A THESIS ENTITLED

"SECONDARY METABOLITES OF THE HEPATICAE"

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By

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"SECONDARY METABOLITES OF THE HEPATICAE"

Leslie J. Harrison

SUMMARY

This thesis consists of ten chapters, the first of which is a General Introduction dealing with i) the nature of secondary metabolites and ii) the terpenoid and aromatic constituents of the Hepaticae (liverworts). This is followed by a discussion of the results of an investigation into the chemical constituents of *Scapania undulata* (Chapter 2). Nine new labdane diterpenoids were isolated and the structures determined by spectroscopic and chemical methods.

Chapter 3 describes the structural elucidation of three sesquiterpenoids (one of which possesses a novel carbon skeleton) from *Chiloscyphus pallescens*. The published structure of chiloscyphone is revised. Also briefly discussed are the constituents of *C. polyanthos* and *Lophocolea bidentata*. Chapter 4 is concerned with the structures of two new sesquiterpenoids, belonging to the zierane class, from *Saccogyna viticulosa*. The structures were determined using spectroscopic methods. The Cope rearrangement of a derived ketone is also considered.

Chapter 5 deals with the constituents of *Anastrepta orcadensis*, *Barbilophozia floerkei* and *Lophozia ventricosa*. The first two species contain dolabellane diterpenoids and two new examples of this class have been obtained from *B. floerkei*. Spectroscopic studies have revealed the

structures, although final details of stereochemistry remain to be determined in one case. L. ventricosa has yielded two known sesquiterpenoids.

The diterpenoids of Plectocolea paroica and Nardia scalaris form the subject matter of Chapter 6. The former species contains four ent-clerodane diterpenoids, one of which has not previously been reported. The malonate half-ester of an ent-kaurane diterpenoid was isolated from the latter species and the structure determined by spectroscopic and chemical methods.

Chapter 7 details the structure determination of a novel eudesmane sesquiterpenoid from Lepidozia reptans using ^{1}H and ^{13}C nmr spectroscopy. Studies of an as yet unidentified sesquiterpenoid from the same source are discussed. Chapter 8 is concerned with the isolation of a muurolane sesquiterpenoid from Marsupella aquatica. The structure was assigned using ^{1}H and ^{13}C nmr spectroscopy.

This is followed by a discussion of the aromatic constituents of Plagiochila species (Chapter 9). Two new dihydrophenanthrenes and an orsellinic acid derivative were isolated from P. spinulosa. P. rutilans yielded a prenylated pyrogallol monomethyl ether. The structure was determined using nmr spectroscopy and confirmed by synthesis.

The final chapter considers the metabolites of Frullania tamarisci. In addition to three known eudesmanolides, a new sesquiterpene alcohol was isolated. This compound has been shown to belong to the rare pacifigorgiane class; the stereochemistry, however, remains partly undetermined.

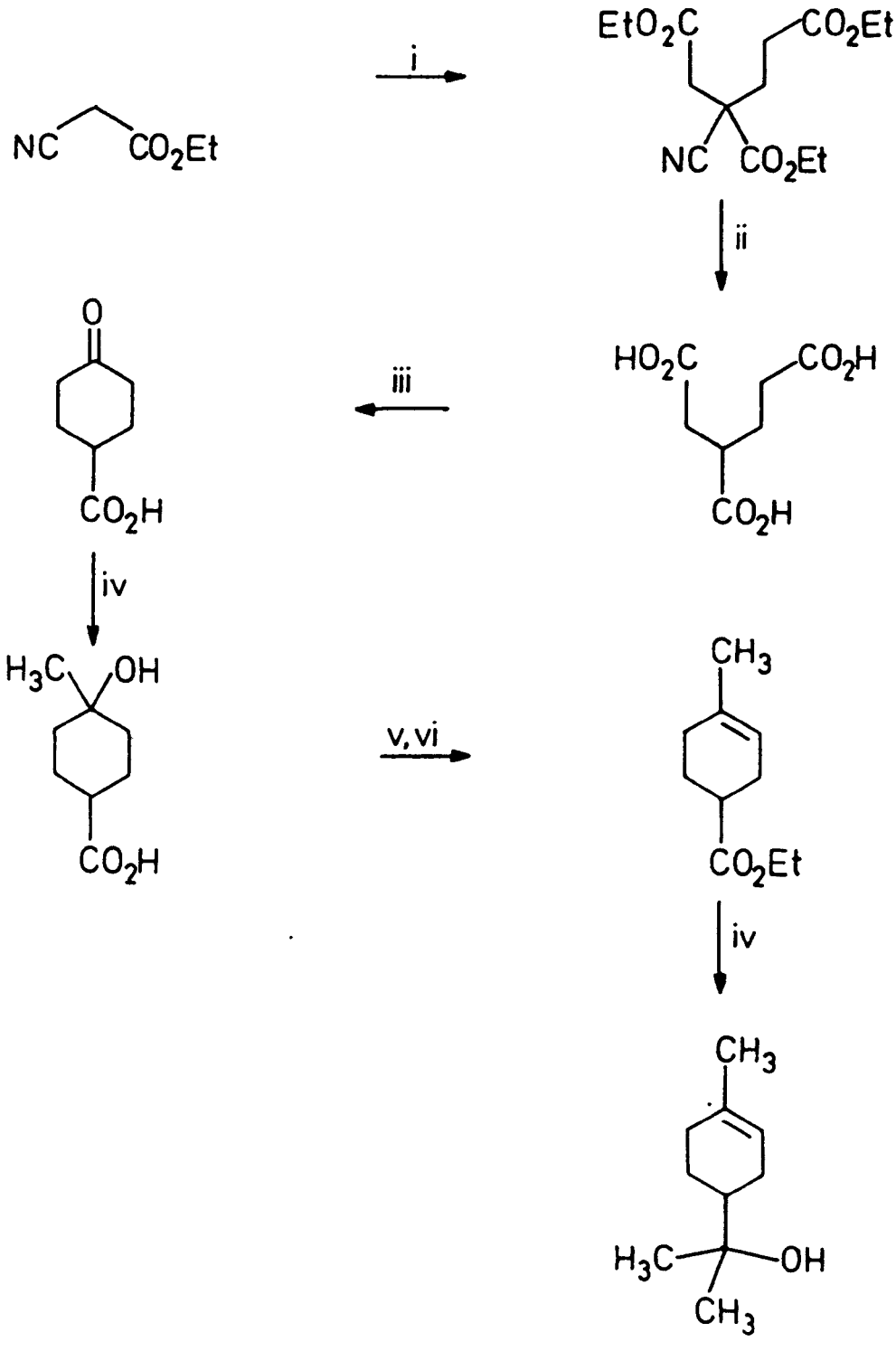
C O N T E N T S

CHAPTER 1

GENERAL INTRODUCTION

Natural product chemistry is an ancient science, food-stuffs, dyestuffs and medicines, for example, all falling within its confines. However, it was not until the eighteenth century that chemists, as opposed to alchemists, began a scientific investigation of extracts obtained from natural sources. They found much of interest.

Firstly, it was necessary to separate and purify the compounds before analysing them. Methods of separation were developed and, undoubtedly, natural product chemistry stimulated the development of today's refined techniques, such as the various analytical and preparative chromatographic methods:
column chromatography, g.c., t.l.c., h.p.l.c., paper chromatography, electrophoresis, ion-exchange, etc. These methods have made possible the isolation of compounds which are present in extremely small amounts. Methods of structure determination have also evolved to deal with small quantities of material. Initially structure elucidation was typically carried out by degradation to obtain smaller fragments of known structure. This early work led to the discovery of many new reactions and rearrangements. However the advent of spectroscopic methods has resulted in dramatic changes in structural elucidation. A large body of data, correlating spectroscopic properties with structure, has been assembled and



(±)- -terpineol.

i) $ICH_2CH_2CO_2Et, \bar{O}Et$; ii) H^+; iii) Ac_2O; iv) MeMgI

v) HBr; $\bar{O}H$; vi) H^+, EtOH.

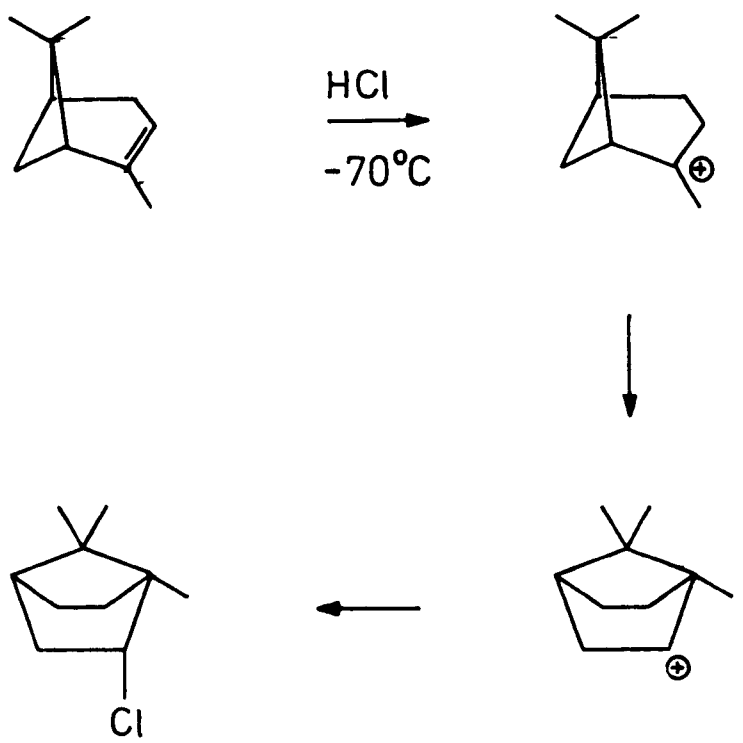
Scheme 1. Perkin's synthesis of α-Terpineol

these data reduce to a minimum the need for chemical manipulation. In fact the determination of structure of compounds present in milligramme quantities is today a matter of routine. In difficult cases the last resort is X-ray crystallography - provided crystals are available.

Natural products traditionally fall into two categories - primary and secondary metabolites - though the border between the two classes is somewhat vague in places. The former, organic compounds which are characteristic of all living systems, include carbohydrates, lipids, amino acids, peptides and proteins, nucleosides, nucleotides and nucleic acids. They are often considered the province of the biochemist.

On the other hand, one of the most productive areas of enquiry for the organic chemist has been that of the chemistry of the secondary metabolites, such as phenols, quinones, terpenes, alkaloids, and the various pigments which organisms - particularly plants and micro-organisms - produce. Studies of the chemistry of terpenoids, for example, were instrumental in the early development of synthetic methods, e.g. Perkin's [1] synthesis of (±)-α-terpineol in 1904 (Scheme 1), and in the recognition of one of the most commonly encountered molecular rearrangements in organic chemistry, the Wagner-Meerwein rearrangement (Scheme 2).

There remains, however, a fundamental and as yet



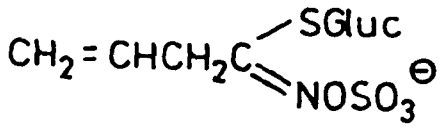
Scheme 2. Wagner-Meerwein Rearrangement.

unresolved problem concerning secondary metabolites - what, if any, is their biological function? Indeed, grafting experiments have shown that plants flourish remarkably well both in the absence of several of their normal, characteristic metabolites and in the presence of many extraneous ones. Some consider these substances to be waste products of metabolism, although many are toxic to the organism which produces them unless they are dissipated into the environment e.g. the volatile monoterpenes produced by many plants, or are sequestered harmlessly in the organism itself.

Others regard secondary metabolites, in particular those from plants, to be important factors in the co-evolution of plants, animals and insects, an idea that is phrased in different terms by various authors[2,3]. Organisms have adopted the production of metabolites, i.e. their enzyme activity, to their living conditions and the production of these compounds cannot be entirely fortuitous. In recent years important discoveries have been made concerning the unexpected biological effects of seemingly uninteresting compounds. We can distinguish several areas of chemical control applied in the Darwinian struggle for survival:

a) Sex-attractants

Biologists realised long ago that scents induce specific animal behaviour, but it was not until 1959,



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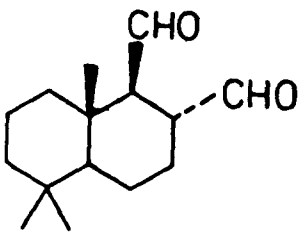

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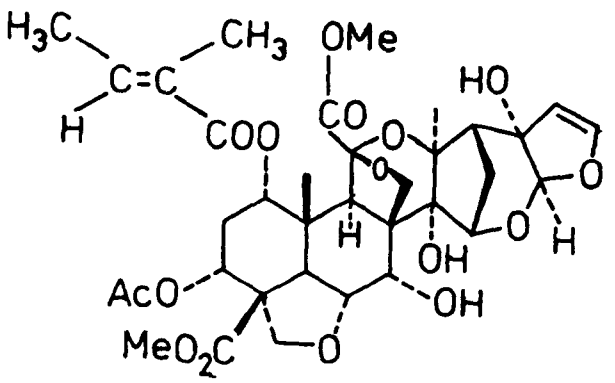

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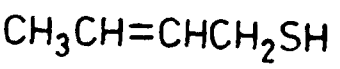


(4)

when the first sex-attractant, bombykol (Fig 1), was isolated from the female silk moth, Bombyx mori[4], that real research started on pheromones, the chemicals acting as attractants. The structures of several specific sex-attractants are known today[5] and some are illustrated below (Fig. 1).

b) Feedants and Antifeedants

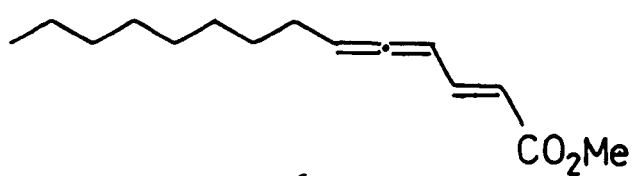
Some plants contain glycosinates e.g. sinigrin (1). These act as antifeedants for most insects, but sinigrin itself is a feeding attractant for the cabbage butterfly, Pieris brassicae. Indeed, the butterfly will only feed upon plants which do contain sinigrin, preferring death rather than eat plants lacking this compound. In this way the cabbage butterfly population is controlled by the availability of a suitable food supply.

Other examples of antifeedants are warburganal (2), a sesquiterpene dialdehyde from the East African tree Warburgia stuhlmanhi[11], which is active against the army worm, and azadirachtin (3) which protects the African neem tree against desert locusts[12].

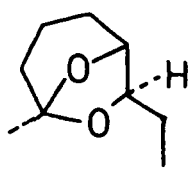
c) Defence and Alarm

A well-known example is the skunk, Mephitis mephitis, which responds to danger by releasing evil smelling thiols, e.g. (4) from its anal glands. The hyaena and deer also release anal exudates under stress. When threatened,

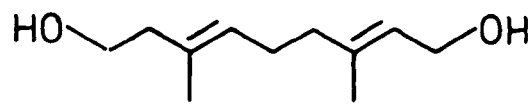
Bombykol[4] (Bombyx mori)



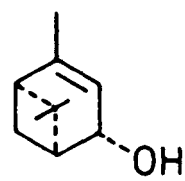
Methyl(E)-2,4,5-tetradecatrienoate[6] (Acanthoscelides obtectus)



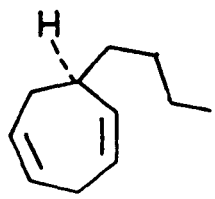
exo-Brevicomin[7]
(Dendroctonus brevicomis)



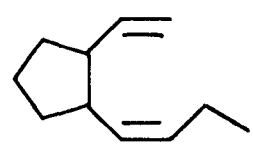
(E,E)-3,7-Dimethyl-2,6-decadien-1,10,diol[8]
(Danaus gilippus)



cis-Verbenol[9]
(Ips typographus)



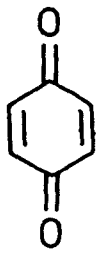
Ectocarpene[10]
(Ectocarpus siliculosus)



Multifidene[10]
(Cutleria multifida)

Fucoserratene[10]
(Fucus serratus)

Fig. 1 Sex attractants isolated from insects and brown algae.



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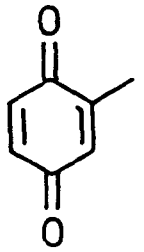

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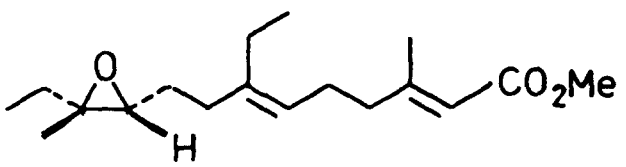

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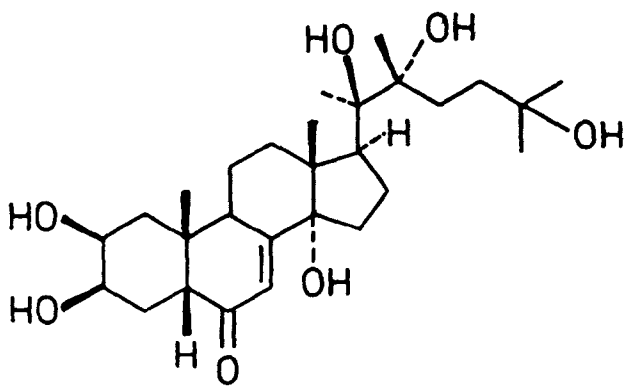
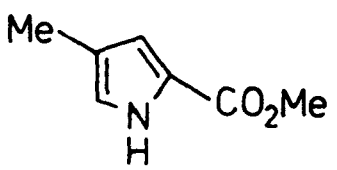


(8)



(9)

the bombardier beetle, Brachynus crepitans, discharges a mixture of simple benzoquinones (5,6) propelled by oxygen released from hydrogen peroxide[13].

d) Development

The change from the juvenile to the adult form in insects is controlled by hormones. Juvenile hormones[14] (7) prevent this change whilst moulting hormones e.g. ecdysone (8) promote it. Although present in the insect, both of these hormone types are also found in plant species e.g. the yew, Taxus baccata, which contains large amounts of β-ecdysone (8)[15]. Presumably their presence allows some sort of ecological control, but the exact nature of this is still unclear.

e) Social behaviour

Processes controlled by secondary metabolites include termite building behaviour, locust accumulation and track indication by ants. The leaf cutting ant, Atta texana, produces a pyrrole derivative (9) by which it marks the route to a food source[16].

It is apparent that many secondary metabolites are of the utmost importance in Nature[17] and undoubtedly many other examples await discovery.

Chemotaxonomy

As the number of plant species examined has increased, chemists have been able to use their knowledge

of the secondary metabolites present to attempt a taxonomical study of various plant families. Taxonomy is related to plant genetic material, closely-related species containing similar genes. It is not, however, feasible to study plant DNA directly and it is therefore necessary to consider features which result from gene expression. Botanists have traditionally used plant morphology to study the interrelationships between various species but this method is not without its disadvantages, perhaps the major one being its subjectiveness. A plant's secondary metabolites are also characteristic of its genetic material; consideration of these should therefore lead to a taxonomic classification complementary to that of the botanist. This is termed chemotaxonomy and is the object of a number of research programmes.

This thesis is concerned with the isolation of secondary metabolites from the Hepaticae (liverworts), the elucidation of their structures and, to a lesser extent, attempts to use these compounds to clarify the inter-relationships within this class of plants.

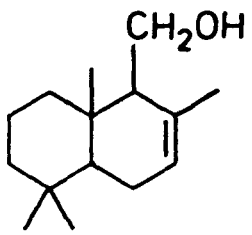
Hepaticae

The Bryophytes are taxonomically placed between the algae and the pteridophytes (ferns), there being approximately 20,000 species known. They are divided into three classes, Musci (mosses, 14,000 species), Hepaticae (liverworts, 6,000 species) and Anthocerotae

(hornworts, 300 species). Of these, the chemically most interesting are the liverworts, this being due to the presence of oil-bodies in their cells. The other two classes are devoid of these structures.

The liverworts are a group of economically unimportant plants and are easily overlooked. They have occasionally found medicinal use, Marchantia polymorpha as a diuretic[18] and Conocephalum conicum against gallstones[18]. Some species of the Hepaticae contain intensely bitter or pungent substances, others induce allergenic contact dermatitis[19] (see p.17) and inhibit the growth of micro-organisms[20]. Despite the presence of such pharmacologically interesting substances, full chemical studies of the Hepaticae have only been carried out during the last twenty years, due mainly to the problems associated with the separation of complex mixtures.

Karl Muller, the eminent bryologist, believed[21] that liverwort oil-bodies contained sesquiterpenoids and that a study of these would yield important taxonomic information, especially as it was established that the characteristic odour of many liverworts is associated with oil-body constituents. Muller listed[21] many qualitative observations on the odour of various species: Leptolejeuna (liquorice); Riella (anise); Solenostoma obovatum (also called Jungermannia obovata) (carrot);



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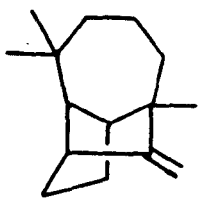

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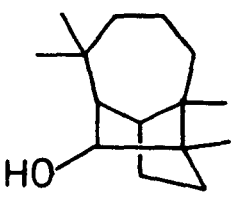


(12)

Lophozia bicrenata (cedar oil); Lophocolea (mossy); Geocalyx (turpentine).

It was not, however, until 1956 that the chemical constituents of the Hepaticae were further investigated, Fujita et al.[22] reporting the presence of sesquiterpene hydrocarbons in the essential oil of Bazzania pompeana. The first isolation of terpenoids in a pure state from the Hepaticae did not occur until over ten years later when Huneck[23] obtained (-)-drimenol (10) from Bazzania trilobata and Huneck and Klein[24] obtained (-)-longifolene (11) and (-)-longiborneol (12) from Scapania undulata.

During the 1970's a large number of papers dealing with liverwort chemistry appeared. These have been summarised at regular intervals. The most recent reviews are those by i) Markham and Porter[25] concerning bryophyte chemistry, viz. lipids, terpenoids, flavonoids, lignins and dihydrostilbenes, prior to 1978; ii) Connolly[26] who has dealt with the terpenoid constituents of some European liverworts; iii) Asakawa[27] who has produced a comprehensive review of the terpenoids, aromatics and lipids of the Hepaticae; iv) Huneck[28] who has reviewed all liverwort metabolites.

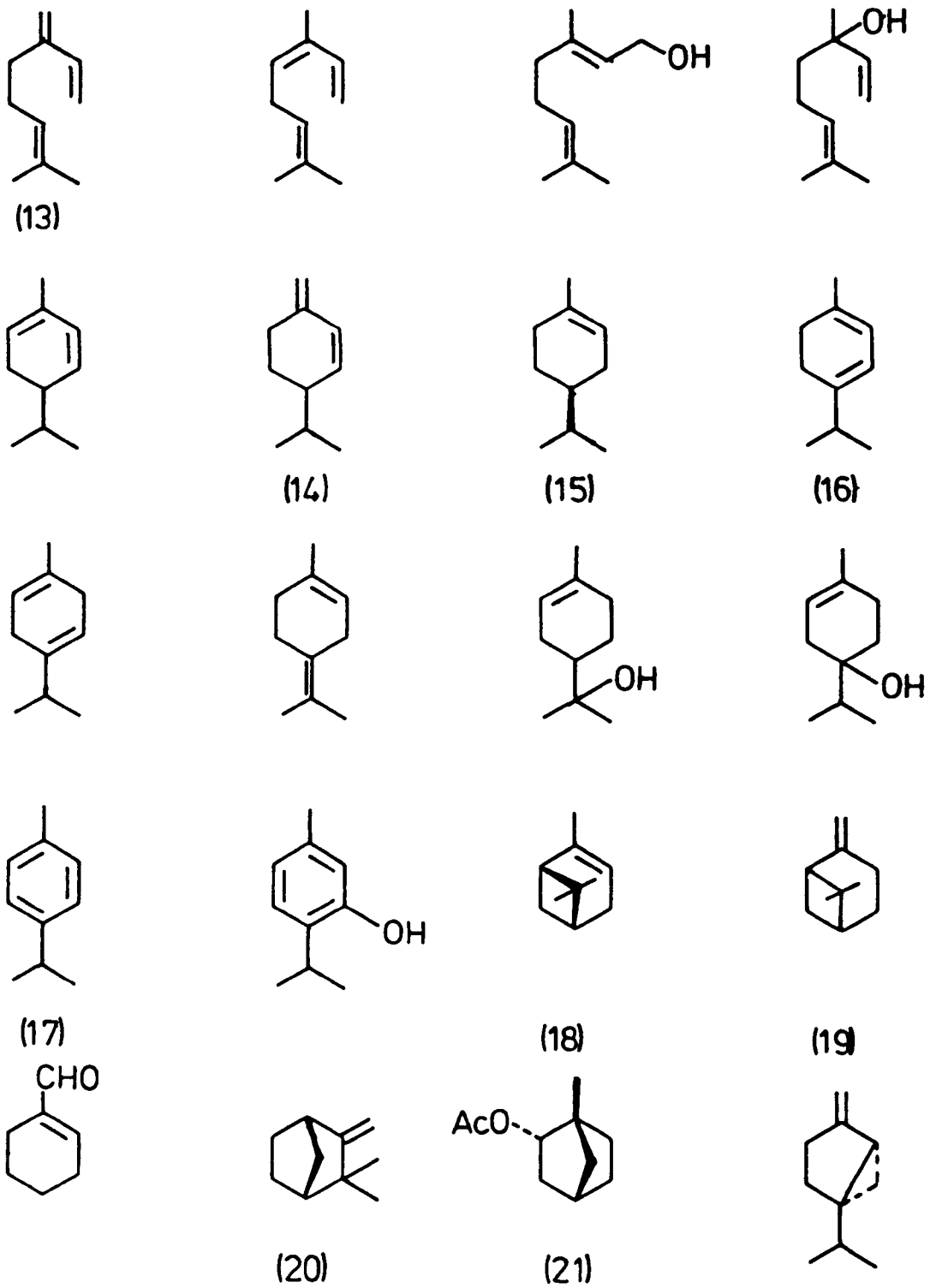


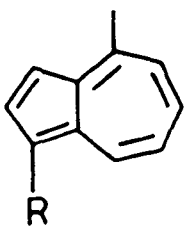
Fig. 2

MONOTERPENOIDS

Relatively few reports on the occurrence of monoterpenoids in the Hepaticae have appeared and these are all based on gc or gcms data except for two studies by Asakawa and co-workers who present[29,30] the only examples of monoterpenoids isolated pure from a liverwort.

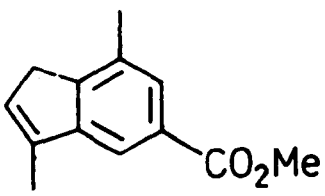
Myrcene (13), β-phellandrene (14), limonene (15), α-terpinene (16), p-cymene (17), α-pinene (18), β-pinene (19) and camphene (20) are the commonest monoterpenoids reported;[31-37] however a number of others have been found and these are illustrated in Fig. 2.

Both optical antipodes of monoterpenes generally occur in higher plants, often as racemates, but some species synthesise only one of the two enantiomers[38]. For the most part, the chiroptical properties of the monoterpenoids found in the Hepaticae have not been clarified. Asakawa's group has published two studies of liverwort monoterpene chirality, Conocephalum conicum[29] containing (-)-limonene (15) and (+)-bornyl acetate (21) whilst Jungermannia exsertifolia[30] contains (+)-limonene (ent-15), (+)-α-pinene (18) and (+)-camphene (20). Thus both enantiomers of limonene occur in liverworts as in higher plants but C.conicum and J. exsertifolia each produces only one of the two enantiomers. There remains much scope for further work in this field.



(22) R=Me

(23) R=CO_2Me



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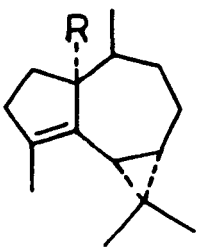
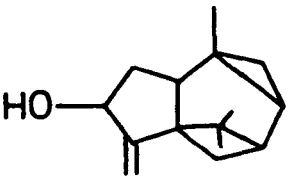
SESQUITERPENOIDS

The presence of sesquiterpenes in liverworts was initially recognised by Fujita et al.[22] through a study of the essential oil of Bazzania pompeana. The first positive identification of a sesquiterpenoid in a liverwort, apart from the guaiazulene-derived aromatic compounds, 1,4-dimethylazulene (22), 1-carbomethoxy-4-methylazulene (23) and 3,7-dimethyl-5-carbomethoxyindene (24) isolated from Calypogeia trichomanis[39-41], was the isolation of (-)-drimenol (10) from Bazzania trilobata[23]. However, the first finding of real importance in the sesquiterpenoid chemistry of liverworts was the discovery in 1967 by Huneck and Klein[24] that Scapania undulata contains (-)-longifolene (11) and (-)-longiborneol (12). The structures were identical in every way with those of the corresponding compounds isolated from vascular plants, except for their absolute configurations. The Scapania sesquiterpenoids are the enantiomers of longifolene and longiborneol derived from Pinus species. Subsequent work (see later) has established that liverwort sesquiterpenoids, with few exceptions, are enantiomeric with those found in vascular plants, an attribute shared with fungi[42] and coelenterates[43].

No attempt will be made here to list all liverwort sesquiterpenoids. Only the most significant ones will be considered; the reader is directed to the recent



(25) R = H
(26) R = OH



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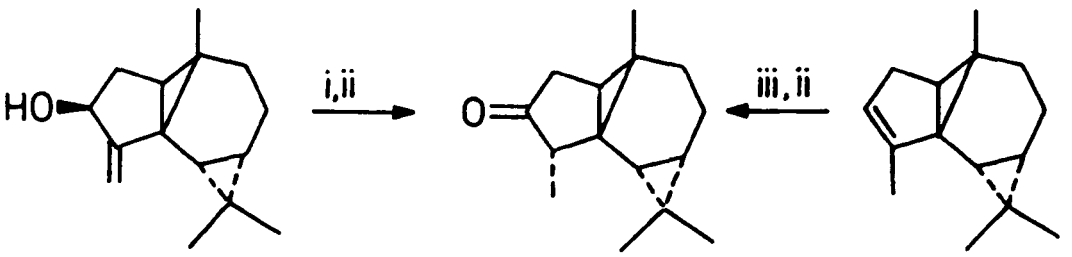
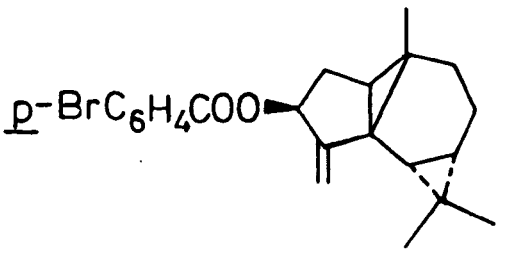


(28) (30)

i. H_2/PtO_2; ii. CrO_3; iii. BH_3.

Scheme 3.



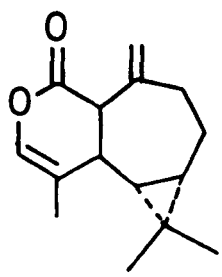
(29)

reviews by Asakawa[27] and Huneck[28] for an exhaustive treatment.

Aromadendranes and Secoaromadendranes

Aromadendranes, also known from higher plants, are mainly found in Mylia, Plagiochila and Porella species belonging to the Jungermanniales. Examples of the basic skeleton are cyclocolorenone (25)[44] and 1-hydroxycyclocolorenone (26)[45] from Plagiochila and Porella species. A modification of this skeleton is found in myliol from Mylia taylorii, originally[46] thought to be (27) but later revised to (28) after an X-ray analysis of the p-bromobenzoate (29)[47]. A related compound, the air-sensitive hydrocarbon anastreptene (30) is common to many Jungermanniales. Proof of structure came from correlation with myliol (Scheme 3)[48].

Another variation of the aromadendrane skeleton is found amongst the metabolites of Plagiochila species. The first to be isolated was plagiochilide[36], a crystalline compound with the formula $C_{15}H_{20}O_2$. Spectral analysis indicated the presence of a lactone [ν_{max} 1760cm^{-1}], an exomethylene grouping [δ_H 4.76, 4.92], two tertiary methyls [δ_H 1.06 (s,6H)], a cyclopropane [δ_H 0,84(m), 0.43 (dd, J 10 Hz)], a vinyl methyl [δ_H 1.74 (d, J 2 Hz)] and a vinyl proton [δ_H 6.24 (d, J 2 Hz)] further deshielded by attachment of the carbon to an ether oxygen. Decoupling studies and chemical degradations established



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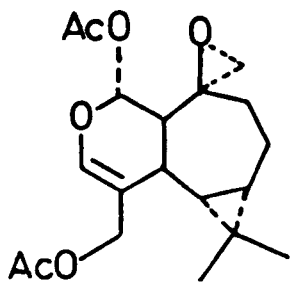
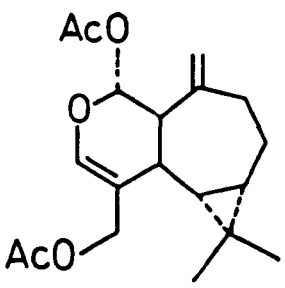
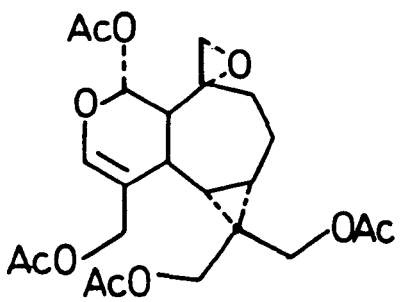


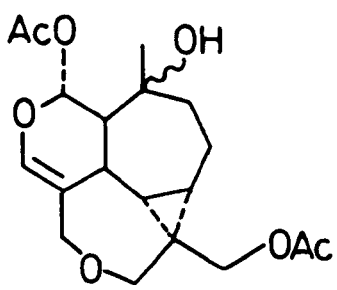
(32)



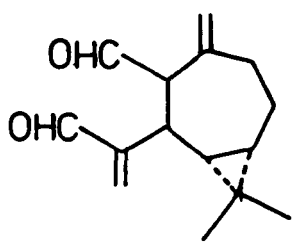
(33)



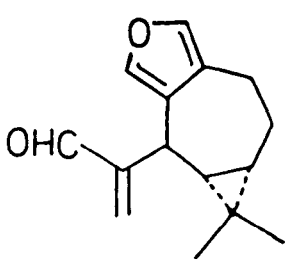
(34)



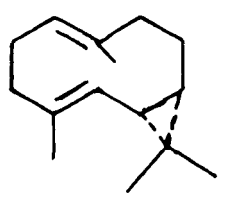
(35)



(36)



(37)



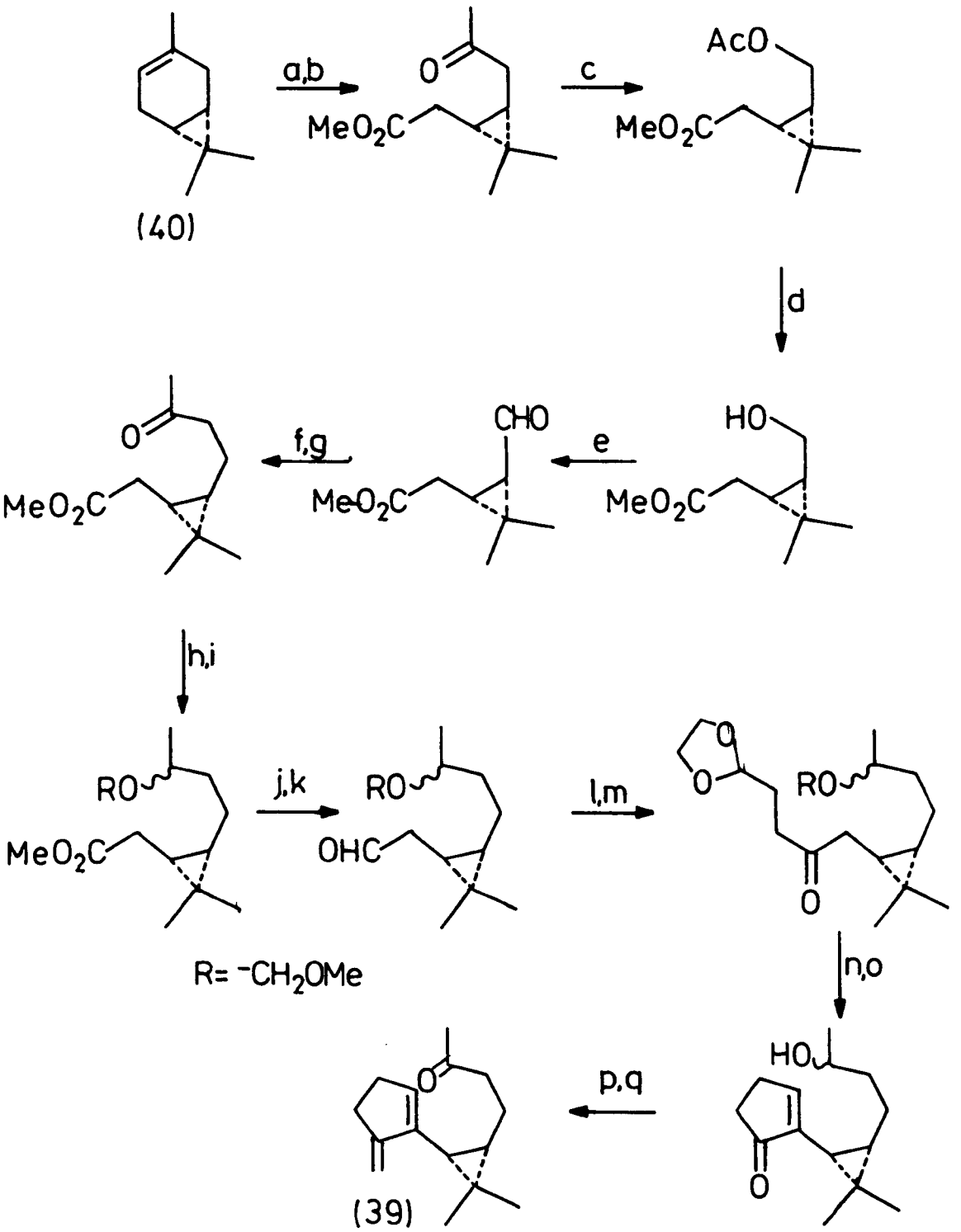
(38)

the structure as (31). The negative Cotton effect observed in the CD spectrum led to the absolute configuration. The Plagiochilaceae have proved to be a rich source of this novel ent-2,3-secoaromadendrane skeleton[49]. A series of ent-2,3-secoaromodendrane hemiacetals, the plagiochilines e.g. (32-35) has been isolated. A few other ent-2,3-secoaromadendranes have been isolated from Plagiochila species e.g. plagiochilal A (≡ hanegokedial) (36)[50] and furanoplagiochilal (37)[44]. The distribution of sesquiterpenoids in fourteen of the world's 1500+ species of Plagiochila has been reported[49]. ent-2,3-Secoaromadendranes are now recognised as common constituents of most Plagiochila species. These and related sesquiterpenoids might be formed from ent-(-)-bicyclo-germacrene (38) which is a co-metabolite.

A rarer modification of the aromadendrane skeleton is exhibited by taylorione (39), an ent-1,10-secoaromadendrane isolated from Mylia taylorii[51]. The structure and absolute configuration were determined by extensive chemical degradation and synthesis[52] from (+)-car-2-ene (40) (Scheme 4).

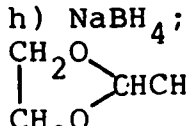
Barbatanes and Trichothecanes

As previously mentioned (p. 12), the Hepaticae elaborate sesquiterpenes enantiomeric to those found in higher plants and, in this respect, are like the fungi[53]. Among the major fungal metabolites are the trichothecanes[54]



a) O_3; b) CH_2N_2; c) $PhCO_3H$; d) KOH/MeOH; e) PCC;

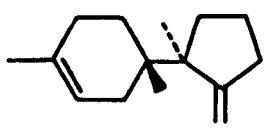
f) Ph_3P = $CHCOMe/CHCl_3$; g) $H_a/PtO_2/EtOH$; h) $NaBH_4$;

i) $(MeO_2)CH_2, P_2O_5$; j) $LiAlH_4$; k) PCC; l)  ;

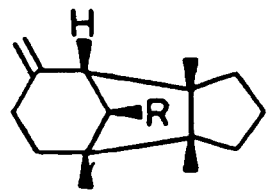
m) PCC; n) HCl/acetone; o) NaOH/MeOH; p) $Ph_3P=CH_2$/THF;

q) $DMSO/C_6H_6$, DCC/PTFA.

Scheme 4. Synthesis of Taylorione.



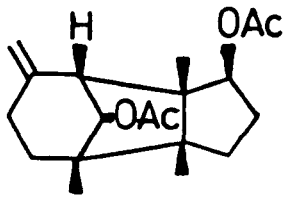
(41)



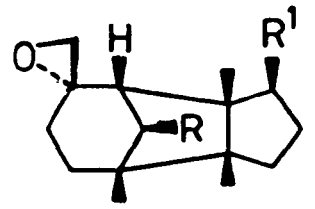
(42) R = OH

(43) R = H

(44) R = OAc

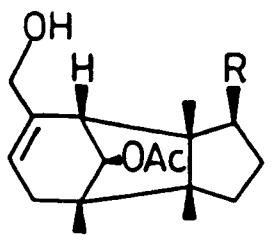


(45)



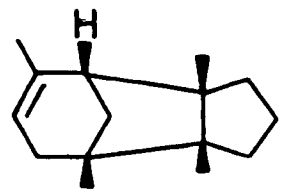
(46) R = OAc, R^1 = H

(47) R = R^1 = OAc

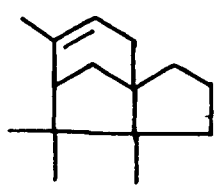


(48) R = H

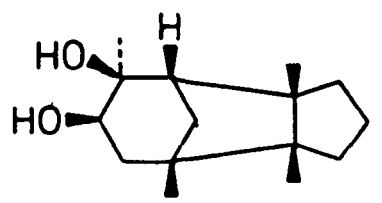
(49) R = OAc



(50)



(51)

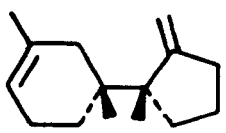


(52)

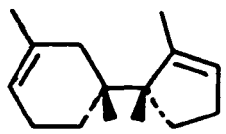
related to trichodiene (41). Liverworts have been shown to produce closely allied substances.

First Connolly et al.[55] isolated a new sesquiterpenoid alcohol, gymnomitrol, from Gymnomitrion obtusum, which they represented as (42). This was accompanied by its parent hydrocarbon gymnomitrene (43) and six other sesquiterpenes with the same skeleton (44-49). The exo double bond of (43) is isomerised under acidic conditions to give isogymnomitrene (50). These structures were established by a combination of ^{1}H nmr and Eu(fod)$_3$ - induced spectral shifts, decoupling experiments and extensive chemical transformations[55]. In 1973, Andersen et al. described the isolation[56] of a number of sesquiterpenes from extracts of Barbilophozia species, two of which were (50) and (43), α- and β-barbatene respectively. At the same time Matsuo et al.[57] isolated a novel tricyclic sesquiterpene, α-pompene, initially represented by structure (51), from Bazzania pompeana. However this structure and that of its double-bond isomer β-pompene were later revised[58], following an X-ray analysis of the p-bromobenzoate of the diol (52) derived from α-pompene using OsO_4. The structures for α- and β-pompene are therefore the same as α- and β-barbatene (isogymnomitrene and gymnomitrene).

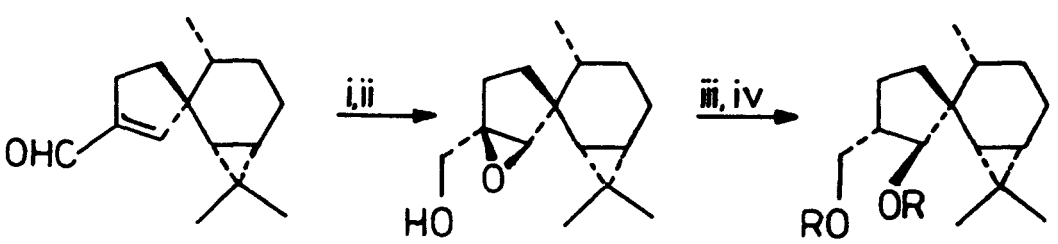
In their paper, Connolly et al. suggested[55] that a plausible biogenetic precursor of the gymnomitrane-type sesquiterpenes was the trichodiene epimer (53). This



(53)



(53a)

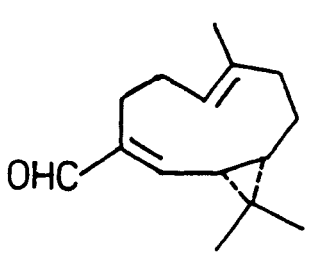


(54)

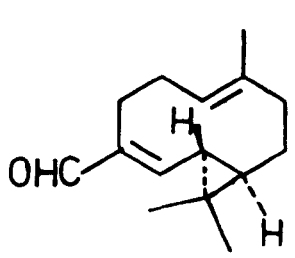
(55) R= OCC_6H_4Br

i. $LiAlH_4$; ii. mcpba; iii. $Li, NH_2(CH_2CH_2)_2NH_2$;
iv. _p-BrC_6H_4COCl, py.

Scheme 5.



(56)



(57)

compound, β-bazzanene, along with its double bond isomer, α-bazzanene (53a) and an alcohol derivative, bazzanenol, were later isolated from Bazzania species[59,60].

Total syntheses have confirmed the structures of (±)-gymnomitrol[61-65], (±)-α- and (±)-β-barbatene[61,65] and (±)-β-bazzanene[65].

Vitranes

A new spiro sesquiterpene aldehyde, vitrenal, has been isolated from Lepidozia vitrea and assigned structure (54) by a combination of chemical degradation (Scheme 5) and spectroscopic evidence[66]. The absolute configuration of vitrenal was established by X-ray analysis of the di-p-bromobenzoate (55). Vitrenal has a unique spiro [4.5] decane skeleton and may be formed from the co-metabolites isobicyclogermacrenal (56)[67] and lepidozenal (57)[68].

Eudesmanes

Eudesmane sesquiterpenoids are very widely distributed in the Hepaticae, particularly in the Jungermanniales. A number of hydrocarbons and alcohols are present (Fig.3) but, more importantly, biologically active eudesmanolides are very common.

Perhaps one of the most significant discoveries relevant to the field of liverwort sesquiterpenoid chemistry was that cases of contact dermatitis, contracted by handling certain European woods, are caused by epiphytic

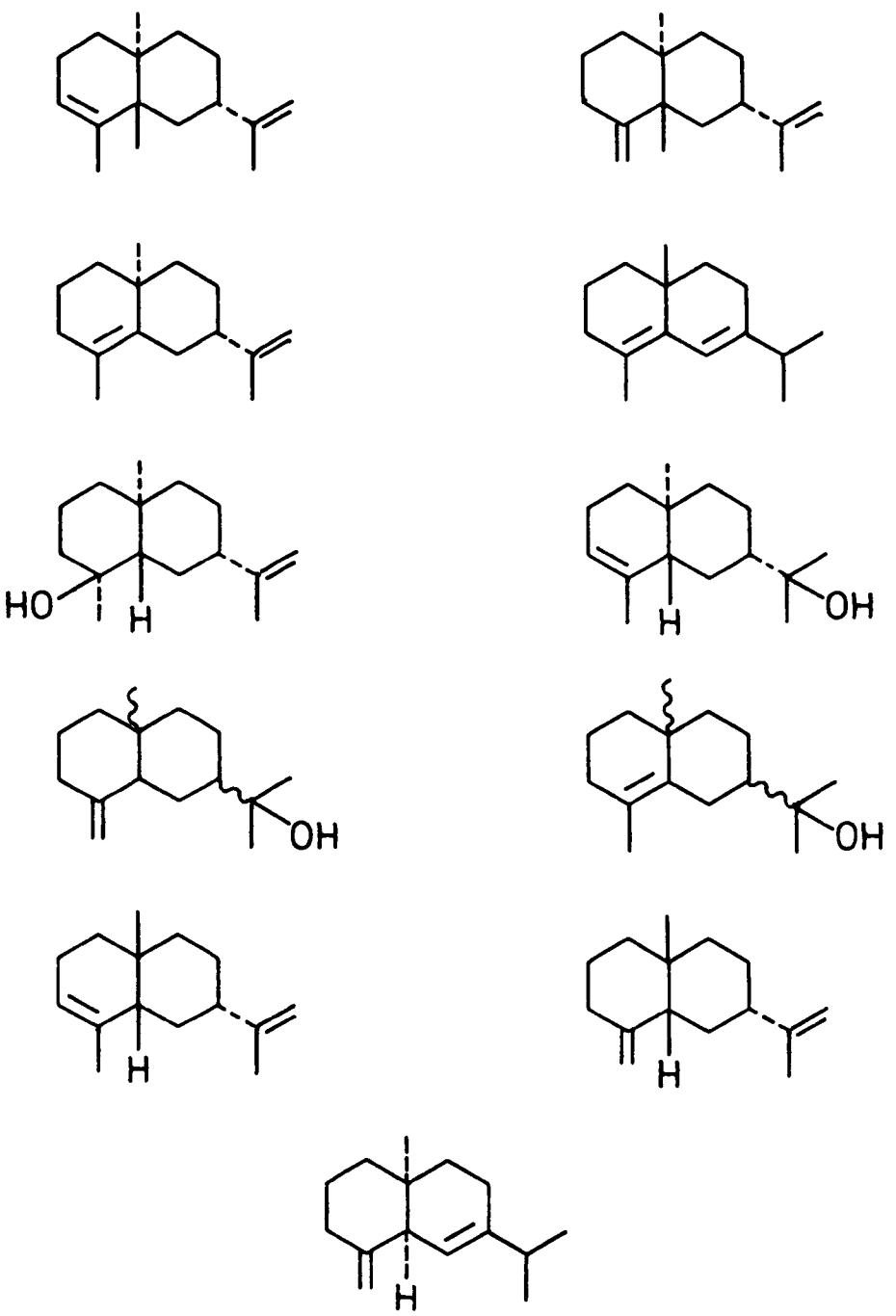
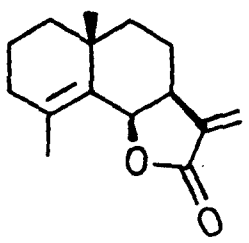
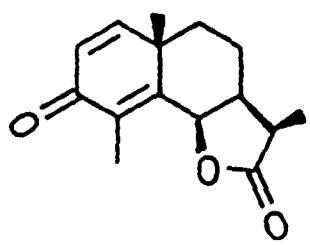


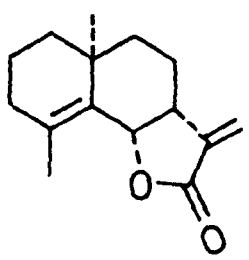
Figure 3. Eudesmanes from the Hepaticae



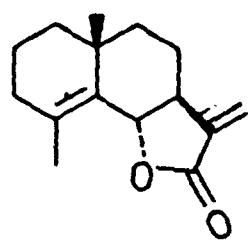
(58)



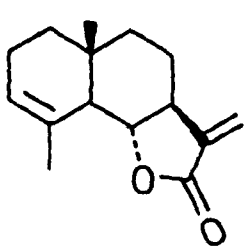
(59)



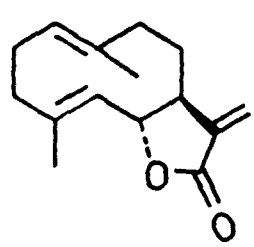
(60)



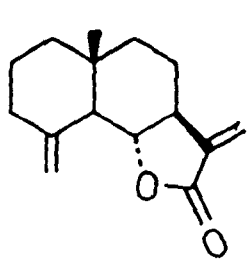
(61)



(62)



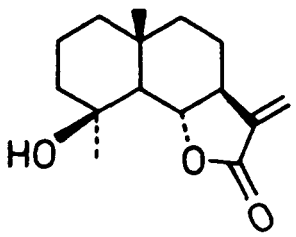
(63)



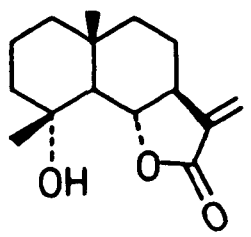
(64)

liverworts (Frullania and Radula species). Le Goulant and Lopes[69] established that allergic reactions could be induced by a component of the ether extract of some of these species. Ourisson and co-workers studied one example and established that the main active principle in Frullania tamarisci was a sesquiterpene α-methylenebutyrolactone. The structure of this compound, (-)-frullanolide (58), was determined by correlation[70] with 6β,11β-santonin (59) and by synthesis[71,72]. Biogenetically (-)-frullanolide (58) may be derived from a germacrene with a β-oriented isopropyl group. Subsequently[70] the ent-germacrene-derived analogue of (58), (+)-ent-frullanolide (60), was isolated from F.dilatata. More recent work has established the structures of a number of Frullania sesquiterpenoid lactones in both the normal and enantio series.

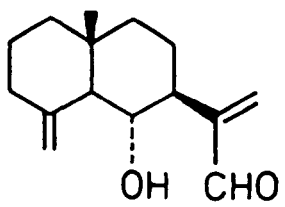
Connolly and Thornton[73] extracted a sample of F. tamarisci from the West of Scotland and isolated (-)-frullanolide (58), γ-cyclocostunolide (61), better known as arbusculin B[74], α-cyclostunolide (62) and costunolide (63), all known compounds. The isolation of costunolide (63) with two of its cyclised isomers, (61) and (62), is of special biogenetic interest as costunolide may be cyclised[75] to a mixture of α-,β- and γ-cyclocostunolides. However, β-cyclocostunolide (64) could not be detected in the extract of F. tamarisci. This compound was later



(65)



(66)



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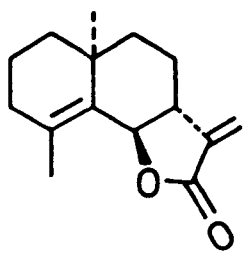
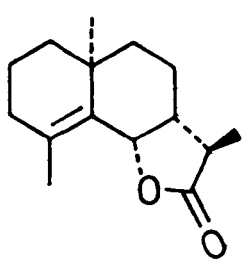
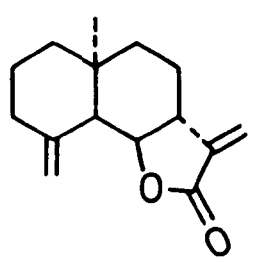


(68)



(69)



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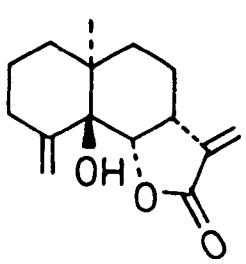

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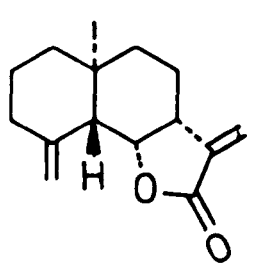

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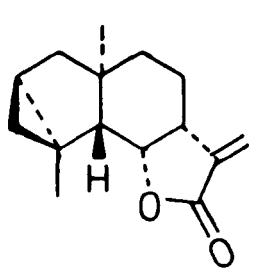
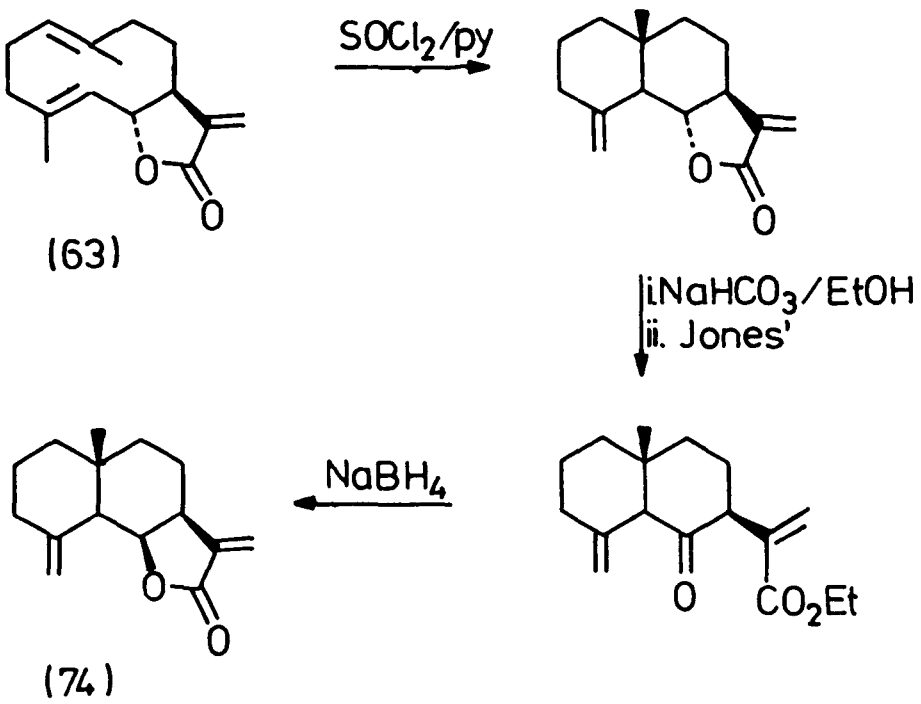


(73)

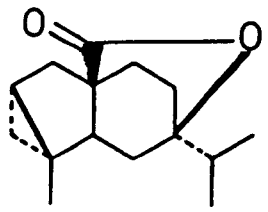
isolated[76] by Asakawa et al. from F. tamarisci subsp. obscura, together with the first 4β-hydroxy-eudesmanolide (65). The latter compound is particularly interesting as the 4α-hydroxyeudesmanolide (66) may be generated from costunolide by reaction with boron trifluoride[77]. A further compound isolated from F. tamarisci subsp. obscura was the closely related hydroxy-aldehyde (67) which Asakawa et al. believe[76] to be the immediate precursor of the eudesmanolides.

Similar members of the ent-eudesmanolide series have been isolated from F.dilatata. An early report[70] described the isolation of (+)-ent-frullanolide (60) from this liverwort and a subsequent study[78] revealed the presence of ent-arbusculin B (68), ent-dihydro-frullanolide (69), ent-cis-β-cyclocostunolide (70) and (+)-ent-5α-hydroxyfrullanolide (71). The absolute stereochemistry of (68) and (69) is antipodal to that of the lactones isolated from F. tamarisci and F.yunnanensis[33]. It is quite interesting that F.dilatata biosynthesises ent-sesquiterpenoids and F. tamarisci elaborates compounds belonging to the normal series although the two species are morphologically similar.

