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"NATURAL PRODUCTS FROM THE HEPATICAE"

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by

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CONTENTS

"NATURAL PRODUCTS FROM THE HEPATICAE"

Malcolm S. Buchanan

SUMMARY

This thesis consists of eight chapters, the first of which is a General Introduction dealing with the nature of secondary metabolites and the terpenoid and aromatic constituents of the Hepaticae (liverworts). This is followed by a discussion of the results of investigations into the chemical constituents of *Herbertus aduncus* and *H. borealis* (Chapter 2). From the former species six herbertane sesquiterpenoids were isolated (two of which are new natural products) and the structures determined mainly using ^{1}H and ^{13}C NMR spectroscopy. Studies of the fatty acid components of the triglycerides and cycloartenol esters of the species are also discussed. The chemical constituents of *H. aduncus* collected from four different sites and one collection of *H. borealis* are compared using ^{1}H NMR and GLC techniques.

Chapter 3 deals with the constituents of *Anastrophyllum donnianum*. Six new sphenolobane diterpenoids were isolated and their structures determined by spectroscopic methods. The constituents of this species, collected from three different sites, are contrasted using GLC and GCMS procedures. The structure of a bicyclogermacrane sesquiterpenoid is also discussed.

The metabolites of *Porella platyphylla* form the subject matter of Chapter 4. Seven methoxylated pinguisanes and a new sacculatane diterpenoid were isolated from one sample of this species while another sample yielded two new pinguisanes.

Their structures were assigned using ^{1}H and ^{13}C NMR spectroscopy.

Chapter 5 describes the structural elucidation of nine new *ent*-kaurane diterpenoids and two new gymnomitrane derivatives from *Jungermannia truncata*. Three known *ent*-kauranes, a pimarane and a halimane were also isolated.

The major part of Chapter 6 is concerned with the structural determination of a cadinane endoperoxide from *Bazzania tricrenata* using spectroscopic methods which included highfield (600 MHz) ^{1}H and phase-sensitive DQF-COSY experiments. The calamenane sesquiterpenoid constituents of this liverwort are also discussed.

The next chapter (Chapter 7) contains a discussion of the structural elucidation of a spirovetivane sesquiterpenoid from *Lepidozia reptans*.

The final chapter (Chapter 8) describes the structural elucidation of two monoterpene hydroperoxides from *Jungermannia obovata*. The monoterpene and sesquiterpene hydrocarbon constituents of this liverwort are also discussed.

CHAPTER 1

GENERAL INTRODUCTION

NATURAL PRODUCT CHEMISTRY

Natural product chemistry is one of the oldest branches of the chemical sciences, its origin dating back to the first decades of the 19th century, or even earlier. After almost 200 years of study, this discipline is still vibrant and evolving, with a wide diversity of natural products being found in the extracts obtained from natural sources. This is illustrative of the array of enzymes involved in the synthesis of a fascinating variety of structures from terrestrial and marine sources. The main reasons for the continuing interests include the challenges offered by the detection, isolation and purification procedures, by the permanently improving methods of structure elucidation, and by the complexities of the biogenetic pathways leading to these compounds. However the most important reason for studying natural product chemistry resides in the biological activity found in some natural products. Natural products from marine, fungal, bacterial, amphibian and insect sources as well as lower and higher plants, have been investigated.

The advent of sophisticated chromatographic techniques, highfield multi-dimensional NMR spectroscopy and new ionization procedures in mass spectroscopy, coupled with X-ray analysis and molecular force-field calculations, have markedly enhanced the isolation and structure elucidation of substantially more complex and diverse natural products. These new techniques have also made possible the isolation of compounds which are present in extremely small amounts.

Many of the medicinal, pharmacological and other biological agents used in the world are either natural products themselves, are derivatives of them, or are modified templates of natural products. Thus there is an enormous potential for

the future development of natural products from the marine and terrestrial environments to produce key biological agents for the future benefit of mankind from renewable resources.

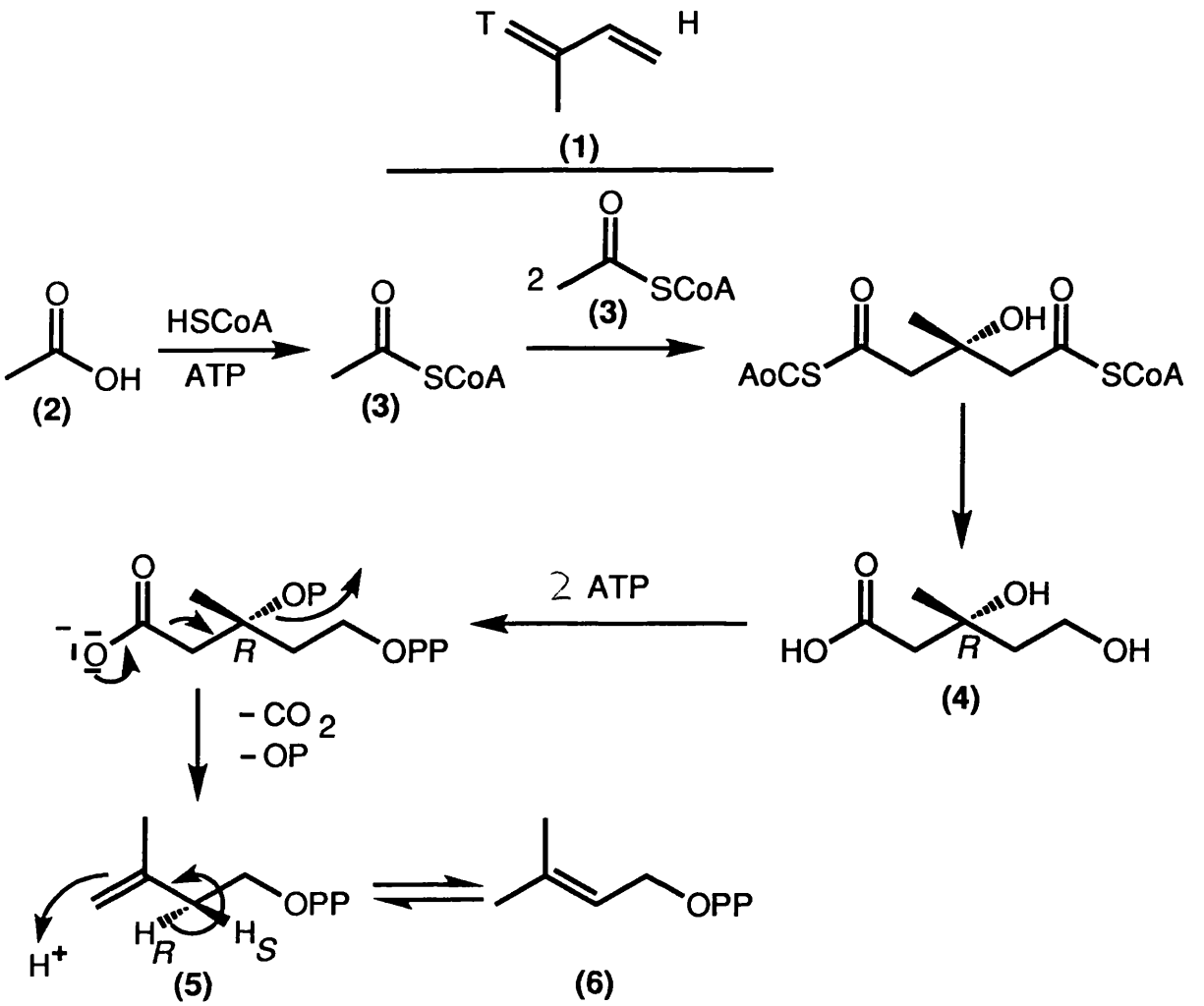
Natural products fall into two categories - primary and secondary metabolites - though the border between the two classes is vague in places. The former are organic compounds which are characteristic of all living systems and include carbohydrates, lipids, amino acids, peptides, proteins, nucleosides, nucleotides and nucleic acids. On the other hand secondary metabolites include phenols, quinones, terpenes, alkaloids, and the various pigments which organisms produce. There remains the fundamental and often unresolved problem of the biological function of secondary metabolites in the organism which produces them. Organisms evolve through changes (mutations) in their genetic make up and these may be positive, negative or neutral. When the change leads to a metabolite with a useful biological function the pathway will be retained and emphasized. Natural selection would be expected to remove biosynthetic pathways leading to products with no useful function. Natural products have been shown to be involved in ecological interactions with many of them being used as defence substances, feeding deterrents, sex-attractants etc.

TERPENOID BIOSYNTHESIS

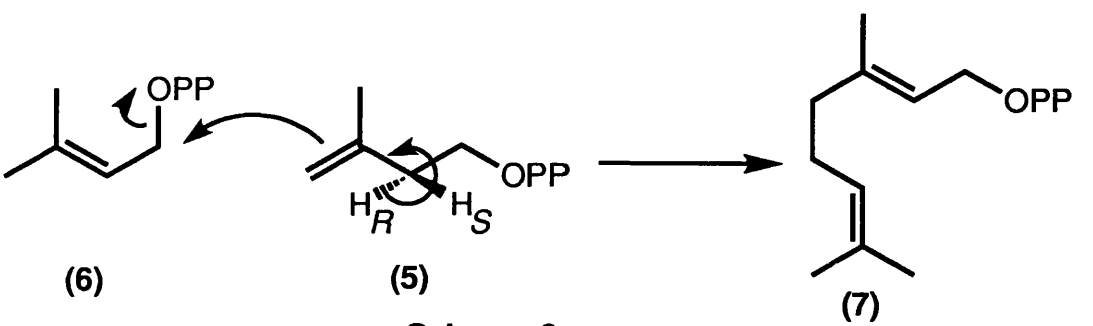
Since the beginning of natural product chemistry, the biogenetic origin of the terpenoids has been the focus of intense interest. From his work which began in 1884, Wallach proposed that the structures of not only the monoterpenes, but other terpene compounds also, can be built from isoprene units (2-methylbuta-1,3-

diene)(**1**)[1]. This concept has come to be known as the Isoprene Rule. It was not until the 1920s and the work of Ruzicka[2] that the validity of this rule for many compounds was established. The Isoprene Rule states that the carbon skeleton of the terpenes is composed of isoprene units (**1**) linked in a regular "head to tail" arrangement or in an irregular arrangement. However, further research unearthed compounds which did not conform to this mode of formation and eventually to overcome this, the Biogenetic Isoprene Rule[2] was conceived. This included the "abnormal" terpenoids and rationalised their formation as proceeding *via* mechanistically feasible shifts and rearrangements (collectively known as migrations) of the "regular" polyisoprenoids. In some cases more than one route is possible and stereochemical and energetic considerations suggest which one is more likely[3].

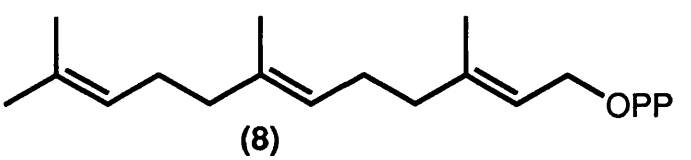
It is now firmly established that the fundamental biogenetic unit is acetic acid (**2**) in the form of acetyl coenzyme A (**3**), which by condensation reactions gives rise to 3(*R*)-mevalonic acid (MVA) (**4**), the immediate precursor of the isoprene unit (**1**) (Scheme 1)[3]. The active form of the isoprene unit, isopentenyl pyrophosphate (IPP) (**5**) and its isomer, dimethylallyl pyrophosphate (DMAPP) (**6**), condense to form geranyl pyrophosphate (GPP) (**7**) (Scheme 2), the precursor of monoterpenoids, which can subsequently condense with additional IPP (**5**) to give farnesyl pyrophosphate (FPP) (**8**) and geranyl geranyl pyrophosphate (GGPP) (**9**), which are the acyclic precursors of the sesquiterpenoids and diterpenoids respectively. The acyclic precursor of the triterpenoids is squalene (**10**), usually as its 2,3-epoxide, and is derived from two farnesyl units joined "head to head". This pathway to the acyclic precursors is stereospecific and controlled by enzymes.



Scheme 1



Scheme 2



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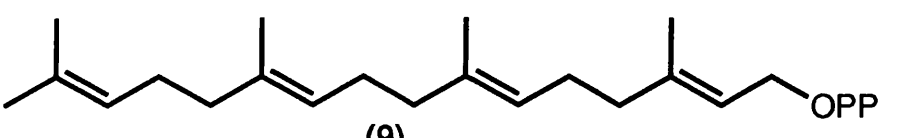

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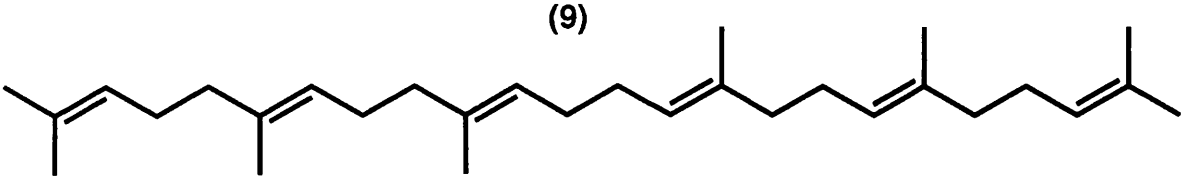
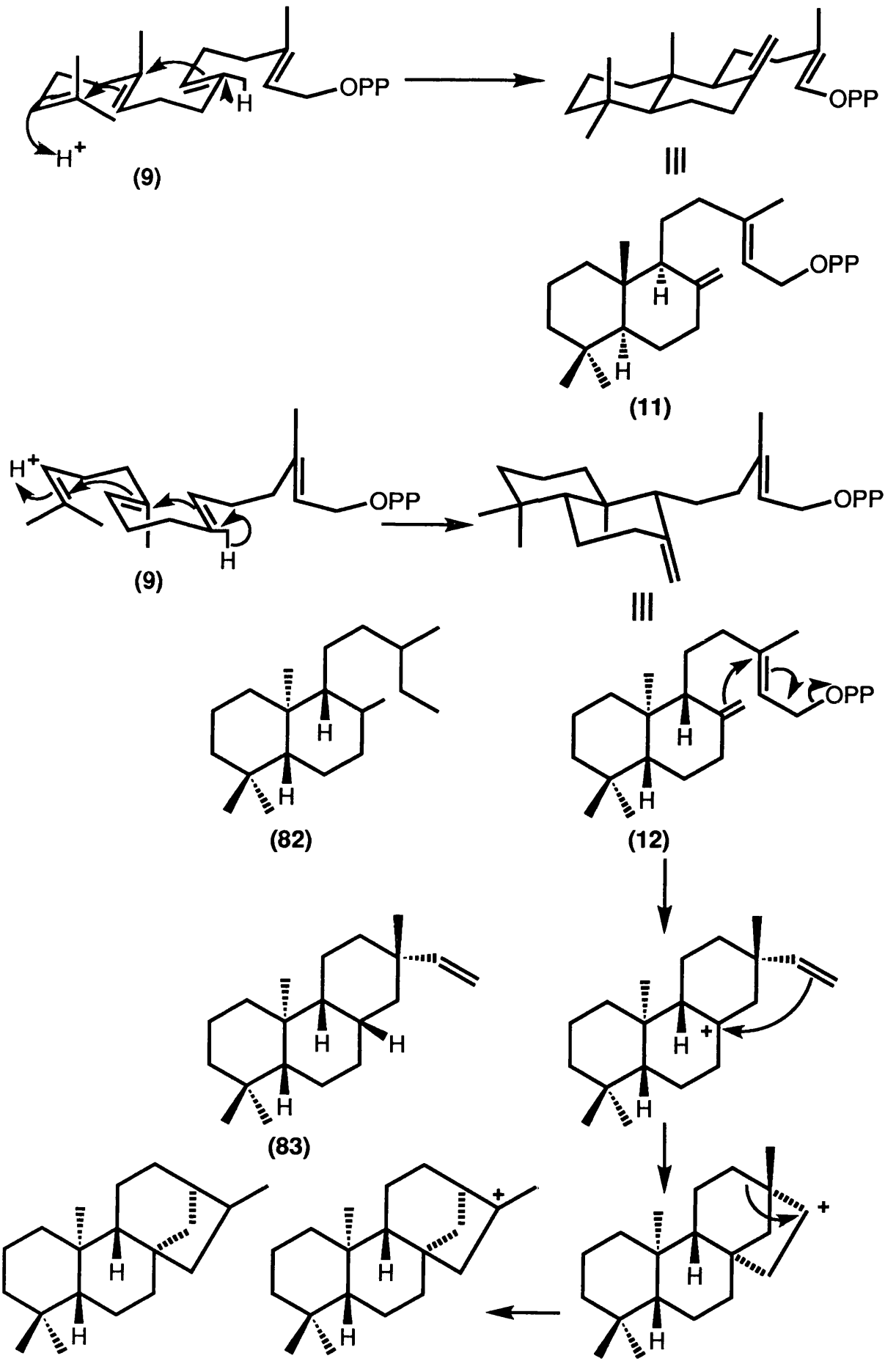


(10)

The acyclic precursors GPP (**7**), *trans*-FPP (**8**) and all *trans*-GGPP (**9**) are converted into the parent terpenoid skeleton by isomerase-cyclase enzymes[3-5]. These enzymes catalyse a multistep process with complete regio- and stereochemical control being maintained at each step. This control is due to the specificity of the three-dimensional arrangement of the active site of the enzyme which will bind specific substrates in a conformation so that there is good orbital overlap for subsequent reaction. For example Scheme 3 gives the mechanistic representations of the cyclizations of GGPP (**9**) to the pyrophosphate esters of the antipodal bicyclic labdadienyl pyrophosphates (**11** and **12**) through the appropriately-folded pseudo-two-chair conformations of GGPP (**9**)[1]. This leads to the 5α,10ß configuration (**11**) or 5ß,10α configuration (**12**) of the AB ring junction. The initial carbocation may undergo additional cyclizations, rearrangements, alkyl shifts and hydride shifts prior to deprotonation to an olefin or nucleophile capture[3-5]. The reactive cationic intermediates are produced ready for reaction by the presence of a divalent cation enzyme co-factor such as Mg^{2+} or Mn^{2+} which neutralises the negative charge of the pyrophosphate. They are stabilized during the enzymatic transformations by ion pairing with pyrophosphate anion and other counter ions. The products of many cyclases are olefins since termination can be achieved by pyrophosphate anion assisted deprotonation leading to elimination. The large number of terpenoid derivatives arises by regio- and stereospecific secondary enzymatic transformations (oxidations, reductions, hydrations, isomerizations, conjugations). It is worth mentioning that the formation of the same terpenoid skeleton in different species may not follow the same biosynthetic pathway[5].


OPP
H
H+
(9)
III
OPP
H
H
(11)
H+
OPP
H
(9)
III
OPP
H
H
(82)
H
H
OPP
(12)
H
H
H
H
(83)
H
H
+
H
H
+
H
H
+
H
H
(74)

Scheme 3

HEPATICAE

The bryophytes are taxonomically placed between the algae and the pteridophytes (ferns etc) in the plant kingdom, there being approximately 20,000 species in the world[6]. They are morphologically divided into three classes, Musci (mosses, 14,000 species), Hepaticae (liverworts, 6,000 species) and Anthocerotae (hornworts, 300 species). Of these the most interesting chemically are the liverworts, due to the presence of oil bodies in their cells. Hornworts and mosses do not contain oil bodies. The liverworts, a group of primitive plants, are considered as an early stage of the evolution of terrestrial green plants. They may have leaves (leafy liverworts) or lack leaves (thalloid liverworts) and have a unique life cycle[7]. Liverworts prefer wet, humus-rich habitats, such as damp rocks and logs, the forest floor, swamps or marshes, or beside streams and pools. Their great dependence on a moist environment is linked to two characteristics : they have flagellated sperm cells, which must swim to the egg cells in the archegonia; and most lack vascular tissues and hence the means for efficient long distance internal transport of fluids.

The chemistry of the liverworts was neglected for a very long time and it is only during the last twenty years that research in this area has developed. Recent progress in analytical methods makes it easier to isolate pure compounds from liverworts and to determine the chemical structures of minute samples. Results have shown that liverworts contain a surprising range[6] of chemical structures and form a remarkable source of new natural products, some with interesting biological activity. It is the special cell organelles, the oil bodies which are a characteristic feature of the liverworts, which store these natural products[6,7]. These oil bodies

elaborate mainly terpenoids and lipophilic aromatics as their major chemical constituents. The oil bodies show great variation in shape, size and number per cell[7]. The two most important types are the segmented oil body [Figure 1, A and B (x 6000)] and the homogeneous oil body [Figure 1, C and D (x 20000)]. Homogeneous oil bodies are made up of one lipid droplet surrounded by a membrane, whereas segmented oil bodies have several to numerous droplets bound within the membrane. The outer membrane is a (double) unit membrane whereas individual droplets are surrounded by a half membrane and are embedded in a protein matrix. In dried specimens the oil bodies disintegrate and the chemical constituents are easily extracted with organic solvents.

In the wild it is often very hard to collect liverworts in sufficient amounts for chemical research, because of the small size of the plant and its tendency to grow intermingled with other liverworts or mosses. In some cases the amount of plant material collected is only just sufficient to isolate and elucidate the structures of the main compounds. Likewise it is often not possible to obtain sufficient plant material for biological tests. Sufficient plant material can sometimes be obtained by growing liverwort cultures *in vitro*[8]. These *in vitro* cultures may provide the only solution to the problem of obtaining sufficient amounts of biologically active compounds, particularly when synthesis proves impossible or uneconomical.

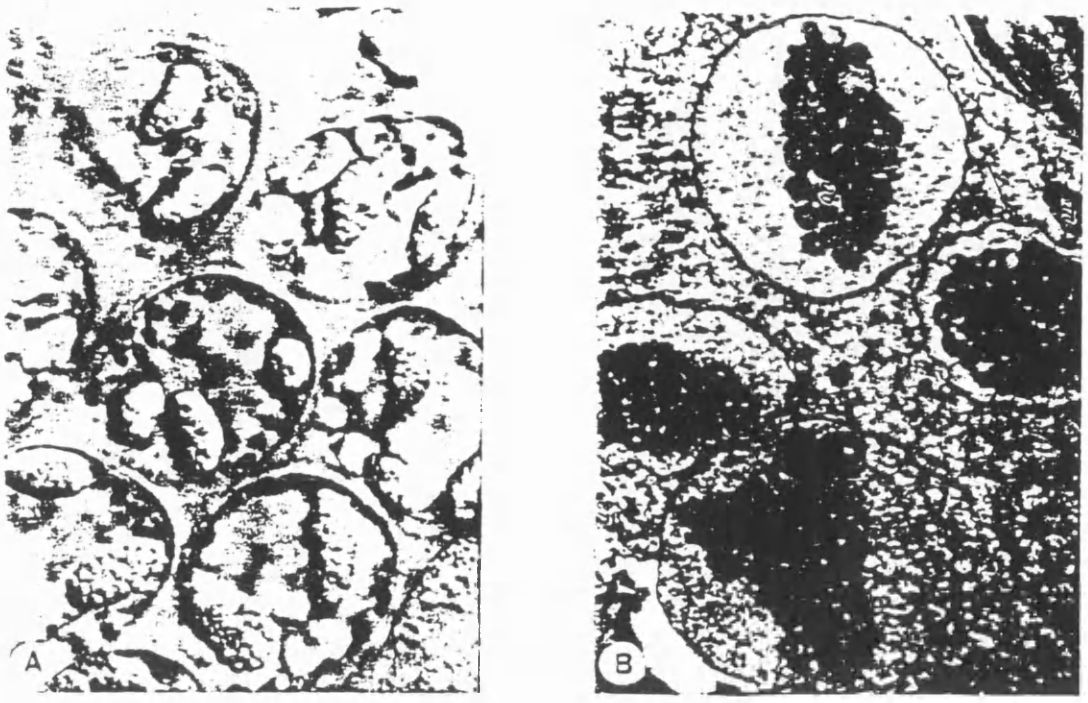
The liverworts are morphologically divided into two subclasses[7] : the Jungermanniidae and the Marchantiidae and these are further divided into seven main orders : Sphaerocarples, Monocleales, Marchantiales (Marchantiidae); Takakiales, Calobryales, Jungermanniales and Metzgeriales (Jungermanniidae). The Jungermanniales (leafy liverworts) are the largest order within the class

Figure 1. Liverwort oil bodies[7].

Segmented

Length of oil bodies: 6-18 µm

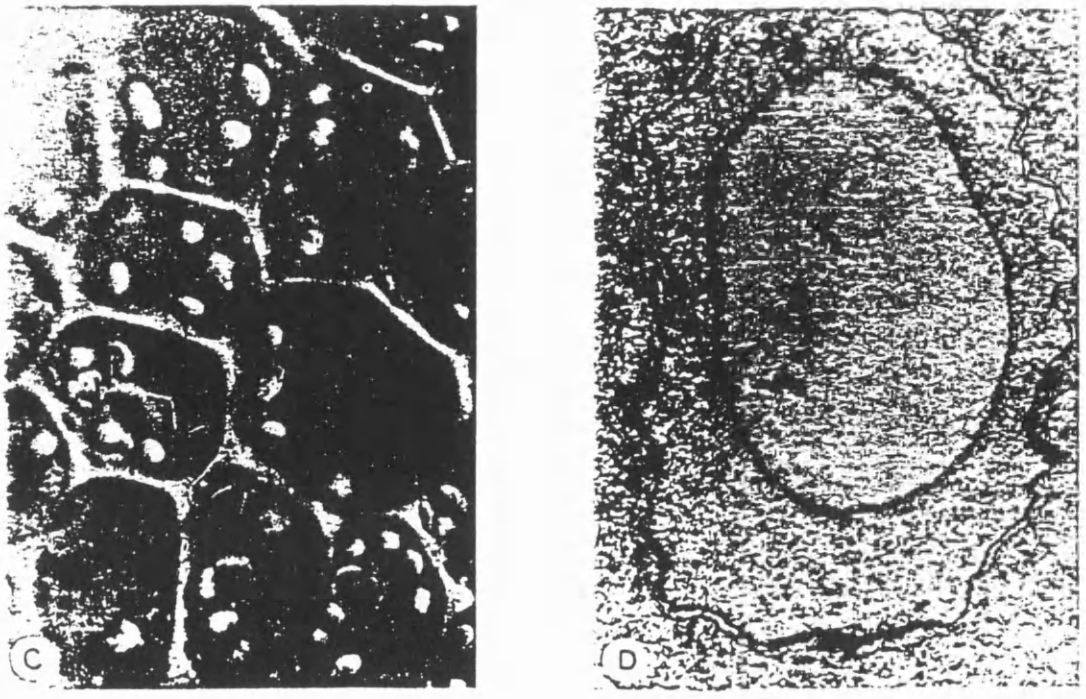
x 6000



Homogeneous

Length of oil bodies: 5-7 µm

x 20000



Hepaticae and contain about 75-80% of all species. The nomenclature for British liverworts followed in this thesis is that used by Smith[9]. Many liverwort species have very wide distributions with some species occurring in more than one continent. Within the subclass Jungermanniidae the oil bodies occur in green photosynthetic cells whereas in the subclass Marchantiidae they are restricted to special 'oil cells' lacking chlorophyll. In some groups of liverworts the oil bodies are absent.

The first chemical investigation of liverworts was carried out by Müller in 1905[10] who reported the presence of sesquiterpenoids in the oil bodies. It was not until 1956 that the chemical constituents of liverworts were further investigated. Fujita *et al*[11] identified sesquiterpene hydrocarbons in the essential oil of *Bazzania pompeana*.

The chemosystematic aspect of the study of the chemical constituents of liverworts is also of interest. The secondary metabolites of a plant are characteristic of its genetic material and so consideration of these should lead to a taxonomic classification. Plant species which produce similar chemical constituents may have similar biosynthetic pathways and thus similar genes which encode the enzymes. However secondary metabolites may also depend on the stage of development of a plant species and on environmental factors and these complicate the issue of chemotaxonomy. Liverwort chemosystematics are studied in several laboratories[6,12].

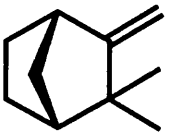
In the following review of liverwort consitituents only the most significant compounds and those with most relevance to this thesis are discussed. For a more comprehensive discussion on liverwort metabolites the reader is directed towards

the following reviews : Markham and Porter[12] concerning bryophyte chemistry prior to 1978; Asakawa[6] who has produced a comprehensive review of the terpenoids, aromatics and lipids of liverworts prior to 1981; Huneck[13] who has reviewed all liverwort metabolites prior to 1981; *Bryophytes : Their Chemistry and Chemical Taxonomy*[14] from 1981 to 1989.

MONOTERPENOIDS

Monoterpenoids have been isolated from the fragrant oils of many plants and are important in the perfumery and flavour industries. Monoterpenoids are also found in many marine organisms, where they are generally halogenated, and as insect pheromones and defence secretions. Relatively few reports of the occurrence of monoterpenoids in the liverworts have appeared and those that have been published are based mainly on their detection by GC or GCMS[6]. This is due to the fact that they are mainly hydrocarbons and generally occur as complex mixtures with sesquiterpene hydrocarbons which are difficult to separate. The first report on the monoterpene composition of a liverwort was by Svensson[15] in 1974 who reported on the monoterpene hydrocarbons from the essential oils of *Jungermannia cordifolia* and *J. obovata*. Among the monoterpenes which were identified by GCMS, camphene (**13**) dominates in *J. cordifolia* while terpinolene (**14**) and limonene (**15**) are the main components of *J. obovata* which has a very characteristic carrot aroma. The interesting homomonoterpenoid alcohol, (-)-2-methylisoborneol (**16**), is responsible for the "mossy" odour of *Lophocolea heterophylla*[16].

Both enantiomers of monoterpenoids occur in higher plants, often as a



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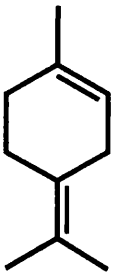

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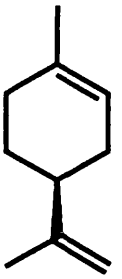

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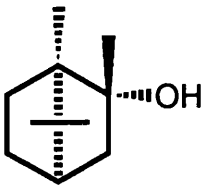

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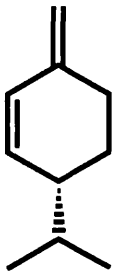

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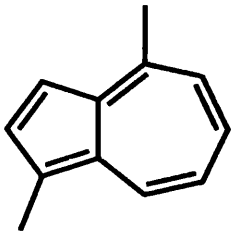

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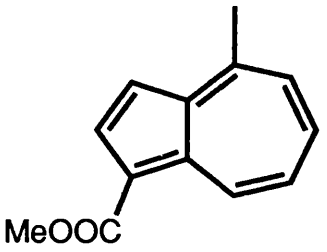

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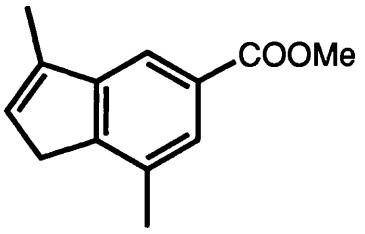

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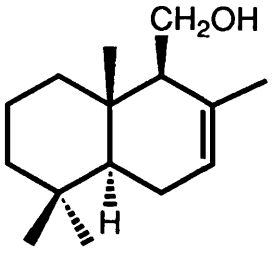

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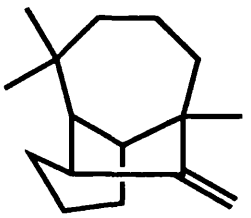

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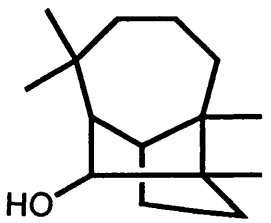

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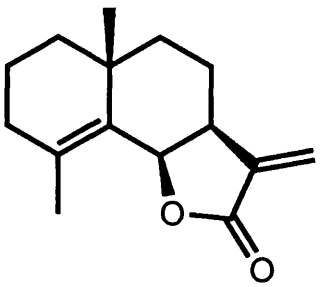


(24)

racemic mixture, but some species synthesize only one of the two isomers[17]. The isolation of (-)-limonene (**15**) from *Conocephalum conicum*[18] and (+)-limonene (*ent*-**15**) from *J. exsertifolia*[19] shows that both enantiomers occur in liverworts. However the absolute configuration of many liverwort monoterpenes remains undetermined. The new and highly sensitive technique of two dimensional gas chromatography (2DGC) should enable progress in this area. It has been used in an analysis of the enantiomeric composition of monoterpene hydrocarbons from the liverwort *Conocephalum conicum*[20]. The results reveal that most monoterpenoids have high optical purity with the exception of ß-phellandrene (**17**) (38% ee).

SESQUITERPENOIDS

The first isolation of terpenoids from a liverwort, apart from the aromatic compounds, 1,4-dimethylazulene (**18**) and 4-methyl-1-methoxycarbonylazulene (**19**), and an indene, 3,7-dimethyl-5-methoxycarbonylindene (**20**) from the blue oil bodies of *Calypogeia trichomanis*[21-23], were the sesquiterpenoids (-)-drimenol (**21**) from *Bazzania trilobata*[24] and (-)-longifolene (**22**) and (-)-longiborneol (**23**) from *Scapania undulata*[25].

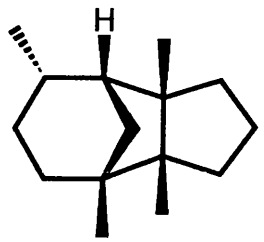
It is interesting that often the absolute configuration of liverwort sesquiterpenoids is opposite to those found in higher evolutionary plants but the same as compounds encountered in lower evolutionary organisms such as the Fungi[26] and Coelenterates (marine invertebrates or their algal symbionts)[27]. This phenomenon was first published in 1967 with the report[25] that *S. undulata* contains (-)-longifolene (**22**) and (-)-longiborneol (**23**), the enantiomers of those found in higher plants. More interestingly *Frullania tamarisci ssp. obscura* produces (-)-

frullanolide (**24**) with the 'normal' 7ß configuration while the morphologically similar *F. dilatata* elaborates the enantiomeric (+)-frullanolide (*ent*-**24**)[28,29]. In general *F. dilatata* biosynthesizes *ent*-sesquiterpenoids while *F. tamarisci ssp. obscura* produces compounds belonging to the 'normal' series[28,30]. There are, however, several exceptions to this trend such as drimanes and guaianes which have only the 'normal' configuration and the eudesmanes and germacranes which have representatives in both the 'normal' and *ent*-series.

The sesquiterpenoids are the most widely distributed of the liverwort metabolites and most sesquiterpenoid skeletons are the same as those found in higher plants. However there are a number of skeletal types that remain unique to the liverworts : gymnomitrane (**25**), 5,10-cycloaromadendrane (**26**), 1,10-secoaromadendrane (**27**), 2,3-secoaromadendrane (**28**), chiloscyphane (**29**), pinguisane (**30**), vitrane (**31**), myltaylane (**32**) and cyclomyltaylane (**33**).

Herbertanes

The herbertanes (**34**) have an unusual carbon skeleton which is similar to the cuparanes (**35**) the difference being that the cyclohexane ring of cuparane has a 3-methyl group whereas in herbertane it has a 4-methyl group. These two skeletal types are examples of the rare occurrence of aromatic sesquiterpenoids in Nature. Cuparene (**36**), the first compound of this skeletal type to be reported, was isolated from the liverwort *Bazzania pompeana* in 1971[31]. In 1975 its absolute configuration was established[32] as *S*-(-)-cuparene (**36**) (wrongly described as *R* in the paper), the enantiomer of that found in higher plants. It has since been discovered that both antipodes of cuparane sesquiterpenoids occur in liverworts.



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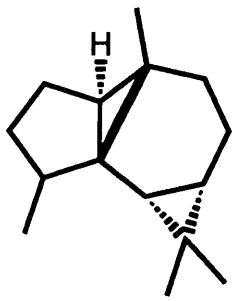

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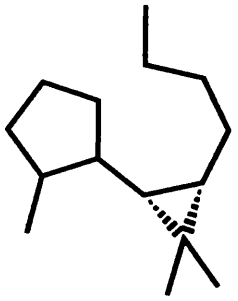

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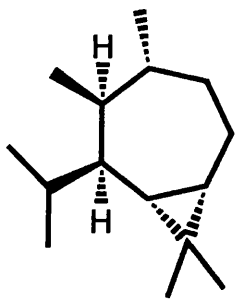

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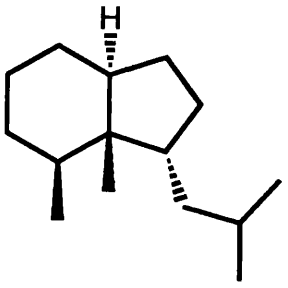

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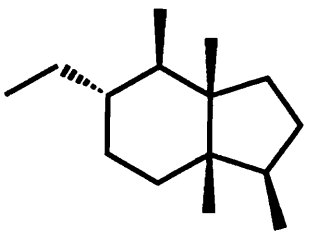

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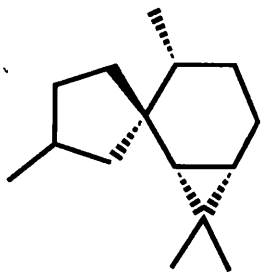

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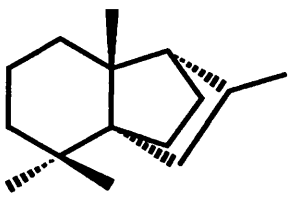

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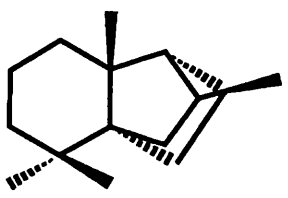

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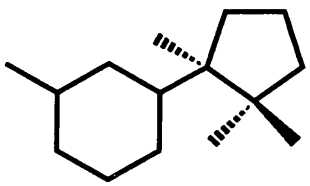

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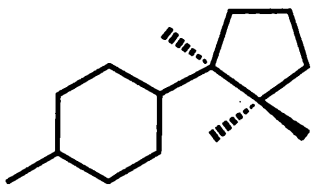

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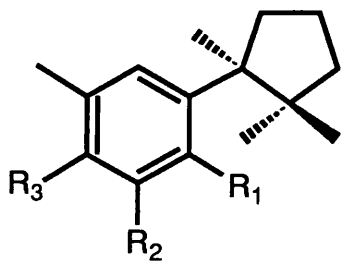


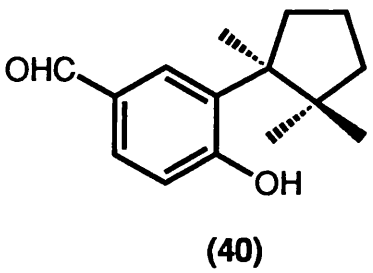
(37) $R_1 = R_2 = R_3 = H$

(38) $R_1 = OH, R_2 = R_3 = H$

(39) $R_1 = R_2 = H, R_3 = OH$

(41) $R_1 = R_2 = OH, R_3 = H$

(44) $R_1 = H, R_2 = R_3 = OH$



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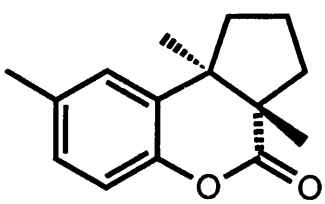

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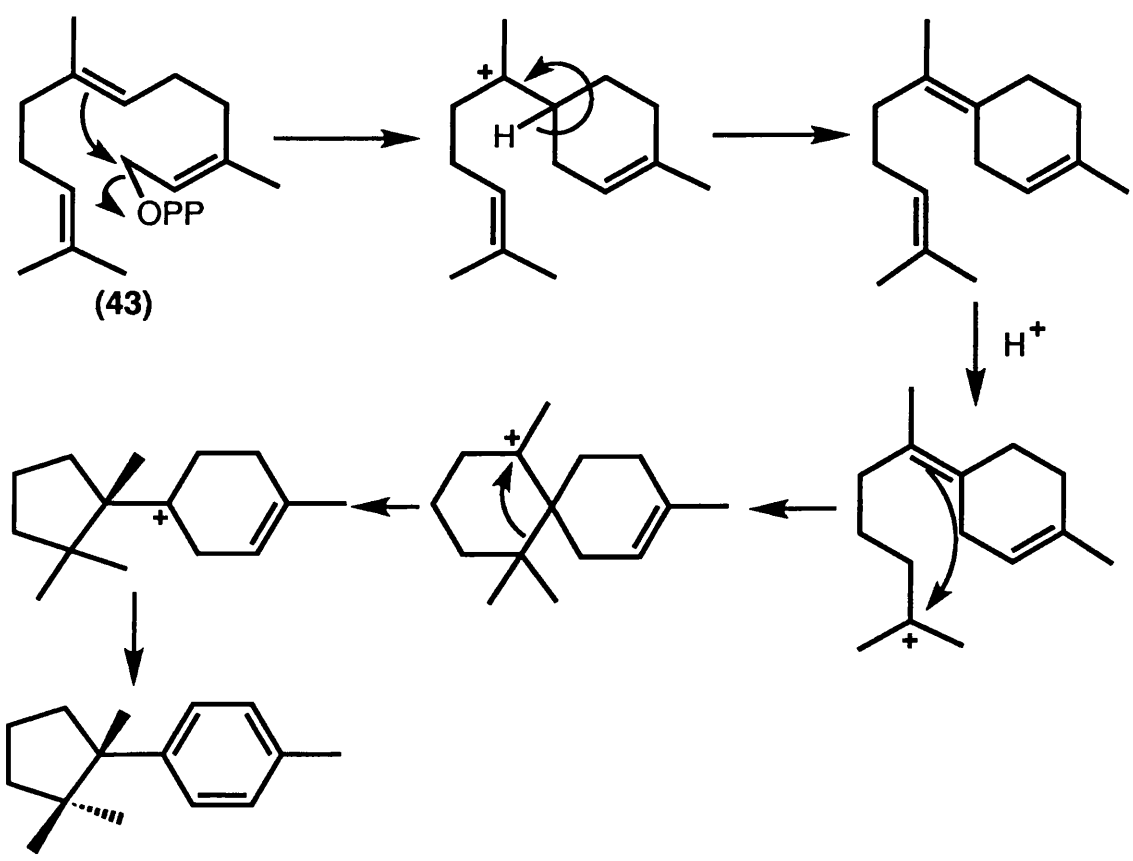


(43)

(36)

Scheme 4

Jungermannia rosulans elaborates several 'normal' cuparane sesquiterpenoids including *R*-(+)-cuparene (*ent*-**36**)[33] (wrongly assigned as *S* in the paper). The herbertanes, which are called isocuparanes by Asakawa[34], form a small group of sesquiterpenoids found largely in liverworts. They have also been found in the Fungi[35].

The first compound in this series (-)-herbertene (**37**) was isolated in 1981 from the leafy liverwort *Herbertus aduncus*[36]. Other compounds of this type of molecule are (-)-α-herbertenol (**38**), (-)-ß-herbertenol (**39**), (-)-α-formylherbertenol (**40**), (-)-herbertenediol (**41**) and (-)-herbertenolide (**42**) from *H. aduncus*[34,37-39] and *H. subdentatus*[34]. These herbertane-type sesquiterpenoids provide another example of the stereospecificity in liverwort sesquiterpenoid biosynthesis since they have the same absolute configuration as the *ent*-cuparanes[32,39]. The enantiomeric form of the herbertanes is not known. Some of them show antifungal properties[39], depressing the growth of certain plant pathogenic fungi. Herbertanes have now also been detected in the *Mastigophora diclados, M. woodsii*[40-43] and *Herbertus acanthelius*[44] species of liverwort.

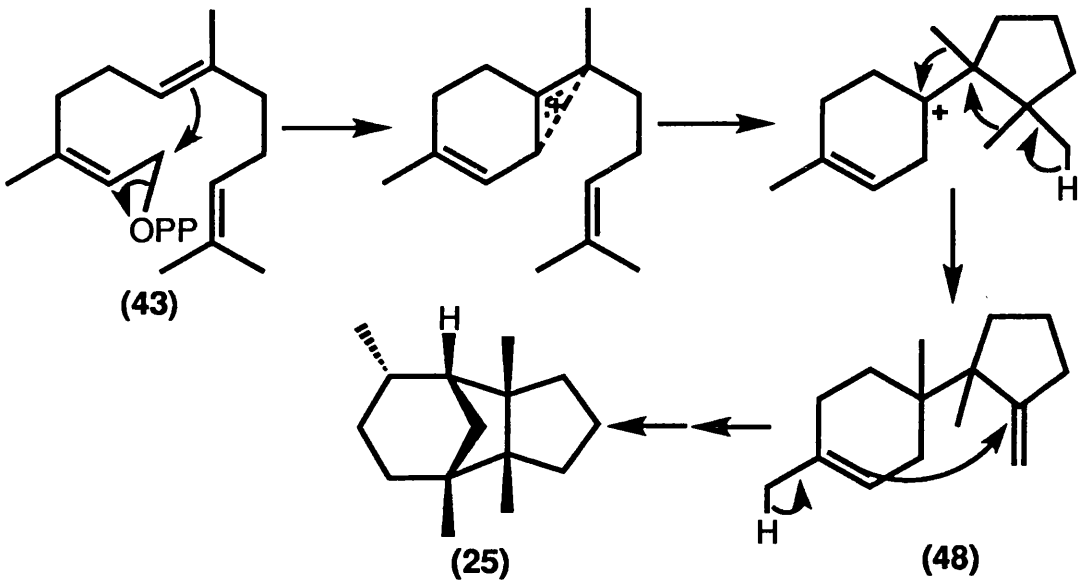
The discovery of (-)-herbertene (**37**) raised some interesting biosynthetic questions since this novel carbon skeleton does not follow the Isoprene Rule. The herbertane skeleton (**34**) may be formed by 1,2-methyl migration of the *ent*-cuparane skeleton (**35**) which is formed by stereospecific cyclization of *cis*-FPP (**43**) (Scheme 4)[14].

Asakawa's group have now isolated four novel herbertane type dimers and a new herbertane monomer, (-)-herbertene-2,3-diol (**44**) from the liverwort *Mastigophora diclados*[42,43]. These dimeric herbertanes are believed to be produced

via phenoxy radicals formed by one electron oxidation of (-)-herbertenediol (**41**) which is a co-metabolite in the plant. These herbertane dimers and some of the monomers exhibit neurotrophic properties[43,45]. The dimers have now been detected in *Herbertus* species[46]. *Mastigophora* species are regarded as being chemically almost identical to *Herbertus* species[41] because both generally produce unique herbertane-type sesquiterpenoids and herbertane dimers. Based on their morphology the Herbertaceae and the Mastigophoreacea are considered to be primitive families in the Jungermanniales[40,47] and the above chemical evidence shows that the species in both these families elaborate a narrow range of terpene skeletons. This is a primitive character[48] and so supports the morphological data.

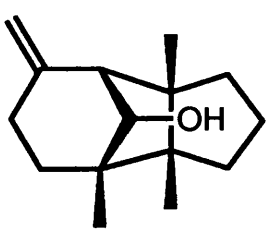
Gymnomitranes

Gymnomitranes (barbatanes)(**25**) were first isolated from the liverwort *Gymnomitrion obtusum*[49] which produces gymnomitrol (**45**), the main constituent, and several other gymnomitranes. The sesquiterpene hydrocarbons gymnomitrene (≡ ß-barbatene ≡ ß-gymnomitrene ≡ ß-pompene)(**46**) and isogymnomitrene (≡ α-barbatene ≡ α-gymnomitrene ≡ α-pompene)(**47**) are widespread in the liverworts[6,13]. The biosynthetic route to the gymnomitrane skeleton is believed to involve cyclisation of *cis*-FPP (**43**) leading *via* a cuparane intermediate to ß-bazzanene (**48**), a diastereoisomer of trichodiene. Cyclisation of **48** affords the gymnomitrane skeleton (**25**) (Scheme 5)[6,50,51]. The most recent examples of this skeleton to be reported include (-)-gymnomitr-3(15)-en-12-ol (**49**), (-)-gymnomitr-3(15)-en-12-al (**50**) and (-)-gymnomitr-3(15)-en-12-oic acid (**51**) from the liverwort *Marsupella emarginata var patens*[52]. The major constituent of the liverwort

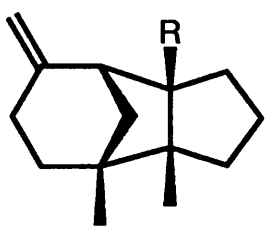


(43) (25) (48)

Scheme 5



(45)

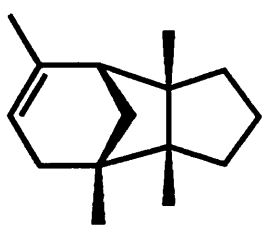


(46) R = Me

(49) R = CH_2OH

(50) R = CHO

(51) R = CO_2H



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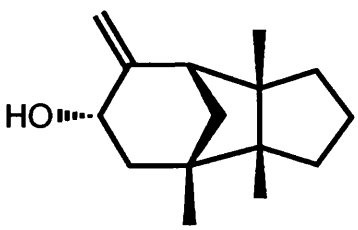

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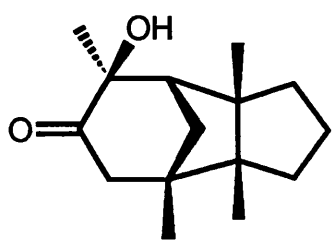


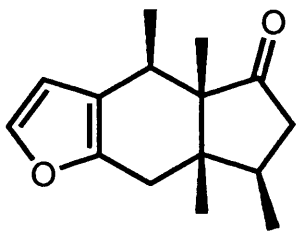
(53)

Reboulia hemisphaerica was found to be (+)-gymnomitr-3(15)-en-4α-ol (**52**)[53]. A later investigation[54] of cultures on the same species resulted in the isolation of 3ß-hydroxygymnomitran-4-one (**53**), together with compound **52**.

Pinguisanes

The first representative of the pinguisane (**30**) type of sesquiterpene is the ketone, pinguisone (**54**), which was isolated from the liverwort *Aneura pinguis* and reported in 1969 by Benesova *et al*[55]. It was soon followed by deoxopinguisone (**55**) from *Ptilidium ciliare*[56]. The pinguisane skeleton is unique and difficult to rationalise simply in terms of the Isoprene Rule. No satisfactory biosynthetic route to this skeleton has yet been proposed.

Pinguisanes are widely distributed in liverworts and occur in the Lejeuneaceae, Porellaceae, Ptilidiaceae, Lepidolaenaceae of the Jungermanniales and Aneuraceae of the Metzgeriales[57]. Recently investigation of *Frullanoides densifolia* and *Trocholejeunea sandvicensis*[58] has provided several new pinguisane derivatives. *F. densifolia* produced two new rearranged pinguisane sesquiterpenoids, spirodensifolin A (**56**) and spirodensifolin B (**57**), together with a new pinguisane-type alcohol, isonaviculol (**58**). Other previously known pinguisanes, also found in this liverwort, include naviculol (**59**), the geometrical isomer of **58**, which has already been reported from *Porella navicularis*[59]. The stereochemistry of the epoxide ring in compound **56** was established by X-ray crystallographic analysis since NOE difference results were ambiguous. Among the pinguisanes isolated from *T. sandvicensis* is the new compound furanopinguisanol (**60**).



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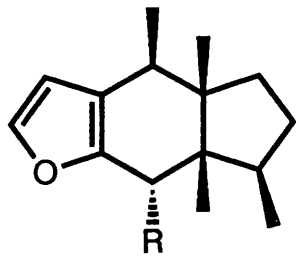
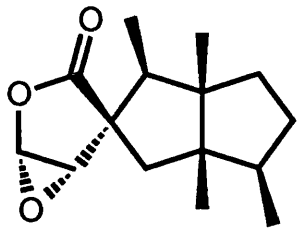


(55) R = H
(60) R = OH



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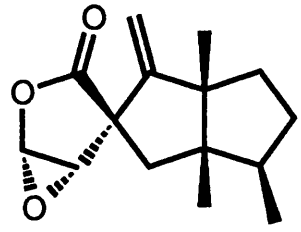

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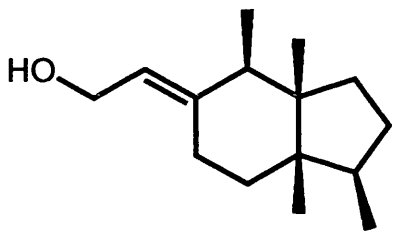
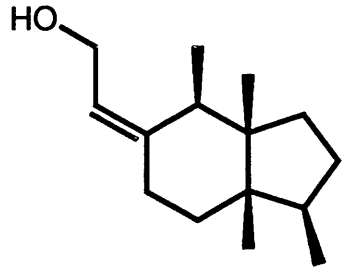


(58)



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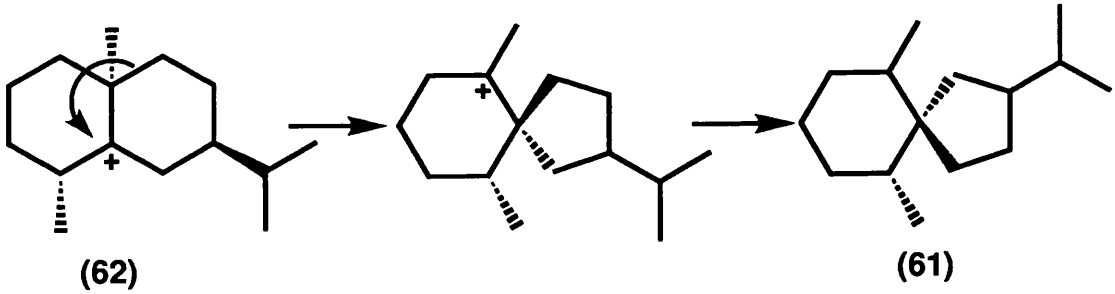
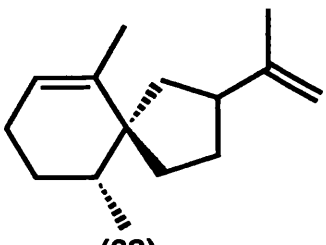


(62) (61)

Scheme 6



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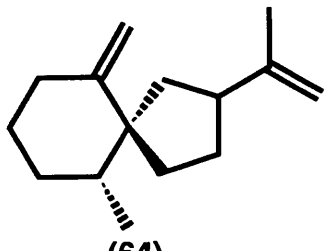


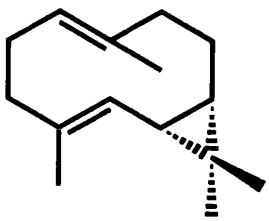
(64)

Spirovetivanes

The spirovetivanes (vetispiranes) (**61**) are found in vetiver oil[60] and also occur as phytoalexins in infected potatoes[61]. This skeleton is derived from the eudesmane skeleton (**62**)[62] by a rearrangement (Scheme 6). Although there have been several reports of this skeleton in higher plants their only occurrence in liverworts to date is in the morphologically similar *Scapania maxima* and *S. robusta*[63,64]. Wu and Lee identified the major sesquiterpene hydrocarbon as being α-spirovetivene (**63**)[63,64] and subsequently detected ß-spirovetivene (**64**) as a minor constituent[64].

Bicyclogermacranes

Bicyclogermacranes, which are also found in higher plants[65,66]; have a *cis*-fused cyclopropane ring junction. (-)-Bicyclogermacra-1(10),4-diene (**65**) is widespread in liverworts and is an important biosynthetic intermediate for a number of sesquiterpenoids including those of the aromadendrane and maaliane-type[6]. (-)-Bicyclogermacra-1(10),4-diene (**65**) belongs to the *ent*-series since its sign of rotation is opposite to that of the same compound in the peel oil of *Citrus* species[6]. Few other representatives of this type have been reported in liverworts. These are *ent*-3ß-acetoxybicyclogermacra-1(10),4-diene (**66**) which occurs in the extracts of *Plagiochila yokogurensis, Pedinophyllum truncatum* and *Scapania ampliata*[6] and most recently bicyclogermacra-1(10),4-dien-13-al (**67**) which has been isolated from *Conocephalum conicum*[67]. The stereoisomeric lepidozanes, which are found in marine organisms[68] as well as liverworts, have a *trans*-fused ring junction. This type of skeleton was first discovered in a liverwort by Matsuo *et*



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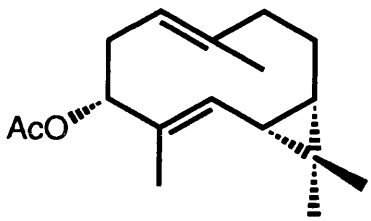

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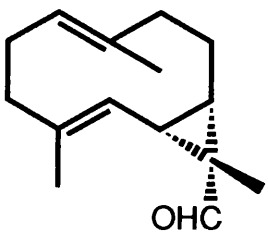

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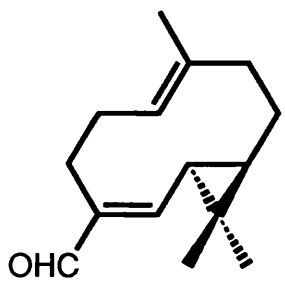

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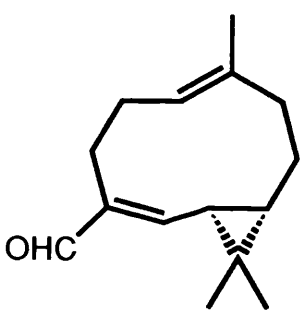

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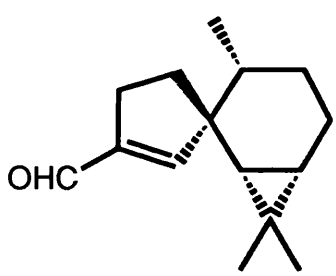

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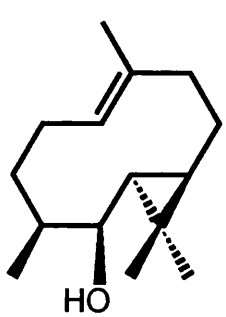


(71)

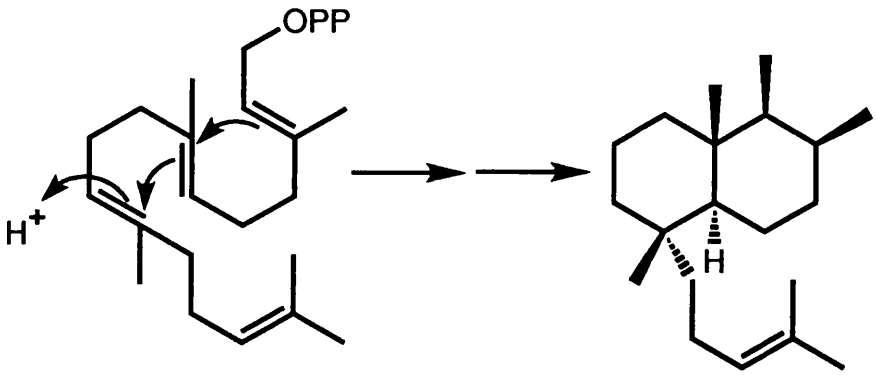
al[69,70] who isolated (-)-lepidozenal (**68**) from *Lepidozia vitrea*. This liverwort also produced (-)-isobicyclogermacra-1(10),4-dien-15-al (**69**)[70,71], a double bond isomer of the bicyclogermacradiene skeleton, and (+)-vitrenal (**70**)[70,72] with an unusual spiro-skeleton unique to the liverworts. All three of these sesquiterpenoids have the *ent*-configuration. The only other example of the lepidozane skeleton in liverworts is (-)-lepidozen-5-ol (**71**) which was isolated from *Trocholejeunea sandvicensis*[58].

DITERPENOIDS

Only certain genera of the liverworts produce diterpenoids and the sacculatane (**72**) and verrucosane (**73**) (and its modified skeletons) types remain unique to the liverworts. Most of the diterpenoids found in liverworts have representatives from both the 'normal' and *ent*-series as in higher plants and only the kauranes (**74**) belong exclusively to the *ent*-series. The first diterpenoid to be isolated from liverworts was the *ent*-kaurane, *ent*-(-)-16ß-hydroxykaurane (**75**), which was reported in *Anthelia julacea* and *A. juratzkana* by Huneck and Vevle in 1970[73]. It shows plant growth inhibitory activity.

