A THESIS ENTITLED

"PHYTOCHEMICAL STUDY OF SOME SCOTTISH LIVERWORTS"

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by

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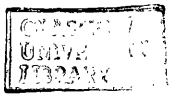

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Finally I would also like to express my sincere gratitude to my family and friends for their help and support.

"PHYTOCHEMICAL STUDY OF SOME SCOTTISH LIVERWORTS"

Summary

This thesis consists of four chapters. The first chapter gives a general introduction dealing with the nature of secondary metabolites and the skeletal types found in the Hepaticae (liverworts). This is followed by a discussion of the chemical constituents found in *Plagiochila spinulosa* (Chapter Two). Six dihydrophenanthrene derivatives, a pentasubstituted benzene, a fusicoccane derivative and three bibenzyl-diterpenoid conjugate compounds were isolated. Among these compounds one dihydrophenanthrene and one bibenzyl-diterpenoid are new and a fusicoccane derivative is isolated for the first time from this species. The structures of these compounds were determined mainly using ^{1}H and ^{13}C NMR spectroscopy. Definitive evidence for the structures of the dihydrophenanthrenes was obtained from NOE difference experiments. The structure of the pentasubstituted benzene derivative was established by a long-range δ_C/δ_H correlation experiment.

Chapter three deals with the chemical constituents of *Barbilophozia barbata*. The sesquiterpenoid hydrocarbon,gymnomitrene, two acorane derivatives, (1S*,10S*)-2R*,3S*-diacetoxy-4,7(11)-acoradien-8-one and (1S*,10S*)-2S*-acetoxy-4,7(11)-acoradien-8-one and a dolabellane diterpenoid, 18-acetoxy-3S,4S;7S,8S-diepoxydolabellane were isolated. The acorane monoacetate is a new compound.

The secondary metabolites of *Frullania tamarisci* form the subject matter of the final Chapter. Six eudesmanolides, frullanolide, α-cyclocostunolide, β-cyclocostunolide, γ-cyclocostunolide, oxyfrullanolide, 5β-hydroxy-4(15)-eudesmadien-6,12-olide, a germacranolide, costunolide and one pacifigorgiane derivative, tamariscol were isolated. Oxyfrullanolide and β-cyclocostunolide are reported for the first time in this species. New compound 5β-hydroxy-4(15)-eudesmadien-6,12-olide was isolated as a mixture with, its 5-epimer, oxyfrullanolide.

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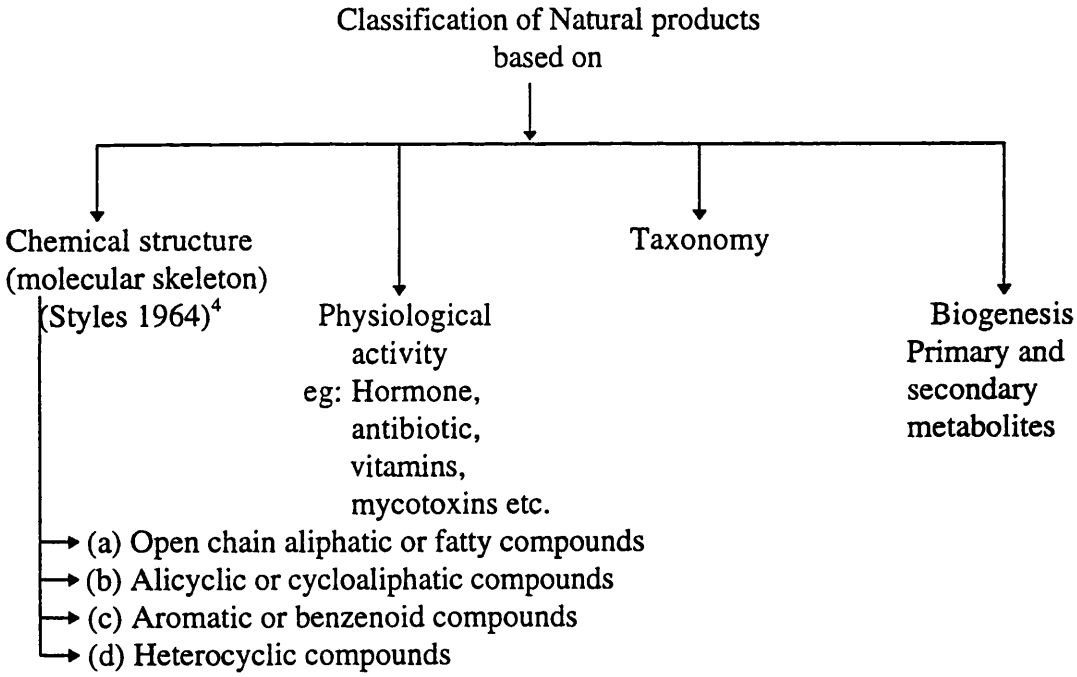
CHAPTER ONE

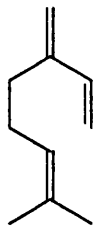
GENERAL INTRODUCTION

The use of plants and plant products for food, shelter etc., is as old as humanity. The earliest medicinal use of plants is found in the Vedic period (over 4000 years BC)[1]. Originally plant products were used either as powders or as crude extracts. With the development of science and technology a great change has occurred in the use of natural products which are now often separated and purified. To develop the potential of plant products there is a great need for the screening of a wide range of plant species. This task presents organic chemists with a considerable challenge.

Natori[3] has presented four different classifications for natural products.



Classification based on **molecular skeleton** is superficial. In fact many compounds belong to more than one group. For example myrcene (1) and geraniol (2) belong to group (a) and p-cymene (3) belongs to group (c) but due to biogenetic considerations they are all treated as group (b).



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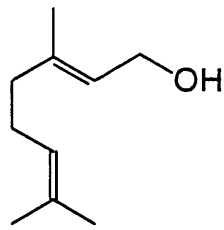

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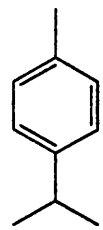


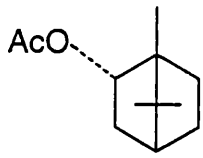
(3)

Many compounds have been isolated from specific plant species or genera or families. For example opium alkaloids occur in *Papaver somniferum.* This has led to the development of **chemotaxonomy** or **chemosystematics,** which attempts to review plant constituents according to plant taxa[5]. Constituents are regarded as markers for evolution and for the classification of plants.

Classification on the basis of biosynthesis: All natural compounds are biosynthesised by enzymatic processes. The major source of carbon is usually glucose, which is photosynthesised in green plants. Natural products are divided into two groups, primary and secondary metabolites. Polysaccharides, proteins, sugars, fats and nucleic acids are the fundamental building blocks of living matter and thus are considered as primary metabolites. Alkaloids, terpenoids, steroids, etc. are known as secondary metabolites because they are formed by specific chemical processes which take place only in certain species or else give different products according to the species. In general such reactions do not appear to be essential for existence. Secondary metabolites have a restricted distribution and are often characteristic of an individual genus or species. In contrast primary metabolites have a broad distribution in all living things and are virtually identical even in those species that are genetically very different.

HEPATICAE

Bryophytes are divided in three classes: Musci (Mosses), Hepaticae (Liverworts) and Anthocerotae (Hornworts). The Hepaticae family is divided into two sub-classes and six orders by most of the authors[2]. The sub-class **Jungermanniidae** consists of three orders: (i) The Calobryales which has only one



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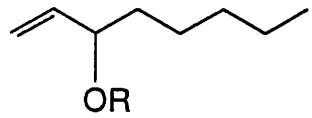
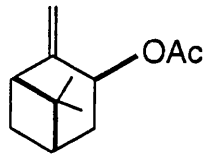


(5):R=H

(6):R=Ac



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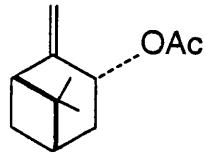

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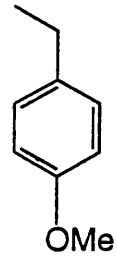

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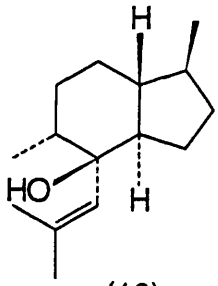

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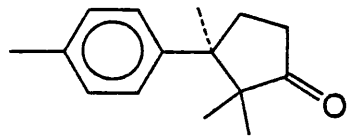

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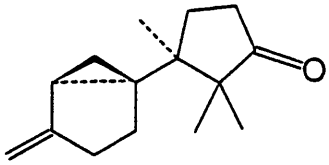

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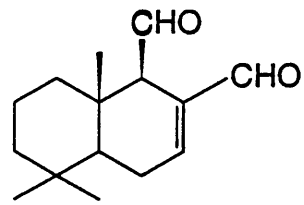

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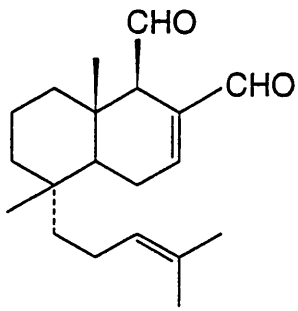

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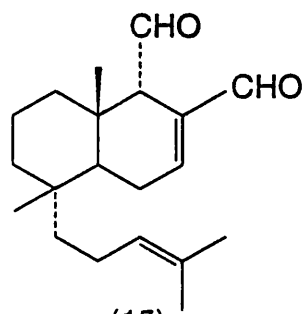


(15)

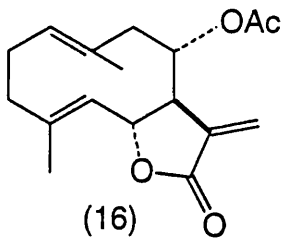
genus *Haplomitrium* with 8 species. Plants of this order are distributed in the southern hemisphere and South east Asia. (ii) The Jungermanniales, the largest order of the Hepaticae, which has 12 families, 250 genera and 3500 species distributed all over the world. (iii) The Metzgeriales, the second largest order of this class, which consists of about 12 family, 30 genera and 600 species and is distributed world-wide. The next sub-class, the **Marchantiidae** also consists of three orders: (I) The Monocleales which has only one genus and whose distribution is confined to New Zealand and tropical America. (ii) The Marchantiales, the third largest order, which has about 14 families, 28 genera and 200 species and is distributed world-wide. (iii) The Sphaerocarpales has three genera and around 25 species. These plants occur in the arid and semi-arid region of the world.

The Hepaticae have a special characteristic among the Bryophytes in that they contain oil bodies in their cells. These oil bodies form a rich source of terpenoids and other metabolites. Since the 1970s there has been much research into the metabolites of the Hepaticae. A wide range of terpenoid and aromatic compounds has been isolated, some of which have biological activity including allergenic, antifungal, antimicrobal, cytotoxic, irritant and plant growth regulating activity and insect antifeedant character[6,7].

Some biological characteristics are:

Characteristic odour

Some liverworts have aromatic compounds with mushroomy, turpentine-like, sweet mossy or woody type odours[6,7]. The mushroom-like odour of *Conocephalum conicum* is due to the presence of (+)-bornyl acetate (4), 1-octen-3-ol (5) and 1-octen-3-yl acetate (6)[9]. *Wiesnerella denudata* also emits a sweet, mushroomy smell due to the presence of (+)-bornyl acetate (4)[8]. The very intense fragrance found in *Targionia hypophylla* is due to the presence of *cis* and *trans*-pinocarveyl acetate (7) and (8)[10]. p-Ethylanisol (9) gives a naphthalene-like odour and occurs in *Leptoleunea elliptica*[11]. Tamariscol (10), isolated by Connolly *et al*[28] from *Frullania tamarisci*, has a sweet, mossy and woody smell. *Plagiochila yokogurensis* also has a mossy-like odour[15]. A strong turpentine-like smell is found in some species of



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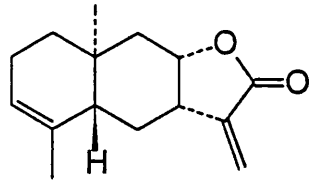

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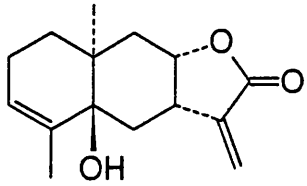

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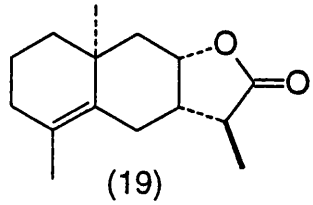

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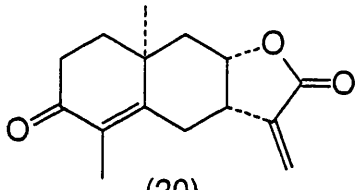

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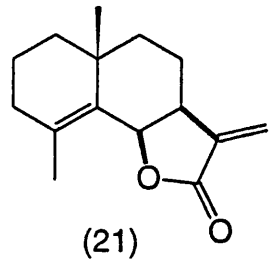

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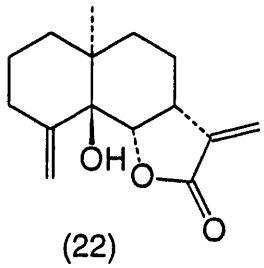

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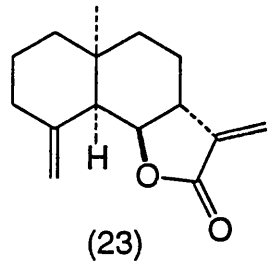

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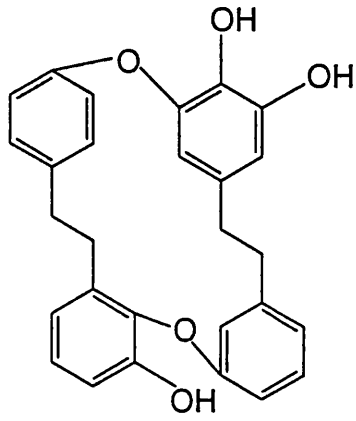

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Porella, while *Jungermannia obovata* has a strong carrot-like odour[18]. *Mannia fragrans* has a distinctive smell due to the presence of volatile terpenoids. R(-)-α-Cuparenone (11) was identified by the Prague group of Herout[18] and a second odoriferous component of this liverwort is grimaldone (12)[23].

Hot and bitter taste

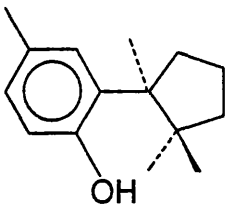
Some species of liverwort are bitter and hot to the taste. The pungency of liverworts is due to the presence of sesquiterpenoid aldehydes and dialdehydes, secoaromadendrane type hemiacetals, germacranolides and eudesmanolides[7,8]. *Porella vernicosa* shows pungency and skin irritant properties due to the presence of tadeonal (13)[13]. *Tricholeopsis sacculata* contains sacculatal (15) and iso-sacculatol (14), which are responsible for the pungency of this plant[14]. *Plagiochila yokogurensis* also shows pungency and growth inhibitory activity[15]. The female gamete of *Conocephalum conicum* contains tulpinolide (16) which is responsible for its pungency[16]. *Chiloscyphus polyanthus* and *Diplophyllum albicans* also show pungency and growth inhibitory characteristic due to the presence of the *ent*-eudesmanolides; diplophyllolide (17), *ent*-5β-hydroxydiplophyllolide (18), *ent*-dihydrodiplophyllin (19) and *ent*-3-oxodiplophyllin (20)[17].

Allergenic contact dermatitis

Some members of the Hepaticae have allergenic properties[6]. Knoch *et al*[41] reported that *Radula* and *Frullania spp.* cause contact dermatitis due to the presence of eudesmane and eremophilane sesquiterpene lactones. Green *et al*[42,43] found that (-) frullanolide (21) is an allergenically active compound. Other *Frullania* compounds, (+)-oxyfrullanolide (22), and (+)-*cis*-β-cyclocostunolide (23) are also allergenically active compounds[44].

Antimicrobial and antifungal activity

Some liverworts show antimicrobial and antifungal activity[7]. Marchantin A (24) from *Marchantia polymorpha*[45] and (-)-α-herbertenol (25), (-)-β-herbertenol



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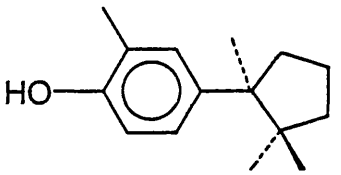

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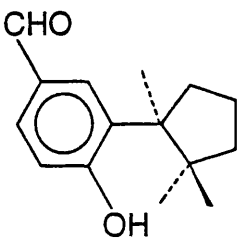

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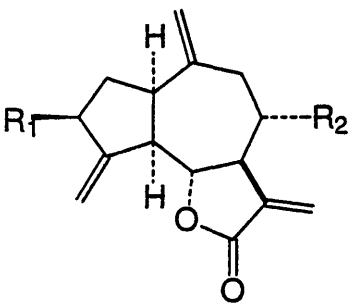


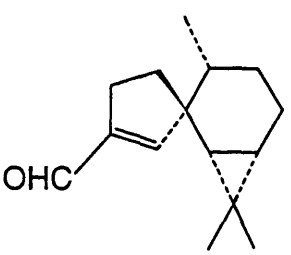
(a): R_1=OH, R_2=H

(b): R_1=OAc, R_2=H

(c):R_1=OAc, R_2=OAc

(d):R_1=OH, R_2=OAc

(28)



(29)

(26) and (-)-α-formylherbertenol (27) from *Herberta adunca*[34-36] have all been reported to be active. Some constituents isolated from *Concephalum conicum Marchantia domingensis, Dumortiera hirsuta, Metzgeria furcata* and *Porella platyphylla* have microbial activity, while some constituents of *Diplophyllum albicans, Lunularia cruciata* and *Porella vernicosa* have antifungal activity[7].

Plant growth regulatory activity

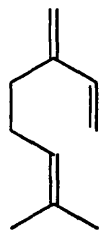
Plant growth regulatory activity is also shown by some liverworts[46]. The *ent*-eudesmanolides (17)-(20) isolated from *Chilocyphus polyanthus* and *Diplophyllum albicans*[17] and the guaianolides (28) from *Conocephalum conicum*[16] and *Weisnerella denudata* show growth inhibitory activity towards the germination and growth of roots of rice. Huneck and Meinuger[47] have tested twenty-nine species of *Hepaticae* and reported that all gave positive results for plant growth regulatory activity. Kodama *et al*[33] reported that the synthetic enantiomer (-)-vitrenal has weak growth promoting properties in contrast to the strong inhibition of growth of rice seedlings shown by (+)-vitrenol (29) from *Lepidozia vitrea*[31,32].

Terpenoids

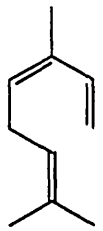
It has been established, on the basis of many studies, that the *Hepaticae* are not only rich in the variety of the common types of terpene skeletons that they produce but also in producing a range of new skeletons many of which are unique to the family. Much scope still remains in this field for the study of the biosynthesis of these skeletal types and for the isolation of the enzyme systems responsible for their production.

Monoterpenoids

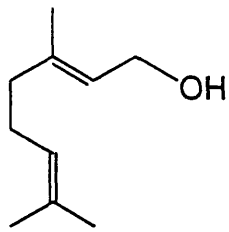
Many liverworts have a characteristic smell due to the presence of monoterpenoids. Monoterpenoids in liverworts have been reported mainly on the basis of detection with GC or GCMS[6]. Generally liverwort monoterpenoids occur in the mixture with sesquiterpenoids. Many monoterpenoids reported in liverworts are



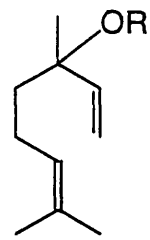
Myrcene
(30)



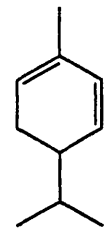
Ocimene
(31)



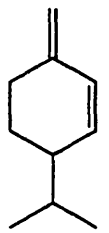
Geraniol
(32)



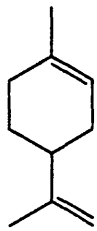
Linalool; R=H
Linalyl acetate; R=Ac
(33)



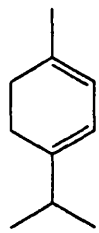
α-Phellandrene
(34)



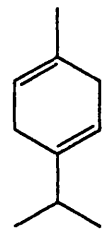
β-Phellandrene
(35)



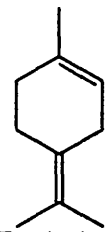
Limonene
(36)



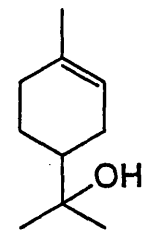
α-Terpinene
(37)



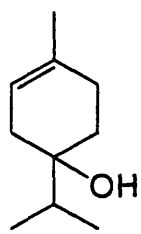
γ-Terpinene
(38)



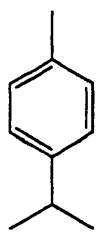
Terpinolene
(39)



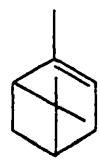
α-Terpineol
(40)



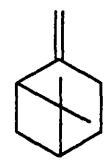
Terpinen-4-ol
(41)



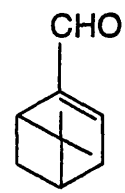
p-Cymene
(42)



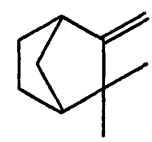
α-Pinene
(43)



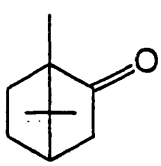
β-Pinene
(44)



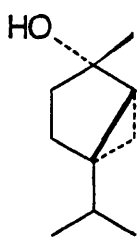
Myrtenal
(45)



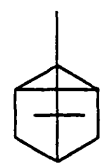
Camphene
(46)



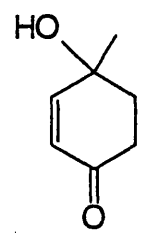
Camphor
(46)



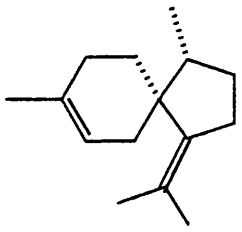
Thujanol
(47)



Tricyclene
(48)



Trisnor derivative
(49)



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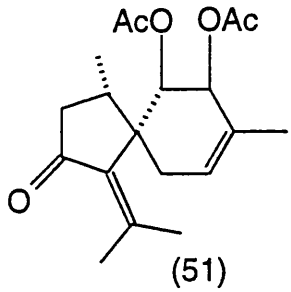

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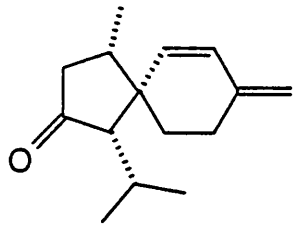

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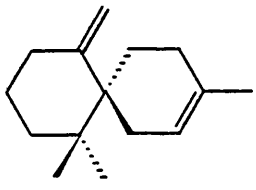

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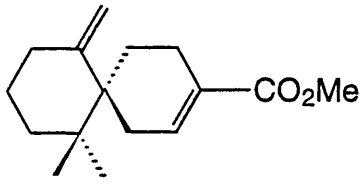

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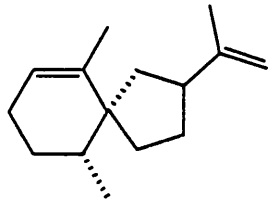

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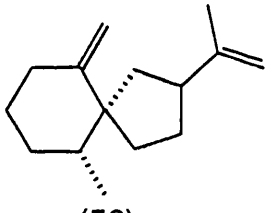

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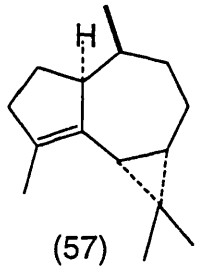

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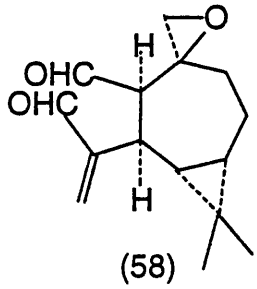

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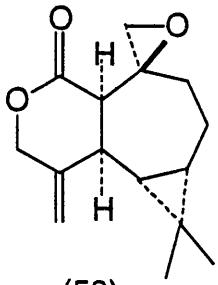

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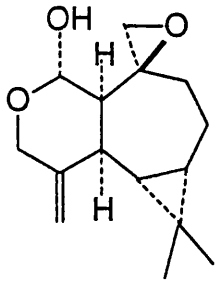

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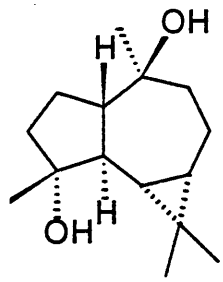

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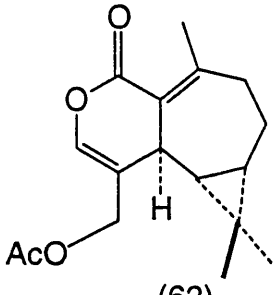

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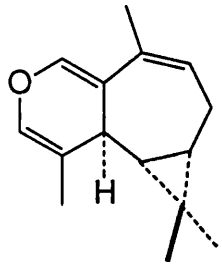


(63)

hydrocarbons but carbonyl compounds and alcohols are also found eg.(30) to (49). Other representatives include myrtenal (45) from *Plagiochilion mayabarae*[48], linalool (33) from *Plagiochila ovalifolia*[6] and α-terpineol (40), terpineol (41), and (-)-thujanol (47) from *Conocephalum conicum*[6,18]. *Bazzania pompeana* and *Porella perrottetiana* contain camphor (46) while (+)-bornyl acetate (4) occurs in *Wiesnerella denudata*[8]. *Cis* and *trans*-pinocarveyl acetates (7) and (8) were reported by Asakawa *et al*[10] from *Targonia hypophylla*. The trisnor derivative (49) occurs in *Jungermannia obovata*[18].

Sesquiterpenoids

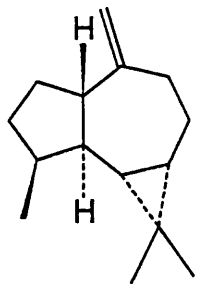
The Hepaticae are well known for producing sesquiterpenoids which are often enantiomeric to those produced in higher plants[120]. Some are also produced in marine organisms. Several unique sesquiterpenoid skeletons have also been found. It is appropriate to give a brief account of the skeletal types found in the Hepaticae.

Acoranes and **Alaskanes**. Andersen *et al*[49] have isolated α-alaskene (50) from *Barbilophozia barbata*. The acorane derivative (51) was recently isolated from Scottish *Barbilophozia barbata*[65]. Recently a new acorane, inflatenone (52), was isolated from *Gymnocolia inflata*[87].

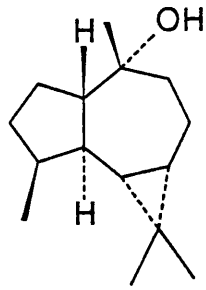
Chamigranes. This skeleton was found in *Scapania undulata* by Andersen et al[53]. Recently the presence of β-chamigrene (53) and methyl chamigrenate (54) were reported in *Omphalanthus filiformis* [111].

Spirovetivanes. This skelton is found in vetiver oil[63]. α-Spirovetivene (55) and its β-isomer (56) have been found in *Scapania maxima* and *Scapania robusta*[64].

