A THESIS ENTITLED

"STUDIES IN NATURAL PRODUCTS"

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" STUDIES IN NATURAL PRODUCTS "

SUMMARY

This thesis consists of four chapters. The first chapter gives a general introduction dealing with the nature of secondary metabolites and the skeletal types found in the *Hepaticae*. This is followed by an examination of the chemical constituents found in *Marsupella* species. Three amorphane (muurolane) type sesquiterpenoids were isolated from *Marsupella aquatica* and their structures shown to be [1R*, 6S*, 10S*] 2R*,3S*-diacetoxyamorpha-4,7(11)-dien-8-one, [1R*, 6S*, 10S*] 2R*,3S*-diacetoxy-7ξ 11ξ-epoxyamorph-4-en-8-one, and [1R*, 6S*, 10S*] 2S*-acetoxyamorpha-4,7(11)-dien-8-one.

The third chapter consists of an investigation of the liverwort *Nardia scalaris*. Two compounds, *ent*-(14S)-kaur-16-en-14-yl hydrogen malonate and the longipinane sesquiterpenoid, marsupellone, were isolated.

The last chapter deals with a discussion of the Annonaceous acetogenins and some other chemical constituents found in *Annonaceous* species. A new acetogenin derivative which contains a γ-lactone ring and an ene-diyne chromophore was isolated for the first time from *Milliusa velutina*.

CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Since last century chemists have been interested in the investigation of extracts obtained from natural sources.This interest has increased over the years with the development of separation and purification methods, such as column chromatography, g.c., t.l.c., h.p.l.c., paper chromatography, ion exchange, etc. Following the separation and purification of compounds, different spectroscopic techniques are used for structure elucidation, such as NMR (including two dimensional NMR spectroscopy ; COSY , HMBC , HSQC) , I.R. , UV and mass spectroscopy. The structural elucidation of complex molecules, often available in small amount, is greatly facilitated by these modern spectroscopic techniques. X-ray analysis and molecular force-field calculations are also readily available.

Traditionally, natural products are considered in two categories : primary and secondary metabolites. However the border between the two classes is somewhat vague in places. The former are the organic compounds which are characteristic of all living systems, such as carbohydrates, lipids, amino acids, peptides, proteins, nucleic acids, nucleosides and nucleotides whereas the secondary metabolites include phenols, quinones, terpenes, alkaloids, aromatics, etc. There is a considerable interest in plant secondary metabolites and many plants have been and are being investigated by chemists in the search for new structural types with useful biological activity.

HEPATICAE

On the evolutionary scale, bryophytes are placed in the lower plants and are taxonomically between the algae and the pteridophytes (ferns). Approximately 20,000 species of them are known[1]. They are divided into three classes : Bryopsida or mosses (bryales) , Hepaticae or liverworts and Anthocerotae or hornworts. While the hornworts comprise some 300 species the liverworts comprise 6,000 species worldwide. The mosses form the largest bryophyte class with 14,000 species. The liverworts are chemically the most interesting plants because, unlike mosses and hornworts, they have oil bodies in their cells, which contain large quantities of secondary metabolites .

The liverworts and mosses form two distinct classes within the Bryophyta. They

differ from each other in rhizoid structure, in the manner of development of sex organs and in the prevailing mode of growth and cell structure of their leaves .

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

1. Jungermanniidae
    - Calobryales
    - Jungermanniales
    - Metzgeriales
2. Marchantiidae
    - Monocleales
    - Marchantiales
    - Sphaerocarpales

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

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

As we mentioned above, the characteristic feature of the Hepaticae is their ability to elaborate large quantities of terpenoids and other metabolites which are stored in special cell organelles, called the oil bodies. Under the light microscope, the oil bodies appear as colourless, rarely brownish or bluish, organelles in the cytoplasm, varying from 2 to 20 μm in length. In dried specimens they disintegrate. Several oil body types can be recognised, the most important ones are the homogenous oil body and the segmented oil body. The difference between them is that while homogenous oil bodies are made up of

one lipid droplet surrounded by a membrane, the segmented oil bodies have several to numerous droplets bounded within the membrane as can be seen in figure 1.1

The distribution of the oil bodies varies. For example, within the subclass Jungermannidae, oil bodies occur in green, photosynthetic cells whereas in the subclass Marchantiidae, they are restricted to special 'oil cells' lacking chlorophyll.

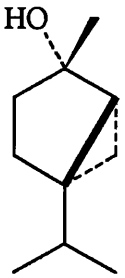
Some characteristic features found in the secondary metabolites also led to a taxonomic classification. For instance, plant species which produce similar chemical constituents might have similar biosynthetic pathways and thus similar genes which encode enzymes. On the other hand, secondary metabolites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

Many liverworts prefer wet, humus-rich habitats, such as damp rocks, the forest floor, swamps or marshes, or beside streams and pools and it is often troublesome to collect them in sufficient amounts for chemical research, due to their small size and their tendency to intermingle with other plants. Muller was the first person to investigate liverworts and to report the presence of sesquiterpenoids in the oil bodies in 1905[6]. Since 1956 , chemists have shown great interest in the chemical constituents of liverworts[1].

MONOTERPENOIDS

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

(-)-Thujanol **(1)** and its epimer have been isolated by Connolly *et al*[18] from the liverwort *Conocephalum conicum* together with α-terpineol **(2)** and terpineol **(3).** *Bazzania pompeana* and *Porella perrottetiana* contain camphor **(4)** while (+)-bornyl acetate **(5)** and (-)-β-sabinene **(6)** occur in *Wiesnerella denudata*[1]. *Frullania,*



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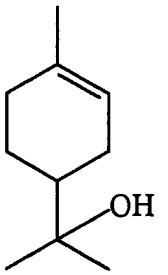

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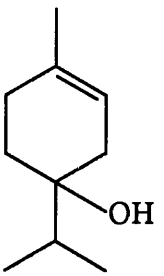

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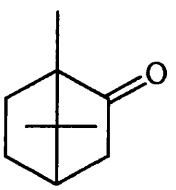

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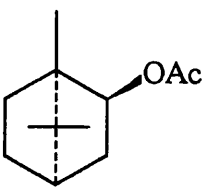

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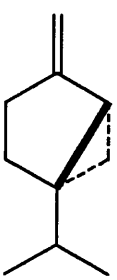

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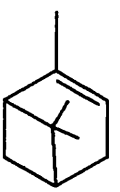

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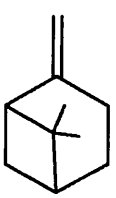

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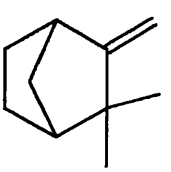

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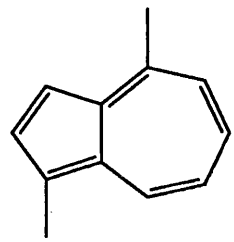
Jungermannia, Plagiochila and *Porella* species belonging to the Jungermanniales emit a turpentine-like odor and they contain α-pinene **(7)**, β-pinene **(8)** and camphene **(9)**.

SESQUITERPENOIDS

Sesquiterpenoids are defined as a group of C_{15} compounds and they are found in many forms of living systems of which higher plants are the principal member[8]. Although the study of sesquiterpenoids was begun in the early years of the nineteenth century, it was only after 1920 that serious progress was made. For many years it was known that certain essential oils developed a blue colour on distillation. Many sesquiterpenoids gave similar blue substances when dehydrogenated and the blue colour was attributed to the presence of a small group of hydrocarbons. The name "azulene" was given to the oils by Piesse in 1804[9]. Those azulenes which were first obtained from natural sources were given trival names. However as their identity with other hydrocarbons was established the superfluous names were discarded.

Some *Calypogeia* species contain characteristic blue oil bodies[10]. Two azulene derivatives, 1,4-dimethylazulene **(10)** and 4-methyl-1-methoxycarbonylazulene **(11)**, have been isolated from *Calypogeia trichomanis*. Further investigation of the essential oil of the same liverwort gave 3,7-dimethyl-5-methoxycarbonylindene **(12)**. While azulenes are often obtained as artefacts during isolation procedures, the azulenes in *Calypogeia* species occur naturally since the oil bodies in intact gametophytes show a blue colour and the solvents immediately become blue when the cultured cells are immersed in suitable solvents[11]. Guaiazulene **(13)** was isolated from *Pellia* species. It was suggested that guaiazulene might be an artefact since azulenes were not detected in the ether extract of the same species, even by GC-MS analysis[1].

The skeleton of the gymnomitranes (barbatanes) was first reported by Connolly *et al*[12] from the liverwort *Gymnomitrion obtusum*, a rich source of gymnomitranes, e.g. gymnomitrol **(14)**. This type of skeleton is not found outwith the Hepaticae. Although the corresponding hydrocarbons α- and β-gymnomitrenes are widespread , oxygenated derivatives are much less common. Recently three derivatives of gymnomitrol have been reported from *Plagiochila trabeculata* by Toyota et al[13]. These are 9α-



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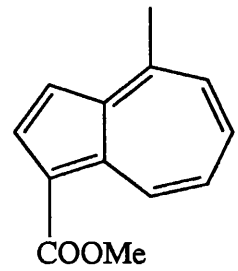

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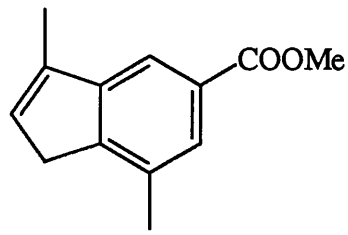

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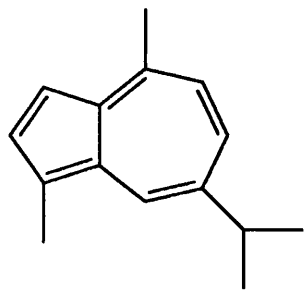

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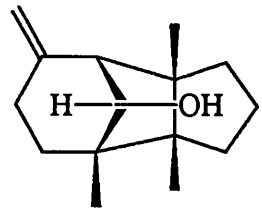

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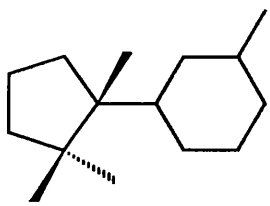

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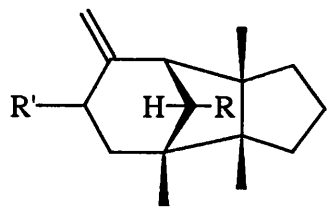
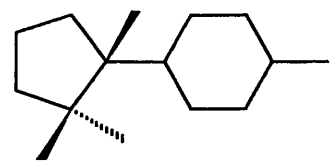




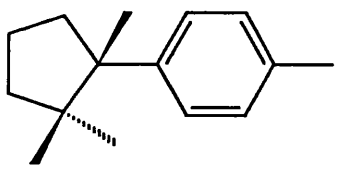
(20)

(15) R= OAc; R'= H, OH

(16) R= O cinn; R'= H, OH

(17) R= OAc; R'= O

(18) R= H; R'= H, OH



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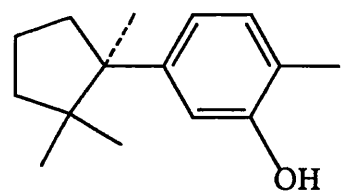

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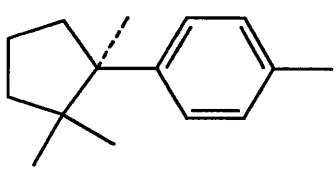

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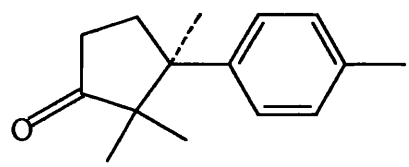


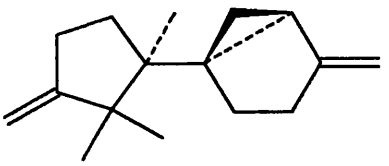
(24)

hydroxygymnomitryl acetate **(15)**, the corresponding cinnamate **(16)** and 9-oxogymnomitryl acetate **(17)**. From *Reboulia hemisphaerica*, a similar 9α-hydroxy derivative **(18)**, lacking the bridge oxygen substituent, was reported by Becker et al[14].

The herbertanes **(19)** have a carbon skeleton which is similar to the cuparanes **(20)**. The difference lies in the position of the methyl on the six-membered ring. These two skeletal types are examples of the rare occurrence of aromatic sesquiterpenoids in nature. (R)-(-)-cuparene **(21)** was the first compound of this skeletal type to be reported and it was isolated from the liverwort *Bazzania pompeana* in 1971[15]. In 1972 Matsuo et al[16] isolated δ-cuparenol **(22)** and cuparene **(23)** from the same liverwort. These two compounds have also been reported from *Marchantia polymorpha*[17]. R(-)-α-cuparenone **(24)**[18] and the cyclocuparane structure, grimaldone **(25)**, are the odoriferous compounds from *Mannia fragrans*[19]. The herbertanes form a small group of sesquiterpenoids found largely in liverworts[20]. They have also been found in the fungi[21]. The first compound in this series (-)-herbertene **(26)** was isolated in 1981 from the leafy liverwort *Herbertus aduncus*[22]. Other compounds of this type molecule are (-)-α-herbertenol **(27)**, (-)-β-herbertenol **(28)**, (-)-α-formylherbertenol **(29)**, (-)-herbertenediol **(30)** and (-)-herbertenolide **(31)** from *H. aduncus*[20,23,24] and *H. subdentatus*[20].

Pinguisanes are widely distributed in liverworts and occur in the *Lejeuneaceae, Porellaceae, Ptilidiaceae, Lepidolaenaceae* of the Jungermanniales and *Aneuraceae* of the Metzgeriales[25]. The first pinguisane sesquiterpenoid was the ketone pinguisone **(32)** which was isolated from the liverwort *Aneura pinguis* in 1969 by Benesova *et al*[26]. The pinguisane skeleton is unique and difficult to rationalise simply in terms of the Isoprene Rule. Investigations of *Frullanoides densifolia* and *Trocholejeunea sandvicensis*[27] have provided several new pinguisane derivatives. *F. densifolia* produced two new rearranged pinguisane sesquiterpenoids, spirodensifolin A **(33)** and spirodensifolin B **(34)**, together with a new pinguisane-type alcohol, isonaviculol **(35)**. Pinguisanes occur with different oxygenation patterns. For example, dehydropinguisanin **(36)**, dehydropinguisenol **(37)** and pinguisenal **(38)** were isolated from *Tricholejeunea sandvicensis*[28].

The spirovetivanes are found in vetiver oil[29] and also occur as phytoalexins in



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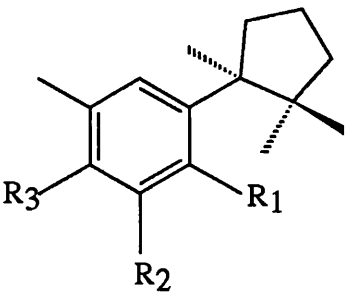
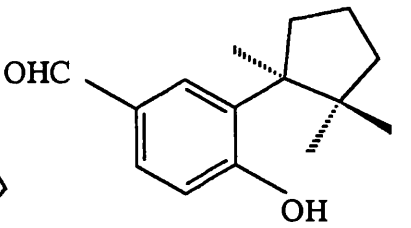
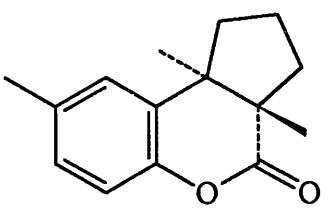




(29)

(26) R_1=R_2=R_3=H
(27) R_1=OH, R_2=R_3=H
(28) R_1=R_2=H, R_3=OH
(30) R_1=R_2=OH, R_3=H



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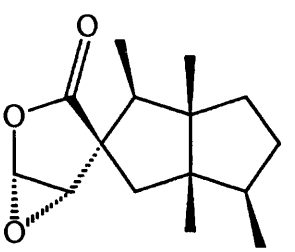

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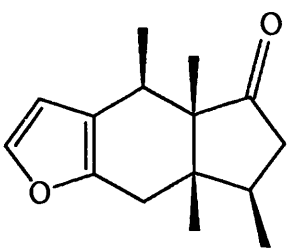

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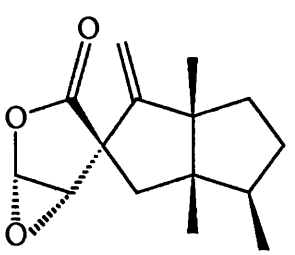

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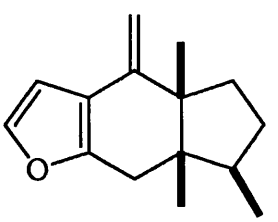

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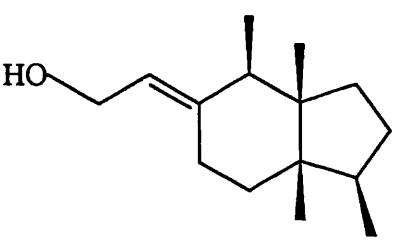

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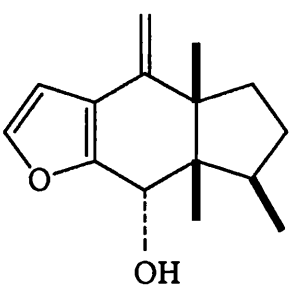

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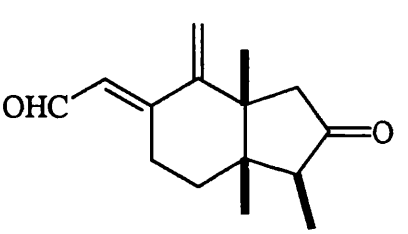


(38)

infected potatoes[30]. α-Spirovetivene **(39)** and its β-isomer have been found in *Scapania maxima* and *Scapania robusta*[31].

Bicyclogermacranes are found in higher plants[32,33]. (-)-Bicyclogermacra-1(10),4-diene **(40)** is widespread in liverworts and some other representatives of this type have been reported. Those are *ent*-3β-acetoxybicyclogermacra-1(10), 4-diene **(41)** which occurs in the extracts of *Plagiochila yokogurensis*, *Pedinophyllum truncatum* and *Scapania ampliata*[1] and bicyclogermacra-1(10),4-dien-13-al **(42)** isolated from *Conocephalum conicum*[34].

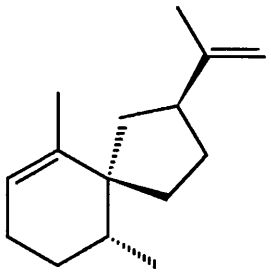
Cadinanes are found in some species of Jungermanniales , Marchantiales and Metzgeriales[1] . *Conocephalum conicum* contains δ-cadinene **(43)**[35] . Andersen *et al*[36] have reported the presence of (-)-γ-cadinene **(44)** in *Scapania undulata*. Three more new cadinane derivatives , (+)-4-muurolen-6α-ol **(45)** , scapanol **(46)** and ent-T-muurolol **(47)** were isolated from Belgian *Scapania undulata*[37].

DITERPENOIDS

Only certain genera of liverworts produce diterpenoids. Labdane, pimarane and kaurane diterpenoids thus far isolated from the Hepaticae belong to the *ent*-series while higher plants produce both normal and ent-labdanes, pimaranes and kauranes. The first diterpenoid isolated from liverworts was *ent*-16β-hydroxykaurane **(48)** from *Anthelia julacea* and *A. juratzkana* by Huneck and Velve in 1970[38].