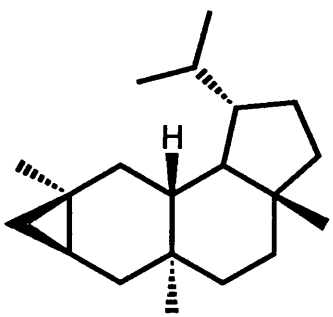
Sacculatanes

The sacculatane skeleton (**72**), being unique to the liverworts, has created interest from a biogenetic point of view and is most likely to be derived from GGPP by a cyclization mechanism similar to that of the drimanes (Scheme 7)[74]. The first report of this skeletal type was in the liverwort *Trichocoleopsis sacculata* from which sacculatal (**76**) and isosacculatal (**77**) were isolated by Asakawa and

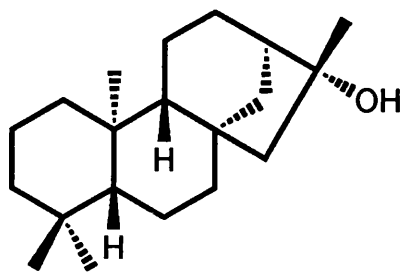


(72)

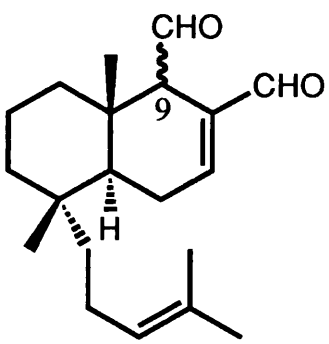
Scheme 7



(73)

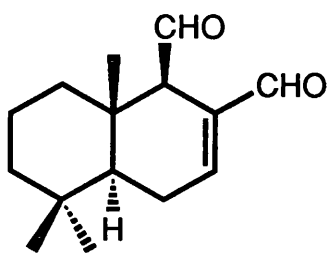


(75)



(76) 9β - **CHO**

(77) 9α - **CHO**



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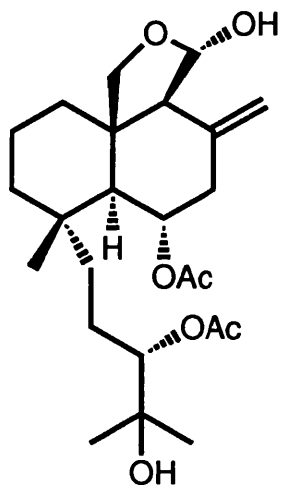
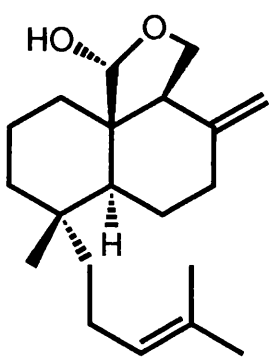


(79)



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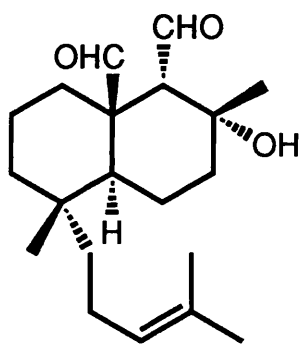


(81)

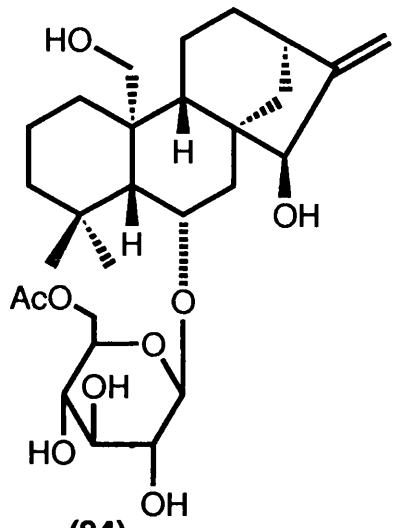
Takemoto[74]. Sacculatal (**76**) is a pungent substance whereas isosacculatal (**77**), the C-9 epimer of **76** is not[6]. This variation of pungency was also observed for polygodial (**78**), a drimane-type sesquiterpene found in several *Porella* species of liverwort, and its C-9 epimer. The structural feature responsible for this intense pungency is the dial system. Total synthesis of sacculatal (**76**)[75] revealed that it has the 'normal' drimane absolute configuration. In addition to its pungency sacculatal (**76**) shows tumour-promoting, antifeedant and piscicidal activity[6].

The sacculatane-type diterpenoids have a widespread occurrence in liverworts and have been found in *Trichocoleopsis*, *Pellia*, *Porella* and *Makinoa* species[6]. The most recent representatives of this type are the two hemiacetals, sacculaplagin (**79**)[76] and sacculaporellin (**80**)[77], isolated from the liverwort *Plagiochila acanthophylla* and *Porella perrottetiana* respectively. Another dialdehyde derivative, 8-hydroxy-9-hydroperrottetianal A (**81**) has been isolated from *Fossombronia pusilla*[78] and shows antibacterial activity.

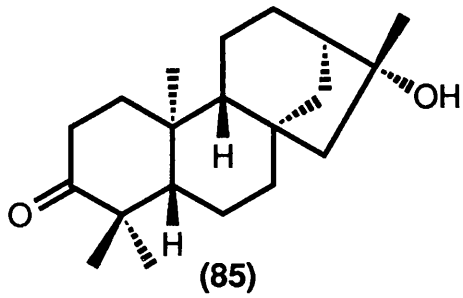
Kauranes

The biogenesis of *ent*-labdane (**82**), *ent*-pimarane (**83**) and *ent*-kaurane (**74**) type diterpenoids can be represented as in Scheme 3, by cyclization of all *trans*-GGPP (**9**).

The kaurane skeleton is commonly found in Nature and most kauranoids belong to the *ent*-series. The polymorphic liverwort *Jungermannia infusca*[79] has an intensely bitter taste which has been shown to be due to the presence of the kaurene glycosides, infuscasides A-E [eg. **84** ≡ infuscaside A]. This is the first report of terpenoid glycosides in liverworts and although these have an intense



(84)



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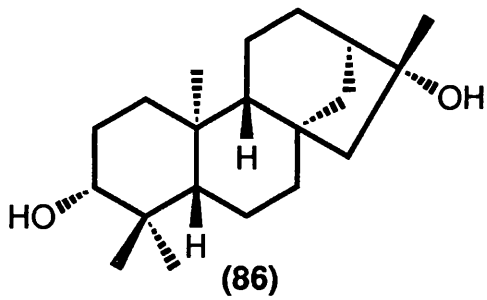

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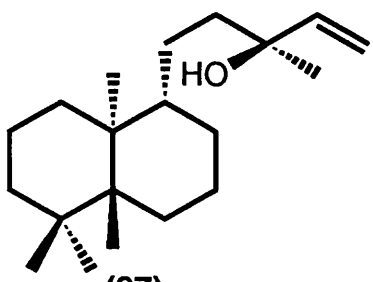

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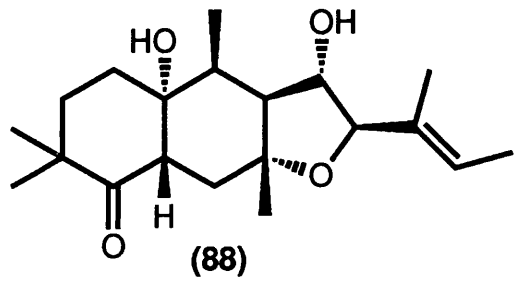

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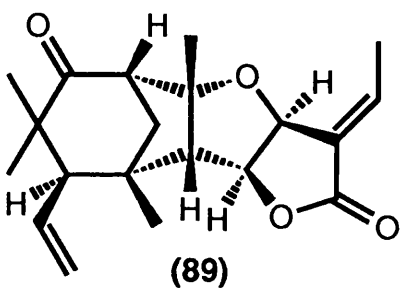

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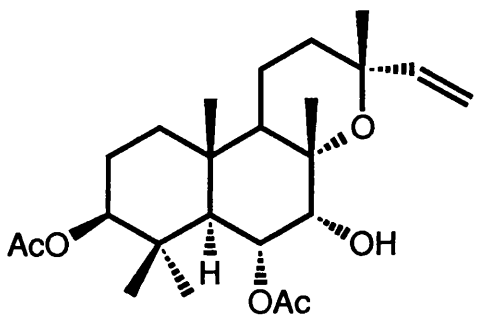

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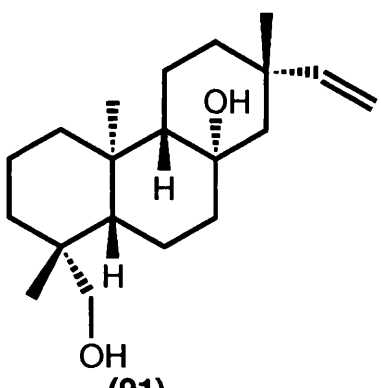

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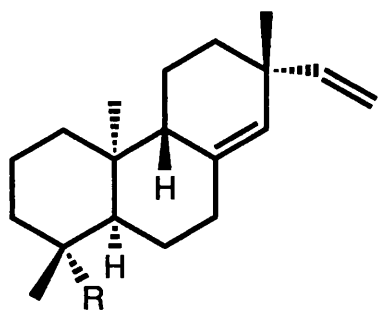
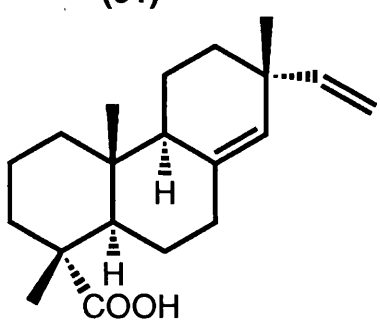
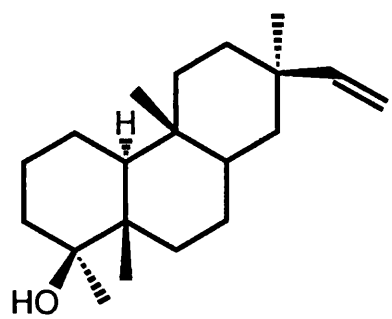


(92) R = CH_2OH

(93) R = COOH



(94)



(95)

bitterness the corresponding aglycones are tasteless. The most recent examples of this skeleton reported from liverworts are *ent*-kauran-16ß-ol-3-one (**85**) and *ent*-kauran-3ß,16ß-diol (**86**) from *Frullanoides densifolia*[58].

Labdanes

Ent-manool (**87**), isolated from *Jungermannia torticalyx* by Matsuo *et al*[80], was the first representative of this skeletal type to be found in liverworts. The labdane skeleton (**82**) is common in Nature. Novel modifications of this skeleton found in liverworts include scapanin G (**88**) from Scottish *Scapania undulata*[81] and the unusual secolabdane pallavicinin (**89**) from *Pallavicinin subciliata*[41]. Several manoyl oxide derivatives (e.g. **90** ≡ hamachilobene A) have been isolated from *Frullania hamachiloba*[82] and belong to the 'normal' series of absolute configuration.

Pimaranes

There are very few representatives of this skeletal type in liverworts. The first to be discovered were the three *ent*-pimaranes, (-)-thermarol (**91**), *ent*-pimara-8(14),15-dien-19-ol (**92**) and *ent*-pimara-8(14),15-dien-19-oic acid (**93**) from the liverwort *Jungermannia thermarum*[83]. No further *ent*-pimaranes have since been reported. Subsequently the 'normal' isopimarane, (-)-sandaracopimaric acid (**94**) was found in the liverwort *Mastigophora diclados*[84]. A recent report reveals the discovery of a rearranged pimarane (**95**) in *Schistochila aligera*[85]. These are the only pimaranes to have been reported in liverworts to date.

Sphenolobanes

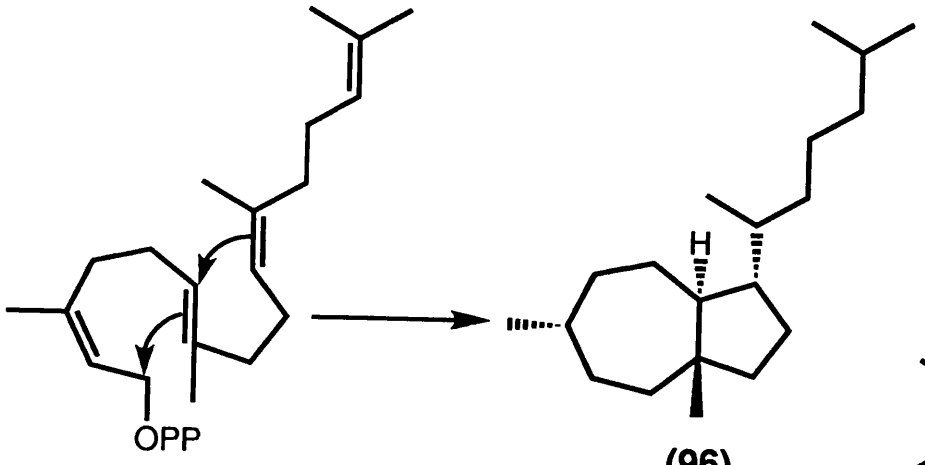
A possible biosynthetic route to the sphenolobane skeleton (**96**) is shown in Scheme 8. Sphenolobanes have been reported in a marine organism (deepwater sponge)[86], the Cistacea (*Halimium viscosum*)[87,88] and the Pinaceae (*Pseudolarix kaempferi*)[89] as well as liverworts (*Anastrophyllum minutum*)[90]. This skeleton was first reported in *P. kaempferi*[89] from which pseudolaric acids A, B and C (**97-99**) were isolated. The sphenolobane derivatives from *H. viscosum*[87,88] are epimeric at C-9 and this modified skeleton is called tormesane [e.g. tormesol (**100**)]. The only occurrence of the sphenolobane skeleton in liverworts to date is in the liverwort *Anastrophyllum minutum (Sphenolobus minutus)*[90] from which six compounds (**101-106**) of this skeletal type were isolated.

TRITERPENOIDS

Triterpenoids are rare in liverworts and some that have been reported may well have come from impurities in the plant material. Since 1982[6] there have been occasional reports of hopanes[53,91] and cycloartanes[40,92,93] as minor constituents. For example cycloart-23-ene-3ß,25-diol (**107**) has been found in *Plagiochila kahsiana (P. peculiaris)*[46] and cycloartenol (**108**) has been found in both *Mylia taylori*[92] and *Lophozia ventricosa*[93]. However it is worth noting that cycloartanes are common in mosses[13].

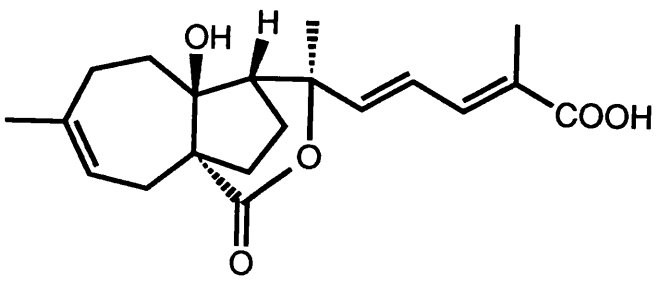
AROMATICS

Bibenzyls and bisbibenzyls are the main types of aromatic constituents found in liverworts. The bibenzyls are the most characteristic components of liverworts

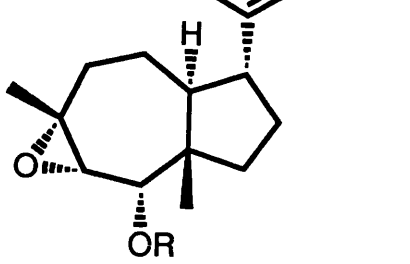


(96)

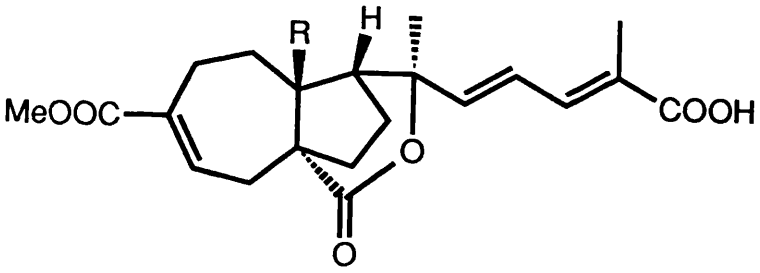
Scheme 8



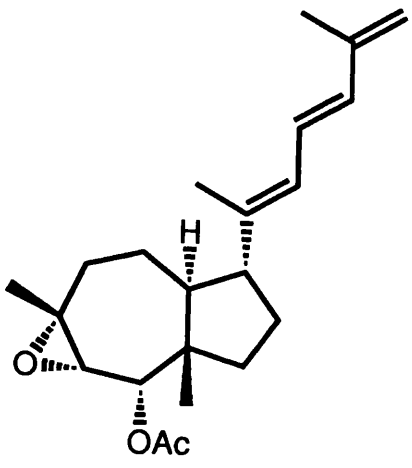
(97)



(101) R = Ac
(102) R = H



(98) R = OAc
(99) R = OH



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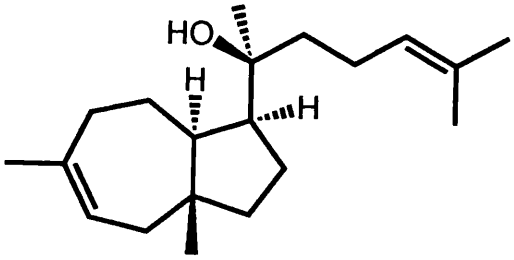

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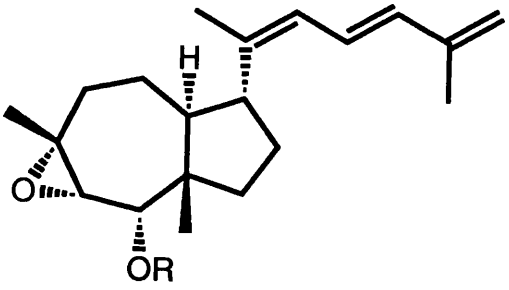
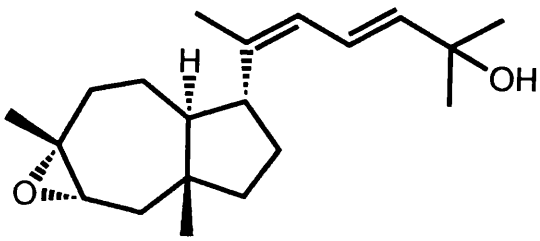


(105) R = Ac
(106) R = H



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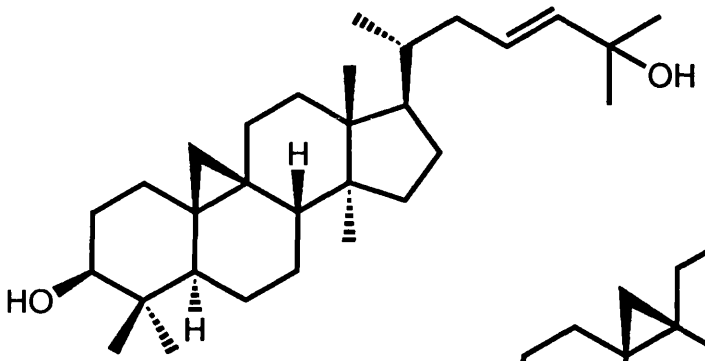

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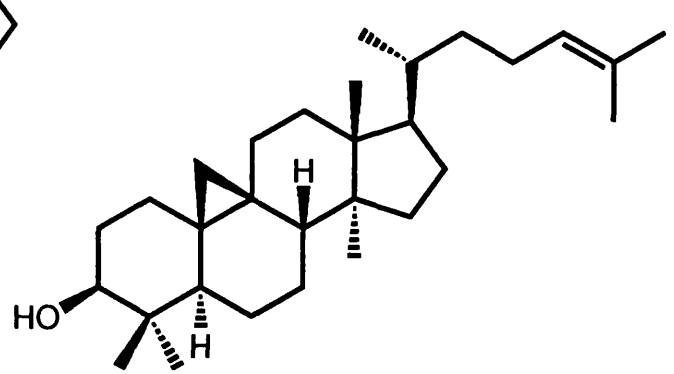
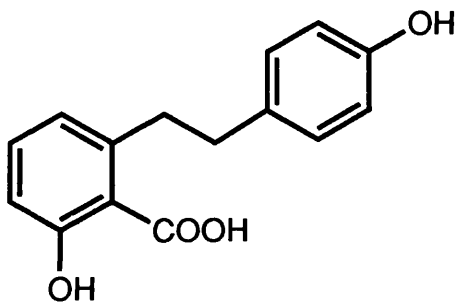
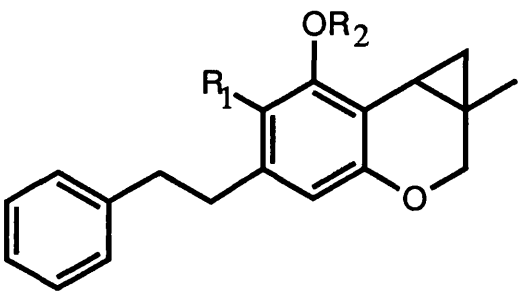


(108)



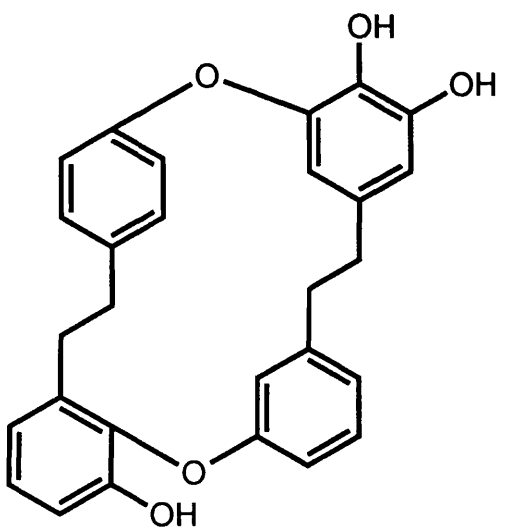
(109)



(110) $R_1 = H$, $R_2 = H$

(111) $R_1 = H$, $R_2 = Me$

(112) $R_1 = COOH$, $R_2 = H$



(113)

and many of them are biologically active. The first representative of the bibenzyl type, lunularic acid (**109**), was isolated from the liverwort *Lunularia cruciata* in 1969[94]. Since the discovery of lunularic acid (**109**) many bibenzyl derivatives have been isolated with various modifications of the basic bibenzyl unit[6,95]. The cyclopropanochroman type is one of the most recent classes to be discovered e.g. radulanins I, J and K (**110-112**) from the liverwort *Radula javanica*[96]. Interestingly *R. javanica* biosynthesizes both enantiomeric forms of these cyclopropanochroman derivatives, radulanins J (**111**) and K (**112**) have the opposite Cotton effect and rotation to radulanin I (**110**).

Bisbibenzyls are two bibenzyl units joined by ether and biphenyl linkages. The various types of bisbibenzyls differ in the nature of linkages and their positions of attachment to the bibenzyl unit[95]. The first example of a bisbibenzyl was marchantin A (**113**) from the liverworts *Marchantia polymorpha, M. paleacea var. diptera* and *M. tosana*[6,97]. It has since proved to be an important constituent of *Marchantia* species[95]. Marchantin A (**113**) and many of the other bisbibenzyls show a wide range of biological activity[98]. Thus far the bisbibenzyl structure is known only in liverworts.

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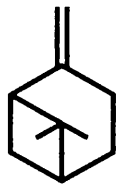
CHAPTER 2

HERBERTUS ADUNCUS AND H. BOREALIS

INTRODUCTION

Herbertus aduncus (Herberta adunca) belongs to the family Herbertaceae of the order Jungermanniales. The genus *Herbertus* was established in 1821 by S.F. Gray[1] and has also been called *"Herberta"*. The original name *"Herbertus"* is retained here as in Smith [2]. Based on their morphology the Herbertaceae are considered to be a primitive family in the Jungermanniales[3]. As well as *H. aduncus* two other species, *H. borealis* and *H. stramineus* grow in Scotland[2]. These Scottish species have not been chemically investigated. However many liverwort species have worldwide distributions and may occur in more than one continent. *H. aduncus* is also found in Japan and Canada and there have been publications dealing with this species from these two countries (Japan[3-9], Canada[6]). These investigations were carried out by the Japanese research groups of Asakawa[3,4,6] and Matsuo[5,7-9] and resulted in the isolation of the following terpenoid constituents : ß-pinene (**1**)[6], ß-selinene (**2**)[4], calamenene (**3**)[4], γ-muurolene (**4**)[3], *ent*-cuparene (**5**)[3,4,6], *ent*-2-hydroxycuparene (≡ δ-cuparenol ≡ herbertusone) (**6**)[3,4,6], 1-hydroxycuparene (iso-δ-cuparenol) (**7**)[3], *ent*-1,2-dihydroxycuparene (**8**)[6], *ent*-α-cuparenone (**9**)[6], (-)-herbertene (**10**)[5,6,9], (-)-α-herbertenol (**11**)[6,7,9], (-)-α-formylherbertenol (**12**)[7,9], (-)-ß-herbertenol (**13**)[6,7,9], (-)-herbertenediol (**14**)[8,9] and (-)-herbertenolide (**15**)[8,9]. These herbertane (isocuparane) and *ent*-cuparane sesquiterpenoids have the same absolute configuration[6,9] and they provide a further significant example of the *ent*-stereospecificity of the biosynthesis of many of the liverwort sesquiterpenoids. Herbertane and *ent*-cuparane sesquiterpenoids have also been found in other *Herbertus* species (*H. sakuraii*[6,10], *H. subdentatus*[6,11,12], *H. acanthelius*[12]). In general the major constituents of *Herbertus* species are herbertane sesquiterpenoids and these are regarded as important chemical markers in the family Herbertaceae[6,13].



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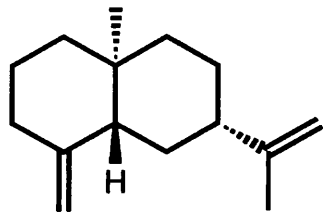

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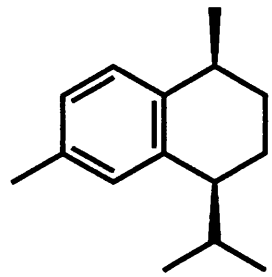

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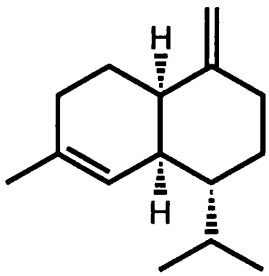

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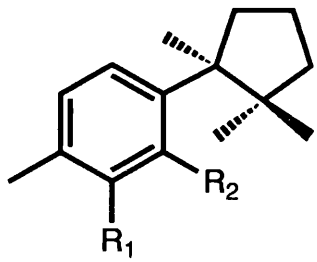
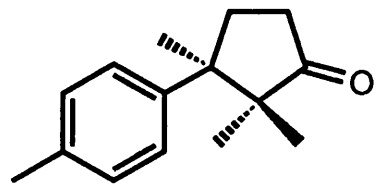


(5) $R_1 = R_2 = H$
(6) $R_1 = OH$, $R_2 = H$
(7) $R_1 = H$, $R_2 = OH$
(8) $R_1 = R_2 = OH$



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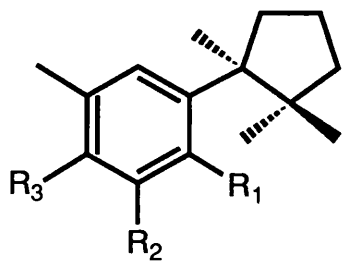
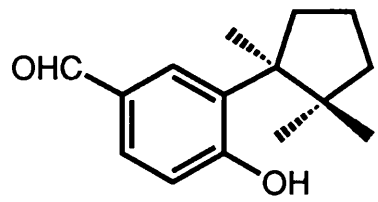


(10) $R_1 = R_2 = R_3 = H$
(11) $R_1 = OH$, $R_2 = R_3 = H$
(13) $R_1 = R_2 = H$, $R_3 = OH$
(14) $R_1 = R_2 = OH$, $R_3 = H$



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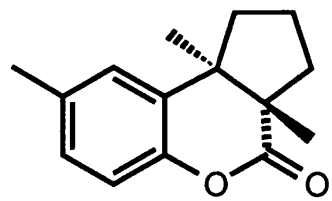


(15)

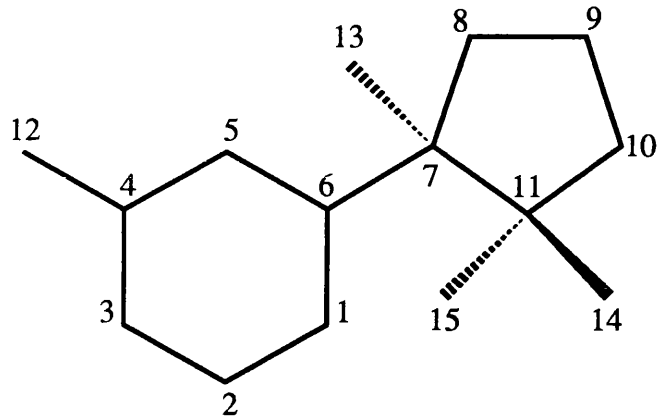
In each case the main component was found to be α-herbertenol (**11**). No *ent*-cuparane type sesquiterpenoids were detected in the Ecuadorian liverworts *H. subdentatus* and *H. acanthelius* and only (-)-α-herbertenol (**11**) was isolated from the latter. Chemical evidence therefore shows that the Herbertaceae elaborate a narrow range of terpene skeletons; this behaviour can be regarded as a primitive character[14] and so supports the morphological data.

The methanolic extract of *H. aduncus* has antifungal activity which has been shown[6] to be due to the presence of the sesquiterpenoid phenols, **11-13**.

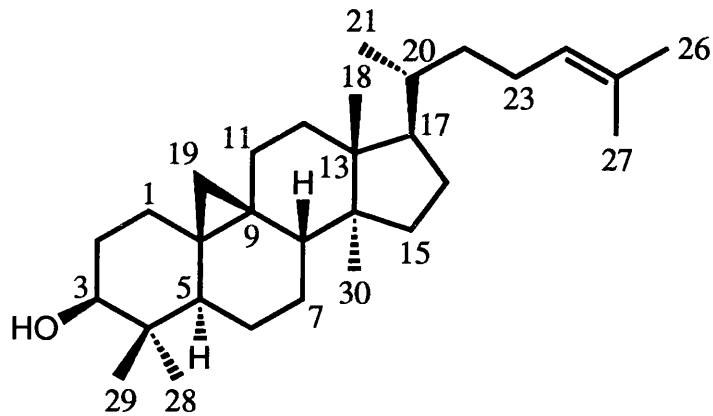
In the previous studies of *H. aduncus* there was no comment on the lipid or di- and triterpenoid content of this liverwort. Fatty acids normally occur in all plants as free acids, polar lipids, neutral lipids (monoglycerides, diglycerides, triglycerides) and other neutral lipids including steryl and wax esters. The occurrence of fatty acids and their derivatives in liverworts has been reviewed by Asakawa (1982)[15] and Karunen (1990)[16].

DISCUSSION

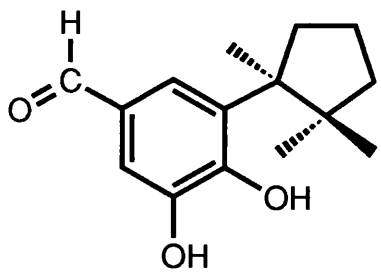
Herbertus aduncus ssp. hutchinsiae was collected at various locations in Scotland and proved to be a rich source of herbertane sesquiterpenoids (Scheme 1). Other major constituents of this liverwort were found to be triglycerides (triacylglycerol) and a mixture of fatty acid esters of the triterpenoid cycloartenol (**16**). The chemical constitutions of four samples of *H. aduncus* obtained from different locations, and one sample of *H. borealis* were compared.



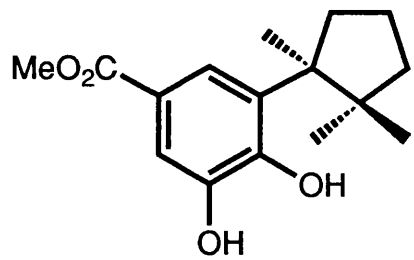
Scheme 1



(16)



(17)



(18)

From a sample of *H. aduncus* collected at Loch Creran in the West of Scotland in September 1991 the following known herbertanes were isolated : (-)-herbertene (**10**), (-)-α-herbertenol (**11**), ß-herbertenol (**13**) and (-)-herbertenediol (**14**). These were readily identified by comparison of their spectroscopic properties with published data[9]. The ^{13}C NMR data for compounds **13** and **14** have not previously been reported and are given in the Experimental. In addition two new herbertanes were isolated, (-)-1,2-dihydroxy-12-oxoherbertene (**17**) and methyl 1,2-dihydroxy-12-herbertenoate (**18**); the former compound is a major component of the extract.

The new herbertane aldehyde **17** is a crystalline compound and has the molecular formula $C_{15}H_{20}O_3$ ($[M]^+$ at m/z 248.1407) as determined by HRMS. Its IR spectrum reveals the presence of hydroxyl groups (ν_{max} 3670, 3590, 3500, 3350, 3200 cm^{-1}) and a carbonyl group (ν_{max} 1670 cm^{-1}). The ^1H NMR spectrum (Figure 1) (see Experimental) has resonances for three tertiary methyls (δ_H 1.44, 1.19, 0.74), two aromatic protons which are *meta*-related [δ_H 7.40 (d, J = 1.9 Hz); 7.24 (d, J = 1.9 Hz)] and an aldehyde proton [δ_H 9.69 (s)]. The ^{13}C NMR spectrum (Figure 2) (see Experimental) has resonances for fifteen carbons and confirmed the presence of an aldehyde [δ_C 193.0 (d)] and a tetrasubstituted benzene ring [δ_C 151.6, 144.4, 133.4, 127.4 (all s); 127.9, 110.7 (both d)]. It contains further signals for three methyls, three methylenes and two quaternary sp^3 carbons. These data reveal that this compound is an aromatic bicyclic sesquiterpenoid containing a 1,2,3,5-tetrasubstituted benzene nucleus with an aldehyde group, two hydroxyls and a cyclopentane ring bearing three tertiary methyls. These data indicate a cuparane or herbertane type of skeleton. Signal

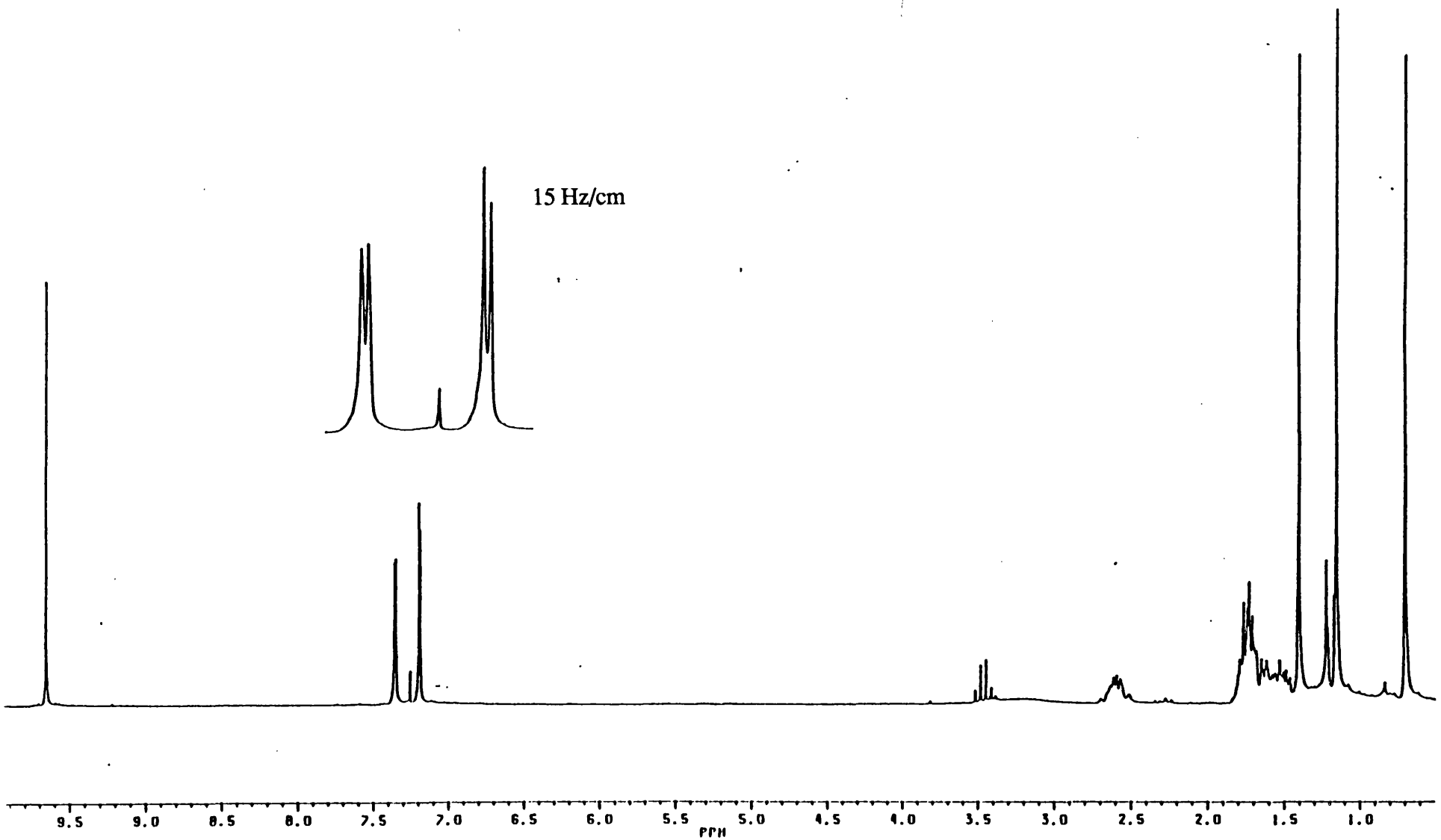


Figure 1. ^{1}H NMR spectrum of compound **17.**

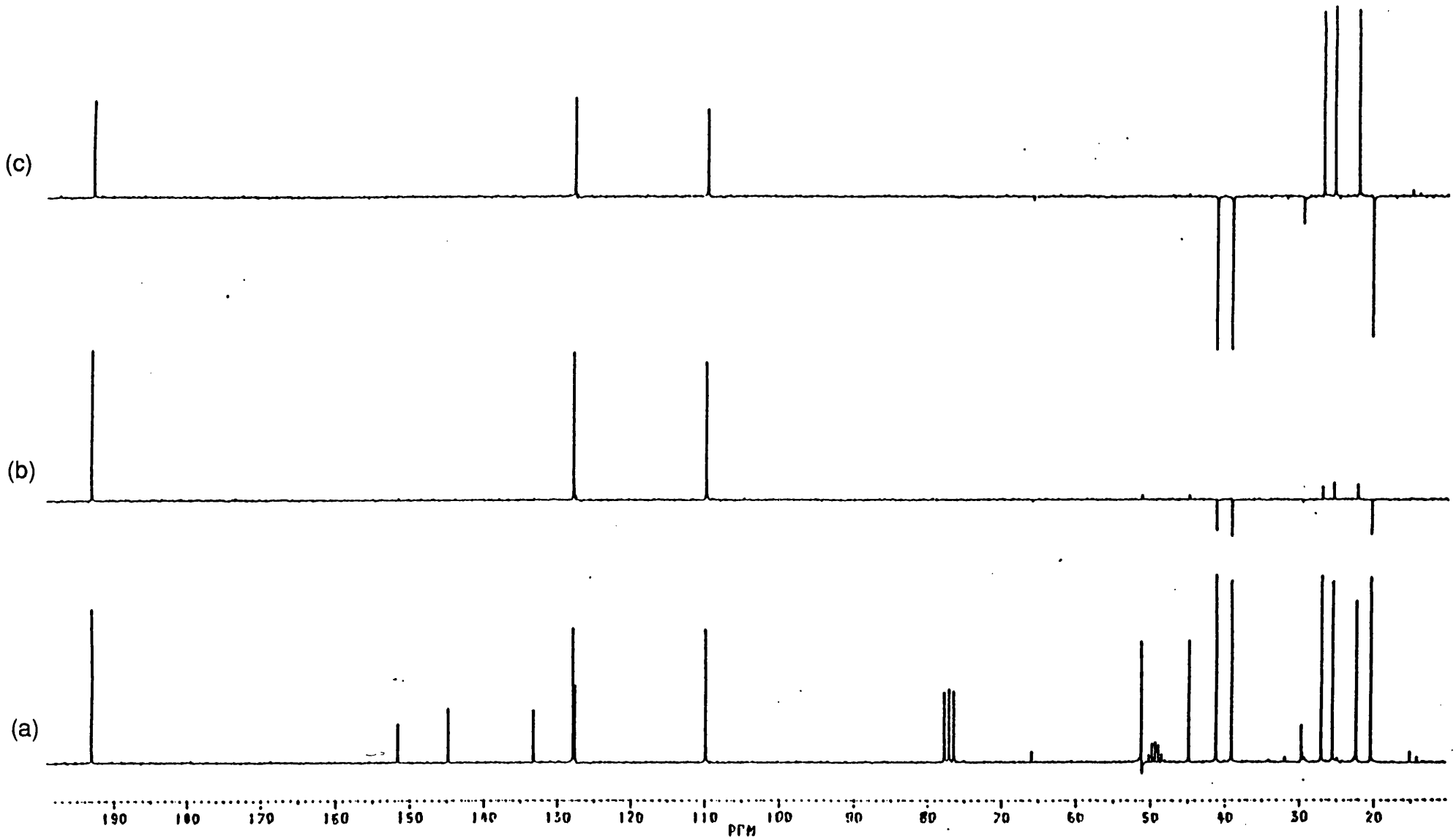


Figure 2. Composite pulse decoupled (CPD) and DEPT spectra of compound **17.**
(a) CPD; (b) 90° DEPT (CH signals only); (c) 135° DEPT (CH_3 and CH signals positive, CH_2 signals negative).

assignments and the position of attachment of the aromatic substituents were established as follows.

One of the tertiary methyl groups has an unusually shielded resonance (δ_H 0.74) and molecular models indicate that this will be 3H-13 as it lies in a shielding zone of the benzene ring. The NOEs observed between 3H-13 (0.7%) and 3H-15 (0.7%) indicate that the resonance at δ_H 1.19 (s) is 3H-15 and therefore the remaining tertiary methyl signal at δ_H 1.44 must be 3H-14. This signal is notably deshielded since the 3H-14 methyl group is *cis* to the aromatic ring and lies in its deshielding zone. A similar situation pertains for H-8α which resonates at δ_H 2.66 (m), further downfield than any of the other methylene protons. The α-configuration of this proton is confirmed by NOEs observed between 3H-13 (0.9%) and H-8α (2.8%). One of the aromatic protons (δ_H 7.40) received NOEs from 3H-14 (1.8%), 3H-13 (1.5%), H-8α (11.5%) and the aldehyde proton (5.5%) indicating that it must be *ortho*-related to the cyclopentane ring and the aldehyde proton. Since the aromatic protons are *meta*-related the above information leads to a 1,2-dihydroxyherbertene skeleton in which the aromatic methyl group has been oxidised to an aldehyde.

Support for this structure comes from the deshielded nature of the aromatic protons in compound **17** [δ_H 7.40 (H-5), 7.24 (H-3)] as compared with those of (-)-herbertenediol (**14**) (δ_H 6.75, 6.59). This is due to deshielding by the aldehyde group. The NOE observed at H-5 (5.5%) on irradiation of the aldehyde proton suggests that the aldehyde group is mainly in the orientation indicated in structure **17** (no NOE was observed at H-3). From the NOE difference experiments we can identify the approximate chemical shifts of 10-Hß, 9-Hß, 8-Hß (1.76 ppm),

10-Hα (1.69 ppm) and 9-Hα (1.55 ppm) in the five-proton multiplet (δ_H 1.45-1.90). The chemical shifts of both aromatic protons, the aldehyde proton and both hydroxyl protons varied depending on the concentration and solvent system used.

In the preferred conformation of compound **17** the benzene ring adopts a sterically more preferable pseudoequatorial orientation and both H-8α and 3H-14 are close to the plane of the benzene ring. There is rotation about the C-6, C-7 bond as indicated by the significant NOEs at H-5 from 3H-13 (1.5%), 3H-14 (1.8%) and H-8α (11.5%). The specific rotation of **17**, $[\alpha]_D$ = -11°, has the same sign as the known herbertanes and it is assumed to belong to the same absolute stereochemical series. Herbertanes generally have small negative rotations[9].

The other new herbertane (**18**) is less polar than **17** and has the molecular formula $C_{16}H_{22}O_4$ ($[M]^+$ at m/z 278.1512). Its IR spectrum shows the presence of hydroxyl groups (ν_{max} 3680, 3600, 3510, 3360, 3200 cm^{-1}) and a carbonyl group (ν_{max} 1690 cm^{-1}). The ^1H and ^{13}C NMR spectra (see Experimental) are very similar to those of **17** except for the presence of a carbomethoxyl group [δ_H 3.87 (s, 3H); δ_C 167.9 (s), 52.1 (q)] and the absence of an aldehyde group. These data indicate that compound **18** is the methyl ester of the acid which presumably arises by oxidation of the aldehyde (**17**).

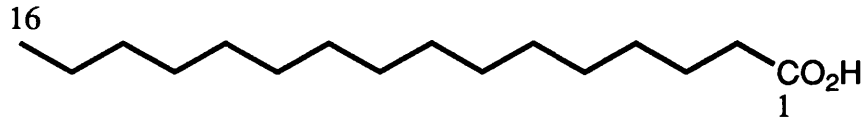
The two new herbertanes **17** and **18** and herbertenediol (**14**) are unstable to preparative TLC on silica gel and may dimerise or oligomerise *via* phenoxy radicals. Herbertane dimers have been reported from liverworts which produce herbertane sesquiterpenoids[17,18].

The composition of the early fractions containing the least polar components of the extract was determined by GLC, using comparison with *n*-alkane standards. This

established the presence of four major sesquiterpene hydrocarbons (several minor mono- and sesquiterpene hydrocarbons were also detected), one of which was identified as herbertene (**10**)[9] from the ^{1}H NMR of these fractions. However, due to lack of appropriate sesquiterpene standards the others could not be identified. Some homologous series of alkanes were also identified, the most predominant being the odd carbon *n*-alkanes, *n*-C_{23}-C_{33} . Two less abundant series, normal and branched, of even carbon alkanes with the same carbon number, C_{22}-C_{32} , were also present. Thus the odd-carbon-numbered *n*-alkanes predominate in agreement with previous reports[15]. This predominance of odd over even *n*-alkanes is also found in higher plants[15].

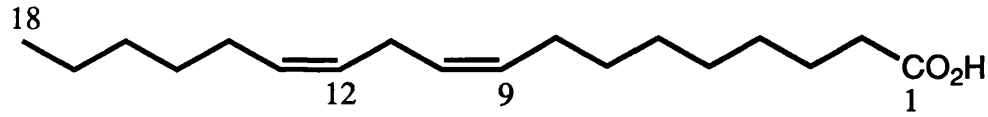
A fatty acid triterpenoid ester mixture was also isolated from the extract. The triterpenoid moiety was easily identified as cycloartenol (**16**) by comparison of its spectroscopic properties with literature values[19] and the free alcohol was itself isolated from a later fraction. In the ester mixture the resonance for H-3 appears at δ_H 4.56 (br dd, J = 10.4, 4.8 Hz) as expected.

In order to analyse the fatty acid components of the esters, the ester mixture was separated by repeated preparative TLC over silica gel (CH_2Cl_2 - hexane) to give four samples of cycloartenol esters with the compositions : **24a**; **24a, 23a**; **23a, 22a, 21a**; **21a, 20a, 19a**. These samples are given in order of increasing polarity. Only one ester (**24a**), the least polar, was isolated in a pure state. By extensive analysis of the ^{13}C NMR spectra (Table 1) of these four samples along with that of cycloartenol (**16**) (see Experimental) and comparison with literature data for fatty acids[20-23] four of the fatty acid constituents were established as 9Z,12Z-octadeca-9,12-dienoic acid (linoleic acid) (**20**)[20]; 9Z,12Z,15Z-octadeca-9,12,15-trienoic acid (α-linolenic acid) (**21**)[20]; 5Z,8Z,11Z,14Z-eicosa-5,8,11,14-tetraenoic acid (arachidonic acid) (**23**) and



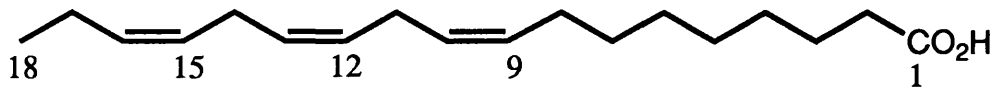
(19)

(19a) Cycloartenol ester of 19



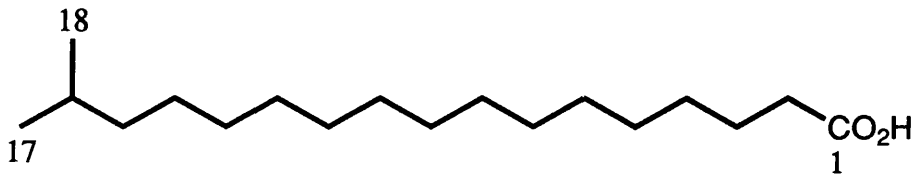
(20)

(20a) Cycloartenol ester of 20



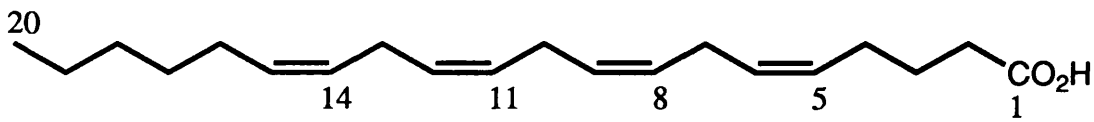
(21)

(21a) Cycloartenol ester of 21



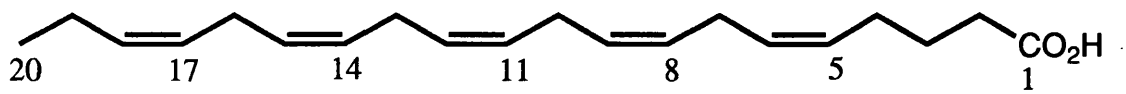
(22)

(22a) Cycloartenol ester of 22



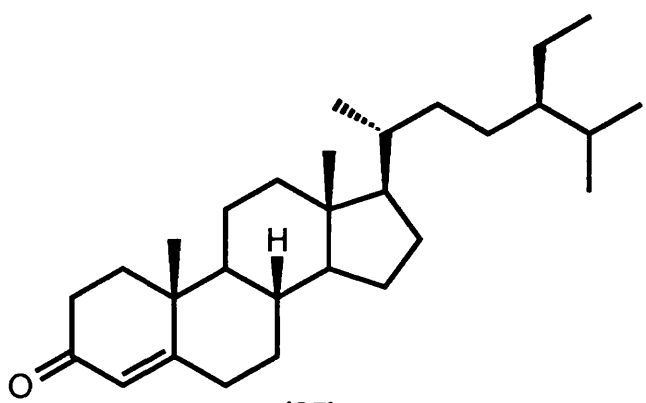
(23)

(23a) Cycloartenol ester of 23



(24)

(24a) Cycloartenol ester of 24



(25)

Table 1. ^{13}C NMR data of fatty acid component of ester mixture.

C	20a	21a	23a	24a
1	173.6 s	173.6 s	173.4 s	173.4 s
2	34.8 t	34.8 t	34.2 t	34.2 t
3	25.1 t	25.1 t	25.0 t	25.0 t
4	29.2 t	29.1 t	26.6 t	26.6 t
5	29.2 t	29.1 t	129.1 d	128.7 d
6	29.2 t	29.1 t	128.6 d	128.5 d
7	29.3 t	29.6 t	25.5 t	25.5 t
8	27.2 t	27.2 t	127.9 d	127.9 d
9	130.1 d	130.3 d	128.7 d	129.1 d
10	128.0 d	127.7 d	25.6 t	25.6 t
11	25.6 d	25.6 t	128.2 d	128.2 d
12	127.9 d	128.3 d	128.2 d	128.2 d
13	130.2 d	128.3 d	25.6 t	25.6 t
14	27.2 t	25.6 t	127.5 d	128.1 d
15	29.6 t	127.1 d	130.5 d	128.1 d
16	31.5 t	131.9 d	27.2 t	25.6 t
17	22.6 t	20.5 t	29.3 t	127.0 d
18	14.1 q	14.3 q	31.5 t	132.0 d
19			22.6 t	20.6 t
20			14.1 q	14.3 q

5Z,8Z,11Z,14Z,17Z-eicosa-5,8,11,14,17-pentaenoic acid (**24**).

In order to confirm the presence of the above fatty acids and identify more of the fatty acids in the ester mixture a sample of the ester mixture was subjected to methanolysis in methanolic hydrochloric acid to yield a mixture of fatty acid methyl esters and triterpene alcohols which were subsequently separated by preparative TLC. From ^{1}H and ^{13}C NMR analysis of the triterpene alcohol mixture the main component was identified as 25-methoxycycloartanol by comparison with published data for cycloartenol (**16**)[19]. This compound is an artifact of the reaction conditions and is possibly a new compound. GLC and GCMS experiments on the fatty acid methyl esters established the presence of six major constituents. Identification was achieved by comparison of the retention times and mass spectral fragmentation patterns with those of authentic specimens. The results are shown in Table 2, with their relative proportions as a percentage of the ester mixture as calculated from the peak area of the GLC.

Table 2. Fatty acid composition of cycloartenol esters.

Fatty acid	**T_R of methyl ester (mins)**	**% Composition**
19	11.64	9
20	15.45	21
21	15.55	33
22	16.57	5
23	20.00	15
24	20.80	7
unidentified	14.87 (others < 1%)	10

These GLC and GCMS data confirmed the presence of the four unsaturated fatty acids (**20, 21, 23** and **24**) previously identified from the ^{13}C NMR spectra of the cycloartenol esters and identified two further fatty acids, hexadecanoic acid (palmitic acid) (**19**) and 16-methylheptadecanoic acid (isostearic acid) (**22**).

One of the fractions obtained from silica gel column chromatography was found to contain triglycerides. The ^{13}C NMR analysis of the triglycerides (see Experimental) suggests that the main fatty acid attached to glycerol is α-linolenic acid (**21**). This was indicated by six olefinic resonances [δ_C 126.9 (d, C-15); 127.5 (d, C-10); 128.1 (d, C-13); 128.2 (d, C-12); 130.1 (d, C-9); 131.8 (d, C-16)] which are characteristic for α-linolenic acid (**21**). These signals were the predominant olefinic resonances in the ^{13}C NMR spectrum of the triglycerides. GLC analysis of the fatty acid methyl esters obtained by methanolysis of the triglycerides confirms the ^{13}C NMR analysis, revealing that α-linolenic acid (**21**) constitutes approximately 61% of the fatty acids present in the triglycerides. The GLC analysis also revealed the presence of small amounts of other fatty acid methyl esters, including the five others of the cycloartenol ester mixture. Both triglycerides and cycloartenol esters are methods of storing fatty acids. Such lipids are mainly kept in globules as energy stores[16].

Also isolated from this liverwort extract was the known steroid sitost-4-en-3-one(≡ ß-sitostenone ≡ stigmast-4-en-3-one) (**25**)[24,25] which was identified by comparison of its ^{1}H NMR data with published data[25] and its ^{13}C NMR data with literature data for stigmastanol[26]. The ^{13}C NMR data for ß-sitostenone (**25**) have not previously been reported and are listed in the Experimental. Sterols are components of nearly all living organisms. Campesterol, stigmasterol and

sitosterol are found in many liverworts[15] and, together with 24-methylcholest-5,22-dien-3ß-ol, have been detected in *H. aduncus*[3,4].

This liverwort (*H. aduncus*) was collected at three other sites in Scotland: Glen Ure, Glen Kinglas and Loch Lomond. The ^{1}H NMR spectra of the crude extracts were compared (Figure 3) with that of the original Loch Creran sample. The main components of each extract are 1,2-dihydroxy-12-oxoherbertene (**17**), α-herbertenol (**11**), cycloartanes and triglycerides. Their yields are shown in Table 3 (see Experimental for calculations, pp. 51-53). From this analysis we can

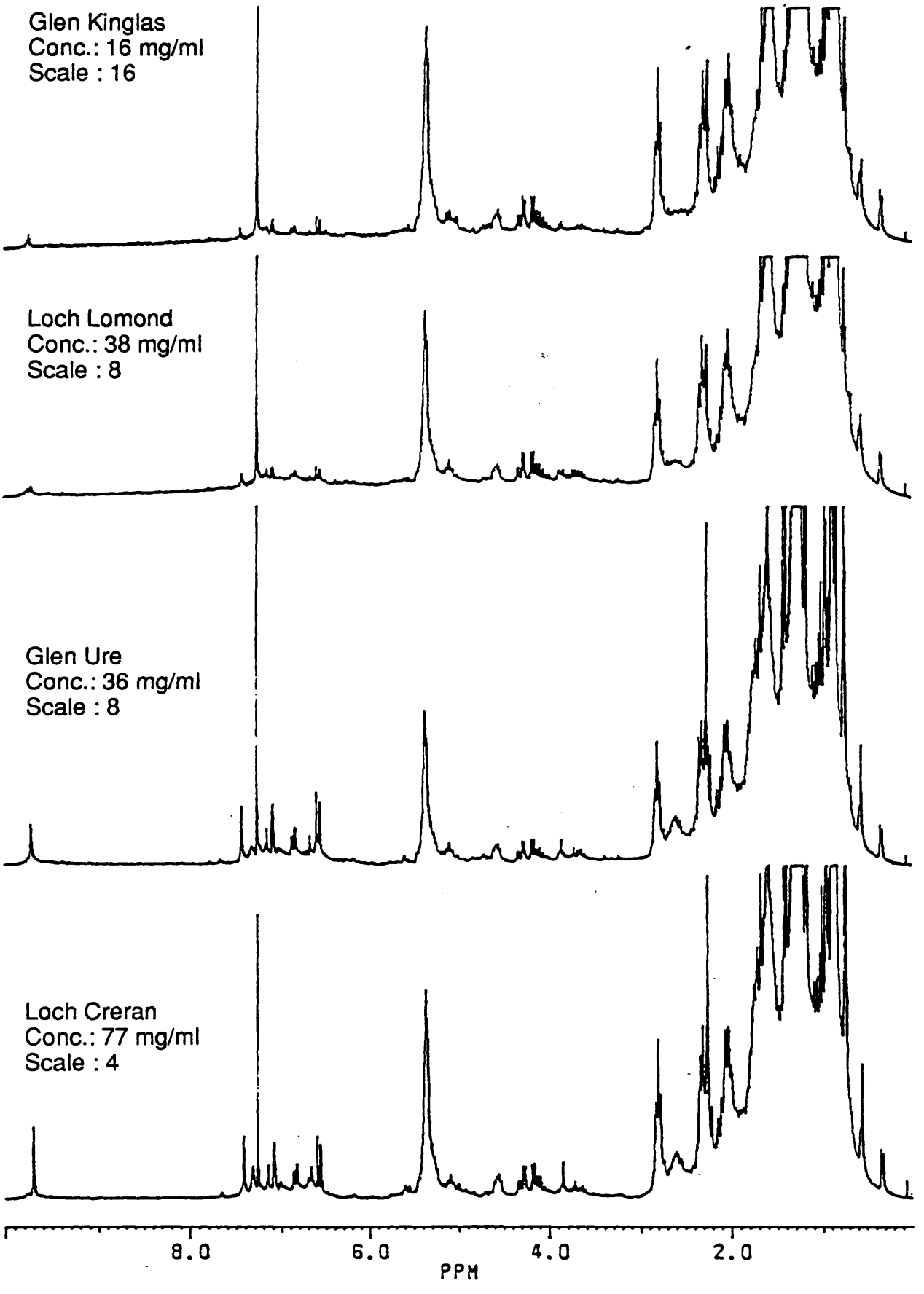
Table 3. Percentage yields of four of the major constituents in *H. aduncus* extracts*.

Collection Site	17	11	Cycloartanes	Triglycerides
Loch Creran	7.1 (10)	7.1 (10)	7.1 (10)	3.6 (5)
Glen Ure	5.7 (8)	8.6 (12)	5.7 (8)	2.8 (4)
Loch Lomond	2.8 (4)	2.8 (4)	5.7 (8)	2.8 (4)
Glen Kinglas	3.6 (5)	3.6 (5)	7.1 (10)	3.6 (5)

* the relative proportions are given in parentheses.

conclude that within the Loch Creran and Glen Ure extracts there is a larger yield of the herbertanes relative to the cycloartanes and triglycerides while in the Glen Kinglas and Loch Lomond samples the reverse is true. Furthermore the Loch Creran sample has the largest content of 1,2-dihydroxy-12-oxoherbertene (**17**), about twice as much as the Loch Lomond and Glen Kinglas extracts. The Glen Ure sample has the largest α-herbertenol (**11**) content, about three times as much

Figure 3. ^{1}H NMR spectra of *Herbertus aduncus* crude extracts collected at four different sites.



as the Loch Lomond and Glen Kinglas extracts. There are several possible explanations for the variation in terpenoid and lipid content, including location and climate.