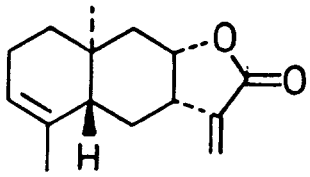
Two new cis-eudesmanolides, ent-β-frullanolide (72) and (+)-brothenolide (73), have beenisolated from F.brotheri[79]. Structure (72), deduced from spectroscopic data, was confirmed (Scheme 6) by preparation



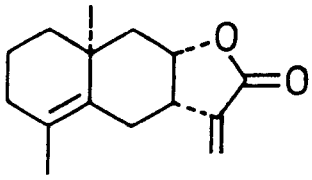
Scheme 6



(77)



(78)

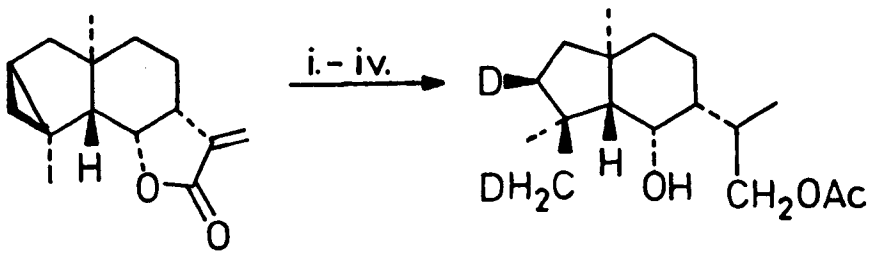


(79)

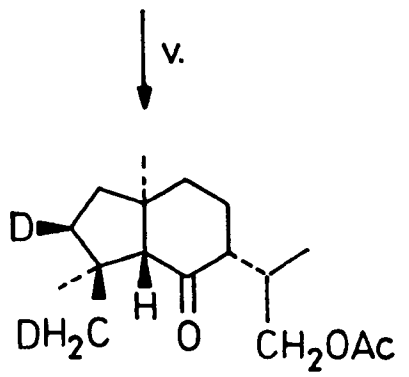
of its enantiomer (74) from (+)-costunolide (63). The β-configuration of the cyclopropane ring of brothenolide (73) was deduced by comparing the ^{1}H n m r spectra of (75) and (76). Cleavage of the cyclopropane ring of (73) by deuteriation followed by $LiAlH_4$ reduction and acetylation gave a deuteriated monoacetate (75) which was then oxidised to the ketone (76). The C-4 methyl signal at 1.20 p p m of monoacetate (75) shifted to 1.34 p p m in ketone (76), indicating that the C-4 methyl group was α-oriented and in the plane of the carbonyl group. Thus, the cyclopropane methylene was established to be β (Scheme 7).

(+)-Crispatanolide (77), a new eudesmane C-14, C-7 δ-lactone, has been isolated from a thalloid liverwort, Makinoa crispata[80]. The molecular structure was established by X-ray crystallographic analysis and the absolute configuration was based upon its positive Cotton effect.

Another series of ent-eudesmanolides with a C-8 oxygen substituent has been isolated from Diplophyllum species. Benesova et al.[81] isolated ent-diplophyllolide from European D. albicans and assigned the structure (78) on the basis of its ^{1}H n m r , c d and i r spectra. Ohta et al.[82] have isolated ent-diplophyllin (79), an isomer of ent-diplophyllolide, from American D. albicans and D. taxifolium together with the 9-hydroxy derivative



(73) (75)

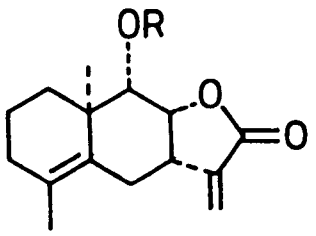


(76)

i. H_2-Pd; ii. D_2-PtO_2; iii. $LiAlH_4$;

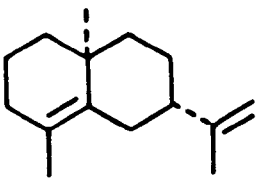
iv. Ac_2O-py; v. Jones'.

Scheme 7.

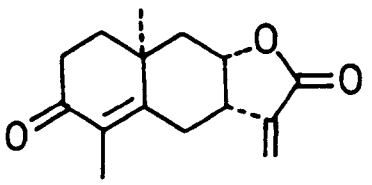


(80) R=H

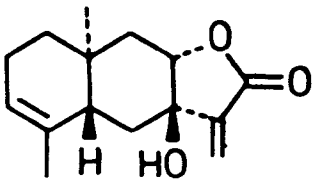
(81) R=Ac



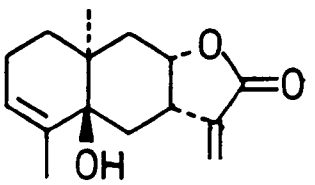
(82)



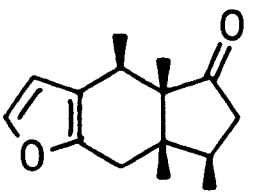
(83)



(84)



(85)



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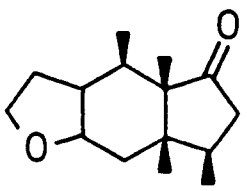
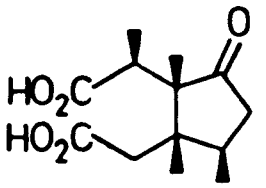


(87)



(88)

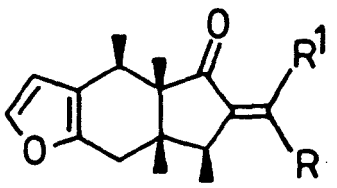
(80) and the corresponding acetate (81). These lactones were accompanied by ent-eudesm-4-ene (82).

Two ent-eudesmanolides, ent-3-oxodiplophyllin (83) and ent-7α-hydroxydiplophyllolide (84), occur in European Chiloscyphus polyanthos[83], together with ent-diplophyllin and ent-diplophyllolide. (84) was originally described as ent-5α-hydroxydiplophyllolide (85) but has since been revised following an examination of the Eu $(fod)_3$-induced shifts in the ^1H n m r spectrum[84].

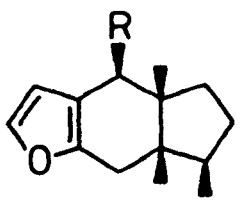
Pinguisanes

Liverwort sesquiterpenoids in this class represent such a highly rearranged skeleton that until biosynthetic evidence is forthcoming it cannot be rationally classified and hence must represent a new class of sesquiterpene. The first representative of this group, the ketone pinguisone (86), was isolated [85] from Aneura pinguis. The basic skeleton was deduced from comparison of the ^1H nmr. spectrum of pinguisone with that of its tetrahydro-derivative (87), the maleic anhydride adduct and the dimethyl ester of the keto dicarboxylic acid (88) resulting from ozonolysis of pinguisone.

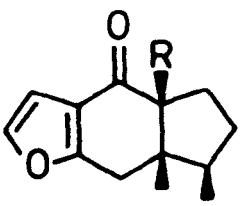
In a later detailed study by Corbella et al.[86] the unusual skeleton of pinguisone was confirmed, especially the lack of the β-methyl group in the furan ring which is found in most furanoid sesquiterpenoids. The absolute stereochemistry of pinguisone was established



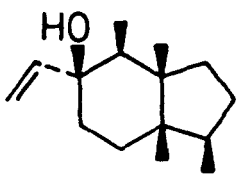
(89) R=C_6H_4Br-p. R^1=H
(90) R=H. R^1 = C_6H_4Br-p



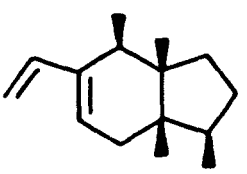
(91) R=Me
(92) R=CO_2Me



(93) R=Me
(94) R=CO_2Me



(95)



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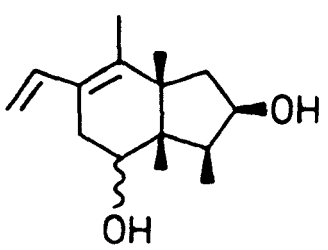

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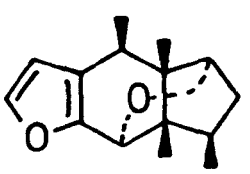

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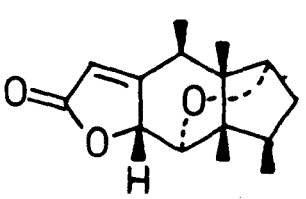
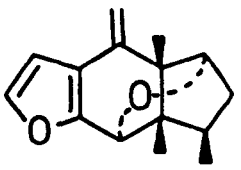


(99)

by X-ray crystallography. Corbella *et al.*[86] prepared the *trans* (E)- and *cis* (Z)-*p*-bromobenzylidene derivatives of pinguisone (structures (89) and (90) respectively), the (Z)-isomer providing suitable crystals. Both the five-and six-membered carbocyclic rings possess unusual conformations. In the cyclohexane ring five carbons (C-4 - C-8) are coplanar whereas the carbons in the cyclopentane ring are in a puckered arrangement, close to the envelope conformation.

The second pinguisane-type sesquiterpene, deoxopinguisone (91), probably better called pinguisane, was isolated from *Ptilidium ciliare*[87], *Trichocoleopsis*[33], *Lejeunea*[88,89] and *Porella*[90] species. Structure (91) was confirmed by correlation with pinguisone (86) by Wolf-Kishner reduction of the latter. Further research[91] on the constituents of *Porella* species resulted in the isolation of three pinguisane -type sesqui- and nor-sesquiterpenes (92, 93, 94). The structures of the new compounds were deduced by spectroscopic methods, chemical degradation and correlation with deoxopinguisone (91).

More recently further examples of this skeletal type have appeared. Pinguisenol (95) and α-pinguisene (96) were isolated from *Porella vernicosa*[92]. The related liverwort *Porella platyphylla* produced[93] β-pinguisenediol (97), pinguisanin (98) and pinguisanolide (99), whilst *Tricholejeunea* species[90] yielded dehydro-



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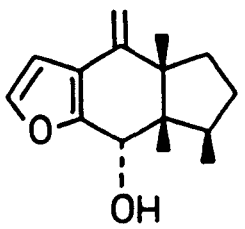

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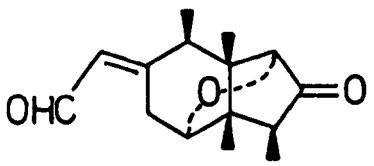

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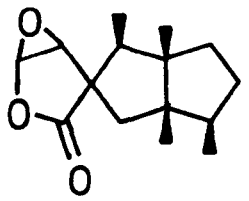

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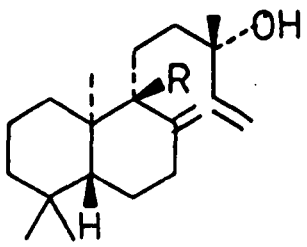
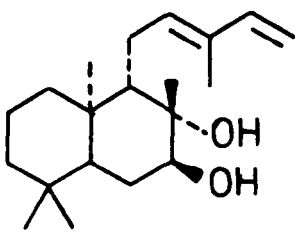
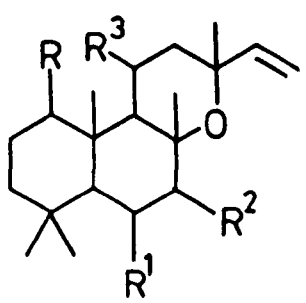


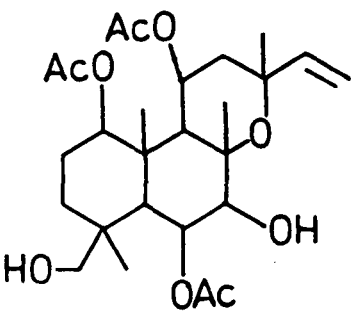
(104) R=H
(105) R=OH



(106)



(107) R=R^1=R^2=R^3=OAc
(108) R=R^1=R^3=OAc;
R^2=OH
(109) R=R^2=OH;
R^1=R^3=OAc



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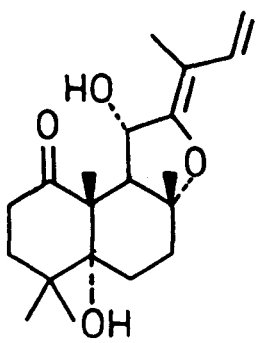


(111)

pinguisanin (100), dehydropinguisenol (101) and pinguisenal (102).

A unique spiro-pinguisane type sesquiterpenoid lactone (103) has recently been isolated from Ptychanthus species[94].

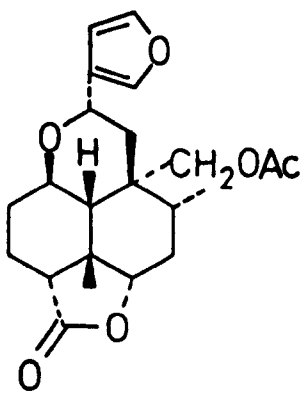
DITERPENOIDS

The distribution of diterpenoids in the Hepaticae is restricted to certain genera. Representatives of two new types, the sacculatanes and verrucosanes, as well as labdanes, pimaranes, kauranes, clerodanes and dolabellanes have been encountered so far.

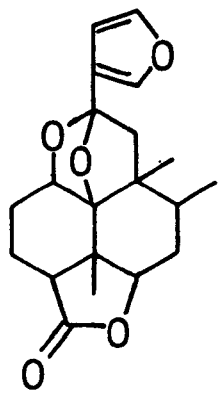
The labdanes, pimaranes and kauranes so far isolated from liverworts belong to the ent-series while higher plants produce both normal and ent-series.

Labdanes

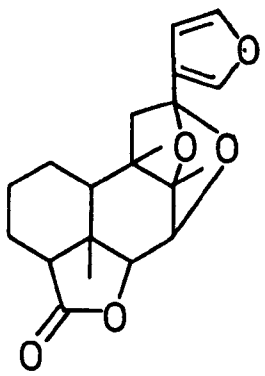
Only a relatively small number of labdanes has been reported form liverworts. Matsuo et al. isolated jungermanool (104),[95] and ent-manool (105)[96] from Jungermannia torticalyx, the structure of the former being determined by a combination of spectroscopic and degradative methods. Porella perrottetiana yielded[97] the novel labdane ent-labda-12(E),14 -diene-7α,8β-diol (106)). Takeda et al.[98,99] have recently isolated the highly oxygenated labdanes (107-110) from Ptychanthus striatus. Another highly oxygenated labdane from a liverwort is scapanin (111), isolated from Scapania



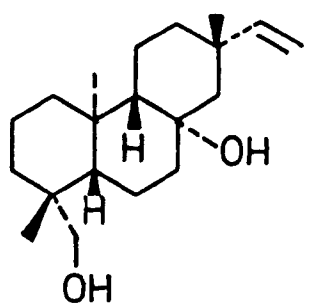
(112)



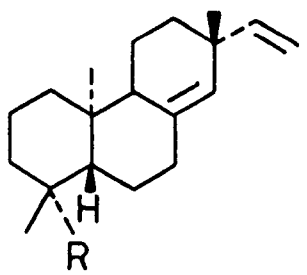
(113)



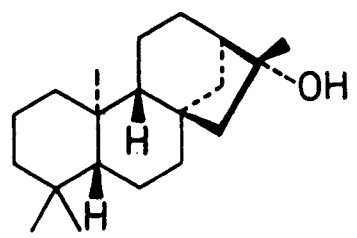
(114)



(115)



(116) R=CH_2OH
(117) R=CO_2H



(118)

undulata by Huneck and Overton[100]. The structure was determined[26] by Connolly and Huneck using spectroscopic and chemical methods. A large number of related labdanes has been found in S. undulata (see p. 42).

Clerodanes

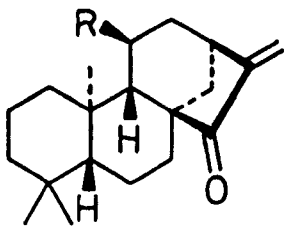
This class of diterpenoid is rare in the Hepaticae. So far only a few examples have been reported (see p. 149), one of which is gymnocolin (112) from Gymnocolea inflata[101]. The structure is based on spectroscopic data and an X-ray crystallographic analysis. The two remaining examples are orcadensin and anastreptin, both isolated[100] from Anastrepta orcadensis, which have been assigned[26] structures (113) and (114) respectively. Their stereochemistries are still undetermined.

Pimaranes

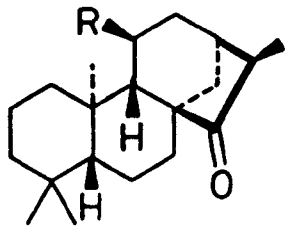
(-)-Thermarol (ent-pimar-15-ene-8β,19-diol) (115), a new ent-pimarane, has been isolated[102] from Jungermannia thermarum, together with the previously known ent- pimara-8(14),15-dien-19-ol (116) and ent-pimara-8(14),15-dien-19-oic acid (117).

Kauranes

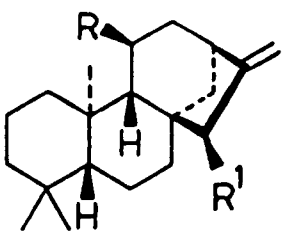
Huneck and Vevle[103] showed that the main component of the waxy coating of the leafy liverworts Anthelia juratzkana and A. julacea was ent- kauran-16β-ol (118).



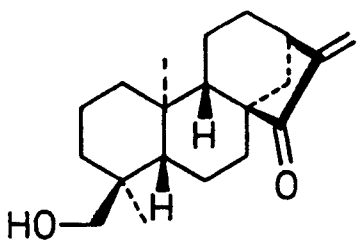
(119) R=OH
(125) R= H
(126) R= OAc



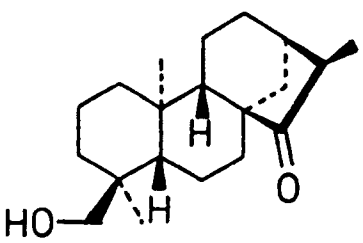
(120) R=OH
(123) R=H
(124) R=OAc



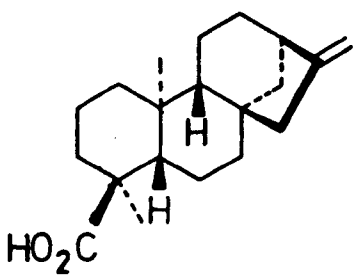
(121) R=R^1=OH
(122) R=OH, R^1=OAc
(127) R=H, R^1=OH
(131) R=R^1=OAc



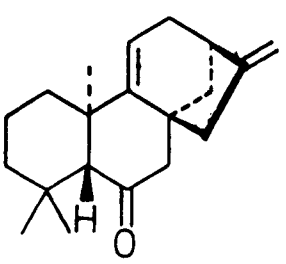
(128)



(129)

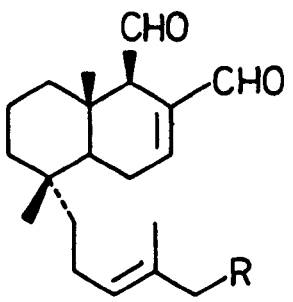


(130)

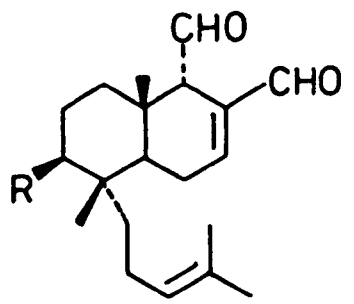


(132)

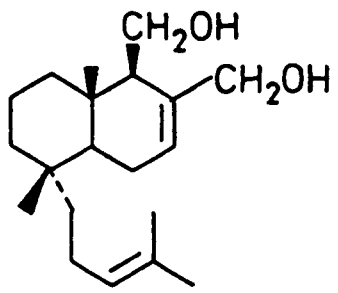
Connolly and Thornton[104] isolated four ent-kauranes from Jungermannia atrovirens (Solenostoma triste), ent - 11α-hydroxykaur-16-en-15-one (119), ent - 11α-hydroxy-(16S)-kauran-15-one (120), ent - kaur-16-ene-11α,15α-diol (121) and ent - 15α-acetoxykaur-16-en-11α-ol (122). Matsuo and Hayashi have reported a similar series of seven ent-kauranes from J.infusca[105,106]. These were ent-(16S)-kauran-15-one (123), ent-11α-acetoxy-(16S)-kauran-15-one (124), ent-kaur-16-en-15-one (125), ent-11α-acetoxykaur-16-en-15-one (126), ent-kaur-16-15α-ol (127), along with (119) and (120). Additionally, Benes et al.[107] isolated (119), (121) and (122) from J.sphaerocarpa, the structure of (121) being established by X-ray crystallographic analysis[108]. Two new ent-kauranes, ent-18-hydroxykaur-16-en-15-one (128) and ent-18-hydroxy-(16S)-kauran-15-one (129) have been isolated from Porella densifolia,[109] together with ent-kaur-16-en-18-oic acid (130). J. gracillima (Solenostoma crenulatum) has yielded[110] (119), (122), (124) and ent-11α, 15α-diacetoxykaur-16-en (131). ent-15α-Hydroxykaura-9(11), 16-dien-6-one (132) has recently been isolated from Czechoslovakian Nardia scalaris[111] whilst (132) has been found[35] in N. compressa.



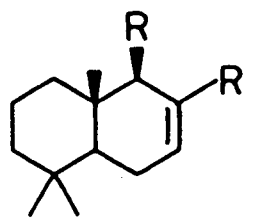
(133) R=H
(138) R=OH



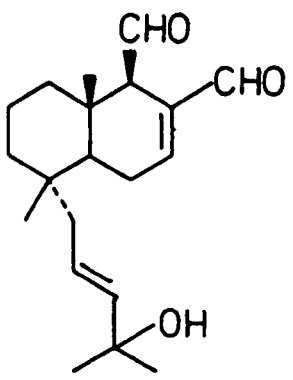
(134) R=H
(140) R=OH



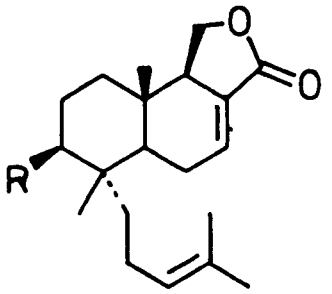
(136)



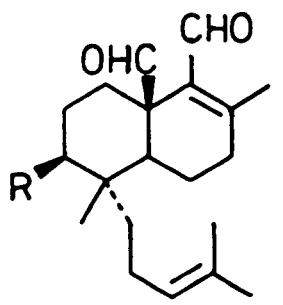
(135) R=CHO
(137) R=CH_2OH



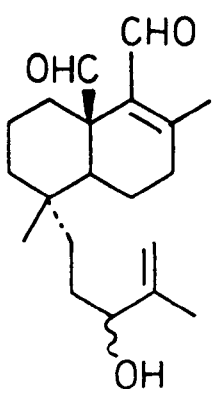
(139)



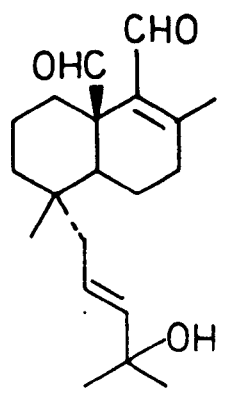
(141) R=H
(142) R=OH



(143) R=H
(144) R=OH



(145)

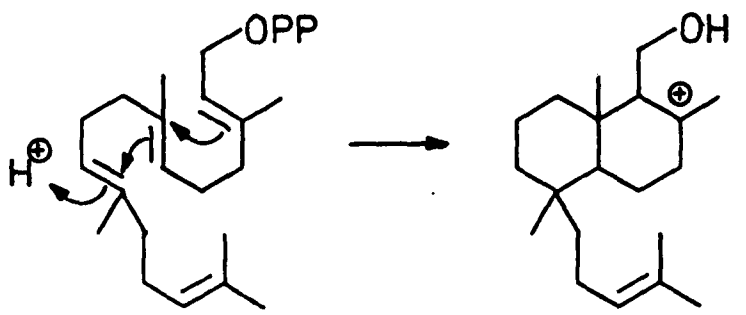


(146)

Sacculatanes

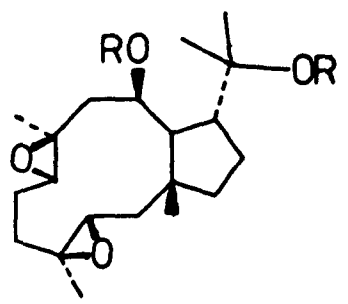
Two unique diterpenoid dialdehydes, the pungent sacculatal (133) and the non-pungent isosacculatal (134), have been isolated from Trichocoleopsis sacculata[112]. The structures were established by spectroscopic correlation with the sesquiterpenoid polygodial (135). Asakawa assigned the absolute stereochemistry of sacculatal by comparing the $Eu(fod)_3$-shifted ^1H n m r spectra of derivative(136) and drimanediol (137). Since both enantiomers of (136) will give the same spectrum, this method is invalid. Subsequent investigation[113] of the pungent metabolites of T.sacculata and Pellia endiviifolia resulted in the isolation of 19-hydroxy-sacculatal (138) and 18-hydroxysacculatal (139) from the former species, and 3-hydroxyisosacculatal (140), sacculatanolide (141) and 3-hydroxysacculatanolide (142) from the latter species.

Two new sacculatanes, perrottetianal A (143) and perrottetianal B (144), have been isolated from Porella perrottetiana[97]. The former is now recognised as a common component of non-pungent Porella species. Surprisingly, it has also been isolated from Makinoa species belonging to the Metzgeriales. Perrottetianal C and perrottetianal D, whose structures are tentatively assigned as (145) and (146) on spectroscopic grounds, have been isolated from European Porella platyphylla[114].



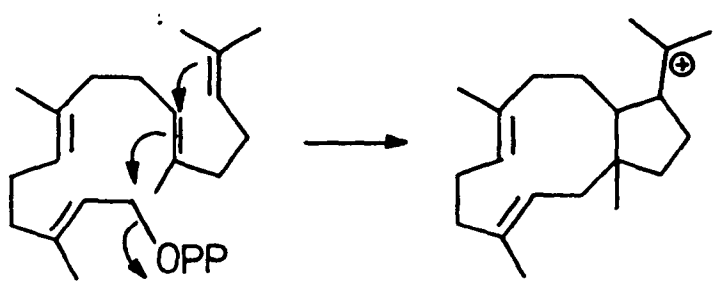
(147)

Scheme 8.



(148) R=Ac

(148a) R=H



(149)

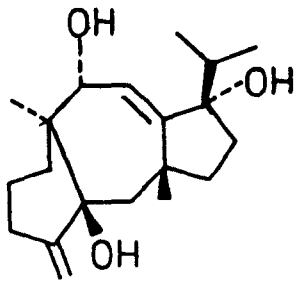
Scheme 9.

The sacculatanes are interesting from a biogenetic point of view. The ring system is the same as that of the drimanes with an additional isoprene unit attached to C-13. They may be formed from geranylgeranyl pyrophosphate (147) by a cyclisation analogous to that of the drimanes (Scheme 8). However, the absolute configurations of the sacculatanes have not been satisfactorily determined. It would be interesting to know whether they are of normal configuration like drimanes from the Hepaticae or whether they belong to the enantio series.

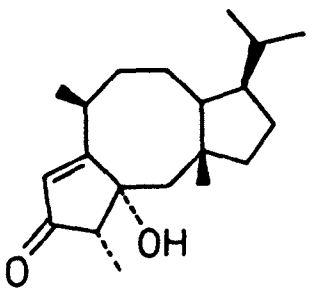
Dolabellanes and Related Compounds

In his recent review[26] of new terpenoids from the Hepaticae, Connolly has reported the occurrence of the dolabellane barbilycopodin (148) (see p. 130) in the extract of Barbilophozia floerkei. The structure was elucidated using a mixture of spectroscopic and chemical methods along with an X-ray analysis of the corresponding diol floerkein B (148a). The absolute configuration of barbilycopodin is still undetermined. This is the first report of a dolabellane from plants, this class having previously been found only in marine organisms[115].

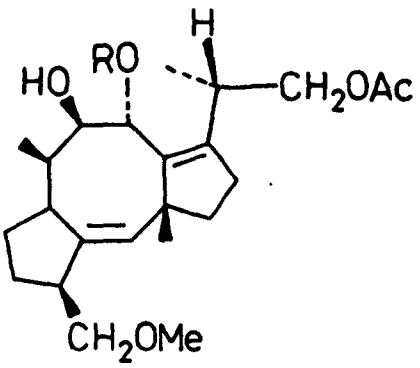
The biogenesis of barbilycopodin can be represented, as in Scheme 9, by cyclisation of all-trans-geranylgeranyl pyrophosphate. Further cyclisation of (149) would lead to the dolastane skeleton e.g. dolatriol



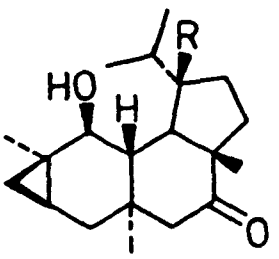
(150)



(151)

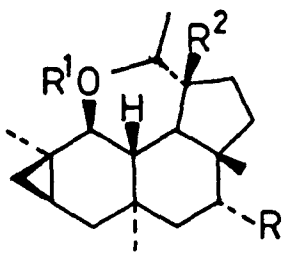


(152)



(156) R=H

(162) R=OH



(153) $R^1=R^2=H$; R =OH

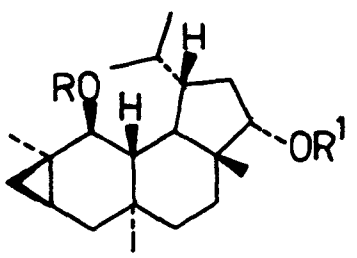
(154) R=OH; $R^1=OCC_6H_4Br$-p; $R^2=H$

(155) $R=R^1=R^2=H$

(157) R=OAc, $R^1=R^2=H$

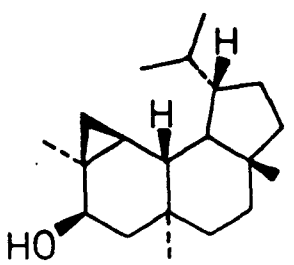
(160) $R=R^2=OH$; $R^1=H$

(161) R=OAc; $R^1=H$; $R^2=OH$



(158) R=H; R^1=Ac

(159) R=Ac, R^1=H

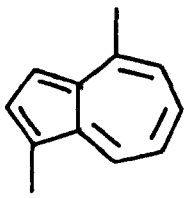


(163)

(150) found in marine organisms[116].

A different cyclisation of (149) affords anadensin (151), a diterpenoid isolated from Anastrepta orcadensis[117]. The structure was assigned following consideration of the ^{1}H and ^{13}C n m r spectra and $Eu(fod)_3$-induced shifts. An X-ray analysis confirmed this and revealed the relative stereochemistry. The absolute configuration remains undetermined. This carbon skeleton is unknown in higher plants but has been found in fungal products e.g. fusicoccin (152)[118] and the cotylenins[119].

An alternative modification of the dolabellane skeleton leads to the verrucosanes isolated from Mylia verrucosa. The structure of (-)-verrucosane-2β-9α-diol (153) was deduced[120] by a combination of chemical degradations and ^{1}H and ^{13}C n m r spectroscopy. An X-ray analysis of the 2-bromobenzoate (154) established the final stereochemistry[121]. Eight other verrucosanes, (-)-verrucosan-2β-ol (155), (-)-2β-hydroxyverrucosan-9-one (156), (-)-9α-acetoxyverrucosan-2β-ol (157), (-)-11α-acetoxyverrucosan-2β-ol (158), (-)-2β-acetoxyverrucosan-11α-ol (159), (-)-verrucosane-2β-,9α,13β-triol (160), (-)-9α-acetoxyverrucosane-2β,13β-diol (161) and (-)-2β,13β-dihydroxyverrucosan-9-one (162) as well as a neoverrucosane, (-)-neoverrucosan-5β-ol (163), have also been isolated from M.verrucosa[122,123].



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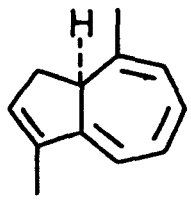
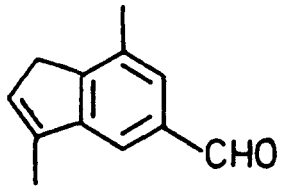


(164)



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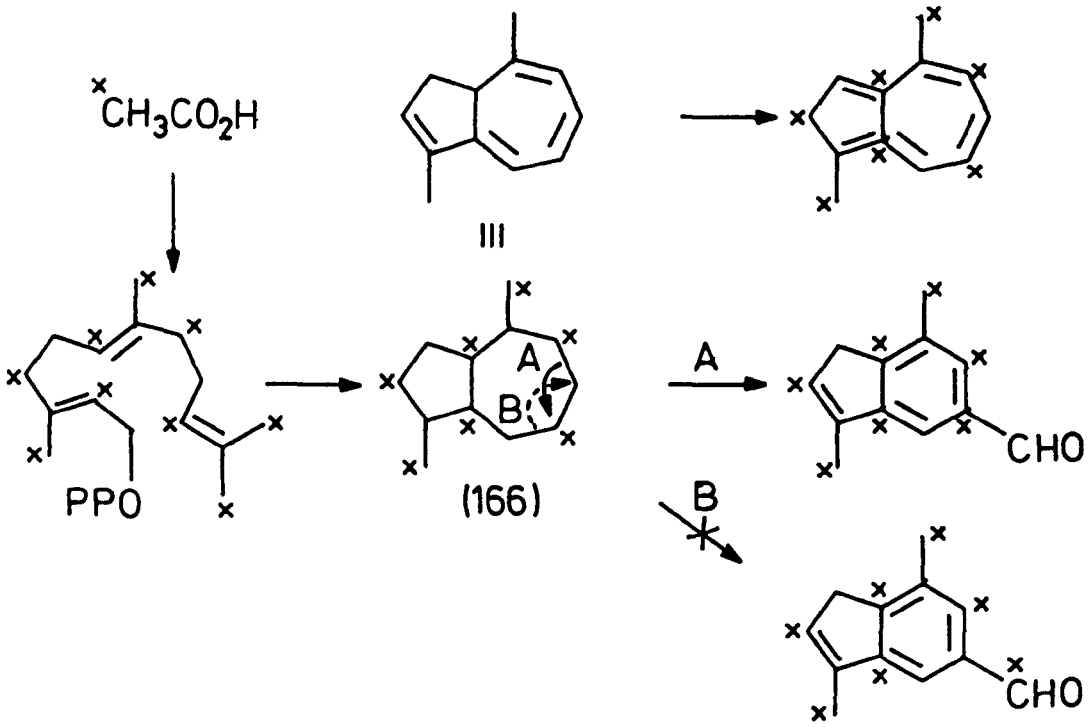


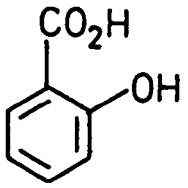
Scheme 10

BIOSYNTHETIC STUDIES

Although the Hepaticae produce a number of rare terpenoid skeletons, little biosynthetic work has been published. Corbella et al.[86] attempted to study the biosynthesis by Aneura pinguis of pinguisone (86) whose structure is difficult to rationalise in terms of the isoprene rule. The published report gives no experimental details of this attempt but merely records the failure to achieve measurable incorporations. Similarly Andersen et al. failed to achieve significant incorporations of [2-^{14}C]-acetate and (±)-[5-^{3}H]-mevalonate using Conocephalum conicum[124].

Recently Takeda et al. have carried out labelling studies using Calypogeia granulata cell cultures[125]. It is significant that the cultured cells produced the same metabolites as the whole plant.

Calypogeia species have previously given the aromatic metabolites 1,4-dimethylazulene (22), 1-carbomethoxy-4-methylazulene (23) and 3,7-dimethyl-5-carbomethoxyindene (24). Takeda et al. have isolated (22) as well as its 3,10-dihydro-derivative (164) and the new 3,7-dimethylindene-5-carboxaldehyde (165). By feeding [2-^{13}C]-acetate they observed a labelling pattern in agreement with that expected for a biogenetic route to 1,4-dimethylazulene (22) via farnesyl pyrophosphate and 3,10-dihydro-1,4-dimethylazulene (164) (Scheme 10). 3,7-Dimethylindene-5-carboxaldehyde (165) is considered to be derived from the hypothetical precursor (166)



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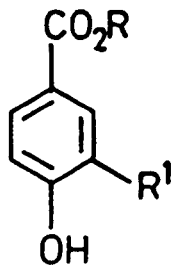
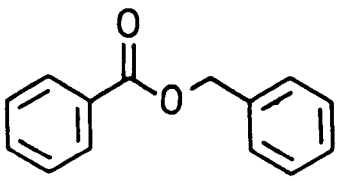


(168) $R=R^1=H$

(171) $R=H;R^1=OH$

(172) $R=Me;R^1=OH$

(173) $R=Me;R^1=OMe$



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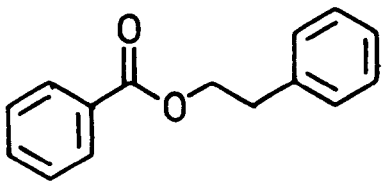
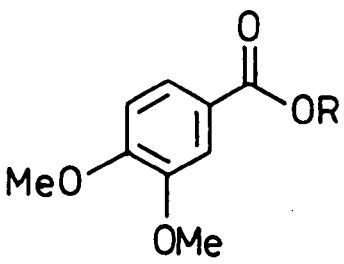
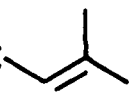
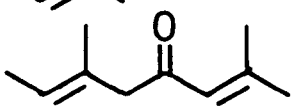
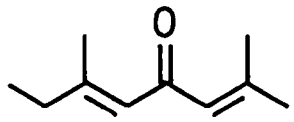


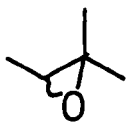
(170)

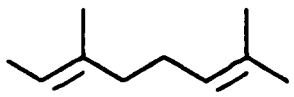


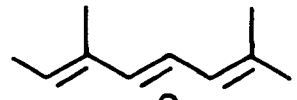
(174) $R=H_2C$ 

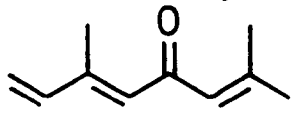
(175) $R=H_2C$ 

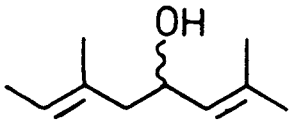
(176) $R=H_2C$ 

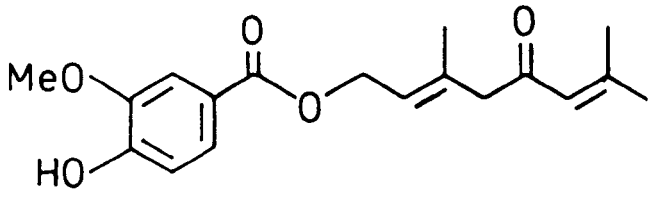
(178) $R=H_2C$ 

(179) $R=H_2C$ 

(180) $R=H_2C$ 

(181) $R=HC$ 

(182) $R=H_2C$ 

(177) 

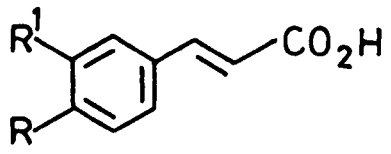
via route A or route B as shown in Scheme 10. Labelling studies showed that the former route is the actual biosynthetic pathway.

AROMATIC COMPOUNDS

Recently several aromatic metabolites of the Hepaticae have been reported.

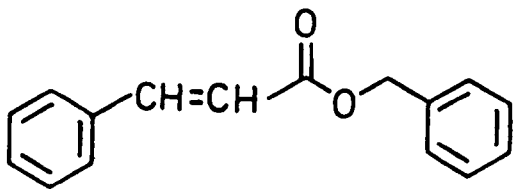
Benzoic Acid Derivatives

O- and p-hydroxybenzoic acids (167,168) have been detected in Asterella lindenbergiana[126]. Benzyl benzoate (169) and β-phenylethylbenzoate (170) have been isolated from the primitive liverwort Isotachis japonica[127]. The liverwort Trichocolea tomentella has been a rich source of benzoic acid derivatives. T. tomentella from Japan[128] produces 3,4-dihydroxybenzoic acid (171) along with its methyl ester (172) and methyl 3-methoxy-4-hydroxybenzoate (173). Also present is a series of prenyl benzoates trichocolein (174), tomentellin (175), isotomentellin (176) and demethoxytomentellin (177). This liverwort collected in Europe[129] also yielded these prenyl benzoates along with five others: epoxytrichocolein A (178), deoxo-tomentellin (179), deoxytomentellin (180), dehydro-tomentellin (181) and oxytomentellin (182).



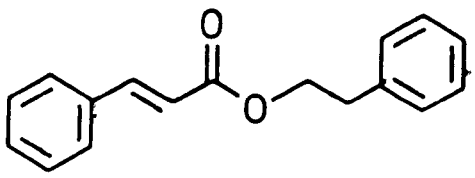
(183) R=OH; R^1=H

(184) R=H; R^1=OH



(185) cis isomer

(186) trans isomer



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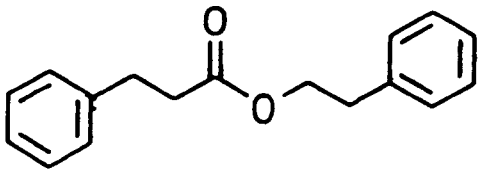

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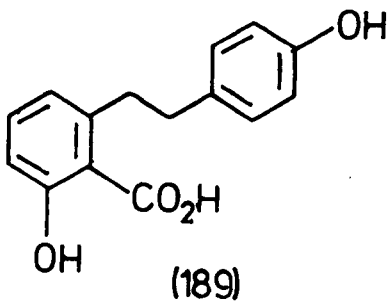

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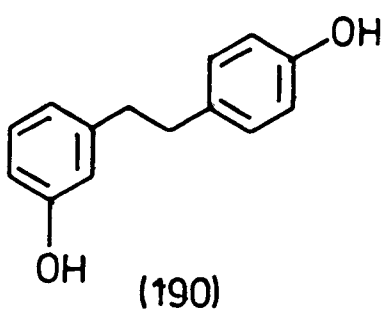

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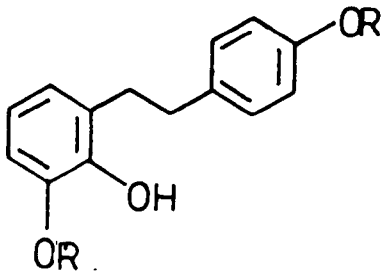
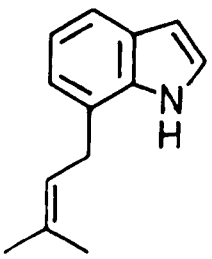
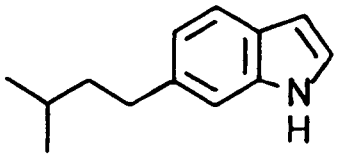


(191) R=Me

(192) R=H



(193)



(194)

Cinnamic Acid Derivatives

Asterella species[126] contain p- and m-coumaric acids (183, 184). Cis- and trans-benzyl cinnamates (185,186), β-phenylethyl cinnamate (187) and its dihydro-derivative (188) have been found in Isotachis japonica[127].

Bibenzyls

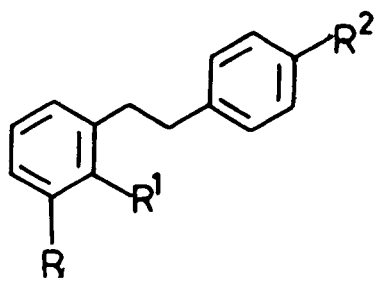
The growth-inhibitory substance lunularic acid (189) and its decarboxylation product lunularin (190) are ubiquitous in liverworts;[130] they have not been detected in lichens, mosses, the Authocerotae or ferns. The occurrence of lunularic acid[131] in some algae seemed to provide chemical evidence for the theory that the Hepaticae might have originated from algae, but subsequent studies revealed its absence from other algae[130].

Pellepiphyllin (191) has been isolated from Pellia epiphylla and P.neesiana [132,133]. Pellia species also produce 2,3,4′-trihydroxybibenzyl (192)[134].

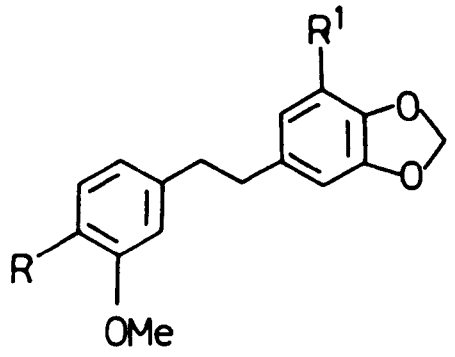
Twenty-five Frullania species have been studied[31,135] and twelve species contain bibenzyl derivatives which are shown in Fig. 4.

Indole Derivatives

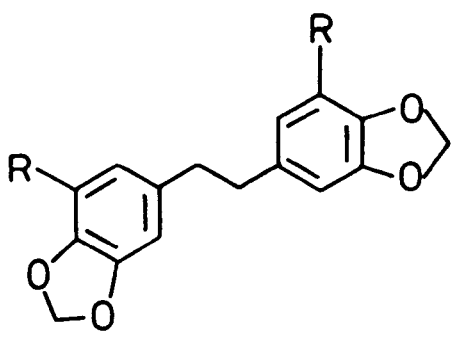
Two prenylated indoles (193,194) have been isolated from Riccardia sinuata[136,137]. These are the only nitrogen-containing compounds isolated from the Hepaticae.



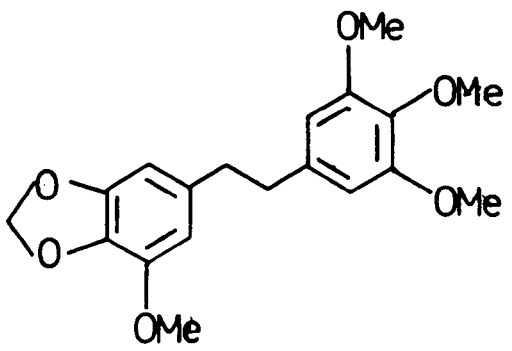
R	R^1	R^2
OMe	H	H
OH	H	OH
OMe	H	OH
OMe	H	OMe
OH	CO_2H	OH
OH	OH	OH
OMe	OH	OMe



R	R^1
H	H
H	OH
H	OMe
OH	OMe



R=H
R=OH



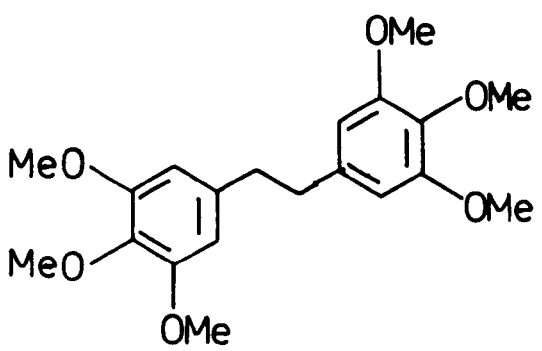
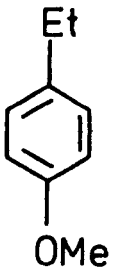


Figure 4. Bibenzyls from Frullania species



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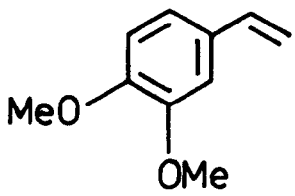

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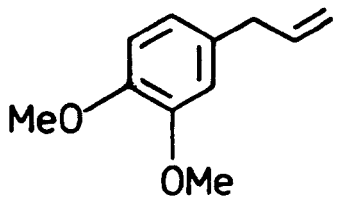

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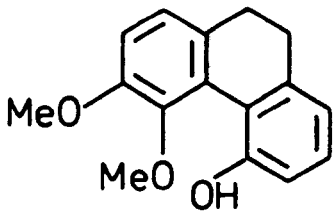

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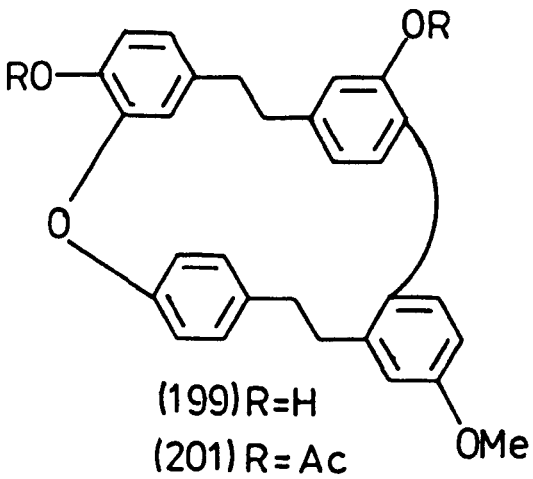
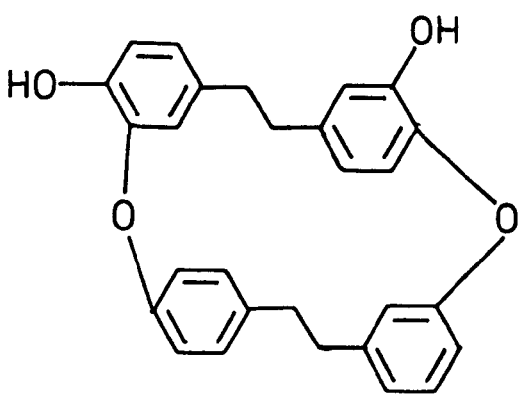


(199) R=H

(201) R=Ac



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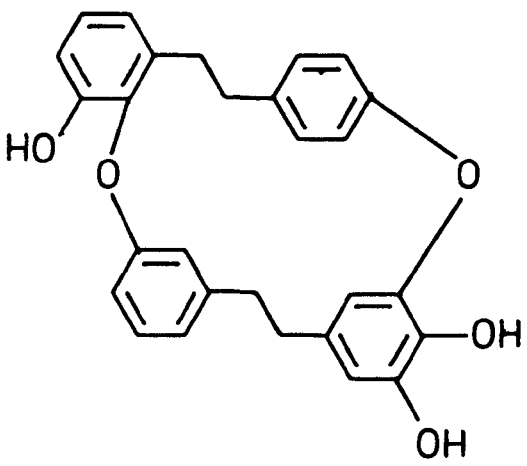


(202)

Miscellaneous

p-Ethylanisole(195) causes the intense odour of Leptolejeunea elliptica[138]. 3,4-Dimethoxystyrene (196) is a minor constituent of Conocephalum conicum[139]. Dimethyl eugenol (197) is the major component of Marchesinia species[32,89].

The structure of 3,4-dimethoxyphenanthren-5-ol (198), a metabolite of Riccardia jackii, has been determined by X-ray analysis[140].

The novel cytotoxic aromatic ethers riccardin A (199) and riccardin B (200) have been isolated from Riccardia multifida and the structure of the former confirmed by X-ray analysis of the diacetate (201)[141,142]. A similar compound, marchantin A (202) has been isolated from Marchantia species[33,141].

CHAPTER 2

THE SCAPANIACEAE

Table 1

Sesquiterpenoids from Scapania undulata[27]

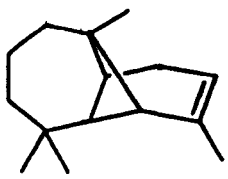
Aequilobene	α-Helmiscapene
α-Alaskene	β-Helmiscapene
Asperene	α-Himachalene
Anastreptene	β-Himachalene
α-Barbatene	γ-Himachalene
β-Barbatene	Isolongifolene
Bazzanene	Longiborneol
α_1-Bisabolene	Longicyclene
α_2-Bisabolene	Longifolene
γ-Cadinene	Longipinanol
Calamenene	α-Longipinene
β-Caryophyllene	β-Longipinene
α-Chamigrene	Sativene
β-Chamigrene	Scapanene
Drimenol	Siberene
Epicubenol	Undulatene
β-Farnesene	α-Ylangene

Introduction

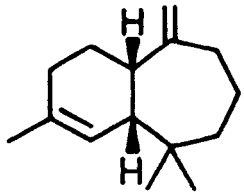
A number of members of the Scapaniaceae have been investigated chemically. The largest genus is *Scapania*, eleven species of which have been examined. The common genus *Diplophyllum* has also been studied although most reports concern *D.albicans*, surely Scotland's commonest liverwort. *Douinia* and *Macrodiplophyllum* have attracted much less attention.

1) *Scapania*

Almost all publications on the metabolites of *Scapania* species are concerned with the presence of sesquiterpenoids. No monoterpenoids have been reported which is surprising since *Scapania* species are extremely rich in metabolites. A large number of sesquiterpenoids has been reported. For convenience these are given in Table 1. Although no single compound is present in all species investigated, a number seem fairly widespread. It appears that the important chemical markers are himachalane, longifolane, longibornane and longipinane sesquiterpenoids. Among the first terpenoids to be isolated from *S. undulata* and indeed from the Hepaticae were (-)-*ent*-longifolene (11) and (-)-*ent*-longiborneol(12) which were shown to be enantiomeric with the same compounds isolated from higher plants[24]. Matsuo *et al*.[143] studied Japanese *S. undulata* and found it to contain the *enantio* sesquiterpenoids (-)-longifolene



(203)



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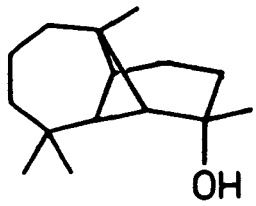

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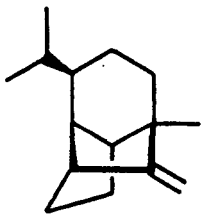

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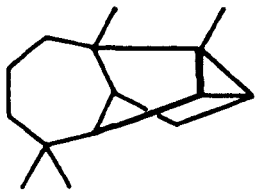

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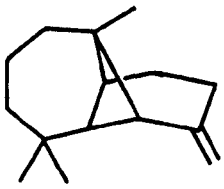

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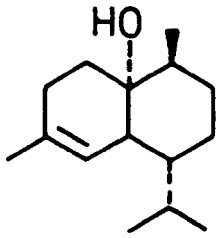

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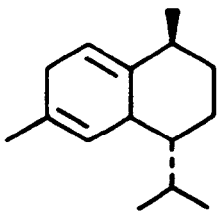
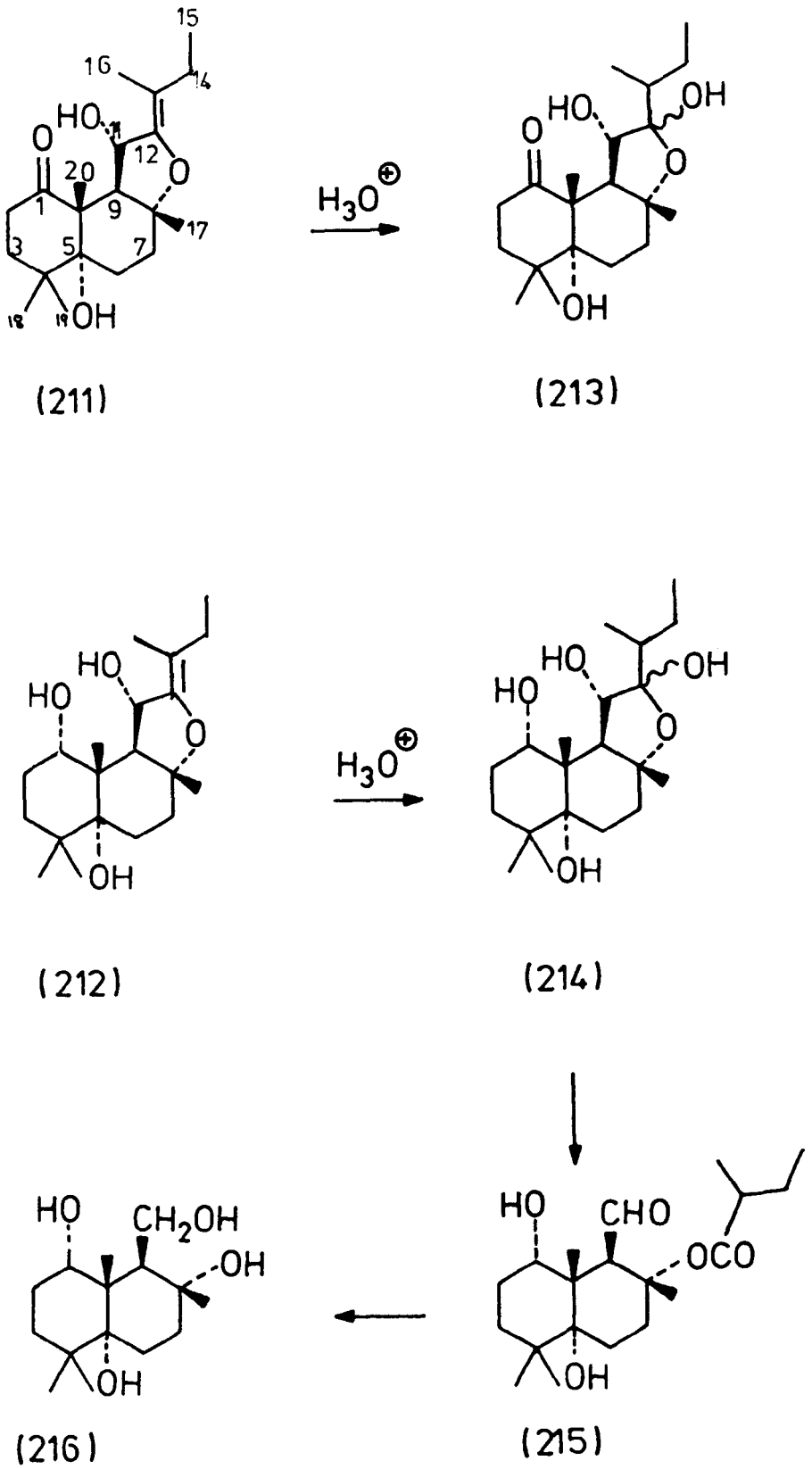


(210)

(11), (-)-longiborneol (12) and (-)-α-longipinene (203) in addition to (+)-α-himachalene (204). Andersen *et al.*[60] subjected the essential oil of European *S.undulata* to detailed study and identified over twenty *enantio* sesquiterpenoids including the novel compound (-)-longipinanol (205) together with sativene (206), (-)-longicyclene (207), (-)-longiborneol (12), (+)-α-longipinene (203) and (-)-β-longipinene (208). Also present were four sesquiterpenes which have remained unidentified - aequilobene, asperene, scapanene and undulatene. Recently Connolly *et al.*[144] have isolated yet another sesquiterpenoid, (+)-*ent*-epicubenol (209) from *S.undulata* collected in Germany. It was identified by comparison of its spectroscopic data with those of (-)-epicubenol (*ent*-209) and by conversion into (+)-*ent*-cubenene (210).

In 1971 Huneck and Overton[100] reported the presence of a crystalline diterpenoid, scapanin, in *S.undulata* collected in Germany. Recently the oxygenated labdane structure (111) has been proposed[26] for scapanin. This structure was assigned on the basis of spectroscopic studies and chemical transformations (Scheme 11). Since scapanin itself is extremely acid sensitive and cannot be chromatographed, most of the work was carried out on the dihydro - and tetrahydro- derivatives, (211) and (212) respectively. Water can easily be added to the enol ether system of (211) and (212) to yield


15
16
14
HO
11
12
O
20
1
9
17
3
5
7
OH
$H_3O^{\oplus}$
OH
(211)
(213)
(212)
(214)
CHO
OCO
CH_2OH
(216)
(215)

Scheme 11

the hydrates (213) and (214). Tetrahydroscapanin hydrate (214) was then oxidised to the aldehydo-ester (215) by sodium metaperiodate. This was then reduced to the tetrol (216) by treatment with $LiAlH_4$.