Aromadendranes and seco-aromadendranes. This type of skeleton has been found in many liverworts e.g. *Porella, Plagiochila, Mylia* and *Diplophyllum* species[6]. Asakawa *et al*[50] have investigated 14 species of *Plagiochila* and have reported that these species contain aromadendrane derivatives. α-Gurjunene (57) occurs in *Plagiochila ovalifolia, Porella vernicosa* and *P. gracillina*[51]. The seco-aromadendrane derivatives, plagiochilal B (58) and plagiochiline J (59) and K (60)



(64)



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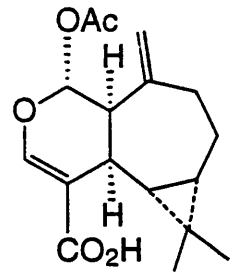

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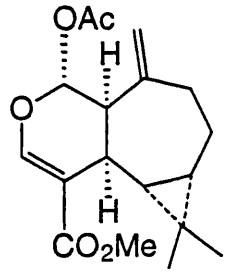

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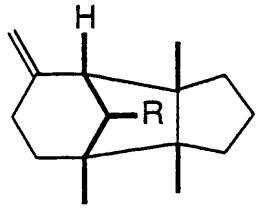
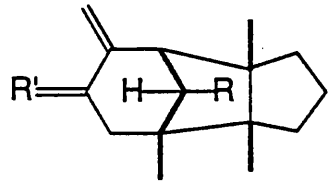


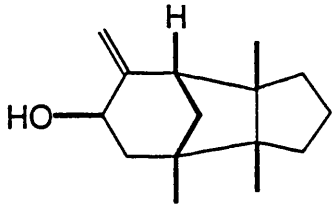
a; R=H
b; R=OH
c; R=OAc

(68)



	R	R'
a;	OH	H,H
b;	OAc	H, α-OH
c;	O cinn.	H, α-OH
d;	OAc	O

(69)



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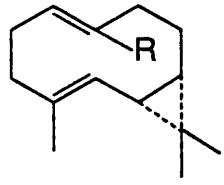
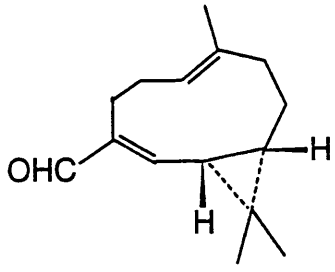


(71) R =Me
(72) R =CHO



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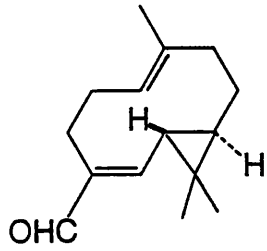

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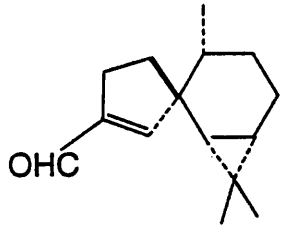

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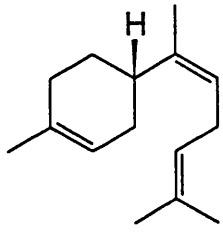

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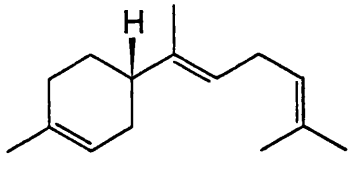

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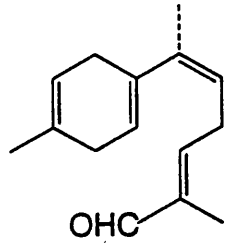

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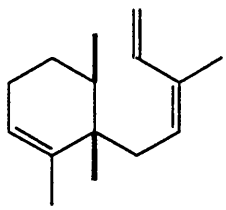

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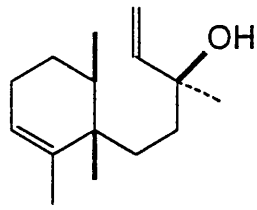

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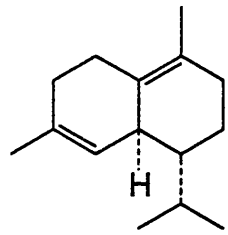

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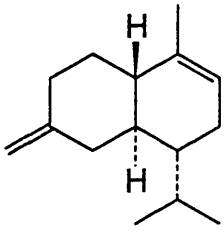

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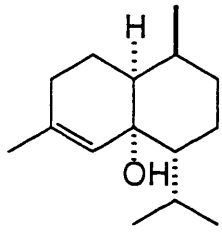

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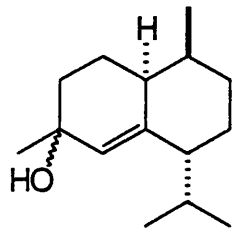

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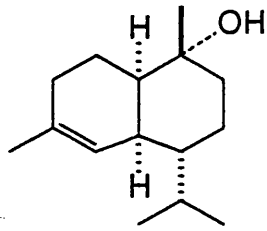

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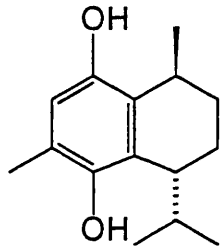

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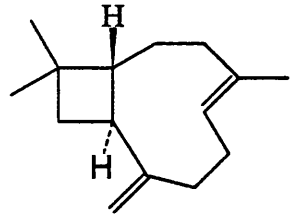

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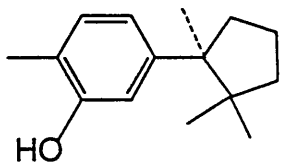

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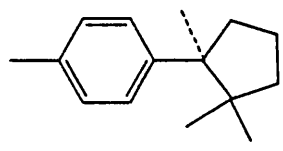

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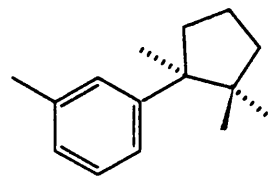


(90)

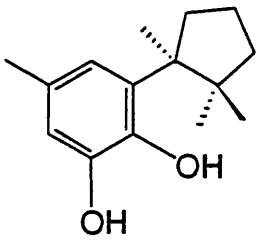
have been obtained from *Plagiochila fruticosa*[92]. Nagashima *et al*[101] isolated *ent*-4β,10α-dihydroxyaromadendrane (61), acetoxyisoplagiochilide (62) and plagiochiline N (63) from *Plagiochila ovalifolia.* The simple aromadendrane, β-diploalbicene (64) and diploalbicanol (65) have been found in *Diplophyllum albicans* and *Diplophylum taxifolium*[39]. Two new seco-aromadendrane derivatives, plagiochiline L(66) and M (67), were isolated by Hashimoto *et al*[110] from *Heteroscyphus planus.*

Gymnomitranes (Barbatanes). This type of skeleton is not found outwith the Hepaticae. The skeleton (68) was first reported by Connolly *et al*[52] from *Gymnomitrion obtusum* which contains a wide range of gymnomitrane derivatives e.g. gymnomitrol (68a). The hydrocarbon gymnomitrene (β-barbatene) and isogymnomitrene (α-barbatene) are widespread in liverworts but the oxygenated derivatives are less common. Toyota *et al*[19] isolated the oxygenated derivative (69) from *Plagiochila trabeculata.* A similar to 9α-hydroxy derivative but lacking the bridge oxygen substituent has been obtained from *Reboulia hemisphaerica* by Becker *et al*[20]. Nagashima *et al*[104] isolated (-)-gymnomitr-8(12)-en-9β-ol (70) from *Marsupella aquatica.*

Bicyclogermacranes and **Vitranes.** Bicyclogermacranes are widespread in liverworts. Asakawa *et al*[50] have isolated bicyclogermacrene (71) from *Plagiochila spp.* Bicyclogermacren-14-al (72) was found in *Concephalum conicum*[18]. Matsuo *et al*[31,32] isolated isobicyclogermacrenal (73), lepidozenal (74) a rare *trans* bicyclo-undecane skeleton and vitrenal (75) from *Lepiodozia vitrea.*

Bisabolanes and **Monocyclofarnesenes.** Andersen et al[53] have isolated *cis*-α-bisabolene (76) and its *trans* isomer (77) from *Scapania undulata.* The new bisabolane derivative, julaceal (78), occurs in European *Anthelia julacea*[54], while the monocyclofarnesenes, striatene (79) and striatol (80) are found in *Ptychanthus striatus*[24-26].

Cadinanes and Calamenanes. These are found in some species of *Jungermanniales, Marchantiales* and *Metzgeriales*[6]. *Conocephalum conicum* contains δ-cadinene (81)[55]. The presence of (-)-γ-cadinene (82) in *Scapania undulata* has been reported by Andersen *et al*[53]. Recently three new cadinane derivatives,



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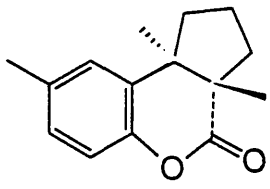

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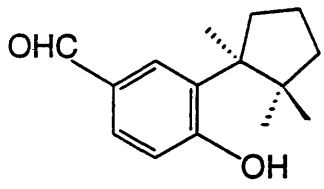

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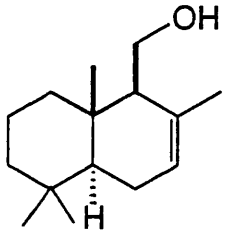

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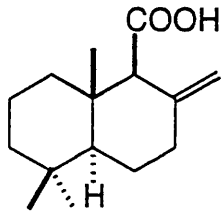

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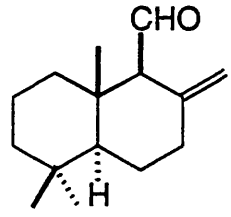

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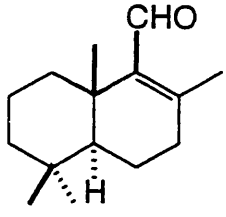

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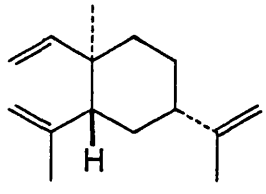

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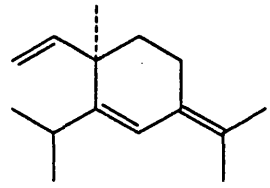

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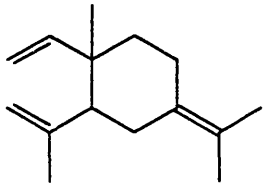

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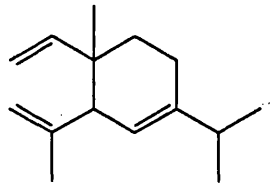

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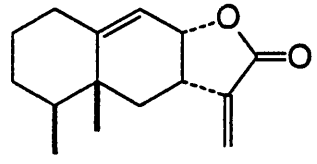

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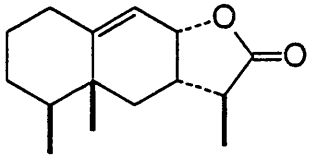

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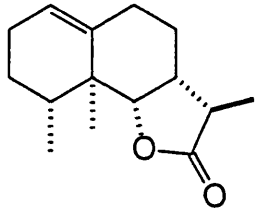

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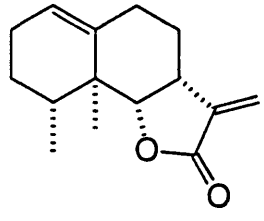

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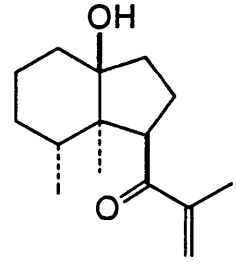


(106)

(+)-4-muurolen-6α-ol (83), scapanol (84) and ent-T-muurolol (85) were isolated from Belgian *Scapania undulata*[107]. A new calamenene type, (+)-5,8-dihydroxycalamenene (86) has been found in *Heteroscyphus planus*[110].

Caryophyllane. Caryophyllene (87) is one of the most common sesquiterpenoids in the Frullaniaceae[57]. It also occurs in *Barbilophozia barbata* and *Scapania undulata*[49].

Cuparanes. Matsuo *et al*[58] have isolated δ-cuparenol (88) and (-)-cuparene (89) from *Bazzania pompiana.* These two compounds have also been reported from *Marchantia polymorpha*[59]. R(-)-α-cuparenone (11)[18] and the cyclocuparane structure, grimaldone (12), are the odoriferous compounds from *Mannia fragrans*[23].

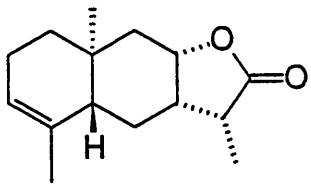
Herbertanes. The herbertane skeleton, which is unique to liverworts, is closely related to cuparane. Several members of this series including (-)-herbertene (90), herbertenediol (91), herbertenolide (92) and the hydroxyaldehyde derivative (93) have been reported from *Herberta adunca*[21,34-36].

Drimanes. These have a bicyclofarnesol skeleton. Asakawa *et al* have reported (-)-drimenol (94) from *Frullania monocera* and *F. osumiensis*[57] and tadeonal (13) [13] from *Porella vernicosa.* Recently the new drimane compounds, albicinc acid (95), albicanal (96) and isoalbicanal (97) were found in *Diplophyllum serrulatum*[98].

Elemanes. β-Elemene (98) was isolated by Ohta *et al*[60] from *Diplophyllum albicans* and *D. taxifolium.* The presence of β-elemene (98) and α-elemene (99) in *Conocephalum conicum* has been reported[9,6]. Two other isomers, γ– and δ-elemene (100) and (101), were found in *Frullania davurica*[57].

Eremophilanes. These are quite rare in liverworts. Eremofrullanolide (102), dihydroeremofrullanolide (103) and the lactones (104) and (105) have been found in *Frullania dilatata*[44,106]. Connolly *et al*[29] isolated the rearranged eremophilane, chiloscypholone (106), from *Chiloscyphus pallescens.*

Eudesmanes. This is the most common skeleton in liverworts and is exemplified by (-)-dihydrodiplophyllolide (107) from *Tritomaria quinquedentata*[87]. Three new



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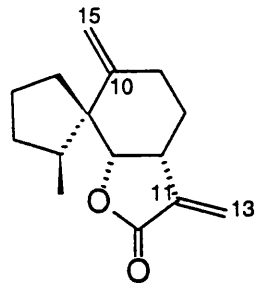
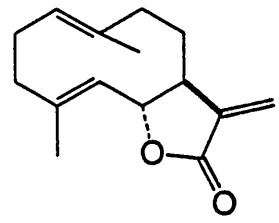


(108)

(109) : 11,13-dihydro

(110) : 10,15-dihydro



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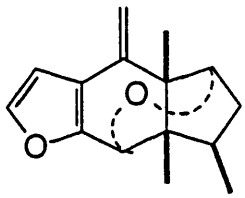

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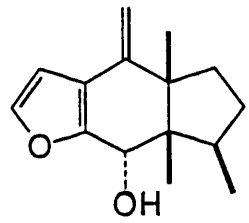

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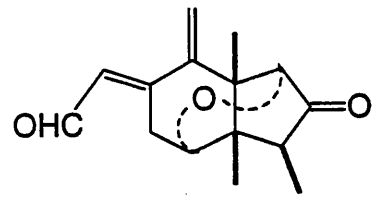

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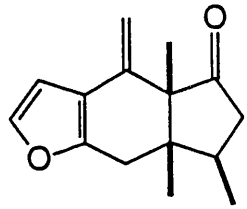

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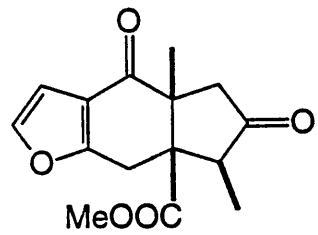

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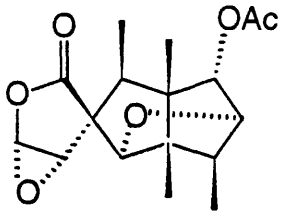

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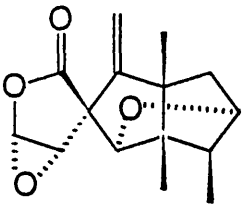

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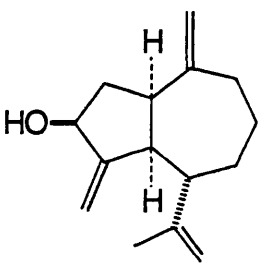

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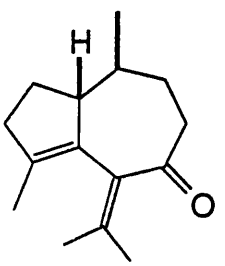

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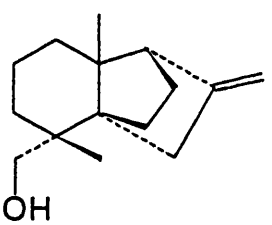

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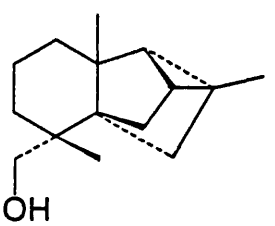

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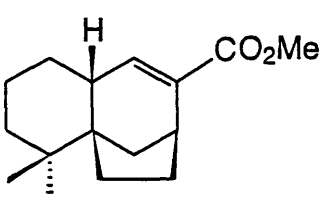

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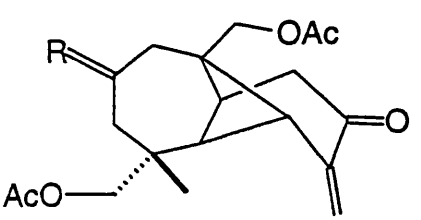
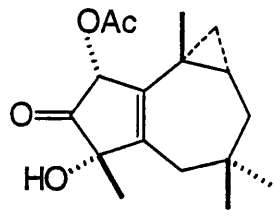


(124): R= H, α-OAc

(125): R= H, β-OAc

(126): R= H, H



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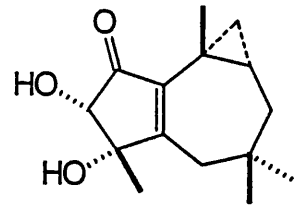

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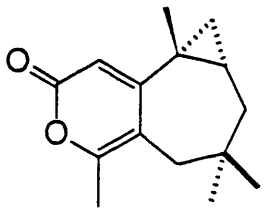
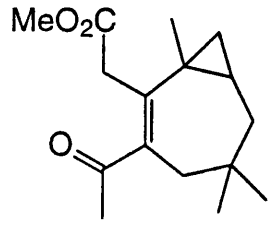


(129)



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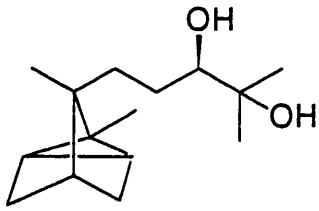
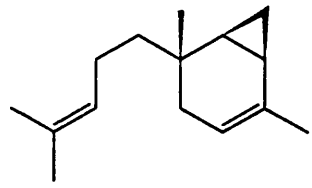
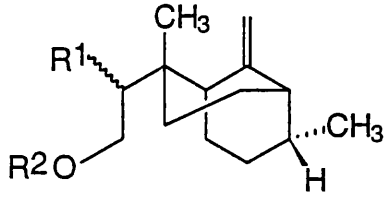


(131)



(132)



	R1	R2
(133):	—OH	H
(134):	····OH	H

rearranged **spiroeudesmane** derivatives, spirodilatanolide A (108), B (109) and C (110) have been reported from *Frullania dilatata*[106].

Germacranolides. Costunolide (111) is present in *Frullania tamarisci*[78], *Marchantia polymorpha* and *Porella japonica*[61]. Asakawa *et al*[16] isolated tulpinolide (16) from *Chiloscyphus polyanthus.*

Guaianes. A series of guaianolides (28) has been isolated by Asakawa *et al*[16] from *Concephalum conicum* and *Weisnerella denudata.*

Pinguisanes. This unique sesquiterpenoid skeleton is difficult to rationalise in terms of the isoprene rule. Pinguisanes are widespread in the liverworts and occur with different oxygenated patterns exemplified by dehydropinguisanin (112), dehydropinguisenol (113) and pinguisenal (114) from *Tricholejeunea sandvicensis*[62], dehydropinguisone (115) from *Plagiochila retrospectans*[101] and bryopterin D (116) from *Bryopteris filicina*[102]. *Frullanoides densifolia* afforded two interesting rearranged pinguisanes, spirodensifolin A (117) and B (118)[90].

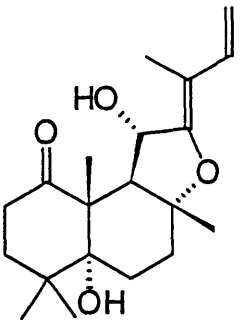
Zierane. Saccogynol (119) was identified in *Saccogyna viticulosa*. This is a rare skeleton, the only other known example being zierone (120)[18].

Tricyclic sesquiterpenoids. Several unusual tricyclic structures have been found. Myltaylenol (121) and cyclomyltaylenol (122) occur in *Mylia taylorii*[37,38], while methyl omphalate (123) has been isolated from *Omphalanthus filiformis*[111].

Gorgiane. This marine skeleton was found in *Frullania tamarisci* as tamariscol (10)[28].

Longipinanes. Examples of this skeleton include 9,11α,14α-triacetoxymarsupellone (124), 9,11β,14α-triacetoxymarsupellone (125) and 9,14α-diacetoxymarsupellone (126) from German *Marsupella emarginata*[88].

Africanes. Sesquiterpenoids (127) and (128) were found in *Porella caespitans* by Toyota *et al*[97]. Recently two interesting 3,4-secoafricanes, secoswartzianin A (129) and B (130) have been found in *Porella swartziana*[109].



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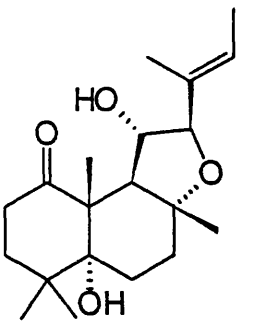

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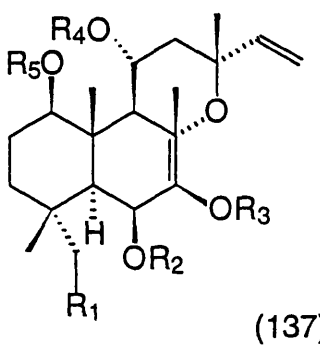
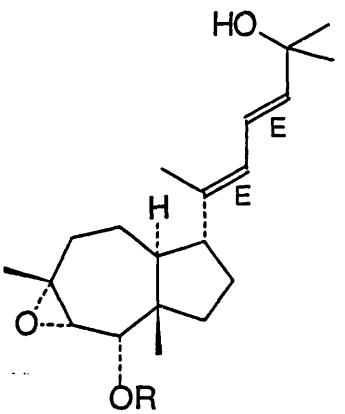


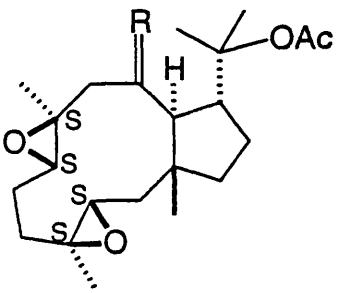
	R_1	R_2	R_3	R_4	R_5
A:	H	Ac	H	Ac	Ac
B;	H	Ac	Ac	Ac	Ac
C:	H	Ac	H	Ac	H
D:	OH	Ac	H	Ac	Ac
E:	H	Ac	H	H	Ac

(137)



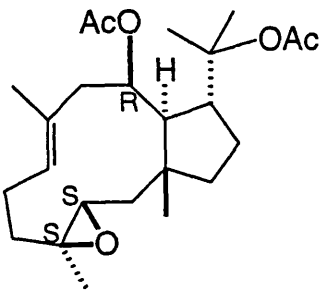
(138): R=Ac

(139): R=H

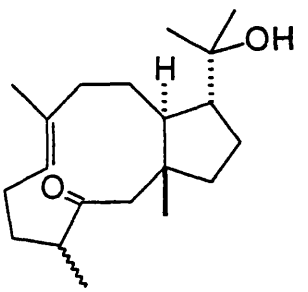


(140): R=H, β–OAc

(142): R = H, H



(141)



(143)

Miscellaneous. A **santalane** type sesquiterpenoid (131) has been reported from *Porella caespitans*[97]. Chinopodene (132) from *Marchantia chinopodia*[100], has a new carbon skeleton. Two new bicyclic sesquiterpenoids, trifarienol A (133) and B (134), are reported from Malaysian *Chilolejeunea trifaria*[114].

Diterpenoids

The occurrence of diterpenoids in liverworts is not as common as that of sesquiterpenoids. The commonly found skeletons are labdane, pimarane, abietane, kaurane, sacculatane, clerodane, dolabellane, verrucosane, neoverrucosane, sphenolobane and fusicoccane.

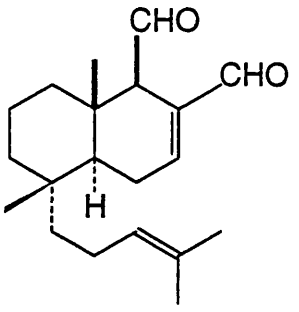
Labdanes. These form the most common skeletons in liverworts. *Scapania undulata* afforded scapanin A (135) and B (136)[67]. Five highly oxygenated derivatives, ptychantins A-E (137) have been isolated from *Ptychanthus striatus*[115].

Sphenolobanes. This type is limited and unique to liverworts. It is exemplified by compounds (138) and (139) found in *Anastrophyllum minutum*[69].

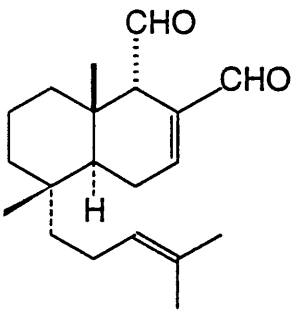
Dolabellanes. The dolabellane skeleton is common in marine organisms. *Barbilophozia floerkei, B. lycopodiales* and *B. attenuata* contain several examples, e.g. barbilycopodin (140), 10R,18-diacetoxy-3S,4S-epoxydolabell-7E-ene (141), 18-acetoxy-3S,4S;7S,8S-diepoxydolabellane (142) and 18-hydroxydolabell-7E-en-3-one (143)[70].

Sacculatanes. This unique skeleton has been reported only in liverworts. Sacculatal (144) and isosacculatal (145) were isolated from *Trichocoleopsis sacculata* by Asakawa and Takemoto[71].

