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"A PHYTOCHEMICAL STUDY OF SOME NEPALESE LIVERWORTS"

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by

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A Phytochemical Study of Some Nepalese Liverworts

Summary

This thesis describes a phytochemical investigation of three members of the Hepaticae family (liverworts) from Nepal. The species examined were *Marchantia paleacea* Betrol subsp. *paleacea*, *Marchantia papillata* Raddi subsp. *grossibarba* (steph) Bishl and *Plagiochasma appendiculatum* Lehm et. Lindenb. *M. paleacea* yielded the bisbibenzyl derivatives marchantins A, C and D, *p*-hydroxybenzaldehyde, 2-hydroxy-3,7-dimethoxyphenanthrene, the corresponding 1,1-dimer and the interesting new compound 3,4-dihydro-8-hydroxy-4-(4-hydroxyphenyl)-isocoumarin. Marchantins A and C and the above phenanthrene were obtained from *M. papillata* while marchantins A and B and riccardin D were isolated from *Plagiochasma appendiculatum.* The structures were assigned from 1D and 2D ^{1}H and ^{13}C NMR spectra and were confirmed, where appropriate, by comparison with published data. The thesis is prefaced by a general review of the Hepaticae and their chemical constituents.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Classification of Natural Products

Natural products have been known since the formation of living beings and have been used in medicine since at least 4000 BC.[91] Initially the use of natural products was totally traditional. Over the years knowledge of the chemistry of natural products has gradually changed from being traditionally descriptive in nature to being mechanistic and predictable. Natural product chemistry is an ancient science. Activities involving the preparation of foodstuffs, colouring matters, fibre, toxins, medicines and stimulants are as old as mankind. The most important reason for studying natural product chemistry resides in the biological activity found in natural products. The true properties of extracts obtained from Nature aroused great curiosity amongst scientists. They began to separate, purify and finally analyse the compounds produced in living cells. Efficient separation techniques e.g. column chromatography, g.c., preparative tlc, hplc, paper chromatography, ion-exchange *etc* were developed to isolate chemical constituents from natural sources. These methods have made it possible to isolate compounds which are present in extremely small amounts. Following the separation and purification of the compounds, different spectroscopic techniques like ^{1}H and ^{13}C NMR (including 2D NMR, COSY,

HMBC, HMQC), IR, UV and MS are used for their structure elucidation. With the help of these techniques the structure elucidation of compounds present in sub-milligram quantities is possible these days. In difficult cases X-ray crystallography may be used, provided the crystals are available.

Many medicinal, pharmacological and other biological agents are either natural products themselves, derivatives of them or modified templates of natural products. Thus the investigation of natural product chemistry is a never ending task for mankind.

According to Natori [95] natural products may be classified on the following basis:

a) Chemical structure: This classification is based on molecular skeleton.[107]

i) *Open–chain aliphatic or fatty compounds:* e.g., fatty acids, sugars, most amino acids.

ii) *Alicyclic or Cycloaliphatic compounds:* e.g., terpenoids, steroids, some alkaloids.

iii) *Aromatic or Benzenoid compounds:* e.g., phenolics, quinones.

iv) *Heterocyclic compounds:* e.g., alkaloids, flavonoids, nucleic acid bases.

Since this is merely a superficial classification, it is obvious that many closely related natural products will belong to more than one class. For example, geraniol (**28**), farnesol (**29**), and squalene (**30**) belong to class *i)* and thymol (**31**) to class *iii)*, but because of biogenetic consideration, they are usually treated with other terpenoids and steroids under class *ii).*[110]

b) ***Physiological Activity*:** Morphine (**32**) (1806), penicillin (**33**) (1939), and prostaglandins (**34**) (1963) are physiologically active factors of plant and animal origin. Hence, a classification based on physiological activity is frequently employed e.g., hormones, vitamins, antibiotics, and mycotoxins. Although compounds belonging to each group have diverse structures and biosynthetic origins, occasionally a close correlation is found between such aspects and activity.

c) ***Taxonomy:*** This is based on comparative morphological studies of plants, i.e., plant taxonomy. Even a single species contains numerous constituents which have closely related structures. For example, the 'opium' from *Papaver somniferum* contains twenty-odd alkaloids such as morphine (**32**), thebaine (**35**), codeine (**36**), and narcotine (**37**), all of which are biosynthesized from the 1-benzylisoquinoline precursor (**38**) by oxidative coupling. Thus alkaloids having these similar structures are characteristic constituents of this plant genus and are designated as opium alkaloids. The advance methods of isolation and micro-characterisation of plant constituents have led to a new field 'chemotaxonomy' or 'chemosystematics' which attempts to review plant constituents according to plant taxa.[49, 87] Thus the constituents contribute to the classification of plants.

d) ***Biogenesis:*** The constituents of all plants are biosynthesised in organisms by enzymatic reactions. A major source of carbon is glucose, which is photosynthesised in green plants. On this basis natural products fall into two

groups, primary and secondary metabolites. The former, organic compounds which are characteristic of all living systems, includes carbohydrates, lipids, amino acids, peptides and proteins, nucleosides, nucleotides, and nucleic acids. On the other hand, secondary metabolites include phenols, quinones, terpenoids, alkaloids and various pigments which organisms produce. Secondary metabolites are wholly or partly derived from primary metabolites. Secondary metabolites are formed by specific chemical processes which take place only in certain species and give different products depending upon the species, whereas primary metabolites have broad distribution in all living things and are virtually identical even in those species that are genetically very different

1.2 Classification of the Hepaticae

The name bryophytes was first introduced by Braun (1864).[99] However, he included algae, fungi, lichens, and mosses. At present the bryophytes are placed between algae (thallophytes) and pteridophytes (ferns).[1] With the exception of a few aquatic forms, bryophytes are truly land-inhabiting plants although they need water to survive. They have adopted themselves to a terrestial life. The bryophytes are known as the *amphibians* of the plant kingdom. They grow frequently in humid and shady places.

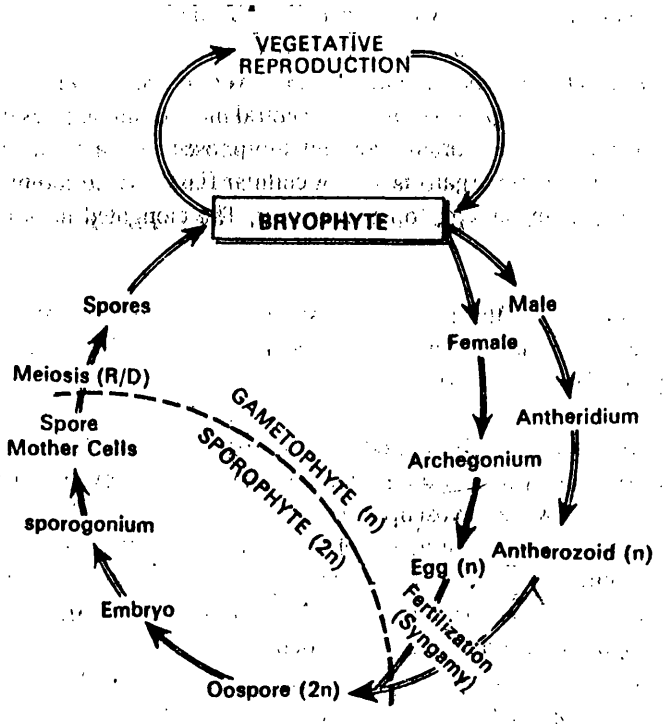
The division of bryophytes includes about 960 genera and 24,000 species.[98] The bryophytes are divided into three classes.

Class I: Hepticopsidia (Hepaticae or Liverworts) which includes 225 genera and 8,500 species.[98]

Class II: Anthocerotopsida (Anthocerotae or Hornworts) which includes 6 genera and 301 species.[98]

Class IV: Bryopsida (Musi or Mosses) which includes 650 genera and 14,000 species.[98]

Of these, the chemically most interesting are liverworts, due to the presence, in their cells, of oil bodies[53] which contain secondary metabolites. Hornworts and mosses do not contain oil bodies. The liverworts and mosses form two distinct classes within the bryophytes. 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. Liverworts may have leaves (leafy liverworts) or may not have leaves (thalloid). They have a unique life cycle.[1,98,127] Most of the liverworts have a chromosome number n=9, while mosses begin with n=11 and hornworts have n=5 or 6. The life cycle of bryophytes is thus different from that of higher plants including pteridophytes. The liverworts contain a surprising range[1] of chemical structures, some of which show interesting biological activity. The oil bodies, a characteristic feature of liverworts, store these natural products and elaborate terpenoids and lipophilic aromatics as their major chemical constituents. Oil bodies (cell organelles) vary in shape, size and number per cell. The main types are the segmented oil body [Figure 1, A and B (x 600)] and the homogeneous oil body [Figure 1, C and D (x 200)].[40] Segmented oil bodies have several to numerous droplets bound within the membrane whereas homogeneous oil bodies are made up of one lipid



Bryophyte. Typical graphic life-cycle

FIG. 1.

Segmented

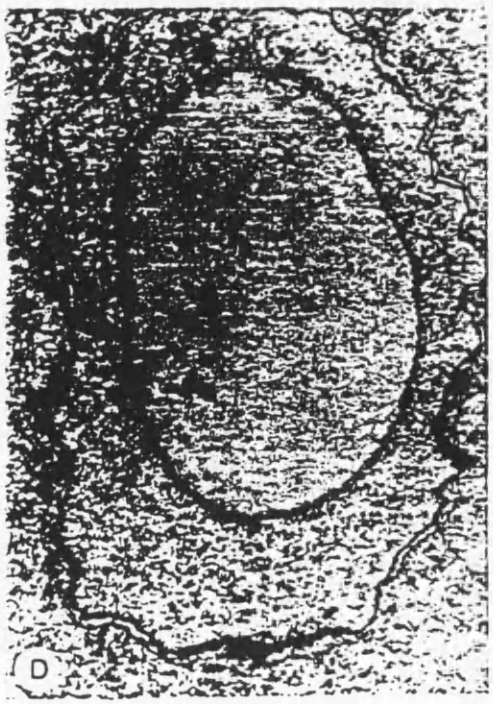
Length of oil bodies: 6-18 µm

x 6000

Homogeneous

Length of oil bodies: 5-7 µm

x 20000



droplet surrounded by a membrane. In dried specimens the oil bodies disintegrate and chemical constituents are easily extracted with organic solvents.

It is often hard to collect liverworts in a reasonable quantity as they have a tendency to grow intermingled with other liverworts and mosses. In some cases the amount of plant material collected is just sufficient to isolate and elucidate the structure of main constituents and is not enough for biological tests. In such cases, *in vitro* culture[33] of liverworts helps to obtain sufficient plant material for further investigation of these secondary metabolites. Reports on the production of secondary metabolites by *in vitro* culture of liverworts are fewer than those for higher plants.

The liverworts are morphologically divided into two sub-classes[53] which are the **Jungermanniidae** and **Marchantiidae.** The general difference between these two sub-classes is the kind of habitat in which they grow. The sub-class Jungermanniidae represents the liverwort adapted to a moist climate while the sub-class Marchantiidae grows in dry climatic regions. Within the sub-class Jungermanniidae the oil bodies occur in green phytosynthetic cells whereas in the sub-class Marchantiidae they are restricted to special oil cells lacking chlorophyll. In some groups of liverworts the oil bodies are absent. These two sub-classes are further divided into following orders.

Sub class Jungermanniidae

Order Calobryales: The order Calobryales consists of a single family, the Calobryaceae with 2 genera, *Calobrium* and *Haplomitrium. Calobrium* includes 8 species of which *Calobrium bulmii* is the best known. *Haplomitrium* is represented by a single species *H. hookeri.*[98] Most of this order occur in the

Southern hemisphere and in South-east Asia. They grow in moist habitats and are very sensitive to desiccation.

Order Jungermanniales: The order Jungermanniales, the largest order of Hepaticae, consists of 40 families with 250 genera and 35,00 species.[53] The Jungermanniales (leafy liverworts) form the largest group within the Hepaticae and contain about 80% of all species. These species are widely distributed around the world.

Order Metzgeriales: The order Metzgeriales, the second largest order of the Hepaticae, consists of 12 families with 30 genera and about 600 species. It includes mainly thalloid liverworts. Distribution is world wide and habitat includes both dry and wet regions

Sub-class Marchantiidae

Order Monocleales: The order Monocleales consists of a single genus (*Monoclea*) whose distribution is confined to New Zealand and tropical America. This order also includes thalloid liverworts.

Order Marchantiales: The order Marchantiales, the third largest order of the Hepaticae, consists of 14 families with about 35 genera and approximately 42 species. These are thalloid liverworts that are adapted to semi-arid and arid areas as well as arctic, alpine, and tropical high mountain regions. These are distributed world wide. *Riccia* and *Marchantia* are two large genera of this order.

Order Sphaerocarpales: The order Sphaerocarpales, a very small order, consists of 2 families with 3 genera and about 25 species. The species occur mainly in the arid and semi-arid regions of the world.

The ***Anthocerotales*** or hornworts (single family, 6 genera, and 301 species) and ***Takakiales*** (single family,single genus and 2 species) are classified within the Hepaticae (Grolle 1983) although arguments have been put forward to remove them from the liverworts. Some features of the Anthocerotales seem to justify the categorising of this order in a different sub class. The anomalous character of this group has been noticed by Leitgeb[77] and Cavers.[41] Howe(1899)[64] placed it in a different class under the name ***Anthocerotae.***

1.3 Constituents of the Hepaticae

For many years the phytochemistry of liverworts was not been taken seriously, perhaps because of the difficulty in their collection. Many liverworts prefer wet, humus-rich habitats like damp rocks, the forest floor, swamp or marshes or beside streams and pools. It is often troublesome to collect them in sufficient amount for chemical research. However, the first chemical investigation of liverworts was carried out by Muller in 1905[92] and he reported the presence of sesquiterpenoids in the oil bodies of several species. It was not until 1956 that the chemical constituents of liverworts were further investigated. Fujita *et al*[50] identified sesquiterpene hydrocarbons in the essential oil of *Bazzania pompeana.* Many papers reviewing the chemistry of liverworts are available these days. Such reviews include:

i) Markam and Porter,[78] concerning bryophyte chemistry viz lipids, terpenoids, flavonoids, lignins, and dihydrostilbenes, prior to 1978.

ii) Connolly,[43] concerning terpenoid constituents of some European liverworts.

iii) Asakawa,[1] concerning a comprehensive review of terpenoids, aromatics and lipids of liverworts prior to 1981.

iv) Huneck,[66] concerning all liverworts metabolites prior to 1981.

v) Asakawa,[2] *Bryophytes: Their Chemistry and Chemical Taxonomy* from 1981 to 1989.

vi) Asakawa,[4] liverwort metabolites until 1995.

The chemical constituents present in liverworts are:

1.3.a Monoterpenoids: Thermal decomposition of almost all terpenoids gives isoprene as one of the products and this led to the suggestion that the skeletal structures of all naturally occurring terpenoids can be formed by head to tail joining of isoprene units (Ruzicka, 1958).[37,103] These terpenoids are classified depending on the number of isoprene units e.g., Monoterpenoids C_{10}, Sesquiterpenoids C_{15}, Diterpenoids C_{20}, Triterpenoids C_{30} etc.

The occurrence of monoterpenoids in liverworts has not been reported in a great detail. Monoterpenoids are responsible for the characteristic odour of many liverworts. They are used in perfumery and the flavour industries. The identification of monoterpenoids in liverworts has been carried our largely by GC and GCMS.[1] They are mainly hydrocarbons and generally occur in complex mixtures with sesquiterpenoid hydrocarbons, which are difficult to separate. However, Asakawa *et al* presented [20,108] examples of pure monoterpenoids

isolated from liverworts. The first detailed report on the monoterpenoid composition of liverworts was published by Svensson[109] in 1974. He described the monoterpenoid hydrocarbons from the essential oils of *Jungermannia cordifolia* and *J. obovata.* Among the monoterpenoids identified by GCMS, camphene (**21**) dominates in *J. cordifolia* while terpinolene (**11**) and limonene (**8**) are the main components in *J. obovata* which has a characteristic carrot aroma.

Examples of monoterpenoids found in liverworts are shown in structures (**1**) to (**27**). Among these myrcene (**1**), β-phellandrene (**7**), limonene (**8**), α-terpinene (**9**), *p*-cymene (**14**), α-pinene (**18**), β-pinene (**19**) and camphene (**21**) are the commonest monoterpenoids reported.[15,63] Connolly *et al* [44] have isolated (-)-thujanol (**39**), and its epimer (**40**) together with α-terpineol (**12**) and terpineol (**13**) from the liverwort *Conocephalum conicum.* They have also isolated the *trisnor* monoterpenoid (**41**), a degradation product of ascaridole (**42**). Myrtenal (**20**) occurs in *Plagiochila mayabarae*[13], linalool (**4**) in *Plagiochila ovalifolia*[1], camphene (**21**) in *Bazzania pompeana* and *Porella perrottetiana*, and (+)-bornyl acetate (**24**) in *Wiesnerella denudata*[12]. *Cis* and *trans*–pinocarveyl acetates (**43**) and (**44**) have been reported in *Targonia hypophylla* by Asakawa *et al.*[22]

Both enantiomers of monoterpenoids occur in higher plants, often as racemic mixtures, but some species synthesize only one of the two isomers.[74] The isolation of (-)–limonene (**8**) from *Concephalum conicum*[108] and (+)–limonene (*ent*-**8**) from *J. exertifolia*[20] shows that both enantiomers occur in liverworts as in higher plants . The absolute configuration of many liverwort monoterpenoids is still undetermined. However, the highly sensitive technique

of two dimensional gas chromatography (2DGC) is being used to make good progress in this field. It has been used in the analysis of the enantiomeric composition of monoterpenoid hydrocarbons from the livewort *Conocephalum conicum.*[124]

1.3.b Sesquiterpenoids: The oil bodies of liverwort species contain sesquiterpenoids which are found in four orders Calobryales, Jungermanniales, Metzgeriales and Marchantiales[1]. However, higher plants are the principal source of sesquiterpenoids.[102] Sesquiterpenoids produced in liverworts are often enantiomeric to those produced in higher plants.[67] The presence of sesquiterpenoids in liverworts was initially recognised by Fujita *et al*[18] through a study of essential oil of *Bazzania pompeana.* Some sesquiterpenoids give a blue colour which was attributed to the presence of a small group of hydrocarbons. The name 'azulene' was given to the oils by Presse[48] in 1804. Some *Calypogeia* species contain characteristic blue oil bodies.[1]

The first important structure contribution to the sesquiterpenoid chemistry of liverworts was made by Huneck and Klein[67] (1967) by the discovery of (-)–longifolene (**45**) and (-)-longiborneol (**46**) in *Scapania undulata.* Other sesquiterpenoids from liverworts include the aromatic compounds, 1,4–dimethylazulene (**47**), 4-methyl-1-methoxycarbonylazulene (**48**) and 3,8-dimethyl-5-methoxycarbonylindene (**49**) from the blue oil bodies of *Calypogeia trichomains,*[65,88,89] and (-)–drimenol (**50**) from *Bazzania trilobata*[71].

There are several sesquiterpenoid skeleta which are unique to liverworts. Gymnomitrane (**51**), aromadendrane (**52**), 1,10-secoaromadendrane

(**53**), 2,3-secoaromadendrane (**54**), chiloscyphane (**55**), pinguisane (**56**), vitrane (**57**), myltaylane (**58**) and cyclomyltaylane (**59**) are obvious examples.

Pinguisane: The pinguisane skeleton is unique and is difficult to rationalise simply in terms of the Isoprene Rule. Pinguisanes are widely distributed in liverworts and occur in the Lejeuneaceae, Porellaceaea, Ptilidiaceae and Lepidolaenaceae of the Jugermanniales and the Aneuraceae of the Metzgeriales.[54]

The first representative of this skeleton, the ketone pinguisone (**60**), was isolated from *Aneura pinguis* by Bensova *et al* in 1969.[35] The unsual skeleton of pinguisone was later confirmed by Corbella *et al.*[47] The second representative of this skeleton, deoxopinguisone (**61**), was isolated from *Ptilidium ciliare*,[76] *Trichocoleopsis*,[17] *Lejeunea*[26,55] and *Porella*[23] *species.* Furthermore, *Porella verniocosa*[21] was found to contain pinguisenol (**62**) and α-pinguisene (**63**). Similarly, *P. platyphylla*[28] was reported to contain β-pinguisenediol (**64**), pinguisanin (**65**) and pinguisanolide (**66**). A unique spiro-pinguisane sesquiterpenoid lactone (**67**) has also been reported in a *Ptychanthus* species.[113] The biosynthesis of 3-oxopinguis-5(10)-en-11,6β-olide (**68**) has been elucidated using axenic cultures of *Aneura pinguis.*[111] It seems likely that three other sesquiterpenoids, β-monocyclonerolidol (**69**) and striatene (**70**) from *Ptychanthus striatus* (Lehm. st. Lindenb) Nees,[113] and trifarienol A (**71**) from *Cheilolejeanea trifaria* are biosynthesised [111] from intermediates on the same pathway. Pinguisone (**60**) [106] and its biosynthetically related compounds have been isolated from many liverworts of the Metzgeriales and Jungermanniales.[6]

1.3.c Diterpenoids: The presence of diterpenoids in liverworts is limited to certain genera. The first diterpenoid, *ent*-16β-hydroxykaurane (**72**), was isolated from *Anthelia julacea* and *A. juartzkana* by Huneck and Velve in 1970.[69] The labdane-, pimaranes- and kaurane-type diterpenoids so far isolated from liverworts belong to the *ent-* series while higher plants produce both enantiomeric series.

Labdanes: The labdane skeleton is common in liverworts. The first example of this skeleton, *ent*-manool (**73**), from *Jungermannia torticalyx*, was reported by Matsuo *et al.*[80] The liverwort *Scapania undulata* was found to contain scapanin A (**74**) and scapanin B (**75**).[72] Scapanin G (**76**) has also been reported from Scottish *Scapania undulata* [72] The unusual secolabdane, pallavicinin (**77**), is a constituent of *Pallavicinin subciliata.*[130]

Pimaranes: This group is not very common in liverworts. The three *ent*-pimaranes, (-)-thermarol (**78**), *ent*-pimara-8(14),15-dien-19-ol (**79**) and *ent*-pimara-8(14),15-dien-19-oic acid (**80**) from the liverwort *Jungermannia thermarum*[83], were the first to be reported. The normal isopimarane, (-)-sandaracopimaric acid (**81**) has been found in the liverwort *Mastigophora diclados*[42] and the presence of the rearranged pimarane (**82**) is reported in *Schistochila aligera.*[93]

Kauranes: Both the normal and *ent*-series are common in higher plants while liverworts produce only the *ent*-series.[1] By 1995, over forty *ent*-kaurane diterpenoids had been isolated from liverworts.[4]. *Ent*-18-hydroxy-16-kauren-15-

one (**83**) and (16*R*)-*ent*-18-hydroxykaurane (**84**) were reported in *Porella densifolia* by Matsuo *et al.*[82] Connolly *et al*[46] isolated four *ent*-kauranes from *Solenostoma triste*. These are *ent*-11α-hydroxykaur-16-en-15-one (**85**), *ent*-11α-hydroxy-(16*S*)-kauran-15-one (**86**), *ent*-kaur-16-ene-11α,15α-diol (**87**) and *ent*-15α-acetoxykaur-16-en-11α-ol (**88**).

Sphenolobanes: This skeleton was first reported in the *Pinaceae (Pseudolarix kaempferi)*[116] from which the pseudolarix acids were isolated. However, the only occurrence of this skeleton in liverworts is in *Anastrophyllum minutum* (*sphenolobus minutum*) [115] from which six compounds (**89-94**) of this skeletal type were isolated.

Sacculatanes: The sacculatane skeleton (**95**) has been reported only in liverworts. The isolation of sacculatal (**96**) and isosacculatal (**97**) from the liverwort *Trichocoleopsis sacculata* was first reported by Asakawa and Takemoto.[7] Sacculatanes have also been further reported in *Trichocoleopsis, Pellia, Porella* and *Makinoa* species.[1] Other representatives of this type are the two hemiacetals, sacculaplagin (**98**)[62] and sacculaporellin (**99**)[24], isolated from the liverworts *Plagiochila acanthophylla* and *Porella perrotteliana* respectively.

1.3.d Triterpenoids: Triterpenoids are quite rare in liverworts. α-Zeorin (**100**) has been isolated from *Reboulia hemisphaerica*[118] and *Plagiochasma rupestre.*[57] Other examples include cycloartenol (**101**) from *Mylia taylori*[112] and *Lophozia ventricosa*[73], cycloart-23-ene-3β,25-diol (**102**) from *Plagiochila kahsiana (P. peculiaris)*[11], ursolic acid (**103**), friedelinol (**104**) and epifriedelinol (**105**) from

Gymmocolea inflata[1] and friedelin (**106**) from *Porella, Frullania* and *Conocephalum* species.

1.3.e Aromatic Compounds: The most common aromatic compounds in liverworts are bibenzyls and bisbibenzyls. The bibenzyls are the most characteristic metabolites of some liverworts and many of them are biologically active. Lunularic acid (**107**), the first representative of bibenzyl type, was isolated from *Lunularia cruciata* in 1965.[123] Lunularic acid (**107**) and lunularin (**108**) are universally distributed in liverworts and in all parts of liverworts.[1] These two compounds have been reported in seventy six species of liverwort.[1,51] Fourteen bibenzyl derivatives have been isolated from *Radula kojana* by Asakawa *et al.*[10] The interesting bibenzyl cannabinoid derivative (**109**) was obtained from *Radula perrottetii.*[120] Another novel compound, the cyclohexenone, 4-(p-methoxyphenethyl)-cyclohex-2-en-1-one (**110**) is a constituent of *Plagiochila longispina.*[105] The cyclopropanochroman type e.g., radulanins I, J and K (**111-113**), is a recent class with variation of the basic bibenzyl unit, from the liverwort *Radula javanica.*[9]

Bisbibenzyl structures are known only in liverworts. Bisbibenzyls are two bibenzyl units joined together by ether and biphenyl linkages. They differ in the linkages and their positions of attachment to the benzyl units.[52] The first bisbibenzyl reported was marchantin A (**114**), isolated from the liverworts *Marchantia polymorpha, M. paleacea var. diptera* and *M. tosona*[1,17] as the major component, together with marchantin B (**115**). Isomarchantin C (**170**) has also been found in liverworts.[8,129] Three other compounds, pakyonol (**171**) from Korean *Mannia fragrans*,[70] and neomarchantins A and B (**172-173**) from

Schistochila glaucescens,[114] provide further representatives of this group. Taiwanese *Mannia subpilosa* contains marchantin N (**174**) and marchantinquinone (**175**).[128]

Bisbybenzyls with a biphenyl link, riccardin A (**116**), riccardin B (**117**), riccardin C (**176**) and riccardin D (**177**), were isolated from *Riccardia multifida.* The structure of the first was confirmed by X-ray analysis of the diacetate (***118***).[14,29] Approximately ten riccardins have been isolated so far.

Plagiochila sciophila contains plagiochins A-D (**178-181**).[59] Similarly, *P. fruticosa* has been reported to contain isoplagiochins A and B (**182-183**),[58] and two unique bisbibenzyls, isoplagiochins C and D (**184-185**).[60] The latter two have two biphenyl links. The first open chain bisbibenzyls isolated were perrottetins E-G (**186-188**) from *Radula perrottetin.*[119] However, perrottetin E was later obtained from about ten other liverworts. Other examples of open-chain bisbibenzyls are paleatins A and B (**154-155**) from *Marchantia paleacea* subsp. *paleacea.*[61] These compounds are linear analogues of the macrocyclic bisbibenzyl ethers, and are therefore possible biogenetic precursors of the marchantins and the riccardins.[4]

Bisbibenzyls show a wide range of biological activity.[106] Marchantins A, B, and C, riccardins A and B, and perrottetin E show cytotoxic activity while marchantins A, D, and E exhibit 5-lipoxygenase and calmodulin inhibitory activity. Marchantin A also shows cardiotonic, antimicrobial, and antifungal properties.[3]

Two prenylated indoles, 6-(3-methyl-2-butenyl)-indole (**119**) and 7-(3-methyl-2-butenyl)-indole (**120**) have been isolated from *Riccardia sinuata.*[34,36] These are the first nitrogen compounds identified in liverworts.

Flavonoids are widely distributed in the liverworts, particularly in the Marchantiales.[1] Most of the flavonoids found in liverworts occur as glycosides. *Frullania, Porella, Plagiochila* and *Trichocolea* species (Jungermanniales) all produce flavonoid glycosides. A few flavonoid aglycones are found in *Corsinia*[101] and *Frullania*[15,18,30] species. Apigenin (**121**) and luteolin (**122**) are the most common flavonoid aglycones.

1.4 Characteristics of the Hepaticae

The biological activities of liverworts are due to the presence of terpenoids and lipophilic aromatic compounds in their oil bodies. Liverworts are occasionally used for medical purposes *e.g. Marchantia polymorpha* as a diuretic and *Conocephalum conicum* as a cure for gallstones.

Odour: The characteristic odour of many liverworts is associated with oil-body constituents. Muller listed[92] many qualitative observations on the odour of various species.: *Leptolejeuna* (liquorice), *Rella* (anise), *Solenostoma obovatum* i.e, *Jungermannia obovata* (carrot), *Lophozia bicrenata* (cedar oil), *Lophocolea* (mossy), *Geocalyx* (turpentine). The mushroom-like odour of *Concephalum conicum* is due to the presence of (+)-bornyl acetate (**123**), 1-octen-3-ol (**124**) and 1-octen-3-yl acetate (**125**).[108] A sweet mushroomy odour of *Wiesnerella denudata* is due to the presence of (+)-bornyl acetate (**123**).[12] The very intense fragrance in *Targionia hypophylla* is due to the presence of *cis* and *trans*-pinocarveyl acetate (**43**) and (**44**) respectively.[22] p-Ethylanisol (**126**) has a naphthalene-like odour and occurs in *Leptoleunea elliptica.*[94] Tamariscol (**127**), isolated by Connolly *et al*[45] from *Frullania tamarisci* , has a mossy and woody smell.

Pungency and bitterness: Some genera of liverworts produce characteristic tastes, e.g. pungent, bitter and hot, depending upon the chemical constituents. Mizutani[90] reported that *Porella vernicosa* contains a very pungent substance, the sesquiterpenoid aldehyde, polygodial (**128**), which is the hot tasting substance of the *P. vernicosa* complex, *P. arbores-vitae, P. fauriei, P. gracillima, P. obtusata* subs. *Macroloba,* and *P. roerii.*[2] Crude extracts of these species contain about 10-30 percent of polygodial. *Jamesoniella autumanalis* contains an intense bitter principle whose taste is very similar to lilac and *Swertia japonica* or the root of *Gentiana scabra var. orientalis.*

Plagiochila species (more than 3000) are divided into two chemo-types: pungent species and non-pungent species. The strong pungency in the former group is mainly due to plagiochiline A (**129**), a sesquiterpene hemiacetal.[1] *Plagiochila yokogurensis* also produces plagiochiline A (**129**) and another pungent hemiacetal, plagiochiline I (**130**).[1] *Plagiochila hattoriana* produces plagiochiline A (**129**) and the bitter hemiacetal, plagiochiline B (**131**).[1] The structures of the bitter diterpenoids scapanin (**74**) from *Scapania undulata,* floerkein B (**133**) from *Barbilophozia floerkei* and anastreptin (**134**) from *Anastrepta orcandensis,* were elucidated by Connolly.[43] The bitter diterpenoid barbilycopodin (**132**) has also been reported from the liverworts, *B. floerkei* and *B. attenuata.*[43, 72]

Two kaurane glycosides, infuscaside A (**135**) and infuscaside B (**136**), have been isolated from *Jungermannia infusca.*[2] They have an intensely bitter taste in contrast to other kaurane glycosides such as stevioside, from the higher plant *Stevia rebaudiana* (Compositae), which are intensely sweet.[5]

Allergenic contact dermatitis: Some liverworts cause intense allergenic contact dermatitis.[2] *Frullania* species are notable in this category. The allergy-inducing substances of *Frullania* species are sesquiterpenoid lactones with an α-methylene-γ-lactone group.[5] Sesquiterpenoids possessing an α–methyl-γ-butyrolactone group do not induce allergy.[2] The sesquiterpene lactones, (+)-frullanolide (**137**), (-)-frullanolide (**138**), (+)-oxyfrullanolide (**139**) and (+)-*cis*-β-cyclocostunolide (**140**), present in *Frullania* species, are all allergenic.[16,56,100] There are more than five hundred *Frullania* species composed of two main chemo-types. Type A contains allergenic sesquiterpenoid lactones with an α-methylene-γ-butyrolactone group while type B does not contain any sesquiterpene lactones.

Antitumour activity: Twenty three species in sixteen genera of liverworts have been screened for antitumour compounds through the agency of the National Cancer Institute, USA.[5] The level of antitumour activity was low with only four species giving positive results. *Conocephalum conicum* and *Wiesnerella denudata* contain some guaianolides which have antitumour activity.

Antimicrobial and antifungal activity: The lipophilic extracts of several liverworts, including species of *Bazzania, Frullania, Marchantia, Plagiochila, Porella* and *Radula* show antimicrobial and antifungal activity. Marchantin A (**114**) from *Marchantia polymorpha*[117], and (-)-α-herbertenol (**141**), (-)-β-herbertenol (**142**) and (-)-α-formylherbertenol (**143**) from *Herberta adunca* [79,85,86] are reported to be active.

