Extractives from the Meliaceae and Icacinaceae

by

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Preface

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, from January 1990 to December 1990, under the supervision of both Dr. D.A. Mulholland and Professor K.H. Pegel.

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

Signed : L-A. Akerman

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B.Sc.(Hons.)

I certify that the above statement is correct

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M.Sc. (Natal), Ph.D. (Natal)

Co-supervisor

Signed : K H Pegel

Prof. K. H. Pegel

Ph.D. (Wits)

Co-supervisor

Acknowledgements

I would like to express my appreciation to my co-supervisor, Dr D. A. Mulholland, for her invaluable assistance and support during the course of this project as well as to my co-supervisor, Professor K.H. Pegel for his helpful advice.

I wish to thank Mr Martin Watson for his assistance with N.M.R. spectroscopy.

I would also like to thank all the other members of the Chemistry department who have assisted me this year.

In addition, I am indebted to Mr. D.D. Fraser for helping me with the documentation of this thesis.

Finally, I acknowledge the financial support of the C.S.I.R. and the University of Natal whose support enabled this work to be performed.

Abstract

The wood, leaf and seed extracts of *Apodytes dimidiata*, *Turraea floribunda* and *Turraea obtusifolia* were examined.

Two new limonoids belonging to the Toonafolin group were isolated from the seeds, two known havanensin-type limonoids were isolated from the leaves and both stigmasterol and sitosterol were isolated from the wood of *Turraea floribunda*.

Sitosterol as well as a limonoid which could not be characterised were isolated from the seeds, phytol, melianone and a protolimonoid similar to sapelin-F were isolated from the leaves and three protolimonoids: melianodiol, melianotriol and 7,8-dihydroturraeanthin 3-acetate were isolated from the wood of *Turraea obtusifolia*.

An ester was isolated from the seeds of *Apodytes dimidiata*.

Appropriate reactions were performed, where possible, on the compounds isolated.

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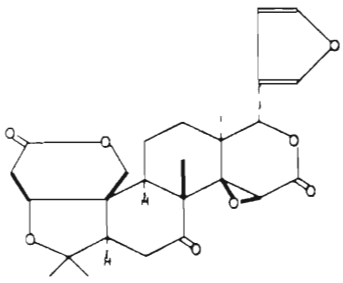
Abbreviations

AcO	acetate
^{13}C N.M.R.	carbon-13 nuclear magnetic resonance
$^{\circ}C$	degrees celsius
conc.	concentrated
d	doublet
^{1}H N.M.R.	proton (^{1}H) magnetic resonance
Hz	Herz
I.R.	infrared
m	multiplet
Me	methyl
mol	mole
M.pt.	melting point
No.	number
ppm	parts per million
q	quartet
s	singlet
t	triplet
T.L.C.	thin layer chromatography
T.M.S	tetramethylsilane
p-TsOH	para-toluenesulphonic acid



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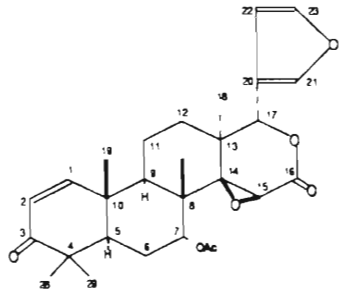

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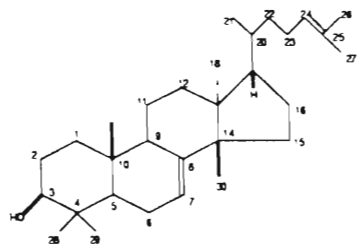

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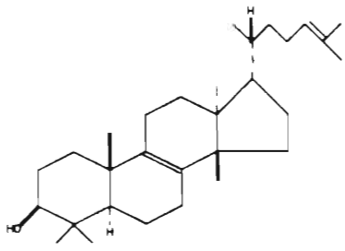

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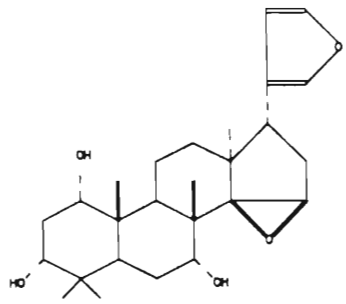

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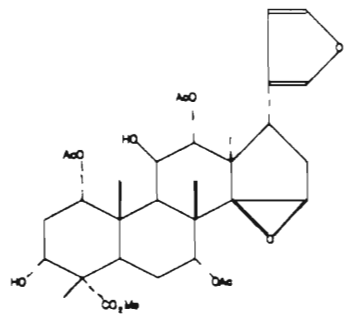

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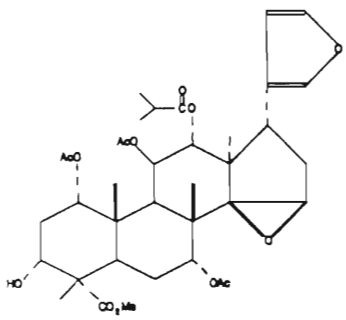

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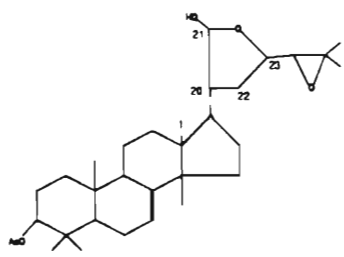

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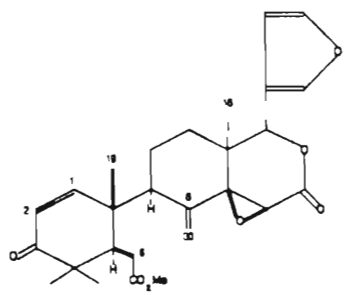

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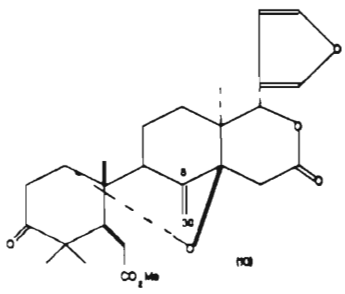

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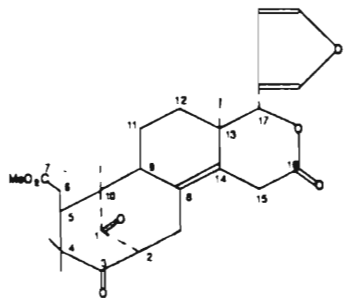

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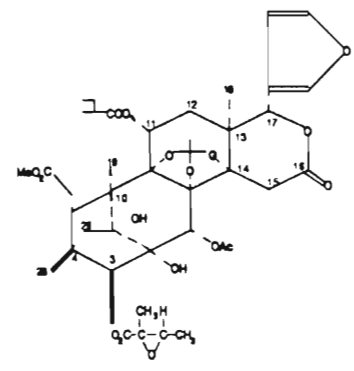

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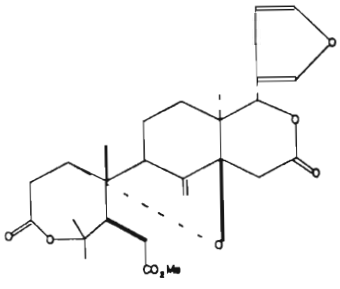

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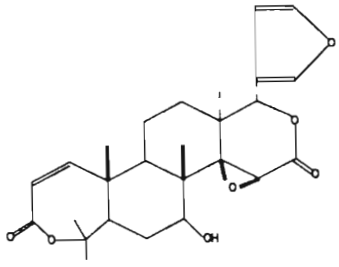

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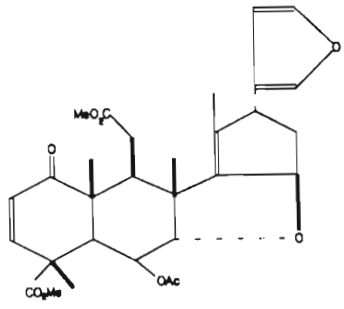

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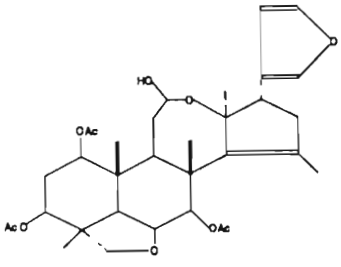

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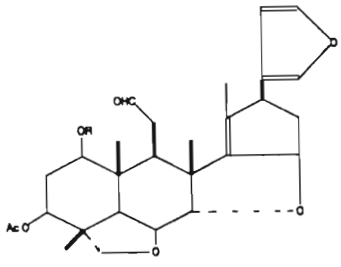

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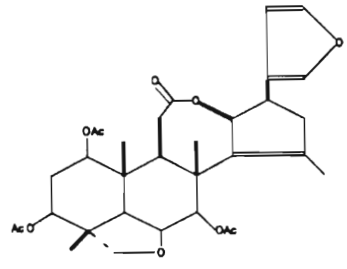

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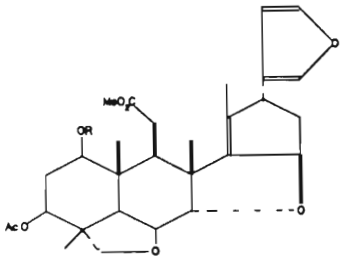

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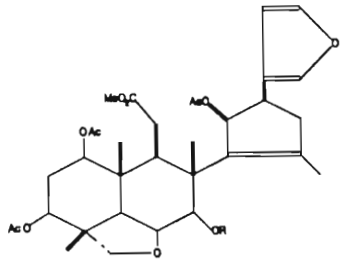

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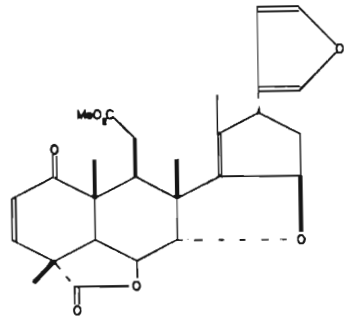

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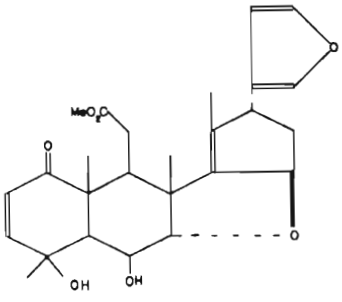

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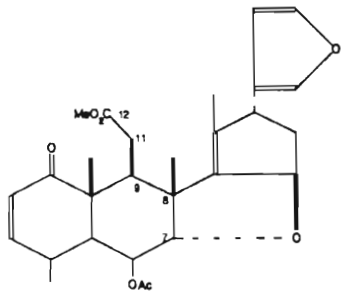

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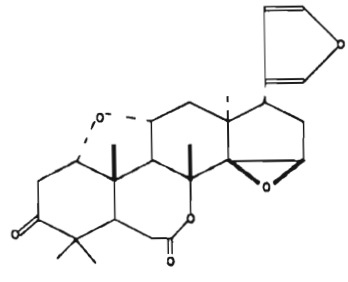

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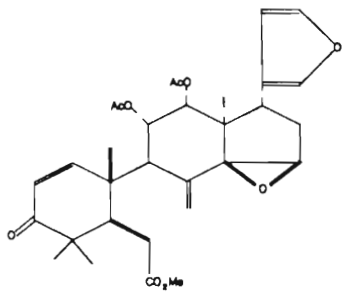

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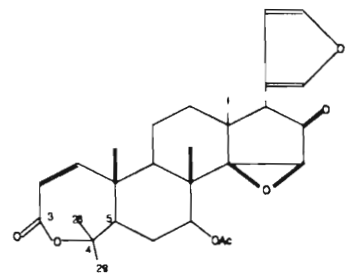

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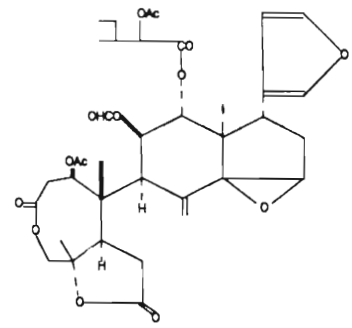

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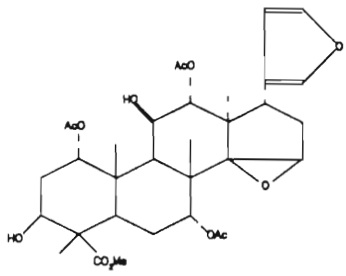

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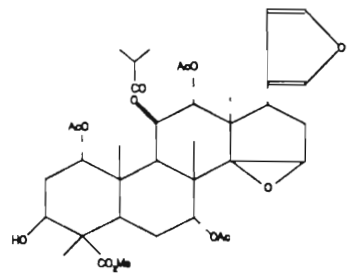

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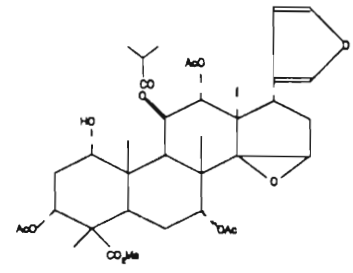

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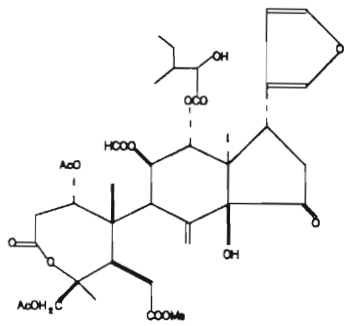

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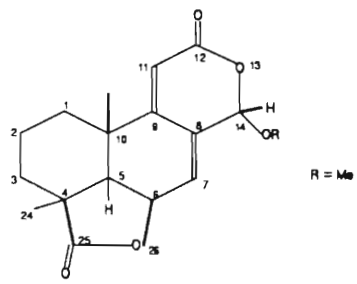

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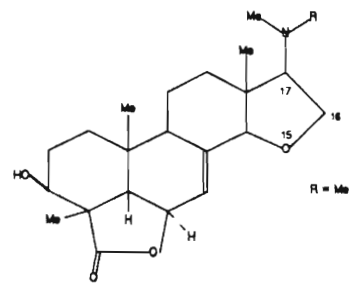

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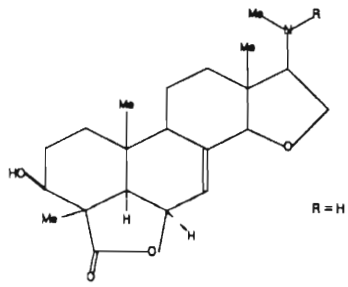

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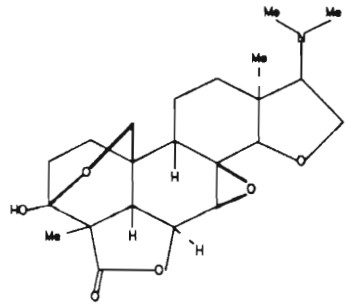

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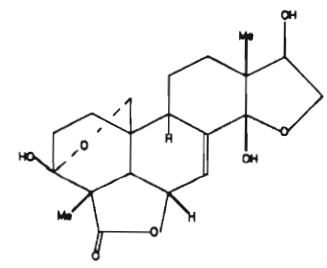

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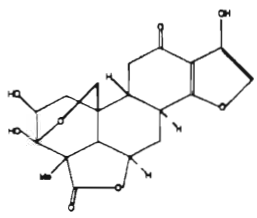

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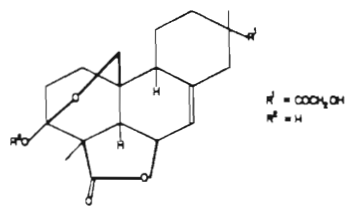

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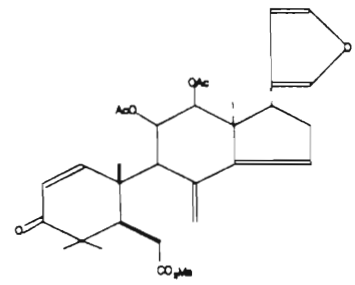

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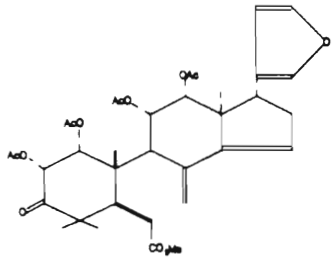

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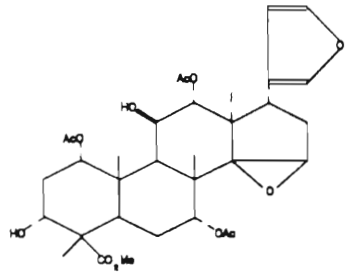

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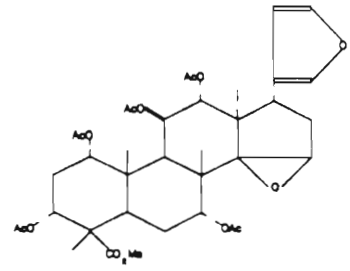

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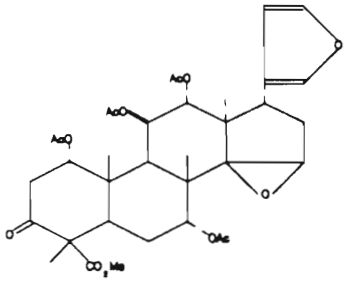

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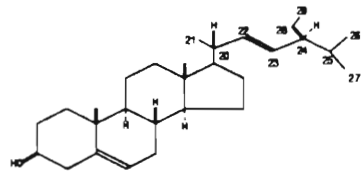

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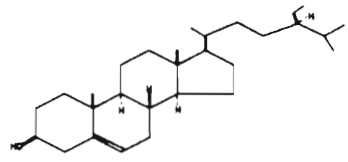

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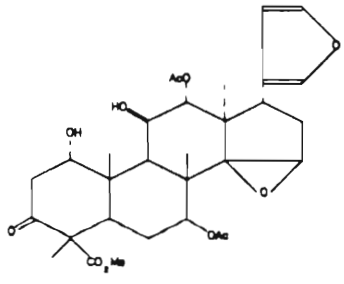

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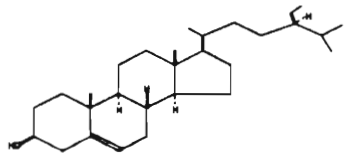

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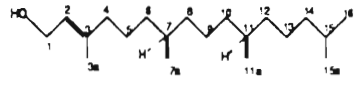

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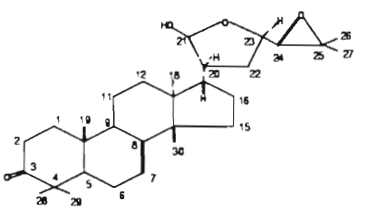

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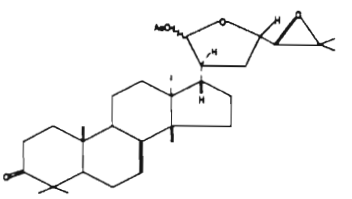

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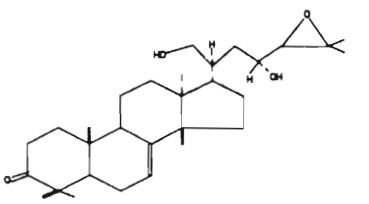

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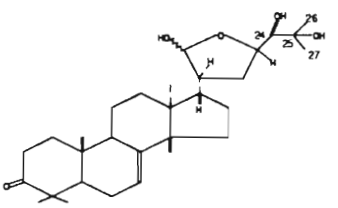

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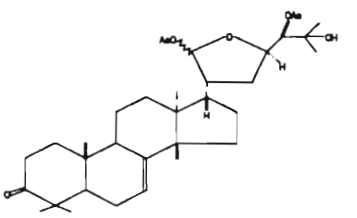
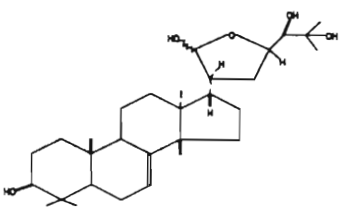


COMPOUND XIa



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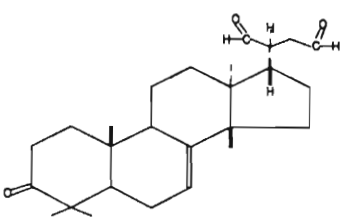

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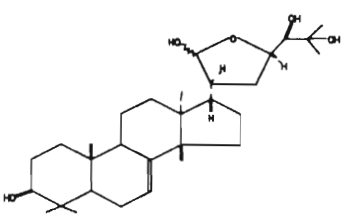

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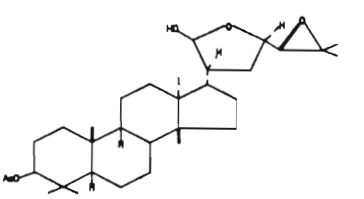
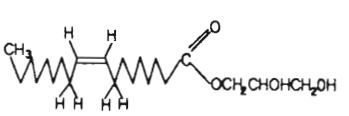


COMPOUND XIII



COMPOUND XIV

Chapter 1 Introduction

1.1 The family Meliaceae

1.1.1 Limonoids

Limonoids are compounds with a C-22 nucleus attached to a furan ring.[1] They are named after the first limonoid ever discovered, called limonin(1). The structure of limonin, an extractive of citrus fruits, was elucidated in 1960 by Arigoni, Barton, Corey and Jeger [2] and may be seen below in Figure 1.1:

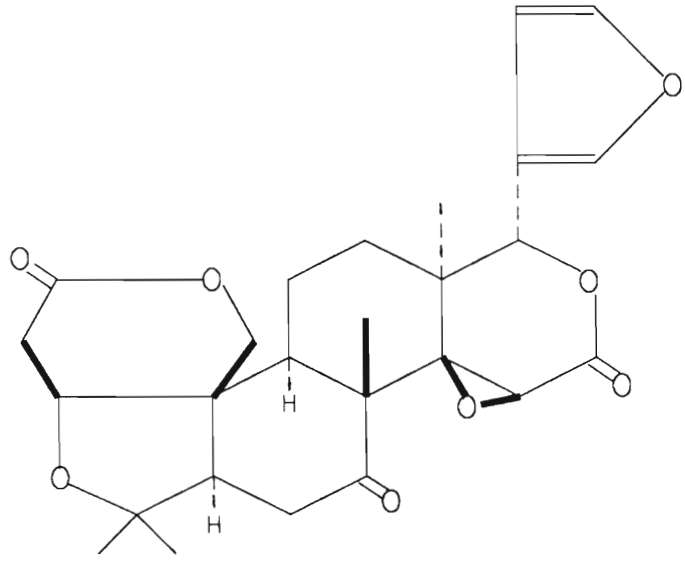


Figure 1.1 Limonin (1)

The first limonoid to be isolated in the Meliaceae family was gedunin(2) . It was isolated in 1960 from *Entandrophragma angolense* [3] which is a timber tree found in West Africa.[4] The structure may be seen overleaf in Figure 1.2, and was only elucidated due to its similarity to limonin.[5]

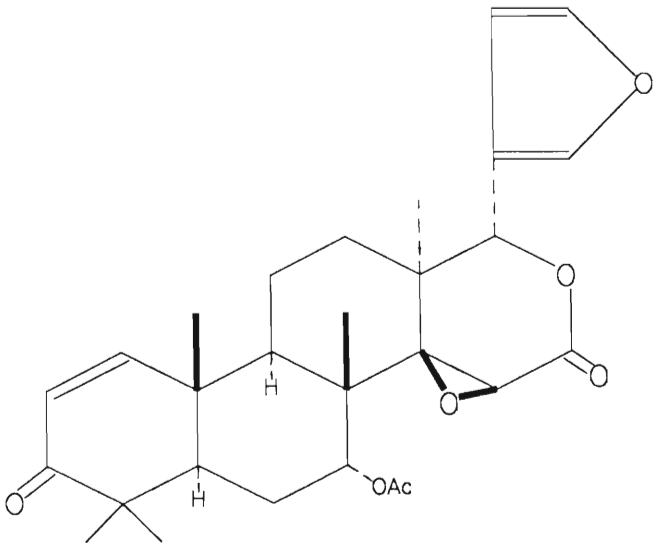


Figure 1.2 Gedunin (2)

The biological reason for the production of limonoids in plants is most probably due to the fact that most limonoids are active as insect antifeedants, although not always directly insecticidal.[6,7] Although it is true that insects would rather starve than eat leaves containing limonoids, the commercial application may be limited due to the fact that insects may adapt to limonoids very quickly so that African insects eat African Meliaceae in preference to similar South American species and vice versa.

Limonoids have also been found to be active against cancer. Citrus limonoids which contain a furan moiety attached to the D-ring lactone have been found to induce the detoxifying enzyme system, glutathione S-transferase (GST).[8] It has been found that the furan moiety is the critical site for enzyme-inducing activity. These GST enzymes catalyse adduct formation between glutathione and electrophiles (eg. reactive carcinogenic species) to water-soluble substances which can be excreted. Hence, enhancement of the activity of GST leads to an increased ability to detoxify carcinogens, implying that these limonoids are potential anticarcinogens.

Gedunin(2), the active constituent of *Azadirachta indica* (Meliaceae family) has been found to be active against malaria, the A-ring appearing to be the important factor.[9]

1.1.2 The biosynthesis of limonoids

Limonoids are derived from such tetracyclic triterpenes as euphol or tirucallol by means of oxidative changes and molecular rearrangements.[3] The former usually involves oxidations of double bonds, or Baeyer-Villiger attacks on ketones as seen in Figure 1.3:

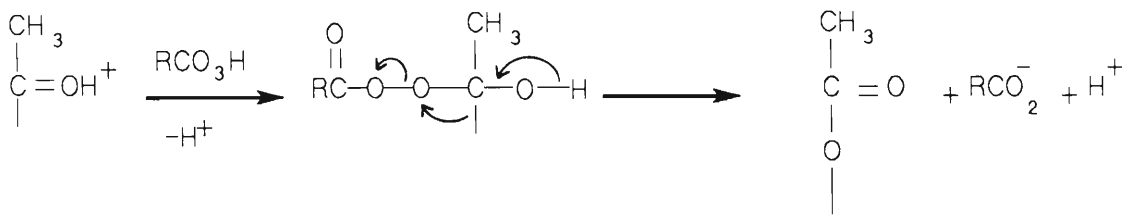


Figure 1.3

Although there is no direct proof for the biosynthesis of limonoids by means of tracer incorporation, there are well supported schemes which are unlikely to be in error. The first scheme follows the definition of a limonoid which states that it is a triterpene derivative in which the side chain has become a furan ring by the loss of four carbon atoms. The name tetranortriterpenoids is thus used interchangeably with limonoids. Hence, the side chain of euphol or tirucallol is first oxidised to form a protolimonoid and eventually forms a β-substituted furan ring as seen in Figure 1.4 overleaf:

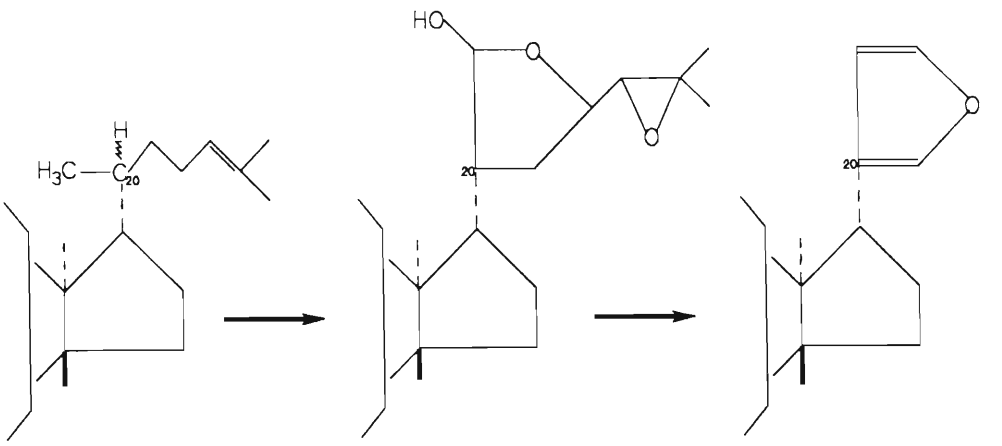


Figure 1.4

These protolimonoids, which are oxidised triterpenes, have also been found to occur biologically and have been isolated in the Meliaceae family and will be discussed later. In the above scheme (Fig 1.4), the C-20 stereochemistry is preserved in the protolimonoids and is usually the 20-S-H configuration (tirucallol) although there are some examples of 20-R-H configuration (euphol).

Arigoni, Barton, Corey and Jeger suggest two processes from a tirucallol / euphol type precursor.[2] The first is the oxidative cleavage of the side chain between C-23 and C-24 and the second apo-euphol type rearrangement where the methyl group migrates from C-14 to C-8.

Halsall and co-workers have proposed a scheme for the conversion of the euphol side chain into the β-substituted furan ring.[10] Their proposal is supported by the occurrence of compounds such as flindissol and turreanthin . The 24-ketone could arise from the 24,25-epoxide by rearrangement or via formation of the glycol and subsequent oxidation of the 24-hydroxyl group.[11] Fission of the C-23, C-24 bond of the side chain is believed to occur by a Baeyer-Villager oxidation analogous to that which occurs in the biosynthesis of nyctanthic acid [12,13] or methyl angolensate,[14] producing the dihydrofuran which, on dehydration, would yield the β-substituted furan as seen in Figure 1.5 overleaf.

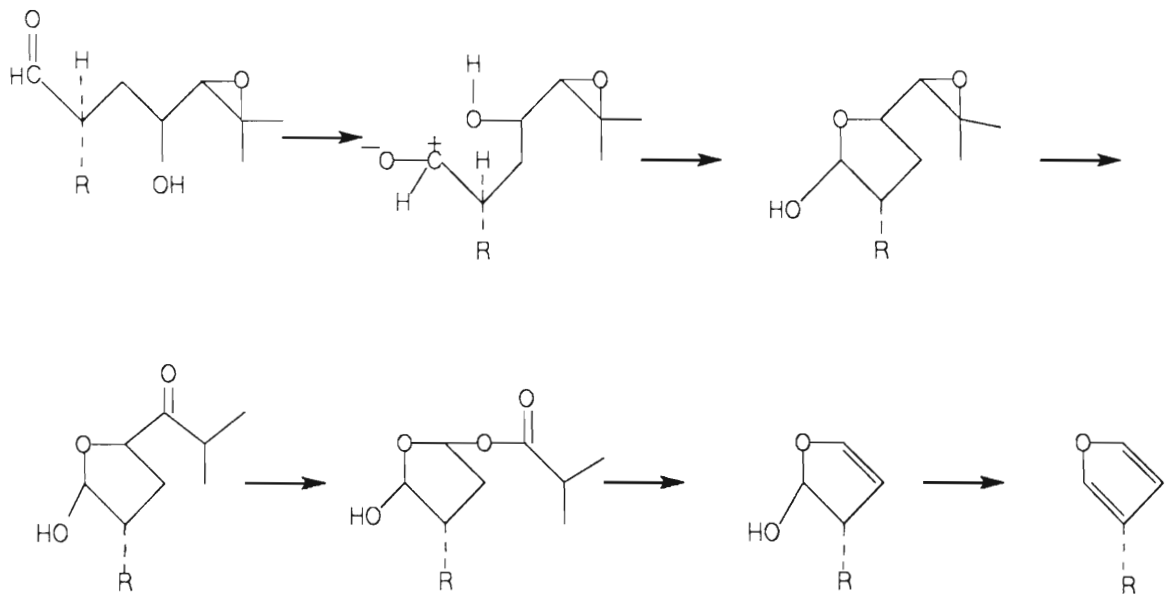


Figure 1.5

Cotterell, Halsall and Wriglesworth have proposed a scheme for the apo-euphol rearrangement[15] which can be seen in Figure 1.6 overleaf. The nuclear double bond is oxidised to an epoxide which undergoes acid-catalysed isomerisation with a shift of a methyl group from C-14 to C-8 giving a 7α-hydroxy-8β-methyl-14-ene compound.

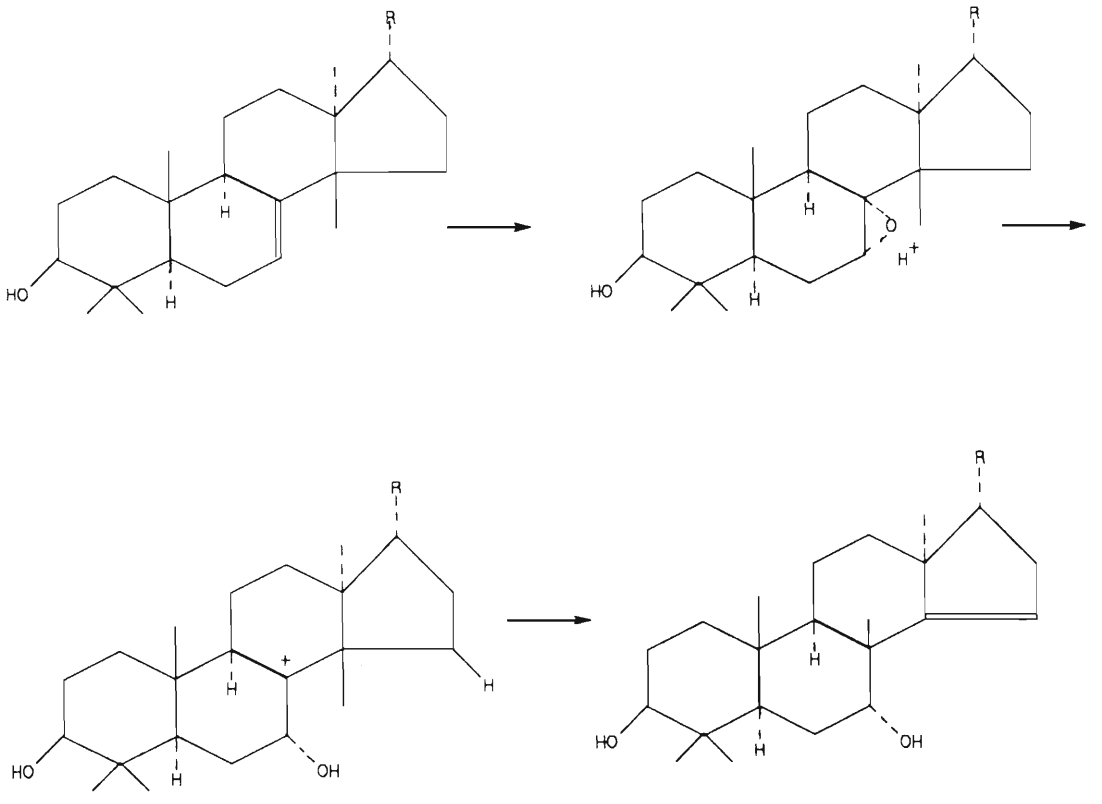


Figure 1.6

However, it is unknown whether or not only one of these isomers can be used in the biosynthesis of limonoids. In Melia, both tirucallol and euphol can be used as starting material, but euphol is more readily used,[16] hence the known protolimonoids with the euphol configuration, occur in Melia.

It is also thought that limonoids are derived from the hypothetical triterpene 7-ene-isomer of tirucallol or the 20-epimer being butyrospermol(3).[17] It has been suggested that this compound can be formed directly from the cationic intermediate resulting from the cyclisation of squalene, without the intervention of euphol(4)[18,15] as seen overleaf in Figure 1.7.

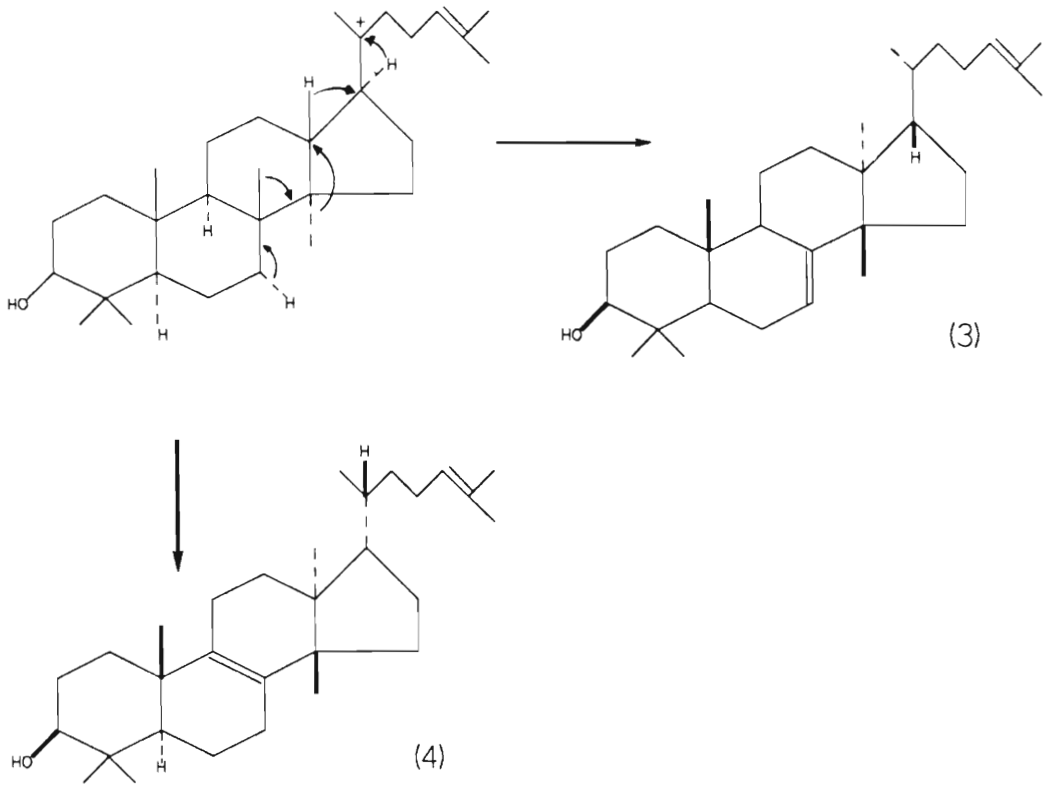


Figure 1.7

1.1.3 Classification of limonoids

Limonoids have been classified according to which of the four rings in the tetracyclic triterpene skeleton has been oxidised.

Subgroup (a) = rings B and D opened

Subgroup (b) = a new ring formed between C-2 and C-30

Subgroup (c) = compounds of subgroup (b) further modified by bridging of ring A.[19]

Ten main groups of limonoids have been found and each will be discussed individually:

Group 1 consists of protolimonoids. Protolimonoids occur in two classes. The first class is like euphol, having a β-methyl group at C-14 and a double bond between carbon atoms 7 and 8. The second class has a 7α hydroxyl group and the double bond has moved to Δ^{14} and the β-methyl group to C-8. This change depends on the opening of the 7,8α- oxide shown below in Figure 1.8, which has been demonstrated in the laboratory.[20,21,22,23,24,25]

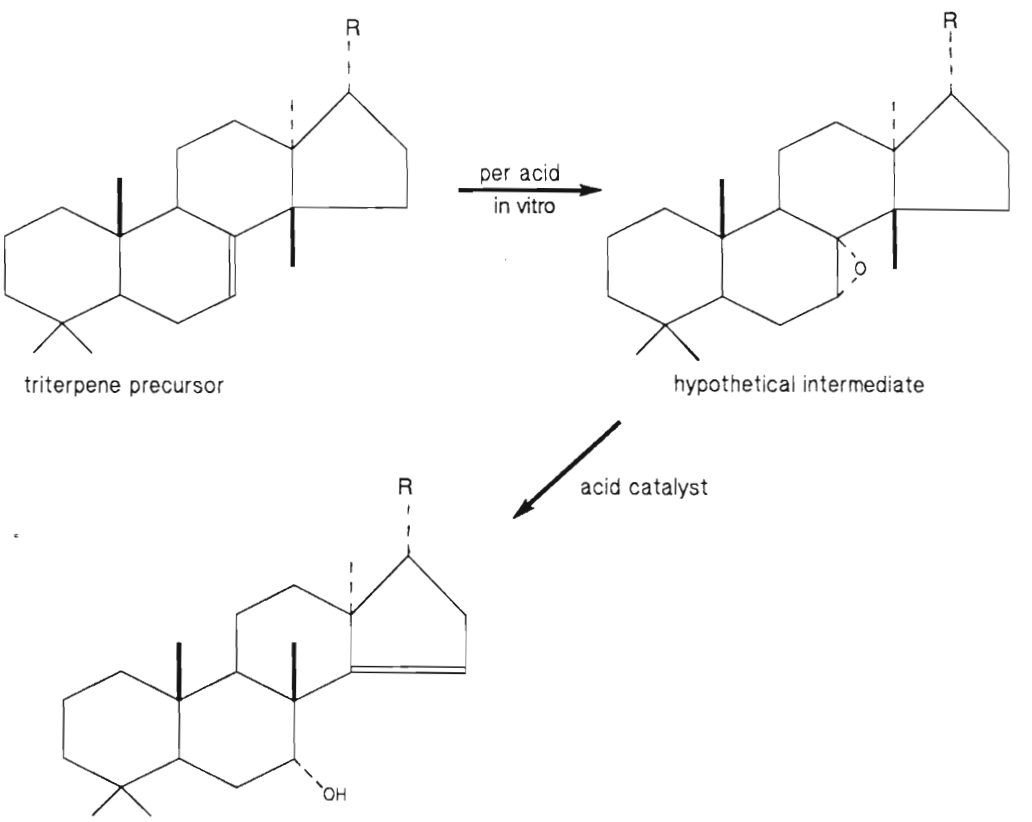


Figure 1.8

The second group is known as the havanensin group. In this group, the compounds contain a furan side chain while all the rings of the nucleus remain intact, as seen overleaf in Figure 1.9:

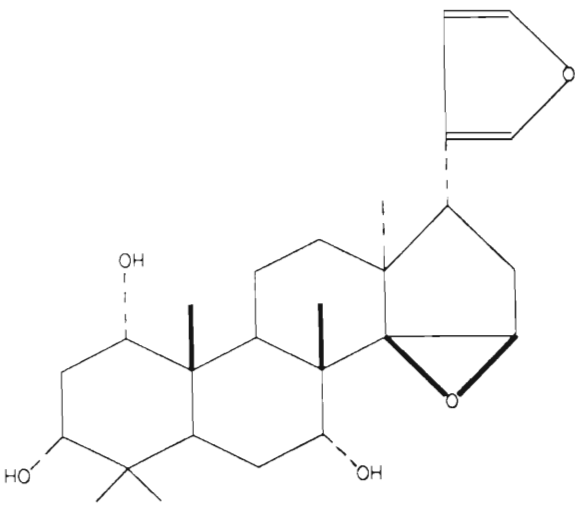


Figure 1.9 Havanensin (5)

Some other limonoids(6,7) obtained from *Turraea floribunda* belong to this group.[26,27] They have a carbomethoxy group at C-4 and appear as follows in Figure 1.10:

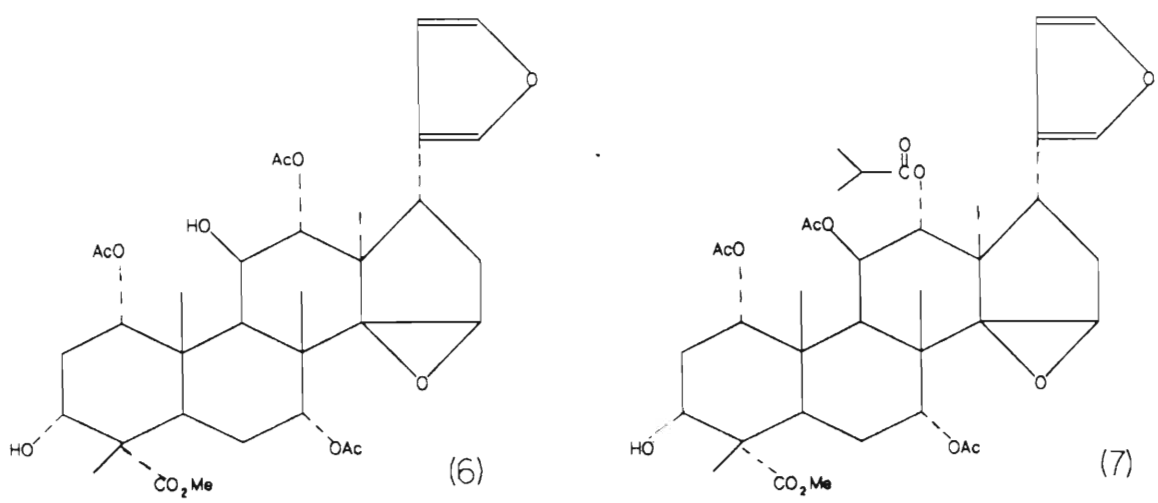


Figure 1.10

The partial synthesis of the furan ring for havanensin-type compounds has been demonstrated in the laboratory by sodium metaperiodate oxidation of turreanthin(8) in the presence of perchloric acid as shown overleaf in Figure 1.11:[20,28]

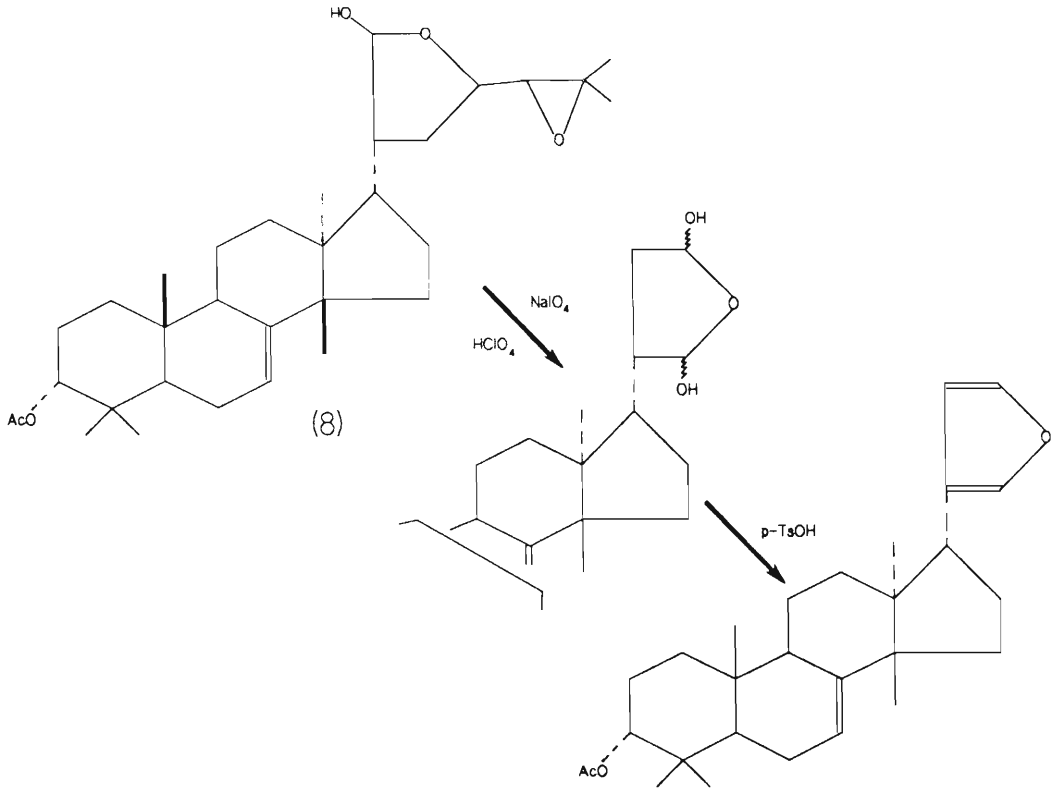


Figure 1.11 The Partial synthesis of Havanensin-type compounds

The third group in which ring D has opened is known as the Gedunin group. The biosynthesis of the lactone ring D follows through a series of potential intermediates between the cyclopentene ring of deoxyhavanensin and the epoxy lactone. This is known since various of these intermediates have been isolated in the Gedunin series. The important step is the allylic oxidation to a 16-ketone, after which formation of the lactone ring occurs. However, if the double bond is first epoxidised, formation of the ketone is blocked and the five-membered ring lactone remains.The scheme can be seen overleaf in Figure 1.12:

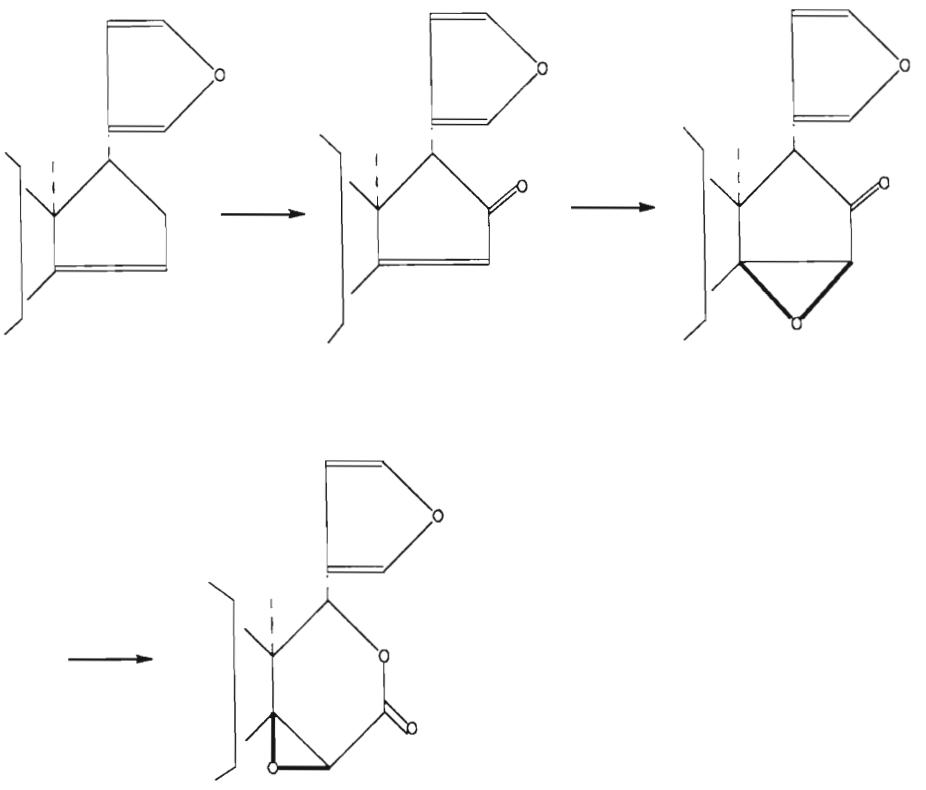


Figure 1.12

This scheme has been demonstrated in the laboratory.[28,29,30,31]

The 4(a) group contains limonoids in which rings B and D are opened, for example: andirobin(9) which can be seen overleaf in Figure 1.13.

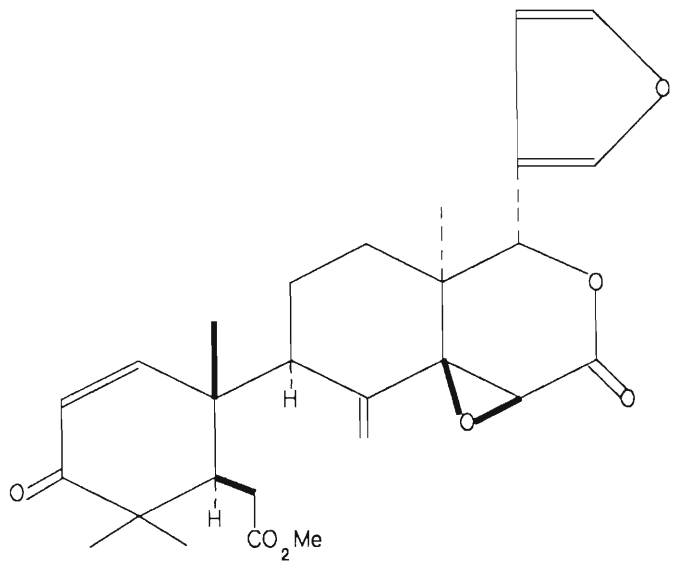


Figure 1.13 Andirobin (9)

It is proposed that ring B is opened to a lactone or ester by oxidation of a 7-keto compound without the formation of a subsequent carbon ring.

The partial synthesis of andirobin(9) has been achieved in the laboratory by Baeyer-Villiger oxidation and the scheme can be seen overleaf in Figure 1.14. [32,33]

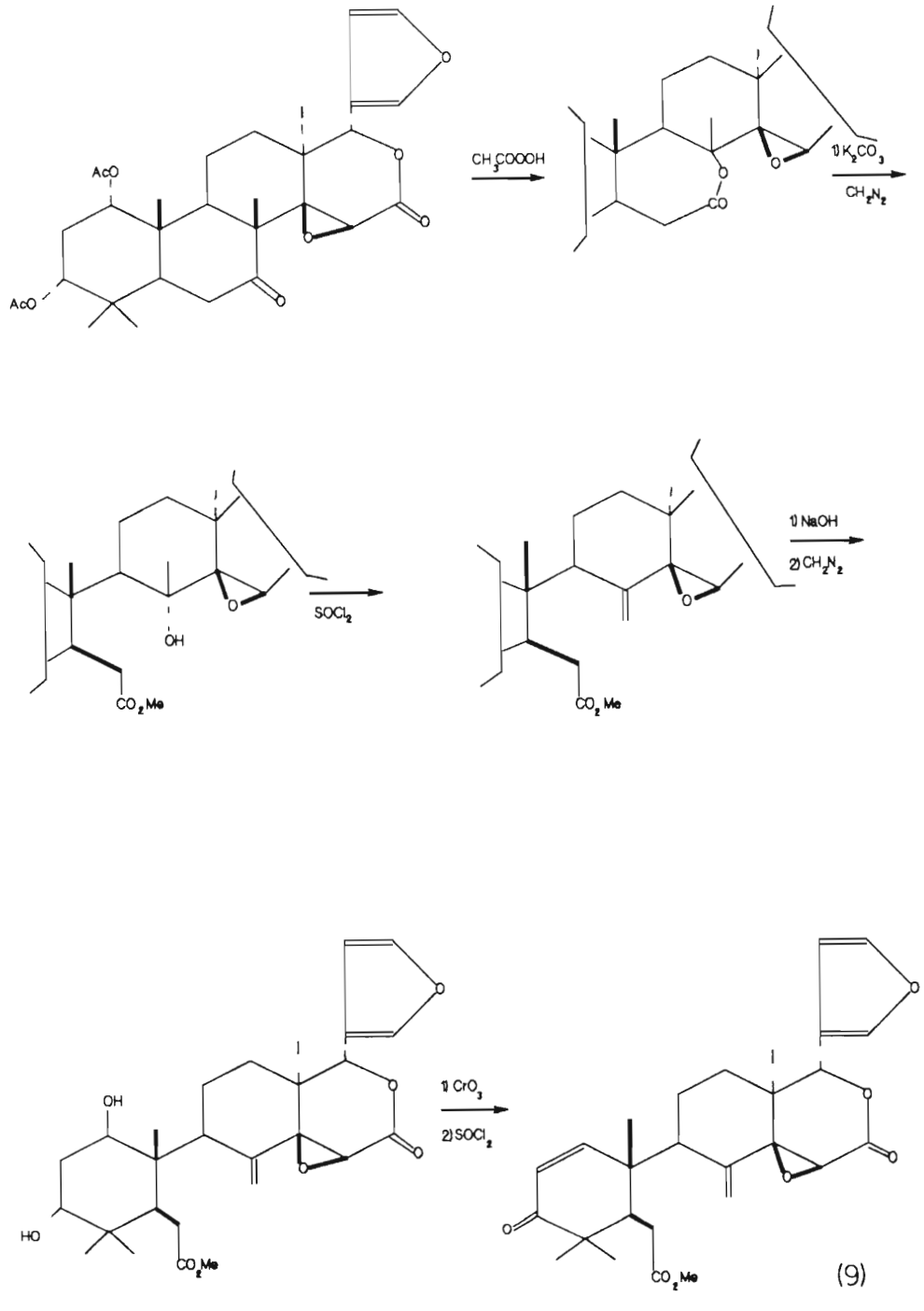


Figure 1.14 The Partial synthesis of Andirobin (9)

Cyclisation can occur spontaneously from a suitable derivative to give a 1,14 oxide from either a 1-hydroxy-14-ene or a 14-hydroxy-1-ene compound.[34] The simplest example of this is methyl angolensate(10) which is one of the most common

limonoids. Cyclisation of the 1α,3α-diol gave the 3α-alcohol which was oxidised to methyl angolensate(10)(Fig. 1.15). [33,34] This alcohol can then be obtained from methyl angolensate by borohydride reduction [35] or rapid reduction with aluminium isopropoxide. Prolonged reduction with isopropoxide leads to equilibration to the equatorial 3β isomer.

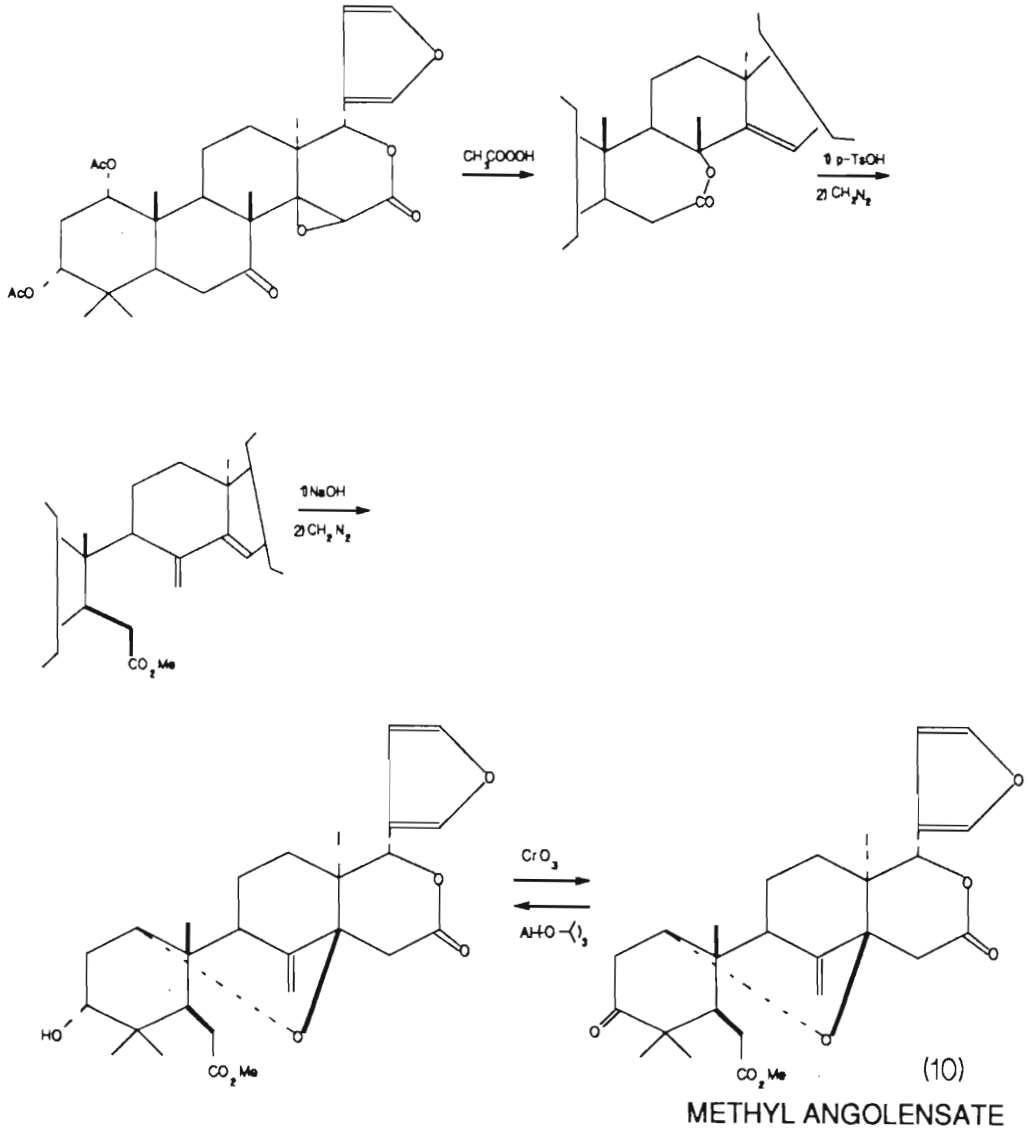


Figure 1.15 The Partial synthesis of Methyl angolensate (10)

The fourth group in which cyclisation forming a new ring B occurs, is known as the mexicanolide group (4 b). Compounds of this group are derived from 1,3- diketo-diene lactones of the andirobin group by spontaneous Michael cyclisation, producing mexicanolide(11) in the simplest case [36,33,37] as seen overleaf in Figure 1.16 . In the synthesis, only mexicanolide(11) with the 8,14 double bond, is produced with

no trace of the 8,30- or 14,15-double bond isomers. These are common in natural products, and it is not known how they are produced.