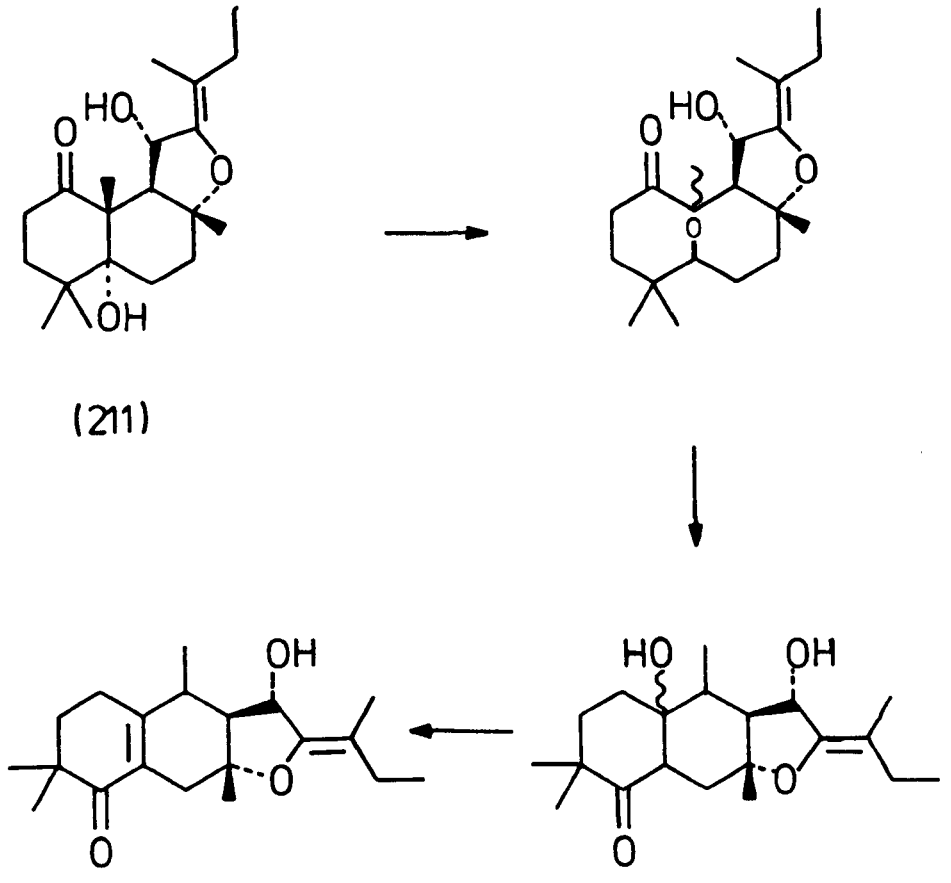
The relative positions of the carbonyl group and the tertiary hydroxyl group were confirmed by treating dihydroscapanin (211) with alkali. The resulting retro-aldol reaction was followed by an aldol condensation and dehydration (Scheme 12) to give the anhydro-iso derivative (217).

The (Z) stereochemistry of the 12,13-double bond as well as the configuration at C-8 were determined by an X-ray crystallographic analysis[26] of dihydroscapanin (211).

It is interesting that while scapanin is the major diterpenoid metabolite of the many European samples of S.undulata examined by Huneck[145] it has so far been found in only two samples collected in Scotland[146].

ii) Diplophyllum

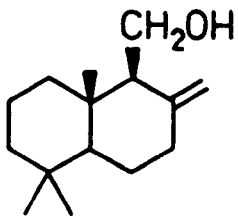
The metabolites of Diplophyllum species are completely different from those contained in Scapania species. Instead of longipinanes etc. as chemical markers, D.albicans and D.taxifolium contain ent-eudesmanolides as the major constituents. In Czechoslovakian D.albicans Benesova et al.[81] discovered ent-diplophyllolide (78), whereas in American D.albicans



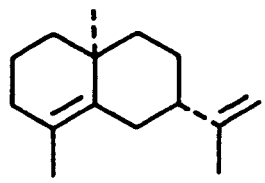
(211)

(217)

Scheme 12.



(218)



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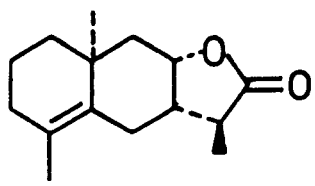

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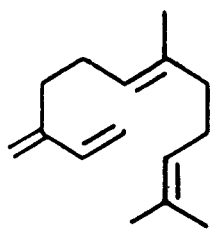

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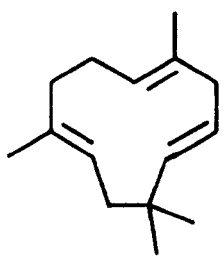


(222)

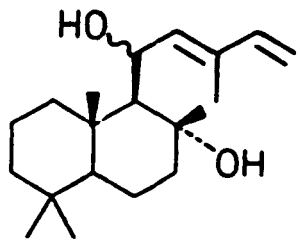
Andersen and co-workers[82] found ent-diplophyllin (79) and ent-9β-acetoxydiplophyllin (81) along with a number of other sesquiterpenoids including anastreptene (30), albicanol (218) and ent-selina-4,11-diene (219). In the same paper Andersen's group reported that D.taxifolium also contained ent-diplophyllin (79) and ent-9β-acetoxy-diplophyllin (81). Recently Asakawa et al.[83] have investigated D.albicans collected in France. The major components of the extract were ent-diplophyllolide (78), ent-diplophyllin (79), ent-9β-acetoxydiplophyllin (81), and the previously unreported ent-dihydrodiplophyllin (220). Thus it appears that ent-eudesmanolides are the chemical markers for Diplophyllum species. So far ent-eudesmanolides have not been detected in any other members of the Scapaniaceae but are present in some members of the Lophocoleaceae (see p. 88).

iii) Macrodiplophyllum

In 1949, M.plicatum was moved from the genus Diplophyllum to the genus Macrodiplophyllum[147]. This decision is supported by a study of the metabolites of M.plicatum[148] which showed that this species has no chemical relationship with Diplophyllum species, the main components being bicyclogermacrene (38), trans-β-farnesene (221) and α-humulene (222).

iv) Douinia

D.ovata has been investigated[149] but no terpenoids were identified.



(223)

DISCUSSION

Scapania undulata

This liverwort was collected in various locations in Scotland and proved to be a rich source of labdane diterpenoids, most of which have the same basic tricyclic skeleton as scapanin (111). So far ten new compounds have been identified. Considerable variation in content was observed suggesting either the existence of different chemical races or some kind of environmental or seasonal control. The liverwort Anastrepta orcadensis has also been reported[26,100] to exist in several chemical races based on analysis of its diterpenoid content. It is convenient to discuss the chemical examination of S.undulata in terms of the extracts obtained from different locations.

Extract A

The plant material for this extract was collected near Aberfoyle in 1980. The three major sesquiterpenoid constituents, ent-longiborneol (12), ent-longipinanol (205) and ent-epicubenol (209), were readily identified by comparison of the spectroscopic properties with those of authentic samples.

The first diterpenoid proved to be labda-12(E), 14-diene-8α, 11β-diol (223), $C_{20}H_{34}O_2$ [m/z 288.2456, M^+-H_2O], m.p. 114-116°C, $[\alpha]_D$ + 24.4° (c, 1.50 in $CHCl_3$), [ν_{max} (CCl_4) 3620 and 3520 cm^{-1}; λ max (EtOH) 234 nm

(ε 7700)]. It has a vinyl group [δ_H 6.40 (dd, J 11, 18 Hz, H-14), 5.19 (d, J 18 Hz, H-15) and 5.04 (d, J 11 Hz, H-15^1), δ_C 112.7 (t,C-15) and 135.8 (d, C-14)], a trisubstituted double bond [δ_H 6.03 (br d, J 10 Hz, H-12), δ_C 133.0 (s, C-13) and 141.6 (d, C-12)], an allylic secondary alcohol [δ_H 5.09 (dd, J 10, 3 Hz, H-11), δ_C 68.6 (d)], a tertiary alcohol [δ_C 75.5 (s,C-8)], a vinyl methyl [δ_H 1.79 (d, J 3Hz, 3H-16), δ_C 12.2 (q)], a tertiary methyl attached to an oxygen-bearing carbon [δ_H 1.44 (s, 3H-17), δ_C 26.7 (q)] and three tertiary methyls [δ_H 0.90 (s), 0 84 (s) and 0.76 (s), δ_C 16.6 (q, C-20), 21.4 (q, C-19) and 33.6 (q,C-18)] which together with two tetrasubstituted carbon atoms, two methine and five methylene groups constitute a bicarbocyclic system. Double irradiation experiments confirmed the allylic nature of the secondary alcohol and the structure of the side-chain. The ^{13}C chemical shifts (Table 2) supported the assignment of the labdane structure (223). The high field ^{13}C chemical shift of the vinyl methyl indicates that it is <u>cis</u> with respect to C-11 and therefore the trisubstituted double bond has the (E) configuration. It is difficult to assign the configuration of the 11-hydroxyl group in view of the uncertain confo rmation of the side-chain. It is tempting, however, to suggest that it is probably (11S), the configuration which is common to all the

Table 2

^{13}C nmr chemical shifts of diterpenoids from Scapania undulata.

Carbon	(223)	(228)	(236)	(238)
1	40.4	42.2	80.4	82.3
2	18.6	18.5	28.3	24.1
3	41.8	44.7	42.2	41.7
4	33.4	34.0	33.5	33.6
5	56.8	58.8	57.0	57.0
6	20.7	69.1	69.5	69.1
7	46.5	48.9	48.9	48.8
8	75.5	80.0	80.1	80.1
9	65.3	61.7	60.5	59.9
10	38.9	36.6	42.2	40.9
11	68.6	28.7	32.0	30.7
12	141.6	82.8	82.7	82.7
13	133.0	136.7	136.8	136.5
14	135.8	118.1	117.9	118.5
15	112.7	12.7	12.8	13.0
16	12.2	13.0	13.0	13.5
17	26.7	26.0	26.1	26.2
18	33.6	33.0	32.4	32.3
19	21.4	23.6	23.4	23.3
20	16.6	17.0	12.2	12.6
$\underline{CH_3}CO$				21.7
$CH_3\underline{CO}$				170.9

Table 2 (cont.)

^{13}C nmr chemical shifts of diterpenoids from *Scapania undulata*.

Carbon	(239)	(240)	(241)	(242)
1	83.6	82.1	78.0	219.6
2	23.9	24.1	26.3	35.2
3	40.4	41.7	41.7	42.9
4	32.0	33.6	33.1	34.0
5	65.7*	57.0	55.0	57.4
6	207.8	69.1	67.0	68.9
7	58.9	47.8	48.7	48.1
8	81.6	81.6	80.0	80.9
9	59.3*	59.6	65.7	58.9
10	40.9	40.9	42.3	54.1
11	29.9	28.0	72.0	71.9
12	83.6	82.1	86.5	88.6
13	135.7	211.3	135.9	134.9
14	119.3	-	117.7	119.6
15	13.0	-	12.2	12.2
16	13.6	24.0	12.8	13.0
17	25.4	27.0	26.7	27.1
18	31.5	32.4	32.1	31.5
19	21.6	23.4	22.9	23.4
20	12.5	13.0	12.8	17.5
$\underline{CH_3}CO$	21.1	21.8		
$CH_3\underline{CO}$	170.6	170.9		

* Signals may be interchanged.

Table 2 (contd.)

^{13}C nmr chemical shifts of diterpenoids from *Scapania undulata*.

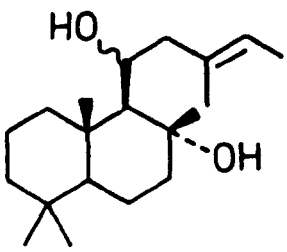
Carbon	(243)	(244)	(245)	(246)
1	218.5	79.7	219.7	78.2
2	35.7	28.2	35.5	26.7
3	34.5*	40.6	38.0	40.4
4	37.6	32.7	32.2	32.5
5	80.3	55.5	52.3	54.5
6	28.0	21.1	21.8	20.9
7	34.7*	40.6	40.0	40.9
8	80.3	80.9	81.1	81.0
9	52.8	59.9	58.3	65.3
10	57.9	42.0	52.8	42.5
11	71.5	31.8	71.9	71.8
12	89.2	82.8	88.6	88.3
13	135.2	136.8	135.3	134.8
14	119.9	117.9	119.3	120.7
15	12.3	12.7	12.4	12.1
16	13.1	13.0	13.1	13.1
17	25.2	25.0	25.8	25.8
18	27.0≠	33.0	31.0	32.9
19	26.7≠	20.9	23.9	20.9
20	17.3	11.6	15.8	12.3

*,≠ Signals may be interchanged.

Table 2 (contd.)

^{13}C nmr chemical shifts of diterpenoids from *Scapania undulata*.

Carbon	(249)	(251)
1	74.0	215.8
2	30.8	35.1
3	24.3	39.1
4	38.4	32.3
5	79.1	53.5
6	26.5	21.8
7	35.8	39.6
8	80.7	80.7
9	50.9	54.1
10	42.8	50.1
11	71.9	30.4
12	90.1	82.5
13	134.5	136.8
14	121.2	117.5
15	12.1	12.8
16	13.2	12.9
17	26.1	25.0
18	28.0	31.5
19	26.1	23.5
20	20.2	15.2



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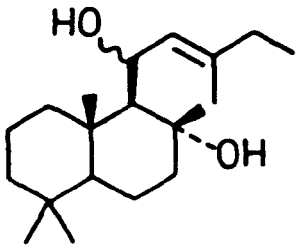

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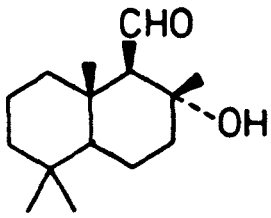
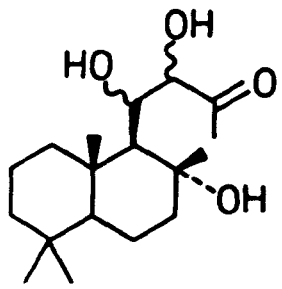


(226)

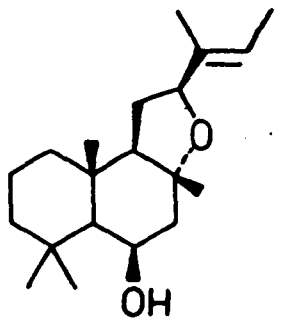
other 11-hydroxy compounds from S.undulata. The configuration at C-8 follows from the degradative experiment described below.

Hydrogenation of the diene system yielded two isomeric olefins. Isomer A has a trisubstituted double bond [δ_H 5.20 (m, H-14)] and two vinyl methyls [δ_H 1.59 (s, 3H-15 and 3H-16)] and is therefore labd-13-ene-8α,11ξ-diol (224), the product of 1,4-addition of hydrogen to diene (223). Isomer B also has a trisubstituted double bond [δ_H 5.74 (br d, J 11 Hz, H-12)] and one vinyl methyl [δ_H 1.67 (d, J 2 Hz, 3H-16)] in addition to an ethyl group [δ_H 1.98 (q, J 7 Hz, 2H-14) and 0.99 (t, J 7 Hz, 3H-15] and must therefore be labd-12-ene-8α,11ξ-diol (225).

Support for the proposed structure (223) and evidence for the C-8 configuration were obtained by osmoylation and periodate cleavage to the known aldehyde 8α-hydroxydriman-11-al (226), the spectroscopic properties of which were identical with those reported in the literature[150]. However the specific rotation of + 26° is lower than the reported literature value of +66° though it suggests that the natural product belongs to the normal series of absolute configuration. Later attempts to repeat the degradation gave either no product or small amounts of a C_{18} compound, [ν_{max} 3600 (free OH) and 1710 cm^{-1}], which has two secondary alcohols



(227)

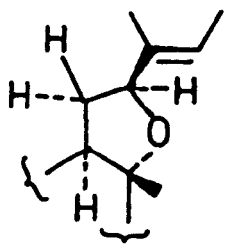


(228)

[δ_H 4.54 (dd, J 6, 11 Hz, H-11) and 4.03 (d, J 6Hz, H-12, δ_C 83.2 and 89.2], a tertiary alcohol [δ_C 73.1 (C-8)] and four tertiary methyls [δ_H 1.06 (s), 0,89 (s), 0.85 (s) and 0.80 (s)]. These data are consistent with structure (227) which can be derived from diene (223) by osmoylation followed by periodate cleavage of the 13,14 bond. Further cleavage of the side-chain would be prevented if the side-chain adopted a conformation which prevented formation of a cyclic periodate ester. However the ir spectrum gives no indication of an interaction between the C-8 hydroxyl group and the C-12 hydroxyl group or of any interaction involving the ketone carbonyl.

Three further new diterpenoids were obtained from this extract. It was apparent from their spectroscopic properties that they all possessed the same basic tricyclic skeleton as scapanin (111) and differed only in pattern of hydroxylation.

The least polar compound, 8α,12(S)-epoxy-labd-13(E)-en-6β-ol (228), $C_{20}H_{34}O_2$ (m/z 3 06.2574), m.p. 170-172°C, $[\alpha]_D$ - 51.1° (c, 2.16 in $CHCl_3$), [ν_{max} (CCl_4) 3620 cm^{-1}], has a trisubstituted double bond [δ_H 5.57 (dqq, J 1.5, 1.5, 5.6 Hz, H-14), δ_C 118.1 (d, C-14) and 136.7 (s, C-13)], a secondary alcohol [δ_H 4.56 (br s, H-6), δ_C 69.1 (d)], a secondary carbon bearing oxygen [δ_H 4.27 (br dd, J 6.3, 9.1 Hz, H-12),

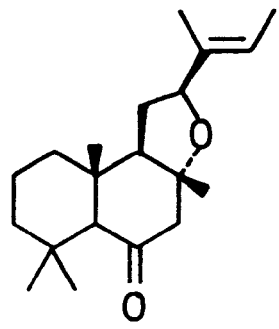


J	Hz
9,11α	4·7
9,11β	13
11α,11β	11
11α,12	6·2
11β,12	9·1

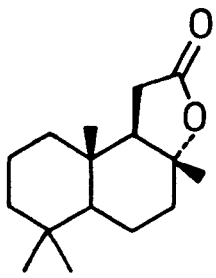
Fig. 5

δ_C 82.8 (d)], a tertiary carbon bearing oxygen [δ_C 80.0 (s)], two vinyl methyls [δ_H 1.60 (s, 3H-16) and 1.59 (d, J 5.8 Hz, 3H-15), δ_C 12.7 (q) and 13.0 (q)], a tertiary methyl attached to an oxygen-bearing carbon [δ_H 1.36 (d, ^{4}J 0.9 Hz, 3H-17), δ_C 26.0 (q)], three tertiary methyls [δ_H 1.19 (s), 1.15 (s) and 0.99 (s), δ_C 17.0 (q, C-20), 23.6 (q, C-19) and 33.0 (q, C-18)] and a methine [δ_H 0.94 (d, J 2.8 Hz, H-5) , δ_C 58.8 (d)] which together with two tetrasubstituted carbon atoms, one methine and five methylene groups constitute a tricyclic system.

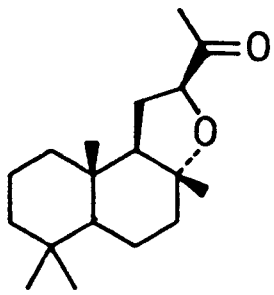
The ^{13}C nmr shifts and multiplicities (See Table 2) suggest a hydroxylabdane skeleton with an 8, 12 ether bridge and a 13,14-double bond. The 360 MHz ^{1}H nmr spectrum revealed all the coupling constants between the protons on C-9, C-11 and C-12. These are shown in Fig. 5 and indicate that both H-9 and H-12 are α-oriented. The (E)-configuration of the double bond follows from the highfield ^{13}C shifts of the vinyl methyls which must be <u>cis</u>. The hydroxyl group must be placed at C-6 since H-5 is a clean doublet (J 2.8 Hz). The magnitude of this coupling indicates that the hydroxyl group must be axial. The strong shielding of H-5 (δ_H0.94) was unexpected and is reminiscent of a cyclopropyl proton (see p. 54). However the corresponding ^{13}C resonance shows no such shielding. The C-8 methyl



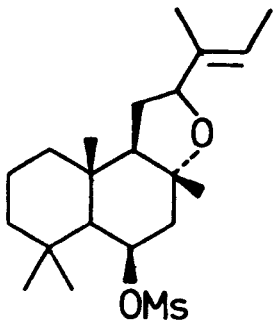
(229)



(230)

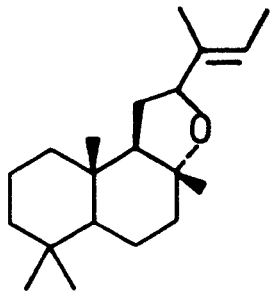


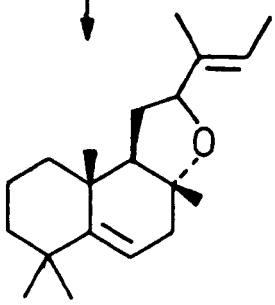
(231)

(228) —X→ 

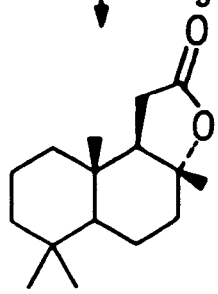
OMs

(232)

$LiAlH_4$ → 

$MsCl,Et_3N$ ↓ 

(233)

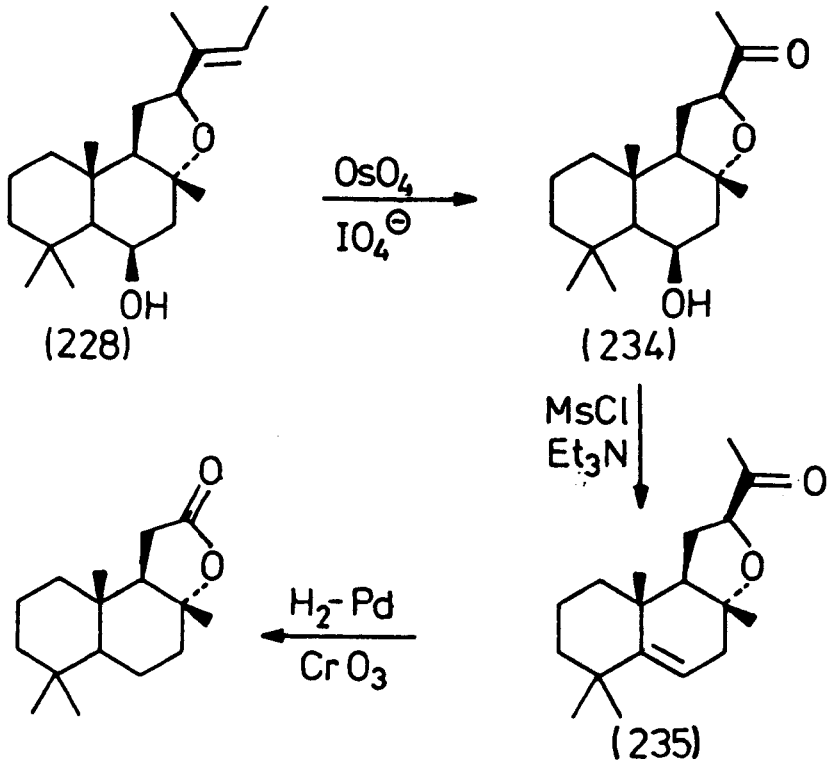
$i.OsO_4;IO_4^{\ominus}$
$ii.CrO_3$ ↓ 

Scheme 13

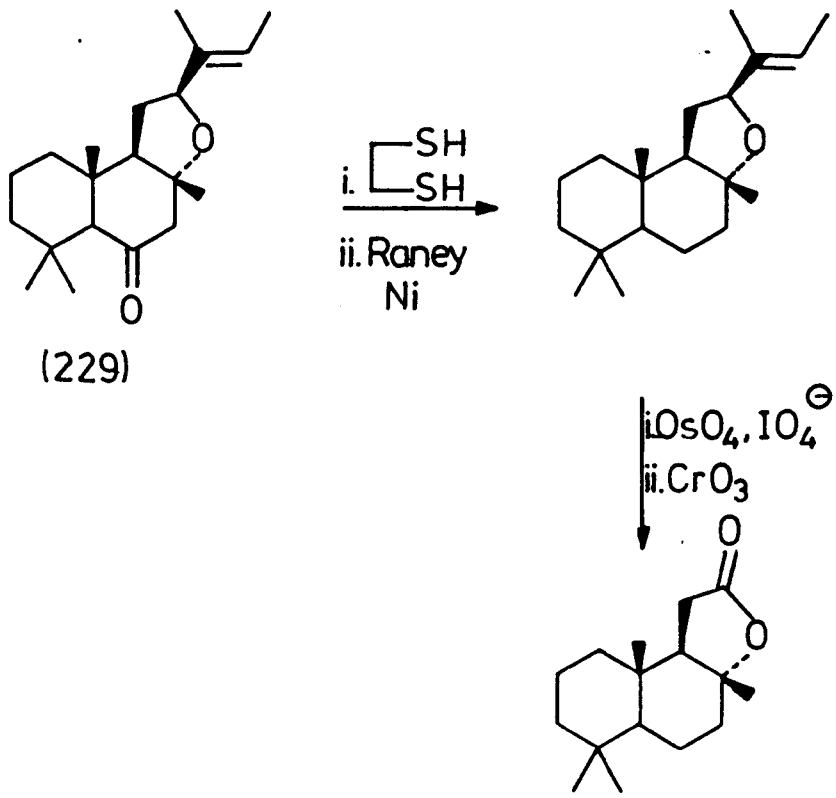
exhibits a long-range W coupling, presumably with H-7α, indicating that it is axial. These arguments led to structure (228).

Oxidation of the 6-hydroxyl group gave the expected ketone 8α, 12(S)-epoxylabd-13(E)-en-6-one (229), $C_{20}H_{32}O_2$ (m/z 304.2415), [ν_{max} (CCl_4) 1715 cm^{-1}], which has a similar ^1H nmr spectrum to the starting alcohol. H-5 appears as a singlet [δ_H 2.12] whereas the C-7 methylene appears as an AB quartet with one component (H-7α) exhibiting further long-range coupling to the C-8 methyl (^4J 0.9 Hz) and to H-5. Coupling across a ketone carbonyl group is not unusual, being found in the case of acetone where free rotation occurs and might be expected to mitigate any long-range coupling.

In an effort to establish the absolute configuration, correlation of (228) with norambreinolide (230) was attempted. It is known that 14,15-bisnor-8α,-12(S)-epoxylabdan-13-one (231) can easily be oxidised to norambreinolide (230) by treatment with almost any oxidant[151]. Scheme 13 was therefore envisaged. However, on treatment with mesyl chloride and triethylamine (by the method of Crossland et al.[152]), (228) gave not the desired mesylate (232) but the elimination product (233) whose structure was supported by its ^1H nmr spectrum which had resonances for a new trisubstituted double bond [δ_H 5.46 (t, J 4 Hz, H-6)] and an allylic methylene group [δ_H 2.30 (d, J 4Hz, 2H-7)]. Since selective reduction of the 5,6-double bond seemed



Scheme 14

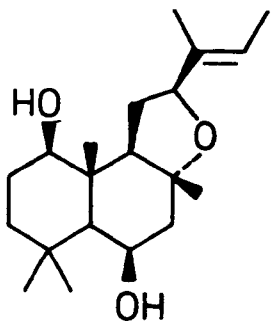


Scheme 15

improbable a new approach was undertaken (Scheme 14).

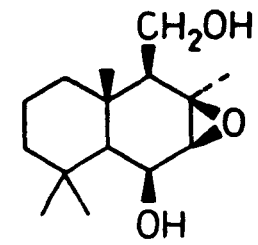
Lemieux-Johnson oxidation[153] of the alcohol (228) gave the required bisnor-compound (234), $C_{18}H_{30}O_2$ (m/z 278), [δ_H 2.25 (s, methyl ketone)]. However, treatment of this keto-alcohol (234) with mesyl chloride and triethylamine failed to give the desired elimination product (235). The approach was modified yet again (Scheme 15). The 6-oxo compound (229) was treated with ethanedithiol-$BF_3.Et_2O$ and the major product isolated by preparative tlc. The ir and uv spectra suggested an α,β-unsaturated ketone [$\nu_{max}(CCl_4)$ 1675 cm^{-1}; λ_{max} (EtOH) 238 nm; δ_C 200.0 (s)]. The ^1H nmr spectrum contained the following resonances: δ_H 5.76 (br s, vinyl proton), 5.32 (br m, vinyl proton), 3.42 (q, J 8 Hz, C<u>H</u>-CH_3), 2.56 (4H, m, thioketal), 1.84 and 1.65 (both br s, vinyl methyls), 1.26 (d, J 8Hz, CH-<u>C</u>H_3), 1.14, 1.08 and 0.84 (tertiary methyls). The sample contained a minor impurity which made analysis of the ^{13}C nmr spectrum of the major product difficult. No structure could be postulated for this compound and this work was abandoned.

The next compound in order of increasing polarity was the diol, 8α, 12(S)-epoxylabd-13(E)-ene-1β,6β-diol (236), $C_{20}H_{34}O_3$ (m/z 322.2534), m.p. 174-176°C, $[\alpha]_D$-57.6° (c, 0.99 in $CHCl_3$), [ν_{max} 3450 and 3330 cm^{-1}], which has a trisubstituted double bond

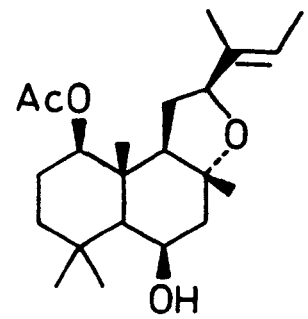


(236)

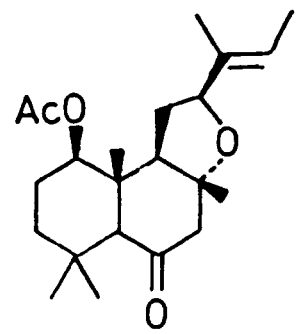
[δ_H 5.57 (dqq, J 1.8, 1.8, 6.7 Hz, H-14), δ_C 117.9 (d, C-14) and 136.8 (s, C-13)], two secondary alcohols [δ_H 4.54 (br d, J 2.4 Hz, H-6), δ_C 69.5 (d); δ_H 3.28 (dd, J 4.7, 11.2 Hz, H-1), δ_C 80.4 (d)], one secondary carbon bearing oxygen [δ_H 4.27 (dd, J 6.4, 9.7 Hz, H-12), δ_C 82.7 (d)], a tertiary carbon bearing oxygen [δ_C 80.1 (s, C-8)], two vinyl methyls [δ_H 1.60 (s, 3H-16) and 1.59 (d, J 6.9 Hz, 3H-15), δc 12.8 (q) and 13.0 (q)], one tertiary methyl attached to an oxygen- bearing carbon δ_H 1.36 (s, 3H-17), δ_C 26.1 (q)], three tertiary methyls [δ_H 1.18 (s), 1.16 (s) and 0.98 (s), δ_C 12.2 (q, C-20), 23.4 (q, C-19) and 32.4 (q, C-18)] and a methine [δ_H 0.85 (d, J 2.8 Hz, H-5), δ_C 57.0 d)] which together with two tetrasubstituted carbon atoms, one methine and four methylene groups again constitute a tricyclic system. These spectroscopic properties indicate that the diol has the same structure as the mono-alcohol (228) with an additional secondary hydroxyl group. Comparison of ^{13}C shifts enabled this extra hydroxyl group to be placed at C-1 (see Table 2). The C-1 hydroxy group is equatorial (H-1 has one large coupling and one smaller coupling indicating that it is axial) and the C-6 hydroxyl group is axial since H-6 exhibits only a small coupling to H-5 which again is at high field. As in the mono-alcohol (228) the proton at the ether terminus, H-12, is coupled to a



(237)



(238)



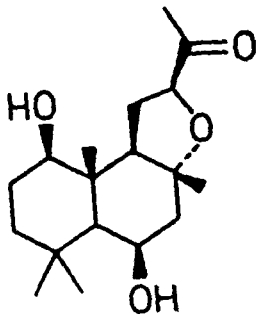
(239)

methylene neighbour and also shows allylic coupling to the vinyl proton. In order to confirm the structure an X-ray analysis was carried out.[234] This showed that the proposed structure (236) is correct. The explanation for the highfield shift of H-5 must lie in shielding by the axial C-H bonds. This effect may be enhanced by the fact that there are large axial 1,3-interactions involving the four axial groups on the top face of the molecule. A similar shielding effect of H-5 has been reported[154] in the drimane sesquiterpenoid dihydro-uvidin B (237).

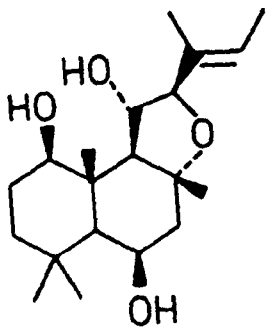
Treatment of diol (236) with acetic anhydride in pyridine gave the 1-monoacetate (238). The ^1H nmr spectrum of the product is similar to that of the starting alcohol except for the presence of an acetate methyl [δ_H 1.98 (s)] and a downfield shift of H-1 to δ_H 4.24.

Oxidation of the acetate (238) with Collins reagent yielded the keto-acetate (239). H-5 now appears as a singlet at δ_H 2.15 whilst the C-7 methylene is an AB quartet (J_{AB} 10.7 Hz). Double resonance experiments demonstrated a long range W-coupling between H-7α and the C-8 methyl which can only occur if the latter is axial.

Attempts to open the ether ring of the keto-acetate (239) by treatment with base were unsuccessful.



(240)



(241)

Sodium borohydride reduction of the keto-acetate (239) yielded the original acetate (238).

Oxidation of the acetate (238) with OsO_4 - $NaIO_4$ gave the bisnorlabdane (240), $C_{20}H_{32}O_5$ (m/z 309.2069, M^+-CH_3CO), [$\nu_{max}(CCl_4)$ 3610, 1740 and 1720 cm^{-1}] with the expected spectroscopic properties [δ_H 2.22 (s), δ_C 27.0 (q) and 211.4(s) (methyl ketone)].

The most polar compound was the triol, 8α,12(R)-epoxylabd-13(E)-ene-1β,6β,11α-triol (241), $C_{20}H_{34}O_4$ (m/z 338.2477), m.p. 178-180°C, $[\alpha]_D$-29.8° (c, 0.02 in MeOH), [ν_{max} (KBr) 3420 and 1640 cm^{-1}], which has a trisubstituted double bond [δ_H 5.64 (dqq, J 1.5, 1.5, 6.8 Hz, H-14], δ_C 117.7 (d, C-14) and 135.9 (s,C-13)], three secondary alcohols [δ_H 4.55 (brs, H-6), δ_C 67.0(d); δ_H 4.25 (dd, J 7.1, 10.3 Hz, H-11), δ_C 72.0 (d); δ_H 3.41 (dd, J 4.8, 11.4 Hz, H-1), δ_C 78.0 (d)], one secondary carbon bearing oxygen [δ_H 4.04 (br d, J 7.1 Hz, H-12), δ_C 86.5 (d)], one tertiary carbon bearing oxygen [δ_C 80.0 (s, C-8)], two vinyl methyls [δ_H 1.70 (d, J 1.0 Hz, 3H-16) and 1.62 (dd, J 1.0, 6.8 Hz, 3H-15), δ_C 12.2 (q) and 12.8 (q)], one tertiary methyl attached to an oxygen-bearing carbon [δ_H 1.44 (s, 3H-17), δ_C 26.7 (q)], three tertiary methyls [δ_H 1.22 (s, 3H-19), δ_C 22,9 (q); δ_H 1.18 (s, 3H-20), δ_C 12.8 (q); δ_H 0.99 (s, 3H-18), δ_C 32.1 (q)] and a highfield methine [δ_H 0.85 (d, J 3.4 Hz, H-5), δ_C 55.0 (d)] which together with two tetra-

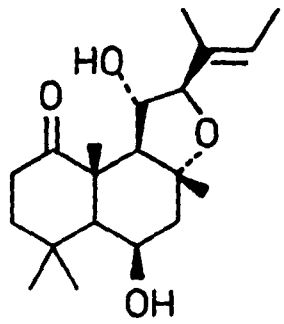
substituted carbon atoms, one methine and three methylene groups again constitute a tricyclic skeleton. The ^1H nmr spectrum is similar to that of the diol (236) apart from the appearance of a new signal due to a secondary hydroxyl which was readily assigned to C-11 by double resonance studies. Thus it is coupled to both H-12 and H-9 [δ_H 1.63 (d, J 10.3 Hz)]. The large couplings of H-11 to H-9 and H-12 indicate that it is β-oriented; the 11-hydroxyl group is therefore α-oriented as in scapanin (111). The triol, therefore, has structure (241).

The triol is the most abundant compound in the extract but was not amenable to chemical transformations. All attempts to protect the alcohol groups selectively were unsuccessful.

These three compounds, the mono-alcohol (228), the diol (236) and the triol (241), represent an interesting pattern of increasing hydroxylation of the labdane skeleton.

Extract B

This extract, from material collected at the same place and time as that which gave extract A but allowed to dry for a few months before extraction, yielded the same labdanes as extract A except for the mono-alcohol (228) which was completely absent. This demonstrates the disadvantage of not extracting the plant material as soon as possible; the enzymes may

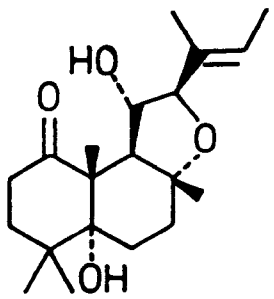


(242)

still be active and this can lead to the loss of metabolites.

Extract C

This extract was also derived from material collected at the same site as above but at a later date. It contained both the mono-alcohol (228) and the diol (236) but lacked the triol (241). However, a small amount of a new labdane was isolated. It proved to be 6β,11α-dihydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (242), $C_{20}H_{32}O_4$ (m/z 336.2301), m.p. 260-261°C, $[\alpha]_D$ + 29.3° (c, 0.28 in MeOH), [ν_{max} (KBr) 3610, 3420 and 1695 cm^{-1}] which has a hydrogen-bonded cyclohexane [δ_C 219.6 (s,C-1)], a trisubstituted double bond [δ_H 5.66 (dqq, J 1.4, 1.4, 6.7 Hz, H-14), δ_C 119.6 (d, C-14) and 134.9 (s, C-13)], two secondary alcohols [δ_H 4.58 (br q, J 3.2 Hz, H-6), δ_C 68.9 (d); δ_H 4.33 (ddd, J 1.4, 6.8, 10.0 Hz, H-11), δ_C 71.9 (d)], a secondary oxygen-bearing carbon [δ_H4.14 (br d, J 6.6 Hz, H-12), δ_C 88.6 (d)], a tertiary oxygen-bearing carbon [δ_C 80.9 (s, C-8)], two vinyl methyls [δ_H 1.72 (br s, 3H-16) and 1.63 (ddd, J 6.7, 1.1, 1.1 Hz, 3H-15), δ_C 12.2(q) and 13.0 (q)] and four tertiary methyls [δ_H 1.55 (s), 1.46 (d, J 0.6 Hz), 1.42 (s) and 1.10 (s), δ_C 17.5 (q, C-20), 23.4 (q, C-19), 27.1 (q, C-17) and 31.5 (q, C-18)], which together with two tetrasubstituted carbon atoms, two methine and three methylene groups again constitute a tricyclic labdane



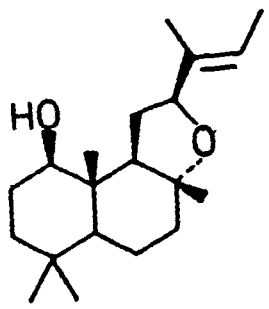
(243)

skeleton. These data led directly to structure (242). This compound is, of course, closely related to the triol (241). Direct interconversion was not attempted because of lack of material. It should be noted, however, that reduction of the 1-oxo group affords a 1α-hydroxyl group (cf. reduction of scapanin (111), p. 37).

Extract D

Plant material collected a few miles from the site of the previous samples afforded the fourth extract.

The first compound was isolated by crystallisation of the crude extract and proved to be a dihydroscapanin, 5α,11α-dihydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (243), $C_{20}H_{32}O_4$ (m/z 336.2308), m.p. 209-211°C, $[\alpha]_D$ + 44.6° (c, 0.86 in $CHCl_3$), [ν_{max} ($CHCl_3$) 3610, 3360 and 1696 cm^{-1}]. It has a hydrogen-bonded cyclohexanone [δ_C 218.5 (s, C-1)], a trisubstituted double bond [δ_H 5.67 (dqq, J 1.5, 1.5, 6.8 Hz, H-14), δ_C 119.9 (d, C-14) and 135.2 (s, C-13)], a secondary alcohol [δ_H 4.23 (m, H-11), δ_C 71.5 (d)], a tertiary alcohol [δ_C 80.3 (s, C-5)], a secondary oxygen-bearing carbon [δ_H 4.23 (m, H-12), δ_C 89.2 (d)], a tertiary oxygen-bearing carbon [δ_C 80.3 (s, C-8)], two vinyl methyls [δ_H 1.74 (br s, 3H-16) and 1.65 (br d, J 6.8 Hz, 3H-15), δ_C 12.3 (q) and 13.1 (q)] and four tertiary methyls [δ_H 1.32 (s), 1.23 (s), 1.19(s) and 1.08(s), δ_C 17.3(q, C-20), 25.2 (q, C-17), 26.7 (q,C-19) and 27.0 (q, C-18)]

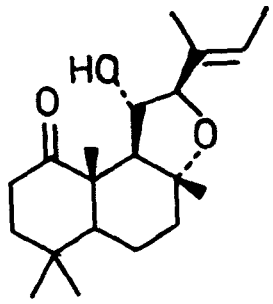


(244)

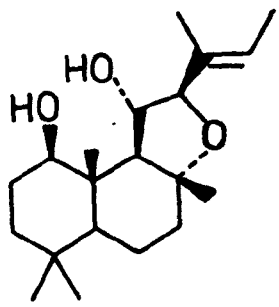
which together with two tetrasubstituted carbon atoms, one methine and four methylene groups again constitute a tricyclic labdane skeleton. These spectroscopic properties showed that this compound differed from scapanin (111) only in the nature of its side-chain which is the same as that of the triol (241). Thus the structure of the dihydroscapanin was established as (243).

Column chromatography of the extract yielded ent-longiborneol(12), ent-longipinanol (205) and four additional new diterpenoids, three of which had the readily recognisable tricyclic labdane skeleton.

The least polar compound was the mono-alcohol, 8α,12(S)-epoxylabd-13(E)-en-1β-ol (244), $C_{20}H_{34}O_2$ (m/z 306.2566), m.p. 132-133°C, $[\alpha]_D$ -48.6° (c, 0.85 in $CHCl_3$), [ν_{max} ($CHCl_3$) 3620 cm^{-1}], which has a tri-substituted double bond [δ_H 5.55 (dqq, J 1.5, 1.5, 7.0 Hz, H-14), δ_C 117.9 (d, C-14) and 136.8 (s, C-13)], a secondary alcohol [δ_4 3.33 (dd, J 6.0, 10.0 Hz, H-1), δ_C 79.7 (d)], a secondary carbon bearing oxygen [δ 4.28 (dd, J 6.4, 9.4 Hz, H-12, δ_C 82.8 (d)], a tertiary carbon bearing oxygen [δ_C 80.9 (s, C-8)], two vinyl methyls [δ_H 1.58 (s, 3H-16) and 1.57 (d, J 7.0 Hz, 3H-15), δ_C 12.7 (q) and 13.0 (q)] and four tertiary methyls [δ_H 1.11 (s), 0.84 (s), 0.83 (s) and 0.80 (s), δ_C 11.6 (q, C-20), 20.9 (q, C-19), 25.0 (q, C-17) and 33.0 (q, C-18)]. These features can readily be accommodated in structure (244).



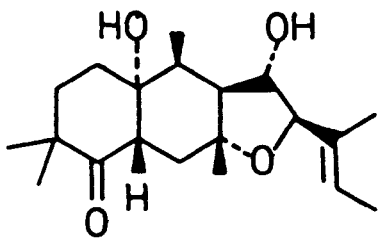
(245)



(246)

The next compound was the ketol, 11α-hydroxy-8α.,12(R)-epoxylabd-13(E)-en-1-one (245), $C_{20}H_{32}O_3$ (m/z 320.2350), m.p. 95-96°C, $[\alpha]_D$ + 9.8° (c, 1.37 in $CHCl_3$), [ν_{max} ($CHCl_3$) 3430 and 1700 cm^{-1}], which has a hydrogen-bonded cyclohexanone [δ_C 219.7 (s, C-1)], a trisubstituted double bond [δ_H 5.64 (dqq, J 1.5, 1.5, 6.7 Hz, H-14), δ_C 119.3 (d, C-14) and 135.3 (s,C-13)], a secondary alcohol [δ_H 5.85 (s, OH) and 4.26 (dd, J 6.8, 10.0 Hz, H-11), δ_C71.9 (d)], a secondary carbon bearing oxygen [δ_H 4.11 (br d, J 6.8 Hz, H-12), δ_C 88.6 (d)], a tertiary carbon bearing oxygen [δ_C 81.1 (s, C-8)], two vinyl methyls [δ_H 1.70 (S, 3H-16) and 1.62 (d, J 6.8 Hz, 3H-15), δ_C 12.4 (q) and 13.1 (q)] and four tertiary methyls [δ_H 1.20 (6H,s) and 1.02 (6H,s) δ_C 15.8 (q, C-20), 23.9 (q, C-19), 25.8 (q, C-17) and 31.0 (q, C-18)]. The ketol was unambiguously assigned structure (245) by comparison of the above data with those of the previously isolated Scapania diterpenoids.

From a later fraction was isolated the corresponding diol, 8α., 12(R)-epoxylabd-13(E)-ene-1β,11α-diol (246), $C_{20}H_{43}O_3$ (m/z 322.2503), m.p. 186-187°C, $[\alpha]_D$ -19.7° (c, 1.95 in $CHCl_3$), [ν_{max}($CHCl_3$) 3610 and 3400 cm^{-1}] which has a trisubstituted double bond [δ_H 5.63 (J 1.3, 1.3, 6.7 Hz, H-14), δ_C 120.7 (d, C-14) and 134.8 (S, C-13)], two secondary alcohols [δ_H4.16 (dd, J 7.1, 10.2 Hz, H-11) δ_C 71.8 (d); δ_H 3.45

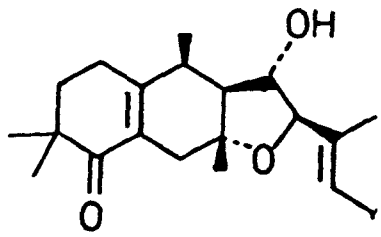


(248)

(dd, J 5.4, 10.4 Hz, H-1), δ_C 78.2 (d)], a secondary carbon bearing oxygen [δ_H 4.03 (br d, J 7.0 Hz, H-12), δ_C 88.3 (d)], two vinyl methyls [δ_H 1.69 (br s, 3H-16) and 1.63 (br d, J 6.7 Hz, 3H-15), δ_C 12.1 (q) and 13.1(q)] and four tertiary methyls [δ_H 1.19 (s), 0.90 (s), 0.86 (s) and 0.80 (s), δ_C 12.3 (q, C-20), 20.9 (q, C-19), 25.8 (q, C-17) and 32.9 (q, C-18)]. These data are readily reconciled with structure (246).

Strong support for the structural assignments of this family of Scapania diterpenoids comes from comparison of the ^{13}C data listed in Table 2.

The most polar compound isolated from the extract has markedly different spectroscopic properties, apart from the resonances due to the side chain, from the other 8,12-epoxylabdanes, suggesting a different carbon skeleton. This compound, scapanin G (248), $C_{20}H_{32}O_4$ (m/z 318. 2207 [M^+-H_2O]), m.p. 125-127°C (subl.), [ν_{max} 3600, 3400 and 1710 cm^{-1}] has a ketone [δ_C 215.4 (s)], a trisubstituted double bond [δ_H 5.56 (dqq, J 1.1, 1.1, 6.8 Hz, H-14), δ_C 120.0 (d, C-14) and 134.5 (s, C-13)], a secondary alcohol [δ_H 3.99 (m, H-11), δ_C 73.7 (d)], a tertiary alcohol [δ_C 79.0 (s)], a secondary carbon bearing oxygen [δ_H 3.91 (m, H-12), δ_C 88.2 (d)], a tertiary carbon bearing oxygen [δ_C80.0 (s, C-8)], three methine groups [δ_H 2.14 (m, H-10), δ_C 40.1 (d); δ_H 2.50 (dd, J 4.4, 10.7 Hz, H-9), δ_C 50.9 (d);



(247)

δ_H 2.63 (dd, J 4.7, 11.4 Hz, H-6), δ_C 47.4 (d)], two vinyl methyls [δ_H 1.64 (s, 3H-16), δ_C 13.0; $_H$ 1.59 (d, J 6.8 Hz, 3H-15), δ_C 11.9], a secondary methyl [δ_H 0.99 (d, J 4.5 Hz, 3H-20)] and three tertiary methyls [δ_H 1.15 (s), 1.09 (s) and 1.00 (s)] which together with three methylene groups and one tetra-substituted carbon atom constitute a tricyclic system.

It is apparent that scapanin G has the same side-chain as the other Scapania diterpenoids with a hydroxyl group attached to C-11; however the presence of a secondary methyl and three methines suggested a different carbon skeleton. It is known[155] that 5α,11α-dihydroxy-8α,12(R)-epoxy labd-13(E)-en-1-one (243) undergoes a retro-aldol initiated rearrangement to (247) when exposed to alkaline conditions. The spectroscopic properties of scapanin G accord well with structure (248) which is the hydrate of (247). Support for this proposal comes from the fact that irradiation of H-10 causes simultaneous collapse of the secondary methyl group and of H-9 (to a clean doublet coupled to H-11). The facile loss of water in the mass spectrometer is to be expected on the basis of this structure.

Definitive proof of the relative stereochemistry of the ring junction and the secondary methyl has not been obtained but the most probable arrangement is shown in structure (248). Models indicate that with a trans

ring junction, ring B can adopt a chair conformation only with the tertiary hydroxyl group α. The observed coupling of 4.4 Hz between H-9 and H-10 can be satisfactorily accommodated by having the secondary methyl group β (axial). In addition, H-9 still retains the 1,3-diaxial relationship with the tertiary hydroxyl which it has in the unrearranged material (243). This is reflected in the similarity of the chemical shifts of H-9 in the two compounds. It is possible that scapanin G (248) is an artefact formed from (243) on the Al_2O_3 column. Surprisingly, treatment of scapanin G with base failed to induce dehydration.

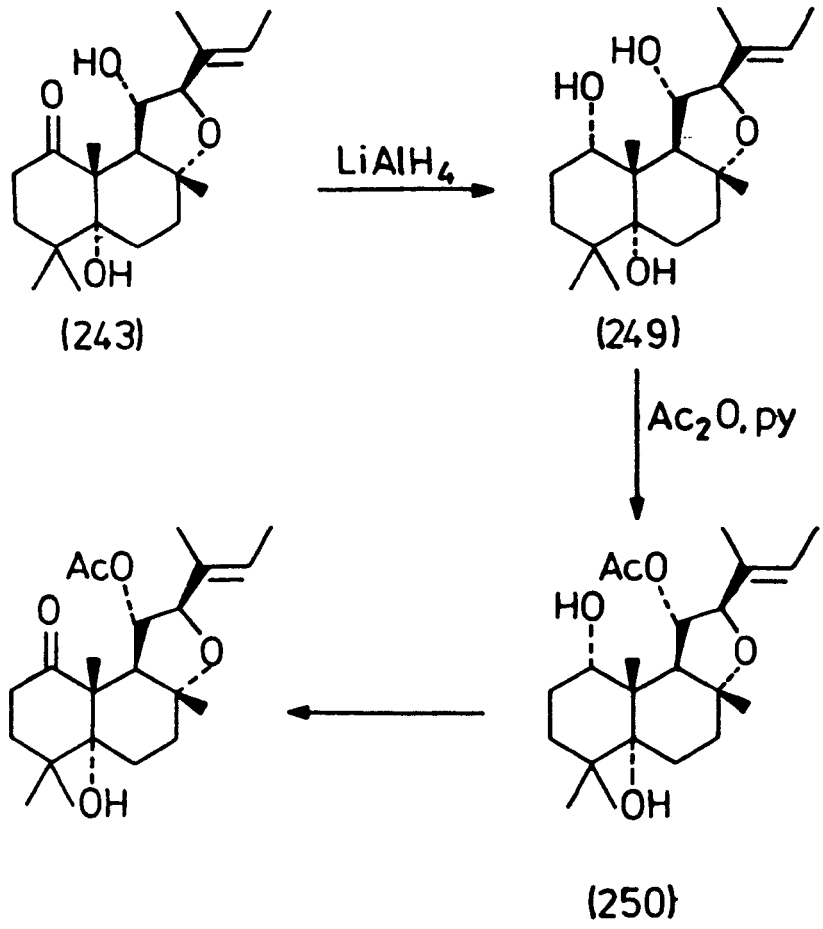
Absolute Configuration of the Scapania Diterpenoids

Ideally the absolute configurations of the previously described diterpenoids of S. undulata should be determined by interrelation within the series and by correlation of one member of the series with a compound of known absolute configuration. This, however, proved to be difficult, both chemically and because of lack of material.

Another method of determining absolute configuration is by circular dichroism (cd), a technique used to measure Cotton effects. These are exhibited by molecules which have either an intrinsically dissymmetric chromophore (e.g. twisted biphenyls) or a dissymmetrically perturbed symmetric chromophore (e.g. chiral ketones). The sign and the amplitude of the Cotton effect are related to the dissymmetry of the molecule. Configuration or conformation can be deduced from Cotton effect curves either by a theoretical treatment or by empirically deduced rules.

The ketone chromophore has been studied to the greatest extent and the results have been interpreted in terms of the octant rule[156,157].

It was decided to determine the absolute configuration of the Scapania diterpenoids by recourse to cd studies of suitable ketones. The natural products which possess a 1-oxo group were obvious candidates for this work; difficulties, however, arose due to the hydrogen-bonding which exists in all of the natural 1-ketones between the carbonyl group and the 11α-hydroxyl group.



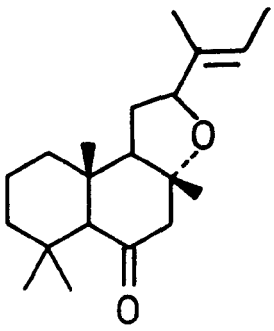
(243) (249) (250)

Scheme 16

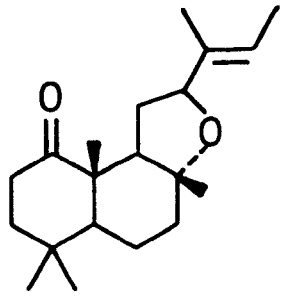
This complicates the cd curve of molecules such as scapanin (111) and makes interpretation impossible. It seemed likely that the cd curve of the 11-acetate of one of the natural 1-ketones should be more amenable to interpretation since the hydrogen bonding would no longer be present. However the 11-hydroxyl group is resistant to acetylation, even in the presence of N,N-dimethyl-aminopyridine, because of the hydrogen-bonding and it was necessary to attempt the less direct route of reduction - acetylation - oxidation (Scheme 16). The work was carried out upon the hydroxy ketone (243) as this was the most readily available natural ketone.

$LiAlH_4$ reduction of (243) gave the expected product 8α, 12(R)-epoxylabd-13(E)-ene-1α,5α,11α-triol (249), m.p. 213-215°C, $C_{20}H_{34}O_4$ (m/z 338), [ν_{max} ($CHCl_3$) 3520 cm^{-1}]. The stereochemistry at C-1 is readily assigned from the ^1H nmr spectrum. H-1 appears as a broad singlet [δ_H 3.78] and hence is equatorial (β). It is interesting to note that H-9 [δ_H 3.25 (d, J 11.0 Hz] is more deshielded than in the other Scapania diterpenoids as a result of the influence of the axial 1-hydroxy group.

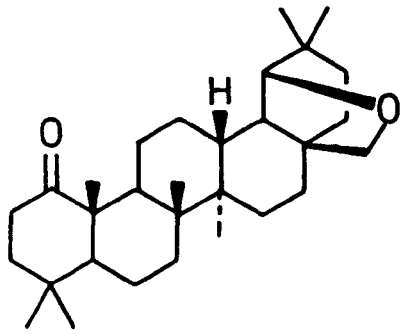
Acetylation of the triol (249) with Ac_2O-pyridine proceeded smoothly at steam bath temperature to give the monoacetate (250) [ν_{max} 3570, 3470 and 1745 cm^{-1}]. The ^1H nmr spectrum of the product is similar to that of the starting alcohol (249) except for the presence of an



(229)



(251)



(252)

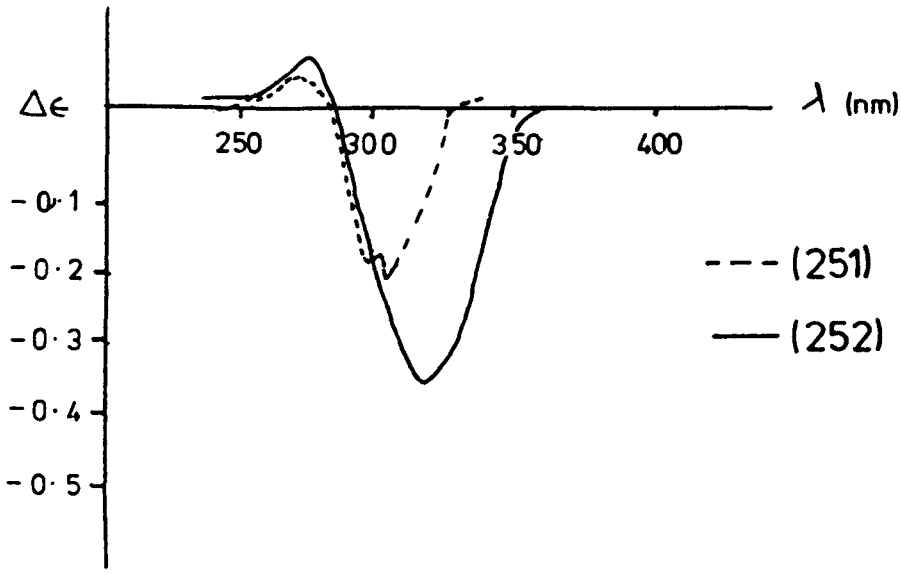


Fig. 6

acetate methyl [δ_H 2.04 (s)] and a downfield shift of H-11 to δ_H 5.61.

Oxidation of the monoacetate (250) proved to be impossible with Jones reagent or Collins reagent. This is presumably due to the strong intramolecular hydrogen- bonding of the 1-hydroxyl group with both the 5-hydroxyl and the 11-acetate.

This approach to a suitable derivative for cd studies was abandoned in favour of the simple 6-ketone (229) and the 1-ketone (251). An examination of models suggested that the former would have a complex cd spectrum and this indeed proved to be the case [$\Delta\varepsilon_{264}$ + 1.07; $\Delta\varepsilon_{290}$ + 0.08; $\Delta\varepsilon_{315}$ -0.03; $\Delta\varepsilon_{325}$ - 0.06]. However the 1-ketone (251), obtained by Jones oxidation of the mono-alcohol (244), gave a simple cd curve which is superimposable upon that of 1-oxo-allobetulin (252) (Fig. 6). Since all known triterpenoids belong to the normal series of absolute configuration it follows that the 1-ketone (251) also belongs to the normal series.

Although no successful interconversions have been carried out within this series of labdane diterpenoids it is extremely unlikely that their absolute configurations will be different from that of the 1-ketone (251).

Summary

The isolation of this series of oxygenated labdanes from S. undulata is interesting for a number of reasons.

Although it is generally accepted that the chemical markers for Scapania species are the ent-longipinane-longifolane sesquiterpenoids (see p. 36), it is apparent that the above labdane diterpenoids constitute a much more characteristic group of chemical markers for S. undulata itself.

These diterpenoids dramatically increase the number of known labdanes from the Hepaticae (see p. 26). The normal absolute configurations of these diterpenoids, when considered alongside the enantio absolute configurations of the other labdanes isolated, indicate that the Hepaticae are similar to higher plants in producing both enantiomeric series of diterpenoids.

S. undulata has been shown to exist in a number of chemical races. There appears to be at least three different enzyme systems present in these races, viz.

i) an enzyme system which initially oxygenates at C-6, as in (228), (236), (241) and (242).

ii) a system which initially oxygenates at C-1, as in (244), (245) and (246).

iii) an enzyme system which causes C-5 oxygenation, as in scapanin (111) and (243).

The reasons for the production of this group of

diterpenoids by S. undulata are unknown. However, since polyoxygenation increases the hydrophilic character of these diterpenoids and since the liverwort is found mainly in mountain streams, they may have some kind of "excretion" role as waste metabolites.

