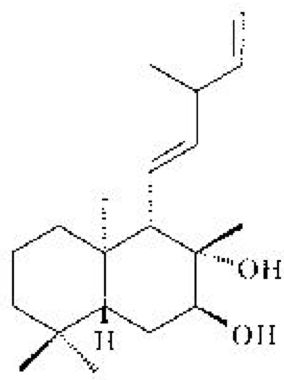

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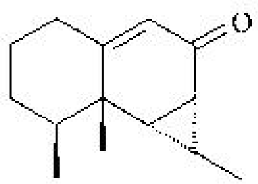

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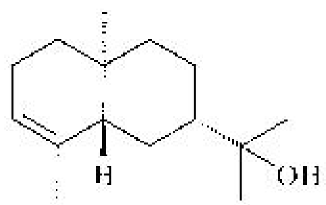

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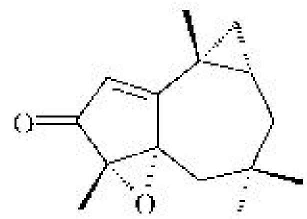

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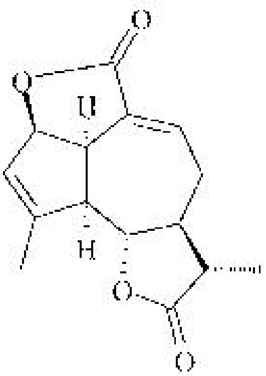

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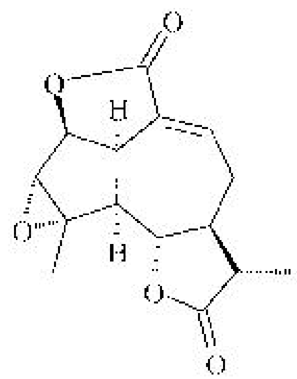

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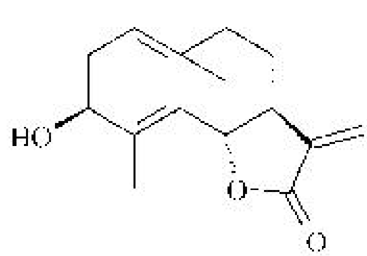

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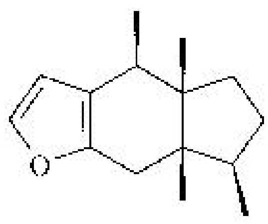

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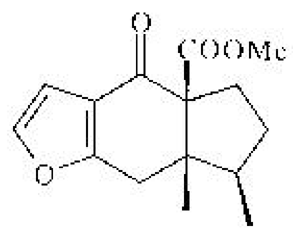

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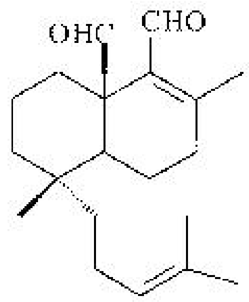

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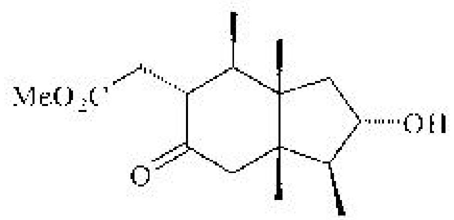

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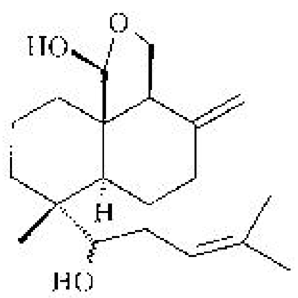

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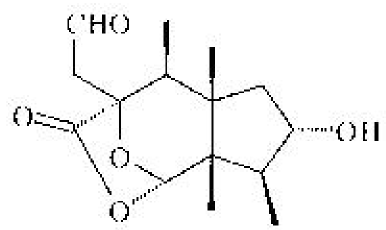

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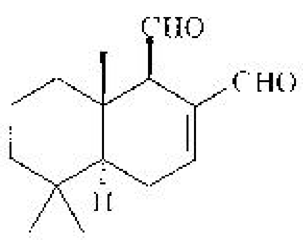

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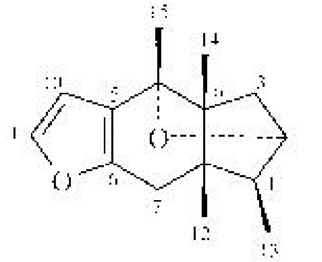

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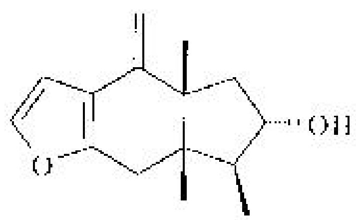


(21)

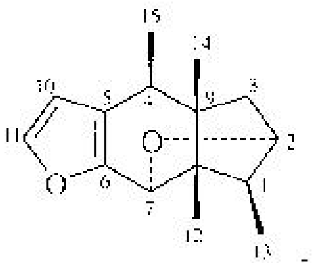
eudesmol (**8**), with related sesquiterpene hydrocarbons and alcohols enantiomeric to those found in higher plants[9].

Another related species called *Porella japonica* has been investigated by Asakawa *et al*[11] and two guaiane-type sesquiterpenoid dilactones, porelladiolide (**10**), 3α,4α-epoxyporelladiolide (**11**) and a germacranolide, 3β-hydroxyeustunolide (**12**) and their related sesquiterpene lactones. Previous examination of this liverwort by Asakawa *et al*[9,11] gave two pinguisanes (**13**) and (**14**), and a sacculatane-type diterpenoid dial (**15**) together with various mono- and sesquiterpene hydrocarbons.

A recent study of the liverwort *P. platyphylla* was carried out by Buchanan *et al*[13] who found a new pinguisane (**16**) and a new sacculatane (**17**) along with the known compounds pinguisanin (**1**), β-pinguisenediol (**3**), porellapinguisanolide (**18**) and perrottetianal B (**5**). *P. platyphylla* produces a wide range of terpenoid metabolites including mono-, sesqui-, di-, and triterpenoids[13,15,16].

RESULTS AND DISCUSSION

The plant material (33 g) was collected at Rowardennan, Loch Lomond. It was ground and extracted with diethyl ether. The crude extract (400 mg) was subjected to flash chromatography followed by preparative TLC. The major constituent, pinguisanin (**1**), present in fractions eluted with 30 % ethyl acetate in light petroleum was obtained as an oil, following purification by preparative TLC (eluent 25 % ethyl acetate in petroleum ether). The molecular formula, $C_{15}H_{20}O_2$ was readily apparent from the ^{13}C-NMR spectrum (Table-1) which showed two tertiary methyl signals (δ_C 25.3 ; δ_C 12.9), two secondary methyls (δ_C 20.0 ; δ_C 12.5), one methylene (δ_C 47.9), six methines, two of which oxygenated (δ_C 79.1 ; δ_C 76.0), two quaternary carbons (δ_C 51.0 ; δ_C 40.4) and two double bonds, one of which is tetra substituted. From its ^1H NMR spectrum the double bond protons were clearly observed at δ_H 6.25 (H-10) and at δ_H 7.34 (H-11), whose shifts revealed the presence of α,β-disubstituted furan ring in the structure. Two oxygenated methines [δ_H 4.37 (H-7, s) ; δ_H 3.95 (H-2, brs)], two secondary methines [δ_H 2.08 (H-1, brq, J=7.4 Hz) ; δ_H 2.58 (H-4, q, J=7.3 Hz)], the methylene protons [δ_H 1.60 (H_a-3, dd, J=14.0 Hz, 2.0 Hz) ; δ_H 1.90 (H_b-3, dd, J= 14.0 Hz, 3.0 Hz)] and the four methyls, two of which are secondary, were clearly observed in the ^1H NMR spectrum. In its IR spectrum, strong double bond ring stretches at 1470 and 1384 cm^{-1} were observed for the furan ring. However no hydroxyl bands were observed, indicating the two oxygenated methines are connected by an ether linkage. Further proof of the structure was also obtained from 2D NMR experiments. Its HMBC spectrum (Table-2) showed correlations between H-7 and the substituted double bond carbons of the furan ring, C-5 and C-6. However no correlation was observed between these two carbons and H-2, the other oxygenated methine. H-4 correlates with C-5 and C-6, as well as with C-15. A ^3J correlation was observed between C-5 and 3H-15. While one of the tertiary methyl carbons, C-12, correlated with H-7, the other, C-14, had correlations to 2H-3. Finally the mass spectrum gave a parent ion peak [M^+] at 232. Pinguisanin (1) is a known compound previously isolated by Asakawa *et al*[5,6].