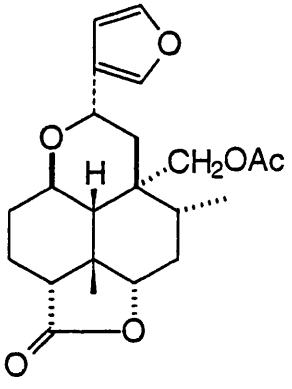
Clerodanes. Representatives of this group continue to appear. The *cis*-clerodanes gymnocolin (146) and ventricosenediolide (147) occur in *Gymnocolia inflata*[72] and *Lophozia ventricosa*[87] respectively. Harrison *et al*[116] found four simple *ent*-clerodanes in *Jungermannia paroica*, i.e. kolavelool (148), Z-*ent*-3β,4β-epoxyclerod-13-en-15-al (149) and its E-isomer (150) and a new natural compound, *ent*-3β,4β-epoxyclerod-14-en-13ξ-ol (151). The seco-clerodane, schistochilic acid (152), is one



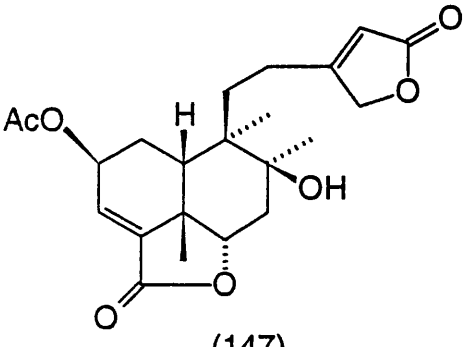
(144)



(145)



(146)



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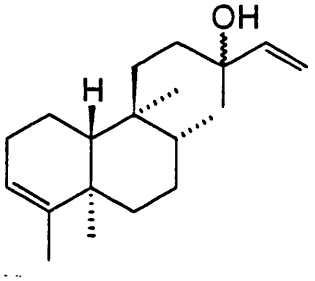

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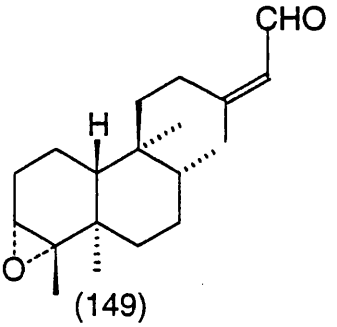

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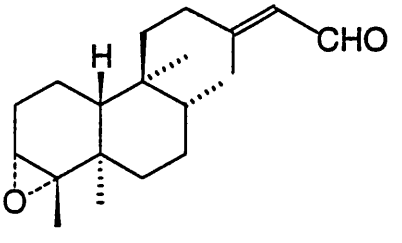

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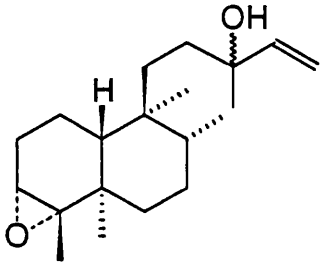

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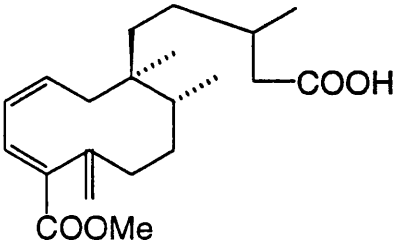
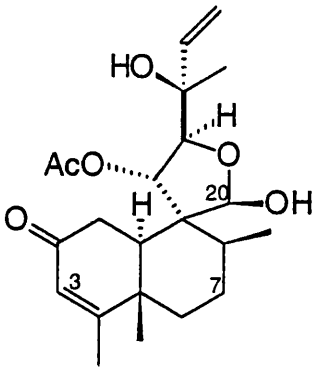
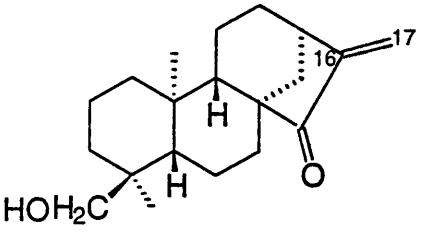


(152)



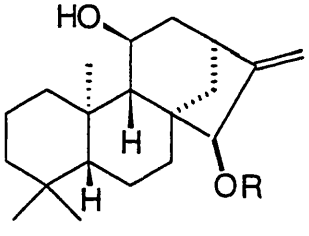
(154)

(153) : 3β, 4β-epoxide
(155) : 20-deoxy
(156) : 7β-hydroxy

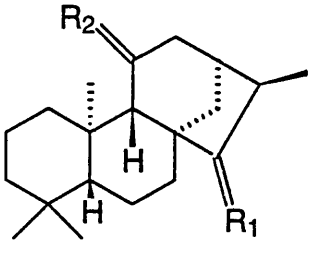


(157)

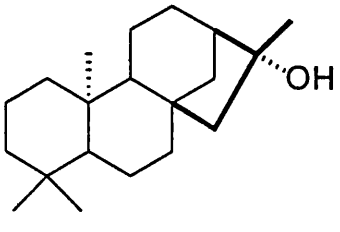
(158) : 16,17-dihydro



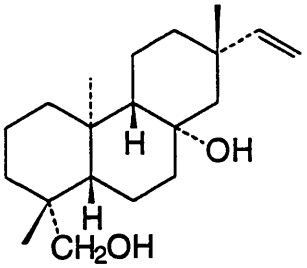
(159) : R = Ac
(160) : R = H



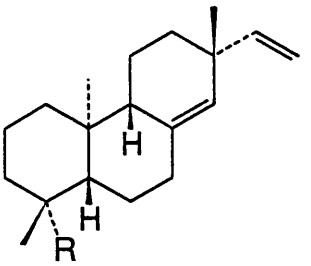
(161) : R_1=O R_2=H, β–OH
(162) : R_1=O R_2=H, β–OH
16,17 didehydro



(163)

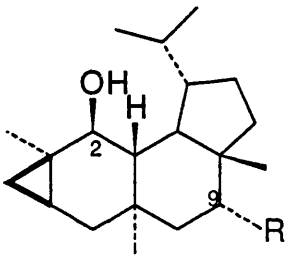


(164)



(165) R= CO_2H

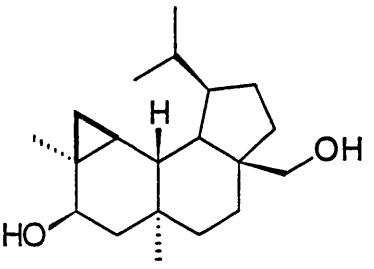
(166) R=CH_2OH



(167): R=H

(168): R=OH

(169): R=OAc



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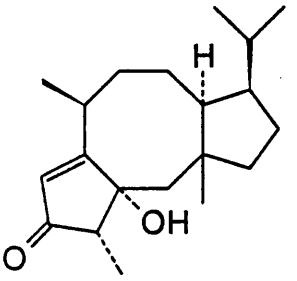
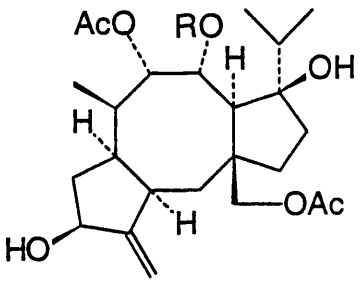
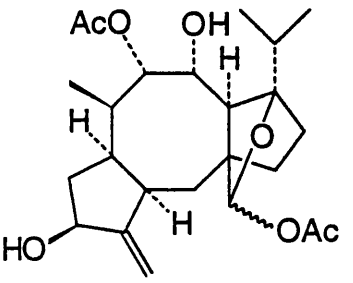


(171)



(172) : R= H

(173) : R= Ac



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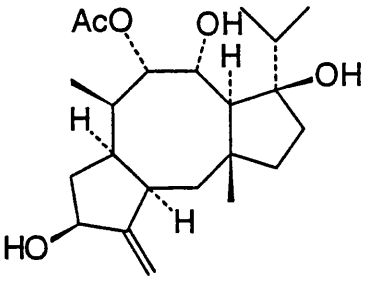

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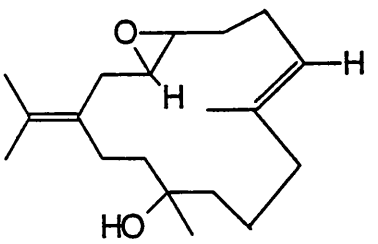

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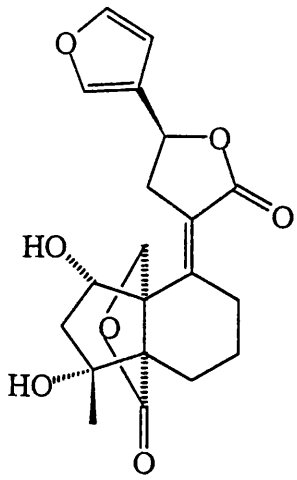

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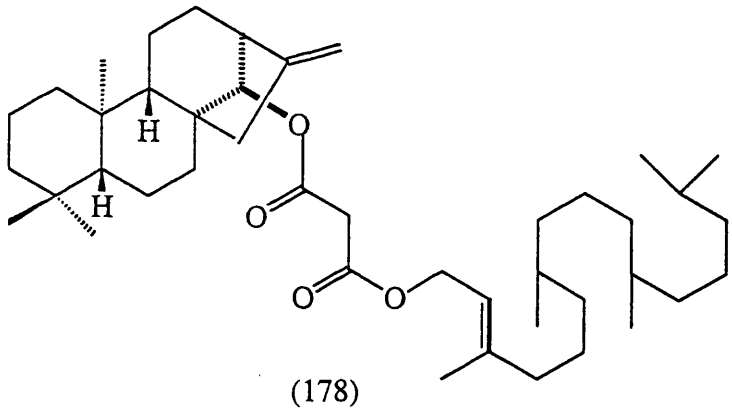

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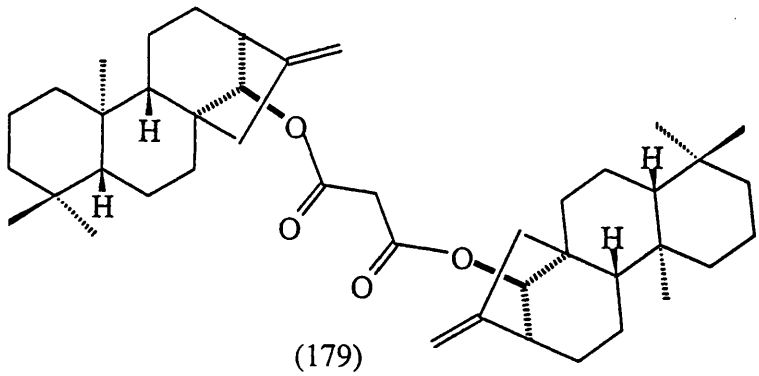
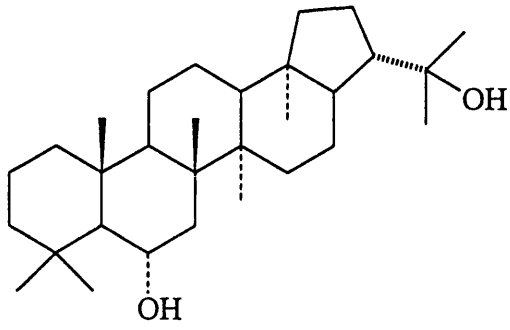


(179)



(180)

of several clerodanes in *Schistochila nobils*[89]. *Heteroscyphus planus* contains four spiroclerodanes, hetroscyphones A (153), B (154), C (155) and D (156)[110].

Kauranes. While both normal and *ent*-kauranes occur in higher plants, liverworts produce only the *ent*-form[6]. The presence of *ent*-18-hydroxykauran-15-one (157) and (16R)-ent-18-hydroxykauran-15-one (158) was reported in *Porella densifolia* by Matsuo *et al*[73]. Connolly *et al*[74] isolated four *ent*-kaurene derivatives, (159)-(162) from *Solenostoma triste*. *Ent*-16β-hydroxykaurane (163) was detected in *Anthelia julacea* by Nagashima *et al*[54].

Pimaranes. A few representatives of this group occur in the Hepaticae, (-)Thermarol (164), *ent*-pimara-8(14),15-dien-19-oic acid (165) and *ent*-pimara-8(14),15-dien-19-ol (166) are examples from *Jungermannia thermarum*[75].

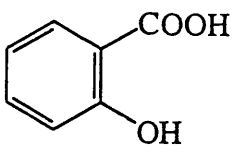
Verrucosanes and **Neoverrucosanes.** *Mylia verrucosa* produces 2β-hydroxyverrucosane (167), 2β,9α-dihydroxyverrucosane (168) and 9α-acetoxy-2β-hydroxyverrucosane (169)[76]. Hashimoto *et al*[110] have found a new neoverrucosane, 13*epi*-neoverrucosan-5β,20-diol (170) in *Heteroscyphus planus.*

Fusicoccane. The first example of this skeleton in liverworts was anadensin (171) from *Anastrepta orcadensis*[77]. Later fusicoplagin A (172), B (173), C (174) and D (175) were isolated from *Plagiochila acanthophylla*[121].

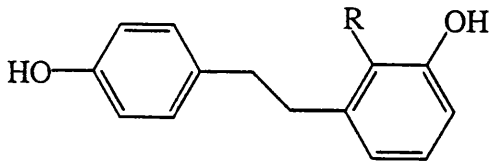
Miscellaneous diterpenoids. The first example of a **Cembrane** type skeleton in liverworts, setiformenol (176), was isolated from *Tetralophozia setiformis*[87]. Jamesoniellide C (177) from *Jamesoniella autumnalis*[119] has a highly rearranged skeleton. The bisditerpenoids (178) and (179) have been reported from *Nardia subclavata*[86].

Triterpenoids

Triterpenoids are rare in the *Hepaticae*. α-Zeorin (180) has been isolated from *Reboulia hemisphaerica*[20] and *Plagiochasma rupestre*[80].

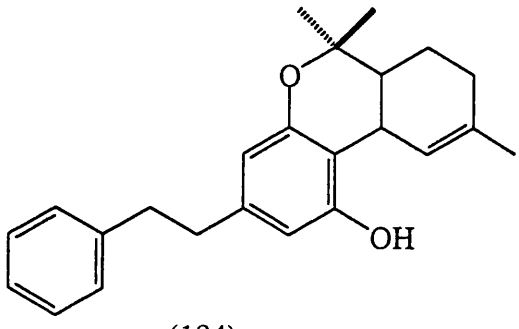


(181)

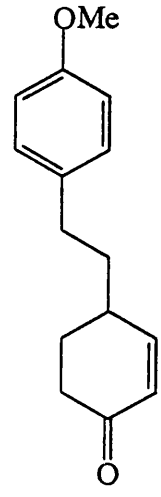


(182): R= COOH

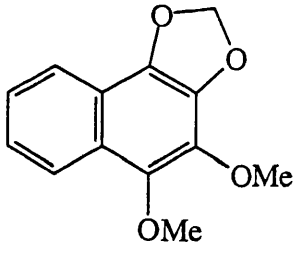
(183): R= H



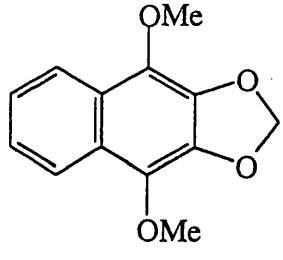
(184)



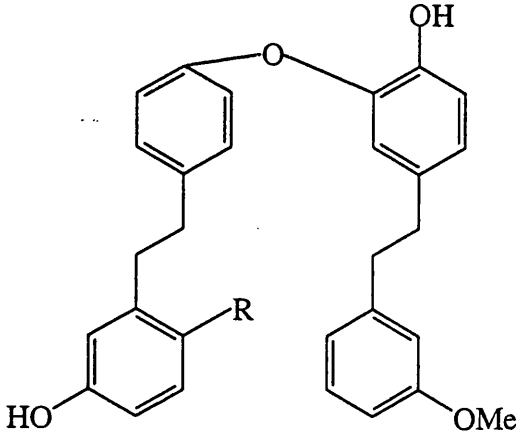
(185)



(186)

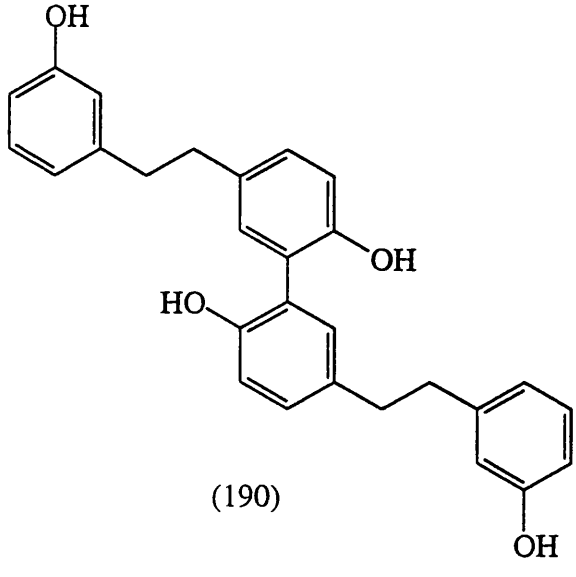


(187)

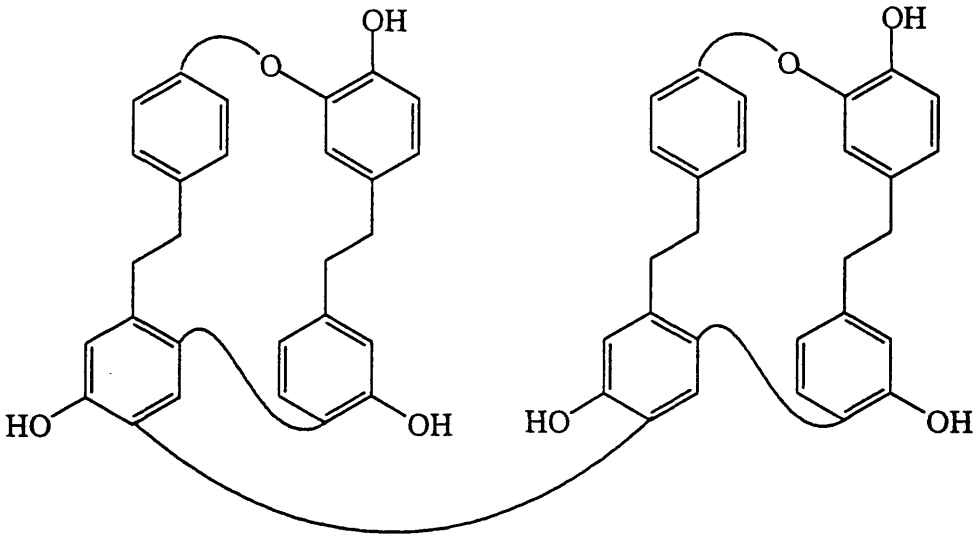


(188): R=H

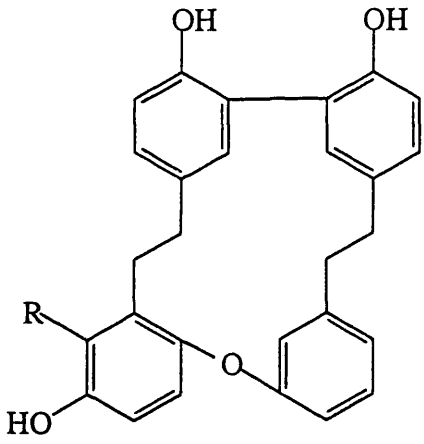
(189): R=OH



(190)

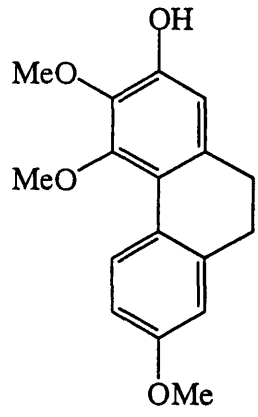


(193)

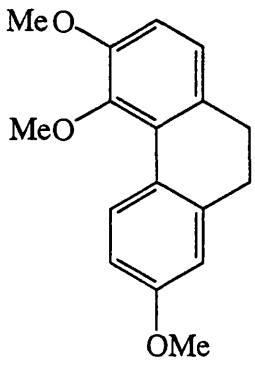


(191) : R=H

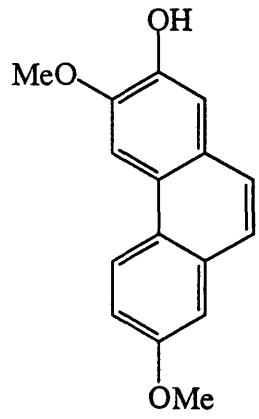
(192) : R = OH



(194)



(195)



(196)

Aromatic compounds

Some aromatic compounds, including mainly bibenzyls and bisbibenzyls occur in liverworts.

Simple benzenes, o-Hydroxybenzoic acid (181) was found in *Asterella lindenbergiana*[81].

Bibenzyls. These are the characteristic metabolites of some liverworts. Lunularic acid (182) and lunularin (183) have been reported in 76 species of the *Hepaticae*[6,82]. Asakawa *et al*[96] have reported fourteen bibenzyl derivatives from *Radula kojana.* A new bibenzyl cannabinoid derivative (184) has been found in *Radula perrottetii*[103]. An interesting new cyclohexenone, 4(-*p*-methoxyphenethyl)-cyclohex-2-en-1-one (185) has been reported from *Plagiochila longispina*[93].

Naphthalenes. Two new naphthalene derivatives, wettstein A (186) and B (187) have been found in *Wettsteinia schusterana*[99].

Bisbibenzyls. These are unique to the Hepaticae and are exemplified by perrottetin E11-methyl ether (188) and 14-hydroxyperrottetin E11-methyl ether (189) from *Pellia endivifolia*[95]. Toyota *et al*[103] have isolated isoperrottetin (190) from *Radula perrottetii.* The macrocyclic isoplagin A (191) and B (192) and the macrocyclic dimer pusilatin A (193) have been reported from *Plagiochila fruticosa*[113] and *Blasia pusilla*[112] respectively.

Phenanthrenes. Several dihydrophenanthrenes, including 2-hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (194) and 3,4,7-trimethoxy-9,10-dihydrophenanthrene (195) from *Plagiochila spinulosa,* have been reported[122]. 3,7-Dimethoxy-2-hydroxyphenanthrene (196) was found in *Marchantia polymorpha*[84].

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CHAPTER TWO

Plagiochila spinulosa (Dicks) Dum.

Plagiochila spinulosa (Dicks) Dum.

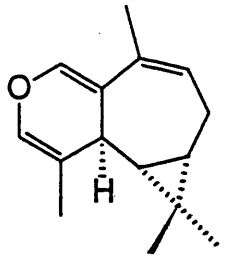
Introduction

The distribution of the nine species of the genus *Plagiochila* in Scotland has been reported[1]. Around 25 species of this genus world-wide have been chemically investigated and many compounds have been reported[10]. Here we present a brief review of recent results. Most of the investigations of this genus show the presence of the 2,3-secoaromadendrane skeleton, e.g. plagiochiline N (1) from *Plagiochila ovalifolia*[2].

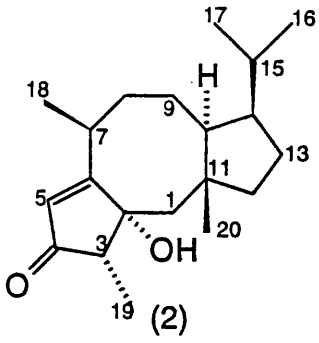
Fusicoccane diterpenoids are well known in fungi. A fusicoccane derivative, anadensin (2), was reported in the Hepaticae (*Anastrepta orcadensis*) for the first time in 1983 by Huneck *et al*[3] and its structure assigned on spectroscopic evidence and by X-ray crystal structure analysis. This compound was later identified in *Plagiochila ovalifolia* by Nagashima *et al*[2]. The presence of the highly oxygenated fusicoccanes, fusicoplagin A-D (3) in *Plagiochila acanthophyla* sub-spp. *japonica* has been reported by Hashimoto *et al*[4]. The bisbibenzyls, isoplagin A and B (4) occur in *Plagiochila fruticosa*[5].

The biosynthetic pathway for the formation of fusicoccane from geranylgeranyl pyrophosphate has been elucidated using deuterium labelled substrates in conjunction with ^{13}C NMR spectroscopy[22]. Three of the four (4R) mevalonoid atoms retained in fuscicoccin (path-A) have been located at C-7, C-15 and C-23 by ^{13}C NMR spectroscopy, following the incorporation of (3 ^{13}C, 4 $^{2}H_2$) mevalonolactone; a 1,2-deuteride shift is demonstrated in the formation of bicyclic intermediate (IV), whilst two consecutive 1,2-deuteride shifts are established during further cyclisation of (IV) to (A) [Scheme I].

Scottish *Plagiochila spinulosa* has been investigated by only Connolly[6,26], his co-workers (Harrison[7] and Salamani[8]) and Rycroft[27]. They have reported the isolation of the dihydrophenanthrene derivatives, (5)- (10), a penta-substituted benzene (11) and the diterpenoid-bibenzyl conjugates spinuloplagins A (12) and B (13). There are only two other reports of dihydrophenanthrenes in liverworts, namely



(1)



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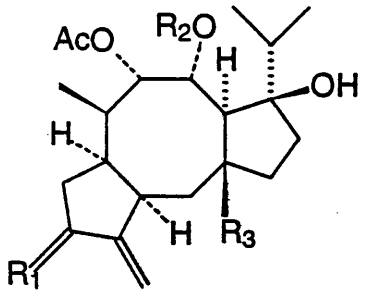
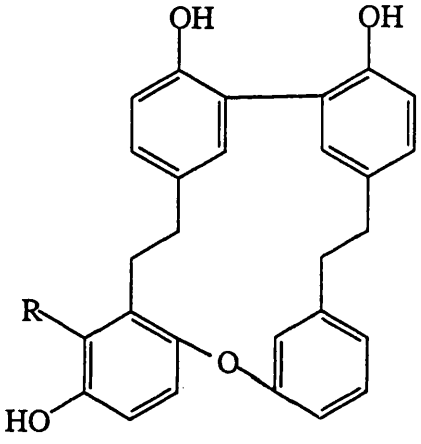
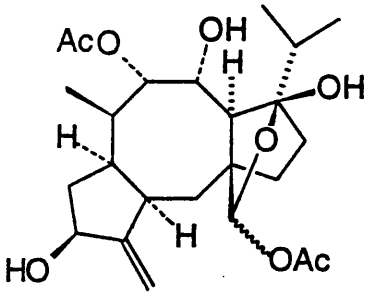


	R_1	R_2	R_3
(3) A=	H, β-OH	H	CH_2OAc
(3) B=	H, β-OH	Ac	CH_2OAc
(3) D=	O	H	CH_3

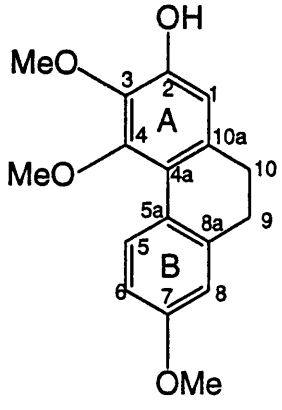


(4) A : R=H

(4) B : R=OH



(3) C



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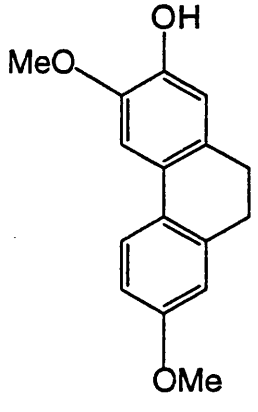

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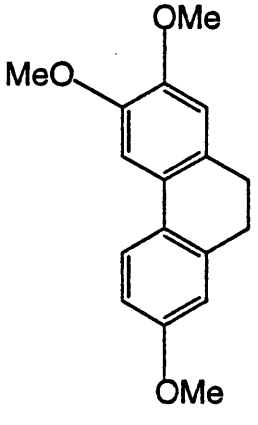