Scapania gracilis

S. gracilis is very common in Scotland but grows in drier locations than S. undulata. Whereas the latter produced a copious amount of bitter-tasting extract, S.gracilis prdduced a much smaller amount of extract (as a percentage of the dry weight of plant material). Indeed, the terpenoid content is very low and only a small amount of (+)-ent-epicubenol (209), identical with a sample obtained from S. undulata, was isolated.

Diplophyllum albicans

D. albicans, collected in the West of Scotland, yielded two compounds which were identified as ent-diplophyllin (79) and ent-diplophyllolide (78) by comparison of their physical properties with literature values. Thus Scottish D. albicans is chemically similar to the French sample studied by Asakawa et al. (see p.40).

EXPERIMENTAL

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 a Perkin Elmer 580 instrument. Ultra-violet (uv) spectra were measured for ethanolic solutions using a Pye Unicam SP 800 spectrophotometer. Mass spectra (ms) were recorded using an MS 12 instrument (low resolution) and an MS 902 S instrument (high resolution). Optical rotations were measured on an Optical Activity AA-100 polarimeter in $CHCl_3$ solution (unless otherwise specified).

Unless otherwise stated, nuclear magnetic resonance (nmr) spectra were recorded for $CDCl_3$ solutions using a Perkin Elmer R.32 instrument (^1H, 90 MHz) or a Varian XL 100 instrument (^{13}C, 25.16 MHz). Chemical shifts were measured using the δ scale with tetramethylsilane as internal standard. Highfield nmr spectra were recorded using either a Bruker WH 360 instrument (^1H, 360 MHz) or a Bruker WP 200 SY instrument (^1H, 200 MHz; ^{13}C 50.32 MHz). Chemical shifts were again measured relative to TMS but the internal standard was $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_C 77.0. All nmr spectra were interpreted using first-order analysis except where stated otherwise. ^{13}C nmr assignments are based on chemical shift rules, multiplicities in SFORD or DEPT spectra, correlation with ^1H chemical shifts and comparison with published data for similar compounds.

All plant material was air-dried, ground and extracted with Et_2O. Extracts were normally chromatographed over a neutral Al_2O_3 (grade III) column and the crude fractions further purified by preparative thin-layer chromatography (tlc) over Merck Kieselgel GF_{254}. Compounds were visualised using uv light or by adsorption of I_2 vapour. Analytical tlc plates were visualised using uv light and by spraying with ceric sulphate - H_2SO_4. Eluants for column chromatography were increasing percentages of Et_2O in petroleum ether followed by EtOAc in Et_2O and finally MeOH. Tlc plates were developed using appropriate concentrations of EtOAc in petroleum ether or MeOH in $CHCl_3$. Solvents were of analytical grade except for column chromatography when bulk solvents were used.

All solvents were removed using a Buchi rotary evaporator and water aspirator. Petroleum ether refers to the fraction boiling between 60°C and 80° C. All reagents and solvents used in chemical reactions were purified according to literature methods. Organic solutions were dried over $MgSO_4$.

"Acidic work-up" means acidification of reaction mixture with dilute HCl, extraction with Et_2O, drying over $MgSO_4$ and removal of solvent.

Scapania undulata

Extract A

The plant material (2.5 kg) collected near Aberfoyle in 1980 yielded 45 g of crude extract.

(-)-ent-Longiborneol (12) and (-)-ent-Longipinanol (205).

Steam distillation of an earlier fraction gave an essential oil from which a mixture of ent-longiborneol (12) and ent-longipinanol (205) could be crystallised (ex cold pentane). Repeated preparative tlc of this mixture gave

a) ent-longiborneol (12), m.p. 106-108°C (ex CH_3CN), [lit.[60] 106-107° C].

δ_H : 3.76 (d, J 5 Hz, C<u>H</u>-OH); 0.94 (6H, s), 0.87 (s) and 0.84 (s), (four tertiary methyls),

and

b) ent-longipinanol (205), m.p. 82-83°C (ex aqueous MeOH), [lit.[60] 82.5-83.0°C].

δ_H : 1.95 - 1.80 (6H, m); 1.35 (s, C<u>H</u>$_3$COH); 0.91 (6H, s) and 0.90 (s), (three tertiary methyls).

(+)-ent-Epicubenol (209)

Steam distillation of a middle fraction gave (+)-ent-epicubenol (209) as an oil.

δ_H : 5.37 (br d, H-4); 1.67 (s, 3H-14); 0.94, 0.86 and 0.78 (3d, J 7 Hz, secondary methyls).

Labda-12(E),14-diene-8α,11ξ-diol (223)

Recrystallisation from petroleum ether - $CHCl_3$ gave labda-12(E),14-diene- 8α,11ξ-diol (223), (110 mg), m.p. 114-116°C, $[\alpha]_D$ + 24.4° (c, 1.50 in $CHCl_3$), m/z 288.2456 (M^+-H_2O, $C_{20}H_{32}O$ requires m/z 288.2453).

ν_{max} : 3620 and 3520 cm^{-1}

λ_{max} : 234 nm (ε 7800)

δ_H : 6.40 (dd, J 11, 18 Hz, H-14); 6.03 (br d, J 10 Hz, $w_{\frac{1}{2}}$ 3 Hz, H-12); 5.19 (d, J 18 Hz, H-15); 5.04 (d, J 11 Hz, H-15^1); 5.09 (dd, J 10, 3 Hz, H-11); 2.80 (br s, OH); 1.79 (d, J 3 Hz, 3H-16); 1.44 (s, 3H-17); 0.90, 0.84 and 0.76 (3s, tertiary methyls).

$\delta_H(C_6D_6)$: 6.46 (dd, J 11, 18 Hz, H-14); 6.14 (br d, J 10 Hz, $w_{\frac{1}{2}}$ 3 Hz, H-12); 5.16 (d, J 10, 3 Hz, H-11); 5.00 (d, J 11 Hz, H-15^1); 3.00 (br s, OH); 1.81 (d, J 3 Hz, 3H-16); 0.99, 0.80 and 0.74 (3s, tertiary methyls).

δ_C : see Table 2 (p.44).

8α,12(s)-Epoxylabd-13(E)-en-6β-ol (228)

Recrystallisation from petroleum ether - $CHCl_3$ gave 8α,12(s)-epoxylabd-13(E)-en-6β-ol (228), (100 mg), m.p. 170-172°C, $[\alpha]_D$ -51.1° (c, 2.16 in $CHCl_3$), m/z 306.2551 ($C_{20}H_{34}O_2$ requires m/z 306.2559).

ν_{max} : 3620 cm^{-1}

δ_H (360 MHz): 5.57 (dqq, J 1.5, 1.5, 5.6 Hz, H-14); 4.56 (br s, H-6); 4.27 (br dd, J 6.3, 9.1 Hz, H-12); 2.12 (dd, J 2.6, 13.5 Hz,

H-7β); 1.88 (ddd, J 4.8, 6.0, 10.8 Hz, H-11α); 1.68 (ddd, J 9.0, 11.0, 13.0 Hz, H-11β); 1.59 (d, J 5.8 Hz, 3H-15); 1.59 (dd, J 4.7, 13.0 Hz, H-9); 1.36 (d, J 0.9 Hz, 3H-17); 1.19, 1.15 and 0.99 (3s, tertiary methyls); 0.94 (d, J 2.8 Hz, H-5).

δ_C : see Table 2 (p.44).

8α ,12(s)-Epoxylabd-13(E)-ene-1β ,6β -diol (236)

Recrystallisation from petroleum ether - $CHCl_3$ gave 8α ,12(s)-epoxylabd-13(E)-ene-1β ,6β -diol (236), (110 mg), m.p. 174-176° C, $[\alpha]_D$ -57.6 (c, 0.99 in $CHCl_3$), m/z 322.2534 ($C_{20}H_{34}O_3$ requires m/z 322.2508).

ν_{max}(KBr) : 3450 and 3330 cm^{-1}.

δ_H(360 MHz) : 5.57 (dqq, J 1.8, 1.8, 6.7 Hz, H-14); 4.54 (br d, J 2.4 Hz, H-6); 4.27 (dd, J 6.4, 9.7 Hz, H-12); 3.28 (dd, J 4.7, 11.2 Hz, H-1); 2.21 (ddd, J 5.2, 6.2, 11.6 Hz, H-11α); 2.12 (dd, J 2.6, 13.3 Hz, H-7β); 1.88 (ddd, J 9.9, 11.6, 13.1 Hz, H-11β); 1.71 (br dd, J 4.1, 13.3 Hz, H-7α); 1.60 (s, 3H-16); 1.59 (d, J 6.9 Hz, 3H-15); 1.36 (s, 3H-17); 1.18, 1.16 and 0.98 (3s, tertiary methyls); 0.85 (d, J 2.8 Hz, H-5).

δ_C : see Table 2 (p.44).

8α,12(R)-Epoxylabd-13(E)-ene-1β,6β,11α-triol (241)

Recrystallisation from MeOH gave 8α,12(R)-epoxylabd-13(E)-ene-1β,6β,11α-triol (241), (130 mg), m.p. 178-180°C, $[\alpha]_D$ -29.8° (c, 0.02 in MeOH), m/z 338.2477 ($C_{20}H_{34}O_4$ requires m/z 338.2457).

ν_{max}(KBr) : 3420 and 1640 cm^{-1}.

δ_H(360 MHz): 5.64 (dqq, J 1.5, 1.5, 6.8 Hz, H-14); 4.55 (br s, H-6); 4.25 (dd, J 7.1, 10.3 Hz, H-11); 4.04 (br d, J 7.1 Hz, H-12), 3.41 (dd, J 4.8, 11.4 Hz, H-1); 2.05 (dd, J 2.4, 13.4 Hz, H-7β); 1.80 (br dd, J 4.2, 13.4 Hz, H-7α); 1.70 (d, J 1.0 Hz, 3H-16); 1.63 (d, J 10.3 Hz, H-9); 1.62 (dd, J 1.0, 6.8 Hz, 3H-15); 1.44 (s, 3H-17); 1.22 (s, 3H-19); 1.18 (s, 3H-20); 0.99 (s, 3H-18); 0.85 (d, J 3.4 Hz, H-5).

δ_C(d_6-dmso): see Table 2 (p.45).

Hydrogenation of Labda-12(E),14-diene-8α,11ξ-diol (223)

A solution of diene (223) (41 mg) in EtOAc (25 ml) was hydrogenated in the presence of 10% Pd-C catalyst (5 mg) for 30 min. The catalyst was filtered off and the solvent removed to give an oily product. Preparative tlc gave two products:

a) the less polar product was labd-13-ene-8α,11ξ-diol (224), (13 mg), m.p. 75-78°C, m/z 290.2610 (M^+-H_2O, $C_{20}H_{34}O$ requires m/z 290.26095).

ν_{max} : 3610 cm^{-1}.

δ_H : 5.20 (m, H-14); 4.28 (br d, J 9 Hz, H-11); 1.59 (s, 3H-15 and 3H-16); 1.34 (s, 3H-17); 1.06, 0.80 and 0.75 (3s, tertiarY methYls).

b) the more polar product was labd-12-ene-8α,11ξ-diol (225), (18 mg), a colourless oil, m/z 290.2610 (M^+-H_2O, $C_{20}H_{34}O$ requires m/z 290.26095).

ν_{max} : 3610 cm^{-1}.

δ_H : 5.74 (br d, J 11 Hz, H-12); 4.88 (dd, J 4, 11 Hz, H-11); 2.74 (br s, OH); 1.98 (q, J 7 Hz, 2H-14); 1.67 (d, J 2 Hz, 3H-16); 1.45 (s, 3H-17); 0.99 (t, J 7 Hz, 3H-15); 0.91, 0.84 and 0.77 (3s, tertiarY methYls).

Periodate-OsO_4 Oxidation of Labda-12(E),14-diene-8α,11ξ-diol (223).

a) To a stirred solution of the diene (223), (40 mg), in aqueous dioxan (10 ml) was added OsO_4 (5 mg); after 10 min, $NaIO_4$ (100 mg) was added, and the mixture stirred for 12 h. Work-up gave an oily residue which yielded, upon preparative tlc, 8α-hydroxydriman-11-al (226), (20 mg), $[\alpha]_D$ + 26° (c, 0.4 in $CHCl_3$) [lit.[150] + 66°].

ν_{max} : 3400 and 1710 cm^{-1}.

δ_H : 10.02 (d, J 2Hz, H-11); 1.40, 1.14, 0.91 and 0.85 (4s, tertiarY methYls).

b) The diene (223) , (60 mg), was treated as above. Work-up gave 14,15-bisnor-8α,11ξ,12ξ-trihydroxylabdan-13-one (227), (11 mg), as a gum, m/z 312 ($C_{18}H_{32}O_4$ requires m/z 312).

ν_{max} : 3600 and 1710 cm^{-1}.

δ_H : 4.54 (dd, J 6, 11 Hz, H-11); 4.03 (d, J 6 Hz, H-12); 2.26 (s, 3H-16); 1.06, 0.89, 0.85 and 0.80 (4s, tertiary methyls).

δ_C : 16.3, 18.2, 20.8, 21.1, 23.9, 26.9, 33.1, 33.7, 37.2, 39.8, 40.4, 42.3, 56.7, 65.1, 73.1, 83.2, 89.2 and 212.1.

Oxidation of 8α, 12(S)-Epoxylabd-13(E)-en-6β-ol (228)

CrO_3 (60 mg), dry pyridine (100 mg) and dry CH_2Cl_2 (5 ml) were stirred together for 15 min. A solution of the alcohol (228), (30 mg), in dry CH_2Cl_2 (3 ml) was added and the mixture stirred for a further 15 min, after which the solution was diluted with Et_2O and filtered through celite. Removal of solvent and preparative tlc gave 8α,12(S)-epoxylabd-13(E)-en-6-one (229), (20 mg), m/z 304.2415 ($C_{20}H_{32}O_2$ requires m/z 304.2402).

ν_{max} : 1715 cm^{-1}.

δ_H(200 MHz): 5.57 (dqq, J 1.5, 1.5, 7.0 Hz, H-14); 4.43 (dd, J 6.4, 9.5 Hz, H-12); 2.68 (d quintets, J 10.8, 0.9 Hz, H-7α); 2.59 (d, J 10.9 Hz, H-7β); 2.27 (dd, J 5.1, 13.4 Hz, H-9); 2.12 (br s, H-5); 1.96 (ddd, J 5.9, 5.9, 11.4 Hz, H-11β);

1.60 (br s, 3H-16); 1.58 (d, J 7.0 Hz, 3H-15); 1.07 (d, J 0.8 Hz, 3H-17); 1.17, 0.94 and 0.86 (3s, tertiary methyls).

Dehydration of 8α,12(S)-Epoxylabd-13(E)-en-6β-ol (228)

To a stirred solution of the alcohol (228), (20 mg), in dry CH_2Cl_2 (2 ml) containing Et_3N (5 drops) at -10°C was added mesyl chloride (2 drops). After 5 min the reaction mixture was diluted with CH_2Cl_2 (15 ml), washed with ice-water (20 ml), cold 10% HCl (20 ml), saturated $NaHCO_3$ solution (20 ml) and brine (20 ml), and dried over $MgSO_4$. The solvent was removed and the residue purified by preparative tlc to yield 8α,12(S)-epox labda-5,13(E)-diene (233), (17 mg), as a gum.

δ_H : 5.58 (br q, J 7 Hz, H-14); 5.46 (t, J 4 Hz, H-6); 4.30 (br dd, J 6, 9, Hz, H-12); 2.30 (d, J 4 Hz, 2H-7); 1.62 (br s, 3H-15 and 3H-16); 1.10 (9H, s) and 1.06 (s, tertiary methyls).

Lemieux-Johnson Oxidation of the Mono-alcohol (228)

To the alcohol (228), (10 mg) in aqueous dioxan (5 ml) was added OsO_4 (3 mg). After 15 min, $NaIO_4$ (50 mg) was added and the mixture stirred for 12 h. The usual work-up gave 14,15-bisnor-6β-hydroxylabdan-13-one (234), (8 mg), as a gum, m/z 278 ($C_{18}H_{30}O_2$ requires m/z 278).

δ_H : 4.62 (m, H-6); 4.32 (t, J 8 Hz, H-12); 2.25 (s, 3H-16); 1.31, 1.19, 1.15 and 0.99 (4s, tertiary methyls).

Attempted Dehydration of 14,15-Bisnor-6β-hydroxy labdan-13-one (234)

To a stirred solution of the alcohol (234), (8 mg), in CH_2Cl_2 (1 ml) containing Et_3N (5 drops) at -10°C was added mesyl chloride (1 drop). After 5 min the reaction was worked up as usual to give a complex mixture of products.

Thioketalisation of 8α,12(S)-Epoxylabd-13(E)-en-6-one (229)

To the ketone (229), (20 mg), dissolved in Et_2O (5 ml) was added ethanedithiol (5 drops) and $BF_3.Et_2O$ (5 drops). After 12 h water was added and the mixture extracted into $CHCl_3$. The organic layer was dried and the solvent removed. Preparative tlc gave the major product as a gum.

ν_{max} : 1675 cm^{-1}.

λ_{max} : 238 nm.

δ_H : 5.76 (1 H, br s); 5.32 (1 H, br m); 3.42 (q, J 8 Hz, C<u>H</u>-CH_3); 2.56 (4 H, m, thioketal); 2.25 (1 H, s) 2.23 (1 H, d, J 5 Hz); 2.05 (1H, s); 1.84 and 1.65 (2 br s, vinyl methyls); 1.26 (d, J 8 Hz, secondary methyl); 1.14, 1.08 and 0.84 (3s, tertiary methyl).

The Mono-Acetate (238)

To the diol (236), (25 mg) in dry pyridine (2 ml) was added Ac_2O (1 ml) and the mixture left overnight. MeOH was added and the mixture evaporated. Preparative tlc

gave 1β-acetoxy -8α,12(S)-epoxylabd-13(E)-en-6β-ol (238) as a gum, m/z 364.2612 ($C_{22}H_{36}O_4$ requires m/z 364.2613).

ν_{max} : 3610 and 1735 cm^{-1}.

δ_H : 5.45 (br q, J 7 Hz, H-14); 4.56 (br s, H-6); 4.51 (br dd, J 8, 14 Hz, H-12); 4.24 (br t, J 7 Hz, H-1); 1.98 (s, CH_3CO); 1.58 (d, J 7 Hz, 3H-15); 1.55 (br s, 3H-16); 1.33, 1.23, 1.18 and 0.96 (4s, tertiary methyls).

δ_C : see Table 2 (p.44).

1β-Acetoxy -8α,12(S)-epoxylabd-13-en-6-one (239)

To Collins reagent (132 mg) in dry CH_2Cl_2 (5 ml) was added the hydroxyacetate (238), (25 mg), and the mixture stirred for 10 h. Dilution with Et_2O, filtration through celite and removal of solvent gave, after preparative tlc, the ketone (239), (20 mg), as a gum, m/z 362.2456 ($C_{22}H_{34}O_4$ requires m/z 362.2457).

ν_{max} : 1730 and 1720 cm^{-1}.

δ_H (360 MHz): 5.55 (dqq, J 1.9, 1.9, 5.7 Hz, H-14); 4.70 (dd, J 4.9, 11.0 Hz , H-1); 4.39 (br t, J 7.9 Hz, H-12); 2.66 and 2.63 (ABq, J_{AB} 10.7 Hz, 2H-7); 2.30 (dd, J 6.2, 12.3 Hz, H-9); 2.15 (s, H-5); 2.03 (s, CH_3CO); 1.59 (d, J 6.0 Hz, 3H-15); 1.59 (s, 3H-16); 1.19, 1.07, 0.96 and 0.95 (4s, tertiary methyls).

δ_C : see Table 2 (p.45).

Base Treatment of 1β -Acetoxy-8α,12(S)-epoxylabd-13(E)-en-6-one (239)

The ketone (239), (5 mg), was stirred for 2 h in 1% ethanolic KOH (2 ml). Acidification and extraction into $CHCl_3$ gave the crude product which was treated with Ac_2O-pyridine. Work-up gave a complex mixture.

$NaBH_4$ Reduction of the Ketone (239)

The ketone (239) (20 mg) and $NaBH_4$ (10 mg) in EtOH (2 ml) were stirred for 3 h. Addition of water and extraction into $CHCl_3$ gave 1β -acetoxy -8α,12(S)-epoxylabd-13(E)-en-6β-ol (238), (17 mg), identical with an authentic sample.

1β -Acetoxy -6β -hydroxy -14,15-bisnor-8α,12(S)-epoxy-labdan-13-one (240)

The hydroxyacetate (240), (30 mg), was dissolved in aqueous dioxan (5 ml) and OsO_4 (7 mg) added. After 20 min. $NaIO_4$ (100 mg) was added and the mixture stirred overnight. The usual work-up followed by preparative tlc gave the bisnor labdane (240), (24 mg), as a gum, m/z 309.2069 (M^+-CH_3CO, $C_{18}H_{29}O_4$ requires m/z 309.2066).

ν_{max} : 3610, 1740 and 1720 cm^{-1}.

δ_H : 4.57 (br s, H-6); 4.47 (dd, J 7, 8 Hz); 4.23 (t, J 8 Hz); 2.22 (s, 3H-16); 2.00 (s, CH_3CO); 1.26, 1.23, 1.18 and 0.97 (4s, tertiary methyls); 0.88 (d, J 3 Hz, H-5).

δ_C : see Table 2 (p.45).

Extract B

The plant material (2.5 kg) collected near Aberfoyle in 1980 was air-dried for three months. Chromatograph of the crude extract (40 g) yielded (-)-ent-longiborneol (12), (-)-ent-longipinanol (205), (+)-ent-epicubenol (209), labda-12(E),14-diene-8α,11ξ-diol (223), 8α,12(S)-epoxylabd-13(E)-ene-1β,6β-diol (236) and 8α,12(R)-epoxylabd-13(E)-ene-1β,6β,11α-triol (241).

Extract C

The plant material (1 kg) was collected near Aberfoyle in September 1982. It yielded a crude extract (25 g). The following compounds were isolated by chromatography.

8α,12(S)-Epox labd-13(E)-en-6β-ol (228)

Crystallisation from petroleum ether - $CHCl_3$ gave the mono-alcohol (228), (50 mg), identical with an authentic sample.

8α,12(S)-Epox labd-13(E)-ene-1β,6β-diol (236)

Crystallisation from petroleum ether - $CHCl_3$ gave the diol (236), (65 mg), identical with an authentic sample.

6β,11α-Dihydroxy-8α,12(R)-Epoxylabd-13(E)-en-1-one (242)

Crystallisation from MeOH gave 6β,11α-dihydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (242), (10 mg), m.p. 260-261°C, $[\alpha]_D$ + 29.3° (c, 0.28 in MeOH), m/z 336.2301 ($C_{20}H_{32}O_4$ requires m/z 336.2300).

ν_{max}(KBr) : 3610, 3420 and 1695 cm^{-1}.

δ_H (200 MHz) : 6.15 (d, J 1.3 Hz, OH); 5.66 (dqq, J 1.4, 1.4, 6.7 Hz, H-14); 4.58 (br q, J 3.2 Hz, H-6); 4.33 (ddd, J 1.4, 6.8, 10.0 Hz, H-11); 4.13 (br d, J 6.6 Hz, H-12); 3.03 (ddd, J 6.5, 12.9, 14.6 Hz,

H-2β); 2.30 (ddd, J 4.4, 4.4, 14.7 Hz, H-2α); 1.72 (br s, 3H-16); 1.63 (ddq, J 6.7, 1.1, 1.1 Hz, 3H-15); 1.46 (d, J 0.6 Hz, 3H-17); 1.55, 1.42 and 1.10 (3s, tertiary methyls); 1.36 (d, J 2.8 Hz, H-5).

δ_C : see Table 2 (p.45) .

Extract D

A crude extract (50 g) was obtained from plant material which was collected near Aberfoyle in August 1981. The extract crystallised from Et_2O to give

5α,11α-Dihydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (243)

The ketone (243), (150 mg), was recrystallised from petroleum ether - $CHCl_3$ as prisms, m.p. 209 - 211°C, $[\alpha]_D$ +44.6° (c, 0.86 in $CHCl_3$), m/z 336.2308 ($C_{20}H_{32}O_4$ requires m/z 336.2300).

ν_{max} ($CHCl_3$) : 3610, 3360 and 1696 cm^{-1}.

δ_H : 5.67 (dqq, J 1.5, 1.5, 6.8 Hz, H-14); 4.23 (m, H-11 and H-12); 2.85 - 2.55 (m, 2H-2); 1.74 (br s, 3H-16); 1.65 (br d, J 6.8 Hz, 3H-15); 1.32, 1.23, 1.19 and 1.08 (4s, tertiary methyls).

δ_C : see Table 2 (p.46)

Chromatography of the remainder of the extract gave the following compounds.

8α,12(S)-Epoxylabd-13(E)-en-1β-ol (244)

Re crystallisation from petroleum ether - Et_2O gave the mono-alcohol (244), m.p. 132-133°C, $[\alpha]_D$ - 48.6° (c, 0.85 in $CHCl_3$), m/z 306.2566 ($C_{20}H_{34}O_2$ requires m/z 306.2559).

ν_{max} ($CHCl_3$): 3620 cm^{-1}

δ_H (200 MHz): 5.5 (dqq, J 1.5, 1.5, 7.0 Hz, H-14); 4.27 (dd, J 6.2, 9.2 Hz, H-12); 3.33 (dd, J 5.8 9.8 Hz, H-1); 2.14 (ddd, J 4.7, 4.7, 11.3 Hz, H-11); 1.58 (S, 3H-16); 1.57 (d, J 7.0 Hz, 3H-15); 1.11, 0.84, 0.83 and 0.80 (4s, tertiary methyls).

δ_C : see Table 2 (p 46).

11α-Hydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (245)

The ketol was recrystallised from petroleum ether - Et_2O as needles, m.p. 95-96°C, $[\alpha]_D$ + 9.8° (C, 1.37 in $CHCl_3$), m/z 320.2350 ($C_{20}H_{52}O_3$ requires m/z 320.2351).

ν_{max} ($CHCl_3$): 3430 and 1700 cm^{-1}

δ_H (200 MHz): 5.85 (d, J 0.9 Hz, 0.4); 5.64 (dqq, J 1.4, 1.4, 6.7 Hz, H-14); 4.25 (dd, J 6.8, 10.0 Hz, H-11); 4.10 (br, d, J 6.8 Hz, H-12)

ν_{max} ($CHCl_3$) : 3600, 3400 and 1710 cm^{-1}

δ_H (200 MHz): 5.56 (dqq, J 1.1, 1.1, 6.8 Hz, H-14); 3.99 (m, H-11); 3.91 (m, H-12); 3.61 (br d, J 12 Hz, OH); 2.71 (s, OH); 2.63 (dd, J 4.7; 11.4 Hz, H-6); 2.50 (dd, J 4.4, 10.7 Hz, H-6); 2.50 (dd, J 4.4, 10.7 Hz, H-9); 2.14 (m, H-10); 1.64 (s, 3H-16); 1.59 (d, J 6.8 Hz, 3H-15); 1.15, 1.09 and 1.00 (3s, tertiary methyls); 0.99 (d, J 4.5 Hz, 3H-20).

δ_C : 11.9 (q), 11.9 (q), 13.0 (q), 24.7 (q), 25.3 (q), 25.3 (q), 31.6 (t), 35.7 (t), 35.8 (t), 40.1 (d), 44.3 (s), 47.4 (d), 50.9 (d), 73.7 (d), 79.0 (s), 80.0 (s), 88.2 (d), 120.0 (d), 134.5 (s) and 215.5 (s).

Reduction of 5α,11α-Dihydroxy-8α,12(R)-epoxylabd-13(E)-en-1-one (243)

The ketone (243), (25mg), was dissolved in dry Et_2O (10ml) and $LiAlH_4$ (50mg) added. After 30 min the usual work-up gave, after recrystallisation from $CHCl_3$, 8α,12(R)-epoxylabd-13(E)-ene-1α,5α,11α-triol (249), (22mg) m.p. 213-215°C, $[\alpha]_D$ +30.8°, m/z 338 ($C_{20}H_{34}O_4$ requires m/z 338).

ν_{max}($CHCl_3$): 3600 and 3420 cm^{-1}

δ_H (200 MHz): 5.60 (ddq, J 1.3, 1.3, 6.7 Hz, H-14); 4.11 (dd, J 6.6, 11.0 Hz, H-11); 4.04 (br d, J 6.6 Hz, H-12); 3.78 (br t, J 3.5 Hz, H-1); 3.24 (d, J 11.0 Hz, H-9); 1.67 (br s, 3H-16); 1.62 (ddq, J 6.8, 0.9, 0.9 Hz, 3H-15); 1.19 (br), 1.00, 0.99 and 0.97 (4s, tertiary methyls).

δ_C : see Table 2 (p. 47)

11α-Acetoxy-8α,12(R)-epoxylabd-13(E)-ene-1α,5α-diol (250)

The triol (249), (20mg), dry pyridine (2ml) and Ac_2O (2ml) were heated on the steam bath for 3h. Addition of MeOH and removal of solvents, followed by preparative tlc gave the monoacetate (250) (21mg) as a gum.

ν_{max} ($CHCl_3$): 3570, 3470 and 1745 cm^{-1}.

δ_H : 5.61 (dd, J 7, 12 Hz, H-11); 5.60 (m, H- 14); 4.37 (d, J 3 Hz, OH); 3.48 (br H-1); 3.43 (d, J 12 Hz, H-9); 3.14 (d, J 3Hz, OH); 2.04 (s, CH_3CO); 1.62 (br s, 3H-16); 1.58 (br d, J 8 Hz, 3H-15); 1.25 (3H ,s), 1.01 (3H,s) and 0.97 (6H, s, tertiary methyls).

Attempted Jones' Oxidation of the Diol (250)

To a stirred solution of the diol (250), (13mg), in acetone (3ml) at 0°C was added Jones reagent (10 drops) and stirring continued for 90 min. Extraction into $CHCl_3$ gave a gum which tlc and ^1H nmr indicated to be starting material.

Attempted Collins' Oxidation of the Diol (250)

CrO_3 (48mg) was added to a stirred solution of pyridine (76 mg) in dry CH_2Cl_2 (3ml) at room temperature. After 15 min the diol (250) (13mg) in dry CH_2Cl_2 (3ml) was added in one portion and stirring continued for a further 60 min. Work-up gave a gum which was identified by tlc and ^1H nmr as starting material.

8α,12(s)-Epoxylabd-13(E)-en-1-one (251)

To a stirred ice-cold solution of the alcohol (244) (50mg) in acetone (6ml) was added Jones reagent (0.5ml). After 5 min, the usual work-up, followed by preparative tlc, gave 8α,12(s)-epoxylabd-13(E)-en-1-one (251) as a crystalline solid, m/z 304.2405 ($C_{20}H_{32}O_2$ requires m/z 304.2402).

δ_H (200 MHz): 5.56 (dqq, J 1.6, 1.6, 7.0 Hz, H-14); 4.32 (m H-12); 2.60 (ddd, J 5.0, 8.6, 15.3 Hz, H-2); 2.28 (ddd, J 5.0, 8.7 15.3 Hz, H-2^1); 1.59 (brs 3H-16);

1.58 (brd, J 7.0 Hz, 3H-15); 1.12, 1.11, 1.01 and0.99 (4s, tertiary methyls).

δ_C : see Table 2 (p.47).

Scapania gracilis

The plant material (2.0kg) was collected near Aberfoyle in September 1980. A crude extract (10.6g) was obtained and this was chromatographed in the usual manner. ^1H Nmr analysis of the fractions indicated the virtual absence of terpenoids. Only fats were present.

i) Ent-Epicubenol (209)

Steam distillation of the combined earlier fractions gave an oil (10mg) which was identified as (+)-ent-epicubenol (209) by comparison with an authentic sample obtained from S. undulata.

Diplophyllum albicans

The plant material (935g) was collected near Aberfoyle in 1980 and a crude extract (3.5g) obtained. Chromatographic separation yielded two compounds

i) ent-Diplophyllin (79)

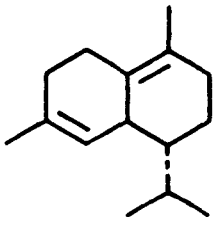
ent-Diplophyllin (79), (499mg) was identical with a sample obtained from Chiloscyphus polyanthos (see p.112).

ii) ent-Diplophyllolide (78)

ent-Diplophyllolide (78), (60mg), was identical with a sample obtained from Chiloscyphus polyanthos (see p 112).

CHAPTER 3

THE LOPHOCOLEACEAE



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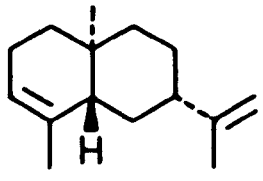

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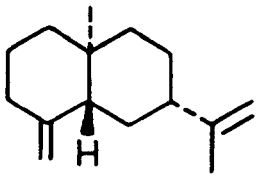

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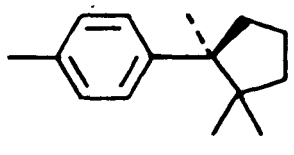

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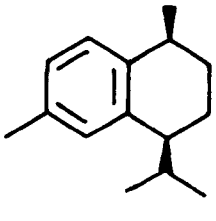

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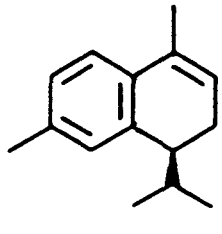

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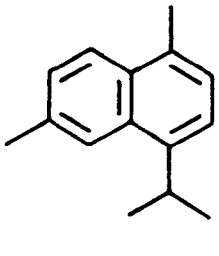
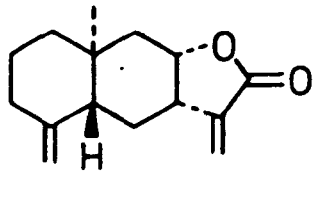
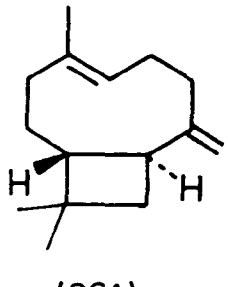


(259)



(260)



(261)

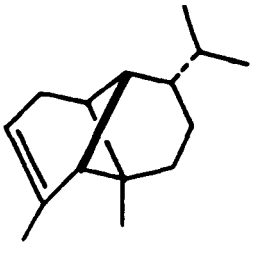
Introduction

Four genera of the Lophocoleaceae have previously been investigated chemically.

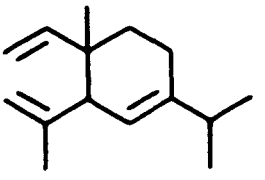
i) Lophocolea

Asakawa et al.[148] have reported that the major components of Japanese L.minor are bicyclogermacrene(38) and an unidentified sesquiterpene alcohol [M^+218]. This species also contains various sesquiterpene alcohols and oxygenated diterpenoids. In contrast, the major component of French L.heterophylla[35] is β-barbatene (43) with various sesquiterpene hydrocarbons also present. Absent from both samples are δ-cadinene (253), caryophyllene (261), longifolene (11) and α-selinene (254) which were reported by Thomas[158] to be present in a cultured sample of L.heterophylla. Recently, Herout and co-workers[159] have investigated Czechoslovakian L. heterophylla and found that the major constituents of the sesquiterpene hydrocarbon fraction were B-barbatene (43) and ent-β-selinene (255). Cuparene (256), cis-calamenene (257), calacorene (258), cadalene (259) and four unidentified sesquiterpenes were also present. The oxygenated sesquiterpenoid fraction consisted mainly of ent-isoalantolactone (260), accompanied by a small amount of an isomeric compound along with a sesquiterpenoid ketone and two alcohols, none of which were unidentified.

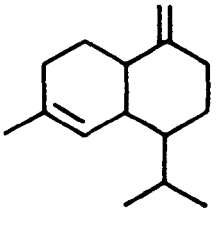
L. heterophylla has a characteristic and very intense "mossy" odour. Herout's group isolated the compound



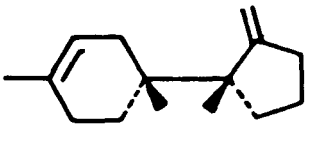
(262)



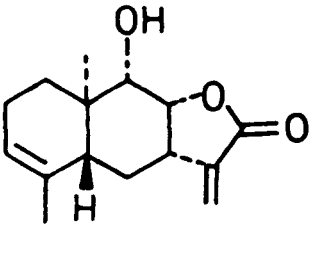
(263)



(264)



(265)



(266)

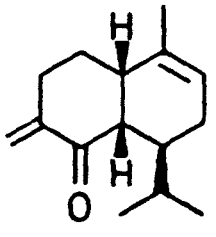
responsible, an alcohol, $C_{12}H_{20}O$, but were unable to deduce its structure.

ii) Heteroscyphus

Only H. bescherellei from Japan has been investigated[16] One sample contained α-copaene (262), β-barbatene (43), δ-elemene (263), calamenene (257), cuparene (256), α-himachalene (204) and calacorene (258), whereas a second contained β-barbatene (43), γ-cadinene (264), bicyclogermacrene (38), cuparene (256), β-bazzanene (265), drimenol (10) and unidentified diterpenoid acetates. H.bescherellei resembles L.minor in its chemical content, both containing β-barbatene (43) and bicyclogermacrene (38) as major components.

iii) Chiloscyphus

Samples of C.polyanthos from France and Wales have been investigated[83,84,160] and found to be similar. The major constituents are the eudesmanolides ent-diplophyllin (79), ent-diplophyllolide (78), ent-3-oxodiplophyllin (83), ent-9β-hydroxydiplophyllolide (266) and ent-7α-hydroxy-diplophyllolide (84). The last-named compound was initially[83] incorrectly described as ent-5α-hydroxydiplophyllolide (85) but this was later revised[26,84] on the basis of the following ^1H nmr spectroscopic evidence. The α-methylene protons [δ_H 5.85 and 6.21] appear as sharp singlets indicating the absence of allylic coupling, and, on addition of Eu(fod)$_3$,



(267)

H-8 and one exomethylene proton move rapidly downfield, closely followed by the other exomethylene proton. C-7 hydroxylated sesquiterpenoid lactones from plants are quite rare[161].

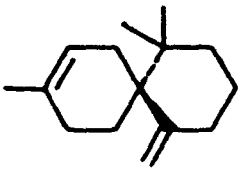
Matsuo and co-workers[162,163] isolated an α,β-unsaturated sesquiterpenoid ketone, chiloscyphone, from a Japanese sample of C.polyanthos. They proposed structure (267) on the basis of spectroscopic (mainly ^{1}H nmr, ir, ord and cd) studies on chiloscyphone and its hydrogenation and dehydrogenation products. At first sight the structure of chiloscyphone is unusual because it belongs to the normal absolute stereochemical series rather than the enantio series typical of most sesquiterpenoid metabolites of the Hepaticae.

Moreover, a closer examination of the published data for chiloscyphone raises doubts as to the validity of the proposed structure (267). The ^{1}H nmr spectrum contains no signals characteristic of an isopropyl group and it is difficult to rationalise the facile loss of a fragment of m/z 69 in the mass spectrometer. Recently Gras[164] has synthesised compound (267) and has shown it to be different from chiloscyphone.

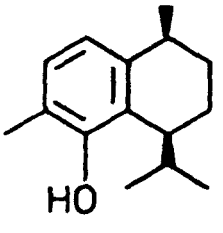
The revision of the structure of chiloscyphone forms part of the subject matter of this Chapter.

iv) Clasmatocolea

Gradstein et al.[89] have studied Colombian

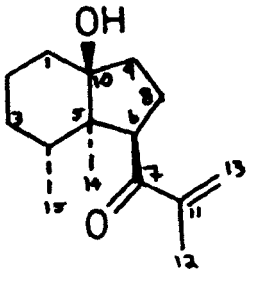


(268)



(269)

Clasmatocolea vermicularis by gcms, the major constituents being ent-diplophyllin (79) and ent-5α-hydroxyfrullanolide (71) along with two unidentified sesquiterpenoid lactones. β-Barbatene (43), β-chamigrene (268) and 5-hydroxycalamenene (269) were also detected.



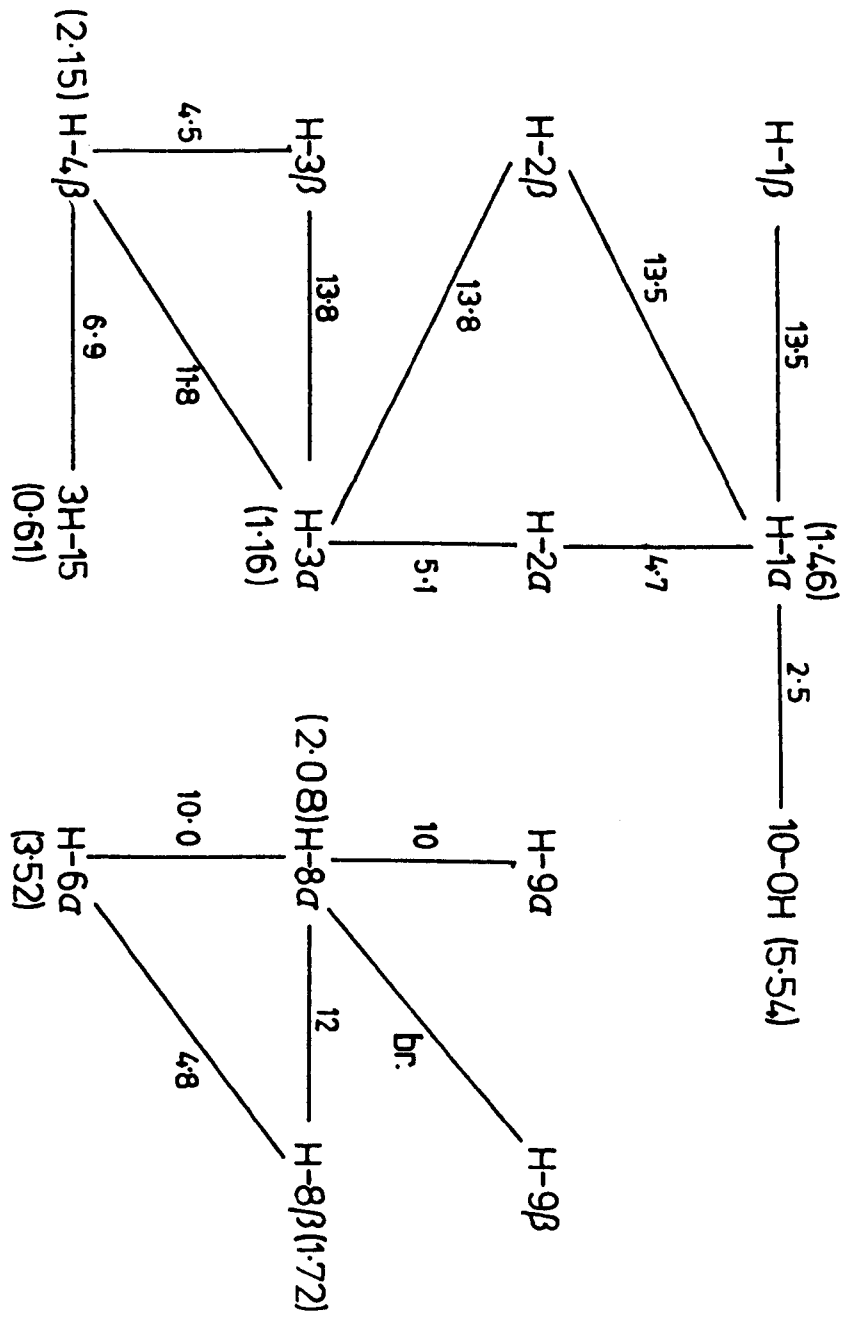
(270)

DISCUSSION

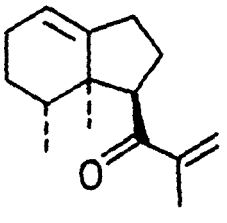
Chiloscyphus pallescens

The liverwort C.pallescens was found growing pure in a well in an abandoned hamlet in Knapdale Forest. The extract contained ent-longiborneol (12) and three new sesquiterpenoids, one of which, chiloscypholone (270), was present in high yield (0.28% of dry weight). Chiloscypholone proved to be an interesting sesquiterpenoid with a novel carbon skeleton[165]. It was readily converted by dehydration to chiloscyphone (271), (see below), which could not be detected in the C.pallescens or, indeed, in the C. polyanthos samples studied.

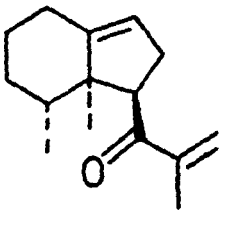
Chiloscypholone (270), $C_{15}H_{24}O_2$ (m/z 236.1769), mp.p. 94-94°C, $[\alpha]_D$ +53.9° (c, 0.86 in $CHCl_3$), [ν_{max} 3400 and 1660 cm^{-1}; λ_{max} (EtOH) 225nm (ϵ8900)], has a secondary methyl [δ_H 0.61 (d, J 6.9 Hz), δ_C 17.4 (q)], a tertiary methyl [δ_H 0.91 (s), S_C 20.7 (q)], an isopropenyl ketone [δ_H 1.89 (dd, J 1.5, 0.8 Hz), δ_C 18.0 (q); δ_H 5.81 (br q, J 1.5 Hz) and 6.04 (br s), δ_C 126.0 (t); δ_C 145.5 (s) and 209.0 (s)], and a strongly bonded tertiary hydroxy-group [δ_H 5.54 (d, ^{4}J 2.5 Hz), δ_C 81.4 (s)] which together with a tetrasubstituted carbon atom, five methylene and two methine groups constitute a bicarbocyclic system. Homonuclear decoupling and first order analysis of the 360 MHz ^{1}H nmr spectrum enabled the assignment of the couplings between the protons shown in Scheme 17 together


H-1β
13·5
(1·46)
H-1α
2·5
10-OH (5·54)
H-2β
13·5
13·8
H-2α
4·7
5·1
H-3β
13·8
(1·16)
H-3α
4·5
11·8
(2·15) H-4β
6·9
3H-15
(0·61)
(2·08) H-8α
10
H-9α
10·0
H-6α
(3·52)
12
br.
4·8
H-8β (1·72)
H-9β

Scheme 17.



(271)

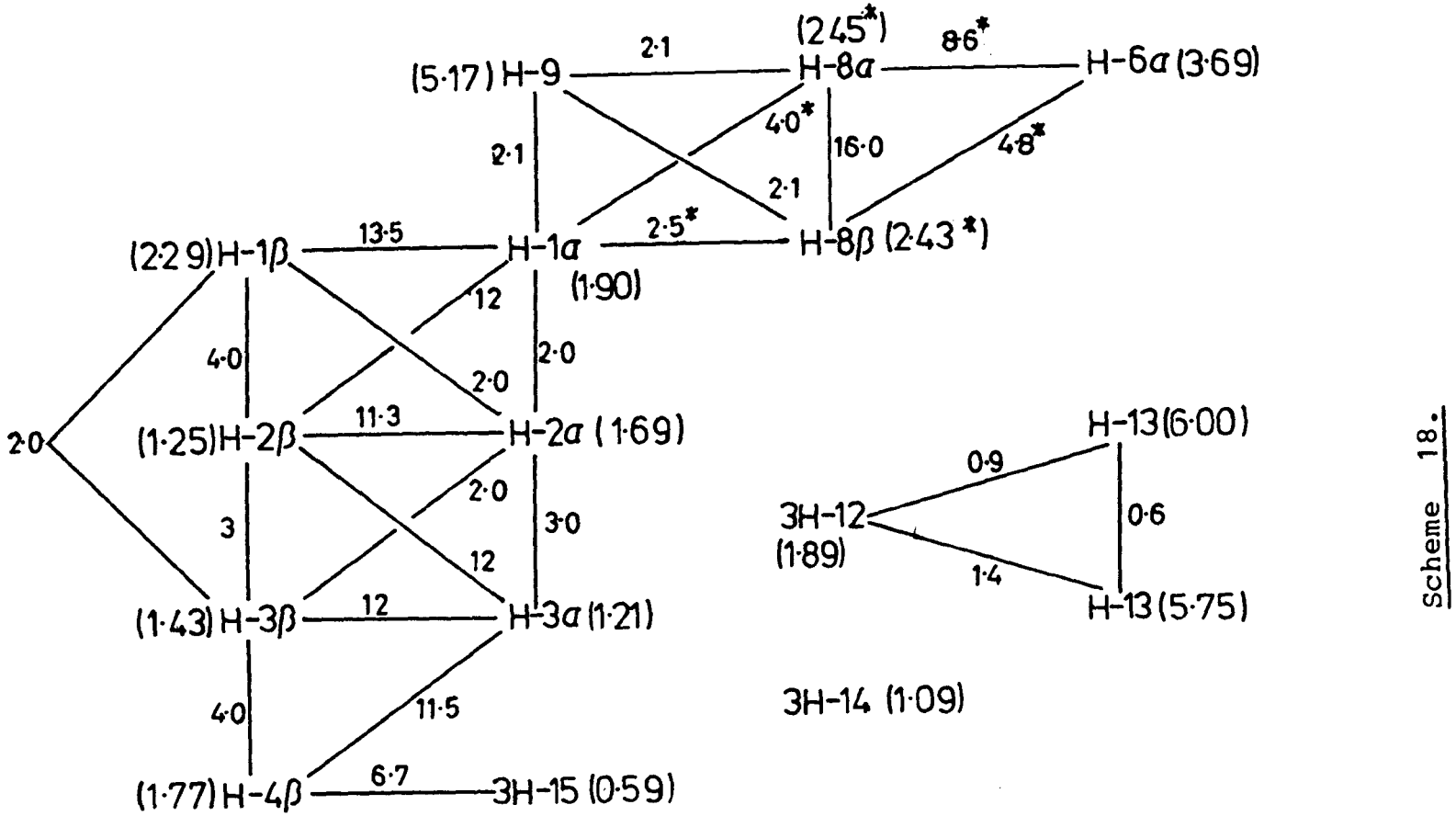


(272)

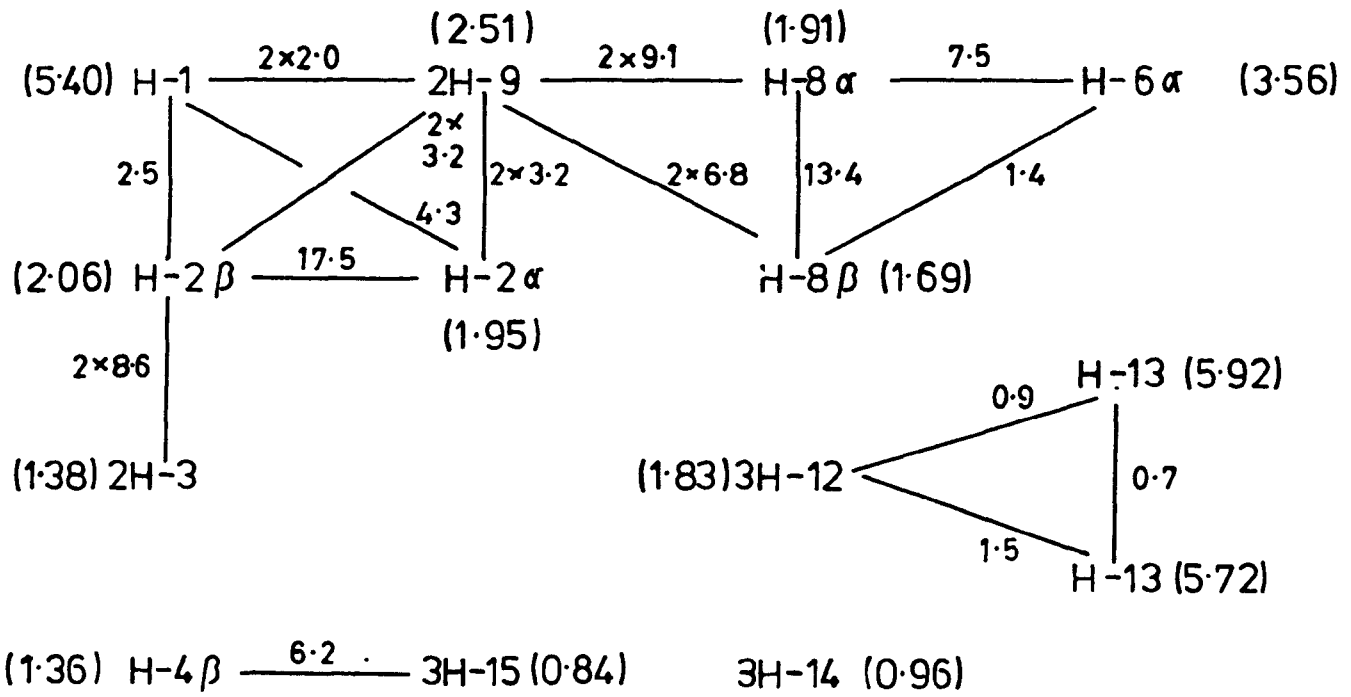
with their eventual assignment. The features described, especially the four bond W coupling involving the bonded tertiary hydroxy-proton, appear to be accomodated uniquely by structure (270). Confirmation came from consideration of the dehydration products, chiloscyphone (271) and isochiloscyphone (272), obtained by treatment of (270) with thionyl chloride in pyridine at -20°C.

Isochiloscyphone (272) $C_{15}H_{22}O$ (m/z 218.1667), oil, has a trisubstituted double bond in addition to the enone system of (270). The assignment of all the proton-proton coupling constants in the molecule, based on decoupling experiments at 360 MHz, is shown in Scheme 18 (asterisked parameters come from second-order calculations). This information leads uniquely to structure (272) for isochiloscyphone, apart from the relative configuration of the isopropenyl ketone. Since the tertiary hydroxy-group in chiloscyphone (270) is <u>trans</u> to the axial 1-H and <u>syn</u> to the isopropenyl ketone, the latter must be β in (272).

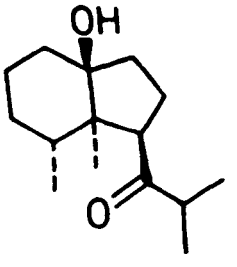
Chiloscyphone (271), $C_{15}H_{22}O$ (<u>m/z</u> 218.1675), $[\alpha]_D$ -24.4° (c, 0.76 in $CHCl_3$), is isomeric with (272) and has identical physical and spectroscopic properties with those published for the compound from <u>C.polyanthos</u>. The proton parameters and connectivity are shown in Scheme 19 and lead to (271) as the revised structure for chiloscyphone. Structure (271) nicely accommodates the loss of a fragment of m/z 69 in the mass spectrometer by α-cleavage.


(5·17) H-9
2·1
(2·45*)
H-8α
8·6*
H-6α (3·69)
2·1
4·0*
16·0
4·8*
2·1
(2·29) H-1β
13·5
H-1α
2·5*
H-8β (2·43*)
(1·90)
12
4·0
2·0
2·0
2·0
(1·25) H-2β
11·3
H-2α (1·69)
3
2·0
3·0
12
(1·43) H-3β
12
H-3α (1·21)
4·0
11·5
(1·77) H-4β
6·7
3H-15 (0·59)
H-13 (6·00)
0·9
3H-12
(1·89)
0·6
1·4
H-13 (5·75)
3H-14 (1·09)

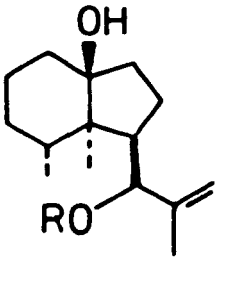
Scheme 18.


(2·51)
(1·91)
(5·40) H-1
2×2·0
2H-9
2×9·1
H-8α
7·5
H-6α (3·56)
2·5
2×3·2
2×3·2
4·3
2×6·8
13·4
1·4
(2·06) H-2β
17·5
H-2α
(1·95)
H-8β (1·69)
2×8·6
H-13 (5·92)
0·9
(1·38) 2H-3
(1·83) 3H-12
0·7
1·5
H-13 (5·72)
(1·36) H-4β
6·2
3H-15 (0·84)
3H-14 (0·96)

Scheme 19.



(273)



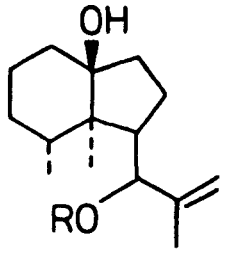
(274) R = H

(275) R = Ac

Chiloscypholone (270) was hydrogenated over a Pd/C catalyst to give dihydrochiloscypholone A (273) $C_{15}H_{26}O_2$ (m/z 238), m.p. 87-88°C [ν_{max} 3400 and 1700 cm^{-1}] which has an isopropyl ketone [δ_H 2.76 (septet, J 6.9 Hz); 1.12 and 1.04 (both d, J 6.9 Hz); δc 222.4 (q)]. The ^1H nmr spectrum is identical to a trace impurity in the 360 MHz ^1H nmr spectrum of chiloscypholone. It therefore seems likely that dihydrochiloscypholone A (273) is a minor metabolite of C.pallescens.

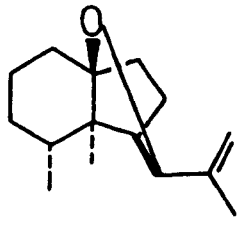
A second dihydro-derivative resulted from $LiAlH_4$ reduction of (270). Dihydrochiloscypholone B (274), $C_{15}H_{26}O_2$ (m/z 220, M^+-H_2O), m.p. 101-103°C [ν_{max} 3620 and 3440 cm^{-1}] has a new secondary hydroxy group [δ_H 4.46 (d, J 6Hz), δ_C 78.9 (d)]. Only one C-7 epimer was observed. It is formulated as shown since hydride should be delivered to the less hindered face of the carbonyl group. The fact that the side-chain is held in one conformation by hydrogen-bonding between the ketone carbonyl group and the C-10 hydroxyl group accounts for the stereospecificity of this reaction.

Treatment of (274) with acetic anhydride in dry pyridine gave a monoacetate (275), $C_{17}H_{28}O_3$ (m/z 262, M^+-H_2O) m.p. 62-64°C, [ν_{max} 3620 and 1735 cm^{-1}], [δ_H 5.98 (d, J 9 Hz, CH-OAc), 1.97 (s, CH_3CO)]. Similarly, treatment of dihydrochiloscypholone B (274) with bromo-benzoyl chloride in pyridine in an attempt to produce an

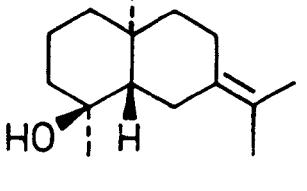


(276) R = OCC_6H_4Br-p

(277) R = $O_2SC_6H_4Br$-p



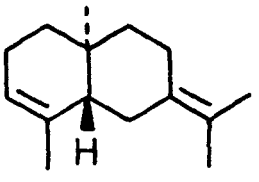
(278)



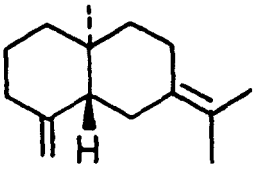
(279)

X-ray derivative, formed the bromobenzoate (276) as an oil, [δ_H 7.85, 7.49 (AA'BB' system, $J_{AB+AB'}$ 8 Hz) and 6.32 (d, J 10 Hz H-7). The analogous reaction using bromobenzenesulphonyl chloride and pyridine formed not the bromobenzenesulphonate (277) but the cyclised derivative (278), $C_{15}H_{24}O$ (m/z 220). The i r spectrum indicated that the product was non-hydroxylic, whilst nmr spectroscopy showed that there were still two oxygen-bearing carbons present [δ_H 4.43 (br s, CH-O), δc 87.0 (s) and 80.1 (d)]. This is consistent with the formation of a tetrahydrofuran ring by displacement of the bromobenzenesulphonyl group of (277) by the C-10 hydroxyl group. The absolute configuration of chiloscypholone (270) has not yet been determined.