(1)

Table-1, ^{13}C and ^1H NMR Data of Compound (1)

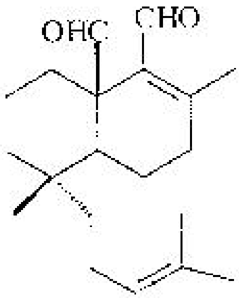
	δ_C	δ_H	MULTIPLICITIES (J, Hz)
1	53.0	2.08	brq (7.4 Hz)
2	79.4	3.95	brs
3	47.9	1.90 (H_β-3) 1.60 (H_α-3)	dd (14.0 Hz, 3.0 Hz) dd (14.0 Hz, 2.0 Hz)
4	39.0	2.58	q (7.3 Hz)
5	122.4		
6	149.1		
7	76.0	4.37	s
8	51.0		
9	40.4		

10	110.1	6.25	d (2.0 Hz)
11	142.7	7.34	d (2.0 Hz)
12	12.9	1.08	s
13	12.5	1.13	d (2.3 Hz)
14	25.3	1.19	s
15	20.0	1.15	d (2.2 Hz)

Table-2. HMBC Correlations of Compound (**1**)

δ_C	C	TYPE	H
53.0	**1**	methine	H_α-3, 3H-12
79.4	**2**	methine	H_α-3, H-1
47.9	**3**	methylene	H-4, H-1, 3H-14
39.0	**4**	methine	H_β-3, 3H-14
122.4	**5**	quaternary	3H-15, H-7, H-10, H-11, H-4
149.1	**6**	quaternary	H-10, H-11, H-7, H-4
76.0	**7**	methine	3H-12, H-2
51.0	**8**	quaternary	H-2, H-7, 3H-14, 3H-12
40.4	**9**	quaternary	H-2, H-7, H-4, H-1, H_β-3, 3H-12
110.1	**10**	double bond	H-11
142.7	**11**	double bond	H-10
12.9	**12**	methyl	H-7
12.5	**13**	methyl	H-1
25.3	**14**	methyl	2H-3
20.0	**15**	methyl	H-4

The other compound present in the fractions was perrottetianal A (**4**), which was previously isolated by Asakawa *et al*[7]. Unfortunately we had very little of this compound and it was very sensitive. Its spectral data were quite similar to those in the literature[7], and a parent ion [M$^+$], 302 m/z, was observed in its mass spectrum. Despite the impurities its ^{1}H NMR spectrum showed a tertiary methyl at δ_H 0.70 (3H-17), one vinyl methyl at δ_H 2.16 (3H-18) and a dimethyl allyl group at δ_H 1.68, δ_H 1.60 (3H-15 and 3H-16, brs) and at δ_H 5.06 (H-13, brt, J=6.4 Hz). The two aldehydes were seen as singlets at δ_H 10.08 (3H-20) and at δ_H 9.85 (3H-19).



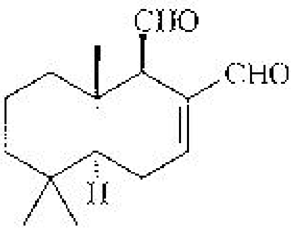
(4)

Porella-1 and *Porella*-2

Two more *Porella* species have been collected in France, in the Vosges, by Prof. J. D. Connolly. The identification of these two *Porella* species is still awaited. Two compounds have been identified in these species polygodial **(19)**, a known sesquiterpene aldehyde, and a new sesquiterpenoid **(20)**, a derivative of pinguisanin.

Polygodial is known to be the pungent substance found in the *Porella vernicosa* complex[4,5]. Its ^{1}H NMR spectrum clearly revealed the two aldehydes at δ_H 9.45 (H-12, s) and at δ_H 9.52 (H-11, d, J=5.0 Hz). The three tertiary methyls were observed as singlets at δ_H 0.91, δ_H 0.94, δ_H 0.95. The proton on the trisubstituted double bond, H-7, appeared at δ_H 7.11 as multiplet. The broad multiplet at δ_H 2.82 belonged to **H-9** attached to the secondary aldehyde. The rest of the methylene signals and one remaining methine proton, H-5, were seen mostly as overlapping signals between δ_H 1.10 and δ_H 2.90. The ^{13}C NMR spectrum of polygodial clearly revealed its molecular formula $C_{15}H_{22}O_2$; δ_C 39.6 (C-1), δ_C 18.0 (C-2), δ_C 41.7 (C-3), δ_C 33.1 (C-4 and C-13), δ_C 49.0 (C-5), δ_C 25.2 (C-6), δ_C 154.2 (C-7), δ_C 138.3 (C-8), δ_C 60.3 (C-9), δ_C 36.9 (C-10), δ_C 201.9 (C-11), δ_C 193.2 (C-12), δ_C 21.9 (C-14), δ_C

15.3 (C-15). The mass spectrum supported the molecular formula with a parent ion peak [M^+] at 234 m/z. The carbonyl stretching vibrations of the two aldehydes in this compound are observed at 1685 cm^{-1} and 1720 cm^{-1} and their C-H vibrations at 2900 cm^{-1} and 2980 cm^{-1}. The compound polygodial **(19)** was identified by comparison of its spectral data with those in the literature[17,18].



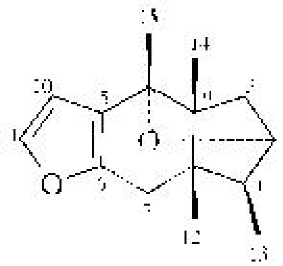
(19)

The new pinguisane type sesquiterpenoid **(20)** was isolated from *Porella*-I species. The spectral data of compound **(20)** were similar to those of pinguisanin **(1)** with regard to the signals of the furan ring, H-10 and H-11, in its ^{1}**H** NMR spectrum. However the rest of the spectrum differed considerably from that of pinguisanin **(1)**. One of the two secondary methyls present in pinguisanin had now became a tertiary methyl in this new compound **(20)**, which showed three tertiary methyls, one of which is more deshielded than the others [δ_H 1.38 (3H-15, s) ; δ_H 1.03 (3H-12, s) ; δ_H 0.92 (3H-14, s)], and one secondary methyl, 3H-13, at δ_H 0.83 as a doublet (J= 7.5 Hz). The two signals of the oxygenated methines in pinguisanin were now reduced to only one in the new compound **(20)**, appearing at δ_H 3.69 as brd (J= 1.5 Hz). In the IR spectrum, the presence of the furan ring was shown by the double bond ring stretches at 1382

cm^{-1} and 1466 cm^{-1}. Again, as in pinguisanin, no hydroxyl group was observed which proved the presence of an ether linkage in the structure. The molecular formula of the new compound **(20)**, $C_{15}H_{20}O_2$, was clearly revealed by its ^{13}C NMR spectrum (Table-3), which showed the four methyl signals [δ_C 21.2 (C-12) ; δ_C 13.2 (C-13) ; δ_C 11.1 (C-14) ; δ_C 24.1 (C-15)], two methylenes [δ_C 32.3 (C-7) ; δ_C 38.0 (C-3)], four methine signals, two of which belonged to the furan ring [δ_C 108.4 (C-10) ; δ_C 141.3 (C-11)], one was the oxygenated methine appearing at δ_C 80.8 (C-2) and the fourth appeared at δ_C 46.6. There was also an oxygenated quaternary carbon, δ_C 77.9 (C-4).