Plant growth regulatory activity: Huneck and Meinunger[68] tested twenty nine species of liverworts for growth regulatory activity using *Lepidium sativum* seedlings as a test system and reported positive results for all of them. The results indicated that *Marchantia polymorpha* promoted the growth of shoots,

Conocephalum conicum and *Plagiochila porelloides* accelerated the growth of roots, *Barbilophozia floerkei, Cephalozia bicuspidata, Lepidozia reptans, Marsupella emarginata* and *Ptilidium pulcherrium* inhibited the growth of both shoots and roots. Kodama *et al* [75] reported that the synthetic enantiomer (-)-vitrenal has weak growth promoting properties in contrast to the strong inhibition of the growth of rice seedlings shown by (+)-vitrenal (**144**) from *Lepidozia vitrea.*[81,84]

CHAPTER TWO

Marchantia paleacea Betrol subsp.*paleacea*

2.1 Introduction:

Systematic Position:

Division – Bryophyte

Class - Hepaticopsida

Order - Marchantiales

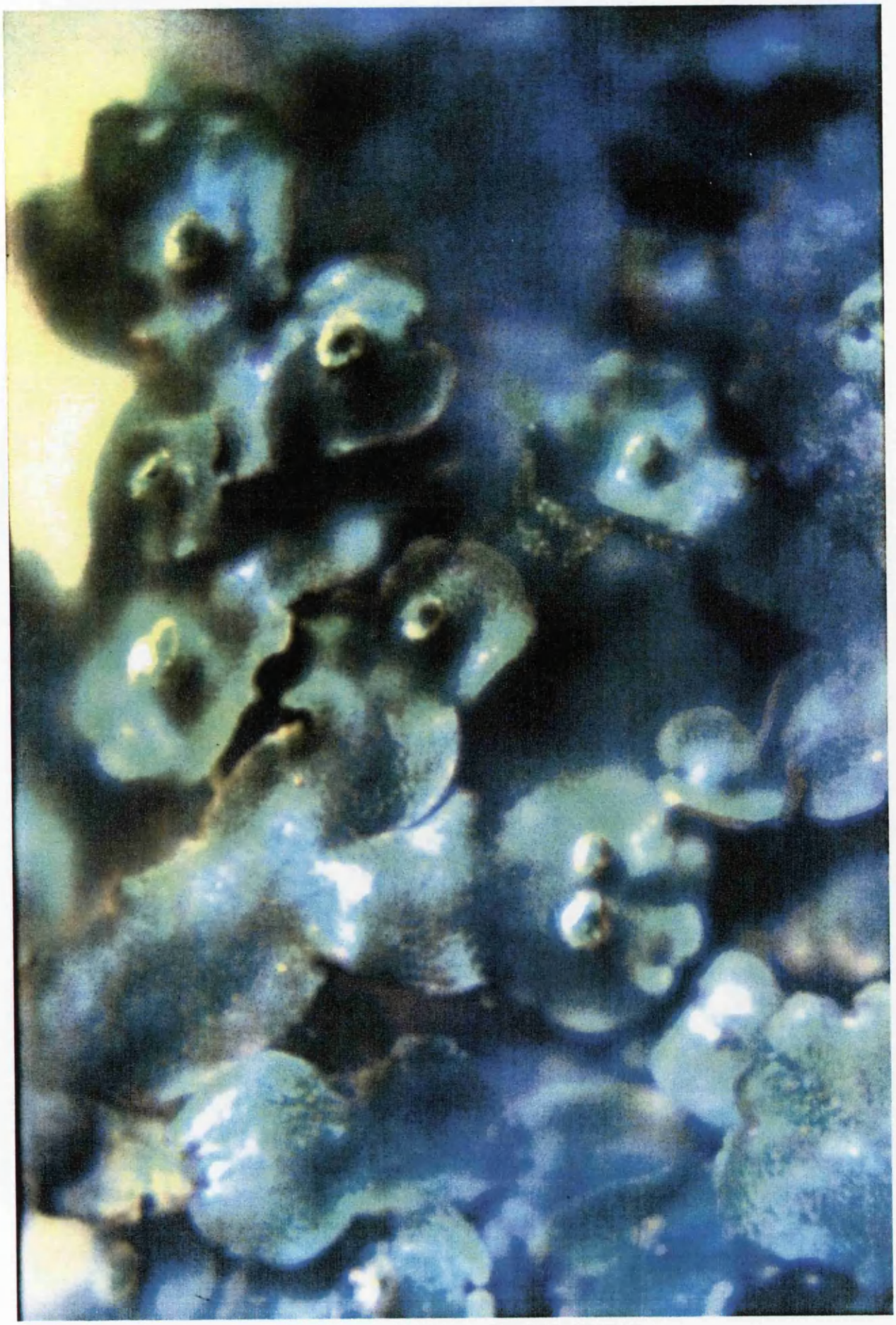
Family - Marchantiaceae

Genus - *Marchantia*

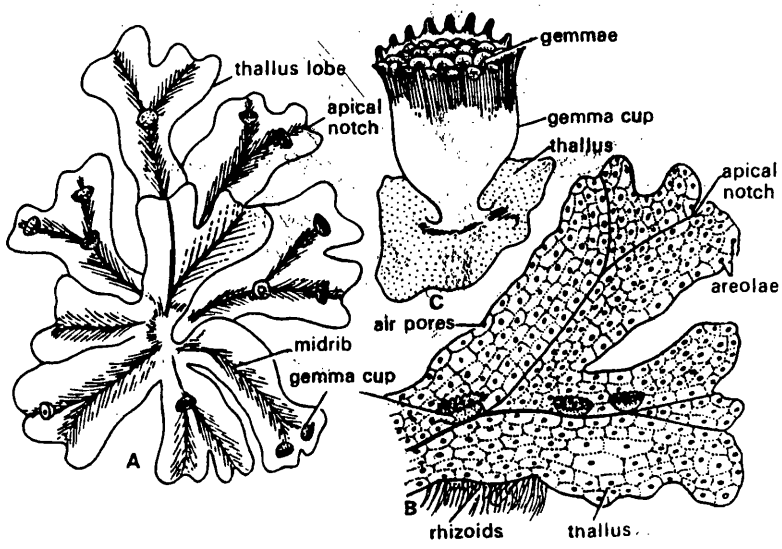
Species - *paleacea* Betrol

Subsp. - *paleacea*

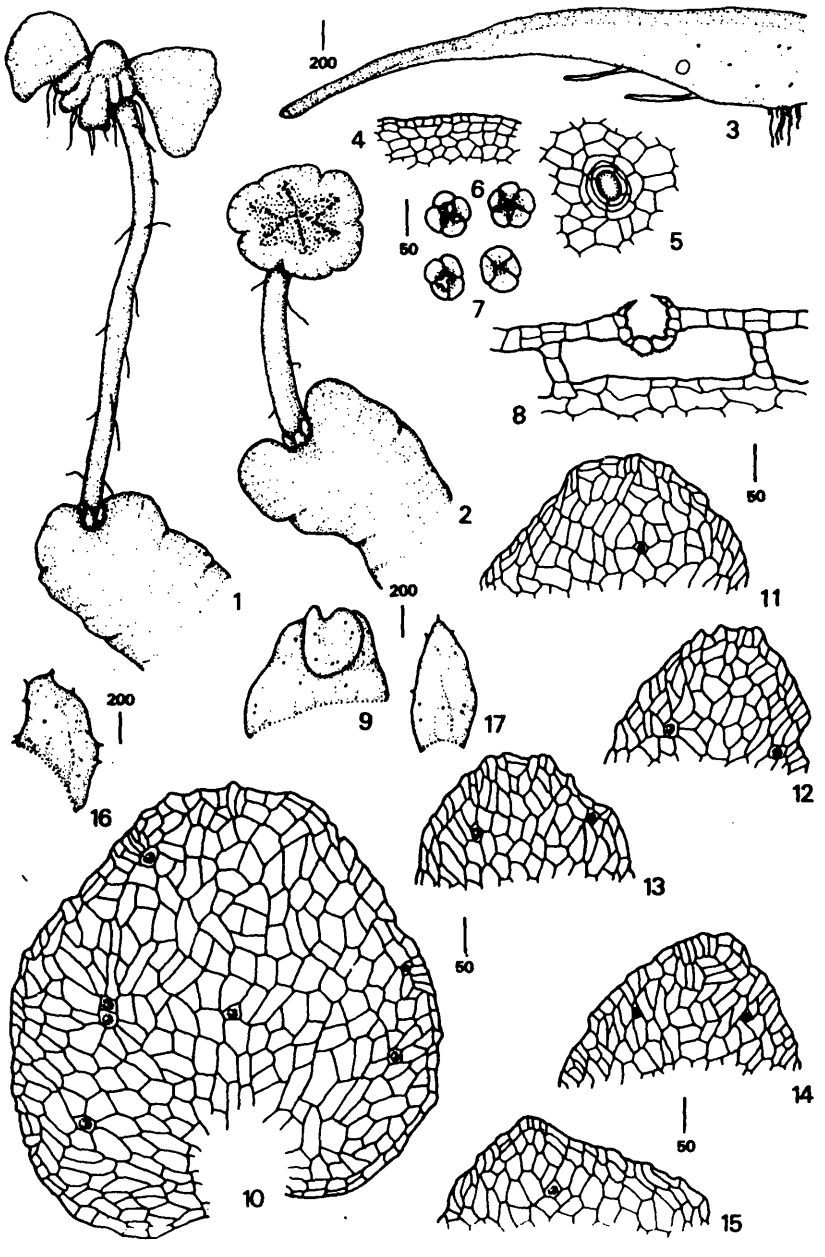
Marchantia is one of the largest genus in the order Marchantiales which is composed of twenty eight genera.[38] The genus *Marchantia* consists of about sixty five species[98] distributed all over the world. *Marchantia polymorpha* is the most common one. *Marchantia* species occur in areas with a humid climate, temperate, tropical or subtropical. They are absent in areas with a long dry season, but a few can stand cold periods. They are able to grow on substrata unsuitable for other plants, e.g. polluted soil, or the ash left by fires. At least 60 % of the species are indifferent to pH; a few prefer acidic or basic substrata; but none seems to be restricted to a narrow pH range. These species are particularly



Marchantia paleacea Betrol subsp. *Paleacea*



Marchantia sp. A, thalli showing habit; B, part of thallus; C, gemma cup on thallus.



. - *Marchantia paleacea* Bertol. subsp. *diptera* (Nees et Mont.) H. Inoue .1: female thallus, 2: male thallus, 3: thallus section, 4: thallus margin, 5: epidermal pore, surface view, 6-7: epidermal pores, inner opening, 8: epidermal pore, section, 9: median scale, 10: appendage of median scale, 11-15: apices of appendages of median scales, 16-17: laminal scales.

adopted for the colonization of bare surfaces in newly created habitats. They are pioneers in humid, disturbed sites resulting from natural perturbations or from human activity. *Marchantia* species have diversification potentialities that are absent in near relatives. Within the same species, populations differ in biochemical and chemical characteristics[25]

The species *Marchantia paleacea* and *Marchantia nepalensis* are identical, as pointed out by Schiffner (1901) and Evans (1917). There was confusion between the subspecies *M. paleacea* Betrol subsp. *diptera* and *M. paleacea* Betrol subsp. *diptera.* prior to 1957, when Hattori recognised the distinction between *"paleacea"* and *"diptera".* Subsp. *paleacea* differs from subsp. *diptera* mainly in the structure of the appendage of the median scales. Non-functional female receptacles are absent in subsp. *paleacea.* 28 % of archegoniophores have sporophytes in subsp. *paleacea* while it is only 20% in the case of subsp. *diptera.* Subsp. *paleacea* and subsp. *diptera* are not found in the same altitudinal belt in the area where their ranges overlap. The former occurs at medium altitude, the latter at low elevations i.e., 0-500 m for subsp. *Diptera,* above 500 m for subsp. *paleacea.*

2.2 Constituents: All Japanese specimens[38] of *M. paleacea,* which have papillae, were referred to as *M. diptera* till 1957, when Hatori distinguished ' *paleacea'* and '*diptera'*. Japanese records of *M.diptera* prior to 1957 belong partly to subsp. *M. paleacea* and partly to subsp. *M. diptera.* Subsp. *paleacea,* a european taxon, was first cited from Asia by Taylor in 1836. However, the species was not generally recognised from Asia until recently.

Several papers are available regarding the chemical constituents of subsp *M. diptera* but little is known about those from subsp *M. paleacea.*

However, the chemical constituents in both of the subsp. may be similar. Most work has been done on the most common species, *M. polymorpha,* which contains a wide range of compounds. As in the case of *M. polymorpha, M. paleacea* subsp. *diptera* contains various macrocyclic bisbibenzyls, i.e., marchantins A-G (**114,115,145,146,147,148,149** respectively).[19,25] Lunularin (**150**), 2-hydroxy-3,7-dimethoxyphenanthrene (**151**), and *para*-hydroxybenzaldehyde (**152**) have also been identified.[25] Cell suspension cultures of *M. paleacea* subsp. *diptera* produced luteolin (**153**).[96] Two novel bisbibenzyls, paleatins A-B (**154-155**), together with the known marchantins A-E, have been isolated from MeOH extract of *M. paleacea* subsp. *diptera.*[61] Two new unstable long chain butenolides, 2-(8'Z,11'Z-hexadecadienyl)-penta-2,4-dien-4-olide (**156**) and 2-(8'Z-hexadecenyl)-penta-2,4-dien-4-olide (**157**) have been isolated[121] from the ether extract of Japanese *M. paleacea* subsp. *diptera.* The isolation of these compounds from a liverwort for the first time is of phylogenic interest. *M. paleacea* subs. *diptera* produces not only *ent*-sesquiterpenoids (*vide infra*) but also an *ent*-diterpenoid, *ent*-labda-7,13E-dien-15-ol (**158**), and phytol (**159**).[25,27,122] Two novel cyclopropane containing *ent*-cuparane sesquiterpenoids, (**160**) and (**161**), have been isolated from *M. paleacea* subsp. *diptera* and *M. polymorpha.*[25,122]

2.3 General Experimental:

Extraction: Extraction of the plant material was carried out by hot percolation using suitable solvents (mostly ether, ethyl acetate or methanol). All the solvents were removed *in vacuo* using a Buchi rotary evaporator.

Chromatography: Extracts were fractionated by column (flash) chromatography (CC) over silica gel (Merck Kieselgel GF_{254}). Each of the crude fractions was further purified either by column chromatography over sephadex LH20 or by preparative thin layer chromatography (PTLC) over silica gel [Merck Kieselgel GF_{254} (0.75 mm thick)]. After each chromatographic separation the fractions recovered from preparative plates and those collected from columns were checked by analytical thin layer chromatography (TLC) over silica gel [Merck Kieselgel GF_{254} (0.25 thick)] precoated on aluminium plates. Analytical and preparative plates were visualized under UV light (254 or 366 nm) or by adsorption of I_2 vapour.

Spectroscopy: Nuclear magnetic resonance spectra (NMR) were measured using Bruker AM 360 (^1H at 360 MHz ; ^{13}C at 90 MHz), DPX 400 and 500 MHz spectrometers. Spectra were recorded for $CDCl_3$ solutions relative to $CHCl_3$ at δ_H 7.25 and $CDCl_3$ at δ_c 77.0 and chemical shifts are reported in ppm. Tabulated ^1H NMR data have coupling constants (J) in Hz given in parenthesis. Signals indicated as 'm' were unresolved or overlapping multiplets. ^1H and ^{13}C NMR assignments are based in general on chemical shift rules or comparison with published data for similar compounds. More definitive ^1H NMR assignments were made by NOE difference and homo-decoupling experiments. ^{13}C NMR multiplicities were obtained from DEPT [90° DEPT (CH signals only); 135° DEPT (CH_3 and CH signals positive, CH_2 signals negative)] or J-modulated ^{13}C (CH and CH_3 signals positive, CH_2 and C signals negative) spectra. HMQC (^1H detected Heteronuclear Multiple Quantum Correlation)[31] and HMBC (^1H detected Heteronuclear Multiple Bond Correlation)[32] experiments were performed using standard Bruker software and are the inverse equivalents of 2D

direct and 2D long-range carbon-proton correlation experiments respectively. Mass spectra (70 ev) were recorded in the continuous scanning mode at low resolution. Accurate mass was determined in the high resolution electric field scanning mode. The machine used was a Jeol JMS-700.

2.4 Materials and Methods (Experimental)

Plant material was collected from Nagarkot (Nepal) at an elevation of 1914m., longitude $27^0 43`$ and latitude $85^0 31`$. Nagarkot is situated at a distance of 32 Km north east of Kathmandu, the capital city of Nepal. The plant was collected from the inner walls of a well in the month of October. 1989. The habitat was a shady place surrounded by tall trees. The plant was identified by Dr. David G. Long, Royal Botanic Garden, Edinburgh where the specimen is deposited.

Sample no. X_2 (Marchantia paleacea)

The sample was dried at room temperature. Dried and powdered material (150 gm.) was subjected to hot percolation for 8 hours using analar MeOH. The MeOH extract (10gm) was recovered after evaporating the solvent and was fractionated by flash chromatography using gradient elution with increasing amounts (5%) of ethyl acetate in petroleum ether.

Please note that the structures of the isolated compounds are presented in the text and are indicated by bold numbers without brackets. All of the compounds were gums or oils. Their spectroscopic properties are presented in the text during the discussion and/or in Tables.

Isolation of marchantin C **8**, *2-hydroxy-3,7-dihydroxyphenanthrene* **6**, *p-hydroxybenzaldehyde* **1**, *and 2,2'-dihydroxy-3,3',7,7'-tetramethoxy-biphenanthrene* **3**:

Fractions 7-10 from the flash column (eluted with 20% ethyl acetate in petroleum ether) were combined and fractionated further by passing through a Sephadex column using MeOH: $CHCl_3$ (1:1) as eluant. Fractions 6-10 thus obtained were combined and refractionated further over Sephadex. Fractions 9-15 thus obtained were combined and subjected to preparative tlc using 30% ethyl acetate in petroleum ether to develop the plate. Nine bands were observed. The first band (x-2, b1) afforded marchantin C **8**, the third band (x-2, b3) 2-hydroxy-3,7-dihydroxyphenanthrene **6**, the fifth band (x-2, b5) p-hydroxybenzaldehyde **1** and the ninth band (x-2, b9) 2,2'-dihydroxy-3,3',7,7'-tetramethoxy-biphenanthrene **3**. The compounds were recovered from the silica by washing with ethyl acetate.

Isolation of marchantin A **7**:

Fractions 14-18 from the original flash column (eluted with 35% to 55% ethyl acetate in petroleum ether) were combined and fractionated further by passing through a Sephadex column using MeOH:$CHCl_3$ (1:1) as eluent. The thirteenth fraction thus obtained was subjected to prep. tlc using 30% ethyl acetate in petroleum ether as eluent. The 1st band (x-2, b1) afforded marchantin A **7**.

Sample no. X_3' (Marchantia paleacea)

The extract of dried and powdered material (150 gm) was made as in case of sample no. X_2 . The crude extract (8gm) was fractionated further by flash chromatography as above.

Isolation of 3,4-dihydro-8-hydroxy-4-(4'-hydroxyphenyl)-isocoumarin **4** *and 2-hydroxy-3,7-dimethoxyphenanthrene* **6***:*

Flash column fractions 6-7 (eluted with 35% to 40% ethyl acetate in petroleum ether) were combined and fractionated further by passing through a Sephadex column using MeOH:$CHCl_3$ (1:1) as eluent. Fractions 9-10 thus obtained were combined and rechromatographed over Sephadex. The resulting fractions 7-13 and 14-16 were combined separately and then subjected to preparative tlc using 30% ethyl acetate in petroleum ether to develop the plates. The fourth band (x-3′, b4) of fractions 7-13 afforded 3,4-dihydro-8-hydroxy-4-(4'-hydroxyphenyl)-isocoumarin **4** and the second band (x-3′, b2) of fractions 14-16 yielded 2-hydroxy-3,7-dimethoxyphenanthrene **6**.

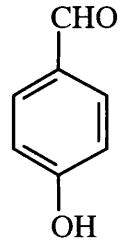
Isolation of marchantin D **9** *:*

Flash column fractions 10-11 (eluted with 55% to 60% ethyl acetate in petroleum ether) were combined and chromatographed over Sephadex as usual. Fractions 8-11 thus obtained were combined and subjected to preparative tlc using 30% ethyl acetate in petroleum ether. The second band (x-3′, b2) of this plate yielded marchantin D **9**. The presence of marchantin C **8** was also noticed in this sample no. X_3' of *M. paleacea.*

2.5 **Results and Discussion:**

p-Hydroxybenzaldehyde **1** *(x-2, b5)*

This compound was obtained in very small amount, as a gum. Its mass spectrum showed a molecular ion at m/z 122, corresponding to $C_7H_6O_2$. A large M-1 peak at m/z 121 suggested the presence of an aldehyde function. The ^1H NMR spectrum was very simple and showed an aldehyde proton at δ_H 9.80 (s) and an AA'BB' system at δ_H 7.74 and 6.88 (J = 8.6 Hz). These data are consistent with the structure of *p*-hydroxybenzaldehyde **1**. Identity was confirmed by comparison with an authentic sample. *p*-Hydroxybenzaldehyde has been previously reported as a constituent of liverworts.[25]

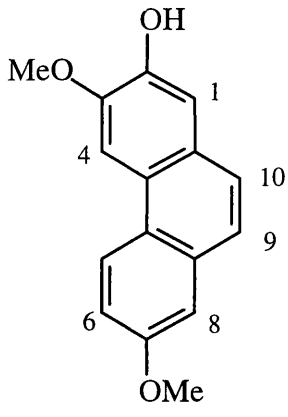


1

2,2'-Dihydroxy-3,3',7,7'-tetramethoxybiphenanthrene **3** *(x-2, b9)*

This compound was also obtained in very small amount. Its ^1H NMR spectrum was reminiscent of that of a phenanthrene (*cf* **6** below). Thus it had two strongly deshielded signals, one an isolated singlet [δ_H 8.05 (s, H-4)] and the other an ortho-coupled doublet [δ_H 8.47 (d, J = 8.2 Hz, H-5)] which formed

part of a 1,2,4-substituted aromatic ring [δ_H 7.22 (dd, J = 8.2,2.7 Hz, H-6), 7.10 (d, J = 2.7 Hz, H-8)]. Other signals in the spectrum included an AB system at δ_H 7.31 and 7.05 (both d, J = 9.0 Hz, H-9 and H-10 respectively), a phenolic hydroxyl proton at δ_H 5.78 (bs) and two methoxyl groups at δ_H 4.14 and 3.87. NOE difference experiments enabled us to arrive at part-structure **2** (no substituent at C-1). Irradiation of the methoxyl group attached to C-3 (δ_H 4.14) afforded a NOE at H-4 while irradiation of the other methoxyl (δ_H 3.87) afforded a NOE at H-8. Because of the low concentration of sample and the large solvent peak NOEs to H-6, which was partially obscured by the solvent, were not detected. NOEs from H-9 to H-8 and to H-10 distinguished between H-9 and H-10. The problem of the substituent at C-1 still remained to be resolved. The mass spectrum provided a ready solution. The parent ion and base peak was observed at m/z 506 with a much less intense ion at m/z 253. These clearly indicate that this compound is a symmetrical dimeric phenanthrene with structure **3**. Such dimers arise by phenolic radical coupling and may be artefacts. Often the coupling involves one of the phenolic oxygens to give an unsymmetrical dimer. Such dimers show doubling of signals. In our case there is no doubling of signals and hence the dimer is symmetrical. Moreover the unsymmetrical dimer would have an additional aromatic proton. This did not appear in our compound.



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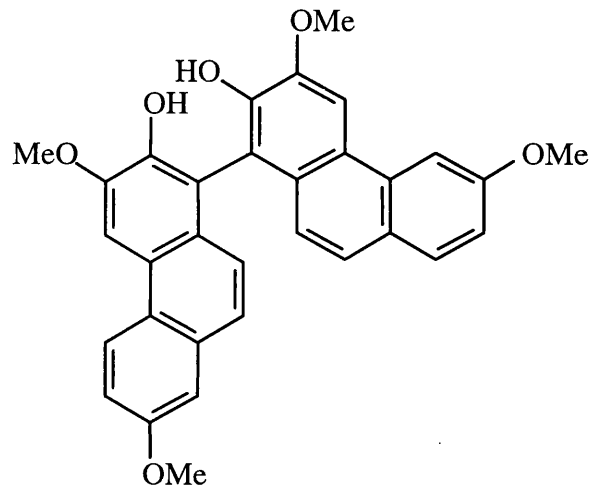
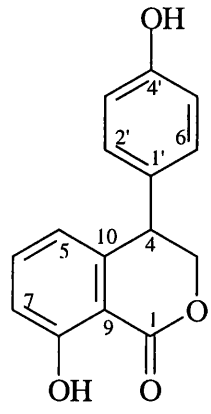


3

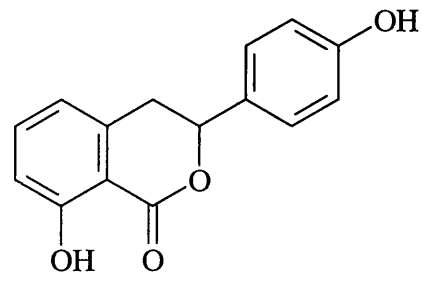
3,4-Dihydro-8-hydroxy-4-(4'-hydroxyphenyl)-isocoumarin 4

Compound **4** was obtained as a gum. Its mass spectrum was relatively simple with few peaks. There was a parent ion at m/z 256, consistent with a molecular formula of $C_{15}H_{12}O_4$, and a fragment ion at m/z 121. Its ^1H NMR spectrum revealed two two aromatic rings, one *para*-disubstituted [δ_H 6.99 and 6.76 (AA'BB', J = 8.6 Hz, H-2',6' and H-3',5')] and the other with three contiguous protons [δ_H 7.33 (t, J = 7.9 Hz, H-6), 6.86 (d, J = 8.4 Hz, H-5) and 6.41 (d, J = 7.5 Hz, H-7)], a bonded hydroxyl proton [δ_H 11.04 (s, 8-OH)], and a three spin system CH-CH_2 [ABX] with the methylene group attached to oxygen [δ_H 4.57 (dd, J = 11.0, 5.0 Hz, H-3), 4.50 (dd, J = 11.0, 8.6 Hz, H-3), 4.21 (dd, J = 5.0, 8.6 Hz, H-4)]. The carbon spectrum [see Table C] confirmed the presence of the two aromatic rings and the CH-CH_2-OR system and additionally revealed the presence of an ester/lactone carbonyl group at δ_C 170.0.

The spin systems were readily observed in the COSY spectrum and the assignment of the protonated carbons followed from the HMQC spectrum. The above data can be assembled to give the 3,4-dihydroisocoumarin structure **4**. Confirmation of the structure of **4** was obtained from correlations in the HMBC spectrum which also permitted the assignment of the non-protonated carbons. Despite its simple structure the 3,4-dihydroisocoumarin **4** is a new natural



4



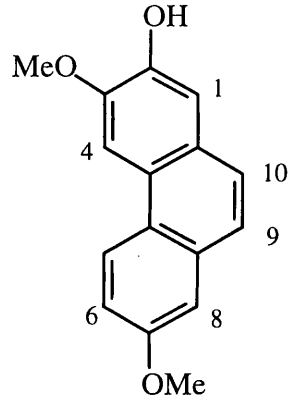
5

product and has not been reported previously. Isocoumarins are usually polyketide in origin and are normally substituted on position 3. The isocoumarin hydrangenol **5**, clearly a derivative of lunularic acid, has been reported in a liverwort by Becker and his colleagues[134]

2-Hydroxy-3,7-dimethoxyphenanthrene* 6 *(x-3', b2)

Compound **6** was also obtained as a gum. Accurate mass analysis indicated a molecular formula $C_{16}H_{14}O_3$ [m/z 254.0943; required 254.0943] and hence ten double bond equivalents. Its ^1H NMR spectrum was reminiscent of that of a phenanthrene bearing three substituents, two methoxyl groups and a phenolic hydroxyl. Thus there is an ABX system [δ_H 7.16 (2H, m, H-6 and H-8), 8.33 (d, J = 8.8 Hz, H-5)] which includes a highly deshielded proton, two isolated aromatic singlets [δ_H 7.81 (s, H-4), 7.25 (s, H-1)] one of which is also

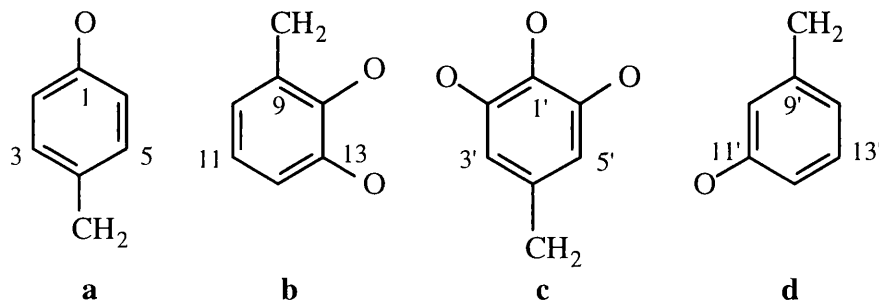
deshielded, an AB system [δ_H 7.52 and 7.47 (both d, J = 8.8 Hz, H-10 and H-9 respectively)], a phenolic hydroxyl [δ_H 5.79 (s)] and two methoxyl groups [δ_H 4.03 and 3.87 (both s, 3-OMe and 6-OMe respectively)]. NOE difference experiments readily revealed the substitution pattern of the phenanthrene nucleus as in **6**. Thus NOEs from H-1 to H-10, H-4 to H-5 and the methoxyl group at δ_H 4.03, H-5 to H-4 and H-6, the other methoxyl to H-6 and H-8 and H-9 to H-8 led to the assignment of structure **6**, 2-hydroxy-3,7-dimethoxyphenanthrene. This compound has been previously reported as a constituent of *Marchantia polymorpha.*[5] The proton NMR shifts are virtually identical with those given above but the carbon resonances were not reported [see Table D].



6

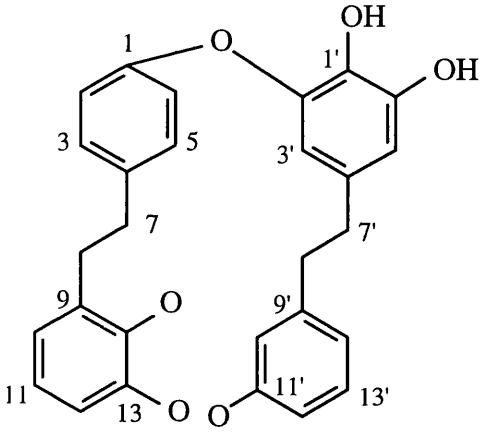
Marchantin A* 7 *(x-2, b 1)

The mass spectrum of this compound, m/z 440, corresponding to $C_{28}H_{24}O_5$, indicated that it was a bisbibenzyl derivative. The carbon and proton NMR data [see Tables A and B] supported this conclusion. The aromatic proton spin systems **a** to **d** were readily deduced from the COSY spectrum.

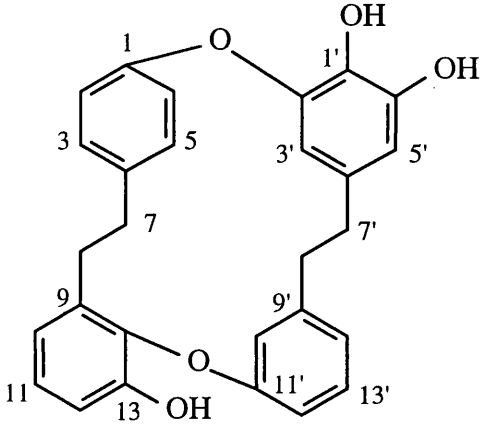


a **b** **c** **d**

Correlations of aromatic protons to the methylene carbons in the HMBC spectrum revealed the positions of attachment of the methylenes to the aromatic ring and hence the bibenzyl units. The shielded nature of H-3' (δ_H 5.25) is characteristic of bisbibenzyls with a C-2' to C-1 ether linkage. Thus the part structure **e** can be assembled. This is reminiscent of marchantin A **7**. Comparison with published data[132] confirmed their identity.



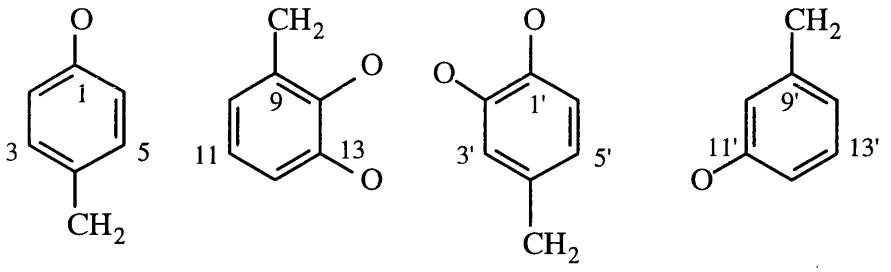
e



7

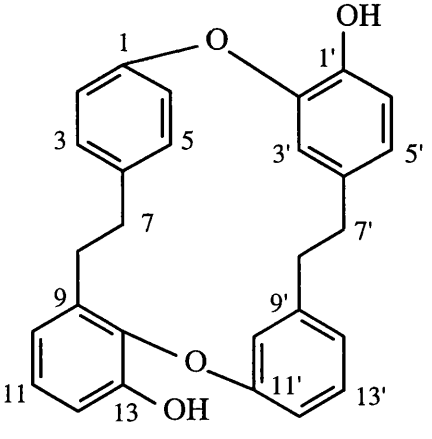
Marchantin C* 8 *(x-2, b1)

This compound was obtained as a gum. In the mass spectrum it showed a parent at m/z 424, consistent with the molecular formula $C_{28}H_{24}O_4$. The proton and carbon spectra were typical of a bisbibenzyl [see Tables A and B]. The COSY spectrum again proved invaluable in deducing the aromatic proton spin systems **a** to **d**.



a **b** **c** **d**

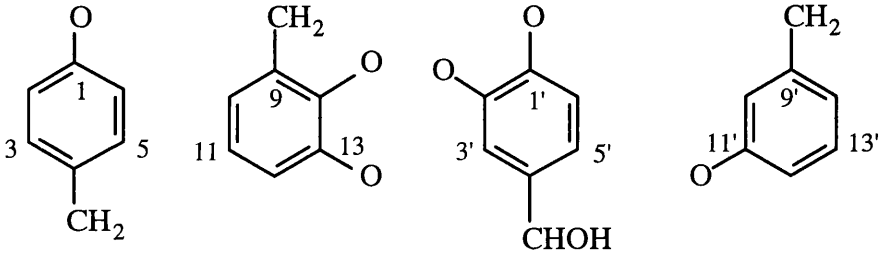
The attachment of the methylene groups was revealed by HMBC correlations of the methylene protons to the protonated aromatic carbons or, equally conveniently, by the correlations of aromatic protons to the methylene carbons. Thus H-3/5 correlated with C-7, H-10 with C-9, H-3' and H-5' with C-7', and H-10' and H-14' with C-8'. As usual the protonated carbons were assigned from the HMQC spectrum. A marchantin type of ether link from C-1 to C-2' was indicated by the shielded chemical shift of H-3' (δ_H 5.53). The presence of only two phenolic hydroxyls (δ_H 4.90 and 5.00) in combination with the substitution pattern described above strongly suggested that this compound was marchantin C **8**. Comparison with published data[132] confirmed this suggestion.



8

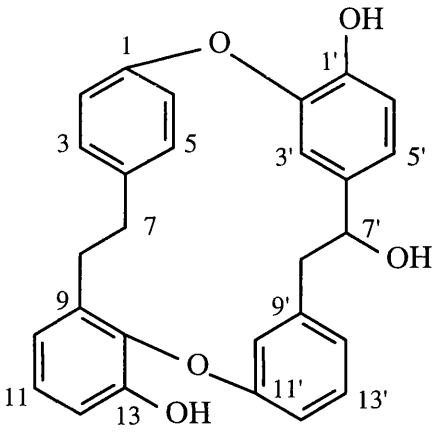
Marchantin D **9** *(x-3′, b2)*

This compound was also obtained as a gum. Its spectroscopic properties [see Tables A and B] indicated that it was a bisbibenzyl derivative. However, the presence of only three methylene groups, together with an oxygenated methine [δ_H 4.03 (dd, J = 10.0, 3.9 Hz)], distinguished it from the other bisbibenzyls. There was some broadening in the proton spectrum and some of the quaternary carbon resonances were weak. Protonated carbons were assigned, as usual, from the HMQC spectrum. The aromatic spin systems **a-d** were apparent from the COSY spectrum.



a **b** **c** **d**

The positions of attachment of the methylene and hydroxy methine groups were readily deduced from correlations in the HMBC spectrum. Thus H-3/5 showed correlations to C-7, H-10 to C-8, H-3'and H-5' to the carbinol carbon C-7' and H-10' and H-14' to C-8. Since H-3' resonated at highfield the usual C-1 to C-2' ether link was assumed. On the assumption that the second ether link was between C-14 and C-11', as is normal for the marchantins, structure **9** was assigned to the compound. This is the structure of marchantin D and comparison with published data[132] confirmed their identity.



9

Tables A-D

TableA

Carbon Shifts of the Bisbibenzyls					
	Marchantin A 7	**Marchantin B 11**	**Marchantin C 8**	**Marchantin D 9**	**Riccardin D 12**
1	153.5	152.7	153.2	153.7	152.7
2	121.5	121.1	121.7	122.1	122.2/122.6
3	130.1	129.5	130.1	129.8	129.2/129.4
4	139.3	139.9	139.5	139.5	140.3
5	130.1	129.5	130.1	129.8	129.2/129.4
6	121.5	121.1	121.7	122.1	122.2/122.6
7	34.3	35.7	35.7	36.2	37.8
8	29.2	29.8	30.7	30.7	35.1
9	136.6	127.1	136.5	137.1	143.4
10	122.3	121.5	122.4	122.3	122.8
11	126.4	112.3	126.4	126.2	130.2
12	114.9	143.3	114.7	114.7	113.4
13	149.2	135.6	149.1	149.1	153.6
14	140.2	139.1	140.1	140.1	121.6
1'	131.4	132.3	143.8	131.7	143.82
2'	147.2	146.3	146.5	147.1	146.5
3'	108.5	107.8	115.9	108.2	116.1
4'	132.8	132.3	133.1	131.7	133
5'	109.9	109.2	122.8	106.1	122.2
6'	144.8	144.1	115.4	145.1	115
7'	34.4	34.2	34.4	84.9	36.6
8'	35.8	35.6	36.2	44.2	37.7
9'	143.3	143.2	142.6	143.1	143.84
10'	115.9	115.3	116.1	117.1	122.5
11'	157.3	156.4	157.1	157.2	131.6
12'	112.5	111.9	112.4	112.2	117.1
13'	129.2	128.9	129.3	128.7	152.9
14'	123.4	123.3	123.7	124.7	117.2

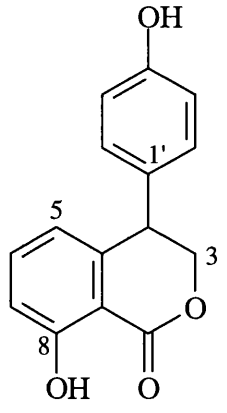
TableB

Proton NMR Shifts of the Bisbibenzyls					
	Marchantin A 7	**Marchantin B 11**	**Marchantin C 8**	**Marchantin D9**	**Riccardin D 12**
2,6	6.45 (obs)	6.62 (d, J = 8.3 Hz)	6.60 (d, J = 8.5 Hz)	6.50 (bd, J = 7.9 Hz)	ca 6.75
3,5	6.81 (d, J = 8.2 Hz)	6.96 (d, J = 8.4 Hz)	6.94 (d, J = 8.5 Hz)	6.87 (d, J = 8.4 Hz)	ca 6.9
7	2.60 (m)	3.02 (m)	3.02 (m)	3.03 (m), 2.94 (m)	2.91 (m), 2.82 (m)
8	2.90 (m)	2.95 (m)	3.01 (m)	2.98 (m), 2.84 (m)	2.91 (m), 2.54 (m)
10	6.90 (dd, J = 7.6, 0.7 Hz)	6.95 (d, J = 8.5 Hz)	7.02 (dd, J = 7.8, 2.5 Hz)	6.95 (dd, J = 7.8, 1.5 Hz)	7.02 (dd, J = 7.7, 1.0 Hz)
11	7.03 (t, J = 7.9 Hz)	6.89 (d, J = 8.5 Hz)	7.15 (t, J = 8.9 Hz)	7.09 (t, J = 7.9 Hz)	7.3 (t, J = 7.9 Hz)
12	6.76 (dd, J = 8.0, 1.0 Hz)		6.87 (dd, J = 8.0, 1.5 Hz)	6.78 (dd, J = 8.0, 1.5 Hz)	6.89 (dd, J = 7.9, 1.1 Hz)
3'	5.25 (d, J= 1.4 Hz)	5.17 (d, J = 1.9 Hz)	5.53 (d, J = 2.0 Hz)	4.9 (d, J = 1.6 Hz)	5.42 (d, J = 2.0 Hz)
5'	6.35 (d, J = 1.4 Hz)	6.50 (d, J = 1.9 Hz)	6.74 (dd, J = 8.1, 2.0 Hz)	6.58 (d. J = 1.8 Hz)	6.73 (dd, J = 8.1, 2.0 Hz)
6'			6.88 (d, J = 8.1 Hz)		6.92 (d, J = 8.1 Hz)
7'	2.90 (m)	2.83 (m)	2.84 (m)	4.03 (dd, J = 10.0, 3.9 Hz)	2.72 (m), 2.57 (m)
8'	2.61 (m)	2.78 (m)	2.27 (m)	3.03 (m), 2.60 (m)	2.65 (m), 2.57 (m)
10'	6.45 (obsc)	6.63 (bs)	6.62 (bt, J = 1.5 Hz)	6.69 (t, J = 2.1 Hz)	6.32 (dd, J = 7.7, 1.6 Hz)
11'					6.82 (d, J = 7.7 Hz)
12'	6.45 (obsc)	6.59 (dd, J = 8.3, 2.7 Hz)	6.54 (dd, J = 8.2, 2.1 Hz)	6.39 (dd, J = 8.2, 2.1 Hz)	
13'	6.85 (t, J = 7.8 Hz)	7.01 (t, J = 7.9 Hz)	6.98 (t, J = 8.9 Hz)	6.82 (t, J = 8.0 Hz)	
14'	6.28 (bd, J = 7.5 Hz)	6.43 (d, J = 7.6 Hz)	6.38 (bd, J = 7.6 Hz)	6.04 (bd, J = 7.5 hz)	6.50 (d, J = 1.6 Hz)

Table C **NMR Data of 3,4-Dihydro-8-hydroxy-4-(4'-hydroxyphenyl)-isocoumarin 4**

	^{13}C (δppm)	^{1}H (δppm)	HMBC
3	73.1	4.53 (dd, J=11.0, 5.0 Hz) 4.50 (dd, J=11.0, 8.0 Hz)	
4	42.9	4.21 (dd, J=5.0, 8.6 Hz)	H-2′, H-6′
1	170.0		H-3
8	162.7		H-6
7	117.0	6.41 (d, J=7.5 Hz)	8-OH
6	136.9	7.33 (t, J=7.9 Hz)	
5	118.6	6.86 (d, J=8.4 Hz)	
10	143.6		H-6, H-3, H-3
9	108.8		8-OH, H-7, H-5
2′, 6′	130.3	6.99 (d, J=8.6 Hz)	H-4, H-3', H-5'
3′, 5′	116.3	6.76 (d, J=8.6 Hz)	H-2′, H-6′
1′	134.0		
4′	155.7		H-2′, H-6′ H-3′, H-5′
OH at C-8		11.05 (s)	

Protonated carbons assigned from HMQC spectrum.