The **sacculatane**-type diterpenoids have a widespread occurrence in liverworts and have been found in *Trichocoleopsis, Pellia, Porella* and *Makinoa* species[1]. Sacculatal **(49)** and isosacculatal **(50)** were isolated from the liverwort *Trichocoleopsis sacculata* by Asakawa and Takemoto[39], the first report of this skeletal type. Sacculaplagin **(51)**[40] and sacculaporellin **(52)**[41] , two hemiacetals, were isolated from the liverworts *Plagiochila acanthophylla* and *Porella perrottetiana.*

The **kaurene** skeleton is commonly found in Nature and *ent*-kauranes occur in higher plants. Liverworts produce only the *ent*-form[1]. *Ent*-18-hydroxykauren-15-one **(53)** and *ent*-(16R)-18-hydroxykauran-15-one **(54)** were isolated from *Porella densifolia*



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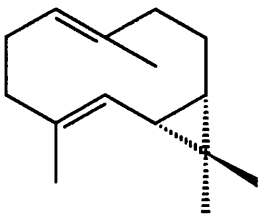

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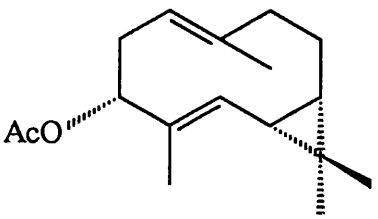

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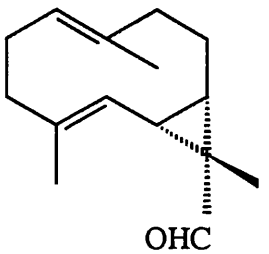

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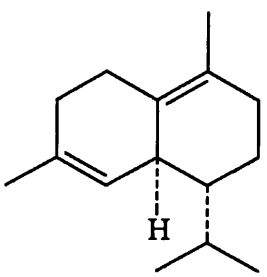

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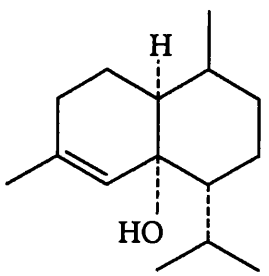

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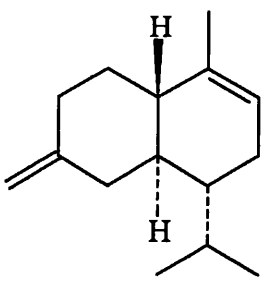

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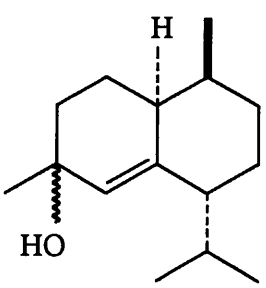

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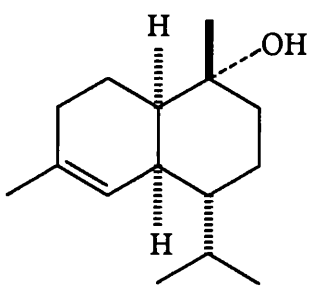

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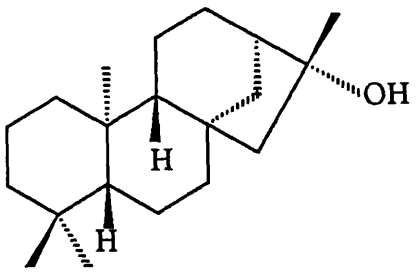

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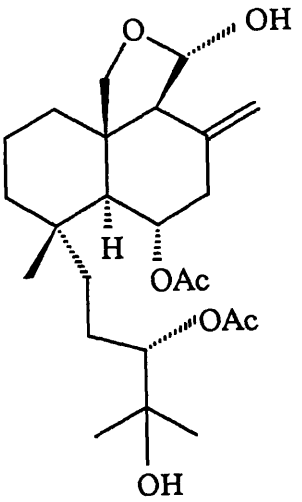

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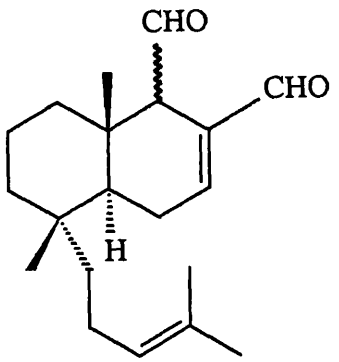
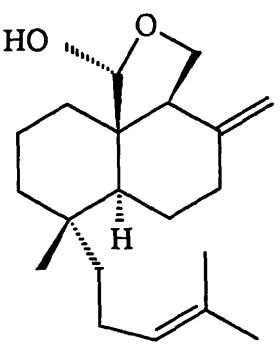


(49) 9-β–CHO
(50) 9-α–CHO



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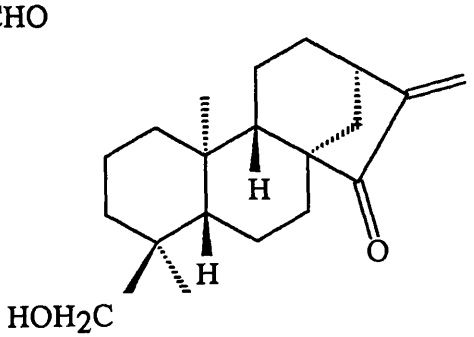
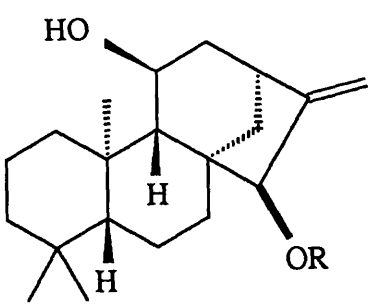
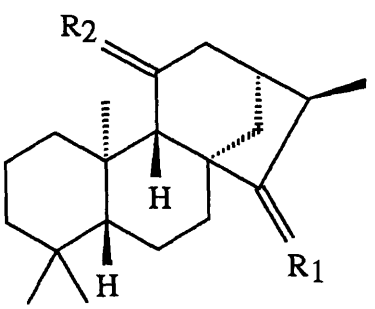


(53)
(54) 16,17-dihydro



(55) R=Ac
(56) R=H



(57) R_1=O R_2=H, β-OH
(58) R_1=O R_2=H, β-OH
16,17 didehydro

by Matsuo et al[42]. Four *ent*-kaurene derivatives **(55-58)** were found in *Solenostoma triste* by Connolly et al[43].

Labdanes are the most common terpenoids found in liverworts. The first representative of this skeletal type, *ent*-manool **(59)**, was isolated from *Jungermannia torticalyx* by Matsuo et al[44]. The liverwort *Scapania undulata* afforded scapanin A **(60)** and B **(61)**[45].

Pimaranes occur quite rarely in liverworts. (-)-Thermarol **(62)**, *ent*-pimara-8(14),15-dien-19-oic acid **(63)** and *ent*-pimara-8(14),15-dien-19-ol **(64)** from the liverwort *Jungermannia thermarum*, are the first representatives of this species[46]. However, subsequently the 'normal' isopimarane, (-)-sandaracopimaric acid **(65)** was found in the liverwort *Mastigophora diclados*[47].

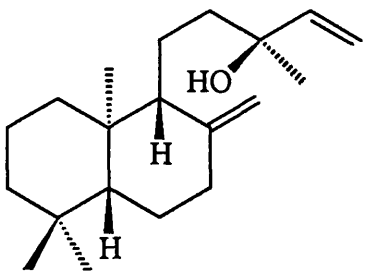
The **sphenolobane** skeleton was first reported in the marine organism sponge, *Pseudolarix kaempferi*[48]. The only occurrence of the sphenolobane skeleton found in liverworts is in the liverwort *Anastrophyllum minutum (Sphenolobus minutus)*[49], e.g. **(66).**

TRITERPENOIDS

Triterpenoids are found rarely in liverworts. While cycloartenol **(67)** has been found in both *Mylia taylorii*[50] and *Lophozia ventricosa*[51], cycloart-23-ene-3β, 25-diol **(68)** has been found in *Plagiochila kahsiana (P. peculiaris)*[52]. Another triterpenoid, α-zeorin **(69)**, has been isolated from *Reboulia hemisphaerica*[14] and *Plagiochasma rupestre*[53].

AROMATICS

The main types of aromatic compounds found in liverworts are bibenzyls and bisbibenzyls. The bibenzyls are the most characteristic and many of them are biologically active. Lunularic acid **(70)**, a growth inhibitor, isolated from the liverwort *Lunularia cruciata* in 1969[54], is the first representative of the bibenzyl type. It occurs widely and has been reported in 76 species of the Hepaticae[1,55]. Many variations of the basic bibenzyl unit[1,56] have been published e.g. the interesting cyclohexenone, 4 (-p-methoxyphenethyl)-cyclohex-2-en-1-one **(71)** from *Plagiochila longispina*[57] and a new bibenzyl cannabinoid derivative **(72)** from *Radula perrottetii*[58]. Another variation is the cyclopropanochroman



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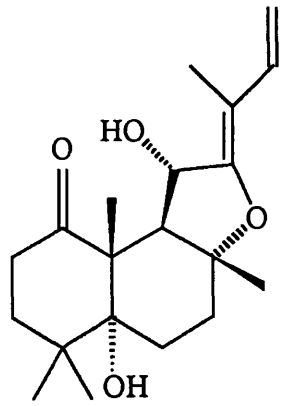

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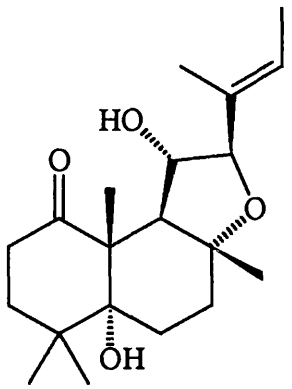

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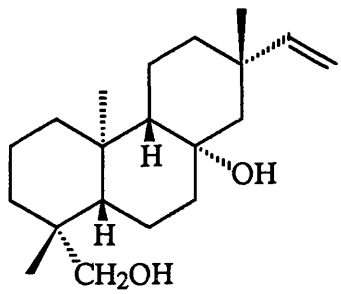

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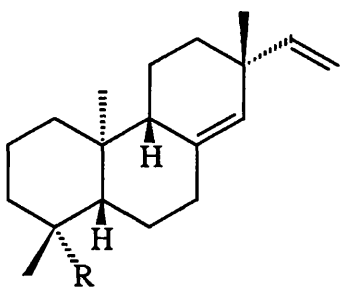
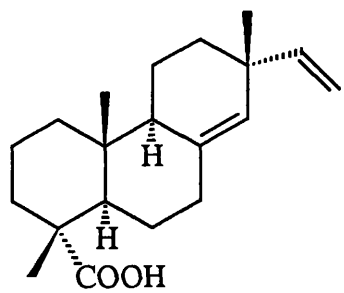


(63) R= CO_2H
(64) R= CH_2OH



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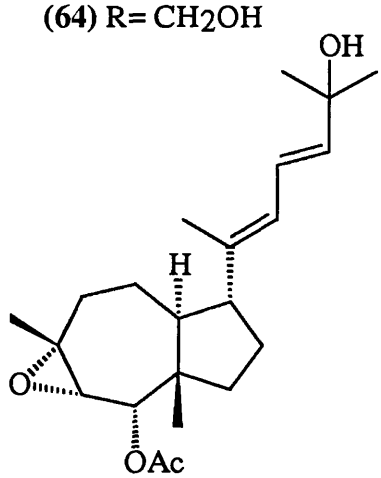

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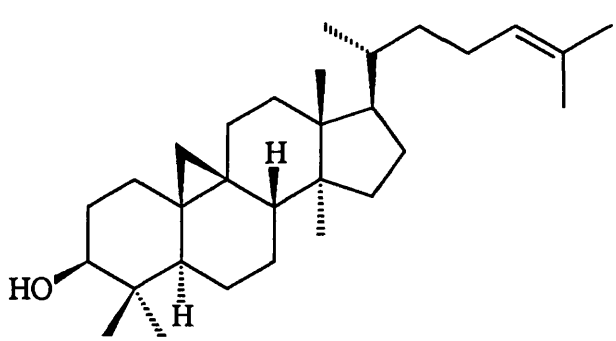

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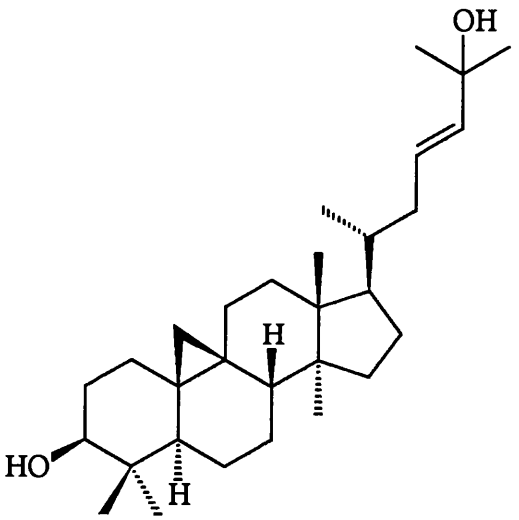

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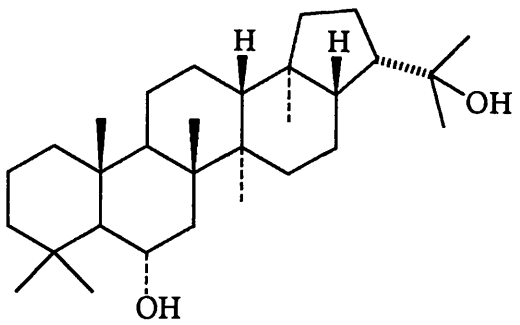

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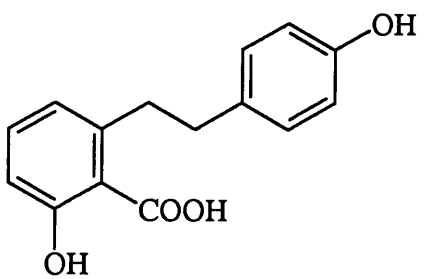

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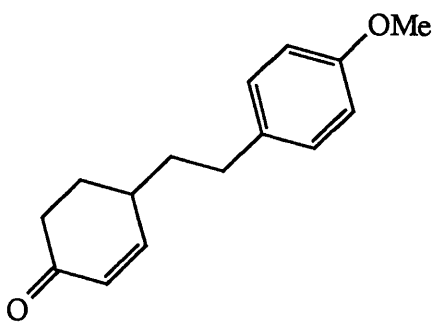

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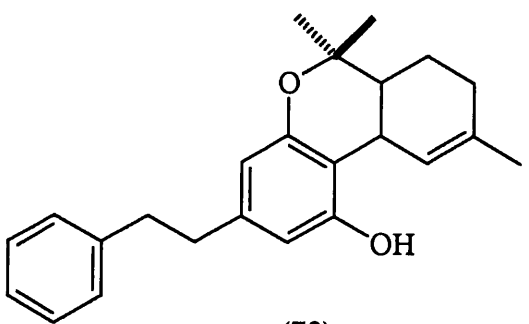

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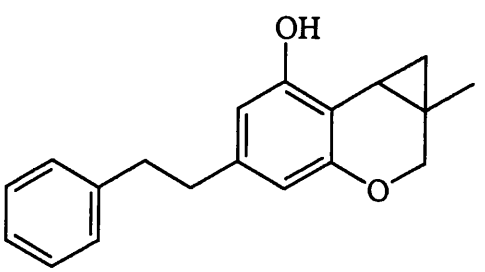

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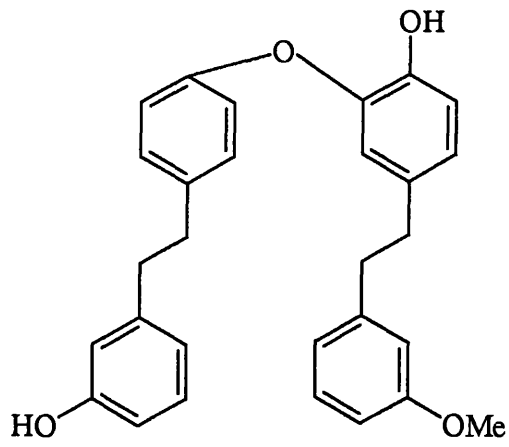

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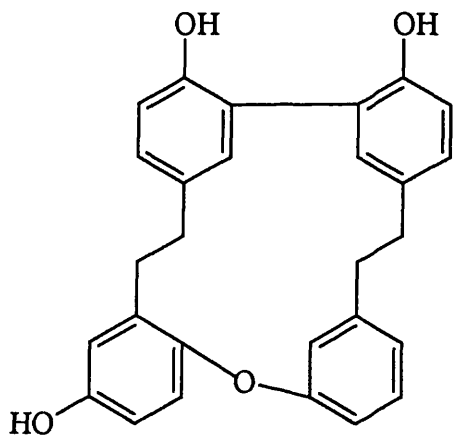

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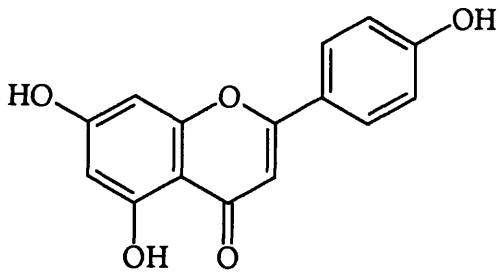

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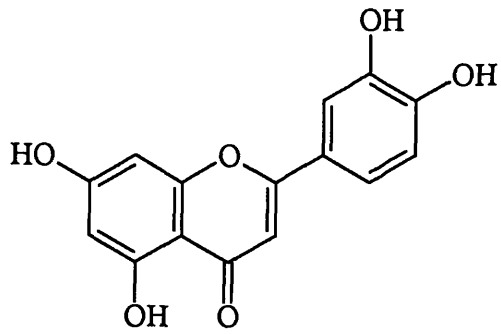

(77)

type e.g. radulanins I (**73**) from the liverwort *Radula javanica*[59].

Bisbibenzyls are two bibenzyl units joined by ether and biphenyl linkages. These compounds are unique to the liverworts and are exemplified by perrottetin E-11`-methyl ether (**74**) from *Pellia endiviifolia*[60], and isoplagin A (**75**) from *Plagiochila fruticosa.*

Flavonoids are widely distributed in the Hepaticae, particularly in the Marchantiales[61]. Most of the flavonoids found in the Hepaticae are flavonoid glycosides, except for a few flavonoid aglycones found in *Corsinia*[62] and *Frullania* species[63, 64, 65]. Apigenin (**76**) and luteolin (**77**) glycosides are the most common flavonoids.

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CHAPTER TWO

Marsupella aquatica

INTRODUCTION

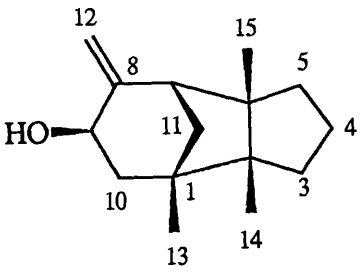
The liverwort *Marsupella aquatica* is very common on rocks in hill streams. It differs from the species *M. emarginata* in a number of small points. It grows taller (3-8 cm), is dull blackish green rather than red brown in colour, and has the leaves more channelled in form and less deeply bifid[1].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

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

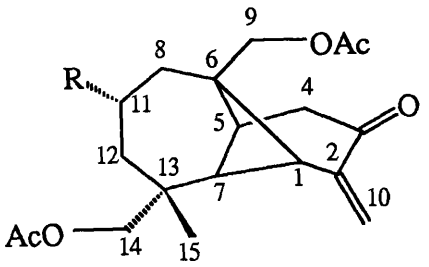
RESULTS AND DISCUSSION

Marsupella aquatica was collected in a burn near Inverary. It was dried, powdered and eluted with diethyl ether. The crude extract was subjected to flash chromatography followed by preparative TLC. Three new amorphane/muurolane type sesquiterpenoids **(12)-(14)** were obtained and their structures assigned on the basis of their spectroscopic properties.

