A Thesis Entitled

"STRUCTURE AND SYNTHESIS OF SOME LIVERWORT METABOLITES"

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by

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Finally, I would like to thank the Algerian Government for the financial support, also my parents, my wife and all members of my family for their forbearance during my research.

SUMMARY

This thesis consists of four chapters. The first chapter, which is a General Introduction, deals with (i) the nature of secondary metabolites and (ii) the terpenoids and aromatic constituents of the liverworts. Chapter 2 describes several efforts to synthesise a lunularic acid derivative which occurs in the liverwort Plagiochila spinulosa. A Wittig reaction of 2- nitroveratraldehyde and 4- methoxybenzylphosphonium chloride afforded a mixture of stilbenes which on reduction gave 2- amino- 3,4,4'- trimethoxybibenzyl. This amino-bibenzyl was subjected to a Sandmeyer reaction in the hope of producing 2- cyano- 3,4,4'- trimethoxybibenzyl. Unfortunately, the desired compound was obtained only in very low yield. The major product was 3,5,6- trimethoxy- 9,10- dihydrophenanthrene, the result of a Pschorr cyclisation.

A similar Wittig route was used to prepare 2- bromo- 3,4,4'- trimethoxybibenzyl. Attempts at carboxylation resulted only in debromination.

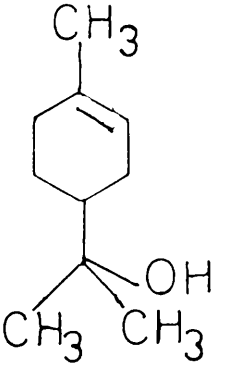
Chapter 3 concerns a discussion of the constituents of Plagiochila spinulosa. Five dihydrophenanthrene derivatives were isolated together with methyl 2- methyl- 3,4- methylenedioxy- 6- methoxybenzoate and an unusual natural product spinuloplagin B which is a diterpenoid- bibenzyl conjugate. A synthesis of 2- hydroxy- 3,7- dimethoxy- 9,10,- dihydrophenanthrene is also described.

The final chapter considers the metabolites of Frullania tamarisci. In addition to the known compounds (-) frullanolide and tamariscol, a new dimeric sesquiterpenoid lactone was isolated.

C O N T E N T S

C H A P T E R 1

GENERAL INTRODUCTION



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The study of natural products has long been a subject of fascination for the organic chemist, foodstuffs, dyestuffs, medicines and stimulants, for example, all falling within its confines. However, it was only in the eighteenth century that chemists began to investigate the extracts obtained from natural sources. The study of natural products provided, in part, the stimulus for the development of modern chromatographic and spectroscopic methods. Nowadays structural elucidation requires only small amounts of material and involves a minimum of chemical transformation.

Natural products traditionally fall into two categories primary and secondary metabolites. Polysaccharides, proteins, fats, lipids, amino acids, nucleosides and nucleic acid are the fundamental building blocks of living matter and are considered to be the primary metabolites. The chemistry of the secondary metabolites, such as phenols, quinones, terpenes, alkaloids and various pigments has been a productive area of research for the organic chemist. Studies of terpenoids, for example, were instrumental in the early development of a synthesis of terpineol (1) in 1904 by Perkin[1].

Organisms have adapted the production of metabolites, i.e. their enzyme activity to their living conditions, and the production of these compounds cannot be entirely fortuitous. Secondary metabolites, in particular those from plants, are considered to be an important factor in the co-evolution of

plants, animals and insects. The importance of chemical control is apparent in areas of survival e.g. defence chemicals, feedants, and antifeedants, the pheromones and sex attractants.

This thesis is concerned with the isolation of secondary metabolites from the Hepaticae (liverworts) and with the attempted synthesis of two of them.

HEPATICAE

The bryophytes are taxonomically placed between the algae and the pteridophytes and there are about 20,000 species in the world. They are morphologically divided into three main groups: (i) Musci (mosses, 14,000 species); (ii) Hepaticae (liverworts, 300 species) and (iii) Anthocerotae (hornworts, 300 species). Liverworts are the most interesting in chemical terms. This interest is probably associated with the presence of oil bodies in their cells. The Musci and Anthocerotae do not contain oil bodies and have been found to be of limited chemical interest.

Liverworts are primitive plants, economically unimportant, and are easily overlooked. Some have found biological use e.g. *Marchantia polymorpha*[2] as a diuretic and *Conocephalum conicum* against gallstones[2]. Some species induce allergenic contact dermatitis[3] and inhibit the growth of micro-organisms[4].

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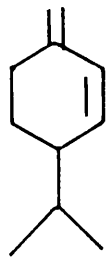

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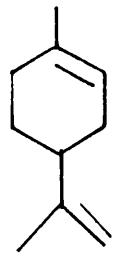

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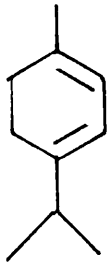

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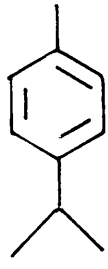

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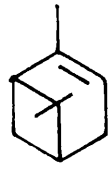

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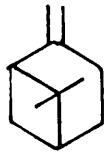

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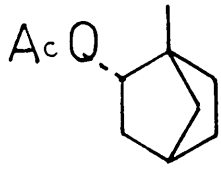

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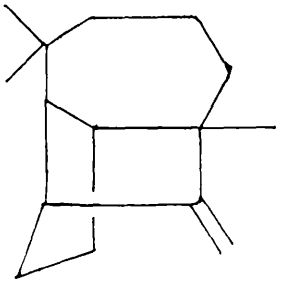
In 1905 the famous bryologist Karl Muller[5] reported that the oil bodies of the Hepaticae contained sesquiterpenoids. Further studies were delayed until 1956, when Fujita et al[6] reported the presence of sesquiterpenoids in the essential oil of Bazzania pompeana. The presence of chemically and pharmacologically interesting substances in the Hepaticae has made the liverworts the object of considerable attention in the past twenty-five years.

The chemistry of the bryophytes has been the subject of regular reviews. The most recent reviews are those by (i) Markham and Porter[7] reviewing the chemical constituents of the bryophytes, viz, lipids, terpenoids. flavonoids, lignans and bibenzyls published prior to 1978, (ii) Connolly[8] who dealt with terpenoid constituents of some European liverworts; (iii) Asakawa[9] who has produced a comprehensive review of liverwort constituents, (iv) Huneck[10] who dealt with all liverwort metabolites.

MONOTERPENOIDS

Reports on the occurrence of monoterpenoids in the Hepaticae are limited. Most of monoterpenoids are detected by gc or gcms. Asakawa and co-workers[11] reported the isolation of pure monoterpenoids from Conocephalum conicum species. The most common monoterpenoids are: Myrcene (2), β-phellandrene (3), limonene (4), α-terpinene (5), p-cymene (6), α-pinene (7), β-pinene (8) and camphene (9).



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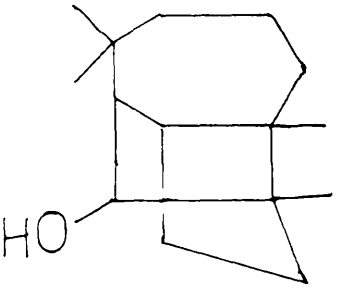

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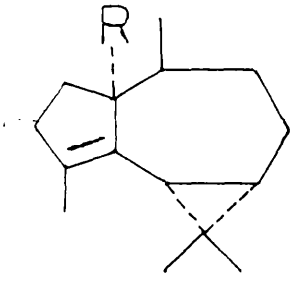


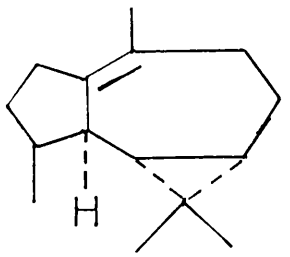
(13) R = H

(14) R = OH

Both optical antipodes of monoterpenoids generally occur in higher plants often as racemates though some species synthesise only one of the two isomers[12]. In general the chiroptical properties of monoterpenoids found in the Hepaticae have not been clarified. Asakawa et al,[11] studied the chirality of monoterpenes from C. conicum which contains (-)- limonene (4) and (+)- bornyl acetate (10), while Jungermannia exsertifolia[13] contains (+)- limonene (ent - 4), α-pinene (7) and camphene (9). 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.

SESQUITERPENOIDS

Liverworts are extremely rich in sesquiterpenoids many of which are enantiomeric to those found in higher plants. The first recognition of sesquiterpenes in liverworts was reported by Fujita et al[6], in a study of the essential oil of Bazzania pompeana. The first positive identification of sesquiterpenes was reported in 1967 by Huneck and Klein[14] who isolated (-)- longifolene (11) and (-)- longiborneol (12) from Scapania undulata. The sesquiterpenoids (11) and (12) are the enantiomers of longifolene and longiborneol derived from Pinus species.



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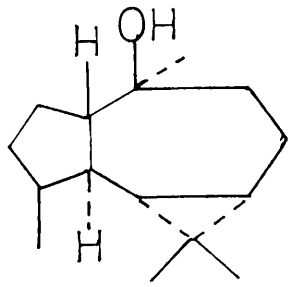

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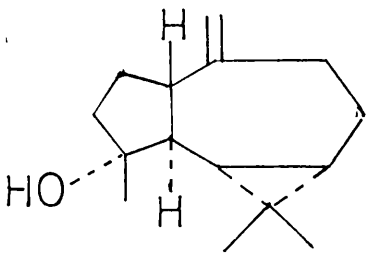

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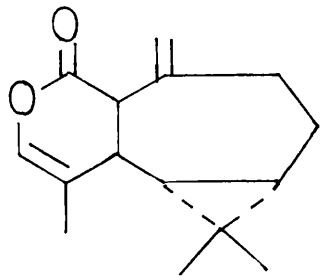

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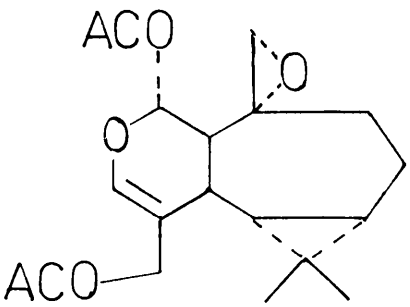

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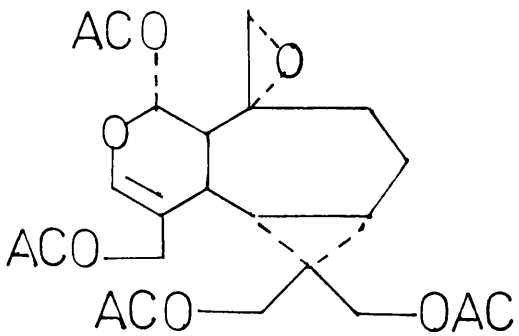

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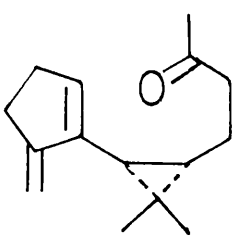

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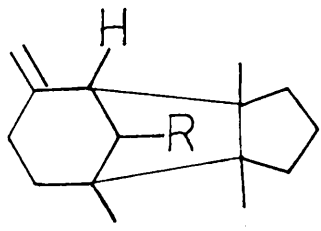
AROMADENDRANES AND SECOAROMADENDRANES

Aromadendrane-type sesquiterpenoids are mainly distributed in Mylia, Plagiochila and Porella species belonging to Jungermanniales. Cyclocolerenone (13)[15] and 1-hydroxycyclocolorenone (14)[16] from Plagiochila and Porella species are the simplest representatives in this type. Another variation of the aromadendrane skeleton is found amongst the metabolites of Plagiochila[17,18] species e.g. ladene (15), globulol (16) and spathulenol (17). The absolute configuration of these compounds is opposite to that found in higher plants.

Sesquiterpenoids with a secoaromadendrane skeleton are also found in livertworts. The first to be isolated from Plagiochila species was plagiochilide (18)[18]. It is a crystalline compound. Its ^{1}H n.m.r. showed 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, J10Hz.)], a vinyl methyl [δ_H 1.74(d, J2.0Hz.)] and a vinyl proton [δ_H 6.24 (d, J2.0Hz.)] and the i.r. indicated the presence of a lactone ($\bar{\nu}_{max}$ 1760 cm^{-1}). These spectroscopic properties indicated structure (18). The Plagiochilae proved to be a rich source of compounds with ent-2,3- secoaromadendrane skeleton[19] e.g. compounds (19, 20).

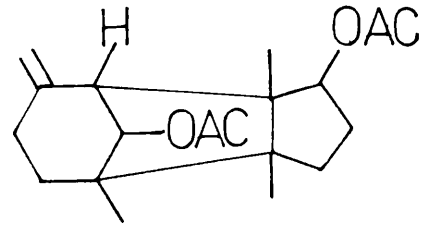
Taylorione (21) from Mylia taylorii[20] was established as an ent-1, 10-secoaromadendrenone. The structure and absolute configuration were determined by extensive chemical degradation and synthesis[21].



(22) R = OH

(23) R = H

(24) R = OAC



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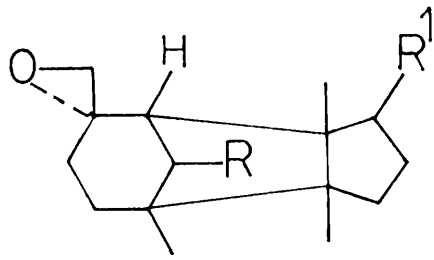
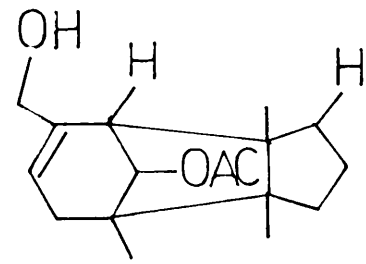


(26) R = OAC, R^1 = H

(27) R = R^1 = OAC



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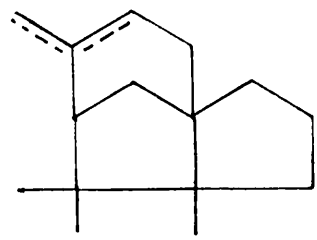

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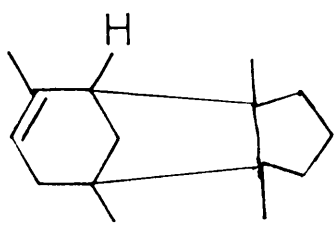

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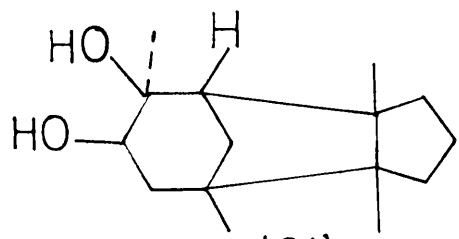

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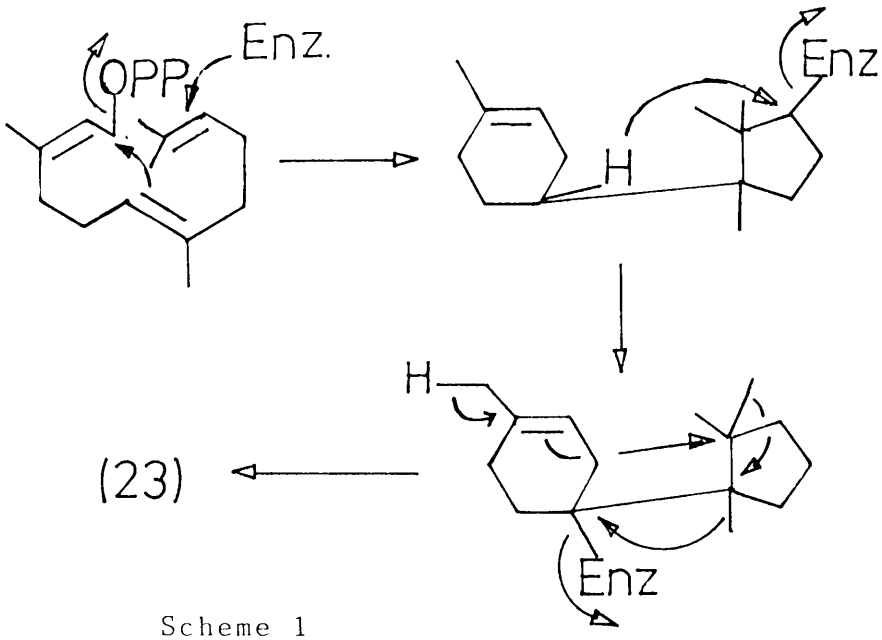
GYMNOMITRANES (BARBATANES) AND TRICHOTHECANES

Connolly et al[21] isolated a new sesquiterpene alcohol gymnomitrol (22) from Gymnomitrion obtusum together with six other sesquiterpenes with the same skeleton (23) - (28). These structures were determined by a combination of ^{1}H n.m.r. and Eu(fod)$_3$ - induced spectral shifts, decoupling experiments, and extensive chemical transformations.

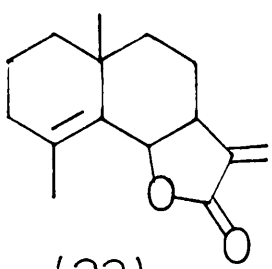
Matsuo et al[22], reported the isolation of new tricyclic sesquiterpenes α- and β- pompene from Bazzania pompeana which were assigned the erroneous structure (29). The spectral data of α- and β- pompenes were identical with those of α- barbatene (α- gymnomitrene) (30) and β- barbatene (β- gymnomitrene) (23) respectively. That α- pompene is identical with α- barbatene (30) has been shown by x-ray analysis of the p-bromobenzoate of the derived diol (31).

Connolly et al[21], suggested that the gymnomitrene skeleton can be derived from an enzyme-bound farnesyl pyrophosphate which leads to a trichodiene-type intermediate and then to the gymnomitrane as shown in Scheme 1 .

Total syntheses[23] have confirmed the structures of (±)- gymnomitrol (22) and α- and β- barbatenes (30, 23).



Scheme 1



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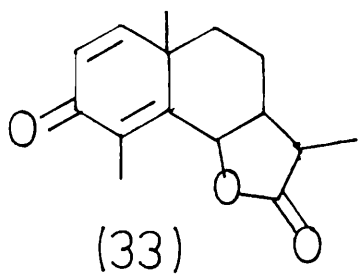

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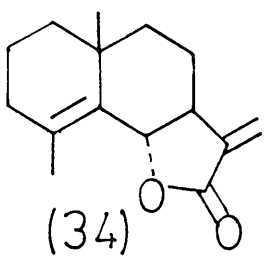

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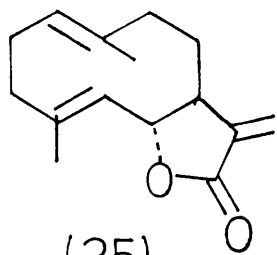

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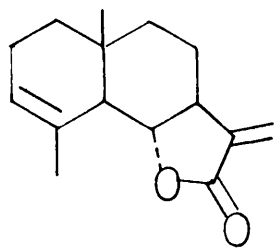
EUDESMANES

This type of sesquiterpenoid is very widely distributed in the Hepaticae, particularly in the Jungermanniales. The eudesmanolides often have biological activity. Ourisson[24] and co-workers isolated (-)- frullanolide (32) whose structure was confirmed by correlation with 6β 11β- santonin (33) and by synthesis[25]. Frullanolide has been shown to be responsible for the high incidence of contact dermatitis among forestry workers.

Connolly and Thornton[26] isolated (-)- frullanolide (32), α- cyclocostunolide (34), (+) costunolide (35) and γ- cyclocostunolide (34) from Scottish Frullania tamarisci. However, β- cyclocostunolide (37) could not be detected in the extract. It was later isolated[27] from the Japanese Frullania tamarisci subsp obscura together with the new eudesmane- type sesquiterpenoids, the hydroxy aldehyde (38) and 4β- hydroxy-eudesmanolide (39). Similar compounds of the ent- eudesmanolide series have been isolated from Frullania dilatata. It is interesting that F. dilatata biosynthesises ent- sesquiterpenoids while F. tamarisci elaborates compounds belonging to the normal series although these two species are morphologically similar.

Two ent- eudesmanolides, ent-3- oxodiplophyllin (40) and ent-7- hydroxydiplopyllolide (41) occur in European Chiloscyphus polyanthos[28.29] together with known compounds.



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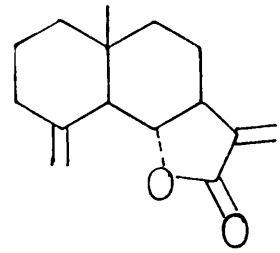

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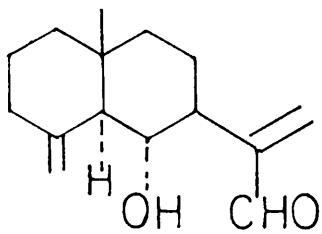

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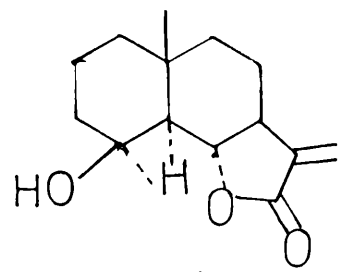

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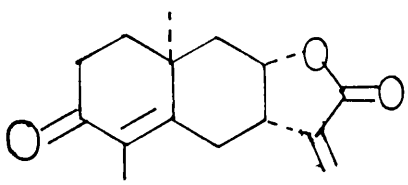

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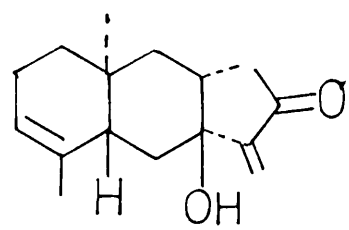

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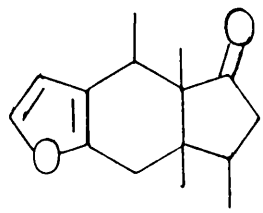

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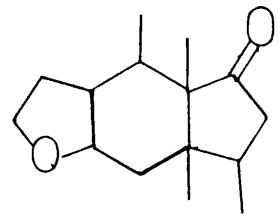

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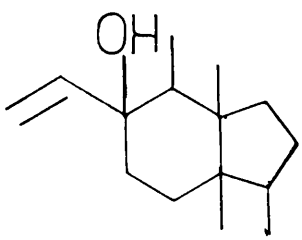
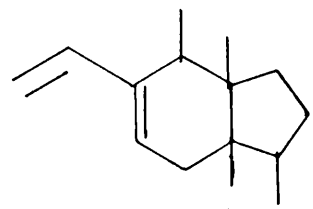


(44)

i



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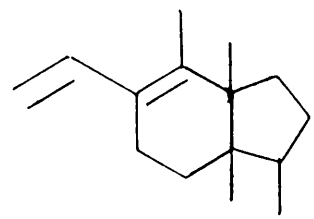
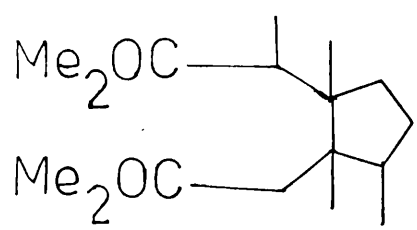


(46)

ii
iii



(47)

(i) p-TsOH/C_5H_6

(ii) O_3, H_2O_2

(iii) CH_2N_2

Scheme 2

The structures (40) and (41) were determined on the basis of the $Eu(fod)_3$-induced shifts in the ^1H n.m.r. spectrum [29].

PINGUISANES

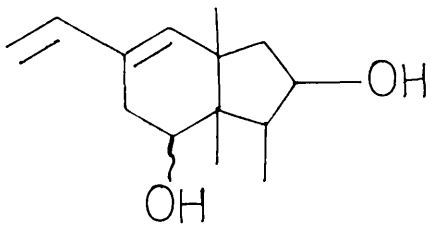
The first representative of this group, pinguisone (42)[30] was found in Aneura pinguis. The basic skeleton was deduced from comparison of the ^1H n.m.r. of pinguisone (42) with that of its tetrahydroderivative (43). Later an x-ray analysis of its p-bromo-benzylidene derivative established the structure and absolute stereochemistry of (42)[31].

Recently, further examples of this category have appeared. Pinguisenol (44) and α-pinguisene (45) have been isolated from Porella vernicosa[32]. A dehydration of (44) with p-TsOH gave two conjugated hydrocarbons (45) and (46), whose ozonolysis without separation followed by methylation afforded dimethyl ester (47). (Scheme 2)

Three pinguisanes (48) - (50) have been isolated from European Porella platyphylla and their structures bases mainly on ^1H and ^{13}C n.m.r. spectroscopic evidence and spectroscopic correlation with the co-metabolite deoxypinguisone (52)[33]. Recent work[34] has shown that the structure (49) should be revised to (51).

DITERPENOIDS

The diterpenoids are the second largest group of terpenoids in bryophytes. The representative types in this category are labdanes, pimaranes, kauranes, together with new types examplified by sacculatanes, verrucosanes, dolabellanes and fusicoccanes.