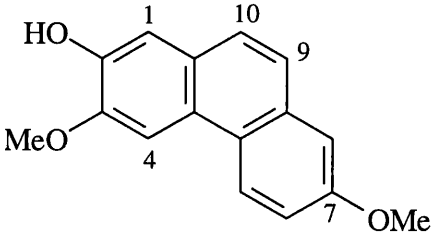


4

Table D NMR Data of 2-Hydroxy-3,7-dimethoxyphenanthrene 6

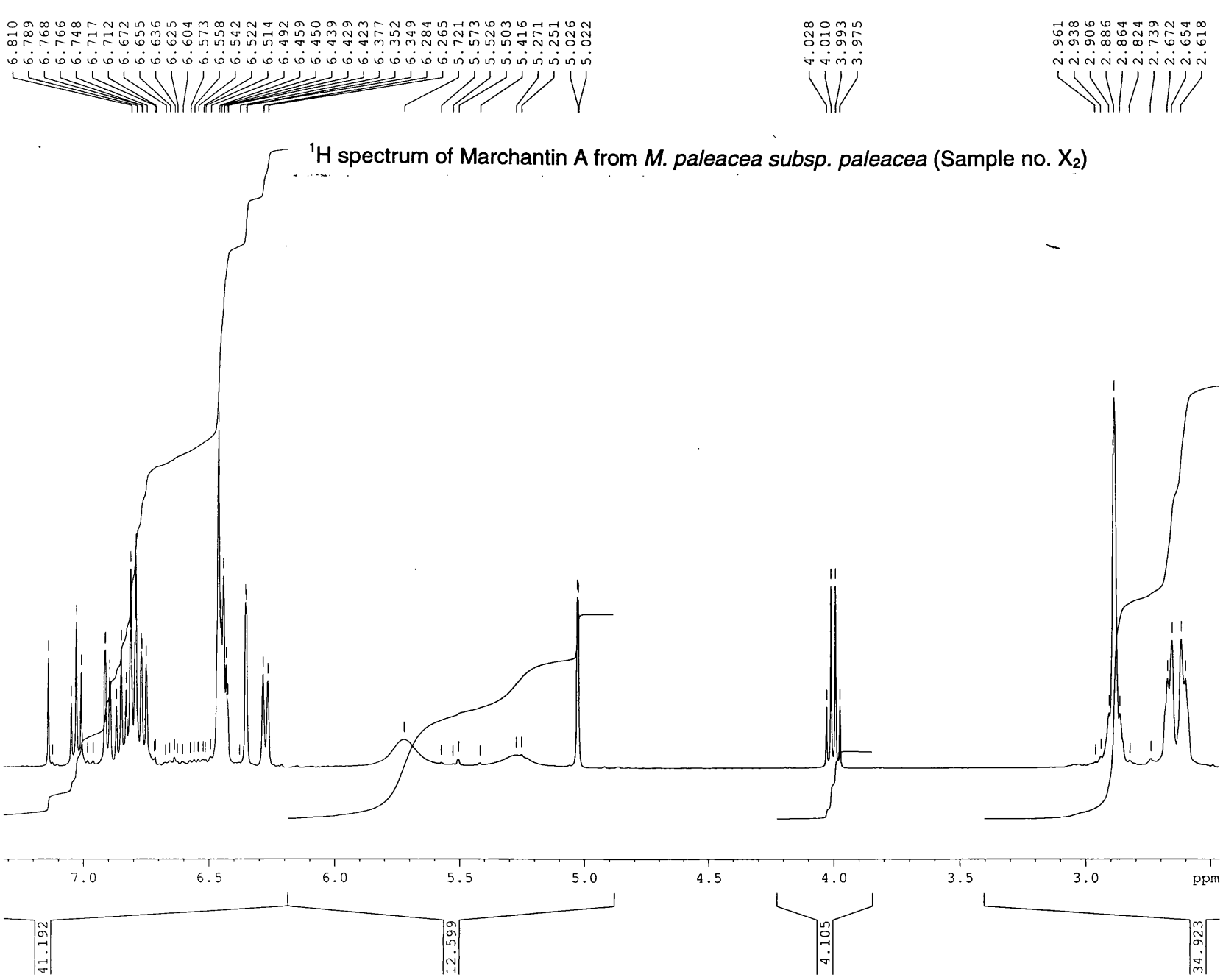
	^{13}C (δ ppm)	^{1}H (δ ppm)	HMBC
1	111.80	7.25 (s)	
2	145.37		H-4, H-1
3	147.7		H-1, H-4, 3-OMe
4	102.5	7.81 (s)	
5	124.0	8.33 (d, J = 8.8 Hz)	
6	117.3	7.16 (m)	
7	157.9		H-5, H-6, H-8
8	108.8	7.16 (m)	
9	127.0	7.52 (d, J=8.8 Hz)	
10	125.2	7.47 (d, J=8.8 Hz)	
11	102.5		
12	132.9*		
13	126.9*		
14	132.2		H-9, H-10, H-5
7-OMe	55.8	3.8	
3-OMe	56.4	4.03	
2-OH		5.74 (s)	

Protonated carbons assigned from HMQC spectrum.



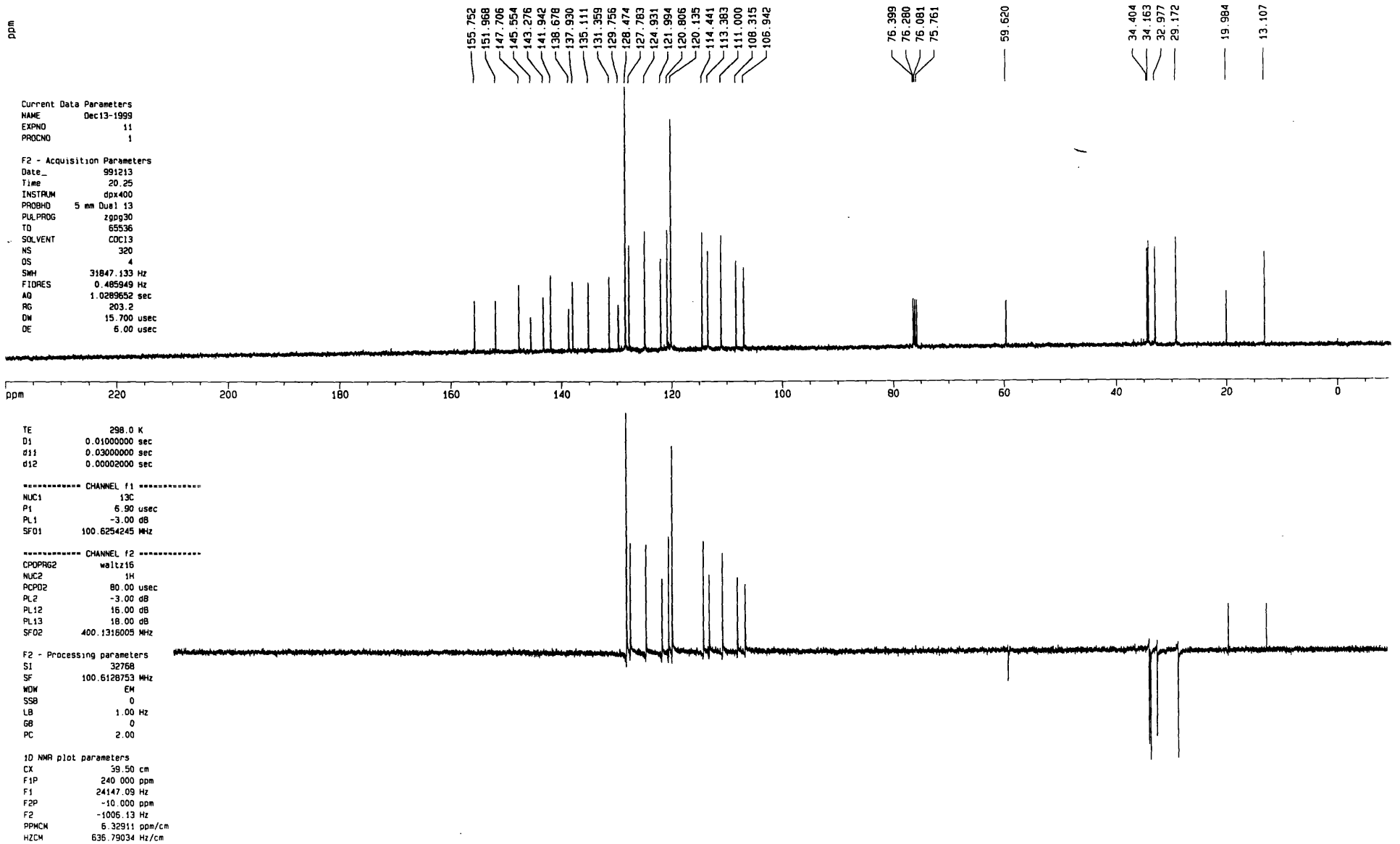
6

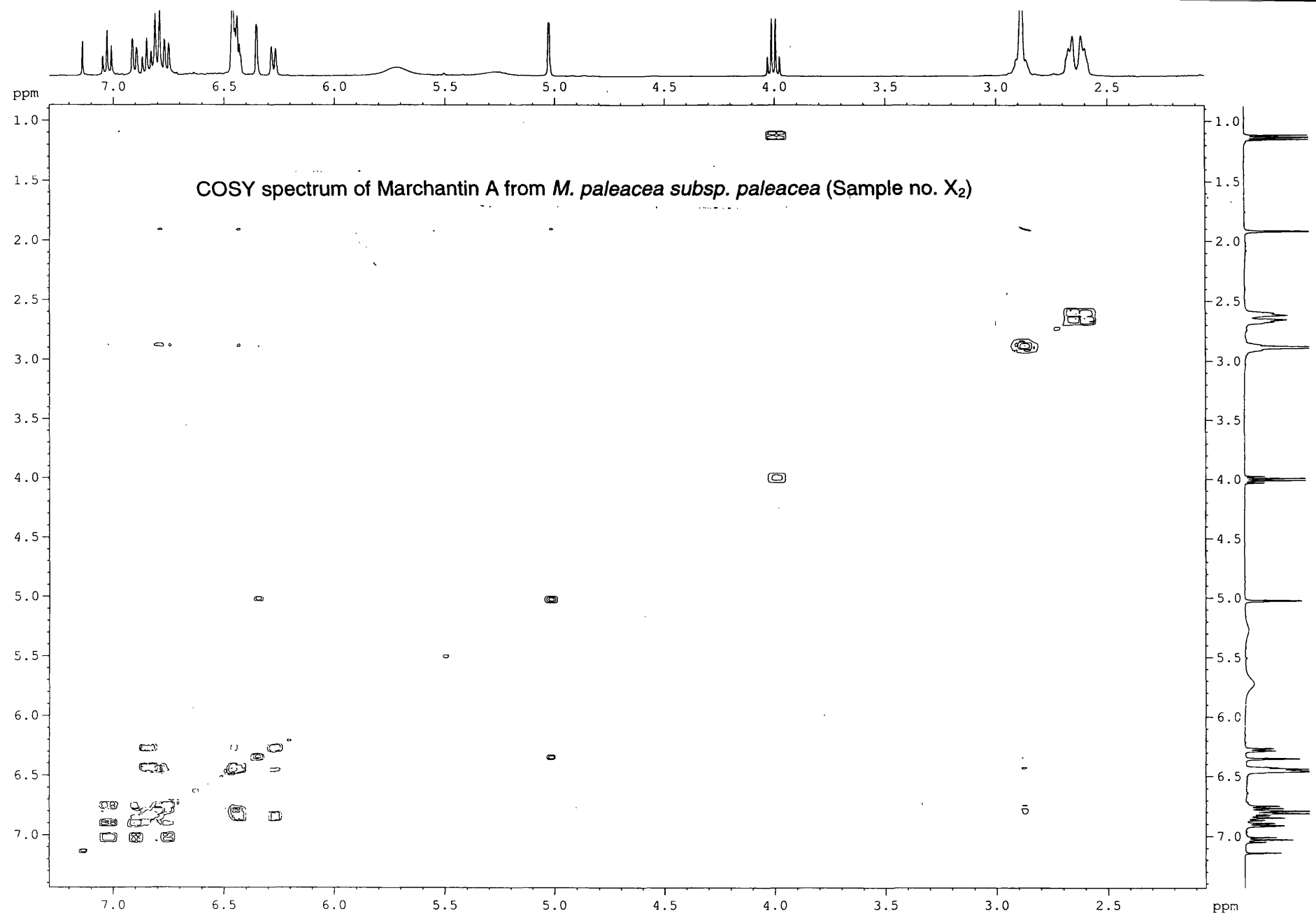
NMR Spectra



^{1}H spectrum of Marchantin A from *M. paleacea subsp. paleacea* (Sample no. X_2)

^{13}C (DEPT) spectrum of Marchantin A from *M. paleace subsp. paleacea* (Sample no. X_2)




COSY spectrum of Marchantin A from *M. paleacea subsp. paleacea* (Sample no. X_2)
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6.0
5.5
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4.0
3.5
3.0
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1.0
1.5
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4.0
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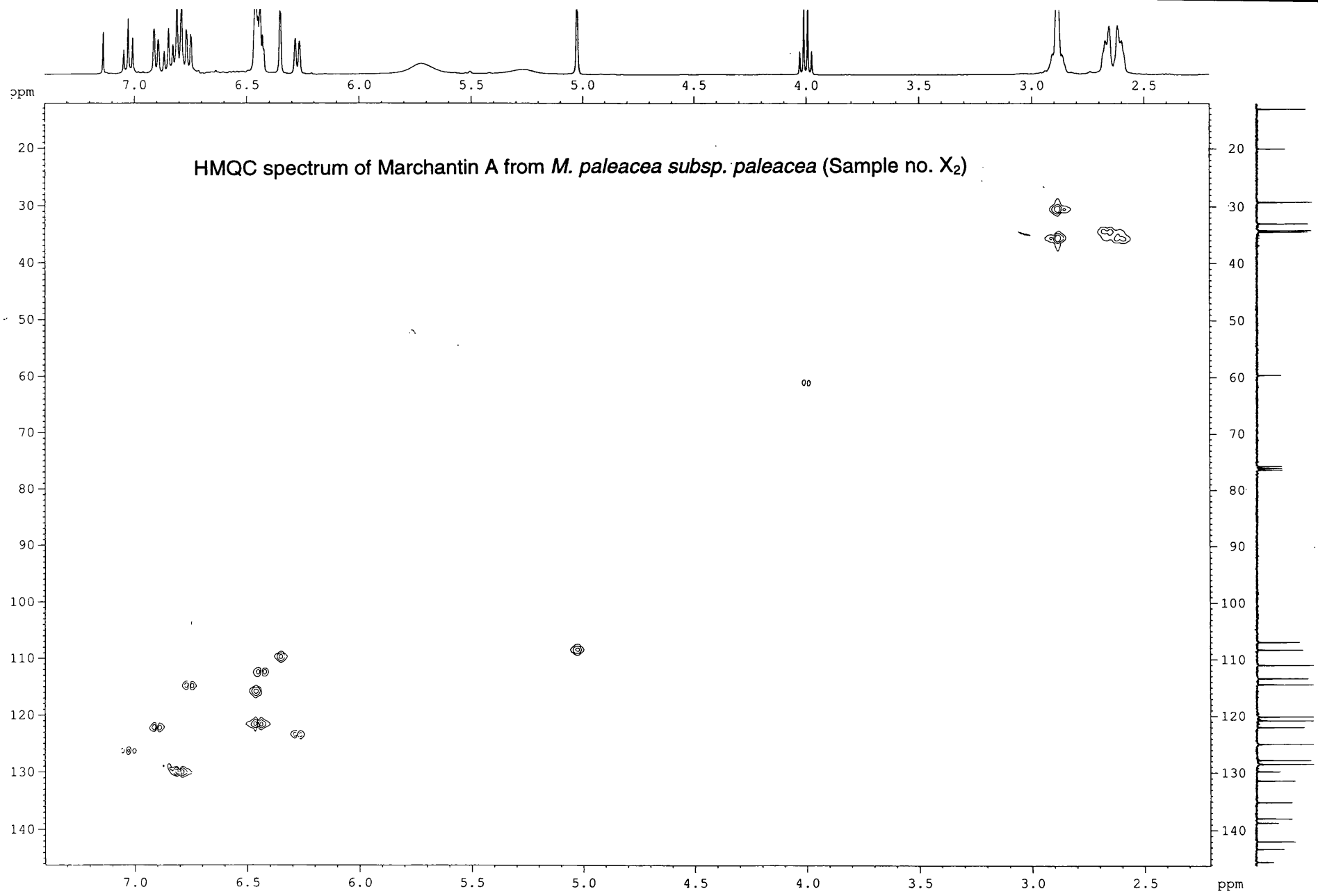
5.5
6.0
6.5
7.0
ppm


HMQC spectrum of Marchantin A from *M. paleacea subsp. paleacea* (Sample no. X_2)
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6.0
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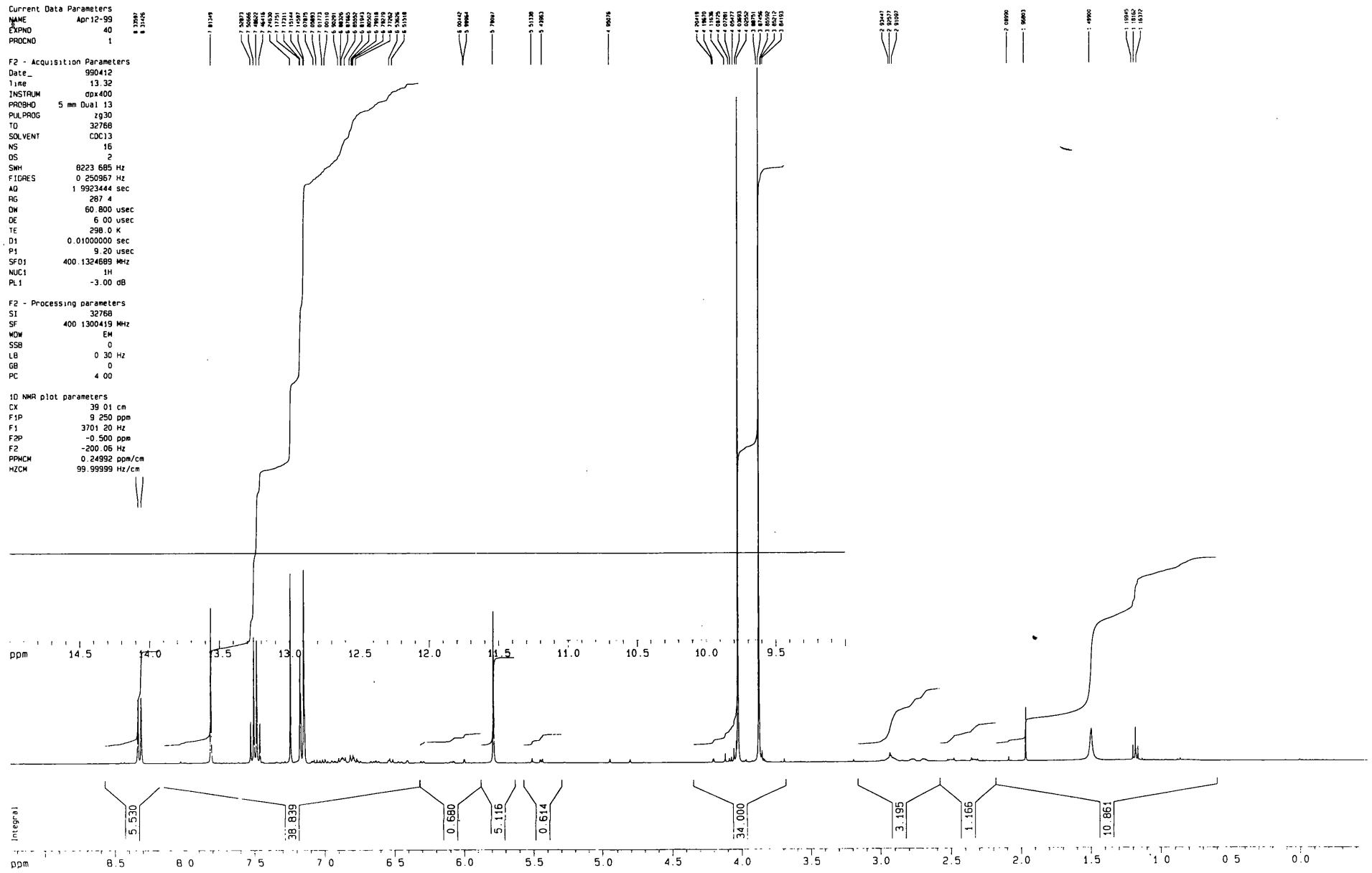
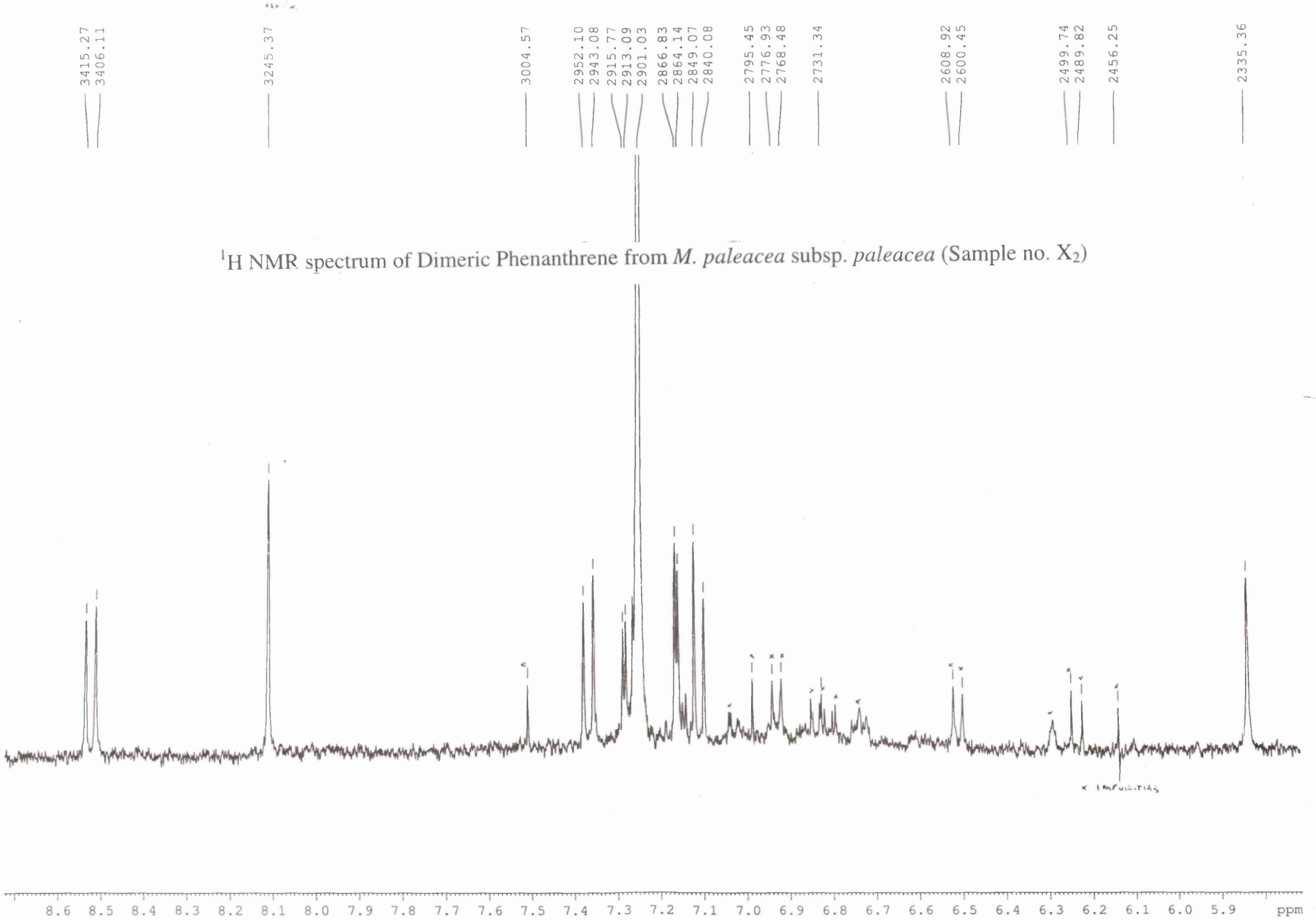
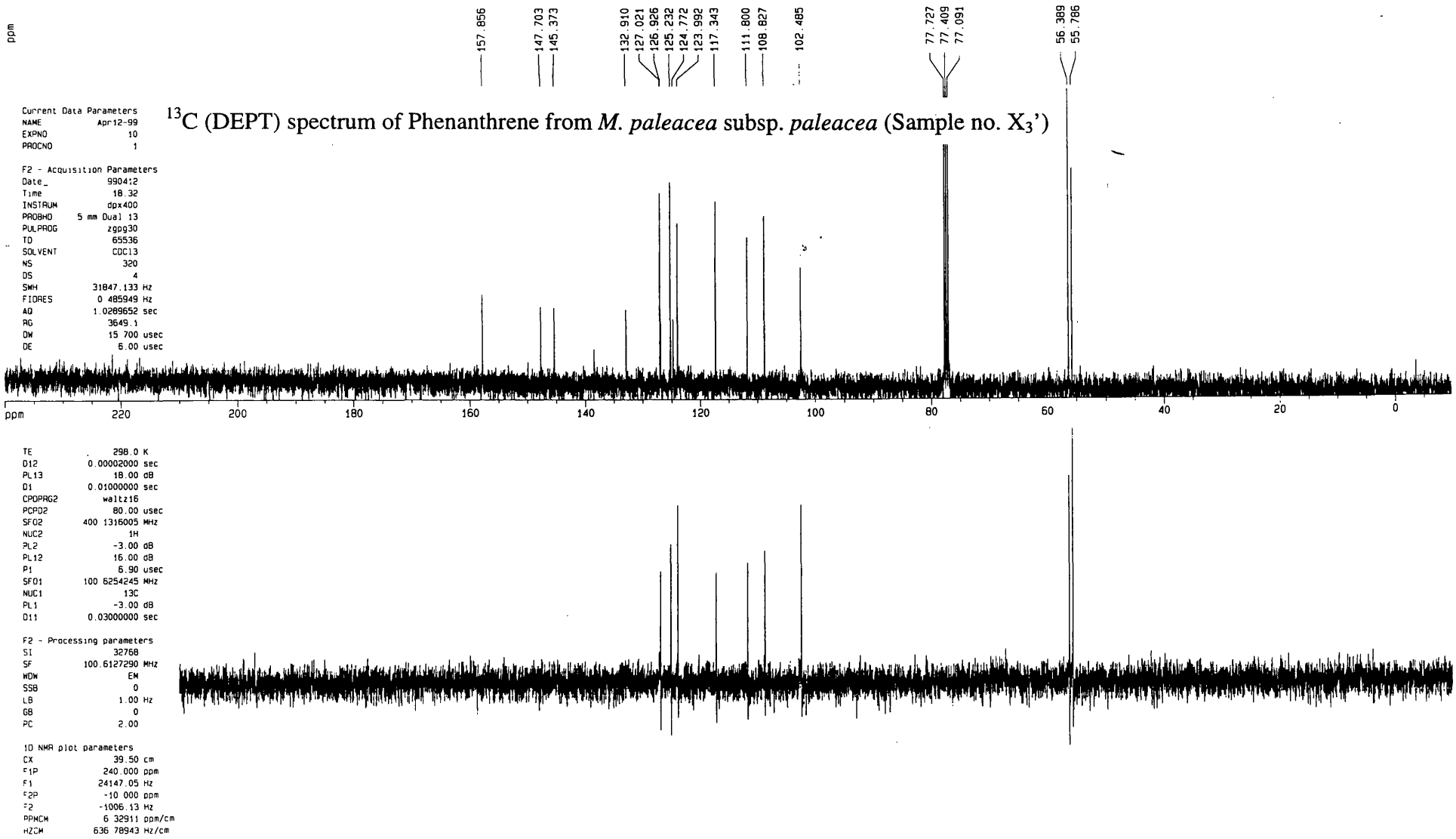
5.0
4.5
4.0
3.5
3.0
2.5
20
30
40
50
60
70
80
90
100
110
120
130
140

^{1}H NMR spectrum of Phenanthrene from *M. paleacea* subsp. *paleacea* (Sample no. X_2)

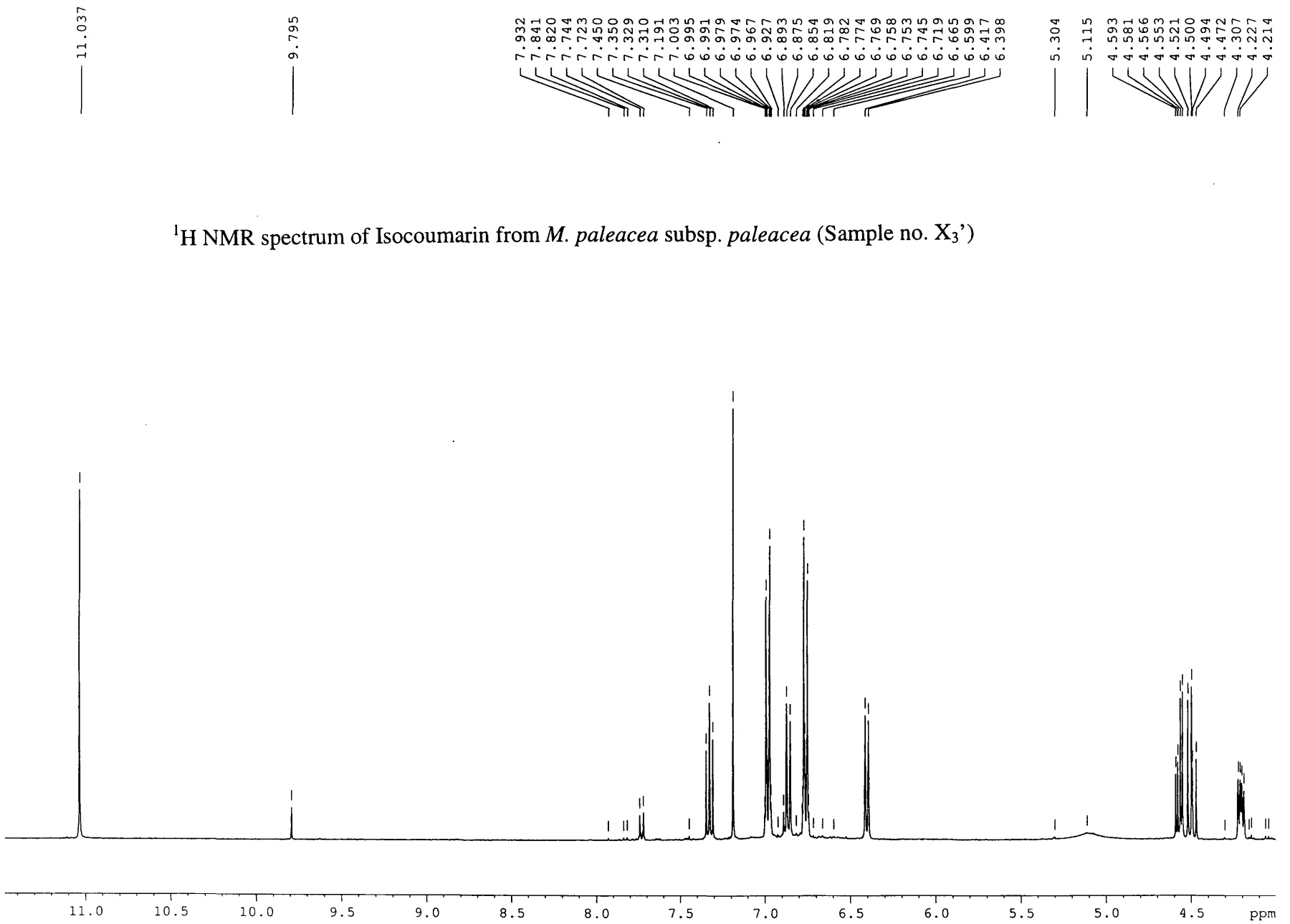




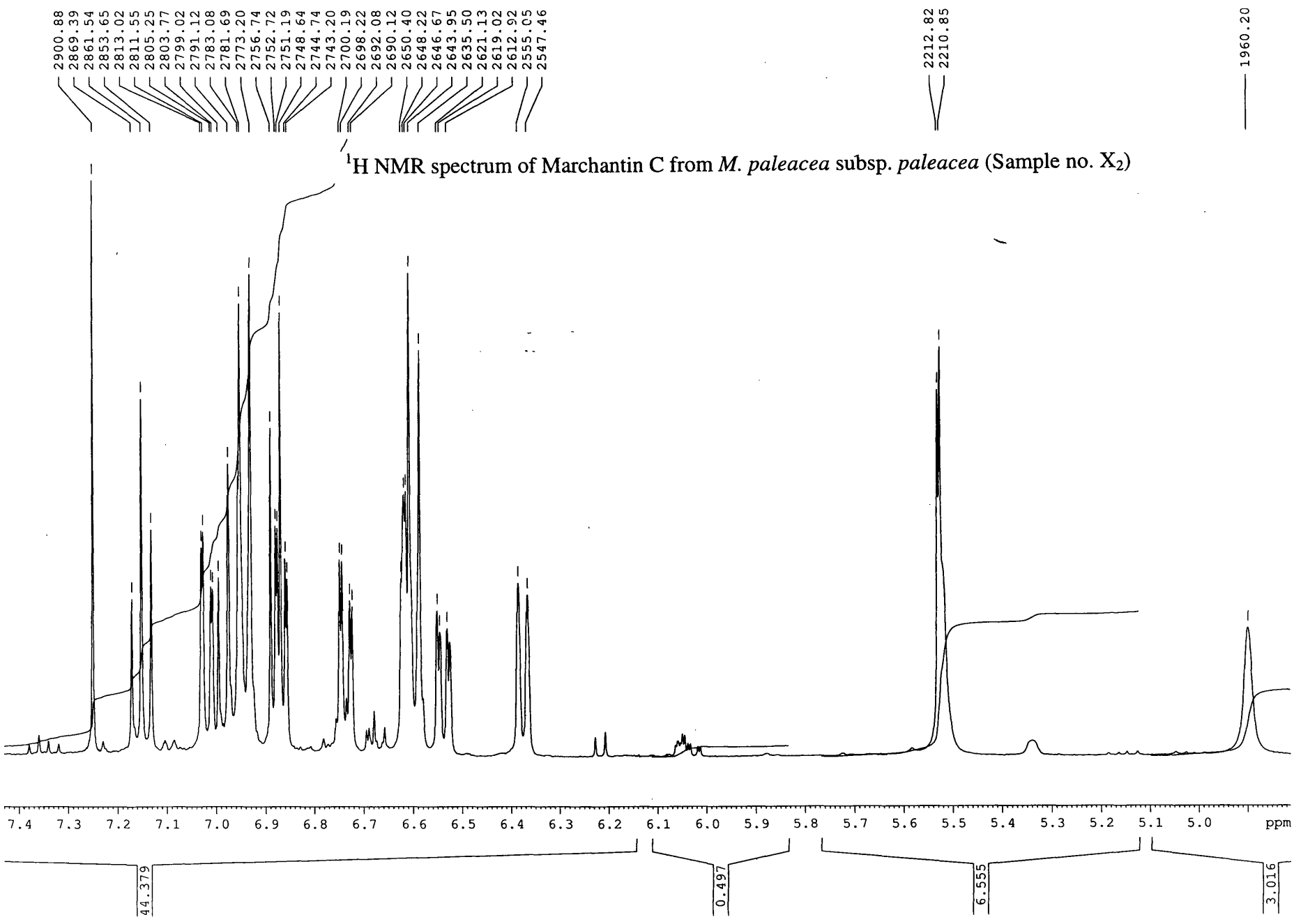
^{1}H NMR spectrum of Dimeric Phenanthrene from *M. paleacea* subsp. *paleacea* (Sample no. X_2)