2D NMR experiments were carried out on the compound **(20)** to confirm its structure. The data from its HMBC spectrum are given in Table-4. The oxygenated quaternary carbon, C-4, had correlations to 3H-15, which is the most deshielded methyl in the ^1H NMR spectrum, 3H-14, 2H-3 and H-2, thus clearly revealing the ether linkage between C-2 and C-4 unlike pinguisanin which has the ether linkage between C-2 and C-7. Some of the other correlations observed belonged to the quaternary carbons C-8 and C-9. C-8 had ^3J correlations to H-2, 3H-14, 3H-13 and did not have any correlation to 3H-15. However C-9 had a ^3J correlation to 3H-15 but no correlation was observed between C-9 and 3H-13. Thus, these results accord with the structure **(20)**, isopinguisanin. The mass spectrum supported the molecular formula by showing a parent ion peak $[M^+]$ at 232 m/z. Unfortunately the compound decomposed with time.

In a previous investigation of *P. platyphylla*, the unstable alcohol **(21)** was isolated[10]. On sitting in $CDCl_3$, it was transformed into isopinguisanin **(20)**. It is interesting to note that the treatment of pinguisanin **(1)** with acid transforms it into isopinguisanin **(20)**.



(20)

Table-3, ^{13}C and ^1H NMR Data of Compound **(20)**

	δ_C	δ_H	**MULTIPLICITIES (J, Hz)**
1	46.6	1.74	overlapping with 2H-3
2	80.8	3.69	brd (J= 1.5 Hz)
3	38.0	1.78	overlapping with H-1
4	87.9		
5	120.4		

6	148.6		
7	32.3	2.38 (H_a-7) 2.56 (H_b-7)	d (J= 17.2 Hz) d (J= 17.2 Hz)
8	42.9		
9	51.9		
10	108.4	6.39	brd (J= 2.0 Hz)
11	141.3	7.25	overlapping with CDCl3
12	21.2	1.03	s
13	13.2	0.83	d (J= 7.5 Hz)
14	11.1	0.92	s
15	24.1	1.38	s

Table-4. HMBC Correlations of Compound **(20)**

δ_C	C	TYPE	H
46.6	**1**	methine	2H-7, 3H-13, 3H-12
80.8	**2**	methine	3H-13, H-1
38.0	**3**	methylene	3H-14
77.9	**4**	quaternary	3H-14, 3H-15, 2H-3, H-2
120.4	**5**	quaternary	3H-15, 2H-7, H-10, H-11
148.6	**6**	quaternary	2H-7, H-10, H-11
32.3	**7**	methylene	3H-12, H-1
42.9	**8**	quaternary	3H-12, 3H-13, 3H-14, 2H-7, H-2
51.9	**9**	quaternary	3H-12, 3H-14, 3H-15, H_b-7, H-2
21.2	**12**	methyl	H_a-7
13.2	**13**	methyl	H-1

Table-5, HMQC Correlations of Compound (**20**)

δ_C	C	δ_H	No
46.6	**1**	1.74	1H
80.8	**2**	3.69	1H
38.0	**3**	1.78	2H
32.3	**7**	2.38 (H_a-7) 2.56 (H_b-7)	2H
108.4	**10**	6.39	1H
141.3	**11**	7.25	1H
21.2	**12**	1.03	3H
13.2	**13**	0.83	3H
11.1	**14**	0.92	3H
24.1	**15**	1.38	3H

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Chapter-12

Miliusa velutina

INTRODUCTION

The Annonaceae form a large family of aromatic trees, shrubs or climbers, which are widely distributed in tropical and sub-tropical regions of both hemispheres[1]. It consists of about one hundred and thirty genera and two thousand three hundred species[2]. They are widely distributed in the tropics, mainly at low elevations in moist forest areas. Asia and Australasia are the centre of the distribution of the Annonaceae[3,4] with about fifty one genera (approximately 950 species). In Africa and Madagascar there are forty genera with approximately 450 species, whereas on the American continent there are about thirty eight genera (740 species).

Annonaceous plants are well recognised by the alternate, extipulate leaves, mostly trimerous flowers, numerous and often truncate free stamens, free carpels and seeds with ruminate endosperm[5,6,7,8]. They are characterized by a great many archaic and extremely primitive features[1,4,7]. These include the occurrence of secretory cells in the leaf parenchyma, the almost universal occurrence of solitary or clustered crystals in the epidermis of the leaf, simple perforations of the vessels and the absence of external glands. There are also some significant anatomical characters of the wood and flowers[9].

The Annonaceae family is of appreciable economic importance as a source of edible fruits, e.g. West Indian custard apple, the North American pawpaw (*Asimina*), sweetsop, cherimoya and ilama (*Annona*)[8]. The genus *Annona* is the most important source of edible fruits and in Africa some species of this genus are also used for medicinal purposes[10]. The barks of annonaceous plants are usually aromatic, astringent and stimulant and the inner layer of the bark is a source of useful fibre[11]. The woods of some Annonaceous plants have been employed for alcohol production[12]. Some of the individual alkaloidal and non-alkaloidal constituents of the Annonaceae are also pharmacologically interesting [e.g. diterpenoids with antibacterial[13] and antitumour[14] activity, oliveroline with anti-Parkinsonian properties[15]; liriodenine with antitumour, antibacterial and antifungal properties[16,17]].

Due to their occurrence in inaccessible low elevation rain forest areas, a relatively small amount of phytochemical work has been carried out on the Annonaceae in comparison with many other plant families of similar size. Some phytochemical information is available on only about 33% of genera and 7% of recognised species[17].

Biogenetically, the family is capable of producing a wide range of interesting secondary plant metabolites (e.g. terpenoids, flavonoids, indolosesquiterpenes, cyclohexane derivatives, aromatic compounds, acetogenins etc.) and is now the focus of much interest by phytochemists.

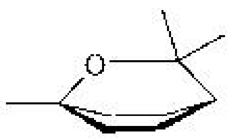
The Annonaceae produces various structural types of monoterpenoids. 1,8 Cineole (**1**) and cuminyl aldehyde (**2**) have been isolated from the oils of *Xylopia aethiopica*[19,20,21], while *Annona squamosa* afforded the monoterpenes borneol (**3**), limonene (**4**) and trans-ocimene (**5**)[22,23]. An interesting benzylated monoterpene (**6**) has been isolated from *Uvaria*. *Cananga odorata* is a rich source of linalool (**7**)[24].

Several sesquiterpenes have been isolated from the genera *Cananga*, *Xylopia*, *Cymbopetalum*, *Annona* and *Artabotrys*. Farnesol (**8**), occurs in the volatile oils of *Cananga odorata*[24] and yingzhaosu A (**9**) and yingzhaosu B (**10**), two unusual monocyclic sesquiterpenes, in *Artabotrys uncinatus* root[25,26]. A tetracyclic sesquiterpane, ishwarane (**11**), was isolated from *Cymbopetalum penduliflorum*[27]. *Annona* species have also been found to contain other sesquiterpenes, e.g. β-caryophyllene (**12**) from *Annona squamosa*[28].