Synthetically, the only way that these compounds have been formed is by hydrogenation of the $\Delta^{8(30)},\Delta^{14}$diene.[38,39] This gives the $\Delta^{8(14)}$- and $\Delta^{8(30)}$- dihydro-derivatives, with possibly a trace of the Δ^{14} compound.

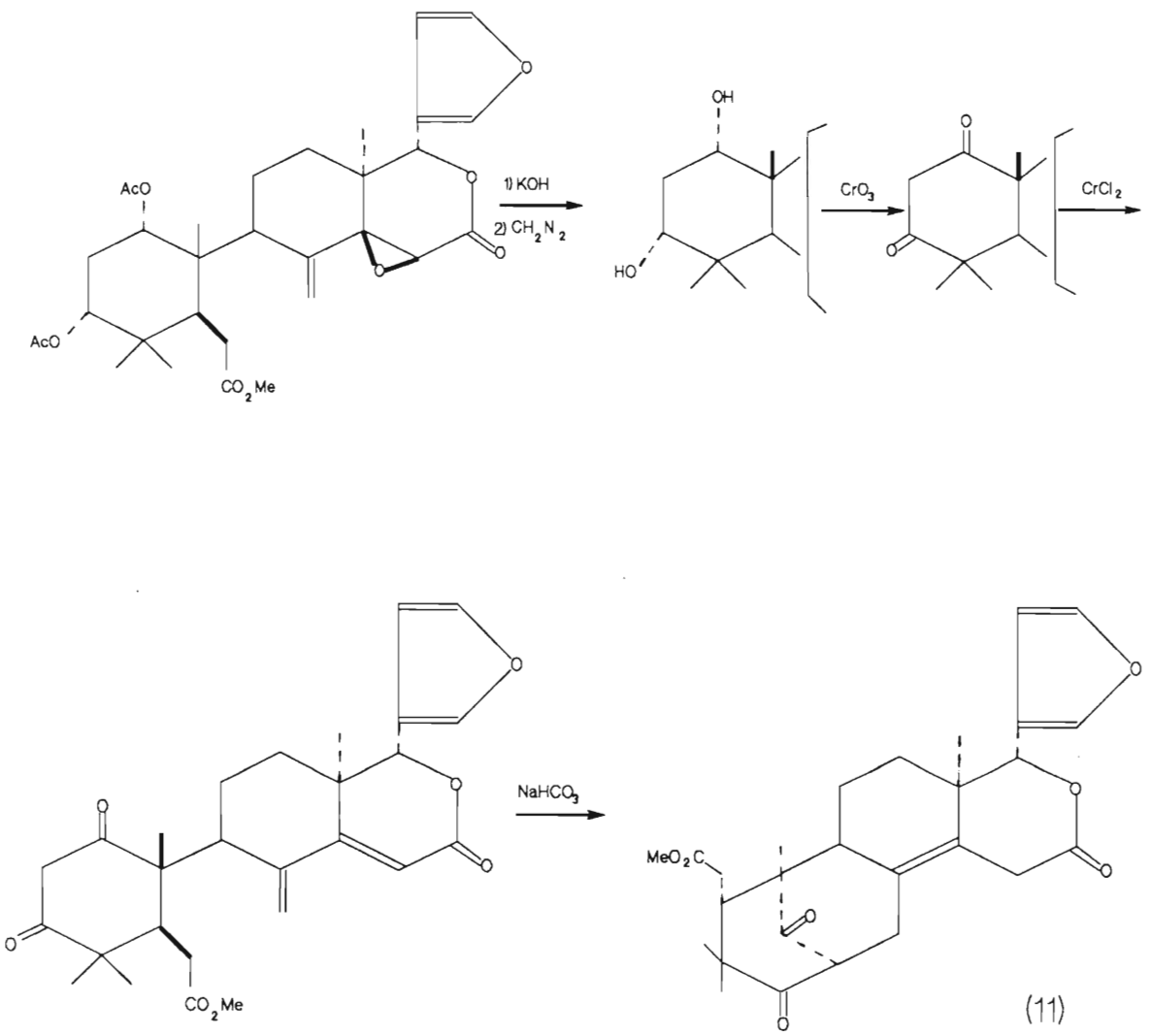


Figure 1.16 The Partial synthesis of Mexicanolide (11)

The $\Delta^{8(14)}$, $\Delta^{8(30)}$ and Δ^{14} compounds, as well as the 8,14 diene are all resistant to catalytic hydrogenation. The Δ^{14}-16-oate system has been reduced to a dihydroderivative by the use of Raney alloy in alkaline solution.[40]

The nomenclature of these compounds is based upon the presumed biosynthesis of mexicanolide(11), in which the original C-30 methyl group becomes the ring member joining C-8 and C-2. It has been proposed that the unsubstituted nucleus of mexicanolide(11) should be called methyl meliacate.[35]

Group 4c is known as the phragmalin group. Entandrophragmin(12), the longest known member of this group, was one of the first limonoids to be isolated from the Meliaceae[41] but the structure was unknown for a long time until elucidated by X-ray crystallography.[42]

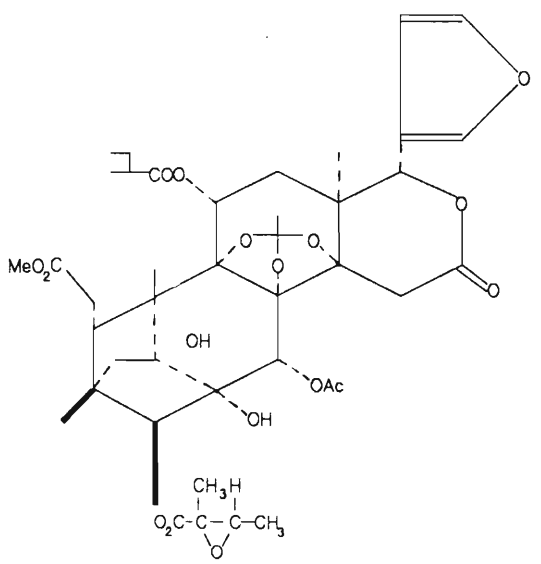


Figure 1.17 Entandrophragmin (12)

The most interesting question about phragmalin-type compounds is how they are formed in the plant. Formally the C-29 methyl group is oxidised, but since the C-1 ketone is also reduced, the transformation from mexicanolide(11) is actually an isomerisation. It has been suggested that the precursor is a hemi-ketal of the *Xylocarpus* type as seen in Figure 1.18a, which yields an oxygen radical.[19] This

can then oxidise C-29 to a radical, which can attack the ketonic form of the C-1 ketal, giving a second oxygen radical, which finally oxidises C-9. Since C-8 oxidation is necessary to produce the original ketal, this scheme explains why phragmalin derivatives are always oxidised at C-8 and C-9 (Fig. 1.18a & b).

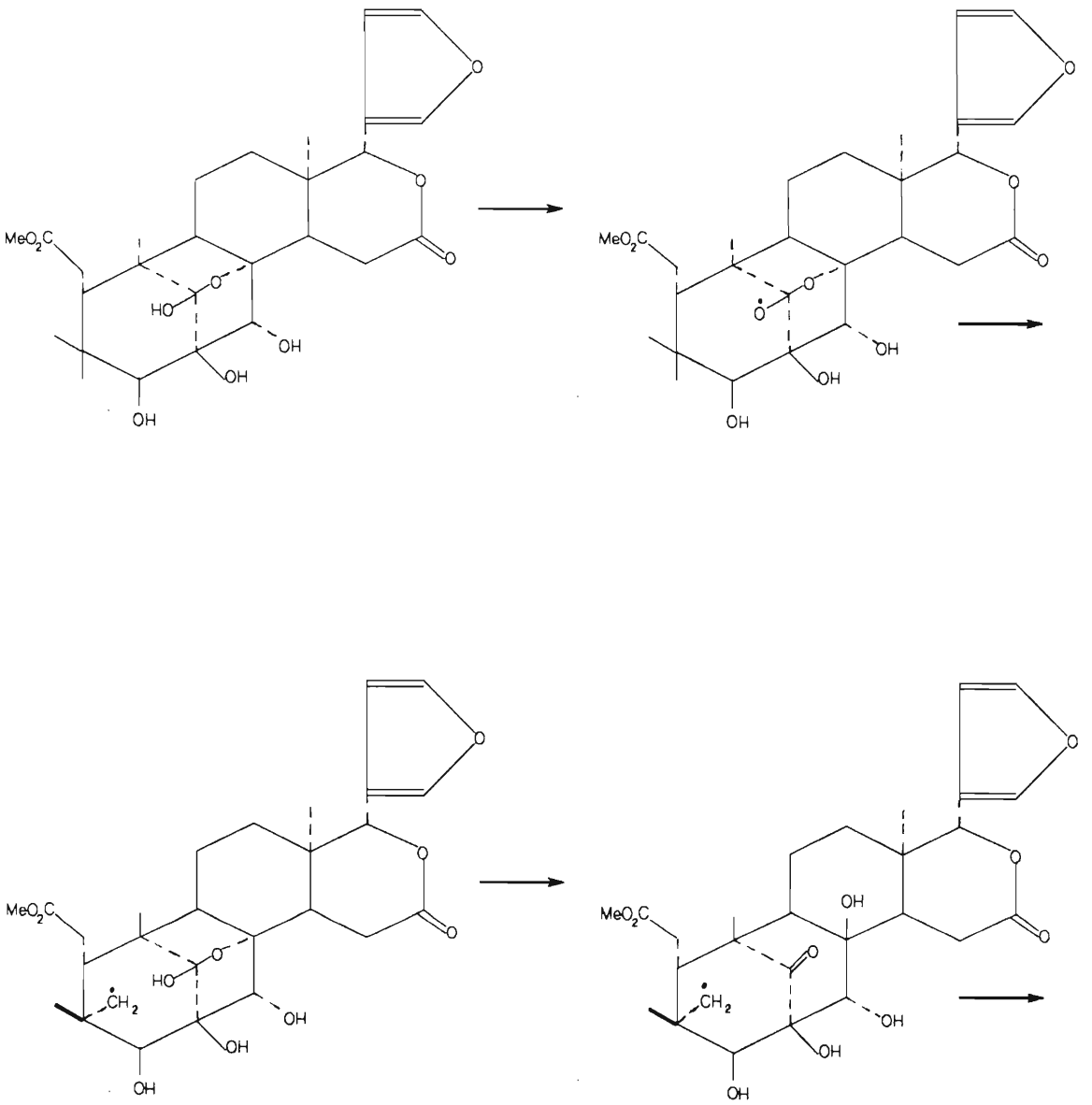


Figure 1.18.a

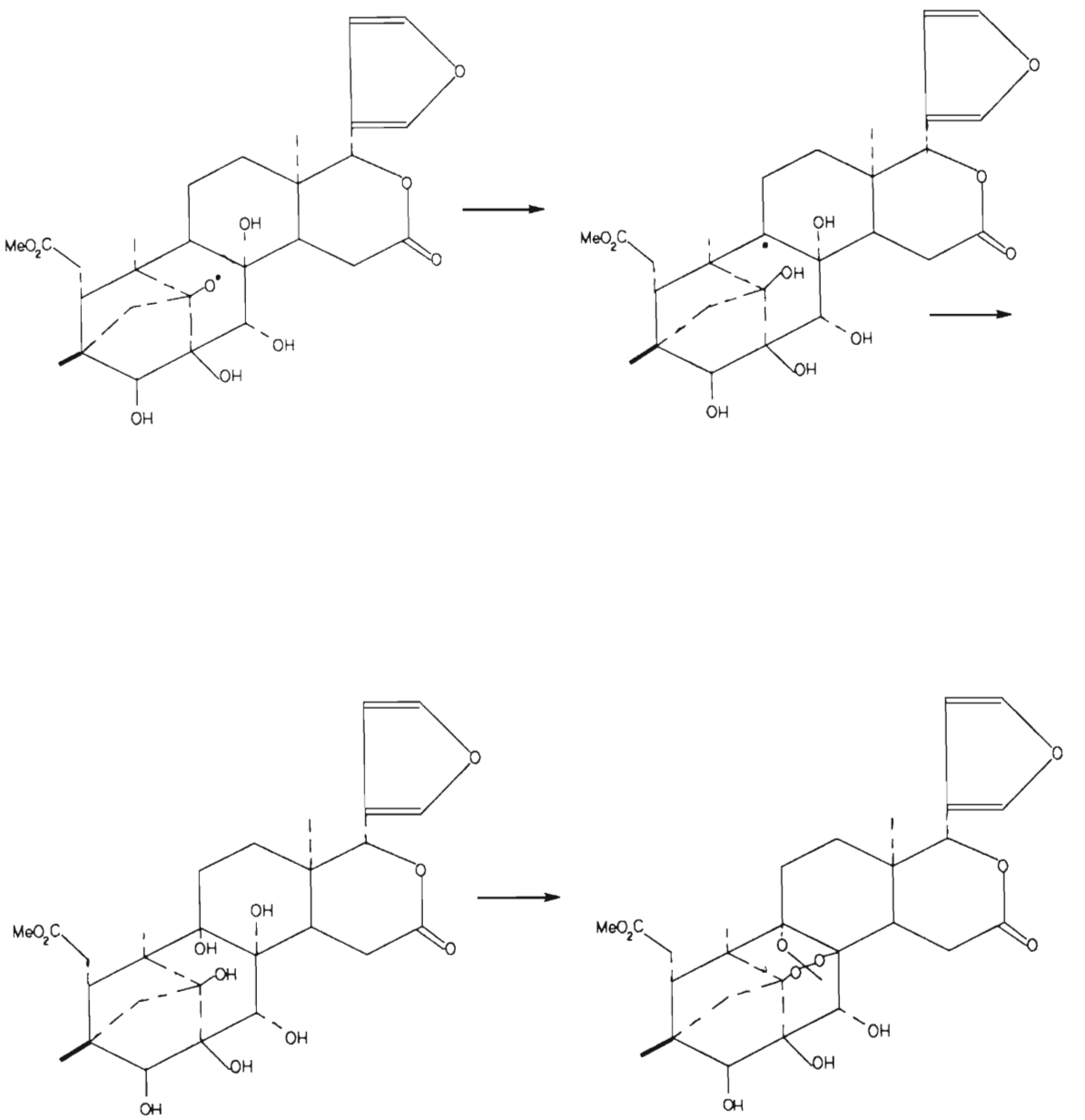


Figure 1.18.b

The fifth group is the methyl ivorensate group in which rings A,B and D are opened and can be seen in Figure 1.19.

This is still a very small group.The original member, methyl ivorensate(13) is found in small amount in *Khaya ivorensis* [43] and was synthesised by the oxidation of

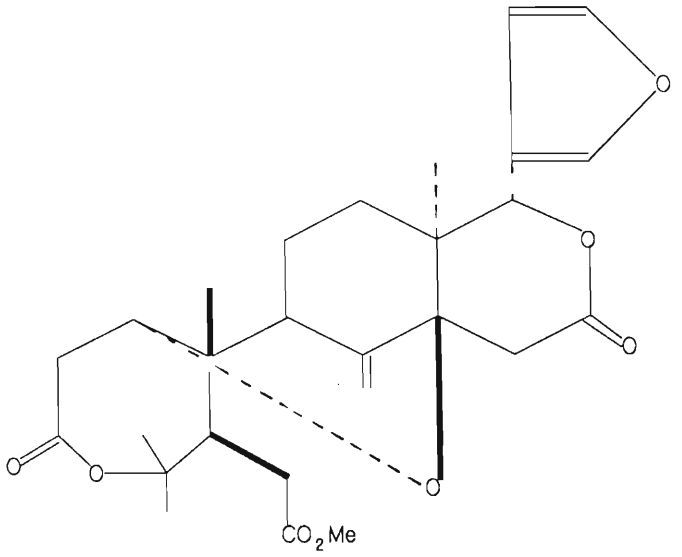


Figure 1.19 Methyl ivorensate (13)

methyl angolensate(10) with perbenzoic acid.[44] It has subsequently been found in *Soymida febrifuga.*[45] *Khaya ivorensis* timber also yielded the ring A lactone corresponding to mexicanolide(11).[44] This was not obtained by oxidation of mexicanolide(11), and is presumably formed by cyclisation of the 1-ketone 3,4-lactone related to methyl ivorensate(13).

The sixth group is known as the obacunol group and has rings A and D opened.

This group, to which limonin(1) belongs, is characteristic of the Rutaceae, but rare in Meliaceae. Obacunol(14) occurs in *Lovoa trichiliodes* [46] and in *Carapa procera*,[47] the structure of which may be seen overleaf in Figure 1.20:

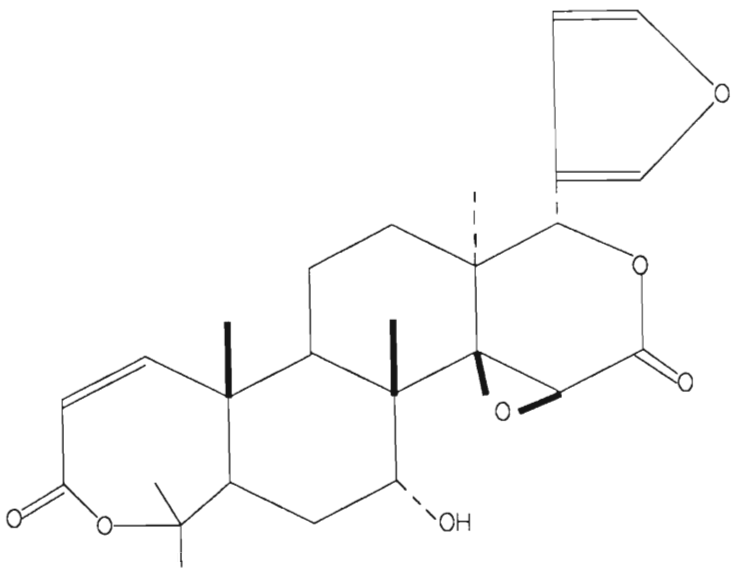


Figure 1.20 Obacunol (14)

The seventh group is known as the nimbin group in which ring C is opened (Fig. 1.21).

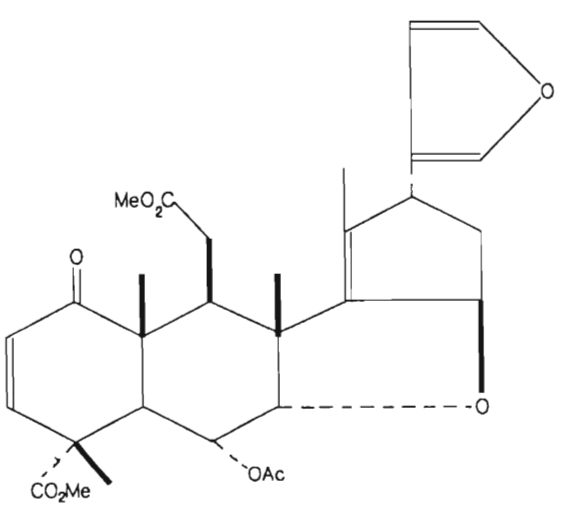


Figure 1.21 Nimbin (15)

This is a large and important group and contains some complex compounds. At one time it was thought to be confined to the closely related genera Melia and Azadirachta, until heudelobin(16), a typical member of the group, was found in the bark of *Trichilia heudelottii*.[48] However, it has been suggested by Professor Taylor, that it would be advisable to repeat this isolation in case of confusion of

extracts. There are no very simple members of the group, the most common feature in addition to the ring C opening is the formation of a tetrahydrofuran ring between C-28 and C-6.

Given that biosynthesis depends on the oxidative opening of ring C, similar to ring opening in other limonoids, the simplest derivative is the long-known salannin(19)[49] (Fig. 1.22b), in which ring C is opened to a methyl ester. The subsequent changes can be rationalised as allylic rearrangement of a Δ^{14}-13-ol, with addition of the 7α-hydroxyl to the allylic cation at C-15. This cyclisation depends on the extra flexibility due to opening ring C, as 7,15 oxides do not occur in other groups. However, the occurrence of C-12 aldehydes in this group suggests that the opening of ring C is not oxidative but hydrolytic. Thus the intermediate will be a 12β-hydroxy 14,15β-oxide, which will open to give a 15β-hydroxy-13-en-12-al (Fig. 1.22a).

In heudebolin(16) (Fig 1.22a), a member of this group, the C-12 aldehyde has cyclised in a hemi-acetal with the 15-hydroxyl group. Related to this again is ohchinolide(18) (Fig 1.22b), in which the hemiacetal has been replaced by a lactone. It has been shown by Ochi[50] by X-ray crystallography that the 15-oxygen in this is β which, since ring D has rotated about C 8-14, corresponds to a 15α-OH configuration in the original intact limonoid. A possible unifying hypothesis would be that ring opening proceeds originally from a 12β- hydroxy- 14,15β- oxide by hydrolysis as outlined above, and that the hydrate of the aldehyde substitutes H-15 allylically with inversion to give compounds similar to heudebolin which may then be oxidised to lactones. Opening of the lactone or hemi-acetal ring C is accompanied by a second allylic substitution at C-15 by the 7α-hydroxy, giving rise to compounds similar to salannin(19)(Fig. 1.22b) and ohchinal(17) (Fig 1.22a).

Nimbolidin(20)[51] represents then an intermediate stage between ohchinolide(18) and salannin(19). The next stage is oxidation of ring A to an 1-oxo compound, with elimination of the 3 substituent, and oxidation of C-28 to a carboxyl. This gives the lactone nimbolide(21),[52] the hydroxy ester form of which is nimbin(15) (Fig 1.21). Decarboxylation at C-4 then gives nimbinene(23) [53] which is oxidised to a C-4

alcohol in nimbandiol(22) (Fig 1.22c). This oxidation is fairly common in terpenes, and can occur during isolation of the compounds especially of C-4 aldehydes.[54]

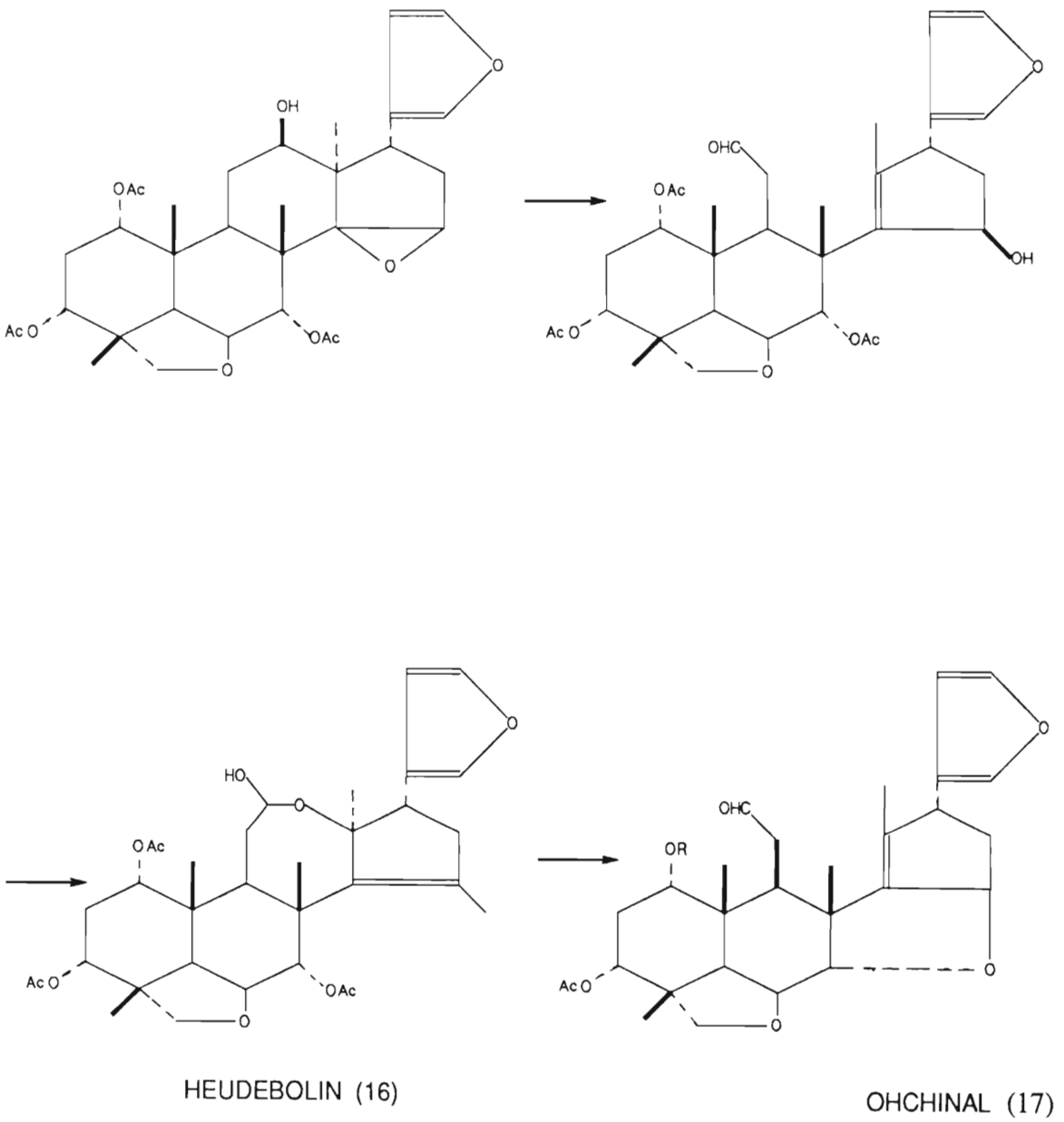


Figure 1.22a

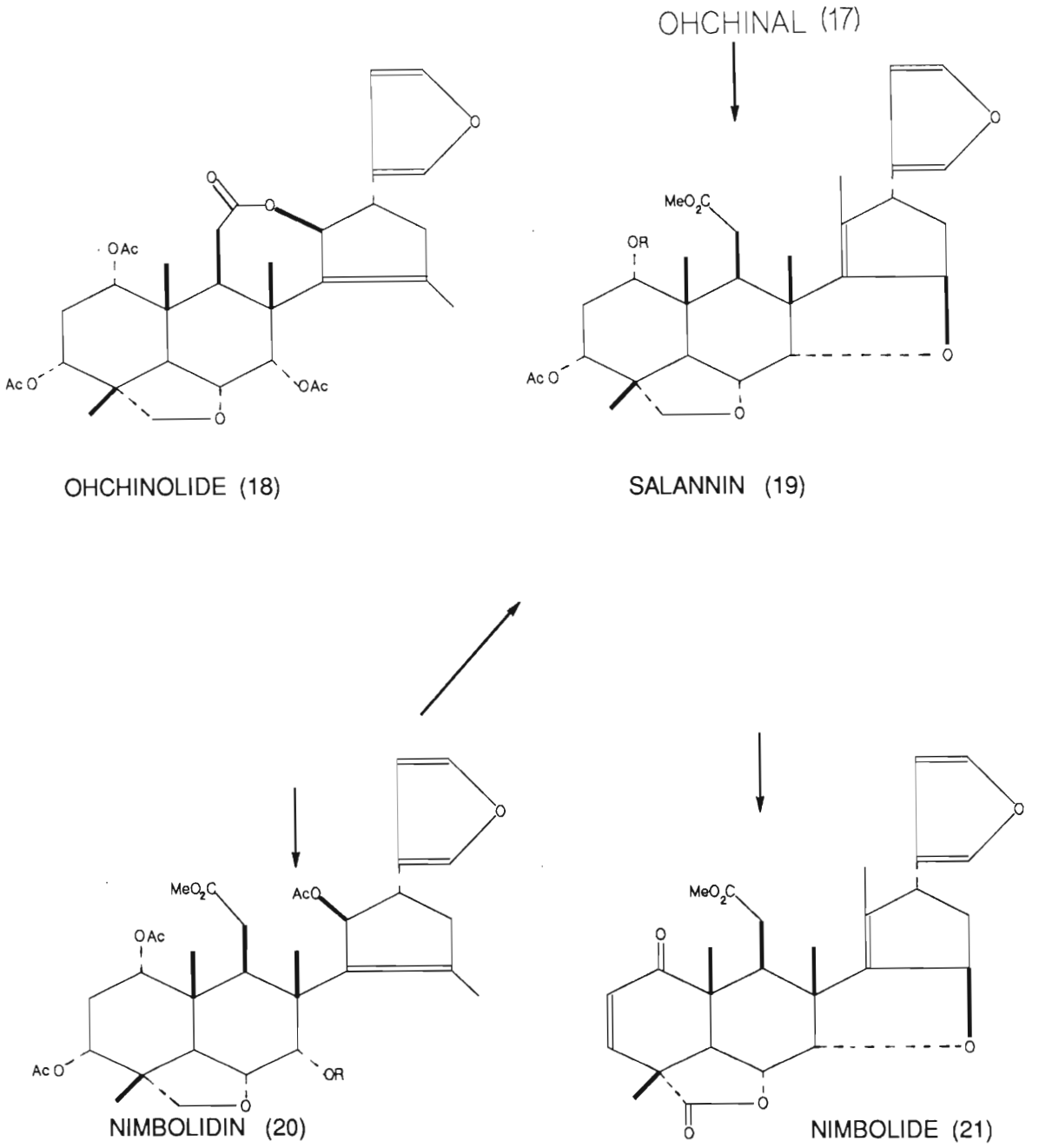

OHCHINAL (17)
OAc
Ac O
OAc
O
OHCHINOLIDE (18)
MeO2C
OR
Ac O
O
SALANNIN (19)
MeO2C
AcO
OAc
Ac O
OR
O
NIMBOLIDIN (20)
MeO2C
O
O
O
NIMBOLIDE (21)

Figure 1.22b

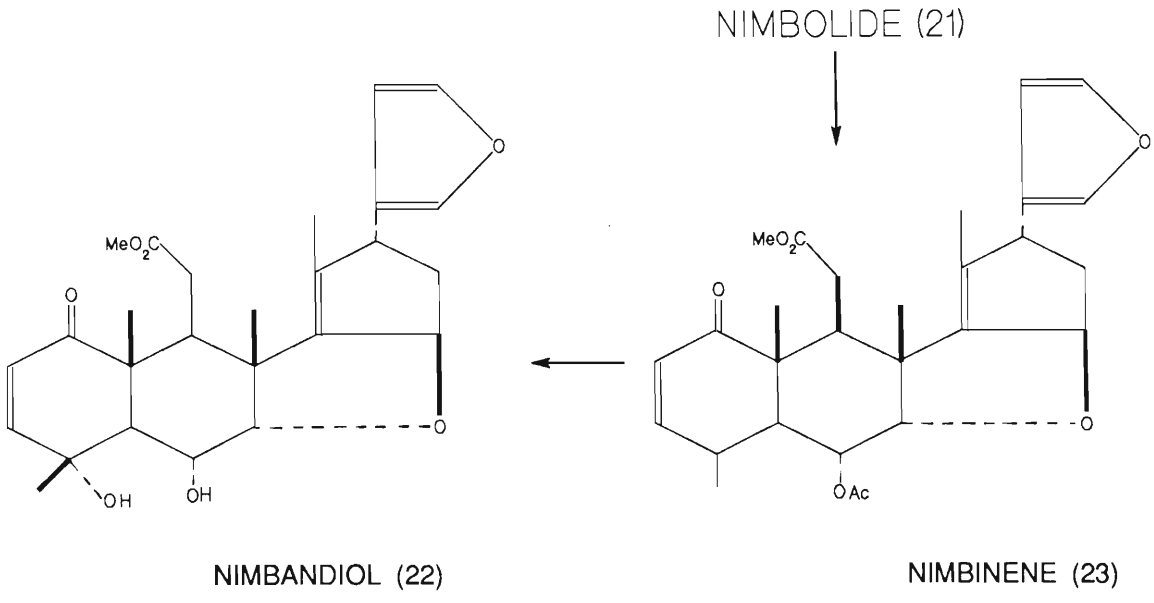


Figure 1.22c

The eighth group is known as the toonafolin group in which ring B is opened. This group has only recently been discovered by Kraus[6] in work on *Toona ciliata*. Toonafolin(24)[55] (Fig 1.23) is the ring B lactone corresponding to cedrelone, with the addition of a 1,11α- ether bridge. The structure was determined straightforwardly by N.M.R. techniques.

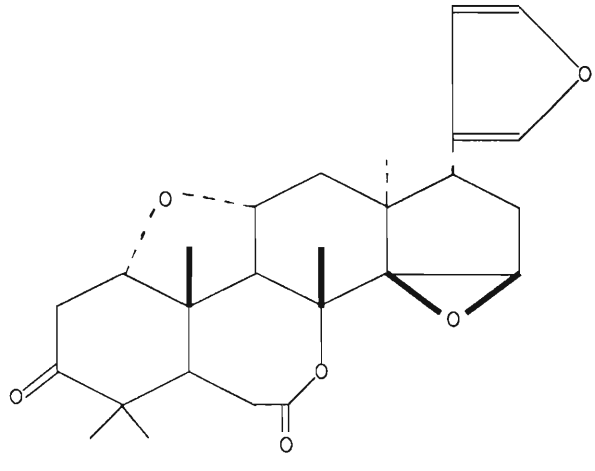


Figure 1.23 Toonafolin (24)

Alkaline hydrolysis opened the lactone, thionyl chloride then gave the expected exomethylene compound. Toonacilin(25)[6] (Fig. 1.24) already has the lactone opened, it has in addition an 11α,12α diacetoxy system. The structure of this was determined by crystallography.

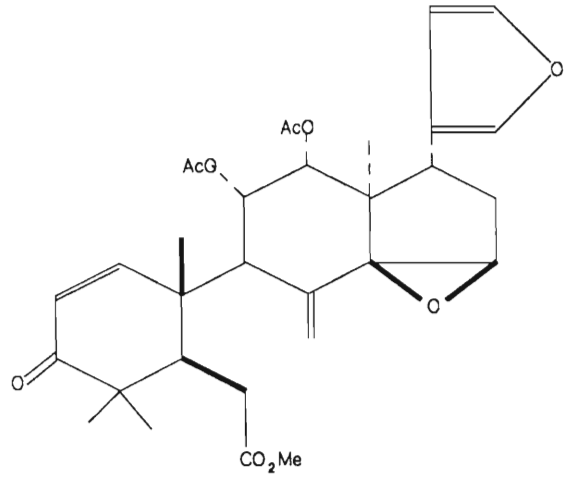


Figure 1.24 Toonacilin (25)

The ninth group is known as the evodulone group in which ring A is opened. This group has also been discovered only recently, although it has for some time been suspected to be precedent to the prieurianin group of limonoids. Members have been discovered in several plants, as minor companions of prieurianin limonoids, in *Carapa* species, and in *Toona sureni.* Evodulone(26) can be seen overleaf in Figure 1.25:

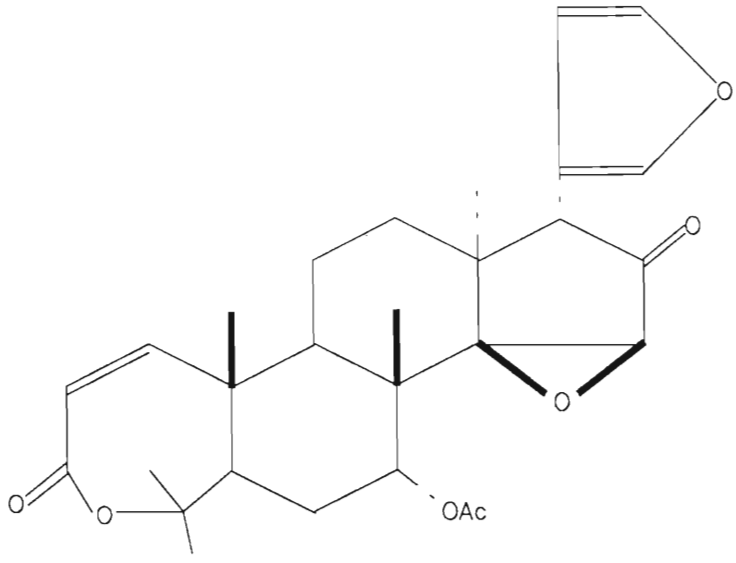


Figure 1.25 Evodulone (26)

The tenth group is the prieurianin group and here both rings A and B are opened (Fig 1.31). The structures of both prieurianin(31)[56] and dregeanin(27) [57] can be seen in Figure 1.31 and Figure 1.26 respectively. A large number of related compounds have also been found, thus making this a major group comparable to the nimbin and havanensin groups. Most members are characterised by possession of the 11β-formyloxy-12α-[3-methyl, 2-hydroxy]valerate system. The biosynthesis and reactions of dregeanin(27) may be seen overleaf in Figure 1.26:

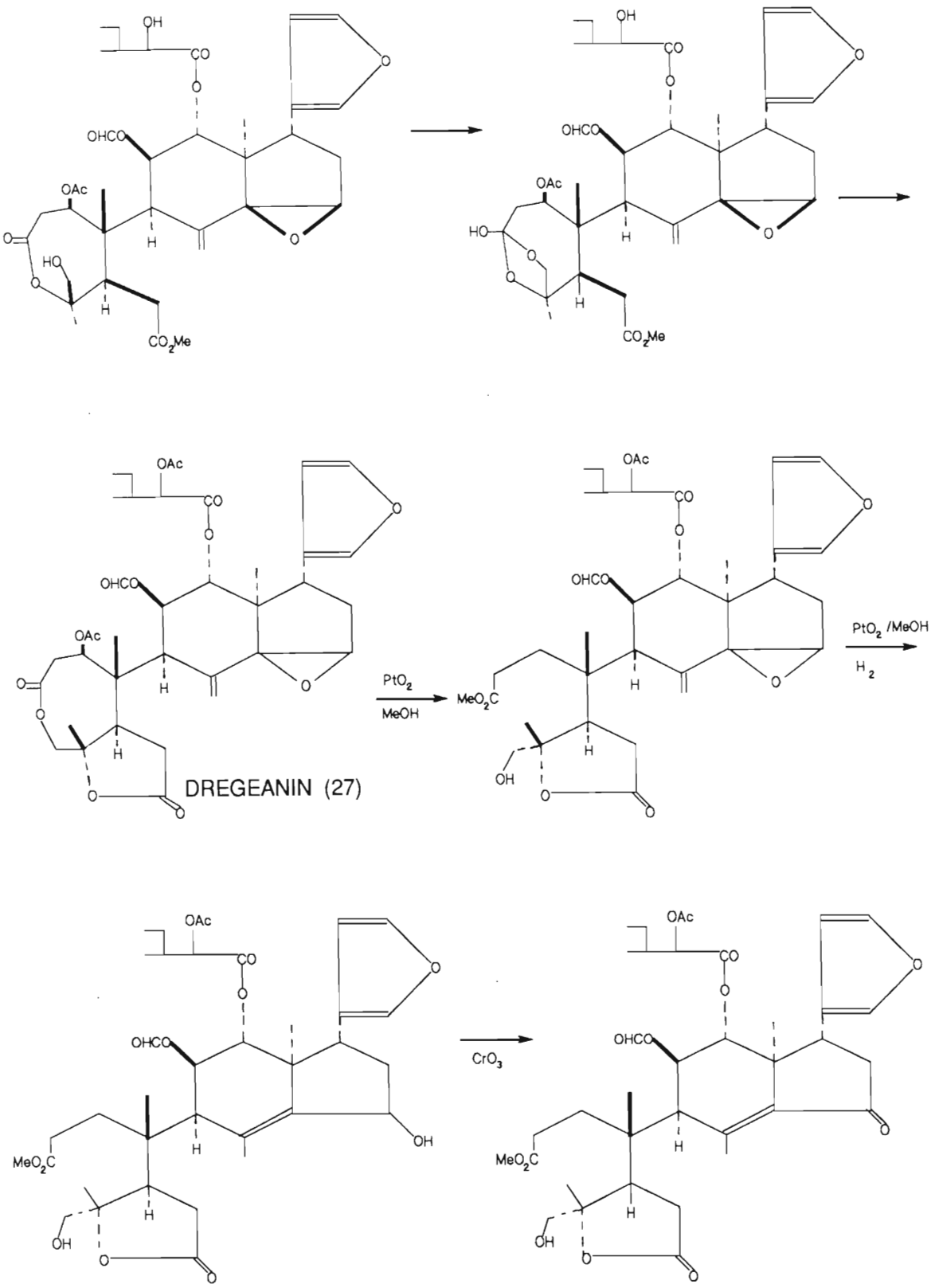


Figure 1.26 The biosynthesis and reactions of Dregeanin (27)

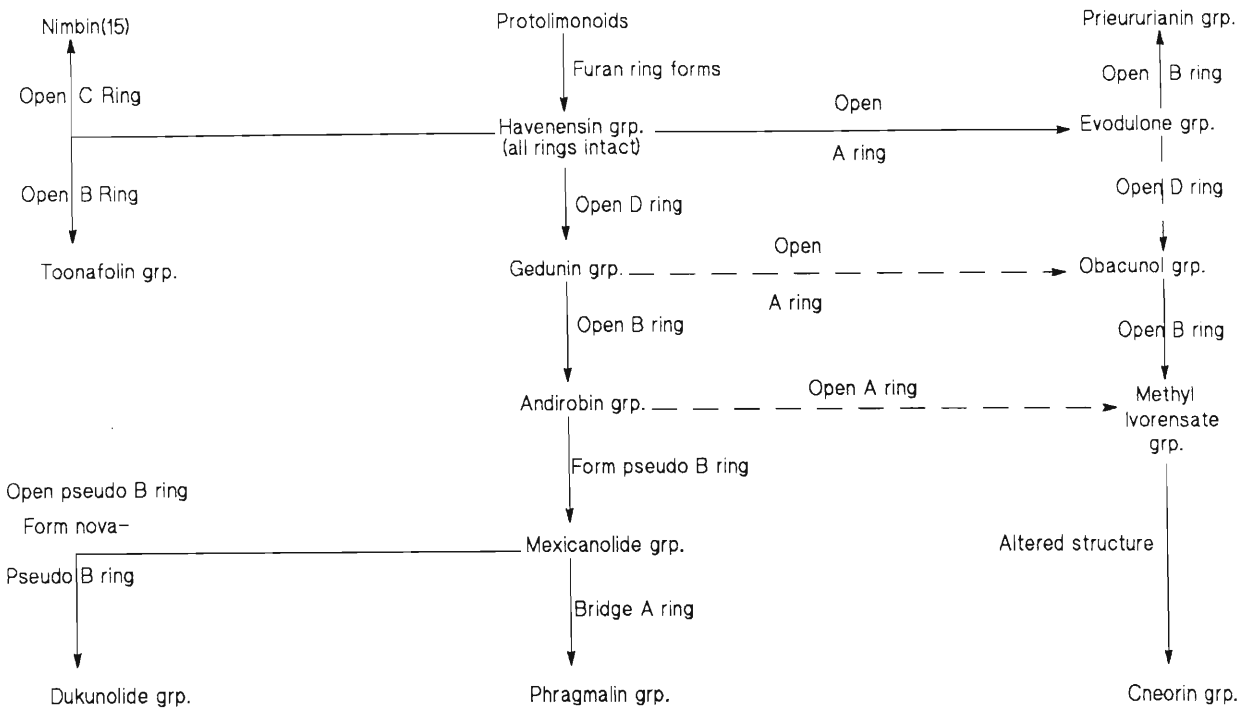


Figure 1.27 Diagram of the biosynthesis of limonoids

1.1.4 The Taxonomic importance of limonoids

Limonoids are of taxonomic importance at two levels: the family level and the generic level. Firstly, at the family level, the limonoids in the Meliaceae, Cneoraceae and Rutaceae show structural variations which are characteristic for the genus.

At the generic level, limonoids can be used to decide the affinities of a particular species. However, this is restricted to the Meliaceae, since limonoids of the other two families do not have major structural changes within the families.

1.1.5 Turraea obtusifolia and *Turraea floribunda*

Both *Turraea obtusifolia* and *Turraea floribunda* belong to the very diverse family, Meliaceae, of which the true Spanish Mahogany is a member.

Turraea obtusifolia, commonly known as the Small Honeysuckle Tree, is a small deciduous tree, only reaching approximately 3m in height, and although it is widespread, it is not common or abundant in any specific area, thus making it extremely difficult to obtain. Only one small tree was located, hence only very small amounts of material were available. To add to this problem, the tree does not bear fruit prolifically, hence making very few seeds available for extraction. This species has, however been found to grow from coastal dunes to rocky hills as the following distribution map shows [58] (Fig. 1.28):

Figure 1.28 The distribution map of *Turraea obtusifolia*

The species *Turraea floribunda* is commonly known as the Wild Honeysuckle tree. It is also a deciduous tree commonly growing to about 3 to 5 metres in height, but can reach 10 to 13 metres in forests and ravines. This species is widely distributed throughout Eastern Africa, being more accessible than *obtusifolia*. The distribution map can be seen below [58] (Fig. 1.29):

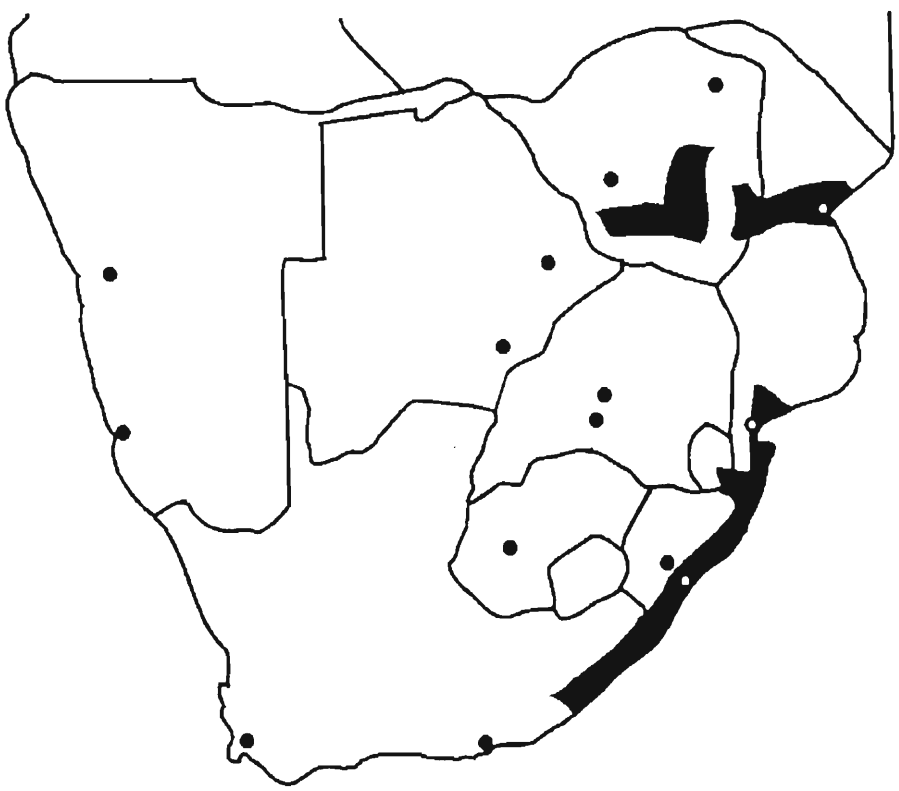
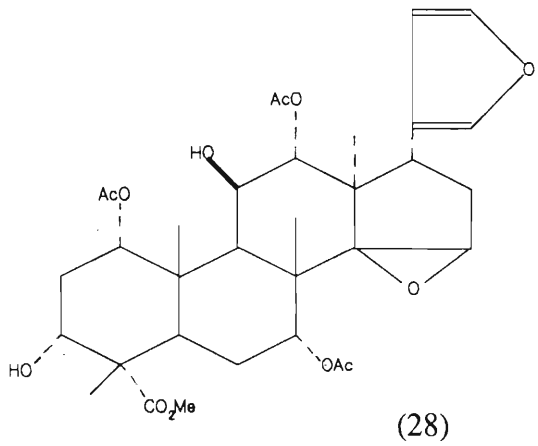


Figure 1.29 The distribution map of *Turraea floribunda*

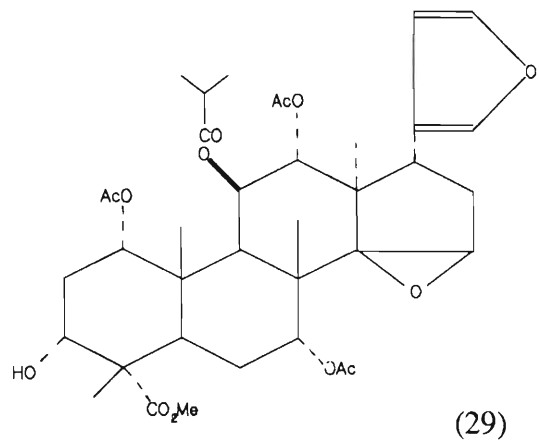
Although an overdose is poisonous, a concoction of the root and bark is used in tribal African medicine to treat rheumatism, dropsy and heart disease, while the bark is used by witchdoctors to induce trances before divining dances.

The reason for studying these two species was due to the fact that limonoids have been found extensively within the Meliaceae family, as well as occurring in the Rutaceae and Cneoraceae families, although the Meliaceae differs from the latter two families in diversity of structures of its limonoids.[59]

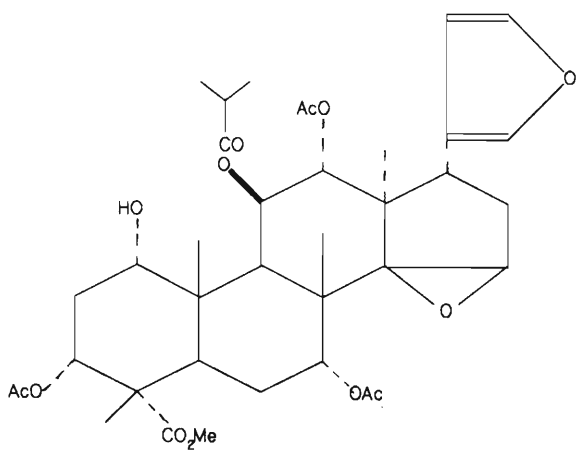
Connolly et al.[27] have recently elucidated the structures of three limonoids occurring in the species *Turraea floribunda*. They may be seen below(28,29,30) (Fig 1.30):



(28)



(29)



(30)

Figure 1.30

Connolly et al.[27] have also isolated the complex limonoid, prieurianin(31), from *Turraea obtusifolia*, the structure of which may be seen overleaf (Fig 1.31):

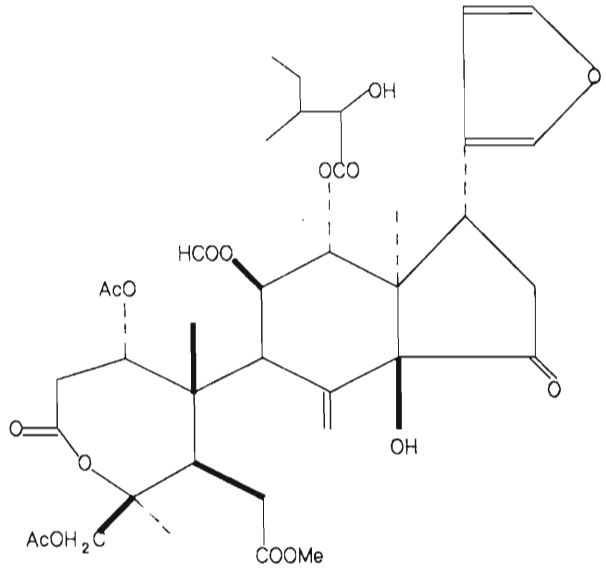


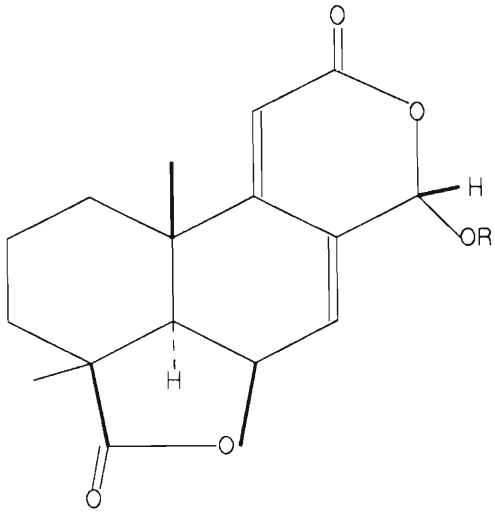
Figure 1.31 Prieurianin (31)

1.2 The family Icacinaceae

The species, *Apodytes dimidiata*, belonging to the family Icacinaceae, is commonly referred to as the White Pear tree and is found growing abundantly along the East Coast of Southern Africa. It is most frequently found as a small bushy tree, 4 to 5 metres tall in coastal evergreen bush, but does reach heights of 20 metres when growing in forest. The Natives of Natal, the Zulus, use the root bark to prepare an infusion which is used as an enema for intestinal parasites.[58]

Previous research on the family of Icacinaceae has yielded interesting compounds including new diterpenes and diterpene-based alkaloids.

Ellestad et al. isolated a C_{17} antifungal terpenoid(32) from an unidentified *Acrostalagmus* species, the structure of which can be seen overleaf [60] in Figure 1.32:

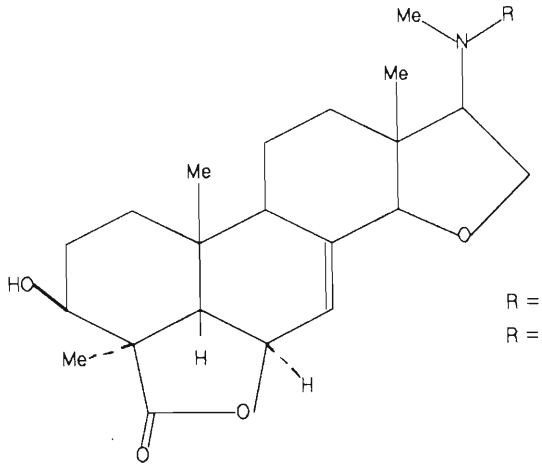


R = Me

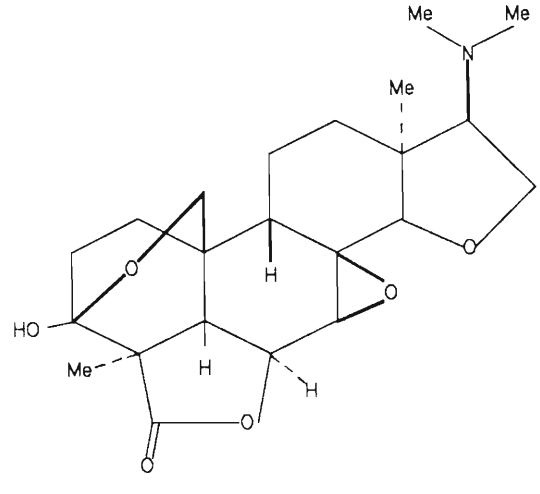
(32)

Figure 1.32

It possesses significant antifungal activity *in vitro* against a number of fungi and *in vivo* against experimental ringworm infections in Guinea pigs. Vanhaelen et al. , reported having isolated and identified two new diterpene-based alkaloids from the leaves and roots of *Icacina guesfeldtii*. These were isolated and identified as icaceine(33) and de-N-methylicaceine(34).[61,62] A previous study had already revealed the presence of icacine(35)[61] in the roots, and together these bases were the first alkaloids with a pimarane skeleton to be isolated from the plants, being found almost exclusively in the leaves (Fig 1.33).



R = Me ICACEINE (33)
R = H De-N-METHYLICACEINE (34)

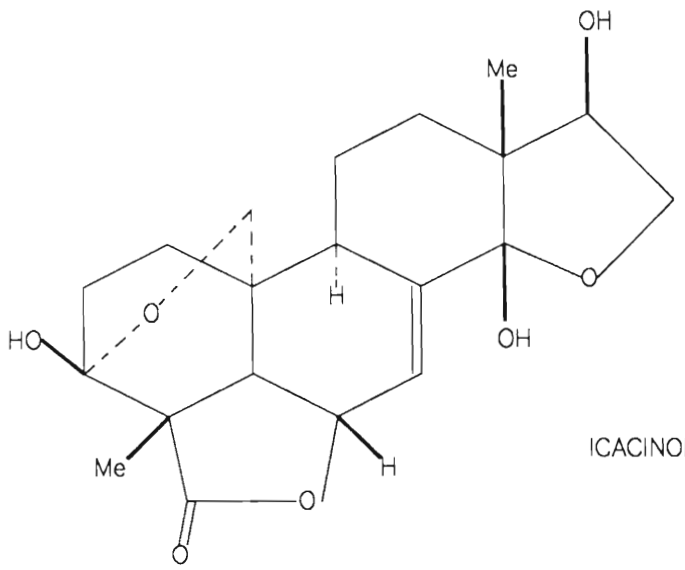


ICACINE (35)

Figure 1.33

Icacina guesfeldtii Ascher is a shrub found in different regions in Tropical Africa. In Lodja (Zaire), the root decoction is used in popular medicine as an anti-convulsant.[61]

Vanhaelen et al. continued their study of biologically active constituents by studying *Icacina claessensis*, also used as a popular medicine in Zaire as an anti-convulsant. A diterpene with a similar alkaloidal skeleton was isolated. This compound was identified as icacinol(36) (Fig 1.34).[62]

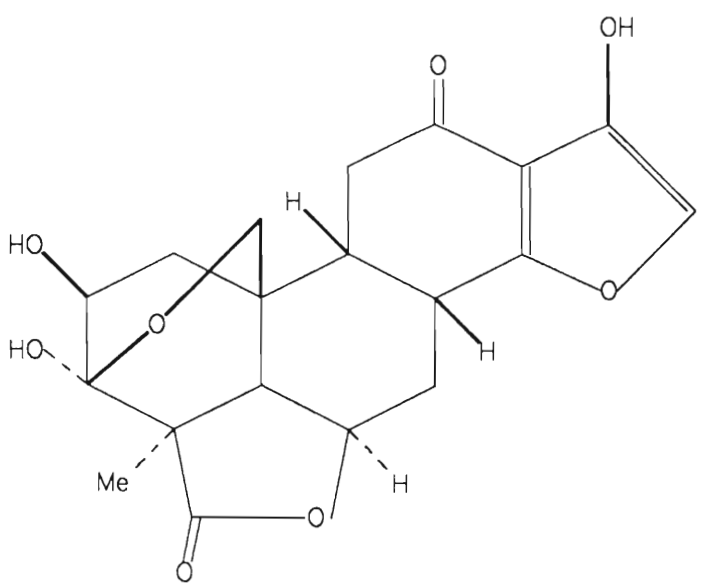


ICACINOL (36)

Figure 1.34

Subsequent study of the species *Icacina mannii* isolated a new diterpene called icacenone(37). *Icacina mannii* is a shrub endemic to Tropical Africa, the root decoctions being used in popular medicine around Kinshasa (Zaire) for treatment of fibrous tumours.[63]

The structure of icacenone(37) ($C_{19}H_{20}O_7$) can be seen below [63] (Fig 1.35):



ICACENONE (37)

Figure 1.35

Both icacinol(36) and icacenone(37) were isolated by Vanhaelen et al.[64] in the species *Icacina senegalensis* which is a shrub endemic to Casamance (Senegal). The roots have been used as a starch source in times of famine, although they have been found to be toxic in some cases. In addition to the two abovementioned compounds, some polar constituents were also isolated in the form of two steryl glucosides, sitosteryl 3-O-β-D-glucopyranoside and stigmasteryl 3-O-β-D-glucopyranoside. Hence the genus, *Icacina*, has been shown to contain novel diterpene lactone structures related to the pimarane skeleton.