^{13}C (DEPT) spectrum of Phenanthrene from *M. paleacea* subsp. *paleacea* (Sample no. X_3')

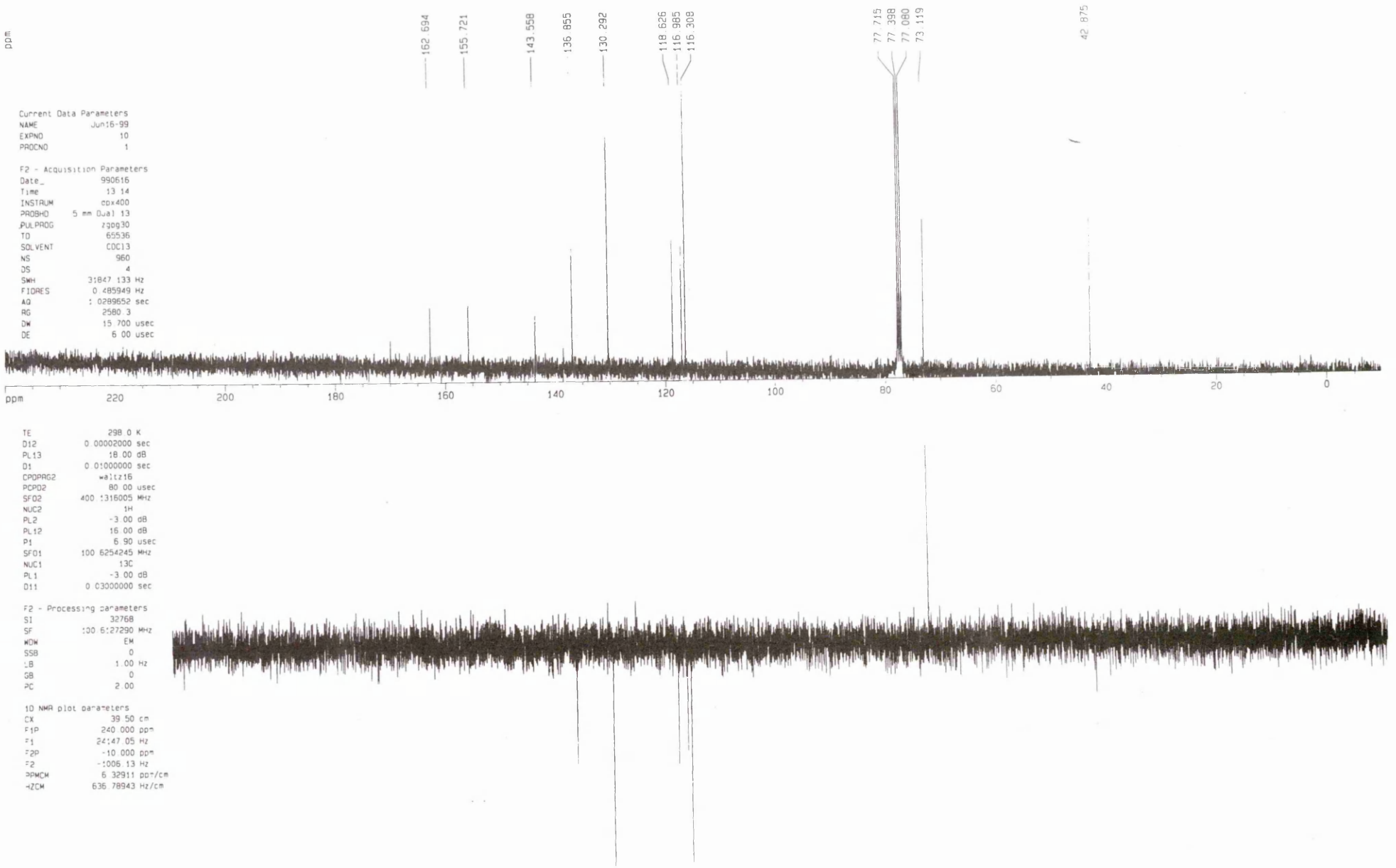


^{1}H NMR spectrum of Isocoumarin from *M. paleacea* subsp. *paleacea* (Sample no. X_3')

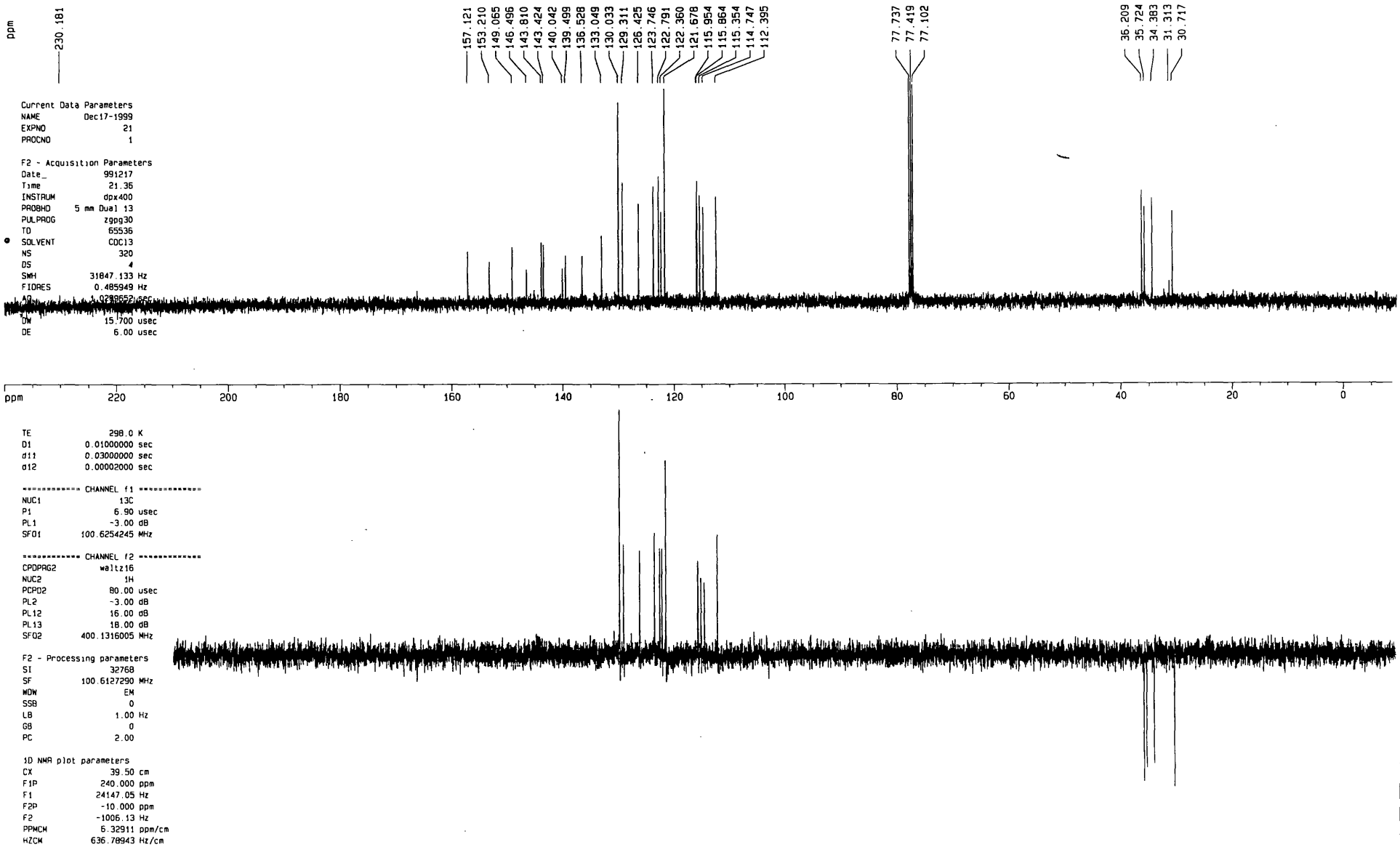


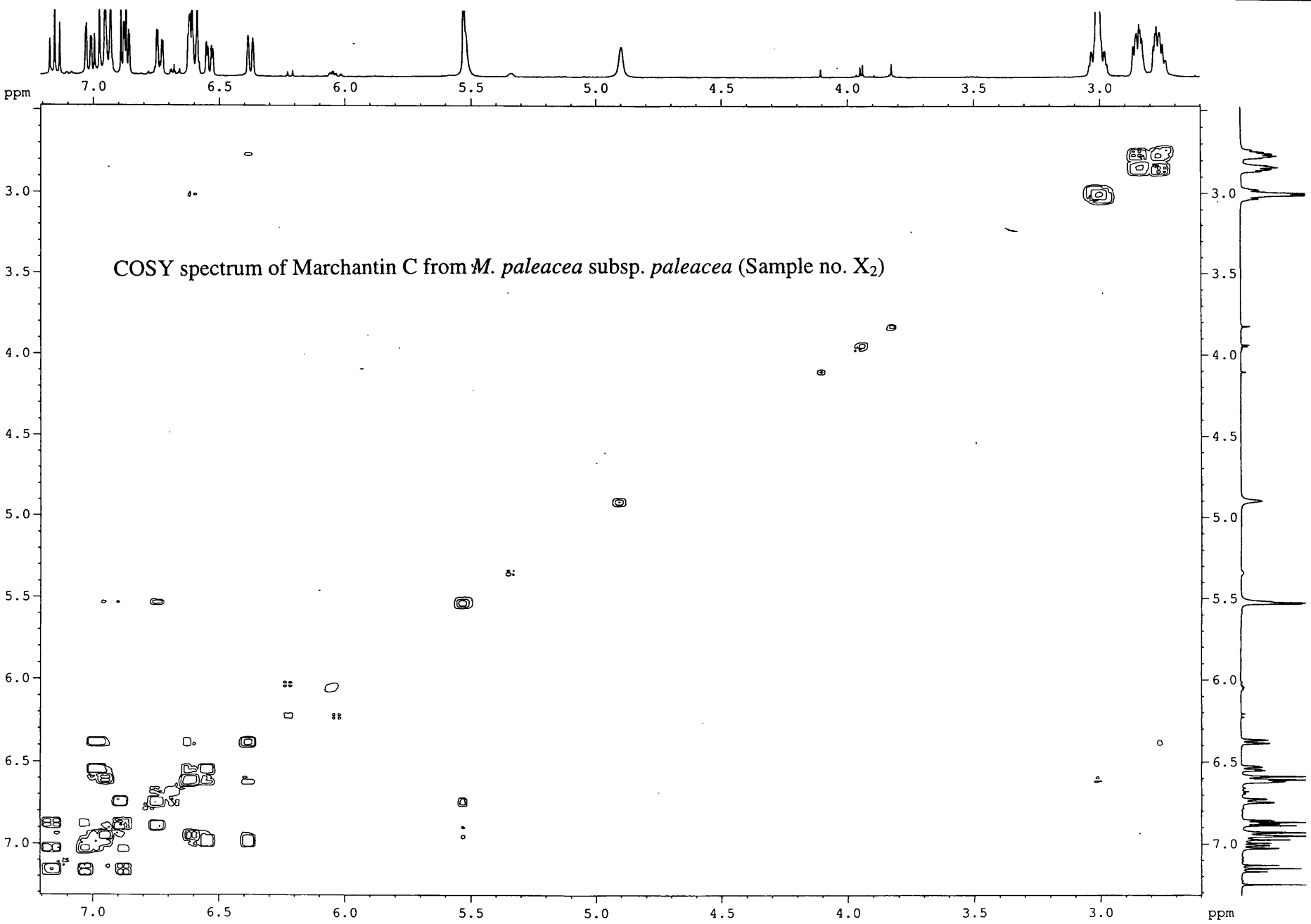
^{1}H NMR spectrum of Marchantin C from *M. paleacea* subsp. *paleacea* (Sample no. X_2)

^{13}C (DEPT) spectrum of Isocoumarin from *M. paleacea* subsp. *paleacea* (Sample no. X_3')

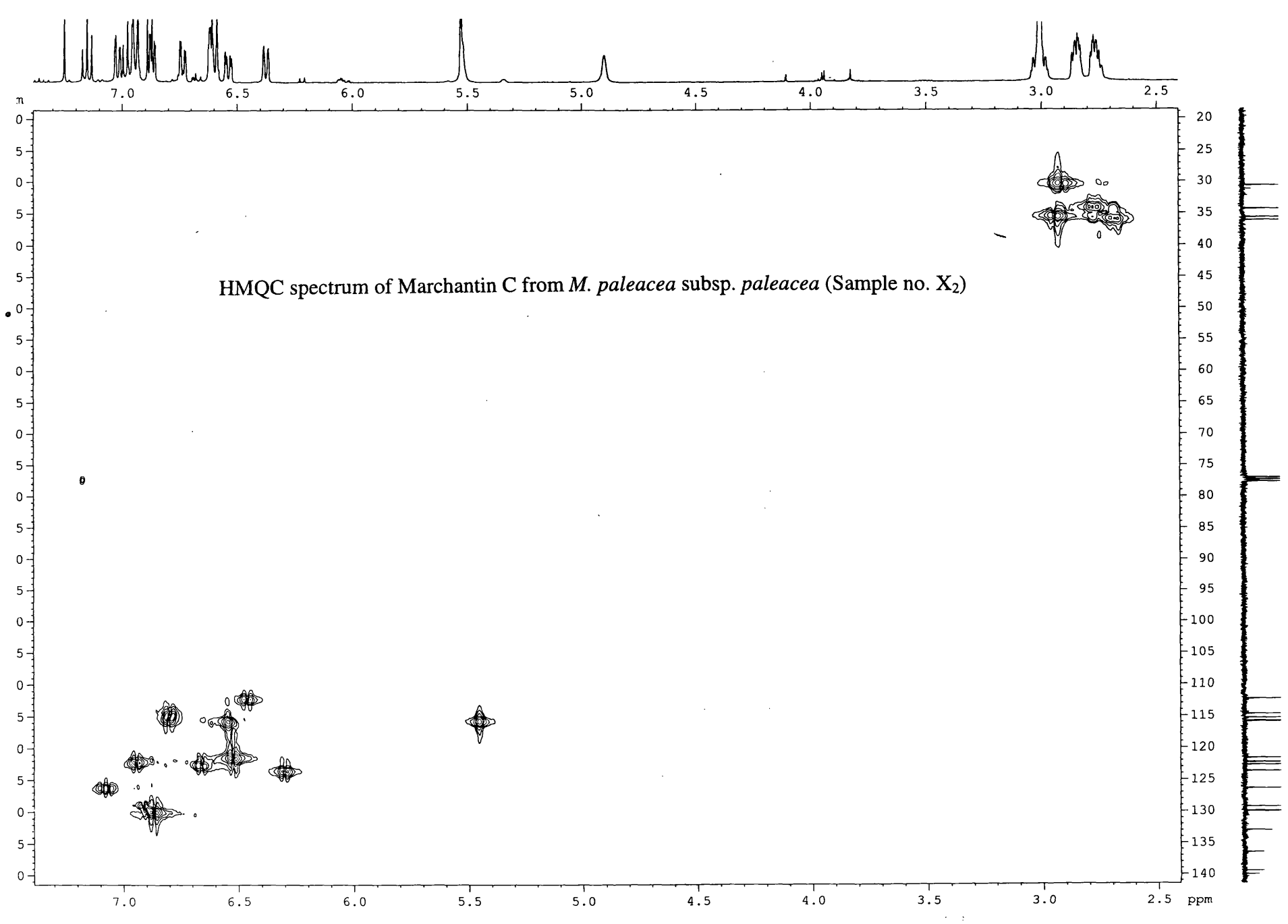


^{13}C NMR spectrum of Marchantin C from *M. paleacea* subsp. *paleacea* (Sample no. X_2)





COSY spectrum of Marchantin C from *M. paleacea* subsp. *paleacea* (Sample no. X_2)


HMQC spectrum of Marchantin C from *M. paleacea* subsp. *paleacea* (Sample no. X_2)
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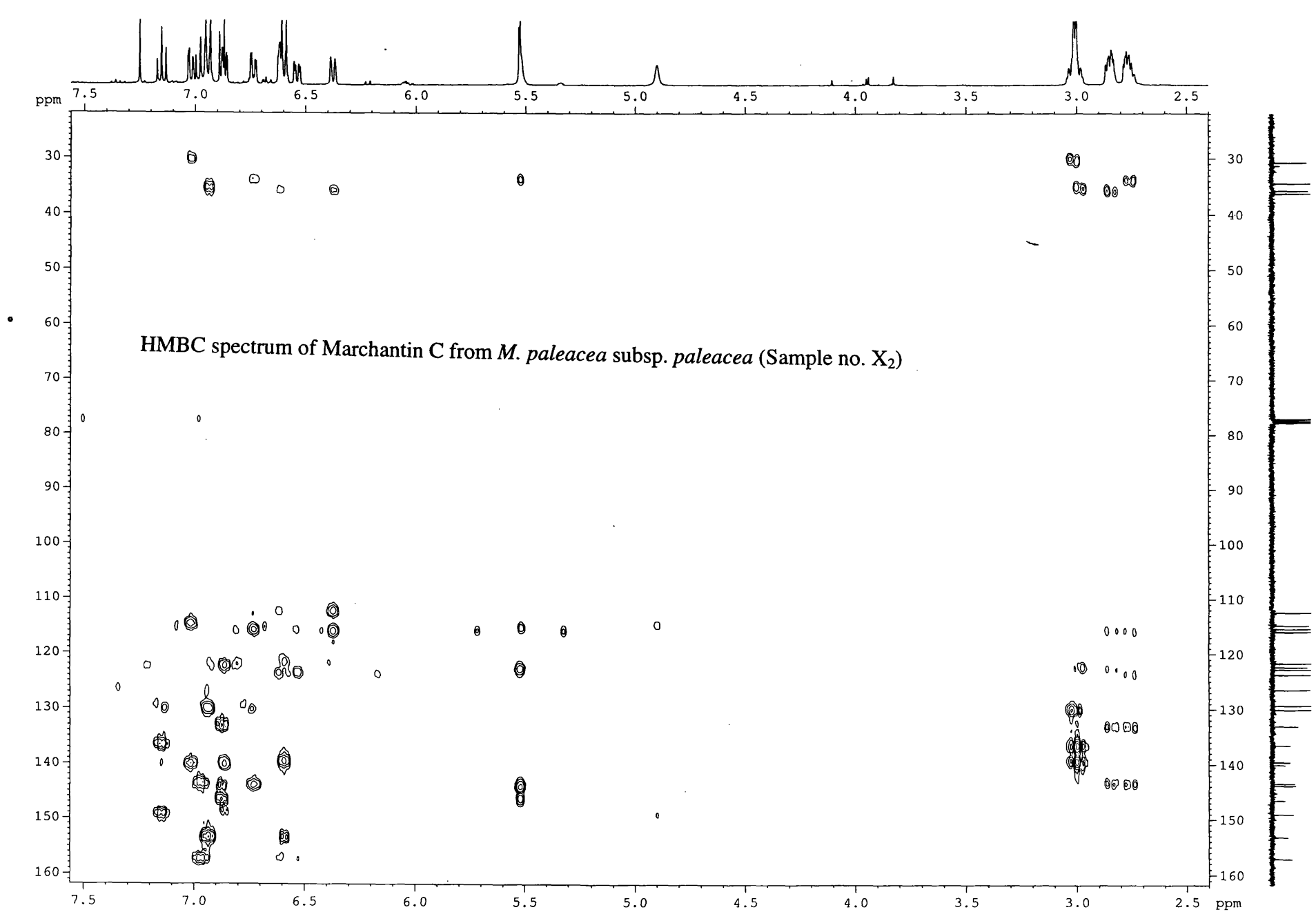
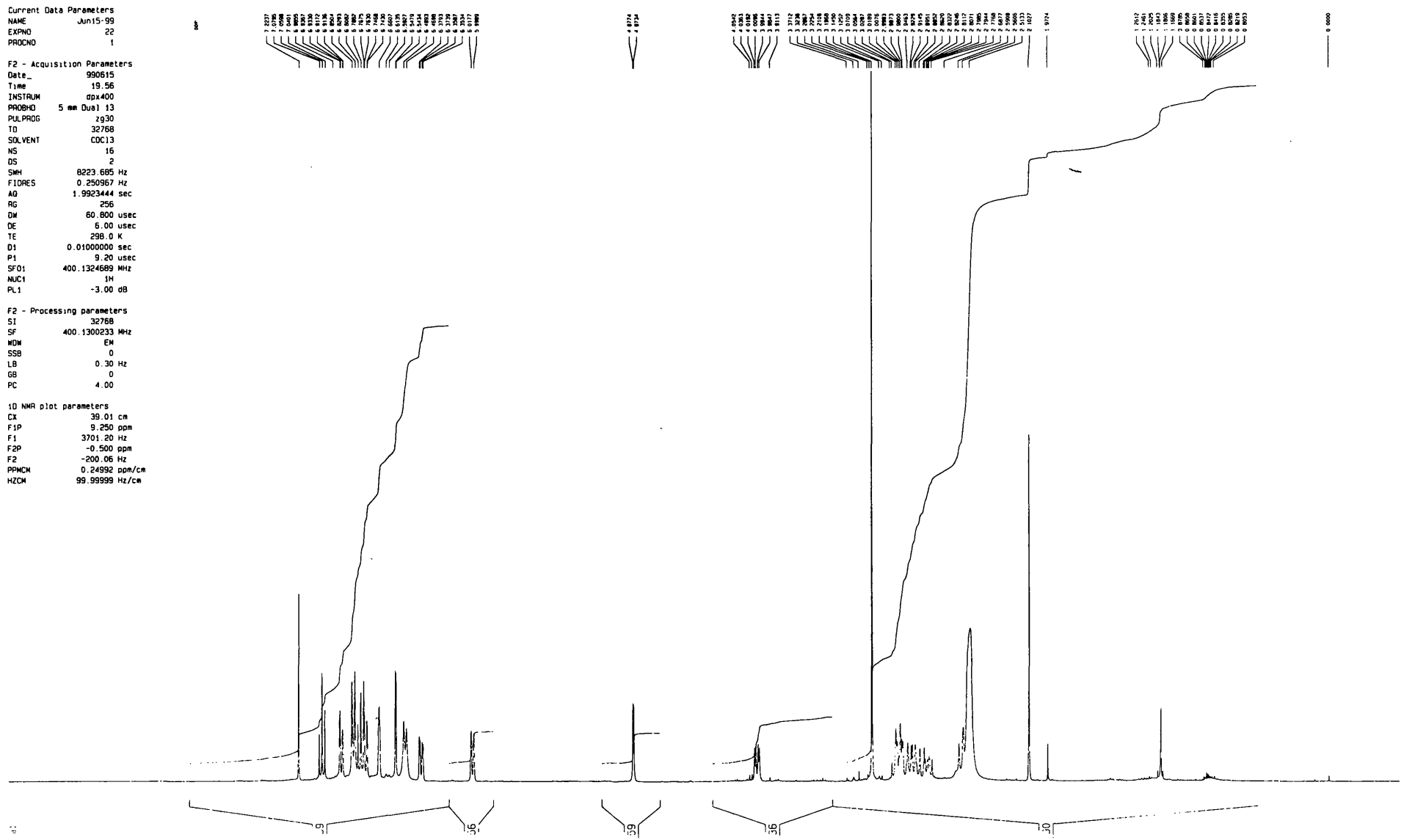
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm

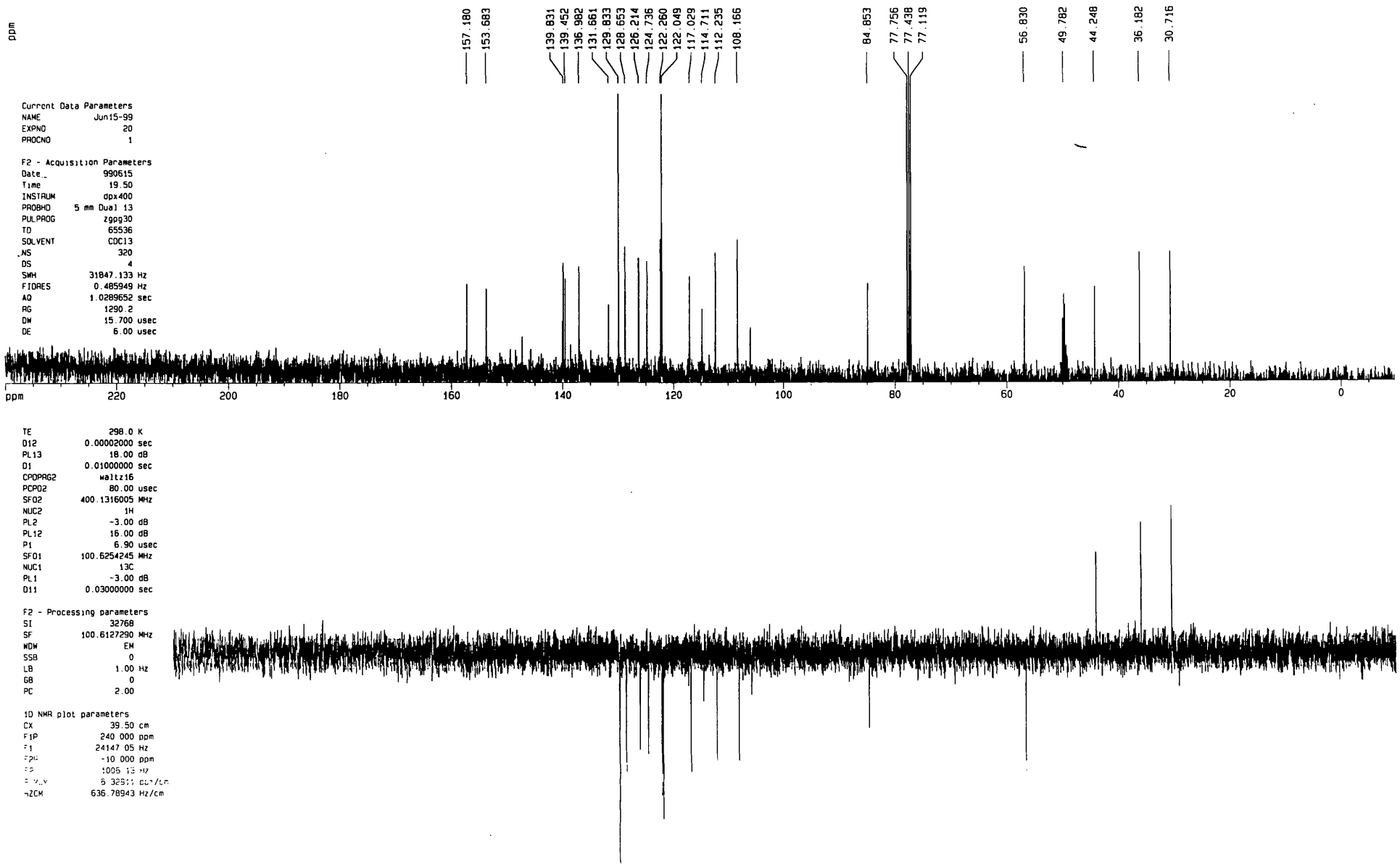


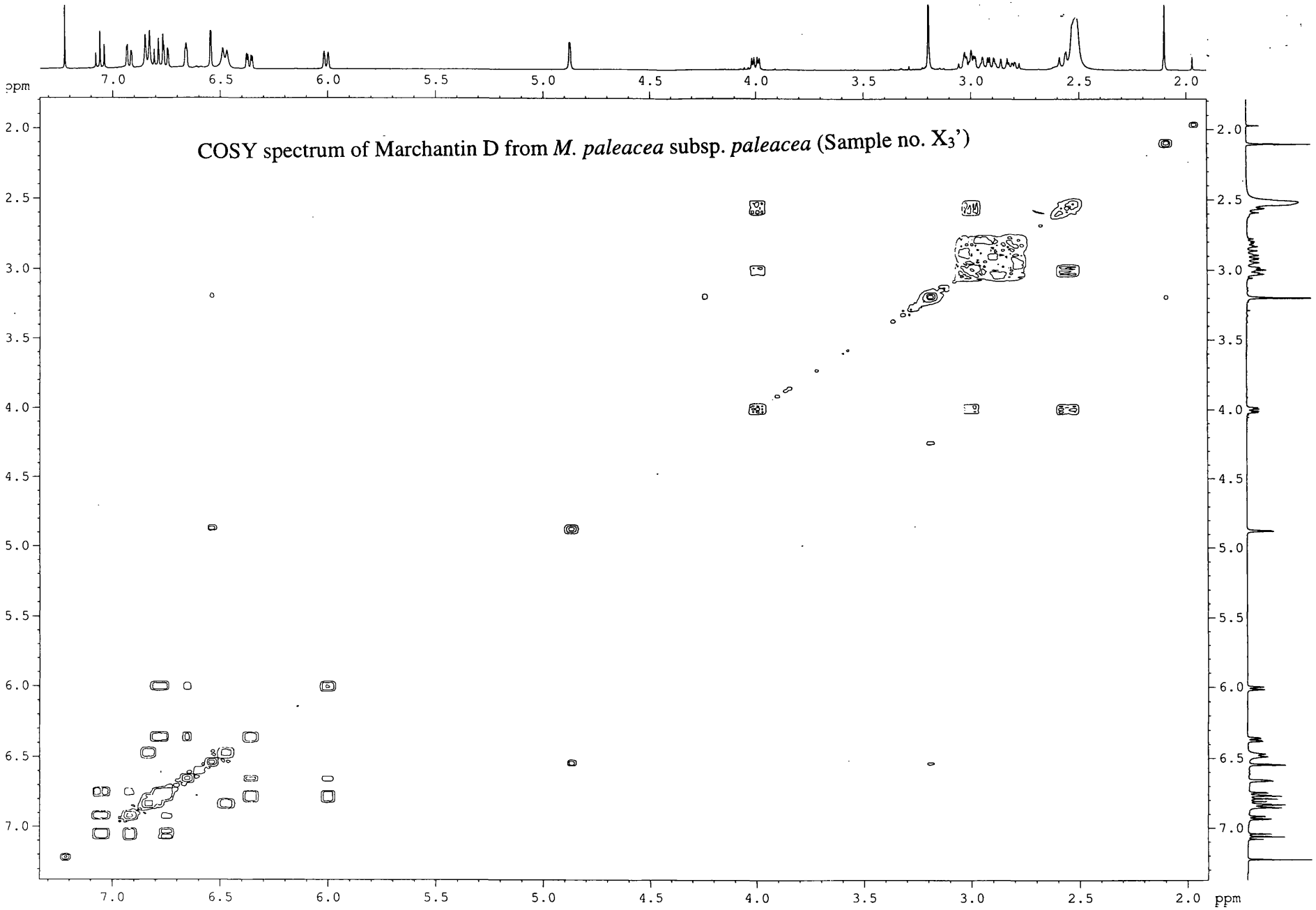
HMBC spectrum of Marchantin C from *M. paleacea* subsp. *paleacea* (Sample no. X_2)

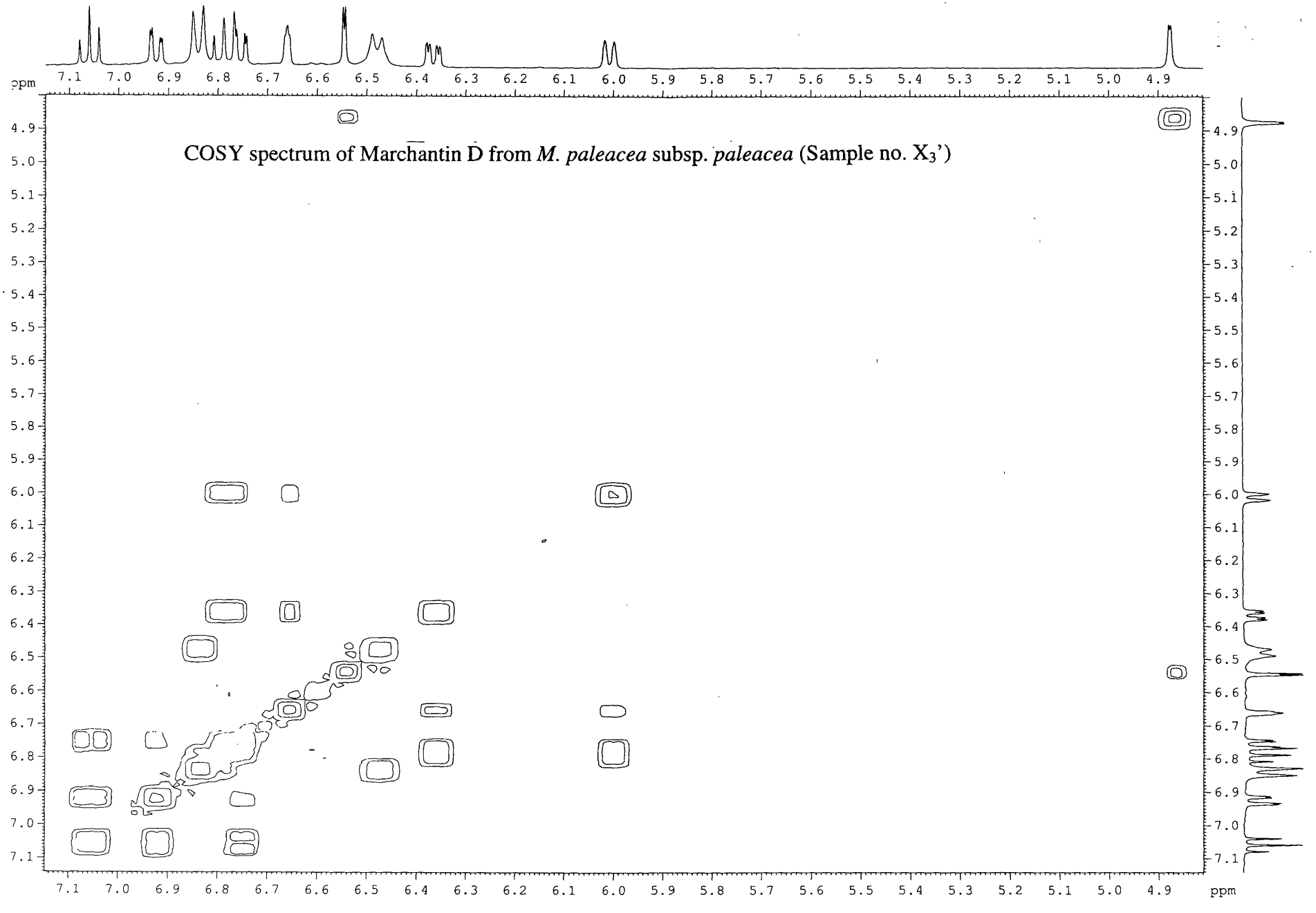
^{1}H NMR spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')



^{13}C (DEPT) spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')

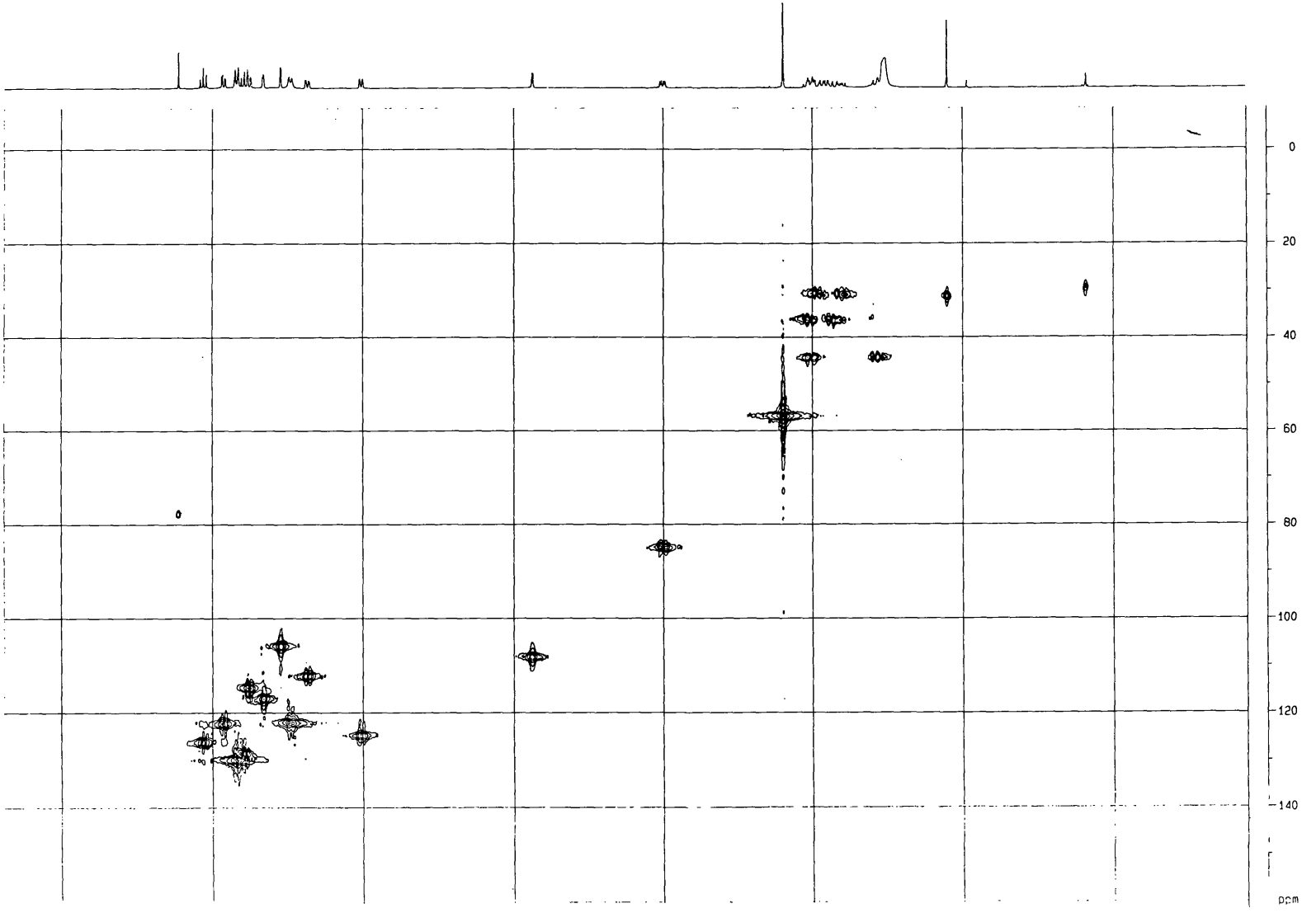



COSY spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')
ppm
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0