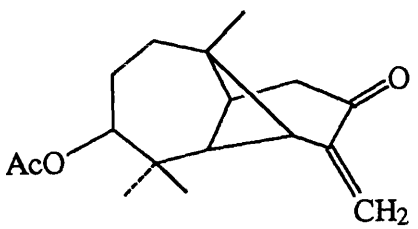
The major constituent **(12)**, present in fractions eluted with 40% ether in light petroleum, was obtained as an oil, following purification by preparative TLC (eluent 35% ether in petrol). The molecular formula $C_{19}H_{26}O_5$ was readily apparent from the ^{13}C NMR spectrum (Table-1) which revealed the presence of six methyl groups (including two acetates), one methylene group, six methine carbons (including two secondary oxygen-bearing carbons and a vinyl carbon), a trisubstituted and a tetrasubstituted double bond, two acetate carbonyls and a ketonic carbonyl. The molecule is thus bicarbocyclic. The IR spectrum confirmed the acetates [1746 cm^{-1}], and suggested the presence of an unsaturated ketone [1686, 1615 cm^{-1}]. This suggestion was supported by a UV absorption band at 248 nm [ε 7000]. The ^1H NMR spectrum (Table-1.) showed *inter alia*



(1)



(2) R = H, α–OAc
(3) R = H, β–OAc
(4) R = H, H



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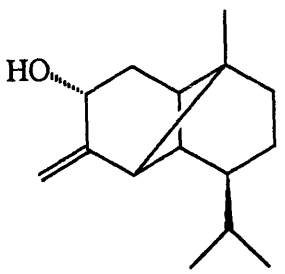

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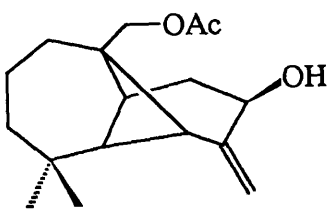

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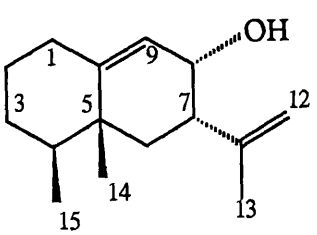
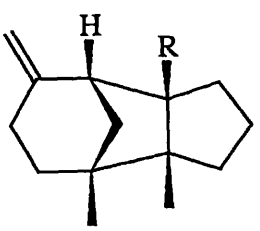
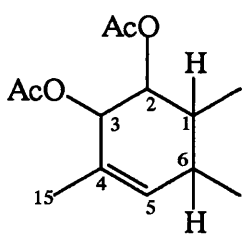


(8)

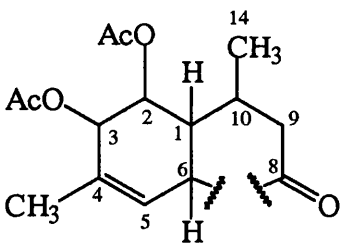


(9) R = CH_2OH
(10) R = CO_2H
(11) R = CHO

a secondary methyl group, three deshielded methines, two of which overlapped, arising from the two secondary acetates and the vinyl proton, and four sharp methyl signals between 2.1 and 1.8 ppm. Two of these methyl signals must belong to the acetates while the other two must arise from vinyl methyls with little or no coupling. The assignment of the proton resonances was aided by double resonance experiments and by NOE difference experiments. The problem of the overlap of signals was resolved by changing the solvent from deuteriochloroform to deuteriobenzene. This resulted in a clear separation of the three deshielded methine resonances which were readily identified by decoupling experiments. Irradiation of H-2 [δ_H 5.30, t, J 3.8 Hz] resulted in the collapse of H-3 [δ_H 5.66, brd, J 3.6 Hz] to a broad singlet. The other coupling is to H-1 which lies underneath the acetate signals as can be seen in the 2D direct C/H correlation spectrum. H-3 is allylic and couples to the vinyl methyl, 3H-15, the vinyl proton H-5 and the other allylic proton H-6. All the couplings are small and therefore it is not immediately apparent which double bond carbon bears the methyl group 3H-15. A strong NOE from H-3 to 3H-15 readily resolved this problem. Irradiation of the hidden resonance for H-1 resulted in loss of the couplings from H-2 and H-6, thus completing the part structure **(A)**. A small coupling with H-3 was also apparent, indicating their W geometry (see below).



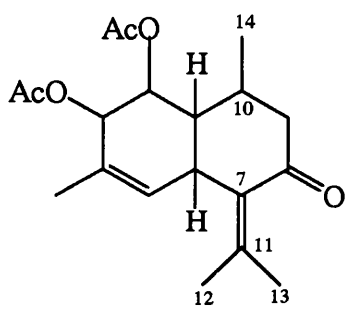
(A)



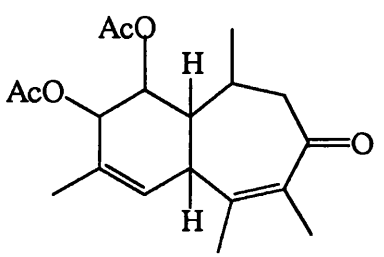
(B)

A further coupling of H-1 to the methine proton H-10 [δ_H 2.58, obscured m] which is partly obscured by one of the methylene protons (2H-9), enables part structure **(A)** to be extended. Irradiation of H-10 and the methylene proton results in collapse of the other methylene signal and the secondary methyl resonance. The methylene protons have no further couplings and the large J_{gem} (15.6 Hz) suggests a situation α to a carbonyl

group. Thus we arrive at part structure **(B)**. A tetrasubstituted double bond bearing two methyl groups remains to be placed to complete the structure. There are two possibilities **(C)** and **(D)**. The former **(C)** is preferred since in the long range 2D C/H spectrum the methyls are mutually correlated as well as showing correlations to C-7 and C-11. Correlation between the methyls in structure **(D)** would represent unusual $^4J_{CH}$ couplings. Other long range correlations which support structure **(C)** are 3H-15 to C-3, C-4 and C-5, 3H-14 to C-9, C-10 and C-1, and H-5 to C-3 and C-15. Surprisingly, no correlations were observed from H-1, H-2, H-3, H-6 and H-10. Structure **(C)** has a cadinane type skeleton with acetates attached to C-2 and C-3. Conjugation of the isopropylidene group at C-7 with the ketone carbonyl causes considerable deshielding of the two methyl singlets 3H-12 and 3H-13 [δ_H 2.03, 1.82 respectively in $CDCl_3$].



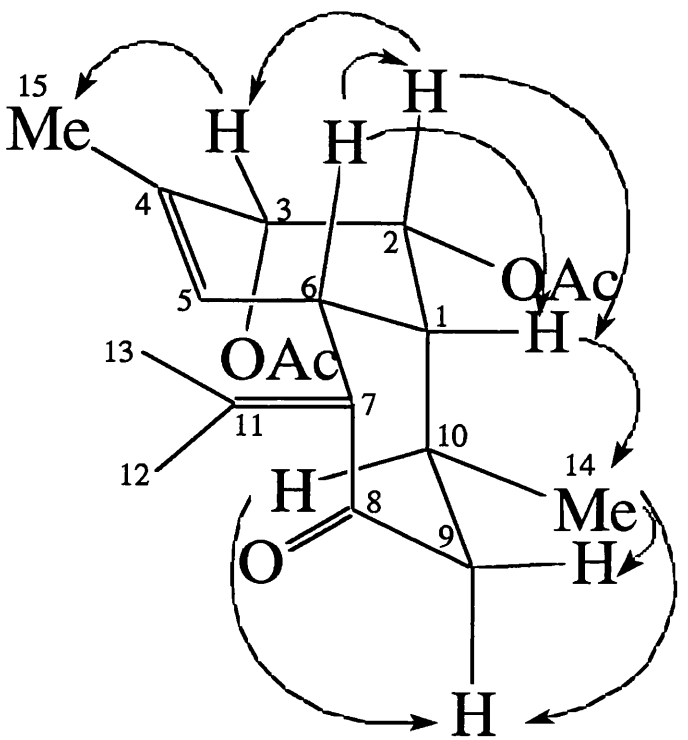
(C)



(D)

The relative stereochemistry of **(12)** was deduced from NOE difference experiments (Table-2). These can be summarised as follows : irradiation of H-3 afforded NOEs at H-2 and the vinyl methyl group 3H-15 ; irradiation of H-6 gave NOEs at H-5, H-1, H-2 and the methyl 3H-13 (confirmed in the deuteriobenzene experiment). Thus the protons attached to C-1, C-2, C-3 and C-6 must all lie on the same side (arbitrarily chosen as the β face) of the molecule, and the AB ring junction must be cis. The small couplings observed for the system H-1, H-2 and H-3 are consistent with this conclusion. Irradiation of H-10 gave NOEs at the secondary methyl group and also at the equatorial proton of the C-9 methylene group while irradiation of the secondary methyl group resulted in NOEs at

H-10, H-1 and 2H-9. Thus the secondary methyl group is also β. The stereochemical findings are indicated in **(12)** and the NOEs are summarised in **(E)**. As expected irradiation of 3H-12 had little effect other than enhancing the intensity of its geminal neighbour 3H-13. Irradiation of the latter afforded a strong NOE at H-6 and a weaker one at H-5. The absolute configuration of **(12)** remains to be determined. The *cis* ring fusion in this compound places it in the amorphane or muurolane group of sesquiterpenoids rather than the cadinane group. Amorphanes and muurolanes are usually distinguished by the relative configuration at C-7, which is missing in the case of **(12)**. A reasonable systematic name for **(12)** is (1R*, 6S*, 10S*)-2R*, 3S*-diacetoxyamorpha-4,7(11)-dien-8-one.



(E)

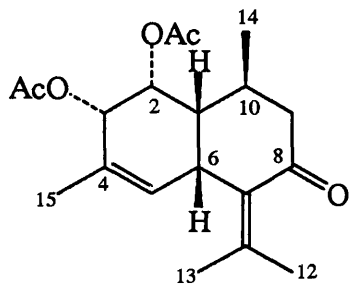
The second compound, the epoxide **(13)**, was obtained from the same fractions as **(12)**. Its spectroscopic properties were very similar to those of **(12)**. In the ^{1}H NMR spectrum (Table-3) the most obvious difference was the upfield shift of two methyl groups

[the sharp vinyl methyls of **(12)**] to 1.42 and 1.15 ppm and separation of the resonances for H-2 [δ_H 5.18, dd, J 4.8, 3.7 Hz], H-3 [δ_H 5.45, brd, J 4.2 Hz] and H-5 [δ_H 5.58, brs]. As above, double irradiation experiments readily identified the various protons. The ^{13}C NMR spectrum (Table-3) revealed the loss of the tetrasubstituted double bond of **(12)** and its replacement by two tertiary carbons at 64.6 and 71.03 ppm, indicative of an epoxide. Thus **(13)** is a 7,11-epoxide of **(12)**. NOE difference experiments (Table-4) support the same relative stereochemistry. The assignment of the *cis* fusion of the rings and the configuration of the secondary methyl group follow readily from NOEs to H-2, H-6 and 3H-14 on irradiation of H-1. The configuration of the epoxide is less easily decided. However, since irradiation of 3H-13 afforded a large NOE (*ca* 11%) at H-6, it is perhaps more likely that the epoxide is α. The proposed stereochemistry is indicated in **(13)**. Compound **(13)** is therefore [1R*, 6S*, 10S*]-2R*, 3S*-diacetoxy-7ξ, 11ξ-epoxyamorph-4-en-8-one. The epoxide **(13)** may be an artefact formed by air oxidation of the enone **(12)**.

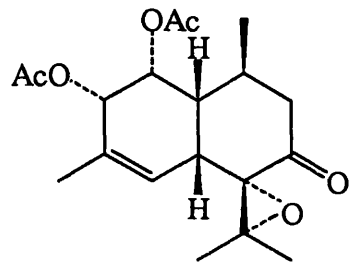
The third compound **(14)** was obtained from column fractions eluted with 25% ether and light petroleum. It was purified by preparative TLC. It is very similar to compound **(12)**, but has only one acetate group. Its molecular formula $C_{17}H_{24}O_3$ was deduced from the ^{13}C NMR spectrum (Table-5). In the ^{1}H NMR (Table-5) spectrum, the secondary acetate methine appeared at δ_H 5.18 as a ddd (J 9.7, 7.4, 3.6 Hz). This suggested that the acetate is attached to C-1, with a methylene group at C-2 and leads to structure **(14)** for this compound. NOE difference experiments (Table-6) confirmed structure **(14)** and the proton NMR assignments shown in the Table-5. The ^{13}C resonances have been assigned by analogy with **(12)**.

The relative stereochemistry of **(14)** is the same as that of **(12)**. Irradiation of H-6 gives NOEs at H-1 and H-2 thus confirming the *cis* ring junction. NOEs from 3H-14 to both the C-9 methylene protons are consistent with the β-configuration of 3H-14. Thus compound **(14)** is [1R*, 6S*, 10S*]-2S*-acetoxyamorpha-4,7(11)-dien-8-one.

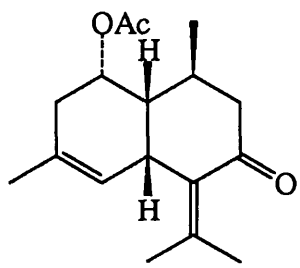
The *Marsupella* compounds are an addition to the small group of amorphane (muurolane) sesquiterpenoids to be isolated from liverworts. The longifolane sesquiterpenoids normally associated with *Marsupella* species were not detected.



(12)



(13)



(14)

GENERAL EXPERIMENTAL

Nuclear Magnetic Resonance Spectra (NMR) were recorded on Bruker WP 200 SY, AM 200 SY (^{1}H at 200.132 MHz and ^{13}C at 50.32 MHz) and AM 360 (^{1}H at 360 MHz and ^{13}C at 90 MHz) and 500 MHz spectrometers. HSQC and HMBC experiments are inverse detected versions of 2D direct and 2D long-range carbon-proton correlation experiments respectively.

Spectra were recorded for $CHCl_3$ relative to δ_H 7.25 and $CDCl_3$ relative to δ_C 77.0 and chemical shifts are reported in ppm. Coupling constants (J) in Hz are given in parenthesis in the tabulated ^1H NMR data. Signals indicated 'm' are unresolved or overlapped multiplets. ^1H and ^{13}C signal assignments are based on general chemical shift rules and comparison with published data for similar compounds. More definitive ^1H NMR assignments were made by NOE difference and homo-decoupling experiments.

Dried and powdered material was extracted with diethyl ether or methanol. The crude extracts were fractionated by column chromatography over silica gel G_{254}. Eluents used for silica gel column chromatography were increasing percentages of diethyl ether or ethyl acetate in light petroleum. Each of the crude fractions was further purified on TLC by using 0.75 mm thick preparative plates over silica gel GF_{254}. Eluents for TLC were increasing percentages of diethyl ether or ethyl acetate in light petroleum. Compounds on TLC and analytical plates were visualised using UV light or iodine vapour. Solvents were evaporated using a Buchi Rotavapor and water aspirator.

High resolution mass spectrometry was used to determine the masses of the compounds.

EXPERIMENTAL

The plant material was collected from a burn beside the Inverary road, approximately one mile west of Arrochar. It was identified by Prof. J.D. Connolly and Dr. D.S. Rycroft. A reference sample is deposited with Dr. Rycroft in the Department of Chemistry, University of Glasgow.

Dried and powdered plant material (1 kg) extracted with diethyl ether by percolation over several hours. The crude extract (3.94 g) showed several spots on analytical TLC (UV). It was then subjected to flash chromatography using a column of silica gel G_{254} and increasing percentages of petroleum ether-diethyl ether as eluent. Compounds from the separated fractions were isolated and purified by preparative thin layer chromatography on silica gel GF_{254}. Purified compounds were subjected to spectroscopic analysis.

[1R*, 6S*, 10S*] 2R*,3S*-diacetoxyamorpha-4,7(11)-dien-8-one (12), $C_{19}H_{26}O_5$,m/z 334 (M^+), 274 (M^+-CH_3CO_2H), 214 (M^+-$2CH_3CO_2H$) colourless oil, λ_{max} 248 nm (log ε = 3.84), IR; 1746, 1686, 1615 cm^{-1}, $[\alpha]_D$-90.2 (c. 1.1 in $CHCl_3$).

^{13}C and ^1H NMR data in Table -1.

[1R*, 6S*, 10S*] 2R*,3S*-diacetoxy-7ξ 11ξ-epoxyamorph-4-en-8-one (13), $C_{19}H_{26}O_6$, colourless oil.

^{13}C and ^1H NMR data in Table -3.

[1R*, 6S*, 10S*] 2S*-acetoxyamorpha-4,7(11)-dien-8-one (14), $C_{17}H_{24}O_3$, m/z 276 (M^+), 216 (M^+-CH_3CO_2H), colourless oil, λ; 246 nm (log ε = 3.77), IR; 1727 cm^{-1}, [α_D-150.5 (c. 3.5 in $CHCl_3$).

^{13}C and ^1H NMR data in Table -5.

Table-1 ^{13}C and ^1H shifts of **(12)**

	^{13}C δ (ppm)	^1H δ (ppm)	Multiplicities (J, Hz)	^{13}C δ(ppm) (C_6D_6)	^1H δ(ppm) (C_6D_6)	Multiplicities (J, Hz)
1	28.3	2.55	m	42.46	1.82	obscure
2	72.78	5.23	overlapped with 5	72.91	5.30	t (3.8)
3	68..0	5.47	brd (4.7)	68.10	5.66	brd (3.6)
4	131.3			131.59		
5	129.42	5.23	overlapped with 2	129.60	5.03	brs
6	42.28	3.7	m	42.00	3.41	brs
7	133.62			134.0		
8	203.20			201.02		
9 9`	50.41	2.42 &2	dd (14.8 & 4.37) under acetate signals		2.55 &1.96	dd (15.6, 11.5)
10	41.78	2	under acetate signals	28.37	2.58	obscure
11	143.7			142.7		
12	23.12	2.03		21.70	1.48	s
13	22.02	1.82		23.22	2.18	s
14	19.78	1.04	d (6.55)	22.00	1.01	d (6.4)

15	20.88	1.63	ddd (2.38, 1.9, 0.28)	19.76	1.50	brs
16	170.62			169.94		
17	21.07	2.05	s		1.85	s
18	170.05			169.32		
19	21.84	2.03	s		1.80	s

Table-2 NOEDIFF Correlations of **(12)**

Irradiation	Enhancement
H-3	H-2, 3H-15
H-5	H-6, 3H-15
H-6	H-5, H-1, H-2, 3H-15
H-10	$H\text{-}9_{(eq)}$, 3H-14
$H\text{-}9_{(eq)}$	H-10
3H-12	H-13
3H-13	H-12, H-6, H-5
3H-15	H-5, H-3
3H-14	H-10, H-1, 2H-9

Table-3 ^{13}C and ^{1}H shifts of **(13)**

	^{13}C (δ ppm)	^{1}H (δ ppm)	Multiplicities (J, Hz)
1	42.58*	2.23	brt
2	72.55	5.18	dd (4.8, 3.7)
3	68.16	5.45	brd (4.2)
4	132.95		
5	124.48	5.58	brs
6	43.06*	2.72	overlapping with 10
7	71.03		
8	206.0		
$9_{(eq)}$ 9`	50.12	2.45 & 2.2	dd (13.9, 4.1) hidden under acetate signals.
10	29.77	2.72	overlapping with 6
11	64.6		
12	20.81	1.47	s
13	19.75	1.2	s
14	19.8	1.14	d (6.5)
15	20.0	1.66	brd (1.7)
16	170.45		
17	21.3	2.05	s
18	170.0		
19	21.0	2.1	s

* May be interchanged

Table-4 NOEDIFF Correlations of **(13)**

Irradiation	Enhancement
H-5	H-6, 3H-15
H-3	H-2, 3H-15
H-2	H-3, H-6, H-1
$H\text{-}9_{(eq)}$	H-10
H-1	H-6, H-2, 3H-14
3H-15	H-3, H-5
3H-12	H-6, H-13
3H-14	H-10, H-1, 2H-9

Table-5 ^{13}C and ^{1}H shifts of **(14)**

	^{13}C δ (ppm)	^{1}H δ (ppm)	Multiplicities (J, Hz)
1	41.3	ca. 2.1	m
2	73.4	5.18	ddd (9.7, 7.4, 3.6)
3	31.8	2.19	brd (7.1)
4	132.8		
5	123.7	4.96	brs
6	42.5	3.73	m
7	132.8		
8	203.4		
9	49.81	2.41	dd (14.6, 4.9)
10	26.9	2.22	m
11	143.9		
12	23.19	2.02	s
13	22.2*	1.8	s
14	22.2*	1.03	d (6.7)
15	22.8*	1.65	s
16	170.6		
17	21.39	2.06	s

* May be interchanged

Table-6 NOEDIFF Correlations of **(14)**

Irradiation	Enhancements
H-2	H-6, H-3, H-1
H-5	H-6, H-13, 3H-15
H-6	H-5, H-2, H-1, 3H-13
$H\text{-}9_{(eq)}$	H-10, H-9`
3H-12	3H-13
3H-13	H-6, H-5, 3H-12
3H-15	H-5, H-3
3H-14	H-10, 2H-9

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CHAPTER THREE

Nardia scalaris

INTRODUCTION

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

In an early investigation of *Nardia scalaris*, Benes *et al*[4,5] isolated an *ent*-kaurane diterpenoid **(1)** and (+)-21α-methoxyserrat-14-en-3-one **(2).**

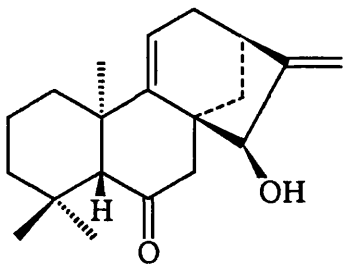
In a later study Connolly *et al*[6] isolated an acidic diterpenoid. It was characterised as its methyl ester, methyl *ent*-kaur-16-en-14-yl malonate **(3).** They have also found the derived ketone **(4).** The same diterpenoid has been reported by Langenbahn *et al*[2] with compound **(5).**

The related species *N. succulenta* contains, in addition to the simple kaurene derivatives *ent*-kaur-16-en-15β-ol[7], *ent*-(16R)-kauran-15-one and *ent*-kaur-16-en-15-one[8], a wide range of terpenoid malonates and half-malonates. Langenbahn *et al*[2] isolated compounds **(6)** and **(7)** from the acidic fraction from *N. succulenta.* They also reported compounds **(8), (9), (10)** and **(11)** from neutral fractions of the same liverwort.