In 1973, Mussini et al.[65] isolated a new diterpenoid from the species *Annona coriacea*. They had previously found 2 new diterpenoids with a Clerodane skeleton by extracting the roots of the same species with acetone.

The structure of this new diterpenoid, annonalide(38) ($C_{20}H_{26}O_6$) is as follows [66] (Fig 1.36):

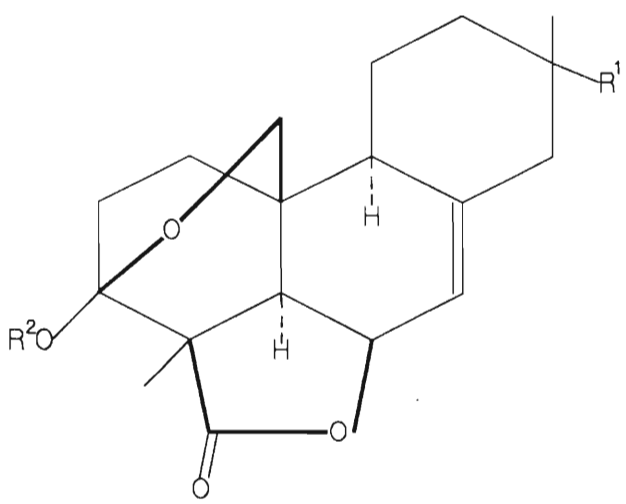


Figure 1.36

Hence, the species *Apodytes dimidiata* belonging to the same family as all the above, could possibly contain similar products as well as some novel ones. This obviously aroused interest in the species since it has never been studied before.

Chapter 2 Extractives from *Turraea floribunda*

2.1 Seeds

The seeds of *Turraea floribunda* were picked during May, 1990 from a tree located in Burman Bush in Durban, South Africa. The seed capsules were approximately 2 cm in diameter and although the tree was fairly large and well established, the seeds did not appear to be very abundant. Once picked, the seed capsules were laid out on newspaper for a few days before they burst open yielding the seeds. The seed coat was woody, very hard and approximately 3mm thick. Arranged in a ring within the capsule were 3 to 4 small and bright orange seeds, each one about the size of a lucky bean (6mm) (Fig.2.1). These bright orange seeds were dried, crushed and extracted as described in the Experimental section (5.2.1).

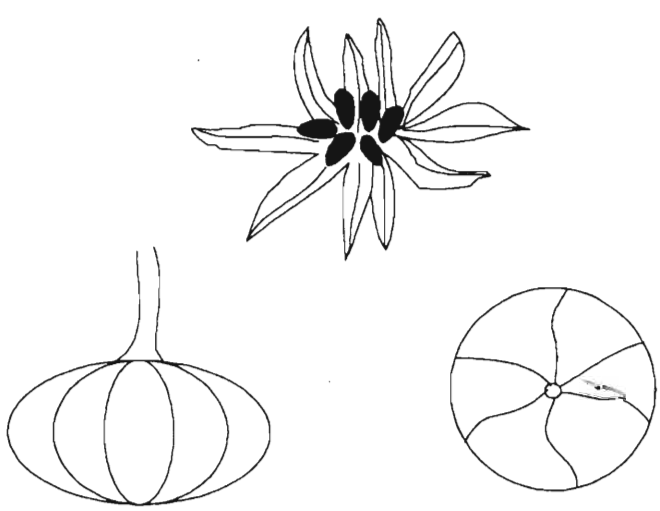


Figure 2.1 The seed capsule of *Turraea floribunda*

Two major components and several minor components were isolated from the hexane extract. The first component, COMPOUND I, was amorphous. The mass

spectrum of COMPOUND I showed that the accurate molar mass of the compound was 538.2562 $g.mol^{-1}$ while the calculated mass was 538.2566 $g.mol^{-1}$. This supported a molecular formula of $C_{31}H_{38}O_8$.

The ^1H N.M.R. spectrum(p.109) showed resonances at δ7.29, 7.14 and 6.20 which are typical of H-21, H-22 and H-23 of a β-substituted furan ring at C-17 which, together with the fact that the compound was isolated from the Meliaceae, suggested that COMPOUND I was possibly a limonoid. The spectrum had a 3-proton singlet at δ3.60 which is typical of a carbomethoxy group and two 3-proton singlets at δ1.90 and 1.70 indicating the presence of two acetate groups. The methyl region showed four further singlets due to methyl groups at δ1.0, 0.95(x2) and 0.80. The double bond region contained several signals.

The ^{13}C N.M.R. spectrum(p.109) showed 31 carbon signals, of which 10 were singlets, 11 were doublets, 3 were triplets and 7 were quartets. The spectrum had a singlet at δ204.0, indicating a keto-group and 3 singlets at δ174.5, 170.6 and 170.2 due to the carbonyl carbon atoms of the two acetate peaks and the carbomethoxy group. The two doublets in the C-O region at δ 76.9 and 72.4 were assignable to the carbon atoms to which the acetate groups were joined. A quartet at δ 52.2 indicated the -O<u>C</u>H$_3$ carbon of the carboxymethyl group. The usual β-substituted furan-ring resonances were present at δ 142.5(d,C-21); 140.3(d,C-23); 123.8(s,C-20) and 111.1(d,C-22). Thus, it was concluded that there were eight oxygen atoms in the molecule.

There were a further six resonances in the double bond region at δ 152.5(d), 148.9(s), 139.3(s), 125.5(d), 123.7(d) and 119.2(t) indicating the presence of three double bonds. The presence of both a carbomethoxy group and a terminal methylene group (triplet at δ119.2) suggested that ring B had been opened to give an 8,30-double bond and a carbomethoxy group at C-7. This is similar to the prieurianin class of compounds wherein the C-8 and C-30 resonances are found at comparable positions.[67] Thus it was proposed that the resonances at δ 139.3(s) and 119.2(t) were ascribable to C-8 and C-30 respectively. There were only two further unassigned

triplets in the spectrum and these were tentatively assigned to C-6 and C-16 as these two carbon atoms are not usually part of double bond systems nor have they acetate groups attached to them. This left C-1, C-2, C-14, C-15, C-11 and C-12 unassigned if we assume the normal limonoid structure with an oxygen function (in this case a keto group) at C-3, geminal dimethyl groups at C-4 and methyl groups in their usual configurations at C-10 and C-13 i.e. β and α respectively.

An acetate group in the usual α-position at C-12 [27] in ring-B opened compounds is known to be shielded by the nearby furan ring. The acetate peak at δ 1.70 was shifted upfield from the normal acetate position so the one acetate peak was assigned to C-12. This left one further acetate group and two double bonds to assign between carbon atoms 1, 2, 11, 14 and 15. Obviously if C-12 had an acetate group attached to it, a double bond between C-11 and C-12 was unlikely. Thus the two acetate groups are bound in a vicinal cis arrangement i.e.11α,12α as revealed in the ^{1}H N.M.R. spectrum.[6] These peaks at δ5.43 (d,J=11Hz,C-11) and 5.75 (d,J=11Hz,C-12) have coupling constants which differ from the usual 11β, 12α arrangement which is common in limonoids(J=3Hz) .[27] The remaining double bond between C-1 and C-2 suggests α,β unsaturation in ring A. However due to the amount of unsaturation in the compound, little resolution was achieved in the u.v. spectrum in which only a broad band from 238 to 284 nm was observed. Thus the structure in Figure 2.2 was tentatively proposed for COMPOUND I.

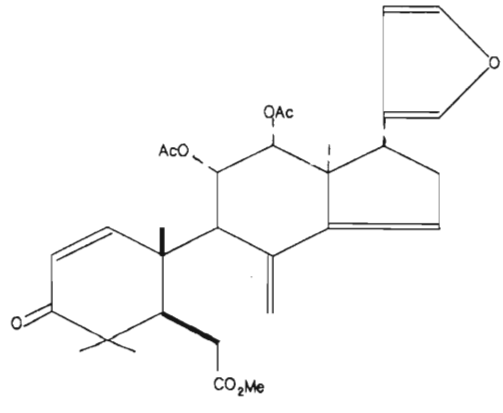


Figure 2.2 The proposed structure of COMPOUND I

More evidence for this structure was obtained by examination of the Cosy(p.110) and Hetcor(p.111) spectra. Use of Cosy and Hetcor spectra together made it possible to assign some peaks in the double bond region in both ^{13}C N.M.R. and ^{1}H N.M.R. spectra.

A doublet at δ6.75 (J=11Hz) was coupled to a doublet at δ6.09 (J=11Hz). No further coupling was noted. These peaks could be assigned to H-1 and H-2 respectively, their coupling constant being very similar to known compounds extracted from this species (J=10Hz).[27] The Hetcor spectrum showed that the doublet at δ152.5 could be ascribed to C-1 and the doublet at δ125.5 to C-2.

Geminal coupling between the two C-30 protons could be seen in the Cosy spectrum The two protons resonated at δ 5.20 (d, J=2Hz) and 4.90 (d, J=2Hz) and differ significantly from toonacilin(25)(δ5.39, 5.21; J_{gem}=1Hz), wherein these values are shifted further downfield because of the epoxide group in the 14,15 position. H-15 was found at δ 5.65(t, J=0.9Hz) and the Hetcor spectrum showed that C-15 resonates at δ 123.7(d). H-12 occurred as a doublet at δ 5.70 (J=11Hz) and was coupled to H-11 at δ 5.43. H-11 was in turn coupled to H-9 at δ 2.77 (d, J=5Hz). The Hetcor then enabled the assignment of C-11, C-12 and C-9 as δ72.4(d), 76.9(d), and 52.4(d) respectively.

The H-15 triplet at δ 5.65 (J=0.9Hz) was coupled to a multiplet at δ 2.35(2H) which was attached to a carbon which gave a triplet in the ^{13}C N.M.R. spectrum(δ 31.9) which was further coupled to a proton attached to a carbon atom which gave a doublet in the ^{13}C N.M.R. spectrum(δ 44.0) i.e. H-15, 2H-16 and H-17.

Unfortunately, the Cosy spectrum was rather complex in the δ 2.0-3.5 region but it also appeared that a further two multiplets in this area (which were attached to carbon atoms giving a doublet and triplet in the Hetcor spectrum) were coupled i.e. H-5 and 2H-6. The infra-red spectrum showed strong, broad absorbtion at 1770 cm^{-1} due to the presence of the acetate groups, as well as a shoulder at 1700 cm^{-1} which supported the α,β- unsaturated ketone .

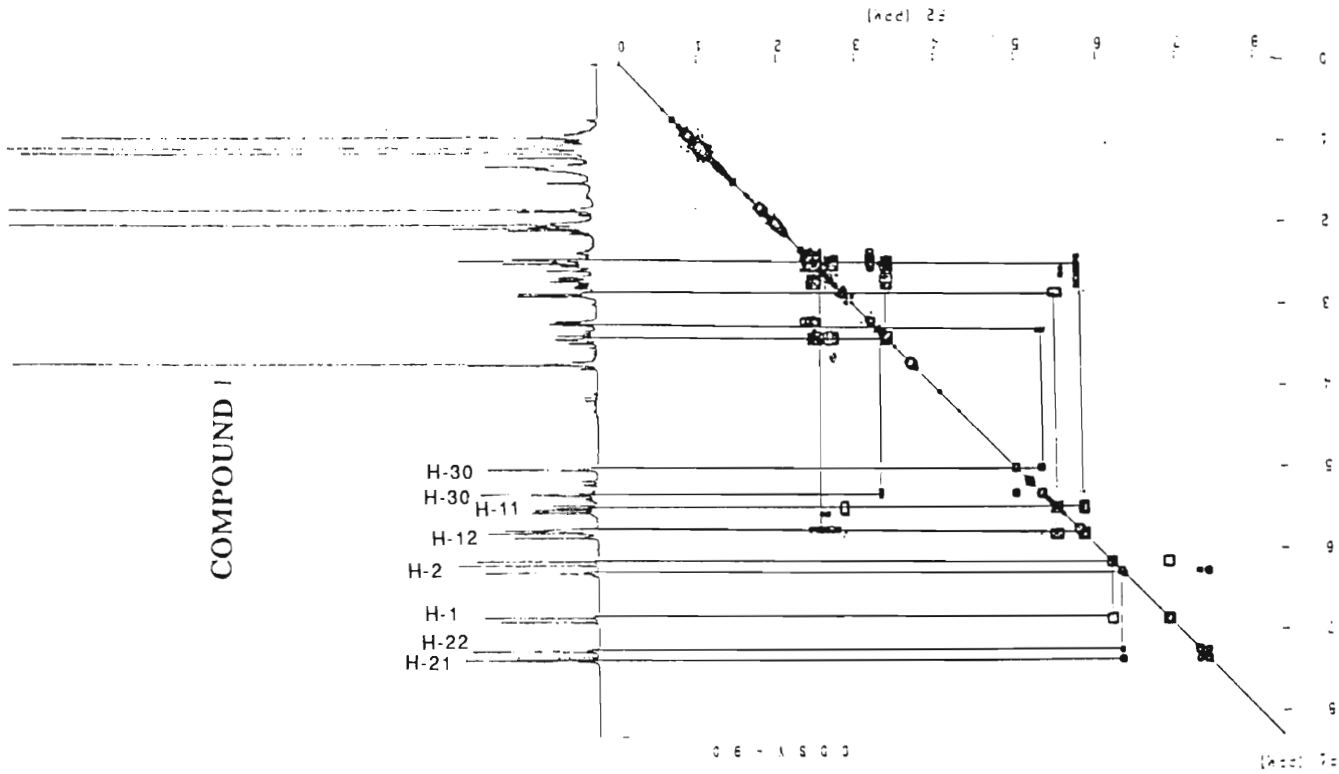


Figure 2.3 The Cosy spectrum of COMPOUND I

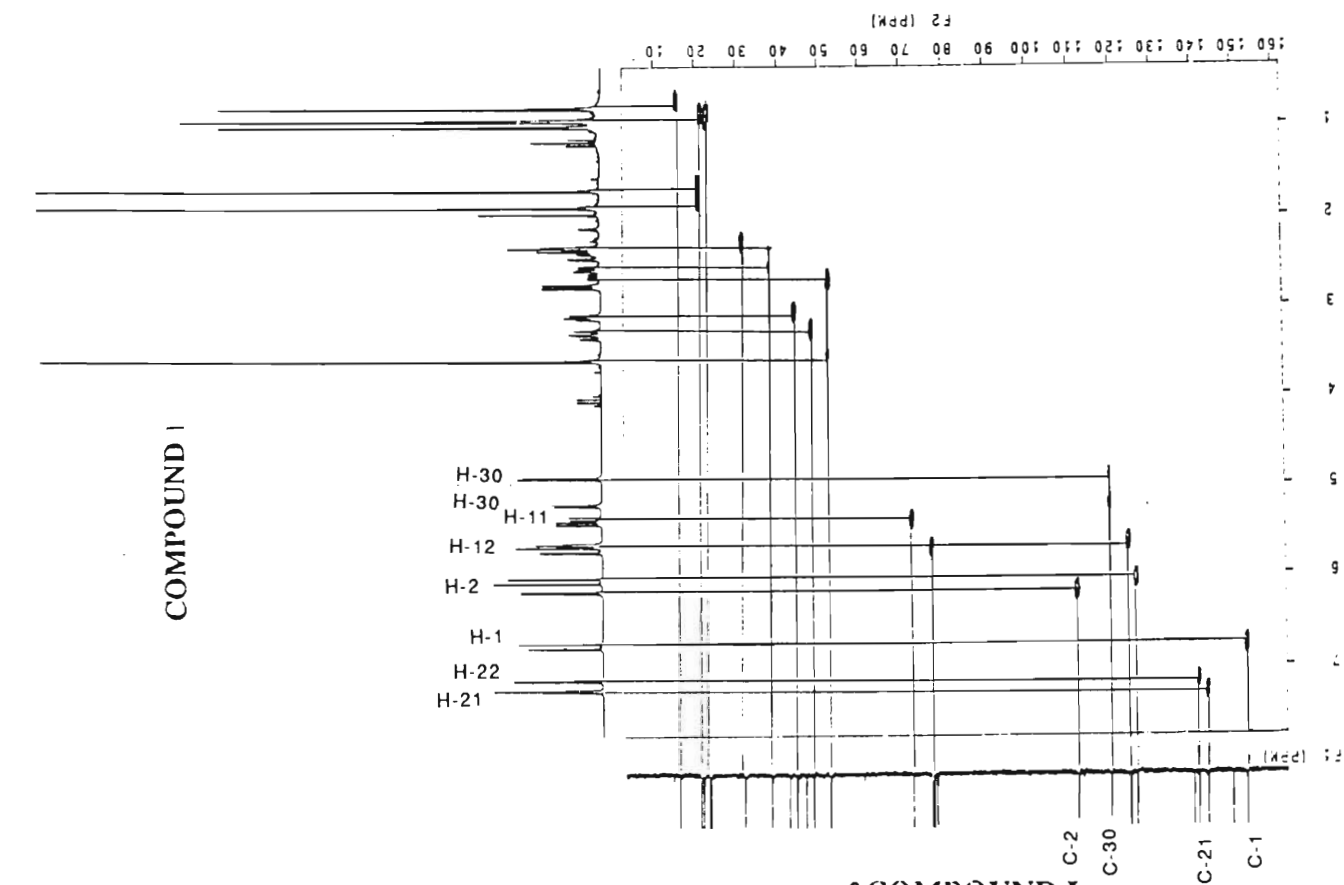


Figure 2.4 The Hetcor spectrum of COMPOUND I

COMPOUND II was obtained as a clear gum. However, on examination of the ^{13}C spectrum, it appeared to be a mixture, possibly containing COMPOUND I as well. By varying the solvent systems used for T.L.C. analysis, it was attempted to separate the mixture into its components, but attempts were met with no success.

Thus an attempt was made to acetylate Compound II as this method is often used to separate a mixture into its individual components. However the acetylation failed suggesting the absence of appropriate hydroxyl groups in the molecule. A sodium borohydride reduction was first performed at -20° C without success. Consequently the reaction was repeated at room temperature. Two reaction products were clearly obtained at different R_f values. These compounds were separated by means of column chromatography.

The compound with the higher R_f value was found to be identical to COMPOUND I in all respects, having not been reduced by sodium borohydride because of α,β unsaturation in ring A.

The other compound was labelled COMPOUND II and it was also amorphous. Due to the small amount isolated, it was impossible to obtain Hetcor or Cosy spectra.

The ^1H N.M.R. spectrum(p.112) showed resonances at δ7.33, 7.27 and 6.25 which are typical of H-21, H-23 and H-22 of a β-substituted furan ring attached to C-17 which indicated that COMPOUND II was also a limonoid. H-1 and H-2 occurred at δ5.0 and 5.34 respectively. They were thus shifted and were not as far downfield as in COMPOUND I(δ6.83(H-1) and 6.14(H-2)). This suggested the absence of the double bond between C-1 and C-2 as in COMPOUND I. The spectrum also had a 3-proton peak at δ3.68 which is typical of a carbomethoxy group as well as four 3-proton peaks at δ2.06, 2.01, 1.97 and 1.80 which indicated the presence of four acetate peaks. The methyl region showed a further four singlets due to methyl groups (δ1.66, 1.36, 1.05 and 0.87). The double bond region again contained several peaks.

The ^{13}C N.M.R. spectrum(p.112) showed 35 carbon peaks of which 11 were singlets, 12 were doublets, 3 were triplets and 9 were quartets.

Most notable was the fact that the two doublets present in COMPOUND I at δ152.5 (C-1) and 125.5 (C-2) were missing and the spectrum had 5 singlets at δ174.8, 171.2, 171.1, 171.0 and 170.5. This indicated two extra acetate groups at C-1 and C-2 as well as the acetate groups at C-11 and C-12 and the presence of the carbomethoxy group at C-7. Five doublets in the C-O region (δ77.5, 77.0, 75.9, 75.1 and 74.0) were assigned to the carbon atoms to which the acetate groups were attached and the remaining one to an hydroxyl group at C-3. This implied that the original compound was similar to COMPOUND I with a keto- group at C-3 and in place of the α,β-unsaturated ketone, two acetate groups at C-1 and C-2. The fact that no hydroxyl groups were present explained why the mixture had not acetylated and could only be separated by means of the sodium borohydride reduction of the keto- group at C-3. The orientation of the 4 acetate groups in this spectrum corresponded to those of other limonoids found [6,68] i.e. two α orientated acetate groups attached to carbon atoms 1 and 2 and similarly two α-orientated groups at C-11 and C-12 . The positions of H-1 and H-2 in the ^{1}H N.M.R. spectrum were consistent with those expected for compounds with two α-orientated acetate groups attached to C-1 and C-2 .[68] A quartet at δ52.2 indicated the carbon atom of the -O<u>C</u>H$_3$ group. The usual β-substituted furan ring resonances were present at δ142.8(d, C-21), 140.5(d, C-23), 124.0(s, C-20) and 111.4(d, C-22). This accounts for 12 oxygen atoms in the molecule.

There were four further resonances in the double bond region at δ150.2(s), 139.3(s), 123.7(d) and 121.5(t) indicating the presence of two double bonds, as compared to δ148.9(s), 139.3(s), 123.7(d) and 119.2(t) in COMPOUND 1.

As in COMPOUND I the presence of a carbomethoxy group and a triplet at δ121.5 indicating a terminal methylene group suggested that ring B had been opened to give an 8,30- double bond with a carbomethoxy group at C-7. Thus it was proposed that the resonances at δ139.3(s) and 121.5(t) are ascribable to C-8 and C-30 respectively.

However, the resonances of C-14 and C-8 are probably interchangeable. There were only two further unassigned triplets at δ37.6 and 33.3 and these were tentatively assigned to C-6 and C-16 as these carbon atoms are not usually part of double bonds nor have acetate groups attached to them.

This left C-14 and C-15 unassigned, if we assume the normal limonoid structure with an oxygen function at C-3, geminal dimethyl groups at C-4, and methyl groups at C-10 and C-13. Hence the resonances at δ123.7(d) and 150.2(s) suggested a double bond between C-15 and C-14 respectively as in COMPOUND I. The structure proposed was thus the reduced form of the original compound ($C_{35}H_{44}O_{12}$)(Fig 2.5). These above mentioned limonoids in which ring B has opened, fall into the Toonafolin group (Group 8) which has been discovered by Kraus in work on *Toona ciliata* .[6] However, both COMPOUND I and COMPOUND II are very rare in not having an oxidatively opened ring D, which places both COMPOUND I and COMPOUND II in a new class of limonoids.

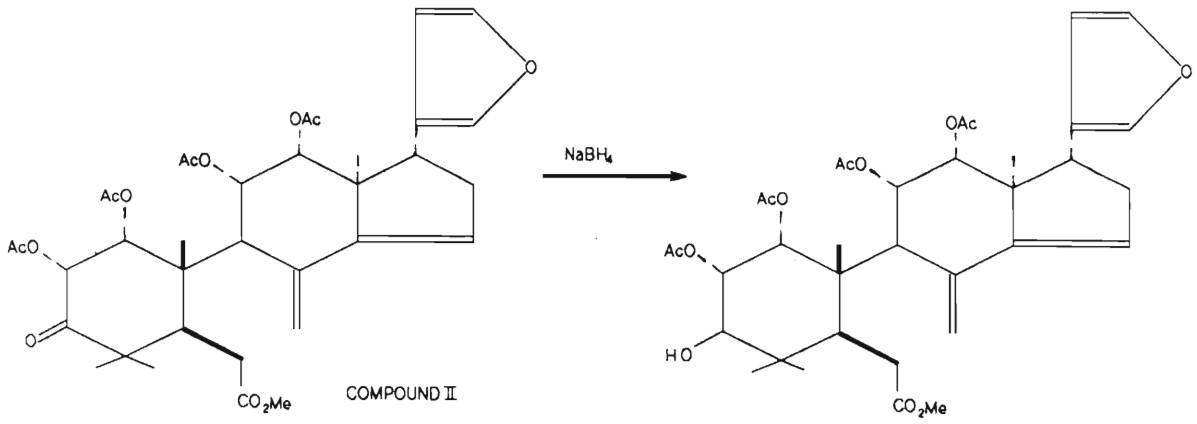


Figure 2.5 The proposed structure of COMPOUND II

2.2 Leaves

The leaves were picked from the same tree located in Burman Bush from which the seeds were obtained. They were then processed and extracted as described in the Experimental section (5.2.2) and from the hexane extract one compound was obtained as a clear gum and labelled as COMPOUND III.

Firstly, COMPOUND III was clearly identified as a limonoid since the typical β-furan ring resonances in the ^{1}H N.M.R. spectrum(p.113) occurred at δ 7.23(H-21), 7.08(H-23) and 6.38(H-22). The four 3-proton peaks in the ^{1}H N.M.R. spectrum at δ 3.58, 2.10, 2.07 and 2.01 were characteristic of a carbomethoxy group and three acetate groups. The methyl region showed a further four singlets due to methyl groups at δ 1.36, 1.28, 1.14 and 0.96. The double bond region contained several peaks.

The ^{13}C N.M.R. spectrum(p.113) showed 33 carbon peaks of which 10 were singlets, 12 were doublets, 3 were triplets and 8 were quartets.

The spectrum had four singlets at δ 175.7, 173.5, 170.6 and 169.9 due to the carbonyl carbon atom of the three acetate groups and the carboxymethyl group. The five doublets in the region in which C-O groups occur at δ 86.9, 74.6, 74.2, 73.9 and 73.3 were assigned to the three carbon atoms to which the acetate groups were joined leaving two peaks which were assigned to carbons joined to hydroxyl groups. A quartet at δ51.8 indicated the -O<u>C</u>H$_3$ carbon of the carboxymethyl group. The usual β-furan ring resonances were present at δ142.6(d, C-21); 140.8(d, C-23); 128.4(s, C-20) and 112.5(d, C-22). On comparison with compounds already elucidated from *Turraea floribunda* ,[27] it was found that this spectral data was identical with that recorded for Compound A(6)[27] previously isolated from this species. COMPOUND III was assigned the structure seen in Fig. 2.6 on p.47.

In this compound, the carbomethoxy group occurred at C-29 and the three acetate groups at C-1, C-7 and C-12, all being α-orientated.[27] The two hydroxyl groups were attached at C-3 and C-11 with the former being α-orientated and the latter

β-orientated.[27] The singlet in the ^{13}C N.M.R. spectrum at δ 73.9 and the doublet at 63.2 represented C-14 and C-15 respectively. This is characteristic of an epoxide group at this 14,15 position which is common to other limonoids found in *Turraea floribunda*. The four remaining singlets in the ^{13}C N.M.R. spectrum at δ 51.4, 47.9, 40.8 and 40.0 were representative of C-4, C-8, C-10 and C-13 respectively; while the remaining doublets at δ 41.2 and 41.1 represented C-9 and C-17. Three triplets are recorded at δ 32.1, 27.6 and 25.3 and these are attributed to methylene groups at C-2, C-6 and C-16 respectively. Finally, the seven quartets occurred in the ^{13}C N.M.R. spectrum at δ 23.7, 21.3, 21.2, 20.7, 18.1, 16.6 and 16.6. Three of these have already been discussed and are the methyl groups attached to the carbomethoxy group at C-29 as well as the two methyl groups on the acetates. The remaining four quartets were assigned to C-4, C-8, C-10 and C-13 (Fig 2.6).

More evidence for this structure was obtained by examination of the Cosy(p.114) and Hetcor(p.115) spectra. From the Hetcor spectrum, the C-O resonances at δ86.9, 74.6, 74.2 and 73.9 appeared to be linked to proton resonances at δ 4.35, 4.58, 5.02 and 3.59 respectively. Referring again to the Hetcor spectrum, the doublet at δ 63.2 representing C-15 appeared to correspond to a resonance at δ 3.53 in the ^{1}H N.M.R. spectrum. The Cosy spectrum clearly showed the coupling of H-21, H-22 and H-23.

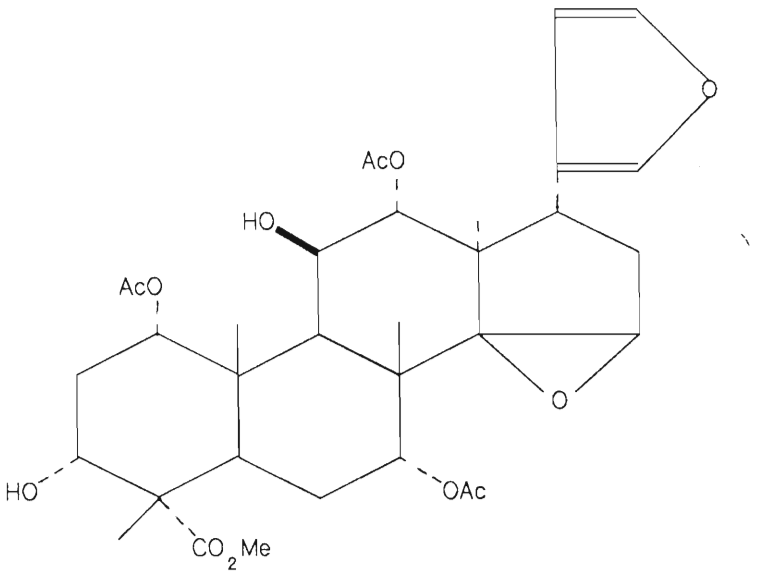


Figure 2.6 The proposed structure of COMPOUND III

COMPOUND III was then acetylated as described in the Experimental section (5.9) and the main product isolated by means of column chromatography. The compound was labelled COMPOUND IIIa and was obtained as a clear gum. The mass spectrum of COMPOUND IIIa showed that the molecular ion occurred at 716 which is to be expected for a molecular formula of $C_{37}H_{48}O_{14}$.

From the ^{1}H N.M.R. spectrum(p.116), it was clear that COMPOUND IIIa is a penta-acetate, since five 3-proton resonances were recorded at δ 1.92; 2.05; 2.11; 2.12 and 2.19. In the ^{13}C N.M.R. spectrum(p.116), the five singlets at δ 174.2; 171.4; 170.5; 169.8 and 169.5 confirmed the five acetate resonances, while the remaining singlet at δ 170.1 represented the carbomethoxy function at C-29. The rest of the data for the compound was identical to COMPOUND III. The structure proposed was thus (Fig 2.7):

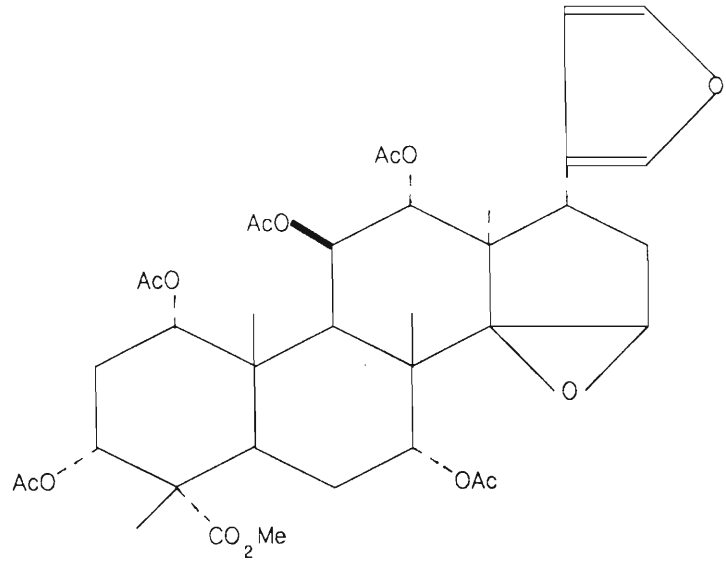


Figure 2.7 The proposed structure of COMPOUND IIIa

A minor product isolated from the acetylation mixture was labelled COMPOUND IIIb and was obtained as a gum. The mass spectrum showed that the molecular ion occurred at 672 which indicated a molecular formula of $C_{35}H_{44}O_{13}$.

In the ^{1}H N.M.R. spectrum(p.119) the most significant feature was the presence of four acetate peaks at δ2.19, 2.17, 2.14 and 2.09. The usual β-furan ring resonances were present at δ7.29, 7.13 and 6.43 which represented H-21, H-23 and H-22 respectively; and the 3-proton carboxymethyl peak was at δ3.68. Finally, four methyl resonances were reported at δ1.35, 1.28, 1.21 and 1.06.

No ^{13}C N.M.R. spectrum was obtained due to the small amount of sample. However, the presence of a keto- group in place of the hydroxyl group at C-3 in COMPOUND III,[27] was confirmed by an infra red spectrum(1745 cm^{-1}).

This limonoid has not been previously reported. The structure proposed can be seen in Figure 2.8 overleaf:

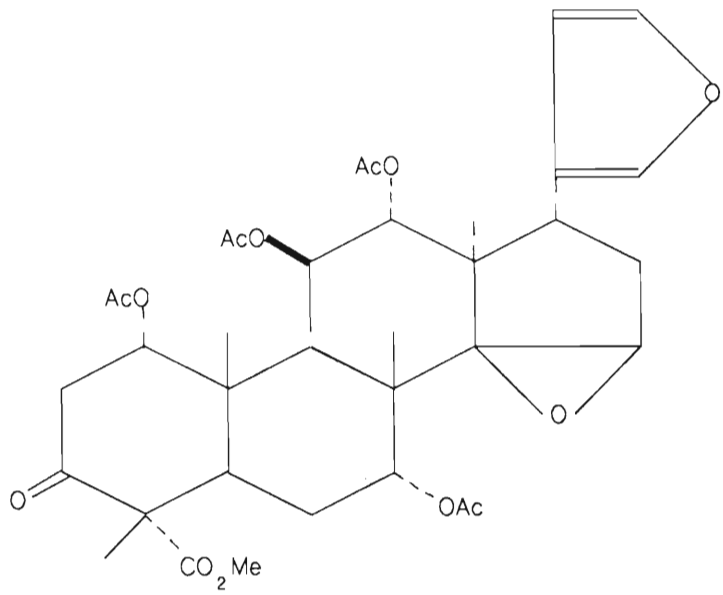


Figure 2.8 The proposed structure of COMPOUND IIIb

2.3 Wood

The wood extract of *Turraea floribunda* was obtained from a plant in the garden of Professor K.H. Pegel. The first compound obtained was labelled COMPOUND IV.

The absence of β-furan ring resonances in the ^{1}H N.M.R. spectrum(p.121) eliminated the possibility of the compound being a limonoid. There were resonances present in the double bond region of the ^{1}H N.M.R. spectrum at δ5.35(1H) and 5.25(2H) and a resonance at 3.68 . The methyl region showed a further six singlets at δ1.26, 1.00, 0.85, 0.85, 0.83 and 0.68 representing 6 methyl groups in the compound.

The ^{13}C N.M.R. spectrum(p.121) indicated that the compound had 29 carbon atoms of which 6 were quartets, 9 were triplets, 11 were doublets and 3 were singlets. This suggested a tetracyclic triterpene. The singlet and doublet at δ 141.2 and 122.0 respectively represented both a quarternary and tertiary carbon atom involved in the double bond. The quarternary carbon atom was further proof of a cyclised structure. Also rather downfield in the ^{13}C N.M.R. spectrum were two doublets at δ 138.7 and 129.6 which represented another double bond. From the Hetcor(p.123),

it appeared that the doublet at δ122.0 corresponded to a signal in the ^{1}H N.M.R. spectrum at δ 5.35 and the doublets in the ^{13}C N.M.R. spectrum at δ138.7 and 129.6 were attached to a multiplet in the ^{1}H N.M.R. spectrum at δ 5.25. The resonance at δ 3.68 which could imply the presence of a proton adjacent to a carbon atom attached to an oxygen group is supported by the doublet at δ 71.9 in the ^{13}C N.M.R. spectrum. This information suggested that the compound was a cyclised triterpene with two double bonds and one hydroxyl group.

Similarities between the ^{1}H N.M.R. spectra of pure stigmasterol and COMPOUND IV were noted and the ^{13}C N.M.R. spectra were compared .[69] They were found to be identical as is shown in Table 2.1. Comparison of the R_f values and melting points were further proof that COMPOUND IV was stigmasterol.

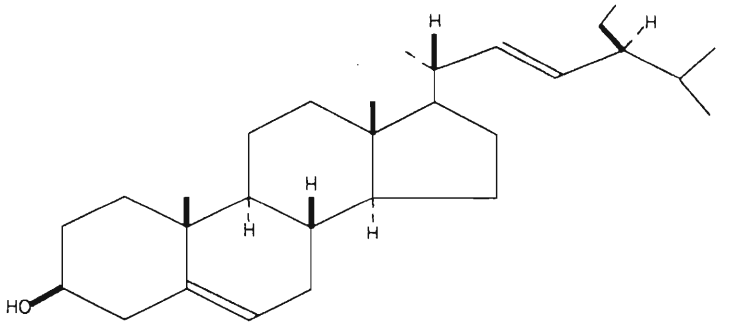


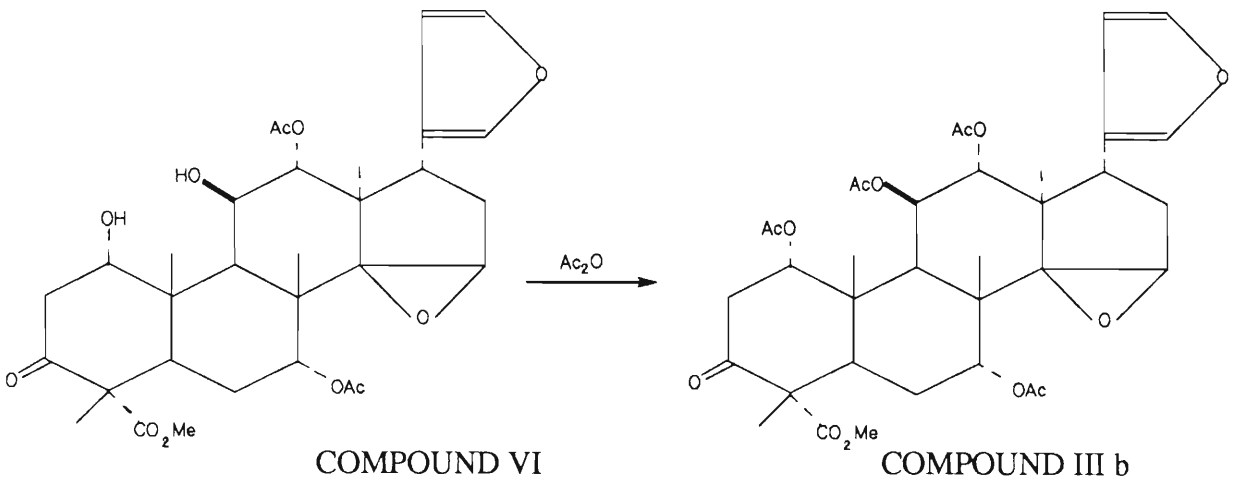
Figure 2.9 The structure of Stigmasterol (COMPOUND IV)

Carbon atom		Stigmasterol[69] (ppm)	COMPOUND IV
1	triplet	37.4	37.4
2	triplet	31.7	31.6
3	doublet	71.8	71.9
4	triplet	42.4	42.3
5	singlet	140.9	141.2
6	doublet	121.7	121.9
7	triplet	31.9	31.9
8	doublet	31.9	31.9
9	doublet	50.3	50.3
10	singlet	36.6	36.6
11	triplet	21.1	21.1
12	triplet	39.8	39.9
13	singlet	42.4	42.4
14	doublet	57.0	57.0
15	triplet	24.4	24.4
16	triplet	28.9	28.3
17	doublet	56.0	56.0
18	quartet	12.2	12.3
19	quartet	19.4	19.4
20	doublet	40.5	40.6
21	quartet	21.1	21.1
22	doublet	138.4	138.7
23	doublet	129.4	129.6
24	doublet	51.3	51.4
25	doublet	31.9	31.9
26	quartet	19.0	19.0
27	quartet	21.1	21.1
28	triplet	25.4	25.4
29	quartet	12.0	12.0

Table 2.1The comparison of COMPOUND IV and Stigmasterol

The second compound found in the wood extract was identified as sitosterol and labelled as COMPOUND V. A T.L.C. analysis showed it to have the same R_f value as sitosterol. The compound formed spindle shaped white crystals from methylene chloride with the same melting point as sitosterol. The spectroscopic data are described more fully in chapter 4, since the same compound was also isolated from *Turraea obtusifolia* and labelled COMPOUND7. [70]

Compound III, identified as COMPOUND A(6)[27] was also isolated from the wood where it has been found previously. In addition, a further compound labelled COMPOUND VI was obtained. Comparison of the spectra of COMPOUND VI (diacetate) with those of COMPOUND IIIb indicated that COMPOUND VI was unacetylated COMPOUND IIIb. COMPOUND VI was thus acetylated and T.L.C. analysis showed that both the compounds had the same R_f value. The spectra have been included.



COMPOUND VI COMPOUND III b

Figure 2.10 The proposed structure of COMPOUND VI

Chapter 3 Extractives from *Turraea obtusifolia*

3.1 Seeds

The species *Turraea obtusifolia* does not occur very abundantly, and hence it was very difficult to locate a tree in Natal. However, some leaves and seeds were donated for extraction by Mr. Geoff Nichols of the Durban Parks, Beach and Recreation Department, who had a small tree in his garden. Another problem with this species is that it does not bear prolifically and hence very few seeds were obtained. The seeds were picked in June 1990.

The seed capsules were of similar appearance to those of *Turraea floribunda* but were only approximately 1.5 cm in diameter. The seed coats differed in that they were soft and paper thin which meant that the seeds could be manually removed without waiting for the capsule to burst open to yield the bright orange seeds. These seeds were then treated as described in the Experimental section (5.3.1).

Due to the small amount and limited availability of seed extract, the quantity of minor components obtained was too insignificant to warrant further work. However, one compound (COMPOUND VII) was isolated in sufficient quantity and purity to allow useful spectra to be obtained. The ^{1}H N.M.R. spectrum(p.125) of the first compound showed no peaks representing a β-substituted furan ring hence eliminating the possibility of COMPOUND VII being a limonoid. No acetoxy or carbomethoxy resonances were observed. Most notable in the ^{1}H N.M.R. spectrum was the doublet at δ 5.40 (J=6Hz) which occurred in the double bond region and the singlet at 3.53. The methyl region showed a further 6 singlets due to methyl groups in the compound at (δ 1.64, 1.50, 1.33, 1.28, 1.04 and 0.71).

The ^{13}C N.M.R. spectrum(p.125) showed 29 carbon atom peaks of which 6 were quartets, 11 were triplets, 9 were doublets and 3 were singlets. A singlet and a doublet occurred at δ 140.9 and 122.1 respectively. This suggested a trisubstituted double bond probably within a cyclised structure.

The doublet at δ 71.9 occurred in the C-O region. This peak together with the ^{1}H N.M.R. spectrum resonance at δ3.53 indicated the presence of an hydroxyl group in the compound.

Thus, it was deduced that COMPOUND VII was a 29 carbon atom cyclised structure containing one double bond and one hydroxyl group. T.L.C. analysis of the compound and pure sitosterol revealed that both had the same R_f value when using a solvent system of methylene chloride : ethyl acetate :: 70:30. It formed clear spindle shaped crystals from methylene chloride and had a melting point corresponding to that of sitosterol.

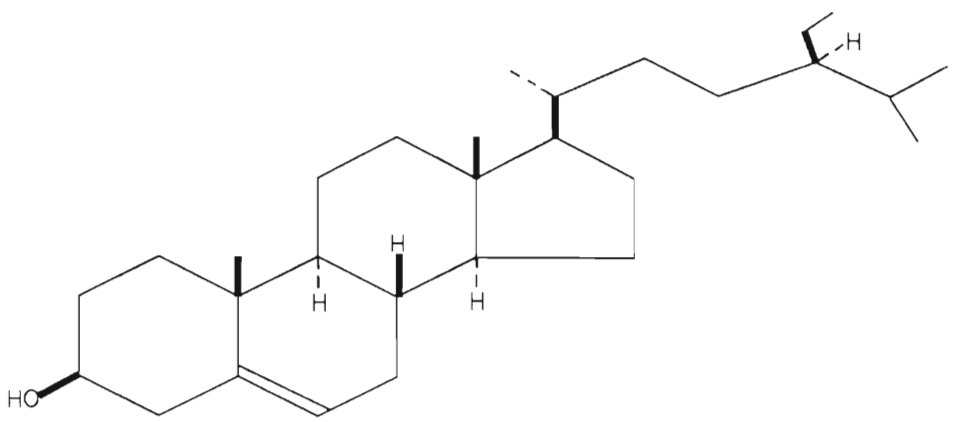


Figure 3.1 The structure of Sitosterol (COMPOUND VII)

^{13}C spectral data were in close agreement, hence COMPOUND VII was identified as sitosterol[70](see Fig 3.1). A table of comparison can be seen (Table 3.1) in which the ^{13}C N.M.R. spectrum of both COMPOUND VII and pure sitosterol are recorded.

Carbon atom		Sitosterol[70]	COMPOUND VII
1	triplet	37.3	37.4
2	triplet	31.8	31.7
3	doublet	71.9	72.0
4	triplet	42.4	42.4
5	singlet	140.9	141.2
6	doublet	121.8	122.1
7	triplet	32.0	32.1
8	singlet	32.0	32.0
9	doublet	50.3	50.2
10	singlet	36.6	36.6
11	triplet	21.1	21.1
12	triplet	39.9	39.9
13	triplet	42.4	42.4
14	doublet	56.8	56.9
15	triplet	24.3	24.4
16	triplet	28.2	28.3
17	doublet	56.2	56.2
18	quartet	11.9	11.9
19	quartet	19.4	19.5
20	doublet	36.2	36.3
21	quartet	19.1	19.1
22	triplet	34.0	34.0
23	triplet	29.3	29.2
24	doublet	50.3	50.2
25	triplet	26.2	26.0
26	quartet	18.8	18.8
27	quartet	19.8	19.9
28	triplet	23.1	23.1
29	quartet	11.9	11.9

Table 3.1

3.2 Leaves

The leaves, picked from the same tree from which the seeds were obtained, were bitter-tasting, indicating the presence of limonoids. Extraction of these leaves was carried out as described in 5.3.2 to yield two major components. The one compound labelled COMPOUND VIII was isolated as a viscous gum. The ^{1}H N.M.R. spectrum(p.126) showed a multiplet at δ5.40 and a doublet at 4.14 (J=6Hz), while five peaks were reported in the methyl region at δ0.88, 0.86, 0.86, 0.85 and 0.83. The remaining resonances in the ^{1}H N.M.R. spectrum gave triplets implying that they were adjacent to methylene groups while the singlet occurring at δ1.67 was assigned to a methyl group attached to a quarternary carbon. The absence of β-substituted furan ring resonances eliminated the possibility of the compound being a limonoid.

Twenty carbon atoms were reported in the ^{13}C N.M.R spectrum(p.126). Of these 20 carbons, 5 were quartets, 10 were triplets, 4 were doublets and one singlet was present. This implied a hydrocarbon chain. Four of the five quartets reported in the ^{13}C N.M.R. spectrum at δ22.8, 22.7, 19.8, 19.8 and 16.2, corresponded to four methyl doublets in the ^{1}H N.M.R. spectrum at δ 0.88, 0.86, 0.86 and 0.85 respectively. This implied that the four methyl groups were attached to tertiary carbon atoms.

The singlet and doublet in the ^{13}C N.M.R. spectrum at δ140.6 and 123.5 occurred in the double bond region while the triplet at 59.5 was shifted further downfield than the others implying an oxygen atom attached to a methylene group. The above information inferred a hydrocarbon chain with 5 methyl groups, a double bond and an oxygen function attached. From the Cosy spectrum(p.127), it was clear that the doublet at δ4.14 was connected to the triplet at δ5.4 which implied that two protons were splitting the tertiary carbon atom involved in the double bond. The compound had the same R_f value and melting point as pure phytol($C_{20}H_{40}O$). Finally, comparison of ^{13}C N.M.R. spectra confirmed the structure of COMPOUND VIII as phytol (Fig. 3.2). A table showing the correlation between COMPOUND VIII and pure phytol[71] may be seen in Table 3.2.

Carbon atom		Phytol[71]	COMPOUND VIII
1	triplet	59.4	59.5
2	doublet	123.4	123.5
3	singlet	139.9	140.5
4	triplet	40.0	40.0
5	triplet	25.3	25.2
6	doublet	36.8	36.8
7	doublet	32.8	32.8
8	triplet	37.5	37.5
9	triplet	24.6	24.6
10	triplet	37.6	37.6
11	doublet	32.9	32.9
12	triplet	37.4	37.4
13	triplet	24.9	24.9
14	triplet	39.5	39.5
15	doublet	28.0	28.1
3a	quartet	16.2	16.2
7a,11a	2x quartet	19.8	19.8
15a	quartet	22.6	22.7
16	quartet	22.7	22.8

Table 3.2

Figure 3.2 The structure of phytol (COMPOUND VIII)

The second compound was isolated as a yellowish gum from methylene chloride and was labelled COMPOUND IX. Evidence for an epimeric mixture was visible in the ^{13}C N.M.R. spectrum(p.128) in the form of two doublets at δ 101.6 and 97.5.

These occurred further downfield than is to be expected from normal C-O bonds and were thus assigned to C-21 which is attached to two oxygen atoms in a protolimonoid structure. H-21 was represented by a peak at δ 5.34 in the ^{1}H N.M.R. spectrum.

Thus COMPOUND IX occurred as the α and β forms of the hemiacetal group attached to C-21. Compounds such as melianone and turraeanthin are known to form C-21 epimeric mixtures in solution.[72] Once acetylated, the two isomers cannot equilibrate, and can thus be isolated.

Infrared spectroscopy(p.149) revealed strong absorption at 3450 cm^{-1} indicating the prescence of an hydroxyl group, 825 cm^{-1} which represents a double bond and 1715 cm^{-1} which is characteristic of a saturated 6-membered ring ketone. T.L.C. analysis showed that this compound had an identical R_f value to that of pure melianone against which it was compared. In order to obtain crystals, it was attempted to recrystallise from chloroform : pentane as described by Lavie et al.[72] but this was unsuccessful.

Compound IX was thus assigned the structure of melianone and can be seen in Figure 3.3 overleaf:

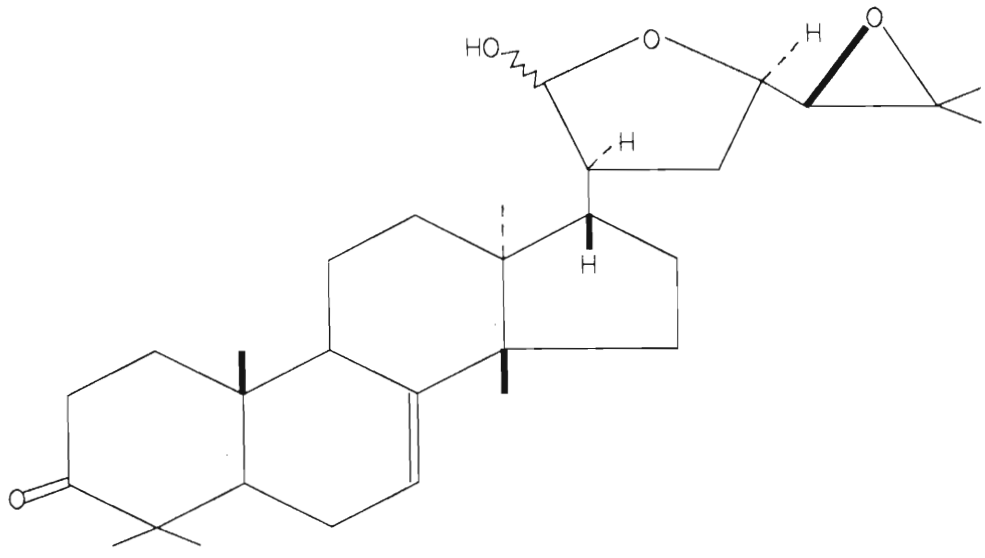


Figure 3.3 The structure of Melianone (COMPOUND IX)

In order to obtain a single isomer, COMPOUND IX was acetylated as described in the Experimental section (5.9) and chromatographed in order to isolate the acetylation product labelled COMPOUND IXa. The mass spectrum of COMPOUND IXa showed that the molecular ion occurred at 512 which indicated a molecular formula of $C_{32}H_{48}O_5$. The ^1H N.M.R. spectrum(p.131) of the acetate showed only one 3-proton acetate group singlet resonance at δ 2.02. This signal was due to the methyl protons of this acetate group attached to C-21. The fact that only a monoacetate was formed was further confirmation of the structure of melianone containing only one hydroxyl group. In the ^1H N.M.R. spectrum, 7 singlets due to methyl groups were visible in the methyl region at δ1.30, 1.27, 1.10, 1.00, 0.99, 0.97 and 0.78. The orientation for 21-OAc was found to be below the plane of the ring and the asymmetric centres of the side chain 23R and 24R.[72]

The ^{13}C N.M.R. spectrum(p.131) of the acetate showed 32 carbon peaks of which 8 were quartets, 8 were triplets, 8 were doublets and 8 were singlets. The singlet occurring at δ217.5 was within the characteristic region for carbonyl functions and thus represented a keto group at the 3-position. The singlet and doublet occurring at δ145.8 and 118.7 respectively represented the double bond between carbon atoms 7 and 8. The singlet at δ 170.9 which represented the carbonyl moiety of the acetate

group at C-21 was absent in the parent compound, COMPOUND IX. As a consequence of this acetate group, an extra quartet should also appear in the ^{13}C N.M.R. spectrum and appropriately eight quartets have been recorded at δ27.4, 25.0, 24.6, 22.9, 21.6, 21.5, 19.6 and 12.8. The 8 triplets reported represented carbon atoms 1,2,6,10,11,15,16 and 22 while the remaining 7 doublets represented carbon atoms 5,9,17,20,21,23 and 24.

In the Hetcor spectrum(p.133), it could be seen that C-21 at δ 101.0 in the ^{13}C N.M.R. spectrum corresponded to a resonance in the ^{1}H N.M.R. spectrum at δ 6.20 unlike 5.34 in the unacetylated parent compound. The proton signal at H-21 was shifted downfield due to the acetate group, which indicated that acetylation occurred at that point.

The doublet at δ 118.7 corresponded to a resonance at δ 5.30 in the ^{1}H N.M.R. spectrum. This was due to the olefinic proton, H-7. This signal in the ^{1}H N.M.R.spectrum of COMPOUND IXa was no longer overlapped by that of H-21 as it was in the parent compound (COMPOUND IX).

At δ 3.90, the H-23 proton signal was split by both H-22 and H-24 protons to form a very symmetric double double doublet which was not as clear in COMPOUND IX. Also noticeable was the doublet representing H-24 at δ 2.70 (J=8Hz) in the ^{1}H N.M.R. spectrum.

Finally, in the Cosy spectrum(p.132) of COMPOUND IXa, the proton signals H-24 and H-23 were clearly coupled since they are adjacent to each other.

The mass spectrum of COMPOUND IXa showed that the molecular ion occurred at 512 followed by a peak at 497. This loss of 15 implied the loss of a methyl group. The next peak at 437 meant a loss of 60 due to the acetate group. The structure was assigned to melianone acetate and can be seen in Figure 3.4.

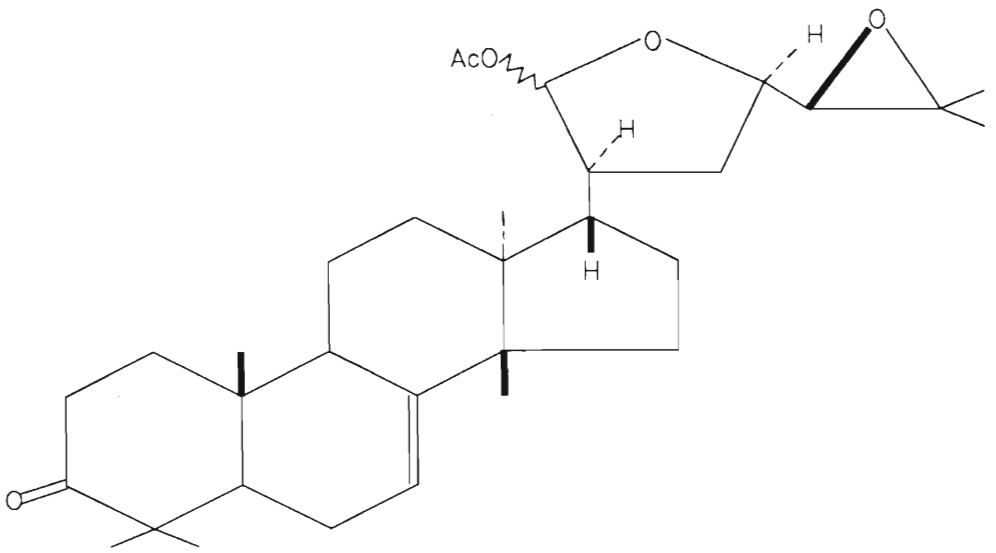


Figure 3.4 The structure of acetylated Melianone (COMPOUND IXa)

An attempt was made to reduce the keto group at C-3 of COMPOUND IXa by means of a sodium borohydride reduction to give COMPOUND IXb. After purification there was only a sufficient amount for a ^{1}H N.M.R. spectrum to be obtained. Most significant in this spectrum was the multiplet at δ3.35 which represented H-3 when a β-orientated hydroxyl group is attached to C-3 [72] and which was absent in COMPOUND IXa. Thus it was inferred that the sodium borohydride reduction was successful.

