EXTRACTIVES FROM THE MELIACEAE AND ANNONACEAE

by

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DOCTOR OF PHILOSOPHY

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PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry and Applied Chemistry, University of Natal, Durban, from May 1995 to September 1997, under the supervision of Prof. D.A. Mulholland.

These studies represents origional 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 : .....

N. Naidoo, M.Sc.

I hereby certify that the above statement is correct.

SIGNED : .....

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ACKNOWLEDGEMENTS

I would like to thank my supervisor Prof. D.A. Mulholland for allowing me this opportunity to undertake the study of Ph.D. Moreover, my deepest appreciation for her support, encouragement, guidance and above all her friendship during the duration of my thesis, may this be only the beginning of a lifetime association.

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Many thanks to Vimla Bigay and Vimlan Naidoo for being my pillar of strength, without whom life would be meaningless.

Για τιν αγαπι τις ζωις μου, Παρασκεβγι. Αναγνωριζω τις ομωρφες στιγμεσπου περασαμε μαζι. Το ξερω οτι εχουμε φτασι σε αυτο το σημιω που
πρεπη ο καθε ενας μας να παρουμε το δικω μας το δρομω, αλλα ποτε μου δεν το ειχα φανταστη οτι θα εχανα μια τοσω καλι φιλι. Θα εισαι παντα στις σκεψις και στις προσεφχες μου και ευχωμε και εγω να ειμαι παντα στις δικες σου. Και τορα αντιω, αγαπι μου, που αυτο θα εισαι παντα για μενα.

Αναγνοριζω τον Θεο που με προσεχε και με εδωσε τη δινάμη να ξεπερασωτις δισκολες στιγμες που με βρικανε αυτο το χρονο.
Η αγαπη, υπομονη, δινάμι και σοφια ειτανε παντα μαζι μου στις δισκολεχωρες. Σε ευχαριστττω παρα πολυ που μου εδωσες τιν ευκερια να σπουδασω ως αυτο το σιμειω.

Μου εδωσε παρα πολυ χαρα που γνωρισα ολους αυτους μεσα σε αυτο τοθεμα και ευχωμε παντα να τους ευλογαει ο Θεος.
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I acknowledge receiving financial support from the University of Natal and FRD which was most essential for the completion of this work.

ABBREVIATIONS

Ac - acetate

br - broad resonance

br s - broad singlet

^{13}C NMR - carbon-13 nuclear magnetic resonance spectroscopy

COSY - correlated nuclear magnetic resonance spectroscopy

d - doublet

dd - doublet of doublets

DEPT - distortionless enhancement by polarization transfer

dt - doublet of triplets

gem - geminal

^{1}H NMR - proton nuclear magnetic resonance spectroscopy

HETCOR - heteronuclear shift correlation nuclear magnetic resonance spectroscopy

Hz - hertz

FTIR/IR - Fourier transformed infrared spectroscopy/infrared spectroscopy

m - multiplet

Me - methyl

Ph - phenyl

ppm - parts per million

q - quartet

s - singlet

t - triplet

t.l.c - thin layer chromatography

ABSTRACT

Members of the Annonaceae and Meliaceae, two plant families which are distributed throughout the world, have been studied during the course of this work. The research is an account of the compounds isolated from six members of the Meliaceae (Australian, Indian and South African) and one from the Annonaceae (South African). These families have attracted special attention of scientists world-wide because of the compounds these plants produce and their medicinal applications. Extracts from these species are known to be widely used in traditional medicine and previous studies have demonstrated their anti-cancer, anti-microbial and other pharmacological activities.

Limonoids and protolimonoids were isolated from the Indian Meliaceae species, *Aphanamixis polystacha* and from the South African Meliaceae species *Turraea obtusifolia*. Extractives from the seeds of *Aphanamixis polystacha* yielded seven limonoids (**2-A** to **2-G**) from the hexane extract, while the methanol extract only yielded sucrose (**2-H**). Three of the limonoids (**2-B**, **2-E** and **2-F**) were novel and the stereochemistry of polystachin, compound (**2-A**), was revised. Extractives from *Turraea obtusifolia* yielded two triterpenoids (**3-A** and **3-B**) and a limonoid (**3-C**) from the hexane extract of the seed.

Several triterpenoids and a benzofuran compound were isolated from the Australian Meliaceae species, *Dysoxylum pettigrewianum*, *Dysoxylum muelleri* and *Aglaia sapindina*. Extractives from *Dysoxylum pettigrewianum*, yielded three triterpenoids (**4-A** to **4-C**) from the hexane extract of the bark. Two of these triterpenoids (**4-B** and **4-C**) were novel. Extractives from *Dysoxylum muelleri* yielded two triterpenoids (**4-D** and **4-E**) from the methylene chloride extract of the wood. Extractives from *Aglaia sapindina* yielded a benzofuran compound (**4-F**) from the hexane extract of the wood. This resulted in the revision of the structure of ferrugin (**4-56**).

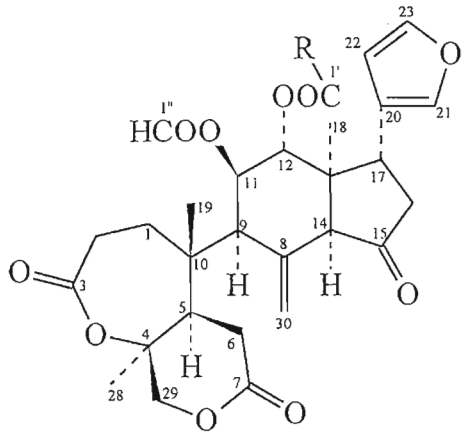
A flavonoid (**5-A**) was isolated from the aqueous extract of the seed of the South African Meliaceae *Trichilia dregeana*.

A polyoxygenated cyclohexane epoxide (**6-A**) was isolated from the root and leaves of the Annonaceae species, *Monanthotaxis caffra*.

The research undertaken involved the collection, extraction, isolation, purification and structural elucidation of these compounds by infrared, mass and nuclear magnetic resonance spectroscopic techniques.

NOMENCLATURE

A. Nomenclature used in chapter one



- **1-1 to 1-53**, where **1** refers to chapter one and **1 to 53** refers to compounds discussed in the literature search. In all cases, H-5 and H-9 are α in the limonoids and protolimonoids discussed.

B. Nomenclature used in chapters two to six

- Chapters two to six have individual nomenclature sections within each chapter.

CHAPTER 1

A brief review of the Triterpenoids

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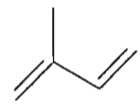
* Classification is mentioned according to structural similarity and oxidative complexity.

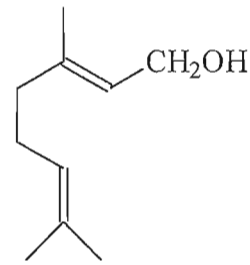
1.1 Biosynthetic considerations

The isoprene unit (**1-1**) is the foundation upon which many naturally occurring plant metabolites are built.



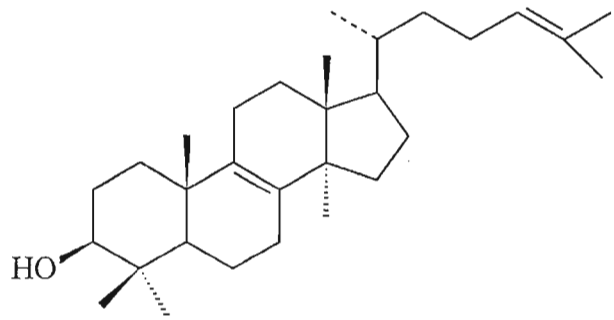
1-1

It was first isolated more than 100 years ago and was eventually found to be an essential unit in the makeup of all terpenoids e.g. geraniol (**1-2**) which is made up of two isoprene units.[1]



1-2

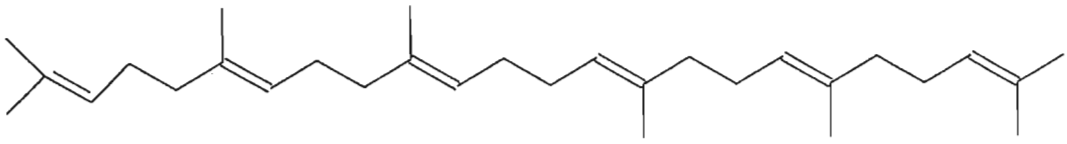
The triterpenoids form a large group of naturally-occurring compounds which are widely distributed throughout the plant kingdom. A small but important group, which includes lanosterol (**1-3**), is of animal origin.



1-3

The triterpenoids are considered to be derived from an acyclic precursor which is cyclised and further elaborated to give different classes of compounds.[1] The acyclic precursor was found to be hexa-ene all-*trans* squalene (**1-4**), and according to the Biogenetic Isoprene Rule, the

different types of triterpenoids are formed according to the conformation that squalene epoxide (**1-5**) adopts, presumably at an enzyme surface prior to cyclisation.[2,3]



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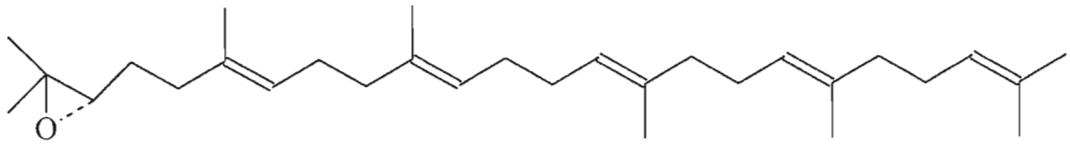
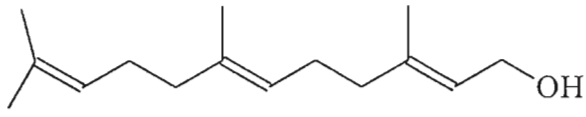


1-5

It was postulated that different types of terpenoids are formed by linear combination of the isoprene units to produce C_{10} monoterpenoids e.g. geraniol (**1-2**), C_{15} sesquiterpenoids e.g. farnesol (**1-6**), C_{20} diterpenoids e.g. geranylgeraniol (**1-7**), C_{30} triterpenoids e.g. squalene (**1-4**) and other terpenoids by the tail-to-tail or tail-to-head condensation reactions.



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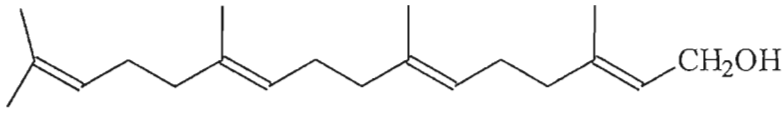
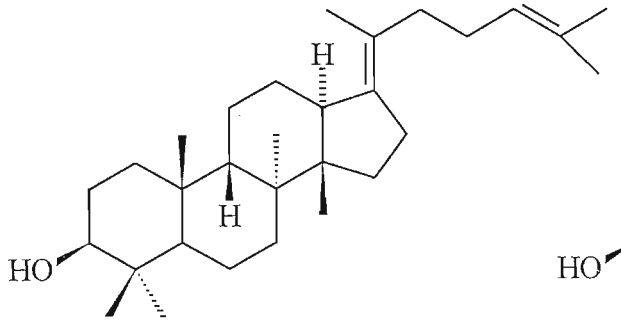


1-7

Tetra- and pentacyclic triterpenoids are formed by the cyclisation of squalene epoxide (**1-5**), depending on its conformation, which, in turn, relies on enzymes catalysing the cyclisation.[2,3,4]

Cyclisation of squalene epoxide in the chair-boat-chair-boat conformation leads to the formation of protosterol (**1-8**) -type compounds whereas the cyclisation of squalene epoxide in the chair-chair-chair-boat conformation leads directly to the formation of the dammarenediols (**1-9**), which could play an important role in the biosynthesis of limonoids (discussed later in this chapter).[1]



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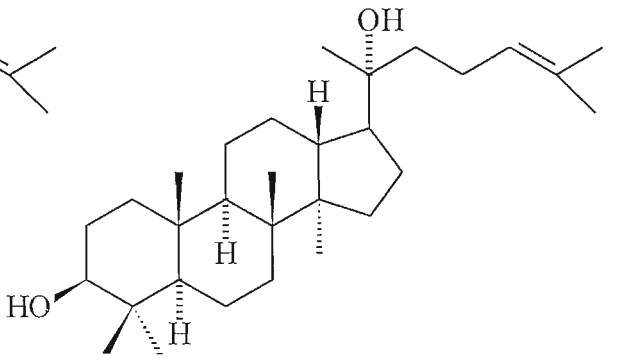
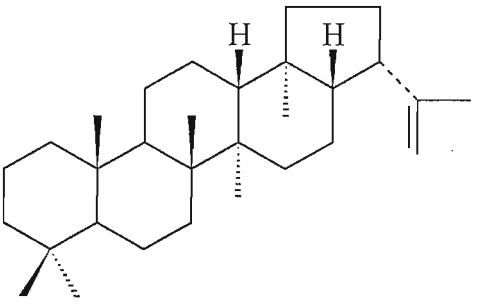


1-9

Cyclisation of squalene via other conformations is also possible, the chair-chair-chair-chair-chair conformation leads to the formation of diplotene (**1-10**) and tetrahymanol (**1-11**), while the chair-chair-chair-chair-boat conformation results in the formation of moretenol (**1-12**) and chair-boat-chair-chair-boat cyclisation yields arborinol (**1-13**).[1]



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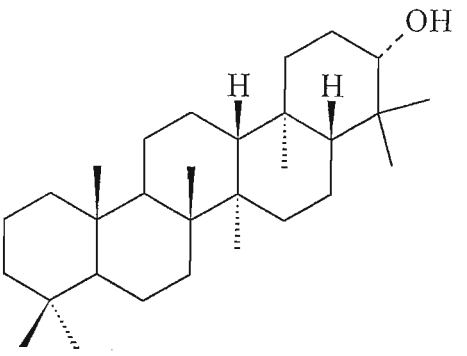

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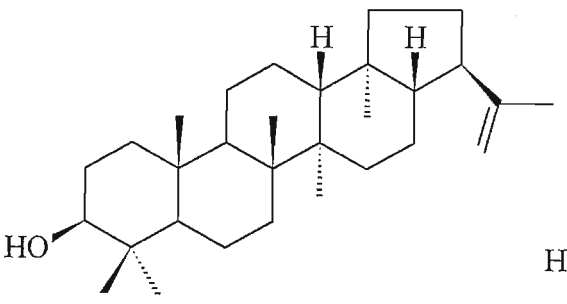

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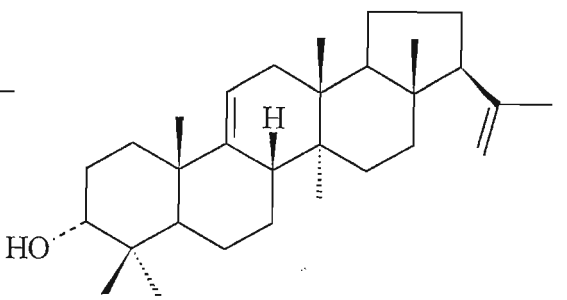


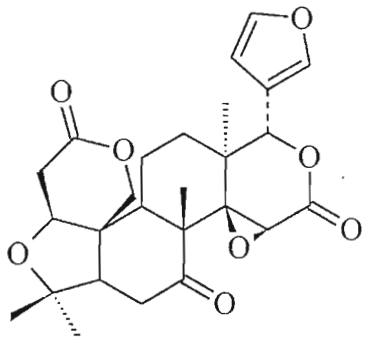
1-13

1.2 Biogenesis of the limonoids

1.2.1 Introduction

Over the past four decades, study of extractives from the families Meliaceae, Rutaceae, Cneoraceae and Simaroubaceae has revealed that certain triterpenoids are peculiar to these families. Research into these substances has constituted a new branch of terpenoid chemistry.

The first group of substances is the tetranortriterpenoids, C-26 compounds found in the families Meliaceae, Rutaceae and Cneoraceae. These compounds are now commonly known as limonoids owing to their relationship to limonin (**1-14**), whose structure was the first to be elucidated, in 1960.[5] Limonin was isolated from the seeds of the genus *Citrus*, which belongs to the Rutaceae family. During the 1950's, gedunin (**1-15**) was isolated from the timber of *Entandrophragma angolense* (Meliaceae),[6] and its structure was elucidated after its relationship to limonin (**1-14**) was recognised.[7]



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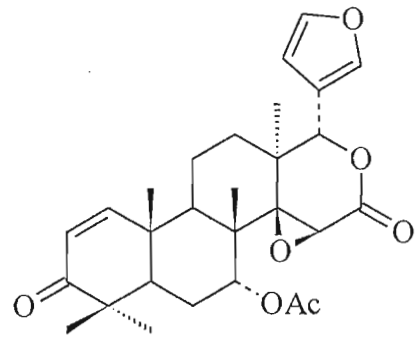
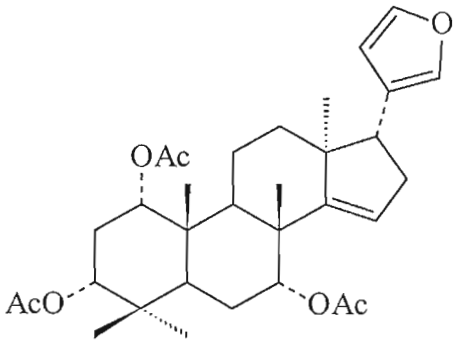


1-15

Since then, many species from the family Meliaceae have been examined, and this research has revealed that limonoids are common in this family.[8] It is with this group of compounds that this work will be largely concerned.

A limonoid is described as a compound with a C-22 nucleus attached to a β–substituted furan ring at the C-17α position. The nature of the nucleus may vary considerably, ranging from a tetracyclic structure with all rings carbocyclic, as in deoxyhavanensin (**1-16**), to more

complex structures where one or more of the rings has been opened oxidatively, such as prieurianin (**1-17**) accompanied by further cyclisations as in utilin (**1-18**).



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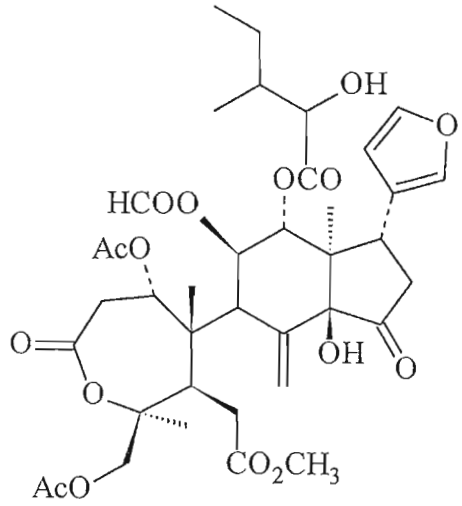

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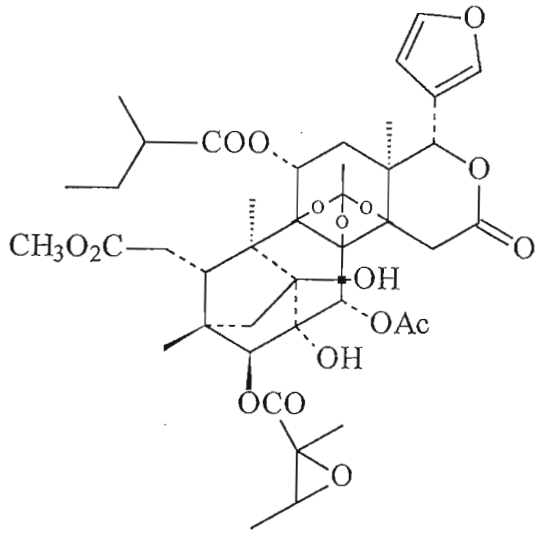
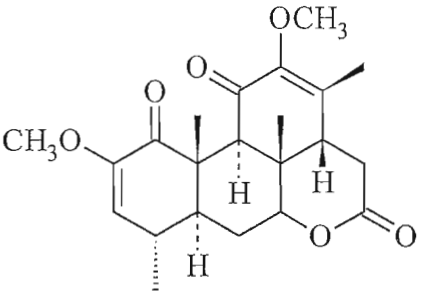


1-18

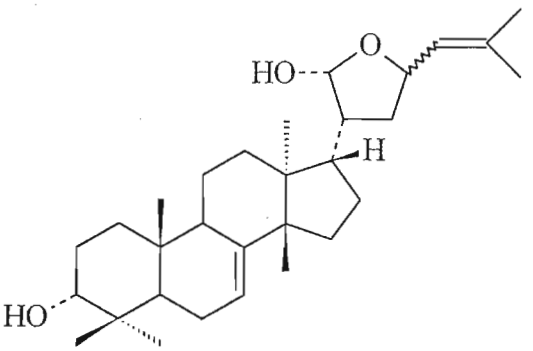
It is apparent that the more complex limonoids are obtained from simpler ones by a series of oxidative steps and cyclisations. These steps will be described in more detail later.

The second group of substances is the decanortriterpenoids, which are C-20 compounds found in plants of the family Simaroubaceae.[8] These are known as quassinoids, owing to their relationship to quassin (**1-19**), whose structure was elucidated in 1962.[9] Soon after this, it was recognised that the quassinoids and limonoids probably shared a biosynthetic pathway. This work will not be concerned with the quassinoids.



1-19

A third group of compounds isolated from the Rutaceae and Meliaceae needs to be mentioned at this point. These are C-30 compounds having a limonoid-like carbocyclic nucleus, but lacking a furan ring, e.g. flindissol (**1-20**). These compounds are referred to as protolimonoids, since it appears that the limonoids (and quassinoids) are derived biogenetically from such compounds.[10] This biogenesis will be described later.



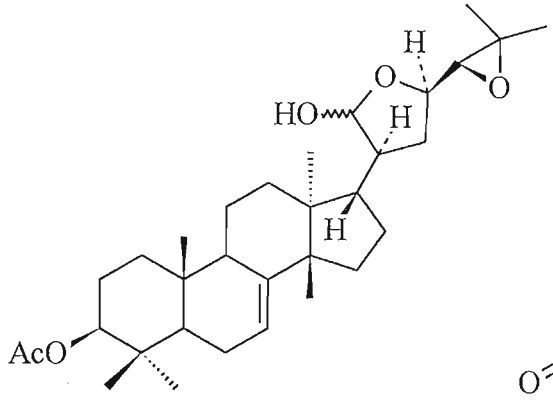
1-20

1.2.2 Classification of compounds from the Meliaceae

The limonoids isolated from the Meliaceae have been classified into nine groups,[8] according to structural similarity and oxidative complexity. Since this classification is useful in discussing the biogenesis of the limonoids, it will be summarised here.

1.2.2.1 Type A (protolimonoids)

The protolimonoids constitute the first major group of compounds isolated from plants in the Meliaceae family. Examples are flindissol (**1-20**), turraeanthin (**1-21**) and grandifoliolenone (**1-22**). These compounds are found both in the Meliaceae and Rutaceae, but often in small quantities. This may indicate that they are either incompletely metabolised intermediates, or by-products of the main biosynthetic pathway.



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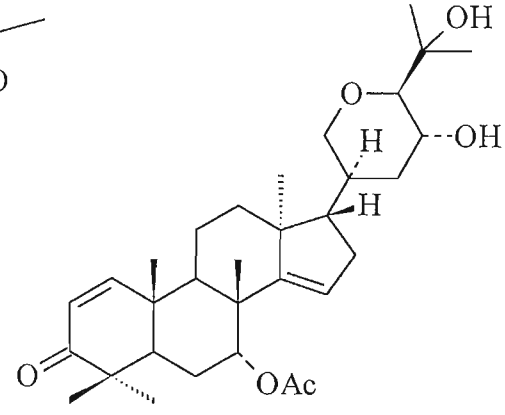
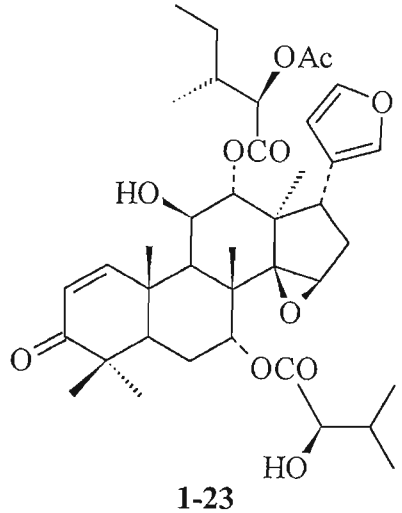


1-22

1.2.2.2 Type B limonoids

These have a tetracyclic nucleus, with all rings carbocyclic, as in deoxyhavanensin (**1-16**). Other, more oxidised, compounds belonging to this group are the heudelottins (**1-23**, **24**, **25**).



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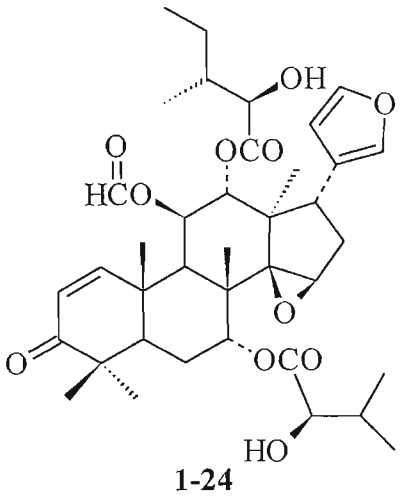

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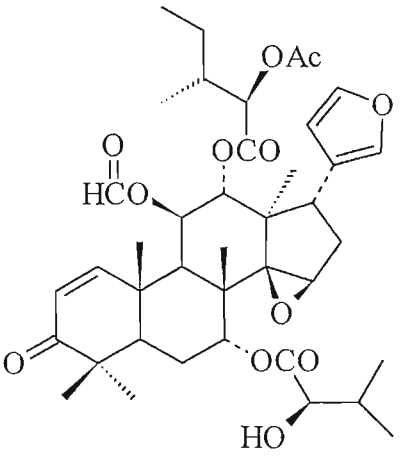
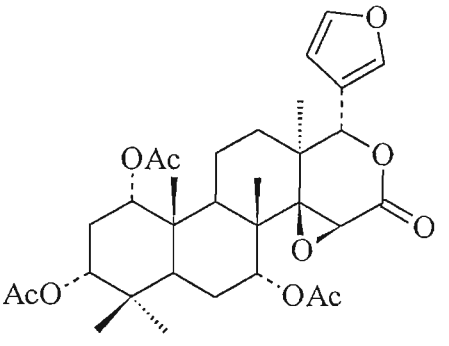


1-25

This group is significant, as it is believed that all the more complex limonoids arise from precursors in this class. They are the simplest true limonoids, and occur in minor quantities in many species. After the type B limonoids, branches arise in the biosynthetic pathway to give three groups, types C, G and H limonoids.

1.2.2.3 Type C limonoids

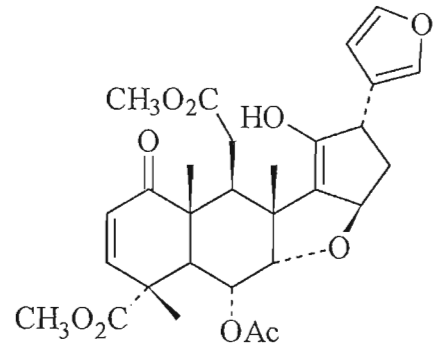
These compounds have rings A, B and C carbocyclic, and ring D oxidised to a lactone. Typical examples are gedunin (**1-15**) and khivorin (**1-26**).



1-26

1.2.2.4 Type G limonoids

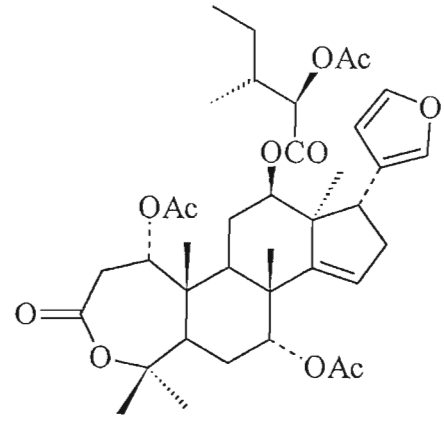
In these compounds, ring C is opened oxidatively, while rings A, B and D remain carbocyclic. Such compounds may undergo further change to give rise to compounds such as nimbin (**1-27**).



1-27

1.2.2.5 Type H limonoids

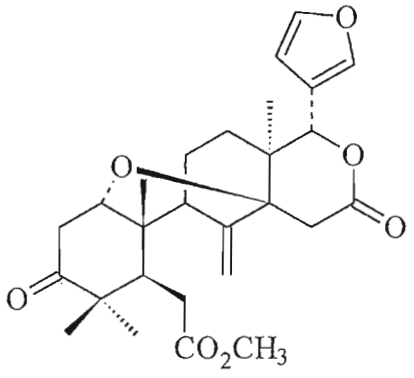
A number of compounds, isolated from *Trichilia dregeana*, constitute this group of limonoids.[11] These have rings B, C and D carbocyclic, and ring A oxidised to a lactone e.g. dregeana 3, (**1-28**).



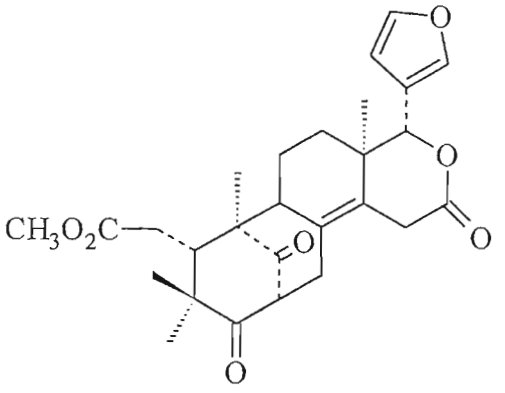
1-28

1.2.2.6 Type D limonoids

This class of limonoids arises from the gedunin (**1-15**) group. These compounds have rings A and C carbocyclic, a ring D lactone, and ring B cleaved. There are two subdivisions in this class, viz. the limonoids related to methyl angolensate (**1-29**), and those similar to mexicanolide (**1-30**). In the latter case, further cyclisation has taken place after opening of ring B, to give a bridged ring system, which is known as a bicyclononanolide skeleton.



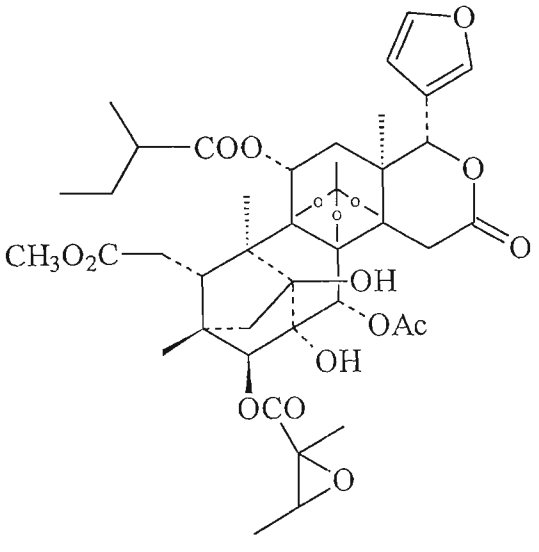
1-29



1-30

1.2.2.7 Type E limonoids

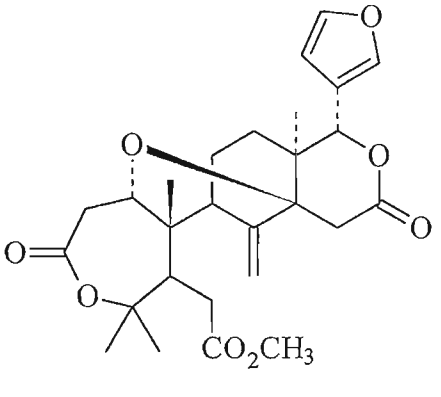
This group is the last in the series arising from the type C limonoids via compounds such as mexicanolide (**1-30**). A typical example of this class is utilin (**1-18**). The characteristic feature of these compounds is ring A bridged by an oxidised C-4 methyl group. In addition, more complex oxygen functions such as hemiorthoesters, may be present. The type E limonoids are the most complex known.



1-18

1.2.2.8 Type J limonoids

These compounds, exemplified by methyl ivorensate (**1-31**), are thought to be derived from type C limonoids.[11] They have ring C carbocyclic, ring B opened and lactones in ring A and D.

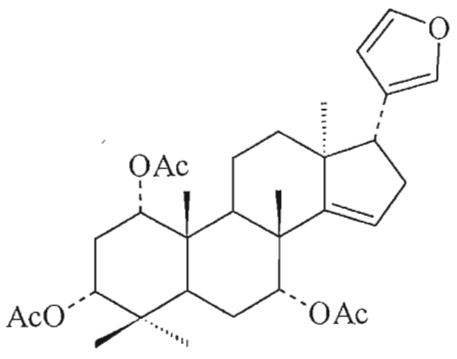


1-31

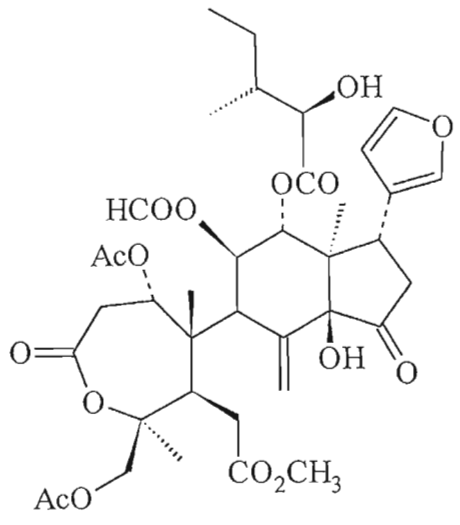
1.2.2.9 Type F limonoids

A typical example of this group is prieurianin (**1-17**). They may be considered to arise from a simple limonoid such as deoxyhavanensin (**1-16**), probably via the type H group, with which

they occur. The prieurianin group limonoids have rings C and D carbocyclic, ring B opened and a ring A lactone.



1-16

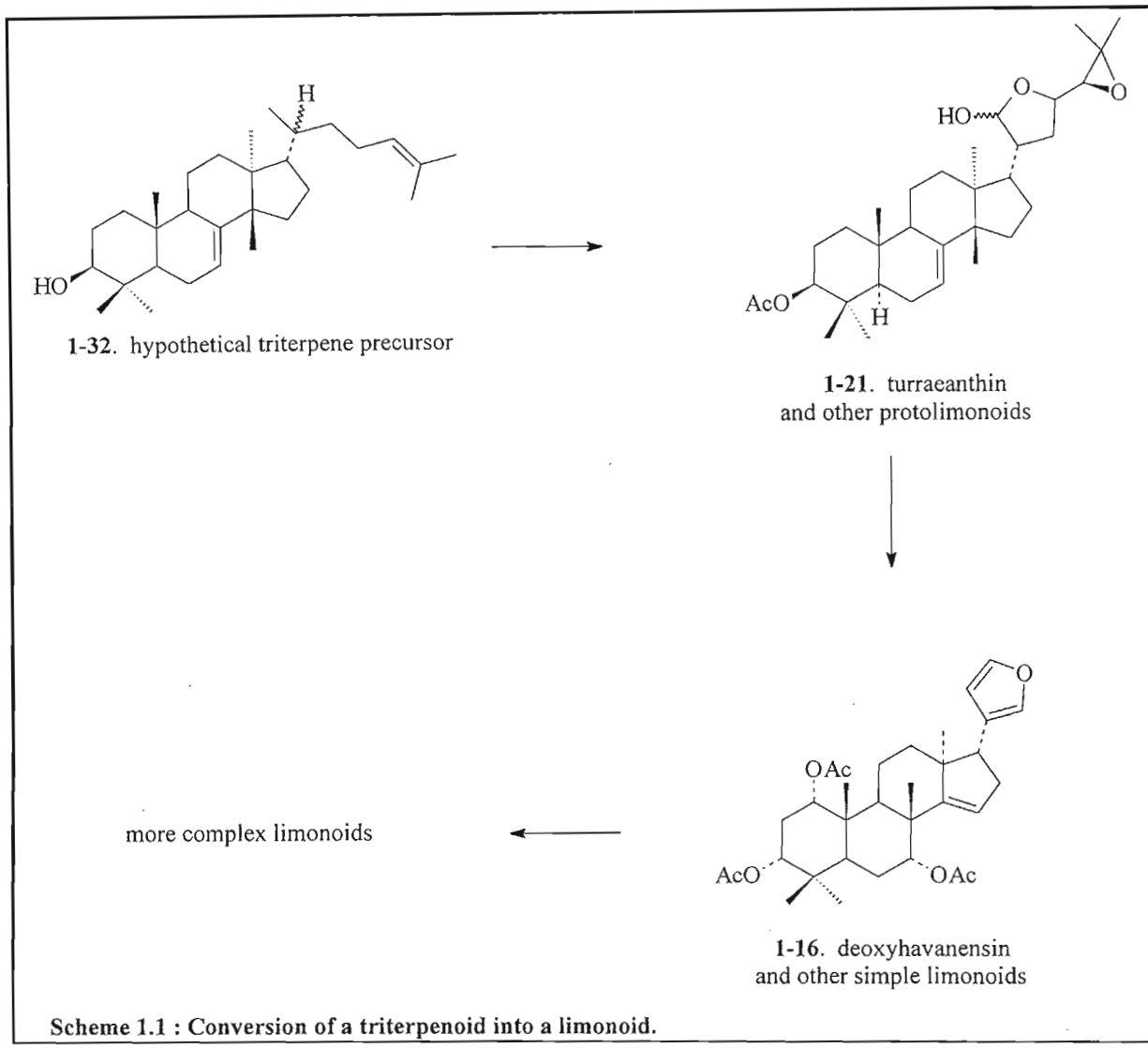


1-17

1.3 Biosynthetic transformations amongst the limonoids

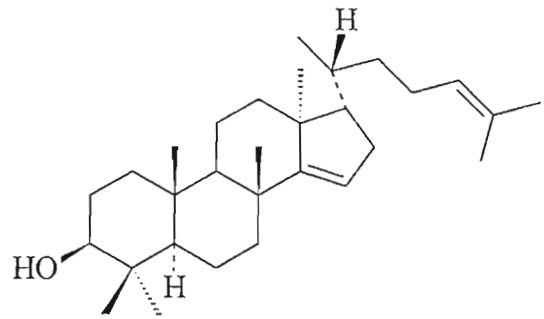
The classic method of studying biosynthetic pathways in living systems is by the feeding of radioactively labelled precursors. This has not been used extensively for the study of the biosynthesis of the limonoids because most limonoids are produced in the timber of large trees which are difficult to handle. Instead, most of the research in this field has been directed towards the isolation of as many different varieties of limonoids as possible. By studying how the types of limonoids are related structurally, it has been possible to propose probable biosynthetic pathways for the major types of limonoids. In many cases, it has been possible to simulate these pathways in the laboratory, thus lending further support to these proposals.

The limonoids are proposed to arise from the triterpene precursor (**1-32**) below;[10] this is oxidised in stages to a protolimonoid such as turraeanthin (**1-21**), which undergoes further changes to give the simple true limonoids, such as deoxyhavanensin (**1-16**). Finally, the simple limonoids are converted into more complex ones (scheme 1.1).



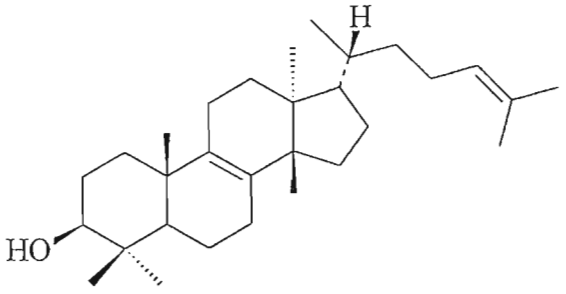
Scheme 1.1 : Conversion of a triterpenoid into a limonoid.

When limonin (**1-14**) was first isolated, Barton *et al.*[5] suggested that limonin (and other limonoids) was derived from a compound with the so-called apo-euphol structure (**1-33**).

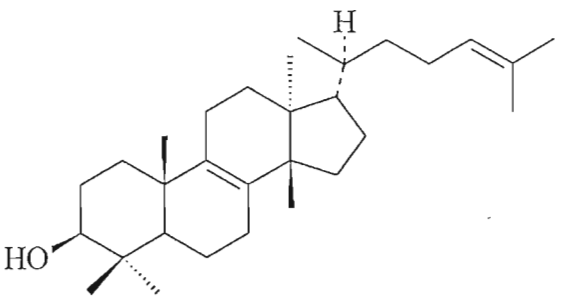


1-33

The apo-euphol structure was in turn thought to be derived from either of the triterpenoids euphol (**1-34**), triucallol (**1-35**) or butyrospermol (**1-36**).[5]



1-34



1-35

For this to occur, two changes needed to be accounted for:

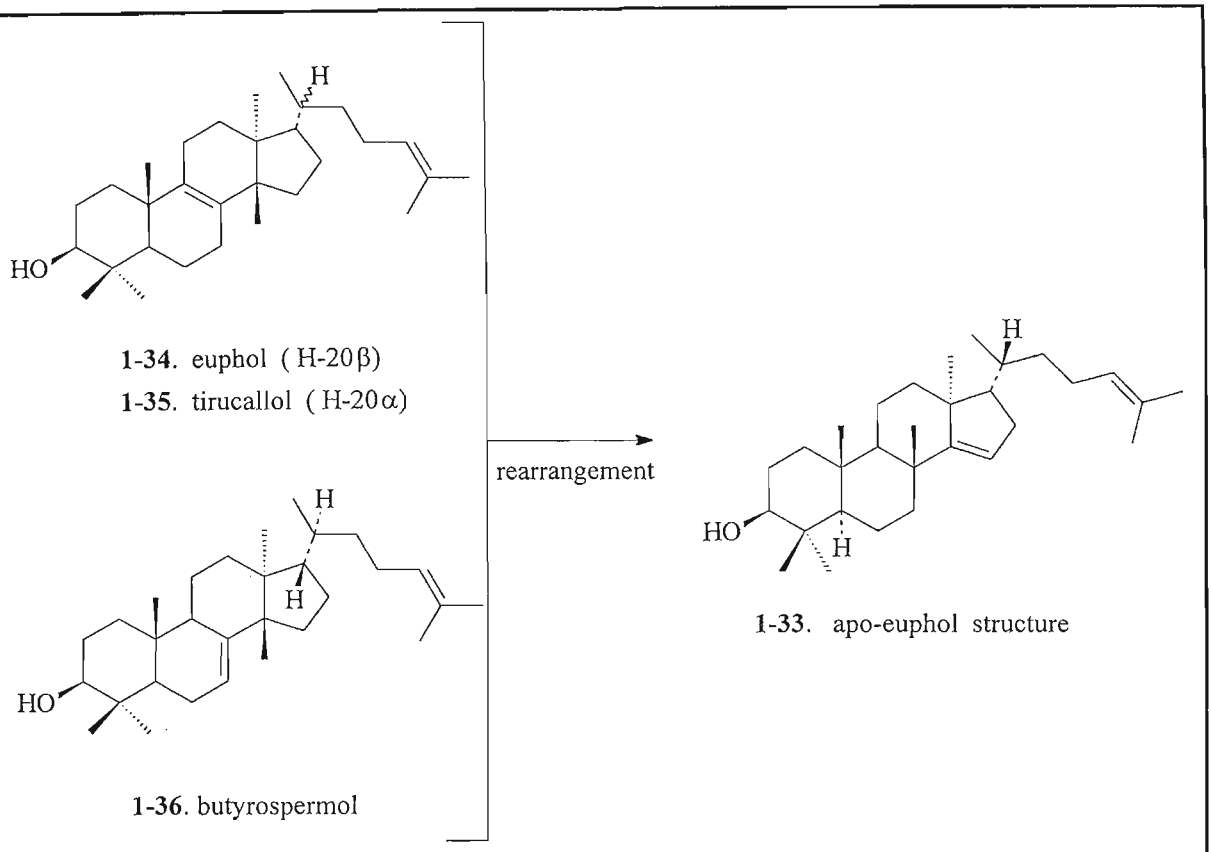
1. migration of the C-14 methyl group to C-8, and formation of a 14,15-double bond (the so-called apo-euphol rearrangement),

2. cleavage of the C-23:C-24 bond and subsequent formation of a β-substituted furan.

Each of these changes will be discussed individually.

1.3.1 The apo-euphol rearrangement

Barton *et al.*[5] suggested that the apo-euphol structure (**1-33**) was derived from either tirucallol (**1-35**) or euphol (**1-34**), by migration of the C-14 methyl group to C-8, or perhaps from butyrospermol (**1-36**), the Δ^7 isomer of euphol, by a similar rearrangement (scheme 1.2). These rearrangements were possibly initiated by oxidative attack on the nuclear double bond.



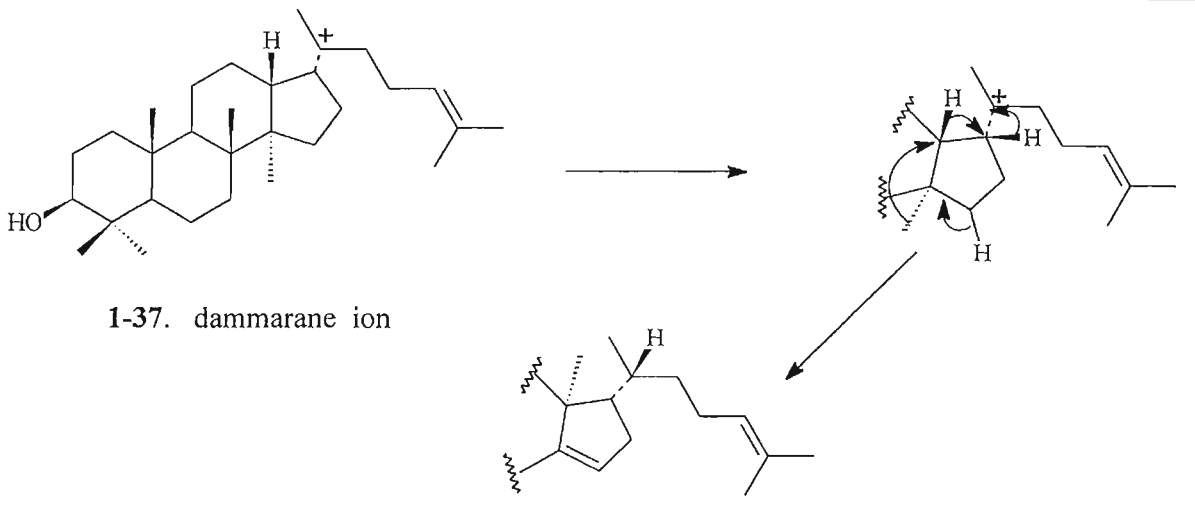
1-34. euphol (H-20β)
1-35. tirucallol (H-20α)

1-36. butyrospermol

1-33. apo-euphol structure

Scheme 1.2 : Rearrangement of euphol (1-34), triucallol (1-35) or butyrospermol (1-36) to yield the apo-euphol structure (1-33).

An alternative formation of the apo-euphol structure, directly from squalene via the dammarane ion (**1-37**) was also proposed:[12,13] (scheme 1.3).

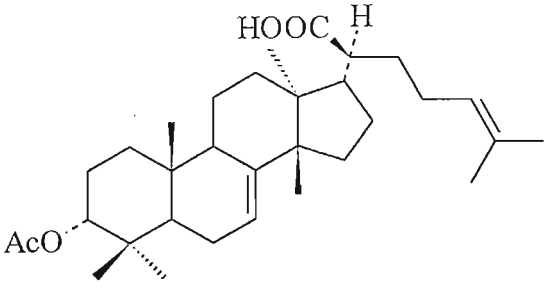


1-37. dammarane ion

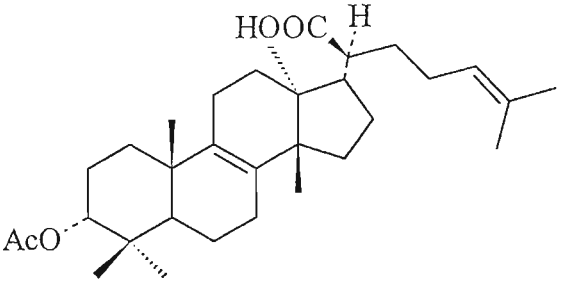
Scheme 1.3 : Formation of the apo-euphol structure via the dammarane ion.

Owing to the fact that C-20 becomes trigonal in the course of the rearrangement, it is not possible to say whether the limonoids arise from the euphol (H-20β) or tirucallol (H-20α) precursor.

The isolation of turraeanthin (**1-21**),[14] a compound having a C-7:C-8 double bond and a side chain more oxidised than butyrospermol (**1-36**), led to the suggestion that the limonoid precursor was tirucalla-7,24-dien-3β-ol, the proposed triterpene precursor (**1-32**) referred to earlier.[14] The hypothetical parent compound (**1-32**) has been found naturally as the 20α-butyrospermol (**1-36**) isomer, along with the isolation of two closely related acids, acetyl-Δ^7-elemolic acid (**1-38**) and acetyl-Δ^8-elemolic acid (**1-39**).[15]

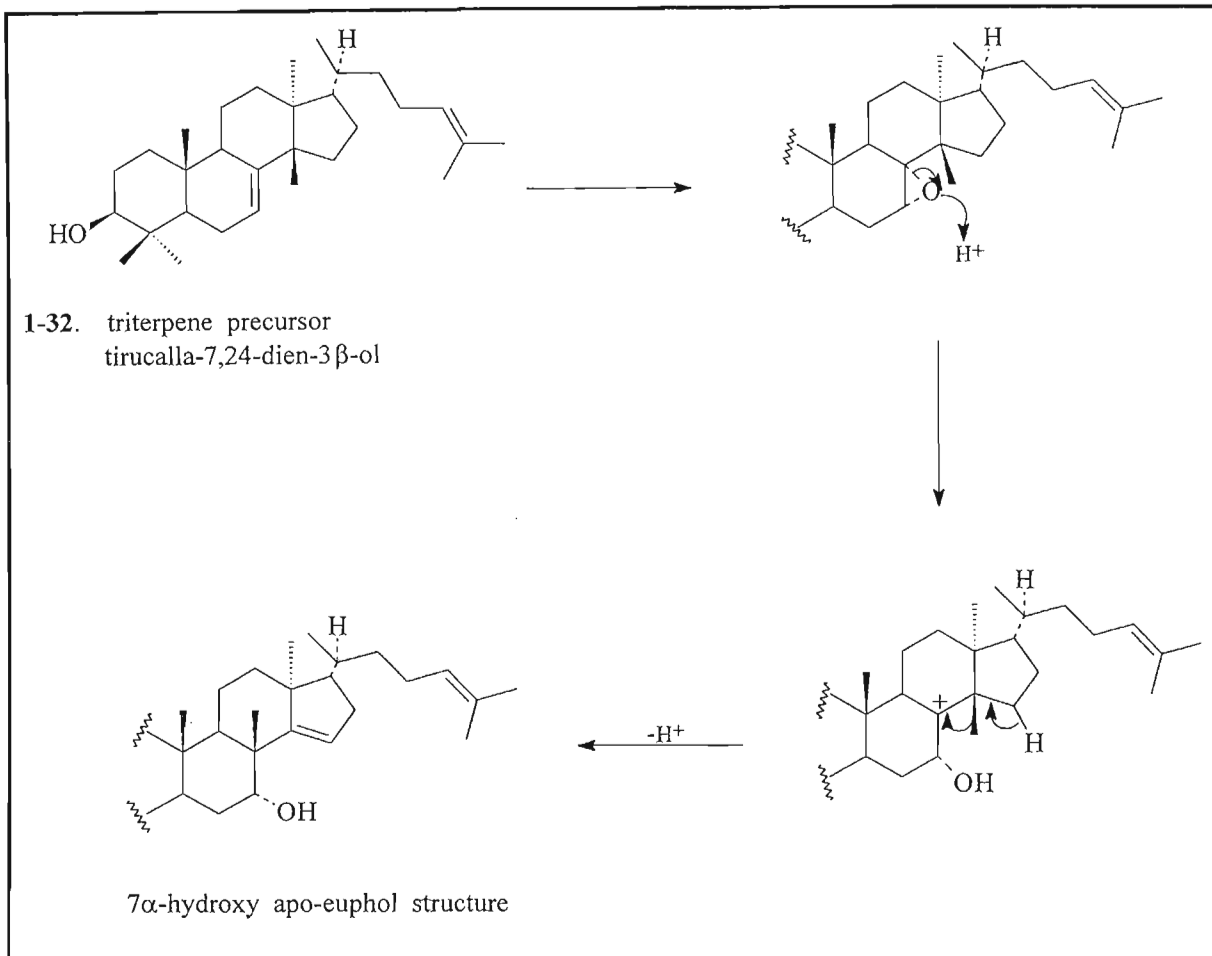


1-38. acetyl-Δ^7-elemolic acid



1-39. acetyl-Δ^8-elemolic acid

Halsall *et al.* have suggested a mechanism whereby the tirucalla-7,24-dien-3β-ol (**1-32**) might be transformed to the apo-euphol structure (**1-33**).[13] They noted that all naturally-occurring compounds having the apo-euphol structure (**1-33**) are oxygenated at C-7. Furthermore, when this takes the form of a hydroxy group, it has the α-configuration. Knowing that epoxy-groups are fairly common amongst the limonoids, they proposed that the biogenesis of the apo-euphol structure (**1-33**) involved the formation of the 7α,8α-epoxide of the hypothetical parent compound (**1-32**), followed by opening of the epoxide ring with rearrangement to give the 7α-hydroxy-apo-euphol structure, scheme 1.4.

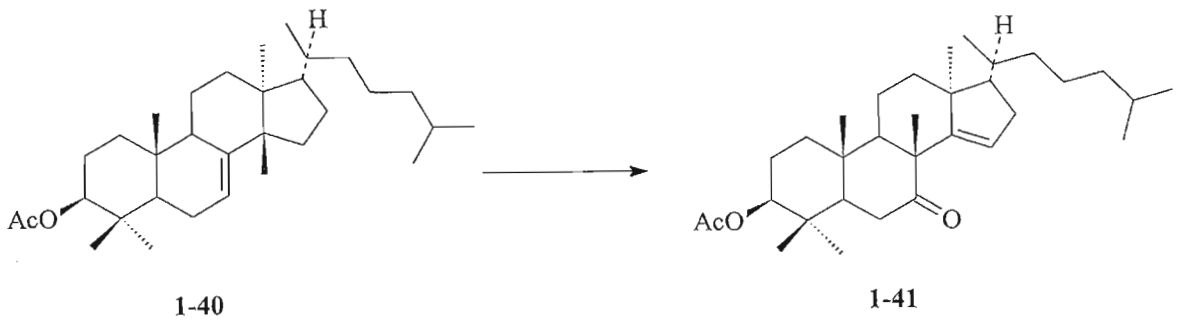


1-32. triterpene precursor
tirucalla-7,24-dien-3β-ol

7α-hydroxy apo-euphol structure

Scheme 1.4 : Formation of the 7α-hydroxy apo-euphol structure from a triterpene.

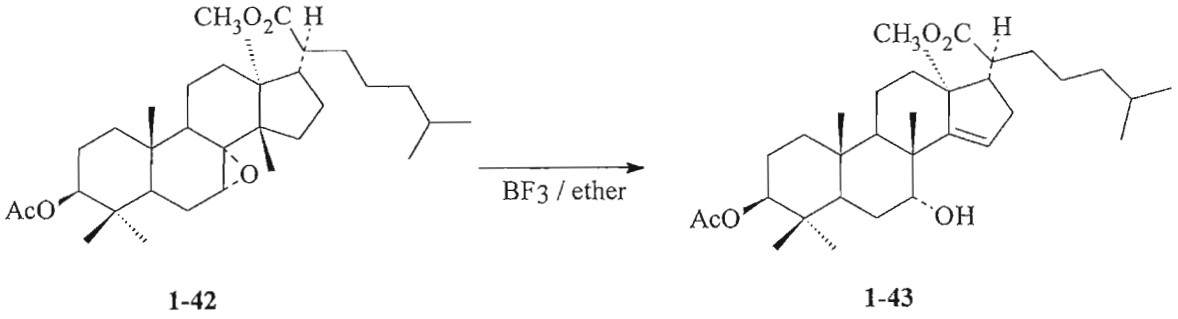
A similar conversion in the euphol series had been carried out by Spring *et al.*[16] They transformed dihydrobutyrospermyl acetate (**1-40**) to 7-oxo-apo-euph-14-en-3β-yl acetate (**1-41**) in the laboratory, scheme 1.5.



1-40 1-41

Scheme 1.5 : Conversion of dihydrobutyrospermyl acetate (1-40) to 7-oxo-apo-euph-14-en-3 β-yl acetate (1-41).

The transformation proposed by Halsall *et al.*[13] was achieved in the laboratory by the rearrangement of the 7α,8α-epoxide of methyl acetyl-Δ^7-dihydroelemolate (**1-42**) with boron trifluoride-etherate to give the 7α-hydroxy-apo-euphol derivative (**1-43**), scheme 1.6.



1-42 1-43

Scheme 1.6 : Rearrangement of 7 α,8α-epoxide of methyl acetyl-Δ^7-dihydroelemolate (1-42) to give the 7 α-hydroxy-apo-euphol derivative (1-43).

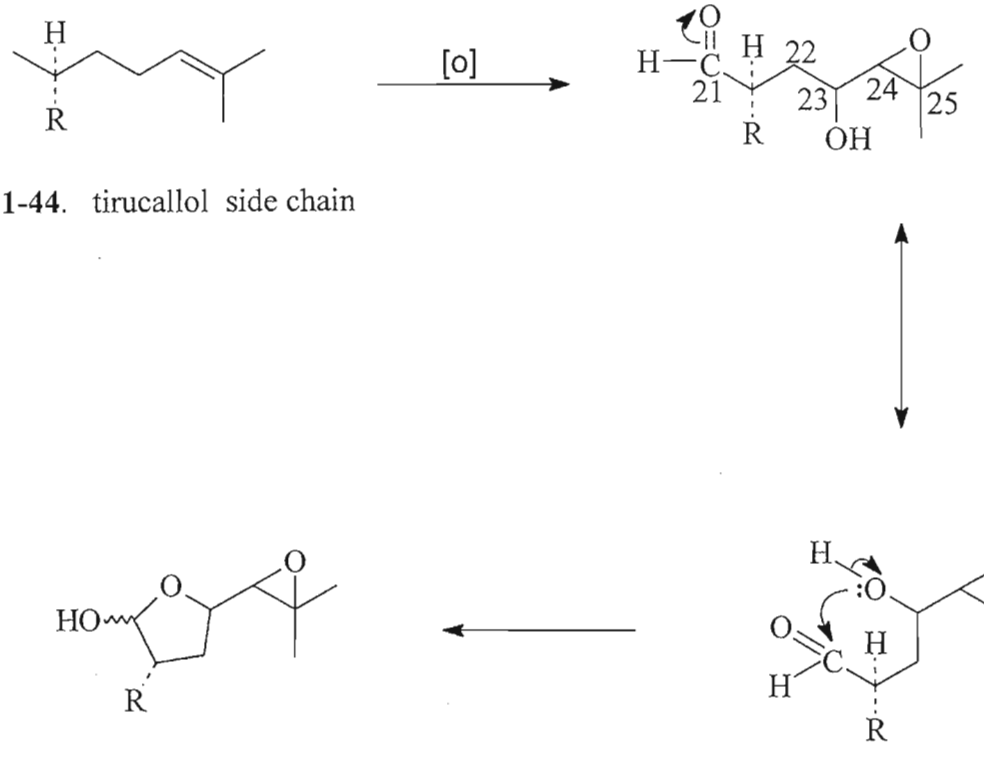
1.3.2 Formation of the furan ring

The modification of the aliphatic side chain in tirucallol (**1-35**), for example, to a β-substituted furan ring will now be considered. Barton *et al.* suggested that cleavage between C-23 and C-24 was necessary to arrive at the β-furan ring of the limonoids, although a mechanism was not suggested.[5]

In this case also, the isolation of turraeanthin (**1-21**) provided some clues to the possible biosynthesis of the furanyl ring, since turraeanthin (**1-21**) and other protolimonoids have side

chains intermediate in complexity between that of tirucallol (**1-35**) and the furans of the limonoids.

Halsall *et al.* proposed that the simple tirucallol side chain (**1-44**) is oxidised in stages to produce an aldehyde group at C-21, a hydroxy group at C-23, and an epoxide in place of the C-24:C-25 double bond.[14] The hydroxy group at C-23 then cyclises onto the aldehyde carbonyl group to form the hemiacetal ring in the turraeanthin side chain (**1-45**), scheme 1.7.



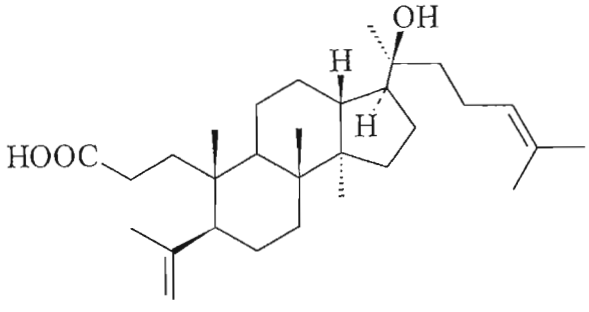
1-44. tirucallol side chain

1-45. turraeanthin side chain

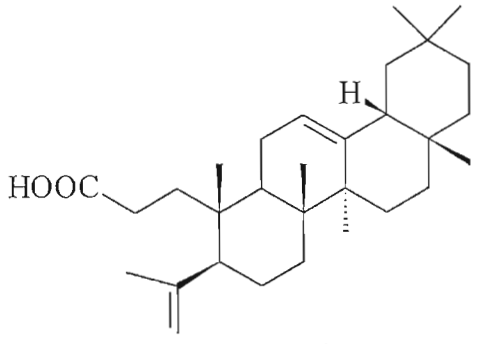
Scheme 1.7 : Formation of the furan ring (1-45) from the tirucallol side chain (1-44).

A model for the oxidation of the turraeanthin side chain (**1-45**) to the furan ring was also outlined by Bevan *et al.*[14] It was proposed that a keto group was formed at C-24, either by rearrangement of the epoxide, or by the formation of the diol from the epoxide and subsequent oxidation of the C-24 hydroxy group to a ketone. The ketone thus formed was envisaged as

undergoing a Baeyer-Villiger oxidative cleavage of the C-23:C-24 bond. Similar cleavages result in the opening of ring A in dammarenolic (**1-46**) and nyctanthic (**1-47**) acids,[17,18] and in the opening of ring B in methyl angolensate (**1-28**) and related compounds. This cleavage results in the loss of the four carbon fragment originally required by Barton *et al.*[5]

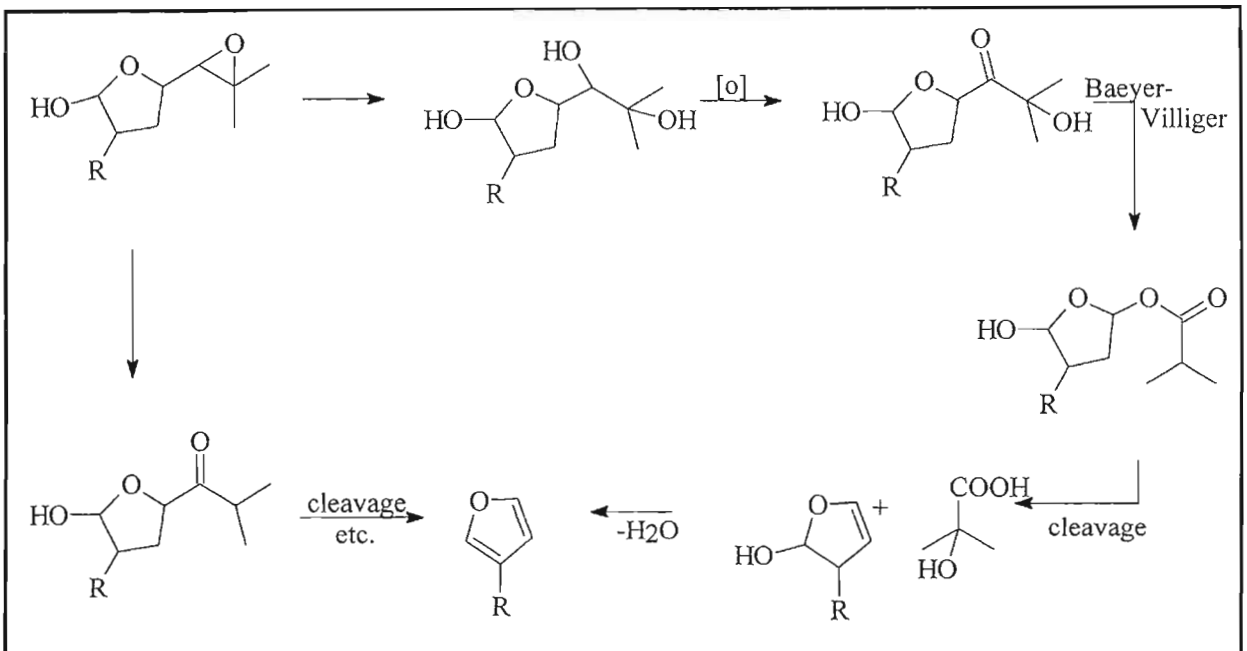


1-46



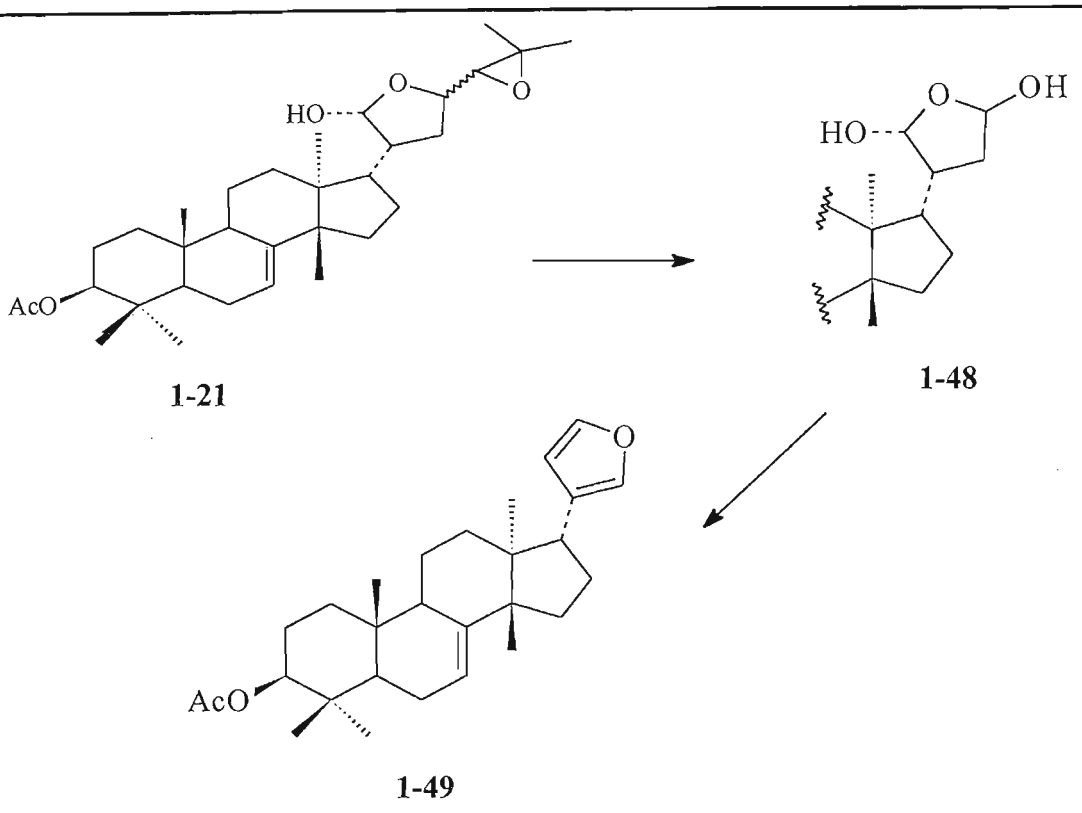
1-47

The Baeyer-Villiger cleavage would result in a dihydrofuran ring, which may be dehydrated to yield a β-substituted furan ring, scheme 1.8.



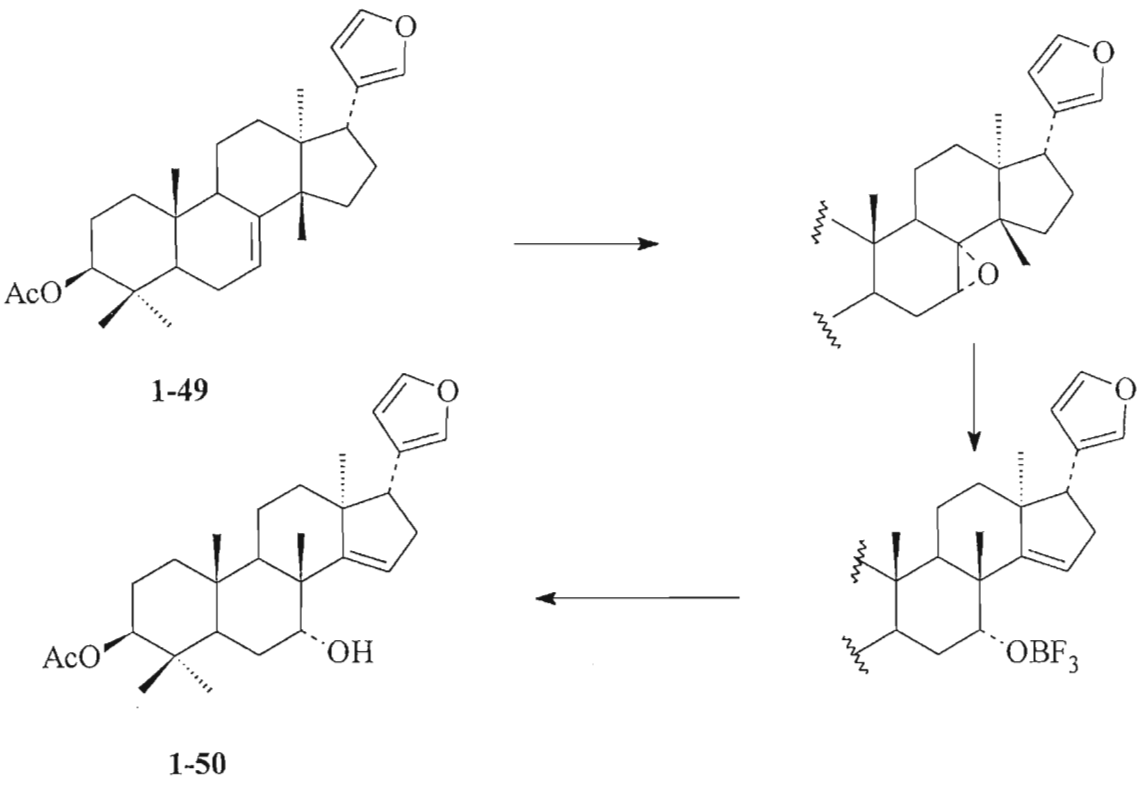
Scheme 1.8 : Baeyer-Villiger oxidation of the turraeanthin side chain (1-45) into a furan ring.

This process has been simulated in the laboratory[19,20] : turraeanthin (**1-21**), on treatment with sodium metaperiodate in aqueous dioxane containing a trace of perchloric acid, was converted into the hemiacetal (**1-48**). This was then dehydrated with *p*-toluenesulphonic acid in benzene, to give the furan ring (**1-49**), scheme 1.9.



Scheme 1.9 : The formation of the furan ring from turraeanthin (1-21) via the hemiacetal (1-48).

The apo-euphol rearrangement was also performed on this compound (**1-49**).[19,20] The 7α,8α-epoxide was formed with monoperphthalic acid at -3^{0}C, and this was rearranged using boron trifluoride-etherate, as before, to obtain the 7α-hydroxy-apo-compound (**1-50**, scheme 1.10.).



Scheme : 1.10 : Apo-euphol rearrangement of compound (1-49) resulting in the formation of the 7α-hydroxy apo-compound (1-50).

These two sets of reactions constitute the transformation of a protolimonoid into a simple limonoid.

Since the apo-euphol rearrangement has been demonstrated with compounds with both oxidised and hydrocarbon side chains, it is difficult to say whether it precedes or follows the formation of the β-furan ring. However, the fact that compounds such as grandifoliolenone (**1-22**) have been isolated, where the apo-euphol rearrangement has taken place and no furan ring is present, together with the fact that no compound with a C-7:C-8 double bond and furan ring has been isolated, tends to suggest that the apo-euphol rearrangement may precede furan ring formation.

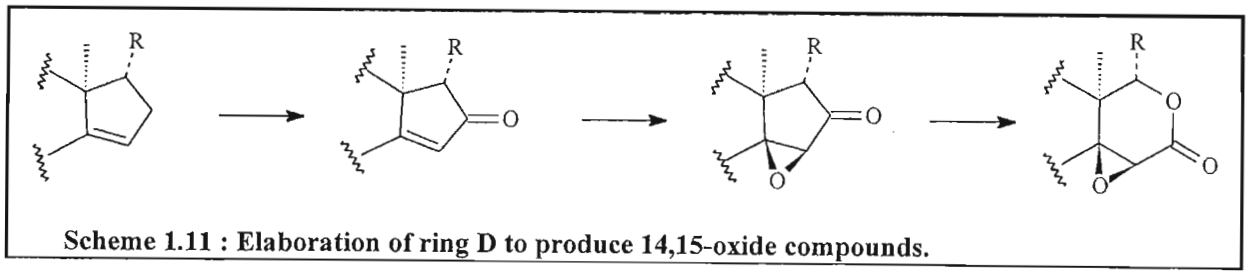
1.3.3 Elaboration of the limonoid structure

The first major step in the biosynthesis of the limonoids, viz. the formation of simple limonoids from triterpene precursors via the protolimonoids, has now been discussed. The next stage to be considered is the conversion of the simple limonoids into more complex ones. Most of the changes, even leading to the most complex limonoids, are oxidative, and it is envisaged that these oxidations are brought about by peroxidase enzymes in the plant. The essentially oxidative nature of the biosynthetic changes has already been seen in the formation of the furan ring. The classification of the limonoids discussed earlier, and the flow-chart drawn up here, will aid in this discussion, as the limonoids were discussed in increasing order of oxidative complexity.

1.3.3.1 Elaboration of ring D

In the classification discussed earlier, it was seen that two modifications of ring D occur. The first is the oxidation of the carbocyclic ring, to obtain an epoxy-lactone, as in gedunin (**1-15**); the second type is where ring D remains carbocyclic, but the C-14:C-15 double bond may be oxidised to give 14,15-oxide compounds and 14-hydroxy, 15-keto compounds, such as the heudelottins (**1-23, -24, -25**) and prieurianin (**1-17**).

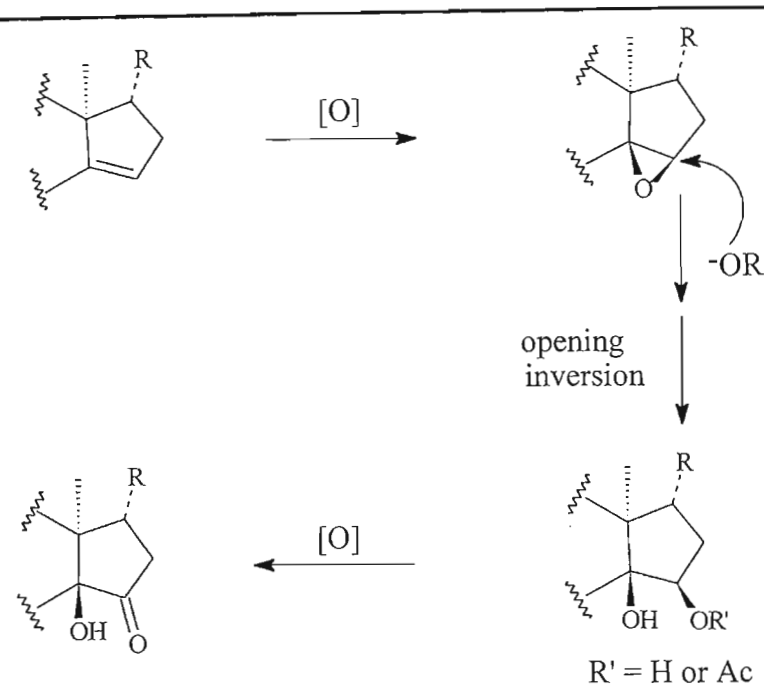
Barton *et al.* have proposed a scheme for the oxidation of ring D to obtain the δ -lactone structure.[5] This involves allylic oxidation of ring D to give a cyclopentenone ring, followed by epoxide formation, and finally, further oxidation of the ketone to give an α,β-epoxy-δ-lactone structure as in gedunin (**1-15**). This sequence is shown in scheme 1.11, below:



Scheme 1.11 : Elaboration of ring D to produce 14,15-oxide compounds.

This theoretical sequence is supported by the fact that compounds representing each stage of the sequence have been isolated. The second type of ring D structure i.e. ring D carbocyclic,

as found in compounds like prieurianin (**1-17**), arises from the oxidation of the C-14:C-15 double bond only, as follows (scheme 1.12) :[21]

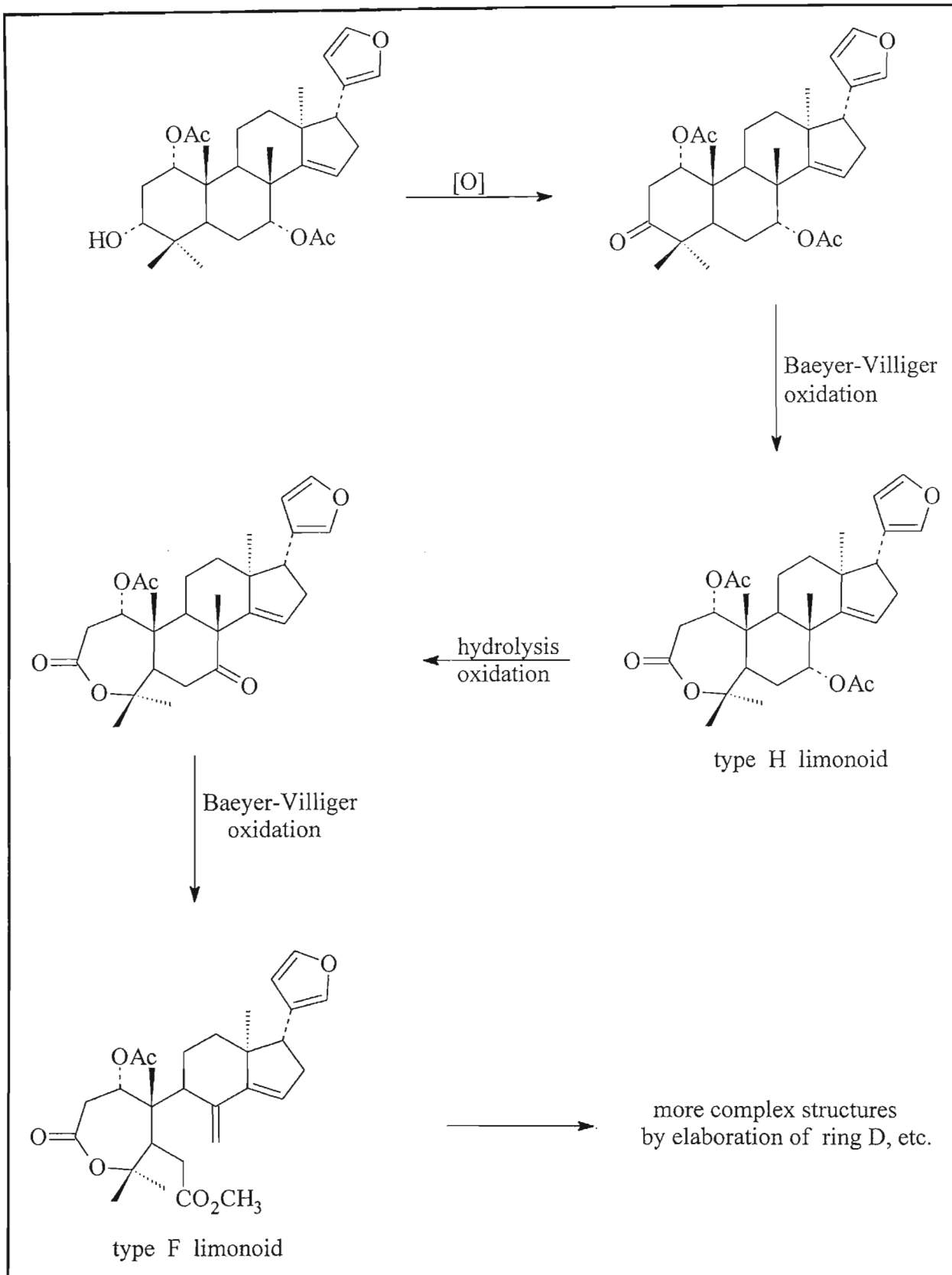


Scheme 1.12 : Elaboration of ring D to produce 14-hydroxy, 15-keto compounds.

In this case too, the pathway is supported by the fact that compounds with all the stages of oxidation shown above have been isolated.

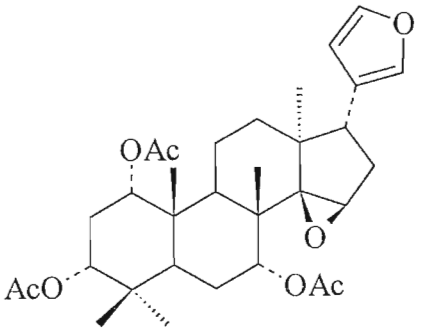
1.3.3.2 Biogenesis of type H and F limonoids

Limonoids related to prieurianin (**1-17**), (type F) and dregeanin (**1-28**), (type H) appear to arise from precursors similar to havanensin (**1-51**) or deoxyhavanensin (**1-16**), by successive oxidative stages.[8] It would appear that oxidation of ring A by a Baeyer-Villiger reaction occurs first (scheme 1.13), since the dregeanin-type limonoids, which seem to be intermediate in complexity between deoxyhavanensin (**1-16**) and prieurianin (**1-17**), have ring B carbocyclic.

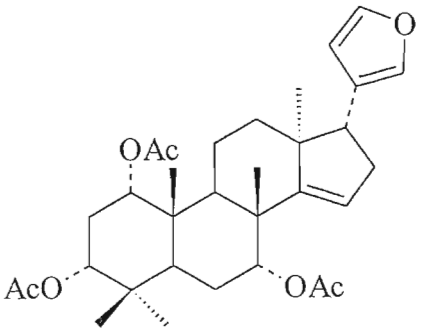


Scheme 1.13 : Biogenesis of type F limonoids.

The oxidation of ring A is followed by a Baeyer-Villiger oxidation and cleavage of ring B, similar to that proposed for the cleavage of the side chain in the furan ring biogensis. This yields the basic skeleton of limonoids related to prieurianin (**1-17**), scheme 1.13.



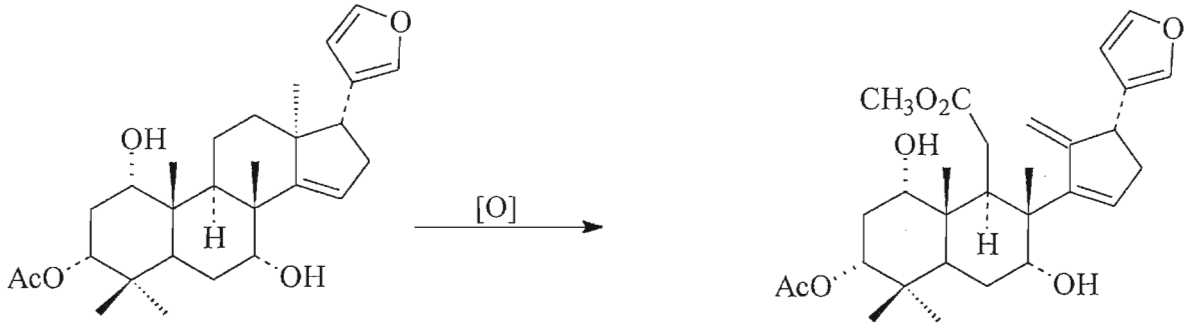
1-51



1-16

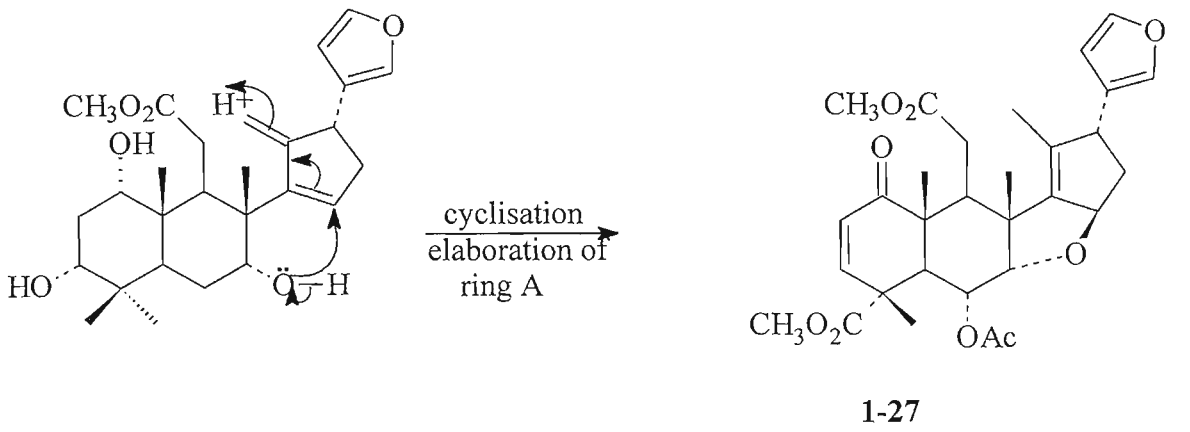
1.3.3.3 Biogenesis of type G limonoids

The type G limonoids, with only ring C opened, appear to arise from a precursor related to deoxyhavanensin (**1-16**) by a Baeyer-Villiger oxidation of ring C,[10] scheme 1.14.



Scheme 1.14 : Type B limonoids oxidised to type G limonoids.

More complex compounds, such as nimbin (**1-27**), arise from this basic skeleton by attack on the double bond at C-15 by the C-7 hydroxy group, and elaboration of ring A[10], scheme 1.15.

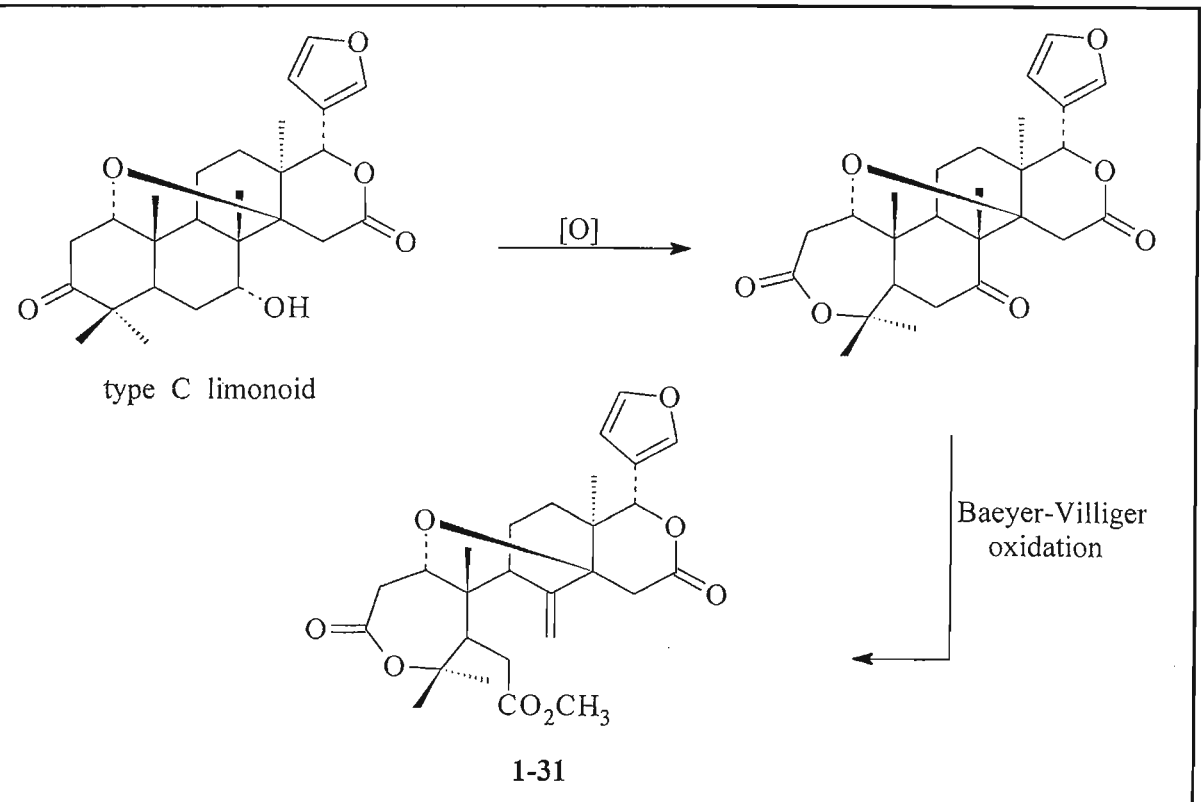


1-27

Scheme 1.15 : Mechanism for the cyclisation and elaboration of ring A.