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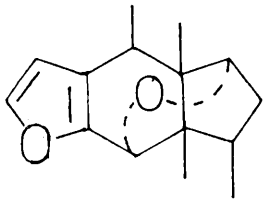

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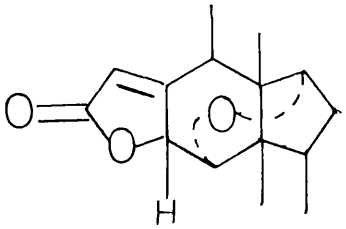

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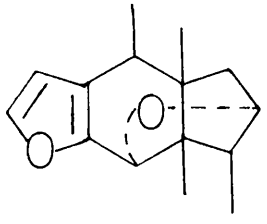

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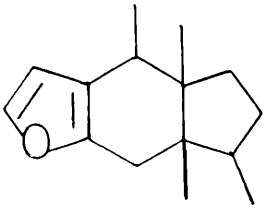

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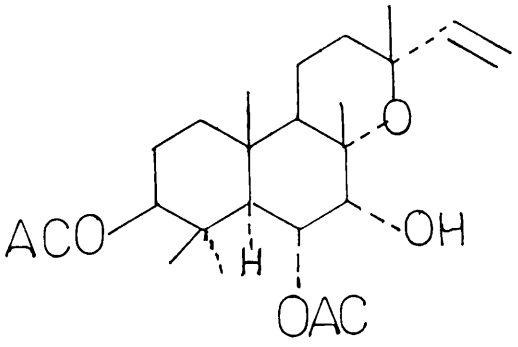

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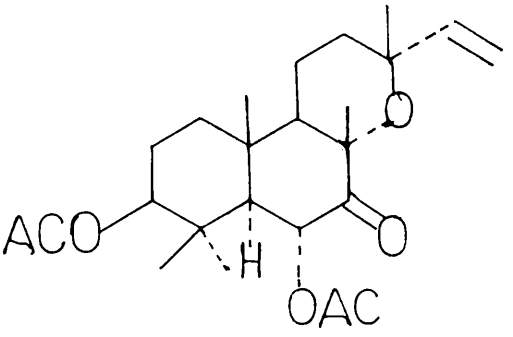

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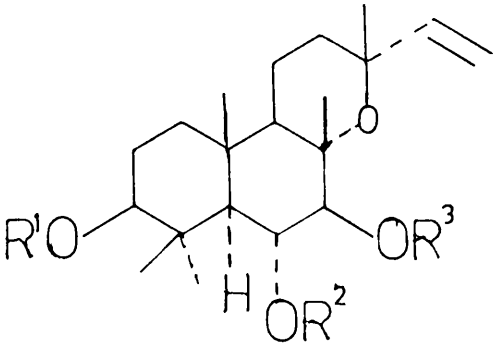
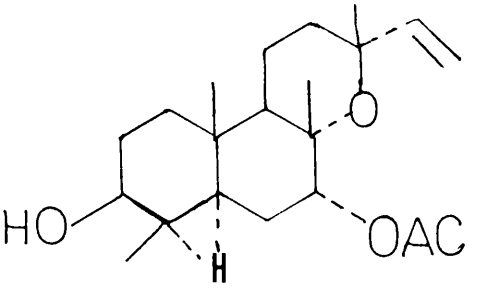




(57)

(55) $R^1 = R^2 = AC, R^3 = H$

(56) $R^1 = R^3 = AC, R^2 = H$

LABDANES

The most recent examples of this skeletal type have been reported by Asakawa et al[35]. Five new labdane-type diterpenoids hamachilobenes A - E have been isolated from the liverwort Frullania hamachiloba. They were shown to be the manoyloxide derivatives (53) - (57) by a combination of ^{1}H and ^{13}C n.m.r. and CD spectra.

KAURENES

All representatives from bryophytes belong to the ent-kaurenes series. Connolly and Thornton[36] isolated four ent-kaurenes from Jungermannia atrovirens

ent - 11α - hydroxykaur - 16 - en - 15 one (58)

ent - 11α - hydroxy - (16S) - kaurane - 15 - one (59)

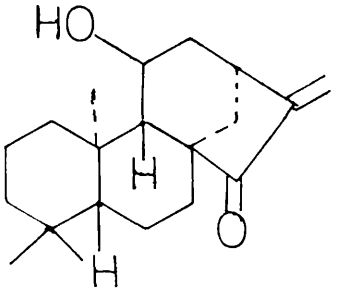
ent - kaur - 16 - ene - 11α, 15α - diol (60) and

ent - 15α - acetoxykaur - 16 - ene - 11α - ol (61).

The structure of (60) was established by x-ray crystallographic analysis[37]. The ent - 15α - hydroxykaura - 9 (11), 16 - diene - 6 - one (62) has recently been isolated from Czechoslovakian Nardia scalaris[38], as well as being found in N. compressa[39].

SACCULATENES

Two unique diterpene dialdehydes, the pungent sacculatal (63) and the non-pungent isosacculatal (64) have been isolated from Trichocoleopsis sacculata[40]. Their structures were determined by spectroscopic and chemical correlation with the sesquiterpene dialdehyde, polygodial (65).



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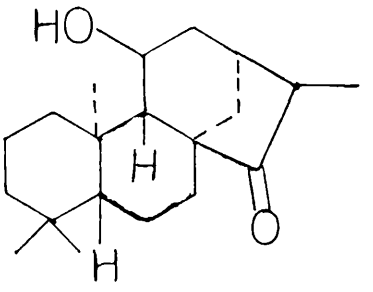

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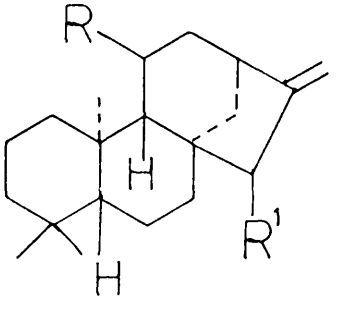
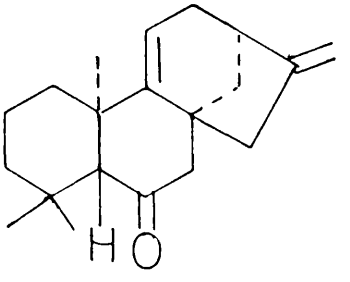


(60) R=R'=OH

(61) R=OH, R'=OAC



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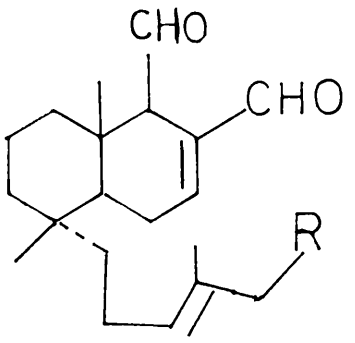
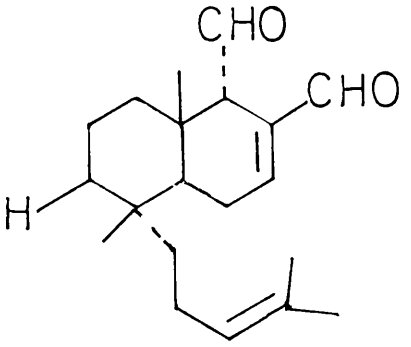


(63) R = H



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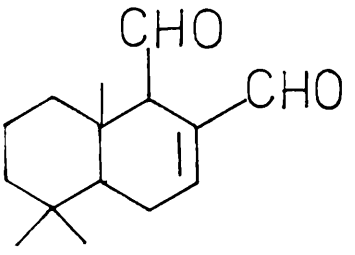

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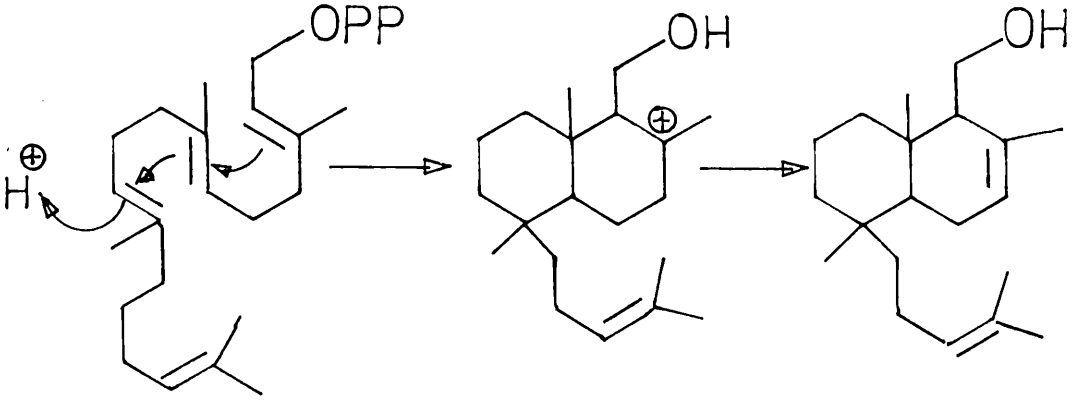


(66)

Scheme 3

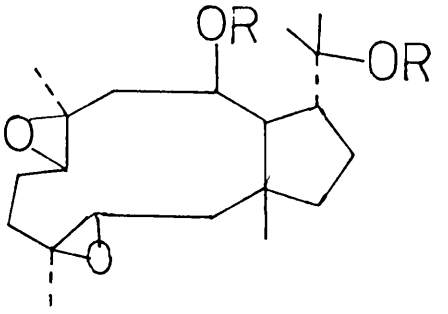
The sacculatanes are interesting from a biogenetic point of view. The ring system is the same as that of the drimene-type sesquiterpenes with an additional isoprene attached to C-15. They may be formed from geranyl-geranyl pyrophosphate (66) by a cyclisation mechanism analogous to that of the drimenes, (scheme 3).

DOLABELLANES

In 1971, Huneck and Overton[41] isolated diterpenoids from Barbilophozia floerkei, B. lycopodioides, Anastrepta orcadensis and Gymnocolea inflata. However, it is only recently that Connolly[42] reported the occurrence of the dolabellane barbilycopodin (67) in the extract of Barbilophozia floerkei as the major constituent. The structure of (67) was identified by spectroscopic and chemical methods along with an x-ray analysis of the corresponding diol floerkein B (67a). This is the first report of a dolabellane from plants. This class has previously been detected in marine organisms[43].

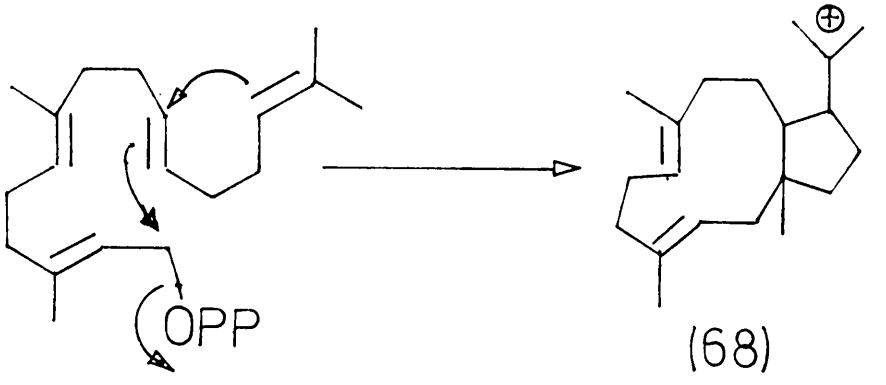
The biosynthesis of barbilycopodin involves cyclisation of all-transgeranyl-geranyl pyrophosphate as shown in scheme 4

Further cyclisation of (68) affords anadensin (69), a diterpenoid isolated from Anastrepta orcadensis[44]. Its structure was assigned on the basis of its ^{1}H and ^{13}C n.m.r. spectroscopy and $Eu(fod)_3$-induced shifts. The stereochemistry of (69) was confirmed by an x-ray analysis.



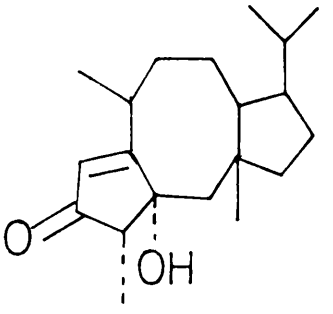
(67) R = AC

(67a) R = H

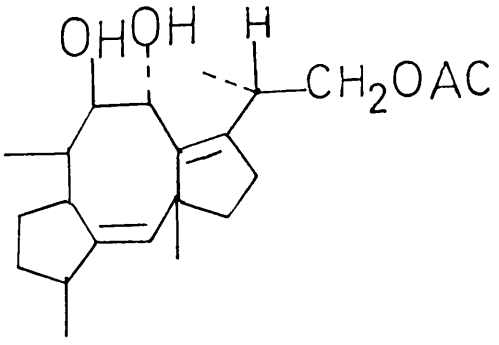


(68)

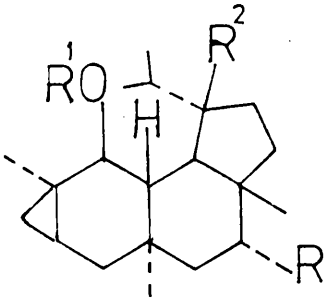
Scheme 4



(69)

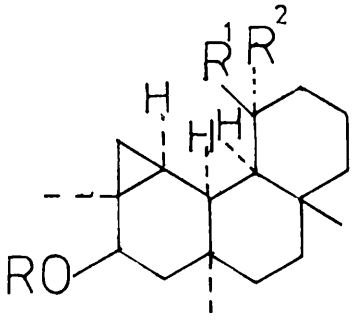


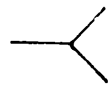
(70)



(71) $R^1 = R^2 = H, R = OH$

(71) $R = OH, R^1 = OC\ HIBr\_P, R^2 = H$



(73) $R^1 =$  , $R^2 = R = H$

This skeleton is unknown in higher plants, but has been found in fungal products e.g. fusicoccin (70)[45] and the cotylenins[46].

VERRUCOSANES

More deep-seated modification of the dolabellane skeleton leads to the verrucosanes which were isolated from Mylia verrucosa. The (-)- verrucosane - 2β, 9α - diol (71)[47] was determined by spectroscopic and chemical data. An x-ray of the 2- bromobenzoate (72) established its stereochemistry.

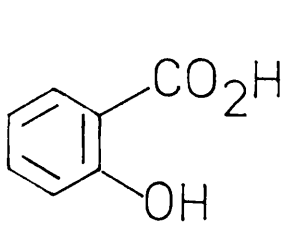
A new verrucosane-type sesquiterpenoid (73) has been isolated from Plagiochila stephensoniana[48]. The structure of (73) elucidated by extensive 2 D n.m.r. spectroscopy, was confirmed and its absolute configuration established by x-ray analysis of the p-bromobenzoyl derivative.

AROMATIC COMPOUNDS

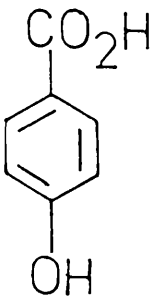
A wide range of aromatic metabolites of the Hepaticae has been reported.

BENZOIC ACID DERIVATIVES

O- and p- hydroxybenzoic acids (74), (75) have been obtained from Asterella lindenbergiana[49]. Two methyl esters (76) and (77) of 3,4- disubstituted benzoic acid have been detected in Trichocolea tomentella[50] together with 3,4- dihydroxy benzoic acid (78).



(74)



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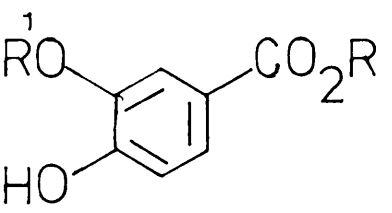
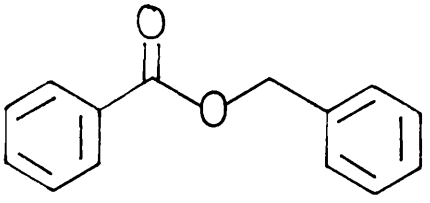


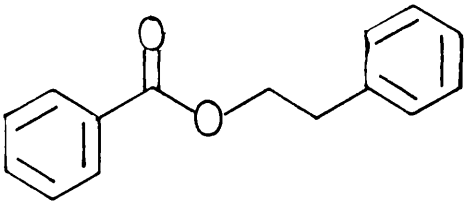
(76) $R = Me, R^1 = H$

(77) $R = R^1 = Me$

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



(79)



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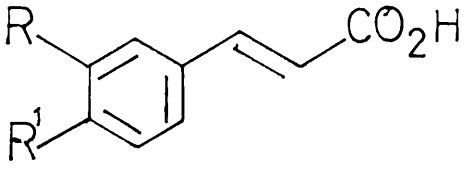
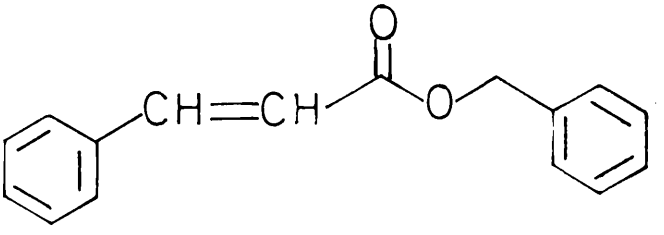


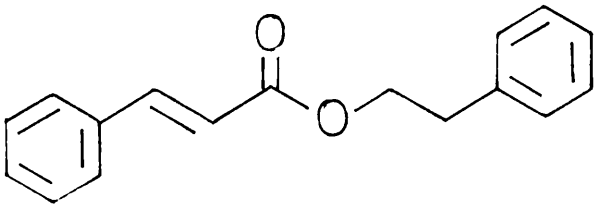
(81) $R = H, R^1 = OH$

(82) $R = OH, R^1 = H$



(83) Cis isomer

(84) Trans isomer



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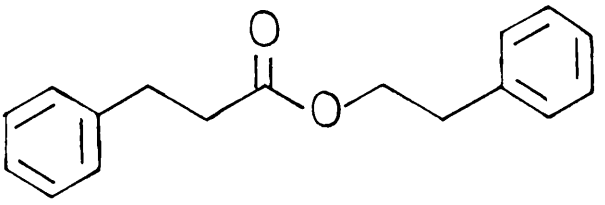
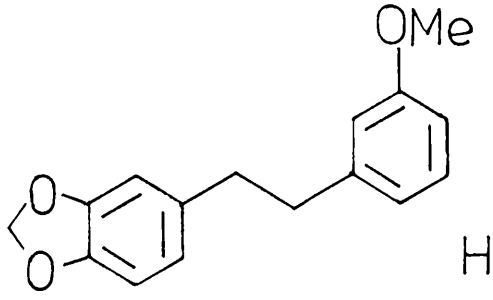


(86)



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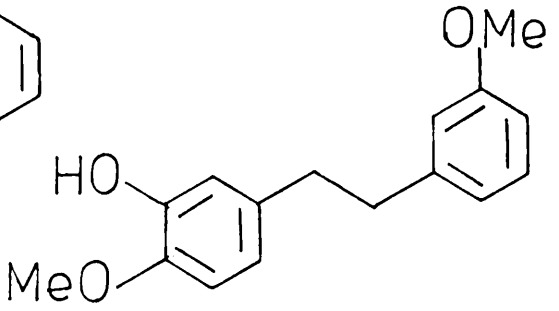

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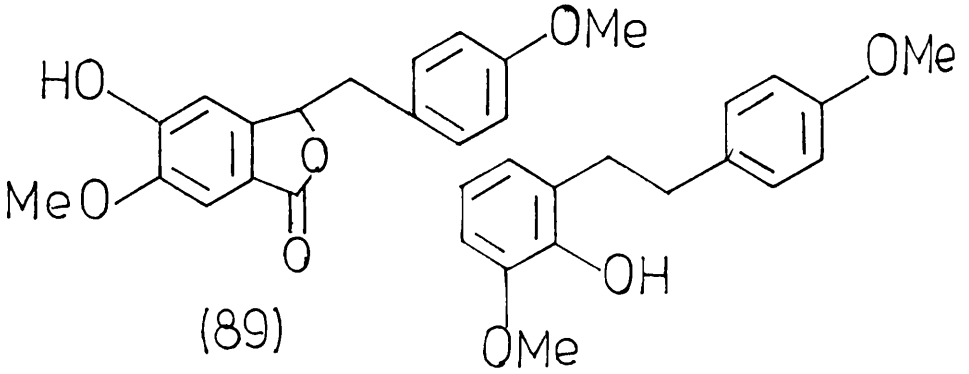


(89)

(90)

Benzyl benzoate (79) and β-phenylethylbenzoate (80) have been isolated from the primitive liverwort Isotachis japonica[23].

CINNAMIC ACID DERIVATIVES

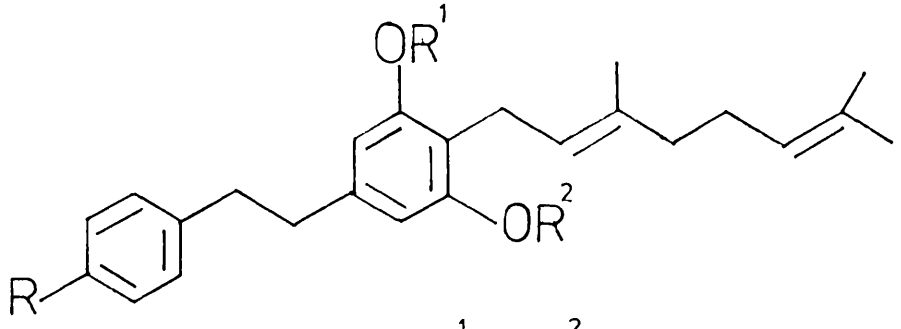
Asterella species[49] contain p- and m- coumaric acids (81), (82) whilst Isotachis japonica[51] contains benzyl trans- and cis- cinnamates (83), (84) together with β- phenylethyl-cinnamate (85) and its dihydro derivative (86).

BIBENZYLS

Frullania species are rich sources not only of sesquiterpene lactones but also of bibenzyl derivatives. Twenty-five Frullania species have been studied[52] and twelve species contain bibenzyl derivatives. Recently, Asakawa et al[53], have reported the isolation of three novel bibenzyl derivatives (87) - (89) from the Australian Frullania falciloba. Their structures were established as 3,4- methylene-dioxy-3'- methoxybibenzyl (87), 3- hydroxy- 4,3'- dimethoxybibenzyl (88) and 3- [4'- methoxybenzyl]- 5,6- dimethoxyphthalide (89) by spectral methods and synthesis.

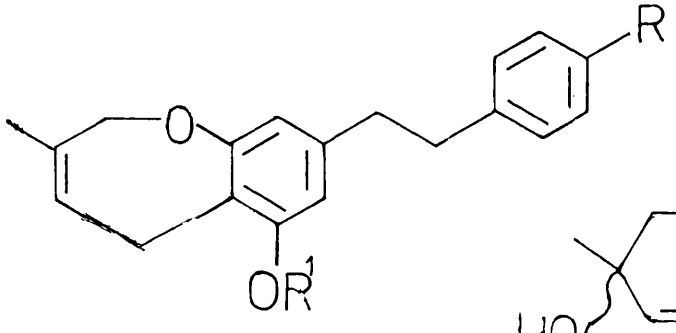
Pellepiphyllin (90) has been isolated from Pellia epiphylla[54].

Radula species contain many bibenzyls incorporating prenyl groups. Seven bibenzyls (91) - (97) have been isolated from R. variabilis. Their structures were established mainly by detailed analysis of ^{1}H n.m.r. and mass spectra[55].