A minor component was also isolated as a gum and labelled COMPOUND X. The amount of sample was small and slightly impure, but similarities to melianone made it possible to elucidate the structure.

The superimposed signals at δ5.35(2H) due to both H-7 and H-21 were present in the ^{1}H N.M.R. spectrum(p.135). Multiplets at δ3.9(H-23) and 2.25(H-22) were also present.

The keto- group at C-3 as in melianone was present in the ^{13}C N.M.R. spectrum(p.135) at δ217.7 and the C-8 singlet and C-7 doublet at δ145.8 and 118.4 respectively. No signals were observed in the hemiacetal region which indicated that a hemiacetal was not present in this compound. The presence of the peaks at δ68.2(d) and 59.6(s) indicated that the epoxide ring between C-24 and C-25 was still intact. Two C-O signals remained at δ71.5(d) and 64.8(t) which implied that both C-23(d) and C-21(t) respectively were attached to hydroxyl groups. Comparison of the ^{13}C N.M.R. spectra of COMPOUND X and sapelin F showed that the two compounds were identical except that the 3-hydroxy group had been replaced by a keto- group in COMPOUND X.[80] This compound has not been reported previously.

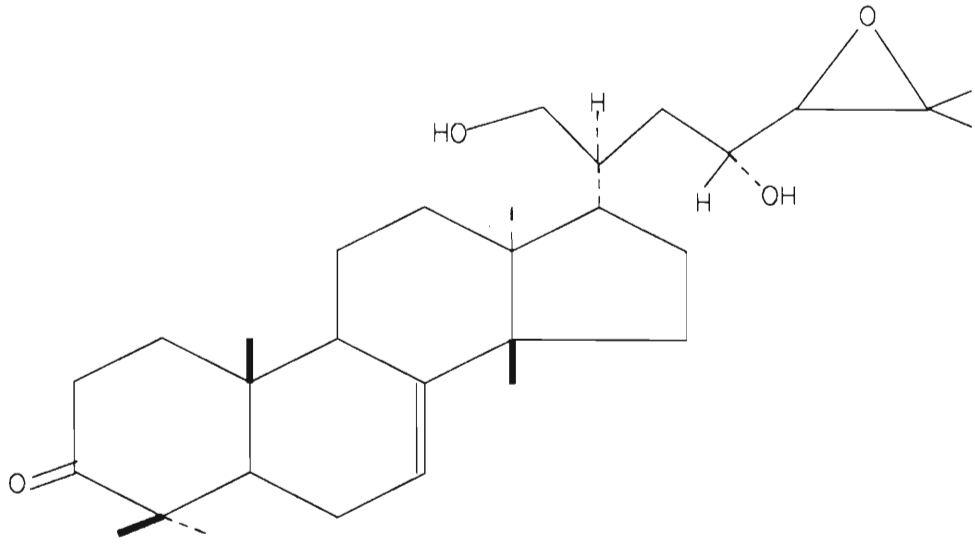


Figure 3.5 The proposed structure of COMPOUND X

3.3 Wood

The wood extract was previously investigated by Professor D.A.H. Taylor who obtained plant material from the Natal Forestry Department.[27] Melianone (COMPOUND IX) as well as three other protolimonoids were isolated from this extract, the first one which was obtained as a clear gum was labelled COMPOUND XI. The mass spectrum of the compound showed that the molecular ion occurred at 488 which agreed with a molecular formula of $C_{30}H_{48}O_5$.

No furan ring resonances were present in the ^{1}H N.M.R. spectrum(p.136), thus eliminating the possibility of COMPOUND XI being a limonoid. As in melianone (COMPOUND IX), H-7(1H) and H-21(1H) were superimposed at δ5.31 which intergrated to two protons. The peak at δ4.50(m,1H) was assigned to H-23 and occurred further downfield than in melianone (δ3.90)[72] where it also occurred as a multiplet due to splitting by neighbouring protons on both C-22 and C-24. At δ1.26, the 6-proton peak indicated the presence of the 2 methyl groups (26 and 27). The remaining 4 peaks in the methyl region at δ1.11, 1.03, 1.01 and 0.83(6H) showed that a further 4 methyl groups were present in the compound at C-4, C10, C-13 and C-14.[72] The ^{13}C N.M.R. spectrum(p.136) had a singlet at δ217.6 (217.3 in melianone) which indicated a keto- group at C-3. The doublet and singlet which represent the 7-8 double bond were identical with those of melianone (δ118.0 and 145.7 respectively). The difference between COMPOUND XI and melianone was apparent from the C-O region. The doublet and singlet representing the epoxide group (C-24,C-25) were no longer present at δ65.1 and 57.2. The doublet and singlet occurred at δ75.0 and 73.7 respectively which implied that the epoxide group had opened and that both C-24 and C-25 were attached to hydroxyl groups as in melianodiol.[79] The doublet at δ96.5 occured in the same position as that for melianone and was assigned to C-21 (hemiacetal carbon). Two doublets remained at δ78.4 and 77.8. The latter peak was assigned to C-23 since it corresponded to H-23 in the Hetcor spectrum. The resonance at δ78.4(d) was attributed to an impurity since it was of low intensity and corresponded to no proton signal in the ^{1}H N.M.R. spectrum.

Only 4 other doublets were recorded in the ^{13}C N.M.R. spectrum at δ52.2, 48.1, 46.1 and 45.0 which would account for carbon atoms 5,9,13 and 23. The outstanding 4 singlets at δ50.5, 47.7, 43.3 and 34.8 were assigned to C-4, C-10, C-13 and C-14. The triplet at δ29.1 was assigned to C-22 ,[1] and the remaining 7 triplet signals at δ37.2, 33.6, 33.0, 30.2, 23.0, 22.9 and 16.4 to C-1, C-2, C-6, C-11, C-12, C-15 and C-16. Seven quartets were recorded as in melianone at δ27.2, 26.4(x2), 24.2, 23.0, 21.3 and 12.4.

A total of 7 quartets, 8 triplets, 9 doublets and 7 singlets were present in the ^{13}C N.M.R. spectrum. The structure proposed was melianodiol[79] which has previously been isolated from *Melia azedarach.*

Further evidence for this structure was found in the Hetcor, Cosy and mass spectra.

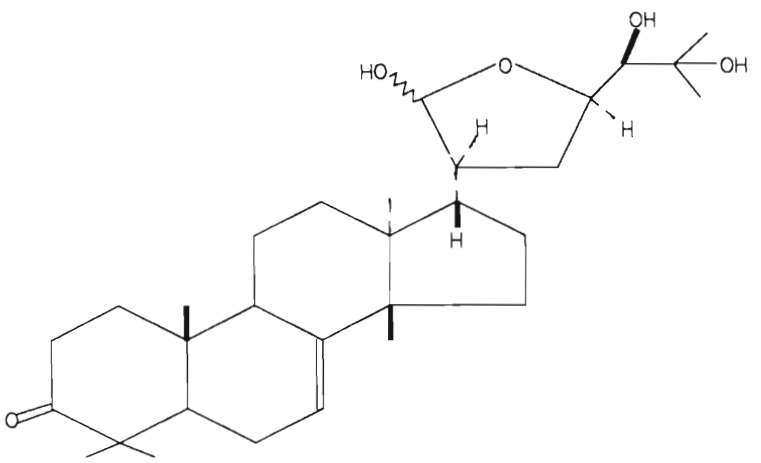


Figure 3.6 The proposed structure of Melianodiol (COMPOUND XI)

The mass spectrum showed that the molecular ion occurred at 488 which was identical for that obtained for melianodiol.[79] The molecular peaks expected for the loss of 1 molecule of water, a methyl group and another molecule of water were observed at 470, 455 and 437.

The Hetcor spectrum(p.138) indicated that the resonance at δ82.0 in the ^{13}C N.M.R. spectrum corresponded to H-23 (δ4.50m) in the ^{1}H N.M.R. spectrum while the doublet at δ77.8 corresponded to a resonance at δ3.15. This latter resonance shifted downfield on acetylation which implied that the proton giving the signal was attached to a carbon atom with an hydroxyl group. This signal system was thus assigned to H-24. The hemiacetal doublet (C-21) at δ102.0 and double bond (C-7) at 122.0 corresponded to the superimposed signals of H-7 and H-21 at δ5.31(2H) as seen in the Hetcor spectrum. The triplet assigned to C-22 (δ28.0) corresponded to the resonance at δ2.10(1H).

The Cosy spectrum(p.137) showed that H-21 (δ5.31) was coupled to H-22 (δ2.10) which was in turn coupled to H-23 (δ4.40) and finally to H-24 (δ3.15).

COMPOUND XI was then acetylated, the reaction mixture separated by means of column chromatography and the main product obtained as a gum (COMPOUND XIa).

The most significant feature in the ^{1}H N.M.R. spectrum(p.139) were the two acetate methyl group singlets at δ2.20 and 2.07. This was to be expected as the acetylation product of COMPOUND XI because only the hydroxyl groups attached to C-21 and C-24 would acetylate under normal conditions. The vinylic proton (H-7) signal still resonated at δ5.30 (1H), but it was no longer overlapped by that of 21-H as in both melianone (COMPOUND IX) and COMPOUND XI. H-21 had been shifted down-field and appeared as a doublet (J=3Hz) at δ6.15. This proved that acetylation occurred at C-21. H-23 remained at δ4.50, but H-24 had been shifted down to 4.85 and was a distinct doublet (J=3Hz) being split only by H-23.

The ^{13}C N.M.R. spectrum(p.139) showed the presence of the two carbonyl carbon atoms of the acetate groups at C-21 and C-24 (δ171.6 and 170.5). The only other two notable features were that C-21 had been shifted downfield from δ96.5 to 100.3 and that C-24 had been shifted from δ75.0 to 76.4. This confirmed that these 2 carbon atoms were the 2 points of acetylation of melianodiol (COMPOUND XI)(See Fig 3.7).

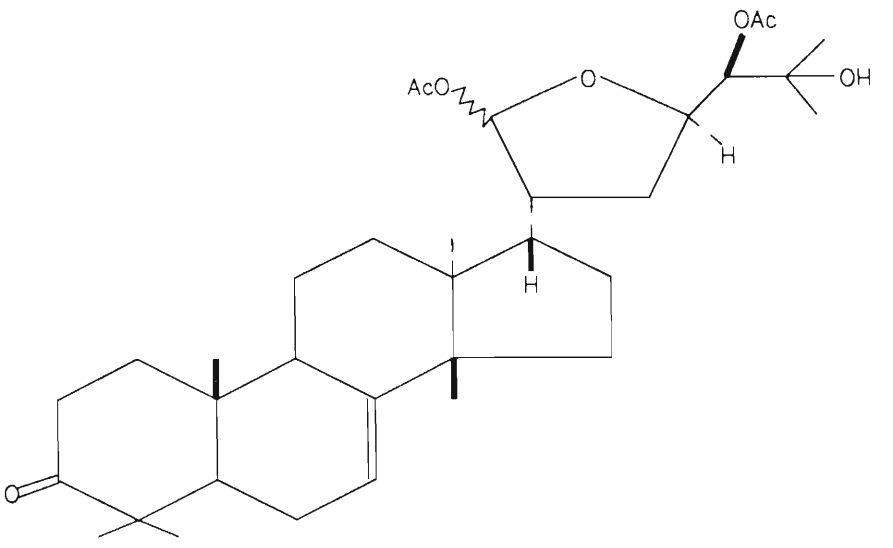


Figure 3.7 The proposed structure of COMPOUND XIa

The keto-group at C-3 of COMPOUND XI was then reduced by means of sodium borohydride, the reaction mixture separated using column chromatography, and the main product, COMPOUND XIb obtained as a gum. The ^{1}H N.M.R. spectrum(p.140) solution was very dilute due to the small amount of sample and there were strong traces of ethyl acetate present in the spectrum. The peaks representing H-7, H-21, H-23 and H-24 were all in the same position as in COMPOUND XI.

The ^{13}C N.M.R. spectrum(p.140) showed the loss of the 3 keto- group at δ217.6 while C-21, C-24 and C-25 remained in the same positions as COMPOUND XI i.e. at δ97.1, 75.2 and 73.8. The two remaining peaks in the C-O region at δ79.4 and 78.9 were assigned to C-23 and C-3. Thus COMPOUND XIb was assigned the expected structure of melianotriol (Fig. 3.8).

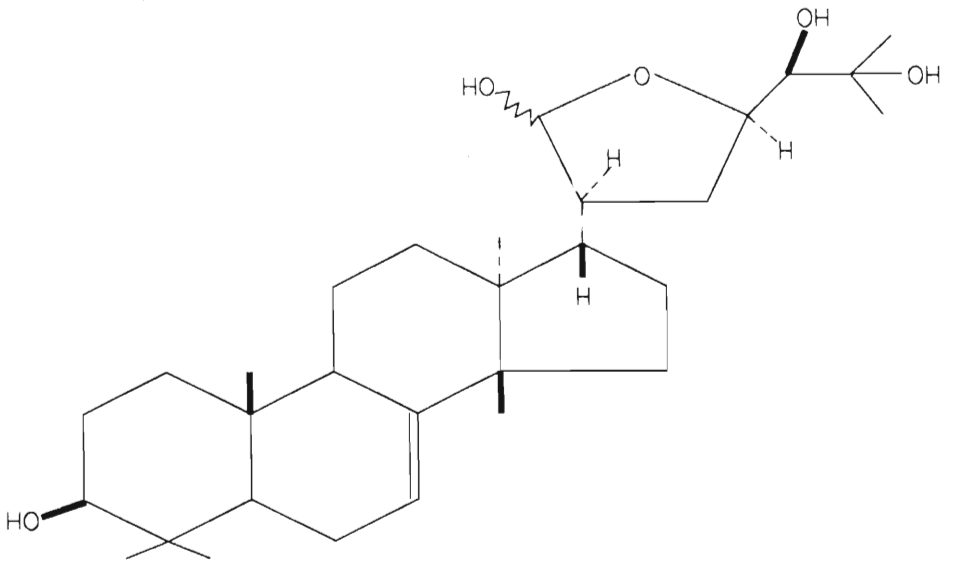


Figure 3.8 The proposed structure of Melianotriol (COMPOUND XIb)

COMPOUND XI was finally oxidised by means of a sodium periodate oxidation, the reaction mixture chromatographed and the main product, COMPOUND XIc isolated as a gum.

The ^{1}H N.M.R. spectrum(p.141) solution was again weak and contaminated with ethyl acetate but the spectrum showed 2 peaks at δ9.65 and 9.90 which indicated the presence of 2 aldehyde groups. The peaks due to 23-H, 24-H and 21-H at δ4.50, 3.15 and 5.30 were absent or had shifted and there were two fewer methyl groups.

The ^{13}C N.M.R. spectrum(p.141) showed 2 doublets at δ204.1 and 200.7 which confirmed the two aldehyde groups. The peak due to the hemiacetal C-21 (δ96.5) was absent as were all the doublets in the C-O region.

In aqueous solution, hemiacetal formation is reversible and the following scheme shows how the reaction product was obtained. The major product with the two aldehyde groups was isolated (Fig.3.9).[79]

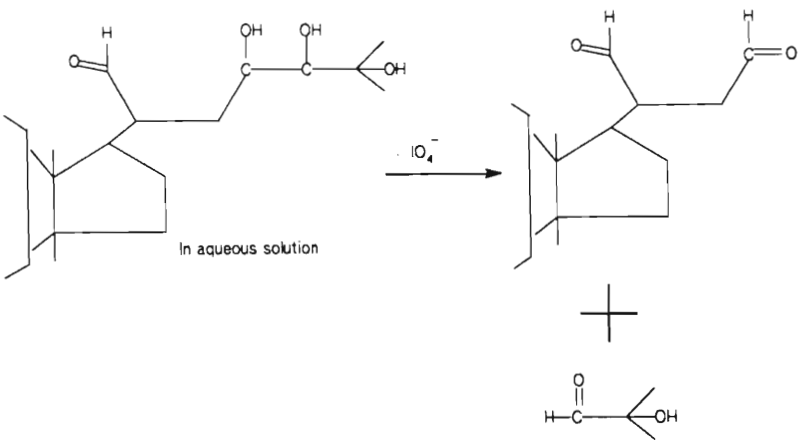


Figure 3.9

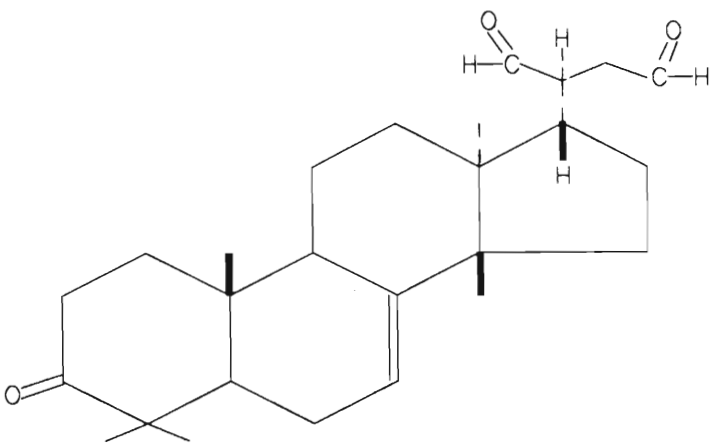


Figure 3.10 The proposed structure of COMPOUND XIc

COMPOUND XII was isolated as a gum. The ^{1}H N.M.R. spectrum(p.142) of COMPOUND XII and COMPOUND XIb were identical, hence the structure was assigned to melianotriol. Both the naturally occurring melianotriol and the synthetic product (COMPOUND XIb) have previously been shown to have comparable biological activity.[80]

Similarly, the ^{13}C N.M.R. spectra(p.142) were identical.

COMPOUND XIII was also isolated as a gum. The ^{1}H N.M.R.spectrum(p.143) was similar to that of melianone (COMPOUND IX) except for the resonance at δ4.68 and an acetoxy singlet at 2.06.

The ^{13}C N.M.R. spectrum(p.143) showed that the 3 keto- group had been lost but the resonance at δ172.0 indicated the presence of an acetate group. Also lost were the double bond resonances representing the double bond between C-7 and C-8. This implied that COMPOUND XIII was the 3-acetoxy analogue of melianone in which the double bond had been hydrogenated, namely 7,8-dihydroturraeanthin. However, small signals in the double bond region of the ^{1}H N.M.R. spectrum indicated that the compound was contaminated with turreanthin (Δ^{7}).

Both 3α- and 3β-acetoxy turraeanthin have previously been isolated and the two isomers can be differentiated using the 3-H resonance. The 3β- proton resonates further downfield than the 3α- proton (δ4.7 as opposed to 4.52) hence the data confirmed a 3β proton i.e.: COMPOUND XIII was identified as 7,8-dihydroturraeanthyl 3-acetate.[73]

Comparison of ^{1}H N.M.R. spectra [59] confirmed the identity of COMPOUND XIII.
(See Appendix 1 on page 158)

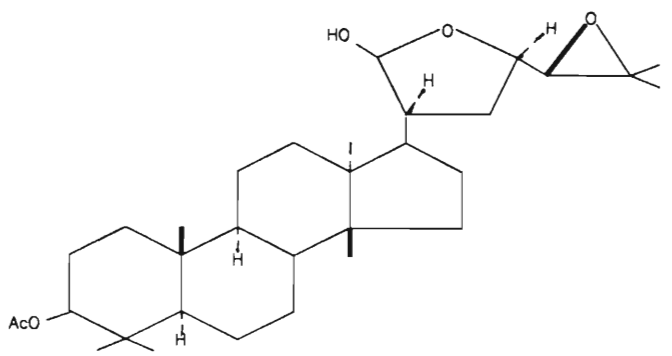
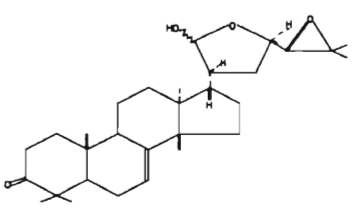


Figure 3.11 The proposed structure of COMPOUND XIII

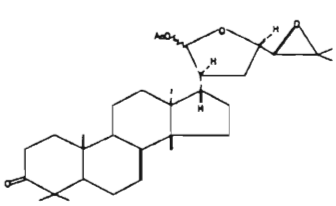
A table showing how the proton resonances H-21, H-7, H-23 and H-24 deviate in the protolimonoids isolated in this chapter can be seen below:

	H-7(ppm)	H-21(ppm)	H-23(ppm)	H-24(ppm)
COMP.IX	5.30	5.30	3.90	2.90
COMP. IXa	5.30	6.20	3.90	
COMP. X	5.35	5.35	3.95	2.78
COMP. XI	5.31	5.31	4.50	3.15
COMP. XIa	5.30	6.13	4.50	4.85
COMP. XIb	5.30	5.30	4.50	3.11
COMP. XIc	5.30	9.65 or 9.90		-
COMP. XII	5.30	5.30	4.50	3.24
COMP. XIII	-	5.40	3.95	2.85

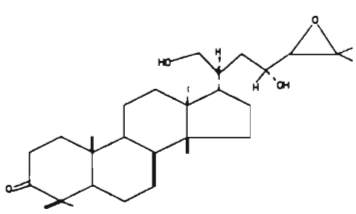
Table 3.3



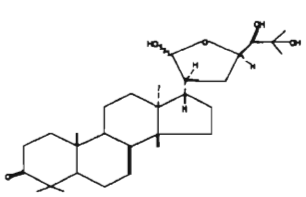
COMPOUND IX



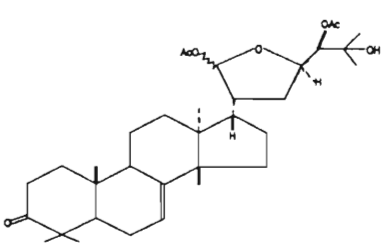
COMPOUND IXa



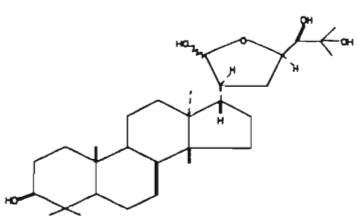
COMPOUND X



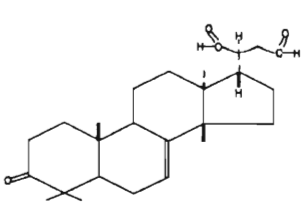
COMPOUND XI



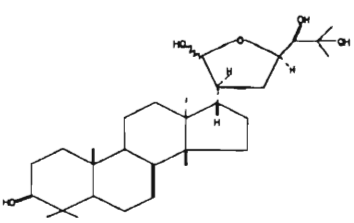
COMPOUND XIa



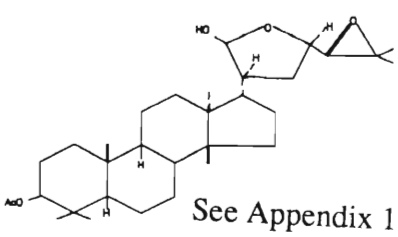
COMPOUND XIb



COMPOUND XIc



COMPOUND XII



COMPOUND XIII

See Appendix 1 on p.158

Chapter 5 Extractives from *Apodytes dimidiata*

4.1 Wood

A branch was removed from the *Apodytes dimidiata* tree located outside the Elizabeth Sneddon theatre on the campus of the University of Natal. A pressing of the leaves was identified by the keeper of the Herbarium in the Department of Biology. The methanolic residue was tested with Dragendorff reagent which is a universal detection method for alkaloids. A faint orangy-red colour was noted being an indication of the presence of alkaloids. This reaction was supplemented by viewing the spot under an ultraviolet lamp. There was fairly strong fluorescence which is characteristic of some alkaloids.[74] The purpose of acidifying the extract was to convert the alkaloids into their salt form. The salts would thus be soluble in the aqueous layer and so the non-polar compounds could be removed in the chloroform layer. By basifying the aqueous solution, the alkaloid salts were reconverted to their free base form. These bases, being more soluble in chloroform could then be extracted into that layer and evaporated down for chromatographic analysis. This was, however unsuccessful since the yield was too small to warrant chromatography. The fact that the Dragendorff test was so faint could indicate that the concentration of alkaloids in the wood was very small. Since there were no aluminium oxide sheets available for T.L.C. analysis, silica gel sheets were used instead. This posed no problem since silica gel as the stationary phase, being acidic, would simply require alkali to be added to the mobile phase, whereas aluminium oxide requires no alkali. It has been suggested [74] that the mobile phase can be made alkaline by adding ammonia to the more polar solvents, or diethylamine or pyridine to the less polar solvents.

The first solvent system used was toluene : ethyl acetate : ethanolamine :: 7:2:7 [75] which gave extremely poor resolution. All that appeared after spraying with Dragendorff was a very faint streak. Truter et al.[75] suggest that although alkaloids are bases, alkali conditions are not always best since the bases get separated from the salts. Hence, it was decided to try both a basic and acidic mobile phase to see which gave the best resolution. The two systems used were chloroform : methanol :: 85:15 and chloroform : acetic acid :: 85:15. The basic system was unsuccessful since many compounds appeared to be superimposed on one another. The acid system was negative for the Dragendorff test, but did have a distinct spot at R_f=0.4 which fluoresced under ultraviolet light.

Employing the solvent system as used by Vanhaelen [63] i.e. toluene : acetone : ethanol : ammonium hydroxide :: 40:40:10:3 proved unsuccessful.

Dry flash chromatography was used next as described in the experimental, [5.1.2] starting with methylene chloride and gradually increasing the polarity with the addition of ethyl acetate followed by methanol in 5% successions. The latter fractions collected appeared to be faintly positive when sprayed with Dragendorff. These latter fractions were combined and run in a solvent system of toluene : acetone : ethanol : ammonium hydroxide :: 40:40:10:3. A faint streak appeared in the range R_f=0.49-0.8 when sprayed with Dragendorff. The Dry Method of column chromatography was again employed starting with toluene : acetone : ethanol : ammonium hydroxide :: 40:40:10:3 and making it progressively more polar by decreasing the toluene and increasing the ethanol.

Truter et al. [75] suggest that for unknown alkaloids, chromatography must first take place in cyclohexane : chloroform : diethylamine :: 5:4:1 on T.L.C. sheets. If the R_f values are greater than 0.3 then the following two solvent systems should be used:

cyclohexane : diethylamine :: 9:1

benzene : ethylacetate :diethylamine :: 7:2:1

If the R_f values are less than 0.3 the following are advised:

chloroform : acetone : diethylamine :: 5:4:1

chloroform : diethylamine :: 9:1

The R_f value was above 0.3 but neither of the systems quoted proved successful.

Once some neutral aluminium oxide preparative layer chromatographic plates had been obtained, it was decided to follow Vanhaelen's method [63] directly despite the fact that small variations in water content can play an important role in the T.L.C. analysis. Using the solvent system toluene : acetone : ethanol : ammonium hydroxide :: 40:40:10:3, seven bands appeared at R_f values of 0.17; 0.28; 0.40; 0.48; 0.57; 0.70; 0.95 respectively.

Further spectroscopic analysis would be futile since only minute quantities of each component were available.

4.2 Leaves

The leaves from the same *Apodytes dimidiata* tree from which the wood was obtained, were dried and extracted with ethanol as performed by Vanhaelen. The method for isolating alkaloids was followed as described in the Experimental section (5.4.1). Unfortunately, the yield was again extremely small. Approximately 1 g of a dark green-brown residue remained after evaporation down on the rotary evaporator. There was a faint positive test for alkaloids with Dragendorff reagent, but was not worth chromatographing since it was very contaminated with chlorophylls.

It was suggested that perhaps when the leaves were crushed, the alkaloids may have reacted with free acids in the plant material and become salts.

4.3 Seeds

The seeds of the same *Apodytes dimidiata* tree were collected when they had fully ripened and fallen to the ground in May. The berries appeared to be small scarlet and very fleshy . The sap from this flesh caused very stubborn staining. After drying, the flesh shrivelled up leaving wrinkled black seeds. Due to the unsuccessful attempt to isolate alkaloids from this tree,[77,78] it was decided to study the hexane extract.

One compound was isolated in sufficient quantity to obtain spectroscopic data. It was labelled COMPOUND XIV and was obtained as an amorphous solid.

The ^{13}C N.M.R. spectrum(p.144) had a singlet at δ174.8 which suggested the presence of an ester group and which was confirmed by infrared absorption at 1740 cm^{-1}. Two doublets were present in the double bond region at δ130.3 and 130.0 confirmed the presence of a double bond. The C-O region had 3 peaks at δ70.3(d), 65.1(t) and 63.5(t). The remaining triplets occurred between δ34.2 and 22.7 with one quartet at (δ14.1(q)). This suggested that COMPOUND XIV consisted of a long hydrocarbon chain.

The ^{1}H N.M.R. spectrum(p.144) showed a triplet at δ5.28(J=4Hz) which confirmed the presence of a double bond. A doublet occurred at δ4.06 (J=6Hz, 2H) as well as two signals at δ3.66(1H) and 3.50(2H). The strong proton signal at δ1.24 and the presence of only one methyl group signal at 0.82 suggested a long hydrocarbon chain. Also present were three individual methylene group signals at δ2.25(2H), 1.90(2H) and 1.54(2H).

Further evidence for the structure was obtained from the Hetcor and Cosy spectra. In the Hetcor spectrum(p.146) the three C-O signals at δ70.3(d), 65.1(t) and 63.5(t) corresponded to the ^{1}H N.M.R. peaks at δ3.66(1H), 4.06(2H) and 3.50(2H) respectively. This suggested one -CH-O- and two -CH_2-O- groups respectively. The triplet at δ22.7 in the ^{13}C N.M.R. spectrum corresponded to the main triplet peak at 1.24 in the ^{1}H N.M.R. spectrum, while the quartet at δ14.1 in the ^{13}C N.M.R. spectrum corresponded to the triplet at 0.82 in the ^{1}H N.M.R. spectrum.

The Cosy spectrum(p.145) showed that the signal at δ3.66 (C-OH) was coupled to both 4.06 (CH_2O) and 3.50 (CH_2O) which implied that they were adjacent to each other in the compound. The strong singlet at δ1.24 was coupled to a triplet at δ0.82. The above evidence implied that the compound was an ester in which glycerol had esterified with a long-chain fatty acid. The individual methylene group signals at δ1.90(2H) and 1.54(2H) were assigned to the two -CH_2 groups in the chain, adjacent to the carbonyl group. However, the fact that the double bond signal at δ5.28 only intergrated to 1 proton and the 2-CH_2 groups assigned to those on either side of the double bond in the fatty acid chain only intergrated to two protons, suggested that a mixture was present. Thus the hydrocarbon chain was a mixture of both the saturated and unsaturated fatty acids.

An infra-red spectrum(p.150) showed strong absorbance at 3450 cm^{-1} which confirmed the presence of free hydroxyl groups, while the strong absorbance at 1740 cm^{-1} represented the carbonyl group of the ester. The hydrocarbon chain was observed at 3000 cm^{-1}.

Further evidence for the structure of COMPOUND XIV was obtained from the mass spectrum in which the molecular ion(M^+) occurred at 356. The subsequent peaks at 338 and 325 represented the loss of H_2O(-18) and -CH_2OH(-31) respectively, while the strong peak at 299 represented the loss of a butyl group(-57) at the end of the chain.

In the case of esters, the most important α-cleavage reactions involve the loss of the alkoxy group from the ester to form the corresponding acylium ion, $RCH=C=O^{+\cdot}$ at 264. This is due to the loss of glycerol ($C_3H_8O_3$, 92 g/mol). By subtracting the mass of glycerol, the carbonyl group of the ester and the terminal methyl group, the remaining figure of 222 g/mol was used to calculate the length of the hydrocarbon chain. Since the mass of an individual methylene group is 14 g/mol, the molecular mass of the remaining molecule was divided by 14 which left a value of 16 with two hydrogens missing (due to the double bond). Therefore the structure was identified as a C_{18} fatty acid. However, the reasonably sized peak at 358 suggested that the

compound was a mixture of both the saturated and unsaturated fatty acid. An estimate of the relative amounts of the saturated and unsaturated fatty acids could not be obtained from the mass spectrum as alkanes are known to give more intense molecular ions than the related alkene.[81] Although it is impossible to predict the position of the double bond using mass spectrometry because it migrates,[81] the most obvious unsaturated C_{18} fatty acid is oleic acid. Both stearic (saturated) and oleic acid are commonly found in all plants and animals. Thus compound XIV is a mixture of both glyceryl oleate and glyceryl stearate.

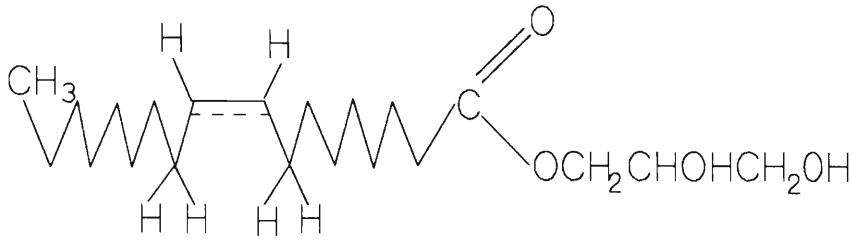
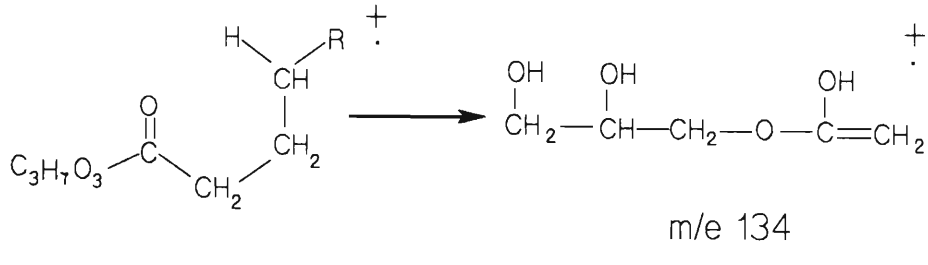


Figure 4.1 The proposed structure of COMPOUND XIV

Evidence for the important β-cleavage reaction known as the McLafferty rearrangement[81] was found in the mass spectrum at 134 which represented the $CH_2OHCHO\text{-}HCH_2OCOH{=}CH_2^{+\cdot}$ fragment.



COMPOUND XIV was then hydrolyzed so as to separate glycerol from the fatty acid mixture and the latter mixture was labelled COMPOUND XIVa. The ^1H N.M.R. spectrum of COMPOUND XIVa showed that the hydroxyl groups of glycerol at δ3.66(1H), 4.06(2H) and 3.50(2H) were no longer present, which

indicated that the ester had been hydrolyzed. A weak, broad absorption was observed at δ8.0. Further evidence for the saturated/unsaturated nature of the fatty acid was observed in the ^{1}H N.M.R. spectrum of COMPOUND XIVa. The main methylene peak at δ1.23 should have intergrated to 11 methylene groups but in fact integrated to 26, the excess being due to the presence of the saturated chain. Hence, the percentage of saturated and unsaturated fatty acids present could be approximately calculated.

$$\% \text{ Oleic acid } = \frac{11}{(11+15)} \times 100 \quad = 42\ \%$$

$$\% \text{ Stearic acid} = \frac{15}{(11+15)} \times 100 \quad = 58\ \%$$

Chapter 5 Experimental

5.1 General

5.1.1 Proton Nuclear Magnetic Resonance Spectroscopy

For fairly large samples, spectroscopic analysis could be carried out using a Varian T-60 N.M.R. spectrometer. The extract was dissolved in deuteriochloroform ($CDCl_3$) followed by the addition of a few drops of tetra-methyl silane (T.M.S.) before the analysis was performed. The T.M.S. served as an internal standard, and absorbances were measured in ppm from T.M.S.

These spectra were also recorded using the proton probe of a Varian CFT-20 Spectrometer at 80 MHz. The high-field ^1H N.M.R. spectra were conducted using a Gemini 200 MHz spectrophotometer at room temperature.

5.1.2 Carbon-13 Nuclear Magnetic Resonance Spectroscopy

These spectra were all recorded at room temperature using a Gemini 200 MHz spectrophotometer. The spectra were recorded with proton noise decoupling and δ-values (ppm) were measured relative to the central peak of the chloroform triplet.

Infra-red spectra were recorded on a Shimadzu infrared spectrophotometer (IR-408) using KBr disks.

Ultra-violet spectra were recorded on a Varian DMS 300 U.V. visible spectrophotometer, using chloroform as solvent.

Melting points were recorded on a Kofler micro-hot stage melting point apparatus.

Optical rotations were recorded at room temperature in chloroform solution on a Perkin-Elmer 141 polarimeter.

Mass spectra were recorded at the Cape Town Technikon by Dr Boshoff but have not been included since they were recorded on photosensitive paper. Accurate mass spectra were not available.

The following spray reagents were prepared in order to test for the presence of various types of compounds:

a) Anisaldehyde spray reagent which is a mixture of 5% H_2SO_4, 5% anisaldehyde and 90% methanol. After spraying the T.L.C. sheet with this reagent, the sheet had to be heated for a few minutes with a blow-drier for detection.

b) Dragendorff reagent for the positive identification of alkaloids was prepared by dissolving 0.85 g of basic Bismuth nitrate in 40 ml of water and 10 ml of glacial acetic acid. This was put into a solution of 8 g of Potassium iodide in 20 ml of water. For spray purposes, 50 ml of the prepared Dragendorff reagent was diluted with a solution of 100 g tartaric acid in 500 ml water.

5.1.3 Chromatography

Analytical thin layer chromatography (T.L.C.) work was performed using 0.2 mm thick aluminium-backed silica gel sheets (Merck Art. 5553). The solvent systems are described individually later in this chapter.

Four different types of column chromatography were used. For the majority of separations, flash chromatography was used. This involved the use of different sized columns ranging from 2 cm to 5 cm in diameter which were prepared by placing a

plug of cotton wool in the area between the stop-cock and the flattened bottom of the column (see Figure 5.1).

This was followed by a thin layer of acid-washed sand and topped with silica gel 60 grain size 0.040-0.053 mm (230 - 400 mesh ASTM, Merck Art. 9385). 30 g of silica to 1 g of extract was the ratio generally used to make up the columns. Lastly, a thin layer of acid-washed sand was placed on top of the silica gel and solvent added. The column was connected to a pump and solvent was pushed through several times to remove air. When the silica was thoroughly saturated with solvent, the solvent level was allowed to reach the top of the sand, the pump switched off, the regulator

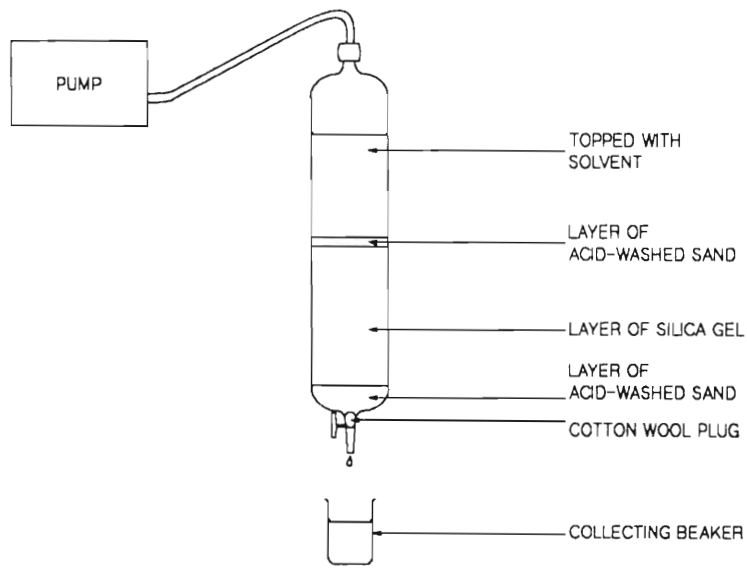


Figure 5.1 Diagram of a chromatographic column

valve opened and the stop-cock closed. The residue was dissolved in solvent and added to the layer of sand by means of a pipette and the walls washed down with solvent until the extract was just below the sand. The column was then topped up with solvent, the pump switched on and fractions collected. As the column progressed, the solvent was made consistently more polar. When the thick columns were used, 30 ml fractions were collected and when thin columns were used, 10 ml fractions were collected.

The second type of column chromatography used on occassion made use of gravity with coarse silica gel 60 grain size 0.2 - 0.5 mm (35-70 mesh ASTM, Merck Art. 7734). The solvent was allowed to pass through the column under gravity.

When compounds appeared to be very difficult to separate on T.L.C., another gravity column was employed. These columns required a fine silica gel 60, particle size less than 0.063 mm (finer than 230 mesh ASTM, Merck Art. 7729).

The fourth type of column chromatography used, was a new method published in the recent edition of Vogel.[76] It is called Dry Column Flash Chromatography and was developed from Flash Chromatography by L.M. Harwood. It is a good method since there is minimal material loss and it is economical in both time and solvents. Pressure was applied rather than vacuum as stated by Harwood.[76] Firstly, the column is eluted with the least polar solvent system in which the extract is soluble. Once the solvent is seen to pass through the receiver, the silica was allowed to dry. The extract was then loaded into the column and the different components eluted by adding successive portions of increasing polarity solvent mixture, allowing the column to be drained after each addition. The volume of the more polar solvent in the solvent system was gradually increased.

5.2 Extractives from *Turraea floribunda*

5.2.1 The seed extract

Turraea floribunda seeds (57.85 g) were ground in a coffee grinder and extracted in a Soxhlet extraction apparatus (see Fig 5.2) using hexane for 8 hours. The hexane extract was then evaporated on a rotary evaporator (see Fig 5.3) to yield a gum (8.58 g). The gum was chromatographed according to the various chromatographic techniques mentioned using a solvent system of methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions.

T.L.C. analysis was conducted using a mixture of methylene chloride : ethyl acetate :: 70:30.

Due to the extremely small amount of starting material, it was very difficult to isolate sufficient quantities of compounds.

Two compounds were isolated in sufficient quantity for analysis. COMPOUND I (40 mg) was obtained as an amorphous material with an R_f value of 0.90 when using a methylene chloride : ethyl acetate :: 70:30 solvent system, while COMPOUND II (a clear, yellowish gum) was only obtained pure after a sodium borohydride reduction. This was performed by preparing a solution of impure COMPOUND II (100 mg) in methanol (20 cm^3) and cooling it to -20 $^{\circ}$C while sodium borohydride (10 mg) was added by stirring. After 15 minutes, the mixture was diluted with acid (HCl, 4M), extracted with $CHCl_3$ and chromatographed (CH_2Cl_2:EtOAc::70:30).

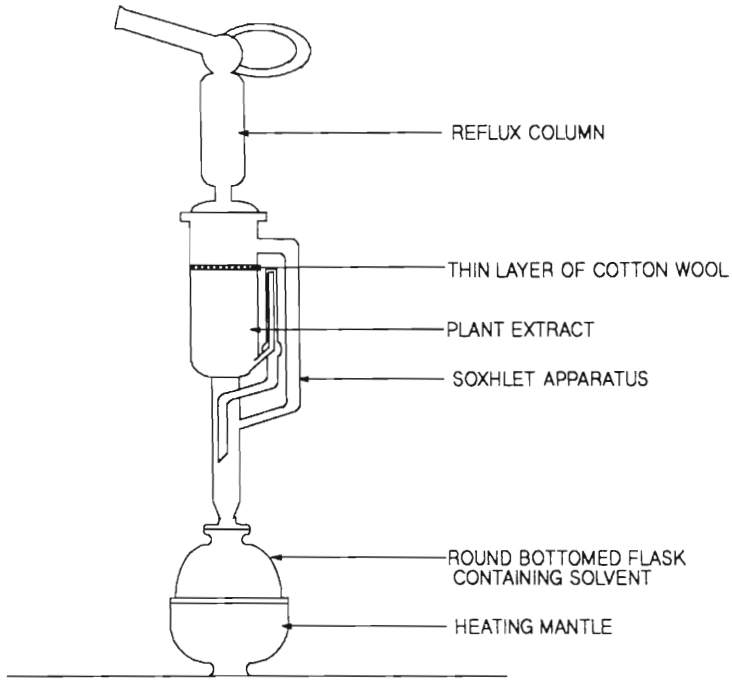


Figure 5.2 Diagram of the Soxhlet extraction apparatus

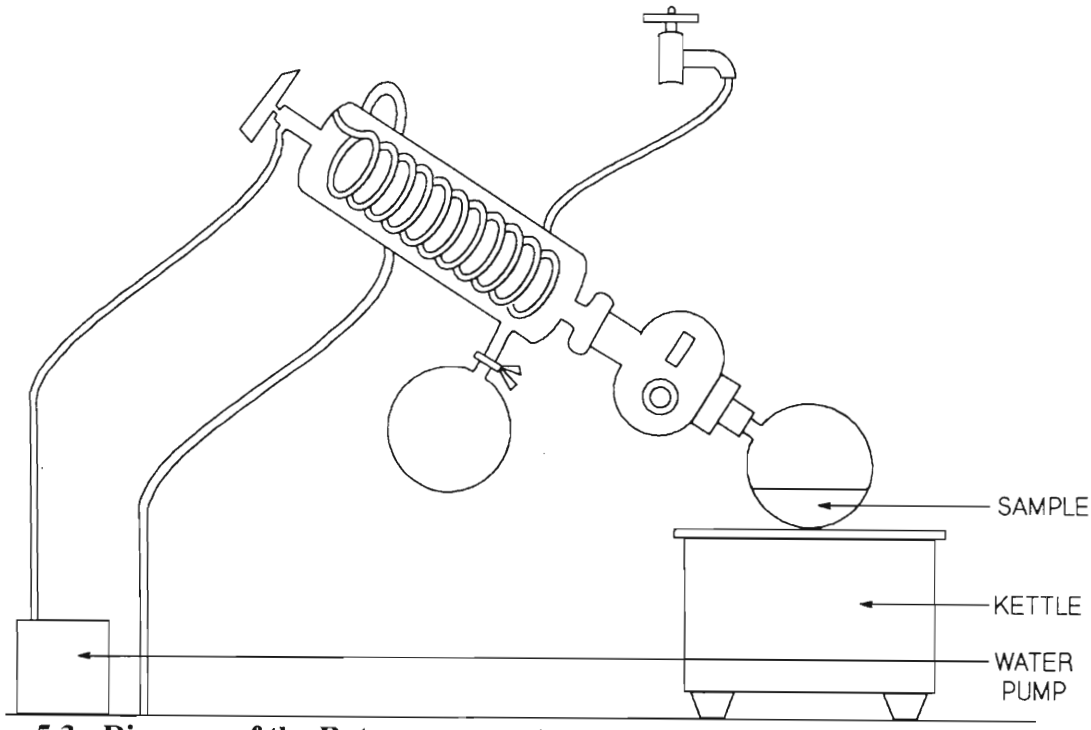


Figure 5.3 Diagram of the Rotary evaporator

5.2.1.1 Physical data of COMPOUND I

Infrared spectrum:

ν max (KBr) 1770(OAc), 1700(α,β unsaturation) cm^{-1}

Mass	Required: $C_{31}H_{38}O_8$	538
	Found:	538

^{13}C N.M.R. spectrum:

δ 204.0(s), 174.5(s), 170.6(s), 170.2(s), 152.4(d), 148.9(s), 142.8(d), 140.5(d), 139.3(s), 125.5(d), 124.1(s), 123.7(d), 119.3(t), 111.4(d), 77.0(d), 72.4(d), 52.4(d), 52.4(s), 52.3(q), 48.2(d), 46.3(s), 44.0(d), 42.2(s), 37.9(t), 31.9(t), 23.2(q), 22.8(q), 21.5(q), 20.9(q), 20.7(q) and 15.6(q).

^{1}H N.M.R. spectrum:

δ 7.29(s,1H); 7.14(s,1H); 6.75(d,J=11Hz,1H); 6.20(s,1H); 6.09(d,J=11Hz,1H); 5.70(d,J=11Hz); 5.65(t,J=0.9Hz); 5.43(d, J=11Hz); 5.20(d, J=2Hz); 4.90(d,J=2Hz); 3.60(s,3H); 3.3(m);3.1(m); 2.77(d,J=5Hz); 2.35(2H); 1.90; 1.70(2xAc); 1.0, 0.95 and 0.80(3xq).

5.2.1.2 Physical data of COMPOUND II (reduced)

^{13}C N.M.R. spectrum:($C_{35}H_{46}O_{12}$)

δ 174.9(s), 171.2(s), 171.1(s), 171.0(s), 170.5(s), 150.2(s), 142.8(d), 140.5(d), 139.3(s), 124.0(s), 123.7(d), 121.5(t), 111.4(d), 77.5(d), 77.0(d), 75.9(d), 75.1(d), 74.0(d), 52.2(q), 51.9(d), 50.4(s), 48.8(d), 47.0(s), 39.1(d), 38.5(s), 37.6(t), 33.3(t), 27.7(q), 24.7(q), 24.5(q), 21.5(q), 21.4(q), 20.9(q), 19.1(q) and 15.0(q).

^{1}H N.M.R. spectrum:

δ 7.34(s,1H); 7.27(s,1H); 6.25(s,1H); 5.34(m); 5.0; 3.69(s,3H); 2.06; 2.01; 1.97; 1.80(4xAc); 1.66; 1.36; 1.04 and 0.87(4xq).

5.2.2 The leaf extract

Turraea floribunda leaves (278.38 g) were dried in an oven at 65°C for 30 minutes and then left in the oven overnight at 45°C. The crisp leaves were ground in a coffee grinder and placed in a mortar. Liquid nitrogen was poured over the crushed leaves so as to freeze them, thus making them crisp and easily ground to fine powder with the pestle. The resulting fine leaf powder was extracted with hexane in a Soxhlet extraction apparatus for 8 hours. The hexane extract was evaporated down on a rotary evaporator to yield a gum (99.03 g). The gum was then chromatographed on a column using the various chromatographic techniques mentioned in 5.1.3. In order to remove all the chlorophylls, column chromatography had to be conducted repetitively starting with methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions. T.L.C. analysis was carried out using a mixture of methylene chloride : ethyl acetate :: 70:30.

Although a number of compounds were isolated, only one was obtained in sufficient quantity to purify thoroughly. The presence of chlorophylls in the extract proved to be a major hindrance which was not experienced in either the wood or seed extraction. COMPOUND III (100 mg) was obtained as a yellow gum and by means of T.L.C.(thin layer chromatography) analysis, it was found to have an R_f value of 0.37 with methylene chloride : ethyl acetate ::70:30 as the solvent system.

5.2.2.1 Physical data of COMPOUND III

^{13}C N.M.R. spectrum: ($C_{33}H_{44}O_{12}$)

δ 175.7(s), 173.4(s), 170.5(s), 170.0(s), 142.5(d), 140.8(d), 128.3(s), 112.4(d), 86.9(d), 74.6(d), 74.1(d), 73.9(d), 73.8(s), 73.2(d), 63.2(d), 51.8(q), 51.4(s), 47.9(s), 41.1(d), 41.0(d), 40.7(s), 39.9(s), 32.1(d), 32.0(t), 27.6(t), 25.3(t), 23.7(q), 21.3(q), 21.2(q), 20.7(q), 18.1(q) and 16.6(2xq).

^{1}H N.M.R. spectrum:

δ 7.23(s,1H); 7.08(s,1H); 6.38(s,1H); 5.02(s,1H); 4.58(m,1H); 4.35(s,1H); 3.59(m,2H); 3.58(s,3H); 3.53(s,1H); 2.95(m,1H); 2.10, 2.07, 2.01(3xAc); 1.36, 1.28, 1.14 and 0.96(4xq).

COMPOUND III was acetylated as described later in this chapter and after column chromatography, a major and minor acetylated product was obtained. COMPOUND IIIa (penta-acetate, 60 mg) had an R_f value of 0.31 in methylene chloride : ethyl acetate ::70:30 as the solvent system and was the major product found. COMPOUND IIIb (tetra-acetate, 20 mg) was the minor product found and was also a clear gum which had an R_f value of 0.31 in methylene chloride : ethyl acetate ::70:30.

5.2.2.1.1 Physical data of COMPOUND IIIa

Mass Required: $C_{37}H_{48}O_{14}$ 716

Found: 716

^{13}C N.M.R. spectrum:

δ 174.2(s), 171.4(s), 170.5(s), 170.1(s), 169.8(s), 169.5(s), 142.8(d), 141.0(d), 128.3(s), 112.6(d), 79.7(d), 76.6(d), 74.5(d), 74.2(s), 73.4(d), 72.4(d), 63.4(d), 52.0(q), 49.6(s), 48.8(s), 40.6(s), 40.4(d), 40.2(s), 40.2(d), 33.3(d), 32.5(t), 25.2(t), 24.6(t), 23.7(q), 21.5(q), 21.4(q), 21.2(q), 21.0(q), 21.0(q), 17.9(q), 16.9(q) and 16.5(q).

^{1}H N.M.R. spectrum:

δ 7.11(s,1H); 6.40(s,1H); 5.19(m,1H); 4.95(m); 4.89(m); 4.65(m); 3.55(s); 3.39(m); 2.19, 2.12, 2.11, 2.05, 1.92 (5xAc); 1.35, 1.25, 1.20 and 1.05(4xq).

5.2.2.1.2 Physical data of COMPOUND IIIb

Infrared spectrum:

ν max(KBr) 1745cm^{-1}(keto group)

Mass Required: $C_{35}H_{44}O_{13}$ 672

Found: 672

^{1}H N.M.R. spectrum:

δ 7.29(s,1H); 7.13(s,1H); 6.43(s,1H); 5.16(m,1H); 4.83(m,1H); 3.68(s,3H); 3.63(s,1H), 3.32(d,J=8Hz,1H); 2.19, 2.17, 2.14, 2.09(4xAc); 1.35, 1.28, 1.21(2x) and 1.06(5xq).

5.2.3 The wood extract

The wood extract (11.25 g) was obtained from Professor Taylor who had extracted plant material provided by Professor K.H. Pegel. Separation of components was by means of repetition of the various column chromatographic procedures described in 5.1.3. The solvent system for the mobile phase was a mixture of methylene chloride and ethyl acetate, starting with methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions. T.L.C. analysis was conducted using a mixture of methylene chloride : ethyl acetate :: 70:30.

T.L.C. analysis of the original extract showed a mixture of components but only two were present in sufficient quantity to obtain pure samples by repeated column chromatography.

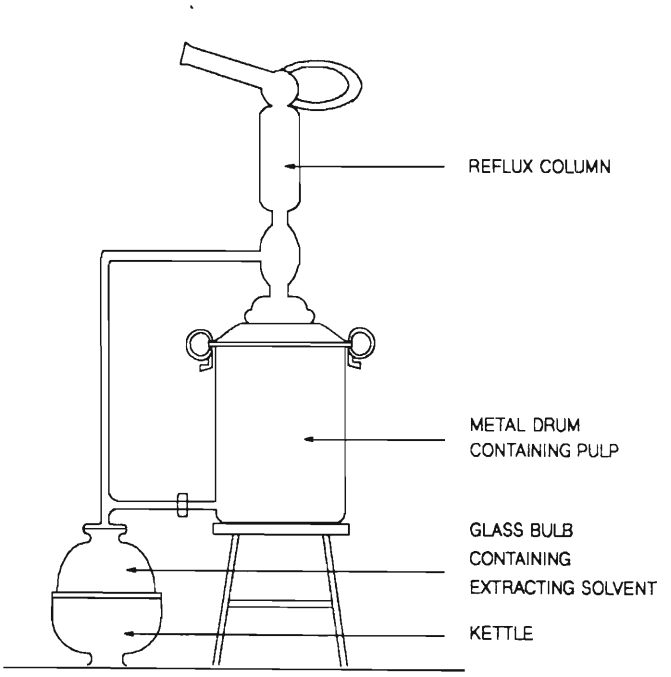


Figure 5.4 Diagram of the extraction apparatus used for wood

COMPOUND IV was obtained as white spindle shaped crystals (400 mg) and similarly COMPOUND V was also obtained as white spindle shaped crystals (350 mg). The R_f values of these two compounds using a methylene chloride : ethyl acetate ::70:30 solvent system were 0.64 and 0.67 respectively.

5.2.3.1 Physical data of COMPOUND IV

Melting point: 170°C

^{13}C N.M.R. spectrum: ($C_{29}H_{48}O$)

141.2(s), 138.4(d), 129.4(d), 121.9(d), 71.9(d), 57.0(d), 56.0(d), 51.4(d), 50.3(d), 42.4(s), 42.3(t), 40.6(d), 39.9(t), 37.4(t), 36.6(s), 31.9(t), 31.9(d), 31.9(d), 31.6(t), 28.3(t), 25.4(t), 24.4(t), 21.1(t), 21.1(q),21.1(q), 19.4(q), 19.0(q), 12.3(q) and 12.0(q).

^{1}H N.M.R. spectrum:

5.35(d,1H); 3.68(m,1H); 3.50(m,1H); 1.26; 1.00; 0.85; 0.85; 0.83 and 0.68(6xq).

5.2.3.2 Physical data of COMPOUND V

Refer to COMPOUND VII since both COMPOUND V and COMPOUND VII are sitosterol.

Melting point: 137°C

The spectroscopic data can be seen as for COMPOUND VII.

5.3 Extractives from *Turraea obtusifolia*

5.3.1 The seed extract

Turraea obtusifolia seeds (17.02 g) were ground in a coffee grinder and extracted in a Soxhlet extraction apparatus using hexane for 8 hours. The hexane extract was then evaporated on a rotary evaporator to yield an almost colourless gum (2.6 g). This gum was chromatographed according to the various chromatographic techniques mentioned using a solvent system of methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions.

T.L.C. analysis was conducted using a mixture of methylene chloride : ethyl acetate :: 70:30.

Due to the extremely small amount of starting material, it was very difficult to isolate sufficient quantities of compounds. However, two structures were elucidated. COMPOUND VII (255 mg) was obtained as clear crystals and COMPOUND VIII (50 mg) as a white amorphous solid with R_f values of 0.67 and 0.43 respectively using a methylene chloride : ethyl acetate ::70:30 solvent system.

5.3.1.1 Physical data of COMPOUND VII

Melting point: 137 °C

^{13}C N.M.R. spectrum: ($C_{29}H_{49}O$)

δ 141.2(s), 122.1(d), 72.0(d), 56.9(d), 56.2(d), 50.2(d), 50.2(d), 42.4(t), 42.4(t), 39.9(t), 37.4(t), 36.6(s), 36.3(d), 34.0(t), 32.1(t), 32.0(s), 31.7(t), 29.2(t), 28.3(t),26.0(t), 24.4(t), 23.1(t), 21.1(t),19.9(q), 19.5(q), 19.1(q), 18.8(q), 11.9(q) and 11.9(q).

^{1}H N.M.R. spectrum:

5.40(d,2H); 3.53(s,1H); 2.32(m,3H); 1.64; 1.50; 1.33; 1.28; 1.04 and 0.71(6xq).

5.3.2 The leaf extract

Turraea obtusifolia leaves (49.27 g) were dried in an oven at 65°C for 30 minutes and then left in the oven overnight at 45°C. The crisp leaves were ground in a coffee grinder and placed in a mortar. Liquid nitrogen was poured over the crushed leaves so as to freeze them, making them crisp and easily ground to fine powder with a pestle. The resulting fine leaf powder was extracted with hexane in a Soxhlet extraction apparatus for 8 hours. The hexane extract was evaporated down on a rotary evaporator to yield a gum (17.55 g). The gum was then chromatographed on a column using flash chromatography (5.1.3). In order to remove all the chlorophylls, column chromatography had to be conducted repetitively starting with methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions. T.L.C. analysis was carried out using a mixture of methylene chloride : ethyl acetate :: 70:30.