A second species, *Herbertus borealis*[2,27], is very rare. Its only known British location is on Beinn Eighe in North West Scotland (this is in fact virtually the entire world population since it is otherwise known from only three small sites in South West Norway). Within the Beinn Eighe plant community it can be locally dominant. *H. borealis* was described as a new species in 1970 (Crundwell)[27] having previously been confused with *H. aduncus*. The Beinn Eighe mountain range lies in a National Nature Reserve and the sample of *H. borealis* used in the present work was collected by arrangement with Scottish National Heritage.

Initial analysis of the *H. borealis* crude extract by TLC, ^{1}H NMR and GLC suggested that herbertanes and lipids were present. As soon as the ground liverwort was extracted with Et_2O problems arose due to polymerisation of unsaturated fats and to facilitate analysis the extract was subjected to vacuum distillation to separate the more volatile terpenoid fraction. The composition of this terpenoid fraction was investigated using GLC methods and comparison with authentic specimens. The mixture was found to consist of mainly two sesquiterpenes, herbertene (**10**) and α-herbertenol (**11**), the latter being present in greater amount (3:4 from GLC peak areas). This was confirmed from the ^{1}H NMR spectrum of the terpenoid mixture which contained readily identifiable signals for both **10** and **11**. The conditions of collection imposed by Scottish National Heritage precluded a second investigation.

In conclusion we can say that the Scottish collections of *H. aduncus* and *H. borealis* also contain herbertane sesquiterpenoids, as in other *Herbertus* species[3-12]. The

present work adds support to the statement[6] that herbertane derivatives are major constituents of the Herbertaceae and can be used as chemical markers of the family.

It is noteworthy that no *ent*-cuparane sesquiterpenoids were identified in Scottish *Herbertus* species and the Matsuo group has not reported any *ent*-cuparanes in their studies[5,7-9] of Japanese collections of *Herbertus* species. The present work confirms that α-herbertenol (**11**) is the most abundant herbertane in *Herbertus* species (see p.28).

GENERAL EXPERIMENTAL

Melting points (m.p.) were determined on a Kofler hot-stage apparatus and are uncorrected. Infra-red (IR) spectra were recorded in CCl_4 solution (unless otherwise stated) on either a Perkin Elmer 580 or Philips 9800 FTIR spectrometer. Ultra-violet (UV) spectra were measured for Et_2O solutions (unless otherwise stated) using a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrometer. Low resolution mass spectra were determined using a VG updated MS12 spectrometer while high resolution mass spectra were determined on a modified Kratos MS9 instrument. Optical rotations were measured on an optical activity AA-100 polarimeter in $CHCl_3$ solution at 20°C.

Gas-liquid chromatographic (GLC) separations were achieved with an Hewlett-Packard 5880A instrument equipped with a CP Sil 5 CB (chrompack) fused silica capillary column (25 m x 0.32 mm I.D. x 0.12 μm) and flame ionization detector (FID). The Grob-type injector was operated in the split mode (50:1) and the helium carrier and make up gas flow rate was 2 ml/min. A linear temperature programme was used in which the column temperature was programmed from 80°C (held at this temperature for 2 minutes) to 240°C (5 mins) at 5°C/min. The injection port and detector temperature were 255°C and 260°C respectively. Exceptions were for the analyses of the monoterpenoids in *Jungermannia obovata* in which two temperature programmes were used; the column temperature was programmed from 50°C (2 mins) to 200°C at 2°C/min and then from 200°C (5 mins) to 250°C (1 min) at 3°C/min. In this case the injector port and detector temperature were both 57°C. Two temperature programmes were also used for the analysis of the non-polar fractions in *Herbertus aduncus*; the

column temperature was programmed from 80°C (2 mins) to 130°C at 2°C/min and then from 130°C (1 min) to 290°C (20 mins) at 10°C/min. The injector port and detector temperatures were both 284°C.

GLC-mass spectrometry (GCMS) analyses were carried out with a Hewlett-Packard 5971 mass selective detector interfaced to a 5890 Series II gas-liquid chromatograph and computer (Vectra QS/16S). Separations were effected with a HPl fused silica capillary column (12.5 m x 0.2 mm I.D. x 0.33 μm). Injection and temperature programming conditions were identical to those for GLC above. Retention times (T_R) from the total ion current (TIC) traces practically match those of the FID chromatograms. All reported retention times (T_R) are taken from the FID chromatograms. Mass spectra (70 ev) were recorded in the continuous scanning mode. GCMS analyses of the monoterpenoids in *J. obovata* were carried out with an AEI MS 30 instrument interfaced to a PE Sigma 3 gas-liquid chromatograph. Separations were carried out with a 25 m BP1 column using a temperature programme of 50°C (3 mins) to 260°C at 5°C/min.

Unless otherwise stated, nuclear magnetic resonance (NMR) spectra were recorded at 298 K and at 4.7 T on Bruker WP200SY and AM200SY spectrometers (^{1}H, 200.132 MHz; ^{13}C, 50.32 MHz). Higher field NMR spectra were recorded using either a Bruker AC300 instrument (^{1}H, 300.13 MHz; ^{13}C, 75.47 MHz) at 7.05 T or with a Varian VXR600S spectrometer (^{1}H, 600 MHz; ^{13}C, 150 MHz) at 14.1 T. Spectra were recorded for $CDCl_3$ solutions (unless otherwise specified) relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0 and chemical shifts are reported in ppm. Occasionally TMS was used as internal standard at δ 0. Tabulated ^{1}H NMR data have coupling constants (*J*) in Hz given in parenthesis. Signals indicated as 'm' were unresolved or

overlapped multiplets. ^{1}H and ^{13}C NMR assignments are based in general on chemical shift rules and comparison with published data for similar compounds. More definitive ^{1}H NMR assignments were by homonuclear decoupling and NOE difference experiments which are discussed when used. ^{13}C NMR multiplicities were obtained from DEPT [90° DEPT (CH signals only); 135° DEPT (CH_3 and CH signals positive, CH_2 signals negative)] or *J*-modulated ^{13}C (CH and CH_3 signals positive, CH_2 and C signals negative) spectra. The ^{1}H and ^{13}C signals of some compounds were confirmed by 2D δ_C /δ_H and δ_H /δ_H correlation experiments and these experiments are specified when used. ^{1}H or ^{13}C signals with similar chemical shifts and the same multiplicity are therefore interchangeable unless otherwise confirmed by 2D δ_C /δ_H and δ_H /δ_H correlation experiments. Homonuclear proton NOE difference experiments were performed by selectively irradiating a single line of a multiplet for 3s and using a 90° observation pulse to minimise selective population transfer (SPT) effects. The NOEs reported are given as percentage enhancement and are the results of scaling up the observed NOEs in inverse proportion to the degree of saturation in order to obtain values equivalent to the result of complete saturation. These NOEs have an accuracy of approximately ± 20%. All 2D δ_C /δ_H correlation experiments[28,29] used polarization transfer [1/2*J* (^{13}C-^{1}H)] and refocussing [1/4*J* (^{13}C-^{1}H)] delay times altered in order to maximise polarization transfer and enhance correlations for direct and long-range coupling constants. For the experiments involved here delays were chosen for direct and long-range couplings equal to 147 Hz and 10 Hz respectively and heteronuclear couplings are absent from both frequency domains. Homonuclear proton couplings (except geminal couplings) are removed from F_1 in the 2D direct δ_C /δ_H correlation experiments by having a BIRD (Bilinear Rotation Decoupling) pulse

sequence in the centre of the evolution period[29]. The one exception to this is the 2D direct δ_C /δ_H correlation of compound **15** from *Anastrophyllum donnianum* where ^{1}H-^{1}H couplings are still present in F_1[28]. The 2D long-range δ_C /δ_H correlation experiments (the pulse sequence used[30] is essentially the same as sequence D reported by Krishnamurthy and Casida[31]) contained both TANGO (Testing for Adjacent Nuclei with a Gyration Operator) and BIRD pulse sandwiches within the pulse sequence. In general both of these pulses discriminate between a proton that is directly bound to a ^{13}C and a distant (or long-range coupled) proton on the basis of the large difference in the size between the one-bond and long-range coupling constants. In the 2D long-range δ_C /δ_H correlation experiments the effect of the TANGO pulse sequence is to reduce the intensity of correlations from protons attached directly to ^{13}C. The effect of the BIRD pulse sequence is to suppress the modulation of the long-range response by the one-bond couplings (the BIRD pulse also achieves suppression of direct bond correlations). The pulse sequence used is the basic CH correlation sequence[28], with delays lengthened to facilitate long-range correlations, and modified by TANGO and BIRD pulses. The HMQC (^{1}H detected heteronuclear Multiple-Quantum Connectivity)[32] and HMBC (Heteronuclear Multiple-Bond Connectivity)[33] experiments were performed on a Bruker AC300 spectrometer (^{1}H, 300.13 MHz; ^{13}C, 75.47 MHz) and are the inverse of the conventional chemical shift correlation experiments. In these techniques the ^{1}H-^{13}C couplings are observed from the side of the much more sensitive proton which is detected during acquisition rather than the low γ ^{13}C nucleus and this greatly enhances the sensitivity of the NMR experiments. The pulse scheme for HMQC contains a BIRD pulse in order to remove large signals caused by protons not directly attached to ^{13}C. Direct and long-range coupling constants have been

chosen to equal 135.1 Hz and 7.1 Hz for HMQC and HMBC experiments. The 2D δ_H /δ_H correlation experiments were performed on a Varian VXR600S spectrometer (600 MHz) and obtained by phase-sensitive double quantum filtered COSY (DQF-COSY)[34].

The solvents used were either of analytical grade or bulk solvents distilled before use. Unless otherwise indicated, all plant material soon after collection was air-dried, ground mechanically and extracted with either MeOH or Et_2O at room temperature. After the solvent was removed these extracts were stored in a fridge if not investigated immediately. The crude extracts were analysed by TLC and ^1H NMR. Extracts were then fractionated by column chromatography (CC) over silica gel (Merck kieselgel GF_{254}). Eluents for silica gel CC were increasing percentages of Et_2O in petroleum ether followed by MeOH in Et_2O and finally MeOH. Each of the crude fractions was further purified by either preparative thin-layer chromatography (PLC) over silica gel [Merck kieselgel GF_{254} (1 mm thick)] or by CC over Sephadex LH-20 gel. TLC plates were developed using various solvent combinations in appropriate concentrations, the most commonly used solvent systems being ; Et_2O in petroleum ether, Et_2O in CH_2Cl_2 and hexane in CH_2Cl_2. After each chromatographic separation the fractions removed from preparative plates and collected from columns were checked by TLC, ^1H NMR and sometimes ^{13}C NMR for their compositions. The total amount in each fraction was not always further chromatographed and therefore many of the actual yields are much greater than indicated in the experimental section.

All solvents were removed using a Buchi rotary evaporator and water aspirator. Petroleum ether refers to the fraction boiling between 40°C and 60°C. All reagents and solvents used in chemical reactions were purified according to literature methods. Analytical TLC was over Merck precoated silica gel 60 F_{254} (0.25 mm thick).

Preparative and analytical plates were visualized under UV light (254 or 366 nm), by adsorption of I_2 vapour or by spraying with 25% H_2SO_4 and then heating.

British liverwort species were collected by Dr D. S. Rycroft and Professor J. D. Connolly and identified by Dr Rycroft. Reference specimens are retained in the Hepaticae collection in the Department of Chemistry. The Malaysian species, *Jungermannia truncata*, was collected by Professor Connolly and identified by Dr R. Grolle (Jena). Reference specimens are located in Jena and Glasgow.

EXPERIMENTAL

A. *Herbertus aduncus* collected at Loch Creran.

The plant material which was collected at Loch Creran in the West of Scotland in September 1991 was ground mechanically and the powder (660 g) extracted with Et_2O to yield a crude extract (15.5 g). The crude extract was subjected to the usual chromatographic procedure to give the following constituents in order of increasing polarity : (i) a mixture of alkanes and terpene hydrocarbons (700 mg); (ii) (-)-herbertene (**10**) (29 mg); (iii) a cycloartenol ester mixture (2.10 g); (iv) (-)-α-herbertenol (**11**) (1.10 g); (v) triglycerides (1.25 g); (vi) ß-herbertenol (**13**) (152 mg); (vii) (-)-herbertenediol (**14**) (410 mg); (viii) ß-sitostenone (**25**) (4 mg); (ix) cycloartenol (**16**) (74 mg); (x) methyl 1,2-dihydroxy-12-herbertenoate (**18**) (11 mg) and (xi) (-)-1,2-dihydroxy-12-oxoherbertene (**17**) (1.32 g).

(i) *Analysis of non-polar fractions.* GLC and ^{1}H NMR of these fractions established that they were composed of (a) three unidentified sesquiterpene hydrocarbons (as well as many other minor mono- and sesquiterpene hydrocarbons); (b) herbertene (**10**); (c) odd carbon *n*-alkanes, *n*-C_{23}-C_{33} ; (d) even carbon *n*-alkanes, *n*-C_{22}-C_{32} and (e) even carbon branched alkanes, C_{22} -C_{32} .

(ii) *(-)-Herbertene* (**10**) was isolated as an oil. $[\alpha]_D$ -59° (c, 0.17 in $CHCl_3$) [lit.[9] $[\alpha]_D$ -48.3 (c, 1.3 in $CHCl_3$)].

T_R (mins) : 12.37

δ_H (lit.[9]) : 7.18 (m, 3H); 7.01 (m, 1H); 2.53 (m, H-8α); 2.36 (s, 3H-12); 1.5-1.9 (m, 5H); 1.28 (d, *J* = 0.7 Hz, 3H-14); 1.09 (s, 3H-15); 0.58 (s, 3H-13).

δ_C (lit.[9]) : 147.5 (s), 136.7 (s), 127.8 (d), 127.3 (d), 126.0 (d), 124.1 (d), 50.5 (s), 44.2 (s), 39.7 (t), 36.8 (t), 26.5 (q), 24.4 (q), 24.3 (q), 21.8 (q), 19.7 (t).

(iii) *Cycloartenol ester mixture* was isolated as an oil.

(a) Analysis of triterpenoid esters. Repeated preparative TLC (CH_2Cl_2 - hexane) gave the following samples in order of increasing polarity : **24a**; **24a, 23a**; **23a, 22a, 21a**; **21a, 20a, 19a.** The samples were analysed by ^{13}C NMR and four fatty acids, **20, 21, 23** and **24,** were identified. The ^{13}C NMR data of the fatty acid component of the ester are shown in Table 1. One of the esters was isolated pure (**24a**) as indicated by its ^{13}C NMR spectrum. The ^{1}H NMR and ^{13}C NMR

data of **24a** are as follows (these data are almost identical for each ester apart from minor differences attributable to variation in the fatty acid chain).

δ_H : 5.37 (m, 10H, vinyl); 5.09 (br t, J = 7.0 Hz, H-24); 4.56 (br dd, J = 10.4, 4.8 Hz, H-3); 2.82 (m, 8H, doubly allylic); 2.31 (t, J = 7.5 Hz, 2H, α to C=O); 1.67, 1.59 (both s, 3H-26 and 3H-27); 0.96 (t, J = 7.5 Hz, 3H, fatty acid terminal methyl); 0.95 (s, 3H); 0.88 (s, 6H); 0.87 (d, J ~ 6Hz, 3H-21); 0.83 (s, 3H); 0.56 (d, J = 4.0 Hz, H-19); 0.33 (d, J = 4.0 Hz, H-19).

δ_C : 130.9 (s, C-25); 125.2 (d, C-24); 80.4 (d, C-3); 52.2 (d, C-17); 48.8 (s, C-14); 47.9 (d, C-8); 47.1 (d, C-5); 45.2 (s, C-13); 39.5 (s, C-4); 36.3 (t, C-22); 35.9 (d, C-20); 35.5 (t, C-15); 32.8 (t, C-12); 31.6 (t, C-1); 29.8 (t, C-19); 28.1 (t, C-16); 26.8 (t, C-2); 26.5 (t, C-11); 25.9 (s, C-10); 25.8 (t, C-7); 25.7 (q, C-27); 25.4 (q, C-28); 24.9 (t, C-23); 20.9 (t, C-6); 20.1 (s, C-9); 19.3 (q, C-30); 18.2 (q, C-21); 18.0 (q, C-18); 17.6 (q, C-26); 15.2 (q, C-29).

For ^{13}C resonances of fatty acid component see Table 1.

(b) Methanolysis of triterpenoid esters. The triterpenoid ester mixture (*ca* 20 mg) was refluxed with acetyl chloride (1 ml) and MeOH (25 ml + 3 ml Et_2O to solubilise the esters) for five hours. The solvents (MeOH and methyl acetate) were evaporated and the products purified by preparative TLC (Et_2O - pet. ether, 20:80) to give a fatty acid methyl ester mixture and a triterpenoid alcohol mixture.

(c) Analysis of triterpenoid alcohols. Analysis of the ^{1}H and ^{13}C NMR spectra of the mixture revealed the presence of 25-methoxycycloartanol which is by far the main product (*ca* 80%).

δ_H : 3.24 (m, H-3); 3.17 (s, OMe); 1.13 (s, 3H-26 and 3H-27); 0.95 (s, 3H); 0.88 (s, 3H); 0.80 (s, 3H); 0.54 (d, J = 4 Hz, H-19); 0.32 (d, J = 4Hz, H-19).

δ_C : 78.8 (d, C-3); 74.7 (s, C-25); 52.5 (d, C-17); 49.1 (q, OMe); 48.8 (s, C-14); 48.0 (d, C-8); 47.1 (d, C-5); 45.3 (s, C-13); 40.5 (s, C-4); 40.3 (t, C-24); 36.8 (t, C-22); 36.1 (d, C-20); 35.6 (t, C-15); 32.9 (t, C-12); 32.0 (t, C-1); 30.4 (t, C-2); 29.9 (t, C-19); 28.1 (t, C-16); 26.5 (t, C-11); 26.0 (t, C-7); 25.4 (q, C-28); 25.0 (q, C-26 and C-27); 21.1 (t, C-6); 20.6 (t, C-23); 20.0 (s, C-9); 19.3 (q, C-30); 18.3 (q, C-21); 18.0 (q, C-18); 14.0 (q, C-29). The C-10 resonance was obscured.

(d) Analysis of fatty acid methyl esters. The fatty acid methyl ester mixture was analysed by GLC and GCMS and the results are shown in Table 2.

(iv) *(-)-α-Herbertenol* (**11**) was isolated as an oil. $[\alpha]_D$ -52° (c, 0.13 in $CHCl_3$) [lit.[9] $[\alpha]_D$ -55.0° (c, 1.8 in $CHCl_3$)].

T_R (mins) : 17.88

δ_H (lit.[9]) : 7.09 (br d, J = 2.2 Hz, H-5); 6.86 (ddq, J = 8.0, 2.2, 0.7 Hz, H-3); 6.57 (d, J = 8.0 Hz, H-2); 4.63 (s, OH); 2.60 (m, H-8α); 2.26 (s, 3H-12); 1.45-1.85 (m, 5H); 1.41 (d, J = 0.7 Hz, 3H-14); 1.18 (s, 3H-15); 0.76 (s, 3H-13).

δ_C (lit.[9]) : 152.2 (s), 133.0 (s), 130.0 (d), 128.9 (s), 127.2 (d), 116.6 (d), 50.9 (s), 44.6 (s), 41.2 (t), 39.4 (t), 26.9 (q), 25.5 (q), 22.8 (q), 20.8 (q), 20.3 (t).

(v) *Triglycerides were isolated as an oil.*

(a) Analysis of triglycerides.

δ_H : 5.34 (m); 4.28 (dd, J = 12.5, 5.0 Hz); 4.12 (dd, J = 12.5, 6.0 Hz); 2.79 (br t, J = 6.0 Hz); 2.30 (t, J = 7.5 Hz); 2.05 (m); 1.61 (m); 1.26 (m); 0.96 (t, J = 7.5 Hz); 0.87 (m).

^{13}C NMR of glycerol unit : δ_C 62.0 (t, 2C); 68.8 (d, 1C).

^{13}C NMR of first two carbons in fatty acid chains : δ_C 173.1 (s, 1C); 172.7 (s, 2C); 33.9 (t, 1C); 33.8 (t, 2C).

^{13}C NMR signals assigned to the α-linolenic acid (**21**) chain : δ_C 173.1/172.7 (s, C-1); 33.9/33.8 (t, C-2); 24.6 (t, C-3); 28.9 (t, C-4); 28.9 (t, C-5); 28.9 (t, C-6); 29.4 (t, C-7); 27.0 (t, C-8); 130.1 (d, C-9); 127.5 (d, C-10); 25.4 (t, C-11); 128.2 (d, C-12); 128.1 (d, C-13); 25.3 (t, C-14); 126.9 (d, C-15); 131.8 (d, C-16); 20.4 (t, C-17); 14.2 (q, C-18).

(b) Methanolysis of triglycerides. The same procedure as above was employed for the triglycerides (40 mg).

(c) Analysis of fatty acid methyl esters. GLC methods identified the methyl esters of **19**(7%), **20**(11%), **21**(61%), **22**(<1%), **23**(5%) and **24**(3%). Their retention

times practically matched those of the identified methyl esters obtained from methanolysis of the cycloartenol ester mixture (Table 2). The approximate yields were determined from GLC peak areas. The remaining components (14%) belong to unidentified peaks with retention times of 11.11 mins (3%), 15.84 mins (4%) and several others (all < 1%).

(vi) *β-Herbertenol* (**13**) was obtained as a solid.

T_R (mins) : 18.65

δ_H (lit.[9]) : 7.09 (s, H-5); 7.05 (dd, *J* = 8.2, 2.2 Hz, H-2); 6.69 (d, *J* = 8.2 Hz, H-1); 4.82 (br s, OH); 2.46 (m, H-8α); 2.25 (s, 3H-12); 1.45-1.90 (m, 5H); 1.24 (s, 3H-14); 1.05 (s, 3H-15); 0.56 (s, 3H-13).

δ_C : 151.4 (s), 139.7 (s), 129.7 (d), 125.6 (d), 122.3 (s), 113.9 (d), 49.9 (s), 44.1 (s), 39.6 (t), 36.9 (t), 26.4 (q), 24.4 (q), 24.2 (q), 19.6 (t), 16.1 (q).

(vii) *(-)-Herbertenediol* (**14**) was obtained as a semicrystalline solid. $[\alpha]_D$ -45° (c, 0.31 in $CHCl_3$) [lit.[9] $[\alpha]_D$ -46.5° (c, 1.4 in $CHCl_3$)].

T_R (mins) : 22.05

δ_H (lit.[9]) : 6.75 (d, *J* = 1.5 Hz, H-5); 6.59 (d, *J* = 1.5 Hz, H-3); 6.15 (br s, OH); 5.63 (br s, OH); 2.68 (m, H-8α); 2.27 (s, 3H-12); 1.52-1.90 (m, 5H); 1.48 (s, 3H-14); 1.25 (s, 3H-15); 0.83 (s, 3H-13).

δ_C : 143.1 (s), 141.2 (s), 133.4 (s), 128.0 (s), 121.8 (d), 113.5 (d), 51.0 (s), 44.7 (s), 40.9 (t), 39.1 (t), 26.8 (q), 25.4 (q), 22.8 (q), 21.1 (q), 20.2 (t).

(viii) *β-Sitostenone* (**25**) was isolated as a gum.

δ_H (lit.[25]) : 5.72 (s, H-4); 1.17 (s, 3H-18); 0.7-1.0 (m, 3H-21, 3H-26, 3H-27 and 3H-29); 0.70 (s, 3H-19).

δ_C : 199.7 (s, C-3); 171.8 (s, C-5); 123.7 (d, C-4); 56.0 (d, C-17); 55.8 (d, C-14); 53.8 (d, C-9); 45.8 (d, C-24); 42.4 (s, C-13); 39.6 (t, C-12); 38.6 (s, C-10); 36.1 (d, C-20); 35.7 (t, C-1); 35.6 (d, C-8); 34.0 (t, C-22); 33.8 (t, C-2); 32.9 (t, C-6); 32.0 (t, C-7); 29.1 (d, C-25); 28.2 (t, C-16); 26.0 (t, C-23); 24.2 (t, C-15); 23.0 (t, C-28); 21.0 (t, C-11); 19.8 (q, C-27); 19.0 (q, C-26); 18.7 (q, C-21); 17.4 (q, C-19); 11.9 (q, C-18 and C-29).

(ix) *Cycloartenol* (**16**) was isolated as a semicrystalline compound from a sterol-containing fraction.

EIMS m/z (rel.int.): 426 $[M]^+$ (3), 218 (18), 175 (16), 135 (20), 107 (35), 95 (47), 69 (100), 58 (81), 41 (85).

δ_H (lit.[19]) : 5.08 (br t, J = 7.0 Hz, H-24); 3.26 (dd, J = 10.5, 4.8 Hz, H-3); 1.66, 1.58 (both s, 3H-26 and 3H-27); 0.94 (s, 6H); 0.87 (s, 3H); 0.86 (d, $J \sim$ 6Hz, 3H-21); 0.79 (s, 3H); 0.53 (d, J = 4.1 Hz, H-19); 0.31 (d, J = 4.1 Hz, H-19).

δ_C (lit.[19]) : 130.9 (s, C-25); 125.2 (d, C-24); 78.8 (d, C-3); 52.2 (d, C-17); 48.7 (s, C-14); 48.0 (d, C-8); 47.1 (d, C-5); 45.2 (s, C-13); 40.4 (s, C-4); 36.3 (t, C-22); 35.8 (d, C-20); 35.5 (t, C-15); 32.8 (t, C-12); 31.9 (t, C-1); 30.3 (t, C-2); 29.9 (t, C-19); 28.1 (t, C-16); 26.4 (t, C-11); 26.0 (t, C-7); 25.7 (q, C-27); 25.4 (q, C-28); 24.9 (t, C-23); 21.1 (t, C-6);

19.9 (s, C-9); 19.3 (q, C-30); 18.2 (q, C-21); 18.0 (q, C-18); 17.6 (q, C-26); 14.0 (q, C-29). The C-10 resonance was obscured (lit.[19] 25.96 ppm).

(x) *Methyl 1,2-dihydroxy-12-herbertenoate* (**18**) was obtained as a gum.

HRMS : m/z 278.1512 $[M]^+$ calculated for $C_{16}H_{22}O_4$: 278.1518.

T_R (mins) : 29.07

ν_{max} (cm^{-1}) : 3680, 3600, 3510, 3360, 3200 (OH); 3010, 2960, 2915, 2885; 1690 (C=O); 1600, 1505, 1460 (aryl C-C); 1435, 1380, 1330, 1300, 1225, 1105, 1010, 760.

λ_{max} (MeOH)(nm): 228, 268, 288.

EIMS m/z (rel.int.): 278 $[M]^+$ (62), 208 (96), 196 (79), 195 (100), 177 (41), 137 (30), 91 (21), 77 (17).

δ_H : 7.66 (d, J = 1.9 Hz, H-5); 7.49 (d, J = 1.9 Hz, H-3); 3.87 (s, OMe); 2.62 (m, H-8α); 1.45-1.90 (m, 5H); 1.41 (s, 3H-14); 1.18 (s, 3H-15); 0.72 (s, 3H-13).

δ_C : 167.9 (s), 148.8 (s), 142.9 (s), 133.2 (s), 124.0 (d), 119.9 (s), 113.7 (d), 52.1 (q), 51.3 (s), 44.8 (s), 41.1 (t), 39.2 (t), 27.0 (q), 25.5 (q), 22.4 (q), 20.3 (t).

(xi) *(-)-1,2-Dihydroxy-12-oxoherbertene* (**17**) was isolated as crystals, m.p. 153-155°C (ex. CH_2Cl_2), $[\alpha]_D$ -11° (c, 1.5 in $CHCl_3$).

HRMS : m/z 248.1407 $[M]^+$ calculated for $C_{15}H_{20}O_3$: 248.1421.

T_R (mins) : 27.97

ν_{max} (cm^{-1}) : 3670, 3590, 3500, 3350, 3200 (OH); 3110, 2960, 2885; 1670 (C=O); 1590, 1505, 1460 (aryl C-C); 1430, 1325, 1290, 1160, 680.

λ_{max}(MeOH)(nm): 232, 292, 310.

EIMS m/z (rel.int.): 248 $[M]^+$ (78), 178 (100), 166 (75), 165 (81), 137 (16), 91 (16), 77 (14).

δ_H : 9.69 (s, H-12); 7.40 (d, *J* = 1.9 Hz, H-5); 7.24 (d, *J* = 1.9 Hz, H-3); 2.66 (m, H-8α); 1.45-1.90 (m, 5H); 1.44 (s, 3H-14); 1.19 (s, 3H-15); 0.74 (s, 3H-13).

δ_C* : 193.0 (d), 151.6 (s), 144.4 (s), 133.4 (s), 127.9 (d), 127.4 (s), 110.7 (d), 51.2 (s), 44.8 (s), 41.1 (t), 39.1 (t), 27.0 (q), 25.5 (q), 22.2 (q), 20.3 (t).

* 3 drops of CD_3OD were added to $CDCl_3$ solution.

B. Comparison of four major chemical constituents in *Herbertus aduncus ssp. hutchinsiae* collected at four different sites in Scotland.

Samples of *H. aduncus* were collected at Glen Ure in September 1991, Glen Kinglas in December 1991 and Loch Lomond in January 1992. The ground samples from Glen Ure (341 g), Glen Kinglas (16 g) and Loch Lomond (147 g) yielded 6.7 g, 0.3 g and 3.5 g of crude Et_2O extract respectively. These extracts were analysed by TLC and ^1H NMR. In order to compare the relative proportions of four of the major constituents [1,2-dihydroxy-12-oxoherbertene (**17**), α-herbertenol (**11**), cycloartanes, triglycerides] ^1H NMR spectra (Figure 3) were measured for a known concentration of each of the extracts [Loch Creran (77 mg/ml), Glen Ure (36 mg/ml), Loch Lomond (38 mg/ml), Glen Kinglas (16 mg

/ ml)] using identical parameters each time. A signal was chosen in the ^{1}H NMR spectrum to represent each of the major constituents {**17** [δ_H 9.69 (s), H-12]; **11** [δ_H 6.57 (d, J = 8.0 Hz, H-2)]; cycloartanes [δ_H 0.31 (d, J ~ 4 Hz, H-19)]; triglycerides [δ_H 4.28 (dd, J = 12.5, 5.0 Hz, 2H)]} and these signals were then integrated and the relative proportions calculated from the signal areas. Processing parameters included a line broadening factor (LB = 0.3 Hz) to increase signal/noise and spectra were processed with the same normalisation constant (i.e. in absolute intensity mode) so that intensities could be compared between different samples. The relative proportions were then converted into percentage yields as follows.

C. Calculation of yield for 1,2-dihydroxy-12-oxoherbertene (**17**) in Glen Ure extract.

This was achieved by taking a known weight of trinitrobenzene (1.9 mg) and of the Glen Ure extract (54.4 mg) and dissolving them both in $CDCl_3$ (0.7 ml). The ^{1}H NMR spectrum was then measured (LB = 0.3 Hz) and the integrated area of the three proton singlet (δ_H 9.35) in trinitrobenzene was set to three and compared with the area of the aldehyde proton singlet (δ_H 9.69) in compound **17**. This revealed that a proton in compound **17** has an area 1.4 times as large as a proton in trinitrobenzene, therefore, there are 1.4 times as many molecules of compound **17** present. Thus there will be 3.1 mg [1.4 x 1.9 x 248(MW of **17**)/213 (MW of trinitrobenzene] of compound **17** in 54.4 mg of extract. The approximate yield of 1,2-dihydroxy-12-oxoherbertene (**17**) in the Glen Ure extract is therefore 5.7%. The yields for each of the major constituents in all four extracts were then calculated relative to 5.7% using the proportions calculated above. The results are

shown in Table 3.

D. *Herbertus borealis.*

The plant material was collected at Beinn Eighe, by permission from the Nature Conservancy Council (Scottish National Heritage), in May 1992. The dry plant material (120 g) was ground and extracted with Et_2O to yield a crude extract (690 mg) which was initially analysed by TLC, ^1H NMR and GLC. The extract was then subjected to vacuum distillation by way of a cold finger fitted to an oil pump. The cold finger was immersed in an oil bath and the heat supplied by a hot plate. The extract (*ca* 160 mg) gave a very small quantity (<1 mg) of a terpene mixture which was then analysed by means of GLC and ^1H NMR. The two main terpenoid components were identified as herbertene (**10**) (T_R = 12.39 mins) and α-herbertenol (**11**) (T_R = 17.83 mins). From GLC peak areas the ratio of **10** : **11** is 3:4. Identifiable ^1H NMR signals for herbertene (**10**) (lit.[9]) : δ_H 7.16 (m, 3H); 2.34 (s, 3H-12); 1.07 (s, 3H-15); 0.56 (s, 3H-13). Identifiable ^1H NMR signals for α-herbertenol (**11**) (lit.[9]) : 6.85 (br d, *J* = 8Hz, H-3); 6.58 (d, *J* = 8Hz, H-2); 4.57 (br s, OH); 2.26 (s, 3H-12); 1.40 (s, 3H-14); 1.18 (s, 3H-15); 0.76 (s, 3H-13).

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CHAPTER 3

ANASTROPHYLLUM DONNIANUM

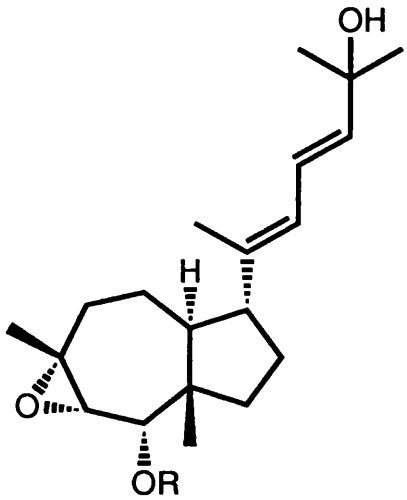
INTRODUCTION

The liverwort *Anastrophyllum donnianum* belongs to the family Lophoziaceae of the order Jungermanniales. The only *Anastrophyllum* species to have been investigated chemically previously is *A. minutum* (*Sphenolobus minutus*)[1]; this liverwort proved to be a rich source of sphenolabane diterpenoids. Sphenolobanes **1** - **6** were isolated[1] along with the sesquiterpene hydrocarbons anastreptene (**7**), ß-barbatene (gymnomitrene) (**8**) and bicyclogermacra-1(10),4-diene (**9**).

DISCUSSION

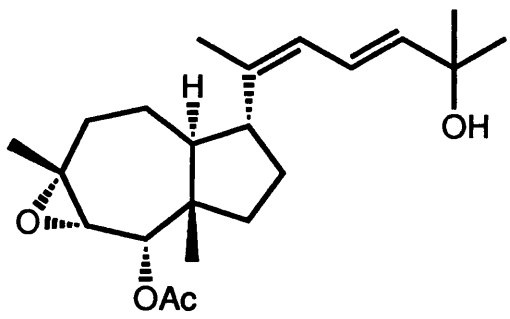
The chemical constituents of *Anastrophyllum donnianum* collected at three sites in Scotland have been investigated. From a sample collected at Beinn Damh, in addition to the known sesquiterpenes anastreptene (**7**)[2,3], *cis*-calamenene (**10**)[4], *cis*-5-hydroxycalamenene (**11**)[4,5], diplophyllin (**12**)[6,7], myliol (**13**)[8,9] and 3α-acetoxybicyclogermacra-1(10),4-diene (**14**)[10,11], we have isolated and elucidated the structures of six new sphenolobane diterpenoids (**15-20**). The extract also contained a mixture of fatty acid esters of cycloartenol and triglycerides. The known compounds, **7** and **10-13** were identified by comparison of their spectroscopic data with published data[2-9].

The sphenolobane diterpenoid (**15**), $C_{20}H_{30}O_2$ (m/z 286 $[M]^+$, from GCMS), the major component of the extract (*ca* 7% of extract), exhibited a characteristic fragment m/z 107 which has been observed in other sphenolobanes[1] and is consistent with a triene side chain as in partial structure **21**. The UV spectrum confirmed the presence of a conjugated triene (λ_{max} 275 nm) and the IR spectrum showed a carbonyl group (ν_{max} 1690 cm^{-1}). The ^1H NMR spectrum (Figure 1) (Table 1) showed the following



(1) R = Ac

(2) R = H



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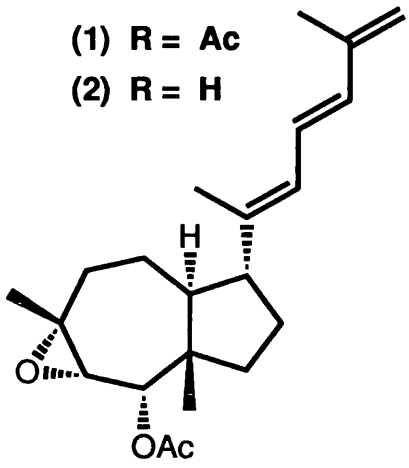
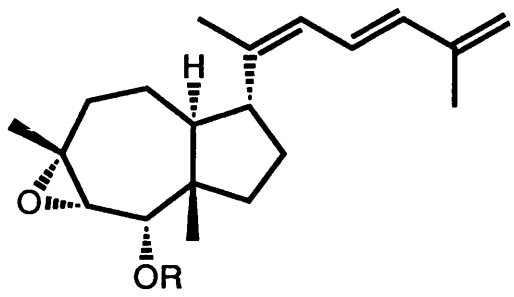


(4)



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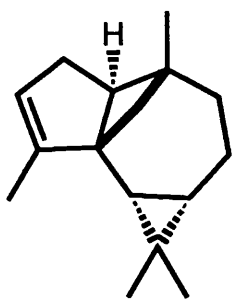
(6) R = H



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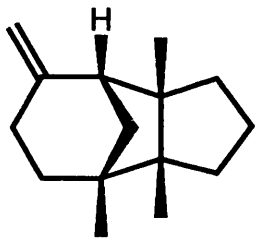

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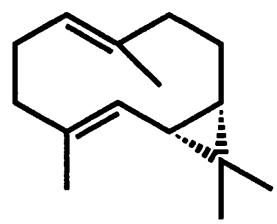

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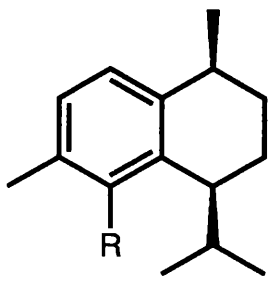

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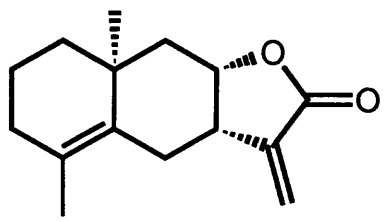
(11) R = OH



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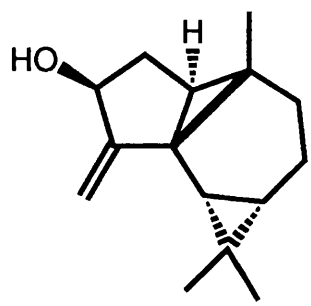

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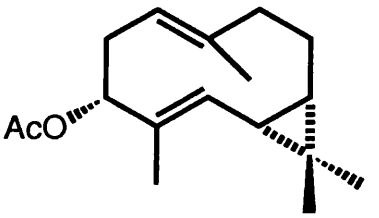

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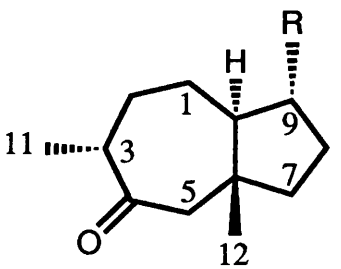
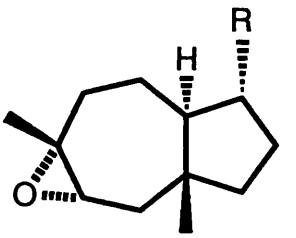




(15) R = (21)

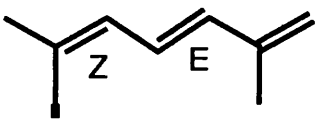
(16) R = (24)

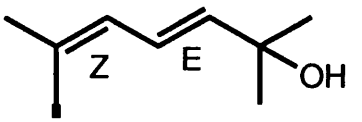
(18) R = (25)

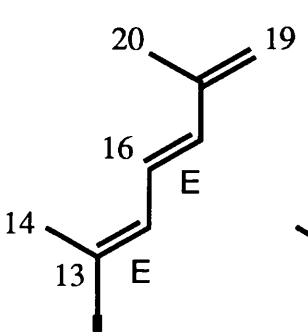
(17) R = (24)

(19) R = (25)

(20) R = (26)

(27) R = 

(28) R = 



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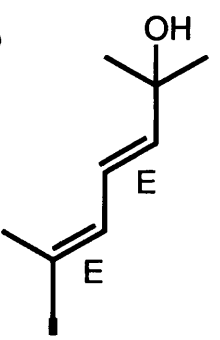

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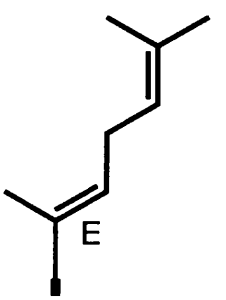

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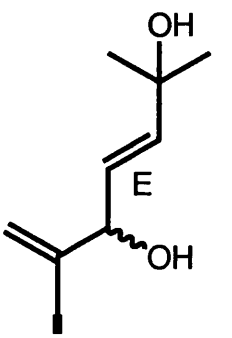

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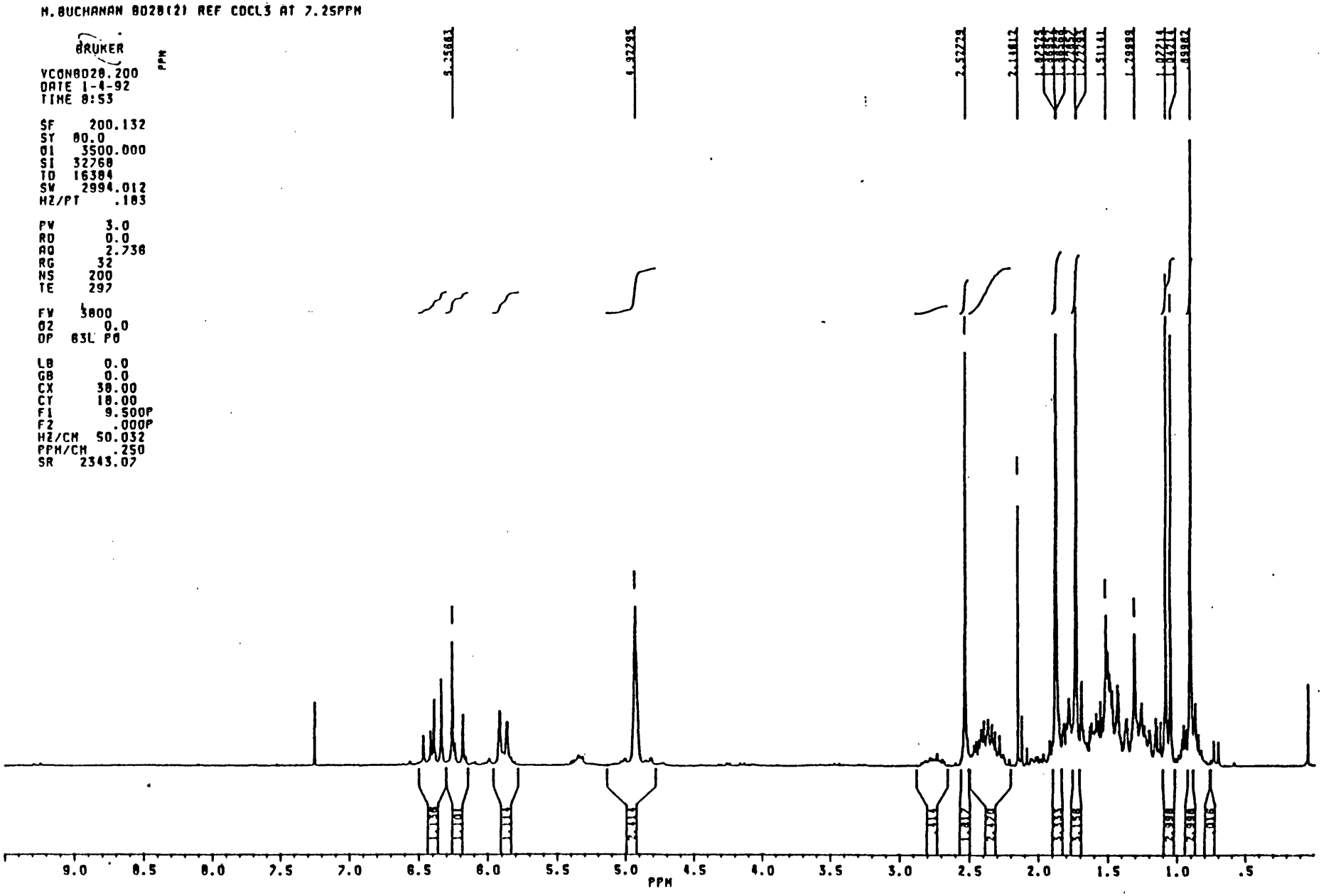


Figure 1. ^{1}H NMR spectrum of compound **15.**

Table 1. ^{1}H NMR data of sphenolobanes 4 and 15[a].

H	4[b]	15[c]
1α	1.90 m	1.10-1.95 m (2H)
1ß	1.18 m	
2α	1.44 m	1.37 m
2ß	1.96 m	1.78 m
3		2.40 m
4	2.85 d (5.5)	
5α		2.53 s (2H)
5ß	5.00 d (5.5)	
7α	1.67 m	1.30-1.65 m (2H)
7ß	1.37 m	
8α	1.85 m	1.10-1.95 m (2H)
8ß	1.52 m	
9	2.38 m	2.33 dd (10.9, 6.4)
10	1.94 m	1.49 m
11	1.31 d (2.5)	1.06 d (7.0)
12	0.99 br s	0.90 s
14	1.74 d (1)	1.72 d (1.1)
15	5.91 br d (11)	5.89 br d (10.5)
16	6.42 dd (11, 15.5)	6.40 dd (15.3, 10.5)
17	6.25 d (15.5)	6.22 d (15.3)
19a	4.95 br s	4.93 br s (2H)
19b	4.96 br s	
20	1.90 s	1.87 br s
22	2.10 s	

[a] Figures in parentheses are coupling constants (J) in Hz.

[b] Data from ref. 1.

[c] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation.

coupling pattern for three olefinic protons : δ_H 5.89 (br d, J = 10.5 Hz, H-15); 6.40 (dd, J = 15.3, 10.5 Hz, H-16) and 6.22 (d, J = 15.3 Hz, H-17). In addition the ^{1}H NMR spectrum contained signals for a tertiary methyl [δ_H 0.90 (s, 3H-12)], a secondary methyl [δ_H 1.06 (d, J = 7.0 Hz, 3H-11)], two vinyl methyls [δ_H 1.72 (d, J = 1.1 Hz, 3H-14); 1.87 (br s, 3H-20)], an isolated methylene [δ_H 2.53 (s, 2H-5)] and an exomethylene [δ_H 4.93 (br s, 2H-19)]. Double resonance experiments established allylic couplings between the vinyl methyl at δ_H 1.72 (d, J = 1.1 Hz, 3H-14) and H-15 [δ_H 5.89 (br d, J = 10.5 Hz)] and also between the vinyl methyl at δ_H 1.87 (br s, 3H-20) and the exomethylene protons [δ_H 4.93 (br s, 2H-19)]. The protons of the exomethylene group [δ_H 4.93 (br s, 2H-19)] and of the isolated methylene group [δ_H 2.53 (s, 2H-5)] are isochronous in $CDCl_3$ but appear as separate signals in C_6D_6 solvent [δ_H 4.88, 4.85 (both br s, 2H-19); 2.38 (d, J = 15 Hz, H-5); 2.28 (d, J = 15 Hz, H-5); ref. C_6D_5H at 7.15 ppm]. The ^{13}C NMR spectrum (Figure 2) (Table 2) revealed the presence of a carbonyl [δ_C 215.3 (s, C-4)], a trisubstituted double bond [δ_H 140.5 (s, C-13); 126.1 (t, C-15)], a disubstituted double bond [δ_C 133.7 (d, C-17); 125.3 (d, C-16)] and an exomethylene [δ_H 142.3 (s, C-18); 115.8 (t, C-19)]. The spectrum showed a further thirteen carbons including four methyls, five methylenes, three methines and one quaternary carbon. These data reveal that compound **15** is a bicyclic diterpenoid ketone with three conjugated double bonds in a side chain represented by partial structure **21**.

A 2D direct δ_C /δ_H correlation experiment (Figure 3) enabled the location of all the hydrogens, with the exception of 2H-1 and 2H-8 since their carbons are isochronous, and the assignment of all the protonated carbons. It was not possible to get any connectivity information for the bicyclic ring system from the ^{1}H NMR

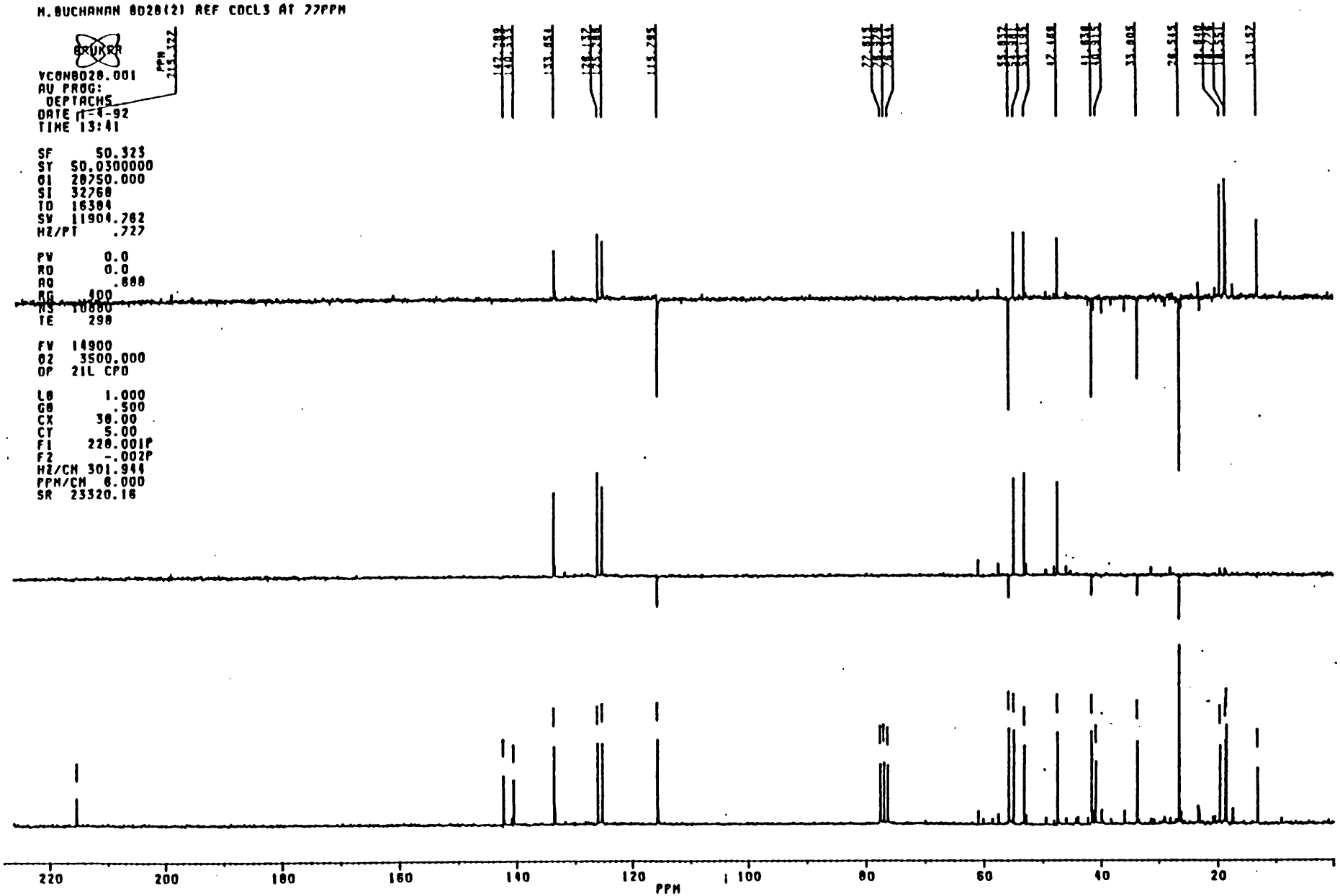


Figure 2. ^{13}C NMR and DEPT spectra of compound **15.**

Table 2. ^{13}C NMR data of sphenolobanes.

C	4[a]	15[b]	16	17
1	23.1 t	26.6 t	26.6 t	23.1 t^{o}
2	34.4 t	33.9 t	33.9 t	39.9 t^{+}
3	60.2 s	47.5 d	47.5 d	60.2 s
4	59.7 d	215.3 s	215.5 s	61.0 d
5	71.8 d	55.8 t	55.9 t	41.2 t^{+}
6	46.7 s	40.9 s	41.0 s	43.9 s
7	35.5 t	41.6 t	41.7 t	36.0 t^{+}
8	25.6 t	26.6 t	26.6 t	26.1 t^{o}
9	52.1 d	53.2 d	53.1 d	52.7 d
10	47.8 d	55.0 d	54.9 d	57.4 d
11	23.3 q	18.8 q	18.8 q	23.4 q
12	16.4 q	19.6 q	19.7 q	17.5 q
13	140.6 s	140.5 s	139.9 s	140.3 s
14	13.2 q	13.2 q	13.1 q	12.9 q
15	126.4 d*	126.1 d	125.2 d*	125.1 d*
16	125.4 d*	125.3 d	122.9 d*	122.9 d*
17	133.8 d*	133.7 d	139.3 d	139.1 d
18	142.4 s	142.3 s	70.9 s	70.9 s
19	115.9 t	115.8 t	29.9 q	29.84 q$^{\#}$
20	18.6 q	18.6 q	29.8 q	29.81 q$^{\#}$
21	170.3 s			
22	20.8 q			

[a] Data from ref. 1.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

[c] ^{13}C NMR was composite pulse decoupled (CPD) only and therefore the multiplicities were not established.

$^{*+o\#}$ Assignments may be interchangeable in each vertical column.

Table 2. ^{13}C **NMR data of sphenolobanes continued...**

C	18	19	20[c]
1	26.5 t[o]	26.0 t[o]	23.0
2	34.0 t	39.9 t[#]	39.5 [#]
3	47.6 d	60.3 s	60.3
4	215.7 s	61.2 d	61.0
5	56.0 t	41.4 t[#]	41.3 [#]
6	40.8 s	43.8 s	43.9
7	41.6 t	36.1 t[#]	36.0 [#]
8	26.4 t[o]	25.7 t[o]	29.2
9	52.8 d	52.4 d	45.9
10	54.6 d	57.1 d	58.5
11	18.9 q	23.4 q	23.4
12	19.7 q	17.5 q	17.8
13	135.8 s[*]	135.9 s[*]	149.8
14	12.4 q	12.1 q	112.1
15	124.8 d[+]	124.8 d[+]	87.8
16	27.1 t	27.0 t	143.3[*]
17	123.3 d[+]	123.4 d[+]	123.7 [*]
18	131.5 s[*]	131.6 s[*]	70.7
19	17.7 q	17.7 q	29.7
20	25.7 q	25.7 q	29.7

[a] Data from ref. 1.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

[c] ^{13}C NMR was composite pulse decoupled (CPD) only and therefore the multiplicities were not established.

*+o# Assignments may be interchangeable in each vertical column.

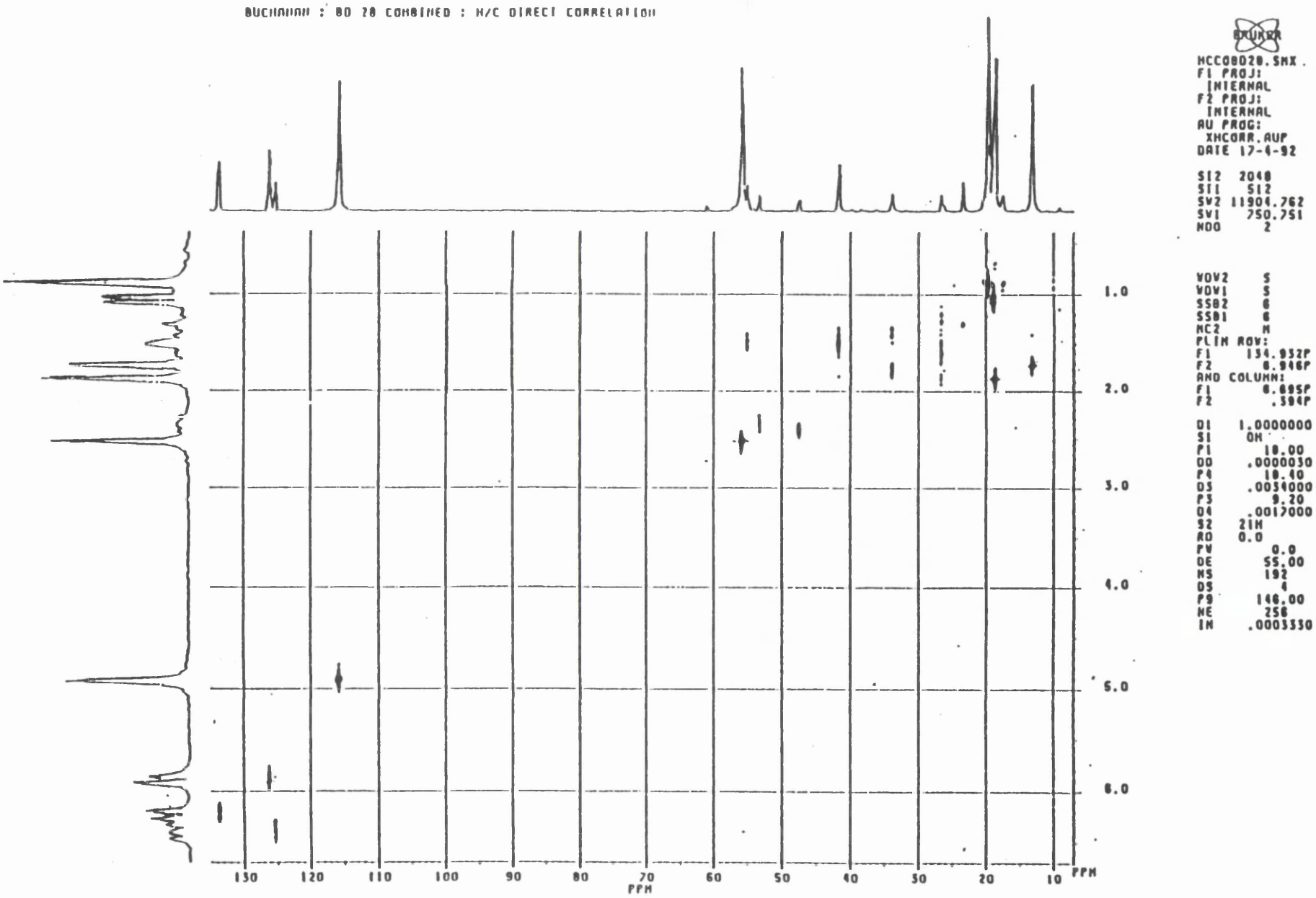


Figure 3. 2D direct δ_C / δ_H correlation spectrum of compound **15.**

spectrum in view of its congested upfield region. Connectivity deduced from a 2D long-range δ_C /δ_H correlation experiment (Figure 4) is shown in Table 3. The protons of the C-12 methyl group (tertiary) showed correlations to C-5, C-7 and C-10 (non-quaternaries) which must arise from $^3J_{CH}$; the remaining correlation, to C-6 (quaternary) must therefore arise from $^2J_{CH}$. There is also a $^3J_{CH}$ correlation of C-12 with 2H-5. The C-5 methylene group is isolated and this feature together with its ^{1}H and ^{13}C chemical shifts [δ_C 55.8 (t); 2.53 (s, 2H)] indicate that it is bonded to the carbonyl carbon [δ_C 215.3 (s, C-4)]. Therefore the correlation of 2H-5 to C-4 is $^2J_{CH}$. The carbonyl carbon (C-4) also has a correlation with the secondary methyl protons 3H-11 and this must be $^3J_{CH}$. These methyl protons (3H-11) also have correlations with C-3 ($^2J_{CH}$) and C-2 ($^3J_{CH}$). This information indicates partial structure **22**. Since the olefinic carbon C-16 (δ_C 125.3) showed no correlations it was not possible to deduce the complete side chain (**21**) previously derived from ^{1}H NMR and MS data. There is however a correlation between the vinyl methyl protons (3H-14) at δ_H 1.72 and the methine carbon at δ_C 53.2 (C-9) indicating that the side chain is attached to one of the rings at this methine carbon. Thus part structure **21** can be expanded to **23**. Combination of **22** and **23** together with the two methylene carbons C-1 and C-8 (δ_C 26.6) leads to the sphenolobane structure **15** for the natural product. The ^{1}H and ^{13}C NMR data of **15** are similar to those of the known sphenolobane, 3α ,4α-epoxy-5α-acetoxysphenoloba-13*E*,16*E*,18-triene (**4**)[1] (see Tables 1 and 2).