COSY spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')

HMQC spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')



Current Data Parameters

NAME	Jun15-99
EXPNO	23
PROCNO	1

F2 - Acquisition Parameters

Date_	990615
Time	19 58
INSTRUM	dpx400
PROBHD	5 mm Dual 13
PULPROG	inv4gp
TD	2048
SOLVENT	CDCl3
NS	8
DS	16
SWH	3306.878 Hz
FIDRES	1.614687 Hz
AQ	0.3097076 sec
RG	13004
DW	151.200 usec
DE	6.00 usec
TE	298.0 K
D12	0.00002000 sec
D2	0.00345000 sec
P16	1000.00 usec
D13	0.00000300 sec
d20	0.00242700 sec
D1	1.34201097 sec
P1	9.20 usec
SFO1	400.1317212 MHz
NUC1	1H
PL1	-3.00 dB
PL2	-3.00 dB
P3	8.40 usec
SFO2	100.6204259 MHz
NUC2	13C
D0	0.00000300 sec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	50.00 %
GPNAM1	sine.100
D16	0.00010000 sec
P2	18.40 usec
GPX2	0.00 %
GPY2	0.00 %
GPZ2	30.00 %
GPNAM2	sine.100
GPX3	0.00 %
GPY3	0.00 %
GPZ3	40.00 %
GPNAM3	sine.100
PL12	15.00 dB
CPDPRG2	garp
PCPD2	66.00 usec
IN0	0.00002920 sec

F1 - Acquisition parameters

ND0	2
TD	128
SFO1	100.6204 MHz
FIDRES	133.775681 Hz
SW	170.177 ppm

F2 - Processing parameters

SI	2048
SF	400.1300233 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40

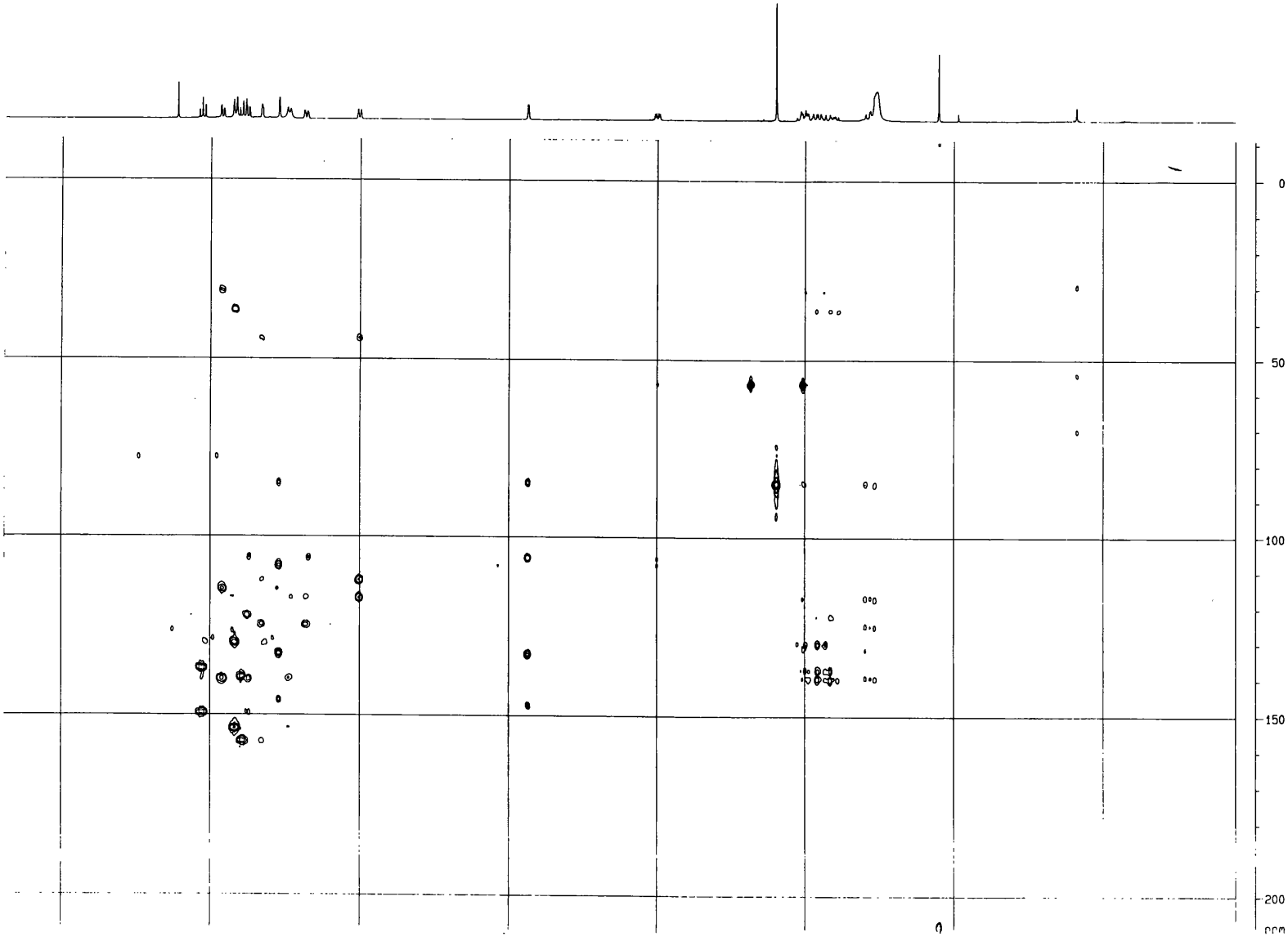
F1 - Processing parameters

SI	512
MC2	QF
SF	100.6127290 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	31.00 cm
CX1	20.00 cm
F2PL0	8.381 ppm
F2L0	3353.53 Hz
F2PH1	0.117 ppm
F2H1	46.67 Hz
F1PL0	151.189 ppm

HMBC spectrum of Marchantin D from *M. paleacea* subsp. *paleacea* (Sample no. X_3')



Current Data Parameters

NAME	Jun15-99
EXPNO	24
PROCNO	1

F2 - Acquisition Parameters

Date_	990615
Time	20 28
INSTRUM	dpx400
PROBHD	5 mm Dual 13
PULPROG	inv4gs1plrnd
TD	2048
SOLVENT	CDCl3
NS	8
DS	16
SWH	3306.878 Hz
FIDRES	1.614687 Hz
AQ	0.3097076 sec
RG	16384
DW	151.200 usec
DE	6.00 usec
TE	298.0 K
D1	1.36114395 sec
P1	9.20 usec
SFO1	400.1317212 MHz
NUC1	1H
PL1	-3.00 dB
D2	0.00350000 sec
P3	8.40 usec
SFO2	100.6227604 MHz
NUC2	13C
PL2	-3.00 dB
D6	0.06500000 sec
D0	0.00000300 sec
P16	1000.00 usec
D16	0.00010000 sec
P2	18.40 usec
D13	0.00000300 sec
IN0	0.00002240 sec

F1 - Acquisition parameters

ND0	2
TD	128
SFO1	100.6228 MHz
FIDRES	174.386154 Hz
SW	221.833 ppm

F2 - Processing parameters

SI	2048
SF	400.1300233 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	512
MC2	QF
SF	100.6127290 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	31.00 cm
CX1	20.00 cm
F2PLO	8.381 ppm
F2LO	3353.53 Hz
F2PHI	0.117 ppm
F2HI	46.67 Hz
F1PLO	210.619 ppm
F1LO	21190.96 Hz
F1PHI	-11.214 ppm
F1HI	-1128.24 Hz
F2PPMCM	0.26550 ppm/cm
F2HZCM	106.67304 Hz/cm
F1PPMCM	11.09164 ppm/cm
F1HZCM	1115.96021 Hz/cm

CHAPTER THREE

Marchantia papillata Raddi subsp. *grossibarba* (steph) Bishl

3.1 Introduction:

Systematic position:

Division - Bryophyte

Class - Hepaticopsida

Order - Marchantiales

Family - Marchantiaceae

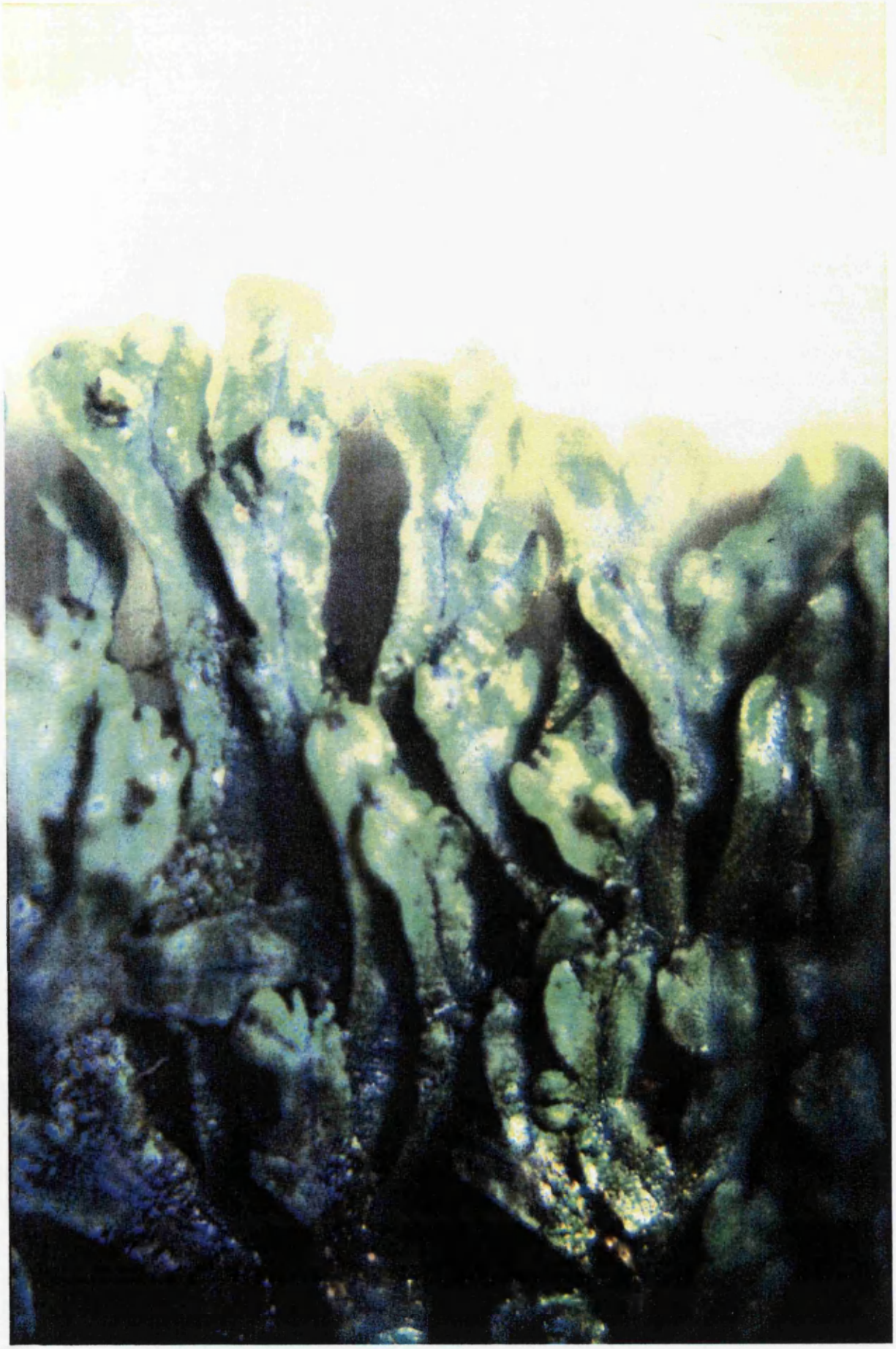
Genus - *Marchantia*

Species - *papillata* Raddi

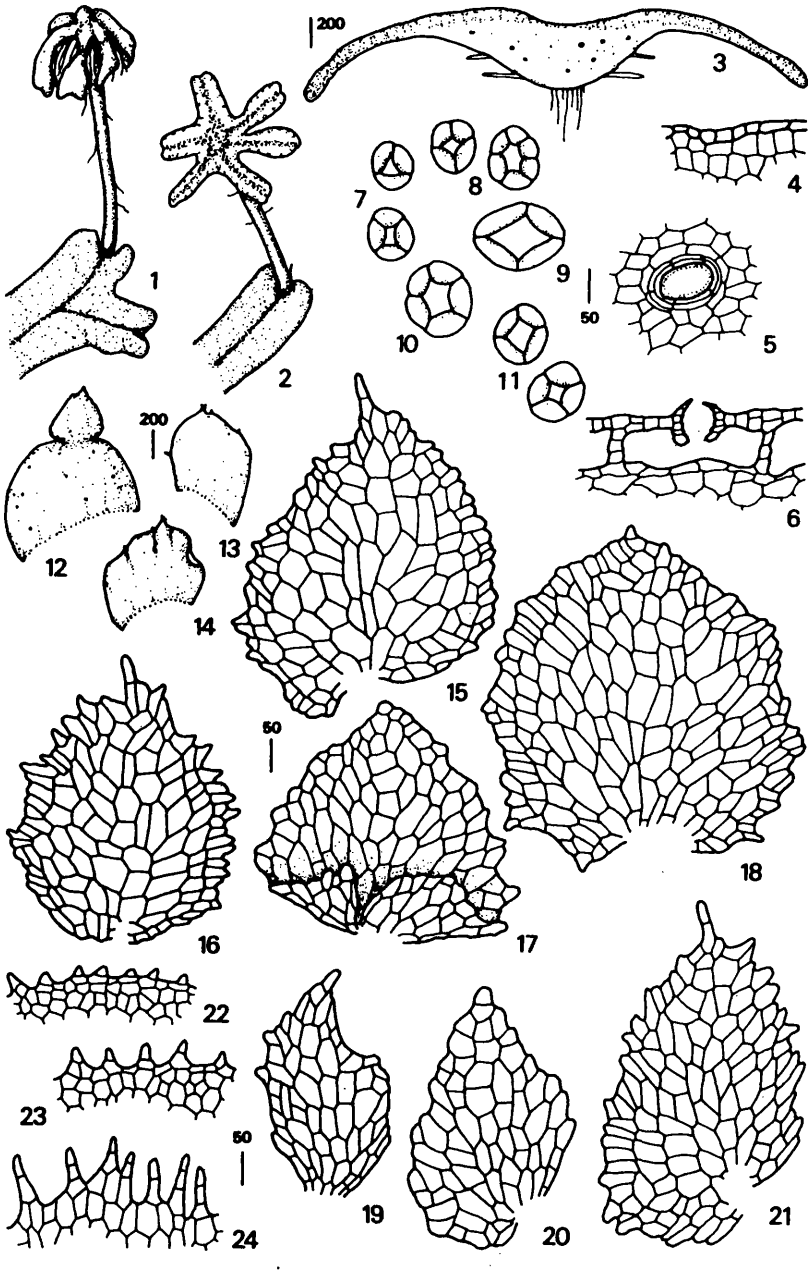
Subsp. - *grossibarba* (steph) Bischl

The taxa of sect. *papillata* are very closely allied and are difficult to distinguish. They have been thoroughly investigated (Bischler 1989) and were subdivided into two subspecies, *M. papillata* subsp. *papillata* and *M. papillata* subsp. *grossibarba.*

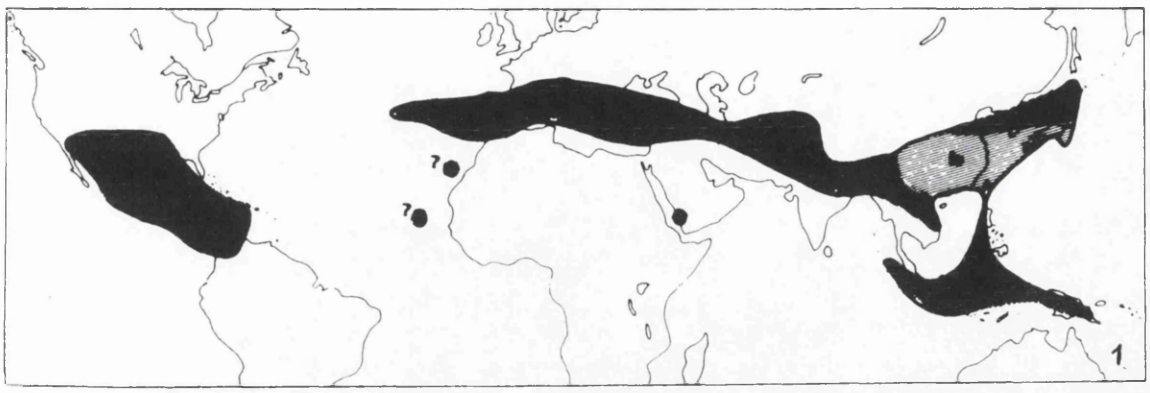
Subsp. *papillata* is known from South America between 2°S and 35°S (Bischler 1984). Unlike subsp. *grossibarba*, it has been collected mainly at low elevations (0-500 m.), and is absent from high elevations in tropical areas. Subsp. *grossibarba* occurs from 30-3000m. These two subspecies are found in Asia, Oceania, Africa and South America. The range includes small and large



Marchantia papillata Raddi subsp. grossibarba (Steph) Bishl



- *Marchantia papillata* Raddi subsp. *grossibarba* (Steph.) Bischl. 1: female thallus, 2: male thallus, 3: thallus section, 4: thallus margin, 5: epidermal pore, surface view, 6: epidermal pore, section, 7-11: epidermal pores, inner openings, 8: median scale, 13-14: laminal scales, 15-21: appendages of median scales, 22-24: cupules.



- *M. paleacea* (map 1) has a circumtethyan range; it is differentiated into two subspecies towards the northeastern border of its distribution (Bischler 1986a).

- *M. papillata* (map 2), limited in Asia to continental areas, is also present in tropical South America.

islands and continental areas. The two subspecies occur in similar habitats, forests and forest margins, woods, shrubby vegetation, cultivated areas and gardens. Subsp. *grossibarba* differs from subsp. *papillata* in its robust appearance. The main differences are found in the structure of the appendage of the median scales. These are orange, triangular, and usually acute with a single cell apically in subsp. *grossibarba*, and purplish, ovate, and with a row of 2-3 cells apically in subsp. *papillata.*

3.2 Constituents: There are no reports regarding the chemical constituents of *Marchantia papillata* Raddi subsp *grossibarba.*

3.3 Materials and Methods (Experimental)

Plant material was collected from Godawari (Nepal) at an elevation of 1487m., longitude 27^0 36` and latitude $85^0$23`. Godawari is situated at a distance of 17 Km southeast of Kathmandu, the capital city of Nepal. The plant was collected from a damp old wall in the month of October, 1989. The habitat was a shady place surrounded by tall trees. The plant was identified by Dr. David G Long, Royal Botanic Garden, Edinburgh where the specimen is deposited.

Sample no. Y_1 (Marchantia papillata)

The extract of dried and powdered material (50 g) was made as in case of sample no. X_2. The extract recovered (5g) was fractionated by flash chromatography as described above.

Please note that the structures of the isolated compounds are presented in the text and are indicated by bold numbers without brackets. All of the

compounds were gums or oils. Their spectroscopic properties are presented in the text during the discussion and/or in Tables.

Isolation of the sesquiterpenoid 11-eudesmen-4α-ol **10***:*

Fraction 10 (eluted with 10% ethyl acetate in petroleum ether) was concentrated and was subjected to prep tlc using 30% ethyl acetate in petroleum ether to develop the plates. Extraction of the second band (Y-1, b2) afforded 11-eudesmen-4α-ol **10**.

Sample no.Y_2 (Marchantia papillata)

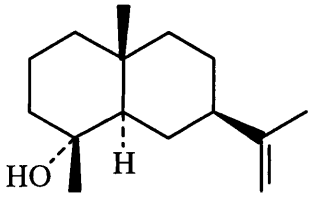
The extraction and chromatographic separation were carried out as for sample no. Y_1. The major constituents recovered from the preparative tlc were identified as the 2-hydroxy-3,7-dimethoxyphenanthrene **6**, marchantin A **7** and marchantin C **8**.

3.4 Results and Discussion:

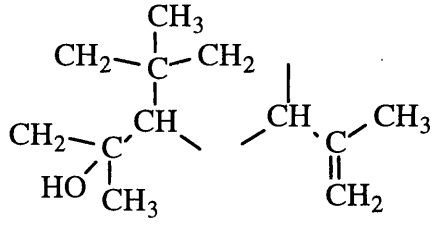
11-Eudesmen-4α-ol **10** *(y-1, b2)*

This compound was obtained as an oil slightly contaminated by fatty material. Its carbon NMR spectrum suggested the molecular formula $C_{15}H_{26}O$. Thus there were resonances for an exomethylene group [δ_C 150.7 (C) and 108.1 (CH_2)], a tertiary carbinol [δ_C 72.3], two methines [δ_C 54.9 and 46.3], a fully substituted carbon [δ_C 34.6], six methylenes [δ_C 44.7, 43.4, 41.1, 26.9, 26.0 and 20.1] and three methyl groups [δ_C 22.7, 21.1 and 18.7]. The proton NMR spectrum confirmed the presence of three methyls [δ_H 0.87, 1.11, 1.74], one of

which was a vinyl methyl, and an exomethylene group [δ_H 4.68 and 4.70(each bs)]. In the HMBC spectrum the methyl at δ_H 0.87 showed correlations with a quaternary carbon, a methine and two methylenes while the second methyl at δ_H 1.11 showed correlations to the same methine, the carbinol carbon and a third methylene. These data enabled the construction of part structure **A**. The HMBC spectrum also revealed an isopropenyl group attached to a methine. Combination of the isopropenyl group with part structure A led to the eudesmene **10** as the most likely structure. A NOE between the two tertiary methyl groups supported their 1,3-diaxial relationship in a trans decalin ring system. Compound **10**, 11-eudesmen-4α-ol, has already been described in the literature and comparison with published spectroscopic data confirmed its identity.[131]



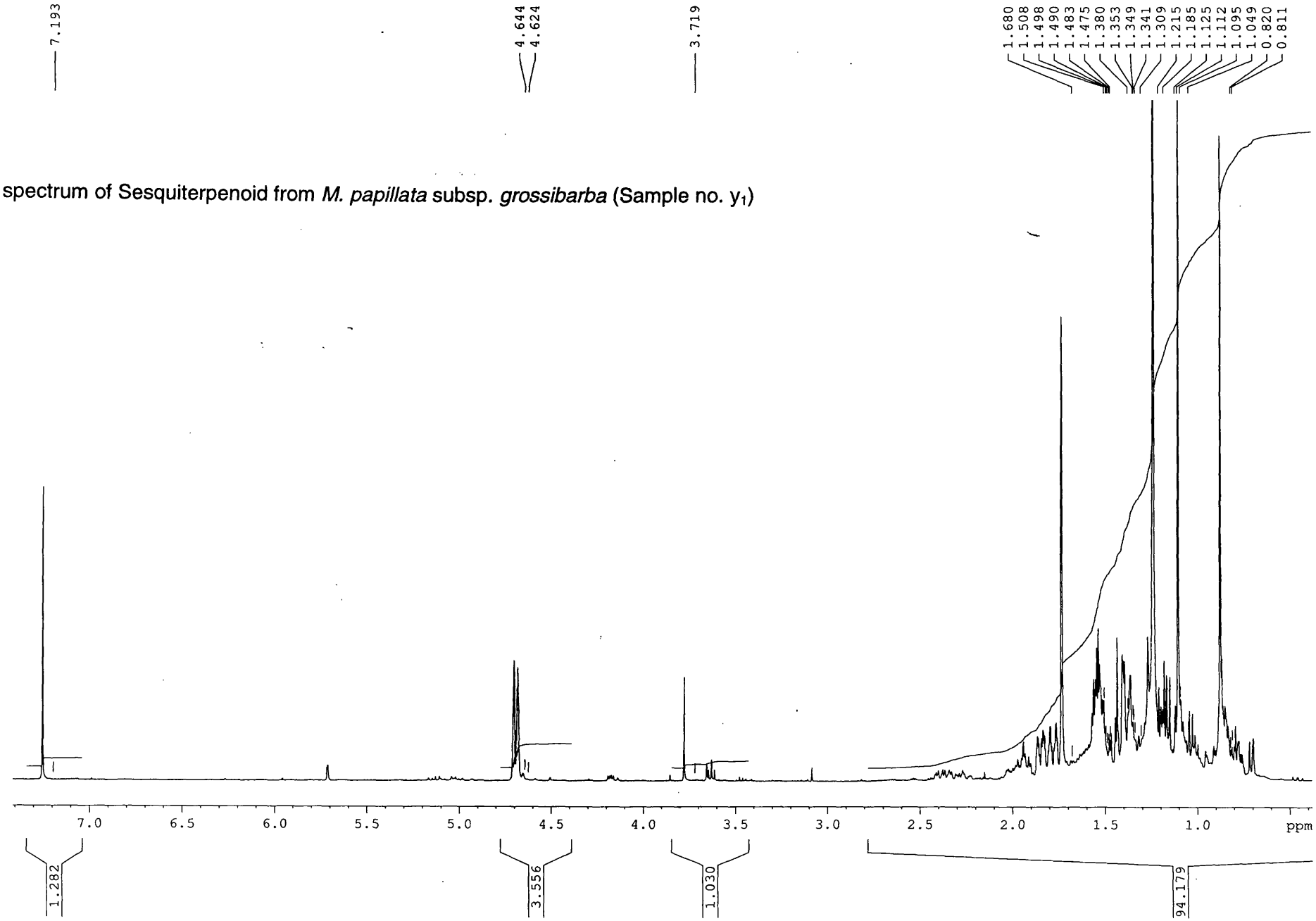
10



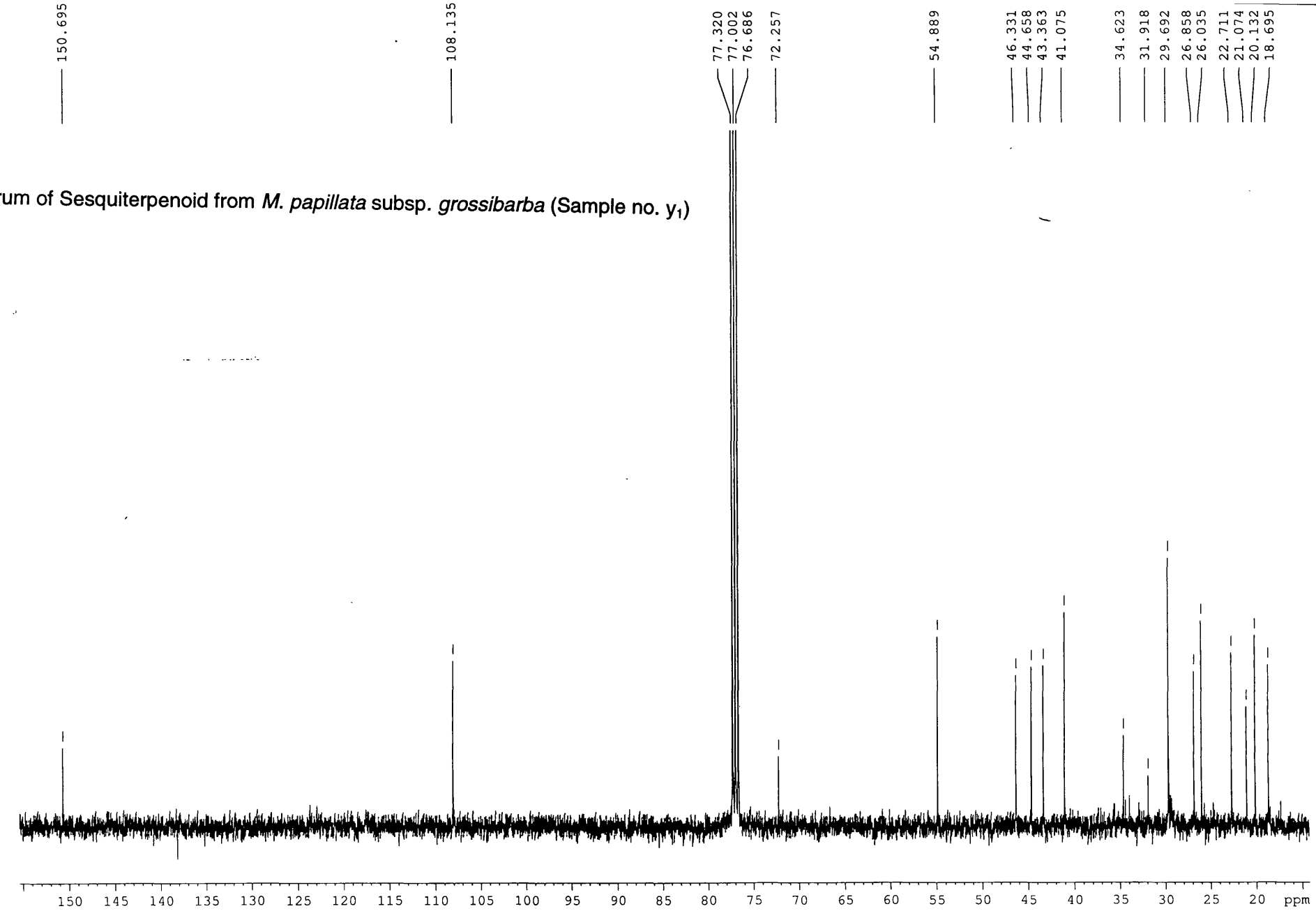
A

NMR Spectra

^{1}H NMR spectrum of Sesquiterpenoid from *M. papillata* subsp. *grossibarba* (Sample no. y_1)



^{13}C spectrum of Sesquiterpenoid from *M. papillata* subsp. *grossibarba* (Sample no. y_1)



CHAPTER FOUR

Plagiochasma appendiculatum Lehm et Lindenb

4.1 Introduction:

Systematic Position:

Division – Bryophyte

Class - Hepaticopsida

Order - Marchantiales

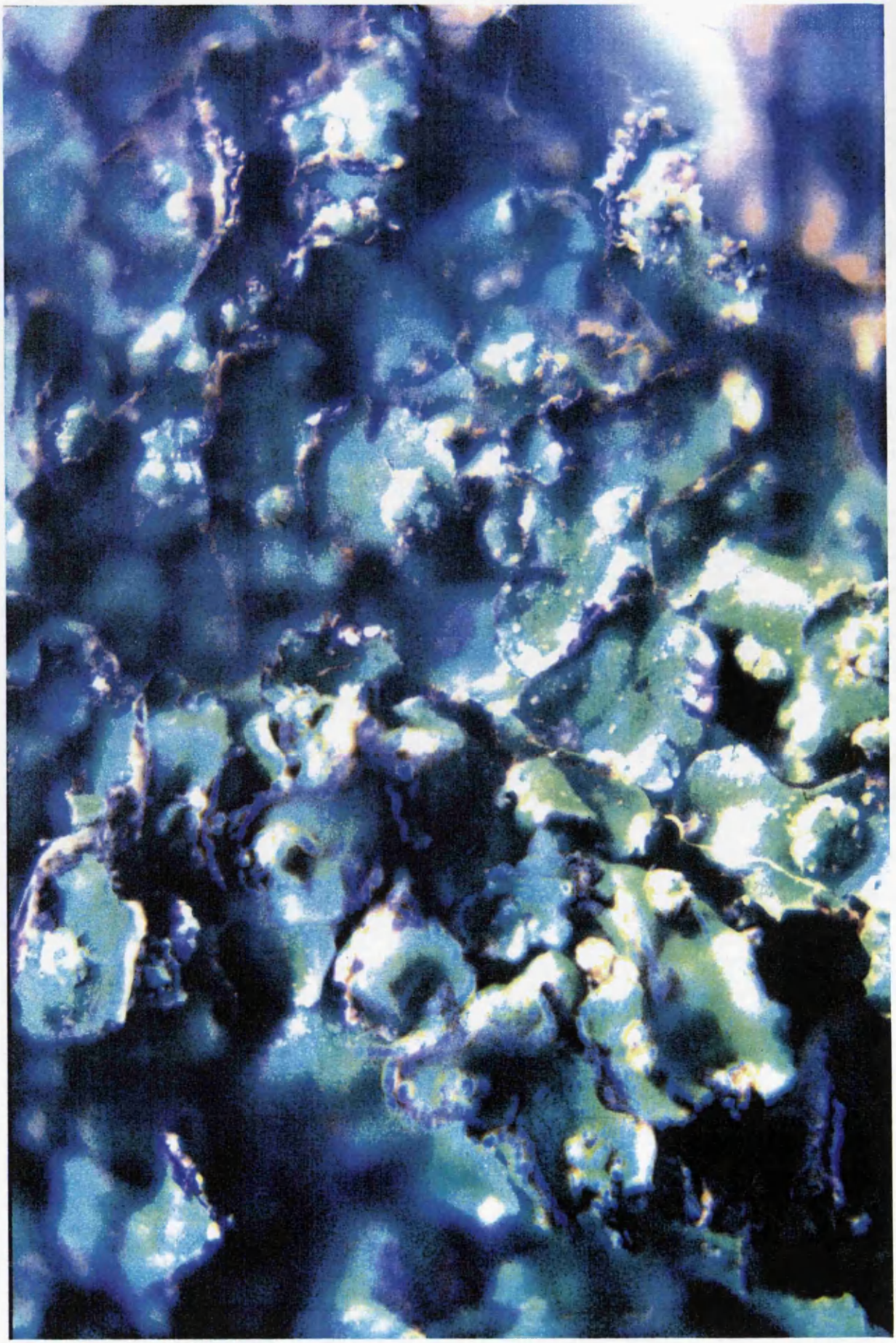
Family - Aytoniaceae

Genus -*Plagiochasma* Lehm et Lindenb

Species - *appendiculatum*

Pande has suggested[97] that *Plagiochasma appendiculatum* has migrated from the Himalayas to the different bryogeographical regions of the Indian subcontinent. Its wide occurrence indicates that it has adjusted well under different environmental conditions. It has been reported that *P. appendiculatum* populations survive well[125] over a pH range of 3.0 to 7.0. Populations of *P. appendiculatum* commonly grow in moist as well in very dry habitats. As reported by Vishvakarma and Kaul,[126] the wider distribution of this species at Pachmari (India) is due to its tolerance of a wider range of soil moisture content.

4.2 Constituents: *Plasiochasma rupestre* contains *ent*-marsupellone (**162**), as reported by Connolly *et al.*[57] This is the first example of a longipinane-type



Plagiochasma appendiculatum Lehm et. Lindenb

5 K.M.
CHANGUNARAYAN
KHANAIGAON
NEUPANE
SANDAHA
GULELICHAUR
KHOLDAHA
GAUSALI
NAGARKOT
MILPANI
KHORSHANIBARI
GAUDOL
PIKHEL
PASHUPATINATH
6 K.M.
TRIBHUVAN
INTERNATIONAL
AIRPORT 6 K.M.
NEW ROAD
BANESWOR
KARKIGAUN
DUWAKOT
JHAUKHEL
CHALING
KHARIPATI
THAPAGAUN
NILBARI
KOLPA
HANUMANTAR
BRAMAYANI
TRIPURESWAR
GOTHATAR
RINANI
KUWADANDA
LAMKHEGWATALA
PAGALKHANA
MAHADEWPOKRI DARA
JITPUR
KOPUNDOL
THAPATHALI
SINAMUGAL
BORE
KUNDOL
PHAIDHOKA
NAKADES
JHAUKHEL
MIN BHAWAN
NAYAGHAT
BAUDHKHA
KOTESHWOR
GOKHUPHAL
GHIMIREGAUN
16 K.M.
BHADGAUN
SURAL
LALITPUR
BRAMAYANI
TATHALI
KATUNJE
IKHUDOL
AMALDOL
TANCOK
MOTITAR
CITRAPUR
KIWAFOK
SURJEBINAYAK
DEUJAGAU
OCU
TIKATHALI
SANETAR
PUWARGAU
NALINCOK
DHANUSAR
MAGAGAU
OKHTHALI
KHUMALTAR
SANAGAU
LUBHU
BASNET GAU
DOKATHALI
BISTGAU
SANGA
DHAPAKHE
CIHAN DARA
SIRADOL
BISUNDOL
KHOKNA
HARISHIDHI
RISAL DARA
GUNDU
DARAGAU
BHAISIPATI
To Banepa-Dhulikhel, Namoboudha
SUNAKOTHI
DARATHOK
CARGHARE
BUNGAMATI
POKHRI THUMKO
SINAGAL
GODAMCHAUR
SISNERI
DHANESWAR
THAIBA
KHALCOK DARA
BAREGAUN
CUNIKHEL
CAPAGAU
BAJRABARAHI
CHAMPI
MANEDODHAN
17 K.M.
BOTANICAL
GARDENS
PYANGGAU
CILU
KHARBOTTAR
GODAWARI
17 KM
BHALEWAR
VALLEY BAUNDRY
KOTKHUGAU
BURUNCLI
BHAGABAN

sesquiterpenoid in the Marchantiales. The same authors also reported the hopane triterpenoid α-zeorin (**100**) and flavonoid apigenin 4',7-dimethyl ether (**163**), in addition to two elemane sesquiterpenoids, elema-1,4(15),11-trien-3,14-olide (**164**) and elema-1,4(15),11-trien-3-al (**165**). The isolation of sesquiterpenoids of the longipinane and elemane class is interesting in terms of the chemotaxonomy of the Hepaticae. Further investigation of *P. rupestre*[104] has led to the isolation of the sesquiterpenoid, eudesma-4(15),11-dien-14-ol (**166**). *P. rupestre* has also been reported to contain luteolin 6,8-di-C-α-L-arabinopyranoside (**167**).[4] Two macrocyclic bisbibenzyls, marchantin H (**168**) and I (**169**), have been obtained from *P. intermedium.*[8] These structures were determined by the total assignment of the ^{1}H and ^{13}C spectra of the compounds.