Although a number of compounds were isolated, only two were obtained in sufficient quantity to purify. The presence of chlorophylls in the extract proved to be a major hindrance which was not experienced in either the wood or seed extraction. COMPOUND VIII (45 mg) was obtained as a clear, viscous residue with an R_f value of 0.89 when using a solvent system of methylene chloride : ethyl acetate ::70:30. COMPOUND IX (350 mg) was obtained as an orange gum and had an R_f value of 0.65 when using the same solvent system. A minor compound was also isolated as a clear gum and was labelled COMPOUND X (25 mg).

5.3.2.1 Physical data of COMPOUND VIII

^{13}C N.M.R. spectrum: ($C_{20}H_{40}O$)

δ 140.6(s), 123.5(d), 59.5(t), 40.0(t), 39.5(t), 37.6(t), 37.5(t), 37.4(t), 36.8(d), 32.9(d), 32.8(d), 28.1(d), 25.2(t), 24.9(t), 24.6(t), 22.8(q), 22.7(q), 19.8(q), 19.8(q) and 16.2(q).

^{1}H N.M.R. spectrum:

δ 5.4(t,1H); 4.14(d,J=8Hz,2H); 1.99(t,4H); 1.67(s,3H); 1.25(t,6H); 0.88, 0.86, 0.86 and 0.85(4xq).

5.3.2.2 Physical data of COMPOUND IX

Infrared spectrum:

ν max (KBr) 3400-3450(OH), 1715(six-membered ring C=O), 825(C=C) cm^{-1}

^{13}C N.M.R. spectrum: ($C_{30}H_{46}O_4$)(C-21 anomeric mixture)

δ 217.3(s), 145.9(s), 118.0(d), 101.6(d), 97.5(d), 78.8(d), 76.7(d), 67.7(d), 65.3(d), 57.7(s), 57.1(s), 51.2(d), 50.6(s), 47.7(s), 47.1(d), 43.3(s), 37.2(t), 34.0(t), 33.6(t), 33.0(t), 32.6(t), 30.2(t), 29.9(t), 26.2(q), 26.0(q), 23.6(q), 23.2(q), 22.0(q), 20.2(q), 16.4(t) and 11.4(q).

^{1}H N.M.R. spectrum:

δ 5.34(s,2H); 3.90(m,1H); 2.90(d,J=8Hz); 2.20(m); 1.30(s,6H); 1.12(m,1H); 1.04(s,9H) and 0.86(m)

COMPOUND IX (200 mg) was then acetylated as described in section 5.9. By means of column chromatography, the main product was isolated as COMPOUND IXa, a gum which had an R_f value of 0.90 in a methylene chloride : ethyl acetate ::70:30 solvent system.

5.3.2.2.1 Physical data of COMPOUND IXa

Mass Required: $C_{32}H_{48}O_5$ 512

Found: 512

m/e 497, 437, 365

^{13}C N.M.R. spectrum:

δ 217.5(s), 170.9(s), 145.8(s), 118.7(d), 101.0(d), 79.2(d), 65.1(d), 57.2(s), 52.5(d), 51.2(s), 50.5(d), 48.4(d), 48.0(s), 47.5(d), 43.7(s), 38.6(t), 35.0(t), 37.4(t), 35.2(s), 33.9(t), 31.9(t), 27.5(t), 27.4(q), 25.0(q), 24.6(q), 24.5(t), 22.9(q), 21.6(q), 21.5(q), 19.6(q), 17.8(t) and 12.8(q).

^{1}H N.M.R. spectrum:

δ 6.20(d, J=3Hz); 5.30(s); 3.90(m,1H); 2.79(d,J=8Hz); 2.02(s,3H); 1.30, 1.27, 1.10, 1.00, 0.99, 0.97 and 0.78(7xq).

The carbonyl group at carbon atom number three in COMPOUND IXa was then reduced. A solution of COMPOUND IXa (255 mg) in methanol (60 cm^3) was stirred with sodium methoxide (25 mg) and sodium borohydride (37.5 mg) for 30 minutes. The reaction mixture was then diluted with aqueous acid (HCl, 4M) and extracted into chloroform. The chloroform was evaporated down and the precipitate chromatographed (CH_2Cl_2:EtOAc::70:30). The resulting product isolated by means of column chromatography was labelled COMPOUND IXb (20 mg) and had an R_f value of 0.85 in a methylene chloride : ethyl acetate ::70:30 solvent system.

5.3.2.2.2 Physical data of COMPOUND IXb

^{1}H N.M.R.spectrum:

δ 3.35(3-H); 1.66(s); 1.60(s); 1.26(s); 1.25(s); 1.14(s); 1.14(s); 1.03(s); 1.00(s); 0.88(s); 0.83(s) and 0.80(s).

5.3.2.2.3 Physical data of COMPOUND X

^{13}C N.M.R. spectrum: ($C_{30}H_{48}O_4$)

δ 217.7(s), 145.8(s), 118.4(d), 71.6(d), 68.2(t), 64.8(d), 59.6(s), 52.4(d), 51.4(s), 49.2(d), 48.5(d), 43.6(d),43.4(s), 38.6(s), 36.8(t), 34.3(s), 33.9(t), 33.2(t), 29.8(t), 28.6(t), 27.5(q), 27.2(t), 25.0(q), 24.6(q), 24.4(t), 22.1(q), 21.7(q), 19.5(q), 18.4(t) and 12.8(q).

^{1}H N.M.R. spectrum:

δ 5.35(m,2H); 3.95(m,1H); 2.78(m); 1.33, 1.32, 1.26, 1.12, 1.05, 1.02 and 1.00(7xq).

5.3.3 The wood extract

The wood extract (21.75 g) was obtained from Professor Taylor who had extracted a plant provided by the Natal Forestry Department. Separation of components was by means of repetition of the various column chromatographic procedures described in 5.1.3. The solvent system for the mobile phase was a mixture of methylene chloride and ethyl acetate, starting with methylene chloride and making it progressively more polar by adding ethyl acetate in 5% successions. T.L.C. analysis was conducted using a mixture of methylene chloride : ethyl acetate :: 70:30.

T.L.C. analysis of the original extract showed a mixture of components but only two main products were present in sufficient quantity to obtain pure samples by repeated column chromatography.

The two compounds were labelled COMPOUND XI (500 mg) and COMPOUND XII (50 mg). Both were colourless gums. The former had an R_f value of 0.23 and

the latter an R_f value of 0.20 when using a methylene chloride : ethyl acetate ::70:30 solvent system. A minor component, COMPOUND XIII (25mg), was isolated in sufficient quantity to obtain a ^1H N.M.R. spectrum.

5.3.3.1 Physical data of COMPOUND XI

Mass Required: $C_{30}H_{47}O_5$ 488

Found: 488

m/e 470, 455, 437, 412, 397, 365

^{13}C N.M.R. spectrum:

δ 217.6 (s), 145.7(s), 118.0(d), 96.5(d), 77.8(d), 75.0(d), 73.7(s), 52.2(d), 50.5(s), 48.1(d), 47.7(s), 46.1(d), 45.0(d), 43.3(s), 37.2(t), 34.8(s), 33.6(t), 33.0(t), 30.2(t), 29.1(t), 27.2(q), 26.4(2xq), 24.2(q), 23.0(q), 23.0(t), 22.9(t), 21.3(q), 16.7(t) and 12.4(q).

^1H N.M.R. spectrum:

δ 5.31(s,2H); 5.26(s,1H); 4.50(m,1H); 3.15(m,1H); 2.25(m); 2.10(m); 1.50(m); 1.26, 1.25, 1.25, 1.11, 1.03, 1.01 and 0.83(7xq).

COMPOUND XI (150 mg) was acetylated (see 5.9) and column chromatography then followed in order to isolate the main product labelled COMPOUND XIa which had an R_f value of 0.58 in methylene chloride : ethyl acetate ::70:30.

5.3.3.1.1 Physical data of COMPOUND XIa

^{13}C N.M.R. spectrum: ($C_{34}H_{51}O_7$)

δ 217.5(s), 171.6(s), 170.5(s), 145.7(s), 118.7(d), 100.3(d), 77.7(d), 77.4(d), 76.4(d), 72.6(s), 52.5(d), 51.1(s), 50.1(d), 48.4(d), 48.0(s), 46.7(d), 43.7(s), 38.6(t), 35.2(s), 35.0(t), 33.9(t), 33.8(t), 31.8(t), 27.6(t), 27.4(q), 27.4(q), 26.8(q), 24.6(q), 24.4(t), 22.9(q), 21.6(q), 21.4(q), 21.0(q) and 12.8(q).

^{1}H N.M.R. spectrum:

δ 6.13(d,1H); 5.30(s,1H); 4.85(d,1H); 4.50; 3.29(s,1H); 2.20, 2.07(2xAc); 1.30, 1.18, 1.10, 1.03, 1.01, 0.99 and 0.80(7xq).

A sodium borohydride reduction was carried out on COMPOUND XI during which 180.9 mg of COMPOUND XI was stirred with 26 mg of $NaBH_4$ in methanol at 20°C for one hour. The reaction was then diluted with water whereupon it turned cloudy. It was then acidified with aqueous acid (HCl, 4M) and was finally extracted into ethyl acetate. The ethyl acetate was subsequently evaporated down on a rotary evaporator to yield a brown residue which was chromatographed (CH_2Cl_2:EtOAc::70:30). After isolation by means of column chromatography, the main product was labelled COMPOUND XIb. It had an R_f value of 0.08 in methylene chloride : ethyl acetate ::70:30.

5.3.3.1.2 Physical data of COMPOUND XIb

^{13}C N.M.R. spectrum: ($C_{30}H_{48}O_5$)

δ 145.9(s), 118.4(d), 97.1(d), 79.4(d), 78.9(d), 75.2(s), 73.8(d), 50.8(d), 50.0(s), 48.9(d), 46.5(s), 45.4(d), 43.6(d), 43.2(d), 39.0(t), 37.2(t), 35.1(t), 34.7(d), 34.2(t), 31.6(t), 27.6(t), 27.3(q), 26.8(q), 26.8(t), 24.0(t), 23.2(q), 17.5(q), 14.7(q), 14.2(q) and 13.0(q).

^{1}H N.M.R. spectrum:

δ 5.30(2H); 5.18(m,1H); 4.50(m,1H); 3.11(m,1H); 1.21, 1.20, 0.91, 0.90, 0.79, 0.77 and 0.67(7xq).

A periodate oxidation was then carried out on COMPOUND XI. A solution of sodium periodate (400 mg) in water (1.5 cm^3) and 70% perchloric acid (1 drop) was added to a solution of COMPOUND XI (180.9 mg) in dioxan (20 cm^3). The mixture was stirred for one hour and then the precipitate of sodium periodate was filtered off and washed with dioxan. Sodium hydrogen carbonate (40 mg) was added to the clear solution, which was concentrated to 10 cm^3 and then poured into water. Extraction with ether yielded a gum which was chromatographed (CH_2Cl_2:EtOAc::70:30) to give a pure product which was amorphous and labelled COMPOUND XIc. It had an R_f value of 0.18 in a methylene chloride : ethyl acetate ::70:30 solvent system.

5.3.3.1.3 Physical data of COMPOUND XIc

^{13}C N.M.R. spectrum:

δ217.6(s), 204.2(d), 200.8(d), 145.3(s), 119.0(d), 52.4(d), 51.0(s), 48.5(d), 48.4(d), 48.4(d), 48.0(s), 43.7(s), 38.6(t), 35.1(t), 35.0(t), 34.0(t), 33.9(s), 32.9(t), 27.7(t), 27.4(q), 24.6(q), 24.5(t), 23.9(q), 21.7(q), 18.1(t) and 12.8(q).

^{1}H N.M.R. spectrum;

δ 9.90(s); 9.70(s); 5.30; 1.18,1.11,1.04,1.01 and 1.00(5xq).

5.3.3.2 Physical data of COMPOUND XII

^{13}C N.M.R. spectrum: ($C_{30}H_{50}O_5$)

δ 145.9(s), 118.4(d), 97.0(d), 79.4(d), 78.9(d),75.1(d), 73.9(s), 50.8(d), 49.0(s), 48.9(d), 46.5(s), 45.4(d), 43.6(d), 43.6(d), 39.0(t), 37.3(t), 35.1(t), 34.2(t), 32.0(d), 31.6(t), 27.6(t), 27.3(q), 26.8(q), 26.8(t), 24.0(t), 23.2(q), 17.5(q), 14.7(q), 14.2(q) and 13.0(q).

^{1}H N.M.R. spectrum:

δ 5.25(s,2H); 4.50(m,1H); 3.25(m,1H); 1.27, 1.25, 0.99, 0.96, 0.85, 0.84 and 0.74(7xq).

5.3.3.3 Physical data of COMPOUND XIII

^{1}H N.M.R. spectrum :

δ 5.40(m,2H); 4.68(1H); 3.95(m,1H); 2.85(d, J=10Hz); 2.06(s, 3H); 0.98,0.91,0.85,0.80,1.01,1.32 and 1.32 (7xq).

5.4 Extractives from *Apodytes dimidiata*

5.4.1 The wood extracts

The wood (20.5 kg) was debarked and then milled. The wood chips were then extracted in large metal drums (as shown below in Figure 5.4) with hexane for approximately 48 hours. The wood chips were subsequently extracted for a further 48 hours with methanol in order to extract the alkaloids. The method as used by On'Okoko et al.[63] was used to purify the alkaloids, in which the residue (after the methanol extraction) was dissolved in chloroform. The chloroform was extracted with 1% HCl solution (7x500 ml). The combined HCl aqueous layers were basified with NH_4OH. This liberated the free alkaloid bases which could be extracted (7x500 ml) into a chloroform layer. The chloroform layer was evaporated down on a rotary evaporator yielding a yellow-brown gum (1.3 g).

Thin layer chromatographic analysis was conducted using precoated 0.2 mm thick aluminium-backed silica gel 60 F254 sheets (Merck Art. 5554) using various solvent systems which are discussed in detail in Chapter 4 .

Preparative layer chromatographic analysis was conducted using precoated neutral alumina plates of 0.25 mm thickness (Merck Art. 5713, Aluminium oxide F-254, Type E.)

5.4.2 The leaf extracts

The leaves (599.9 g) were placed in an oven for 30 minutes at a temperature of 65 $^{\circ}$C and then left in the oven at 45 $^{\circ}$C overnight. The crisp leaves were crushed in a coffee grinder, removed and placed in a mortar. Liquid nitrogen was poured over the leaves so as to freeze them and make them crisp. They could then be ground to a fine powder using a pestle. This leaf powder was extracted in a Soxhlet for 12 hours with refluxing ethanol.

The extract was chromatographed using various solvent systems as described in Chapter 4.

5.4.3 The seed extracts

The seeds (155.5 g) were collected and allowed to dry in air. The dried seeds were ground in a coffee grinder after which, they were treated similarly to the leaves by being crushed to a fine powder using a mortar and pestle.

The seed powder was extracted in a Soxhlet for 24 hours with refluxing hexane. The hexane extract was evaporated down to yield a gum (55.32 g). Part of the extract was redissolved in hexane and left standing for 5 days. A white precipitate formed and this was filtered off. An N.M.R. analysis of this precipitate indicated that it was merely a long-chain fatty acid mixture, not worthy of further consideration.

The remaining extract was chromatographed using flash chromatography and a solvent system of ethyl acetate and methylene chloride in varying proportions, starting with pure methylene chloride and making the system progressively more polar by adding ethyl acetate in 5 % successions.

A single major component was obtained after repeated column chromatography. The product, a pale lemon yellow gum (45 mg), was labelled COMPOUND XIV

5.4.3.1 Physical data of COMPOUND XIV

Infrared spectrum:

ν max(KBr) 3450(OH), 3000(hydrocarbon chain), 1740(carbonyl)cm^{-1}

Mass	Required: $C_{21}H_{40}O_4$	356
	Found:	356

m/e 358, 356, 338, 325, 299, 264, 222, 134

^{13}C N.M.R. spectrum:

δ 174.8(s), 130.3(d), 130.0(d), 70.3(d), 65.1(t), 63.5(t), 34.2(t), 34.0(t), 32.0(t), 32.0(t), 29.8(t), 29.6(t), 29.4(t), 29.3(t), 29.2(t), 29.0(t), 27.3(t), 27.2(t), 24.9(t), 22.7(t) and 14.1(q).

^{1}H N.M.R. spectrum:

δ 5.28(t), 4.06(m), 3.66(m), 3.50(m), 2.25(m), 1.90(m), 1.54(m), 1.24(s), 1.20(s) and 0.82(m).

COMPOUND XIV (30 mg) was then hydrolyzed in a round-bottomed flask by adding 20 ml of 10% NaOH solution and allowing the mixture to reflux gently for 40 minutes. The mixture was cooled and acidified (H_2SO_4, 2M) until the fatty acid precipitated and could be filtered off. This fatty acid portion of the ester was labelled COMPOUND XIVa.

5.4.3.2 Physical data of COMPOUND XIVa

Melting point: 39°C

^{1}H N.M.R. spectrum:

δ 5.33(t), 2.32(t), 1.99(m), 1.60(m), 1.27(s), 1.23(s) and 0.85(m).

5.5 The acetylation procedure

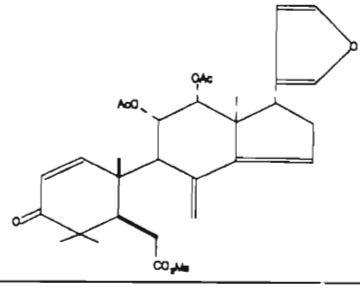
Acetylation was performed in an excess of acetic anhydride in pyridine. For every 100 mg of sample, 1 cm^3 of acetic anhydride and 1 cm^3 of pyridine were used.

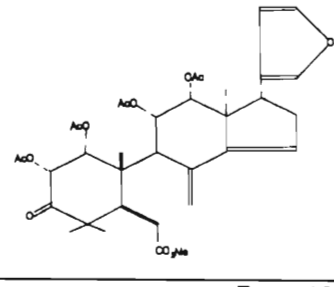
The material to be acetylated was dissolved in the appropriate amount of pyridine in a small round-bottomed flask. The relative amount of acetic anhydride was then added, the flask warmed on a water bath for a short while and was then left to stand overnight under anhydrous conditions (12 hours). When the reaction was complete, the excess acetic anhydride was destroyed with methanol.

The pyridine was then removed by azeotropic distillation with toluene, and the last traces eliminated by distillation with methanol.

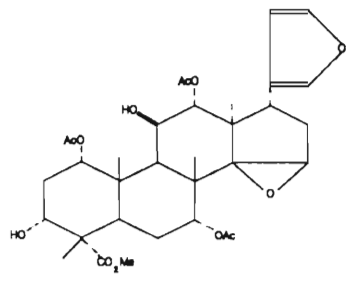
Tables

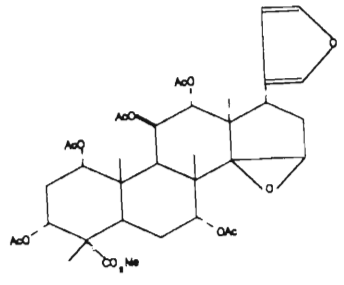
Carbon atom	COMPOUND I	Carbon atom	COMPOUND II
1	152.4(d)	14	150.2(s)
2	125.5(d)	15	123.7(d)
3	204.0(s)	20	124.0(s)
14	148.9(s)	21	142.8(d)
15.	123.7(d)	22	111.4(d)
21	142.8(d)	23	140.5(d)
22	111.4(d)	30	121.5(t)
23	140.5(d)	5x OAc at C-1,2,11	174.9(s)
20	124.1(s)	and 12	171.2(s)
30	119.3(t)	and 1x CO_2Me	171.1(s)
2xOAc+1xCO_2Me	174.5(s)	"	171.0(s)
"	170.6(s)	"	170.5(s)
"	170.2(s)	CO_2Me	52.2(q)
5 doublets	76.95(d)	2x triplets	37.6(t)
"	74.95(d)	"	33.3(t)
"	52.4(d)	8x doublets	77.5(d)
"	48.2(d)	"	77.0(d)
"	44.0(d)	"	75.9(d)
2 triplets	37.90(t)	"	75.1(d)
"	31.5(t)	"	51.9(d)
4 singlets	139.3(s)	"	48.8(d)
"	52.4(s)	"	39.1(d)
"	46.3(s)	"	24.0(d)
"	42.2(s)	4x singlets	139.3(s)
6 quartets	23.18(q)	"	50.4(s)
"	22.8(q)	"	47.0(s)
"	21.5(q)	"	38.5(s)
"	20.8(q)	8x quartets	27.7(q)
"	20.7(q)	"	24.7(q)
"	15.6(q)	"	24.5(q)
CO_2Me	52.3(q)	"	21.5(q)
		"	21.4(q)
		"	20.9(q)
		"	19.1(q)
		"	15.0(q)



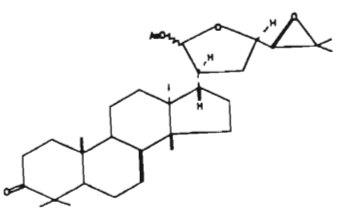


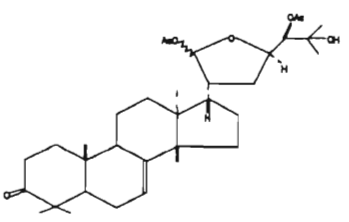
Carbon atom	COMPOUND III	Carbon atom	COMPOUND IIIa
20	128.3(s)	20	128.3(s)
21	142.5(d)	21	142.8(d)
22	112.4(d)	22	112.6(d)
23	140.8(d)	23	141.0(d)
3x OAc + 1x	175.7(s)	5x OAc + 1x	174.2(s)
CO_2Me	173.4(s)	CO_2Me	171.4(s)
"	170.5(s)	"	170.5(s)
"	170.0(s)	"	170.1(s)
9x doublets	86.9(d)	"	169.8(s)
"	74.6(d)	"	169.5(s)
"	74.1(d)	9x doublets	79.7(d)
"	73.9(d)	"	76.6(d)
"	73.2(d)	"	74.5(d)
"	63.2(d)	"	73.4(d)
"	41.1(d)	"	72.4(d)
"	41.0(d)	"	63.4(d)
"	32.1(d)	"	40.4(d)
5x singlets	73.8(s)	"	40.2(d)
"	51.4(s)	"	33.3(d)
"	51.8(s)	5x singlets	74.2(s)
"	40.7(s)	"	49.6(s)
"	39.9(s)	"	48.8(s)
3x triplets	32.0(t)	"	40.6(s)
"	27.6(t)	"	40.2(s)
"	25.3(t)	3x triplets	32.5(t)
CO_2Me	51.8(q)	"	25.2(t)
7x quartets	23.7(q)	"	24.6(t)
"	21.3(q)	CO_2Me	52.0(q)
"	21.2(q)	9x quartets	23.7(q)
"	20.7(q)	"	21.5(q)
"	18.1(q)	"	21.4(q)
"	16.6(2xq)	"	21.2(q)
		"	21.0(q)
		"	21.0(q)
		"	17.9(q)
		"	16.9(q)
		"	16.5(q)



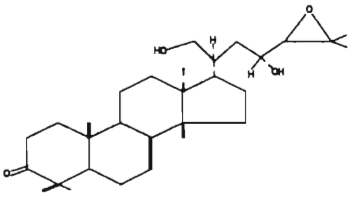


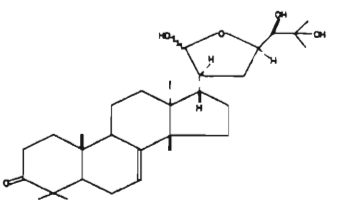
Carbon atom	COMPOUND IXa	Carbon atom	COMPOUND XIa
3	217.5(s)	3	217.5(s)
7	118.7(d)	7	118.7(d)
8	145.8(s)	8	145.7(s)
21	101.0(d)	21	100.3(d)
23	79.2(d)	24	76.4(d)
24	65.1(d)	25	72.6(s)
25	57.2(s)	6x doublets	77.7(d)
4x singlets	51.2(s)	"	77.4(d)
"	48.0(s)	"	52.5(d)
"	43.7(s)	"	50.1(d)
"	35.2(s)	"	48.4(d)
4x doublets	52.5(d)	"	46.7(d)
"	50.5(d)	4x singlets	51.1(s)
"	48.4(d)	"	48.0(s)
"	47.5(d)	"	43.7(s)
8x triplets	38.6(t)	"	35.2(s)
"	35.0(t)	7x triplets	38.6(t)
"	34.4(t)	"	35.0(t)
"	33.9(t)	"	33.9(t)
"	31.9(t)	"	33.8(t)
"	27.5(t)	"	31.8(t)
"	24.5(t)	"	27.6(t)
"	17.8(t)	"	24.4(t)
8x quartets	27.4(q)	9x quartets	27.4(q)
"	25.0(q)	"	27.4(q)
"	24.6(q)	"	26.8(q)
"	22.9(q)	"	24.6(q)
"	21.6(q)	"	22.9(q)
"	21.5(q)	"	21.6(q)
"	19.6(q)	"	21.4(q)
"	12.8(q)	"	21.0(q)
OAc	170.9(s)	"	12.8(q)
		OAc	171.6(s)
		OAc	170.5(s)



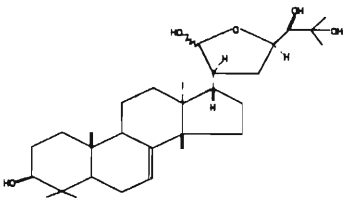


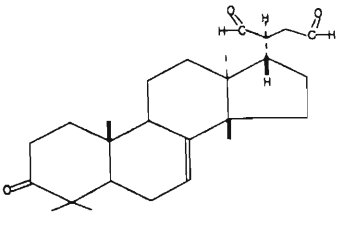
Carbon atom	COMPOUND X	Carbon atom	COMPOUND XI
3	217.7(s)	3	217.6(s)
7	118.4(d)	7	118.0(d)
8	145.8(s)	8	145.7(s)
24	68.2(d)	21	96.5(d)
25	59.6(s)	23	77.8(d)
5x doublets	71.6(d)	24	75.0(d)
"	52.4(d)	25	73.7(s)
"	49.2(d)	4x doublets	52.2(d)
"	48.5(d)	"	48.1(d)
"	43.6(d)	"	46.1(d)
4x singlets	51.4(s)	"	45.0(d)
"	43.4(s)	4x singlets	50.5(s)
"	38.6(s)	"	47.7(s)
"	34.3(s)	"	43.3(s)
9x triplets	64.8(t)	"	34.8(s)
"	36.8(t)	8x triplets	37.2(t)
"	33.9(t)	"	33.6(t)
"	33.2(t)	"	33.0(t)
"	29.8(t)	"	30.2(t)
"	28.6(t)	"	29.1(t)
"	27.2(t)	"	23.0(t)
"	24.4(t)	"	22.9(t)
"	18.4(t)	"	16.7(t)
7x quartets	27.5(q)	7x quartets	27.2(q)
"	25.0(q)	"	26.4(q)
"	24.6(q)	"	26.4(q)
"	22.1(q)	"	24.2(q)
"	21.7(q)	"	23.0(q)
"	19.5(q)	"	21.3(q)
"	12.8(q)	"	12.4(q)





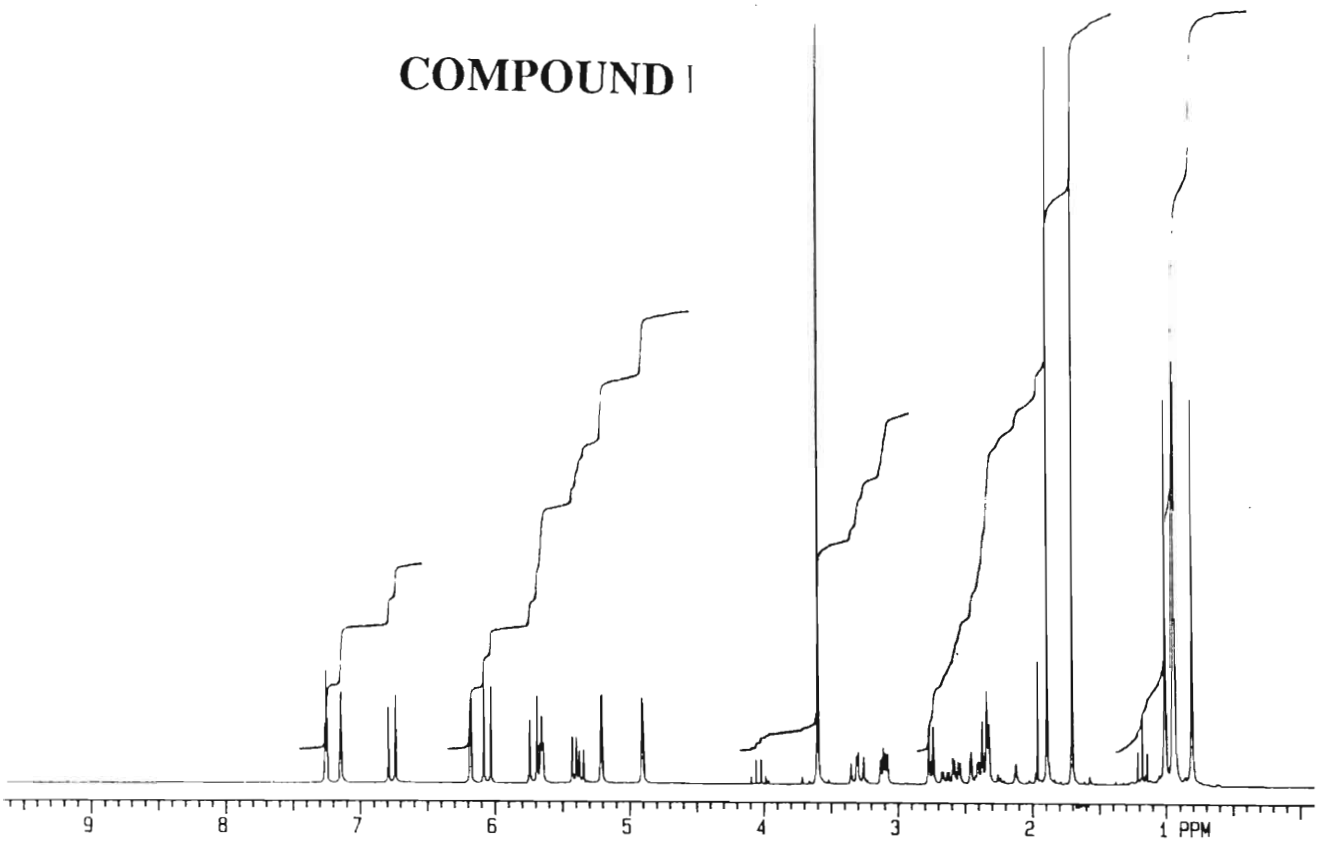
Carbon atom	COMPOUND XIb	Carbon atom	COMPOUND XIc
7	118.4(d)	3	217.6(s)
8	145.9(s)	7	119.0(d)
21	97.1(d)	8	145.3(s)
24	75.2(d)	21+23	204.2(d)
25	73.8(s)	"	200.8(d)
8x doublets	79.4(d)	4x singlets	51.0(s)
"	78.9(d)	"	48.0(s)
"	50.8(d)	"	43.7(s)
"	48.9(d)	"	33.9(s)
"	45.4(d)	4x doublets	52.4(d)
"	43.6(d)	"	48.5(d)
"	43.2(d)	"	48.4(d)
"	34.7(d)	"	48.4(d)
2x singlets	50.0(s)	8x triplets	38.6(t)
"	46.5(s)	"	35.1(t)
8x triplets	39.0(t)	"	35.0(t)
"	37.2(t)	"	34.0(t)
"	35.1(t)	"	32.9(t)
"	34.2(t)	"	27.7(t)
"	31.6(t)	"	24.5(t)
"	27.6(t)	"	18.1(t)
"	26.8(t)	5x quartets	27.4(q)
"	24.0(t)	"	24.6(q)
7x quartets	27.3(q)	"	23.9(q)
"	26.8(q)	"	21.7(q)
"	23.2(q)	"	12.8(q)
"	17.5(q)		
"	14.7(q)		
"	14.2(q)		
"	13.0(q)		

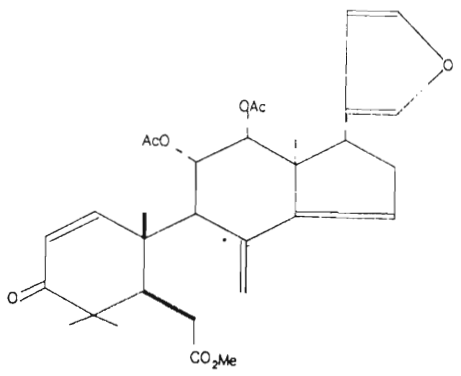




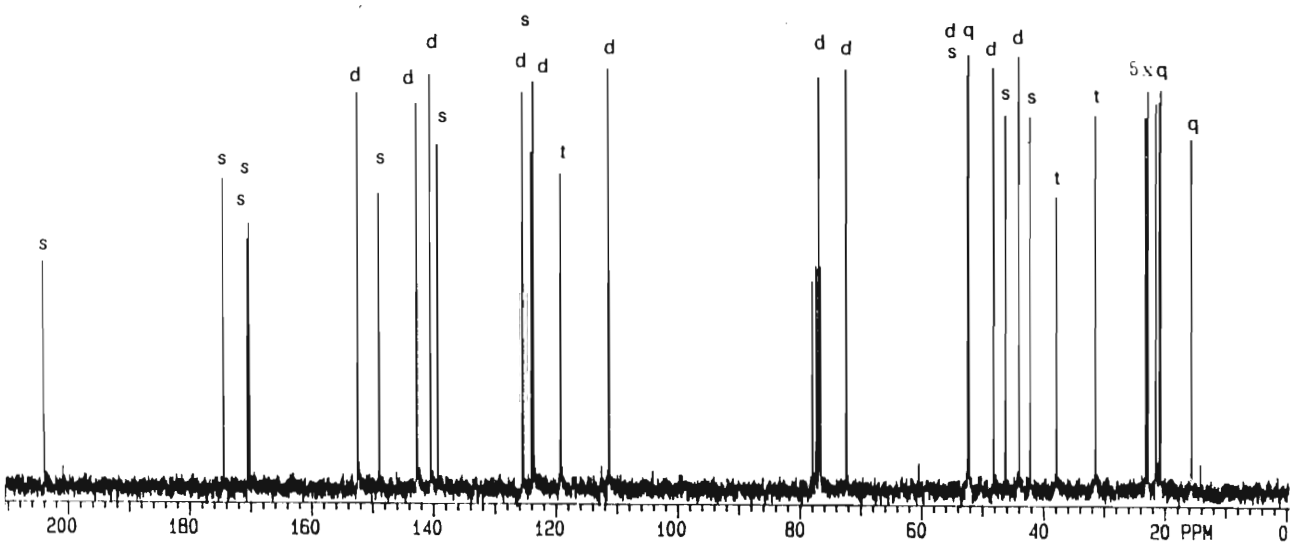
Spectra

COMPOUND I

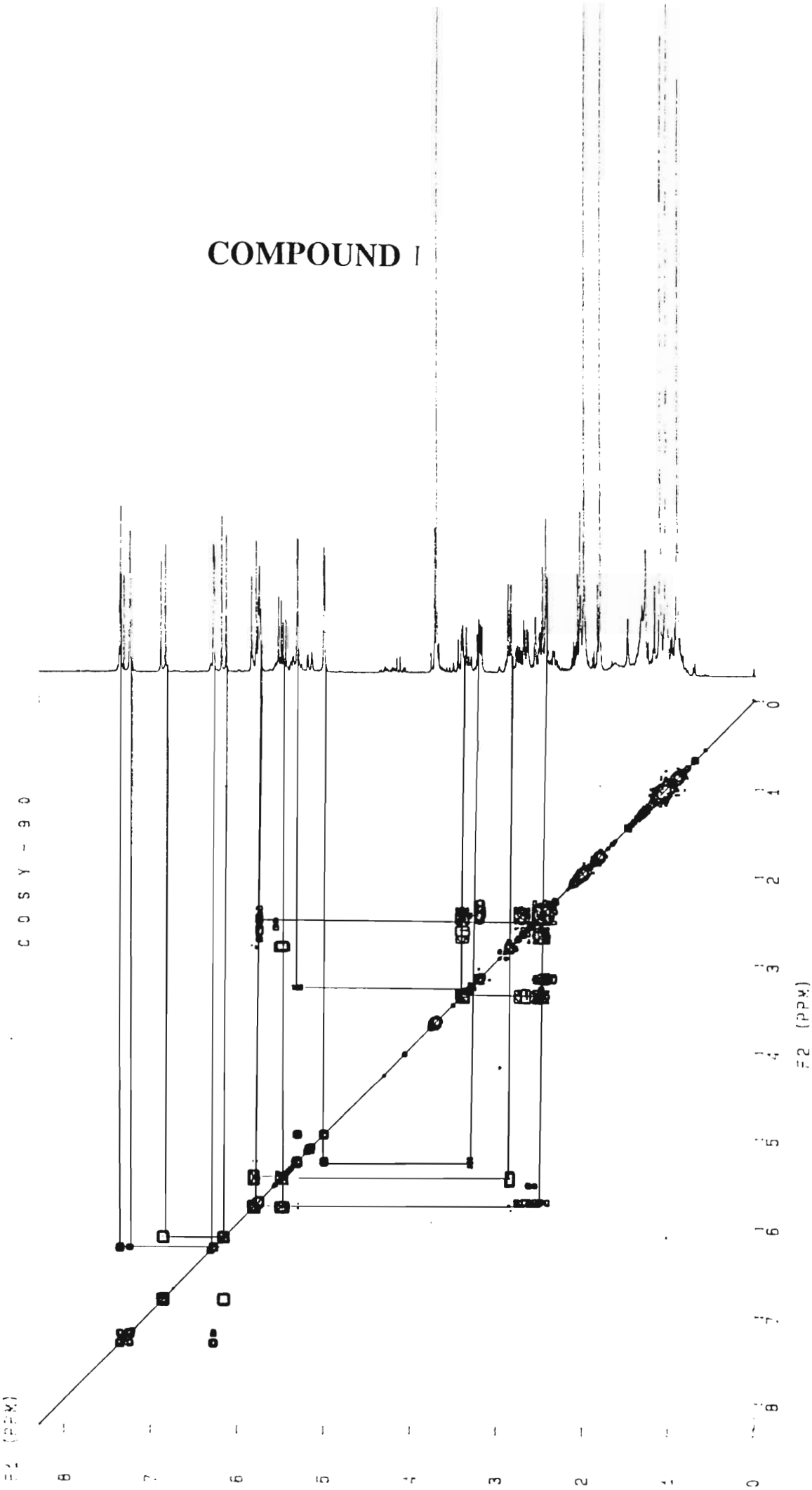




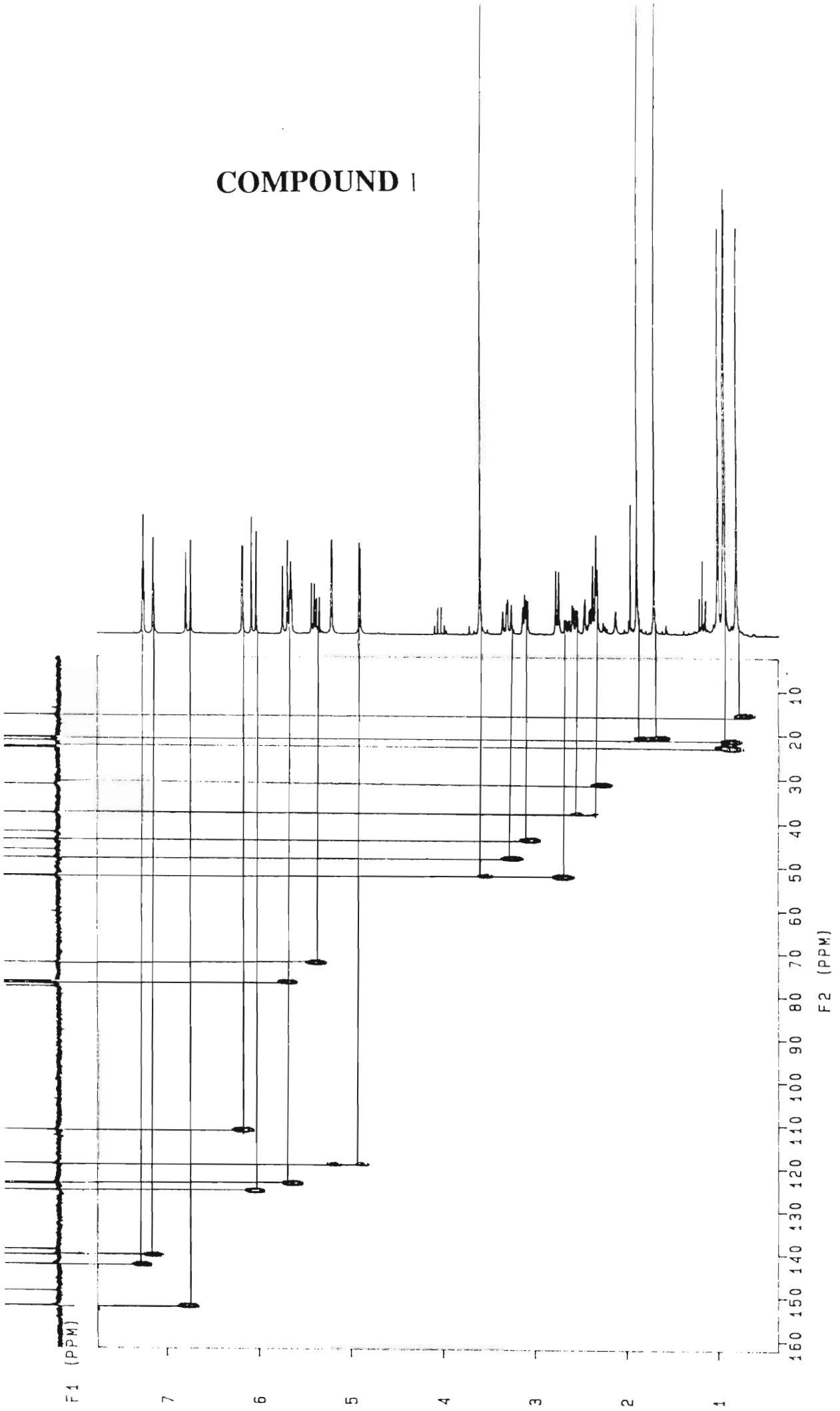
COMPOUND I



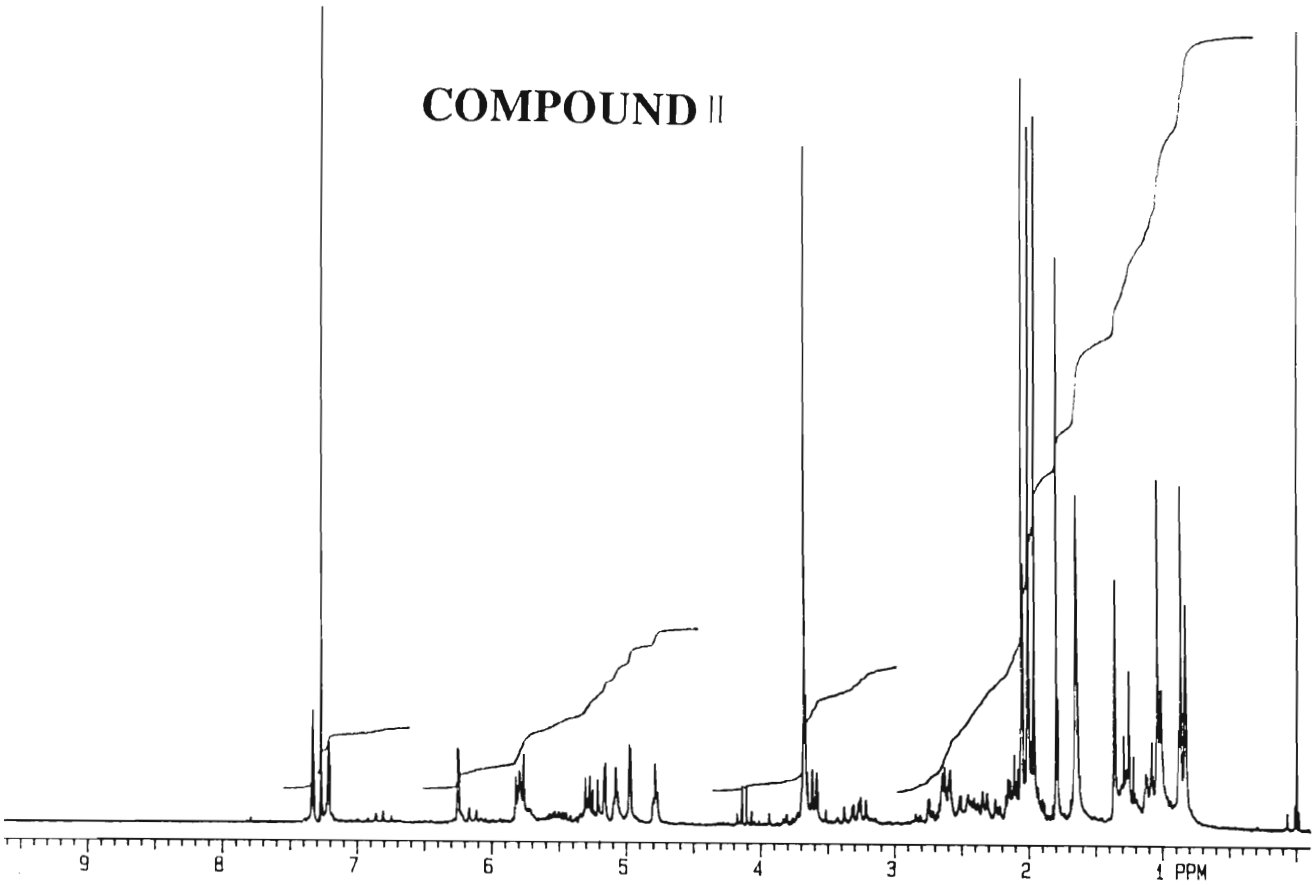
COMPOUND I

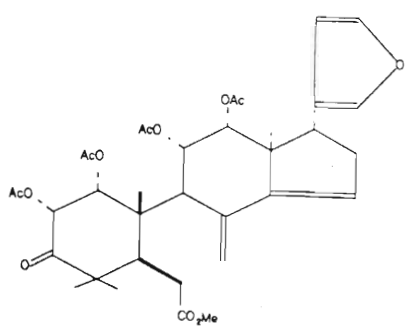


COMPOUND I

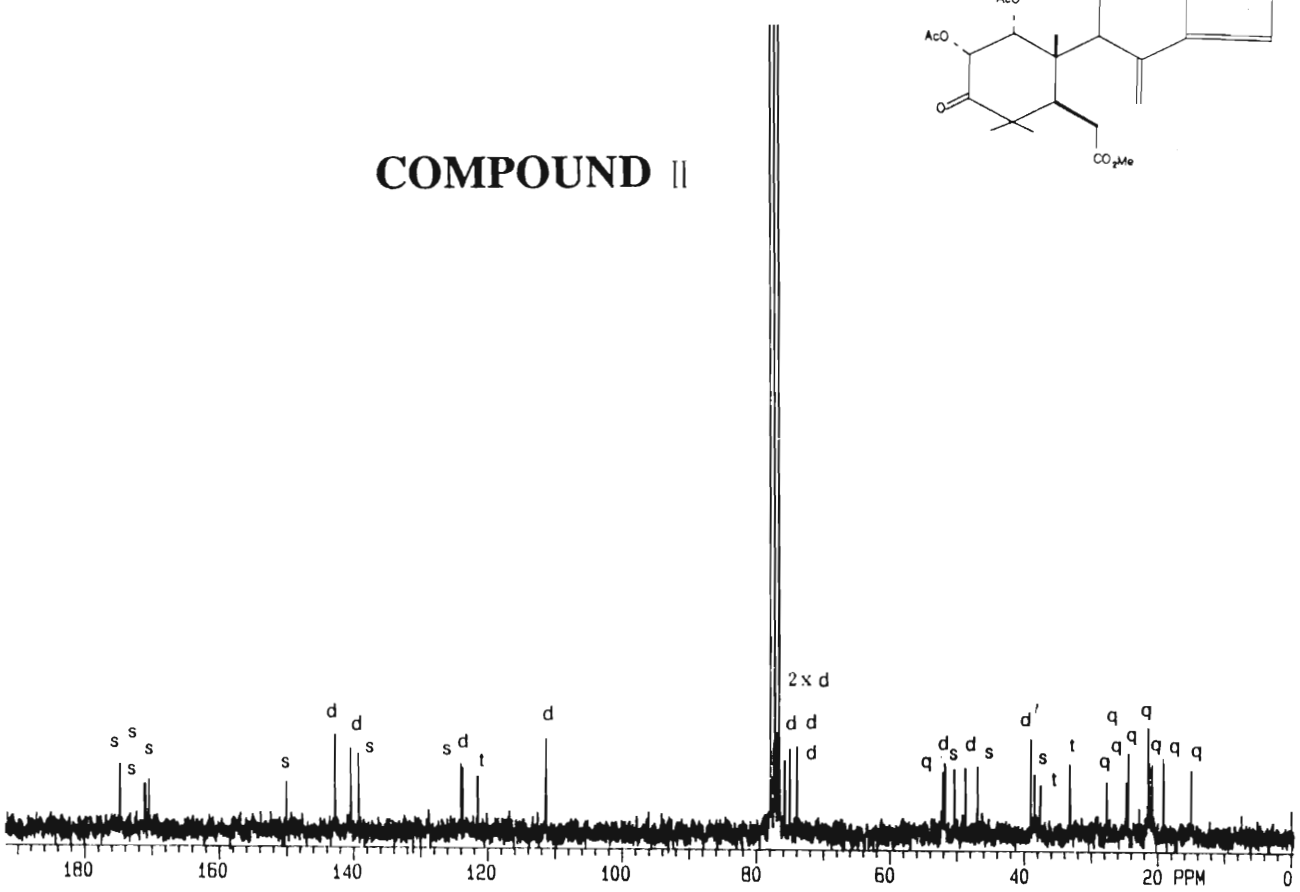

F1 (PPM)
7
6
5
4
3
2
1
F2 (PPM)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160