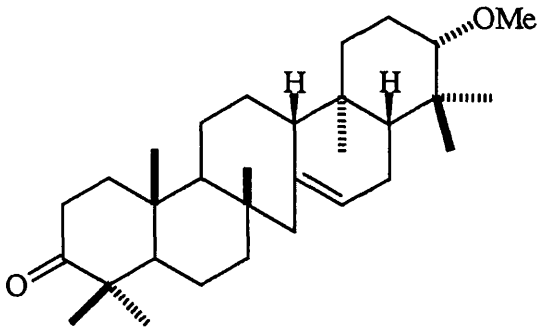
Similar terpenoid esters were found in *N. scalaris* by the same authors[2] and were recognised as derivatives of *ent*-kaurene alcohols with the functional group predominantly at C-14, though derivatives with functionalization at C-15 were also observed[2]. Compounds **(12), (13), (14), (15)** and **(16)** have been reported from the fraction of *N. scalaris*[2] while the neutral fractions afforded the compounds **(17), (18A)** and **(18B).**

RESULTS AND DISCUSSION

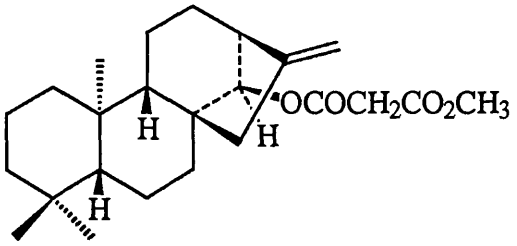
In a continuation of the chemical investigation of *Nardia scalaris*, we have isolated a diterpenoid which has been shown by chemical and spectroscopic methods to be *ent*-(14S)-kaur-16-en-14-yl hydrogen malonate **(19),** which was previously characterised as its


H
OH
O

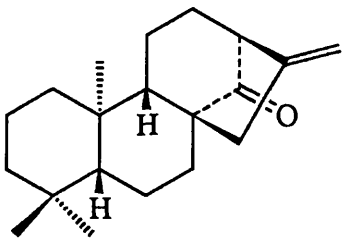
(1)


OMe
H
H
O

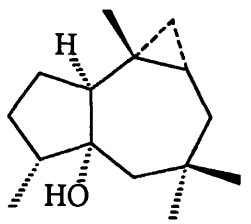
(2)


OCOCH2CO2CH3
H
H
H

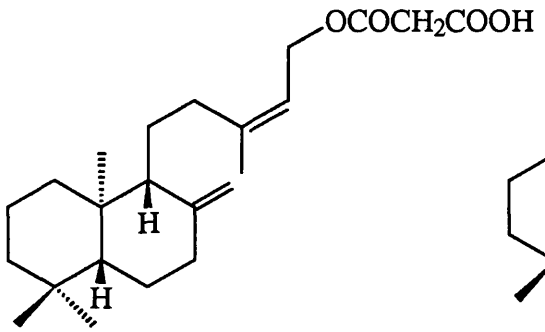
(3)


H
O
H

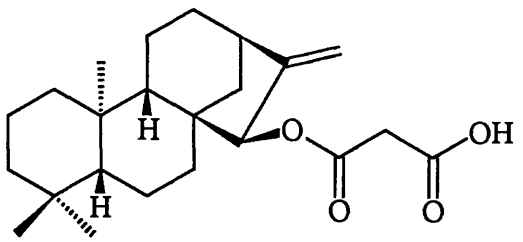
(4)


H
HO

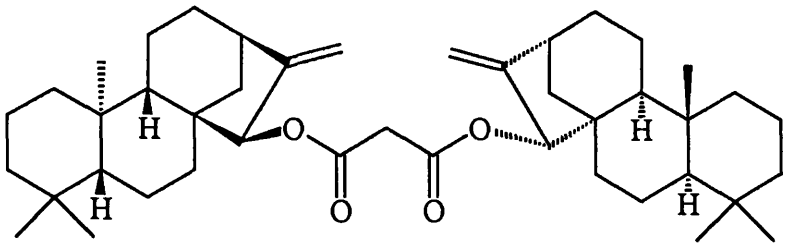
(5)


OCOCH2COOH
H
H

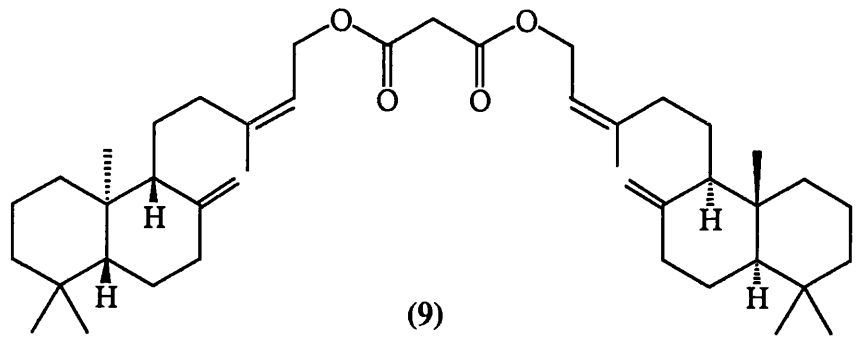
(7)


H
O
OH
H
O
O

(6)


H
O
O
H
H
O
O
H

(8)



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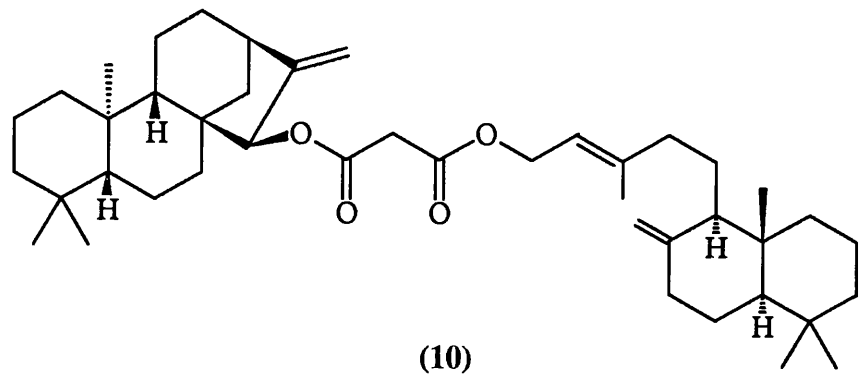

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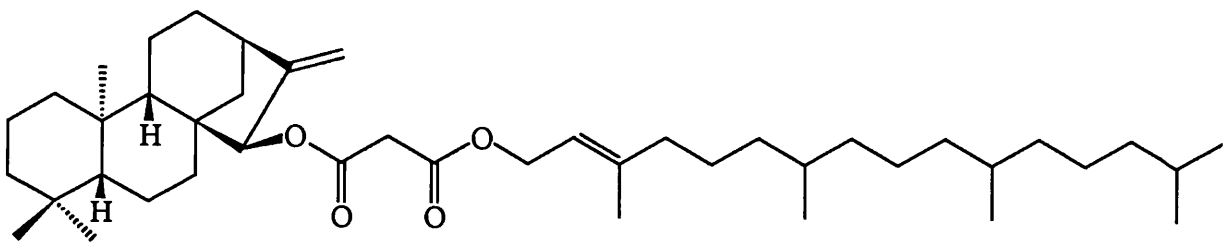

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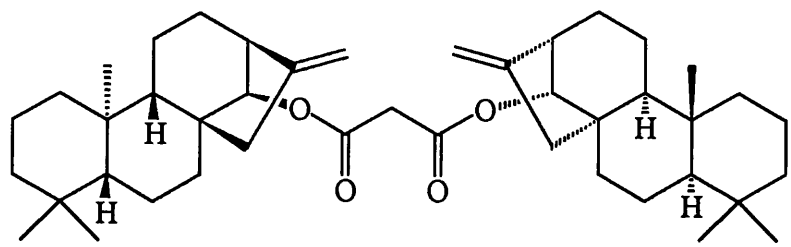

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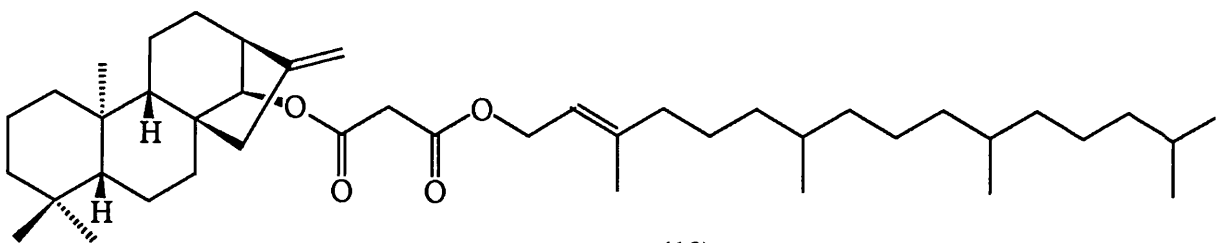

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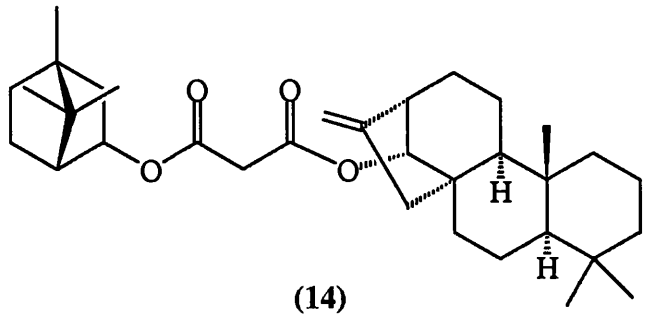

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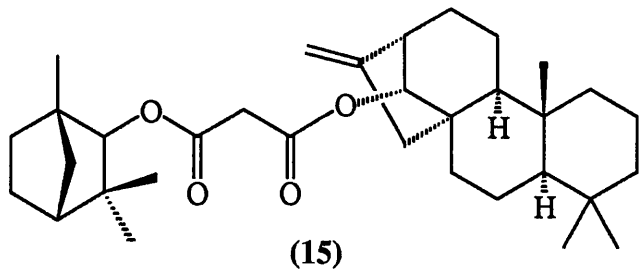

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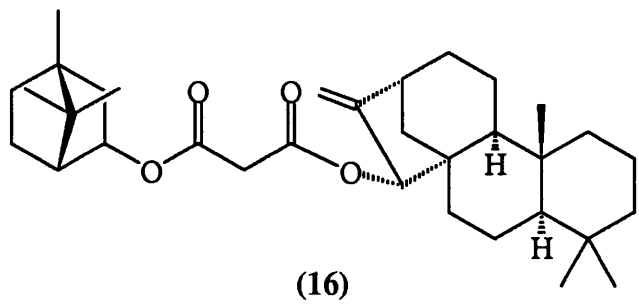

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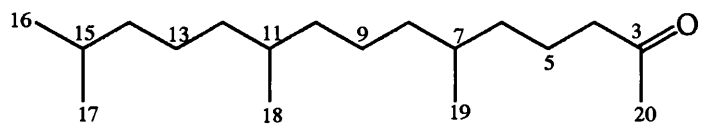

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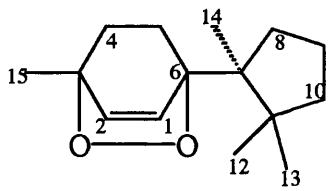
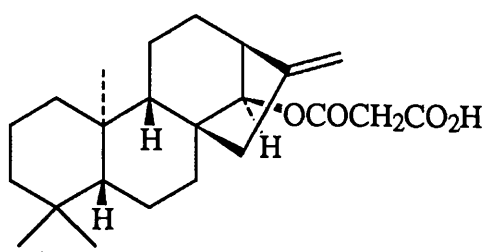
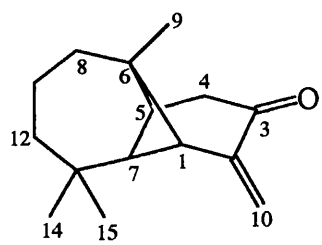


(18A) and (18B)



(19)



(20)

methyl ester (3) by Connolly *et al*[6]. The compound was isolated from the acidic fraction of *Nardia scalaris* whose analytical plate eluted with diethyl ether revealed the presence of one spot. Its ^{13}C NMR spectrum suggested the molecular formula $C_{23}H_{34}O_4$ which is also supported by the mass spectrum (m/z=374).

In its ^{13}C NMR spectrum (Table-1), it has exomethylene carbons [δ_C 151.9 (C-16) and 105.5 (C-17)], a hydrogen malonyl residue [δ_C 167.0 (C-21), 41.8 (C-22), 170.5 (C-23)], a secondary oxygen bearing carbon [δ_C 81.6 (C-14)], a methine carbon [δ_C 49.0 (C-13)], three tertiary methyl carbons [δ_C 33.6 (C-18), 21.5 (C-19) and 17.9 (C-20)] together with three tetrasubstituted carbons, two methines and eight methylene groups. These data suggested that the compound was a tetracyclic diterpenoid of the kaurene class and comparison with literature values confirmed this[6].

Its ^1H NMR spectrum (Table-1) shows the exomethylene protons at δ_H 4.83 (brs, 2H-17), the methylene protons of the hydrogen malonyl residue at δ_H 3.40 (s, 2H-22), a methine at δ_H 2.68 (brs H-13), a hydrogen on the oxygen-bearing carbon at δ_H 5.41 (brs, H-14) and three tertiary methyls at δ_H 0.79 (s, 3H-18), 0.82 (s, 3H-19) and 1.05 (s, 3H-20). The IR spectrum revealed the presence of a carboxylic acid at 1733 cm^{-1} (carbonyl) and a broad band at 3370 cm^{-1} due to the O-H stretching vibration. The compound was subjected to an exchange experiment with D_2O to identify the carboxylic acid proton. However, the exchange occurred at the α-position [δ_H 3.40 (2H-22)] of the malonic acid.

It seemed likely that the secondary ester function was attached to C-14[6]. Support for this assignment was obtained from the NOE difference experiments (Table-2) ; irradiation of H-13 resulted in a NOE at H-14, while irradiation of H-14 gave a reasonable NOE at H-13 (1.5 %) and a substantial NOE at Me-20 (3.5 %). Irradiation of the exomethylene protons (H-17) gave an enhancement of H-13. Irradiation of Me-20 caused a 5 % increase in the intensity of H-14, thus confirming the position of attachment of the malonate and the configuration at C-14. Irradiation of Me-20 also identified Me-19. Thus the compound is *ent*-(14S)-kaur-16-en-14-yl hydrogen malonate **(19).** This compound has already been reported from the same liverwort by Langenbahn *et al*[2]

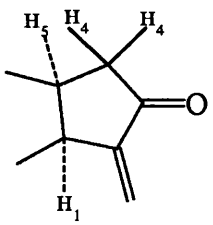
The neutral fraction of the same liverwort afforded an *ent*-longipinane type sesquiterpenoid, $C_{15}H_{22}O$ (m/z 218), which was isolated by means of combination of column and preparative thin layer chromatography on silica gel. The spectroscopic data revealed that the compound was a tricyclic α,β-unsaturated sesquiterpene ketone with a ketone group conjugated with an exocyclic methylene [1707 cm^{-1}, 1626 cm^{-1} ; δ_H 4.93, and δ_H 5.86, (2H-10), both d, J 1.65 Hz.], a methylene adjacent to the carbonyl group [δ_H 2.70, and δ_H 2.48 (2H-4), both dd, J 18.9 and 2.6 Hz.], three methines [δ_H 1.36 (H-7), s; δ_H 2.28 (H-5), dt, J 6.8 and 3.1 Hz. ; δ_H 2.81 (H-1), d, J 6.8 Hz.], and three tertiary methyls [δ_H 0.76 (3H-9), s, and 0,91 (3H-14 and 3H-15), both s].

The ^{13}C NMR spectrum in deuteriobenzene (Table-3) revealed the presence of three methyl groups [δ_C 23.5 (C-9) ; δ_C 28.3 and 28.0 (C-14 and C-15)], four methylenes [δ_C 45.2 (C-4) ; δ_C 41.6 (C-12) ; δ_C 40.0 (C-8) ; δ_C 22.0 (C-11)], an exomethylene group [δ_C 151.5 (C-2) ; δ_C 115.4 (C-10)], a carbonyl [δ_C 199.4 (C-3)], three methine carbons [δ_C 59.6 (C-7) ; δ_C 48.0 (C-1) ; δ_C 37.7 (C-5)], and two tetrasubstituted carbons [δ_C 43.0 (C-6) ; δ_C 33.5 (C-13)].

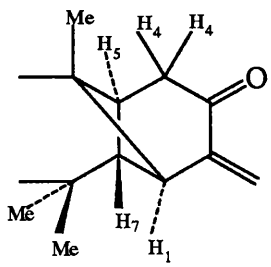
In deuteriobenzene solution, three well resolved methyl resonances were observed [δ_H 0.60 (3H-9), s, δ_H 0.70 (3H-14), s and δ_H 0.76 (3H-15), s] and there was less overlap of the methylene protons. Changes in chemical shift were also observed for some of the other resonances, e.g. the exomethylene protons [δ_H 6.11 and δ_H 4.67 (2H-10), both d, J 1.95 Hz], the methylene adjacent to the carbonyl group [δ_H 2.29 and δ_H 2.53 (2H-14), both dd, J 18.8 and 4.2 Hz] and the methine protons [δ_H 2.53 (H-1), d, J 6.92 Hz, δ_H 1.77 (H-5), dt, J 6.78 and 2.58 Hz, and δ_H 1.16 (H-7), s]. With the exception of one of the exomethylene protons (which is deshielded by 0.25 ppm), the above protons are shielded in deuteriobenzene relative to deuteriochloroform and must therefore lie "behind" and relatively close to the carbonyl group[9].

Initially the spectroscopic properties suggested the part structure **(A)**. The IR band at 1707 cm^{-1} seemed consistent with such a proposal. However, it soon became apparent that this part structure was not tenable . In the HMBC spectrum (in C_6D_6) (Table-4), the highest field methyl correlates with both the H_1 methine carbon at 48.3 ppm

and the H_5 methine carbon at 38.5 ppm. Thus both of these carbons must be three bonds away from the methyl proton. The methine singlet H_7 correlates with the exomethylene quaternary carbon. This must be a $^3J_{CH}$ since H_1 is already occupying the allylic position. NOE experiments (Table-6) provided further evidence against part structure **(A).** Irradiation of the methyl at highest field gave a substantial NOE (3 %) at one of the methylene protons (δ_H 2.53) adjacent to the carbonyl group. Irradiation of the methyl at δ_H 0.76 gave NOEs at H_5 (3 %) and H_7 (4 %), while irradiation of the third methyl at δ_H 0.70 resulted in NOEs at H_1 (2.2 %) and H_7 (3.5 %). These results can be rationalised by having a longipinane skeleton as in **(B)** with the three methines attached to the cyclobutane ring. H_1 and H_5 are not vicinal but four bonds apart and their coupling of 6.5 Hz arises from a $^4J_{HH}$ coupling with ideal W geometry. The dihedral angles between "H_7 and H_1" and "H_7 and H_5" are 90° and hence no coupling is observed. Thus structure **(20)** was assigned to this sesquiterpenoid. Unfortunately it is a known compound, marsupellone, isolated by Matsuo *et al*[9] from the liverwort *Marsupella emarginata* in 1979



(A)



(B)

EXPERIMENTAL

Plant material was collected at the edge of a small road in Dartmoor, Devon, and identified by Dr D.S. Rycroft. Dried and powdered material (44.96 gr) gave a crude extract (1.28 gr) on extraction with methanol. Ether and aqueous NaCl were added to the crude methanol extract and, on separation, this mixture gave 461.5 mg crude ether extract. The crude ether extract was then separated into acid and neutral fractions by

extraction with dilute Na_2CO_3. Evaporating the ether layer gave the neutral fraction (312 mg). After acidification, the aqueous part was again subjected to an extraction with diethyl ether to give an acidic fraction (74.5 mg).