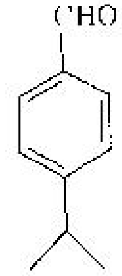
The diterpenoids present in the Annonaceae appear to be a major feature of the chemistry of the genera *Xylopia* and *Annona*, e.g. (**13-17**), and only one diterpenoid, polyalthic acid (**16**), from *Polyalthia fragrans*, has been reported from other genera[29]. About thirty kaurane, trachylobane, kolavane and labdane diterpenoids have been reported[18,30].

The occurrence of triterpenoids in the Annonaceae has not been widely investigated. Sitosterol has frequently been isolated from various species in the family[18]. Several pentacyclic triterpenes have been reported, mostly from the genus Uvaria and friedelin (**18**) has been isolated from the leaves of *Annona squamosa*[31]. The tetracyclic triterpenoid polycarpol (**19**) was discovered by Cavé *et al*[32] simultaneously in the barks of *Greenwayodendron (Polyalthia) oliveri* and *Meiocarpidium lepidotum*. Later it was found to be present in several other species.

Flavonoids are one of the most widespread and numerous groups of naturally occurring compounds[33]. Among the genera of the Annonaceae, *Uvaria* is a particularly important source of flavanones and dihydrochalcones. The African



(1)



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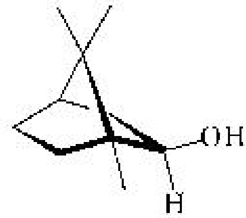

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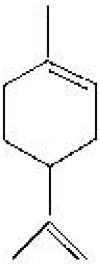

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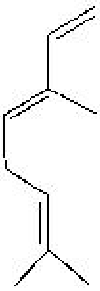

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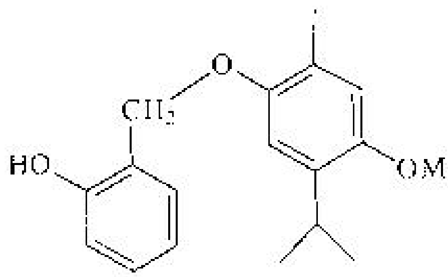

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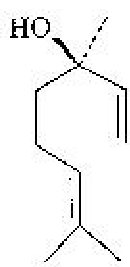

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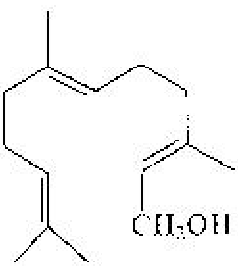

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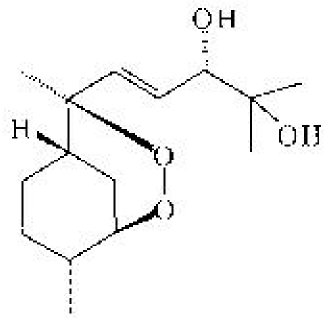

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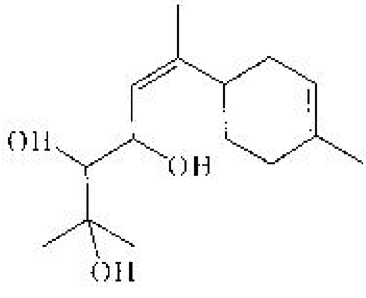

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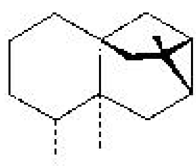

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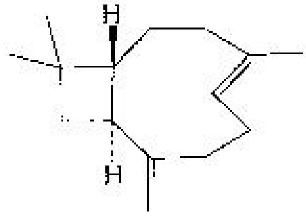

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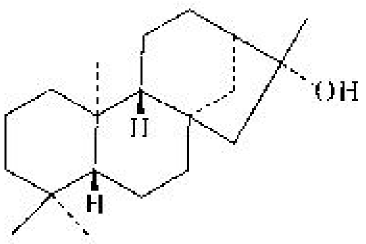

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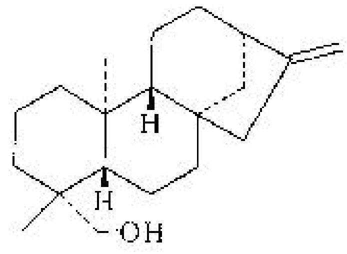

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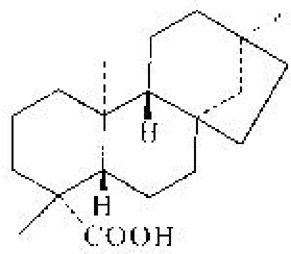

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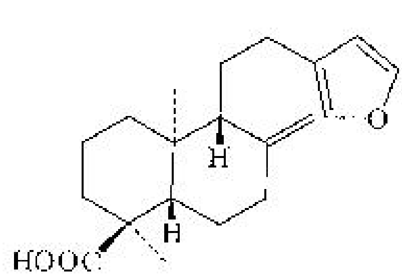

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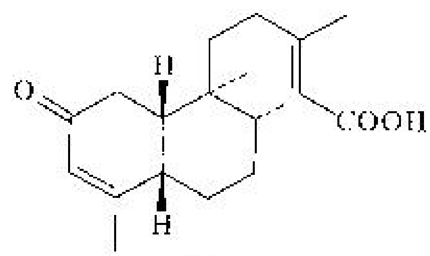

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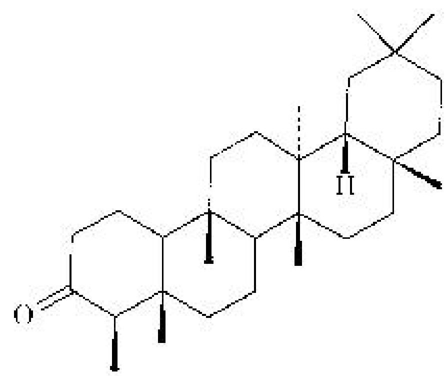
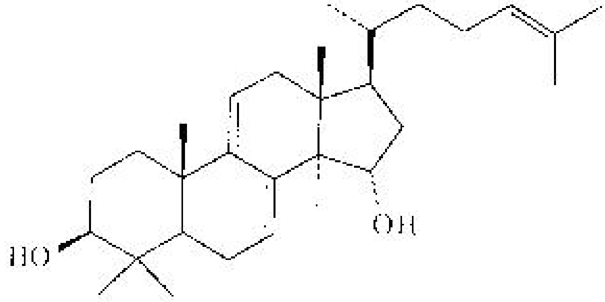


(18)



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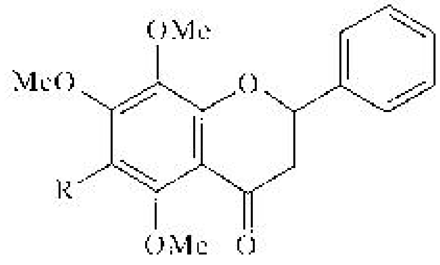
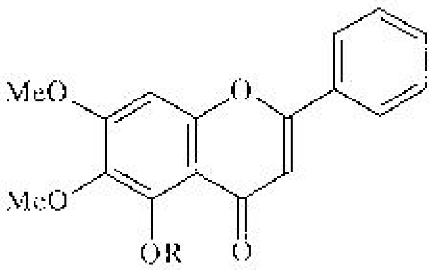
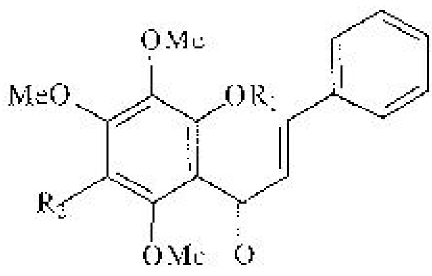