COMPOUND II

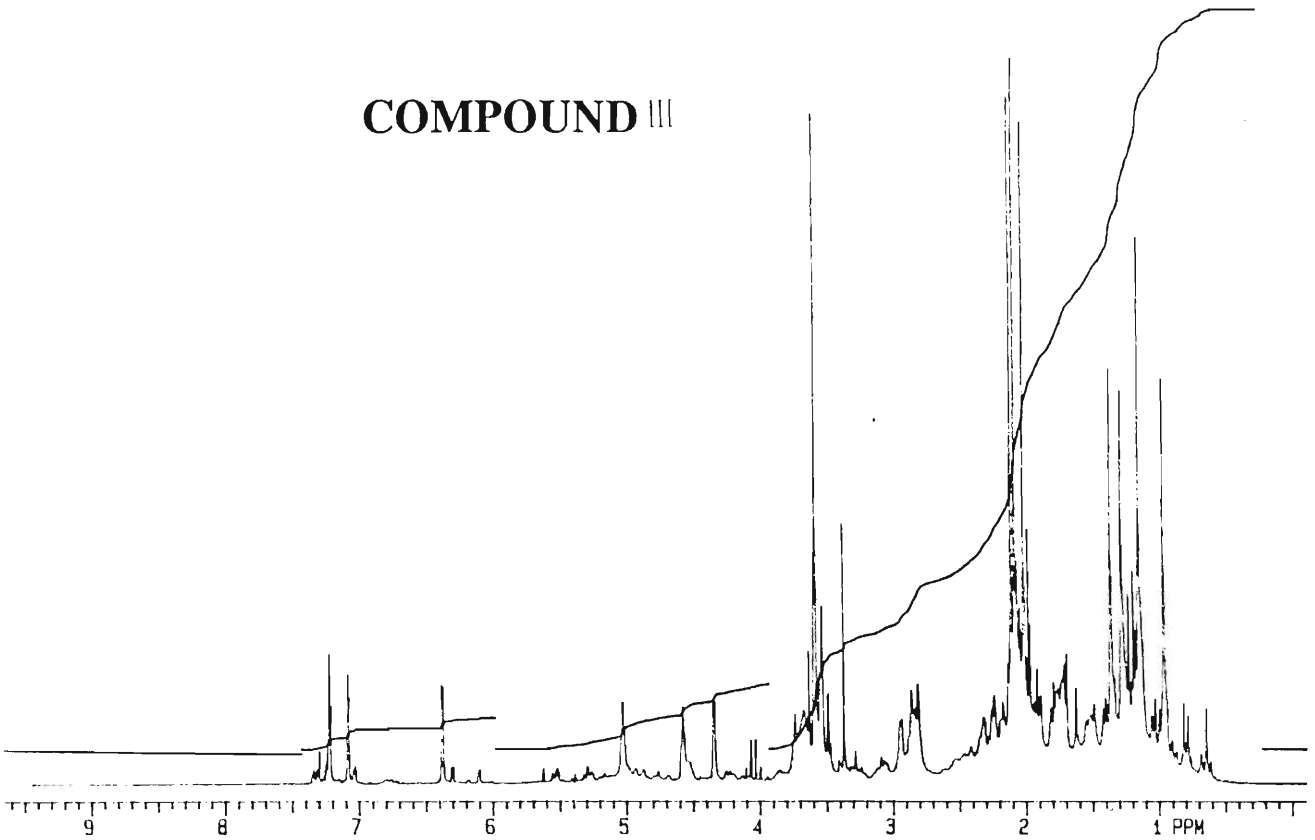


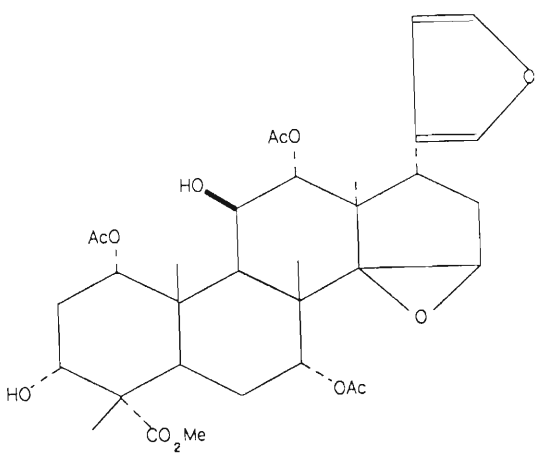


COMPOUND II

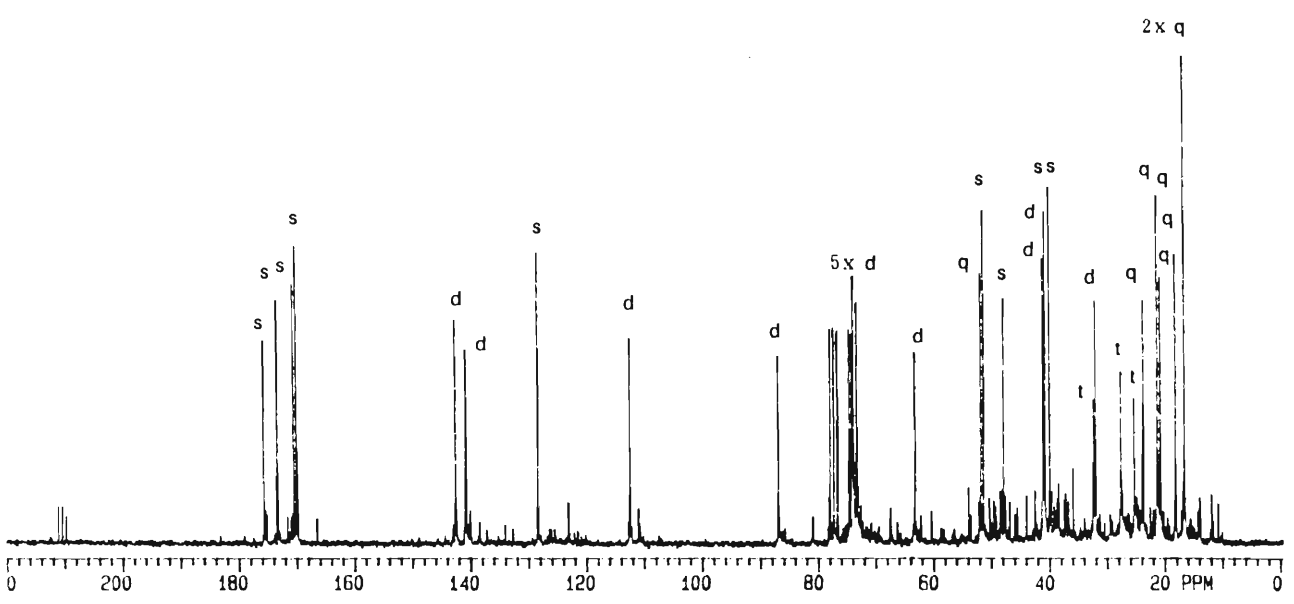


COMPOUND III

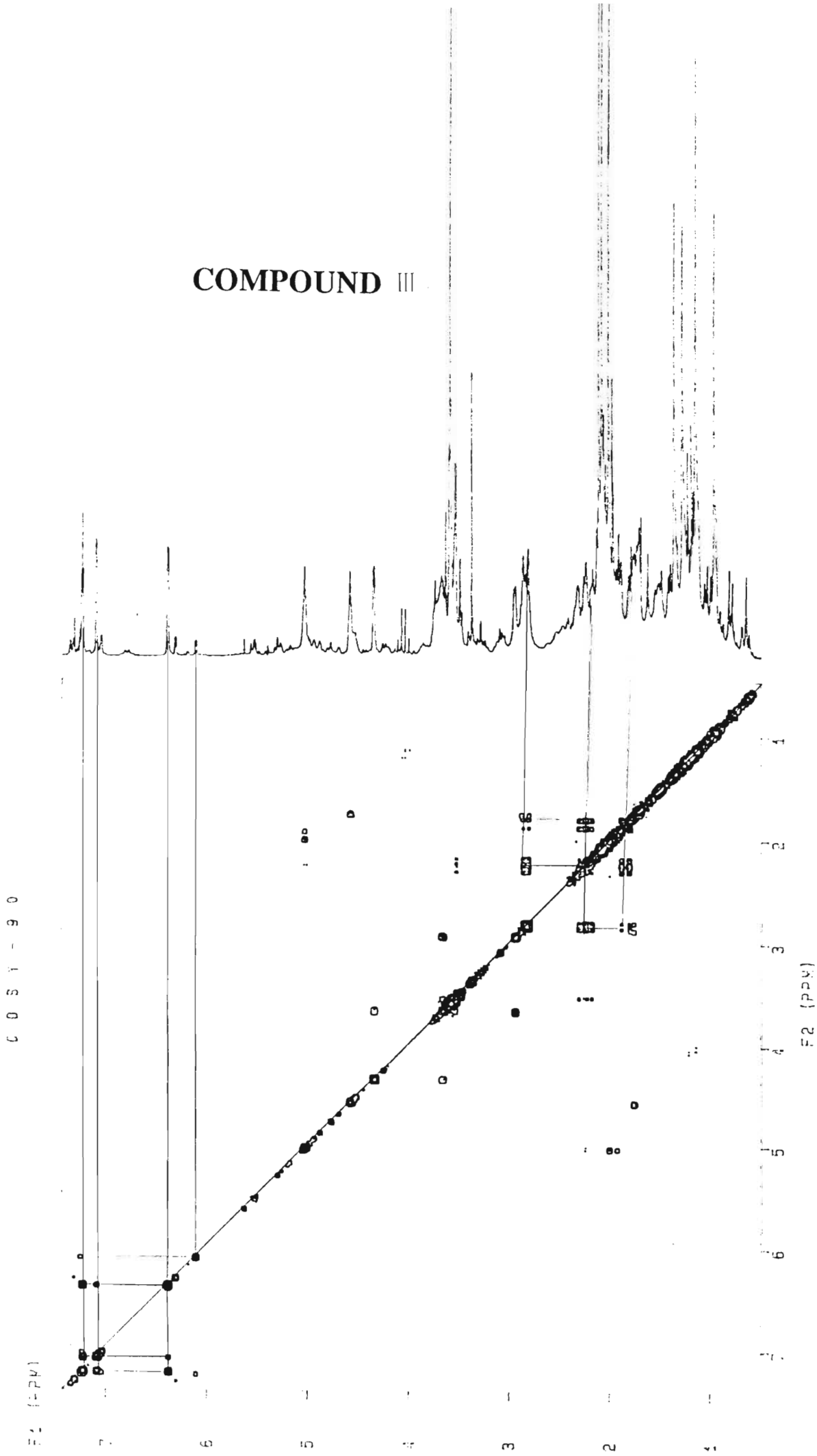




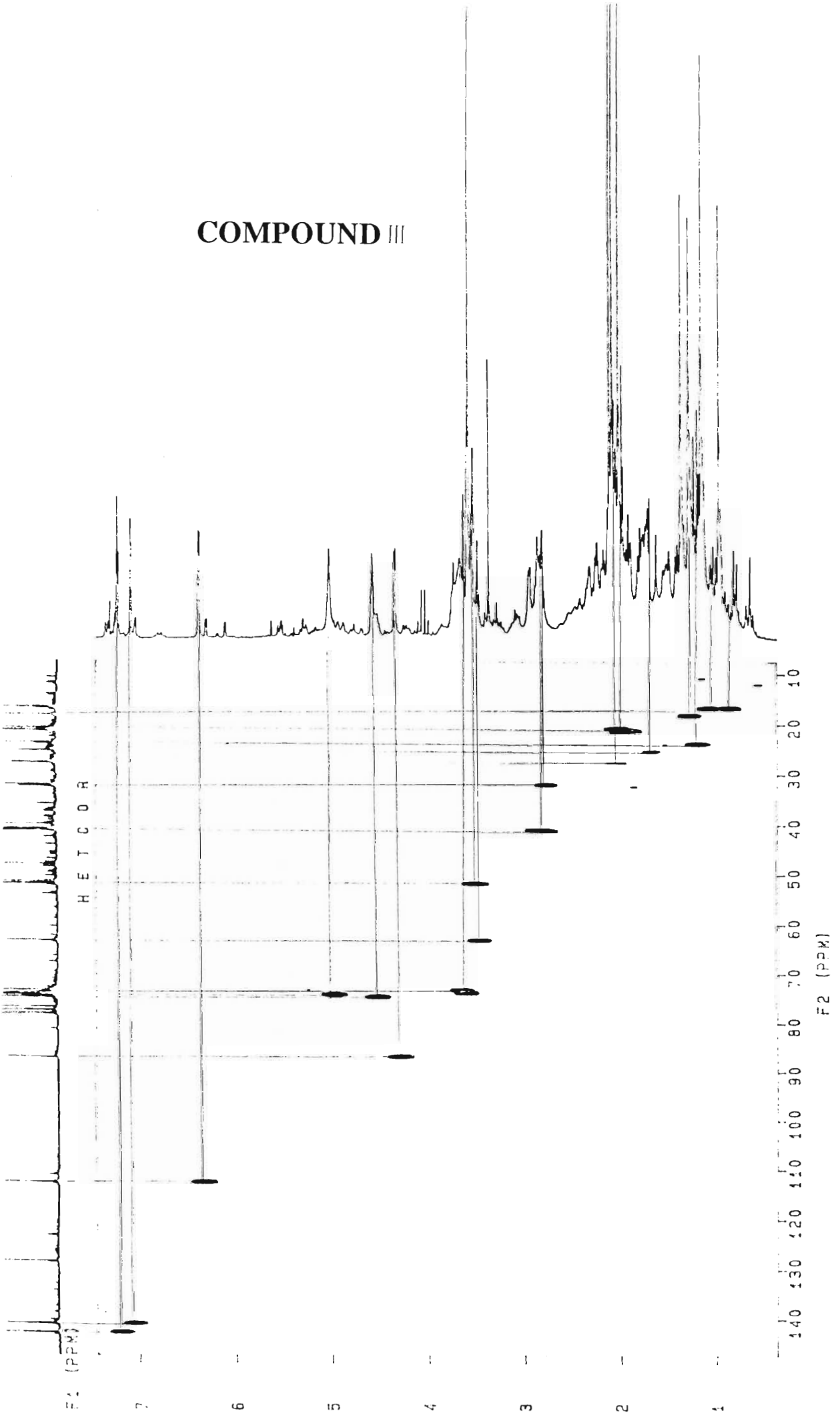
COMPOUND III



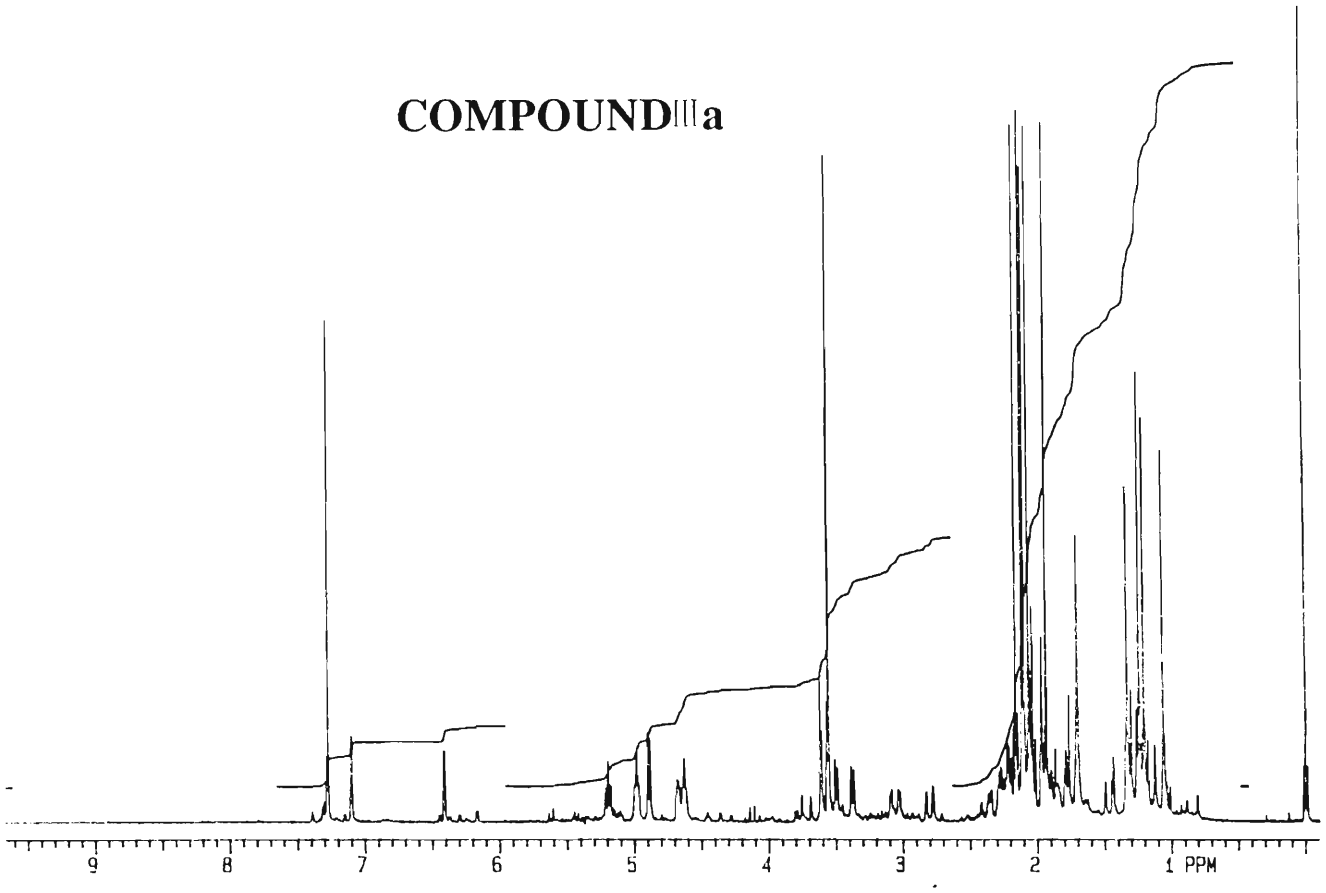
COMPOUND III

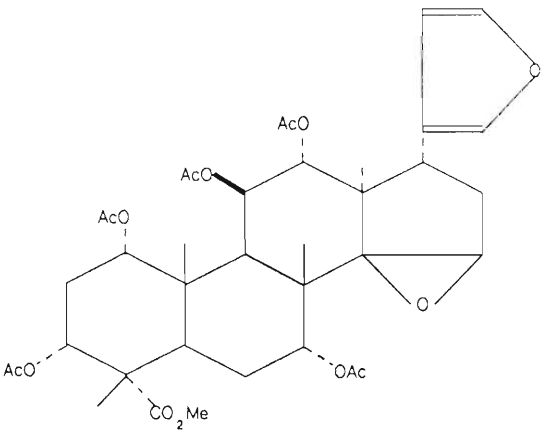


COMPOUND III

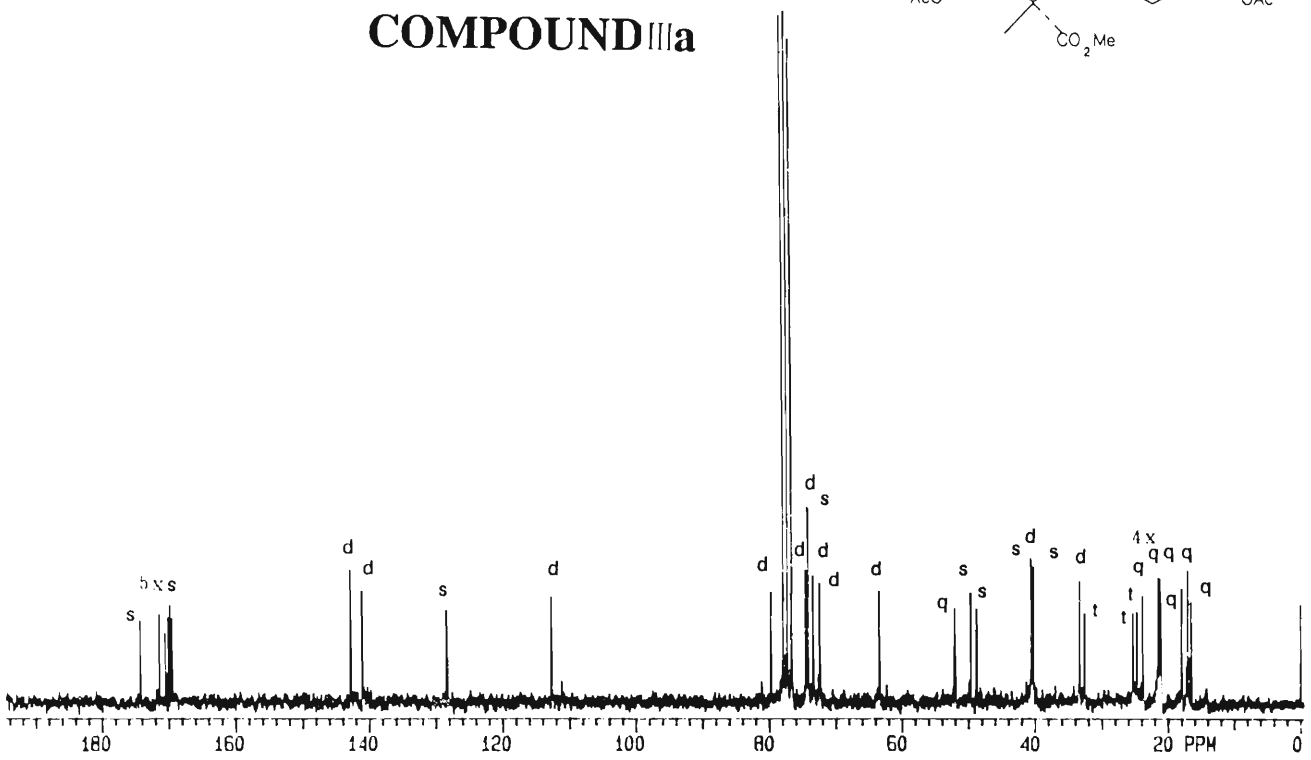


COMPOUND IIIa



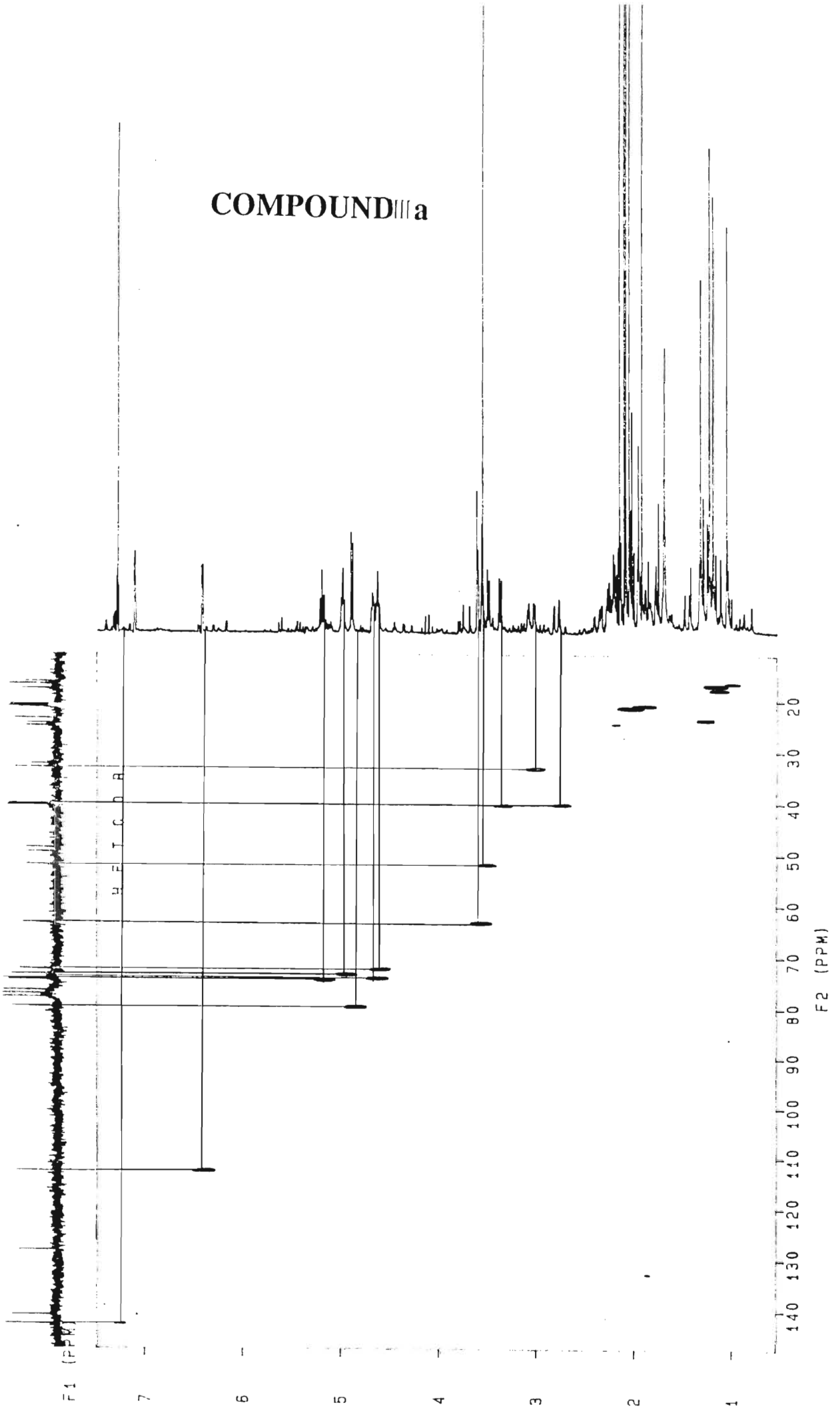


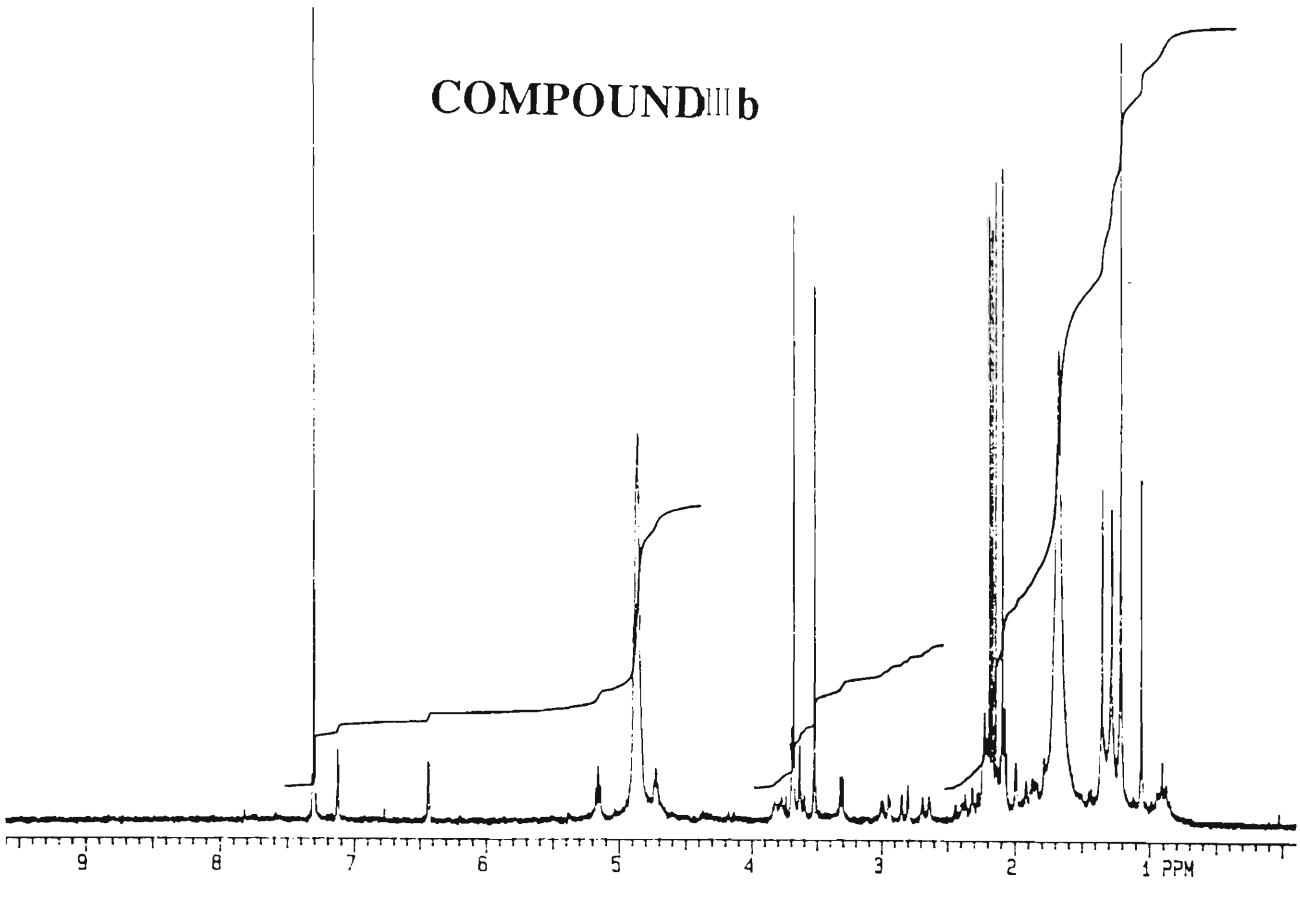
COMPOUND IIIa

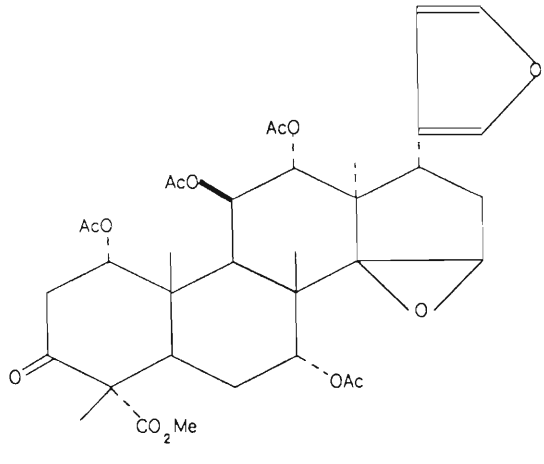


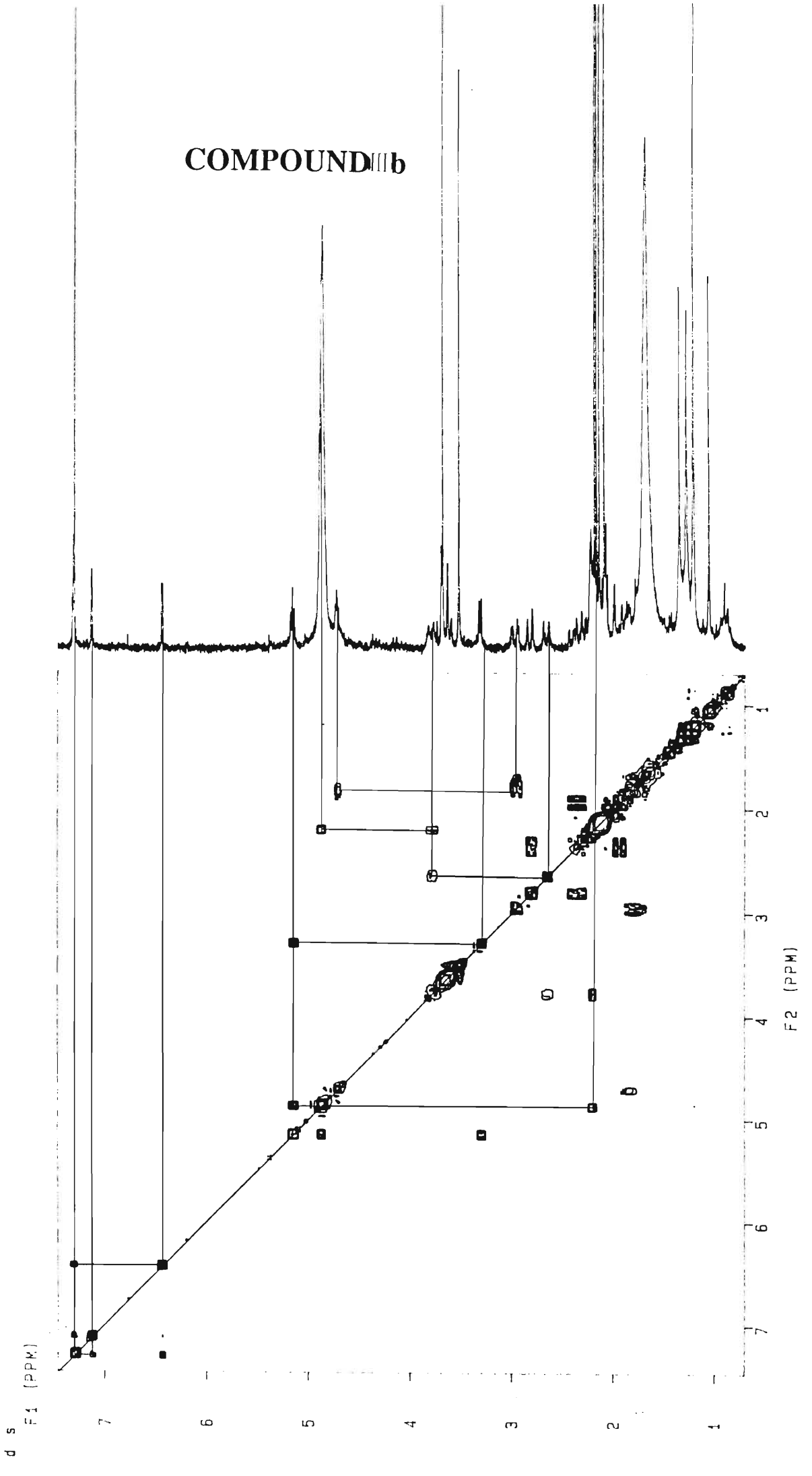
COMPOUND IIIa
COSY-90
F1 (PPM)
F2 (PPM)
1
2
3
4
5
6
7

COMPOUND III a

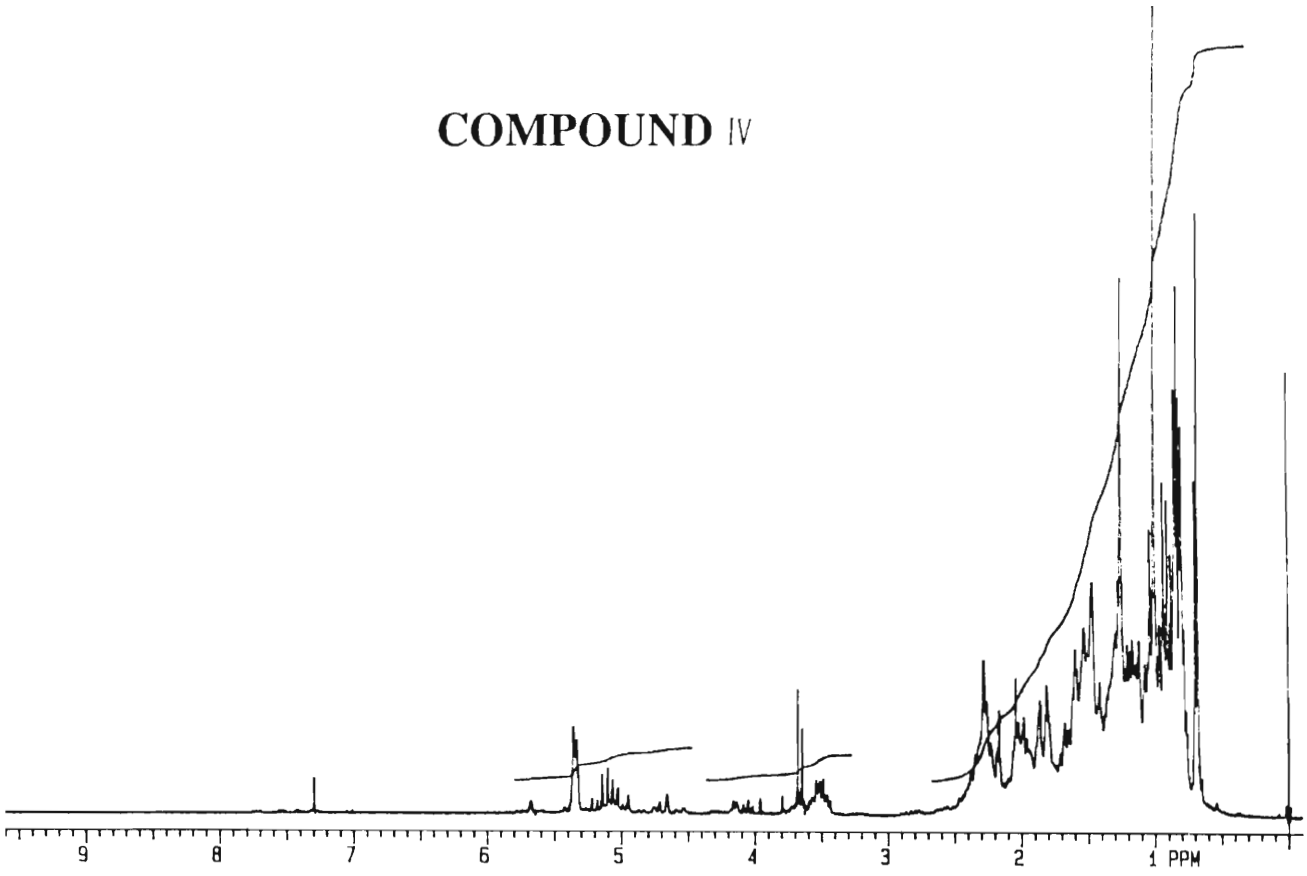


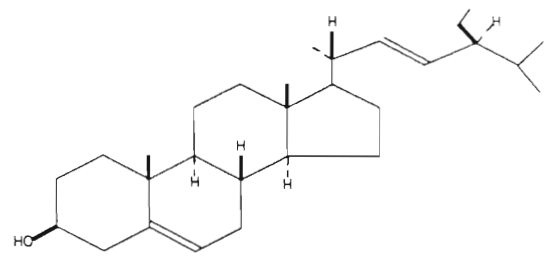

COMPOUND III b
9
8
7
6
5
4
3
2
1 PPM


AcO
AcO
AcO
O
O
OAc
CO2Me

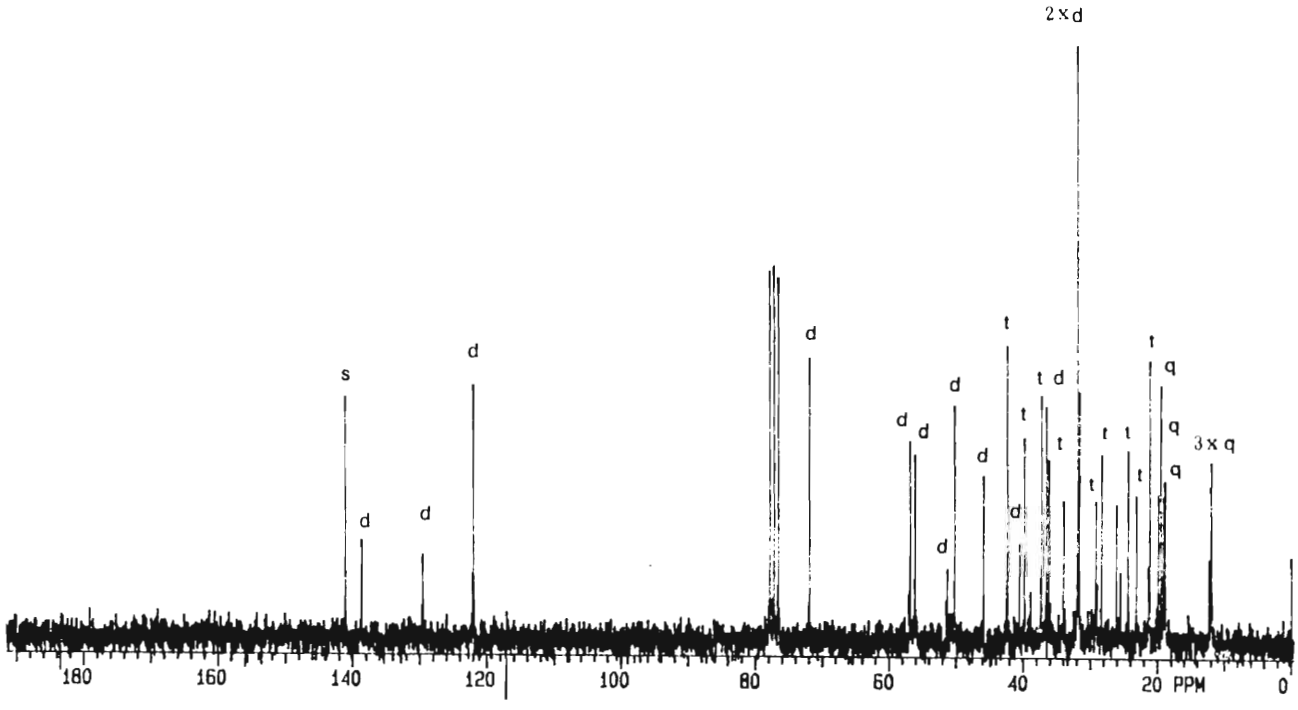

COMPOUND IIIb
COSY-90
F1 (PPM)
F2 (PPM)
d s

COMPOUND IV



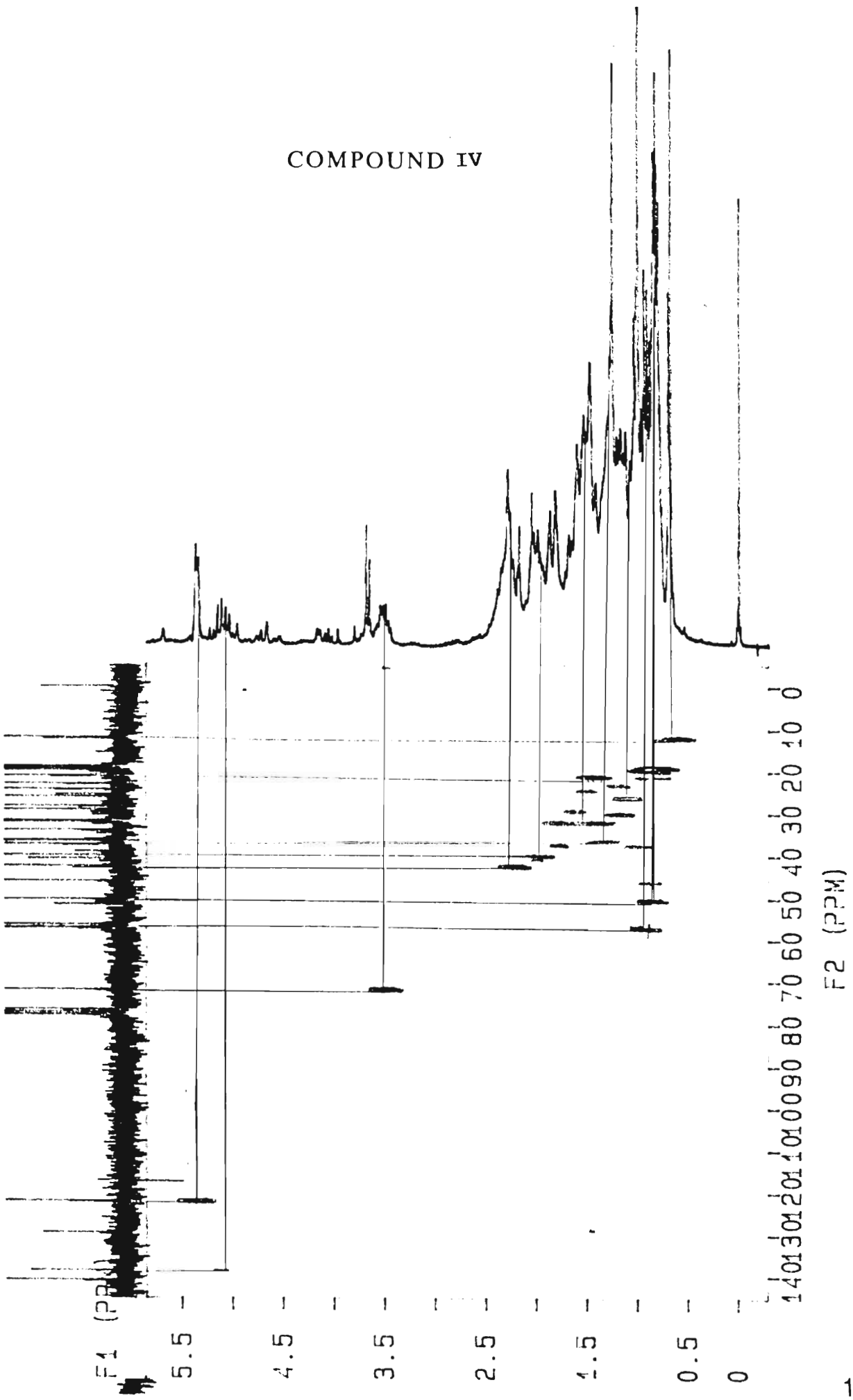


COMPOUND IV

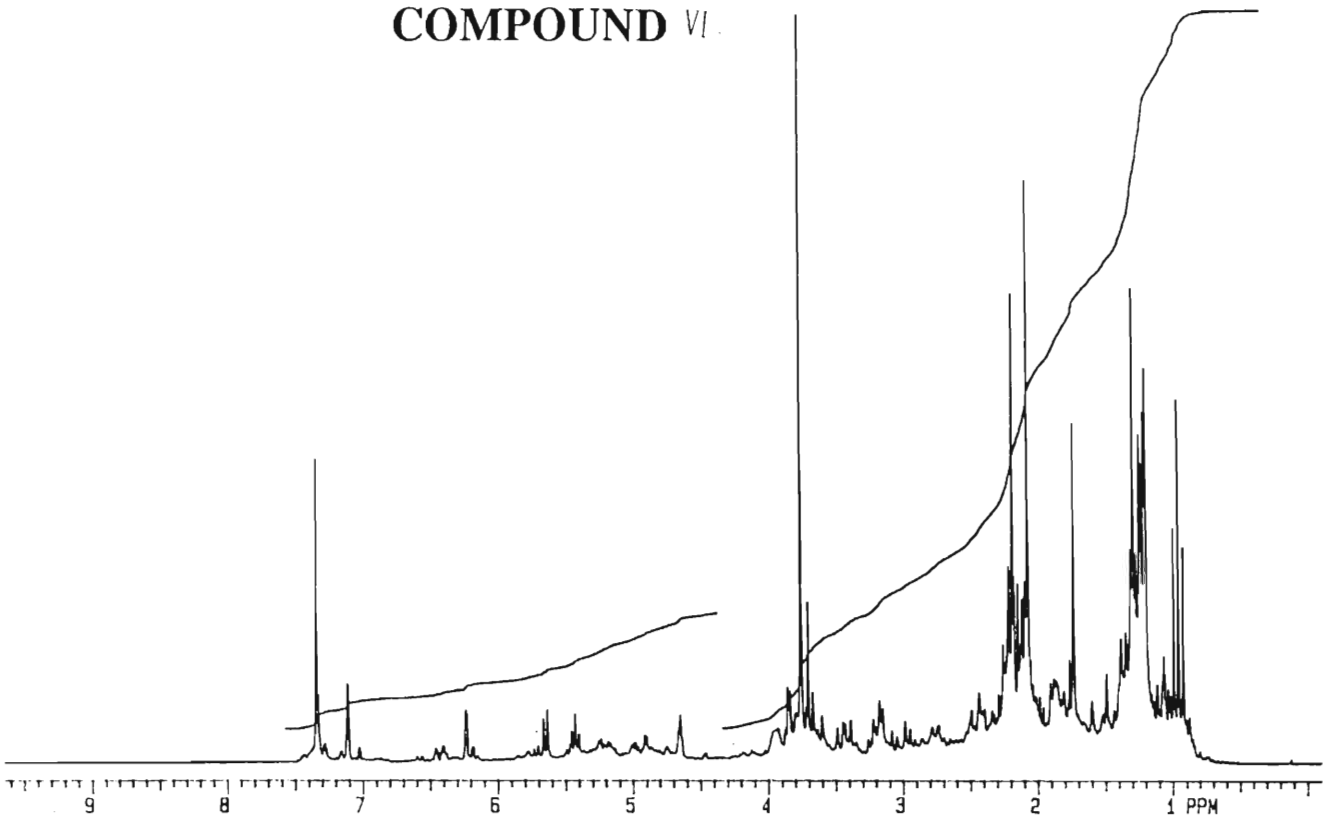


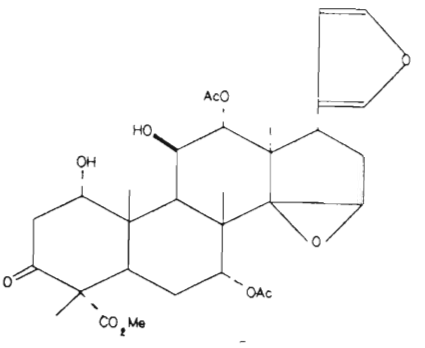
F1 (PPM)
5.5
4.5
3.5
2.5
1.5
0.5
0
5.55.04.54.03.53.02.52.01.51.00.5 0
F2 (PPM)
COMPOUND IV

COMPOUND IV

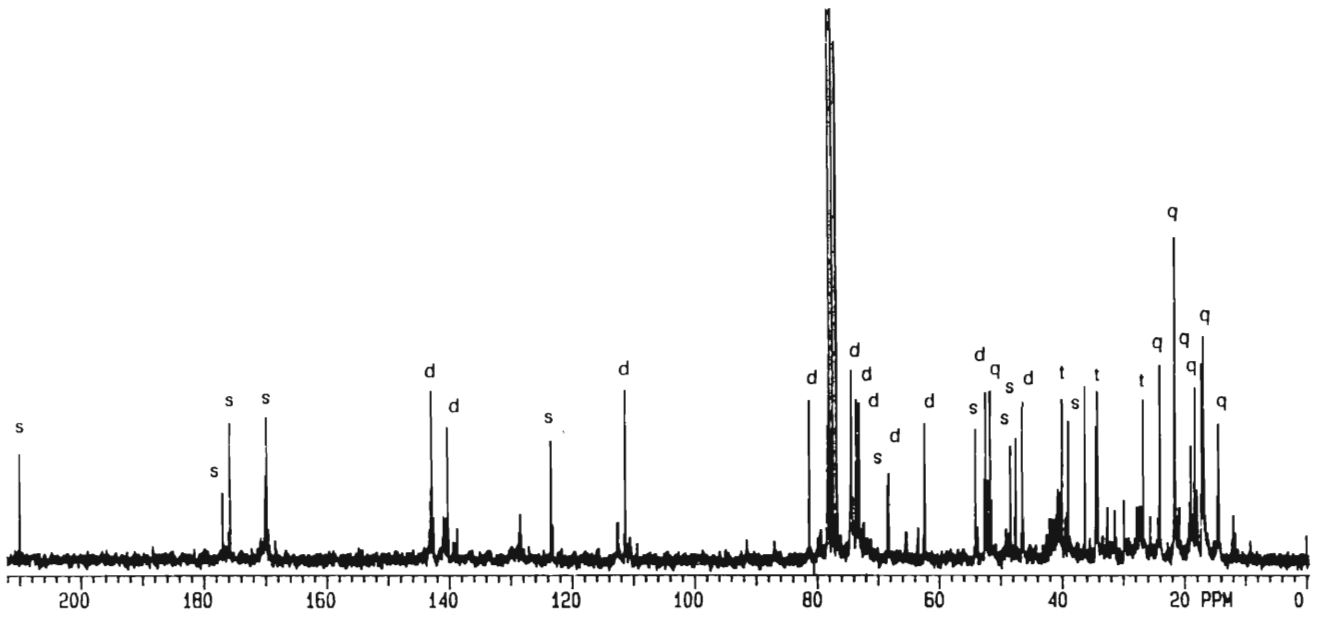

F1 (PPM)
5.5
4.5
3.5
2.5
1.5
0.5
0
F2 (PPM)
140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