The acidic fraction, which seemed to be pure from its analytical plate, was examined by NMR spectroscopy techniques. However the analytical plate of the neutral extract showed a few spots, one of them major. Purification was done by preparative thin layer chromatography on silica gel GF_{254}. As eluent, 10% ethyl acetate and light petroleum ether were used. From the preparative plate, the major band afforded a colourless oil (15 mg) which was subjected to spectroscopic analysis.

After evaporation, the major band we gained was 15 mg. Afterwards, the purified compound was subjected to spectroscopic analysis.

***ent*-(14S)-kaur-16-en-14-yl-hydrogen malonate (19)** : 58.1 mg, $C_{23}H_{34}O_4$, m/z 374, IR ; 1733, 3370 cm^{-1}.

^{13}C and ^{1}H NMR data in Table-1.

Marsupellone (20) : 15 mg, $C_{15}H_{22}O$, m/z 218, IR ; 1707, 1626 cm^{-1}.

^{13}C and ^{1}H NMR data in Table-3.

Table-1 ^{13}C and ^{1}H shifts of **(19)**

	δ_C (ppm)	δ_H (ppm)	Multiplicities (J, Hz)
1	40.2		
2	18.6		
3	41.8		
4	33.2		
5	56.1		
6	19.7		
7	33.1		
8	48.3		
9	59.0		
10	39.2		
11	17.2		

12	32.7		
13	49.0	2.68	brs
14	81.6	5.41	brs
15	45.5		
16	151.9		
17	105.5	4.83	brs
18	33.6	0.79	s
19	21.5	0.82	s
20	17.9	1.05	s
21	167.0		
22	40.8	3.40	s
23	170.5		

Table-2 NOEDIFF correlations of **(19)**

Irradiation	Enhancements
3H-19	3H-20
3H-20	H-14, 3H-19
H-13	H-14
2H-17	H-13
H-14	H-13, 3H-20

Table-3 ^{13}C and ^{1}H shifts of **(20)**

	^{13}C δ (ppm)	^{1}H δ (ppm)	Multiplicities J (Hz)	^{13}C δ (ppm) (C_6D_6)	^{1}H δ (ppm) (C_6D_6)	Multiplicities J (Hz)
1	47.0	2.81	d (6.8)	48.0	2.53	d (6.92)
2	150.5			151.5		
3	201.2			199.4		
4 4`	44.7	2.70 2.48	dd (18.9, 2.6)	45.2	2.29 2.53	dd (18.8, 4.2)
5	37.2	2.28	dt (6.8, 3.1)	37.7	1.77	dt (6.78, 2.58)
6	42.5			43.0		

7	59.2	1.36	s	59.6	1.16	s
8	39.4			40.0		
9	23.1	0.76	s	23.5	0.60	s
10 10`	115.6	5.86 4.93	d (1.65)	115.4	6.11 4.67	d (1.95)
11	21.4			22.0		
12	41.1			41.6		
13	33.1			33.5		
14	27.5*	0.91	s	28.0*	0.70	s
15	27.8*	0.91	s	28.3*	0.76	s

* May be interchanged

Table-4 HMBC of **(20)** in C_6D_6

δ_C (ppm)	C	Type	H
47.6	1	methine	2H-10, H-5
151.5	2		2H-10, H-7
199.4	3	carbonyl	2H-10, H-1, 2H-4, H-5
45.2	4	methylene	
37.7	5	methine	H-1, 2H-4
43.0	6		2H-4, H-7
59.6	7	methine	2H-4, 3H-14, 3H-15, (2H-8)
40.0	8	methylene	H-7, 3H-14, 3H-15
23.5	9		H-1, H-5
115.4	10		H-1
22.0	11		
41.6	12	methylene	2H-8, 3H-9
33.5	13		3H-14, 3H-15
27.9	14		
28.3	15		

Table-5 HSQC of **(20)** in C_6D_6

C	δ_H (ppm)	No
1	2.81	1H
4	2.70 and 2.48	2H
5	2.28	1H
7	1.36	1H
8		2H
9	0.76	3H
10	5.86 and 4.93	2H
11		2H
12		2H
14	0.91	3H
15	0.91	3H

Table-6 NOEDIFF Correlations of **(20)** in C_6D_6

Irradiation	Enhancements
H-10`	H-10
H-10	H-10`, H-1
H-1	H-10
H-4	H-4`, H-5, 3H-9
H-4`	H-5, H-7, H-1, H-4
H-5	H-7
H-7	H-5, H-1, 3H-14, 3H-15
Methyl at 0.70	H-1, H-7
Methyl at 0.76	H-5, H-7
3H-9	H-4, H-5, 2H-8

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CHAPTER FOUR

Miliusa velutina

INTRODUCTION

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

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

The Annonaceae are closely related to the Magnoliaceae and the Eupomatiaceae (the latter have sometimes been included in the Annonaceae). They can be distinguised from the Magnoliaceae by the absence of stipules, reduced, whorled perianth, dorsal microsporangia and ruminate endosperm , and from the Eupomatiaceae by the absence of diaphragms composed of stone-cells in the pith, by the bast not being stratified, and by the presence of typical bordered pits in the wood parenchyma in the latter.

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

interesting [e.g. diterpenoids with antibacterial[13] and antitumour[14] activity; oliveroline with antiparkinsonian properties[15]; liriodenine with antitumour, antibacterial and antifungal properties[16,17]].

Due to their occurrence in inaccessible low elevation rain forest areas, a relatively small amount of phytochemical work has been carried out on the Annonaceae in comparison with many other plant families of similar size. Some phytochemical information is available on only about 33% of genera and 7% of recognised species[18].

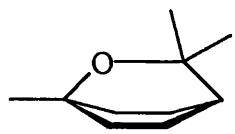
Biogenetically, the family is capable of producing a wide range of interesting secondary plant metabolites (e.g. terpenoids, flavonoids, indolosesquiterpenes, cyclohexane derivatives, aromatic compounds, acetogenins etc.) and is now the focus of much interest by phytochemists.

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

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

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

The occurrence of triterpenoids in the Annonaceae has not been widely investigated. Sitosterol has frequently been isolated from various species in the family[18].



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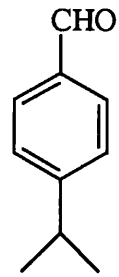

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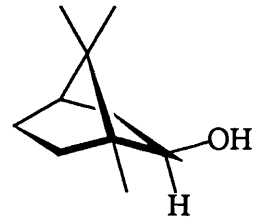

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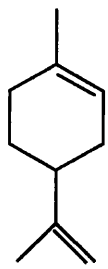

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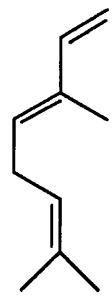

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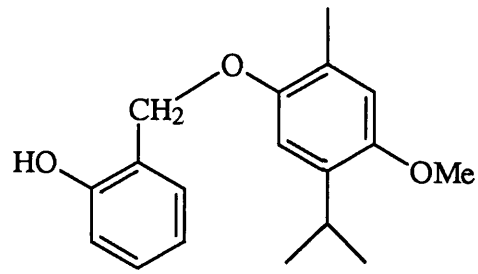

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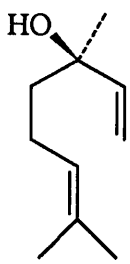

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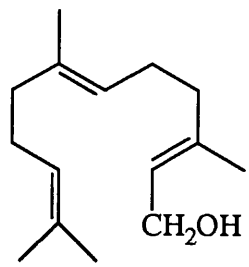

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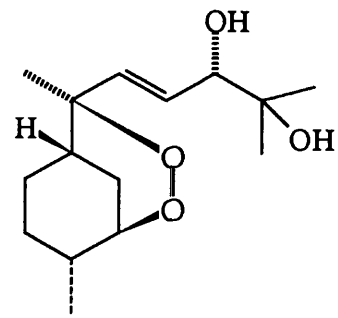

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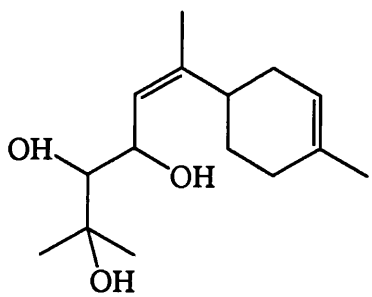

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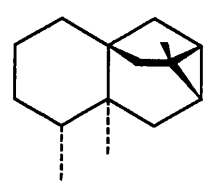

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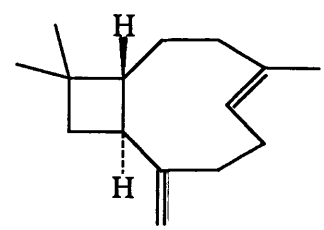

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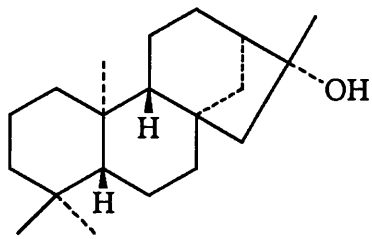

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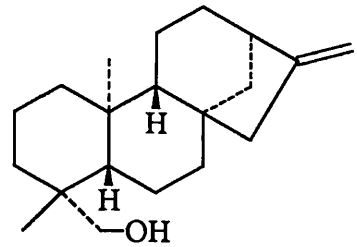

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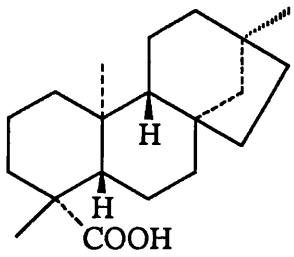

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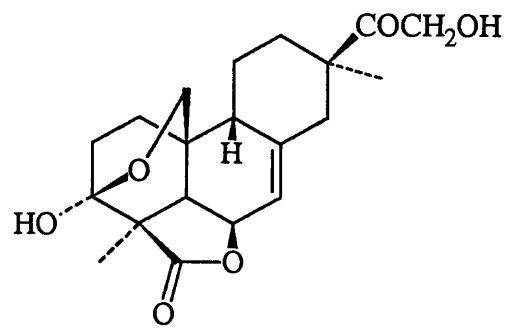

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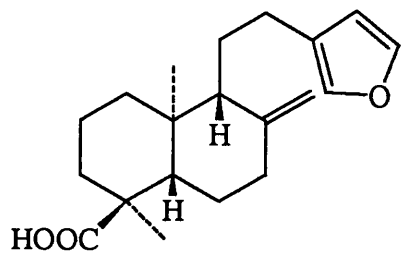

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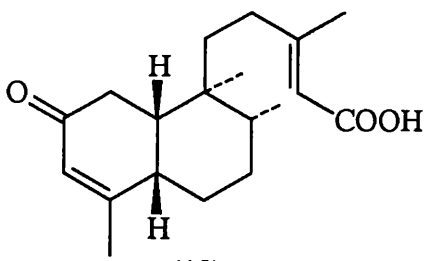

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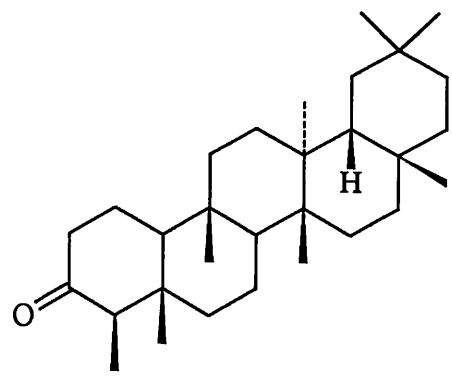

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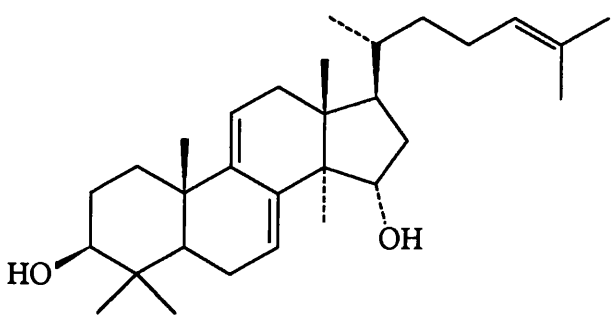

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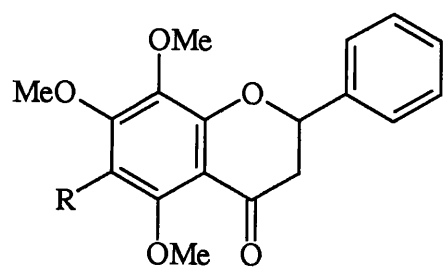
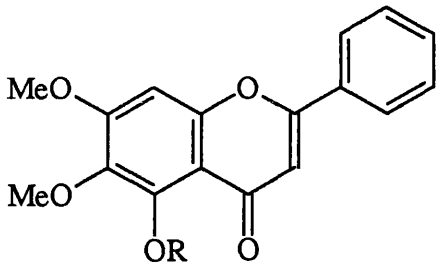
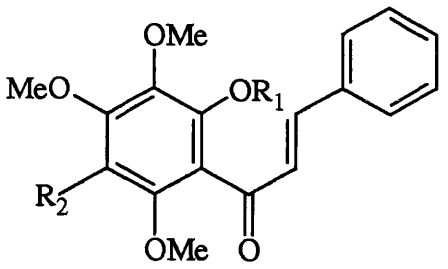


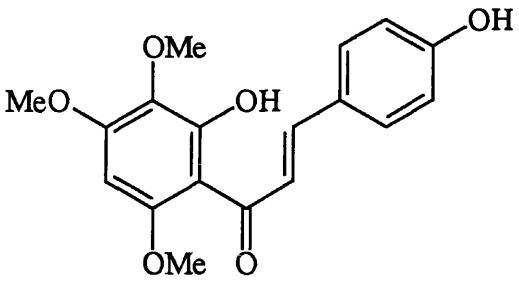
(21) R=H
(27) R=Me



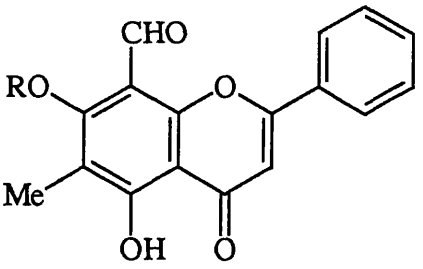
(22) R=Me
(23) R=H



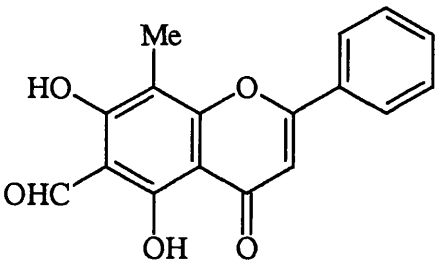
(24) R_1=R_2=H
(25) R_1=Me, R_2=H
(28) R_1=H, R_2=OMe



(26)



(29) R=H
(30) R=Me



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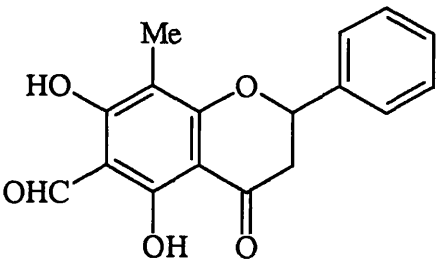

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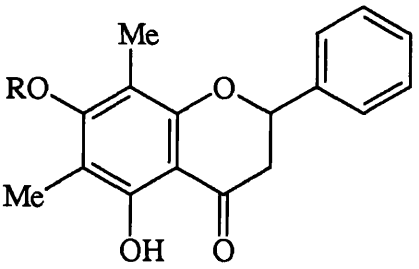
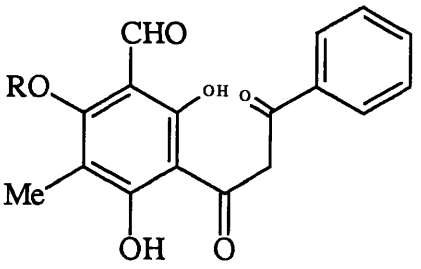
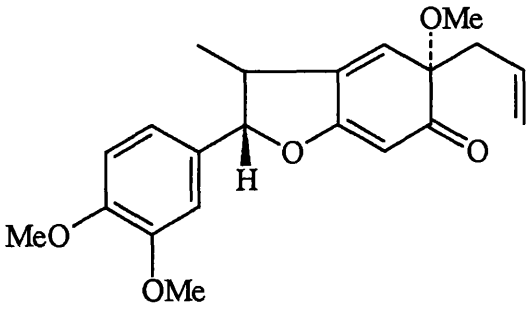


(33) R=Me
(34) R=H



(35) R=H
(36) R=Me



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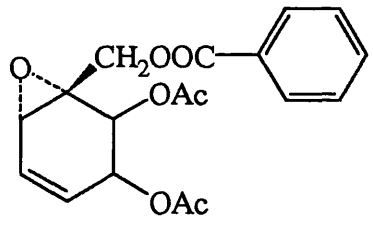

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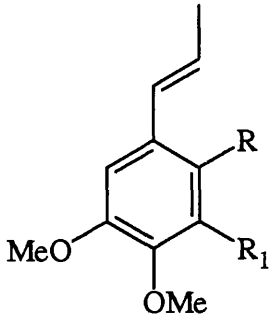
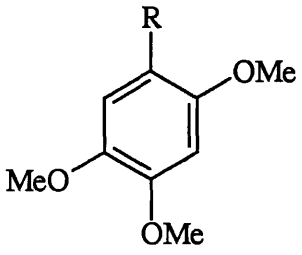
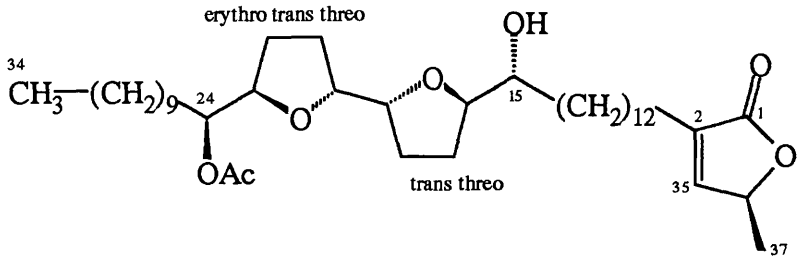


(39) R= OMe, R_1= H
(40) R= H, R_1= OMe
(41) R= R_1= OMe



(42) R= $-CH=CH_2$
(43) R= $-CH(CH_2OH)OCH_2Me$
(44) R= $-CHO$



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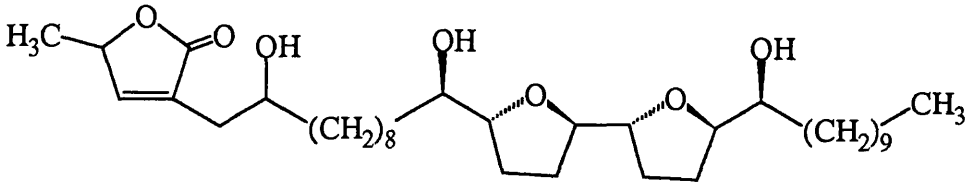


(46)

Several pentacyclic triterpenes have been reported, mostly from the genus Uvaria and friedelin **(19)** has been isolated from the leaves of *Annona squamosa*[31]. The tetracyclic triterpenoid polycarpol **(20)** was discovered by Cavé *et al*[32] simultaneously in the barks of *Greenwayodendron (Polyalthia) oliveri* and *Meocarpidium lepidotum.* Later it was found to be present in several other species.