4.3.1 Materials and Methods (experimental)

Plant material was collected from Godawari (Nepal) at an elevation of 1487m., longitude 27^{0} 36` and latitude $85^{0}$23`. Godawari is situated at a distance of 17 Km southeast of Kathmandu, the capital city of Nepal. The plant was collected from a damp old wall in the month of October, 1989. The habitat was a shady place surrounded by tall trees. The plant was identified by Dr. David G Long, Royal Botanic Garden, Edinburgh where a specimen is deposited.

The sample was dried at room temperature. Dried and powdered material (460 g) was subjected to hot percolation with diethyl ether for 8 hours. Evaporation of the solvent afforded a crude ether extract (5g). The same plant material was then percolated further using analar MeOH for 8 hours to give a crude MeOH extract (25g). A portion of the MeOH extract (12g) was fractionated by flash chromatography using gradient elution with increasing

ethyl acetate in petroleum ether. Similarly, a portion (4.5 g) of the ether extract was also subjected to flash chromatography.

Please note that the structures of the isolated compounds are presented in the text and are indicated by bold numbers without brackets. All of the compounds were gums or oils. Their spectroscopic properties are presented in the text during the discussion and/or in Tables.

Isolation of Riccardin D **12** *and Marchantin A* **7** *(from MeOH extract)*

Flash column fractions 15-17 (eluted with 55 % to 65% ethyl acetate in petroleum ether) were combined and fractionated further by passing through a Sephadex column using MeOH:$CHCl_3$ (1:1) as eluent. Fractions 9-15 thus obtained were combined and again chromatographed over Sephadex. Fractions 6-11 from this column were combined and then subjected to preparative tlc using 30% ethyl acetate in petroleum ether. Extraction of the first band with ethyl acetate yielded riccardin D **12** while extraction of the second band gave marcantin A **7**.

Isolation of Marchantin B **11** *(from MeOH extract)*

The flash column fractions 19-21 (eluted with 65% to 70% ethyl acetate in petroleum ether) were combined and chromatographed over Sephadex as usual. The 13th fraction gave almost pure marchantin B **11**.

Isolation of riccardin D **12** *(from ether extract)*

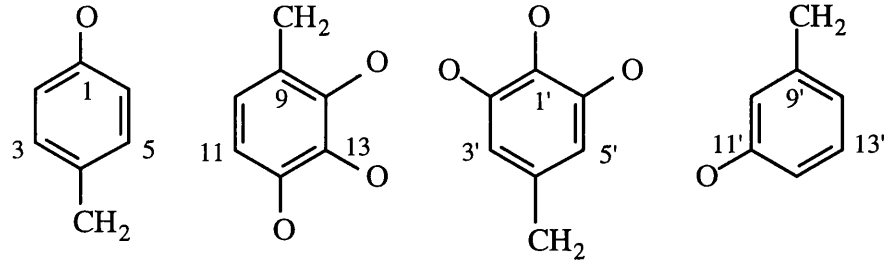
The flash column fraction of the ether extract, eluted with 45% of ethyl acetate in petroleum ether, was subjected to preparative tlc using 30% ethyl

acetate in petroleum ether as eluent. The fourth band (f-12,b 4) afforded riccardin D **12.**

Results and Discussion

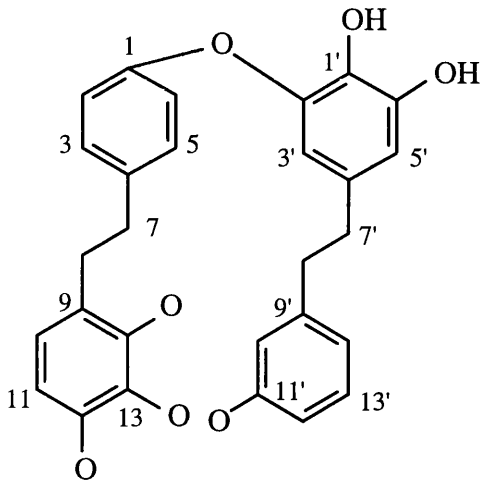
Marchantin B 11 *(f19-21, f13)*

This compound was obtained as a gum. Its mass spectrum gave a parent ion at m/z 456, corresponding to the molecular formula $C_{28}H_{24}O_6$, which suggested a bisbibenzyl derivative. The proton and carbon NMR data [see Tables A and B] were consistent with the presence of four aromatic rings and two CH_2-CH_2 groups. Moreover signals for four phenolic hydroxyl groups were visible in the proton spectrum. The aromatic proton spin systems **a-d** were readily identified in the COSY spectrum.



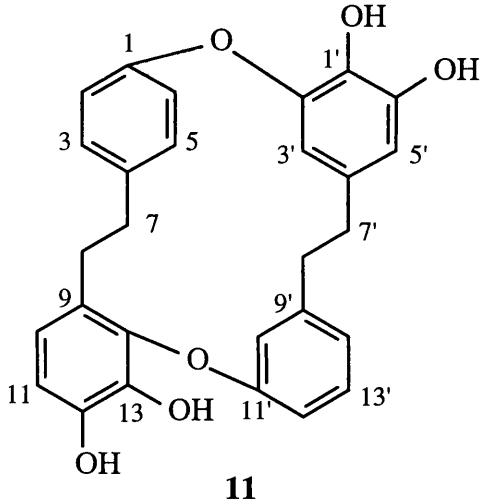
a **b** **c** **d**

The ROESY and NOE difference spectra readily allowed the placement of the methylene groups. Thus the protons attached to C-7/8 showed NOEs to H-3/5 and H-10 while those attached to C-7'/8' showed NOEs to H-3', H-5', H-10' and H-14'. A significant NOE from H-3' to H-2/6 indicated the position of the top ether link , from C-2' to C-6, between the two bibenzyl moieties. These data are summarised in part structure **e.**



e

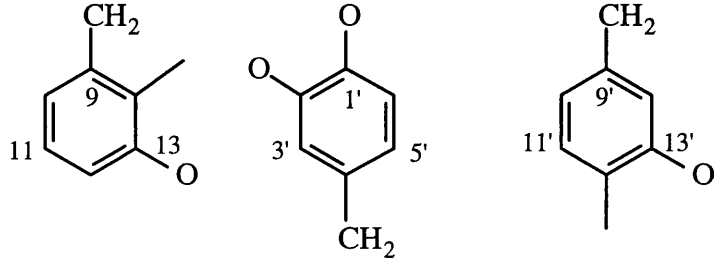
Correlations of the methylene protons in the HMBC spectrum confirmed part structure **e** and allowed the assignment of most of the quaternary carbons. The protonated carbons were assigned from correlations in the HMQC spectrum. The position of the final ether link was more difficult to deduce in view of the overlap of three of the hydroxyl protons and the resultant uncertainty of the HMBC correlations of these protons. However part structure **e** suggested that the compound was marchantin B **11**. Comparison of spectroscopic data with literature values[132] confirmed this suggestion.



11

Riccardin D **12** *(f-12, b4)*

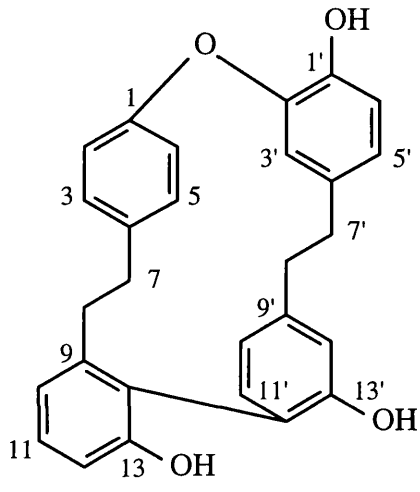
This compound was also obtained as a gum. Its mass spectrum showed a parent ion at m/z 424, consistent with the molecular formula $C_{28}H_{24}O_4$. Its spectroscopic properties [see Tables A and B] suggested a bisbibenzyl structure but the presence of three phenolic hydroxyls in the proton NMR spectrum precluded the typical marchantin bis-ether linkage. There was some broadness in the proton spectrum suggesting restricted rotation. This was also apparent in the carbon spectrum where the protonated carbons of the expected *para*-disubstituted benzene all had different chemical shifts. Only broad resonances were visible in the proton spectrum instead of the typical AA'BB' system. The coupling patterns **a-c** of the other rings were visible in the COSY spectrum. The chemical shift of H-3' [δ_H 5.35] indicates the same kind of ether linkage as in the other marchantins and hence the probability the remaining aromatic ring being *para*-disubstituted.



a **b** **c**

Correlations between H-10 and C-8, H-3' and H-5' and C-7' and H-10' and H-14' and C-8' confirmed the position of attachment of the methylene groups to the aromatic rings. As expected, no correlations were observed from the broad aromatic proton resonances to the remaining methylene C-7. The lack of oxgens necessitated the presence of a carbon-carbon bond between two of the aromatic rings. Riccardin D[133] **12** has such a carbon-carbon bond. Comparison of spectroscopic data revealed their identity. The original structural elucidation of riccardin D does not discuss broadening of proton signal or problems with carbon resonances, only overlapping of signals. In the original work[133] the

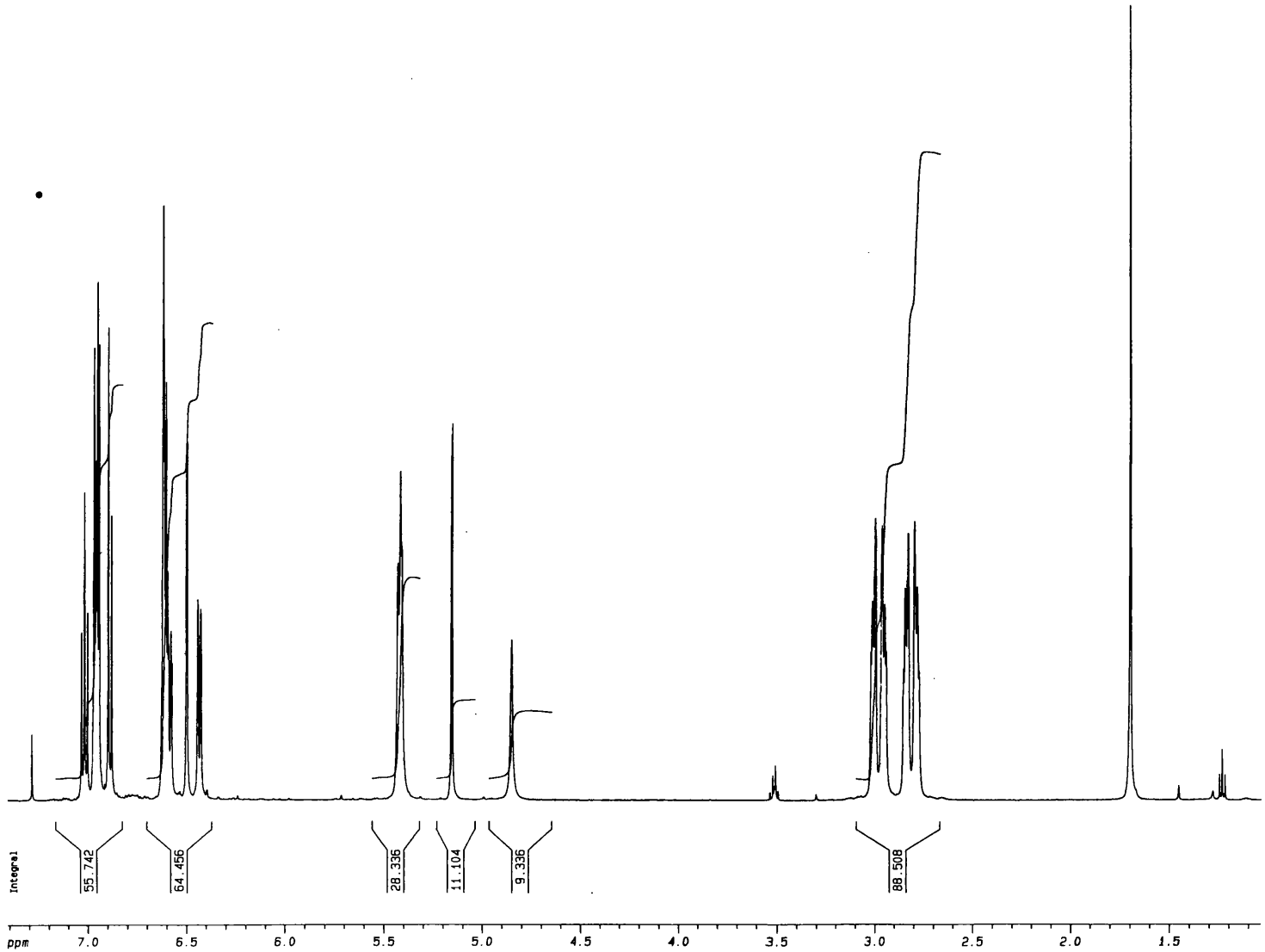
problem was removed by making derivatives whose spectra showed normal *p*-disubstituted benzene resonances.



12

NMR Spectra

^{1}H spectrum of Marchantin B from *Plagiochasma appendiculatum*



Current Data Parameters

NAME	rita1
EXPNO	1
PROCNO	1

F2 - Acquisition Parameters

Date_	990326
Time	19.52
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	zg
TD	32768
SOLVENT	CDCl3
NS	16
DS	2
SWH	6009.615 Hz
FIDRES	0.183399 Hz
AQ	2.7263477 sec
RG	101.6
DW	83.200 usec
DE	10.50 usec
TE	300.0 K
D1	1.00000000 sec
P1	5.00 usec
SFO1	500.1323164 MHz
NUC1	1H
PL1	2.00 dB

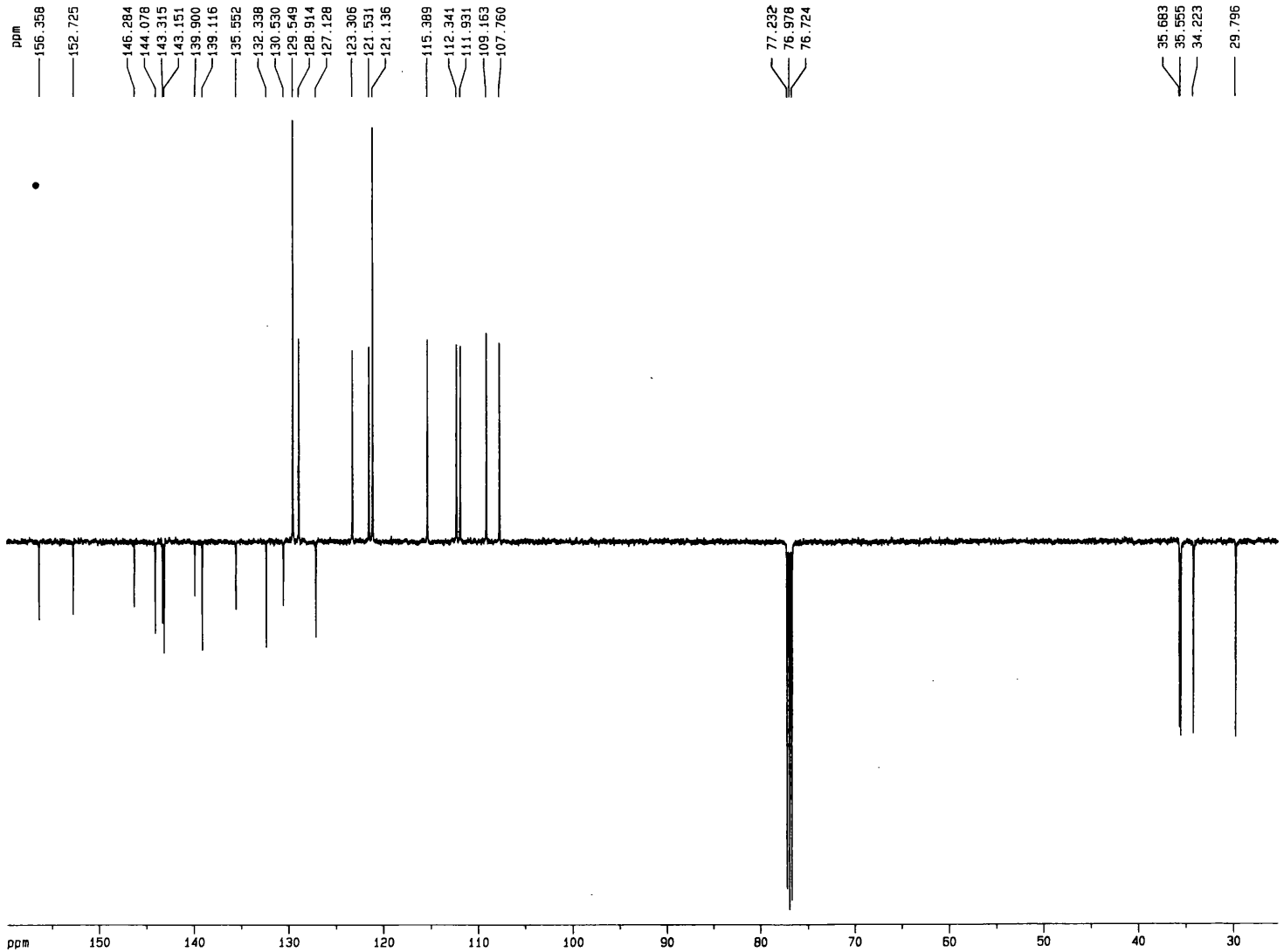
F2 - Processing parameters

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

1D NMR plot parameters

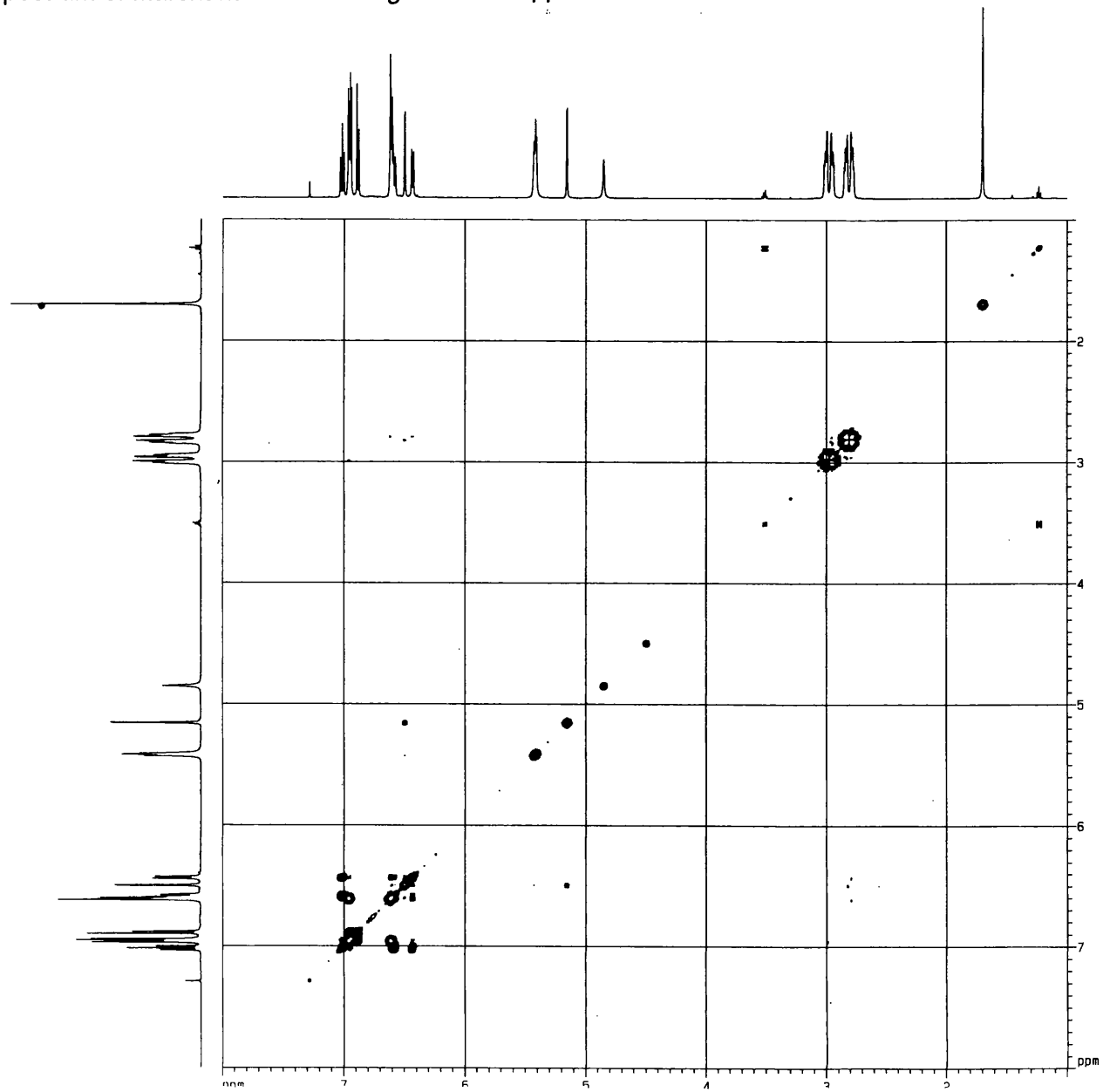
CX	30.00 cm
F1P	7.411 ppm
F1	3706.67 Hz
F2P	1.038 ppm
F2	519.02 Hz
PPMCM	0.21245 ppm/cm
HZCM	106.25497 Hz/cm

J mod spectrum of Marchantin B from *Plagiochasma appendiculatum*



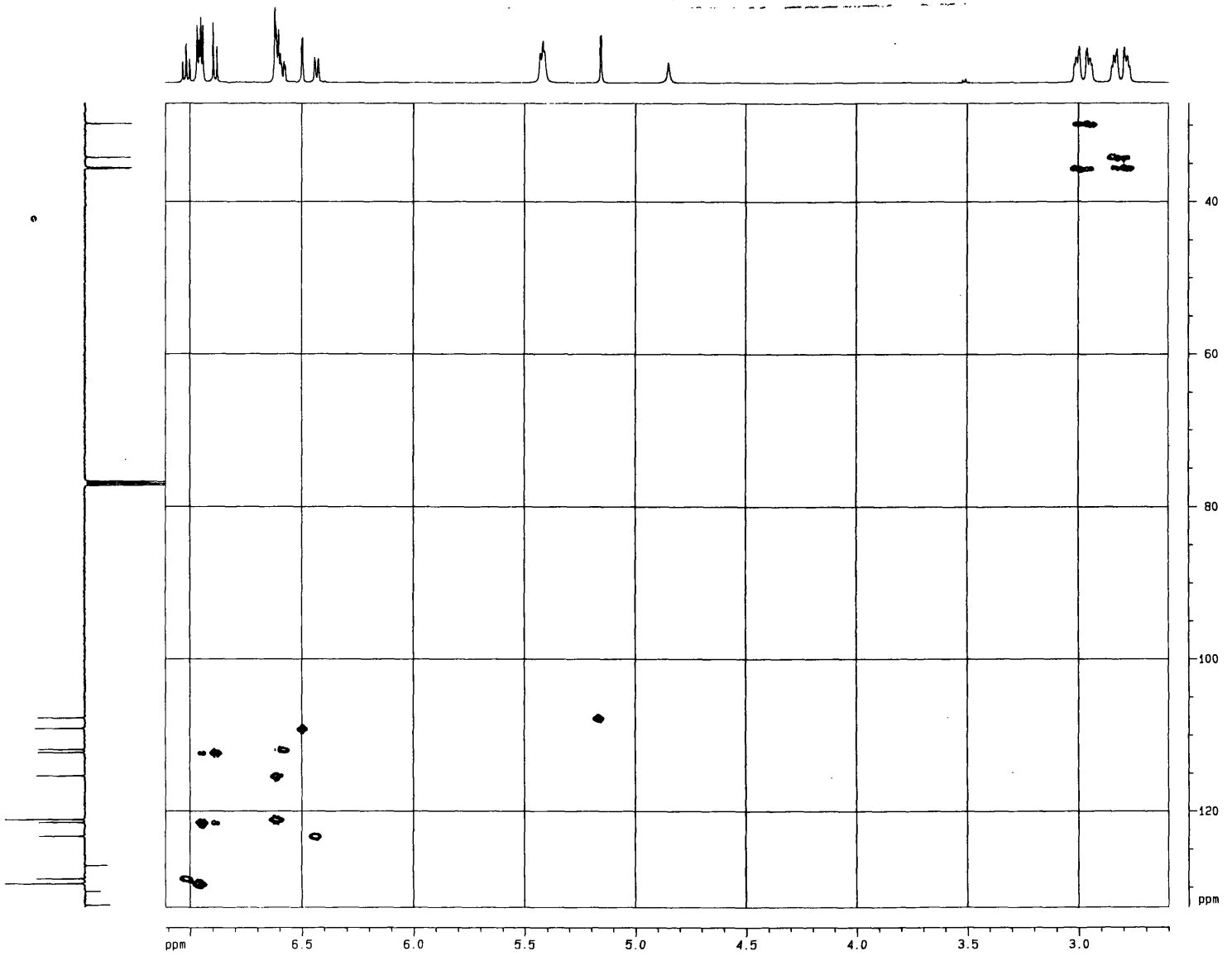
Current Data Parameters	
NAME	rita1
EXPNO	5
PROCNO	1
F2 - Acquisition Parameters	
Date_	990327
Time	13.42
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	jmod
TD	32768
SOLVENT	C6D6
NS	7063
DS	8
SWH	30303.031 Hz
FIDRES	0.924775 Hz
AQ	0.5407220 sec
RG	8192
DW	16.500 usec
DE	13.00 usec
TE	300.0 K
P1	10.00 usec
DELTA	0.00001273 sec
D20	0.00750000 sec
PL12	25.00 dB
D1	3.00000000 sec
CPDPRG2	waltz16
PCPD2	100.00 usec
SFO2	500.1323164 MHz
NUC2	1H
PL2	120.00 dB
D13	0.00000300 sec
SFO1	125.7728299 MHz
NUC1	13C
PL1	-4.00 dB
P2	20.00 usec
F2 - Processing parameters	
SI	32768
SF	125.7577978 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40
1D NMR plot parameters	
CX	30.00 cm
F1P	159.785 ppm
F1	20094.27 Hz
F2P	25.333 ppm
F2	3185.81 Hz
PPMCM	4.48175 ppm/cm
HZCM	563.61554 Hz/cm

COSY spectrum of Marchantin B from *Plagiochasma appendiculatum*



Current Data Parameters	
NAME	rita1
EXPNO	2
PROCNO	1
F2 - Acquisition Parameters	
Date_	990326
Time	20.04
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	cosygp
TD	2048
SOLVENT	CDCl3
NS	1
DS	16
SWH	3501.401 Hz
FIDRES	1.709668 Hz
AQ	0.2925044 sec
RG	812.7
DW	142.800 usec
DE	10.50 usec
TE	300.0 K
D1	1.00000000 sec
P1	9.50 usec
SFO1	500.1322506 MHz
NUC1	1H
PL1	2.00 dB
D0	0.00000300 sec
P16	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
IN0	0.00028560 sec
F1 - Acquisition parameters	
ND0	1
TD	256
SFO1	500.1323 MHz
FIDRES	13.677346 Hz
SW	7.001 ppm
F2 - Processing parameters	
SI	1024
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.00
F1 - Processing parameters	
SI	1024
MC2	QF
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
2D NMR plot parameters	
CX2	20.00 cm
CX1	20.00 cm
F2PLO	8.000 ppm
F2LO	4001.29 Hz
F2PHI	1.000 ppm
F2HI	499.88 Hz
F1PLO	8.000 ppm
F1LO	4001.29 Hz
F1PHI	1.000 ppm
F1HI	499.88 Hz
F2PPMCM	0.35005 ppm/cm
F2HZCM	175.07002 Hz/cm
F1PPMCM	0.35005 ppm/cm

HMQC spectrum of Marchantin B from *Plagiochasma appendiculatum*



Current Data Parameters

NAME	rita1
EXPNO	3
PROCNO	1

F2 - Acquisition Parameters

Date_	950326
Time	20.21
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	inv4gp
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	3501.401 Hz
FIDRES	1.709668 Hz
AQ	0.2925044 sec
RG	5160.6
DW	142.800 usec
DE	13.00 usec
TE	300.0 K
D12	0.00002000 sec
D2	0.00345000 sec
P16	2000.00 usec
D13	0.00000300 sec
d20	0.00142700 sec
D1	1.00000000 sec
P1	9.50 usec
SFO1	500.1322506 MHz
NUC1	1H
PL1	2.00 dB
PL2	-4.00 dB
P3	10.00 usec
SFO2	125.7865420 MHz
NUC2	13C
D0	0.00000300 sec
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
GPX3	0.00 %
GPY3	0.00 %
GPZ3	40.00 %
GPNAM3	sine.100
PL12	16.50 dB
CPDPRG2	garp
PCPD2	80.00 usec
IN0	0.00002840 sec

F1 - Acquisition parameters

ND0	2
TD	256
SFO1	125.7665 MHz
FIDRES	68.772011 Hz
SW	139.987 ppm

F2 - Processing parameters

SI	1024
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.00

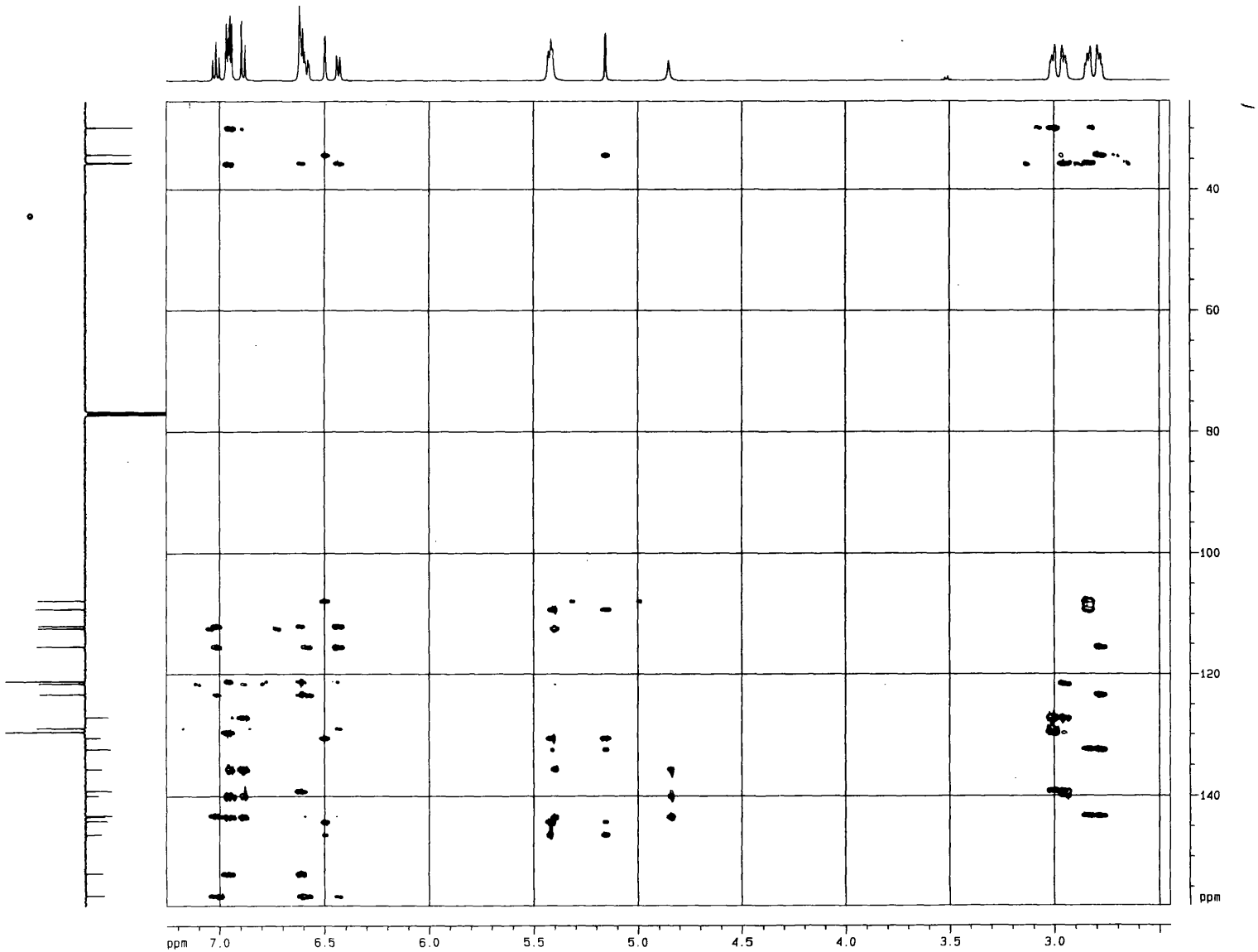
F1 - Processing parameters

SI	1024
MC2	QF
SF	125.7577885 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PLO	7.112 ppm
F2LO	3556.77 Hz
F2PHI	2.593 ppm
F2HI	1300.01 Hz
F1PLO	132.632 ppm
F1LO	16679.56 Hz
F1PHI	27.988 ppm
F1HI	3[illegible]85 Hz
F2PPMCM	0.18049 ppm/cm
F2HZCM	90.27048 Hz/cm
F1PPMCM	5.27721 ppm/cm
F1HZCM	663.65015 Hz/cm

HMBC spectrum of Marchantin B from *Plagiochasma appendiculatum*



Current Data Parameters

NAME	rita1
EXPNO	4
PROCNO	1

F2 - Acquisition Parameters

Date_	990326
Time	23.21
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	inv4gplplrnd
TD	2048
SOLVENT	CDCl3
NS	32
DS	16
SWH	3501.401 Hz
FIDRES	1.709668 Hz
AQ	0.2925044 sec
RG	4597.6
DW	142.800 usec
DE	13.00 usec
TE	300.0 K
D1	1.00000000 sec
P1	9.50 usec
SFO1	500.1322506 MHz
NUC1	1H
PL1	2.00 dB
D2	0.00345000 sec
P3	10.00 usec
SFO2	125.7677995 MHz
NUC2	13C
PL2	-4.00 dB
D6	0.08000000 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
IN0	0.00002485 sec