COMPOUND VI

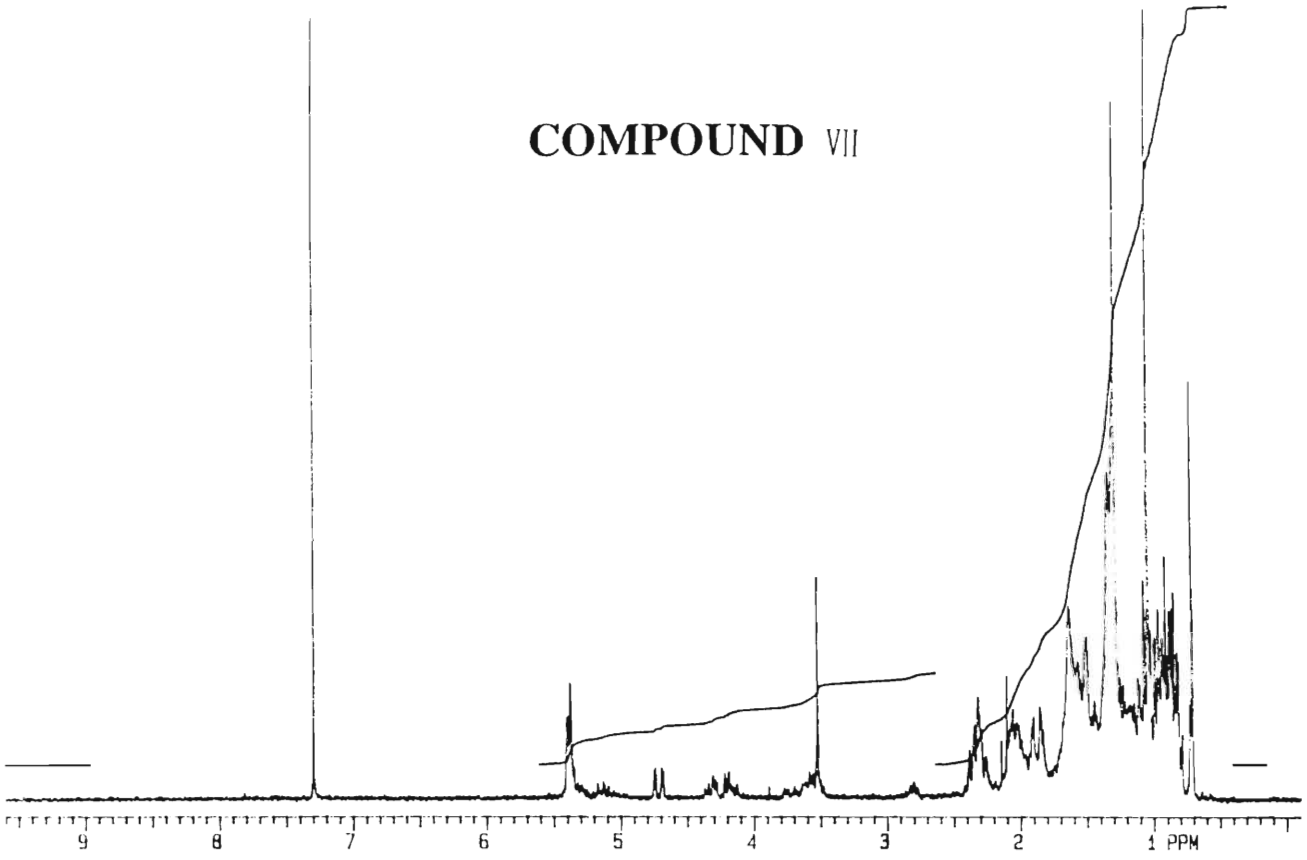


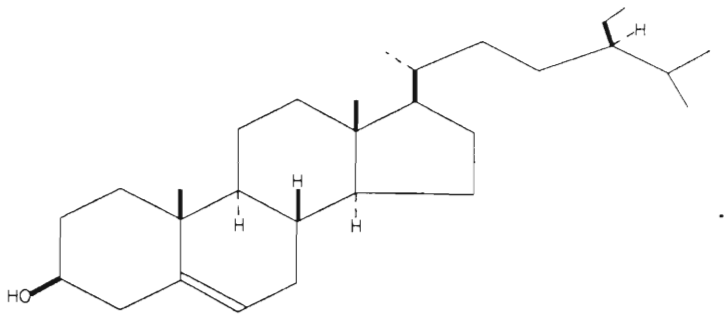


COMPOUND VI

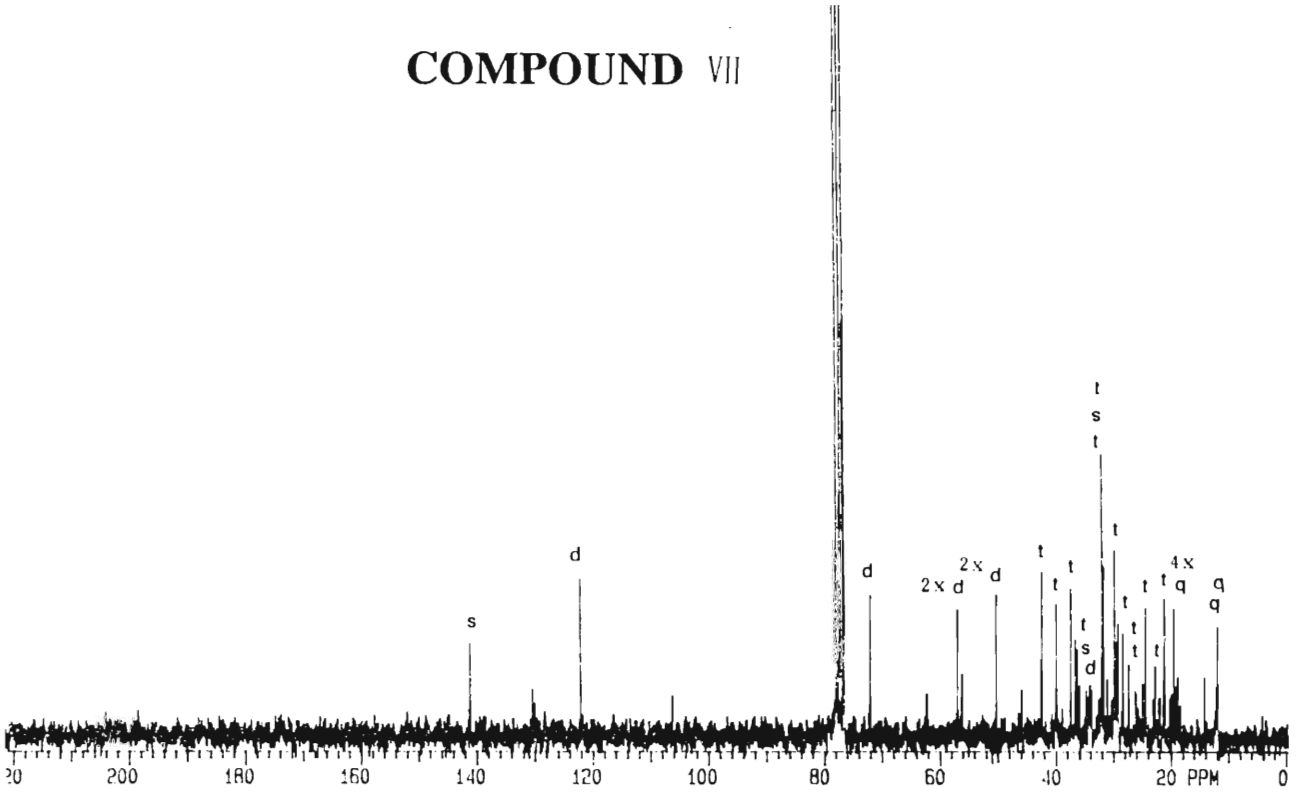


COMPOUND VII

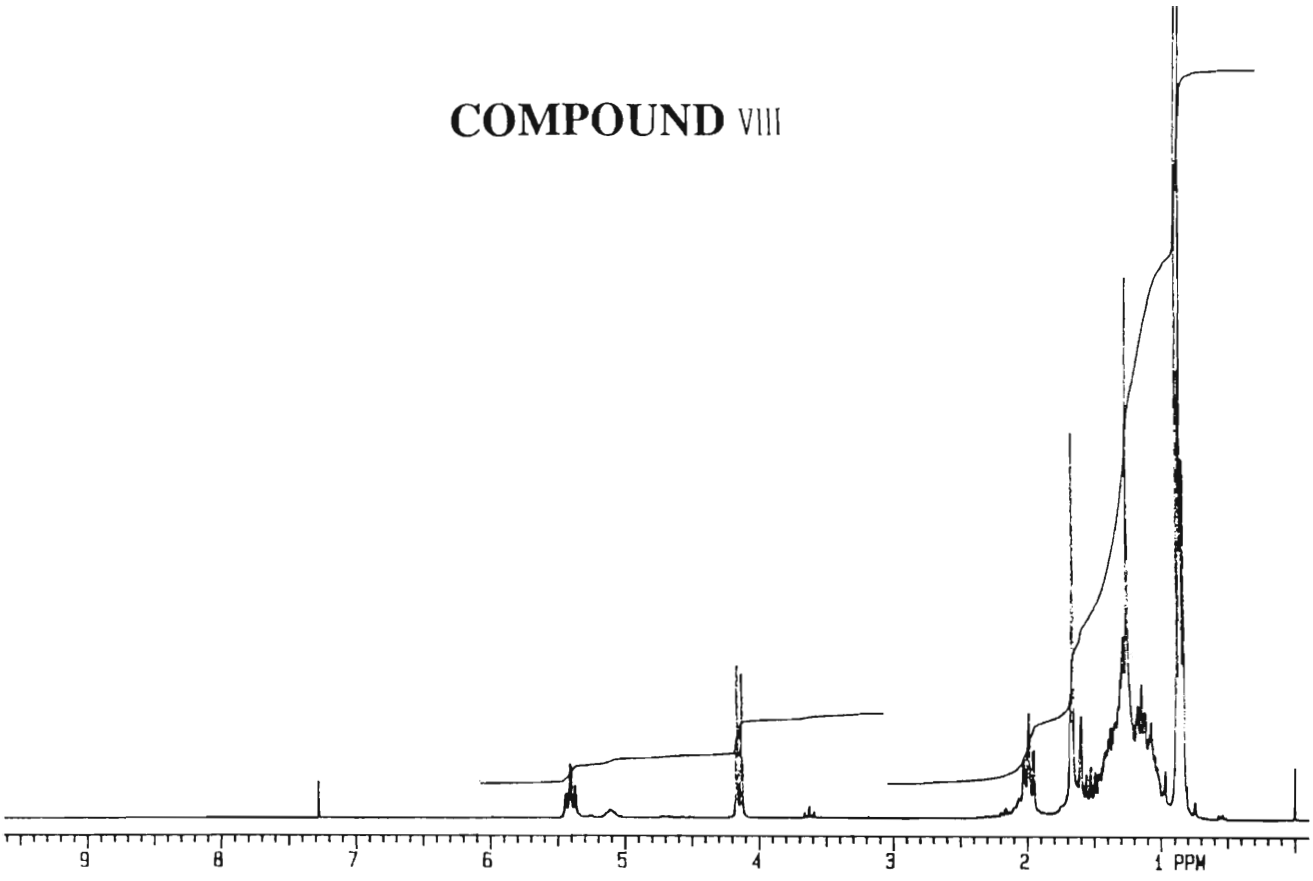




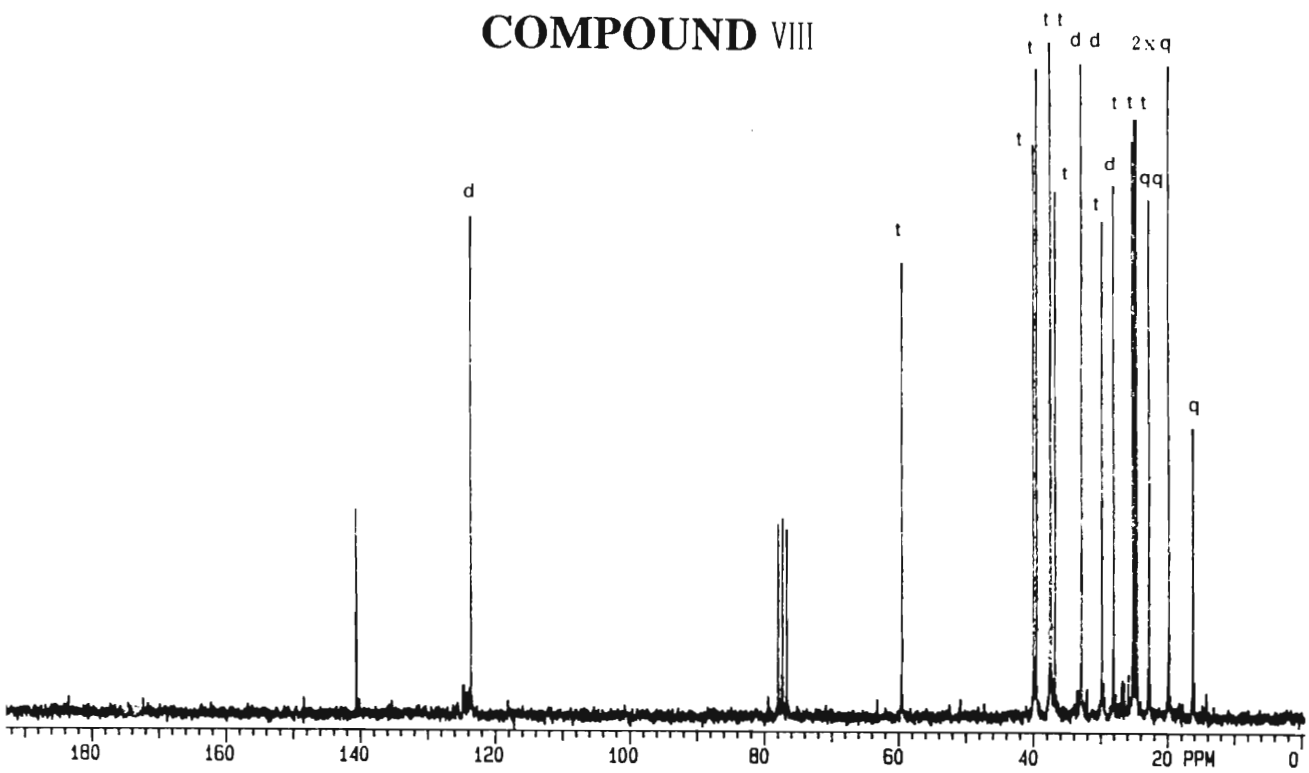
COMPOUND VII



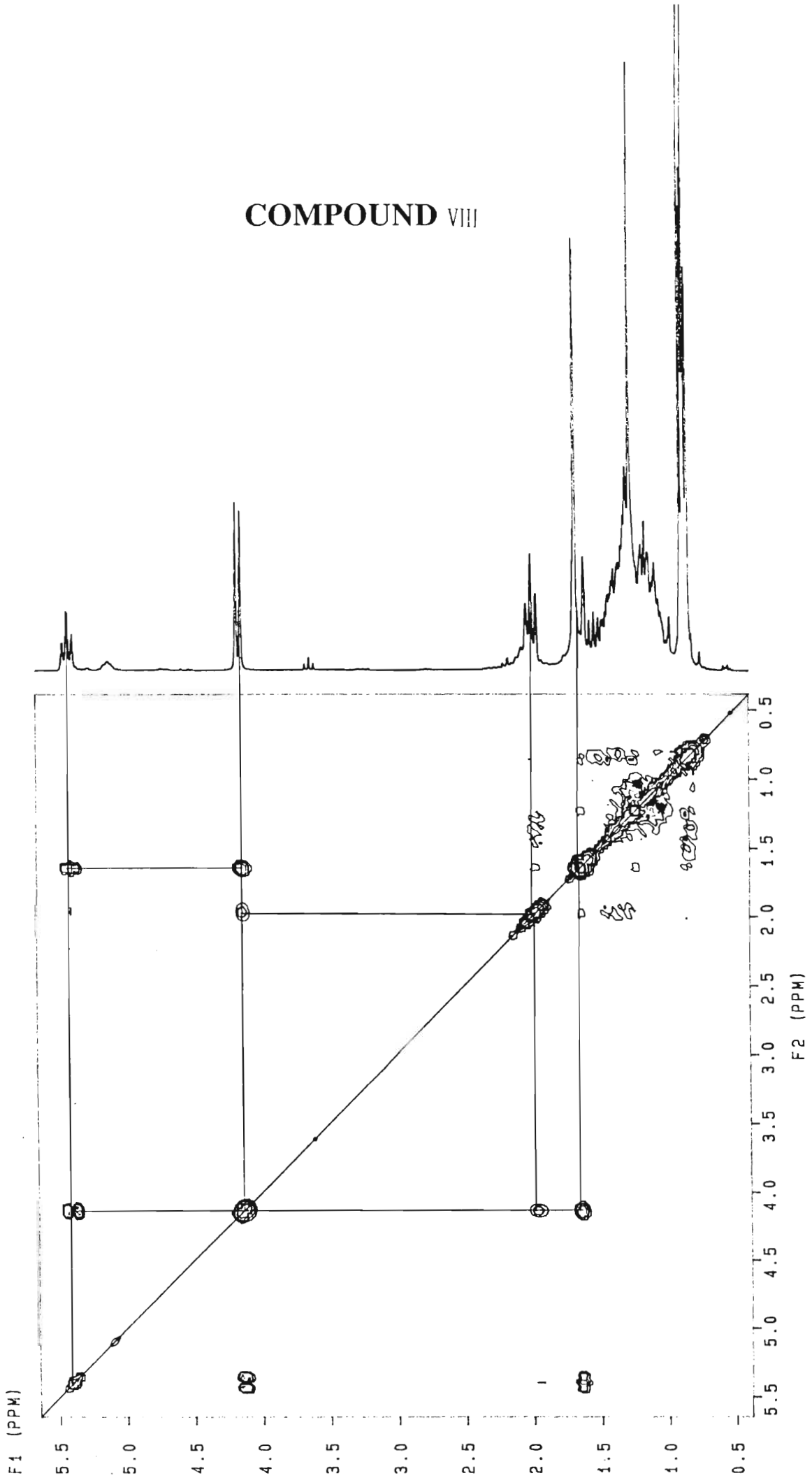
COMPOUND VIII



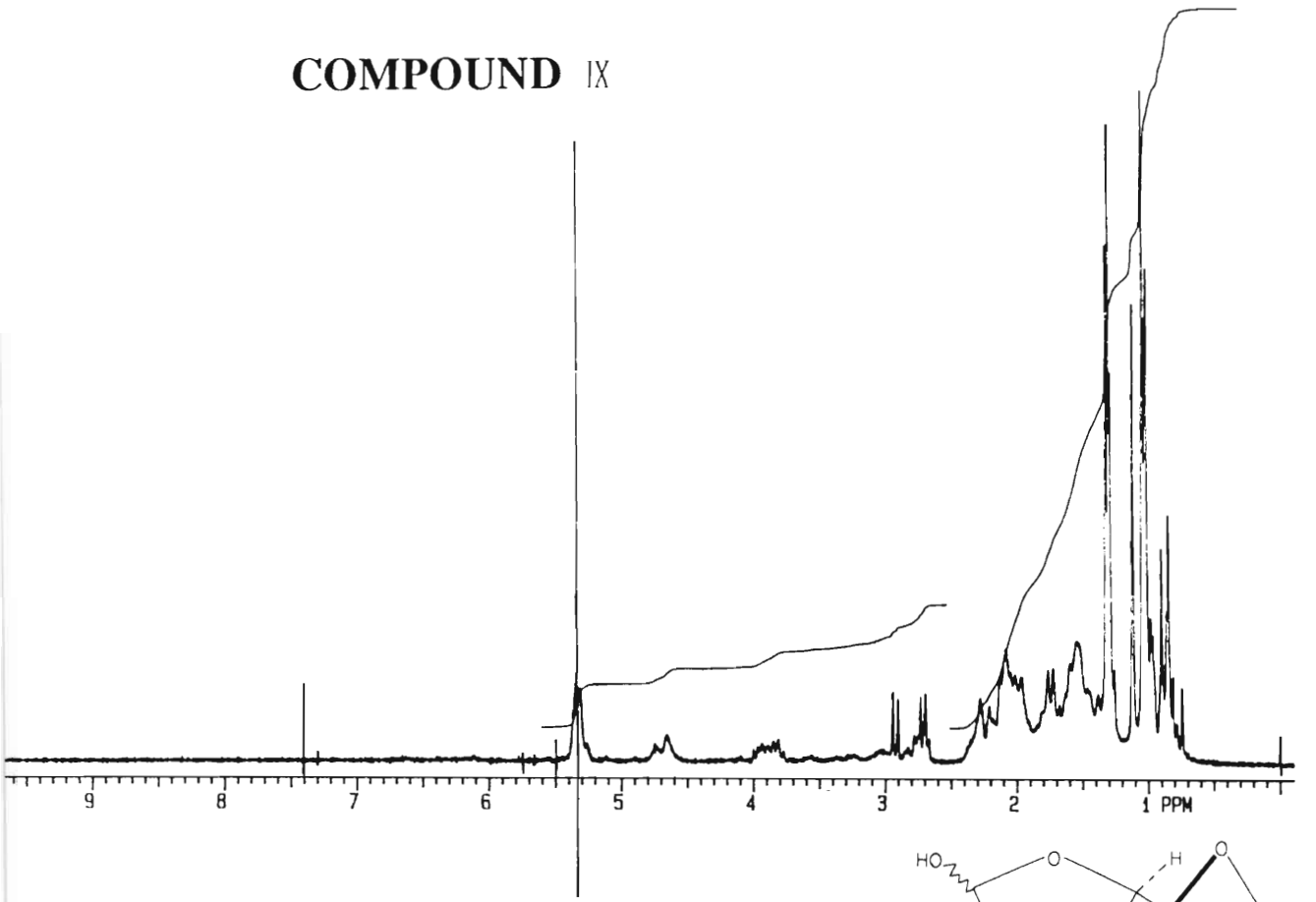
COMPOUND VIII

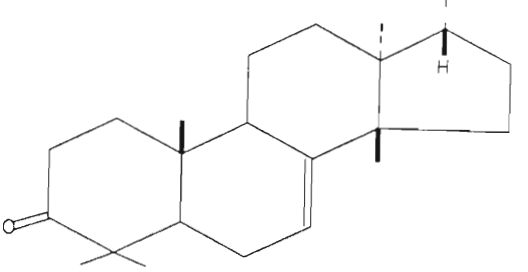


COMPOUND VIII

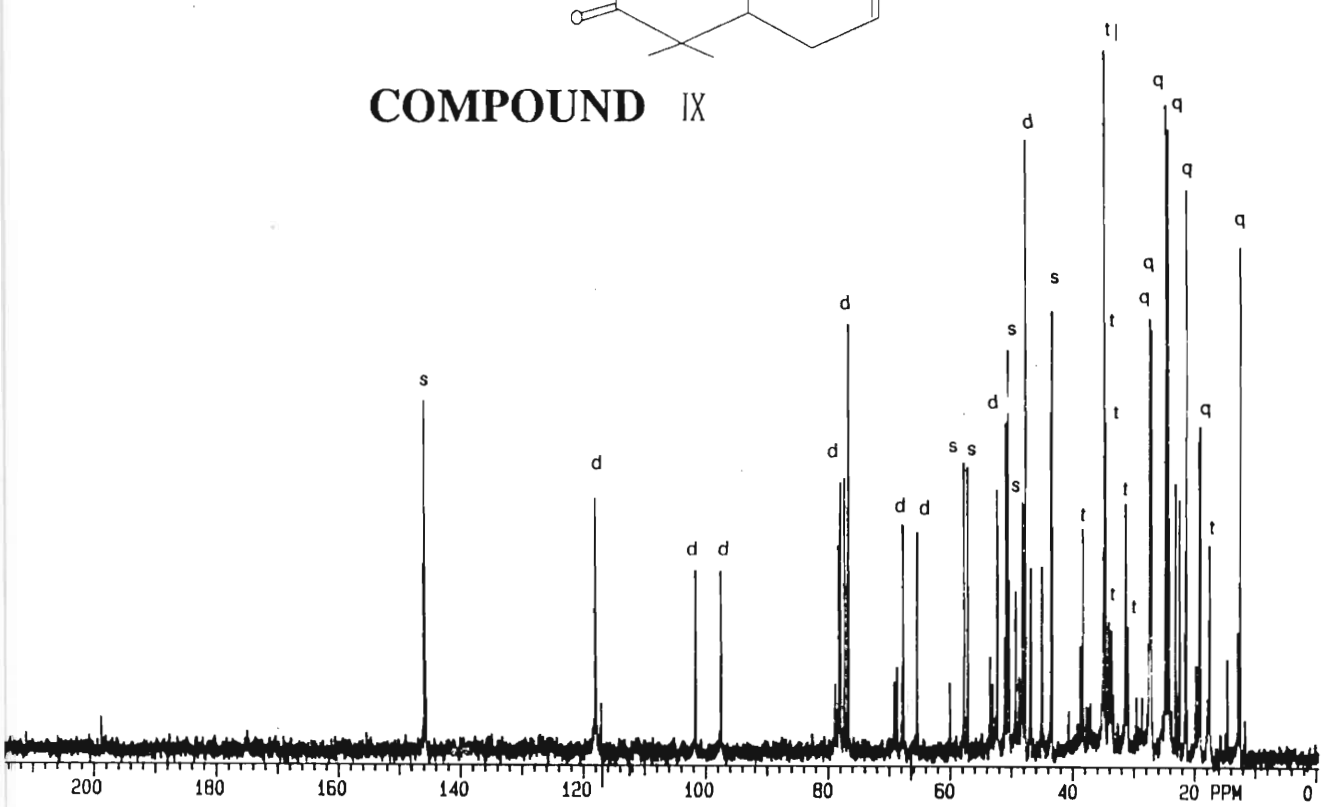


COMPOUND IX

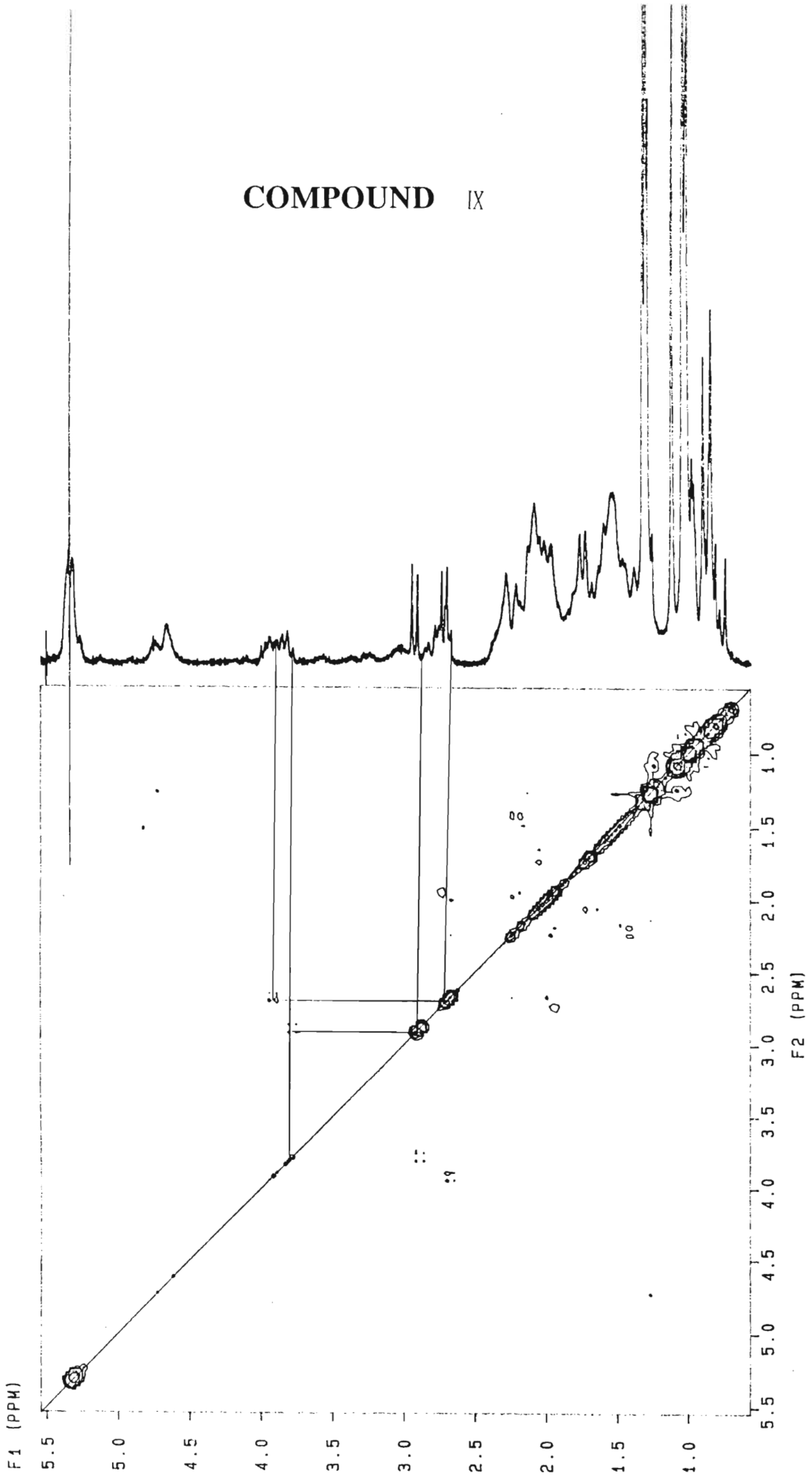




COMPOUND IX

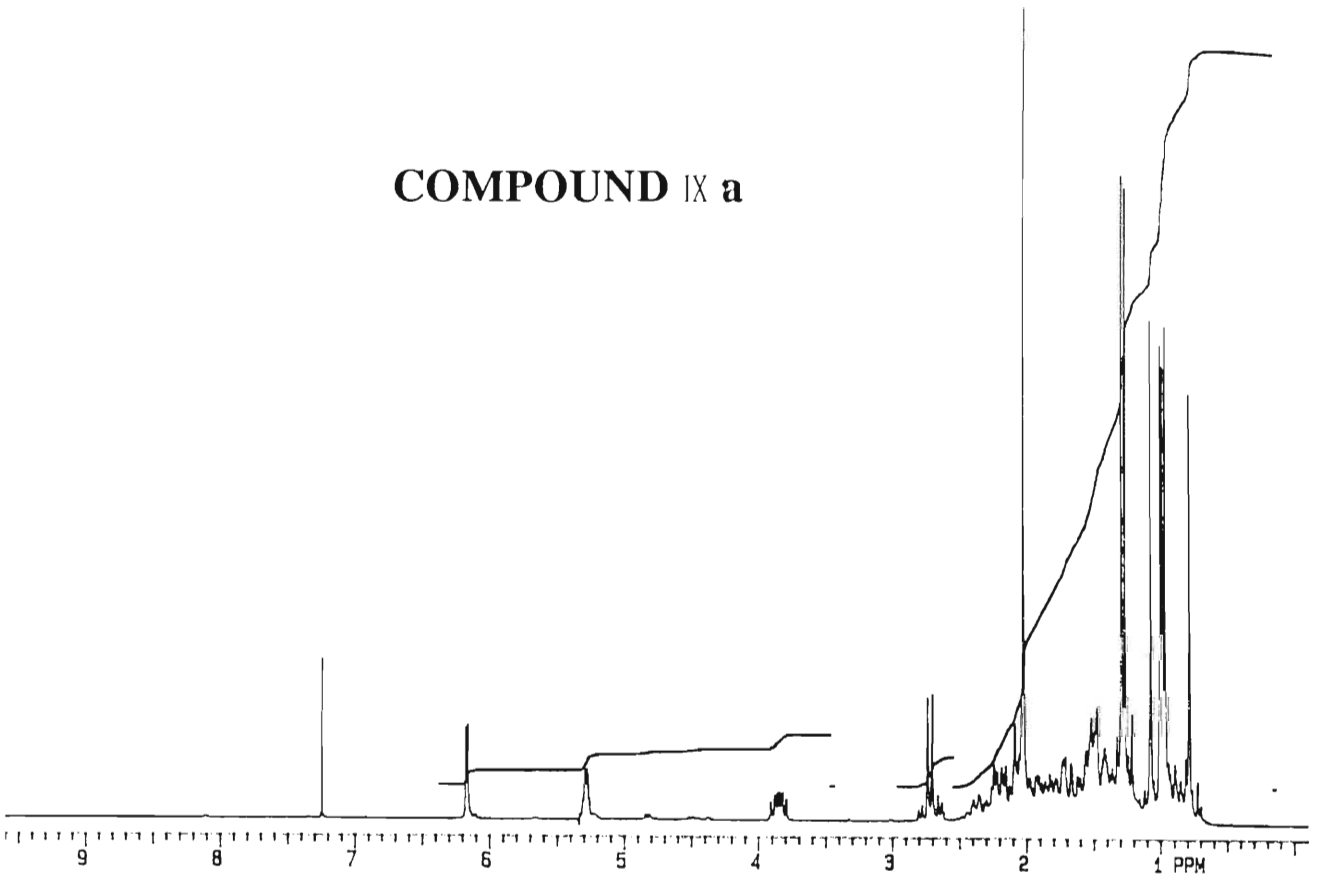


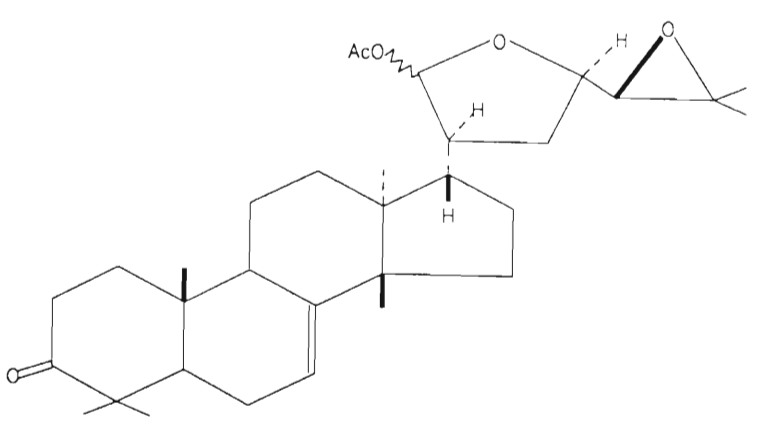
COMPOUND IX


F1 (PPM)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F2 (PPM)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

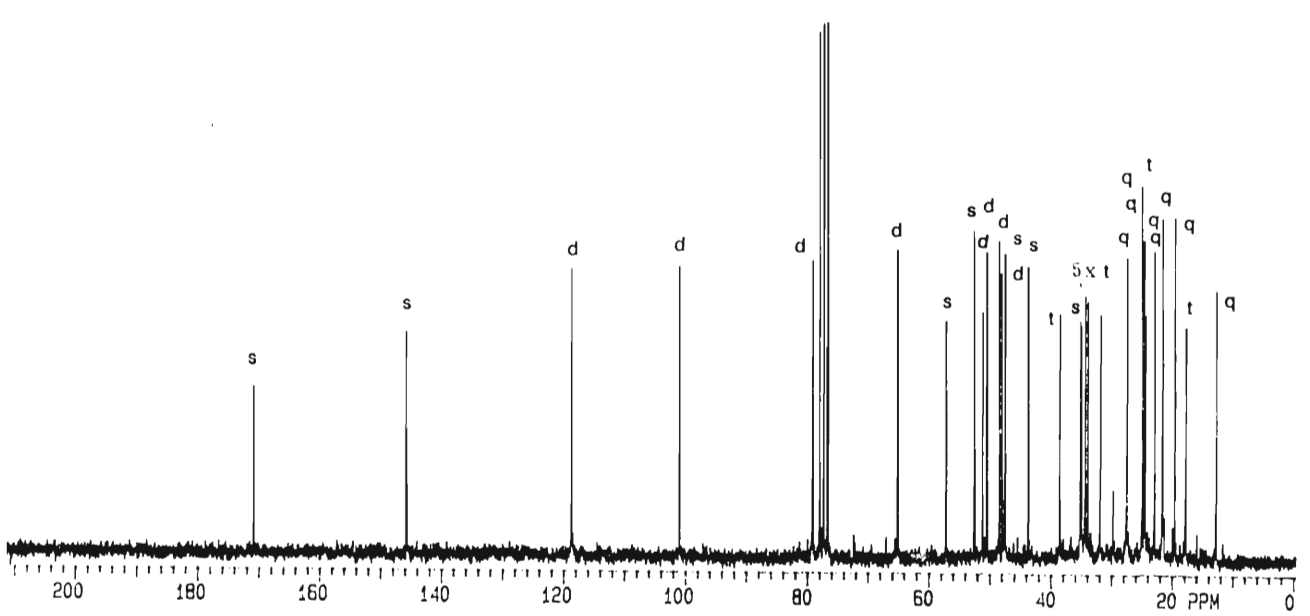
COMPOUND IX
F1 (PPM)
F2 (PPM)

COMPOUND IX a

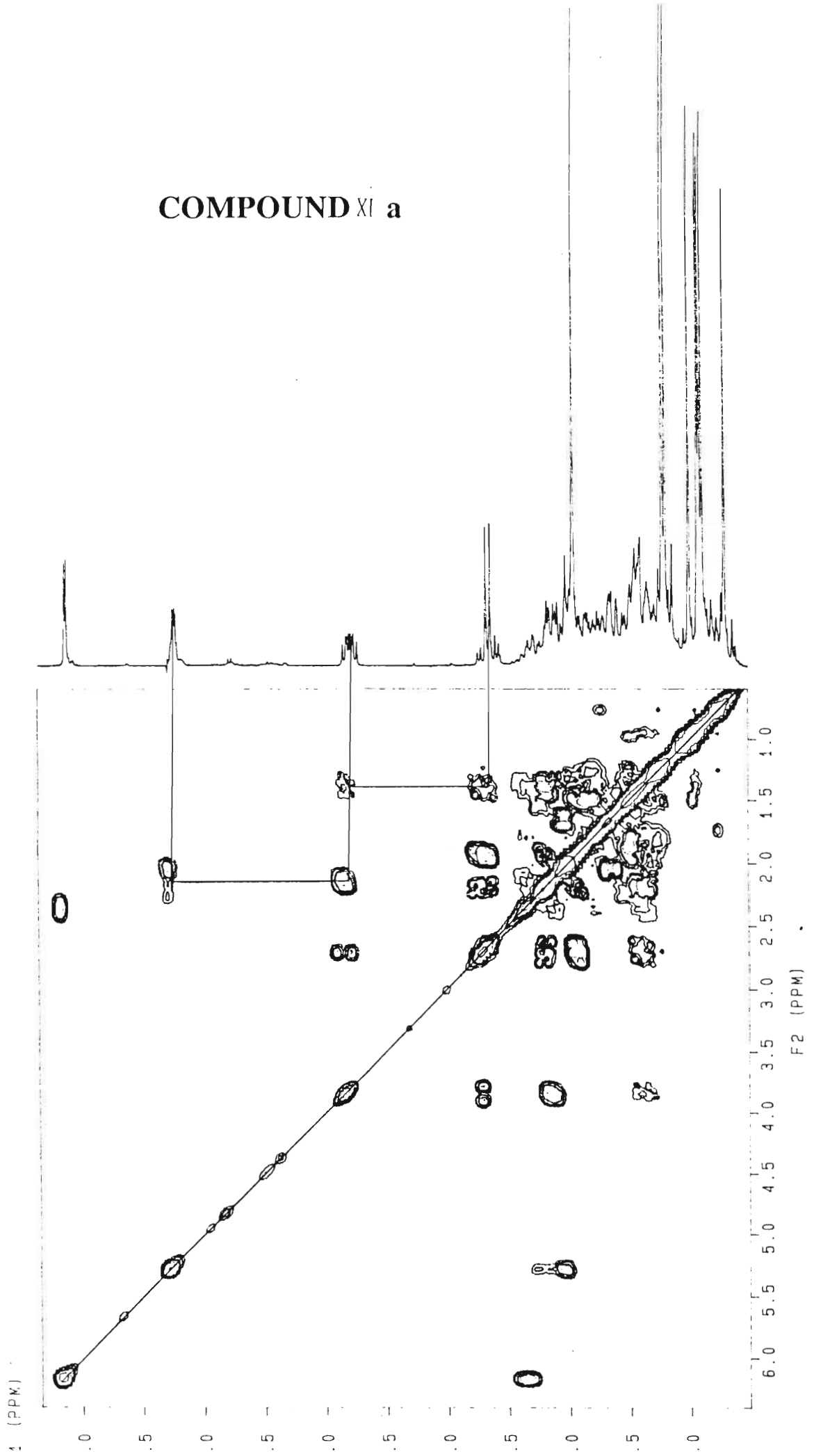




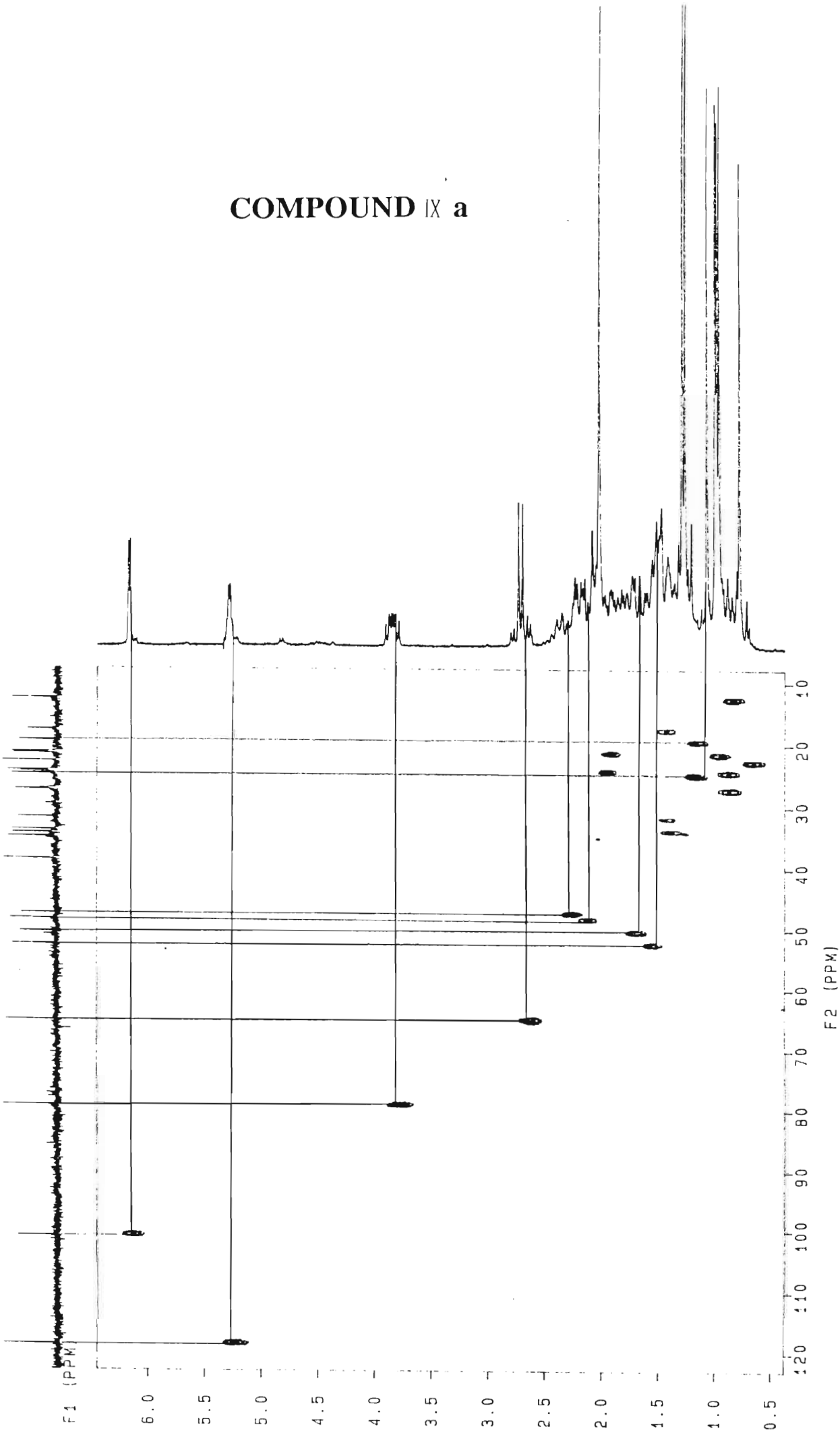
COMPOUND IX a

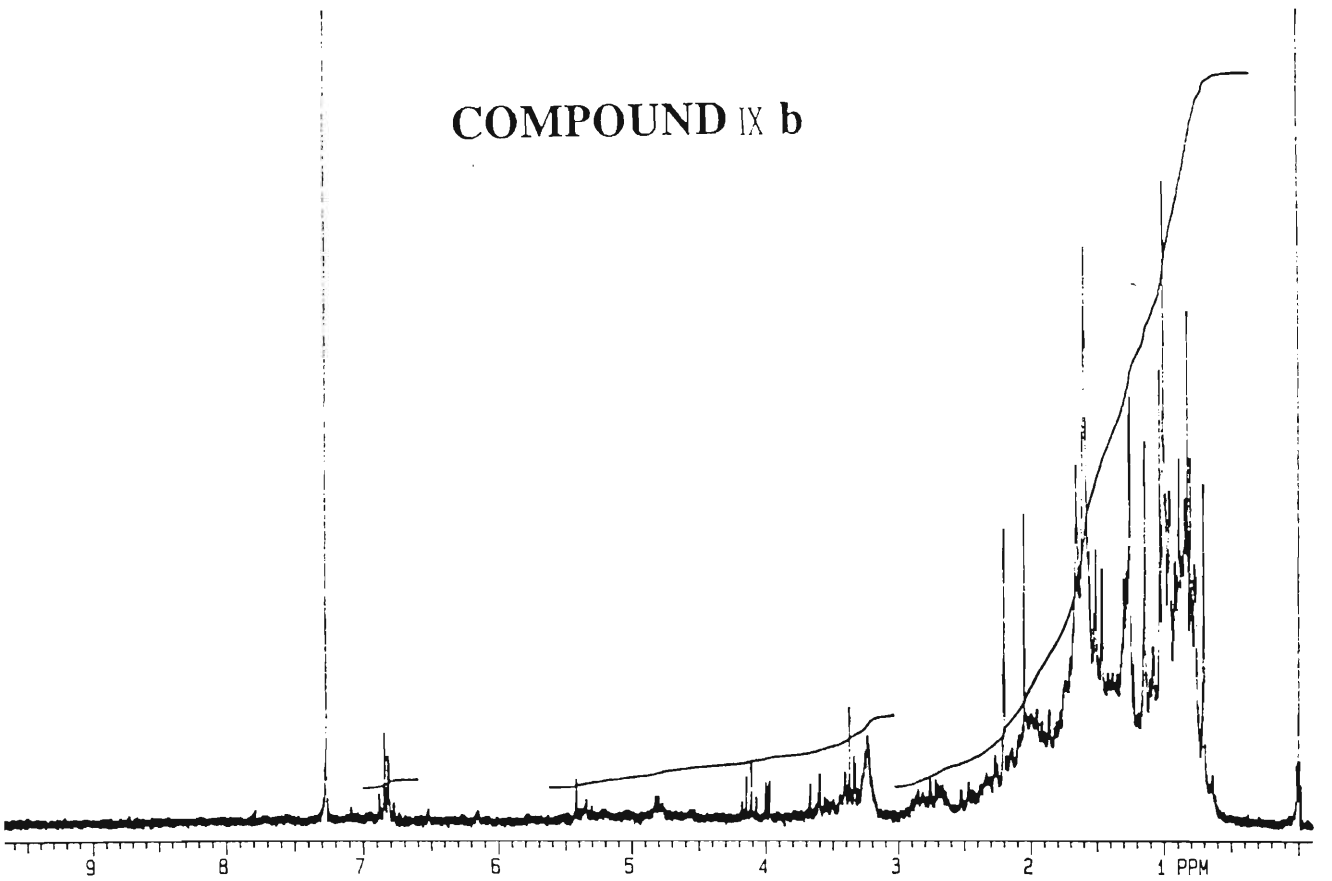


COMPOUND XI a

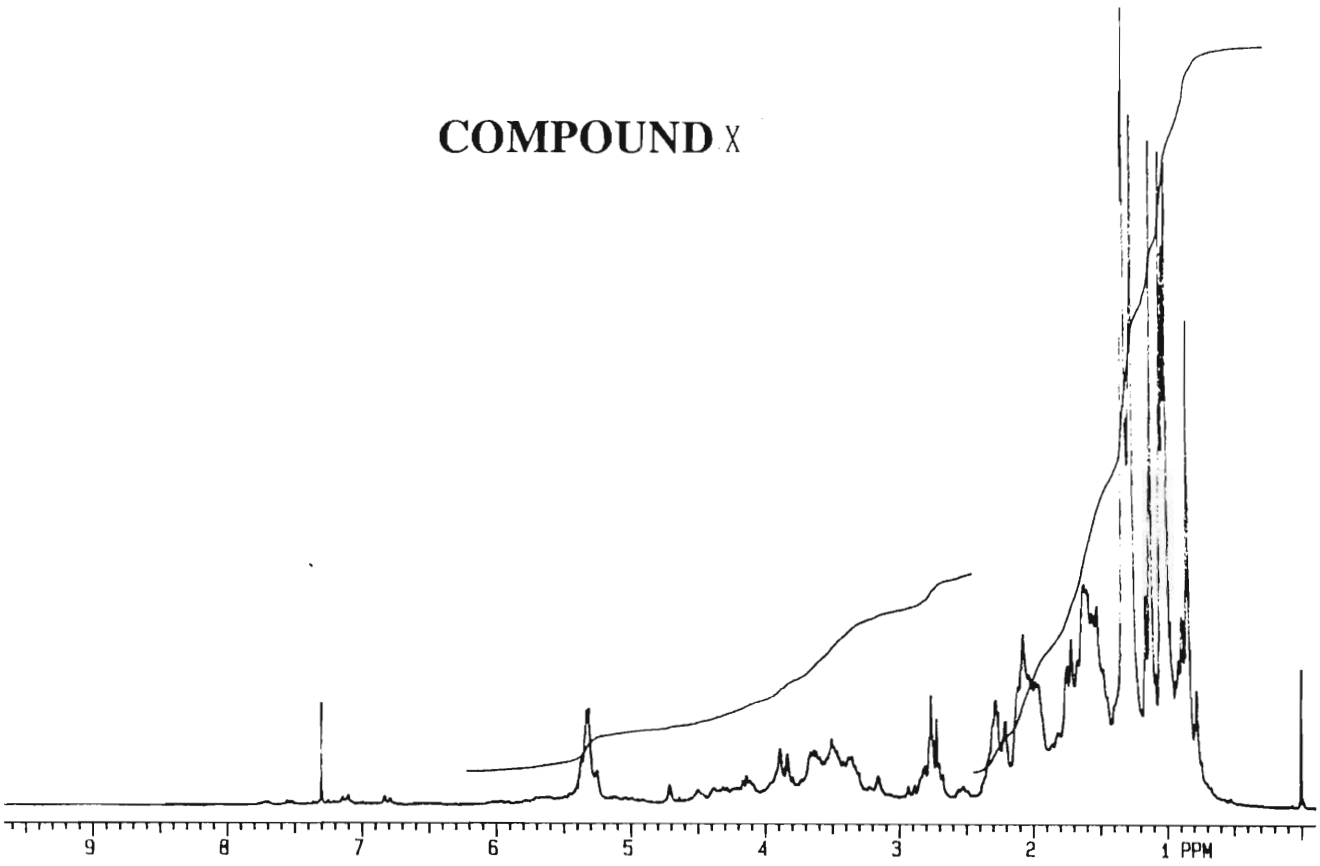


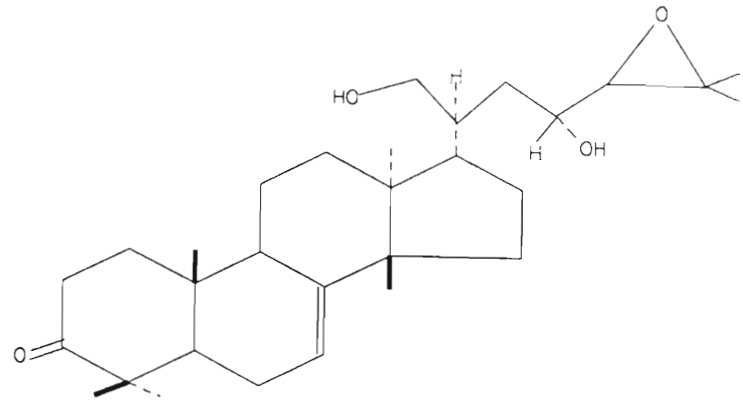
COMPOUND IX a



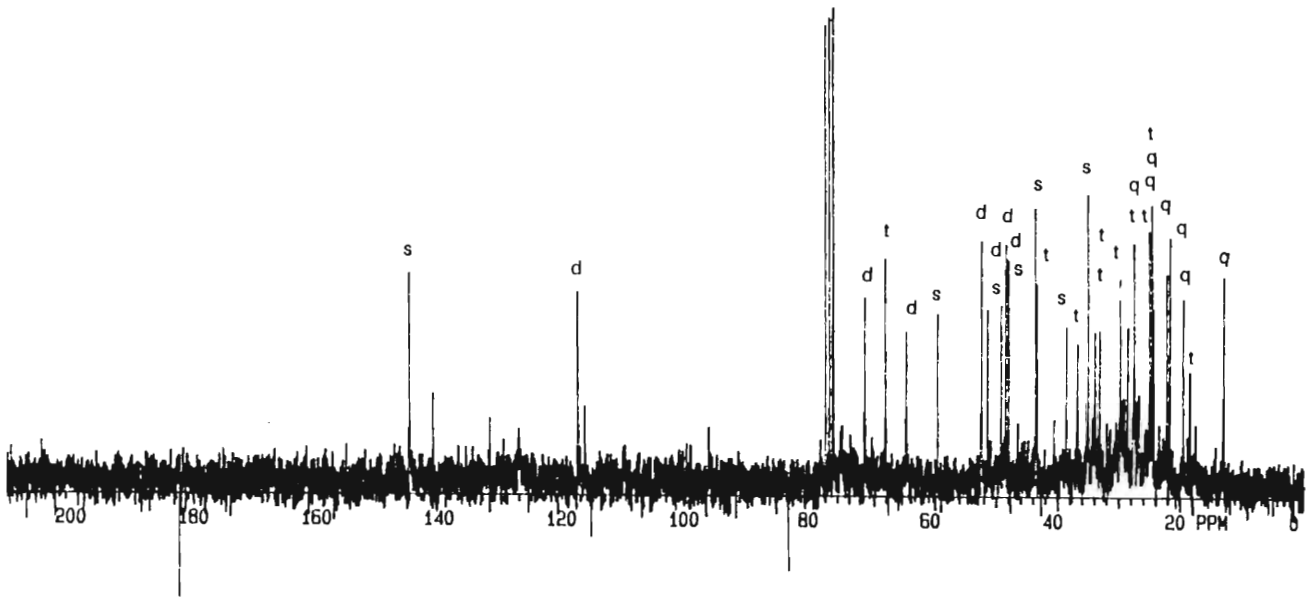

COMPOUND IX b
9
8
7
6
5
4
3
2
1 PPM

COMPOUND X

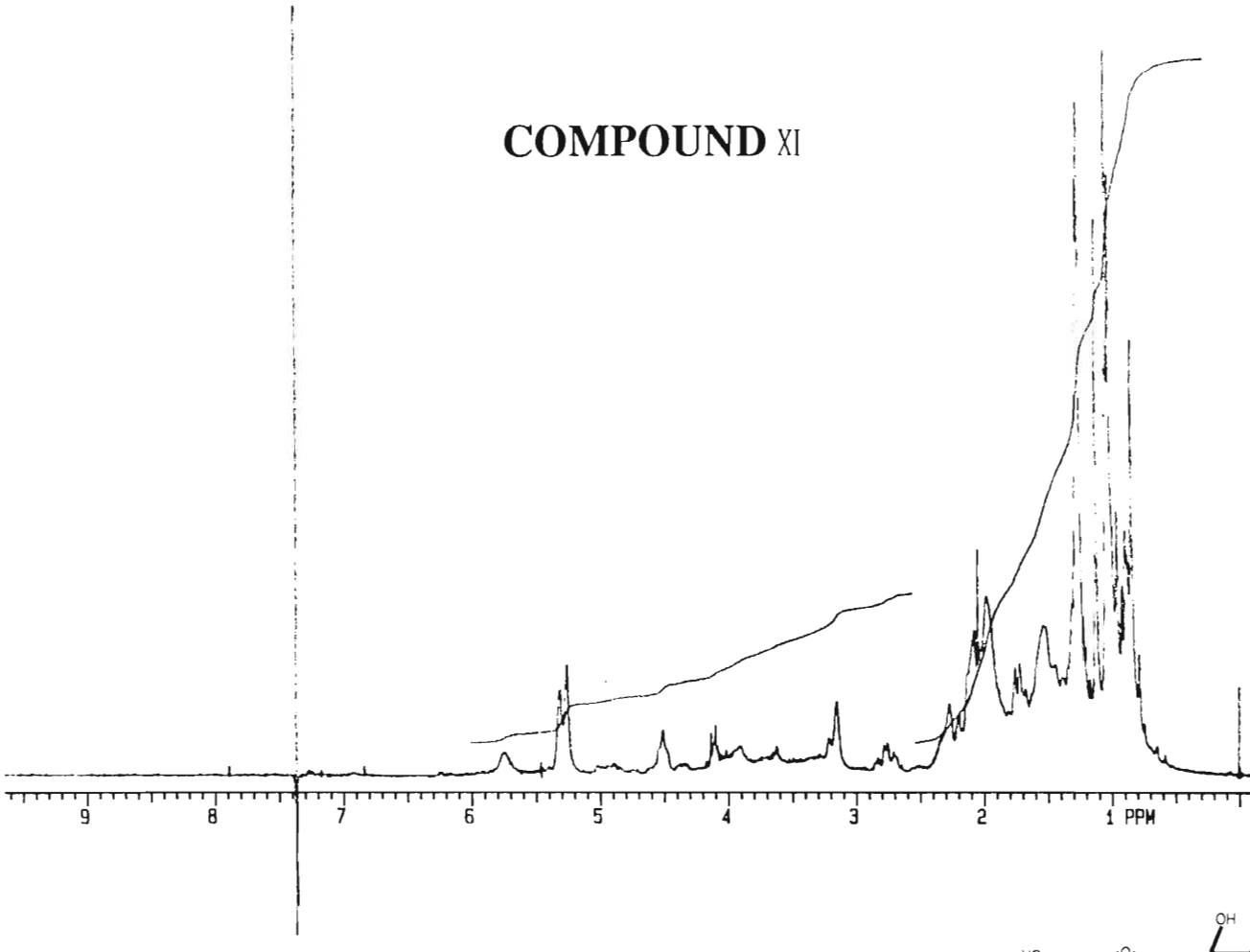


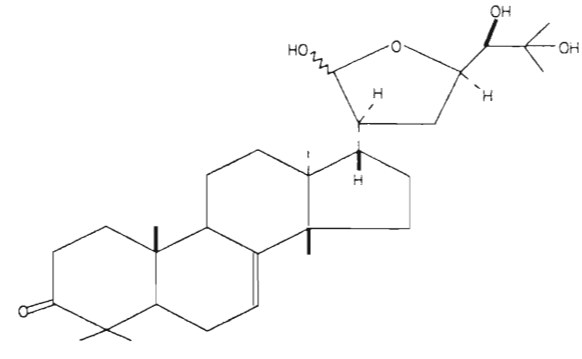


COMPOUND X

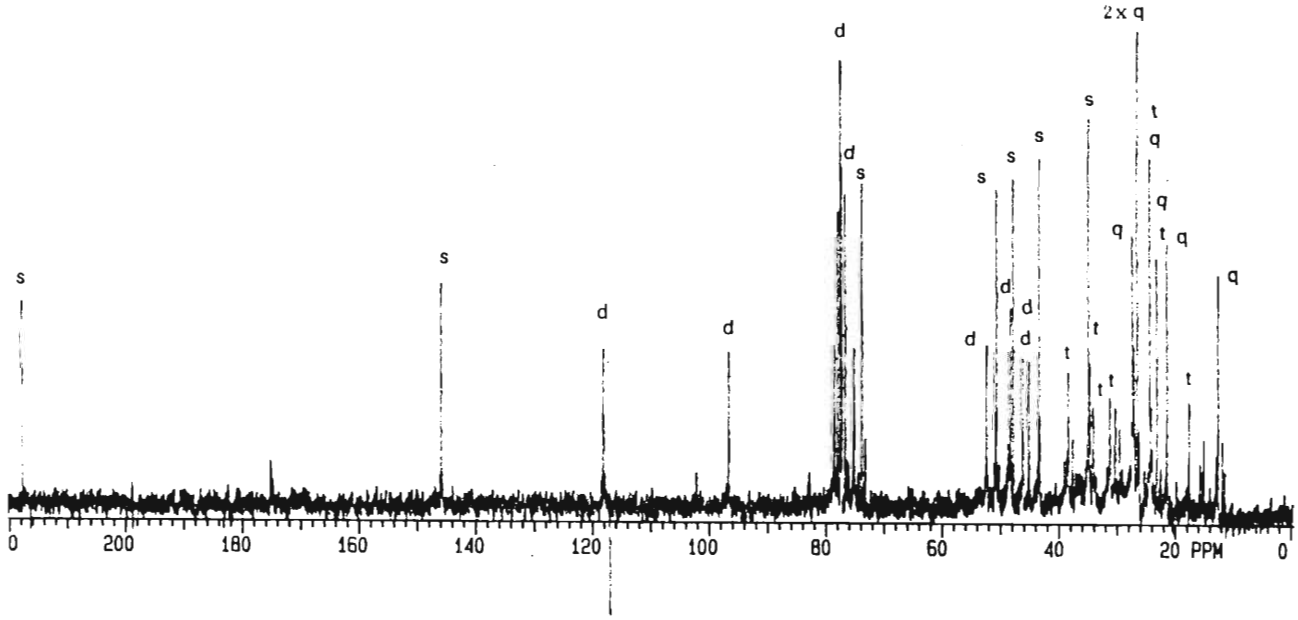


COMPOUND XI





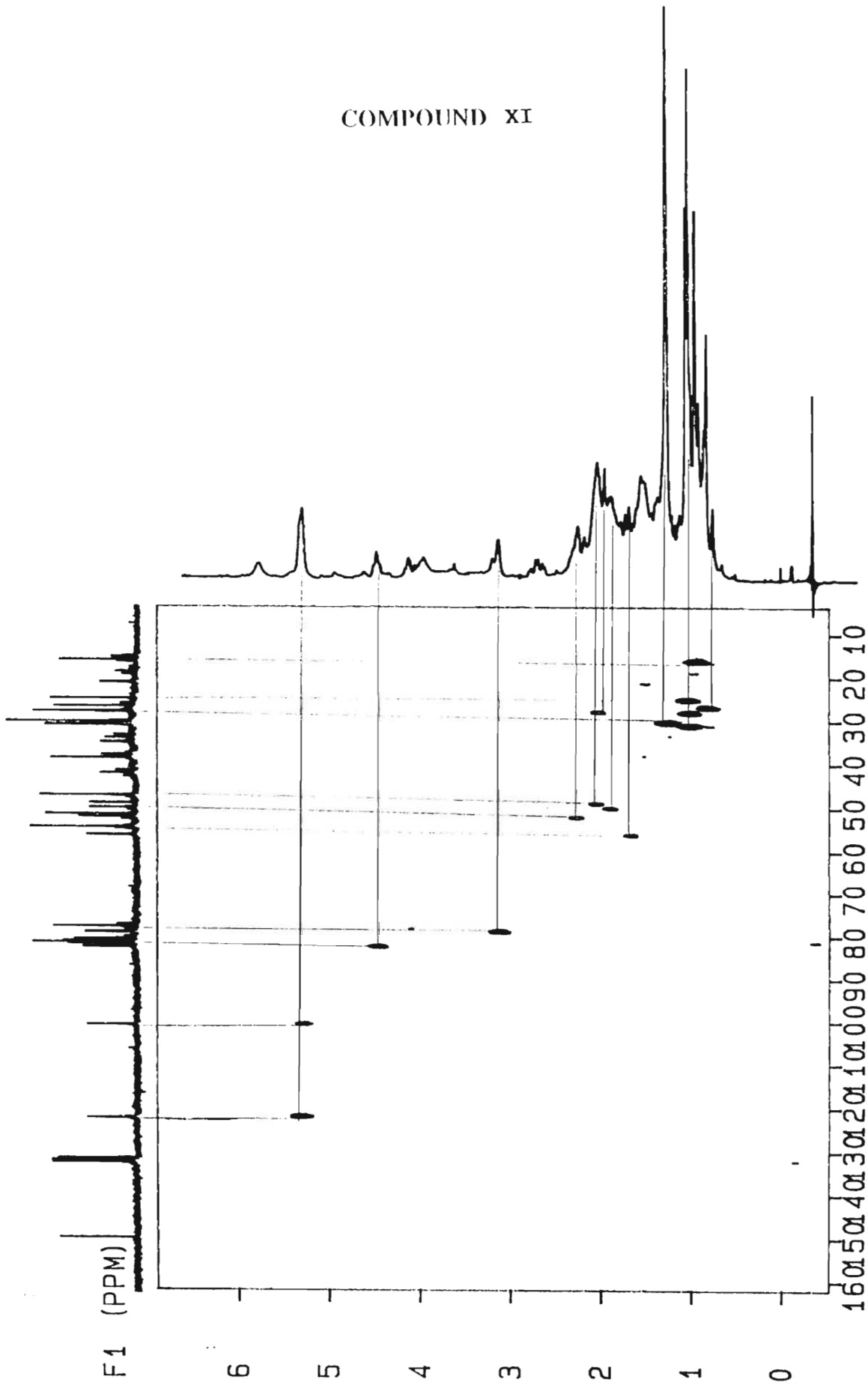
COMPOUND XI



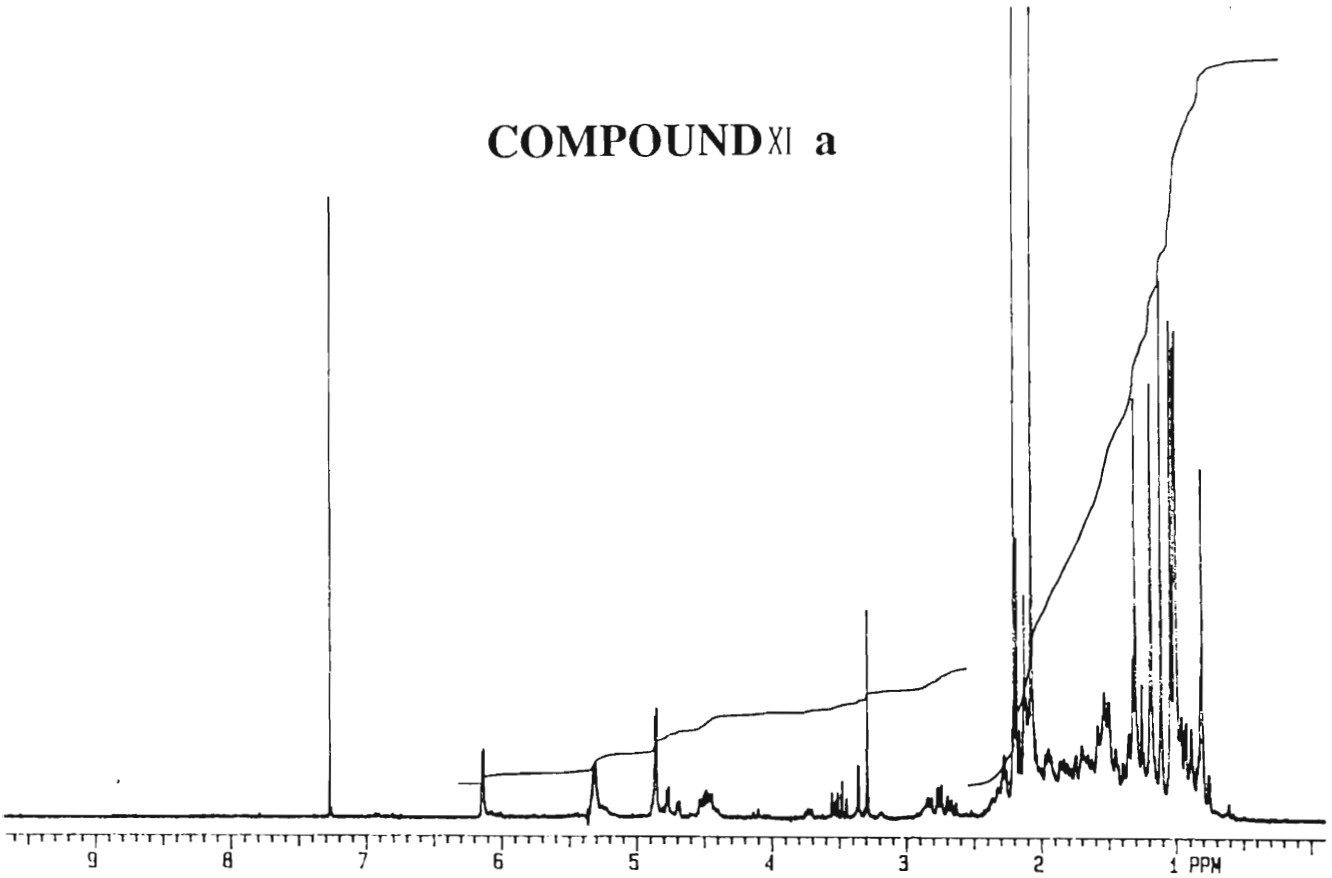
COMPOUND XI

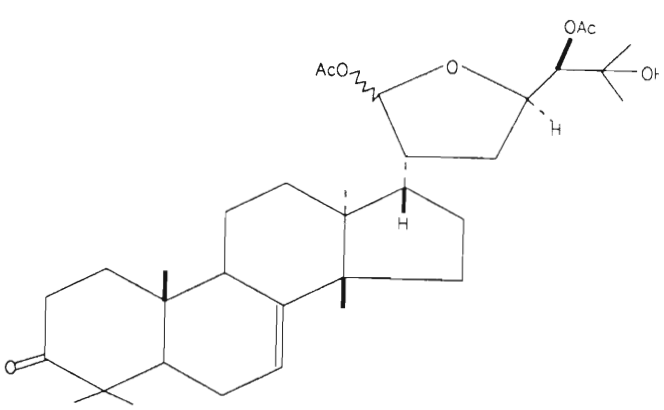
F1 (PPM)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 (PPM)
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

COMPOUND XI

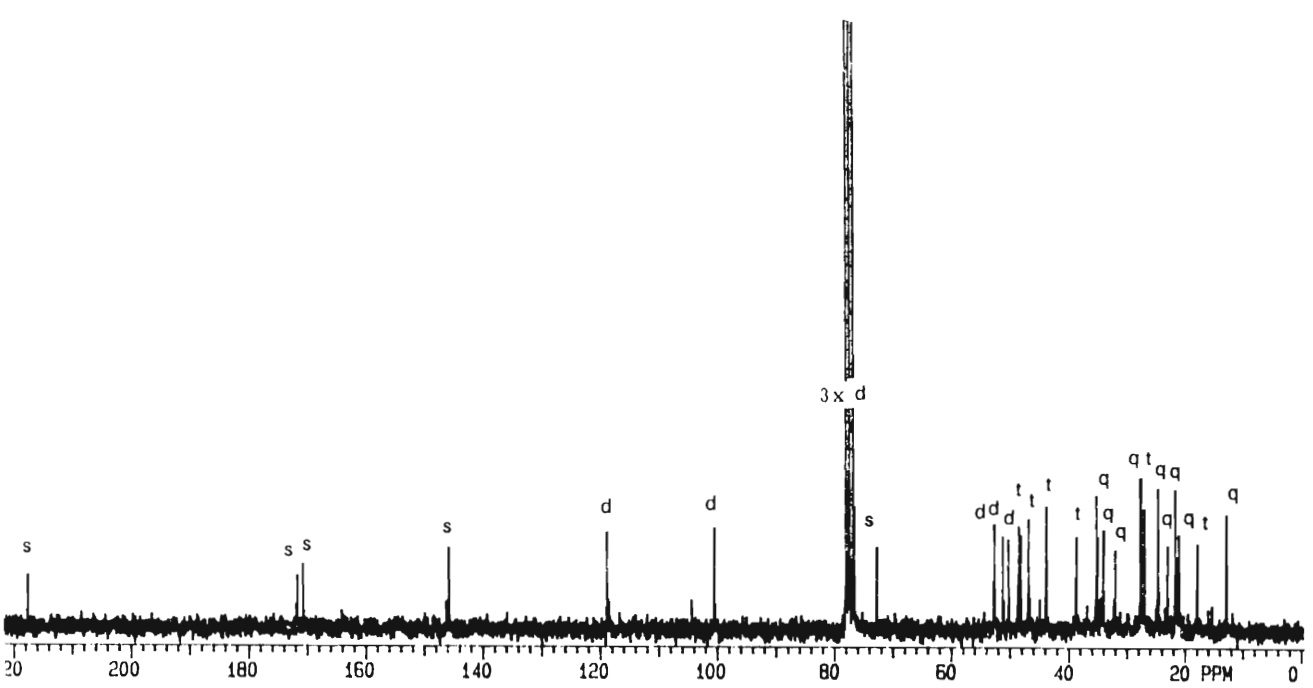

F1 (PPM)
6
5
4
3
2
1
0
F2 (PPM)
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