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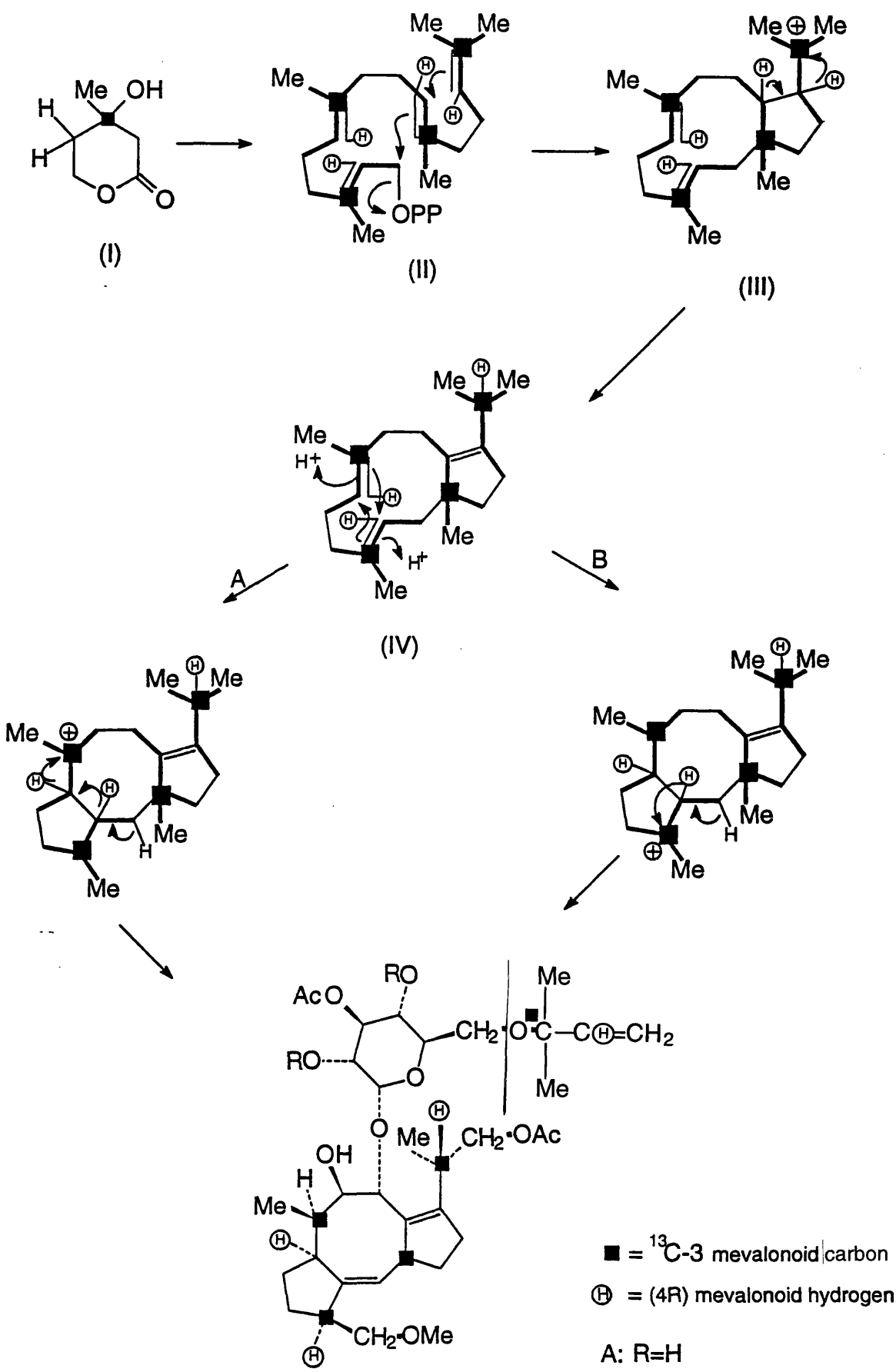
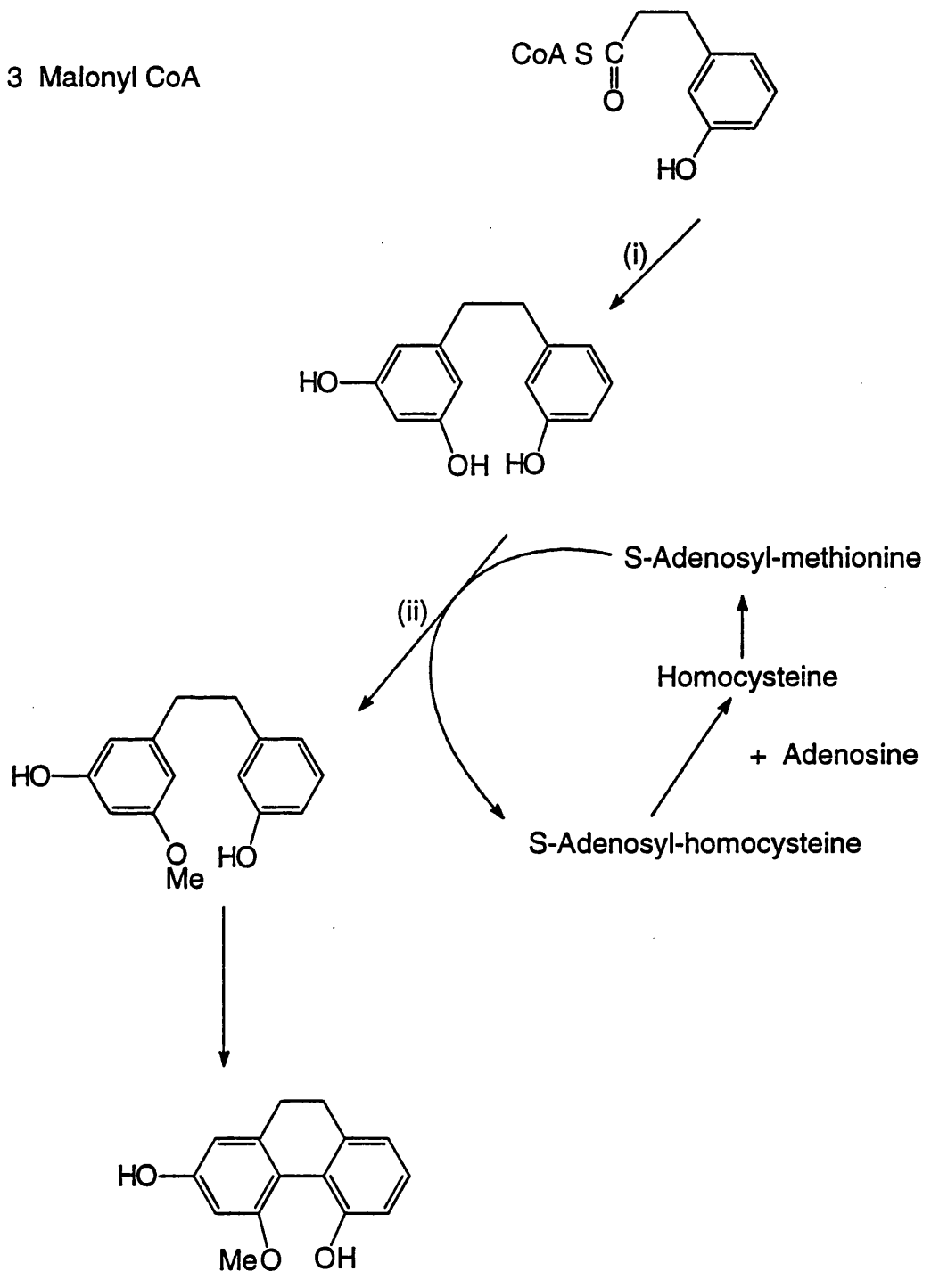
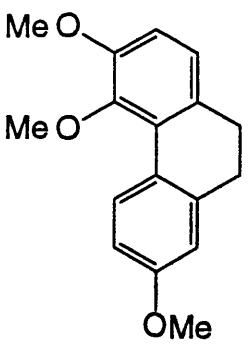

Me OH
H
H
O
O
(I)
Me Me
Me
OPP
Me
(II)
Me ⊕ Me
Me
Me
(III)
Me H Me
Me
H+
Me
H+
Me
(IV)
A
B
Me H Me
Me ⊕
H
Me
H
Me
Me H Me
Me
H
Me
H
⊕
Me
RO
AcO
CH2 O C C H =CH2
RO
O
Me
Me
O
Me
H
CH2 OAc
OH
H
Me
CH2 OMe
■ = 13C-3 mevalonoid carbon
Ⓗ = (4R) mevalonoid hydrogen
A: R=H
B: R=OAc

Scheme I


3 Malonyl CoA
CoA S C
O
HO
(i)
HO
OH HO
S-Adenosyl-methionine
(ii)
Homocysteine
+ Adenosine
HO
O
Me
HO
S-Adenosyl-homocysteine
HO
MeO
OH

Scheme II



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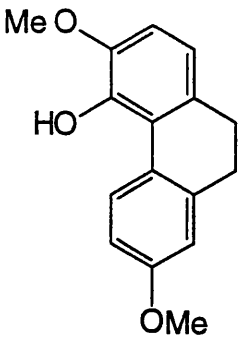
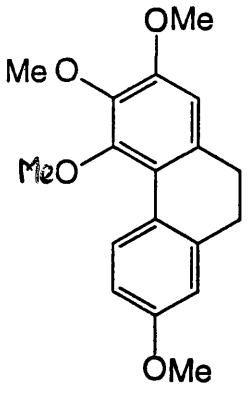
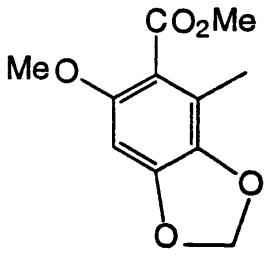


(9)



(10)



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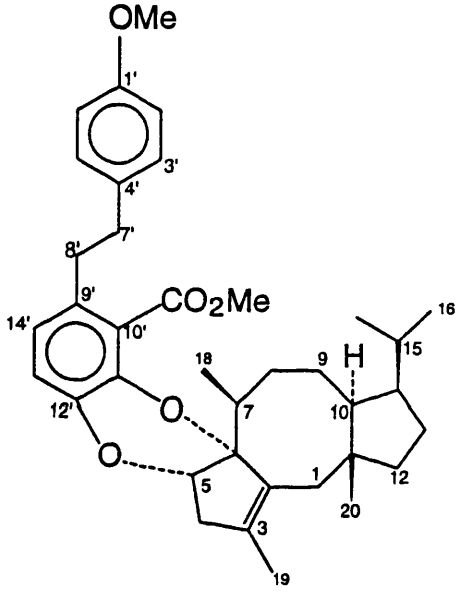
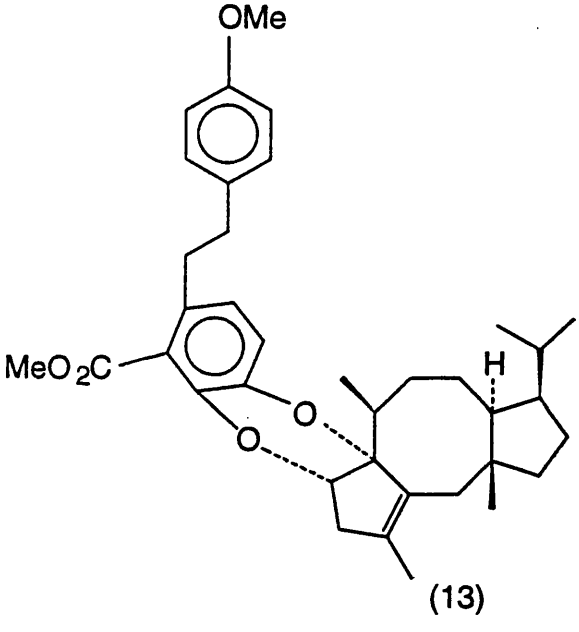


(12)



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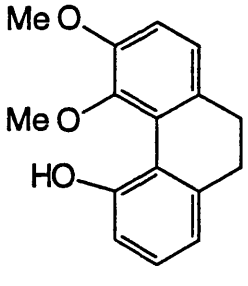

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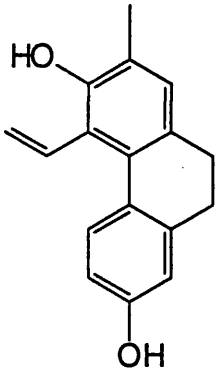

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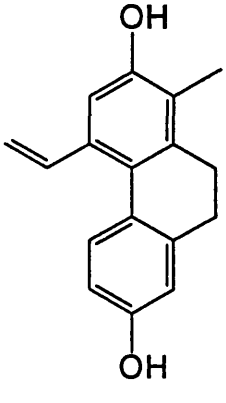

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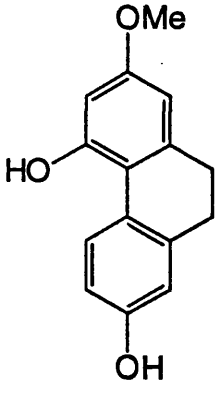


(17)

the isolation of 5-hydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (14) from *Riccardia jackii*[9] and 3,7-dihydroxy-1,2-dimethoxy-9,10-dihydro-5H-phenanthro(4,5-bcd)pyran-5-one from the Indian liverwort *Asterella angusta*[21]. Dihydrophenanthrene derivatives occur in higher plants[11,16&19], in orchids[13,17,18] and in aquatic plants[20]. The dihydrophenanthrenes (15) and (16), isolated from an aquatic plant, have antitumour activity[20]. Recently Lee *et al*[23] tested the antitumor activity of the compounds isolated from the orchid *Dendrobium nobile* and found that the dihydrophenanthrene derivative (17) is active. A phenolic hydroxyl group is essential for the activity.

The biosynthesis of 9,10-dihydrophenanthrene derivatives has been studied by Müller *et al*[24]. They stated that the dihydrophenanthrene pathway proceeds via phenyl propionic acid derivatives. m-Hydroxyphenyl propionyl-CoA and malonyl CoA are the substrates of the bibenzyl synthase reaction (i) [Scheme II]. In the next step, monomethylation of the bibenzyl is carried by S-adenosyl-methionine and S-adenosyl-homocysteine is also produced in this step. This reaction is catalysed by a specific *O*-methyltransferase (ii). Further oxidative coupling leads to the 9,10-dihydrophenanthrene derivative (iii).

Nagashima *et al*[2] and Asakawa[10] have suggested that *Plagiochila* species exist in eight chemotypes, according to their secondary metabolites i.e. (I) 2,3-secoaromadendrane type, (II) bibenzyl type, (III) cuparane and iso-cuparane type, (IV) bibenzyl-cuparane and isocuparane type, (V) gymnomitrane-bicyclogermacrene type, (VI) bicyclogermacrene-spathulenol type, (VII) pinguisane type and (VIII) sesquiterpenoid lactone type. Dihydrophenanthrene derivatives have not been reported in *Plagiochila* spp. and thus *P. spinulosa* may represent a further chemotype, namely (IX) the dihydrophenanthrene type.

Results and Discussion

P. spinulosa was collected from the Aberfoyle Forest in Scotland and investigated as a continuation of previous work. In the present work, a new bibenzyl-fusicoccane conjugate spinuloplagin C (20) has been isolated along with the previously reported spinuloplagins A (12) and B (13). Six dihydrophenanthrenes [(5), (7)-(10) and (18)] and a pentasubstituted benzene (11) were also found and definitive evidence for their structures was obtained from NOE difference experiments and in the case of the benzenoid derivative, a long-range δ_C/δ_H correlation experiment. The fusicoccane diterpenoid, anadensin (2) was isolated for the first time from this species. The presence of anadensin in this species is interesting but perhaps not entirely unexpected. Compounds were identified by spectroscopic analysis and comparison of the data with those of previously reported compounds.

One of the major constituents of the extract was found in fraction four, eluted with ether and petroleum ether. It was shown to be 2-hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (5), m.p. 139-142^{0}C (ex ether- pet. ether), on the basis of its spectroscopic properties (Table I). Its ^{13}C NMR spectrum revealed the presence of seventeen carbons including three methoxyls [δ_C 55.1, 60.0 and 61.0], only one of which has a neighbouring proton, two aliphatic methylenes [δ_C 30.0 and 30.1], four aromatic methines [δ_C 110.0 (C-1), 111.6 (C-6), 113.0 (C-8) and 128.3 (C-5)] and eight other aromatic quaternary carbons [δ_C 120.3 (C-4a), 125.3 (C-5a), 134.9 (C-10a), 139.0 (C-8a), 139.4 (C-3), 147.5 (C-2), 150.5 (C-4) and 157.9 (C-7)], including four oxygenated carbons [C-3, C-2, C-4 and C-7]. The molecular formula of (5), $C_{17}H_{18}O_4$, was deduced from the ^{13}C spectrum and the mass spectrum (m/z 286). Its nine double bond equivalents are satisfied by a dihydrophenanthrene structure. The presence of a four proton multiplet at δ_H 2.71 (2H-9 and 2H-10) is consistent with this proposal[4,11,13,18,19]. Other signals in the ^{1}H NMR spectrum include three methoxyls at δ_H 3.74 (s, at C-4), 3.83 (s, at C-7) and 3.96 (s, at C-3), a phenolic hydroxyl proton at δ_H 5.76 (s, at C-2, exchangeable with D_2O), an aromatic singlet at δ_H 6.62 (s, H-1)[13], and a three spin aromatic system [δ_H 6.81 (dd, J=2.8, 8.5

Table I

^{1}H and ^{13}C NMR data

2-Hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene

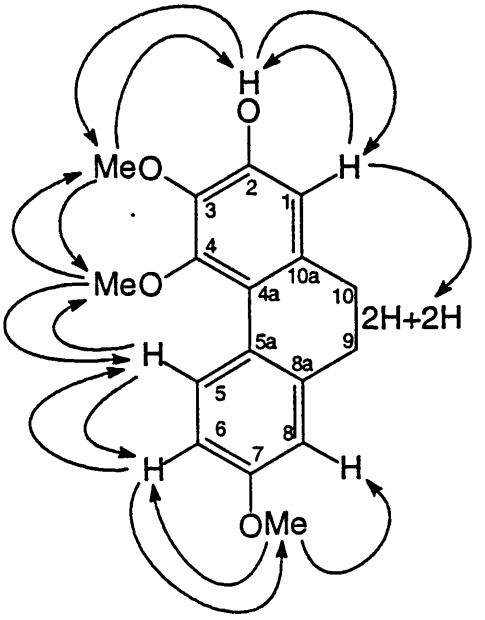
	δ_H	δ_C
1	6.62 (s)	110.0 (d)
2		147.5 (s)
3		139.4 (s)
4		150.5 (s)
4a		120.3 (s)
5a		125.4 (s)
5	8.18 (d, J= 8.5 Hz)	128.3 (d)
6	6.81 (dd, J=8.5, 2.8 Hz)	111.6 (d)
7		157.9 (s)
8	6.76 (d, J=2.8 Hz)	113.0 (d)
8a		139.0 (s)
9 and 10	2.71 (br m)	30.0 & 30.1(both t)
10a		134.9 (s)
OH at C-2	5.76 (s)	
OMe at C-3	3.96 (s)	55.1, 60.0 and 61.1 (all q)
OMe at C-4	3.74 (s)	
OMe at C-7	3.83 (s)	

Table II

2-Hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene

NOEs % →

Irradiation ↓	Saturation%(approx)	H-1 6.62	OH at C-2 5.76	OMe at C-3 3.96	OMe at C-4 3.74	H-5 8.18	H-6 6.81	H-8 6.76	OMe at C-7 3.83	H-9 &10 2.71
3.74 (OMe at C-4)	70			0.1		4.9				
3.83 (OMe at C-7)	75						15.8			
3.96 (OMe at C-3)	69		3.4		0.3					
5.76 (OH at C-2)	36	1.9		0.8						
6.62 (H-1)	69		1.4							1.4
6.81 (H-6)	36					11.3			1.7	
8.18 (H-5)	44				1.2		9.9			



(NOE 5)

Hz, H-6), 6.76 (d, J=2.8 Hz, H-8) and 8.18 (d, J=8.5 Hz, H-5)]. The lowfield shift of H-5 is consistent with its position in a dihydrophenanthrene with an oxygen substituent at C-4[13,14]. Therefore there must also be substitution at C-7. These data lead 2,3,4,7-tetra substituted dihydrophenanthrene structure for (5). NOE difference experiments readily confirmed structure (5) and enabled the identification of the methoxyl groups. The NOEs are summarised in Table II and structure (NOE 5). The substitution pattern of ring A was established as follows. Irradiation of the aromatic proton H-1(δ_H 6.62) afforded NOEs at the methylene (δ_H 2.71, 1.4%) and at the phenolic hydroxyl proton (1.4%). The phenolic hydroxyl proton (δ_H 5.76) gave NOEs at H-1 (1.9%) and at δ_H 3.96 (methoxyl attached at C-3, 0.8%). Irradiation of this methoxyl (δ_H 3.96) resulted in NOEs at the phenolic hydroxyl (3.4%) and the at δ_H 3.74 (methoxyl attached at C-4, 0.3%). As expected irradiation of the C-4 methoxyl affected the C-3 methoxyl and gave a reasonable NOE at H-5 (δ_H 8.18, 4.9%). The substitution of ring B follows from the couplings of the protons and also from NOEs to H-6 (δ_H 6.81) and H-8 (δ_H 6.76) on irradiation of the remaining methoxyl at δ_H 3.83 (attached to C-7) and to H-6 and the C-4 methoxyl on irradiation of H-5. The carbon assignments (Table I) are based on comparison with data reported for similar compounds[13,16].

The second compound, m.p. 74-76^{0}C (ex petroleum ether- $CHCl_3$), was found in the fourth portion of fraction two. Its NMR spectral data (see Experimental) indicate its structure as 3,4,7-trimethoxy-9,10-dihydrophenanthrene (8). Its ^{13}C NMR spectrum contains seventeen carbons including three methoxyls at [δ_C 55.1 56.0 and 60.0], two methylenes [δ_C 29.5 and 30.5], five aromatic methines [δ_C 110.0 (C-1), 111.6 (C-6), 113.1 (C-8), 122.8 (C-2) and 129.5 (C-5)] and seven quaternary carbons [δ_C 125.3 (C-4a), 128.0 (C-5a), 131.4 (C-10a), 140.5 (C-8a), 146.5 (C-3), 152.0(C-4) and 158.4 (C-7)], including three oxygenated carbons [C-3, C-4 and C-7]. These carbon signals and the mass spectrum (m/z 270) lead to the molecular formula $C_{17}H_{18}O_3$, consistent with a dihydrophenanthrene. The ^{1}H NMR spectrum shows the expected four proton multiplet signal at δ_H 2.73 (2H-9 and 2H-10). Three

methoxyls signals are present at δ_H 3.68 (at C-4), 3.83 (atC-7) and 3.88 (at C-3) in the spectrum. An AB system at δ_H 6.74 (d, J=8.2 Hz, H-1) and δ_H 6.92 (d, J=8.2 Hz, H-2) with *ortho* coupling indicates there are methoxyl groups at C-3 and C-4 in ring A. A three spin aromatic system [δ_H 6.83 (dd, J=2.8, 8.6 Hz, H-6), 6.76 (d, J=2.8 Hz, H-8) and 8.36 (d, J=8.6 Hz, H-5)] as in (5) above is consistent with oxygen substitution at C-4 and a methoxyl group at C-7. These data lead to the 3,4,7-trimethoxy-9,10-dihydrophenanthrene structure (8).

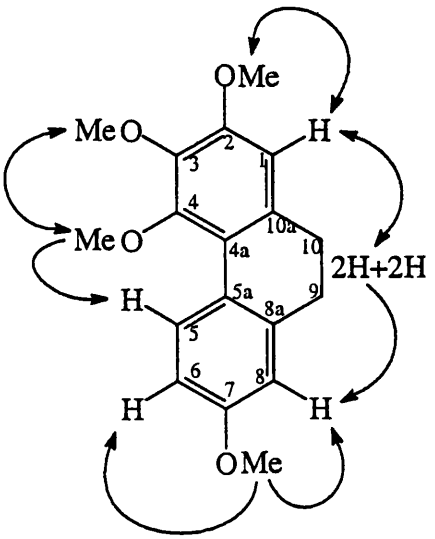
The third compound, m.p. 118-120^{0}C (ex petroleum ether), was obtained from the fifth portion of fraction two which was eluted with ether and petroleum ether. From its spectroscopic properties (see experimental) it was identified as 4-hydroxy-3,7-dimethoxy-9,10-dihydrophenanthrene (9). Sixteen carbons are present in the ^{13}C NMR spectrum of this compound including two methoxyls [δ_C 55.1 and 56.2], two methylenes [δ_C 29.6 and 30.5], five aromatic methines [δ_C 108 (C-1), 111.2 (C-6), 113.1 (C-8), 118.1 (C-2) and 129.5 (C-5)] and seven quaternary carbons [δ_C 120.6 (C-4a), 125.5(C-5a),, 131.5 (C-10a), 140.1(C-8a), 142.7 (C-3a), 145.6 (C-4) and 158.2 (C-7)], including three oxygenated carbons [C-3, C-4 and C-7]. These spectroscopic properties and the mass spectrum (m/z 256) lead to the molecular formula $C_{16}H_{16}O_3$ and to a dihydrophenanthrene structure (9). Its ^{1}H NMR spectrum contains the expected multiplet at δ_H 2.75 (2H-9 and 2H-10). Two methoxyls at δ_H 3.83 (at C-7) and 3.91 (at C-3) and one phenolic hydroxyl at δ_H 6.2 (s, exchangeable with D_2O, attached at C-4) appear in the spectrum. The coupling pattern of protons at δ_H 8.36 (d, J=8.6 Hz, H-5), 6.86 (dd, J=8.6, 2.8 Hz, H-6) and 6.78 (d, J=2.8 Hz, H-8) reveal as above, an oxygen substituent at C-7 in ring B. The AB system at δ_H 6.68 (d, J=8.1 Hz, H-1) and 6.73(d, J=8.1 Hz, H-2) pointed to the presence of two substituents in ring A. The downfield shift of H-5 is also consistent with oxygen substituent at C-3 and C-4. Since both methoxyls have a neighbouring proton (δ_C 55.1 and 56.2), the phenolic hydroxyl must be attached to C-4. Thus the structure of this compound is established as 4-hydroxy-3,7-dimethoxy-9,10-

Table III

2,3,4,7-Tetramethoxy-9,10-dihydrophenanthrene

NOEs% →

Irradiation ↓	saturation . % (approx)	H-1 6.57	OMe atC-2 3.87	OMe atC-3 3.90	OMe atC-4 3.76	H-5 8.21	H-6 6.81	OMe atC-7 3.83	H-8 6.76	2H-9 & 2H-10 2.74
2.73 (H-9,10)	22	12.4							8.0	
2.74 (H-9,10)	22	7.2							14.4	
3.76 (OMe at C-4)	63			0.5		4.1				
3.83 (OMe at C-7)	67						7.0		6.6	
3.87 (OMe at C-2)	66	14.4								
3.90 (OMe at C-3)	61				0.5					
6.57 (H-1)	71		3.4							1.8

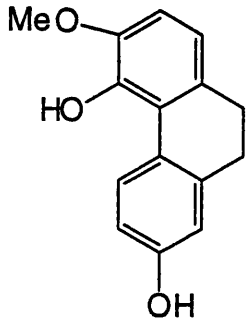


(NOE 10)

dihydrophenanthrene (9). The carbon shifts are assigned by comparison with those previously reported for similar compounds[13,16].

Three other dihydrophenanthrenes were also isolated from the extract as minor metabolites. One was found in the fourth portion of fraction three, eluted with ether and petroleum ether. On spectroscopic evidence it was identified as 2,3,4,7-tetramethoxy-9,10-dihydrophenanthrene (10), $C_{18}H_{20}O_4$, m/z 300, m.p. 134-138^{0}C. The presence of a four proton multiplet at δ_H 2.74 (2H-9 and 2H-10) in the ^{1}H NMR spectrum reveals that it is a dihydrophenanthrene. The spectrum also has four methoxyls at δ_H 3.76 (attached at C-4), 3.83 (attached at C-7), 3.87 (attached at C-2) and 3.90 (attached at C-3). An aromatic singlet at δ_H 6.57 (s, H-1)[13] and a three spin aromatic system [δ_H 6.81 (dd, J=2.8, 8.7 Hz, H-6), 6.77 (d, J=2.8 Hz, H-8) and 8.21 (d, J=8.7 Hz. H-5)] are consistent with the proposed 2,3,4,7-tetrasubstituted dihydrophenanthrene structure for (10). NOE difference experiments, which are summarised in Table III and structure (NOE 10), confirmed this structure and the position of the methoxyl groups. On irradiation, the four proton multiplet (δ_H 2.73, H-9 and 10) afforded NOEs at H-1 (δ_H 6.57, 12.4%) and H-8 (δ_H 6.76, 8.0%) and thus confirmed the presence of a proton (δ_H 6.57) at C-1. NOEs are also observed at H-6 (δ_H 6.81, 7.0%) and at H-8 (δ_H 6.76, 6.6%) on irradiation of the methoxyl protons at δ_H 3.83 (attached to C-7), thus confirming the position of this methoxyl and the substitution pattern of this compound as in (10).