The second compound, ent-eudesm-7(11)-en-4α-ol (279), $C_{15}H_{26}O$ (m/z 222.1980), m.p. 162-163°C [lit.[166] 164-166°C] $[\alpha]_D$ -7.9° (c, 0.71 in $CHCl_3$), [ν_{max} 3600 cm^{-1}], has two tertiary methyls, one of which is attached to a carbon bearing oxygen [δ_H 1.12 (s, 3H-15) and 0.95 (s, 3H-14), δ_C 18.1 (q) and 22.0 (q)], two vinyl methyls [δ_H 1.68 (dd, J 2.0, 1.2 Hz) and 1.65 (t, J 1.4 Hz); δ_C 20.2 (q) (2 x CH_3)], a tetrasubstituted double bond [δ_C 131.4 and 120.9] and a tertiary hydroxyl group [δ_C 72.2(s)] which together with a tetrasubstituted carbon atom, one methine and six methylene groups constitute a bicarbocyclic system. Homonuclear decoupling and first-order analysis of the 360 MHz ^1H nmr spectrum enabled the assignment of the



(280)



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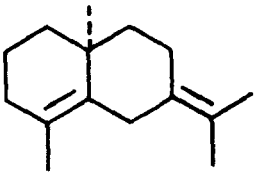
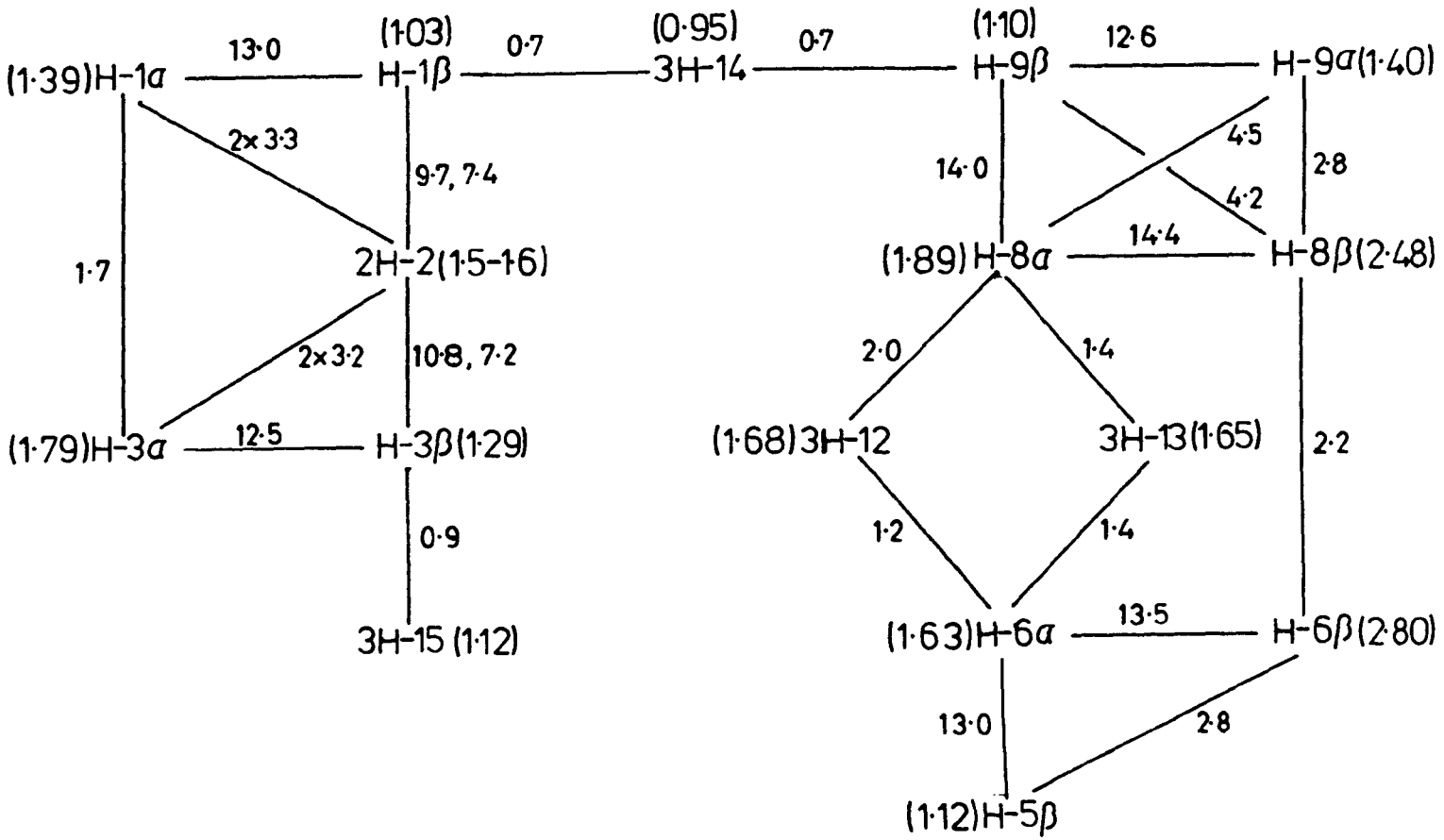


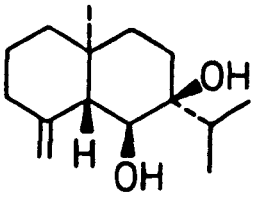
(282)

couplings between the protons shown in Scheme 20 together with their eventual assignments (chemical shifts in parentheses). That the C-4 methyl group is axial follows from the existence of a W coupling between the C-4 methyl protons and the 3β-proton. Further evidence is provided by dehydration of (279) to give two compounds. The ^1H nmr spectrum of this mixture indicated that it consisted of eudesma-3, 7(11)-diene (280)[167] and eudesma-4(14), 7(11)-diene (281)[168]. If the hydroxyl group in (279) was axial then eudesma-4(5), 7(11)-diene (282) should have been a major product; none, however, was observed. Preparative tlc over $AgNO_3$-impregnated SiO_2 afforded pure ent-eudesma-4(14), 7(11)-diene (281), $C_{15}H_{24}$ (m/z 204), as an oil, $[\alpha]_D$ - 97.6 (c, 0.78 in $CHCl_3$) [lit.[167] $[\alpha]_D$ -110°], [δ_H 4.70 and 4.46 (br s, exomethylene protons); 1.63 (s, vinyl methyls); 0.75 (s, tertiary methyls)] whose spectroscopic properties are identical with the literature values[167].

Recently, Bohlmann et al.[169] have published the isolation of eudesm-7(11)-en-4α-ol (ent-279) from Acritopappus prunifolius (Compositae). The spectroscopic data are similar to those in Scheme 20 but several discrepancies exist. Bohlmann reports H-8β at δ_H 2.39 as compared with δ_H 1.89 in our assignment and fails to observe any coupling between the C-4 methyl group and the 3α proton. It is possible that Bohlmann's sample was not pure


(1·39)H-1α
13·0
(1·03)
H-1β
0·7
(0·95)
3H-14
0·7
(1·10)
H-9β
12·6
H-9α(1·40)
2×3·3
9·7, 7·4
14·0
4·5
4·2
2·8
1·7
2H-2(1·5-1·6)
(1·89)H-8α
14·4
H-8β(2·48)
2×3·2
10·8, 7·2
2·0
1·4
(1·79)H-3α
12·5
H-3β(1·29)
(1·68)3H-12
3H-13(1·65)
2·2
0·9
1·2
1·4
3H-15 (1·12)
(1·63)H-6α
13·5
H-6β(2·80)
13·0
2·8
(1·12)H-5β

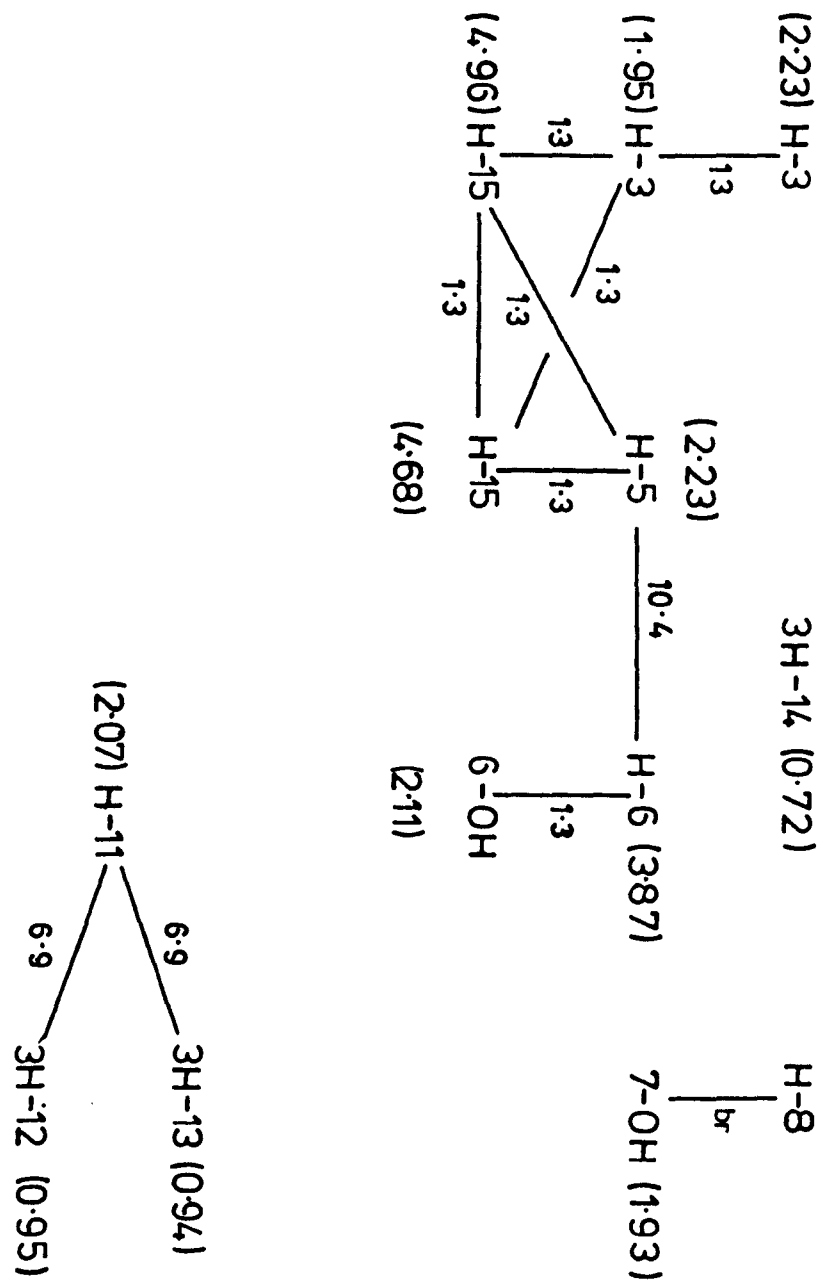
Scheme 20.



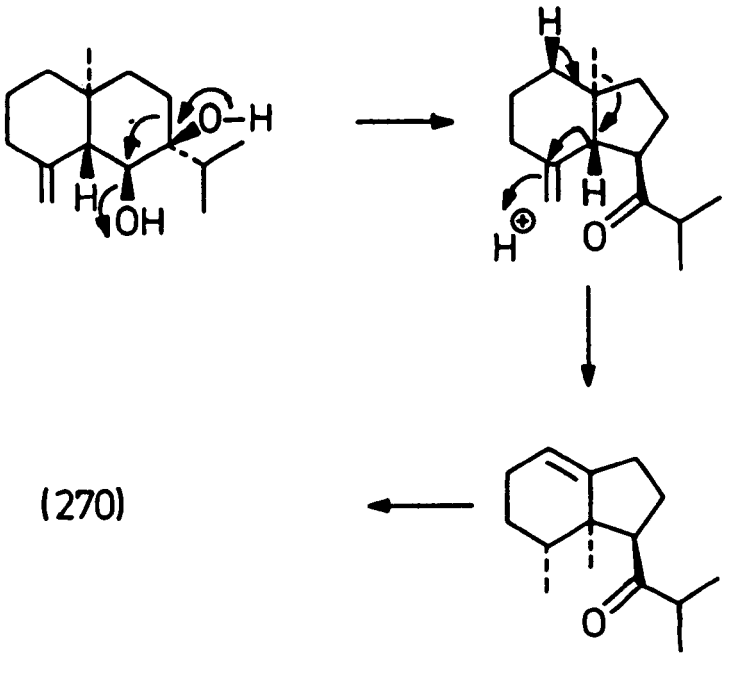
(283)

The third new sesquiterpenoid, ent-eudesm-4(15)-ene-6α,7α-diol (283), $C_{15}H_{26}O_2$ (m/z 238.1930), m.p. 110-112°C, $[\alpha]_D$ + 98.4° (c, 0.93 in $CHCl_3$), [ν_{max} 3600 and 3570 cm^{-1}], has two secondary methyls [δ_H 0.95 (d, J 6.9 Hz) and 0.94 (d, J 6.9 Hz), δ_C 16.4 (q) and 17.9 (q)], one tertiary methyl [δ_H 0.72 (br s)], an exomethlyene [δ_H 4.96 (q, J 1.3 Hz) and 4.68 (q, J 1.3 Hz), δ_C 106.4 (t) and 148.1 (s)], a secondary alcohol [δ_H 3.87 (dd, J 10.4, 1.3 Hz) and 2.11 (d, J 1.3 Hz, ON) δ_C 67.5 (d)] and a tertiary alcohol [δ_H 1.93 (br s), δ_C 75.3 (s)] which together with a tetrasubstituted carbon atom, two methine and five methylene groups again constitute a bicarbocyclic system. Homonuclear decoupling and first order analysis of the 360 MHz ^1H n m r spectrum enabled the assignment of the couplings between the protons shown in Scheme 21 together with their eventual assignments (chemical shifts in parentheses). The C-6 hydroxyl-group is equatorial because H-6 exhibits a large coupling (J 10.4 Hz) to H-5 and must therefore be axial. The C-7 hydroxyl proton is coupled to H-8α and this can only occur when the C-7 hydroxyl-group is axial allowing a four-bond W coupling. The absolute configuration of (283) has not been determined, though it is anticipated that it will belong to the enantio series.

The isolation of this diol is of interest since it is an attractive candidate for ring-contraction and

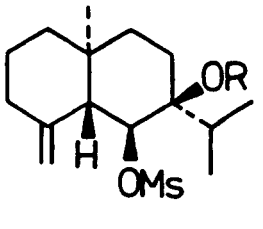

(2·23) H-3
(1·95) H-3
1·3
(4·96) H-15
1·3
1·3
(4·68)
H-15
1·3
(2·23)
H-5
10·4
H-6 (3·87)
6-OH
(2·11)
3H-14 (0·72)
H-8
br
7-OH (1·93)
(2·07) H-11
6·9
6·9
3H-12 (0·95)
3H-13 (0·94)

Scheme 21.



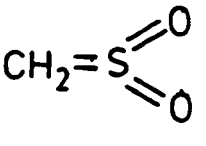
(270)

Scheme 22



(284) R=H

(286) R=Ms



(285)

methyl migration processes which would lead, after dehydrogenation, to the chiloscypholone carbon skeleton with the correct stereochemistry (Scheme 22). An attempt was made to induce the ring-contraction *via* the intermediacy of the monomesylate (284). Unfortunately because of the hindered nature of the secondary hydroxyl group it proved impossible to prepare this mesylate under normal conditions (mesylchloride in pyridine). The more vigorous method of Crossland *et al.*[152] in which the alcohol is added to a mixture of mesyl chloride and Et_3N (thought to involve (285) as the active species) formed the dimesylate (286). Lack of material prevented further work.

In addition to the above compounds, an aldehyde [δ_H 9.57 (s)] was isolated. This decomposed before it could be further investigated.

Lophocolea bidentata

The major component of the ether extract of *L. bidentata* collected in the West of Scotland was a crystalline compound, m.p. 61-62°C, identified as *ent*-diplophyllolide (78) by comparison with an authentic sample.

Chiloscyphus polyanthos

The major components of *C. polyanthos* from the West of Scotland were the known eudesmanolides *ent*-diplophyllolide (78), *ent*-diplophyllin (79) and *ent*-7α-hydroxydiplophyllolide (84).

Summary

The species of the Lophocoleaceae would appear to fall into two categories - those containing sesquiterpenoid lactones and those lacking lactones. Lophocolea heterophylla, L. bidentata, Chiloscyphus polyanthos and Clasmatocolea vermicularis belong to the former category whereas L. minor Heteroscyphus bescherellei and Chiloscyphus pallescens belong to the latter. Two discrepancies are apparent - French L.heterophylla and Japanese C. polyanthos which do not contain lactones. In the latter case identification, based upon morphological features, is difficult. Further study is necessary.

EXPERIMENTAL

Chiloscyphus pallescens

The plant material (200g) was collected in Knapdale Forest in the West of Scotland. The crude extract (12g) was chromatographed in the usual manner and a number of compounds isolated.

i) Chiloscypholone (270)

Recrystallisation from cold pentane gave chiloscypholone (560mg) as prisms, m.p. 93-94°C, $[\alpha]_D$ + 53.9° (c, 0.86 in $CHCl_3$), m/z 236.1769 ($C_{15}H_{24}O_2$) requires m/z 236.1776.

ν_{max} : 3400 and 1660 cm^{-1}

λ_{max} : 225 nm (ε 8900)

δ_H (360 MHz): see Scheme 17 (p. 93).

δ_C : 17.4 (q), 18.0 (q), 20.7 (q), 20.7 (t), 27.9 (t), 29.5 (t), 29.6 (d), 30.0 (t), 38.4 (t), 52.0 (d), 53.8 (s), 81.4 (s), 126.0 (t), 145.5 (s) and 209.0 (s).

ii) ent-Longiborneol (12)

ent-Longiborneol (12), recrystallised from cold pentane, m.p. 104-106°C, $[\alpha]_D$ - 17.7° (c, 0.48 in $CHCl_3$), [lit.[24] m.p. 107-108°C, $[\alpha]_D$ - 19°], was identical with an authentic sample obtained from Scapania undulata.

iii) ent-Eudesm-7(11)-en-4α-ol (279)

The alcohol (279), (100mg), was recrystallised

from hexane as prisms, m.p. 162-163°C [lit.[166] 164-166°C], $[\alpha]_D$ - 7.9° (c, 0.71 in $CHCl_3$), m/z 222.1980 ($C_{15}H_{26}O$ requires m/z 222.1984).

ν_{max} : 3600 cm^{-1}

δ_H (360 MHz): see Scheme 20 (p.100)

δ_C : 18.1 (q), 20.1 (t), 20.2 (q), 20.2 (q), 22.0 (q), 24.6 (t), 25.4 (t), 34.8 (s), 40.9 (t), 43.5 (t), 45.2 (t), 55.6 (d), 72.2 (s), 120.9 (s) and 131.4 (s).

iv) ent-Eudesm-4(15)-ene-6α,7α-diol (283)

Recrystallisation from hexane gave the diol (283), 75 mg, as needles, m.p. 110-112°C, $[\alpha]_D$ + 98.4° (C, 0.93 in $CHCl_3$), m/z 238.1930 ($C_{15}H_{26}O_2$ requires m/z 238.1993).

ν_{max} : 3600 and 3570 cm^{-1}

δ_H (360 MHz) : see Scheme 21 (p.102)

δ_C : 16.4 (q), 16.7 (q), 17.9 (q), 22.7 (t), 24.0 (t), 34.0 (d), 34.9 (t), 37.0 (s), 37.9 (t), 41.5 (t), 52.3 (d), 65.7 (d), 75.3 (s), 106.4 (t) and 148.1 (s).

Hydrogenation of Chiloscypholone (270)

Chiloscypholone (270), (50mg), in EtOAc (15ml) was stirred with 10% Pd-C for 30 min under hydrogen. Filtration and removal of solvent gave dihydrochiloscypholone A (273), (50mg), m.p. 87-88°C (ex pentane), m/z 238

($C_{15}H_{26}O_2$ requires m/z 238).

ν_{max} : 3400 and 1700 cm^{-1}

δ_H : 5.38 (d, ^4J 2.5 Hz, 10-OH); 2.76 (septet, J 6.9 Hz, H-11); 1.12 and 1.04 (2d, J 6.9 Hz, 3H-12 and 3H-13); 0.86 (s, 3H-14); 0.64 (d, J 6.9 Hz, 3H-15).

δ_C : 17.3 (q), 17.7 (q), 18.7 (q), 20.1 (t), 20.5 (q), 27.6 (t), 29.5 (d), 29.5 (t), 29.9 (t), 38.2 (t), 43.1 (d), 53.6 (s), 57.2 (d), 81.5 (s) and 222.4 (s).

Dihydrochiloscypholone B (274)

To a solution of $LiAlH_4$ (250 mg) in anhydrous Et_2O (15 ml) was added crystalline chiloscypholone (270), (100 mg). When all the material had dissolved, wet Et_2O was added to destroy excess hydride. An acidic work-up gave dihydrochiloscypholone B (274), (95 mg), recrystallised from cold pentane, m.p. 101-103°C, m/z 220 (M+ - H_2O, $C_{15}H_{24}O$ requires m/z 220).

ν_{max} : 3620 and 3440 cm

δ_H : 4.98 and 4.82 (2 br s, 2H-13); 4.46 (d, J 6Hz, H-7); 2.45 (br s, 2 OH); 1.71 (br s, 3H-12); 0.91 (d, J 6.9 Hz, 3H-15); 0.78 (s, 3H-14).

δ_C : 19.0 (q), 19.9 (q), 20.3 (q), 20.7 (t), 25.6 (t), 30.1 (d), 30.6 (t), 31.1 (t),

36.4 (t), 49.7 (d), 51.5 (s), 78.8 (d), 82.3 (d), 111.7 (t) and 147.6 (s).

Dihydrochilosocypholone B Acetate (275)

To the diol (274), (50 mg), in dry pyridine (3ml) was added Ac_2O (1ml) and the solution left overnight. Removal of solvents gave the acetate (275), (59 mg), m.p. 62-64°C, m/z 262 (M^+-H_2O, $C_{17}H_{26}O_2$ requires m/z 262).

ν_{max} : 3620 and 1735 cm^{-1}

δ_H : 5.98 (d, J 9 Hz, H-7); 5.00 (br, s, H-13); 4.92 (d, J 1.5 Hz, H-13'); 1.97 (s, CH_3CO); 1.63 (s, 3H-12); 0.92 (d, J 7 Hz, 3H-15); 0.79 (s, 3H-14).

δ_C : 17.2 (q), 18.8 (q), 19.1 (q), 20.5 (t), 21.5 (q), 22.8 (t), 30.1 (d), 30.3 (t), 31.7 (t), 36.3 (t), 48.2 (d), 50.2 (s), 81.2 (d), 82.9 (s), 116.3 (t), 143.3 (s), and 169.9 (s).

The Bromoacetate (276)

A solution of diol (274), (25mg), and p-bromobenzoyl chloride (26mg) in dry pyridine (2 ml) was left overnight. An acidic work-up followed by preparative tlc gave the oily bromobenzoate (276), (20 mg).

ν_{max} : 3610 and 1735 cm^{-1}

δ_H : 7.87 and 7.49 (AA'BB' system, $J_{AB+AB'}$

8 Hz); 6.32 (d, J 10 Hz, H-13); 5.07 (br s, H-13'); 4.93 (d, J 1 Hz, H-7); 1.68 (br s, 3H-12); 0.78 (s,3H-14); 0.66 (d, J 7 Hz, 3H-15).

Cyclisation of Dihydrochiloscypholone B (274)

The above reaction was repeated using p-brosyl chloride (30 mg). Preparative tlc gave the cyclic ether (278), (19 mg), as an oil, m/z 220 ($C_{15}H_{24}O$ requires m/z 220).

ν_{max} : 2960, 2940 and 910 cm^{-1}

δ_H : 5.20 (br s, H-13); 4.86 (br s, H-13'); 4.44 (br s, H-7); 1.58 (br s, 3H-12); 1.47 (s, 3H-14); 0.86 (d, J 7Hz 3H-15).

δ_C : 12.1 (q), 17.2 (q), 19.7 (q), 20.2 (t), 21.9 (t), 27.0 (t), 29.4 (t), 30.1 (d), 34.5 (t), 45.4 (d), 50.7 (s), 80.1 (d), 87.0 (s), 108.9 (t) and 144.7 (s).

Dehydration of Chiloscypholone (270)

To a stirred solution of the alcohol (270), (70mg), in dry pyridine (5 ml) at -20°C was added freshly distilled $SOCl_2$ (10 drops). After 3 min the solution was allowed to come to room temperature and was poured into ice-cold $NaHCO_3$ solution. Extraction into $CHCl_3$ gave a residue which was purified by preparative tlc

over $AqNO_3$ - impregnated SiO_2. Two major products were isolated.

i) Chiloscyphone (271), oil, $[\alpha]_D$ - 24.4° (c, 0.76 in $CHCl_3$) lit. $[\alpha]_D$ - 45.7° (dioxan)], m/z 218.1675 ($C_{15}H_{22}O$ requires m/z 218.1671).

ν_{max} : 1670 cm^{-1}

λ_{max} : 220 nm (ϵ8920)

δ_H (360 MHz): see Scheme 19 (p.96).

δ_C : 17.6 (q), 17.6 (q), 20.7 (q), 25.4 (t), 26.1 (t), 27.1 (t), 29.2 (t), 33.1 (d), 49.8 (s), 52.7 (d), 117.3 (d), 123.7 (t), 146.0 (s), 146.5 (s) and 206.5 (s).

ii) Isochiloscyphone (272), oil, m/z 218.1667 ($C_{15}H_{22}O$ requires m/z 218.1671).

ν_{max} : 1675 cm^{-1}

λ_{max} : 220 nm (ϵ 8960)

δ_H (360 MHz): see Scheme 18 (p.95).

δ_C : 18.0 (q), 18.2 (q), 19.9 (q), 26.3 (t), 27.2 (t), 31.4 (t), 35.4 (t), 35.4 (d), 52.4 (d), 55.4 (s), 118.1 (d), 124.0 (t), 146.8 (s), 149.1 (s) and 205.1 (s).

Dehydration of ent-Eudesm-7(11)-en-4 -ol (279)

To a stirred solution of the alcohol (279), (60mg) in dry pyridine (5ml) at ice temperature was added freshly

distilled $SOCl_2$ (10 drops). After 3 min the solution was warmed to room temperature and was poured into ice-cold $NaHCO_3$ solution (10ml). Extraction into $CHCl_3$ gave an approximately 1:1 mixture of two compounds (by integration of ^1H nmr signals).

δ_H : 5.31 (m, vinyl proton); 4.70 and 4.46 (2 br s, exomethylene group); 1.63 (s, vinyl methyl); 0.81 and 0.75 (2s, tertiary methyls).

Preparative tlc over $AgNO_3$-impregnated SiO_2 gave ent-eudesma-4(15), 7(11)-diene (281), (10mg), as an oil, $[\alpha]_D$ - 97.6° (C, 0.75 in $CHCl_3$) [lit.[167] $[\alpha]_D$ - 110°], m/z 204 ($C_{15}H_{24}$ requires m/z 204).

^{H} : 4.70 and 4.46 (2 br s, 2H-15); 1.63 (s, vinyl methyls); 0.75 (s, 3H-14).

Mesylation of ent-Eudesm-(4,15)-ene-6 ,7 -diol (283)

a) To a solution of the diol (283), (20mg), in dry pyridine (1 ml) at room temperature was added freshly distilled mesyl chloride (0.05 ml), and the mixture left overnight. An acidic work-up gave only starting material (by tlc and ^1H nmr).

b) To a solution of the diol (283), (20 mg), in dry CH_2Cl_2 (2 ml) containing Et_3N (5 drops) at -10°C was added mesyl chloride (1 drop). After 5 min an acidic work-up followed by preparative tlc gave the dimesylate (286), the ir spectrum of which lacked hydroxyl and carbonyl stretch. Other attempts to esterify selectively

the secondary alcohol by reducing the amount of mesyl chloride added and/or shortening the reaction time met with no success.

Chiloscyphus polyanthos

The plant material (150 g) was collected near Aberfoyle in central Scotland and yielded a crude extract (1.9g) which was chromatographed. Three sesquiterpenoid lactones were isolated.

i) ent-Diplophyllin (79)

ent-Diplophyllin (79), (20mg), was isolated as an oil, $[\alpha]_D$ - 73.6° (c, 0.57 in $CHCl_3$) [lit.[82] m.p. 30-31.5°C, $[\alpha]_D$ - 108°], m/z 232.1460 ($C_{15}H_{20}O_2$ requires m/z 232.1463).

ν_{max} : 1775 cm^{-1}

δ_H : 6.22 and 5.60 (2 d, J 3 Hz, 2H-12), 4.48 (q, J 7 Hz, H-8); 3.00 (m, H-7); 1.64 (s, 3H-15); 1.06 (s, 3H-14).

δ_C : 18.8 (t), 19.2 (q), 26.8 (q), 27.7 (t), 31.9 (t), 33.5 (s), 37.1 (t), 41.1 (d), 42.6 (t), 76.4 (d), 121.3 (t), 127.2 (s), 131.3 (s), 140.5 (s) and 170.8 (s).

ii) ent-Diplophyllolide (78)

Recrystallisation from aqueous EtOH gave ent-diplophyllolide (78), (15mg), m.p. 61-62°C, $[\alpha]_D$ - 124° (c, 0.10 in $CHCl_3$) [lit.[81] m.p. 60-62°C, lit.[82] $[\alpha]_D$ + 132°

for diplophyllolide], m/z 232.1462 ($C_{15}H_{20}O_2$ requires m/z 232.1463).

ν_{max} : 1775 cm^{-1}

δ_H : 6.12 and 5.57 (both br s, 2H-12); 5.37 (br s, H-3); 4.50 (dt, J 2.0, 5.0 Hz, H-8); 2.98 (m,H-7); 1.61 (d, J 2.0 Hz, 3H-15), 0.86 (s, 3H-14).

δ_C : 17.2 (q), 21.1 (q), 22.2 (t), 27.5 (t), 30.9 (s), 37.9 (t), 41.0 (t), 41.2 (d), 43.9 (d), 77.0 (d), 120.2 (t), 122.3 (d), 142.1 (s) and 170.6 (s).

iii) ent-7α-Hydroxydiplophyllolide (84)

The hydroxylactone (84), (22mg), was recrystallised from hexane, m.p. 82-83°C, $[\alpha]_D$- 70.3° (c, 0.94 in $CHCl_3$) [lit.[83] m.p. 72-74°C, $[\alpha]_D$ - 36.1°], m/z 248.1413 ($C_{15}H_{20}O_3$ requires m/z 248.1412).

ν_{max} : 3600 and 1785 cm^{-1}

δ_H : 6.04 and 5.69 (both s, 2H-12); 5.32 (br s, H-3); 4.22 (d, J 4.0 Hz, H-8); 3.51 (br s,-OH); 1.60 (br s, 3H-15); 0.85 (s, 3H-14).

δ_C : 16.9 (q), 21.0 (q), 22.2 (t), 31.2 (s), 33.6 (t), 37.7 (t), 38.1 (t), 40.5 (d), 75.4 (s), 82.0 (d), 120.6 (t), 122.4 (d), 132.7 (s), 145.1 (s) and 169.1 (s).

Lophocolea bidentata

The crude extract(1.5 kg)was obtained from plant material (150g) collected near Drymen and was chromatographed to yield one sesquiterpenoid.

i) ent-Diplophyllolide (78)

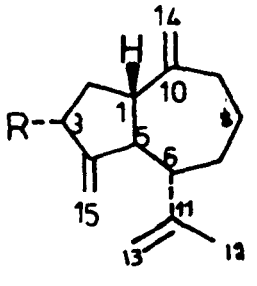
Recrystallisation from aqueous EtOH gave ent-diplophyllolide (78), (40mg), identical with a sample obtained from Chiloscyphus polyanthos.

CHAPTER 4

THE GEOCALYCACEAE

Introduction

No species from this rather uncommon family have previously been studied.



(287) R=OH

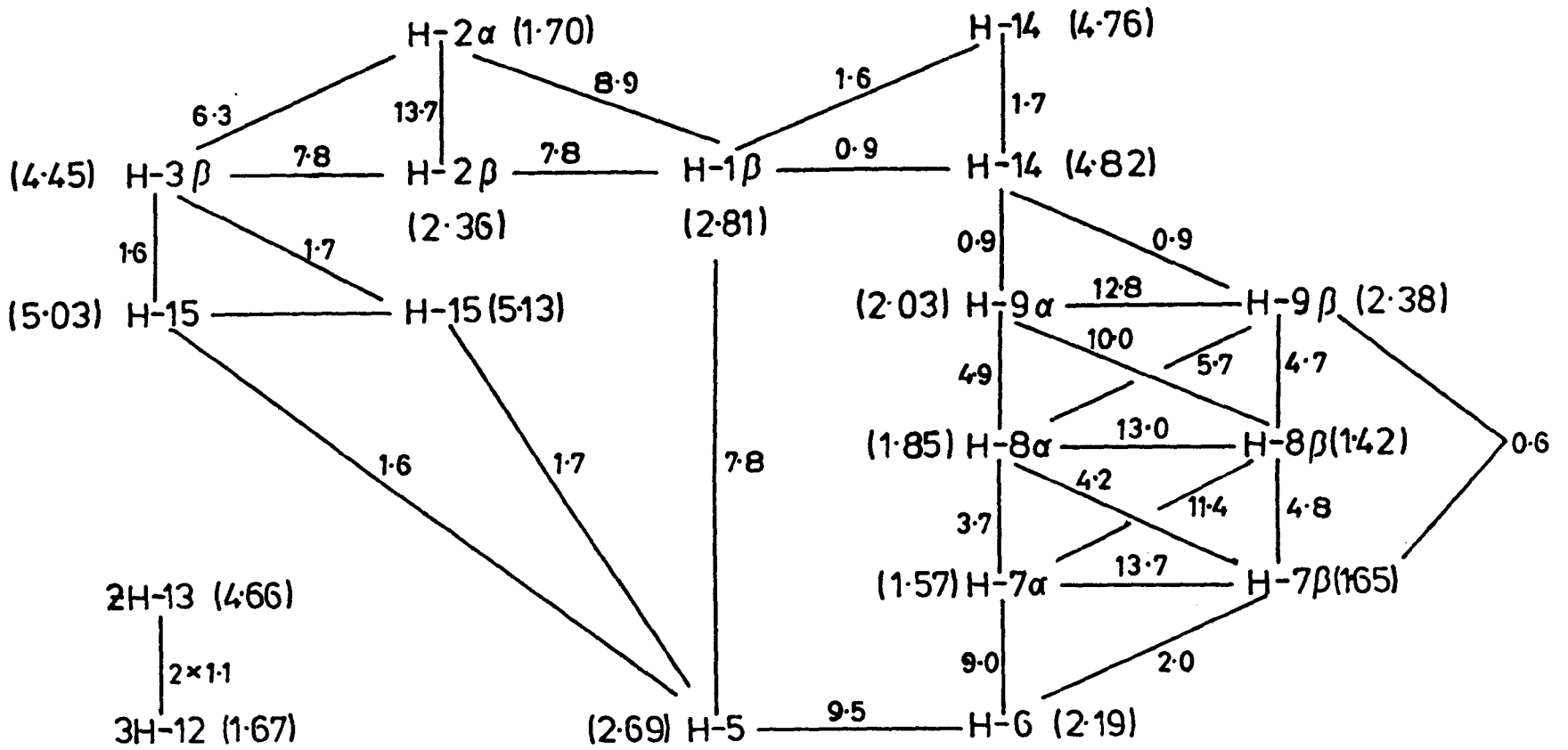
(288) R=H

Discussion

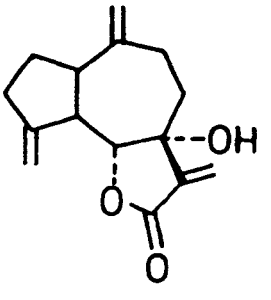
The chemical constituents of Saccogyna viticulosa collected on Arran have been investigated. Two sesquiterpenoids with an unusual carbon skeleton, the alcohol saccogynol (287) and the corresponding hydrocarbon deoxysaccogynol (288) were isolated.

The major constituent, saccogynol (287), $C_{15}H_{22}O$, (m/z 218.1667), oil, $[\alpha]_D$ + 14.4° (c, 0.90 in $CHCl_3$), [ν_{max} 3610 cm^{-1}], has a secondary alcohol [δ_H 4.45 (br t, J 7 Hz), δ_C 73.8 (d)], two exomethylene groups [δ_H 5.13 (dt, J 0.7, 1.7 Hz) and 5.03 (br t, J 1.6 Hz), δ_C 109.7 (t) and 156.2 (s); δ_H 4.82 (dq, J 1.7 0.9 Hz) and 4.76 (br dd, J 1.6, 1.7 Hz), δ_C 111.4 (t) and 150.5(s)*] and an isopropenyl group [δ_H 4.66 (2H, m), δ_C 110.2 (t) and 151.7 (s)*; δ_H 1.67 (t, J 1.1 Hz), δ_C 20.0 (q)] (asterisked values may be interchanged) which together with three methine and four methylene groups constitute a bicarbocyclic system. The assignment of all of the proton - proton couplings together with their eventual assignments (Scheme 23, chemical shifts in parentheses) based on homonuclear decoupling experiments at 360 MHz led to structure (287) for saccogynol.

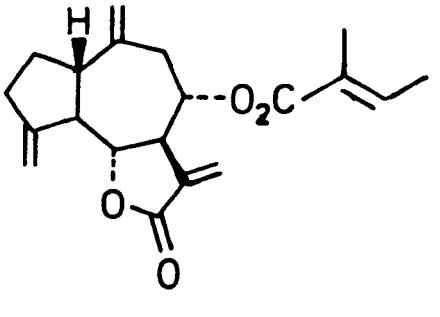
The relative stereochemistry was assigned following consideration of the magnitudes of the coupling constants. The value for $J_{1,5}$ (7.8 Hz) does not, in itself, define the ring-junction as trans, a similar value (9 Hz) also being observed in the case of cis-guaianolides e.g. 7 -


H-2α (1·70)
H-14 (4·76)
6·3
13·7
8·9
1·6
1·7
(4·45) H-3β
7·8
H-2β
7·8
H-1β
0·9
H-14 (4·82)
(2·36)
(2·81)
1·6
1·7
0·9
0·9
(5·03) H-15
H-15 (5·13)
(2·03) H-9α
12·8
H-9β (2·38)
10·0
5·7
4·7
4·9
(1·85) H-8α
13·0
H-8β (1·42)
0·6
1·6
1·7
7·8
4·2
11·4
4·8
3·7
(1·57) H-7α
13·7
H-7β (1·65)
2H-13 (4·66)
9·0
2·0
2×1·1
3H-12 (1·67)
(2·69) H-5
9·5
H-6 (2·19)

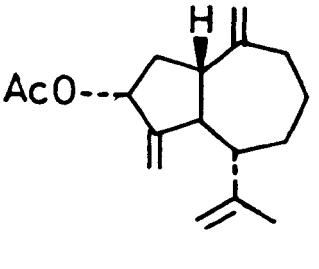
Scheme 23.



(289)



(290)



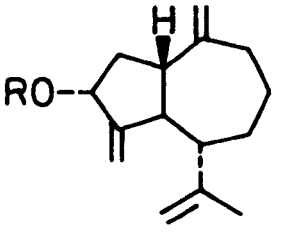
(291)

hydroxy-3-deoxyzaluzanin C (289)[170]. However, the degree of conformational flexibility conferred upon the molecule by a cis ring-fusion would be expected to reduce the size of $J_{1,2\alpha}$ to approximately 3-4 Hz. Since both $J_{1,2}$ values are large this would suggest that the less flexible trans junction is present. It is also difficult to reconcile the ring B couplings, especially the ^4J coupling between H-7β and H-9β, with a cis-fused molecule. Hence a trans fused system is proposed.

Relatively few examples of trans fused guaiane derivatives appear in the literature and hence it was difficult to find a suitable model system with which to compare the spectroscopic data for (287). One example, however, is ferreyanthuslactone (290)[171]. Here $J_{1,5}$ is 9 Hz and this, along with $J_{5,6}$ (10 Hz) and $J_{1,2}$ (2 x 9 Hz), is in good agreement with the values observed for saccogynol.

It is relatively straightforward to determine the stereochemistries at C-3 and C-6. The large value of $J_{5,6}$ 9.5 Hz) indicates the trans disposition of the relevant protons. The C-3 hydroxyl group must be α-oriented in order that H-3 possesses two large couplings. In the C-3 epimer, models indicate that the value of $J_{2\beta,3\alpha}$ is approximately zero.

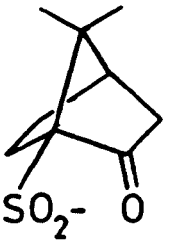
Upon standing with Ac_2O in pyridine, saccogynol (287) formed the oily acetate (291), $C_{17}H_{24}O_2$ (m/z 260),

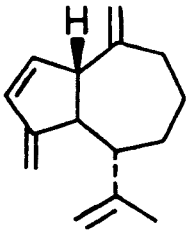


(292) R=<u>p</u>-BrC_6H_4CO-

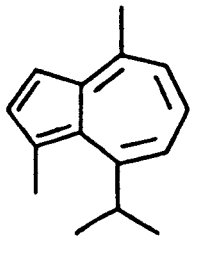
(293) R=<u>p</u>-$BrC_6H_4SO_2$-

(294) R=





(295)



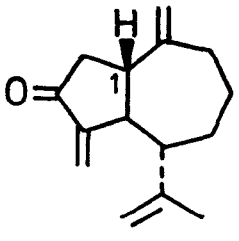
(296)

[ν_{max} 1740 cm^{-1}], whose ^{1}H and ^{13}C nmr spectra showed the expected deshielding of H-3 and C-3 [δ_H 5.48 (ddt, J 8.6, 5.8, 1.8 Hz), δ_C 75.4 (d)].and the appearance of signals characteristic of an acetate group [δ_H 2.04 (s), δ_C 21.2 (q) and 170.7 (s)].

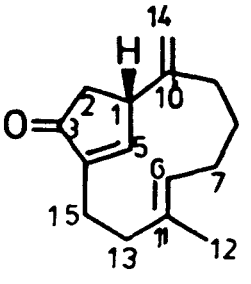
In an attempt to prepare a suitable heavy-atom X-ray derivative, the alcohol (287) was treated with p-bromobenzoyl chloride in pyridine. The resultant oily p-bromobenzoate (292), [ν_{max} 1740 cm^{-1}] again exhibited the expected deshielding of H-3 [δ_H 5.70 (ddt, J 6.0, 8.0, 1.5 Hz)] and signals characteristic of a para-disubstituted benzene ring [δ_H 7.87 and 7.54 (AA'BB' system, $J_{AB+AB'}$ 9 Hz)].

However, efforts to prepare the p-bromobenzene-sulphonate (293) and D-camphorsulphonate (294) of saccogynol were unsuccessful. Attempted preparative tlc of the crude reaction products on SiO_2 resulted in the appearance of a blue colour and their irreversible adsorption onto the chrmmatographic support. It seems likely that the elements of the sulphonic acid are easily eliminated from (293) and (294) to yield the olefin (295) which rearranges on SiO_2 with concomitant dehydrogenation to form an azulene derivative e.g. (296). The same result was observed during chromatography of the crude reaction product from dehydration of saccogynol.

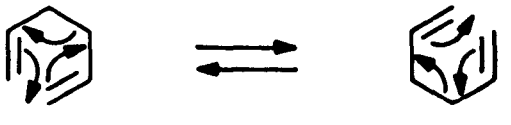
Oxidation of saccogynol (287) readily took place



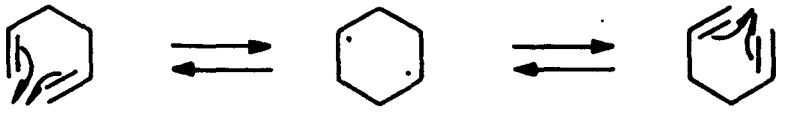
(297)



(298)



Scheme 25

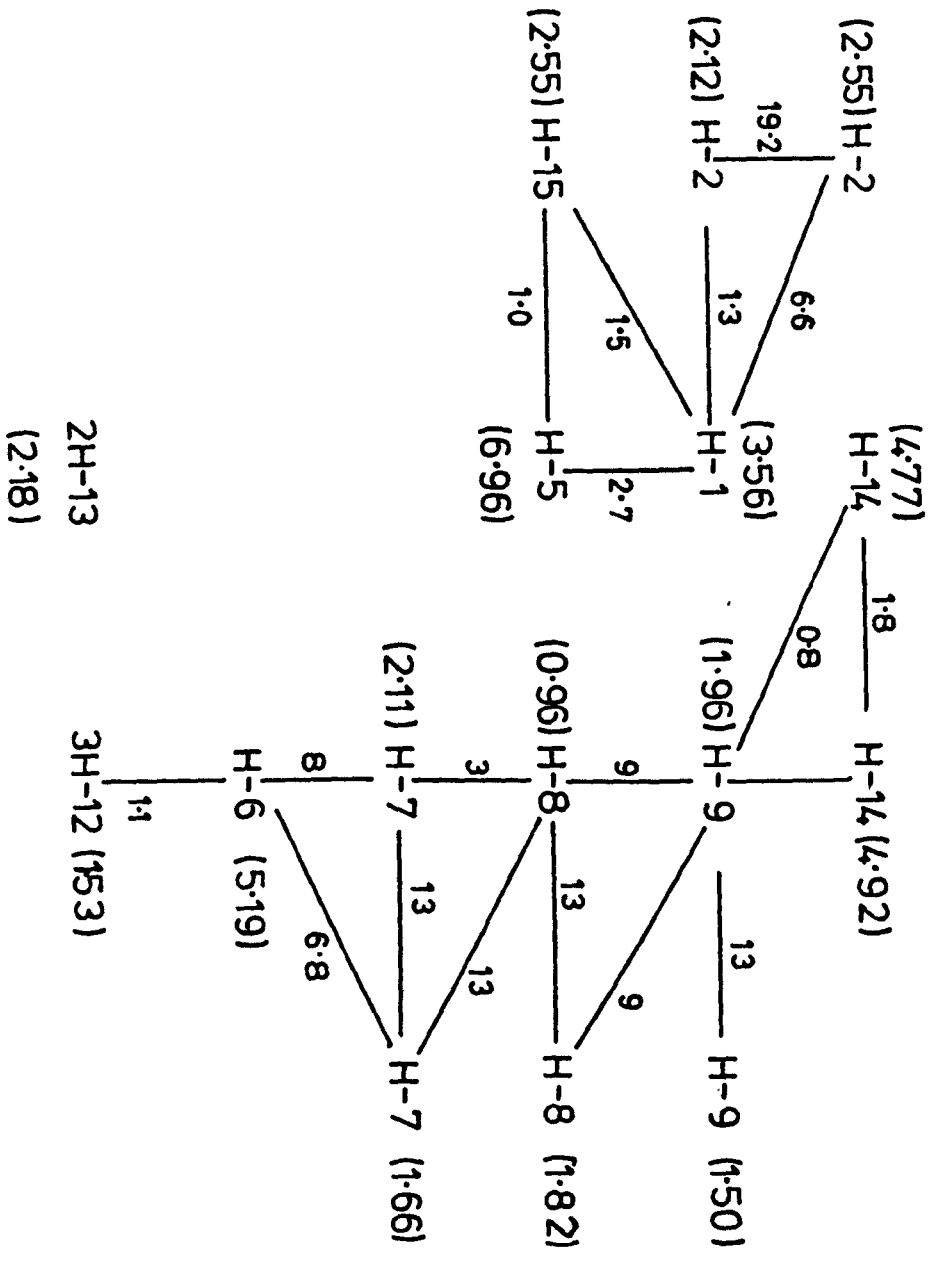


(299)

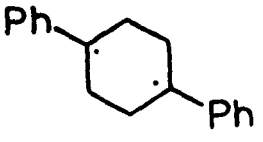
Scheme 26

on treatment of the alcohol with activated MnO_2[172] or Collins' reagent[173]. The product was not the expected enone (297) but rather a crystalline compound, m.p. 125-127°C, $C_{15}H_{20}O$ (m/z 216), $[\alpha]_D$ + 197.8° (c, 0.91 in $CHCl_3$), [ν_{max} 1700 cm^{-1}; λ_{max} 249 nm (ϵ 2600)] having a vinyl methyl [δ_H 1.53 (d, J 1.1 Hz), δ_C 14.8 (q)], an exomethylene group [δ_H 4.92 (br t, J 1.8 Hz) and 4.77 (dd, J 1.8, 0.8 Hz), δ_C 113.1 (t) and 150.6 (s)], an α-substituted cyclopentenone [δ_H 6.96 (dd, J 2.7, 1.0 Hz), δ_C 144.4 (s), 162.22(d) and 209.0 (s)] and a tri-substituted olefin [δ_H 5.19 (br t, J 7.5 Hz), δ_C 128.3 (d) and 134.2 (s)].which along with one methine and six methylene groups constitute a bicarbocyclic system. Consideration of the 360 MHz ^1H nmr spectrum and homo-nuclear decoupling experiments allowed the assignment of the proton-proton coupling constants together with the eventual assignments shown in Scheme 24 (chemical shifts in parentheses). The results are consistent with structure (298) derived from enone (297) by a Cope rearrangement.

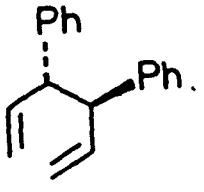
The Cope rearrangement of hexa-1,5-dienes[174] to isomeric forms is generally regarded as being a [3,3] sigmatropic rearrangement (Scheme 25). However the possible intervention of a cyclohexane-1,4-diyl intermediate (299) was recognised[175] (Scheme 26) and recently received experimental support from the work of Dewar and Wade[176] who showed that phenyl substituents in the 2- or 2,5-


(2·55) H-2
19·2
(2·12) H-2
6·6
1·3
1·5
(2·55) H-15
1·0
H-5
(6·96)
2·7
(3·56)
H-1
(4·77)
H-14
1·8
0·8
(1·96) H-
9
H-14 (4·92)
9
(0·96) H-8
13
H-9 (1·50)
13
9
13
H-8 (1·82)
(2·11) H-7
3
8
H-6 (5·19)
13
6·8
13
H-7 (1·66)
2H-13
(2·18)
1·1
3H-12 (1·53)

Scheme 24.



(300)



(301)

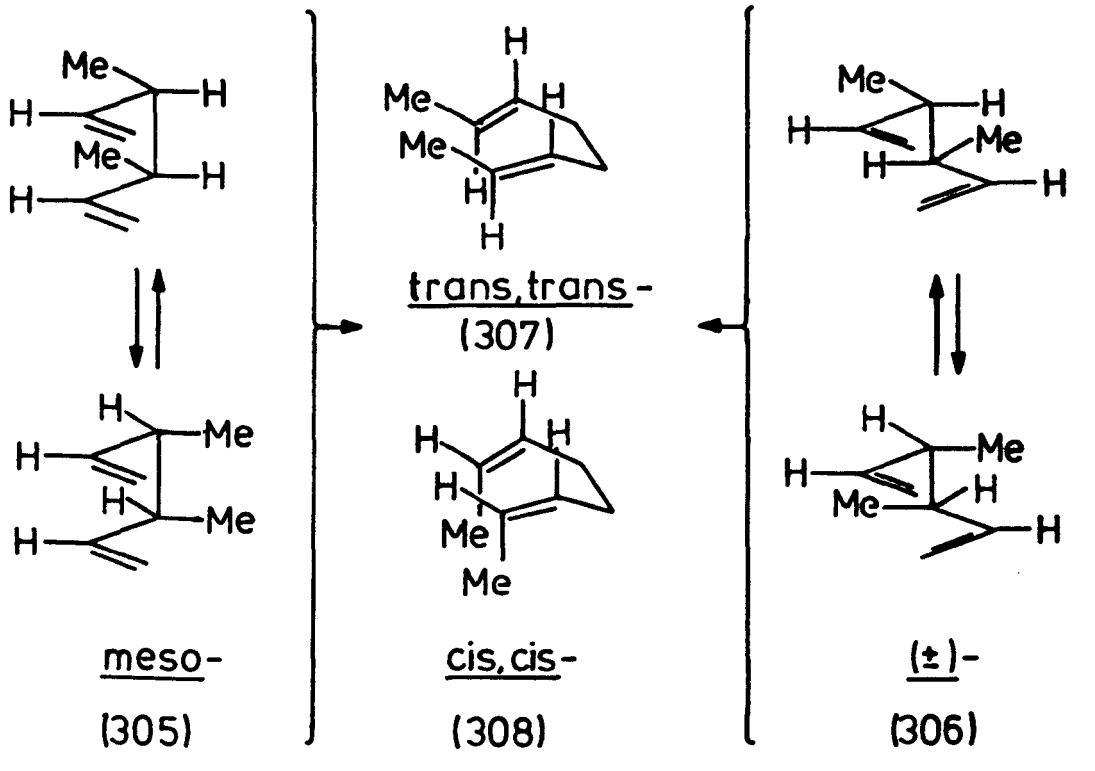
(302)

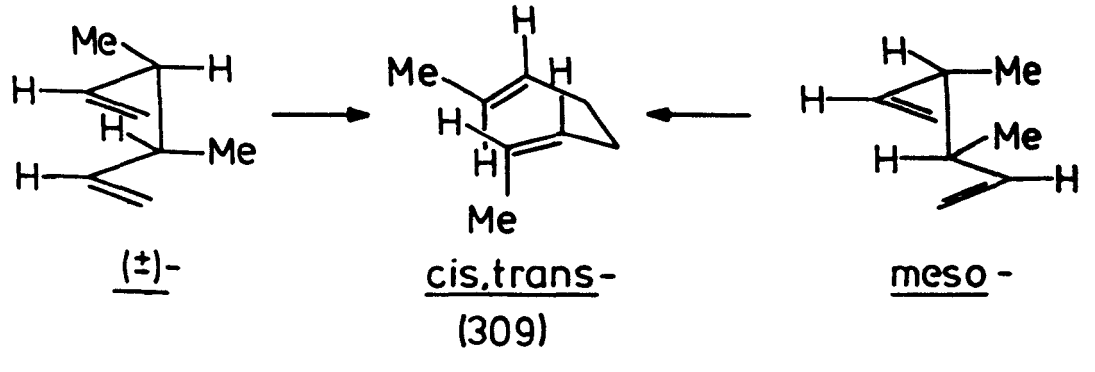
(303)

(304)

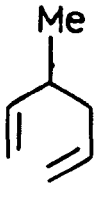
positions which should promote stabilisation of the radical centres of a species such as (300) did indeed significantly enhance the rate of the Cope rearrangement. However, Lutz and Berg[177] established that (±)-3,4-diphenylhexa-1,5-diene (301), a substrate with both phenyl groups positioned in such a way as to preclude participation in cyclohexane-1,4-diyl delocalisation, also exhibited a significant rate enhancement. This activation by unsaturated substituents in the 3- and/or 4-positions is typical of Cope rearrangements and is usually interpreted as stabilisation of a transition state resembling a pair of weakly interacting allyl radicals (302). Thus it seems likely that whereas the unsubstituted hexa-1,5-diene and its derivatives with conjugative substituents in positions 1,3,4 or 6 follow the pericyclic route, systems with radical stabilising substituents in positions 2 or 5 prefer to react via the diradicaloid pathway.

In suprafacial-suprafacial[3,3] sigmatropic processes exemplified by the vast majority of Cope rearrangements, two possible geometries have been considered for the cyclic transition state, the chairlike arrangement (303) and the boatlike arrangement (304). It is clear that, for molecules which can readily adopt either arrangement, the former geometry is strongly favoured. Moreover, of two alternative chairlike arrangements, the one which minimises 1,3-pseudodiaxial interactions is preferred.





Scheme 27



(310)

This has been demonstrated (Scheme 27)[178] for the Cope rearrangements of meso-3,4-dimethylhexa-1,5-diene (305) and (±)-3,4-dimethylhexa-1,5-diene (306). If a boatlike transition state is involved, the meso-isomer will give trans, trans-octa-2,6-diene (307) and/or cis, cis-octa-2,6-diene (308) and the (±)-isomer will give cis, trans-octa-2,6-diene (309). If the chairlike arrangement is preferred in the transition state, the outcome will be the reverse - the (±) isomer will give a mixture of trans, trans - and cis, cis-, while the meso-isomer will give cis, trans-octa-2,6-diene.

When heated for 6h at 225°C the meso-isomer yielded predominantly (99.7%) cis, trans-isomer. The (±)-isomer, after 18 h at 180°, yielded trans, trans-(90%) and cis, cis-octa-2,6-diene (10%). This shows that the chairlike transition state is favoured.

The (±)-isomer has the choice between a chairlike transition state in which both methyl groups are axial (leading to the cis, cis-isomer) and one in which both methyl groups are equatorial (leading to the trans,trans-isomer). Since mainly trans, trans-octa-2,6-diene is formed the latter transition state must be preferred.

The majority of Cope rearrangements proceed only at elevated temperature e.g. 3-methylhexa-1,5-diene (310) rearranges at 210-260°C in the gas phase[179]. A number, however, proceed at lower temperature, normally as a

(311) (312)

Scheme 28

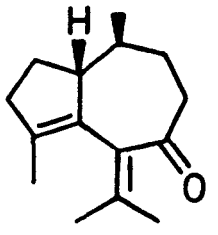
(313) (314)

Scheme 29

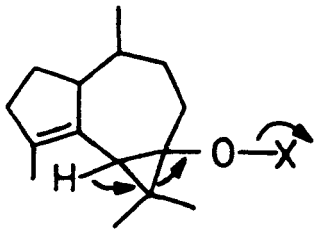
result of some special feature of the system e.g. relief of ring-strain as in the case of cis-1,2-divinylcyclopropane (311)[180] which rearranged to cyclohepta-1,4-diene (312) at -20°C (Scheme 28) or transition-metal catalysis exhibited by cis-1,2-divinylcyclobutane (313)[181] which was quantitatively converted to cis-cyclo-octa-1,5-diene (314) (Scheme 29) at 24°C in the presence of a nickel catalyst bearing the tri-(2-biphenyl)phosphite ligand, as opposed to the purely thermal process[182] which requires temperatures of 80-120°C.

The Cope rearrangement of the enone (298) occurs rapidly at room temperature. A model of (298) indicates that the hexa-1,5-diene system is held in the energetically most favourable quasi-chair conformation, thus the entropy factor is reduced. Also an electron-withdrawing group at the 2-position causes a large rate ehhancement; Wehrli et al.[193] found that cyano- or carbomethoxy-substituents at positions 2 or 5 caused a dramatic acceleration ($\times 10^5$ at 150°C). Whilst it is likely that neither of these factors alone would be enough to cause the reaction to proceed at such a low temperature, together they are sufficient.

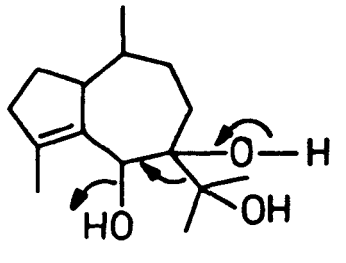
Also present in the extract was the hydrocarbon deoxysaccogynol (288). Unfortunately it proved impossible to purify since decomposition occurred when it was subjected to chromatography over $AgNO_3$-impregnated SiO_2.