The stereochemistry of the side chain was deduced as follows: the 16*E* geometry is indicated by the large vicinal coupling (J = 15.3 Hz) between H-16 and H-17. The shielded nature of the C-14 methyl carbon (δ_C 13.2) is consistent with a 13*E* configuration. Furthermore the shielded character of the H-9 proton [δ_H 2.33 (dd, J

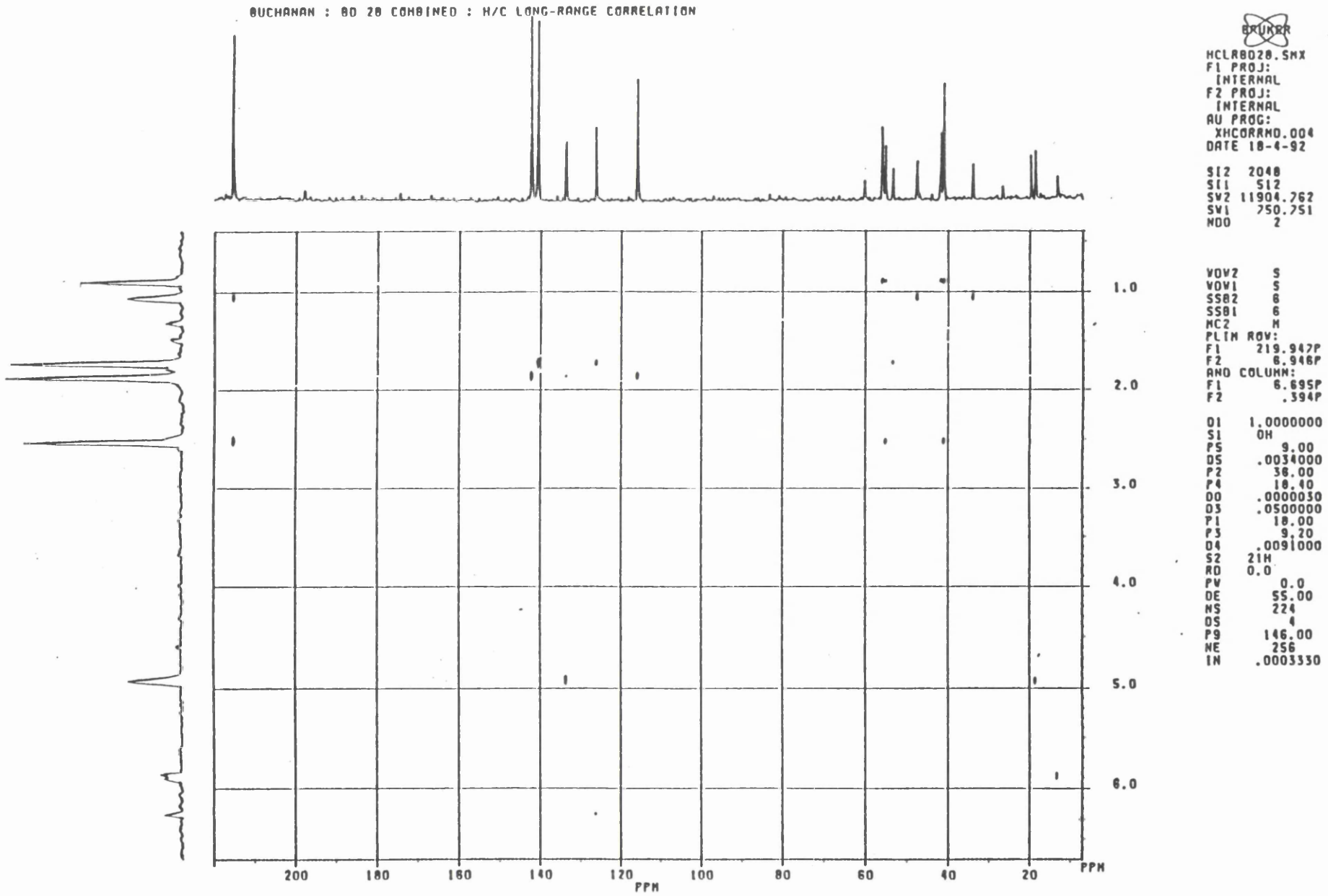
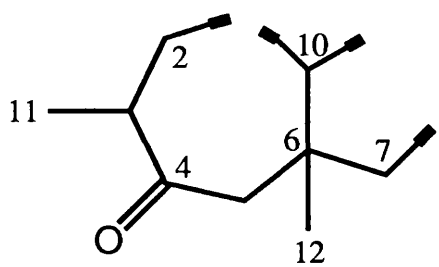


Figure 4. 2D long-range correlation spectrum of compound **15.**

Table 3. 2D long-range δ_C / δ_H correlations of compound 15.

H	Correlated C
2H-5	4,6,10,12
3H-11	2,3,4
3H-12	5,6,7,10
3H-14	9,13
H-15	14
H-17	15
2H-19	17,20
3H-20	17,18,19



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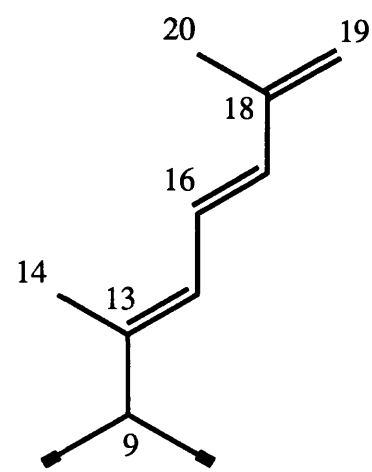


(23)

= 10.9, 6.4 Hz)] supports this as sphenolobanes with the same side chain stereochemistry exhibit this feature[1]. Thus compound **15** is sphenoloba-13*E*,16*E*,18-trien-4-one. The sample of compound **15** decomposed before any NOE difference experiments could be carried out, therefore the stereochemistry of the bicyclic ring indicated in structure **15** was established by analogy with compound **16** which is discussed as follows.

The IR spectrum of the next compound **16**, $C_{20}H_{32}O_2$ ([M]$^+$ at m/z 304.2401), revealed the presence of hydroxyl (ν_{max} 3600 cm^{-1}) and ketonic carbonyl groups (ν_{max} 1690 cm^{-1}). A ready loss of water was observed in the mass spectrum (m/z 286). The ^{1}H NMR spectrum (see Experimental) shows signals for an isolated methylene [δ_H 2.53 (s, 2H-5)], a vinyl methyl [δ_H 1.70 (d, J = 1.3 Hz, 3H-14)], a secondary methyl [δ_H 1.07 (d, J = 7.0 Hz, 3H-11)] and three tertiary methyls, two of which are attached to an oxygenated carbon [δ_H 0.90 (s, 3H-12); 1.34 (s, 3H-19 and 3H-20)]. The ^{13}C NMR spectrum (Table 2) showed twenty carbons : five methyls, five methylenes, three methines, four olefinic carbons [δ_C 139.9 (s, C-13); 139.3 (s, C-17); 125.2, 122.9 (both d, C-15 and C-16)], a carbonyl carbon [δ_C 215.5 (s, C-4)], a quaternary [δ_C 41.0 (s, C-6)] and a quaternary carbon bearing an oxygen [δ_C 70.9 (s, C-18)]. These NMR data are similar to those of compound **15** and it is apparent that the only differences lie in the nature of the side chain which contains the conjugated diene system as in part structure **24** [λ_{max} 240 nm; δ_H 5.81 (br d, J = 10.8 Hz, H-15), 6.44 (dd, J = 15.3, 10.8 Hz, H-16), 5.72 (d, J = 15.3 Hz, H-17)]. The mass spectrum of **16** shows a base peak at m/z 107 which arises from the side chain of **16** by dehydration. As above the 13*E*,16*E* geometry of the side chain is indicated by the coupling pattern of the olefinic protons, the shielded nature of the C-14 methyl signal (δ_C 13.1) and the shielded

character of the H-9 resonance (δ_H 2.32). The coupling pattern of H-9 [δ_H 2.32 (dd, J = 10.8, 6.0 Hz)] indicates that it has one coupling near zero Hz and thus a dihedral angle close to 90° with one of its adjacent protons. The H-3 proton assignment [δ_H 2.42 (dqd, J = 11.0, 7.0, 3.2 Hz)] was confirmed by homonuclear decoupling. The side chain stereochemistry is supported by the NOEs observed between H-9 (8.2%) and H-15 (6.7%) and between 3H-14 (3.2%) and H-16 (10.0%). NOE difference experiments were further used to establish the relative stereochemistry of the bicyclic nucleus. The NOEs between H-9 (5.0%) and 3H-12 (1.3%) and between 3H-12 (0.8%) and H-3 (7.0%) indicate that these are on the same side of the molecule. When either H-9 or 3H-12 was irradiated there was no NOE at H-10 suggesting that the five- and seven-membered rings are *trans*-fused. On the basis of the above findings compound **16** is 18-hydroxysphenoloba-13*E*,16*E*-dien-4-one.

Compound **17**, $C_{20}H_{32}O_2$ (m/z 304.2390 $[M]^+$) is isomeric with **16** and contains a hydroxyl group (ν_{max} 3605 cm^{-1}) and a conjugated diene (λ_{max} 240 nm). The mass spectrum shows ready loss of water (m/z 286) and characteristic side chain cleavage (m/z 107). These data suggest the same side chain as in **16** (partial structure **24**). The resonances of this side chain are readily identified in the ^1H NMR spectrum [δ_H 5.79 (br d, J = 10.8 Hz, H-15); 6.43 (dd, J = 15.3, 10.8 Hz, H-16); 5.71 (d, J = 15.3 Hz, H-17); 1.67 (d, J = 1.1 Hz, 3H-14)]. The resonances of the hydroxylated C-18 appears at δ_C 70.9 (s). Other ^{13}C NMR signals (Table 2) include five methyls, five methylenes, six methines and three quaternary carbons. The resonances at δ_C 60.2 (s, C-3), 61.0 (d, C-4) and δ_H 2.74 (br t, J = 7.0 Hz, H-4) are consistent with the presence of a trisubstituted epoxide. The proton, H-4, forms an AMX spin system with two protons of a methylene group [δ_H 2.27 (dd, J = 13.7, 7.0 Hz, H-5ß); 2.01 (br dd, J =

13.7, 7.0 Hz, H-5α)]. The proton at δ_H 2.27 was assigned the ß-configuration on the basis of the NOEs it receives from 3H-12 (3.0%) and H-4 (5.1%), therefore the signal at δ_H 2.01 is 5α . It is apparent that the ketonic carbonyl group of compound **16** has been replaced by a 3,4-epoxide in **17**. The chemical shift of 3H-11 [δ_H 1.31 (s)] supports this proposal. The ^{1}H NMR chemical shifts of the two other tertiary methyls reveal that they also are attached to oxygenated carbons [δ_H 1.33 (s, 3H-19 and 3H-20)]. The 13*E*,16*E* geometry of the side chain again follows from the NMR data [δ_C 12.9 (q, C-14); δ_H 2.34 (br dd, *J* = 11.0, 6.0 Hz, H-9); *J* (16,17) = 15.3 Hz] and is supported by NOEs observed between H-9 (9.1%) and H-15 and between 3H-14 (1.8%) and H-16. The relative stereochemistry of compound **17** was established by NOE difference experiments. The NOEs observed between H-9 (9.0%) and 3H-12, 3H-12 and 3H-11 (1.0%), 3H-12 (1.6%) and H-4 (12.0%) and between H-4 and 3H-11 (1.0%) indicate that these protons are on the same side of the bicyclic ring system. These NOEs also reveal a 3α ,4α-epoxide. Saturation of 3H-12 did not give a NOE at H-10, consistent with a *trans*-fusion of the bicyclic system. Thus compound **17** is 3α ,4α-epoxy-18-hydroxysphenoloba-13*E*,16*E*-diene.

The molecular formula of compound **18** is $C_{20}H_{32}O$, as determined by GCMS. The IR spectrum showed the presence of a carbonyl group (ν_{max} 1695 cm^{-1}). The ^{1}H NMR spectrum (see Experimental) contains signals for two vinyl protons [δ_H 5.09 (m, H-15 and H-17)], an allylic methylene [δ_H 2.68 (m, 2H-16)], an isolated methylene [δ_H 2.52 (s, 2H-5)], three vinyl methyls [δ_H 1.54 (d, *J* = 1.0 Hz, 3H-19/20); 1.61 (br s, 3H-19/20); 1.68 (d, *J* = 1.1 Hz, 3H-14)], a tertiary methyl [δ_H 0.88 (s, 3H-12)] and a secondary methyl [δ_H 1.07 (d, *J* = 7.0 Hz, 3H-11)]. The ^{13}C NMR spectrum (Table 2) displayed twenty carbons : five methyls, six methylenes, three methines, four olefinic

carbons [δ_C 135.8, 131.5 (both s, C-13 and C-18); 124.8, 123.3 (both d, C-15 and C-17)], a carbonyl carbon [δ_C 215.7 (s, C-4)] and a quaternary carbon. These NMR data are remarkably similar to those of **15**. Comparison revealed that the only differences lie in the side chain which contains two isolated double bonds and can be represented by partial structure **25**. The base peak at m/z 109 in the mass spectrum of **18** is consistent with the presence of this side chain. Thus compound **18** is sphenoloba-13*E*,17-dien-4-one. The 13*E* configuration follows from the shielded chemical shift of the C-14 methyl group (δ_C 12.4).

Compound **19**, $C_{20}H_{32}O$, is isomeric with **18**. Characteristic cleavage of the side chain in the mass spectrum results in the base peak at m/z 109 which indicates the presence of partial structure **25**. The ^1H NMR spectrum (see Experimental) and the ^{13}C NMR spectrum (Table 2) contain the signals for the bicarbocyclic ring system of **17** and the side chain of **18**. The 13*E* geometry of the side chain is again supported by the shielded C-14 signal [δ_C 12.1 (q)]. These data lead to 3α ,4α-epoxysphenoloba-13*E*,17-diene for compound **19**.

The polar compound **20** is very labile and only limited spectroscopic data were collected for it. This compound was isolated from a very weakly UV active band on a preparative plate and therefore it does not have any conjugation. The ^1H (see Experimental) and ^{13}C (Table 2) NMR data revealed the presence of an epoxide similar to compounds **17** and **19** [δ_C 60.3 (C-3), 61.0 (C-4); δ_H 2.75 (br t, J = 7.0 Hz, H-4), 1.31 (s, 3H-11)]. The epoxide methine is again part of an AMX spin system with the C-5 methylene protons [δ_H 2.28 (dd, J = 13.8, 7.0 Hz, H-5ß); 2.03 (br dd, J = 13.8, 7.0 Hz, H-5α)]. The ^{13}C NMR spectrum displayed twenty carbons : twelve saturated carbons, four olefinic carbons [δ_C 149.8 (C-13), 112.1 (C-14), 143.3, 123.7 (C-16 and

C-17)], and four carbons bearing an oxygen function [δ_C 87.8 (C-15), 70.7 (C-18), 61.0 (C-4), 60.3 (C-3)]. This information is clearly consistent with a sphenolobane possessing the same bicarbocyclic ring system as compounds **17** and **19** but with a modified side chain which contains the following groups : an exomethylene [δ_C 149.8 (C-13); 112.1 (C-14); δ_H 5.15, 5.03 (both br s, 2H-14)]; an allylic alcohol [δ_C 87.8 (C-15); δ_H 4.73 (br d, J = 7.4 Hz, H-15)] whose carbinol methine proton couples vicinally with one proton of a *trans*-disubstituted double bond [δ_C 143.3, 123.7 (C-16 and C-17); δ_H 5.93 (dd, J = 15.7, 0.8 Hz, H-17); 5.63 (dd, J = 15.7, 7.4 Hz, H-16)], a tertiary hydroxyl group [δ_C 70.7 (C-18)] and two tertiary methyl groups [δ_H 1.32 (s, 3H-19 and 3H-20)]. These data lead to part structure **26** for the side chain. Thus compound **20** is a 3α ,4α-epoxy-15,18-dihydroxysphenoloba-13(14),16*E*-diene.

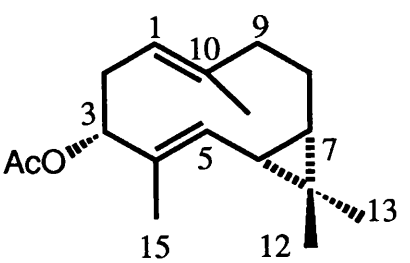
The relative stereochemistry of sphenolobanes **15** and **18** are assumed to be the same as in **16** and the relative stereochemistry of sphenolobanes **19** and **20** is assumed to be the same as in **17** in view of their co-occurrence in *A. donnianum*. The instability of these compounds did not permit stereochemical studies.

The sphenolobanes **15-20** are unstable when subjected to preparative TLC on silica gel and undergo oxidation of the side chain. It was possible to isolate compound **20** from a polar band during purification of compound **17** on a preparative plate. Gel chromatography over Sephadex LH-20 (CH_2Cl_2 - pet. ether, 3:2) was used in the purification of compound **15** which was particularly unstable to silica gel. Small amounts of compounds **16** and **17** were isolated because they are unstable to silica gel and these two compounds are much more abundant than indicated in the experimental section.

The compounds **15** and **16** co-occur with very small amounts of their $\Delta^{13(15)}$

geometrical isomers, compounds **27** and **28**, which could not be isolated. Evidence for their presence is as follows. In the ^{1}H NMR spectra of compounds **15** and **16** minor deshielded multiplets for H-9 appear at δ_H 2.78 and δ_H 3.00 consistent with the presence of 13(15)*Z* double bonds in compounds **27** and **28** respectively[1]. The corresponding signals for the $\Delta^{13(15)}$ and $\Delta^{16(17)}$ double bonds are also visible, the signals for the latter double bond confirming the 16*E* configuration.

Also isolated from the Beinn Damh extract was a minor constituent which was independently identified as **14** before we discovered that it is a known compound, having been discovered in the extracts of several liverworts[10,11]. Detailed double resonance and NOE difference experiments (Table 4) enabled a chain of coupling between the protons to be established and permitted full proton assignment (Scheme 1). The ^{1}H NMR data in Scheme 1 is more complete than that reported by Asakawa and his colleagues[10]. The ^{13}C NMR data, which has not been reported previously, is given in the Experimental. These and other spectroscopic data (see Experimental) indicate that compound **14** is a bicarbocyclic sesquiterpenoid acetate with a skeleton related to bicyclogermacrane.



(14)

Table 4. NOEs of compound **14**.

Irradiated proton	NOE at proton (%)													
	1	5	9α	2α	2β	8β	9β	15	14	8α	6	12	13	7
3				1.5	1.5			1.3						
1		1.6	3.1	4.0						1.5				
5										8.0			1.4	
9α	5.9						25.6			4.4				
14					6.1		5.7							2.6
7						4.4	3.1		0.6		4.3	1.1		

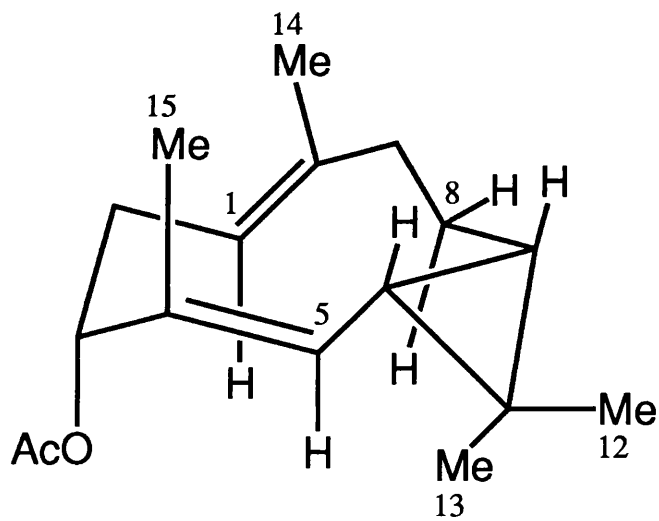
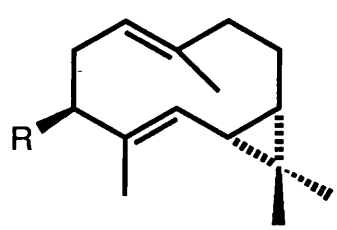


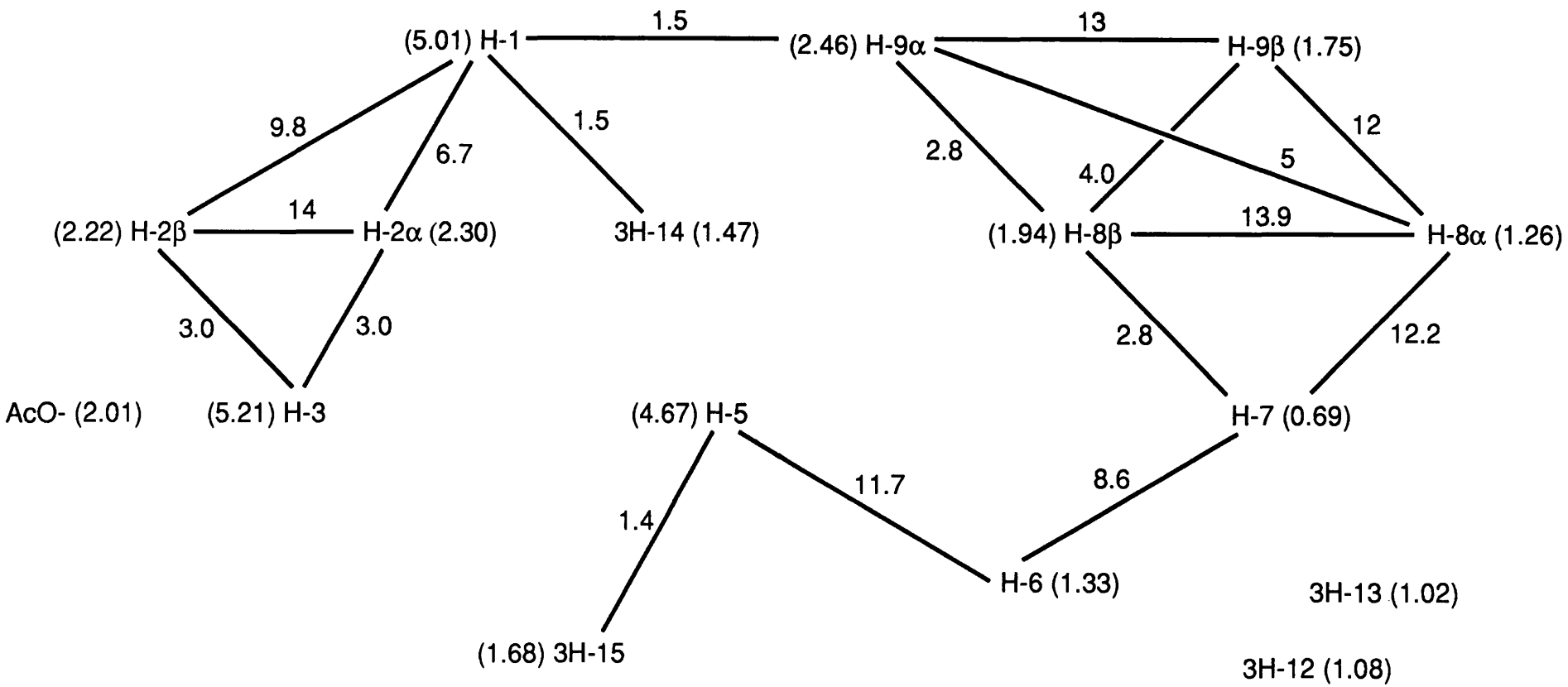
Figure 5



(29) R = OAc

(30) R = OH

Scheme 1. Chemical shifts and coupling constants of compound **14**[*].



[*] Chemical shifts in parenthesis.

The vicinal coupling (J = 8.6 Hz) of the cyclopropane protons (H-6 and H-7) and the substantial NOE (4.3%) between them established the *cis*-fused junction of the cyclopropane ring. Thus the bicyclic skeleton is a bicyclogermacrane rather than a lepidozane (*trans*-fused ring junction). The large J (H-5, H-6) = 11.7 Hz implies a *trans* periplanar relationship between H-5 and H-6. The *trans* geometries of both the trisubstituted double bonds were deduced not only from the shielded nature of the olefinic methyl carbons (δ_C 15.7 and 21.1)[12,13] but also from the lack of NOEs between the olefinic methyl groups and the olefinic protons. Thus compound **14** is a *trans, trans*-1(10),4-bicyclogermacradiene. The location of the acetate group was established to be C-3 by the triplet signal (J = 3.0 Hz) of H-3 and decoupling experiments. Separate decouplings at δ_H 5.01 (ddquin., J = 9.8, 6.7, 1.5 Hz, H-1) and δ_H 5.21 (t, J = 3.0 Hz, H-3) showed that both these protons have vicinal couplings to the two C-2 methylene protons at δ_H 2.22 (ddd, J = 14, 9.8, 3.0 Hz, H-2ß) and δ_H 2.30 (ddd, J = 14, 6.7, 3.0 Hz, H-2α).

The coupling and NOE difference information shown in Scheme 1 and Table 4 respectively is consistent with the configuration of the double bonds and the conformation of the ten-membered ring elucidated for bicyclogermacra-1(10),4-diene (*ent*-**9**)[14-16]. The most important NOEs which indicate this conformation for compound **14** (Figure 5) are those between (i) H-1 and H-5 (1.6%) (ii) H-1 and H-8α (1.5%) (iii) H-5 and H-8α (8.0%) and (iv) H-5 and 3H-13 (1.4%). This conformation has also been reported for other *trans, trans*-1(10),4-bicyclogermacradienes[12]. The *trans* double bonds have a crossed orientation in order to minimise transannular non-bonding interactions. In view of this conformation and the appearance of H-3 [δ_H 5.21 (t, J = 3.0 Hz)] the acetate group is axial (α) at

C-3 and on the same side of the cyclodecadiene ring as the fused cyclopropane. Further support for this conclusion came from the NOE at 3H-15 (1.3%) upon irradiation of H-3 and also comparison of the ^{13}C NMR data of **14** with those of similar compounds. The C-3 epimer (**29**) and its corresponding alcohol (**30**) have been isolated from the soft coral *Parerythropodium fulvum*[17] and their ^{13}C NMR data are very similar to those of **14**. The only significant difference is a shielding (*ca* 4-5 ppm) of an olefinic methyl carbon (C-15) in **29** and **30** relative to **14**. This is explained by the equatorial (ß) acetate/hydroxyl eclipsing the olefinic methyl (C-15) and thus shielding it (γ-effect). These data lead unequivocally to 3α-acetoxybicyclogermacra-1(10),4-diene for compound **14**. The absolute configuration of **14** isolated here has not been determined.

The GLC of the crude extract from Beinn Damh showed that anastreptene (**7**) and sphenoloba-13*E*,16*E*,18-trien-4-one (**15**) are the most abundant terpenoid constituents of the extract. Two other samples of *A. donnianum* were collected in Scotland from sites in Knoydart and the Fannich hills. The samples were very small and their crude extracts were analysed by GLC and GCMS using the known compounds isolated from the Beinn Damh extract as standards. The GLC trace of the crude extract of the Knoydart sample revealed the presence of anastreptene (**7**) and compound **15**. It is evident that these compounds are not the most abundant in the extract which contains other main components, as yet unidentified. The GLC and GCMS analyses of the Fannich's sample revealed the presence of the five sphenolobanes **15-19** isolated from the Beinn Damh extract. Anastreptene (**7**), the major constituent, and diplophyllin (**12**) were also identified. The mass spectra of several other GLC peaks in the trace of the Fannich's extract are

consistent with the presence of several sphenolobanes, presumably new, since they have molecular ions in the correct region for diterpenoids and bare peaks at m/z 107 characteristic of the side chain **21.**

The above results indicate a close chemical similarity for the three samples of *A. donnianum* and a similar pattern is obtained from *A. minutum*[1]. Thus their morphological classification and their chemistry are consistent. The variation of the relative concentrations of main sphenolobane constituents may be due to a number of factors such as genetic differences, location, climate, period of collection, etc.

It should be noted that the Beinn Damh sample of *A. donnianum* was collected from the same patch of ground as *Bazzania tricrenata* (Chapter 6). *Cis*-calamenene (**10**) and *cis*-5-hydroxycalamenene (**11**) are the main constituents of *B. tricrenata.* Therefore in view of the small amount of **10** and **11** isolated from *A. donnianum* it is possible their presence is due to contamination with *B. tricrenata.*

EXPERIMENTAL

Anastrophyllum donnianum collected at Beinn Damh.

The plant material was collected at Beinn Damh in September 1991. The sample was dried, ground (206 g) and extracted with Et_2O to give a crude extract (2.85 g). The crude extract was separated into twenty fractions by flash column chromatography on silica gel. Further separation by either preparative TLC or chromatography over Sephadex LH-20 gave in order of increasing polarity the following constituents : anastreptene (**7**) (90 mg), *cis*-calamenene (**10**) (2 mg) (see

p. 149), fatty acid ester mixture (235 mg), *cis*-5-hydroxycalamenene (**11**) (2 mg) (see p. 150), 3α-acetoxybicyclogermacra-1(10),4-diene (**14**) (6 mg), sphenoloba-13*E*,17-dien-4-one (**18**) (5 mg), 3α ,4α-epoxysphenoloba-13*E*,17-diene (**19**) (3 mg), sphenoloba-13*E*,16*E*,18-trien-4-one (**15**) (75 mg), triglycerides (200 mg), diplophyllin (**12**) (4 mg), myliol (**13**) (14 mg), 18-hydroxysphenoloba-13*E*,16*E*-dien-4-one (**16**) (11 mg), 3α,4α-epoxy-18-hydroxysphenoloba-13*E*,16*E*-diene (**17**) (5 mg) and 3α,4α-epoxy-15,18-dihydroxysphenoloba-13(14),16*E*-diene (**20**) (2 mg).

Analysis of fatty acid ester mixture. ^{1}H and ^{13}C NMR analysis of the ester mixture identified the major alcohol component of the esters as cycloartenol by analogy with published data for this compound[18] and also the data collected for the cycloartenol esters in *Herbertus aduncas ssp. hutchinsiae* (Chapter 2, Table 1 and pp. 44 and 45). Fatty acids were indicated by the following ^{1}H NMR data:

δ_H : 5.36 (m, vinyl); 2.79 (m, doubly allylic); 2.28 (m, α to C=O); 2.04 (m, allylic); 1.30, 1.24 (both m, saturated).

The following NMR data could be assigned to the cycloartenol component :

δ_H : 5.08 (m, H-24); 4.57 (m, H-3); 1.66, 1.59 (both s, 3H-26 and 3H-27); 0.88 (s, 6H); 0.87 (d, $J \sim 6$ Hz, 3H-21); 0.83 (s, 3H); 0.56 (d, $J = 4.0$ Hz, H-19); 0.32 (d, $J = 4.0$ Hz, H-19).

δ_C : 130.7 (s, C-25); 125.2 (d, C-24); 80.2 (d, C-3); 52.2 (d, C-17); 48.7 (s, C-14); 47.8 (d, C-8); 47.1 (d, C-5); 45.2 (s, C-13); 39.4 (s, C-4); 36.3 (t, C-22); 35.8 (d, C-20); 35.5 (t, C-15); 32.8 (t, C-12); 31.5 (t, C-1); 29.7 (t, C-19); 28.1 (t, C-16); 26.8 (t, C-2);

26.4 (t, C-11); 25.9 (s, C-10); 25.8 (t, C-7); 25.7 (q, C-27); 25.4 (q, C-28); 24.9 (t, C-23); 20.9 (t, C-6); 20.0 (s, C-9); 19.2 (q, C-30); 18.2 (q, C-21); 17.9 (q, C-18); 17.6 (q, C-26); 15.1 (q, C-29).

This ester mixture was not further investigated.

3α-Acetoxybicyclogermacra-1(10),4-diene (**14**) was isolated as an oil.

HRMS : m/z 262.1944 $[M]^+$ calculated for $C_{17}H_{26}O_2$: 262.1933.

T_R (mins) : 18.94

$\nu_{max}(CHCl_3)(cm^{-1})$: 2930, 2860; 1730 (C=O); 1455, 1375; 1255 (C-O).

EIMS m/z (rel.int.) : 262 $[M]^+$ (18), 220 (14), 202 (23), 175 (56), 159 (46), 152 (53), 145 (28), 132 (46), 121 (62), 109 (100), 95 (26), 81 (50).

δ_H (lit.[10]) : see Scheme 1.

δ_C : 170.2 (s, C=O); 143.5 (s, C-10); 126.3 (d, C-1/5); 126.1 (s, C-4); 119.8 (d, C-1/5); 79.0 (d, C-3); 37.1 (t, C-9); 30.6 (t, C-2); 30.3 (d, C-7); 29.1 (q, C-12); 27.0 (t, C-8); 26.8 (d, C-6); 21.4, 21.1 (both q, C-14/-OAc); 20.3 (s, C-11); 15.7, 15.4 (both q, C-13 and C-15).

Sphenoloba-13E,17-dien-4-one (**18**) was obtained as an oil.

T_R (mins) : 25.85

$\nu_{max}(CHCl_3)(cm^{-1})$: 3020, 2930, 2860; 1695 (C=O); 1460, 1385.

GCMS m/z(rel.int.) : 288 $[M]^+$ (27), 109 (100), 95 (35), 82 (26), 67 (30), 55 (35), 41 (44).

δ_H : 5.09 (m, H-15 and H-17); 2.68 (m, 2H-16); 2.52 (s, 2H-5); 2.42 (m, H-3); 2.25 (m, H-9); 1.68 (d, *J* = 1.1 Hz, 3H-14); 1.61 (br s, 3H-19/20); 1.54 (d, *J* = 1.0 Hz, 3H-19/20); 1.0-1.90 (m, 9H); 1.07 (d, *J* = 7.0 Hz, 3H-11); 0.88 (s, 3H-12).

δ_C : see Table 2.

3α,4α-Epoxysphenoloba-13E,17-diene (**19**) was isolated as an oil.

T_R (mins) : 25.18

GCMS m/z(rel.int.) : 288 $[M]^+$ (24), 109 (100), 93 (73), 79 (57), 67 (62), 55 (67), 43 (86).

δ_H : 5.07 (m, H-15 and H-17); 2.74 (br t, *J* = 7.0 Hz, H-4); 2.67 (m, 2H-16); 2.26 (dd, *J* = 13.6, 7.0 Hz, H-5ß); 2.25 (m, H-9); 2.02 (br dd, *J* = 13.6, 7.0 Hz, H-5α); 1.68 (br s, 3H-14); 1.61, 1.51 (both br s, 3H-19 and 3H-20); 1.10-1.90 (m, 9H); 1.31 (s, 3H-11); 0.88 (s, 3H-12).

δ_C : see Table 2.

Sphenoloba-13E,16E,18-trien-4-one (**15**) was isolated as a gum.

T_R (mins) : 28.12

$\nu_{max}(CHCl_3)(cm^{-1})$: 3020, 2940, 2870; 1690 (C=O); 1460, 1385, 1230.

λ_{max} (nm) : 275

GCMS m/z(rel.int.) : 286 $[M]^+$ (31), 107 (100), 93 (41), 77 (16), 55 (29), 41 (28).

δ_H : see Table 1.

δ_C : see Table 2.

18-Hydroxysphenoloba-13E,16E-dien-4-one (**16**) was obtained as a gum.

HRMS : m/z 304.2401 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402.

T_R (mins) : 28.82

$\nu_{max}(CHCl_3)(cm^{-1})$: 3600 (OH); 3020, 2970, 2935, 2870; 1690 (C=O); 1460, 1385, 970.

λ_{max} (nm) : 240

EIMS m/z(rel.int.) : 304 $[M]^+$ (43), 286 (9), 233 (38), 205 (19), 177 (18), 161 (25), 149 (24), 133 (29), 125 (50), 107 (100), 93 (67), 81 (64).

δ_H : 6.44 (dd, *J* = 15.3, 10.8 Hz, H-16); 5.81 (br d, *J* = 10.8 Hz, H-15); 5.72 (d, *J* = 15.3 Hz, H-17); 2.53 (s, 2H-5); 2.42 (dqd, *J* = 11.0, 7.0, 3.2 Hz, H-3); 2.32 (dd, *J* = 10.8, 6.0 Hz, H-9); 1.70 (d, *J* = 1.3 Hz, 3H-14); 1.34 (s, 3H-19 and 3H-20); 1.20-2.00 (m, 9H); 1.07 (d, *J* = 7.0 Hz, 3H-11); 0.90 (s, 3H-12).

δ_C : see Table 2.

3α,4α-Epoxy-18-hydroxysphenoloba-13E,16E-diene (**17**) was isolated as a gum.

HRMS : m/z 304.2390 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402.

T_R (mins) : 28.22

$\nu_{max}(CHCl_3)(cm^{-1})$: 3605 (OH); 3020, 2980, 2930, 2860, 1460, 1380, 1220, 970.

λ_{max} (nm) : 240

EIMS m/z(rel.int.) : 304 $[M]^+$ (3), 286 (2), 179 (24), 161 (43), 135 (37), 121 (52), 107 (92), 95 (100).

δ_H : 6.43 (dd, *J* = 15.3, 10.8 Hz, H-16); 5.79 (br d, *J* = 10.8 Hz,

H-15); 5.71 (d, *J* = 15.3 Hz, H-17); 2.74 (br t, *J* = 7.0 Hz, H-4); 2.34 (br dd, *J* = 11.0, 6.0 Hz, H-9); 2.27 (dd, *J* = 13.7, 7.0 Hz, H-5ß); 2.01 (br dd, *J* = 13.7, 7.0 Hz, H-5α); 1.67 (d, *J* = 1.1 Hz, 3H-14); 1.15-1.95 (m, 9H); 1.33 (s, 3H-19 and 3H-20); 1.31 (s, 3H-11); 0.90 (s, 3H-12).

δ_C : see Table 2.

3α,4α-Epoxy-15,18-dihydroxysphenoloba-13(14),16E-diene (**20**) was obtained as a gum.

δ_H : 5.93 (dd, *J* = 15.7, 0.8 Hz, H-17); 5.63 (dd, *J* = 15.7, 7.4 Hz, H-16); 5.15, 5.03 (both br s, 2H-14); 4.73 (br d, *J* = 7.4 Hz, H-15); 2.75 (br t, *J* = 7.0 Hz, H-4); 2.28 (dd, *J* = 13.8, 7.0 Hz, H-5ß); 2.03 (br dd, *J* = 13.8, 7.0 Hz, H-5α); 1.32 (s, 3H-19 and 3H-20); 1.31 (s, 3H-11); 0.90 (s, 3H-12).

δ_C : see Table 2.

GLC analysis of *Anastrophyllum donnianum* collected in Knoydart.

The plant material was collected in May 1992. The ground material (2.4 g) was extracted with Et_2O to give a crude ether extract (19 mg). GLC of the extract identified anastreptene (**7**) (T_R = 9.96 mins) and sphenoloba-13*E*,16*E*,18-trien-4-one (**15**) (T_R = 28.10 mins). Unidentified peaks with retention times of 38.77 and 41.59 minutes are the main constituents of this extract.

GLC and GCMS analyses of *Anastrophyllum donnianum* collected from the Fannich hills.

The plant material was collected in May 1993. Analysis of the crude extract by GLC and GCMS identified the following compounds : anastreptene (**7**) (T_R = 9.87 mins, m/z 202 $[M]^+$); diplophyllin (**12**) (T_R = 21.53 mins, m/z 232 $[M]^+$); 3α,4α-epoxysphenoloba-13*E*,17-diene (**19**) [T_R = 25.07 mins, m/z 109 (100%)]; sphenoloba-13*E*,17-dien-4-one (**18**) [T_R = 25.73 mins*, m/z 288 $[M]^+$, 109 (100%)]; sphenoloba-13*E*,16*E*,18-trien-4-one (**15**) [T_R = 28.00 mins, m/z 286 $[M]^+$, 107 (100%)]; 3α,4α-epoxy-18-hydroxysphenoloba-13*E*,16*E*-diene (**17**) [T_R = 28.35 mins, m/z 286 $[M-18]^+$, 107 (100%)]; 18-hydroxysphenoloba-13*E*,16*E*-dien-4-one (**16**) [T_R = 28.97 mins, m/z 286 $[M-18]^+$, 107 (100%)]. Several other GLC peaks were scanned by GCMS but the compounds remain unidentified. Their GLC retention times, together with their heaviest and most significant ions in the GCMS are as follows: T_R = 25.73 mins*, m/z 286 (8%), 107 (100%); T_R = 26.26 mins, m/z 286 (57%), 107 (73%); T_R = 26.61 mins, m/z 286 (38%), 107 (100%); T_R = 27.39 mins, m/z 286 (48%), 107 (100%).

* These are separate peaks on the TIC trace but the same peak on the FID chromatogram due to a better column in the GCMS (T_R is taken from the FID chromatogram).

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CHAPTER 4

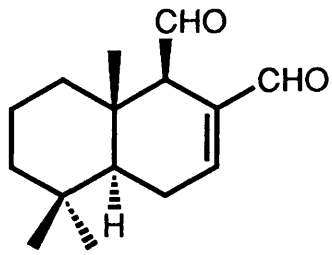
PORELLA PLATYPHYLLA

INTRODUCTION

Porella platyphylla belongs to the family Porellaceae of the order Jungermanniales. The *Porella* species of liverwort are rich sources of drimane, pinguisane, guaiane, pseudoguaiane, germacrane, aromadendrane and striatane-type sesquiterpenoids[1]. The genus has been divided into two major chemotypes, pungent and non-pungent[2]. The former type produces an intensely pungent drimane-type sesquiterpene, polygodial (**1**), and related compounds; in addition it usually also produces aromadendranes and pinguisanes. The non-pungent type does not produce drimanes but usually produces large amounts of pinguisanes. The Porellaceae family has been further divided into five chemotypes[1,3] : (i) drimane, pinguisane and aromadendrane, (ii) sacculatane only, (iii) pinguisane only, (iv) pinguisane and sacculatane, (v) pseudoguaiane and sacculatane. It should be noted that type (iv) has been characterised differently in a later paper[4].

P. platyphylla produces a wide range of terpenoid metabolites including mono-, sesqui-, di-, and triterpenoids. Chemical investigations of this liverwort have identified the monoterpenoids limonene, ß-phellandrene, α-terpinene, terpinolene, *p*-cymene, α-pinene, ß-pinene, camphene and ß-sabinene[1]. All the sesquiterpenoids elaborated by this liverwort are of the pinguisane-type[1-3,5]. Many of the original structures proposed for pinguisanes isolated from *P. platyphylla* and other liverworts are dubious and some have subsequently been revised[5,6]. The diterpenoid constituents isolated from this liverwort are perrottetianals A-D (**2-5**)[7,8]. The triterpenoid, friedelin[9], has also been isolated.

The major constituent of European *P. platyphylla* is pinguisanin (**6**)[2,6] which is also the major constituent of *Ptilidium pulcherrimum*[10] and *Trocholejeunea*



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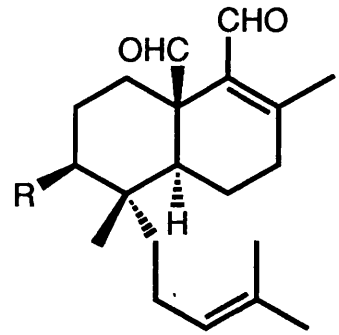
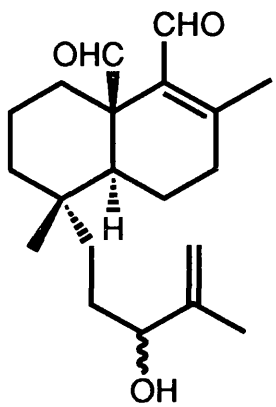


(2) **R = H**
(3) **R = OH**



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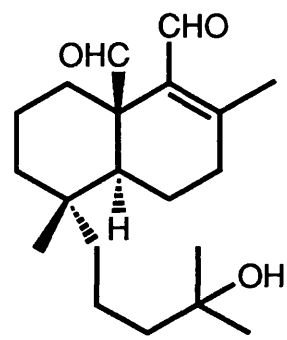

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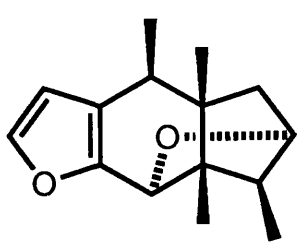

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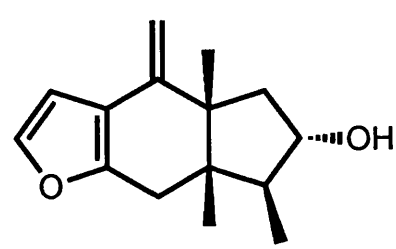


(7)

sandvicensis[11]. Pinguisanin (**6**) is one of the pinguisanes whose structure has been revised[5,6]. The investigations to date on *P. platyphylla* indicate that it belongs to the non-pungent type of *Porella* species and to chemotype (iv) of the Porellaceae.

P. platyphylla displays antimicrobial activity against gram-positive bacteria; however, the active substances have not been isolated[12].

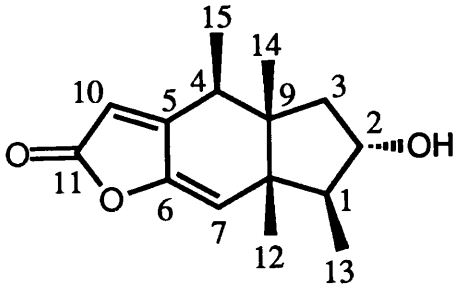
This liverwort was investigated in order to try and isolate the previously reported[5] crystalline, unstable alcohol **7** and also to compare the chemical constituents isolated from the samples collected at different locations in Yorkshire.

DISCUSSION

The liverwort *Porella platyphylla* was collected from various locations in Yorkshire and proved to be a rich source of pinguisane sesquiterpenoids. The chemical examination of *P. platyphylla* is discussed in terms of the extracts obtained from different locations.

Extract A

The plant material for this extract was collected near Langcliffe, Yorkshire in April 1990, stored in the dry state for six months and then powdered. Half of it was extracted with Et_2O using a Soxhlet extractor. The major sesquiterpenoid, pinguisanin (**6**), was readily identified by comparison of its spectroscopic properties with published data[6]. Among the minor constituents present in this extract it was possible to isolate two new pinguisanes **8** and **9** from the more polar fractions obtained by column chromatography. Other minor constituents were not investigated further.



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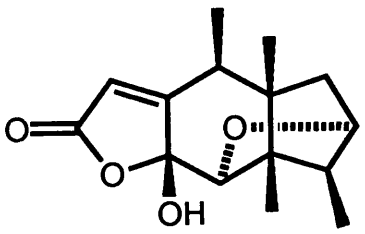

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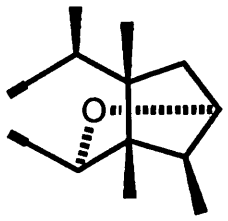

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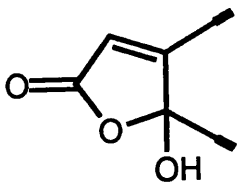

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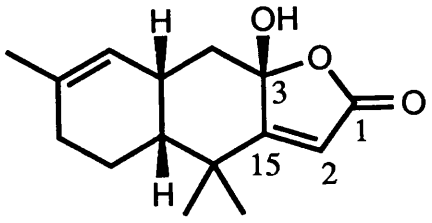

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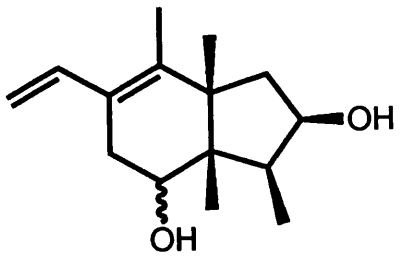

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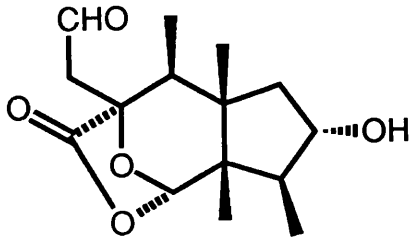

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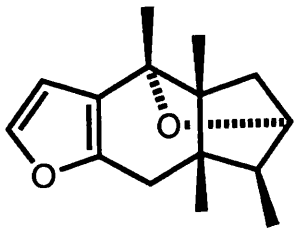


(15)

Compound **8** has a parent ion in the mass spectrum at m/z 248 $[M]^+$, corresponding to $C_{15}H_{20}O_3$. The IR spectrum exhibited the presence of a hydroxyl group at υ_{max} 3625 cm^{-1} and an enol lactone (υ_{max} 1784, 1675 cm^{-1}). The UV spectrum suggested the presence of a conjugated dienone system (λ_{max} 282 nm). The ^1H NMR spectrum (see Experimental) indicates the presence of two tertiary methyl and two secondary methyl groups [δ_H 0.74, 0.92 (both s, 3H-12 and 3H-14); 1.05 (d, J = 6.9 Hz, 3H-15); 1.24 (d, J = 6.8 Hz, 3H-13)], a vinyl proton [δ_H 5.42 (d, J = 2.1 Hz, H-7)], a one proton multiplet [δ_H 5.74 (dd, J = 2.3, 2.1 Hz, H-10)] assigned to the α-proton of a butenolide group, and a methine proton attached to a carbon-bearing oxygen [δ_H 3.93 (ddd, J = 9.0, 7.6, 3.1 Hz, H-2)], whose coupling is consistent with α-attachment of the hydroxyl at C-2[13]. Also present are the AB protons of an ABX system [δ_H 1.76 (br dd, J = 14.8, 3.1 Hz, H-3α); 1.94 (dd, J = 14.8, 9.0 Hz, H-3ß)] arising from the C-3 methylene protons, a broad quintet (J = 7.3 Hz, H-1) at δ_H 2.20 and a quartet of doublets (J = 6.8, 2.3 Hz, H-4) at δ_H 3.20. The ^{13}C NMR spectrum (see Experimental) confirms the presence of the above functional groups. Thus there are resonances for two trisubstituted double bonds [δ_C 148.2, 159.9 (both s); 111.3, 114.8 (both d)], a carbonyl [δ_C 170.6 (s)] and an oxygenated methine (δ_C 78.0). The remaining resonances include four methyls, one methylene, two methines and two quaternary carbons. These spectral data suggest that this compound is a bicyclic sesquiterpene alcohol with a butenolide group. Structure **8** is consistent with the evidence presented above.

The second minor constituent isolated from the extract (**9**) displayed a molecular ion peak at m/z 264 $[M]^+$, indicating a molecular formula of $C_{15}H_{20}O_4$.

The IR spectrum showed the presence of a carbonyl, possibly a butenolide group (υ_{max} 1760, 1650 cm^{-1}) and also a hydroxyl group (υ_{max} 3420, 3260 cm^{-1}). The presence of a hydroxyl group is supported by a $[M-18]^+$ fragment ion at m/z 246. The UV spectrum has λ_{max} 220 nm. The ^1H NMR spectrum (see Experimental) shows resonances for two tertiary methyls and two secondary methyl groups [δ_H 1.11 (s, 3H-12); 1.21 (s, 3H-14); 0.99 (d, J = 7.4 Hz, 3H-13); 1.24 (d, J = 7.5 Hz, 3H-15)], a vinyl proton [δ_H 5.78 (s, H-10)] and two protons attached to oxygenated carbons [δ_H 3.77 (s, H-7); 3.86 (br s, H-2)]. The spectrum also shows a broad doublet of triplets (J = 13.9, 1.5 Hz, H-3α) at δ_H 1.43 and a doublet of doublets (J = 13.9, 2.0 Hz, H-3ß) at δ_H 1.73 due to the C-3 methylene protons, a broad quartet of triplets (J = 7.4, 2.0 Hz) at δ_H 1.93 due to H-1 and a quartet (J = 7.5 Hz) at δ_H 2.60 due to H-4. The ^{13}C NMR spectrum (see Experimental) showed the presence of a trisubstituted double bond, a carbonyl [δ_C 171.6, 168.7 (both s, C-5 and C-11); 117.5 (d, C-10)], a quaternary sp^3 carbon substituted by two oxygens [δ_C 105.5 (s, C-6)] and two oxygenated methine groups [δ_C 85.3 (C-7), 79.6 (C-2)] together with four methyls, one methylene, two methines and two quaternary sp^3 carbon atoms. Partial structure **10** is obviously present as the spectral data for this portion are very similar to those of pinguisanin (**6**) (see Table 2 and Experimental)[6]. The remaining portion has partial structure **11** (γ-hydroxybutenolide) since the spectroscopic data compare very well with those of furodysinin lactone (**12**)[14] which contains the same moiety. Compound **12** showed IR absorptions at ν_{max} ($CHCl_3$) 3580, 3300, 1745 and 1640 cm^{-1} and a UV band at λ_{max} (EtOH) 221 nm. The ^1H NMR ($CDCl_3$) contains a one proton singlet at δ_H 5.67 assigned to H-2 while the ^{13}C NMR ($CDCl_3$) includes resonances at δ_C 174.76

(s, C-1), 169.82 (s, C-15), 115.27 (d, C-2) and 104.91 (s, C-3). Combination of **10** and **11** leads to structure **9** with the stereochemistry at the hemiketal carbon still to be established. The configuration at C-6 was deduced by consideration of the allylic coupling between H-4 and H-10. Allylic coupling is large ($J \sim 3.0$ Hz) when the allylic C-H bond is orthogonal to the plane of the double bond, and small ($J < 0.5$ Hz) when it lies in the plane of the double bond[15-17]. Models reveal that when the 6-OH is in the ß-configuration the six-membered ring prefers a chair conformation and the allylic C-4, H-4 bond is in the plane of the double bond whereas in the alternative α-configuration the C-H bond is almost orthogonal to the plane of the double bond regardless of the conformation of the ring. In the latter case a significant allylic coupling would be expected while in the former case this coupling will be virtually zero as seen for compound **9**. Thus the hydroxyl group is ß.

In some of the ^{13}C NMR spectra (recorded at room temperature) recorded for compound **9** not all of the signals were observed and other signals appeared broad. This broadening effect is noticeable for the resonances associated with (C-5/C-11), C-10, C-4 and C-6 with this last hemiketal carbon resonance (C-6) often missing completely. The ^{13}C NMR spectra with sharp signals can mean either a slow exchange process in which the spectra are the superposition of spectra due to each species present or a fast exchange process where the observed spectrum may be considered as due to a single species whose NMR parameters (chemical shifts and coupling constants) are the averages of those for the individual species (suitably weighted to take account of unequal populations). In the present example whether or not the system is in the fast or slow exchange regimes there

cannot be much of the C-6 epimer or the acid tautomers present as there is no allylic coupling [^4J (H-4, H-10)] and there are only fifteen carbon signals whose chemical shifts accord with the hydroxylactone form (**9**). It was not possible to tell whether the sharp signals indicated a fast or slow exchange system as no variable temperature experiments were carried out. In the ^{13}C NMR spectra with broad signals there must be exchange proceeding at a rate comparable with the timescale of the NMR observation. This exchange process could involve either or both epimerisation at C-6 or keto-acid/hydroxylactone tautomerism. We already know there is very little of the minor isomers present but the broadness could still arise from exchange broadening caused by the minor isomers. In the case of C-6 the fact that the signal is so broad that it can be lost in the noise[18] suggests that the exchange involves a large chemical shift between the exchanging species, which would be the case for keto-acid/hydroxylactone tautomerism.

Pinguisanes **8** and **9** are probably formed by autoxidation of pinguisanin (**6**) through a radical mechanism involving molecular oxygen which converts the furan ring into a butenolide.

Extract B

This extract was obtained from the other half of the ground plant material used in extract A. This plant material was collected in April 1990, ground in October 1990, and extracted at room temperature, with Et_2O in May 1991. Some of the more polar fractions were examined for the presence of the two new pinguisanes, **8** and **9**, discovered in extract A, to enable further structural studies to be carried out. Unfortunately pinguisanes **8** and **9** were not detected and the only compound isolated was the unstable ß-pinguisenediol (**13**) which was identified

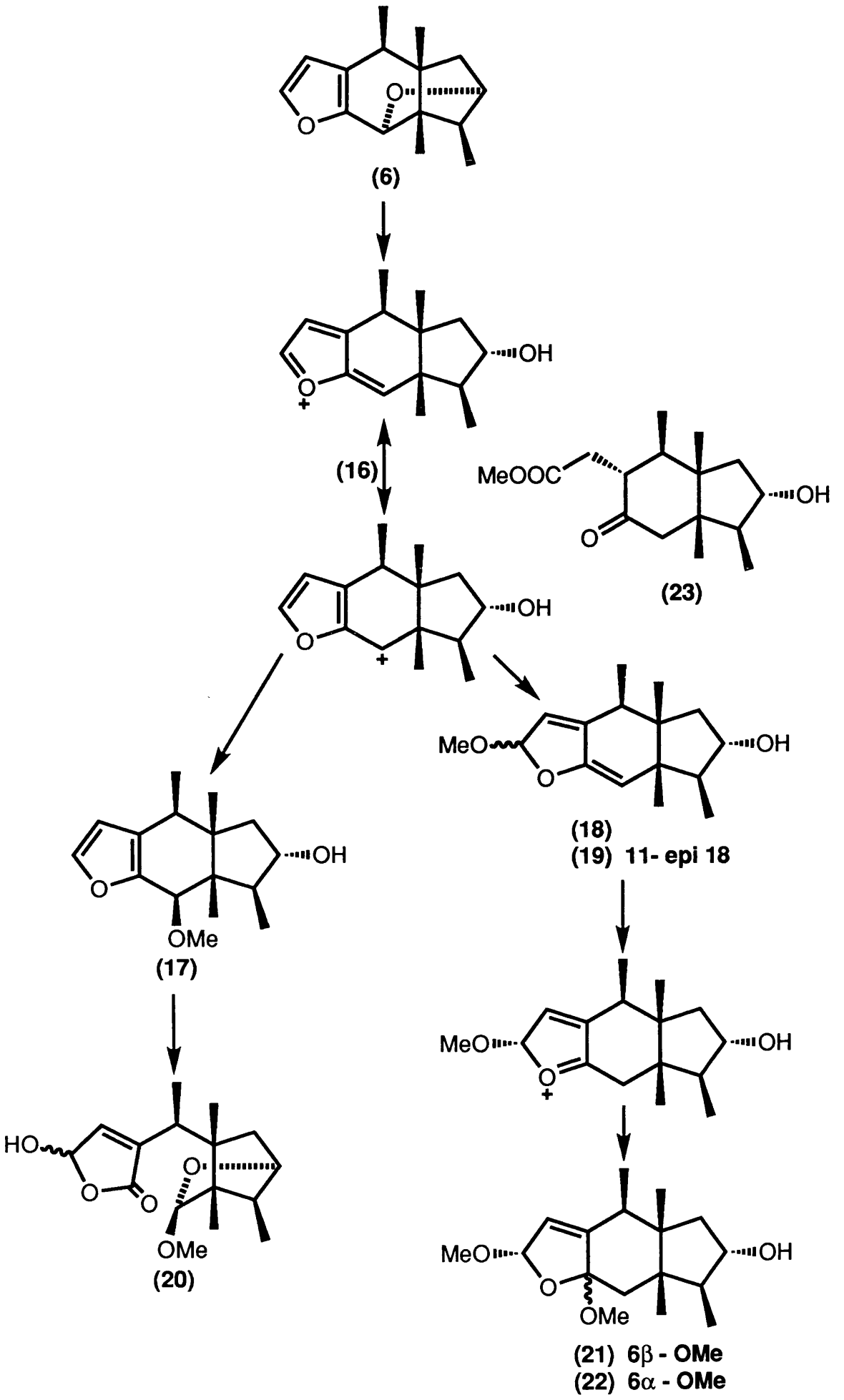
by comparison of its ^{1}H NMR data with published data[2]. The long storage time of the ground liverwort is probably a factor in the failure to detect **8** and **9**.