The fourth portion of fraction three also afforded another dihydrophenanthrene, 2,3,7-trimethoxy-9,10-dihydrophenanthrene (7), as an oil. Seventeen carbons are present in the ^{13}C NMR spectrum of this compound including three methoxyls [δ_C 55.3 (q), 55.9 (q) and 56.1 (q)], two methylenes [δ_C 28.6 (t), 29.7 (t)], five aromatic methines [δ_C 106.7 (d), 111.3 (d), 112.1 (d), 113.5 (d) and 124.0 (d)] and seven quaternary carbons [δ_C 119.0 (s), 129.0 (s), 134.8 (s), 138.5 (s), 143.0 (s), 145.0 (s) and 156.4 (s)], of which three are oxygenated carbons [C-2, C-3



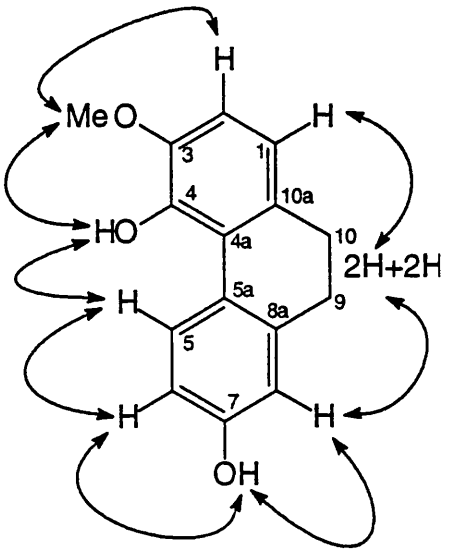
(18)

Table IV

4,7-Dihydroxy-3-methoxy-9,10-dihydrophenanthrene

NOEs %

Irradiation ↓ (δ_H)	saturation . % (approx)	H-1 6.72	H-2 6.69	OMe at C-3 3.91	OH at C-4 6.19	H-5 8.31	H-6 6.76	OH at C-7 4.68	H-8 6.71	H-9 & H-10 2.73
8.31 (H-5)	38				2.3		3.9			
6.76 (H-6)	31					7.4		2.2		
6.72 (H-1)	38									0.9
6.71 (H-8)	67							1.5		0.6
6.70 (H-2)	100			0.8						0.5
6.19 (OH at C-4)	58			0.2		2.0				
4.68 (OH at C-7)	51						2.4		3.0	
3.91 (OMe at C-3)	79		6.6		1.1					
2.73 (2H-9 & 2H-10)	77	14.7							0.7	

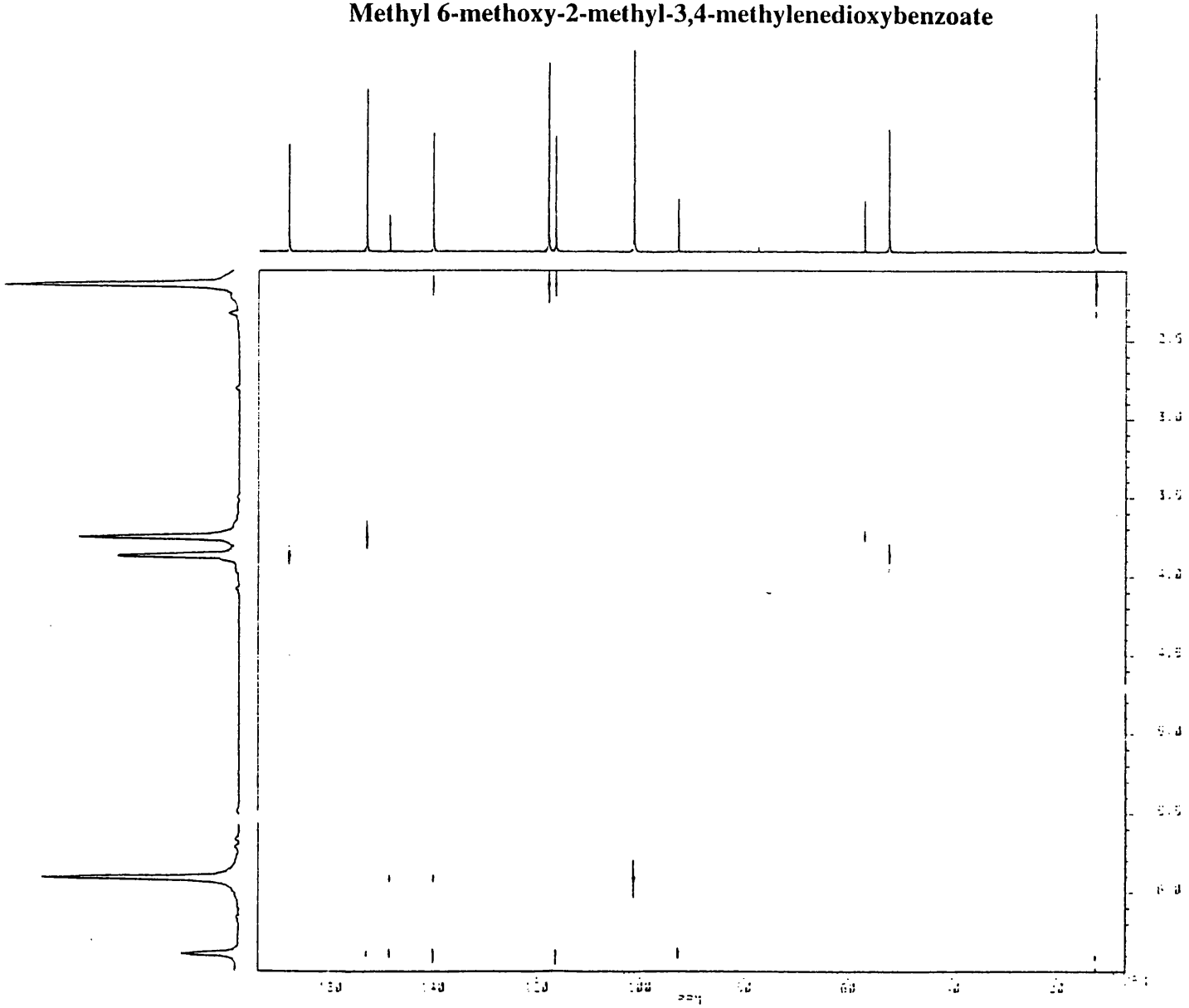


(NOE 18)

and C-7]. These spectroscopic properties and the mass spectrum (m/z 270) lead to the molecular formula $C_{17}H_{18}O_3$. Signals for three methoxyls appear in the ^1H NMR spectrum at δ_H 3.83, 3.89 and 3.92 together with a four proton multiplet at δ_H 2.80 (2H-9 and 2H-10) and two aromatic singlets [δ_H 6.73 (s, H-1) and 7.18 (s, H-4)]. A three spin aromatic system [δ_H 7.54 (d, J=8.4 Hz, H-5), 6.83 (dd, J=2.8, 8.4 Hz, H-6) and 6.77 (d, J=2.8 Hz, H-8) is consistent with an oxygen substituent at C-7 as above. The chemical shift of H-5 is less deshielded in comparison with the other dihydrophenanthrenes indicating that there is no oxygen substitution at C-4. Since all the methoxyl signals in the ^{13}C NMR spectrum appear around δ_C 55, they have at least one proton neighbour. Since of H-1 and H-4 resonate as singlets in the proton spectrum the oxygen substituent must be at C-2 and C-3. The spectroscopic properties of (7) conform to those reported previously[8].

The most polar compound was found in the first portion of fraction six. Spectral features identified it as 4,7-dihydroxy-3-methoxy-9,10-dihydrophenanthrene (18) $C_{15}H_{14}O_3$, m/z 242. The characteristic four proton multiplet at δ_H 2.73 (2H-9 and 2H-10) revealed the 9,10-dihydrophenanthrene system. The proton spectrum shows two phenolic hydroxyls [δ_H 4.68 (s, OH at C-7) and δ_H 6.19 (s, OH at C-4); both exchangeable with D_2O] and a methoxyl [δ_H 3.91 (s, OMe at C-3). The presence of an AB system [δ_H 6.72 (d, J=8.1 Hz, H-1) and δ_H 6.69 (d, J=8.1 Hz, H-2)] with *ortho* coupling indicate that there are two substituents in ring A. The three aromatic proton spin system [δ_H 8.31 (d, J=8.5 Hz, H-5), δ_H 6.76 (dd, J=8.5, 2.8 Hz, H-6) and δ_H 6.71 (d, J=2.8 Hz, H-8)] is consistent with the presence of oxygen substituents at C-7 and at C-4. These data lead to a 3,4,7-trisubstituted dihydrophenanthrene structure for (18). NOE difference experiments readily confirmed the structure (18) and enabled the identification of the phenolic hydroxyl groups. The NOEs are summarised in Table IV and structure (NOE 18). The substitution pattern of the compound was established as follows. Irradiation of the four proton multiplet at δ_H 2.73, (2H-9 and 2H-10) shows NOEs at δ_H 6.72 (14.7%) and at H-8 (δ_H 6.71, 0.7%) indicating the attachment of the proton at δ_H 6.72 to C-1.

Methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate



(fig 1)

$\delta c/\delta_H$ long-range Correlation spectrum of compound (11)

Table V

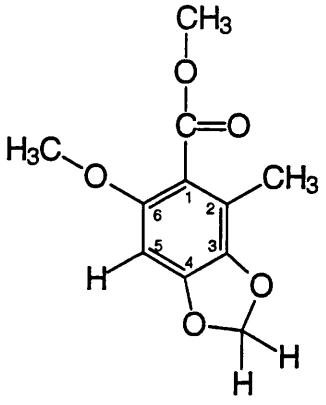
δ_H/δ_C long-range correlation intensities for compound (11)

$\delta_H \rightarrow$ / $\downarrow \delta_C$	δ_H 2.14 (s, Me)	δ_H 3.74 (s, OMe)	δ_H 3.86 (s, CO_2Me)	δ_H 5.90 (s, 2H)	δ_H 6.38 (s, H)
δ_C 168.1 (s)			2.7		
δ_C 153.0 (s)		4.1			0.5
δ_C 148.5 (s)				0.6	1.0
δ_C 140.0 (s)	3.1			0.6	1.5
δ_C 117.7 (s)	4.8				
δ_C 116.2 (s)	3.0				1.0
δ_C 101.3 (t)				5.0	
δ_C 92.8 (d)					1.4
δ_C 56.8 (q)		1.3			
δ_C 52.2 (q)			3.3		
δ_C 12.5 (q)	6.0				

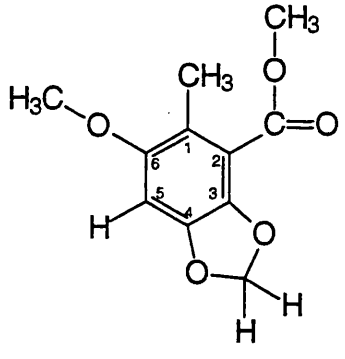
Table VI

^{13}C and ^{1}H NMR data of compound (11)

	δ_C	δ_H
C-1	116.2 (s)	
C-2	117.7 (s)	
C-3	140.0 (s)	
C-4	148.5 (s)	
C-5	92.8 (d)	6.38 (s, H)
C-6	153.0 (s)	
$\underline{C}O_2Me$ at C-1	168.1 (s)	
$CO_2\underline{Me}$ at C-1	52.2 (q)	3.86 (s, 3H)
Me at C-2	12.5 (q)	2.14 (s, 3H)
Methylenedioxy at C-3,4	101.3 (t)	5.90 (s, 2H)
OMe at C-6	56.8 (q)	3.74 (s, 3H)



(11)



(19)

This is confirmed by the irradiation of H-1 which saturated the H-2 (δ_H 6.69) signal and gave a NOE was observed at δ_H 2.73 (H-9 and 10, 0.9%). Irradiation of the phenolic proton at δ_H 4.68 afforded NOEs at δ_H 6.76 (H-6, 2.4%) and δ_H 6.71 (H-8, 3.0%) indicating that the hydroxyl group at δ_H 4.68 is attached to C-7. Irradiation of the remaining phenolic hydroxyl at δ_H 6.19 gave NOEs at δ_H 8.31 (H-5, 2.0%) and at the methoxyl group at δ_H 3.91 (0.2%) revealing the substitution pattern of ring A as in structure (18).

Fraction three afforded a second major compound as an amorphous solid, m.p. 79-80^{0}C, eluted with light petroleum and ether, which was identified as methyl 6-methoxy-2-methyl-3,4-methylenedioxybenzoate (11), on the basis of its spectroscopic properties [^{1}H, ^{13}C, 2D long-range δ_H/δ_C correlation (fig 1)]. Its ^{13}C spectrum has eleven carbons (Table VI) including an aromatic methyl [δ_C 12.5 (q)], two methoxyls [δ_C 56.8 (OMe at C-6) and 52.2 (CO_2Me at C-1)], a methylenedioxy [δ_C 101.3(at C-3,4)], an aromatic methine [δ_C 92.8 (C-5)], three oxygenated aromatic carbons [δ_C 140.0 (s), 148.5 (s) and 153.0 (s)] and three quaternary carbons. These properties and the mass spectrum (m/z 224) reveal the molecular formula $C_{11}H_{12}O_5$. The ^{1}H NMR spectrum (Table VI) contains single aromatic proton [δ_H 6.38 (s, H-5)] which pointed to a benzene ring structure with pentasubstitution. The strongly shielded nature of this proton suggests that it lies between two oxygen substituents. The ^{1}H spectrum also contains an aromatic methyl at δ_H 2.14 (at C-2), two methoxyls [δ_H 3.74 (at C-6) and δ_H 3.86 (CO_2Me at C-1)] and a methylenedioxy[26] at δ_H 5.98 (at C-3,4). These spectroscopic properties lead to structures (11) or (19). A 2D long-range δ_H/δ_C correlation experiment (fig1) confirmed the proposed structure (11) and the results are summarised in Table V. The oxygenated carbon at δ_C 140.0 (C-3) correlates with the methyl group at δ_H 2.14, the methylene group at δ_H 5.90 and with the aromatic proton at δ_H 6.38, confirming its position at C-3 *ortho* to the methyl group at C-2. The oxygenated carbon δ_C 153.0 (C-6) correlates with the methoxyl protons at δ_H 3.74 and with the aromatic proton at δ_H 6.38 but does not show any correlation with methyl protons (δ_H 2.14) which are more than three bond

away from this carbon. This confirms that the methoxyl group attached as in structure (11).

A diterpenoid was identified in the fourth portion of fraction two of the extract, eluted with petroleum ether and ether. It was identified as anadensin (2), m.p. 169-172^{0}C (ex MeOH). There are twenty carbon shifts in the ^{13}C NMR spectrum, namely five methyls, five methylenes, six methines and four quaternary carbons [see Experimental]. A singlet carbon resonating at δ_C 209.2 (C-4) revealed the presence of a ketonic carbonyl group and one at δ_C 81.4 (C-2) shows that C-2 has an oxygen substituent. There is also a tetrasubstituted double bond [δ_C 128.3 (d, C-5) and 187.3 (s, C-6)]. From the ^{13}C NMR and mass spectra (m/z 304) it is possible to deduce the molecular formula as $C_{20}H_{32}O_2$ and hence this molecule is tricyclic with a ketone and double bond. The ^{1}H NMR spectrum shows the presence of one tertiary methyl [δ_H 0.71 (3H-20)], four secondary methyls [δ_H 0.71 and 0.86 (3H-16, 3H-17), 1.11 (3H-18) and 1.20 (3H-19)], an ABq system at δ_H 2.23 and 1.84 (J=14.4 Hz, 2H-1), a tertiary alcohol at δ_H 1.77 (at C-2), a one proton quartet at δ_H 2.65 (J=7.6 Hz, H-3), a one proton multiplet at δ_H 2.87 (H-7) and a vinyl proton at δ_H 5.95 (H-5). These carbon and proton shifts agree with those reported for anadensin[3] and thus this compound is identified as anadensin (2).

The third portion of fraction two, eluted with petroleum ether and ether, afforded a bibenzyl-fusicoccane conjugate that was identified as spinuloplagin A (12), m.p. 89^0-90^{0}C (ex MeOH), whose structure has been established by X-ray analysis[26]. The ^{13}C and ^{1}H NMR shifts [Tables VII and VIII] were assigned by 2D δ_H/δ_C direct correlation[26] and NOE difference experiments. The ^{13}C NMR spectrum (Table 7) and the mass spectrum (m/z 572) are consistent with the molecular formula $C_{37}H_{48}O_5$. The presence of six aromatic protons, an AA′ BB′system [δ_H 6.80 (d, J=8.7 Hz, H-2′ and H-6′), δ_C 113.7 (d, C-2′ and 6′); δ_H 7.06 (d, J=8.5 Hz, H-3′and H-5′)], δ_C 129.3 (d, C-3′ and 5′)] and an *ortho* coupled system [δ_H 6.75 (d, J=8.3 Hz, H-13′), 118.7 (d, C-13′) and δ_H 6.55 (d, J=8.3 Hz, H-14′), δ_C 120.7 (d, C-14′)], a four-proton multiplet [δ_H 2.75 (m, 2H-7′, 2H-8′); δ_C 37.0 and 35.0 (both t, C-7′ and 8′)] and six

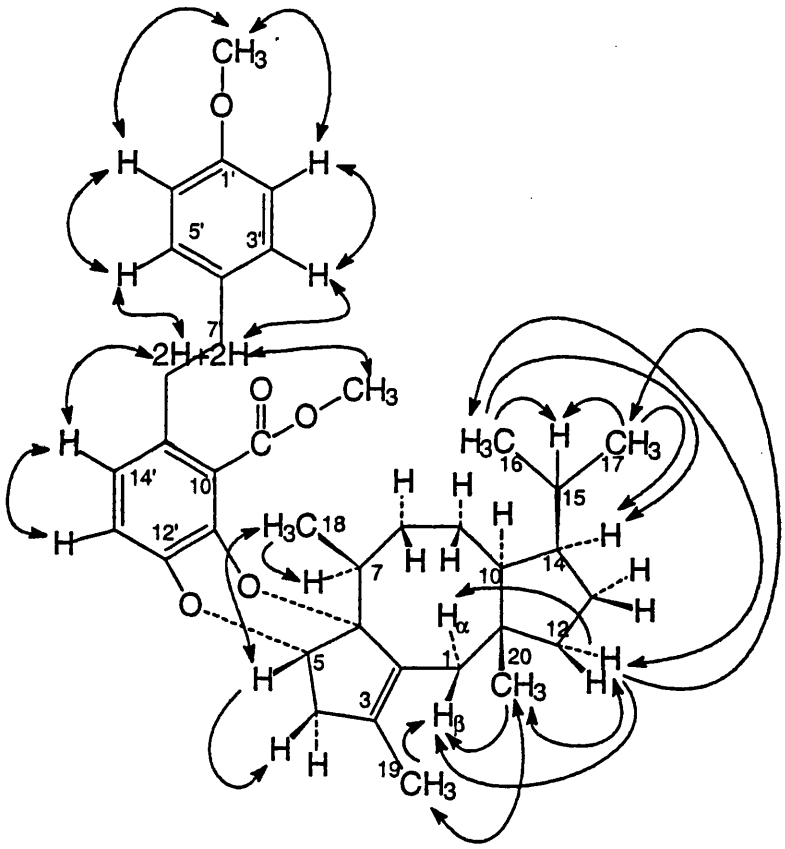
Table VII

Spinuloplagins	δc		
	A**	B	C
C-1′	157.4 (s)	157.7 (s)	157.8 (s)
C-2′ and C-6′	113.7 (d-2)	113.7 (d-2)	113.3 (d-2)
C-3′ and C-5′	129.3 (d-2)	129.3 (d-2)	129.3 (d-2)
C-4′	134.0 (s)	134.0 (s)	134.0 (s)
C-7′	37.0 (t)	37.0 (t)	37.0 (t)
C-8′	35.0 (t)	35.7 (t)	35.5 (t)
C-9′	132.6 (s)	131.1 (s)	130.9 (s)
C-10′	123.3 (s)	123.8 (s)	122.4 (s)
C-11′	144.6 (s)	145.1 (s)	146.4 (s)
C-12′	140.2 (s)	139.4 (s)	140.7 (s)
C-13′	118.7 (d)	117.4 (d)	117.8 (d)
C-14′	120.7 (d)	122.2 (d)	120.7 (d)
$\underline{C}O_2Me$ at C-10′	168.2 (s)	168.2 (s)	168.3 (s)
$CO_2\underline{Me}$ at C-10′	51.9 (q)	52.1 (q)	52.1 (q)
OMe at C-1′	55.2 (q)	55.2 (q)	55.3 (q)
C-1	38.5 (t)	38.5 (t)	35.9 (t)
C-2	137.5 (s)	137.9 (s)	89.1 or 90.9 (s)
C-3	136.2 (s)	136.2 (s)	138.9 (s)
C-4	40.8 (t)	40.6 (t)	121.4 (d)
C-5	74.9 (d)	74.9 (d)	41.4 (t)
C-6	94.1 (s)	93.3 (s)	89.1 or 90.9 (s)
C-7	33.7(d)	33.7 (d)	34.9 (d)
C-10 and C-14	46.9 (d) and 48.8 (d)	46.9 (d) and 48.6 (d)	39.3 (d) and 46.9 (d)
C-11	45.6 (s)	45.7 (s)	44.0 (s)
C-12	44.1 (t)	44.1 (t)	42.9 (t)
C-15	28.0 (d)	28.0 (d)	28.5 (d)
C-16	20.0 (q)	20.0 (q)	
C-17	24.6 (q)	24.5 (q)	11.0, 20.1, 20.2
C-18	16.8 (q)	16.7 (q)	23.8 and 24.5
C-19	15.5 (q)	15.3 (q)	(all q)
C-20	19.3 (q)	19.2 (q)	
t	23.0 (t)	22.9 (t)	24.4 (t)
t	24.8 (t)	24.5 (t)	26.3 (t)
t	30.1 (t)	30.1 (t)	29.1 (t)

** Assignment based on 2D δ_H/δ_C correlation experiment[26]

Table VIII

Spinuloplagins	δ_H		
	A	B	C
H-2′ and H-6′	6.80 (d, J=8.7 Hz)	6.80 (d, J=8.7 Hz)	6.81 (d, J=8.3 Hz)
H-3′ and H-5′	7.06 (d, J=8.5 Hz)	7.06 (d, J=8.4 Hz)	7.07 (d, J=8.3 Hz)
2H-7′ and 2H-8′	2.75 (m)	2.74 (m)	2.75 (m)
H-13′	6.75 (d, J=8.3 Hz)	6.71 (d, J=8.2 Hz)	6.72 (d, J=8.2 Hz)
H-14′	6.55 (d, J=8.3 Hz)	6.59 (d, J=8.2 Hz)	6.61 (d, J=8.2 Hz)
CO_2Me at C-10′	3.86 (s)	3.89 (s)	3.85 (s)
OMe at C-1′	3.78 (s)	3.77 (s)	3.76 (s)
H-1α	1.78 (d, J=14.3 Hz)	1.86 (d, J=13.8 Hz)	
H-1β	2.40 (d, J=14.3 Hz)	2.42 (d, J=13.8 Hz)	
H-4β	2.52 (dd, J=8.0, 16.4 Hz)	2.52 (dd, J=8.4, 16.4 Hz)	5.31 (br m, H-4)
H-5	4.85 (br m)	4.89 (br t, J=7.7 Hz)	2.30 and 2.45 (both m, 2H-5)
H-7	2.32 (m)	2.29 (m)	2.00 (m)
H-10 and H-14	2.1 (m)	2.1(m)	2.08 (m, H-14)
2H-12	1.42 (m)	1.42 (m)	
H-15	1.72 (m)	1.72 (m)	1.72 (m)
3H-16	0.83 (d, J=6.5 Hz)	0.83 (d, J=6.6 Hz)	0.82 (d, J=5.8 Hz)
3H-17	0.89 (d, J=6.5 Hz)	0.89 (d, J=6.6 Hz)	0.85 (d, J=5.8 Hz)
3H-18	1.10 (d, J=6.5 Hz)	1.10 (d, J=6.9 Hz)	1.1 (d, J=6.8 Hz)
3H-19	1.60 (s)	1.60 (s)	1.60 (s)
3H-20	0.72 (s)	0.72 (s)	



(NOE 12)

Table IX

Spinuloplagin A

NOEs % →

Irradiation ↓ at δ_H	Saturation % (approx)	0.72 3H-20	0.83 3H-16	0.89 3H-17	1.10 3H-18	1.42 H-12	1.60 3H-19	1.72 H-15	1.78 H-1α	2.10 H-14	2.32 H-7	2.40 H-1β	2.52 H-4	2.75 4H-7' & 8'	3.78 OMe at H-1'	3.86 CO_2Me at H-10'	4.84 H-5	6.55 H-14'	6.75 H-13'	6.80 H-2' & 6'	7.06 H-3' & 5'
0.72	66					6.6	3.3					3.6									
0.83	45					4.1		5.9		2.0											
0.89	41							4.3		5.3											
1.10	40										7.7						8.5				
1.42	100	1.5	1.0	0.4					1.5	2.4		2.3									
1.60	91	1.3										4.8									
2.40	51	0.9				2.5	2.9		15.3												
2.75	60															0.3		14.5			10.0
3.78	70																			7.6	
3.86	69													0.3							0.5
4.84	50				1.0								4.8								
6.55	44													1.3					1.5		
6.75	60																	5.1			
6.80	81														2,3						7.4
7.06	84													0.7						5.2	

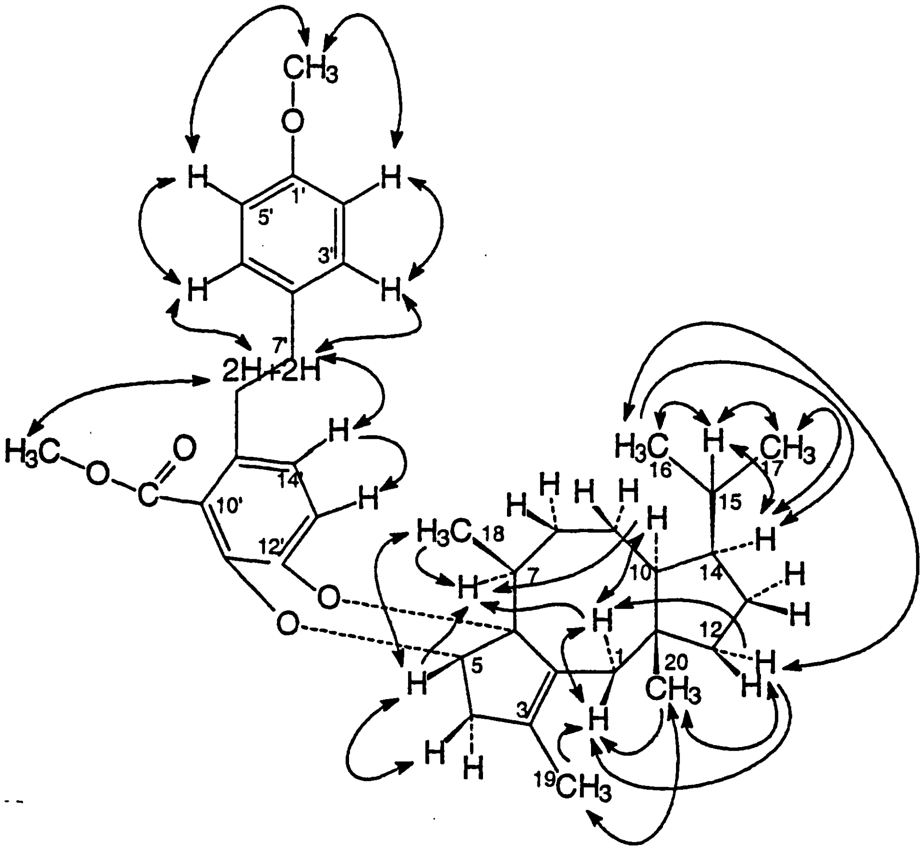
aromatic quaternary carbons [Table VII] clearly indicate a bibenzyl system with four substituents, two of which are a methoxyl [δ_H 3.78 (s), δ_C 55.2 (q), OMe at C-1′] and a carbomethoxyl [δ_H 3.86 (s, CO_2Me at 10′), δ_C 51.9 (q, $CO_2\underline{Me}$), δ_C 168.2 (s, $\underline{C}O_2Me$)]. In addition the ^1H and ^{13}C NMR spectra also contain a tertiary methyl [δ_H 0.72 (s, 3H-20), δ_C 19.3 (q, C-20)], three secondary methyls [δ_H 0.83 and 0.89 (both d, J=6.5 Hz, 3H-16 and 3H-17), δ_C 20.0 (q, C-16), δ_C 24.6 (q, C-17) and δ_H 1.10 (d, J=6.5 Hz, 3H-18), δ_C 16.8 (q, C-18)] and a vinyl methyl [δ_H 1.60 (s, 3H-19), δ_C 15.5 (q, C-19)]. Other identifiable shifts include δ_H 1.78 and 2.40 (both d, J=14.3 Hz, H-1α and H-1β), δ_C 38.5 (t, C-1); δ_H 2.52 (dd, J=8.0, 16.4 Hz H-4β), δ_C 40.8 (t, C-4); δ_H 4.85 (br m, H-5), δ_C 74.9 (d, C-5); δ_C 94.1 (s, C-6); δ_H 2.32 (m, H-7), δ_C 33.7 (d, C-7); δ_H 2.1 (m, H-10 and H-14), δ_C 46.9 and 48.8 (both d, C-10 and C-14); δ_H 1.42 (m, 2H-12), δ_C 44.1 (t, C-12); δ_H 1.72 (m, H-15), δ_C 28.0 (d, C-15). These data are associated with the diterpenoid moiety. The chemical shifts of two aromatic carbons (C-11′ and C-12′) and of two carbons (C-5 and C-6) of the diterpenoid unit indicate that they are involved in ether links. The molecular formula shows only two more oxygen atoms are available and these can be accommodated in the ether links as in structure (12). The orientation of the attachment of aromatic ring was revealed by the X-ray analysis. NOE difference experiments helped to verify this structure and confirm some of the stereochemistry. The results are summarised in Table IX and structure (NOE 12). Irradiation of the tertiary methyl 3H-20 (δ_H 0.72) shows NOEs to H-1β (δ_H 2.40, 3.6%), the vinyl methyl 3H-19 (δ_H 1.60, 3.3%) and H-12 (δ_H 1.42 6.6%). The secondary methyl 3H-16 (δ_H 0.83), on irradiation, afforded NOEs at H-12 (4.1%), H-15 (δ_H 1.72, 5.9%), and H-14 (δ_H 2.1, 2.0%). As expected, irradiation of 3H-17 (δ_H 0.89) gave NOEs at H-15 (4.3%) and H-14 (5.3%), while irradiation of the remaining secondary methyl 3H-18 (δ_H 1.10) showed NOEs at H-7 (δ_H 2.32, 7.7%) and H-5 (δ_H 4.84, 8.5%). The vinyl methyl 3H-19, on irradiation, afforded NOEs at 3H-20 (1.3%) and H-1β (4.8%). These data confirmed the structure of the diterpenoid unit. In addition irradiation of the aromatic protons at δ_H 6.80 (H-2′ and 6′) afforded NOEs at the methoxyl group (δ_H 3.78, 2.3%) and H-3′, 5′ (δ_H 7.06, 7.4%), while irradiation of the four proton multiplet (δ_H 2.75 2H-7′ and 2H-8′) showed NOEs at the carbomethoxyl (δ_H 3.86, 0.3%), H-14′ (δ_H 6.55, 14.5%) and

Table X

Spinuloplagin B

NOEs %

Irradiation ↓ at δ_H	Saturation % (approx)	0.72 3H-20	0.83 3H-16	0.89 3H-17	1.10 3H-18	1.42 H-12	1.60 3H-19	1.72 H-15	1.86 H-1α	2.10 14	2.29 H-7	2.42 H-1β	2.52 H-4	2.74 4H-7' & 8'	3.77 OMe at H-1'	3.89 CO_2Me at H-10'	4.89 H-5	6.59 H-14'	6.71 H-13'	6.80 H-2' & 6'	7.06 H-3' & 5'
0.72	58					7.5	2.4.					2.1									
0.83	42					4.4		6.2		1.4											
0.89	47					1.9		6.1		4.0											
1.10	31										8.3						5.4				
1.42	51	1.0	0.7						1.2	1.8		1.8									
1.72	35		1.1	1.5						1.7											
1.86	31									5.1	3.5	17.3									
2.10	50			1.0				2.0	2.4		6.8										
2.42	36	0.6							15.4												
2.52	18																7.7				
2.74	24															0.7		10.0			8.0
3.77	69																			6.2	
3.89	64													0.3							
4.89	39				3.2						6.0		7.5								
6.59	37													1.5					1.4		
6.80	42														3.3						9.5
7.06	70													0.7						2.8	

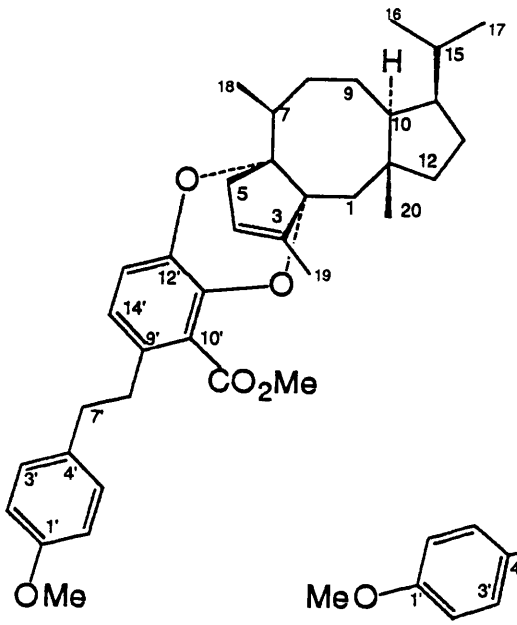


(NOE 13)

H-3′ and H-5′ (δ_H 7.06, 10%), confirming the substitution pattern in the bibenzyl unit of the structure. All these results are totally consistent with structure (12) for spinuloplagin A. It is interesting to notice that there are no NOEs observed between the bibenzyl and the diterpenoid moieties.