Flavonoids are one of the most widespread and numerous groups of naturally occurring compounds[33]. Among the genera of the Annonaceae, *Uvaria* is a particularly important source of flavanones and dihydrochalcones. The African species, *Monanthotaxis (Popowia) cauliflora,* has been found to be a good source of simple flavonoids. From the stem bark of this species, 5,7,8-trimethoxyflavanone **(21)** ; 5,6,7-trimethoxyflavone **(22)** ; 5-hydroxy-6, 7-dimethoxyflavone **(23)** and three chalcones **(24), (25)** and **(26)** have been isolated. Kanakugin **(27)** and kanakugiol **(28)** have been recorded from the ripe fruit of this species[34].

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

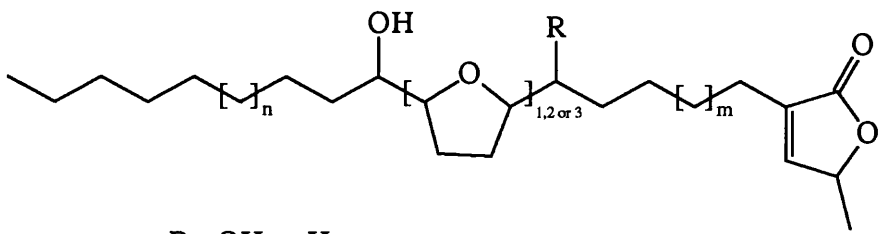
The chemistry of non-flavonoid aromatic compounds isolated from Annonaceae is very heterogeneous. Compounds range from simple benzyl derivatives to more complex structures such as the neolignan **(37)** from *Duguetia surinamensis* or the cyclohexene derivative **(38)** from *Uvaria catocarpa*[42]. Benzyl or benzoyl derivatives, together with highly oxidized cyclohexane derivatives, appear to be very common in the chemistry of the genus *Uvaria.*

Several propenyl benzene and vinyl benzene derivatives have been recorded from annonaceous plants. Asarone **(39),** *trans*-isoelemicin **(40)** and *trans*-isomyristicin **(41)** have been isolated from the bark of *Guatteria gaumeri*[43], and 2,4,5-trimethoxy-styrene **(42)** from *Pachypodanthium confine*[44], *P. staudtii*[45,46] and *Duguetia eximia*[47]. An

aromatic compound, pachysontol **(43)**, has been found by Bevalot *et al*[46] in *P. staudtii* and asaraldehyde **(44)** has been reported from *Guatteria gaumeri*[43] and *P. staudtii*.

Apart from those compounds mentioned above, the Annonaceae family is the subject of considerable interest because of the presence of the acetogenins, a group of compounds with a range of useful biological properties such as cytotoxic, antitumoral, antiparasitic, pesticidal, antimicrobial and immunosuppresive activities. The field of investigation of acetogenins expanded greatly with the number of isolated and reported acetogenins increasing rapidly[48]. In 1990[49] thirty one and in 1993[50] sixty one acetogenins were described as part of two reviews and now they number more than 160[48].

Annonaceous acetogenins constitute a series of C-35/C-37 natural products of polyketide origin derived from fatty acids. Their structure is characterised by a long alkyl chain bearing a terminal unsaturated γ-methyl-γ-lactone (sometimes rearranged to a γ-lactone containing an acetonyl group α to the lactone carbonyl), one, two or three tetrahydrofuran rings and some oxygenated substituents along the chain, particularly α to a tetrahyrofuran, and in some cases double bonds and/or epoxides[48] :



R= OH or H

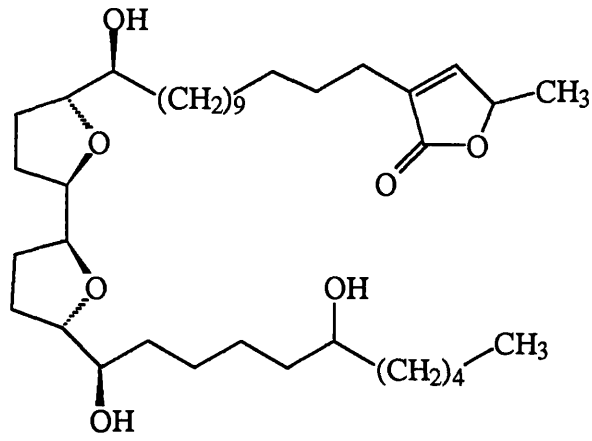
The first annonaceous acetogenin, uvaricin **(45)**, a new antitumor agent was isolated by Jolad *et al*[51] from the roots of *Uvaria acuminata* (Annonaceae) in 1982. Uvaricin is a fatty acid lactone which contained a number of original structural characteristics, particularly a bis-tetrahydrofuran pattern flanked by hydroxyls and a terminal unsaturated lactone. Dabrah and Sneden[52,53] and Cortes *et al*[54], two years later, described four new products presenting the same structural characteristics. These products found in species belonging to the family of the Annonaceae formed a new class of natural compounds called acetogenins. In 1991, two more acetogenins, molvizarin **(46)** and motrilin **(47)**, were reported by Cortes *et al*[55].

Classification of the acetogenins of the Annonaceae is done according to the number and arrangement of the tetrahydrofuran rings along the alkyl chain as proposed by Cave *et al*[48].

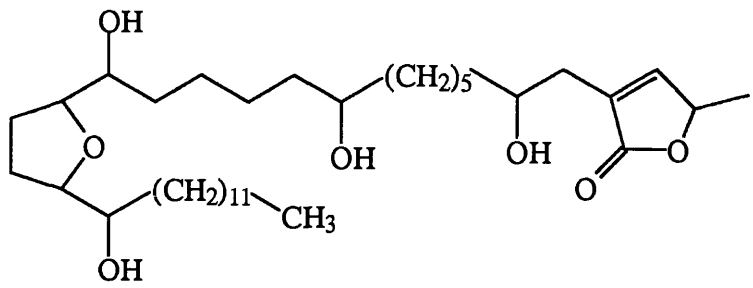
The first isolated acetogenin, uvaricin **(45)**, was stated to be a new antitumour agent[51]. Since then many studies have appeared describing cytotoxic, antitumour, antiparasitic, pesticidal, antimicrobial, antifungal and immunosuppressive properties of various acetogenins. For instance, molvizarin **(46)** and motrilin **(47)** exhibited cytotoxic activity. There are some other cytotoxic acetogenins from the seeds of *Annona muricata* such as annonacin **(48)**, annonacinone **(49)**[56,57] and murisolin **(50)**[58,59]. The acetogenins senegalene **(51)**, squamocin **(52)** and molvizarin **(46)** from *Annona senegalensis*[60,61] and ulacins from *Rollina ulei*[62] exhibit antiparasitic activity. Pesticidal activity of acetogenins can be seen in annonin **(53)**[64], bullatacin **(54)**[65,63], asimicin **(55)**[49,66], squamocin **(52)**[67], goniothalamicin **(56)**[49,68] and sylvaticin **(57)**[69]. Oils of some *Annona* seeds are traditionally used to get rid of lice in the scalp[67], as for example, the ground seeds of *Annona reticulata* in Vietnam[70]. In some countries of South America, ground bark or seeds of some species of Annonaceae are spread on soils as pesticides[48]. Interesting immunosuppressive activity has been observed in the acetogenins isolated from *Annona muricata*[71].

RESULTS AND DISCUSSION

In the present work, extraction of *Miliusa velutina* afforded a complex oily mixture. Flash chromatography and extensive preparative TLC failed to yield pure compounds. However spectroscopic analysis of fractions enabled us to identify some of the structural features present in these compounds. The IR spectrum of later fractions showed characteristic absorptions of γ-lactone (1764 cm^{-1}, C=O), free and intramolecularly bonded hydroxyl bands at 3606 cm^{-1} and 3446 cm^{-1}, respectively, a disubstituted acetylenic stretch at 2232 cm^{-1} and olefinic CH stretches at 3026 cm^{-1} and 3010 cm^{-1}. The UV spectrum was indicative of an ene-diyne chromophore [λ_{max} 283 (ε 3228), 267 (3980), 253 (3060), 239 (3048) nm and strong end absorption]. The lack of both strong IR absorption at 1750 cm^{-1} and a UV absorption maximum at around 215.5 nm indicated that the γ-lactone ring is not α,β-unsaturated. The spectroscopic properties



(47)



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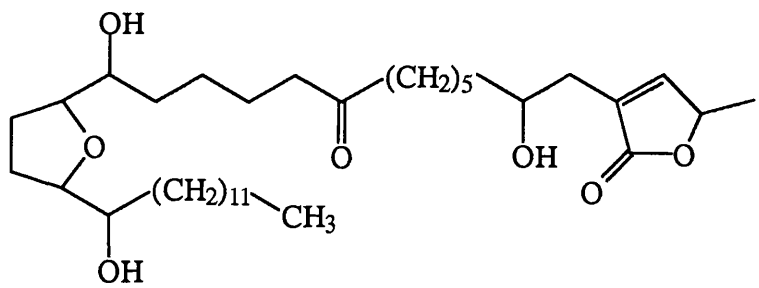

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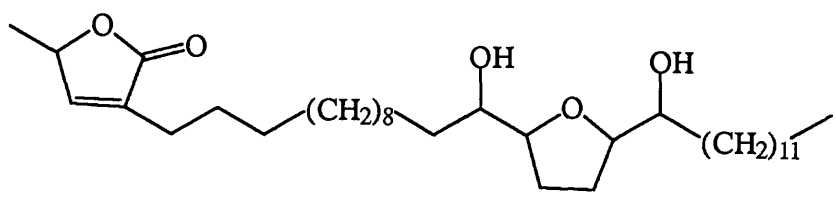

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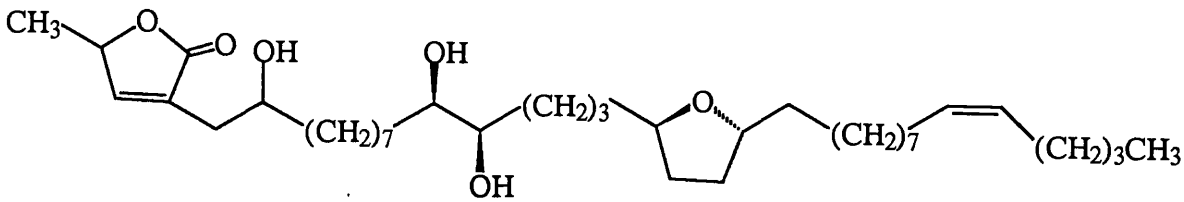

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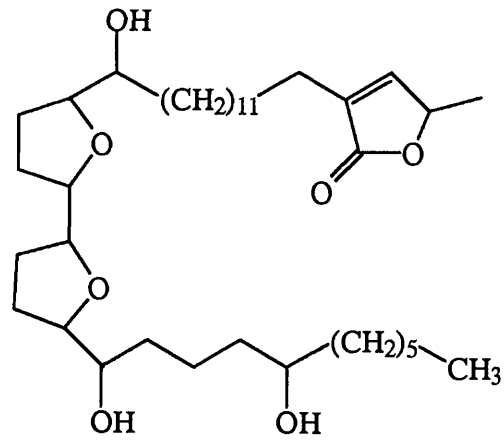

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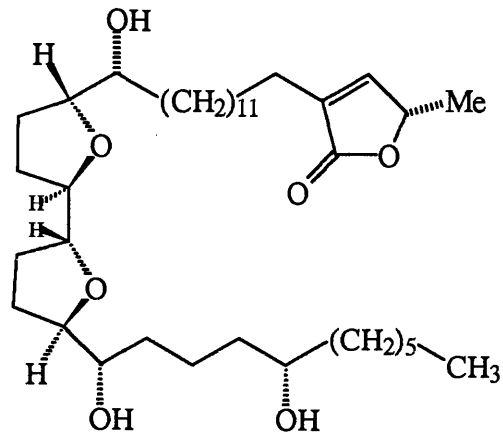

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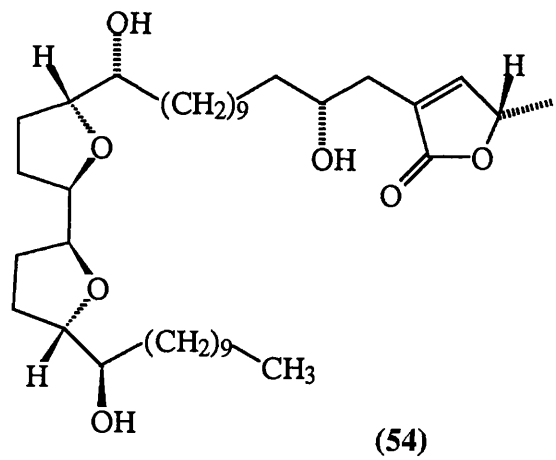

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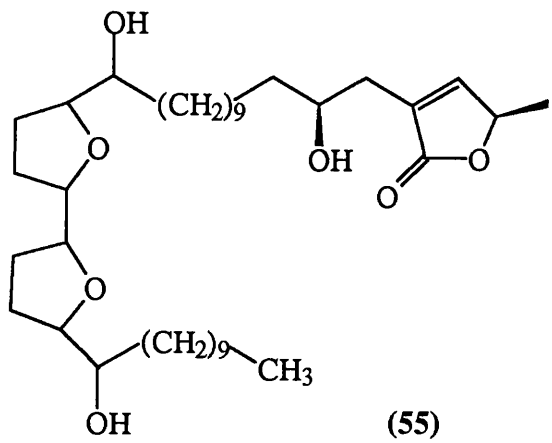

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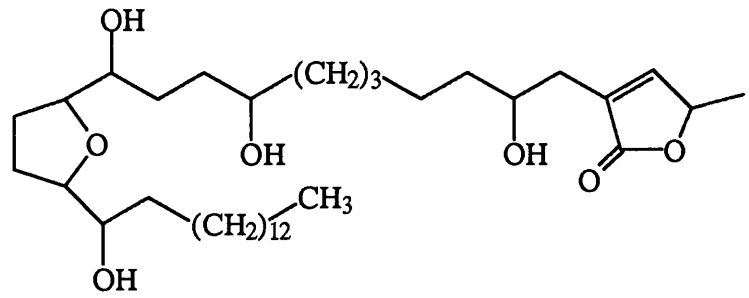

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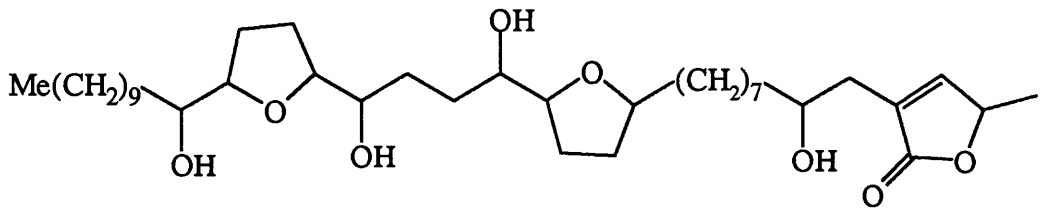

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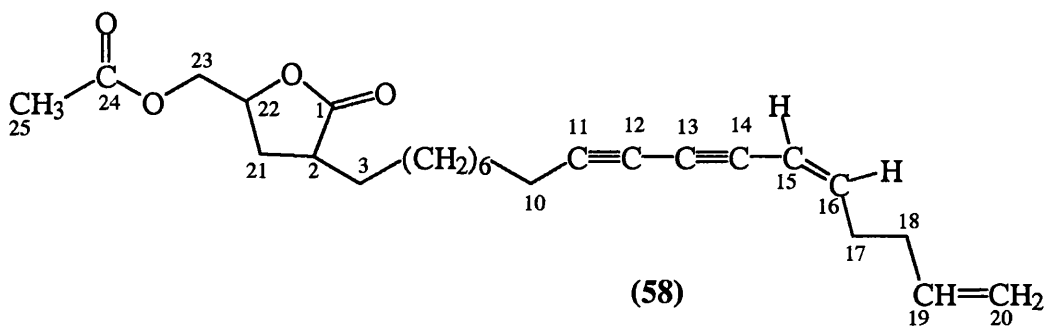

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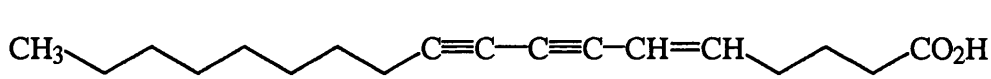

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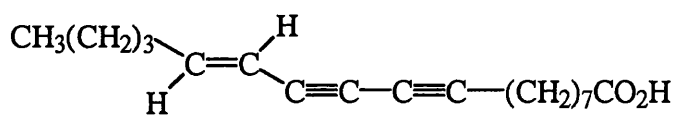

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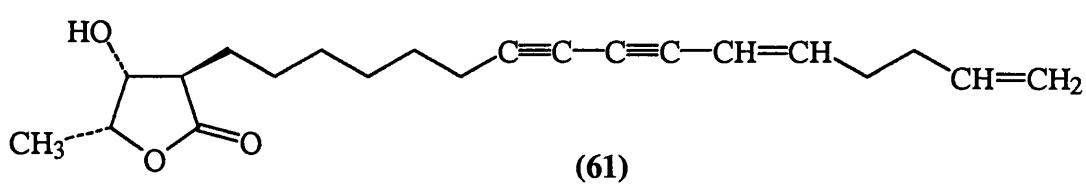


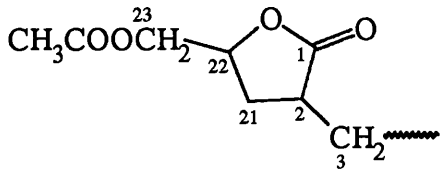
(61)

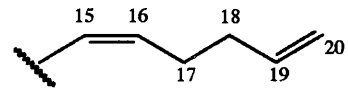
of the earlier fractions indicated the presence of the same γ-lactone system but absence of the ene-diyne chromophore.

Acetylation of a portion (5 g) of the extract in the usual way, followed by flash chromatography, afforded a range of fractions. After multiple elution preparative TLC, several bands were obtained, one of which proved to be pure as judged by its ^{1}H and ^{13}C NMR spectra (Table 1). Finally, the analysis of its COSY, HMBC, and HSQC spectra led us to the structure **(58).**

The compound was isolated from the fraction eluted with 40% ether and light petroleum. Its ^{13}C NMR spectrum showed that there are 25 carbon atoms in the structure and indicated a molecular formula of $C_{25}H_{34}O_4$. Although at first no satisfactory mass spectral data were obtained, the mass spectrum of the same compound obtained from a later fraction (60% ether and light petroleum) supported the molecular formula derived from the ^{13}C NMR spectrum. Its ^{13}C NMR spectrum (Table-1) revealed the presence of a methyl group (acetate), five methine carbons [including a terminal vinyl group], four acetylene carbons, thirteen methylene groups (including a terminal vinyl group and an oxygen bearing carbon) and two carbonyls, one of which is the acetate.

The IR spectrum revealed the presence of the γ-lactone (1764 cm^{-1}) and acetylenic absorption as discussed above. The ene-diyne chromophore was also present [λ_{max} 283 (ε 3228), 267 (3980), 253 (3060), 239 (3048) nm]. The ^{1}H NMR spectrum (Table-1) showed signals for a primary acetate group 2H-23 [δ_H 4.29 (dd, 12.1 and 3.4 Hz) ; 4.15 (dd, 12.1 and 5.4 Hz)] coupled to a methine hydrogen on a carbon bearing oxygen [δ_H 4.75 (ddt, 8.4, 4.8 and 4.9 Hz)]. The COSY spectrum readily revealed that this methine is coupled to a methylene group 2H-21 [δ_H 2.25 and 2.1 (both m)] which in turn is coupled to a methine H-2 [δ_H 2.68 (dq, 5.3 and 9.0 Hz)]. The COSY spectrum indicated that the H-2 is also coupled to the methylene group 2H-3 [δ_H 1.88 and 1.5 (both m)] which probably forms part a methylene chain. Evidence from the HMBC spectrum confirms that the above coupled system belongs to a γ-lactone ring as shown in part structure **(A).**





(A) **(B)**

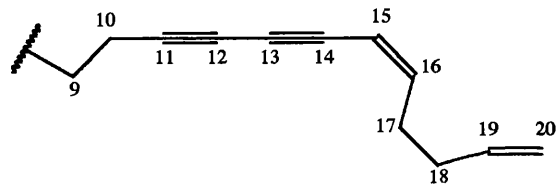
Thus the lactone carbonyl carbon has $^{3}J_{CH}$ correlations from H-22, 2H-21 and 2H-3, and a $^{2}J_{CH}$ correlation from H-2 while the acetate carbonyl carbon correlates with 2H-23 and the acetate methyl protons. The carbons with directly attached protons were assigned using an HSQC experiment (Table-3).