COMPOUND XI a





COMPOUND XI a



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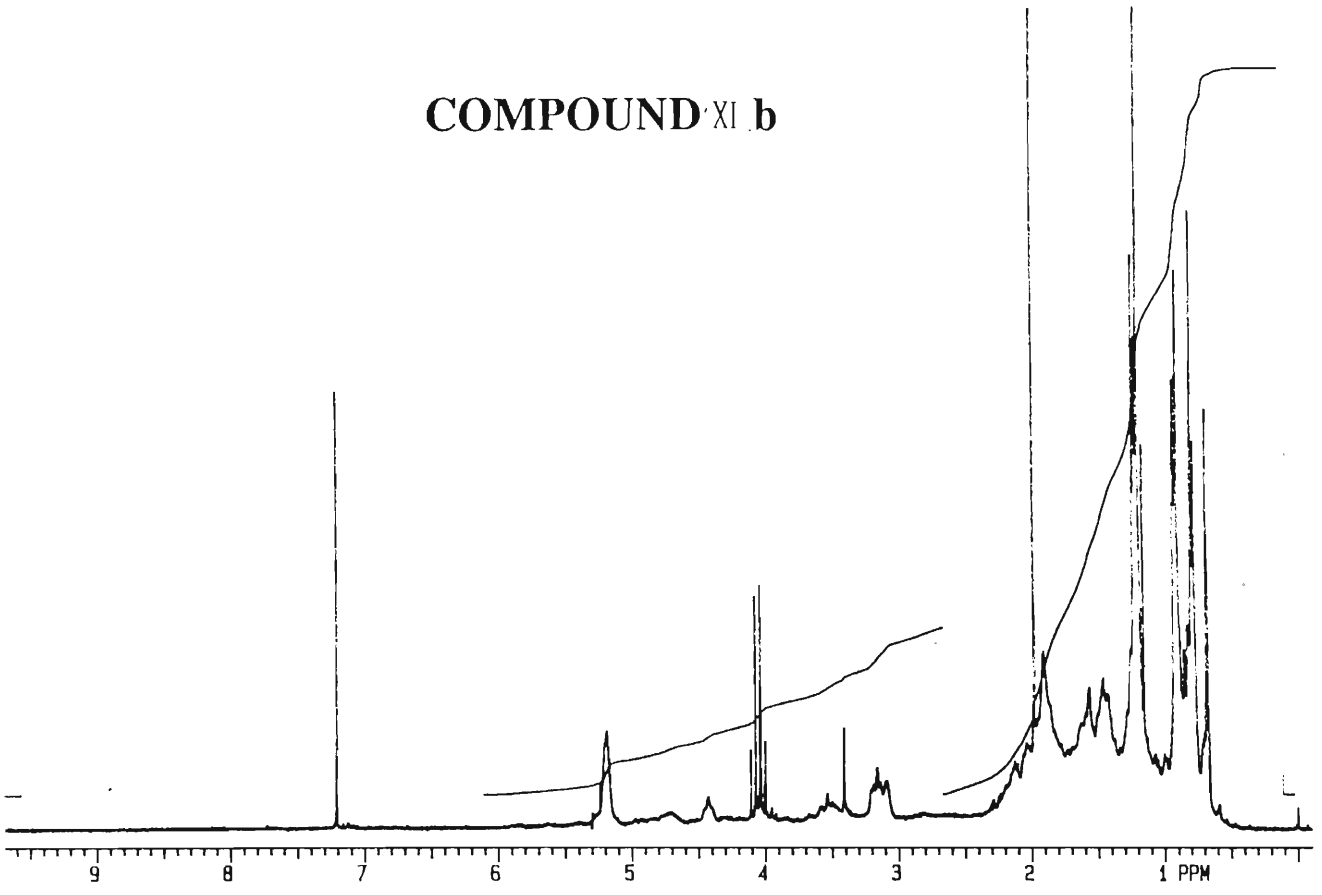

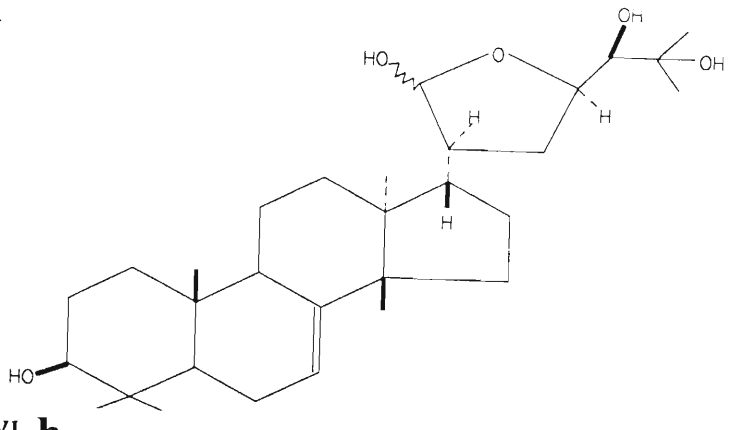

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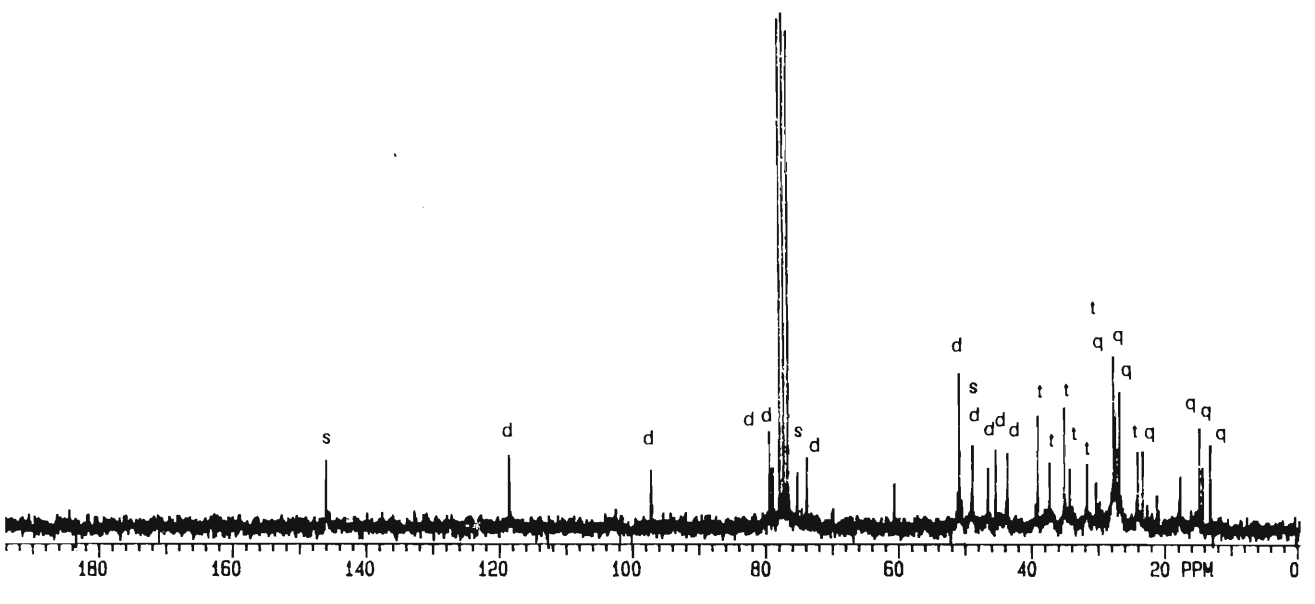

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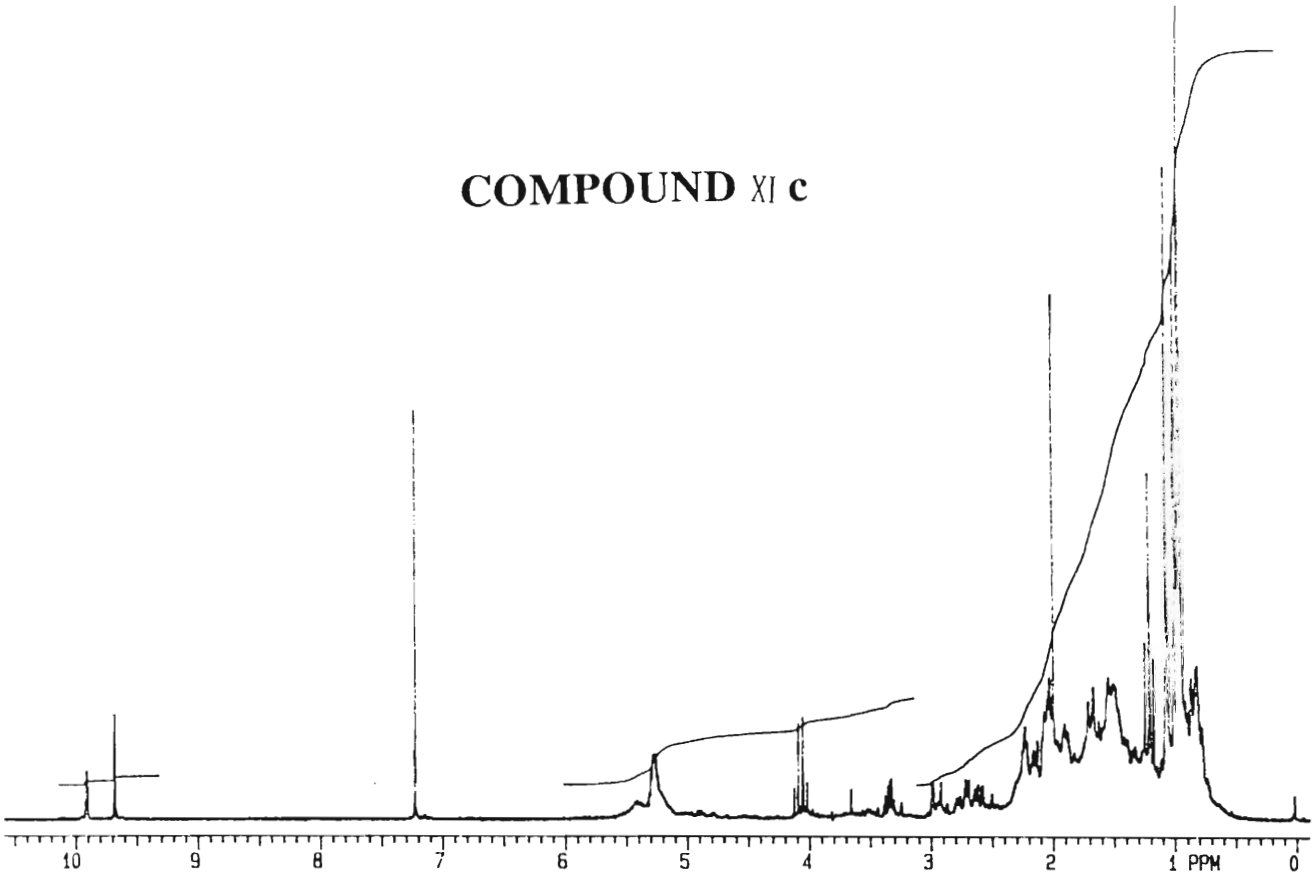

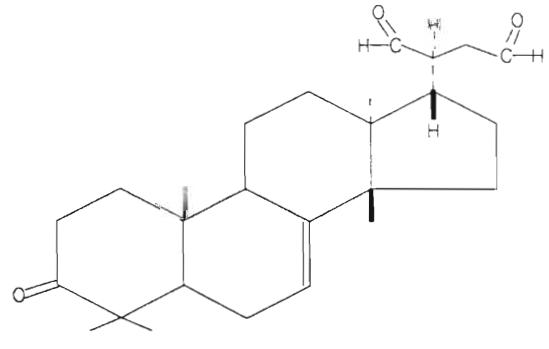

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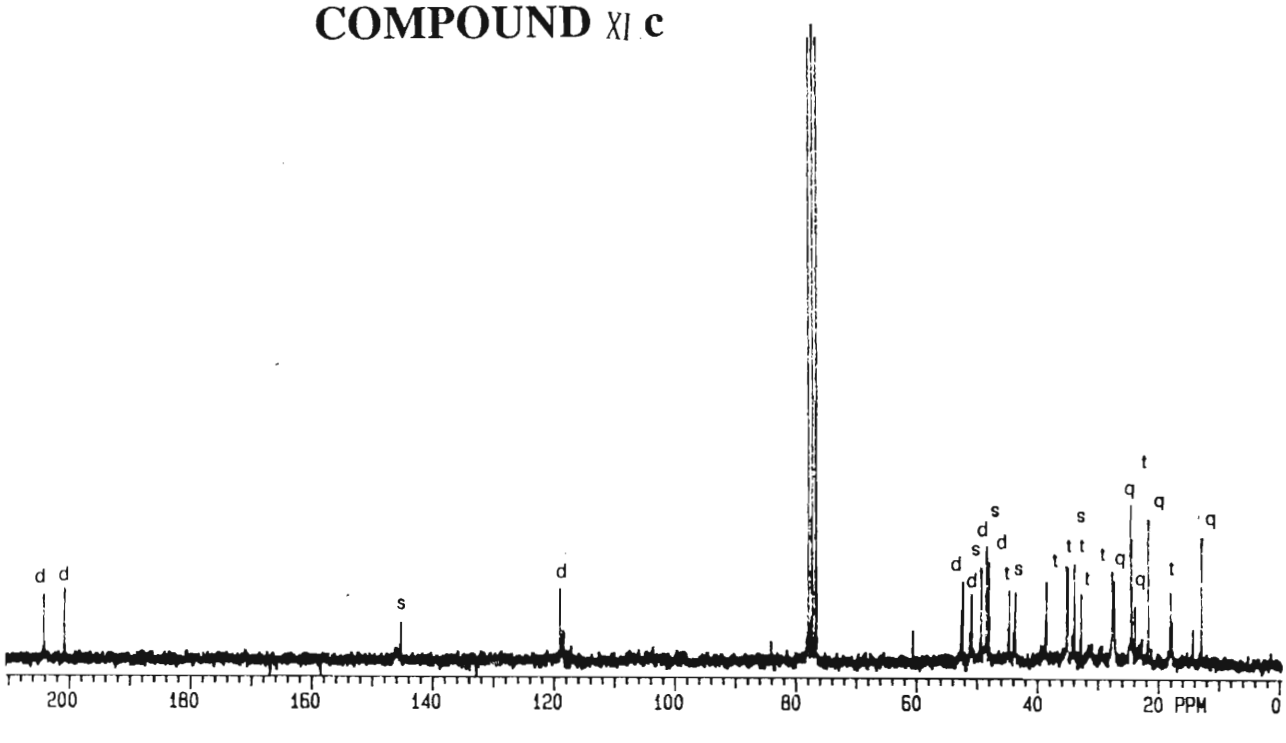
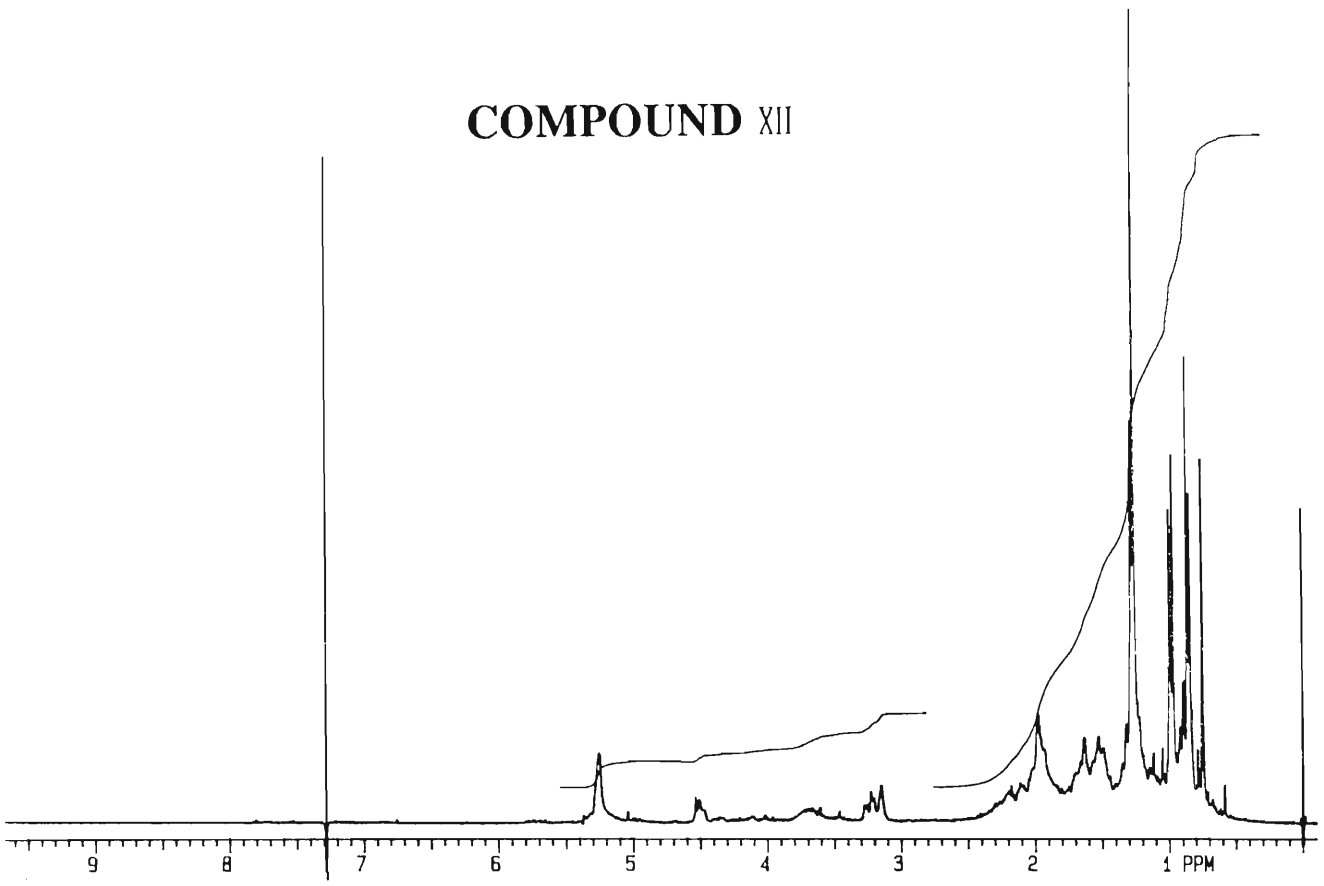
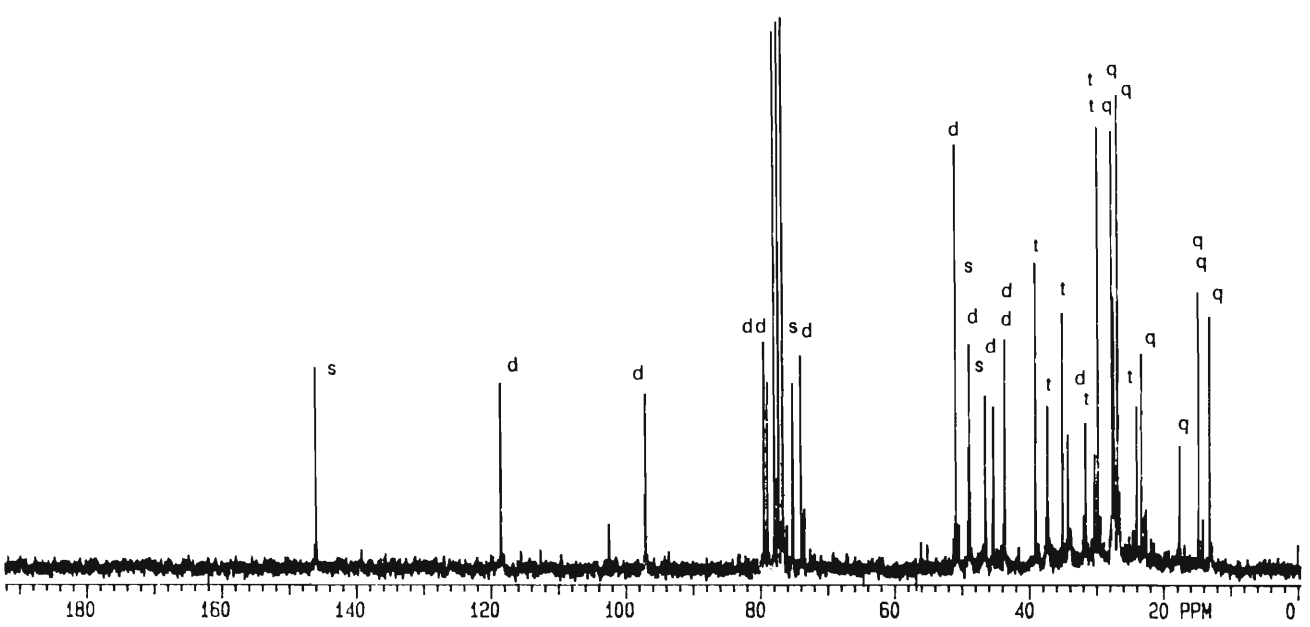


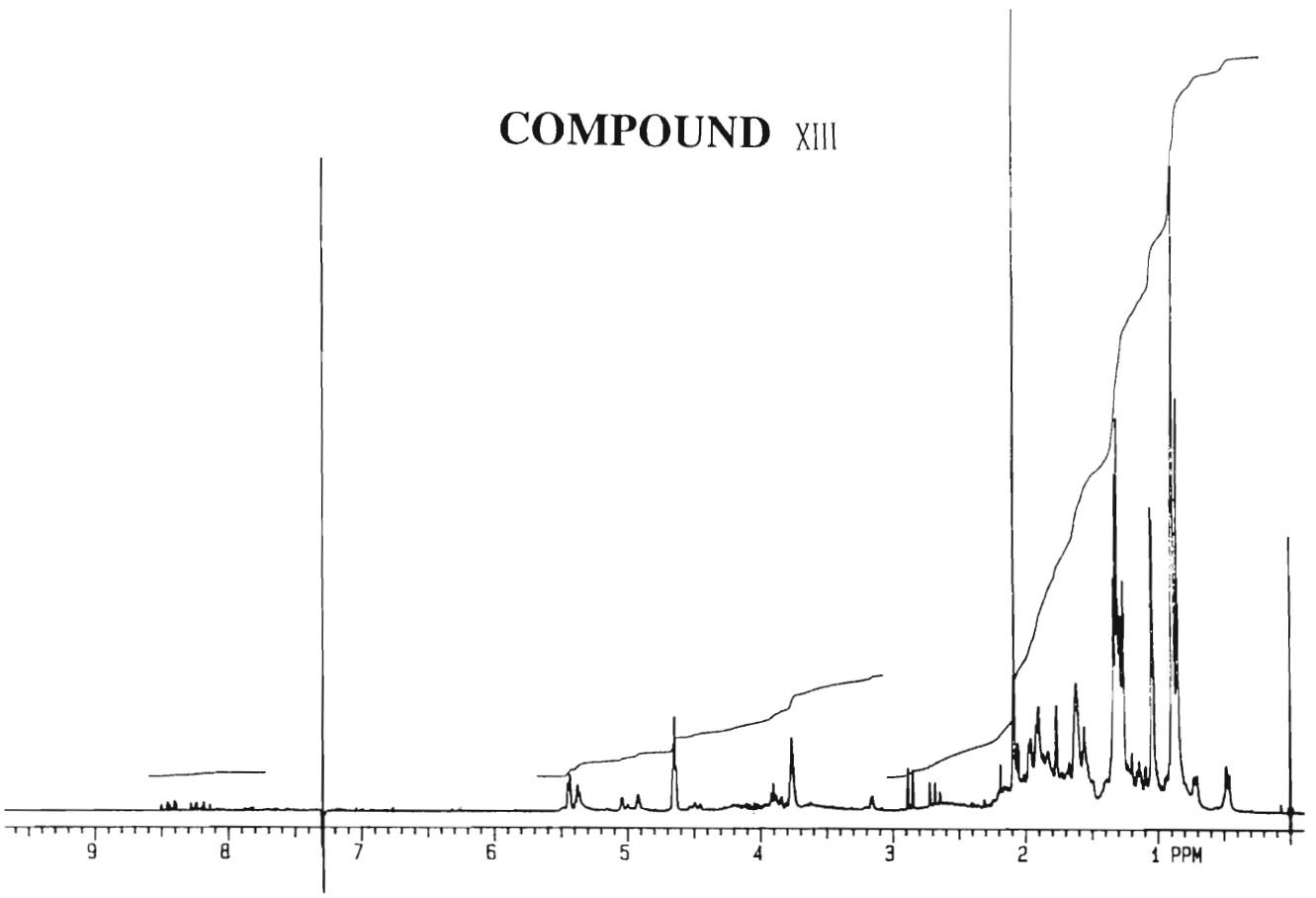
COMPOUND XII

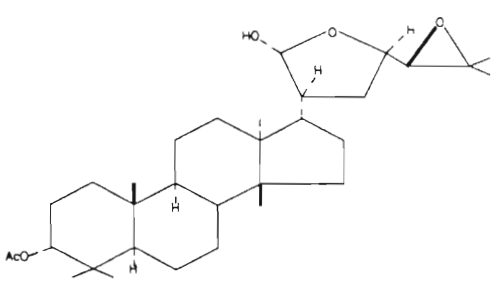


COMPOUND XII

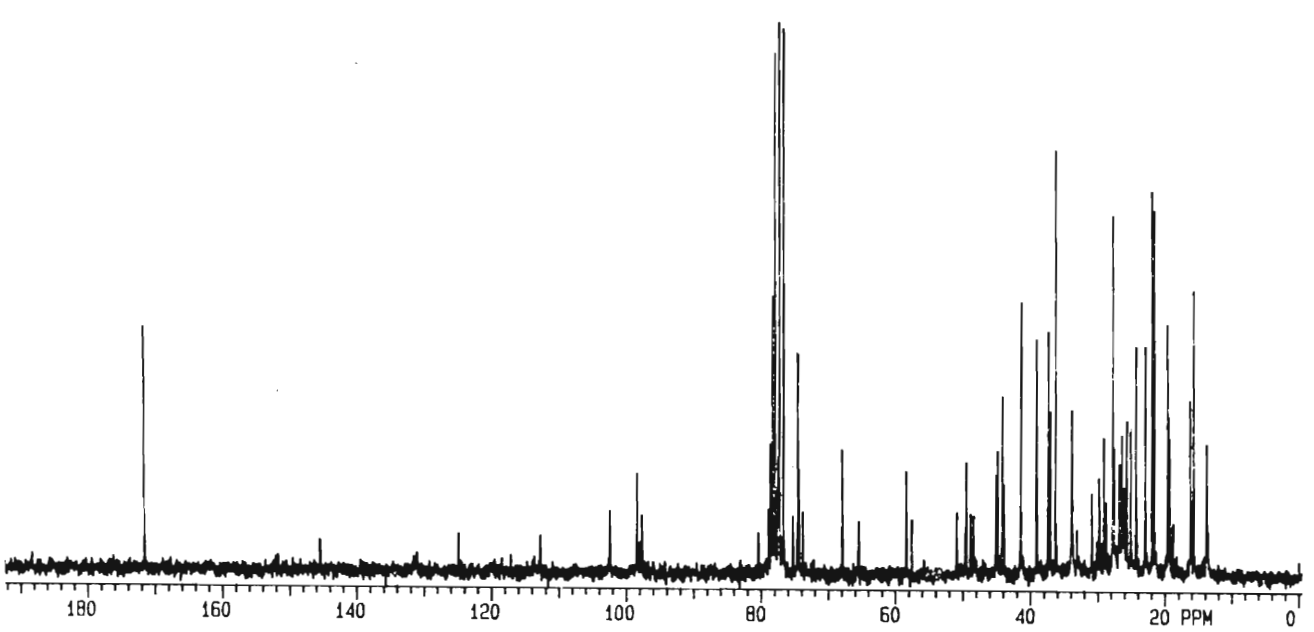


COMPOUND XIII

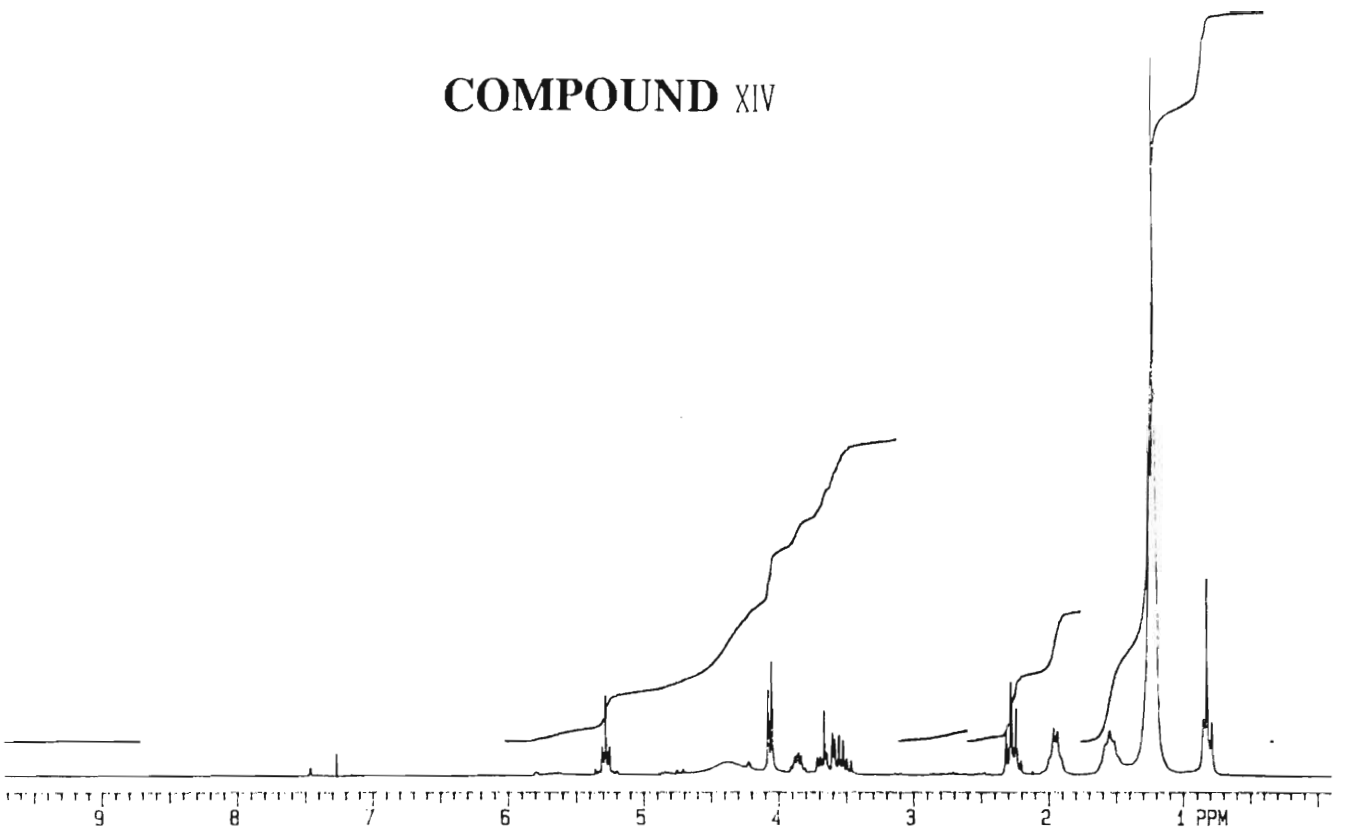


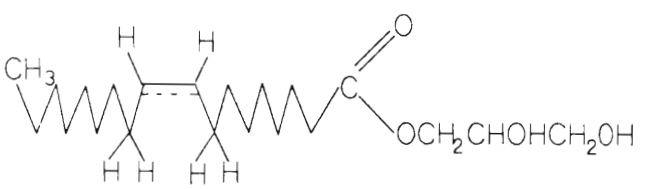


COMPOUND XIII



COMPOUND XIV





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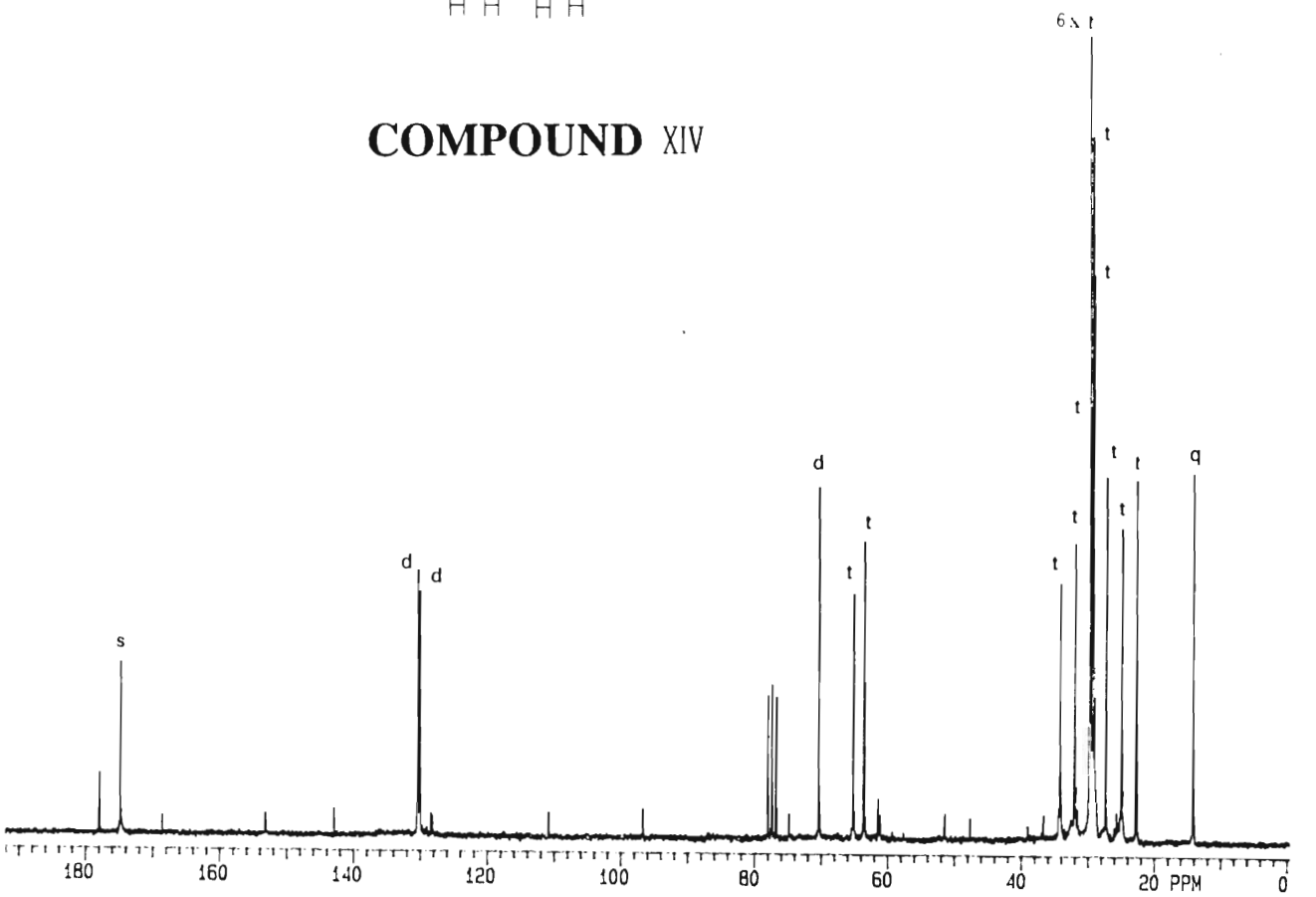

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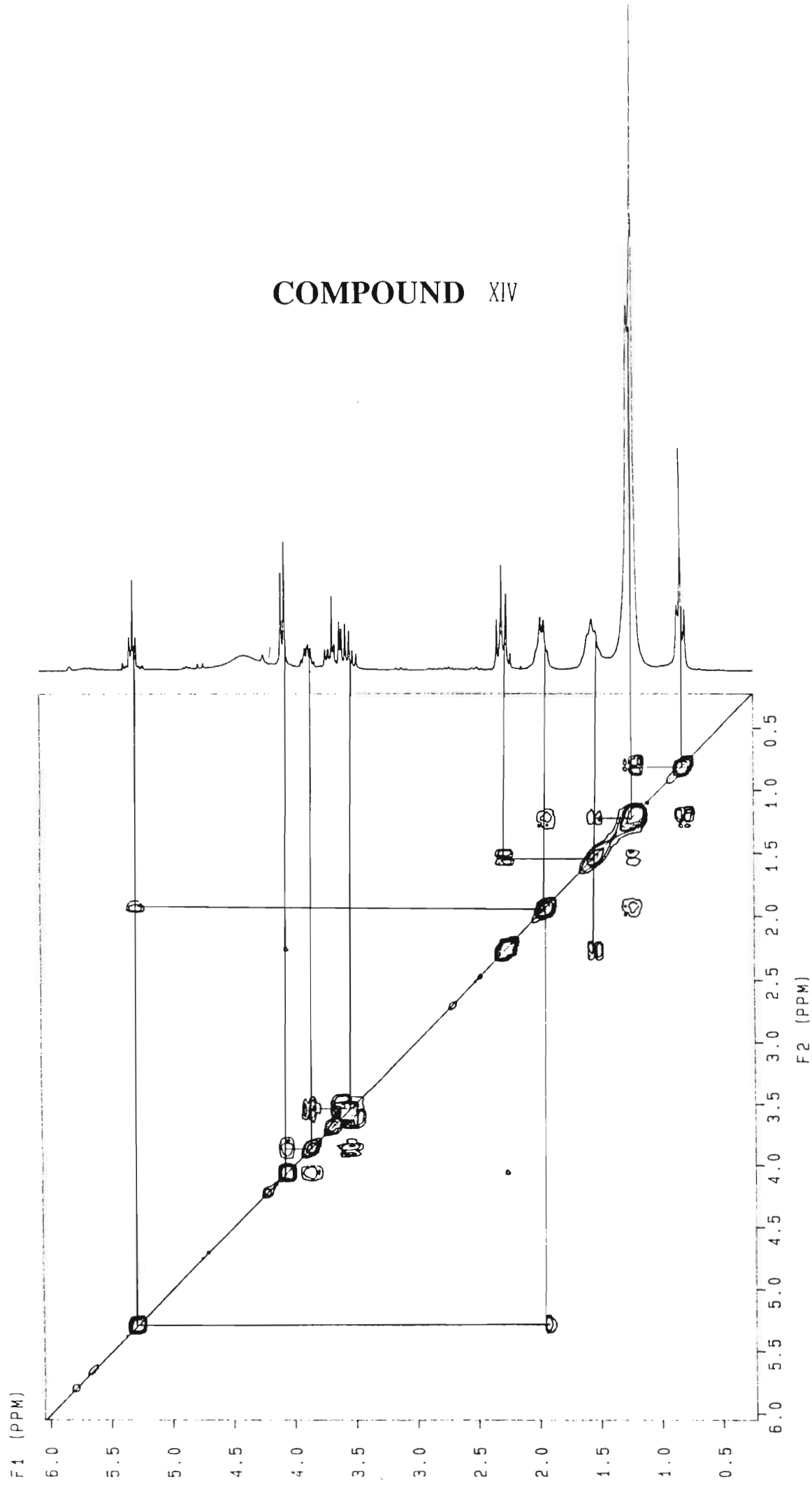

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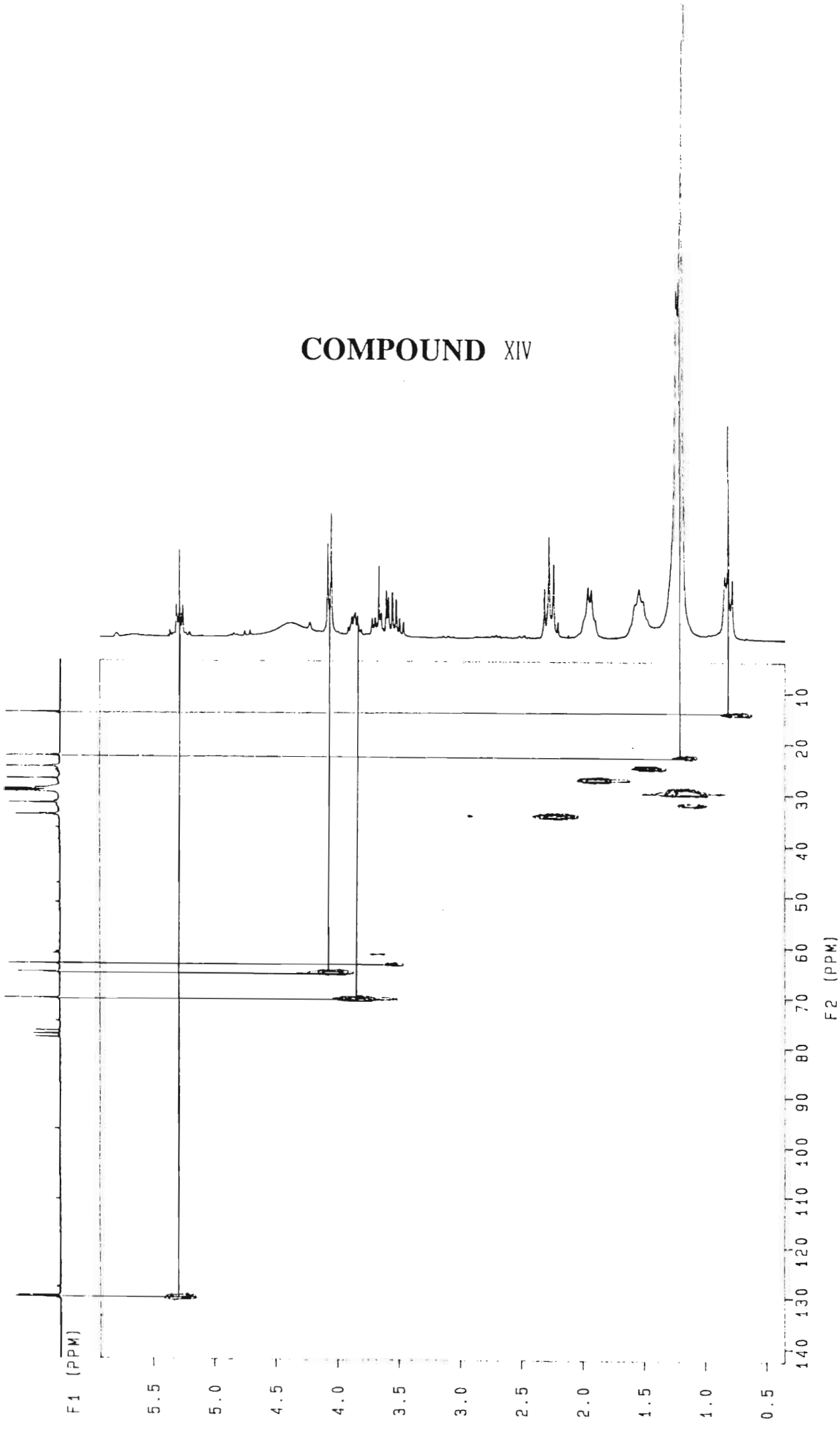
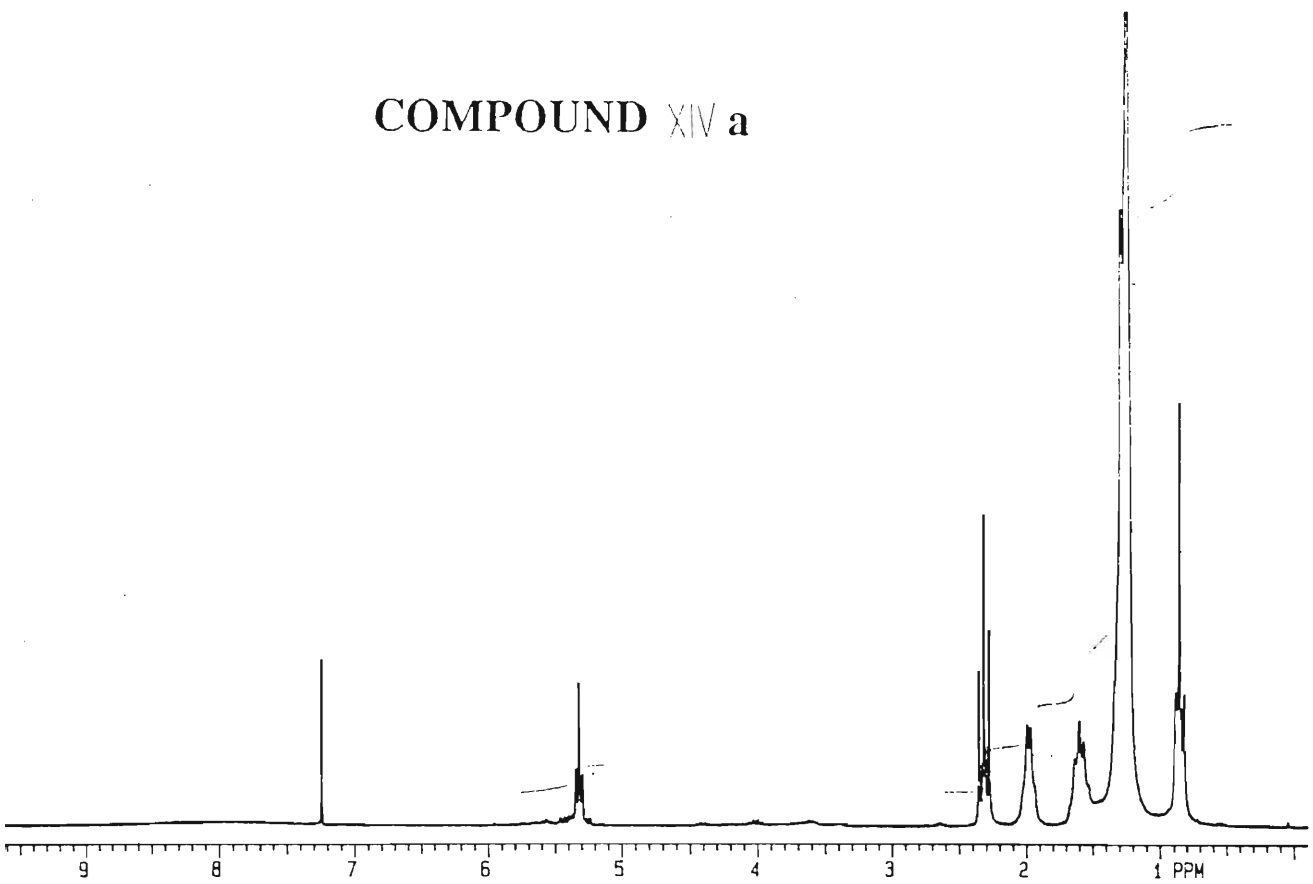


COMPOUND XIV a



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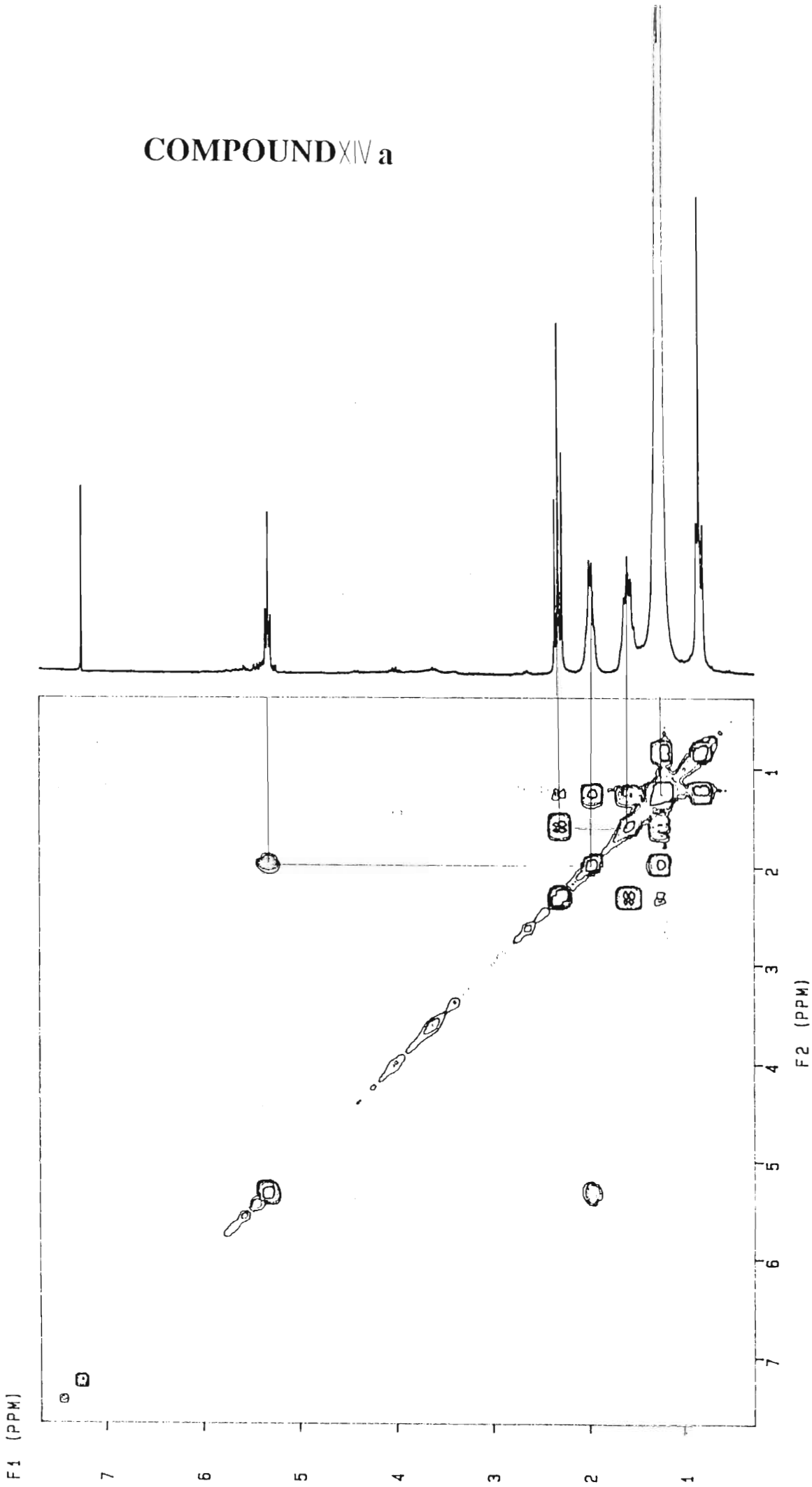
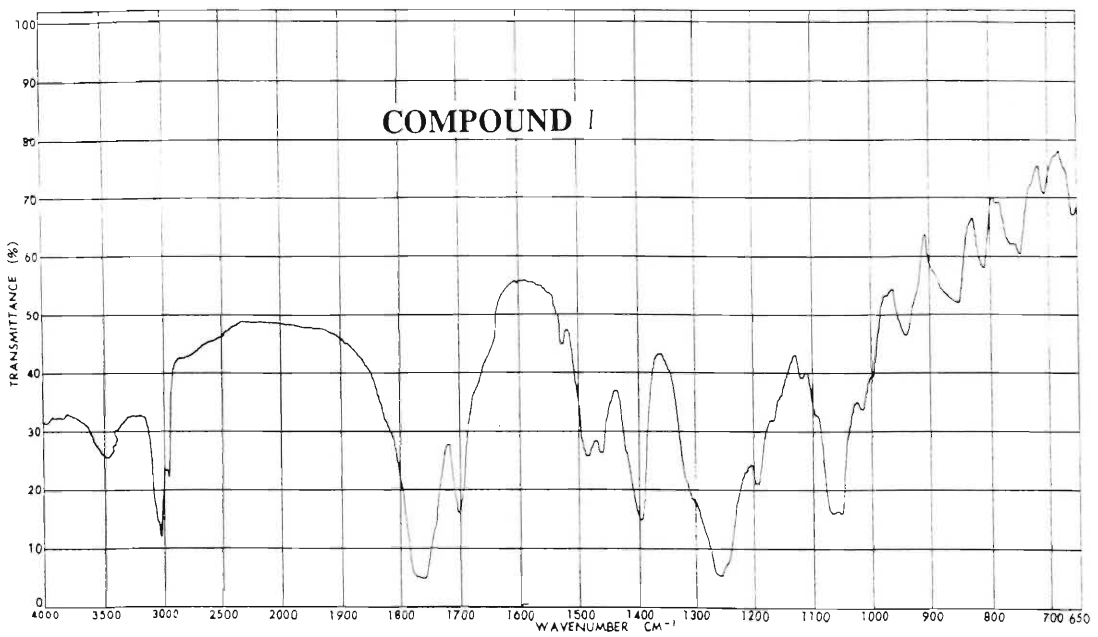
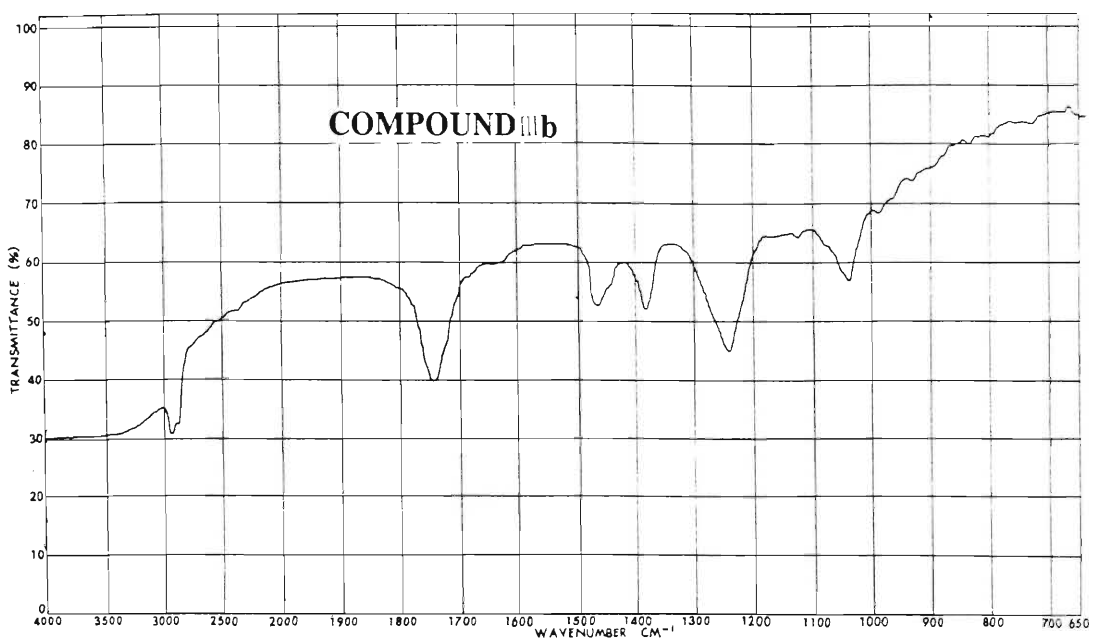
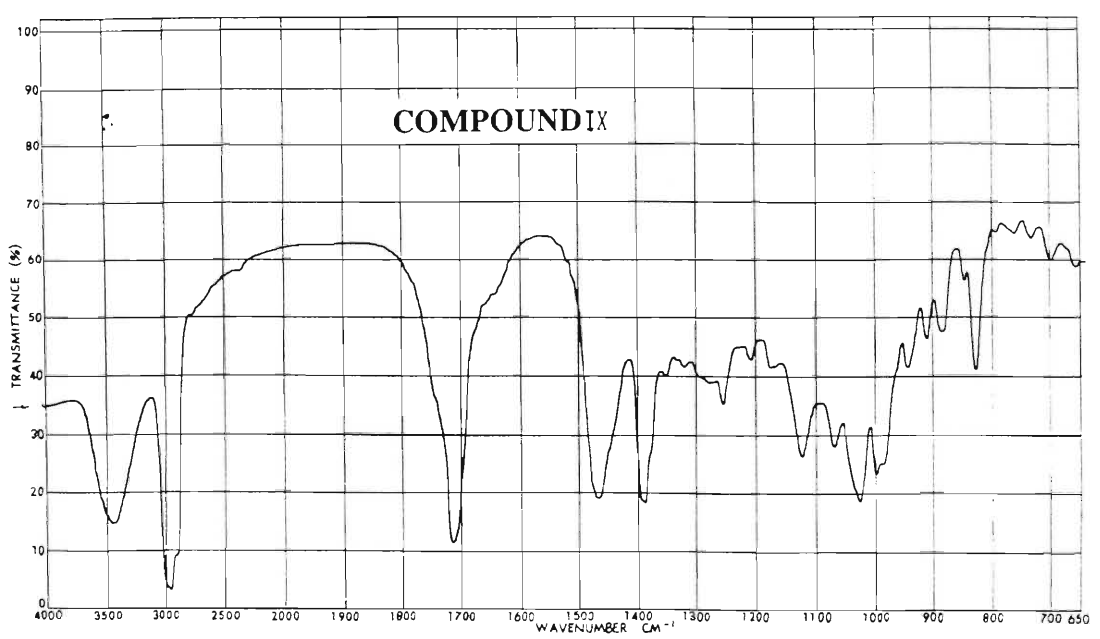
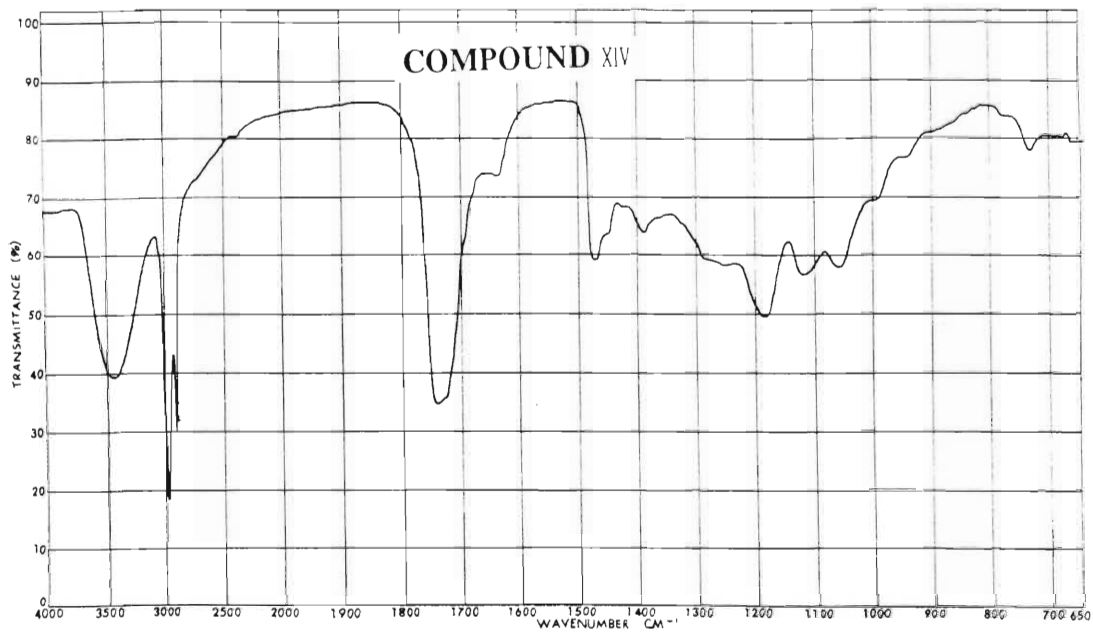



COMPOUND I
TRANSMITTANCE (%)
WAVENUMBER CM⁻¹


COMPOUND IIIb
TRANSMITTANCE (%)
WAVENUMBER CM⁻¹


COMPOUND IX
TRANSMITTANCE (%)
WAVENUMBER CM⁻¹


COMPOUND XIV
TRANSMITTANCE (%)
100
90
80
70
60
50
40
30
20
10
0
4000
3500
3000
2500
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
650
WAVENUMBER CM⁻¹

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This compound has since been re-isolated in larger quantities from a different source and has been found to be 3-acetoxy-7-deacetoxy-glabretal.[82]