UNIVERSITY OF NATAL

EXTRACTIVES FROM THE MELIACEAE

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EXTRACTIVES FROM THE MELIACEAE

by

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Submitted in partial fulfilment

of the requirements for the degree of

Master of Science

in the

Department of Chemistry

University of Natal

Durban

1991

DECLARATION

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban under the supervision of Doctor D.A. Mulholland.

These studies represent original work by the author and have not been submitted in any other form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

Signed: .....

T.V. MONKHE

I hereby certify that the above statement is correct.

Signed: .....

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December 1991

ACKNOWLEDGEMENTS

I would like to express my appreciation for the generous help and guidance given to me by Doctor D.A. Mulholland throughout the course of this investigation.

In addition I wish to thank:

Mr M. Watson of the University of Natal, Pietermaritzburg for running the NMR spectra.
Dr Boshof of the Cape Town Technikon for running the mass spectra.
Professor D.A.H. Taylor for supplying the plant material.
Professor F.M. Dean for his help in finding the structure of compound E.
Mrs M. du Plessis for typing this thesis so cheerfully and capably.
My parents for all their support throughout my studies.

SUMMARY

The leaves of *Aphanamixis polystacha, Trichilia dregeana, Ekebergia capensis*, the bark of *Cedrela odorata* and the heartwood and bark of the Australian tree *Aglaia ferruginaea* were extracted.

Two diterpenes, 13-epimanoyl oxide and Aphanamixol, were isolated from the *T. dregeana* and *A. polystacha* extracts, respectively.

The *E. capensis* extract yielded lupeol and kaempferol 3-O-glucoside.

(-)-Epicatethin was isolated from the *C. odorata* extract.

The bark extract of *A. ferruginaea* yielded a novel compound named compound E and the heartwood yielded two compounds compound F and compound G which were similar to glabretal but have not been reported before.

ABBREVIATIONS

AcO	acetate
^{13}C-nmr	carbon-13 magnetic resonance
^{0}C	degrees celcius
conc.	concentrated
d	doublet
GGPP	geranylgeraniol pyrophosphate
GLU	glucoside
^{1}H-nmr	hydrogen-1 magnetic resonance
Hz	Hertz
lit	literature
m	multiplet
Me	Methyl
m/e	mass to charge ratio
m.p.	melting point
NMR	Nuclear Magnetic Resonance
p.	page
p.l.c.	preparative layer chromatography
pmr	proton magnetic resonance
pp	pages
ppm	parts per million
q	quartet
s	singlet
t	triplet
t.l.c.	thin layer chromatography
TMS	tetramethylsilane
TsOH	p-toluenesulphonic acid
UDP	uridine diphosphate

CONTENTS

CHAPTER 1

1 DITERPENES FROM THE MELIACEAE

1.1 INTRODUCTION

This chapter describes the isolation of two diterpenes: 13-epimanoyl oxide [8] from the leaves of *Trichilia dregeana* and aphanamixol (*ent*-13E-labden-8α-15-diol) [9] from the leaves of *Aphanamixis polystacha*. Both trees belong to the Meliaceae family.

1.2 OCCURRENCE AND BIOGENESIS OF DITERPENES

Diterpenes are by definition C_{20} compounds consisting of four isoprene (C_5H_8) units. However, several naturally occurring compounds containing either fewer than 20 or more than 20 carbon atoms are known at present, and which are related to diterpenoids and hence, are best treated along with the related C_{20} compounds.

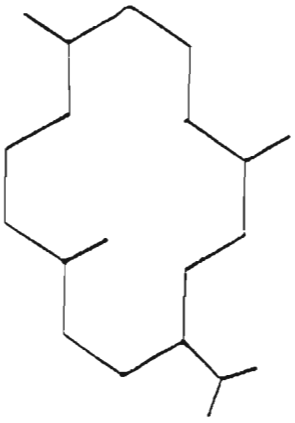
Diterpenoids are widely distributed in the plant and animal kingdoms.[1] Using the Biogenetic Isoprene Rule, the terpene structures could be deduced by accepted reaction mechanisms from acyclic precursors such as geraniol, geranylgeraniol, etc.

Diterpenoids which were first investigated were resin acids occurring in rosin, the steam non-volatile residue from the traditional manufacture of turpentine from the oleoresinous exudate of trees belonging to the family of *Pinacea*.

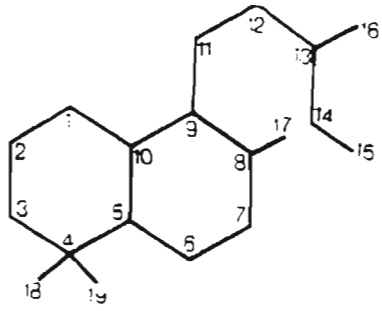
Of the 170 carbon frameworks known for diterpenoids at present, seven (Figure 1) of these account for some 50 percent of the known diterpenes. Labdanes account for the largest number. Labdanes are also widely distributed though entirely restricted to the higher plants.

Reactions of diterpenoids were studied in detail up until 1965, essentially as part of structure elucidation activity. With the widespread use of spectral methods of determining structures, chemical degradations and transformations in diterpenoids, in

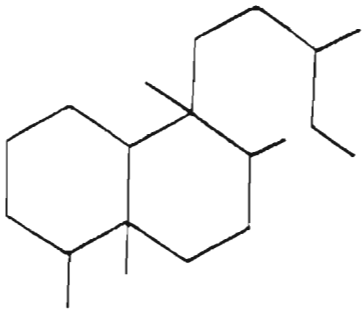
common with other natural products, have become minimal. The absolute stereochemistry of all important diterpenoids is known at present.



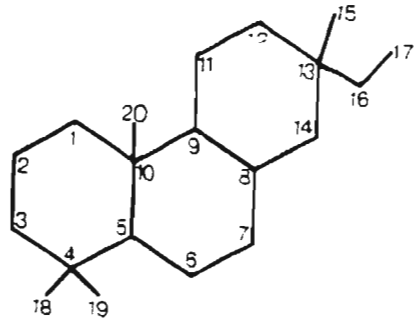
Cembrane



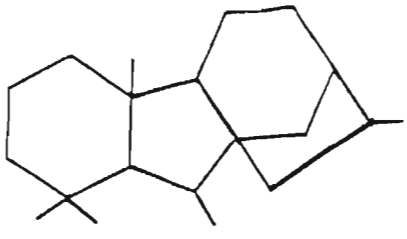
Labdane



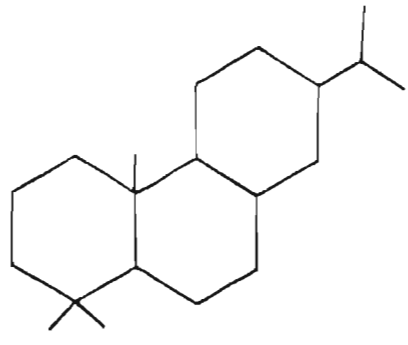
Clerodane



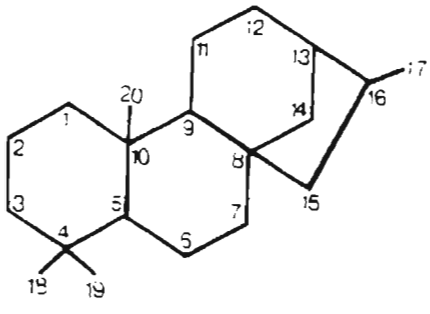
Pimarane



Gibberellane



Abietane



Kaurane

Figure 1

Attack of an electrophilic species such as H^+ at the C-14, C-15 olefinic linkage in geranylgeraniol pyrophosphate (GGPP) can trigger cyclization leading to a mono-, bi- or tricyclic system (Figure 2) depending on the conformation of the substrate ordered by the cyclase enzyme.

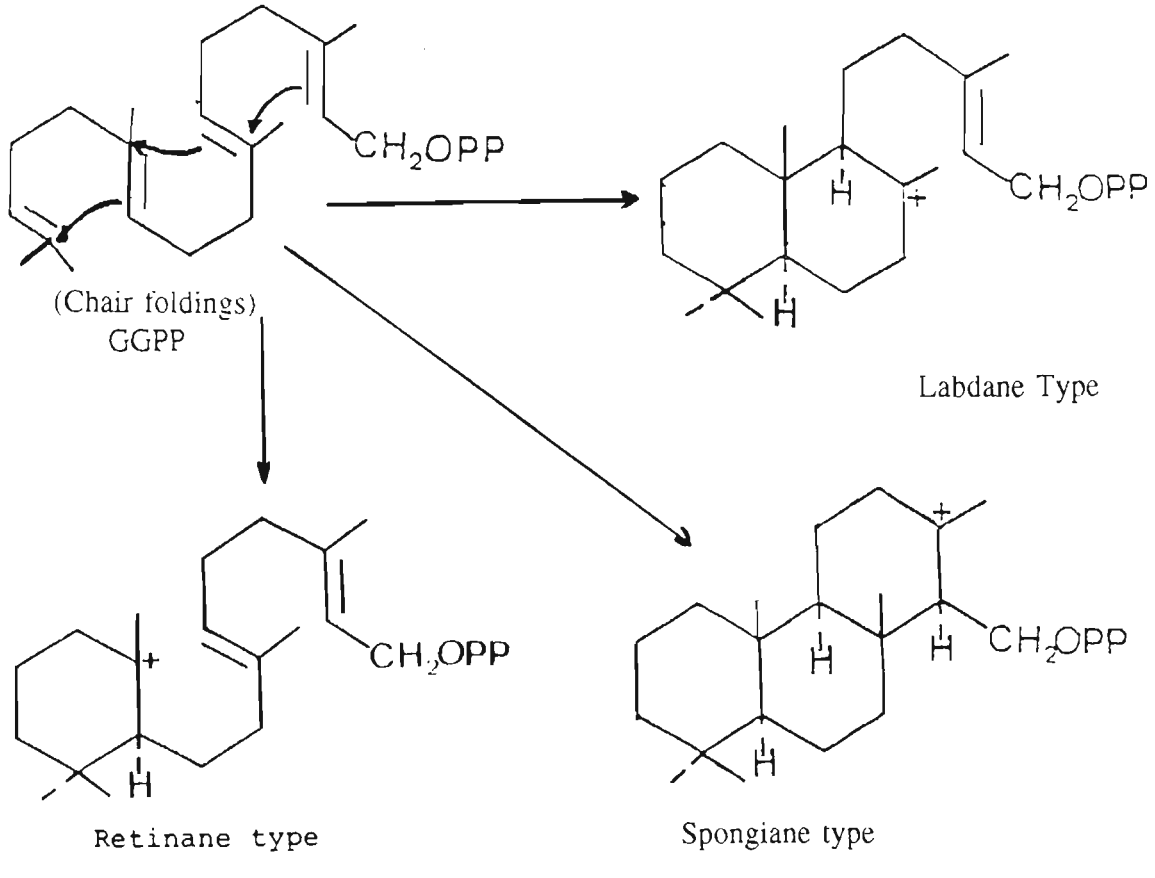


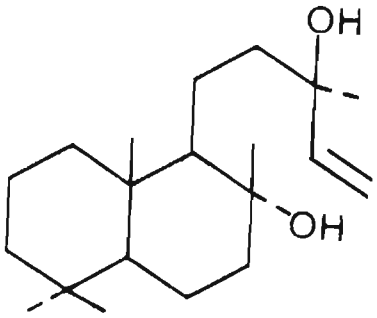
Figure 2

The stereochemistry at ring junctions of labdanes is consistent with the concerted antiparallel addition mechanism. The absolute stereochemistry of the resulting cyclic diterpenes will be dictated by the nature of the cyclase (and unlike triterpenes and steroids, for many diterpene classes both antipodal types occur in nature.)

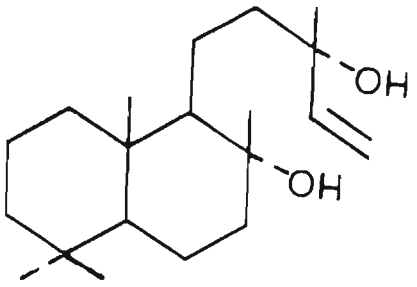
All known labdanes have either an olefinic linkage involving C-8 or have an oxygen functionality on this carbon or one of its immediate neighbours, and this is fully consistent with the involvement of the bicyclic ion (Figure 2) in the genesis of these diterpenoids. Both chiralities at C-13 prevail and even cases of occurrence of both

epimers in the same plant as is the case of the Clary Sage (*Salvia sclarea L*) oil in which sclareol [1] and 14-episclareol [2] co-occur, are known.

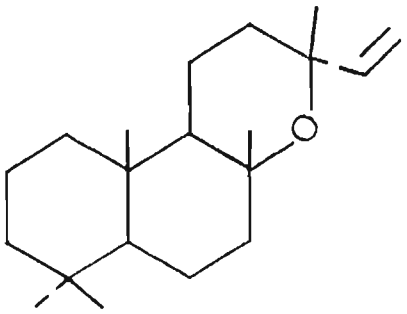
A large number of cyclic ethers based on labdane framework have been isolated, as examples of such structures, manoyl oxide [3], first isolated from *Dacrydium colensoi*, and marrubin [4], are cited.



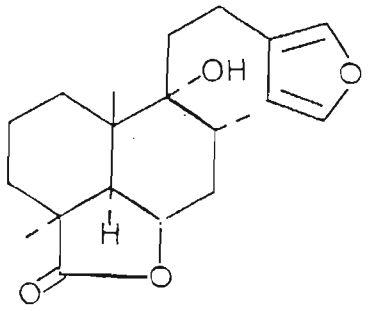
Sclareol [1]



13-Episclareol [2]



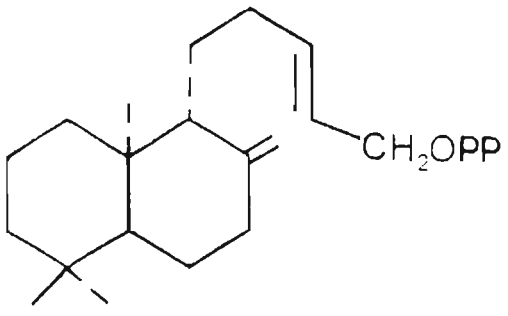
Manoyl oxide [3]



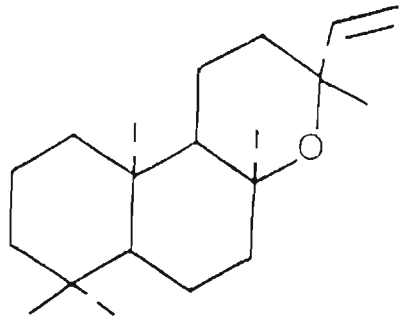
Marrubin [4]

Figure 3

There has not been much activity in the area of biosynthesis of labdanes and related bicyclic diterpenoids. A single investigation relates to the incorporation of [15-^{3}H]-(-)-copalol pyrophosphate [5] by the fungus *Gibberella fujikuroi*[2], resulting in 0.0012 percent label in the metabolite (-)-13-epimanoyl oxide (oleoryl oxide) [6].



(-)-Copalol pyrophosphate [5]

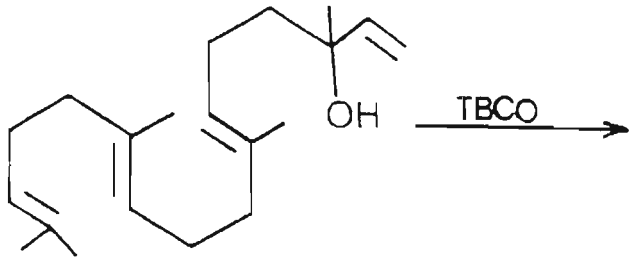


Oleoryl oxide [6]

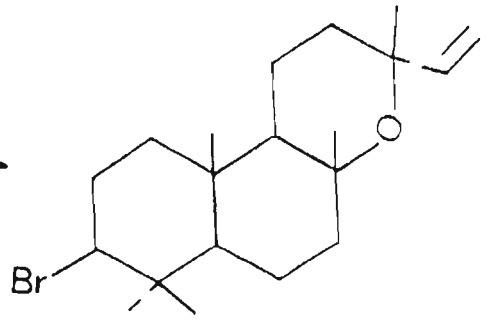
This indicates that the allylic hydroxyl rearrangement (of geraniol or linalool type) can occur at the bicyclic stage.

1.3 PARTIAL SYNTHESIS OF 13-EPIMANOYL OXIDE FROM GERANYL-LINALOOL

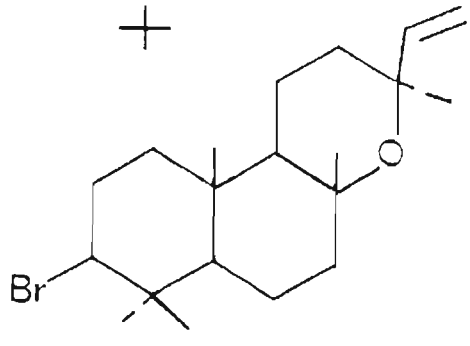
An interesting biomimetic synthesis involves brominative cyclization of geranyl-linalool [7] (Figure 4) in the presence of 2,4,4,6-tetrabromocyclohexa-2,5-dienone (TBO) to afford the tricyclic ethers (Figure 4) (cf. manoyl oxide [3] and 13-epimanoyl oxide [8]) though in poor (2% for each isomer) yield.



Geranyl-linalool [7]



Manoyl oxide [3]



13-epimanoyl oxide [8]

Figure 4

1.4 13-EPIMANOYL OXIDE : AN EXTRACTIVE FROM THE LEAVES OF *TRICHILIA DREGEANA*

1.4.1 INTRODUCTION

Trichilia dregeana (also referred to as *T. chirindensis* Swynnerton, E.G. Baker) is a very large tree reaching heights of 30 m or more; occurring in evergreen forests. This species, which belongs to the Meliaceae family, is found in the eastern parts of South Africa and it extends northwards to tropical Africa.[3]

1.4.2 13-EPIMANOYL OXIDE : EXTRACTIVE FROM *T. DREGEANA*

Samples of the leaves of *T. dregeana* were collected from a tree on the University of Natal campus. Compound A was isolated from the hexane extract and was found to have a molar mass of 290 g mol^{-1} and this, together with the carbon/hydrogen analysis indicated a molecular formula of $C_{20}H_{34}O$. This formula corresponded to many compounds belonging to the labdane group of diterpenoids. This compound did not acetylate.

The infrared spectrum (p. 66) of compound A showed a very intense absorption band at 2950 cm^{-1} due to a C-H stretching. The bands at 1645 and 1190 cm^{-1} indicated the presence of an olefinic group and an ether group respectively. The three bands at 1000, 975 and 940 cm^{-1} suggested a terminal methylene group.

The ^{13}C-nmr spectrum of compound A (p. 69) showed peaks due to five methyl groups at δ15.87(q), δ21.28(q), δ23.93(q), δ32.76(q) and δ33.30(q). The ^{13}C-nmr spectrum also showed two C-O resonances at δ73.35(s) and 76.15(s). As there was only one oxygen atom in the molecule, this was a further indication of the presence of an ether linkage. The peaks at δ149.02(d) and δ109.74(t) confirmed the presence of a terminal methylene group.

The molecular formula and the presence of only one double bond indicated that three rings were present. A further two doublets (δ56.54 and δ58.58) seven triplets (δ15.87,

δ18.63, δ19.90, δ34.85, δ39.39, δ42.11 and δ43.11) and two singlets (δ33.30 and δ36.84) were present, accounting for the 20 carbon atoms.

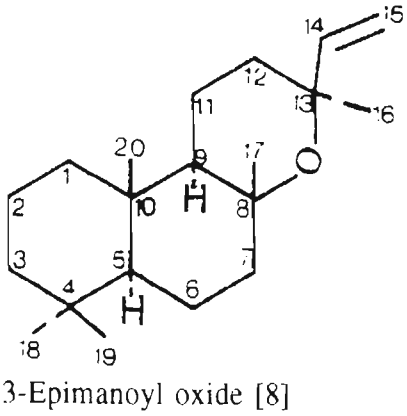
The ^{1}H-nmr spectrum (p. 68) showed that the compound contained five tertiary methyl groups having δ values of 0.66, 0.72, 0.79, 1.06 and 1.05.

The 2-D Hetcor spectrum (p. 72) showed that the two proton resonance at δ4.83 was due to the terminal methylene group. The Cosy spectrum (p. 71) showed that this resonance was coupled to a resonance at δ5.95 (1H). This supported the -CH=CH_2 group suggested by the infrared spectrum and the ^{13}C-nmr spectrum.

The absence of further coupling in the Cosy spectrum of the quadruplet at δ5.95 indicated that the vinyl grouping was attached to a carbon bearing no protons.

The 2-D Hetcor spectrum also showed correlation between the methyl protons at δ0.66, δ0.72, δ0.79, δ1.06 and δ1.15 and the carbon peaks at δ15.87(q), δ21.28(q) δ33.80(q), δ32.75(q) and δ23.93(q) respectively. The multiplet centred at δ2.14 in the ^{1}H-nmr spectrum was correlated to δ34.85(t) in the ^{13}C-nmr spectrum.

A literature search[4,5] indicated that the compound might be 13-epimanoyl oxide [8]. This was confirmed by comparison of literature and experimental ^{1}H and ^{13}C-nmr data and optical rotation data. The melting point (97-100^{0}C) of compound A agreed with that given in the literature.
From the literature all carbon resonances were assigned as in Table 1 (p. 56).



13-Epimanoyl oxide [8]

1.5 APHANAMIXOL : EXTRACTIVE FROM *APHANAMIXIS POLYSTACHA*

1.5.1 INTRODUCTION

Aphanamixis polystacha (Wall) J.N. Parker (Synonyms, *Amoora rohituka* et Arn, *Aphanamixis rohituka* Roxb. Pierre) is an Indo-Malayan member of the Meliaceae family. It is a useful timber tree in Bengal. The seed, bark and leaves are used in the classical system of Indian medicine.[6]

1.5.2 APHANAMIXOL : EXTRACTIVE FROM *A. POLYSTACHA*

The leaves of *A. polystacha*, obtained from a tree on the University of Natal campus, were finely ground and extracted with refluxing hexane in a Soxhlet extractor. A green viscous extract was obtained. Repeated flash-column chromatography using fine silica gel yielded a pure crystalline compound named compound B.

The mass spectrum (p. 75) of compound B gave M^+: 308 g mol^{-1} suggesting a molecular formula $C_{20}H_{36}O_2$. This was confirmed by carbon/hydrogen analysis. Compound B formed a monoacetate on acetylation.

The infrared spectrum (p. 74) provided important information regarding the functional groups of compound B. The absorption bands at 3300 and 1675 cm^{-1} indicated the presence of a hydroxyl group and a tertiary methyl group respectively. The two bands at 1465 and 1395 cm^{-1} are due to a gem-dimethyl group.

The ^{13}C-nmr and Adept spectra (p. 77 and p. 78) of compound B showed five quartets at δ33.48, δ23.96, δ21.56, δ16.49 and δ15.53. The ^{13}C-nmr spectrum (p. 77) also showed peaks at δ141.23(s) and δ123.58(d) indicating the presence of a double bond. A further 8 triplets (δ39.83, δ44.59, δ43.00, δ42.07, δ39.83, δ23.60, δ20.61 and δ18.48), 2 doublets (δ61.31, δ56.23) and 3 singlets (δ74.35, δ39.34, δ33.33) were present, accounting for 20 carbon atoms.

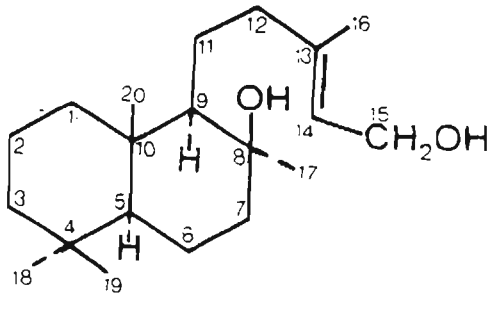
The ^{1}H-nmr spectrum showed the presence of 5 tertiary methyl groups at δ0,81 (6H), δ0.89, δ1.51 and δ1.71. The ^{1}H-nmr spectrum also showed a hydroxyl proton resonance at δ3.49 indicated by its disappearance on addition of D_2O. The 2-D Cosy spectrum (p. 79) showed that the triplet at δ5.46 (1H) was coupled to the doublet at δ4.16 (2H). No further coupling between the two peaks at δ5.46 and δ4.16 with any other proton.

The Hector spectrum (p. 80) correlated the methyl peaks in the ^{1}H-nmr spectrum at δ0.81 (2 x CH_3), δ0.89, δ1.51 and δ1.71 to the carbon peaks at δ33.48 and δ21.56, δ23.96, δ16.50 and δ15.52 respectively. The doublet at δ4.16 (2H) and the triplet at δ5.46 (1H) corresponded to the carbon peaks at δ59.38(t) and δ123.58(d) respectively. Evidence from the Cosy and Hetcor spectra and the fact that a monoacetate could be formed suggested the following group:

$$C{=}CH\text{-}CH_2OH$$

The molecular formula and the presence of only one double bond indicated the presence of 2 rings. A literature[4,5,6,7] survey indicated that compound B might be aphanamixol [9]. This was confirmed by comparison of literature and experimental melting point values, ^{1}H and ^{13}C-nmr data.

From the literature all carbon resonances were assigned as in Table 2 (p. 57).



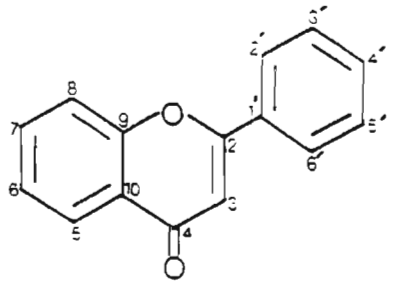
Aphanamixol [9]

CHAPTER 2

2 FLAVONOIDS FROM THE MELIACEAE

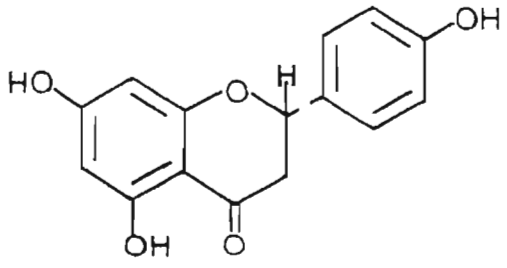
2.1 OCCURRENCE AND BIOSYNTHESIS OF FLAVONOIDS

Flavonoids are ubiquitous in plants and occur as precursors for condensed tannins. They occur as aglycones and glycosides.[8] The system used in numbering flavonoids is shown in structure [10].

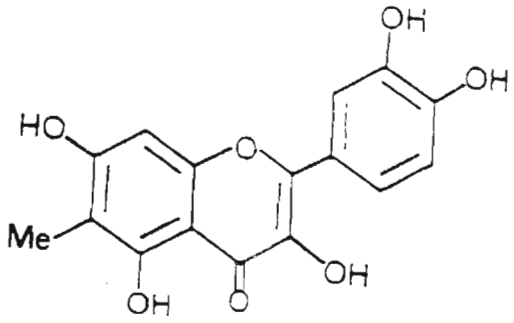


[10]

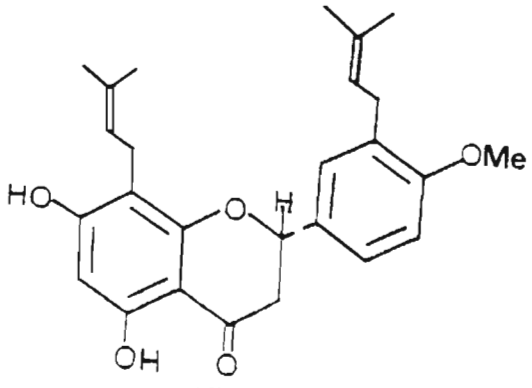
Three flavonoids from the Meliaceae have been reported so far, naringenin [11] from *Soymida febrifuga*,[9] pinoquercetin [12] from *Amoora rohituka*[10] and nimbaflavone [13] from *Azadirachta indica*.[11]



Naringenin [11]

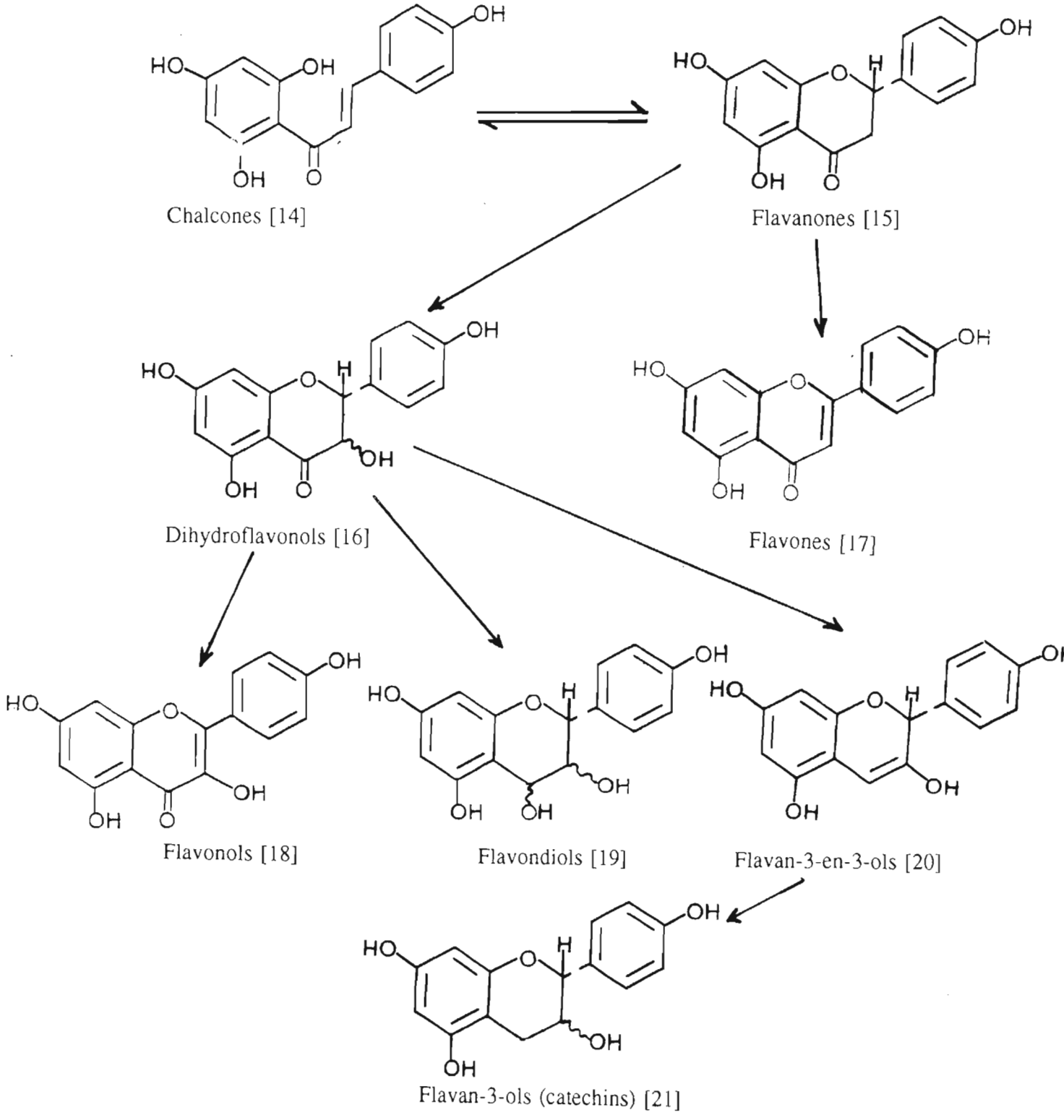


Pinoquercetin [12]



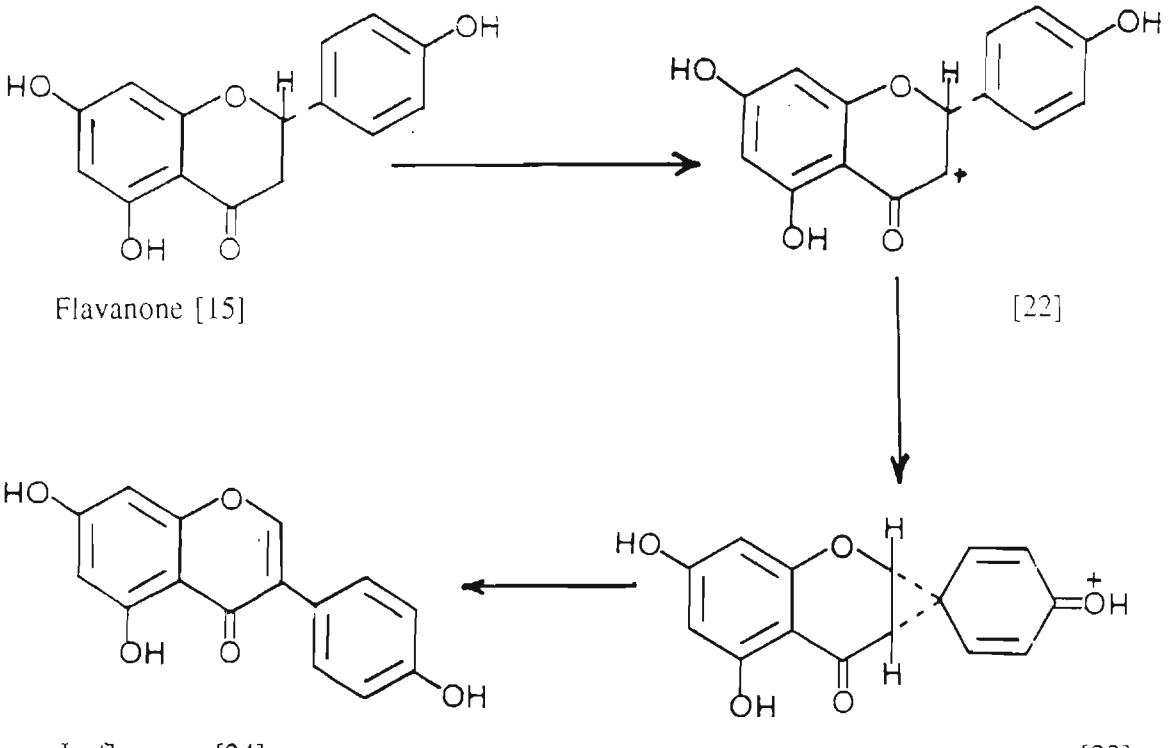
Nimbaflavone [13]

All classes of flavonoids derive their C-15 carbon skeleton from 4-coumaroyl-CoA and three units of malonyl-CoA. The basic biosynthetic process leads to the formation of flavanone via the chalcone structure[12,13,14]



Scheme 1

The dihydroflavonols could be formed by a direct oxygenation of the flavanones. Direct dehydration of the dihydroflavonols leads to flavonols and direct reduction leads to flavandiols. Catechins and epicatechins are formed from flavanones by the reduction pathway leading by way of flavan-3-en-3-ols (Scheme 1). In these conversions the original R-configuration at C-2 persists but there is no fixed configuration at the other centres in ring C. Thus both catechin (3R) and epicatechin (3S) configurations are found in flavan-3-ols.[13]



Flavanone [15] [22]

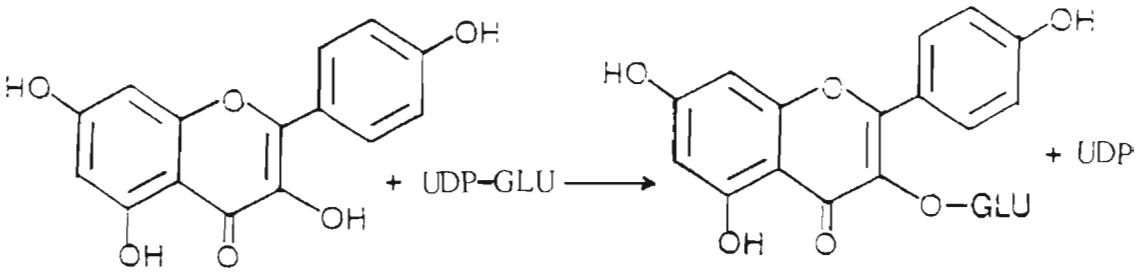
Isoflavones [24] [23]

Scheme 2[13]

The carbonium ion [22] and the spirodienone [23] are possible intermediates in the formation of isoflavonoids[13] [24] (Scheme 2)

Further variety occurs within each category as a result of the differences in oxygenation of rings A and B and from derivatization reactions especially O-methylation and O- and C- glycosylation.

The glucosylation of flavonols at the 3-O-position is catalysed by UDP-glucose; flavonol 3-O-glucosyl-transferase (Scheme 3).



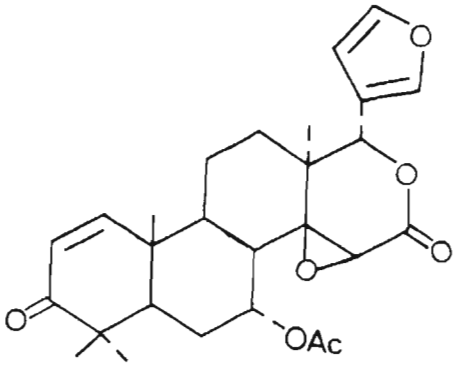
Kaempferol [18] Kaempferol 3-O-glucoside [25]

Scheme 3

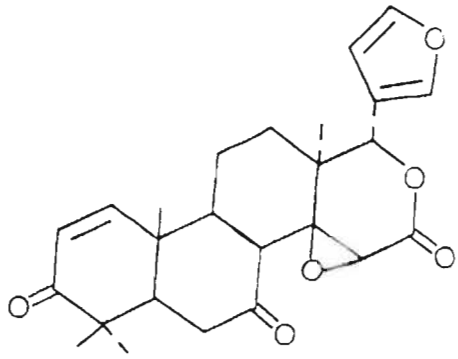
2.2 EXTRACTIVE FROM *CEDRELA ODORATA*

2.2.1 INTRODUCTION

The wood and bark of *Cedrela odorata* has been examined previously for limonoids. Several limonoids were found including gedunin [19] and 7-deacetoxy-7-oxogedunin [20].



Gedunin [19]



7-Deacetoxy-7-oxogedunin [20]

2.2.2 (-)-EPICATECHIN: EXTRACTIVE FROM *C.ODORATA*

The bark of *C. odorata* was obtained from the Ngoye forest in Zululand. Compound C was isolated from the methanol extract and was found to have a molar mass of 290 g mol^{-1}, and this suggested a molecular formula $C_{15}H_{14}O_6$ indicating that this compound was possibly a flavonoid.

The infrared spectrum (p. 88) of compound C showed absorption bands at 3400, 1610 and a small band at 2900 cm^{-1} indicating the presence of a hydroxyl group, an aromatic ring and an aliphatic C-H group respectively. The absence of carbonyl and double bond C=C resonances suggested that compound C was a catechin-type compound. The bands at 1390, 1280 and 110 cm^{-1} in the fingerprint region were due to C-O stretches indicating the possible presence of hydroxyl and/or ether groups. The band at 1450 cm^{-1} was due to a C-H deformation. The band at 815 cm^{-1} was possibly due to an isolated aromatic C-H or two adjacent aromatic C-H groups.

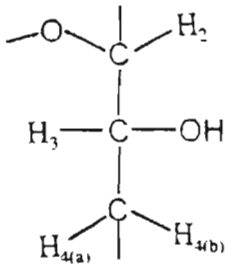
From the ^{13}C-nmr spectrum (p. 91) it could be seen that the compound had resonances at δ28.71(t), δ68.45(d) and δ82.68(d). This together with the absence of a C-4 carbonyl resonance confirmed that compound C was a catechin-type compound with the above mentioned resonances being due to C-4, C-3 and C-2, respectively.

The ^{1}H-nmr spectrum (p. 90) showed a pair of doublets at δ5.91 and δ6.06 (J = 2.3 Hz) typical of H-6 and H-8 protons in a phloroglucinol ring A system.[15] The substitution pattern of ring B could be determined from the ^{1}H-nmr spectrum.

H-2′ appeared as a singlet at δ6.93 and H-5′ appeared as a pair of doublets at δ6.80 (J = 3.1 Hz) and δ6.79 (J = 3.1 Hz).

The integral of the broad resonance at δ8.3 indicated four phenolic protons. Addition of D_2O to the compound indicated that the broad singlet at δ4.30 was due to a hydroxyl group. This accounts for five of the six oxygen atoms. The sixth oxygen atom is the pyran ether oxygen.

From the Hetcor and Cosy spectra, the positions of resonances due to H-2, H-3 and H-4(a) and (b) could be determined in the ^{1}H-nmr spectrum. It was found that H-2 occurred as a doublet (J = 7.7 Hz) at δ4.60. H-3 occurred as a multiplet at δ4.04 and the H-4(a) and H-4(b) protons each gave rise to four peaks centred at δ2.94 and δ2.56, respectively. This confirmed the following sequence:



$J_{2,3}$ = 7.7 Hz

$J_{3,4(a)}$ = 5.3 Hz

$J_{3,4(b)}$ = 8.3 Hz

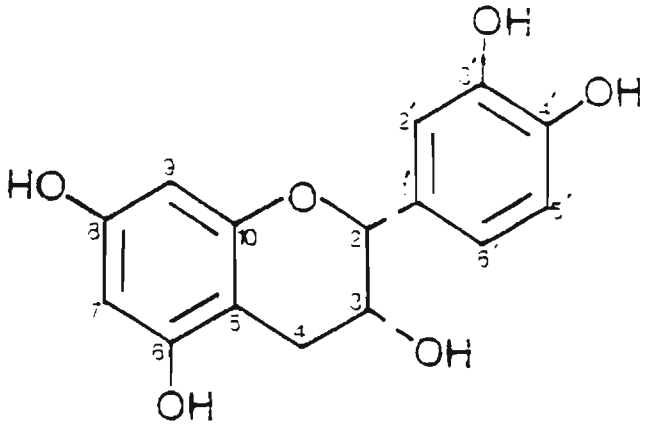
$J_{4(a),4(b)}$ = 16.0 Hz

The ^{13}C-nmr spectrum showed five resonances in the region δ140-160 indicating carbon atoms in the aromatic rings joined to oxygen atoms. These resonances were assigned to C-5, C-7, C-9, C-3′ and C-4′ from previous ^{1}H-nmr data. Doublets at δ95.60 and δ96.41 were assigned to C-8 and C-6 respectively using the Hetcor spectrum (p. 94). These resonance positions are typical for C-6 and C-8 in a phloroglucinol ring A system.

Singlets at δ100.76 and δ132.25 were assigned to C-10 and C-1′ respectively, and doublets at δ115.50, δ116.04 and δ120.31 to C-2′, C-5′ and C-6′.

A literature search indicated that the compound might be (-)-epicatechin. This was confirmed by comparison of literature and experimental ^{1}H and ^{13}C-nmr data and optical rotation data.

From the literature all carbon resonances were assigned as in Table 3 (p. 58).



(-)-Epicatechin [26]

2.3 KAEMPFEROL 3-0-GLUCOSIDE : EXTRACTIVE FROM *EKEBERGIA CAPENSIS*

2.3.1 INTRODUCTION

Ekebergia capensis (*E. meyeri* Presl ex. DC.) commonly known as Cape Ash is a small species of African trees belonging to the Meliaceae, tribe Trichiliae. *E. capensis* is a medium to large evergreen, widespread in eastern Africa from Sudan to the Cape. The timber is not durable but the tree is cultivated for shade. The tree has fleshy, berry-like, almost spherical fruit, turning pink to bright red when mature. The bark is used as an emetic and in the treatment of dysentery. A decoction of the root is said to relieve headaches and chronic coughs, while the leaves provide a remedy for intestinal worms.[3] The timber and bark have been previously examined for limonoids[16], and ekebergolactones were found in very small amounts (Prof D.A.H. Taylor, personal communication).

2.3.2 KAEMPFEROL 3-0 GLUCOSIDE : EXTRACTIVE FROM *E. CAPENSIS*

The leaves of *E. capensis* were obtained from the Durban Botanic Gardens in Natal. Compound D was isolated from the methanol extract of the leaves.

The infrared spectrum (p. 96) of compound D showed a broad absorption band at 3400 cm^{-1} and a sharp band at 2925 cm^{-1} due to hydroxyl groups and an intramolecular 'chelate' H-bonded hydroxyl group. The band at 1650 cm^{-1} suggested an aryl or α,β-unsaturated ketone. The bands at 1605 and 1513 cm^{-1} were due to benzene rings. The bands in the fingerprint region at 1370 and 1170 cm^{-1} were due to hydroxyl groups and a C-O stretching which was due to a keto- group and/or an ether group. The band at 840 cm^{-1} was due to 2 adjacent aromatic C-H groups and/or an isolated aromatic C-H group.

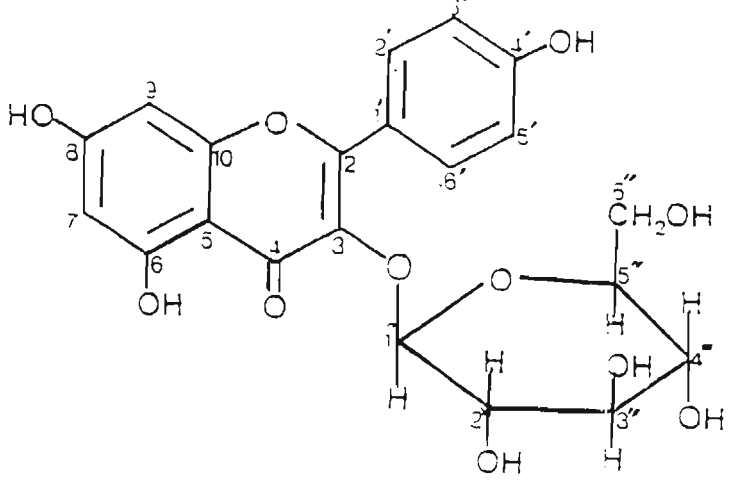
The ^{1}H-nmr spectrum (p. 97) showed a pair of doublets at δ6.23 and δ6.46 typical of H-6 and H-8 protons in a phloroglucinol ring A system of flavonoids.[15] Ring B substitution pattern could be determined from the ^{1}H-nmr spectrum. The doublet at δ6.90 (2H) (J = 8.94 Hz) suggested H-3′ and H-5′ protons *ortho* to oxygen in a *para* substituted benzene ring. The doublet at δ8.04 (2H) (J = 8.93 Hz) corresponded to the H-2′ and H-6′ protons. The sharp singlet at δ12.60 corresponded to an H-bonded phenolic proton. The broad resonance centred at δ10.3 was due to phenolic hydroxyl protons. The broad peak at δ4.3 was due to a hydroxyl proton. This was shown by addition of D_2O. The doublet at δ5.45 (1-H) (J = 7.18 Hz) is due to a C-1" proton in a hexose sugar. This resonance position and J value was typical of the C-1" proton in a β-linked glucose moiety attached to the C-3 hydroxyl group of flavonoids.[15] The peaks in the region δ3.0-4.0 were characteristic of the sugar moiety. This evidence suggested that compound D might be a flavonoid 3-0-β-D-glucoside.

Compound D was hydrolysed. T.l.c. of the hydrolysis mixture against a standard sample of glucose proved that the sugar was glucose.

The ^{13}C-nmr spectrum (p. 98) indicated 21 carbon atoms. The singlet at δ177.4 was typical of C-4 in a flavonone.[17] The singlets at δ164.1, δ161.1, δ150.8, δ156.4 and δ156.3 typical of C-7, C-5, C-4′. C-2 and C-9 of a flavonol containing a phloroglucinol ring A structure and a para-substituted ring B.[17] The C-8 and C-6 doublets occurred in their usual positions of δ93.6 and δ98.7 respectively as did the C-3 and C-10 singlets at δ133.0 and δ104.1 respectively. The singlets at δ121.0 and δ159.8 were tentatively assigned to C-1′ and C-4′ of the *para* substituted aromatic ring. C-2′ and C-6′ which are meta to the *para* substituent occurred as a 2-carbon doublet at δ130.7 and C-3′ and C-5′ which are in the *ortho* position were assigned to the 2-carbon doublet at δ115.0. The resonance position of δ101.4 for the C-1" of the glycoside indicated that the sugar was a β-D-glucose in the C-3 hydroxyl position.[17] The other glucose resonances occurred at δ77.2(d), δ76.5(d), δ74.2(d), δ70.1(d) and δ61.0(t).