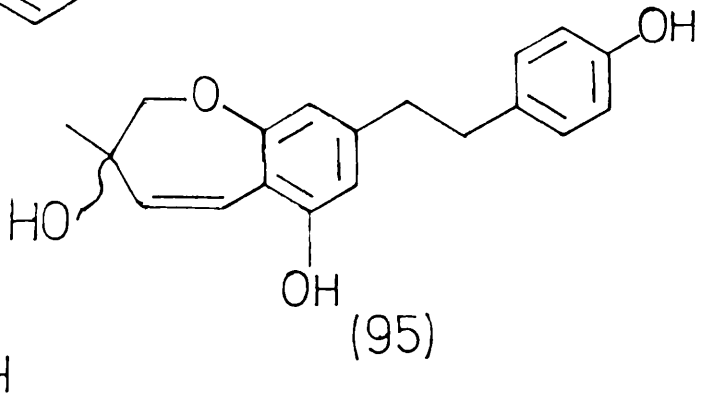
(91) $R = R^1 = R^2 = H$

(92) $R = OH,\ R^1 = R^2 = H$



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

(94) $R = OH,\ R^1 = H$



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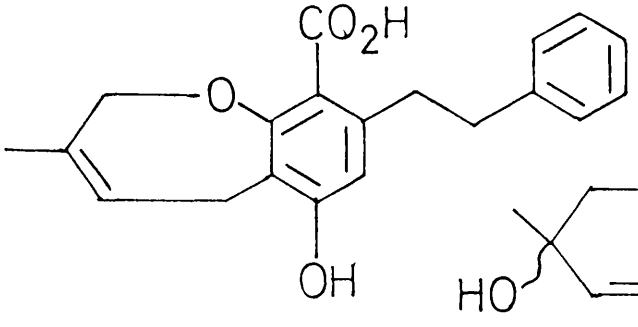

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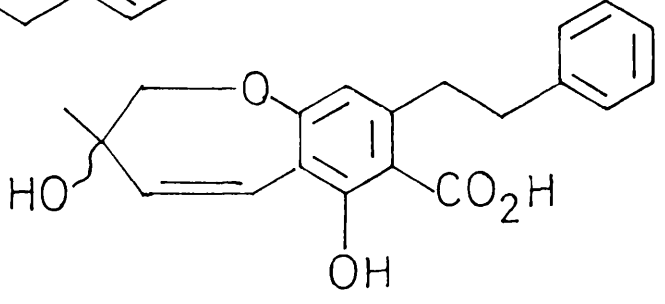

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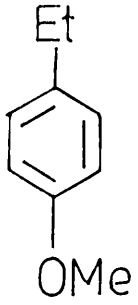

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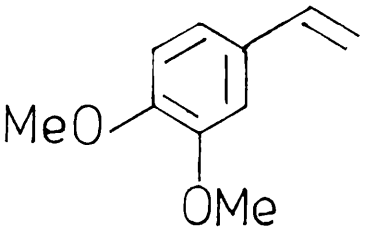

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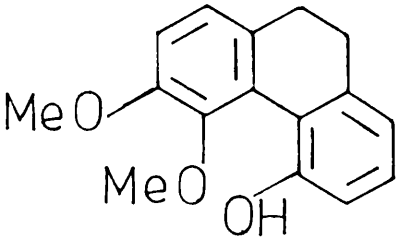

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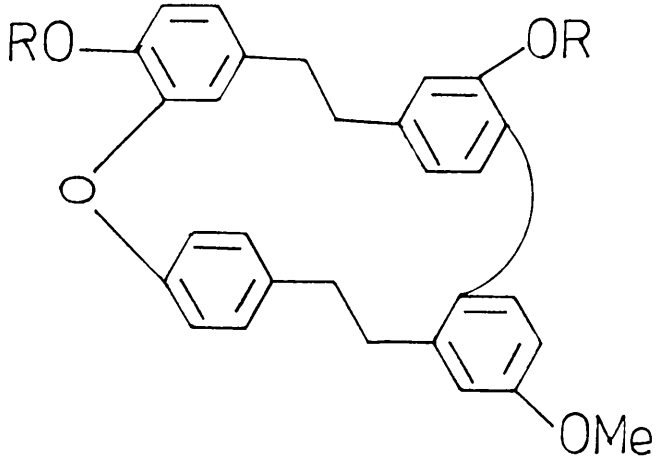
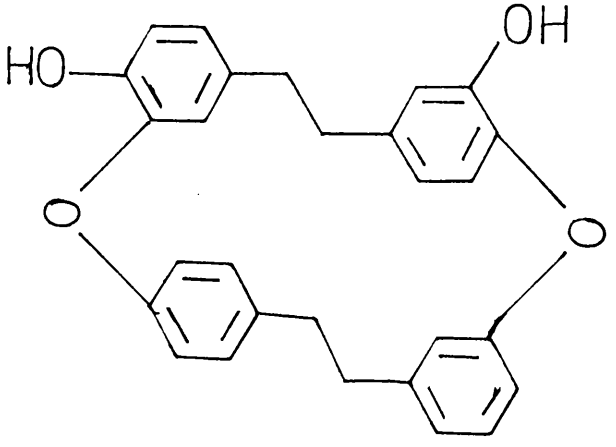


(101) R = H

(102) R = AC



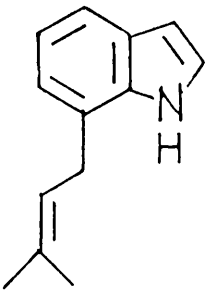
(103)

The p- ethylanisole (98) isolated from Loptolejeunes elliptica[56] causes an intense mold-like odour. Conocephalum conicum[57] produces 1- vinyl- 3,4- dimethoxybenzene (99) as a minor component. The 3,4- dimethoxy- 5- hydroxy- 9, 10- dihydrophenanthrene (100) was isolated from Riccardia jackii and its structure established by x-ray crystallographic analysis[58]. The novel aromatic ethers riccardin A (101) and riccardin B (103) have been isolated from Riccardia multifida. The structure of the former was established by x-ray analysis of the diacetate (102)[59].

INDOLE DERIVATIVES

The unusual indole derivatives (104), (105) have been isolated from Riccardia sinuata[56,57]. These are the first nitrogen-containing compounds in the Hepaticae.

Two new sulphur-containing acrylates, isotachin A (106) and isotachin B (107) were detected in the liverwort Isotachis japonica[60]. Their structures were determined by chemical and spectral methods.



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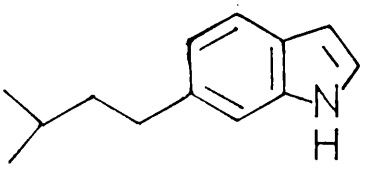

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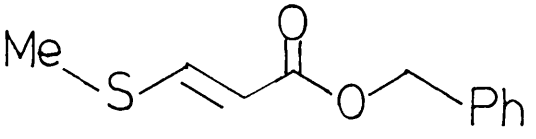

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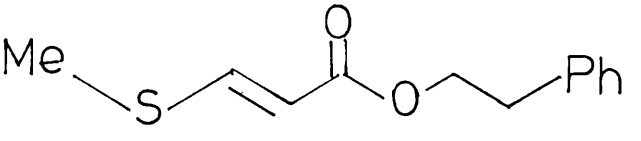
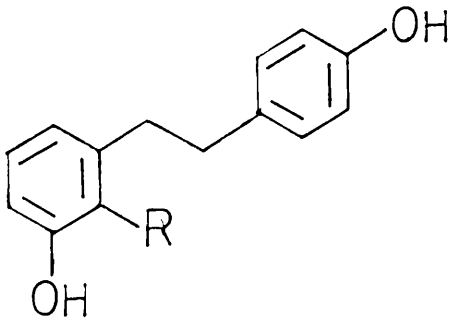


(107)

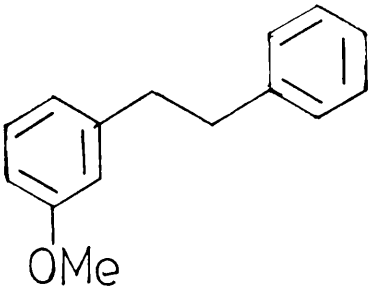
CHAPTER 2



(108) R = CO_2H

(109) R = H

(111) R = OH



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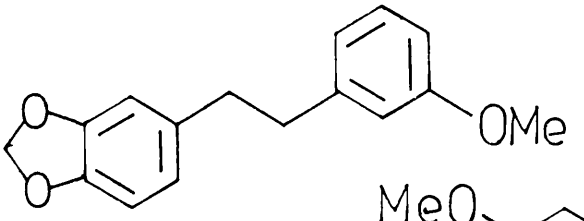

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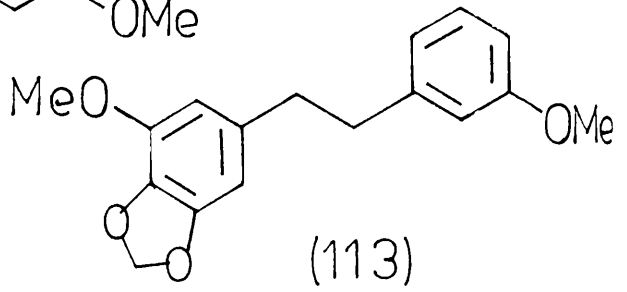

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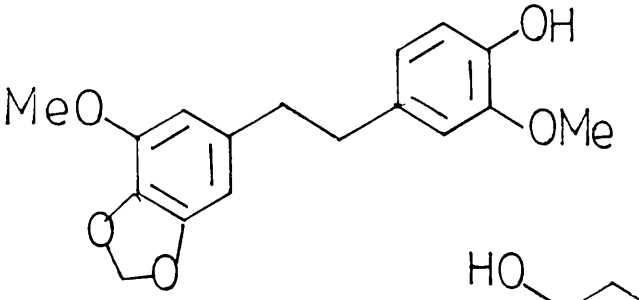

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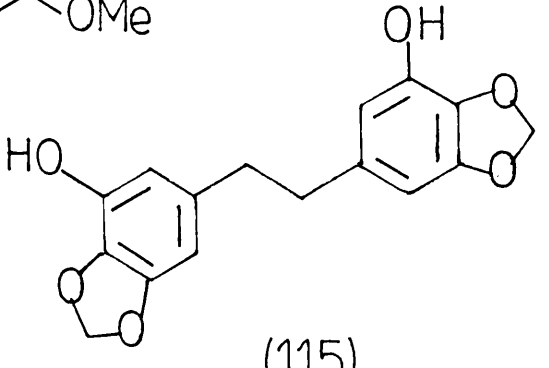


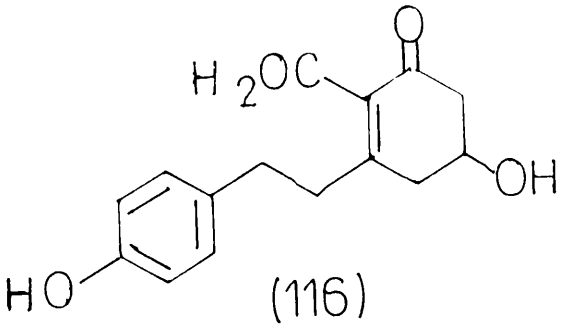
(115)

INTRODUCTION

Lunularic acid (3,4'- dihydroxybibenzyl-2- carboxylic acid) (108) was first isolated by Valio et al[61], in 1960 as a growth inhibitor and dormancy factor in the Middle East strain of the liverwort Lunularia cruciata. It has since been found in numerous other liverworts. Compound (108) was tested in a number of higher plant systems by Fries[62] for plant growth inhibitory activities. It was observed that it had an effect at higher concentration, but no effect at low concentration. Lunularic acid (108) and the corresponding decarboxylated derivative lunularin (109) have been detected[63] in 76 species of Hepaticae, but do not occur in the Anthocerotae or algae.

In addition to compound (108) numerous further bibenzyls have been isolated from liverwort species. 3- Methoxybibenzyl (110)[64] was isolated from Frullania dilata var anamola while 2,4,4'- trihydroxybibenzyl (111)[65] was isolated from Pellia endiviifolia and its structure proved by synthesis[66]. Later Asakawa[67] found four new related bibenzyls (112) - (115) from three Frullania species. Compounds (112) and (113) were isolated from F. ericoides. The former compound has been found also in Australian F. falciloba and its structure confirmed by synthesis[53]. Compound (113) has also been synthesised[67]. The bibenzyl (114) was obtained from F. boninicola while compound (115) was isolated from F. paruistipula.

The most significant compound in this series, prelunularic acid (preLNA)(116)[68] was isolated from the suspension



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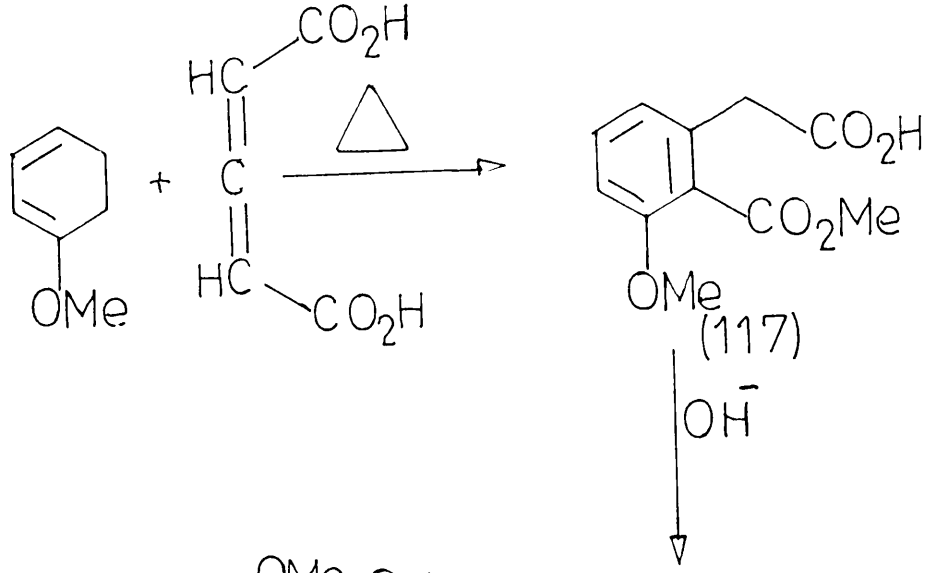

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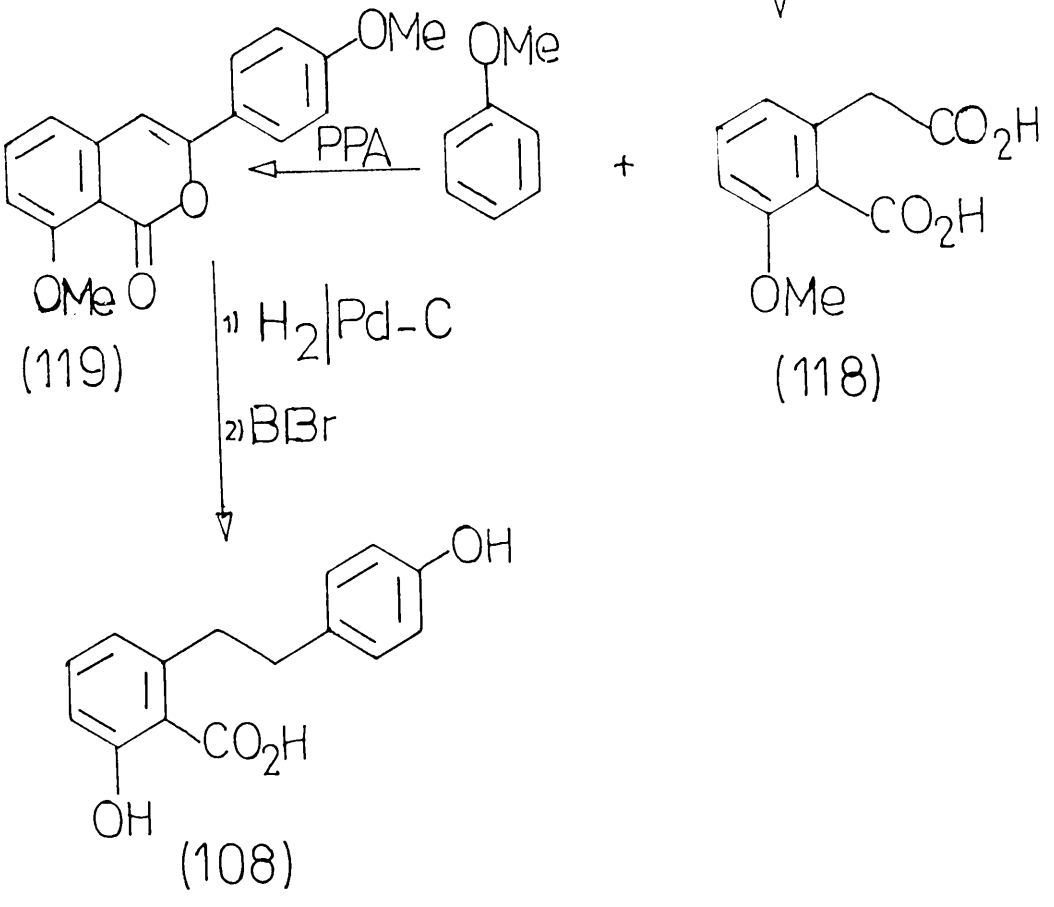


(119)

(118)

(108)

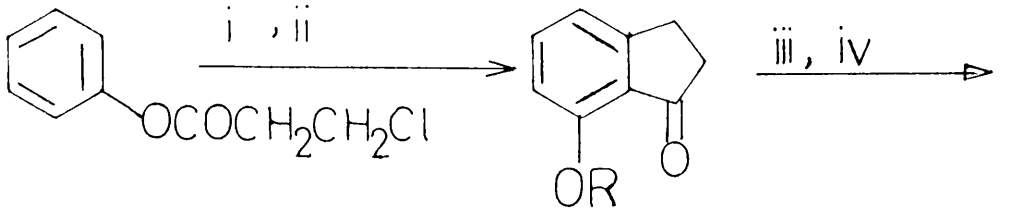
Scheme 5

cultured cells of Marchantia polymorpha. Its structure was determined by spectroscopic and chemical means.

Prelunularic acid (116) has been shown to be converted into lunularic acid (108) under acidic or basic conditions. For example approximately 0.3% of preLNA (116) in water was converted in one day at room temperature, and 9% on boiling for 30 minutes. The isolation of (116) is significant for the biosynthesis of (108) and stilbenoids in general.

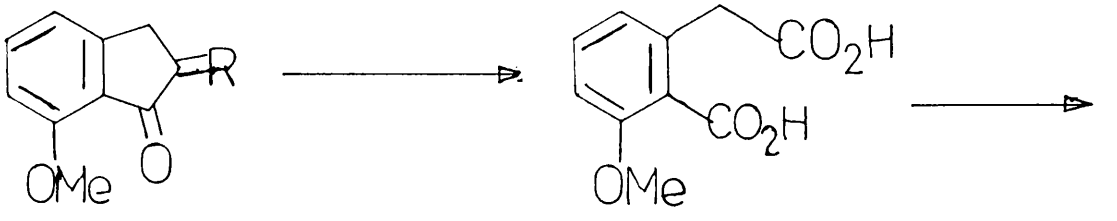
Two syntheses of lunularic acid have been reported. The first was by Arai et al[69]. Heating a mixture of 1-methoxy cyclo- hexa- 1,3- diene with allene- 1,3- dicarboxylate in a sealed t e gave dimethyl 3- methoxyhomophthalate (117). The hydrolysis of (117) gave 3- methoxyhomophthalic acid (118). Condensation of (118) with anisole in polyphosphoric acid gave the isocoumarin (119) in 81% yield. Catalytic hydrogenation of (119) followed by treatment with BBr_3 afforded (108). Its ^1H n.m.r. was identical with that of the natural acid (scheme 5).

In 1977 Huneck and Schreiber[70] devised another synthesis of (108)(scheme 6). Phenyl β-chloropropionate was converted into the corresponding hydrindanone (120) by Friedel-Crafts cyclisation. Methylation with methyl iodide in acetone and K_2CO_3 afforded (121) which was converted into the oximo-ketone (122) on treatment with isoamyl nitrite. Compound (122) in 30% formaldehyde-water yielded the diketone (123)



(120) R = H

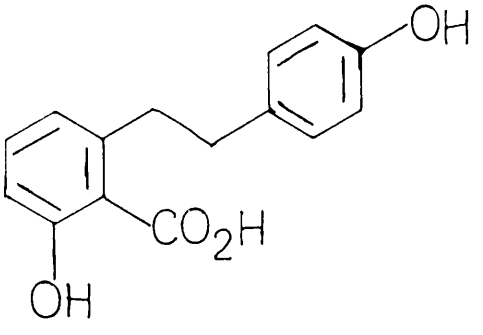
(121) R = CH_3



(122) R = NOH

(123) R = O

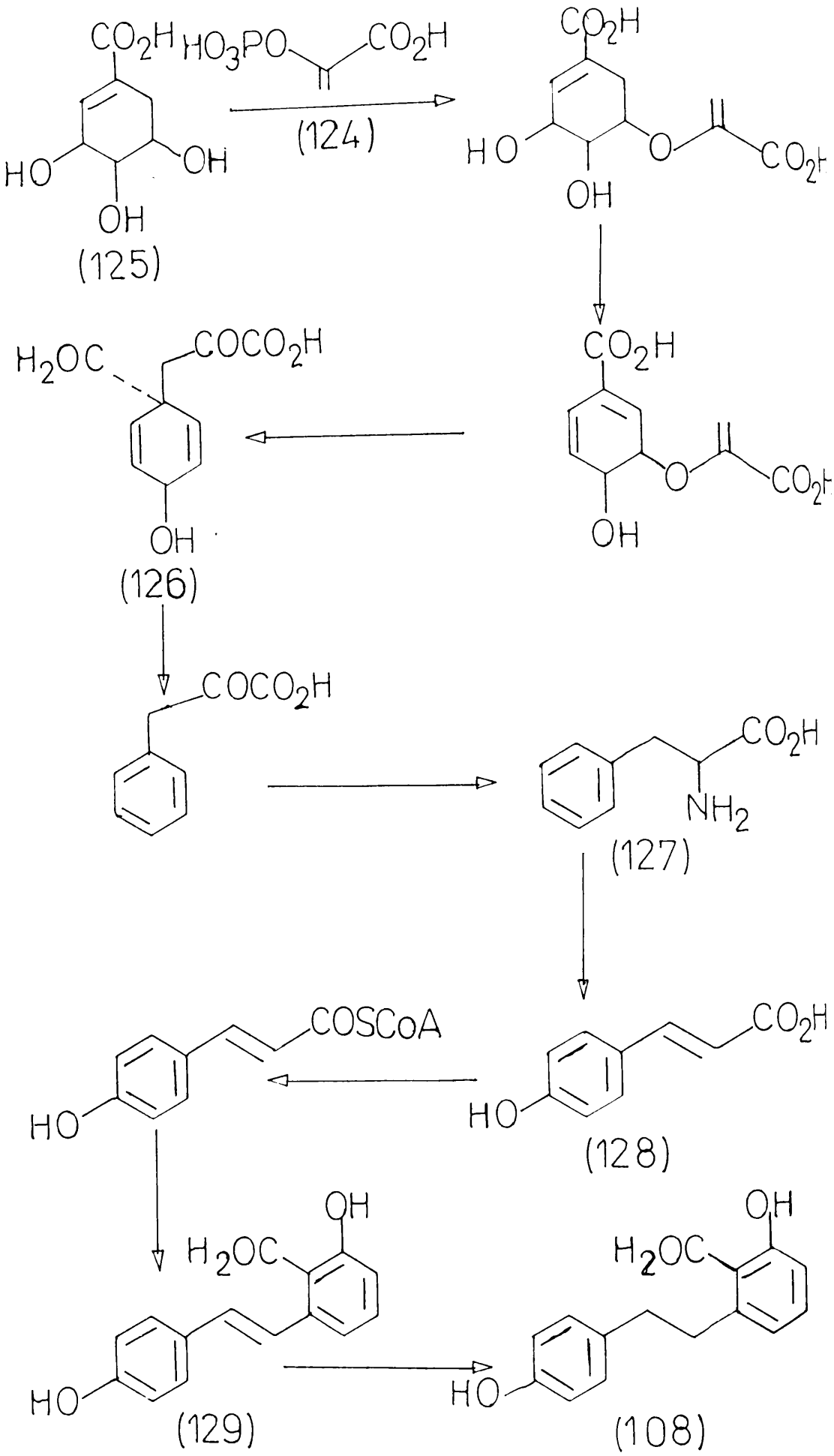
(118)



(108)

(i) $AlCl_3$; (ii) MeI; (iii) isoamyl nitrite; (iv) HCHO/HCl

Scheme 6


CO2H
HO3PO
CO2H
(124)
HO
OH
OH
(125)
CO2H
HO
O
CO2H
OH
CO2H
O
CO2H
OH
H2OC
COCO2H
OH
(126)
COCO2H
CO2H
NH2
(127)
COSCoA
CO2H
HO
HO
(128)
OH
H2OC
OH
H2OC
HO
HO
(129)
(108)

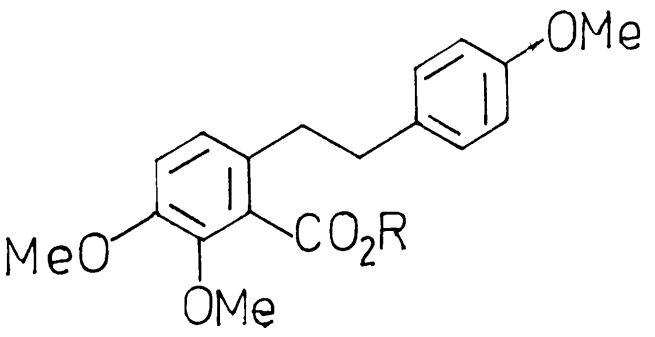
Scheme 7

which was transformed into the homophthalic acid (118) by 15% H_2O_2 in glacial acetic acid. The conversion of the homophthalic acid (118) into lunularic acid followed the same route as in (scheme 5).

The biosynthesis of the various aromatic compounds that have been recently isolated from the Hepaticae and Anthocerotae is not always clearly established. Stilbenes, bibenzyls and phenanthrenes are believed to be derived from shikimic acid and acetate units. The biosynthesis of lunularic acid (108) has been investigated and the results show that this bibenzyl derivative (108) is synthesised in vivo[71] via the phenyl propanoid-polymalonate pathway. Reaction of pyruvate (124) with shikimic acid (125) followed by a process not unrelated to the Claisen rearrangement leads to prephenate (126). This is a biological example a symmetry-allowed pericyclic process. Decarboxylation of (126) is followed by aromatisation and reductive transamination to give phenylalanine (127). The stereospecific elimination of ammonia yields trans-p-coumaric acid (128). Condensation of p-coumaroyl-Co-A with a triketide unit, then cyclisation and aromatisation of the polyketide chain followed by the reduction of the olefinic protons in (129) affords lunularic acid (108) (Scheme 7). The main metabolite of lunularic acid in Lunularia cruciata is its decarboxylation product[72] lunularin (109).