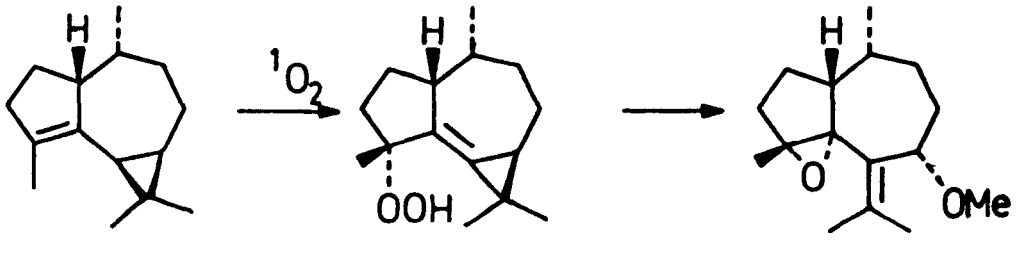
(315)



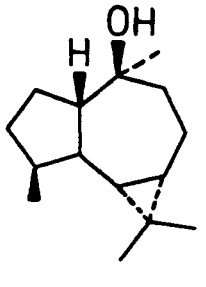
Scheme 30



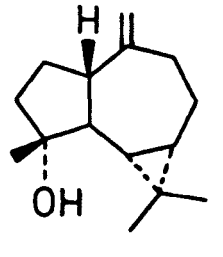
Scheme 31



Scheme 32



(316)



(317)

Glc indicated that the mixture contained mainly one component. The ^{1}H nmr spectrum (see Experimental) is similar to that of saccogynol (287) except that it lacked the secondary hydroxyl resonance. The ^{13}C nmr spectrum is also consistent with structure (288).

Saccogynol (287) and deoxysaccogynol (288) are two new members of the rare zierane class of sesquiterpene. Indeed only one other natural product possessing this skeleton, zierone (315), has previously been reported[184]. It is probable that this skeleton arises by cleavage of the cyclopropane ring in an aromadendrane precursor (Scheme 30) or a 1,2-isopropyl shift in a guaiane precursor (Scheme 31). Zierane sesquiterpenes have been synthesised[185] by sensitised photo oxygenation of gurjunene (Scheme 32).

The absolute configuration of the Saccogyna sesquiterpenoids has not been determined. Previously aromadendrane sesquiterpenoids e.g. ent-globulol (316)[49] and ent-spathulenol (317)[49] have been isolated from the Hepaticae and have proved to be the enantiomers of the compounds isolated from higher plants. It is expected that saccogynol also belongs to the enantio series.

EXPERIMENTAL

Saccogyna viticulosa

The plant material (200g) was collected on Arran. The crude extract (9g) was subjected to the usual chromatographic separation to give two major compounds.

i) Deoxysaccogynol (288)

The first column fraction contained mainly deoxysaccogynol (288). The terpenoid material was isolated by steam distillation but further purification by preparative tlc was unsuccessful. Chromatography over $AgNO_3$-impregnated SiO_2 resulted in decompositon of the material. The following spectral data were obtained on a mixture of deoxysaccogynol (288) and an unidentified compound (approximately 10% of the latter by glc using a 1% OV-1 column at 80°C).

ν_{max} : 2930, 1645 and 890 cm^{-1}

δ_H : 4.83 (2H, m, exomethylene protons);
4.71-4.65 (4H, m, exomethylene protons);
1.71 (t, J 1.1 Hz, vinyl methyl).

δ_C : 20.0 (q), 29.0 (t), 30.1 (t), 31.3 (t), 34.7 (t), 36.8 (t), 48.4 (d), 49.3 (d), 52.4 (d), 106.8 (t), 109.6 (t), 110.3 (t), 150.1 (s), 152.8 (s) and 154.4 (s).

ii) Saccogynol (287)

Steam distillation and preparative tlc of a later fraction gave saccogynol (287), (185 mg), oil, $[\alpha]_D$ + 14.4°

(C, 0.90 in $CHCl_3$), m/z 218.1667 ($C_{15}H_{22}O$ requires m/z 218.1671).

ν_{max} : 3610, 2930, 1645 and 890 cm^{-1}

δ_H : see Scheme 23 (p.117)

δ_C : 20.0 (q), 29.9 (t), 34.6 (t), 36.1 (t), 39.0 (t), 46.6 (d), 48.2 (d), 50.0 (d), 73.8 (d), 109.7 (t), 110.2 (t), 111.4 (t), 150.5 (s), 151.7 (s) and 156.2 (s).

Acetylation of Saccogynol (287)

To a solution of saccogynol (287), (25 mg), in dry pyridine (2 ml) was added Ac_2O (1 ml). After 12 h MeOH (10 ml) was added and the solvent removed to give the acetate (291), (26 mg), as a gum, m/z 260 ($C_{17}H_{24}O_2$ requires m/z 260).

ν_{max}: 2930, 1740 and 890 cm^{-1}.

δ_H : 5.48 (ddt, J 5.8, 8.6, 1.8 Hz, CH OAc); 5.13 and 5.06 (2 br s, exomethylene group); 4.81 and 4.75 (2 br s, exomethylene group); 4.67 (2H, br s, exomethylene group); 2.06 (s. OAc); 1.67 (br s, vinyl methyl).

δ_C : 20.4 (q), 21.2 (q), 29.7 (t), 34.5 (t), 35.9 (t), 36.3 (t), 46.9 (d), 48.1 (d), 50.7 (d), 75.4 (d), 110.3 (t), 111.5 (t), 112.1 (t), 149.6 (s), 151.2 (s), 151.5 (s) and 170.7 (s).

Saccogynol Bromobenzoate (292)

To a solution of saccogynol (287), (20 mg), in dry pyridine (1 ml) was added p-bromobenzoyl chloride (23 mg). After 24 h an acidic work-up gave, after preparative tlc, the oily p-bromobenzoate (292), (30mg).

ν_{max} : 1740 and 890 cm^{-1}

δ_H : 7.87 and 7.54 (AA'BB' system, $J_{AB+AB'}$ 9Hz); 5.70 (ddt, J 6.0, 8.0, 1.5 Hz, CHOR); 5.21 and 5.13 (2br s, exomethylene); 4.81 (2H, m, exomethylene); 4.66 (2H, br s, exomethylene); 1.67 (br s, vinyl methyl)

Attempted Preparation of Saccogynol p-Bromobenzenesulphonate(293

To saccogynol, (20 mg), in dry pyridine (2 ml) was added p-bromobenzenesulphonyl chloride (25 mg). After 12h solvent was removed under reduced pressure. Upon preparative tlc decomposition occurred and a blue colour appeared. No material was recovered.

Attempted Preparation of Saccogynol D-Camphorsulphonate (294)

The above experiment was repeated using D-camphorsulphonyl chloride in place of p-bromobenzene-sulphonyl chloride. The same result was observed.

Dehydration of Saccogynol (287)

To a stirred solution of saccogynol (20 mg) in dry pyridine (2 ml) at 0°C was added freshly distilled $SOCl_2$ (7 drops). After 5 min the solution was warmed

to room temperature, poured onto a mixture of ice and saturated $NaHCO_3$, and extracted with Et_2O to give a gummy residue which turned blue upon preparative tlc. No material was recovered.

Oxidation of Saccogynol (287)

a) Saccogynol (30 mg) and activated MnO_2 (100 mg), prepared according to the method of Goldman[186], were stireed overnight in dry benzene (10 ml). Filtration through celite yielded a mixture of compounds from which, the major product the rearranged ketone (298), was isolated by preparative tlc. Sublimation under reduced pressure gave crystals, (20mg), m.p. 125-127°C, $[\alpha]_D$ + 147.8° (C, 0.91 in $CHCl_3$), m/z 216 ($C_{15}H_{20}O$ requires m/z 216).

ν_{max} : 1700 cm^{-1}

λ_{max} : 249 nm (ϵ 2600)

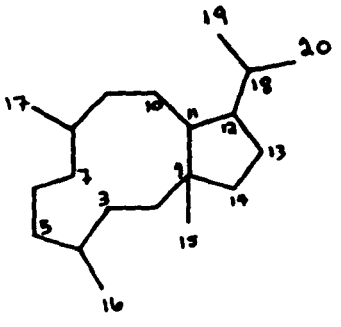
δ_H (360 MHz): See Scheme 24 (p.121)

δ_C : 14.8 (q), 23.0 (t), 26.1 (t), 26.1 (t0, 33.1 (t), 36.9 (t), 41.0 (t), 47.7 (d), 113.1 (t), 128.3 (d), 134.2 (s), 144.4 (s), 150.6 (s), 162.2 (d) and 209.0 (s).

b) CrO_3 (83 mg) and dry pyridine (131 mg) were stirred for 15 min in dry CH_2Cl_2 (5 ml). Saccogynol (25 mg) in dry CH_2Cl_2 (5 ml) was added in one portion and stirring continued for a further 15 min. The usual work-up gave, after preparative tlc, the enone (298).

CHAPTER 5

THE LOPHOZIOIDEAE



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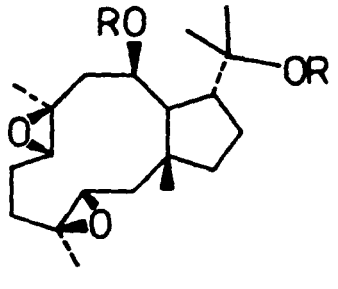
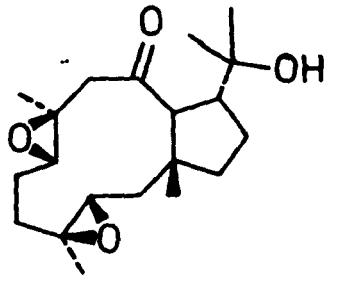


(148) R = Ac

(148a) R=H



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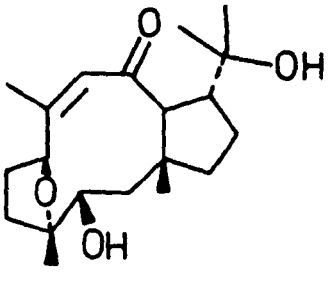

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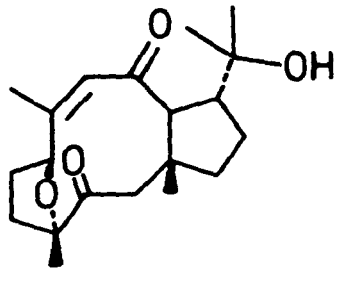
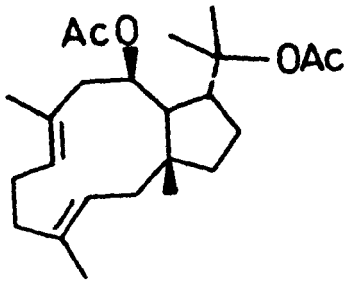


(321)

Introduction

The family Jungermanniaceae is divided into subfamilies, the largest of which is Lophozioideae. (The subfamily Jungermannioideae is considered in Chapter 6). Four of the eleven genera which constitute the Lophozioideae have been previously investigated and it appears that, in most cases, diterpenoids are the characteristic metabolites. In 1971, Huneck and Overton[100] isolated diterpenoids from Barbilophozia floerkei, B. lycopodioides, Anastrepta orcadensis and Gymnocolea inflata. However it is only recently that the structures of these diterpenoids have been determined by Connolly and Huneck[26]. The Barbilophozia diterpenoids proved to have the dolabellane skeleton (318) (see p.30) previously known only from marine sources. The major constituent, barbilycopodin (148), was identified by spectroscopic and chemical studies which were carried out upon the derived diol, floerkein B (148a). Oxidation of floerkein B gave the corresponding ketol (319), the ir of which possesses a carbonyl band at 1687 cm^{-1} indicating that the ketone is part of a medium ring. Mild base treatment of the ketol (319) resulted in β-elimination followed by trans annular opening of the second epoxide to give the ether (320) which could be oxidised to the diketone (321). X-ray analysis of floerkein B (148a) revealed the relative stereochemistry. Reduction of barbilycopodin to the



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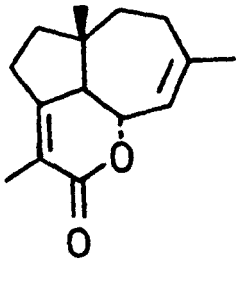


(323)

corresponding diene (322) in a low and variable yield was achieved using a Zn-Cu couple. A minor product was a mono-epoxide which is also present in the plant extract . Hercynolactone(323) a new sesquiterpenoid belonging to the uncommon carotane class has been isolated from B. lycopodioides and B. hatcheri and its structure and relative configuration determined by X-ray analysis[187].

The liverwort Anastrepta orcadensis proved to be an interesting source of diterpenoids and has been shown to exist in three distinct chemical races. From German and Scottish samples of A.orcadensis were isolated the novel clerodanes anastreptin (114) and orcadensin (113) respectively[100]. Their structures were assigned following spectroscopic studies but the stereochemistries are still undetermined. Another sample from Germany contained[117] anadensin (151) (see p.31), the structure and relative stereochemistry of which was determined by X-ray analysis.

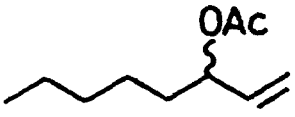
The final diterpenoid-containing liverwort in this group, Gymnocolea inflata, contains a number of bitter components[101]. One of these, gymnocolin, was assigned the cis-clerodane stucture (112) on the basis of spectroscopic studies and an X-ray analysis[101]. The absolute configuration remains undetermined.

A diterpenoid has been reported[148] from Lophozia Cornuta and L. incisa following a gcms investigation of these species. However, no structure has been proposed.

Only a few sesquiterpenoids have been reported from this subfamily. Anastreptene (30), an air-sensitive aromadendrane derivative, was first isolated[48] from Anastrepta orcadensis (see p.13) and has since been shown to be a common liverwort metabolite. The characteristic liverwort sesquiterpene β-barbatene (43) is common among Barbilophozia species.

Huneck[134] has reported the isolation of two unidentified sesquiterpenoids, ventricosin A and ventricosin B, from Lophozia ventricosa (see p.137).

This chapter deals mainly with an investigation of various samples of Anastrepta orcadensis and Barbilophozia floerkei, the latter yielding two novel dolabellanes. Also reported are the structures of ventricosins A and B.



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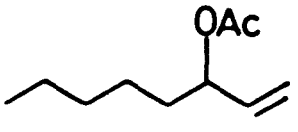
DISCUSSION

Anastrepta orcadensis

A sample of A. orcadensis, collected near Bridge of Orchy in Scotland, contained no diterpenoids. However, a sample collected in the German Democratic Republic (GDR) contained both barbilycopodin (148) and anadensin (151) which were readily identified by comparison of their ^1H nmr specta with those of authentic samples. This result suggested that the sample consisted of a mixture of liverworts and probably contained Barbilophozia species. The lack of purity was further indicated by the isolation of chiloscypholone (270) (see p.92) from the extract. By employing preparative tlc on $AgNO_3$ - impregnated SiO_2, it proved possible to isolate anastreptene (30) and B-barbatene (43) from the non-polar column fractions. These were identified by comparison of their ^1H nmr spectra with the reported values. The ^{13}C nmr spectra of these compounds have been measured for the first time (see Experimental).

The final compound, also isolated from the non-polar fractions by preparative tlc over $AgNO_3$ - impregnated SiO_2, was oct-1-en-3-yl acetate (324), an oil, [ν_{max} 1740 cm^{-1}]. It has a vinyl group [δ_H 5.79 (ddd, J 5.5, 9.0, 17.0 Hz, H-2), δ_C 116.5 (t, C-1) and 136.7 (d, C-2)], the resonances due to 2H-1 overlapping with those of an allylic oxygen-bearing methine [δ_C 74.9 (d, C-3)]. The



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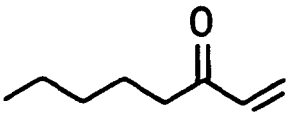


(326)

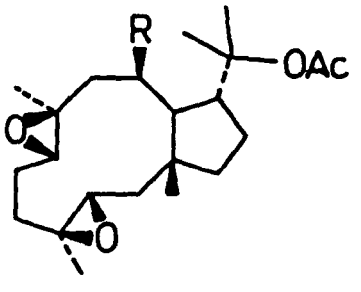
presence of an acetate group [δ_H 2.04 (s), δ_C 21.2 (q) and 170.3 (s)] and signals characteristic of an n-alkyl chain suggested structure (324). Comparison of the ^1H nmr spectrum with literature[188] values confirmed this identification. Oct-1-en-3-yl acetate (324) has recently been detected in the liverwort Conocephalum conicum by gcms[29]. Unfortunately, the sample of (324) decomposed before the optical rotation could be measured.

Alk-1-en-3-ols are comonly encountered in Nature. Oct-1-en-3-ol (325) and the corresponding ketone (326) are responsible for the odour of the cultivated mushroom[189], and the flavour of oxidised dairy products[190.191]. Among the precursors which have been proposed for oct-1-en-3-ol are the hydroperoxides of linoleic acid and arachidonic acid.[191]

Barbilophozia floerkei

Three samples of B.floerkei were investigated, one collected in the Harz Mountains in the GDR and one each in southern Scotland (Loch Doon) and central Scotland (Stirling). Whereas barbilycopodin (148) was present in both Scottish samples, the German sample lacked this diterpenoid. Two new dolabellanes have been isolated from the German and the southern Scottish samples.

The first sample to be investigated was that from Loch Doon in southern Scotland. The major constituent of the Et_2O extract proved to be barbilycopodin (148)

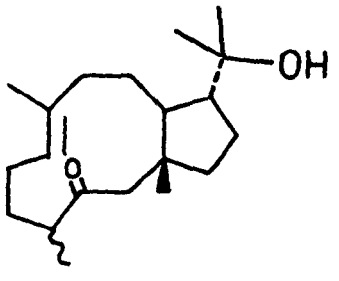


(148) R = OAc

(327) R = H

which was identical with an authentic sample. Also present was a smaller amount of a less polar diterpenoid, 18-acetoxy-3(S*), 4(S*), 7(S*), 8(S*)-diepoxydolabellane, 10-deacetoxybarbilycopodin (327), m.p. 89-91°C, $C_{22}H_{36}O_4$ (m/z 364.2636), $[\alpha]_D$ - 16.8° (C, 1.43 in $CHCl_3$), [ν_{max} 1730 cm^{-1}]. It has two epoxides [δ_H 3.05 (dd, J 4, 8 Hz, H-3) and 2.78 (br d, J 8 Hz, H-7), δ_C 61.1 (s), 61.5 (s), 61.6 (d) and 64.9 (d)], an acetate group [δ_H 1.96 (3H, s), δ_C 21.9 (q) and 170.3 (s)], a tertiary oxygen-bearing carbon [δ_C 84.9 (s)] and five tertiary methyls [δ_H 1.55, 1.49, 1.39, 1.31 and 1.19 (5S), δ_C 17.1 (q), 18.3 (q), 23.1 (q), 24.1 (q) and 26.1 (q)] which together with one fully substituted carbon, two methine and seven methylene groups constitute a bicarbocyclic system. The ^1H nmr spectrum is similar to that of barbilycopodin (148), the main difference being the absence of the signals due to the C-10 acetoxy group of barbilycopodin. This suggested that the new compound is simply 10-deacetoxybarbilycopodin (327). Comparison of the ^{13}C shifts of (327) with those of barbilycopodin (148) confirmed this supposition. Both C-8 and C-12 have moved downfield in (327) whereas C-11 and C-9 have moved upfield.

The second sample of B.floerkei examined was collected near Stirling in Scotland. Only barbilycopodin (148) was isolated from the extract.



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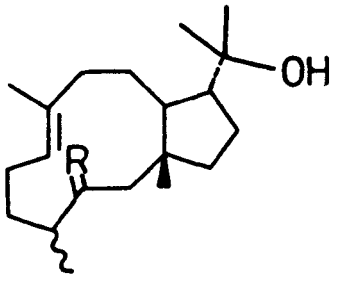
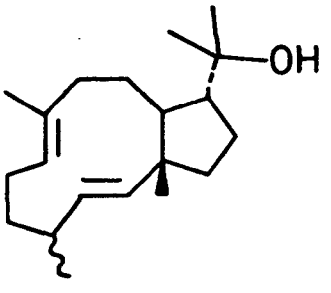
Examination of the extract of German B.floerkei revealed the presence of two dolabellane diterpenoids. The major constituent is 18-hydroxydolabell-7(E)-en-3-one (328), an oil, $C_{20}H_{34}O_2$ (m/z 306.2594), $[\alpha]_D$ +52.9° (c, 1.43 in $CHCl_3$), [V_{max} 3610 and 1703 cm^{-1}]. It has a ketone [S_c 214.6 (s, C-3)], a trisubstituted double bond [δ_H 5.03 (dd sextets, J 7.0, 8.0, 1.0 Hz, H-7), δ_c 126.1 (d, C-6) and 136.7 (s, C-8)], an isolated methylene group [δ_H 2.41 and 2.26 (ABq, J_{AB} 18.6 Hz, 2H-2)], a methine [δ_H 2.28 (dd, J 4, 8, 6.8 Hz, H-4)], a vinyl methyl [δ_H 1.63 (d, J 1.5 Hz, 3H-17), δ_c 16.6 (q)], a dimethyl carbinol group [δ_H 1.26 and 1.25 (2s, 3H-19 and 3H-20), δ_c 26.6 (q), 29,3(q) and 74.0 (s)], a secondary methyl [δ_H 1.11 (d, J 6.8 Hz, 3H-16), δ_c 24.2 (q)] and a tertiary methyl [0.85 (s, 3H-15), δ_c 17.8 (q)].

Assuming a dolabellane skeleton, these data are accommodated by structure (328). The presence of an isolated methylene group served to position the carbonyl group at C-3 (the analogous position (C-13) in the cyclopentane ring is excluded since the ir spectrum [V_{max} 1703 cm^{-1}] clearly indicates the presence of a medium ring ketone). The trans nature of the double bond was deduced from the highfield position [δ_c 16.6 (q)] of C-17 in the ^{13}C nmr spectrum. Definitive proof for the position of the trisubstituted double bond is lacking but biogenetic analogy suggests a preference for the 7,8 position rather than 8,9. The stereochemistry of the secondary methyl



(328) R=O

(330) R= N-NHC$_6$H$_4$CH$_3$



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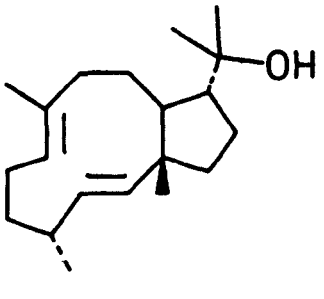

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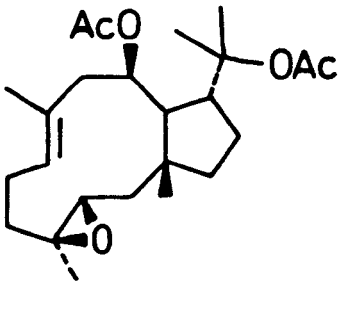


(332)

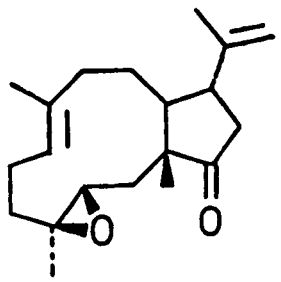
remains undetermined. Although H-4 can be clearly observed in the ^{1}H nmr spectrum [δ_H 2.28 (ddq, J 4, 8, 7 Hz)], the conformation of the molecule is unknown and therefore the relative configuration of C-4 cannot be assigned with any certainty.

An attempt was made to convert the ketone (328) into the diene (329) via the tosylhydrazone (330)[192] to enable comparison with the known[193] compound (331). However, the tosylhydrazone could not be formed under mild conditions; more vigorous conditions led to the decomposition of the material.

A minor constituent of this extract proved to be the mono-epoxide (332), previously isolated from the Zn-Cu couple reduction of barbilycopodin (148) (see p.131). The 3,4 position of the epoxide followed from a comparison of the chemical shift of the epoxide proton, H-4 [δ_H 2.98 (dd, J 3.4, 10.0 Hz)] with the corresponding values for the epoxides (333), (334) and (335) (Table 3). It can be seen that the higher-field epoxide resonance [$\delta_H \sim 2.75$] is due to H-7 whereas the lower-field signal [$\delta_H \sim 3.00$] is due to H-3.

Lophozia ventricosa

The final liverwort to be examined was L.ventricosa which was collected in the Harz Mountains, GDR. Two sesquiterpenoids, previously reported as ventricosin A and ventricosin B by Huneck et al.[134], were isolated from



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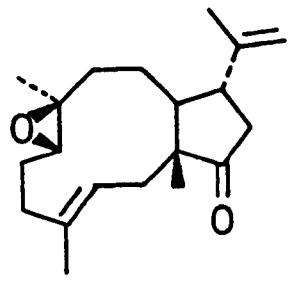

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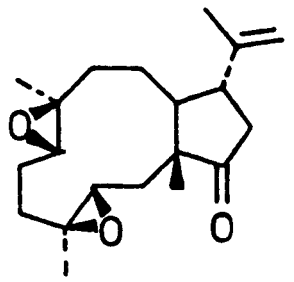
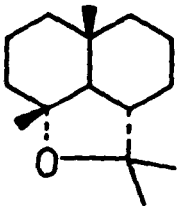


(335)

Table 3

	(333)	(334)	(335)
H-3	2·99	5·42	3·07
H-7	5·10	2·75	2·80



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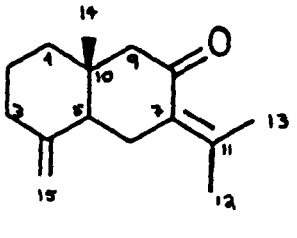

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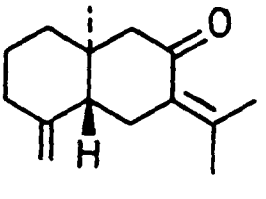
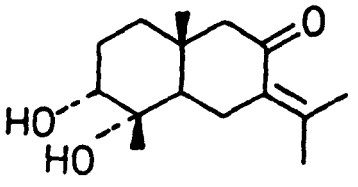


(338)

the Et_2O extract and were identified as known compounds.

Ventricosin B, a crystalline compound, m.p. 64-65°, $C_{15}H_{26}O$, $[\alpha]_D$ -36.1° (c, 1.16 in $CHCl_3$) has two tertiary oxygen - bearing carbons [δ_C 78.6 (s) and 81.4 (s)] and four tertiary methyls [δ_H 1.26, 1.07, 0.98 and 0.85 (4s), δ_C 17.9 (q), 22.8 (q), 25.8 (q) and 30.8 (q)] which together with one fully substituted carbon atom, two methine and six methylene groups constitute a tricyclic system. The identify of this compound with ent-maalioxide (336), previously isolated from Plagiochila acanthophylla subspecies japonica by Matsuo et al.,[194] was readily established by comparison of the spectroscopic data with the literature values.

The second compound, ventricosin A, was obtained as an oil, $C_{15}H_{22}O$, $[\alpha]_D$ - 80.5° (c, 1.41 in $CHCl_3$), [ν_{max} 1685 cm^{-1}; λ_{max} 246 nm (ϵ 7950)]. The ^1H nmr spectrum (see Experimental) is identical with the literature values for eudesma - 4 (15), 7 (11)-dien-8-one which has previously been isolated in both enantiomeric forms, the (+)-form (337) from Atractylodes japonica by Endo et al.[195] and the (-)-form (338) from Asarum caulescens by Endo et al.[196] and from Peteravenia schultzii by Bohlmann et al.[197]. The last two groups incorrectly assigned their isomer to the normal series. The magnitude and sign of the specific rotation of the compound from L.ventricosa indicate that it belongs to the enantio series and is therefore (338).



(339)

The ^{13}C nmr spectrum of this compound has not previously been reported. Comparison of the ^{13}C chemical shifts with those of cuauhtemone (339)[198] (Table 4) support the structural assignment.

Table 4

^{13}C nmr chemical shifts of eudesmane sesquiterpenoids

Carbon	(338)	(339)
1	37.0	32.9
2	23.4	25.7
3	41.4	74.3
4	149.1	73.1
5	47.1	45.6
6	29.3	25.7
7	131.5	131.2
8	201.8	202.0
9	57.6	60.2
10	38.0	36.3
11	144.1	144.1
12	22.2	22.7
13	23.2	23.4
14	17.3	18.6
15	107.0	21.4

Anastrepta orcadensis

The plant material was collected in the Harz Mountains in the G D R. The usual chromatographic separation of the crude extract (10.8g) gave a number of compounds.

i) Anastreptene (30)

Preparative tlc of the early fractions over $AgNO_3$ - impregnated SiO_2 gave anastreptene, (15mg), as a low-melting solid. The spectroscopic data were identical with those of the literature[48].

δ_C : 12.6 (q), 14.9 (q), 15.9 (q), 15.9 (q), 18.1 (s), 19.0 (t), 19.3 (s), 20.8 (d), 29.5 (d), 30.3 (d), 30.9 (t), 30.9 (t), 40.2 (s), 122.8(d) and 142.5 (s).

ii) β-Barbatene (43)

Preparative tlc of the early fractions over $AgNO_3$ - impregnated SiO_2 gave β-barbatene (17 mg) as an oil.

δ_H : 4.58 (br s, exomethylene); 1.02, 0.89 and 0.83 (3s, tertiary methyls).

δ_C : 23.4 (q), 24.8 (q), 27.5 (q), 22.5 (q) 28.7 (t), 35.5 (t), 37.1 (t), 38.1 (t), 43.1 (s), 46.8 (t), 54.1 (s), 55.4 (s), 56.0 (d), 107.5 (t) and 152.0 (s).

iii) Oct-1-en-3-yl Acetate (324)

Preparative tlc of a more polar fraction over $AgNO_3$ - impregnated SiO_2 gave the acetate (324), (33mg), as an oil.

ν_{max} : 1740 cm^{-1}

δ_H : 5.79 (ddd, J 5.5, 9, 17 Hz, H-2); 5.40 - 5.05 (m, 2H-1 and H-3); 2.04 (s, OAc).

δc : 14.0 (q), 21.2 (q), 22.6 (t), 24.8 (t), 31.6 (t), 34.2 (t), 74.9 (d), 116.5 (t), 136.7 (d) and 170.3 (s).

iv) Barbilycopodin (148)

From a late fraction was isolated, by crystallisation, barbilycopodin, (40mg), identical with an authentic sample from Barbilophazia floerkei.

v) Anadensin (151)

Anadensin (25mg) was isolated from a late fraction. It crystallised from MeOH as prisms m.p. 179-181°C [lit.[117] m.p. 181-182°C], ^1H nmr spectrum identical with that of an authentic specimen.

Barbilophozia floerkei

Extract A

Plant material (300g) was collected at Loch Doon in south-west Scotland and the crude extract (9.8g) subjected to the usual chromatographic purification to give two compounds.

i) 18-Acetoxy-3(S*), 4(S*), 7(S*), 8(S*)-diepoxy-dolabellane; 10-Deacetoxybarbilycopodin (327)

The mono-acetate (327), (43mg), crystallised from hexane as prisms, m.p. 89-91°C, $[\alpha]_D$ - 16.8° (C, 1.43 in $CHCl_3$), m/z 364.2636 ($C_{22}H_{36}O_4$ requires m/z 364.2613).

ν_{max} : 1730 cm^{-1}

δ_H : 3.05 (dd, J 4, 8Hz, H-3); 2.78 (d, J 8 Hz, H-7); 1.96 (s, OAc); 1.55, 1.49, 1.39, 1.31 and 1.19 (5s, tertiary methyls).

δ_C : 17.0 (q), 17.5 (q), 21.3 (q), 22.7 (q), 23.2 (t), 23.6 (q), 23.6 (q), 25.5 (t), 26.0 (q), 37.2 (t), 40.4 (t), 41.6 (t), 44.3 (s), 45.2 (d), 45.2 (t), 55.5 (d), 58.8 (s), 61.3 (s), 62.4 (d), 65.6 (d), 75.4 (d), 84.4 (s), 170.2 (s) and 170.3(s).

Extract D

The extract (5.6g) from a sample of B.floerkei collected in the G D R was chromatographed as usual to yield two compounds.

i) 10,18-Diacetoxy-3(S*), 4(S*)-epoxydolabell-7(E)-ene(332)

Crystallisation from a crude fraction gave the mono-epoxide (332), m.p. 161-162°C, $[\alpha]_D$ - 75°

δ_H (360 MHz): 5.42 (ddd, J 1.8, 7.2, 11.2 Hz, H-10); 5.20 (br d, J 12.0 Hz, H-7); 2.98 (dd, J 3.4, 10.0 Hz, H-3); 2.09 and 1.99 (2s, OAc); 1.69 (br s, 3H-17);

1.53, 1.51, 1.45 and 1.21 (4s, tertiary methyls).

δ_C : 16.2 (q), 16.8 (q), 21.5 (q), 22.8 (q), 23.0 (q), 23.9 (q), 24.3 (t), 25.7 (t), 25.8 (q), 38.6 (t), 42.0 (t), 42.2 (t), 44.4 (s), 44.4 (d), 45.7 (t), 54.8 (d), 62.3 (s), 63.8 (d), 76.6 (d), 84.6 (s), 128.6 (d), 130.4 (s), 170.4 (s) and 170.8 (s).

ii) 18-Hydroxydolabell-7(E)-en-3-one (328)

The ketone, (30 mg), was an oil, $[\alpha]_D$ + 52.9° (c, 1.43 in $CHCl_3$), m/z 306.2594 ($C_{20}H_{34}O_2$ requires m/z 306.2559).

ν_{max} : 3610 and 1703 cm^{-1}

δ_H (360 MHz): 5.03 (dd sextets, J 7.0, 8.0, 1.0 Hz, H-7): 2.41 and 2.26 (ABq, J_{AB} 18.6 Hz, 2H-2); 2.28 (ddq, J 4, 8, 7 Hz, H-4); 1.63 (d J 1.5 Hz, 3H-17); 1.26 and 1.25 (2s,3H-19 and 3H-20); 1.11 (d, J 6.8 Hz, 3H-16); 0.85 (s, 3H-15).

δ_C : 16.6 (q), 17.8 (q), 24.2 (q), 25.6(t), 25.6 (t), 26.6 (q), 29.3 (t), 29.3 (q), 34.1 (q), 37.7 (t), 39.5(t), 43.3 (t), 43.6 (d), 44.5 (s), 49.0 (t), 58.4 (d), 74.0 (s), 126.1 (d), 136.7(s) and 214.6 (s).

Lophozia ventricosa

From plant material which was collected in the Harz Mountains, G D R in September 1981 was obtained a crude extract (38g). The usual chromatographic separation gave two compounds.

i) ent-Maalioxide (336)

Low pressure sublimation gave ent-maalioxide (336), m.p. 64-65°C, $[\alpha]_D$ - 36.1° (c, 1.16 in $CHCl_3$) [Lit.[194] m.p. 66°C, $[\alpha]_D$ - 34.5°].

δ_H : 1.26, 1.07, 0.98 and 0.85 (4s, tertiary methyls).

δ_C : 17.9 (q), 21.3 (t), 22.3 (t), 22.8 (q), 25.8 (q), 27.5 (t), 30.8 (q), 34.2 (s), 40.7 (t), 41.1 (t), 43.1 (d), 43.4 (t), 58.4 (d), 78.6 (s) and 81.4 (s).

ii) ent-Eudesma-4(15),7(11)-dien-8-one (338)

The ketone was obtained as an oil, $[\alpha]_D$ - 80.5° (c, 1.41 in $CHCl_3$) [lit.[197] - 87.4°].

ν_{max} : 1685 cm^{-1}

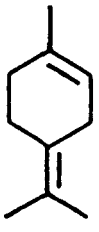
λ_{max} : 246 nm (ε 7950)

δ_H : 4.95 and 4.60 (2 br s, exomethylene); 2.24 (s, 2H-9); 1.96 and 1.78 (2 br s, vinyl methyls); 0.73 (s, 3H-14).

δ_C : see Table 4 (p. 140).

CHAPTER 6

THE JUNGERMANNIOIDEAE



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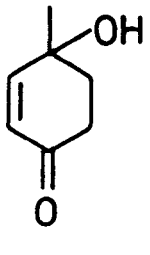

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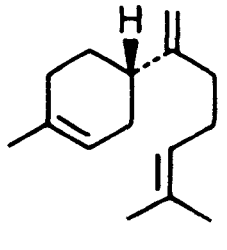

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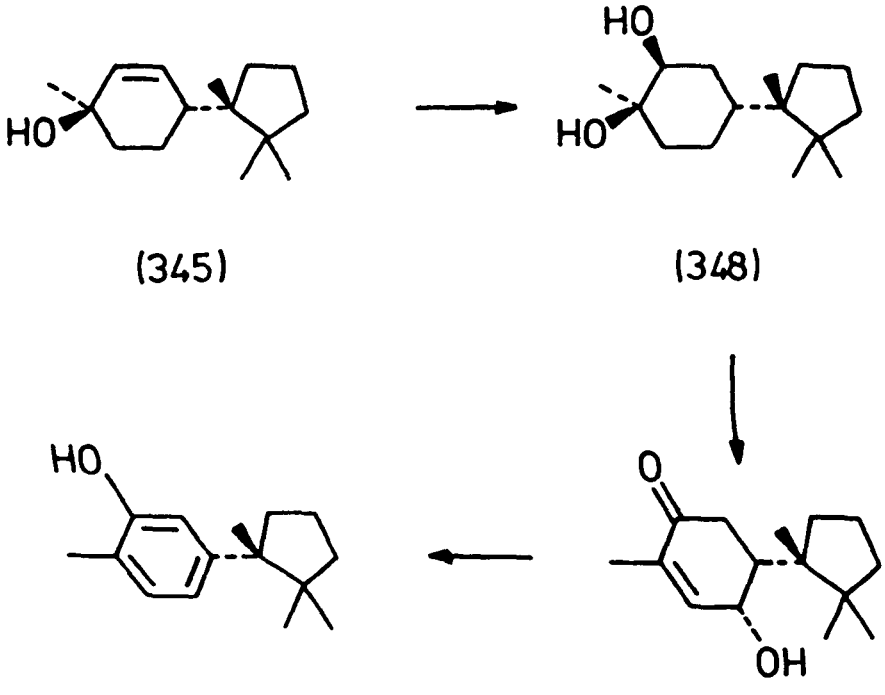
Introduction

The other main subfamily of the Jungermanniaceae is the Jungermannioideae. The major genus is Jungermannia, members of which appear in a number of guises in the literature. The most recent terminology will be used and synonyms given where appropriate . As is the case for the Lophozioideae, diterpenoids appear to be the significant chemical markers in most species. However, Jungermannia and Nardia species are pleasant - smelling, indicative of monoterpenoids. Indeed, J.obovata (Solenostoma obovatum) was one of Muller's[21] original list of liverworts with characteristic odours (see p. 9). In this case the sweet, carrot-like smell appears to be due to large amounts of limonene (15), terpinolene (340) and γ-terpinene (341)[34,199]. A number of other monoterpenes are also widespread among Jungermannia species, including myrcene (13), α-pinene (18), α-terpinene (16) and camphene (20). In the case of J.exsertifolia the chirality of some of the monoterpenoid constituents has been determined[30] (see p.11). The degraded monoterpenoid (342) has been isolated from J. obovata[26].

Sesquiterpenoids have only rarely been reported from the Jungermannioideae. β-Barbatene (43), β-bisabolene (343) and cuparene (256) are present in Nardia species[148] whilst the main sesquiterpenoid components of Jungermannia species are cuparanes. Cuparene is present in both J.infusca[32] and J.rosulans[200], the latter species also



(345) (348)

(344) (346)

Scheme 33

(347) (349)

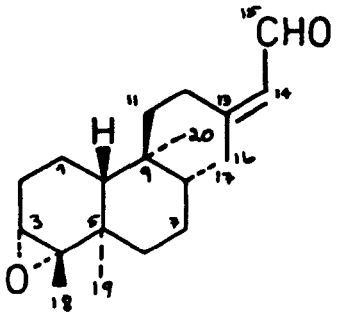
containing 2-hydroxycuparene (344), cuprenenol (345) and rosulantol (346). The metabolites of J. rosulans are of interest for two reasons. Cuprenenol (345) and rosulantol (346) are at a lower oxidation level than cuparene (347) and 2-hydroxycuparene (344) and provide an insight into the biogenesis of the cuparanes. Matsuo[200] has shown that rosulantol (346) is easily converted into 2-hydroxycuparene (344) and has also transformed cuparenenol (345) into the diol (348). A possible biogenetic pathway is illustrated in Scheme 33.

Cuparane sesquiterpenoids are quite common in the Hepaticae, (-)-cuparene (256) and (S)-2-hydroxycuparene (349) belonging to the ent series of absolute configuration having been isolated from Bazzania pompeana[201] and Marchantia polymorpha[33]. In contrast, the sesquiterpenoid metabolites of J. rosulans belong to the normal series. Unfortunately the presence of cuparene (256) in J. infusca[32] was detected by gcms and the chirality is unknown. Indeed this has been the method of analysis used for all other Jungermannia species investigated, apart from the isolation[202] of ent-longifolene (11) from J. exsertifolia subsp. cordifolia. Hence the Hepaticae produce both enantiomers of cuparanes.

Diterpenoids are very common metabolites of the Jungermannioideae, kauranes being the commonest but labdanes and pimaranes having also been identified. All

of the diterpenoids reported so far belong to the enantio series of absolute configuration and are reviewed in Chapter 1 (see p. 26).

This chapter is devoted to an investigation of the liverworts. Jungermannia paroica and Nardia scalaris. Both species have yielded diterpenoids.



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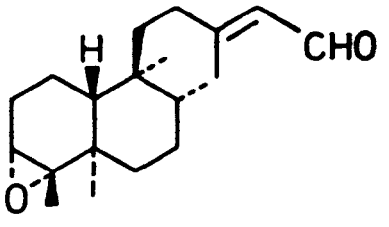

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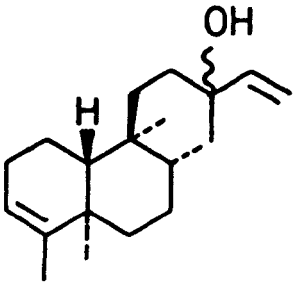
Discussion

Jungermannia paroica (Plectocolea paroica)

J. paroica is, in general, a rather rare species (less so in the West of Scotland) and is found only in small quantities. The plant material was collected at the Devil's Pulpit near Bearsden and, despite there being only 30g of plant material available, it was possible to isolate sufficient quantities of the major components to enable their identification. The liverwort has an extremely pleasant odour and it is probable that a number of mono-terpenoids are present. These, however, were not investigated.

Four diterpenoids were isolated and all proved to be simple clerodanes. Two of the metabolites were epoxy α,β -unsaturated aldehydes which differed only by isomerism around the olefinic bond. Their spectroscopic properties suggested a clerodane skeleton and the compounds proved to be identical with ent-3β, 4β-epoxyclerod-13(14) (Z)-en-15-al (350) and the corresponding (E)-isomer (351), both isolated previously from Solidago serotina[203]. The ^{13}C chemical shifts of these compounds have not been published but readily support the assignment of the double bond configuration. In the (Z)-isomer (350) the methyl group C-16 is at low field [δ_C 25.3] and the methylene C-12 at high field [δ_C 26.7] with respect to the corresponding resonances in the (E)-isomer (351), [δ_C 17.8 and 34.5 respectively].



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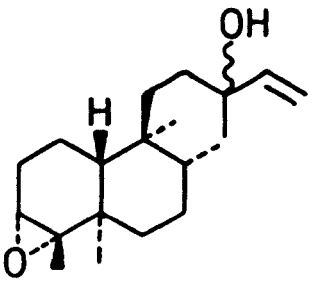
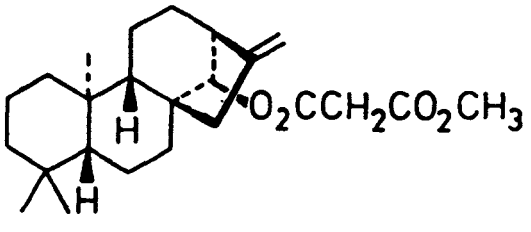


(353)

The third compound is the dienol ent-cleroda-3,14-dien-13ξol, kolavelool (352), previously isolated from Hardwickia pinnata[204] and Solidago elongata[205].

The final compound is ent-3β,4β-epoxyclerod-14-en-13-ol (353), $C_{20}H_{34}O_2$ (m/z 306.2568), an oil, $[\alpha]_D$ -29.6° (c, 1.07 in $CHCl_3$), [ν_{max} 3430 cm^{-1}]. It has a vinyl group [δ_H 5.92 (dd, J 10, 17 Hz, H-14), 5.23 (dd, J 2, 17 Hz, H-15) and 5.06 (dd, J 2, 10 Hz, H-15'), δ_C 112.0 (t, C-15) and 145.0 (d, C-14)], a tertiary alcohol [δ_C 73.3 (s, C-13)], an epoxide [δ_H 2.91 (br s, $W_{\frac{1}{2}}$5Hz,H-3), δ_C 62.2 (d, C-3) and 66.5 (s, C-4)] and five methyls (δ_H 1.27 (s, 3H-16), 1.15 (s, 3H-18), 1.02 (s, 3H-19), 0.65 (d, J 6 Hz, 3H-17) and 0.63 (s, 3H-20), δ_C 15.9 (q), 16.8 (q), 18.7 (q), 19.7 (q) and 27.6 (q)] which together with two fully substituted carbon atoms, six methylene and two methine groups constitute a bicarbocyclic system. The structure readily followed from comparison of the above spectroscopic data with those of the co-metabolites. It was obvious that the new compound possesses the side-chain of (352) and the ring system of(350) and (351). This reasoning led to structure (353).

The absolute configurations of (352), (350) and (351) have been assigned in the literature. All belong to the enantio series. Unfortunately no rotations are given for the last two compounds (350) and (351). Since kolavelool (352) from J. paroica has the same magnitude and sign of specific rotation as the literature values

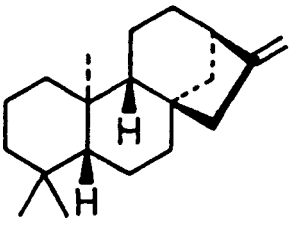


(354)

it must also belong to the enantio series. No direct correlation of the other three clerodanes was achieved but they are assumed to be ent.

Nardia scalaris

The plant material was collected at Loch Lomond and analytical tlc revealed the presence in the Et_2O extract of a major component. The inability to recover this compound from chromatographic adsorbents (Al_2O_3 and SiO_2) combined with the low Rf value (0.05 in 3% MeOH-$CHCl_3$) suggested that the compound may be acidic. However the compound failed to be extracted into aqueous $NaHCO_3$ or 10% NaOH solution. Following treatment of the crude extract with ethereal CH_2N_2 analytical tlc indicated that the initial polar compound had been replaced by one of larger Rf. The extract was then subjected to column chromatography (Al_2O_3) and preparative tlc to yield methyl ent-kaur-16-en-14(S)-yl malonate (354), $C_{24}H_{36}O_4$ (m/z 388.2617), m.p. 66-69°C, $[\alpha]_D$ - 49.4° (c, 0.34 in $CHCl_3$), [ν_{max} 1756 and 1735 cm^{-1}]. It has an exomethylene group [δ_H 4.84 (br s, 2H-17), δ_C 105.3 (t, C-17) and 152.2 (s, C-16)], a methyl malonyl residue [δ_H 3.71 (s, 3H-24), δ_C 52.3 (q); δ_H 3.37 (s, 2H-22), δ_C 41.6 (t); δ_C 166.2 (s) and 167.1 (s)], a secondary oxygen-bearing carbon [δ_H 5.39 (br s, $w_{\frac{1}{2}}$ 4 Hz, H-14), δ_C 81.1 (d)], a methine [δ_H 2.66 (br s, $w_{\frac{1}{2}}$ 8 Hz, H-13), δ_C 49.0 (d)] and three



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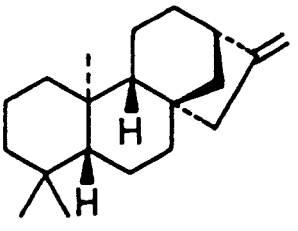

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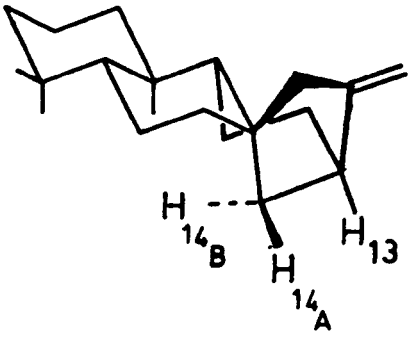
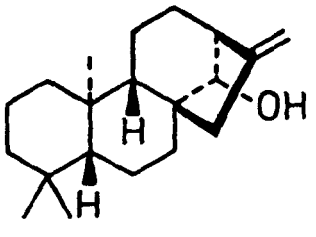


Fig. 7

tertiary methyl groups [δ_H 1.05 (s), 0.83 (s) and 0.79 (s), δ_C 17.9 (q, C-20), 21.6 (q, C-19) and 33.6 (q, C-18] which together with three tetrasubstituted carbon atoms, two methine and eight methylene groups constitute a tetracarbocyclic system. These data suggested that the compound is a tetracyclic diterpenoid of the kaurene (355) or phyllocladene (356) class. There are only two possibilities, C-14 and C-15, for the position of attachment of the secondary ester group since the corresponding methine resonance is a broad singlet indicating that it has no methylene neighbour and since the derived ketone (358) (see below) is a cyclopentanone. C-15 is easily discounted as double irradiation demonstrated the lack of allylic coupling between the methine and the exomethylene protons and therefore the oxygen function must be attached to C-14. Support for this assignment was obtained by irradiation of H-14 which caused sharpening of a methine signal (H-13, br s, $w_{\frac{1}{2}}$ 8 Hz) at δ_H 2.66 which in turn is coupled to the exomethylene protons. Inspection of models shows that the dihedral angle between H-13 and $H\text{-}14_A$ is approximately 40^o and that between H-13 and $H\text{-}14_B$ is approximately $80\text{-}90^o$ regardless of the conformation of ring C (Fig. 7). This requires coupling constants of approximately 5 Hz and approximately 0 Hz respectively. Since H-14 is a broad singlet it follows that the ester substituent has replaced $H\text{-}14_A$. The resonances for the



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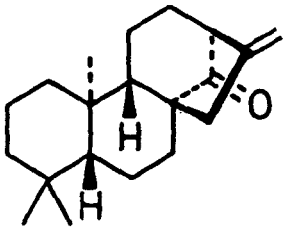


(358)

C-15 methylene group appear at δ_H 2.29 (dt, J 2.7, 16.9 Hz) and δ_H 2.06 (br d, J 16.9 Hz) and show the expected long-range coupling to the exomethylene protons.

The ^{13}C chemical shifts of the malonate (see Table 5) are consistent with the proposed structure (354). Introduction of the oxygen function at C-14 causes the expected downfield shifts of C-8, C-13 and C-14 relative to kaurene (355)[206]. Models indicate that C-7 and C-15 should be shielded by the oxygen function and this is observed in the ^{13}C nmr spectrum. The only other differences are a deshielding of C-9 (3 ppm) and a slight shielding of C-12 (0.7 ppm).

Treatment of the malonate (354) with $LiAlH_4$ in Et_2O afforded the expected ent-kaur-16-en-14(S)-ol (357), m.p. 80-85°C, $C_{20}H_{32}O$ (m/z 288.2456), $[\alpha]_D$ - 145.3° (c, 0.09 in $CHCl_3$), [ν_{max} 3630 cm^{-1}]. The ^1H nmr spectrum is consistent with this structure (357), H-14 [δ_H 4.13 (br s)] experiencing a characteristic shielding of 1.3 ppm upon removal of the ester function. The ^{13}C nmr spectrum (Table 5) also accords well with this structure. As expected C-14 moves upfield while C-13 and, to a lesser extent, C-8, move downfield with respect to the malonate (354).

Oxidation of the alcohol with Jones reagent yielded ent-kaur-16-en-one (358), m.p. 127-129°C, $C_{20}H_{30}O$ (m/z 286.2296), $[\alpha]_D$ - 8.7° (c, 0.60 in $CHCl_3$),

Table 5

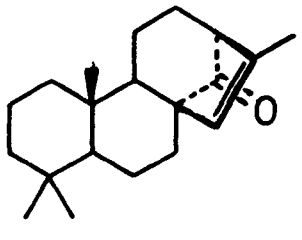
^{13}C nmr chemical shifts of Kaurenes

Carbon	(355)	(354)	(357)	(358)
1	41.3	40.3	40.4	40.6
2	18.7	18.7	18.7	18.6
3	42.3	41.9	41.9	41.9
4	33.3	33.2	33.2	33.2
5	56.1	56.2	56.4	55.2
6	20.3	19.7	19.8	19.6
7	40.4	33.1	33.1	38.2
8	44.2	48.3	49.4	50.4
9	56.1	59.1	58.8	64.8
10	39.3	39.3	39.4	39.5
11	18.1	17.2	17.6	17.2
12	33.3	32.6	32.3	32.1
13	44.2	49.0	51.9	55.0
14	39.9	81.1	76.3	220.5
15	49.2	45.6	44.8	44.6
16	156.0	152.2	153.0	144.3
17	102.8	105.3	106.5	107.0
18	33.7	33.6	33.7	33.7
19	21.7	21.6	21.6	21.3
20	17.6	17.9	18.1	15.4
21		166.2		
22		167.1		
23		41.6		
24		52.3		

[ν_{max} 1745 (cyclopentanone) cm^{-1}]. Again the nmr spectrum was particularly useful in confirming the kaurene skeleton and eliminating the possibility of a phyllocladene. C-20 is shielded (2.7 ppm) on formation of the ketone. Models indicate that H-14 and the C-10 methyl group are close together, hence it is expected that removal of this interaction will have a noticeable effect upon the C-20 resonance. This effect is the counterpart of the Nuclear Overhauser Effect (NOE) which is observed in ^{1}H nmr spectroscopy. The C-7 resonance has moved to lower field upon removal of the γ-gauche interaction with the C-14 oxygen. C-9 has moved further downfield upon oxidation of the C-14 alcohol whereas C-12 is essentially unchanged.

In the ^{1}H nmr spectrum of the ketone (358), H-5 is observed at high field [δ_H 0.73 (dd, J 2.3, 11.2 Hz)] while the corresponding ^{13}C resonance at 55.2 ppm is readily identified by its extremely small residual coupling in the SFORD ^{13}C nmr spectrum (irradiation at 0 ppm). A similar effect for H-5 is observed in the diterpenoids from Scapania undulata (see p.54). The magnitude of the residual splitting in the off-resonance ^{13}C nmr spectrum (irradiation at 0 ppm) provides a ready means of assigning C-5, C-9 and C-13. Since H-13 has the lowest field chemical shift its corresponding carbon resonance has the largest residual coupling.

In order to determine the absolute configuration of



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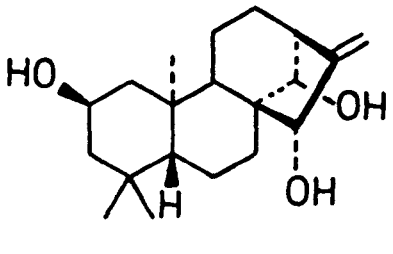
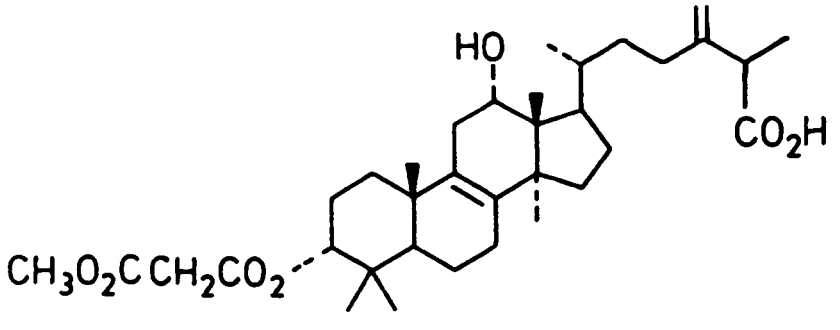


(360)

these compounds the cd spectrum of the ketone (358) was measured. The observed negative Cotton effect [$\Delta\epsilon_{305}$-1.17] is in agreement with predictions for the ketone (358) using the simple octant rule[156]. A satisfactory kaurenone model system could not be found. However, the octant rule predicts a negative Cotton effect for isophyllocladen-14-one (359) and this is indeed observed [$\Delta\epsilon_{290}$-1.29][207]. Comparison of models suggests that similar Cotton effects should be observed for (358) and (359). Hence the Nardia diterpenoid belongs to the enantio series.

Summary

These results for Jungermannia and Nardia species are consistent with previous work on these genera, the major metabolites being ent-diterpenoids. This, however, is the first report of clerodanes from the Jungermannioideae. Previously this class of diterpenoids has only been found in the closely related subfamily of Lophozioideae (see Chapter 5). On the other hand, kauranes are very common constituents of Jungermannia and Nardia species, only one example, ent-kauran-16β-ol (118), having been isolated from another genus[103]. Whilst oxygenation is the rule rather than the exception in kauranoid diterpenoids regardless of their source, it is seldom that this oxygenation occurs at the 14-position. A number of bitter polyhydroxylated kaurenes e.g. (360) exhibiting 14-



(361)

hydroxylation have been isolated from the fern Pteris plumbaea[208] and from Isodon shikokianus. Some of these diterpenoids possess antifeedant activity.

Malonates have not previously been reported from the Hepaticae and in general they are uncommon. A few examples such as the malonate of polyporenic acid A (361) are known[210].

EXPERIMENTAL

Jungermannia paroica (Plectocolea paroica)

J. paroica (30g), collected at the Devil's Pulpit near Bearsden, gave a crude extract (460mg) which was subjected to the usual chromatographic separation. Four diterpenoids were isolated.

i) ent-Cleroda-3,14-dien-13-ol,Kolavelool (352)

Repeated preparative tlc gave the oily alcohol (352), (25 mg), $[\alpha]_D$ - 40.0° (c,1.15 in $CHCl_3$), [lit.[204] -40.4°], m/z 290 ($C_{20}H_{34}O$ requires m/z 290).

ν_{max} : 3410 cm^{-1}

δ_H : 5.86 (dd, J 10, 17 Hz, H-14); 5.16 (dd, J 2, 17 Hz, H-15); 5.15 (br s, H-3); 5.01 (dd, J 2, 10 Hz, H-15[1]); 2.80 (br s, OH); 1.95 (br m, 2H-2); 1.51 (d, J 1 Hz, 3H-18); 1.21 (s, 3H-16); 0.92 (s, 3H-19); 0.71 (d, J7 Hz, 3H-17); 0.65 (s, 3H-20).

δ_C : 15.9 (q), 18.0 (q), 18.2 (t), 18.5 (q), 19.9 (q), 26.9 (t), 27.5 (t), 27.7 (q), 31.8 (t), 35.3 (t), 36.2 9d), 36.8 (t), 38.1 9s), 38.3 (s), 46.3 (d), 73.5 (s), 111.9 (d), 120.4 (d), 144.5 (s) and 145.0(s).

ii) ent-3β, 4β-Epoxyclerod-13(14)(Z)-en-15-al (350)

The aldehyde (27mg) was isolated as an oil, $[\alpha]_D$ -31.8° (c, 1.08 in $CHCl_3$), m/z 304 ($C_{20}H_{32}O_2$ requires m/z 304).

ν_{max} : 1675 cm^{-1}

λ_{max} : 238 nm (ϵ 8900)

δ_H : 9.86 (d, J 8 Hz, H-15); 5.86 (br d, J 8 Hz, H-14); 2.93 (br s, $w_{\frac{1}{2}}$ 5 Hz, H-3); 1.97 (br s, 3H-16); 1.16 (s, 3H-18); 1.04 (s, 3H-19); 0.90 (d, J 6 Hz, 3H-17); 0.66 (s, 3H-20).

δ_C : 15.6 (t), 16.0 (q), 16.8(q), 18.4 (q), 19.7 (q), 25.3 (q), 26.7 (t), 28.1 (t), 28.3 (t), 36.2 (d), 37.1 (t), 37.3 (s), 37.8 (t), 39.6 (s), 48.0 (d), 62.0 (d), 66.4 (s), 127.8 (d), 165.4 (s) and 190.3(d).

iii) ent-3β,4β-Epoxyclerod-13(14)(E)-en-15-al (351)

The aldehyde (351), (25mg) was isolated as an oil, $[\alpha]_D$ - 41.0° (c, 1.30 in $CHCl_3$), m/z 304 ($C_{20}H_{32}O_2$ requires m/z 304).