1.3.3.4 Biogenesis of type J limonoids

These have the gedunin (**1-15**) ring D lactone, a ring A lactone, and ring B opened. This arrangement seems to have arisen from the oxidation of ring A, and oxidative cleavage of ring B, starting from a type C limonoid. An example of the probable biogenesis of methyl ivorensate (**1-31**) is shown below, scheme 1.16.[10]

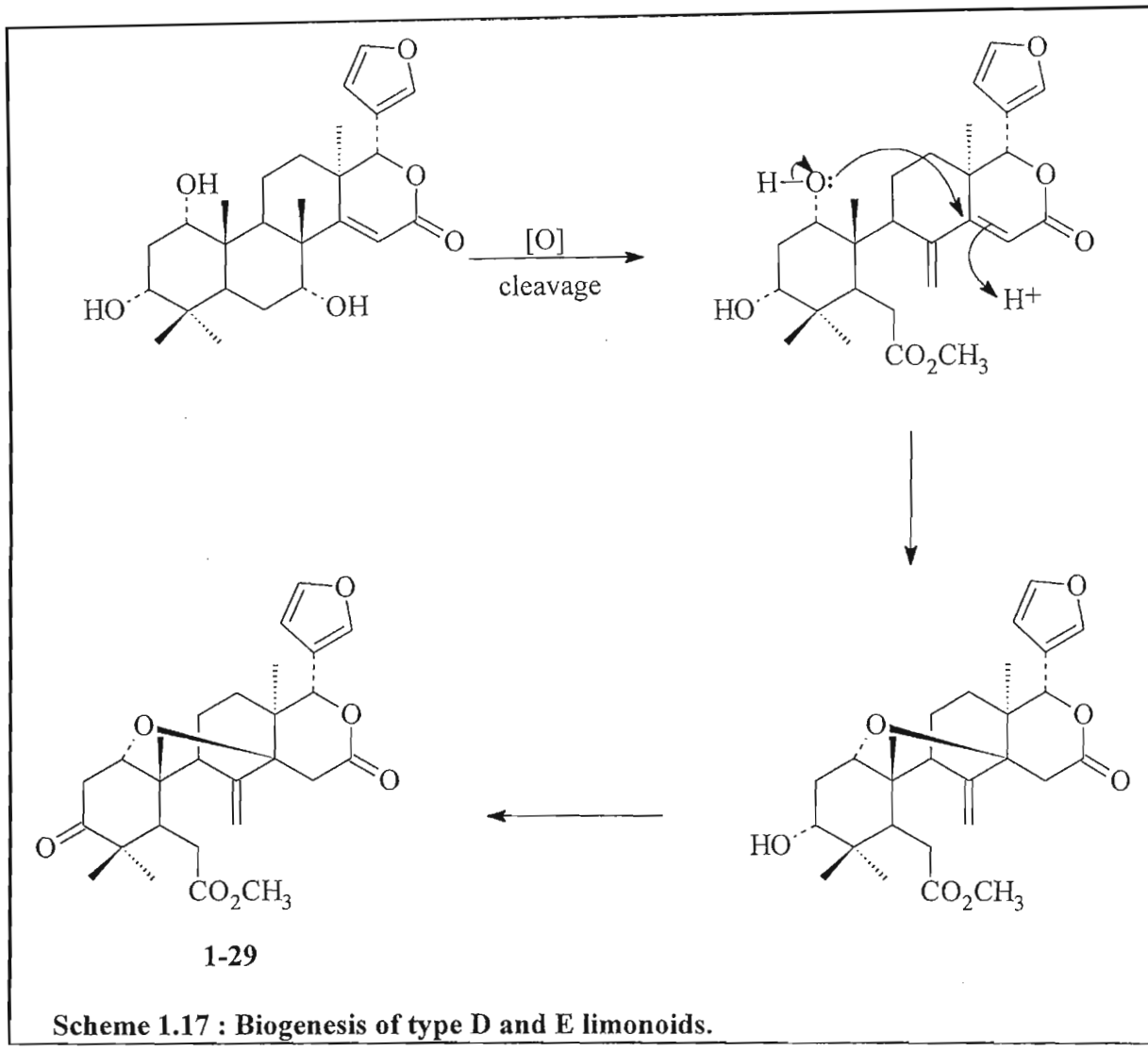


1-31

Scheme 1.16 : The probable biogenesis of methyl ivorensate.

1.3.3.5 Biogenesis of type D and E limonoids

The type D compounds appear to arise from the gedunin (**1-15**) group of limonoids by oxidative opening of ring B, as proposed for the biogenesis of the prieurianin (**1-17**) limonoids. Subsequent cyclisations then occur to yield the various structures found in this class.[8]

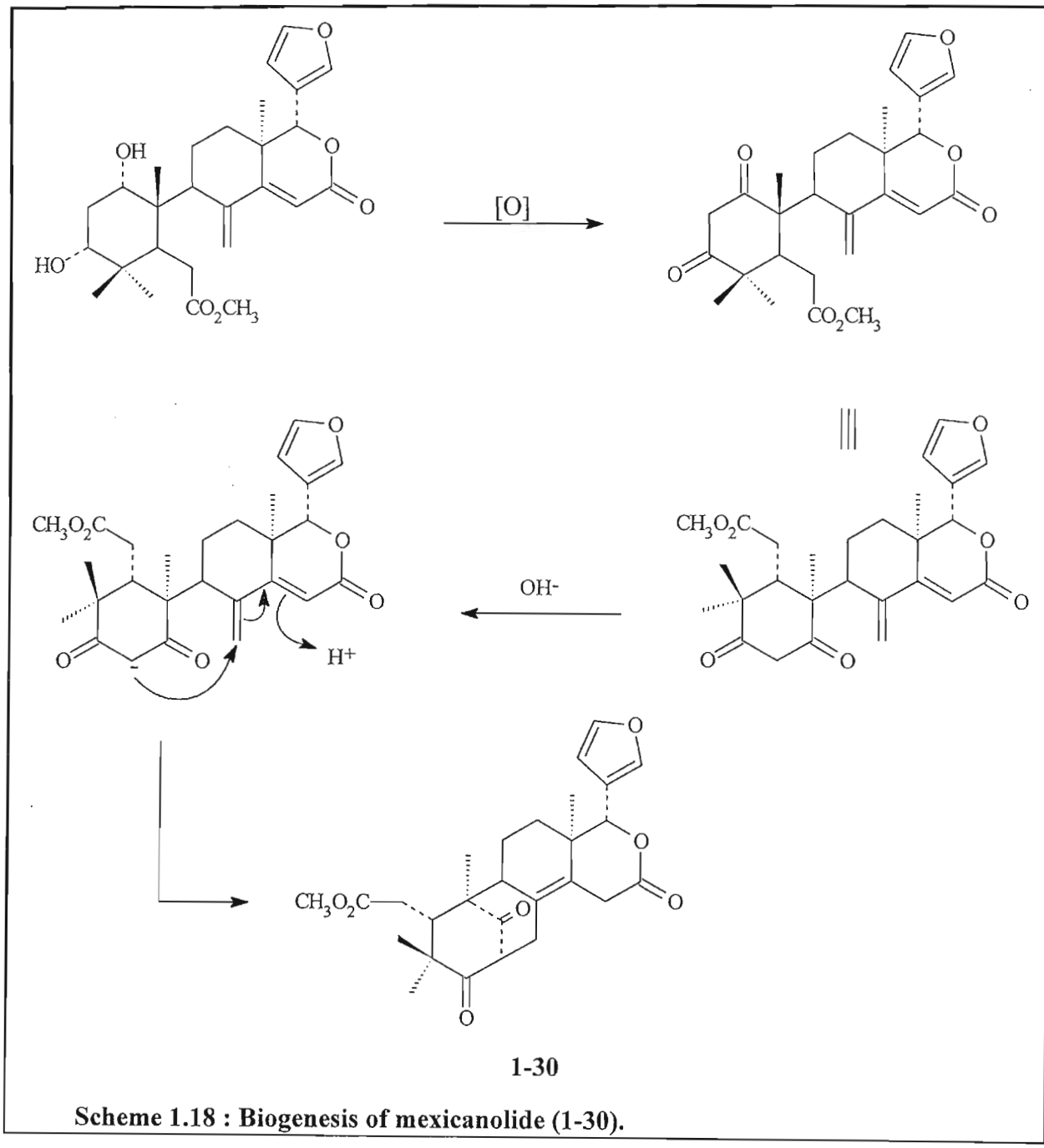


1-29

Scheme 1.17 : Biogenesis of type D and E limonoids.

In the methyl angolensate (**1-29**) subgroup, after opening of ring B, the hydroxy group at C-1 attacks the double bond at C-14, to form a 1,14-bridge[8], scheme 1.17.

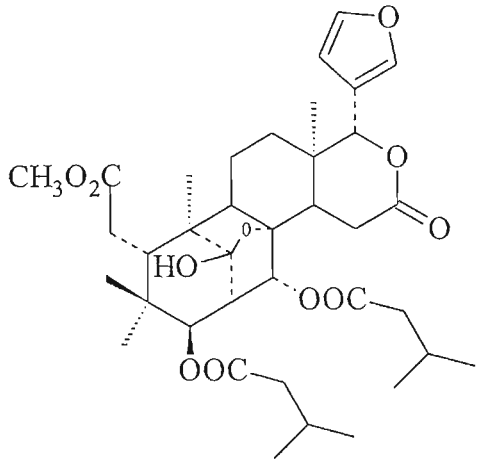
In order to achieve the mexicanolide (**1-30**) structure, it was proposed that after opening of ring B and oxidation of the hydroxy moieties at C-1 and C-3 to carbonyls, rotation about the C-9:C-10 bond occurrs, followed by a Michael-type addition of C-2 to the double bond at C-30[8,10], scheme 1.18.



1-30

Scheme 1.18 : Biogenesis of mexicanolide (1-30).

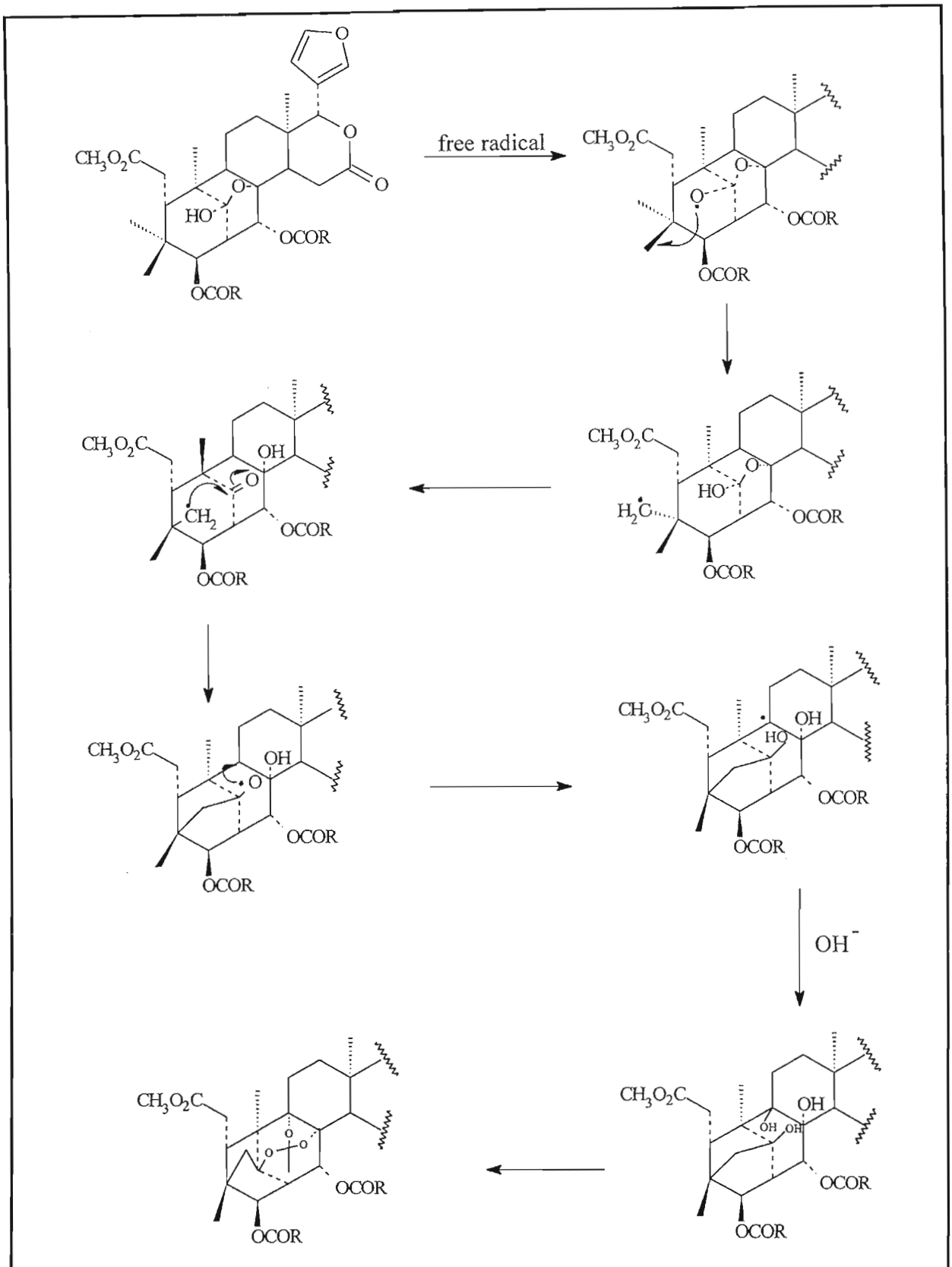
It is thought that type E limonoids, with a bridged ring A, arise from compounds such as xyloccensin B (**1-52**), which, although they belong to the mexicanolide group, have structures intermediate between the type D and E limonoids.[8]



1-52

A mechanism for the formation of the ring A bridge has been proposed,[8] starting with a compound such as xyloccensin B (**1-52**), which has a C-1:C-8 hemiacetal link. The reaction sequence was proposed to be initiated by the formation of an oxygen centred radical at C-1 (by attack of a peracid radical). The oxygen centred radical abstracts a hydrogen atom from one of the methyl groups at C-4 (C-29), thus forming a carbon centred radical there, which attacks C-1 again, the hemiacetal bridge having opened. This would form the bridge across ring A. The oxygen centred radical left at C-1 could then react further, oxidising C-9 and eventually forming a hemi-*ortho*-ester. These changes are shown below in scheme 1.19.

The transformations leading from deoxyhavanensin (**1-16**) to the ring D lactones of gedunin (**1-15**), and the subsequent conversion of gedunin (**1-15**) to compounds like mexicanolide (**1-30**) and methyl angolensate (**1-29**), have been achieved in the laboratory.[22-26] This fact, together with the fact that the reactions are either spontaneous cyclisations or simple oxidations, confirms that these are most probably the biosynthetic routes that occur *in vivo*. The biogenesis of the type E limonoids is at the moment a hypothesis. The existance of compounds such as xyloccensin B (**1-52**), seems to indicate that it is probable, but it appears that further study of this pathway is necessary.

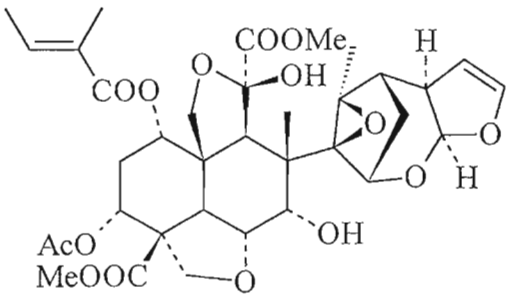


Scheme 1.19 : Mechanism for the formation of a ring A bridge.

1.4 Bioactivity of limonoids

Limonoids display a wide range of biological activities, including insect anti-feedant, growth regulating, anti-fungal, bactericidal, antiviral and anti-leukaemic properties.[27]

Limonoids may act as insect anti-feedants, e.g. azadirachtin (**1-53**),[28] which is known to affect over 200 species of insects and mites,[29] although azadirachtin (**1-53**) does not appear to be directly insecticidal.



1-53

Havanensin (**1-51**) - type compounds, as well as limonoids of the prieurianin (**1-17**) - type have been found to exhibit anti-cancer activity.[30]

Limonin (**1-14**) is among the *Citrus* limonoids whose biological properties have been extensively investigated. These compounds were found to affect glutathione S-transferase (GST) activity,[31-33] the enzyme responsible for the detoxification of xenobiotics (e.g. carcinogens). Limonin was also tested as a tumour development inhibitor. It showed inhibitor activity against benzo[a]pyrene - induced neoplasma of mice.[34]

Information on the biological activity of several limonoids is available.[35-37]

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FOREWORD TO EXPERIMENTAL

(Chapters 2 to 6)

A. Nuclear magnetic resonance spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded at the University of Natal, Durban on a 300 MHz Gemini spectrometer, and at the Universty of Nijmegen, Holland on a 400 MHz Bruker spectrometer, using $CDCl_3$, CD_3OD or D_2O as solvents. The spectra were referenced against the central line of the deuteriochloroform signal at δ_C 77.0 ppm, the chloroform singlet at δ_H 7.24 ppm, the deuteriomethanol signal at δ_C 49.0 ppm, the methanol singlet at δ_H 4.10 ppm or δ_H 3.34 or the deuterium oxide singlet at δ_H 4.61 ppm.

B. Infrared spectroscopy

Infrared spectra were recorded with a Nicolet Impact 400 D spectrometer which was calibrated against an air background, using KBr discs or NaCl cells with chloroform as solvent.

C. Melting points

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

D. Optical rotations

Optical rotations were measured at room temperature in chloroform using an Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (4 X 200 mm) unjacketed flow tube. Concentrations are quoted in g/ml.

E. General chromatography

Silica gel (0.2 mm) containing fluorescent indicator (F_{254}) on aluminium backed plates (Merck: Art 5554) was used for t.l.c analysis and silica gel (Merck Art: 9385, 0.040-0.053 mm particle size, 2300-400 mesh ASTM) was used for flash chromatography. The t.l.c plates were

developed using anisaldehyde: conc.H_2SO_4: methanol [1:2:97] as spray reagent, followed by heating.

The solvent system employed was a mixture of hexane, dichloromethane and ethyl acetate in the appropriate optimized ratios for the particular separation.

F. Mass spectrometry

GC/MS spectra were recorded using a Finnigan 1020 GC/MS spectrometer using both injection and solid probe methods. High resolution mass spectra were recorded by Dr. P. Boshoff at the Cape Technikon.

G. Extraction of plant material

Wood samples were milled, while bark and seed samples were ground in a coffee grinder before soxhlet extraction with an appropriate solvent (see individual chapters for the specific solvent used) for 12 hours. The gum obtained after extraction was then chromatographed on a gravity column and further purification was achieved by repeated chromatography on the gravity column.

H. Acetylation reaction

The compound to be acetylated was dissolved in pyridine (3ml) and treated with acetic anhydride (3ml). The mixture was temporarily heated and allowed to stir overnight at room temperature.

CHAPTER 2

Extractives from *Aphanamixis polystacha*

Index

Chapter 2

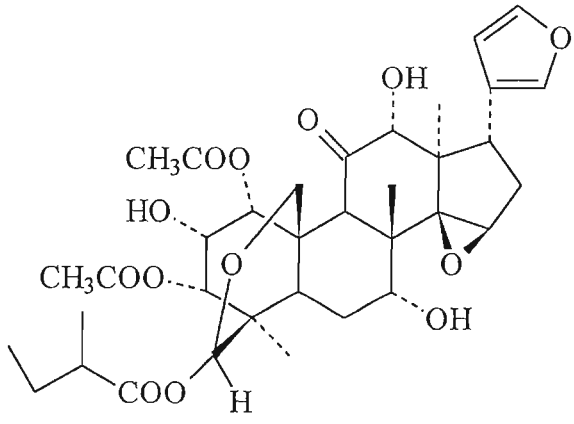
Extractives from *Aphanamixis polystacha*

2.1 Introduction

The genus *Aphanamixis* belongs to the Meliaceae family and is indigeneous to the Indian sub-continent. Within the genus *Aphanamixis*, two species have been previously examined, namely *Aphanamixis grandifolia* and *Aphanamixis polystacha*. A literature search was undertaken to become acquainted with the type of compounds naturally synthesised by the genus *Aphanamixis*.

2.1.1 *Aphanamixis grandifolia*

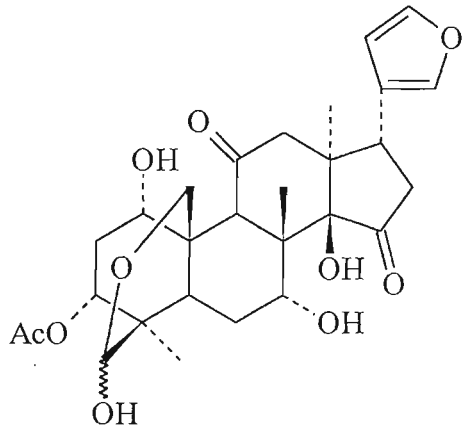
Five compounds, of which three were limonoids and two sesquiterpenoids were reported to have been isolated from the species *Aphanamixis grandifolia*. Polonsky *et al.* (1978)[1] reported the isolation and structure of aphanastatin* (**2-1**) from the seeds of the Eastern Himalayan plant *Aphanamixis grandifolia*. The author reported that the extracts prepared from the seeds markedly inhibited the growth of the murine lymphocytic leukemia P388 cells.



2-1

* The Dictionary of Natural Products incorrectly reports that aphanastatin was isolated from *Aphanamixis grandiflora*. An examination of the paper revealed that aphanastatin was isolated from the species *Aphanamixis grandifolia* and that *Aphanamixis grandiflora* does not exist as a separate species.

Polonsky *et al.* (1979)[2,3] reported the isolation of two limonoids, named amoorastatone (**2-2**) and amoorastatin (**2-3**). These compounds belong to type B limonoids (chapter 1, section 1.2.2.2) and have the characteristic pentacyclic nucleus, with all rings carbocyclic. These compounds have a similar skeletal structure to aphanastatin (**2-1**) but display a greater potency to inhibit cell growth of the murine lymphocytic leukemia P388 cells.



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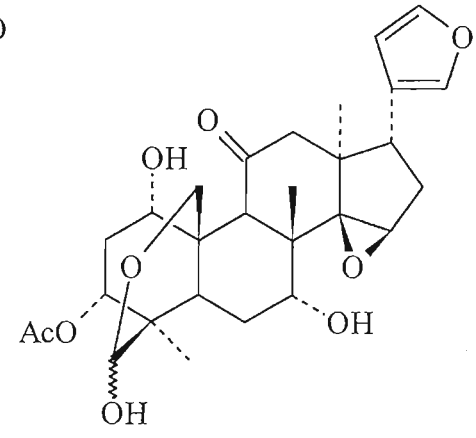
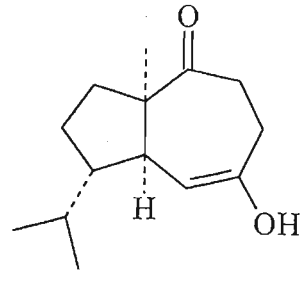


2-3

Compounds (**2-2**) and (**2-3**) were found to exhibit significant antihelmintic and insect antifeedant properties.

Nishizawa *et al.* (1984)[4] isolated two sesquiterpenoids, named aphanamol I (**2-4**) and aphanamol II (**2-5**) from the dried fruit peel of *Aphanamixis grandifolia*; these compounds exhibited minor toxic properties and have been traditionally employed as fish or dart arrow poisons.



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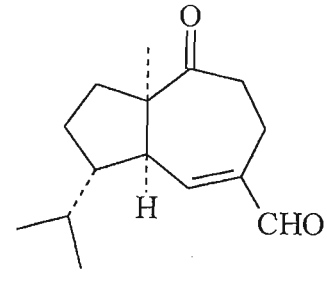

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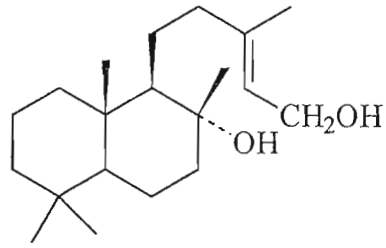
2.1.2 *Aphanamixis polystacha*

Aphanamixis polystacha (Wall.) J.N. Parker {synonyms, *Amoora rohituka* Wight et Arn, *Aphanamixis rohituka* (Roxb)}, is an Indo-Malayan member of the Meliaceae family.

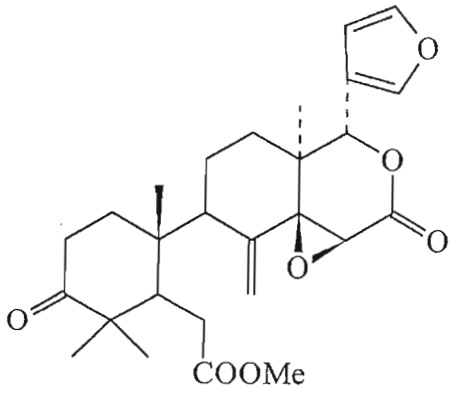
Fifteen compounds, of which eleven were limonoids, two were triterpenes, a diterpene and a flavanone were previously isolated from *Aphanamixis polystacha*.

Chandrasekharan *et al.* (1968)[5] isolated a labdane diterpenoid, named aphanamixol (**2-6**) from the fruit of *Aphanamixis polystacha*. Monkhe (1991)[20] also isolated this compound from the leaves of the species.



2-6

Chatterjee (1970)[6] isolated a limonoid, named aphanamixinin (**2-7**), and a triterpenoid, named turraeanthin (**2-8**) from the fruit-shell of *Aphanamixis polystacha*.



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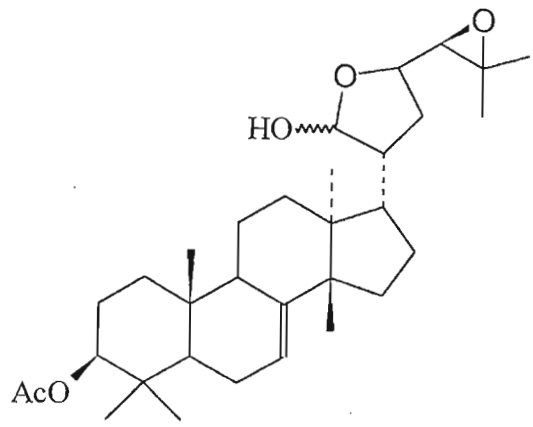
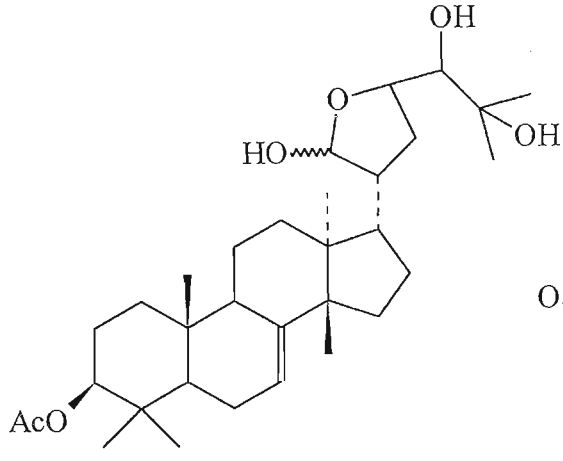


2-8

Kundu *et al.* (1985)[7] isolated a tetracyclic triterpenoid, named aphananin (**2-9**) from the fruit of the species. Aphananin (**2-9**) is the product of epoxide ring opening in aphanamixin (**2-8**) to produce the diol derivative. Aphananin (**2-9**) also possesses the hemiacetal system which is

considered to be a biogenetic intermediate between butyrospermol (**1-35**, chapter 1, section 1.3.1) and C_{26}-triterpenoids.



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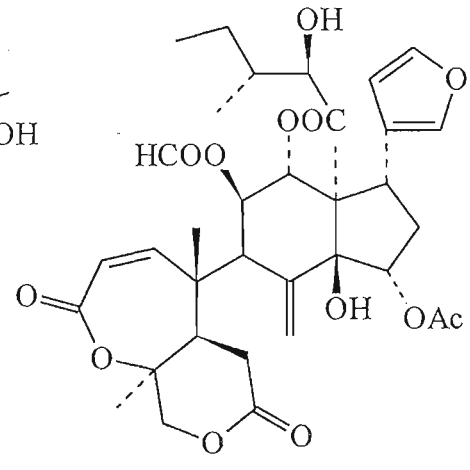
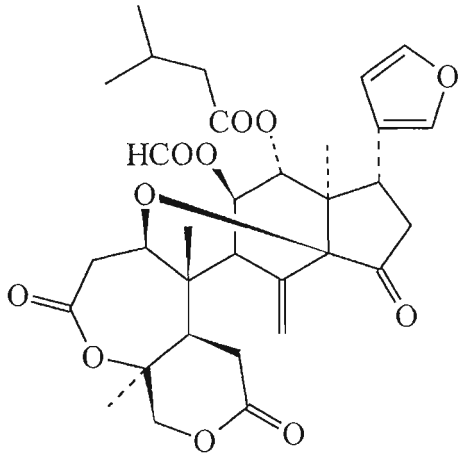


2-10

Jolad *et al.* (1981)[8] reported the isolation of hispidin C (**2-10**), Mulholland *et al.* (1979)[9] isolated polystachin (**2-11**), Brown *et al.* (1978)[10] isolated rohituka 4 (**2-12**), rohituka 5 (**2-13**), rohituka 6 (**2-14**), rohituka 8 (**2-15**), and rohituka 7 (**2-16**), Gullo *et al.* (1975)[11] reported the crystal structure of prieurianin (**2-17**) and Connolly *et al.* (1976)[12] isolated rohitukin (**2-18**) from the fruit of *Aphanamixis polystacha*. These compounds belong to type F limonoids (chapter 1, section 1.2.2.9) which have rings C and D carbocyclic, ring B opened and ring A present as a lactone.



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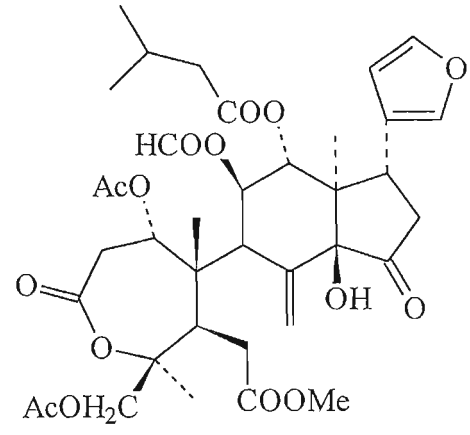

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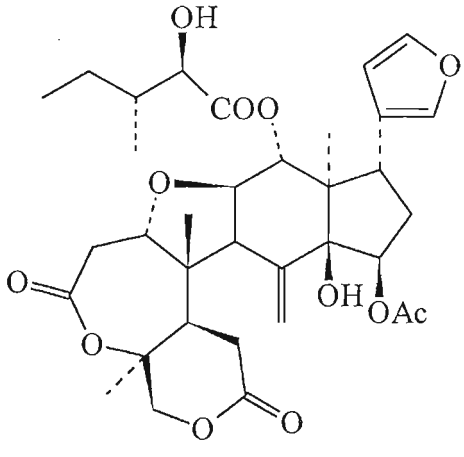

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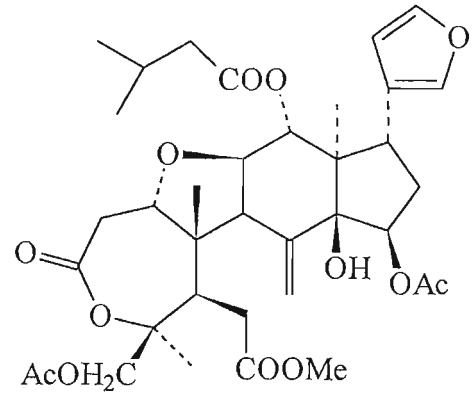

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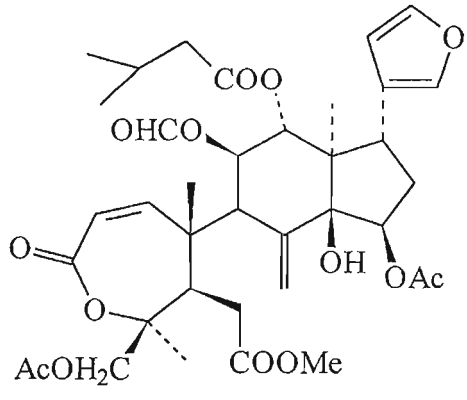

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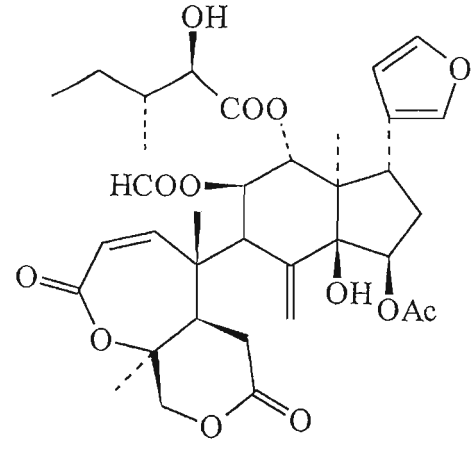

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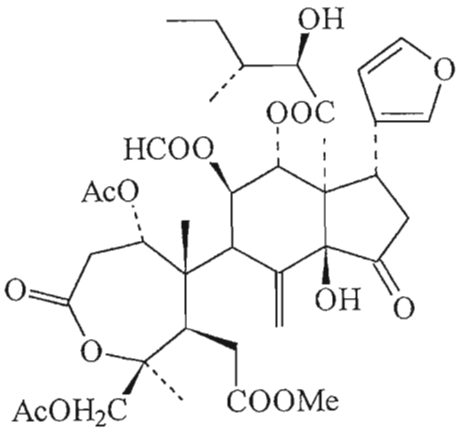

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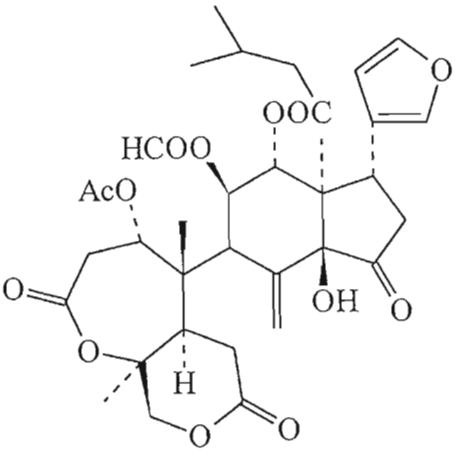
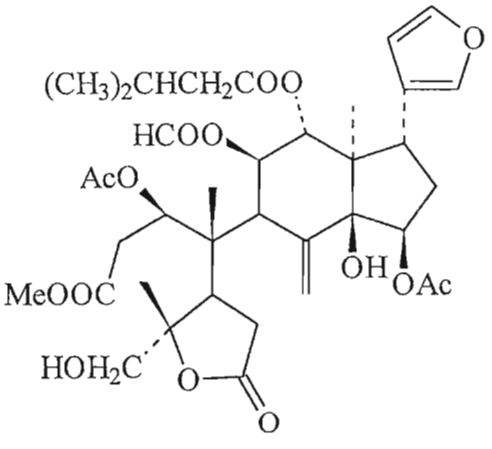


2-18

MacLachlan *et al.* (1982)[13] isolated rohituka 1 (**2-19**) and rohituka 2 (**2-20**). These compounds belong to type F limonoids (chapter 1, section 1.2.2.9) and have ring A cleaved , rings C and D carbocyclic, ring B opened have a γ-lactone.



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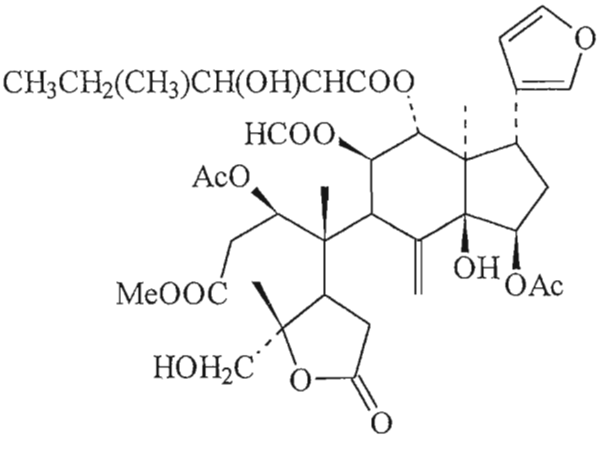
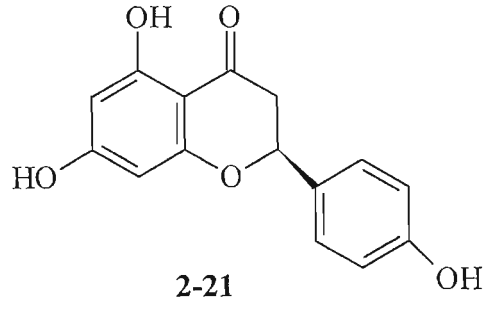


2-20

Gupta *et al.* (1980)[14] reported the isolation of the 4',7-di-Me ether, 5-*O*-α-$_L$-rhamnopyranoside derivative of naringenin (**2-21**) from the ethanol extract of the fruit of *Aphanamixis polystacha*. This was the only reported isolation of a flavanone from *Aphanamixis polystacha*.



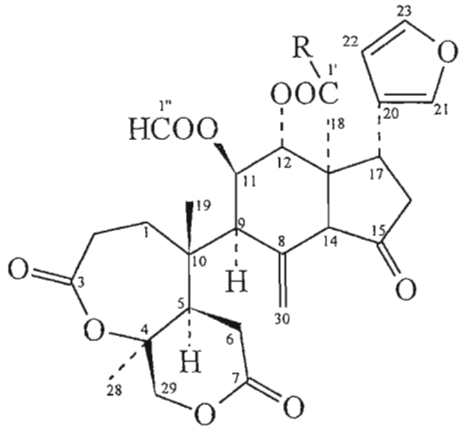
2-21

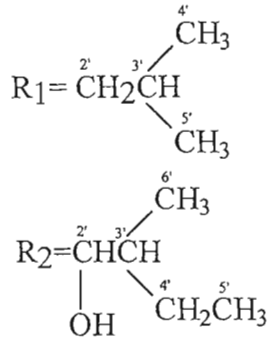
2.2 Results and Discussion

Several specimens of *Aphanamixis polystacha* are found cultivated in Durban. The seeds were gathered during late spring from a tree located at the University of Natal campus. The hexane extract yielded seven compounds of which three (**2-B**, **2-E** and **2-F**) were novel. The stereochemistry of the known compound (**2-A**) was revised, while the investigation of the methanol extract only yielded sucrose (**2-H**).

A re-examination of the species was undertaken firstly to attempt to isolate compounds, polystachin (**2-A**), rohituka 1 (**2-19**) and rohituka 2 (**2-20**) which were previously found in *Aphanamixis polystacha* in order to prove their stereochemistry at carbon atom 1. These compounds have been published with an oxygen atom at C-1β. This is different from other known compounds with the 1,14-oxide bridge or 1-acetates. Therefore it was envisaged that NOE experiments could be performed on these compounds in order to confirm the stereochemistry at C-1. Unfortunately, compounds rohituka 1 (**2-19**) and rohituka 2 (**2-20**) were not isolated during our investigation of the species. The second aim was to assign carbon NMR shifts of compounds previously isolated but whose carbon NMR data had not been fully assigned and finally to search for novel compounds from this species.

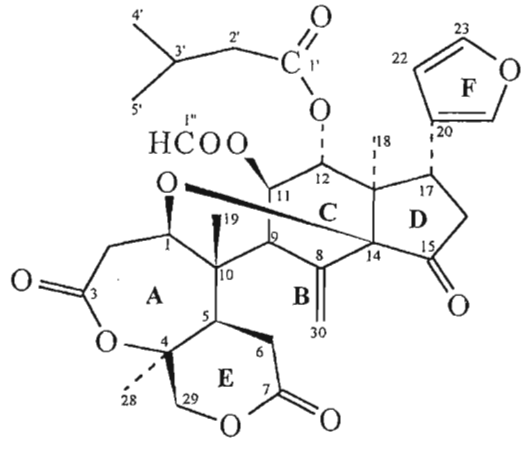
2.3 Nomenclature used in this chapter





- **2-1 to 2-21**, where **2** refers to chapter two and **1 to 21** refers to compounds discussed in the literature search. In all cases, H-5 and H-9 are α in the limonoids discussed.
- **2-A to 2-H**, where **2** refers to chapter two and **A to H** refers to compounds isolated from *Aphanamixis polystacha* during my research. In all cases, H-5 and H-9 are α in the limonoids isolated.
- 2-A1, where 2 refers to chapter two, A to compound isolated from *Aphanamixis polystacha* during my research and 1 to spectrum 1 of the compound concerned.

2.3.1 Structural elucidation of compound 2-A



polystachin (according to the literature)

FAB mass spectrometry of compound (**2-A**), (spectrum 2-A1) showed the $[MH]^+$ peak to be at *m/z* 599, which corresponded to a molecular formula of $C_{32}H_{38}O_{11}$. The literature search suggested that this compound was polystachin[9].

Although the compound is known, its elucidation will be discussed in detail, as the other compounds isolated from this species have a similar structure.

The IR spectrum (spectrum 2-A2) showed a band at 2961 (saturated C-H stretching), 1754 (broad band, C=O stretching), 1474 (C-H deformation), 1383 (methyl group symmetrical deformation), 1151, 1081 (C-O stretching) and 758 (C-H out-of -plane deformation) cm^{-1}.

The molecular formula indicated the presence of eleven oxygen atoms. The ^{13}C NMR spectrum (spectrum 2-A4) showed resonances for the carbon atom of the keto group at C-15 (δ 205.3), two lactone carbonyl carbon atoms (δ 172.3, 172.3), the side chain ester carbon atom at C-1′ (δ 167.6), the formate ester carbon atom at C-1″ (δ 160.2), the C-1,C-14 oxygen linked carbon atoms at δ 73.6 and 87.4 and the carbon atoms linked to the furan ring oxygen at δ 140.6 and δ 142.9.[9,10,15]

The ^1H NMR spectrum (spectrum 2-A3) displayed resonances ascribable to a β-substituted furan ring (ring F) protons at δ 6.20 (H-22), 7.23 (H-21) and 7.33 (H-23). These proton signals were seen to be coupled to each other in the COSY spectrum (spectrum 2-A5). The HETCOR spectrum (spectrum 2-A6) correlated the proton resonance at δ 6.20 to the carbon resonance at δ 110.4 (C<u>H</u>), δ 7.23 to δ 140.6 (C<u>H</u>), and δ 7.33 to δ 142.9 (C<u>H</u>). The quaternary carbon atom resonance at δ 121.7 was assigned to C-20.[9,10,15]

In ring A, proton H-1 was split by protons H-$2_{a\&b}$ resulting in a triplet at δ 3.79 (J= 9.3 Hz) in the proton spectrum. The COSY spectrum showed H-1 coupled to multiplets H-$2_{a\&b}$ (δ 3.18 and δ 2.96). The HETCOR spectrum correlated H-1 to the carbon atom resonance at δ 73.6 (C<u>H</u>O) which was indicative of a carbon atom linked to an oxygen atom and H-$2_{a\&b}$ to the carbon atom at δ 38.3 (C<u>H</u>$_2$). The lactone carbonyl carbon atom which occurred at C-3 resonated at δ 172.3 (<u>C</u>) and has been mentioned previously. The C-4 quaternary carbon atom resonance occurred at δ 78.6.[15]

In ring E, proton H-5 was split by protons H-$6_{a\&b}$ and yielded a multiplet at δ 2.91 in the proton spectrum. The COSY spectrum showed H-5 coupled to multiplets H-$6_{a\&b}$ (δ 2.55 and 2.29).

The HETCOR spectrum correlated H-5 to the carbon atom resonance at δ 40.7 (CH) and H-$6_{a\&b}$ to the carbon resonance at δ 32.7 (CH_2). The C-7 lactone carbonyl carbon resonance occurred at δ 172.3. Protons H-$29_{a\&b}$ split each other only, and resulted in a pair of doublets at δ 4.18 (J=12 Hz) and δ 4.08 (J=12.0 Hz) in the proton spectrum. The COSY spectrum confirmed the coupling between H-$29_{a\&b}$. The HETCOR spectrum correlated H-$29_{a\&b}$ to the carbon atom resonance at δ 74.3 (CH_2) which was indicative of a carbon atom attached to an oxygen atom. Ring B is usually opened in these types of compounds,[9,10] either giving a C-7:C-29 lactone as in this case, or ring E opened with a carbomethoxy group at C-7. The characteristic 2H-30 vinyl protons occurred superimposed at δ 5.47. The HETCOR spectrum correlated 2H-30 to the carbon atom resonance at δ 118.9 (CH_2). The quaternary carbon atom C-8 was assigned to the resonance at δ 134.5 in the double bond region of the carbon spectrum while the other quaternary carbons, C-10 and C-13, were assigned to resonances at δ 49.9 and δ 48.9 respectively.[15]

In ring C, H-9 was split by H-11 resulting in a doublet at δ 2.70 (J=5.4 Hz) in the proton spectrum. The coupling of the formyl proton was not observed. The H-11 proton was split by H-9, H-12 and the formate proton and occurred as a multiplet at δ 5.32. Proton H-12 was split by H-11 and occurred as a doublet at δ 6.03 (J= 10.8 Hz). The COSY spectrum confirmed the coupling between H-9, H-11 and H-12. The coupling constants confirmed the C-11β and C-12α substitution pattern.[15] The HETCOR spectrum was used to correlate the H-9 proton signal to the carbon resonance at δ 55.3 (CH), H-11α to the carbon resonance at δ 72.5 (CH) and H-12β to the carbon resonance at δ 72.8 (CH). The quaternary carbon atom resonance for C-14 occurred at δ 87.4 (C) and this was confirmed by a long range HETCOR experiment (spectrum 2-A7). The spectrum showed long range coupling between C-14 and one of the H-30 protons.

In ring D, the C-15 resonance occurred at δ 205.3. The protons H-$16_{a\&b}$ were split by the proton H-17 (δ 3.86) and occurred as multiplets at δ 2.84 and δ 2.36 in the proton spectrum. The COSY spectrum confirmed the coupling between H-$16_{a\&b}$ and H-17. The HETCOR spectrum was used to correlate proton resonances $H_{16a\&b}$ and H-17 to the carbon-13 NMR atom resonances at δ 41.1 (CH_2) and δ 36.8 (CH).

The ester occurring at C-12α was identified as the 3'-methyl butyrate ester and was identified

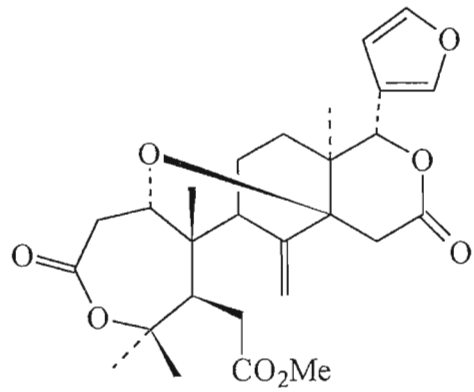
by H-2′ and H-3′ occurring as a cluster of resonances between δ 1.75 - 1.90. Proton resonances for H-4′ and H-5′ occurred at δ 0.75 and 0.85 in the proton spectrum. The COSY spectrum confirmed the coupling between these protons, while the HETCOR spectrum correlated protons H-2′ (δ 1.70) to the carbon atom resonance at δ 24.8 (<u>C</u>H), H-3′ (δ 1.65) to δ 43.0 (<u>C</u>H_2), H-4′ (δ 0.75) and H-5′ (δ 0.85) to methyl carbon resonances at δ 22.4 and 22.3.[15]

The formate proton resonance, H-1″ occurring at δ 8.11 in the proton spectrum was correlated to a carbon resonance at δ 160.2 (<u>C</u>H) using the HETCOR spectrum.

Finally the three methyl proton resonances for H-18, H-19 and H-20 were assigned to carbon atom resonances at δ 12.2 (<u>C</u>H_3), 22.2 (<u>C</u>H_3) and 29.1 (<u>C</u>H_3) respectively.[15] Comparison of the ^{1}H NMR chemical shifts and coupling constant values with those published by Mulholland[9], confirmed polystachin as the compound isolated.

Several compounds isolated from *Aphanamixis polystacha* possessed the 1α-oxide, e.g. (**2-12, 13, 14, 17** and **18**) which led us to question the stereochemistry at C-1 of polystachin (**2-A**) which was published as 1β-oxide[9].

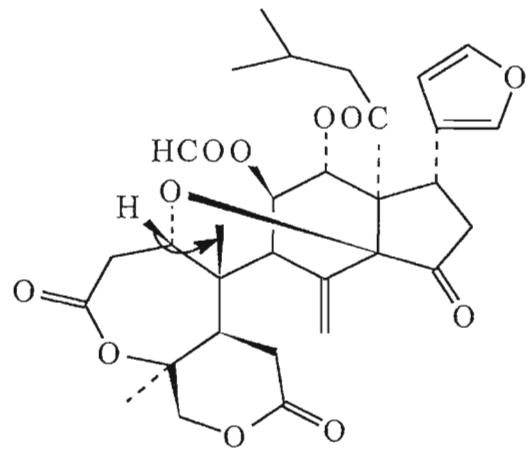
The original argument by Taylor *et al.*[9] was based on a comparison of a model of polystachin (**2-A**) with that of methyl ivorensate (**2-22**) which possessed the 1α-oxide. It was argued that in a 1α-14β-oxide linkage of compound (**2-A**) there was hindrance between C-6 and the substituents at C-11 and C-12 which would not be present in a 1β-14β oxide linkage of compound (**2-A**).[9] This hindrance was lacking in methyl ivorensate (**2-22**) which does not have substituents at C-11 and C-12. Hence, it was concluded that polystachin (**2-A**) had the 1β-14 oxide linkage. Taylor *et al.*[9] also argued that the model predicated dihedral angles in keeping with a 1α-H isomer and a coupling constant ($W_{1/2}$ = 16 Hz) significantly greater than that found in methyl ivorensate (**2-22**, $W_{1/2}$ = 10 Hz).



2-22

NOE experiments (spectrum 2-A8) irradiating proton H-1 of compound (**2-A**) resulted in a positive signal for the 3H-19 resonance at δ 1.02[15]. This proved that H-1 was β and the stereochemistry at C-1 had been incorrectly assigned previously. Thus the structure of polystachin is reassigned as **2-A** in line with the other compounds with the 1,14-oxide.

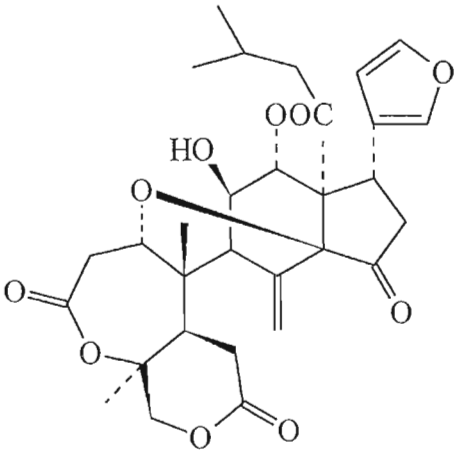
An examination of a model of polystachin with the 1α–14β oxide did not show hindrance between 2H-6 and substituents at C-11 and C-12 as had been proposed.[9] It is possible that the 7,29-lactone ring could influence the conformation of ring A resulting in the different observed $W_{1/2}$ values.



2-A (revised structure of polystachin)

2.3.2 Structural elucidation of compound 2-B

FAB mass spectrometry of compound (**2-B**), (spectrum 2-B1) showed the $[MH]^+$ peak to be at *m/z* 571, which corresponded to a molecular formula of $C_{31}H_{38}O_{10}$ for **2-B**. The peak at *m/z* 553 is indicative of the loss of a water molecule $[MH\text{-}H_20]^+$, while the peak at *m/z* 593 corresponds to the gain of a sodium ion $[MH+Na]^+$.



2-B

The IR spectrum (spectrum 2-B2) showed a broad band at 3413 (-OH stretching), 2960 (saturated C-H stretching), 1745 (broad band, C=O stretch) and 1464 (C-H deformation) cm^{-1}.

The ^1H NMR spectrum (spectrum 2-B3) showed the presence of furanyl ring protons H-21 (s), H-22 (s) and H-23 (s) at δ 7.22, δ 6.21 and δ 7.33 which confirmed that compound (**2-B**) was a limonoid. The presence of two H-30 vinylic protons which resonated at δ 5.36 and δ 5.34 and the absence of any carbomethoxy group proton resonance between δ 3.5-3.8 implied ring B was opened with a possible C-7:C-29 lactone being present. The proton spectrum also confirmed the presence of the 3′-methyl butyrate ester proton resonances, as well as three methyl singlets (δ 1.83, 1.10 and 0.79) which occurred in the upfield region of the spectrum.

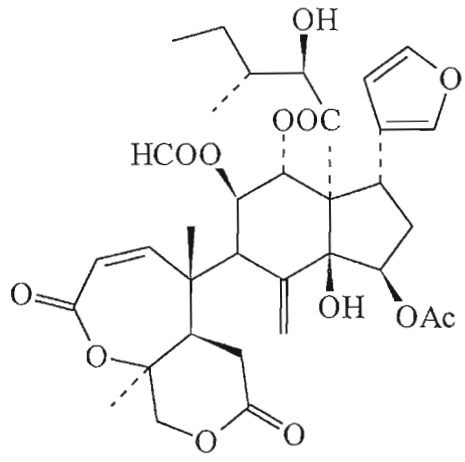
The ^{13}C NMR spectrum (2-B4) of compound (**2-B**) displayed a keto group carbon resonance at δ 205.9, three ester carbonyl carbon resonances at δ 174.0, 173.6 and 168.2 and five signals representing carbon attached to oxygen in the region δ70-90. The carbon chemical shifts of compound (**2-B**) were very similar to those of compound (**2-A**).

The COSY spectrum (2-B5) confirmed that the H-9α, H-11α and H-12β coupling system was

still intact, while the HETCOR spectrum (2-B6) confirmed that C-11 was still attached to an oxygen atom. Compound (**2-B**) seemed to be closely related to compound (**2-A**) but the resonance due to the formyl group proton at C-11β which occurred in (**2-A**) at δ 8.11 was now missing. Hence, a hydroxy group was positioned at C-11. A literature search revealed that compound (**2-B**) was novel. Compound (**2-B**) was named *rohituka 12*.

2.3.3 Structural elucidation of compound 2-C

Mass spectrometry of compound (**2-C**) (spectrum 2-C1) showed the $[M]^+$ peak to be at *m/z* 732. The peak at *m/z* 672 ($[M-60]^+$) which corresponded to a molecular formula of $C_{35}H_{44}O_{13}$ was due to the loss of an acetic acid molecule. This indicated that an acetate group was present. The literature search suggested that the compound was rohituka-7.[10]



2-C

The IR spectrum (spectrum 2-C2) showed significant bands at 3402 (-OH stretching) and 1747 (broad band, C=O) cm^{-1}.

The ^1H NMR spectrum (spectrum 2-C3) contained resonances ascribable to furanyl ring protons at δ 7.15, 6.24 and 7.34, which confirmed that the compound was a limonoid. The $\Delta^{8,30}$ -double bond C=C$\underline{H}_2$ proton resonances were present at δ 5.18 and 5.21 which indicated an opened ring B and the C-7:C-29 lactone modification. The proton spectrum indicated the presence of three different esters in the molecule, one due to the acetyl group indicated by the acetyl group

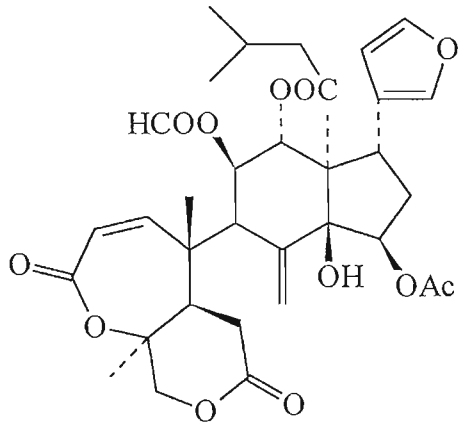
methyl proton resonance at δ 2.10, one to the formate ester proton at δ 7.90 and the other to the ester at C-12α. A triplet at δ 0.77, a doublet at δ 0.84, multiplets at δ 0.94, 1.18, 1.49 and a broad singlet at δ 3.13 were seen to be coupled in the COSY spectrum (spectrum 2-C5) and could be assigned to a 2-hydroxy-3-methylpentanoate ester.

Compound (**2-C**) seemed to have a similar skeletal framework to polystachin (**2-A**), but housed a different ester at C-12, lacked the ketone carbonyl resonance in the carbon spectrum (spectrum 2-C4) and resonances for the 1,14-epoxide linkage were absent. The COSY spectrum (2-C5) displayed coupling between H-15, H-$16_{a\&b}$ and H-17 thereby indicating substitution of the keto group at C-15 which was previously present in **2-A**. The HETCOR spectrum (spectrum 2-C6) correlated H-15 to a carbon atom attached to an oxygen. This implied that the acetate group was now present at C-15.

A doublet at δ 7.53 (J=11.4 Hz) in the COSY spectrum was coupled to a doublet at δ 6.05 (J=12.1 Hz) which was indicative of $\Delta^{1,2}$-double bond.[15] Finally, the change in chemical shift of C-14 from δ 87.0 in polystachin (**2-A**) to δ 79.0 in compound (**2-C**) indicated the presence of a tertiary alcohol at this position.[15] Hence, compound (**2-C**) was confirmed to be the known compound rohituka-7-acetate. King *et al.* (1983)[21] determined the stereochemistry of rohituka-7- acetate by X-ray crystallography.

2.3.4 Structural elucidation of compound 2-D

FAB mass spectrometry of compound (**2-D**) (spectrum 2-D1) showed the molecular ion peak $[MH]^+$ to be at *m/z* 643 which corresponded to a molecular formula of $C_{34}H_{45}O_{12}$ for compound (**4-D**). A literature search suggested that the compound could be rohituka-9.[10]



2-D

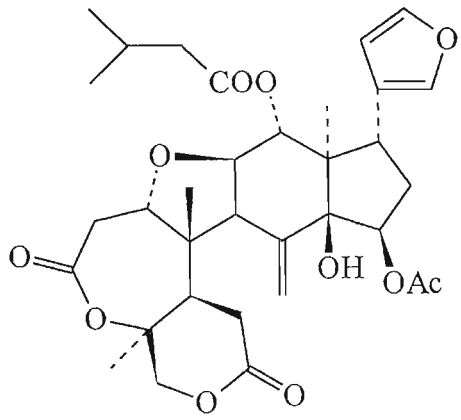
The IR spectrum (spectrum 2-D2) showed significant bands at 3500 (O-H stretching) and 1732 (broad band, C=O) cm^{-1}.

The ^{1}H NMR spectrum (spectrum 2-D3) was similar to spectrum 2-C3, compound (**2-C**), except for the presence of a different ester at C-12. A close inspection of the proton spectrum revealed that the ester was the 3′-methyl butyrate ester, with H-2′ and H-3′ occurring as a cluster of resonances between δ 1.70-1.90 and H-4′ and H-5′ occurring as doublets between δ 0.75-0.85.

The ^{13}C NMR spectrum (spectrum 2-D4), COSY spectrum (spectrum 2-D5) and HETCOR spectrum (spectrum 2-D6) were used to correlate and verify the other proton and carbon atom chemical shifts and thus confirm rohituka-9[10] as the compound isolated.

2.3.5 Structural elucidation of compound 2-E

FAB mass spectrometry of compound (**2-E**) (spectrum 2-E1) showed the molecular ion peak $[MH]^{+}$ to be at *m/z* 615, which corresponded to a molecular formula of $C_{33}H_{42}O_{11}$ for compound (**2-E**). A literature search suggested that the compound was novel.



2-E

The IR spectrum (spectrum 2-E2) showed significant bands at 3500 (O-H stretching) and 1743 (broad band, C=O) cm^{-1}.

The ^1H NMR spectrum (spectrum 2-E3) seemed to be very similar to the ^1H NMR spectrum (spectrum 2-D3) of compound (**2-D**), except for the absence of the $\Delta^{1,2}$ –double bond and the formate proton which occurred as a singlet at δ 7.94 in compound (**2-D**).

The COSY spectrum (spectrum 2-E5) indicated that H-1(δ 4.07), possibly with an oxygen substituent, was coupled to H-2_a (δ 3.11) and H-2_b (δ 2.80). The HETCOR spectrum (2-E6) correlated H-1 to the carbon atom resonance at δ 78.1, which confirmed C-1 was attached to an oxygen atom. The HETCOR spectrum correlated the H-11 resonance at δ 4.07 (1H) to a carbon atom resonance at δ 79.6, which indicated H-11 was also attached to an oxygen atom. Compounds containing a 1,11-oxide linkage were previously isolated from *Aphanamixis polystacha* (**2-13**, **2-14**).[10] Comparison of the chemical shifts of compound (**2-E**) to those published by Brown *et al.* (1978)[10] for compounds containing 1α,11β-oxides confirmed that the 1α,11β-oxide was present. The ester occurring at C-12 was identified as the 3′-methyl butyrate and was identified by H-2′ and H-3′ occurring as a cluster of proton resonances between δ 1.75 - 1.90. Proton resonances at H-4′ and H-5′ occurred as resonances at δ 0.75 and 0.85 in the proton spectrum. The COSY spectrum confirmed the coupling between these protons, while the HETCOR spectrum correlated protons H-2′ (δ 1.70) to the carbon atom resonance at δ 24.8 (CH), H-3′ (δ 1.65) to δ 43.0 (CH_2), H-4′ (δ 0.75) and H-5′ (δ 0.85) to methyl carbon atom

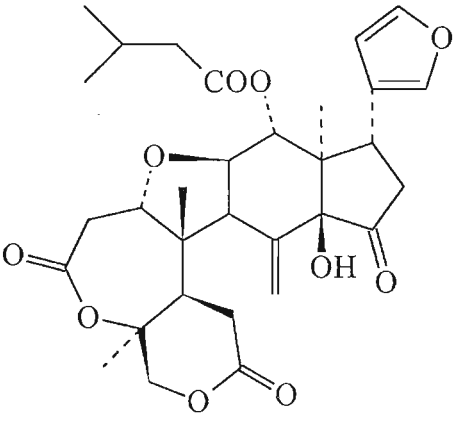
resonances at δ 22.4 and 22.3.[15]

Hence, a novel limonoid (**2-E**) was isolated, which contained a 1α,11β-oxide, a 3'-methyl butyrate ester at C-12α and a C-15β acetate. Compound (**2-E**) was named *rohituka 13*.

2.3.6 Structural elucidation of compound 2-F

FAB mass spectrometry of compound (**2-F**) (spectrum 2-F1) showed the molecular ion peak $[MH]^+$ to be at *m/z* 571, which corresponded to a molecular formula of $C_{31}H_{38}O_{10}$ for **2-F**.

The peak at *m/z* 553 was indicative of the loss of a water molecule $[MH\text{-}H_2O]^+$.



2-F

The IR spectrum (spectrum 2-E2) showed significant bands at 3500 (O-H stretching) and 1728 (broad band, C=O) cm^{-1}.

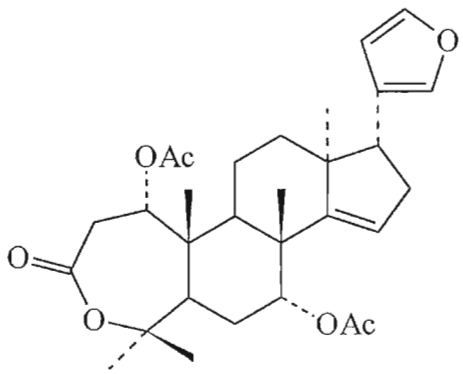
The ^1H NMR spectrum (spectrum 2-F3) seemed to be very similar to the ^1H NMR spectrum (spectrum 2-E3) of compound (**2-E**), except for the absence of the acetyl methyl group proton singlet.

The absence of the acetyl methyl group proton resonance from the proton spectrum 2-F3, and the presence of a ketone carbon resonance at δ 209.3 in the ^{13}C NMR spectrum (spectrum 2-F4) implied that a keto group was now present at C-15. This explains the downfield shift of one of the H-30 vinylic protons from δ 5.13 in compound (**2-E**) to δ 6.21 in compound (**2-F**). Hence, the above structure was proposed for compound (**2-F**).

Comparison of the proton and carbon chemical shifts of compounds (**2-E**) and (**2-F**) confirmed the presence of the 1α,11β-oxide linkage and the 3′-methyl butyrate ester at C-12α. A literature search showed that compound (**2-F**) was novel. Compound (**2-F**) was named *rohituka 14*.

2.3.7 Structural elucidation of compound 2-G

Mass spectrometry of compound (**2-G**), (spectrum 2-G1) showed the molecular ion peak $[M]^+$ to be at *m/z* 512, which corresponded to a molecular formula of $C_{30}H_{40}O_7$. The peak at *m/z* 452 $[M\text{-}CH_3COOH]^+$ was indicative of the loss of an acetic acid molecule. A literature search suggested that the compound was kihadalactone A (**2-G**) previously isolated from *Phellodendron amurense*.[16]



2-G

The IR spectrum (spectrum 2-G2) showed significant peaks at 1728 (broad band, C=O) and 1250, 1020 (C-O stretch) cm^{-1}.

The ^1H NMR spectrum (spectrum 2-G3) showed resonances for the protons of five tertiary methyl groups (δ 0.75, 1.17, 1.21, 1.40 and 1.51), two acetyl groups (δ 2.01 and 2.05), two oxygen bearing methines [δ 4.82,d (J=7.0 Hz), 5.19 br.s], a vinylic proton at δ 5.36 br.s and the characteristic β-substitued furanyl protons (δ 7.37, 7.23 and 6.26). Five of the seven oxygens were accounted for by the furan and two acetyl groups, the sixth and seventh oxygens must, therefore, be present as a lactone in view of the presence of an extra signal in the ester carbonyl region of the ^{13}C NMR spectrum (spectrum 2-G4).

The ^{13}C NMR spectrum confirmed the presence of the two acetyl esters with carbonyl carbon resonances at δ 169.7 (C) and 170.0 (C), the lactone carbonyl carbon atom resonance at 173.0

(C), the C14-15 double bond resonances at δ 158.7 (C) and δ 119.1 (CH) and signals due to the furan ring at δ 124.5 (C), 139.7 (CH), 111.0 (CH) and 142.6 (CH). The ^{13}C NMR spectrum also indicated the presence of carbon atoms attached to an oxygen at δ 85.5 (C) , 74.3 (CH) and δ 71.0 (CH). The HETCOR spectrum (spectrum 2-G6) correlated H-7 to the carbon atom at δ 74.3 and H-1 to the carbon atom at δ 71.0. The ^{13}C NMR spectrum also confirmed the presence of seven CH_3 resonances, five due to the tertiary methyl groups (δ 19.8, 15.2, 23.6, 34.4 and 27.2) and two to the acetyl methyl group carbon atoms (δ 20.7 and 21.1). The HETCOR spectrum correlated the above mentioned tertiary methyl group carbon atom resonances to H-18, H-19, H-28, H-29 and H-30 respectively. The two acetyl methyl group resonances were assigned to proton NMR resonances at δ 2.01 and 2.11.[16]

The COSY spectrum indicated the familiar coupling system between H-15,16 and 17, thus confirming the location of the double bond between C-14 (δ 158.7) and C-15 (δ 119.1). All that remained was to add the two acetate groups to the molecule. One of the acetate groups was placed at C-7α as an oxygenated substituent is necessary at this position on biosynthetic grounds.[9]

Since the two oxygen bearing methines at δ 4.82 and δ 5.19 were not coupled to each other in the COSY spectrum, they could not be alongside each other at positions C-6 and C-7. The downfield resonance at δ 3.12 (2H-2) indicated a proton in close promixity to the deshielded group (*i.e.* the carbonyl group at C-3). This resonance was coupled to the resonance at δ 4.82 which indicated the presence of the acetyl group at C-1.

A search through the literature indicated that the compound was kihadalactone A, first isolated by Kishi *et al.* (1992)[16] from *Phellodendron amurense*. A comparison of the ^{13}C NMR and ^{1}H NMR chemical shifts of kihadalactone A with those of compound (**2-G**) verified the structure of the compound. These types of compounds (type H, chapter 1) were previously isolated from *Trichilia dregeana*, and can be considered precursors to the more complex limonoids (**2-A to 2-F**) which were isolated earlier in this chapter.

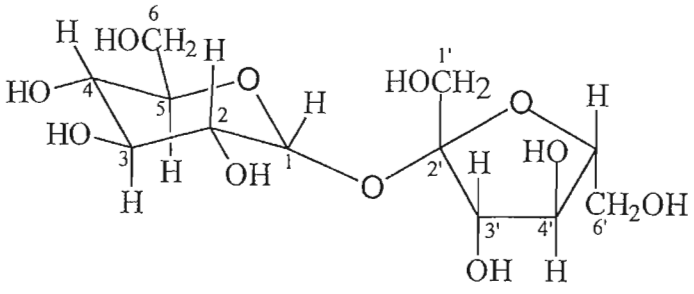
2.3.8 Structural elucidation of compound 2-H

This compound was isolated in a crystalline form from the methanol extract of the seed, of which it constituted a substantial part of the extract. It was found to be readily soluble in water and thus, all the subsequent NMR spectroscopic data were collected using D_2O as a solvent.

The ^1H NMR spectrum (spectrum 2-H1) was typical of a sugar and contained several multiplets in the region δ 3.2-3.8, a triplet at δ 3.86 and two doublets at δ 4.03 and δ 5.22.

The ^{13}C NMR spectrum (spectrum 2-H2) contained twelve carbon resonances, indicating that the compound was a disaccharide.

A comparison of NMR data suggested that the compound was sucrose.[17] The ^1H NMR spectrum of a sample of sucrose was identical to the spectrum of compound (**2-H**) and the physical data (melting point and optical rotation) also matched the literature values.



2-H

Resonances were assigned according to Richards.[18] Doublets at δ 5.22 (J=3.8 Hz) and δ 4.03 (J=8.7 Hz) were ascribed to the protons H-1 and H-3′ respectively, the triplet at δ 3.86 (J=8.4 Hz) was due to the proton H-4′, the multiplet at δ 3.75 belonged to the proton H-5′, the multiplet at δ 3.68 to H-5, the broad singlet at δ 3.63 was assigned to the protons H-6′ and H-6, the triplet at δ 3.57 (J=9.6 Hz) to H-3, the singlet at δ 3.49 to 2H-1′, the multiplet at δ 3.36 was due to the proton H-2, and the triplet at δ 3.28 (J=9.3 Hz) to H-4.

2.4 Experimental

Aphanamixis polystacha (Wall.) J.N. Parker {synonyms, *Amoora rohituka* Wight et Arn, *Aphanamixis rohituka* (Roxb)}, is an Indo-Malayan member of the Meliaceae family.

Several specimens of *Aphanamixis polystacha* are found cultivated in Durban. The seeds were gathered during late spring from a tree located at the University of Natal campus. The milled seeds (604.67 g) were extracted successively with hexane and methanol in the manner explained in the "Foreword to Experimental" section. Since the seed material obtained from *Aphanamixis polystacha* had an oily constituency, the hexane extract was subject to partitioning. The hexane extract was evaporated to approximately 200ml and then extracted with aqueous methanol (3 X 50 ml, 90%). The methanolic solution was then diluted with water to 50% and extracted with chloroform (3 X 50 ml). The chloroform extract was then subjected to column chromatography. The extractions provided 15.36 g and 8.09 g of extract, respectively.

2.4.1 Physical data of compound 2-A

polystachin

Yield : 6.4 mg

Mass spectrum (spectrum 2-A1) :

(FAB) *m/z* 599 $[MH]^+$

621 $[MNa]^+$

Infrared spectrum (spectrum 2-A2) :

υ_{max} (NaCl) : 2961 (saturated C-H stretching),
1754 (broad band, C=O stretching),
1474 (C-H deformation),
1383 (methyl group symmetrical deformation),
1151, 1081 (C-O stretching) and
758 (C-H out-of -plane deformation) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-A3

^{13}C NMR spectrum : spectrum 2-A4

COSY spectrum : spectrum 2-A5

HETCOR spectrum : spectrum 2-A6

Long-range HETCOR spectrum : spectrum 2-A7

NOE spectrum : spectrum 2-A8

Optical rotation : $[\alpha]_D = -74.3^0$ ($CHCl_3$, $c = 3.8 \times 10^{-4}$)

^{1}H and ^{13}C NMR chemical shifts of compound (**2-A**) : Table 2.5.1

2.4.2 Physical data of compound 2-B

rohituka 12

Yield : 4.4 mg

Mass spectrum (spectrum 2-B1) :

(FAB) *m/z* 571 $[MH]^+$

553 $[MH-H_2O]^+$

469 $[MH-C_5H_9O_2]^+$

593 $[MNa]^+$

Infrared spectrum (spectrum 2-B2) :

υ_{max} (NaCl) : 3413 (-OH stretching),

2960 (saturated C-H stretching),

1745 (broad band, C=O stretching),

1463 (C-H deformation) and

1291 (C-O stretching) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-B3

^{13}C NMR spectrum : spectrum 2-B4

COSY spectrum : spectrum 2-B5

HETCOR spectrum : spectrum 2-B6

Optical rotation : $[\alpha]_D = -79.6^0$ ($CHCl_3$, $c = 4.4 \times 10^{-4}$)

^{1}H and ^{13}C chemical shifts of compound (**2-B**) : Table 2.5.2

2.4.3 Physical data of compound 2-C

rohituka 7

Yield : 3.2 mg
Mass spectrum (spectrum 2-C1) :

m/z 732 $[M]^+$
672 $[M-CH_3COOH]^+$

Infrared spectrum (spectrum 2-C2) :

υ_{max} (NaCl) : 3402 (-OH stretching),
2961 (saturated C-H stretching),
1747 (broad band, C=O stretching),
1464 (C-H deformation),
1291 (-C=C- stretch),
1089 (C-O stretching) and
756 (=C-H out of plane bending) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-C3

^{13}C NMR spectrum : spectrum 2-C4

COSY spectrum : spectrum 2-C5

HETCOR spectrum : spectrum 2-C6

Optical rotation : $[\alpha]_D = -42.7^0$ ($CHCl_3$, $c = 2.4 \times 10^{-4}$)

^{1}H and ^{13}C chemical shifts of compound (**2-C**) : Table 2.5.3

2.4.4 Physical data of compound 2-D

rohituka 9

Yield : 3.2 mg

Mass spectrum (spectrum 2-D1) :

m/z 643 $[MH]^+$
665 $[MNa]^+$

Infrared spectrum (spectrum 2-D2) :

υ_{max} (NaCl) : 3500 (-OH stretching),
2960 (saturated C-H stretching),
1731 (broad band, C=O stretching),
1465 (C-H deformation),
1375 (methyl group symmetrical deformation),
1071 (C-O stretching),
756 (=C-H out of plane bending) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-D3

^{13}C NMR spectrum : spectrum 2-D4

COSY spectrum : spectrum 2-D5

HETCOR spectrum : spectrum 2-D6

Optical rotation : $[\alpha]_D = -48.6^0$ ($CHCl_3$, $c = 3.9 \times 10^{-4}$)

^{1}H and ^{13}C chemical shifts of compound (**2-D**) : Table 2.5.4

2.4.5 Physical data of compound 2-E

rohituka 13

Yield : 3.7 mg

Mass spectrum (spectrum 2-E1) :

m/z 615 $[MH]^+$

Infrared spectrum (spectrum 2-E2) :

υ_{max} (NaCl) : 2959, 2926 (saturated C-H stretching),
1742 (broad band, C=O stretching),
1465 (C-H deformation),
1371 (methyl group symmetrical deformation),
1075 (C-O stretching) and
756 (=C-H out of plane bending) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-E3

^{13}C NMR spectrum : spectrum 2-E4

COSY spectrum : spectrum 2-E5

HETCOR spectrum : spectrum 2-E6

Optical rotation : $[\alpha]_D = -45.1^0$ ($CHCl_3$, $c = 6.6 \times 10^{-4}$)

^{1}H and ^{13}C chemical shifts of compound (**2-E**) : Table 2.5.5

2.4.6 Physical data of compound 2-F

rohituka 14

Yield : 2.3 mg

Mass spectrum (spectrum 2-F1) :

m/z 571 $[MH]^+$
553 $[MH-H_2O]^+$

Infrared spectrum (spectrum 2-F2) :

υ_{max} (NaCl) : 2960, 2931 (saturated C-H stretching),
1728 (broad band, C=O stretching),
1463 (C-H deformation) and
743 (=C-H out of plane bending) cm^{-1}.

^{1}H NMR spectrum : spectrum 2-F3

^{13}C NMR spectrum : spectrum 2-F4

COSY spectrum : spectrum 2-F5

HETCOR spectrum : spectrum 2-F6

Optical rotation : $[\alpha]_D = -51.2^0$ ($CHCl_3$, $c = 3.2 \times 10^{-4}$)

^{1}H and ^{13}C chemical shifts of compound (**2-F**) : Table 2.5.6

2.4.7 Physical data of compound 2-G

kihadalactone A

Yield : 1.2 mg

Mass spectrum (spectrum 2-G1) :

m/z 512 $[M]^+$
497 $[M-CH_3]^+$
452 $[M-CH_3COOH]^+$

Infrared spectrum (spectrum 2-G2) :

υ_{max} (NaCl) : 2959 (saturated C-H stretching),
1728 (broad band, C=O stretching),
1462 (C-H deformation),
1371 (methyl group symmetrical deformation) and
1072 (C-O stretching),

^{1}H NMR spectrum : spectrum 2-G3

^{13}C NMR spectrum : spectrum 2-G4

COSY spectrum : spectrum 2-G5

HETCOR spectrum : spectrum 2-G6

Optical rotation : $[\alpha]_D = +31.7^0$ ($CHCl_3$, $c = 2.8 \times 10^{-4}$), lit[16] : 28.4^0

^{1}H and ^{13}C chemical shifts of compound (**2-G**) : Table 2.5.7

2.4.8 Physical data of compound 2-H

sucrose, β-D-Fructofuranosyl α–D-glucopyranoside

Yield : 3.45g (cryst. MeOH)

Melting Point : 184-186°C (lit.value 185-187°C)

^{1}H NMR spectrum : spectrum 2-H1

^{13}C NMR spectrum : spectrum 2-H2

Optical Rotation : $[\alpha]_D = +65.9^0$ (H_2O, $c = 1.0 \times 10^{-4}$), lit[19] : $+66.5^0$

^{1}H NMR and ^{13}C NMR data of compound (**2-H**) : Table 2.5.8

2.5 List of tables of ^{1}H and ^{13}C NMR chemical shifts.

Table 2.5.1 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-A.

Atom Number	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)
1	H-1 (*t*), 3.79, *J*=9.3Hz	73.6 (*d*)
2	H-2_a (*m*), 3.18 H-2_b (*m*), 2.96	38.3 (*t*)
3		172.3 (*s*)
4		78.6 (*s*)
5	H-5 (*m*), 2.91	40.7 (*d*)
6	H-6_a (*m*), 2.55 H-6_b (*m*), 2.29	32.7 (*t*)
7		172.3 (*s*)
8		134.5 (*s*)
9	H-9 (*d*), 2.70, *J*=5.4Hz	55.3 (*d*)
10		49.9 or 48.9 (*s*)
11	H-11 (*m*), 5.32	72.5 (*d*)
12	H-12 (*d*), 6.03, *J*=10.8Hz	72.8 (*d*)
13		49.9 or 48.9 (*s*)
14		87.4 (*s*)
15		205.3 (*s*)
16	H-16_a (*m*), 2.84 H-16_b (*m*), 2.36	41.1 (*t*)
17	H-17 (*m*), 3.86	36.8 (*d*)
18	H-18 (*s*), 0.88	12.2 (*q*)
19	H-19 (*s*), 1.02	22.2 (*q*)
20		121.7 (*s*)
21	H-21 (*s*), 7.23	140.6 (*d*)
22	H-22 (*s*), 6.20	110.4 (*d*)
23	H-23 (*s*), 7.33	142.9 (*d*)
28	H-28 (*s*), 1.98	29.1 (*q*)
29	H-29_a (*d*), 4.18, *J*=12.0Hz H-29_b (*d*), 4.08, *J*=11.7Hz	74.3 (*t*)
30	H-30 (*s*), 5.47	118.9 (*t*)
1′		167.6 (*s*)
2′	H-2′ (*m*), 1.70	24.8 (*d*)
3′	H-3′ (*m*), 1.65	43.0 (*t*)
4′	H-4′ (*m*), 0.75	22.4 (*q*)
5′	H-5′ (*m*), 0.85	22.3 (*q*)
1″	H-1″ (*s*), 8.11	160.2 (*d*)

Table 2.5.2 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-B.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*dd*), 3.69, *J*=7.2, 10.4Hz	74.3 (*d*)
2	H-2_a (*m*), 3.13 H-2_b (*m*), 2.85	38.7 (*t*)
3		173.8 (*s*)*
4		79.4 (*s*)
5	H-5 (*m*), 2.89	41.2 (*d*)
6	H-6_a (*m*), 2.58 H-6_b (*m*), 2.31	32.4 (*t*)
7		172.6 (*s*)*
8		135.9 (*s*)
9	H-9 (*d*), 2.70, *J*=10.8Hz	56.1 (*d*)
10		50.7 (*s*) or 48.9 (*s*)
11	H-11 (*m*), 3.96	74.9 (*d*)
12	H-12 (*d*), 5.81, *J*=9.9Hz	77.5 (*d*)
13		50.7 (*s*) or 48.9 (*s*)
14		87.4 (*s*)
15		205.9 (*s*)
16	H-16_a (*m*), 2.78 H-16_b (*m*), 2.32	41.2 (*t*)
17	H-17 (*m*), 3.85	37.0 (*d*)
18	H-18 (*s*), 0.79	15.5 (*q*)
19	H-19 (*s*), 1.10	22.7 (*q*)
20		122.3 (*s*)
21	H-21 (*s*), 7.22	140.7 (*d*)
22	H-22 (*s*), 6.21	110.6 (*s*)
23	H-23 (*s*), 7.33	142.9 (*d*)
28	H-28 (*s*), 1.83	28.2 (*q*)
29	H-29_a (*d*), 4.21, *J*=11.7Hz H-29_b (*d*), 3.99, *J*=11.7Hz	73.9 (*t*)
30	H-30_a (*s*), 5.36 H-30_b (*s*), 5.34	117.68 (*s*)
1′		168.2 (*s*)*
2′	H-2′ (*m*), 1.70	43.1 (*t*)
3′	H-3′ (*m*), 1.65	24.9 (*d*)
4′		22.4 (*q*)
5′		22.5 (*q*)

* carbon atoms could be interchangable.

Table 2.5.3 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-C.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*d*), 7.53, *J*=11.4Hz	152.8 (*d*)
2	H-2 (*d*), 6.05, *J*=12.1Hz	120.3 (*d*)
3		166.7 (*s*)*
4		79.0 (*s*)
5	H-5 (*m*), 2.64	51.8 (*d*)
6	H-6_a (*m*), 2.65 H-6_b (*m*), 2.51	29.5 (*t*)
7		172.2 (*s*)*
8		140.4 (*s*)
9	H-9 (*d*), 3.01, *J*=8.4Hz	51.0 (*d*)
10		43.5 (*s*)
11	H-11 (*m*), 5.56	72.4 (*d*)
12	H-12 (*d*), 6.15, *J*=10.6Hz	76.0 (*d*)
13		50.8 (*s*)
14		84.5 (*s*)
15	H-15 (*m*), 5.59	71.0 (*d*)
16	H-16_a (*m*), 2.41 H-16_b (*m*), 2.00	36.5 (*t*)
17	H-17 (*t*), 3.88, *J*=9.6Hz	39.5 (*d*)
18	H-18 (*s*), 0.91	13.4 (*q*)
19	H-19 (*s*), 1.63	24.2 (*q*)
20		123.3 (*s*)
21	H-21 (*s*), 7.15	140.5 (*d*)
22	H-22 (*s*), 6.25	110.5 (*s*)
23	H-23 (*s*), 7.30	142.7 (*d*)
28	H-28 (*s*), 1.66	27.0 (*q*)
29	H-29_a (*d*), 4.20, *J*=12.1Hz H-29_b (*d*), 4.05, *J*=12.0Hz	74.6 (*t*)
30	H-30_a (*s*), 5.21 H-30_b (*s*), 5.16	119.4 (*s*)
1′		175.0 (*s*)*
2′	H-2′ (*m*), 3.13	74.8 (*d*)
3′	H-3′ (*m*), 1.49	37.7 (*d*)
4′	H-4′ (*m*), 0.94	23.0 (*t*)
5′	H-5′ (*m*), 0.77	11.4 (*q*)
6′	H-6′ (*m*), 0.84	15.3 (*q*)
1″	H-1″ (*s*), 7.90	159.6 (*d*)
CH_3COO-		169.5 (*s*)
CH_3COO-	2.10 (*s*)	20.9 (*q*)

* carbon atoms could be interchangable.

Table 2.5.4 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-D.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*d*), 7.57, *J*=12.3Hz	153.1 (*d*)
2	H-2 (*d*), 6.05, *J*=12.1Hz	120.0 (*d*)
3		172.5 (*s*)*
4		79.1 (*s*)
5	H-5 (*m*), 2.17	51.1 (*d*)
6	H-6_a (*m*), 2.42 H-6_b (*m*), 2.61	32.6 (*t*)
7		172.2 (*s*)*
8		140.7 (*s*)
9	H-9 (*m*), 2.94	51.9 (*d*)
10		43.4 (*s*)
11	H-11 (*m*), 5.55	71.7 (*d*)
12	H-12 (*d*), 6.07, *J*=6.9Hz	74.3 (*d*)
13		50.5 (*s*)
14		84.5 (*s*)
15	H-15 (*m*), 5.54	72.5 (*d*)
16	H-16_a (*m*), 2.41 H-16_b (*m*), 2.00	36.4 (*t*)
17	H-17 (*m*), 3.87	39.5 (*d*)
18	H-18 (*s*), 0.95	13.4 (*q*)
19	H-19 (*s*), 1.09	24.2 (*q*)
20		123.5 (*s*)
21	H-21 (*s*), 7.15	140.4 (*d*)
22	H-22 (*s*), 6.24	110.6 (*s*)
23	H-23 (*s*), 7.30	142.7 (*d*)
28	H-28 (*s*), 1.67	27.0 (*q*)
29	H-29_a (*d*), 4.24, *J*=11.4Hz H-29_b (*d*), 4.01, *J*=11.4Hz	74.7 (*t*)
30	H-30_a (*s*), 5.18 H-30_b (*s*), 5.12	119.1 (*s*)
1''	H-1'' (*s*), 7.93	159.7 (*d*)
1'		166.8 (*s*)*
2'	H-2' (*m*), 1.70	42.7 (*t*)
3'	H-3' (*m*), 1.65	24.68 (*d*)
4'	H-4' (*m*), 1.75	22.7 (*q*) or 22.3 (*q*)
5'	H-5' (*m*), 1.70	22.7 (*q*) or 22.3 (*q*)
CH_3COO-		169.5 (*s*)
CH_3COO-	2.10 (*s*)	20.7 (*q*)

* carbon atoms could be interchangable.