Extract C

Chromatography of extract C, from the liverwort collected near Clapham, Yorkshire in November 1990, resulted in the isolation of the main constituent pinguisanin (**6**)[6]. From the minor constituents present in this extract, porella-pinguisanolide (**14**)[13] and isopinguisanin (**15**)[5] were isolated. The known compounds **6** and **14** were identified by comparison of their spectroscopic data with published data for these compounds[6,13]. Porellapinguisanolide (**14**), was first isolated from the liverwort *Porella cordaeana*[13] and there is no previous report of it in *P. platyphylla*. Isopinguisanin (**15**) has previously been reported from *P. platyphylla*[5], however, its spectroscopic data were not published and are given in the Experimental. Compound **15** was identified by comparison of its spectroscopic data with unpublished data[19]. Isopinguisanin (**15**) is believed to be an artifact, produced from compounds such as pinguisanin (**6**) in acidic conditions, with the allylic cation **16** as intermediate. It was also formed by exposure of the crystalline, unstable alcohol **7** to acid[5]. Although other minor constituents were present in this extract, it was not possible to isolate them.

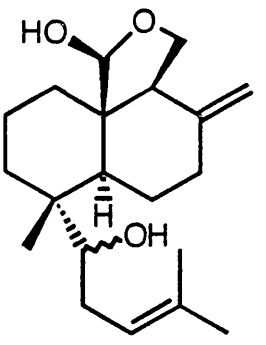
Extract D

This sample of *P. platyphylla* was collected near Ingleton, Yorkshire in August 1992 and was extracted with methanol using a Soxhlet extractor. This liverwort extract was prepared by Mr. G. Hoizey, Reims, France as part of a


(6)
OH
(16)
MeOOC
OH
(23)
OH
MeO
OH
(18)
(19) 11- epi 18
OH
OMe
(17)
MeO
OH
HO
OMe
(20)
MeO
OH
MeO
OH
OMe
(21) 6β - OMe
(22) 6α - OMe

Scheme 1

summer project. Methanol was used in order to maximise the yield of extract. The crude extract afforded a series of seven methoxylated pinguisanes (**17-23**) and a new sacculatane-type diterpenoid (**24**). The seven methoxylated pinguisanes are probably artifacts. These were isolated with the known compounds pinguisanin (**6**)[6], ß-pinguisenediol (**13**)[2], porellapinguisanolide (**14**)[13], perrottetianal B (**3**)[20] and atraric acid (**25**)[21-23] which were identified by comparison of their spectroscopic data with those of published data[2,6,13,20-22]. There is no known previous report of ^{13}C NMR data for ß-pinguisenediol (**13**) and these are given in the Experimental. Atraric acid (**25**) is a structural component of lichen depsides[21,22]



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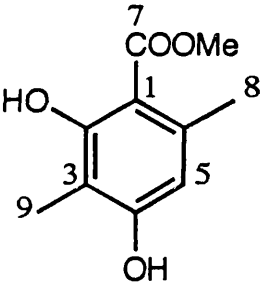


(25)

and is also the major odoriferous constituent of oakmoss *Evernia prunastri*[23]. Benzoic acid derivatives of this type have not been detected in liverworts previously and the isolation of such a minute amount (< 1mg) of **25** from *P. platyphylla* suggests that it may have come from a different source, e.g. a moss or lichen contaminent. Pinguisanin (**6**) and methoxylated pinguisanes **18** and **19** are the main constituents.

The least polar methoxylated pinguisane (**17**) in the series has the molecular formula $C_{16}H_{24}O_3$ (m/z 264.1744 $[M]^+$). The IR spectrum shows the presence of

a hydroxyl group (ν_{max} 3364 cm^{-1}). The ^1H NMR spectrum (Table 1) contains the signals for two protons of an α,ß-disubstituted furan ring [δ_H 7.33 (d, J = 1.9 Hz, H-11); 6.23 (br d, J = 1.9 Hz, H-10)], two tertiary methyl groups [δ_H 0.94 (s, 3H-12); 0.83 (s, 3H-14)], two secondary methyl groups [δ_H 1.17 (d, J = 7.2 Hz, 3H-15); 0.98 (d, J = 7.0 Hz, 3H-13)], a methoxyl group [δ_H 3.48 (s, 7-OMe)], one allylic proton which is coupled with a secondary methyl [δ_H 2.91 (br q, J = 7.2 Hz, H-4)] and two methine protons attached to carbons bearing oxygen [δ_H 3.96 (ddd, J = 9.0, 7.0, 1.9 Hz, H-2); 3.72 (br s, H-7)]. The ^{13}C NMR spectrum (Table 2) also shows the presence of a disubstituted furan [δ_C 148.8 (s, C-6); 120.4 (s, C-5); 142.3 (d, C-11); 109.1 (d, C-10)], two oxygenated methines [δ_C 79.2 (d, C-2); 74.8 (d, C-7)] and a methoxyl group [δ_C 59.1 (q, 7-OMe)] in addition to resonances for two quaternary sp^3 carbons, two methines, one methylene and four methyls. The above data indicated that **17** is a bicyclic sesquiterpene alcohol with an α,ß-disubstituted furan ring and a methoxyl group. Decoupling experiments established the connectivity of the protons and led to the two partial structures **26** and **27**. The spectral data are very similar to those of the co-metabolite pinguisanin (**6**), (see Table 2 and Experimental)[6], except for the presence of methoxy and hydroxy groups and the absence of an ether function. The position of attachment of both the methoxyl and hydroxyl groups was established by NOE difference experiments (Table 3). The NOEs between the 7-OMe (2.0%) and H-7 (7.0%) confirm the attachment of the methoxyl group at C-7. Thus the hydroxyl group is attached to C-2, the coupling pattern of H-2 (ddd, J = 9.0, 7.0, 1.9 Hz) indicates an α-configuration of the hydroxyl group[13]. Additional support for structure **17** comes from the mass spectrum which reveals a very intense fragment

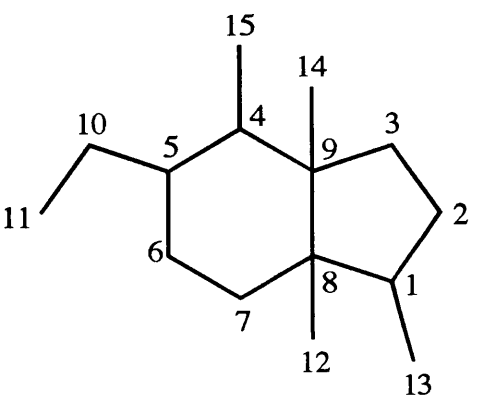
Table 1. ^{1}H **NMR data of pinguisanes**[a].

H	17	18	19
1	1.80 quin. (7.0)	2.05 quin. (7.0)	2.04 quin. (7.0)
2	3.96 ddd (9.0,7.0,1.9)	3.82 ddd (9.2,7.0,3.2)	3.80 ddd (9.2,7.0,3.4)
3	1.59 dd (14.1,1.9) α	1.67 dd (14.8,3.2) α	1.67 dd (14.8,3.4) α
	1.90 dd (14.1,9.0) ß	1.87 dd (14.8,9.2) ß	1.85 dd (14.8,9.2) ß
4	2.91 br q (7.2)	2.86 br qt (6.7,2.3)	2.87 br qt (6.7,2.0)
7	3.72 br s	4.65 br s	4.63 br s
10	6.23 br d (1.9)	5.53 ddd (2.5,1.9,1.5)	5.55 ddd (2.3,1.8,1.5)
11	7.33 d (1.9)	5.95 br dd (2.1,1.5)	5.82 br t (1.5)
12	0.94 s	0.79 s	0.76 s
13	0.98 d (7.0)	0.97 d (7.0)	0.96 d (7.0)
14	0.83 s	0.68 s	0.60 s
15	1.17 d (7.2)	1.15 d (6.7)	1.13 d (6.7)
6-OMe			
7-OMe	3.48 s		
11-OMe		3.38 s	3.38 s
OH	1.72 br s	1.98 br s	2.22 br s

[a] Figures in parentheses are coupling constants (*J*) in Hz.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

* Assignments may be interchangeable.



Pinguisane skeleton

Table 1. ^{1}H NMR data of pinguisanes continued...

H	20[b]	21[b]	22
1	2.32 br q (7.5)	2.72 br quin. (7.2)	2.75 br quin. (7.0)
2	4.05 m	3.88 ddd (9.3,7.8,3.1)	3.89 ddd (9.2,7.9,3.1)
3	1.79 br dd (14.0,1.3)	1.71 dd (14.8,3.1) α	1.71 dd (14.8,3.1) α
	1.72 br dd (14.0,1.6)	1.88 dd (14.8,9.3) ß	1.89 dd (14.8,9.2) ß
4	2.86 br q (7.0)	2.64 qt (6.7,1.9)	2.64 br q (6.8)
7	5.25 s	2.06 d (14.3) α	2.12 d (14.2) α
		1.48 d (14.3) ß	1.27 d (14.2) ß
10	6.99 br d (1.4)	5.53 dd (1.9,0.7)	5.53 t (1.4)
11	6.10 d (1.4)	5.77 dd (1.9,0.7)	5.37 t (1.2)
12	0.80 s	0.63 s	0.63 s*
13	0.97 d (7.5)	0.97 d (6.8)	0.97 d (6.8)
14	1.13 s	0.70 s	0.62 s*
15	1.12 d (7.0)	1.09 d (6.7)	1.09 d (6.8)
6-OMe		3.02 s	3.12 s
7-OMe	3.35 s		
11-OMe		3.39 s	3.47 s
OH	4.81 br s	1.61 br s	1.57 br s

[a] Figures in parentheses are coupling constants (J) in Hz.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

* Assignments may be interchangeable.

Table 2. ^{13}C NMR data of pinguisanes.

C	6[a]	17	18	19
1	53.0 d	46.8 d	52.1 d	51.8 d
2	79.4 d	79.2 d	78.1 d	78.0 d
3	47.8 t	45.1 t	43.3 t	43.4 t
4	38.9 d	34.2 d	35.3 d	35.4 d
5	122.4 s	120.4 s	143.4 s *	143.0 s *
6	149.1 s	148.8 s	154.7 s *	154.7 s *
7	75.9 d	74.8 d	100.4 d#	100.7 d#
8	51.1 s	46.2 s°	48.2 s°	47.9 s°
9	40.3 s	52.1 s°	48.4 s°	48.4 s
10	110.1 d	109.1 d	119.9 d#	120.2 d#
11	142.6 d	142.3 d	109.5 d#	108.9 d#
12	12.9 q	14.9 q+	19.2 q+	19.2 q+
13	12.5 q	14.7 q+	12.4 q+	12.5 q+
14	25.2 q	15.6 q+	15.3 q+	15.3 q+
15	20.0 q	12.6 q+	12.3 q+	12.1 q+
6-OMe				
7-OMe		59.1 q		
11-OMe			54.3 q	54.7 q

[a] These data agree with those in ref 6.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

o+*# Assignments may be interchanged in each vertical column.

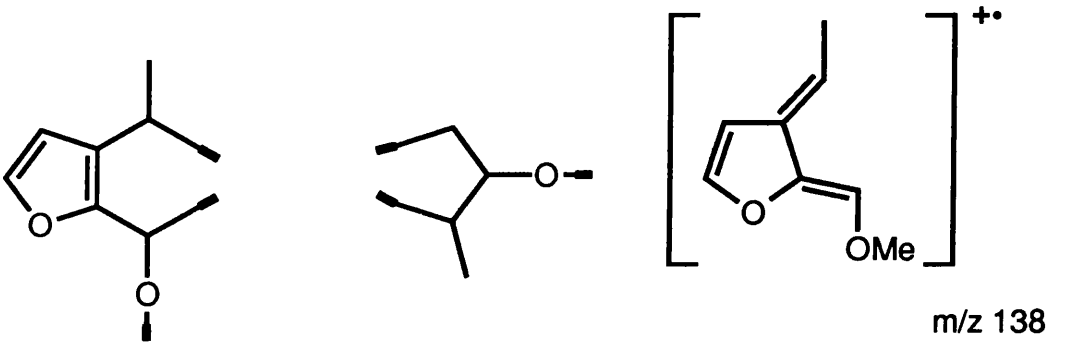
Table 2. ^{13}C NMR data of pinguisanes continued...

C	20[b]	21[b]	22
1	46.0 d	46.0 d	46.0 d
2	79.3 d	78.2 d	78.2 d
3	45.2 t	44.0 t	44.1 t
4	35.5 d	35.5 d	35.6 d
5	141.2 s	148.4 s	148.1 s
6	171.8 s	111.8 s	109.7 s
7	105.5 d	43.2 t	43.4 t
8	54.0 s	47.4 s	47.3 s
9	43.6 s	49.1 s	49.1 s
10	146.1 d	121.6 d	120.9 d
11	96.4 d	108.0 d	106.2 d
12	10.1 q	20.1 q	20.1 q
13	11.8 q	12.2 q	12.2 q
14	19.2 q	14.6 q	14.3 q
15	19.0 q	12.0 q	11.9 q
6-OMe		49.2 q	49.7 q
7-OMe	55.2 q		
11-OMe		54.5 q	56.0 q

[a] These data agree with those in ref 6.

[b] Assignments confirmed by 2D direct and long-range δ_C / δ_H correlation experiments.

o+*# Assignments may be interchanged in each vertical column.



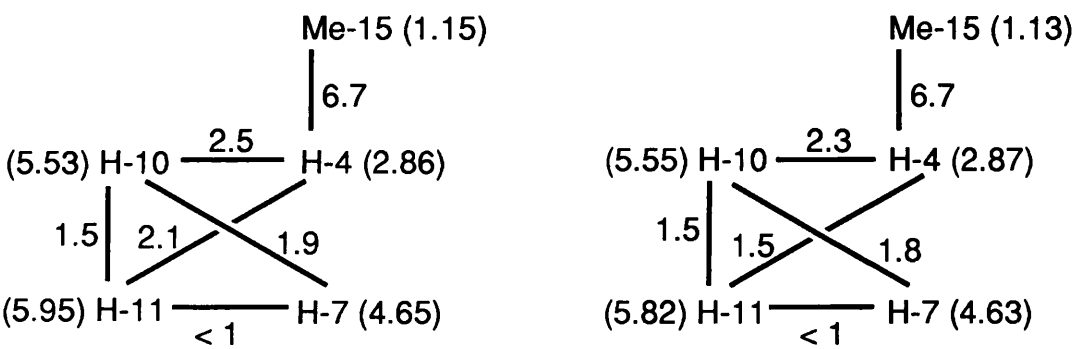
(26) (27) (28)

Table 3. NOEs of compound **17.**

NOE at proton (%)

Irradiated proton	10	7	7-OMe	4	1	3α	15	13	14
7			2.0		2.3			0.8	
7-OMe		7.0							
4					10.7	3.6	1.8		
15	6.7			11.6		7.4			2.4

Scheme 2. Chemical shifts and coupling constants of compounds **18** and **19**[*].



(18) **(19)**

[*] Chemical shifts in parenthesis.

ion at m/z 138 (**28**) due to a retro-Diels-Alder reaction, typical of furano condensed pinguisanes[11,24,25]. The configuration of the methoxyl group at C-7 was more difficult to establish. However examination of molecular models, in conjunction with NOE difference results (Table 3) and coupling information, suggested a preferred conformation in which the six-membered ring is a boat with the secondary methyl at C-4 and the methoxyl at C-7 both pseudoequatorially and ß-orientated. There is a small but significant coupling involving H-4, H-7 and H-10, resulting in broadening of their resonances. In this conformation there is favourable geometry for these couplings [^5J (H-10, H-7) was established as 0.5 Hz]. The structure **17**, 6,11-epoxy-2α-hydroxy-7ß-methoxypinguisa-5,10-diene, is proposed for this compound.

In order of increasing polarity the next methoxylated pinguisane (**18**) in the series has the molecular formula $C_{16}H_{24}O_3$ (m/z 264.1734 $[M]^+$). The IR spectrum of **18** shows the presence of a hydroxyl group (ν_{max} 3428 cm^{-1}) and the UV spectrum (λ_{max} 263.4 nm) suggests the presence of a conjugated diene. The ^1H NMR spectrum (Table 1) has resonances for two secondary methyl groups [δ_H 1.15 (d, J = 6.7 Hz, 3H-15); 0.97 (d, J = 7.0 Hz, 3H-13)], two tertiary methyl groups [δ_H 0.79 (s, 3H-12); 0.68 (s, 3H-14)], a methoxyl group [δ_H 3.38 (s, 11-OMe)], two olefinic protons [δ_H 5.53 (ddd, J = 2.5, 1.9, 1.5 Hz, H-10); 4.65 (br s, H-7)] and an allylic proton [δ_H 2.86 (br qt, J = 6.7, 2.3 Hz, H-4)]. Also present is the AB portion [δ_H 1.87 (dd, J = 14.8, 9.2 Hz, H-3ß); 1.67 (dd, J = 14.8, 3.2 Hz, H-3α)] of an ABX system involving an oxygenated methine [δ_H 3.82 (ddd, J = 9.2, 7.0, 3.2 Hz, H-2)], which is further coupled to a vicinal proton [δ_H 2.05 (quin., J = 7.0 Hz)], a strongly deshielded methine [δ_H 5.95 (br dd, J = 2.1, 1.5 Hz, H-11)] and

a hydroxyl proton [δ_H 1.98 (br s, OH)]. The ^{13}C NMR spectrum (Table 2) also suggested the presence of an acetal and two trisubstituted double bonds [δ_C 119.9, 109.5, 100.4 (all d, C-7, C-10 and C-11); 154.7, 143.4 (both s, C-5 and C-6)], a methoxyl [δ_C 54.3 (q, 11-OMe)] and a secondary alcohol [δ_C 78.1 (d, C-2)]. In addition there are resonances for four methyls, one methylene, two methines and two quaternary carbons. These data led to structure **18** with only the configuration at C-11 to be established.

The next compound (**19**) is only slightly more polar than **18** and has the same molecular formula $C_{16}H_{24}O_3$ (m/z 264.1714 $[M]^+$). The spectroscopic properties of **19** are virtually identical to those of **18** (see Experimental and Tables 1 and 2). The spin-spin couplings were assigned from decoupling experiments and the couplings between H-4, H-7, H-10, H-11 and 3H-15 are shown in Scheme 2. The main difference between the two compounds is that H-11 and 3H-14 are slightly more shielded in **19** and H-11 has a smaller homoallylic coupling to H-4 (Scheme 2). These data indicate that these two compounds are epimeric at C-11.

In order to establish the relative stereochemistry and distinguish between the epimers NOE difference experiments were carried out (Tables 4 and 5). The large NOEs observed between the methoxyl protons and H-11 confirmed that the methoxyl group is attached to C-11 while the NOEs observed between (i) 3H-15 and 3H-14 (ii) 3H-14 and 3H-12, and (iii) 3H-12 and 3H-13 indicate that all the methyl groups have the expected ß-orientation of pinguisanes. NOE difference experiments did not prove to be very helpful in establishing the stereochemistry at C-11. Neither were the chemical shift differences of 3H-14 and H-11 between **18** and **19** useful in this respect. If these differences are due to the 11-OMe stereochemistry one might also have

Table 4. NOEs of compound **18.**

Irradiated proton	NOE at proton (%)												
	11	10	7	2	11-OMe	4	1	3β	3α	15	13	12	14
11		3.0			1.7								
11-OMe	8.2												
4							12.2		2.7	2.2			
1			1.7			18.7					2.4		
15		10.4				9.8			6.1				1.8
12			13.8	8.6				4.2			1.3		0.9
14								6.6	1.6	2.1		0.7	

Table 5. NOEs of compound **19.**

Irradiated proton	NOE at proton (%)												
	11	10	7	2	11-OMe	4	1	3β	3α	15	13	12	14
11		3.8			1.6								
11-OMe	7.8												
4							14.9		3.1	2.1			
1			2.0	2.0		18.8					2.5		
15		11.3				9.8			6.5				1.8
12			15.8	8.7				4.3			1.8		1.2
14								5.8	1.2	2.3		0.4	

expected significant changes in the 11-OMe and H-4 chemical shifts, which however do not occur. From the data of **18** and **19** (Scheme 2) the homoallylic coupling ^5J (H-4, H-11) is apparently dependent on whether the protons (H-4 and H-11) are syn or anti to each other, but literature data could not be found which would enable this to be used for assignment.

Compounds **18** and **19** are therefore 6,11-epoxy-2α-hydroxy-11ß-methoxy-pinguisa-5(10),6-diene and its C-11 epimer. These two compounds have the six-membered ring in a half chair conformation with 3H-15 pseudoequatorial and C-9 the out of plane atom. This conformation permits the observed allylic coupling between H-4 and H-10 (Scheme 2). The large NOEs observed between H-4 and H-1 are particularly indicative of the pseudoequatorial nature of 3H-15 and also the puckered (near envelope) conformation of the cyclopentane ring.

Compound **20** shows no parent ion peak in its mass spectrum. The IR spectrum exhibits the presence of hydroxyl (ν_{max} 3300 cm^{-1}) and butenolide (ν_{max} 1780 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) contains the signals for two tertiary methyl groups [δ_H 1.13 (s, 3H-14); 0.80 (s, 3H-12)], two secondary methyl groups [δ_H 1.12 (d, J = 7.0 Hz, 3H-15); 0.97 (d, J = 7.5 Hz, 3H-13)], a methoxyl group [δ_H 3.35 (s, 7-OMe)], an olefinic proton [δ_H 6.99 (br d, J = 1.4 Hz, H-10)] and an allylic proton coupled to a secondary methyl [δ_H 2.86 (br q, J = 7.0 Hz, H-4)]. Also present are two acetal protons [δ_H 5.25 (s, H-7); 6.10 (d, J = 1.4 Hz, H-11)], one of which is probably allylic, and an ether proton [δ_H 4.05 (m, H-2)]. The ^{13}C NMR spectrum (Table 2) exhibited the signals due to an α,ß-unsaturated lactone [δ_C 171.8 (s, C-6); 146.1 (d, C-10); 141.2 (s, C-5)], a methoxyl [δ_C 55.2 (q, 7-OMe)], an acetal [δ_C 105.5 (d, C-7)], a hemiacetal [δ_C 96.4 (d, C-11)] and an ether terminus carbon [δ_C 79.3 (d, C-2)]. The

^{13}C NMR spectrum indicated the presence of sixteen carbons including further signals for four methyls, one methylene, two methines and two quaternary carbons. These data suggest that compound **20** has the molecular formulae $C_{16}H_{24}O_5$. Proton decoupling studies of **20** revealed some connectivity. Irradiation of the multiplet at δ_H 4.05 (H-2) sharpened the signal at δ_H 2.32 (H-1) and simplified the signals at δ_H 1.79 and 1.72 changing them into a distinct AB system (J = 14.0 Hz). The couplings of H-2 are 1.6 [^3J (H-2, H-3)], 1.3 [^3J (H-2, H-3)] and 2.1 [^3J (H-2, H-1)] Hz. Irradiation of the olefinic proton at δ_H 6.99 (H-10) sharpened the quartet at δ_H 2.86 (H-4). These data, along with the 2D direct δ_C /δ_H correlation (which enabled the assignments of all protons and carbons), suggested structure **29** with the attachment of the hydroxyl and methoxyl groups outstanding. The olefinic hydrogen [δ_H 6.99 (br d, J = 1.4 Hz, H-10)] is placed ß to the carbonyl group because of its lowfield chemical shift. The 2D long-range δ_C /δ_H correlation (Table 6) confirmed structure **29** and also established positions of attachment of the hydroxyl and methoxyl groups. The protons of the methoxyl group (δ_H 3.35) have correlations ($^3J_{CH}$) to the carbon at δ_C 105.5 (C-7) which in turn correlated with H-2 (δ_H 4.05) (^3J *via* ether oxygen) and 3H-12 (δ_H 0.80). These results indicate the methoxyl attachment to C-7 and also confirm an ether linkage between C-2 and C-7. The hydroxyl group must therefore be attached to C-11, as part of a hydroxybutenolide ring. The methoxyl attachment site was further supported by NOEs observed between H-7 (7.0%) and 7-OMe (3.4%). The stereochemistry at C-7 was established as *R* on the basis of the very large NOEs observed between H-4 (21.4%) and H-7 (19.4%). On irradiation of H-4 there is a -ve NOE, arising from the three spin effect[26], at the 7-OMe (-0.5%). In the reverse experiment irradiation of the 7-OMe affords a -ve NOE at H-4 (-0.7%).

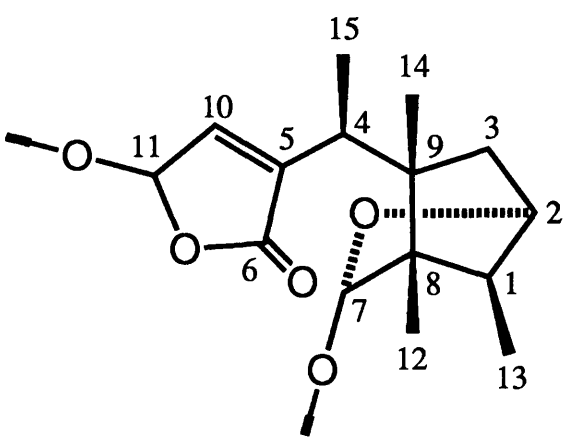
Table 6. 2D long-range δ_C / δ_H correlations for compound 20.

H	Correlated C
H-1	3,9
H-2	7,8,9
2H-3*	1,4,9 (14,15)+
H-4	5,9,10 (14,15)+
H-7	1,2,7-OMe
H-10	5,6,11
3H-12	1,7,8,9
3H-13	1,2,8
(3H-14, 3H-15)#	3,4,5,8
7-OMe	7

* H-3α and H-3ß are very close in chemical shift.

+ C-14 and C-15 are very close in chemical shift.

3H-14 and 3H-15 have overlapping signals.



(29)

In the ^{1}H and ^{13}C NMR spectra of compound **20** some of the signals are broad and, in some spectra, these resonances are observed as doubled signals. The signals most affected are those associated with 7-OMe, H-7, 3H-12, 3H-14, 3H-15, C-10 and C-11. It is clear that there is a relatively slow exchange (on the NMR timescale) between the C-11 epimers of the hemiacetal function. H-11 appears relatively unaffected. GLC analysis of compound **20** resulted in only one peak on the chromatogram (T_R = 27.10 mins); similar analysis of the TMS ether of **20** gave two peaks (T_R = 27.35, 27.70 mins) in the ratio 4:5. This confirms the presence of the C-11 epimers.

Compound **20** contains an oxa-2,2,1-bicycloheptane system with an *exo*-methoxyl substituent at C-7 and an *endo*-substituent at C-9 containing the hydroxybutenolide group. This arrangement accounts for the significant four-bond W coupling (^{4}J) between H-3α and H-1 since there is close to ideal planar W geometry.

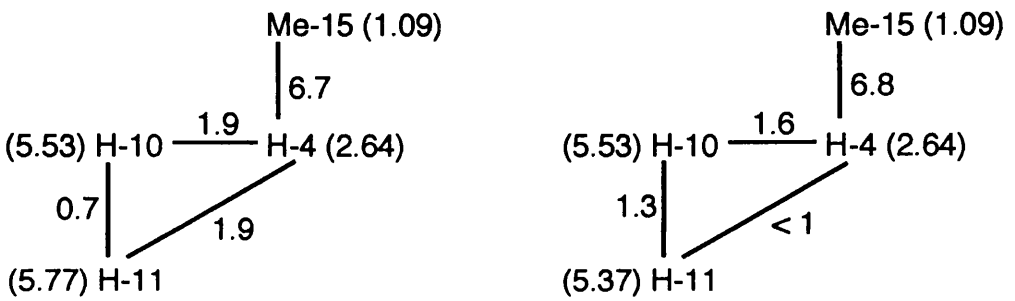
Compound **21** is the next methoxylated pinguisane in the sequence. It shows the presence of a hydroxyl group (ν_{max} 3351 cm^{-1}) in its IR spectrum. The ^{1}H NMR spectrum (Table 1) contains the signals for two secondary methyl groups [δ_H 1.09 (d, J = 6.7 Hz, 3H-15); 0.97 (d, J = 6.8 Hz, 3H-13)], two tertiary methyl groups [δ_H 0.70 (s, 3H-14); 0.63 (s, 3H-12)], two methoxyl groups [δ_H 3.39 (s, 11-OMe); 3.02 (s, 6-OMe)], an isolated methylene group [δ_H 2.06 (d, J = 14.3 Hz, H-7α); 1.48 (d, J = 14.3 Hz, H-7ß)], a vinyl proton [δ_H 5.53 (dd, J = 1.9, 0.7 Hz, H-10)] and an allylic proton attached to an acetal carbon [δ_H 5.77 (dd, J = 1.9, 0.7

Hz, H-11)]. Also present is an AB portion of an ABX spin system [δ_H 1.71 (dd, J = 14.8, 3.1 Hz, H-3α); 1.88 (dd, J = 14.8, 9.3 Hz, H-3ß)] involving a proton attached to an oxygenated carbon [δ_H 3.88 (ddd, J = 9.3, 7.8, 3.1 Hz, H-2)], with a vicinal neighbour [δ_H 2.72 (br quin., J = 7.2 Hz)], and a hydroxyl group [δ_H 1.61 (br s, OH)]. The ^{13}C NMR spectrum (Table 2) also suggested the presence of a trisubstituted double bond [δ_C 121.6 (d, C-10); 148.4 (s, C-5)], two allylic acetals [δ_C 108.0 (d, C-11); 111.8 (s, C-6)], a secondary alcohol [δ_C 78.2 (d, C-2)] and two methoxyl groups [δ_C 54.5 (q, 11-OMe); 49.2 (q, 6-OMe)]. The remaining signals arise from four methyls, two methylenes, two methines and two quaternary carbons making a total of seventeen carbons. These data along with 2D direct and long-range δ_C / δ_H correlation and NOE difference experiments allowed complete assignment of all proton and carbon resonances and led to the pinguisane structure **21**. The assignment of the quaternary carbons C-8 and C-9 is based on their 2D long-range δ_C / δ_H correlations (Table 7). Thus C-9 (δ_C 49.1) correlates with 3H-15, 3H-14 and 3H-12 while C-8 (δ_C 47.4) correlates with 3H-13, 3H-14 and 3H-12. The relative stereochemistry was deduced from NOE difference experiments. Thus NOEs observed between (i) 3H-12 (1.4%) and H-3ß(6.0%) (ii) 3H-12 (0.7%) and 3H-13 (1.4%) (iii) 3H-14 (1.9%) and H-3ß (6.0%), and (iv) 3H-14 (1.1%) and 3H-15 (1.4%) confirm the *cis* junction between the five- and six-membered rings and the ß-orientation of the four methyl groups normally found in pinguisanes. The NOEs between H-11 (3.9%) and the 6-OMe (0.8%) suggest that the methoxyl groups are on opposite sides of the molecule. The fact that there are no NOEs observed between the 6-OMe and H-1, H-4 or H-7α suggests that the C-6 methoxyl group is ß-orientated and therefore the C-11 methoxyl group α-orientated. These

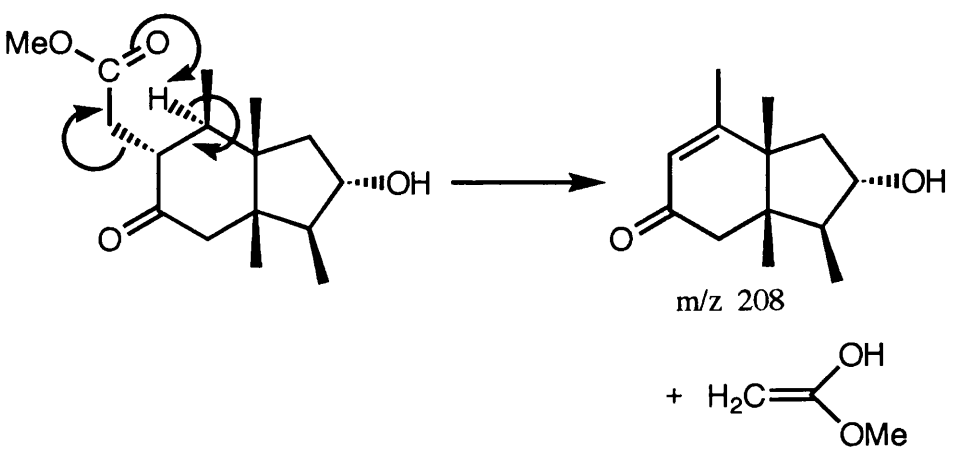
Table 7. 2D long-range δ_C / δ_H correlations for compound 21.

H	Correlated C
H-1	12
3H-12	1,7,8,9
3H-13	8
3H-14	8,9
3H-15	5,9
11-OMe	11

Scheme 3. Chemical shifts and coupling constants of compounds **21**and **22**[*].



[*] Chemical shifts in parenthesis.



Scheme 4

arguments lead to stereostructure **21**.

Compound **22** is slightly more polar than **21**. The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of the two are almost identical suggesting they are stereoisomers. The stereostructure of **22** was clarified by the NOEs between 11-OMe (0.5%) and 6-OMe (0.3%) and between 6-OMe (0.6%) and H-4 (2.4%). These results indicate that both methoxyl groups are α-orientated.

Both compounds **21** and **22** have their six-membered ring in a twist conformation. This was indicated by the NOEs between (i) 3H-15 and H-3α (ii) 3H-15 and H-10, and (iii) H-7ß and H-1. The pinguisane conformation is a result of the *cis* junction of the five- and six-membered rings and the mutual repulsion of the four ß-orientated methyls. As a result H-1 and H-4 are close in space and afford large NOEs. This is a common situation in pinguisanes (see Tables 3-5). In compound **21**, with a ß-orientated C-6 methoxyl, a pseudoequatorial orientation of the 2,5-dihydrofuran ring is adopted whereas in compound **22**, with an α-orientated C-6 methoxyl, a pseudoaxial orientation occurs. Decoupling experiments carried out on **21** and **22** revealed the couplings involving H-11, H-10 and H-4 shown in Scheme 3. The allylic coupling ^{4}J (H-4, H-10) and, in particular, the homoallylic coupling ^{5}J (H-4, H-11) are smaller for **22**, indicating that the pseudoaxial orientation of the 2,5-dihydrofuran causes a less favourable geometry. Maximum allylic coupling (J ~ 3.0 Hz) is observed when the allylic C-H bond is perpendicular to the C=C plane[15-17].

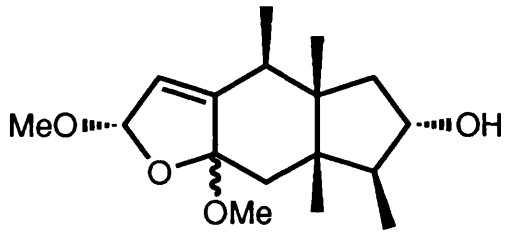
The last in the series of methoxylated pinguisanes is compound **23** which has the molecular formula $C_{16}H_{26}O_4$ (m/z 282.1829 $[M]^+$) indicating four double bond equivalents. The IR spectrum shows the presence of a hydroxyl group (ν_{max} 3467

cm^{-1}), a six-membered ketone (ν_{max} 1717 cm^{-1}) and a saturated ester group (ν_{max} 1740 cm^{-1}). The ^1H NMR spectrum (see Experimental) has signals for two tertiary methyl groups [δ_H 1.01 (s, 3H-14); 0.76 (s, 3H-12)], two secondary methyl groups [δ_H 0.96 (d, J = 6.7 Hz, 3H-15); 0.92 (d, J = 6.9 Hz, 3H-13)], a methoxyl group [δ_H 3.65 (s, 11-OMe)], a secondary alcohol [δ_H 3.85 (ddd, J = 9.0, 7.4, 2.8 Hz, H-2)] and a hydroxyl proton [δ_H 1.57 (br s, OH)]. The ^{13}C NMR spectrum (see Experimental) confirmed the presence of a six-membered ring ketone [δ_C 210.4 (s, C-6)], a methyl ester [δ_C 173.6 (s, C-11); 51.7 (q, 11-OMe)] and a secondary alcohol [δ_C 77.9 (d, C-2)]. The molecule is therefore bicarbocyclic. The five-membered ring portion of **23** was easily identified as the signal pattern of the ^1H NMR spectrum reveals signals similar to other pinguisanes that contain this structural unit[13]. This was confirmed by spin-spin decoupling which also gave the following additional information. Irradiation of the signal at δ_H 2.74 (dddd, J = 12.2, 8.4, 4.0, 0.8 Hz, H-5) caused the double doublet signals at δ_H 2.31 (J = 13.6, 0.8 Hz, H-7ß) and 2.29 (J = 16.2, 4.0 Hz, H-10) to collapse to doublets and the double quartet signal at δ_H 2.10 (J = 12.2, 6.7 Hz, H-4) to collapse to a quartet. There is a small coupling (J = 0.8 Hz) between H-9 and H-7ß which involves non-ideal W geometry. The situation is made more favourable by the intervening sp^2 hybridised carbon atom. The H-7ß proton has a geminal coupling (J = 13.6 Hz) to the doublet at δ_H 2.21 (H-7α), indicating an isolated methylene group. The vicinal coupling ^3J (H-5, H-4) of 12.2 Hz indicates a dihedral angle close to zero or 180°. Inspection of models indicate that the latter is more favourable since the C-4 methyl and the methyl acetate substituents are then both equatorial. The H-5 methine is also vicinally coupled to the C-10 methylene [δ_H 2.60 (dd, J = 16.2, 8.4

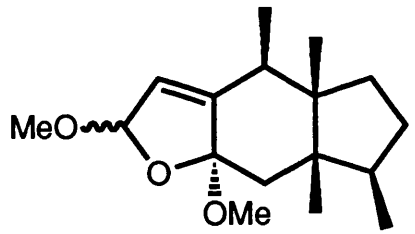
Hz, H-10); 2.29 (dd, J = 16.2, 4.0 Hz, H-10)]. The structure of **23** was therefore established as methyl 2α-hydroxy-6-oxo-11-pinguisanoate. Further evidence for the methyl acetate side chain of **23** came from the MS which shows a peak at m/z 208 arising from McLafferty rearrangement (Scheme 4).

The stereochemistry at C-5 was confirmed by NOE difference experiments. Thus NOEs are observed between H-5 and 3H-14 (1.2%) confirming the suggestion that H-5 and H-4 are *trans* diaxial and the methyl acetate side chain is α-orientated. The normal ß-configuration of all the methyls was confirmed by the NOEs observed between (i) 3H-12 and 3H-13 (1.5%) (ii) 3H-12 and 3H-14 (1.9%) (iii) H-5 and 3H-14 (1.2%) and (iv) H-5 and 3H-15 (0.8%). The six-membered ring in compound **23** has a chair conformation indicated by the following evidence. The NOEs observed between 3H-12 and both H-7ß (5.8%) and H-7α (4.3%) indicate that H-7ß must be axial and H-7α equatorial. The NOE between H-4 and H-1 (9.8%) implies that H-4 is axial.

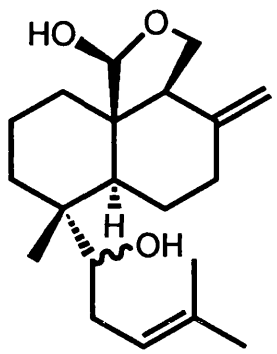
Recently three methoxylated pinguisane artifacts, without the hydroxyl group attached to C-2, have been isolated from *Trocholejeunea sandvicensis*[27]. Two of them (**30** and **31**) are similar to compounds **21** and **22** in that they also have methoxyl groups attached to C-6 and C-11. Compound **30** has the methoxyl groups in the same orientation as **22** (6α-OMe, 11α-OMe) however the orientation of the methoxyl groups in **31** (6α-OMe, 11ß-OMe) is different from that of **21** (6ß-OMe, 11α-OMe). If these are artifacts it is possible that all the isomers formed have not been isolated in both cases. Earlier work on the methanol extract of *Porella platyphylla*[5] resulted in the isolation of several methoxylated artifacts some of which have been obtained again in the present work (**18** and **19**). The



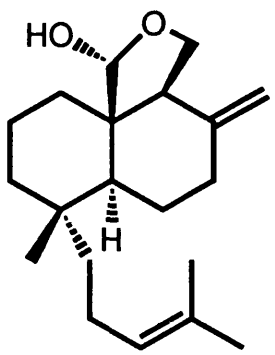
(21) 6β - OMe
(22) 6α - OMe



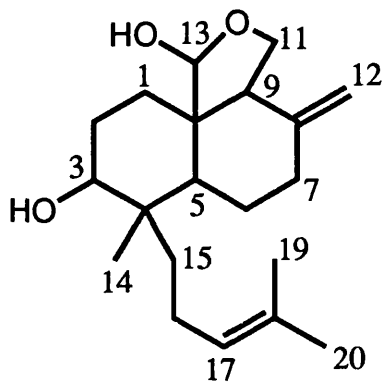
(30) 11α - OMe
(31) 11β - OMe



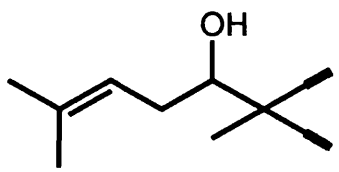
(24)



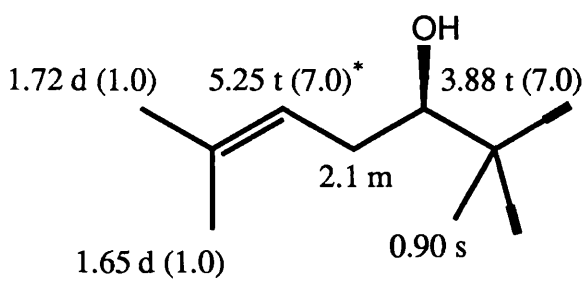
(32)



(33)



(34)



* Slightly split triplet.

Scheme 5

suggestion was made that these compounds arose by reaction of methanol with the cationic species, **16**. Treatment of pinguisanin (**6**) with methanol, silica gel and a trace of acid resulted in the formation of methoxylated products[5].

Scheme 1 shows how the pinguisanes **17-22** may have been produced. Like pinguisanin (**6**), methoxylated pinguisanes (**17-19, 21** and **22**) are easily detected by analytical TLC since their spots turn red immediately when sprayed with 25% H_2SO_4. On storage in the freezer for a year compound **17** was transformed completely into **20**. Compound **23**, the most polar of the pinguisane artifacts, may have been formed from the corresponding acid, by acid catalysed esterification with methanol. This pinguisane acid is a new natural product.

A crystalline diterpenoid (**24**), $C_{20}H_{32}O_3$ (m/z 320.2332 $[M]^+$), was isolated from this extract. The IR spectrum indicated the presence of a hydroxyl group (ν_{max} 3600, 3500 cm^{-1}). The ^1H NMR spectrum (Figure 1) (Table 8) contained signals for a tertiary methyl [δ_H 0.93 (s, 3H-14)], an exomethylene [δ_H 5.00 (br s, H-12*Z*); 4.86 (br s, H-12*E*)], an oxygenated methylene [δ_H 4.30 (dd, J = 9.2, 1.6 Hz, H-11ß); 3.88 (dd, J = 9.2, 6.1 Hz, H-11α)], two oxygenated methines [δ_H 5.31 (d, J = 10.0 Hz, H-13); 3.44 (ddd, J = 7.6, 4.8, 2.8 Hz, H-15)], one of which (δ_H 5.31) is coupled to a hydroxyl proton at δ_H 2.42 (d, J = 10.0 Hz, 13-OH) and is presumably associated with a hemiacetal (*vide infra*), and a dimethylallyl group [δ_H 5.17 (t sept., J = 7.5, 1.4 Hz, H-17); 1.74 (br s, 3H-19); 1.64 (br s, 3H-20)]. The mass spectrum gave an $[M-H_2O]^+$ fragment ion at m/z 302 and also confirmed the presence of a dimethylallyl group by the fragment ions at m/z 69 and m/z 233. The ^{13}C NMR spectrum (Figure 2) showed the presence of twenty carbons (Table 8); three methyls, six methylenes, two methines, two quaternary carbons, four sp^2

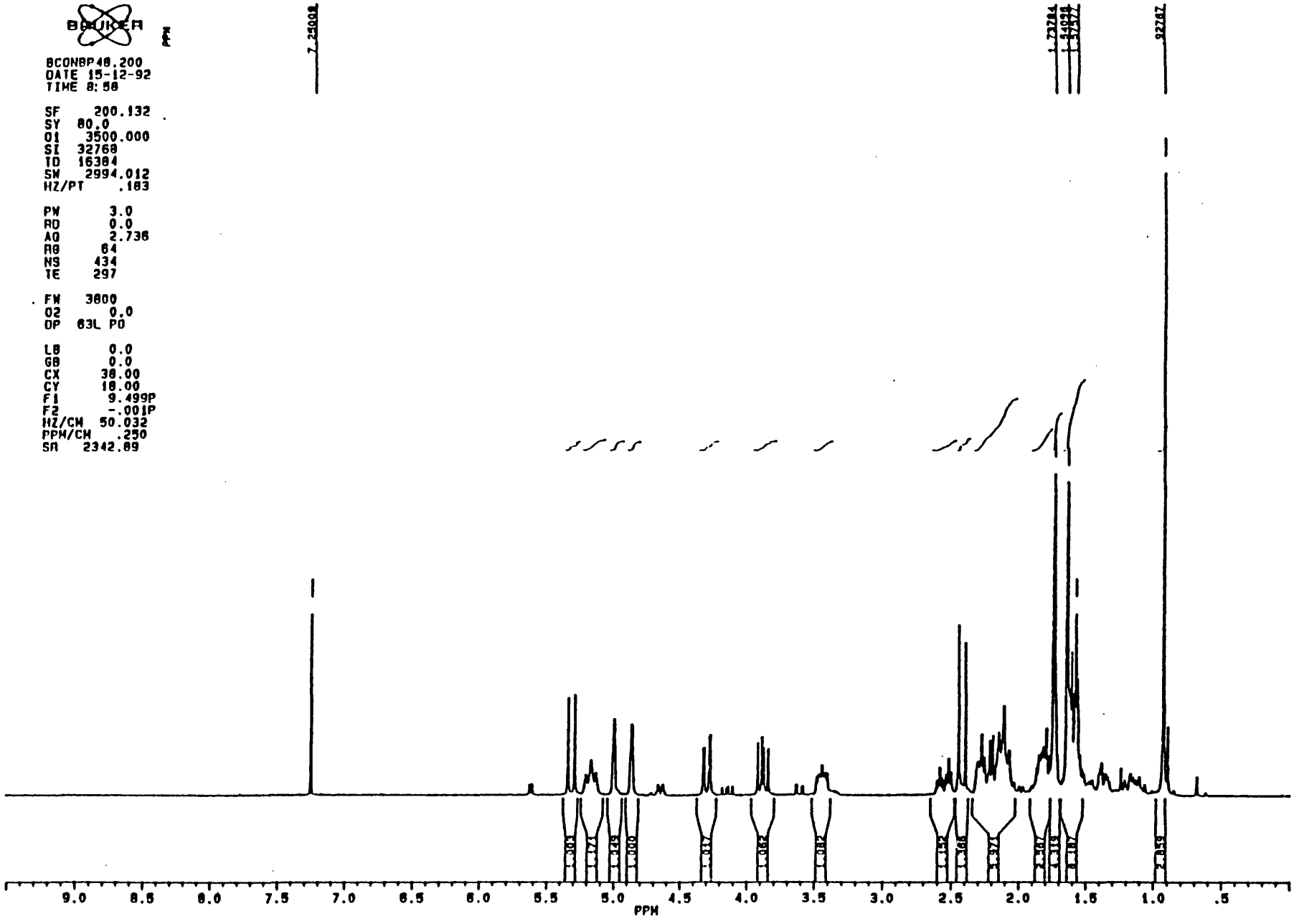


Figure 1. ^{1}H NMR spectrum of compound **24.**

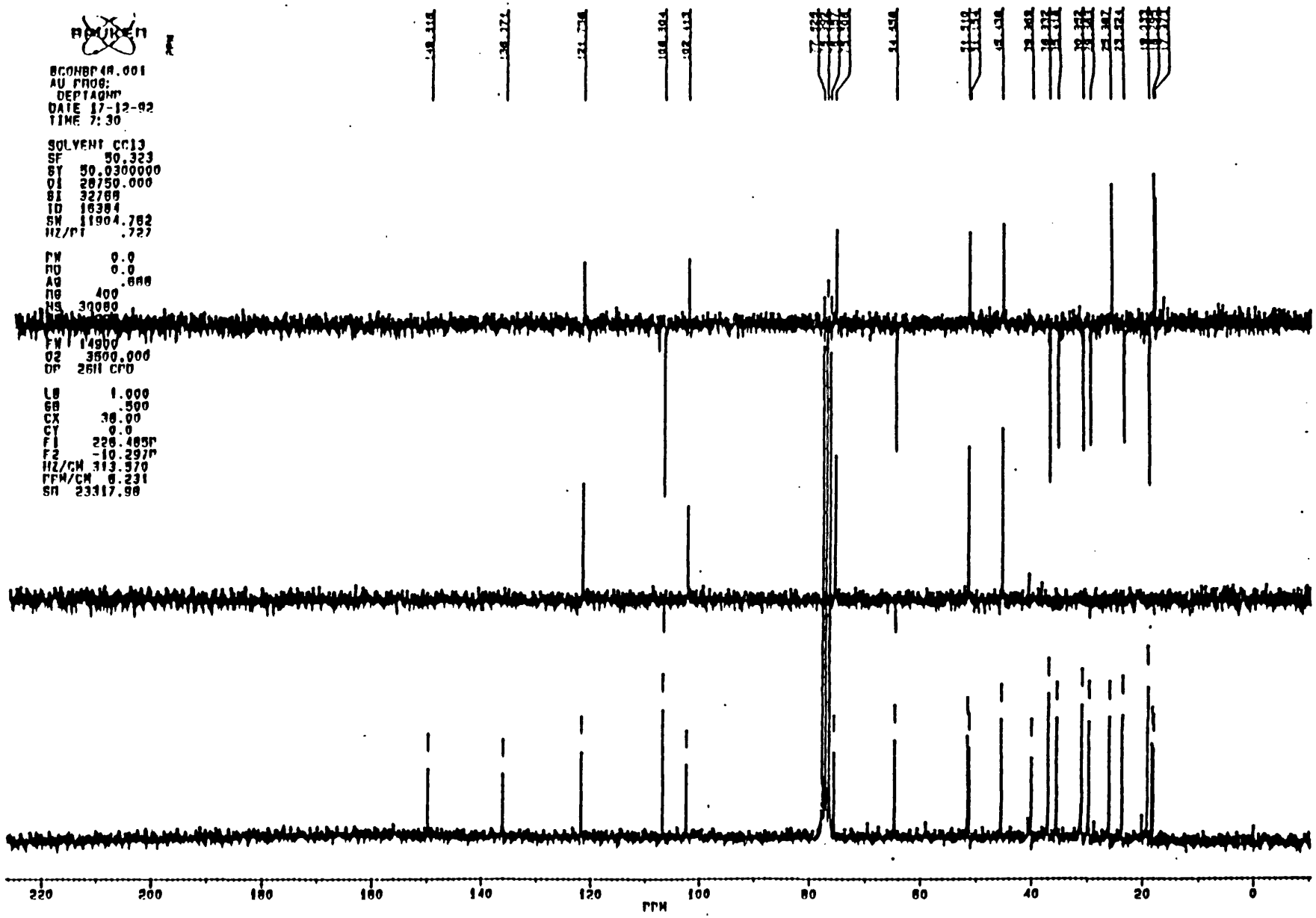


Figure 2. ^{13}C NMR and DEPT spectra of compound **24.**

Table 8. ^{1}H and ^{13}C NMR data of Sacculatanes 24 and 32[a].

	24[b]		32[c]	
Site.	δ_C	δ_H	δ_C	δ_H
1α	37.0 t	1.83 m	37.4 t	
ß		1.75 m		
2α	19.0 t	1.60 m (2H)	19.5 t	
ß				
3α	30.9 t	1.35-1.64 m (2H)	37.6 t	
ß				
4	39.9 s		36.1 s	
5	45.3 d	2.23 dd (12.0, 4.7)	49.0 d	1.66 dd (12.5, 3.7)
6α	23.5 t	1.80 m (2H)	21.8 t	1.72 m (1H), 1.80 dq (1H)
ß				
7α	35.3 t	2.10 m	35.3 t	
ß		2.55 br dt (13.2, 4.0)		2.58 ddd (13.2, 3.7, 3.7)(1H)
8	149.7 s		149.7 s	
9	51.5 d	2.29 dd (6.1, 1.6)	51.6 d	2.26 br d (6.0)
10	51.1 s		51.5 s	
11α	64.7 t	3.88 dd (9.2, 6.1)	64.9 t	3.90 dd (9.5, 6.6), 4.30 dd (8.8, 1.5)
ß		4.30 dd (9.2, 1.6)		

Table 8. continued.....

12*Z*	106.8 t	5.00 br s	107.0 t	4.87 br s
12*E*		4.86 br s		5.00 br s
13	102.4 d	5.31 d (10.0)	102.5 d	5.31 d (10.3)
14	18.3 q	0.93 s	21.3 q	0.93 s
15	75.5 d	3.44 ddd (7.6, 4.8, 2.8)	37.4 t	
16α	29.6 t	2.12 m (2H)	28.8 t	1.84-1.97 m (2H)
ß				
17	121.7 d	5.17 t sept. (7.5, 1.4)	125.0 d	5.10 dd (6.6, 6.6)
18	136.0 s		131.1 s	
19	17.9 q	1.74 br s	17.6 q	1.69 s
20	26.0 q	1.64 br s	25.7 q	1.61 s
13-OH		2.42 d (10.0)		2.35 d (9.5)

[a] Figures in parentheses are coupling constants (*J*) in Hz.

[b] Assignments were confirmed by a 2D direct δ_C / δ_H correlation experiment.

[c] Data from ref. 28.

carbons [δ_C 149.7 (s, C-8); 136.0 (s, C-18); 121.7 (d, C-17); 106.8 (t, C-12)], an oxygenated methylene [δ_C 64.7 (t, C-11)], a hemiacetal carbon [δ_C 102.4 (d, C-13)] and an oxygenated methine [δ_C 75.5 (d, C-15)].

The ^{1}H NMR and ^{13}C NMR spectral data of **24** are similar to those of sacculaporellin (**32**) (Table 8) apart from the presence of an extra hydroxyl. Sacculaporellin (**32**) is a sacculatane diterpenoid hemiacetal which has been isolated from the liverwort *Porella perrottetiana*[28]. The presence of perrottetianal B (**3**) in this extract led to the initial suggestion that the new diterpenoid has plane structure **33**. This proposal is untenable since separate irradiation of both the carbinol methine proton [δ_H 3.44 (ddd, J = 7.6, 4.8, 2.8 Hz, H-15)] and the vinyl proton H-17 [δ_H 5.17 (t sept., J = 7.5, 1.4 Hz)] caused changes in the multiplet at δ_H 2.12 associated with 2H-16. The 2D direct δ_C / δ_H correlation spectrum (Figure 3) confirmed that only the C-16 methylene protons resonated at δ_H 2.12. Irradiation at δ_H 2.12 (2H-16) caused collapse of both H-15 and H-17 into broad singlets and also simplified the methyl signal (3H-20) at δ_H 1.64 (br s) as a result of removal of a homoallylic coupling. These results led to the partial structure **34**. The ^{1}H NMR data compared very well with those of the same partial structure of clausantalene (Scheme 5), a sesquiterpenoid from the roots of *Clausena indica* (Rutaceae)[29]. The above arguments led to structure **24** (without regard for stereochemistry) for this diterpenoid.

The assignment of the carbons in sacculatanes **24** and **32** was assisted by comparison with data on other known sacculatanes[30-32]. A 2D direct δ_C / δ_H correlation experiment was also carried out for compound **24** (Figure 3). Comparison of the ^{13}C NMR data for **24** and **32** (Table 8) reveals that C-15 has the

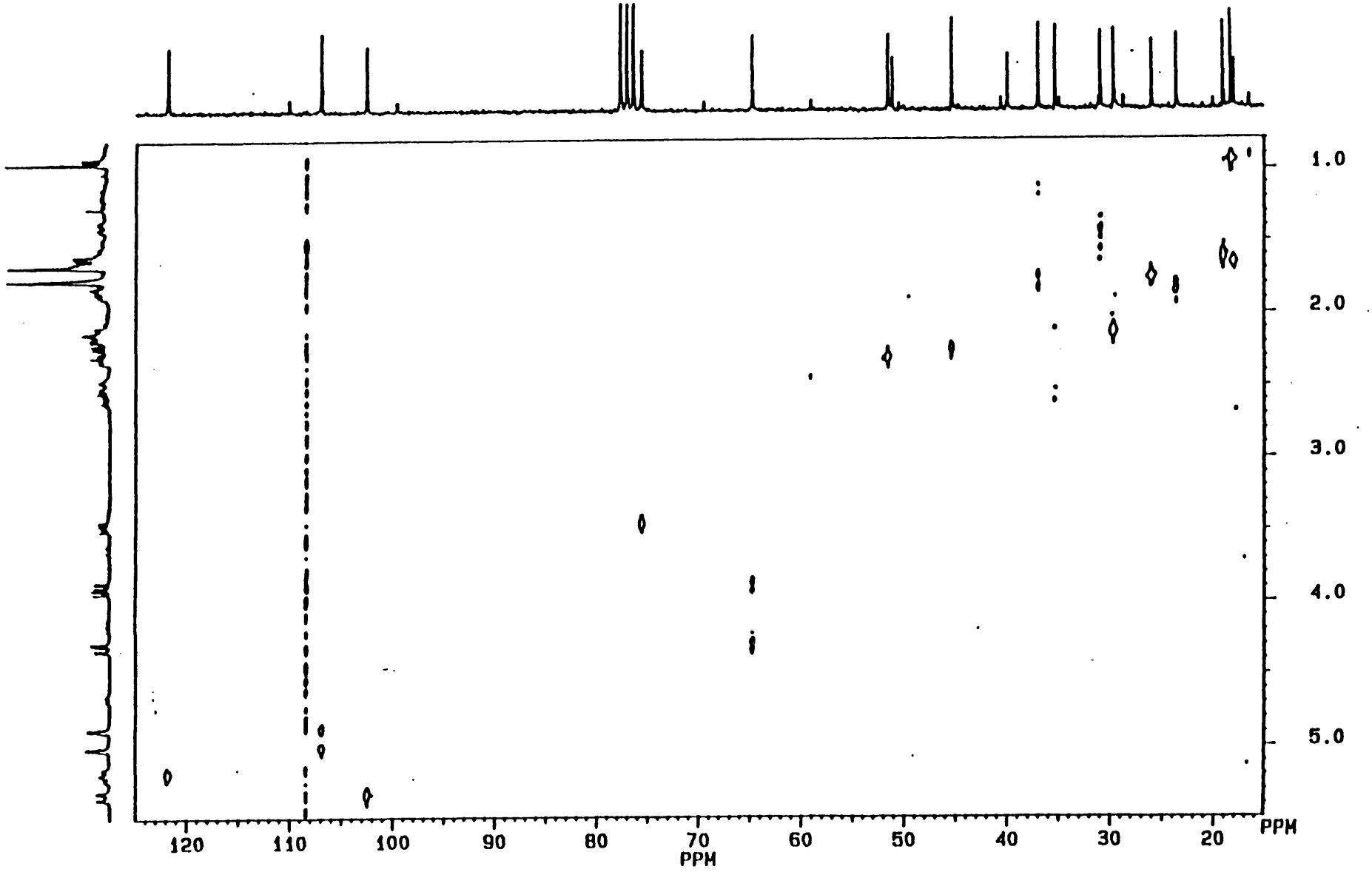


Figure 3. 2D direct δ_C / δ_H correlation spectrum of compound **24.**

large downfield shift (38.1 ppm) expected from hydroxylation (α-effect), C-16 (0.8 ppm) and C-4 (3.8 ppm) both have smaller deshieldings (ß-effect) and C-17 (3.3 ppm), C-14 (3.0 ppm), C-5 (3.7 ppm) and C-3 (6.7 ppm) are all shielded by the γ-effect. The deshielding of C-6 (1.7 ppm) is probably due to a syn-diaxial interaction between the 15-OH and C-6 (δ-effect).