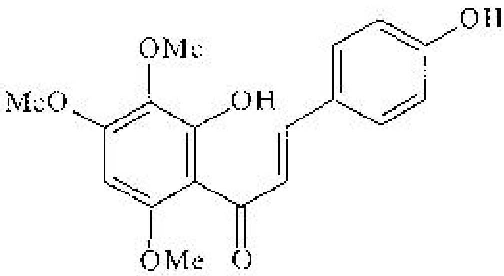
(20) R=H
(26) R=Me



(21) R=Me
(22) R=H



(23) R_1=R_2=H
(24) R_1=Me, R_2=H
(27) R_1=H, R_2=OMe



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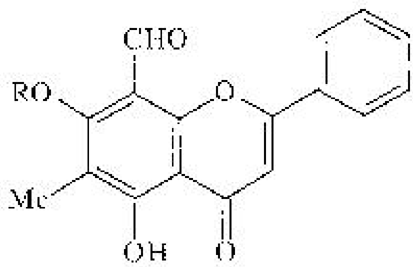
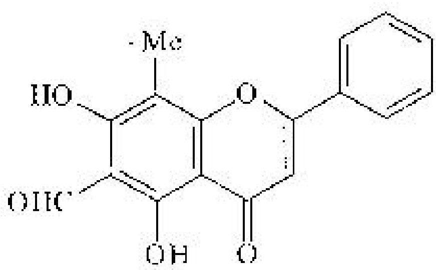


(28) R=H
(29) R=Me



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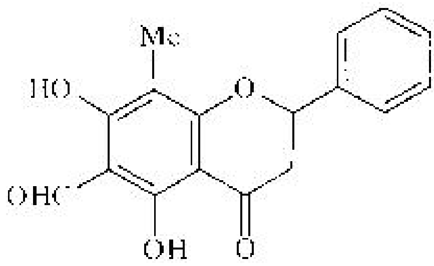

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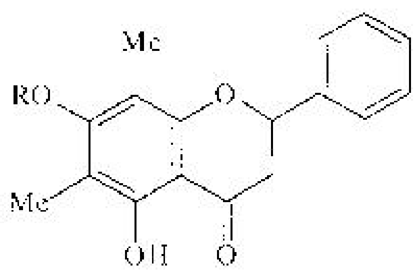
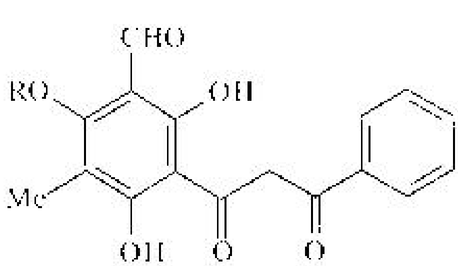


(32) R=Me
(33) R=H



(34) R=H
(35) R=Me

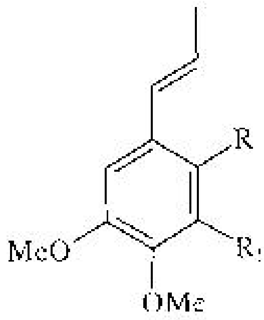
species, *Monanthotaxis (Popowia) cauliflora*, has been found to be a good source of simple flavonoids. From the stem bark of this species, 5,7,8-trimethoxyflavanone (**20**), 5,6,7-trimethoxyflavone (**21**), 5-hydroxy-6,7-dimethoxyflavone (**22**) and three chalcones (**23**), (**24**) and (**25**) have been isolated. Kanakugin (**26**) and kanakugiol (**27**) have been recorded from the ripe fruit of this species[34].

C-methyl and C-formyl flavonoids have been reported from the Asian species *Unona (desmos) lawii*. They include three flavones : unonal (**28**), unonal 7-methyl ether (**29**) and isounonal (**30**)[35], three flavonones : lawinal (**31**), desmethoxy matteucinol (**32**) and desmethoxymatteucinol-7-methyl ether (**33**)[36], and two dibenzomethanes : (**34**) and (**35**)[36,37]. Flavonoids have also been reported from *Cananga*[38,39], *Annona*[40] and *Pachypodanthium*[41].

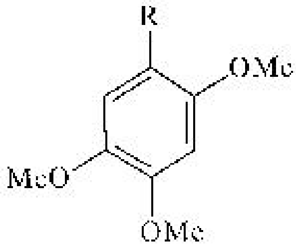
Several propenyl benzene and vinyl benzene derivatives have been recorded from annonaceous plants. Asarone (**36**), *trans*-isoelemicin (**37**) and *trans*-isomyristicin (**38**) have been isolated from the bark of *Guatteria gaumeri*[42] and 2,4,5-trimethoxystyrene (**39**) from *Pachypodanthium confine*[43], *P. staudtii*[44,45] and *Duguetia eximia*[16]. An aromatic compound pachysontol (**40**), has been found by Bevalot *et al*[45] in *P. staudtii* and asaraldehyde (**41**) has been reported from *Guatteria gaumeri*[42] and *P. staudtii*.

Apart from those compounds mentioned above, the Annonaceae family is the subject of considerable interest because of the presence of the acetogenins, a group of compounds with a range of useful biological properties such as cytotoxic, antitumoral, antiparasitic, pesticidal, antimicrobial and immunosuppressive activities. The field of investigation of acetogenins expanded greatly with the number of isolated and reported acetogenins increasing rapidly[17]. In 1990[18] thirty one and in 1993[17] sixty one acetogenins were described as part of two reviews and now they number more than 160[47].

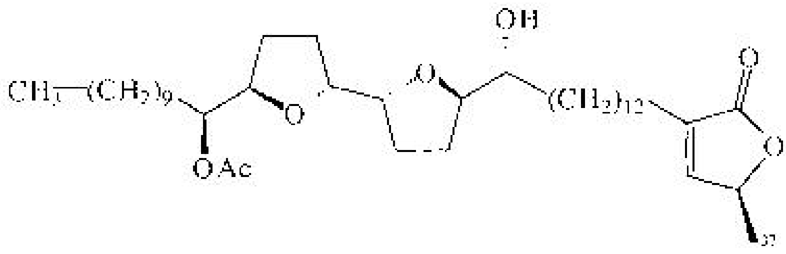
Annonaceous acetogenins constitute a series of C-35/C-37 natural products of polyketide origin derived from fatty acids. Their structure is characterised by a long alkyl chain bearing a terminal unsaturated γ-methyl-γ-lactone (sometimes rearranged to a γ-lactone containing an acetonyl group α to the lactone carbonyl), one, two or three tetrahydrofuran rings and some oxygenated substituents along the chain, particularly α to a tetrahyrofuran, and in some cases double bonds and/or epoxides[17]:



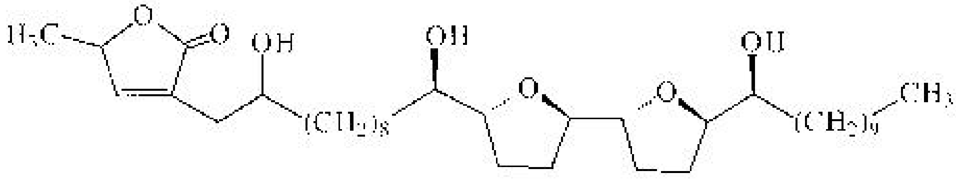
(36) R= OMe, R_1= H
(37) R= H, R_1= OMe
(38) R= R_1= OMe