A literature[14,16] search indicated that compound D might be kaempferol 3-0-glucoside. This was confirmed by comparison of literature and experimental ^{1}H and ^{13}C-nmr data.

From the literature[14,16] all carbon resonances could be assigned as in Table 4 (p. 59).



Kaempferol 3-O-glucoside [25]

2.4 COMPOUND E: EXTRACTIVE FROM *AGLAIA FERRUGINAEA*

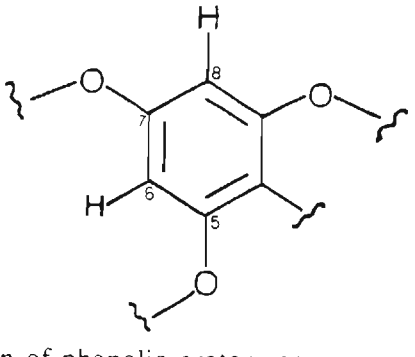
The bark of the Australian tree *Aglaia ferruginaea*, which belongs to the Meliaceae family, was finely ground and extracted with refluxing hexane for 24 hours in a Soxhlet extractor.

Analytical thin layer chromatography of the green extract indicated the presence of several compounds. Repeated flash-column chromatography using fine silica gel yielded a pure compound named compound E.

The accurate molar mass of 434.1713 g mol^{-1} corresponded to the molecular formula $C_{26}H_{26}O_6$. The accurate mass spectrum showed a major peak at m/e 300.0986 g mol^{-1} corresponding to the molecular formula $C_{17}H_{16}O_5$. This corresponds to a loss of a methoxystyrene fragment. Major peaks were also found at m/e 135.0448 and m/e 181.0503 g mol^{-1} corresponding to the molecular formulae $C_8H_7O_2$ and $C_9H_9O_4$. The peak at m/e 135.0448 g mol^{-1} indicated the loss of a further ArC(OH)=COH fragment.

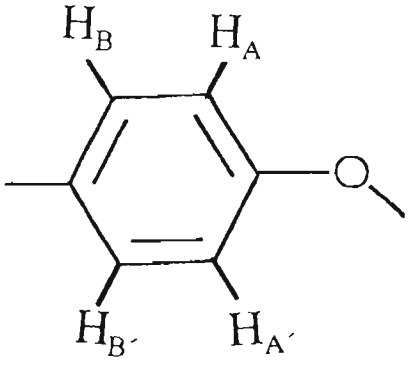
The ^{1}H-nmr spectrum (p. 107) of compound E resembled that of a flavonoid.[15] The two hydroxyl proton resonances at δ1.75 and δ3.35 were indicated by their disappearance on addition of D_2O. The three resonances in the region δ3.7-3.9 each integrating to three protons were due to three methoxyl groups.

The two doublets at δ6.14 and δ6.29 (J=2.0Hz) each integrating to one proton are typical of H-6 and H-8 of a phloroglucinol ring A system in flavonoid compounds.[15]

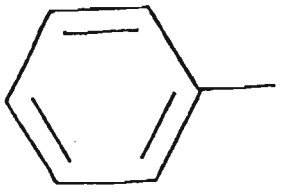


There was no sign of phenolic proton resonances in the ^{1}H-nmr spectrum.

The doublet at δ6.67 (2H, J=9.0 Hz) with *ortho*-splitting suggested protons *ortho*- to an oxygen atom in a *para*-substituted benzene ring. This doublet was part of an AA´BB´ system. The other was found at δ7.12 (J=9.0 Hz).



Integration of the peaks in the aromatic region indicated a further five aromatic protons and therefore an unsubstituted benzene ring.



The ^{1}H-nmr spectrum also showed four further resonances : a doublet at δ4.80, a four line resonance at δ4.0, a six line resonance at δ2.7 and a four line resonance at δ2.2 each integrating to one proton.

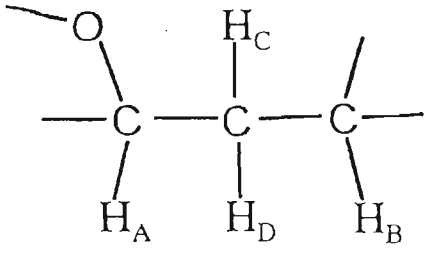
The ^{13}C-nmr spectrum (p. 108) showed 28 resonances. The resonance at δ30.0 was due to an impurity from the solvents which we regularly find.

From the ^{13}C-Adept spectrum (p. 108), multiplicities could be assigned to all carbon resonances.

The Hetcor spectrum (p. 111) correlated the carbon resonance at δ79.03(d) to a doublet in the ^{1}H-nmr spectrum at δ4.8 indicating a H_A-C-O grouping. The carbon

resonance at δ53.21(d) corresponded to the four line proton resonance at δ4.0 due to a proton referred to as H_B. The carbon resonance at δ36.3(t) corresponded to the two four line proton resonances at δ2.2 and δ2.7 due to non-equivalent CH_2 protons, H_C and H_D.

The arrangement of the protons H_A, H_B, H_C and H_D could be established from the Cosy spectrum (p. 110). H_A was coupled to the non-equivalent CH_2 protons H_C and H_D, though there was only a slight coupling between H_A and H_D. Protons H_C and H_D were in turn coupled to proton H_B. The protons H_A and H_B were not further coupled. This suggested the following arrangement:



Since the molecular formula of compound E was $C_{26}H_{26}O_6$, 14 double bond equivalents were indicated. Taking into account the phloroglucinol ring, the two aromatic rings and ring C of a typical flavonoid system, we were left with one double bond equivalent to account for.

The infrared, ^{13}C-nmr and ^{1}H-nmr spectra did not suggest any alkene double bond. Therefore an extra ring had to be found.

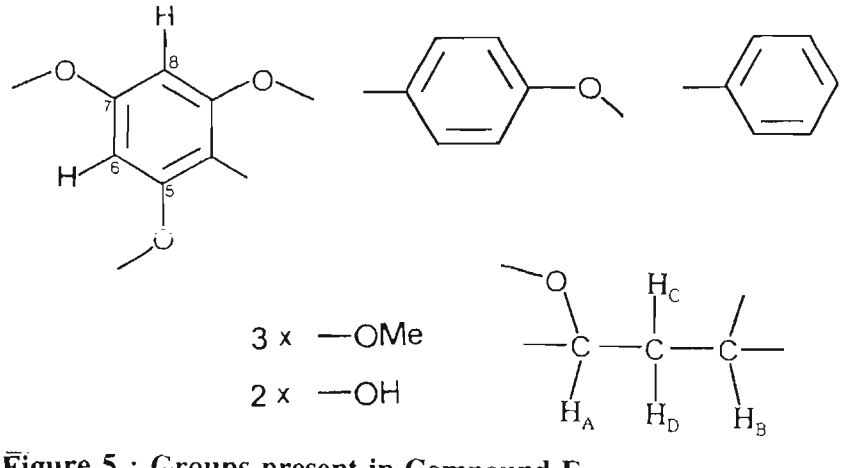
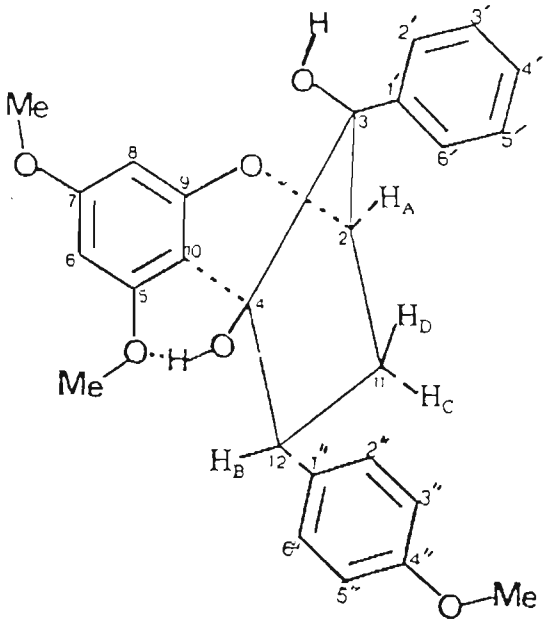


Figure 5 : Groups present in Compound E

When all groups present (Fig. 5) were arranged, the only possible structure was the isoflavonoid [27].

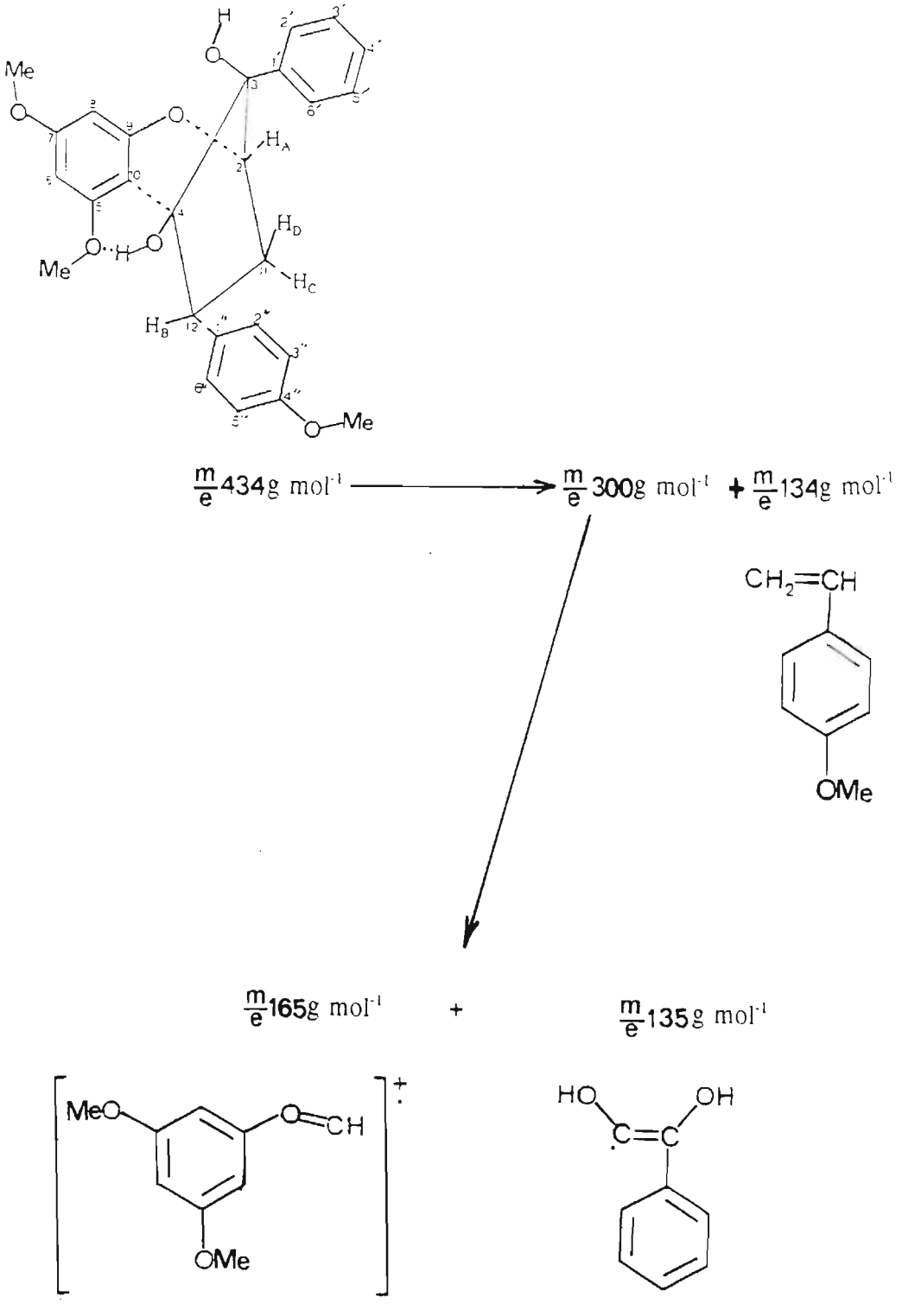


COMPOUND E [27]

The H-bonded hydroxyl proton would resonate at a lower field (δ3.35) than the other.

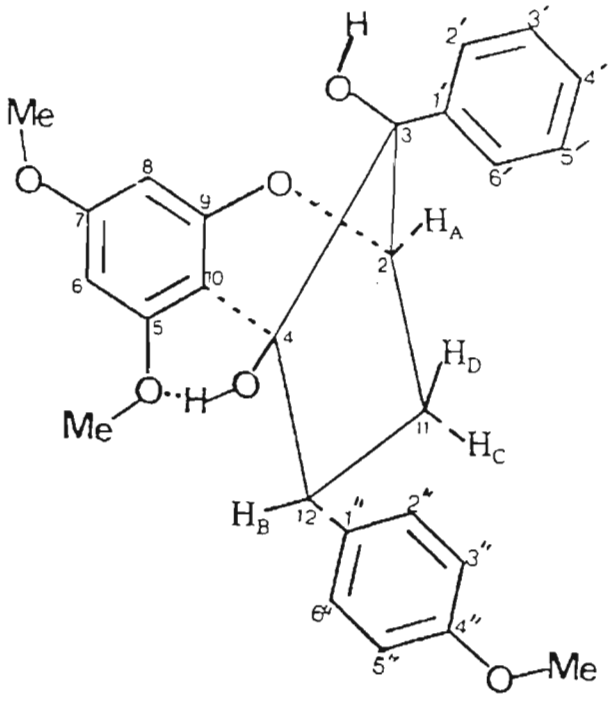
The mass spectrum (p. 108) of compound E showed major peaks at m/e 300, 135, 134 and 165 g mol-1.

Since the molar mass of compound E was 434 g mol^{-1} the peak at m/e 300 g mol^{-1} suggested the loss of a p-methoxystyrene (m/e 134 g mol^{-1}) fragment. The fragment with m/e 300 g mol^{-1} in turn loses a fragment (m/e 135 g mol^{-1}) fragment leaving a residual fragment with m/e 165 g mol^{-1}. The peak at m/e 165 g mol^{-1} and m/e 135 g mol^{-1} corresponded to the molecular formulae $C_9H_9O_3$ and ArC(OH)=C-OH respectively. This was confirmed by accurate mass measurements of the peaks at m/e 300 and 165 g mol^{-1}.



Scheme 4 : Fragmentation pattern

All structures were supported by accurate mass measurements.



COMPOUND E [27]

Table 5

Experimental J. value (Hz)	Angles (measured)
J_{AC} = 6.0	~30^0
J_{AD} = 1.1	90^0-100^0
J_{BC} = 14.0	~140^0
J_{BD} = 6.6	~10^0
J_{CD} = 13.8	geminal

Dreiding models of compound E were constructed to ascertain the stereochemistry at positions 2, 3, 4 and 12. H_B and the anisyl groups, C-2, C-11 and C-2-H_A bonds were interchanged and the angles H_A-H_C, H_A-H_D, H_B-H_C and H_B-H_D of the various combinations were measured (Table 5).

The coupling constants were also measured (Table 5).

The structure that fitted the experimental J values was the structure where the stereochemistry at C-2 was [R] and C-11 was α with respect to the cyclopentane ring to give angles H_B-H_C and H_B-H_D equal to ~140^0 and ~10^0 respectively.

The angles agreed with those calculated using the Karplus equation. It can be postulated that the p-methoxystyrene compound would be added across C-2 and C-4 in a Diels-Alder cyclo-addition type of reaction to give the 'syn' product.

If this was the case, C-12 would be expected to be on the α side of the isoflavonoid structure as has been shown to be the case with C-11. If the stereochemistry at C-2 was [R] and C-12 was α (with respect to the isoflavonoid structure) then C-3 would have to be β.

Further evidence for this structure was obtained by studying long range W coupling between H_A and the hydroxyl proton on C-4 and H_C and the same hydroxyl proton.

If a W course one way round the cyclopentane ring was traced, we found that we could accommodate long range coupling between the C-4 hydroxyl proton and H_A. Similarly H_C was coupled with the C-4 hydroxyl proton. These long range couplings did not result in any splitting of the resonance peaks in the ^{1}H-nmr spectrum but could be detected in the Cosy spectrum (p. 113).

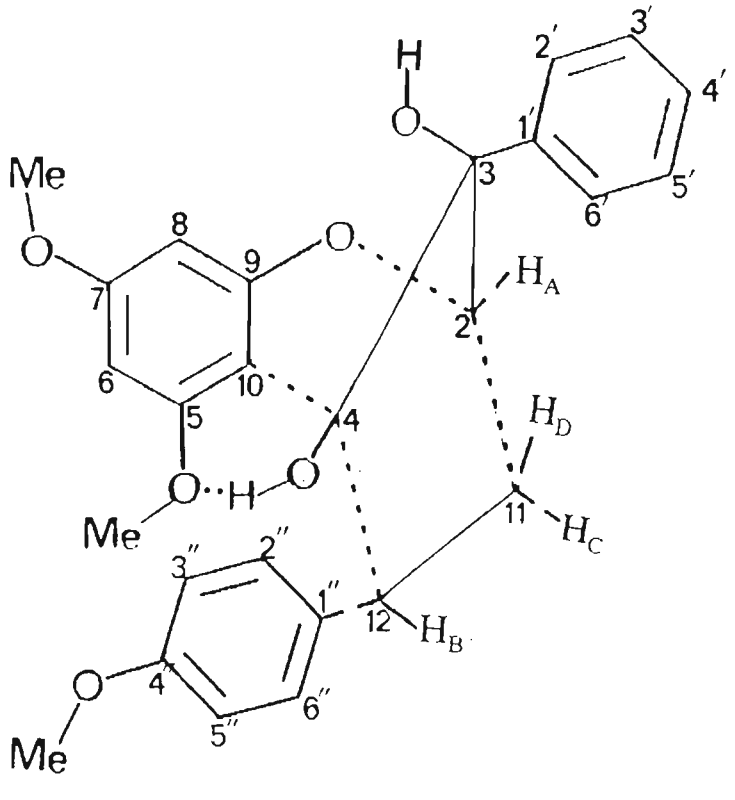
In compounds where aromatic rings are in close proximity to one another, marked effects in the ^{1}H-nmr spectrum have been reported. As there were no strong effects observed in the ^{1}H-nmr spectrum (p. 110) of compound E, it was safe to reject a geometry with these two substituents on the same side of the cyclopentane ring and away from the phloroglucinol ring.

The phloroglucinol nuclear protons were at a slightly higher field than is normal for flavonoid compounds (for example δ6.14 (H-6) and δ6.29 (H-8) in compound E but δ6.2 (H-6) and 6.4 (H-8) in orobol).[15] This might indicate slight shielding by one of the aryl substituents. If this was the case, then shielding would be provided by the

anisyl group when on the same side of the cyclopentane ring as the phloroglucinol ring since the angle between the phloroglucinol ring and the phenyl ring would be closer to 90^0, whereas the angle between the phloroglucinol ring and the anisole ring (when on the same side of the cyclopentane ring) would be very much less than 90^0. This was further evidence for the stereochemistry at position C-12.

It is not yet known whether the OH groups are in a *cis*- or *trans*-position with respect to each other. Further material needs to be extracted so that an attempt may be made to produce crystals for X-ray analysis of this new compound.

This type of isoflavonoid with a C-2:C-4 bridged structure has not been isolated previously.



COMPOUND E [27]

The carbon resonances were assigned as in Table 6 (p. 60).

CHAPTER 3

3. PROTOLIMONOIDS AND A TRITERPENE FROM THE MELIACEAE

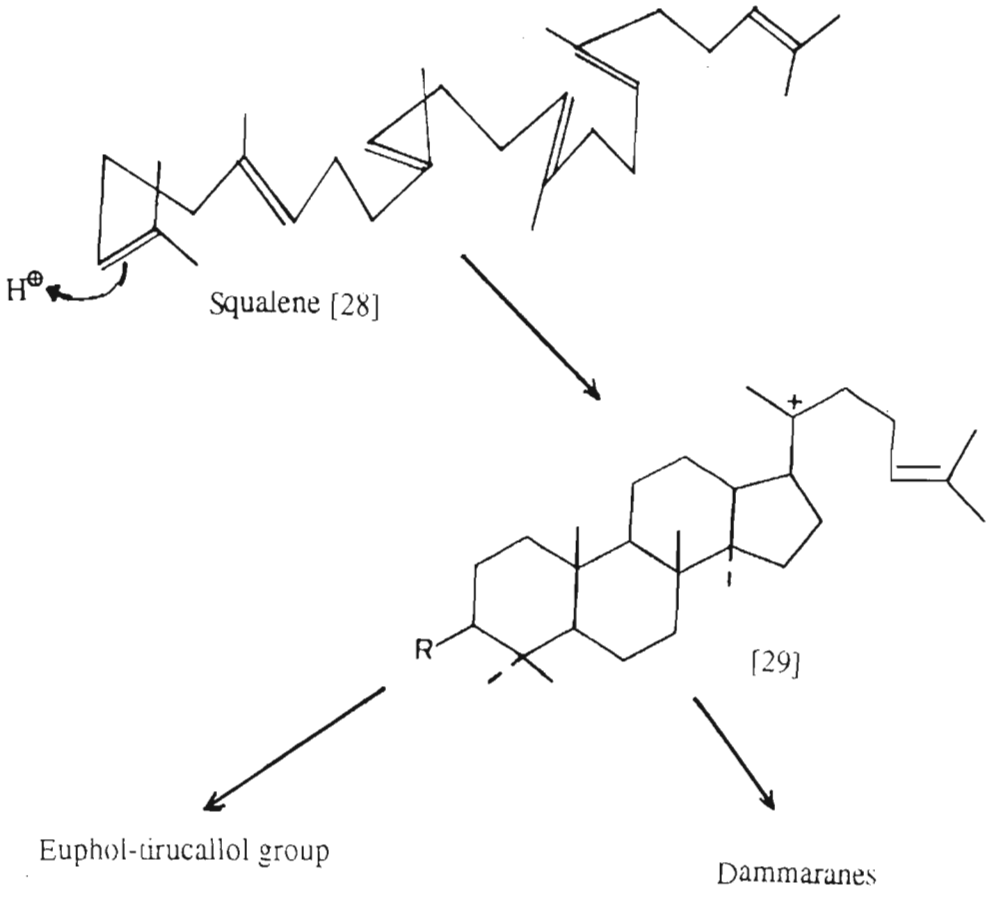
3.1 BIOGENESIS OF TRITERPENES AND PROTOLIMONOIDS

It has been proposed[18] that the structure and configuration of each basic triterpene skeleton is derivable, according to the principles of the Biogenetic Isoprene Rule,by cyclization of all-*trans* squalene folded in a specific conformation.

Cyclisation of *trans*-squalene in the chair-chair-chair-boat sequence leads to the formation of two groups of triterpenes.

Group A

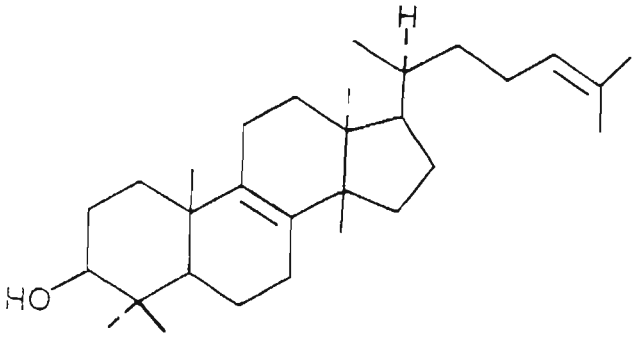
Attack by $H^{\oplus}$ on squalene in the chair-chair-chair-boat conformation leads to the cation [29] as shown in Scheme I.



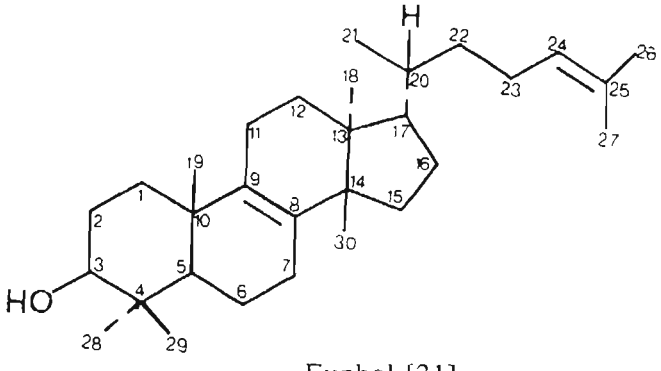
Scheme 5: Chair-chair-chair-boat sequence (A)

Cation [29], without rearrangement leads to members of the dammarane group.

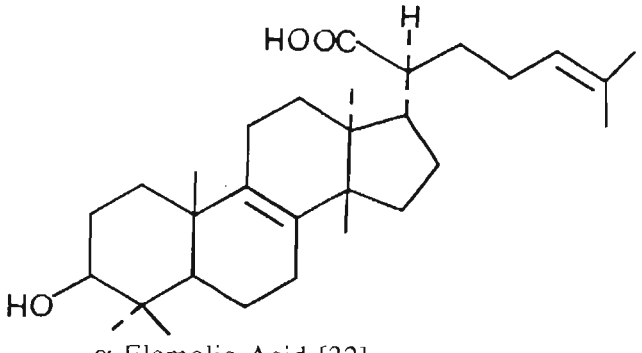
Rearrangement of cation [29] leads to members of the euphol-tirucallol group. Tirucallol [30] and euphol [31] and differ only in configuration at C-20.



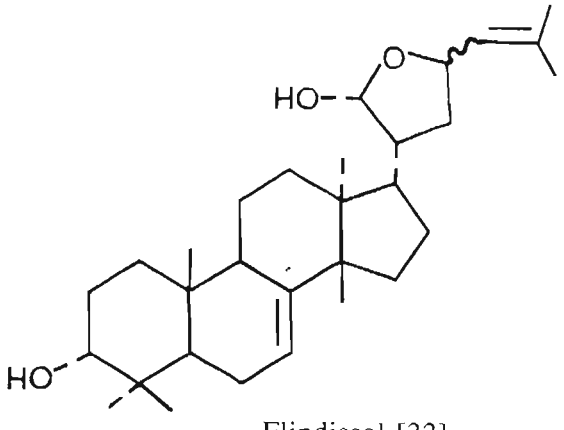
Tirucallol [30]



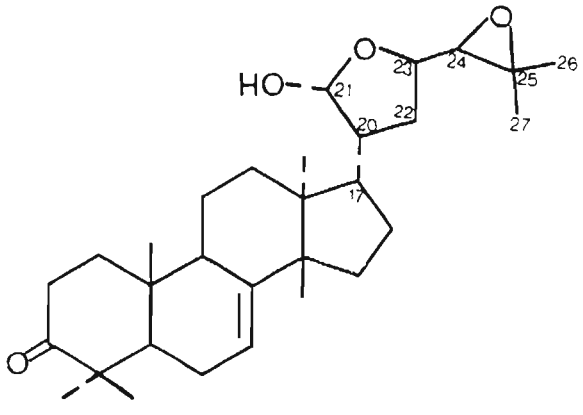
Euphol [31]



α-Elemolic Acid [32]

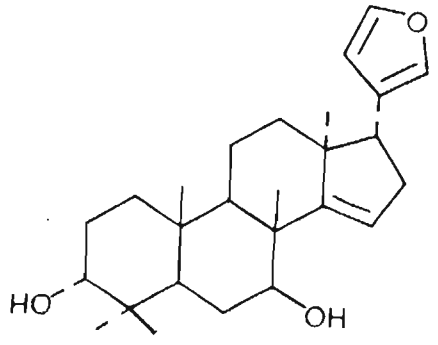


Flindissol [33]



Melianone [34]

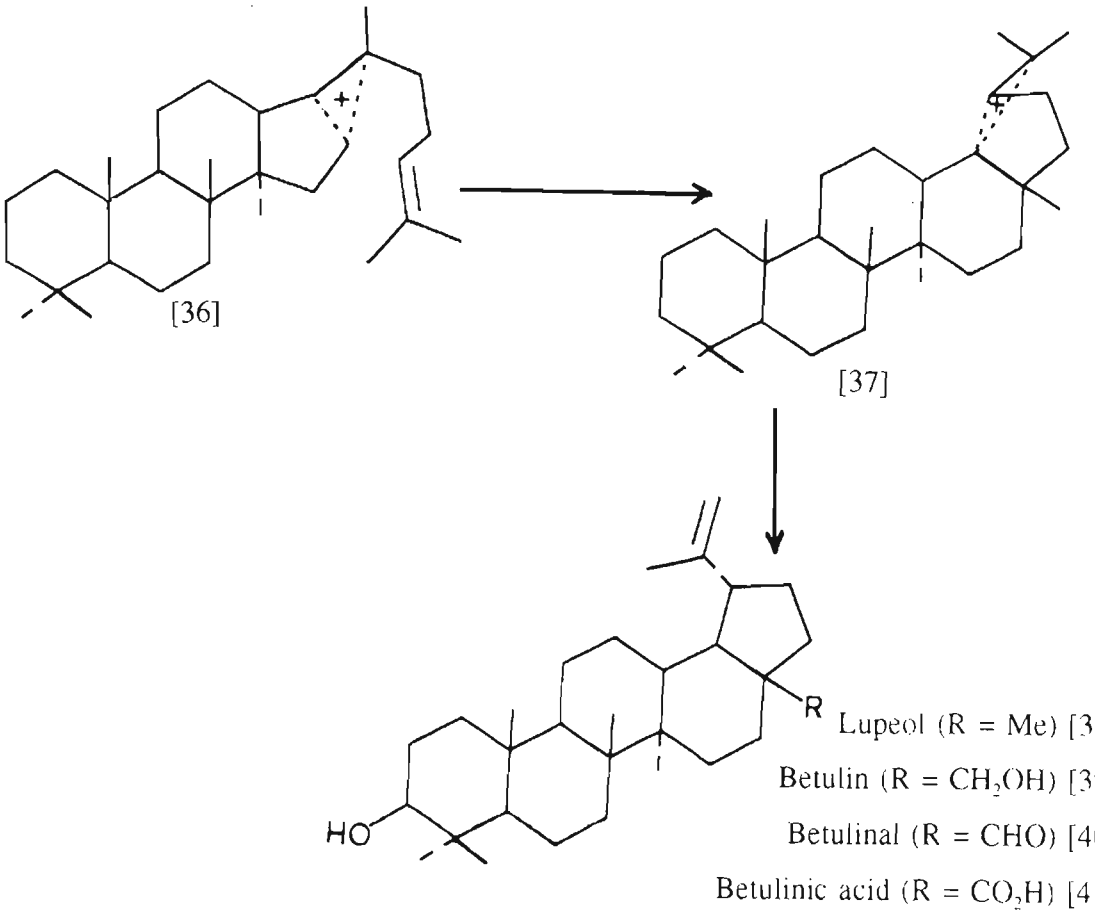
Removal of the terminal four carbon atoms of the side-chain, migration of the C-14 methyl group to C-8 (concerted with oxidative attack at C-7) and proton from C-15 furnishes the intermediate [35] which leads to limonoid compounds.



[35]

Group B

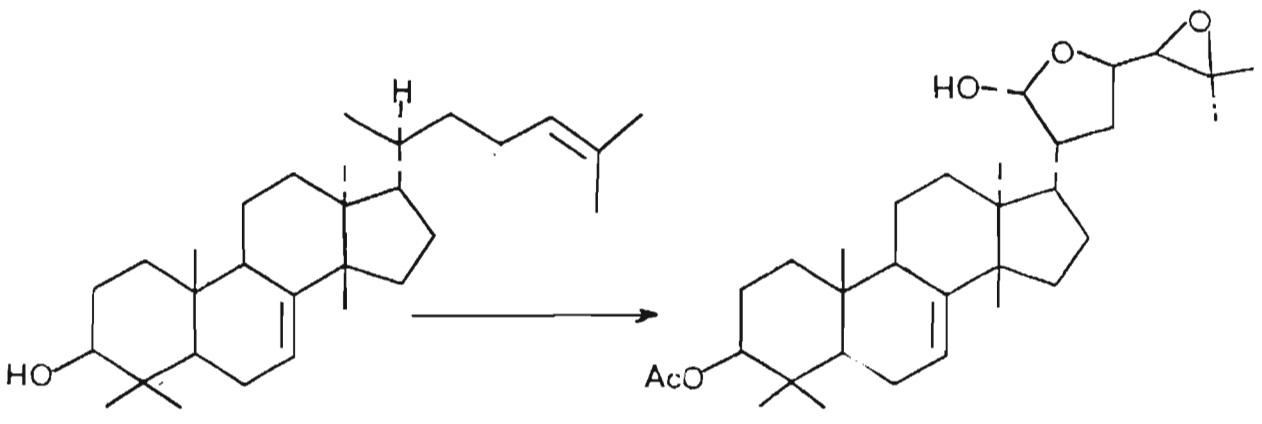
Conversion of the initially formed ion (Scheme 2) leads directly to the lupane group, exemplified by lupeol [38], betulin [39], betulinal [40] and betulinic acid [41].



Scheme 6: Chair-chair-chair-boat sequence (B)

Limonoids have been classified by Taylor[19] according to which of the four carbocyclic rings have been oxidatively opened. The precursors of limonoids, the protolimonoids, are usually included in the system.

Limonoids appear to arise from the euphane-tirucallane group of triterpenes. The hypothetical triterpene precursor [42][20] is oxidised in stages to a protolimonoid such as turraeanthin [43]. This undergoes further changes to give true limonoids.



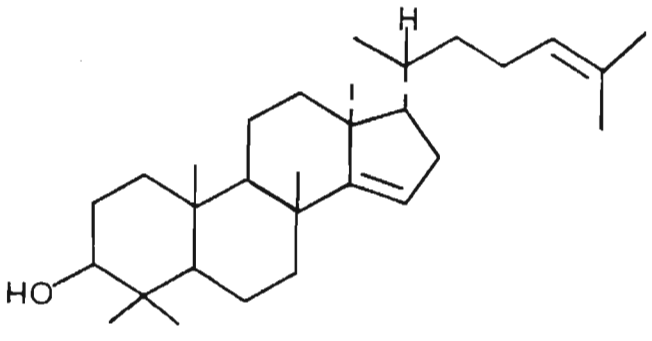
Hypothetical triterpene Precursor [42]

Turraeanthin [43]

Limonoids

Scheme 7:

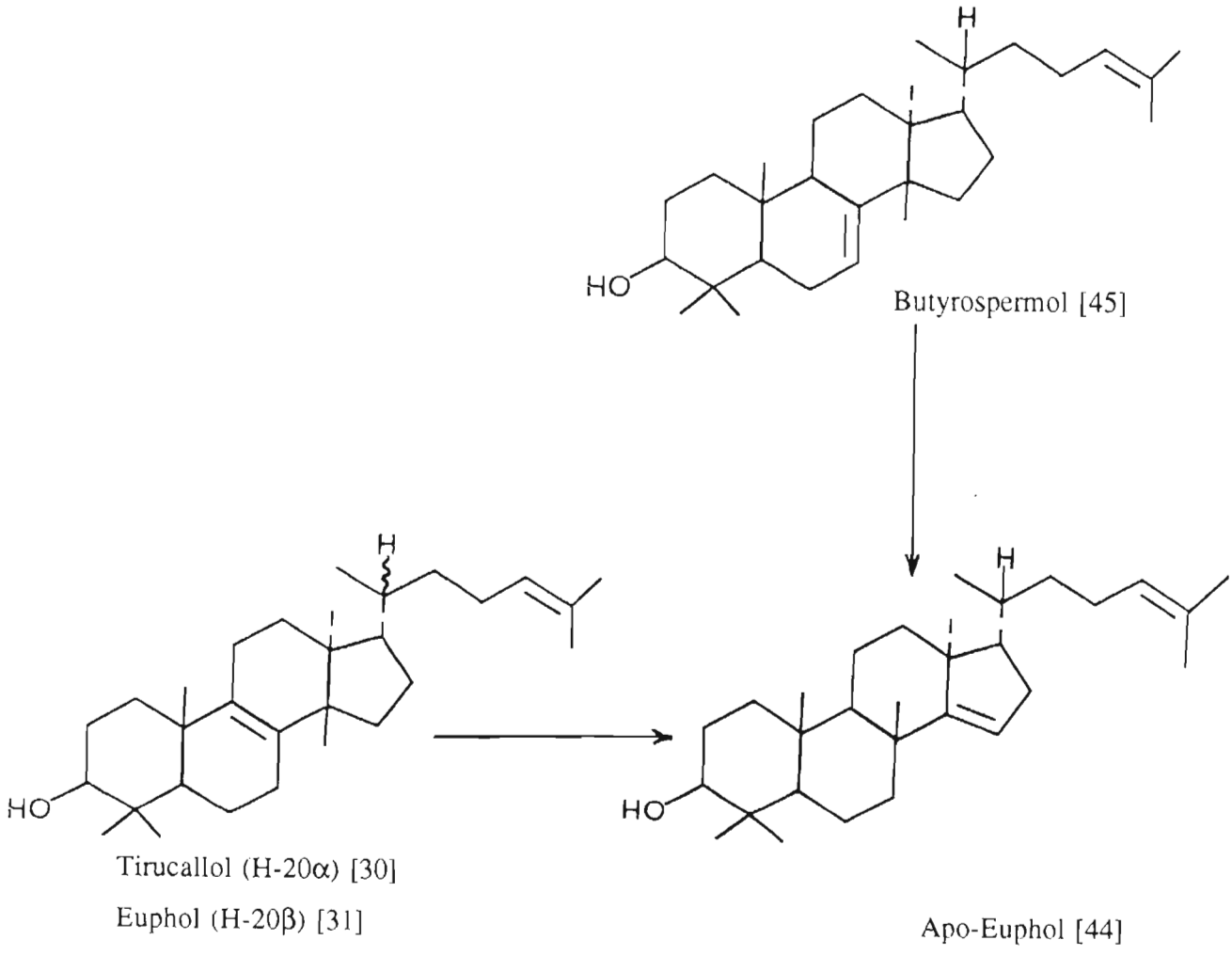
It has been suggested[21] that protolimonoids and limonoids were derived from a compound with the so-called apo-euphol structure [44].



Apo-euphol [44]

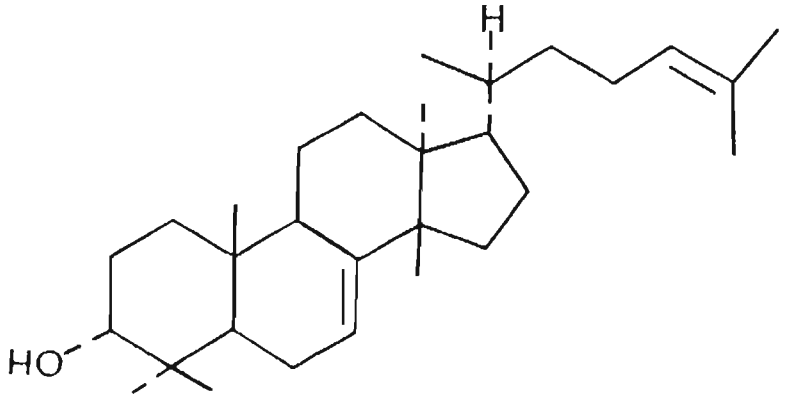
THE APO-EUPHOL REARRANGEMENT

It was originally suggested[21,22] that apo-euphol [44] could be biosynthesized either from tirucallol [30] or from euphol [31] or its Δ^7 isomer, butyrospermol [45], by the migration of the C-14 methyl group to C-8.[23,24]



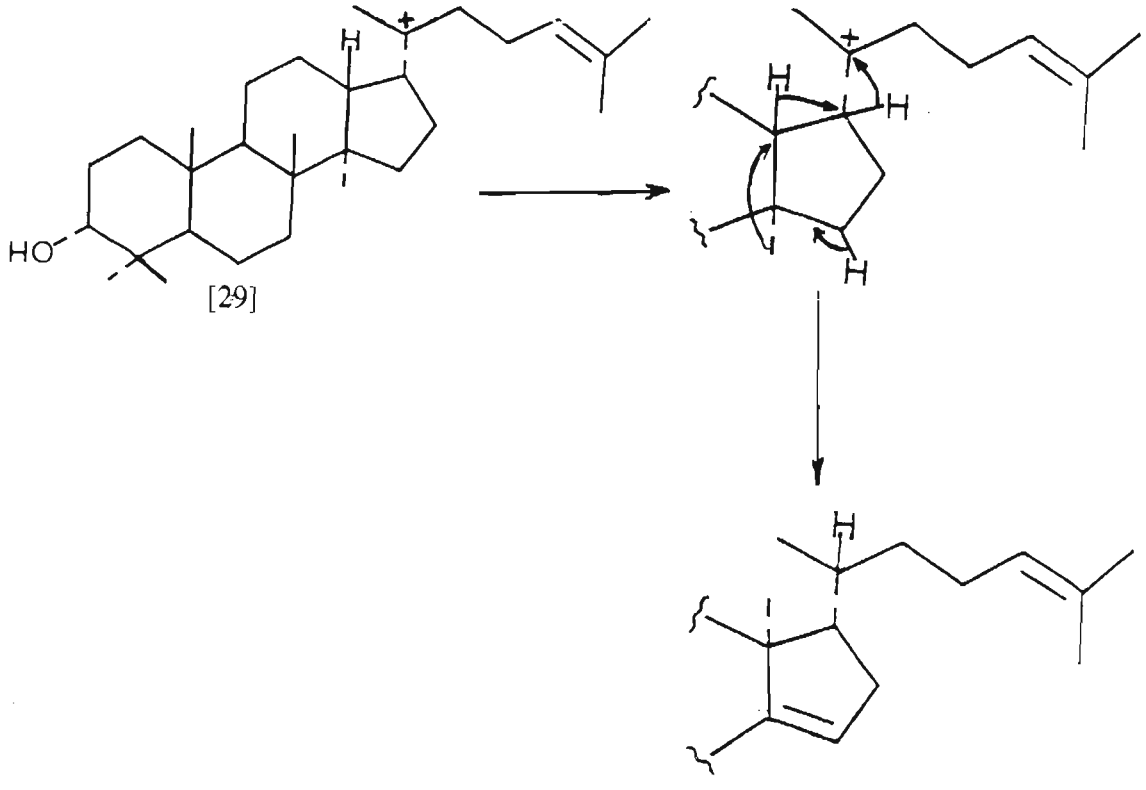
Scheme 8: **Formation of the apo-euphol structure from euphol, butyrospermol, tirucallol**

Tirucalla-7,24-dien-3-ol (possibly the 3α-ol isomer [42]) has been suggested as being the possible precursor of the protolimonoids.[22]



Tirucalla-7,24-dien-3α-ol [42]

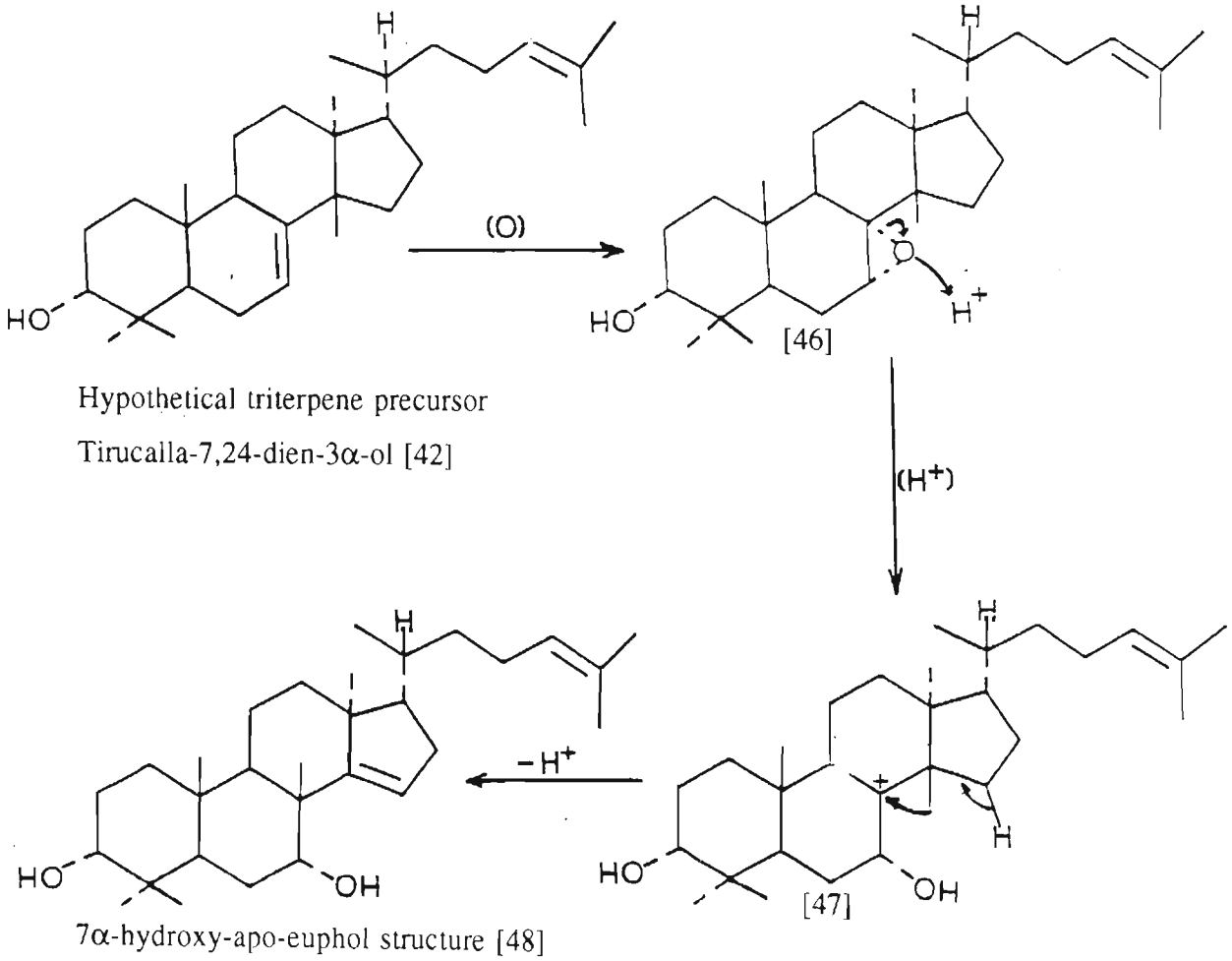
It has also been suggested[22,25] that the apo-euphol structure could be derived directly from squalene via the dammarane ion [29].



Scheme 9: Formation of the apo-euphol structure from the dammarane ion.

There are two classes of protolimonoids: the first, like euphol, has a double bond at Δ^7 and a β-methyl group at C-14; the second, the so-called apo-group, has a α-hydroxyl group at C-7, the double bond at Δ^{14} and a β-methyl group at C-8.[19] The side chains appear to be the same in both groups of protolimonoids as there is apparently no specific stage at which the apo-change occurs.

Spring *et al*[26] suggested that the C-14 methyl group migration could be brought about by an oxidative rearrangement. This oxidative rearrangement has been reproduced experimentally[27,28] by oxidation of the Δ^7 double bond to form a 7-α-epoxide. The change in the apo-group of protolimonoids is due to the opening of the 7-α-epoxide. [22,27,28,29,30,31].