F1 - Acquisition parameters

ND0	2
TD	512
SFO1	125.7678 MHz
FIDRES	39.298290 Hz
SW	159.983 ppm

F2 - Processing parameters

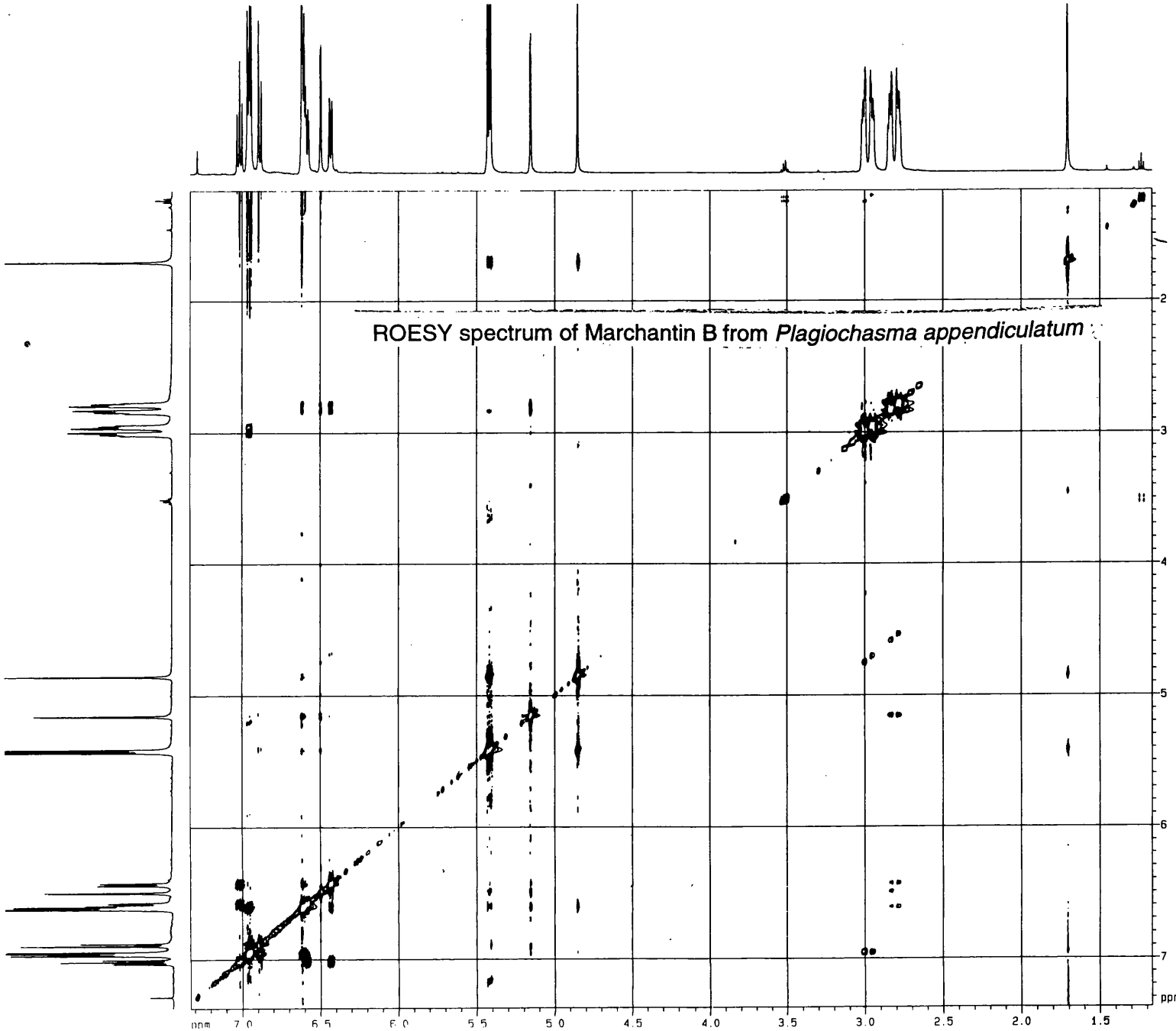
SI	1024
SF	500.1300000 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	1024
MC2	QF
SF	125.7577815 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PLO	7.255 ppm
F2LO	3628.58 Hz
F2PHI	2.449 ppm
F2HI	1224.78 Hz
F1PLO	158.097 ppm
F1LO	19881.99 Hz
F1PHI	25.445 ppm
F1HI	3199.86 Hz
F2PPMCM	0.19225 ppm/cm
F2HZCM	96.15175 Hz/cm
F1PPMCM	6.63264 ppm/cm
F1HZCM	834.10651 Hz/cm



Current Data Parameters

NAME	rita1
EXPNO	7
PROCNO	1

F2 - Acquisition Parameters

Date_	990402
Time	18.38
INSTRUM	spect
PROBHD	5 mm Multinu
PULPROG	roesyst
TD	4096
SOLVENT	CDCl3
NS	56
DS	16
SWH	3501.401 Hz
FIDRES	0.854834 Hz
AQ	0.5849588 sec
RG	362
DW	142.800 usec
DE	10.50 usec
TE	300.0 K
L0	1
13	256
D1	1.00000000 sec
D11	0.03000000 sec
D12	0.00002000 sec
PL1	2.00 dB
P1	9.50 usec
SF01	500.1322506 MHz
NUC1	1H
D0	0.00000300 sec
PL11	28.00 dB
P15	200000.00 usec
IN0	0.00028560 sec

F1 - Acquisition parameters

ND0	1
TD	512
SF01	500.1323 MHz
FIDRES	6.838673 Hz
SW	7.001 ppm

F2 - Processing parameters

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

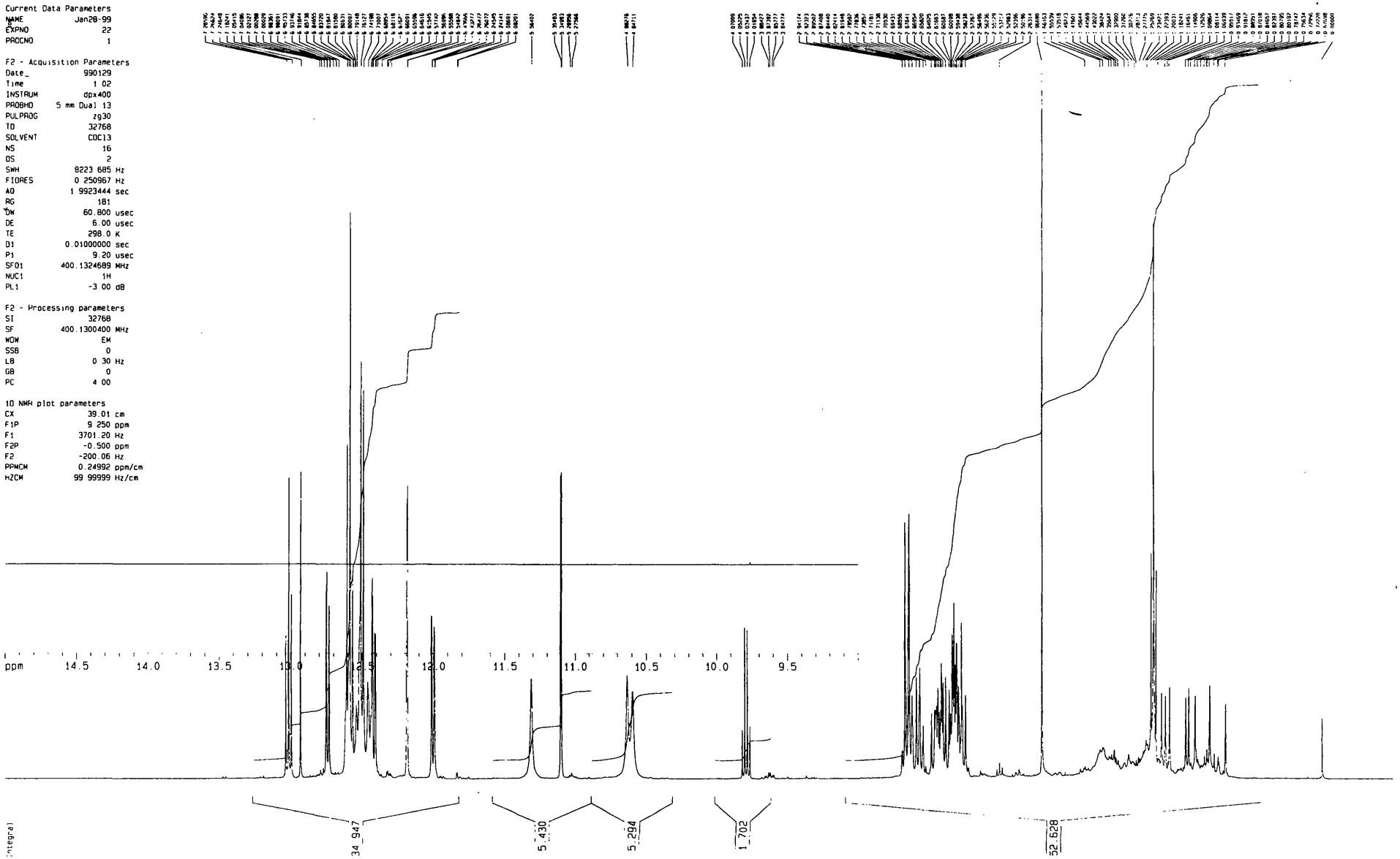
F1 - Processing parameters

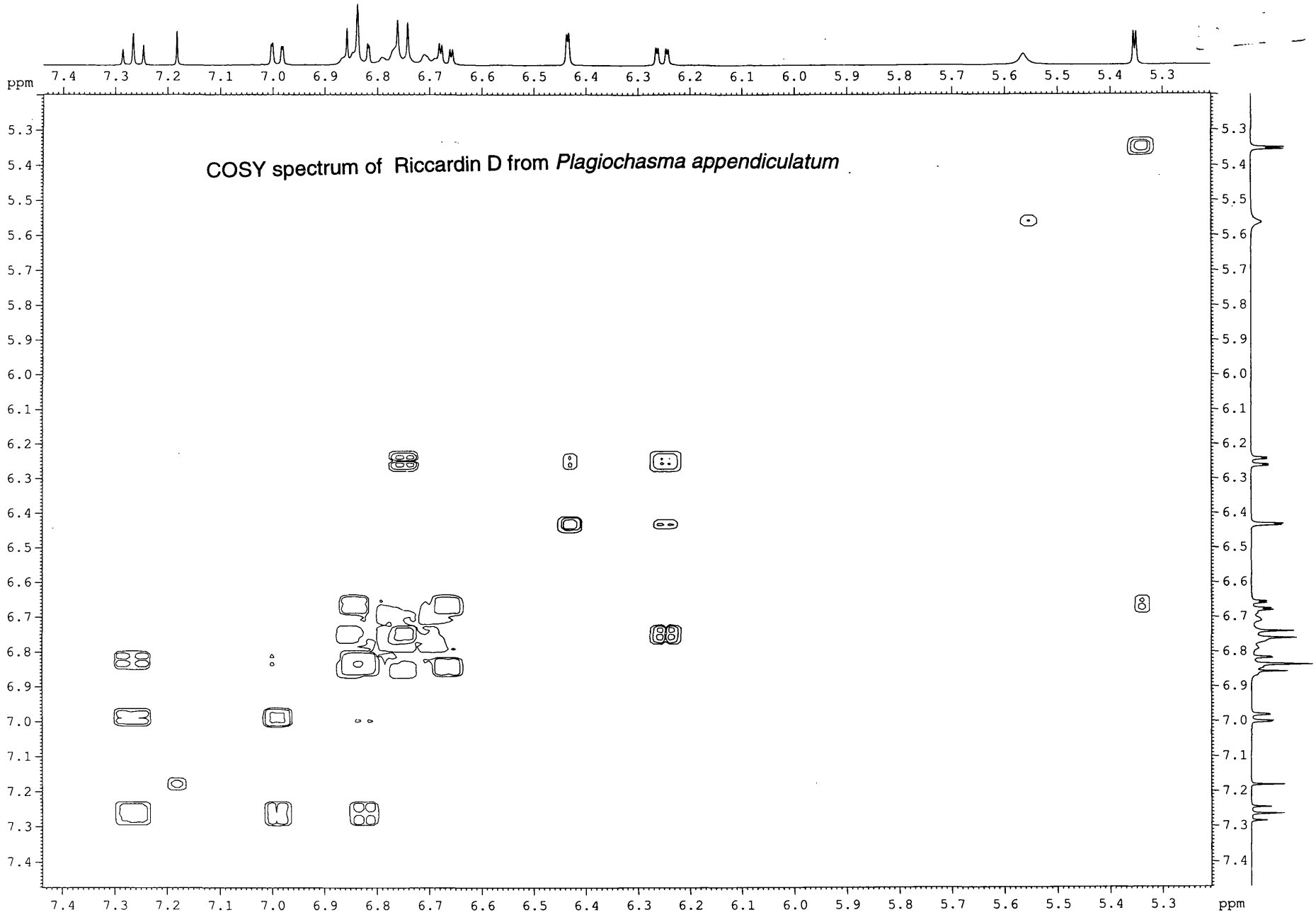
SI	1024
MC2	States-TPPI
SF	500.1300000 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

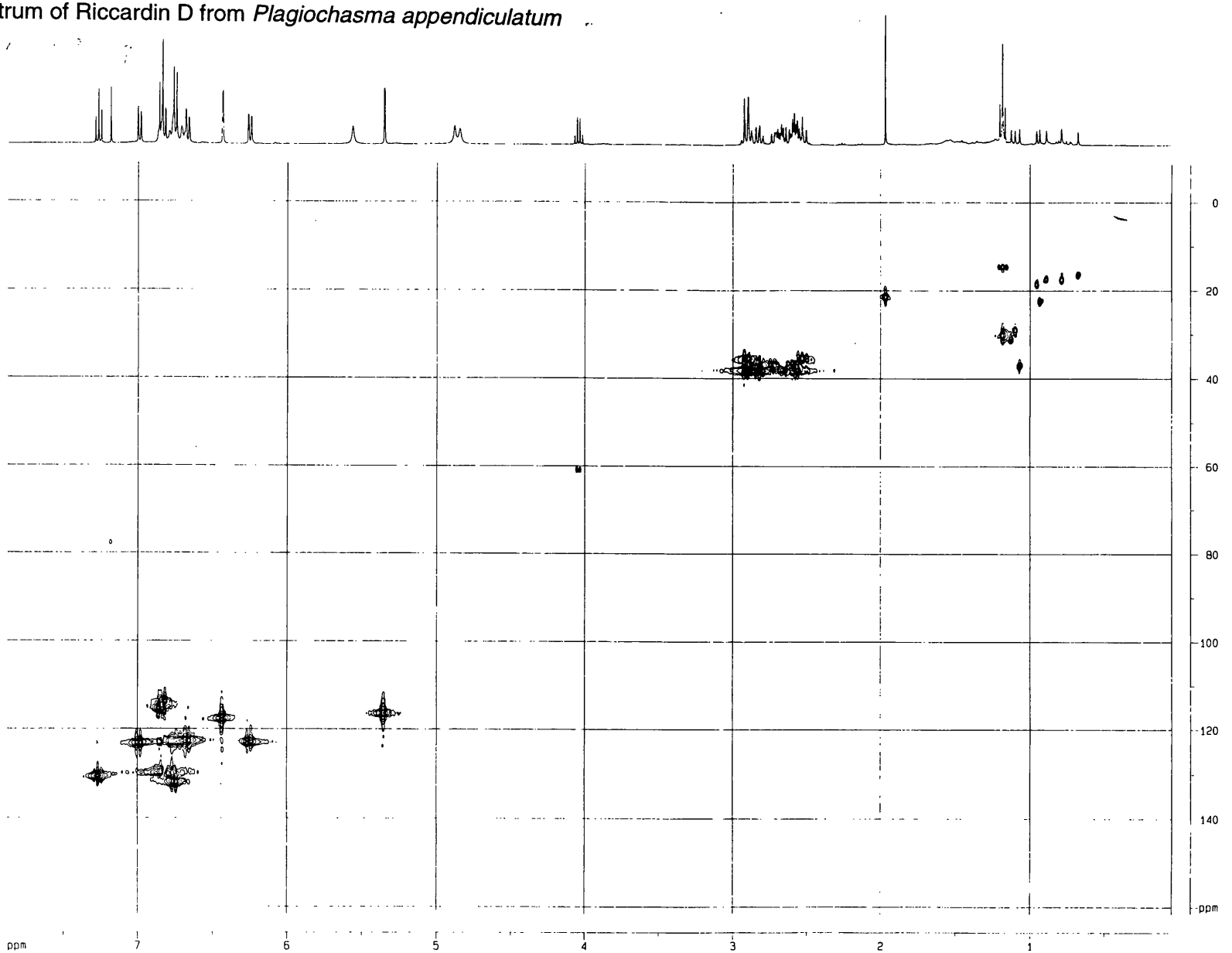
CX2	26.00 cm
CX1	22.00 cm
F2PLO	7.330 ppm
F2LO	3666.19 Hz
F2PHI	1.167 ppm
F2HI	583.66 Hz
F1PLO	7.371 ppm
F1LO	3686.71 Hz
F1PHI	1.164 ppm
F1HI	581.95 Hz
F2PPMCM	0.23706 ppm/cm
F2HZCM	118.55892 Hz/cm
F1PPMCM	0.28218 ppm/cm
F1HZCM	141.12535 Hz/cm

^{1}H NMR spectrum of Riccardin D from *Plagiochasma appendiculatum*




COSY spectrum of Riccardin D from *Plagiochasma appendiculatum*
ppm
7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2 6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3
5.3 5.4 5.5 5.6 5.7 5.8 5.9 6.0 6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 7.0 7.1 7.2 7.3 7.4
ppm

HMQC spectrum of Riccardin D from *Plagiochasma appendiculatum*



Current Data Parameters

NAME	Jan28-99
EXPNO	23
PROCNO	1

F2 - Acquisition Parameters

Date_	990129
Time	1.04
INSTRUM	dpx400
PROBHD	5 mm Dual 13
PULPROG	inv4gp
TD	2048
SOLVENT	CDCl3
NS	4
DS	16
SWH	3132.832 Hz
FIDRES	1.529703 Hz
AQ	0.3269108 sec
RG	20642.5
DW	159.600 usec
DE	6.00 usec
TE	298.0 K
D12	0.00002000 sec
D2	0.00345000 sec
P16	1000.00 usec
D13	0.00000300 sec
d20	0.00242700 sec
D1	1.32357895 sec
P1	9.20 usec
SFO1	400.1316293 MHz
NUC1	1H
PL1	-3.00 dB
PL2	-3.00 dB
P3	8.40 usec
SFO2	100.6204259 MHz
NUC2	13C
D0	0.00000300 sec
GPX1	0.00 %
GPY1	0.00 %
GPZ1	50.00 %
GPNAM1	sine.100
D16	0.00010000 sec
P2	18.40 usec
GPX2	0.00 %
GPY2	0.00 %
GPZ2	30.00 %
GPNAM2	sine.100
GPX3	0.00 %
GPY3	0.00 %
GPZ3	40.00 %
GPNAM3	sine.100
PL12	15.00 dB
CPDPRG2	garp
PCPD2	66.00 usec
INO	0.00002920 sec

F1 - Acquisition parameters

NDO	2
TD	128
SFO1	100.6204 MHz
FIDRES	133.775681 Hz
SW	170.177 ppm

F2 - Processing parameters

SI	2048
SF	400.1300400 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40

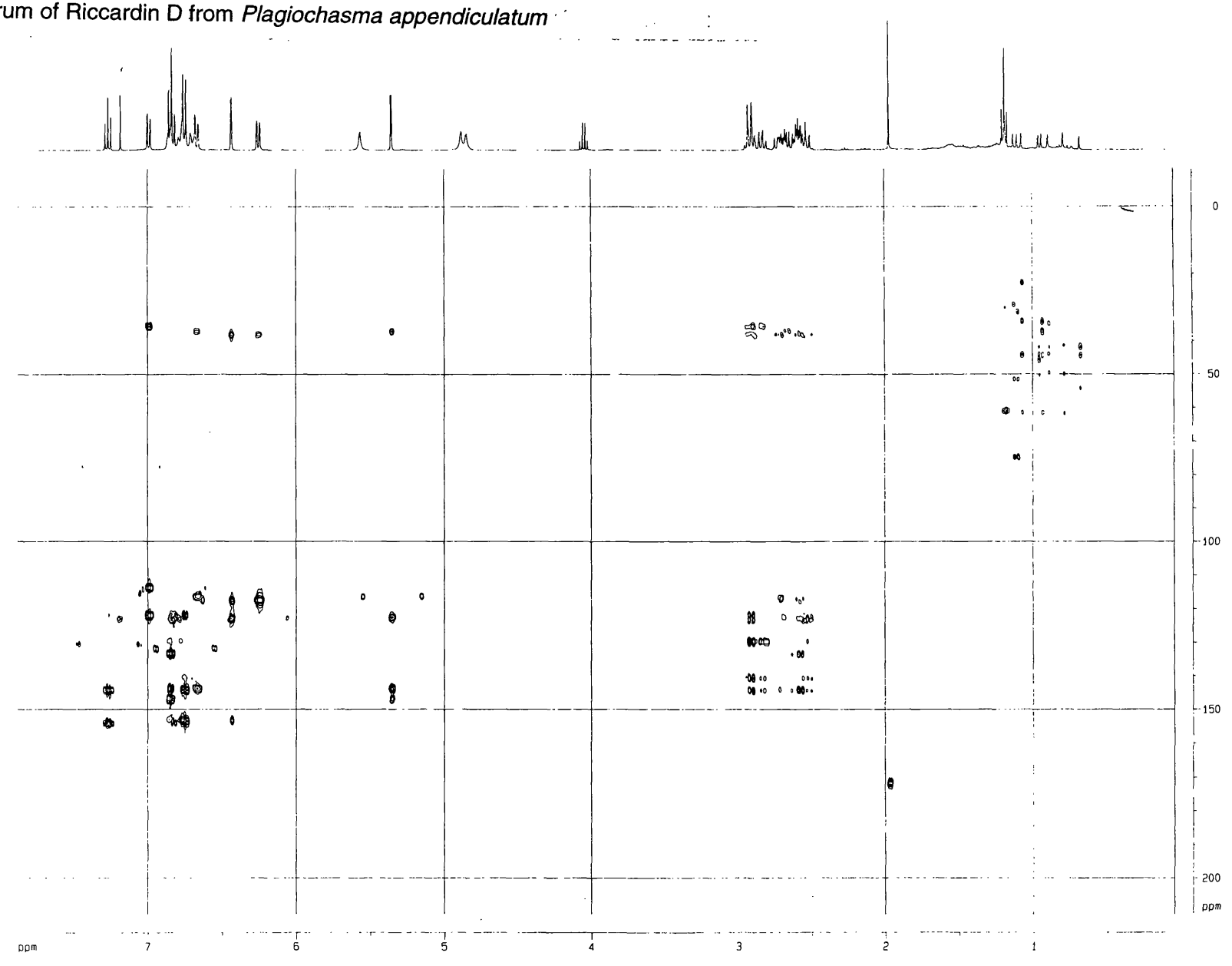
F1 - Processing parameters

SI	512
MC2	QF
SF	100.6127290 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	31.00 cm
CX1	20.00 cm
F2PLO	7.877 ppm
F2LO	3151.79 Hz
F2PHI	0.047 ppm
F2HI	18.97 Hz
F1PLO	161.589 ppm
F1LO	16257.86 Hz
F1PHI	-8.589 ppm
F1HI	-864.11 Hz
F2PPMCM	0.25256 ppm/cm
F2HZCM	101.05869 Hz/cm
F1PPMCM	8.50885 ppm/cm
F1HZCM	855.09888 Hz/cm

HMBC spectrum of Riccardin D from *Plagiochasma appendiculatum*



Current Data Parameters

NAME	Jan28-99
EXPNO	24
PROCNO	1

F2 - Acquisition Parameters

Date_	990129
Time	1 20
INSTRUM	dpx400
PROBHD	5 mm Dual 13
PULPROG	inv4gslplrnd
TD	2048
SOLVENT	CDCl3
NS	4
DS	16
SWH	3132 832 Hz
FIDRES	1.529703 Hz
AQ	0 3269108 sec
RG	16384
DW	159 600 usec
DE	6.00 usec
TE	298 0 K
D1	1.34271204 sec
P1	9 20 usec
SFO1	400 1316293 MHz
NUC1	1H
PL1	-3 00 dB
D2	0.00350000 sec
P3	8.40 usec
SFO2	100.6227604 MHz
NUC2	13C
PL2	-3.00 dB
D6	0.06500000 sec
D0	0 00000300 sec
P16	1000.00 usec
D16	0.00010000 sec
P2	18 40 usec
D13	0 00000300 sec
IN0	0 00002240 sec

F1 - Acquisition parameters

ND0	2
TD	128
SFO1	100.6228 MHz
FIDRES	174.386154 Hz
SW	221.833 ppm

F2 - Processing parameters

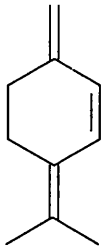
SI	2048
SF	400.1300400 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1 40

F1 - Processing parameters

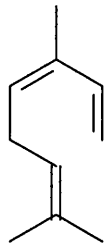
SI	512
MC2	QF
SF	100 6127290 MHz
WDW	QSINE
SSB	2
LB	0 00 Hz
GB	0

2D NMR plot parameters

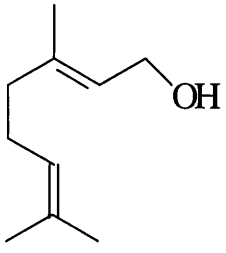
CX2	31 00 cm
CX1	20 00 cm
F2PLO	7 877 ppm
F2LO	3151 79 Hz
F2PHI	0 047 ppm
F2HI	18 97 Hz
F1PLO	210 619 ppm
F1LO	21190 96 Hz
F1PHI	-11 214 ppm
F1HI	-1128 24 Hz
F2PPMCM	0 25256 ppm/cm
F2HZCM	101.05869 Hz/cm
F1PPMCM	11 09154 ppm/cm
F1HZCM	1115 96021 Hz/cm



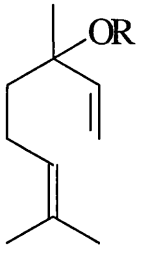
(1) Myrcene



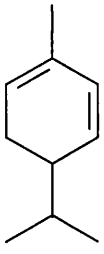
(2) Ocimene



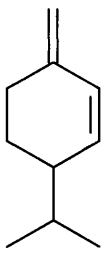
(3) Geraniol



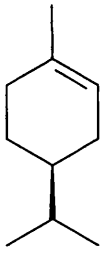
(4) Linalool; R=H
(5)Linalyl acetate; R=Ac



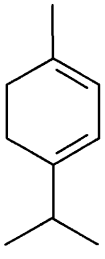
(6) α-Phellandrene



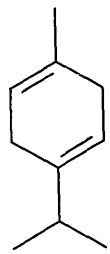
(7) β-Phellandrene



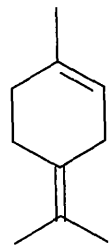
(8) Limonene



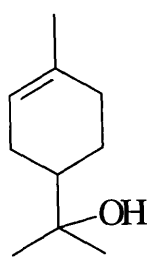
(9) α-Terpinene



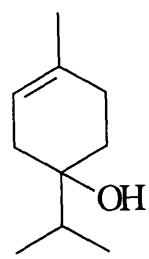
(10) γ-Terpinene



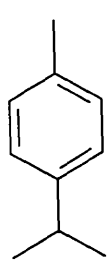
(11)Terpinolene



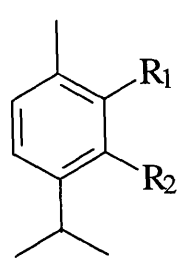
(12) α–Terpineol



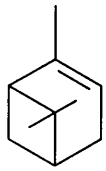
(13) Terpinen-4-ol



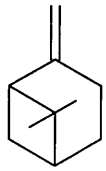
(14) p-Cymene



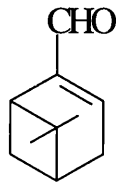
(15) Carvacrol; R_1=OH, R_2=H
(16) Thymol; R_1=H, R_2=OH
(17) Thymyl acetate; R_1=H, R_2=OAc



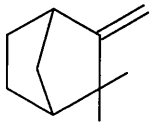
(18) α-Pinene



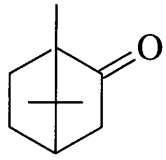
(19) β-Pinene



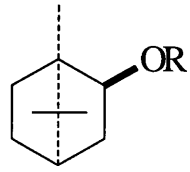
(20) Myrtenal



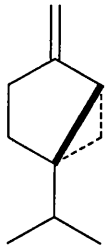
(21) Camphene



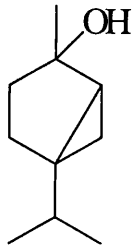
(22) Camphor



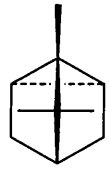
(23) Borneol; R=H
(24) Bornyl acetate; R=Ac



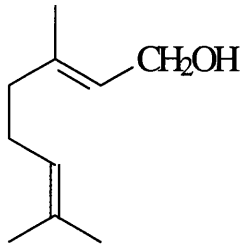
(25) β–Sabinene



(26) Thujanol



(27) Tricyclene



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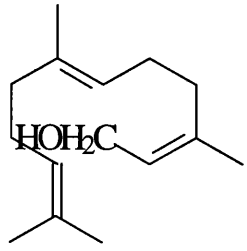

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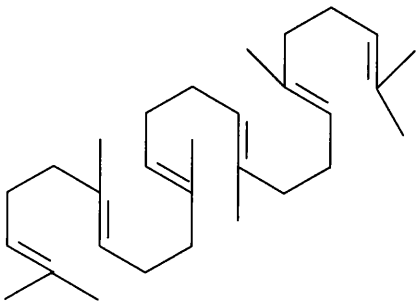

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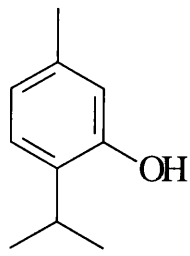

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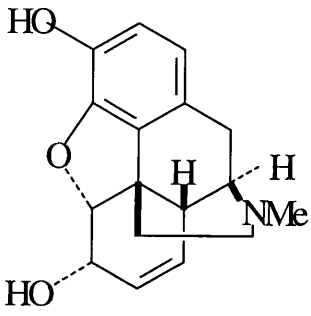

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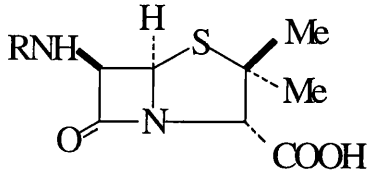

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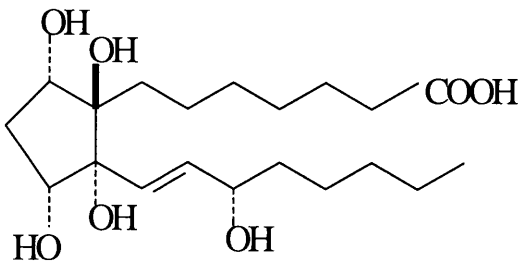

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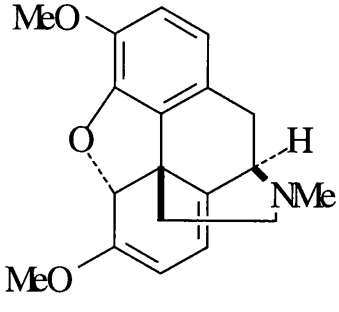

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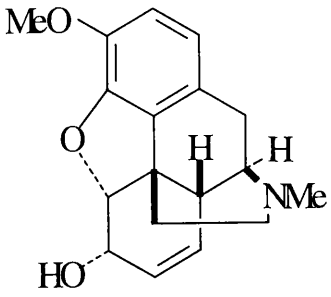

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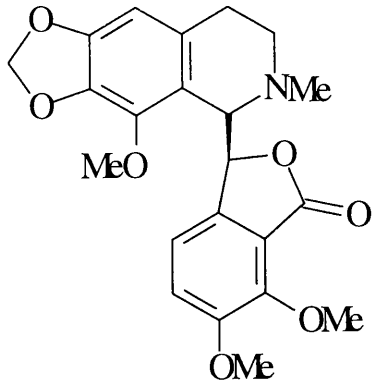

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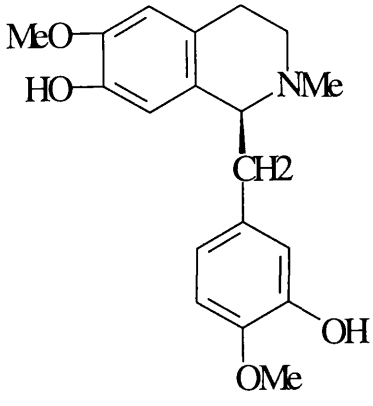

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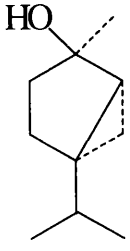

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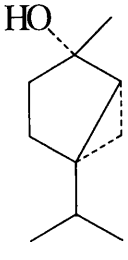

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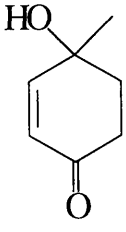

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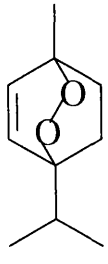

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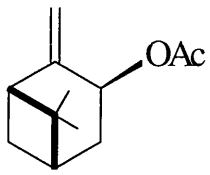

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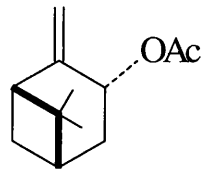

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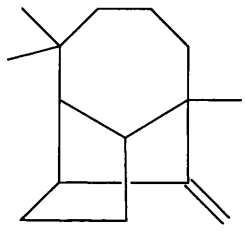

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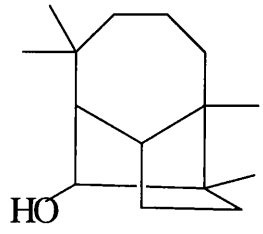

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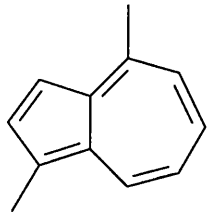

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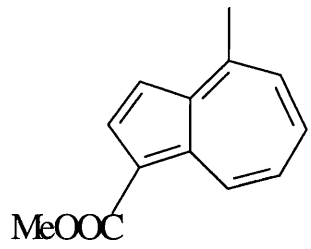

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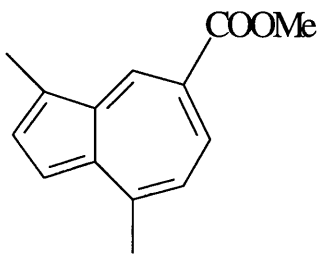

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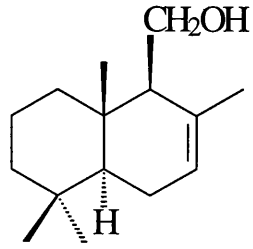

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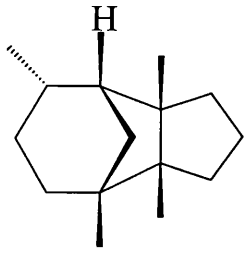

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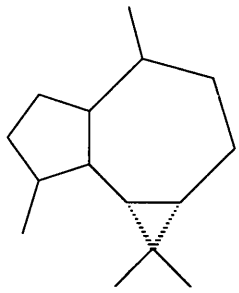

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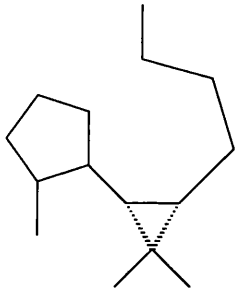

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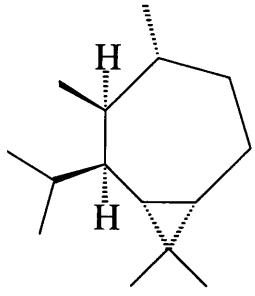

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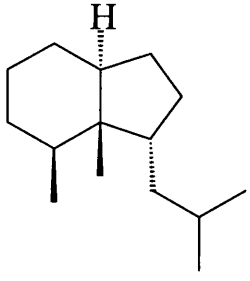

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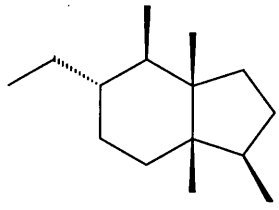

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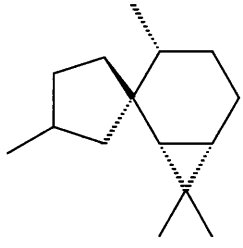

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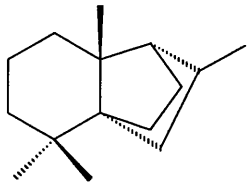

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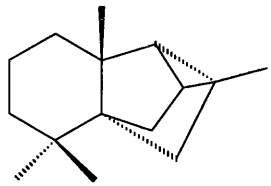

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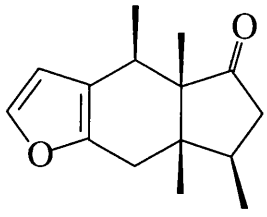

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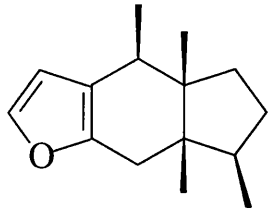

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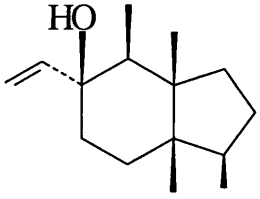

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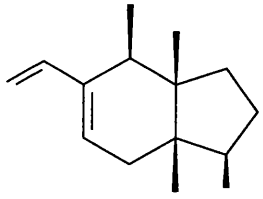

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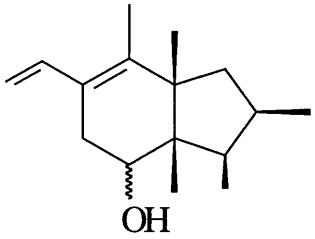

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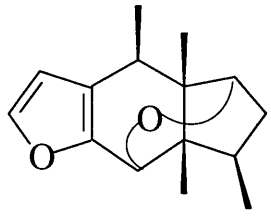

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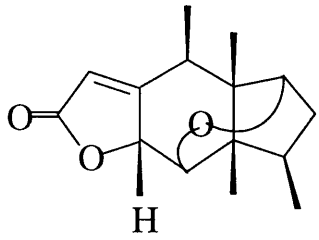

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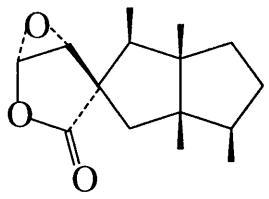

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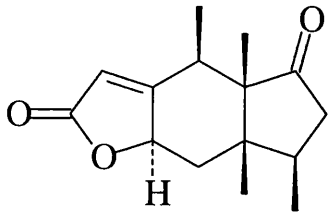

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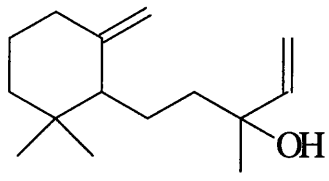

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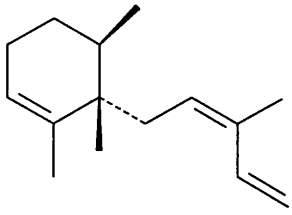

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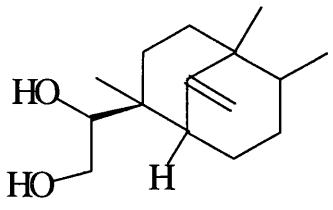