In order to support the side chain hydroxyl group attachment site from NOE difference experiments, it was necessary to identify the protons of the C-6 methylene group. These assignments were made by irradiating the signal at δ_H 2.55 (br dt, J = 13.2, 4.0 Hz, H-7ß) which resulted in significant changes at δ_H 2.10 and δ_H 1.80 and removal of a small allylic coupling to one of the exomethylene protons (H-12*E*). Analysis of the 2D direct δ_C / δ_H correlation (Figure 3) led to the identification of H-7α at δ_H 2.10 and 2H-6 at δ_H 1.80. Subsequent irradiation at δ_H 2.23 (dd, J = 12.0, 4.7 Hz, H-5) also resulted in change at δ_H 1.80 (2H-6). The proton, H-7α, also showed allylic coupling to both exomethylene protons (H-12*E* and H-12*Z*). The NOEs between (i) H-15 (3.3%) and H-17 (5.9%), (ii) H-15 and H-5 (2.0%), (iii) H-15 and 2H-16 (1.4%), (iv) H-15 and 2H-6 (5.0%), and (v) H-15 (5.5%) and 3H-14 (2.0%) confirms α-attachment of the isopentenyl moiety and hydroxyl attachment at C-15.

On dilution of the NMR sample of compound **24** the hydroxyl proton at C-13 [δ_H 2.42 (d, J = 10.0 Hz)] varies little in chemical shift, indicating intramolecular hydrogen bonding. Whereas the hydroxyl proton at C-15 loses its coupling to H-15 showing that intermolecular exchange of the hydroxyl proton has increased (chemical exchange decoupling).

The stereochemical assignments of **24** are based largely on NOE difference

results. The NOEs observed between H-13 and H-1ß (2.4%) and between H-13 and H-2ß (8.6%) indicate that the hydroxyl group on the hemiacetal carbon must be ß-orientated. It is interesting that in compounds **24** and **32** the hydroxyl group at C-13 has the opposite stereochemistry. The evidence for the α-attachment of the hydroxyl at C-13 in compound **32** is a NOE observed between H-13 and H-6ß[28]. In compound **24** the methyl group attached to C-4, the hemiacetal and the exomethylene moieties must all be on the same side of the decalin system because of the NOEs between (i) 3H-14 (2.8%) and H-13 (11.0%), (ii) H-12*Z* (10.3%) and H-11ß (11.7%), and (iii) H-12*E* (9.4%) and H-7ß (6.9%). Related NOEs supported this conclusion including negative NOEs arising from the three spin effect[26]. Thus there are negative NOEs between (i) H-12*Z* (-0.5%) and H-7ß (-0.6%), (ii) H-12*E* (-1.8%) and H-11ß (-1.5%), and (iii) H-12 *Z* (-1.1%) and H-11α (-1.6%). These data are consistent with a *trans* decalin in a chair-chair conformation. The configuration at C-15 remains undetermined but compound **24** appears to be a single isomer on the basis of its spectroscopic data.

This sample of *P. platyphylla* (extract D) produced large amounts of pinguisanes along with sacculatane diterpenoids. Polygodial (**1**) and related compounds were not detected. Thus this sample of *P. platyphylla* belongs to the non-pungent type of *Porella* species and to chemotype (iv) of the Porellaceae in common with previous studies (see p. 78). The lack of complete data precluded any valid deductions about extracts A-C. The unstable alcohol **7** was not detected in any of the above extracts.

EXPERIMENTAL

Extract A

The plant material was collected near Langcliffe, Yorkshire in April 1990. Six months later the material was ground (460 g) and half of it (230 g) was extracted with Et_2O using a Soxhlet extractor. This yielded a crude extract (4.64 g) which was divided into several fractions by column chromatography on silica gel. The separate fractions were subjected to preparative TLC to give the following constituents in order of increasing polarity : pinguisanin (**6**) (50 mg) (see Table 2 and p. 103), 2α-hydroxypinguisa-5(10),6-dien-11,6-olide (**8**) (20 mg) and 2α, 7α-epoxy-6ß-hydroxypinguis-5(10)-en-11,6-olide (**9**) (10 mg).

2α-Hydroxypinguisa-5(10),6-dien-11,6-olide (**8**) was isolated as a semicrystalline solid.

ν_{max} (cm^{-1}) : 3625 (OH); 2970, 2930, 2870; 1784 (C=O); 1675, 1605 (C=C).

λ_{max} (nm) : 282

EIMS m/z (rel.int.) : 248 $[M]^+$(20), 215 (18), 176 (46), 175 (58), 161 (41), 148 (40),121 (23), 119 (25), 105 (36), 91 (47), 53 (91), 43 (92), 41 (100).

δ_H : 5.74 (dd, *J* = 2.3, 2.1 Hz, H-10); 5.42 (d, *J* = 2.1 Hz, H-7); 3.93 (ddd, *J* = 9.0, 7.6, 3.1 Hz, H-2); 3.20 (qd, *J* = 6.8, 2.3 Hz, H-4); 2.20 (br quin., *J* = 7.3 Hz, H-1); 1.94 (dd, *J* = 14.8, 9.0 Hz, H-3ß); 1.76 (br dd, *J* = 14.8, 3.1 Hz, H-3α); 1.24 (d, *J* = 6.8 Hz, 3H-13); 1.05 (d, *J* = 6.9 Hz, 3H-15); 0.92, 0.74

(both s, 3H-12 and 3H-14).

δ_C : 170.6 (s, C-11); 159.9, 148.2 (both s, C-5 and C-6); 114.8, 111.3 (both d, C-7 and C-10); 78.0 (d, C-2); 51.0 (d, C-1); 50.5, 49.0 (both s, C-8 and C-9); 43.2 (t, C-3); 36.1 (d, C-4); 18.7, 15.3, 12.7, 12.0 (all q, C-12, C-13, C-14 and C-15).

2α ,7α -Epoxy-6β-hydroxypinguis-5(10)-en-11,6-olide (**9**) was obtained as a semicrystalline material.

ν_{max}(KBr)(cm^{-1}) : 3420, 3260 (OH); 2930; 1760 (C=O); 1650 (C=C).

λ_{max}(EtOH)(nm) : 220

EIMS m/z (rel.int.) : 264 $[M]^+$ (1), 246 (1), 218 (4), 137 (27), 121 (28), 109 (46), 97 (76), 91 (33), 79 (41), 67 (48), 53 (71), 41 (100).

δ_H* : 5.78 (s, H-10); 3.86 (br s, H-2); 3.77 (s, H-7); 2.60 (q, *J* = 7.5 Hz, H-4); 1.93 (br qt, *J* = 7.4, 2.0 Hz, H-1); 1.73 (dd, *J* = 13.9, 2.0 Hz, H-3ß); 1.43 (br dt, *J* = 13.9, 1.5 Hz, H-3α); 1.24 (d, *J* = 7.5 Hz, 3H-15); 1.21 (s, 3H-14); 1.11 (s, 3H-12); 0.99 (d, *J* = 7.4 Hz, 3H-13).

δ_C* : 171.6, 168.7 (both s, C-5 and C-11); 117.5 (d, C-10); 105.5 (s, C-6); 85.3 (d, C-7); 79.6 (d, C-2); 53.6 (d, C-1); 51.1 (s, C-8); 45.9 (t, C-3); 44.3 (d, C-4); 42.3 (s, C-9); 24.3, 15.2, 13.2, 11.4 (all q, C-12, C-13, C-14 and C-15).

* Solvent : $CDCl_3$ + 3 drops of CD_3OD.

Extract B

The other half of the ground material (230 g) collected near Langcliffe, Yorkshire in April 1990 was extracted with Et_2O in May 1991 to yield 2.1 g of crude extract. PLC of a polar fraction obtained by column chromatography on silica gel gave ß-pinguisenediol (**13**) (20 mg) (see p. 107).

Extract C

This sample of plant material was collected near Clapham, Yorkshire in November 1990. In January 1991 the material was ground (252 g) and extracted with Et_2O to give a crude extract (2.68 g) which was chromatographed by a combination of CC and PLC to give in order of increasing polarity: pinguisanin (**6**) (400 mg) (see Tables 2 and p. 103), isopinguisanin (**15**) (< 1 mg) and porellapinguisanolide (**14**) (4 mg) (see p. 108).

Isopinguisanin (**15**) was obtained as a gum.

ν_{max} (cm^{-1}) : 2960, 2930, 1715, 1550; 1250, 1220, 1110, 980 (C-O).

EIMS m/z (rel.int.) : 232 $[M]^+$ (4), 175 (13), 121 (24), 109 (33), 91 (40), 64 (100), 55 (52), 48 (93), 41 (80).

δ_H : 7.25 (obscured by $CHCl_3$, H-11); 6.39 (d, J = 1.9 Hz, H-10); 3.70 (br s, H-2); 2.58 (d, J = 17.3 Hz, H-7); 2.39 (dd, J = 17.3, 0.8 Hz, H-7); 1.78 (m, 3H); 1.38 (s, 3H-15); 1.03, 0.92 (both s, 3H-12 and 3H-14); 0.83 (d, J = 7.5 Hz, 3H-13).

δ_C : 148.6 (s, C-6); 141.3 (d, C-11); 120.4 (s, C-5); 108.4 (d, C-10); 80.9 (d, C-2); 78.0 (s, C-4); 52.0 (s, C-9); 46.6 (d, C-1); 42.9 (s, C-8); 38.1, 32.3 (both t, C-3 and C-7); 24.1, 21.2, 13.3, 11.1 (all q, C-12, C-13, C-14 and C-15).

Extract D

The plant material was collected near Ingleton, Yorkshire in August 1992. The ground plant material (336 g) was extracted with methanol using a Soxhlet extractor. This yielded 14 g of extract and 8.2 g of this was divided into ten fractions by CC on silica gel using a petroleum ether - Et_2O gradient. Further chromatography of these fractions by PLC on silica gel yielded the following constituents in order of increasing polarity: pinguisanin (**6**) (300 mg); methyl 2,4-dihydroxy-3,6-dimethylbenzoate (atraric acid) (**25**) (< 1 mg); 11,13-epoxy-8(12),17-sacculatadiene-13ß,15-diol (**24**) (30 mg); 6,11-epoxy-2α-hydroxy-7ß-methoxy-pinguisa-5,10-diene (**17**) (29 mg); perrottetianal B (**3**) (5 mg); 6,11-epoxy-2α-hydroxy-11-methoxypinguisa-5(10),6-diene (**18**) (54 mg); 6,11-epoxy-2α-hydroxy-11-methoxypinguisa-5(10),6-diene (**19**) (11-epi **18**) (104 mg); 2α,7-epoxy-11-hydroxy-7*R*-methoxy-6,7-secopinguis-5(10)-en-6,11-olide (**20**) (60 mg); 6,11-epoxy-11α,6ß-dimethoxy-2α-hydroxypinguis-5(10)-ene (**21**) (9 mg); 6,11-epoxy-11α,6α-dimethoxy-2α-hydroxypinguis-5(10)-ene (**22**) (3 mg); ß-pinguisenediol (**13**) (57 mg); porella-pinguisanolide (**14**) (18 mg) and methyl 2α-hydroxy-6-oxo-11-pinguisanoate (**23**) (16 mg).

Pinguisanin (**6**) was isolated as a gum.

δ_H (lit.[6]) : 7.33 (d, *J* = 1.9 Hz, H-11); 6.25 (dd, *J* = 1.9, 0.6 Hz, H-10); 4.36 (br s, H-7); 3.95 (br t, *J* = 2.0 Hz, H-2); 2.58 (q, *J* = 7.3 Hz, H-4); 2.07 (br q, *J* = 7.4 Hz, H-1); 1.90 (dd, *J* = 13.8, 2.7 Hz, H-3ß); 1.59 (br dd, *J* = 13.8, 1.3 Hz, H-3α); 1.18 (s, 3H-14); 1.14 (d, *J* = 7.3 Hz, 3H-15); 1.13 (d, *J* = 7.4 Hz, 3H-13); 1.07 (s, 3H-12).

δ_C (lit.[6]) : see Table 2.

Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (atraric acid) (**22**) was isolated as a semicrystalline solid.

HRMS : m/z 196.0744[M]$^+$ calculated for $C_{10}H_{12}O_4$: 196.0736.

ν_{max} (cm^{-1}) : 3584, 3416 (OH); 2928; 1736 (C=O); 1655.

λ_{max}(EtOH)(nm) : 305, 270, 220.

EIMS m/z (rel.int.) :196[M]$^+$ (42), 164 (100), 149 (23), 136 (95), 107 (13), 95 (9), 79 (17).

δ_H (lit.[21]) : 12.02 (s, 2-OH); 6.20 (s, H-5); 5.03 (br s, 4-OH); 3.91 (s, OMe); 2.45 (s, 3H-8); 2.09 (s, 3H-9).

δ_C (lit.[22]) : 172.6 (s, C=O); 163.2 (s, C-2); 158.0 (s, C-4); 140.1 (s, C-6); 110.5 (d, C-5); 108.4 (s, C-3); 105.3 (s, C-1); 51.8 (q, OMe); 24.1 (q, C-8); 7.6 (q, C-9).

11,13-Epoxy-8(12),17-sacculatadiene-13β,15-diol (**24**) was isolated as a crystalline compound and recrystallised from MeOH, m.p. 130-135° C.

HRMS : m/z 320.2332[M]$^+$ calculated for $C_{20}H_{32}O_3$: 320.2351.

ν_{max}($CHCl_3$)(cm^{-1}) : 3600, 3500 (OH); 2972, 2934 ; 1700, 1600 (C=C); 1400.

EIMS m/z (rel.int.) : 320[M]$^+$ (1), 302 (11), 233 (42), 221 (10), 204 (11), 187 (59), 175 (100), 161 (42), 145 (23), 133 (27).

δ_H : see Table 8.

δ_C : see Table 8.

6,11-Epoxy-2α-hydroxy-7β-methoxypinguisa-5,10-diene (**17**) was isolated as a semi-crystalline solid.

HRMS : m/z 264.1744 [M]$^+$ calculated for $C_{16}H_{24}O_3$: 264.1725.

ν_{max} (cm^{-1}) : 3364 (OH); 2971, 2938, 2880, 2824, 1765.

λ_{max} (nm) : 219

EIMS m/z (rel.int.) : 264[M]$^+$ (2), 233 (3), 191 (5), 175 (6), 152 (3), 138 (100), 123 (10), 99 (18).

δ_H : see Table 1.

δ_C : see Table 2.

Perrottetianal B (**3**) was obtained as an unstable oil.

δ_H (lit.[20]) : 10.11 (s, H-11); 9.84 (s, H-13); 5.15 (br t, J = 7.5 Hz, H-17); 3.43 (m, H-3); 2.84 (m, 1H); 2.14 (s, 3H-12); 1.75 (s, 3H-19); 1.64 (s, 3H-20); 0.71 (s, 3H-14).

δ_C (lit.[20]) : 204.5 (d, C-13); 190.1 (d, C-11); 158.6 (s, C-8); 138.5 (s, C-9); 136.4 (s, C-18); 121.4 (d, C-17); 75.5 (d, C-3); 50.4 (s, C-10); 47.8 (d, C-5); 39.5 (s, C-4); 35.9 (t, C-15); 31.4 (t, C-7);

30.3, 29.7 (both t, C-1 and C-2); 26.0 (q, C-20); 19.5, 18.8 (both q, C-14 and C-12); 18.5 (t, C-6); 18.0 (q, C-19); 16.3 (t, C-16).

6,11-Epoxy-2α -hydroxy-11-methoxypinguisa-5(10),6-diene (**18**) was obtained as a gum.

HRMS : m/z 264.1734$[M]^+$ calculated for $C_{16}H_{24}O_3$: 264.1725.

ν_{max} (cm^{-1}) : 3428 (OH); 2971, 2934, 2878, 2836, 1798; 1680, 1631 (C=C).

λ_{max} (nm) : 263.4

EIMS m/z (rel.int.) : 264$[M]^+$ (19), 192 (37), 191 (100), 177 (28), 163 (35), 149 (20), 109 (22), 97 (24), 91 (31), 43 (40), 41 (58).

δ_H : see Table 1.

δ_C : see Table 2.

6,11-Epoxy-2α -hydroxy-11-methoxypinguisa-5(10),6-diene (**19**) (11-epi **18**) was obtained as a gum.

HRMS : m/z 264.1714$[M]^+$ calculated for $C_{16}H_{24}O_3$: 264.1725.

ν_{max} (cm^{-1}) : 3413 (OH); 2970, 2936, 2878, 2834, 1736; 1680, 1630 (C=C).

λ_{max} (nm) : 263.6

EIMS m/z (rel.int.) : 264$[M]^+$ (16), 192 (35), 191 (100), 177 (28), 163 (39), 149 (21), 109 (21), 105 (26), 97 (33), 91 (35), 43 (41), 41 (57), 28 (62).

δ_H : see Table 1.

δ_C : see Table 2.

2α ,7-Epoxy-11-hydroxy-7R-methoxy-6,7-secopinguis-5(10)-en-6,11-olide (**20**) was isolated as a semicrystalline mixture of C-11 epimers.

HRMS : m/z 265.1440 $[M-OCH_3]^+$ calculated for $C_{15}H_{21}O_4$: 265.1439.

T_R (mins) : 27.10

ν_{max} (cm^{-1}) : 3300 (OH); 2990, 2950, 2870, 2800; 1780 (C=O); 1475.

λ_{max} (nm) : 206

EIMS m/z (rel.int.) : 265 $[M-OCH_3]^+$ (1), 236 (1), 218 (1), 203 (1), 185 (1), 169 (2), 149 (2), 137 (3), 128 (7), 109 (100), 99 (23), 81 (5).

δ_H : see Table 1.

δ_C : see Table 2.

Preparation and GLC analysis of tetramethylsilyl (TMS) ethers of **20**. About 0.2 mg of **20**, 15 μL of dry pyridine and 30 μL N,O-bistrimethylsilyltrifluoroacetamide (BSTFA) were added to a microbiological T-tube which was then corked and heated at 80℃ for 30 minutes. The reagents were evaporated off and the products analysed by GLC which indicated the presence of two TMS ethers with retention times 27.35 and 27.70 minutes.

11α,6β-Dimethoxy-6,11-epoxy-2α-hydroxypinguis-5(10)-ene (**21**) was isolated as a gum.

HRMS : m/z 265.1788 $[M-CH_3]^+$ calculated for $C_{16}H_{25}O_3$: 265.1804.

ν_{max} (cm^{-1}) : 3351 (OH); 2971, 2842, 2832.

EIMS m/z (rel.int.) : 265 $[M-CH_3]^+$ (9), 251 (5), 237 (47), 219 (100), 187 (28), 163 (27), 135 (19), 121 (56), 109 (53), 97 (49), 91 (26), 81 (17).

δ_H : see Table 1.

δ_C : see Table 2.

11α,6α-Dimethoxy-6,11-epoxy-2α-hydroxypinguis-5(10)-ene (**22**) was isolated as a gum.

HRMS : m/z 265.1791 $[M-CH_3]^+$ calculated for $C_{16}H_{25}O_3$: 265.1804.

EIMS m/z (rel.int.) : 265 $[M-CH_3]^+$ (6), 251 (2), 237 (42), 219 (100), 187 (27), 163 (21), 156 (24), 149 (26), 136 (43), 121 (47), 109 (27), 97 (31), 81 (12).

δ_H : see Table 1.

δ_C : see Table 2.

β-Pinguisenediol (**13**) was obtained as an oil.

δ_H (lit.[2]) : 6.69 (dd, *J* = 17.3, 11.0 Hz, H-10); 5.13 (dd, *J* = 17.3, 1.4 Hz, H-11*E*); 4.99 (dd, *J* = 11.0, 1.4 Hz, H-11*Z*); 3.73 (dd, *J* = 10.1, 5.9 Hz, H-7); 3.73 (td, *J* = 7.6, 4.5 Hz, H-2); 2.56 (br ddq, *J* = 15.4, 5.9, 1.2 Hz, H-6); 2.18-1.70 (m, 4H); 1.76 (br t, *J* = 1.6 Hz, 3H-15); 1.09 (d, *J* = 7.0 Hz, 3H-13); 0.94, 0.92 (both s, 3H-12 and 3H-14).

δ_C : 138.7 (s, C-4); 135.1 (d, C-10); 124.4 (s, C-5); 112.1

(t, C-11); 78.4 (d, C-2); 72.1 (d, C-7); 51.9, 48.4 (both s, C-8 and C-9); 44.8 (d,C-1); 44.3 (t, C-3); 30.8 (t, C-6); 22.0 (q, C-15); 15.5, 15.1, 14.6 (all q, C-12, C-13 and C-14).

Porellapinguisanolide (**14**) was isolated as a gum.

δ_H (lit.[13]) : 9.66 (dd, *J* = 3.4, 1.0 Hz, H-11); 5.46 (s, H-7); 3.95 (br td,*J* = 9.2, 4.9 Hz, H-2); 2.91 (dd, *J* = 16.6, 1.0 Hz, H-10); 2.77 (dd, *J* = 16.6, 3.4 Hz, H-10); 2.65 (dq, *J* = 8.8, 6.9 Hz, H-1); 2.42 (q, *J* = 7.3 Hz, H-4); 1.87 (dd, *J* = 14.8, 9.6 Hz, H-3ß); 1.63 (dd, *J* = 14.8, 4.9 Hz, H-3α); 0.98 (d, *J* = 6.9 Hz, 3H-13); 0.93 (d, *J* = 7.3 Hz, 3H-15); 0.93 (s, 3H-14); 0.88 (s, 3H-12).

δ_C (lit.[13]) : 197.5 (d, C-11); 172.8 (s, C-6); 107.0 (d, C-7); 79.7 (s, C-5); 77.8 (d, C-2); 49.0 (t, C-10); 47.8 (s, C-8/9); 45.9 (d, C-1); 45.7 (s, C-8/9); 45.1 (t, C-3); 42.1 (d, C-4); 19.0, 14.4, 12.0, 9.8 (all q, C-12, C-13, C-14 and C-15).

Methyl 2α-hydroxy-6-oxo-11-pinguisanoate (**23**) was obtained as a gum.

HRMS : m/z 282.1829 $[M]^+$ calculated for $C_{16}H_{26}O_4$: 282.1831.

ν_{max} (cm^{-1}) : 3467 (OH); 2973, 2880; 1740, 1717 (C=O).

EIMS m/z (rel.int.) : 282 $[M]^+$ (1), 263 (15), 250 (14), 232 (19), 209 (20), 208 (92), 190 (25), 176 (24), 148 (30), 134 (35), 108 (53), 69 (47), 55 (97), 41 (100).

δ_H : 3.85 (ddd, *J* = 9.0, 7.4, 2.8 Hz, H-2); 3.65 (s, 11-OMe);

2.74 (dddd, J = 12.2, 8.4, 4.0, 0.8 Hz, H-5); 2.60 (dd, J = 16.2, 8.4 Hz, H-10); 2.31 (dd, J = 13.6, 0.8 Hz, H-7ß); 2.29 (dd, J = 16.2, 4.0, H-10); 2.21 (d, J = 13.6 Hz, H-7α); 2.10 (dq, J = 12.2, 6.7 Hz, H-4); 1.91 (dd, J = 14.6, 9.0 Hz, H-3ß); 1.82 (br quin., J = 7.0 Hz, H-1); 1.69 (dd, J = 14.6, 2.8 Hz, H-3α); 1.57 (br s, OH); 1.01 (s, 3H-14); 0.96 (d, J = 6.7 Hz,3H-15); 0.92 (d, J = 6.9 Hz, 3H-13); 0.76 (s, 3H-12).

δ_C : 210.4 (s), 173.6 (s), 77.9 (d), 51.7 (q), 51.2 (s), 48.8 (d), 48.0 (d), 47.1 (t), 46.8 (s), 44.7 (t), 42.3 (d), 31.9 (t), 19.8 (q), 15.4 (q), 14.6 (q), 11.9 (q).

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CHAPTER 5

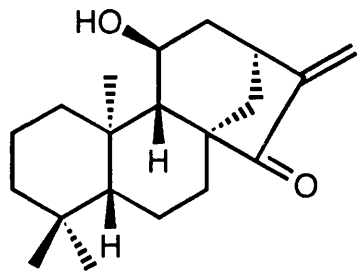
JUNGERMANNIA TRUNCATA

INTRODUCTION

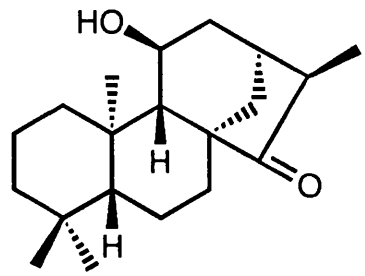
Jungermannia truncata belongs to the family Jungermanniaceae of the order Jungermanniales. *J. truncata* has been investigated once previously (collected in Japan) and was found to contain two *ent*-kaurane diterpenoids, *ent*-11α-hydroxy-16-kauren-15-one (**1**) and *ent*-16(*S*)-11α-hydroxykauran-15-one (**2**)[1]. The *Jungermannia* species of liverwort are rich sources of diterpenoids.

DISCUSSION

The major compound of this collection of *Jungermannia truncata* (from Malaysia) is the new natural product *ent*-7ß-hydroxy-16-kauren-15-one (**3**) which was present in very high yield (about 20% of the crude extract). This liverwort proved to be a rich source of *ent*-kaurane diterpenoids (Scheme 1) and a further eight new compounds (**4-11**) were isolated as well as the previously known *ent*-16-kauren-15-one (**12**)[1,2], *ent*-15α-hydroxy-16-kaurene (**13**)[2] and *ent*-11α-hydroxy-16-kauren-15-one (**1**)[1,3]. Other new compounds include the two gymnomitranes **14** and **15**. In addition the extract of *J. truncata* contains two further types of diterpenoid, a pimarane and a halimane. Pimara-9(11),15-dien-19-ol (**16**) is a new constituent of liverworts though it has been found in the Korean medicinal plant, *Acanthopanax koreanum*[4]. The halimane derivative, pleuroziol (**17**) has been isolated previously from the liverwort *Pleurozia gigantea*[5] collected in East Malaysia. This type of diterpenoid is rare in Nature. The known compounds were identified by comparing their spectroscopic data with published data[2-5]. For the known *ent*-kaurenes identification was helped by comparing ^{13}C NMR data with published



(1)



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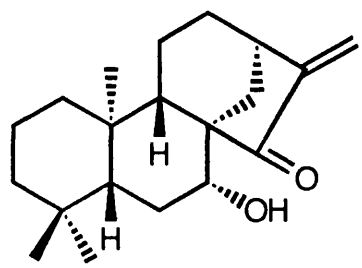

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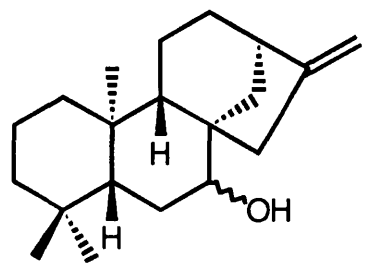
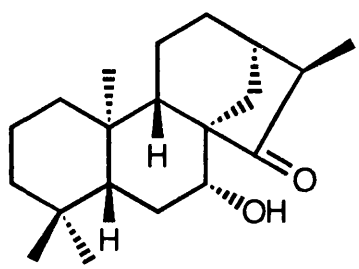
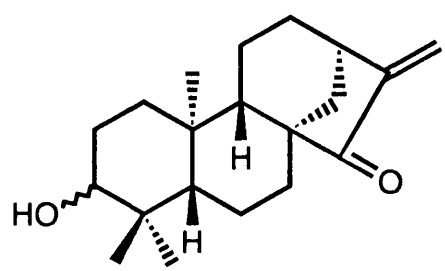


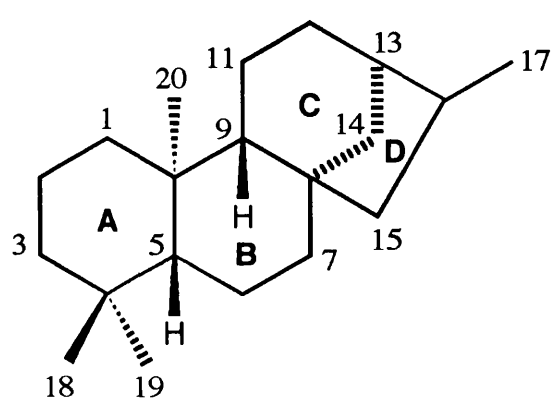
(4) 7α - OH
(21) 7β - OH



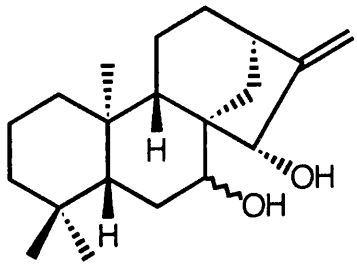
(5)



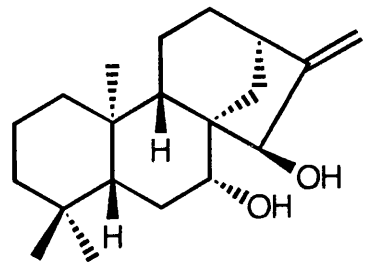
(6) 3β - OH
(20) 3α - OH



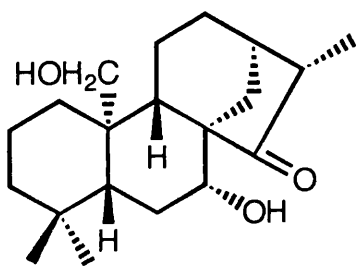
Scheme 1



(7) 7α - OH
(23) 7β - OH



(8)



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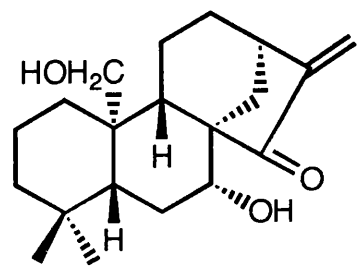

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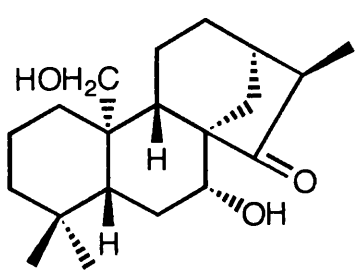

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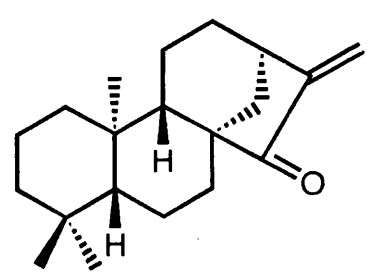

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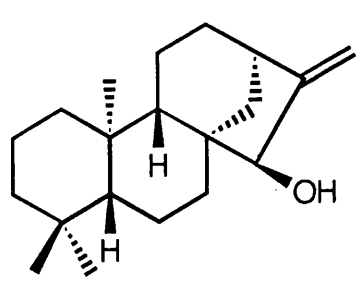

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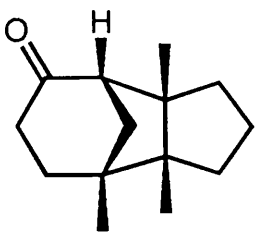

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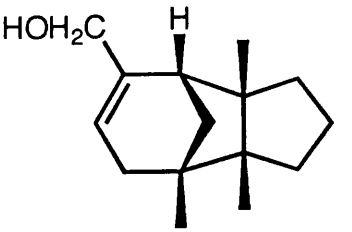

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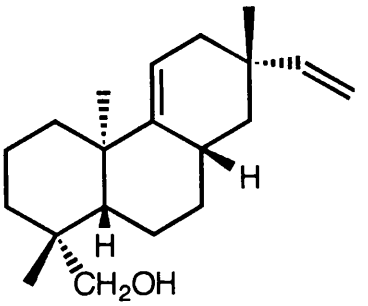

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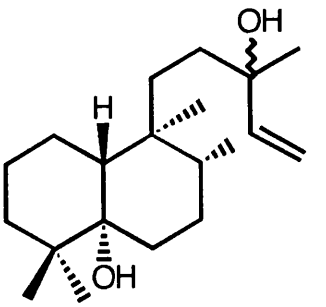

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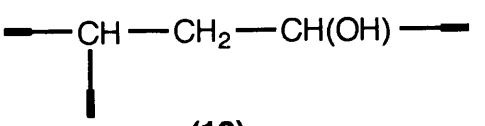

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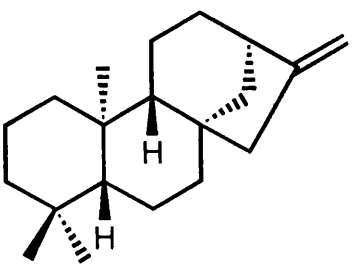

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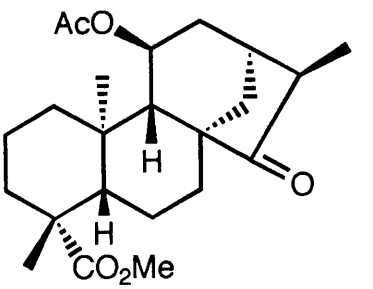

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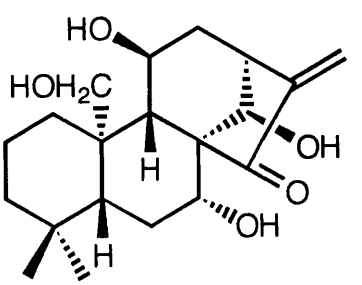


(24)

data[6,7].

The major constituent (**3**) of this extract is a crystalline compound and has the molecular formula $C_{20}H_{30}O_2$ (m/z 302.2239 $[M]^+$, 284.2120 $[M-18]^+$) indicating six double bond equivalents. The IR spectrum indicated the presence of a hydroxyl (ν_{max} 3625, 3486 cm^{-1}) and an α,ß-unsaturated carbonyl group in a five-membered ring (ν_{max} 1727, 1645 cm^{-1}). The UV spectrum showed a band at λ_{max} 232 nm, supporting the presence of a conjugated carbonyl. The ^1H NMR spectrum (Figure 1) (see Experimental) contained signals for three tertiary methyl groups (δ_H 0.80, 0.86, 1.06), two deshielded protons at δ_H 3.05 (m) and 4.03 (dd, J = 12.6, 4.5 Hz), the latter attached to the carbon bearing the hydroxyl group, two exomethylene protons [δ_H 5.91 (t, J = 1.1 Hz); 5.23 (t, J = 1.1 Hz)] and a hydroxyl proton [δ_H 2.78 (br s), exchangeable with D_2O]. The ^{13}C NMR spectrum (Figure 2) indicated the presence of twenty carbons (Table 1) : three methyls, seven methylenes, three methines, three quaternary carbons, one carbonyl carbon [δ_C 210.0 (s)], one oxygenated methine (δ_C 70.5) and two olefinic carbons [δ_C 149.1 (s), 114.5 (t)]. The ^1H chemical shifts revealed that the exomethylene protons were conjugated with the carbonyl group. The ^{13}C shifts of the exomethylene carbons and the carbonyl group support this conclusion. The above spectral data indicate that compound **3** is tetracarbocyclic and suggest a hydroxylated 16-kauren-15-one structure. Evidence for this framework will be presented below. The location and stereochemistry of the hydroxyl group were determined as follows. The multiplicity and coupling constants [δ_H 4.03 (dd, J = 12.6, 4.5 Hz)] of the carbinol methine proton indicate that the hydroxyl group is equatorial and must be attached to C-1, C-3 or C-7. Irradiation of the carbinol methine proton caused the

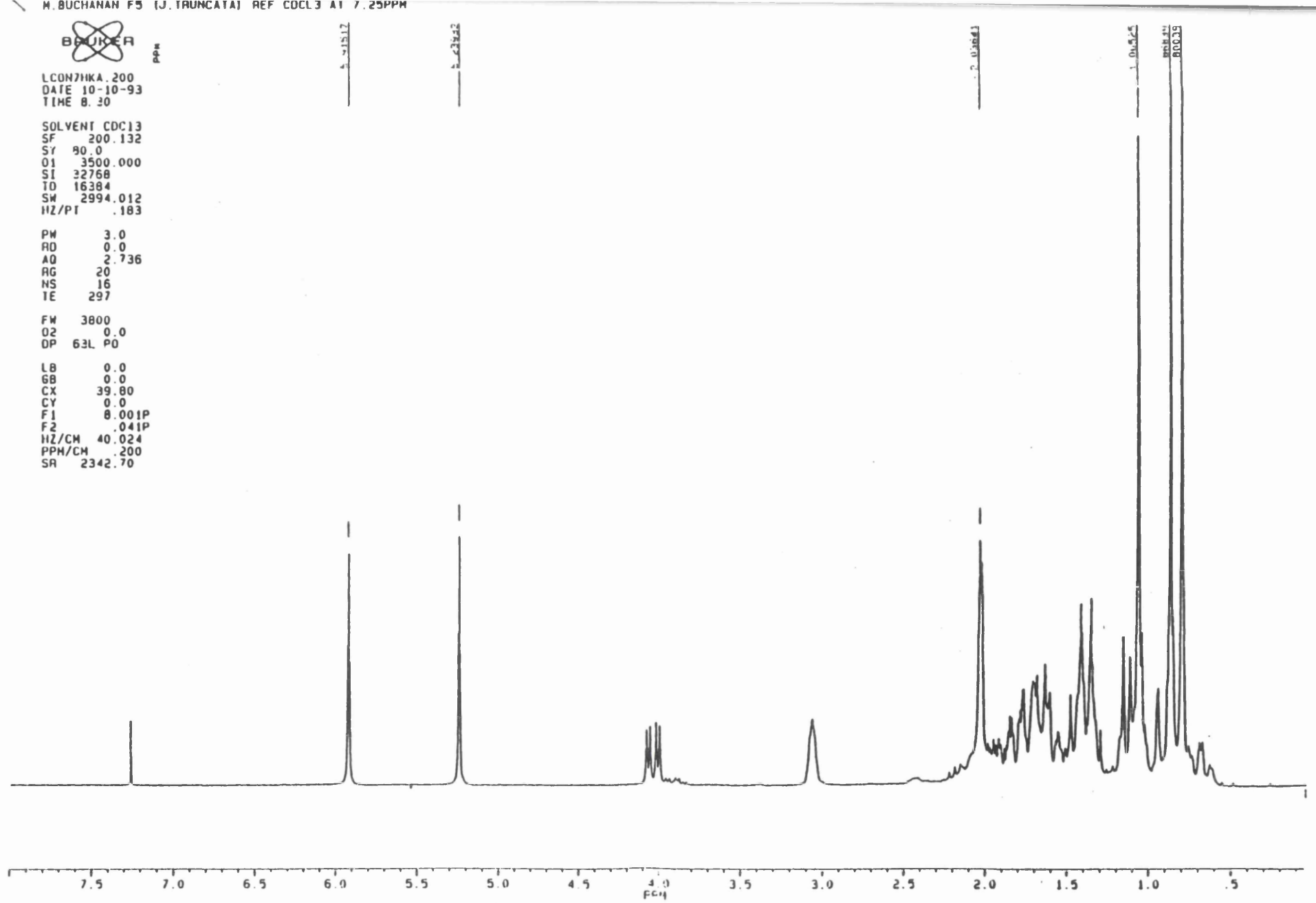


Figure 1. ^{1}H NMR spectrum of compound **3.**

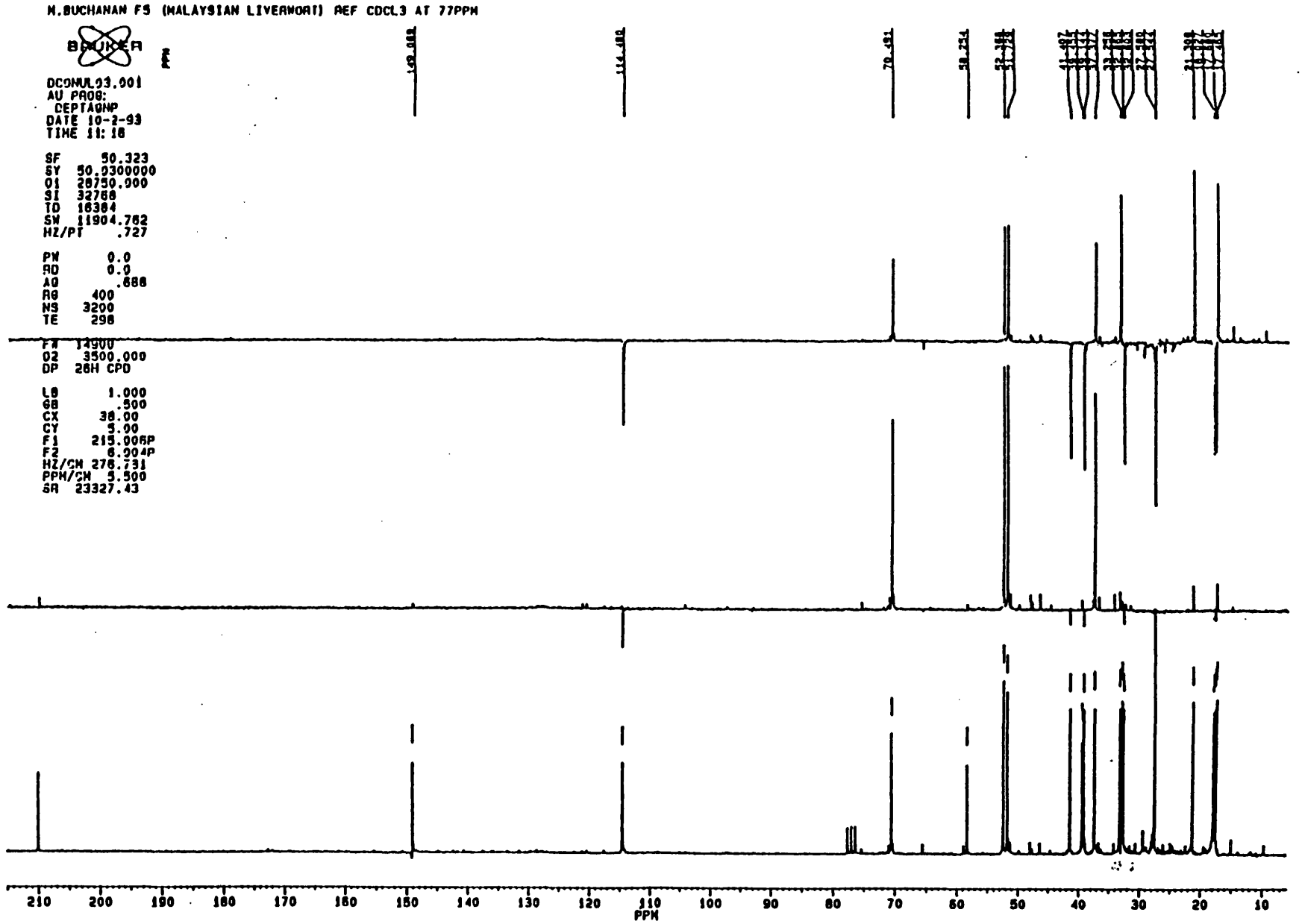


Figure 2. ^{13}C NMR and DEPT spectra of compound **3.**

Table 1. ^{13}C NMR data of *ent*-kauranes.

C	1	3	4	5	6
1	39.4 t	39.1 t	40.2 t	39.1 t	32.5 t*
2	18.3 t	18.0 t^{+}	18.5 t^{+}	18.2 t^{+}	25.0 t
3	41.7 t	41.4 t	41.8 t	41.6 t	75.8 d
4	33.2 s	32.9 s	33.2 s	33.1 s	37.5 s
5	55.2 d	52.4 d	53.1 d	52.8 d	48.1 d
6	18.3 t	27.5 t*	29.4 t*	28.3 t	18.1 t^{+}
7	33.5 t	70.5 d	75.2 d	71.6 d	32.4 t*
8	50.6 s	58.3 s	49.8 s	58.5 s	52.4 s
9	64.4 d	51.7 d	55.9 d	51.8 d	52.2 d
10	38.7 s	39.5 s	39.3 s	39.3 s	39.8 s
11	66.1 d	17.7 t^{+}	17.9 t^{+}	17.7 t^{+}	18.2 t^{+}
12	41.2 t	32.6 t	33.6 t	25.2 t	33.4 t
13	36.8 d	37.4 d	43.3 d	34.3 d	38.1 d
14	36.6 t	27.6 t*	30.5 t*	28.3 t	36.6 t
15	210.1 s	210.0 s	43.2 t	224.2 s	209.3 s
16	150.3 s	149.1 s	155.1 s	48.3 d	149.5 s
17	112.9 t	114.5 t	108.4 t	9.9 q	114.4 t
18	33.5 q	33.3 q	33.5 q	33.4 q	28.4 q
19	21.5 q	21.3 q	21.5 q	21.4 q	22.0 q
20	17.5 q	17.5 q	17.7 q	17.8 q	17.3 q

[a] Data from ref. 7.

[b] Data from ref. 1.

[c] Data from ref. 13.

[d] Data from ref. 14 (solvent C_5D_5N).

$^{+*}$ Assignments may be interchangeable in each vertical column.

Table 1. ^{13}C **NMR data of *ent*-kauranes continued...**

C	7	8	9	10	11
1	40.1 t	40.1 t	34.4 t	34.5 t	34.1 t
2	18.5 t	18.5 t	18.6 t^{+}	18.6 t^{+}	18.5 t^{+}
3	41.7 t	41.7 t	41.6 t	41.6 t	41.5 t
4	33.1 s	33.0 s	33.0 s	33.2 s	32.9 s
5	52.6 d	52.5 d	53.0 d	52.9 d	53.0 d
6	27.9 t*	27.4 t*	29.9 t	29.7 t	29.1 t
7	73.8 d	72.1 d	71.2 d	71.4 d	71.9 d
8	52.7 s	51.1 s	58.9 s	58.3 s	58.3 s
9	53.0 d	45.6 d	51.6 d	52.1 d	52.0 d
10	39.5 s	38.6 s	42.6 s	42.8 s	42.5 s
11	17.9 t	17.9 t	18.3 t^{+}	18.4 t^{+}	18.2 t^{+}
12	33.2 t	33.2 t	28.1 t	32.0 t	24.0 t
13	41.4 d	40.0 d	37.0 d	37.8 d	34.6 d
14	28.5 t*	29.2 t*	27.0 t	28.6 t	28.3 t
15	84.4 d	75.5 d	226.0 s	209.6 s	224.7 s
16	160.8 s	157.8 s	46.8 d	149.7 s	48.5 d
17	107.7 t	105.0 t	15.3 q	114.6 t	10.0 q
18	33.6 q	33.5 q	34.0 q	34.0 q	34.0 q
19	21.6 q	21.6 q	22.4 q	22.4 q	22.3 q
20	17.7 q	17.7 q	61.3 t	61.3 t	61.2 t

[a] Data from ref. 7.

[b] Data from ref. 1.

[c] Data from ref. 13.

[d] Data from ref. 14 (solvent C_5D_5N).

$^{+}$* Assignments may be interchangeable in each vertical column.

Table 1. ^{13}C **NMR data of *ent*-kauranes continued...**

C	12	13	19[a]
1	39.6 t	40.2 t	40.5 t
2	18.4 t^{+}	18.6 t^{+}	18.7 t^{+}
3	41.8 t	41.9 t	42.1 t
4	33.2 s	33.2 s	33.3 s
5	55.3 d	55.3 d	56.3 d^{*}
6	18.5 t^{+}	19.8 t	20.3 t
7	33.5 t	36.4 t	41.3 t
8	52.55 s	45.7 s	44.3 s
9	52.61 d	46.3 d	56.1 d^{*}
10	40.1 s	38.9 s	39.4 s
11	18.1 t^{+}	18.0 t^{+}	18.2 t^{+}
12	32.4 t	33.3 t	33.3 t
13	38.1 d	40.0 d	44.0 d
14	36.7 t	38.7 t	39.9 t
15	211.0 s	82.4 d	49.2 t
16	149.6 s	158.3 s	156.0 s
17	114.3 t	104.7 t	102.8 t
18	33.5 q	33.5 q	33.7 q
19	21.5 q	21.6 q	21.7 q
20	17.5 q	17.5 q	17.6 q

[a] Data from ref. 7.

[b] Data from ref. 1.

[c] Data from ref. 13.

[d] Data from ref. 14 (solvent C_5D_5N).

$^{+*}$ Assignments may be interchangeable in each vertical column.

Table 1. ^{13}C **NMR data of *ent*-kauranes concluded.**

C	20[b]	23[c]	24[d]
1	37.96 t	40.12 t	34.7 t
2	26.94 t	18.59 t	19.0 t
3	78.55 d	41.94 t	41.9 t
4	38.73 s	32.71 s	33.3 s
5	54.17 d	49.36 d	53.8 d
6	18.29 t	26.75 t	30.0 t
7	32.25 t	73.07 d	75.4 d
8	52.20 s	51.47 s	60.1 s
9	52.39 d	46.28 d	67.0 d
10	39.61 s	39.25 s	44.0 s
11	18.17 t	17.53 t	65.4 d
12	33.50 t	33.35 t	39.8 t
13	37.99 d	42.87 d	47.1 d
14	36.52 t	35.14 t	77.5 d
15	210.79 s	81.22 d	207.7 s
16	149.37 s	158.64 s	151.8 s
17	114.54 t	108.50 t	113.3 t
18	28.20 q	33.35 q	34.2 q
19	15.39 q	21.55 q	22.9 q
20	17.45 q	17.24 q	60.3 t

[a] Data from ref. 7.

[b] Data from ref. 1.

[c] Data from ref. 13.

[d] Data from ref. 14 (solvent C_5D_5N).

[+*] Assignments may be interchangeable in each vertical column.

neighbouring equatorial proton signal at δ_H 1.81 (ddd, J = 12.6, 4.5, 1.8 Hz) to collapse to a doublet of doublets (J = 12.6, 1.8 Hz) and the neighouring axial proton signal at δ_H 1.38 (q, J = 12.6 Hz) to collapse to a triplet (J = 12.6 Hz). Thus the neighbouring methylene has only one further coupling. These results reveal the presence of partial structure **18** and thus establish the hydroxyl group as 7α. Comparison of the ^{13}C NMR data of **3** with those of *ent*-16-kaurene (**19**)[7] and *ent*-3ß-hydroxy-16-kauren-15-one (**20**)[1] (Table 1) support this structural assignment. The expected chemical shift differences arising from the introduction of a 7α-hydroxyl group are readily identified. Relative to **20** C-7 of **3** is strongly deshielded (38.3 ppm, α-effect) as are C-8 (6.1 ppm, ß-effect) and C-6 (9.2 ppm, ß-effect) while C-14 is strongly shielded (8.9 ppm, γ-gauche interactions). Chemical shifts of ring A of **3** were similar to those of **19** apart from the shielding of C-5 (3.7 ppm) in **3** which is expected[8] for a carbon γ-*trans* to a 7α-hydroxyl group.

Homonuclear decoupling and NOE difference experiments were carried out in order to assign some of the proton signals and to establish the relative stereochemistry. The tertiary methyl groups were readily assigned by comparison of ^{1}H NMR data with those of other *ent*-kauranes with no functional groups on rings A and B[9]. These assignments were supported by the NOEs observed between 3H-20 (1.2%) and 3H-19 (3.3%). Decoupling at δ_H 3.05 (H-13) resulted in the collapse of both exomethylene proton resonances to doublets by removal of an allylic coupling (1.1 Hz). This irradiation also resulted in change in the spectrum at δ_H 2.03 and δ_H 1.92. The signal at δ_H 2.03 is assigned to 2H-14 as it is a two proton multiplet and also NOEs are observed between 2H-14 (6.7%)

and 3H-20 (3.5%) whereas the signal at δ_H 1.92 is assigned H-12α on the basis of its coupling pattern (tdd, J = 13.4, 7.0, 3.0 Hz) and the 6.7% NOE it receives from 3H-20. The more deshielded exomethylene proton at δ_H 5.91 is assigned to H-17*Z* as it experiences a greater deshielding effect from the magnetic anisotropy of the carbonyl. This assignment was confirmed by the NOE observed between H-13 and H-17*E* (3.0%). The shielded proton signal at δ_H 0.68 (td, J = 12.4, 3.4 Hz) is assigned to H-1ß on the basis of its coupling pattern and its NOE with H-9ß (9.3%). Irradiation of H-1ß also gave a NOE (18.0%) at H-1α [δ_H 1.74 (m)]. Homonuclear decoupling of H-1ß caused changes at δ_H 1.74 (H-1α) and also at δ_H 1.66 which was identified as H-2α (br q, J = 14.0 Hz) on the basis of a NOE (10.0%) from 3H-20. The NOEs observed at H-5ß (6.6%) and H-9ß (3.9%) on irradiation of the carbinol methine proton (H-7ß) (Figure 3) identify the chemical shifts of these protons [δ_H 0.88 (dd, J = 12.6, 1.8 Hz, H-5ß); 1.13 (br d, J = 8.5 Hz, H-9ß)] and further support the 7α-equatorial configuration of the hydroxyl group. The coupling and NOE difference information along with the inspection of models clearly indicate that the two six-membered rings A and B have chair conformations and are *trans*-fused. Ring C has a flattened chair conformation as is indicated by the coupling pattern of H-9ß (br d, J = 8.5 Hz). The H-9ß, H-11α dihedral angle is close to 90°. All the new *ent*-kauranes in the subsequent discussion appear to have this same conformation for ring C. The B/C ring junction is *cis*-fused. The five-membered ring D has an envelope conformation with C-14 being the out of plane atom. Proton H-13 is α-orientated and effectively equatorial in ring C. This confirms the relative stereochemistry indicated in structure **3**. Although the absolute stereochemistry of compound **3** has not been established we assume that

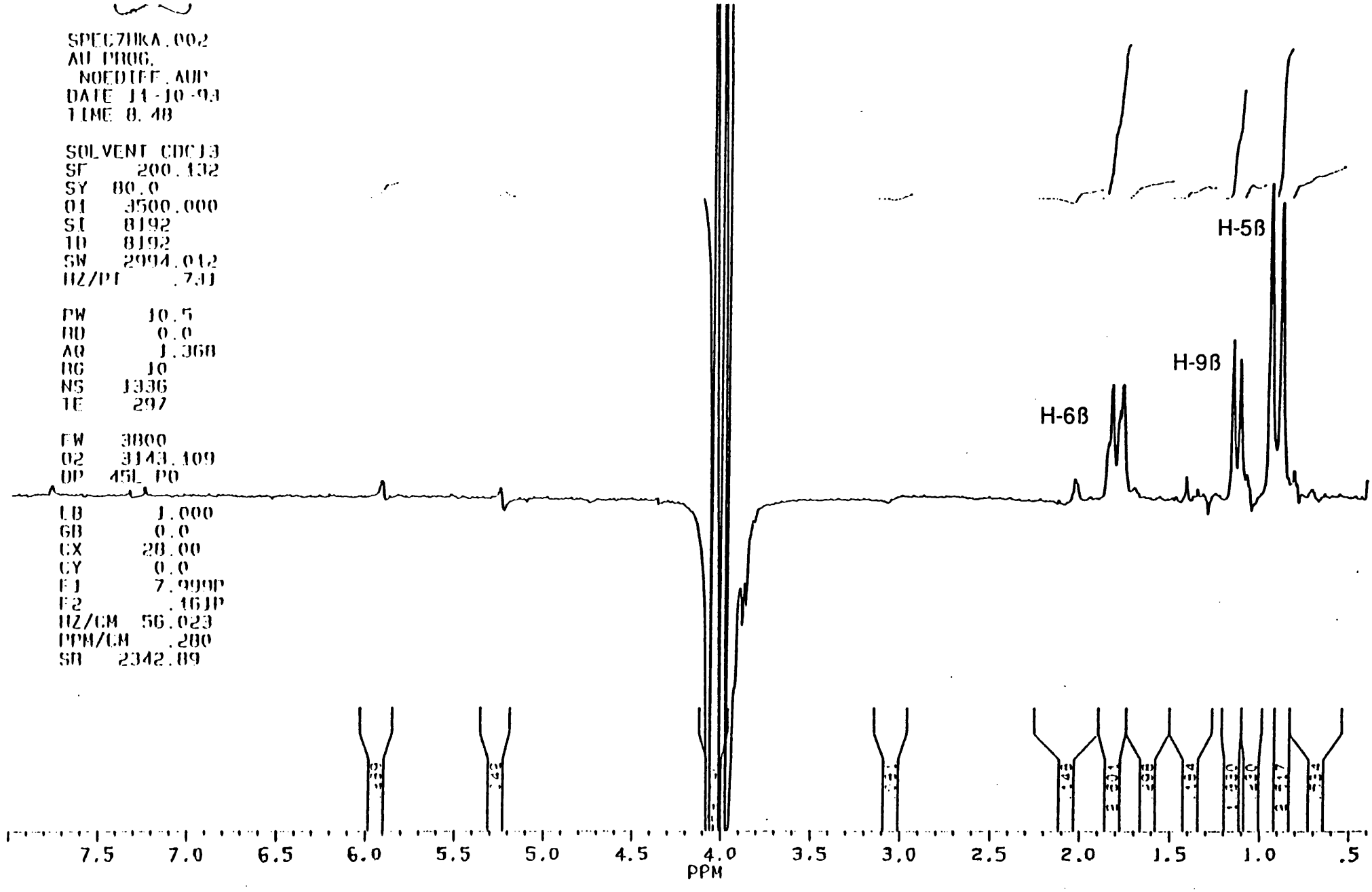


Figure 3. NOE difference spectrum of compound **3** for irradiation at δ_H 4.03 ppm (H-7ß).

it, with the known kaurenes **1** and **12** and the new kauranes (**4-11**) belong to the *enantio*-series on the basis of the co-occurrence of the known *ent*-kaurene **13**[2]. The sign of the specific rotation of **13** (-44°) is the same as for **3** (-112°). In general *ent*-kauranes have negative rotations.

Compound **4** revealed an intense molecular ion peak at m/z 288.2456 and an $[M-18]^+$ fragment at m/z 270.2348. The molecular formula is therefore $C_{20}H_{32}O$. The IR spectrum of **4** showed the presence of a hydroxyl group (ν_{max} 3627, 3509 cm^{-1}). Its ^1H NMR spectrum (see Experimental) contained signals for three tertiary methyl groups (δ_H 1.02, 0.86, 0.81), an exomethylene group [δ_H 4.82 (m), 4.76 (m)] and an oxygen-bearing methine [δ_H 3.46 (dd, J = 12.2, 4.2 Hz)]. The ^{13}C NMR data (Table 1) for **4** indicated the presence of twenty carbons, comprising three methyls, eight methylenes, three methines, an oxygen-bearing methine (δ_C 75.2), an exomethylene moiety [δ_C 155.1 (s), 108.4 (t)] and three quaternary carbons. These spectral data indicate that compound **4** is a tetracarbocyclic diterpenoid, presumably a hydroxylated *ent*-16-kaurene derivative. Comparison of the ^{13}C NMR data of **4** with those of *ent*-16-kaurene (**19**)[7] (Table 1) indicates a close relationship. As for compound **3** the differences can be interpreted in terms of shifts caused by the presence of a 7α-hydroxyl group in **4**. Thus there is a large deshielding of C-7 (33.9 ppm, α-effect) and smaller deshieldings of C-6 (9.1 ppm, ß-effect) and C-8 (5.5 ppm, ß-effect). Expected shieldings of C-15 (6.0 ppm, γ-effect) and C-14 (9.4 ppm, γ-effect) are also observed. The location and relative stereochemistry of the hydroxyl functional group were supported by the following evidence. Homonuclear decoupling of the carbinol methine proton (dd, J = 12.2, 4.2 Hz) as above revealed the presence of

partial structure **18**. Irradiation of H-7ß afforded NOEs at H-9ß (1.5%) and H-5ß (5.1%) consistent with the 7α-equatorial nature of the hydroxyl group. The absence of a C-15 carbonyl relative to **3** results in deshielding of C-16 (6.0 ppm) and shielding of both C-17 (6.1 ppm) and the exomethylene protons [0.41 ppm (H-17*Z*), 1.15 ppm (H-17*E*)]. The exomethylene proton at δ_H 4.76 is H-17*E* since it receives a NOE from H-15ß (1.7%). The signals for H-7ß (0.57 ppm), H-13 (0.37 ppm) and 2H-14 (*ca* 0.4 ppm) are also shielded in **4** relative to compound **3**. Homonuclear decoupling reveals that both exomethylene protons have a 2.7 Hz allylic coupling to H-15α [δ_H 2.65 (dt, J = 16.8, 2.7 Hz)] along with smaller couplings to H-13 [δ_H 2.68 (m)] and H-15ß [δ_H 1.92 (br d, J = 16.8 Hz)]. This indicates that the allylic C-15, H-15α bond is nearly perpendicular to the plane of the double bond, the optimum geometry for allylic coupling[10]. Thus compound **4** is *ent*-7ß-hydroxy-16-kaurene and is a new natural product. The C-7 epimer, *ent*-7α-hydroxy-16-kaurene (**21**), has been isolated from *Sideritis candicans*[11], a plant widely dispersed on the Canary Isles.