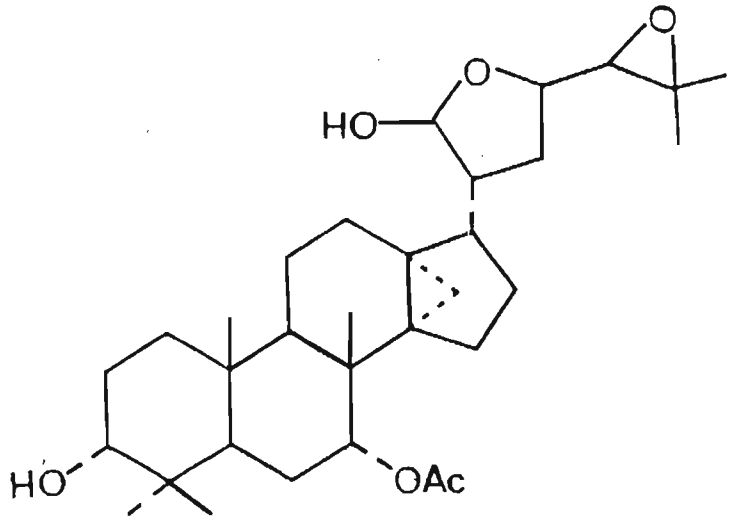
Hypothetical triterpene precursor
Tirucalla-7,24-dien-3α-ol [42]

7α-hydroxy-apo-euphol structure [48]

Scheme 10: Hypothetical formation of the apo-structure

Glabretal [49][32] occupies an intermediate position between the two classes of protolimonoids as it has the 7α-hydroxyl group and the 8β-methyl, but the 14,15-double bond is replaced by a 13,14-cyclopropane ring.

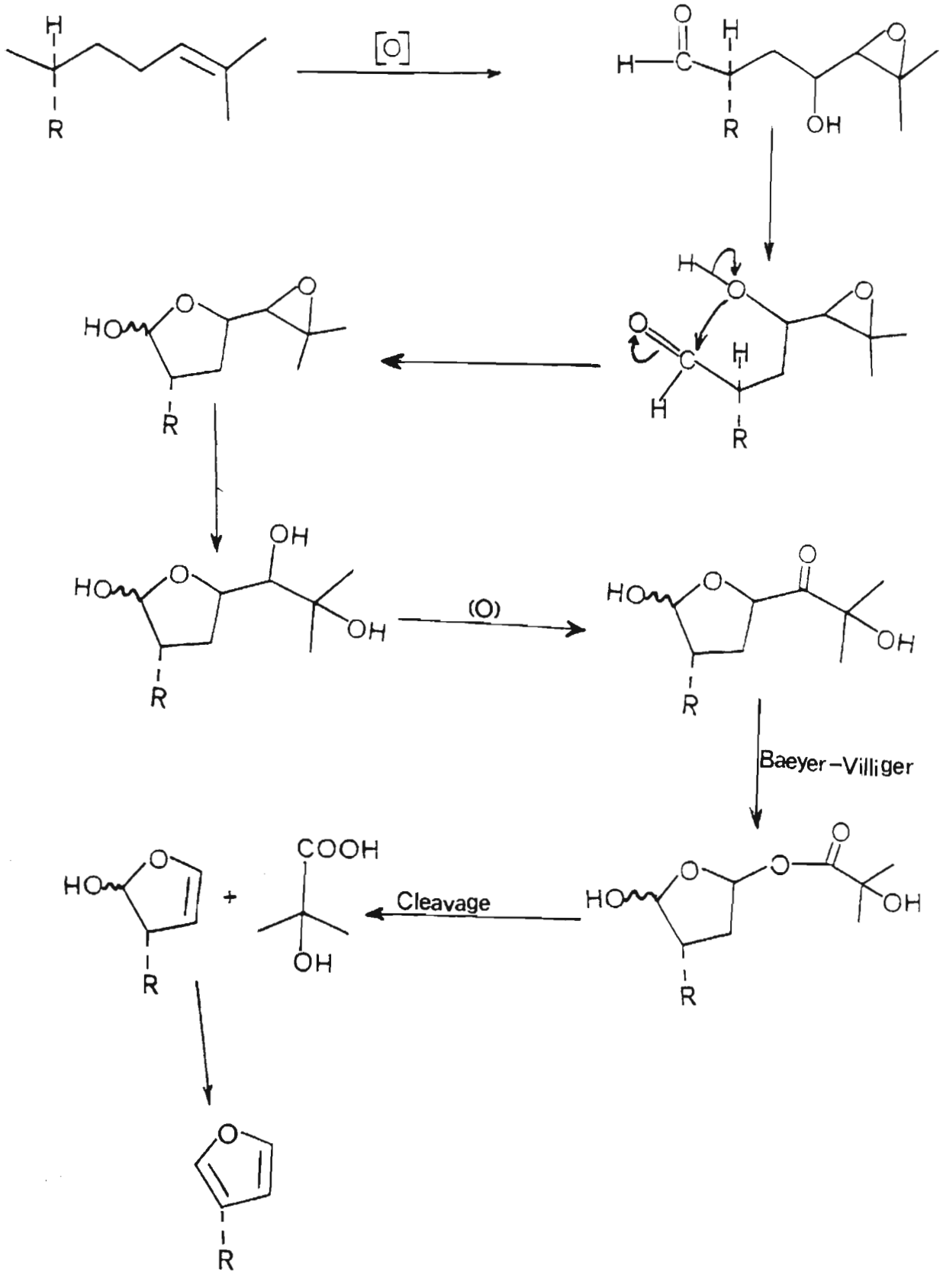
A carbonium ion was suggested,[19] which could either lose a proton at C-15 or capture the C-13 angular methyl, in which case the latter reaction would presumably be enzymatically determined. The cyclopropane ring might possibly be a normal intermediate in the apo-change.[19]



Glabretal [49]

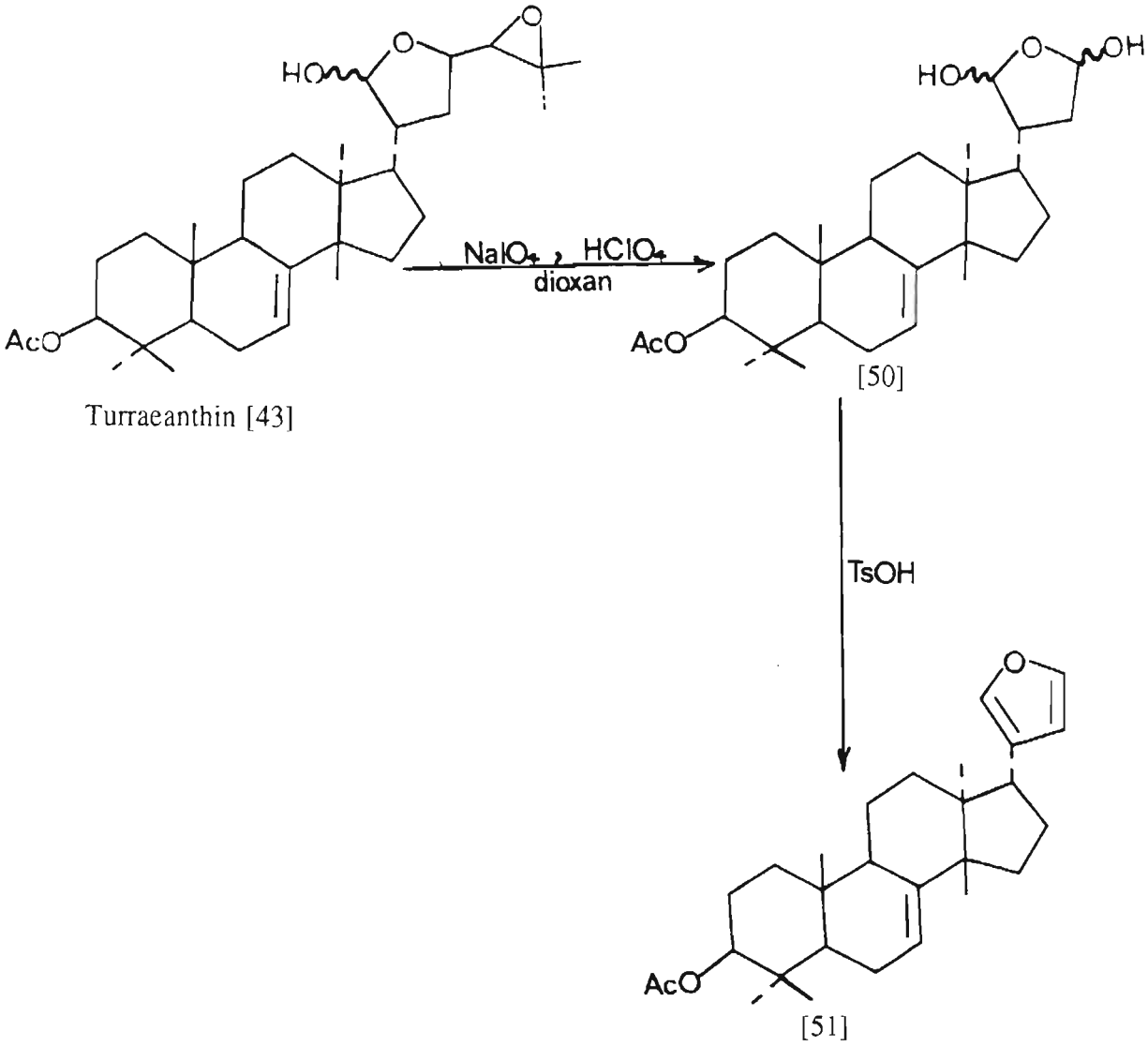
Furan ring formation

The stage at which the oxidation of the terpenoid side-chain occurs is not yet known. It was proposed[32] that the simple tirucallol side chain is oxidised in stages to produce an aldehyde group at C-21, a hydroxyl group at C-23 and an epoxide in place of the C-24:C-25 double bond. The hydroxyl and the carbonyl groups then cyclise to form the hemi-acetal ring in the turraeanthin side chain. Further oxidation may then lead to the furan ring being formed.



Scheme 11: Furan ring formation (A)

Reactions leading to the furan ring (from turraeanthin [43] side chain) have been performed in the laboratory.[27,28] Turraeanthin [43], treated with sodium metaperiodate in aqueous dioxan containing a trace of perchloric acid gave a product which was mainly the labile cyclic hemi-acetal [50]. Treatment of this with toluene-p-sulphonic acid in benzene gave the β-substituted furan ring [51].



Turraeanthin [43]

[50]

[51]

Scheme 12: Furan ring formation (B)

The apo-euphol rearrangement was also performed on turraeanthin [43][27,28] (see Scheme 10).

3.2 PROTOLIMONOIDS FROM *AGLAIA FERRUGINAEA*

The powdered heartwood of the Australian tree *Aglaia ferruginaea* was extracted with refluxing hexane. Two new compounds which have not been reported before, named compound F and compound G, were isolated from the hexane extract.

The structure of compound F

The accurate molar mass of compound F was found to be 530.3573 g mol^{-1} corresponding to the molecular formula $C_{32}H_{50}O_6$.

The molecular formula indicated 8 double bond equivalents. The ^{13}C-nmr spectrum (p. 114) indicated one carbonyl group (δ171.04(s)) which on comparison with the ^{1}H-nmr spectrum (p. 113) was found to be due to an acetate group. Since no alkene double bonds were present, this indicated that the compound had seven rings.

The mass spectrum of compound F showed peaks at m/e 512 (M^+-18), 470 (M^+-60), 452 (M^+-60-18), 399 (M^+-60-71) and 381 (M^+-18-60-71).

The loss of the fragment of 71 $gmol^{-1}$ is typical of compounds having the melianone-type side-chain.[19,25] This was confirmed by the ^{13}C- and ^{1}H-nmr spectra.

The ^{13}C-nmr spectrum of compound F showed that compound F consisted of a mixture of two stereoisomers, hence the pairing of some resonance peaks in both the ^{13}C- and ^{1}H-nmr spectra. It was possible to identify which peaks were paired as the two isomers were not present in equal proportions. The δ value for the minor isomer peak is given below in brackets.

The resonance at δ102.09 (δ98.16)(d) in the ^{13}C-nmr spectra was due to the carbon atom joined to two oxygen atoms. This is typical of C-21 in a melianone-type compound.

The doublets at δ78.39 (δ77.21), δ78.07 and δ74.31 (δ74.18) were due to three carbon atoms joined to oxygen atoms.

The ^{13}C-nmr spectrum also showed a pair of carbon resonances at δ67.66(d) (δ65.32(d)) and δ58.08(s) (δ57.31(s)) confirming the presence of an epoxide ring in a melianone-type side-chain.

The main features of the ^{1}H-nmr spectrum of compound F were a doublet at δ5.45 (1H), a triplet at δ4.65 (1H), a multiplet at δ3.9 (1H), a resonance at δ3.75 (1H), a broad resonance at δ3.65, a doublet at δ2.88 (δ2.71) (1H) and an intense singlet at δ2.09 (3H).

The ^{1}H-nmr spectrum also showed two resonances at δ0.48 (1H) and δ0.71 (1H) due to methylene protons in a cyclopropane ring.[33] The sharp resonance peak at δ2.09 (3H, s) confirmed the presence of an acetate group.[33]

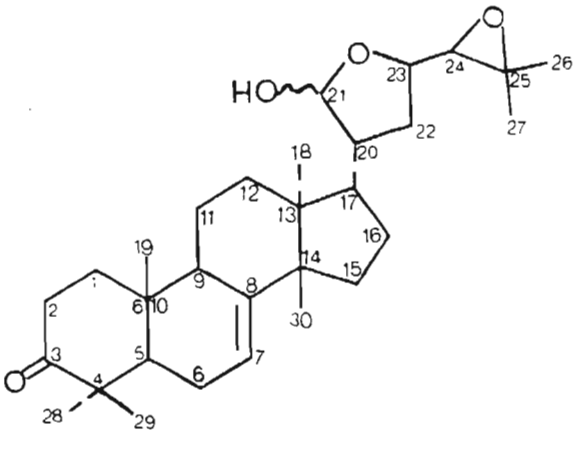
The Hetcor spectrum (p. 117) showed that the proton resonance at δ5.45 (1H, d) corresponded to the carbon resonance at δ102.9 (δ98.16)(d). This is ascribable to H-21.

The proton resonance at δ4.65 (1H, t) and δ3.75 (1H) corresponded to the carbon resonances at δ77.21(d) and δ74.18(d) respectively.

The doublet at δ2.88 (δ2.71) (1H) in the ^{1}H-nmr spectrum corresponded to the carbon resonance at δ67.66 (δ65.32)(d). This is typical of H-24 in a melianone-type side-chain.

Two resonances at δ0.48 (1H) and δ0.71 (1H) in the ^{1}H-nmr spectrum due to cyclopropane methylene protons, corresponded to the carbon resonance at δ13.7(t).

The Cosy spectrum (p. 116) showed that the multiplet at δ3.9 (1H) was coupled to the doublet at δ2.88 (δ2.71) (1H). These resonances were ascribable to H-23 and H-24 respectively.



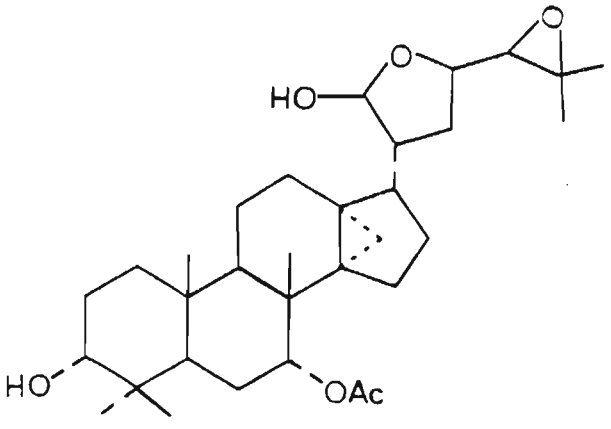
Melianone [34]

Table 7

Proton	Compound F (δ) [44]	Melianone (δ) [28]
H-21	5.45	5.38
H-23	3.9	3.90
H-24	2.88 (2.71)	2.90
3H-26	1.31	1.30
3H-27	1.31	1.30

Table 7 shows how the proton resonances due to the side-chain in compound F compared with those of melianone [34].

The molecular formula $C_{32}H_{50}O_6$, the presence of the melianone side-chain, the cyclopropane ring, an acetate group and the presence of seven rings suggested a compound similar to glabretal [49].[32]

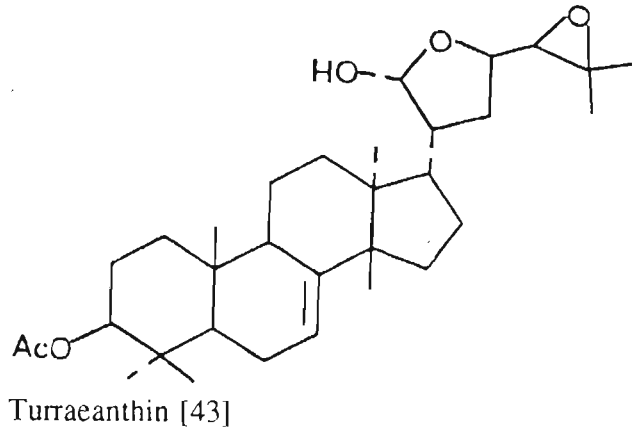


Glabretal [49]

The ^{1}H-nmr spectrum of glabretal [49] showed resonances at δ3.46 (H-3β) and δ5.04 (H-6β) indicative of a 3α-hydroxyl group and a 7α-acetate group respectively.

Compound F was acetylated and its ^{1}H-nmr spectrum compared with that of glabretal [49]. The ^{1}H-nmr spectrum of compound F-acetate gave a resonance peak in the same position as that due to an H-7β of glabretal. Compound F yielded a triacetate with peaks at δ2.09, δ2.08 and δ2.06. H-7β shifted on acetylation from δ3,75 to the same position as in glabretal [49][32].

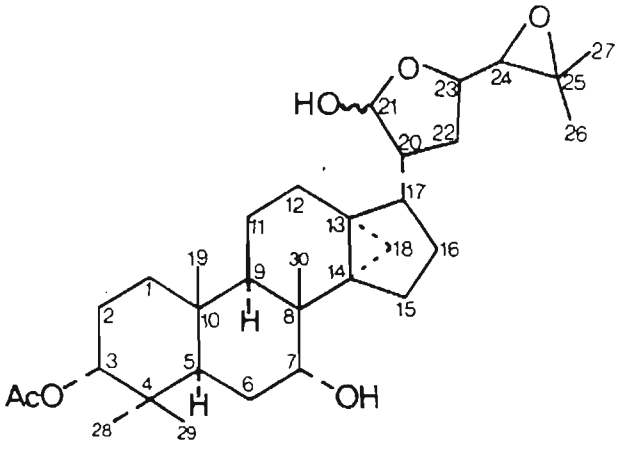
Using literature[34] values for H-3β and H-3α in 3α- and 3β-acetoxy-turraeanthin isomers, the stereochemistry at position 3 of compound F could be deduced from the ^{1}H-nmr spectrum.



Turraeanthin [43]

The two turraeanthin isomers can be differentiated using the H-3 resonances, firstly, since the 3β proton resonates further downfield than the 3α proton (δ4.7 as opposed to 4.52), and secondly, since the bandwidth of the 3α proton resonance is larger than that of the 3β proton (15 Hz as opposed to 7 Hz).

The H-3 proton resonance of compound F was in agreement with the data for a 3β proton, resonating at δ4.65 (bandwidth:12 Hz). Hence structure [52] was proposed for compound F.



Compound F [52]

The structure of compound G

The molar mass of compound G was found to be 570 g mol^{-1} suggesting the molecular formula $C_{35}H_{54}O_6$.

The infrared spectrum of compound G (p. 124) showed a strong absorption band at 3425 cm^{-1} due to hydroxyl groups. The strong band at 2925 cm^{-1} was due to C-H stretchings in CH_3 and CH_2 groups. The strong band at 1707 cm^{-1} suggested an α,β-unsaturated ester group. The weak band at 1645 was due to the C=C group. The absorption bands in the fingerprint region at 1260 cm^{-1} and 1040 cm^{-1} were due to an epoxide C-O stretching and a C-OH stretching respectively.

The mass spectrum (p. 122) showed peaks at m/e 552 (M^+-18), 534 (M^+-18-18), 470 (M^+-100) and 452 (M^+-18-100) g mol^{-1}.

The ^{13}C-nmr spectrum of compound G (p. 124) showed resonances at δ136.68(d) and δ129.22(s) indicative of a double bond.

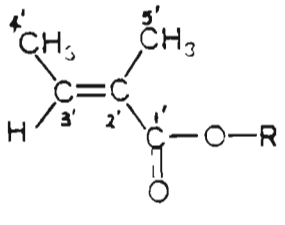
The ^{1}H-nmr spectrum of compound G (p. 123) indicated that compound G was very similar to compound F except for the presence of a quartet at 6.86 (1H) due to an alkene proton, a doublet at δ1.81 (3H) and a resonance at δ1.86 (3H).

The Cosy spectrum of compound G (p. 126) showed that the alkene proton resonance at δ6.86 (1H,q,J = 7.06 Hz) was coupled to the methyl proton resonance at δ1.81 (3H, d J = 7.06 Hz) and long range coupled to the methyl proton resonance at δ1.86 (3H, S) (J too small to be measured). This indicated the following grouping

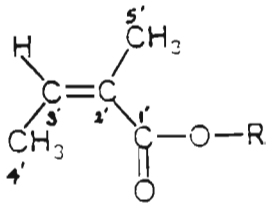
CH_3-CH=C-CH_3

The sharp resonance at 2.09 (3H, S) indicative of the 3α-acetate group in compound F was not present in the ^{1}H-nmr spectrum of compound G.

The resonances due to H-3β were in exactly the same positions in the ^{1}H-nmr spectra of both compounds. This suggested that the compounds differed on the 3α-ester substituent. The presence of the group CH_3-CH=C-CH_3 and an ester group suggested the following esters:



Angelate ester



Tiglate ester

A comparison of literature[35] values of ^{13}C-nmr (Table 8a) and ^{1}H-nmr (Table 8b) with experimental values of the tiglate ester showed that the ester present was the tiglate one.

Table 8a

Carbon	Experimental (δ)	Lit[35] value (δ)
C-1′	167.67(s)	169.5 (169.8)*
C-2′	129.09(s)	129.5 (129.1)+
C-3′	136.68(d)	137.0
C-4′	14.45(q)	14.5
C-5′	12.20(q)	12.2

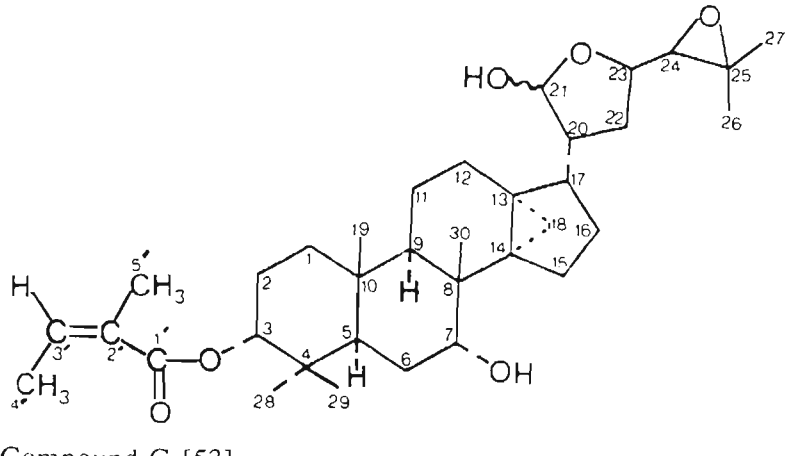
*,+ The two C-1′ and C-2′ literature[35] values given each carbon may be interchanged.

Table 8b

Proton	Experimental		Literature[35] values	
H-3′	δ6.86(q)	J = 7.06 Hz	δ6.89(q)	$J_{3',4'}$ = 7.30 Hz
H-4′	δ1.81(d)	J = 7.06 Hz	δ1.89(d)	$J_{4',3'}$ = 7.30 Hz
H-5′	δ1.86(s)		δ1.93(d)	$J_{5',3'}$ = 1.51 Hz

Ferguson and co-workers[32] isolated a compound from the heartwood of *Guarea glabra* similar to compound G with a 7α-acetate group instead of the 7α-hydroxyl group but also having a tiglate ester at C-3.

Compound F and compound G have not been isolated previously



Compound G [53]

3.3 LUPEOL : EXTRACTIVE FROM *EKEBERGIA CAPENSIS*

Compound H was isolated from the hexane extract of the leaves of *E. capensis*. The melting point of compound H was found to be in the range 215-217^{0}C.

The mass spectrum of compound H (p. 132) showed an M^+ peak at m/e 426 g mol^{-1} suggesting a molecular formula $C_{30}H_{50}O$.

The ^{1}H-nmr spectrum of compound H (p. 133) showed 6 methyl proton resonances at δ0.759, δ0.787, δ0.828, δ0.944, δ0.966 and δ1.029. The multiplet at δ3.25 (1H) was due to a proton attached to a carbon atom joined to an oxygen atom (H-C-O).[33] The resonance at 3.62 was due to a hydroxyl proton and was indicated by its disappearance on addition of D_2O. This accounted for the only oxygen atom in the molecular formula.

A terminal methylene group was indicated by the resonances at δ4.69 (1H, d, J = 2.4 Hz) and δ4.57 (1H, d, J = 2.4 Hz) due to the non-equivalent geminal protons on the terminal methylene group. The coupling constant, J = 2.4 Hz, is typical of the splitting of geminal protons.[33]

The ^{13}C-nmr spectrum (p. 134) indicated 30 carbon atoms. The ^{13}C Adept spectrum showed seven quartets (δ14.55, δ15.40, δ15.99, δ16.14, δ18.02, δ19.33 and δ28.04); eleven triplets (δ18.33, δ20.94, δ25.15, δ27.43, δ27.48, δ29.88, δ34.33, δ35.64, δ38.77, δ40.07 and δ109.63); six doublets (δ38.11, δ48.09, δ48.38, δ50.52, δ55.40 and δ79.20) and six singlets (δ37.25, δ40.92, δ42.93, δ43.11, δ79.30 and δ151.40).

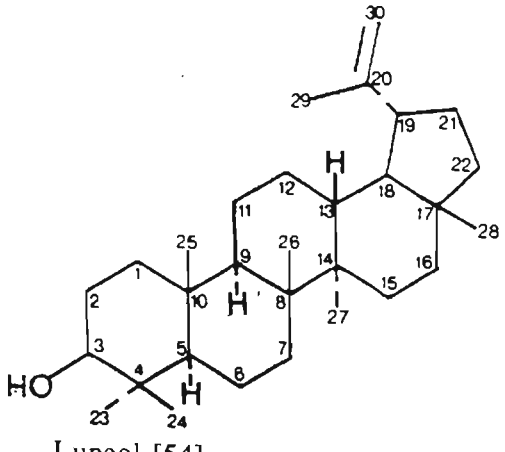
The presence of a terminal methylene group was confirmed by the ^{13}C-nmr spectrum which gave a singlet at δ151.4 and a triplet at δ109.6. The resonance at δ79.2 confirmed the presence of a hydroxyl group.

The molecular formula $C_{30}H_{50}O$ indicated six double bond equivalents. The presence of one alkene double bond indicated the presence of 5 rings.

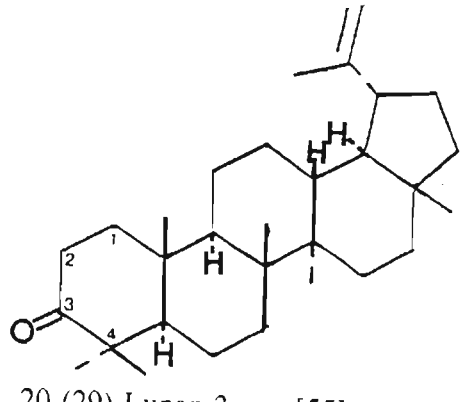
A literature[4] survey was conducted and several compounds with a molecular formula $C_{30}H_{50}O$ were found. The molecular formula $C_{30}H_{50}O$, the melting point of 215-217^{0}C, the presence of 5 rings, the presence of a hydroxyl group and a terminal methylene group suggested that compound H was lupeol (20-(29)-lupen-3-ol) [54]. From the literature all carbon resonances were assigned as in Table 9 (p. 61).

Lupeol [54] was oxidised using chromium trioxide to give 20-(29)-lupen-3-one. The ^{13}C-nmr spectrum of 20-(29)-lupen-3-one (p. 139) showed a resonance at δ218.2(s). This resonance was ascribable to the keto-group at C-3.

The resonances at δ3.62 (hydroxyl proton) and δ3.25 (H-3), in the ^{1}H-nmr spectrum of lupeol (p. 133), were missing in the ^{1}H-nmr spectrum of 20-(29)-lupen-3-one (p. 138). The resonance at δ79.2(d) had disappeared.



Lupeol [54]



20-(29)-Lupen-3-one [55]

CHAPTER 4

4 EXPERIMENTAL

4.1 GENERAL

Melting points of crystalline compounds were determined on the Kofler micro hot-stage melting point apparatus and are uncorrected.

Infrared Spectra were recorded from KBr discs with a Shimadzu IR-408 spectrophotometer.

High resolution mass spectrometric data and accurate masses were recorded at the Cape Town Technikon by Dr Boshof on photosensitive paper. These spectra have not been reproduced in this thesis. The mass spectra in chapter 5 were recorded at the University of Natal, Durban, on a Finnigan 1020 Automated GC/MS.

^{1}H and ^{13}C-nmr spectra were recorded at the University of Natal, Pietermaritzburg, on a Varian Gemini 200 MHz spectrometer. All spectra were recorded in deuteriochloroform except those for the flavonoids which were recorded in acetone-d_6 ((-)-epicatechin) and in DMSO (kaempferol 3-O-glucoside). The δ-values (ppm) are relative to TMS (δ=0).

Progress of all reactions and column chromatographic separations was monitored by thin-layer chromatography on silica gel.

4.2 CHROMATOGRAPHIC TECHNIQUES

4.2.1 THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (t.l.c.) was conducted using pre-coated 0.2 mm thick aluminium-backed silica gel 60 (Merck. Art. 5553). The most commonly used solvent systems were hexane:ethyl acetate (60:40) and methylene chloride:ethyl acetate

However, various other ratios of these solvents were employed depending on the polarity required.

The spots on the plates were visualised by spraying with the spray reagent comprising anisaldehyde:conc. sulphuric acid:methanol in a ratio of 1.25:2.5:96.25. Coloured spots were formed after the plates had been heated at 110^{0}C for one to two minutes.

4.2.2 COLUMN CHROMATOGRAPHY

Four methods of column chromatography were used:

a) With some extracts, a rough separation was first obtained by using a gravity-column packed with Merck. Art. 7733 course silica gel. Most of the chlorophyll was removed using this type of column chromatography.

b) The second type involved a gravity-column packed with Merck. Art. 9385 (0.040-0.063 mm particle size) silica gel.

c) Flash-column chromatography using columns packed with Merck. Art. 9385 silica gel were employed. This was the most commonly used column chromatographic technique.

d) A gravity-column packed with fine Merck. Art. 7729 silica gel was also used for those compounds whose spots on analytical t.l.c. were close together.

The solvent system was generally a mixture of hexane and ethyl acetate or methylene chloride and ethyl acetate, the ratios of which were chosen to give the desired compound(s) an R_f value of approximately 0.35[36] for flash-columns and approximately 0.50 for gravity-columns, when tested by t.l.c.

4.2.3 PREPARATIVE LAYER CHROMATOGRAPHY (P.L.C.)

P.l.c. was performed using 20 x 20 cm glass plates pre-coated to a thickness of 2 mm with Merck. Art. 5745 silica gel. The solvent systems used were mixtures of hexane and ethyl acetate. Narrow strips on either sides of the plates were sprayed with the anisaldehyde reagent and heated to reveal the individual bands, which were then scraped off and extracted with methylene chloride. This technique was not found to be successful because of the similarities of the R_f values of some compounds.

4.3 EXTRACTIVE FROM *TRICHILIA DREGEANA*

4.3.1 THE EXTRACTION OF THE LEAVES OF *T. DREGEANA*

Dried leaves of *T. dregeana* (358 g) were extracted for 24 hours with refluxing hexane in a Soxhlet extractor. Analytical t.l.c. indicated the presence of several compounds. Preliminary gravity-column chromatography over course silica gel removed most of the chlorophyll. Repeated flash column chromatography yielded a pure crystalline compound, 13-epimanoyl oxide (~100 mg). All eluent fractions were monitored by analytical t.l.c.

<u>13-Epimanoyl oxide</u>

Yield : 12 mg, 0.028%

Melting point : 98-100°C

Mass spectrum : M^+= 290 g mol^{-1}; $C_{20}H_{34}O$

Infrared spectrum : ν_{max} 1645 (olefinic grouping), 1190 (ether group) 975, 940, 100 (CH_2 = CHR) cm^{-1}

^{1}H-nmr spectrum:

δ0.66 (3H, s, 3H-20); 0.72 (3H, s, 3H-19); 0.79 (3H, s, 3H-18); 1.06 (3H, s, 3H-16); 1.15 (3H, s, 3H-17); 2.14 (2H, m, H-12); 4.83 (2H, t, H-15); 5.95 (1H, q, H-14)

C/H Analysis: Found : C : 82.67% H : 11.82%

Calculated for : C : 82.40 H : 11.89%

^{13}C-nmr spectrum: The data obtained from the ^{13}C-nmr spectrum of 13-epimanoyl is presented in Table 1 (p. 56).

4.4 EXTRACTIVE FROM *APHANAMIXIS POLYSTACHA*

4.4.1 EXTRACTION OF THE LEAVES OF *A. POLYSTACHA*

The leaves of *A. polystacha* were dried in the oven at 70°C for 20 minutes and left overnight at 40°C. The dried leaves (3519 g) were finely ground using a coffee grinder and extracted for 24 hours with refluxing hexane in a Soxhlet extractor. Gravity-column chromatography was performed using coarse silica gel to remove the chlorophyll. Flash-column chromatography yielded a pure white crystalline compound (265 mg).

Aphanamixol

Yield : 265 mg, 0.075%

Melting point : 124-127°C

Mass spectrum : M^+= 308 g mol^{-1}; $C_{20}H_{36}O_2$

C/H Analysis: Found : C : 82.67% H : 11.82%

Calculated for : C : 82.40 H : 11.89%

Infrared spectrum : ν_{max} 3300 (OH); 1675 (tertiary methyl group); 1465, 1395 (gem-dimethyl groups) cm^{-1}

^{1}H-nmr spectrum:

δ0.81 (6H, s, H-18, 19); 0.89 (3H, s, H-20); 1.15 (3H, s, H-17); 1.71 (3H, s, H-16); 3.49 (OH); 4.16 (2H, d, J = 7.0 Hz, H-15); 5.46 (1H, t, J = 7.0 Hz, H-14)

^{13}C-nmr spectrum: The data obtained from the ^{13}C-nmr spectrum of aphanamixol are presented in Table 2 (p. 57).

4.5 EXTRACTIVE FROM *CEDRELA ODORATA*

4.5.1 EXTRACTION OF THE BARK OF *C. ODORATA*

The powdered bark of *C. odorata* (780 g) was milled and firstly extracted with refluxing hexane and then with refluxing methanol. A red-brown viscous methanol extract was obtained. Repeated flash-column chromatography yielded a pure red gum, (-)-epicatechin (110 mg).

(-)-Epicatechin

Yield	:	110 mg, 0.014%
Mass spectrum	:	M^+290 g mol^{-1}; $C_{15}H_{14}O_6$
Infrared spectrum	:	ν_{max} 3400 OH; 1610 (aromatic ring); 2900 (aliphatic C-H) cm^{-1}
UV spectrum:		λ_{max} 229.1, 279.9 nm

^{1}H-nmr spectrum:

2.56 (1H, m, H-4b); 2.94 (1H, m, H-4a); 4.30 (OH); 4.04 (1H, m, H-3); 4.60 (1H, d, J = 4.0 Hz, H-2); 5.91 (1H, d, J = 2.3 Hz, H-6); 6.06 (1H, d, J = 2.3 Hz, H-8); 6.79 (1H, d, J = 3.1 Hz, H-6′); 6.80 (1H, d, J=3.1 Hz, H-5′); 6.93 (1H, s, H-2′); δ8.3 (phenolic protons);

^{13}C-nmr spectrum: The data is presented in Table 3 (p. 58).

4.6 EXTRACTIVES FROM *EKEBERGIA CAPENSIS*

4.6.1 EXTRACTION OF THE LEAVES OF *E. CAPENSIS*

The leaves of *E. capensis* (376 g) were dried in the oven at 70^{0}C for 20 minutes and left overnight at 40^{0}C. The dried leaves were extracted first with refluxing hexane and then with refluxing methanol. Repeated column chromatography of the hexane extract yielded a pure compound, lupeol. Lupeol was crystallized from acetone (615 mg).

Water was added to the dry crude methanol extract. The aqueous solution was extracted with chloroform to remove chlorophyll. The solution was then extracted

with a 2:1 mixture of chloroform and acetone. Repeated flash column chromatography of the chloroform:acetone extract yielded a pure gum, kaempferol 3-O-glucoside (156 mg).

Lupeol

Yield : 615 mg, 0.16%

Melting point : 215-217^{0}C

Mass spectrum : M^+426 g mol^{-1}; $C_{30}H_{50}O$

^{1}H-nmr spectrum:

3.25 (1H, H-3); 3,62 (OH); 0.76 (3H, s, H-23); 0.79 (3H, s, H-28); 0.83 (3H, s, H-24); 0.94 (3H, s, H-27); 0.97 (3H, s, H-25);); 1.03 (3H, s, H-26); 1.68 (3H, s, H-29); 4.57 (1H, d, J = 2.4 Hz, H-30b); δ4.69 (1H, d, J = 2.4 Hz, H-30a)

^{13}C-nmr Spectroscopy: The data is presented in Table 9.

Kaempferol 3-O-glucoside

Yield : 156 mg, 0.041%

Mass spectrum : M^+448 g mol^{-1}; $C_{21}H_{20}O_{11}$

Infrared spectrum : ν_{max} 3400 OH; 2925 (intramolecular 'chelate' H-bonded OH group); 1650 (α,β unsaturated ketone); 1605, 1513 (aromatic ring) cm^{-1}

^{1}H-nmr spectrum:

4.03 (OH); 5.45 (1H, d, J = 7.2 Hz, H-1"); 6.23 (1H, d, J = 1.8 Hz, H-6); 6.46 (1H, d, J = 1.3 Hz, H-8); 6.90 (2H, d, J = 8.9 Hz, H-3′, 5′); 8.04 (2H, d, J = 8.9 Hz, H-2′, 6′); δ12.6 (5-OH); 10.3 (phenolic OH)

^{13}C-nmr spectrum: The data obtained from the ^{13}C-nmr spectrum of kaempferol 3-O-glucoside is presented in Table 4 (p. 59).

4.6.3 ACID HYDROLYSIS OF KAEMPFEROL 3-0-GLUCOSIDE

Kaempferol 3-O-glucoside (100 mg) was mixed with 6% aqueous hydrochloric acid (10 ml) using a minimum of methanol to effect complete solution. The solution was heated on a steam bath for one hour and then cooled and extracted by shaking with

ether (2 x 10 ml). The ether layer, after drying over sodium sulphate, yielded kaempferol on evaporation.

Kaempferol

Mass spectrum : M^+270 g mol^{-1}; $C_{15}H_{10}O_5$

UV spectrum : λ_{max} 221.2, 266.4, 366.6 nm

^{1}H-nmr spectrum:

6.18 (1H, d, J = 1.9 Hz, H-6); 6.43 (1H, d, J = 1.8 Hz, H-8); 6.91 (2H, d, J = 9.5 Hz, H-3′, 5′); 8.10 (2H, d, J = 9.2 Hz, H-2′, 6′); δ9.4 (phenolic OH)

4.6.4 CHROMIUM TRIOXIDE OXIDATION OF LUPEOL

Chromium trioxide (250 mg) was added to a magnetically stirred mixture of pyridine (10 cm^3) and methylene chloride (10 cm^3). The flask was fitted with a drying tube containing calcium chloride and stirring was continued for 15 minutes. A solution of lupeol (100 mg) in methylene chloride (15 cm^3) was added. The mixture was stirred for 12 hours at room temperature. The mixture was poured into water (15 cm^3) and the aqueous solution extracted with ether (3 x 10 cm^3). The organic fractions were combined and taken to dryness under pressure. 20(29)-Lupen-3-one was obtained.

(20)29-Lupen-3-oxide

Mass spectrum : M^+424 g mol^{-1}; $C_{30}H_{48}O$

UV spectrum : λ_{max} 238.0 nm

^{1}H-nmr spectrum:

0.87 (3H, s, H-28); 0.80 (3H, s, H-23); 0.93 (3H, s, H-24); 0.96 (3H, s, H-27); 1.02 (3H, s, H-25); 1.68 (3H, s, H-29); 1.05 (3H, s, H-26); 4.58 (1H, d, J = 2.4 Hz, H-30b); δ4.69 (1H, d, J = 2.4 Hz, H-30a)

^{13}C-nmr spectrum: The data is presented in Table 10 (p. 62).

4.7 EXTRACTIVES FROM *AGLAIA FERRUGINAEA*

4.7.1 EXTRACTION OF THE BARK OF *A. FERRUGINAEA*

The powdered bark of the Australian tree, *A. ferruginaea*, (85 g) was milled and extracted with hexane for 24 hours in a Soxhlet extractor. Analytical t.l.c. of the hexane extract indicated the presence of several compounds. Flash column chromatography of the hexane extract yielded a pure gum, named compound E (41 mg).

Compound E

Yield	:	41 mg, 0.048%
Mass	:	434.1713 g mol^{-1}; $C_{26}H_{26}O_6$
Mass spectrum	:	m/e 434, 400, 135, 181, 165
Infrared spectrum	:	ν_{max} 3424 (OH); 2925 (aliphatic C-H); 1615, 1515 (aromatic ring) cm^{-1}
UV spectrum	:	λ_{max} 238.7, 272.7 nm

^{1}H-nmr spectrum:

1.75 (OH); 2.2 (1H, m, H-11b); 3.34 (OH); 2.70 (1H, m, H-11a); 2.70 (1H, m, H-11a); 3.34 (OH); 3.71 (3H, s, 4"-OMe); 3.84 (3H, s, 7-OMe); 3.90 (3H, s 5"-OMe); 4.0 (1H, m, H-12); 4.81 (1H, d, J = 6.02 Hz, H-2); 6.67 (2H, d, J = 9.0 Hz, H-3", 5"); 6.14 (1H, d, J = 2.0 Hz, H-6); 6.29 (1H, d, J=2.0 Hz, H-8); δ7.12 (2H, d, J=9.0 Hz, H-2", 6");

^{13}C-nmr spectrum: The data from ^{13}C-nmr spectrum of compound E is presented in Table 6 (p. 60).

4.7.2 EXTRACTION OF THE WOOD OF *A. FERRUGINAEA*

The heartwood of *A. ferruginaea* (778 g) was milled and extracted with refluxing hexane for 24 hours in a Soxhlet extractor. Flash-column chromatography of the hexane extract yielded two pure compounds, named compound F (51 mg) and compound G (30 mg).

Compound F

Yield	:	51 mg, 0.0066%
Mass	:	530.3573 g mol^{-1}; $C_{32}H_{50}O_6$
Mass spectrum	:	m/e 530 (M^+); 512 (M^+-18); 470 (M^+-60); 452 (M^+-60-18); 399 (M^+-60-71); 381 (M^+-18-60-71)

^{1}H-nmr spectrum:

0.48 (1H, H-18b); 0.71 (1H, H-18a); 2.09 (3H, acetate group); 2.88 (2.71)(1H, H-24); 3.65 (OH); 3.75 (1H, H-7); 3.9 (1H, H-23); 4.65 (1H, H-3); δ5.45 (1H, H-21)

^{13}C-nmr spectrum: The data from ^{13}C-nmr spectrum of compound F is given in Table 11 (p. 63).

Compound G

Yield	:	30 mg, 0.0039%
Mass	:	570 g mol^{-1}; $C_{35}H_{54}O_6$
Mass spectrum	:	m/e 552 (M^+-18); 534 (M^+-18-18); 470 (M^+-100); 452 (M^+-18-100)

^{1}H-nmr spectrum:

0.48 (1H, H-18b); 0.70 (1H, H-18a); 1.81 (3H, d, J = 7.06 Hz, H-4′); 1.86 (3H, s, H-5′); 2.87(2.70) (1H, d, J = 7.06 Hz, H-24); 3.77 (1H, H-7); 4.69 (1H, H-3); 5.45 (1H, H-21); δ6.86 (1H, q, J=7.06 Hz, H-3′)

^{13}C-nmr spectrum: The data from ^{13}C-nmr spectrum of compound G is presented in Table 12 (p. 64).

4.7.3 ACETYLATION OF COMPOUND F

To a solution of compound F (100 mg) in pyridine (5 cm^3) was added acetic anhydride (5 cm^3). The mixture was briefly warmed on a steam bath and left to stand overnight. Methanol (10 cm^3) was added and the solvent removed under reduced pressure. Addition of toluene (2 x 10 cm^3), and evaporation under reduced pressure removed the remaining traces of pyridine, then methanol (2 x 10 cm^3) was added in order to remove remaining traces of toluene. T.l.c. showed that all compound F had been acetylated. Compound F triacetate was obtained as a gum.

Compound F triacetate

Mass : 614 g mol^{-1}; $C_{36}H_{54}O_8$

^{1}H-nmr spectrum:

2.06 (3H, s, CH_3-CO-O-); 2.08 (3H, s, CH_3-CO-O-); 2.09 (3H, s, CH_3-CO-O-); 3.9 (1H, H-23); 4.65 (1H, H-3); 5.00 (1H, H-7); δ6.28 (1H, H-21)

4.8 TABLES

TABLE 1: ^{13}C-NMR DATA FOR 13-EPIMANOYL OXIDE

C atom	δ
1	39.39(t)
2	18.63(t)
3	42.23(t)
4	33.30(s)
5	56.54(d)
6	19.90(t)
7	43.11(t)
8	76.15(s)
9	58.58(d)
10	36.84(s)
11	15.87(t)
12	34.85(t)
13	73.35(s)
14	148.02(d)
15	109.74(t)
16	33.40(q)
17	23.93(q)
18	32.75(q)
19	21.28(q)
20	15.87(q)

TABLE 2: ^{13}C-NMR DATA FOR APHANAMIXOL

C atom	δ
1	39.83(t)
2	18.48(t)
3	42.07(t)
4	33.33(s)
5	56.23(d)
6	20.61(t)
7	43.00(t)
8	74.35(s)
9	61.31(d)
10	39.34(s)
11	23.60(t)
12	44.59(t)
13	141.23(s)
14	123.58(d)
15	59.38(t)
16	16.50(q)
17	23.96(q)
18	33.48(q)
19	21.56(q)
20	15.52(q)

TABLE 3: ^{13}C-NMR DATA FOR (-)-EPICATECHIN

C atom	δ
2	82.68(d)
3	68.45(d)
4	28.71(t)
5	157.99(s)*
6	96.41(d)
7	157.56(s)*
8	95.60(d)
9	157.14(s)*
10	100.76(s)
1′	132.25(s)
2′	115.50(d)
3′	146.00(s)
4′	146.00(s)
5′	116.04(d)
6′	120.31(d)

*Resonance positions for C-5, C-7 and C-9 may be interchanged.