The ^{1}H NMR and COSY spectra also show the presence of a terminal vinyl group [δ_H 5.08 (brd, 17.0 Hz), 5.01 (brd, 10.3 Hz) and 5.85 (ddt, 17.0, 10.3 and 6.5 Hz)] coupled to a methylene group 2H-18 [δ_H 2.20 (q, 7.4 Hz)]. This in turn is coupled to an allylic methylene group 2H-17 [δ_H 2.49 (brq, 7.4 Hz)] associated with a cis-disubstituted double bond [δ_H 6.1 (H-16), dt, 10.8 and 7.4 Hz ; δ_H 5.5 (H-15), brd, 10.8 Hz]. H-15 shows no further vicinal coupling . This information leads to a second part structure **(B).**

The HMBC spectrum (Table-2) confirmed part structure **(B).** The C-18 methylene protons correlated with C-19, C-20, C-17 and C-16, while the C-17 methylenes correlated with C-19 and C-18, both the olefinic carbons C-16, C-15 and one of the acetylenic carbons C-12, *via* a long-range correlation through the double bond. Thus the acetylenes [δ_H 78.38 (C-13) ; 65.10 (C-14) ; 84.96 (C-11) ; 71.85 (C-12)] are conjugated with the disubstituted double bond as indicated previously by the ene-diyne UV chromophore and the lack of further vicinal coupling of H-15 (brd). In the HMBC spectrum, H-16 correlates with C-14 and C-13 while H-15 correlates with all the ethynyl carbons. The correlation of H-15 with C-11 represents a $^{5}J_{CH}$ across the conjugated system.

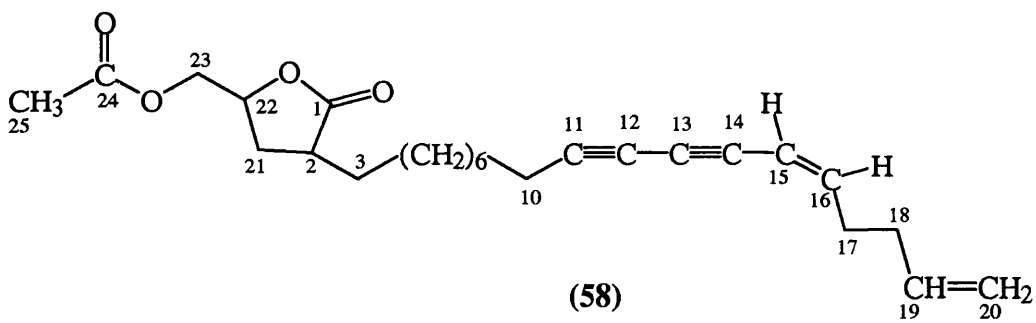
The other end of the conjugated system was readily revealed by inspection of the COSY and HMBC spectra. H-15 showed long range correlation ($^{7}J_{CH}$) with a methylene group 2H-10 at δ_H 2.35 (t, 6.98 Hz) which is clearly coupled to a second methylene 2H-9 at δ_H 1.56 (quintet, 7.69 Hz). These methylene protons show some remarkable

long-range correlations (up to $^{8}J_{CH}$) in the HMBC spectrum with 2H-10 correlating with all the carbons of the conjugated system. Correlations to C-12 were observed from the C-9 methylene protons. These results lead to part structure (C)



(C)

The two part structures (A) and (C) incorporate all the functionality of the natural product, leaving only the five overlapping methylene groups in the ^{1}H NMR spectrum to be accommodated. The COSY spectrum shows that both the C-9 and the C-3 methylene are further coupled in the methylene envelope and so the part structures can be joined as shown in (**58**) to complete the structure.



(**58**)

The *Miliusa* compound (**58**) differs from the compounds normally classified as "acetogenins" in several respects. It has a smaller alkyl chain which lacks oxygenation and there is no unsaturation in the lactone ring. It represents a further addition to the wide range of unsaturated fatty acid derivatives which contain isolated or conjugated acetylenic units. The ene-diyne chromophore occurs widely, often with both the Z and the E forms present. For example, the acids (**59**) and (**60**) have been isolated from the root bark and/or the seed oil of *Paramacrolobium caeruleum, Exocarpus cupressiformis* and other species[72,73]. The compound which bears the closest relationship to (**58**) is sapranthin

(**61**)[74] from the bark of *Sapranthus palanga* (Annonaceae)). It is a C_{21} molecule, lacks the primary hydroxyl group but is oxygenated on the lactone ring. The ^{1}H NMR, ^{13}C NMR, COSY, HMBC and HSQC spectra of compound (**58**) can be seen at the end of the chapter.

EXPERIMENTAL

The plant material was obtained from Bangladesh. A reference sample of the plant is deposited in the herbarium of the University of Dhaka. The dried powdered bark (1.1 kg) was extracted with diethyl ether to give a crude extract (22 g) as a mobile oil. Preliminary analytical TLC showed the presence of several compounds which absorbed strongly in the UV, accompanied by less polar compounds which lacked UV absorption. A portion of the extract (10g) was subjected to flash chromatography using increasing concentrations of ether in light petroleum as eluent. Fractions with similar TLC profiles were combined. Extensive preparative TLC failed to yield pure compounds though spectroscopic analysis of the fractions enabled us to identify some of the structural features present these compounds. In time it also became apparent that some of the compounds were not stable in air.

A portion of the extract (5g) was subjected to acetylation in the usual way. The compounds in the acetylated extract were separated by flash chromatography on a column of silica gel G_{254} by using the same eluent. Compounds from separated fractions were purified by preparative thin layer chromatography (TLC) on silica gel GF_{254}. Further purification was done by using High Pressure Liquid Chromatgraphy (HPLC). Eluents for TLC were increasing percentages of diethyl ether in light petroleum. From the multiple elution preparative TC, several bands were obtained and one of which proved to be pure as judged by its ^{1}H and ^{13}C NMR spectra. The compound was analysed by COSY, HSQC and HMBC NMR experiments, which were done on a 500 MHz Brucker instrument. Compound (**58**) was obtained as an oil (m/z 398)

Compounds were visualised by using UV light or iodine vapour. Solvents were evaporated using a Buchi Rotavapor. The purified compounds were subjected to spectroscopic analysis. The ^{13}C and ^{1}H NMR chemical shifts and the direct and long-

range carbon-proton correlations of **(58)** are given below (the numbers refer to decending order of the carbon chemical shifts) :

TABLE-1 ^{13}C and ^{1}H shifts of **(58)**

	^{13}C δ(ppm)	^{1}H δ(ppm)	Multiplicities (J, Hz)
1	178.78		
2	39.02	2.68	dq (5.25, 9.04)
3 3`	31.00	1.88 ca 1.5	m m
4	29.15*	ca 1.3	
5	29.15*		
6	28.90*	ca 1.4	
7	28.71*		
8	27.15*		
9	28.14	1.56	q (7.69)
10	19.54	2.35	t (6.98)
11	84.96		
12	71.85		
13	78.38		
14	65.10		
15	108.55	5.50	brd (10.8)
16	146.60	6.10	dt (10.8, 7.4)
17	29.82	2.49	brq (7.41)
18	32.78	2.20	q (7.39)
19	137.55	5.85	ddt (17.0, 10.3, 6.5)
20 20`	115.13	5.08 5.01	brd (17.0) brd (10.3)
21	30.02	2.25 & 2.1	m
22	77.23	4.75	ddt (8.4, 4.8, 4.9)
23 23`	65.49	4.29 4.15	dd (12.1, 3.4) dd (12.1, 5.4)
24	170.54		
25	20.73	2.12	s

* May be interchanged

TABLE-2 HMBC Correlations of **(58)**

δ_C (ppm)	C	Type	H
178.78	1	carbonyl	22, 2, 21, 21`, 3, 3`
39.02	2	methine	22, 21, 21`, 3, 3`
31.00	3	methylene	2, 21, 21`
84.96	11	acetylene	17, 15
71.85	12	acetylene	15
78.38	13	acetylene	16, 15
65.10	14	acetylene	16, 15, 17
108.55	15	vinyl	16, 17, 10
146.60	16	vinyl	17, 10, 18
29.82	17	methylene	16, 19, 15, 18, 14
32.78	18	methylene	16, 19, 20, 17
137.55	19	vinyl	17, 18, 20
115.13	20	vinyl	18
30.02	21	methylene	22, 23, 23`, 2
77.23	22	methine	23, 23`, 2, 21, 21`
65.49	23	methylene	21, 21`
170.54	24	carbonyl	23, 23`, 25
20.73	25	acetate	

TABLE-3 HSQC Correlations of **(58)**

	C	δ_H (ppm)	No.
39.02	2	2.68	1H
31.00	3	1.88 and 1.5	2H
108.55	15	5.50	1H
146.60	16	6.10	1H
29.82	17	2.49	2H
32.78	18	2.20	2H
137.55	19	5.85	1H
115.13	20	5.08 and 5.01	2H
30.02	21	2.25 and 2.1	2H
77.23	22	4.75	1H
65.49	23	4.29 and 4.15	2H
20.73	25	2.12	3H

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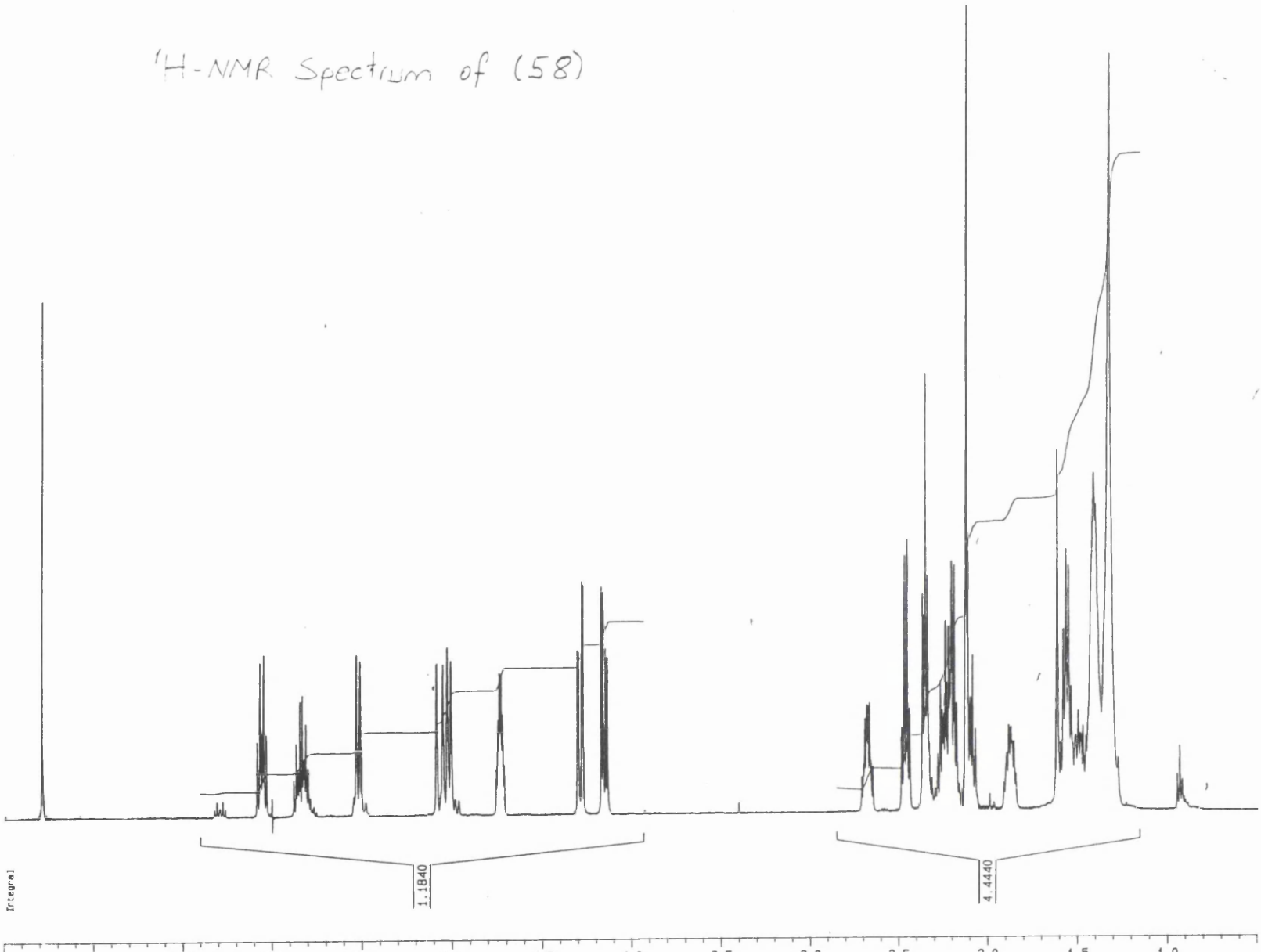
F60_3 1 1 v jmn

^{1}H-NMR Spectrum of (58)



Current Data Parameters

NAME	F60_3
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

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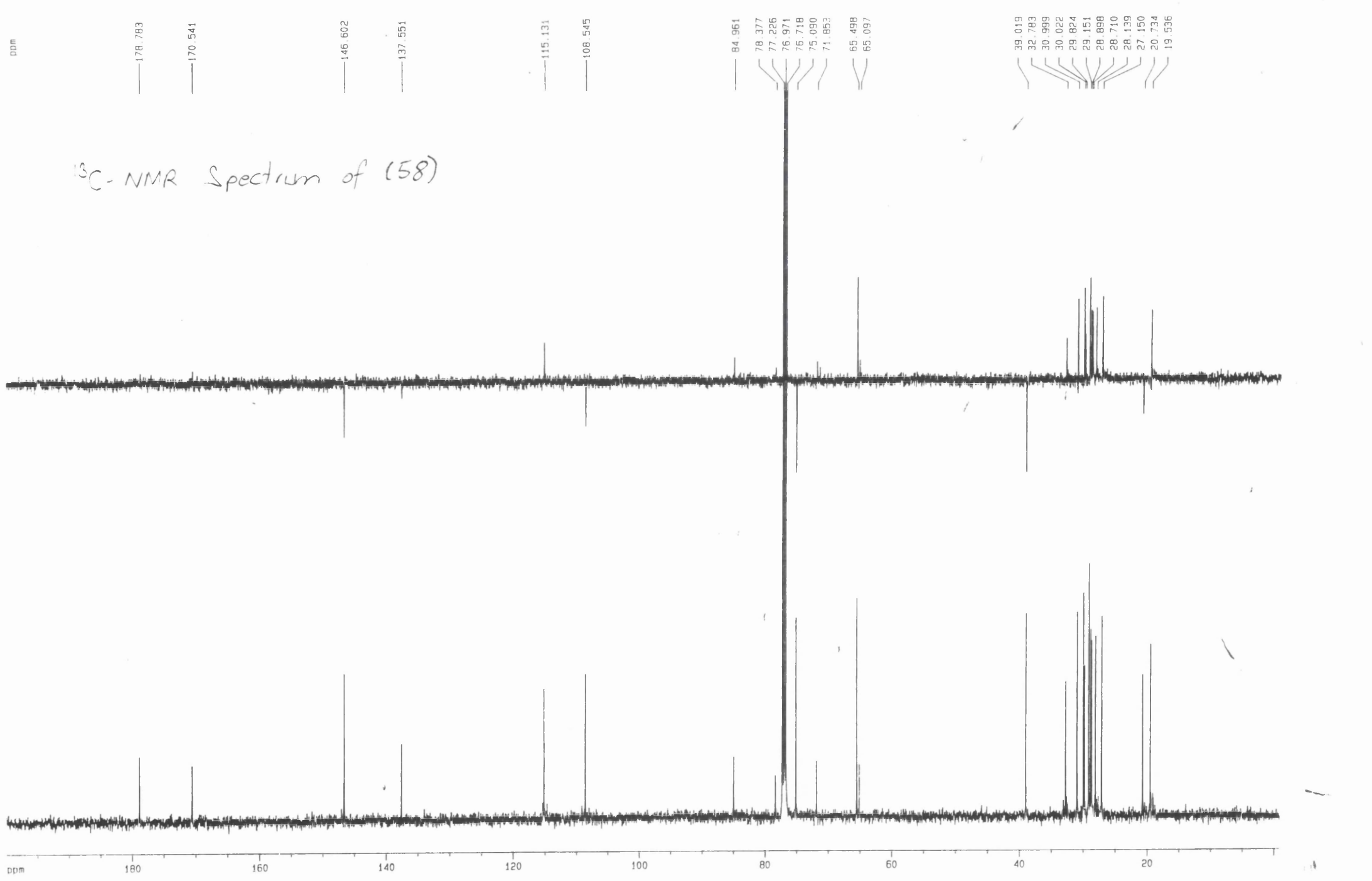
PROBHD	5 mm Multinu
PULPROG	zg
TD	8192
SOLVENT	CDCl3
NS	1
DS	0
SWH	6009.615 Hz
FIDRES	0.733596 Hz
AQ	0.6816244 sec
RG	90.5
DW	83.200 usec
DE	10.50 usec
TE	300.0 K
D1	1.00000000 sec
P1	5.00 usec
DE	10.50 usec
SFO1	500.1330008 MHz
NUC1	1H
PL1	2.00 dB

F2 - Processing parameters

SI	16384
SF	500.1300000 MHz
WDW	no
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

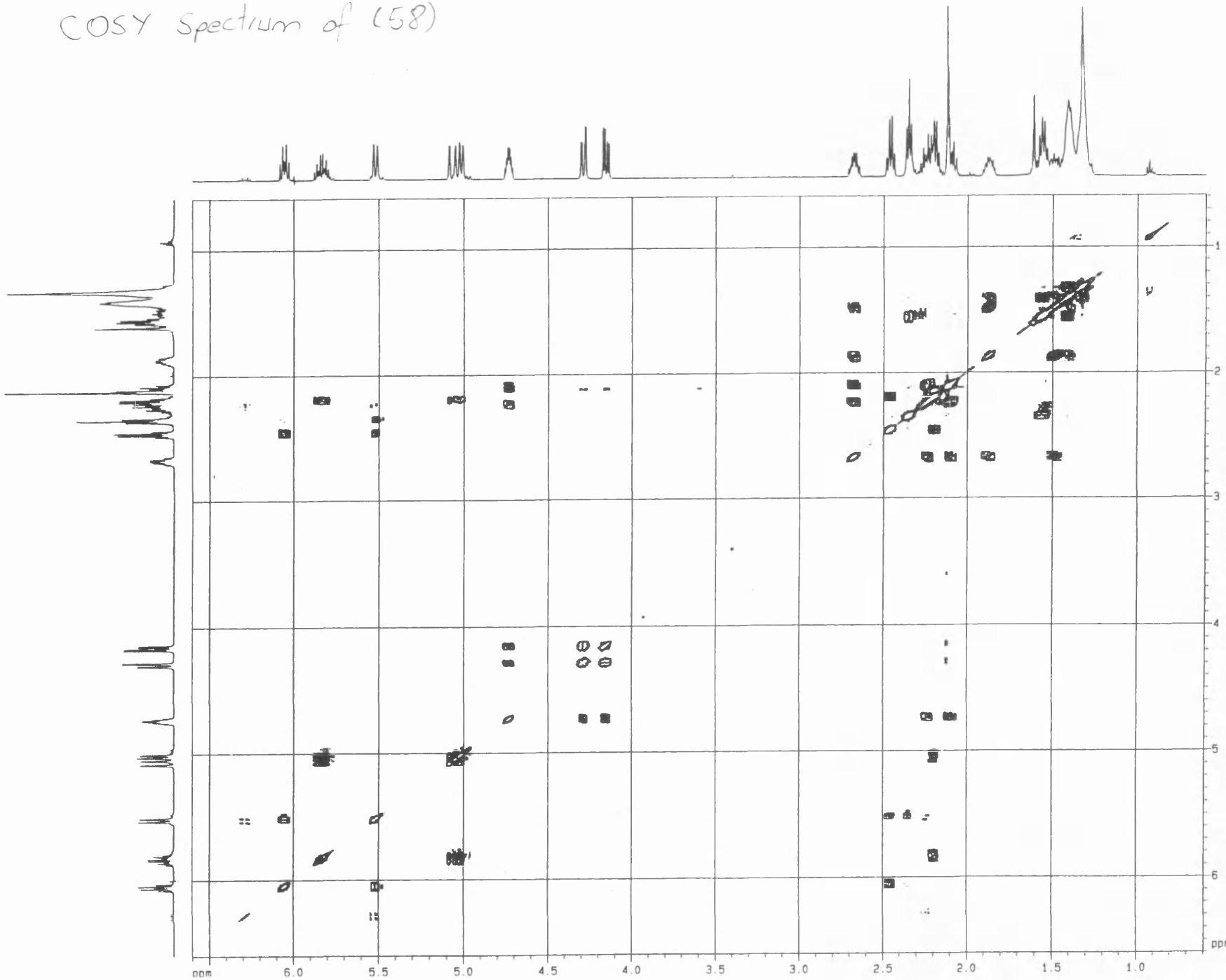
1D NMR plot parameters

CX	30.00 cm
F1P	7.500 ppm
F1	3750.98 Hz
F2P	0.500 ppm
F2	250.07 Hz
PPMCM	0.23333 ppm/cm
HZCM	116.69700 Hz/cm