The second bibenzyl-diterpenoid conjugate, spinuloplagin B (13), $C_{37}H_{48}O_5$ (m/z 572)[8], was isolated from the first portion of fraction three. The ^{13}C and ^{1}H NMR spectra of this compound are extremely similar to spinuloplagin A (Table VII and VIII), the main differences being in the shifts of one ring of the bibenzyl portion. The bibenzyl and diterpenoid portion appear to be identical to those in spinuloplagin A and therefore the only possible difference between spinuloplagin A and B is in the regiochemistry of the formation of the ether linkages. This leads to structure (13) for spinuloplagin B, where the oxygen of C-11′ is attached to C-5 and the oxygen of C-12′ is attached to C-6. The results of NOE difference experiments are very similar to those of spinuloplagin A and are summarised in Table X and structure (NOE 13). The NOEs are consistent with the proposed structure but, as for spinuloplagin A, no NOEs were observed between the two units.

The least polar among all the compounds isolated was found in the third portion of fraction two and identified a new bibenzyl fusicoccane conjugate, spinuloplagin C (20), whose structure was assigned on the basis of spectroscopic evidence (^{13}C and ^{1}H NMR spectra (Tables VII and VIII), NOE difference experiments and decoupling experiments) and comparison with spinuloplagins A and B. The ^{13}C NMR spectrum and the mass spectrum (m/z 572) are consistent with the molecular formula $C_{37}H_{48}O_5$. The ^{13}C and ^{1}H NMR spectrum of this compound are similar to spinuloplagins A and B with some differences in the chemical shifts of the diterpenoid unit indicating that this has been modified. Its ^{1}H spectrum contains five methyls [δ_H 0.80 (s, 3H-20), 0.82 and 0.85 (both d, J=5.8 Hz, 3H-16 and 3H-17), 1.10 (d, J=6.8 Hz, 3H-18) and 1.60 (s, 3H-19)] and a vinyl proton [δ_H 5.31 (m, H-4)]. Other identifiable shifts are δ_H 2.30 and 2.45 (both m, H-5a and H-5b) and δ_H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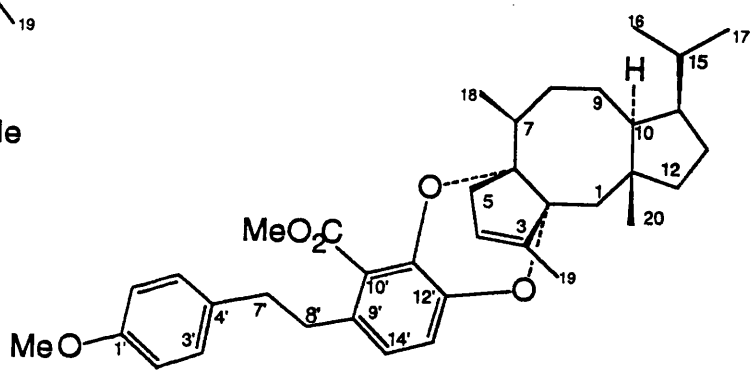
17
15
H
18
7
9
10
12
5
3
1
20
19
O
12'
14'
9'
10'
CO2Me
7'
3'
4'
1'
OMe

(20)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15
H
18
7
9
10
12
5
3
1
20
19
O
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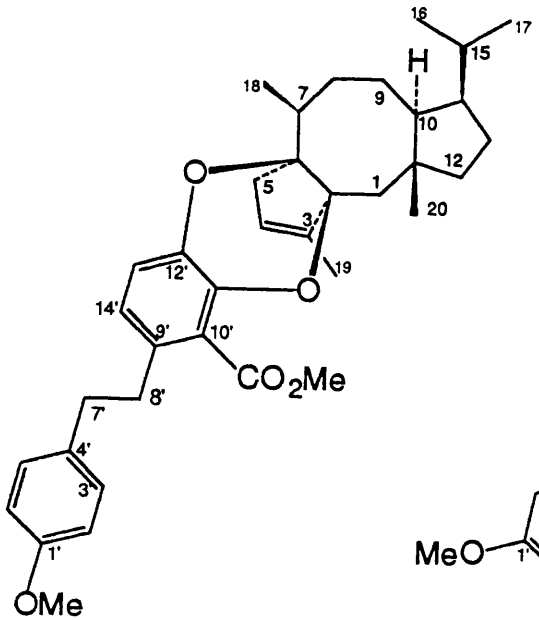
10'
12'
9'
14'
7'
8'
4'
3'
1'
MeO

(21)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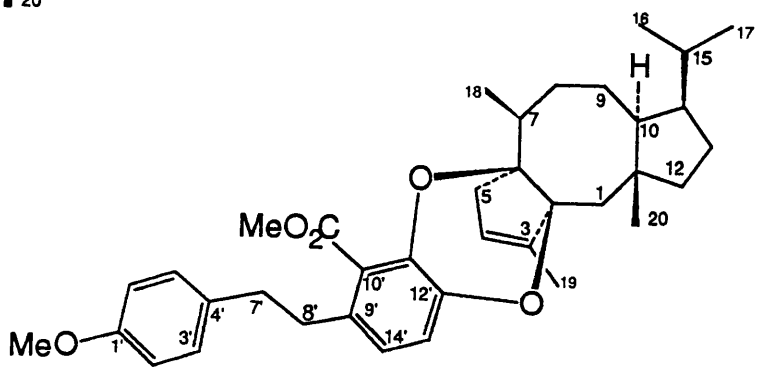
17
15
H
18
7
9
10
12
5
3
1
20
19
O
12'
14'
9'
10'
CO2Me
8'
7'
4'
3'
1'
OMe

(22)


16
17
15
H
18
7
9
10
12
5
3
1
20
19
O
MeO2C
10'
12'
9'
14'
7'
8'
4'
3'
1'
MeO

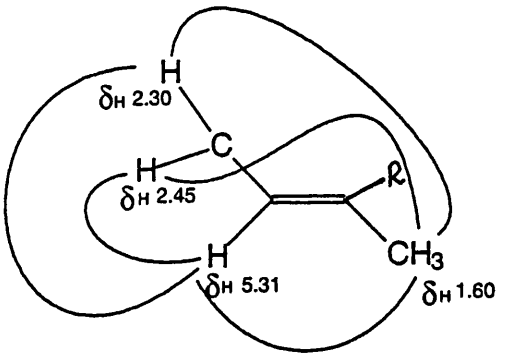
(23)

Table XI

Spinuloplagin C

Homonuclear decoupling experiments

Irradiation	effects
δ_H 1.60 (3H-19)	δ_H 5.31 becomes dd, J=3 and 1.5 Hz δ_H 2.30 becomes dd, J=16.2 and 3.2 Hz δ_H 2.45 becomes dd, J=16.0 and 1.5 Hz
δ_H 5.31 (H-4)	effects seen at δ_H 2.30, δ_H 2.45 and δ_H 1.60 in decoupling difference spectra. δ_H1.60 becomes dd, J=2.5 and 1.5 Hz.



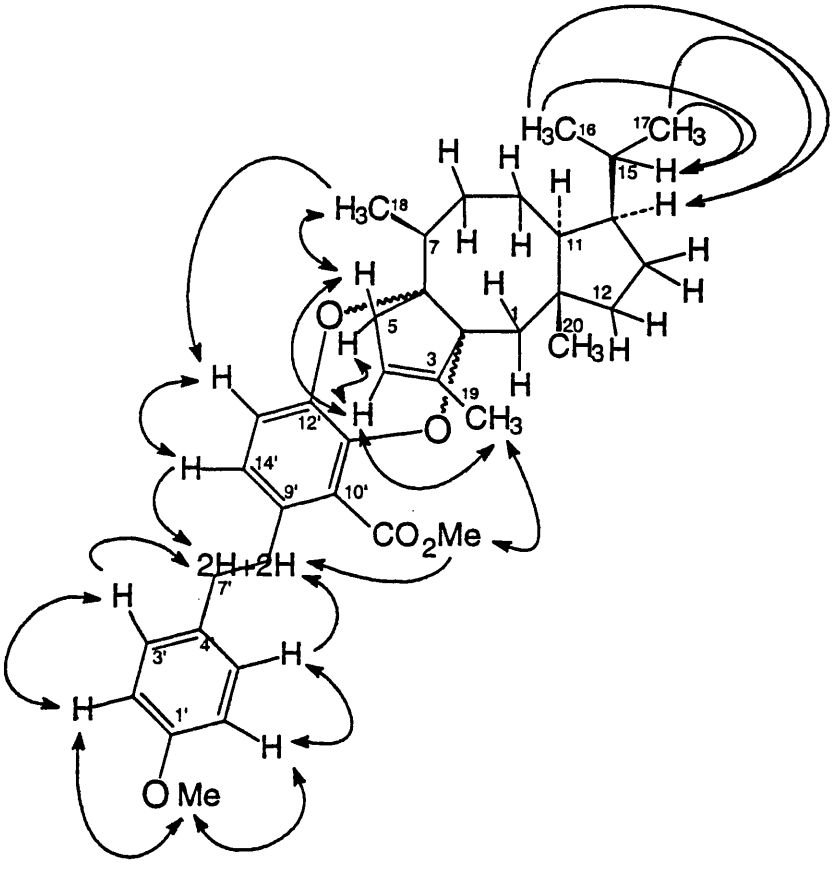
(A)

Table XII

Spinuloplagin C

NOEs % →

Irradiation ↓	Saturation % (app)	δ_H 1.10 (3H-18)	δ_H 1.28	δ_H 1.38	δ_H 1.45	δ_H 1.52	δ_H 1.55 - 1.58	δ_H 1.60 (3H-19)	δ_H 1.68 - 1.80	δ_H 1.72 (H-15)	δ_H 2.00 (H-7)	δ_H 2.08 (H-14)	δ_H 2.10	δ_H 2.09 - 2.16	δ_H 2.30 (H-5a)	δ_H 2.45 (H-5b)	δ_H 2.75 (2H-9 & 2H-10)	δ_H 3.76 (O Me)	δ_H 3.85 (CO_2 Me)	δ_H 5.31 (H-4)	δ_H 6.61 (H-14′)	δ_H 6.72 (H-13′)	δ_H 6.81 (H-2′, 6′)	δ_H 7.06 (H-3′, 6′)
δ_H 0.80	83		3.3	5.1		3.8	2.4		5.9				2.1											
δ_H 0.85	100		3.3			2.4				7.6		6.6												
δ_H 1.10	53		5.8		4.1						5.2				5.2							2.2		
δ_H 1.60	69												4.7	5.6					0.3	6.7				
δ_H 2.30	38	1.1														18.2				3.5				
δ_H 2.45	35														3.5					4.3				
δ_H 5.31	67							1.3							2.5	1.7								
δ_H 3.76	83																						7.9	
δ_H 3.85	80							0.3									0.3							
δ_H 6.61	44																1.4					5.3		
δ_H 6.72	49																				3.6			
δ_H 6.81	95																	2.2						6.6
δ_H 7.06	94																0.7						6.6	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15
H3C18
7
11
12
1
20
CH3
5
3
19
O
12'
14'
9'
10'
CO2Me
2H+2H
7'
3'
4'
1'
O Me

(NOE 20)

1.72 (m, H-15). Homonuclear decoupling experiments involving irradiation at δ_H 1.60 and δ_H 5.31 simplified the signals attributable to part structure (A) (Table XI) and enabled the couplings to be measured. This fragment can be accommodated in the diterpenoid unit as in (20). This change in the structure of the diterpenoid unit and the ^{13}C shifts of the oxygenated carbons [δ_C 89.1 and 90.9 (both s, C-2 and C-6)] indicate that the ether linkages are now between the bibenzyl and C-2 and C-6 of the diterpenoid unit, which leads to four possible structures (20)-(23). NOE difference experiments are consistent with the structures (20) and (22). The results are summarised in Table XII and (NOE 20). Irradiation of the secondary methyl 3H-18 (δ_H 1.10) gave NOEs at H-7 (δ_H 2.00, 5.2%), H-5a (δ_H 2.30, 5.2%), and at the aromatic proton H-13′ (δ_H 6.72, 2.2%). Examination of molecular model shows that the last NOE is consistent with both (20) and (22). These assignments are supported by other small NOEs. Irradiation of the carbomethoxyl group (δ_H 3.85) produced a NOE at the vinyl methyl group 3H-19 (δ_H 1.60, 0.3%). On irradiation of the vinyl methyl NOEs were observed at the vinyl proton H-4 (δ_H 5.31, 6.7%) and at the carbomethoxyl (δ_H 3.85, 0.3%). The largest change in the ^{13}C spectrum of spinuloplagin C relative to spinuloplagins A and B is in the shift of C-10 ($\Delta\ \delta$ ~9 ppm). The X-ray structure of spinuloplagin A shows that H-10 is situated over the 8-membered ring, and it is possible that a ring attached at C-2 and C-6 on the α face of spinuloplagin C could exert a steric shielding on C-10. It is difficult to exclude the possibility that attachment of the bibenzyl to the β face of the molecule as in (22) would result in shielding of C-10 by the five membered ring carbons. Thus (20) and (22) remain as possible structures for spinuloplagin C.

General Experimental

Nuclear Magnetic Resonance Spectra (NMR) were recorded on Bruker WP 200 SY and AM 200 SY spectrometers, ^{1}H at 200.132 MHz and ^{13}C at 50.32 MHz. Spectra were recorded for $CDCl_3$ relative to δ_H 7.25 and δc 77.0 and chemical shifts are reported in ppm. Tabulated ^{1}H NMR data have coupling constant (J) in Hz given in parenthesis. Signals indicated 'm' were unresolved or overlapped multiplets. ^{1}H and ^{13}C signals assignments are based on general chemical shift rule and comparison with publish data for similar compounds. More definitive ^{1}H NMR assignments were made by NOE difference experiments and NOEs were calculated for hundred percent in the tables. ^{13}C NMR multiplicities were obtained from DEPT experiments.

Melting points (m.p.) were determined on a Kofler hot-stage apparatus and are uncorrected.

Dried and powdered plant material was extracted with diethyl ether or methanol. The crude extracts were fractionated by column chromatography over silica gel G_{254}. Eluents for silica gel column chromatography were increasing percentages of diethyl ether or ethyl acetate in light petroleum. Each of the crude fractions was further purified on TLC over silica gel GF_{254} (0.75 mm thick plate). Eluents for TLC were increasing percentagesof 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.

Some NMR spectra of *Plagiochila* compounds (10), (7), (18) and (20) were also run at 360 MHz.

Experimental

Plant material was collected from the Aberfoyle Forest in Scotland and identified by Prof. J.D.Connolly and Dr. D.S.Rycroft. A reference sample is deposited with Dr. Rycroft in the Department of Chemistry, University of Glasgow. Dried and powdered plant material (221g) gave a crude extract (4.4g) on extraction with diethyl

ether. Compounds were separated by flash chromatography using a column of silica gel G_{254} and pet. ether - diethyl ether as eluent. Fractions were collected as follows:

Fraction No	Pet. ether	Diethyl ether	
1	100	0	five portions each of 50 ml
2	95	5	„ „ „
3	90	10	four fractions „ „
4	80	20	„ „ „
5	70	30	„ „ „
6	60	40	„ „ „
7	50	50	„ „ „
8	40	60	„ „ „
9	20	80	„ „ „
10	0	100	„ „ „

Compounds from the separated fraction were isolated and purified by preparative thin layer chromatography on silica gel GF_{254}. Eluents for chromatography were increasing percentages of diethyl ether in light petroleum. Purified compounds were subjected to spectroscopic analysis.

2-Hydroxy-3,4,7-trimethoxy-9,10-dihydrophenanthrene (**5**), (250 mg), m.p. 139-142^{0}C (ex ether- pet. ether), $C_{17}H_{18}O_4$, m/z 286, was isolated from fraction four.

^{13}C and ^1H NMR data as Table I

3,4,7-Trimethoxy-9,10-dihydrophenanthrene (8), (14mg), m.p. 74-76^{0}C (ex petroleum ether- $CHCl_3$), $C_{17}H_{18}O_3$, m/z 270, was found in the fourth portion of fraction two.

δ_H : 2.73 (m, 2H-9 & 2H-10), 3.68 (s, OMe at C-4), 3.83 (s, OMe C-7), 3.88 (s, OMe at C-3), 6.74 (d, J=8.2 Hz, H-1), 6.76 (d, J=2.8 Hz, H-8), 6.83 (dd, J=2.8, 8.6 Hz, H-6), 6.92 (d, J=8.2 Hz, H-2) and 8.36 (d, J=8.6 Hz, H-5)

δ_C : 29.5 (t), 30.5 (t), 55.1 (q), 56.0 (q), 60.0 (q), 110.0 (d, C-1), 111.6 (d, C-6), 113.1 (d, C-8), 122.8 (d, C-2), 129.5 (d, C-5), 125.3 (s, C-4a), 128.0 (s, C-5a), 131.4 (s, C-10a), 140.5 (s, C-8a), 146.5 (s,C-3), 152.0(s,C-4) and 158.4 (s,C-7).

4-Hydroxy-3,7-dimethoxy-9,10-dihydrophenanthrene (9), (12 mg), m.p. 118-120^{0}C (ex petroleum ether), $C_{16}H_{16}O_3$, m/z-256, was isolated from the fifth portion of fraction two.

δ_H : 2.75 (m, 2H-9 and 2H-10), 3.83 (s, OMe at C-7), 3.91 (s, OMe at C-3), 6.20 (s, OH, exchangeable with D_2O attached at C-4), 6.68 (d, J=8.1 Hz, H-1), 6.73 (d, J=8.1 Hz, H-2), 6.78 (d, J=2.8 Hz, H-8), 6.86 (dd, J=8.6, 2.8 Hz, H-6) and 8.36 (d, J=8.6 Hz, H-5).

δ_C : 55.1 (q), 56.2 (q), 29.6 (t), 30.5 (t), 108 (d, C-1), 111.2 (d, C-6), 113.1 (d, C-8), 118.1 (d, C-2), 129.5 (d, C-5) 120.6 (s, C-4a), 125.5 (s, C-5a), 131.5 (s, C-10a), 140.1 (s, C-8a), 142.7 (s, C-3a), 145.6 (s, C-4) and 158.2 (s, C-7).

2,3,4,7-Tetramethoxy-9,10-dihydrophenanthrene (10), (2mg), m.p. 134-138^{0}C, $C_{18}H_{20}O_4$, m/z 300, was isolated from the fourth portion of fraction three.

δ_H (360 MHz) : 2.74 (m, 2H-9 and 2H-10), 3.76 (s, OMe attached at C-4), 3.83 (s, OMe attached at C-7), 3.87 (s, OMe attached at C-2), 3.90

(s, OMe attached at C-3), 6.57 (s, H-1), 6.76 (d, J=2.8 Hz, H-8), 6.81 (dd, J=2.8, 8.7 Hz, H-6) and 8.21 (d, J=8.7 Hz, H-5).

2,3,7-Trimethoxy-9,10-dihydrophenanthrene (7), (2 mg), as an oil, $C_{17}H_{18}O_3$, m/z 270, was isolated from the fourth portion of fraction three.

δ_H (360 MHz) : 2.80 (m, 2H-9 and 2H-10), 3.83, 3.89 and 3.92 (all s, OMe at C-2, C-3 and C-7), 6.73 (s, H-1), 6.77 (d, J=2.8 Hz, H-8), 6.83 (dd, J=2.8, 8.4 Hz, H-6), 7.18 (s, H-4) and 7.56 (d, J=8.4 Hz, H-5).

δ_C : 28.6 (t), 29.7 (t), 55.3 (q), 55.9 (q), 56.1 (q), 106.7 (d), 111.3 (d), 112.1 (d), 113.5 (d), 119.0 (s), 124.0 (d), 129.0 (s), 134.8 (s), 138.5 (s), 143.0 (s), 145.0 (s) and 156.4 (s).

4,7-Dihydroxy-3-methoxy-9,10-dihydrophenanthrene (18), (4 mg), $C_{15}H_{14}O_3$, m/z 242, was isolated from the first portion of fraction six.

δ_H at 360 MHz : 2.73 (m, 2H-9 & 2H-10), 3.91(s, OMe attached at C-3), 4.68 (s, phenolic OH at C-7, exchangeable with D_2O), 6.19 (s, phenolic OH at C-4, exchangeable with D_2O), 6.76 (dd, J=8.5 and 2.8 Hz, H-6), 6.71 (d, J=2.8 Hz, H-8), an AB system [6.72 (d, J=8.1 Hz, H-1) and 6.69 (d, J=8.1 Hz, H-2)] and 8.31(d, J=8.5 Hz, H-5).

Methyl 6-methoxy2-methyl-3,4-methylenedioxybenzoate (11), (70 mg) was isolated from the fraction three as an amorphous solid m.p. 79^0-80^{0}C (lit[8] m.p. 80^{0}C), $C_{11}H_{12}O_5$ (m/z-224).

^{13}C and ^1H NMR data as Table VI

Anadensin (2), (12 mg), $C_{20}H_{32}O_2$, m/z 304, m.p. 169-172^{0}C (ex MeOH), was isolated from the fourth portion of fraction two.

δ_H: 0.71 (s, 3H-20), 0.71 and 0.86 (both d, J=6.6Hz, 3H-16 and 3H-17), 1.11 (d, J=6.6Hz, H-18), 1.77 (s, OH at C-2), 1.20

(d, J=6.6Hz, 3H-19), 2.23 and 1.84 (both d, J=14.4 Hz, 2H-1), 2.65 (J=7.6 Hz, H-3), 2.87 (m, H-7) and 5.95 (s, H-5).

δ_C: 9.8 (q, C-19), 18.6(q, C-16), 20.2 (q, C-17), 21.5 (t), 23.1 (q, C-20), 23.3 (q, C-18), 23.3 (t), 28.3 (d), 29.7(d, C-7), 37.8 (t), 43.6 (s, C-11), 44.1 (t), 48.5 (d), 48.5 (d), 49.5 (d, C-3), 51.1(t, C-1), 81.4 (s, C-2), 128.3 (d, C-5), 187.3 (s, C-6), and 209.2 (s, C-4).

Spinuloplagin A (12), (40 mg), $C_{37}H_{48}O_5$, m/z 572, m.p. 89-90^{0}C (from MeOH)was isolated from the third portion of fraction two.

^{13}C and ^1H NMR data as Table VII and VIII

Spinuloplagin B (13), (8.2 mg), $C_{37}H_{48}O_5$, m/z 572, was isolated from the first portion of fraction three.

^{13}C and ^1H NMR data as Table VII and VIII

Spinuloplagin C (20), (4.0 mg), $C_{37}H_{48}O_5$, m/z 572, was isolated from the third portion of fraction two.

^{13}C and ^1H NMR data as Table VII and VIII

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CHAPTER THREE

Barbilophozia barbata (Schimidal) Loesk.

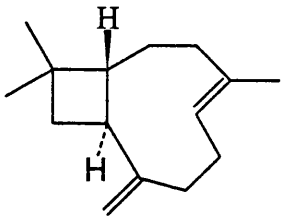
Barbilophozia barbata (Schimidal) Loesk.

INTRODUCTION

The distribution of eight species of the genus *Barbilophozia* in Scotland has been reported[1]. Very few reports have appeared on the chemical investigation of this genus. Some basic skeletons that have been found world-wide are dolabellane, acorane, carotane, gymnomitrane (barbatane), fusicoccane, maaliane, aromadendrane, eudesmane, caryophyllane and calamenane. In 1972 Huneck and Overton[2] studied the German *B. barbata, B. floerkei* and *B. lycopodioides* and reported the presence of the diterpenoids barbilophozin, floerkein A and B and barbilycopodin respectively. These were later shown to be dolabellane derivatives. In a G.L.C. study Andersen *et al*[4] examined these species and *B. attenuata* and found that *B. barbata* contained caryophyllene (1), gymnomitrene and isogymnomitrene (3) and (2), α-cedrene (4), α-alaskene (5) and calamenene (6), *B. floerkei* contained gymnomitrene, isogymnomitrene and bazzanene (7) and *B. lycopodioides* and *B. attenuata* contain gymnomitrene.

The gymnomitrane type skeleton was first reported by Connolly *et al*[3] in *Gymnomitrion obtusum* and is exemplified by gymnomitrol (3; 11-OH) whose structure was derived by spectroscopic and chemical analysis[3,5]. Gymnomitrane derivatives are widespread in the *Jungermanniales*[12]. The biosynthetic derivation of gymnomitrane was proposed by Connolly *et al*[5,12]. In their view, it is synthesised from an enzyme-bound farnesyl pyrophosphate via a tricodiene intermediate which leads to gymnomitrene via a concerted cyclization (Scheme I).