Table 2.5.5 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-E.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*m*), 4.07	78.1 (*d*)
2	H-2_a (*m*), 3.11 H-2_b (*m*), 2.65	37.00 (*t*) or 37.05 (*t*)
3		172.6 (*s*)*
4		79.4 (*s*)
5	H-5 (*m*), 2.74	43.8 (*d*)
6	H-6_a (*m*), 2.73 H-6_b (*m*), 2.33	31.3 (*t*)
7		171.4 (*s*)*
8		140.3 (*s*)
9	H-9 (*d*), 3.16, *J*=6.6Hz	53.8 (*d*)
10		49.5 (*s*)
11	H-11 (*m*), 4.07	79.6 (*d*)
12	H-12 (*d*), 5.74, *J*=8.1Hz	73.6 (*d*)
13		51.8 (*s*)
14		84.0 (*s*)
15	H-15 (*m*), 5.47	74.2 (*d*)
16	H-16_a (*m*), 2.33 H-16_b (*m*), 1.95	37.00 (*t*) or 37.05 (*t*)
17	H-17 (*m*), 3.77	38.8 (*d*)
18	H-18 (*s*), 0.81	13.3 (*q*)
19	H-19 (*s*), 1.16	16.0 (*q*)
20		123.9 (*s*)
21	H-21 (*s*), 7.13	142.4 (*d*)
22	H-22 (*s*), 6.22	110.8 (*s*)
23	H-23 (*s*), 7.26	143.1 (*d*)
28	H-28 (*s*), 1.65	26.6 (*q*)
29	H-29_a (*d*), 4.21, *J*=11.4Hz H-29_b (*d*), 3.98, *J*=11.4Hz	72.3 (*t*)
30	H-30_a (*s*), 5.13 H-30_b (*s*), 5.05	117.0 (*s*)
1′		169.6 (*s*) or 167.5 (*s*)
2′	H-2′ (*m*), 1.70	24.8 (*d*)
3′	H-3′ (*m*), 1.65	43.0 (*t*)
4′	H-4′ (*m*), 0.75	22.4 (*q*) or 22.3 (*q*)
5′	H-5′ (*m*), 0.85	22.4 (*q*) or 22.3 (*q*)
CH_3COO-		169.5 (*s*) or 167.5 (*s*)
CH_3COO-	2.10 (*s*)	21.2 (*q*)

* carbon atoms could be interchangable.

Table 2.5.6 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-F.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*m*), 4.01	78.3 (*d*)
2	H-2_a (*m*), 3.10 H-2_b (*m*), 2.77	37.1 (*t*)
3		172.7 (*s*)*
4		79.5 (*s*)
5	H-5 (*m*), 2.32	43.7 (*d*)
6	H-6_a (*m*), 2.73 H-6_b (*m*), 2.33	31.2 (*t*)
7		171.4 (*s*)*
8		139.1 (*s*)
9	H-9 (*d*), 3.19, *J*=9.9Hz	54.6 (*d*)
10		48.1 (*s*)
11	H-11 (*m*), 4.13	79.6 (*d*)
12	H-12 (*d*), 5.75, *J*=7.8Hz	73.2 (*d*)
13		51.7 (*s*)
14		79.5 (*s*)
15		209.3 (*s*)
16	H-16_a (*m*), 2.77 H-16_b (*m*), 2.34	42.0 (*t*)
17	H-17 (*m*), 3.78	34.8 (*d*)
18	H-18 (*s*), 0.84	13.0 (*q*)
19	H-19 (*s*), 1.14	16.6 (*q*)
20		122.7 (*s*)
21	H-21 (*s*), 7.20	140.5 (*d*)
22	H-22 (*s*), 6.21	110.5 (*s*)
23	H-23 (*s*), 7.33	142.7 (*d*)
28	H-28 (*s*), 1.65	26.7 (*q*)
29	H-29_a (*d*), 4.23, *J*=11.1Hz H-29_b (*d*), 3.98, *J*=11.4Hz	72.7 (*t*)
30	H-30_a (*s*), 6.21 H-30_b (*s*), 5.20	120.9 (*s*)
1'		167.6 (*s*)
2'	H-2' (*m*), 1.82	43.2 (*t*)
3'	H-3' (*m*), 1.82	25.24 (*d*)
4'	H-4' (*m*), 0.90	22.4 (*q*) or 22.3 (*q*)
5'	H-5' (*m*), 0.88	22.4 (*q*) or 22.3 (*q*)

* carbon atoms could be interchangable.

Table 2.5.7 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-G.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*t*), 4.82, *J*=7.0Hz	71.0 (*d*)
2	H-2_a (*m*), 3.12 H-2_b (*m*), 3.17	34.9 (*t*)
3		173 (*s*)
4		85.5 (*s*)
5	H-5 (*m*), 2.52	44.1 (*d*)
6	H-6 (*m*), 1.95	26.4 (*t*)
7	H-7 (*br.s*), 5.19	74.3 (*d*)
8		41.9 (*s*)
9	H-9 (*m*), 2.56	36.0 (*d*)
10		44.3 (*s*)
11	H-11_a (*m*), 1.42 H-11_b (*m*), 1.57	16.3 (*t*)
12	H-12 (*m*), 1.57	32.9 (*t*)
13		47.1 (*s*)
14		158.7 (*s*)
15	H-15 (*br.s*), 5.36	119.1 (*d*)
16	H-16_a (*m*), 2.35 H-16_b (*m*), 2.45	34.3 (*t*)
17	H-17 (*t*), 2.79, *J*=7.5Hz	51.3 (*d*)
18	H-18 (*s*), 0.75	19.8 (*q*)
19	H-19 (*s*), 1.17	15.2 (*q*)
20		124.5 (*s*)
21	H-21 (*s*), 7.23	139.7 (*d*)
22	H-22 (*s*), 6.26	111.0 (*s*)
23	H-23 (*s*), 7.37	142.6 (*d*)
28	H-28 (*s*), 1.51	23.6 (*q*)
29	H-29 (*s*), 1.40	34.4 (*q*)
30	H-30 (*s*), 1.21	1.21 (*q*)
CH_3COO-	2.01 (*s*)	20.7 (*q*)
CH_3COO-	2.11 (*s*)	21.1 (*q*)
CH_3COO-		169.7 (*s*)
CH_3COO-		170.0 (*s*)

Table 2.5.8 : ^{1}H and ^{13}C NMR chemical shifts of compound 2-H.		
Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
1	H-1 (*d*), 5.22, *J*=3.8Hz	100.2 (*d*)
2	H-2 (*m*), 3.36	79.1 (*d*)
3	H-3 (*t*), 3.57, *J*=9.6Hz	80.6 (*d*)
4	H-4 (*t*), 3.28, *J*=9.3Hz	77.3 (*d*)
5	H-5 (*m*), 3.68	80.5 (*d*)
6	H-6 (*br.s*), 3.63	68.2 (*t*)
1′	H-1′ (*br.s*), 3.49	69.4 (*t*)
2′		111.7 (*s*)
3′	H-3′ (*d*), 4.03, *J*=8.7Hz	84.5 (*d*)
4′	H-4′ (*t*), 3.86, *J*=8.4Hz	82.1 (*d*)
5′	H-5′ (*m*), 3.75	89.4 (*d*)
6′	H-6′ (*br.s*), 3.63	70.4 (*t*)

2.6 References

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CHAPTER 3

Extractives from *Turraea obtusifolia*

Index

Chapter 3

Extractives from *Turraea obtusifolia*

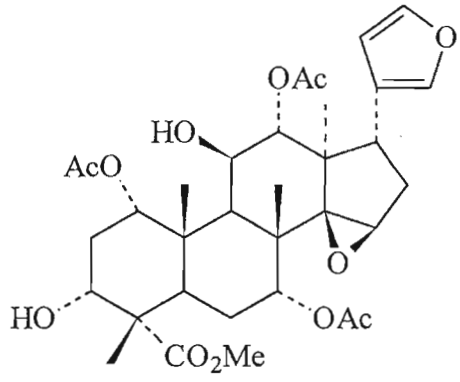
3.1 Introduction

The genus *Turraea* belongs to the Meliaceae family and is indigeneous to Africa and the Indian Ocean Islands. Within the genus *Turraea*, seven species have been examined previously, namely *Turraea floribunda*, *Turraea mombasana*, *Turraea robusta*, *Turraea villosa*, *Turraea nilotica*, *Turraea obtusifolia* and *Turraea holstii*. A literature search was undertaken to become acquainted with the type of compounds naturally produced by the genus *Turraea*.

3.1.1 *Turraea floribunda*

Several limonoids were reported to have been isolated from the species *Turraea floribunda*. Akinniyi *et al.* (1986)[1] reported the isolation and structure of compound (**3-1**) and several closely related derivatives from the bark of the species.

Fraser *et al.* (1994)[2] isolated turraflorin B (**3-2**) and two other closely related limonoids from the bark of the species. These compounds were reported to have 11α, 12α-substituents, but this is now considered incorrect and their structures are being revised as 11β, 12α. (Mulholland, pers.comm.).



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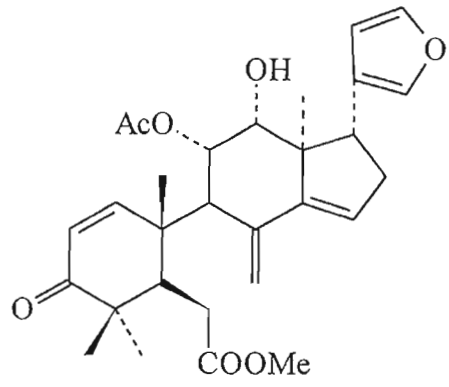

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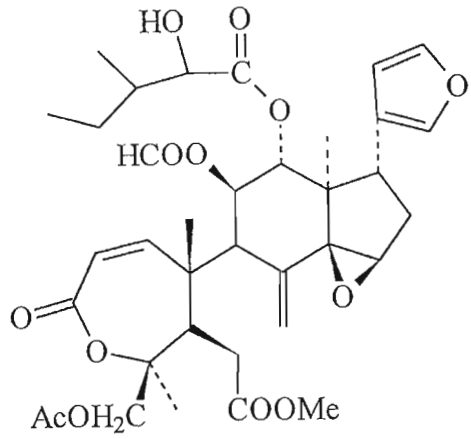
3.1.2 *Turraea mombasana*

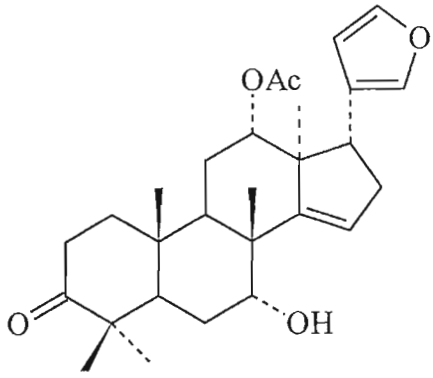
Adul *et al.* (1993)[3] isolated mombasol (**3-3**) from the root bark of *Turraea mombasana*.



3-3

3.1.3 *Turraea robusta*

Rajab *et al.* (1988)[4] isolated mzikonone (**3-4**), while Bentley *et al.* (1992)[5] isolated mzikonol (**3-5**) from the root bark of *Turraea robusta*.



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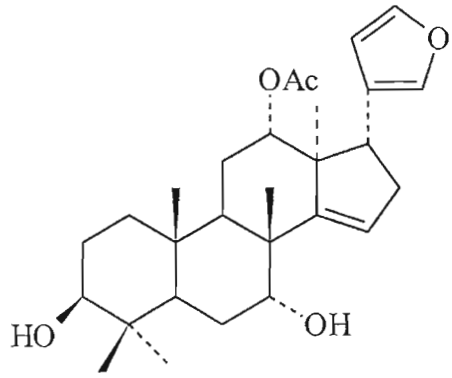
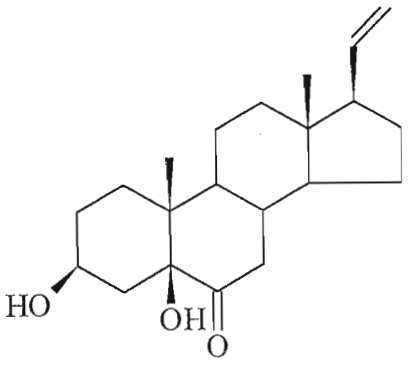


3-5

3.1.4 *Turraea villosa*

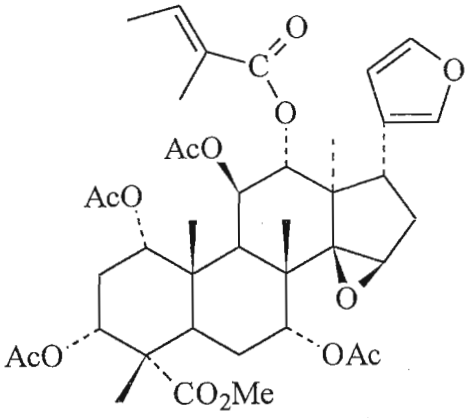
Chiplunkar *et al.* (1993)[6] isolated 3β,5β-dihydroxypregn-20-en-6-one (**3-6**) from the aerial parts of the plant.



3-6

3.1.5 *Turraea nilotica*

Bentley *et al.* (1995)[7] isolated nilotin (**3-7**) from the root bark of the species, while Mulholland *et al.* (1988)[8] isolated niloticin (**3-8**) and other closely related compounds from the bark and wood of *Turraea nilotica*.



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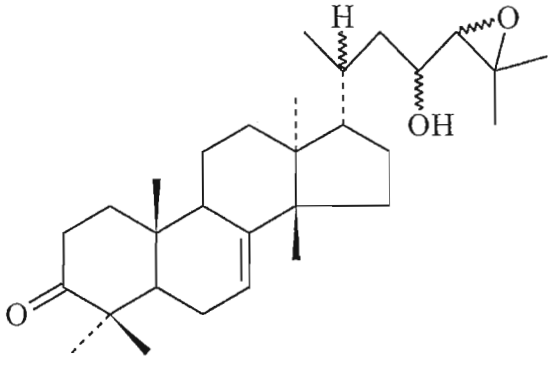

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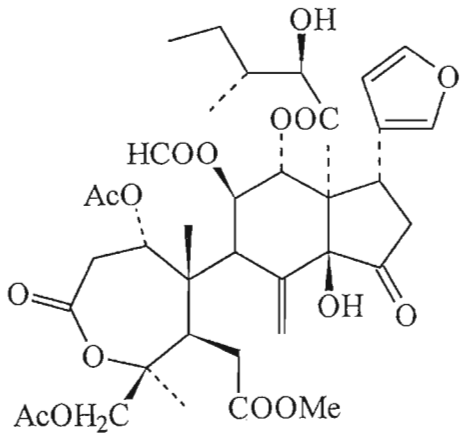
3.1.6 *Turraea obtusifolia*

Akinniyi *et al.* (1986)[1] isolated prieurianin (**3-9**), while Mulholland *et al.* (1993)[9] isolated 7-deacetylglabretal-3-acetate (**3-10**) from the leaves of *Turraea obtusifolia*.



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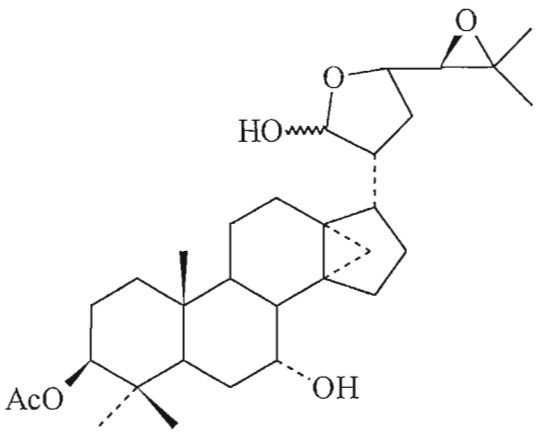

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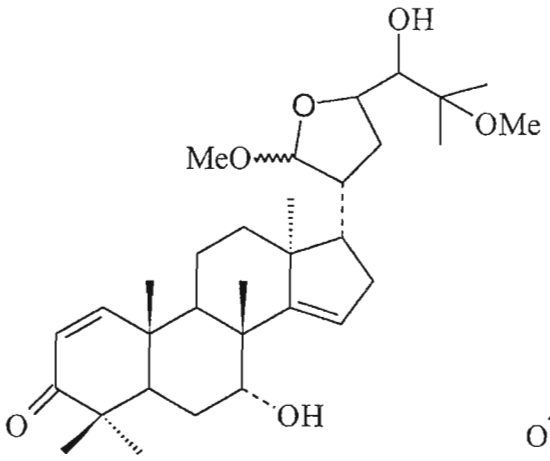
3.1.7 *Turraea holstii*

Monkhe (1997)[17] isolated two protolimonoids (**3-11** and **3-12**) and eight limonoids (**3-13** to **3-20**) from the rootbark and stembark of the species.



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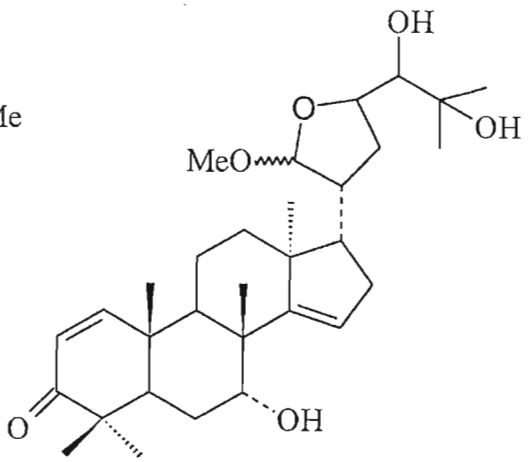

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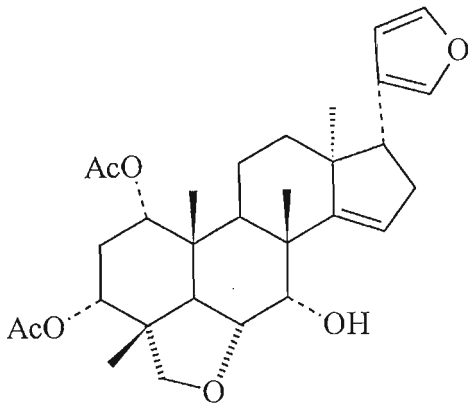

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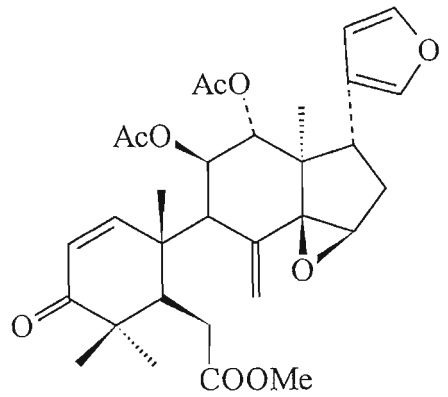

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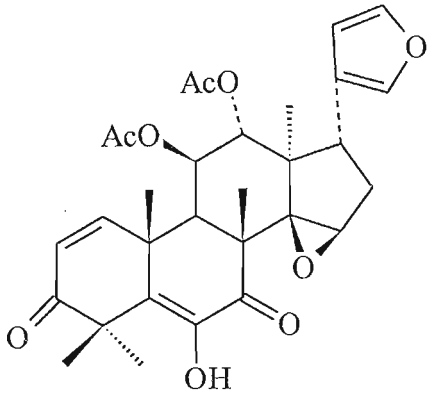

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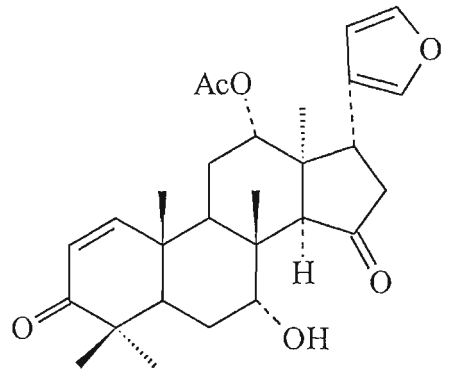

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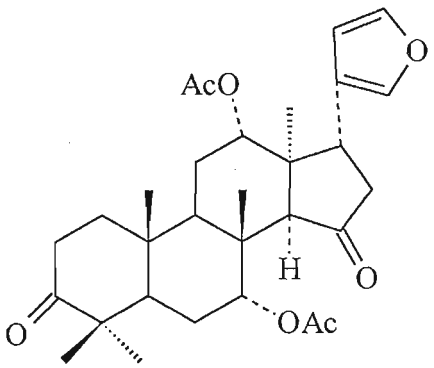

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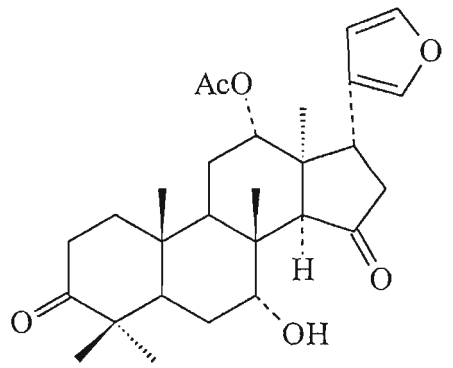

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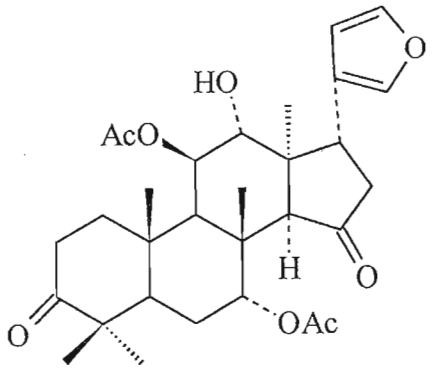

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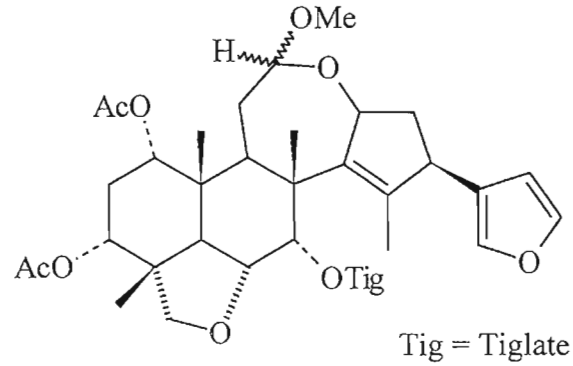


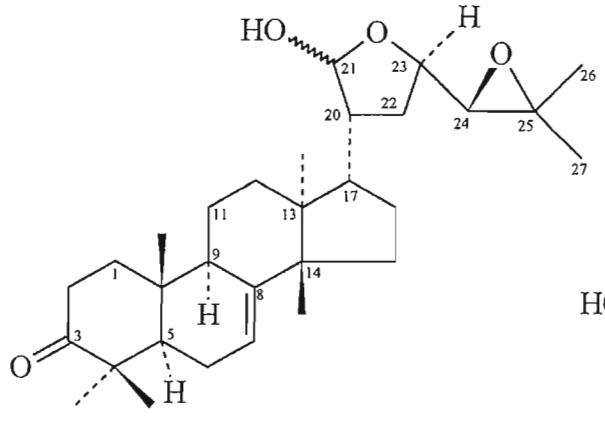
Tig = Tiglate

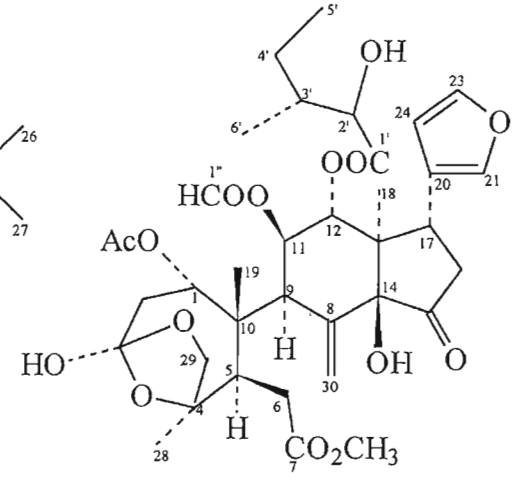
3-20

3.2 Results and discussion

The species *Turraea obtusifolia* is a small bush, hence it was very difficult to collect sufficient seeds for extraction. However, some seeds were donated for extraction by Mr Geoff Nichols, who had a small plant in his garden and a larger plant was found growing in Kirstenbosch Gardens from which most of the seed were obtained. The seed capsules were approximately 1.5cm in diameter. The seed coats were soft and paper thin and contained bright orange seeds. Two protolimonoids, melianone (**3-A**) and turraeanthin (**3-B**), as well as a limonoid, nymania-1 (**3-C**) were isolated from the seeds of *Turraea obtusifolia*.

3.3 Nomenclature of compounds discussed in this chapter





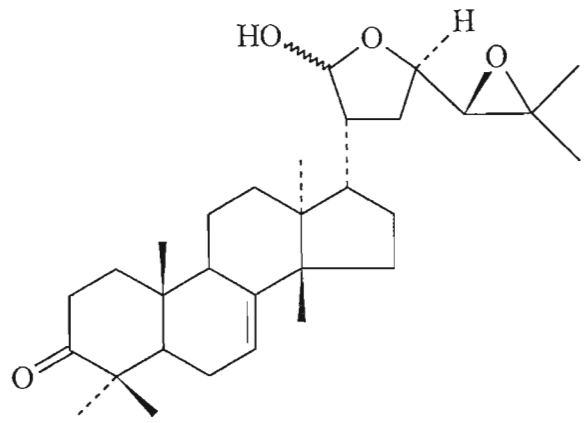
- **3-1 to 3-21**, where **3** refers to chapter three and **1 to 21** refers to compounds discussed in the literature search.
- **3-A to 3-C**, where **3** refers to chapter three and **A to C** refers to compounds isolated from *Turraea obtusifolia* during my research. In all cases, H-5 and H-9 are α in the limonoids and protolimonoids discussed.
- 3-A1, where 3 refers to chapter three, A to compound isolated from *Turraea obtusifolia* during my research and 1 to spectrum 1 of the compound concerned. In all cases, H-5 and H-9 are α in the limonoids and protolimonoids isolated.

3.3.1 Structural elucidation of compound 3-A

Mass spectrometry of compound (**3-A**), (spectrum 3-A1) showed the $[M]^+$ peak to be at *m/z* 470, which corresponded to a molecular formula of $C_{30}H_{46}O_4$. The loss of a methyl group was indicated by the $[M\text{-}CH_3]^+$ peak at *m/z* 455 and the loss of the epoxide side chain by cleavage of the C23-C24 bond gave the $[M\text{-}C_4H_7O]^+$ peak at *m/z* 399.

Infrared spectroscopy (3-A2) revealed strong absorptions at 3430 cm^{-1} which indicated the presence of a hydroxy group and 1707 cm^{-1} which was characteristic of the carbonyl stretch of a saturated 6-membered ring ketone.

Compound (**3-A**) appeared to occur as an epimeric mixture as many peaks were paired in the ^{13}C NMR spectrum (spectrum 3-A4, and expansion, spectrum 3-A5), for example the resonances at δ 101.8 (C-H) and 97.8 (C-H) were both ascribable to C-21 in the two different epimers. The resonance of H-21 appeared at δ 5.34 in the ^1H NMR spectrum (spectrum 3-A3). Thus, compound (**3-A**) occurred as a mixture of C-21 epimers.[10,11]



3-A

The ^{13}C NMR spectrum showed a resonance at δ 216.9 which was characteristic of a keto group carbon atom at the C-3. The resonances that occurred at δ 145.7 (C) and 118.2 (C-H) represented carbon atoms of the double bond between carbon atoms 7 and 8.[10]

Akerman (1990)[10] correlated C-21 at δ 101.8 in the ^{13}C NMR spectrum to the resonance at δ 5.34 (H-21) in the ^{1}H NMR spectrum. The resonances at δ 118.7 (C-H) corresponded to a resonance at δ 5.30 in the ^{1}H NMR spectrum, which confirmed the presence of the olefinic proton, H-7 ($W_{1/2}$=8 Hz). The ^{1}H NMR spectrum also showed a multiplet at δ 3.90 which represented the H-23 proton signal, split by both 2H-22 and H-24. Also noticeable, were two doublets representing H-24 at δ 2.80 and 2.70 for the two epimers.

The above spectral data indicated that compound (**3-A**) was the common protolimonoid, melianone. Hemiacetal groups open and close causing the two epimers to interconvert in solution, therefore it is not possible to separate the two forms. Separation of the mixture is possible by first performing an acetylation reaction. This was not attempted as compound (**3-A**) was obtained in small amounts.

3.3.2 Structural elucidation of compound 3-B

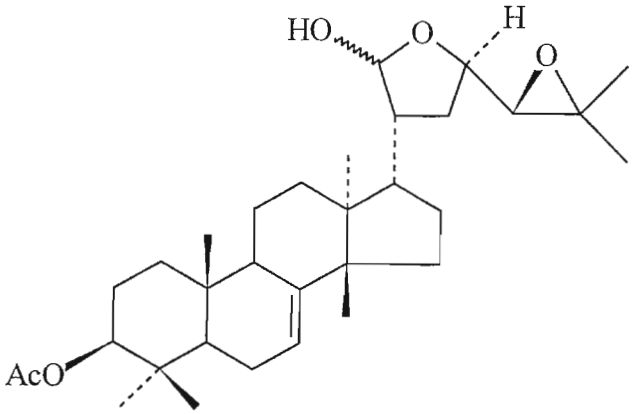
Mass spectrometry (spectrum 3-B1) of compound (**3-B**) showed the $[M]^+$ peak to be at *m/z* 514 which corresponded to a molecular formula of $C_{32}H_{50}O_5$. The loss of a methyl group was indicated by the $[M\text{-}CH_3]^+$ peak at 499.

The infrared spectrum (3-B2) displayed bands at 3428 (OH stretch), 1728 and 1248 (carbonyl and C-O stretch of the acetyl group) cm^{-1}.

The ^{1}H NMR spectrum (spectrum 3-B3) and ^{13}C NMR spectrum (spectrum 3-B4, and expansion, spectrum 3-B5) also indicated the presence of an epimeric mixture.

The compound exibited similar resonances in the ^{1}H NMR spectrum to compound (**3-A**) with an additional acetyl methyl group proton peak at δ 2.00 and a multiplet at δ 4.50 ascribable to H-3α ($W_{1/2}$=18 Hz). The ^{13}C NMR spectrum indicated the absence of the keto group carbon resonance which was present in compound (**3-A**), but the presence of the acetate carbonyl carbon resonance at δ 171.0.

Other than this distinct difference, both the ^{1}H and ^{13}C NMR spectra showed resonances as for compound (**3-A**). This indicated that compound (**3-B**) was the 3β-acetyl derivative of melianone (**3-A**). The literature search revealed that this compound was a commonly known protolimonoid, turraeanthin.



3-B

Both 3-epi-turraeanthin have previously been isolated and the two isomers can be differentiated using the 3-H proton resonance. The 3β- proton ($W_{1/2}$=9 Hz) resonates further downfield than the 3α- proton (δ 4.70 as opposed to δ 4.52) and a large $W_{1/2}$ coupling of 18 Hz indicates a 3β substituent present at C-3.[10]

3.3.3 Structural elucidation of compound 3-C

Infrared spectroscopy (3-C2) revealed strong absorptions at 3439 cm^{-1}, which indicated the presence of a hydroxy group, 1245 cm^{-1} and 1030 cm^{-1}, which represented a C-O linkage, and at 1724 cm^{-1} which was characteristic of a keto group carbonyl stretch.

The proton spectrum (spectrum 3-C3) of compound (**3-C**) appeared to be very similar to that of polystachin (**2-A**, chapter 2). The presence of the formyl proton resonance at δ 7.93, three furanyl proton resonances at δ 7.37, δ 7.20 and δ 6.25, the vinylic proton resonances at δ 6.05 and δ 6.04 and a carbomethoxy group methyl proton resonance at δ 3.70 indicated a limonoid with ring B opened. An acetyl methyl proton resonance at δ 2.00 and the three characteristic methyl proton resonances were readily assigned by comparison of this spectrum with that of polystachin.

By comparison with spectra of related limonoid compounds (chapter 2), the doublet at δ 5.98 (J=13.5 Hz) was assigned to H-12. The two signals for 2H-30 were located at δ 6.05 and δ 6.04, the double doublet centred at δ 5.37 (J=8.5, 10.6 Hz) to H-11 and the double doublet at δ 5.21 (J=3.2, 12.5 Hz) to H-1. After assigning the doublet at δ 4.19 (J=8.2 Hz) to H-9, the doublet at δ 4.11 (J=8.6 Hz) and δ 3.62 (J=8.6 Hz) were assigned to 2H-29. The remaining double doublet at δ 3.97 (J=7.4, 9.3 Hz) in this region was assigned to H-17. The positions assigned to H-9, H-11, H-12 system were confirmed by the COSY spectrum (spectrum 3-C5). The coupling constants indicated the usual 11β, 12α - substitution. The positioning of the formate at C-11 was confirmed by long-range coupling between the formate proton and H-11 seen in the COSY spectrum.

The nature of the substituent at C-12α posed the next question. The distinctive triplet and doublet between δ 0.50 - 0.90 in the ^1H NMR spectrum implied that a 2′-hydroxy, 3′-methylpentanoyl ester was present.

The HETCOR spectrum (spectrum 3-C6) was used to correlate the proton to carbon resonances and identified the resonance at δ 4.40 to be due to the hydroxy group proton at C-3, as no corresponding carbon resonance could be found.

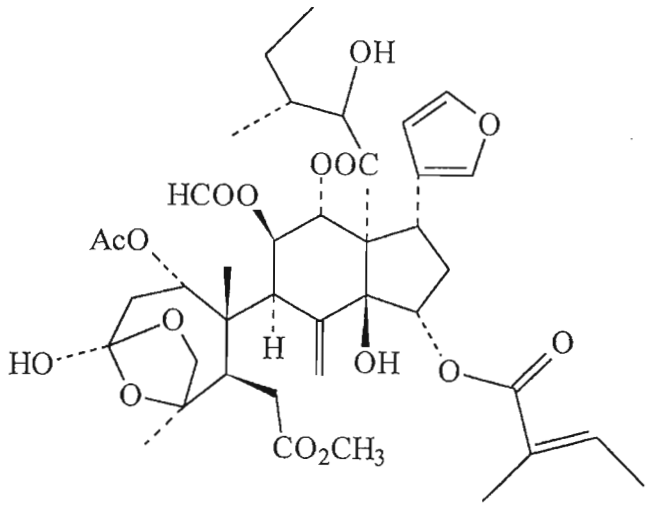
The carbon spectrum (spectrum 3-C4) indicated the presence of the furanyl ring by the characteristic peaks at δ 123.4 (C), 140.5 (CH), 110.6 (CH) and 143.0 (CH), the carbomethoxy group by the resonance (CH_3) at δ 53.0 and the C-15 keto group carbon resonance at δ 207.3.

The existence of the same ring D skeleton as polystachin was confirmed by the C-14 hydroxy group and C-15 keto carbon resonances [δ 80.7 (C) and δ 207.3 (C) respectively], assigned by comparison with the compounds studied in the previous chapter. Finally, the nature of the side chain was confirmed to be a 2′-hydroxy, 3′-methylpentanoyl ester, by comparison of all the carbon resonances of C-1′ to C-6′ with those of the compounds previously studied.

Since this compound possessed no 1,14-oxide linkage (absence of the C-14 quaternary carbon at δ 87.0) or α,β-unsaturated lactone in ring A (absence of H-1 and H-2 as a pair of doublets between δ 6.00 - 7.50) the acetate group was placed at C-1.

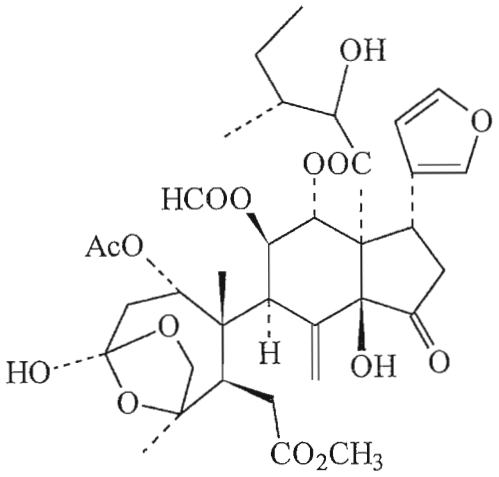
In compounds such as polystachin (**2-A**), a C-7:C-29 lactone was formed, but this was not the case for compound (**3-C**), as the carbomethoxy group was now present. The proton spectrum could provide no further information on the structure of compound (**3-C**), and it was the carbon spectrum (spectrum 3-C4) that provided the required information.

In the previous chapter, the study of the carbon spectra of polystachin and related compounds indicated that C-3 occurred as a singlet at δ 168.0 -174.0, as expected for a lactone carbonyl carbon. However, in compounds such as hispidin A (**3-21**), isolated from *Trichilia hispida*,[14] a hemiorthoester was formed between C-29 and C-3, shifting the position of C-3 upfield to δ 119.0. This C-3 resonance was also found in the carbon spectrum for compound (**3-C**) at δ 119.5 (C), thereby indicating the presence of a C-29:C-3 hemiorthoester in ring A. Thus, structure (**3-C**) was proposed for this compound.



3-21

The literature search revealed that this compound was nymania 1, previously isolated from *Nymania capensis*.[15,16] A comparison between the ^{1}H and ^{13}C NMR chemical shifts of nymania 1 and compound (**3-C**) verified the structure of compound (**3-C**). Compound (**3-C**) decomposed after NMR analysis and a mass spectrum was not obtained.



3-C

3.3.3.1 The chemotaxonomic significance of nymania-1 (compound 3-C)

Nymania was recognised early in the eighteenth century as belonging to the Meliaceae. However, not all subsequent authors have agreed with this conclusion, and at various times it has been placed in different families. This is on account of the superficial differences from other members of the Meliaceae family. MacLachlan *et al.* (1982)[15] isolated a known limonoid prieurianin (**3-9**), together with four other novel complex limonoids, including nymania-1 (**3-C**) from the bark and timber of *Nymania capensis*.

Prieurianin is a highly characteristic marker substance, which is widely distributed in the subfamily Meliodeae of the Meliaceae, having been found in *Trichilia prieuriana*, *Guarea guidonia*, *Ekebergia pterophylla* and *Turraea obtusifolia*. This provided good chemotaxonomic evidence for including *Nymania* in the subfamily Meliodeae.[18] Thus the finding of nymania-1 in the seed of *Turraea obtusifolia* is a further important chemotaxonomic link between the genera *Nymania* and *Turraea*.

3.4 Experimental

Milled seed (46.66 g) of *Turraea obtusifolia* was extracted with hexane in the manner explained in the "Foreword to Experimental" section. Chromatographic separation of the seed extract (2.43 g) yielded three amorphous compounds (**3-A**), (**3-B**) and (**3-C**).

3.4.1 Physical data of compound 3-A

Melianone, 21R(21S),23R:24S,25-diepoxy-21-hydroxy-tirucall-7-en-3-one

Yield : 2.4 mg

Mass spectrum (spectrum 3-A1) :

m/z $[M]^+$ - 470.3287

$[M-CH_3]^+$ - 455

$[M-C_4H_7O]^+$ - 399

Infrared spectrum (spectrum 3-A2) :

υ_{max} (NaCl) : 3430 (O-H stretching),
1707 (sharp band, C=O stretching),
1456 (C-H deformation),
1386 (methyl group symmetrical deformation),
1251 (C-O-C epoxy stretching) and
755 (C-H out-of -plane deformation) cm^{-1}.

^{1}H NMR chemical shifts (spectrum 3-A3) :

δ5.34(1H, *br s*, H-21), 5.30(1H, *br s*, H-7), 3.90(1H, *m*, H-23); 2.80(1H, *d*, *J*=8Hz, H-24), 2.70(1H, *d*, *J*=8Hz, H-24).

^{13}C NMR chemical shifts (spectrum 3-A4) and (spectrum 3-A5, expansion of spectrum 3-A4) :

δ 216.3 (C), 145.7 (C), 145.6 (C), 118.2 (CH), 118.1 (CH), 101.6 (CH), 97.8 (CH), 78.8 (CH), 77.2 (CH), 67.7 (CH), 65.3 (CH), 58.1 (C) and 57.3 (C).*

* Due to the pairing of many peaks in the carbon spectrum only the major downfield peaks are listed.

3.4.2 Physical data of compound 3-B

Turraeanthin, 3β-acetoxy-21R(21S),23R:24S,25-diepoxy-tirucall-7-en-21-ol

Yield : 2.8 mg

Mass spectrum (spectrum 3-B1) :

m/z $[M]^+$ - 514.3653

$[M-CH_3]^+$ - 499

Infrared spectrum (spectrum 3-B2) :

υ_{max} (NaCl) : 3428 (O-H stretching),
1728 (sharp band, C=O stretching),
1458 (C-H deformation),
1248 (C=C) and
756 (C-H out-of -plane deformation) cm^{-1}.

^{1}H NMR chemical shifts (spectrum 3-B3) :

δ5.25(1H, *br s*, H-21), 5.23(1H, *br s*, H-7), 4.50(1H, *m*, H-3), 3.90(1H, *m*, H-23); 2.80(1H, *d*, *J*=8Hz, H-24), 2.70(1H, *d*, *J*=8Hz, H-24) and 2.00 (3H, *s*, OAc),

^{13}C NMR chemical shifts (spectrum 3-B4) and (spectrum 3-B5, expansion of spectrum 3-B4) :

δ 171.0 (C), 145.6 (C), 145.5 (C), 118.2 (CH), 117.9 (CH), 101.8 (CH), 97.8 (CH), 78.5 (CH), 67.7 (CH), 65.3 (CH), 58.0 (C) and 57.3 (C).

* Due to the pairing of many peaks in the carbon spectrum only the major downfield peaks are listed.

3.4.3 Physical data of compound 3-C

nymania-1

Yield : 4.4 mg

Infrared spectrum (spectrum 3-C2) :

υ_{max} (NaCl) : 3439 (OH stretching)
2963 (saturated C-H stretching),
1724 (broad band, C=O stretching),
1383 (methyl group symmetrical deformation),
1245, 1030 (C-O stretching) and
756 (C-H out-of -plane deformation) cm^{-1}.

Optical rotation : $[\alpha]_D = -80.7^\circ$ ($CHCl_3$, c= 4.8 x 10^{-4})

^{1}H NMR chemical shifts (spectrum 3-C3) : Table 3.1

^{13}C NMR chemical shifts (spectrum 3-C4) : Table 3.1

COSY spectrum : spectrum 3-C5

HETCOR spectrum : spectrum 3-C6

3.5 Table of ^{1}H and ^{13}C NMR chemical shifts for compound 3-C

Table 3.1 : ^{1}H and ^{13}C NMR chemical shifts.

Atom Number	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)
1	H-1 (*dd*), 5.21, *J*= 3.2, 12.5 Hz	70.5 (*d*)
2	H-2_a (*dd*), 2.03, *J*=3.2, 13.0 Hz H-2_b (*t*), 2.55, *J*=13.2 Hz	39.8 (*t*)
3		119.5 (*s*)
4		82.6 (*s*)
5	H-5 (*d*), 2.95, *J*=10.7 Hz	48.9 (*d*)
6	H-6_a (*d*), 1.71, *J*=17.8 Hz H-6_b (*dd*), 2.70, *J*=9.7, 17.6 Hz	33.6 (*t*)
7		175.8 (*s*)
8		142.6 (*s*)
9	H-9 (*d*), 4.19, *J*=8.2 Hz	48.6 (*d*)
10		50.5 (*s*) or 49.4 (*s*)
11	H-11 (*dd*), 5.37, *J*=8.5, 10.6 Hz	71.2 (*d*)
12	H-12 (*d*), 5.98, *J*=13.5 Hz	74.7 (*d*)
13		50.5 (*s*) or 49.4 (*s*)
14		80.7 (*s*)
15		207.3 (*s*)
16	H-16_a (*dd*), 2.94, *J*=8.5,18.8Hz H-16_b (*dd*), 2.30, *J*=9.6,19.0Hz	41.5 (*t*)
17	H-17 (*dd*), 3.97, *J*=7.4, 9.3 Hz	35.1 (*d*)
18	H-18 (*s*), 0.98	13.2 (*q*)
19	H-19 (*s*), 1.25	16.6 (*q*)
20		123.4 (*s*)
21	H-21 (*s*), 7.20	140.5 (*d*)
22	H-22 (*s*), 6.25	110.6 (*d*)
23	H-23 (*s*), 7.37	143.0 (*d*)
28	H-28 (*s*), 1.40	28.8 (*q*)
29	H-29_a (*d*), 3.62, *J*=8.6 Hz H-29_b (*d*), 4.11, *J*=8.6	73.5 (*t*)
30	H-30_a (*s*), 6.05, H-30_b (*s*), 6.04	123.4 (*s*)
1′		175.0 (*s*)
2′	H-2′ (*m*), 3.20	74.1 (*d*)
3′	H-3′ (*m*), 1.52	38.3 (*d*)
4′	H-4′ (*m*), 1.16	23.2 (*t*)
5′	H-5′ (*t*), 0.78, *J*=7.4 Hz	11.7 (*q*)
6′	H-6′ (*d*), 0.86, *J*=4.7 Hz	15.3 (*q*)
1″	H-1″ (*s*), 7.93	160.9 (*d*)
CH_3COO		169.7 (*s*)
$\mathbf{CH_3}COO$	2.00 (*s*)	21.1 (*q*)
$COO\mathbf{CH_3}$	3.70 (*s*)	53.0 (*q*)

3.6 References

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Chapter 4

Investigation of the Australian Meliaceae: Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

Index

Chapter 4

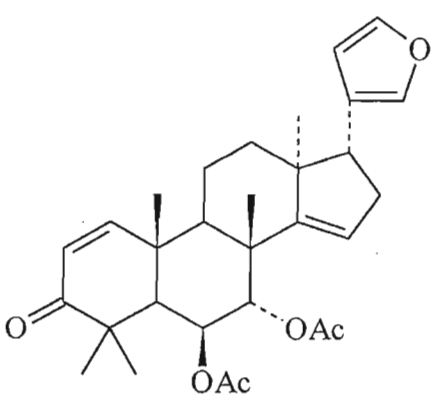
Investigation of the Australian Meliaceae: Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

4.1 Introduction

The genus *Dysoxylum* belongs to the Meliaceae family and is indigeneous to Australasia and the South Pacific Ocean Islands. Within the genus *Dysoxylum*, thirteen species have been examined previously, namely *Dysoxylum binectariferum*, *D. malabaricum*, *D. fraseranum*, *D. alliaceum*, *D. acutangulum*, *D. pettigrewianum*, *D. roseum*, *D. spectabile*, *D. richii*, *D. cumingianum*, *D. muelleri*, *D. lenticellare* and *D. schiffnerii*. A literature search was undertaken to become acquainted with the type of compounds naturally produced by the genus *Dysoxylum*.

4.1.1 *Dysoxylum binectariferum*

Dysobinin (**4-1**), a tetranortriterpenoid was isolated by Singh *et al.* (1976)[1] from the fruits of the species, while Naik *et al.* (1988)[2] isolated rohitukine (**4-2**) from the stem bark of *Dysoxylum binectariferum*.



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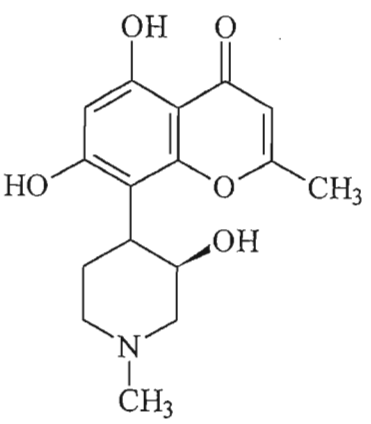

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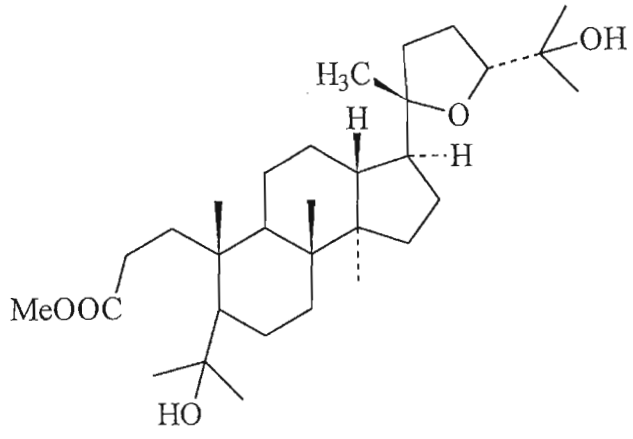
4.1.2 *Dysoxylum malabaricum*

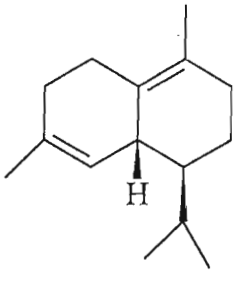
Govindachari *et al.* (1994)[3] isolated dymalol (**4-3**) from the leaves of the species.



4-3

4.1.3 *Dysoxylum fraseranum*

Nagasampagi *et al.* (1968)[4] isolated δ-cadinene (**4-4**), Paknikar *et al.* (1962)[5] isolated α-elemene (**4-5**) and Gough (1964)[6] isolated δ-elemene (**4-6**) from the fruit of the species.



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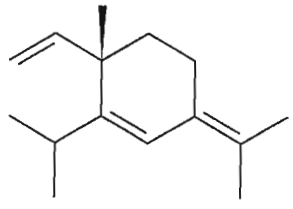

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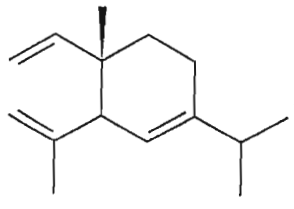

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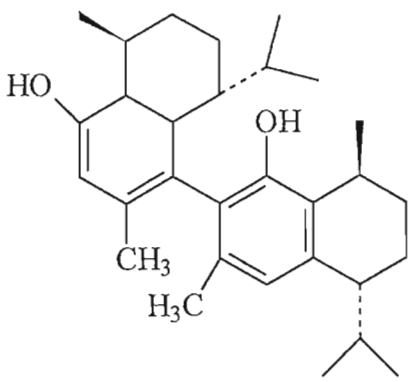
4.1.4 *Dysoxylum alliaceum and acutangulum*

Nishizawa *et al.* (1985)[7] isolated bicalamenene (**4-7**) from the dried peel of *Dysoxylum alliaceum*, while Nishigawa *et al.* (1983)[8] isolated 2-hydroxycalamenene (**4-8**) from the dried seeds of *Dysoxylum alliaceum* and *Dysoxylum acutangulum*.



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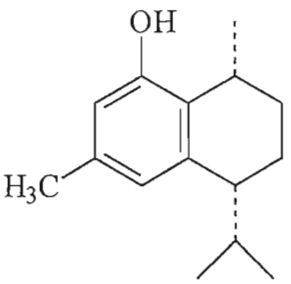
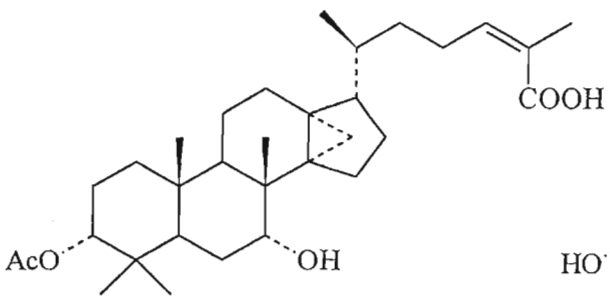


4-8

4.1.5 *Dysoxylum pettigrewianum*

Mulholland *et al.* (1994)[9] isolated dysoxylic acid A (**4-9**) and several related compounds, as well as 3α-hydroxy-7,24*Z*-tirucalladien-26-oic acid (**4-10**) from the hexane extracts of the wood of the species. The hexane extract of the bark was investigated in the present work.



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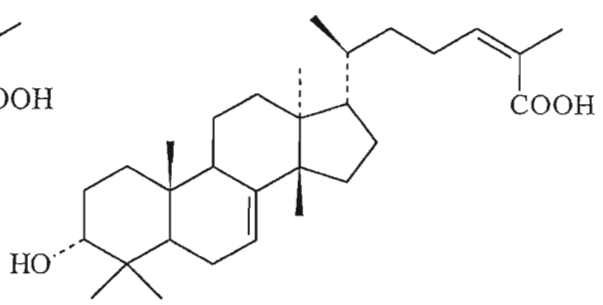

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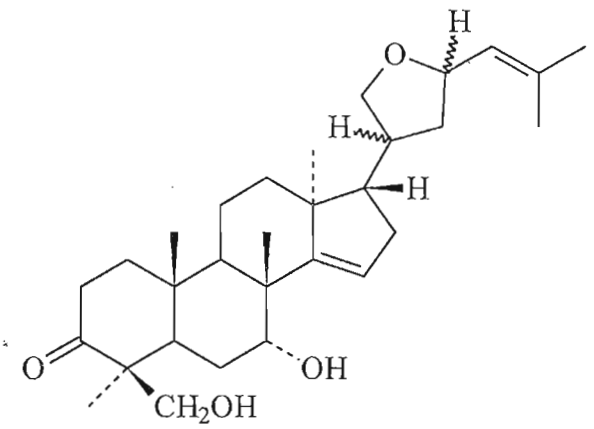
4.1.6 *Dysoxylum roseum*

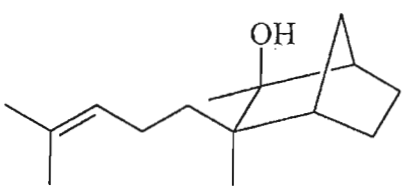
Adesanya *et al.* (1991)[10] isolated dysonone B (**4-11**) and several derivatives from the leaves of *Dysoxylum roseum*.



4-11

4.1.7 *Dysoxylum spectabile*

Russel *et al.* (1994)[11] isolated β-santal-10-en-3-ol (**4-12**) from the fruits of *Dysoxylum spectabile*. Monkhe (1997)[51] isolated two diterpenoids (**4-13**) and (**4-14**) and two limonoids (**4-15**) and (**4-16**) from the bark of the species.



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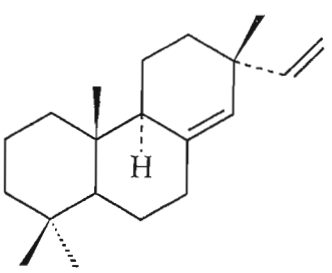

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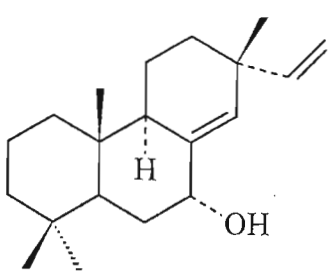

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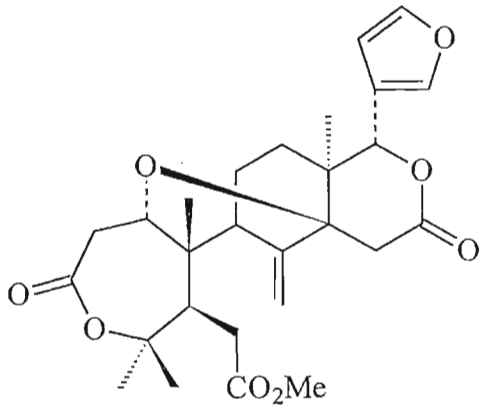

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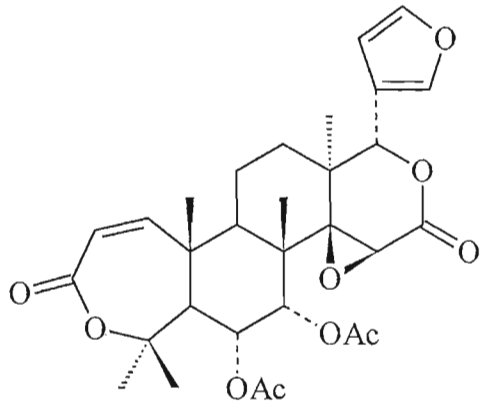

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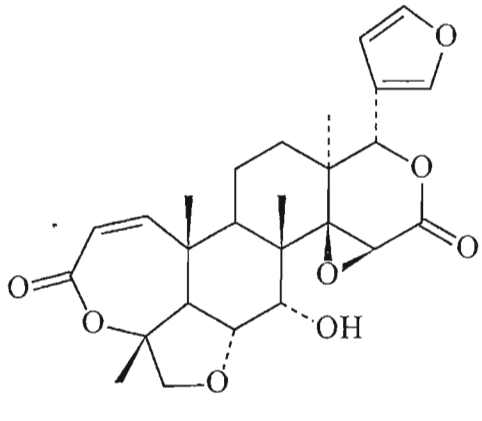
4.1.8 *Dysoxylum richii*

Jogia *et al.* (1987)[12,13] isolated dysoxylin (**4-17**), obacunol (**4-18**) and dysoxylone (**4-19**) from the leaves of *Dysoxylum richii*. Aalbersberg *et al.* (1991)[14] isolated richenol (**4-20**) and richenoic acid (**4-22**) from the fruits of the species. Singh[15] *et al.* (**4-21**) isolated the 4-hydroxy derivative of richenoic acid also from the fruit of the species.



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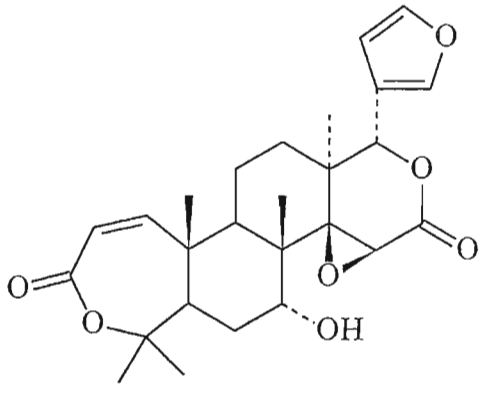

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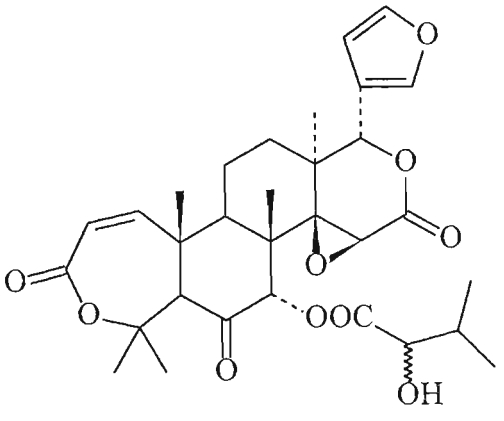

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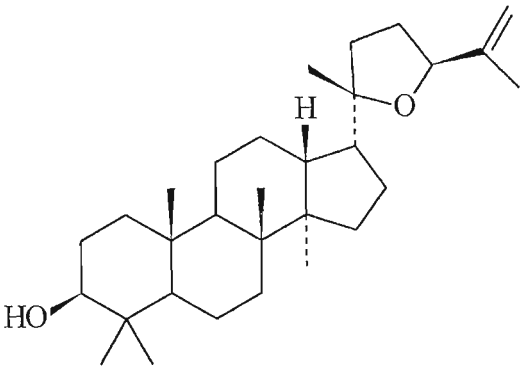

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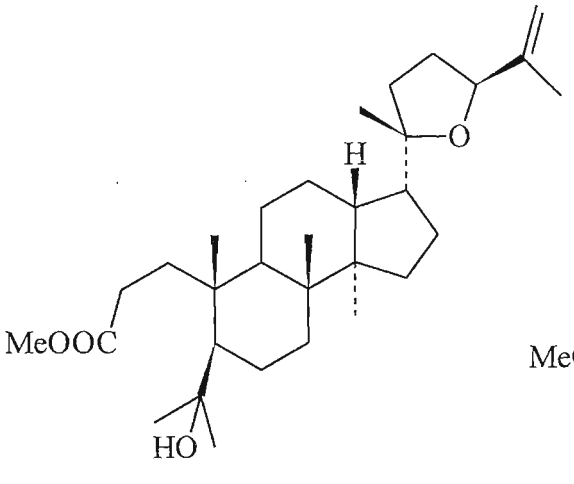

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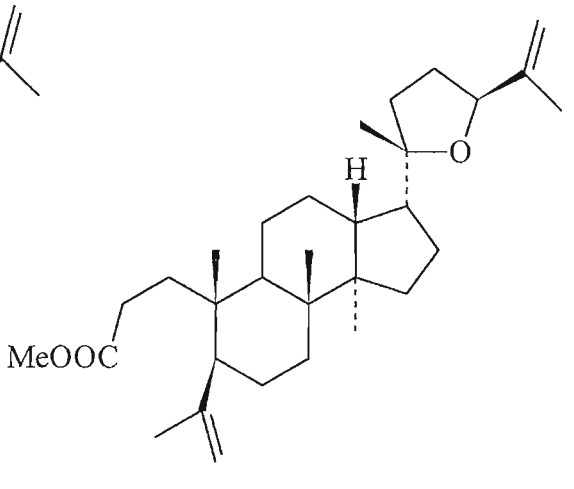

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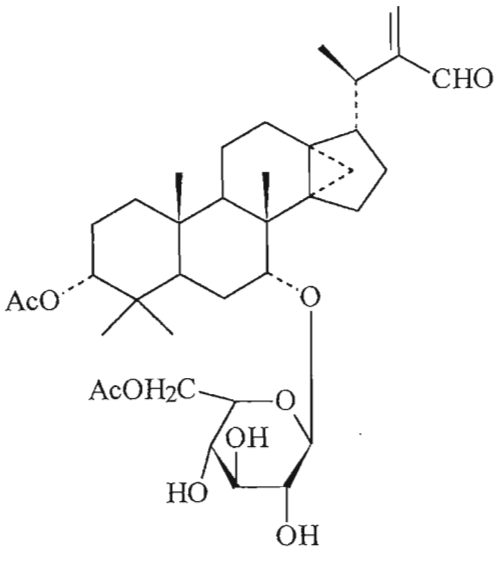
4.1.9 *Dysoxylum cumingianum*

Kashiwada *et al.* (1992) isolated cumindyoside A (**4-23**)[16], cumingianoside B (**4-24**)[16], cumindyoside B (**4-25**)[17] and cumingianoside F (**4-26**)[17] from the leaves of the species.



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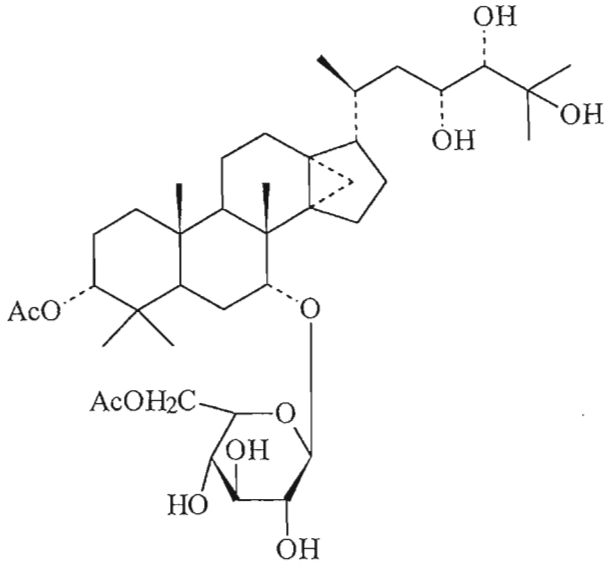

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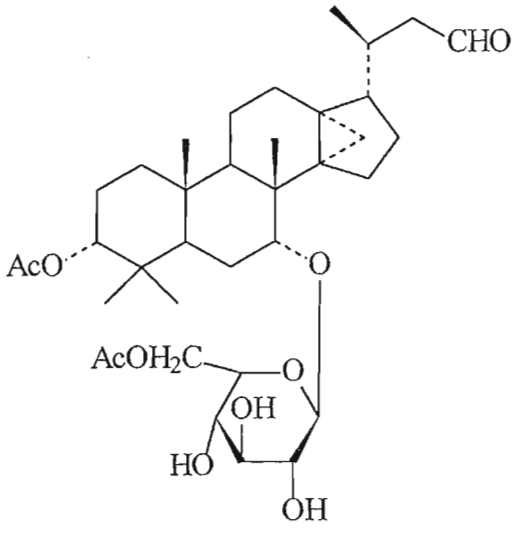

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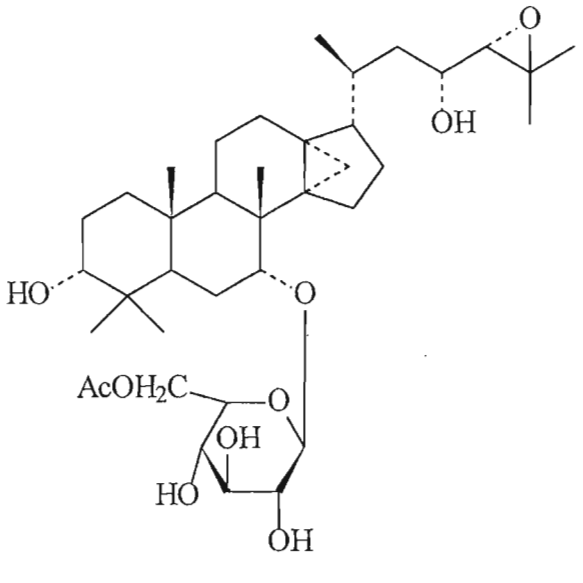
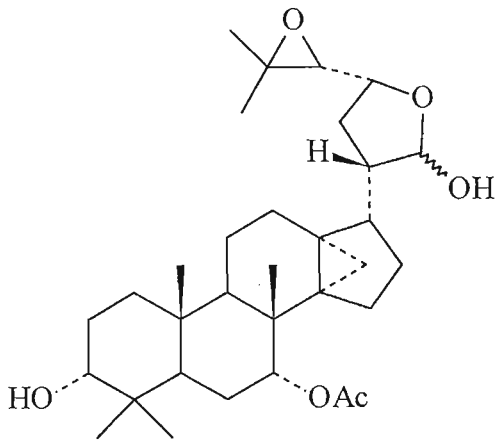


4-26

4.1.10 *Dysoxylum muelleri*

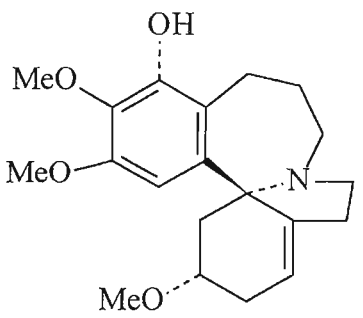
Mulholland *et al.* (1996)[18] isolated several glabretal (**4-27**) type compounds from the hexane extract of the wood and bark of *Dysoxylum muelleri*. The dichloromethane extract of the wood and bark was investigated in the present work.



4-27

4.1.11 *Dysoxylum lenticellare*

Aladesanmi *et al.* (1994)[19] isolated 2α-methoxycomosivine (**4-28**) from the stem of the species, while Aladesanmi *et al.* (1991)[20] isolated 2α-hydroxydyshomerythrine (**4-29**) from the heartwood of the species. Aladesanmi *et al.* (1988) isolated lenticellarine (**4-30**)[21], dysazecine (**4-31**)[22], dysoxyline (**4-32**)[22] and ferrubietolide (**4-33**)[23] from the leaves of the species.



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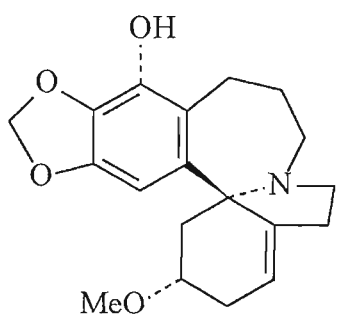

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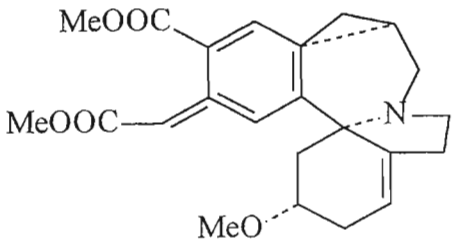

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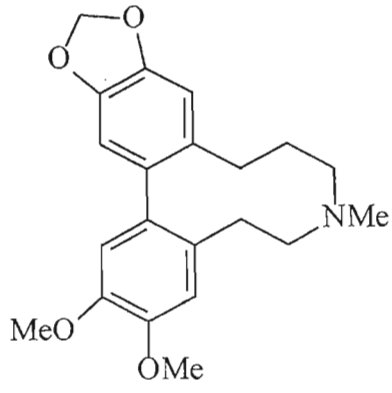
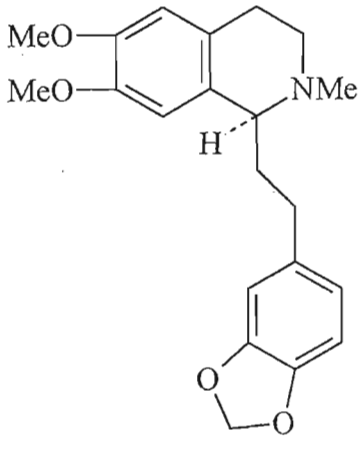
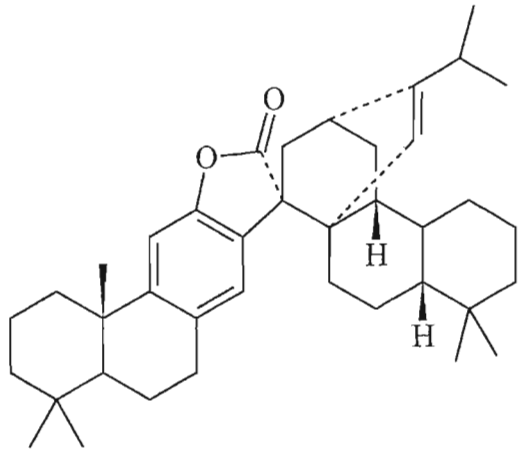


4-31



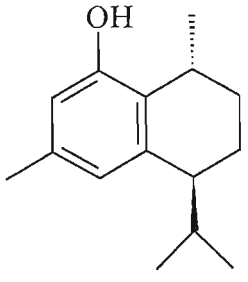
4-32



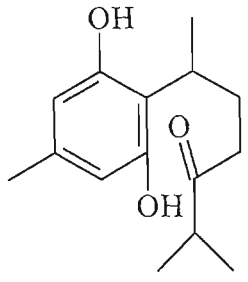
4-33

4.1.12 *Dysoxylum schiffnerii*

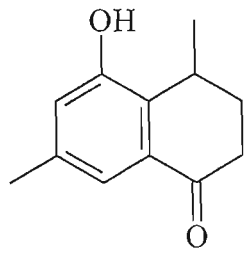
Iourine (1996)[52] isolated three sesquiterpenoids (**4-34**), (**4-35**) and (**4-36**) from the hexane extract of the wood.



4-34



4-35

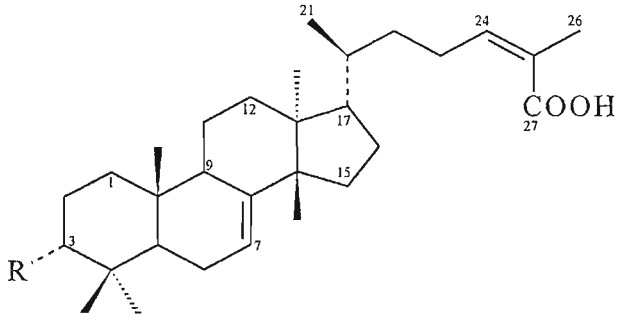


4-36

4.2 Extractives from *Dysoxylum pettigrewianum*

In this work the hexane extract of the bark of *Dysoxylum pettigrewianum* was examined. The plant material was collected in Australia by Professor D.A.H. Taylor. The investigation yielded three compounds (**4-A**, **4-B** and **4-C**). The first compound (**4-A**) was identified as the known masticadienonic acid which was found previously in the wood[9], the other two compounds (**4-B** and **4-C**) were novel.

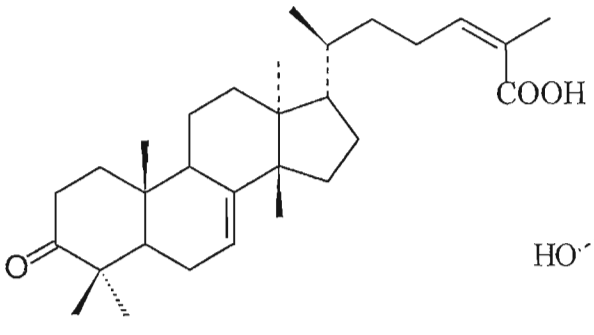
4.2.1 Nomenclature



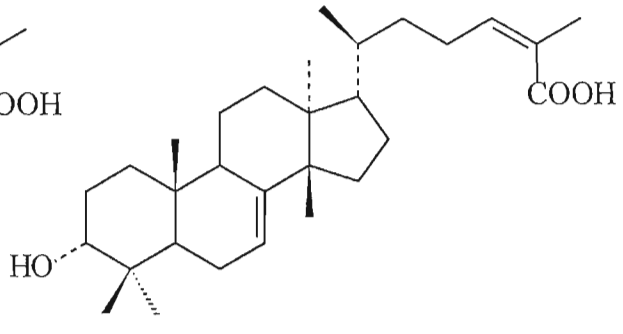
- **4-1 to 4-56**, where **4** refers to chapter four and **1 to 56** refers to compounds discussed in the literature search. In all cases, H-5 and H-9 are α in the protolimonoids and limonoids discussed.
- **4-A to 4-C**, where **4** refers to chapter four and **A to C** refers to compounds isolated from *Dysoxylum pettigrewianum* during this research.
- 4-A1, where 4 refers to chapter four, A to compound isolated from *Dysoxylum pettigrewianum* during this research and 1 to spectrum 1 of the compound concerned.

4.2.2 Introduction

Masticadienonic acid (**4-37**) together with its corresponding alcohol, masticadienolic acid (**4-38**) were initially reported[24,25] as extractives from gum mastic.

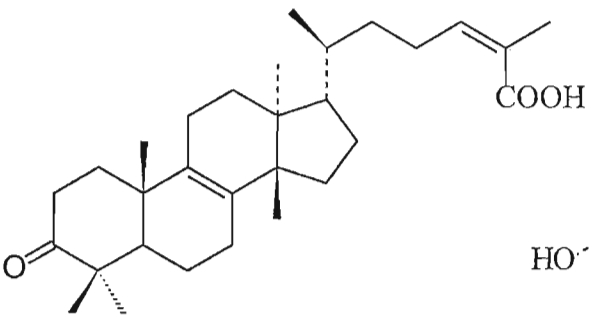


4-37

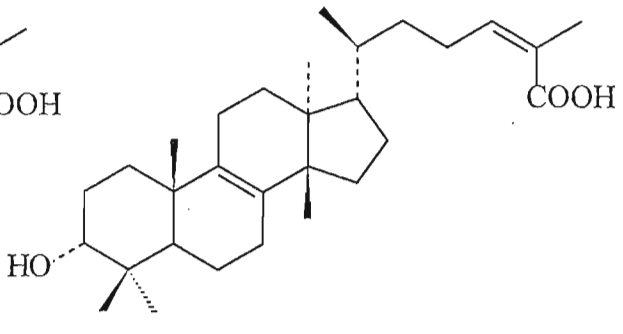


4-38

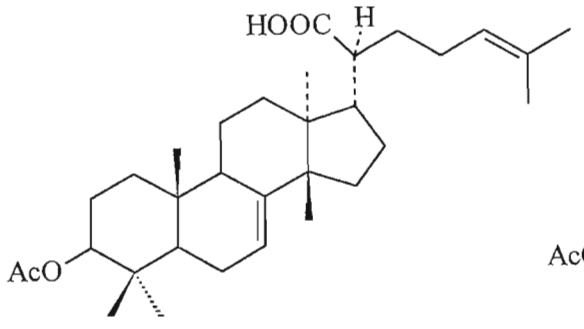
The corresponding Δ^8 isomers [isomasticadienonic (**4-39**) and isomasticadienolic (**4-40**) acids] have also been reported.[26,27] The acids are characterized by a tetracyclic tirucallane skeleton with a Δ^7 or Δ^8 double bond, a Δ^{24} double bond together with a carboxylic acid group at C-26, in which case it is α,β-unsaturated, or C-21 as in the elemolic acids (**4-41**, **4-42**).[28]



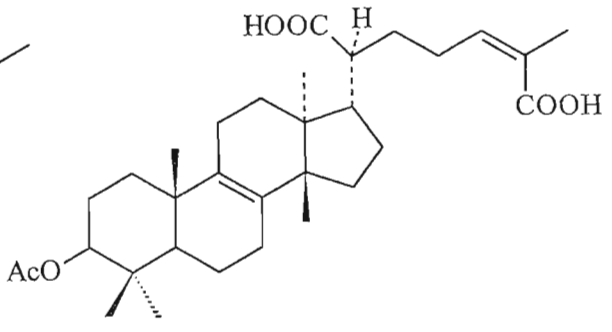
4-39



4-40

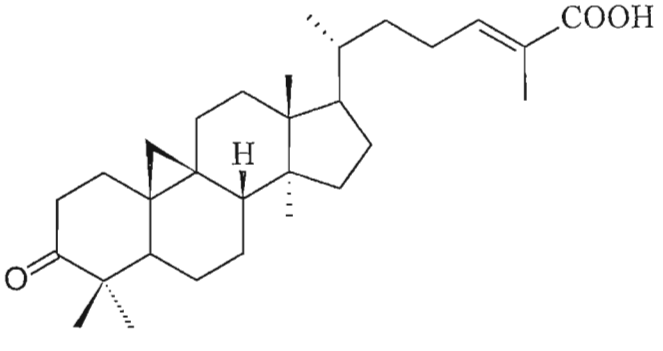


4-41



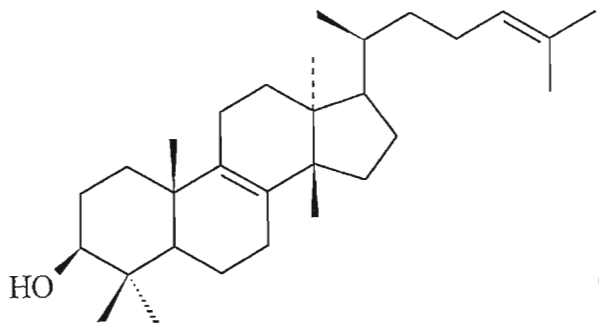
4-42

Mangiferonic acid (**4-43**)[29] is a cycloartane with a C-9, 10, 19-cyclopropane ring.

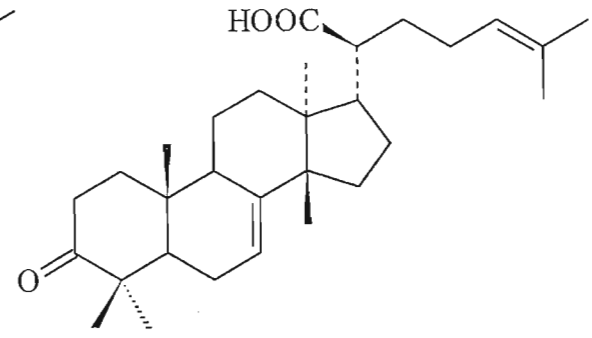


4-43

Biogenetically, the masticadienonic and elemadienolic acid series is assumed to be derived from the same Δ^7 isomer of tirucallol (**4-44**) by oxidation of the C-26 and C-21 methyl groups respectively.[30] The 3α-hydroxy group may be derived from the normal 3β-hydroxy group by oxidation to the ketone function, which was present both in the β-elemonic (**4-45**) acid and masticadienonic (**4-37**) and isomasticadienonic (**4-39**) acids. Subsequent reduction of the carbonyl group to the 3α-hydroxy group with, in some instances, isomerisation of the Δ^7 double bond occur at some stage during the biosynthetic process.

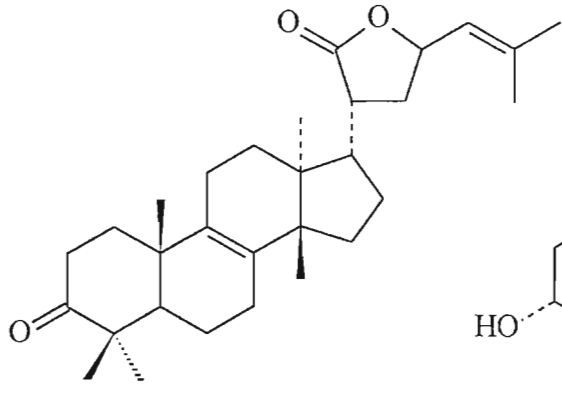


4-44

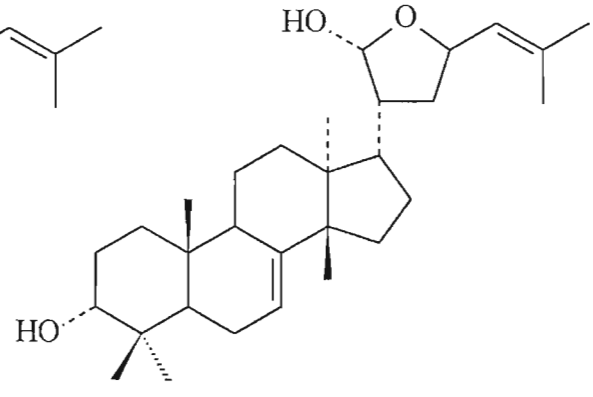


4-45

It was shown[30] that isoflindissone (**4-46**) which is the Δ^8 -3-keto-21-lactone analogue of flindissol (**4-47**) was converted via hydrogenolysis to β-elemonic acid (**4-45**). Flindissol is considered to be an intermediate in the biosynthetic process leading to the formation of the more oxidised triterpenoids, such as limonoids, from those with an intact side chain, and was the first reported compound which contained the potential furan ring precursor and the rest of the side chain intact.



4-46



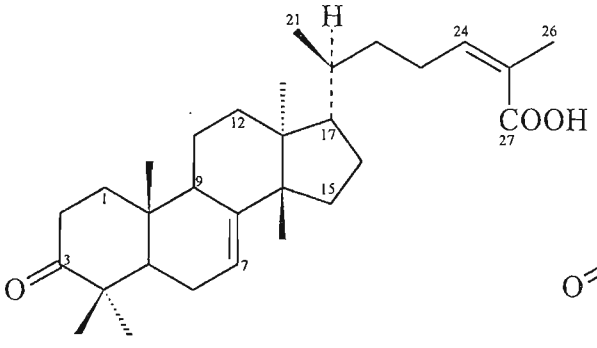
4-47

The presence of hydroxyl substituents at C-7 and a β-axial methyl group at C-8 in compounds (**4-B**) and (**4-C**) suggests that they share a common biosynthetic pathway with limonoids. The presence of the C-13,14,18 cyclopropyl ring suggests that this may represent an intermediate stage in the biosynthetic process leading to the formation of the limonoids. Glabretal (**4-27**)[31], for example, which is thought to occupy an intermediate position between the two groups of

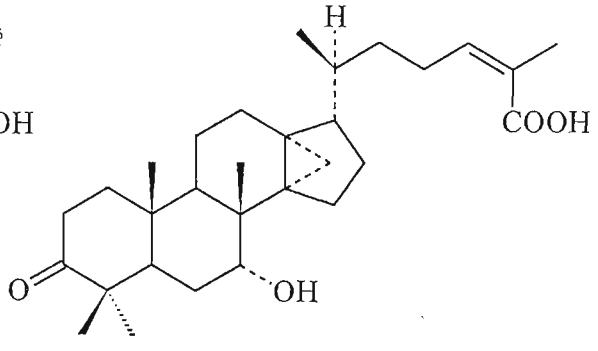
protolimonoids, has a 7α-acetyl group, a 8β-methyl group and the C-13, 14, 18 cyclopropyl ring. It is possible that the cyclopropyl ring containing compounds may be a normal intermediates in the apo-change but under laboratory conditions they would not be likely to survive the conditions of acid catalysis used to open the 7,8-oxide ring.

4.2.3 Results and discussion

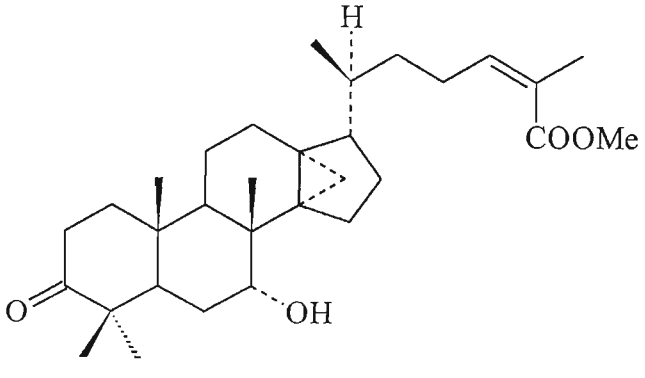
Compound (**4-A**) was identified as the known masticadienonic acid. Compound (**4-B**) possess a 7α-hydroxy substituent instead of the Δ^7 double bond of masticadienonic acid and a C-13,14,18 cyclopropane ring as compared to compound (**4-A**). Compound (**4-C**) was a derivative of compound (**4-B**) having a carbomethoxy group instead of the carboxylic acid side chain.



4-A



4-B



4-C

4.2.3.1 Structural elucidation of compound (4-A)

Mass spectrometry of compound (**4-A**), (spectrum 4-A1) showed the $[M]^+$ peak to be at *m/z* 454, which corresponded to a molecular formula $C_{30}H_{46}O_3$. The mass spectrum indicated the loss of

a methyl group $[M\text{-}CH_3]^+$ by the peak at *m/z* 439 and the loss of a methyl group and a water molecule $[M\text{-}CH_3\text{-}H_2O]^+$ by the peak at *m/z* 421. The presence of the an α,β-unsaturated carboxylic acid (1706 cm^{-1} and 2955 cm^{-1}) was indicated in the infrared spectrum (spectrum 4-A2).

The molecular formula of compound (**4-A**) suggested an equivalent of eight double bonds which corresponded with a tetracyclic molecule with two double bonds and two carbonyl groups. The methyl distribution profile (five tertiary, one secondary, one vinylic) suggested a lanostane or an euphane/triucallane skeleton.

The ^1H NMR spectrum (spectrum 4-A3) of compound (**4-A**) displayed a triplet at δ 6.07 (1H, *J*= 8 Hz) and a broad triplet at δ 5.33 which suggested the possibility of two double bonds being present. This was confirmed in the ^{13}C NMR spectrum (spectrum 4-A4) which had resonances at δ 117.8 (CH) and δ 147.3 (CH), and quaternary carbon resonances at δ 145.9 and δ 125.9. Also present in the proton spectrum was a triplet of doublets at δ 2.78 ascribable to the H-2 axial proton when a keto-group is present at C-3. A resonance at δ 217.1 in the ^{13}C NMR spectrum was assignable to the C-3 keto group. A vinyl methyl group was indicated by a downfield-shifted methyl proton resonance at δ 1.92. Five other tertiary methyl group proton resonances occurred at δ 1.25, 1.12, 1.05, 1.01 and 0.81 (each s, 3H) and a secondary methyl group was indicated by a three-proton doublet at δ 0.89. The additional carbonyl carbon resonance at δ 173.4 in the carbon spectrum was attributed to the carboxylic acid group carbonyl carbon.