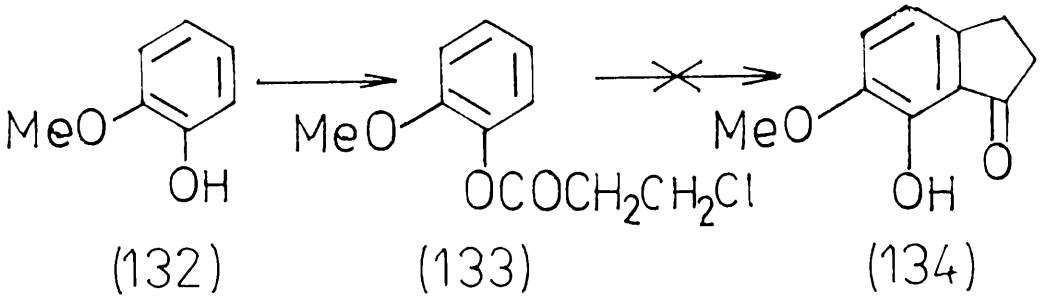
The novel bibenzyl (130) was obtained from Plagiochila spinulosa by Connolly[73]. It is clearly a derivative of lunularic acid. Its structure was determined on the basis of its spectroscopic properties. Its ^{1}H n.m.r. showed an AA'BB' quartet [δ_H 7.08(d, J8.8Hz) and 6.83(d, J8.8Hz)] and an AB quartet [6.84(d, J8.5Hz) and 6.81(d, J8.5Hz)] and benzylic methylene protons at δ_H 2.76. Accurate mass measurement confirmed its molecular formula as $C_{19}H_{22}O_5$ [m/$_z$ 330.1478] and gave useful fragments at m/$_z$ 209 ($C_{11}H_{13}O_4^+$) and the base peak at 121 ($C_8H_9O^+$).

This chapter deals with the attempted synthesis of the lunularic acid derivative (131) to enable further evaluation of its biological activity.



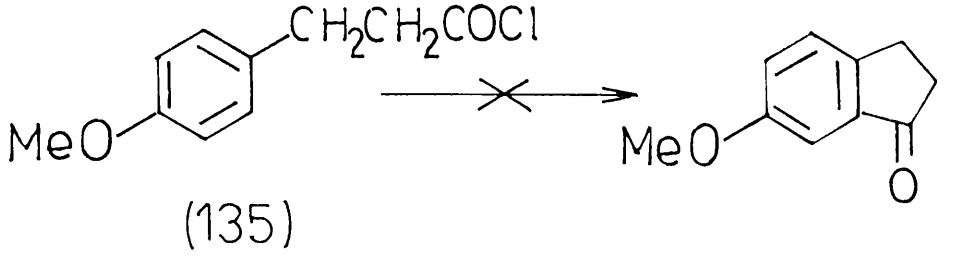
(130) R = Me

(131) R = H



(132) (133) (134)

Scheme 8



(135)

Scheme 9

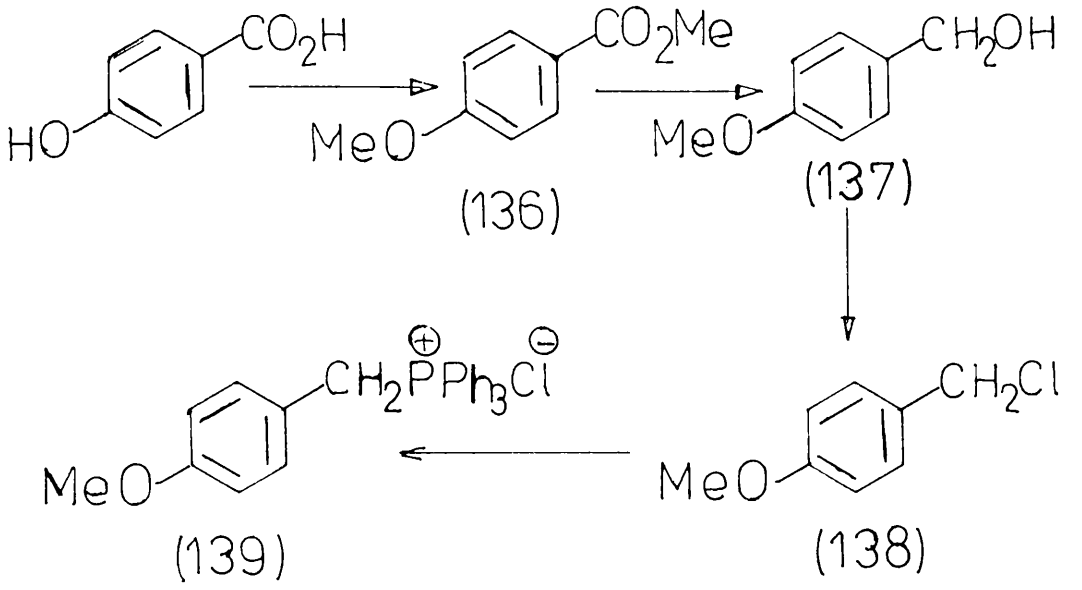
DISCUSSION

Our aim was to obtain by synthesis 3,4,4'- trimethoxybibenzyl-2-carboxylic acid (131).

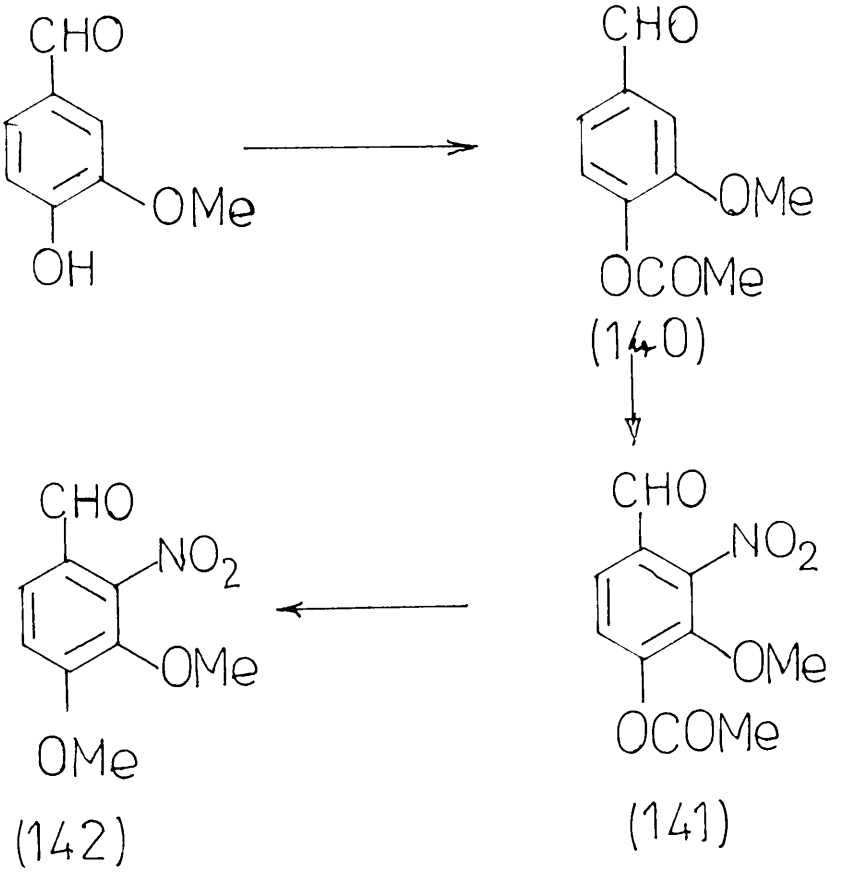
First the Huneck[70] approach was attempted. Reaction of guaiacol (132) with β-Chloropropionyl chloride in presence of $POCl_3$ afforded the chloroester (133) in good yield. Unfortunately Friedel-Crafts cyclisation of (133) to give the desired hydrindanone (134) failed despite several efforts - (scheme 8). Under similar conditions 4- methoxyphenylpropionyl chloride (135) also failed to cyclise, (scheme 9). This approach was therefore abandoned.

The next approach we considered was construction of the bibenzyl system using a Wittig reaction. We intended introducing the carboxyl group via a cyano derivative. Thus we prepared 4- methoxybenzyltriphenylphosphonium chloride (139), (scheme 10), and 2- nitroveratraldehyde (142),(scheme 11) as appropriate precursors.

The esterification of 4- methoxybenzoic acid was carried out using excess dimethyl sulphate in refluxing acetone and potassium carbonate for 5 hours. The ^1H n.m.r. spectrum of the methoxy methyl ester (136) showed an AA' BB' quartet for four aromatic protons at δ_H 7.58 and 6.55 (J8.8Hz.), in addition to two methyl signals at δ_H 4.02 (OMe) and 3.95 (CO_2Me).



Scheme 10



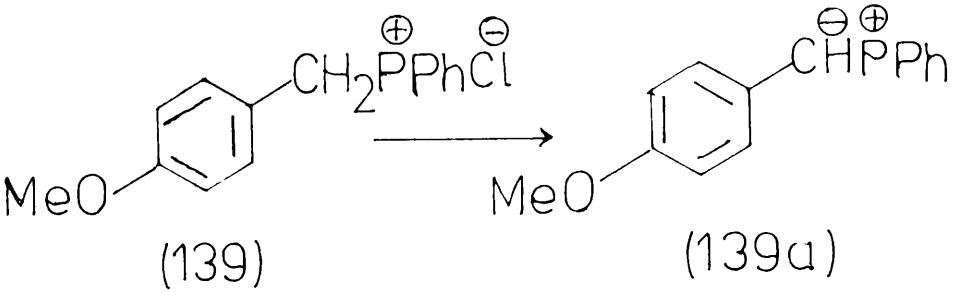
Scheme 11

Methyl 4- methoxybenzoate (136) was easily reduced to the corresponding alcohol (137) using lithium aluminium hydride in dry ether. The reduction was vigorous and the lithium aluminium hydride was added in small portions. The excess of lithium aluminium hydride was conveniently destroyed by addition of aqueous sodium sulphate. The ^1H n.m.r. spectrum of the product (137) (ν_{max} 3390 cm^{-1}) showed loss of the methyl ester and the appearance of a two proton singlet at δ_H 4.45 due to the benzylic methylene group.

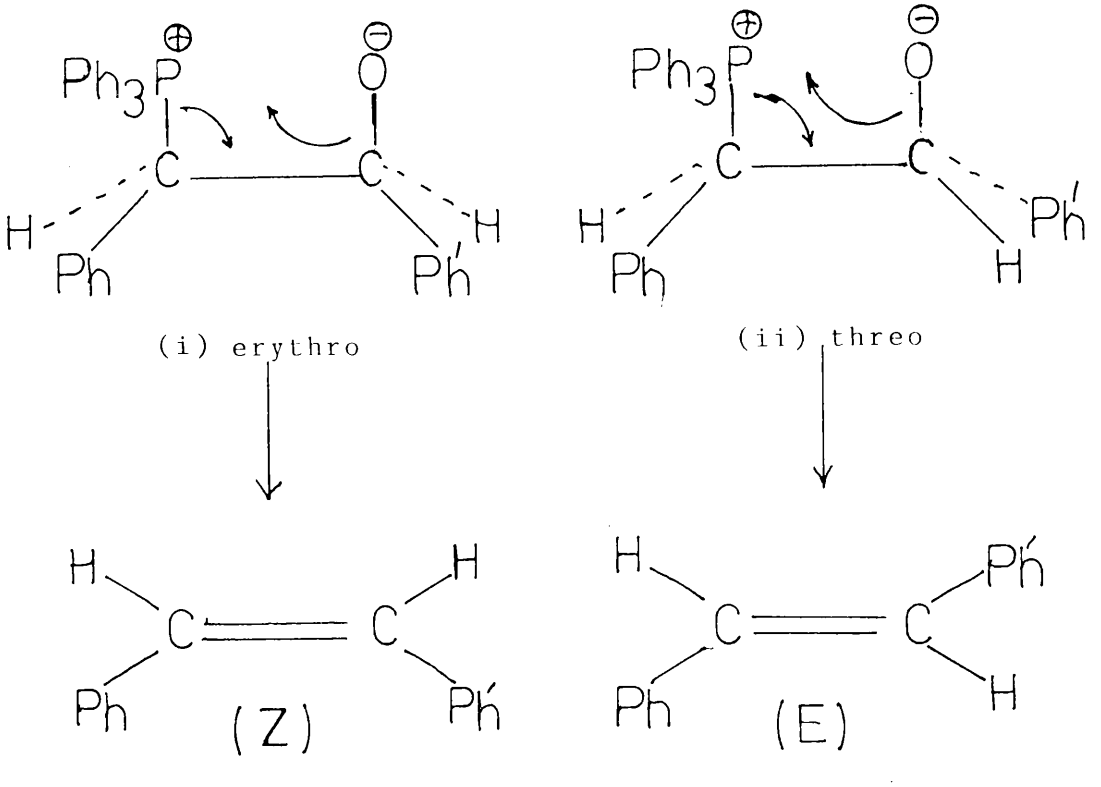
The benzyl chloride (138) was transformed into the Wittig reagent (139) by treatment with triphenyl phosphine under anhydrous conditions in toluene. The ^1H n.m.r. spectrum of (139) showed the expected coupling of the benzylic methylene protons with phosphorous [δ_H 5.32(d, J_{PH}14Hz.)].

The preparation of 2- nitroveratraldehyde (142) is described in scheme 11. Acetylation of vanillin with acetic anhydride in dry pyridine and stirring overnight led after crystallisation from water to vanillin acetate (140). [ν_{max} (CCl_4) 3020, 1760, 1700 and 1600 cm^{-1}]. Its ^1H n.m.r. spectrum showed the loss of the hydroxyl group and the appearance of an acetate at δ_H 2.35(3H, S).

Vanillin acetate (140) was nitrated at -5^o -0^o by red fuming nitric acid (d. 1.50) with vigorous stirring. The yield is dependent on the strength of the acid (d. 1.50) and its age. It was found necessary to use carefully dried vanillin acetate for the nitration, otherwise it was difficult to remove all odour of nitric acid from the product.



Scheme 12



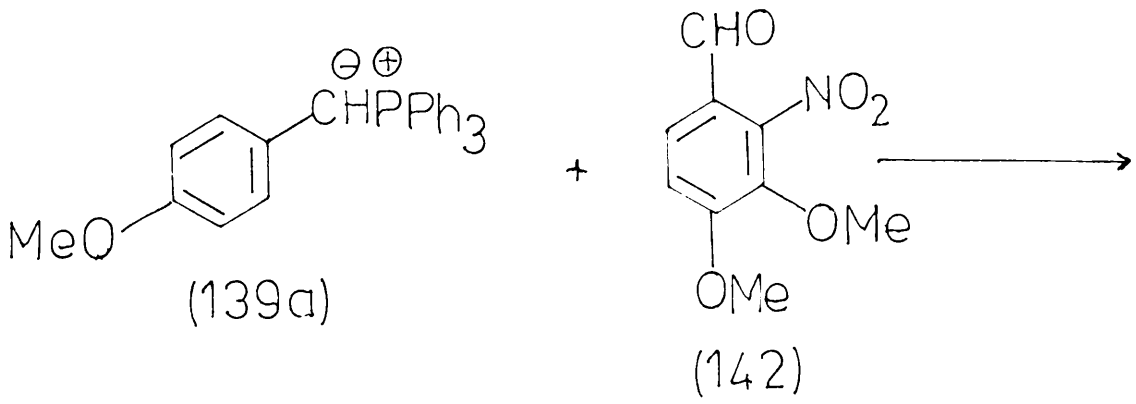
+ $Ph_3P=O$

Scheme 13

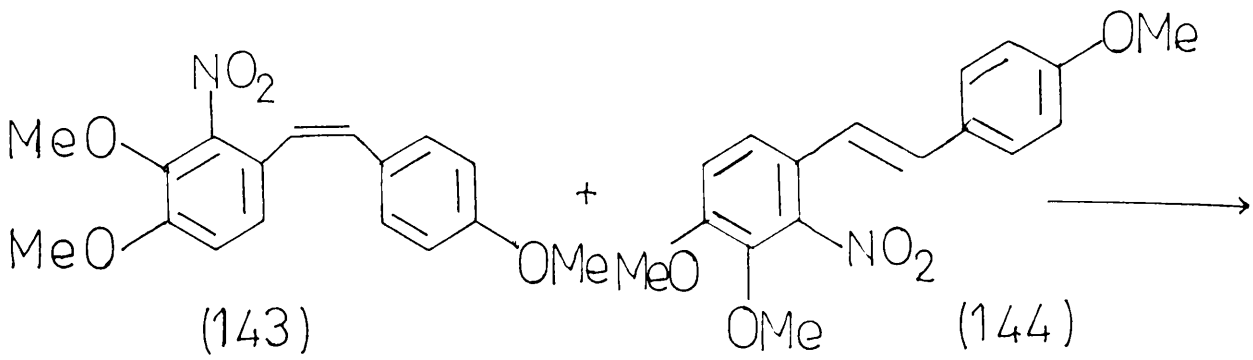
Nitration of vanillin acetate (140) under these conditions gives mainly the 2- nitro derivative (141). A small portion of the 6- nitro derivative can be isolated from the mother liquor. The structure of the 2- nitro compound (141) was readily assigned on the basis of its ^1H n.m.r. which shows two ortho- coupled aromatic protons as an AB quartet at δ_H 7.85 and 7.58 (J9.8Hz.) together with the expected signals for the aldehyde at δ_H 10.15, the methoxy group at δ_H 3.95 and the acetate methyl at δ_H 2.32. Mass spectrometry confirmed its molecular formula ($C_{10}H_9NO_6$: m/z 239).

The acetate (141) was converted to 2- nitroveratraldehyde (142) following the method of MacDonald[74], using dimethyl sulphate in the presence of strong base (50% sodium hydroxide). It was found necessary to use an excess of dimethyl sulphate and to maintain the pH at about 11. Ammonia was added to destroy the excess of the reagent. On cooling the solution to 0°C the product (142) precipitated. Its ^1H n.m.r. spectrum confirmed the presence of two methoxy groups at δ_H 4.00 and 3.92 , the aldehyde singlet at δ_H 9.77 and an AB quartet at δ_H 7.64 and 7.12 (J8.7Hz.) for the aromatic protons.

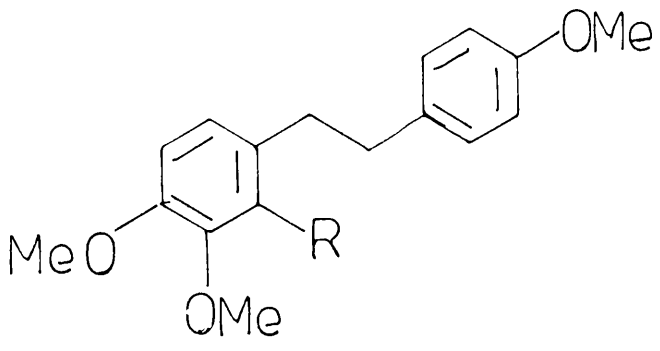
The first step of the Wittig reaction involves the formation of a carbanion by deprotonation of the phosphonium chloride (139), (scheme 12). The ylid (139a) is a good nucleophile and should react easily with the aldehyde. There are two possible transition states: (i) erythro, and (ii) threo, (scheme 13), which lead to two isomeric products (143) and (144) as shown in scheme 13 .



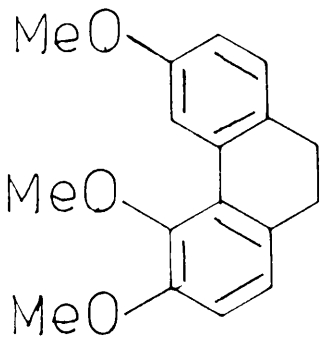
(139a) (142)



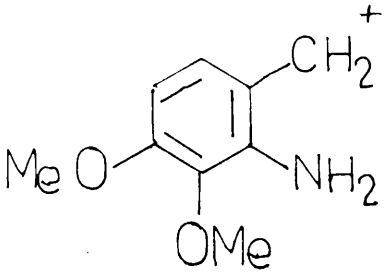
(143) (144)



(145) R = NO_2
(146) R = NH_2
(147) R = CN
(131) R = CO_2H



(148)



(149)

Scheme 14

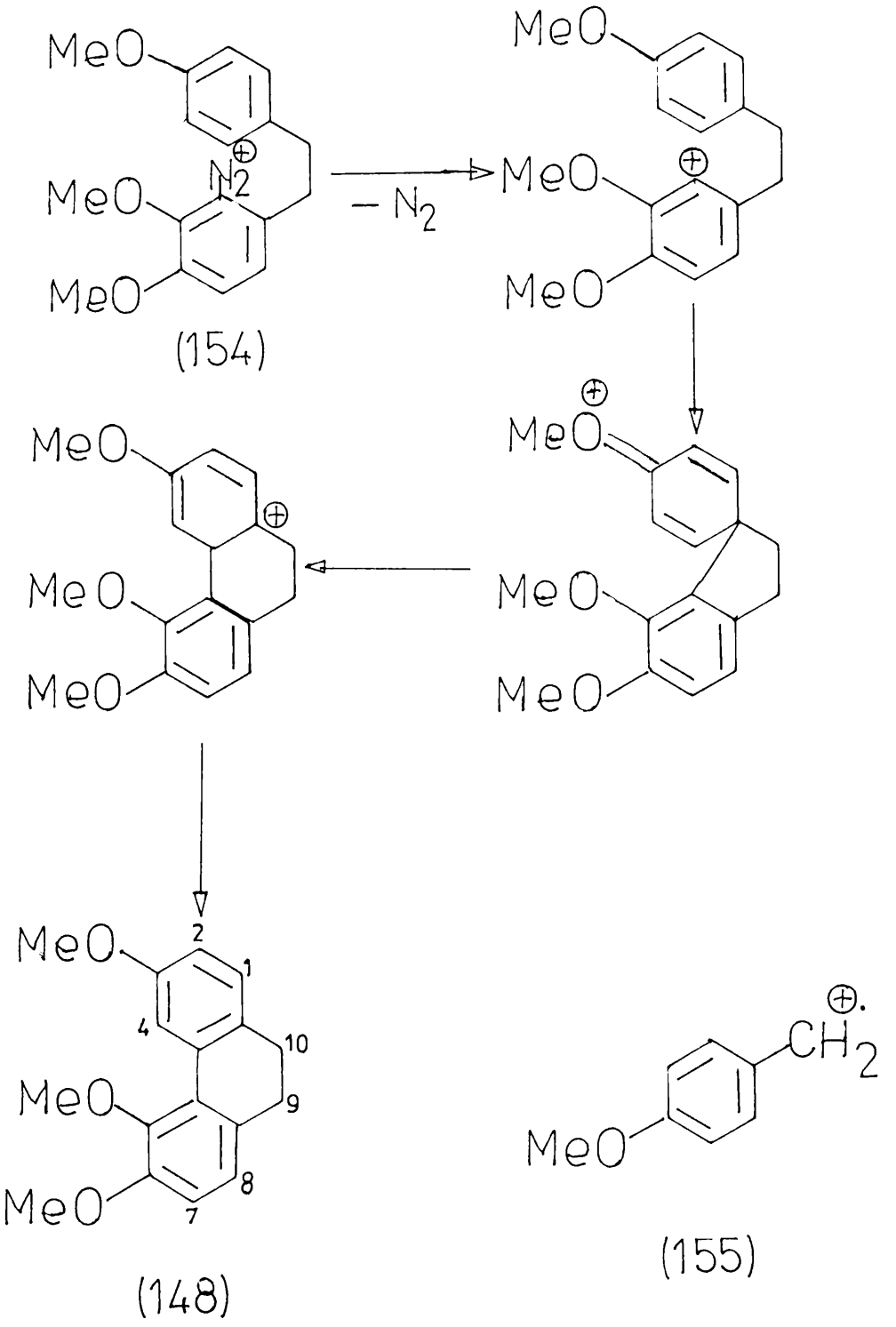
On addition of butyl lithium to the phosphonium chloride (139) a deep-red colour appeared, presumably indicating the formation of the ylid (139a). Reaction of the aldehyde (142) with the ylid (139a) gave the desired stilbene as a mixture of Z- (143) and E- (144) forms, the former predominating since studies[75] with non-stabilised ylides have shown that (Z-) alkene normally predominated. In the ^1H n.m.r. spectrum of the mixture the olefinic protons of the Z-isomer appeared as an AB quartet at δ_H 6.60 and 6.25(J11.95Hz). Its aromatic protons appeared at δ_H 7.09(d, J8.4Hz., $H_{2,6}$), 6.94(d, J8.6Hz., $H_{3,5}$); 6.79(d, J8.7Hz., H_5), and 6.72(d, J8.7Hz., H_6), and its three methoxy groups at δ_H 3.93, 3.86 and 3.75 (each 3H, S). The high resolution mass spectrum of the 2- nitrostilbene mixture confirmed the molecular formula $C_{17}H_{17}NO_5$(m/z 315.2352). The ^1H n.m.r. spectrum of the stilbene mixture showed the formation of the (143) and (144) in a ratio of 3:1. The E- isomer appears similar to the Z-form except the large coupling constant of 16Hz. of the olefinic protons.

The nitrostilbene mixture was converted to the corresponding bibenzyl (145), by hydrogenation in methanol over 5% Pd-C. Filtration of the catalyst and removal of solvent *in vacuo* afforded the 2- nitrobibenzyl (145) in high overall yield. The structure accorded with its ^1H n.m.r. spectrum which shows the loss of olefinic protons and the appearance of a broad multiplet at δ_H 2.77 arising from the four benzylic methylene protons.