High resolution mass spectrometry (HRMS) of compound **5** gave the molecular formula $C_{20}H_{32}O_2$ (m/z 304.2421 $[M]^+$) indicating five double bond equivalents. The mass spectrum also contains a $[M-18]^+$ fragment ion peak at m/z 286.2307. The IR spectrum showed the presence of a hydroxyl group (ν_{max} 3625, 3470 cm^{-1}) and a five-membered ketone (ν_{max} 1740 cm^{-1}). The ^1H NMR spectrum (see Experimental) of **5** revealed signals for three tertiary methyl groups (δ_H 0.81, 0.87, 1.07), a secondary methyl group [δ_H 1.09 (d, J = 7.0 Hz)] and a methine group bearing an oxygen function [δ_H 3.94 (dd, J = 12.4, 4.6 Hz)]. The ^{13}C NMR spectrum (Table 1) contained signals for twenty carbons : four methyls, seven

methylenes, four methines, one oxygen-bearing methine (δ_C71.6), three quaternary carbons and a five-membered ketone (δ_C 224.2). The ^{1}H and ^{13}C NMR spectra resembled those of **3**, except for the presence of a methyl group in place of the exomethylene group at C-16 and suggested that this compound has an *ent*-7ß-hydroxykauran-15-one skeleton. The position of the secondary methyl group was confirmed as follows. Homonuclear decoupling of the readily recognised H-13 multiplet (δ_H 2.46) caused the quintet at δ_H 2.21 (J = 7.0 Hz), which must be H-16, to collapse to a quartet (J = 7.0 Hz). Irradiation of H-16 confirms its coupling to the secondary methyl group [δ_H 1.09 (d, J = 7.0 Hz)]. Irradiation of H-13 also removes the small couplings from a pair of methylene protons at δ_H 1.95 (br dd, J = 12.1, 4.5 Hz) and 2.09 (dd, J = 12.1, 1.3 Hz) which must be 2H-14. Examination of models indicates that H-14*S* has a dihedral angle of 75° with H-13 and it is therefore assigned to the signal at δ_H 2.09. Thus H-14*R*, which has a dihedral angle of about 45° with H-13, is assigned to the signal at δ_H 1.95. The protons associated with the C-12 methylene group are hidden in the methylene envelope at *ca* δ_H 1.6. In general the stereochemistry of the C-17 methyl group is readily determined by consideration of J (H-13, H-16) and NOEs from H-13. For a 17α-methyl J (H-13, H-16ß) is close to zero Hz (dihedral angle ~ 90°) and a significant NOE is expected for 3H-17 from H-13 whereas for a 17ß-methyl J (H-13, H-16α) is *ca* 7 Hz (dihedral angle ~ 30°) and it is H-16α which will experience a large NOE from H-13. Since in the case of **5**, J (H-13, H-16) is 7.0 Hz and irradiation of H-13 affords a large NOE at H-16 the 17-methyl group is ß and the configuration at C-16 is *ent-S*. Comparison of ^{1}H and ^{13}C NMR data of **5** with those of **22**[12] [δ_C 221.6 (s, C-15), 49.1 (d, C-16), 10.8 (q, C-17); δ_H 1.22 (d, J = 7.0

Hz, 3H-17)] which contains the same ring D supported the structural assignment. A 7α-equatorial configuration of the secondary hydroxyl group was again evident from the NOEs observed between H-7ß and both H-5ß (5.5%) and H-9ß (2.5%). Thus compound **5** is *ent*-16(*S*)-7ß-hydroxykauran-15-one.

The molecular formula of the next compound **6** was established as $C_{20}H_{30}O_2$ ($[M]^+$ at m/z 302.2247) by HRMS. The IR, UV and MS spectra showed the presence of a hydroxyl group (ν_{max} 3630, 3500 cm^{-1}; m/z 284 $[M-18]^+$) and a conjugated enone system (ν_{max} 1725, 1650 cm^{-1}; λ_{max} 229 nm). The ^1H NMR and ^{13}C NMR data (see Experimental and Table 1) indicated the presence of three tertiary methyl groups (δ_H 1.09, 0.95, 0.84), an oxygen-bearing methine [δ_C 75.8; δ_H 3.40 (br t, J = 2.5 Hz)] and a ketone conjugated with an exomethylene group [δ_C 209.3 (s), 149.5 (s), 114.4 (t); δ_H 5.93 (t, J = 1.1 Hz), 5.24 (t, J = 1.1 Hz)] and suggested that **6** is a further *ent*-hydroxy-16-kauren-15-one derivative. Comparison of the ^{13}C NMR data (Table 1) of **6** with those of *ent*-3ß-hydroxy-16-kauren-15-one (**20**)[1] (Table 1) indicates that these compounds are similar. Differences in the chemical shifts can be rationalised in terms of these compounds being C-3 epimers. The differences in the chemical shifts of C-2 (1.9 ppm), C-3 (2.8 ppm) and C-4 (1.2 ppm) between the epimers **6** and **20** are small as expected since the change in chemical shift at the α- and ß- carbons on hydroxylation does not directly reflect the stereochemistry. In contrast there is significant shielding of the γ- carbons, C-5 (6.1 ppm) and C-1 (5.5 ppm) in **6** relative to **20**, due to γ- gauche interactions between the axial hydroxyl (*ent*-3α) group and the axial protons on these γ-carbons. As expected, on the basis of γ-gauche interactions of the hydroxyl group, there is a shielding of C-19 (6.6 ppm) and very little difference in chemical shift of C-18

(0.2 ppm) in **20** relative to **6**.

Additional evidence for the location and stereochemistry of the hydroxyl group was available as follows. The multiplicity and coupling constant values (br t, J = 2.5 Hz) of the carbinol methine proton is consistent with its equatorial nature. The broadness of the signal is presumably due to long-range W couplings. Homonuclear decoupling of this proton caused the axial proton at δ_H 1.93 (td, J = 13.0, 2.5 Hz, H-2α) to collapse to a triplet (J = 13.0 Hz) and caused change in the methylene envelope at δ_H 1.56 (H-2ß). The NOEs observed at 3H-19 (1.1%) and 3H-18 (0.5%) on irradiation of H-3α provide final confirmation for the siting (*ent*-3α) of the hydroxyl group at C-3. Further homonuclear decoupling experiments revealed the following information. The exomethylene protons, both triplets (J = 1.1 Hz), are geminally coupled and have allylic couplings to H-13. The exomethylene proton at δ_H 5.24 is assigned H-17*E* as it receives a 2.5% NOE from H-13. There is no noticeable coupling between H-14*S* and H-13 indicating a dihedral angle close to 90°. A vicinal coupling (J = 2.6 Hz) between H-13 and one of the protons on C-12 was also established. The NOE observed at 3H-20 (2.5%) from H-14*S* lends support to the typical kaurane relative stereochemistry indicated in structure **6**. The above spectroscopic data established the structure of **6** as *ent*-3α-hydroxy-16-kauren-15-one. The C-3 epimer (**20**) of **6** has been isolated from the liverwort *Jungermannia vulcanicola*[1].

Compound **7** has the molecular formula $C_{20}H_{32}O_2$ ($[M]^+$ at m/z 304.2386) indicating five double bond equivalents. The MS showed $[M-18]^+$ and $[M-36]^+$ fragments at m/z 286.2307 and 268.2204 respectively. The IR spectrum of **7** showed hydroxyl absorptions (ν_{max} 3600, 3405 cm^{-1}). The ^1H and ^{13}C NMR data

(see Experimental and Table 1) revealed signals for three tertiary methyl groups (δ_H 1.03, 0.89, 0.82), two oxygen-bearing methines [δ_C 84.4, 73.8; δ_H 4.07 (br s), 3.85 (dd, J = 12.4, 4.7 Hz)] and an exomethylene group [δ_C 160.8 (s), 107.7 (t); δ_H 5.15 (br s), 5.07 (br s)]. These data indicate a dihydroxytetracarbocyclic diterpenoid with a basic *ent*-16-kaurene skeleton. Furthermore, comparison of the ^{13}C NMR data (Table 1) of **7** and *ent*-15ß,7α-dihydroxy-16-kaurene (**23**)[13] reveals strong similarities. Differences in chemical shift can be explained if **7** and **23** are C-7 epimers. γ-Gauche interactions of the C-7ß hydroxyl group result in shielding of C-5 (3.2 ppm), C-9 (6.7 ppm) and C-15 (3.2 ppm) in **23** (*ent*-7α) relative to **7** (*ent*-7ß) and also the shielding of C-14 (6.6 ppm) in **7** relative to **23**. Further confirmation of positions and stereochemistry of the two hydroxyl groups were obtained as follows. Irradiation of the carbinol methine proton at δ_H 4.07 (H-15) resulted in NOEs at H-9ß (6.2%) and H-17*Z* (1.8%) indicating that the proton is H-15ß and hence that one of the hydroxyl groups has an α-configuration at C-15 (no NOE was observed at H-14*R*). H-15ß appears as a broad singlet and may have small allylic couplings to the exomethylene protons and a small ^4J coupling to H-14*S*. The other hydroxyl group is attached equatorially to C-7 in view of the spin system: δ_H 3.85 (dd, J = 12.4, 4.7 Hz, H-7ß); 1.83 (ddd, J = 12.4, 4.7, 1.5 Hz, H-6ß); 1.41 (q, J = 12.4 Hz, H-6α) and 0.88 (dd, J = 12.4, 1.5 Hz, H-5ß). These data indicate the presence of partial structure **18**. Irradiation of δ_H 3.85 (H-7ß) resulted in NOEs at H-9ß (1.6%) and H-5ß (4.0%). On the basis of the above findings compound **7** is *ent*-15ß,7ß-dihydroxy-16-kaurene. The C-7 epimer of **7**, *ent*-15ß,7α-dihydroxy-16-kaurene (**23**) has been isolated from the pungent liverwort *Plagiochila pulcherrima*[13].

HRMS of compound **8** gave the same molecular formula $C_{20}H_{32}O_2$ ($[M]^+$ at m/z 304.2396) as that of **7**. Compound **8** also showed an $[M-18]^+$ fragment at m/z 286.2299. Its IR spectrum showed the presence of hydroxyl groups (ν_{max} 3630, 3424 cm^{-1}). The ^1H and ^{13}C NMR spectra (see Experimental and Table 1) were very similar to those of **7** and showed signals for three tertiary methyl groups (δ_H 1.03, 0.86, 0.81), two oxygen-bearing methines [δ_C 75.5, 72.1; δ_H 4.45 (br t, J = 2.5 Hz), 3.56 (dd, J = 11.6, 4.5 Hz)] and an exomethylene group [δ_C 157.8 (s), 105.0 (t); δ_H 5.09 (m), 4.96 (br d, J = 2.5 Hz)]. Comparison of the ^{13}C NMR data (Table 1) of **8** and **7** suggested they were epimeric at C-15. Thus there is a substantial shielding of C-9 (7.4 ppm) in **8** relative to **7** as a result of a γ-gauche interaction of the 15ß-hydroxy group. C-15 is also more shielded (8.9 ppm) in **8** and this can be explained by loss of intramolecular hydrogen bonding. For C-7 in **8** the shielding from loss of intramolecular hydrogen bonding is counterbalanced by a deshielding from the removal of a γ-gauche interaction with the 15α-hydroxy group. This explains why there is only a small shielding of C-7 (1.7 ppm) in **8** compared with **7**.

The presence of partial structure **18** and thus a 7α-equatorial hydroxyl group was again evident from the spin system : δ_H 3.56 (dd, J = 11.6, 4.5 Hz, H-7ß); 1.80 (ddd, J = 11.6, 4.5, 1.7 Hz, H-6ß); 1.37 (q, J = 11.6 Hz, H-6α) and 0.87 (br d, J = 11.6 Hz, H-5ß). NOEs were observed at H-5ß (5.8%) and H-9ß (3.6%) when the proton at δ_H 3.56 (H-7ß) was irradiated. Homonuclear decoupling revealed small allylic couplings between the exomethylene protons and H-13 and also a larger allylic coupling (2.5 Hz) between the exomethylene protons and the carbinol methine proton (H-15α) at δ_H 4.45 (br t, J = 2.5 Hz). This confirms the siting of

the second hydroxyl group at C-15. The exomethylene protons, which also have a geminal coupling of 0.5 Hz, were readily distinguished by a NOE difference experiment. Irradiation of H-13 affords a NOE (2.6%) at H-17*E* (δ_H 4.96) and simultaneously identified 2H-14 as a multiplet at δ_H 1.56. Confirmation of the relative stereochemistry of the hydroxyl group at C-15 as ß was obtained by irradiation of H-15. This resulted in a NOE (3.1%) at H-14*R*. The size of the allylic coupling (2.5 Hz) between H-15α and the exomethylene protons suggests that the C-15, H-15α bond is nearly orthogonal to the double bond plane[10]. The above results reveal structure **8** as *ent*-15α,7ß-dihydroxy-16-kaurene.

The next fraction of the extract contained a mixture of three compounds (**8, 9** and **10**). The GLC of this mixture established the presence of three diterpenoids in the ratio 1(**8**) : 3(**9**) : 1(**10**). It was evident from the ^{1}H and ^{13}C NMR spectra of the mixture that one of the minor diterpenoids in the mixture was **8**. Careful analysis of the NMR data enabled the structures of the remaining two diterpenoids to be established. There was too little material to attempt further separation.

Compound **9**, the most abundant diterpene of the mixture, gave a molecular ion at m/z 320 by the GCMS. The ^{1}H NMR spectrum (see Experimental) showed the signals for two tertiary methyl groups (δ_H 0.90, 0.85), a secondary methyl group [δ_H 1.08 (d, J = 7.6 Hz)], an oxygenated methylene group [δ_H 4.05 (br s, 2H)] and an oxygenated methine group [δ_H 3.95 (dd, J = 11.8, 4.8 Hz)]. The ^{13}C NMR spectrum (Table 1) showed twenty carbons : three methyls, seven methylenes, four methines and oxygenated methylene (δ_C 61.3), an oxygenated methine (δ_C 71.2), a ketonic carbonyl (δ_C 226.0) and three quaternary carbons. These data suggest compound **9** has a hydroxylated *ent*-kaurane skeleton in which one of the tertiary

methyls is oxidised to a hydroxymethyl group. The signal at δ_H 4.05 (br s, 2H) was attributed to the methylene of a hydroxymethyl group which can be present at one of three possible positions, C-18, C-19 and C-20[14,15]. The signal at δ_H 3.95 (dd, J = 11.8, 4.8 Hz) indicates the presence of an equatorial secondary hydroxyl group.

Comparison of the ^{1}H and ^{13}C NMR data (see Experimental and Table 1) of **9** with *ent*-16(*S*)-7ß-hydroxykauran-15-one (**5**) showed similarities. There are the expected shieldings of C-12 (2.9 ppm) and C-17 (5.4 ppm) in **5** relative to **9** which are explained by γ-gauche interactions of the C-16 methyl group. The attachment of the hydroxymethyl group at C-10 was indicated by the deshielding of C-20 (43.5 ppm) and C-10 (3.3 ppm) and by the ^{13}C chemical shifts of the methyls attached to C-4 [δ_C 22.4 (q, C-19); 34.0 (q, C-18); 33.0 (s, C-4)] which compare well with published data[13]. The similarity of the ^{13}C NMR chemical shifts (Table 1) in rings A and B of **9** with *ent*-7ß,11α,14(*S*),20-tetrahydroxy-16-kauren-15-one (**24**)[14] provides further support.

The methine H-16 was identified within a multiplet of overlapping signals by NOE difference and homonuclear decoupling experiments. Decoupling of the secondary methyl group [δ_H 1.08 (d, J = 7.6 Hz)] causes a broad quartet at δ_H 2.15 (J = 7.6 Hz, H-16) to collapse into a broad singlet. In an NOE difference experiment irradiation of the secondary methyl group (3H-17) showed the position of the broad quartet of H-16, although the intensities were distorted by selective population transfer. The configuration at C-16 is *ent*-16*R* since J (H-13, H-16) is *ca* zero Hz (see p. 119). The secondary methyl at C-16 is therefore α. On the basis of these findings structure **9**, *ent*-16(*R*)-7ß,20-dihydroxykauran-15-one, is proposed for this compound.

Compound **10**, the other less abundant component of this mixture, did not give a molecular ion when analysed by GCMS. The ^{1}H NMR spectrum (see Experimental) gave the signals for two tertiary methyl groups (δ_H 0.92, 0.87), a hydroxymethyl group [δ_H 4.05 (br s, 2H)], an oxygenated methine group [δ_H 4.12 (dd, J = 11.7, 5.1 Hz)] and an exomethylene group [δ_H 5.94 (br s), 5.26 (br s)]. The ^{13}C NMR spectrum (Table 1) exhibited the signals for two methyls, seven methylenes, three methines, three quaternaries, a methine bearing a hydroxyl group (δ_C 71.4), a methylene bearing a hydroxyl group (δ_C 61.3), two sp^2 carbons [δ_C 149.7 (s), 114.6 (t)] and a carbonyl carbon [δ_C 209.6 (s)]. These data resembled those of *ent*-16(*R*)-7ß,20-dihydroxykauran-15-one (**9**) except for the presence of the exomethylene group in place of the secondary methyl group at C-16. The ^{13}C NMR chemical shifts of C-15, 16 and 17 and the ^{1}H NMR chemical shifts of the exomethylene protons show that the exomethylene group is conjugated with the carbonyl group. Comparison of the ^{13}C NMR data of **10** with those of **9** and **3** (Table 1) supported this structural assignment. Virtually identical chemical shifts are observed for rings A and B of **10** and **9** and for rings C and D of **10** and **3**. When the hydroxymethyl group signal of **9** and **10** (δ_H 4.05) was irradiated a NOE was observed at 3H-19 supporting the presence of a hydroxymethyl at C-10 in both **9** and **10**. On the above evidence the structure of **10** was established as *ent*-7ß,20-dihydroxy-16-kauren-15-one.

Compound **11** has the molecular formula, $C_{20}H_{32}O_3$ ($[M]^+$ at m/z 320.2332). The IR spectrum of **11** indicates the presence of hydroxyl groups (ν_{max} 3630, 3584, 3458 cm^{-1}) and a five-membered ring ketone (ν_{max} 1734 cm^{-1}). Its ^{1}H NMR spectrum (see Experimental) contained signals for two tertiary methyl groups

(δ_H 0.90, 0.84), a secondary methyl group [δ_H 1.10 (d, J = 7.0 Hz), a hydroxymethyl group [δ_H 4.04 (s, 2H)] and an oxygen-bearing methine [δ_H 4.00 (dd, J = 11.7, 4.9 Hz)]. The ^{13}C NMR spectrum (Table 1) contained signals for twenty carbons : three methyls, seven methylenes, four methines, an oxygenated methylene (δ_C 61.2), an oxygenated methine (δ_C 71.9), a carbonyl [δ_C 224.7 (s)] and three quaternary carbons. Compound **11** has the same molecular formula as **9** and they both contain the same functional groups. Comparison of their ^1H and ^{13}C NMR data shows that they are very similar except for some differences in the ^{13}C NMR chemical shifts in rings C and D which suggest that **11** is the C-16 epimer of **9**. There is a shielding of C-12 (4.1 ppm) and C-17 (5.3 ppm) in **11** relative to **9** as predicted on the basis of γ-gauche interactions of the C-16 methyl group. This structural proposal is supported by the similarity of the chemical shifts of rings C and D of **11** with those of *ent*-16(*S*)-7ß-hydroxykauran-15-one (**5**). The position and stereochemistry of the hydroxyl groups of **11** were determined as follows. A 7α-equatorial configuration for the secondary hydroxyl group was evident from the following information : δ_H 4.00 (dd, J = 11.7, 4.9 Hz, H-7ß); 1.77 (ddd, J = 11.7, 4.7, 1.8 Hz, H-6ß); 1.46 (q, J = 11.7 Hz, H-6α) and 1.06 (dd, J = 11.7, 1.8 Hz, H-5ß). This again indicates the presence of partial structure **18**. As usual irradiation of H-7ß (δ_H 4.00) affords NOEs at H-9ß (2.0%) and H-5ß (6.0%). The attachment of the hydroxymethyl group (C-20) to C-10 was confirmed by the NOEs observed at H-14*S* (13.0%), H-6α (9.0%) and 3H-19 (3.2%) on irradiation at δ_H 4.04. Homonuclear decoupling experiments assigned the signal at δ_H 2.23 (quin., J = 7.0 Hz) to H-16. Irradiation of H-13 affords a NOE (3.5%) at this signal which must therefore be H-16α (see p. 119). Thus the secondary methyl

group is ß and the configuration at C-16 is *ent*-16*S*. This evidence leads to *ent*-16(*S*)-7ß,20-dihydroxykauran-15-one (**11**) as the structure of the natural product.

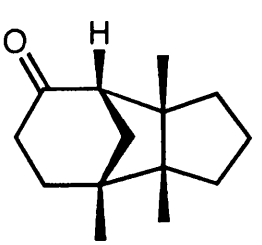
Although *ent*-kaurenes **1**, **12** and **13** are known[1-3] their ^{13}C NMR data have not been reported and these have therefore been listed in Table 1. Only one of the three known *ent*-kauranes that were isolated, *ent*-11α-hydroxy-16-kauren-15-one (**1**), has been isolated previously from *J. truncata*[1]. This was one of the first *ent*-kaurane derivatives to be isolated from a liverwort[3].

The next two components of the extract proved to be sesquiterpenoid derivatives with a gymnomitrane skeleton. The molecular formula of **14** was determined as $C_{14}H_{22}O$ ($[M]^+$ at m/z 206.1663) by HRMS. The IR spectrum showed the presence of a six-membered ketone (ν_{max} 1707 cm^{-1}). The ^1H NMR spectrum (see Experimental) has signals for three tertiary methyls (δ_H 1.07, 0.99, 0.95) while the ^{13}C NMR spectrum (Table 2) shows fourteen carbons : three methyls, six methylenes, one methine, three quaternary carbons and a carbonyl carbon [δ_C215.2 (s)]. Compound **14** is therefore a tricyclic norsesquiterpene with three tertiary methyls and one ketone group. Comparison of the ^{13}C NMR data (Table 2) of **14** with those of 3(15)-gymnomitrene (**25**)[16] revealed considerable similarity and suggested that **14** has a norgymnomitrane skeleton with a carbonyl group replacing the exomethylene at C-3. The resonance at δ_H 2.21 (d, J = 4.5 Hz) was assigned to H-2. Inspection of models revealed that H-2 has a dihedral angle of 90° with H-1*R* [δ_H 1.70 (d, J = 12.1 Hz)] and therefore couples only with H-1*S* [δ_H 2.08 (ddd, J = 12.1, 4.5, 2.9 Hz)]. This situation has been seen in other gymnomitranes, e.g. gymnomitrol (**26**)[17] in which H-2 appears as a sharp singlet because the relevant torsion angle with H-1*R* is near 90°. Thus compound **14** is

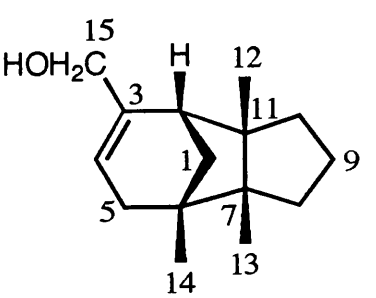
Table 2. ^{13}C NMR data of gymnomitranes.

C	14	15	25[a]	27[a]
1	41.8 t	42.8 t	46.8 t	89.3 d
2	63.3 d	47.0 d	56.0 d	56.9 d
3	215.2 s	143.5 s	152.0 s	141.9 s
4	33.6 t	121.2 d	28.7 t	120.3 d
5	37.1 t	40.3 t	37.1 t	41.2 t
6	43.7 s	43.9 s	43.1 s	47.4 s
7	54.4 s	55.5 s	54.1 s	56.4 s
8	36.0 t	37.2 t	35.9 t	38.7 t
9	26.5 t	27.2 t	27.5 t	27.7 t
10	37.8 t	38.3 t	38.1 t	39.7 t
11	55.9 s	58.4 s	55.4 s	58.4 s
12	27.3 q	27.5 q	27.5 q	26.9 q
13	24.8 q	24.7 q	24.8 q	24.4 q
14	23.5 q	23.6 q	23.4 q	20.2 q
15		67.3 t	107.5 t	64.4 t

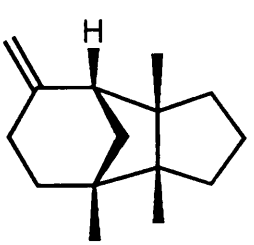
[a] Data from ref 16.



(14)



(15)



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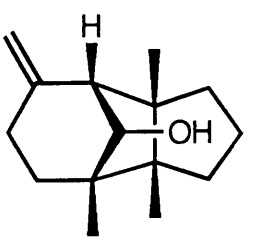
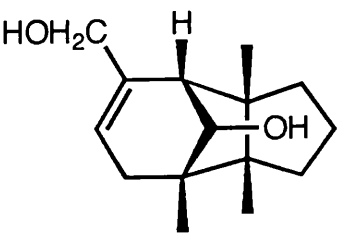


(26)



(27)

15-nor-3-gymnomitrone. This is the first example of a norgymnomitrane derivative as a natural product.

HRMS of compound **15** revealed the molecular formula $C_{15}H_{24}O$ ($[M]^+$ at m/z 220.1818). The IR spectrum showed the presence of a hydroxyl group (ν_{max} 3619, 3349 cm^{-1}). The ^1H NMR spectrum (see Experimental) contained signals for three tertiary methyls (δ_H 1.00, 0.91, 0.85), two protons attached to an oxygenated carbon [δ_H 3.98 (m, 2H)] and an olefinic proton [δ_H 5.48 (m)]. The ^{13}C NMR spectrum (Table 2) showed the presence of a trisubstituted double bond [δ_C 143.5 (s), 121.2 (d)] and a primary alcohol (δ_C 67.3). There were further signals for three methyls, five methylenes, one methine and three quaternary carbons. These data indicate a tricyclic sesquiterpenoid containing three tertiary methyl groups, a trisubstituted double bond and a primary alcohol. These data are consistent with structure **15**, 3-gymnomitren-15-ol. Comparison of the ^{13}C NMR data of **15** with those of 3(15)-gymnomitrene (**25**)[16] and 3-gymnomitrene-1,15-diol (**27**)[16] (Table 2) supported this proposal. Moreover the ^1H NMR spectrum of **27**[16] includes signals at δ_H 3.98 (br s, 2H) and δ_H 5.44 (br s, 1H) which are very similar to the two low field signals in the ^1H NMR spectrum of **15**. Confirmation of structure **15** was provided by homonuclear decoupling and NOE difference experiments. As expected (*vide supra*) H-2 (δ_H 1.81) resonates as a doublet (J = 4.4 Hz) and couples only with H-1*S*. The protons associated with the C-1 methylene group appear at δ_H 1.93 (ddd, J = 10.8, 4.4, 1.0 Hz, H-1*S*) and 1.43 (d, J = 10.8 Hz, H-1*R*). Irradiation of H-2 affords NOEs at 2H-15 (1.3%) and 3H-12 (1.3%) supporting the attachment of the hydroxymethyl group to C-3. The olefinic proton (H-4) exhibits allylic coupling to the hydroxymethyl protons (2H-15),

together with vicinal couplings to the methylene protons H-5α [δ_H 2.22 (m, J_{gem} = 18.8, J_{vic} = 3.4 Hz)] and H-5ß [δ_H 1.93 (br dd, J_{gem} = 18.8, J_{vic} = 3.4 Hz)]. Both these methylene protons at C-5 also show homoallylic coupling with the hydroxymethyl protons. In addition H-5α has a further small W coupling with H-1*S* (J = 1.0 Hz). On the basis of the above results compound **15** is 3-gymnomitren-15-ol.

EXPERIMENTAL

Jungermannia truncata, collected in Malaysia in January 1993, was ground after being air-dried. The ground material, which was contaminated with soil, was extracted with Et_2O to give a crude extract (2.2 g) which was chromatographed on silica gel using the solvent system petroleum ether-Et_2O gradient to give seven fractions. Each of these fractions was rechromatographed on silica gel preparative plates to give the following constituents in order of increasing polarity : *ent*-16-kauren-15-one (**12**) (12 mg), *ent*-15α-hydroxy-16-kaurene (**13**) (45 mg), 15-nor-3-gymnomitrone (**14**) (6 mg), *ent*-7ß-hydroxy-16-kaurene (**4**) (4 mg), 3-gymnomitren-15-ol (**15**) (7 mg), pimara-9(11),15-dien-19-ol (**16**) (6 mg), pleuroziol (**17**) (3 mg), *ent*-7ß-hydroxy-16-kauren-15-one (**3**) (380 mg), *ent*-16(*S*)-7ß-hydroxykauran-15-one (**5**) (1 mg), *ent*-3α-hydroxy-16-kauren-15-one (**6**) (7 mg), *ent*-15ß,7ß-dihydroxy-16-kaurene (**7**) (4 mg), *ent*-15α,7ß-dihydroxy-16-kaurene (**8**) (30 mg); a mixture (3 mg) of **8**, *ent*-16(*R*)-7ß,20-dihydroxykauran-15-one (**9**) and *ent*-7ß,20-dihydroxy-16-kauren-15-one (**10**); *ent*-11α-hydroxy-16-kauren-15-one (**1**) (1 mg) and *ent*-16(*S*)-7ß,20-dihydroxykauran-15-one (**11**) (1 mg).

Ent-16-kauren-15-one (**12**) was obtained as a gum.

EIMS m/z (rel.int.) : 286 $[M]^+$ (19), 271 (12), 218 (25), 149 (38), 109 (33), 95 (47), 81 (52), 69 (53), 55 (71), 43 (100), 28 (76).

δ_H (lit.[2]) : 5.92 (t, *J* = 1.1 Hz, H-17*Z*); 5.23 (t, *J* = 1.1 Hz, H-17*E*); 3.02 (m, H-13); 2.40 (d, *J* = 14.1 Hz, H-14*S*); 1.07 (s, 3H-20); 0.86 (s, 3H-18); 0.80 (s, 3H-19).

δ_C : see Table 1.

Ent-15 α-hydroxy-16-kaurene (**13**) was obtained as a semicrystalline solid, $[\alpha]_D$ -44° (c, 1.00 in $CHCl_3$) [lit.[2] $[\alpha]_D$ -70°].

EIMS m/z(rel. int.) : 288 $[M]^+$ (21), 273 (25), 270 (13), 255 (32), 230 (42), 215 (19), 149 (28), 109 (33), 91 (51), 81 (50), 69 (56), 55 (75), 41 (100), 28 (70).

δ_H (lit.[2]) : 5.08 (m, H-17*Z*); 4.94 (dt, *J* = 2.9, 0.9 Hz, H-17*E*); 3.72 (m, H-15α); 2.64 (m, H-13); 1.98 (d, *J* = 12.0 Hz, H-14*S*); 1.02 (s, 3H-20); 0.98 (br dd, *J* = 12.0, 5.0 Hz, H-14*R*); 0.84 (s, 3H-18); 0.79 (dd, *J* = 11.8, 2.0 Hz, H-5ß); 0.79 (s, 3H-19).

δ_C : see Table 1.

15-Nor-3-gymnomitrone (**14**) was isolated as a gum.

HRMS : m/z 206.1663 $[M]^+$ calculated for $C_{14}H_{22}O$: 206.1671.

T_R (mins) : 14.87

ν_{max} (cm^{-1}) : 2955, 2872; 1707 (C=O).

EIMS m/z (rel.int.) : 206 $[M]^+$ (15), 188 (23), 137 (25), 121 (15), 110 (46), 95

(100), 81 (48).

δ_H : 2.36 (m, 2H-4), 2.21 (d, *J* = 4.5 Hz, H-2); 2.08 (ddd, *J* = 12.1, 4.5, 2.9 Hz, H-1*S*); 1.70 (d, *J* = 12.1 Hz, H-1*R*); 1.07 (s, 3H); 0.99 (s, 3H); 0.95 (s, 3H).

δ_C : see Table 2.

Ent-7β-hydroxy-16-kaurene (**4**) was obtained as a semicrystalline solid.

HRMS : m/z 288.2456 $[M]^+$ calculated for $C_{20}H_{32}O$: 288.2453.

T_R (mins) : 28.17

ν_{max} (cm^{-1}) : 3627, 3509 (OH); 3069, 2930, 2867; 1657 (C=C).

EIMS m/z (rel.int.) : 288 $[M]^+$ (100), 270 (52), 255 (16), 190 (24), 164 (28), 149 (16), 123 (42), 109 (31), 91 (40), 79 (53).

δ_H : 4.82 (m, H-17*Z*); 4.76 (m, H-17*E*); 3.46 (dd, *J* = 12.2, 4.2 Hz, H-7ß); 2.68 (m, H-13); 2.65 (dt, *J* = 16.8, 2.7 Hz, H-15α); 1.92 (br d, *J* = 16.8 Hz, H-15ß); 1.80 (ddd, *J* = 12.2, 4.2, 1.7 Hz, H-6ß); 1.78 (br d, *J* = 12.4 Hz, H-9ß); 1.67 (m, H-14*R*); 1.37 (q, *J* = 12.2 Hz, H-6α); 1.02 (s, 3H-20); 0.86 (s, 3H-18); 0.86 (dd, *J* = 12.2, 1.7 Hz, H-5ß); 0.81 (s, 3H-19); 0.71 (td, *J* = 13.0, 4.0 Hz, H-1ß).

δ_C : see Table 1.

3-Gymnomitren-15-ol (**15**) was obtained as a gum, $[\alpha]_D$ + 27° (c, 0.167 in $CHCl_3$).

HRMS : m/z 220.1818 $[M]^+$ calculated for $C_{15}H_{24}O$: 220.1827.

T_R (mins) : 16.98

ν_{max} (cm^{-1}) : 3619, 3349 (OH); 2932, 1728, 1462, 1383, 1375.

EIMS m/z (rel. int.) : 220 $[M]^+$ (15), 189 (12), 136 (9), 124 (52), 111 (13), 106 (39), 95 (100), 79 (42), 67 (16).

δ_H : 5.48 (m, H-4); 3.98 (m, 2H-15); 2.22 (m, H-5α); 1.93 (br dd, *J* = 18.8, 3.4 Hz, H-5ß); 1.93 (ddd, *J* = 10.8, 4.4, 1.0 Hz, H-1*S*); 1.81 (d, *J* = 4.4 Hz, H-2); 1.43 (d, *J* = 10.8 Hz, H-1*R*); 1.00 (s, 3H-12); 0.91 (s, 3H-13); 0.85 (s, 3H-14).

δ_C : see Table 2.

Pimara-9(11),15-dien-19-ol (**16**) was obtained as a gum.

T_R (mins) : 27.64

GCMS m/z (rel.int.) : 288 $[M]^+$ (18), 273 (86), 257 (100), 220 (18), 189 (74), 161 (25), 147 (24), 133 (38), 119 (58), 105 (70), 91 (87), 79 (61), 67 (43), 55 (37), 41 (44).

δ_H (lit.[4]) : 5.81 (dd, *J* = 17.5, 10.7 Hz, H-15); 5.35 (dt, *J* = 5.0, 2.4 Hz, H-11); 4.92 (dd, *J* = 17.5, 1.5 Hz, H-16*E*); 4.85 (dd, *J* = 10.7, 1.5 Hz, H-16*Z*); 3.84 (d, *J* = 10.8 Hz, H-19); 3.53 (d, *J* = 10.8 Hz, H-19); 1.03 (d, *J* = 0.6 Hz, 3H-20); 0.97 (s, 3H-17), 0.96 (s, 3H-18).

δ_C (lit.[4]) : 151.3 (s, C-9); 150.3 (d, C-15); 115.8 (d, C-11); 109.1 (t, C-16); 65.0 (t, C-19); 46.3 (d, C-5); 41.6 (t, C-14); 41.1 (t, C-1); 38.4 (s, C-4); 37.8 (s, C-10); 37.6 (t, C-12); 35.5 (t, C-3); 34.8 (s, C-13); 29.0 (d, C-8); 26.9 (t, C-7); 26.5 (q, C-18); 26.0 (q,

C-20); 22.5 (q, C-17); 19.1 (t, C-6); 18.0 (t, C-2).

Pleuroziol (**17**) was isolated as a gum.

T_R (mins) : 26.25

ν_{max} (cm^{-1}) : 3615, 3490 (OH); 2925, 2880, 1725.

δ_H (lit.[5]) : 5.89 (dd, *J* = 17.4, 10.7 Hz, H-14); 5.20 (dd, *J* = 17.4, 1.3 Hz, H-15*E*); 5.06 (dd, *J* = 10.7, 1.3 Hz, H-15*Z*); 1.31 (s, 3H-16); 0.97, 0.85 (both s, 3H-19 and 3H-20); 0.82 (s, 3H-18); 0.77 (d, *J* = 6.4 Hz, 3H-17).

δ_C (lit.[5]) : 145.0 (d, C-14); 111.9 (t, C-15); 76.7 (s, C-5)*; 73.4 (s, C-13); 40.8 (d, C-10); 38.8 (s, C-4); 38.4 (s, C-9); 36.9 (t, C-3); 36.4 (d, C-8); 35.1 (t, C-12); 31.9 (t, C-6); 31.3 (t, C-11); 27.6 (q, C-16); 26.3 (t, C-7); 24.4 (q, C-19); 24.0 (q, C-18); 22.0 (t, C-1); 21.5 (t, C-2); 17.5 (q, C-20); 15.8 (q, C-17).

* literature value as signal obscured[5].

δ_C (C_6D_6)$^+$: 145.7 (d, C-14); 111.5 (t, C-15); 76.0 (s, C-5); 73.0 (s, C-13); 41.2 (d, C-10); 39.0 (s, C-4); 38.7 (s, C-9); 37.1 (t, C-3); 36.7 (d, C-8); 35.5 (t, C-12); 32.4 (t, C-6); 31.6 (t, C-11); 28.2 (q, C-16); 26.8 (t, C-7); 24.7 (q, C-19); 24.1 (q, C-18); 22.5 (t, C-1); 21.8 (t, C-2); 18.0 (q, C-20); 16.2 (q, C-17).

$^+$ Values relative to C_6D_6 at 128.0 ppm.

Ent-7β-hydroxy-16-kauren-15-one (**3**) was obtained as a crystalline compound and recrystallised from petroleum ether-Et_2O, m.p. 142-144°C $[\alpha]_D$ -112° (c, 1.16 in $CHCl_3$).

HRMS : m/z 302.2239 $[M]^+$ calculated for $C_{20}H_{30}O_2$: 302.2246.

ν_{max} (cm^{-1}) : 3625, 3486 (OH); 3082, 2992, 2932, 2870; 1727 (C=O); 1645 (C=C).

λ_{max} (EtOH)(nm) : 232

EIMS m/z (rel.int.) : 302 $[M]^+$ (100), 274 (44), 246 (15), 225 (21), 180 (20), 165 (30), 152 (40), 135 (61), 109 (49), 91 (45), 79 (52).

δ_H : 5.91 (t, *J* = 1.1 Hz, H-17*Z*); 5.23 (t, *J* = 1.1 Hz, H-17*E*); 4.03 (dd, *J* = 12.6, 4.5 Hz, H-7ß); 3.05 (m, H-13); 2.78 (br s, OH); 2.03 (m, 2H-14); 1.92 (tdd, *J* = 13.4, 7.0, 3.0 Hz, H-12α); 1.81 (ddd, *J* = 12.6, 4.5, 1.8 Hz, H-6ß); 1.74 (m, H-1α); 1.66 (br q, *J* = 14.0 Hz, H-2α); 1.65 (m, H-12ß); 1.38 (q, *J* = 12.6 Hz, H-6α); 1.13 (br d, *J* = 8.5 Hz, H-9ß); 1.06 (s, 3H-20); 0.88 (dd, *J* = 12.6, 1.8 Hz, H-5ß); 0.86 (s, 3H-18); 0.80 (s, 3H-19); 0.68 (td, *J* = 12.4, 3.4 Hz, H-1ß).

δ_C : see Table 1.

Ent-16(S)-7β-hydroxykauran-15-one (**5**) was isolated as a semicrystalline solid.

HRMS : m/z 304.2421 $[M]^+$ calculated for $C_{10}H_{32}O_2$: 304.2402.

T_R (mins) : 31.38

ν_{max} (cm^{-1}) : 3625, 3470 (OH); 2940, 2880; 1740 (C=O).

EIMS m/z (rel.int.) : 304 $[M]^+$ (100), 286 (25), 271 (15), 246 (63), 152 (39), 123 (56), 109 (54), 93 (32), 81 (56).

δ_H : 3.94 (dd, *J* = 12.4, 4.6 Hz, H-7ß); 2.46 (m, H-13); 2.21 (quin., *J* = 7.0 Hz, H-16α); 2.09 (dd, *J* = 12.1, 1.3 Hz, H-14*S*); 1.95 (br dd, *J* = 12.1, 4.5 Hz, H-14*R*); 1.80 (ddd, *J* = 12.4, 4.6, 1.8 Hz, H-6ß); 1.37 (q, *J* = 12.4 Hz, H-6α); 1.09 (d, *J* = 7.0 Hz, 3H-17); 1.07 (s, 3H-20); 1.02 (br d, *J* = 8.8 Hz, H-9ß); 0.93 (dd, *J* = 12.4, 1.8 Hz, H-5ß); 0.87 (s, 3H-18); 0.81 (s, 3H-19); 0.68 (td, *J* = 13.0, 4.0 Hz, H-1ß).

δ_C : see Table 1.

Ent-3α-hydroxy-16-kauren-15-one (**6**) was isolated as a gum.

HRMS : m/z 302.2247 $[M]^+$ calculated for $C_{20}H_{30}O_2$: 302.2246.

T_R (mins) : 32.06

ν_{max} (cm^{-1}) : 3630, 3500 (OH); 2940, 2860; 1725 (C=O); 1650 (C=C).

λ_{max}(EtOH)(nm) : 229

EIMS m/z (rel.int.) : 302 $[M]^+$ (100), 284 (43), 269 (64), 246 (20), 149 (85), 136 (29), 121 (39), 107 (42), 91 (57).

δ_H : 5.93 (t, *J* = 1.1 Hz, H-17*Z*); 5.24 (t, *J* = 1.1 Hz, H-17*E*); 3.40 (br t, *J* = 2.5 Hz, H-3α); 3.03 (m, H-13); 2.40 (d, *J* = 11.9 Hz, H-14*S*); 1.93 (td, *J* = 13.0, 2.5 Hz, H-2α); 1.88 (tdd, *J* = 13.2, 6.1, 2.6 Hz, H-12); 1.68 (m, H-12); 1.56 (m, H-2ß); 1.32 (m, H-14*R*); 1.09 (s, 3H-20); 0.95 (s, 3H-18); 0.84 (s, 3H-19).

δ_C : see Table 1.

Ent-15β,7β-dihydroxy-16-kaurene (**7**) was isolated as a semicrystalline solid.

HRMS : m/z 304.2386 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402.

T_R (mins) : 31.52

ν_{max} (cm^{-1}) : 3600, 3405 (OH); 3071, 2930, 2869.

EIMS m/z (rel.int.) : 304 $[M]^+$ (100), 286 (96), 271 (42), 258 (30), 268 (4), 162 (37), 131 (24), 122 (64), 105 (49), 91 (91), 83 (24), 71 (25).

δ_H : 5.15 (br s, H-17*Z*); 5.07 (br s, H-17*E*); 4.07 (br s, H-15ß), 3.85 (dd, *J* = 12.4, 4.7 Hz, H-7ß); 3.83 (br s, OH); 2.81 (m, H-13); 2.04 (br dd, *J* = 12.2, 5.3 Hz, H-14*R*); 1.83 (ddd, *J* = 12.4, 4.7, 1.5 Hz, H-6ß); 1.79 (br d, *J* = 13.5 Hz, H-1α); 1.65 (br d, *J* = 12.2 Hz, H-14*S*); 1.42 (m, H-2ß); 1.41 (q, *J* = 12.4 Hz, H-6α); 1.03 (s, 3H-20); 0.98 (br d, *J* = 9.0 Hz, H-9ß); 0.89 (s, 3H-18); 0.88 (dd, *J* = 12.4, 1.5 Hz, H-5ß); 0.82 (s, 3H-19); 0.69 (td, *J* = 13.5, 3.7 Hz, H-1ß).

δ_C : see Table 1.

Ent-15α,7β-dihydroxy-16-kaurene(**8**) was isolated as a semicrystalline solid.

HRMS : m/z 304.2396 $[M]^+$ calculated for $C_{20}H_{32}O_2$: 304.2402.

T_R (mins) : 31.71

ν_{max} (cm^{-1}) : 3630, 3424 (OH); 2930, 2870.

EIMS m/z (rel.int.) : 304 $[M]^+$ (98), 286 (50), 271 (25), 246 (63), 152 (40), 137 (22), 123 (100), 109 (88), 91 (88), 81 (91).

δ_H : 5.09 (m, H-17*Z*); 4.96 (br d, *J* = 2.5 Hz, H-17*E*); 4.45 (br t, *J* = 2.5 Hz, H-15α); 3.56 (dd, *J* = 11.6, 4.5 Hz, H-7ß); 2.69 (m, H-13); 1.80 (ddd, *J* = 11.6, 4.5, 1.7 Hz, H-6ß); 1.56 (m, 2H-14); 1.37 (q, *J* = 11.6 Hz, H-6α); 1.30 (br d, *J* = 8.5 Hz, H-9ß); 1.03 (s, 3H-20); 0.87 (br d, *J* = 11.6 Hz, H-5ß); 0.86 (s, 3H-18); 0.81 (s, 3H-19).

δ_C : see Table 1.

Ent-16(R)-7β,20-dihydroxykauran-15-one (**9**) was isolated as part of a gummy mixture which also contained compounds **8** and **10**.

T_R (mins) : 34.50

GCMS m/z(rel.int.) : 320 $[M]^+$ (14), 302 (4), 292 (17), 274 (100), 259 (16), 203 (19), 163 (30), 149 (55), 125 (42), 109 (53), 81 (58), 55 (81), 41 (92).

δ_H : 4.05 (br s, 2H-20); 3.95 (dd, *J* = 11.8, 4.8 Hz, H-7ß); 2.15 (br d, *J* = 13.0 Hz, H-1α); 2.15 (br q, *J* = 7.6 Hz, H-16ß); 1.08 (d, *J* = 7.6 Hz, 3H-17); 0.90 (s, 3H-18); 0.85 (s, 3H-19); 0.61 (td, *J* = 13.0, 5.0 Hz, H-1ß).

δ_C : see Table 1.

Ent-7β,20-dihydroxy-16-kauren-15-one (**10**) was isolated as part of a gummy mixture which also contained compounds **8** and **9**.

T_R (mins) : 34.82

δ_H : 5.94 (br s, H-17*Z*); 5.26 (br s, H-17*E*); 4.12 (dd, *J* = 11.7, 5.1

Hz, H-7ß); 4.05 (br s, 2H-20); 3.08 (m, H-13); 2.15 (br d, *J* = 13.0 Hz, H-1α); 0.92 (s, 3H-18); 0.87 (s, 3H-19); 0.61 (td, *J* = 13.0, 5.0 Hz, H-1ß).

δ_C : see Table 1.

Ent-11α-hydroxy-16-kauren-15-one (**1**) was obtained as a gum.

T_R (mins) : 31.05

GCMS m/z(rel.int.) : 302 $[M]^+$ (29), 287 (80), 217 (12), 178 (24), 164 (34), 146 (83), 137 (16), 119 (39), 91 (62), 81 (54), 69 (61), 55 (100), 41 (94).

δ_H (lit.[3]) : 5.86 (t, *J* = 1.0 Hz, H-17*Z*); 5.25 (t, *J* = 1.0 Hz, H-17*E*); 4.04 (br d, *J* = 4.6 Hz, H-11α); 3.03 (m, H-13); 2.37 (d, *J* = 12.1 Hz, H-14*S*); 2.13 (ddd, *J* = 14.5, 4.9, 3.1 Hz, H-12α); 1.94 (dd, *J* = 14.5, 4.4 Hz, H-12ß); 1.87 (m, H-1α); 1.40 (m, H-14*R*); 1.37 (br s, H-9ß); 1.00 (s, 3H-20); 0.88 (s, 3H-18); 0.80 (s, 3H-19).

δ_C : see Table 1.

Ent-16(S)-7ß,20-dihydroxykauran-15-one (**11**) was isolated as a gum.

HRMS : m/z 320.2332 $[M]^+$ calculated for $C_{20}H_{32}O_3$: 320.2351.

T_R (mins) : 35.13

ν_{max} (cm^{-1}) : 3630, 3584, 3458 (OH); 2928, 2870; 1734 (C=O).

EIMS m/z(rel.int.) : 320 $[M]^+$ (7), 274 (48), 191 (12), 173 (12), 163 (14), 149 (29), 137 (23), 123 (28), 109 (44), 95 (100).

δ_H : 4.04 (s, 2H-20); 4.00 (dd, *J* = 11.7, 4.9 Hz, H-7ß); 2.46 (m, H-13); 2.23 (quin., *J* = 7.0 Hz, H-16α); 2.20 (br d, *J* = 12.0 Hz, H-14*S*); 2.08 (m, H-14*R*); 1.77 (ddd, *J* = 11.7, 4.9, 1.8 Hz, H-6ß); 1.52 (br d, *J* = 12.5 Hz, H-9ß); 1.46 (q, *J* = 11.7 Hz, H-6α); 1.10 (d, *J* = 7.0 Hz, 3H-17); 1.06 (dd, *J* = 11.7, 1.8 Hz, H-5ß); 0.90 (s, 3H-18); 0.84 (s, 3H-19); 0.60 (td, *J* = 13.0, 4.5 Hz, H-1ß).

δ_C : see Table 1.

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CHAPTER 6

BAZZANIA TRICRENATA

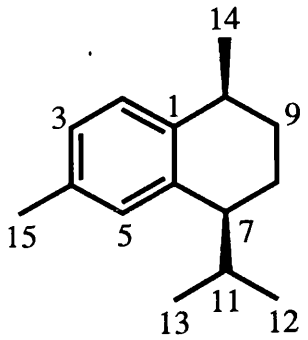
INTRODUCTION

Bazzania tricrenata belongs to the family Lepidoziaceae of the order Jungermanniales. *Bazzania* species of liverwort are rich sources of sesquiterpenoids. The sesquiterpene hydrocarbons ß-barbatene (gymnomitrene)[1-3], ß-bazzanene[1], calamenene (**1**)[1-3], ß-chamigrene[2] and cuparene[1-3] along with oxygenated sesquiterpenes (-)-drimenol[1-3], gymnomitrol[1,2] and 5-hydroxycalamenene (**2**)[1-3] have been reported already from *B. tricrenata.*

DISCUSSION

The ether extract of this plant was subjected to a combination of CC and PLC on silica gel to give a new peroxide with a cadinane skeleton (**3**), together with the known sesquiterpenoids, *cis*-calamenene (**1**)[1-6] and *cis*-5-hydroxycalamenene (**2**)[1,2,4,7,8] which are the main constituents of this extract, isobicyclogermacra-1(10),4-dien-15-al (**4**)[9,10], lepidozenal (**5**)[9,11] and a mixture of *cis*-2-hydroxycalamenene (**6**)[4] and *trans*-2-hydroxycalamenene (**7**)[12]. Isobicyclogermacra-1(10),4-dien-15-al (**4**) is unstable and, on standing, is oxidised to 1,10-epoxyisobicyclogermacr-4-en-15-al (**8**), a stable solid. The known compounds **1, 2, 4** and **5** were identified by comparison of their spectroscopic data with literature values[4-11]. The ^{13}C NMR data for lepidozenal (**5**) have not previously been reported and are listed in the Experimental.

Preparative TLC of an early fraction afforded a band whose GLC, ^{1}H NMR and ^{13}C NMR spectra clearly indicate the presence of a 2:1 mixture of sesquiterpenoids (**6** and **7**). Further efforts at separation of this mixture failed.



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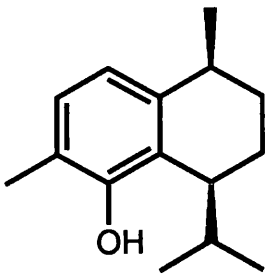

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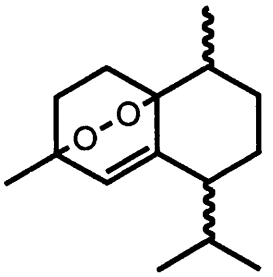

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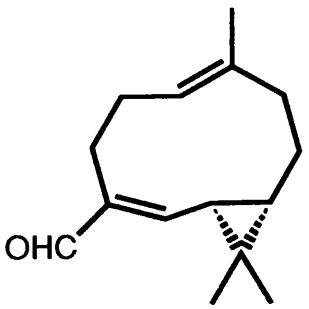

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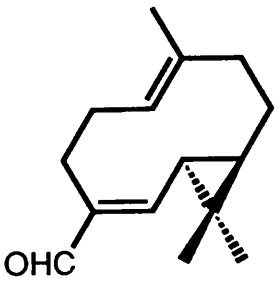

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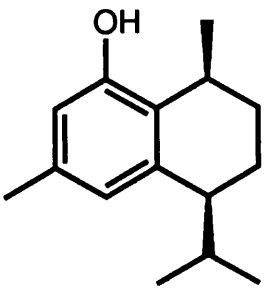

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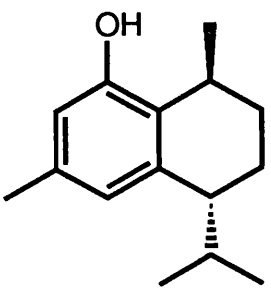

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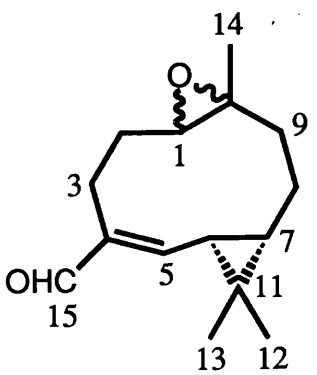

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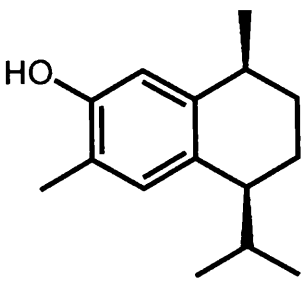

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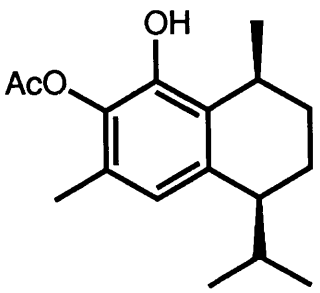


(10)

More detailed analysis of the ^{1}H and ^{13}C NMR data (see Experimental) of this mixture (**6** and **7**) and subsequent comparison with published ^{1}H and ^{13}C NMR data for the hydroxycalamenenes[4,12] revealed that the mixture consisted of *cis*- and *trans*-2-hydroxycalamenenes (**6** and **7**). The ^{1}H NMR spectrum of the mixture contained resonances for two pairs of aromatic protons [δ_H 6.70 (br s), 6.42 (br s), **6**; δ_H 6.58 (br s), 6.42 (br s), **7**] in addition to an aromatic methyl signal [δ_H 2.24 (s), **6** and **7**]. Homonuclear decoupling of the aromatic methyl signal resulted in a sharpening of the aromatic proton resonances into doublets revealing a *meta* coupling of 1.7 Hz for both **6** and **7**. This confirms the hydroxyl group attachment at C-2 in the calamenene skeleton. Further proton assignments were made by homonuclear decoupling (see Experimental). The ^{13}C NMR data for *cis*-2-hydroxycalamenene (**6**) have not been reported previously and are listed in the Experimental.

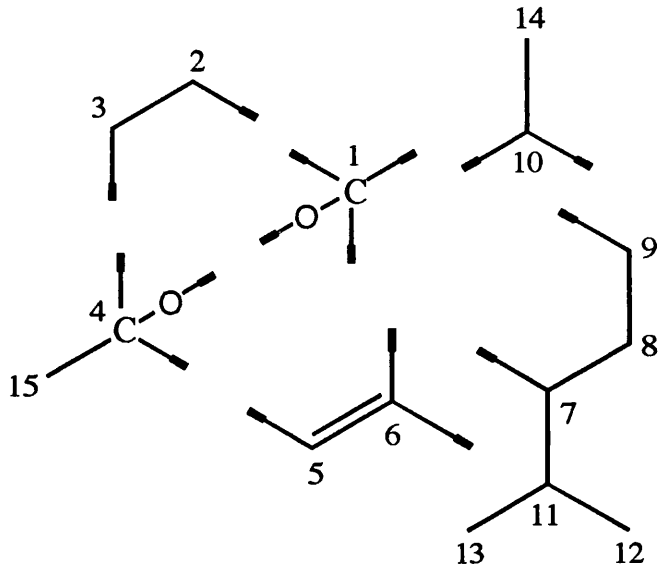
Both of these epimers have previously been found in Nature, *cis*-2-hydroxy-calamenene (**6**) in the liverwort *Bazzania trilobata*[3,4] and *trans*-2-hydroxy-calamenene (**7**) in the seeds of the Indonesian plants *Dysoxylum acutangulum* and *D. alliaceum*[12]. The absolute configuration of **7** from these Indonesian plants was shown to be 7*R*, 10*S*[12]. This compound displays significant toxicity against fish and also has antibacterial activity[12]. Only two other representatives of this skeletal type have been found in liverworts. These are *cis*-3-hydroxycalamenene (**9**) and *cis*-3-acetoxy-2-hydroxycalamenene (**10**) which have been reported from the liverwort *Lophocolea heterophylla*[13,14]. The absolute stereochemistry of **9** is 7*S*, 10*S*. This was presumably deduced from comparison of its optical rotation with that of the same compound isolated from *Eremophila drummondi* whose absolute

configuration was established by X-ray analysis[14]. *Cis*-calamenene (**1**) isolated from *B. trilobata* is the 7*S*, 10*S* (-) isomer (**1**) while the same compound isolated from *B. japonica* is the 7*R*,10*R* (+) isomer (*ent*-**1**)[4]. *Cis*-5-hydroxycalamenene (**2**) from *B. trilobata* has been assigned the 7*S*,10*S* absolute configuration on CD arguments[8].

It is interesting that in our investigations of *B. tricrenata* we have found both *cis*- and *trans*- calamenenes. *Trans*-calamenenes have not previously been found in liverworts. Unfortunately the absolute configuration of the calamenenes isolated from *B. tricrenata* in the present work was not established.

The molecular formula of compound **3**, $C_{15}H_{24}O_2$, was established by HRMS ($[M]^+$ at m/z 236.1783). The ^1H NMR spectrum (600 MHz) (see Experimental) indicated the presence of an olefinic proton [δ_H 5.98 (d, J = 2.4 Hz, H-5)], a tertiary methyl group attached to a carbon bearing oxygen [δ_H 1.37 (s, 3H-15)] and three secondary methyl groups [δ_H 0.96 (d, J = 6.8 Hz, 3H-12/13); 0.95 (d, J = 6.8 Hz, 3H-14); 0.79 (d, J = 6.8 Hz, 3H-12/13)]. The ^{13}C NMR spectrum (see Experimental) contained fifteen carbons, including a trisubstituted double bond [δ_C 148.4 (s, C-6); 126.3 (d, C-4)] and two oxygen-bearing quaternary carbons [δ_C 79.3 (C-4); 75.3 (C-1)]. The remaining resonances consist of four methyls, four methylenes and three methines. The compound is therefore tricyclic.

Analysis of the phase-sensitive DQF-COSY spectrum (600 MHz) (*vide infra*) enabled ^1H chemical shifts to be assigned to the remaining protons and established most of the proton connectivity. Several of the multiplets could be identified in the 600 MHz ^1H NMR spectrum and their coupling constants measured. From the above data the partial structures shown in Scheme 1 could be deduced.



Scheme 1

The phase-sensitive DQF-COSY spectrum (600 MHz) was of particular help in assigning the secondary methyl groups. The secondary methyl groups at δ_H 0.96 and 0.79 both correlate to H-11 [δ_H 2.06 (sept. d, J = 6.8, 3.8 Hz)] and hence must belong to the isopropyl group. Therefore the secondary methyl group at δ_H 0.95 must be 3H-14. The IR spectrum showed a strong absorption at ν_{max} 1215 cm^{-1} (C-O stretch) but lacked any absorptions due to hydroxyl functions. The MS contained fragments at m/z 220.1826 (2%) and 204.1867 (48%) due respectively to loss of an oxygen atom and molecular oxygen. This information led to the connection of the partial structures in Scheme 1 to build a cadinane skeleton with an endoperoxide bridge between the C-1 and C-4 positions as depicted in structure **3**. This structure was supported by a NOE difference experiment. Irradiation of H-5 resulted in NOEs at 3H-15 (0.9%), H-11 (5.0%) and the isopropyl methyl at δ_H 0.79 (1.0%). The absence of a NOE at the other isopropyl methyl (δ_H 0.96) suggests a preferred conformation (rotamer) of the isopropyl group. The higher field isopropyl methyl (δ_H 0.79) is probably lying in a shielding zone of the double bond. Compound **3** contains a dioxa-2,2,2-bicycloctene system. Other compounds

containing this system have similar ^{13}C NMR[15,16] and MS[17] properties to those of **3**. The cyclohexane ring of **3** is flexible and can exist in boat or twist conformations. The highfield ^{1}H NMR (600 MHz) did not provide the solution to the relative stereochemistry of the methyl at C-10 and the isopropyl at C-7 because the resonances for three of the protons of the C-8 and C-9 methylene groups and also H-10 overlap with other signals and are too complex to interpret. However the large allylic coupling (J = 2.4 Hz) between H-5 and H-7 indicates that H-7 must be almost orthogonal to the double bond plane[18]. Thus the isopropyl group at C-7 is pseudoequatorial.