The COSY spectrum (spectrum 4-A5) indicated that the downfield-shifted methyl group (δ 1.92) was long ranged coupled to the alkene proton triplet at δ 6.07 (H-24).

Since only one methyl group (at C-26 or C-27) was deshielded in the proton spectrum of compound (**4-A**), it was assumed that the other was oxidised to the carboxylic acid. The alkene resonance at δ 147.3 (CH) in the carbon spectrum was correlated to the H-24 triplet at δ 6.07 in the HETCOR spectrum (spectrum 4-A6), while the quaternary carbon resonance at δ 125.9 was assigned to C-25.

The HETCOR spectrum correlated the alkene proton (H-7) at δ 5.33 (*bt*) to the carbon resonance at δ 117.8 (CH), while the quaternary carbon resonance at δ 145.9 was assigned to C-8.[27]

The above spectral information together with the observed melting point (180-182^{0}C) confirmed that compound (**4-A**) was the Z-isomer of masticadienonoic acid.[24]

The HETCOR spectrum indicated that the H-27 methyl group proton resonance at δ 1.92 correlated to a carbon (CH_3) resonance at δ 20.6. The chemical shift of the C-27 methyl group had previously been used to ascertain the stereochemistry of the Δ^{24} double bond. If the double bond has an E-configuration, the methyl group resonates at about δ 13.7 in the ^{13}C NMR spectrum since it is shielded by steric interaction with the C-23 methylene group, whereas the Z-configuration results in deshielding of the C-27 methyl group, in which the effect of the methylene group would not be pronounced. The chemical shift (δ 20.6) of the C-27 methyl group indicated Z-stereochemistry of the Δ^{24} double bond.[32]

Use was made of the HETCOR spectrum of compound (**4-A**) in conjunction with the COSY spectrum for assignment of chemical shifts to carbon atoms around the C-20 chiral centre. The C-21 secondary methyl group protons (*d*, δ 0.89), which correlated with a carbon methyl resonance at δ 18.3, were seen to be coupled in the COSY spectrum to a proton (m, δ 1.50, H-20) which correlated with a carbon (CH) resonance at δ 36.0 (C-20). Further correlation was observed between H-20 and C-22 (CH_2, δ 35.6) and H-20 and C-17 (CH, δ 52.3). The C-20 resonance was in accordance with those reported for tirucallane derivatives wherein H-20 is α-orientated.[27] The COSY spectrum also showed that H-7 (bt, δ 5.33) was coupled to a multiplet centred at δ 2.10 (2H-6) which corresponded to a carbon resonance at δ 24.2 (CH_2, C-6) in the HETCOR spectrum. The 2H-6 resonance was coupled further to H-5 (δ 1.75) which correlated with a carbon resonance at δ 52.8 (CH, C-5) in the HETCOR spectrum.

The HETCOR spectrum also indicated that the H-2_{ax} triplet of doublets (δ 2.78) correlated with a carbon resonance at δ 34.0 (CH_2, C-2) and was seen to be coupled in the COSY spectrum to a multiplet centred at δ 1.96 (2H-1) which was attached to a methylene carbon atom which resonated as a (CH_2) at δ 38.6. H-24 (δ 6.10), besides being longed-ranged coupled to H-27, was also coupled to a multiplet at δ 2.50 (2H-23), which correlated with a carbon (CH_2) resonance at δ 26.8 (C-23), which was further coupled to a multiplet at δ 1.41 (2H-22) which corresponded to a resonance at δ 35.6 (CH_2) which was assigned to C-22 in the carbon spectrum.

4.2.3.2 Structural elucidation of compound (4-B)

Mass spectrometry of compound (**4-B**), (spectrum 4-B1) showed the $[M]^+$ peak to be at *m/z* 452, which corresponded to a molecular formula $C_{30}H_{46}O_4$. The mass spectrum also showed the loss of a methyl group $[M\text{-}CH_3]^+$ with a peak at *m/z* 437.

Infrared analysis (spectrum 4-B2) showed absorption bands for an α,β-unsaturated carboxylic acid (1702 cm^{-1} and 3952 cm^{-1}). This information suggested that compound (**4-B**) was also similar to masticadienonic acid (**4-A**). Application of the double bond equivalence formula gave an equivalent of eight double bond equivalents.

The proton spectrum of compound (**4-B**), (spectrum 4-B3) displayed a one proton triplet at δ 6.06 (*J*=8.0 Hz) ascribable to H-24 of the α,β-unsaturated carboxylic acid side chain, as in compound **4-A**. This was confirmed in the ^{13}C NMR spectrum (spectrum 4-B4) which had resonances related to the double bond (C-24:C-25) at δ 147.3 (C**H**, C-24) and δ 125.9 (**C**, C-25) while the C-26 carboxylic acid carbonyl carbon resonance occurred at δ 173.4 (**C**). A sharp triplet at δ 3.78 ($W_{1/2}$=8.0 Hz, 1H) in the proton spectrum was ascribable to H-7β since the chemical shift and value of the half width is typical for the proton when a hydroxy substituent is present at C-7α.[33] The ^{13}C NMR spectrum showed that C-7 occurred as a resonance at δ 74.1 (C**H**). A keto group at C-3 was indicated in the proton spectrum by the triplet of doublets at δ 2.50, ascribable to H-2_{ax}, and the C-3 resonance which occurred at δ 217.5 in the carbon spectrum.

Four tertiary methyl proton resonances were evident at δ 1.12, 1.03, 1.02 and 0.99 (each *s*, 3H) and vinyl and secondary methyl groups were indicated by resonances at δ 1.92 (*s*, 3H) and δ 0.92 (*d*, 3H) respectively. The proton spectrum also had a pair of doublets at δ 0.53 (1H, *J*=4.5 Hz) and δ 0.68 (1H, *J*=4.2 Hz) characteristic of the methylene protons in a tetrasubstituted cyclopropane ring.[31]

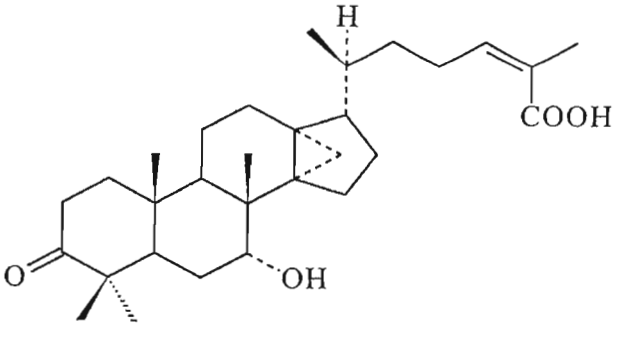
This was confirmed in the ^{13}C NMR spectrum in which the characteristic upfield chemical shift of the cyclopropane methylene carbon was evident at δ 16.9 (CH_2).

The above spectral information confirmed that compound (**4-B**) possessed, in addition to the side chain and 3-keto group of masticadienonic acid (**4-A**), a 7α-hydroxy group and a cyclopropanyl ring.

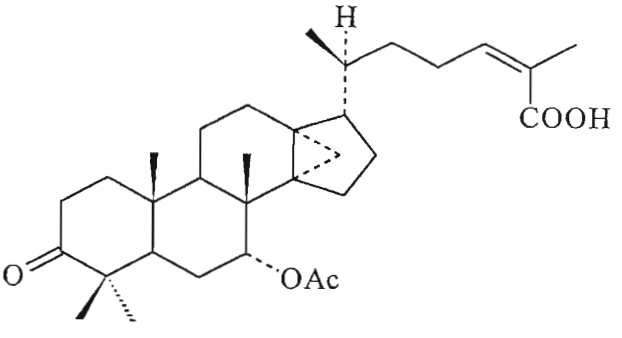
The cyclopropanyl ring has been previously observed in the C-9, 10 and 19 position, as in the cycloartane derivatives (**4-43**)[34,35,36] and also at the C-13,14 and 18 position as in glabretal (**4-27**).[31] The cyclopropanyl methylene carbon chemical shift is deshielded in cycloartane derivatives, resonating at about δ 29.5 ($\underline{C}H_2$),[32] but occurs at about δ 16.0 ($\underline{C}H_2$)[37] in the glabretal-type compounds. The cyclopropanyl methylene protons are also distinguishable in both systems. These protons occur further downfield (between δ 0.6 and δ 0.8) in cycloartane derivatives[32] but occur between δ 0.4 and δ 0.6 in glabretal derivatives.[37] The proton and carbon chemical shifts in compound (**4-B**) suggested placement in the C-13,14,18 position.

The COSY spectrum (spectrum 4-B5) of compound (**4-B**) showed coupling between the cyclopropanyl methylene protons (δ 0.53, 0.68, each *d*, H-18_a, H-18_b). Longe range coupling was observed between 3H-27 (*s*, δ 1.92) and H-24 (*t*, δ 6.06) in the COSY spectrum. The H-24 resonance was also coupled to a multiplet at δ 2.50 (2H-23) which corresponded to a carbon resonance at δ 26.9 ($\underline{C}H_2$, C-23). The latter was coupled to 2H-22 (*m*, δ 1.42) which corresponded to the resonance at δ 35.5 ($\underline{C}H_2$, C-22). The carbon resonance of the C-27 methyl group (δ 20.6) suggested that the Δ^{24} double bond the Z-stereochemistry. Also evident from the COSY spectrum was the coupling between the C-21 secondary methyl group protons (3H, *d*, δ 0.92) and H-20 (*m*, δ 1.50). Thus structure (**4-B**) was assigned to this compound.

Acetylation of compound (**4-B**) resulted in the known 7α-acetatyl derivative (**4-48**, spectrum 4-B7).[9]



4-B



4-48

4.2.3.3 Structural elucidation of compound (4-C)

Compound (**4-C**) decomposed after the ^{1}H NMR spectrum (spectrum 4-C1) and COSY spectrum (4-C2) had been recorded. Nevertheless, the tentative structure (**4-C**) was proposed. Compound (**4-C**) had a very similar proton and COSY spectrum to that obtained for compound (**4-B**).

The proton spectrum of compound (**4-C**) displayed a three proton singlet at δ 3.71 which is indicative of a carbomethoxy group proton resonance. The H-24 triplet which occurred at δ 6.06 (J=8.0Hz) in the α,β-unsaturated carboxylic acid side chain in compound **4-A**, had now shifted to δ 5.90 in compound (**4-C**) which indicated that the carboxylic acid group had been converted into a methyl ester. The broad triplet at δ 3.78 ($W_{1/2}$=8.0 Hz, 1H) in the proton spectrum was ascribable to H-7β since the chemical shift and value of the coupling constant is typical for the proton when a hydroxy substituent is present at C-7α.[33] A 3-keto group was indicated in the proton spectrum by the triplet of doublets at δ 2.45, ascribable to H-2_{ax} (as in the proton spectrum of compound **4-B**).

Four tertiary methyl proton resonances were evident at δ 1.08, 1.04, 1.01 and 0.93 (each *s*, 3H) and vinyl and secondary methyl group were indicated by resonances at δ 1.87 (*s*, 3H) and δ 0.93 (*d*, 3H) respectively. The proton spectrum also had a pair of doublets at δ 0.53 (1H, J=5.1 Hz) and δ 0.67 (1H, J=3.9 Hz) characteristic of the methylene protons in a tetracyclic cyclopropane ring.[31]

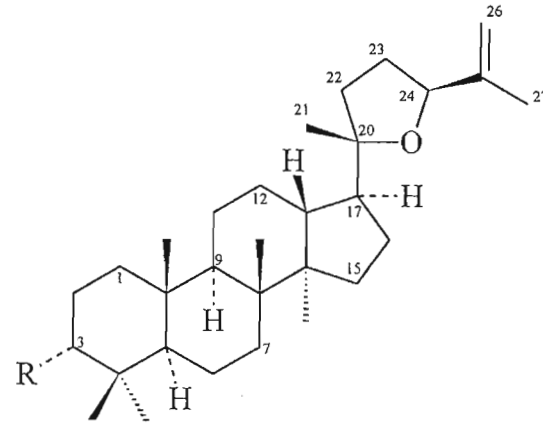
The above spectral information confirmed that compound (**4-C**) only differed from compound (**4-B**), by the presence of the carbomethoxy group at C-27 instead of the carboxylic acid group.

The COSY spectrum of compound (**4-C**) showed coupling between the cyclopropyl ring methylene protons (δ 0.53, 0.67, each *d*, H-18_a, H-18_b). Longe range coupling was observed between the 3H-27 (*s*, δ 1.92) and H-24 (*t*, δ 5.90) resonances. The H-24 proton signal was also coupled to a multiplet at δ 2.45 (2H-23).

4.3 Extractives from *Dysoxylum muelleri*

In this work the dichloromethane extract of the wood of *Dysoxylum muelleri* was examined. The plant material was collected in Australia by Professor D.A.H. Taylor. The investigation yielded two compounds (**4-D** and **4-E**). The first compound (**4-D**) was identified as the known compound cabraleone, while compound (**4-E**) was identified as the known compound richenone. Both compounds were isolated previously from *Dysoxylum richii*.[44] This was the first report of these two dammarane type compounds being isolated from *Dysoxylum muelleri*.

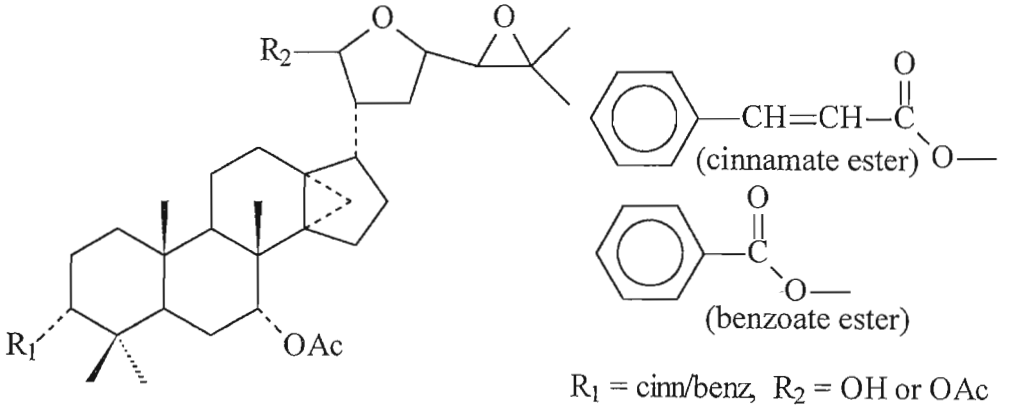
4.3.1 Nomenclature



- **4-D to 4-E**, where **4** refers to chapter four and **D to E** refers to compounds isolated from *Dysoxylum muelleri* during my research.
- 4-D1 and 4-E1, where 4 refers to chapter four, D and E to compounds isolated from *Dysoxylum muelleri* during my research and 1 to spectrum 1 of the compound concerned.

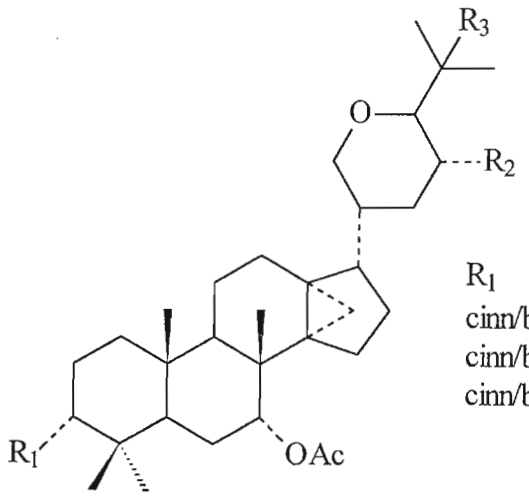
4.3.2 Introduction

Dysoxylum muelleri[40] has been previously investigated and yielded compounds of the type **4-49** and **4-50**, possess a glabretal-type[31] carbon nucleus and of the lanostone type **4-51**, protolimonoid compounds.



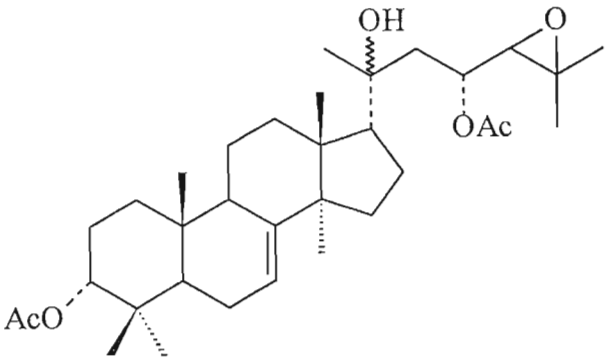
R_1 = cinn/benz, R_2 = OH or OAc

4-49



R_1	R_2	R_3
cinn/benz	OH	OH
cinn/benz	OAc	OH
cinn/benz	OAc	OAc

4-50

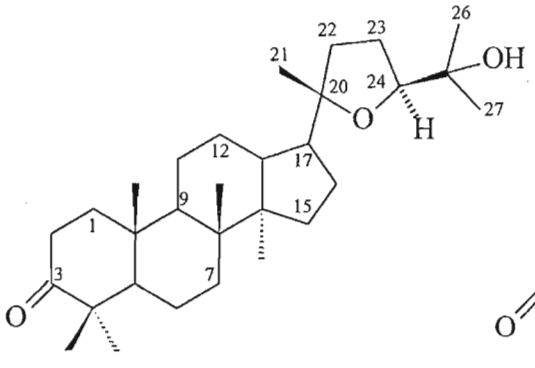


4-51

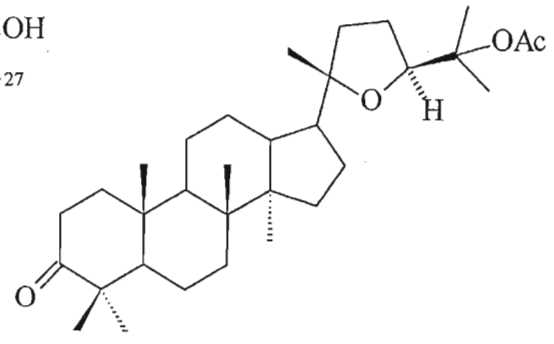
The biogenetic path-way which results in the synthesis of dammarane compounds was discussed in Chapter 1.

4.3.3 Results and Discussion

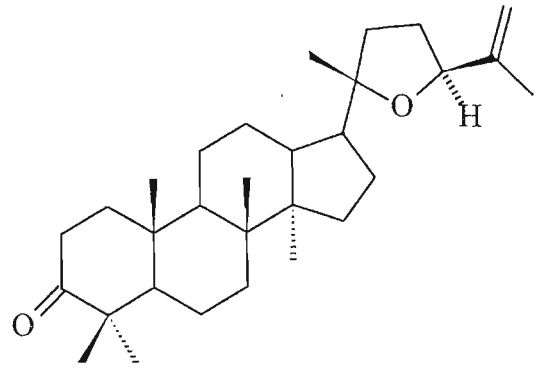
Compounds (**4-D**) and (**4-E**) were identified as dammarane-type triterpenoids. Compound (**4-D**) was acetylated yielding the derivative (**4-52**), while compound (**4-E**) was identified as the dehydrated analogue of (**4-D**).



4-D



4-52



4-E

4.3.3.1 Structural elucidation of compound (4-D)

Mass spectrometry of compound (**4-D**), (spectrum 4-D1) showed the $[M]^+$ peak to be at *m/z* 458, which corresponded to a molecular formula $C_{30}H_{50}O_3$. The mass spectrum showed a $[M\text{-}CH_3]^+$ peak at *m/z* 443 which indicated the loss of a methyl group and the peak at *m/z* 440 indicated the loss of a water molecule, $[M\text{-}H_20]^+$.

An extensive literature search indicated that the compound could be cabraleone (**4-D**) [41,42,43].

Infrared analysis (spectrum 4-D2) confirmed the presence of the hydroxy group absorbance at 3500cm^{-1} and a keto group absorbance at 1704cm^{-1}.

The molecular formula indicated the presence of three oxygen atoms in the compound. The mass and infrared spectra indicated the presence of a hydroxy group (C-25), which accounted for one oxygen atom. The ^{13}C NMR (spectrum 4-D4) spectrum indicated the presence of a keto group carbon (C-3) resonance at δ 218.1 in the ^{13}C NMR spectrum. The third oxygen atom was assigned to the ether oxygen atom between carbon atoms C-20 and C-24. The ^{1}H NMR spectrum (spectrum 4-D3) displayed a one proton triplet at δ 3.72 which was ascribable to a oxygen bonded methine proton (H-24); this was confirmed in the HETCOR spectrum (spectrum 4-D6) which correlated the proton resonance to the carbon resonance at δ 83.2 (CH). The C-O region of the ^{13}C NMR spectrum showed two other fully substituted carbon atom resonances at δ 86.4 and δ 71.4 which were assigned to C-20 and C-25 respectively.

A triplets of doublets at δ 2.45 in the proton spectrum was attributable to the axial H-2 proton, the chemical shift and splitting pattern was characteristic of the proton when a keto group is present at C-3.

Eight tertiary methyl group proton resonances were observed at δ 0.99, 1.08, 1.12, 1.13, 1.21, 0.93, 1.03 and 0.99 (each 3-H,s) respectively.

Comparison of the ^{1}H and ^{13}C chemical shifts with literature values confirmed the structure of compound (**4-D**) as cabraleone.[44]

The stereochemistry at C-20 and C-24 was determined by Rao *et al.*[41] on the basis of X-ray crystallographic analysis. The presence of the hydroxy group at C-25 was confirmed by acetylation with acetic anhydride/pyridine producing the monoacetate (**4-52**). The ^{1}H NMR spectrum of the acetylated product (**4-52**) (spectrum 4-D7) showed that the proton (H-24) resonating at δ 3.73 had undergone a shift from δ 3.73 in (**4-D**) to δ 3.90 in the acetate.

4.3.3.2 Structural elucidation of compound (4-E)

Mass spectrometry of compound (**4-E**), (spectrum 4-E1) showed the $[M]^{+}$ peak to be at *m/z* 440, which corresponded to a molecular formula $C_{30}H_{48}O_2$. The peak at *m/z* 425 was due to the loss of a methyl group $[M\text{-}CH_3]^{+}$, while the peak at *m/z* 399 was due to the loss of an isopropenyl group $[M\text{-}C_3H_5]^{+}$.

Infrared analysis (spectrum 4-E2) showed the presence of a keto group carbonyl stretch absorbance at 1704 cm^{-1} and olefinic group absorbances at 1650 and 1640 cm^{-1}.

The ^{13}C NMR spectrum (spectrum 4-E4) showed the presence of 30 carbon atoms and confirmed the presence of the isopropenyl group whose alkenyl carbon atoms resonated at δ 146.2 (C) and δ 110.4 (CH_2)respectively.

The ^{1}H NMR spectrum (spectrum 4E-3) showed six methyl group proton resonances at δ 0.86, 0.92, 0.99, 1.02, 1.06 and 1.15 respectively, and one vinylic methyl group at δ 1.69. In addition, a multiplet at δ 2.45, a doublet of doublets at δ 4.22 (J=3.4 and 8.9 Hz) and two singlets at δ 4.75 and δ 4.96, each integrating to one proton, were observed. The doublet of doublets centred at δ 4.22 indicated the presence of a proton on a carbon bearing an oxygen functionality and the singlets at δ 4.75 and δ 4.96 suggested the presence of a set of olefinic methylene protons.

The NMR spectra indicated that compound (**4-E**) was very similiar to compound (**4-D**), but dehydration of the sidechain hydroxy group had occurred to give a isopropenyl group. Hence, the above structure was proposed for compound (**4-E**).[44]

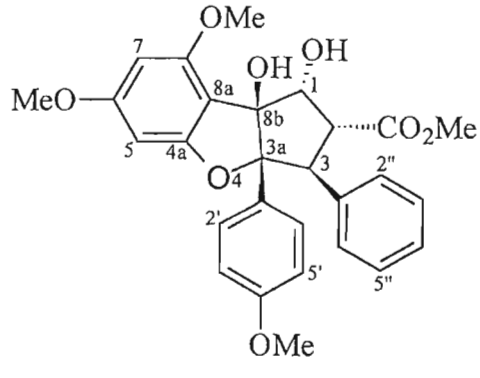
The COSY spectrum (spectrum 4-E5) and HETCOR spectrum (spectrum 4-E6) were used to confirm data published in the literature.[44]

Compound (**4-D**) has been found to be active against the growth of *Bacillus subtilis* and *Lemma*; while compound (**4-E**) inhibited the growth of both the bacteria *Staphylococcus aureus* and *Bacillus subtilis*[45].

4.4 Extractives from *Aglaia sapindina*

The hexane extract of the wood sample of *Aglaia sapindina* (Meliaceae), which was obtained by Professor D.A.H. Taylor from the Atherton Tableland in Queensland, was examined during the study of the Australian Meliaceae. A single compound (**4-F**) was isolated from this species. A literature search indicated that the species *Aglaia sapindina* had not been examined previously. Based on the NMR spectra obtained for compound (**4-F**), the structure of ferrugin (**4-56**) previously isolated from *Aglaia ferruginaea*[50] was revised.[53]

4.4.1 Nomenclature



- **4-F**, where **4** refers to chapter four and **F** refers to compounds isolated from *Aglaia sapindina* during my research.

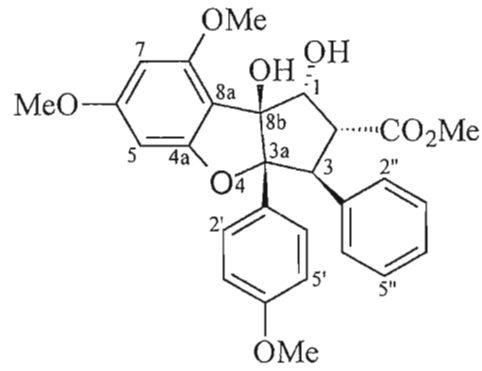
- 4-F1, where 4 refers to chapter four, F to compound isolated from *Aglaia sapindina* during this research and 1 to spectrum 1 of the compound concerned.

4.4.2 Results and Discussion

4.4.2.1 Structural elucidation of compound (4-F)

Mass spectrometry of compound (**4-F**), (spectrum 4-F1) showed the $[M]^+$ peak to be at *m/z* 492, which corresponded to a molecular formula of $C_{28}H_{28}O_8$.

An intensive literature search of compounds isolated from the *Aglaia* species indicated that the compound was the benzofuran, methyl rocaglate[47,48] (compound **4-F**), isolated previously from *Aglaia odorata*[47]. The structure of rocaglamide, the related amide (**4-55**) had been determined by X-ray crystallography[49].



4-F

A strong IR spectrum (spectrum 4-F2) absorbance for an ester carbonyl group stretch occurred at 1744cm^{-1}, the ^{13}C NMR spectrum (spectrum 4-F4) carbonyl carbon signal at δ 170.5 as well as a three-proton singlet at δ 3.60 in the ^{1}H NMR spectrum (spectrum 4-F3) clearly indicated the presence of a methyl ester group attached to C-2.

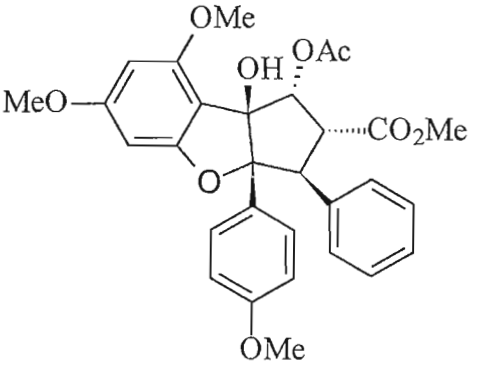
The ^{13}C NMR spectrum also indicated the presence of four oxygen bonded aromatic carbon atoms (δ 164.1, 160.9, 158.7 and 156.9) which were assigned to carbon atoms C-4′, C-8, C-6 and C-4_a.[47] . The ^{1}H NMR spectrum indicated the presence of three methoxy group proton

resonances at δ 3.85, 3.82, and 3.69 respectively and these were positioned at C-6, C-8 and C-4′. The remaining oxygen atom attached to a quaternary carbon atom was assigned to C-3_a (δ 101.9).[47] Thus, the four attachments of oxygen to aromatic carbons would be the three methoxy groups and the ether oxygen at C-4_a. Two hydroxy groups were indicated by their disappearance of their proton resonances upon addition of D_2O. One of these broad resonances disappeared on acetylation and an acetyl group methyl proton resonance was observed at δ 1.79 in the ^{1}H NMR spectrum (spectrum 4-F7) of the acetylated product (compound **4-53**). This indicated the presence of a secondary hydroxy group at C-1, (δ 79.5).[47] The other hydroxy group proton which resonated at δ 3.61 did not acetylate, hence it was placed at carbon atom C-8_b (δ 107.7).[47]

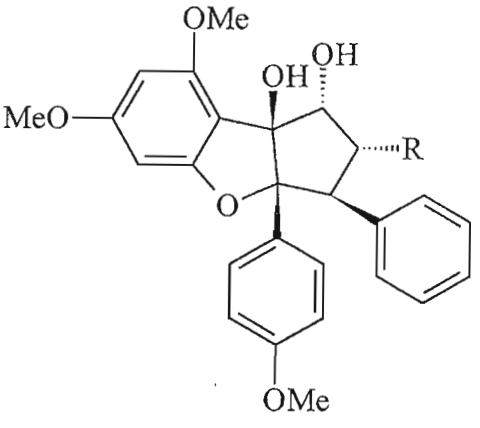
The COSY spectrum (spectrum 4-F5) showed the presence of the coupling system, -C_1H_a-C_2H_b-C_3H_c-, with H_a (*d*, δ 5.01, *J*=6.6 Hz) being coupled to H_b (*dd*, δ 3.91, *J*=6.6 Hz, *J*=14.2 Hz) and H_b being coupled to H_c (*d*, δ 4.29, *J*=14.4 Hz). Protons H_a and H_b were not further coupled. A model showed that these coupling constants indicated a 1α, 2α, 3β-substitution pattern. The HETCOR spectrum (spectrum 4-F6) indicated that the resonances for the three carbon atoms mentioned in the above sequence were δ 79.5 (CH) , 50.5 (CH) and 54.9 (CH), respectively.

Three benzenoid rings were found to be present in this compound. Firstly, a phloroglucinol residue was indicated by a pair of doublets at δ 6.26 (*J*=2.1 Hz) and δ 6.10 (*J*=1.8 Hz) which were typical of H-5 and H-7 in a phloroglucinol ring[45] and this was confirmed by resonances in the ^{13}C NMR spectrum at δ 92.6 (CH) and 89.5 (CH), typical for C-7 and C-5, respectively[46,47]. Secondly, the cluster of aromatic resonances between δ 6.5-7.2 integrated to nine aromatic protons. The COSY spectrum showed coupling between doublets at δ 7.17 (2H, *J*=9.00 Hz) and δ 6.65 (2H, *J*=9.00 Hz), which indicated a *para* substituted benzene ring, these resonances were assigned to H-2′,6′ and H-3′,5′, respectively.[47] The multiplets at δ 6.88, δ 7.07 and δ 7.08 confirmed the presence of an unsubstituted phenyl group and these resonances were assigned to H-2″,6″, H-3″,5″ and H-4″ respectively.[47] . The above data corresponded with that in the literature, hence compound (**4-F**) was confirmed to be methyl rocaglate.[47,48] The stereochemistry at C-8_b and C-3_a have been determined previously by X-ray crystallography for rocaglamide (**4-55**) the related amide.[49]

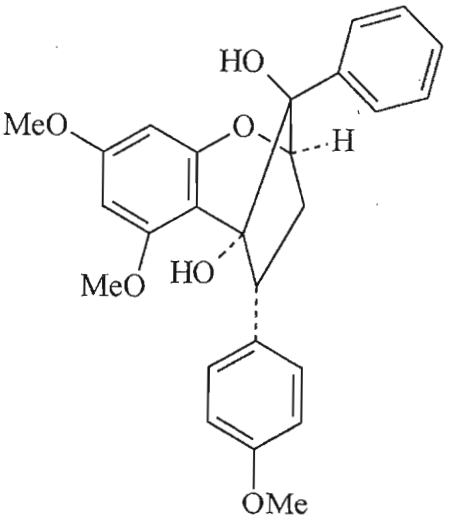
The structure of ferrugin[50] (**4-56**) isolated from *Aglaia ferruginaea*[51] was re-examined due to the similarity of its ^{1}H and ^{13}C NMR spectra to those of methyl rocaglate (**4-F**). The structure previously assigned was found to be incorrect and was revised to be that of rocaglaol (**4-54**). In ferrugin, the hydroxy group did not acetylate using Ac_2O/py and thus the possibility of a secondary hydroxy group was not originally considered.



4-53



R=COOH (**4-54**)
R=$CONH_2$ (**4-55**)



4-56

The acetylation reaction was repeated using methyl rocaglate, (**4-F**) , and again initially no reaction occurred. A re-examination of the reaction mixture after one week showed that acetylation had occurred (compound **4-53**, spectrum 4-F7). The reaction product was isolated

and found to be the 1α-acetate. The H-1β doublet had shifted from δ 5.01 (J=6.6 Hz) in methyl rocaglate to δ 6.04 (J=6.6 Hz) in the acetate, thereby confirming the hydroxy group at C-1. An investigation of a model of rocaglaol (**4-54**) showed that the secondary hydroxy group at C-1 would be sterically hindered by the hydroxy group at C-8_b. This would account for the fact that the secondary hydroxy group did not acetylate readily.

Several rocaglamide derivatives isolated from *Aglaia elliptica* and *A. harmsiana* have exhibited strong insecticidal activity towards neonate larvae of the polyphagous pest insect *Spodoptera littoralis* when incorporated into an artificial diet with LC_{50} values varying from 0.8-19.7 ppm.[54]

4.5 EXPERIMENTAL

Australian Meliaceae species were collected by Prof. D.A.H. Taylor in Australia. Voucher specimens for the Australian collection are housed in the Forest Herbarium, Oxford, UK. (Voucher file DAHT, No. 325-329).

4.5.1 Extractives from Dysoxylum pettigrewianum

Milled bark of *Dysoxylum pettigrewianum* (96.56g) was extracted with hexane in the manner explained in the "Foreword to Experimental" section. Chromatographic separation of the bark extract (3.15g) yielded a single crystalline compound (**4-A**) and two amorphous compounds (**4-B**) and compound (**4-C**). Acetylation of compound (**4-B**) yielded its derivative, compound (**4-48**).

4.5.1.1 Physical data of compound 4-A

3-Oxo-7,24Z-tirucalladien-26-oic acid

Yield : 30.4 mg

Melting point :180-182 °C

Mass spectrum (spectrum 4-A1) :

m/z 454 $[M]^+$

439 $[M-CH_3]^+$

421 $[M-CH_3-H_2O]^+$

393 $[M-CH_3-HCOOH]^+$

Infrared spectrum (spectrum 4-A2) :

υ_{max} (NaCl) : 2955 ($-CH_3$, $-CH_2$ stretching),

1706 (α,β – unsaturated carboxylic acid, C=O stretch),

1456 (C-H deformation),

1384 (methyl group symmetrical deformation) and

1248 (C=C stretch) cm^{-1}.

Optical rotation : $[\alpha]_D$= -22° ($CHCl_3$, *c*= 3.4 x 10^{-4})

^{1}H NMR chemical shifts (spectrum 4-A3) :

δ6.07(1H, *t*, *J*=8 Hz, H-24), 5.33(1H, *bt*, H-7β), 2.78(1H, *m*, H-2), 1.92, 1.25, 1.12, 1.05, 1.01, 0.81 (each 3H, *s*, 6 x CH_3), 0.89 (3H, *d*, CH_3)

^{13}C NMR chemical shifts (spectrum 4-A4) : Table 4.1

COSY spectrum : spectrum 4-A5

HETCOR spectrum : spectrum 4-A6

4.5.1.2 Physical data of compound 4-B

20S-3-Oxo-7α-hydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholest-24Z-en-26-oic acid

Yield : 16.3 mg

Mass spectrum (spectrum 4-B1) :

m/z 452 $[M]^+$

437 $[M-CH_3]^+$

391 $[M-CH_3-HCOOH]^+$

373 $[M-CH_3-HCOOH-H_2O]^+$

Infrared spectrum (spectrum 4-B2) :

υ_{max} (NaCl) : 2952, 2871 ($-CH_3$, $-CH_2$ stretching),

1702 (α,β – unsaturated carboxylic acid, C=O stretch),

1456 (C-H deformation),

1386 (methyl group symmetrical deformation) and

1248 (C=C stretch) cm^{-1}.

Optical rotation : $[\alpha]_D$= -3.97° ($CHCl_3$, c= 2.6 x 10^{-4})

^{1}H NMR chemical shifts (spectrum 4-B3) :

δ6.06 (1H, *t*, *J*=8 Hz, H-24), 3.78 (1H, *bt*, H-7β), 0.68 (1H, *d*, *J*=4.2Hz, H-18_a), 0.53(1H, *d*, *J*=4.5Hz, H-18_b), 1.92, 1.40, 1.10, 1.05, 0.96 (each 3H, *s*, 5 x CH_3), 0.83 (3H, *d*, CH_3)

^{13}C NMR chemical shifts (spectrum 4-B4) : Table 4.1

COSY spectrum : spectrum 4-B5

HETCOR spectrum : spectrum 4-B6

4.5.1.2.1 Physical data of compound 4-48 (acetylation product)

Yield : 8.23 mg

^{1}H NMR (spectrum 4-B7) chemical shifts :

δ6.85 (1H, *t*, *J*=8 Hz, H-24), 5.05 (1H, *bt*, H-7β), 0.67 (1H, *d*, *J*=4.2Hz, H-18_a), 0.38 (1H, *d*, *J*=4.5Hz, H-18_b), 2.00 (3H, *s*, $C\underline{H}_3COO$)

4.5.1.3 Physical data of compound 4-C

20S-3-Oxo-7α-hydroxy-4α,4β,8β-trimethyl-14,18-cyclo-5α,13α,14α,17α-cholest-24Z-en-26-methylcarboxylate

Yield : 1.6 mg

^{1}H NMR chemical shifts (spectrum 4-C1) :

δ5.90 (1H, *t*, *J*=8 Hz, H-24), 3.78 (1H, *bt*, H-7β), 3.71 (3H, *s*, -$COOCH_3$), 0.67 (1H, *d*, *J*=3.9Hz, H-18_a), 0.53(1H, *d*, *J*=5.1Hz, H-18_b), 1.87, 1.08, 1.04, 1.01, 0.93 (each 3H, *s*, 5 x CH_3), 0.93 (3H, *d*, CH_3)

COSY spectrum : spectrum 4-C2

4.5.2 Extractives from *Dysoxylum muelleri*

Milled wood of *Dysoxylum muelleri* (146.38g) was extracted with dichloromethane in the manner explained in the "Foreword to Experimental" section. Chromatographic separation of the wood extract (1.65g) yielded two amorphous compounds, compounds (**4-D**) and (**4-E**). Compound (**4-D**) was acetylated to yield its derivative, compound (**4-52**).

4.5.2.1 Physical data of compound 4-D

cabraleone

Yield : 37.6 mg

Mass spectrum (spectrum 4-D1) :

m/z 458 $[M]^+$

443 $[M-CH_3]^+$

440 $[M-H_2O]^+$

Infrared spectrum (spectrum 4-D2) :

υ_{max} (NaCl) : 3500 (-OH stretching),
2962 ($-CH_3$, $-CH_2$ stretching),
1704 (C=O stretch),
1457 (C-H deformation) and
1375 (methyl group symmetrical deformation) cm^{-1}.

Optical rotation : $[\alpha]_D$= 54° ($CHCl_3$, 3.3 x 10^{-4})

^{1}H NMR (spectrum 4-D3) and ^{13}C NMR (spectrum 4-D4) chemical shifts : Table 4.2

COSY spectrum : spectrum 4-D5

HETCOR spectrum : spectrum 4-D6

4.5.2.1.1 Physical data of compound 4-52 (acetylation product)

Yield : 12.5 mg

^{1}H NMR (spectrum 4-D7) chemical shifts :

δ3.90(1H, t, *J*=7.1 Hz, H-24), 1.98(3H, s, -OOCCH$_3$), 1.21, 1.13, 1.12, 1.08, 1.03, 0.99, 0.99, 0.93 (each 3H, s, 8 x CH$_3$).

4.5.2.2 Physical data of compound 4-E

(20S,24S)-Epoxy-25(26)-ene-dammaran-3-one

Yield : 42.3 mg

Mass spectrum (spectrum 4-E1) :

m/z 440 $[M]^+$

425 $[M-CH_3]^+$

399 $[M-C_3H_5]^+$

Infrared spectrum (spectrum 4-E2) :

υ_{max} (NaCl) : 2917 (-CH$_3$, -CH$_2$ stretching),
1704 (C=O stretching),
1457 (C-H deformation) and
1375 (methyl group symmetrical deformation) cm^{-1}.

Optical rotation : $[\alpha]_D$= 57° ($CHCl_3$, 3.5 x 10^{-4})

^{1}H NMR (spectrum 4-E3) and ^{13}C NMR (spectrum 4-E4) chemical shifts : Table 4.2

COSY spectrum : spectrum 4-E5

HETCOR spectrum : spectrum 4-E6

4.5.3 Extractives from *Aglaia sapindina*

Milled wood of *Aglaia sapindina* (302.33g) was extracted with hexane in the manner explained in the "Foreword to Experimental" section. Chromatographic separation of the wood extract (2.05g) yielded a single amorphous compound (**4-F**) which was acetylated yielding its derivative, compound (**4-53**).

4.5.3.1 Physical data of compound 4-F

methyl rocaglate

Yield : 41.2mg

Mass spectrum (spectrum 4-F1) :

m/z 492 $[M]^+$

Infrared spectrum (spectrum 4-F2) :

υ_{max} (NaCl) : 3488 (-OH stretching),
1744 (C=O stretch),
1628, 1514 (C=C, aromatic stretching) and
1156 (tertiary hydroxy stretch) cm^{-1}.

Optical rotation : $[\alpha]_D$= -35° ($CHCl_3$, 4.5 x 10^{-4})

^{1}H NMR (spectrum 4-F3) and ^{13}C NMR (spectrum 4-F4) chemical shifts : Table 4.3

COSY spectrum : spectrum 4-F5

HETCOR spectrum : spectrum 4-F6

4.5.3.2 Physical data of compound 4-53 (acetylation product)

Yield : 7.67mg

^{1}H NMR (spectrum 4-F7) chemical shifts : Table 4.3

4.6 List of tables of ^{1}H and ^{13}C NMR chemical shifts for compounds 4-A to 4-F

Table 4.1 : ^{13}C NMR chemical shifts of compounds (4-A) and (4-B).

Atom Number	^{13}C chemical shift (ppm) of compound (4-A)	^{13}C chemical shift (ppm) of compound (4-B)
1	38.6 (*t*)	39.5 (*t*)
2	34.0 (*t*)*	33.8 (*t*)*
3	217.1 (*s*)	217.5 (*s*)
4	47.9 (*s*)**	46.9 (*s*)
5	52.8 (*d*)	52.2 (*d*)
6	24.2 (*t*)	24.4 (*t*)**
7	117.8 (*d*)	74.2 (*d*)
8	145.9 (*s*)	37.7 (*s*)***
9	48.5 (*d*)	44.6 (*d*)
10	34.9 (*s*)	35.0 (*s*)***
11	18.2 (*t*)	17.5 (*t*)
12	33.6 (*t*)*	25.0 (*t*)**
13	43.5 (*s*)	32.9 (*s*)
14	51.1 (*s*)**	36.9 (*s*)***
15	34.9 (*t*)*	27.8 (*t*)****
16	28.2 (*t*)	27.5 (*t*)****
17	52.3 (*d*)	46.7 (*d*)
18	12.7 (*q*)	16.3 (*t*)
19	21.9 (*q*)***	15.9 (*q*)
20	36.0 (*d*)	36.4 (*d*)
21	18.3 (*q*)	18.6 (*q*)
22	35.6 (*t*)	35.5 (*t*)*
23	26.8 (*t*)	26.9 (*t*)****
24	147.3 (*d*)	147.3 (*d*)
25	125.9 (*s*)	125.9 (*s*)
26	173.4 (*s*)	173.4 (*s*)
27	20.6 (*q*)***	20.6 (*q*)
28	21.6 (*q*)***	20.8 (*q*)*****
29	24.5 (*q*)***	19.7 (*q*)*****
30	27.4 (*q*)	26.3 (*q*)

* carbon atoms could be interchangable.

** carbon atoms could be interchangable.

*** carbon atoms could be interchangable.

**** carbon atoms could be interchangable.

***** carbon atoms could be interchangable.

Table 4.2 : ^{13}C NMR chemical shifts of compounds (4-D) and (4-E).				
C atom no.	*^{1}H cmpd 4-D*	*^{13}C cmpd 4-D*	*^{1}H cmpd 4-E*	*^{13}C cmpd 4-E*
1		39.9 (*t*)		39.9 (*t*)
2		34.1 (*t*)		34.1 (*t*)
3		218.1 (*s*)		218.2 (*s*)
4		47.4 (*s*)		47.4 (*s*)
5		55.3 (*d*)		55.3 (*d*)
6		19.6 (*t*)		19.7 (*t*)
7		34.6 (*t*)		34.6 (*t*)
8		40.3 (*s*)		40.3 (*s*)
9		49.5 (*d*)		49.8 (*d*)
10		36.8 (*s*)		36.8 (*s*)
11		22.1 (*t*)		22.3 (*t*)
12		26.1 (*t*)		25.8 (*t*)
13		43.1 (*d*)		43.1 (*d*)
14		50.0 (*s*)		50.0 (*s*)
15		31.4 (*t*)		31.4 (*t*)
16		27.4 (*t*)		26.9 (*t*)
17		50.1 (*d*)		50.1 (*d*)
18	0.99 (3H)*s*	16.4 (*q*)	1.01 (3H)*s*	15.3 (*q*)
19	1.08 (3H)*s*	15.1 (*q*)	1.08 (3H)*s*	16.2 (*q*)
20		86.4 (*s*)		86.6 (*s*)
21	1.12 (3H)*s*	23.6 (*q*)	1.17 (3H)*s*	26.6 (*q*)
22		35.7 (*t*)		31.5 (*t*)
23		25.7 (*t*)		31.3 (*t*)
24	3.73 (1H, 7.1)*t*	83.3 (*d*)	2.24 (1H, 5.4, 9.3)*dd*	82.8 (*d*)
25		71.4 (*s*)		146.2 (*s*)
26	1.13 (3H)*s*	24.3 (*q*)	4.78 (1H)*s* 5.00 (1H)*s*	110.4 (*t*)
27	1.21 (3H)*s*	26.7 (*q*)	1.71 (3H)*s*	17.8 (*q*)
28	0.93 (3H)*s*	27.5 (*q*)	0.94 (3H)*s*	26.8 (*q*)
29	1.03 (3H)*s*	21.0 (*q*)	1.04 (3H)*s*	21.0 (*q*)
30	0.99 (3H)*s*	16.4 (*q*)	1.01 (3H)*s*	16.2 (*q*)

Table 4.3 : ^{1}H and ^{13}C shifts for compound 4-F and acetylated product (4-53).

ATOM NUMBER	^{1}H (ppm) - (**4-F**)	^{1}H (ppm) - (**4-53**)	^{13}C (ppm)
1	5.01 (*d*, 6.6)	6.04 (*d*, 6.6)	79.5 (*d*)
2β	3.91 (*dd*, 6.6, 14.4)	4.05 (*dd*, 6.6, 14.4)	51.9 (*d*)
3	4.29 (*d*, 14.4)	4.42 (*d*, 14.4)	55.1 (*d*)
3a			101.9 (*s*)
5	6.26 (*d*, 2.1)	6.26 (*d*, 2.1)	89.5 (*d*)
7	6.10 (*d*, 1.8)	6.10 (*d*, 1.8)	92.6 (*d*)
8a			107.7 (*s*)
8b			93.6 (*s*)
1′			126.4 (*s*)
2′,6′	7.17 (*d*, 9.0)	7.17 (*d*, 9.0)	127.7 (*d*)
3′,5′	6.65 (*d*, 9.0)	6.65 (*d*, 9.0)	112.7 (*d*)
1″			136.9 (*s*)
2″,6″	6.88 (*m*)	6.95 (*m*)	127.8 (*d*)
3″,5″	7.07 (*m*)	7.07 (*m*)	128.9 (*d*)
4″	7.08 (*m*)	7.07 (*m*)	126.5 (*d*)
4a,6,8,4′			156.9 (*s*)
			158.7 (*s*)
			160.9 (*s*)
			164.1 (*s*)
OMe-6	3.85 (*s*)	3.85 (*s*)	55.8, 55.7, 55.1 (*q*)
OMe-8	3.82 (*s*)	3.75 (*s*)	
OMe-4′	3.69 (*s*)	3.65 (*s*)	
CO_2Me	3.63 (*s*)	3.60 (*s*)	51.9 (*q*)
CH_3COO		1.80 (*s*)	
OH	3.61 (*br s*), 1.79		
C=O			170.5 (*s*)

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CHAPTER 5

Extractives from *Trichilia dregeana*

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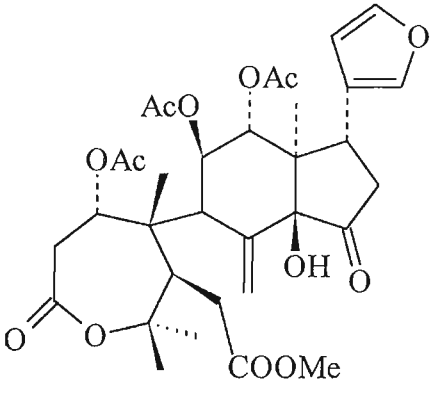
CHAPTER 5

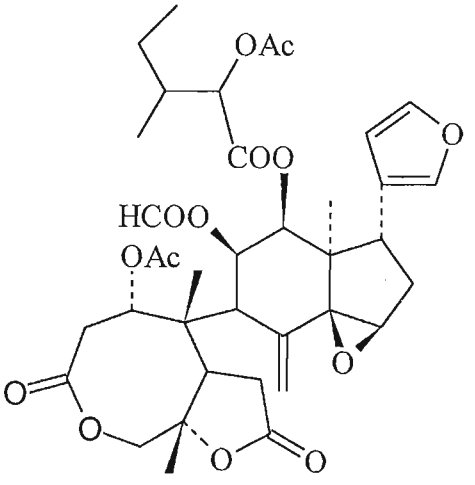
Extractives from *Trichilia dregeana*

5.1 Introduction

The red seeds of *Trichilia dregeana* have been previously examined for limonoids and several limonoids have been found including dregeana 1, 2 (**5-1**)[1], 3, 4 and dregeanin (**5-2**)[2,3]. The dregeana type compounds 1, 3 and 4 are closely related to each other and are similiar in structure to (**5-1**). The red seeds of *Trichilia dregeana* were obtained from a tree located on the grounds of the University of Natal, Durban. The aim of the present investigation was to isolate compounds that could be responsible for the red colour prevalent in the seed coatings.



5-1



5-2

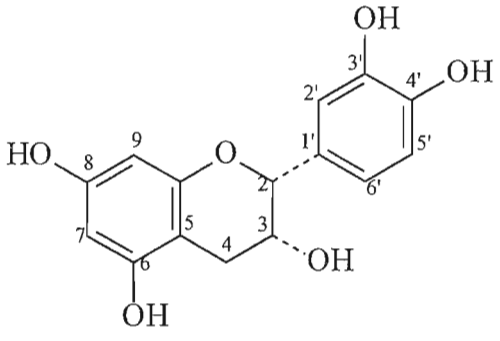
5.2 Results and Discussion

The aqueous extract of the seeds was freeze dried. The red freeze dried extract was dissolved in methanol and the red coloured material was precipitated with dichloromethane. A crude ^{1}H

NMR spectrum (spectrum 5-A2) of the red precipitate dissolved in D_2O implied the presence of a flavonoid/sugar mixture.

TLC revealed the presence of two spots. Separation of the extract using column chromatography resulted in the isolation of a flavonoid (**5-A**) and a mixture of sugars. The sugar mixture was not further investigated.

5.3 Nomenclature of compounds discussed in this chapter



- **5-1 to 5-2**, where **5** refers to chapter five and **1 to 2** refers to compounds discussed in the literature search.
- **5-A**, where **5** refers to chapter five and **A** refers to the compound isolated from *Trichilia dregeana* during my research.
- 5-A1, where 5 refers to chapter five, A to compound isolated from *Trichilia dregeana* during this research and 1 to spectrum 1 of the compound concerned.

5.3.1 Structural elucidation of compound 5-A

Mass spectrometry of compound (**5-A**) showed the $[M]^+$ peak to occur at *m/z* 290, which corresponded to a molecular formula of $C_{15}H_{14}O_6$.

The IR spectrum (spectrum 5-A1) of compound (**5-A**) showed an absorption band at 3332cm^{-1}, which indicated the presence of a hydroxyl group, while the absorption band at 1620cm^{-1} indicated the presence of an aromatic ring. The absence of carbonyl or alkene absorbances suggested that compound **5-A** was a catechin-type compound. The bands at 1390cm^{-1} and

1280cm^{-1} were due to C-O stretches which indicated the possible presence of hydroxyl and/or ether groups; the band at 1467cm^{-1} was due to a C-H deformation. The band at 815cm^{-1} was possibly due to an isolated aromatic C-H or two adjacent aromatic C-H groups.

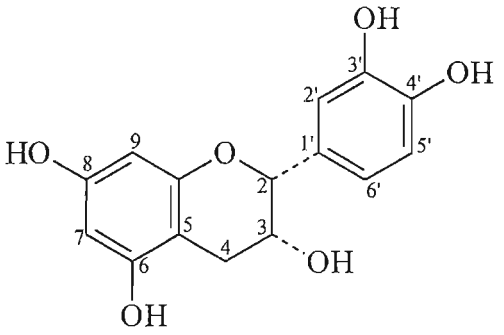
The ^{13}C NMR spectrum (spectrum 5-A3) displayed resonances at δ28.71 (CH_2), δ68.45 (CH) and δ82.68 (CH). This, together with the absence of a C-4 carbonyl resonance, confirmed that compound (**5-A**) was a catechin-type compound with the above mentioned resonances being due to C-4, C-3 and C-2 respectively[7].

The ^{1}H NMR spectrum (spectrum 5-A2) displayed a pair of doublets at δ5.96 (J=2.4 Hz) and δ5.89 (J=2.4 Hz) typical of H-7 and H-9 protons in a phloroglucinol ring A system[4]. The remaining aromatic proton resonances were assigned to H-2′ which appeared as a doublet at δ6.88 (J=1.8 Hz), H-5′ and H-6′ were assigned to the doublet at δ6.77 (J=7.7 Hz) and double doublet at δ6.75 (J=8.2, 1.9 Hz) respectively. These resonances are typical for a 1′,3′,4′-trisubstituted phenyl group[6].

The HETCOR spectrum (spectrum 5-A5) and COSY spectrum (spectrum 5-A4) located the positions of resonances due to H-2, H-3, H-4$_a$ and H-4$_b$ in the proton spectrum. It was found that H-2 occurred as doublet at δ4.60 (J=7.5 Hz), H-3 occurred as a multiplet at δ4.04 and H-4$_a$ and H-4$_b$ protons each gave rise to double doublets centred at δ2.50 and δ2.91, respectively. This confirmed the following sequence -C^{4}H$_a$ H$_b$-C^{3}HOH-C^{2}HO- ($J_{2,3}$=7.7Hz, $J_{3,4a}$=5.3Hz, $J_{3,4b}$=8.3Hz, $J_{4a,4b}$=16.0Hz).

The carbon spectrum displayed five resonances in the region δ140-160 which indicated carbon atoms in an aromatic ring joined to oxygen atoms. Since the proton resonances due to H-7, H-9, H-2′, H-5′ and H-6′ were already assigned, the remaining carbon resonances were assigned using the HETCOR spectrum. The resonances at δ95.60 (CH) and δ96.41 (CH) were assigned to C-7 and C-9. Resonances due to the fully substituted carbon atoms at δ100.76 and δ132.25 were assigned to C-10 and C-1′ and the doublets at δ115.50, δ116.04 and δ120.31 to C-2′, C-5′ and C-6′. Thus structure (**5-A**) was assigned to this compound which was confirmed by comparison of literature and experimental ^{1}H and ^{13}C NMR data and optical rotation data[5].

A literature search indicated that the compound (**5-A**) was (-)-epicatechin[5]. Although (-)-epicatechin has been reported isolated in many species, this is the first reported isolation of (-)-epicatechin in *Trichilia dregeana*.



5-A

5.4 Experimental

The red seeds of *Trichilia dregeana* (247g) were extracted with hot water. Dichloromethane was added to the water extract which yielded a red precipitate. The impure precipitate was purified by flash-column chromatography which yielded a pure red gum, (-)-epicatechin (180mg).

5.4.1 Physical data of compound 5-A

(-)Epicatechin, (2R,3R)-3,3',4',5,7-pentahydroxyflavan

Yield : 180mg

Mass Spectrum : EIMS *m/z* 290.0812 ($[M]^+$, $C_{15}H_{14}O_6$, req. 290.0790)

Infrared spectrum (spectrum 5-A1) :

υ_{max} (NaCl) : 3332 (-OH stretching),
1620 (aromatic ring),
2900 (aliphatic C-H),
1390 and 1280 (C-O stretching) and
815, (aromatic C-H) cm^{-1}.

Optical rotation : $[\alpha]_D = -66^0$ (EtOH, $c = 4.41 \times 10^{-3}$) ($lit^5 : -68^0$)

^{1}H NMR chemical shifts (spectrum 5-A2) : Table 5.1

^{13}C NMR chemical shifts (spectrum 5-A3) : Table 5.1

COSY spectrum : spectrum 5-A4

HETCOR spectrum : spectrum 5-A5

5.5 Table of ^{1}H and ^{13}C shifts for compound 5-A.

Table 5.1 : ^{1}H and ^{13}C shifts for compound **5-A**

ATOM NUMBER	*^{1}H NMR (ppm)*	*^{13}C NMR (ppm)*
2	H-2 (*d*), 4.60, *J*=7.5 Hz	82.6 (*d*)
3	H-3 (*m*), 4.04	68.4 (*d*)
4	H-4_a (*dd*), 2.91, *J*=5.4, 16.2 Hz	28.7 (*t*)
	H-4_b (*dd*), 2.50, *J*=16.2 Hz	
5		157.9 (*s*)*
6		96.4 (*d*)
7	H-7 (*d*), 5.89, *J*=2.4 Hz	157.5 (*s*)*
8		95.6 (*d*)
9	H-9 (*d*), 5.96, *J*=2.4 Hz	157.1 (*s*)*
10		100.7 (*s*)
1′		132.2 (*s*)
2′	H-2′ (*d*), 6.88, *J*=1.8 Hz	115.5 (*d*)
3′		146.0 (*s*)
4′		146.0 (*s*)
5′	H-5′ (*d*), 6.77, *J*=7.7 Hz	116.0 (*d*)
6′	H-6′ (*d*), 6.75, *J*=8.2, 1.9 Hz	120.3 (*d*)
-OH	3.80 (*s*)	

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

5.6 References

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Extractives from *Monanthotaxis caffra*

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Chapter 6

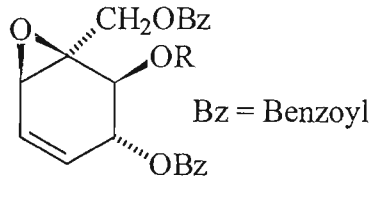
Extractives from *Monanthotaxis caffra*

6.1 Introduction

The genus *Monanthotaxis* belongs to the Annonaceae family and is indigeneous to Africa. Within the genus *Monanthotaxis*, two species have been examined previously, namely *Monanthotaxis buschananii* and *Monanthotaxis cauliflora*. A literature search was undertaken to become acquainted with the type of compounds naturally produced by the genus *Monanthotaxis*.

6.1.1 *Monanthotaxis buschananii*

Liang *et al.* (1988)[1] isolated several oxygenated cyclohexane epoxide derivatives (**6-1** to **6-11**) from the stem bark of the species.



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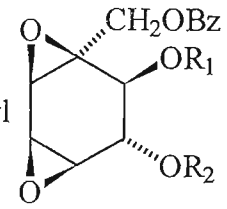
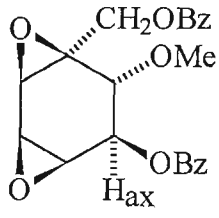
6-2 R=H



6-3 R_1=Me, R_2=Bz
6-4 R_1=R_2=Ac
6-5 R_1=Ac, R_2=Bz



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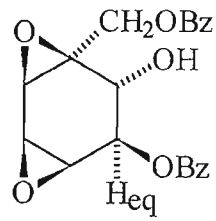

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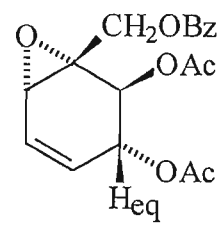

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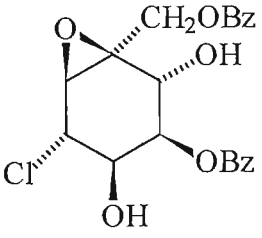

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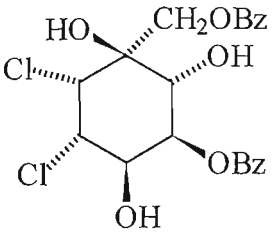

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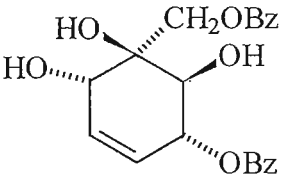
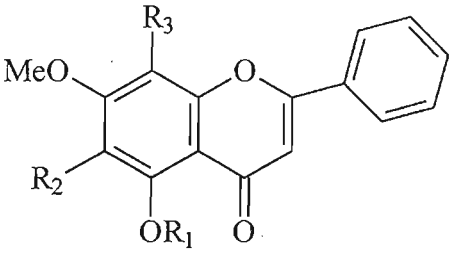


6-11

6.1.2 *Monanthotaxis cauliflora*

Panichpol *et al.* (1978)[2] isolated several flavonoids (**6-12** to **6-20**), while Waterman *et al.* (1979)[3] isolated two alkaloids (**6-21** and **6-22**) from the stem of the species.



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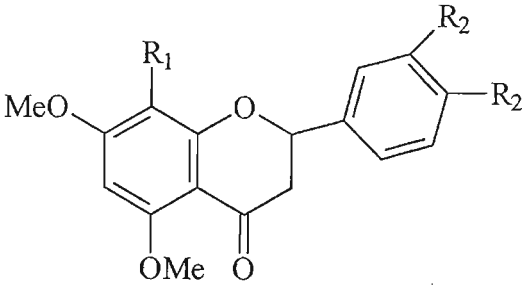
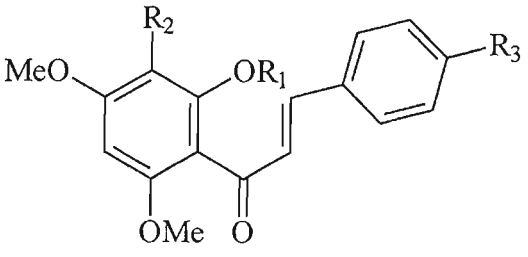
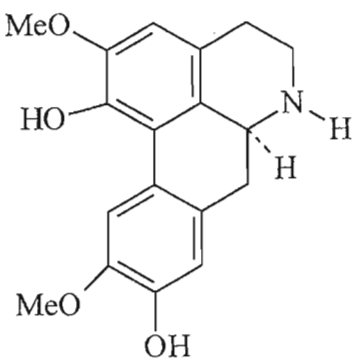
6-13 R_1=R_3=H, R_2=OMe
6-14 R_1=R_2=H, R_3=OMe



6-15 R_1=OMe, R_2=H
6-16 R_1=H, R_2=OMe



6-17 R_1=R_2=R_3=H
6-18 R_1=R_3=H, R_2=OMe
6-19 R_1=Me, R_2=OMe, R_3=H
6-20 R_1=H, R_2=OMe, R_3=OH



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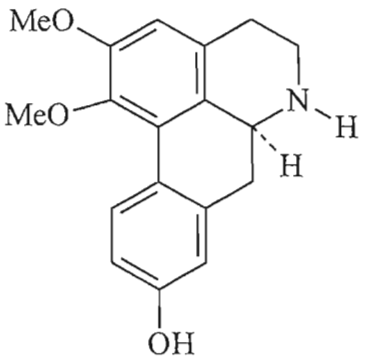
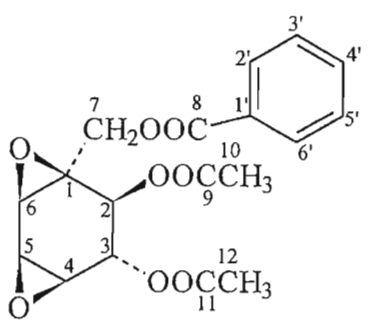


6-22

6.2 Results and discussion

The species *Monanthotaxis caffra* is a plant located in Northern Natal. The plant was collected for extraction by Mrs Anne Hutchings, of the Department of Botany, University of Zululand. The plant is used medicinally by the Zulu tribe of Kwa-Zulu/Natal. The roots are smoked against hysteria and administered as emetics against bad dreams. It is also used to keep cattle strong and fat and as a medicinal charm to attract young women. The leaf and root bark of the plant was examined. Crotepoxide (**6-A**) was isolated from the leaf and root of the species. Compound (**6-A**) was previously isolated from the species *Croton macrostachys* and *Monanthotaxis buschananii*.[1,4]

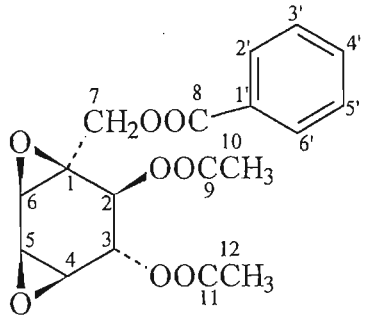
3.3 Nomenclature of compounds discussed in this chapter



- **6-1 to 6-22**, where **6** refers to chapter six and **1 to 22** refers to compounds discussed in the literature search.

- **6-A**, where **6** refers to chapter six and **A** refers to compounds isolated from *Monanthotaxis caffra* during this research.
- 6-A1, where 6 refers to chapter six, A to compound isolated from *Monanthotaxis caffra* during this research and 1 to spectrum 1 of the compound concerned.

6.3.1 Structural elucidation of compound 6-A



6-A

FAB mass spectrometry of compound (**6-A**), (spectrum 6-A1) showed the $[MH\text{-}CH_3COOH]^+$ peak to be at *m/z* 303, which corresponded to a molecular formula of $C_{16}H_{14}O_6$ for this fragment. In conjunction with the ^{13}C and ^1H NMR spectra, a molecular formula of $C_{18}H_{18}O_8$ was proposed for the molecule. This indicated ten double bond equivalents. The mass spectrum also indicated loss of the side chain at C-1, by the peak at *m/z* 227 which was ascribable to a $[MH\text{-}PhCOOCH_2]^+$ ion.

Infrared spectroscopy (6-A2) revealed strong absorptions at 2916 cm^{-1} which indicated the presence of an aromatic (CH) stretch, 1753 and 1726 cm^{-1} for ester carbonyl group stretch and a band at 1273 which indicated the C-O stretch.

The ^1H NMR spectrum (spectrum 6-A3) displayed a doublet at δ 5.69 (J=9.0 Hz) and a double doublet resonance at δ 4.97, (J=9.0, J=1.5 Hz). These resonances were coupled in the COSY spectrum (spectrum 6-A5) and were correlated to the carbon resonances at δ 69.4 (CH) and δ 70.4 (CH) in the HETCOR spectrum (spectrum 6-A6). These resonances were assigned to H-2 and H-3 of compound **6-A**.

Two acetyl proton resonances at δ 2.10 and δ 2.01 were correlated to the resonances at δ 170.1 and δ 170.0 in the long-range HETCOR spectrum (spectrum 6-A7). The carbon resonances at

δ 69.4 (CH) and δ 70.4 (CH) suggested that these carbon atoms were bonded to oxygen atoms, hence the two acetate groups were positioned at C-2 and C-3.

The proton double doublet at δ 3.08 (J=1.5, 3.6 Hz) was coupled to the double doublet at δ 3.43 (J=3.9, 2.7 Hz) which in turn was coupled to the doublet at δ 3.64 (J=2.7 Hz) in the COSY spectrum. These protons were correlated to carbon atom resonances at δ 52.6 (CH), δ 48.1 (CH) and δ 53.8 (CH) in the HETCOR spectrum. These protons were assigned to positions H-4, H-5 and H-6. Two extra rings were required according to the molecular formula and two oxygen atoms were still to be placed. The upfield ^{13}C NMR (spectrum 6-A4) chemical shifts [δ 59.4 (C), δ 53.8 (CH), δ 52.6 (CH), δ 48.1 (CH)] suggested carbon atoms bonded to oxygen in epoxide groups. Since six oxygen atoms were accounted for by the three ester groups the two remaining oxygen atoms were, thus, located as epoxide ring positioned at C-4 : C-5 and C-6:C-1 carbon atoms. Therefore, the quaternary carbon resonance at δ 59.4 was assigned to C-1 due to its bond to the epoxide oxygen and its correlation to the H-7 protons in the long-range HETCOR spectrum. The H-7 protons were coupled to each other in the COSY spectrum and were correlated with the resonance at δ 62.5 (CH_2) in the HETCOR spectrum. The long-range HETCOR spectrum also displayed coupling between the H-7 protons and the ester carbonyl carbon resonance at δ 166.0 which was assigned to C-8.

The proton spectrum showed resonances due to aromatic ring protons in the region δ 7.3 - 8.1. These resonances integrated to five protons and the splitting pattern of two double doublets at δ 7.44 (2H, J=7.8, 7.5 Hz) and δ 7.57 (1H, J=7.5, 7.3 Hz), and a doublet at δ 8.01 (2H, J=7.8 Hz) suggested the presence of a mono-substituted benzene ring. This was confirmed by the ^{13}C NMR spectrum which displayed equivalent resonances for C-2′ and C-6′ at δ 129.8 (CH) and C-3′ and C-5′ at δ 128.6 (CH). The resonance for the carbon atom C-4′ occurred at δ 133.6 (CH) at a lower intensity than the other two resonances, hence the phenyl group was positioned at C-8. The assignment of the stereochemistry of compound (**6-A**) was based on X-ray crystallographic data.[5]

6.4 Experimental

Milled root (18.45g) of *Monanthotaxis caffra* was extracted with hexane in the manner explained in the "Foreword to Experimental" section. Chromatographic separation of the root extract (3.17 g) yielded a single crystalline compound, (**6-A**).

6.4.1 Physical data of compound 6-A

Crotepoxide

Yield : 5.2 mg

Melting point : 152° lit.[4] 152-153°

Mass spectrum (spectrum 6-A1) :

FAB *m/z* $[MH\text{-}OAc]^+$ - 303

$[MH\text{-}PhCOOCH_2]^+$ - 227

Infrared spectrum (spectrum 6-A2) :

υ_{max} (NaCl) : 2916 (aromatic CH stretch)
2848 (saturated C-H stretching),
1753, 1726 (broad band, C=O stretching),
1372 (methyl group symmetrical deformation),
1273, 1236 (C-O stretching) and
713 (C-H out-of -plane aromatic bend) cm^{-1}.

Optical rotation : $[\alpha]_D = 73.7°$ ($CHCl_3$, $c = 2.8 \times 10^{-4}$)

^{1}H NMR chemical shifts (spectrum 6-A3) : Table 6.1

^{13}C NMR chemical shifts (spectrum 6-A4) : Table 6.1

COSY spectrum : spectrum 6-A5

HETCOR spectrum : spectrum 6-A6

Long-range HETCOR : spectrum 6-A7

6.4.1 Table of ^{1}H and ^{13}C NMR chemical shifts for compound 6-A

Table 6.1 : ^{1}H and ^{13}C NMR chemical shifts of compound 6-A.

Atom Number	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)
1		59.4 (*s*)
2	H-2 (*d*), 5.69, *J*=9.0 Hz	69.4 (*d*)
3	H-3 (*dd*), 4.97, *J*=9.0, 1.5 Hz	70.4 (*d*)
4	H-4 (*dd*), 3.08, *J*=1.5, 3.6 Hz	52.6 (*d*)
5	H-5 (*dd*), 3.43, *J*=3.9, 2.7 Hz	48.1 (*d*)
6	H-6 (*d*), 3.64, *J*=2.7 Hz	53.8 (*d*)
7	H-7_a (*d*), 4.55, *J*=12.3 Hz H-7_b (*d*), 4.22, *J*=12.3 Hz	62.5 (*t*)
8		166.0 (*s*)
9		170.1 (*s*)
10	H-10 (*s*), 2.10	20.7 (*q*)
11		170.0 (*s*)
12	H-12 (*s*), 2.01	20.6 (*q*)
1′		129.1 (*s*)
2′	H-2′ (*d*), 8.01, *J*=7.8 Hz	129.8 (*d*)
3′	H-3′ (*dd*), 7.44, *J*=7.8, 7.5 Hz	128.6 (*d*)
4′	H-4′ (*dd*), 7.57, *J*=7.5, 7.3 Hz	133.6 (*d*)
5′	H-5′ (*dd*), 7.44, *J*=7.8, 7.5 Hz	128.6 (*d*)
6′	H-6′ (*d*), 8.01, *J*=7.8 Hz	129.8 (*d*)

6.5 References

1. Liang, G.Y., Gray, A.I., Thomas, D.W., and Waterman, P.G. (1988) *Phytochemistry*, **27**, 3857.

2. Panichpol, K. and Waterman, P.G. (1978) *Phytochemistry*, **17**, 1363.

3. Waterman, P.G. and Pootakahm, K. (1979) *Planta Med.*, **37**, 247.

4. Kupchan, S.M., Hemingway, R.J., Coggon, P., McPhail, A.T. and Sim, G.A. (1968) *J.A.C.S.*, **90**, 2982.

5. Coggon, P., McPhail, A.T. and Sim, G.A. (1969) *J.C.S.(B)*, 534.

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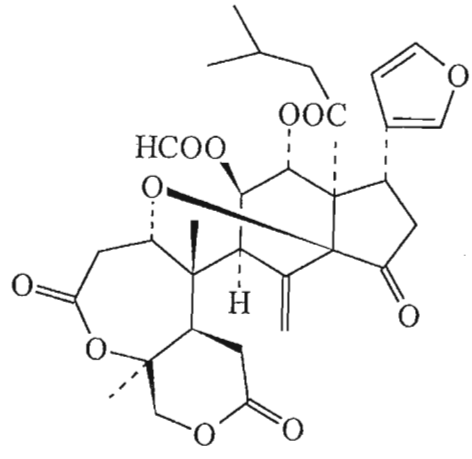
Extractives from *Aphanamixis polystacha*

Compound 2-A



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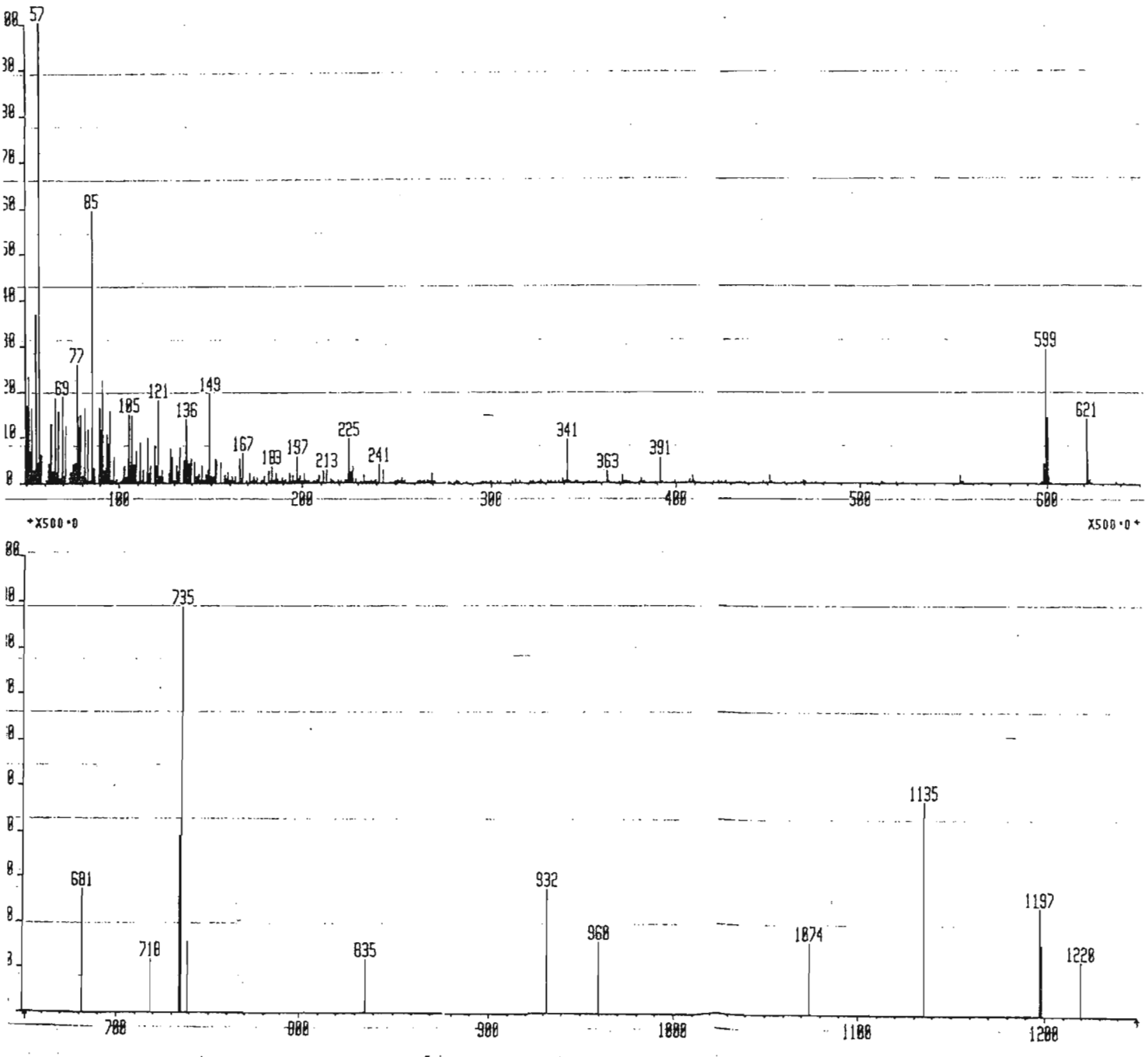

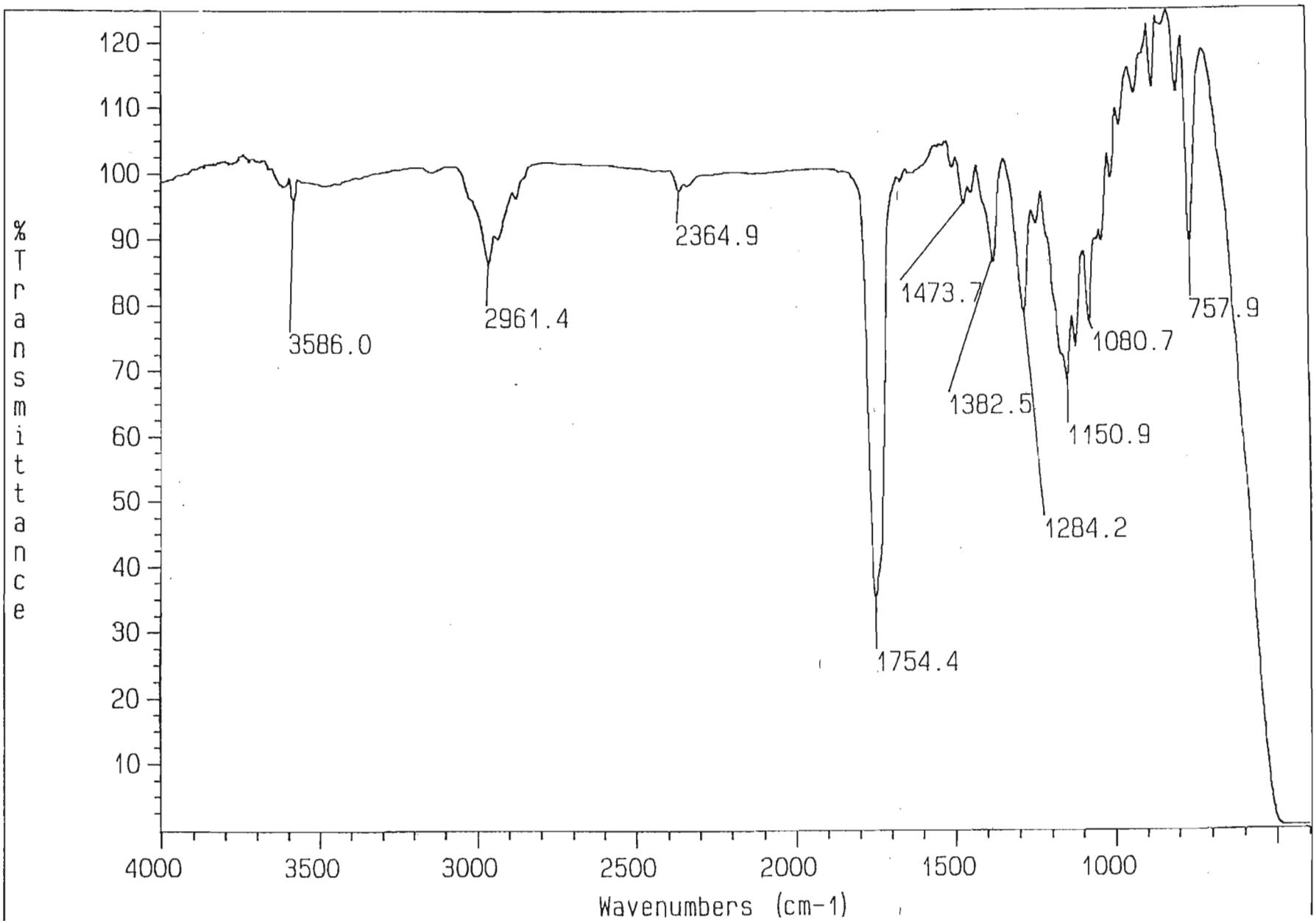

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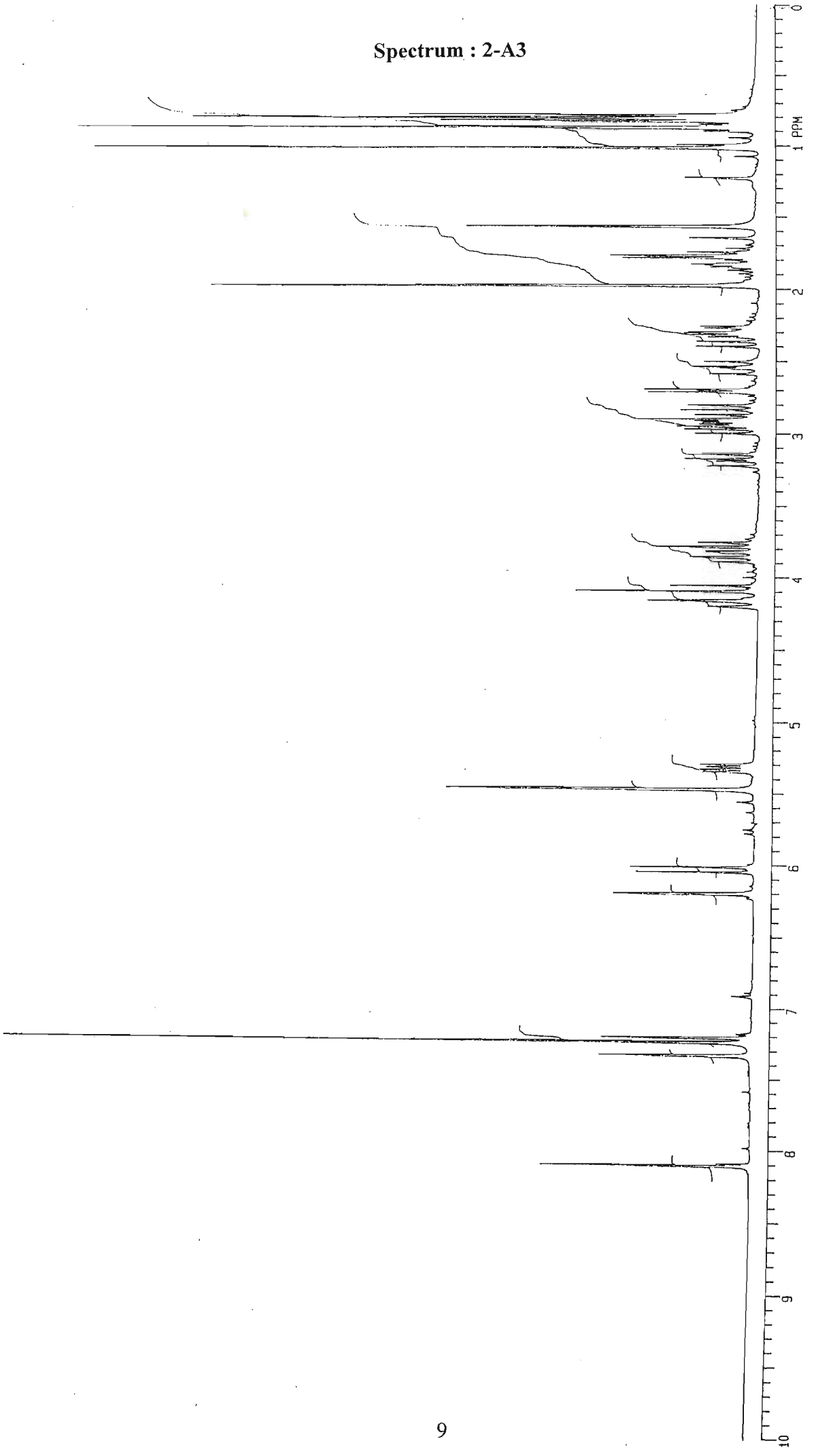
Spectrum : 2-A3