The aromatic protons of (145) were observed as an AB quartet at δ_H 7.04 and 6.92(J8.7Hz.) and an AA'BB' system at δ_H 6.8 and 6.83(J8.9Hz). The remaining signals of the three methoxy groups appeared at δ_H 3.92, 3.87 and 3.77 . (145) showed a molecular ion at m/$_z$ 317.2681 in the high resolution mass spectrum ($C_{17}H_{19}O_5$ requires 317.1263)

The next step involved reduction of the nitrobibenzyl (145) to the corresponding amino compound (146). Attempts to achieve this by prolonged hydrogenation in EtOAc over 5% Pd/C were unsuccessful. Lithium aluminium hydride proved to be a much more convenient reagent for the reduction, which readily afforded the aminobibenzyl (146) as a crystalline solid. Its i.r. spectrum showed the presence of primary amino bands at $\bar{\nu}_{max}$ 3450 cm^{-1}. Its ^1H n.m.r. spectrum revealed the characteristic AA'BB' [δ_H 7.08(d, J8,7Hz.); 6.81(d, J8.7Hz.)] and AB [δ_H 6.78(d, J8.6Hz.), 6.35(d, J8.5Hz.)] systems for the aromatic protons, three methoxy groups at δ_H 3.81, 3.80 and 3.76; and a broad multiplet at δ_H 2.81 due to the benzylic methylenes. The signal for the amino protons appeared as a singlet (exchangeable with D_2O) at δ_H 2.16. Accurate mass measurement of (146) confirmed its molecular formula as $C_{17}H_{21}NO_3$ (m/$_z$ 287.1521). The base peak m/$_z$ 166 is due the fragment (149).

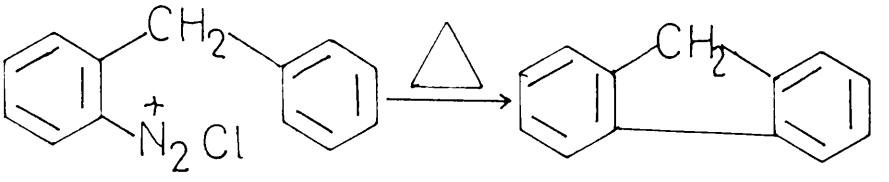
The amino compound (146) was subjected to typical Sandemeyer conditions in the hope of obtaining the nitrile (147). Thus a solution of the diazonium salt of (146) and potassium cyanide was added to a hot solution of CuCN at 85°C.


MeO
MeO
MeO
N2
(154)
− N2
MeO
MeO
MeO
MeO
MeO
MeO
MeO
MeO
MeO
2
1
4
10
9
8
7
(148)
CH2
MeO
(155)

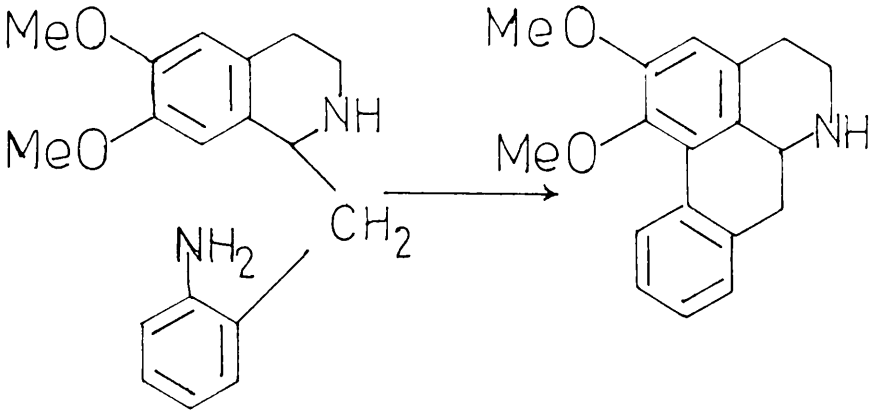
Scheme 15

Unfortunately the major product proved to be the dihydrophenanthrene (148), the result of a Pschorr ring closure. Intramolecular cyclisation of diazonium salts, best known as the Pschorr cyclisation, dates back to the work of Pschorr[76] in 1896. In fact the first example of this type of reaction (150) - (151) was reported by Fischer and Schmidt[(77)] in 1894. The reaction has found more modern application in a synthesis of aporphine[78] alkaloids. The alkaloid (153) was formed in good yield by diazotising the amine (152) in presence of copper salts. It has been reported that in the presence of copper salts[79] diazonium salts of cis-stilbene give phenanthrenes rather than nitriles in 60-80% yield.

Only one possible dihydrophenanthrene product can arise from the diazonium salt (154) since the positions 2'- and 6'- are similar. A suggested mechanism is drawn in (scheme 15). Attack of the electrophile on C-3 or C-5 of the top ring i.e. meta to the methoxy group is unfavourable in electronic terms. Initial ipso attack followed by migration and aromatisation may be a more likely mechanism. The structure of the dihydrophenanthrene (148) was assigned on the basis of its ^{1}H n.m.r. The structure (148) has five aromatic protons [δ_H 8.12(d, J2.7 Hz.); 7.18(d, J8.2Hz.), 6.96(d, J8.2Hz.), 6.82(dd, J2.7, 8.2Hz.) and 6.78(d, J 8.2 Hz.)]. The doublet at δ_H8.12 is characteristic of a proton at C-4 in this system. It has a meta coupling (J2.72Hz.) to the signal at δ_H 6.82 and the latter has a further ortho coupling (J2.7, 8.2Hz.) to the proton of C-1 at



(150) (151)

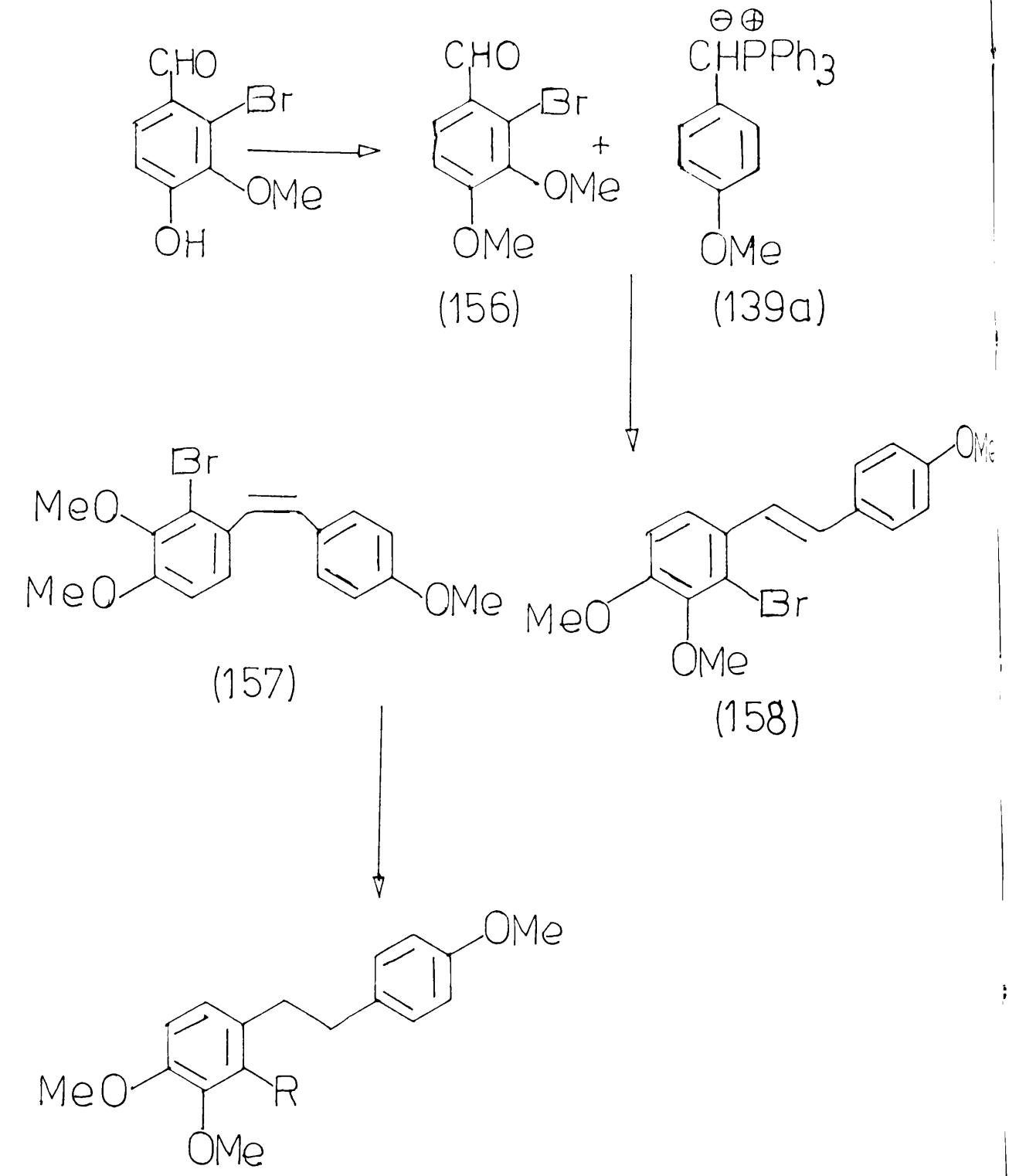


(152) (153)

δ_H 6.96(d, J8.2Hz.). There are also three methoxy groups [δ_H 3.90, 3.84, and 3.73 each (3H, s)] and an A_2B_2 system [δ_H 2.73 (4H, m)]. Accurate mass measurement confirmed the molecular formula [$C_{17}H_{18}O_3$ = $m/_z$ 270.3015 as the base peak].

In addition to cyclised product (148) the desired nitrile (147) was obtained in very low yield. Its structure was confirmed by the spectroscopic data, in particular an i.r. band at $\bar{\gamma}_{max}$ 2220 cm^{-1} and a ^{13}C resonance at δ_C 115.2 arising from the cyano group. The ^1H n.m.r. spectrum showed the expected AA' BB' [δ_H 7.10(d, J8.7Hz.), 6.99(d, 8.5Hz.) and AB [6.85(d, J6.21Hz.); 6.81(d, J6.5Hz.)] systems, three methoxyls at δ_H 4.00, 3.84 and 3.77 and a broad multiplet at δ_H 2.84 for the benzylic protons. The molecular formula $C_{18}H_{19}NO_3$ was established by high resolution mass spectrometry. The parent ion is at $m/_z$ 297.2362, ($C_{18}H_{19}NO_3$ requires 297.3570) and the base peak at $m/_z$ 121.0653 is due to the fragment (155) ($C_8H_9O^+$).

Attempted hydrolysis of the nitrile (147) in 40% sodium hydroxide at reflux for 3 hours failed to produce acidic product (131). The nitrile was recovered unchanged. The failure of this reaction and the problem of the low yield in the formation of the nitrile led us to abandon this route in favour of direct carboxylation of an aromatic bromo-precursor.



(159) R = Br

(131) R = CO_2H

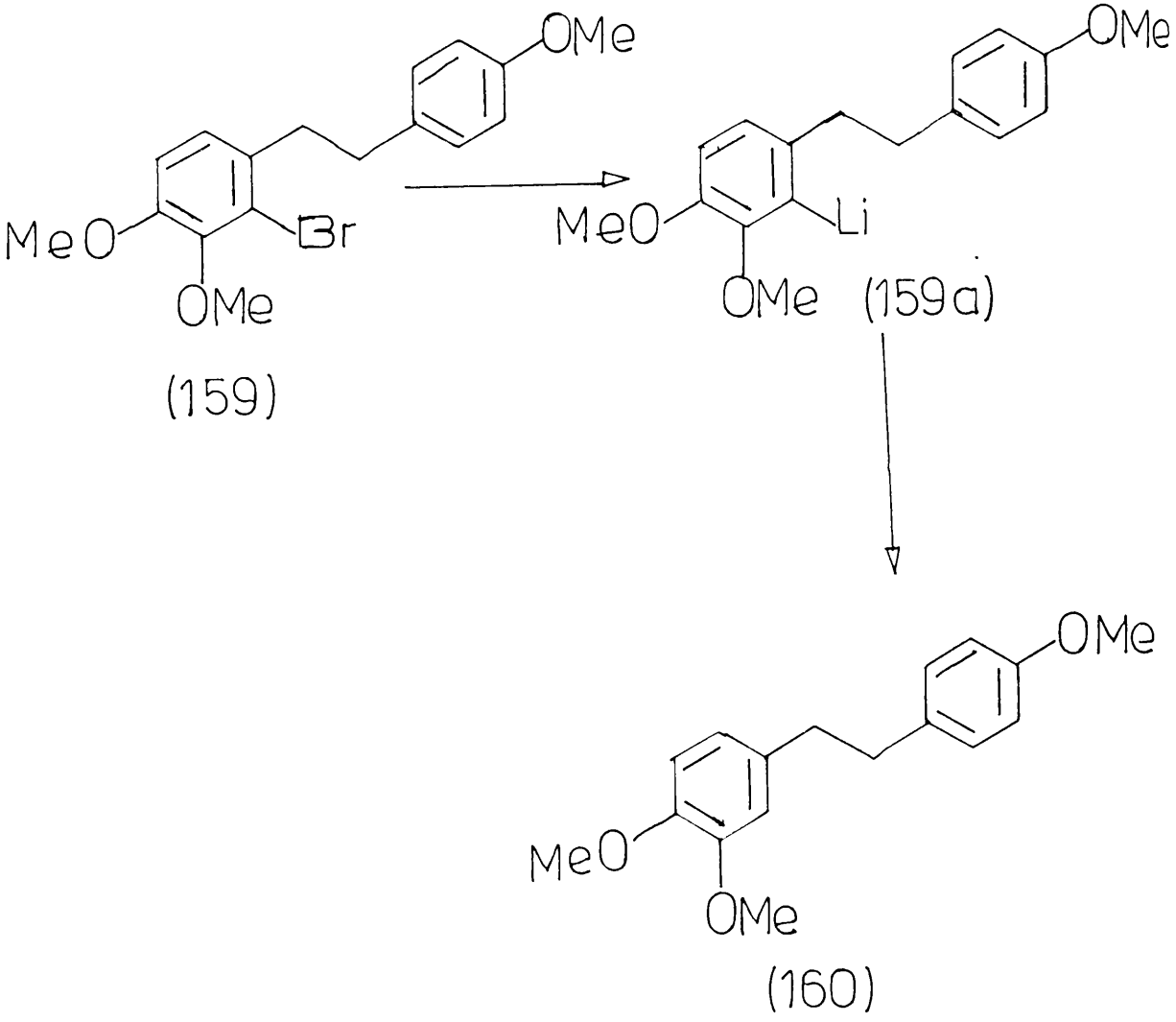
(160) R = H

Scheme 16

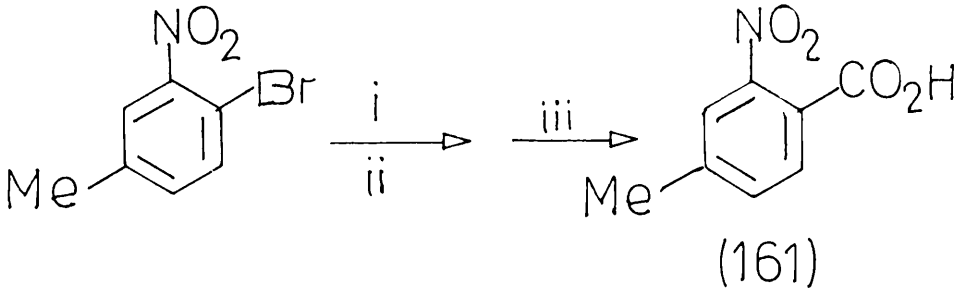
2-Bromovanillin was converted to bromoveratraldehyde (156) using methyl iodide and anhydrous potassium carbonate. The ^1H n.m.r. spectrum of (156) confirmed the presence of two methoxy groups [δ_H 3.98 and 3.80]. The aldehyde (δ_H 10.75) and two ortho-coupled aromatic protons [δ_H 7.71 and 6.95, AB q, J9.8Hz.] The mass spectrum confirmed the molecular formula $C_9H_9BrO_3$ [$m/_z$ 246 as the base peak].

A Wittig reaction of the aldehyde (156) with the ylid (139a) gave the desired stilbene product as a mixture of Z (157) and E - (158) isomers. In this sequence the isomer (158) predominated, on the evidence of the ^1H n.m.r spectrum of the mixture. It showed a pair of doublets (J16.6Hz.) at δ_H 7.35 and 6.68 for the olefinic protons. The aromatic protons appeared as AA' BB' [δ_H7.44 and 7.08] and AB[δ_H 6.90 and 6.86] systems while the three methoxy groups resonated at δ_H 3.88, 3.86 and 3.79. Mass spectrometry gave the molecular formula as the base fragment ($m/_z$ 348 $C_{17}H_{17}BrO_3$) (Scheme 16).

The 2- bromobibenzyl (159) was obtained from the stilbene mixture in good yield by hydrogenation for 8 hours. Its ^1H n.m.r. spectrum confirmed its structure [δ_H 7.43 (d, J8.8Hz., $H_{3'}$, $_{5'}$), 7.14(d, J8.6Hz., $H_{2'}$, $_{6'}$); 6.84(d, J8.6Hz., H_5); 6.81(d, 8.9Hz, H-6)]; and three singlets of methoxy groups at 3.85, 3.84 and 3.76 and a broad multiplet at δ_H 2.86. The mass spectrum confirmed its molecular formula ($m/_z$ 350, $C_{17}H_{19}BrO_3$ requires 350).



Scheme 17



(i) C_4H_9Li; (ii) THF/-100°; (iii) CO_2

Scheme 18

The bromobibenzyl (159) was subjected to the typical halogen metal exchange conditions in the hope of producing the desired carboxylic acid (131). Thus, n-butyl lithium was added to the bromo-compound (159) in dry THF and the solution stirred under nitrogen at - 78°C for 1 hour to form the lithio-derivative (159a) (Scheme 17). The reaction mixture was then quenched with carbon dioxide gas for 1 hour. Unfortunately the major product obtained proved to be the debromobibenzyl (160). None of the desired carboxylic acid (131) was detected. The debromobibenzyl (160) was obtained in reasonable yield and its structure followed readily from its spectroscopic properties. Its mass spectrum confirmed the molecular formula with the base peak at $m/_z$ 272 ($C_{17}H_{19}O_3$) and major fragments at $m/_z$ 151 and 121. In the ^1H n.m.r. spectrum the typical AA' BB' system of the top ring again appeared [δ_H 7.08(d, J8.6Hz.), 6.89(d, J8.7Hz.)]. The substitution pattern of the other ring [δ_H 6.79(d, J8.1Hz.), 6.72(dd, J8.0, 1.8Hz), 6.62(d, J1.8Hz)] clearly revealed the loss of the bromo substituent. The remaining features of the spectrum included three methoxyl groups [δ_H 3.85, 3.83 and 3.77] and a broad singlet (δ_H 2.86) arising from the four benzylic protons.

The failure of the carboxylation step is disappointing. Normally lithiation followed by quenching with carbon dioxide should give reasonable yields of carboxylic acid products.

4- Bromo- 3- nitro toluene[80] for example, afforded the corresponding acid (161) in 80% yield (scheme 18). Clearly moisture has been allowed to enter the system prior to the carbon dioxide. The formation of the debromobibenzyl (160) proves beyond doubt that lithio intermediate (159a) was formed.

EXPERIMENTAL

General Experimental

Melting-points (m.p.) which are uncorrected, were determined on a Kofler hot-stage apparatus. Infrared (i.r.) spectra were recorded in CCl_4 solution on either Perkin Elmer 580 or 257 instruments. Mass spectra (m.s.) were recorded using an MS12 instrument (low resolution) and an MS902S instrument (high resolution). Unless otherwise stated, nuclear magnetic resonance (n.m.r.) spectra were recorded for $CDCl_3$ solutions at 90 MHZ, on a Perkin-Elmer R.32 instrument. Highfield n.m.r. spectra were recorded using a Bruker WP200SY instrument (^1H, 200 MHZ, ^{13}C 50.32 MHZ). Chemical shifts were measured using the δ scale with tetramethylsilane as internal standard or relative to $CHCl_3$ at δ_H 7.25 or $CDCl_3$ at δ_C 77.0. Column chromatography was carried out on Merck silica HF_{254}. Kieselgel GF_{254} was used for preparative thin layer chromatography. Analytical t.l.c. plates were visualised using u.v. light (254 or 350n.m.) and by spraying with ceric sulphate H_2SO_4. Eluants for column chromatography were increasing percentages of Ethyl acetate in petroleum ether. All solvents and reagents used were of analytical grade except for column chromatography when bulk solvents were used. Solvents were removed using a Büchi rotary evaporator and water aspirator. Petroleum ether refers to the fraction boiling between 60°C and 80°C. Organic solutions were dried over anhydrous magnesium sulphate or sodium sulphate.

Methyl 4-methoxybenzoate (136)

A mixture of 4- hydroxybenzoic acid (20 g, 0.144 mole), anhydrous potassium carbonate K_2CO_3 (50 g) and dimethyl sulphate (40 ml) in acetone (200 ml) was refluxed for 5 hr. The acetone was removed and the residue diluted with water (250 ml) and ether (250 ml). The organic layer was washed with concentrated ammonia solution (2 x 50 ml) and then water (50 ml). The ethereal layer was dried over anhydrous sodium sulphate and evaporated to give a white precipitate of methyl-4- methoxy benzoate (136) (20.9 g, 87%); m.p. 51°C-52°C.

δ_H: 7.58(d, J8.2Hz.), 6.55(d, J8.2Hz.), 4.02(S, $-CO_2\underline{CH}_3$), 3.95(S, $-O\underline{CH}_3$).

M.S.: 166(M^+). 135(M^+- OCH_3, base peak), 107(M^+- CO_2Me). 31($-OCH_3$).

4- Methoxybenzyl alcohol (137)[A]

A solution of methyl 4- methoxybenzoate (136) (20.8 g, 0.125 mole) in dry ether (50 ml) was added over a period of 20 min to a solution of lithium aluminium hydride (6.5 g, 0.175 mole) in dry ether (20 ml). Gentle refluxing was continued for 3 hr. The chilled mixture was then decomposed by careful addition of aqueous sodium sulphate. The aqueous phase was extracted thoroughly with ether and the combined organic layers were washed with 5% sodium bicarbonate and dried over sodium sulphate. Distillation afforded (137)

[15.6 g, 90%] as a viscous oil, b.p. 80/0.2 mm)

δ_H: 7.15(d, J8.6 Hz.), 6.86(d, J8.5Hz.), 4.45(S, $-C\underline{H}_2OH$), 3.71(S, $-OC\underline{H}_3$), 3.68(S, exchangeable with D_2O).

$\bar{\nu}_{max}$ 3450 cm^{-1}

4- Methoxybenzyl chloride (138)[8]

A stirred solution of alcohol (137) (15.6 g, 0.118 mole) and dimethyl aniline (12 g, 0.099 mole) in anhydrous toluene (80 ml) was slowly treated with redistilled thionyl chloride (9.2 ml 0.126 mole), in toluene (15 ml). The mixture was warmed to room temperature, and then heated under reflux for 1 hr. The organic layer was separated, washed, dried and evaporated to give a brown oil which on distillation yielded 4- methoxybenzyl chloride (138) (15.3, 89%) as a colourless oil b.p. 95/0.2 mm.

δ_H: 7.42(d, J8.6Hz.), 6.88(d, J8.7Hz.), 4.54(S, $-C\underline{H}_2Cl$) 3.64(S, $-OC\underline{H}_3$).

4- Methoxybenzyltriphenylphosponium chloride (139)

The benzyl chloride (138) (15 g, 0.10 mole) and triphenylphosphine (29 g, 0.11 mole) were stirred and heated in anhydrous toluene under N_2 at 60°C for 46 hr. The hygroscopic precipitated phosphonium chloride (139) (36 g, 84%) was separated by filtration, washed with light petroleum ether, then dried *in vacuo*. It had m.p. 137°C.

δ_H: 7.60(15H, br m, $-P\underline{h}_3P$), 6.89(d, J9.0Hz.), 6.58(d, J8.9Hz.), 5.21(d, 14.0Hz.), 3.62(S, $-OC\underline{H}_3$).

Preparation of Acetyl vanillin (140)

Vanillin (10 g) in pyridine (10 ml) and acetic anhydride (12 ml) were stirred at room temperature overnight. Methanol (8 ml) was added, the temperature was increased to 50°C for 1 hr. Cooling and addition of water gave a white precipitate which was filtered and dried under pump pressure to yield the acetate (140) (11.8 g, 93.4% yield), m.p. 75°C-76°C.

δ_H: 10.11(S, $-C\underline{H}O$), 7.68(dd, J8.7, 2.7Hz.), 7.41(d, J8.9 Hz.), 7.31(d, J8.8Hz), 3.93(S, $-OC\underline{H}_3$), 2.35(S, $-OCO\ C\underline{H}_3$).

M.S.: 194(M^+), 151($M^+ - COCH_3$, base peak), 43($M^+ - 152$)

2-Nitroacetylvanillin (141)[81]

Dry acetyl vanillin (140) (11.2 g, 0.057 mole) was stirred in an ice bath. Red fuming nitric acid (15 ml, d 0.5) was slowly added in small portions. The temperature was maintained below 0°. The solid dissolved to give a dark-red solution. When the addition of nitric acid had been completed, the contents of the flask were poured on to ice. The crude product separated as a yellow precipitate. It was collected by filtration and well washed with water.

Crystallisation from aqueous ethanol afforded 2- nitroacetyl-vanillin (10.2 g, 73%) m.p. 70 - 72°C.