TABLE 4: ^{13}C-NMR DATA FOR KAEMPFEROL 3-O-GLUCOSIDE

C atom	δ
2	156.3(s)
3	133.0(s)
4	177.4(s)
5	161.1(s)
6	98.7(d)
7	164.1(s)
8	93.6(d)
9	156.3(s)
10	104.1(s)
1′	121.0(s)
2′	130.7(d)
3′	115.0(d)
4′	159.8(s)
5′	115.0(d)
6′	130.7(d)
1"	101.4(d)
2"	74.2(d)
3"	77.2(d)
4"	70.1(d)
5"	76.5(d)
6"	61.0(t)

TABLE 6: ^{13}C-NMR DATA FOR COMPOUND E

C atom	δ
2	79.2(d)
3	103.4(s)
4	107.7(s)
5	163.8(s)
6	92.4(d)
7	160.9(s)
8	94.6(d)
9	158.5(s)
10	89.3(s)
11	36.3(t)
12	53.2(d)
1′	138.6(s)
2′	128.0(d)
3′	127.6(d)
4′	126.2(d)
5′	127.6(d)
6′	128.0(d)
1"	126.7(s)
2"	128.9(d)
3"	112.6(d)
4"	156.9(s)
5"	112.6(d)
6"	128.9(d)
5-Me	55.6(q)
7-Me	55.7(q)
4"-Me	55.0(q)

TABLE 9: ^{13}C-NMR DATA FOR LUPEOL

Carbon Atom	δ (ppm)
1	38.80
2	27.50
3	79.30
4	38.96
5	55.43
6	18.36
7	34.35
8	40.92
9	50.55
10	37.25
11	20.97
12	25.18
13	38.13
14	42.93
15	27.46
16	35.67
17	43.11
18	48.11
19	48.41
20	151.40
21	29.91
22	40.10
23	28.06
24	15.42
25	16.16
26	16.01
27	14.58
28	18.05
29	19.35
30	109.65

TABLE 10: ^{13}C-NMR DATA FOR (20)29-LUPEN-3-ONE

Carbon atom	δ (ppm)
1	27.38
2	218.23
3	218.23
4	47.29
5	54.84
6	21.48
7	34.11
8	40.72
9	49.73
10	36.83
11	19.64
12	25.08
13	38.11
14	42.84
15	33.51
16	35.48
17	42.94
18	47.91
19	48.18
20	150.74
21	29.78
22	39.94
23	26.62
24	21.01
25	15.96
26	15.76
27	14.45
28	17.99
29	19.29
30	109.41

TABLE 11: ^{13}C-NMR DATA FOR COMPOUND F

Carbon atom	δ (ppm)
3	78.07(d)
7	74.18(d)
18	13.77(t)
21	102.09 (98.16)(d)
23	78.39 (77.12)(d)
24	67.66 (65.32)(d)
25	58.08 (57.31)(s)
Acetate C=O	171.04(s)
Acetate Me	21.49(q)
C-Me(q)	15.60
	15.71
	19.25
	19.48
	19.57
	21.56
	21.89
	25.02
	27.70

TABLE 12: ^{13}C-NMR DATA FOR COMPOUND G

Carbon atom	δ(ppm)
3	78.11(d)
7	74.22(d)
18	13.73(t)
21	102.05 (98.18)(d)
23	78.43 (77.29)(d)
34	67.64 (65.28)(d)
25	58.09 (57.34)(s)
1′	167.67(s)
2′	129.09(s)
3′	136.68(d)
4′	14.450(q)
5′	12.20(q)
C-Me(q)	27.91
	27.59
	25.04
	21.88
	19.62
	19.50
	19.25
	15.79