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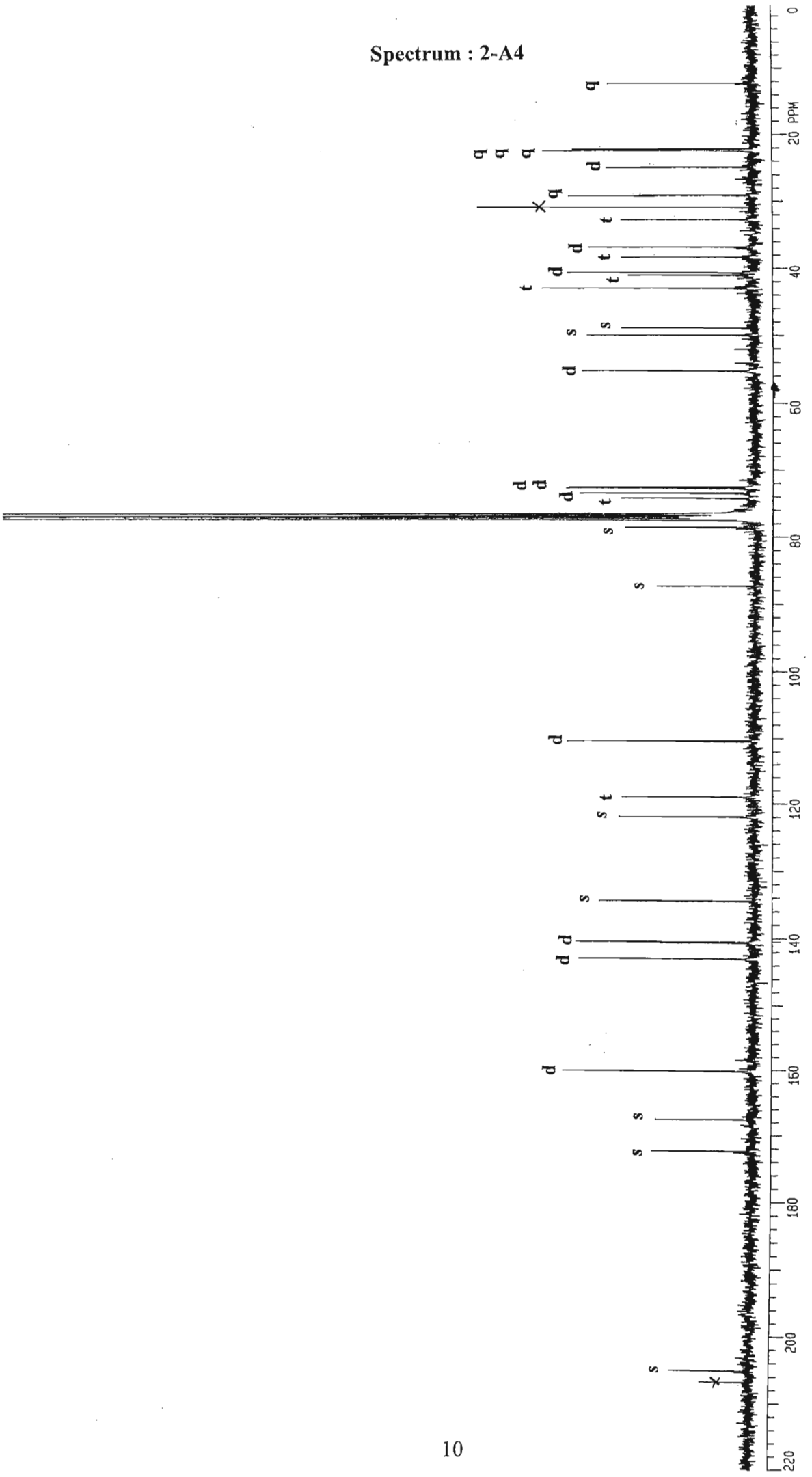

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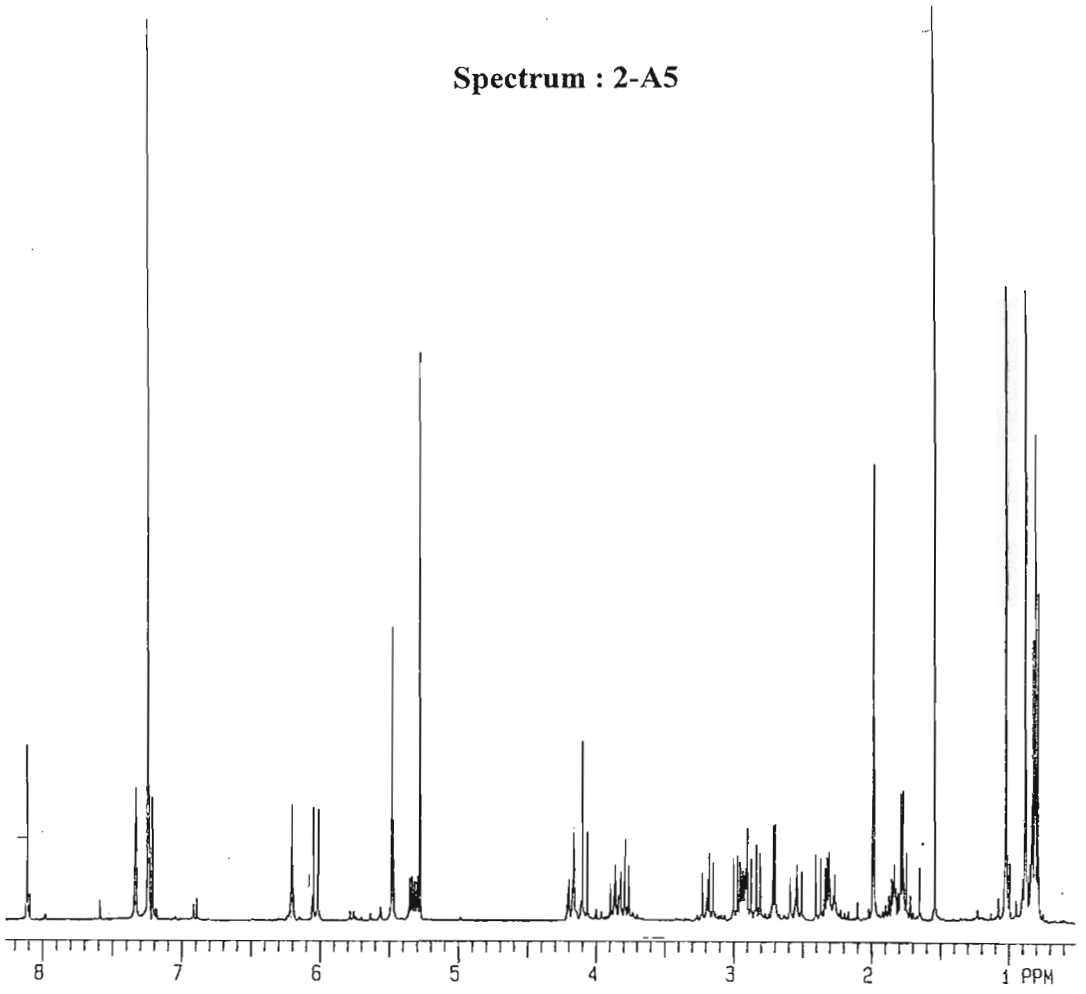

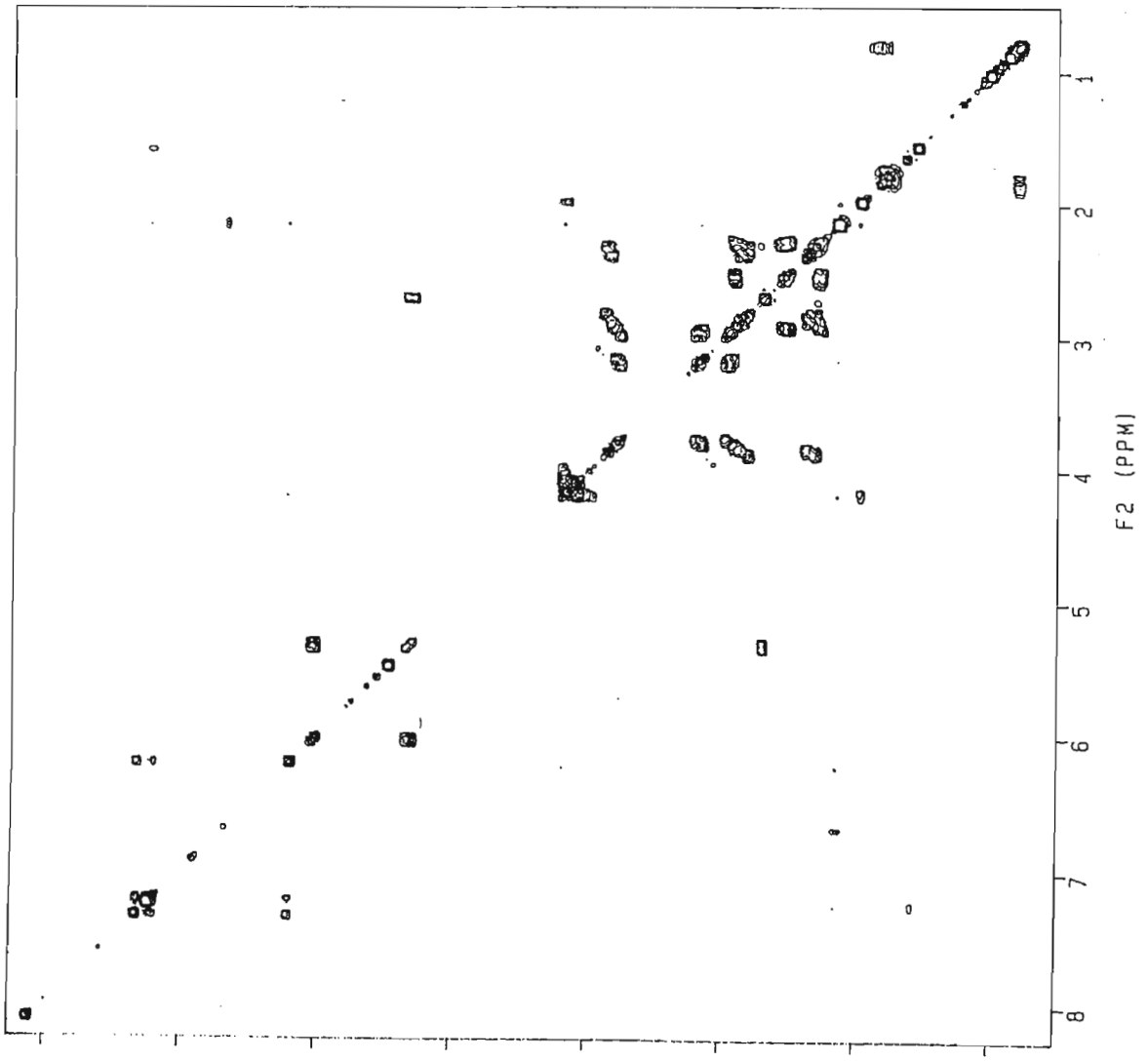

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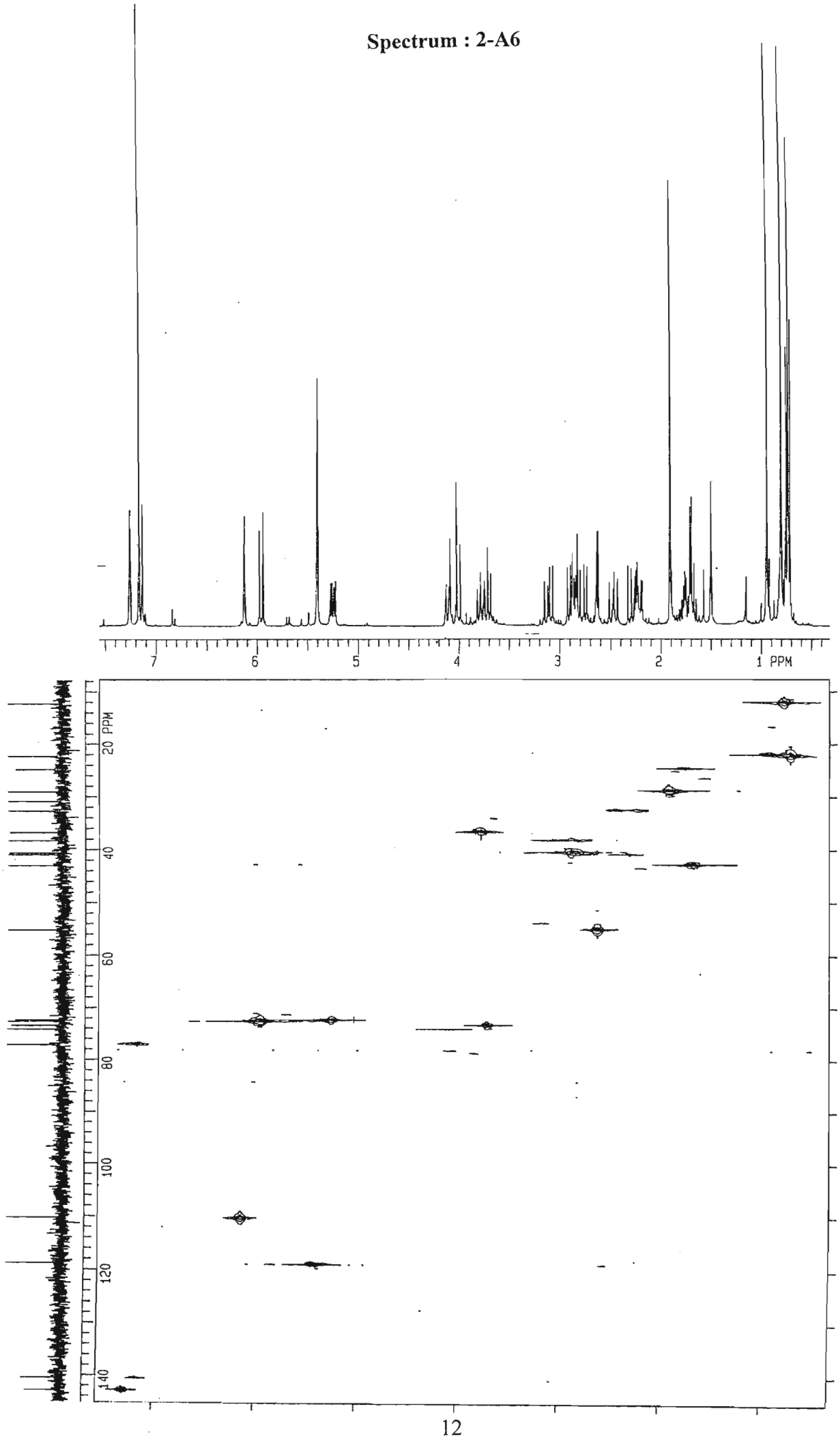

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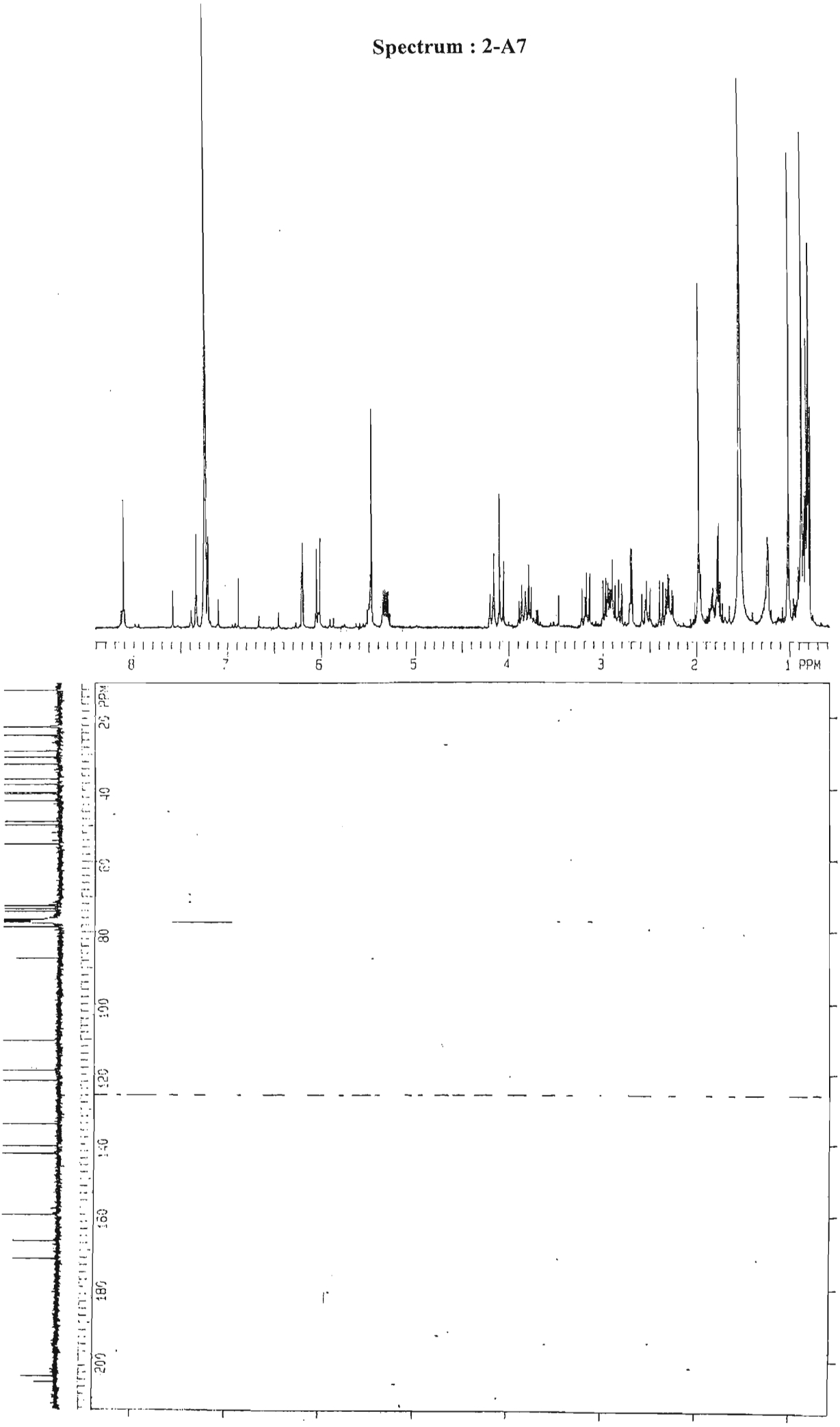

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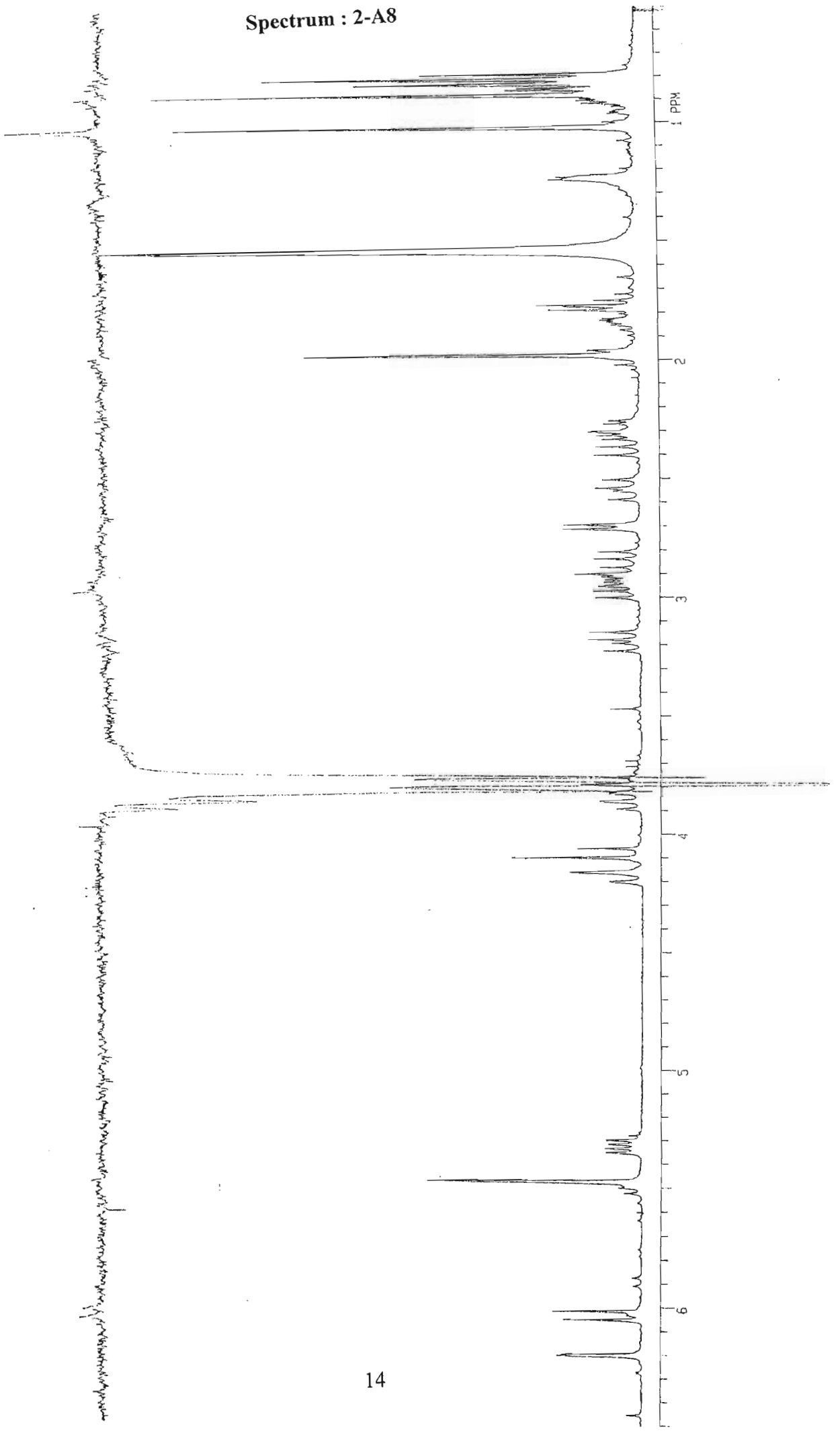
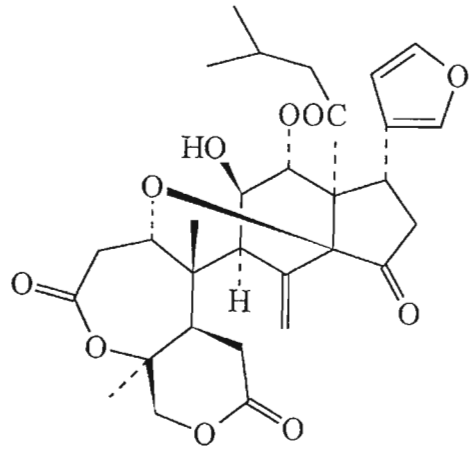


Extractives from *Aphanamixis polystacha*

Compound 2-B



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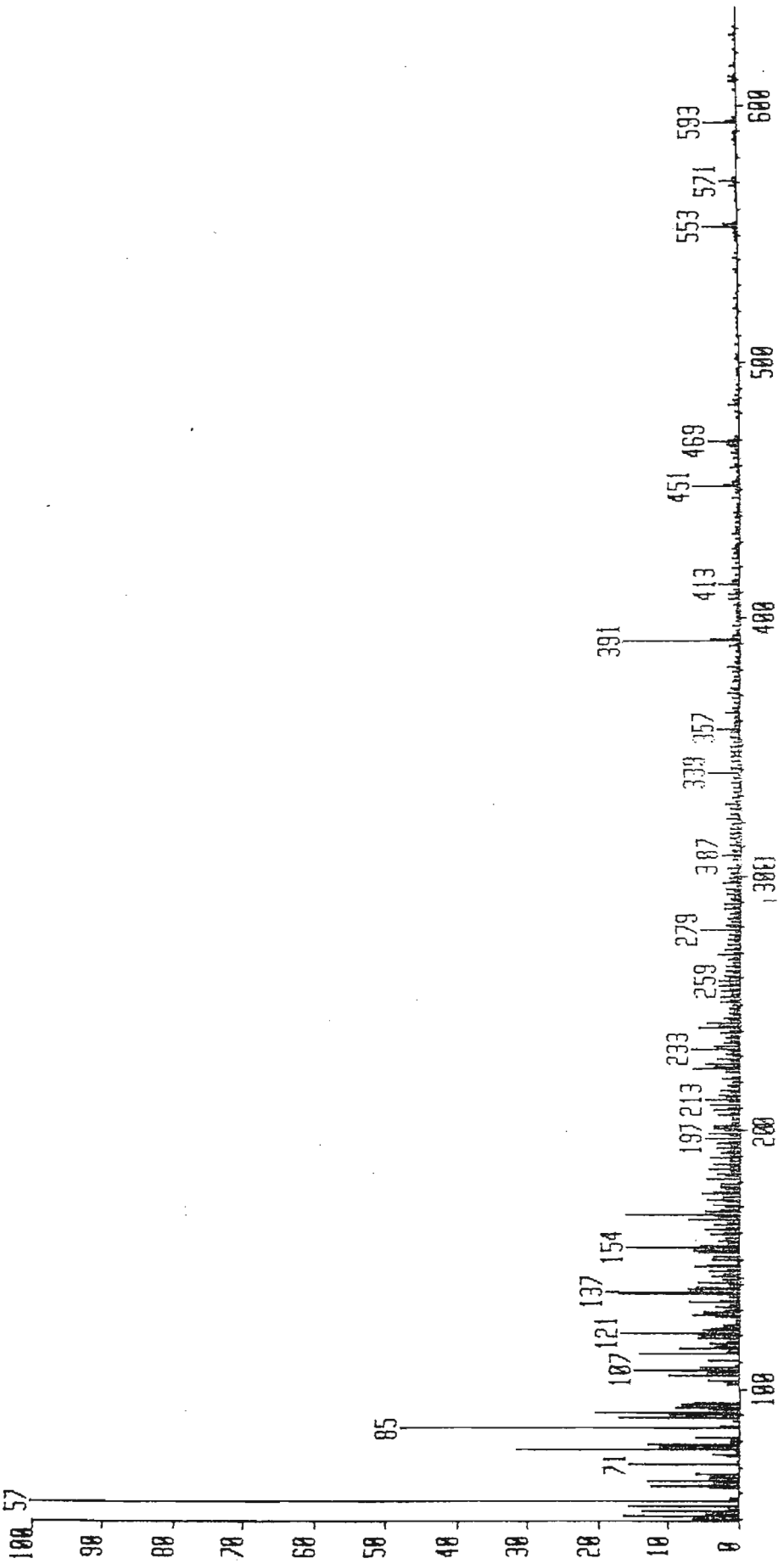

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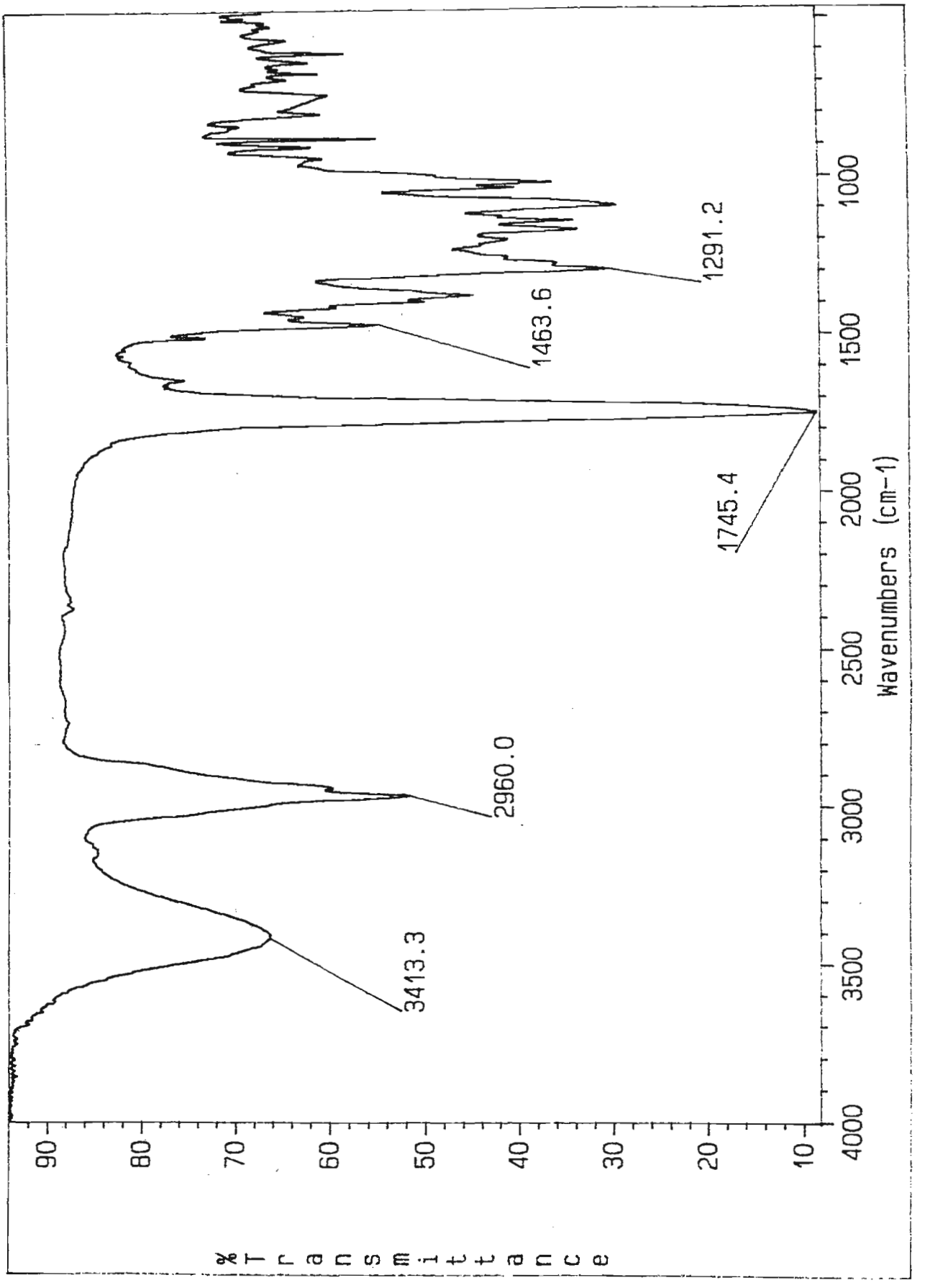

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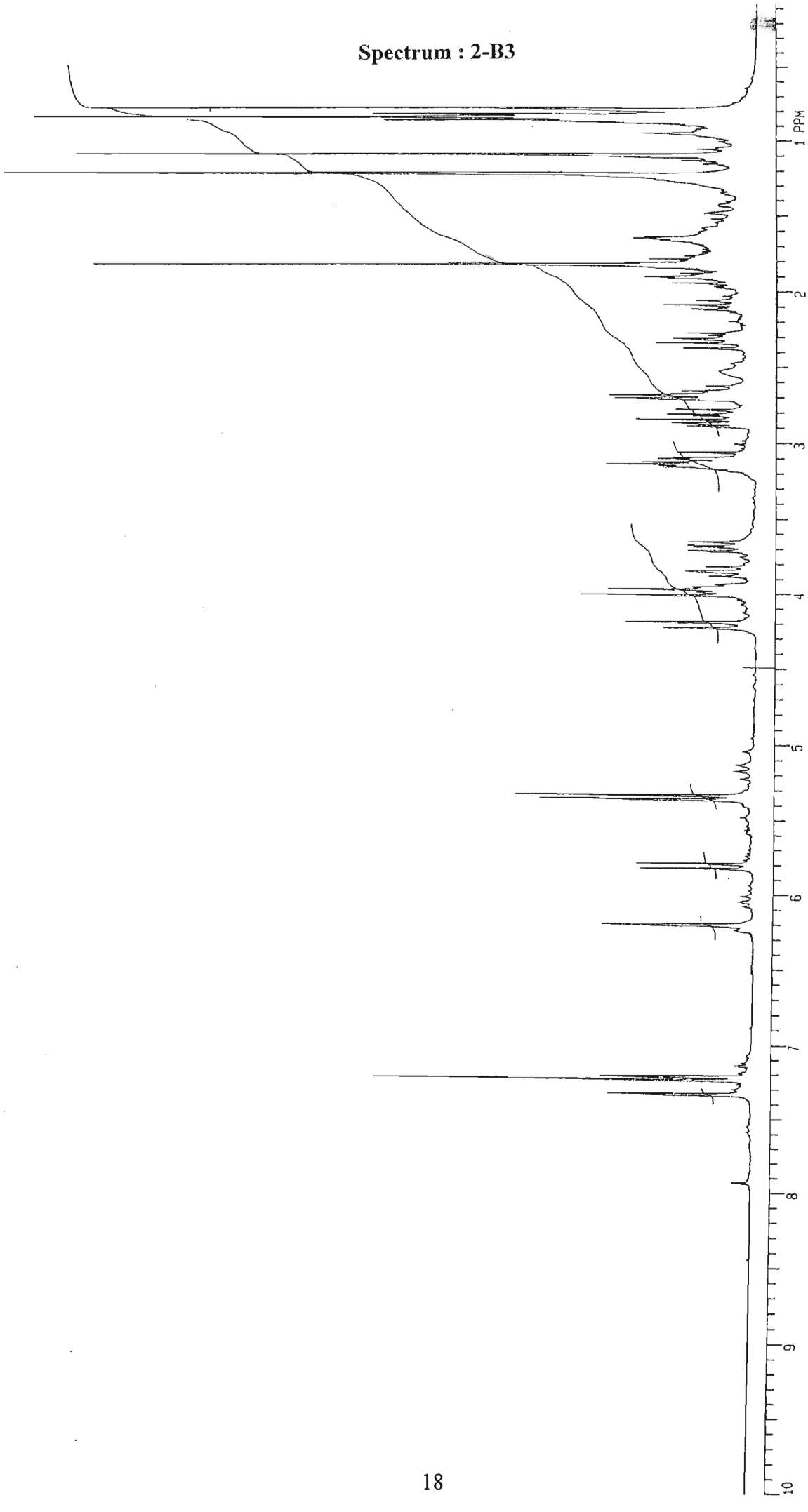

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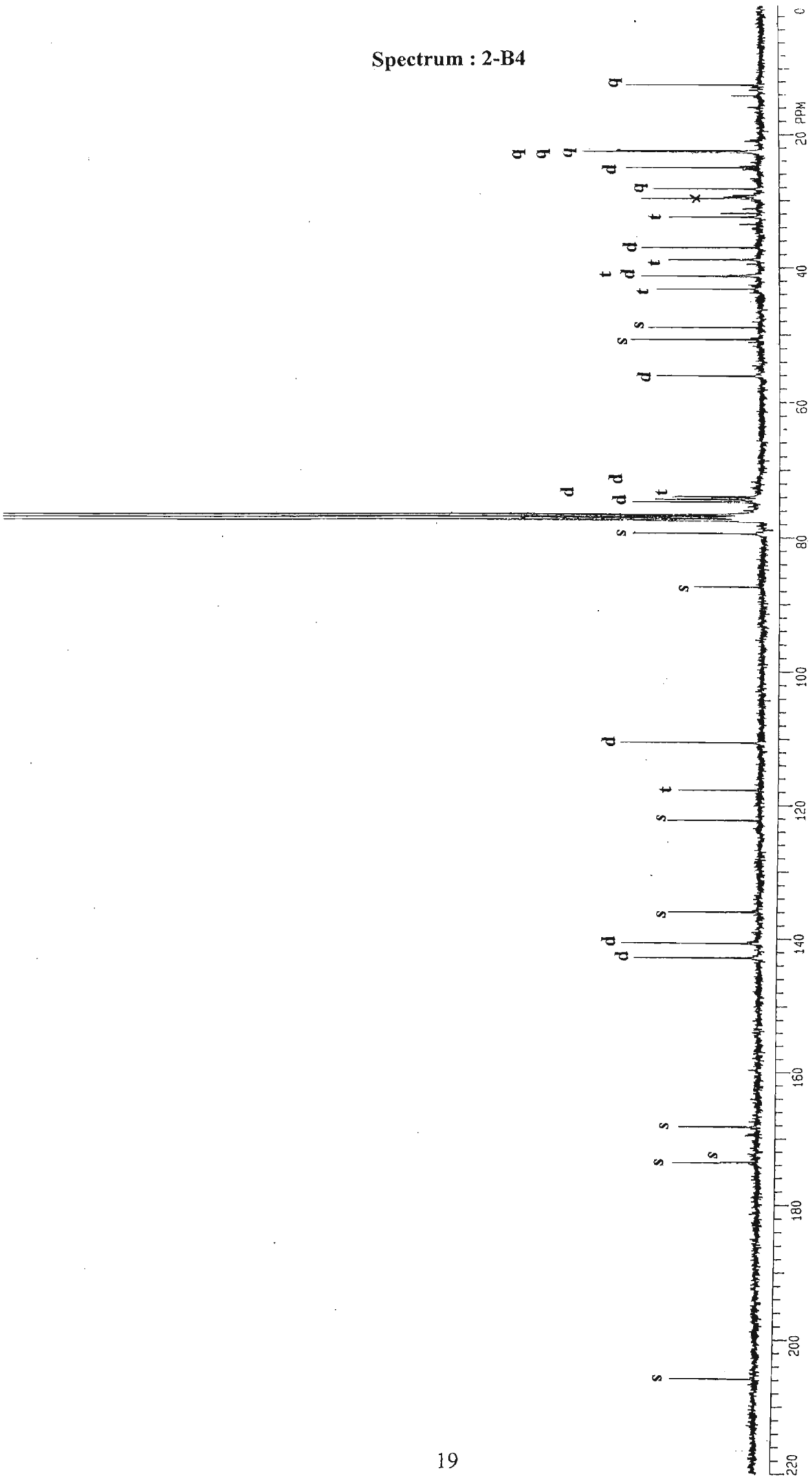

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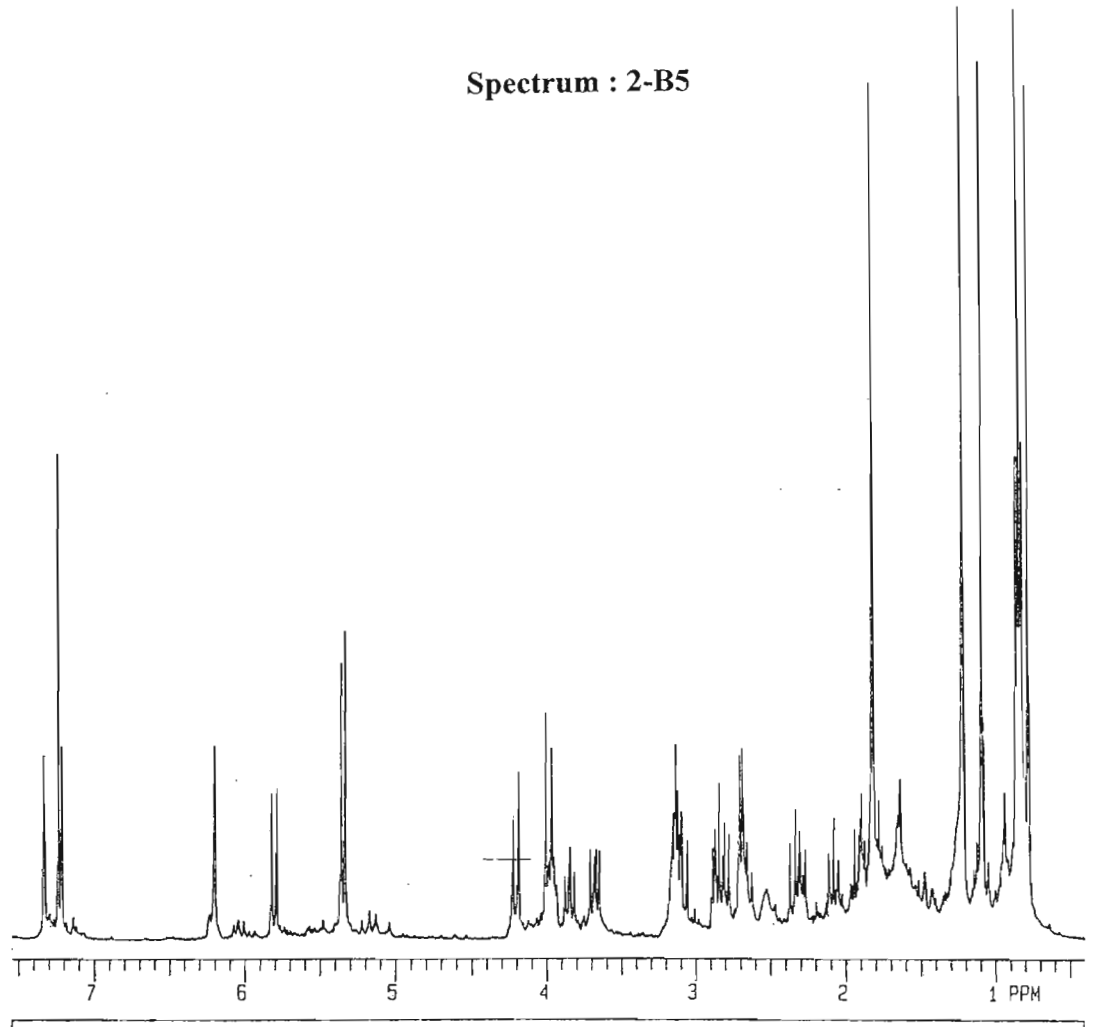

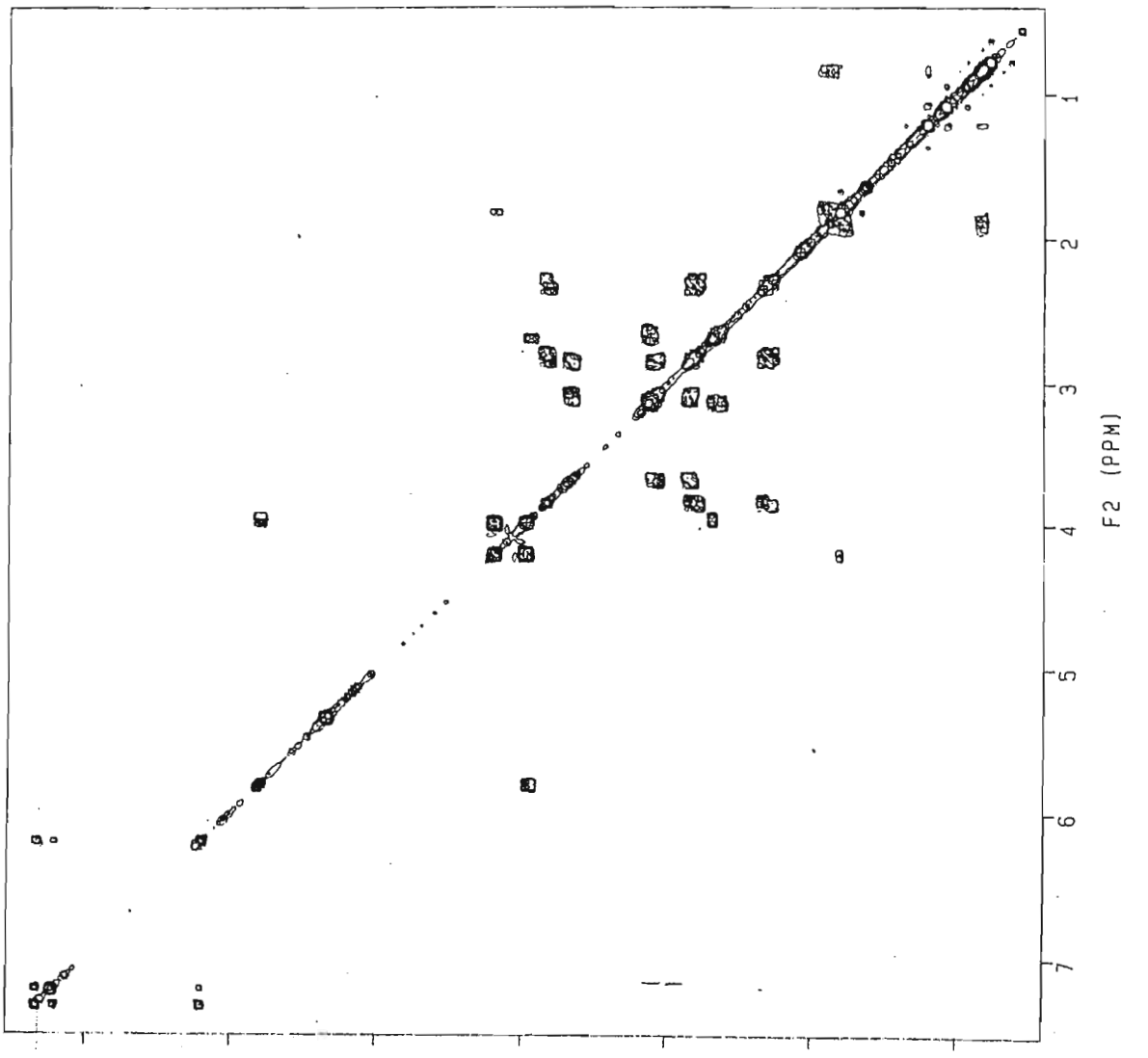

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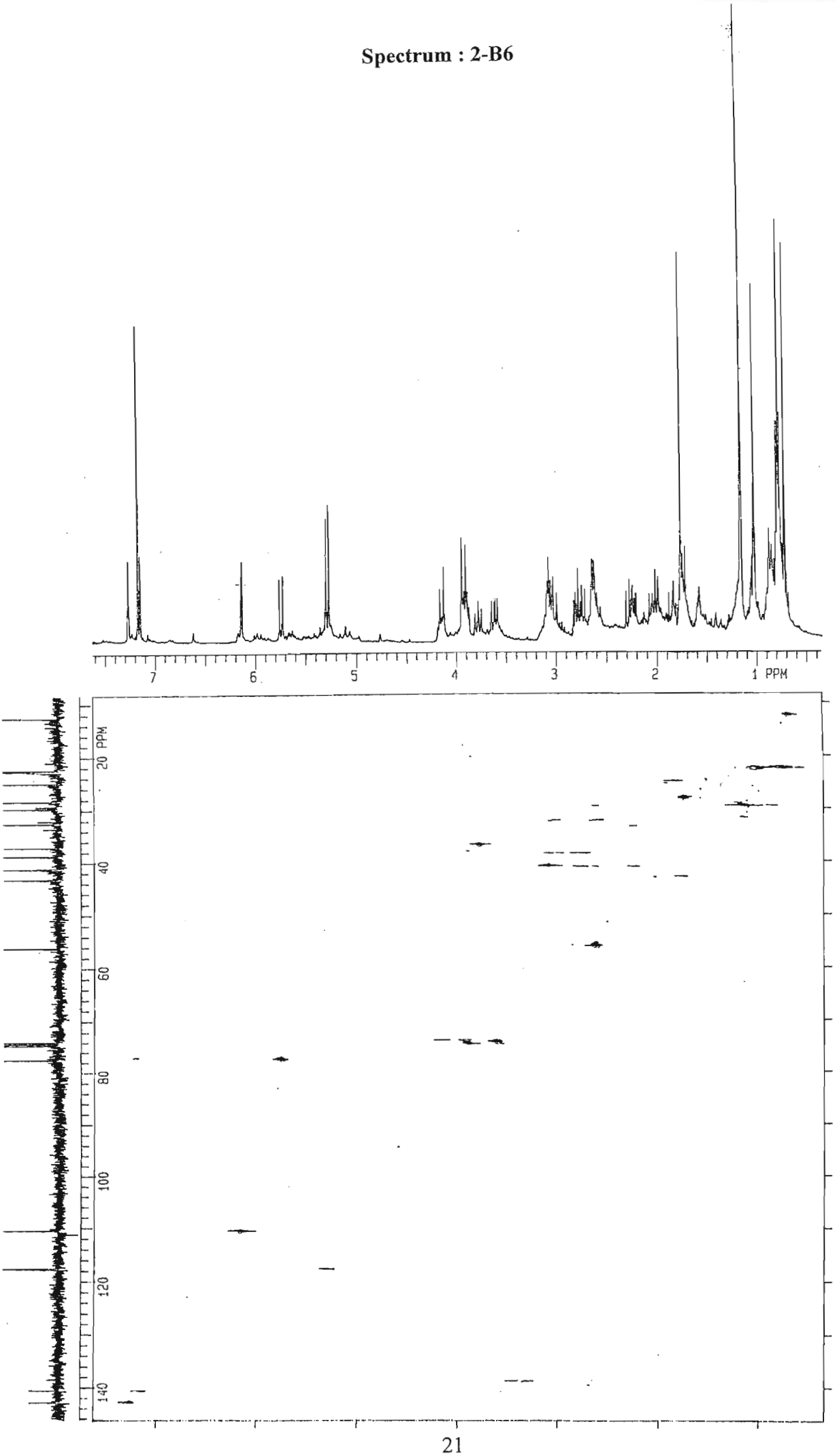
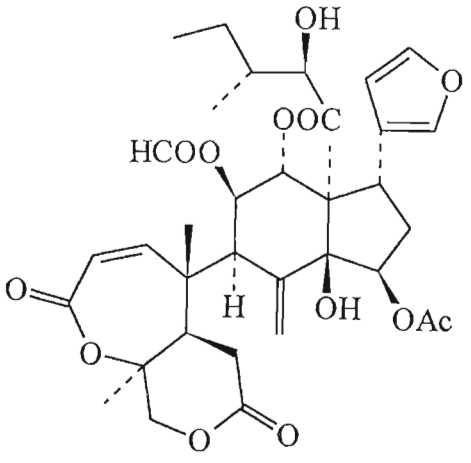
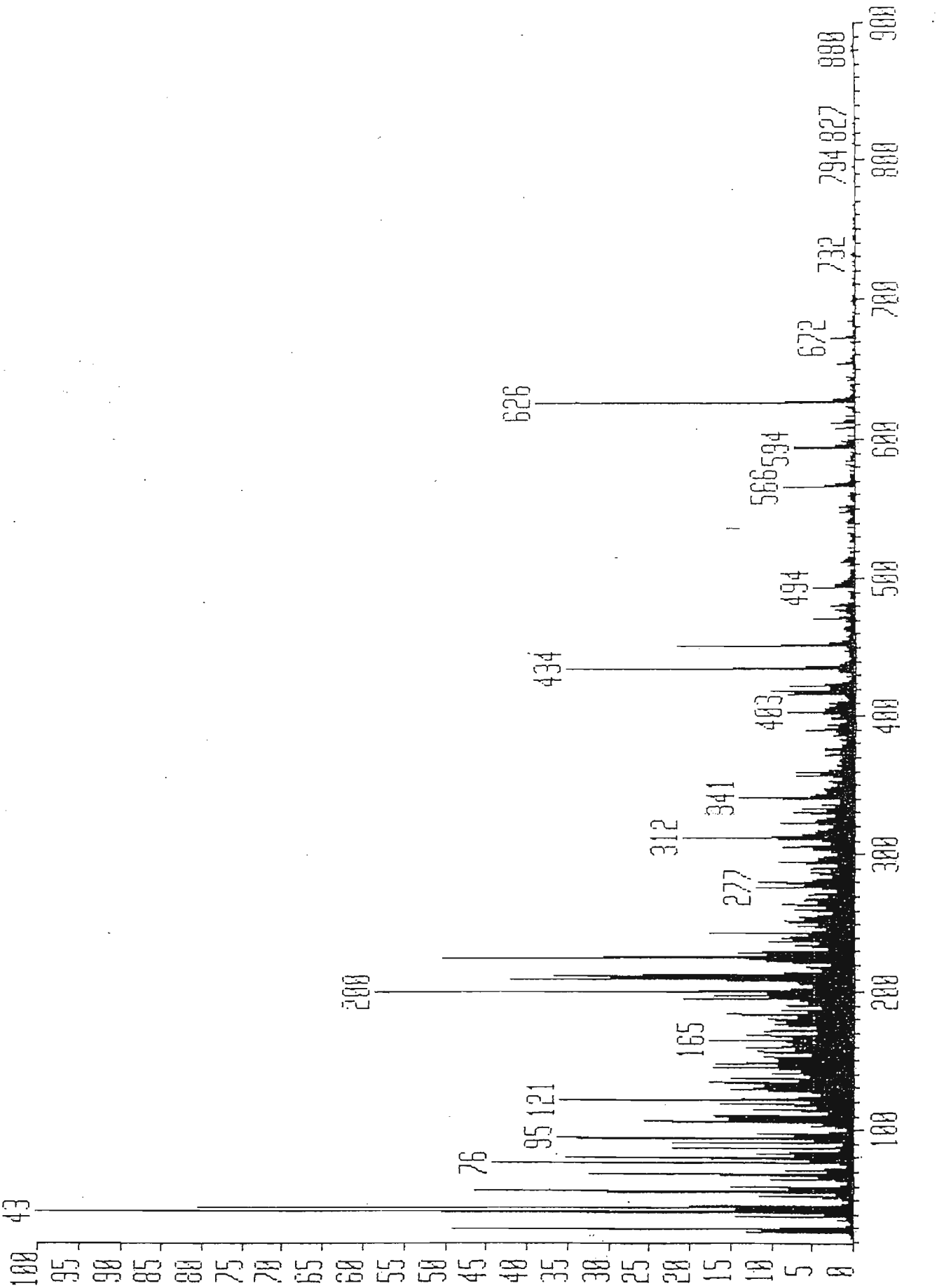


Extractives from *Aphanamixis polystacha*

Compound 2-C



Spectrum : 2-C1



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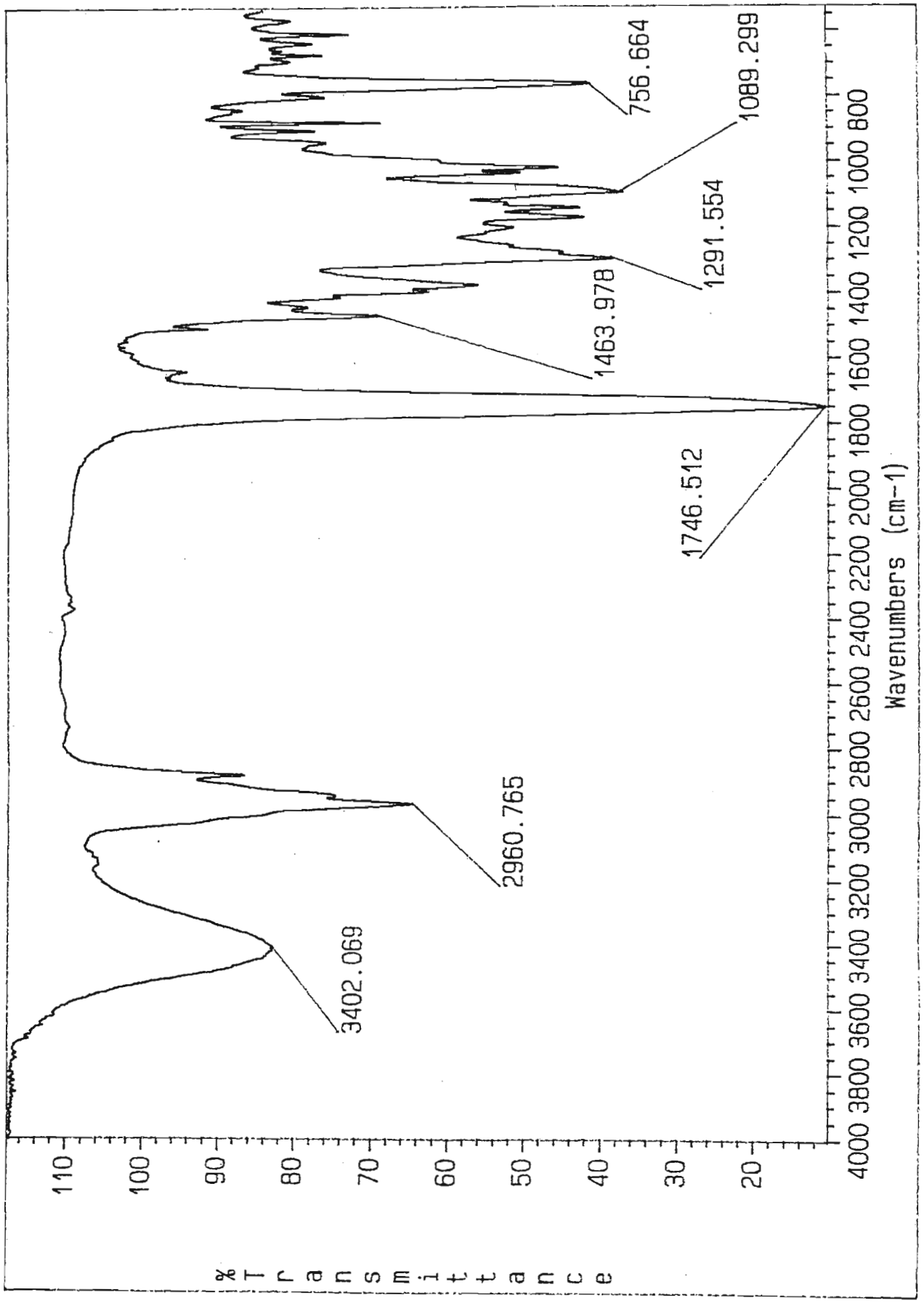
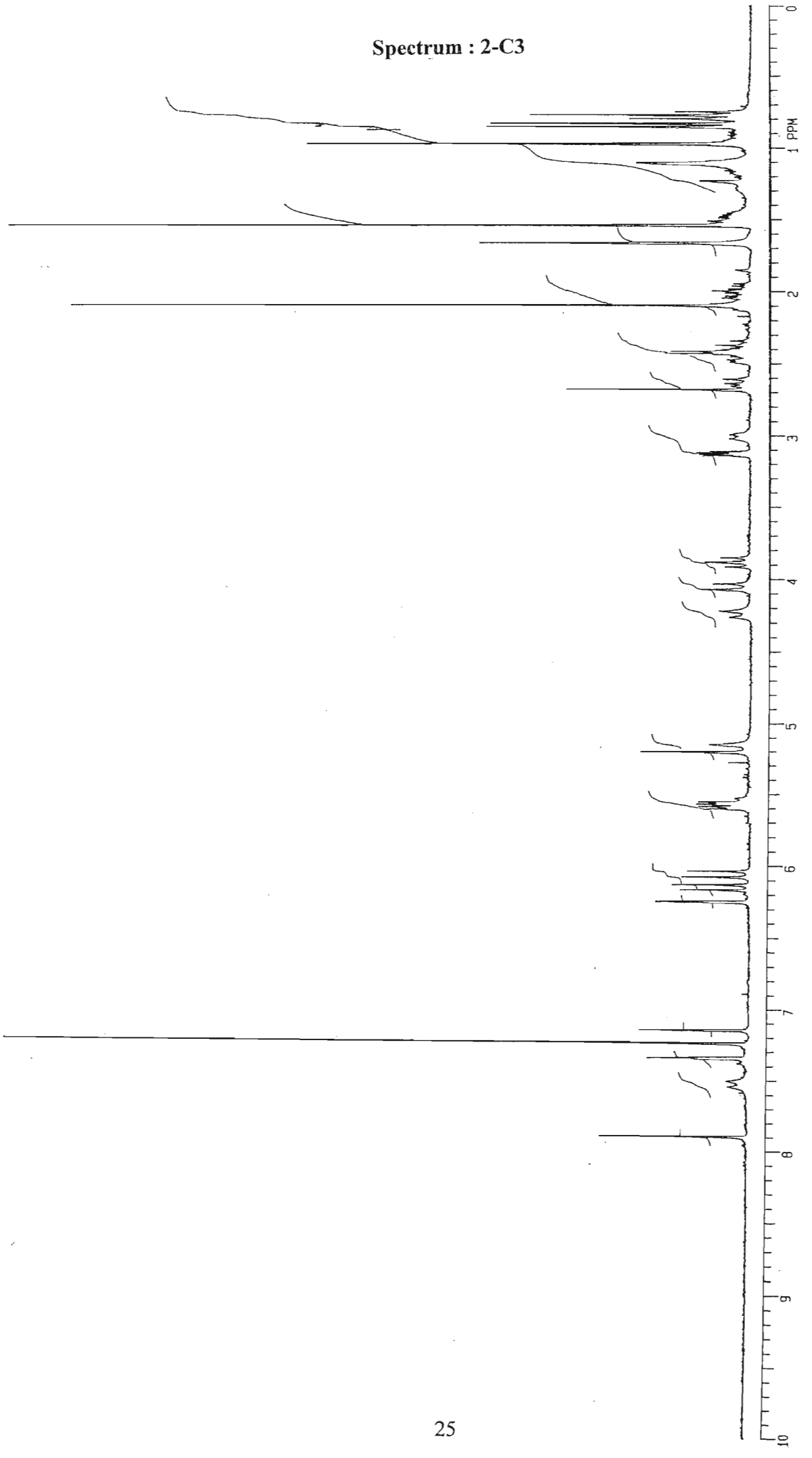
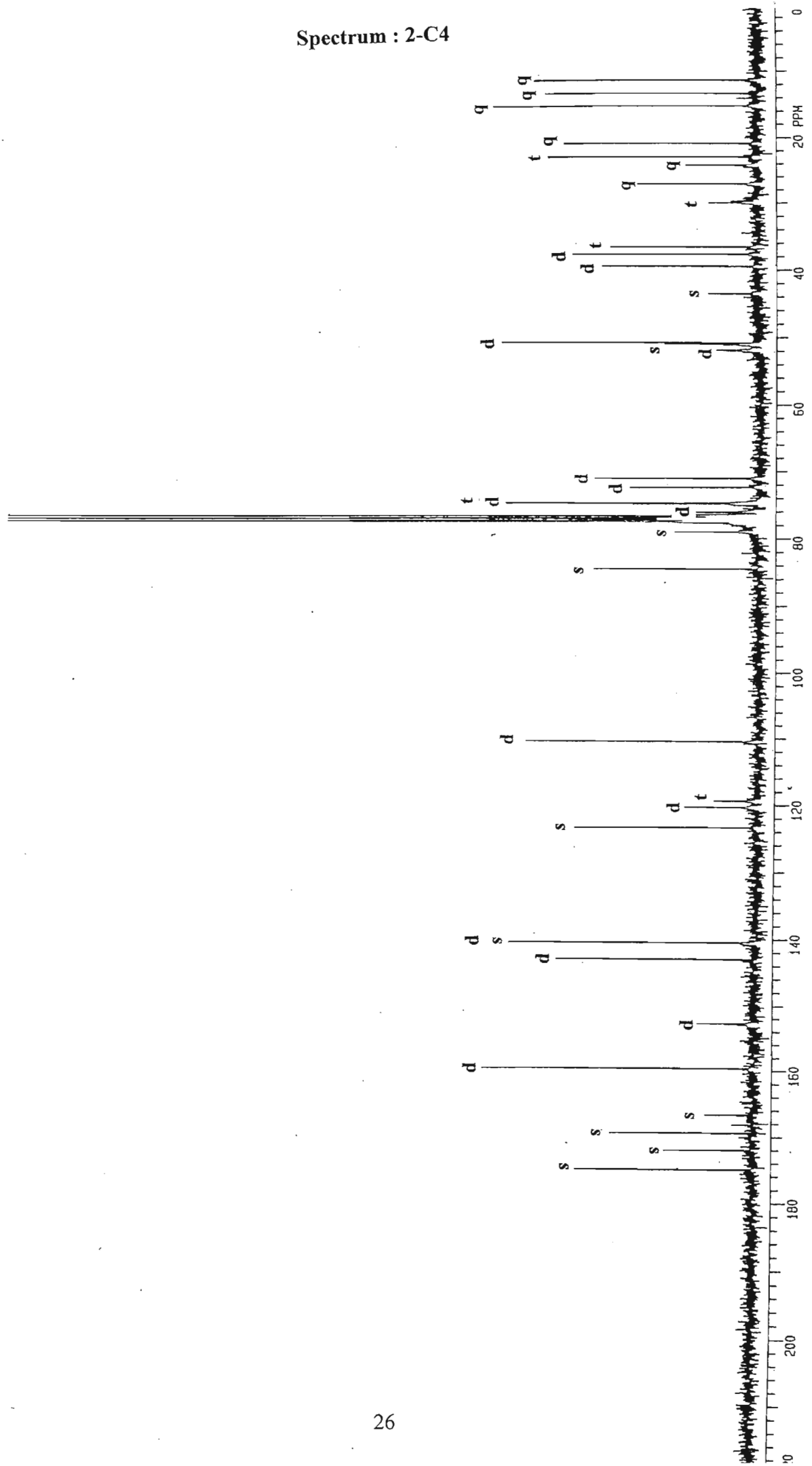