δ_H: 10.15(S, -CHO), 7.85(d, 9.8Hz.), 7.58(d, 9.9Hz.), 3.95(S, $-OCH_3$) 2.32(S, $-COCH_3$)

M.S. 239(M^+), 196($M^+ - COCH_3$), 43(M^+ - 196, base Peak)

2- Nitroveratraldehyde (142)[74]

2- Nitroacetylvanillin (141) (11 g) in water (50 ml) and dimethyl sulphate (30 ml) were stirred vigorously while 50% aqueous sodium hydroxide (18 ml) was added dropwise during one hour, the pH being kept at about 8 and the temperature at 35° - 42° C throughout. Further dimethyl sulphate (10 ml) was then added followed by more alkali until the pH was 11. After further stirring for 20 min., the mixture was cooled, conc. ammonia (10 ml) was added and the reaction left at 0°C. The product was filtered off, washed with water (50 ml) and crystallised from ether to give 2- nitroveratraldehyde (142) (8.2 g, 84% yield), m.p. 61 - 62.5 °C [Lit, 63.5 - 64°].

δ_H: 9.77(S, -CHO), 7.64(d, J8.7Hz.), 7.12(d, J8.7Hz.) 4.00(S, $-OCH_3$), 3.92(S, $-OCH_3$)

M.S. 211(M^+), 165($M^+ - NO_2$), 137(166-CO).

2- Nitro- 3,4,4'- trimethoxystilbene (143)[83]

To 4- methoxybenzyltriphenylphosphonium chloride (139) (9.92 g, 0.02 mole) in dry THF (50 ml) was added n. butyl lithium (12 ml, d, 0.693) and the mixture stirred under nitrogen at -50°C for 1 hr. 2-Nitroveratraldehyde (142), (5g, 0.023 mole) in THF (15 ml) was added and the stirring was continued for 2 hours at room temperature. The mixture was then acidified with dilute hydrochloric acid and extracted with ether (2 x 40 ml). Evaporation of the solvent afforded a pale yellow oil (7.8 g) which showed mainly 2 spots on t.l.c. of r.f. = 0.5 and 0.2. These spots were separated by flash column chromatography in EtOAC: Petrol (0 - 100%). Fractions 7, 8 and 9 were found to contain a single crystalline product (r.f. = 0.2) identical with triphenylphosphine oxide. Fractions (1 - 6) were due to the stilbene as a mixture of Z - (143) and E - (144) forms [3.8 g, 50.94%].

δ_H: 7.09(d, J8.39Hz.), 6.94(d, J8.51Hz.), 6.79(d, 8.7Hz.) 6.72(d, J8.85), 6.60(d, J11.87Hz.), 6.25(d, J11.8Hz.) 3.93(S, $-OC\underline{H}_3$), 3.86(S, $-OC\underline{H}_3$), 3.75(S, $-OC\underline{H}_3$)

δ_C: 158.99(s), 152.06(s), 140.74(s), 133.31(d), 130.23(d), 128.41(s), 125.15(d), 122.73(s), 120.43(d), 114.10(s) 113.61(d), 62.05(q), 56.16(q), 55.11(q).

M.S. $m/_z$ 315.1102 ($C_{17}H_{17}NO_5$ requires 315.1107)

2-Nitro- 3,4,4', trimethoxybibenzyl (145)

2-Nitro- 3,4,4'- trimethoxystilbene mixture (3.5 g, 0.011 mole) in methanol (100 ml) and 5% Pd-C (0.4 g) was stirred under hydrogen for 2 hr. Filtration of the catalyst and evaporation of the solvent afforded the bibenzyl (145) (3.2 g, 93%) as an oil.

δ_H: 7.04(d, J8.99Hz.), 6.92(d, J8.82Hz.), 6.89(d, J8.74Hz.) 6.83(d, J8.62Hz.), 3.92(S, $-OC\underline{H}_3$), 3.87(S, $-OC\underline{H}_3$), 3.77(S, $-OC\underline{H}_2$) and 2.77(4H, br m).

δ_C: 157.94(s), 151.35(s), 140.52(s), 132.72(s), 129.29(d), 125.23(d), 124.91(d), 123.84(s), 113.80(d), 113.73(d), 101.65(s), 61.94(q), 55.54(q), 55.13(q), 35.00(t), 32.93(t).

M.S. $m/_z$ 317.2681, ($C_{17}H_{19}NO_5$ requires 317.1263)

2- Amino- 3,4,4'- trimethoxybibenzyl (146)

Lithium aluminium hydride (6 g) was carefully added in small portions to a solution of the 2- nitrobibenzyl (145) (3 g, 9.46×10^{-3} mole) in dry ether (50 ml). Gentle refluxing was continued for 2 hr. The reaction was worked up by adding aqueous sodium sulphate followed by hydrochloric acid (5 N, 10 ml). The aqueous phase was extracted thoroughly with ether, and the combined organic layers washed with 5% sodium bicarbonate and dried anhydrous magnesium sulphate. The product (146) was obtained as a yellow solid (1.9 g, 70% yield) m.p. 92°C.

δ_H: 7.08(d, J8.73Hz.), 6.81(d, J8.7Hz.), 6.78(d, J8.54Hz.), 6.35(d, J8.48Hz.), 3.82(S, $OC\underline{H}_3$), 3.80(S, $-OC\underline{H}_3$), 3.78(S, $-OC\underline{H}_3$), 2.95(br. m. 4H), 2.16(S, $N\underline{H}_2$, exchangeable with D_2O).

δ_C: 157.84(s), 151.02(s), 138.53(s), 133.87(s), 129.29(d), 129.26(d), 123.85(d), 119.87(s), 118.24(d), 113.74(d), 113.57(d), 101.66(s), 59.76(q), 55.56(q), 55.16(q), 34.60(t), 33.14(t).

M.S. $m/_z$ 287.1513 ($C_{17}H_{21}NO_3$ requires 287.1521).

$\bar{\nu}_{max}$: 3420 cm^{-1}.

2- Cyano- 3,4,4'- trimethoxybibenzyl (147)[82]

2- Amino- 3,4,4'- trimethoxybibenzyl (146) (1 g) was diazotised in dilute HCl (10 ml, 2 N) with sodium nitrite (0.5 g, 7.2×10^{-3} mole) at 0^oC. The resulting diazonium salt solution was added over 75 min with stirring, simultaneously with potassium cyanide (0.6 g, 9.2×10^{-3} mole) in H_2O (8 ml) to a hot solution of CuCN-KCN at 80^o - 5^o [obtained by adding $NaHSO_4$ (0.3 g) and potassium hydroxide (0.5 g) in H_2O (10 ml) to cupric sulphate $CuSO_4.5H_2O$ (0.8 g) and NaCl (1 g) in H_2O (7 ml) at 85^o, cooling, decanting the supernatant liquid, diluting with H_2O (8 ml) and adding potassium cyanide (0.5 g)] The mixture was stirred for 30 mn at 85^o. Cooled and filtered then the filtrate residue was extracted with EtOAC (2 x 30 ml) which yielded a crude product of (0.8 g) and showed mainly 2 spots on t.l.c. These spots were separated by

flash column chromatography in EtOAC; petroleum gave the nitrile (147) in very low yield (0.025 g, 2.5%) and the cyclised product (148) in good yield (0.5 g, 54%).

(i) the nitrile (147); n.m.r. signals at.

δ_H: 7.10(d, J8.7Hz.), 6.99(d, J8.53Hz.), 6.85(d, J6.34Hz.) 6.81(d, J6.58Hz.), 4.00(S, $-OC\underline{H}_3$), 3.84(S, $-OC\underline{H}_3$), 3.77(S, $-OC\underline{H}_3$), 2.84(br m, 4H)

δ_C: 158.01(s), 150.55(s), 137.72(s), 132.76(s), 129.43(d), 129.36(d), 124.61(d), 116.73(d), 115.21(s), 113.77(d), 61.54(q), 56.00(q), 55.11(q), 36.29(t)

M.S. $m/_z$ 297.2362 ($C_{18}H_{19}NO_3$ requires 297.1365)

ir. : 2220 ; 1610 cm^{-1}

(ii) The cyclised compound (148)

δ_H: 8.12(d, J2.72Hz.), 7.18(d, J8.26Hz.), 6.96(d, J8.20 Hz.) 6.82(dd, J2.69, 8.24Hz.), 6.78(d, J8.2 Hz.), 3.90(S, $OC\underline{H}_3$), 3,84(S, $OC\underline{H}_3$), 3.73(S, $-OC\underline{H}_3$), 2.73(br, 4H).

δ_C: 158.34(s), 152.14(s), 147.00(s), 133.40(s), 132.33(s) 130.96(s), 129.44(d), 129.41(s), 123.06(d) 113.62(d) 110.99(d), 60.19(q), 55.93(q), 55.16(q), 29.75(t) 29.24(t)

M.S.: $m/_z$ 270.3015 ($C_{17}H_{18}O_3$ requires 270.1256)

Preparation of 2- bromoveratraldehyde (156)

2- Bromovanillin (7.8 g, 0.033 mole) was heated in acetone (50 ml) with iodomethane (30 ml) and anhydrous potassium carbonate (50 g, 0.36 mole) under reflux on water bath for 6 hr. The inorganic material was removed by filtration; the residue was washed 3 times with acetone and evaporated. To the crystalline residue 10% sulphuric acid (10 ml) was added and dried at room temperature. Chromatography over alumina in $CHCl_3$ yielded prisms of 2- bromoveratraldehyde (156) (6 g, 74%); m.p. 75 - 77°.

δ_H: 10.75(S, -CHO), 7.71(d, J9.8Hz.), 6.95(d, J9.8Hz.), 3.98(S, $-OCH_3$), 3.80(S, $-OCH_3$)

M.S.: 244(M^+. base peak), ($C_9H_9BrO_3$ requires 244).

2- Bromo- 3,4,4'- trimethoxystilbene

A mixture of 4- methoxybenzyltriphenylphosphonium chloride (139) (8 g, 0.019 mole) and n-butyl lithium (10 ml, d 0.693) was stirred at -50° in dry THF (50 ml) under N_2 for 1 hr. To this a solution of 2- bromoveratraldehyde (156) (5 g, 0.020 mole) in THF (10 ml) was added, and the mixture stirred at room temperature for 18 hr. The mixture was then acidified with dilute HCl and extracted with ether (50 ml). The organic layer on evaporation gave a yellow oil which showed mainly 3 spots on t.l.c. Column chromatography in petrol: EtOAC afforded a mixture of Z- (157) and E- (158) of

2- bromo- 3,4,4'- trimethoxystilbene (6 g, 84%), and triphenylphosphine oxide (1.5 g) together with the unchanged phosphonium chloride (139) (1 g).

δ_H: 7.44(d, J8.67Hz.), 7.35(d, J16.65Hz.), 7.08(d, J8.67Hz.), 6.90(d, J8.87Hz.), 6.86(d, J8.77Hz.), 6.68(d, J8.88Hz.), 3.88(S, $-OC\underline{H}_3$), 3.86(S, $-OC\underline{H}_3$), 3.79(S, $-OC\underline{H}_3$)

δ_C: 159.56(s), 152.42(s), 146.63(s), 131.54(d), 132.28(d), 129.91(s), 127.63(d), 125.31(s), 121.62(d), 119.72(s), 114.25(d), 113.61(d), 113.32(d), 111.7(d), 56.32(q), 55.67(q), 55.43(q)

M.S. $m/_z$ 348(M^+, base peak); ($C_{17}H_{17}BrO_3$ requires 348).

2- Bromo- 3,4,4'- trimethoxybibenzyl (159)

2- Bromo- 3,4,4'- trimethoxystilbene mixture (0.6 g, 0.017 mole) in EtOAC (200 ml) and 10% Pd-C (0.3 g) was stirred under hydrogen for 8 hr. Filtration of the catalyst, and evaporation of the solvent afforded the bibenzyl (5.8 g, 96%). Recrystallisation from hexane yielded (159) (4 g) as prisms m.p. 88 - 90°.

δ_H: 7.43(d, J8.79Hz.), 7.14(d, J8.62Hz.), 6.84(d, J8.65Hz.), 6.81(d, J8.98Hz.) 3.85(S, $-OC\underline{H}_3$), 3.84(S, $-OC\underline{H}_3$), 3.76(S, $-OC\underline{H}_3$), 2.86(br m, 4H)

M.S. 350(M^+), 229 ($M^+ - C_8H_{10}O$), 121(M^+ - 229, base peak)

3,4,4'- Trimethoxybibenzyl- 2- carboxylic acid (131)

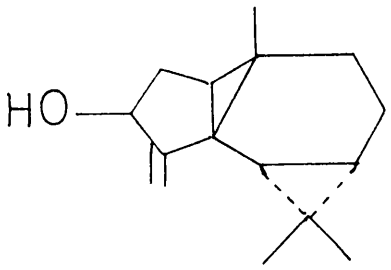
2- Bromo- 3,4,4'- trimethoxybibenzyl (159) (1 g, 2.85 x 10^{-3} mole) in dry THF and n- butyl lithium (15 ml, d, 0.647) were stirred at -78°C under N_2 for 1 hr. Dry carbon dioxide gas was bubbled into this solution for 1 hr. Water (30 ml) was added, and the THF removed by evaporation. The residue was acidified with dil HCl and extracted with EtOAC (2 x 50 ml). The organic layer was evaporated to yield a solid material (0.88 g). Crystallization from ether afforded the debromobibenzyl (160) (0.7 g) m.p. 90 - 91° . No trace of the carboxylic acid (131) was found.

δ_H: 7.08(d, J8.65Hz., $H_{-3',5'}$), 6.82(d, J8.75Hz., $H_{2',6'}$), 6.79(d, J8.07Hz., H-5), 6.72(dd, J806, 1.8Hz., H_6), 6.62(d, J1.85Hz., H-2), 3.84, 3.81, 3.77(each 3 H, s) and A_2B_2 at δ_H 2.82(4H, br)

δ_C: 159.24(s), 157.24(s), 149.21(s), 147.31(s) 134.66(d), 133.91(d), 130.90(d), 129.53(d) 127.62(d), 120.43(d), 119.61(d), 113.82(s) 59.74(q), 55.97(q), 55.36(q), 37.80(t) 37.28(t)

M.S. 272(M^+), 151(M^+ - 121, base peak), 121(M^+ - 151).

CHAPTER 3



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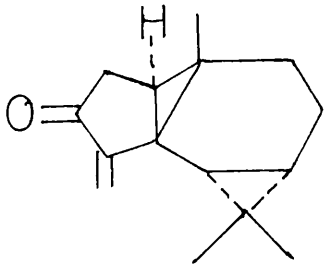

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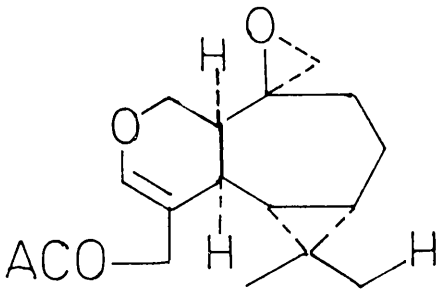


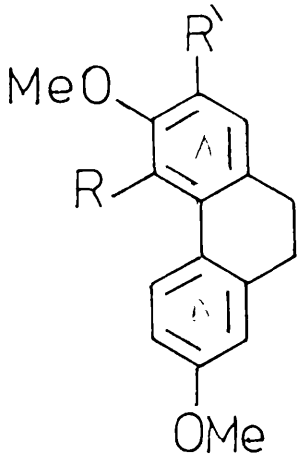
(164)

INTRODUCTION

Plagiochilaea consists of two main groups Mylia and Plagiochila, although some authorities place Mylia in a separate subfamily of the Jungermannialeae. Plagiochila belongs to Jungermanniales and more than 1500 species are known in the world. From Japan 20 species have been reported[17].

Mylia species have proved to be a very rich source of terpenoids. Mylia taylorii, for example, contains sesquiterpenoids of the aromadendendrane class, myliol (162), dehydromylione A (163) and taylorine (21). These might suggest that there is a similarity between M. taylorii and Plagiochila species which also contain aromadendranes and secoaromadendranes. Asakawa et al[17] reported the investigation of fourteen Plagiochila species and showed that the characteristic constituents are ent- secoaromadendranes e.g. plagiochilide (18). It is known that there are two chemical types, one with the sharp, pungent substance plagiochiline A (164), e.g. P. asplenioides, P. hattoriana, P. pulcherrima and the other type which lacks this pungent substance e.g. P. acanthophyle subsp. Japonica and P. arbuscula.

Phenanthrene and dihydrophenanthrene derivatives are rare amongst the Hepaticae. 3, 4-Dimethoxy- 5- hydroxy- 9, 10- dihydrophenanthrene (100) was the first example isolated from the liverwort Riccardia jackii[38]. Connolly[73] isolated the dihydrophenanthrene derivatives (165) - (170)



(165) R = OMe, R' = OH

(165a) R = OMe, R' = OAC

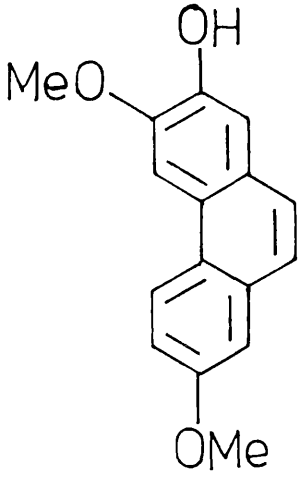
(166) R = R' = OMe

(167) R = OMe, R' = H

(168) R = OH, R' = H

(169) R = H, R' = OH

(170) R = H, R' = OMe



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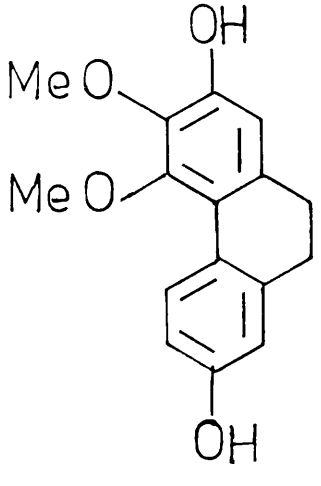

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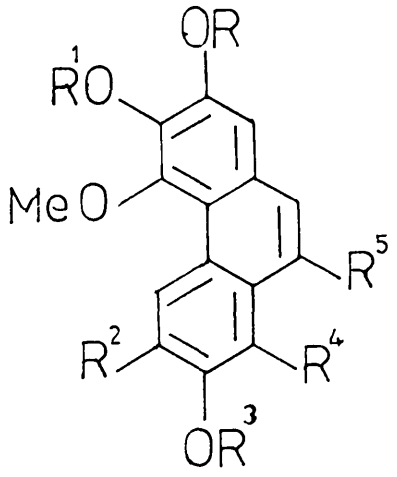
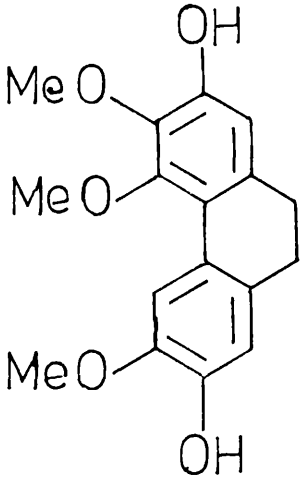


(173) $R = R^1 = Me, R^2 = R^3 = R^4 = H, R^5 = OH$

(174) $R = R^3 = R^4 = R^5 = H, R^1 = Me, R^2 = OMe$



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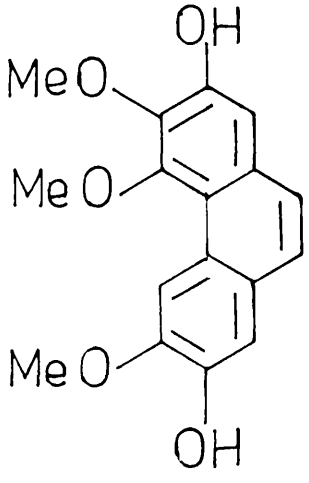
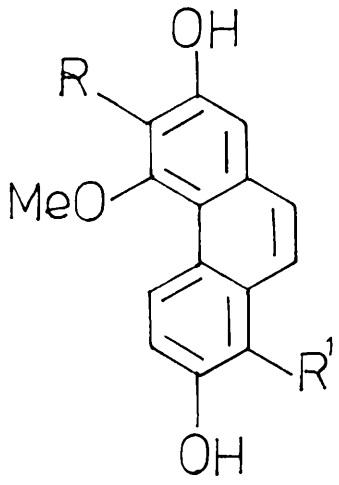


(176)

from the Scottish liverwort Plagiochila spinulosa (vide infra). Recently Asakawa[84] isolated a new phenanthrene derivative (171) from the Indian liverwort Marchantia polymorpha.

A large number of papers deal with the isolation of phenanthrene and dihydrophenanthrene derivatives from higher plant families. Majumder[85] reported the isolation of erianthridin (172) a new 9, 10- dihydro- phenanthrene derivative from the orchids Eria carinata and Eria stricta. From the orchid Bulbophyllum gymnopus, Majumder[86] also isolated another new phenanthrene derivative, gymnopusin (173) together with 2,7- dihydroxy- 3,4,6- trimethoxyphenanthrene (174) and the 9, 10- dihydro derivative (175). Stermitz[87] found a large number of phenanthrenes and the dihydrophenanthrene derivatives (175) and (176) - (178) in Oncidium cebollata, a peyote- replacement plant.

A reinvestigation of Plagiochila spinulosa was undertaken in order to characterise fully the aromatic constituents and to obtain sufficient quantities for biological testing. This chapter describes the isolation and spectroscopic analysis of several dihydrophenanthrenes and other aromatic compounds from P. spinulosa. The synthesis of 2-hydroxy- 3,7- dimethoxy- 9,10,- dihydrophenanthrene (169) is also described.



(177) $R = OMe, R^1 = H$

(178) $R = H, R^1 = OMe$

DISCUSSION

Constituents of Plagiochila spinulosa

Preliminary studies of the constituents of P. spinulosa by Harrison[89] afforded 2- hydroxy- 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (165) as the major component accompanied by 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (167) and the pentasubstituted benzene derivative methyl 6-methoxy- 3,4- methylenedioxy- 2- methylbenzoate (189). In the present work these compounds were again obtained.

It was clear from the ^1H n.m.r. spectrum of the major compound (165), $C_{17}H_{18}O_4$, $m/_z$ 286, that it is a dihydrophenanthrene with three methoxyl groups (δ_H 3.96, 3.83, and 3.73) and a phenolic hydroxyl (δ_H 5.67, s, exchangeable with D_2O). The protons arising from the two methylene groups appear as a complex multiplet at δ_H 2.69. A highly deshielded aromatic proton [δ_H 8.17 (d, J8.4Hz)] must be attached to C-5 (or C-4). It has an ortho- neighbour [δ_H 6.80 (dd, J8.5, 2.9Hz)] which is in turn coupled to a meta- proton [δ_H 6.76 (d, J2.9Hz)]. These data readily established the substitution pattern of ring B which must have an oxygen substituent at C-7. Ring A must have three oxygen substituents since the remaining aromatic proton is present as a singlet [δ_H 6.11]. This proton is probably H-1 since it couples with one of the methylene groups at 360 MHz. Thus the compound is a trimethyl ether (165) of 2,3,4,7- tetrahydroxy- 9, 10- dihydrophenanthrene.

The ^{13}C n.m.r. shifts of (165) reveal that two of the methoxyl groups [δ_C 61.1 and 60.0] have no neighbouring aromatic protons and are situated at C-3 and C-4. Small shifts of H-1 in the acetate (165a) [δ_H 6.71] and methyl ether (166) [δ_H 6.58] suggested that the phenolic hydroxyl group is situated at C-2 and that the natural compound is 2- hydroxy- 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (165). Confirmation of this was obtained from the fully coupled ^{13}C n.m.r. spectrum of (165) which also provided evidence for the assignment of all the ^{13}C resonances. C-1, C-2, and C-3 all lost long-range couplings with the phenolic hydroxyl proton following D_2O exchange.

The second compound, the trimethoxy dihydrophenanthrene (167), $C_{17}H_{18}O_3$, $m/_z$ 270.1259, has the same substitution pattern in ring B as evidenced by the presence of the spin system H-5 [δ_H 8.37 (d, J8.6Hz)], H-6 [δ_H 6.83 (dd, J8.6, 2.8Hz)] and H-8 [$_H$ 6.77 (d, J2.8Hz)]. Ring (A) contains an AB system [δ_H 6.93 (d, J8.1Hz, H-1) and δ_H 6.75 (d, J8.1Hz, H-2)] which must involve H-1 and H-2 since the former is coupled with a methylene group and neither proton is strongly deshielded. Furthermore one methoxyl signal (δ_C 60.0 attached to C-4) has no proton neighbour. The second compound is therefore, 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (167)

An interesting pentasubstituted benzene derivative (189), $C_{11}H_{12}O_5$, $m/_z$ 224, ν_{max} 1733 cm^{-1} was also isolated. Its spectroscopic properties revealed the presence of an aromatic methyl group [δ_H 2.14 (d, J0.5Hz.), δ_C 12.5], a methoxyl group [δ_H 3.75 (d, J0.3Hz.), δ_C 57.0] a carbomethoxyl [δ_H 3.87 (s), δ_C 52.1], a methylenedioxy group [δ_H 5.92 (2H, s), δ_C 92.9 (t)] and an aromatic proton [δ_H 6.29 (s); δ_C 101.3 (d)]. The ^{13}C chemical shift of the methoxyl indicating that it has a proton neighbour. This is also confirmed by the observation of a small long-range coupling (J0.3Hz.) between the methoxyl and the aromatic proton. The ^1H chemical shift of the aromatic proton is consistent with the presence of two ortho- oxygen functions. Finally a long-range coupling (J0.5Hz.) between the aromatic proton and the methyl group favours a para relationship and leads to the structure methyl 2- methyl- 3,4- methylenedioxy- 6- methoxybenzoate (189) for the natural product. This compound is clearly related to orsellenic acid (190) a common polyketide secondary metabolite.