CHAPTER 5

5. SPECTRA

5.1 13-EPIMANOYL OXIDE

5.1.1 Infrared spectrum

5.1.2 Mass spectrum

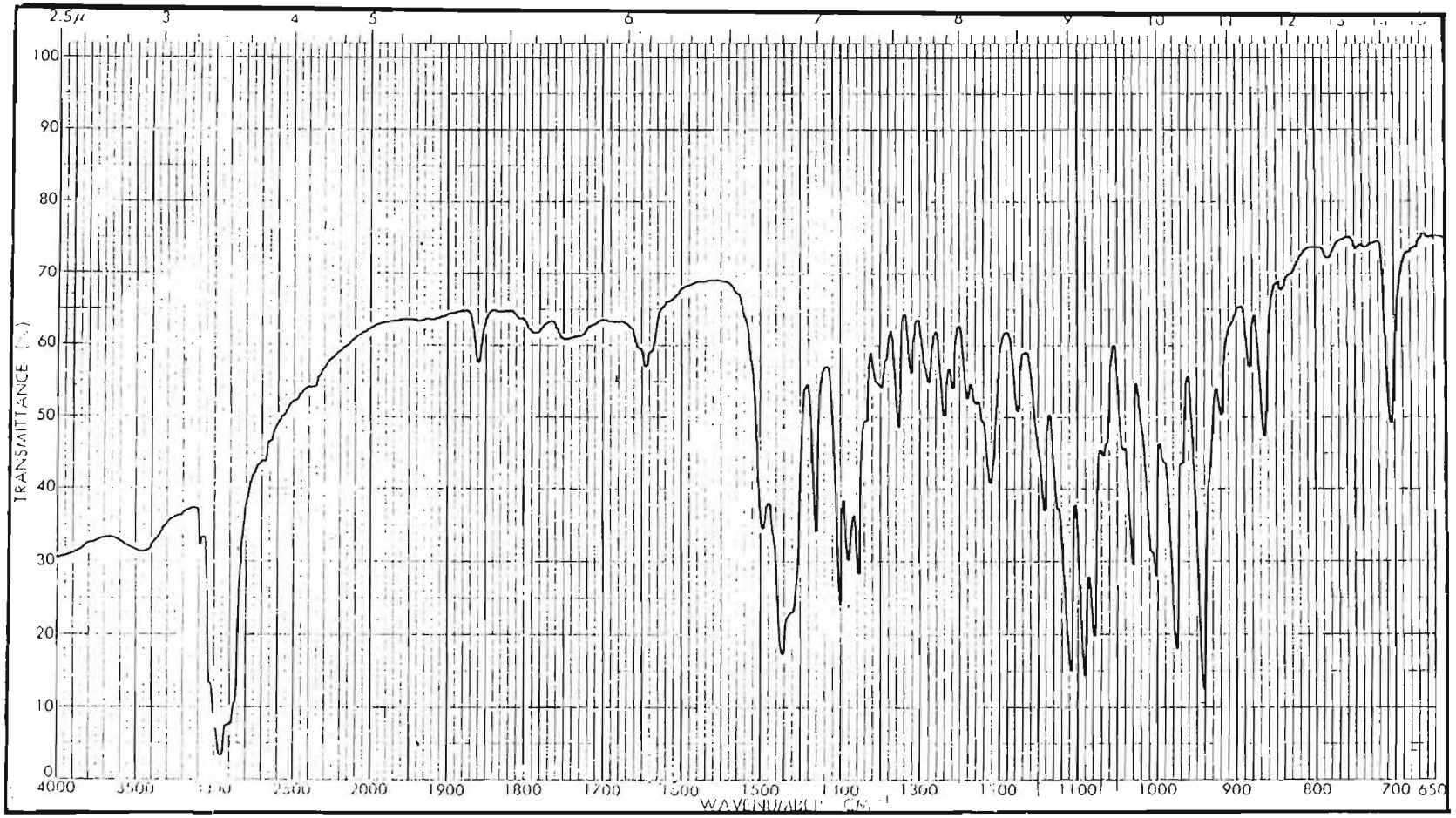
5.1.3 ^{1}H-nmr spectrum

5.1.4 ^{13}C-nmr spectrum

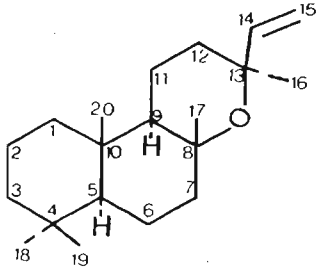
5.1.5 ^{13}C-Adept spectrum

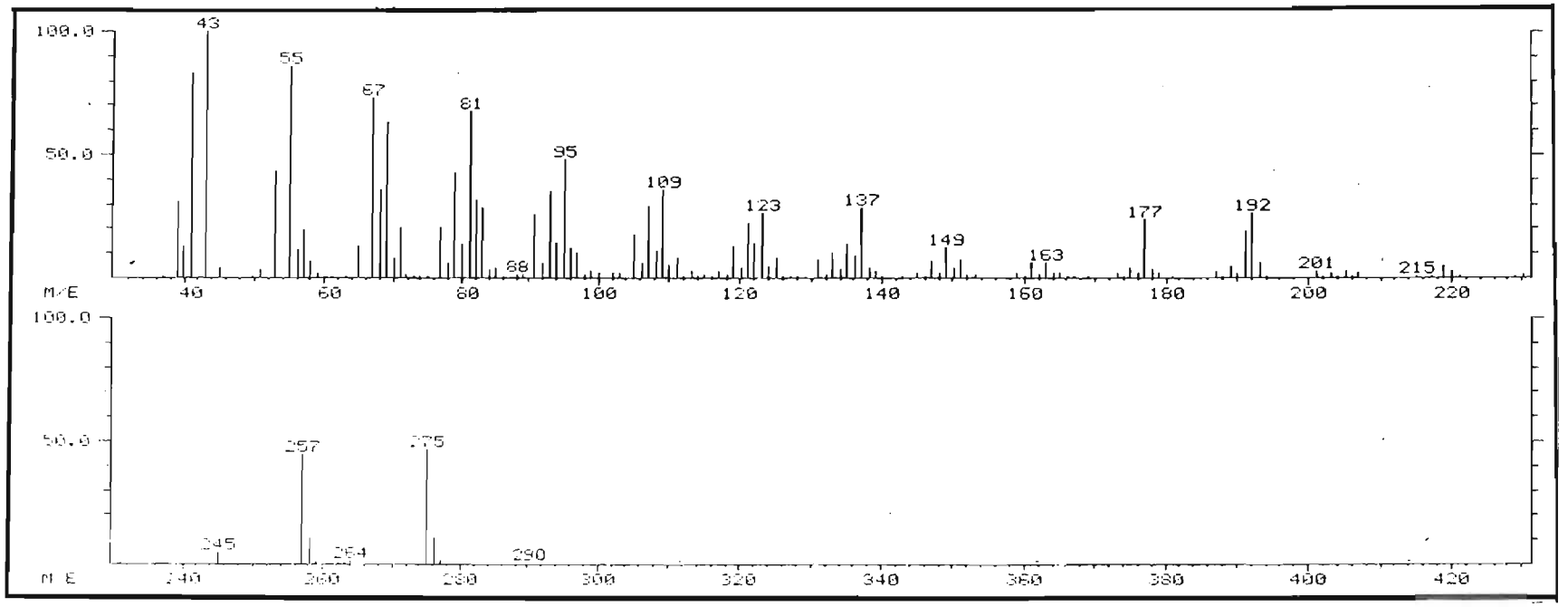
5.1.6 Cosy spectrum

5.1.7 Hetcor spectrum

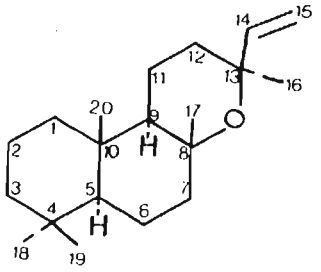


5.1.1 Infrared spectrum

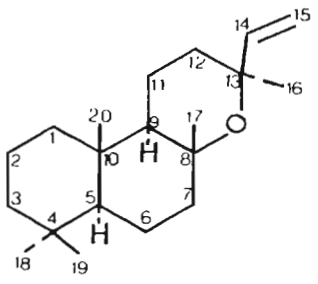




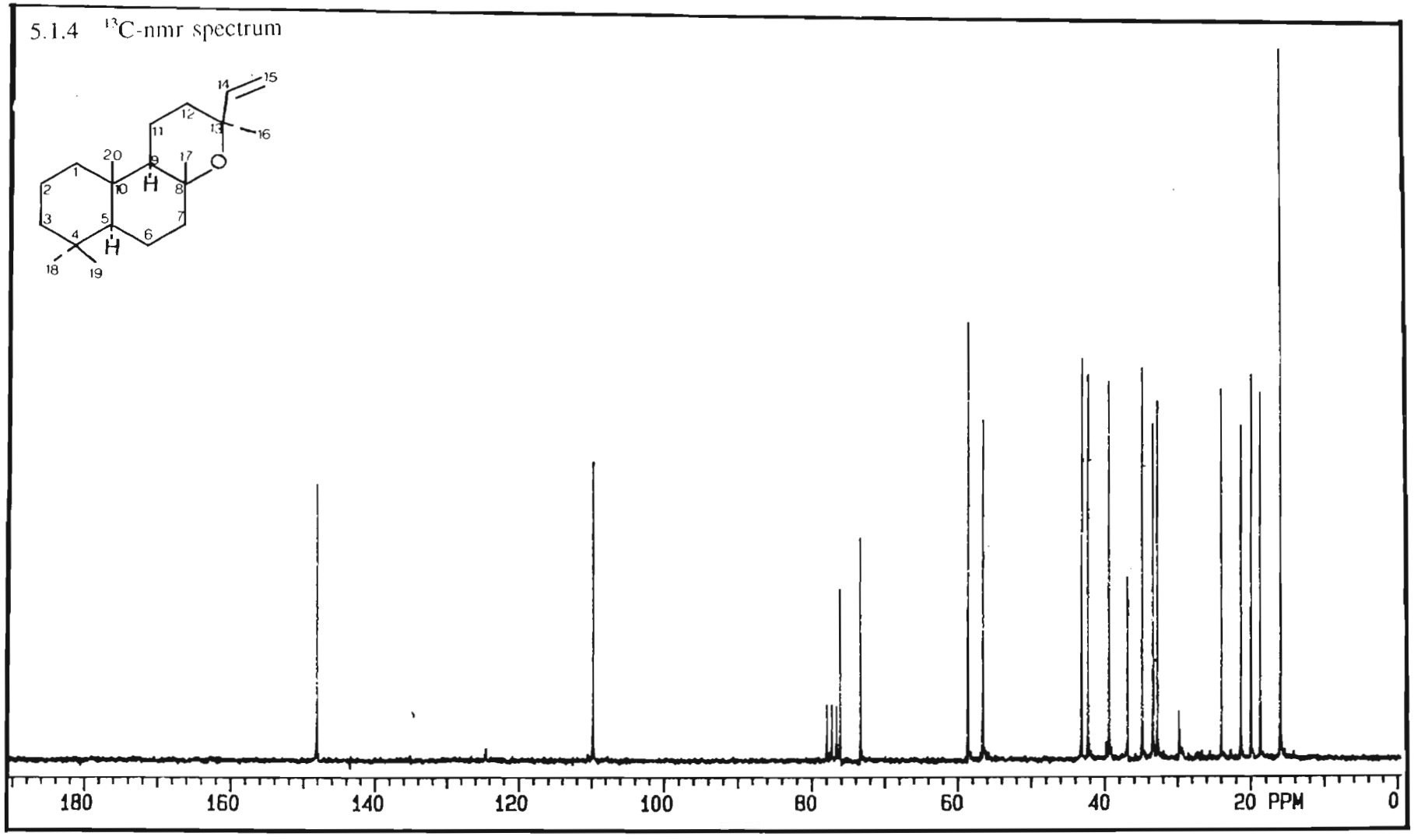
5.1.2 Mass spectrum



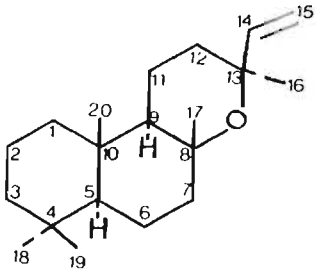
5.1.3 ^{1}H-nmr spectrum

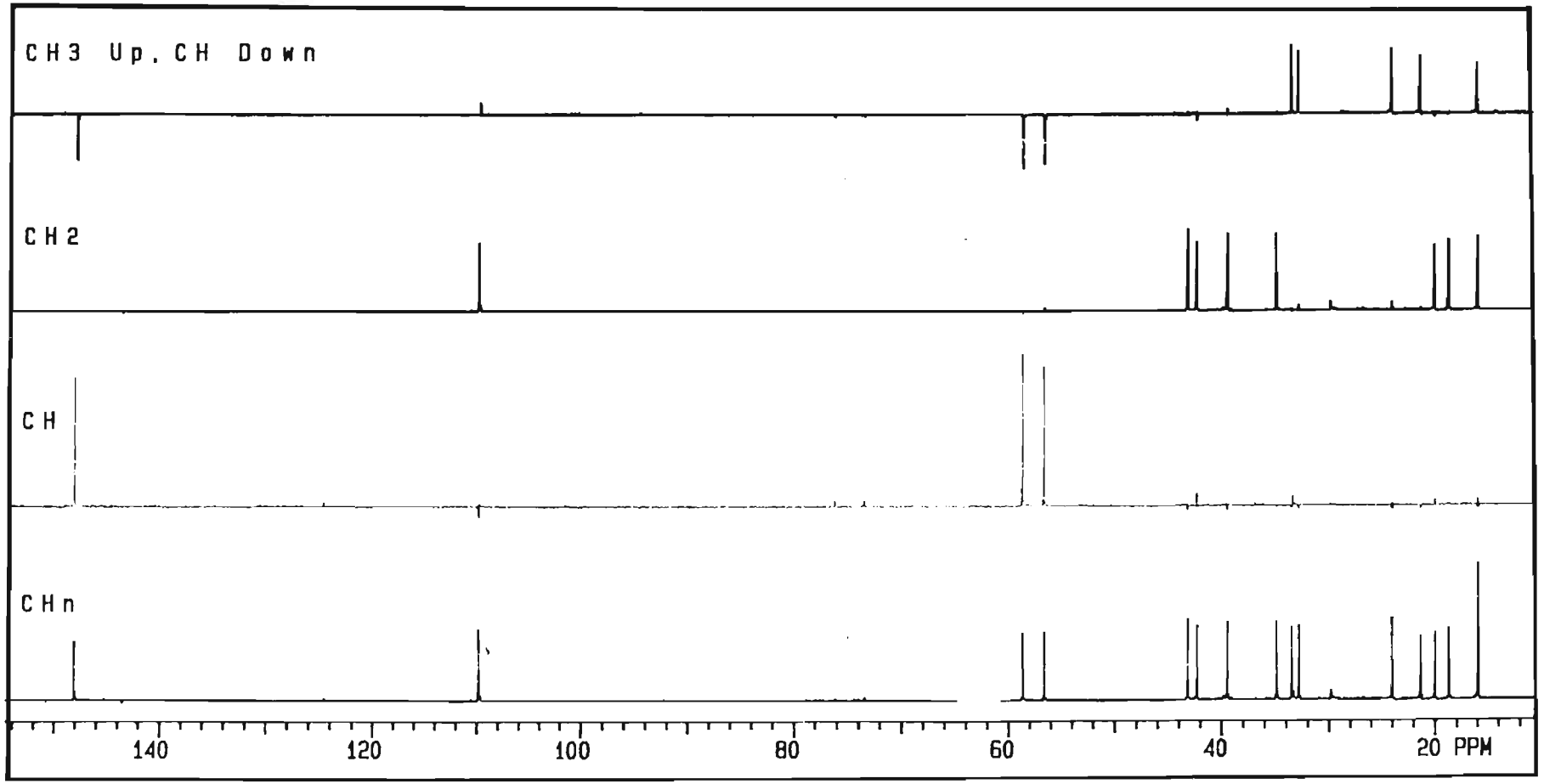


5.1.4 ^{13}C-nmr spectrum

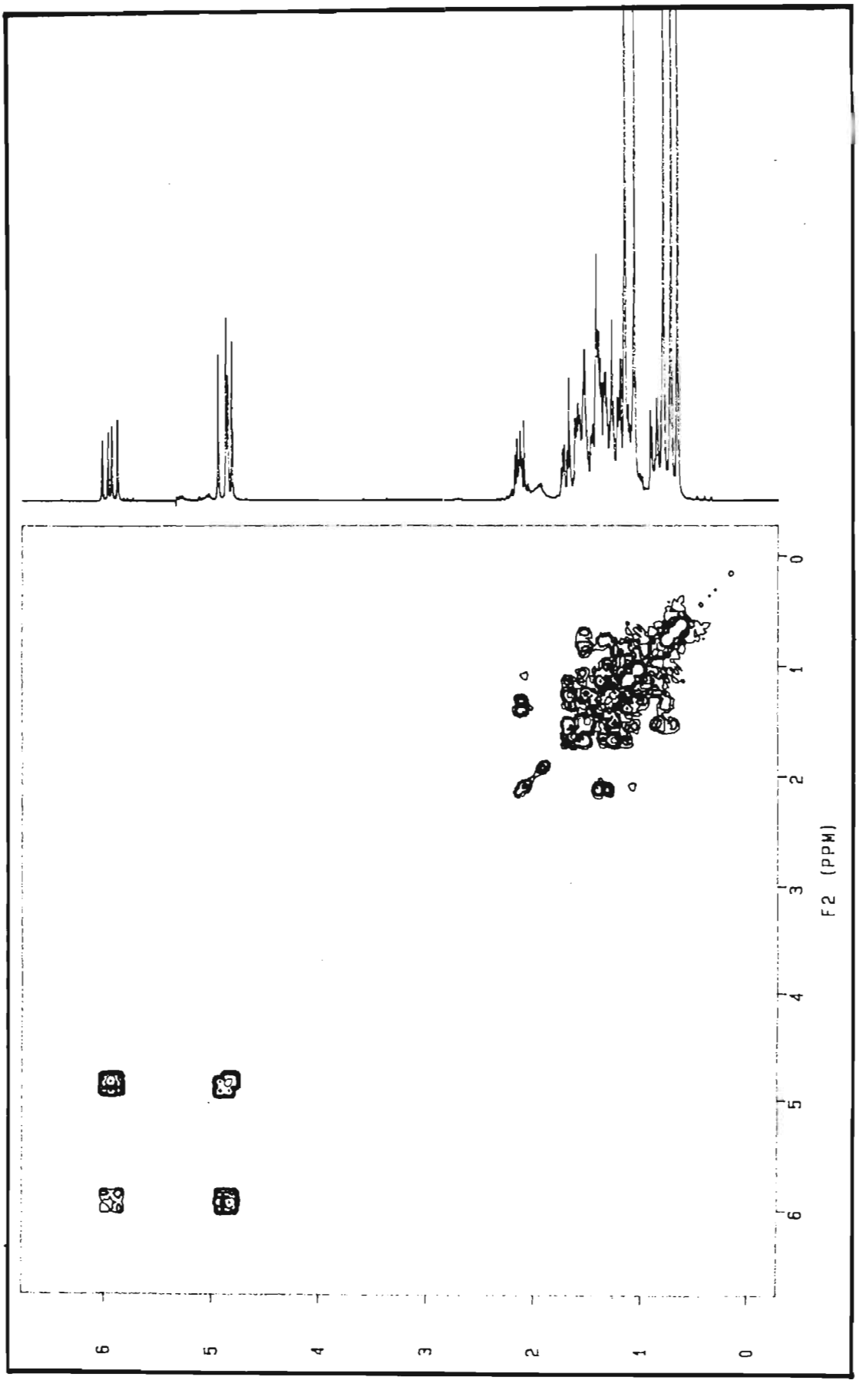


5.1.5 ^{13}C-Adept spectrum

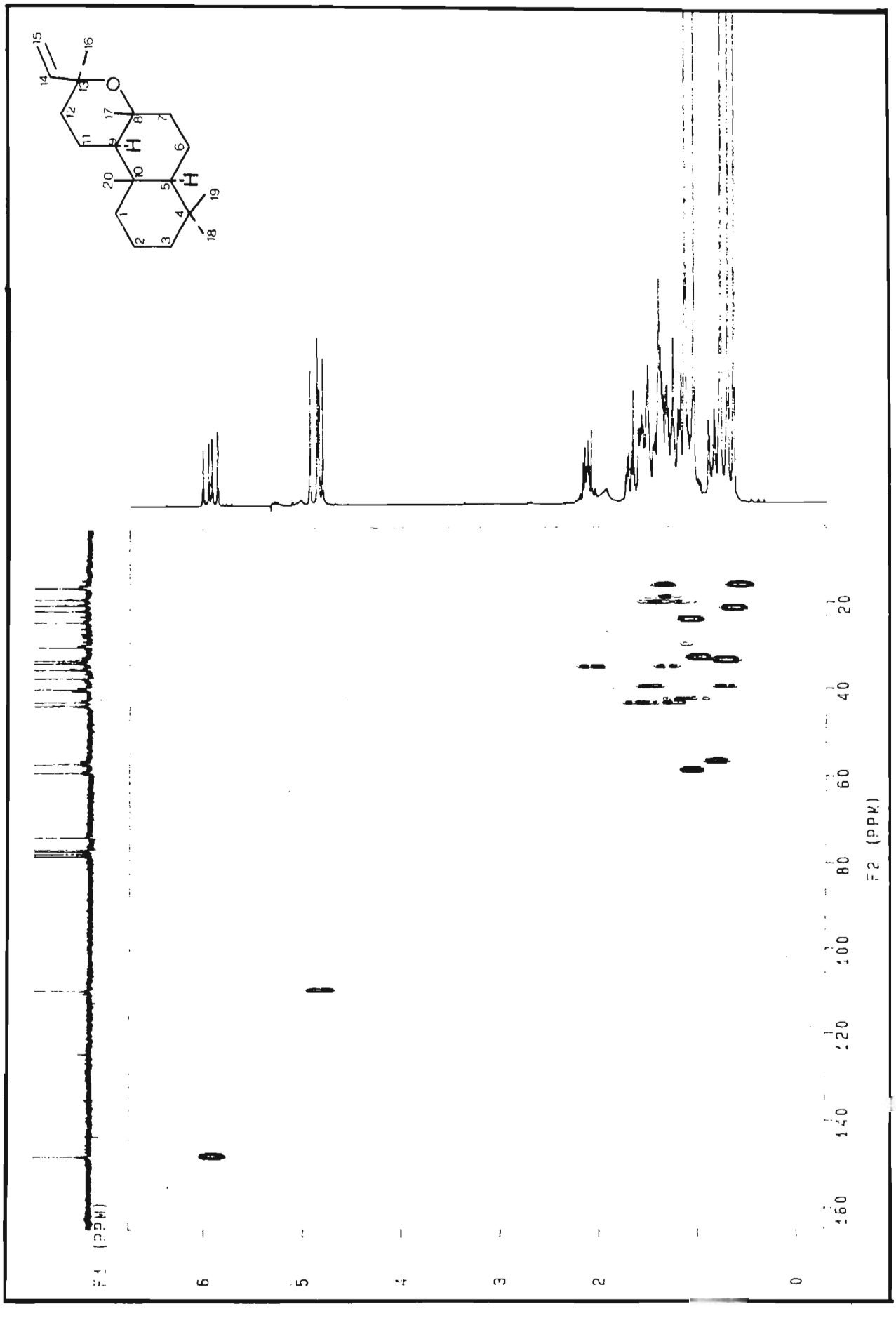




5.1.6 Cosy spectrum



5.1.7 Hetcor spectrum



5.2 APHANAMIXOL

5.2.1 Infrared spectrum

5.2.2 Mass spectrum

5.2.3 ^{1}H-nmr spectrum

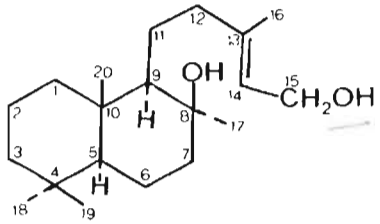
5.2.4 ^{13}C-nmr spectrum

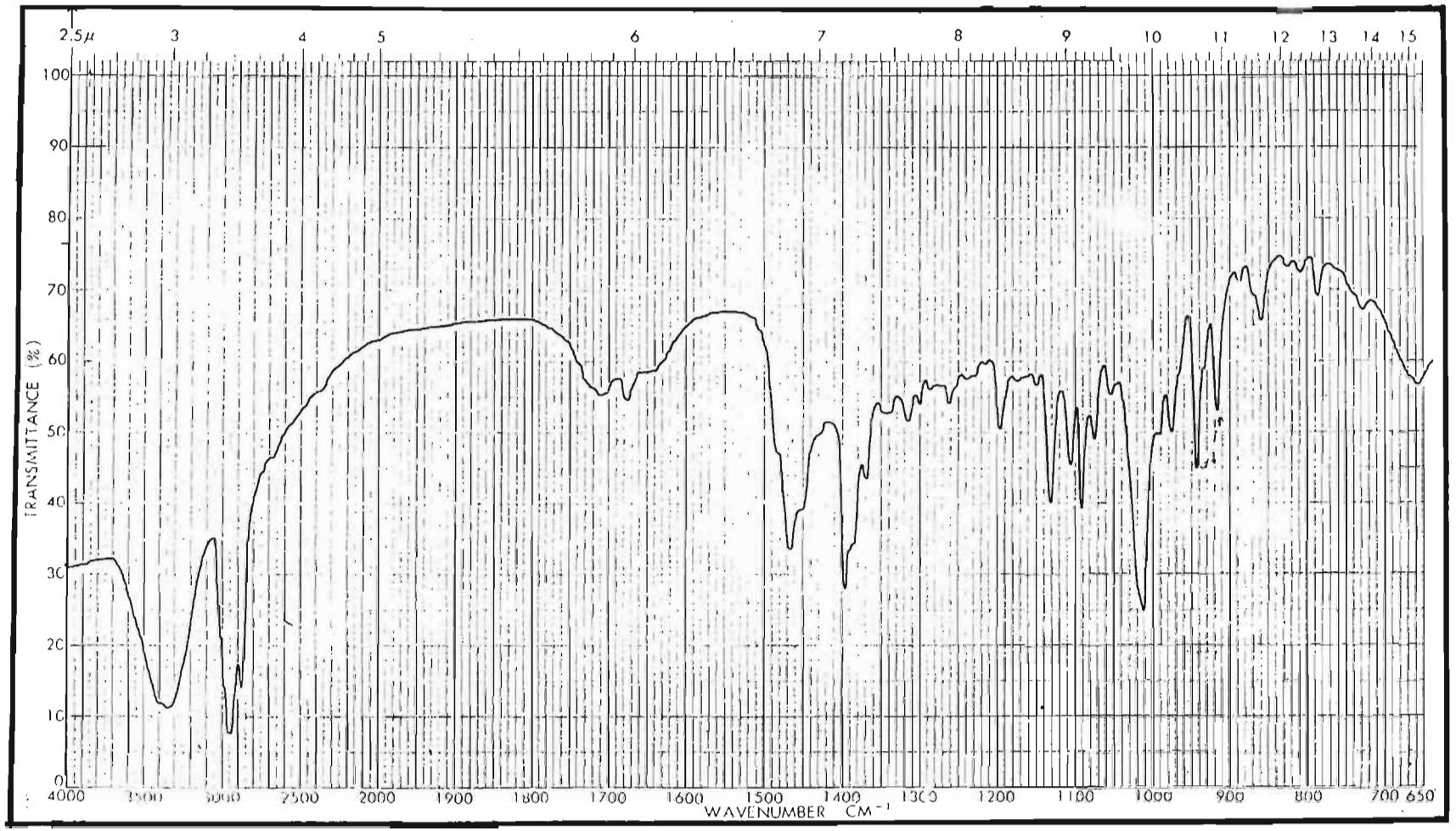
5.2.5 ^{13}C-Adept spectrum

5.2.6 Cosy spectrum

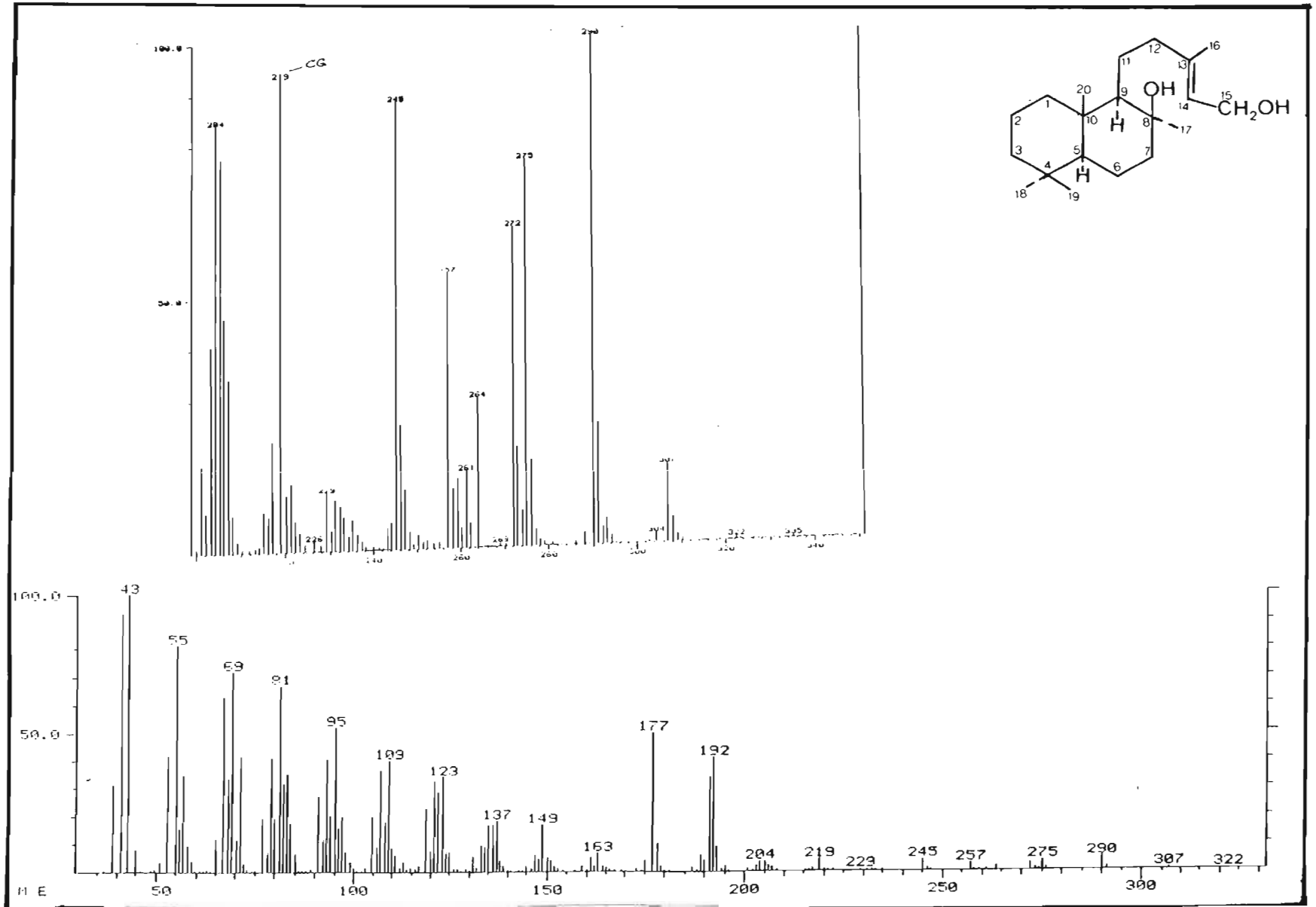
5.2.7 Hetcor spectrum

5.2.1 Infrared spectrum

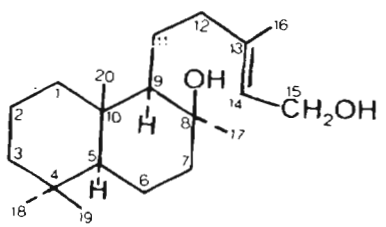




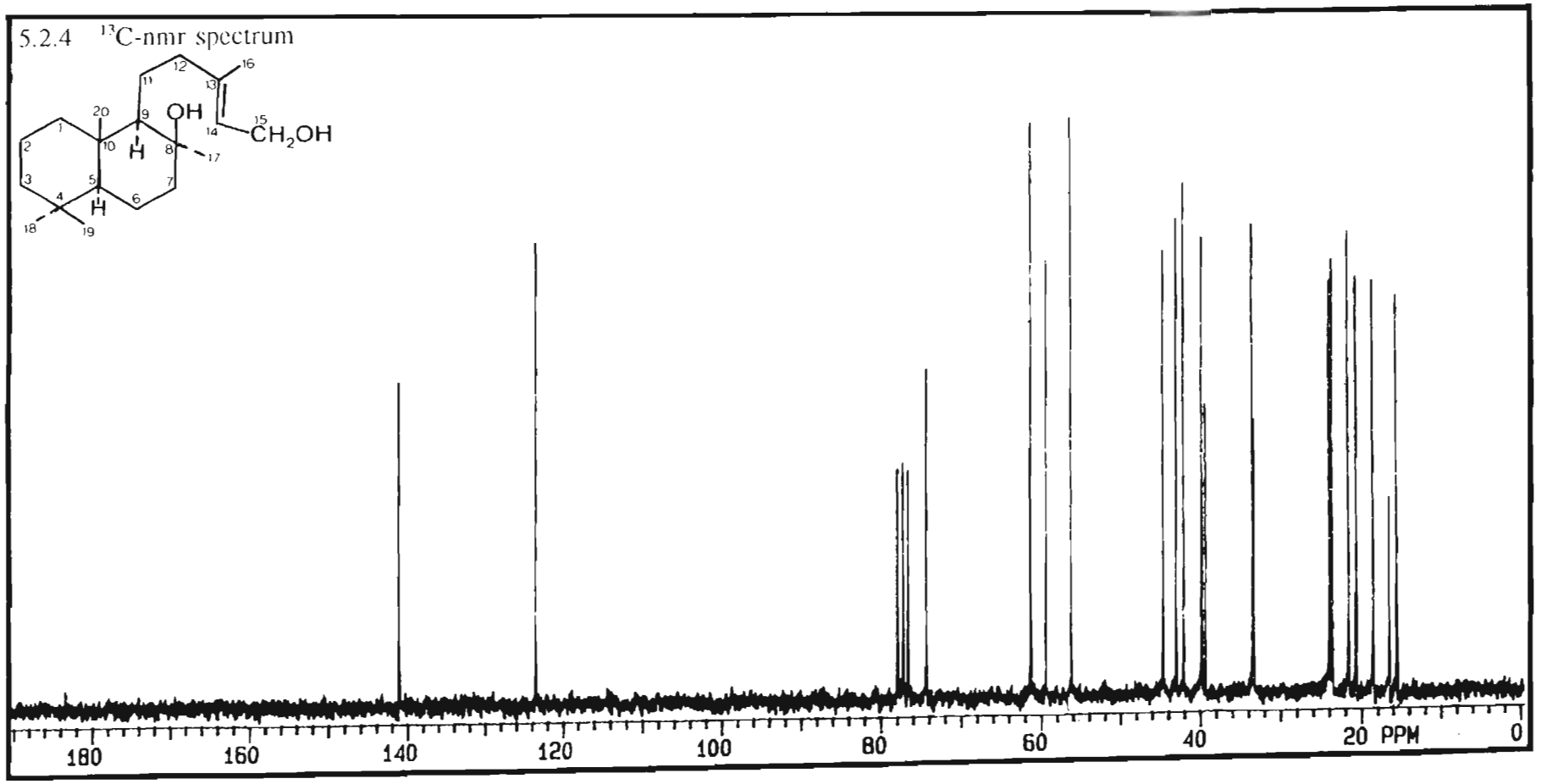
5.2.2 Mass spectrum



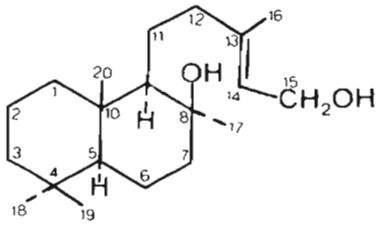
5.2.3 ^{1}H-nmr spectrum

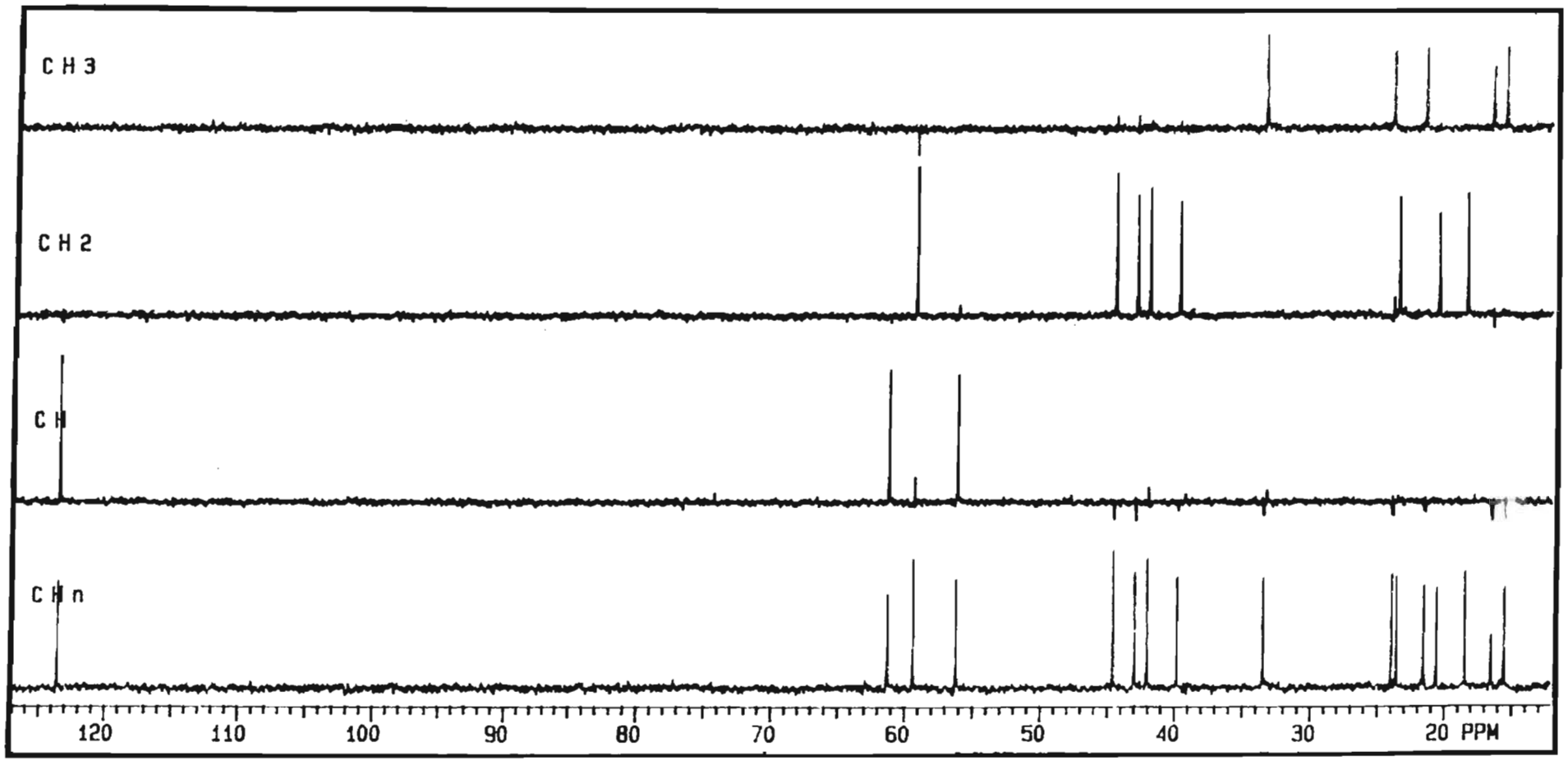


5.2.4 ^{13}C-nmr spectrum

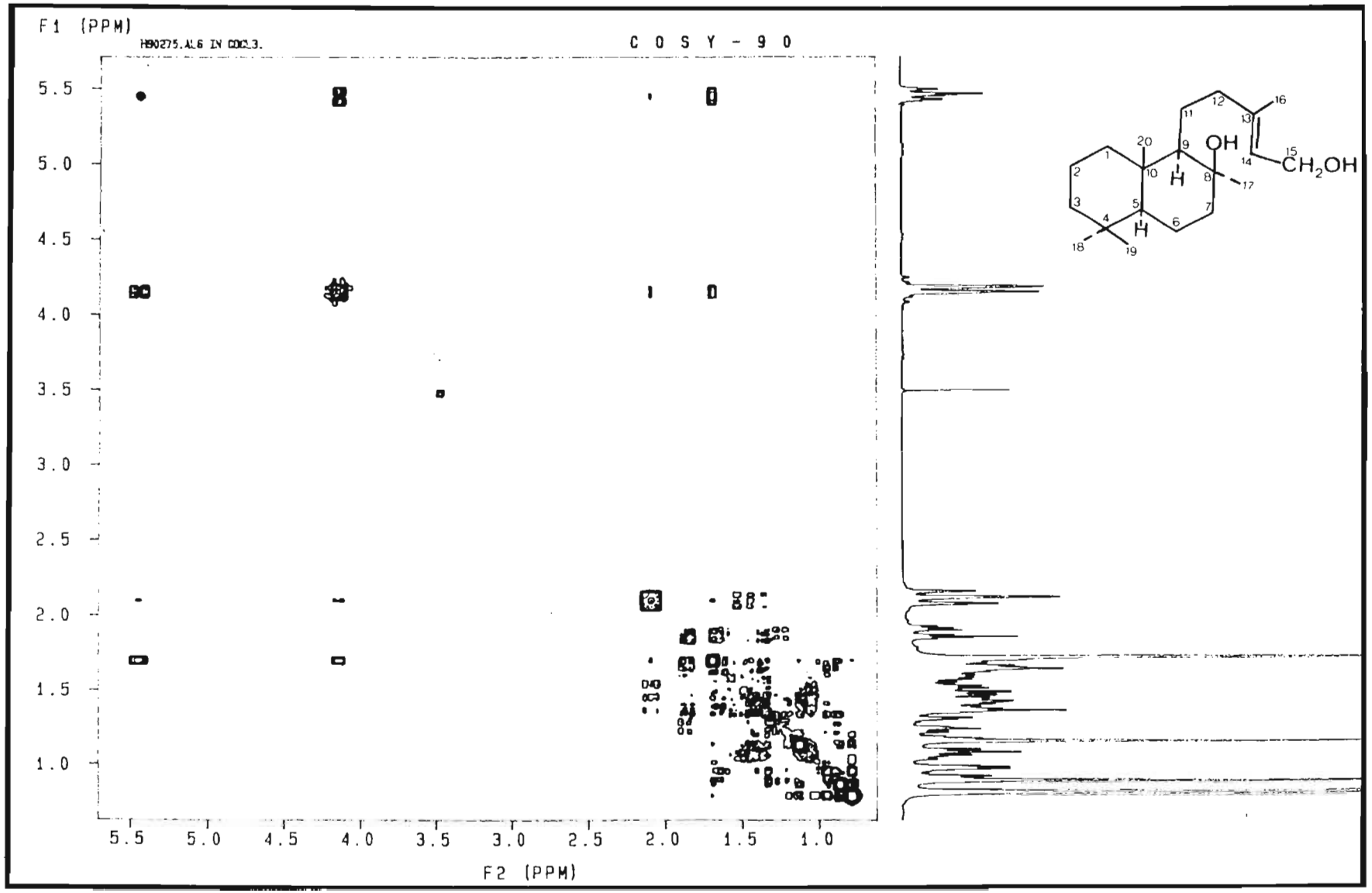


5.2.5 ^{13}C-Adept spectrum

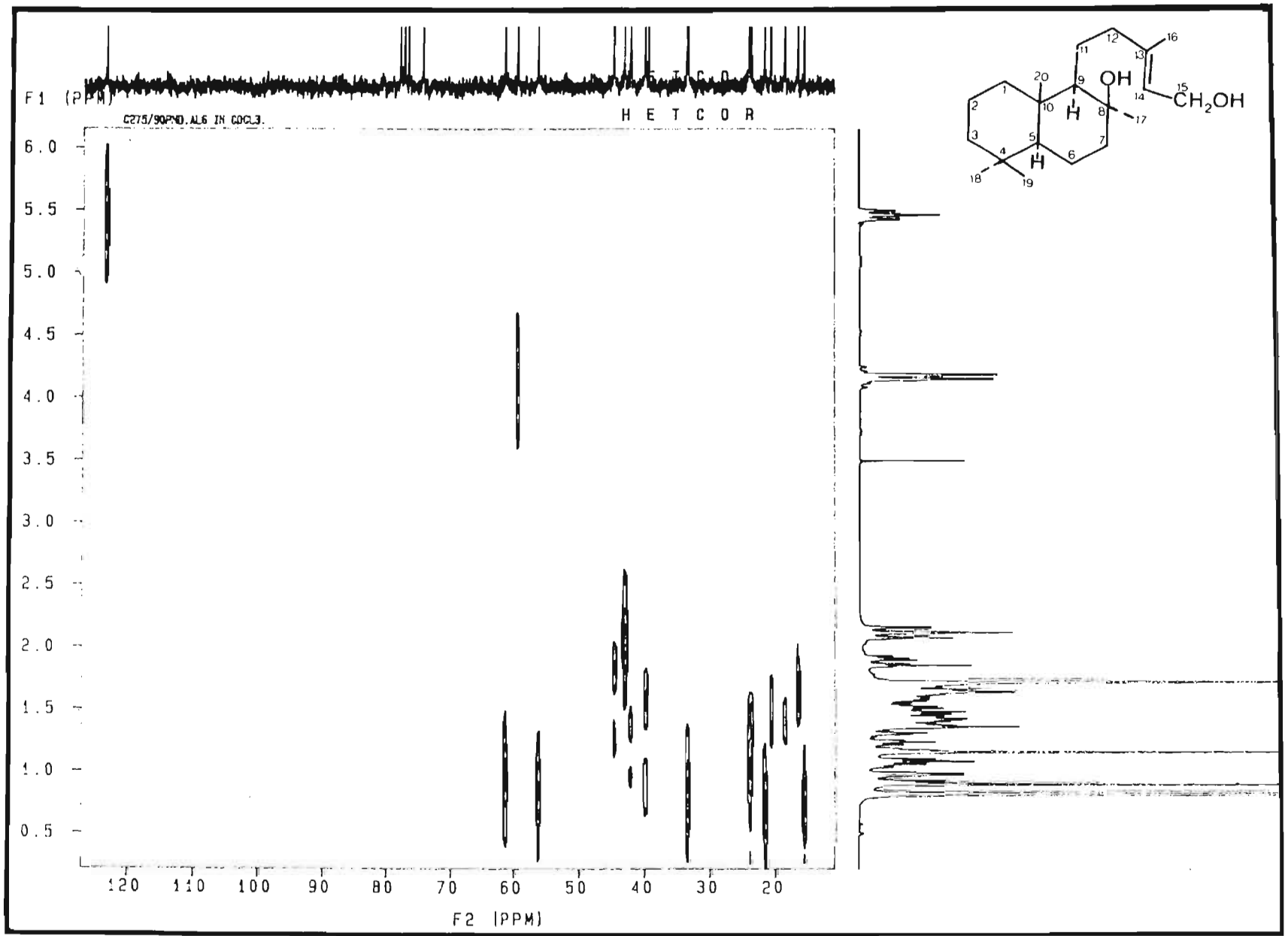




5.2.6 Cosy spectrum



5.2.7 Hetcor spectrum



5.3 APHANAMIXOL 15-MONOACETATE

5.3.1 ^{1}H-nmr spectrum

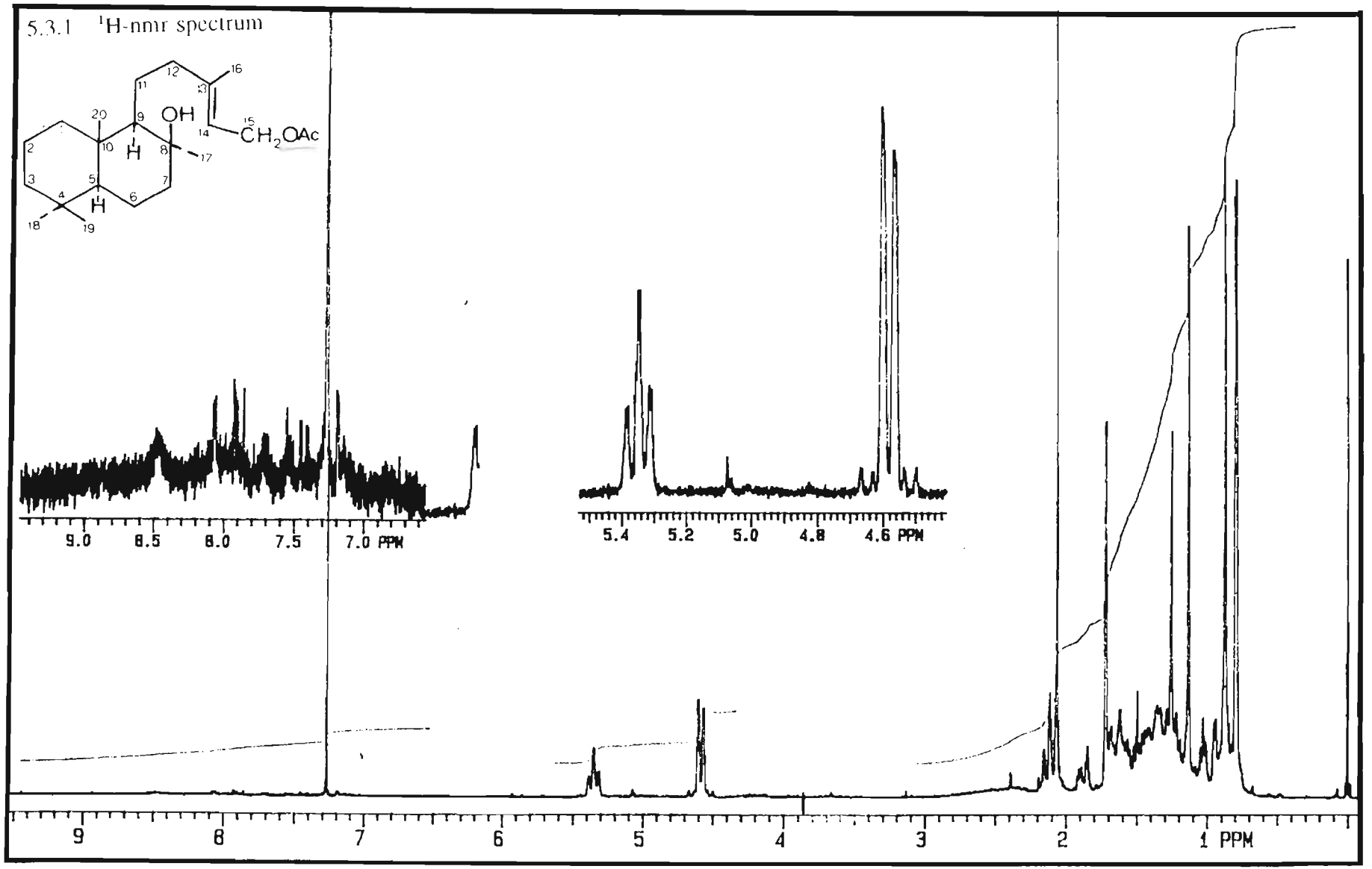
5.3.2 ^{13}C-nmr spectrum

5.3.3 ^{13}C-Adept spectrum