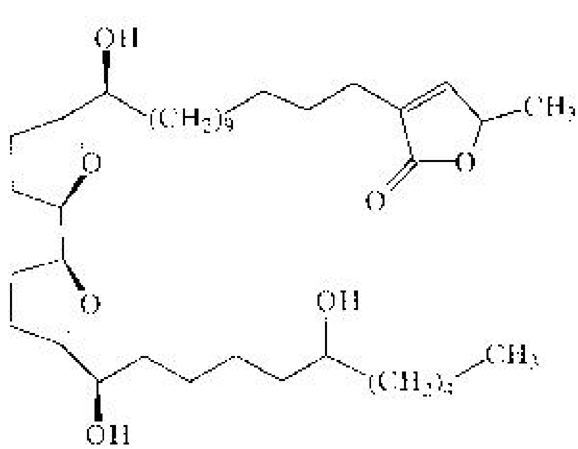
(39) R= $-CH=CH_2$
(40) R= $-CH(CH_2OH)OCH_2Me$
(41) R= $-CHO$



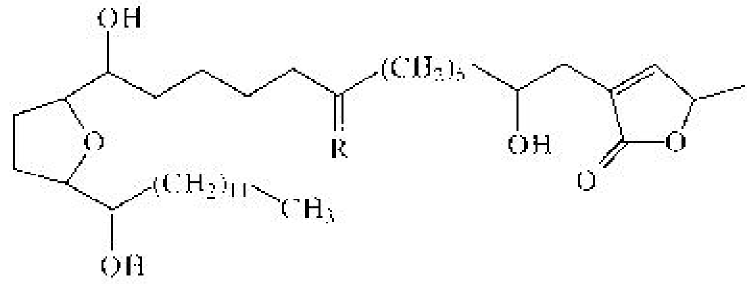
(42)



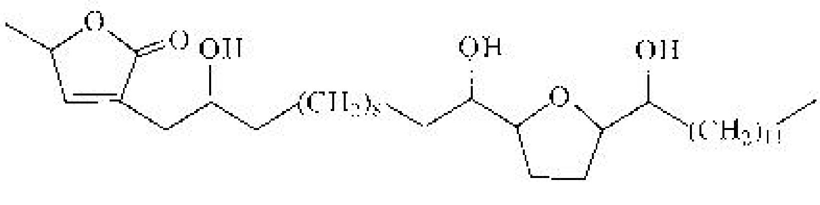
(43)



(44)



(45) R= H, OH
(46) R= O



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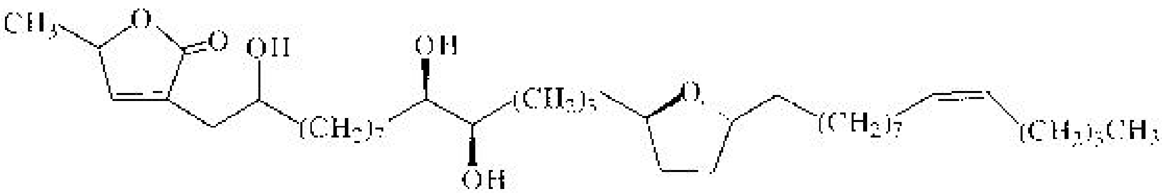

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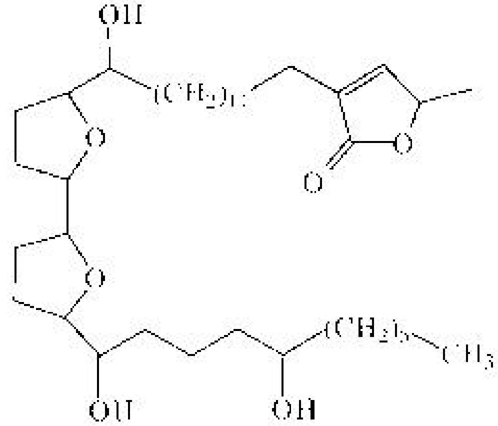

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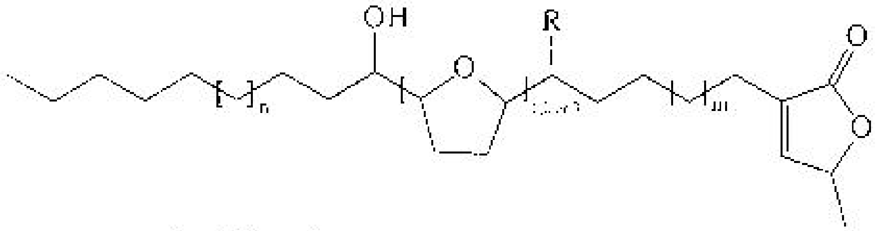
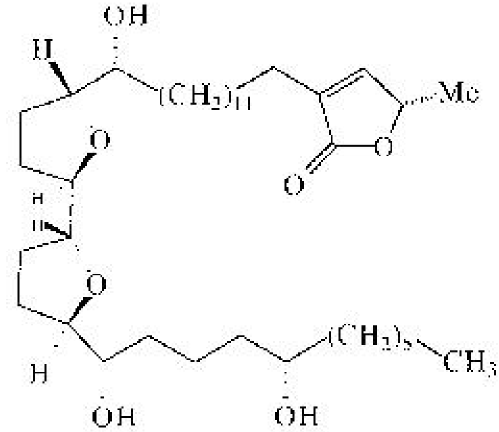


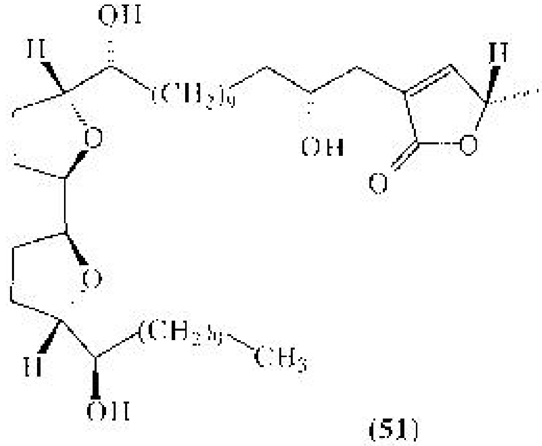
R= OH or H

The first annonaceous acetogenin, uvaricin **(42)**, a new antitumor agent was isolated by Jolad *et al*[50] from the roots of *Uvaria acuminata* (Annonaceae) in 1982. Uvaricin is a fatty acid lactone which contained a number of original structural characteristics, particularly a bis-tetrahydrofuran pattern flanked by hydroxyls and a terminal unsaturated lactone. Dabrah and Sneden[51,52] and Cortes *et al*[53], two years later, described four new products presenting the same structural characteristics. These products found in species belonging to the family of the Annonaceae formed a new class of natural compounds called acetogenins. In 1991, two more acetogenins, molvizarin **(43)** and motrilin **(44)**, were reported by Cortes *et al*[54]. Classification of the acetogenins of the Annonaceae is done according to the number and arrangement of the tetrahydrofuran rings along the alkyl chain as proposed by Cave *et al*[17].