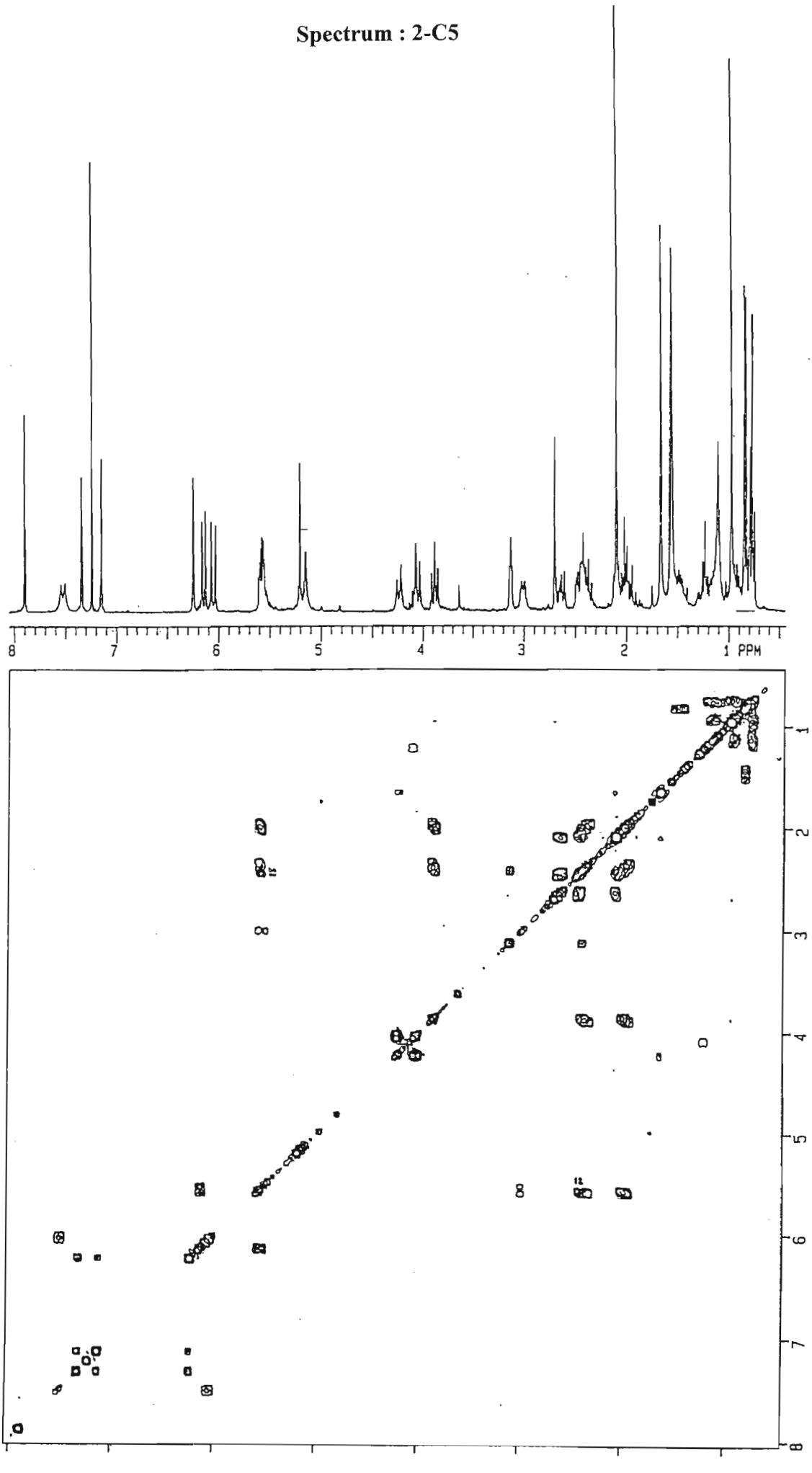
Spectrum : 2-C3



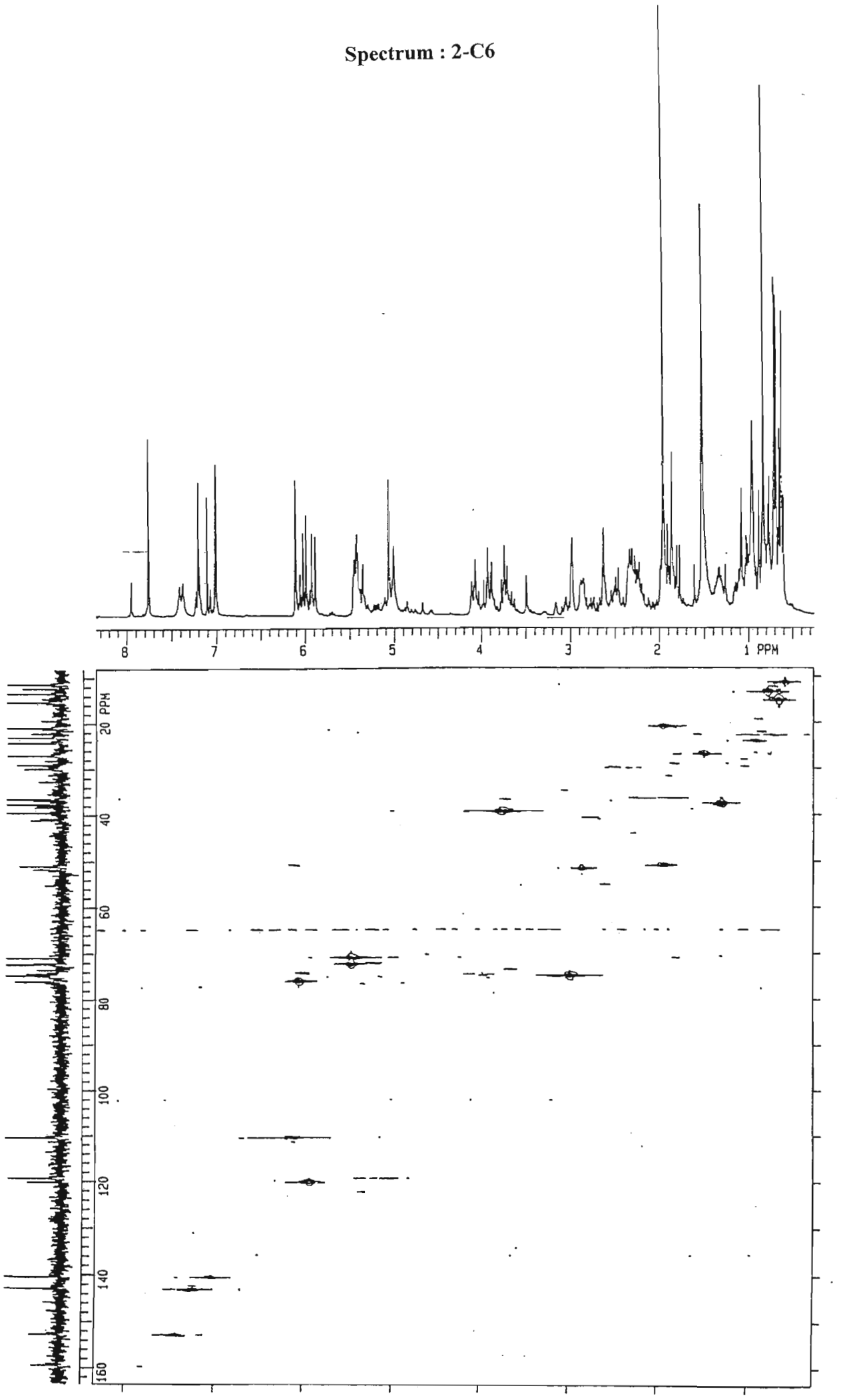
Spectrum : 2-C4



Spectrum : 2-C5

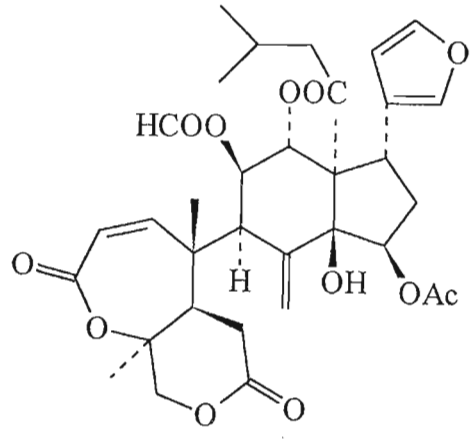


Spectrum : 2-C6

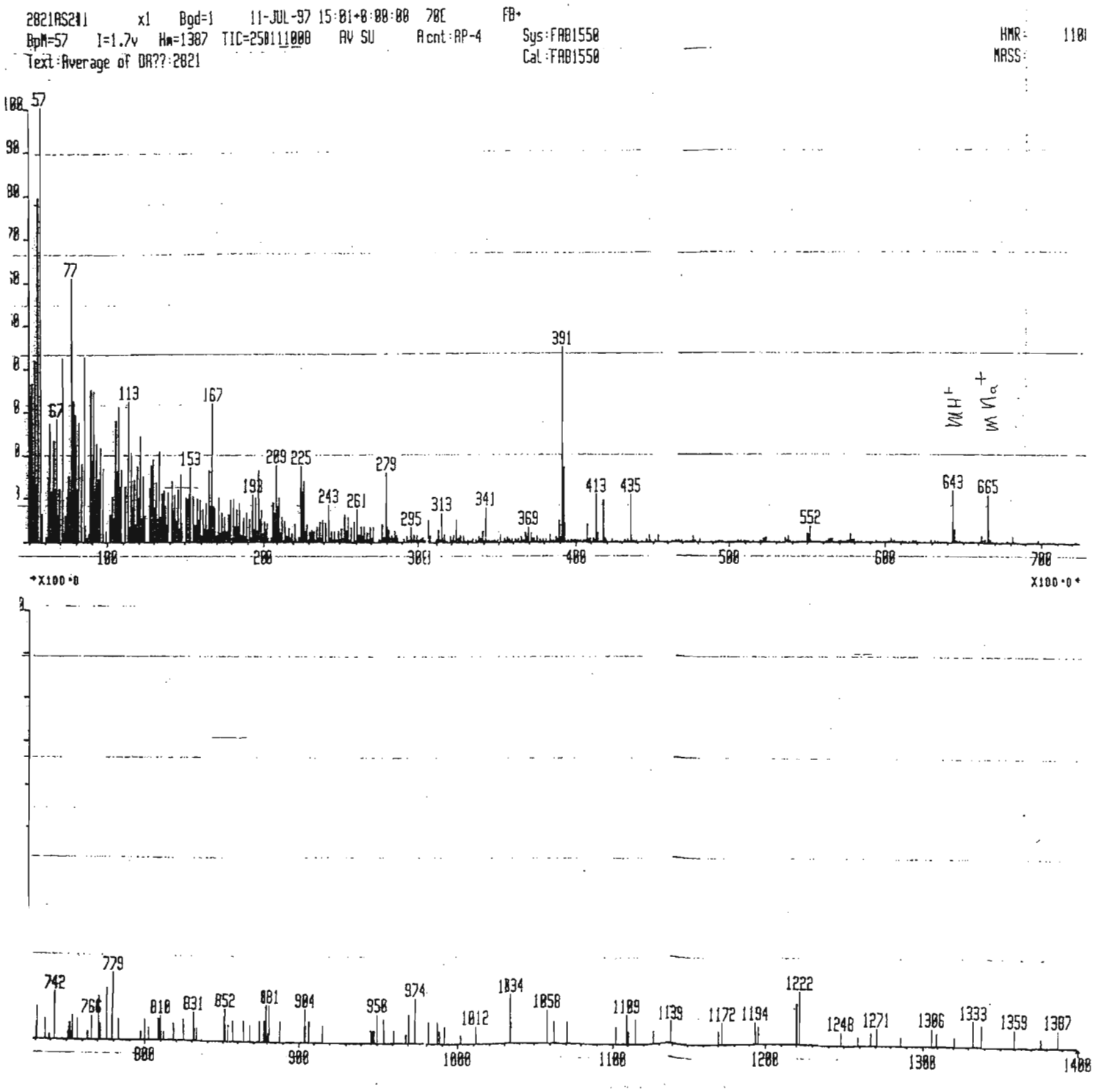


Extractives from *Aphanamixis polystacha*

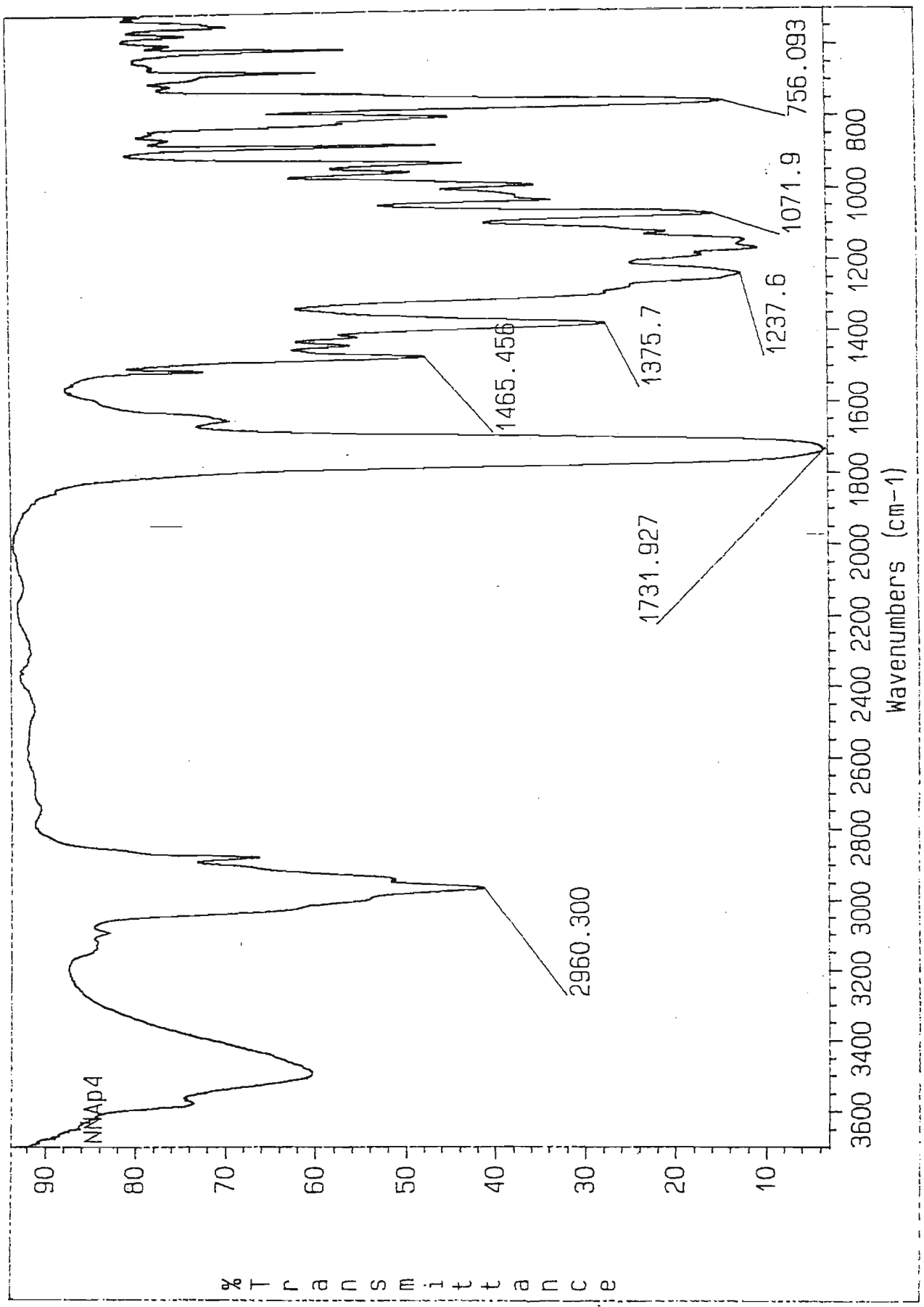
Compound 2-D



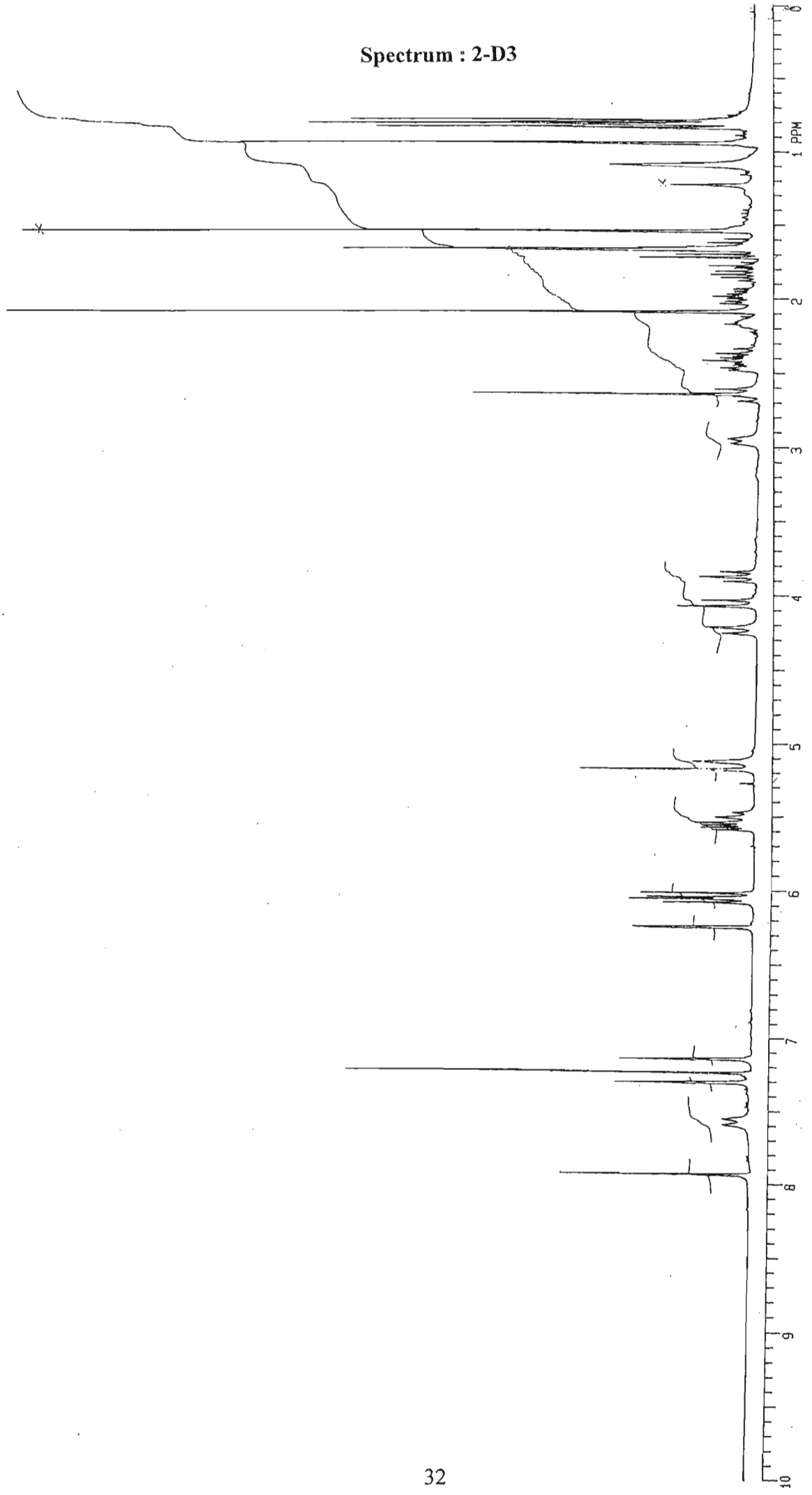
Spectrum : 2-D1



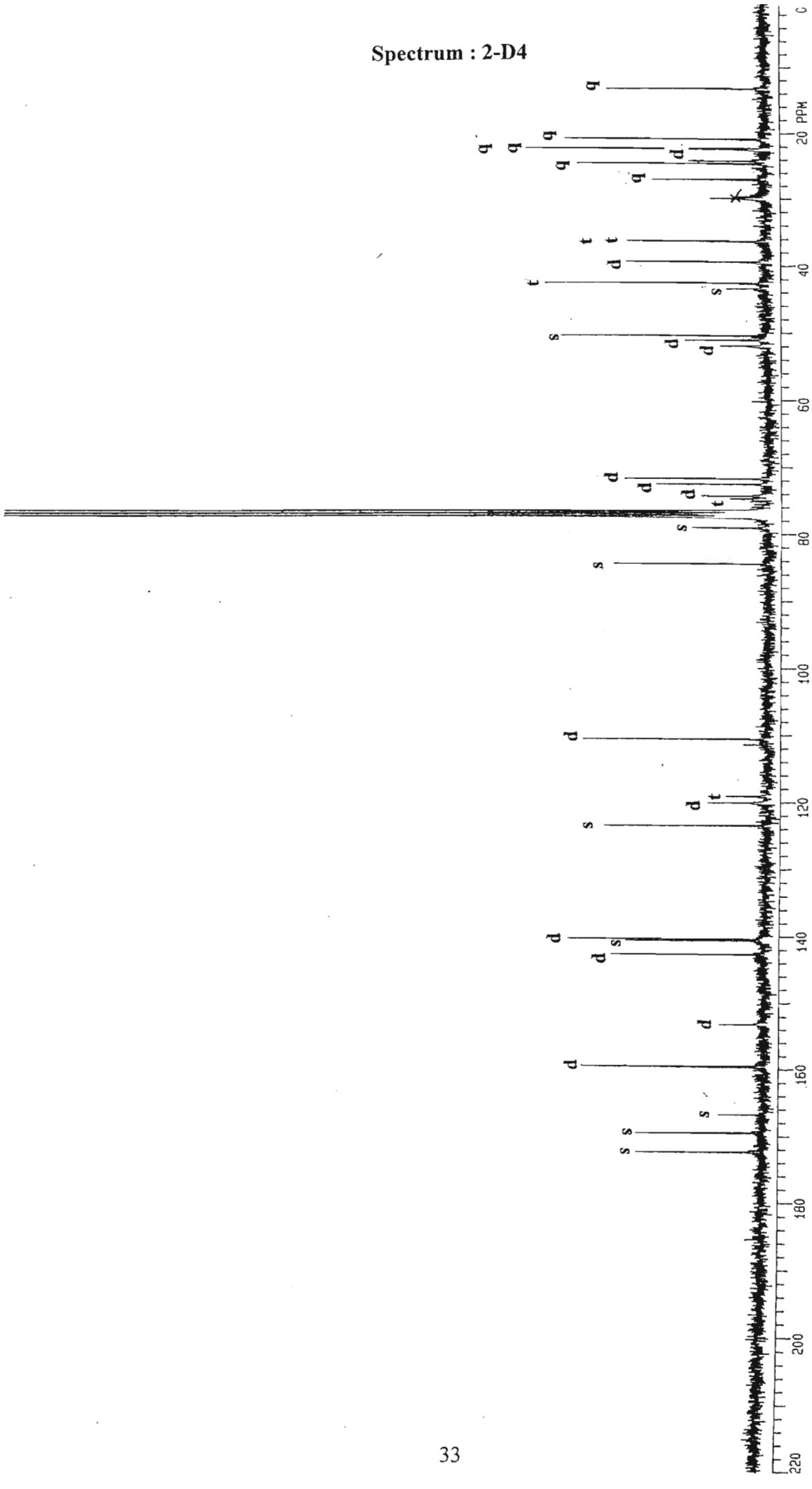
Spectrum : 2-D2



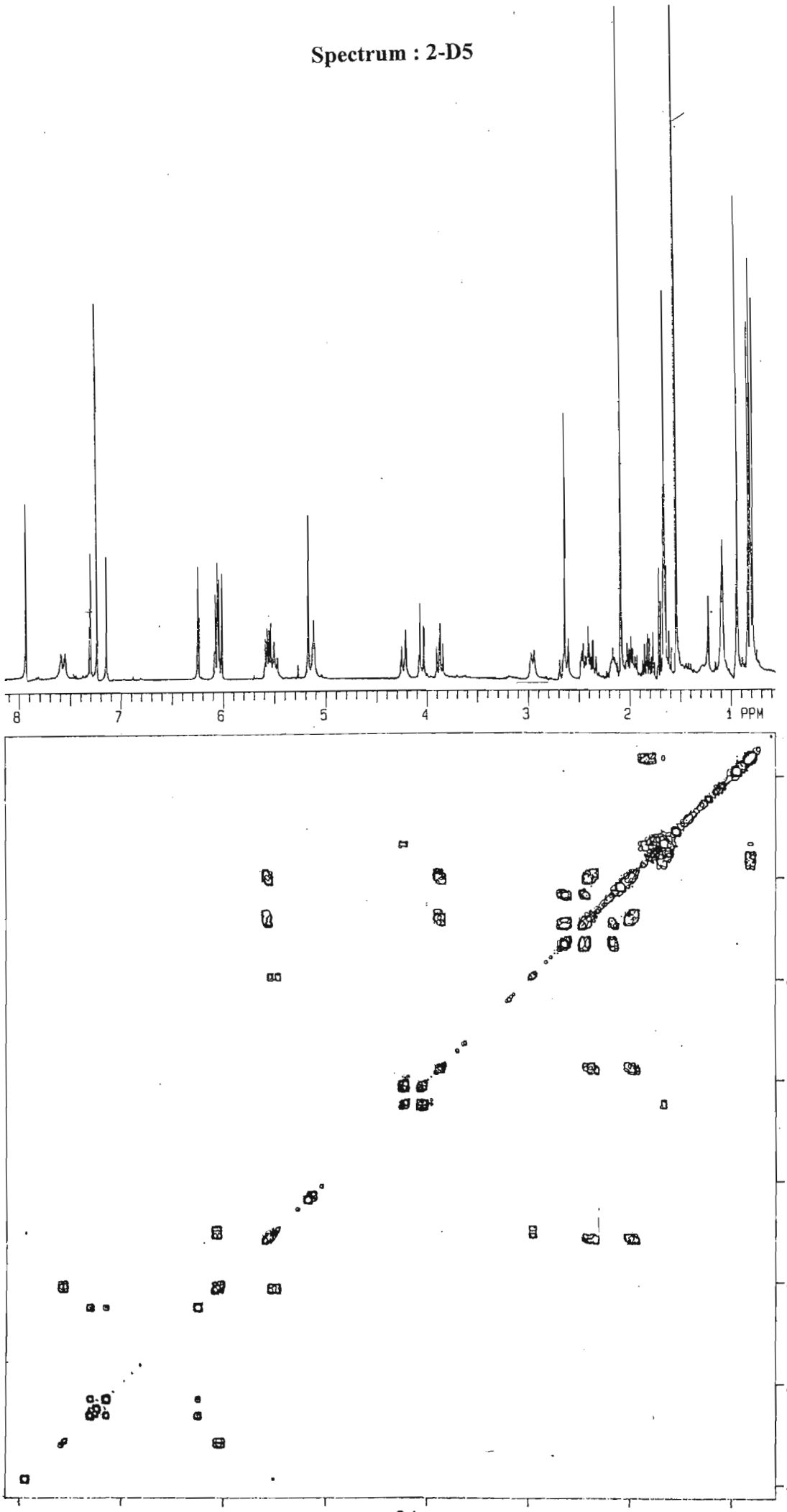
Spectrum : 2-D3



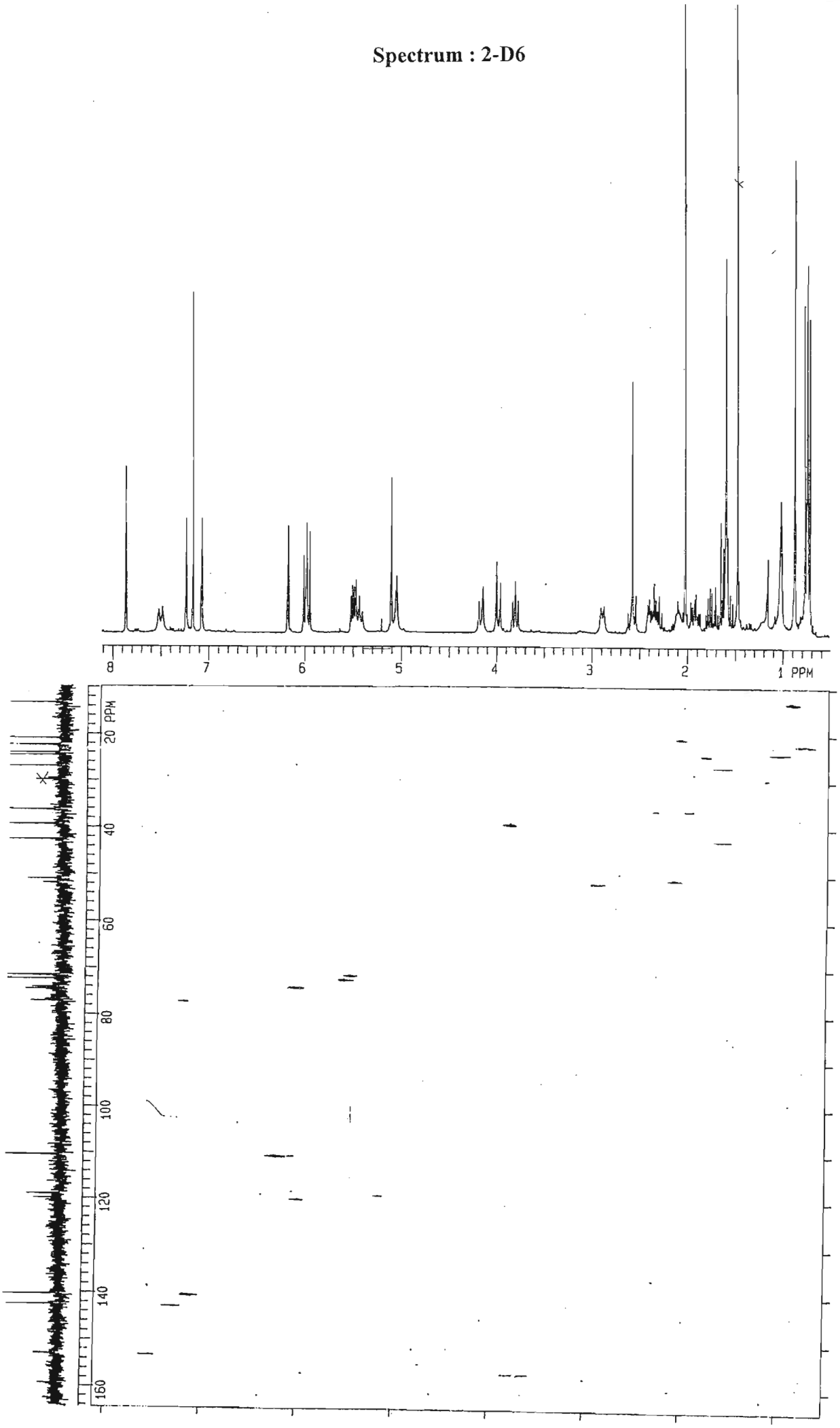
Spectrum : 2-D4



Spectrum : 2-D5

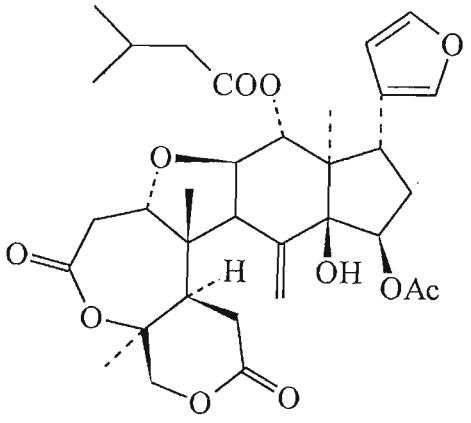


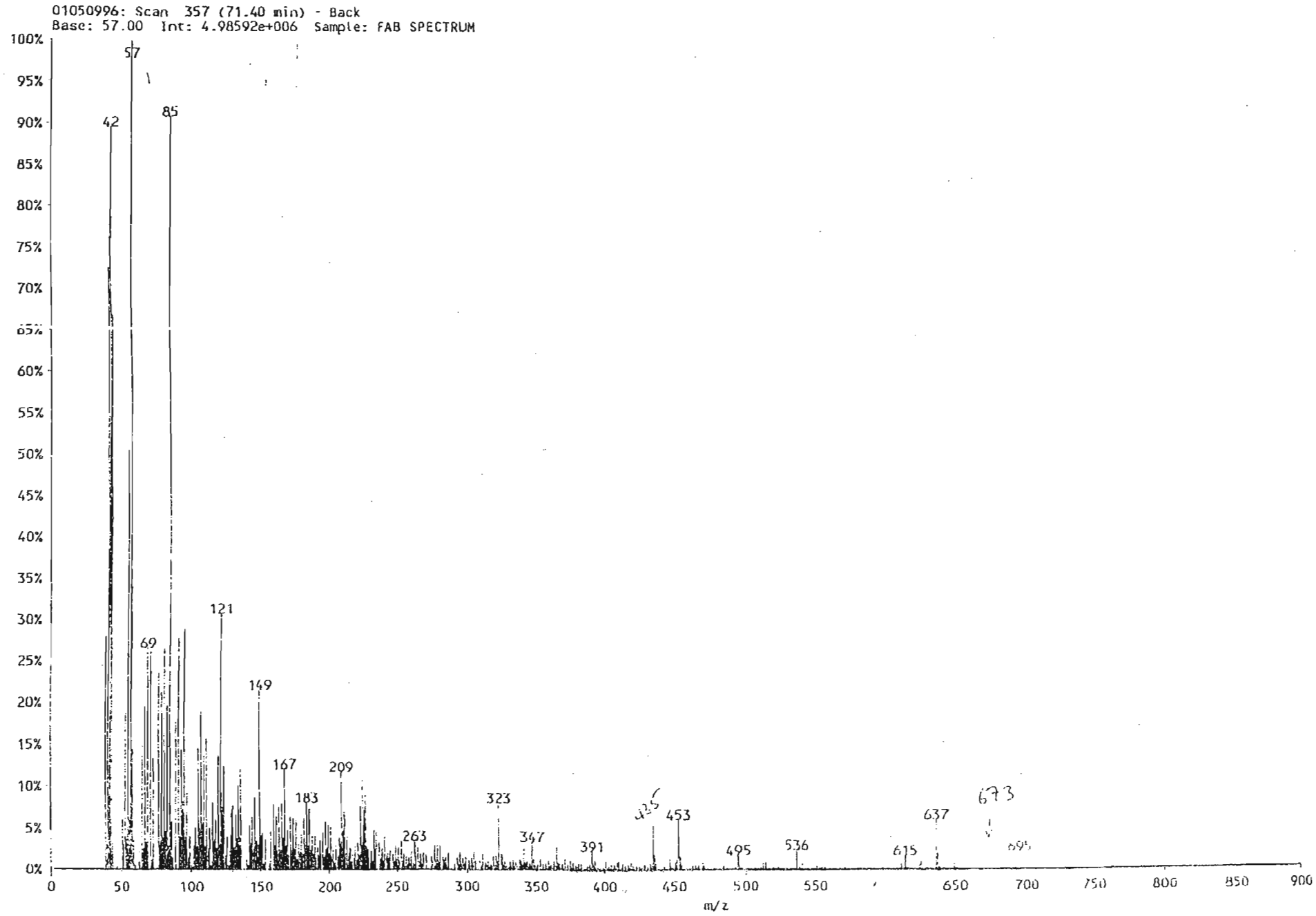
Spectrum : 2-D6



Extractives from *Aphanamixis polystacha*

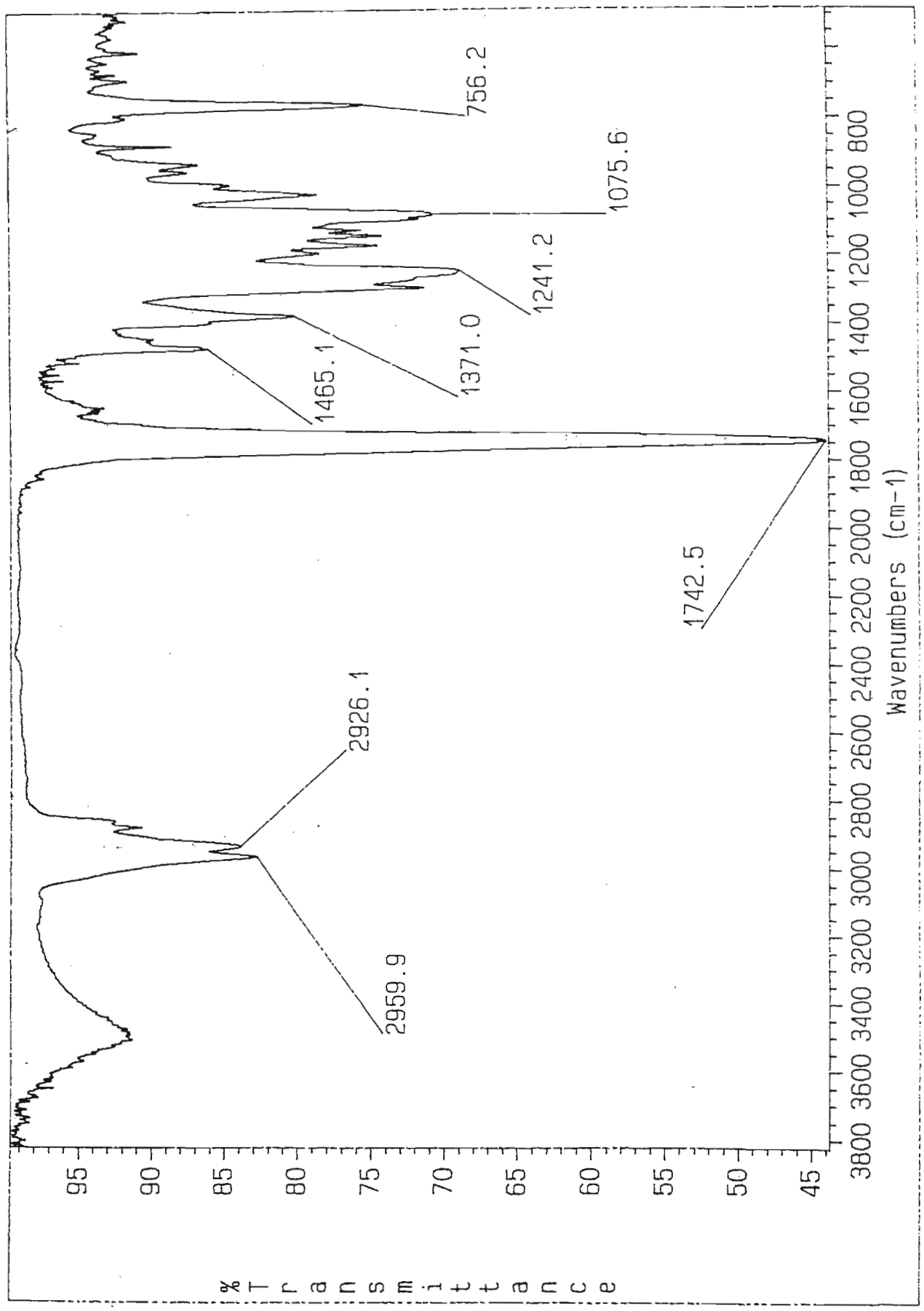
Compound 2-E



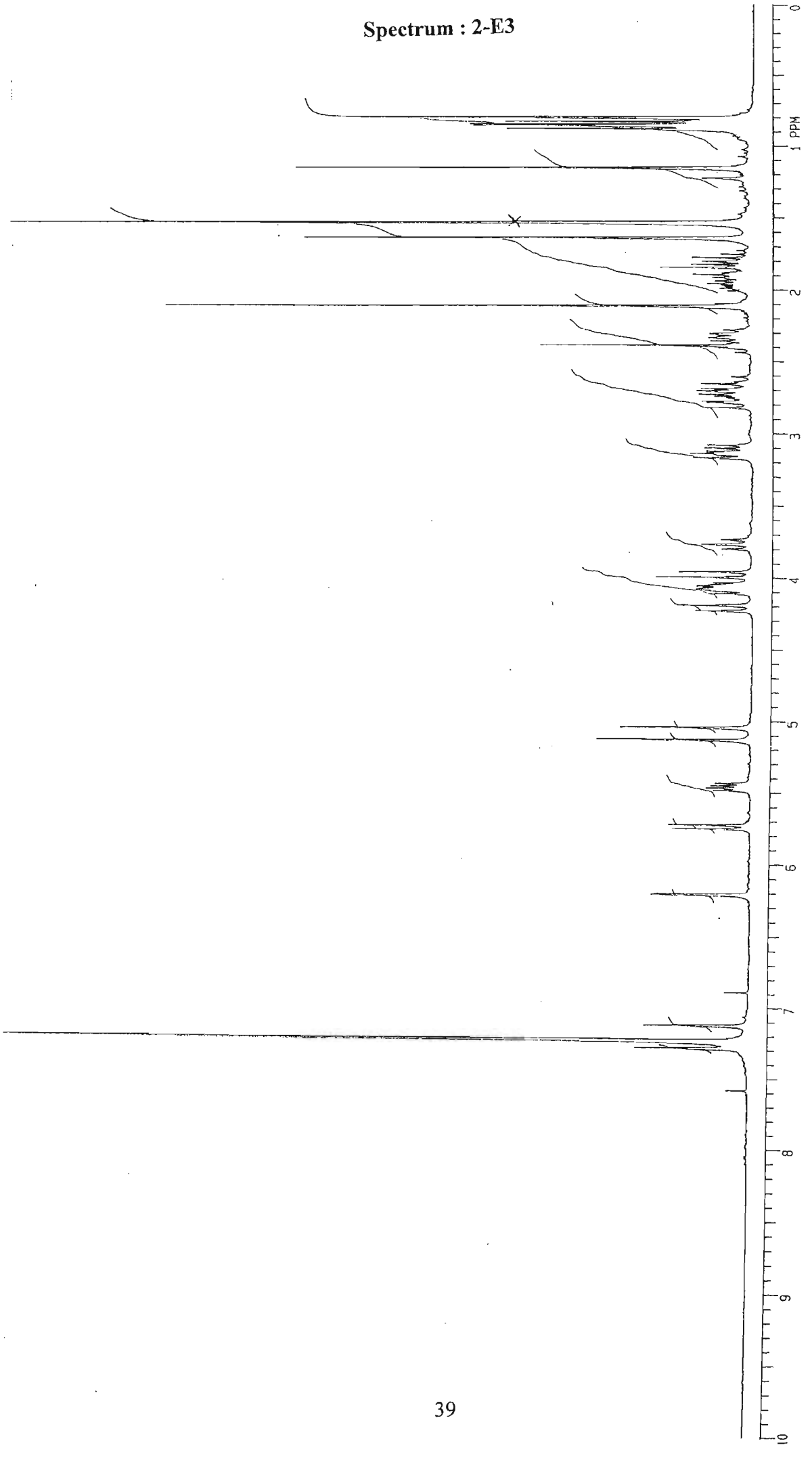


Spectrum : 2-E1

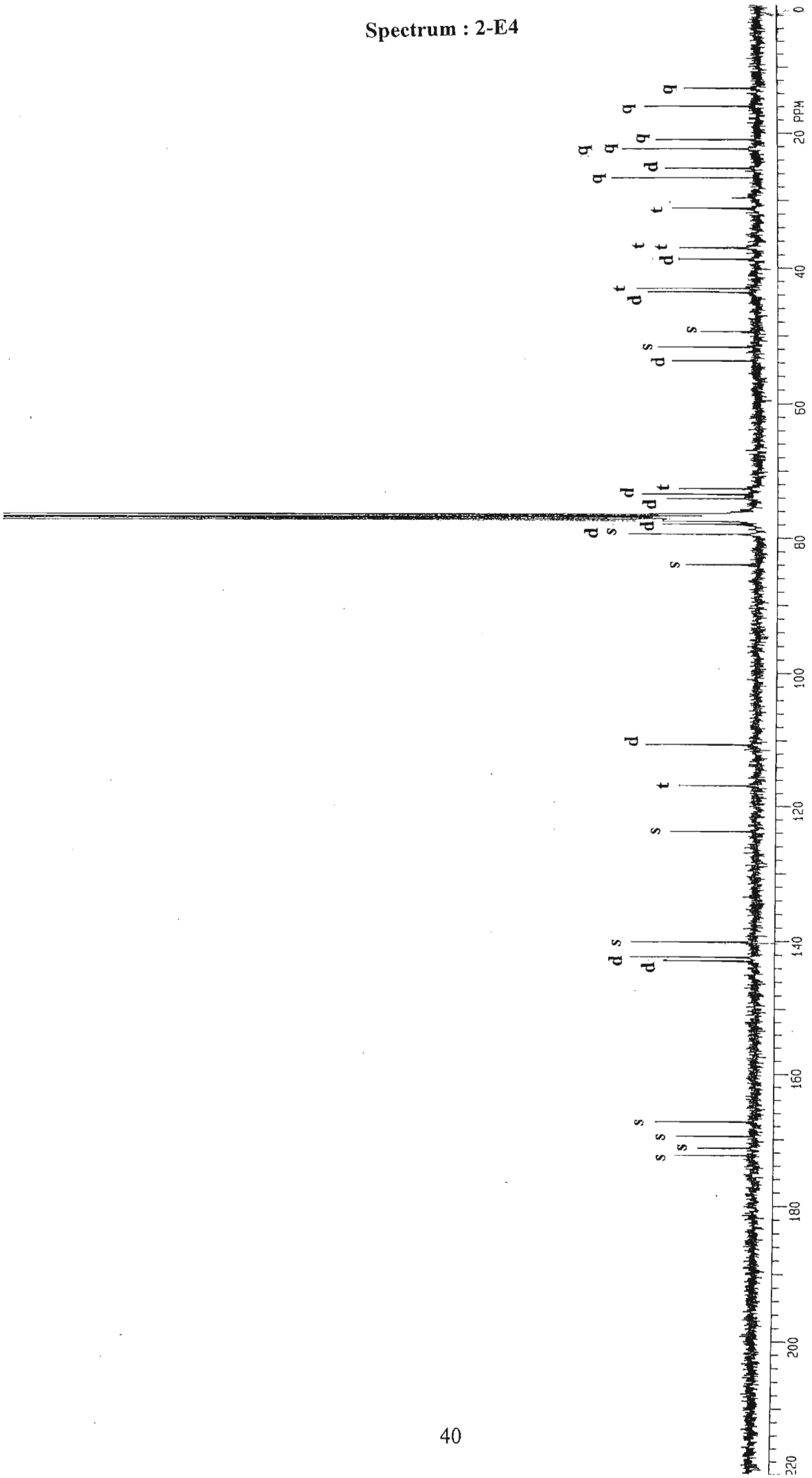
Spectrum : 2-E2



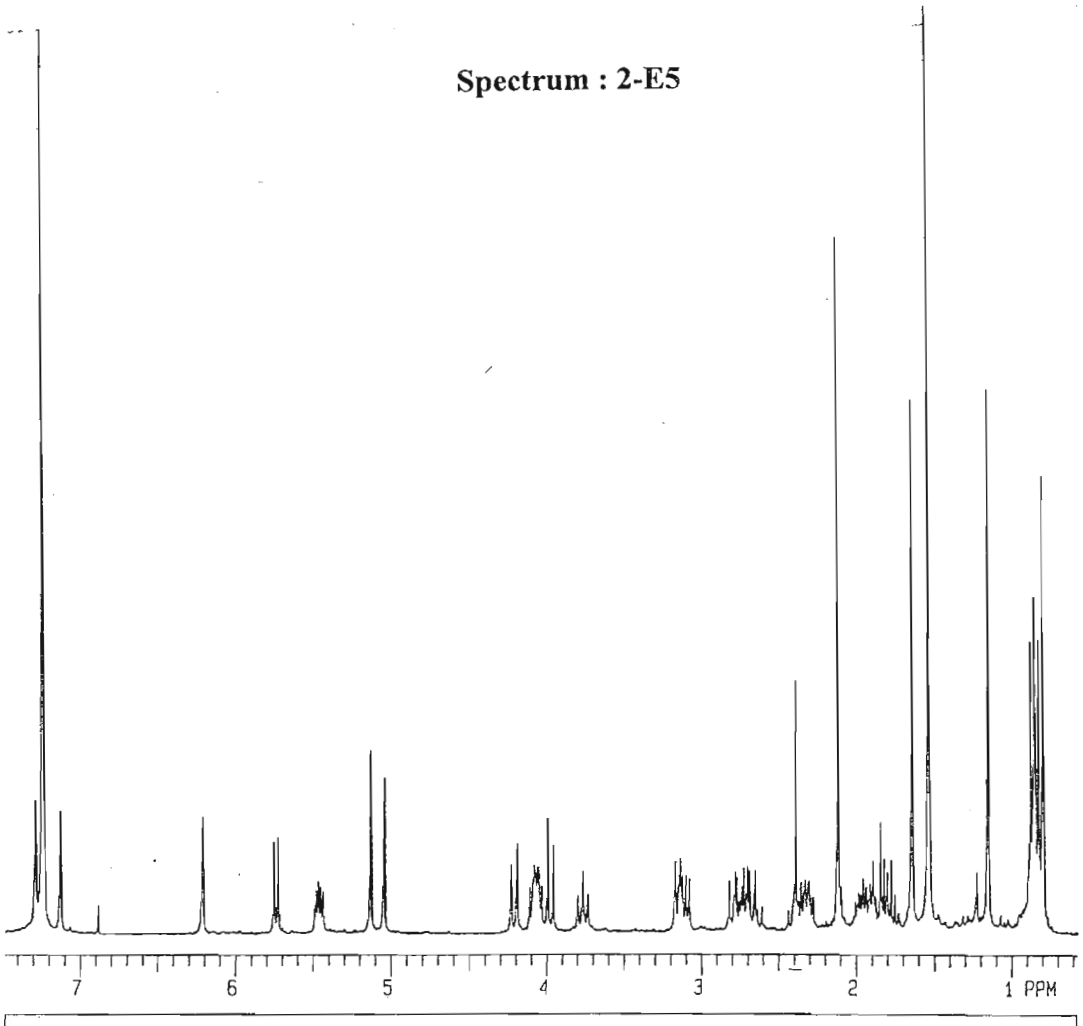
Spectrum : 2-E3

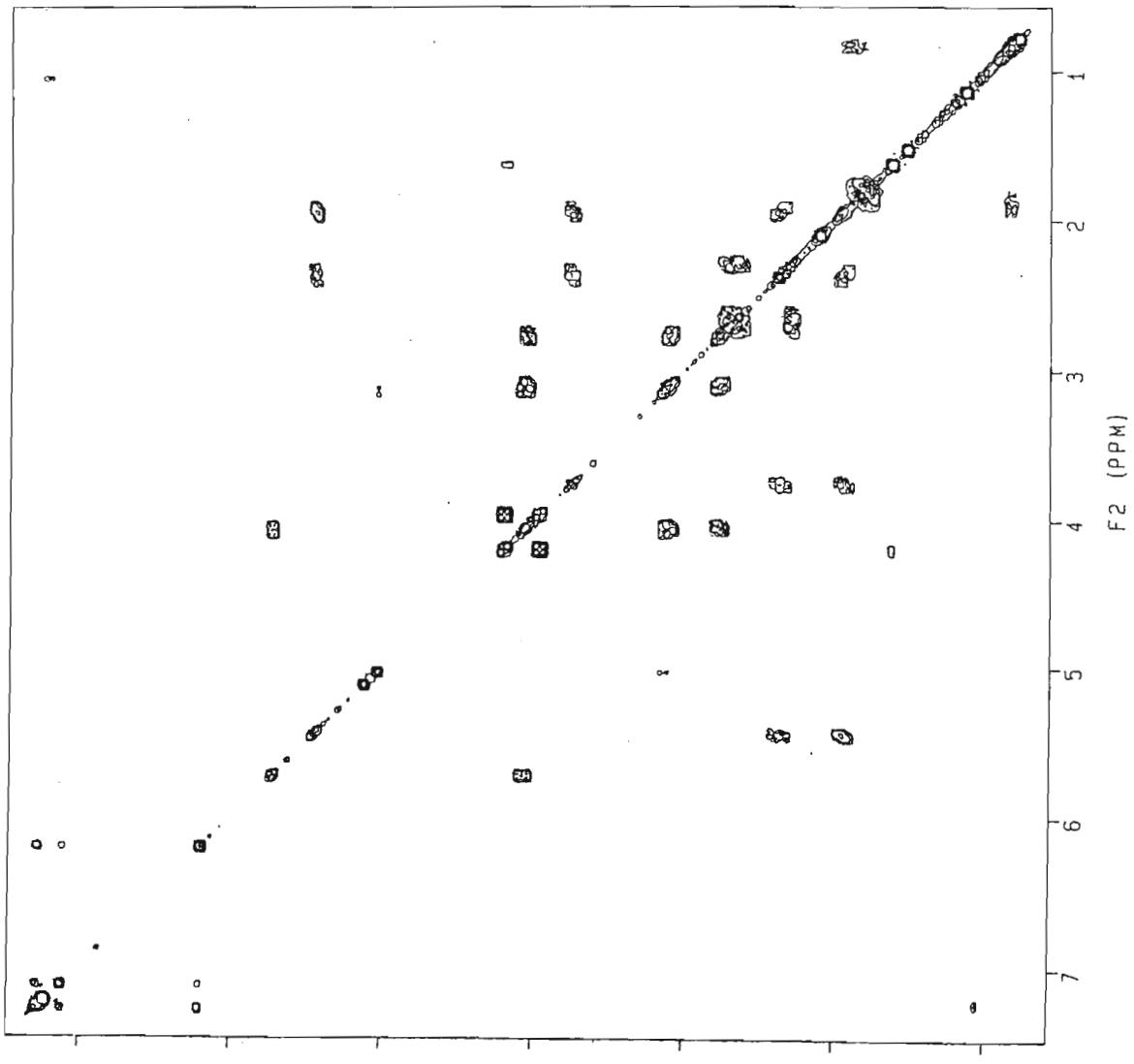


Spectrum : 2-E4

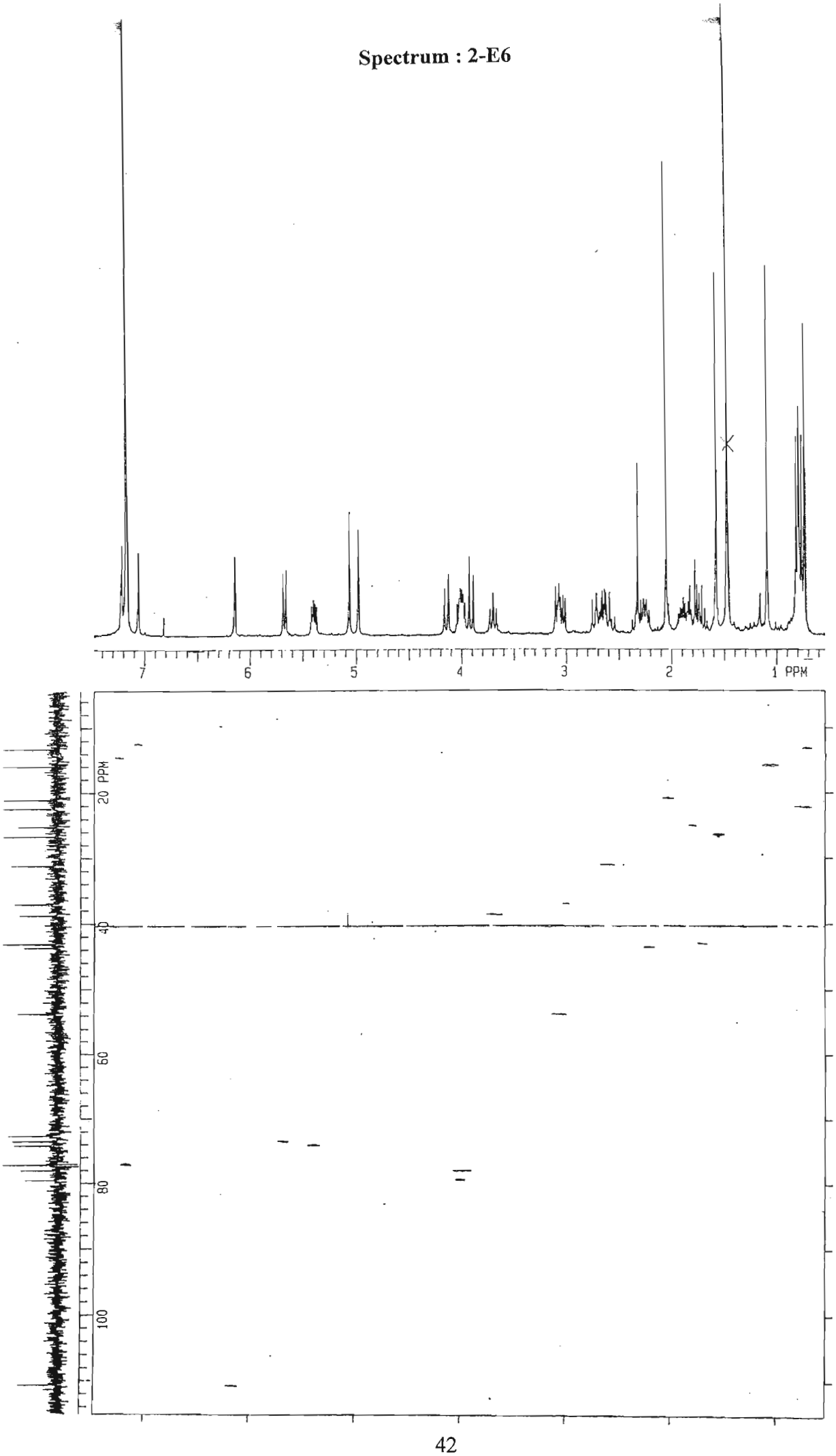


Spectrum : 2-E5



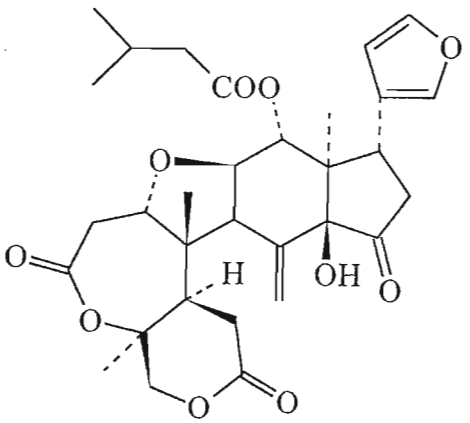


Spectrum : 2-E6

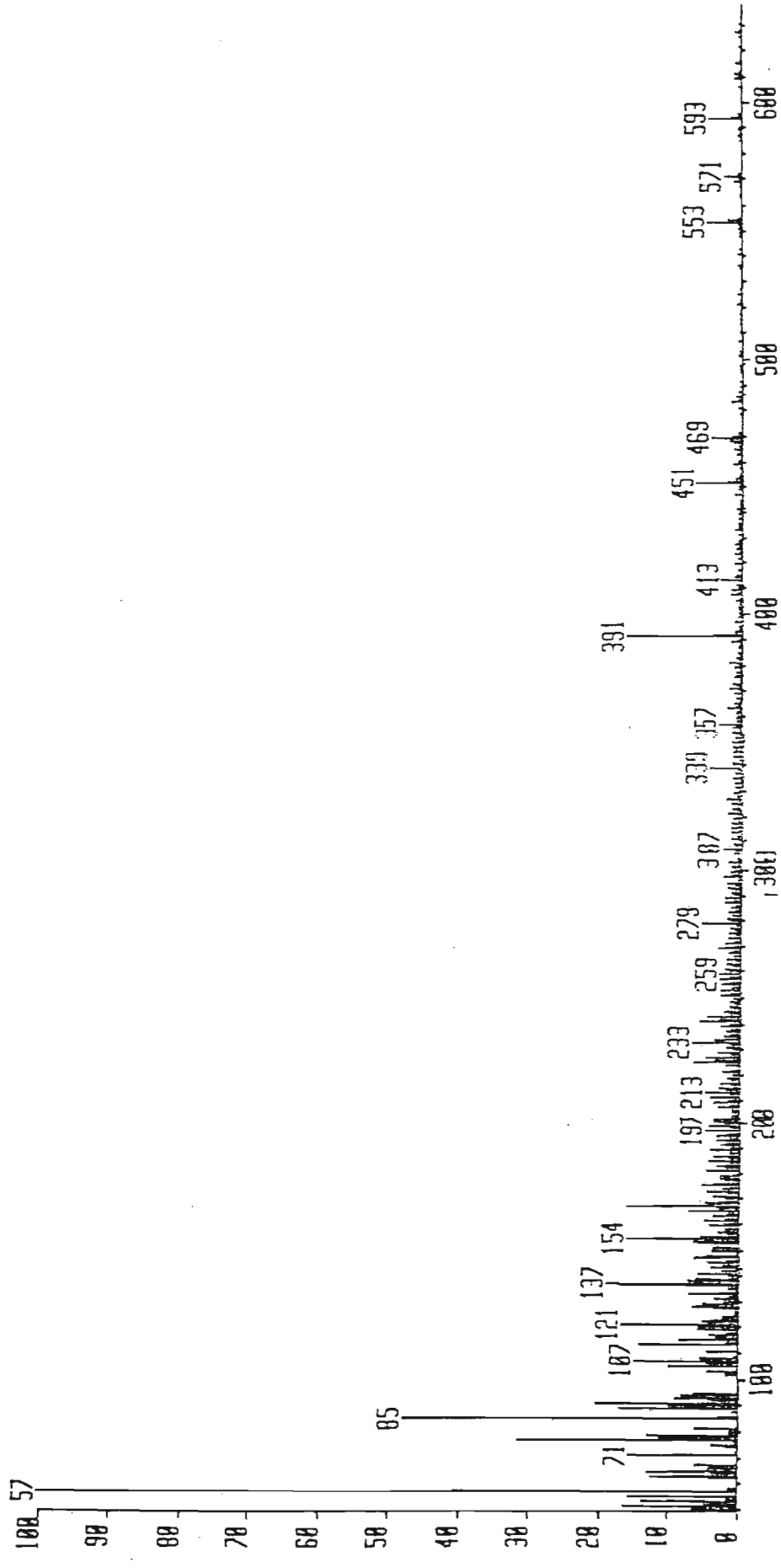


Extractives from *Aphanamixis polystacha*

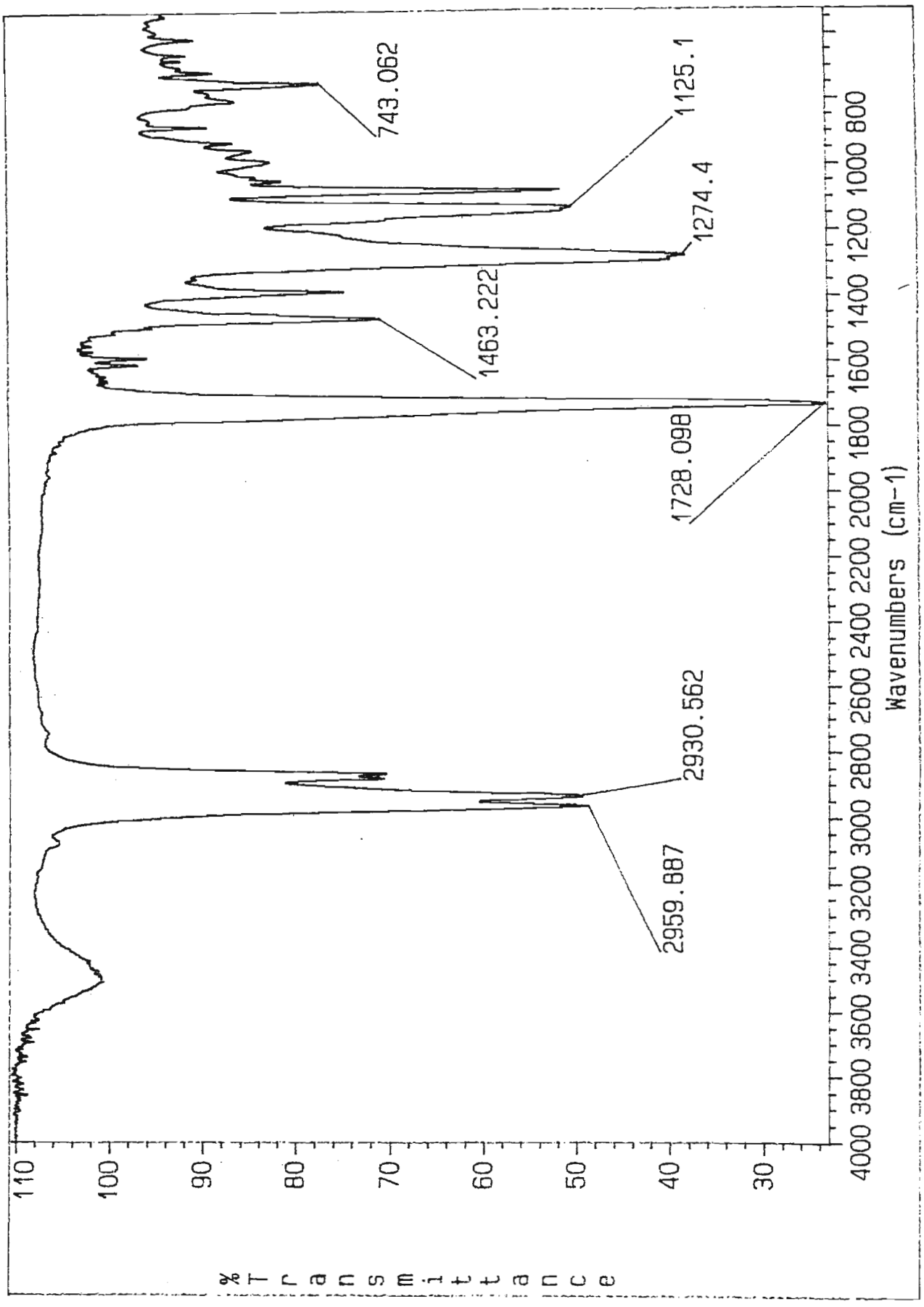
Compound 2-F



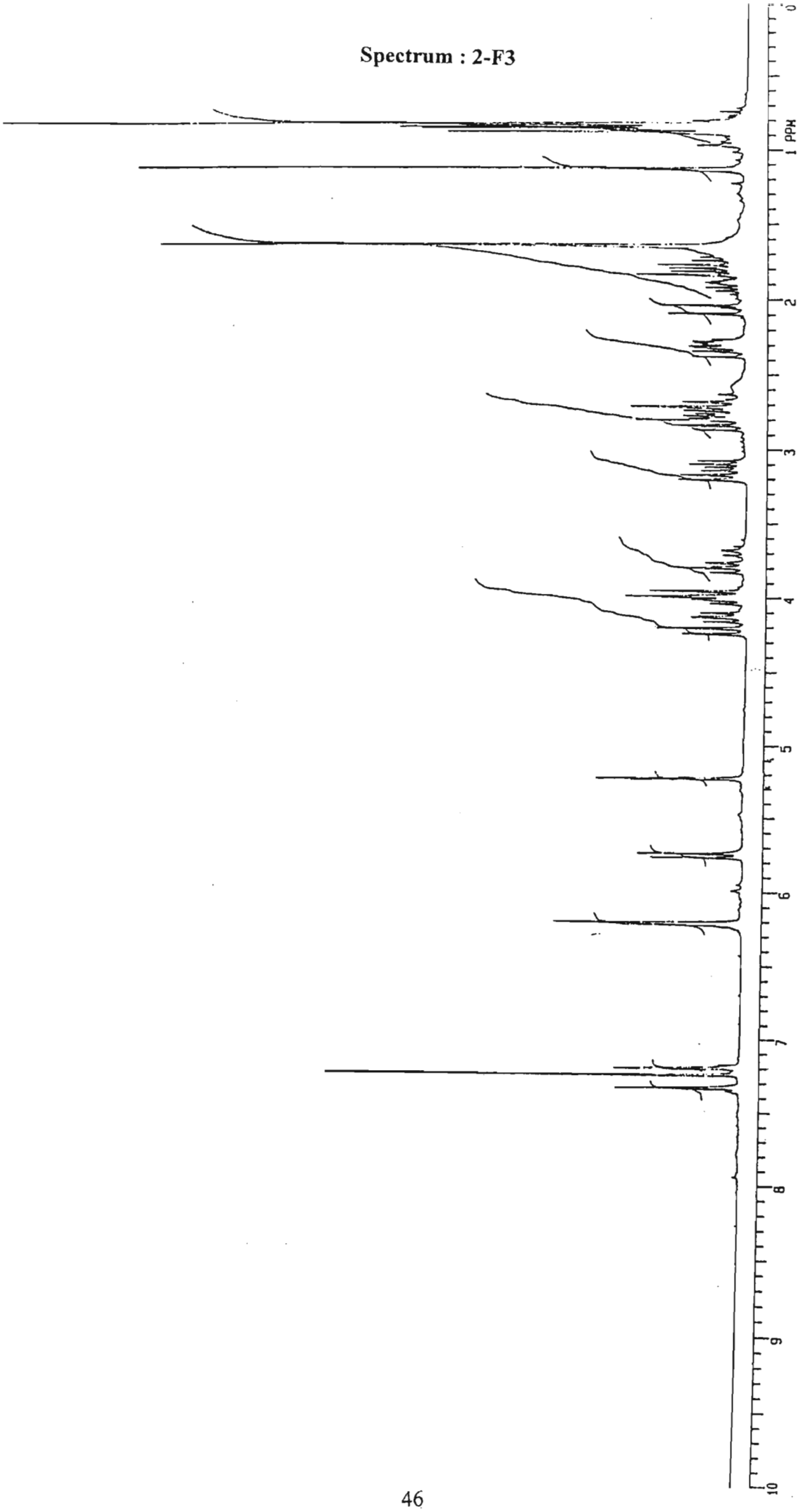
Spectrum : 2-F1



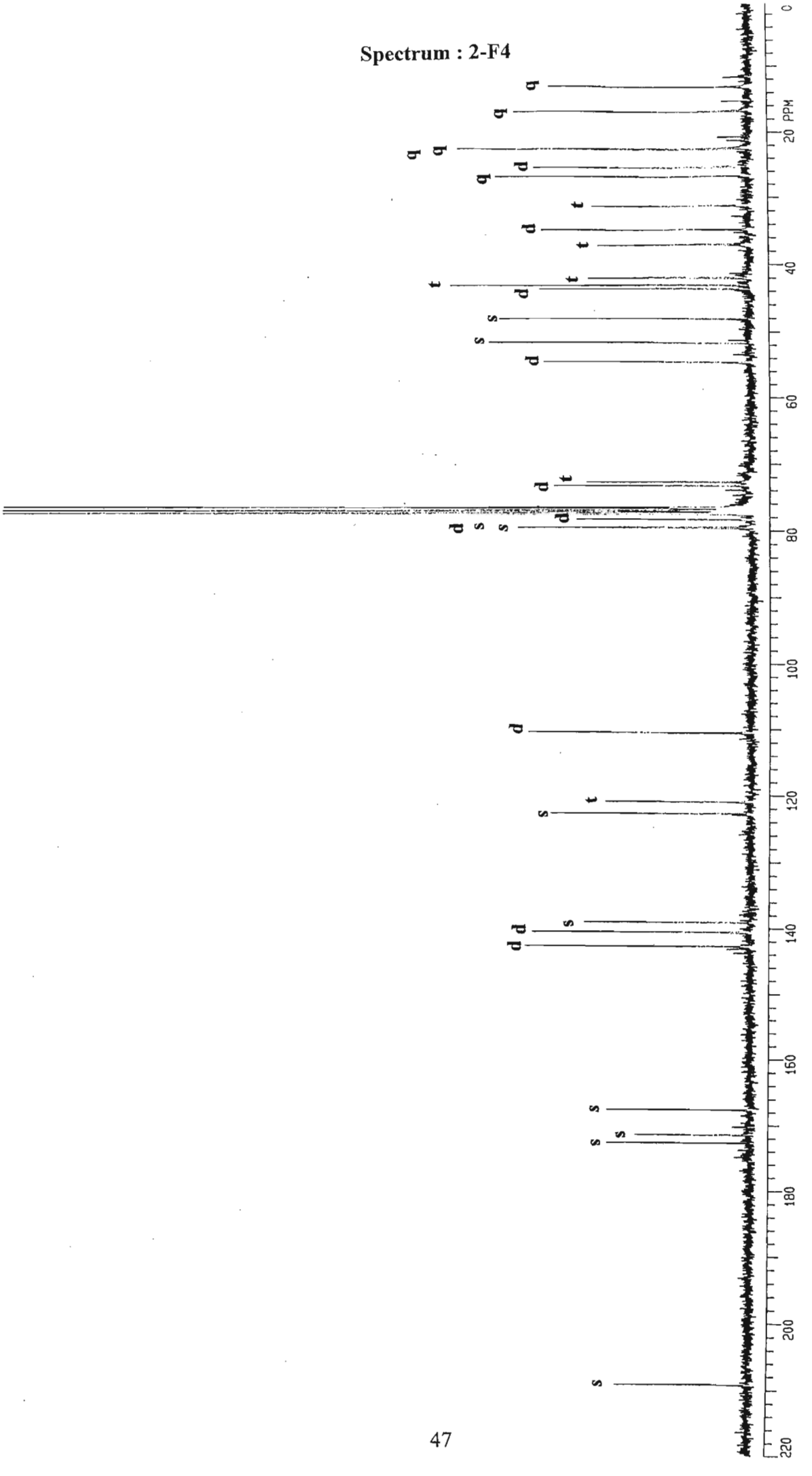
Spectrum : 2-F2



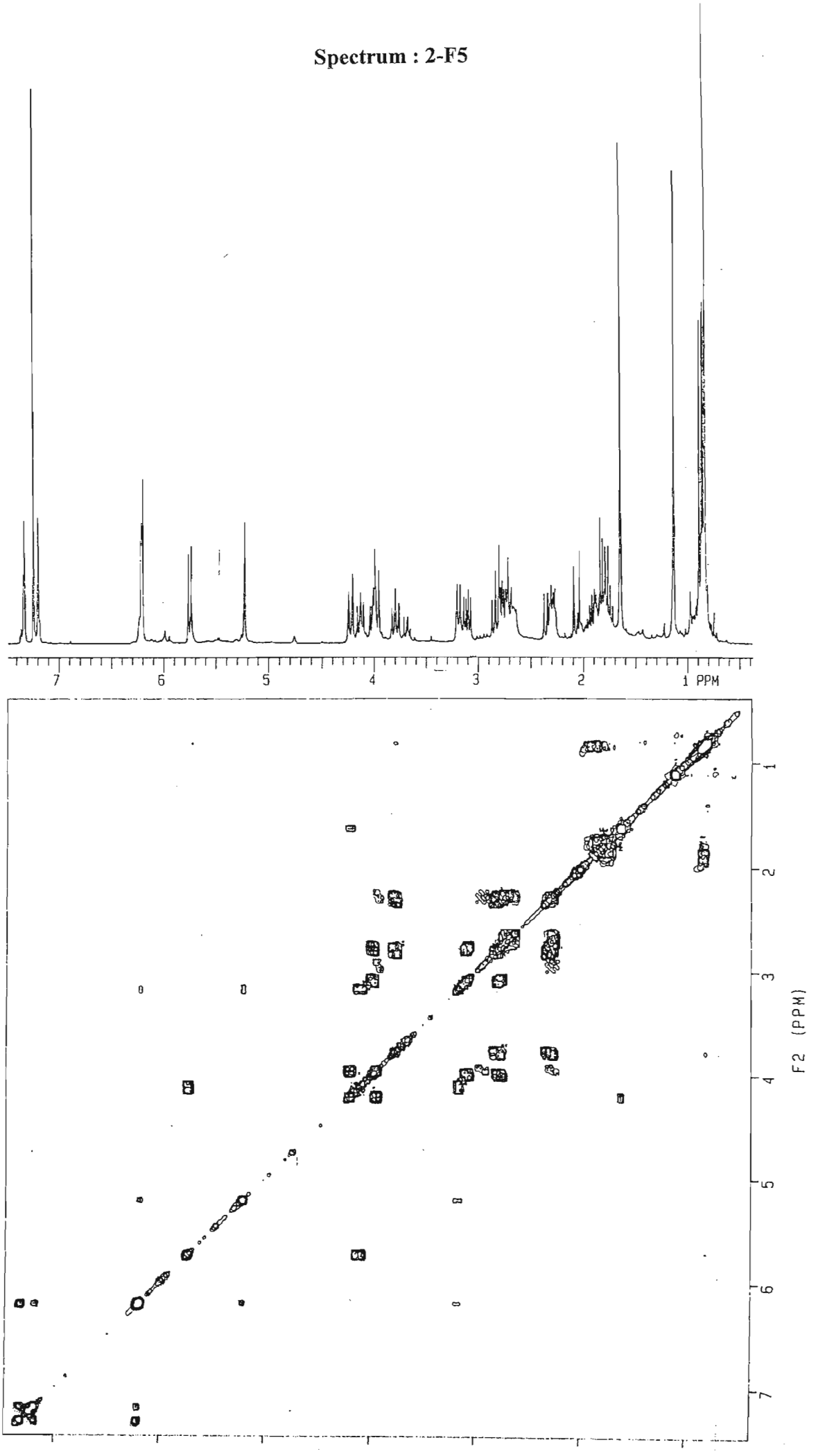
Spectrum : 2-F3



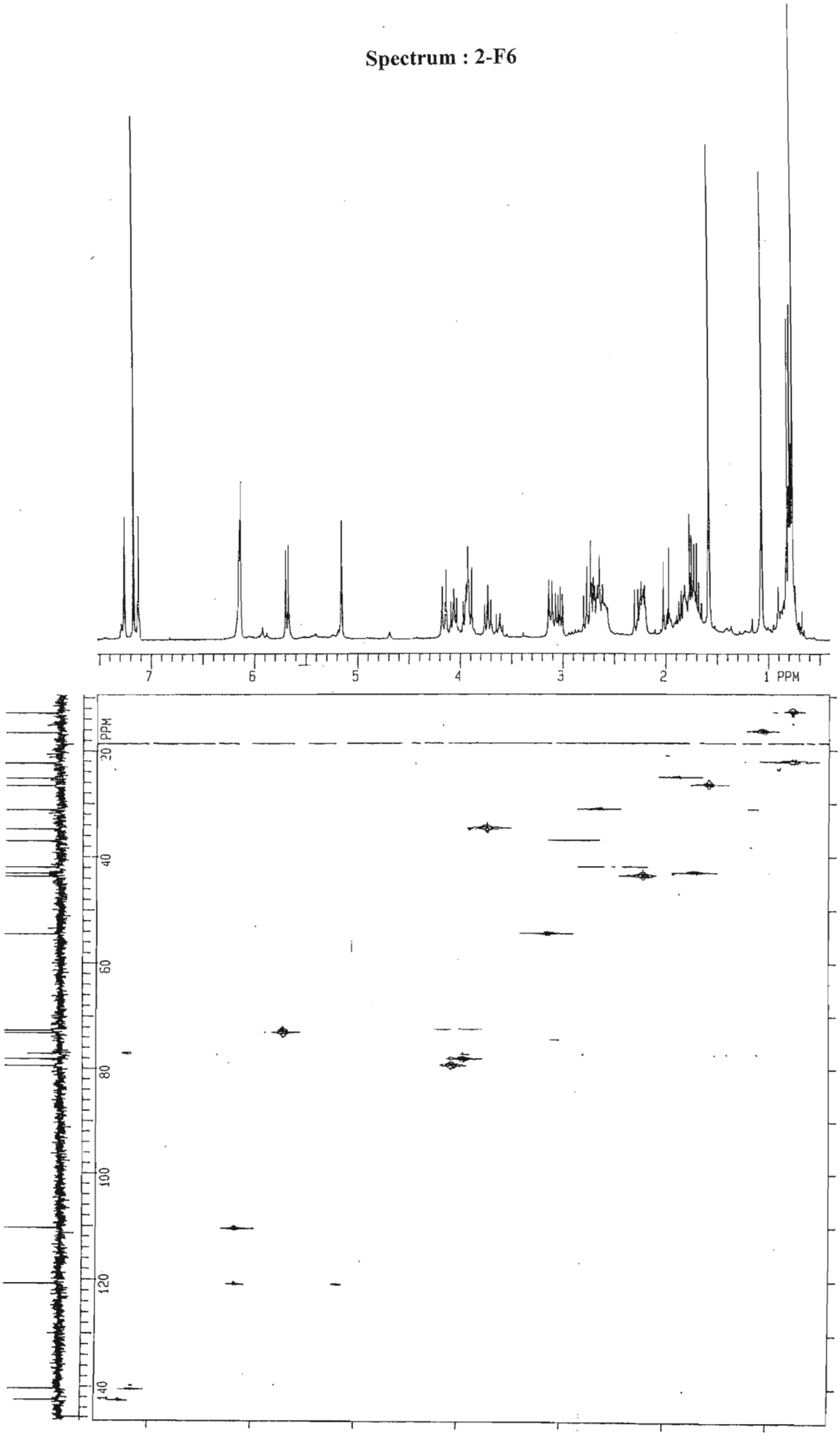
Spectrum : 2-F4



Spectrum : 2-F5

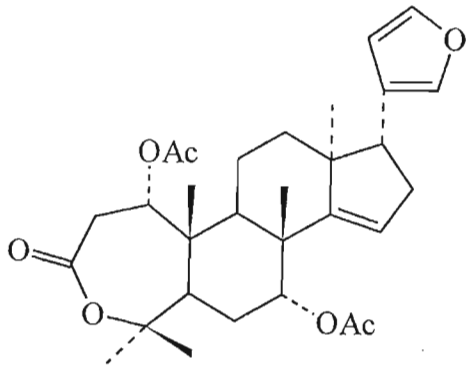


Spectrum : 2-F6

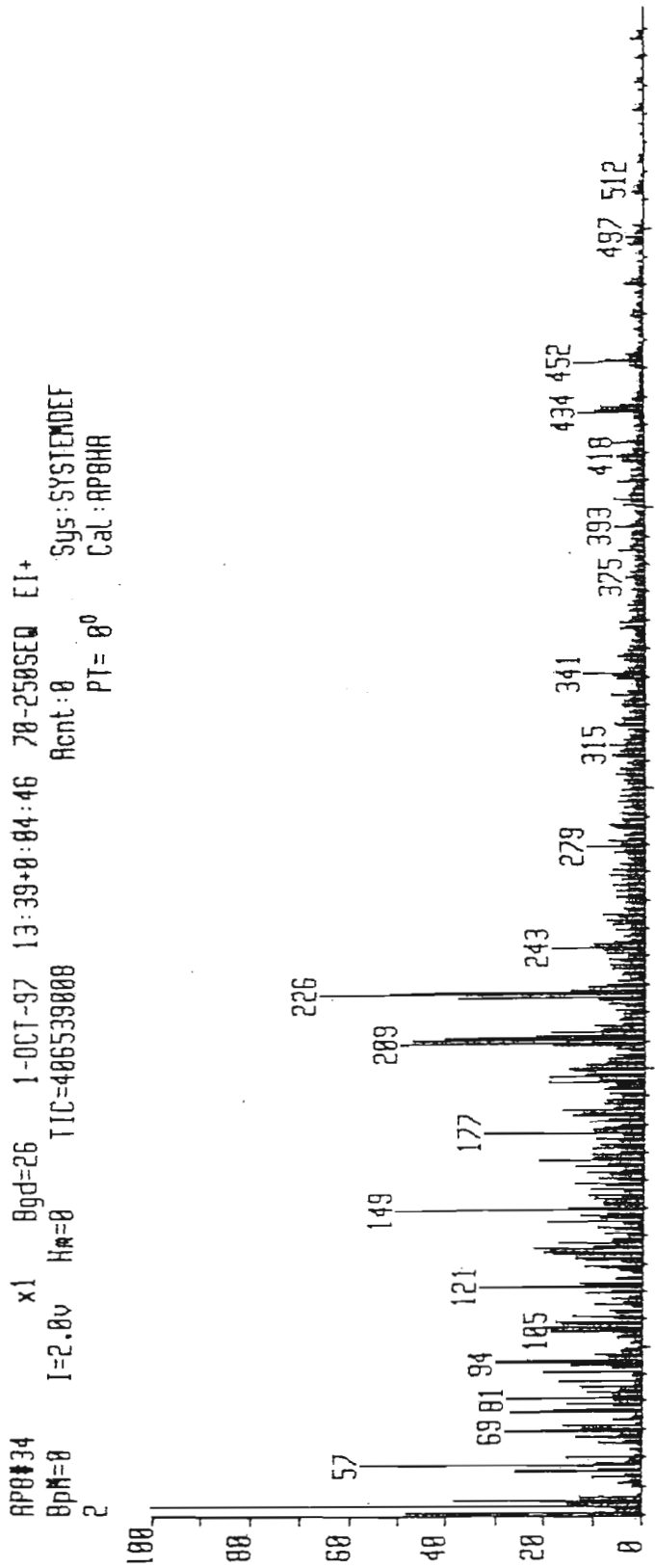


Extractives from *Aphanamixis polystacha*

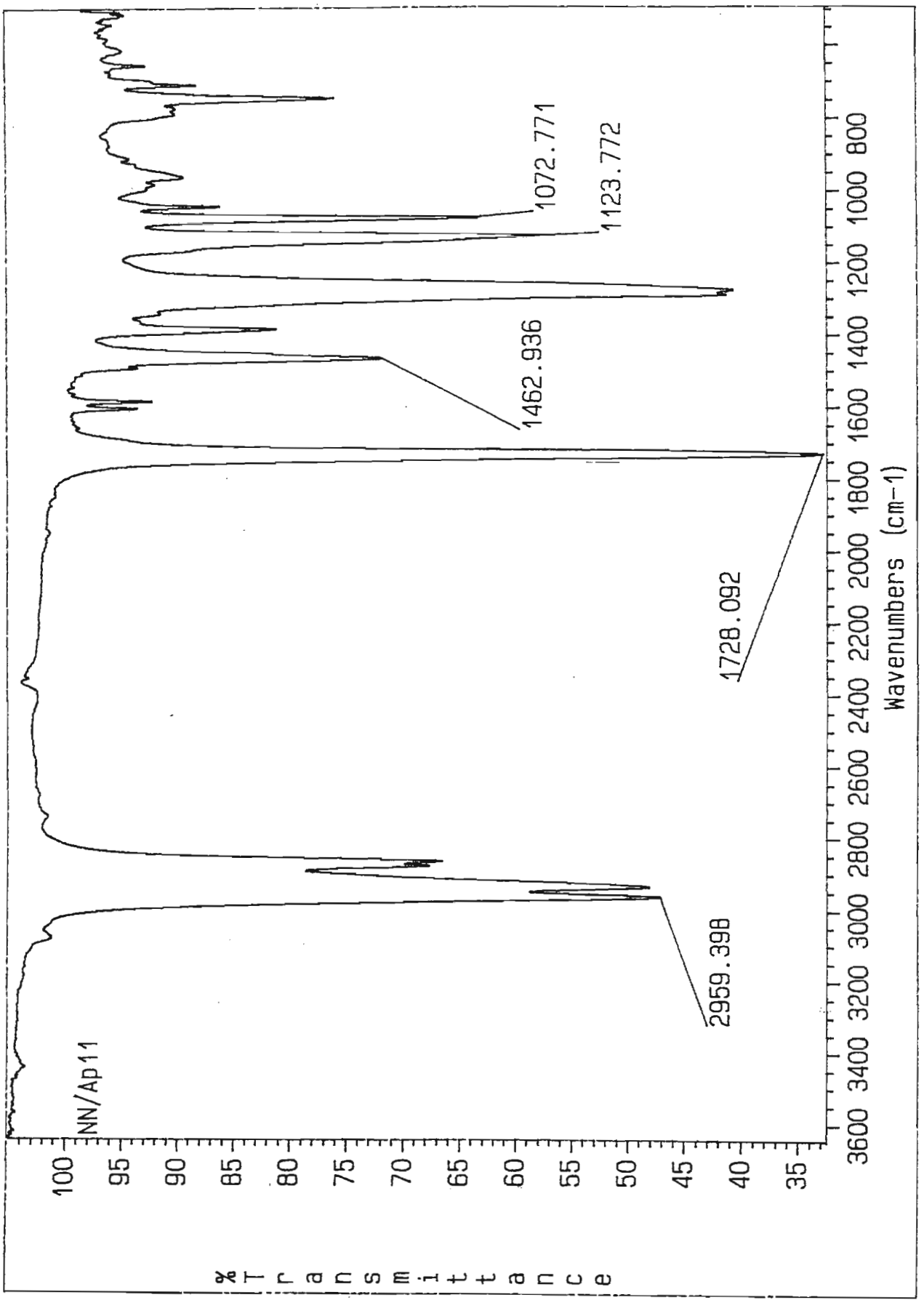
Compound 2-G



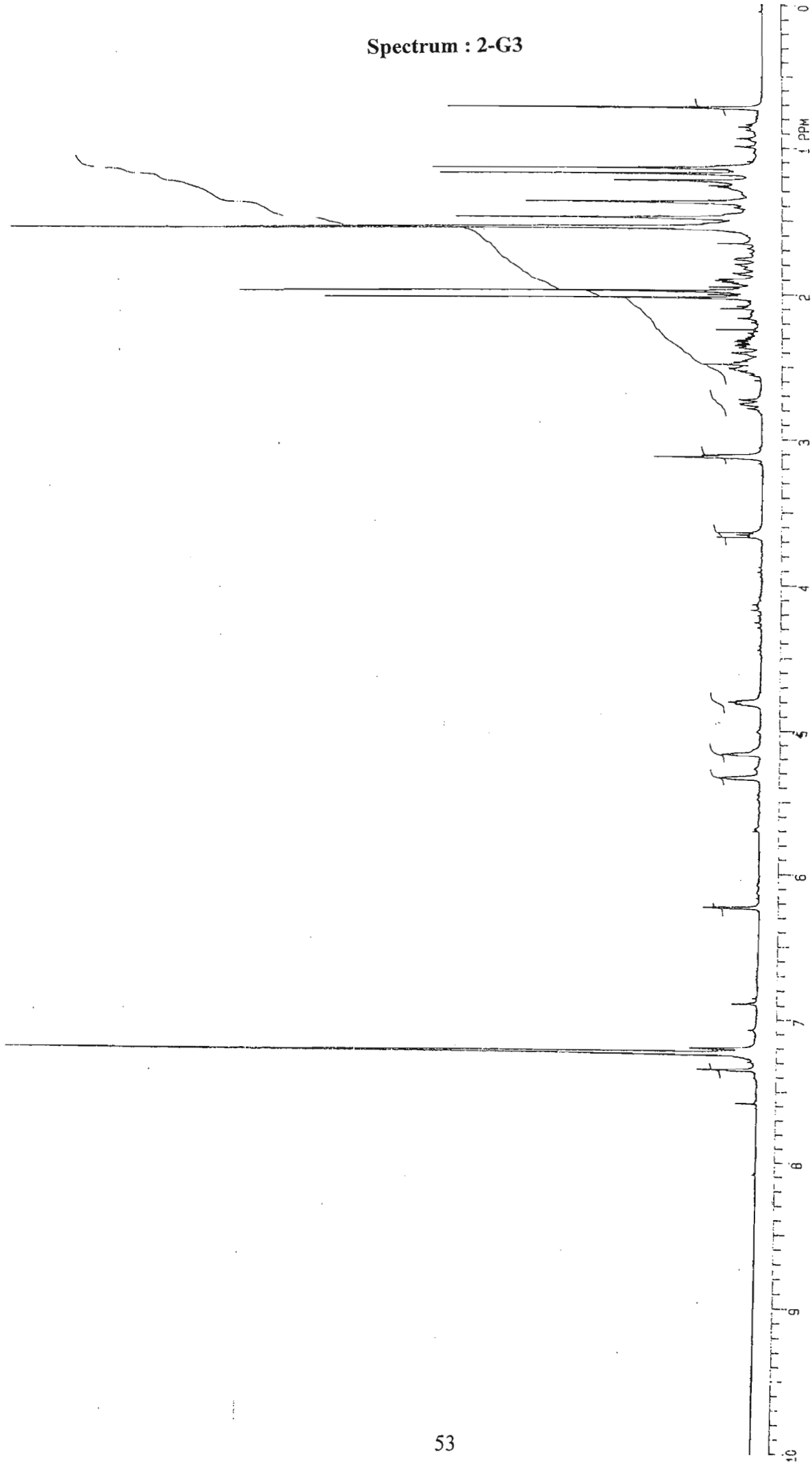
Spectrum : 2-G1



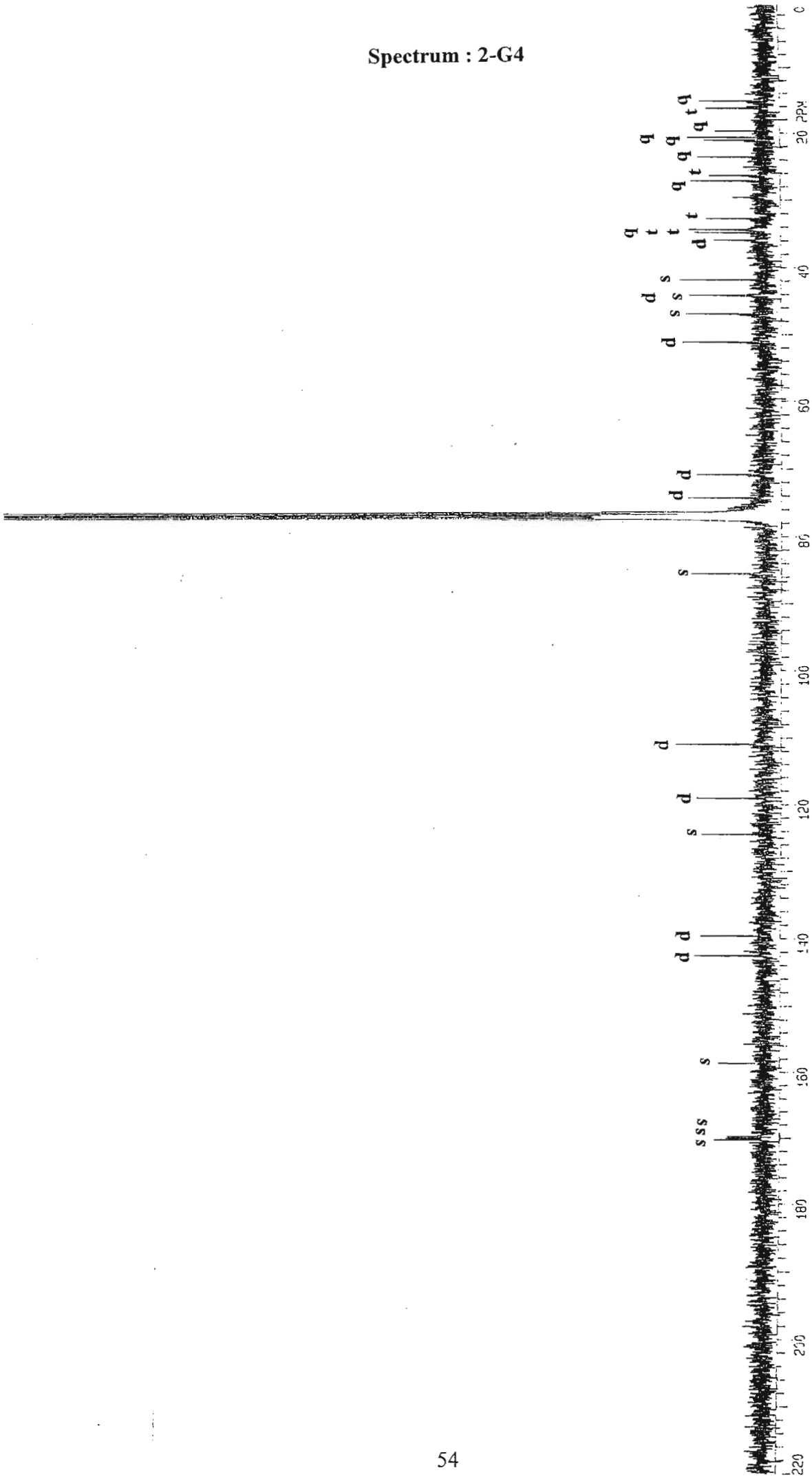
Spectrum : 2-G2



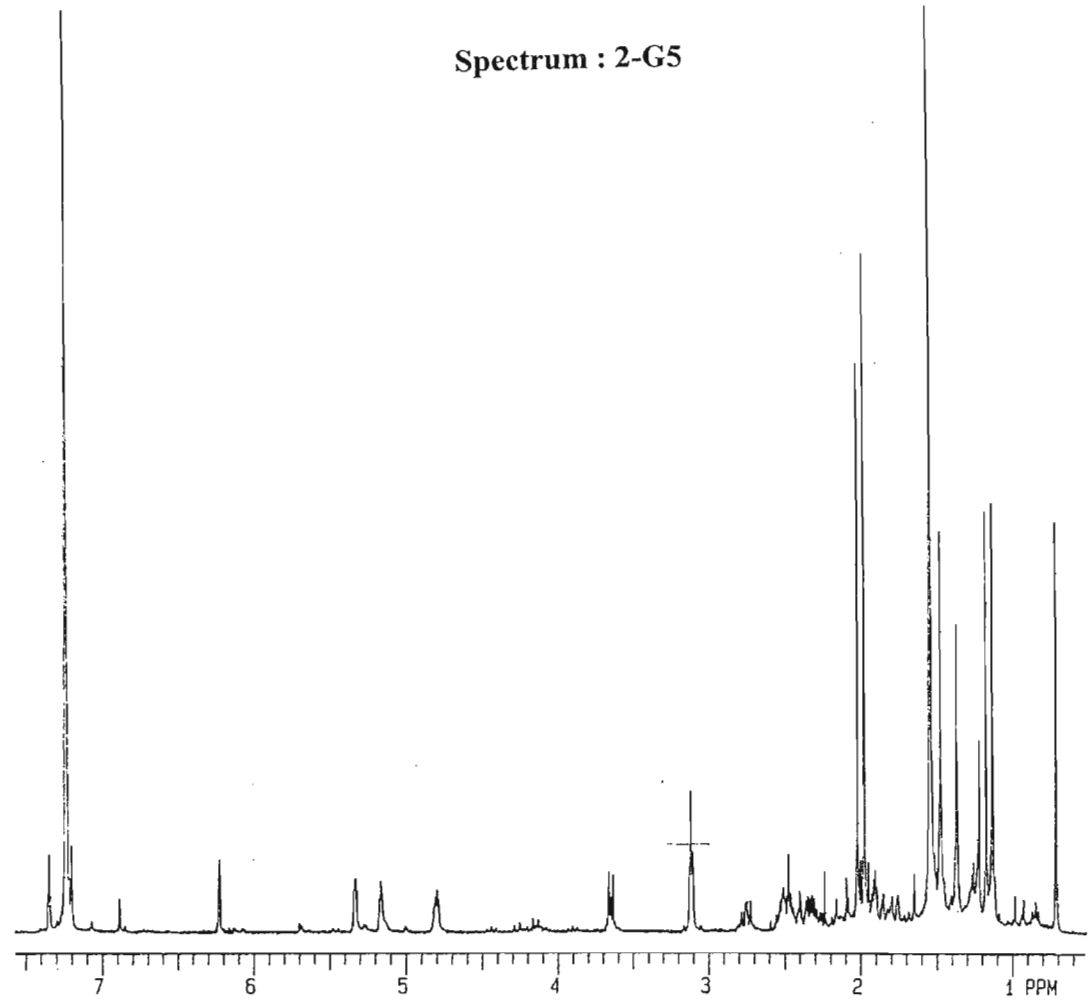
Spectrum : 2-G3

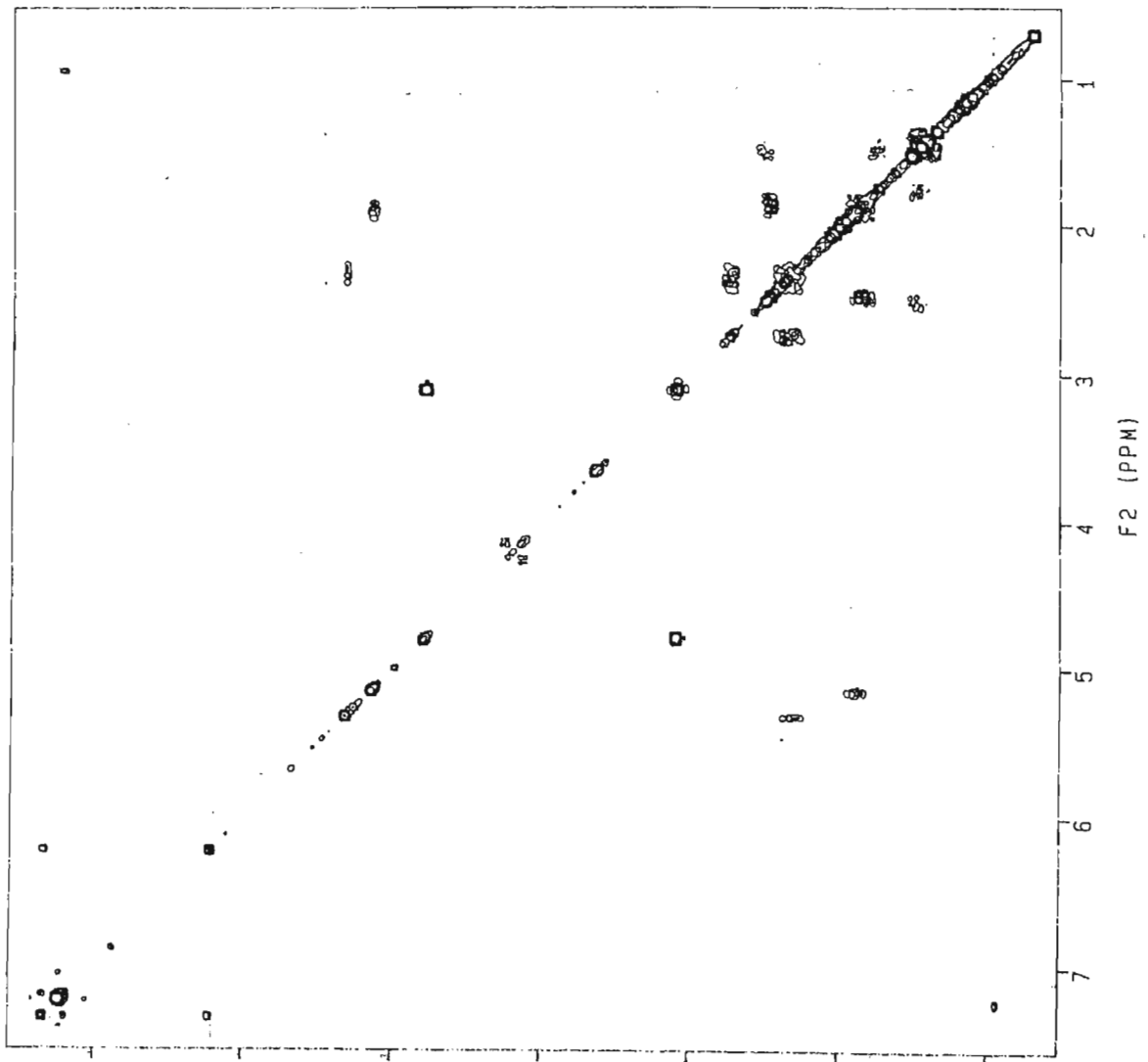


Spectrum : 2-G4

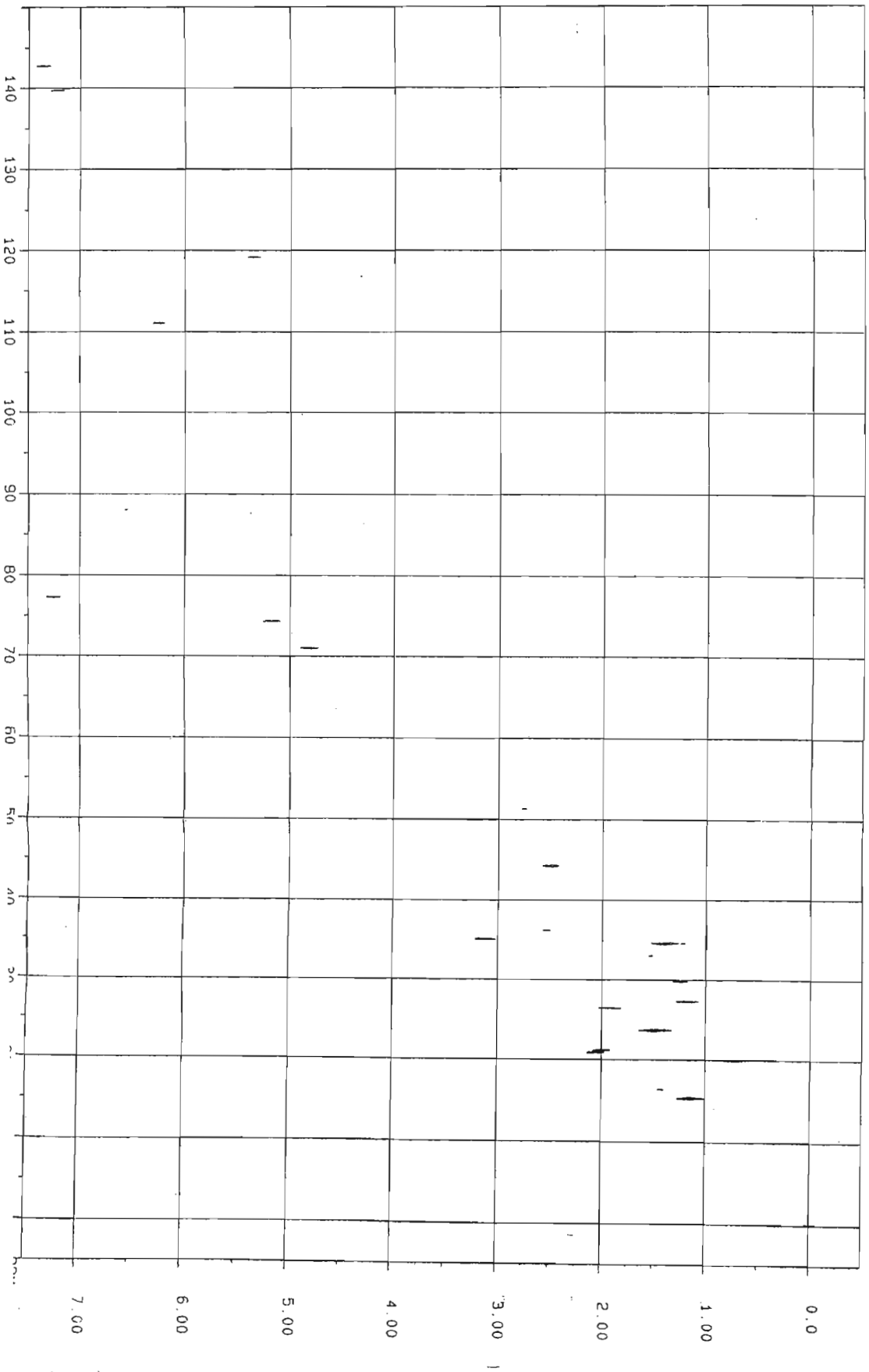


Spectrum : 2-G5



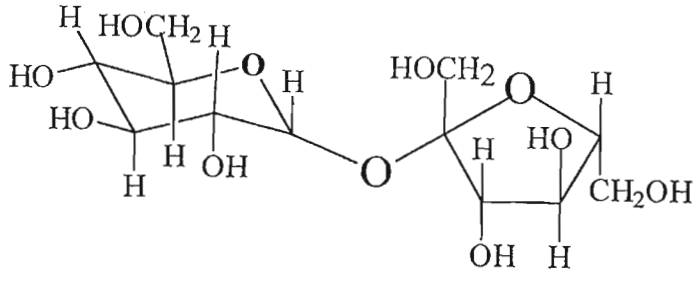


Spectrum : 2-G6

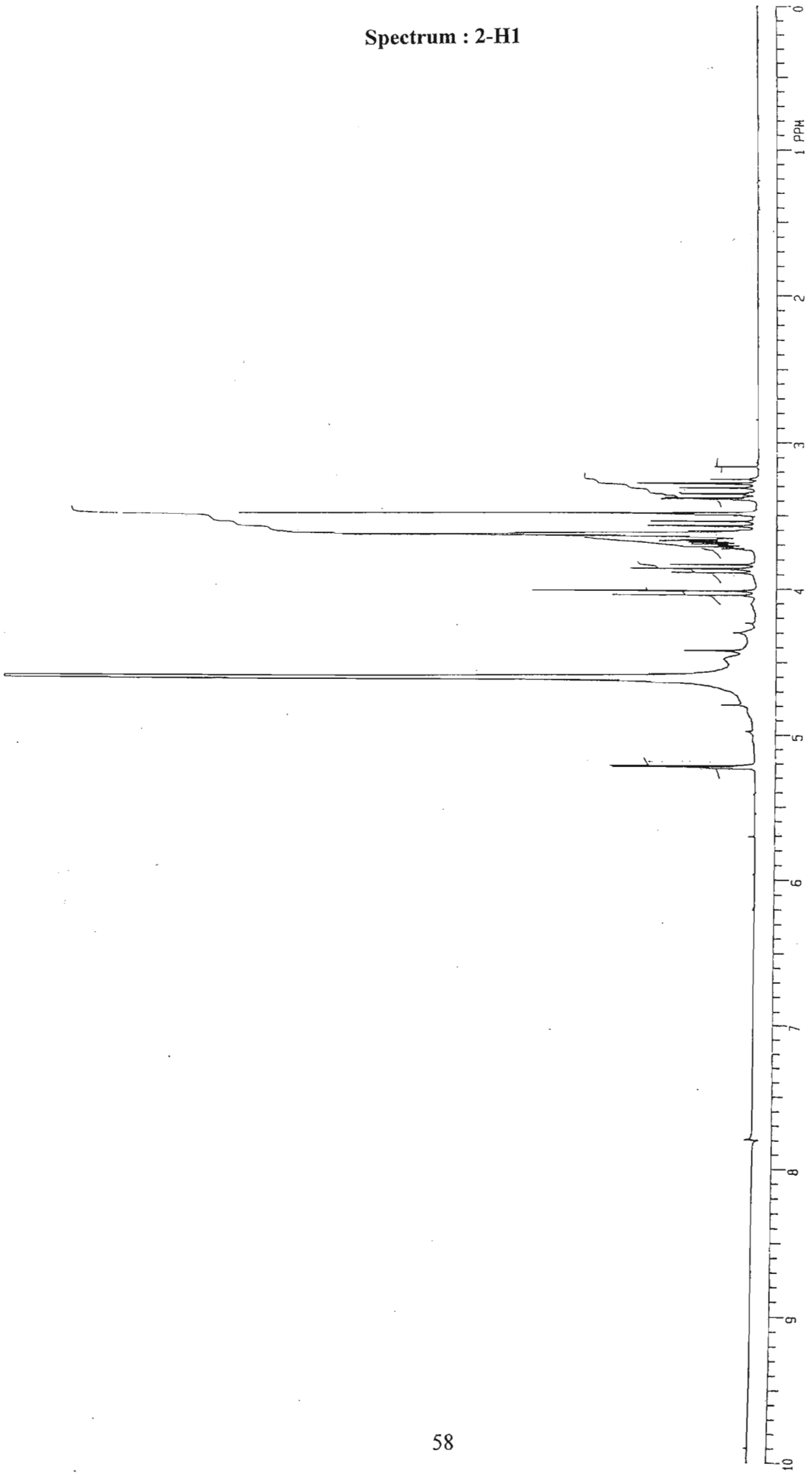


Extractives from *Aphanamixis polystacha*

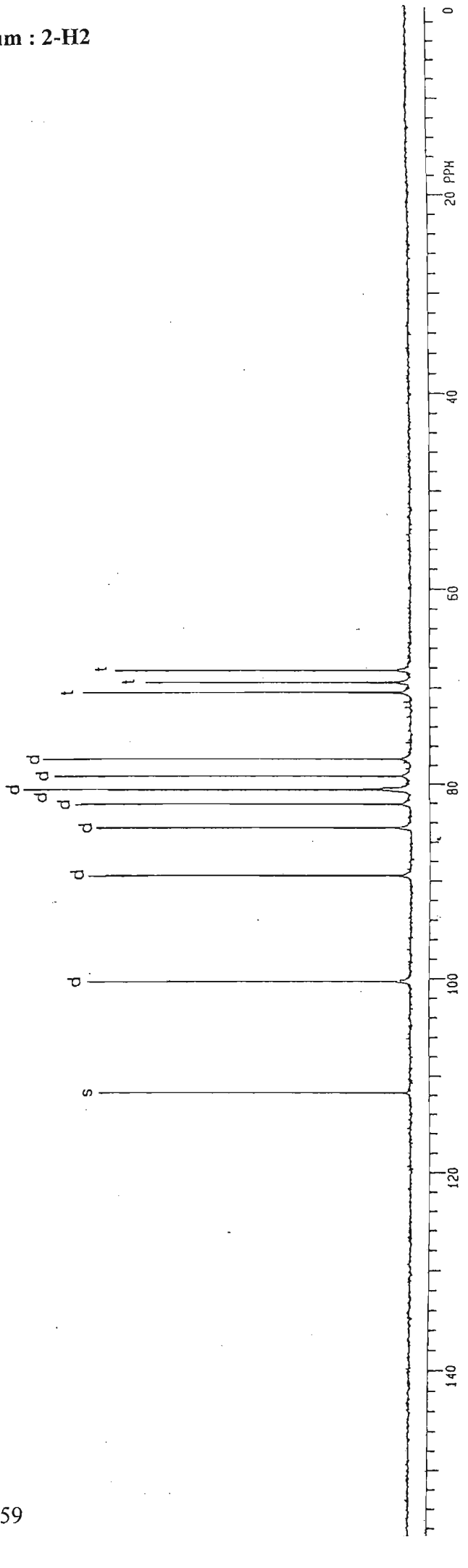