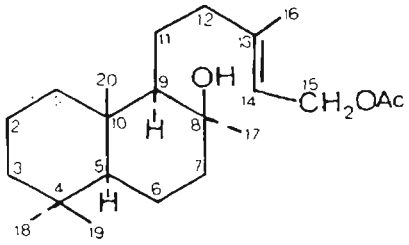
5.3.4 Cosy spectrum

5.3.5 Hetcor spectrum

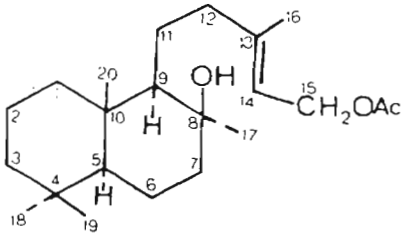
5.3.1 ^{1}H-nmr spectrum

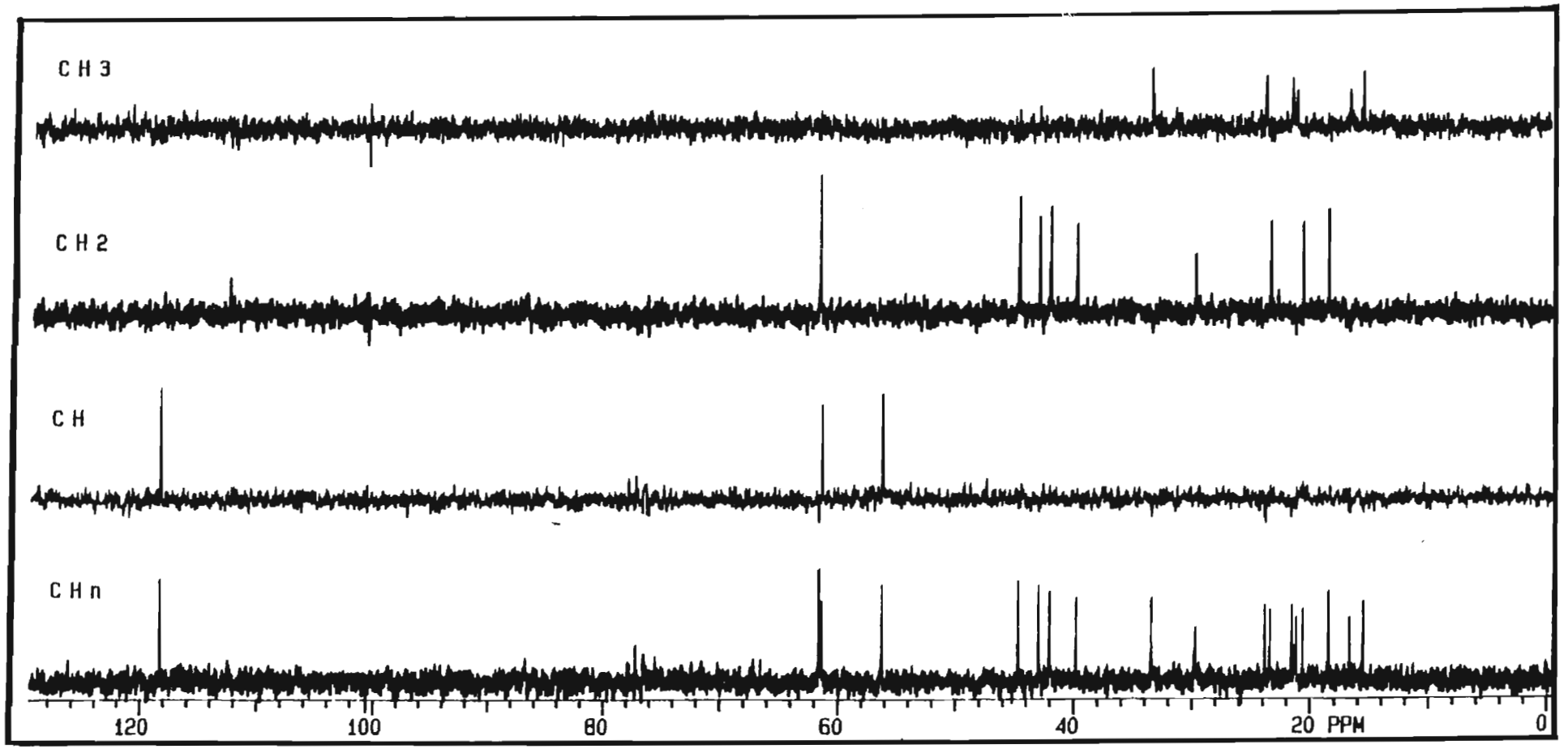


5.3.2 ^{13}C-nmr spectrum

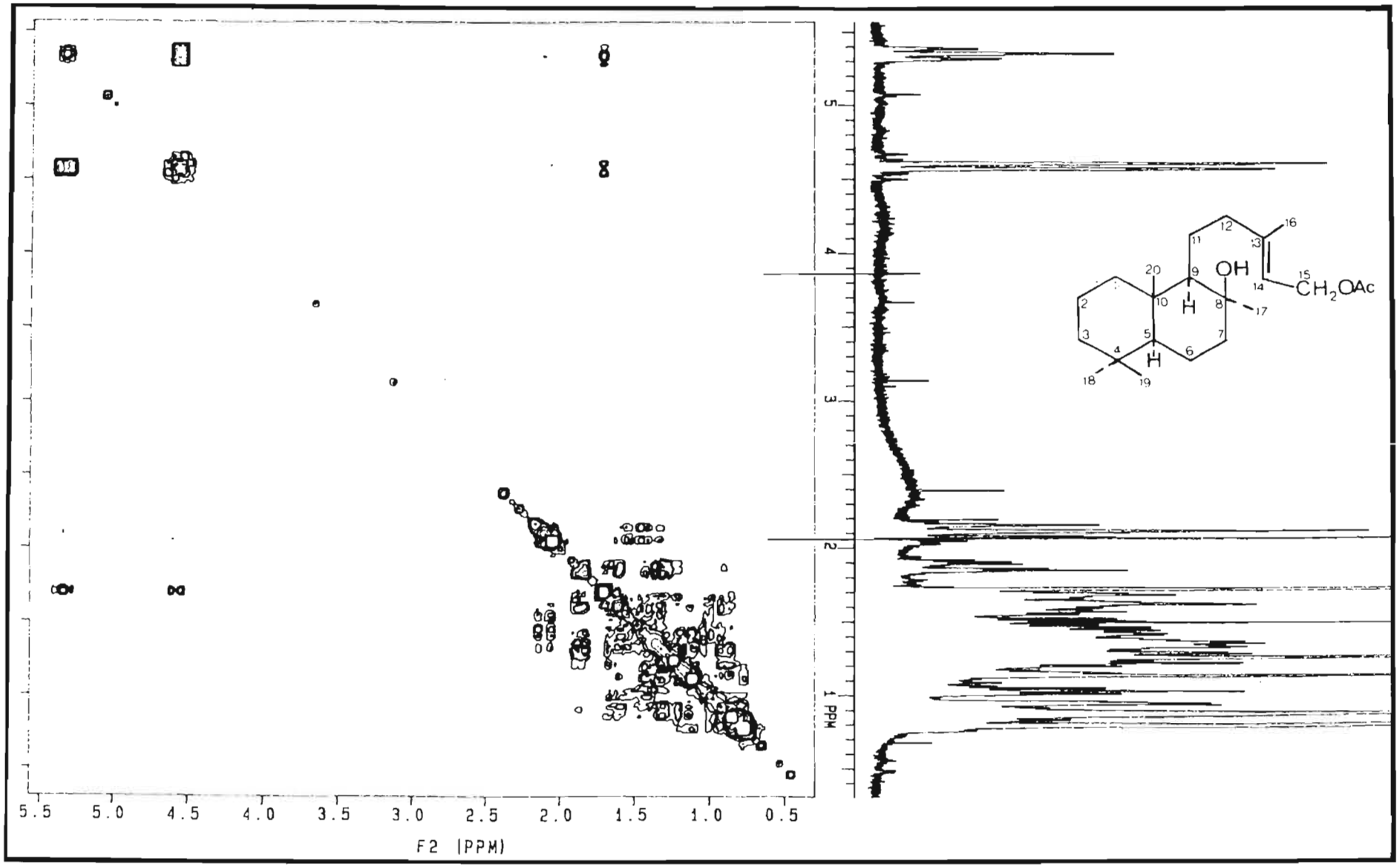


5.3.3 ^{13}C-Adept spectrum

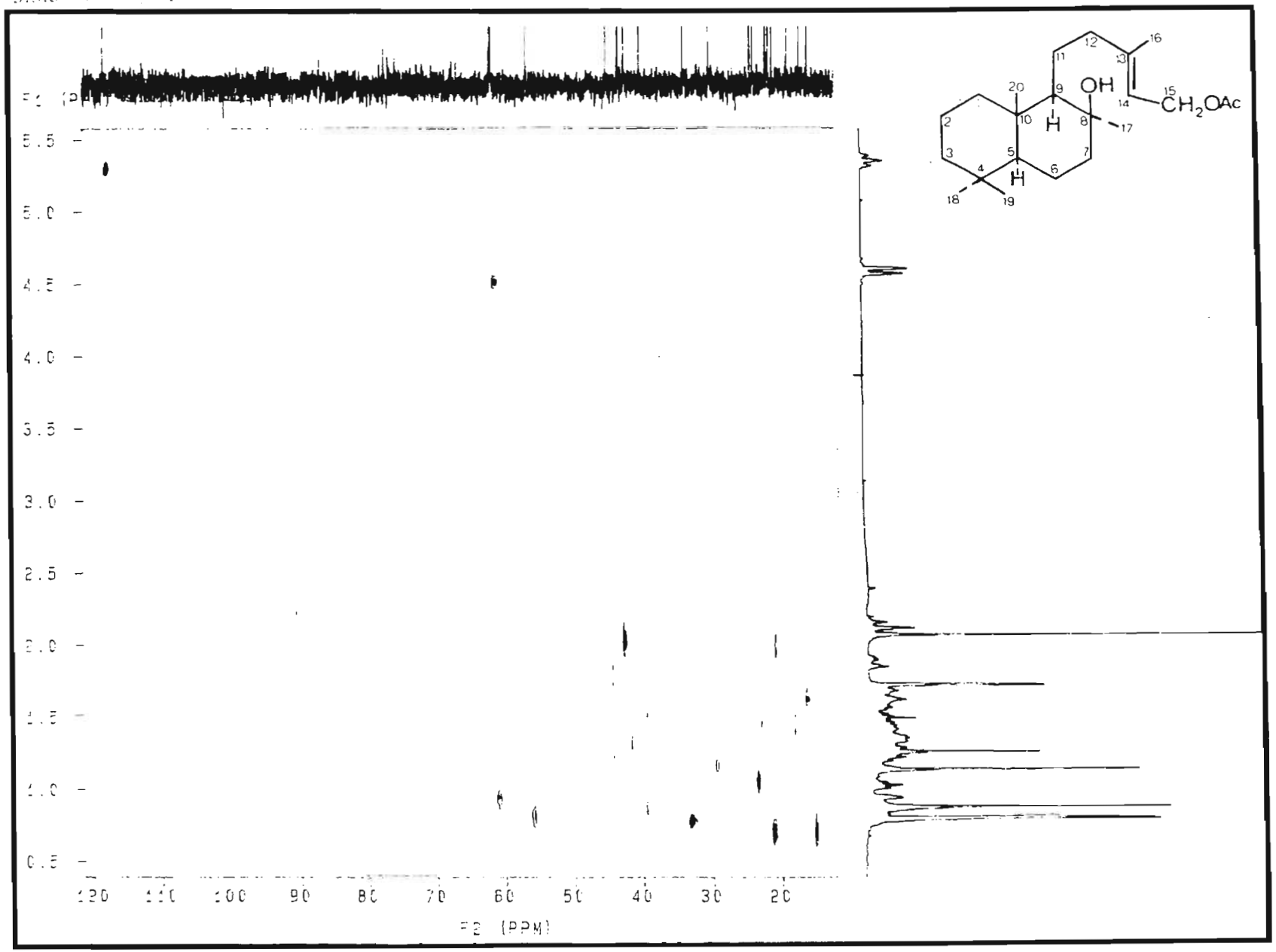




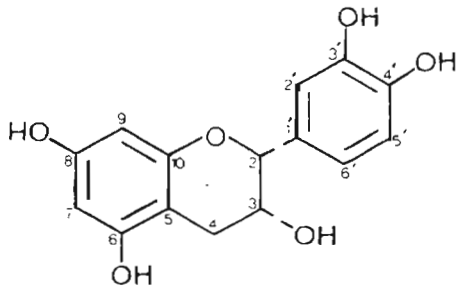
5.3.4 Cosy spectrum

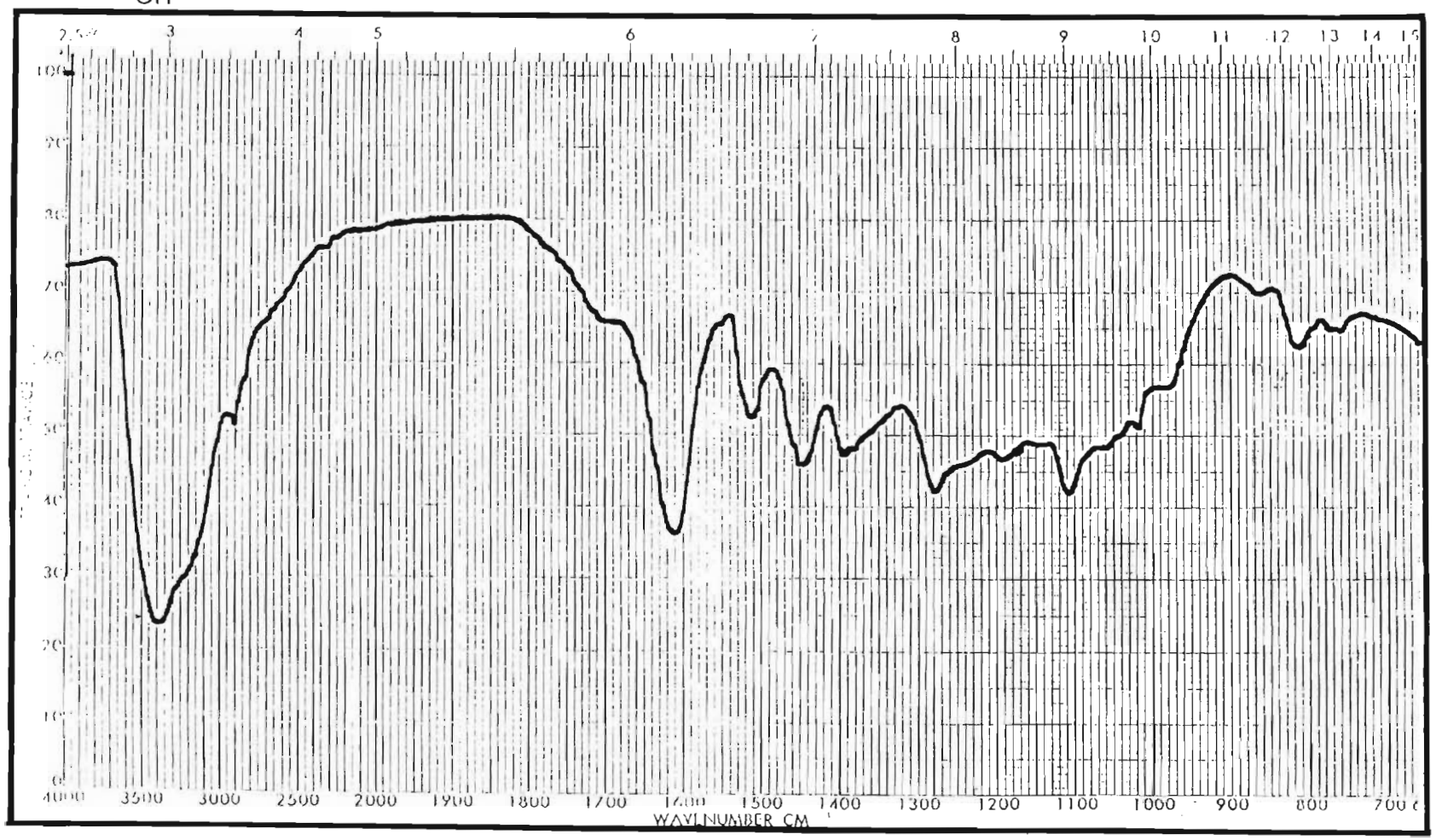


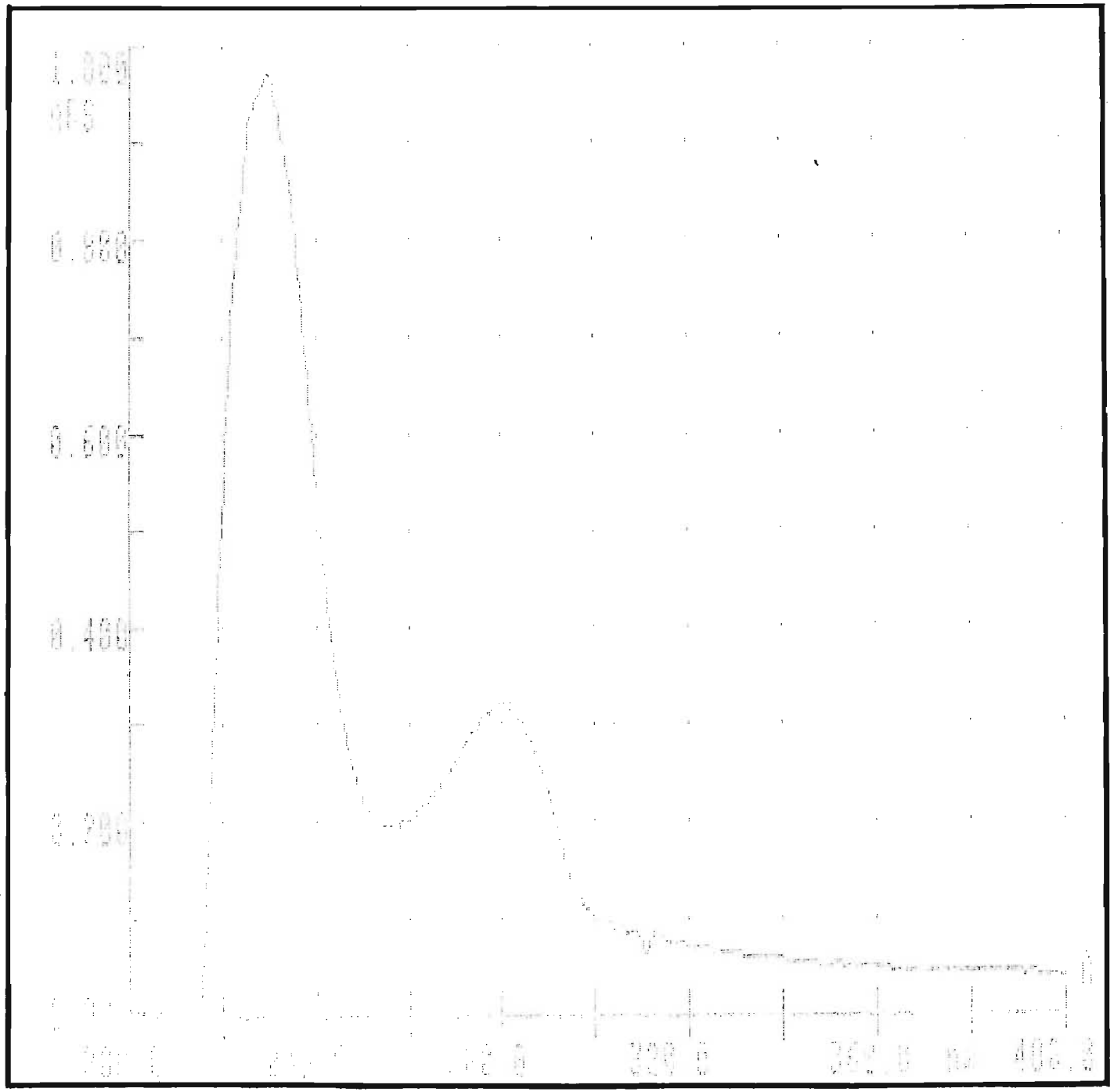
5.3.5 Hetcor spectrum



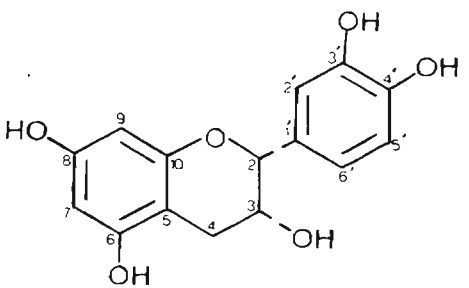
5.4.1 Infrared spectrum



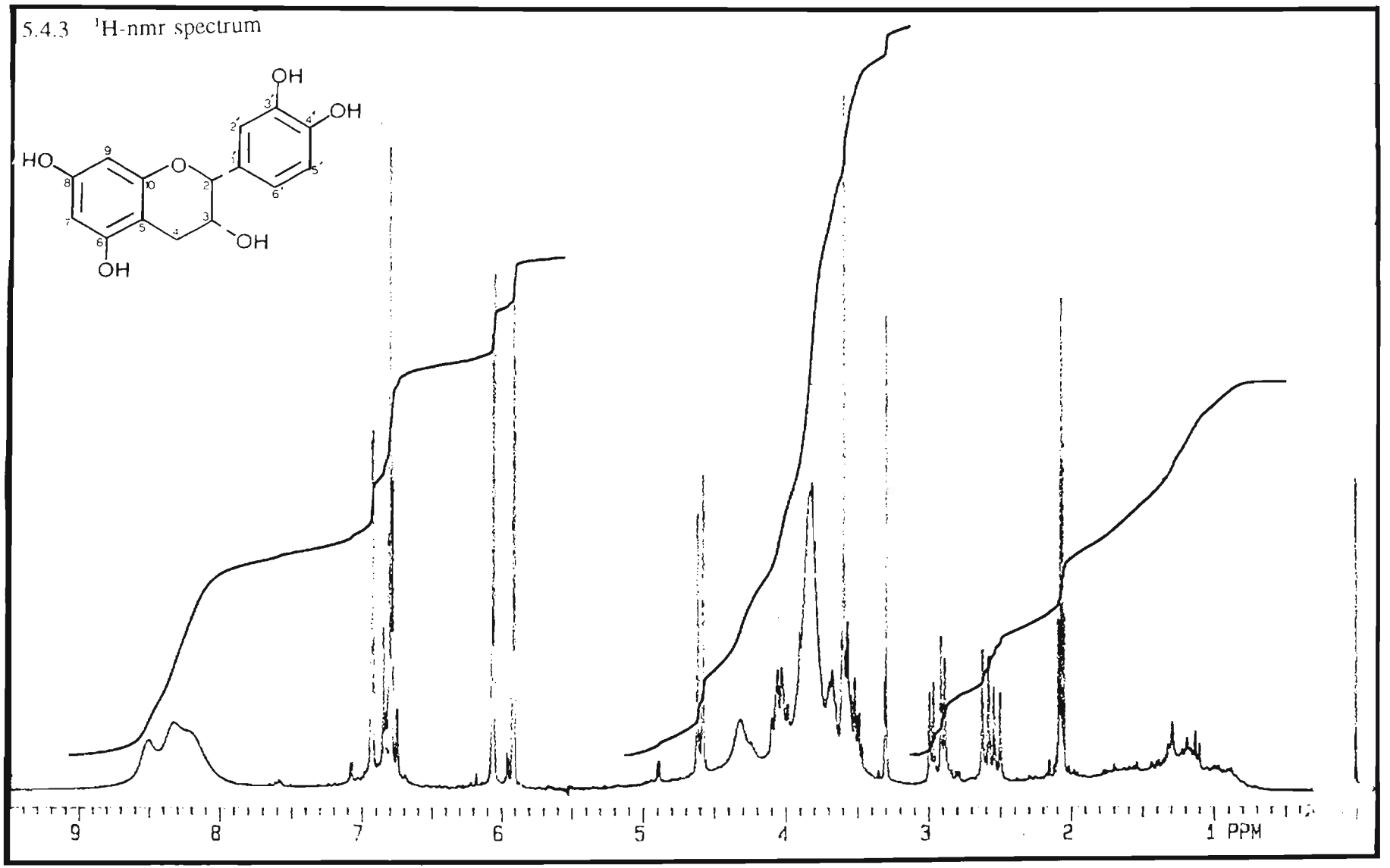




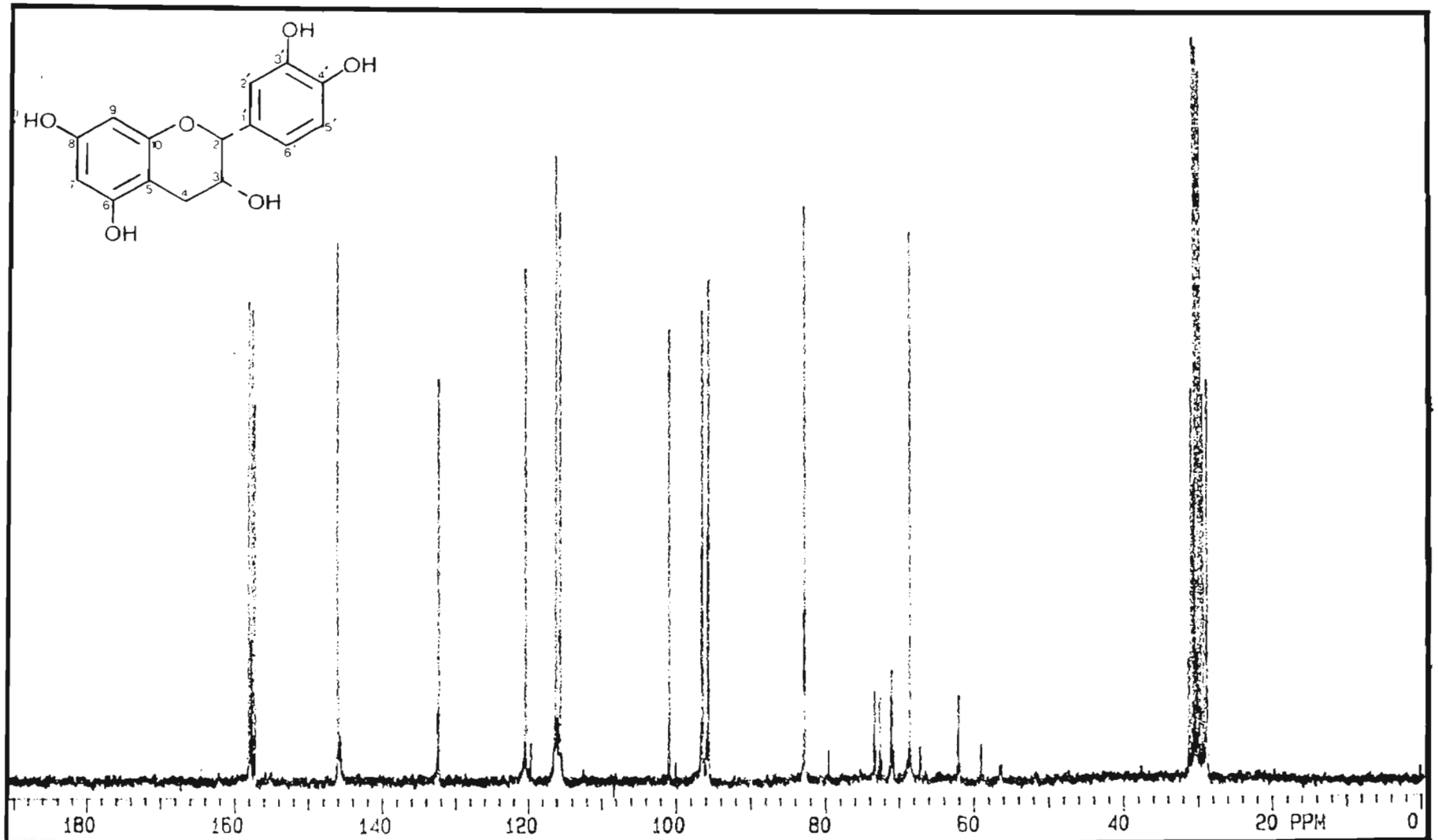
5.4.2 UV spectrum



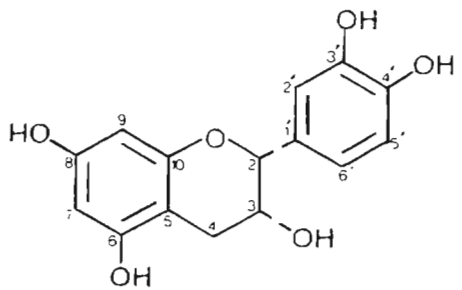
5.4.3 ^{1}H-nmr spectrum

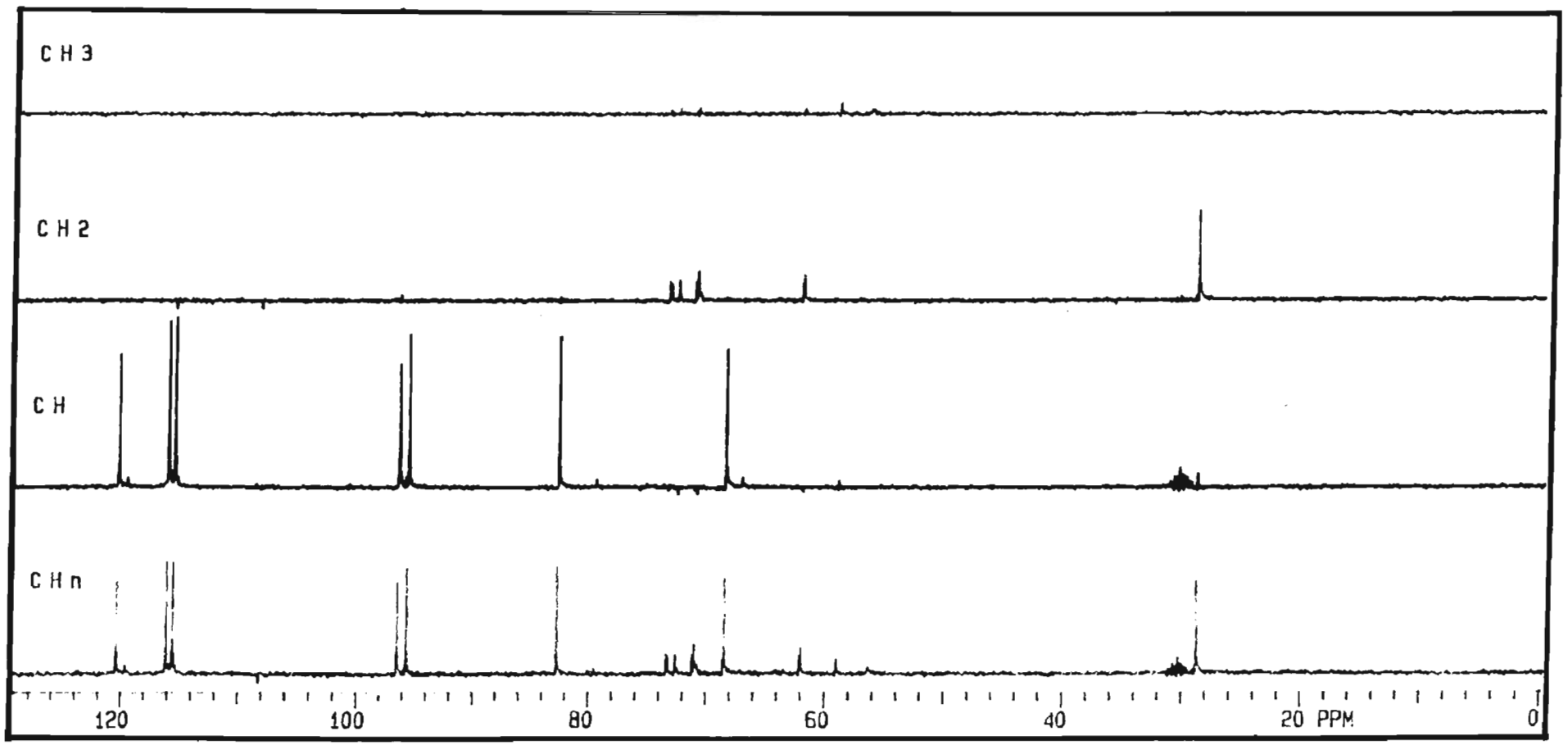


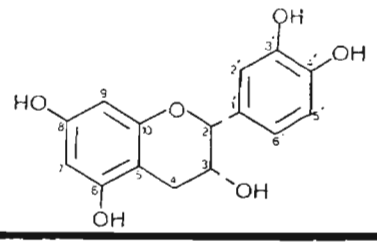
5.4.4 ^{13}C-nmr spectrum



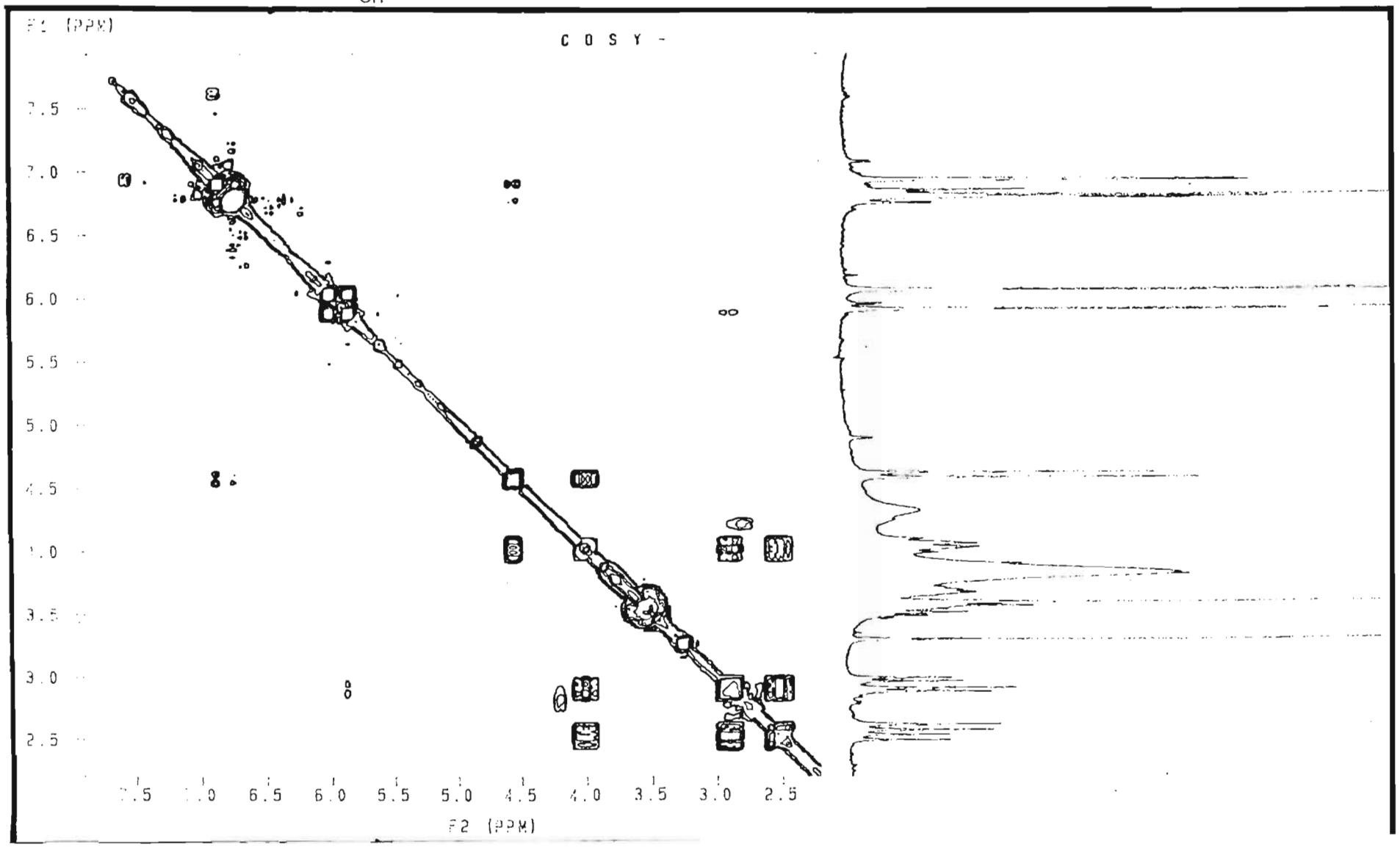
5.4.5 ^{13}C-Adept spectrum



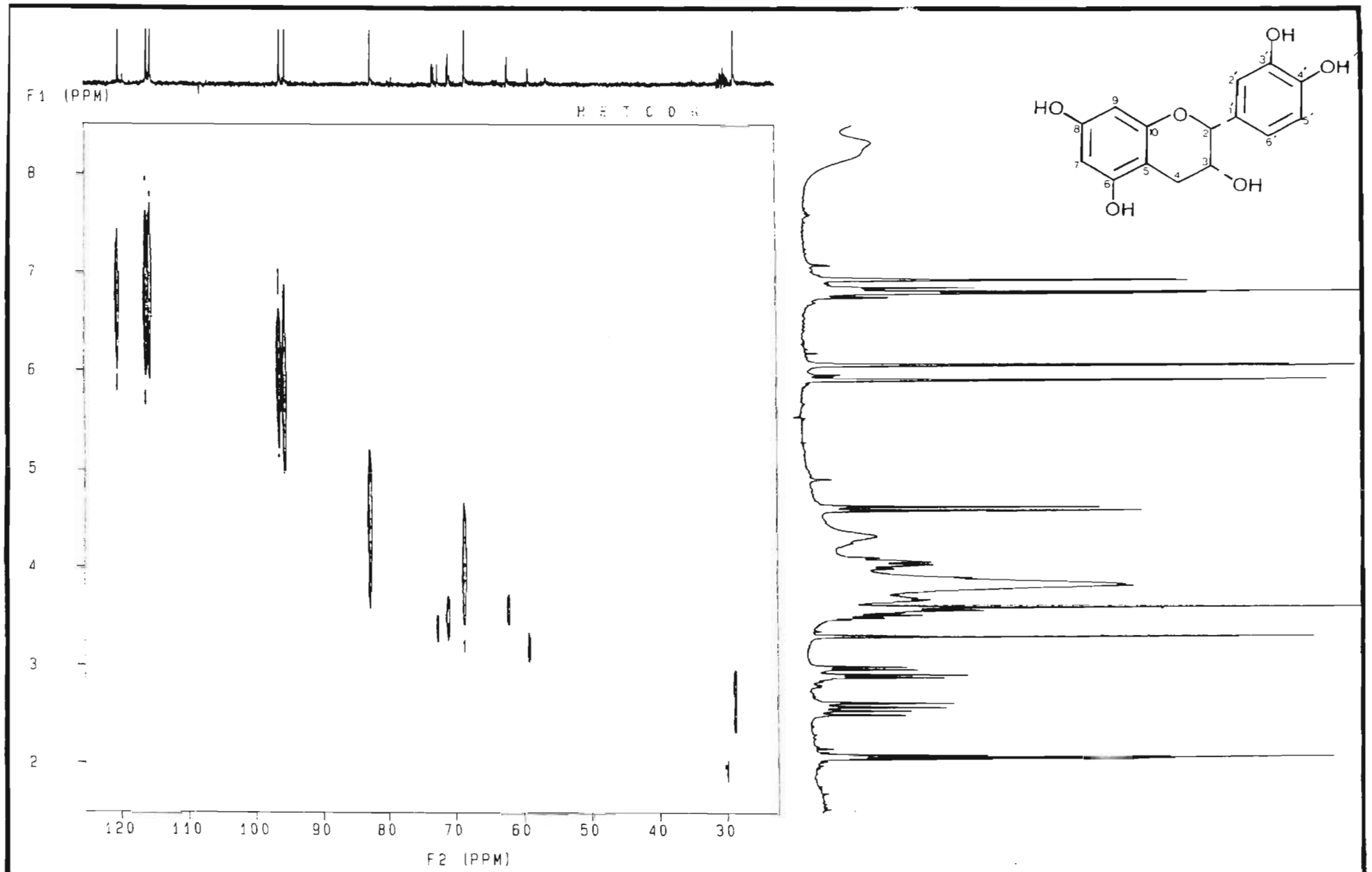




5.4.6 Cosy spectrum



5.4.7 Hetcor spectrum



5.5 KAEMPFEROL 3-O-GLUCOSIDE

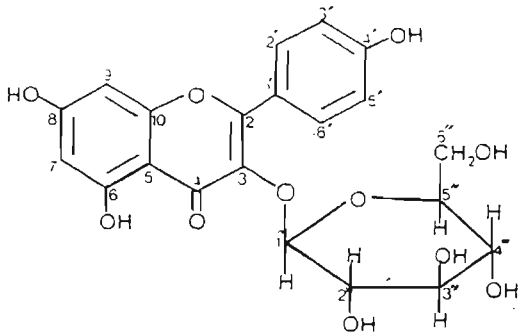
5.5.1 Infrared spectrum

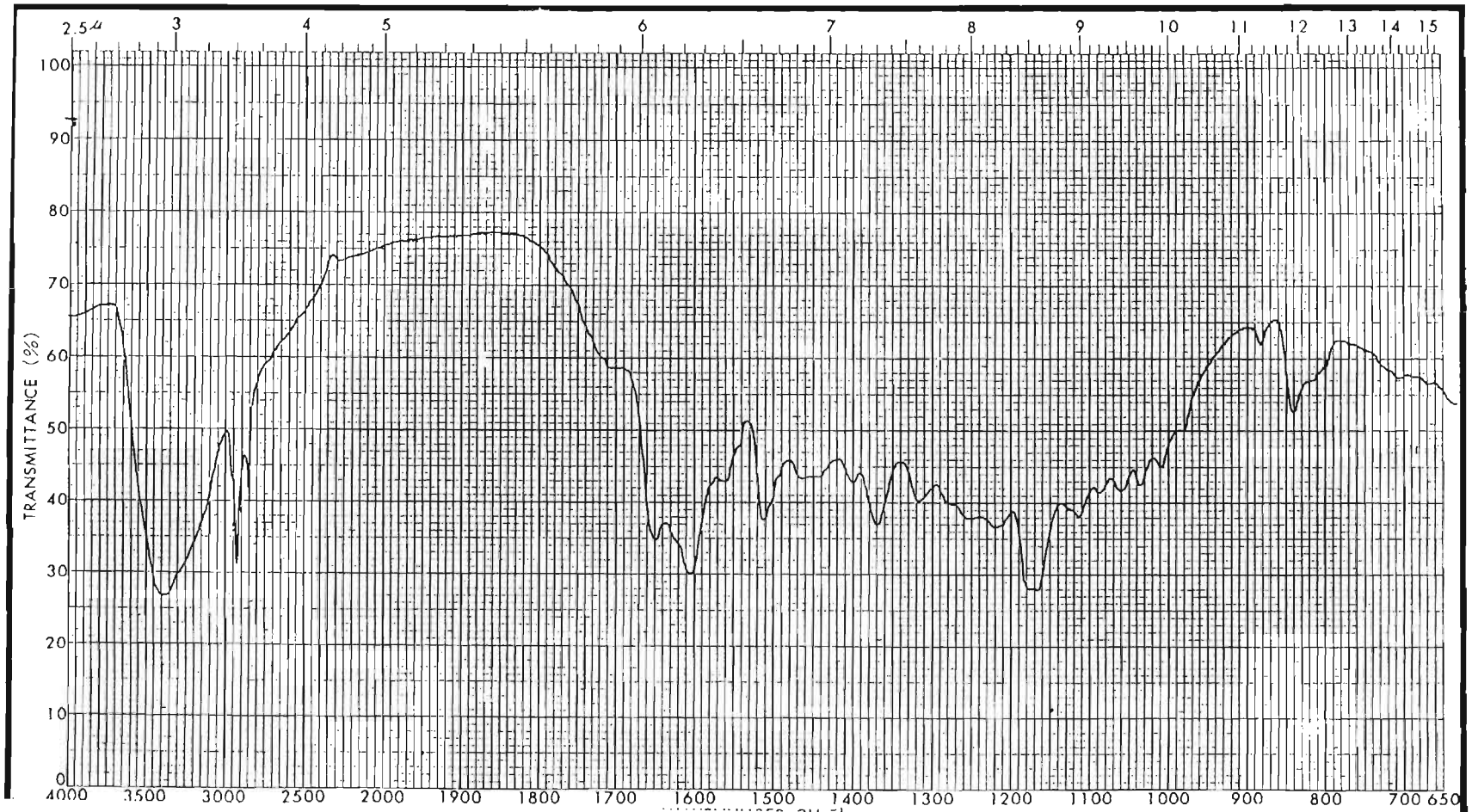
5.5.2 ^{1}H-nmr spectrum

5.5.3 ^{13}C-nmr spectrum

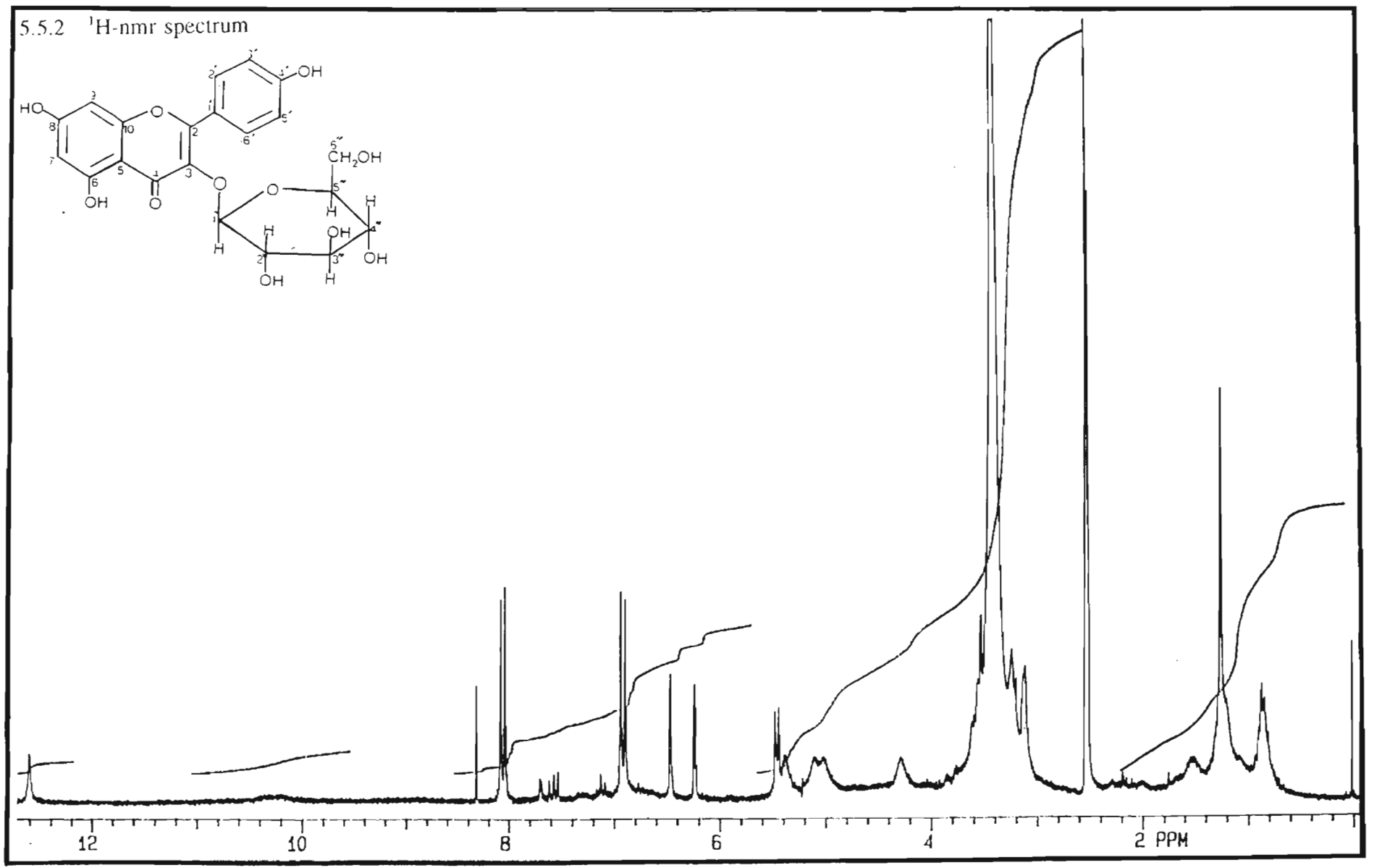
5.5.4 ^{13}C-Adept spectrum

5.5.1 Infrared spectrum

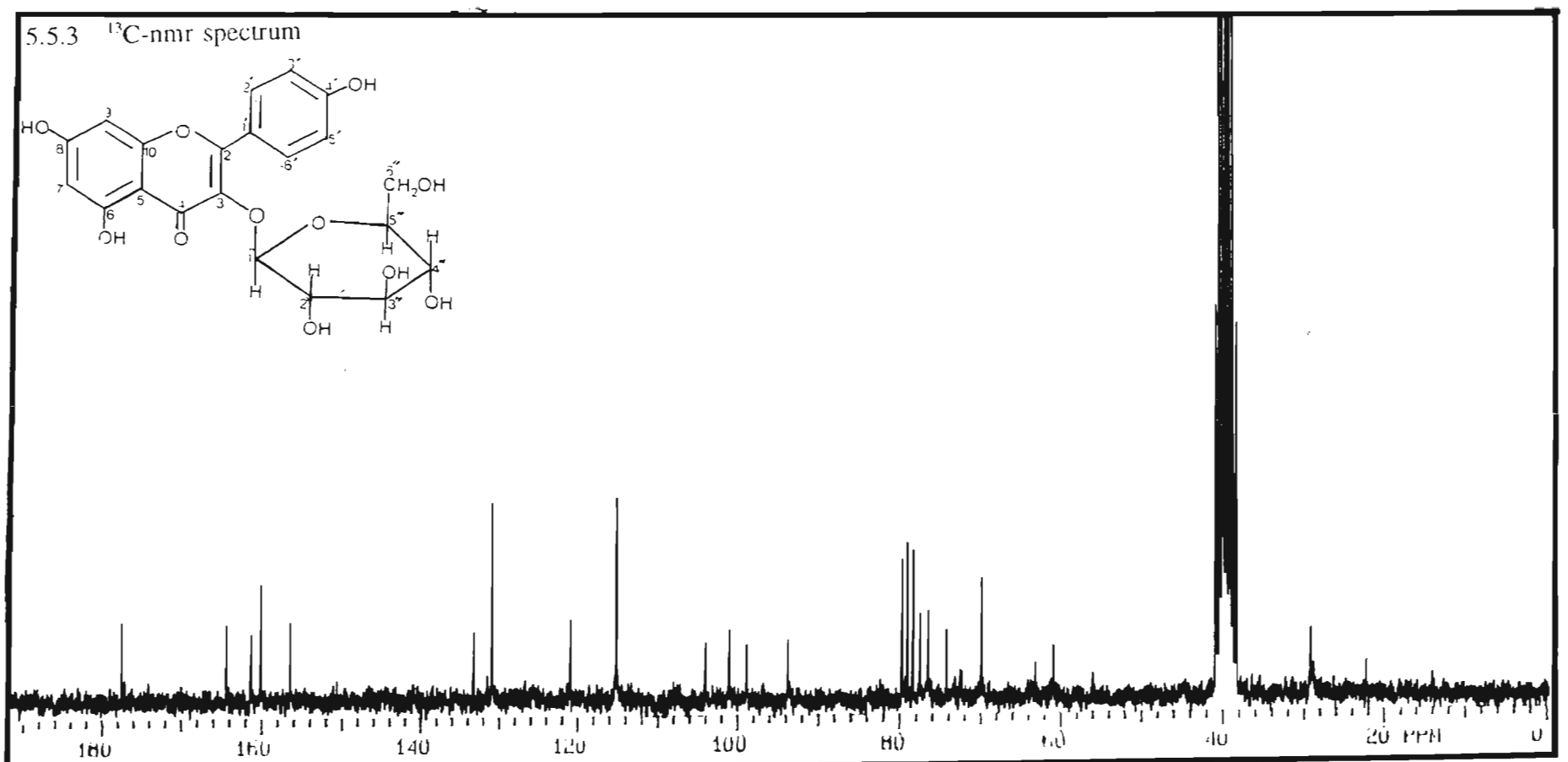




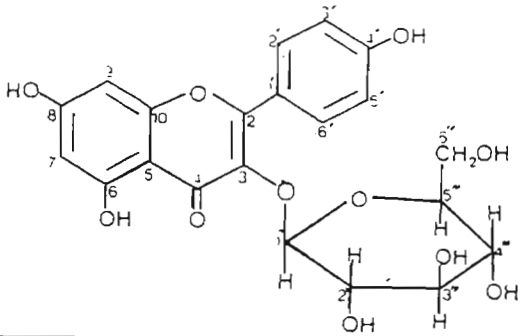
5.5.2 ^{1}H-nmr spectrum

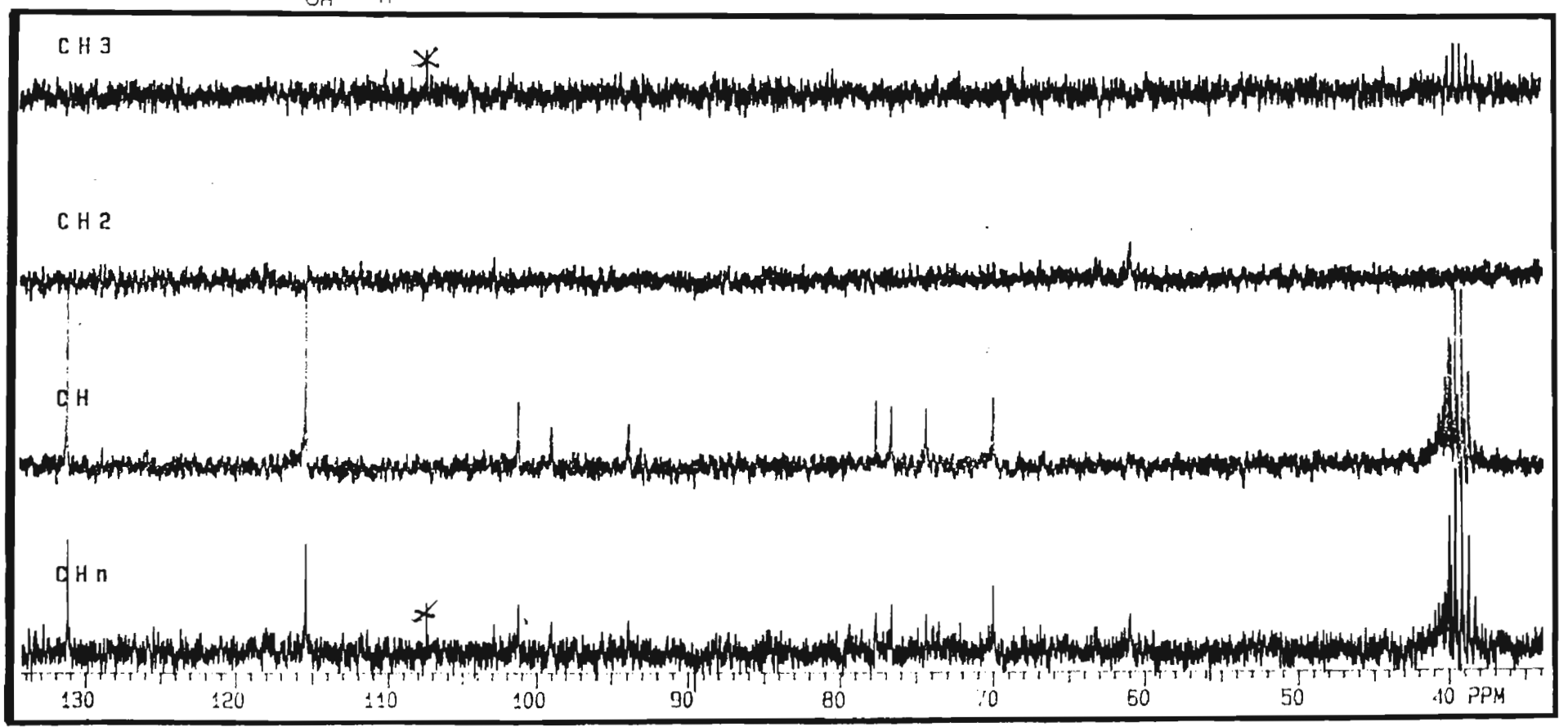


5.5.3 ^{13}C-nmr spectrum



5.5.4 ^{13}C-Adept spectrum

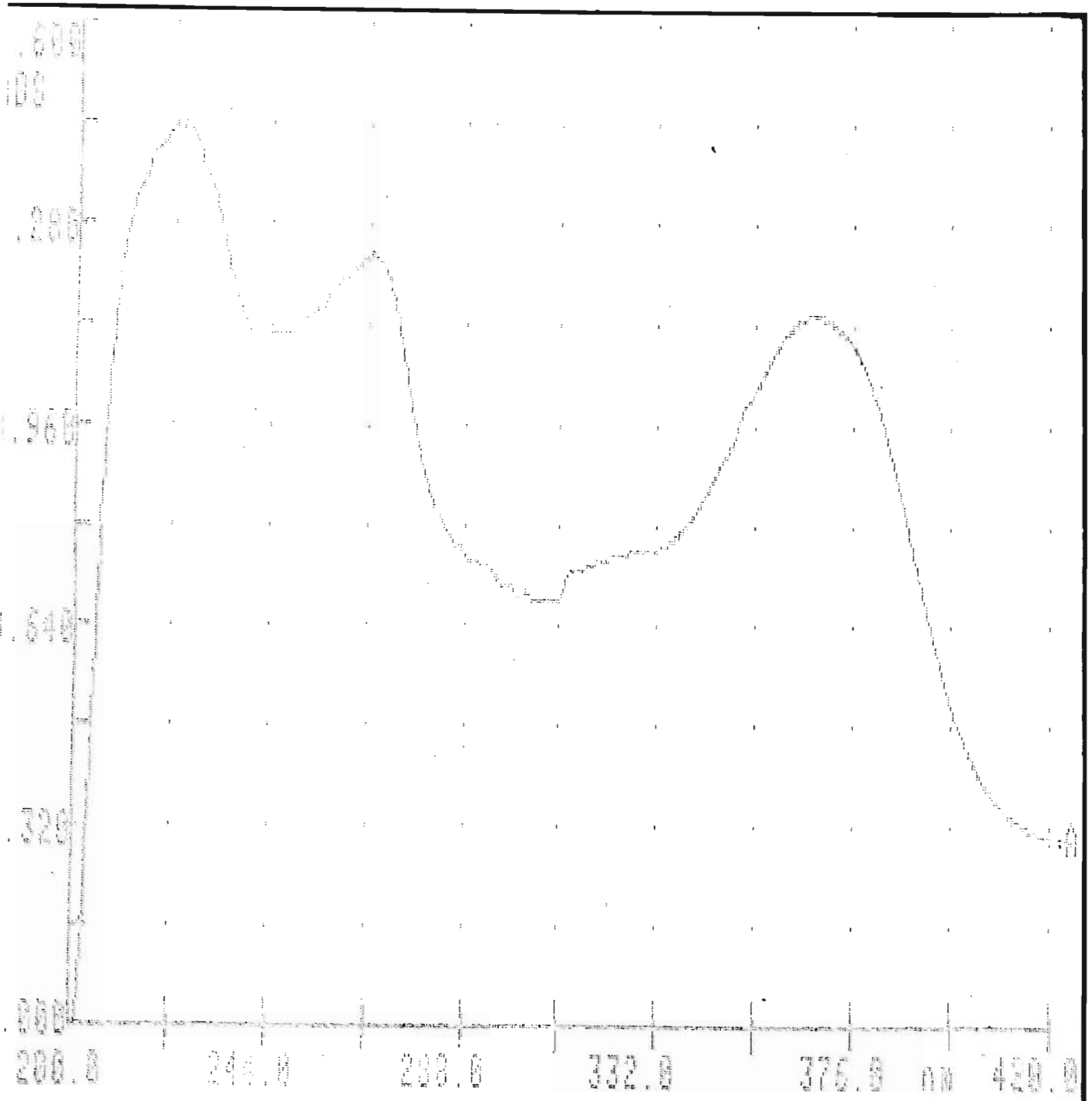




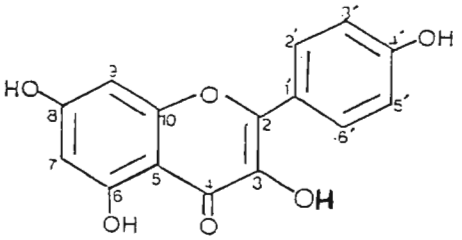
5.6 KAEMPFEROL

5.6.1 UV spectrum

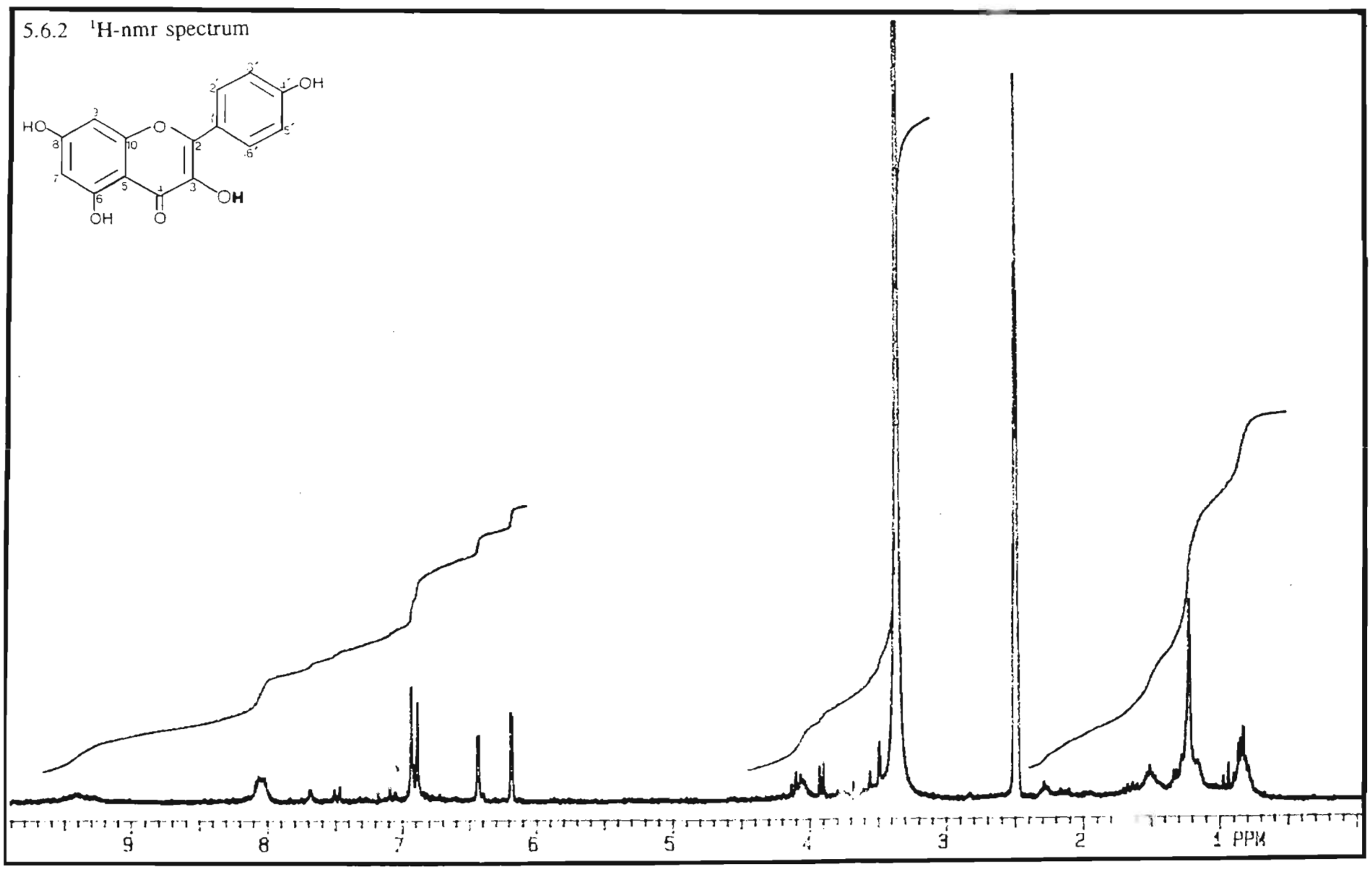