In 1978 Andersen *et al*[11] reported the presence of anastreptene (8) in the essential oil of *B. lycopodioides* and *B. attenuata.* The presence of the sesquiterpenoid lactone, hercynin (9), in *B.barbata* was reported by Huneck *et al*[6,10]. On investigation of Scottish and German liverworts Huneck *et al*[7] found that Scottish *B. floerkei* and German *B. attenuata* contained barbilycopodin (10) and 10-deacetoxybarbilycopodin (11) while German *B.floerkei* afforded barbilycopodin (10), the related monoepoxide (12) and 18-hydroxydolabell-7E-en-3-one (13). Barbilycopodin is present in both German and Scottish *B. lycopodioides.* The



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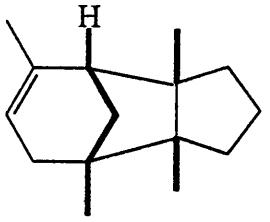

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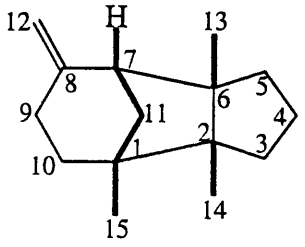

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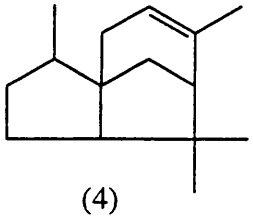

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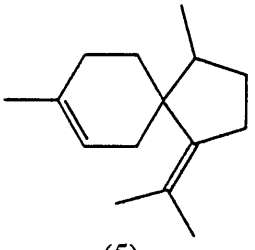

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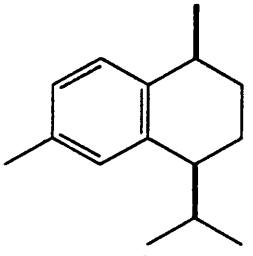

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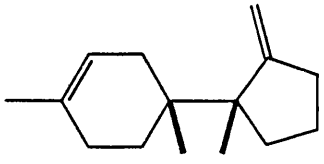

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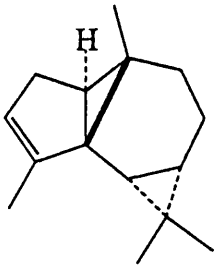

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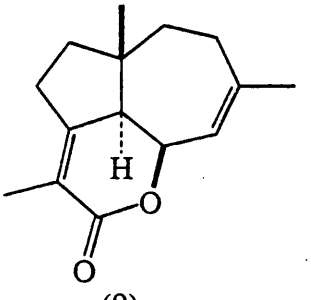

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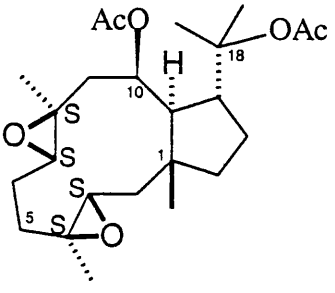

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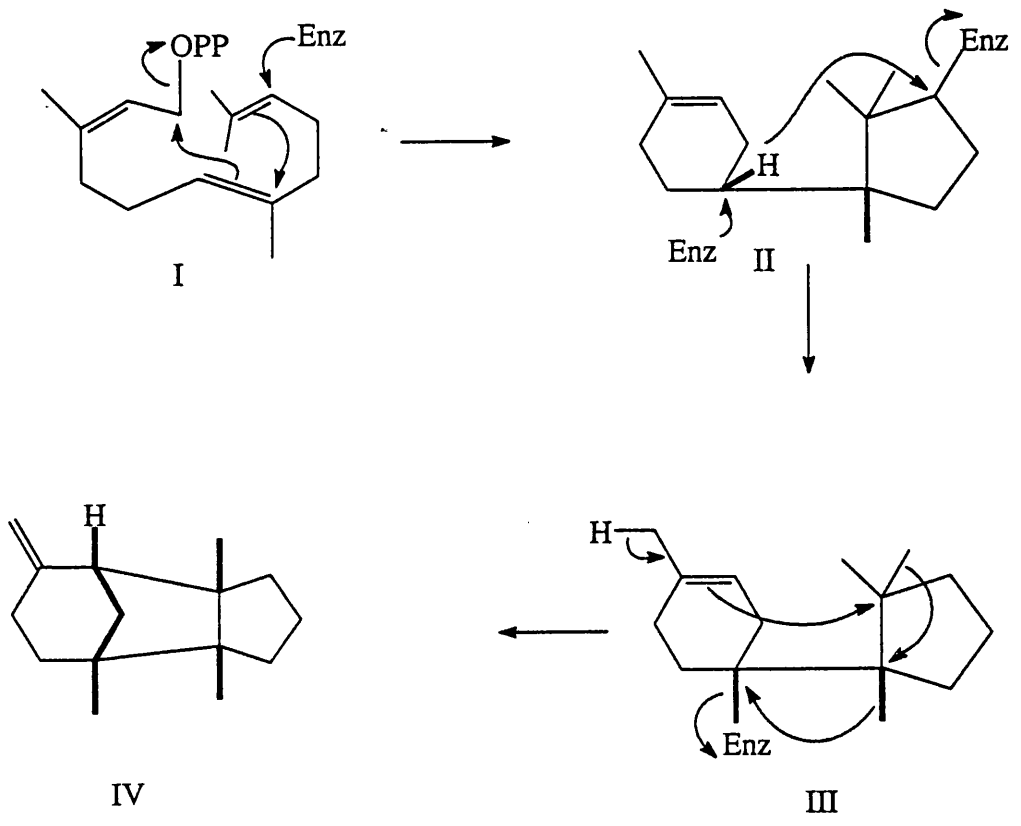
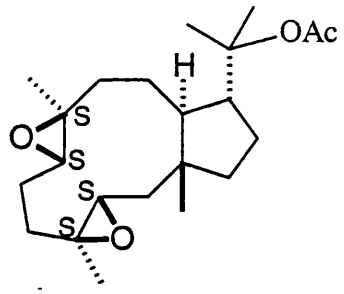


Scheme I



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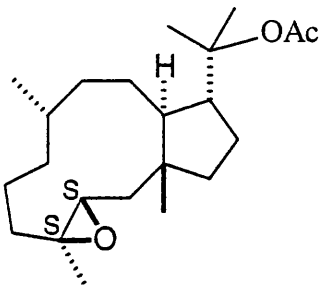

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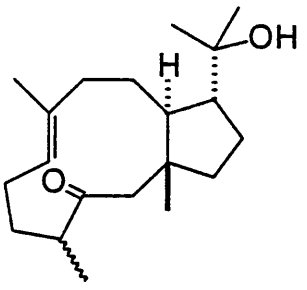

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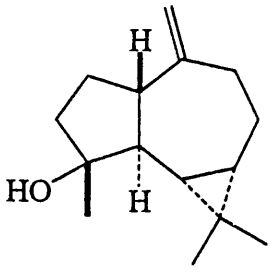

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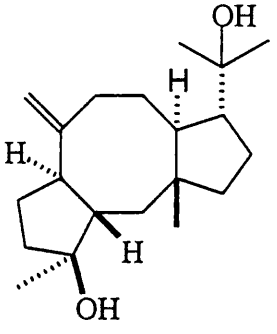

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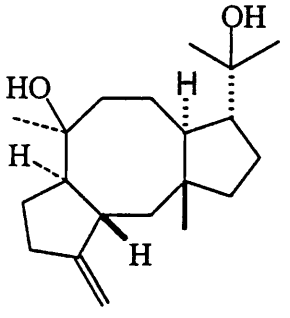
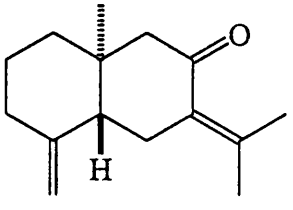


(15B)



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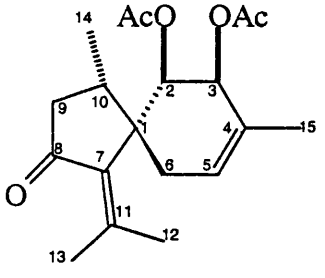

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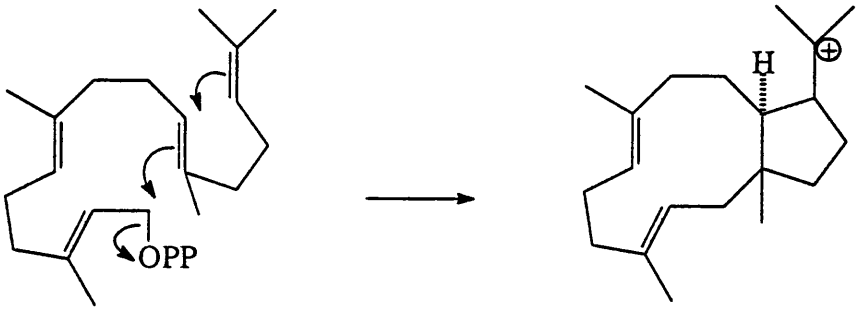
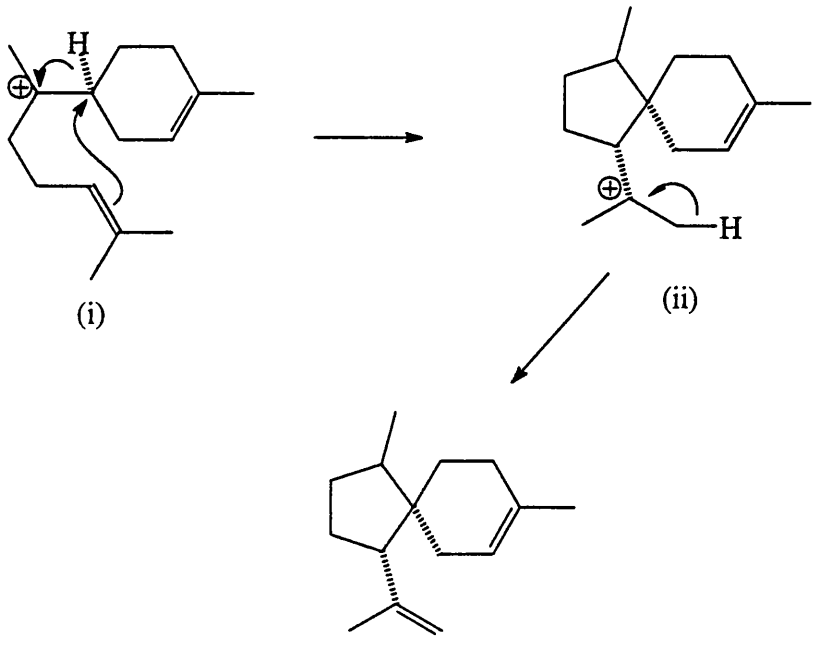


Scheme II



(i) (ii) (iii)

Scheme III

structures of these compounds were confirmed by spectroscopic and X-ray analyses. Barbilycopodin is biosynthesised by cyclisation of all-trans geranyl-geranyl pyrophosphate [Scheme II][10].

In a recent study making use of the modern 2D NMR spectroscopy and X-ray analysis, Tori *et al*[8] have identified (-)-spathulenol (14) in *B. hatcheri* and *B. floerkei* and the new compounds, barbifusicoccin A and B (15) and *ent*-eudesma-4(15),7(11)-dien-8-one (16), in *B. floerkei* along with the previously reported compounds from same species.

Recently Connolly *et al*[9] reported the presence in *B. barbata* of an acorane derivative, (1S*,10S*)-2R*,3S*-diacetoxy-4,7(11)-acoradien-8-one (17) whose structure was confirmed by spectroscopic analysis (^{1}H, ^{13}C, 2D NMR, NOE). Marshall *et al*[13,14] have proposed the biosynthetic pathway for the formation of acorane derivatives. The biosynthesis involves an initial formation of a bisaboloyl cation followed by 1-2 hydrogen shift and cyclization of C-6 to C-10. Finally, deprotonation gives α-acoradiene (Scheme-III).

The above reports show that the genus *Barbilophozia* has a rich biochemical capability, illustrated by the production of such a wide range of skeletal types.

Results and Discussion

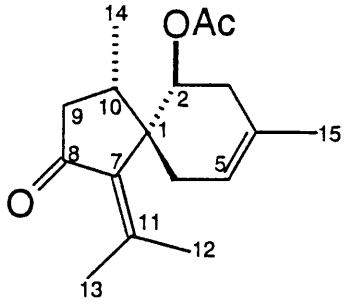
In the present work, the ether extract of *Barbilophozia barbata* afforded gymnomitrene (β-barbatene) (3), 18-acetoxy-3S,4S;7S,8S-diepoxydolabellane (11), (1S*,10S*)-2R*,3S*-diacetoxy-4,7(11)-acoradien-8-one (17) and a new compound whose structure has been established as (1S*,10S*)-2S*-acetoxy-4,7(11)-acoradien-8-one (18) from spectroscopic analysis.

Compound one, gymnomitrene (β-babatene) (3), was isolated from the first non polar fraction, as a gum. Its ^{1}H spectrum contains three tertiary methyl groups at δ_H 0.84, 0.90 and 1.03 (all s, 3 x 3H) and an exomethylene at δ_H 4.58 (br s, 2H-12). The ^{13}C spectrum of this compound shows resonances for fifteen carbons, of which three are methyl carbons [δ_C 23.3 (C-15), 24.7 (C-14) and 27.5 (C-13)] and seven are methylene carbons [δ_C 27.4(t, C-4), 28.7(t, C-9), 35.4(t, C-3), 36.9(t, C-10), 37.9(t, C-5), 46.7(t, C-11) and107.4(t, C-12)]. There are also one methine [δ_C 55.9(d, C-7)] and four quaternary carbons [δ_C 42.9(s, C-1), 54.0(s, C-2), 55.3(s, C-6) and 151.9(s, C-8)] in the ^{13}C spectrum. The above data and the mass spectrum (m/z 204) established the molecular formula as $C_{15}H_{24}$. The molecule is therefore tricyclic. Comparison of these spectroscopic properties with those reported[4,5&9] for β-gymnomitrene (β-barbatene) revealed thier identity. Compound (3) is therefore β-gymnomitrene (β-barbatene).

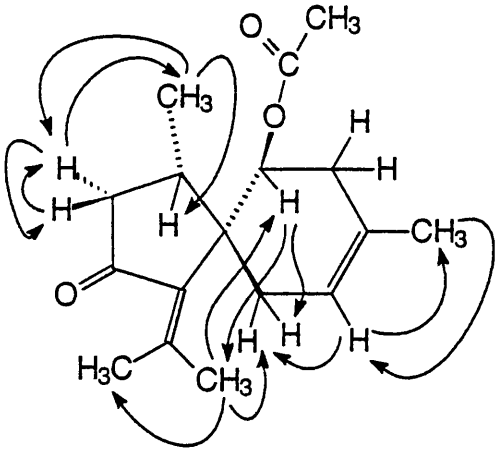
Compound two, (1S*,10S*)-2S*-acetoxy-4,7(11)-acoradien-8-one (18), solid, m.p. 78-83^{0}C, was isolated from the second fraction. Its^{13}C spectrum revealed 17 carbons including a ketonic carbonyl [δ_C 206.9 (s, C-8)] and an acetate [δ_C 170.3 (s), 21.3 (q) at C-2]. In addition there are four quaternary, three methine, three methylene and four methyl carbons (see Table I). The ^{13}C spectrum and mass spectrum (m/z 276) reveal the molecular formula as $C_{17}H_{24}O_3$. Since there are two double bonds [δ_C 119.3 (d, C-5), 132.4 (s, C-11), 133.9 (s, C-7) and 150.9 (s, C-4)], in addition to the two carbonyls, the molecule is bicyclic and appears to be a

Table I

	δ_H	δ_C
1	-	50.0 (s)
2	5.64 (dd, J=10.1, 6.2 Hz)	74.3 (d)
3	-	33.6 (t)
4	-	150.9 (s)
5	5.28 (br m)	119.3 (d)
6	2.35 (dd, J=7.9, 2.8 Hz 1H-6a) and 1H-6b in protons near 2.44	37.0 (t)
7	-	133.9 (s)
8	-	206.9 (s)
9	1.93 (dd, J=17.1, 4.2 Hz -a) 2.56 (dd, J=17.0, 7.2 Hz -b)	46.9 (t)
10	in protons near 2.4	34.3 (d)
11	-	132.4 (s)
12	1.88 (s)	23.1 (q)
13	2.25 (s)	24.0 (q)
14	1.14 (d, J=6.9 Hz)	21.3 (q)
15	1.70 (s)	19.9 (q)
-<u>C</u>OOCH$_3$ at C-2	-	170.3 (s)
-COO<u>CH$_3$</u> at C-2	1.99 (s)	22.7 (s)


14
OAc
9
10
2
1
15
8
7
5
O
11
12
13

(18)


O
CH3
C
O
CH3
H
H
H
H
H
H
CH3
O
H
H
H
H3C
CH3

(18 NOE)

Table II

(1S*,10S*)-2S*-acetoxy-4,7(11)-acoradien-8-one **NOEs % →**

Irradiation ↓	saturation % (approx.)	δ_H1.14 (3H-14)	δ_H 1.70 (3H-15)	δ_H 1.88 (3H-12)	δ_H 1.93 (H-9a)	δ_H 2.25 (3H-13)	δ_H 2.32 (H-6a)	δ_H 2.30 - 2.45	δ_H 2.56 (H-9b)	δ_H 5.28 (H-5)	δ_H 5.64 (H-2)
δ_H 5.64 (H-2)	63			3.7			5.2				
δ_H 5.28 (H-5)	67		1.2				2.2				
δ_H 1.93 (H-9a)	100	0.3							7.2		
δ_H 1.88 (3H-12)	73					1.3				1.6	14.5
δ_H 1.70 (3H-15)	44							1.7		7.4	
δ_H 1.14 (3H-14)	69				6.6			9.1			

sesquiterpenoid acetate. The ^1H spectrum shows the presence of five methyl groups, one secondary [δ_H 1.14 (d, J=6.9 Hz, 3H-14)], one vinyl [δ_H 1.70 (s, 3H-15)], two isopropylidene [δ_H 1.87 and 2.25 (both s, 3H-12 and 3H-13)] and one acetate [δ_H 1.99 (s, OAc)]. One isopropylidene methyl is shifted downfield due to deshielding by the neighbouring ketonic group as in (17). The vinyl proton resonates at δ_H 5.28 (br m, H-5). The large geminal coupling of a methylene group at δ_H 1.97 (dd, J=17.0, 2.8 Hz, H-9a) and δ_H 2.56 (dd, J=17.0, 7.6 Hz, H-9b), indicates that it is α to a carbonyl group. One proton of an allylic methylene group appears at δ_H 2.35 (dd J=7.9, 2.8 Hz, H-6a) and its methylene neighbour is obscured and resonates around δ_H 2.44. The proton attached to the carbon bearing the ester group resonates at δ_H 5.64 (dd, J=10.1, 6.2 Hz, H-2). Other proton shifts are shown in Table-1. These data are very similar to those of compound (17) and suggested that compound two is (1S*,10S*)-2S*-acetoxy-4,7(11)-acoradien-8-one (18). NOE difference experiments provided confirmation and are summarised in Table II and in structure (18 NOE). Irradiation of the downfield proton at δ_H 5.64 (H-2) afforded NOEs at δ_H 2.32 (H-6a, 5.2%) and δ_H 1.87 (one isopropylidene methyl 3H-12, 3.7%). Irradiation of the isopropylidene methyl 3H-12 afforded NOEs at H-2 (14.5%) and with the other isopropylidene methyl at δ_H 2.25 (1.3%). Irradiation of the secondary methyl (δ_H 1.14, 3H-14) gave NOEs at δ_H 1.93 (H-9a, 6.6%) and δ_H 2.42 (H-10, 9.1%) in the methylene envelope. As expected irradiation of H-9a affected the secondary methyl 3H-14 and gave NOEs at δ_H 2.56 (H-9b, 7.2%). NOEs were observed at δ_H 1.70 (vinyl methyl, 1.2 %) on irradiation of the vinyl proton at δ_H 5.28 (H-5) and also at δ_H 2.32 (H-6a, 2.2%). The vinyl methyl on irradiation gave a NOE at the vinyl proton (7.4%). All these experiments support structure (18) and confirm the proton arrangements. The carbon shifts were assigned by comparison with those of (17)[9]. The absolute cofiguration of (17) remains to be determined.

The next compound, (1S*,10S*)-2R*,3S*-diacetoxy-4,7(11)-acoradien-8-one, (17), $C_{19}H_{26}O_5$, m.p. 139-144^{0}C (ex Et_2O-petroleum ether), was isolated from the third fraction. The ^1H spectrum of this compound shows a secondary methyl at δ_H

1.09 (d, J=6.7 Hz, 3H-14), a vinyl methyl at δ_H 1.68 (s, 3H-15) and two isopropylidene methyls at δ_H 1.88 and 2.23 (both s, Me-12, Me-13). Two methylene protons, resonating at δ_H 1.93 (dd, J=17.1 and 4.2 Hz, H-9a) and δ_H 2.56 (dd, J=17.0 and 7.2 Hz, H-9b), have a large geminal coupling (17 Hz) which indicate that they are α to a carbonyl group. The ^1H spectrum also shows a vinyl proton at δ_H 5.57 (m, H-5), an allylic methylene [δ_H 2.30 (m, H-6a) and 2.44 (m, H-6b)] and two oxygen bearing methines at δ_H 5.69 (d, J=4.3 Hz, H-2) and 5.60 (m, H-3). These spectroscopic features readily revealed the identity of this compound with that previously isolated by Connolly *et al*[9] from the same species.

The last and most polar of the isolated compounds, 18-acetoxy-3S,4S;7S,8S-diepoxydolabellane (11), m.p. 89-91^{0}C (ex hexane), $C_{22}H_{36}O_4$, m/z 364, was found in the fourth fraction. The ^1H NMR spectrum of this compound shows five tertiary methyls at δ_H 1.19, 1.31, 1.39, 1.48 and 1.54 (all s) and one acetate methyl at δ_H 1.96 (s). One proton doublet resonates at δ_H 2.77 (d, J=9.5 Hz, H-7) and one at 3.04 (dd, J=2.6 and 9.2 Hz, H-3). These data are the same as those of 10-deacetoxy barbilycopodin (11), isolated by Huneck *et al*[7] and Connolly *et al*[9] from same plant. Thus this compound is 18-acetoxy-3S,4S;7S,8S-diepoxydolabellane (11).

Experimental

The plant material was collected near Aberfoyle and identified by Prof. J.D.Connolly and Dr. D.S.Rycroft. A reference sample of the plant is deposited with Dr. D.S.Rycroft in the Department of Chemistry, University of Glasgow. The dried powdered plant material (106g) was extracted with diethyl ether to give a crude extract (784mg). The compounds in the extract were separated by flash chromatography on a column of silica gel G_{254} with increasing proportions of diethyl ether in light petroleum. The following fractions were collected;

Fraction No	Pet. ether	Diethyl ether	
1	100	0	four portions, each of 50 ml
2	95	5	,, ,, ,,
3	90	10	,, ,, ,,
4	85	15	,, ,, ,,
5	80	20	,, ,, ,,
6	70	30	,, ,, ,,
7	60	40	,, ,, ,,
8	40	60	,, ,, ,,
9	20	80	,, ,, ,,
10	0	100	,, ,, ,,

Compounds from separated fractions were further purified by preparative thin layer chromatography (TLC) on silica gel GF_{254}. Eluents for TLC were increasing

percentages of diethyl ether in light petroleum. Compounds were visualised using UV light or iodine vapour. Solvents were evaporated using a Buchi Rotavapor. The purified compounds were subjected to spectroscopic analysis.

Compound 1 :

β-Barbatene (3), (21 mg), was isolated from the first fraction, as a gum, $C_{15}H_{24}$, m/z 204.

δ_H : 0.84, 0.90 and 1.03 (all s, C-Me), 4.58 (br m, 2H-12) .

δc : 23.3 (q, C-15), 24.7 (q, C-14), 27.4 (t, C-4), 27.5 (q, C-13), 28.7 (t, C-9), 35.4 (t, C-3), 36.9 (t, C-10), 37.9 (t, C-5), 42.9 (s, C-1), 46.7 (t, C-11), 54.0 (s, C-2), 55.3 (s, C-6), 55.9 (d, C-7), 107.4 (t, C-12), 151.9(s, C-8).

Compound 2 :

(1S*,10S*)-2S*-Acetoxy-4,7(11)-acoradien-8-one (18), (2 mg), solid m.p. 78-83^{0}C $C_{17}H_{24}O_3$, m/z 276, ν_{max} 1734 cm^{-1} (acetate), 1698 cm^{-1} (ketone)and 1608 cm^{-1} (C=C), was isolated from the second fraction.

^{1}H and ^{13}C data see Table I.

Compound 3 :

(1S*,10S*)-2R*,3S*-Diacetoxy- 4,7(11)-acoradien-8-one (17), (5.9 mg), m.p. 139-144^{0}C (ex diethyl ether - petroleum ether), [lit[9] m.p. 139-142^{0}C], $C_{19}H_{26}O_5$,m/z 334, was isolated from the third fraction.

δ_H : 1.09 (d, J=6.7 Hz, 3H-14) , 1.68 (s, 3H-15), 1.88 (s, 3H-12), 1.93 (d, J=17.1, 4.2Hz, H- 9a), 1.98 and 2.08 (both s, 2OAc), 2.23 (s, 3H-13), 2.30 (m, H-6b), 2.38 (m, H-10) , 2.44 (m, H-6a), 2.56 (dd, J=17.0, 7.2 Hz, H-9b), 5.57 (m, H-5), 5.60 (m, H-3), 5.69 (d, J=4.3 Hz, H-2).

Compound 4 :

18-Acetoxy-3S,4S;7S,8S-diepoxydolabellane (11), (8 mg), m.p. 89-91^{0}C(ex hexane) [lit[7] m.p. 89-91^{0}C], $C_{22}H_{36}O_4$, m/z 364, was isolated from the fourth fraction.

δ_H: 1.19, 1.31, 1.39, 1.48 and 1.54 (all s, C-Me), 1.96 (s, C$\underline{H_3}$COO), 2.77(d, J=9.5, H-7), 3.04 (dd, J=2.6, 6.6 Hz, H-3).

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CHAPTER FOUR

Frullania tamarisci (L) Dom.

Frullania tamarisci (L) Dom.

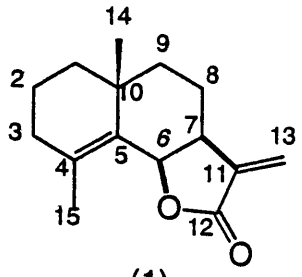
Introduction

The genus *Frullania* is a rich source of many types of terpenoids[1,3]. A lot of chemical work has been carried out since 1969, following the report of Knoch *et al*[2] that the allergenic dermatitis among European timber workers is due to the sesquiterpenoid lactone (-) frullanolide (1) from *Frullania tamarisci* which often grows on the bark of trees. In 1981 Asakawa *et al*[3] investigated 25 species of *Frullania* and they reported that fourteen species of this genus produce allergenic-inducing lactones and eighteen species contain bibenzyls. The sesquiterpenoid lactones and bibenzyls were obtained as major components and these are valuable chemical markers of *Frullania* species. On the basis of chemical constituents Asakawa *et al*[3] have classified the *Frullania* genus into five chemotypes; sesquiterpenoid lactone-bibenzyl type, sesquiterpenoid lactone type, bibenzyl type, monoterpene type and cyclocolorenone type. Ouirce *et al*[4] have reported that *F. tamarisci* and *F. dilatata* are considered to be the cause of eczema. It is interesting that the sesquiterpenoid lactones from *F. dilatata* have the opposite absolute configuration to those of *F. tamarisci*[3,8,16].

Frullania tamarisci is quite common in Scotland and is one of only five species of this genus which are found in Scotland[5]. Scottish *Frullania tamarisci* was investigated in 1973 for the first time by Connolly and Thornton[6] and they isolated three enantiomeric sesquiterpenoid lactones, frullanolide (1), α-cyclocostunolide (2), γ-cyclocostunolide (3) and one germacranolide, costunolide (4).

A biogenetic derivation of eudesmanolides has been suggested by Asakawa *et al*[16]. Costunolide is the precursor of the cyclised lactone, which is derived from trans - trans farnesyl pyrophosphate. It is not possible to determine whether the ring oxidation occurs before or after construction of C-12 oxygenated group. Sesquiterpenoid aldehydes may be the precursors of such lactones [Scheme I].