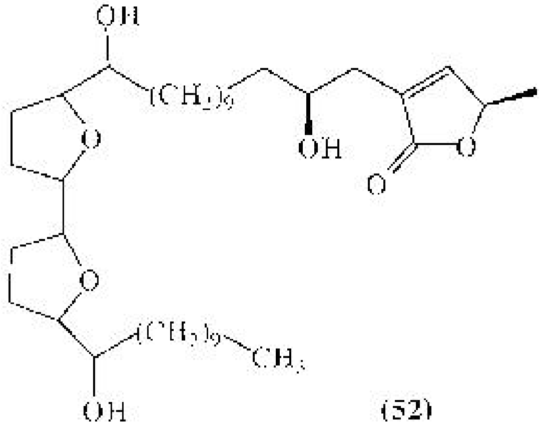
The first isolated acetogenin, uvaricin **(42)**, was stated to be a new antitumour agent[50]. Since then many studies have appeared describing cytotoxic, antitumour, antiparasitic, pesticidal, antimicrobial, antifungal and immunosuppressive properties of various acetogenins. For instance, molvizarin **(43)** and motrilin **(44)** exhibited cytotoxic activity. There are some other cytotoxic acetogenins from the seeds of *Annona muricata* such as annonacin **(45)**, annonacinone **(46)**[55,56] and murisolin **(47)**[57,58]. The acetogenins senegalene **(48)**, squamocin **(49)** and molvizarin **(43)** from *Annona senegalensis*[59,60] and ulacins from *Rollina ulei*[61] exhibit antiparasitic activity. Pesticidal activity of acetogenins can be seen in annonin **(50)**[63], bullatacin **(51)**[64,62], asimicin **(52)**[58,65], squamocin **(49)**[66], goniothalamicin **(53)**[58,67] and sylvaticin **(54)**[68]. Oils of some *Annona* seeds are traditionally used to get rid of lice in the scalp[66], as for example, the ground seeds of *Annona reticulata* in Vietnam[69]. In some countries of South America, ground bark or seeds of some species of Annonaceae are spread on soils as pesticides[17]. Interesting immunosuppressive activity has been observed in the acetogenins isolated from *Annona muricata*[70].



(50)



(51)



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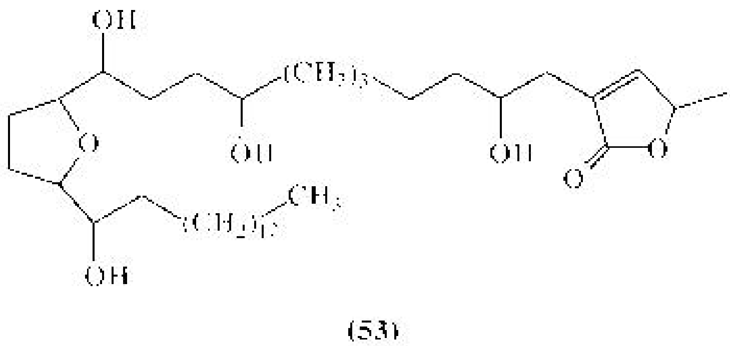

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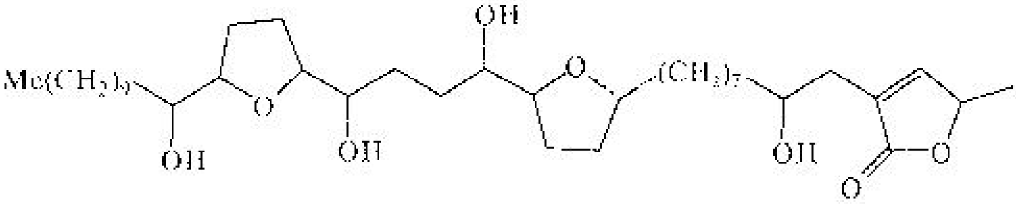

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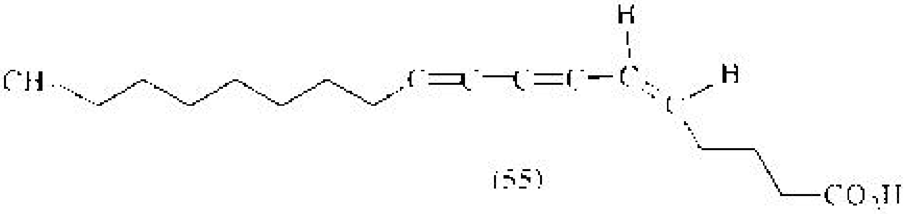

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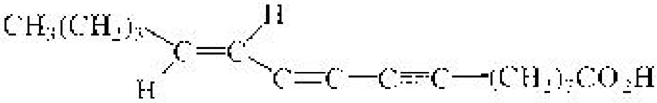

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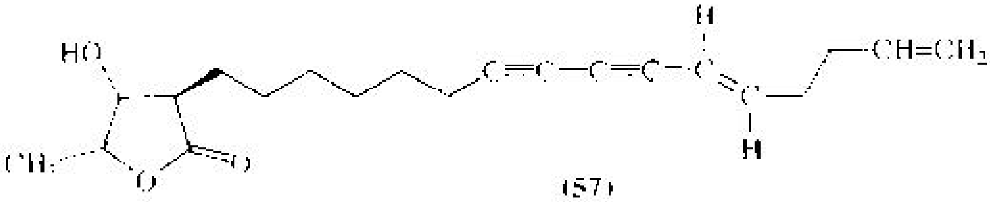

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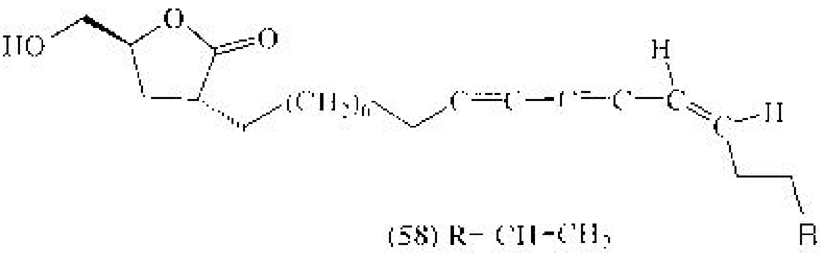
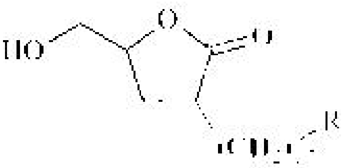