5.6.2 ^{1}H-nmr spectrum



5.6.1 UV spectrum



5.6.2 ^{1}H-nmr spectrum



5.7 COMPOUND E

5.7.1 Infrared spectrum

5.7.2 Mass spectrum

5.7.3 UV spectrum

5.7.4 ^{1}H-nmr spectrum

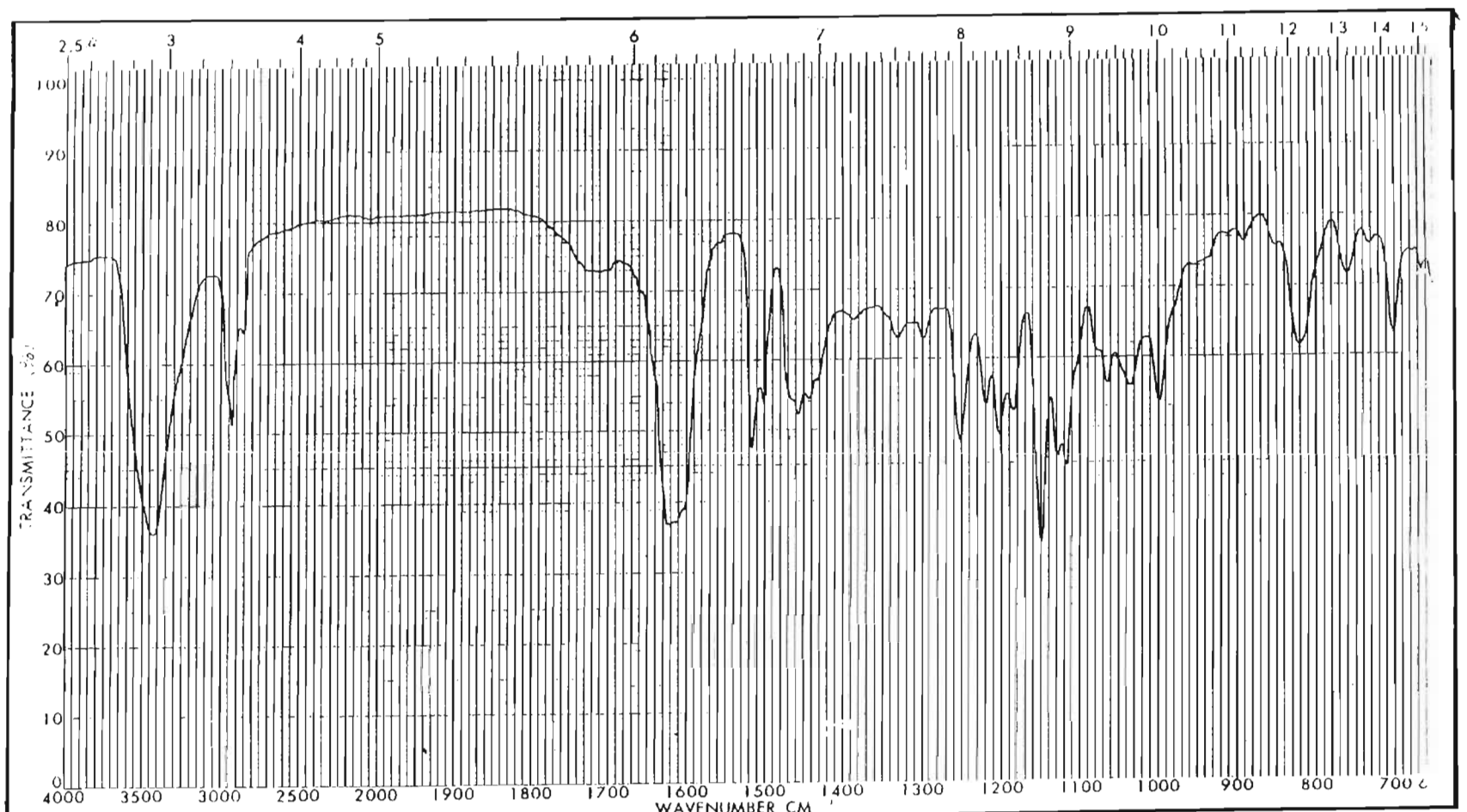
5.7.5 ^{13}C-nmr spectrum

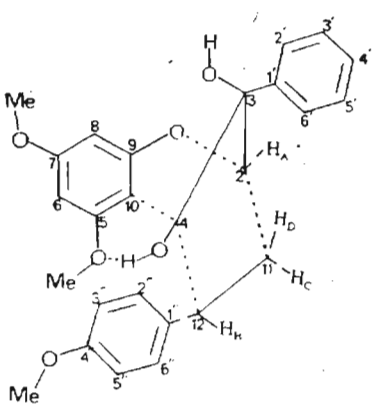
5.7.6 ^{13}C-Adept spectrum

5.7.7 Cosy spectrum

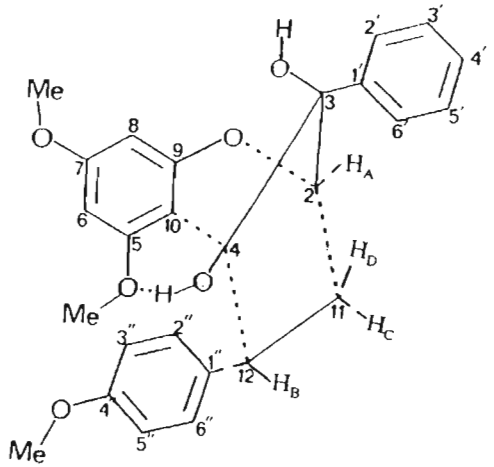
5.7.8 Hetcor spectrum

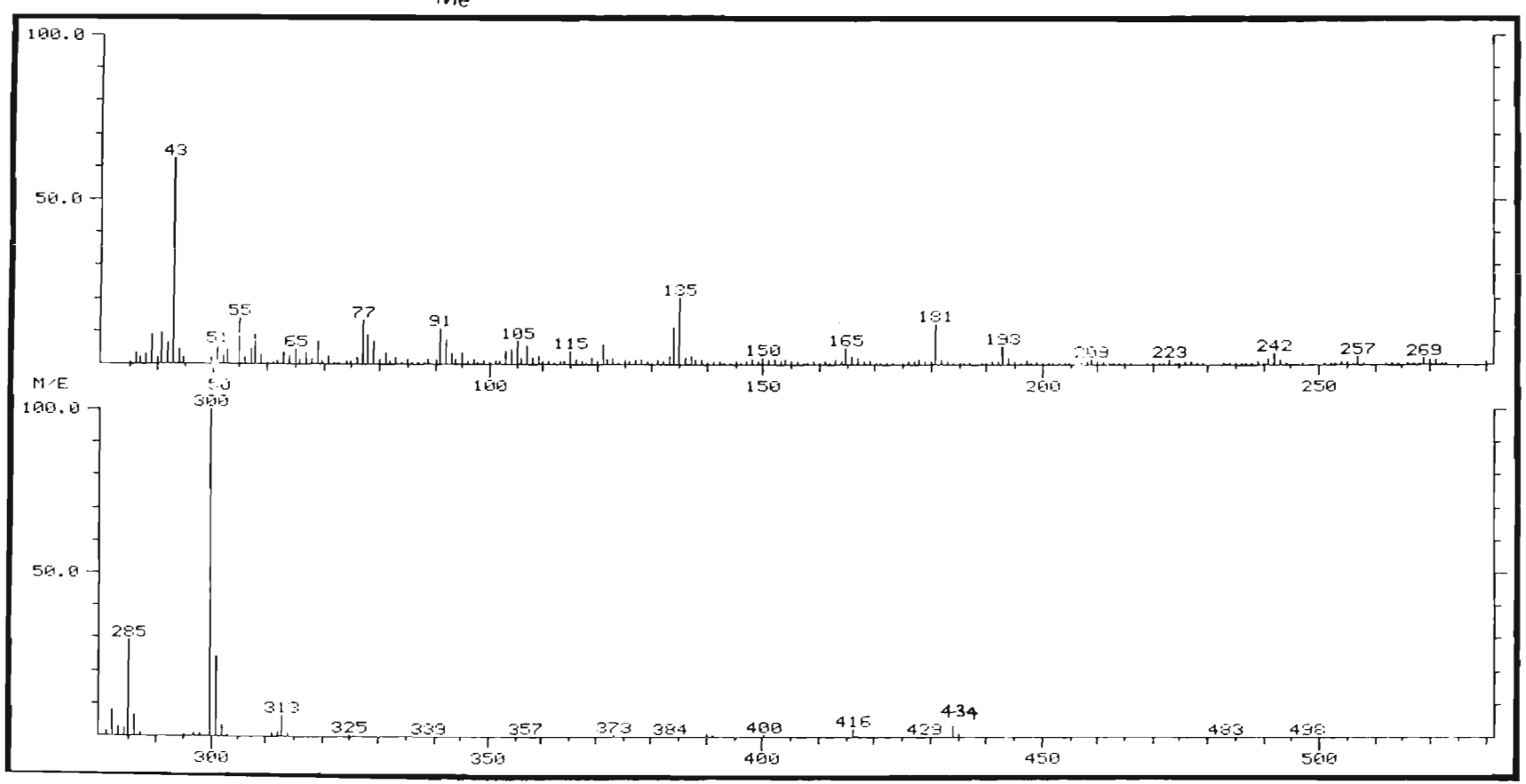
5.7.1 Infrared spectrum



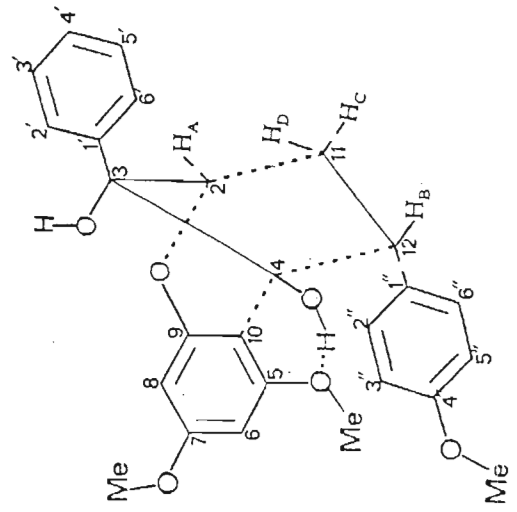


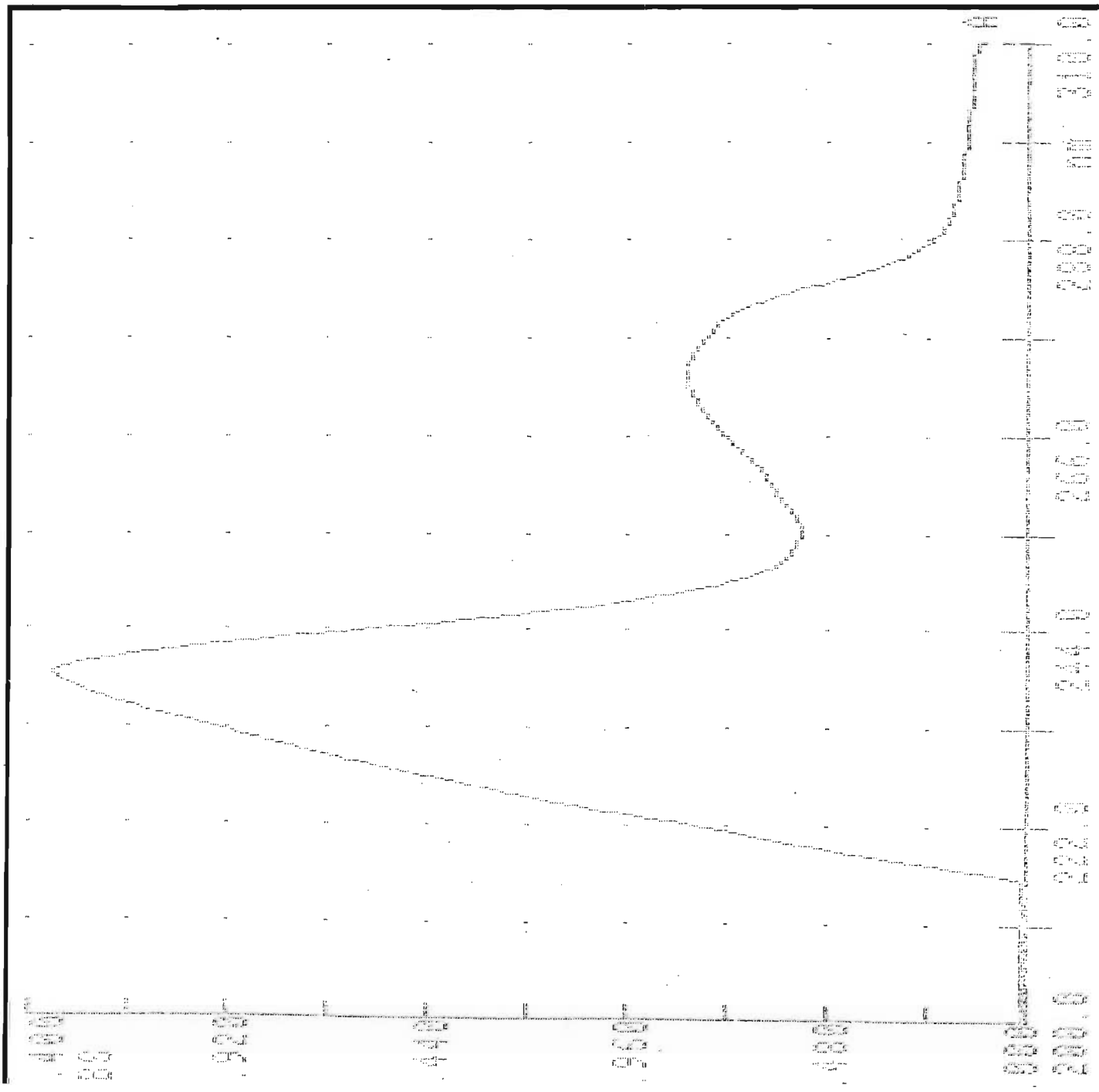
5.7.2 Mass spectrum



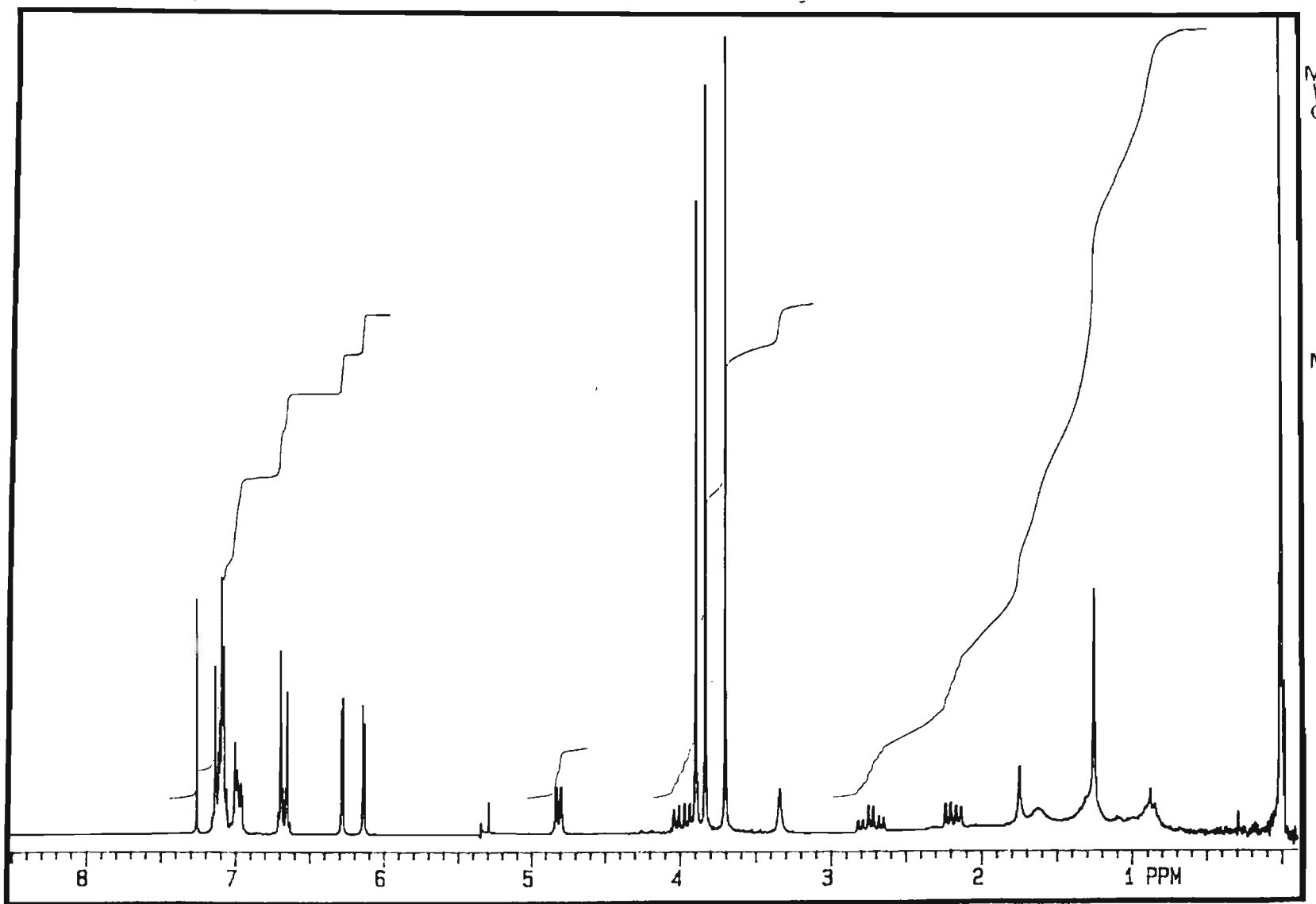


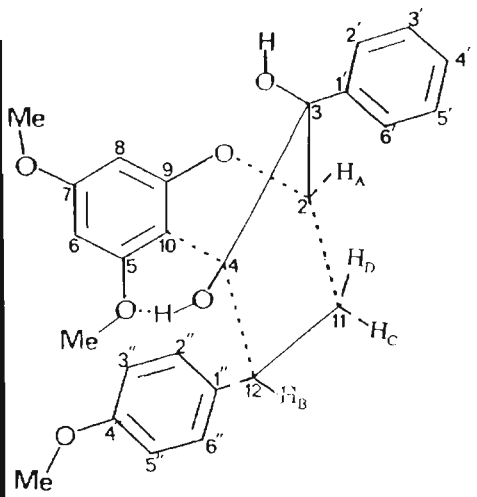
5.7.3 UV spectrum



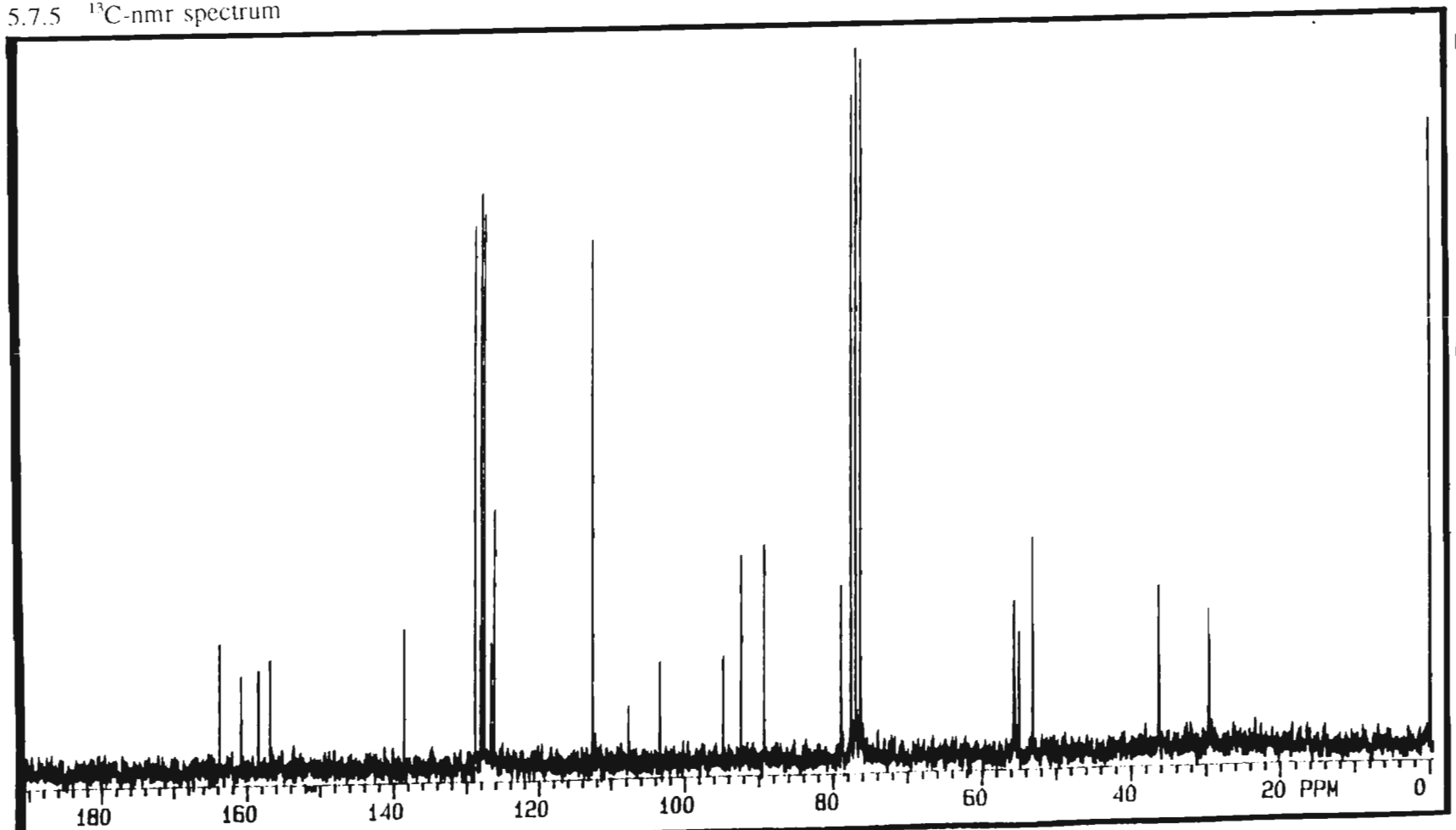


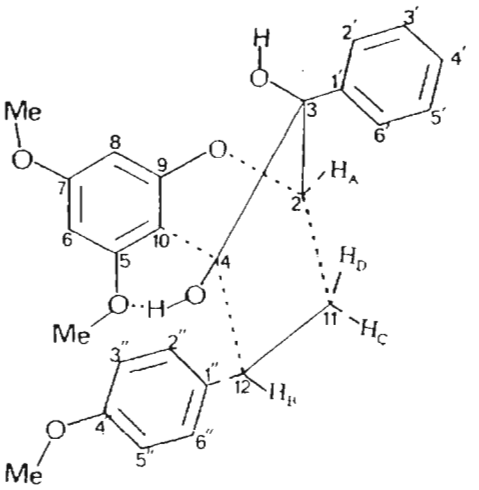
5.7.4 ^{1}H-nmr spectrum

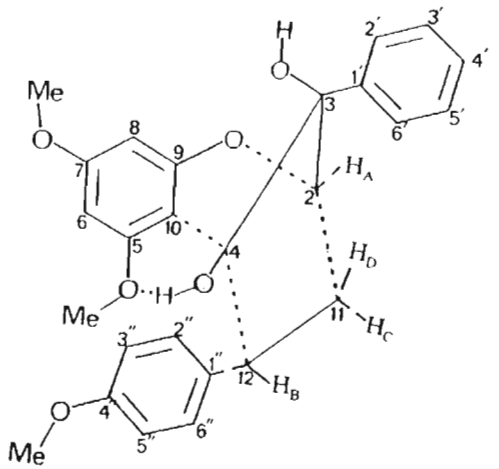




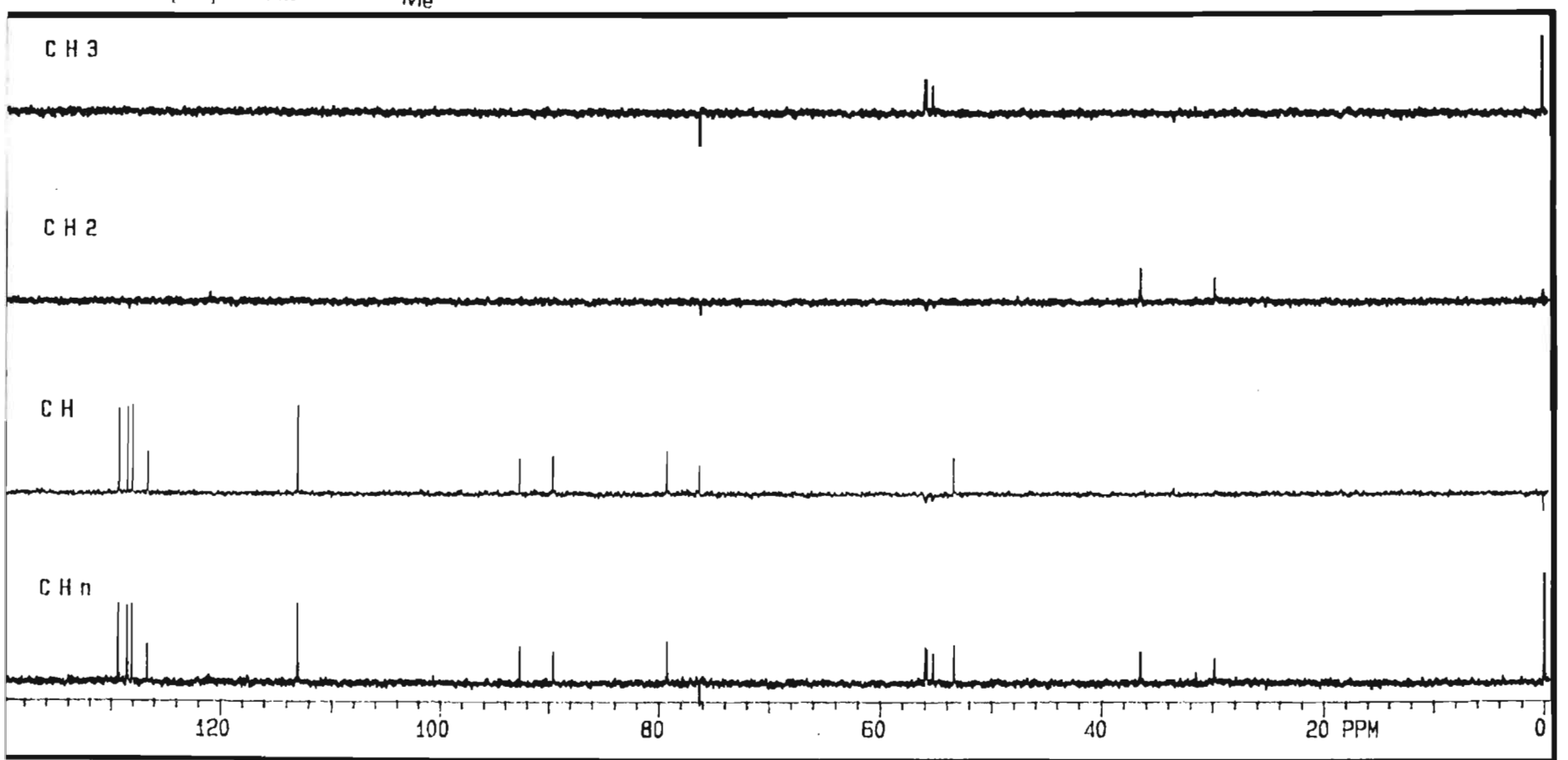
5.7.5 ^{13}C-nmr spectrum



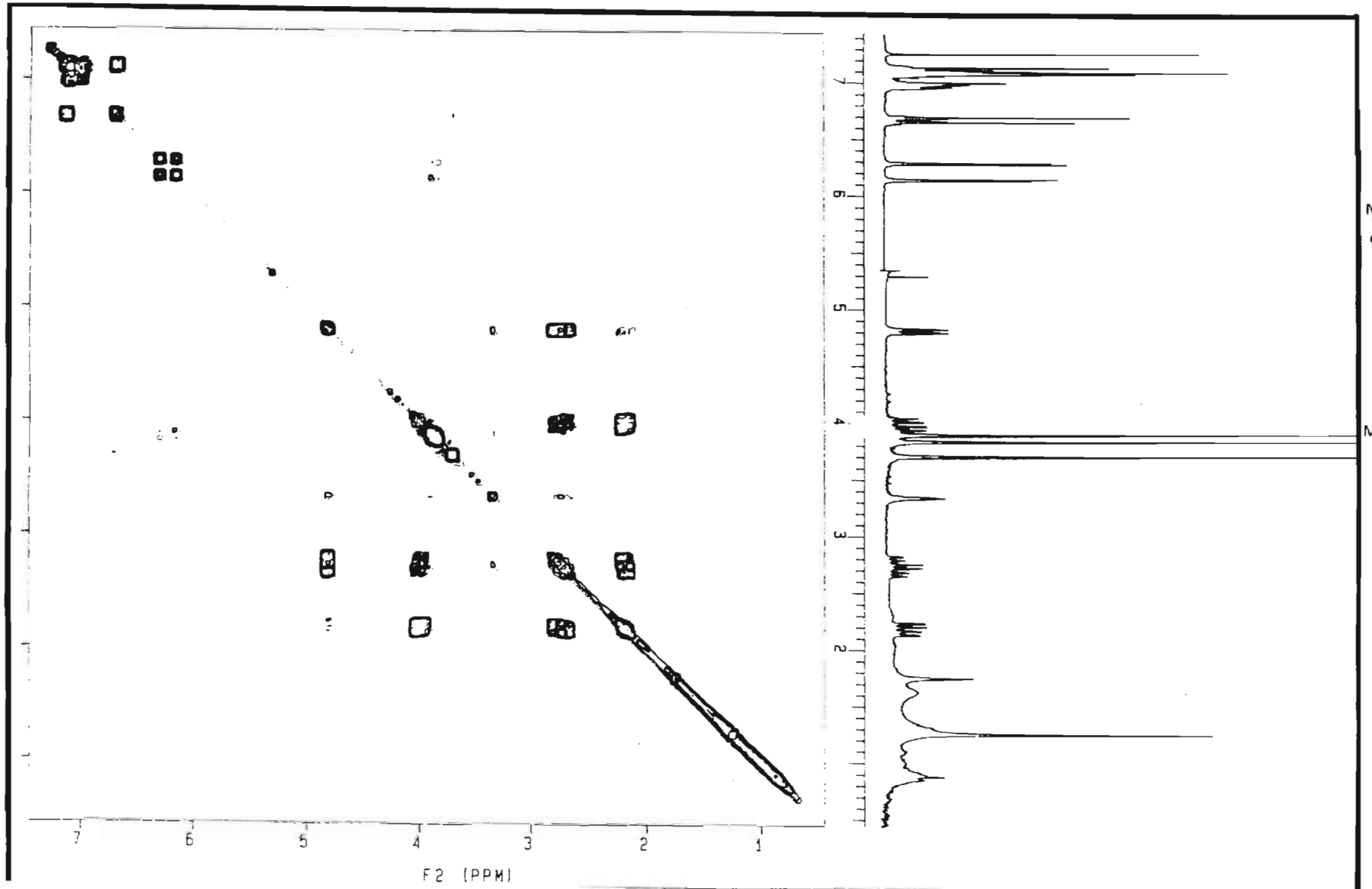


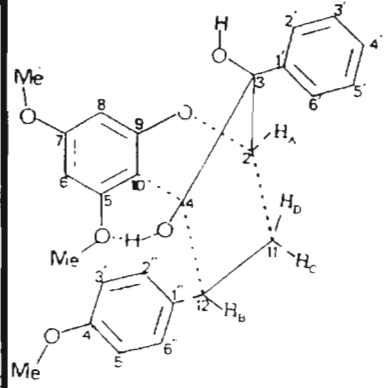


5.7.6 ^{13}C-Adept spectrum

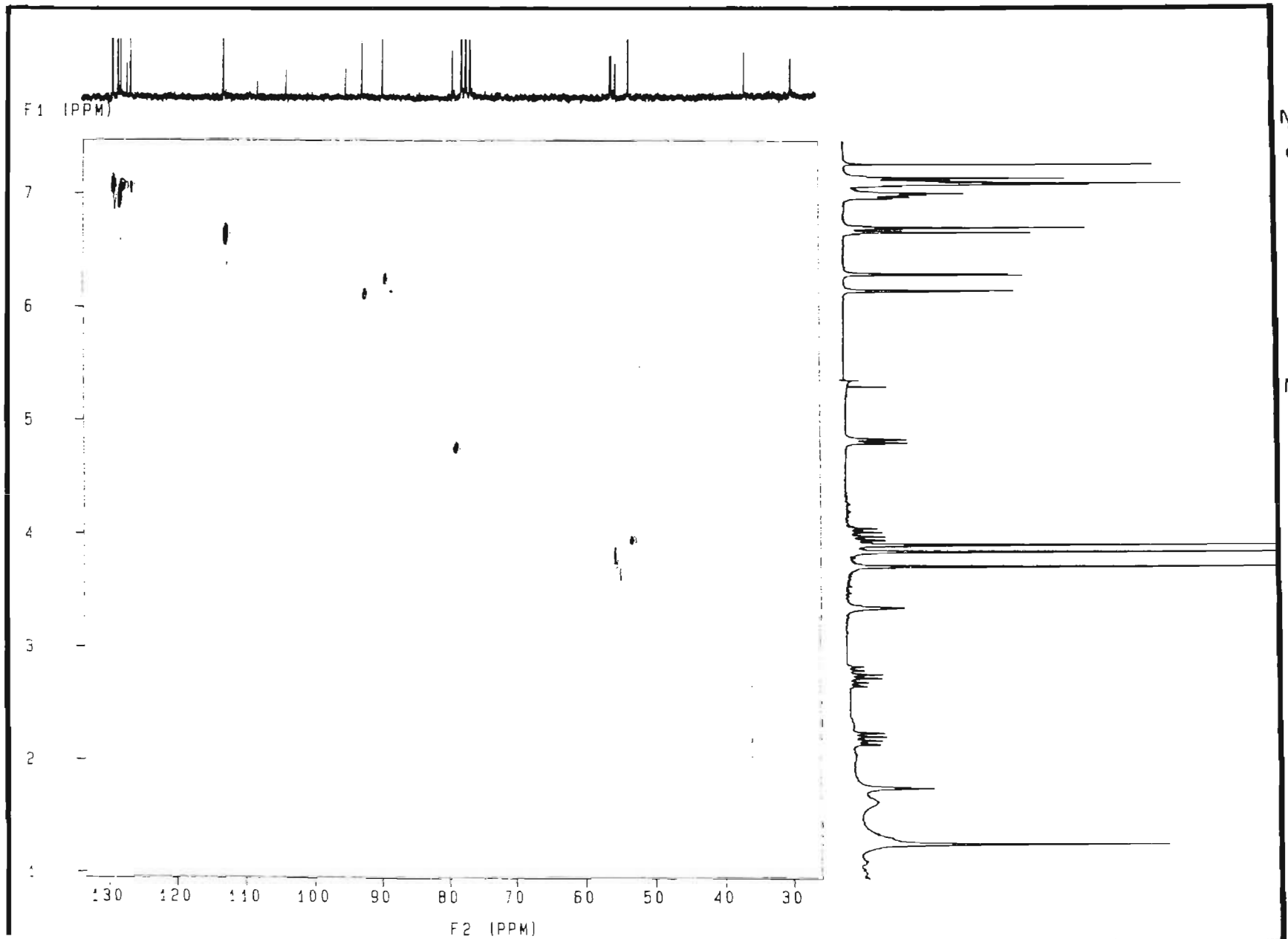


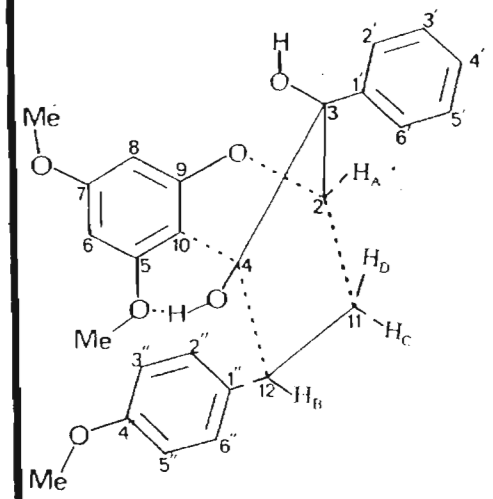
5.7.7 Cosy spectrum



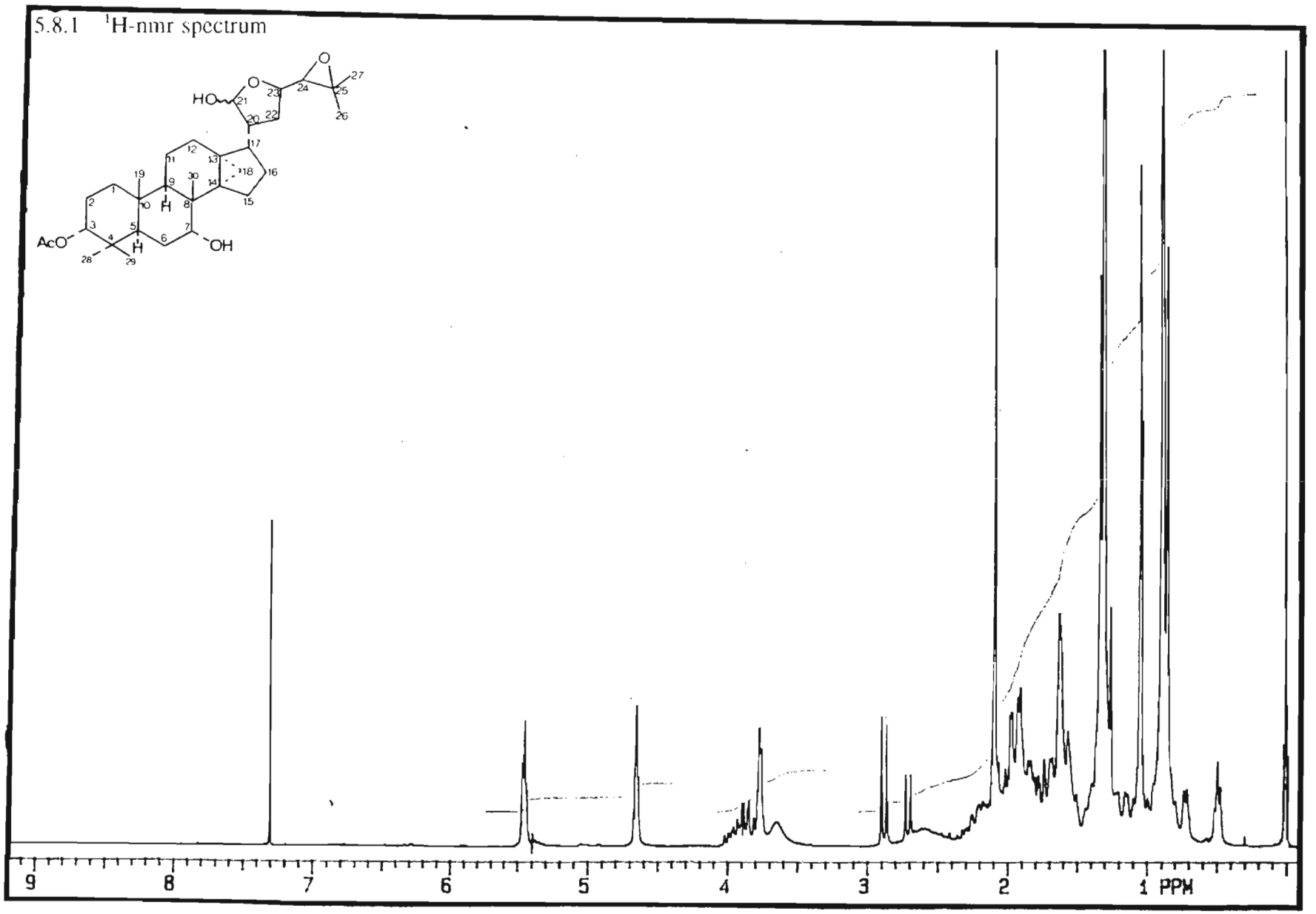


5.7.8 Hetcor spectrum

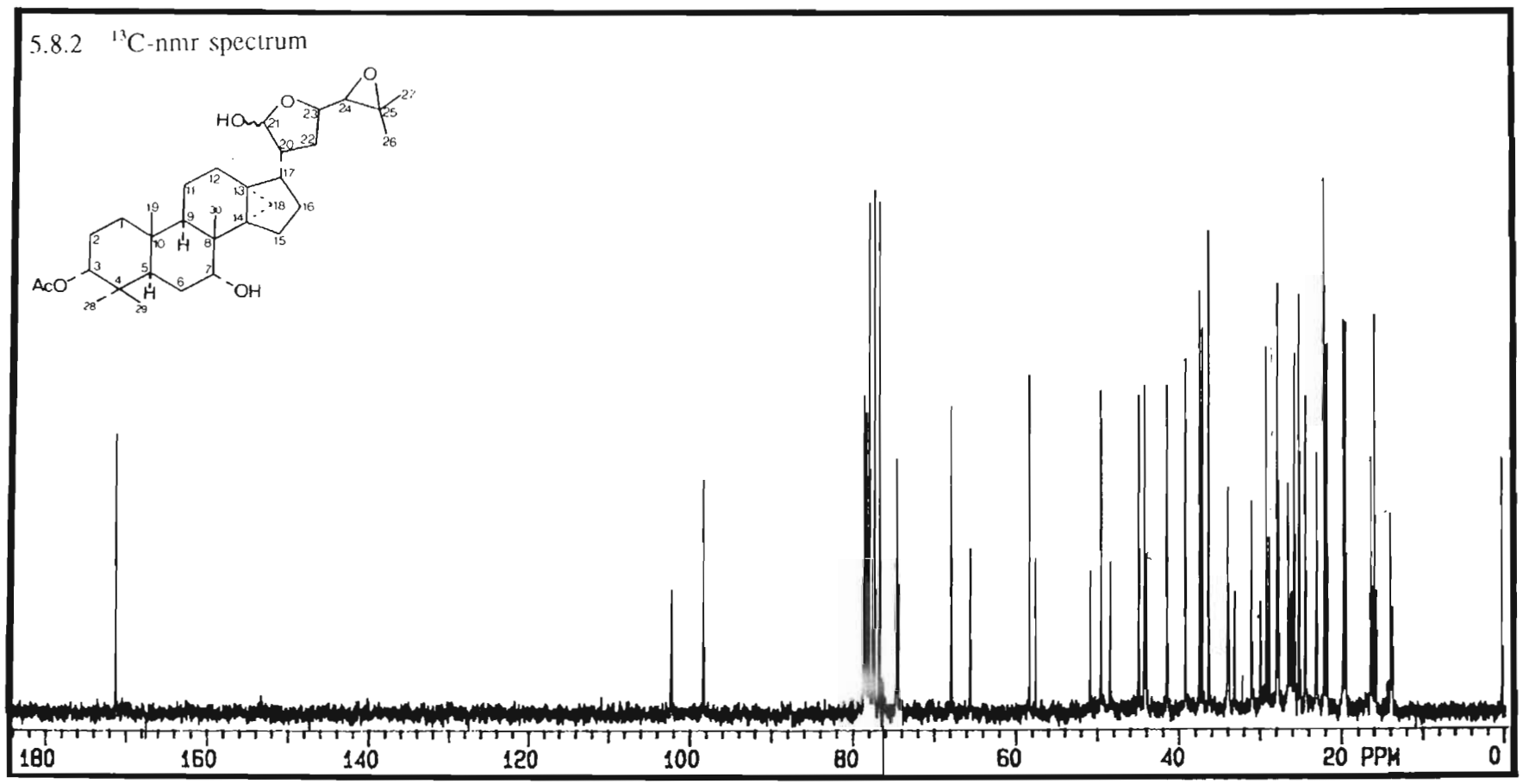




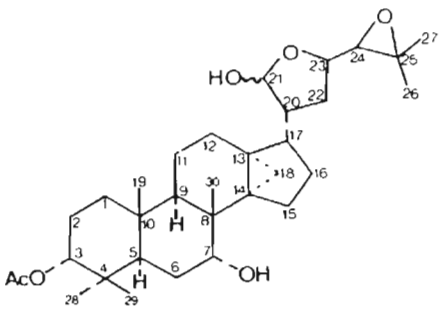
5.8.1 ^{1}H-nmr spectrum

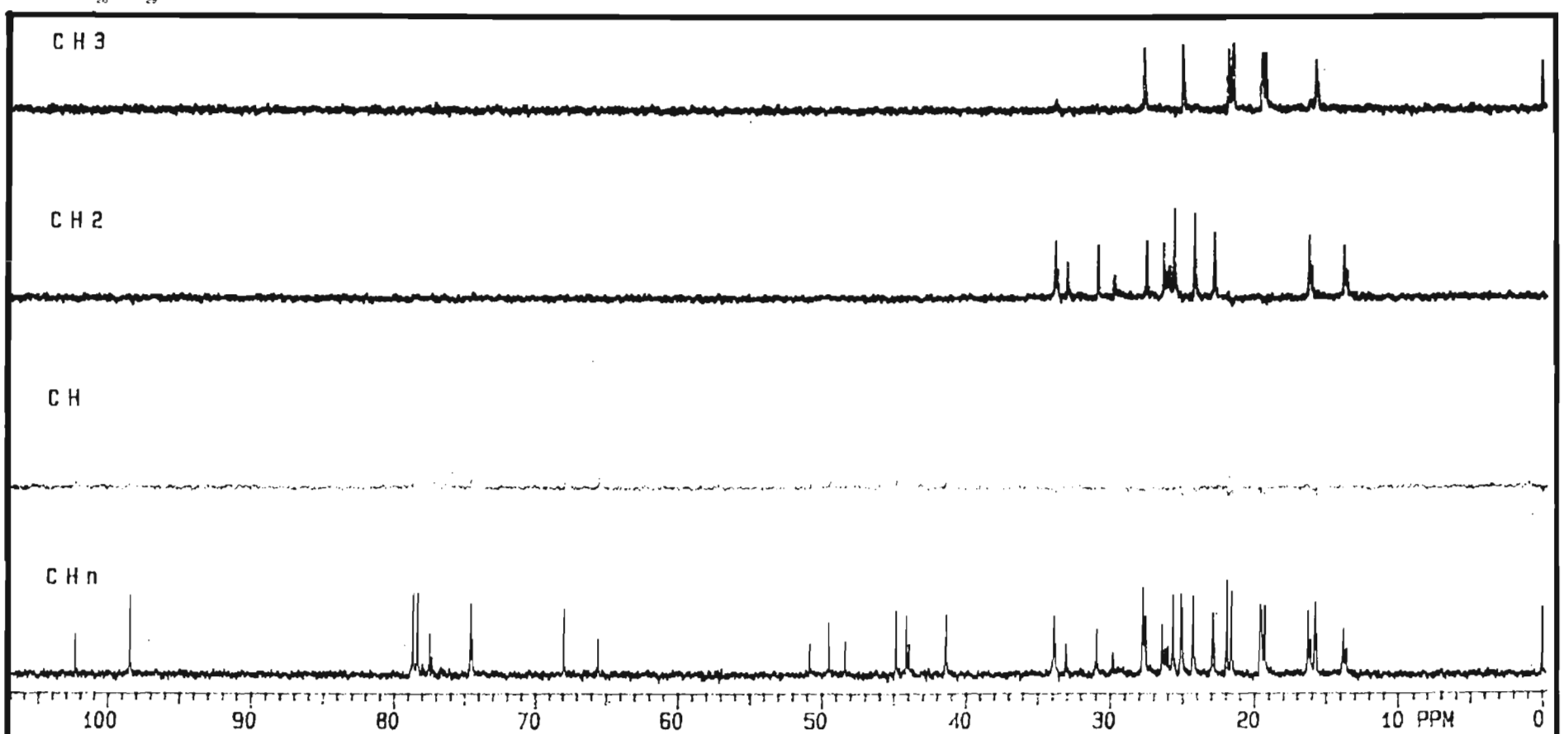


5.8.2 ^{13}C-nmr spectrum

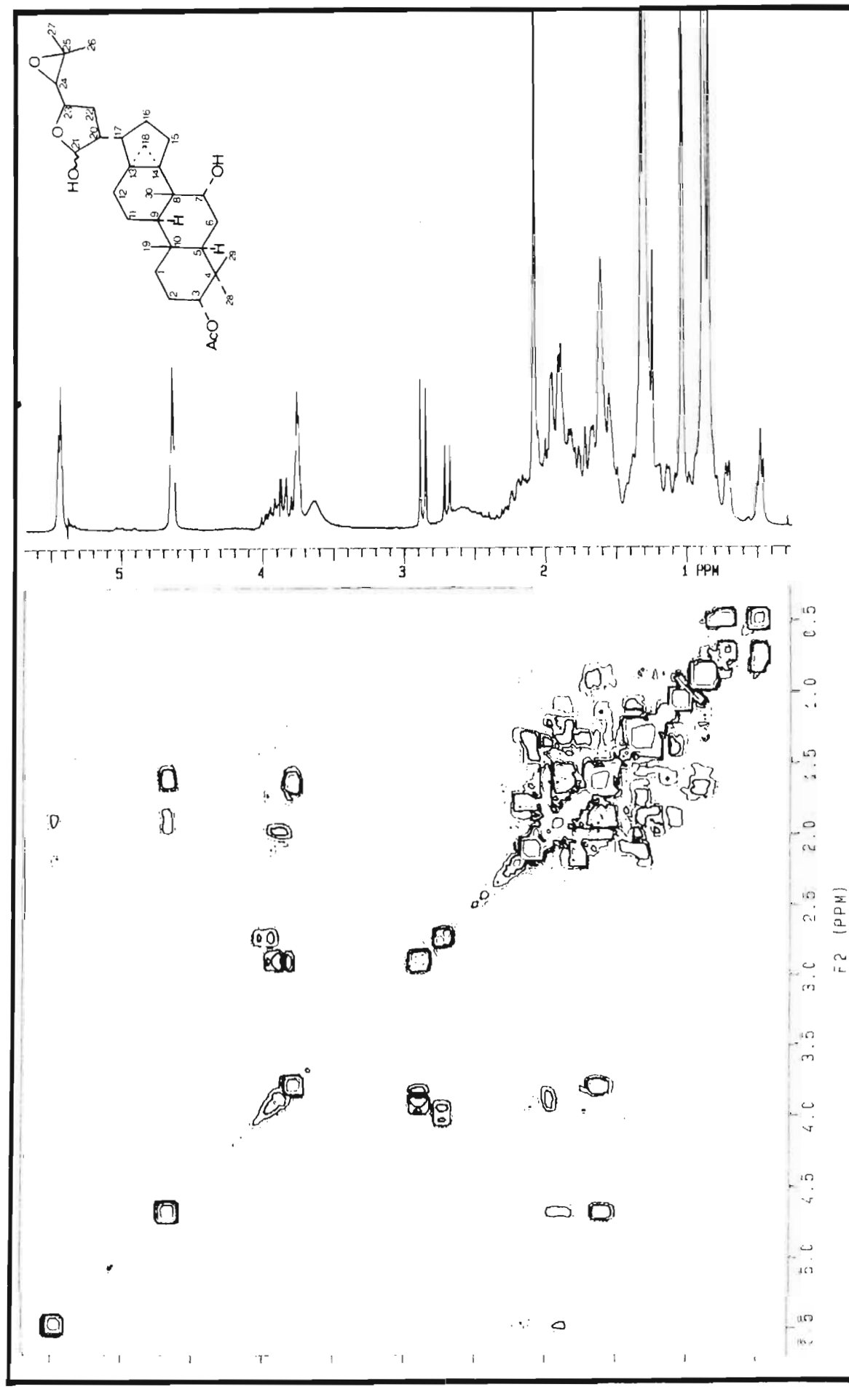


5.8.3 ^{13}C-Adept spectrum

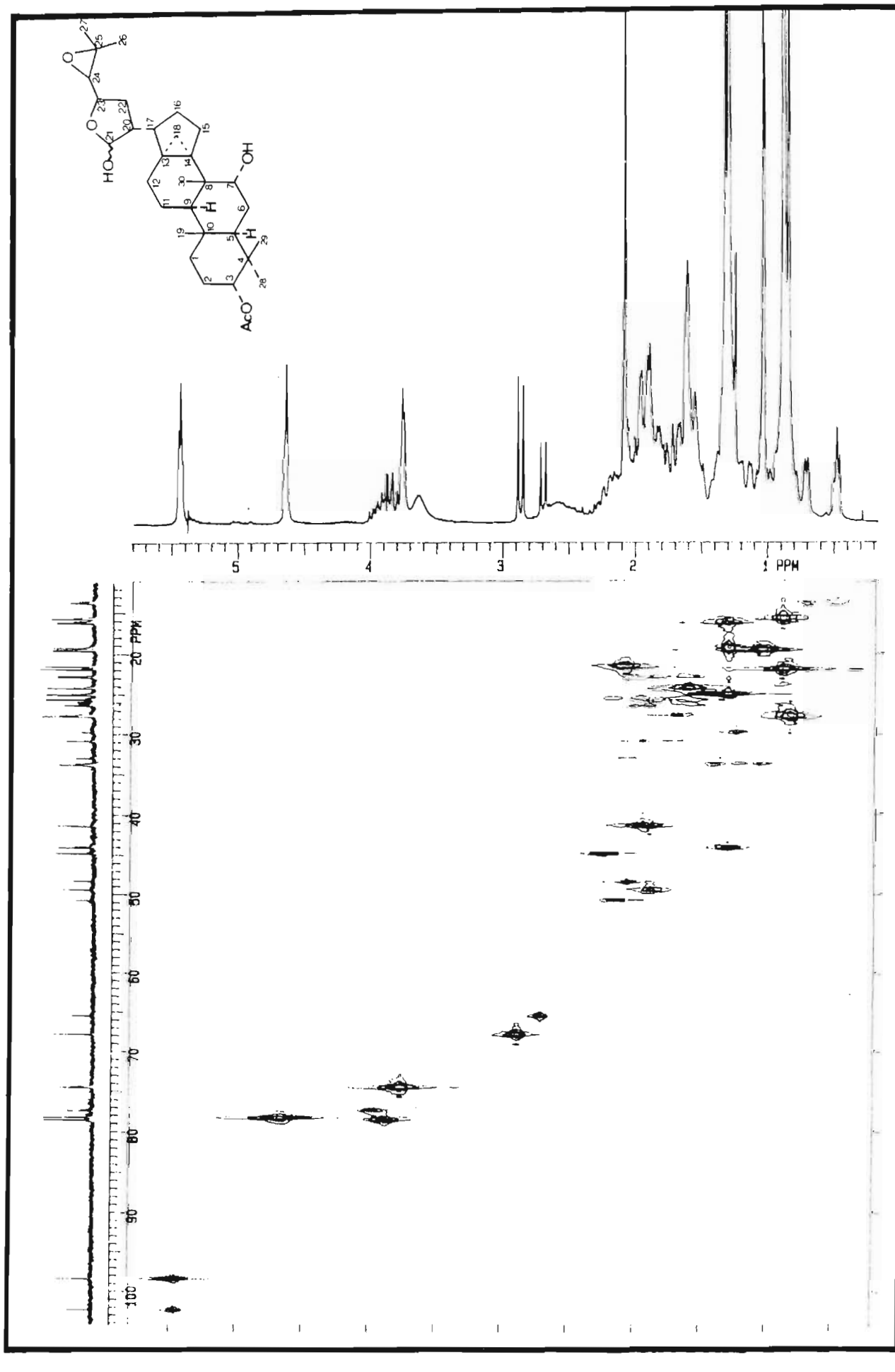




5.8.4 Cosy spectrum



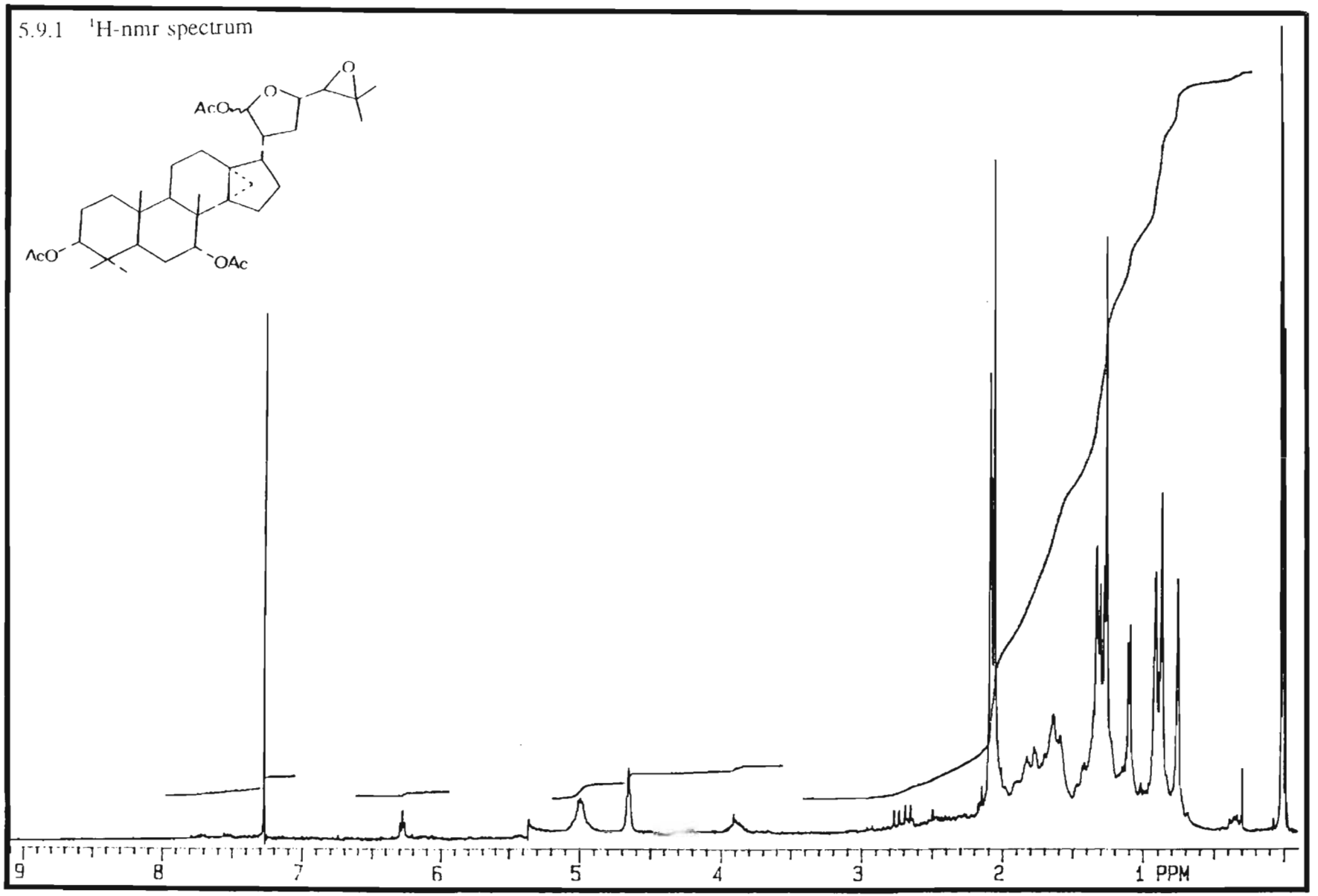
5.8.5 Hetcor spectrum



5.9 COMPOUND F-ACETATE

5.9.1 ^{1}H-nmr spectrum

5.9.1 ^{1}H-nmr spectrum



5.10 COMPOUND G

5.10.1 Infrared spectrum