Further work on P. spinulosa by Ifeadike and Singh[73] resulted in the isolation of the lunularic acid derivative (131), whose atempted synthesis has been described earlier, together with the most interesting and unusual constituent spinuloplagin A (192), whose structure was determined by x-ray analysis.[73] Several other compounds were obtained.

These appeared to be dihydrophenanthrenes. In our re-investigation we have isolated and characterised the dihydrophenanthrenes (165) and (167) - (170) and in addition, have obtained spinuloplagin B (191), an isomer of (192), whose structure will be described below.

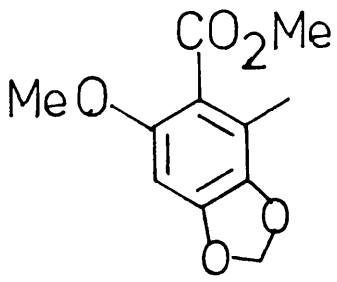
The dihydrophenanthrenes (167) - (170), readily identifiable by the presence of a four proton multiplet for the methylene protons, have the same substitution pattern in ring B as the previous compounds (165) and (170) i.e. a methoxyl group attached to C-7 (see experimental section). The changes in ring A are easily deduced by considering the ^{1}H aromatic proton signals and the ^{13}C chemical shifts of the methoxyl signals.

Compound (169), $C_{16}H_{16}O_3$, $m/_z$ 256.1088, has a phenolic hydroxyl [δ_H 5.42 (s, exchangeable with D_2O)], a methoxyl and two aromatic proton signals [δ_H 7.45 and 6.66]. The deshielded proton must be attached to C-4. The singlet nature of the aromatic proton signal indicates that the oxygen functions are at C-2 and C-3. A decision between a 2- hydroxy- 3- methoxy and a 2- methoxy- 3- hydroxy arrangement was reached by synthesis (see later). The compound is therefore 2- hydroxy- 3,7- dimethoxy- 9, 10- dihydrophenanthrene (169).

The next compound (170), $C_{17}H_{18}O_3$ $m/_z$ 270, is a trimethoxy dihydrophenanthrene. None of the methoxyl groups has a ^{13}C chemical shift in the region of 60 ppm and thus all have at least one ortho proton neighbour. The aromatic protons of ring A again appear as singlets [δ_H 7.19 (H-4) and 6.73 (H-1)]. The compound is therefore 2,3,7- trimethoxy- 9, 10- dihydrophenanthrene (170) and is the methyl ether of (169) above.

The final dihydrophenanthrene (168), $C_{16}H_{16}O_3$ is isomeric with (169). Ring A again has two oxygen substituents, a methoxyl and a phenolic hydroxyl [δ_H 3.56 (s, exchangeable with D_2O)]. The strongly deshielded nature of H-5 [δ_H 8.37 (d, J8.7Hz.)] reveals that there is an oxygen substituent on C-4. The fact that it is the hydroxyl group follows from the absence of a methoxyl with a deshielded ^{13}C chemical shift. Thus the compound is 4- hydroxy- 3,7- dimethoxy- 9, 10- dihydrophenanthrene (168). In agreement with this proposal the ring A protons resonate as an ABq (J8.1Hz) at δ_H 6.74 and 6.69. The ^{13}C chemical shifts of most of the above compounds have been measured and are reported in the experimental section. Complete assignments have been made only for certain compounds.

Careful chromatography of the mother liquors of spinuloplogin A (192) afforded, in minor amount, an isomeric compound which we name spinuloplagin B (191) $C_{37}H_{48}O_5$, $m/_z$ 572.3527. It is clearly a bibenzyl-diterpenoid conjugate



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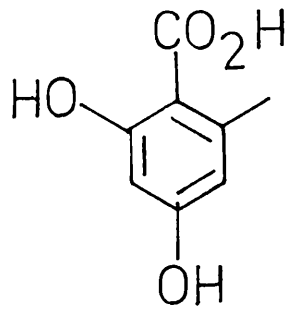

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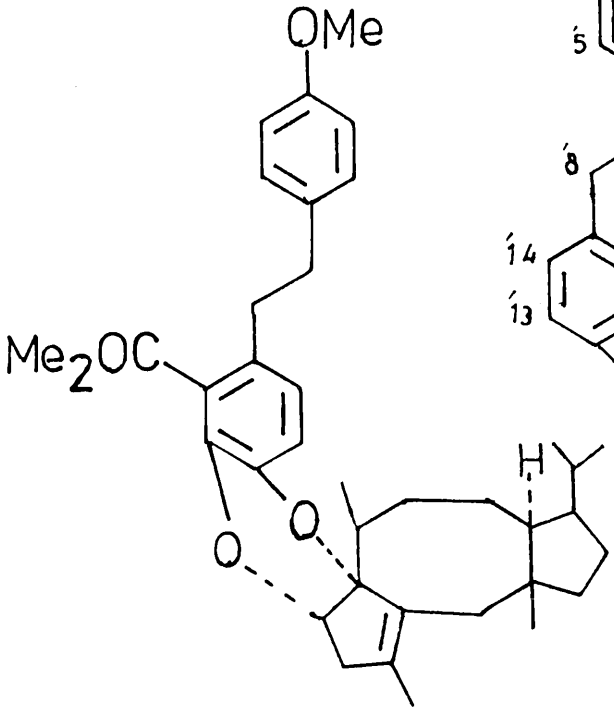
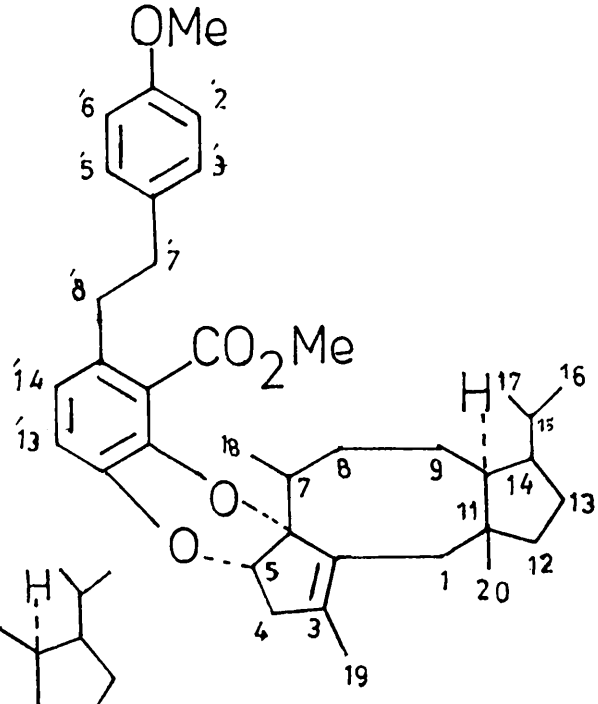


(191)



(192)

and its spectroscopic properties are very similar to those of (192). The ^{1}H and ^{13}C chemical shifts of the two compounds are listed in table for comparison. The shifts of the diterpenoid portion are virtually identical in the two cases. Only in the case of the lower ring of the bibenzyl ring are differences apparent. Thus the protons of the AB system shift from δ_H 6.76 and 6.56 in A to δ_H 6.72 and 6.60 in B. Comparison of the ^{13}C spectrum reveals small shift differences in all of the carbon of the lower aromatic ring. The absence of ^{13}C shift in the diterpenoid portions of (191) and (192) excludes the possibility of configurational differences. The only remaining possibility is regioisomerism i.e. in the formation of the dioxane ring oxygen-3 has become bonded to C-5 of the diterpenoid and oxygen-4 to C-6. Thus spinuloplagin B has structure (191).

Spinuloplagin A and B are unusual natural products. It is not immediately apparent how the dioxan ring is formed. A similar type of system has been reported[90] in nilgherron A (193) which is a diterpenoid dimer. The formation of these compounds is perhaps similar to the formation of the coumarinolignan[91] (194) which has been synthesised by both chemical and enzymatic oxidation of a dihydroxycoumarin in the presence of a phenylpropene.

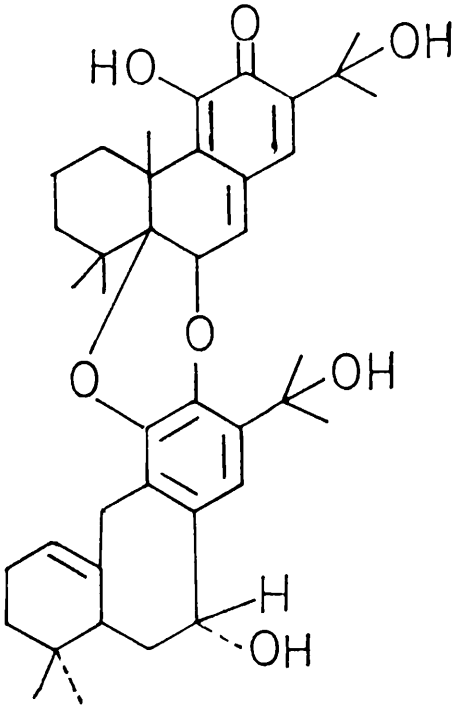
^{1}H n.m.r. chemical shifts of spinuloplagins A (192) and B (191)

H	(191)	(192)
3′, 5′	7.05 (d, J8.7 Hz.)	7.07 (d, J8.7Hz.)
2′, 6′	6.80 (d, J8.7Hz.)	6.80 (d, J8.7Hz.)
13′	6.72 (d, J8.3 Hz.)	6.76 (d, J8.3Hz.)
14′	6.60 (d, J8.3Hz.)	6.56 (d, J8.3Hz.)
5	4.89 (t, J7.75Hz.)	4.87 (t, J7.75Hz.)
OCH_3	3.89 (s)	3.87 (s)
CO_2CH_3	3.77 (s)	3.78 (s)
7′, 8′	2.75 (m, 4 H)	2.76 (m, 4 H)
19	1.60 (s)	1.61 (s)
18	1.11 (d, J7.0Hz.)	1.11 (d, J6.9Hz.)
16	0.89 (d, J6.6Hz.)	0.90 (d, J6.7Hz.)
17	0.83 (d, J6.6Hz.)	0.84 (d, J6.6Hz.)
20	0.72 (s)	0.73 (s)

^{13}C n.m.r. chemical shifts of spinuloplagins A (192) and B (191)

C	(191)	(192)
$\underline{C}O_2Me$	168.3	168.3
1′	157.8	157.8
11′	145.1	144.6
12′	139.4	140.2
2 }	137.9	137.5
3	136.2	136.2
9′	134.0	134.0
4′	131.1	132.6
3′, 5′	129.3 (2d)	129.3 (2d)
14′	122.2 (d)	120.7 (d)
10′	119.1	122.3
13′	117.5 (d)	118.8 (d)
2′, 6′	113.7 (2d)	113.7 (2d)
6	93.2	94.1
5	74.9	74.7
$O\underline{C}H_3$	55.2	55.2
$CO_2\underline{C}H_3$	52.1	51.9
d	48.6	48.6
d	47.0	46.9
s	45.7	45.7
t	44.1	44.1

C	(191)	(192)
t	38.6	38.5
7′	37.0	37.0
8′	35.6	35.7
d	33.8	33.7
t	30.2	30.1
d	28.0	28.0
t + q	24.6	24.6
t	23.0	23.0
q	20.0	20.1
q	19.3	19.3
q	16.7	16.8
q	15.4	15.4



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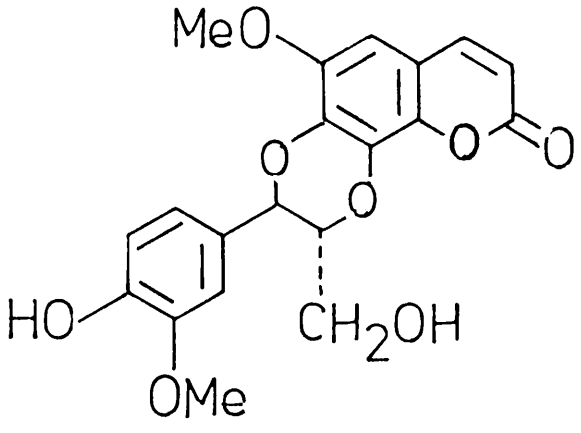

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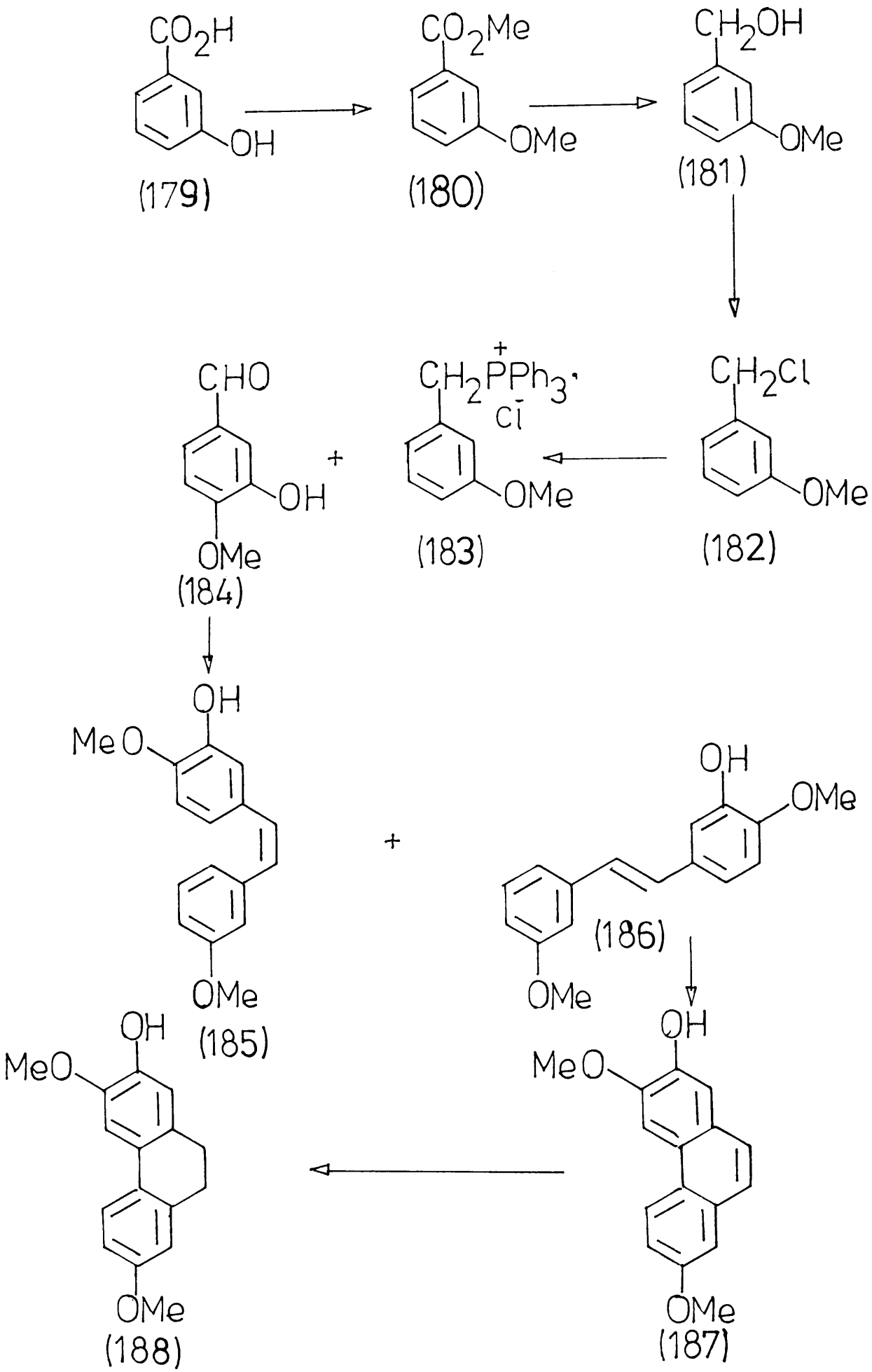
Scheme 19


CO2H
OH
(179)
CO2Me
OMe
(180)
CH2OH
OMe
(181)
CH2Cl
OMe
(182)
CH2PPh3+ Cl-
OMe
(183)
CHO
OH
OMe
(184)
OH
MeO
OMe
(185)
OH
OMe
OMe
(186)
OH
MeO
OMe
(187)
OH
MeO
OMe
(188)

Synthesis of 2- hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene

The liverwort Plagiochila spinulosa, a less common species in Scotland, has yielded 2- hydroxy- 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (165) and the related dihydrophenanthrenes (166) - (170). Since 2- hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene (169) showed some activity in antifungal tests, we decided to synthesise it in order to obtain sufficient quantities for further biological evaluation.

3-Hydroxybenzoic acid (179) was methylated using dimethyl sulphate and anhydrous potassium carbonate in acetone to give methyl 3- methoxybenzoate (180) [δ_H 3.88 and 3.80 (each 3H, s, oMe)]. Reduction of (180) with lithium aluminium hydride yielded the desired 3- methoxybenzyl alcohol (181) [δ_H 4.45 (2H, s)] which was readily converted to the corresponding benzyl chloride (182) [δ_H 4.46 (2H, s)] by reaction with redistilled thionyl chloride. The benzyl chloride (182) was transformed into the phosphonium chloride (183) by treatment with triphenylphosphine under anhydrous conditions in toluene for 46 hours. Its ^{1}H n.m.r. spectrum showed the expected coupling of the benzylic methylene protons with phosphorous [δ_H 5.42 (d, J14Hz.)]. A Wittig reaction of isovanillin (184) with the phosphonium chloride (183) afforded the expected stilbene as a mixture of Z- (185) and E- (186) isomers. The ^{1}H n.m.r. spectrum showed overlapping aromatic

protons at [δ_H 7.35 - 6.75] and a broad signal at $_H$ 5.66 exchangeable with D_2O, together with two methoxyl groups [δ_H 3.88 and 3.86]. The mass spectrum showed a molecular ion at $m/_z$ 256 corresponding to the molecular formula $C_{16}H_{16}O_3$.

The stilbene mixture was transformed into the corresponding phenanthrene (187) by photocyclisation with a 450 W u.v. lamp in an immersion well type quartz reactor under dry conditions in methanol. $+I_2$ The mixture was then washed with sodium thiosulphate to remove excess iodine. Flash column chromatography followed by prep. t.l.c. afforded the desired phenanthrene (187) in low yield. Its spectroscopic properties were consistent with structure (187). Thus, the i.r. spectrum showed a broad band at $\bar{\nu}_{max}$ 3410 cm^{-1}. In the ^1H n.m.r. spectrum, the aromatic protons [δ_H 7.45 - 6.80] were not resolved, but a singlet at δ_H 5.42, exchangeable with D_2O, and two methoxy groups [δ_H 3.84 and 3.78] were apparent. The mass spectrum showed the expected molecular ion at $m/_z$ 254 ($C_{16}H_{14}O_3$ requires 254).

Hydrogenation of phenanthrenes should occur at the 9, 10- double bond[88]. The phenanthrene (187) was converted to the corresponding dihydrophenanthrene (188) by hydrogenation in presence of 10% palladised charcoal in acetic acid. The reaction was allowed to continue for three days. After separation by prep. t.l.c., the 9,10- dihydrophenanthrene (188) was obtained in 10% overall yield. Its structure was confirmed by its ^1H n.m.r. spectrum which showed the appearance

of a new broad multiplet signal at δ_{II} 2.46 due to four protons of C-9 and C-10 and which was identical with that of the natural product.

E X P E R I M E N T A L

Plagochila spinulosa, collected near Aberfoyle (Scotland) was dried, ground and extracted in a Soxhlet apparatus with hexane, Chromatography of the crude extract over alumina (grade H deactivated) afforded an initial separation. Subsequent purification by preparative t.l.c. afforded seven natural products.

(i) 2- Hydroxy- 3,4,7- trimethoxy- 9,10- dihydrophenanthrene (165), (120 mg) was the major component. It was purified by crystallisation from chloroform: petroleum followed by prep. t.l.c. and sublimation at 92°/0.1 mm. It had m.p. 132° - 35° [lit. 135 - 139°][89].

δ_H: 8.17 (d, J8.4Hz., H-5), 6.84 (dd, J2.7, 8.8Hz., H-6), 6.76 (d, J2.9Hz., H-8), 6.62 (s, H-1), 5.67 (s, exchangeable with D_2O), 3.96 (s, -$OC\underline{H}_3$), 3.73 (s, -$OC\underline{H}_3$), 2.69 (m., 4H).

M.S.: $m/_z$ 286 (M^+ base peak), ($C_{17}H_{18}O_4$ requires 286).

(ii) 2- Hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene (169),(14 mg) was purified by prep. t.l.c. followed by sublimation at 105°/0.3 mm as an oil.

δ_H: 8.18 (d, J8.7Hz., H-5), 7.45 (s, 1H), 7.367(d, J2.7Hz., H-8), 7.08 (dd, J2.6, 8.5Hz., H-6), 6.66 (s, 1H), 5.42 (s, exchangeable with D_2O), 3.82 (s, -$OC\underline{H}_3$), 3.71 (s, -$OC\underline{H}_3$), 2.65 m, 4H).

≡ (188) h61

M.S.: $m/_z$ 256.1088 ($C_{16}H_{16}O_3$ requires 256.2994).

(iii) 2,3,7- Trimethoxy- 9,10- dihydrophenanthrene (170), (9 mg.) was purified by prep. t.l.c., followed by sublimation at 85°/0.3 mm as an oil.

δ_H: 7.58 (d, J8.8Hz., H-5), 7.28 (s, 1H), 7.22 (s, 1H), 6.92 (d, J2.7Hz., H-8), 6.78 (dd, J2.6, 7.6Hz., H-6), 3.88 (s, $-OC\underline{H}_3$), 3.86 (s, $-OC\underline{H}_3$), 3.76 (s, $-OC\underline{H}_3$), 2.84 (m, 4H).

M.S.: $m/_z$ 270 (M^+, base peak), ($C_{17}H_{18}O_3$ requires 270).

(iv) 3,4,7- Trimethoxy- 9,10- dihydrophenanthrene (167), (12 mg.), m.p. 91 - 92°, was purified by prep. t.l.c. followed by sublimation (twice) at 90°/0.2 mm, then crystallistion from ethyl acetate:ether.

δ_H: 8.37 (d, J8.6Hz., H-5), 6.94 (d, J8.5Hz.), 6.83 (dd, J8.6, 2.8Hz., H-6), 6.76 (d, J2.7Hz., H-8), 6.68 (d, J8.6 Hz.), 3.88 (s, $-OC\underline{H}_3$), 3.84 (s, $-OC\underline{H}_3$), 3.78 (s, $-OC\underline{H}_3$), 2.76 (m, 4H)

M.S.: $m/_z$ 270.1259 ($C_{17}H_{18}O_3$ requires 270.1256).

(v) 4- Hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene (168), (8 mg.) was purified by prep. t.l.c. It could not be crystallised.

δ_H: 8.37 (d, J8.7Hz., H-5), 7.46 (d, J8.5Hz.), 6.86 (d, J2.6Hz., H-8), 6.74 (d, J8.2Hz.), 6.69 (d, J8.0Hz.), 6.65 (dd, J2.4, 8.6Hz., H-6), 3.84 (s, $-OC\underline{H}_3$), 3.65 (s, $-OCH_3$), 3.56 (s, exchangeable with D_2O).

(vi) Methyl 2- methyl- 3,4- methylenedioxy- 6- methoxybenzoate (189), (8 mg.) was isolated as an amorphous solid. It was purified by prep. t.l.c. followed by sublimation at 86°/0.3 mm, and had m.p. 80°.

δ_H: 6.29 (s, 1H), 5.92 (s, 2H), 3.87 (s, $-CO_2CH_3$) 3.75 (s, $-OC\underline{H}_3$), 2.02 (s, 3H).

M.S.: $m/_z$ 224 (M^+, base peak), ($C_{11}H_{12}O_5$ requires 224)

(vii) Spinuloplagin B (191), (8 mg.) was isolated and purified by prep. t.l.c. as an oil [see table for spectroscopic properties].

Methyl 3- methoxybenzoate (180)

A mixture of 3- hydroxybenzoic acid (20 g., 0.144 mole), anhydrous potassium carbonate (50 g., 0.5 mole) and dimethyl sulphate (40 ml.) in dry acetone (200 ml.) was refluxed for 5 hr. The acetone was removed and the residue diluted with

H_2O (250 ml) and ether (250 ml). The organic layer was washed with concentrated ammonia solution (2 x 40 ml), H_2O (50 ml) and then dried over anhydrous magnesium sulphate. The solvent was evaporated to yield a yellow oil which on distillation at $75^{o}/0.2$ mm gave methyl 3- methoxybenzoate (180) (21 g, 87%).

δ_H: 7.64 (dd, J2.7Hz., 2.8Hz. H-2), 7.41 (dd, J8.1, 8.9Hz. H-5) 7.16 (ddd, J2.4, 3.1, 8.4 Hz. H-4 or H-6), 7.12 (ddd, J2.5, 2.7, 8.4 Hz. H-4 or H-6), 3.88 (s, $-OC\underline{H}_3$), 3.80 (s, $-OC\underline{H}_3$).