(58) R= $CH=CH_2$

(59) R= CH_2CH_3

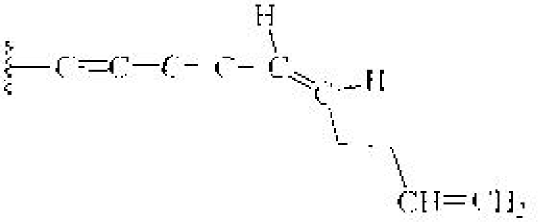


(60) R= $CH=CH_2$

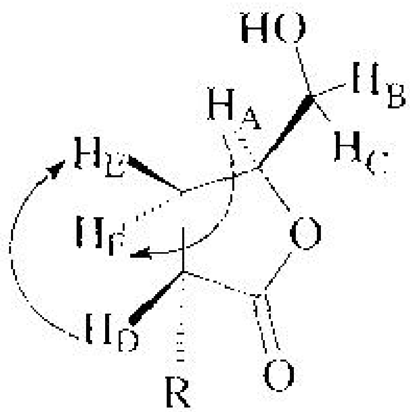
(61) R= CH_2CH_3

RESULTS AND DISCUSSION

In the present work, we have examined the ether extract of *Miliusa velutina* and after using extensive preparative TLC, we were able to identify some of the compounds present in this extract. One of the two compounds was identified as compound **(58)**, which was previously isolated by us as its acetate[74]. Its IR spectrum showed characteristic absorptions for γ-lactone (1764 cm^{-1}), free and intramolecularly bonded hydroxyl bands at 3606 cm^{-1} and 3446 cm^{-1}, respectively, a disubstituted acetylenic stretch at 2232 cm^{-1} and olefinic CH stretches at 3026 cm^{-1} and 3010 cm^{-1}. Its ^{1}H NMR spectrum showed a terminal vinyl group at δ_H 5.79(ddt, J= 17.0, 10.3, and 6.8 Hz); δ_H 4.98(brd, J= 8.7 Hz) and at δ_H 5.02(brd, J=17.2 Hz), and a cis disubstituted double bond at δ_H 6.01(dt, J= 10.8, 7.4 Hz), and at δ_H 5.48(brd, J= 10.8 Hz). The oxygenated methine on the γ-lactone was observed at δ_H 4.58 as multiplet and the methylene protons next to the hydroxyl were seen at δ_H 3.84(brd, J= 12.3 Hz) and at δ_H 3.64(brd, J= 12.3 Hz). Examination of this compound by GCMS gave a base peak at m/z 129, which belongs to the part structure **(A)** of compound **(58)** as shown below. Finally the CI$^+$ gave a peak at 374 m/z (M+NH$_4$)$^+$, which gives the mass of the compound **(58)**, 356 m/z, consistent with the molecular formula $C_{23}H_{32}O_3$. Analysis of COSY and HMBC correlations led to the structure **(58)**. Recent NOE difference experiments permitted the assignment of the relative stereochemistry as in **(58)**. Irradiation of the oxygenated methine proton on the γ-lactone ring (H_A) afforded NOEs at H_B, H_E and H_F as shown in part structure **(B)** below. Irradiation of H_B gave NOEs at H_E and H_C, while H_E gave NOEs at H_F and H_A. The other proton of the methylene group in the lactone ring, H_G, gave NOEs at H_D and H_E. Finally, irradiation of H_D afforded a NOE at H_F.



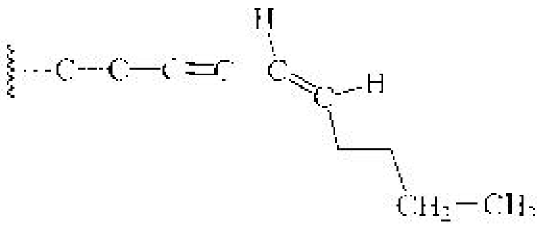
(A)



(B)

Compound **(58)** differs from the compounds normally classified as "acetogenins" in several respects. It has a smaller alkyl chain, which lacks oxygenation, and there is no unsaturation in the lactone ring. It represents a further addition to the wide range of unsaturated fatty acid derivatives, which contain isolated or conjugated acetylenic units. The ene-diyne chromophore occurs widely, often with both the Z and the E forms present. For example, the acids **(55)** and **(56)** have been isolated from the root bark and/or the seed oil of *Paramacrolobium caeruleum*, *Exocarpus cupressiformis* and other species[71,72]. The compound which bears the closest relationship to **(58)** is sapranthin **(57)**[73] from the bark of *Sapranthus palanga* (Annonaceae). It is a C_{21} molecule, lacks the primary hydroxyl group but is **oxygenated on the lactone ring.**

The spectral data of the new compound **(59)** were quite similar to those of compound **(58)**. The only difference was the disappearance of the signals of the terminal vinyl group, which has now been saturated, in the ^1H NMR spectrum of **(59)**. Other than this, we were able to see the cis disubstituted double bond at δ_H 6.02(dt, J= 10.8, 7.5 Hz) and at δ_H 5.45(brd, J= 10.8 Hz), the oxygenated methine on the γ lactone at δ_H 4.59 as a multiplet and the methylene protons attached to the hydroxyl at δ_H 3.85(brd, J= 12.4 Hz) and δ_H 3.61(brd, J= 12.2 Hz). In the GCMS of **(59)**, the most abundant ion, whose mass was 131 *m/z*, belongs to the part structure **(C)** as shown below. Finally the CI^+ analysis of this compound gave the parent ion peak at 358 m/z, corresponding to the molecular formula, $C_{21}H_{34}O_3$.



(C)

Another band containing a mixture of two non UV active compounds was obtained from the preparative TLC of the crude extract of *M. velutina* and their structures have been established by means of spectroscopy, GCMS and CI^+ analysis. Both compounds had the γ-lactone ring but they did not contain any acetylene or disubstituted double bond in their fatty chain attached to the γ-lactone ring. One of the two compounds has been identified as compound **(60)** containing a terminal vinyl group, and the other had fully saturated fatty chain and identified as **(61)**. The CI^+ analysis of the mixture showed two parent ion peaks at 380 and at 382, consistent with the molecular formula $C_{24}H_{44}O_3$ and $C_{24}H_{46}O_3$. The close value of the two masses suggested that the vinyl group in compound **(60)** was saturated in **(61)**. These compounds contain an extra methylene group relative to **(58)** and **(59)**.

EXPERIMENTAL

The plant material was obtained from Bangladesh. A reference sample of the plant is deposited in the herbarium of the University of Dhaka. The dried powdered bark (1.1 kg) was extracted with diethyl ether to give a crude extract (22 g) as a mobile oil. Preliminary analytical TLC showed the presence of several compounds which absorbed strongly in the UV, accompanied by less polar compounds which lacked UV absorption. The crude ether extract (80 mg) was then subjected to preparative TLC for further purification using 30% ethyl acetate and petroleum ether as eluent. A large UV active band was obtained which turned out to be a mixture on examination of the ^1H NMR spectrum. Due to the similarity of the polarities of the two compounds present in this mixture, it was difficult to get a total separation. However, by running the mixture with 25% ethyl acetate and petroleum ether on a preparative plate for a few days we were able to get a separation. These two compounds were then subjected to spectroscopic and GCMS analysis. The GCMS analysis were carried out with a Hewlett-Packard 5971 mass selective detector interfaced to a 5890 Series II gas-liquid chromatograph and computer (Vectra QS/16S).

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GENERAL EXPERIMENTAL

Nuclear Magnetic Resonance Spectra (NMR) were recorded on Bruker WP AM 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) and AM 360 (^{1}H at 360 MHz and ^{13}C at 90 MHz). HSQC and HMBC experiments are inverse detected versions of 2D direct and 2D long-range carbon-proton correlation experiments respectively. Spectra were recorded for $CHCl_3$ relative to δ_H 7.25 and $CDCl_3$ relative to δ_C 77.0 and chemical shifts are reported in *ppm*. Coupling constants (J) in Hz are given in parenthesis in the tabulated ^{1}H NMR data. Signals indicated 'm' are unresolved or overlapped *multiplets*. ^{1}H and ^{13}C signal assignments are based on general chemical shift rules and comparison with published data for similar compounds. More definitive ^{1}H NMR assignments were made by NOE difference experiment. Infra-red (IR) spectra were recorded in $CHCl_3$ solution on either a Perkin Elmer 580 or Philips 9800 FTIR spectrometer. Ultra-violet (UV) spectra were measured in ethanol solutions using a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrometer. High resolution mass spectra were determined on a modified Kratos MS9 instrument.

Dried and powdered materials were extracted with diethyl ether or methanol. The crude extracts were fractionated by column chromatography over silica gel G_{254}. Eluents used for silica gel column chromatography were increasing percentages of diethyl ether or ethyl acetate in light petroleum. Some of the crude fractions were chromatographed on LH20, eluting with methanol and chloroform (1:1), in order to get rid of the massive amount of chlorophyll and fat that they contained. Further purification was done on TLC by using 0.75 mm thick preparative plates over silica gel GF_{254}. Eluents for TLC were increasing percentages of diethyl ether or ethyl acetate in light petroleum. Compounds on TLC and analytical plates were visualised using UV light or iodine vapour. Solvents were evaporated using a Buchi Rotavapor and water aspirator. The Solvents used were either of analytical grade or bulk solvents distilled before use.