ν_{max} : 1675 cm^{-1}

λ_{max} : 237 nm (ϵ 8600)

δ_H : 9.95 (d, J 8 Hz, H-15); 5.89 (br d, J 8 Hz, H-14); 2.94 (br s, $W_{\frac{1}{2}}$ 5 Hz; H-3); 2.18 (br s, 3H-16); 1.17 (s, 3H-18); 1.05 (s, 3H-19); 0.88 (d, J 6 Hz, 3H-17); 0.66 (s, 3H-20).

δ_C : 15.4 (t), 15.9 (q), 16.8 (q , 17.8 (q), 18.6 (q), 19.7 (q), 28.1 (t), 28.3 (t), 34.5 (t), 36.2 (d), 36.2 (t), 37.1 (t),

37.3 (s), 39.2 (s), 48.0 (d), 62.1(d) 66.4 (s), 127.3 (d), 164.9 (s) and 191. 2 (d).

iv) ent-3β,4β-Epoxyclerod-14-en-13ξ-ol (353)

The epoxyalcohol (353), (33mg), was isolated as an oil, $[\alpha]_D$ - 29.6° (c, 1.07 in $CHCl_3$), m/z 306.2568 ($C_{20}H_{34}O_2$ requires m/z 306.2559).

ν_{max} : 3430 cm^{-1}

δ_H : 5.92 (dd, J 10, 17Hz, H-14); 5.23 (dd, J 2, 17Hz, H-15); 5.06 (dd, J 2, 10 Hz, H-15^1); 2.91 (br s, $W_{\frac{1}{2}}$ 5 Hz, H-3); 1.27 (s, 3H-16); 1.15 (s, 3H-18); 1.02 (s, 3H-19); 0.65 (d, J 6 Hz, 3H-17); 0.63 (s, 3H-20).

δ_C : 15.3 (t), 15.9 (q), 16.8 (q), 18.7 (q), 19.7 (q), 27.6 (q), 28.2 (t), 28.2 (t), 31.9 (t), 35.5 (t), 35.9 (d), 37.2 (t), 38.7 (s), 47.7 (d), 62.2 (d), 66.5 (s), 73.3 (s), 112.0 (t) and 145.0 (d).

Nardia scalaris

The plant material (200g) was collected at Loch Lomond in April 1983. The crude extract (4.3 g) in Et_2O was treated with excess ethereal diazomethane for 30 min. The usual chromatographic separation gave one major compound.

i) Methyl ent-kaur-16-en-14(S)-yl malonate (354)

The malonate (354), (25mg), was obtained as a crystalline material, m.p. 66-69°C, (ex pentane), $[\alpha]_D$ -49.4° (c, 0.34 in $CHCl_3$), m/z 388.2617 ($C_{24}H_{36}O_4$ requires m/z 388.2613).

ν_{max} : 1756 and 1735 cm^{-1}

δ_H (200 MHz): 5.39 (br s, $w_{\frac{1}{2}}$ 4 Hz, H-14); 4.84 (br s, 2H-17); 3.72 (s, 3H-24); 3.37 (s, 2H-22); 2.66 (br s, $w_{\frac{1}{2}}$ 8 Hz,H-13); 2.29 (dt, J 2.7, 16.9 Hz, H-15); 2.06 (br d, J 16.9 Hz, H-15'); 1.05, 0.82 and 0.79 (3s, tertiary methyls).

δ_C : see Table 5 (p.154).

$LiAlH_4$ Reduction of the Malonate (354)

To the malonate (354), (20mg), in dry Et_2O (5ml) was added $LiAlH_4$ (100 mg). After 10 min the usual work-up followed by preparative tlc gave ent-kaur-16-en-14(S)-ol (357), (13mg0, m.p. 80-85°C, $[\alpha]_D$ - 145.3° (c,0.09 in $CHCl_3$), m/z 288.2456 ($C_{20}H_{32}O$ requires m/z 288.2453).

ν_{max} : 3630 cm^{-1}

δ_H : 4.90 (br s, 2H-17); 4.13 (br s, H-14); 2.51 (br s, H-13); 2.24 (dt, J 3, 17 Hz, H-15); 1.90 (dt, J 3, 17 Hz, H-15'); 0.93, 0.80 and 0.75 (3s, tertiary methyls).

δ_C : see Table 5 (p.154).

Jones Oxidation of the Alcohol (357)

A solution of the alcohol (357), (13mg), in dry acetone (2ml) at 0°C was stirred with Jones reagent (12 drops) for 15 min. $CHCl_3$ extraction gave the crystalline ketone (358), (9mg), m.p. 127-129°C (ex pentane), $[\alpha]_D$ -8.7° (C, 0.60 in $CHCl_3$), cd $\Delta\epsilon_{305}$ - 1.17, m/z 286.2296 ($C_{20}H_{30}O$ requires m/z 286.2297).

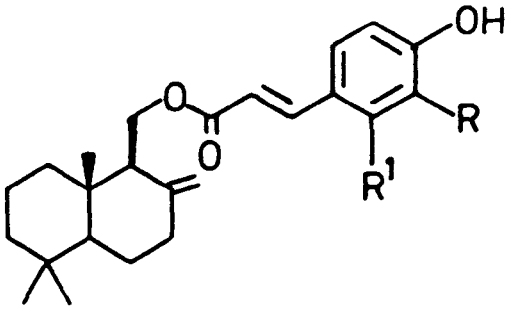
ν_{max} : 1745 cm^{-1}

δ_H (200 MHz): 4.92 (m, 2H-17); 2.80 (br s, H-13); 2.51 (br d, J 17.1 Hz, H-15); 2.29 (dt, J 17.1, 2.8 Hz, H-15^1); 0.87, 0.85 and 0.81 (3 s, tertiary methyls); 0.73 (dd, J 2.3, 11.2 Hz, H-5).

δ_C : see table 5 (p. 154)

CHAPTER 7

THE LEPIDOZIACEAE



(362) R=OH, R^1=H

(363) R=H, R^1=OH

Introduction

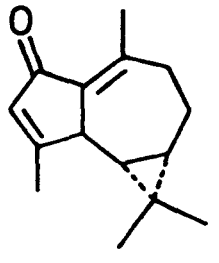
The major genera of Lepidoziaceae are Bazzania, Lepidozia and Kurzia of these, Bazzania and Lepidozia species have been quite widely investigated.

Bazzania species have proved to be rich sources of sesquiterpenoids. Five species have now been studied.

Bazzania japonica[201] contains two major components, albicanyl caffeate (362) and (S)-2-hydroxycuparene (349) as well as a smaller amount of albicanyl 2,4-dihydroxy-cinnamate (363). The compounds have also been isolated from B. pompeana[201] which contains many sesquiterpenoids; α-barbatene (50), β-barbatene (43), β-bazzanene (265), bazzanenol[211] and cuparene (256) have all been isolated. The extracts of B.japonica and B.pompeana are reported[201] to be nearly identical. Albicanyl caffeate and its isomer which have not been isolated from other liverworts, are distinctive chemical markers for the two species.

Although lacking the esters (362) and (363), B.tricrenata produces the same sesquiterpenes as B.japonica and B.pompeana. B. tricrenata from Europe[60] differs from the Japanese variety[201] in that the former contains bazzanene (265) and gymnomitrol (42). Also present in both samples are 5-hydroxycalamenene (269) and drimenol (10). B. trilobata[60,201] is chemically very similar to B. tricrenata.

The final species to be examined, B.tridens[201],



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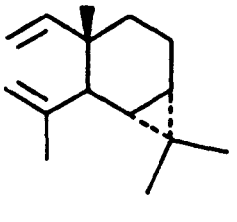

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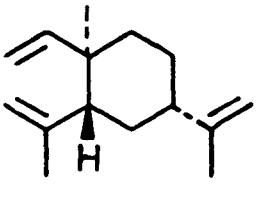
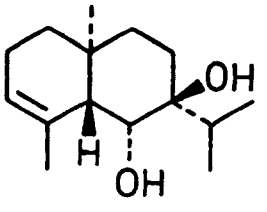


(366)

contains the same sesquiterpene hydrocarbons as the other Bazzania species. However the major component is a novel aromadendrane, tridensenone (364). The structural determination was based on spectroscopic data.

It therefore appears that Bazzania species fall into three categories, i) those containing albicanyl cinnamates, ii) those containing drimenol (10) and 5-hydroxycalamenene (269), and iii) those containing aromadendranes.

Lepidozia species comprise the other main genus of the Lepidoziaceae. L.filamentosa[148], L.reptans[160], L.vitrea[148] and L.subtransversa[160] have previously been studied. ent-Bicyclogermacrene (38) and β-barbatene (43) are the commonest metabolites with bicycloelemene (365) and β-elemene (366) also fairly widespread. ent-Spathulenol (317)[148] has been isolated from L.filamentosa whilst Matsuo et al. have isolated three novel sesquiterpene aldehydes, vitrenal (54)[66], isobicyclogermacrenal (56)[67] and lepidozenal (57)[68] from L.vitrea. Asakawa[148] has reported the presence of a number of unidentified sesquiterpene hydrocarbons and alcohols in Lepidozia species following a gcms study.



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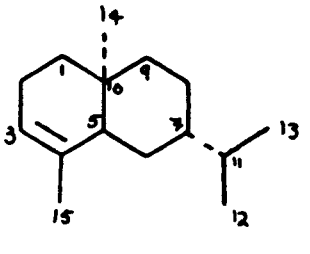

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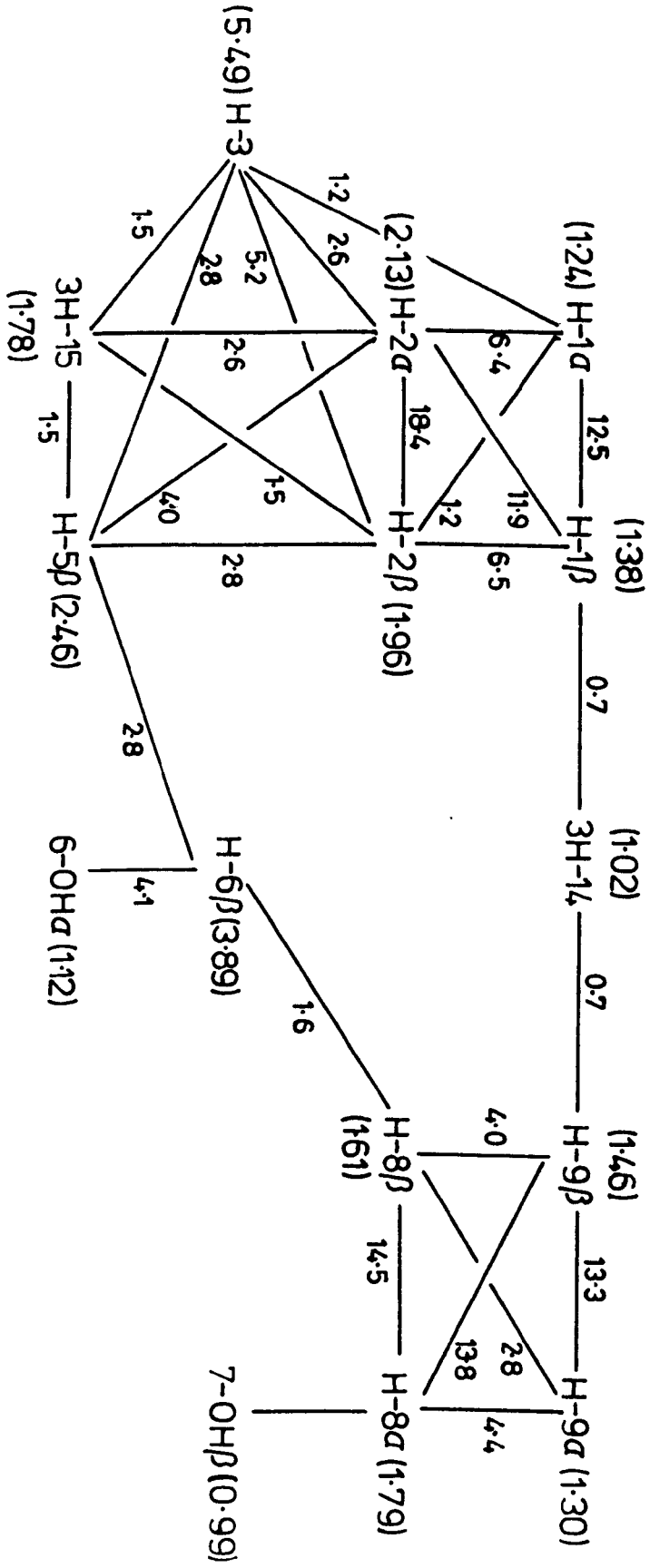
DISCUSSION

Lepidozia reptans

A sample of L.reptans collected in the Harz Mountains in the G D R gave, as the major constituent, the novel ent-eudesm-3-ene-6β,7α-diol (367), m.p. 14-95°C, $C_{15}H_{26}O_2$ (m/z 220, M^+-H_2O), $[\alpha]_D$ + 34.6° (C, 2.2 in $CHCl_3$), [ν_{max} 3620 cm^{-1}] which has a trisubstituted double bond (δ_H 5.94 (ddddq, J 1.2, 2.6, 2.8, 5.2, 1.5 Hz, H-3), δ_C 124.3 (d, C-3) and 132.8 (s, C-4)], a secondary alcohol [δ_H 3.89 (ddd, J 1.6, 2.8, 4.0 Hz, H-6) and 1.12 (d, J 4.1 Hz, OH), δ_C 75.8 (d)], a tertiary alcohol [δ_H 0.99 (s, OH), δ_C 74.9 (s)], a vinyl methyl [δ_H 1.78 (dq, J 2.6, 1.5 Hz, 3H-15), δ_C 20.5 (q)], a tertiary methyl [δ_H 1.02 (t, J 0.7 Hz, 3H-14), δ_C 18.1 (q)] and an isopropyl group [δ_H 2.00 (septet, J 6.8 Hz,H-11), δ_C 32.8 (d); 0.95 and 0.92 (each d, J 6.8 Hz, 3H-12 and 3H-13), δ_C 15.9 (q, two methyls)] which together with one tetrasubstitued carbon atom, one methine and four methylene groups constitute a bicarbocyclic system. Homonuclear decoupling experiments and first-order analysis of the 360 MHz ^1H nmr sepctrum enabled the assignment of all of the proton-proton couplings shown in Scheme 34 together with their eventual assignments (chemical shifts in parentheses). The data indicated a eudesmene skeleton (368). Since H-11 is a clean septet the tertiary hydroxyl group must be placed at C-7; the isopropyl group must be α-oriented since the

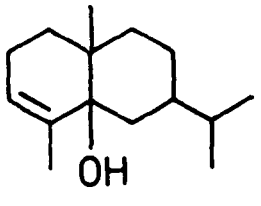

(5·49) H-3
(2·13) H-2α
(1·24) H-1α
(1·38)
H-1β
3H-15
(1·78)
H-2β (1·96)
H-5β (2·46)
(1·02)
3H-14
H-6β (3·89)
6-OHα (1·12)
(1·46)
H-9β
H-8β
(1·61)
H-9α (1·30)
H-8α (1·79)
7-OHβ (0·99)
1·2
2·6
1·5
5·2
2·8
2·6
12·5
18·4
1·5
6·4
1·2
11·9
6·5
4·0
1·5
2·8
0·7
2·8
4·1
1·6
0·7
4·0
14·5
13·3
2·8
13·8
4·4

Scheme 34.

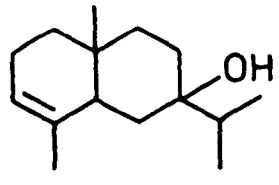
opposite configuration at C-7 would allow a ^4J W-coupling between H-8α and H-11. Such a coupling is not observed. However a very small coupling is observed between H-8α and the 7-hydroxyl proton, thus confirming the C-7 configuration. The secondary alcohol is in a sterically hindered environment since the hydroxyl proton is only slowly exchanged upon addition of D_2O. The removal of a 4Hz coupling to the hydroxyl proton simplified H-6β to a doublet of doublets. That H-6β is equatorial is shown by the magnitude of $J_{5\beta,6\beta}$ (2.8 Hz) and the presence of a ^4J coupling between H-6β and H-8β. Decoupling experiments revealed further long-range couplings notably between H-1β and 3H-14, and H-9β and 3H-14; H-2α and H-2β both exhibit homoallylic coupling to H-5 and to the vinyl methyl. Inspection of models confirms that structure (367) is consistent with these data. The absolute configuration of the diol has not been established but it is assumed to belong to the enantio series.

Various chemical transformations were attempted in order to confirm the proposed structure. The molecule was resistant both to acetylation and oxidation. This is perhaps not surprising in view of the hindered nature of the hydroxyl groups. An attempted pinacol rearrangement yielded many products; the ir spectrum of the crude reaction mixture contained no carbonyl band:

The diol (367) has also been isolated from a sample



(369)



(370)

of L.reptans collected near Catrine in south-west Scotland. A less polar sesquiterpenoid alcohol was also obtained from the extract but could not be purified due to the presence of a minor co-metabolite with the same Rf value.

The ^{1}H and ^{13}C nmr spectra of this alcohol indicated the presence of a trisubstituted double bond [δ_H 5.34 (1H,m), δ_C 121.5 (d) and 139.7 (s)], a tertiary alcohol [δ_C 85.4 (s)], a vinyl methyl [δ_H 1.73 (br s), δ_C, 20.6(q)] and three other methyl groups [δ_H 0.94- 0.82 (9H), δ_C 16.0 (q), 17.7 (q) and 17.7(q)]. The spectroscopic data suggest that the compound could be a eudesmene with a tertiary hydroxyl substituent e.g. (369) or (370). Further study is necessary to establish the structure.

EXPERIMENTAL

Lepidozia reptans

The plant material was collected in the Harz Mountains in the G D R and the crude extract (6.8g) subjected to the usual chromatographic separation. One main component was isolated.

i) ent-Eudesm-3-ene-6β,7α-diol (367)

Crystallisation from pentane gave the diol, (168mg), m.p. 94-95°C, $[\alpha]_D$ + 34.6° (C, 2.2 in $CHCl_3$), m/z 220 (M^+-H_2O, $C_{15}H_{24}O$ requires m/z 220).

ν_{max} : 3620 cm^{-1}

δ_H (360 MHz): see Scheme 34 (p.166).

δ_C : 15.9 (q), 15.9 (q), 18.1 (q), 20.5 (q), 23.0 (t), 27.8 (t), 31.5 (s), 32. 8 (d), 35.3 (t), 39.3 (t), 45.3 (d), 71.7 (d), 74.9 (s), 124.3 (d) and 132.8 (s).

A further collection of L.reptans (200g) was made near Catrine in south-west Scotland in Spring,1983. The usual chromatographic separation of the crude extract (6g) yielded two major constituents.

i) The Mono-Alcohol

Repeated preparative tlc gave the impure mono-alcohol, (40mg), as an oil.

ν_{max} : 3610 cm^{-1}

δ_H : 5.23 (m, vinyl proton); 1.73 (br s, vinyl methyl); 0.94-0.83 (9H, three methyls).

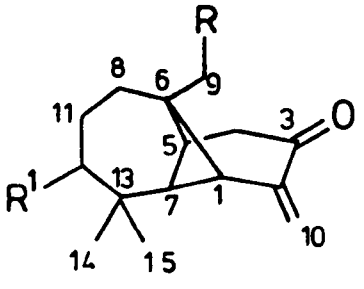
S_c : 16.0 (q), 17.7 (q), 17.7 (q), 20.6 (q), 23.2 (t), 27.4 (t), 35.9 (t), 37.7 (d), 38.7 (t), 45.3 9t), 49.2 9s), 85.4 (s), 121.5 (d) and 139.7 (s).

ii) ent-Eudesm-3-ene-6β,7α-diol (367)

Crystallisation from pentane gave the diol (367), (30mg), identical with an authentic sample.

CHAPTER 8

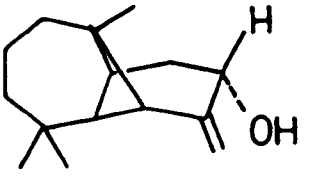
THE GYMNOMITRIACEAE



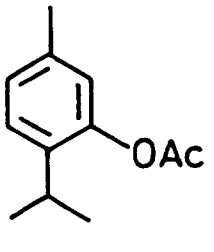
(371) $R=R^1=H$

(372) $R=OAc$, $R^1=H$

(375) $R=H$, $R^1=OAc$



(373)



(374)

Introduction

Three species of the Gymnomitriaceae have previously been studied.

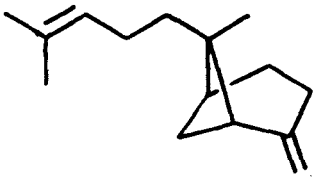
i) Marsupella

Matsuo et al.[212] investigated M. emarginata subspecies tubulosa collected in Japan, isolating three new oxygenated ent-longipinane sesquiterpenoids, (-)-marsupellone (371), (+)-acetoxymarsupellone (372) and (+)-marsupellol (373) from the MeOH extract. The structures were determined mainly by spectroscopic methods. Marsupellone (371) was correlated with ent-α-longipinene (203) proving that the absolute configuration of (371) is antipodal to longipinanes from higher plants.

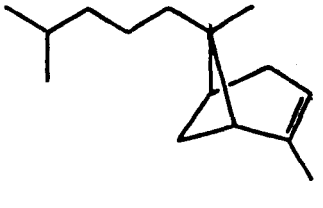
The same liverwort collected in Wales was shown also to contain marsupellone (371) and marsupellol (373) by Asakawa et al.[32] using gcms. The major component, however, is an unidentified sesquiterpene alcohol [M^+ 220]. Also present were the monoterpenoids p-cymene (17) β-phellandrene (14) and thymyl acetate (374) as well as the sesquiterpenoids β-barbatene (43), β-chamigrene (268) and cyclocolorenone (25).

Recently, Huneck et al.[213] examined the constituents of German M. aquatica and isolated a new longipinane derivative, ent-12α-acetoxylongipin-2(10)-en-3-one (375) (which could also be called 12β-acetoxymarsupellone), the structure of which followed from spectroscopic and chemical studies.

Thus it seems that ent-longipinanes are the chemical markers for Marsupella species.



(376)



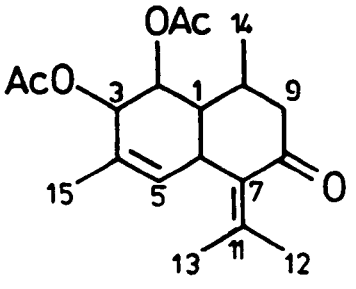
(377)

ii) Gymnomitrion

Asakawa et al.[148] have investigated the Japanese liverwort Gymnomitrion corallioides and have found the major constituents to be trans-β-bergamotene (376) and three unidentified sesquiterpenoid alcohols. The minor components of the extract were trans-α-bergamotene (377), bicyclogermacrene (38), cuparene (256) and C_{18}-C_{25} n-alkanes. This is the only report of bergamotane sesquiterpenes from the Hepaticae.

The absolute stereochemistries have not been determined since the compounds were identified in the course of a gcms stud· of the plant extract.

In contrast, Connolly's[55] study of Scottish G. obtusum led to the discovery of a new class of sesquiterpenoids, the gymnomitranes, of which gymnomitrol (42) and β-barbatene (gymnomitrene) (43) are the best-known examples. Further examples are given in Chapter 1 (p.16).



(378)

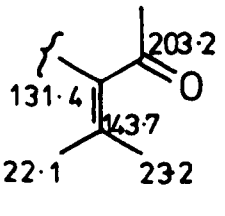


Fig.8

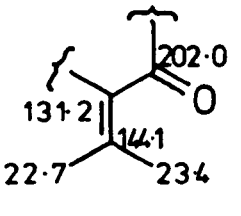


Fig. 9

Discussion

Marsupella aquatica

M. aquatica, regarded by some authorities as a variety of M.emarginata and by others as a distinct species, was collected in Glencoe where it is a common inhabitant of the mountain streams. Examination of the extract yielded a novel sesquiterpenoid $C_{19}H_{26}O_5$, [ν_{max} 1735 cm^{-1}; λ_{max} 247 nm]. It has an isopropenyl ketone [δ_H 2.01 (s, 3H-12), δ_C 23.2 (q); δ_H 1.82 (s, 3H-13), δ_C 22.1 (q); δ_C 131.4 (s, C-7), 143.7 (s, C-11) and 203.2 (s, C-8)], a trisubstituted double bond [δ_H 5.23 (1H, m, H-5), δ_C 129.5 (d, C-5) and 133.7 (s, C-4)], two secondary oxygen-bearing carbons [δ_H 5.48 (dd, J 2, 3.7 Hz, H-3), δ_C 68.0 (d); δ_H 5.23 (m, H-2), δ_C 72.8 (d)], two acetates [δ_H 2.10 (s) and 2.03 (s), δ_C 21.1 (q), 21.9 (q), 170.1 (s) and 170.6 (s)], one vinyl methyl [δ_H 1.64 (dd, J 1.6, 2.0 Hz, 3H-15), δ_C 20.9 (q)] and a secondary methyl [δ_H 1.04 (d, J 6.6 Hz, 3H-14), δ_C 19.8 (q)] which together with one methylene and three methine groups constitute a bicarbocyclic system. These features, in conjunction with the ^{13}C shifts and multiplicities (Table 6) suggest a cadinene (or stereoisomer) skeleton (378). The ^{13}C shifts of the enone system (Fig.8) are in good agreement with the similar system (Fig.9) observed in cuauhtemone (339).[198]

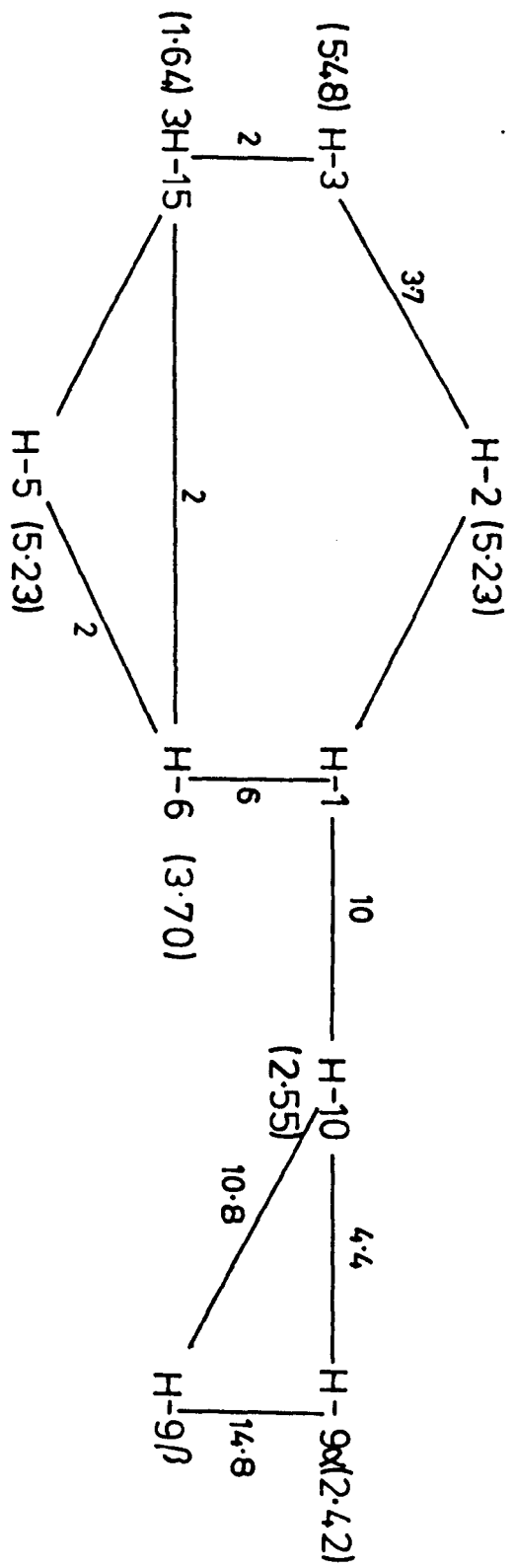
Consideration of the proton-proton couplings in the 360 MHz ^1H nmr spectrum (Scheme 35, chemical shifts in parentheses) permitted partial assignment of stereochemistry.

Table 6

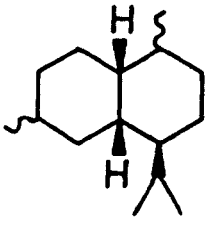
^{13}C nmr chemical shifts of a Marsupella sesquiterpenoid

Carbon	(385)
1	42.3*
2	72.8
3	68.0
4	133.7
5	129.5
6	41.8*
7	131.4
8	203.2
9	50.4
10	28.3
11	143.7
12	23.2
13	22.1
14	19.8
15	20.9
$\underline{CH_3}$ CO	21.1 21.9
CH_3 $\underline{CO}$	170.1 170.6

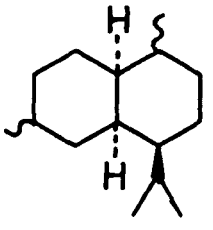
* Assignments may be reversed.


(5·48) H-3
(1·64) 3H-15
H-2 (5·23)
H-5 (5·23)
H-1
H-6 (3·70)
H-10
(2·55)
H-9α (2·42)
H-9β
3·7
2
2
2
6
10
10·8
4·4
14·8

Scheme 35.



(379)



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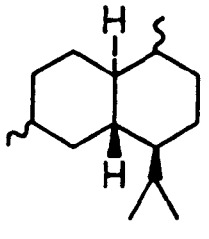

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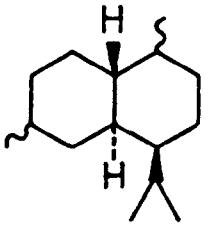

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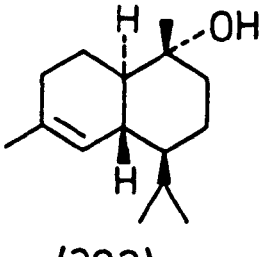

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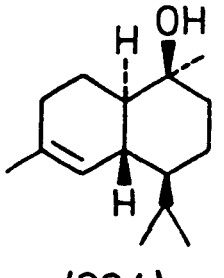

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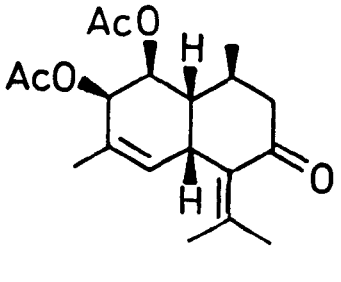

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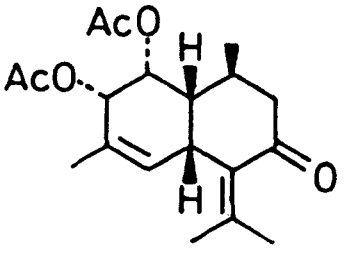

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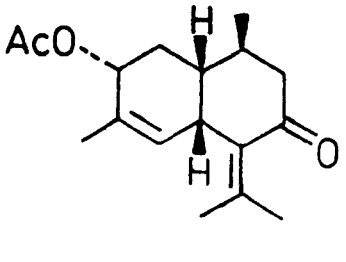

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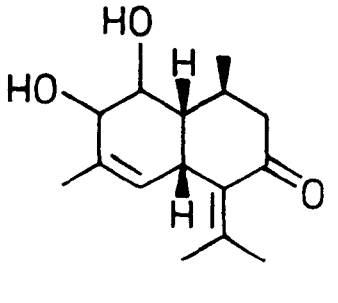
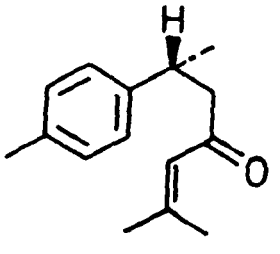


(388)

The magnitude of $J_{1,6}$ (6 Hz) indicates that the rings are cis-fused and thus the compound is a muurolane (379) (or amorphane (380)) and not a cadinane (381) or bulgarane (382). The corresponding value of $J_{1,6}$ in (-)-α-cadinol (383) and (+)-T-cadinol (384), both of which are trans-fused, is 12 Hz.[214] From the large value of $J_{1,10}$ (10 Hz) it follows that the C-10 methyl group is equatorial (β). This is confirmed by the values of $J_{9\beta,10}$ (10.8 Hz) and $J_{9\alpha,10}$ (4.4 Hz). The value of $J_{2,3}$ (3.7 Hz) does not allow an unambiguous assignment of the relative stereochemistries of C-2 and C-3. It does, however, indicate the cis disposition of the acetoxy groups as a trans arrangement would be expected to have the acetates equatorial and hence to give rise to a much larger value for $J_{2,3}$. These arguments lead to two alternative structures (385) and (386), 2β, 3β-diacetoxy-4,7(11)-muuroladien-8-one and 2α, 3α-diacetoxy-4,7(11)-muuroladien-8-one for the diacetate. On available evidence it is not possible to choose between them. The ^{1}H nmr spectrum is in agreement with that reported for 3-acetoxymuurola-4,7(11)-dien-8-one (387) although Bohlmann[215] does not establish beyond doubt the configuration of the secondary acetate.

Treatment of (385) with MeOH-K_2CO_3 resulted in ester solvolysis to give the diol (388). The ^{1}H nmr spectrum showed the expected upfield shifts for H-2 and H-3 (δ_H 3.97 and 4.05 respectively), in addition to the loss of the acetate signals. Lack of material prevented further study of the compound.



(389)

The isolation of a muurolane from Marsupella aquatica indicates that this species differs from the previously investigated Marsupella species which produce characteristic ent-longipinanes (see p.171). However, since few Marsupella species have been examined it is difficult to draw any firm conclusions.

Gymnomitrion obtusum

A study of the constituents of G. obtusum, collected at the same site as the sample previously investigated by Connolly et al. (see p.172), resulted in the isolation of gymnomitrol (42) and the corresponding acetate (44) along with ent-ar-turmerone (389). The last-named compound has previously been isolated from higher plants but this is the first isolation of the enantio form. The spectroscopic data for (389) (see Experimental) are in accord with the literature values [216,217] apart from the specific rotation which is of the opposite sign.

A few bisabolane sesquiterpenoids have previously been isolated from the Hepaticae, e.g. (-)-β-bisabolene (343)[82] but they are rather uncommon.

EXPERIMENTAL

Marsupella aquatica

The plant material (1.1 kg) was collected in Glencoe in September 1980. The crude extract (10.3 g) was chromatographed to give one compound.

i) 2,3-Diacetoxy-4,7(11)-muuroladien-8-one

Repeated preparative tlc gave the enone (385), (35 mg), as a gum.

ν_{max} : 1735 cm^{-1}.

λ_{max} : 247 nm.

δ_H (360 MHz) : see Scheme 35 (p.175).

δ_C : see Table 6 (p.174).

De-acetylation of the Enone (385)

The enone (20 mg) was stirred overnight with dry MeOH (10 ml) and anhydrous K_2CO_3 (200 mg). Filtration and removal of solvent gave a gummy residue which was purified by preparative tlc to give the diol (388), (11 mg) as an unstable gum.

δ_H (360 MHz) : 5.16 (br s, H-5); 4.05 (br d, J 3.9 Hz, H-3); 3.97 (t, J 4.3 Hz, H-2); 3.54 (m, H-6); 2.52 (2H, m); 2.04 and 1.83 (each s, OAc); 1.80 (ddd, J 0.5, 1.4, 2.0 Hz, 3H-15); 1.08 (d, J 6.6 Hz, 3H-14).

Gymnomitrion obtusum

The plant material (900 g) was collected at Ben Lawers in 1972. The crude extract was chromatographed to yield three compounds.

i) Gymnomitrol Acetate (44)

δ_H : 4.76 and 4.71 (2H, exomethylene); 4.68 (s, CH OAc); 2.37 (1H, s); 2.03 (s, OAc); 1.12, 1.04 and 0.85 (3s, tertiary methyls).

δ_C : 20.0 (q), 21.4 (q), 24.0 (q), 27.2 (t), 27.9 (t), 28.2 (q), 36.7 (t), 37.2 (t), 38.3 (t), 46.7 (s), 54.8 (s), 55.1 (s), 60.2 (d), 92.4 (d), 110.3 (t), 149.3 (s) and 170.0 (s).

ii) Gymnomitrol (42)

δ_H : 4.65 and 4.63 (2H, exomethylene); 3.70 (s, CH OH); 2.32 (s); 1.21, 1.07 and 0.97 (3s, tertiary methyls).

δ_C : 19.8 (q), 24.7 (q), 27.2 (t), 28.3 (t), 28.8 (q), 37.0 (t), 37.2 (t), 38.5 (t), 47.5 (s), 54.3 (s), 55.3 (s), 62.7 (d), 91.7 (d), 108.9 (t) and 151.2 (s).

iii) ent-ar-Turmerone (389)

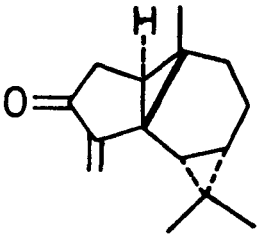
Preparative tlc gave ent-ar-turmerone, (40 mg), as an oil, $[\alpha]_D$ - 42.5° (C, 0.06 in $CHCl_3$), m/z. 216 ($C_{15}H_{20}O$ requires m/z 216).

δ_H : 7.0 (4H, s, Ar-H); 5.9 (m, vinyl proton); 3.25 (sextet, J 7 Hz); 2.6-2.4 (m); 2.29 (s, CH_3-Ar); 2.09 and 1.83 (vinyl methyls); 1.21 (d, J 7 Hz, secondary methyl).

δ_C : 20.7 (q), 21.0 (q), 22.0 (q), 27.6 (q), 35.3 (d), 52.7 (t), 124.2 (d), 126.7 (d), 126.7 (d), 129.1 (d), 129.1 (d), 135.5 (s), 143.8 (s), 154.9 (s) and 199.7 (s).

CHAPTER 9

THE PLAGIOCHILACEAE



(390)

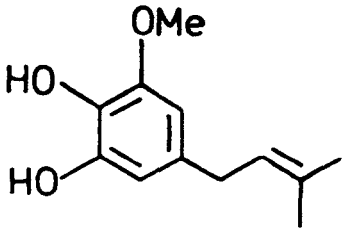
Introduction

The two main genera of the Plagiochilaceae are *Mylia* and *Plagiochila*, although some authorities place *Mylia* in a separate subfamily of Jungermanniaceae.

Mylia species have proved to be a rich source of terpenoids. *Mylia taylorii* contains sesquiterpenoids of the aromadendrane class, myliol (28), dihydromylione A (390) and taylorione (39) (see p.13). These suggest a similarity between *M.taylorii* and *Plagiochila* species which also contain aromadendranes and secoaromadendranes (see below). In contrast, *M. verrucosa*[122,123] has been found to contain a new class of diterpenoids, the verrucosanes, exemplified by 2β, 9α-dihydroxyverrucosane (153) (see p.31). It should be noted that one of the unidentified diterpenoids isolated from *M.anomola* by Benesova *et al.*[218] appears to be (153) (by comparison of the reported spectroscopic data).

Plagiochila species have been studied by Asakawa[49] and he has shown that the characteristic constituents are *ent*-secoaromadendranes e.g. plagiochilide (31) (see p.13). However he has only investigated 1% of the known *Plagiochila* species and, as is shown in the discussion, it is by no means certain that these terpenoids will prove to be ubiquitous amongst *Plagiochila* species.

This chapter deals with the isolation of aromatic metabolites from *Plagiochila* species and with the synthesis of one of these compounds.



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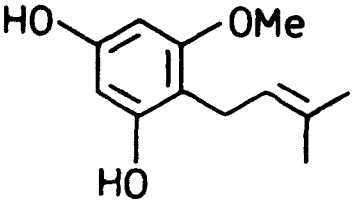

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DISCUSSION

Contrary to the published results which indicate Plagiochila species to be a rich source of sesquiterpenoids, an investigation of Scottish P.porellioides which is an extremely widespread liverwort, revealed a singular absence of terpenoids.

Plagiochila rutilans

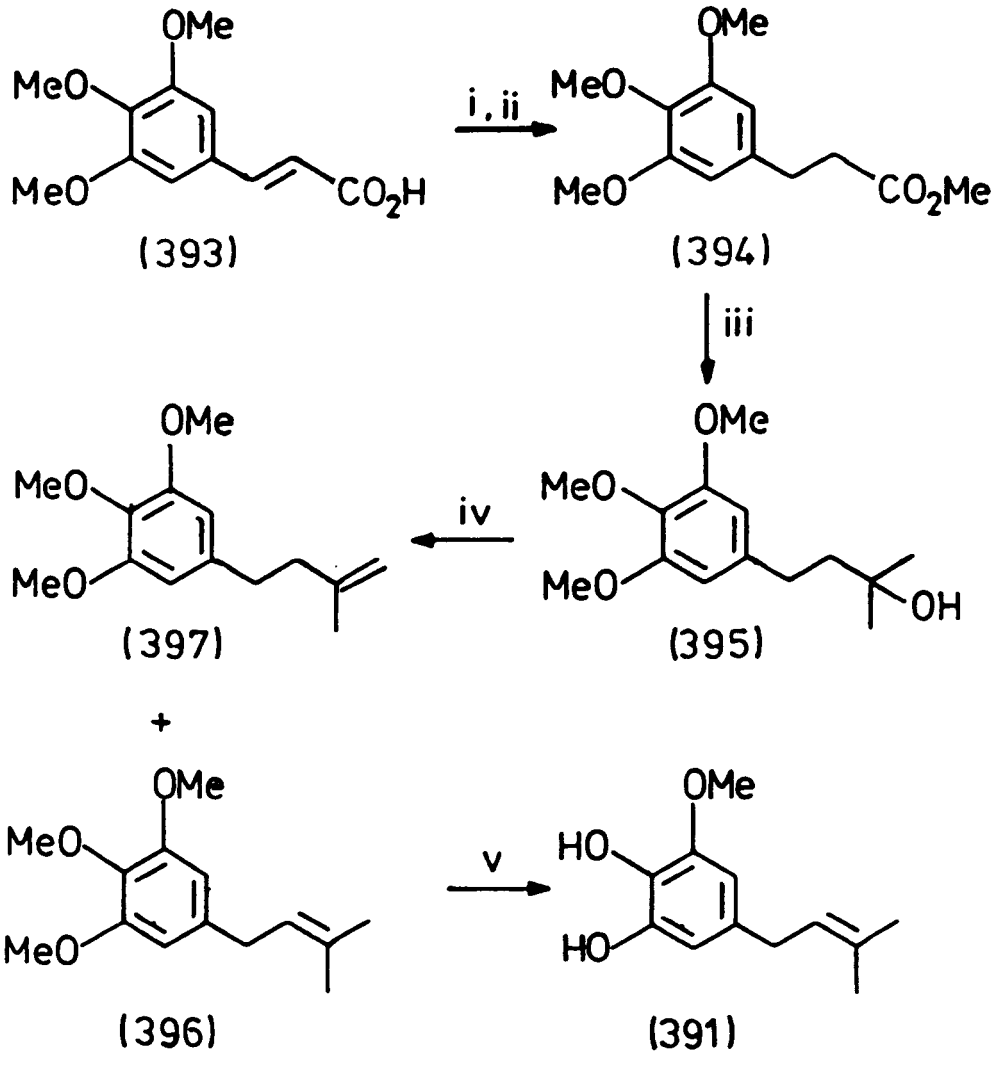
Extraction of a Cuban liverwort, Plagiochila rutilans, yielded a crystalline compound, 1-[3,4-dihydroxy-5-methoxyphenyl]-3-methylbut-2-ene (391), m.p. 94-95°C, $C_{12}H_{16}O_3$ (m/z 208) which has a tetrasubstituted aromatic ring [S_H 6.29 and 6.20 (both 1H, d, J Hz)], a vinyl proton [δ_H 5.20 (t, J8Hz)], two hydroxyls [δ_H 4.70 (2H, br s)], a methoxyl group [δ_H 3.80 (s)], a benzylic methylene group [δ_H 3.2 (2 H, d, J8Hz)] and two vinyl methyls [δ_H 1.7 (6H, s)]. Double resonance experiments indicated that the benzylic methylene group is coupled to the vinyl proton. Thus the compound has a dimethylallyl group attached to an aromatic ring containing one methoxyl and two hydroxyl groups. The presence of two meta-coupled aromatic proton signals indicated that the aromatic ring is unsymmetrically substituted. These data can be accommodated by structures (391) and(392). However the ^{13}C nmr spectrum (Table 7), in particular the chemical shifts of the oxygenated aromatic carbons support the former structure. This structure (391) was confirmed by

Table 7

^{13}C nmr chemical shifts of aromatic compounds.

Carbon	(391)	(395)
1	28.0	31.2
2	122.0	45.7
3	133.0	70.3
4	25.8	29.4
5	17.8	29.4
1'	127.9	136.2
2'	97.4	105.4
3'	148.5*	153.2
4'	137.2	138.4
5'	146.9*	153.2
6'	107.7	105.4
OMe	56.0	56.1

* May be interchanged



i. CH_2N_2: ii. H_2-Pd: iii. MeMgI;

iv. $SOCl_2$-py; v. $LiAlH_4$- C_6H_6.

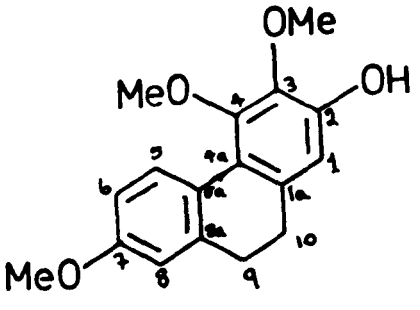
Scheme 36.

synthesis (Scheme 36).

Commercially available 3,4,5-trimethoxycinnamic acid (393) was chosen as the starting point. Methylation with diazomethane followed by catalytic hydrogenation afforded oily methyl 3-[3,4,5-trimethoxyphenyl]-propionate (394). A Grignard reaction involving MeMgI and the ester (394),gave, as the major product, the desired alcohol (395) which has spectroscopic properties consistent with the proposed structure. Thus it has resonances at δ_H 6.42 (2H, s, Ar-H), 3.84 (9H, s, OMe)., 2.63 (2H,t, J 7 Hz, Ar$\underline{CH_2}CH_2$), 1.75 (2H, t, J 7Hz, Ar $CH_2\underline{CH_2}$) and 1.27 (6H, s, tertiary methyls). The ^{13}C nmr resonances are listed in Table 7.

Treatment of the alcohol (395) with $SOCl_2$ in pyridine gave a red, oily product. Preparative tlc afforded the major component, the ^1H nmr spectrum of which indicated the presence of the double bond isomers (396) [δ_H 5.2 (vinyl proton)] and (397) [δ_H 4.75 (exomethylene)] in a ratio of 3:1. Separation of the desired isomer (396) was achieved by preparative tlc over $AgNO_3$ - impregnated SiO_2.

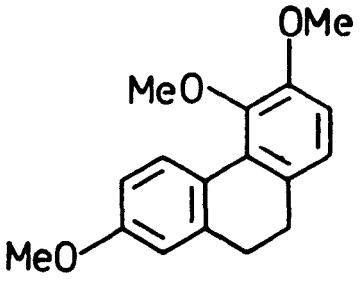
The final step involved partial demethylation of (396) using fresh $LiAlH_4$ in refluxing benzene. The trimethyl ether was converted smoothly into the diol (391) which was obtained, after purification, as a pale-yellow solid identical with the natural product (^1H nmr, tlc, ms).



(398)

A less common species, P.spinulosa, has been examined and three aromatic compounds, including two 9,10-dihydrophenanthrenes, isolated.

The major constituent and also the most polar was a phenol, m.p. 135-139°C, $C_{17}H_{18}O_4$ (m/z 286.1185), [ν_{max} 3620 cm^{-1}]. It has four aromatic protons [δ_H 8.18 (d, J 8.7 Hz), 6.81 (dd, J 2.8, 8.7 Hz), 6.77 (d, J 2.6 Hz) and 6.62 (s)], a phenolic hydroxyl group [δ_H 5.74 (1 H, s, exchangeable with D_2O), three methoxyl groups [δ_H 3.97, 3.83 and 3.74 (each 3H, s)] and an A_2B_2 system [δ_H 2.71 (4H, m)]. These data are consistent with a 9,10-dihydrophenanthrene system (398). The doublet at δ_H 8.18 is characteristic of a proton at C-5 in this system. It has an ortho coupling (J 8.7 Hz) to the signal at δ_H 6.81 and the latter has a further meta coupling (J 2.8 Hz) to the proton at δ_H 6.77. Hence one aromatic ring of the 9,10-dihydrophenanthrene is monosubstituted at C-7. The other aromatic ring must therefore be trisubstituted. The final aromatic proton resonance at δ_H 6.62 is characteristic of a proton at C-1 or C-8 in the 9,10-dihydrophenanthrene system. Hence the compound is a 2,3,4,7-tetrasubstituted-9,10-dihydrophenanthrene (398). Acetylation of the phenol gave a mono-acetate, the ^1H nmr at which indicated a deshielding of H-1 by 0.09 ppm. Hence the phenolic hydroxyl group must be in the trisubstituted ring. Unfortunately the existence of an

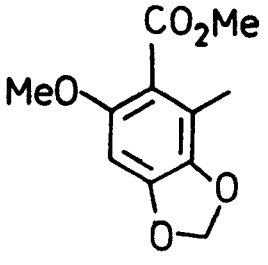


(399)

acetylation shift does not help to determine the position of the hydroxyl group as both ortho and meta protons are deshielded by approximately 0.1 ppm upon acetylation.

A minor metabolite was 3,4,7-trimethoxy-9,10-dihydrophenanthrene (399), $C_{17}H_{18}O_3$ (m/z 270). It has five aromatic protons [δ_H 8.36 (d, J 8.7 Hz), 6.92 (dt, J 8.2, 0.8 Hz), 6.83 (dd, J 2.8, 8.7 Hz), 6.78 (d, J 2.8 Hz) and 6.75 (d, J 8.2 Hz)], three methoxyl groups [δ_H 3.88, 3.84 and 3.68 (3s)] and an A_2B_2 system [δ_H 2.74 (4H, m)] again suggesting a 9,10-dihydrophenanthrene system. The lowfield resonance (δ_H8.36) due to a proton at C-4 or C-5 has an ortho coupling to a proton which has in turn a meta coupling. Thus one aromatic ring is monosubstituted at C-7. The other aromatic ring is therefore disubstituted. The remaining aromatic resonances are due to two mutually ortho protons and, since one of these has a small coupling (J 0.8 Hz) to the methylene group at C-10, this compound is readily formulated as (399).

The final compound is a pentasubstituted benzene $C_{11}H_{12}O_5$ m/z 204 [V_{max} 1733 cm^{-1}] which has an aromatic proton [δ_H 6.29 (s), δ_C 101.3 (d)], a methylenedioxy group [δ_H 5.58 (s), δ_C 92.9 (t)], two methoxyl groups [δ_H 3.87 and 3.75 (2s), δ_C 52.1 (q) and 57.0 (q)] an aromatic methyl [δ_H 2.14 (s), δ_C 12.5 (q)], an ester carbonyl [δ_C 168.0 (s)] and five substituted benzene



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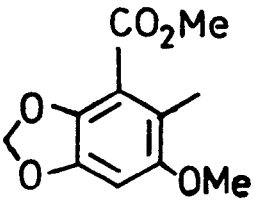

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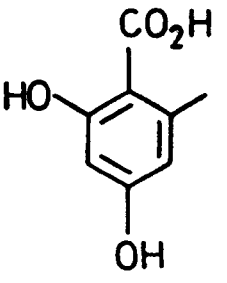

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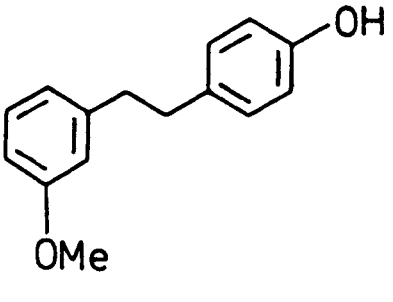

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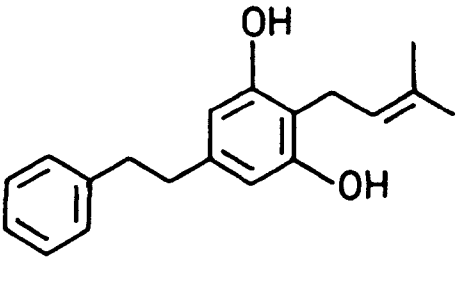

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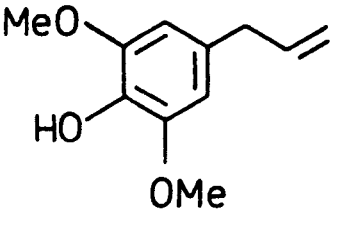


(405)

carbons [δ_c 116.5 (s), 117.7 (s), 140.1 (s), 148.5 (s) and 153.0 (s)]. The strong shielding experienced by the unsubstituted aromatic carbon indicates that it is ortho to two oxygen-bearing carbons. This allows only two possible isomers, (400) and (401). It has so far proved impossible to decide between these two. Efforts to obtain a satifactory fully coupled ^{13}C nmr spectrum were thwarted by lack of material. Structure (400) is perhaps preferable since it is a derivative of orsellinic acid (402), a well-known natural product although still unknown from liverworts.

Summary

The isolation of these aromatic compounds from Plagiochila species indicates that ent-2,3-secoaromadendranes are not always the characteristic chemical markers in this genus. Previously, only one aromatic compound, 3-methoxy-4'-hydroxybibenzyl (403), has been reported from a Plagiochila species (with the exception of the ubiquitous lunularic acid and lunularin). Dihydrophenanthrene derivatives are rare amongst the Hepaticae, 3,4-dimethoxy-5-hydroxy-9,10-dihydrophenanthrene (198) being the only example. Simple prenylated benzenes are unknown from liverworts but a few prenylated bibenzyls have been isolated e.g. (404) from Radula complanata[219] along with the oxygenated allylbenzenes (197) and (405)[129].

EXPERIMENTAL

Plagiochila rutilans

The plant material (11g) was collected in Cuba and the crude extract chromatographed over SiO_2 using an n-hexane-Et_2O gradient to give a crystalline compound.

i) 1-[3,4-Dihydroxy-5-methoxyphenyl]-3-methylbut-2-ene(391)

Recrystallisation from n-hexane-Et_2O gave the phenol (391), (17.5mg), m.p. 94-95°C, m/z 208 ($C_{12}H_{16}O_3$ requires m/z 208).

ν_{max} (KBr) : 3500 cm^{-1}

λ_{max} (MEOH): 207 (log ϵ 4.10) and 291 nm (log ϵ 3.30).

δ_H : see p.182

δ_C : see Table 7.

Methylation of 3,4,5-Trimethoxycinnamic Acid (393)

The acid (1.1g) in MeOH (25ml) was treated with excess CH_2N_2. Removal of solvent gave the crystalline methyl ester(1.02g), m.p. 99-100° C (ex petroleum ether - EtOAc), m/z 252.

δ_H : 7.59 and 6.33 (2d, vinyl protons); 6.74 (2H,s, Ar-H); 3.79 (3H, s, CO_2Me); 3.87 (9H,s , ArOMe).

Hydrogenation of Methyl 3,4,5-Trimethoxycinnamate

The methyl ester (1.02g) in Et_2O (25ml) was hydrogenated over 10% Pd-C catalyst. The product (394) (0.98g), a yellow oil, was homogeneous by tlc. ^1H Nmr

spectroscopy showed that the alkene protons had disappeared and a new resonance was found at δ_H 2.8 (4H, m, CH_2-CH_2).

Grignard Reaction on the Dihydro-ester (394)

The dihydro-ester (394), (0.98g), in Et_2O was added with stirring over 30 min to MeMgI in dry Et_2O. After 12h. the ethereal solution was transferred to a separating funnel and the remaining solid was digested with dilute H_2SO_4. The aqueous layer was added to the separating funnel. An acidic work-up afforded a red oil (0.91g). Preparative tlc gave the major product, the alcohol (395), (574mh), as an oil, m/z 254.

δ_C : see table 7 (p.183).

Dehydration of the Alcohol (395)

To a stirred solution of the alcohol (395), (574mg), in dry pyridine (2ml) at 0°C was added redistilled $SOCl_2$ (1.1 equivalents). After 5 min the reaction mixture was warmed to room temperature and added to a cold solution of $NaHCO_3$. The usual work-up afforded a brown oil. Preparative tlc over SiO_2 and $AgNO_3$- impregnated SiO_2 gave the olefin (396).

δ_H : see p.184 .

Partial Demethylation of the Olefin (396)

The alkene (396), (30mg) in dry benzene (20ml) was refluxed with fresh $LiAlH_4$ (9mg) for 12h. An acidic work-up gave, after preparative tlc, a pale-yellow solid, identical with the natural product (391) by tlc, ^1H nmr and ms.

Plagiochila spinulosa

The plant material (200g) was collected at Kilmaha near Loch Awe. The crude extract (9g) was chromatographed to yield three compounds.

i) 3,4,7-Trimethoxy-9.10-dihydrophenanthrene (399)

The dihydrophenanthrene (399) was isolated as an impure solid (7mg).

δ_H (360 MHz): see p.186 .

ii) The Trimethoxy-9,10-dihydrophenanthrol (398)

The phenol (398), (20mg), was recrystallised from petroleum ether -$CHCl_3$ as needles, m.p. 135-139°C, m/z 286.1185 ($C_{17}H_{18}O_4$ requires m/z 286.1205).

ν_{max} : 3620 cm^{-1}

δ_H (360 MHz) : see p.185 .

iii) Methyl Methoxy Methylenedioxymethylbenzoate (400)

The methyl ester (15mg) was isolated as an amorphous solid, m/z 204.

ν_{max} : 1733 cm^{-1}

S_H : see p.186 .

S_C : 12.5 (q), 52.1 (q), 57.0 (q), 92.9 (t), 101.3 (d), 116.5 (s), 117.7 (s), 140.1 (s), 148.5 (s), 153.0 (s) and 168.0 (s).

Chapter 10

THE FRULLANIACEAE

Introduction

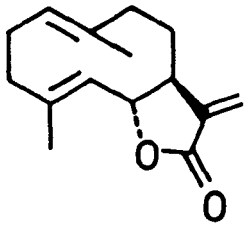
The family Frullaniaceae consists of the large genus Frullania and the smaller genus Jubula.

Frullania

Although not the first liverworts to be studied, those of the genus Frullania provided the impetus for the investigation of the Hepaticae. For the past twenty-seven years it has been apparent that an allergenic contact dermatitis which affects lumberjacks in France, Canada, and the USA is due to Frullania species which grow on the bark of trees[69]. Of the many species known, eleven have been reported to be contact sensitising[220].

It was not until 1969 that Ourisson and co-workers[221] published the structure of the active component, (-)-frullanolide (58), from F.tamarisci. Surprisingly this compound does not possess the ent-stereochemistry which is characteristic of liverwort sesquiterpenoids. Even more surprisingly, the enantiomeric (+)-frullanolide (60) was subsequently isolated from F.dilatata[70]. Since then a number of other compounds have been isolated from Frullania species (see Chapter 1, p.17).

Asakawa et al.[31] have investigated twenty-five Frullania species and have concluded that they fall into five categories depending on the classes of metabolites found.