5.10.2 Mass spectrum

5.10.3 ^{1}H-nmr spectrum

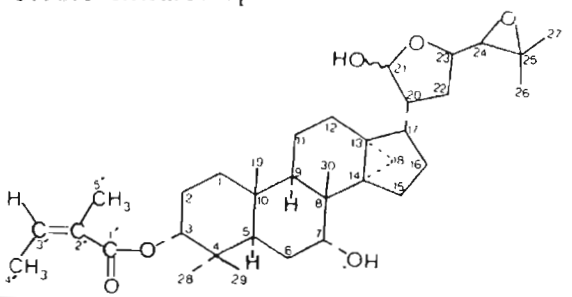
5.10.4 ^{13}C-nmr spectrum

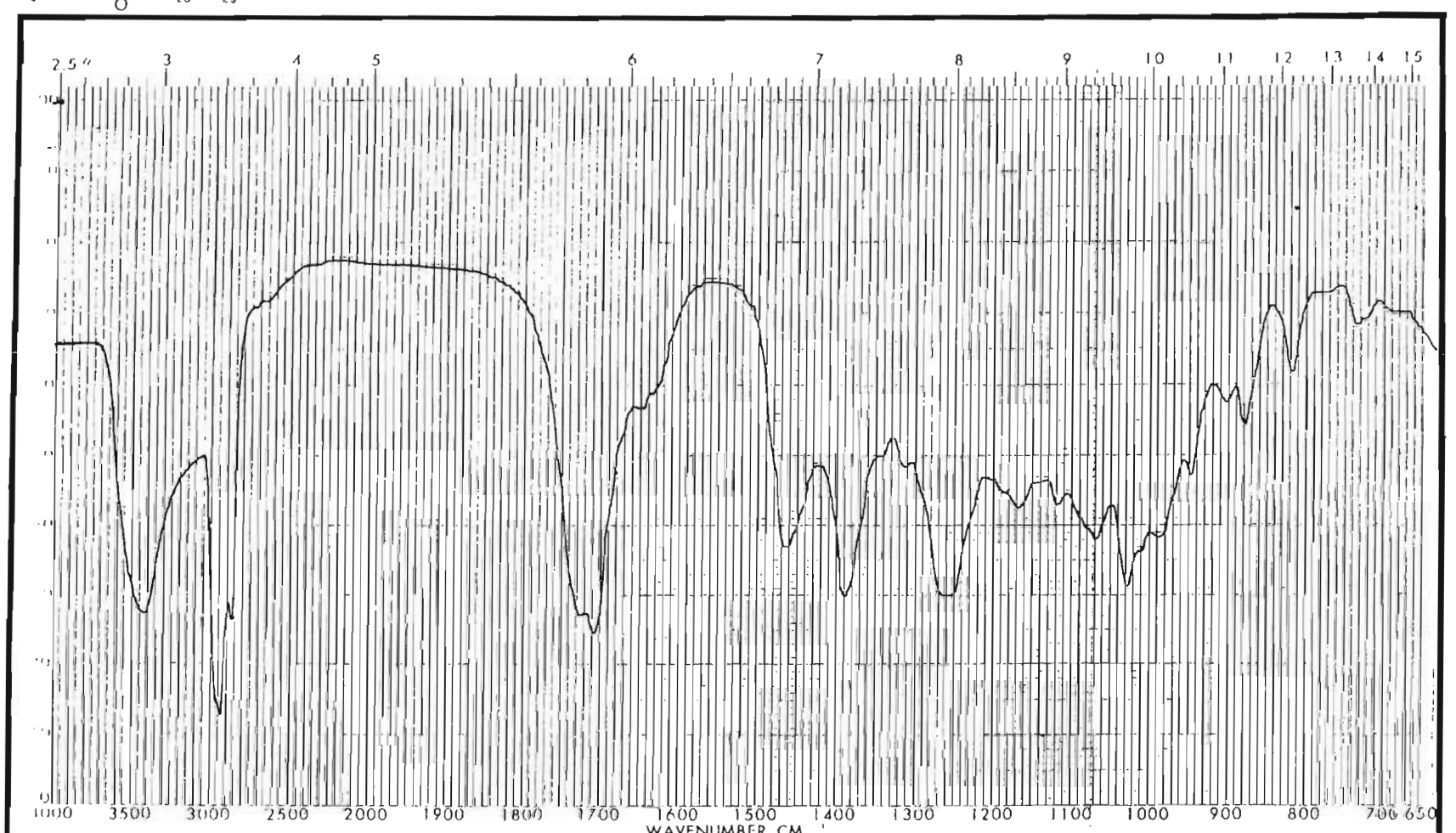
5.10.5 ^{13}C-Adept spectrum

5.10.6 Cosy spectrum

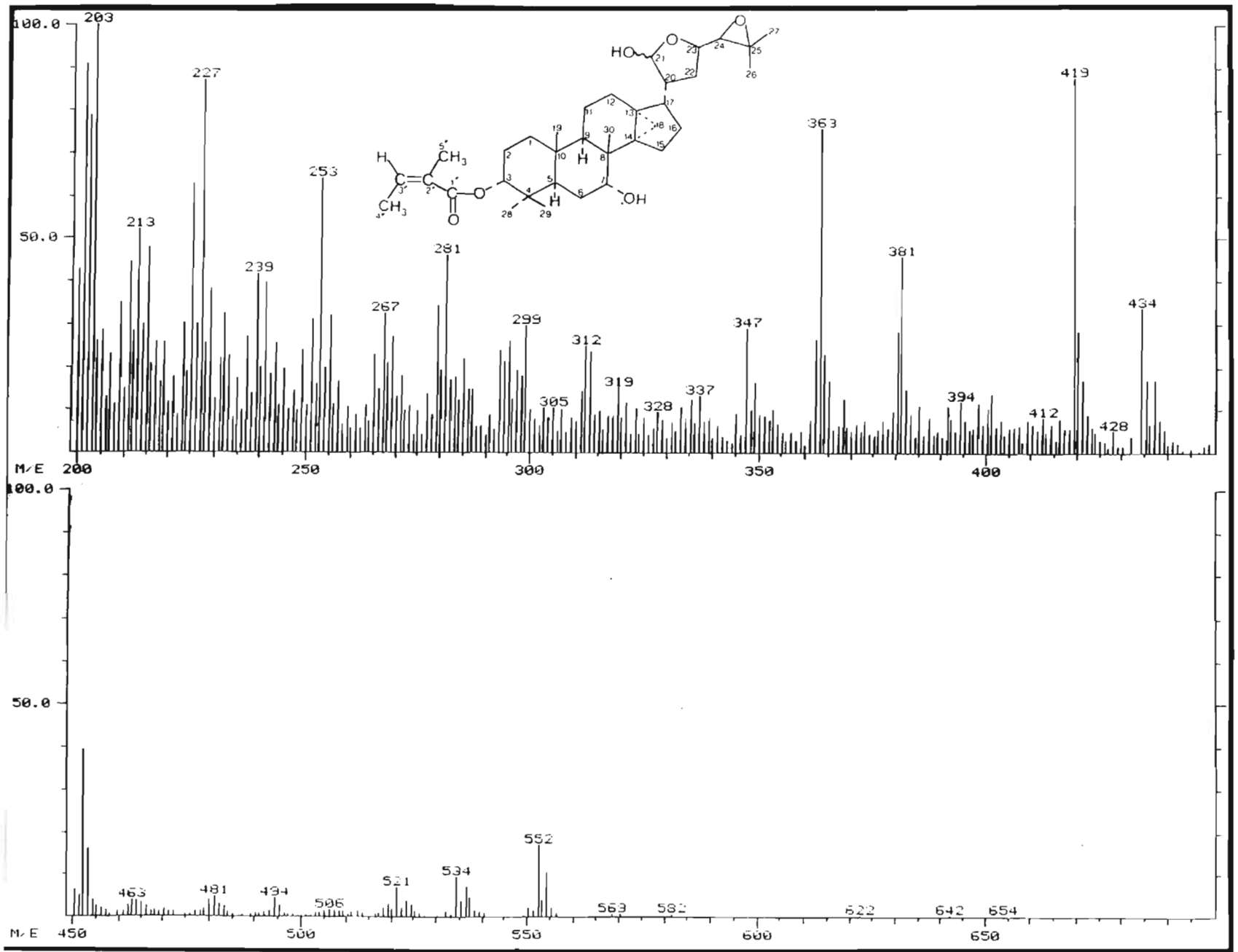
5.10.7 Hetcor spectrum

5.10.1 Infrared spectrum

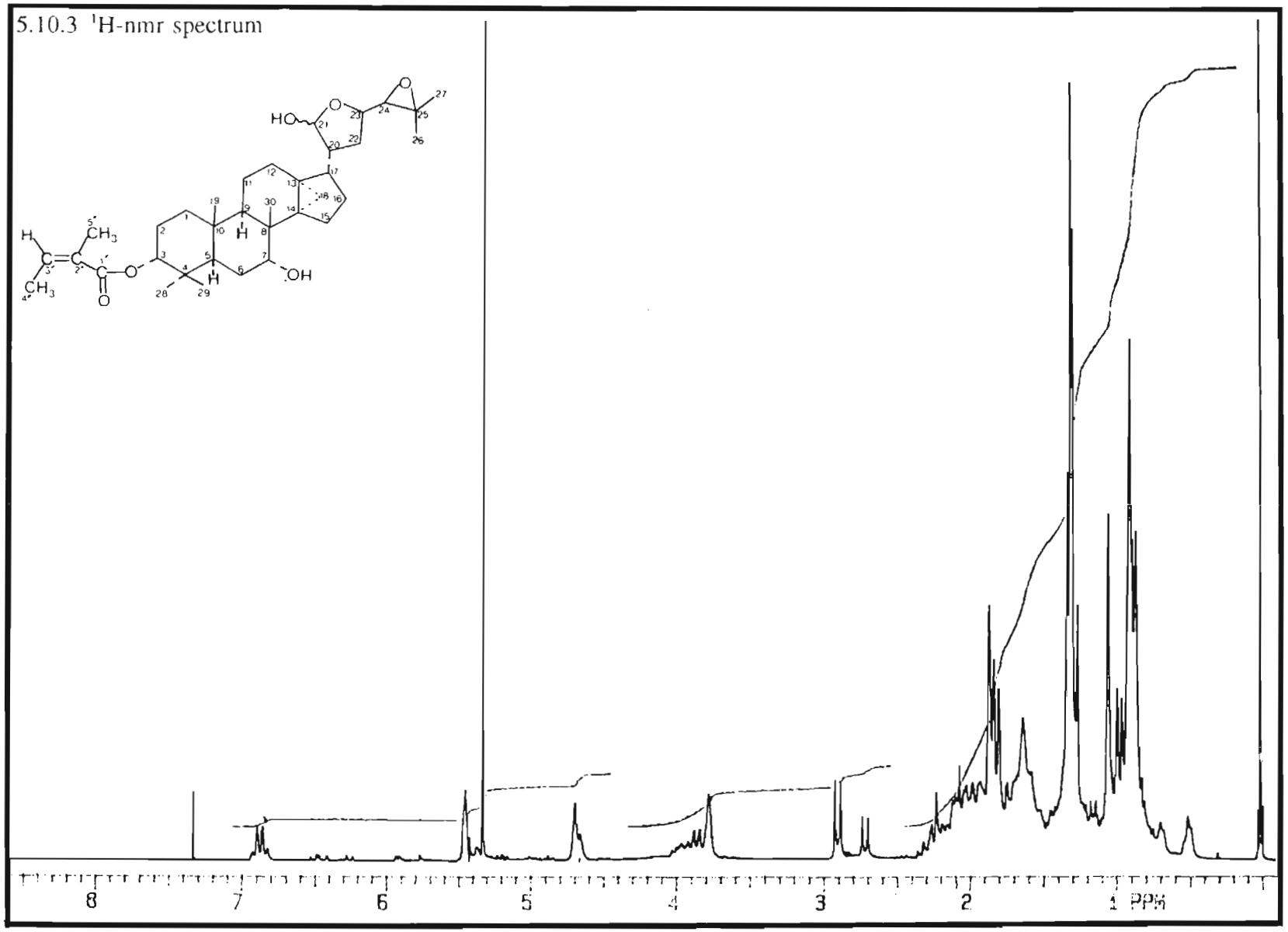




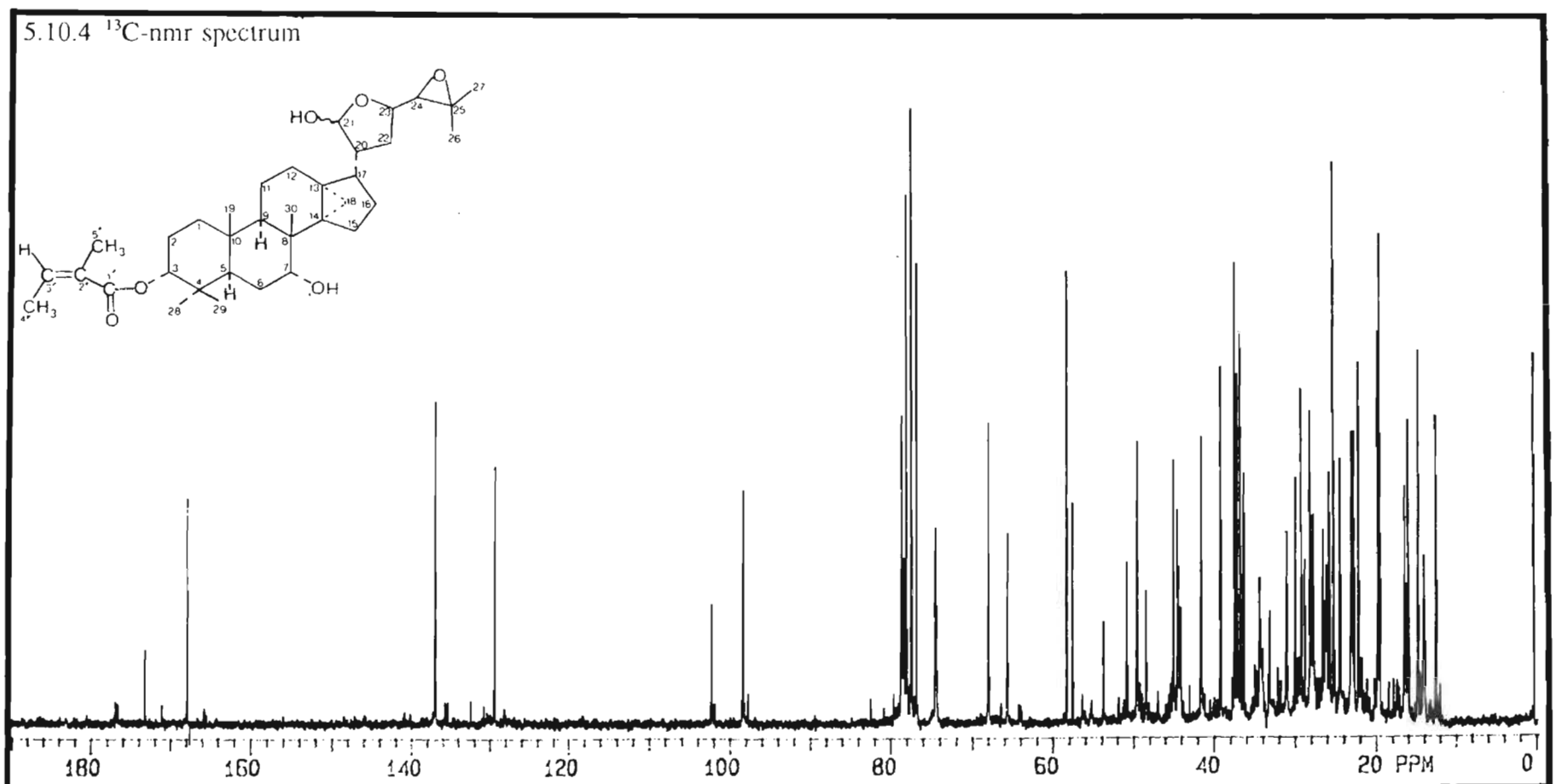
5.10.2 Mass spectrum



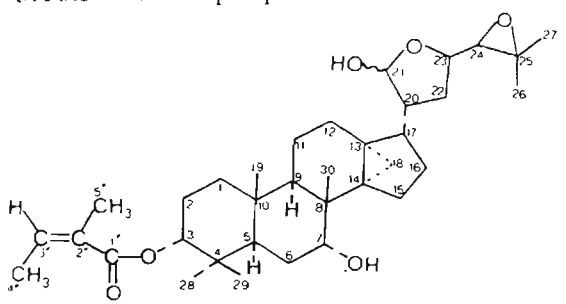
5.10.3 ^{1}H-nmr spectrum

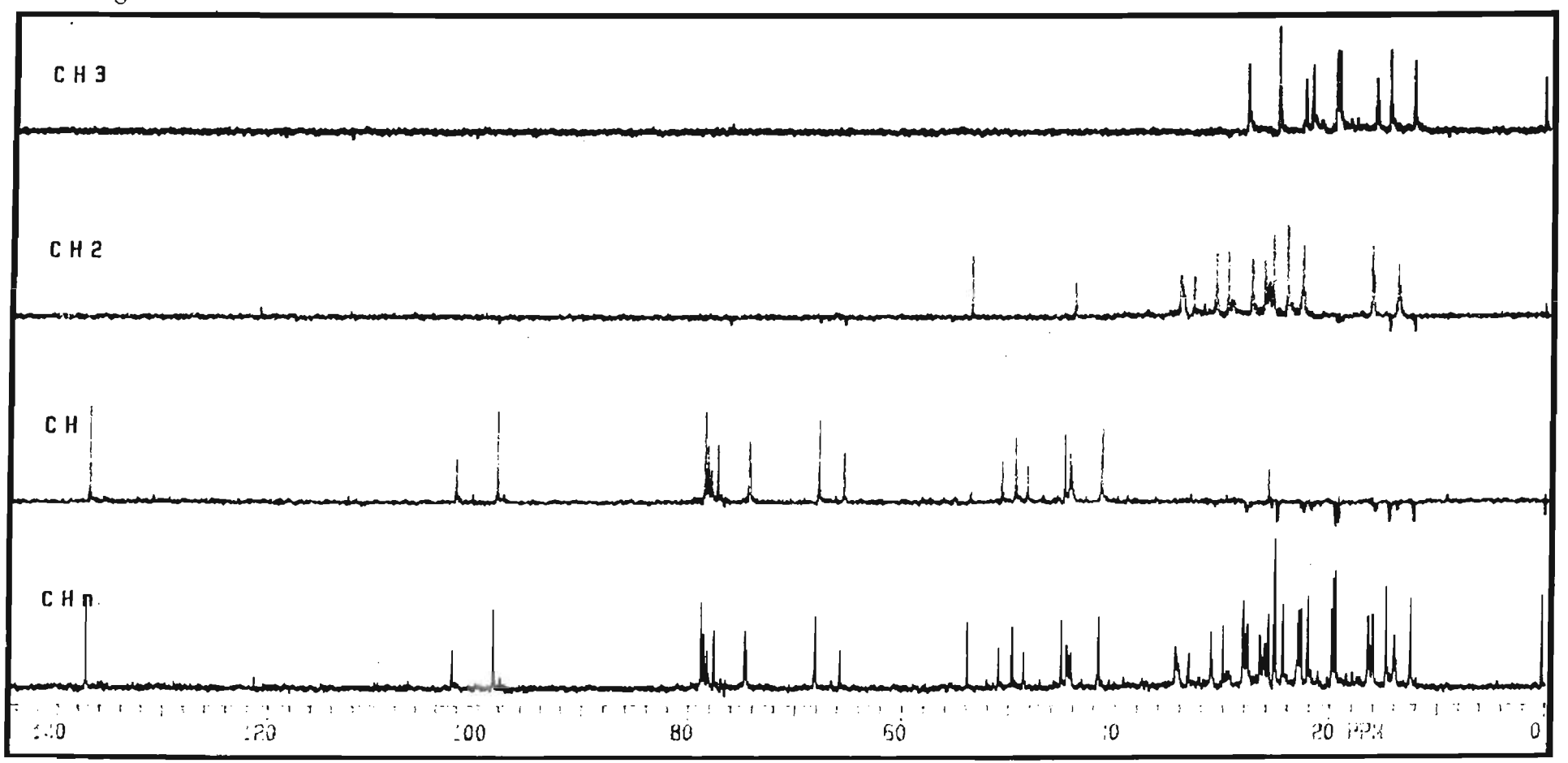


5.10.4 ^{13}C-nmr spectrum

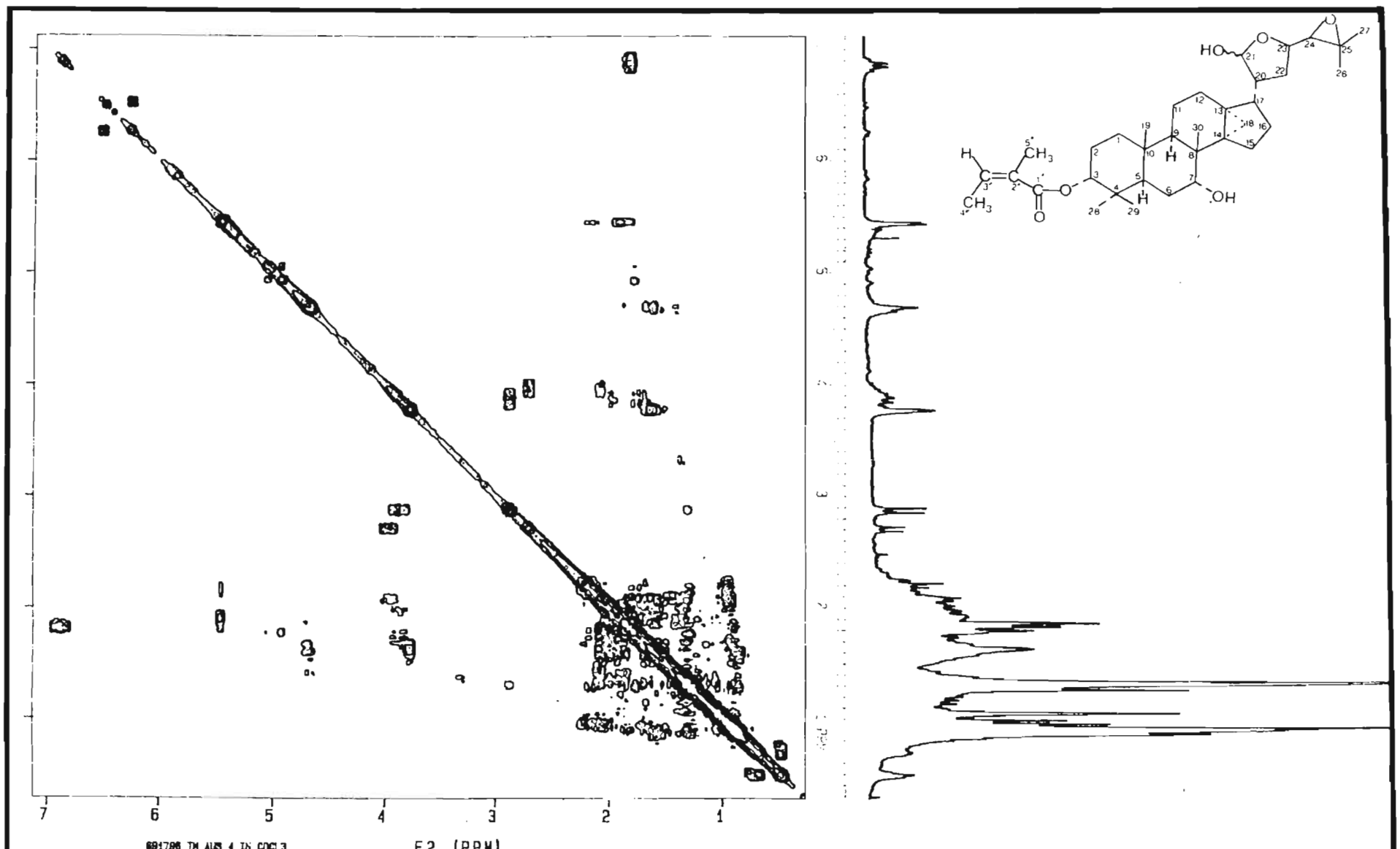


5.10.5 ^{13}C-Adept spectrum

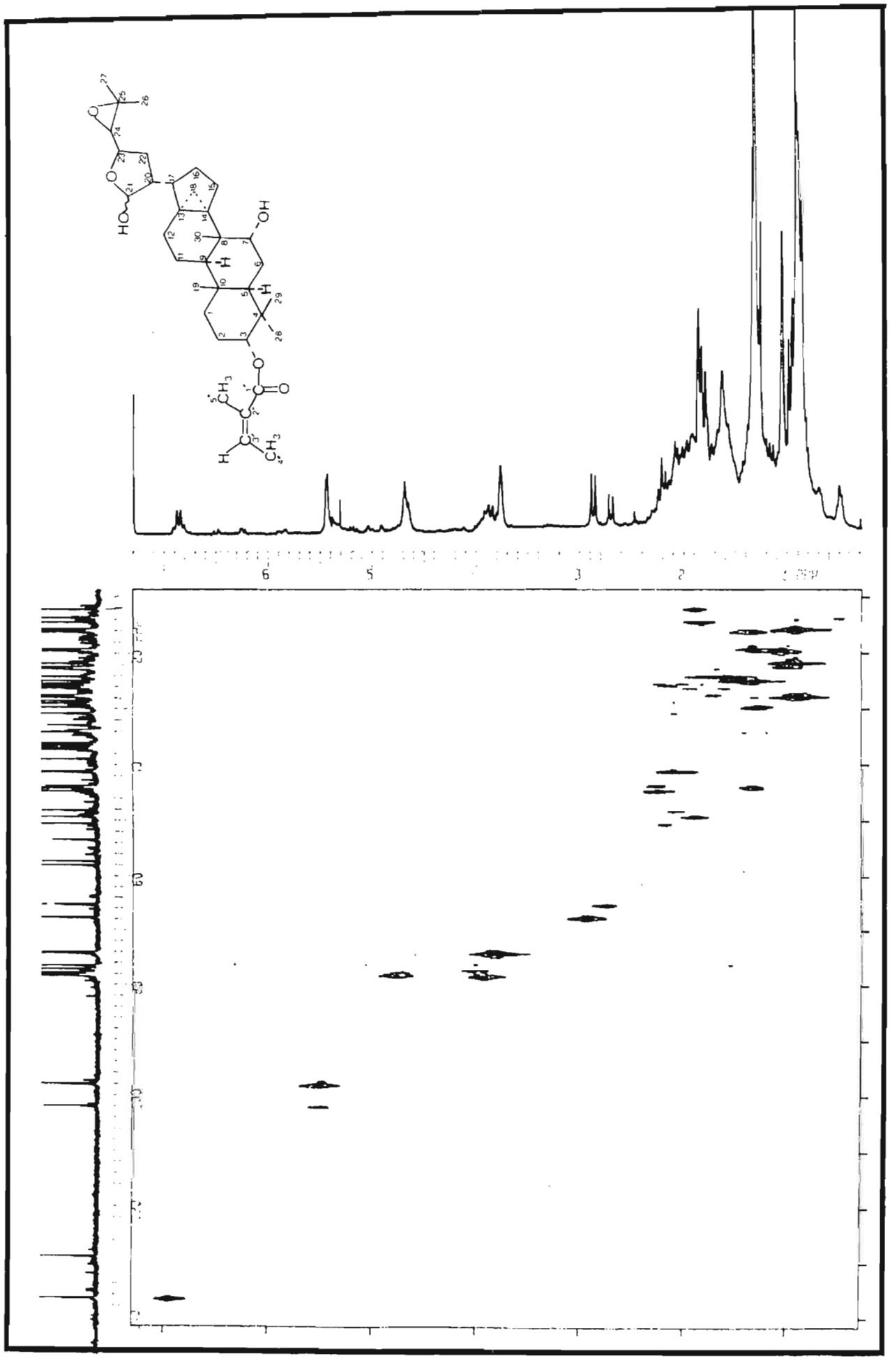




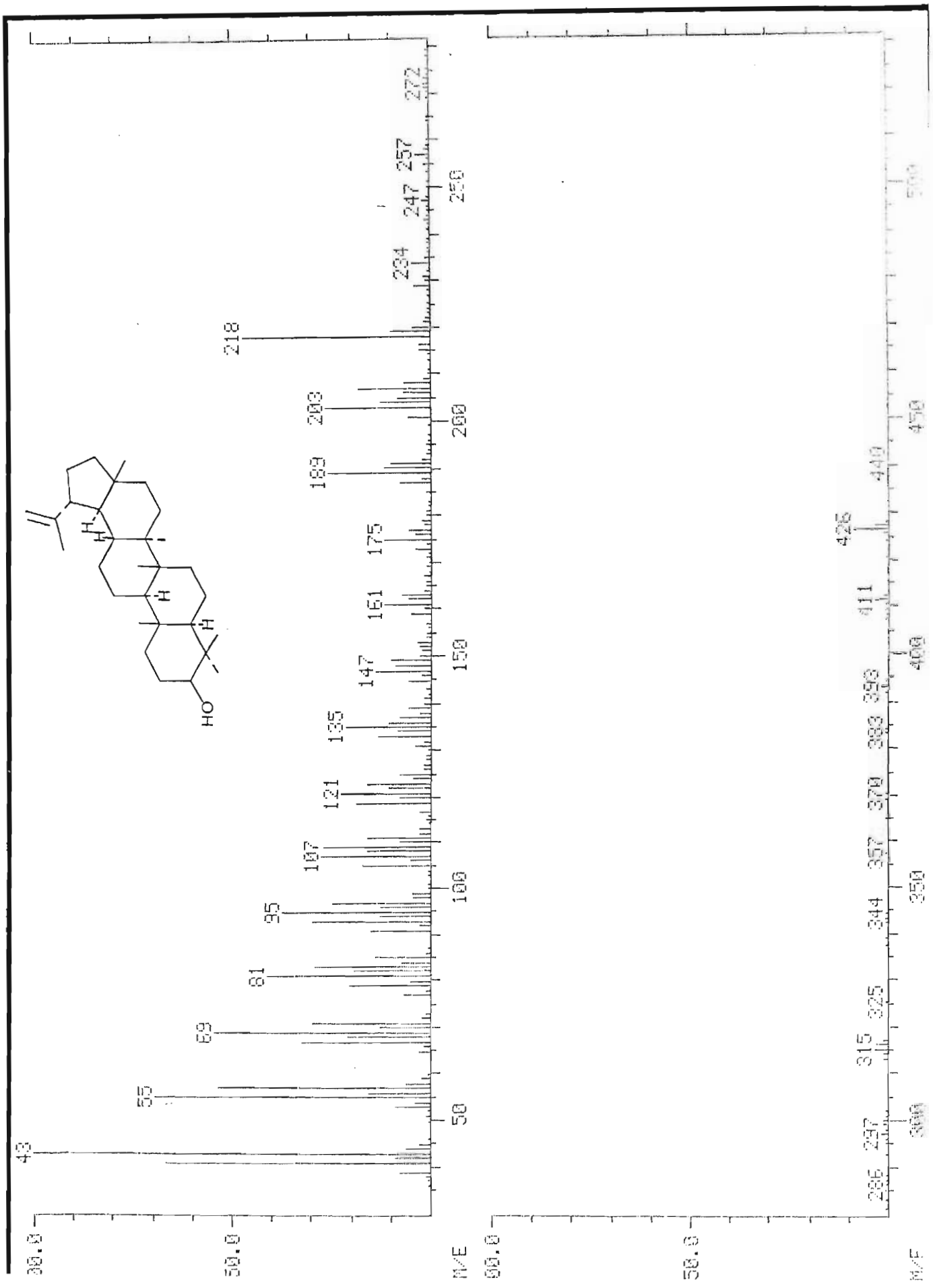
5.10.6 Cosy spectrum



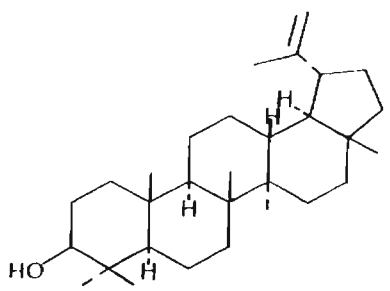
5.10.7 Hetcor spectrum



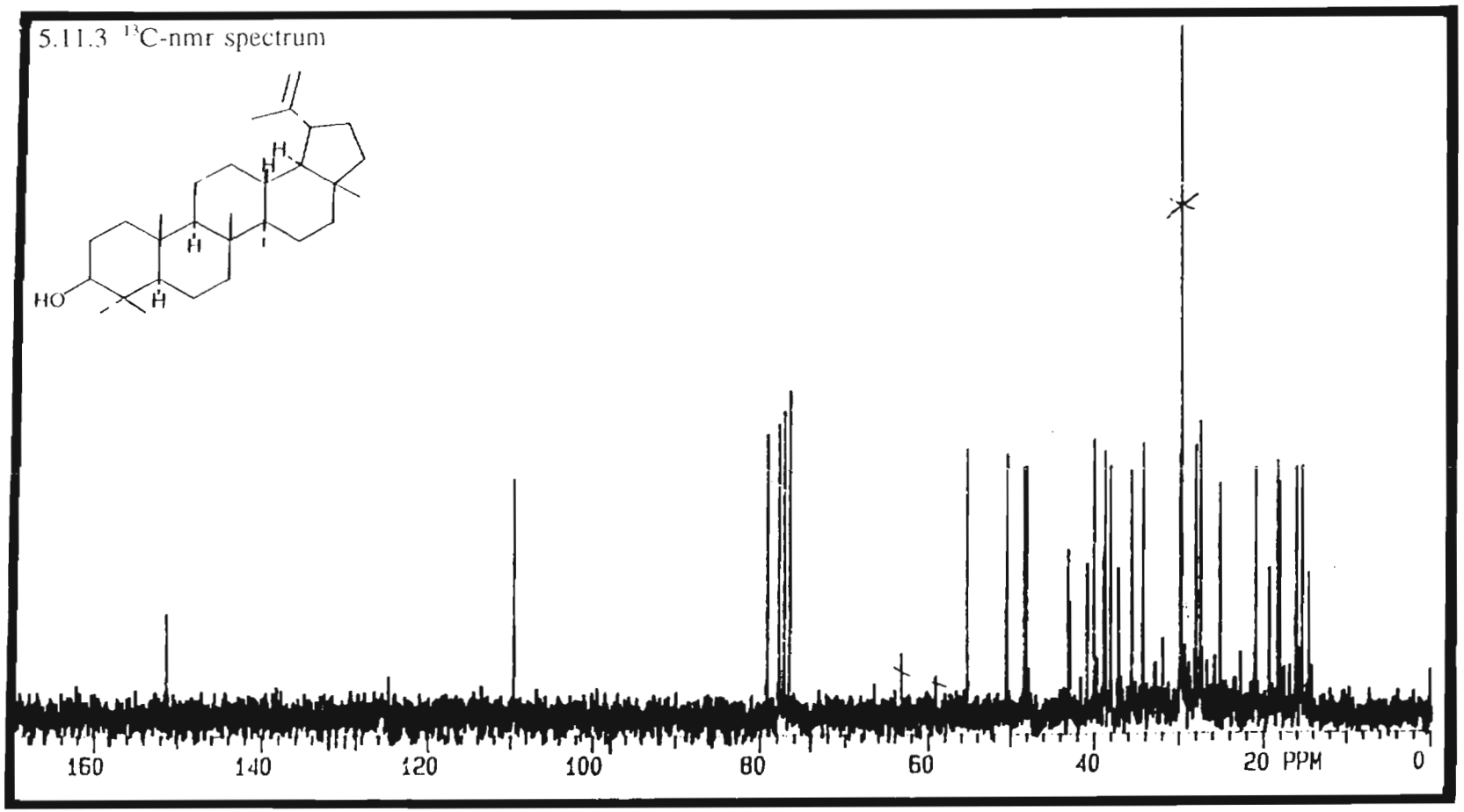
11.1 Mass spectrum



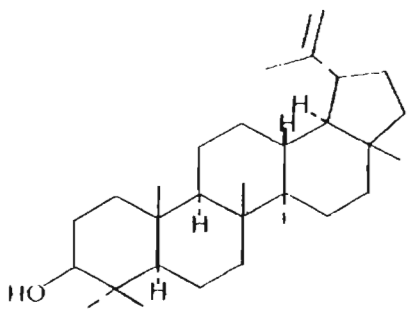
5.11.2 ^{1}H-nmr spectrum

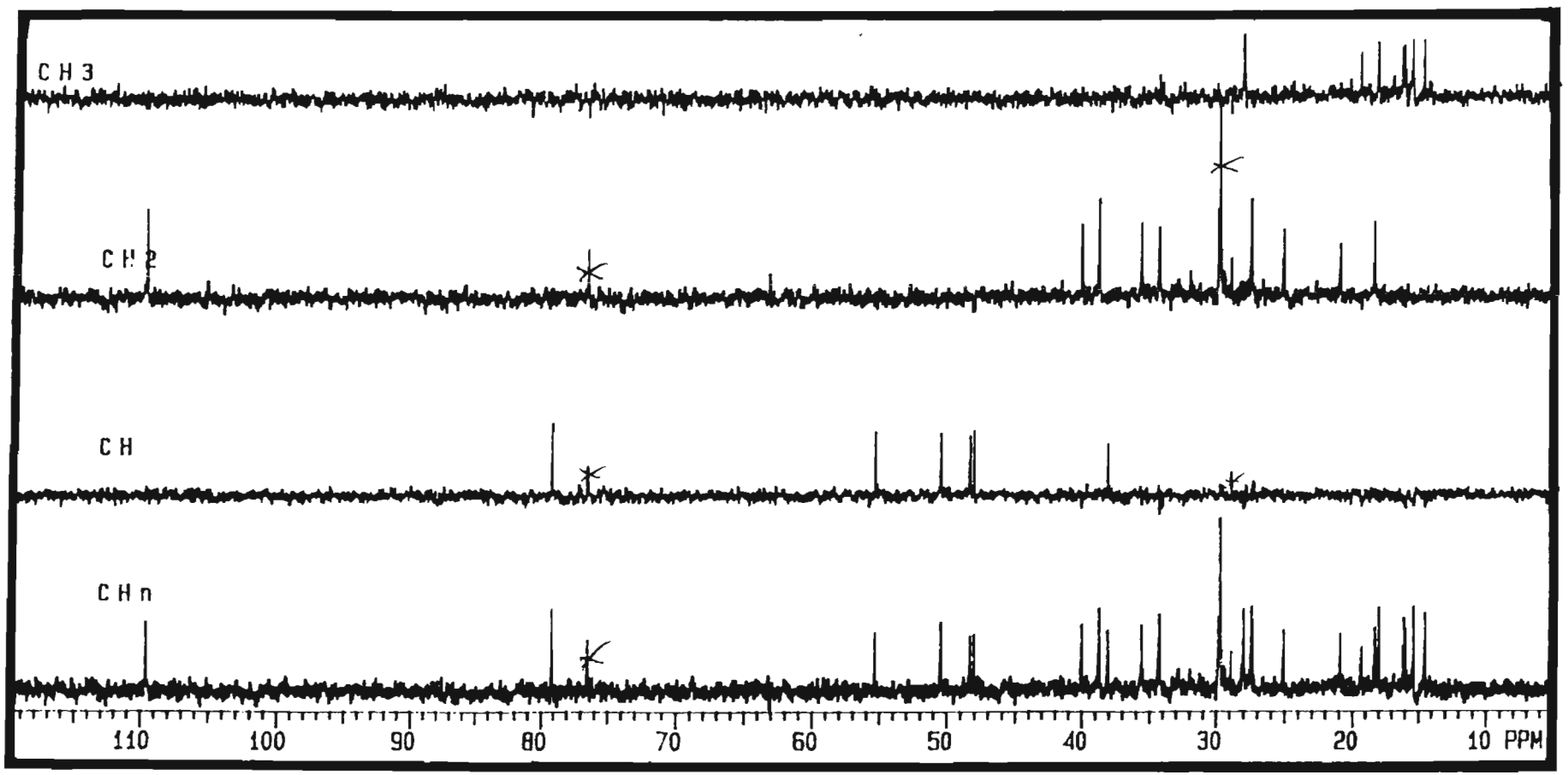


5.11.3 ^{13}C-nmr spectrum



5.11.4 ^{13}C-Adept spectrum





5.12 (20)29-LUPEN-3-ONE

5.12.1 UV spectrum

5.12.2 ^{1}H-nmr spectrum

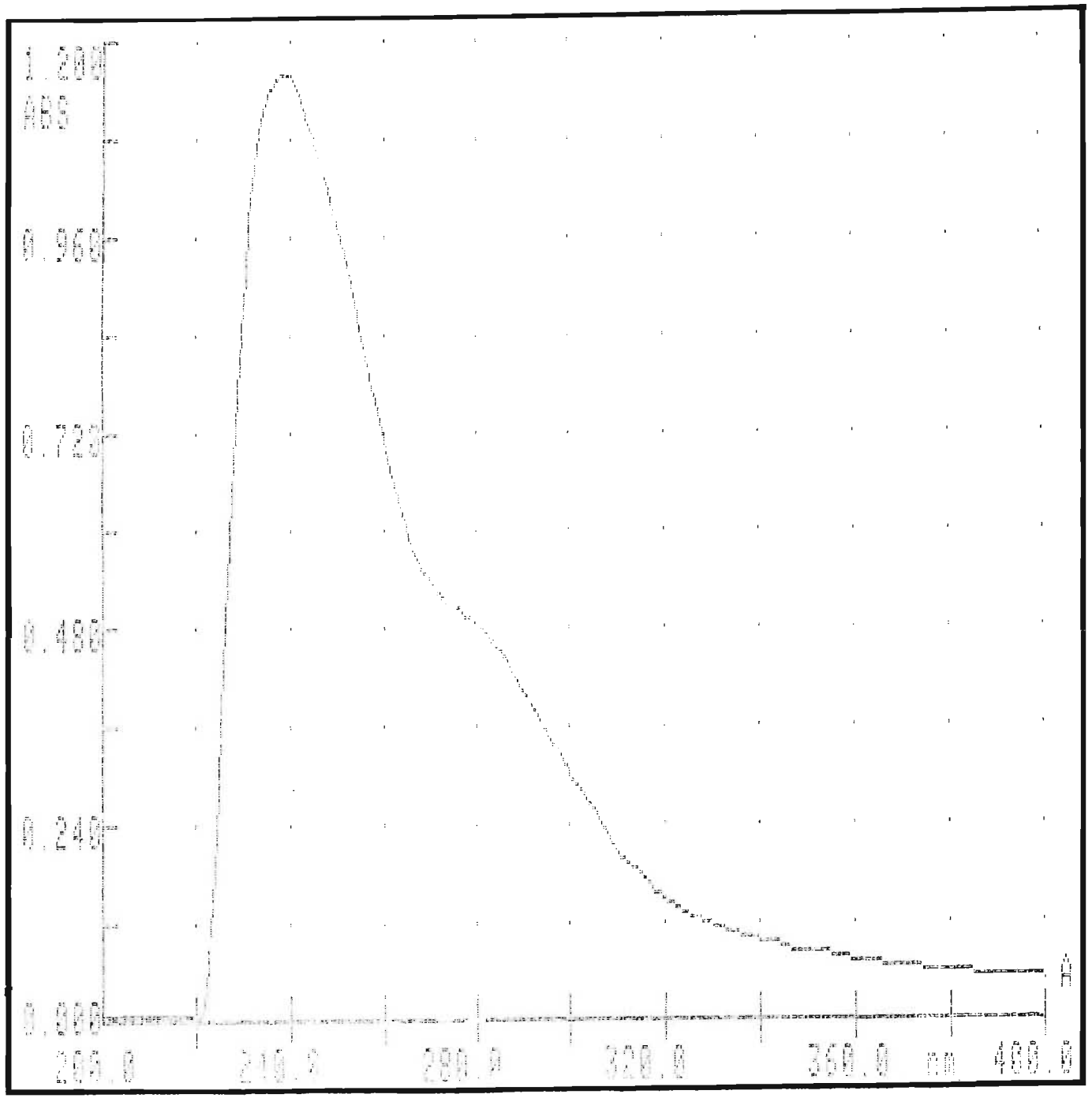
5.12.3 ^{13}C-nmr spectrum

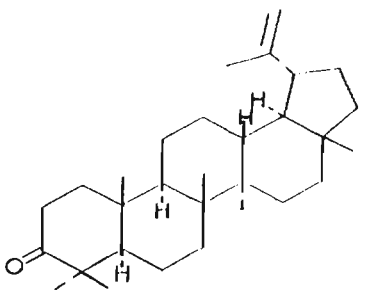
5.12.4 ^{13}C-Adept spectrum

5.12.5 Cosy spectrum

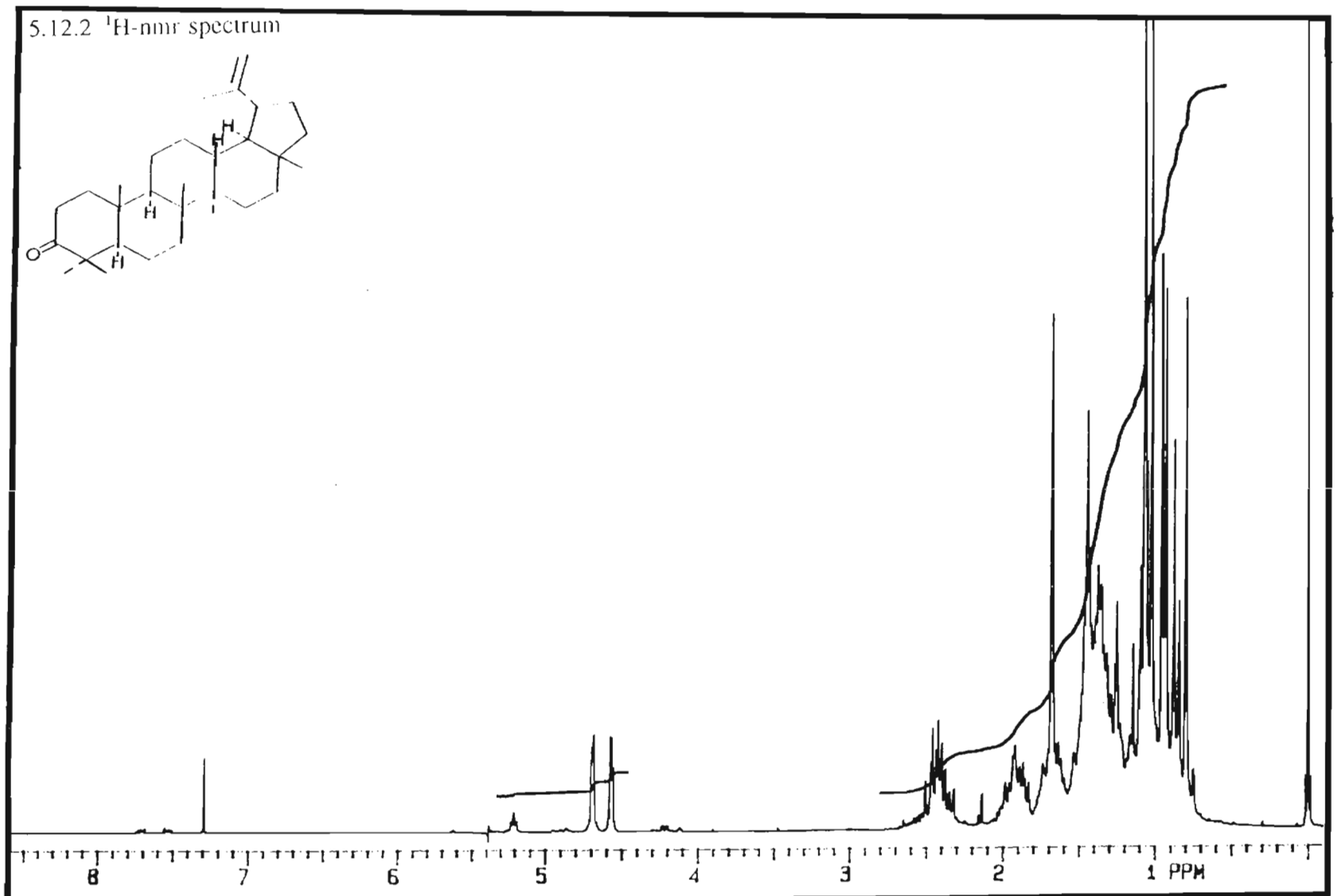
5.12.6 Hetcor spectrum

5.12.1 UV spectrum

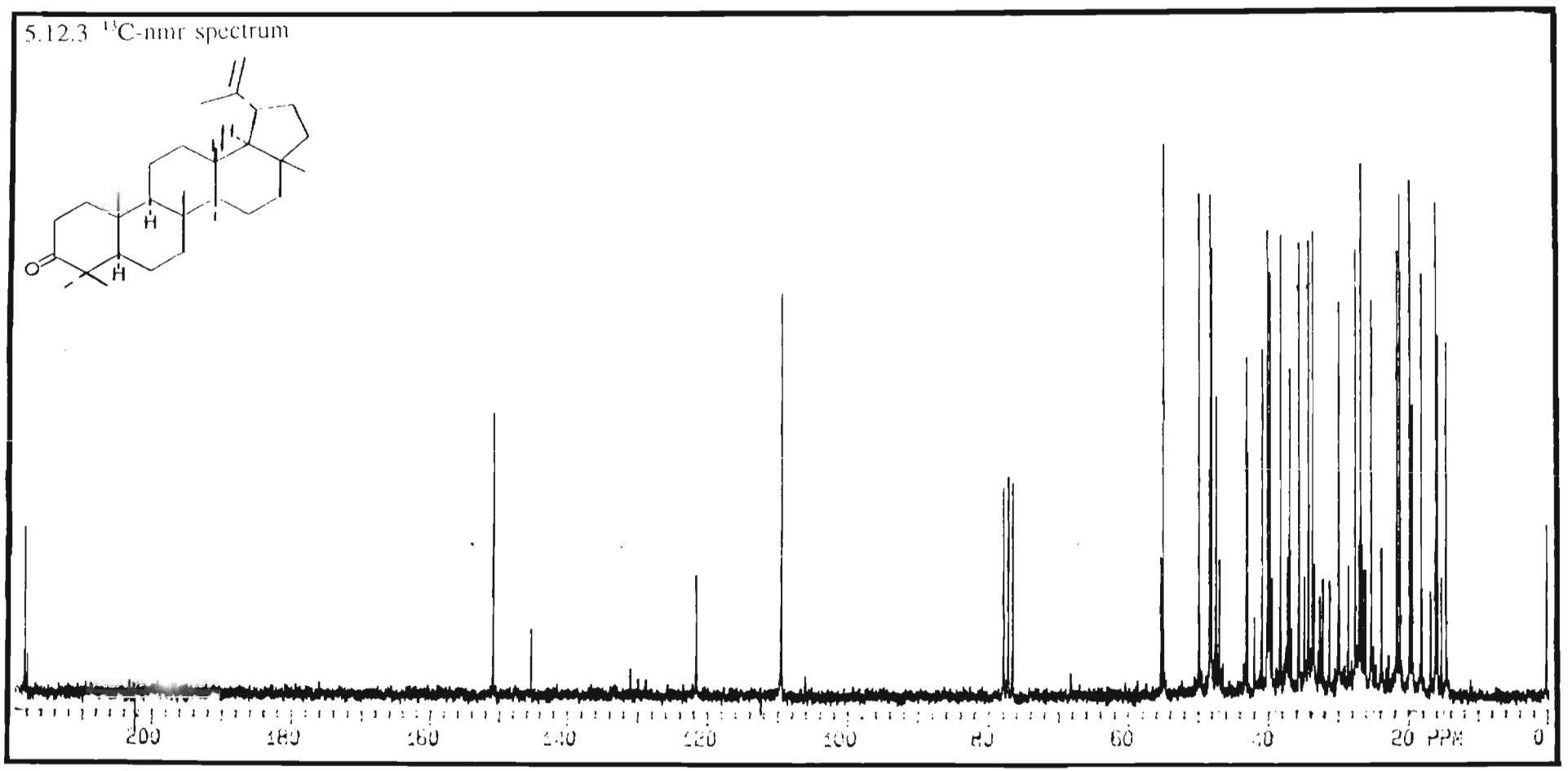




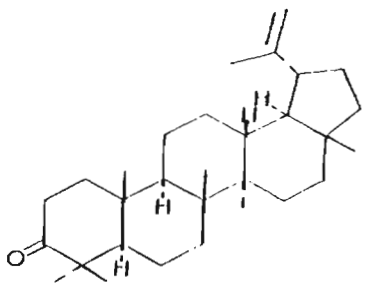
5.12.2 ^{1}H-nmr spectrum

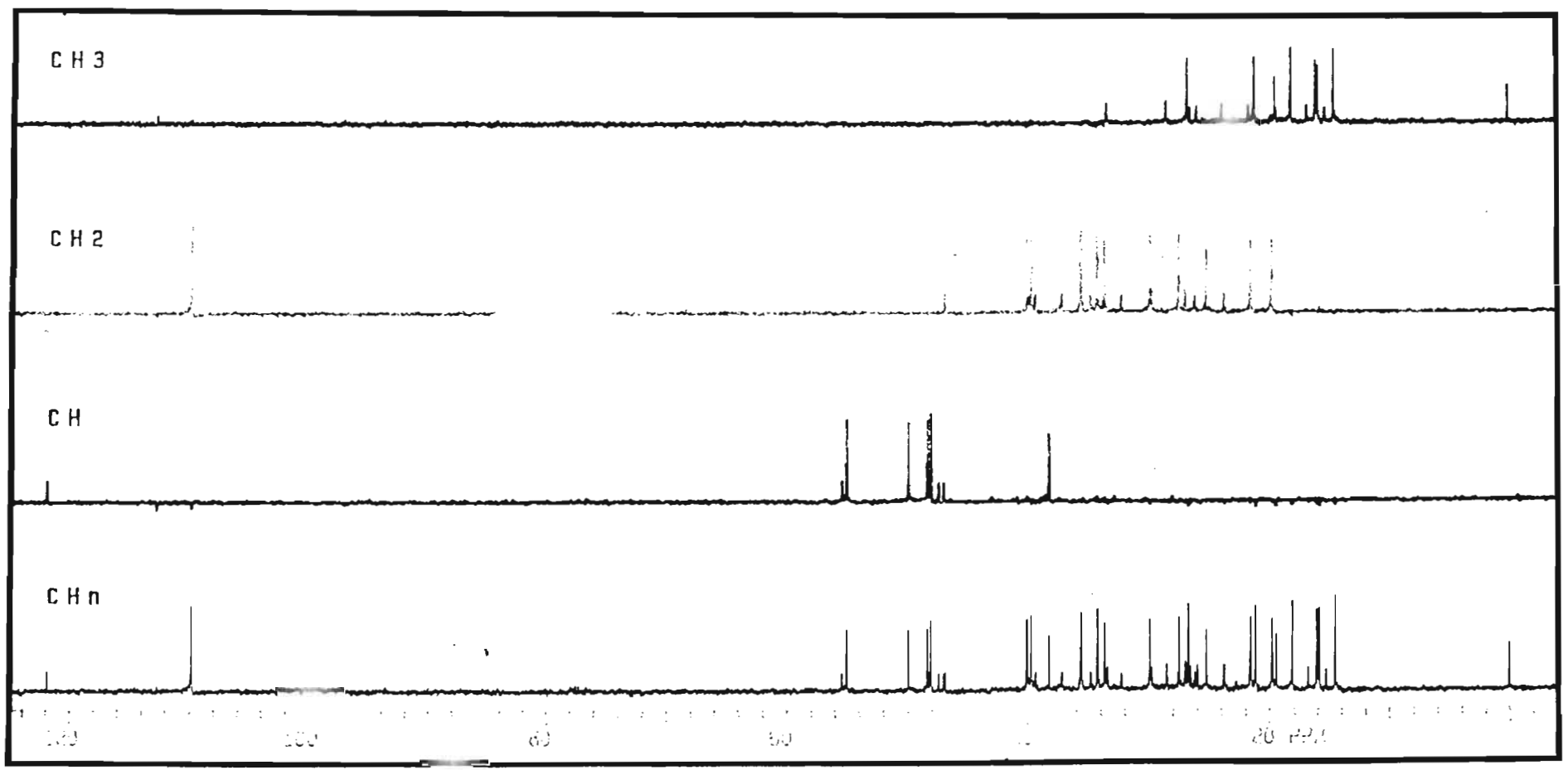


5.12.3 ^{13}C-nmr spectrum



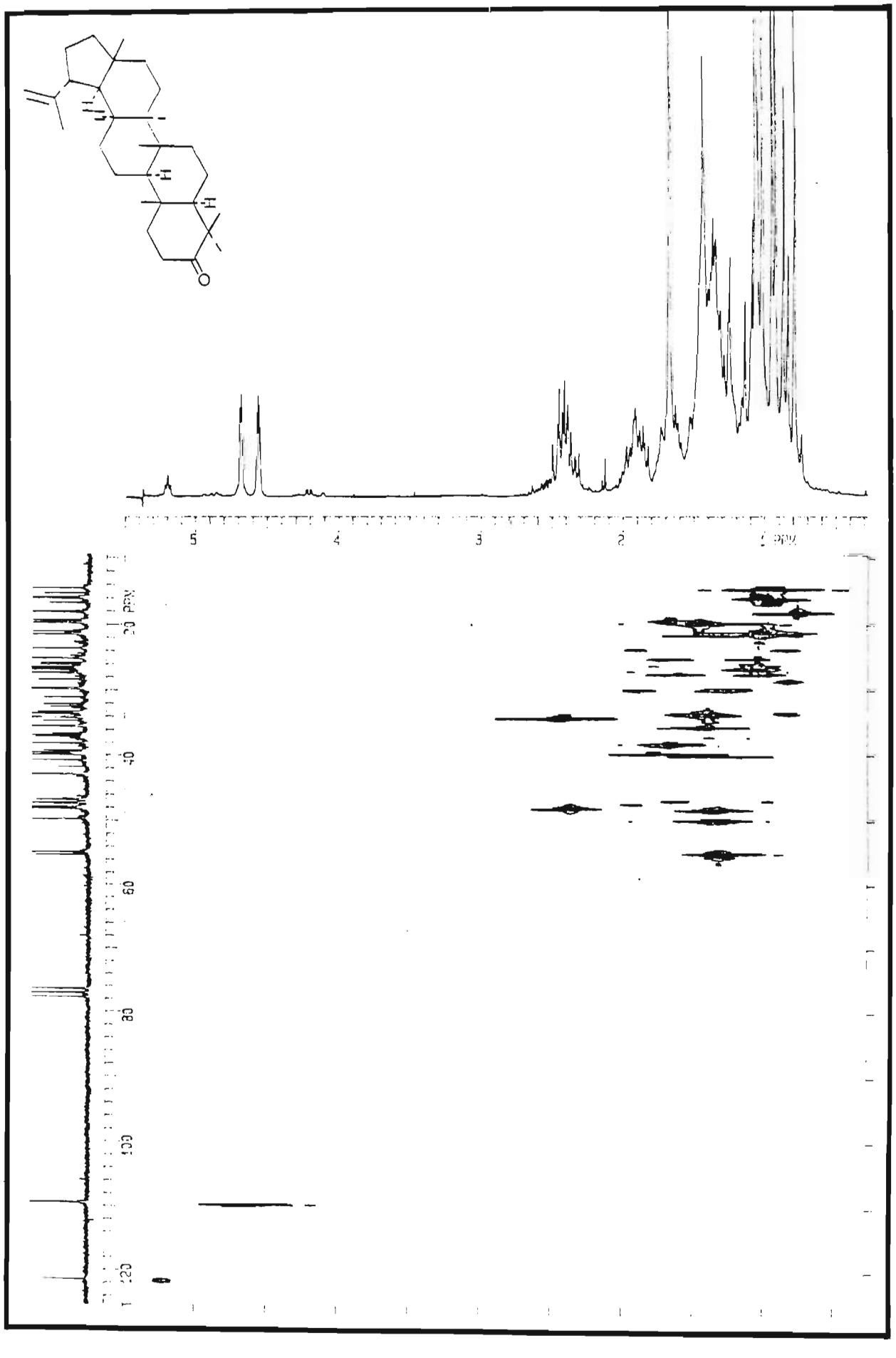
5.12.4 ^{13}C-Adept spectrum





5.12.5 Cosy spectrum

5.12.6 Hetcor spectrum



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