^{13}C- NMR Spectrum of (58)

COSY Spectrum of (58)



Current Data Parameters

NAME	F60_3
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date_	970327
Time	17.56
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	cosygp
TD	4096
SOLVENT	CDCl3
NS	2
DS	4
SWH	3004.808 Hz
FIDRES	0.733596 Hz
AQ	0.6816244 sec
RG	362
DW	166.400 usec
DE	10.50 usec
TE	300.0 K
D1	1.00000000 sec
P1	9.50 usec
D0	0.00000300 sec
P15	2000.00 usec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	10.00 %
GPNAM1	sine.100
D16	0.00010000 sec
P0	5.00 usec
D13	0.00000300 sec
GPX2	0.00 %
GPY2	0.00 %
GPZ2	10.00 %
GPNAM2	sine.100
DE	10.50 usec
SFO1	500.1318005 MHz
NUC1	1H
PL1	2.00 dB
IN0	0.00033280 sec

F1 - Acquisition parameters

ND0	1
TD	512
SFO1	500.1316 MHz
FIDRES	5.868770 Hz
SW	6.008 ppm

F2 - Processing parameters

SI	1024
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

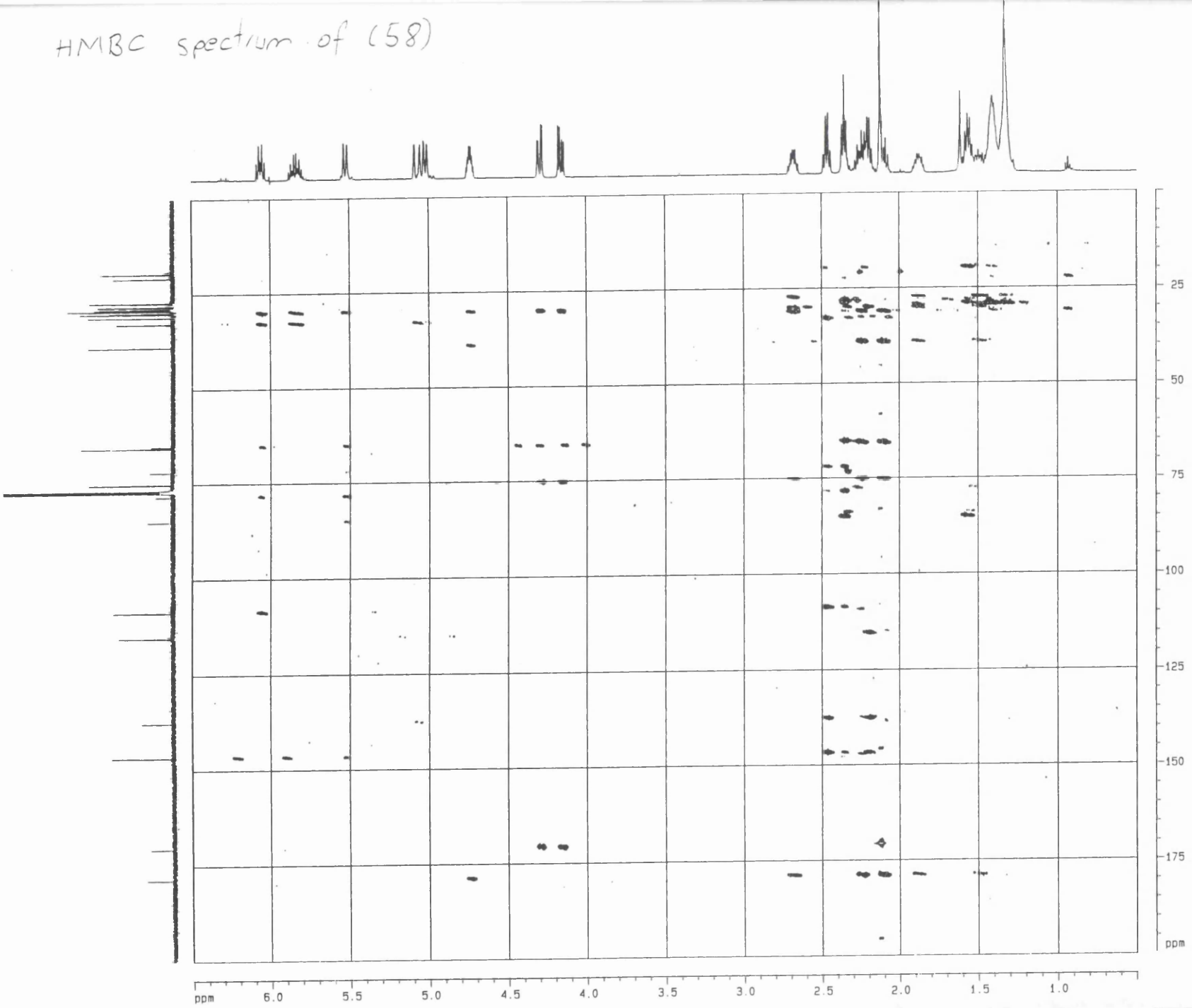
F1 - Processing parameters

SI	1024
MC2	QF
SF	500.1297564 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	27.00 cm
CX1	20.00 cm
F2PLO	6.504 ppm
F2LO	3302.57 Hz
F2PHI	0.596 ppm
F2HI	298.96 Hz
F1PLO	6.500 ppm
F1LO	3300.96 Hz
F1PHI	0.592 ppm
F1HI	296.05 Hz
F2PPMCM	0.22252 ppm/cm
F2HZCM	111.28918 Hz/cm
F1PPMCM	0.30040 ppm/cm

HMBC spectrum of (58)



NAME	F60_3
EXPNO	5
PROCNO	1

F2 - Acquisition Parameters

Date_	970327
Time	20.24
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	inv4gp1p1rnd
TD	2048
SOLVENT	C6D6
NS	16
DS	8
SWH	3004.808 Hz
FIDRES	1.467191 Hz
AQ	0.3408372 sec
RG	32768
DW	166.400 usec
DE	13.00 usec
TE	300.0 K
D1	1.50000000 sec
P1	9.50 usec
D2	0.00345000 sec
P3	10.00 usec
SFO2	125.7703148 MHz
NUC2	13C
PL2	-4.00 dB
D6	0.07000000 sec
D0	0.00000300 sec
P16	2000.00 usec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	50.00 %
GPNAM1	sine.100
D16	0.00010000 sec
P2	19.00 usec
GPX2	0.00 %
GPY2	0.00 %
GPZ2	30.00 %
GPNAM2	sine.100
D13	0.00000300 sec
GPX3	0.00 %
GPY3	0.00 %
GPZ3	40.00 %
GPNAM3	sine.100
DE	13.00 usec
SFO1	500.1317505 MHz
NUC1	1H
PL1	2.00 dB
IN0	0.00001988 sec

F1 - Acquisition parameters

ND0	2
TD	512
SFO1	125.7665 MHz
FIDRES	49.127525 Hz
SW	200.000 ppm

F2 - Processing parameters

SI	1024
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	0.10

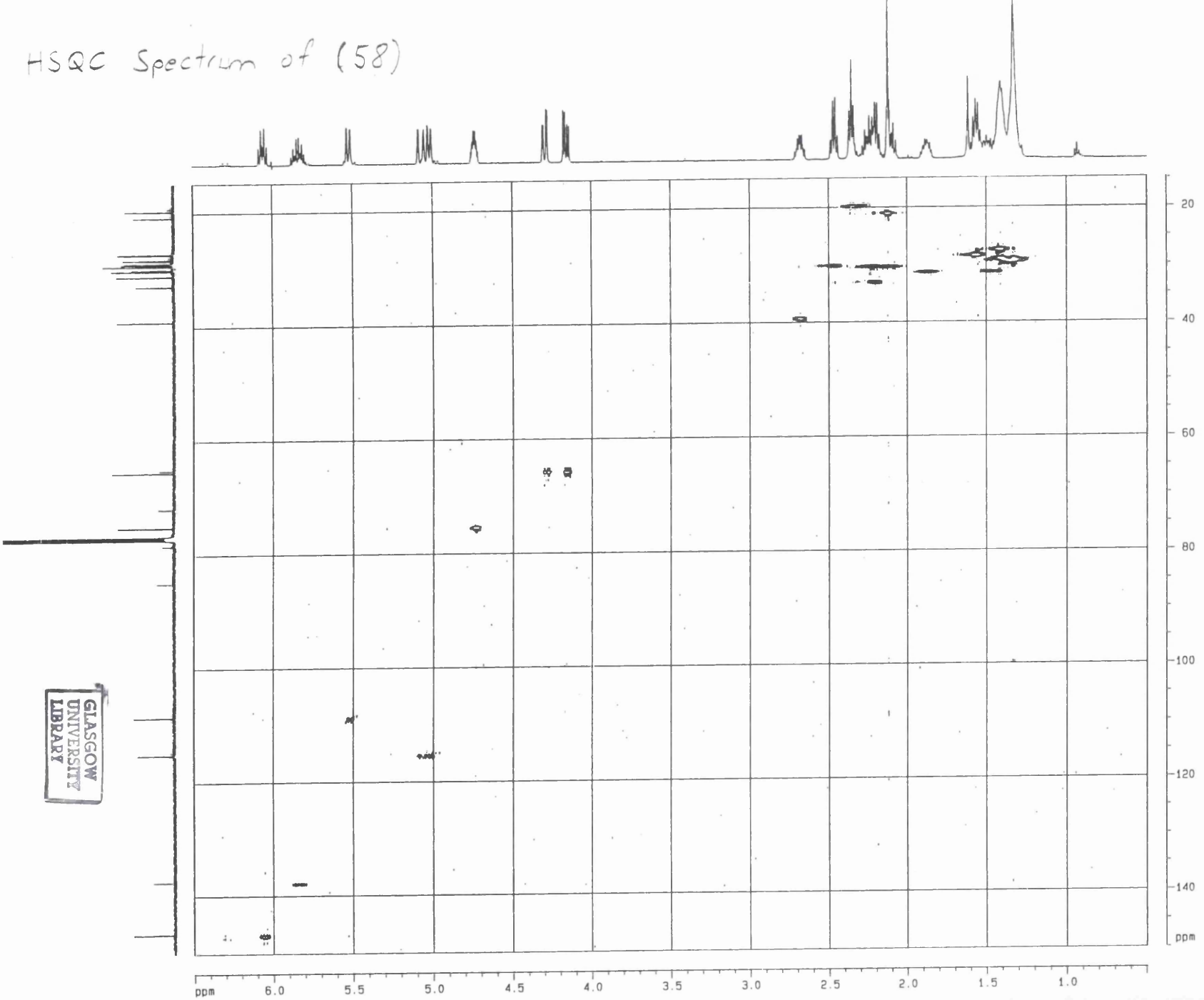
F1 - Processing parameters

SI	1024
MC2	QF
SF	125.7539405 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PLO	6.504 ppm
F2LO	3252.86 Hz
F2PHI	0.496 ppm
F2HI	248.05 Hz
F1PLO	200.000 ppm
F1LO	25150.79 Hz
F1PHI	-0.020 ppm
F1HI	-2.51 Hz
F2PPMCM	0.24032 ppm/cm
F2HZCM	120.19230 Hz/cm
F1PPMCM	10.00100 ppm/cm

HSQC Spectrum of (58)



PROCNO 1

F2 - Acquisition Parameters

Date_	970327
Time	18.27
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	inviedgptp
TD	4096
SOLVENT	CDCl3
NS	8
DS	8
SWH	3004.808 Hz
FIDRES	0.733596 Hz
AQ	0.6816244 sec
RG	32768
DW	166.400 usec
DE	10.50 usec
TE	300.0 K
D13	0.00000300 sec
D24	0.00345000 sec
P16	1200.00 usec
D16	0.00010000 sec
d20	0.0021470 sec
D4	0.00172000 sec
d21	0.0004130 sec
D11	0.03000000 sec
PL12	16.50 dB
D1	1.00000000 sec
P1	9.50 usec
PL2	-4.00 dB
P2	19.00 usec
P4	18.00 usec
SFO2	125.7703148 MHz
NUC2	13C
P3	9.00 usec
D0	0.00000300 sec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	30.00 %
GPNAM1	sine.100
GPX2	0.00 %
GPY2	0.00 %
GPZ2	80.00 %
GPNAM2	sine.100
GPX3	0.00 %
GPY3	0.00 %
GPZ3	20.00 %
GPNAM3	sine.100
DE	10.50 usec
SFO1	500.1317505 MHz
NUC1	1H
PL1	2.00 dB
CPDPRG2	garp
PCPD2	80.00 usec
IN0	0.00000994 sec

F1 - Acquisition parameters

ND0	4
TD	512
SFO1	125.7703 MHz
FIDRES	49.129028 Hz
SW	200.000 ppm

F2 - Processing parameters

SI	2048
SF	500.1300000 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00

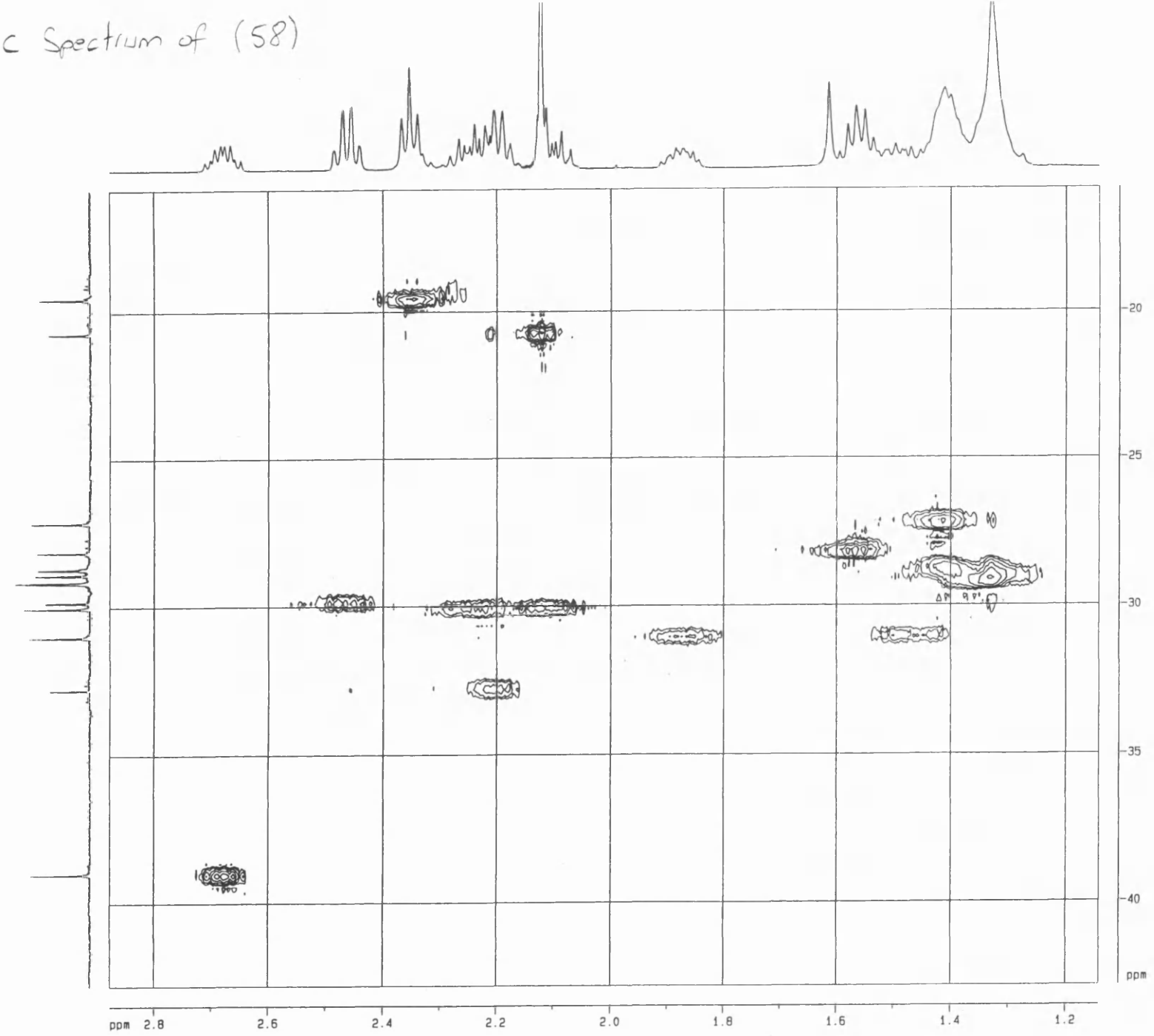
F1 - Processing parameters

SI	1024
MC2	TPPI
SF	125.7577390 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PLO	6.504 ppm
F2LO	3252.86 Hz
F2PHI	0.496 ppm
F2HI	248.05 Hz
F1PLO	150.005 ppm
F1LO	18864.29 Hz
F1PHI	14.835 ppm
F1HI	1865.65 Hz
F2PPMCM	0.24032 ppm/cm
F2HZCM	120.19230 Hz/cm
F1PPMCM	6.75849 ppm/cm
F1HZCM	849.93019 Hz/cm

HSQC Spectrum of (58)



PROCNO 1

F2 - Acquisition Parameters

Date_	970327
Time	18.27
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	inviedgptp
TD	4096
SOLVENT	CDCl3
NS	8
DS	8
SWH	3004.808 Hz
FIDRES	0.733596 Hz
AQ	0.6816244 sec
RG	32768
DW	166.400 usec
DE	10.50 usec
TE	300.0 K
D13	0.00030300 sec
D24	0.00345000 sec
P16	1200.00 usec
D16	0.00010000 sec
d20	0.0021470 sec
D4	0.00172000 sec
d21	0.0004130 sec
D11	0.03000000 sec
PL12	16.50 dB
D1	1.00000000 sec
P1	9.50 usec
PL2	-4.00 dB
P2	19.00 usec
P4	18.00 usec
SFO2	125.7703148 MHz
NUC2	13C
P3	9.00 usec
D0	0.00000300 sec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	30.00 %
GPNAM1	sine.100
GPX2	0.00 %
GPY2	0.00 %
GPZ2	80.00 %
GPNAM2	sine.100
GPX3	0.00 %
GPY3	0.00 %
GPZ3	20.00 %
GPNAM3	sine.100
DE	10.50 usec
SFO1	500.1317505 MHz
NUC1	1H
PL1	2.00 dB
CPDPRG2	garp
PCPD2	80.00 usec
IN0	0.00000984 sec

F1 - Acquisition parameters

ND0	4
TD	512
SFO1	125.7703 MHz
FIDRES	49.129028 Hz
SW	200.000 ppm

F2 - Processing parameters

SI	2048
SF	500.1300000 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00

F1 - Processing parameters

SI	1024
MC2	TPPI
SF	125.7577850 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PLO	2.878 ppm
F2LO	1439.41 Hz
F2PHI	1.141 ppm
F2HI	570.83 Hz
F1PLO	42.785 ppm
F1LO	5380.54 Hz
F1PHI	15.829 ppm
F1HI	1990.63 Hz
F2PPMCM	0.06947 ppm/cm
F2HZCM	34.74309 Hz/cm
F1PPMCM	1.34779 ppm/cm
F1HZCM	169.49522 Hz/cm