3- Methoxybenzyl alcohol (181)[c]

A solution of methyl 3- methoxybenzoate (180) (21 g, 0.126 mole) in dry ether (50 ml) was added over a period of 20 min to a solution of lithium aluminium hydride (5g, 0.14 mole) in dry ether (10 ml). Gentle reflux was continued for 3 hr. The chilled mixture was then decomposed by careful addition of aqueous sodium sulphate. The aqueous phase was extracted thoroughly with ether and the combined organic layers were washed with 5% sodium bicarbonate and dried over sodium sulphate. Distillation afforded (181) (13 g, 75%) as a viscous oil. b.p. 93^{o} - $95^{o}/0.3$ mm.

δ_H: 7.26 - 6.62 (4H), 4.98 (br, exchangeable with D_2O), 4.45 (s, $-C\underline{H}_2OH$), 3.52 (s, $-OC\underline{H}_3$)

3- Methoxybenzyl Chloride (182):

A stirred solution of 3-methoxybenzyl alcohol (181) (13 g, 0.094 mole) and dimethylaniline (15 g, 0.124 mole) in anhydrous toluene (100 ml) was slowly treated with redistilled thionyl chloride (0.100 mole) in toluene (10 ml). The mixture was warmed to room temperature, and then heated under reflux for 1 hr; cooled and acidified with dilute hydrochloric acid. The organic layer was separated, washed, dried and evaporated to give a brown oil which on distillation afforded 3- methoxybenzyl chloride (182) (11.8 g, 80%) as a colourless oil b.p. 95°.

δ_H: 7.32 - 6.84 (4H), 4.46 (s, $-C\underline{H}_2Cl$), 3.72 (s, $-OC\underline{H}_3$)

3- Methoxybenzyl triphenylphosphonium chloride (183):

The benzyl chloride (182) (11 g, 0.07 mole) and triphenylphosphine (20 g, 0.076 mole) were stirred and heated in anhydrous toluene under N_2 at 60° for 46 hr. The precipitated hygroscopic phosphonium chloride (183) (16 g, 54%) was separated by filtration, washed with light petroleum ether, then dried in vacuo. It had m.p. 254° - 56°.

δ_H: 7.68 (brm, 15H), 7.28 - 6.74 (4H), 5.42 (d, J14Hz. $-CH_2$), 3.52 (s, $-OC\underline{H}_3$).

3- Hydroxy- 4, 3'- dimethoxystilbene:

3- Methoxybenzylphosphonium chloride (183) (1.7 g, 4.06 x 10^{-3} mole) and sodium hydride NaH (6 g, 0.25 mole) were stirred as a suspension in dry THF (30 ml) under nitrogen for 2 hours. A solution of 3- hydroxy- 4- methoxybenzaldehyde (isovanillin) (184) (0.9 g, 5.92 x 10^{-3} mole) was added in dry THF (8 ml) and the mixture was stirred for 18 hours at ambient temperature. The mixture was acidified with dil. HCl and extracted with ether (2 x 50 ml). The ether extract on evaporation gave a pale yellow oil (2 g, 84%) which showed three main spots [r.f. = 0.5, 0.3 and 0.2] on an analytical t.l.c. These spots were separated by flash column chromatography, eluting with light petroleum containing increasing proportions of ethyl acetate. 10 Fractions were collected. Fractions 2 - 6 were combined and crystallised from $CHCl_3$ - light petroleum to give a crystalline product m.p. 123° - 124°C (R.f. 0.5) corresponding to the stilbene mixture.

δ_H: 7.36 - 6.72 (9H), 5.66 (s, exchangeable with D_2O), 3.88 (s, $-OC\underline{H}_3$), 3.86 (s, $-OC\underline{H}_3$).

M.S.: $m/_z$ 256 (base peak, $C_{16}H_{16}O_3$ requires 256)

Fractions 7 - 9 contained unchanged isovanillin (R.f. 0.3) and Ph_3 P = O (R.F. 0.2).

2- Hydroxy- 3,7- dimethoxyphenanthrene (187)

The stilbene mixture (2 g, 0.023 mole) was dissolved in methanol (600 ml) containing iodine (1 g) and irradiated for 24 hr with a 450w u.v. lamp in an immersion well type quartz reactor under dry N_2. The methanol was evaporated and the brown residual oil dissolved in EtoAC (100 ml) and washed sodium thiosulphate (2 x 50 ml). The organic layer was then washed with brine, dried over magnesium sulphate and evaporated to give a brown solid (1.5 g) which showed 2 main spots on t.l.c. These spots were separated by flash column chromatography in petroleum: EtoAC followed by prep. t.l.c. The more polar spot proved to be the phenanthrene (187) (0.4 g, 20%) m.p. 125°C.

δ_H: 7.45 - 6.80 (aromatic protons, 7H), 5.42 (s, exchangeable with D_2O), 3.84 (s, $-OC\underline{H}_3$), 3.78 (s, $-OC\underline{H}_3$).

δ_C: 145.30 (s), 144.93 (s), 129.15 (s), 128.78 (s), 126.66 (d), 124.95 (s), 121.73 (s), 119.84 (d), 117.03 (s), 114.73 (d), 113.95 (d), 111.62 (d), 110.92 (d), 110.43 (d), 55.96 (q), 55.21 (q).

M.S.: m/z 254 ($C_{16}H_{14}O_3$ requires 254).

2-Hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene (188)

The crude phenanthrene (187) (0.4 g, 1.5 x 10^{-3} mole) and 10% palladised charcoal (200 mg) in acetic acid were stirred under hydrogen at room temperature for 3 days. The reaction mixture was filtered and the charcoal washed with dilute acetic acid. The solution was made alkaline with sodium bicarbonate, extracted with EtOAC, dried and evaporated to leave a yellow oil. Preparative t.l.c. gave 3 main spots.

Bands 1 and 3 were not investigated further.

Band 2: 2- hydroxy- 3,7- dimethoxy- 9,10- dihydrophenanthrene (188) (0.04 g, 10%).

δ_H: 7.52 - 7.12 (5 H), 4.30 (s, exchangeable with D_2O) 3.84 (s, $-OC\underline{H}_3$), 3.80 (s, $-OC\underline{H}_3$), 2.46 (4H, m).

M.S.: $m/_z$ 256 ($C_{16}H_{16}O_3$ requires 256).

CHAPTER 4

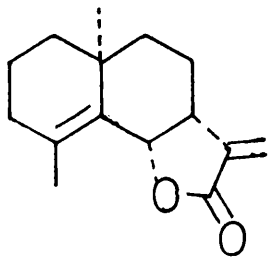
INTRODUCTION

The Frullaniaceae consists mainly of the larger genus *Frullania* and the smaller *Jubula*.

Frullania

Frullania species are very rich sources of sesquiterpene lactones and bibenzyls, and there are more than 1000 species known in the world. *Frullania* species are very interesting from the viewpoint of medicinal chemistry since they produce sesquiterpene lactones which cause intense allergenic contact dermatitis[24] and have antitumoral and plant growth regulatory activity . Of the many species known, eleven have been reported to be contact sensitising[93]. Ourisson and co-workers[94] isolated the active component (-)- frullanolide (32) from *Frullania tamarisci*. The enantiomeric compound (+)-frullanolide (195) was also isolated from *Frullania dilatata*[24]. Since then, a large number of other compounds have been reported from *Frullania* species. Connolly and Thornton[26] published the isolation of the compounds (34) - (36) together with (32) from the Scottish liverwort *F. tamarisci*.

Asakawa *et al*[52] have investigated twenty-five *Frullania* species and concluded that they can be divided into five chemotypes depending on the classes of metabolites found.



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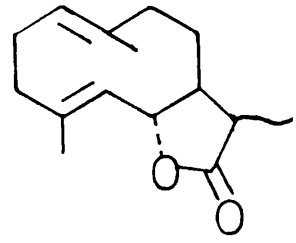

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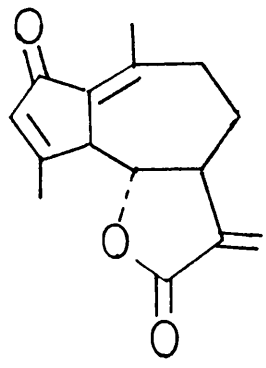

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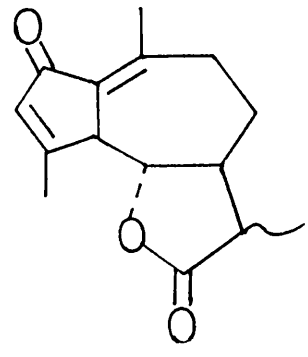

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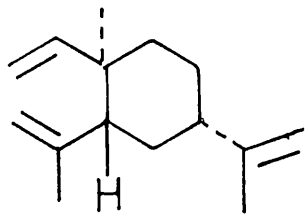

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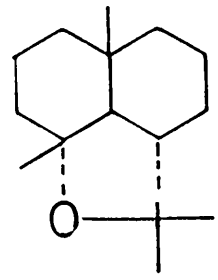


(200)

(i) Sesquiterpene Lactone- Bibenzyl type:

This type is typified by F. tamarisci and F. dilatata. Sesquiterpene lactones such as frullanolide (195), γ - cyclocostunolide (34) and costunolide (35) are the major components together with bibenzyls and other sesquiterpenoids as minor components.

(ii) Sesquiterpene Lactone type:

This category produces a large quantity of sesquiterpene lactones but no bibenzyls. These sesquiterpenoids are usually germacranolides e.g. (35), (196) and guaianolides e.g. (197) - (198) rather than eudesmanolides.

(iii) Bibenzyl type:

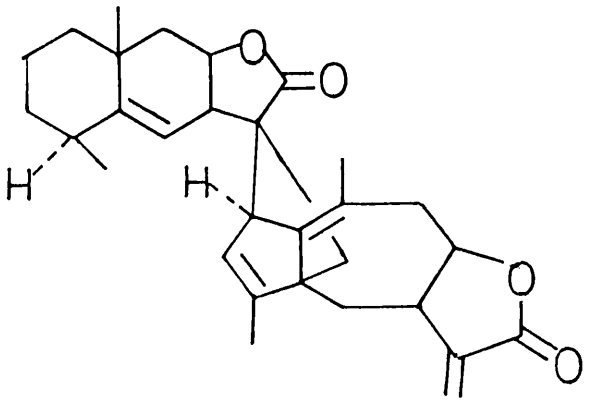
The Frullania species of this type contain bibenzyls e.g. (87) as major constituents and lack sesquiterpenoid lactones.

(iv) Monoterpene type:

Monoterpene hydrocarbons are present in thirteen of twenty-five Frullania species. Most of the species contain α- and β- pinenes (7) - (8) and camphene (9), but they do not contain any bibenzyls or sesquiterpenoid lactones.

(v) Cyclocolorenone type:

This category contains only one member, Frullania diversitexta. It produces mainly cyclocolorenone (13) and a few diterpenoid acetates.



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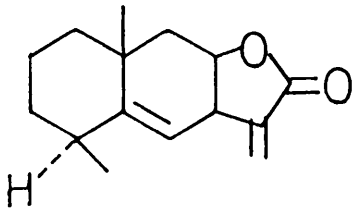

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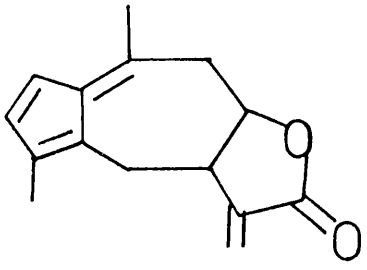

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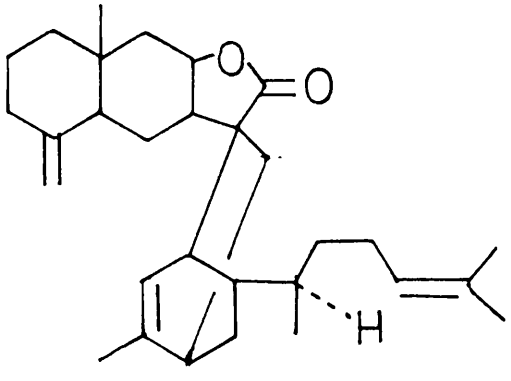

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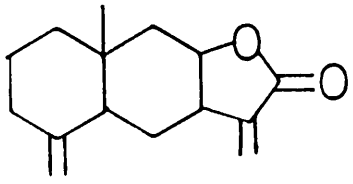

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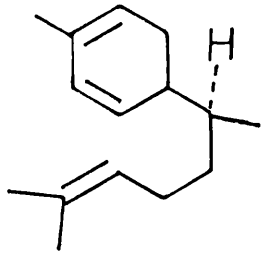


(206)

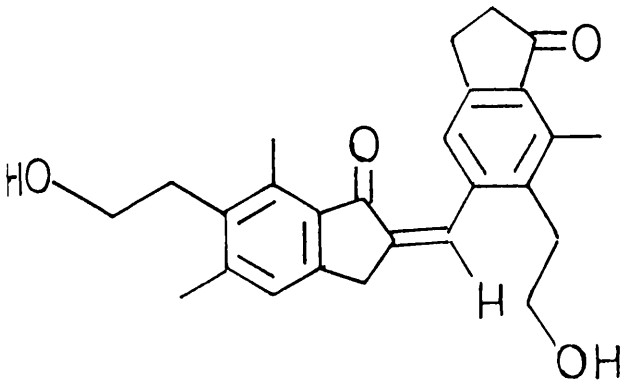
Jubula

Jubula japonica contains sesquiterpenoids, cyclocolorenone (13), β- elemene (199) and maalioxide (200) all of which have been detected by gcms[95].

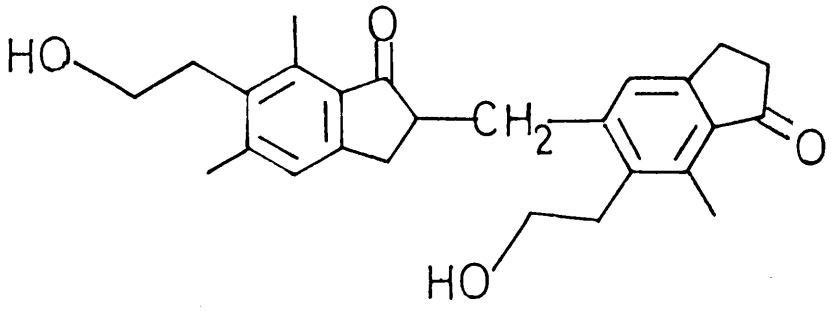
Dimeric sesquiterpenoids are not uncommon in the plant kingdom. The most common mode of dimerisation involves the Diels Alder reaction. The dimer (201) from the roots of Rudbeckia laciniata, recently published by Jakupovic[96], illustrates this point. It clearly arises by Diels Alder reaction of the precursors (202) and (203). The dimer (204) from Helenium antumnale[97] provides another example. It has been synthesised by reaction of isoalantolactone (205) with zingiberene (206).

Aldol condensation provides another possible mechanism of dimerisation[98]. The dinorsesquiterpenoid dimers monachosorins A (207), B (208) and C (209) can be formally derived by aldol condensation of 6- (2- hydroxyethyl)- 5,7- dimethylindan- 1- one (210) and the 5- formyl compound (211). They occur in the fronds of Monachosorum arakii along with mukagolactone (212).

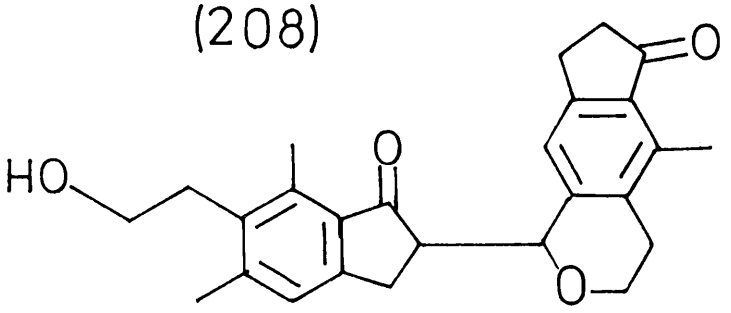
During our work on Frullania tamarisci we isolated a new dimeric sesquiterpenoid formed by a different kind of mechanism. Its structure forms the basis of this section.



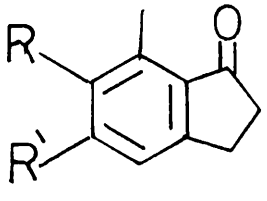
(207)



(208)

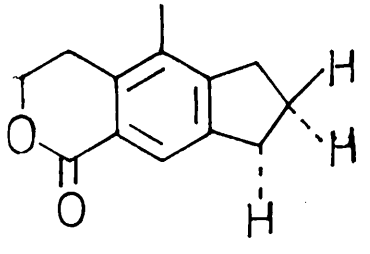


(209)

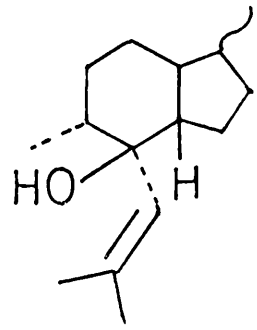


(210) $R = CH_2CH_2OH$, $R' = CH_3$

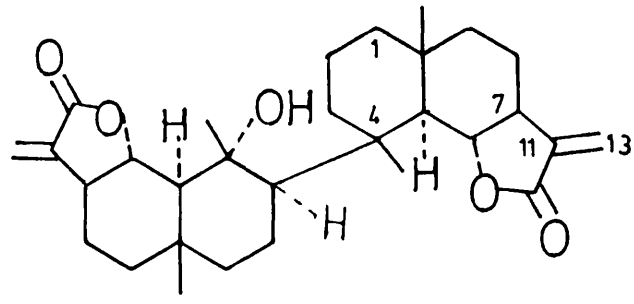
(211) $R = CH_2CH_2OH$, $R' = CHO$



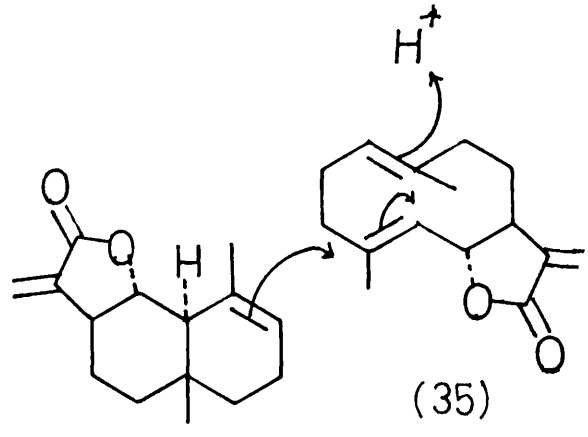
(212)



(213)



(214)



(35)

(36)

DISCUSSION

The Scottish liverwort Frullania tamarisci was previously investigated by Connolly and Thornton[26]. Further work by Connolly et al[99] yielded, additionally to the previous known compounds, the unusual sesquiterpenoid tamariscol (213) as the major constituent. In the present work, both (-)- frullanolide (32) and tamariscol (213) were obtained. The alcohol (213), a pungent oil, $C_{15}H_{16}O$ ($m/_z$ 222), [$\bar{\nu}_{max}$ 3610 cm^{-1} (free hydroxyl)] has in its ^1H n.m.r. spectrum signals for a trisubstituted double bond [δ_H 5.12 (m, J1.4Hz.], two vinyl methyls [1.92 (d, J1.2Hz.) and 1.79 (d, J1.6Hz.)] and two secondary methyls [δ_H 0.96 (d, J6.6Hz.) and 0.89 (d, J6.6Hz.)]. These together with four methine and four methylene groups constitute a bicarbocyclic system. The structure of (213) was eventually solved using 2 D INADEQUATE.

An interesting dimeric sesquiterpenoid lactone (214), $C_{30}H_{42}O_5$ ($\bar{\nu}_{max}$ 3580 and 1765 cm^{-1}) was also isolated. The ^1H n.m.r. spectrum of (214) is similar to that of the sesquiterpenoid lactone (36) especially with respect to the protons associated with the trans-fused exomethylene γ- lactone. Thus the exomethylene protons appear as doublets at δ_H 6.65 (J3.2Hz.), 6.48 (J3.2Hz.), 5.42 (J3.0Hz.) and 5.34 (J3.0Hz.) consistent with the presence of typical conjugated lactone systems. The lactone terminii, H-6 and H-'6, are overlapping triplets at δ_H 3.96 and 4.04 (each t, J11.2Hz.).

A triplet of quartets (J11.2, 3.2Hz.) can be assigned to the protons attached to C-7 and C-7'. Finally there are two proton doublets at δ_H 1.94 and 1.90 (J11.2Hz.) due to H-5 and H-5'. Double irradiation experiments readily confirmed the assignments made above. The other features of the ^1H n.m.r. spectrum include a hydroxyl proton [δ_H 3.2 (br, exchangeable with D_2O)] and four tertiary methyl groups at δ_H 1.26, 1.25, 1.06 and 1.05. These data, in conjunction with the molecular formula and the ^{13}C spectrum, clearly indicate that the dimer consists of two eudesmanolide systems with the same trans-fused lactone as in γ- cyclocostunolide (36) and with C-4 fully substituted in both monomeric units. The ^{13}C spectrum [see experimental] reveals signals for thirty carbons, confirming that the dimer is not symmetrical. There are five doublets, nine triplets and three singlets among the non-oxygenated carbons together with one tertiary oxygenated carbon . This tally suggests that the two units are joined together as in (214). The formation of (214) is perhaps best envisaged as involving costunolide and γ- cyclocostunolide, both known constituents of F. tamarisci. The electrophile resulting from cyclisation of costunolide may attack the double bond of α- cyclocostunolide instead of losing a proton. Subsequent attack by water then gives the dimer.

Determination of the relative stereochemistry of the dimer poses some problems. It is not possible to observe H-3' and to measure its couplings. It seems reasonable, however, to assume that the sesquiterpenoid substituent will be equatorial while H-3' is axial. NoE difference experiments failed to provide a definitive answer. Significant NoEs were observed between H-7, 7' and H-5, H5' and between H-6, 6' and the highfield methyl signals (3H-14, 14'). Irradiation of H-6, 6' also apparently gave NoEs at 3H-15, 15' although it is not possible to be certain that both methyls were affected. The reverse experiment is difficult to do without irradiating other protons. It seems likely, however, that the stereochemistry of dimer (214) is as shown.

This dimer is the first to be reported from the Hepaticae.

EXPERIMENTAL

Plant material (1 kg) was collected in the West of Scotland. After drying, it was finely ground and extracted in a Soxhlet apparatus first with ether and then methanol to give a combined crude extract (35 g). A large scale chromatography over alumina (grade H deactivated) in a solvent system of Et_2O:EtOAC (100% - 0% ether), followed by a sephadex column of (i) CH_2Cl_3:hexane (4:1) and (ii) $CHCl_3$:MeOH (1:1) and followed also by prep. t.l.c. afforded the following compounds.

(-)- Frullanolide (32) : It was recrystallised from $CHCl_3$-light petroleum as needles m.p. 73° - 74° [lit. 74° - 76°][26] (450 mg).

δ_H: 6.17 (d, J1Hz., H-13), 5.46 (d, J1Hz., H-13'), 5.12 (d, J5Hz., H-6), 1.88 (brm., H-7), 1.28 (s, 3H-15), 1.13 (s, 3H-14).

Tamariscol (213): It was obtained as a pungent oil (130 mg), $\bar{\nu}_{max}^{(CCl_4)}$ 3610 cm^{-1}.

δ_H: 5.12 (m, J1.4Hz., H-10), 1.92 (d, J1.2Hz., 3H-12), 1.79 (d, J1.6Hz., 3H-13), 0.96 (d, J6.6Hz., secondary methyl), 0.89 (d, J6.6Hz., secondary methyl).

Dimeric lactone (214): This was the most polar. It was re-crystallised from MeOH and had m.p. 207° - 208° (25 mg).

$\nu_{max}^{(CCl_4)}$ 3580 cm^{-1}, 1765 cm^{-1} (γ-lactone).

δ_H: 6.65 (d, J3.2Hz., H-13, 13'), 6.48 (d, J3.2Hz., H-13', 13'), 5.42 (d, J3.2Hz., 2H, exomethylene protons) 5.34 (d, J2.9Hz., 2H, exomethylene protons), 4.04 (t, J11.19Hz., H-6), 3.96 (t, J11.24Hz., H-6'), 2.59(2H, tq, J11.0, 3.3Hz.), 3.2 (br, exchangeable with D_2O), 1.94 (d, J11.3Hz., H-5), 1.90 (d, J10.71Hz., H-5), 1.27 (s, $-C\underline{H}_3$), 1.26 (s, $-C\underline{H}_3$), 106 (s, $-C\underline{H}_3$) 1.05 (s, $-C\underline{H}_3$).

δ_C: 170.1 (s), 169.6 (s), 139.4 (s), 138.5 (s), 117.8 (t), 116.9 (t), 82.1 (d), 81.9 (d), 71.6 (s), 59.7 (d), 55.7 (d), 53.1 (d), 51.1 (d), 49.3 (d), 45.1 (s,t), 45.0 (t), 42.3 (s), 41.8 (t), 40.8 (t), 40.5 (t), 38.0 (s), 34.7 (t), 24.4 (q), 23.8 (q), 22.8 (t), 22.5 (t), 22.2 (t), 21.4 (q), 19.0 (q), 18.1 (t)

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