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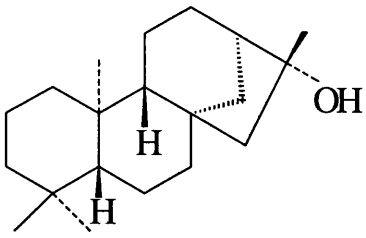

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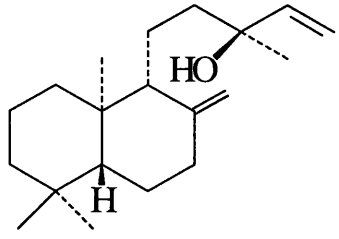

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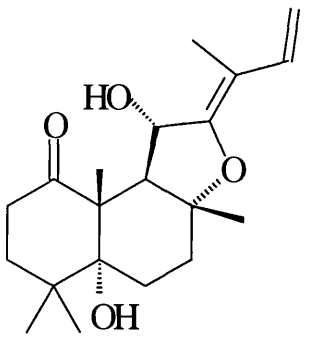

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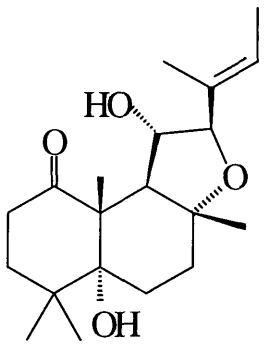

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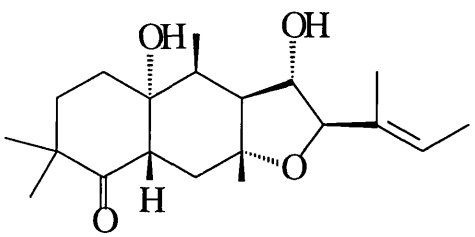

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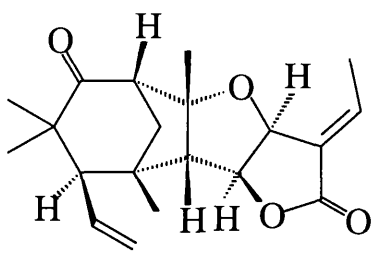

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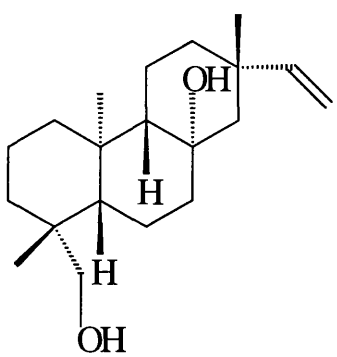

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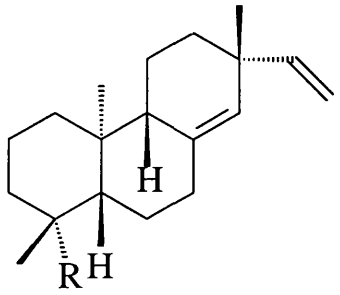
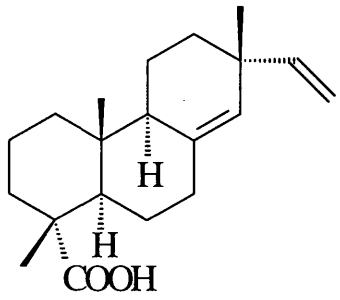


(79) R=CH_2OH

(80) R=COOH



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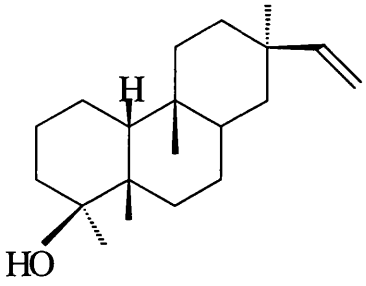

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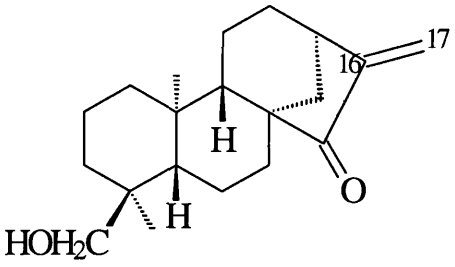
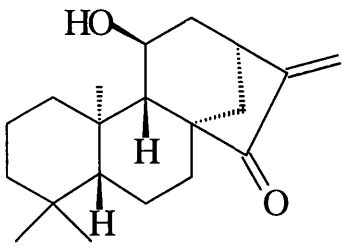


(83)

(84) 16,17-dihydro



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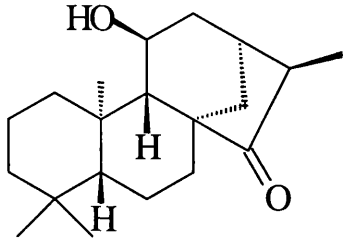

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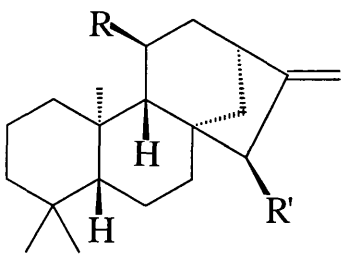
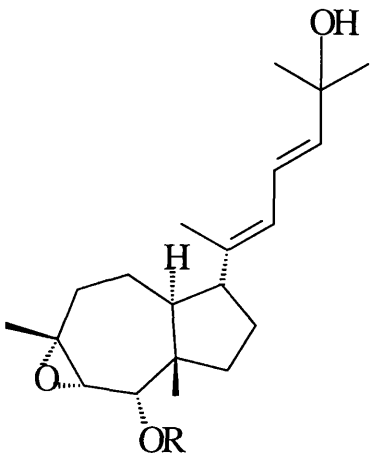
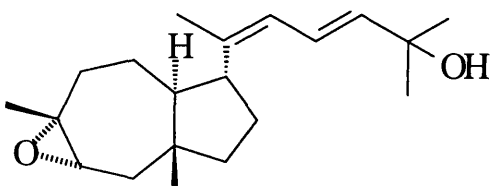


(87) R=R'=OH
(88) R=OH, R'=OAc



(89) R=Ac
(90) R=H



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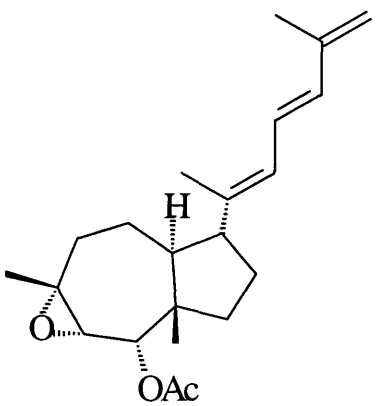

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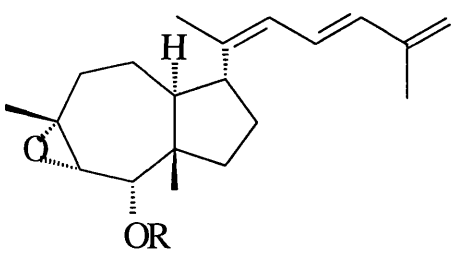
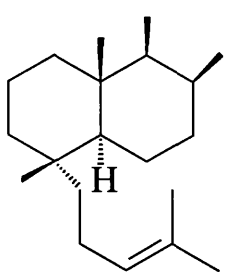


(93) R=Ac
(94) R=H



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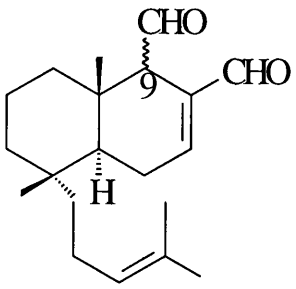
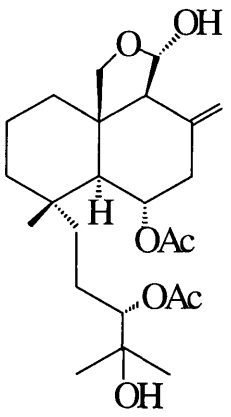


(96) 9β-CHO
(97) 9α-CHO



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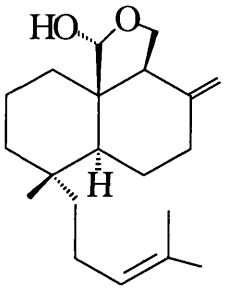

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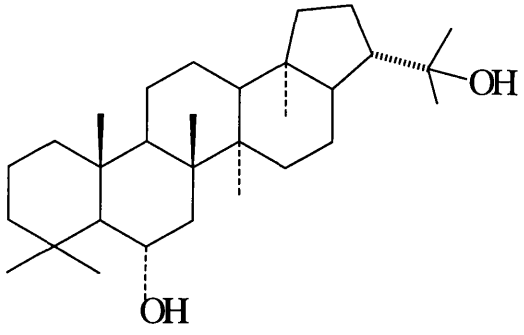

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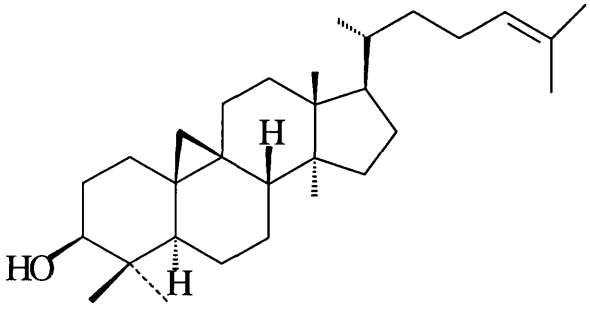

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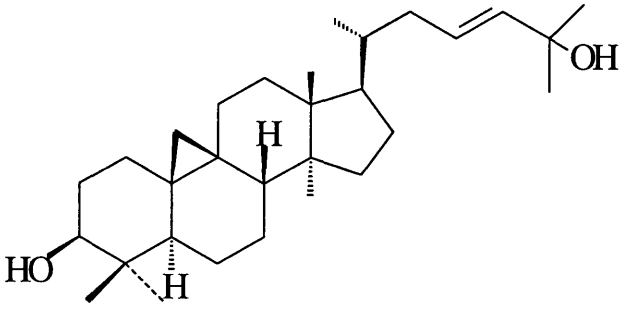

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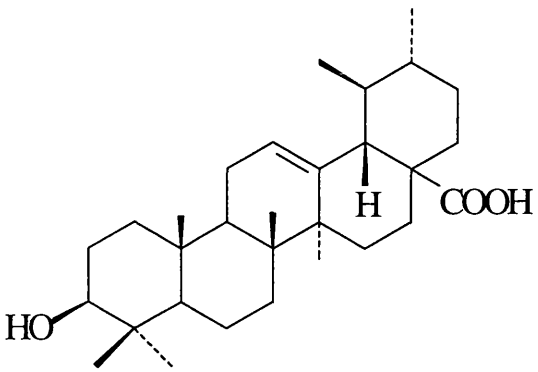

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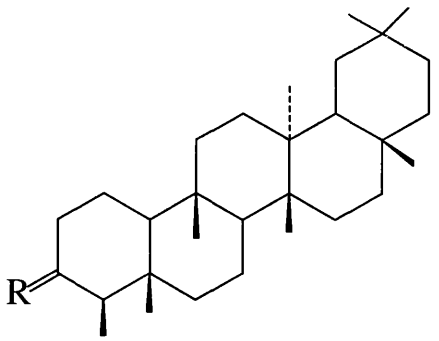
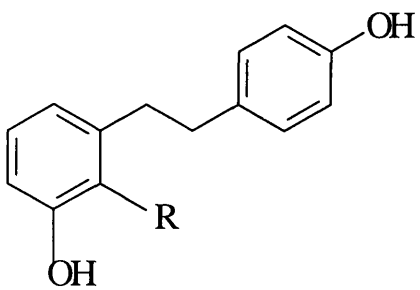
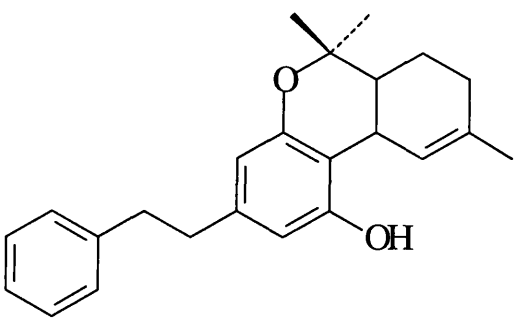


(104) R=H, α-OH
(105) R=H, β-OH
(106) R=O



(107) R=COOH
(108) R=H



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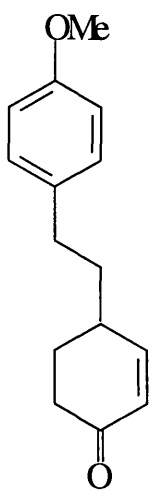

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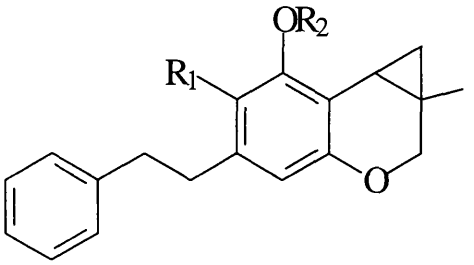
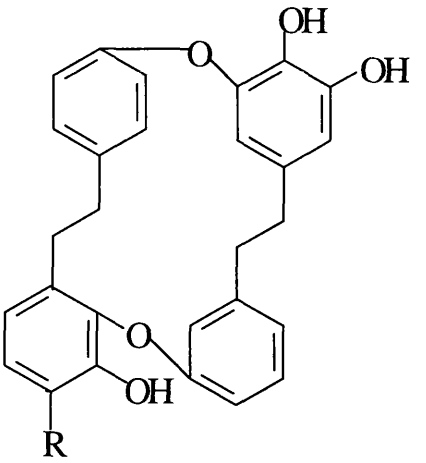
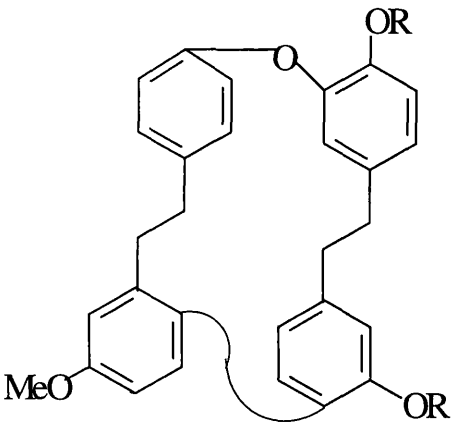


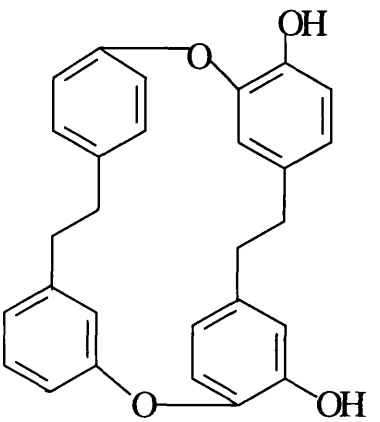
(111) R_1=H, R_2=H
(112) R_1=H, R_2=Me
(113) R_1=COOH, R_2=H



(114) R=H
(115) R=OH



(116) R=H
(118) R=Ac



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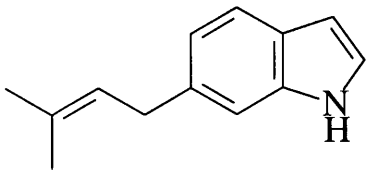

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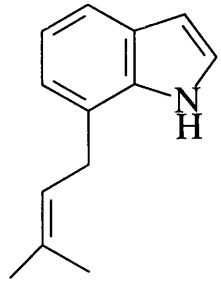

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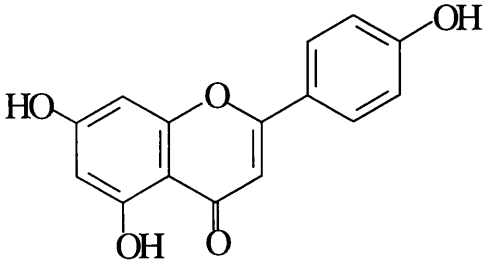
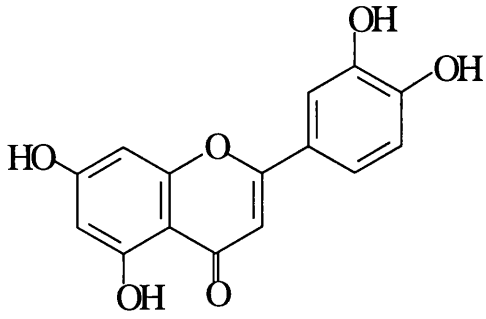


(121)



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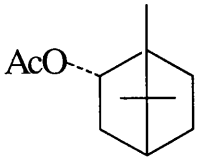

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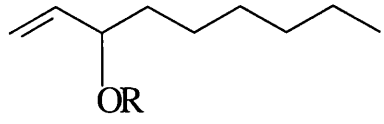
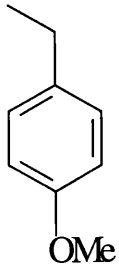


(124) R=H
(125) R=Ac



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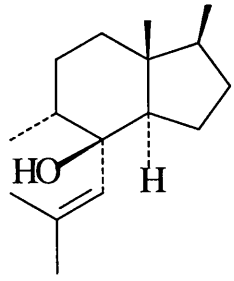

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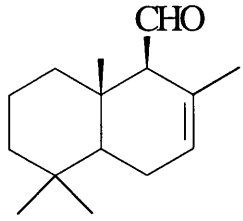

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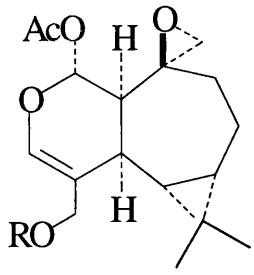
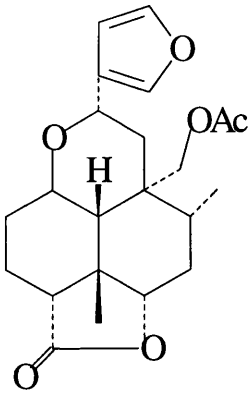


(129) R=Ac
(130) R=H



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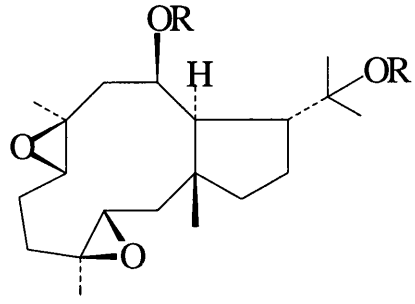
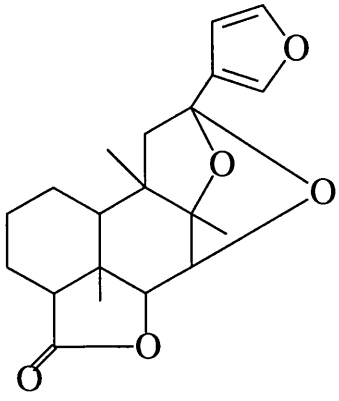


(132) R=Ac
(133) R=H



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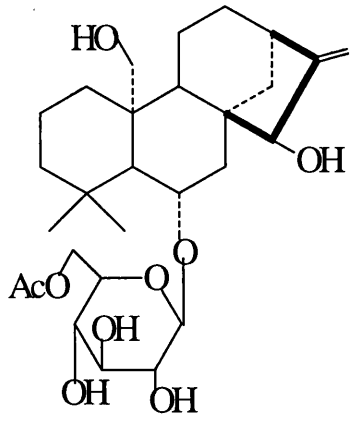

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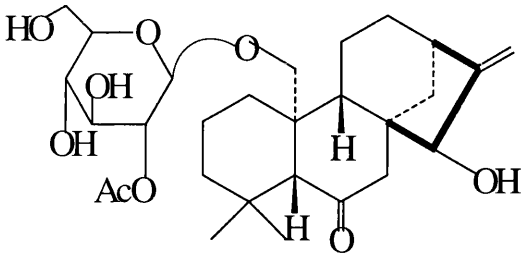

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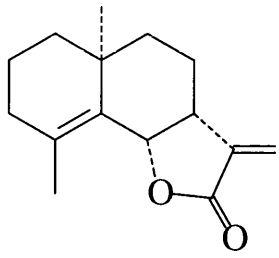

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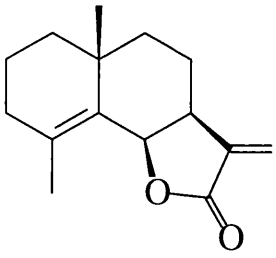

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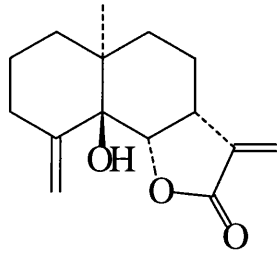

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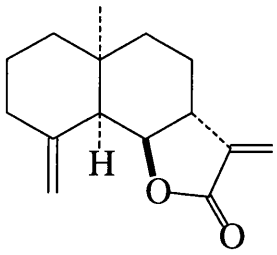

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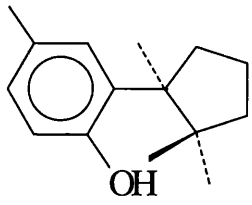

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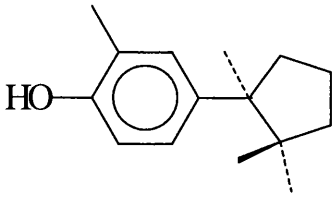

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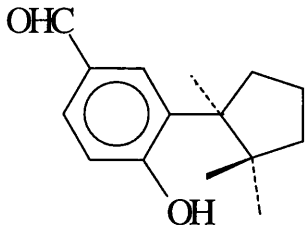

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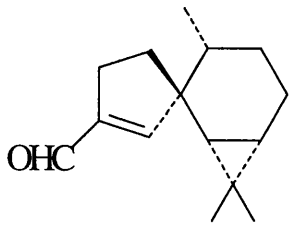

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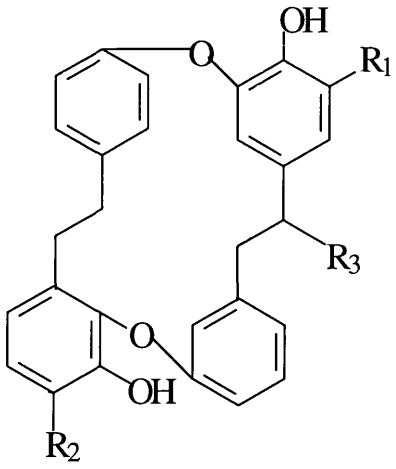
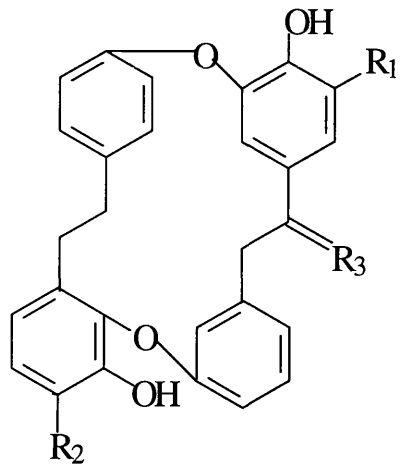
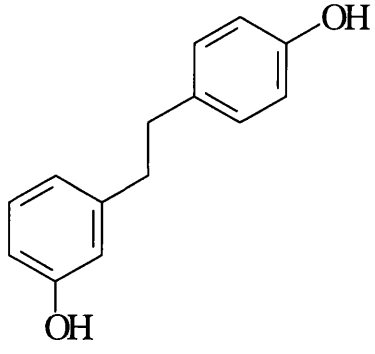


(145) R_1=R_2=R_3=H
(146) R_1=R_3=OH, R_2=H
(147) R_1=OH, R_2=H, R_3=OMe



(148) R_1=R_2=OH, R_3=H,OH
(149) R_1=OH, R_2=H, R_3=O



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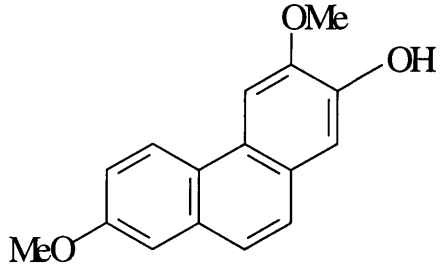

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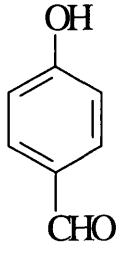

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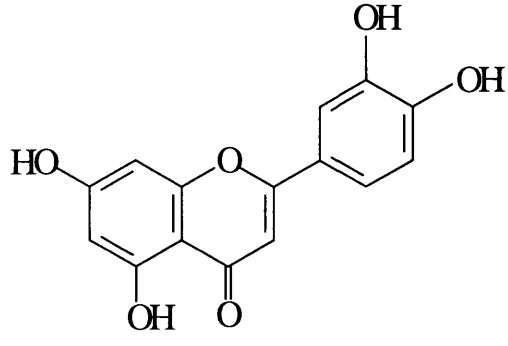

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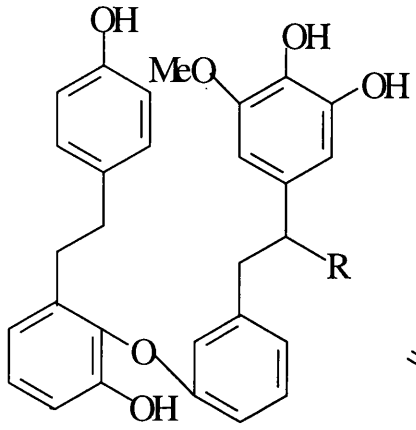
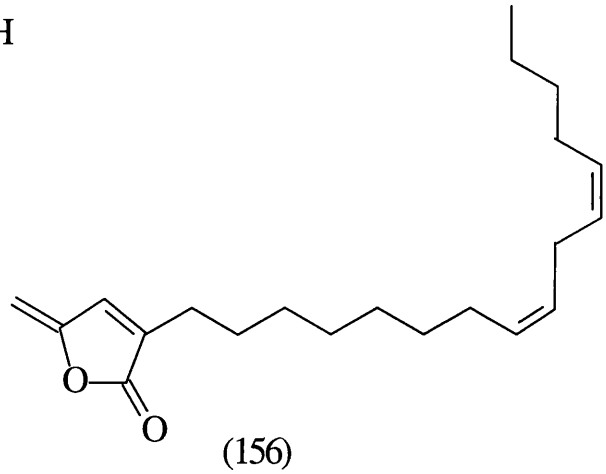


(154) R=OMe
(155) R=H



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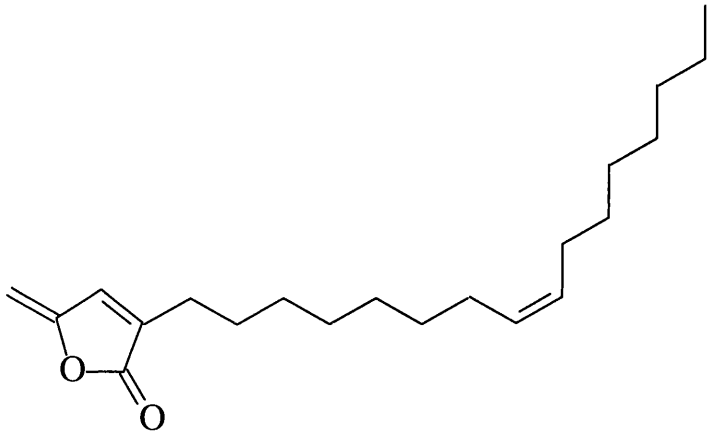

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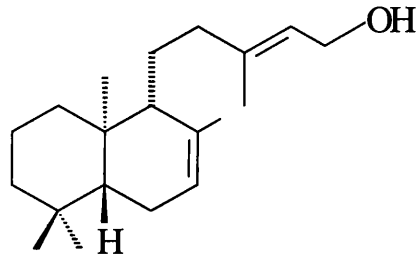

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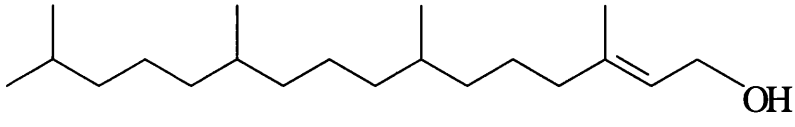

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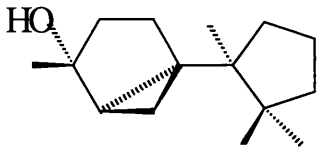

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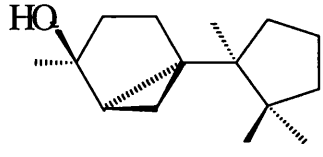

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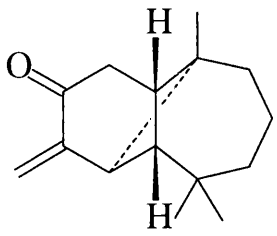

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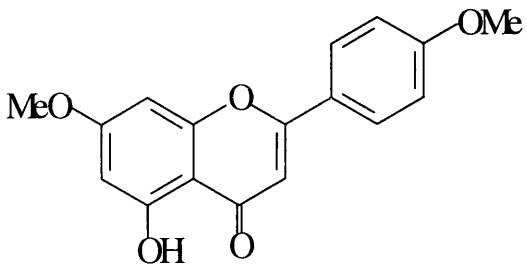

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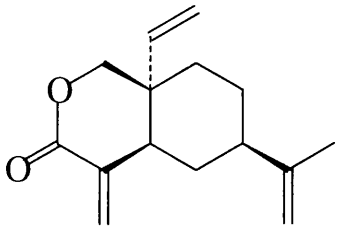

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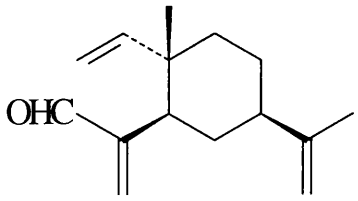

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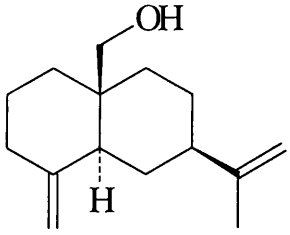

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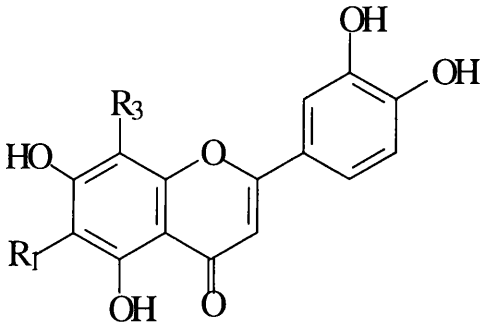
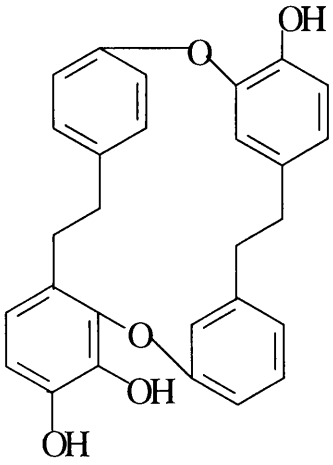


(167) R_1=R_3=Arabinose



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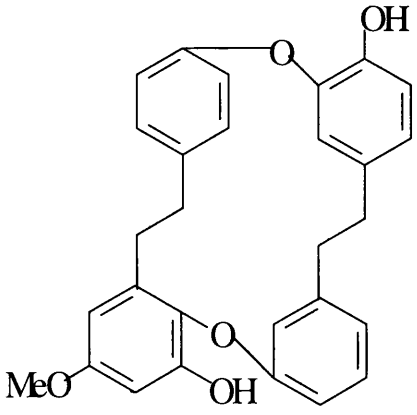

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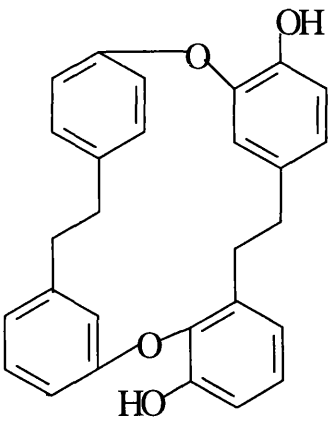

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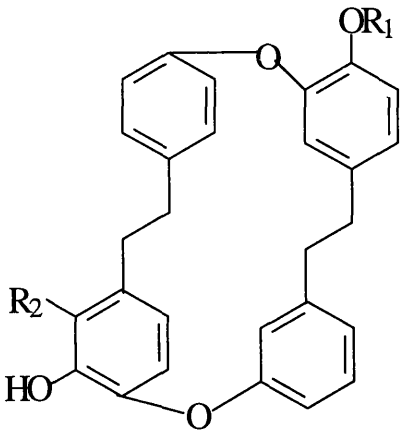
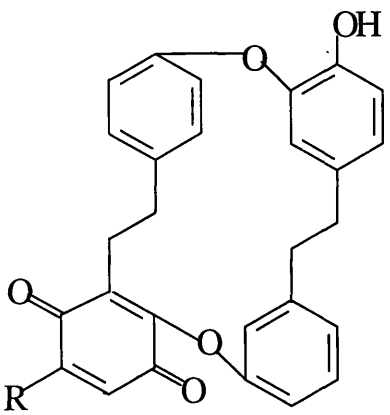
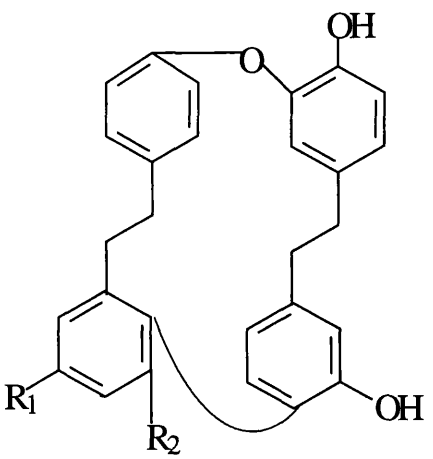


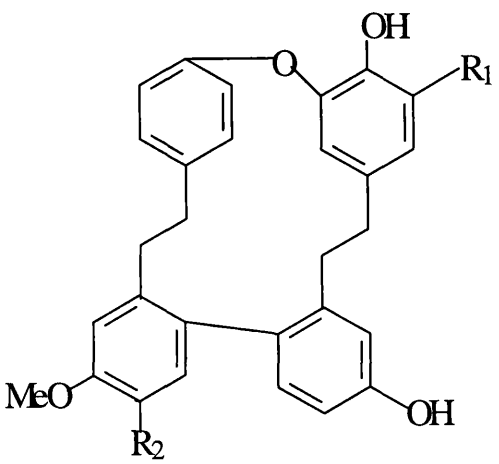
(171) R_1=Me, R_2=H
(172) R_1=R_2=H
(173) R_1=H, R_2=OH



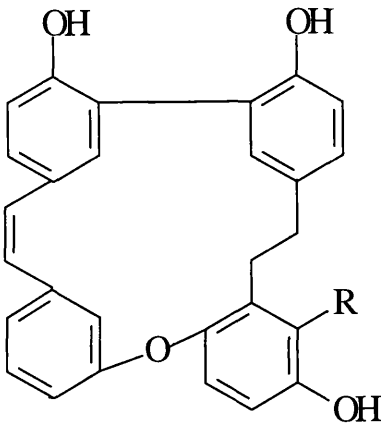
(174) R=OMe
(175) R=H



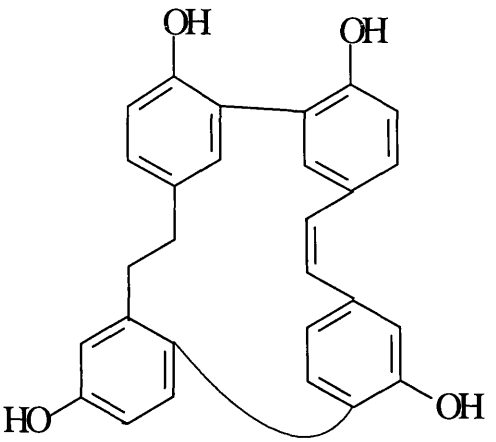
(176) R_1=OH, R_2=H
(177) R_1=H, R_2=OH



(178) R_1=R_2=OH
(179) R_1=OH, R_2=H
(180) R_1=H, R_2=OH
(181) R_1=R_2=H



(182) R=H
(183) R=OH



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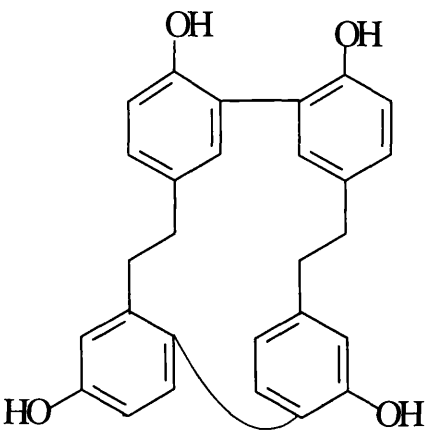
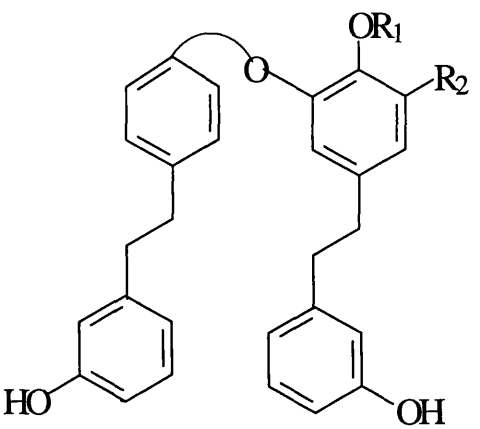


(185)



(186) R_1=R_2=H
(187) R_1=H, R_2=OH
(188) R_1=Me, R_2=OH

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