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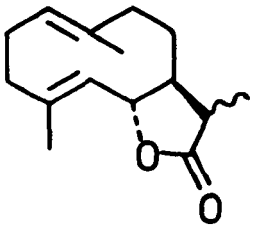

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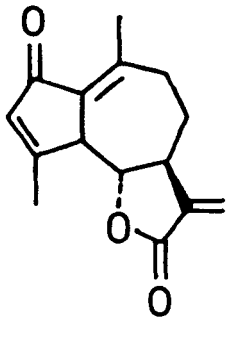

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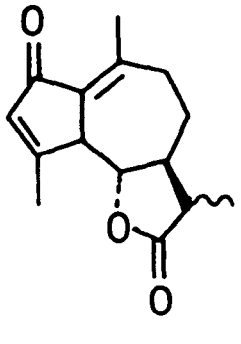

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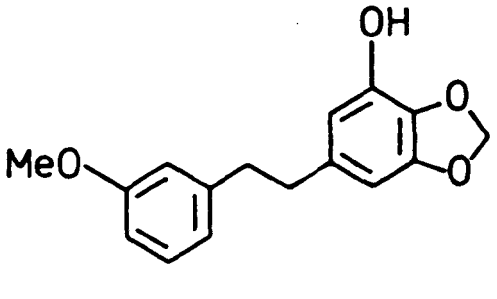

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a) Sesquiterpene Lactone - Bibenzyl Type

This category contains, among others, F.tamarisci and F.dilatata. Sesquiterpene lactones such as frullanolide (60), γ -cyclocostunolide (61) and costunolide (63) are the major components with smaller amounts of other sesquiterpenes and bibenzyls present.

b) Sesquiterpene Lactone Type

The Frullania species of this group contain no bibenzyls; sesquiterpene lactones are present but are of the germacranolide (406, 407) and guaianolide (408, 409) types, rather than eudesmanolides.

c) Bibenzyl Type

Liverworts in this category contain large amounts of bibenzyls e.g (410) but no lactones, and few sesquiterpenes.

d) Monoterpene Type

F.fragifolia is unusual in that it contains mainly monoterpene hydrocarbons (13,14, 19, 20) but no bibenzyls or sesquiterpene lactones.

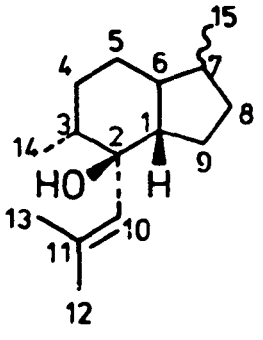
e) Cyclocolorenone Type

This category also contains only one member, F. diversitexta, which produces mainly cyclocolorenone (25) together with a few diterpenoid acetates.

In addition to sesquiterpenoid lactones, bibenzyls and cyclocolorenone,most Frullania species produce many sesquiterpene hydrocarbons and alcohols. β-Caryophyllene (261) is the most widespread sesquiterpene hydrocarbon in Frullania species, with β-elemene (366), α-selinene (254), β-selinene(255), γ-cadinene (264) and α-cuparene (262), also widely distributed. As might be expected α- and β-selinenes from F.tamarisci have the opposite absolute configuration to those from F.dilatata.

Jubula

Jubula japonica produces sesquiterpenoids, cyclocolorenone (25), β-elemene (366) and maalioxide (336) having been detected by gcms[32].



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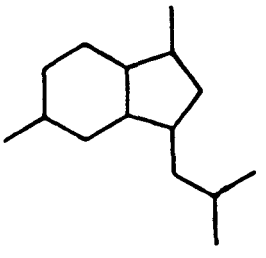

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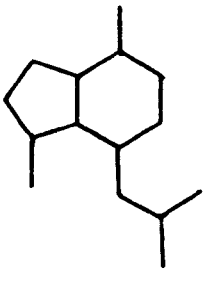

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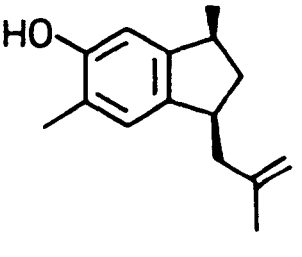

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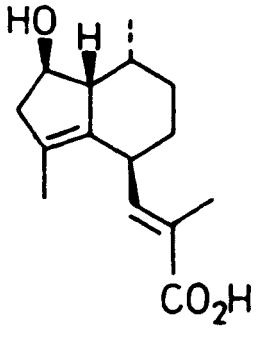

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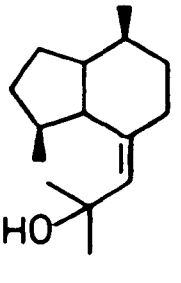
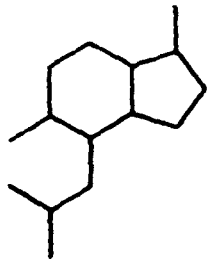


(416)

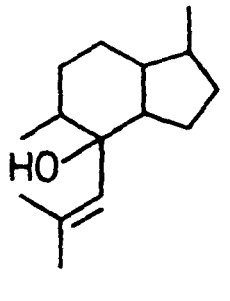
Results

Frullania tamarisci

This liverwort, abundant in Scotland, was previously investigated by Connolly and Thornton[73] (see p. 19) who found (-)-frullanolide (58), α-cyclocostunolide (62), γ-cyclocostunolide (61) and costunolide (63). Reinvestigation has yielded these compounds apart from α-cyclocostunolide (62). However the major constituent is tamariscol (411), a pungent oil, $C_{15}H_{26}O$ (m/z 222.1991), $[\alpha]_D$ + 19.7° (c, 1.1 in $CHCl_3$), [ν_{max} 3620 (free hydroxyl) cm^{-1}] which has a trisubstituted double bond [δ_H 5.07 (m, J 1.3 Hz), δ_C 121.9 (d) and 136.4 p.p.m. (s)], a tertiary alcohol [δ_C 79.0 p.p.m. (s)], two vinyl methyls [δ_H 1.88 (d, J 1.2 Hz), 1.75 (d, J 1.5 Hz); δ_C 20.3 (q) and 28.5 p.p.m. (q)] and two secondary methyls [δ_H 0.92 (d, J 6.6 Hz), 0.88 (d, J 6.6 Hz); δ_C 15.4 (q) and 19.2 p.p.m. (q)] which together with four methine and four methylene groups constitute a bicarbocyclic system. The presence of an isobutenyl group and two secondary methyls along with consideration of the other ^{13}C multiplicities suggested a ring-contracted cadinane (412) or a ring-contracted guaiane (413) skeleton. Both skeletons are known in nature, mutisianthol (414)[222] being an example of the former, valerenolic acid (415)[223] and valerenenol (416)[224] examples of the latter. Since a relatively large amount (ca. 1g) of the alcohol was



(417)



(418)

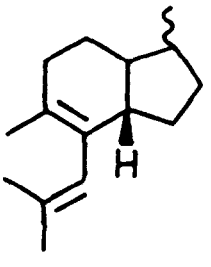
available, a 2-dimensional ^{13}C nmr spectrum was obtained utilising the INADEQUATE pulse sequence (see p.204). This technique established the basic carbon skeleton as (417), hence the gross structure of the alcohol is (418). It also unambiguously assigned all of the ^{13}C resonances apart from C-12 and C-13 (see Table 8).

The 360 MHz ^{1}H nmr spectrum of (411) is not adequately resolved to allow the stereochemistry to be determined. Addition of $Eu(fod)_3$ spreads out some of the signals, the fastest moving being those due to the vinyl proton, a vinyl methyl, a secondary methyl and its associated methine, and one other methine, presumably the ring junction proton to the hydroxyl group. At 360 MHz the shifted sp ectrum is hopelessly broad, but at 100 MHz a few spectral features can be observed. H-3 (ddq, J 4.5, 12.0, 6.5 Hz) has a large couplingto a neighbouring proton (H-2) and is therefore axial. The hydroxyl group must be equatorial to account for the large shift of H-3 on addition of $Eu(fod)_3$. H-1 (dt, J 8.0, 11.0 Hz) is also axial as indicated by its large couplings which also suggests that the ring-junction is trans. Hence the relative stereochemistry of the alcohol may be as shown in (411). The C-7 methine resonance could not be located even at 360 MHz in different solvents and thus it was not possible to observe the necessary couplings for assignment of relative configuration.

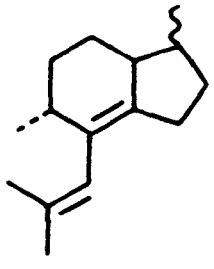
Table 8

^{13}C nmr chemical shifts of a sesquiterpenoid from *Frullania tamarisci*

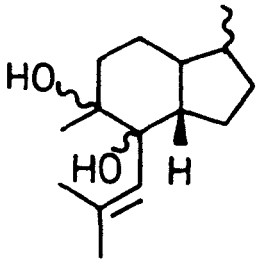
Carbon	(411)
1	59.0
2	79.0
3	46.0
4	33.3
5	30.6
6	50.3
7	40.1
8	32.2
9	24.3
10	121.9
11	136.4
12	28.5
13	20.3
14	15.4
15	19.2



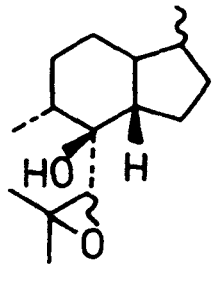
(419)



(420)



(421)



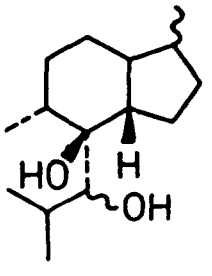
(422)

Chemical manipulation of tamariscol (411) initially proved difficult. The double bond was resistant to catalytic hydrogenation and osmoylation, while ozonolysis gave mainly polymeric material.

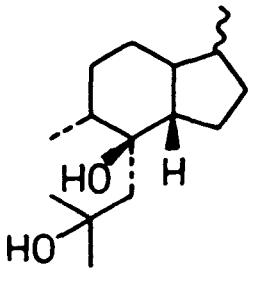
Facile dehydration using $SOCl_2$ gave a high yield of a mixture (glc) of two compounds, assumed to be (419) and (420) from the vinyl resonances in the ^1H nmr spectrum. These could not be separated. When treated with OsO_4 (1 molar equivalent) one product was isolated. The presence of an isobutenyl group [δ_H 5.10 (septet, J 1.3 Hz, H-10), δ_C 122.0 (d) and 136.2 (s), δ_H 1.89 (d, J 1.3 Hz) and 1.75 (d, J 1.3 Hz), δ_C 18.9 (q) and 24.5 (q)], two tertiary hydroxyl groups [δ_C 75.0 (s) and 80.8 (s)], a tertiary methyl [δ_H 1.14 (s), δ_C 28.3 (q)] and a secondary methyl [δ_H 0.93 (d, J 6.6 Hz), δ_C 19.8 (q)] indicated that osmoylation of the tetrasubstituted double bond in (419) had occurred producing diol (421).

Tamariscol (411) was converted in high yield to a mixture of epimeric epoxides (422) by m-chloroperbenzoic acid. The appearance of resonances characteristic of epoxide protons [δ_H 2.59 (s) and 2.56 (s)] indicated that epoxidation had occurred. The epimeric epoxides were not separated since they possessed very similar Rf values.

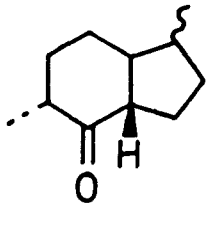
Attempts to open the epoxide ring using aqueous acid or aqueous base were unsuccessful. $LiAlH_4$ could



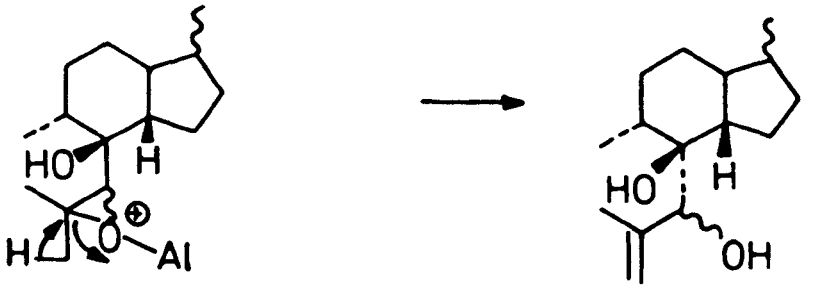
(423,424)



(425)



(426)



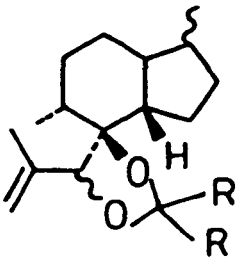
(427)

Fig. 10

be used to reduce the epoxide to a mixture of the three alcohols (423-425). The 1,3-diol (425) was not further investigated. All attempts to oxidise or acylate the vicinal diols (423, 424) were thwarted by the strong intramolecular hydrogen-bonding. Periodate cleavage of these diols gave a small amount of oily material. The infra-red spectrum [V_{max} 1715 (cyclohexanone) cm^{-1}] and mass spectrum [m/z 166] supported formation of the expected indanone (426) but this material could not be sufficiently purified for complete characterisation.

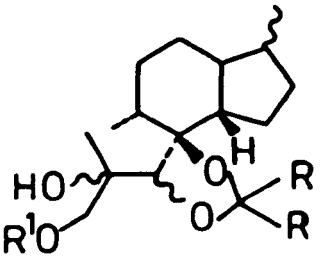
$LiAlH_4$ in refluxing benzene did not reduce the epoxides, producing instead the epimeric allylic alcohols (427) which were separable by preparative tlc. Their formation can be rationalised in the following manner: $LiAlH_4$ is not soluble in benzene (it is in ethereal solvents) and hence does not act as a reducing agent. Instead, an aluminium species acts as a Lewis acid and brings about the rearrangement shown in Fig. 10. The more common Lewis acid, $BF_3.Et_2O$, gave a different product, a volatile oil which readily turned blue upon exposure to oxygen or acid. $BF_3.Et_2O$ is a stronger acid and presumably caused more extensive rearrangement.

These alcohols (427) contain more functionality than other tamariscol derivatives and were used for all remaining experiments especially with a view to preparing a suitable derivative for X-ray analysis.



(428) R=Me

(429) R,R=O

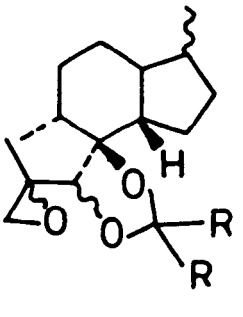


(430) R=Me, R^1=H

(431) R,R=O, R^1=H

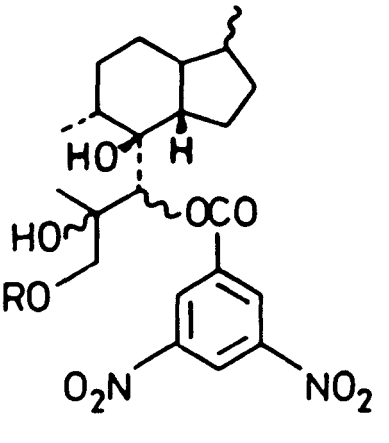
(432) R=Me, R^1 = OCC_6H_4Br-p

(433) R,R=O, R^1 = OCC_6H_4Br-p



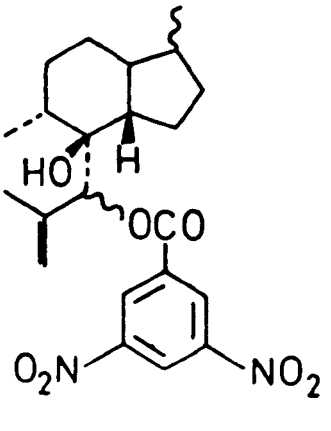
(434) R=Me

(435) R,R=O



(437) R=H

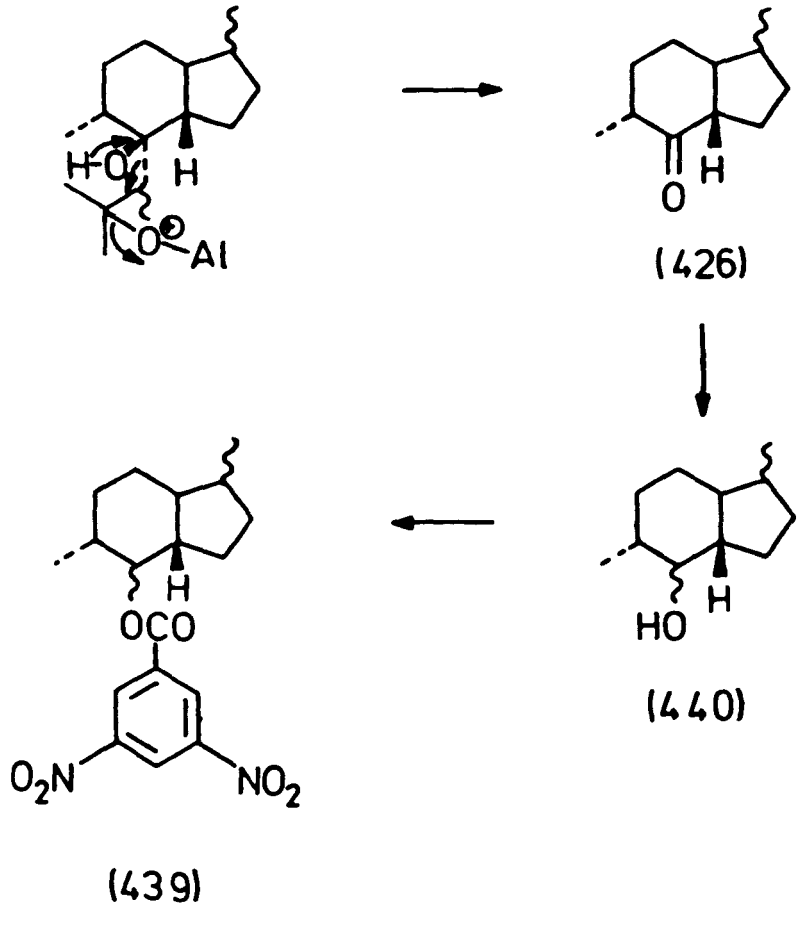
(438) R= OCC_6H_4Br-p



(436)

Again the secondary hydroxyl group is not amenable to oxidation. Jones' and Collins' reagents as well as active MnO_2 were tried without success. It was however, possible to make cyclic derivatives of the diol system. The acetonide (428) (from treatment of the diol (427) with acetone and anhydrous $CuSO_4$) and the carbonate (429) (prepared by treating the diol (427) with N,N^1-carbonyldiimidazole in refluxing benzene) were formed quantitatively and could be further transformed into the diols (430) and (431) using OsO_4. One epimer predominated in both cases. Both diols reacted with p-bromobenzoyl chloride in pyridine to give the corresponding bromobenzoates, (432) and (433), neither of which were suitable for X-ray analysis. Replacement of the bromobenzoate group by p-bromobenzenesulphonate (reputed to produce better crystals) was unsuccessful; the less polar products lacked uv absorption and were probably the epoxides (434) and (435).

The allylic alcohols (427) formed 3,5-dinitrobenzoates (436) on standing with 3,5-dinitrobenzoyl chloride in pyridine. The less polar alcohol (427) was esterified to give the corresponding dinitrobenzoate (436) [ν_{max} : 1735 cm^{-1}] which was converted into a triol (437) using OsO_4 (one epimer was predominant). Treatment of (437) with p-bromobenzoyl chloride in pyridine gave the expected primary bromobenzoate (438), an amorphous solid.

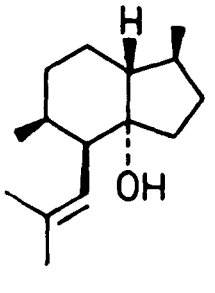


(426)

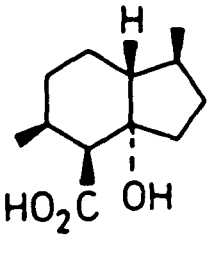
(440)

(439)

Scheme 37.



(441)



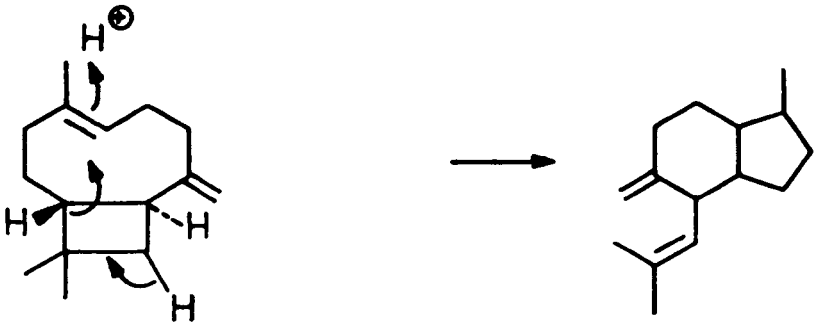
(442)

Also isolated from the dinitrobenzoylation reaction of (427) was a crystalline compound m.p.198-201°C, whichhas a secondary dinitrobenzoate [δ_H 9.20 (m, 3H), and 4.81 (t, J 9.9 Hz)] and two secondary methyls [δ_H0.99 (d, J 6.5 Hz); 0.93 (d, J 6.4 Hz)]. These data are consistent with (439), a hexahydrodimethylindanol dinitrobenzoate. Consideration of the mechanism of formation of the diols (427) from the epoxides (422) on treatment with $LiAlH_4$ - benzene suggests that another pathway exists for decomposition of the inital adduct between the epoxide and the Lewis acid. A retro-aldol reaction (Scheme 37) would generate the ketone (426) which would subsequently be reduced to the hexa-hydrodimethylindanol (440). This product was not observed when purifying the diols (427); presumably it has the same Rf as the less polar diol (427).

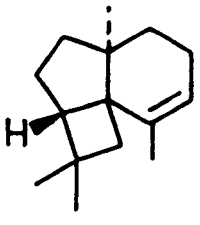
Biogenesis of Tamariscol (411)

Tamariscol (411) possesses an unusual carbon skeleton . of which only one other example has been recorded. Fenical and co-workers[225] isolated pacifigorgiol (441) from the gorgonian Pacifigorgia adamsii and determined the structure by X-ray analysis of the derived acid (442). Subsequently, pacifigorgiol (441) was synthesised by Clardy[226].

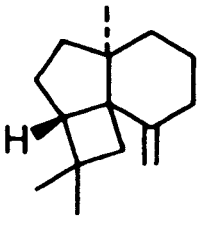
No suggestions have been made in the literature



Scheme 38.



(443)



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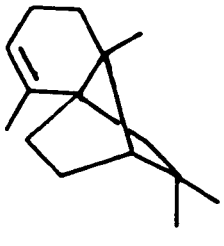

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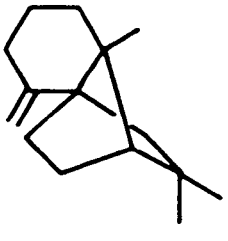

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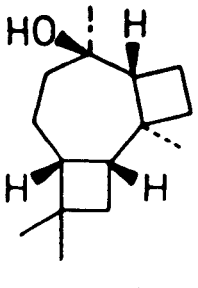

(447)

concerning the biogenesis of the novel carbon framework of pacifigorgiol (441). However, the skeleton is easily derived by rearrangement of a caryophyllene derivative (Scheme 38). This pathway is given support by the presence of β-caryophyllene (261) in the Frullaniaceae (see p.). A small number of other cyclised caryophyllanes have been reported previously. Examples are α- and β-panasinene[227], (443) and (444), neoclovene (445)[228] and β-neoclovene (446)[227], and (447)[228].

The 2-Dimensional INADEQUATE Pulse Sequence

The determination of the carbon skeleton of a molecule would be simplified if it were possible to measure all ^{1}J (^{13}C-^{13}C) values arising from molecules containing two contiguous anisochronic ^{13}C nuclei. However a number of practical problems present themselves.

Since the natural abundance of ^{13}C is 1.1% it follows that measurement of ^{1}J (^{13}C-^{13}C) values involves observation of only 0.012% of the sample. The lines which result from ^{13}C-^{13}C couplings are present in the ^{13}C nmr spectrum as weak satellites accompanying the intense signals of molecules containing isolated ^{13}C atoms. These satellites are often obscured by the noise around the base of the central resonance which is caused by spinning sidebands, incomplete ^{1}H decoupling and impurities.

Synthetic enrichment using double labelling techniques[229] is possible only in a few exceptional cases; hence experimental methods which facilitate the measurement of ^{1}J (^{13}C-^{13}C) data are of interest.

Freeman *et al.*[230] have developed the INADEQUATE pulse sequence for this purpose. It is based on the idea that by suitable treatment of the spin system consisting of the AX system of the coupled ^{13}C nuclei and the intense signal of the compound with only one ^{13}C, the main signal can be supressed. This is possible if

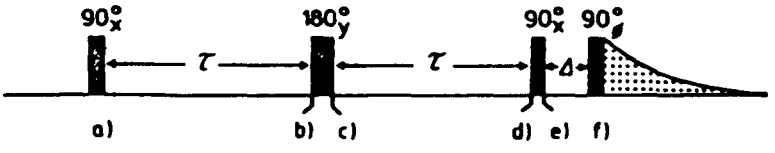
90°x
180°y
90°x
90°
τ
τ
Δ
a)
b) c)
d) e) f)

Fig 11

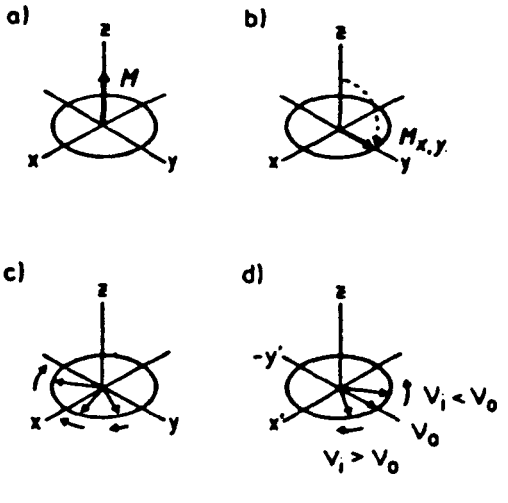
a)
z
M
x
y
b)
z
Mx,y
x
y
c)
z
x
y
d)
z
-y'
x'
Vi < Vo
Vo
Vi > Vo

Fig 12

a 90° phase difference exists between the transverse magnetisation of the satellites and that of the main signal, allowing the selection of the AX magnetisation for detection. This is achieved using the pulse sequence shown in Fig.11.

For the nucleus of interest, the resonance signals of different Larmor frequency present in the spectral window form the macroscopic magnetisation $\underline{M}$ of magnitude M_o parallel to the external field B_o (Fig.12a). A strong radio-frequency field B_1, an RF pulse, produced by a radio-frequency coil on the X-axis, carries $\underline{M}$ away from the Z-axis. The duration and power of the RF pulse determine the direction of $\underline{M}$ after the pulse. If a 90°_x pulse is applied, $\underline{M}$ points along the positive y-axis (Fig. 12b). The longitudinal or Z-magnetisation is thus transformed into a transverse magnetisation. The Larmor frequencies of the various nuclear magnetic moments present vary and $\underline{M}$ now splits into its components (Fig 12c).

In describing nmr experiments the concept of the rotating frame is convenient. It uses a co-ordinate system K' that rotates in the same sense and with the same frequency, ν_o, as the rotating field vector of the RF field. Within the rotating frame vectors that correspond to signals with frequencies $\nu_i > \nu_o$ rotate clockwise, whereas those corresponding to signals with $\nu_i < \nu_o$ rotate anti-clockwise; a signal with $\nu_i = \nu_o$ is

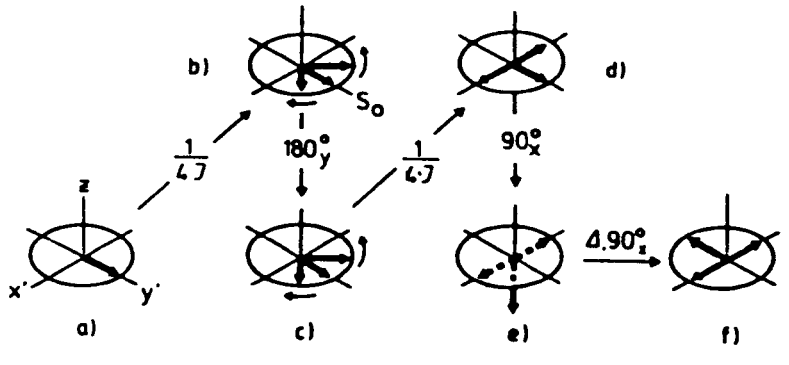

Fig 13

. Phase shifts for the selective detection of $^{13}C,^{13}C$-satellites by the INADEQUATE sequence

ϕ [a]	S_0 [b]	S [c]	Ψ [d]
+x	−y	+x	+x
+y	+x	−y	−y
−x	+y	−x	−x
−y	−x	+y	+y

[a] Phase of read pulse; [b] Phase of main signal; [c] Phase of satellite magnetization; [d] Detector phase.

Table 9

static in the rotating frame (Fig 12 d).

The Larmor frequency determines the chemical shift of the signal whereas the orientation at the beginning of data accumulation determines the phase with respect to the rotating frame and,therefore, the signal phase. The adjustable detector phase allows the selection of certain components of the transverse magnetisation for detection.

Returning to the INADEQUATE pulse sequence, after the initial 90°_{x} pulse (Fig 13 a) ^{13}C-^{13}C coupling leads to a fanning out of the ^{13}C satellites of the A nucleus, which after $\tau = 1/(4J)$ have a phase difference of 90°, whereas the magnetisation S_o of the main signals of molecules with only one ^{13}C nucleus is static on the y axis (Fig. 13 b). The non-selective 180°_{y} pulse leaves the rotational sense of the satellites unchanged (Fig. 13c) and, after the time 2τ, a phase difference of 180° results (Fig 13d). During this time the main signal retains its direction along the y axis; respective inhomogeneity contributions are refocussed by the 180°_{y} pulse. The second 90°_{x} pulse generates <u>double quantum coherence</u>[231] that cannot be detected directly and directs S_o in the z direction (Fig. 13e). Afte a short delay (10μs), transverse magnetisation of the ^{13}C satellites is transformed into detectable magnetisation by a 90° pulse of variable phase (Fig 13f) (see Table 9). The

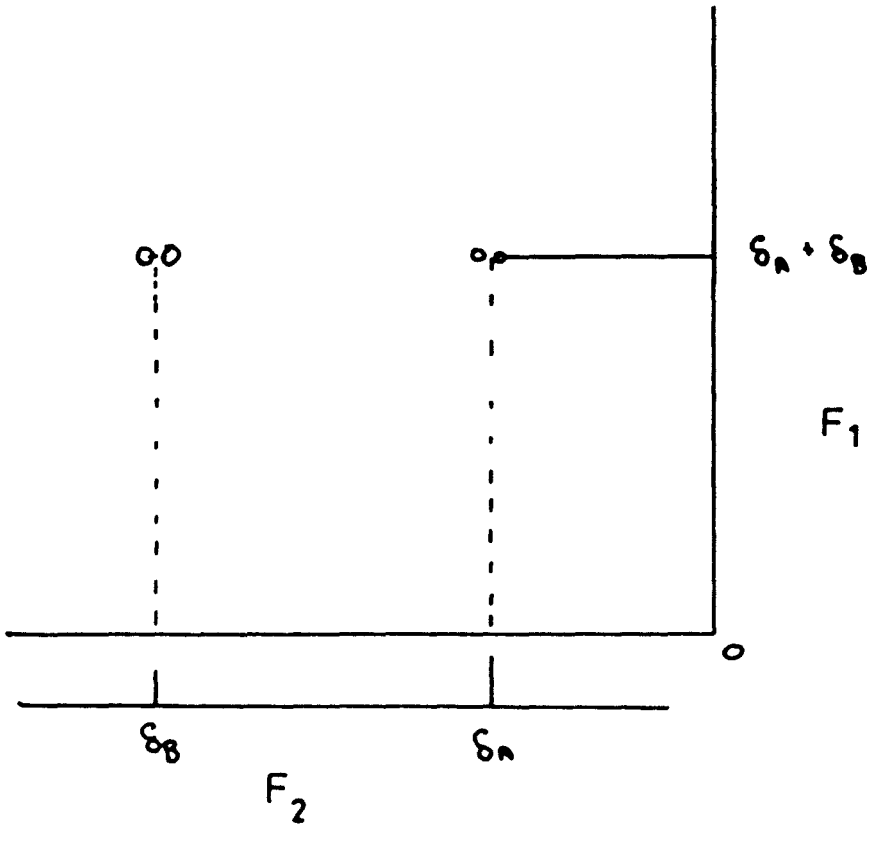

Fig 14.

doublet components have opposite phase, but can be refocussed using an additional spin-echo sequence $1/(4J)-180^{\circ}_{x}-1/(4J)$. ^{1}H-broadband decoupling is applied during the whole experiment.

If the magnitudes of the coupling constants are sufficiently different they may be assigned to specific pairs of carbon resonances simply by picking out the repeated splittings. Adjacent carbon sites are thus identified directly. However, in many cases, the molecular framework is too complex for this method to be applicable as each carbon site can have up to four directly bonded neighbours, and the $^{13}C-^{13}C$ couplings may be close in magnitude or not clearly resolved. Consequently an independent method of assignment is needed.

For this purpose several versions of the INADEQUATE pulse sequence have been proposed[232,233]. A two-dimensional Fourier transform experiment is performed in which both A and X carry information about the frequency of the double-quantum coherence, thus identifying them as part of the same coupled spin system. The double-quantum frequency (ν_{DQ}) occurs at the algebraic sum of the two chemical shifts. The spectrum is displayed as a two-dimensional array with ν_{DQ} in the F_1-dimension and the normal ^{13}C chemical shifts in the F_2-dimension (Fig.14).

An example of a 2-D INADEQUATE spectrum is given

in Fig. 15. Fig. 16 shows various AX spectra obtained by taking slices through the 2-D spectrum parallel to the F_2-axis. Analysis of these AX spectra leads directly - and unambiguously - to the carbon skeleton of tamariscol (411).

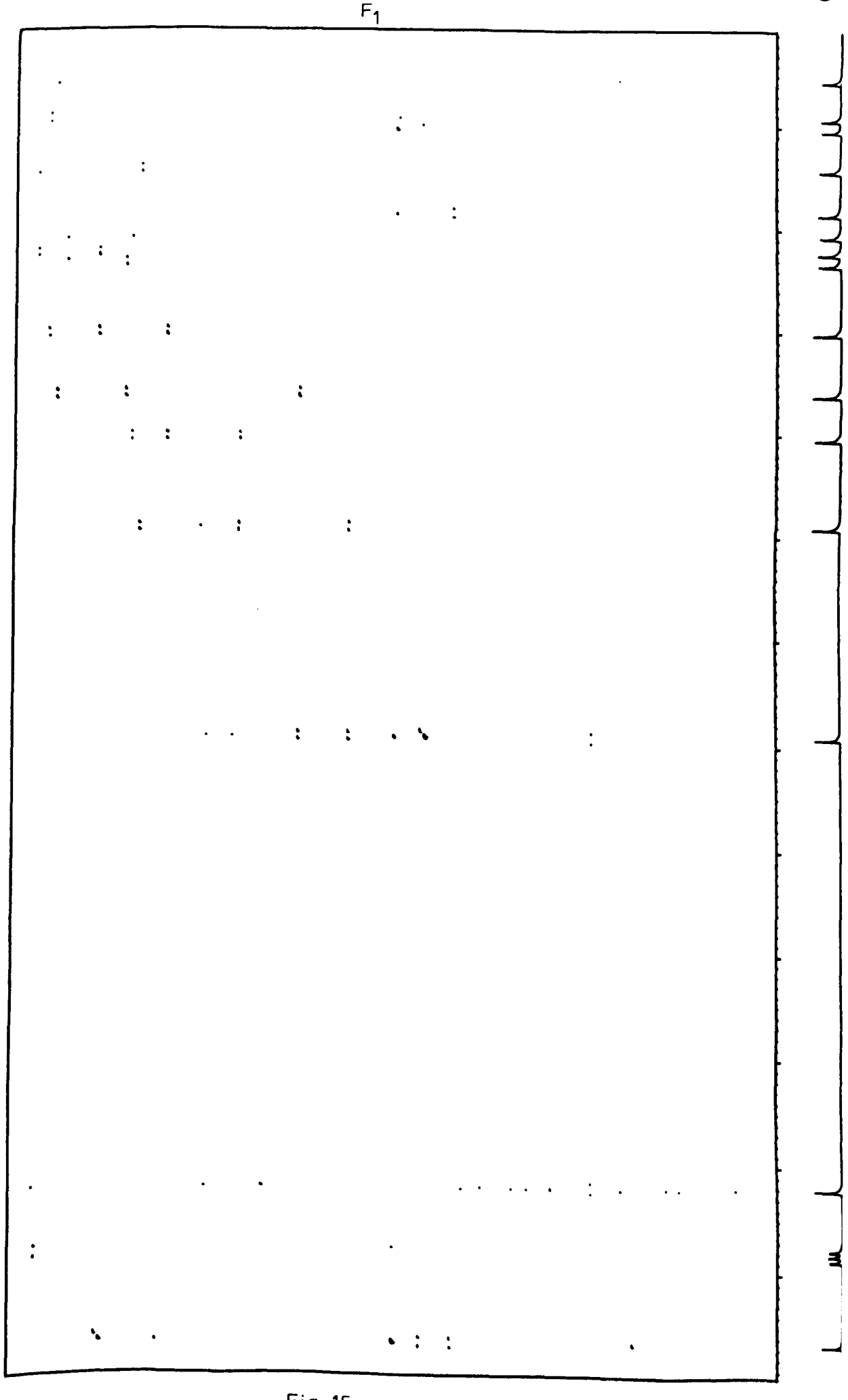

Fig 15

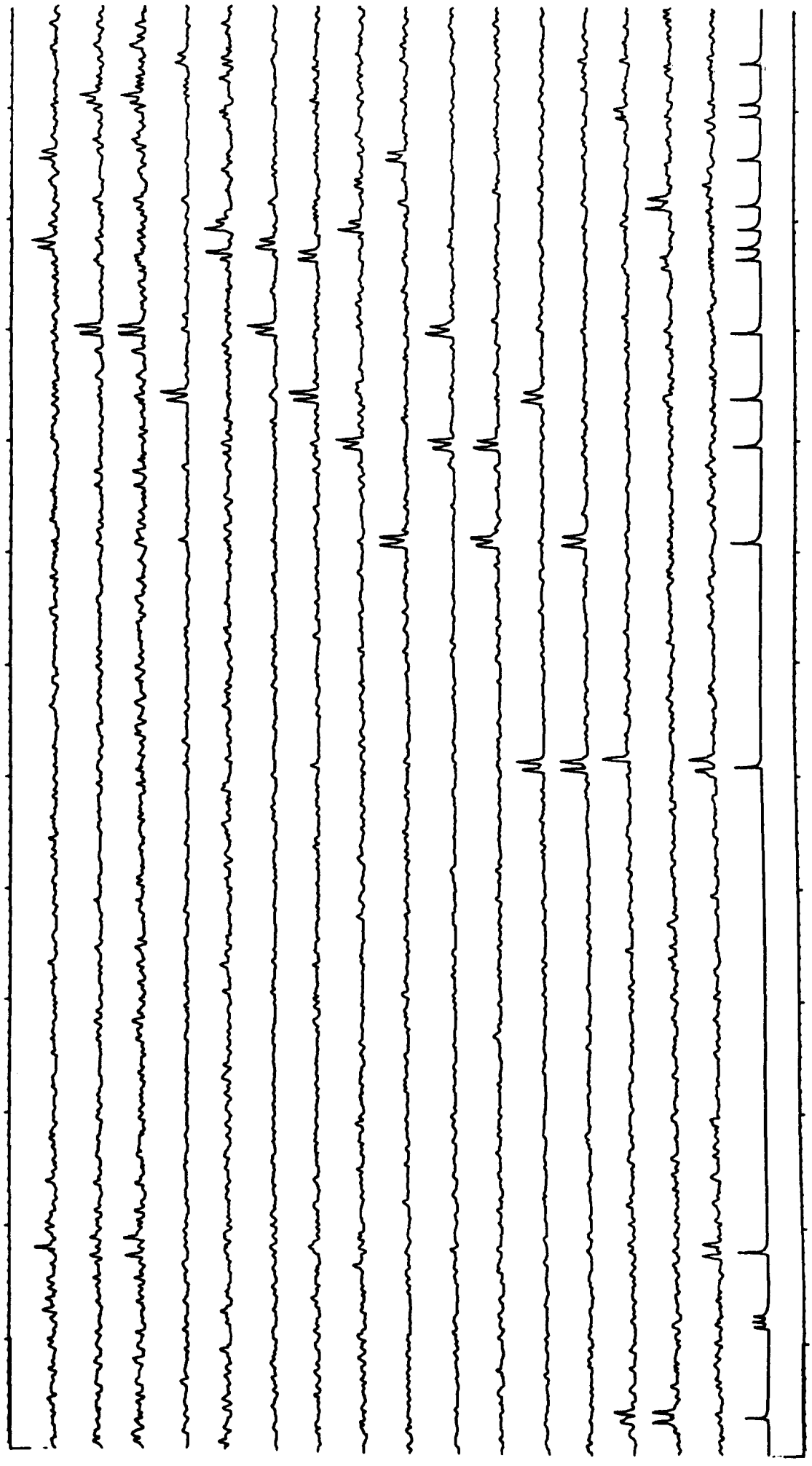

Fig 16

EXPERIMENTAL

Frullania tamarisci

Plant material (2.5 kg) was collected in the West of Scotland. A chromatographic separation of the crude extract (60 g) yielded the following compounds.

i) (-)-Frullanolide (58)

Recrystallisation from petroleum ether - $CHCl_3$ gave (-)-frullanolide (58), (750 mg), m.p. 75-77°C, $[\alpha]_D$ - 111.4 (C, 1.7 in $CHCl_3$) [lit.[73] m.p. 77°C, $[\alpha]_D$ -113°], m/z 232.1467 ($C_{15}H_{20}O_2$ requires m/z 232.1463).

δ_H : 6.00 (d, J 1Hz, H-13); 5.47 (d, J 1Hz, H-13^1); 5.13 (d, J 5 Hz, H-6); 2.85 (br m, H-7); 1.73 (s, 3H-15); 1.05 (s, 3H-14).

δ_C: 18.3 (t), 19.3 (q), 25.1 (t), 25.9 (q), 32.6 (s), 33.1 (t), 37.8 (t), 39.1 (t), 41.0 (d), 75.6 (d), 119.7 (t), 128.8 (s), 137.9 (s), 142.5 (s) and 170.3 (s).

ii) γ-Cyclocostunolide (61)

Recrystallisation from petroleum ether - $CHCl_3$ gave γ-cyclocostunolide (61), (127mg), as needles, m.p. 56-87°C, [lit.[73] m.p. 87-88°C], m/z 232.1462 ($C_{15}H_{20}O_2$ requires m/z 232.1463).

δ_H : 5.98 (d, J 3 Hz, H-13); 5.34 (d, J 3 Hz, H-13^1); 4.42 (br d, J 12 Hz, H-6); 1.84 (br s, 3H-15); 1.09 (s, 3H-14).

δ_C : 18.8 (q), 19.8 (q), 23.1 (t), 26.1 (q), 34.3 (t), 37.0 (s), 40.6 (t), 41.2 (t), 50.2 (d), 83.1 (d), 117.7 (t), 126.3 (s), 130.1 (s), 139.4 (s) and 169.7 (s).

iii) Costunolide (63)

Recrystallisation form MeOH gave costunolide (63), (50 mg), as needles, m.p. 104-105°C [lit.[73] m.p. 106-107°C].

δ_H : 6.23 (d, J 3Hz, H-13); 5.52 (d, J 3 Hz, H-13[1]), 4.86 (1 H, m); 4.72 (1 H, d J 9 Hz); 4.56 (1H, t, J 9 Hz); 1.71 (d, J 1Hz) and 1.42 (d, J 1Hz, two vinyl methyls).

iv) Tamariscol (411)

Steam distillation of an early fraction followed by preparative tlc afforded tamariscol (411), (810mg), as an oil, $[\alpha]_D$ + 19.7° (C, 1.1 in $CHCl_3$), m/z 222.1991 ($C_{15}H_{26}O$ requires m/z 222.1984).

ν_{max} : 3620 cm^{-1}

δ_H (360 MHz) : 5.07 (m, J 1.3 Hz, H-10); 1.88 (d, J 1.2 Hz, 3H-12); 1.75 (d, J 1.5 Hz, 3H-13); 0.92 (d, J 6.6 Hz, secondary methyl); 0.88 (d, J 6.6 Hz, secondary methyl).

δ_C : see Table 8 (p. 197).

Attempted Hydrogenation of Tamariscol (411)

The alcohol (411), (30 mg), in EtOAc (15 ml) was stirred with 10% Pd-C catalyst (30mg) for 7h. Removal of catalyst and evaporation of solvent gave only starting material.

Attempted Osmoylation of Tamariscol (411)

Tamariscol (411), (30mg), in dry pyridine (2ml) was treated with OsO_4 (50 mg) overnight. The usual work-up gave only starting material.

Attempted Lemieux-Johnson Oxidation of Tamariscol (411)

Following the method of Lemieux and Johnson, tamariscol (411), (45mg), was dissolved in aqueous dioxan (5 ml) kept at 0°C, OsO_4 (5 mg) added and the mixture stirred for 15 min. $NaIO_4$ (100 mg) was then added and stirring continued for 12h. Work-up gave only starting material.

Ozonolysis of Tamariscol (411)

a) Tamariscol (411), (30 mg), in EtOAc (15 ml) was cooled to -78°C and ozone passed through the solution until a blue colour persisted. After 1h. solvent was removed in a stream of nitrogen, acetic acid (10ml) and zinc dust (300 mg) added and the mixture stirred for 4h at room temperature. Filtration and removal of

solvent yielded mainly polymeric material.

b) Tamariscol (411), (35mg), in MeOH (10ml) at -78°C was ozonised as above. While still at -78°C the solution was flushed with nitrogen until the blue colour had disappeared. Dimethyl sulphide (2ml) was added and the solution stirred for 2h at -20 C, 30 min. at 0 C and a further 30 min. at room temperature, after which solvent was removed. The product consisted mainly of polymeric material.

Dehydration of Tamariscol (411)

To a stirred, ice-cold solution of the alcohol (411), 100 mg), in dry pyridine (4 ml) was added freshly-distilled thionyl chloride (12 drops). After 5 min the mxiture was allowed to come to room temperature, poured into ice-cold $NaHCO_3$ solution (15ml) and extracted with Et_2O. The organic layer was dried ($MgSO_4$) and solvent removed to give an oily material (90 mg) which glc (1% OV-1, 70°C) showed to be a mixture of two compounds.

ν_{max} : 2950, 1600 and 1580 cm^{-1}

λ_{max} : 225 nm.

δ_H : 5.59 and 5.50 (br s, vinyl protons); 1.75 (br s, vinyl methyl); 1.50 (br s, vinyl methyl); 1.05-0.90 (secondary methyls).

Osmoylation of the Diene Mixture (419, 420)

A solution of the diene mixture (419, 420), (90mg), OsO_4 (101 mg), 0.9 molar equivalents) and dry pyridine (1ml) in Et_2O (5ml) was left overnight. Work-up and preparative tlc on $AgNO_3$ -impregnated SiO_2 gave a crystalline diol (421) (11mg).

δ_H (200MHz) : 5.10 (septet, J 1.3 Hz, H-10); 1.89 (d, J 1.3 Hz) and 1.75 (d, J 1.3 Hz) vinyl methyls; 1.14 (s, 3H-14); 0.93 (d, J 6.6 Hz, 3H-15).

δ_C : 18.9 (q), 19.8 (q), 23.7 (t), 24.5 (q), 25.2 (t), 28.3 (q), 31.8 (t), 36.5 (t), 39.2 (d), 49.5 (d), 51.9 (d), 75.0 (s), 80.8 (s), 122.0 (d) and 136.2 (s).

Periodate Oxidation of Diol (421)

The diol (421), (10mg), in aqueous MeOH (5 ml) was stirred with $NaIO_4$ (30mg) for 4h. The solution was diluted with water and extracted with Et_2O. No product was isolated.

Epoxidation of Tamariscol (411)

To a solution of tamariscol(411), (346mg), in dry CH_2Cl_2 (10ml) was added dropwise, with stirring, a solution of pure m-chloroperbenzoic acid (259 mg, 1.1 equivalents)

in dry CH_2Cl_2 (5ml). After completion of the reaction (monitored by tlc) a solution of sodium sulphite was added to destroy unreacted peracid. The mixture was extracted into Et_2O, washed with $NaHCO_3$ solution and brine, dried ($MgSO_4$) and evaporated to give the epoxides (422) (359mg).

ν_{max} : 3570, 1460, 1380 and 1240 cm^{-1}

δ_H : 2.56, 2.59 (s, epoxide protons); 1.45, 1.41 (s, tertiary methyls); 1.21 (br s); 0.96-0.85 (secondary methyls).

$LiAlH_4$ Reduction of Epoxides (422)

To the epoxides (422), (100mg), in dry Et_2O (20ml) was added $LiAlH_4$ (200mg) and the mixture refluxed for 5h. Wet Et_2O was added to destroy unreacted hydride, then the mixture was acidified with ice-cold 10% HCl and extracted with Et_2O. The organic layer was washed with $NaHCO_3$ solution and dried ($MgSO_4$). Removal of solvent gave a residue which gave three products (423), (424) and (425), upon preparative tlc.

i) δ_H : 3.64 (d, J 7 Hz, collapses to singlet on addition of D_2O, H-10); 1.06 (d, J 6Hz, secondary methyl); 1.00-0.89, (3d, secondary methyls).

ii) δ_H : 3.65 (d, J 7Hz, collapses to singlet on addition of D_2O,H-10); 1.04-0.94 (four secondary methyls)

iii) δ_H : 1.30 (s, 3H-12 and 3H-13); 1.00-0.93 (two secondary methyls).

Attempted Acid Hydrolysis of the Epoxides (422)

To the epoxides (422), (10mg) in dioxan (2ml) was added 20% HCl (2ml) and the mixture stirred for 6h Extraction into $CHCl_3$ (10ml) yielded starting material.

Attempted Base Hydrolysis of the Epoxides (422)

To the epoxides (422), (10mg) in dioxan (2ml) was added 30% NaOH solution (2ml). After stirring for 6h the mixture was extracted into $CHCl_3$ (10ml) to yield only starting material.

Attempted Jones Oxidation of the Diol (423)

The less polar secondary alcohol (423) (5mg) in ice-cold acetone (1ml) was treated with Jones reagent (5 drops). After stirring for 5min the mixture was extracted into $CHCl_3$. Only starting material was obtained.

Attempted Bromobenzoylation of the Diol (423)

To the less polar diol (423), (10mg), in dry pyridine (1ml) was added p-bromobenzoyl chloride (20mg). After 12h solvent was removed; tlc indicated that no reaction had occurred.

Cleavage of the Diol (424)

The more polar diol (424), (25mg) was dissolved in Et_2O (5ml) and stirred with periodic acid dihydrate (100mg) for 5h. Filtration and evaporation of solvent left an oily residue (5mg), m/z 166 ($C_{11}H_{18}O$ requires m/z 166), ν_{max} 1715 cm^{-1}.

Attempted purification by distillation resulted in decomposition of material.

Rearrangement of Epoxides (422)

To the epoxides (422) (359 mg) in dry benzene (15ml) was added $LiAlH_4$ (400mg) and the mixture refluxed under argon until tlc indicated that all of the starting maerial had been consumed. Moist Et_2O was added to destroy unreacted hydride, the mixture was acidified with ice-cold 10% HCl and extracted into Et_2O. After drying ($MgSO_4$) the solution was evaporated to give an oily residue. Preparative tlc gave two epimeric alcohols (427).

i) the more polar compound (138mg), mp. 110-113°C (ex pentane) m/z 238.1939 ($C_{15}H_{26}O_2$ requires m/z 238.1933).

ν_{max} : 3620 and 3580 cm^{-1}

δ_H : 4.90 (br s, H-12); 4.82 (br s, H-12^1); 4.30 (br d, J 6 Hz, H-10); 2.48 (br s, C-2 OH); 2.05 (d, J 6Hz, C-10 OH); 1.86 (br s, 3H-13); 1.05-0.89 (3H-14, 3H-15).

ii) the less polar compound (116mg), m.p. 112-114°C, (ex pentane) m/z 238.1938 ($C_{15}H_{26}O_2$ requires m/z 238.1933).

ν_{max} : 3620 and 3580 cm^{-1}

δ_H : 4.95 (br s, H-12); 4.85 (br s, H-12^1); 4.30 (d, J 6Hz, H-10); 2.32 (s, C-2 OH); 2.12 (d, J 6Hz, C-10 OH); 1.85 (br s, 3H-13); 1.04-0.80 (3H-14, 3H-15).

$BF_3.Et_2O$ Catalysed Rearrangement of Epoxides (422)

The epoxides (422), (10mg), in dry Et_2O (2ml) at 0°C were treated with $BF_3.Et_2O$ (3 drops). After 10 min. the mixture was washed with water, dried ($MgSO_4$) and solvent removed to give a volatile oil which rapidly turned blue upon exposure to the atmosphere.

Attempted Oxidation of the Diol (427)

i) The more polar diol (427) (10mg) in dry acetone (1ml) at 0°C was heated with Jones' reagent (5 drops). After stirring for 5 min. $CHCl_3$ extraction gave starting material.

ii) The more polar diol (427), (11mg), in dry CH_2Cl_2 (1ml) was stirred with Collins reagent (100mg) for 1h. Filtration through Celite and removal of solvent gave only starting material.

iii) The more polar diol (427), (15mg), in dry benzene (5ml) was stirred overnight with activated MuO_2 (50mg). Filtration through Celite and removal of solvent gave only starting material.

Acetonide (428) Formation

The less polar diol (427), (50mg), was dissolved in dry acetone (5ml) and anhydrous $CuSO_4$ (100mg) added. After stirring overnight, the salt was filtered off and solvent removed to give the acetonide (428) (56mg).

δ_H : 5.37 (br s, H-12); 5.14 (br s, H-12^1); 4.53 (s,H-10); 1.89 (br s, 3H-13); 1.52, 1.33 (2s, acetonide methyls); 1.23 (d, J 6Hz, secondary methyl); 0.91 (d, J 6Hz, secondary methyl).

Osmoylation of the Acetonide (428)

To the acetonide (428), (55mg), in Et_2O (5ml) was added dry pyridine (1ml) and OsO_4 (70mg). After 12h the solution was diluted with Et_2O and stirred with aqueous sodium metabisulphite until the organic layer was colourless. The mixture was extracted with EtOAc to give

a mixture of two compounds; preparative tlc gave the diol (430), (35mg), as the major product.

The Acetonide Bromobenzoate (432)

To the diol (430), (25mg), in dry pyridine (2ml) was added p-bromobenzoyl chloride (30mg). After 12h the solvent was removed and the residue chromatographed to give the oily p-bromobenzoate (432) (32mg).

ν_{max} : 3600 and 1745 cm^{-1}

δ_H : 7.90 and 7.66 ($AA'BB'$ system, $J_{AB+AB'}$ 9Hz, aromatic protons); 4.37 (s, H-10); 4.42 and 4.03 (ABq, J 12Hz, 2H-10); 1.41 (s, methyls); 1.32 (s, tertiary methyl); 1.00-0.84 (3H-14 and 3H-15).

Attempted Formation of Acetonide Brosylate

To the acetonide diol (430) (1mg) in dry pyridine (0.1ml) was added p-bromobenzenesulphonyl chloride (1mg). After 12h removal of solvent gave a gummy residue. Analytical tlc showed one spot which was uv inactive.

The Carbonate (429)

The more polar diol (427), (60mg), and N,N^1-carbonyl-diimidazole (150mg) were refluxed overnight in dry benzene (20ml). The solution was washed with water (4x20ml), dried ($MgSO_4$) and evaporated to give the

carbonate (429) (62mg), m.p. 93-96°C (ex pentane).

δ_H : 5.17 (br s, 2H-12); 4.92 (s,H-10); 1.88 (br s, 3H-13); 1.00 (d, J 6 Hz, secondary methyl); 0.92 (d, J 6 Hz, secondary methyl).

δ_C : 14.7 (q), 19.3 (q), 20.3 (q), 23.6 (t), 30.1 (t), 30.4 (t), 32,3 (t), 32.5 (t), 39.9 (d), 44.1 (d) 49.0 (d), 53.0 (d), 81.9 (d), 90.8 (s), 117.9 (t), 140.0 (s), and 154.9 (s).

The Carbonate Diol (431)

To the carbonate (429), (60mg), in Et_2O (10ml) was added dry pyridine (1ml) and OsO_4 (70mg). After 12h. the usual work-up, followed by preparative tlc, gave the carbonate diol (431) (55mg) as a gum.

δ_H : 4.62 (s, H-10); 3.76 and 3.44 (ABq, J_{AB} 11 Hz, 2H-12); 1.31 (s, 3H-13); 1.01-0.94 (3H-14 and 3H-15).

The Carbonate Bromobenzoate (433)

A solution of the carbonate diol (431), (20mg), and p-bromobenzoyl chloride (30mg) in dry pyridine (2ml) was left overnight. Removal of solvent and preparative tlc gave the carbonate bromobenzoate (433) (19mg) as an oil.

ν_{max} : 3610, 1790, 1725 and 1590 cm^{-1}.

Attempted Formation of the Carbonate Brosylate

To the carbonate diol (431) (1mg) in dry pyridine (0.1ml) was added p-bromobenzenesulphonyl chloride (1mg). Removal of solvent gave a residue. Analytical tlc indicated the presence of one major product which lacked uv absorption.

The Dinitrobenzoate (436)

To the less polar alcohol (427), (97mg), in dry pyridine (2ml) was added 3,4-dinitrobenzoyl chloride (110 mg). After 12h., the solvent was removed and the mixture chromatographed to give i) the dinitrobenzoate (436) (140mg), m.p. 127-129°C (ex hexane) m/z 432 ($C_{22}H_{28}N_2O_7$ requires m/z 432).

ν_{max} : 3620 and 1740 cm^{-1}

δ_H : 9.15 (m, 3H, aromatic protons); 5.83 (s, H-10); 5.16 (br s, H-12); 5.05 (br s, H-12^1); 1.95 (br s, 3H-13); 1.06 (d, J 6 Hz, secondary methyl); 0.84 (d, J 6Hz, secondary methyl).

and ii) the hexahydro dimethylindanol dinitrobenzoate (439) (10mg) m.p. 149-154°C.

δ_H (200MHz): 9.20 (m, 3H, aromatic protons); 4.81 (t, J 9.9 Hz, H-2); 0.99 (d, J 6.5 Hz, secondary methyl); 0.93 (d, J 6.4 Hz, secondary methyl).

The Dinitrobenzoate Diol (437)

To the dinitrobenzoate (436) (70mg) in Et_2O (5ml) were added dry pyridine (1ml) and OsO_4 (50mg). After 15h the usual work-up gave, after preparative tlc, the more polar diol (437) (50mg). Recrystallisation from petroleum ether - $CHCl_3$ gave needles m.p. 198-200°C,

ν_{max}: 3620 and 1735 cm^{-1}

δ_H : 9.15 (3H, m, Ar-H); 5.83 (s, CHO-Aryl); 5.17 and 5.06 (2br s, exomethylene); 1.95 (s, vinyl methyl); 1.05 and 0.95 (2d, J 6.6 Hz, secondary methyls).

The Dinitrobenzoate Bromobenzoate (438)

To the dinitrobenzoate diol (437) (30mg) in dry pyridine (2ml) was added p-bromobenzoyl chloride (30mg) and the mixture left overnight. Removal of solvent and preparative tlc gave the dinitrobenzoate bromobenzoate (438) (40mg), an amorphous solid.

δ_H ($CDCl_3$): 9.20 (3H,m,Ar-H); 7.96 and 7.64 (AA'BB' system, $J_{A+AB'}$ 8 Hz, bromobenzoate); 5.96 (s, CH-O); 4.50 and 4.30 (AB system, J_{AB} 10Hz, CH_2-O); 1.45 (s, tertiary methyl).

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