It is possible that compound **3** is produced from a cadinane derivative *via* a photooxidation involving 1,4-cycloaddition of singlet oxygen[17]. Some dioxetanes (endoperoxides) are reported to be unstable and are easily converted mainly to the corresponding diepoxides[17]. There was no indication of this happening with compound **3**, which is stable.

Compound **8** has the molecular formula $C_{15}H_{22}O_2$ ($[M]^+$ at m/z 234.1630, HRMS) indicating five double bond equivalents. Its IR and UV spectra showed the presence of a conjugated carbonyl group (ν_{max} 1678 cm^{-1}; λ_{max} 262 nm). The ^{1}H NMR spectrum (see Experimental) has signals for three tertiary methyl groups (δ_H 1.23, 1.17, 0.93), an olefinic proton [δ_H 6.48 (br d, J = 9.4 Hz)] and a formyl group [δ_H 9.36 (d, J = 1.1 Hz)]. The ^{13}C NMR spectrum contains fifteen carbons (see Experimental) which include three methyls, four methylenes, two methines, a methine bearing an oxygen (δ_C 62.9), two olefinic carbons [δ_C 144.0 (s), 154.9 (d)], one formyl carbon [δ_C 193.7 (d)], one quaternary carbon and a quaternary carbon bearing an oxygen (δ_C 60.0). These data are very similar to those of **4** (see

Experimental) except for the loss of the trisubstituted double bond [δ_C 134.5 (s), 124.4 (d); δ_H 5.05 (br t, J = 7.5 Hz)] and the appearance of a trisubstituted epoxide moiety [δ_C 62.9 (d), 60.0 (s); δ_H 2.96 (dd, J = 11.1, 3.0 Hz)]. The above spectral data indicates that compound **8** is 1,10-epoxyisobicyclogermacr-4-en-15-al. Compound **8** is clearly an artifact and may not be present in the original extract.

The sesquiterpenoid aldehydes **4** and **5**, which we have also isolated from *Lepidozia reptans* (see Chapter 7), have been found in *L. vitrea*, from where they were first reported[9]. Compound **4** has been found in *Bazzania japonica*[19]. It is interesting from a chemosystematics point of view that *B. tricrenata*, *B. japonica*, *L. vitrea* and *L. reptans*, all members of the Lepidoziaceae family, biosynthesize the same sesquiterpene aldehydes (**4** and **5**) which have not yet been found in any other liverwort species.

EXPERIMENTAL

The air-dried and ground liverwort (254 g; collected from a site at Beinn Damh in the North West of Scotland in September 1991) was immersed in Et_2O for 1 week. Removal of the solvent afforded a thick green oil (3.2 g) which was subjected to the usual chromatographic separation to give in order of increasing polarity a sesquiterpene hydrocarbon/lipid mixture (150 mg), *cis*-calamenene (**1**) (30 mg), *cis*-5-hydroxycalamenene (**2**) (67 mg), isobicyclogermacra-1(10),4-dien-15-al (**4**) (4 mg), lepidozenal (**5**) (7 mg); a 2:1 mixture (13 mg) of *cis*-2-hydroxy-calamenene (**6**) and *trans*-2-hydroxycalamenene (**7**); 1,4-peroxycadin-5-ene (**3**) (15 mg) and 1,10-epoxyisobicyclogermacr-4-en-15-al (**8**) (5 mg).

Analysis of sesquiterpene hydrocarbon/lipid mixture. An aliquot of the first fraction was subjected to GLC analysis and this revealed the presence of four main sesquiterpene hydrocarbons in the mixture : T_R = 9.92, 11.39, 13.23 and 13.69 mins (comparison with GLC of *n*-alkane standards indicates that the peaks are in the sesquiterpene hydrocarbon region). It is evident from ^1H NMR analysis that about half the fraction consists of lipid and it is likely that one of the sesquiterpene hydrocarbons is *cis*-calamenene (**1**) which was subsequently isolated from the next fraction. The other sesquiterpene hydrocarbons remain unidentified since they could not be separated by PLC and there were no authentic samples for GLC comparison.

Cis-calamenene (**1**) was isolated as an oil.

T_R (mins) : 13.23

δ_H (lit.[4]) : 7.04 (d, *J* = 7.9 Hz, H-2); 7.02 (s, H-5) ; 6.93 (br d, *J* = 7.9 Hz, H-3); 2.85 (m, H-10); 2.60 (m, H-7); 2.29 (s, 3H-15); 2.28 (m, H-11); 1.4-1.9 (m, 2H-8 and 2H-9); 1.24 (d, *J* = 7.0 Hz, 3H-14); 1.02 (d, *J* = 6.8 Hz, 3H-13); 0.76 (d, *J* = 6.8 Hz, 3H-12).

δ_C (lit.[6]) : 134.4 (s, C-4); 128.6 (d, C-5); 128.5 (d, C-2); 126.2 (d, C-3); 43.6 (d, C-7); 32.5 (d, C-11); 31.0 (d, C-10); 28.7 (t, C-9); 23.3 (t, C-8); 21.4, 21.2, 19.6, 17.5 (all q, C-12, C-13, C-14 and C-15).

The C-1 and C-6 resonances were not observed due to weakness of sample and poor signal to noise ratio [lit.[6] 140.2 (s, C-1); 140.0 (s, C-6)].

Cis-5-hydroxycalamenene (**2**) was obtained as an oil.

δ_H (lit.[8]) : 6.92 (d, *J* = 7.8 Hz, H-3); 6.74 (d, *J* = 7.8 Hz, H-2); 4.60 (s, OH); 2.86 (br sextet, *J* = 7.0 Hz, H-10); 2.72 (td, *J* = 6.9, 4.1 Hz, H-7); 2.20 (s, 3H-15); 2.02 (octet, *J* = 6.9 Hz, H-11); 1.70-2.15 (m, 2H-8 and 2H-9); 1.29 (d, *J* = 7.0 Hz, 3H-14); 0.94 (d, *J* = 6.9 Hz, 3H-13); 0.91 (d, *J* = 6.9 Hz, 3H-12).

δ_C (lit.[8]) : 151.4 (s, C-5); 141.5 (s, C-1); 127.8 (d, C-3); 127.1 (s, C-6); 120.3 (d, C-2); 119.4 (s, C-4); 38.4 (d, C-7); 32.5 (d, C-11); 31.4 (d, C-10); 27.3 (t, C-9); 23.9 (t, C-8); 23.6 (q, C-14); 21.5, 20.3 (both q, C-12 and C-13); 15.9 (q, C-15).

Isobicyclogermacra-1(10),4-dien-15-al (**4**) was obtained as an oil.

δ_H (lit.[10]) : 9.25 (d, *J* = 1.0 Hz, H-15); 6.28 (br d, *J* = 9.5 Hz, H-5); 5.05 (br t, *J* = 7.5 Hz, H-1); 2.75 (dt, *J* = 11.7, 3.4 Hz, H-3); 1.3-2.3 (m, 7H); 1.24 (d, *J* = 1.4 Hz, 3H-14); 1.17, 1.15 (both s, 3H-12 and 3H-13); 0.7-1.1 (m, 2H).

δ_C (lit.[10]) : 194.6 (d), 156.3 (d), 143.0 (s), 134.5 (s), 124.4 (d), 39.9 (t), 38.3 (d), 29.9 (d), 28.6 (q), 27.8 (t), 23.54 (t), 23.51 (t), 21.3 (s), 17.4 (q), 15.9 (q).

Lepidozenal (**5**) was isolated as an oil.

ν_{max}($CHCl_3$)(cm^{-1}) : 2925; 1665 (C=O); 1615 (C=C).

λ_{max} (nm) : 261

EIMS m/z (rel.int.) : 218 $[M]^+$ (3), 203 (3), 189 (5), 175 (6), 162 (3), 149 (49), 135

(8), 133 (9), 121 (12), 105 (14), 97 (40), 83 (59), 69 (58), 57 (73), 43 (74), 28 (100).

δ_H (lit.[9]) : 9.30 (br s, H-15); 6.33 (br d, *J* = 10.2 Hz, H-5); 5.01 (br t, *J* = 7.0 Hz, H-1); 2.65 (ddd, *J* = 12.0, 4.0, 2.9 Hz, 1H); 2.45 (m, 1H); 1.65-2.35 (m, 6H); 1.62 (d, *J* = 1.2 Hz, 3H-14); 1.21 (s, 3H-12/13); 1.20 (m, H-6); 1.10 (s, 3H-12/13); 0.55 (ddd, *J* = 12.0, 5.0, 3.0 Hz, H-7).

δ_H (C_6D_6)*(lit.[11]) : 9.38 (d, *J* = 1.0 Hz, H-14); 5.97 (br d, *J* = 10.1 Hz, H-5); 5.00 (br t, *J* = 7.5 Hz, H-1); 2.94 (ddd, *J* = 12.0, 4.0, 3.0 Hz, 1H); 2.43 (m, 1H); 1.95-2.30 (m, 3H); 1.86 (td, *J* = 12.9, 3.0 Hz, 1H); 1.65 (br dq, *J* = 13.0, 3.0 Hz, 1H); 1.40 (d, *J* = 1.2 Hz, 3H-15); 0.95 (s, 3H); 0.92 (dd, *J* = 10.0, 5.0 Hz, H-6); 0.90 (s, 3H); 0.72 (tdd, *J* = 14.0, 12.0, 3.0 Hz, 1H); 0.13 (ddd, *J* = 11.6, 5.0, 3.0 Hz, H-7).

* δ_H relative to C_6D_5H at δ_H 7.20.

δ_C : 194.3 (d, C-15); 158.1 (d, C-5); 141.0 (s, C-4); 133.1 (s, C-10); 125.8 (d, C-1); 40.0 (t, C-9); 37.8, 34.5 (both d, C-6 and C-7); 26.6, 23.9, 22.7 (all t, C-8, C-3 and C-2); 23.3 (s, C-11); 22.3, 21.4 (both q, C-12 and C-13); 15.4 (q, C-14).

Cis-2-hydroxycalamenene (**6**) was isolated as part of an oily mixture which also contains compound **7**.

T_R (mins) : 18.92

δ_H (lit.[4]) : 6.70, 6.42 (both br s, H-3 and H-5); 4.64 (br s, OH); 3.05 (m,

H-10); 2.70 (m, H-7); 2.35 (m, H-11); 2.24 (s, 3H-15); 1.5-2.1 (m, 2H-8 and 2H-9); 1.21 (d, J = 7.0 Hz, 3H-14); 1.04 (d, J = 6.9 Hz, 3H-13); 0.68 (d, J = 6.8 Hz, 3H-12).

δ_C : 153.0 (s, C-2); 141.4 (s, C-6); 135.7 (s, C-4); 126.7 (s, C-1); 120.8 (d, C-5); 112.8 (d, C-3); 43.2 (d, C-7); 30.8 (d, C-11); 28.8 (t, C-9); 26.5 (d, C-10); 21.2, 21.1, 20.5, 16.3 (all q, C-12, C-13, C-14 and C-15); 17.2 (t, C-8).

Trans-2-hydroxycalamenene (**7**) was isolated as part of an oily mixture which also contains compound **6**.

T_R (mins) : 18.81

δ_H (lit.[12]) : 6.58, 6.42 (both br s, H-3 and H-5); 4.64 (br s, OH); 3.05 (m, H-10); 2.70 (m, H-7); 2.35 (m, H-11); 2.24 (s, 3H-15); 1.5-2.1 (m, 2H-8 and 2H-9); 1.19 (d, J = 7.0 Hz, 3H-14); 0.98 (d, J = 6.8 Hz, 3H-13); 0.82 (d, J = 6.8 Hz, 3H-12).

δ_C (lit.[12]) : 153.0 (s, C-2); 141.2 (s, C-6); 135.0 (s, C-4); 125.9 (s, C-1); 122.9 (d, C-5); 113.3 (d, C-3); 43.0 (d, C-7); 33.2 (d, C-11); 27.2 (t, C-9); 26.6 (d, C-10); 22.1, 21.2, 21.1, 19.6 (all q, C-12, C-13, C-14 and C-15); 19.1 (t, C-8).

1,4-Peroxycadin-5-ene (**3**) was isolated as an oil.

HRMS : m/z 236.1783 $[M]^+$ calculated for $C_{15}H_{24}O_2$: 236.1776.

T_R (mins) : 18.62

$\nu_{max}(CHCl_3)(cm^{-1})$: 3020, 2960, 2930, 2870; 1215 (C-O).

EIMS m/z (rel.int.) : 236 $[M]^+$ (2), 220 (2), 218 (6), 208 (2), 205 (7), 204 (48), 189 (14), 175 (19), 161 (100), 149 (19), 133 (6), 119 (30), 105 (60), 91 (54), 81 (52).

δ_H (600 MHz) : 5.98 (d, *J* = 2.4 Hz, H-5); 2.45 (br ddt, *J* = 11.5, 6.0, 3.0 Hz, H-7); 2.15 (ddd, *J* = 12.8, 9.6, 3.8 Hz, H-2/3); 2.06 (sept. d, *J* = 6.8, 3.8 Hz, H-11); 2.01 (m, H-10); 2.01 (m, H-2/3); 1.79 (br ddt, *J* = 13.7, 9.2, 6.4 Hz, H-9); 1.68 (m, H-8); 1.48 (td, *J* = 12.2, 4.1 Hz, H-2/3); 1.37 (s, 3H-15); 1.26 (m, H-9); 1.25 (m, H-2/3); 1.20 (m, H-8); 0.96 (d, *J* = 6.8 Hz, 3H-12/13); 0.95 (d, *J* = 6.8 Hz, 3H-14); 0.79 (d, *J* = 6.8 Hz, 3H-12/13).

δ_C : 148.4 (s, C-6); 126.3 (d, C-5); 79.3 (s, C-4); 75.3 (s, C-1); 39.4 (d, C-7); 30.6 (d, C-11); 29.9, 26.7, 21.9, 19.3 (all t, C-2, C-3, C-8 and C-9); 27.4 (d, C-10); 22.0, 21.4, 16.9, 15.3 (all q, C-12, C-13, C-14 and C-15).

1,10-Epoxyisobicyclogermacr-4-en-15-al (**8**) was obtained as a semicrystalline solid.

HRMS : m/z 234.1630 $[M]^+$ calculated for $C_{15}H_{22}O_2$: 234.1620.

$\nu_{max}(CHCl_3)(cm^{-1})$: 2930; 1678 (C=O); 1628 (C=C); 1250 (C-O-C); 850.

λ_{max}(EtOH)(nm) : 262

EIMS m/z (rel.int.) : 234 $[M]^+$ (19), 219 (20), 205 (9), 191 (35), 179 (8), 163 (24), 149 (35), 135 (23), 119 (37), 107 (54), 91 (90), 79 (100).

δ_H : 9.36 (d, *J* = 1.1 Hz, H-15); 6.48 (br d, *J* = 9.4 Hz, H-5); 2.96 (dd, *J* = 11.1, 3.0 Hz, H-1); 2.76 (dt, *J* = 13.0, 3.9 Hz, H-3);

1.23 (s, 3H-14); 1.17, 0.93 (both s, 3H-12 and 3H-13).

δ_C : 193.7 (d), 154.9 (d), 144.0 (s), 62.9 (d), 60.0 (s), 39.9 (t), 38.6 (d), 28.8 (d), 28.4 (q), 27.8 (t), 23.9 (s), 22.0 (t), 20.5 (t), 17.3 (q), 15.5 (q).

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CHAPTER 7

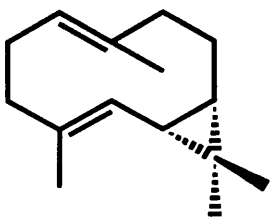
LEPIDOZIA REPTANS

INTRODUCTION

Lepidozia reptans belongs to the family Lepidoziaceae of the order Jungermanniales. The sesquiterpene hydrocarbons α-barbatene[1], ß-barbatene (gymnomitrene)[1,2], bicyclogermacra-1(10),4-diene (**1**)[2], ß-cubebene[2], cuparene (**2**)[2], ß-elemene[2], δ-elemene[2] and α-longipinene[1], and the sesquiterpene diol, (+)-eudesm-3-ene-6ß,7α-diol (**3**)[3] and an unknown sesquiterpenoid mono-alcohol[3] have previously been reported from *L. reptans*. An investigation of the structure of this mono-alcohol forms the subject matter of this chapter. Studies on the plant growth regulatory activity of bryophyte extracts revealed that *L. reptans* (collected in Germany) retards the growth of the shoot and the root of germinating *Lepidium sativum* seeds[4]; the compounds responsible for the activity were not identified.

DISCUSSION

The Et_2O extract of *Lepidozia reptans* was chromatographed on silica gel to give two major sesquiterpenoid components, eudesm-3-ene-6ß,7α-diol (**3**)[3] and a sesquiterpenoid mono-alcohol (**4**)[3], and four minor compounds, bicyclogermacra-1(10),4-diene (**1**)[2,5], cuparene (**2**)[2,6,7], isobicyclogermacra-1(10),4-dien-15-al (**5**)[8,9] and lepidozenal (**6**)[10,11]. The diol is easily detected on an analytical TLC plate as it produces a blue spot when sprayed with 25% H_2SO_4. The known compounds were identified by comparing their spectroscopic data with published data[3,5-7,9,11]. The full ^{13}C NMR data for cuparene (**2**) have not been reported and are therefore listed in the Experimental. The sesquiterpene aldehydes **5** and **6** are labile compounds.



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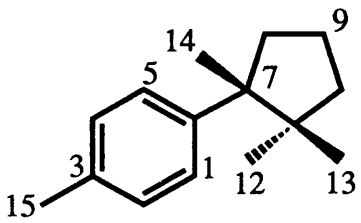

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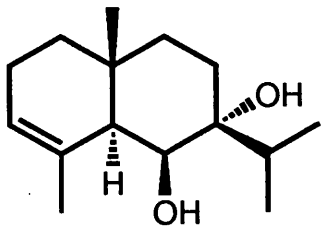

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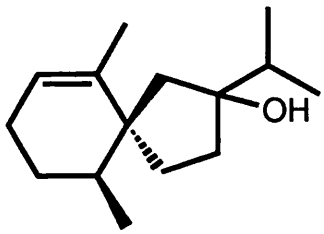

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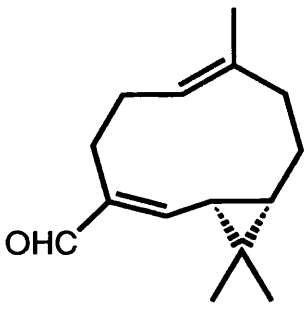

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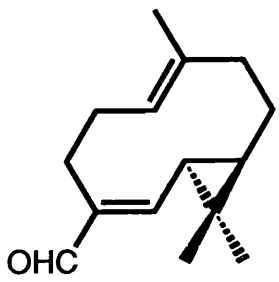

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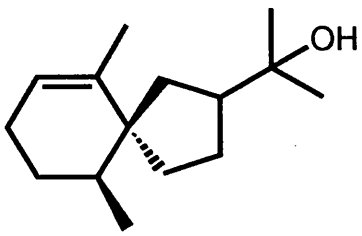

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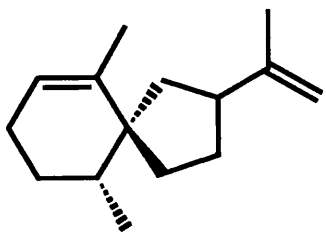

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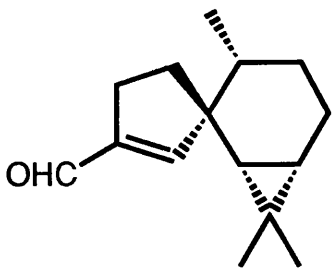


(9)

Compound **4** is an oil and has the molecular formula $C_{15}H_{26}O$ ($[M]^+$ at m/z 222.1981) as determined by HRMS. The IR spectrum and MS show the presence of a hydroxyl group [ν_{max} 3610 cm^{-1}; m/z 204.1878 $[M-18]^+$ (49%)]. The ^1H (600 MHz) and ^{13}C (75.47 MHz) NMR spectra (Table 1) indicated the presence of three secondary methyl groups [δ_H 0.95 (d, J = 6.9 Hz); 0.93 (d, J = 6.9 Hz); 0.89 (d, J = 7.0 Hz)], a vinyl methyl [δ_H 1.78 (dd, J = 3.5, 1.5 Hz)], a trisubstituted double bond [δ_C 139.6 (s), 121.5 (d); δ_H 5.31 (tq, J = 3.7, 1.5 Hz)] and a tertiary-hydroxyl-bearing carbon (δ_C 85.4). The ^{13}C NMR spectrum contains fifteen carbons. These spectroscopic data are identical with those reported previously for the unknown mono-alcohol[3]. This information indicates that compound **4** is a bicyclic sesquiterpene alcohol containing a trisubstituted double bond. The partial structures shown in Scheme 1 were derived from detailed analysis of phase-sensitive DQF-COSY (^1H, 600 MHz) (Table 2) and HMQC (^1H, 300.13 MHz; ^{13}C, 75.47 MHz) NMR spectra. The presence of an isopropyl group was indicated by the fact that there are three secondary methyl groups and only two sp^3 hybridised methine carbons (δ_C 37.7 and 37.8) and so two of the secondary methyl groups must be attached to the same methine carbon. Analysis of the 600 MHz ^1H NMR spectrum confirmed this since a septet signal (J = 6.9 Hz) was visible at δ_H 1.64. An AB system [δ_H 1.73 (d, J = 15.2 Hz), 1.59 (dm, obscured, J = 15.2 Hz)], clearly visible in the phase-sensitive DQF-COSY and HMQC spectra, indicated the presence of an isolated methylene group. The olefinic proton has vicinal coupling (J = 3.7 Hz) to geminal methylene protons at δ_H 1.97 and 1.89 and allylic coupling (J = 1.5 Hz) to the vinyl methyl. A homoallylic coupling (J = 3.5 Hz) was also detected between the vinyl methyl and one of the methylene protons. The joining

Table 1. ^{1}H (600 MHz) and ^{13}C (75.47 MHz) NMR data of compound 4[a,b].

Site	δ_C	δ_H
1	121.5 d	5.31 tq (3.7, 1.5)
2a	23.3 t	1.97 m[#]
b		1.89 m[#]
3a	27.4 t	1.65 m
b		1.35 m
4	37.7 d	1.63 m
5	49.2 s	
6a	45.3 t	1.73 d (15.2)
b		1.59 dm (15.2)
7	85.4 s	
8	38.7 t	1.5 - 1.7 m (2H)
9a	35.9 t	2.15 m
b		1.54 m
10	139.6 s	
11	37.8 d	1.64 sept. (6.9)
12	17.6 q	0.93 d (6.9)*
13	17.6 q	0.95 d (6.9)*
14	20.5 q	1.78 dd (3.5, 1.5)
15	16.0 q	0.89 d (7.0)

[a] Assignments were confirmed by phase-sensitive DQF-COSY, HMQC and HMBC experiments.

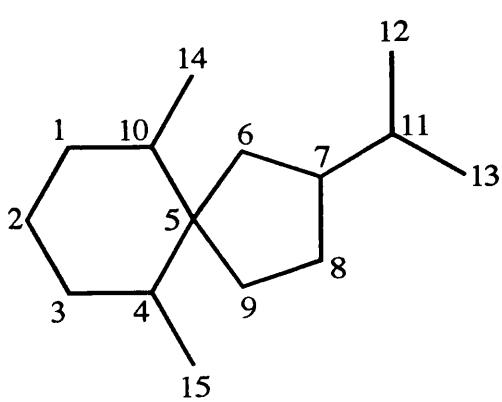
[b] Figures in parentheses are coupling constants (J) in Hz.

* Values may be interchanged.

J_{gem} = 17.8 Hz

Table 2. Phase-sensitive DQF-COSY data for compound 4 (600 MHz).

H	Correlated H
H-1	H-2a, H-2b, 3H-14
H-2a	H-1, H-2b, H-3a, H-3b, 3H-14
H-2b	H-1, H-2a, H-3a, H-3b, 3H-14
H-3a	H-2a, H-2b, H-3b
H-3b	H-2a, H-2b, H-3a
H-4	3H-15
H-6a	H-6b
H-6b	H-6a
(H-8a, H-8b)	H-9a
H-9a	(H-8a, H-8b), H-9b
H-9b	H-9a
H-11	(3H-12, 3H-13)
(3H-12, 3H-13)	H-11
3H-14	H-1, H-2a, H-2b
3H-15	H-4



Spirovetivane skeleton

of these partial structures was permitted by the long-range correlations observed in the HMBC spectrum (Table 3). Long-range correlations are assumed to occur over two or three bonds. The starting point for the connection of the partial structures is the isopropyl group. The methyl protons (3H-12 and 3H-13) show correlations with the carbinol carbon (C-7) thus establishing a bond between C-7 and C-11. The quaternary carbon C-5 (δ_C 49.2) has $^3J_{CH}$ correlations from 3H-14, 3H-15 and H-1 thus establishing the 4,5 and 5,10 bonds. The formation of the 3,4 bond and closure of the six-membered ring follows from a $^3J_{CH}$ correlation of C-3 (δ_C 27.4) with 3H-15. Three methylene carbons remain to be placed. One proton (H-9a) attached to the methylene carbon at δ_C 35.9 (C-9) correlates with C-10 [δ 139.6(s)]. This must be a $^3J_{CH}$ and hence there must be a bond from C-5 to C-9. Then the 2H-9 to C-7 correlations must arise from $^3J_{CH}$ and establish the C-7 to C-8 bond. The H-9b to C-6 correlation must also arise from $^3J_{CH}$ and establishes the C-5 to C-6 bond. The 6,7 bond falls by default. These data reveal that compound **4** is 1(10)-spirovetivene-7-ol. Comparison of the ^{13}C NMR data of compound **4** with 1(10)-spirovetivene-11-ol (**7**)[12] and α-spirovetivene (**8**)[13] showed similar chemical shifts for the six-membered cyclohexene ring and so supports the above conclusions.

Information obtained from NOE difference experiments, run at 600 MHz, led to the provisional assignment of the relative stereochemistry shown in **4**. A Fieser model suggests that the five-membered ring is relatively flexible but the six-membered ring is rather less so, showing a preference for half-chair forms. The NOE difference results were interpreted by Dr. I.H. Sadler (Edinburgh University) as follows. Irradiation of 3H-14 gives NOEs at H-9a and H-6b. This suggests H-

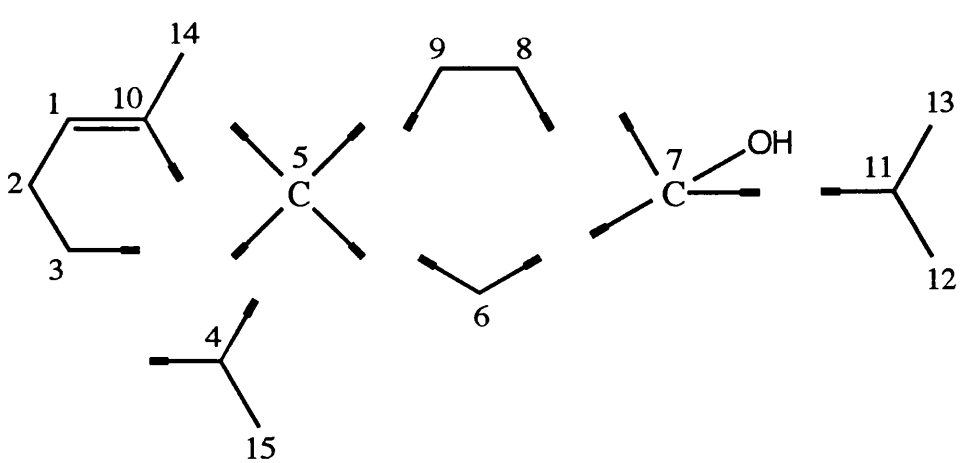
Table 3. Long-range correlations observed in the HMBC spectrum of compound 4 (^{1}H, 300.13 MHz; ^{13}C, 75.47 MHz).

H	Correlated C
H-1	2,3,5,14
H-3b	1,2,(4,11)$^{+}$,5,15
H-6a	(4,11)$^{+}$, 5, 10
H-9a	(4,11)$^{+}$, 5,7,8,10
H-9b	6,7
3H-12, 3H-13*	(4,11)$^{+}$, 7, (12,13)$^{\#}$
3H-14	1,5,10
3H-15	3,(4,11)$^{+}$, 5

* 3H-12 and 3H-13 have overlapping signals.

\+ C-4 and C-11 are very close in chemical shift.

\# C-12 and C-13 are isochronous.



Scheme 1

9a and H-6b are on the top face of the five-membered ring as drawn in **4**. Irradiation of 3H-15 gives a significant NOE at H-6a and a much smaller NOE at H-6b. This indicates that C-15 lies on the C-6 side of the six-membered ring. Irradiation of either set of isopropyl methyl protons gives NOEs at H-6a and H-6b while 3H-14 also appears to be enhanced. The floppiness of the five-membered ring enables the isopropyl methyls to be close to both C-6 protons and also near 3H-14 if they lie above the ring. Thus the isopropyl group is tentatively placed above the five-membered ring.

(-)-Lepidozenal (**6**)[10,14] and (-)-isobicyclogermacra-1(10),4-dien-15-al (**5**)[8,14] were first found in the liverwort *Lepidozia vitrea*, the source of (+)-vitrenal (**9**)[14,15]. All three aldehydes have plant growth inhibitory activity[8,10,14,15]. The reported plant growth activity of *L. reptans*[4] may well be associated with the presence of **5** and **6**. The optical antipode of **5** has been detected in *Aristolochia manshuriensis*[16].

EXPERIMENTAL

The plant material was collected in a wood near Loch Ard in the West of Scotland in June 1992. The air-dried material was ground (1.2 kg) six weeks later and then immersed in Et_2O at room temperature for two weeks. The resultant crude extract (11.15 g) was subjected to flash column chromatography followed by PLC both on silica gel to yield the following constituents in order of increasing polarity : cuparene (**2**) (22 mg), bicyclogermacra-1(10),4-diene (**1**) (33 mg), iso-bicyclogermacra-1(10),4-dien-15-al (**5**) (3 mg) (see p.150), lepidozenal (**6**) (3 mg) (see p.150), 1,(10)-spirovetivene-7-ol (**4**) (86 mg) and eudesm-3-ene-6ß,7α-diol (**3**)

(80 mg).

Cuparene (**2**) was isolated as an oil.

T_R (mins)	:	12.88
δ_H (lit.[6])	:	[7.25 (m, 2H); 7.09 (m, 2H)]*; 2.60 (m, H-8α); 2.32 (s, 3H-15); 1.4-1.9 (m, 5H); 1.26 (s, 3H-12); 1.06 (s, 3H-13); 0.56 (s, 3H-14).
*AA'BB' spin system[17]	:	$J_{AB} + J_{AB'} = 8.3$ Hz, $J_{AA'} + J_{BB'} = 4.5$ Hz.
δ_C	:	144.5 (s, C-6); 134.7 (s, C-3); 128.2 (d, C-2 and C-4); 126.9 (d, C-1 and C-5); 50.2 (s, C-7); 44.2 (s, C-11); 39.7, 36.8 (both t, C-8 and C-10); 26.4, 24.4, 24.3 (all q, C-12, C-13 and C-14); 20.8 (q, C-15); 19.7 (t, C-9).

1,(10)-Spirovetivene-7-ol (**4**) was isolated as an oil.

HRMS	:	m/z 222.1981 $[M]^+$ calculated for $C_{15}H_{26}O$: 222.1984.
ν_{max} (cm^{-1})	:	3610 (OH).
EIMS m/z(rel.int.)	:	222 $[M]^+$ (4), 204 (49), 189 (14), 179 (47), 161 (100), 147 (19), 121 (61).
δ_H (600 MHz)	:	see Table 1.
δ_C (75.47 MHz)	:	see Table 1.

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CHAPTER 8

JUNGERMANNIA OBOVATA

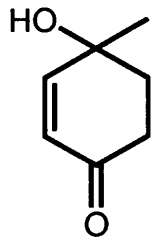
INTRODUCTION

Jungermannia obovata belongs to the family Jungermanniaceae of the order Jungermanniales. The characteristic odour of many liverworts is associated with oil body constituents. The liverwort *J. obovata* has a characteristic sweet, carrot-like smell due to its monoterpenoid content. The monoterpenoids detected in this liverwort include limonene[1,2], myrcene[2], α-terpinene[2], γ-terpinene[2], terpinolene[2], *p*-cymene[2], α-pinene[1,2], and ß-pinene[1]. Most recently a trisnormonoterpenoid (**1**) has been isolated[3]. Unlike *J. obovata* most of the Jungermanniaceae produce diterpenoids which are significant chemical markers. Sesquiterpenoids are uncommon in this family, though *ar*-curcumene and α-cedrene have been detected in *J. obovata*[1].

DISCUSSION

The liverwort *Jungermannia obovata* was extracted with diethyl ether while in moist condition and when the extract was chromatographed on silica gel a monoterpene/sesquiterpene hydrocarbon fraction was obtained together with two oxygenated monoterpenoids (**2** and **3**).

The ^{1}H and ^{13}C NMR spectra of the least polar fraction indicated the presence of lipid and a terpene hydrocarbon mixture. GLC confirmed the mono- and sesquiterpene hydrocarbon nature of the fraction. Subsequent GCMS analysis led to the identification of some of the monoterpenoids by comparison of mass spectral fragmentation patterns and ^{13}C NMR data with published data[4,5]. The results are shown in Table 1. The approximate percentage composition was obtained from the peak areas of the GLC. Two of these monoterpenoids (**7** and



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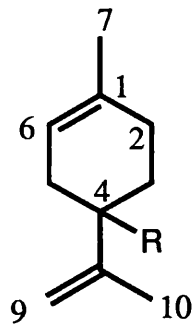
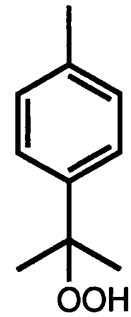


(2) R = OOH
(10) R = OH



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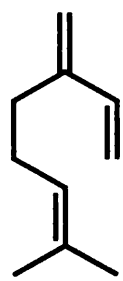

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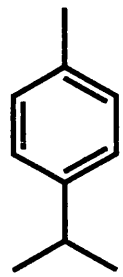

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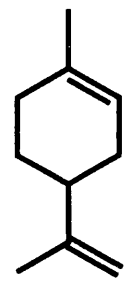

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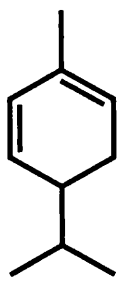

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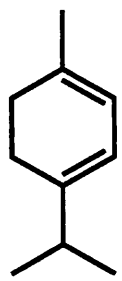

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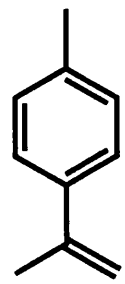

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9) have not been reported previously in *J. obovata*.

Table 1. Chemical composition of least polar fraction.

Component	T_R(mins)	$[M]^+$(m/z)	% Composition
Myrcene (**4**)	6.04	136	<1
p-Cymene (**5**)	7.01	134	13
Limonene (**6**)	7.71	136	13
α-Phellandrene (**7**)	8.37	136	13
α-Terpinene (**8**)	9.61	136	5
p-Isopropenyltoluene (**9**)	11.59	132	<1
Major unidentified sesquiterpene hydrocarbon	27.06	204	19
Other unidentified terpene hydrocarbons	23.41, 28.64 29.55, 29.76 31.74 (Others <1%)	-	19
Lipid	-	-	18

The molecular formula, $C_{10}H_{16}O_2$ ($[M]^+$ at m/z 168.1135), of compound **2** was determined by HRMS. Its IR spectrum showed hydroxyl absorption (ν_{max} 3600 cm^{-1}, strong and broad). The ^1H NMR spectrum (Figure 1) (see Experimental) indicates the presence of two vinyl methyl groups [δ_H 1.81 (t, J = 1.1 Hz); 1.66 (br s)], a vinyl proton [δ_H 5.25 (m)], two exomethylene protons [δ_H 5.02 (q, J = 1.1 Hz, 2H)] and one proton at δ_H 7.27 (s) which is exchangeable with D_2O. The ^{13}C NMR spectrum (Figure 2) showed ten carbons (Table 2). There

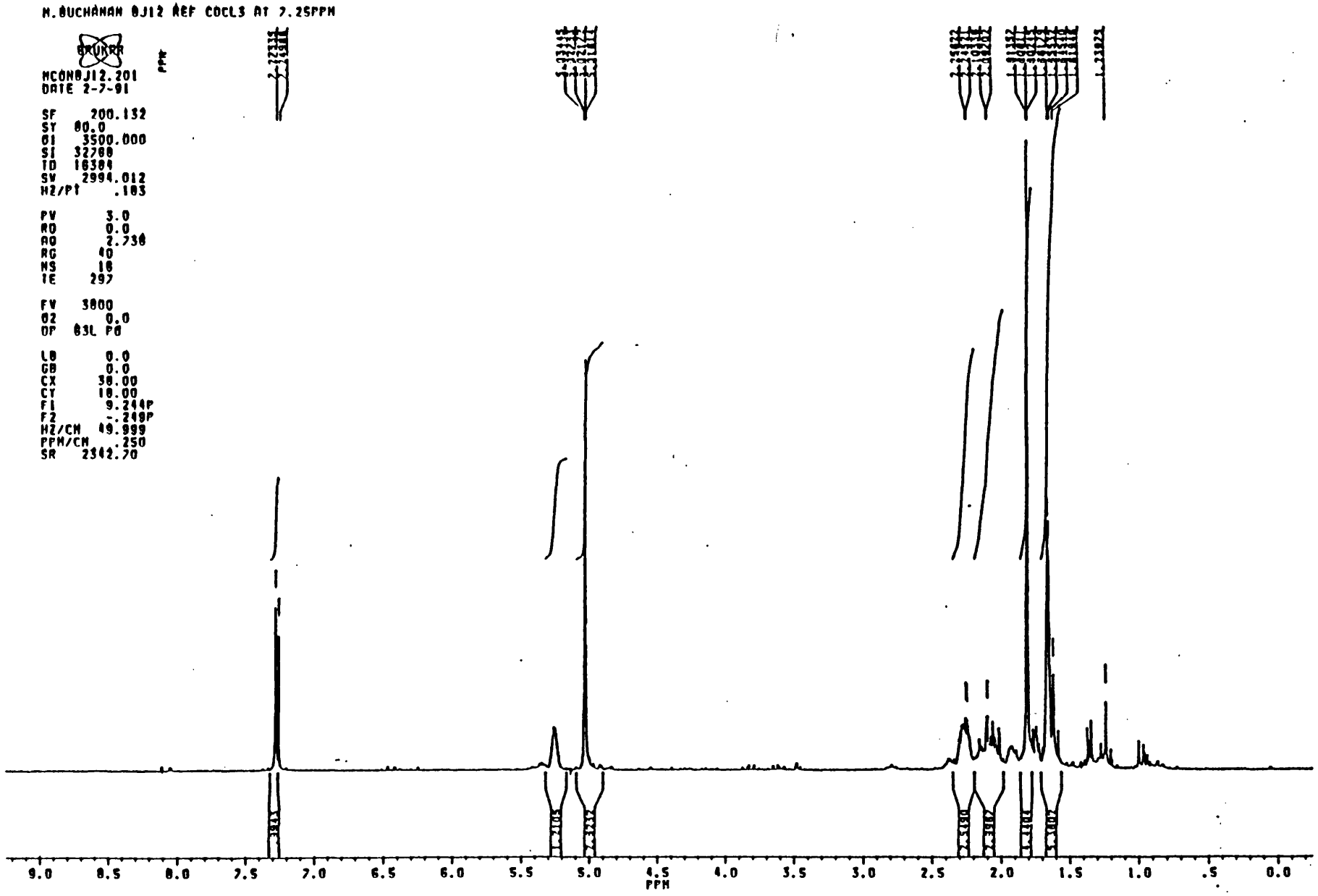

Figure 1. ^{1}H NMR spectrum of compound **2.**

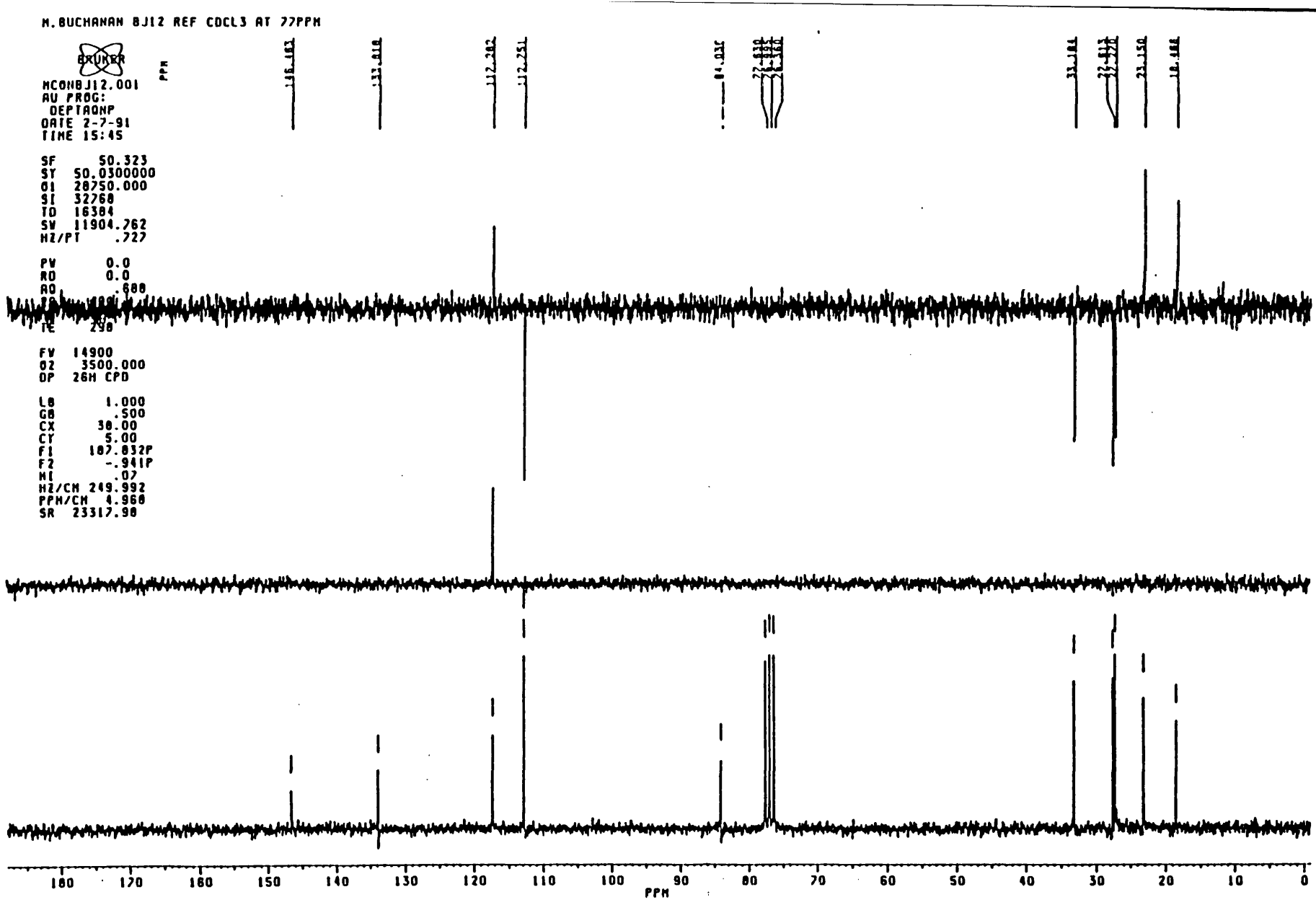

Figure 2. ^{13}C NMR and DEPT spectra of compound **2.**

are resonances for a trisubstituted double bond [δ_C 133.8 (s), 117.3 (d)], a disubstituted double bond [δ_C 146.5 (s), 112.8 (t)], an oxygenated quaternary carbon (δ_C 84.0), three methylene carbons and two methyl groups.

Table 2. ^{13}C NMR data of monoterpenoids.

C	10[a]	2	3
1	133.6 s	133.8 s	137.2 s
2	27.4 t	27.6* t	129.2 d
3	32.1 t	27.3* t	125.4 d
4	72.1 s	84.0 s	141.5 s
5	37.2 t	33.2 t	125.4 d
6	118.4 d	117.3 d	129.2 d
7	23.2 q	23.2 q	21.0 q
8	150.4 s	146.5 s	83.8 s
9	109.7 t	112.8 t	26.1 q
10	18.7 q	18.5 q	26.1 q

[a] Data from ref.9.
* Assignments interchangeable.

One of the oxygens cannot be bonded to a carbon and this suggests the presence of a hydroperoxide group. HRMS gave ions at m/z 152.1185 (14%) for $C_{10}H_{16}O$, 151.1119 (47%) for $C_{10}H_{15}O$, 150.1044 (11%) for $C_{10}H_{14}O$, 136.1235 (12%) for $C_{10}H_{14}$, 135 (24%) and 134 (13%) which reflect fragmentations of M^+-O, M^+-HO, M^+-H_2O, M^+-O_2, M^+-HO_2 and M^+-H_2O_2 respectively. These MS data support the presence of a hydroperoxy group[6,7]. The ^{1}H NMR signal at δ_H 7.27 (s) can be assigned to a hydroperoxy group since hydroperoxy protons appear

characteristically at low field[6,8]. Thus compound **2** is a hydroperoxy monocyclic monoterpenoid diene of the menthane type. NOE difference experiments supported a *para*-menthane with the hydroperoxide attached to C-4 as in **2**. The *p*-menthane arrangement was indicated by the NOEs observed between H-6 and 2H-5 (2.5%) and between 2H-9 and 2H-5 (1.6%). These NOE difference experiments identified the protons at C-5 as an AB type system, with additional small couplings at δ_H 2.20 (m) and 2.33 (m). The deshielded nature of these protons agrees with their allylic position next to an oxygenated carbon. NOEs observed between 2H-9 (3.1%) and 3H-10 (1.7%) and between H-2 (6.0%) and 3H-7 (1.0%) readily distinguished the vinyl methyl resonances.

Initially we thought that the MS peak at m/z 152.1185 (14%) for $C_{10}H_{16}O$ was the parent ion and that compound **2** was the alcohol, 4-hydroxy-*p*-mentha-1(6),8-diene (**10**). However this compound is known and comparison of its ^1H and ^{13}C NMR data[9] with those of **2** (Table 2) showed clearly that they are different. The most significant difference is the deshielding (11.9 ppm) of the oxygenated carbon (C-4) in **2** relative to **10**. This peak at δ_C 84.0 (s) is at lower field than for an ordinary tertiary allylic alcohol and is more characteristic of an allylic carbon bearing a hydroperoxy group[7,10,11]. Furthermore there is an upfield shift in the position of C-8 (3.9 ppm) and a downfield shift of C-9 (3.1 ppm) in going from the alcohol (**10**) to the hydroperoxide (**2**). This upfield ß-shift and downfield γ-shift is characteristic for allylic hydroperoxides relative to allylic alcohols[11]. On the basis of the above information compound **2** is 4-hydroperoxy-*p*-mentha-1(6),8-diene. The absolute configuration remains undetermined.

Compound **3** has the molecular formula $C_{10}H_{14}O_2$ ($[M]^+$ at m/z 166.0991).

The ^1H NMR spectrum (Figure 3) (see Experimental) shows resonances which suggest a *para*-disubstituted benzene ring [AA'BB' spin system[12]: δ_H 7.35 (2H), 7.18 (2H), both complex multiplets, $J_{AB} + J_{AB'} = 8.3$ Hz, $J_{AA'} + J_{BB'} = 4.0$ Hz]. The two proton multiplet at δ_H 7.18 also has an additional small coupling ($J = 0.6$ Hz) to an aromatic methyl [δ_H 2.34 (br s)]. The spectrum also contained two other methyls with identical chemical shifts [δ_H 1.59 (s, 6H)] and a broad one proton singlet at δ_H 7.28. The ^{13}C NMR spectrum (Figure 4) (Table 2) confirms the presence of the *para*-disubstituted benzene ring [δ_C 141.5 (s), 137.2 (s), 129.2 (d, 2C), 125.4 (d, 2C)], an oxygenated quaternary carbon (δ_C 83.8) and three methyl groups [δ_C 26.1 (q, 2C), 21.0 (q)]. It is evident from these spectral data that compound **3** is 8-hydroperoxy-*p*-mentha-1,3,5-triene. The MS indicates the presence of two oxygens and there is only one oxygenated carbon and this suggested the hydroperoxide group. This is supported by the signal at δ_H 7.28 (br s) in the ^1H NMR spectrum as such low field signals are characteristic of a hydroperoxy group[6,8]. Moreover the MS has ions at 150 (10%), 149 (2%), 148 (1%), 134 (13%) and 133 (100%) which reflect fragmentations of M^+-O, M^+-HO, M^+-O_2 and M^+-HO_2 respectively and supports the presence of a hydroperoxy group[6,7].

At first, as in the case for compound **2**, compound **3** was thought to have been the corresponding alcohol derivative but ^{13}C NMR spectral comparison with abietane-type diterpenoids[13] containing the relevant part structure reveals that the oxygenated carbon (C-8) in compound **3** is substantially more deshielded (*ca* 12 ppm) than expected for an alcohol. Carbons bearing a hydroperoxy group characteristically resonate at lower field than the corresponding alcohol. As

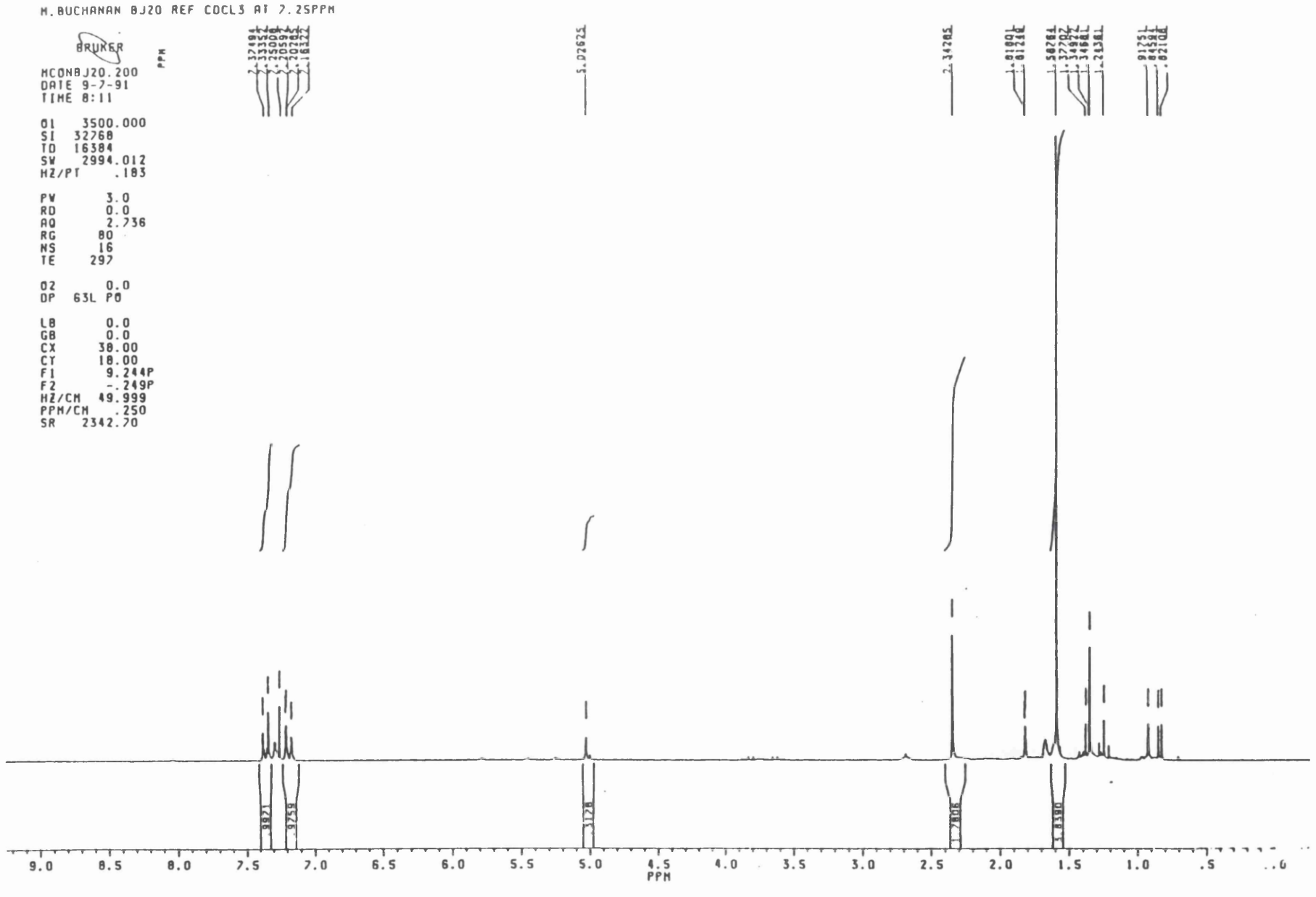

Figure 3. ^{1}H NMR spectrum of compound **3.**

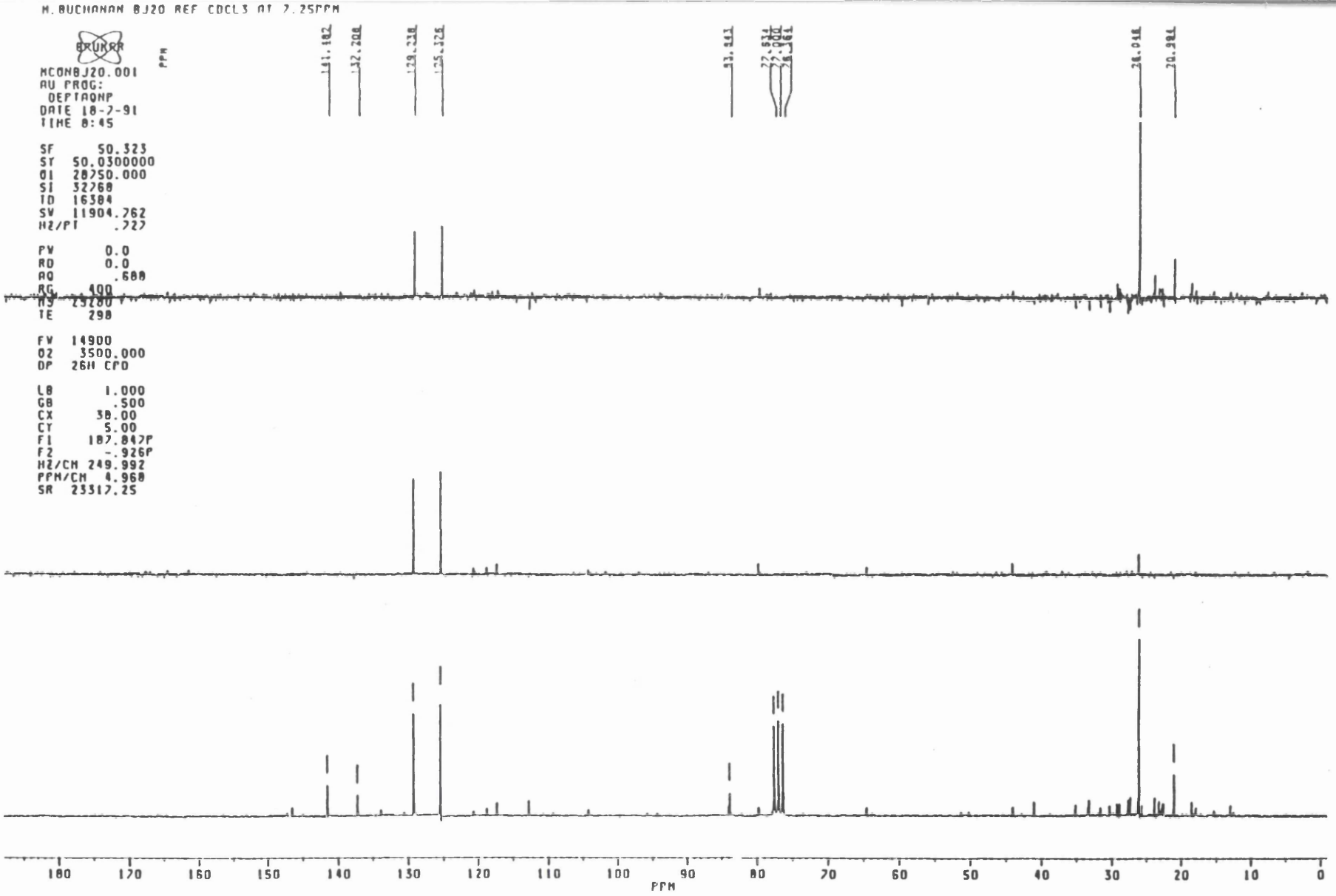

Figure 4. ^{13}C NMR and DEPT spectra of compound **3.**

expected, the hydroperoxide group also shields the isopropyl methyl carbons (*ca* 6 ppm)[13].

Both of these compounds (**2** and **3**) are new natural products and this is the first report of monoterpene hydroperoxides in liverworts. These compounds are presumably produced by a reaction involving ground state (enzymatic) or singlet O_2 with a double bond in a monoterpene hydrocarbon co-metabolite[8].

EXPERIMENTAL

The plant material was collected at Loch Doon, West of Scotland, in June 1991 and immersed while moist in Et_2O in order to capture the volatile components (i.e. it was not dried and ground). The crude extract (1.7 g) was difficult to work with as rapid polymerisation of unsaturated fats occurred giving an insoluble mass. It was possible to redissolve 445 mg of crude extract which was subjected to the usual chromatographic separation to give in order of increasing polarity a monoterpene/sesquiterpene hydrocarbon mixture (8 mg), 4-hydroperoxy-*p*-mentha-1(6),8-diene (**2**) (30 mg) and 8-hydroperoxy-*p*-mentha-1,3,5-triene (**3**) (33 mg).

Analysis of the monoterpene/sesquiterpene hydrocarbon mixture. GLC, GCMS and ^{13}C NMR analyses identified the monoterpenoids shown in Table 1.

4-Hydroperoxy-p-mentha-1(6),8-diene (**2**) was isolated as a gum.

HRMS : m/z 168.1135 $[M]^+$ calculated for $C_{10}H_{16}O_2$: 168.1150.

ν_{max} (cm^{-1}) : 3600 (OH); 2970, 2930; 1550 (C=C).

EIMS m/z (rel.int.) : 168 (7), 152 (14), 151 (47), 150 (11), 149 (51), 136 (12), 135 (24), 134 (13), 133 (27), 123 (32), 107 (85), 93 (100), 81 (74).

δ_H : 7.27 (s, OOH); 5.25 (m, H-6); 5.02 (q, J = 1.1 Hz, 2H-9); 2.20 (m, H-5); 2.33 (m, H-5); 1.6 - 2.2 (m, 2H-2 and 2H-3); 1.81 (t, J = 1.1 Hz, 3H-10); 1.66 (br s, 3H-7).

δ_C : see Table 2.

8-Hydroperoxy-p-mentha-1,3,5-triene (**3**) was obtained as a gum.

HRMS : m/z 166.0991 $[M]^+$ calculated for $C_{10}H_{14}O_2$: 166.0994.

EIMS m/z(rel. int.) : 166 $[M]^+$ (1), 150 (10), 149 (2), 148 (1), 134 (13), 133 (100), 119 (15), 105 (37), 91 (40), 85 (20), 65 (19).

δ_H : 7.35 (m, 2H); 7.28 (br s, OOH); 7.18 (m, 2H); 2.34 (br s, 3H-7); 1.59 (s, 3H-9 and 3H-10).

δ_C : see Table 2.

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