In further investigations of *F. tamarisci* Connolly *et al*[7] found the pacifigorgiane sesquiterpenoid, tamariscol (5), and they assigned its structure and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8
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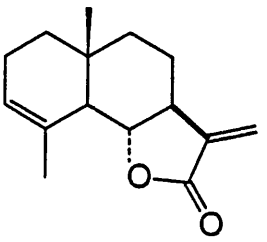
13
6
5
4
11
15
O
12
O

(1)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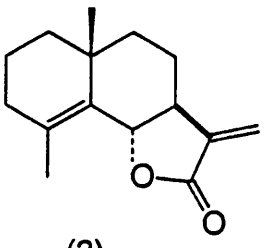
(2)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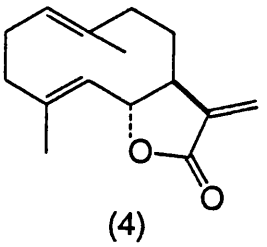
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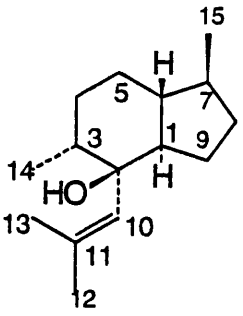
(4)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3
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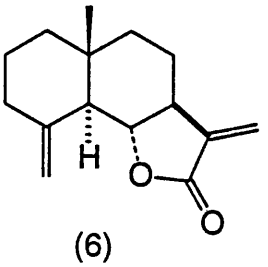
9
14
HO
H
13
10
11
12

(5)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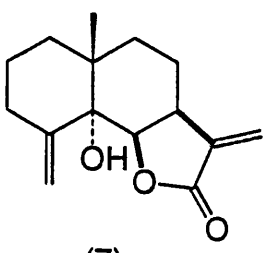
O

(6)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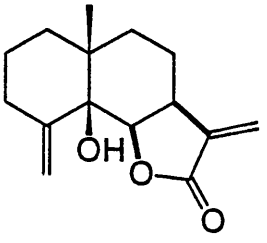
O

(7)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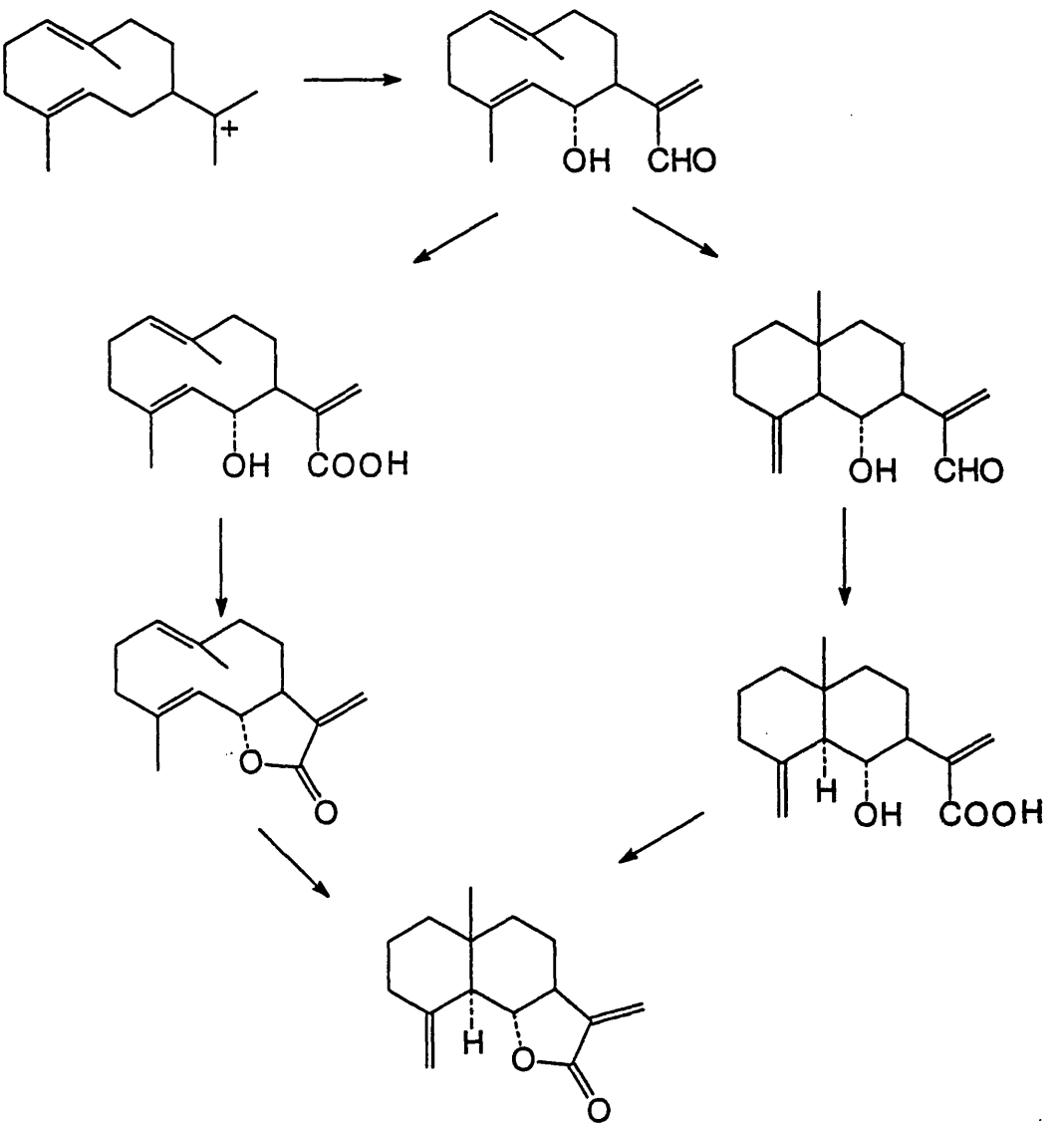
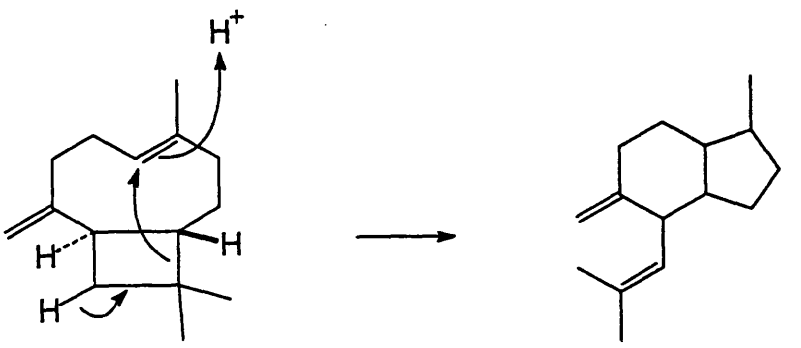
O

(8)



Scheme I



Scheme II

relative configuration on the basis of ^{13}C (including 2D INADEQUATE) and ^{1}H NMR spectroscopy. They also suggested a possible biogenetic derivation of the pacifigorgiane carbon skeleton from a caryophyllene precursor. [Scheme II]. The pacifigorgiane skeleton is usually found in marine organisms.

Results and Discussion

In a continuation of the chemical investigation of *Frullania tamarisci* in the present work, the methanol extract afforded β-cyclocostunolide (6), isolated for the first time in this species, along with the previously isolated compounds. Finally a small amount of a mixture of two compounds of similar polarity was obtained. The ^{1}H and ^{13}C NMR data of the major component were consistent with the data for oxyfrullanolide[8] (7). The minor component is very closely related and can only be the corresponding 5-epimer (8). These two compounds are isolated for the first time from this species.

Compound one, m.p.73-75^{0}C (ex petroleum ether- $CHCl_3$), was isolated from first portion of fraction two of the extract, eluted with petroleum ether and ethyl acetate and was identified as frullanolide (1). Its ^{13}C NMR spectrum contains fifteen carbons, two methyls [δ_C 19.3 (C-14) and 26.0 (C-15)], six methylenes [δ_C 18.1, 25.0, 33.1, 38.0, 39.1 and 120.1], two methines [δ_C 41.2 (C-7) and 76.0 (C-6)] and five quaternary carbons [δ_C 33.0 (C-10), 128.4 (C-4), 138.4 (C-5), 142.2 (C-11) and 171.0 (C-12)]. These data and the mass spectrum (m/z 232) indicated the molecular formula $C_{15}H_{20}O_2$ and pointed towards a sesquiterpenoid lactone structure for (1). The ^{1}H NMR spectrum shows a tertiary methyl at δ_H 1.07 (s, 3H-14), a vinyl methyl at δ_H 1.75 (s, 3H-15), a one proton multiplet at δ_H 2.92 (m, H-7), a one proton doublet at δ_H 5.23 (d, J=5.6 Hz, H-6), a one proton doublet at δ_H 5.58 (d, J=1 Hz, H-13a) and a one proton doublet at δ_H 6.14 (d, J=1 Hz, H-13b). All these spectroscopic characteristic are identical to those reported for frullanolide (1)[6,9]. Carbon assignments are based on comparison with the previously reported data for similar compounds[14].

Compound two, m.p. 84-86^{0}C (ex MeOH), was also obtained from the first portion of fraction two of the extract, eluted with petroleum ether and ethyl acetate, and was identified as α-cyclocostunolide (2). The ^{13}C NMR revealed the presence of two methyls [δ_C 17.3 (C-14) and 23.6 (C-15)], five methylenes [δ_C 21.4 (C-8), 22.7

(C-2), 37.7 (C-1), 39.1 (C-9) and 116.4 (C-13)], four methines [δ_C 51.1 (C-7), 51.4 (C-5), 82.2 (C-6), 122.3 (C-3)] and four quaternary carbons [δ_C 36.0 (C-10), 132.9 (C-4), 139.3 (C-11) and 171.0 (C-12)]. The ^{13}C spectrum and the mass spectrum (m/z 232) lead to molecular formula $C_{15}H_{20}O_2$ and suggest a sesquiterpenoid lactone structure for (2). The proton spectrum of this compound consists of one tertiary methyl at δ_H 0.90 (s, 3H-14), one vinyl methyl at δ_H 1.83 (s, 3H-15), one proton at δ_H 3.87 (t, J=11.2 Hz, H-6), a two proton multiplet at δ_H 5.38 (m, H-3 and H-13a) and a one proton doublet at δ_H 6.05 (J=3.1 Hz, H-13b). These spectroscopic properties are identical to those reported for α-cyclocostunolide (2)[6,10,11].

Compound three, an oil, $C_{15}H_{26}O$ (m/z 222), was also isolated from the first portion of fraction two and was recognised as tamariscol (5) on the basis of its spectroscopic properties. Its ^1H and ^{13}C spectra contain two secondary methyls [δ_H 0.86 (d, J=6.5 Hz, 3H-14) and 0.91 (d, J=6.5 Hz, 3H-15); δ_C 15.0 (q, C-14) and 19.0 (q, C-15)], two vinyl methyls [δ_H 1.74 (d, J=1.3 Hz, 3H-12) and 1.87(d, J=1.3 Hz, 3H-13); δ_C 28.2 (q,C-12) and 20.0 (q, C-13)], a trisubstituted double bond [δ_H 5.05 (m, H-10); δ_C 121.5 (d, C-10) and δ_C 136.4 (s, C-11)], a tertiary alcohol [δ_C 79.2 (s, C-2)] together with four methylenes [δ_C 24.0 (C-9), 30.0 (C-5), 32.0 (C-8) and 33.0 (C-4)] and four methines [δ_C 39.5 (C-7), 45.4 (C-3), 50.0 (C-6) and 58.3 (C-1)]. All these spectroscopic features are identical with those reported for tamariscol (5)[7].

Compound four, m.p. 79-82^{0}C (ex petroleum ether- $CHCl_3$), $C_{15}H_{20}O_2$ (m/z 232), was also present in the first portion of fraction two and on the basis of its NMR and mass spectrum it was identified as γ-cyclocostunolide (3). Its ^{13}C and ^1H spectra show two methyls [δ_H 1.11 (s, tertiary methyl, 3H-14) and δ_H 1.86 (s, vinyl methyl, 3H-15); δ_C 20.0(q), δ_C 26.0(q)], two methines [δ_H 2.56 (m, H-7); δ_C 50.2 (d,C-7) and δ_H 4.54 (d, J=11.3 Hz, H-6); δ_C 83.4 (d, C-6)], an exomethylene [δ_H 5.34 and 6.13 (both d, J=3.0 Hz, 2H-13); δ_C 118.0 (t, C-13), δ_C 139.4 (s, C-11)]

together with five methylenes [δ_C 19.0 (C-2), 23.1 (C-1), 34.3 (C-3), 41.0 (C-8) and 41.1 (C-9)], a tetrasubstitued double bond [δ_C 127.0 and 130.0 (both s, C-4 and C-5)] and two quaternary carbons [δ_C 170.5 (C-12) and 37.1 (s, C-10)]. These data correspond with those of γ-cyclocostunolide (3) [6,12,14].

Compound five, m.p. 102-106^{0}C (ex MeOH), $C_{15}H_{20}O_2$ (m/z 232), was isolated from the fourth portion of fraction two and identified as costunolide (4). Its spectroscopic data include two vinyl methyls [δ_H 1.40 (s, 3H-14) and 1.68 (s, 3H-15); δ_C 16.0 (q, C-14), δ_C 136.9 (s, C-10) , δ_C 17.3 (q, C-15), δ_C 140.0 (s,C-4)], two vinyl protons [δ_H 4.72 (m, H-5) and 4.80 (m, H-1); δ_C 127.2 (d, C-5) and 127.0 (d, C-1)], an oxygenated methine [δ_H 4.57 (t, J=8.5 Hz, H-6); δ_C 81.9 (d, C-6)] and an exomethylene [δ_H 5.50 and 6.23 (both d, J=3.0 Hz, 2H-13); δ_C 119.6 (t, C-13) and 141.4 (s, C-11)] along with four methylenes [δ_C 26.1 (t, C-8), 27.9 (t, C-2), 29.6 (t, C-9) and 39.4 (t, C-3)], a methine [δ_C 50.3 (d, C-7)] and a quaternary carbon [δ_C 170.4 (s, C-12)]. These spectroscopic data identify this compound as costunolide (4) [6,14].

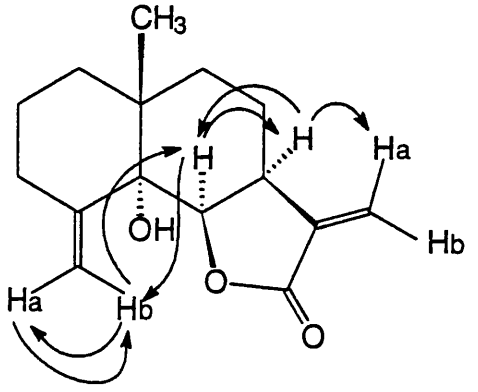
Elution of first portion of fraction four with petroleum ether and ether afforded a mixture of two components of the same polarity. On the basis of spectroscopic evidence the major component, $C_{15}H_{20}O_3$ (m/z 248), m.p. 176-178^{0}C (ex hexane), was identified as oxyfrullanolide (7). The ^{1}H and ^{13}C NMR spectra of this compound show one tertiary methyl [δ_H 0.98 (s, 3H-14); δ_C 18.1 (q, C-14), δ_C 36.7 (s, C-10)], two exomethylenes [{δ_H 5.03 and 5.31(both br s, H-15a and H-15b); δ_C 119.6 (t, C-15), δ_C 147.0 (s, C-4)} and { 5.56 and 6.09 (both dd, J=1.1, 0.3 Hz, H-13a and H-13b); δ_C 111.6 (t, C-13), δ_C 141.5 (s, C-11)}], a one proton multiplet [δ_H 3.27 (br m, H-7); δ_C 38.9 (d, C-7)], an oxygenated methine [δ_H 4.26 (d, J=4.6 Hz H-6); δ_C 80.1 (d, C-6)] together with a tertiary alcohol [δ_C 74.2 (s, C-5)], five methylenes [δ_C 21.8 (t, C-1), 24.3 (t, C-2), 31.8 (t, C-9), 32.0 (t, C-8) and 36.4 (t, C-3)] and a quaternary carbon [δ_C 170.7 (s, C-12)]. This spectroscopic information is

Table I

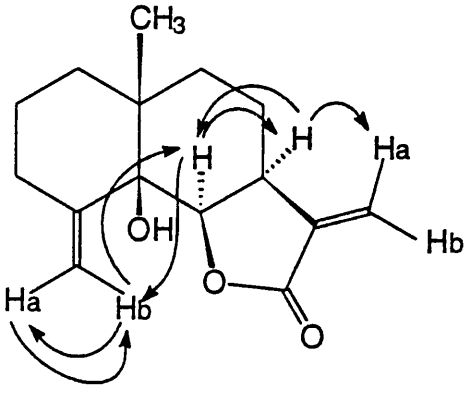
NOEs% →

Irradiation (δ_H) ↓	Saturation % (approx)	δ_H 1.56	δ_H 1.68 - 1.76	δ_H 1.78-1.85	δ_H 1.85-2.00	δ_H 2.1 - 2.25	δ_H 3.27 H-7	δ_H 4.26 H-6 major	δ_H 4.88 H-6 minor	δ_H 5.03 H-15a major	δ_H 5.30 H-15b major, H-15a minor	δ_H 5.41 H-15b minor	δ_H 5.56 H-13a
5.41 H-15b minor	20								4.0		11.8		
5.30 H-15b major, H-15a minor	95							4.3		15.8		1.7	
5.03 H-15a major	82					4.1					20.8		
4.88 H-6 minor	13						6.1					2.3	
4.26 H-6 major	58	2.2					4.4				3.4		
3.27 H-7	54		1.8					4.0	0.9				1.8
0.98 3H-15	42	2.5	2.6	4.4	4.4								

major- Oxyfrulanolide (7), minor- 5b-hydroxy-4(15)-eudesmadien-6,12-olide.



(NOE 7)



(NOE 8)

consistent with that of (+)-oxyfrullanolide (*ent*-7) reported from *F. dilatata*[8]. The carbon assignments are based on comparison with previously reported data for similar compounds[14]. NOE difference experiments also confirm this structure for (7) and are summarised in structure (NOE 7) and Table I. Irradiation of proton H-6 (δ_H 4.26) afforded NOEs at H-7 (δ_H 3.27, 4.4%) and H-15b (δ_H 5.31, 3.4%). As expected irradiation of H-7 shows NOEs at H-6 (4.0%) and H-13a (δ_H 5.56, 1.8%). A NOE is also observed at H-6 (4.3%) on irradiation of H-15b, which also gives a large NOE at H-15a (δ_H 5.03, 15.8%). On irradiation H-15a afforded large NOEs at H-15b (20.8%) and methylene protons at δ_H 2.10 (4.1%). Irradiation of the tertiary methyl (δ_H 0.98) shows NOEs at various methylene protons.

Spectroscopic data also suggested that the minor component could only be the 5-epimer of (7), that is 5β-hydroxy-4(15), 11(13)-eudesmadien-6,12-olide (8), $C_{15}H_{20}O_3$. The proton spectrum of this component contains one tertiary methyl at δ_H 0.98 (s, 3H-14), two exomethylenes [{δ_H 5.29 and 5.41(both m, H-15a and H-15b)} and {δ_H 5.57 and 6.09 (both d, J=1.1 Hz, H-13a and H-13b)}], a one proton multiplet [δ_H 3.27 (br m, H-7)] and an oxygenated methyne [δ_H 4.88 (d, J=4.6 Hz, H-6)]. The coupling constant of H-6 reveals that the lactone is *cis* fused as in (7). Inspection of models indicates that the deshielding of H-6 and H-15 relative to (7) can be rationalised by a change in the configuration at C-5, leading to structure (8) for this minor component. NOE difference experiments [structure (NOE 8) and Table-I] also support structure (8). Irradiation of proton H-6 at δ_H 4.88 gives small NOEs at H-7 (δ_H 3.27, 0.8%) and at H-15b (δ_H 5.41, 0.3%). As expected a NOE is observed at H-6 (0.5%) on irradiation of H-7 which also gives a NOE at H-13a (δ_H 5.56, 1%). Irradiation of H-15b afforded NOEs at H-6 (0.8%) and at H-15a (δ_H 5.29, 15.1%).

The final and most polar compound, β-cyclocostunolide (6), m.p. 66-68^{0}C (ex petroleum ether- $CHCl_3$), $C_{15}H_{20}O_2$ (m/z 232), was found in fraction six. The

proton spectrum of this compound shows a tertiary methyl at δ_H 0.80 (s, 3H-14), two sets of exomethylene proton as sharp doublets [δ_H 4.85 and 4.98 (both d, J=1.3 Hz, 2H-15) and δ_H 5.40 and 6.08 (both d, J=3.2 Hz, 2H-13)] and an oxygenated methine at δ_H 4.02 (t, J=10.9 Hz, H-6). The coupling constants indicate the axial-axial arrangement of the protons H-5, H-6 and H-7 is as in structure (6). These data are identical with those previously reported for β-cyclocostunolide (6)[6,10,15]. This is the first time that β-cyclocostunolide has been found in *F. tamarisci.*

Experimental

The liverwort, *Frullania tamarisci* was collected near Aberfoyle (Scotland) and identified by Prof. J. D. Connolly and Dr. D. S. Rycroft. A reference sample is kept with Dr. Rycroft in the Department of Chemistry, University of Glasgow. A methanol extract (2.57g) was used in this investigation. Compounds were separated by flash chromatography using a column of silica gel G_{254} and petroleum ether - ethyl acetate mixtures as eluent. The following fractions were collected:

Fraction No	Petroleum ether	Ethyl acetate	
1	100	0	four portions, each of 50 ml
2	95	5	,, ,, ,,
3	90	10	,, ,, ,,
4	80	20	,, ,, ,,
5	70	30	,, ,, ,,
6	60	40	,, ,, ,,
7	50	50	,, ,, ,,
8	40	60	,, ,, ,,
9	30	70	,, ,, ,,
10	20	80	,, ,, ,,
11	10	90	,, ,, ,,
12	0	100	,, ,, ,,

Compounds from the separated fractions were purified by preparative thin layer chromatography on silica gel GF_{254}. Eluents for chromatography were increasing percentages of ethyl acetate in petroleum ether. Compounds were visualised using

UV light or by absorption of iodine vapour. Evaporation of solvent was carried out using a Buchi Rotavapor. The purified compounds were subjected to spectroscopic analysis.

Compound one:

Frullanolide (1), (239 mg), m.p.73-75^{0}C (from petroleum ether -$CHCl_3$), [lit.[6] m.p. 74-76^{0}C, ex MeOH], $C_{15}H_{20}O_2$, m/z 232, was isolated from the first portion of fraction two.

δ_H : 1.07 (s, 3H-14), 1.75 (s, 3H-15), 2.92 (m, H-7), 5.23 (d, J=5.6 Hz, H-6), 5.58 (d, J=1 Hz, H-13a) and 6.14 (d, J=1 Hz, H-13b).

δ_C : 18.1(t,), 19.3 (q, C-14), 25.0 (t), 26.0 (q, C-15), 33.0 (s, C-10), 33.1 (t), 38.0 (t), 39.1 (t), 41.2 (d, C-7), 76.0 (d, C-6), 120.1 (t, C-13), 128.4 (s, C-4), 138.4 (s, C-5), 142.2 (s, C-11) and 171.0 (s, C-12).

Compound two:

α-Cyclocostunolide (2), (25 mg), m.p 84-86^{0}C (from MeOH), [lit.[6] m.p. 82-83^{0}C, ex MeOH], $C_{15}H_{20}O_2$, m/z 232, was isolated from the first portion of fraction two.

δ_H : 0.90 (s, 3H-14), 1.83 (s, 3H-15), 3.87 (t, J=11.2 Hz, H-6), 5.38 (m, H-3 and H-13a), 6.05 (d, J=3.1 Hz, H-13b)

δ_C : 17.3 (q, C-14), 21.4 (t,C-8), 22.7 (t, C-2), 23.6 (q, C-15), 36.0 (s, C-10), 37.7 (t, C-1), 39.1 (t, C-9), 51.1 (d, C-7), 51.4 (d, C-5), 82.2 (d, C-6), 116.4 (t, C-13), 122.3 (d, C-3), 132.9 (s, C-4), 139.3 (s, C-11) and 171.0 (s, C-12).

Compound three:

Tamariscol (5), (29mg), $C_{15}H_{26}O$, m/z 222, was isolated from the first portion of fraction two as an oil.

δ_H : 0.86 (d, J=6.5 Hz, 3H-14), 0.91 (d, J=6.5 Hz, 3H-15), 1.74(d, J=1.3 Hz, 3H-12), 1.87(d, J=1.3 Hz, 3H-13) and 5.05 (m, H-10).

δ_C : 15.0 (q, C-14), 19.0 (q, C-15), 20.0 (q, C-13), 24.0 (t, C-9), 28.2 (q,C-12), 30.0 (t, C-5), 32.0 (t, C-8), 33.0 (t, C-4), 39.5 (d, C-7), 45.4 (d, C-3), 50.0 (d, C-6), 58.3 (d, C-1), 79.2 (s, C-2), 121.5 (d, C-10), and 136.4 (s, C-11).

Compound four:

γ-Cyclocostunolide (3), (30 mg), m.p. 79-82^{0}C (from petroleum ether- $CHCl_3$) [lit.[6] m.p. 86-87^{0}C, ex MeOH], $C_{15}H_{20}O_2$, m/z 232, was isolated from the first portion of fraction two

δ_H : 1.11 (s, 3H-14), 1.86 (s, 3H-15), 2.56 (m, H-7), 4.54 (d, J=11.3 Hz, H-6), 5.34 and 6.13 (both d, J=3.0 Hz, 2H-13).

δ_C : 19.0 (t, C-2), 20.0(q, C-14), 23.1(t, C-1), 26.0(q, C-15), 34.3(t, C-3), 37.1(s, C-10), 41.0(t, C-8), 41.1(t, C-9), 50.2 (d,C-7), 83.4 (d, C-6), 118.0 (t, C-13), 127.0(s, C-4), 130.0(s, C-5) 139.4 (s, C-11) and 170.5 (s, C-12).

Compound five:

Costunolide (4), (17.3mg), m.p. 102-106^{0}C (from MeOH) [lit.[6] m.p. 103-105^{0}C, ex MeOH], $C_{15}H_{20}O_2$, m/z 232, was isolated from the fourth portion of fraction two.

δ_H : 1.40 (s, 3H-14), 1.68 (s, 3H-15), 4.57 (t, J=8.5 Hz, H-6), 4.72 (m, H-5), 4.80 (m, H-1), 5.50 and 6.23 (both d, J=3.0 Hz, 2H-13).

δ_C : 16.0 (q, C-14), 17.3 (q, C-15), 26.1 (t, C-8), 27.9 (t, C-2), 29.6 (t, C-9), 39.4 (t, C-3), 50.3 (d, C-7), 81.9 (d, C-6), 119.6 (t, C-13), 127.0 (d, C-1), 127.2 (d, C-5), 136.9 (s, C-10), 140.0 (s, C-4), 141.4 (s, C-11), and 170.4 (s, C-12).

Compound six:

Oxyfrullanolide (7), (8 mg), $C_{15}H_{20}O_3$, m/z 248, m.p. 176-178^{0}C (from hexane), [lit.[8] m.p 179-180^{0}C, ex hexane], ν_{max} 1764 cm^{-1} (lactone), 3588 cm^{-1} (broad, OH) and 1670 cm-1 (C=C), was isolated from the first and second portion of fraction four.

δ_H : 0.98 (s, 3H-14), 3.27 (br m, 1H-7), 4.26 (d, J=4.6 Hz, H-6), 5.03 and 5.31(both br s, H-15a and H-15b), 5.56 and 6.09 (both dd, J=1.1, 0.3 Hz, H-13a and H-13b).

δ_C : 18.1 (q, C-14), 21.8 (t, C-1), 24.3 (t, C-2), 31.8 (t, C-9), 32.0 (t, C-8), 36.4 (t, C-3), 36.7 (s, C-10), 38.9 (d, C-7), 74.2 (s, C-5), 80.1 (d, C-6), 111.6 (t, C-13), 119.6 (t, C-15), 141.5 (s, C-11), 147.0 (s, C-4) and 170.7 (s, C-12).

Compound seven:

5β-Hydroxy-4(15), 11(13)-eudesmadien-6,12-olide (8), $C_{15}H_{20}O_3$, was obtained as the minor component of a mixture with compound six .

δ_H : 0.98 (s, 3H-14), 3.27 (br m, H-7), 4.88 (d, J=4.6 Hz, H-6), 5.29 and 5.41(both m, H-15a and H-15b), 5.57 and 6.09 (both d, J=1.1 Hz, H-13a and H-13b).

Compound eight

β-Cyclocostunolide (6), (6.3 mg), $C_{15}H_{20}O_2$, m/z 232, m.p. 66-68^{0}C (from petroleum ether- $CHCl_3$), [lit.[10] m.p. 66.5-67^{0}C, ex ether], ν_{max} 1766 cm^{-1} (lactone) and 1654 cm^{-1} (C=C), was found in fraction six.

δ_H : 0.80 (s, 3H-14), 4.02 (t, J=10.9 Hz, H-6), 4.85 and 4.98 (both d, J=1.3 Hz, 2H-15) and δ_H 5.40 and 6.08 (both d, J=3.2 Hz, 2H-13).

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