Compound 2-H



Spectrum : 2-H1

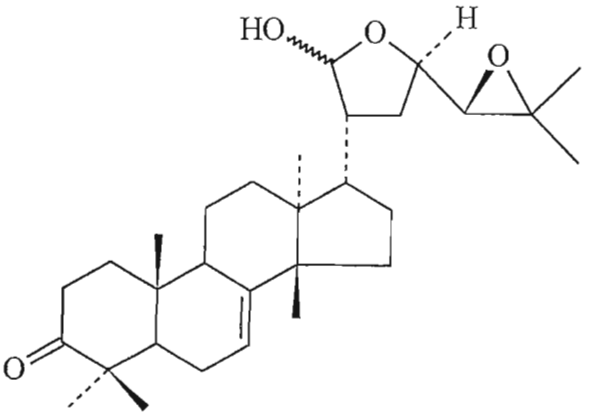


Spectrum : 2-H2

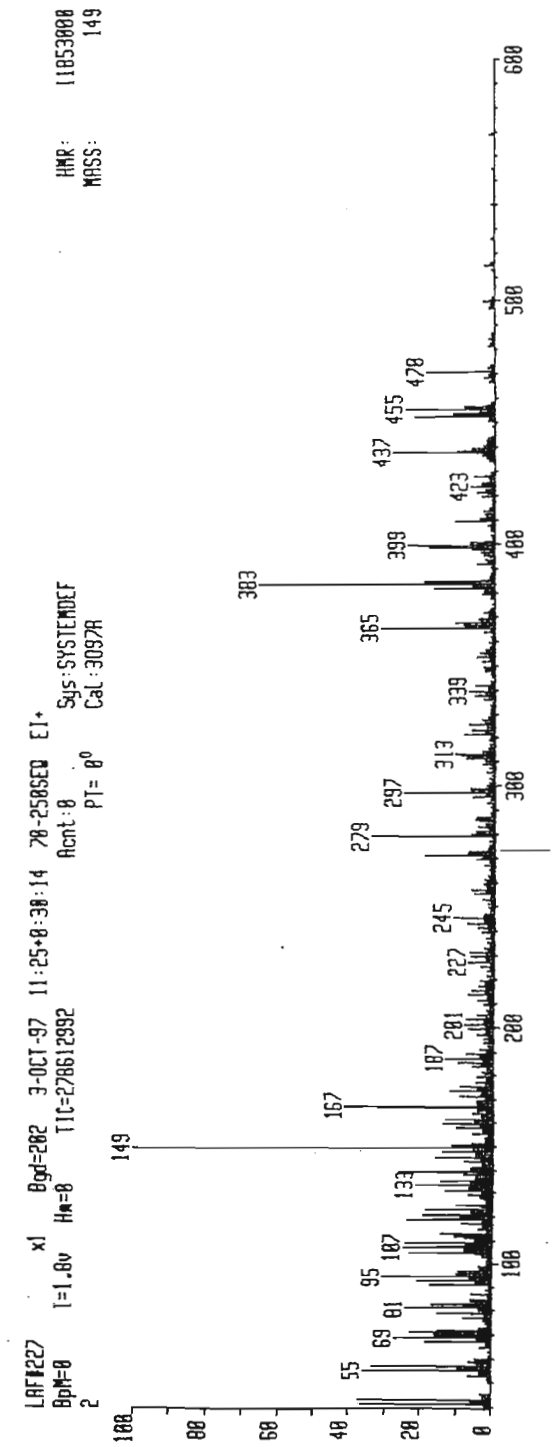


Extractives from *Turraea obtusifolia*

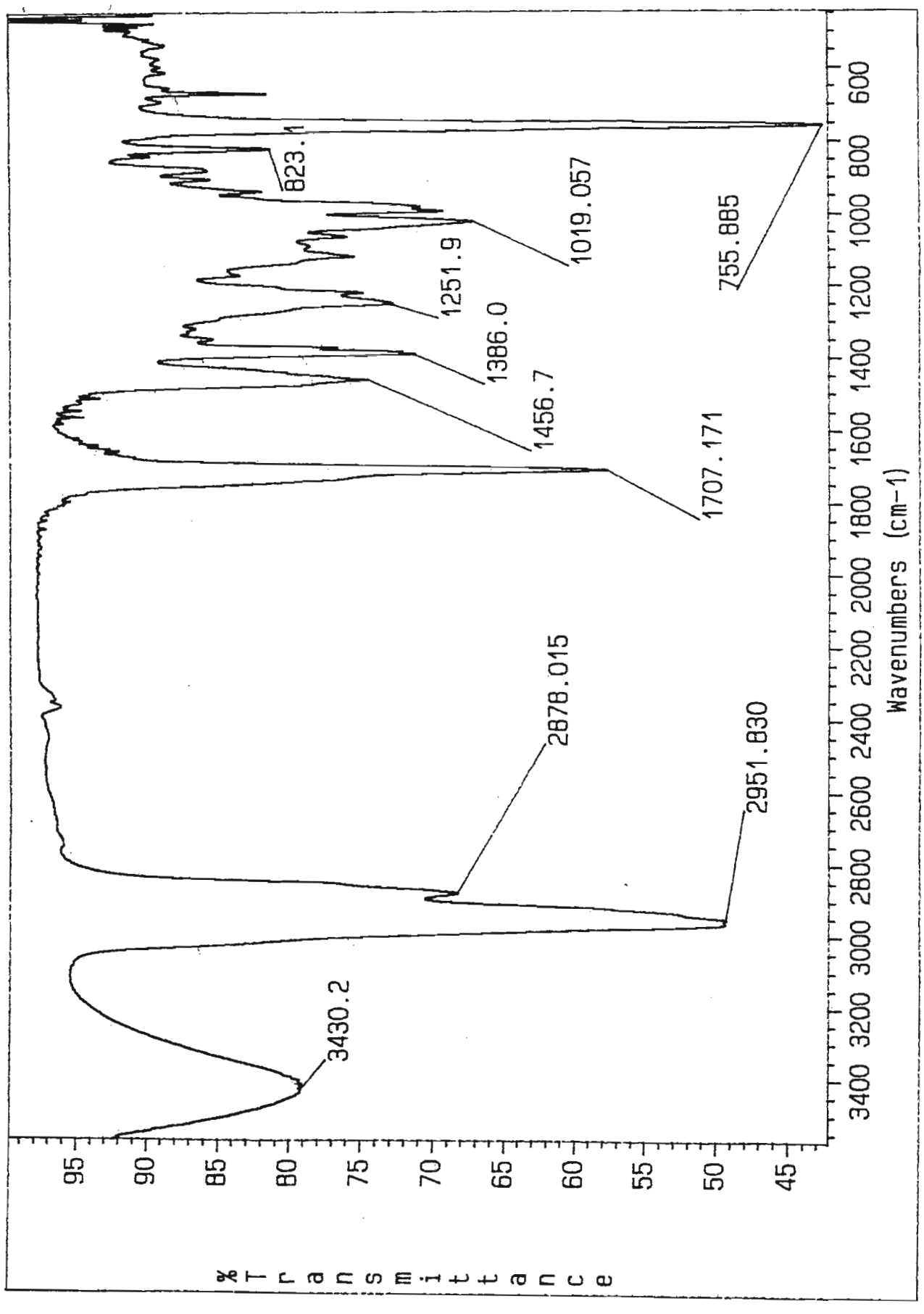
Compound 3-A



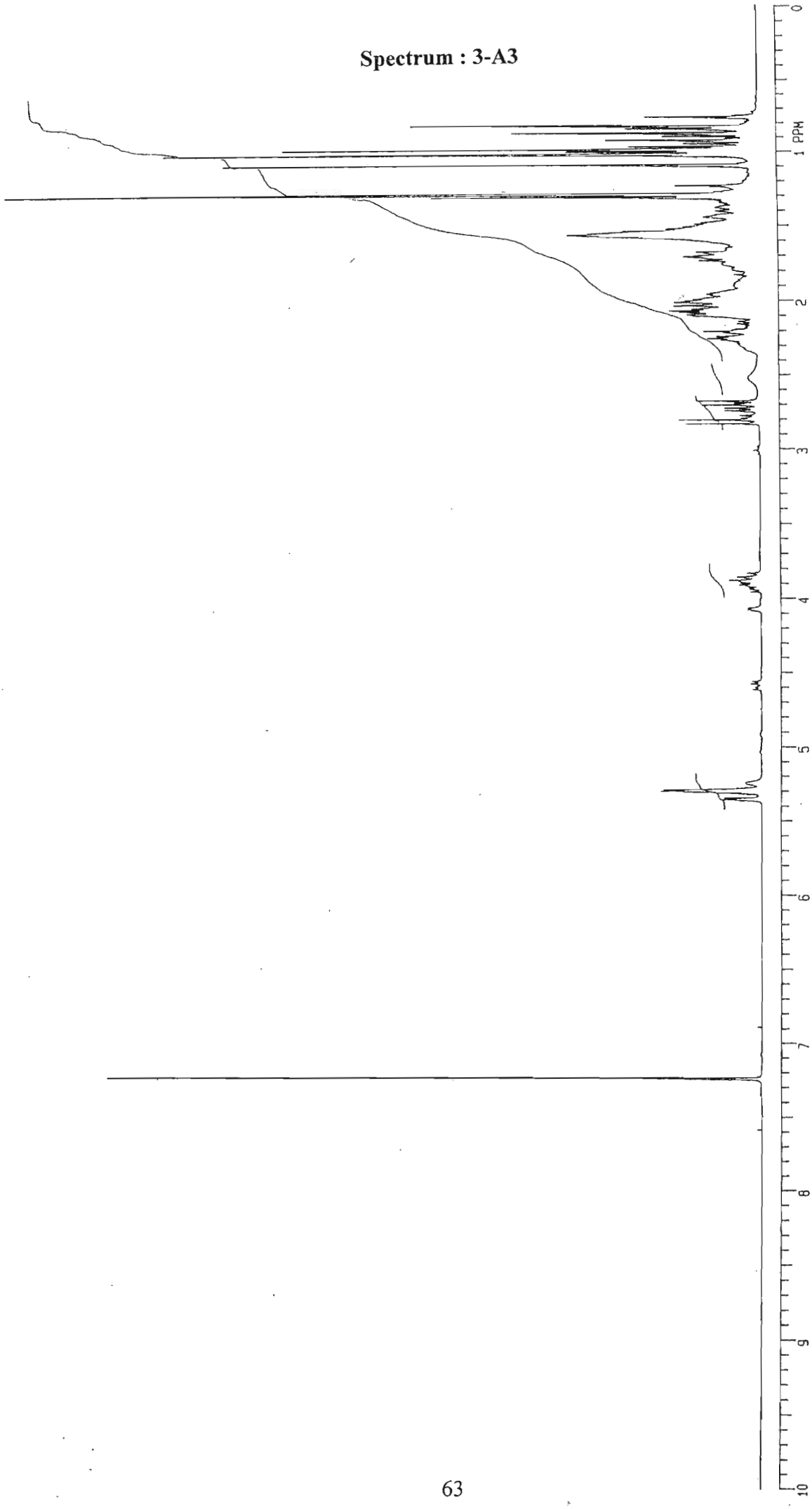
Spectrum : 3-A1



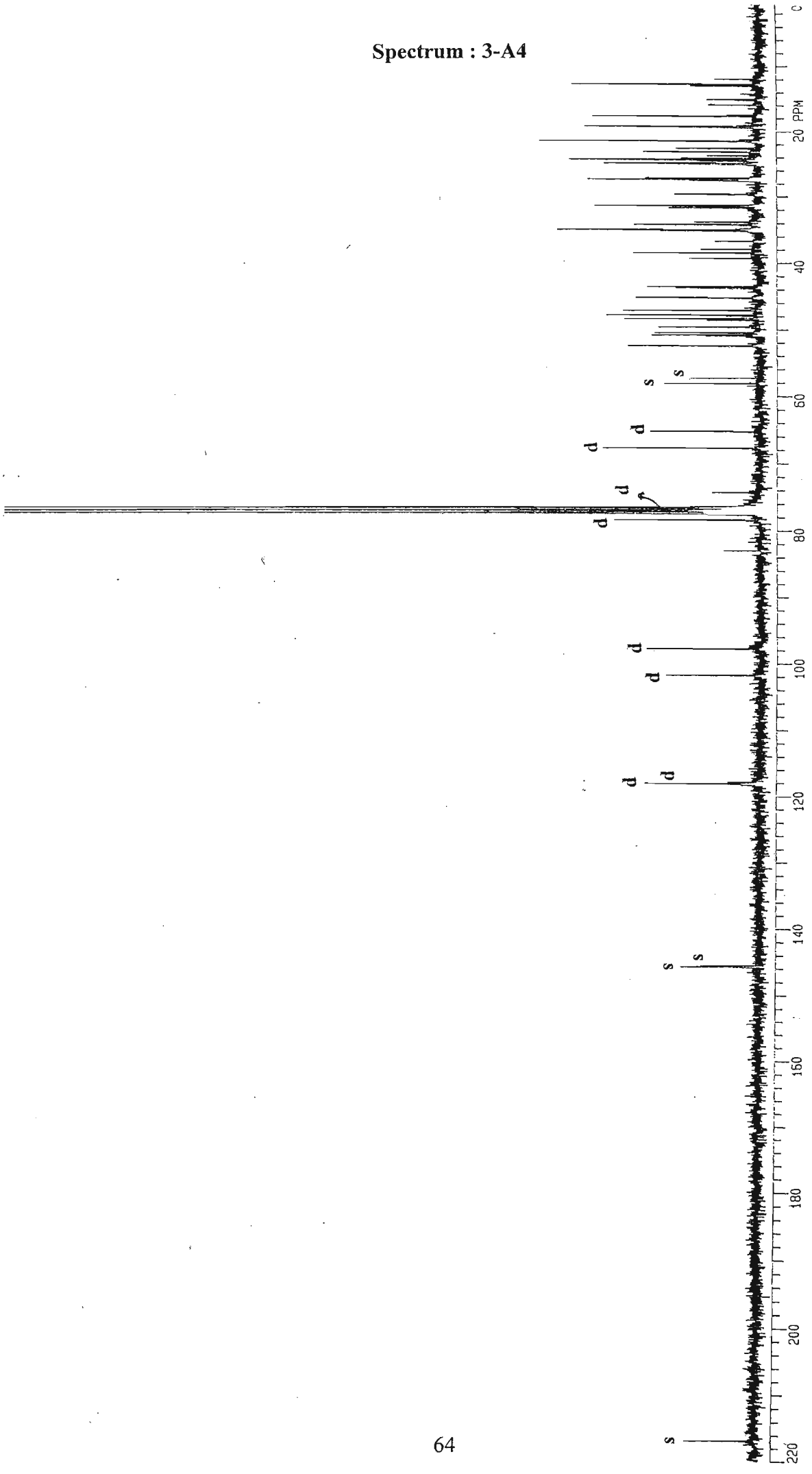
Spectrum : 3-A2



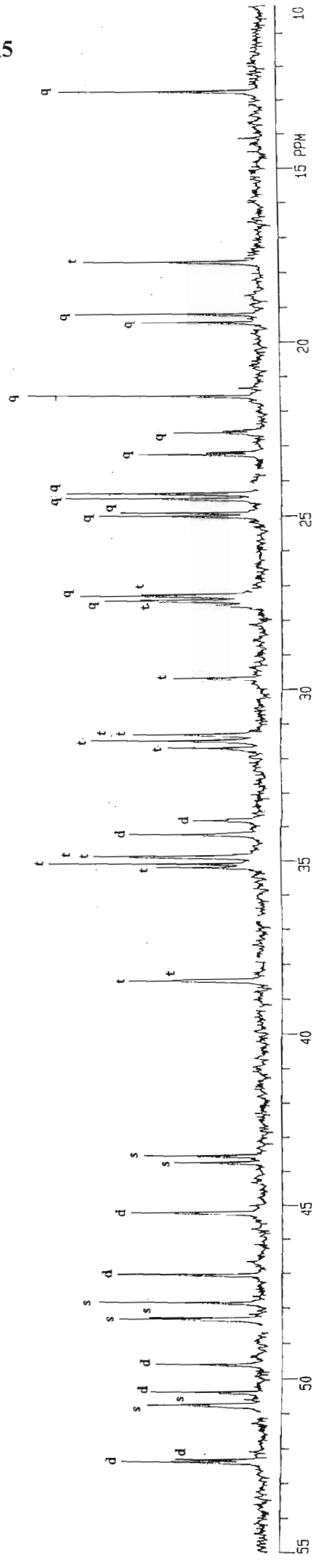
Spectrum : 3-A3



Spectrum : 3-A4

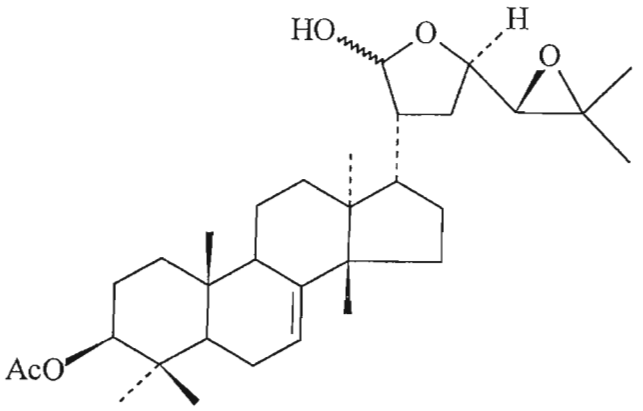


Spectrum : 3-A5

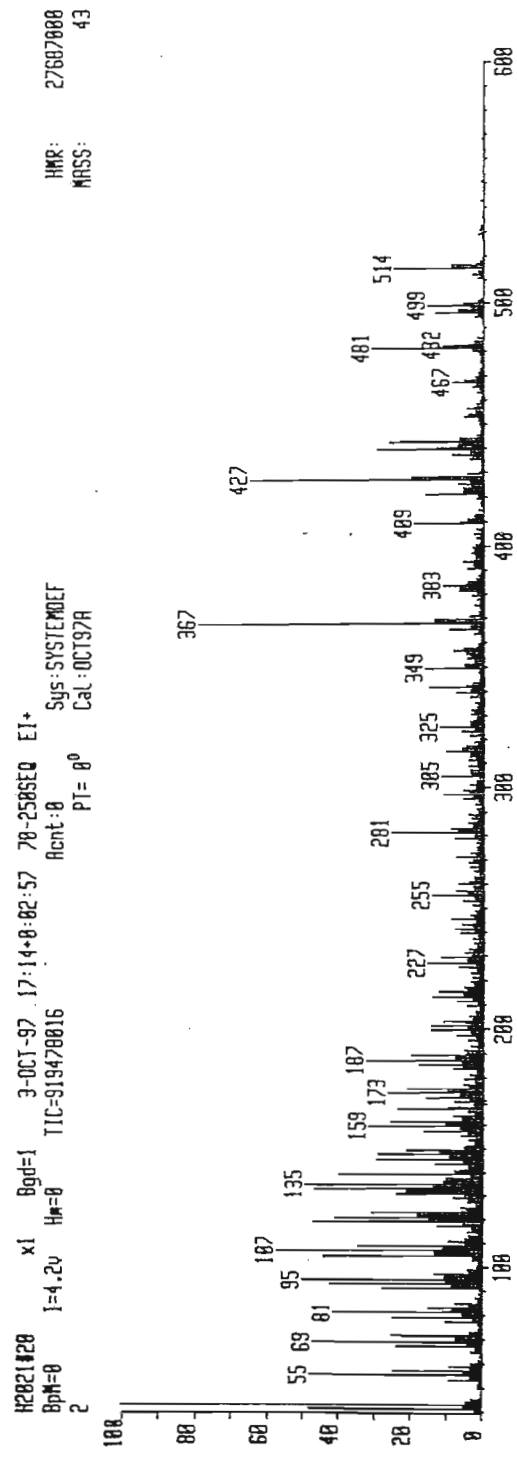


Extractives from *Turraea obtusifolia*

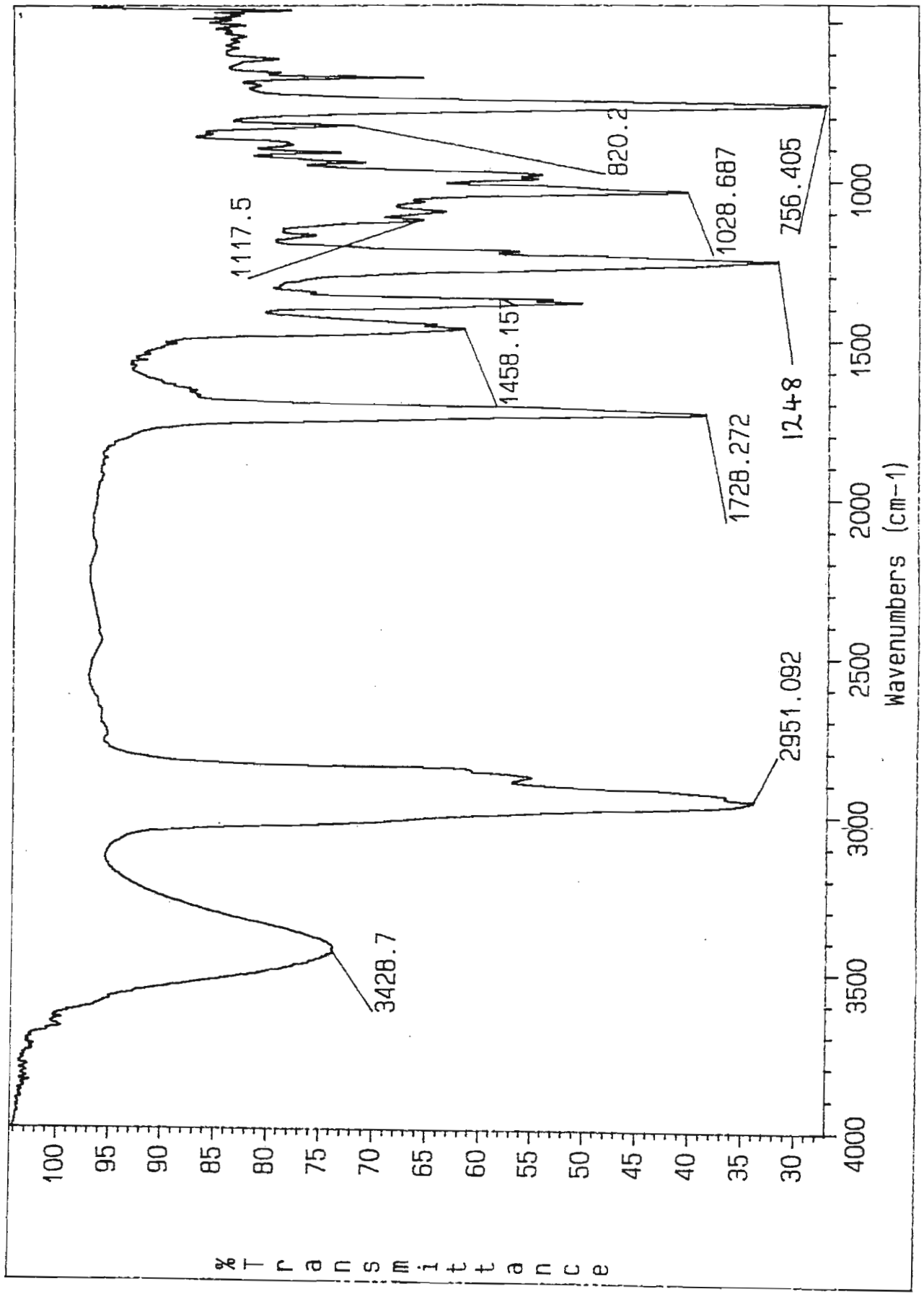
Compound 3-B



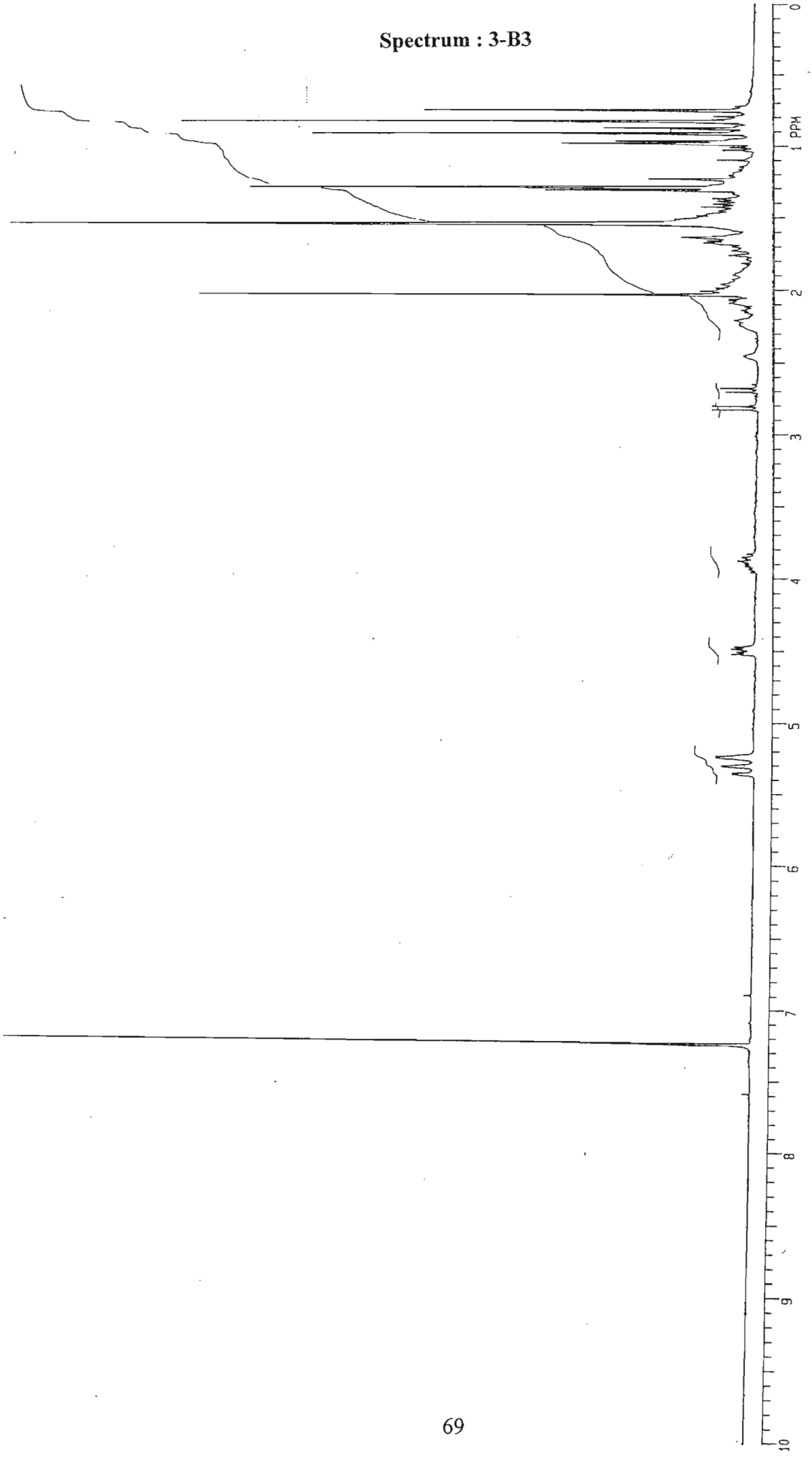
Spectrum : 3-B1



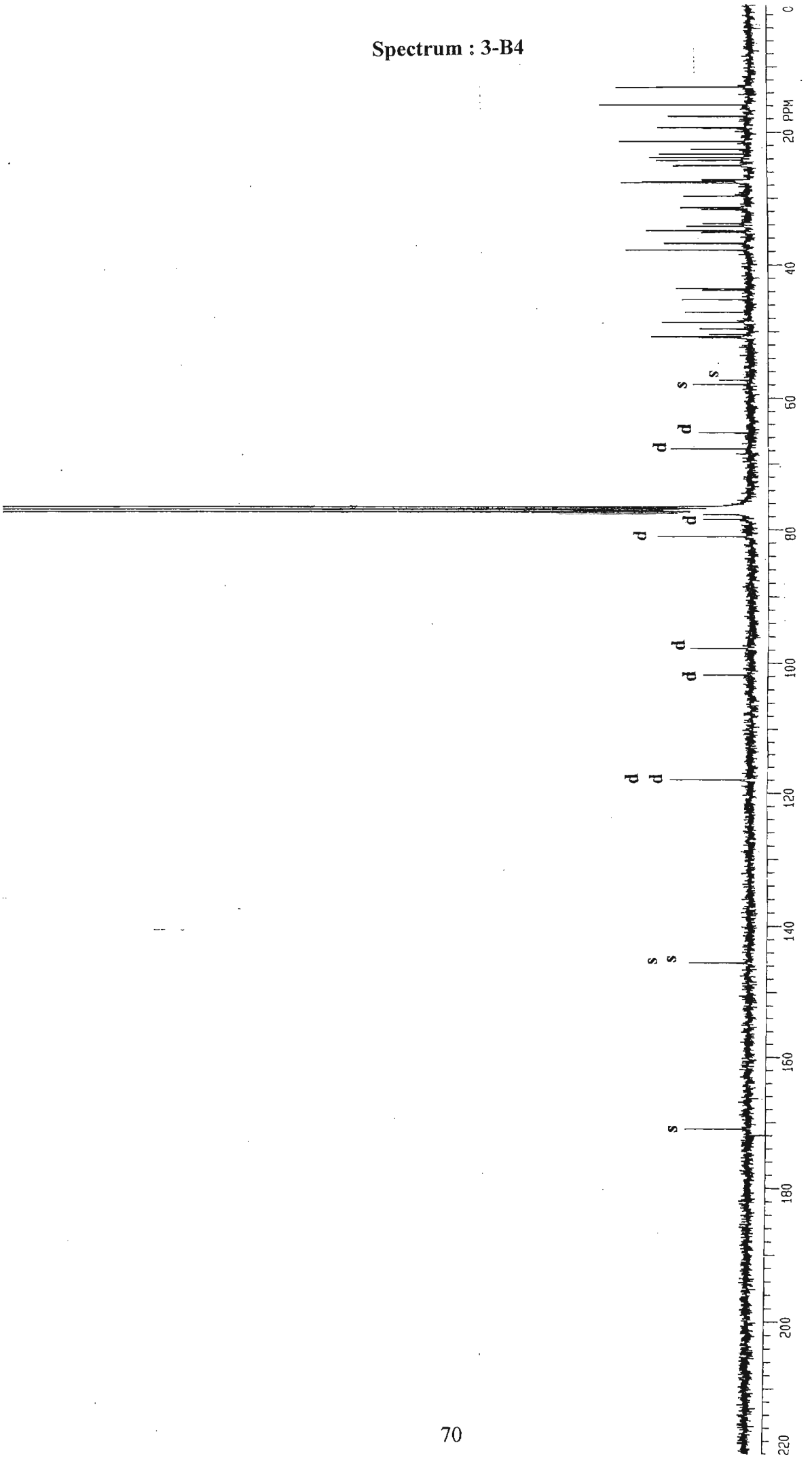
Spectrum : 3-B2



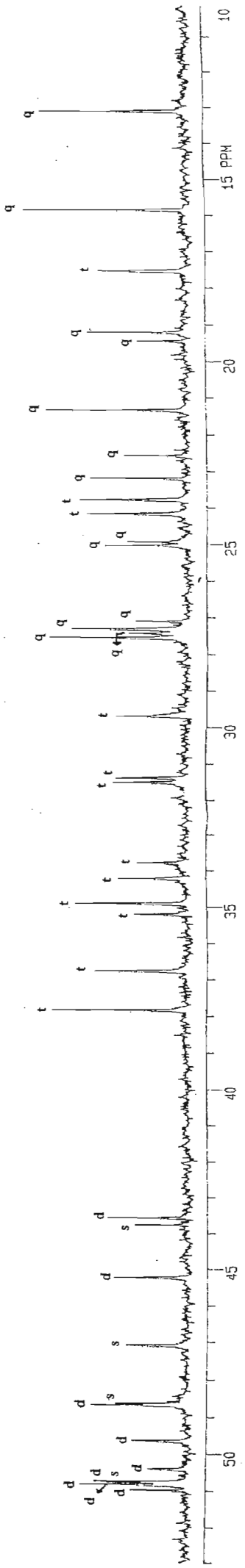
Spectrum : 3-B3



Spectrum : 3-B4

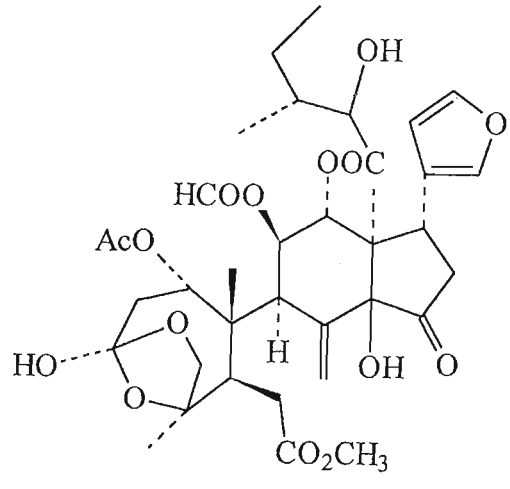


Spectrum : 3-B5

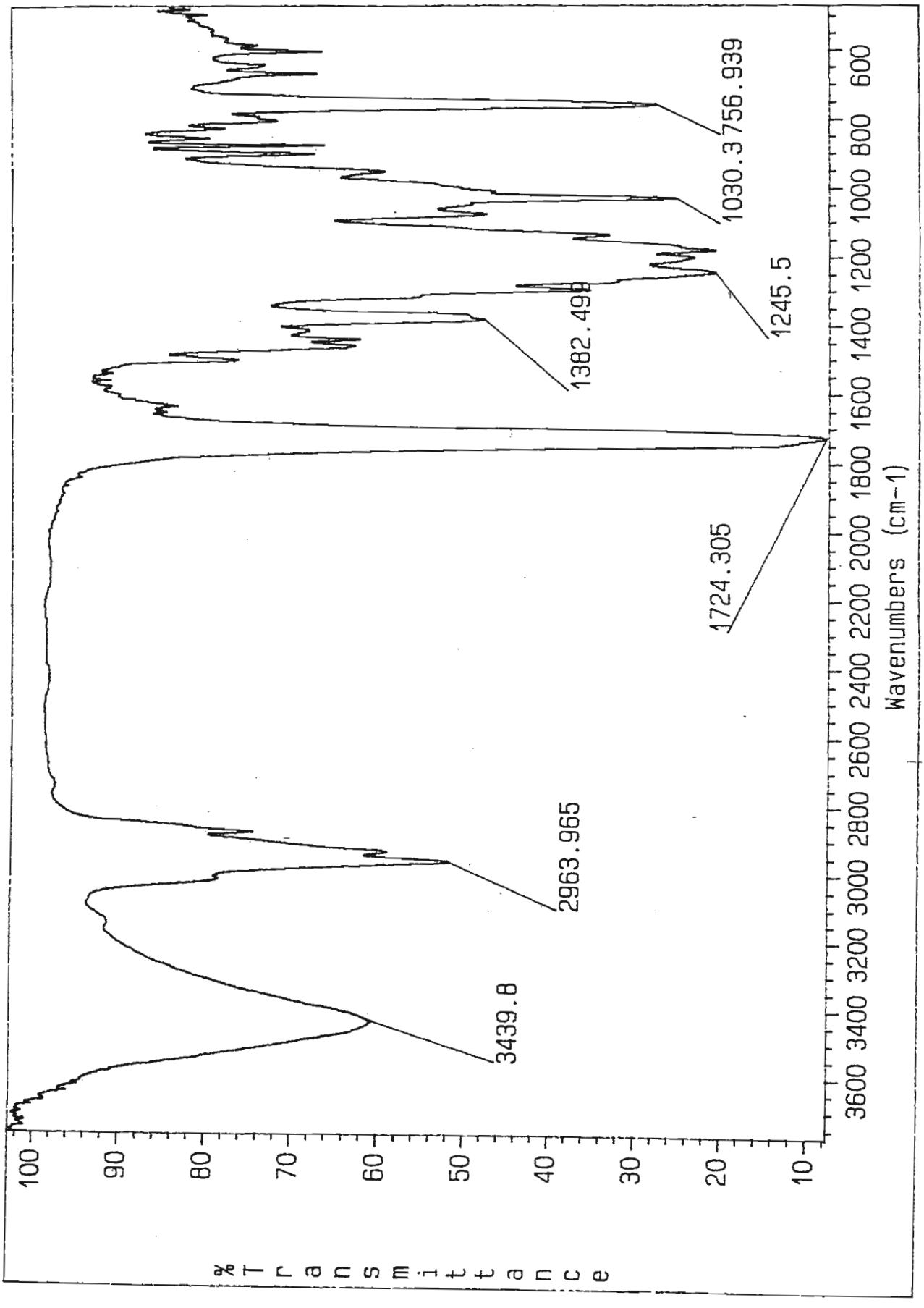


Extractives from *Turraea obtusifolia*

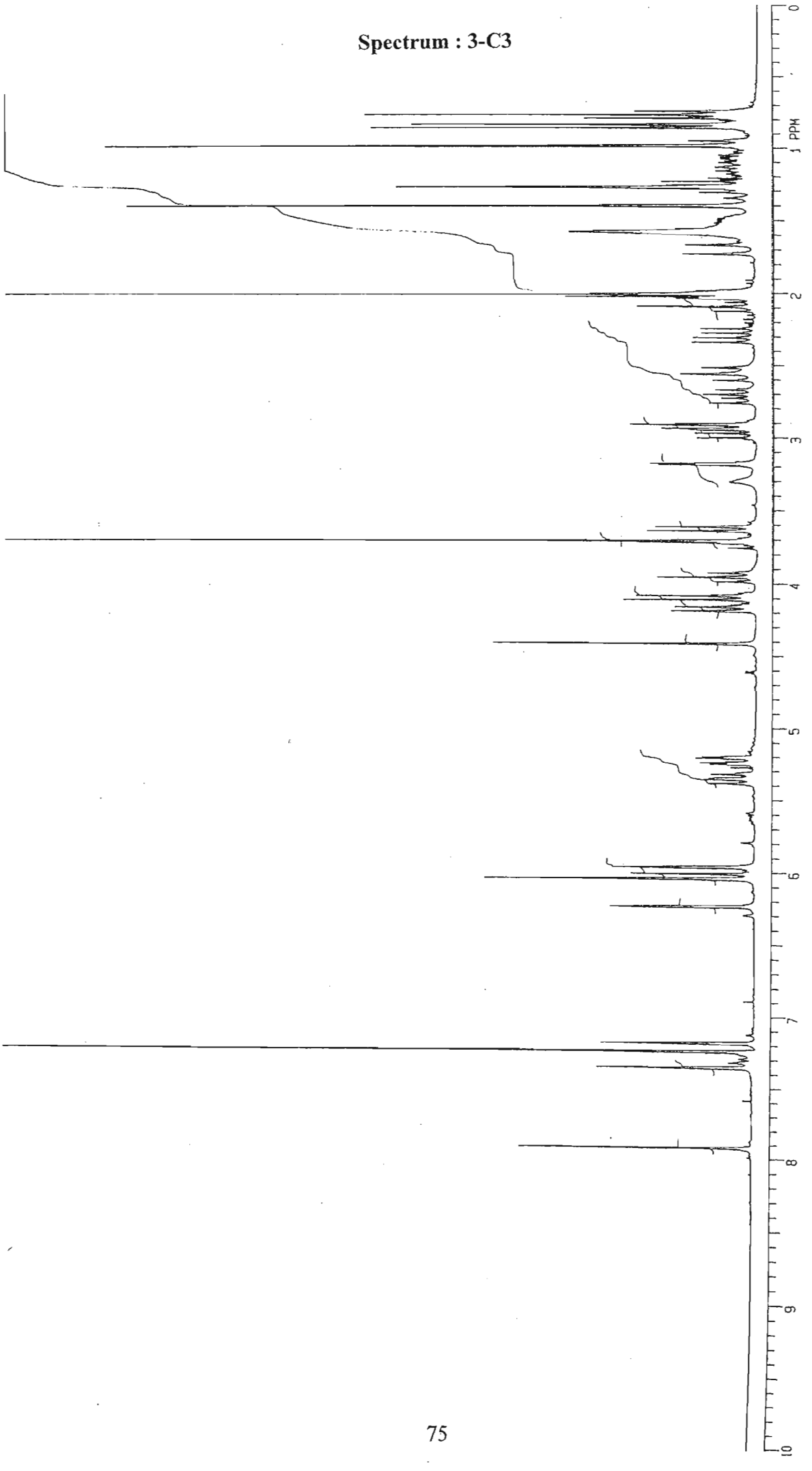
Compound 3-C



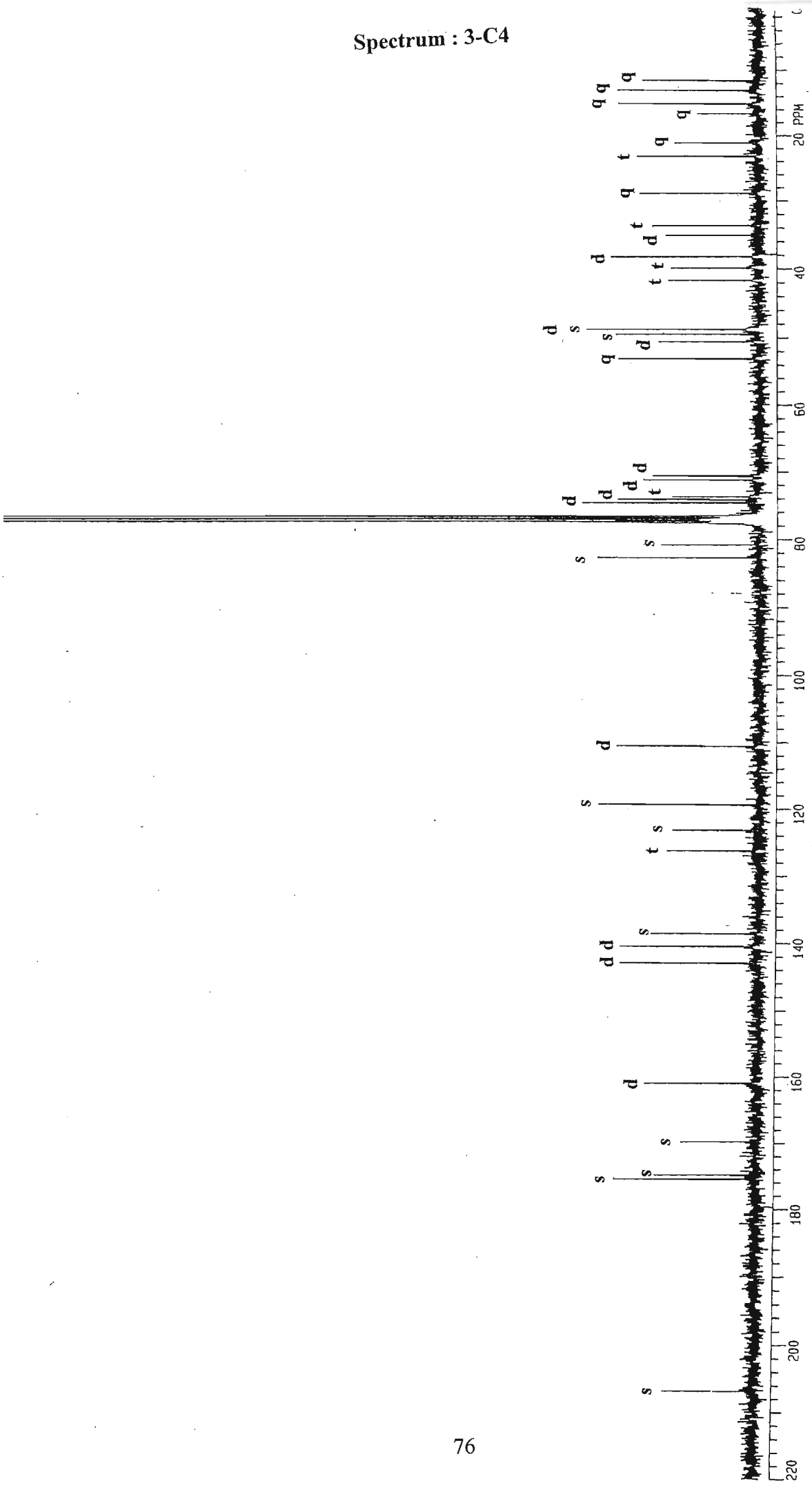
Spectrum : 3-C2



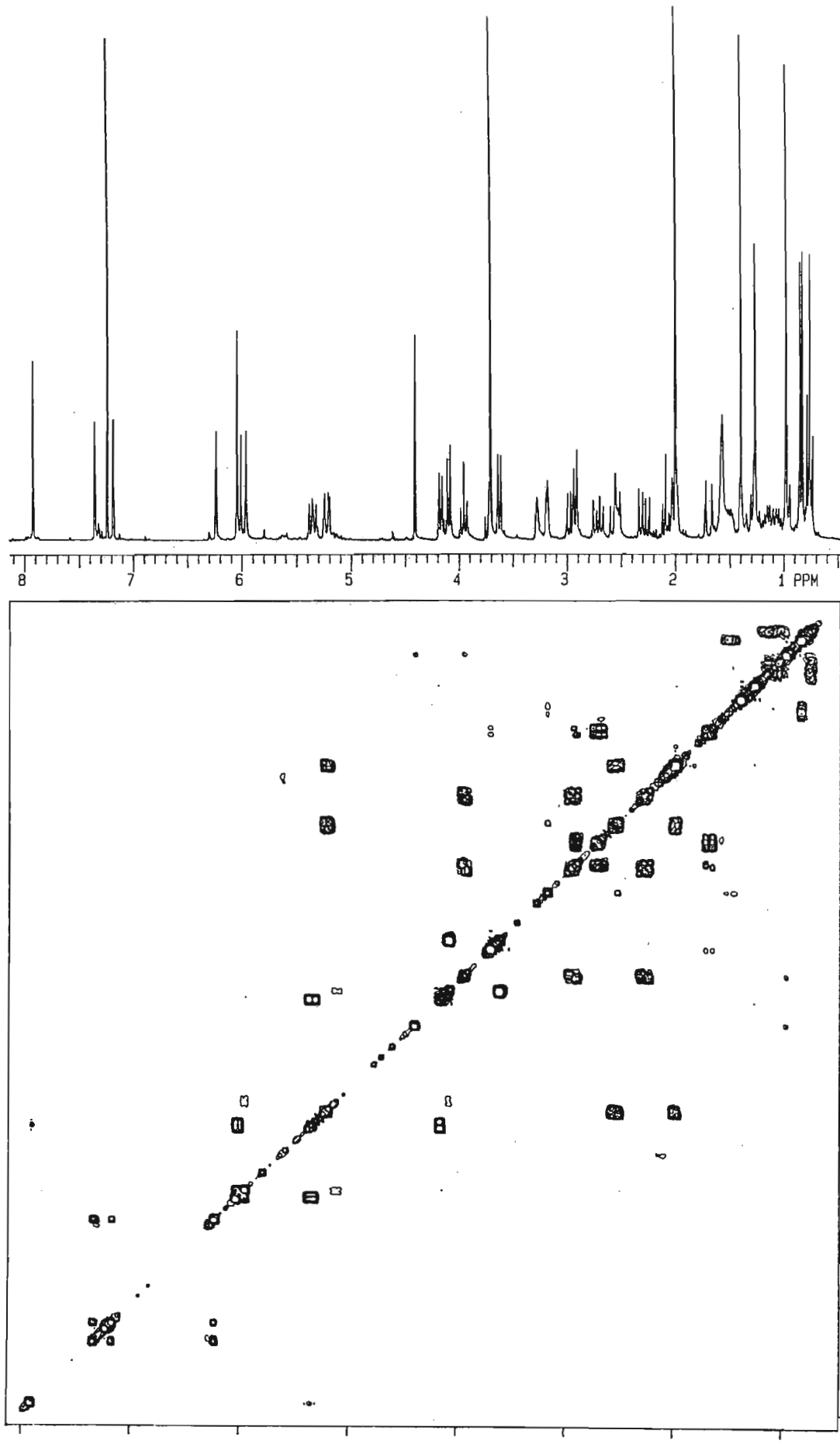
Spectrum : 3-C3



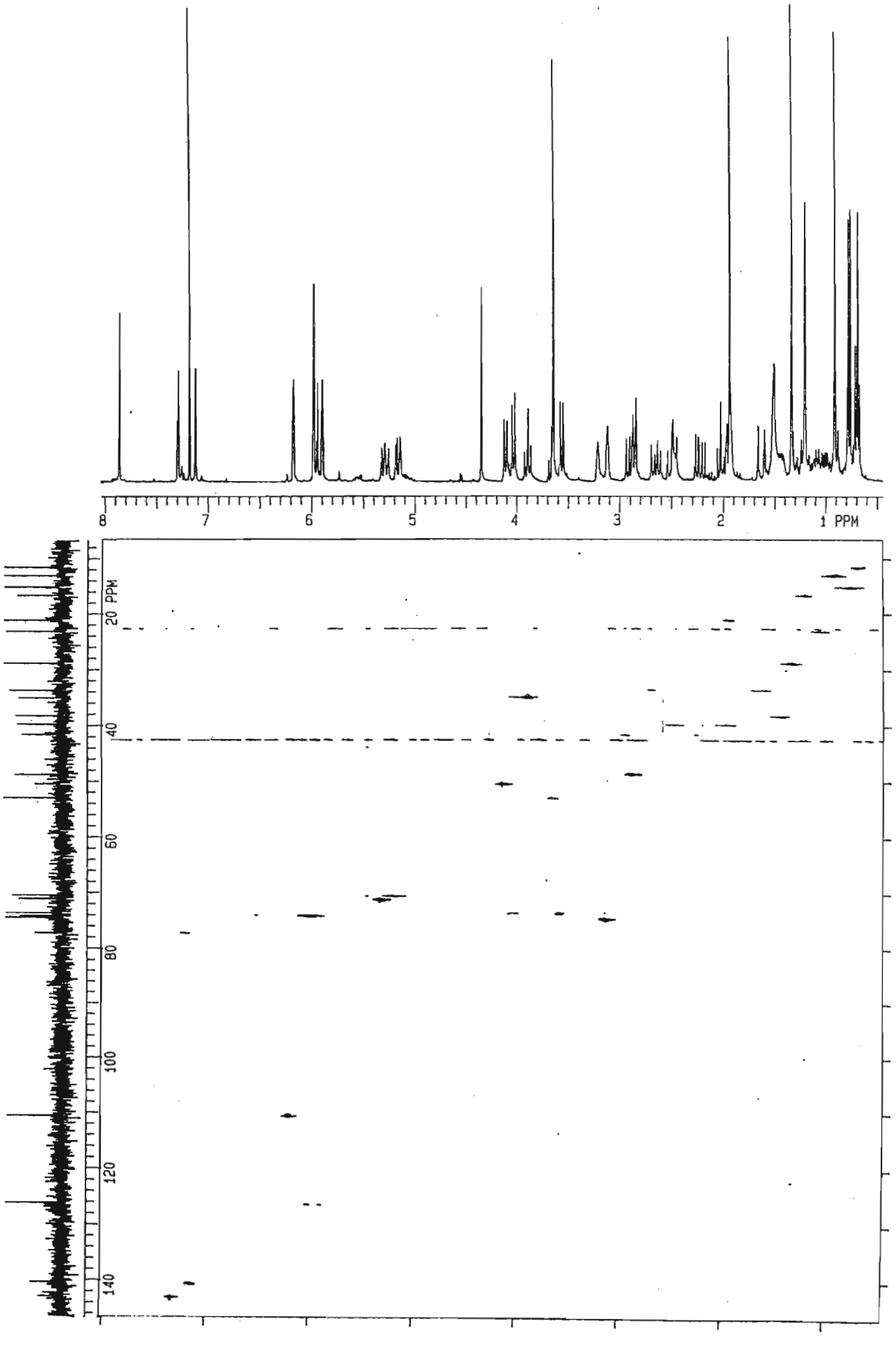
Spectrum : 3-C4



Spectrum : 3-C5

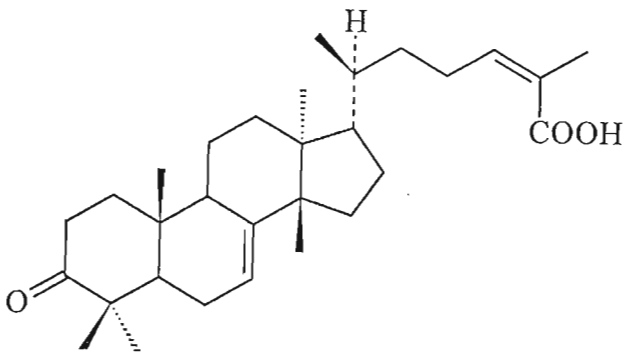


Spectrum : 3-C6

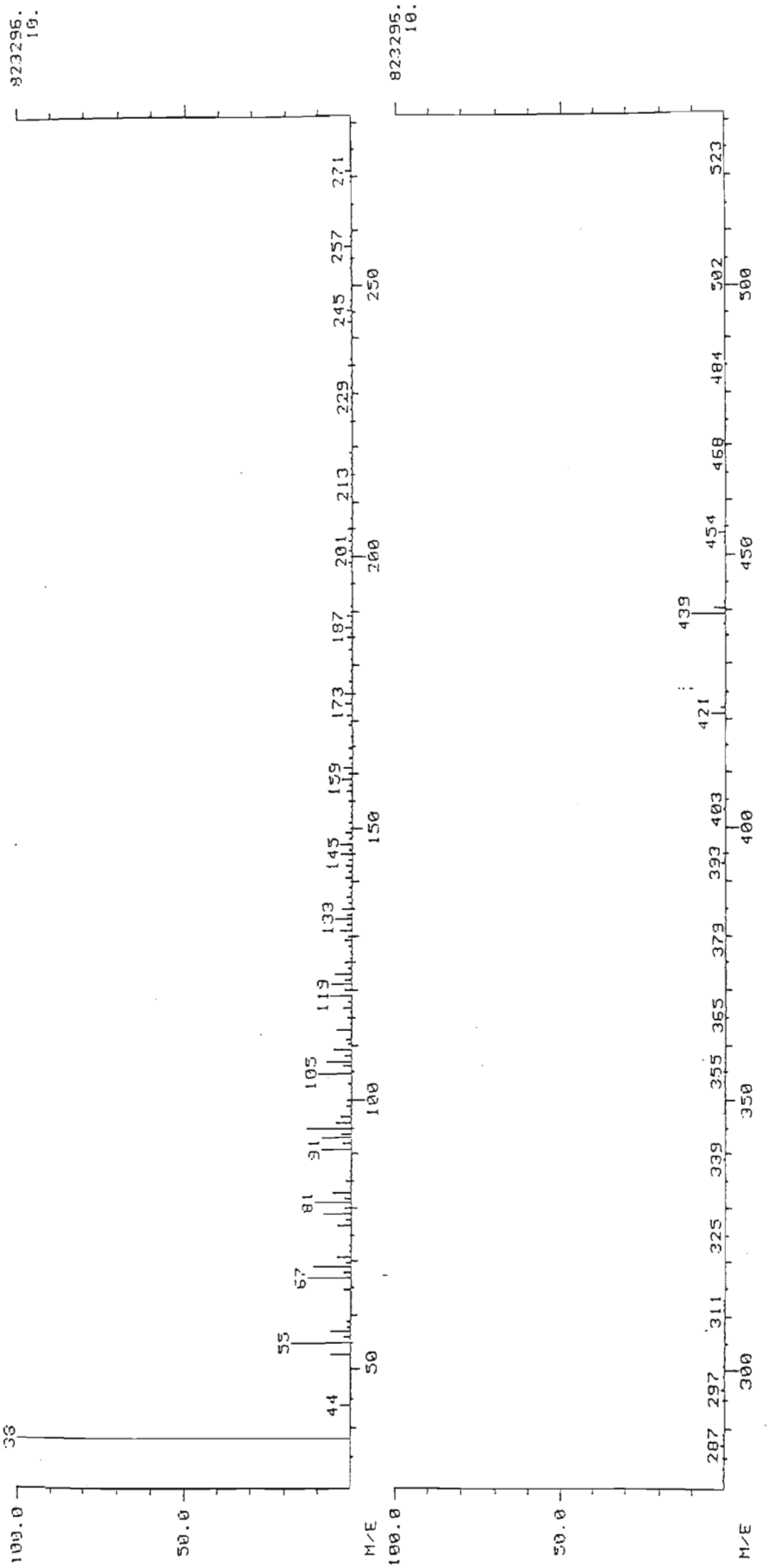


Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

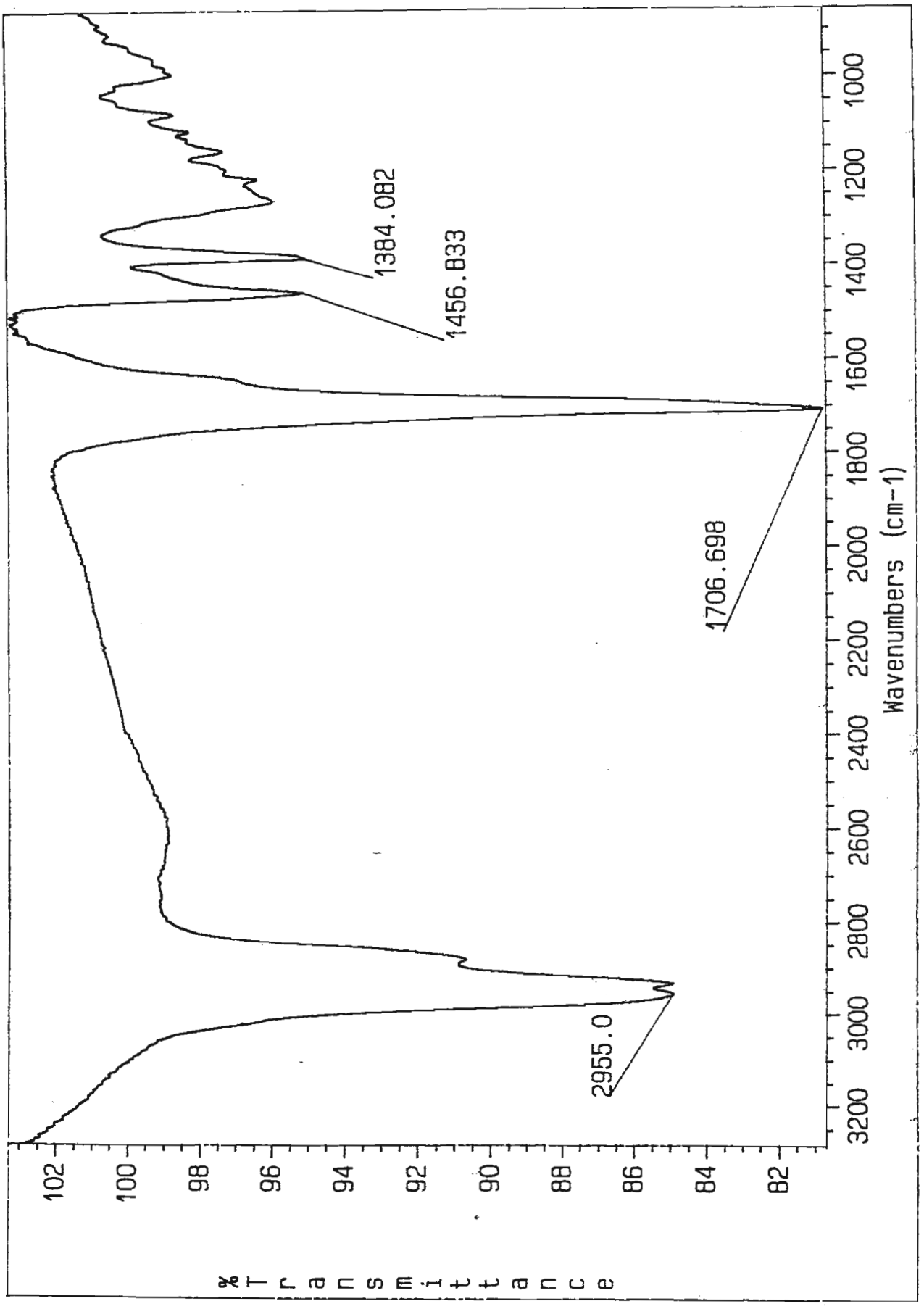
Compound 4-A



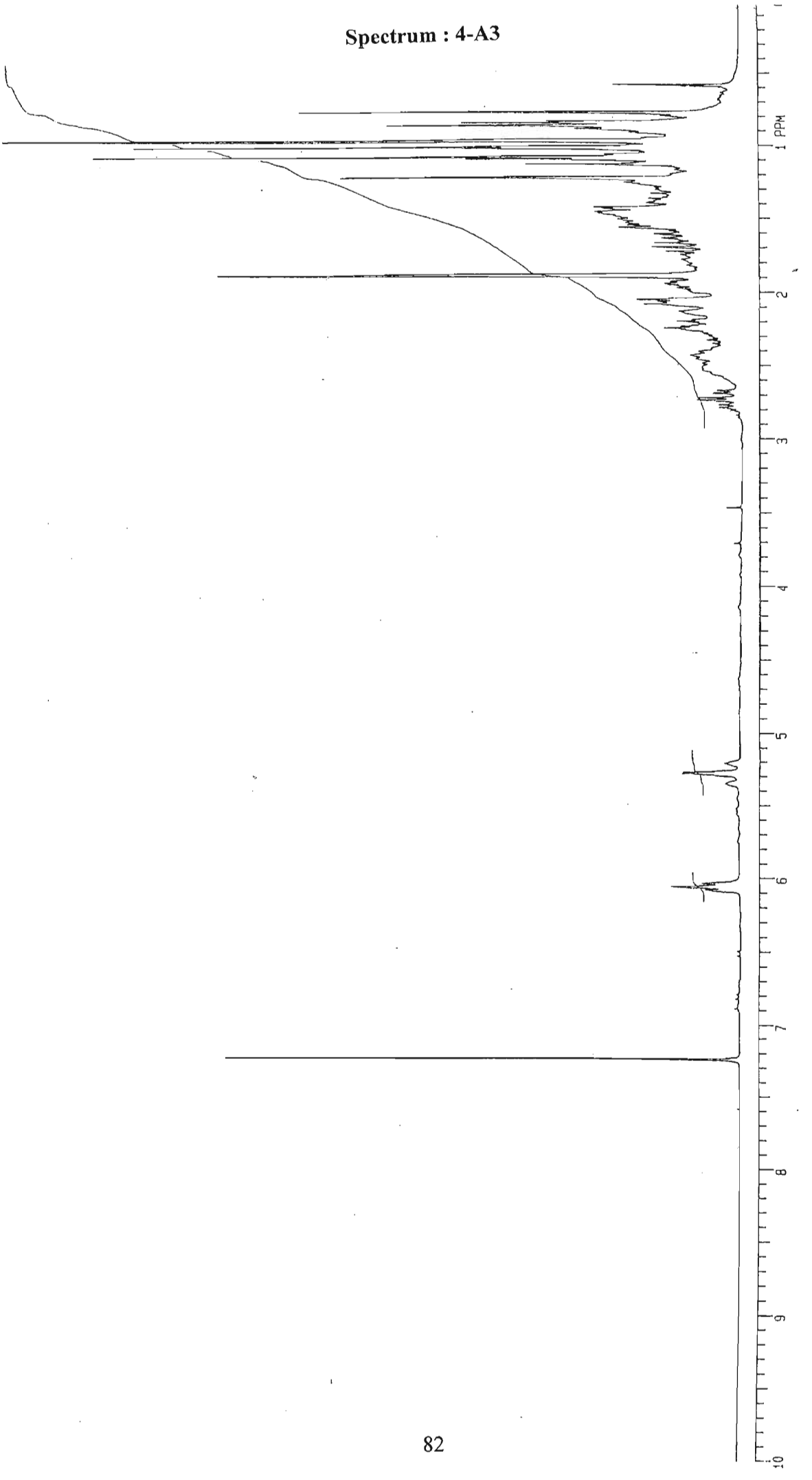
Spectrum : 4-A1



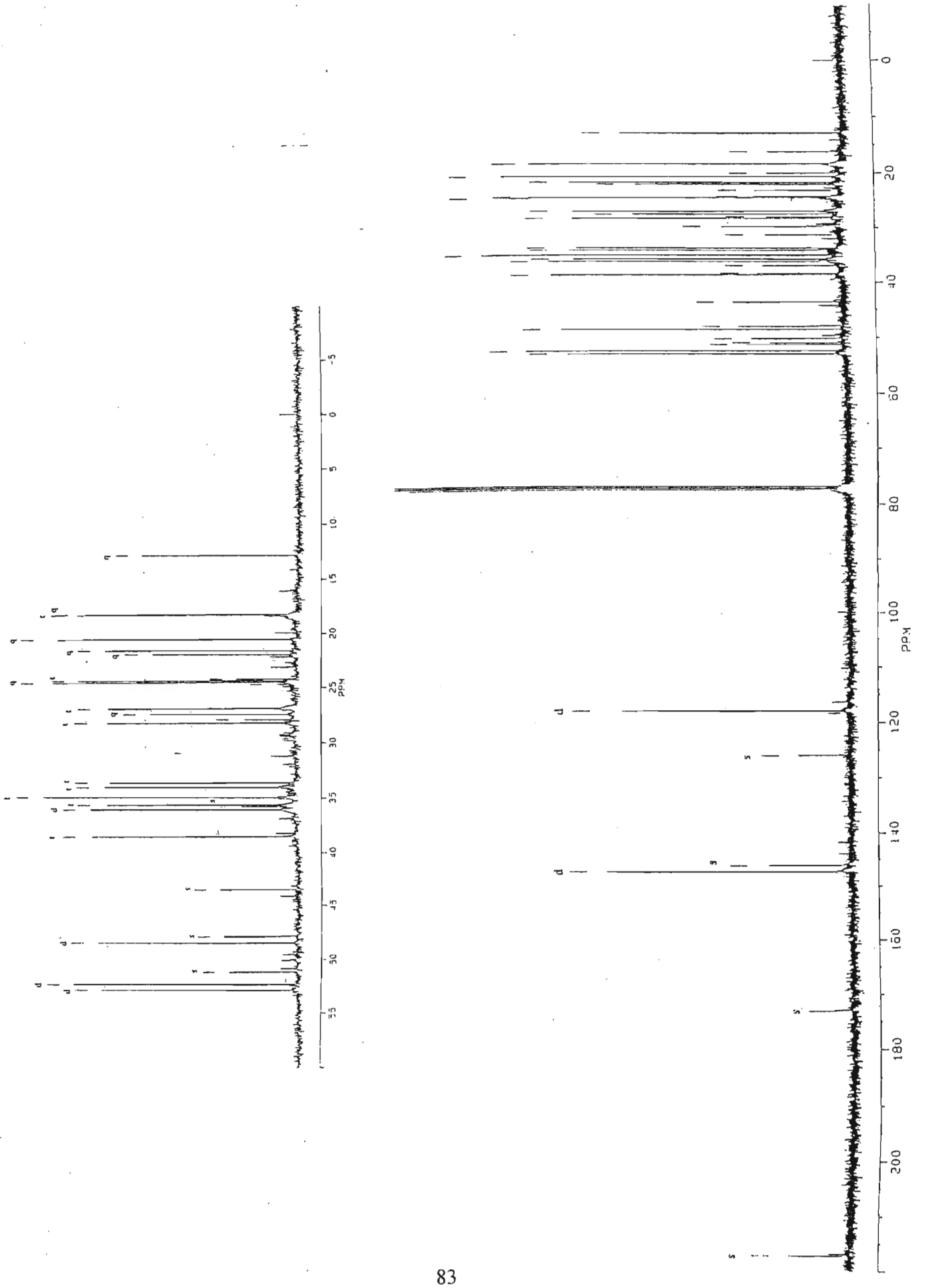
Spectrum : 4-A2



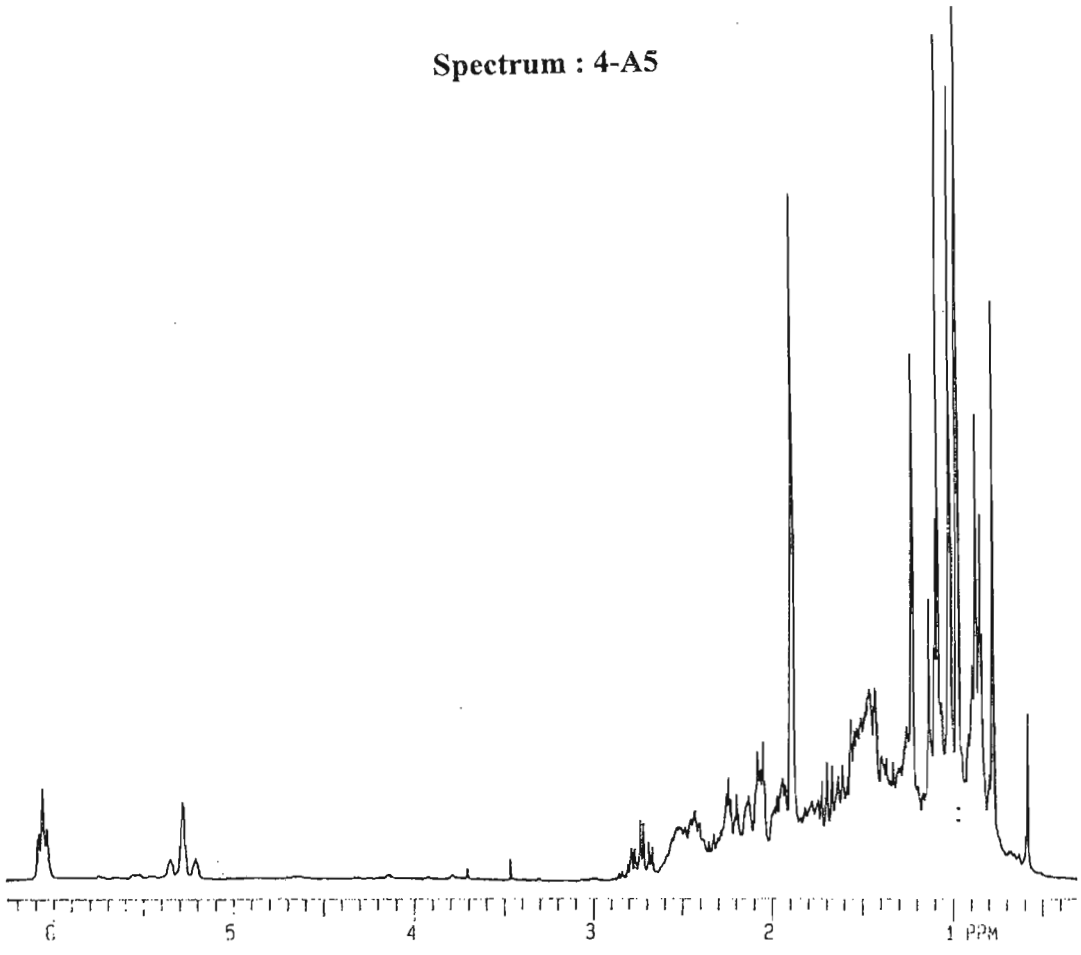
Spectrum : 4-A3

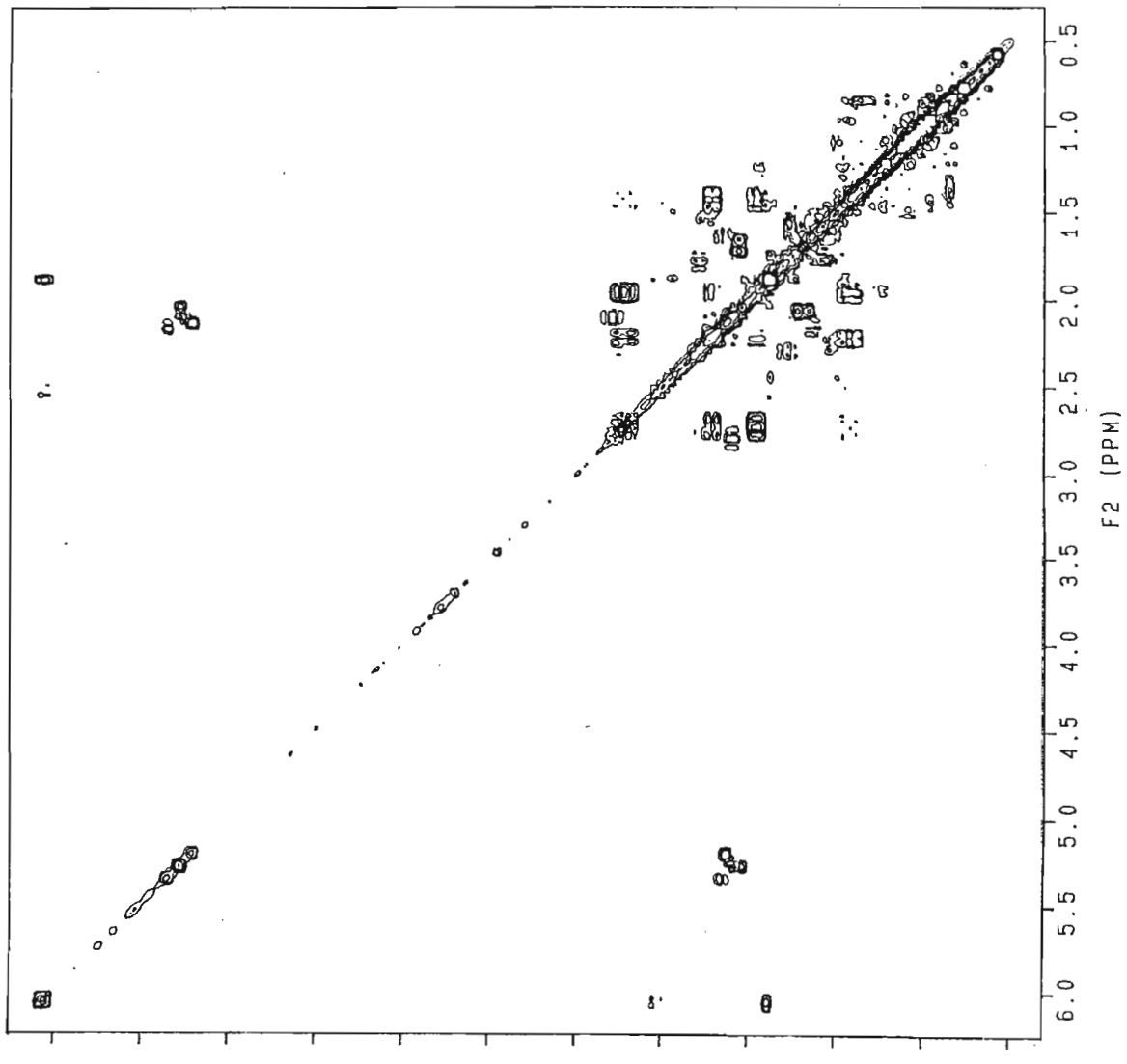


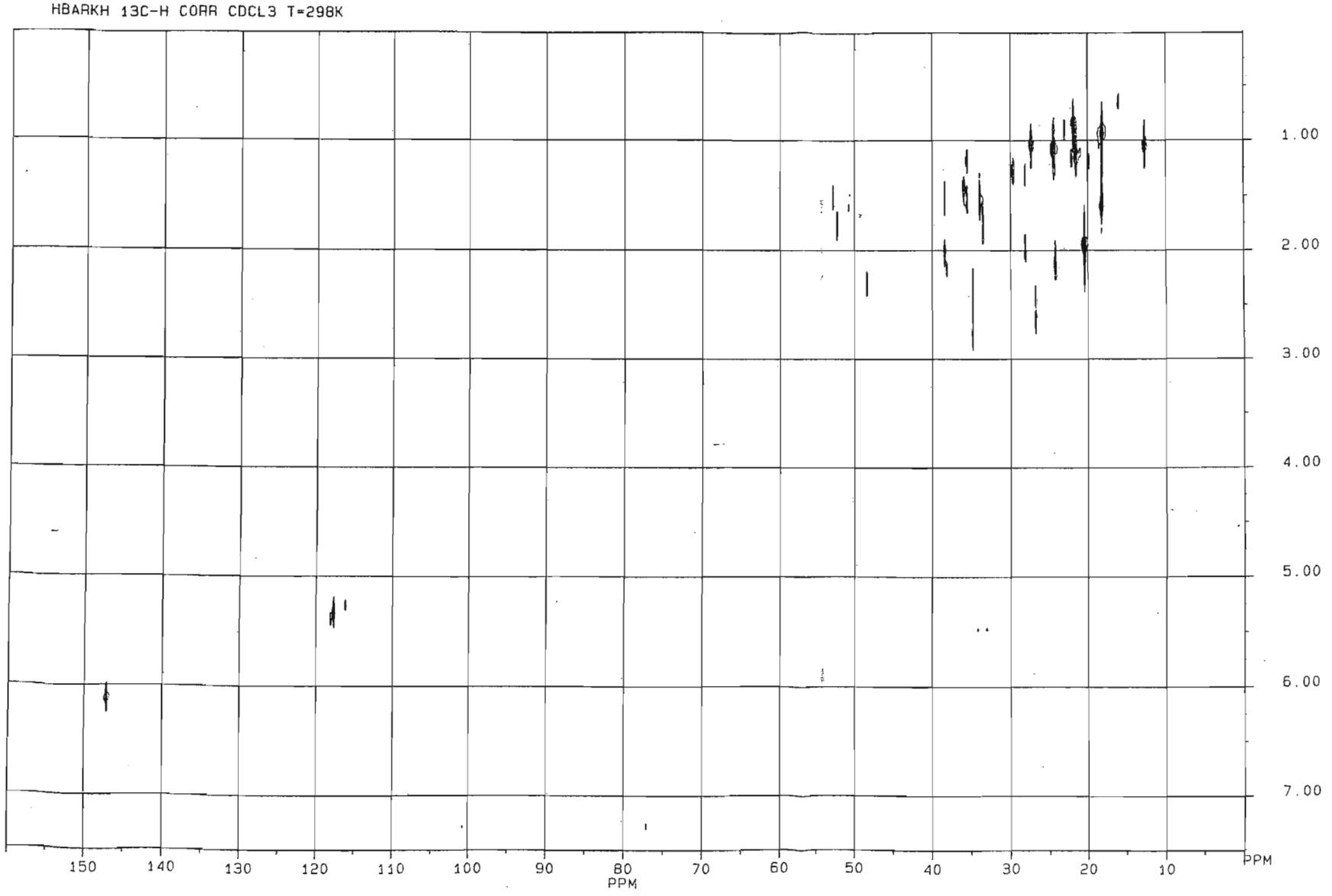
Spectrum : 4-A4



Spectrum : 4-A5







BRUKER

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AU PROG:
XHCORR.AUR
DATE 10-7-97

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SW2 18518.519
SW1 1794.688
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MC2 M
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F1 160.041P
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AND COLUMN:
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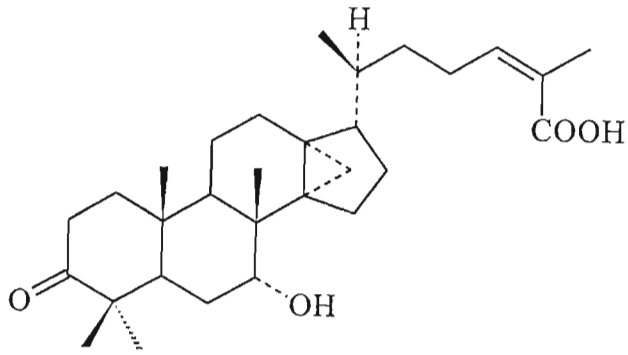
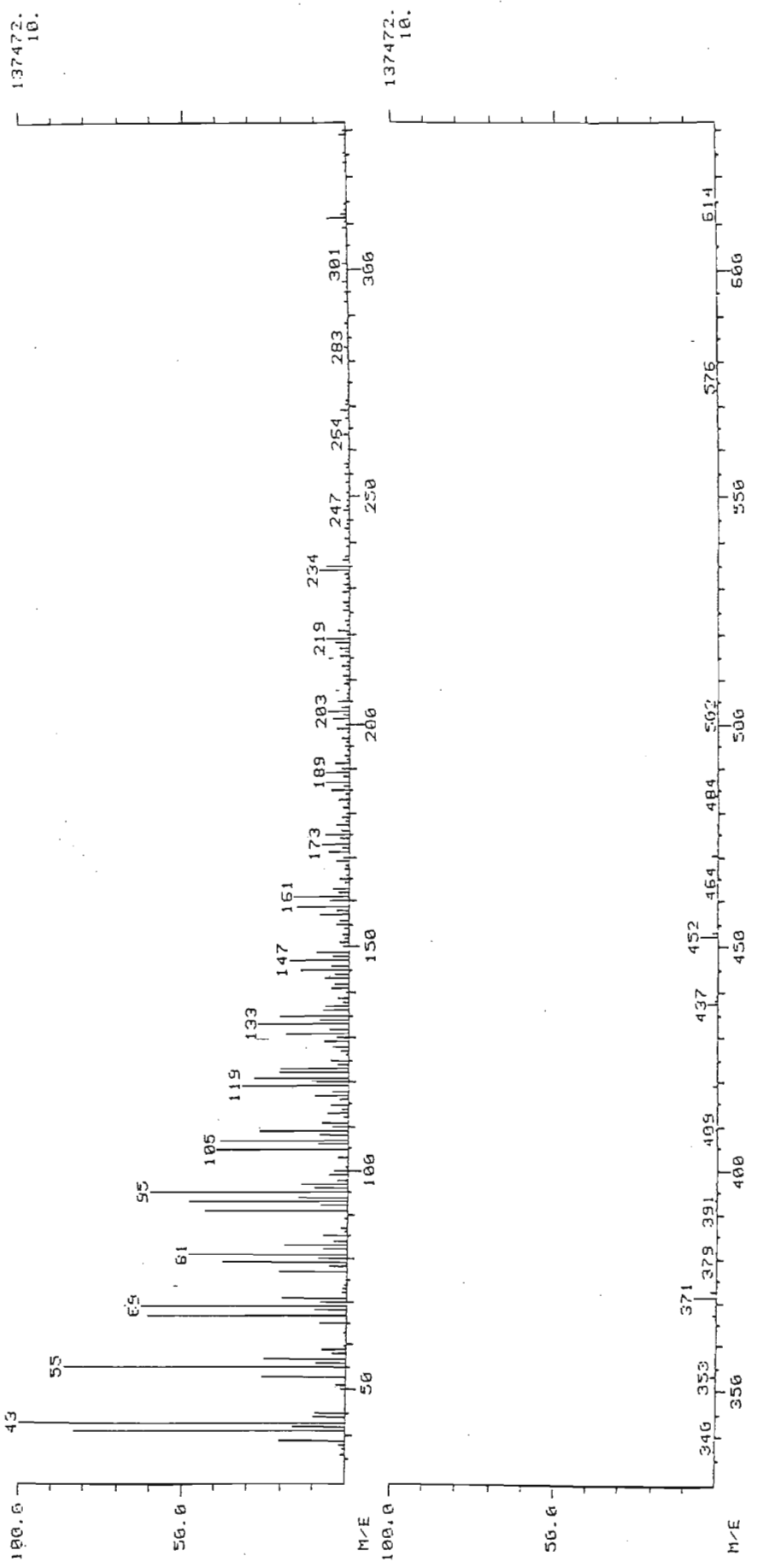
Spectrum : 4-A6

Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

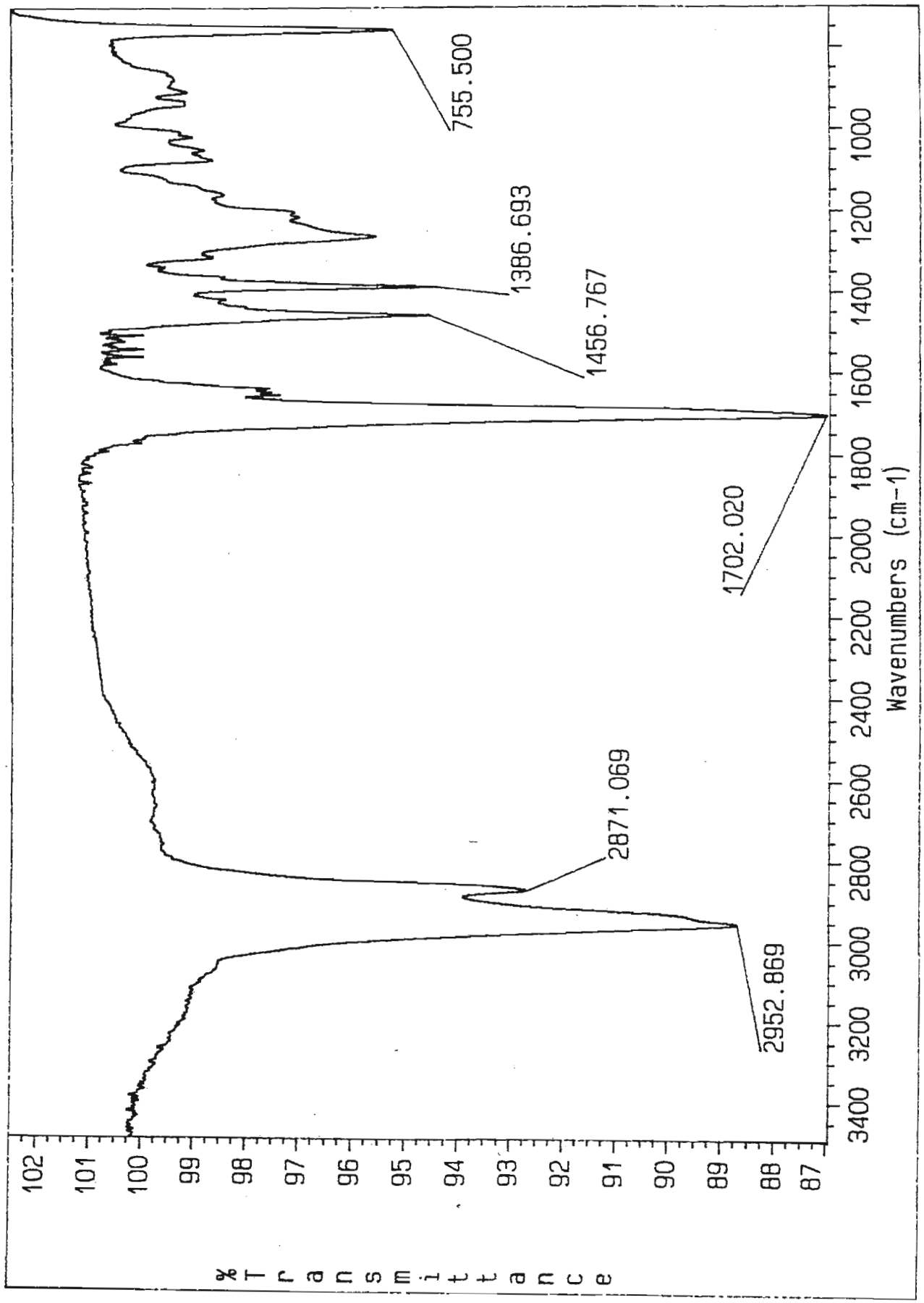
Compound 4-B



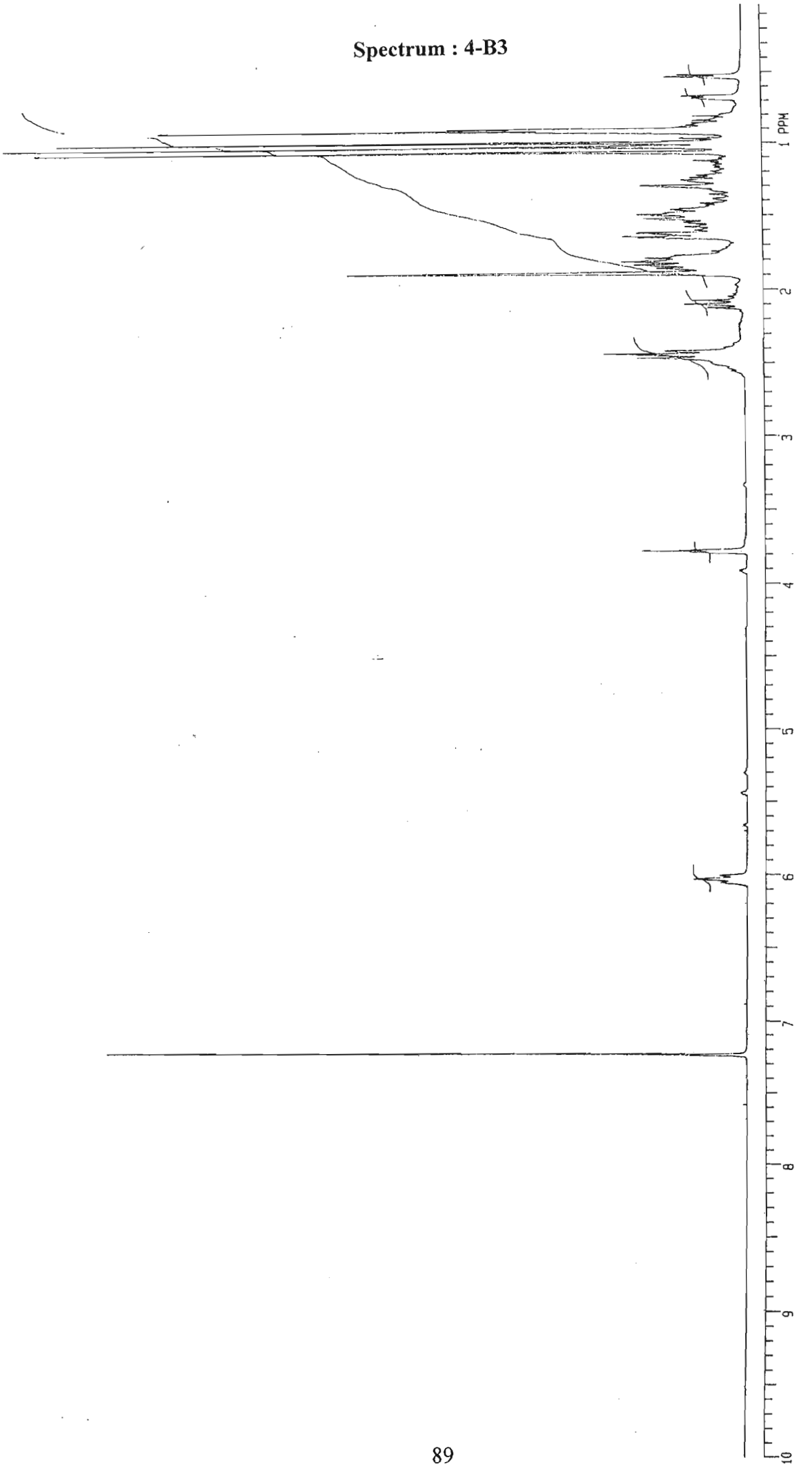
Spectrum : 4-B1



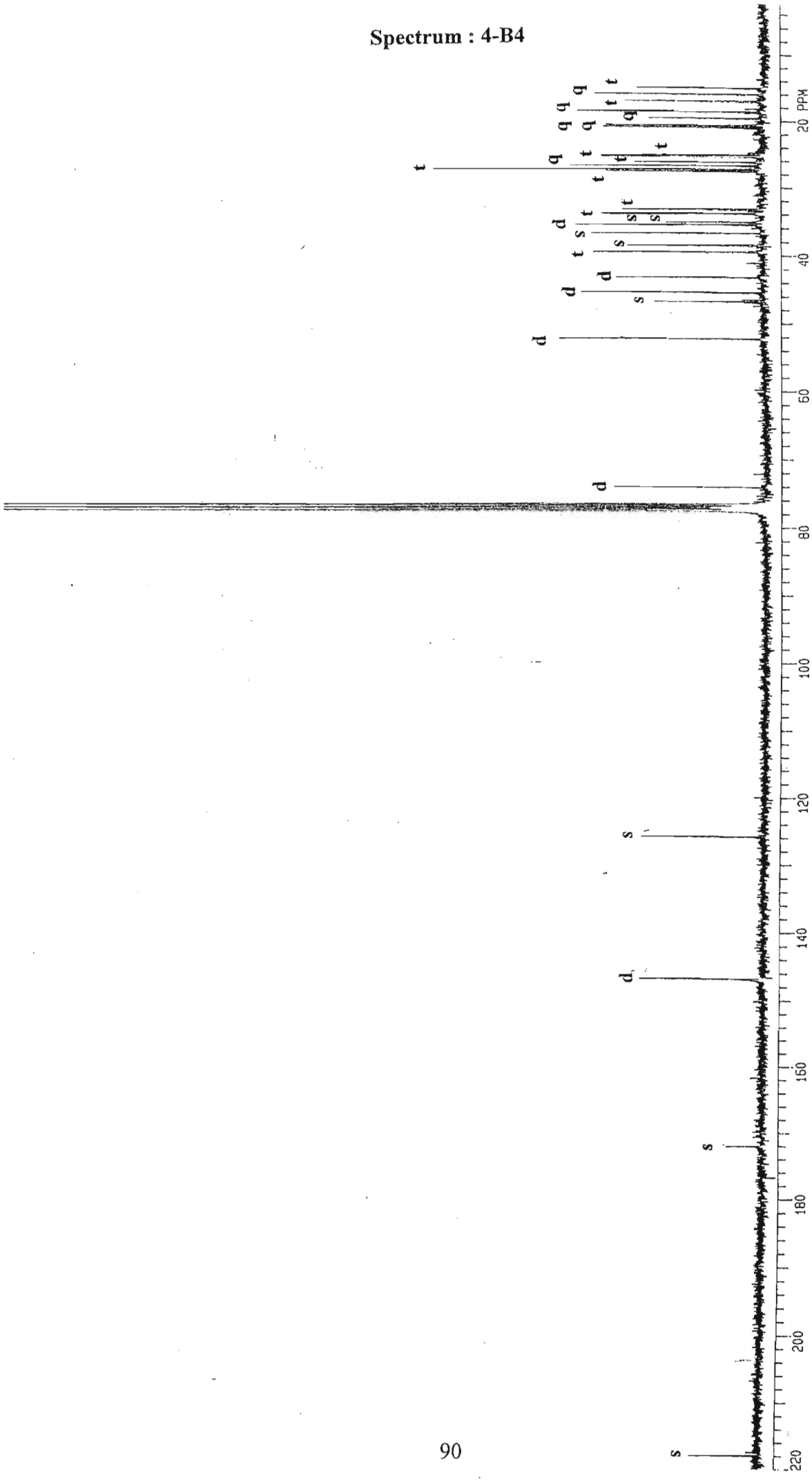
Spectrum : 4-B2



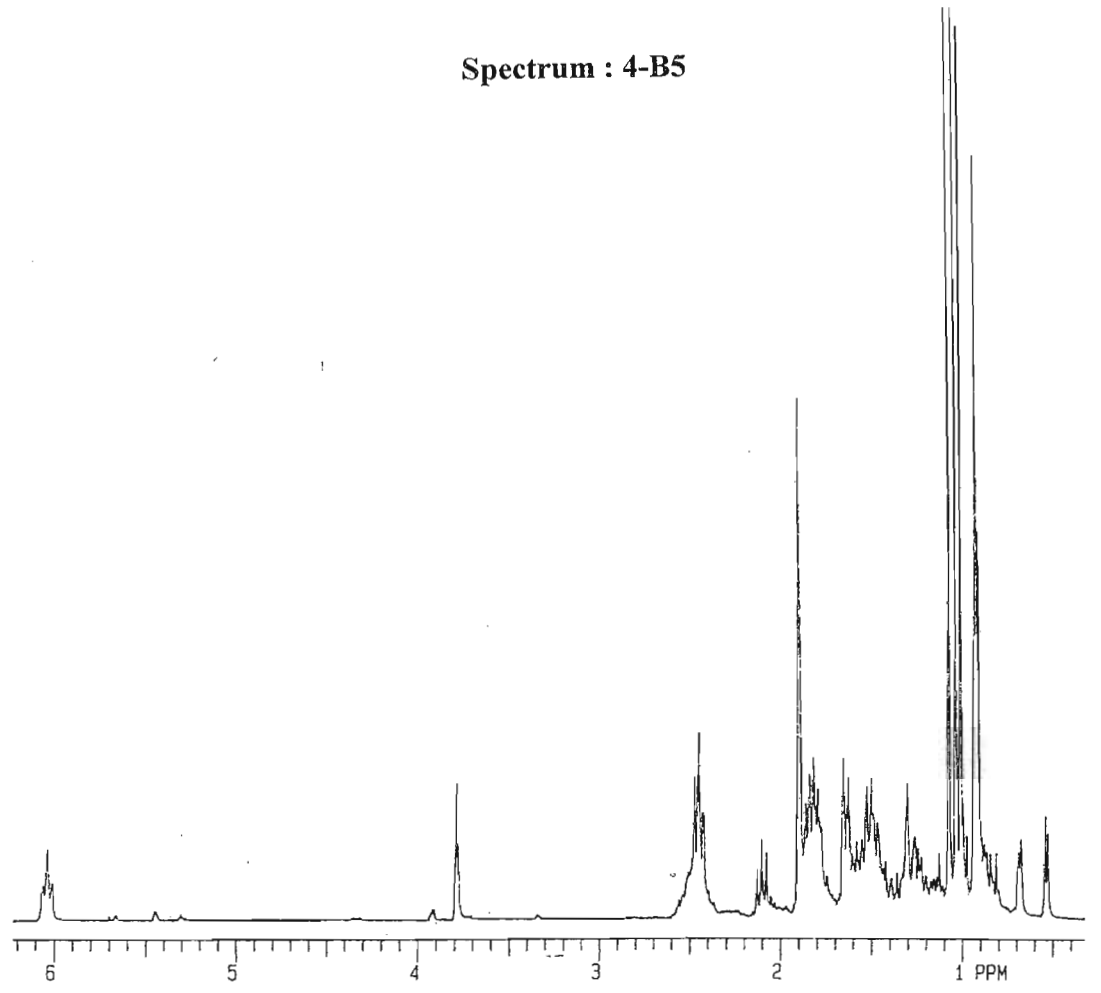
Spectrum : 4-B3

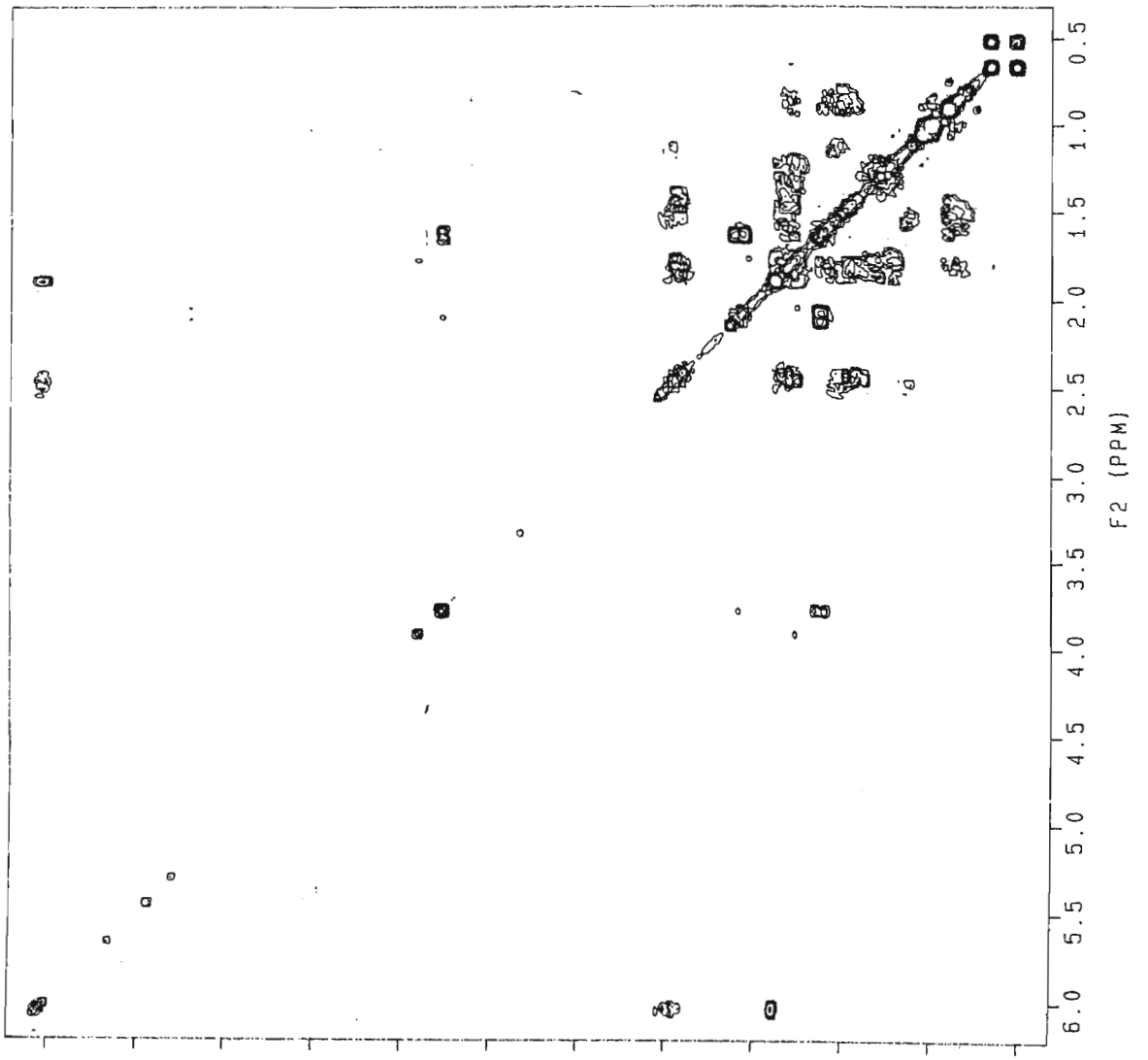


Spectrum : 4-B4

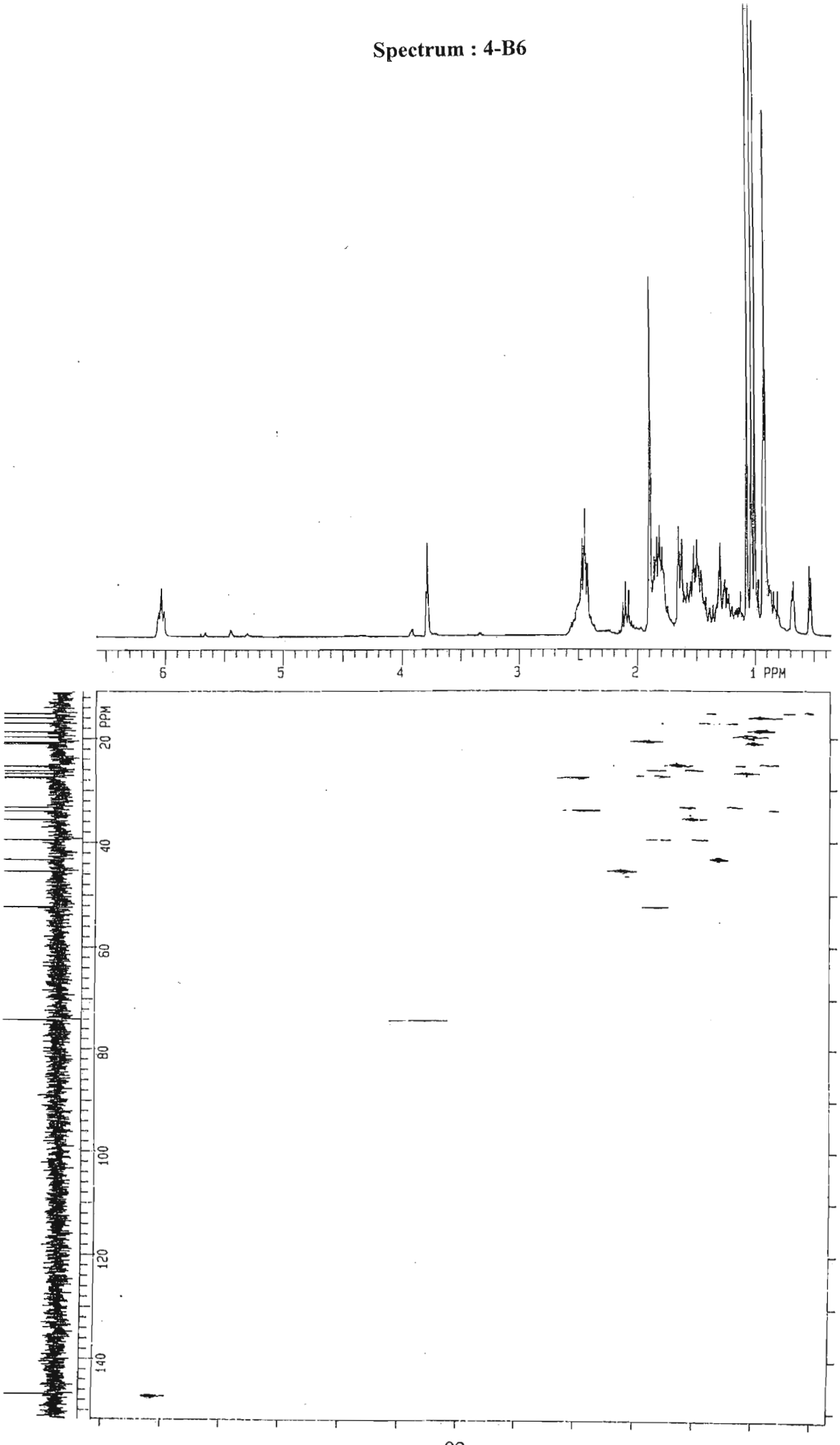


Spectrum : 4-B5

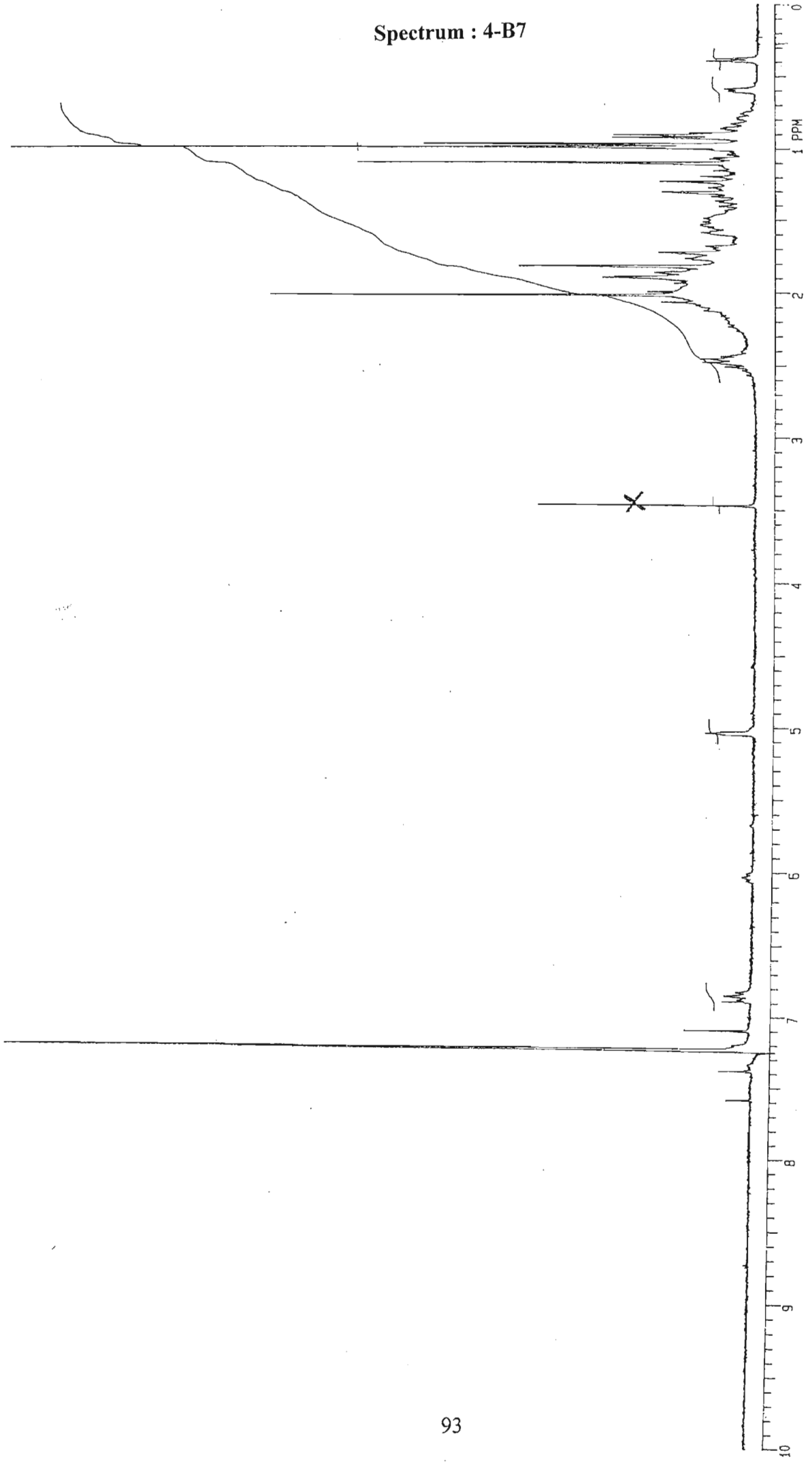




Spectrum : 4-B6

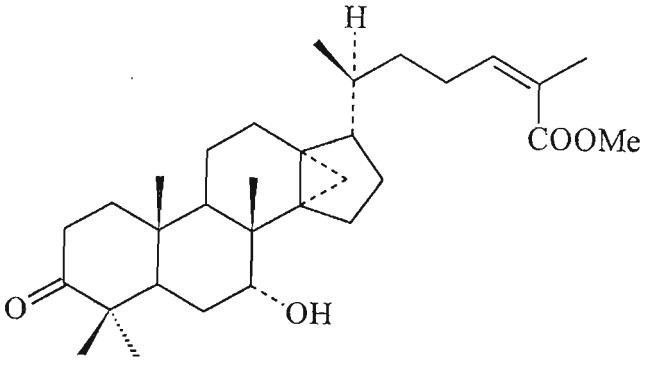


Spectrum : 4-B7

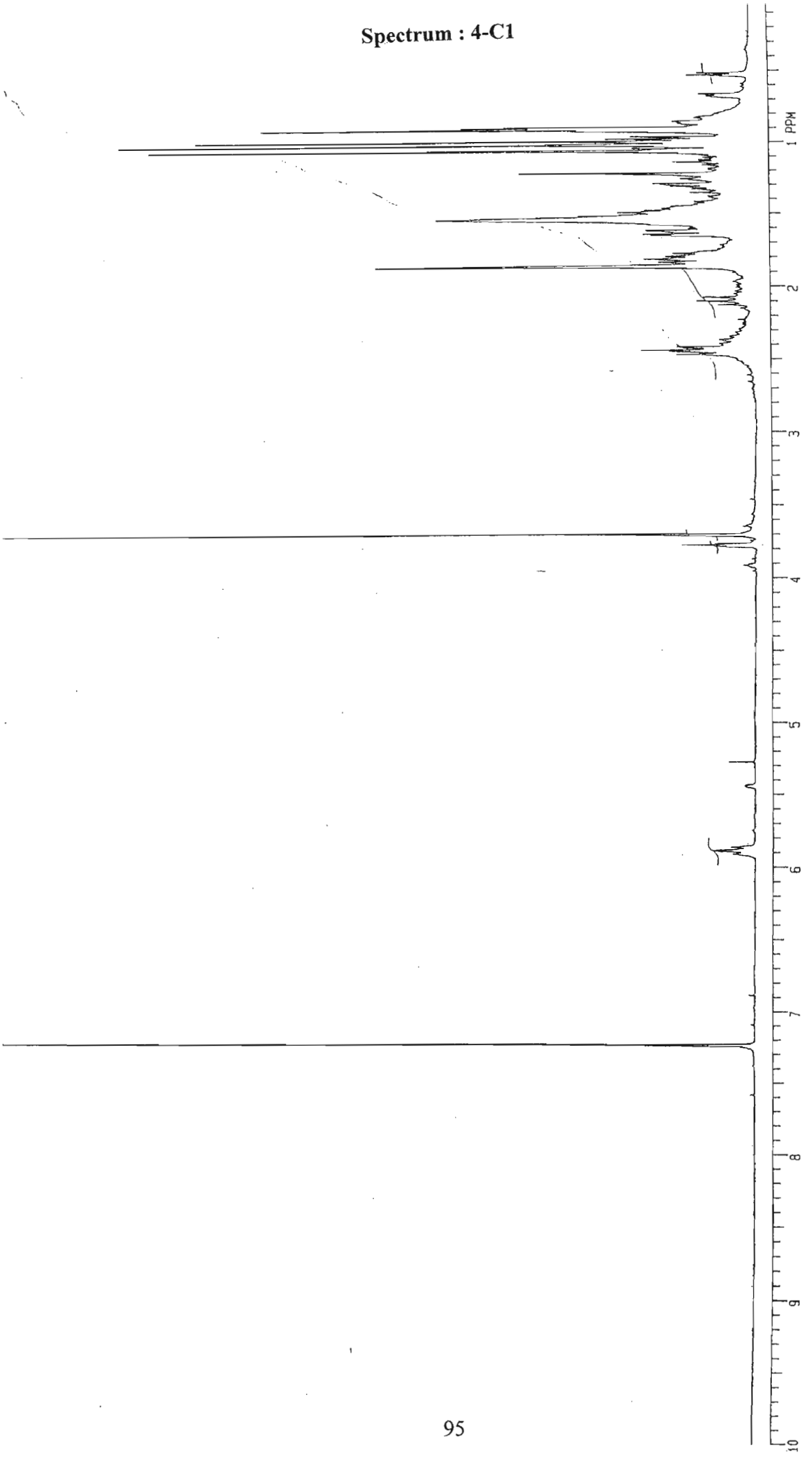


Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

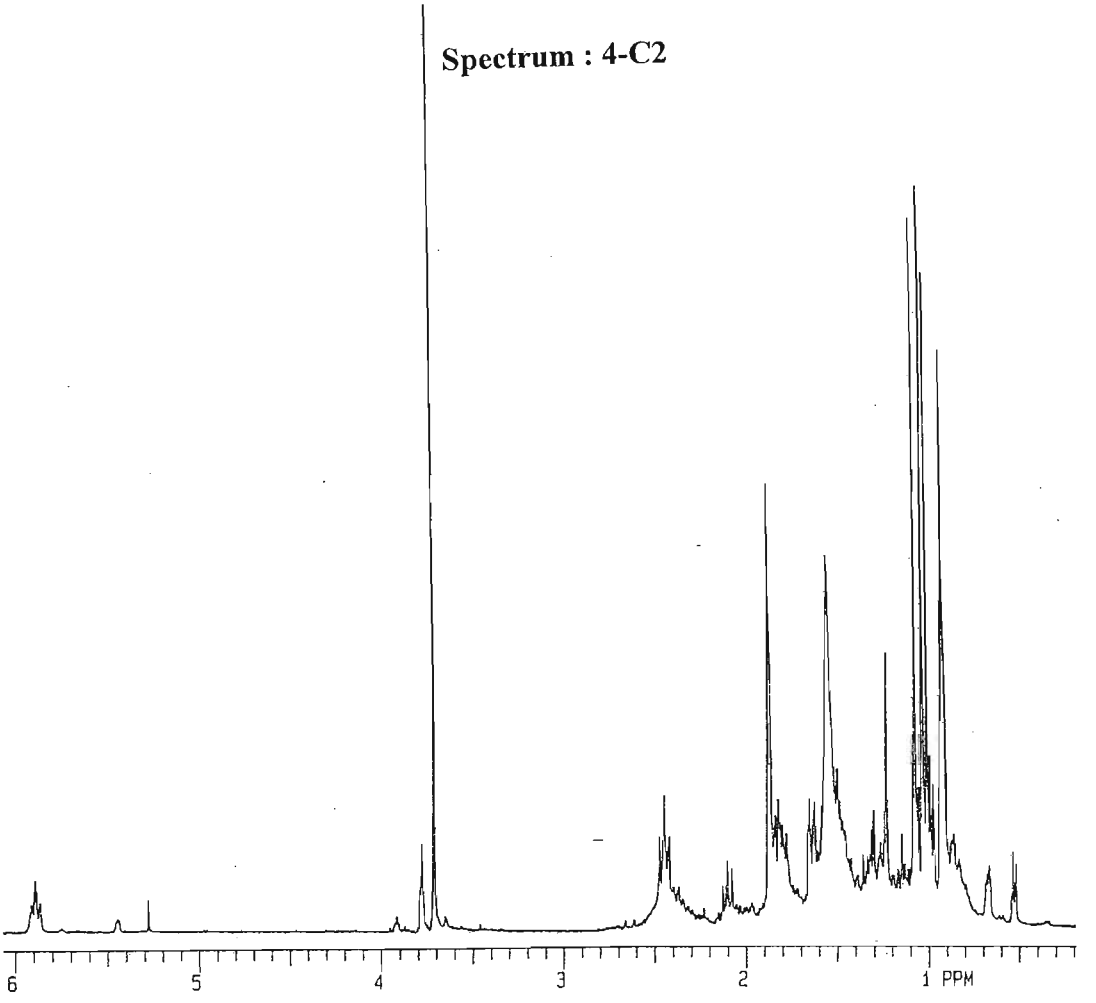
Compound 4-C

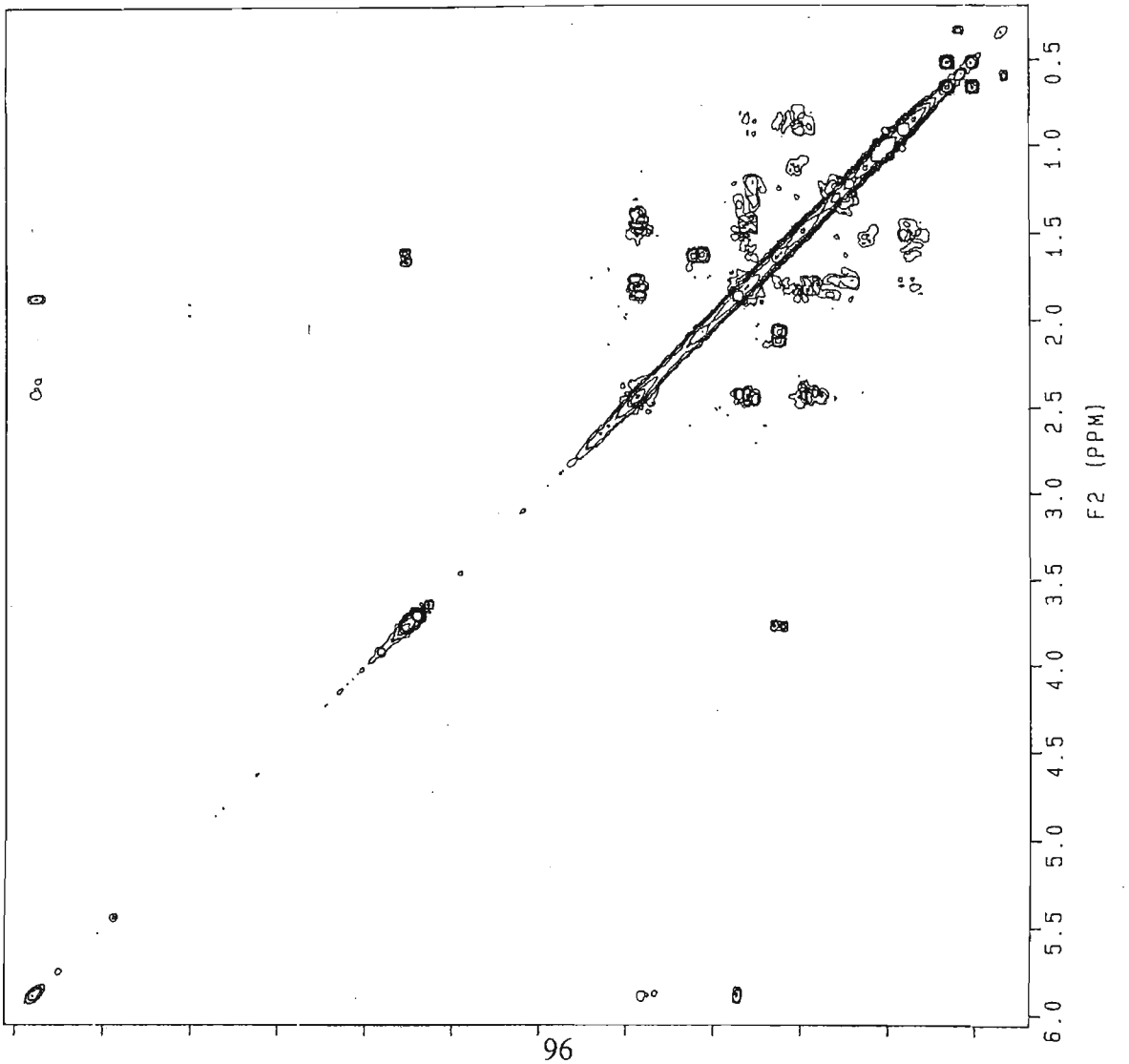


Spectrum : 4-C1



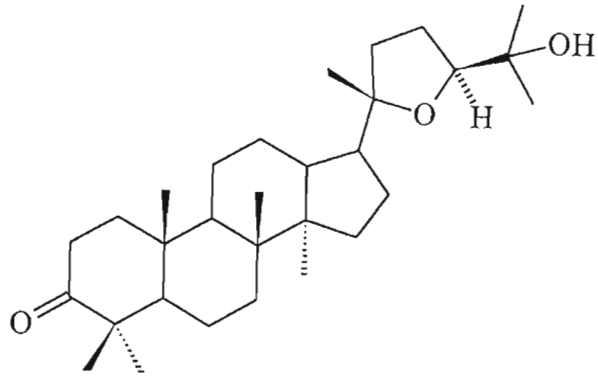
Spectrum : 4-C2



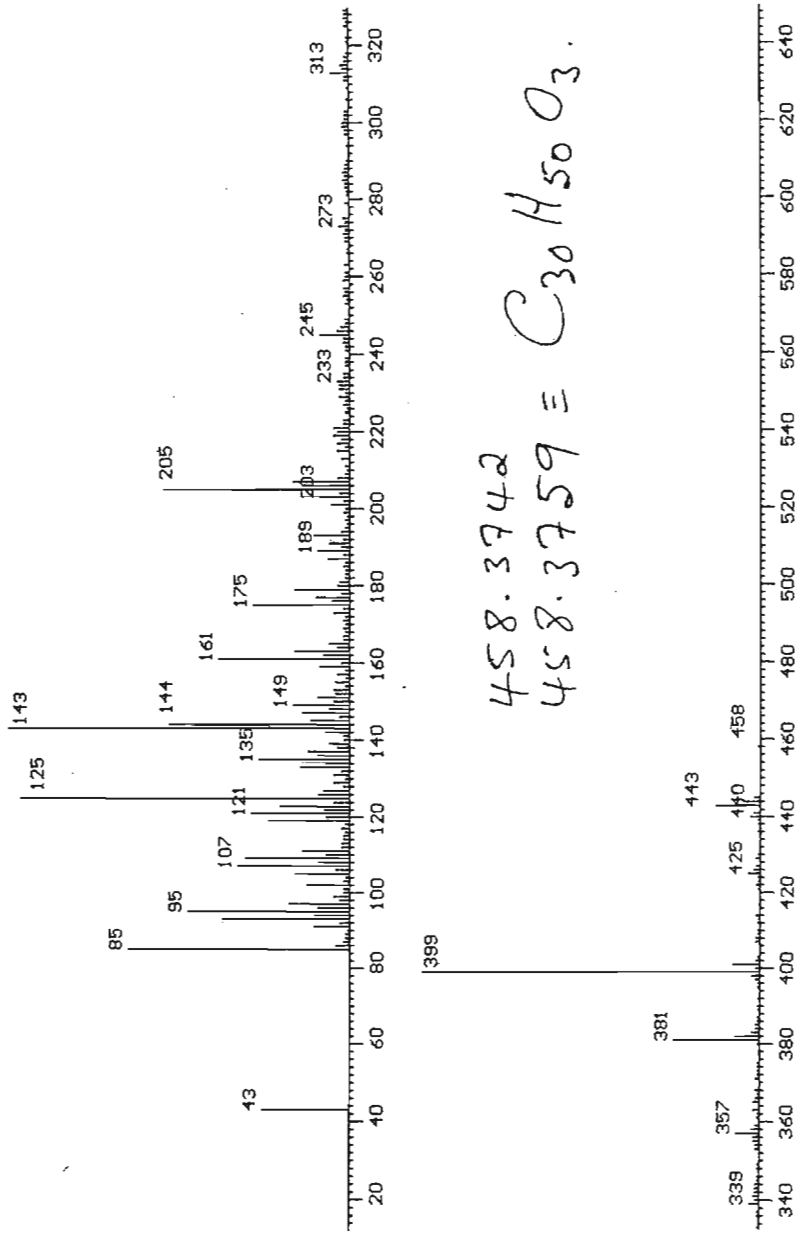


Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

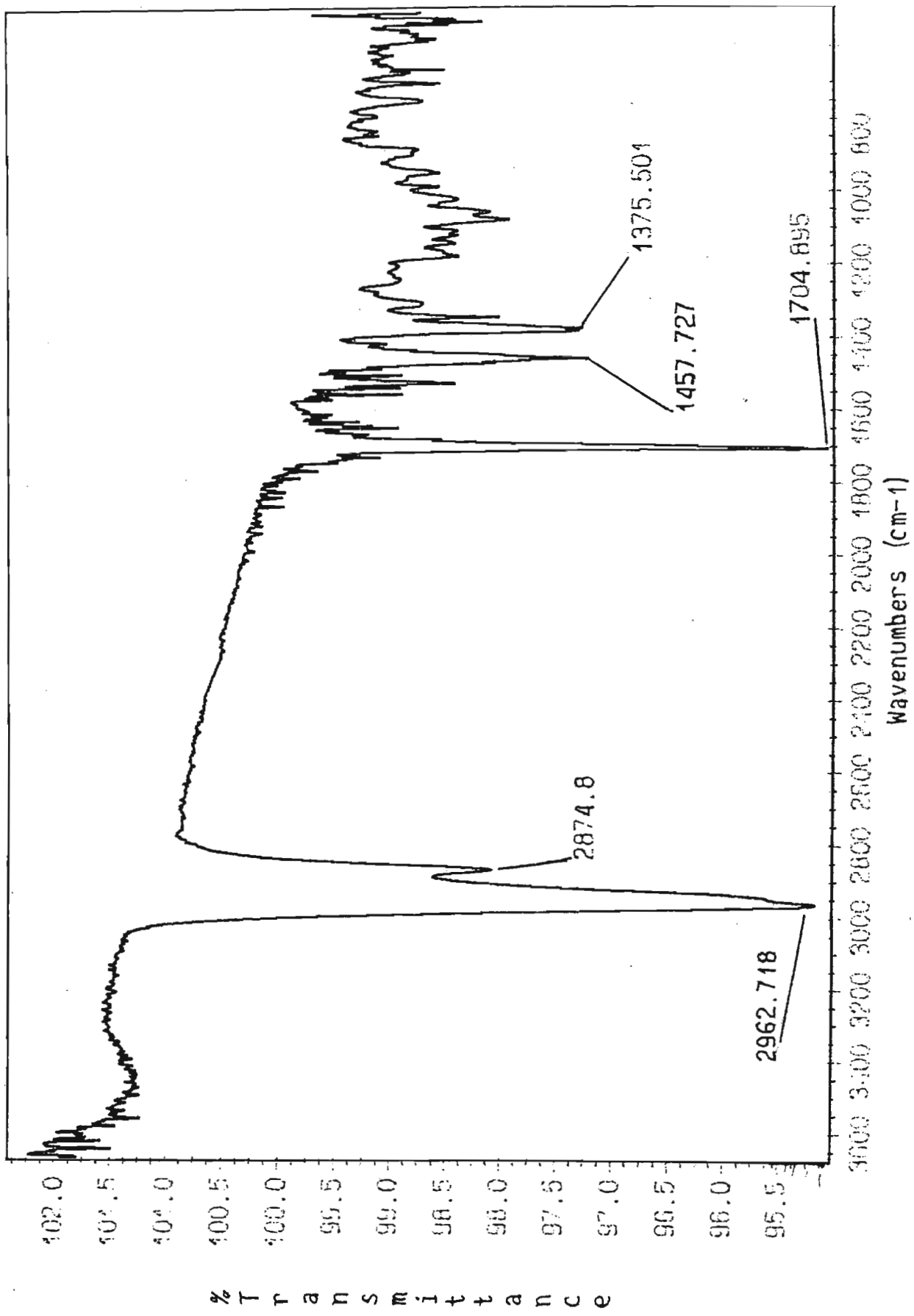
Compound 4-D



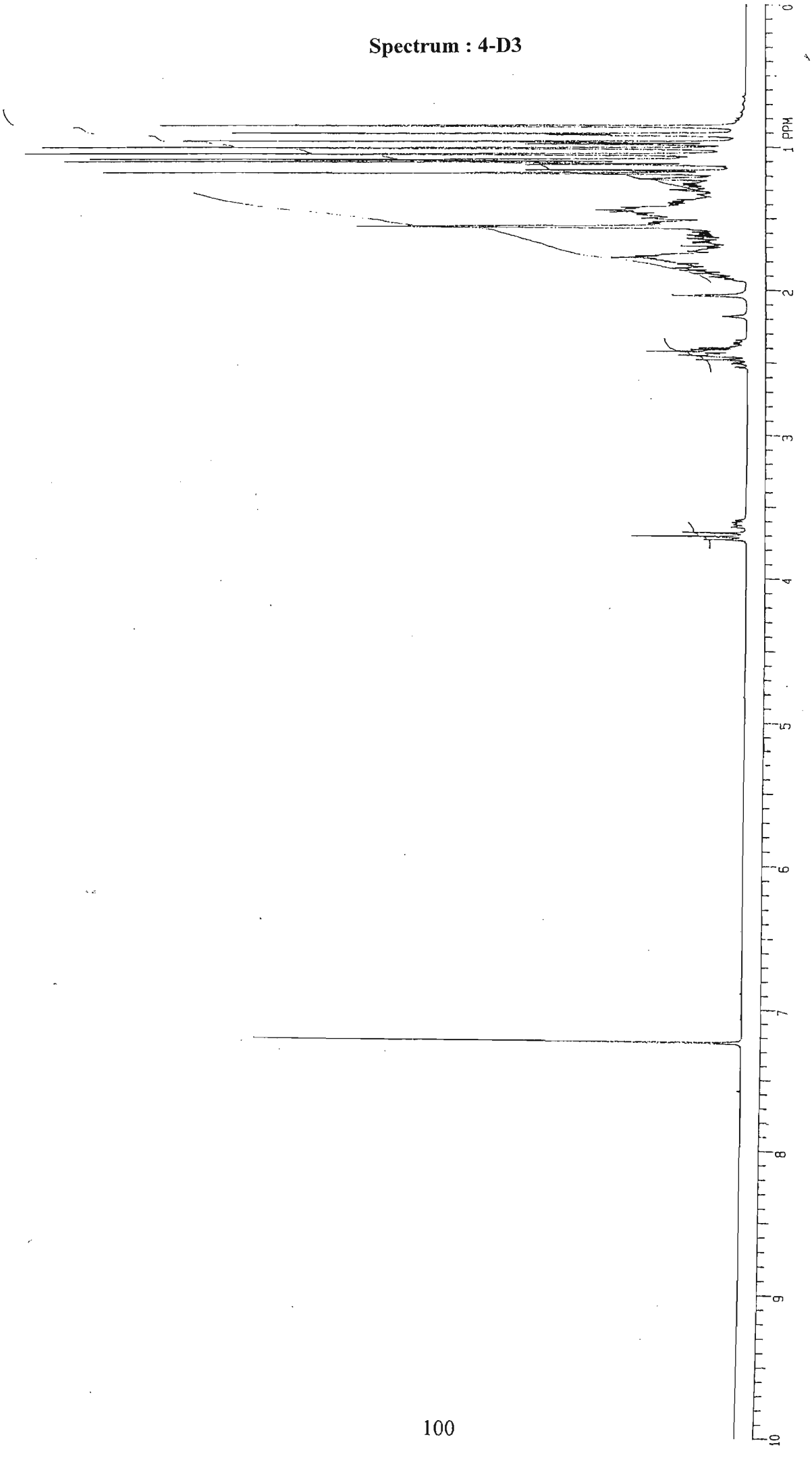
Spectrum : 4-D1



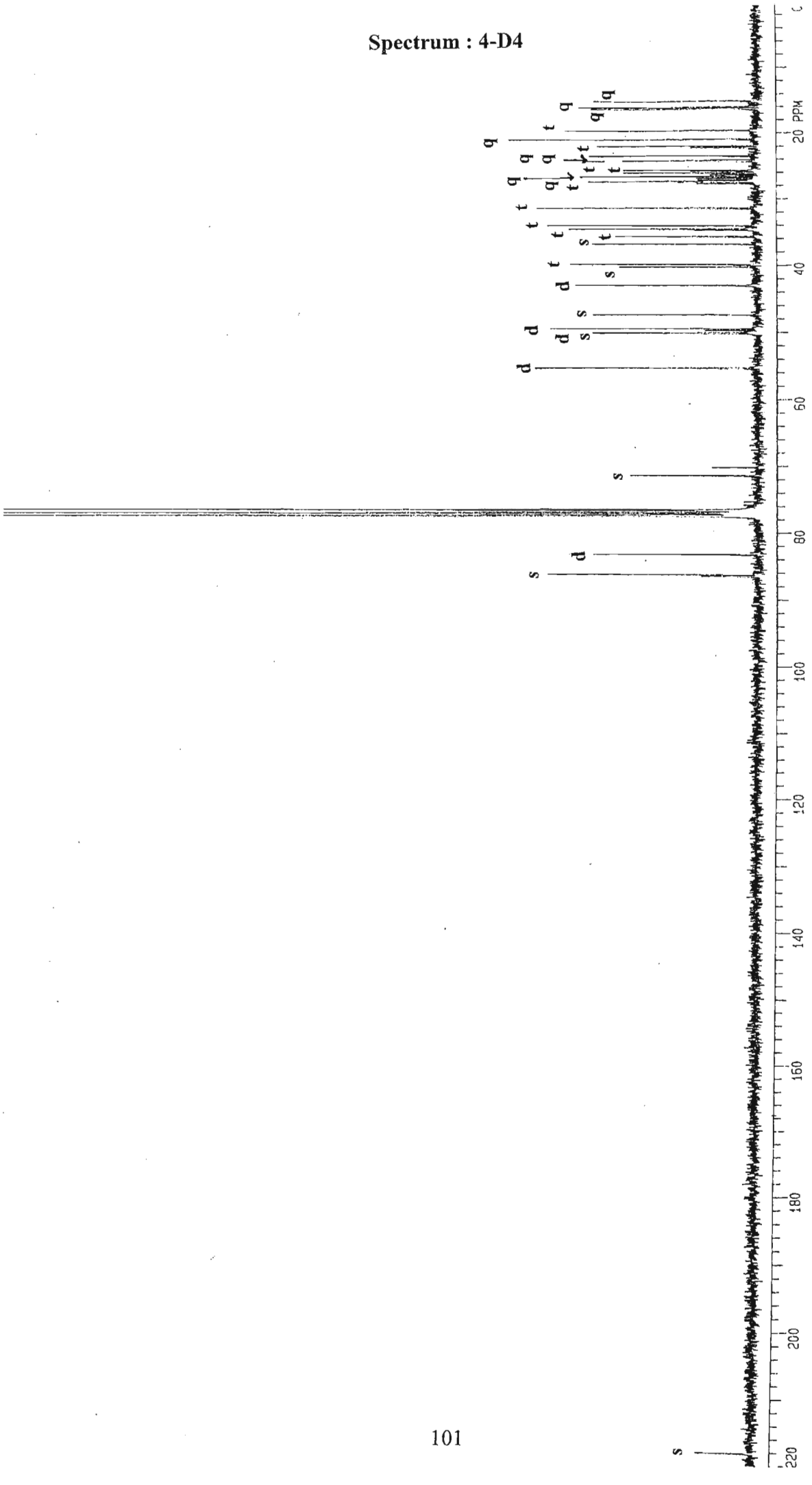
Spectrum : 4-D2



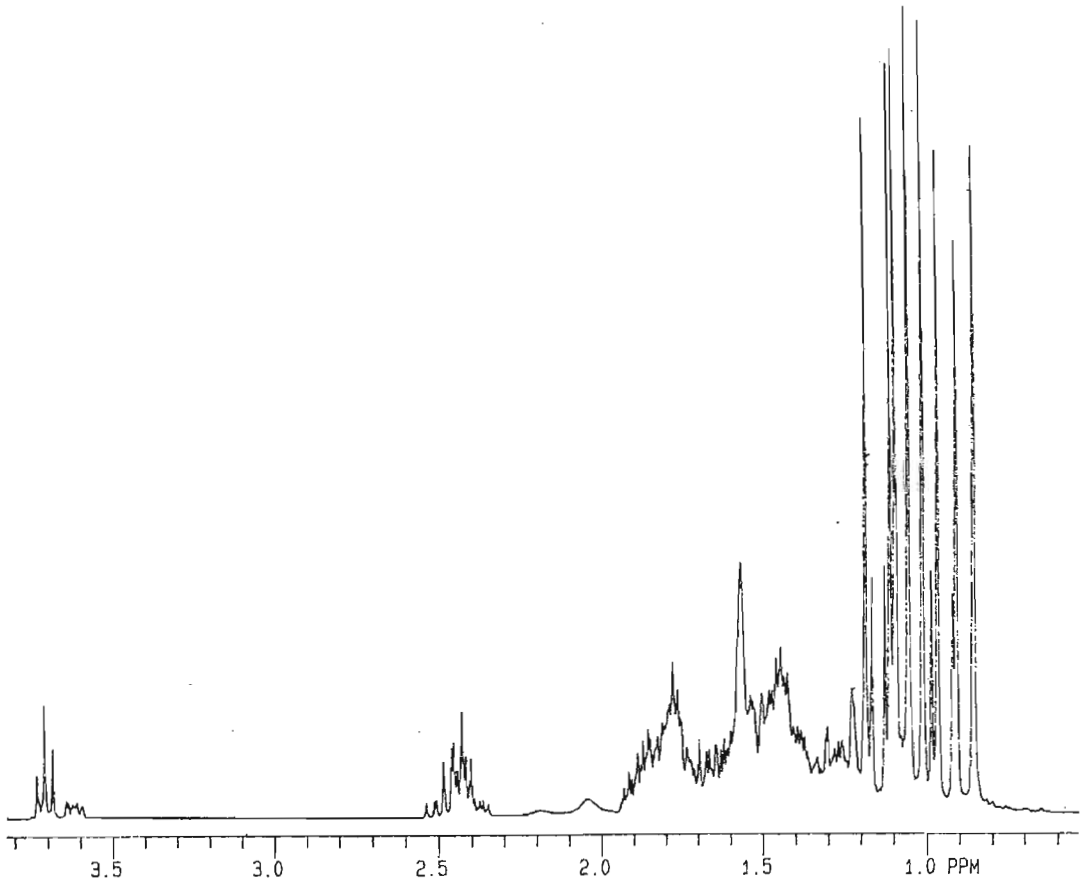
Spectrum : 4-D3

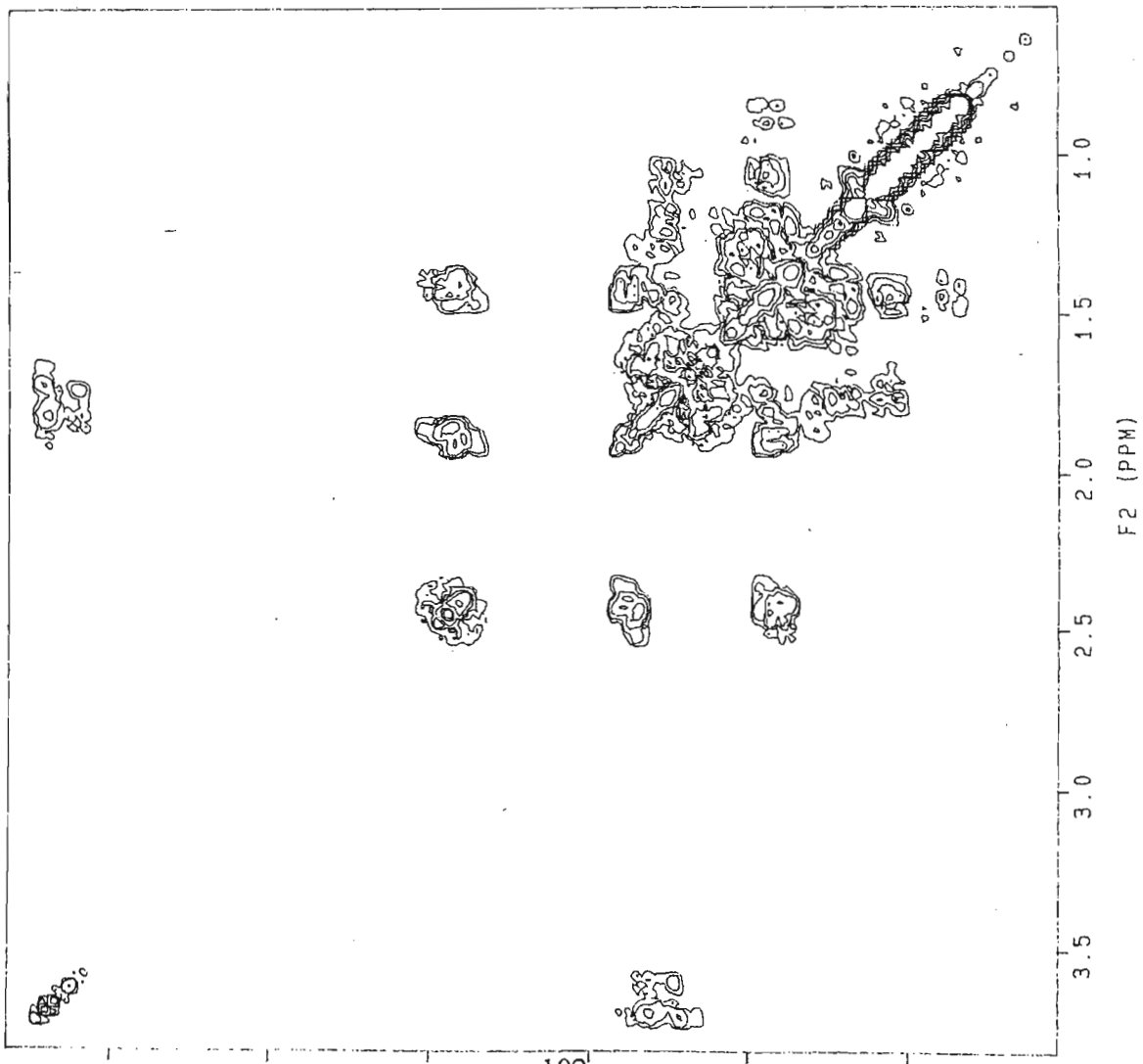


Spectrum : 4-D4

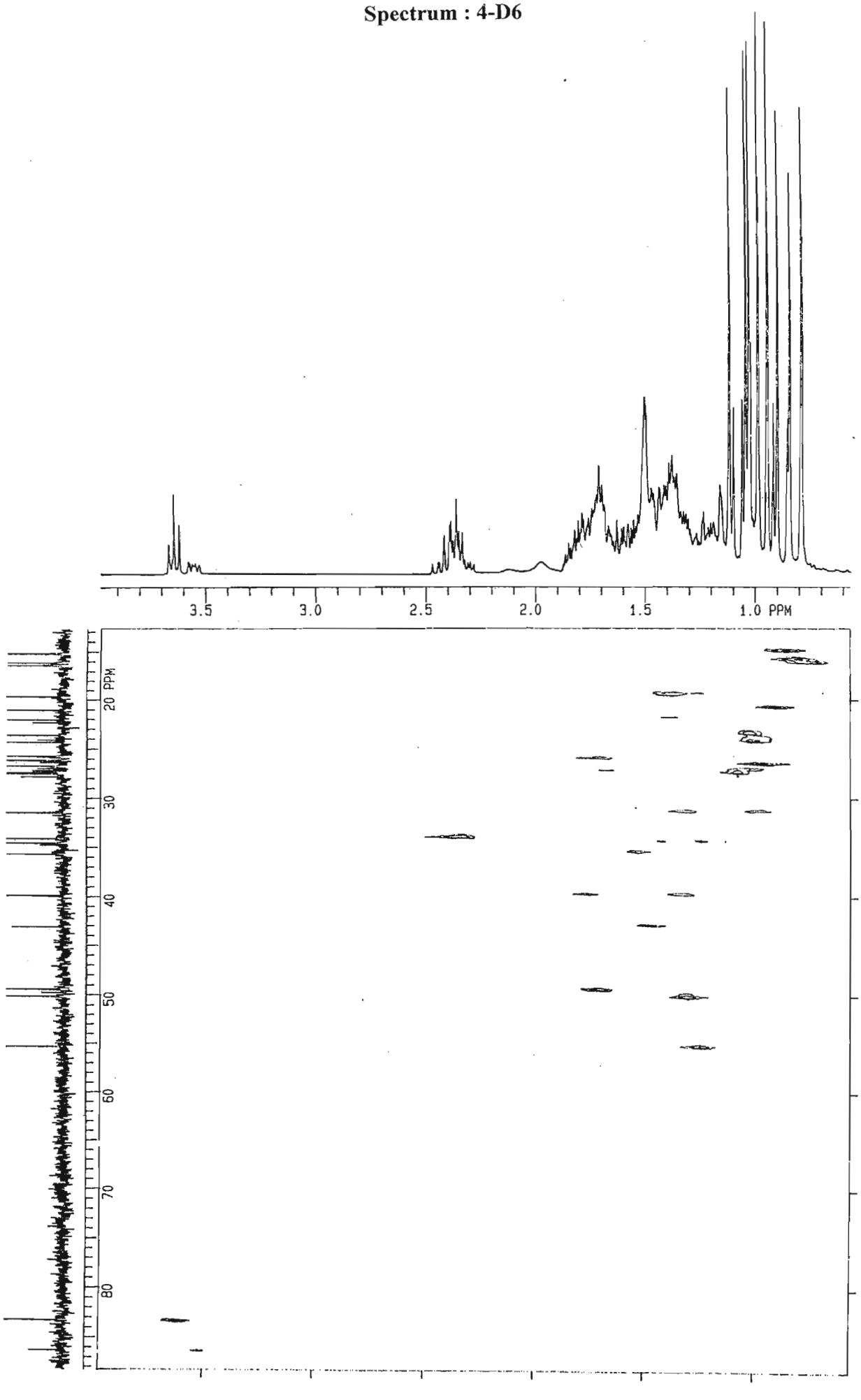


Spectrum : 4-D5

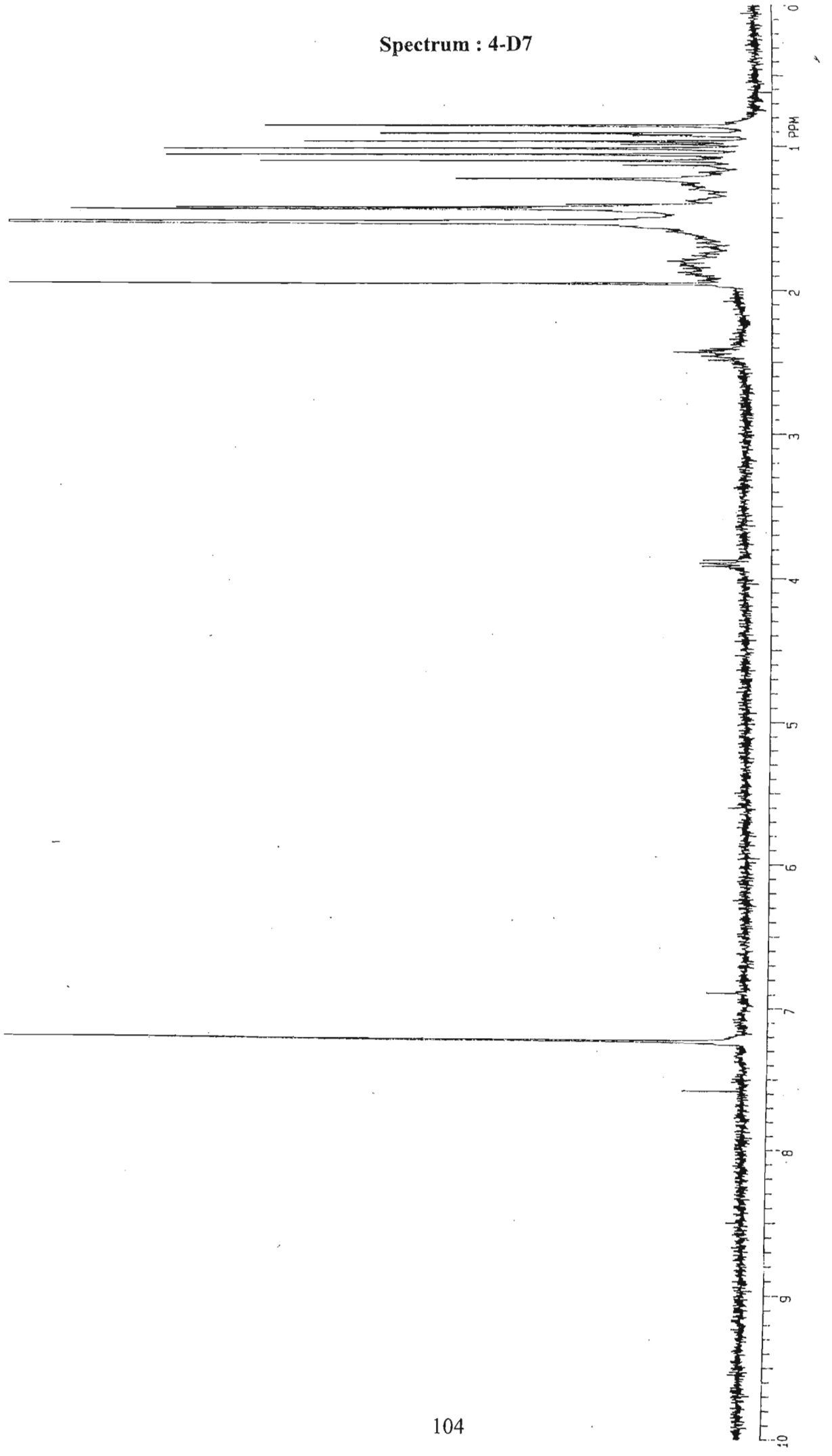




Spectrum : 4-D6

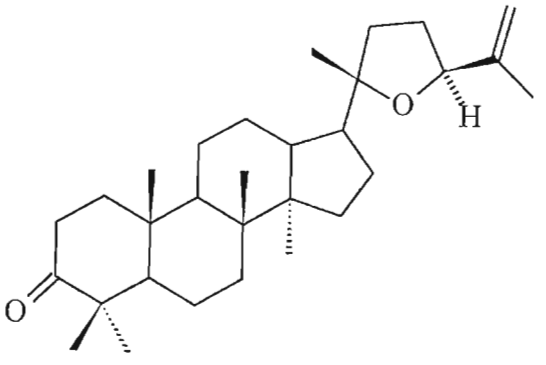


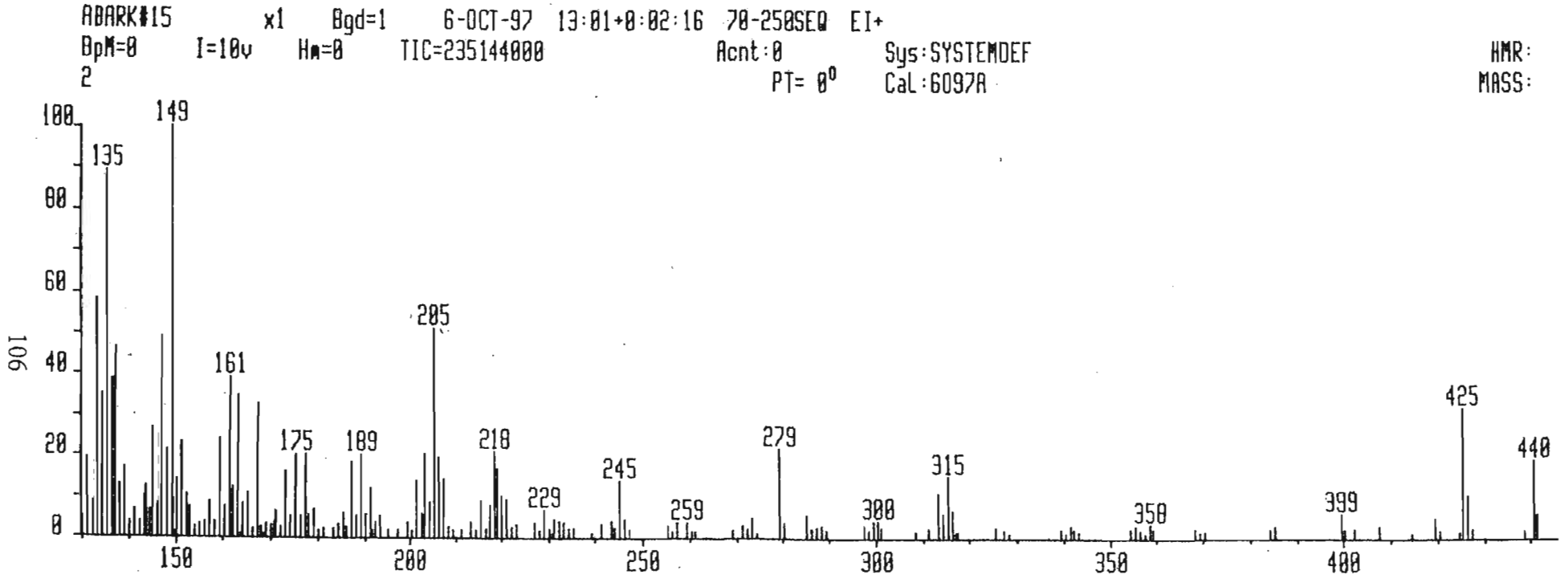
Spectrum : 4-D7



Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

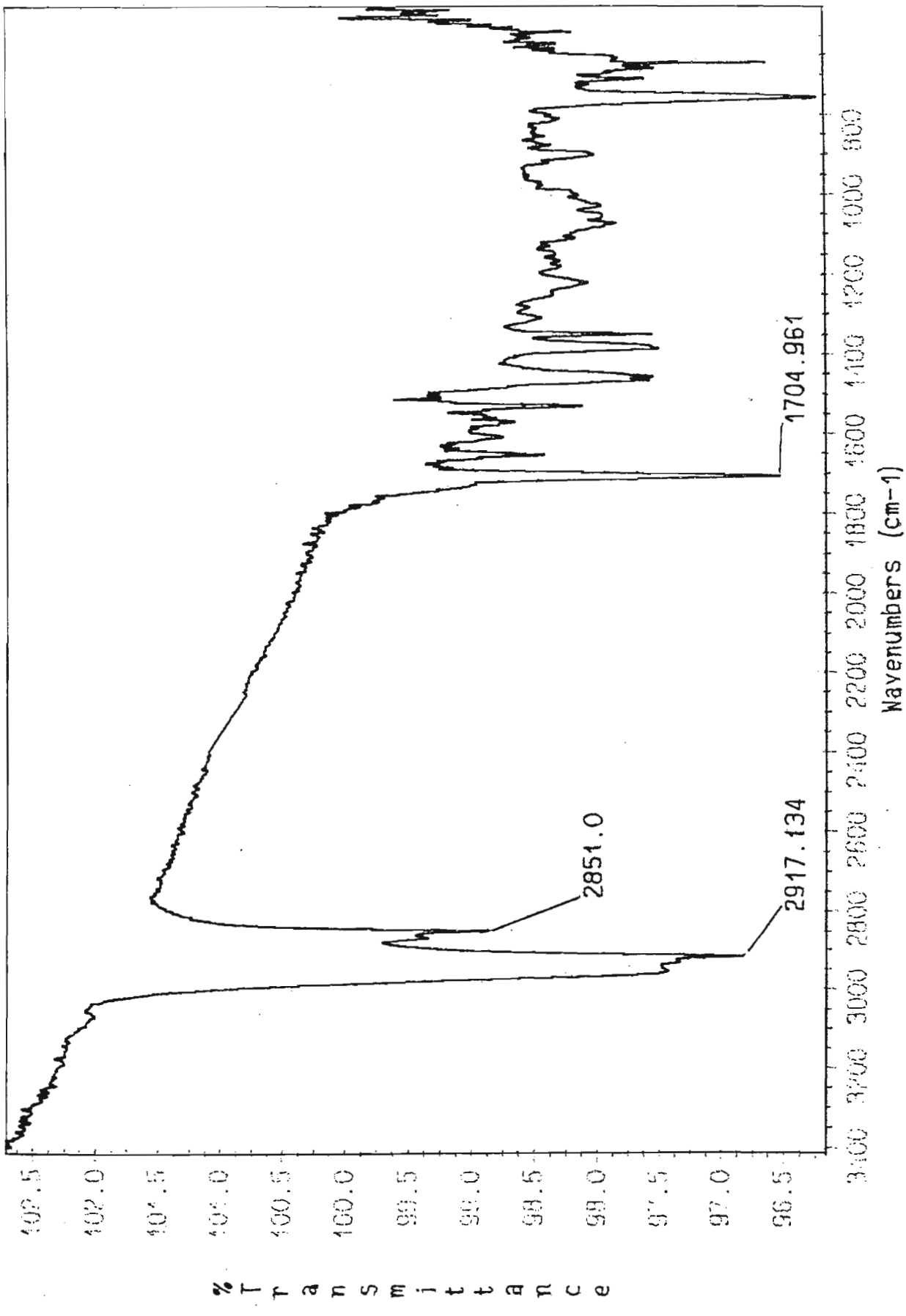
Compound 4-E



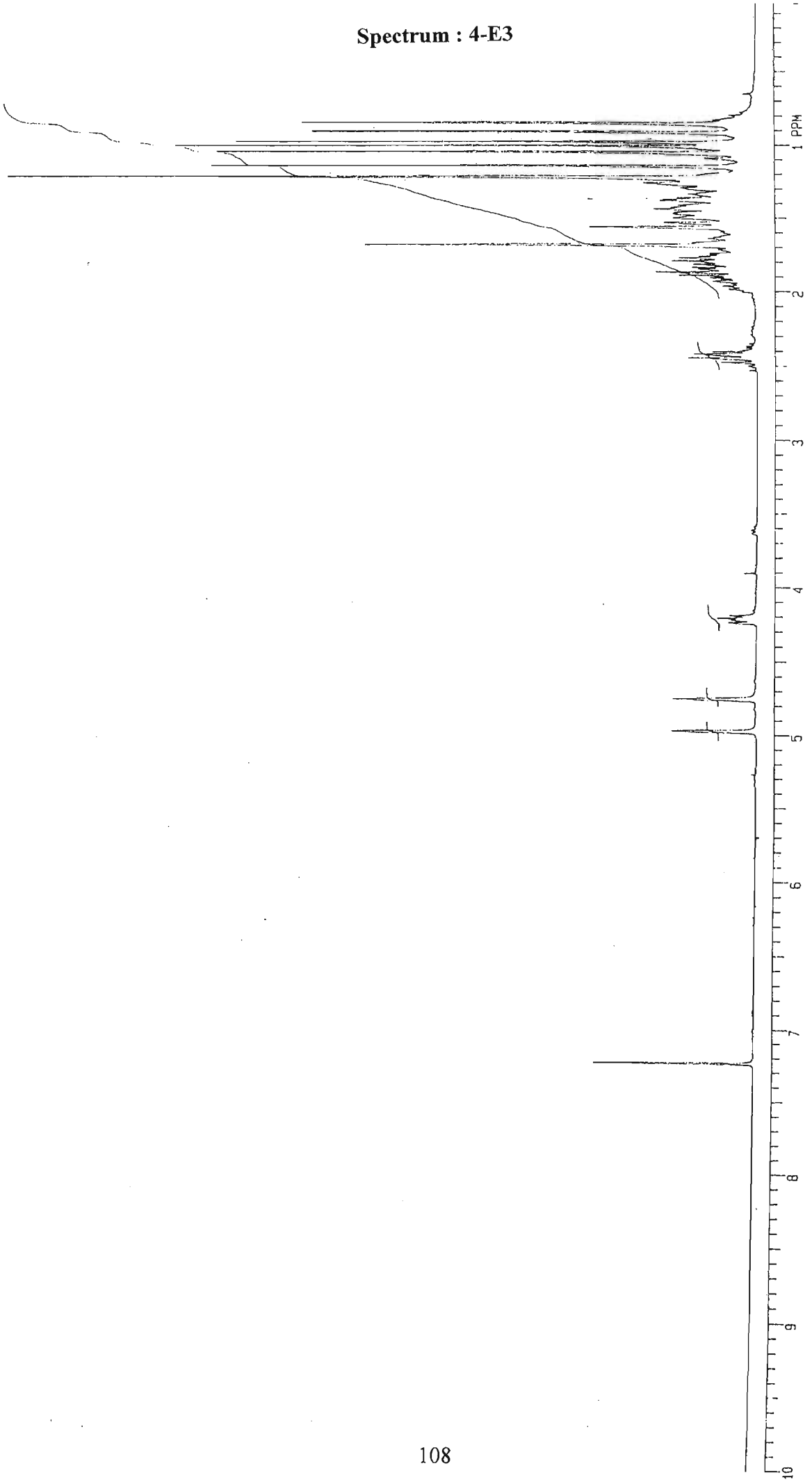


Spectrum : 4-E1

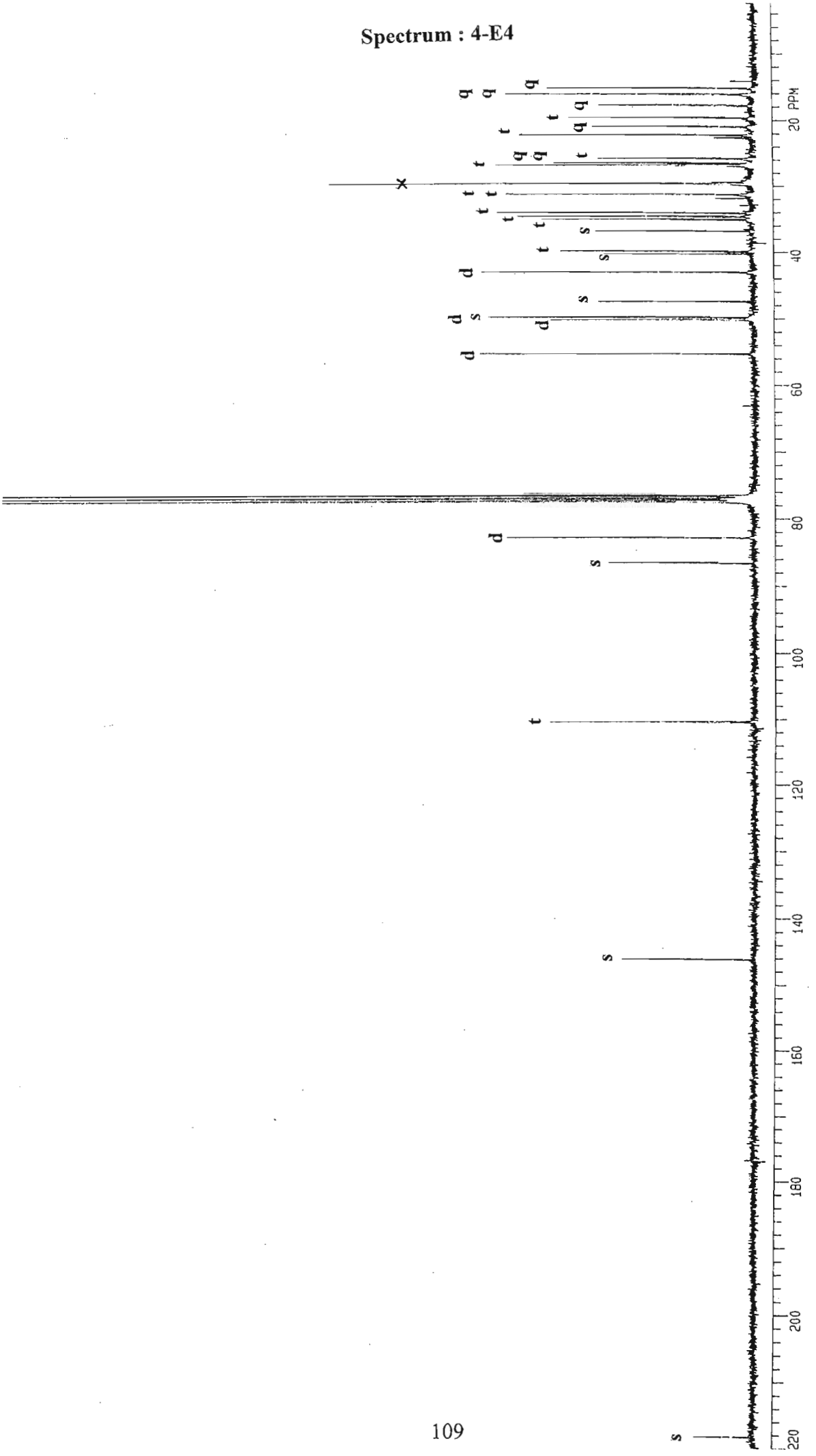
Spectrum : 4-E2



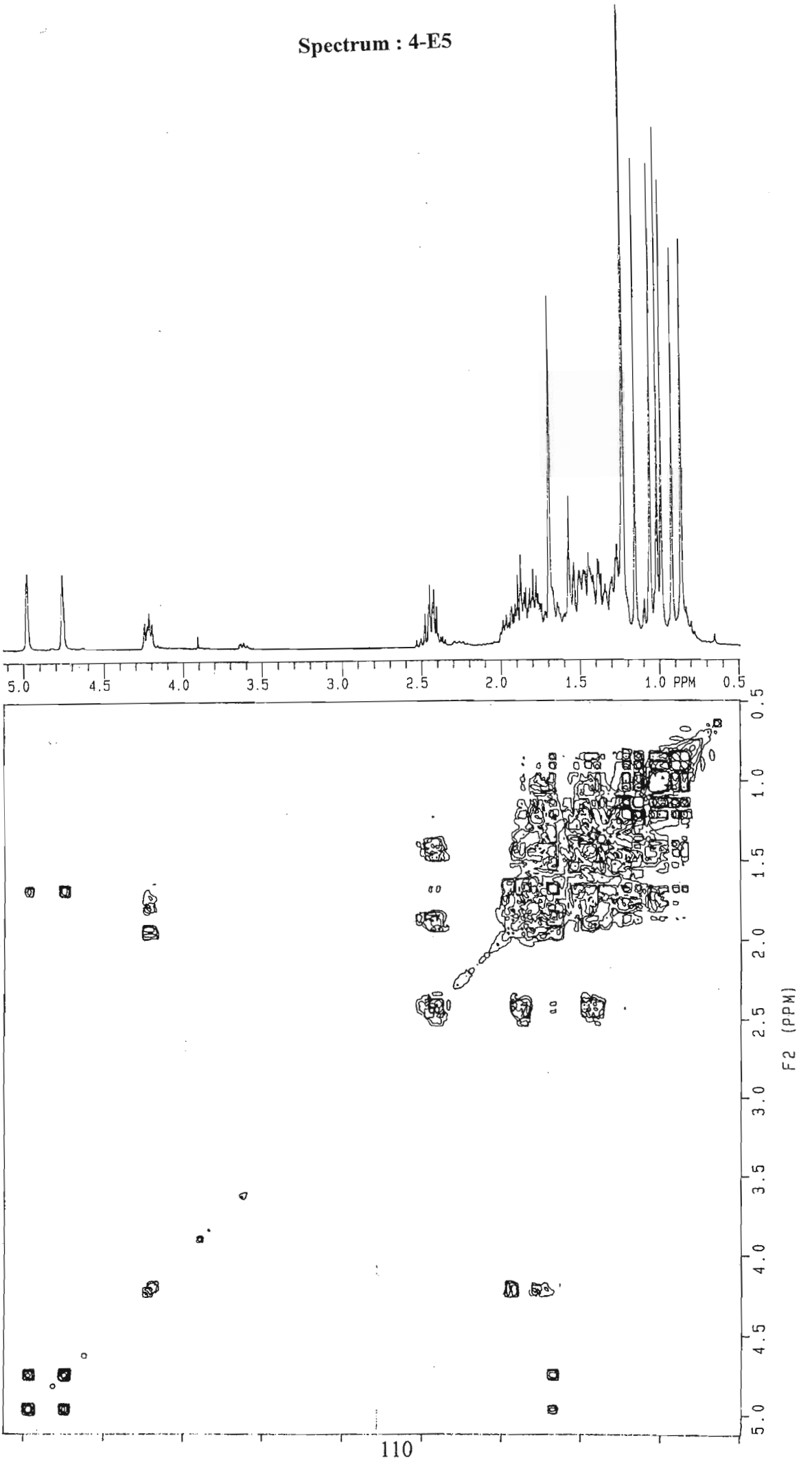
Spectrum : 4-E3



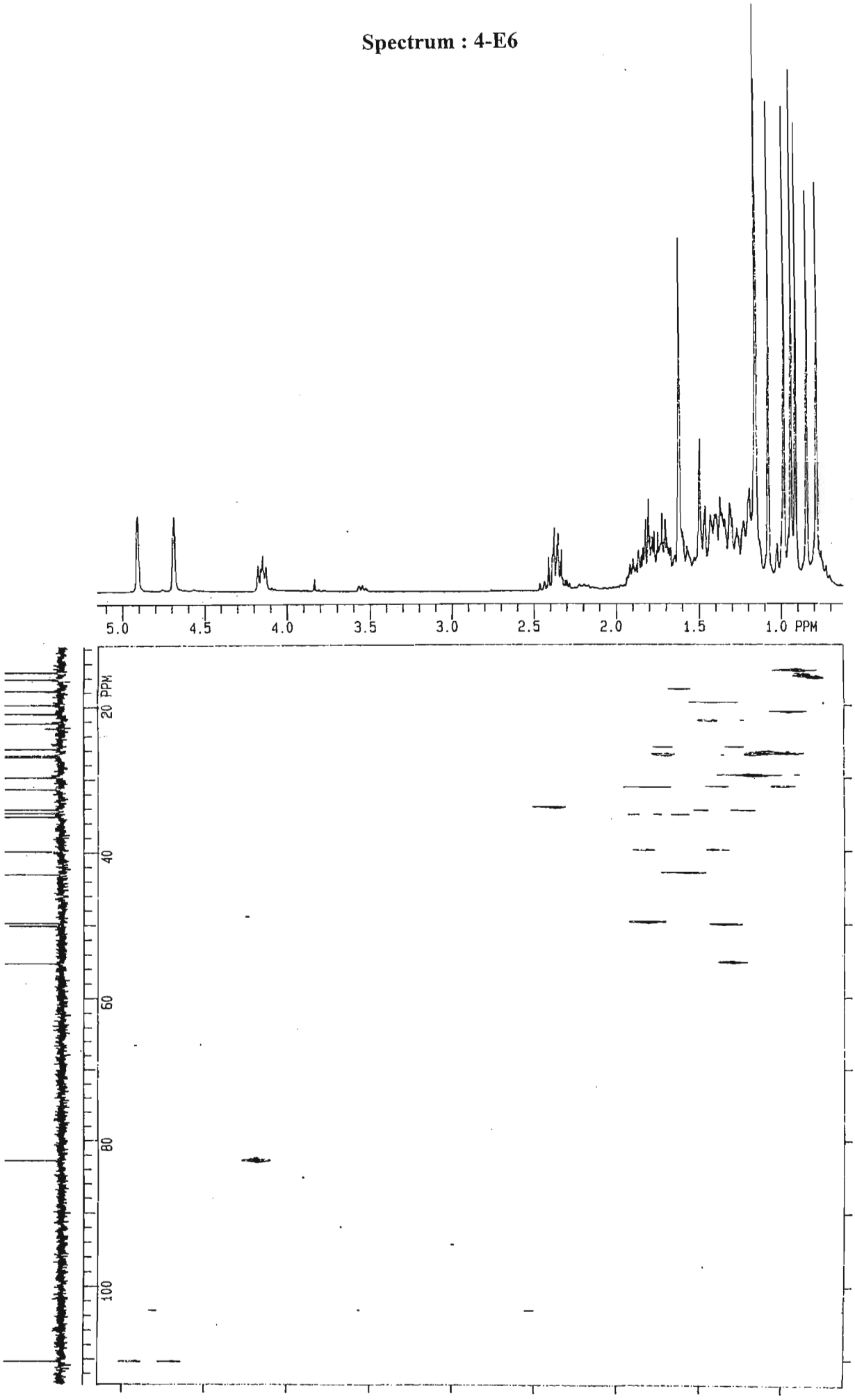
Spectrum : 4-E4



Spectrum : 4-E5

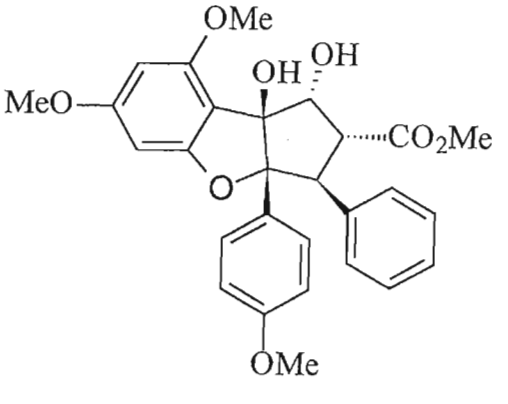


Spectrum : 4-E6

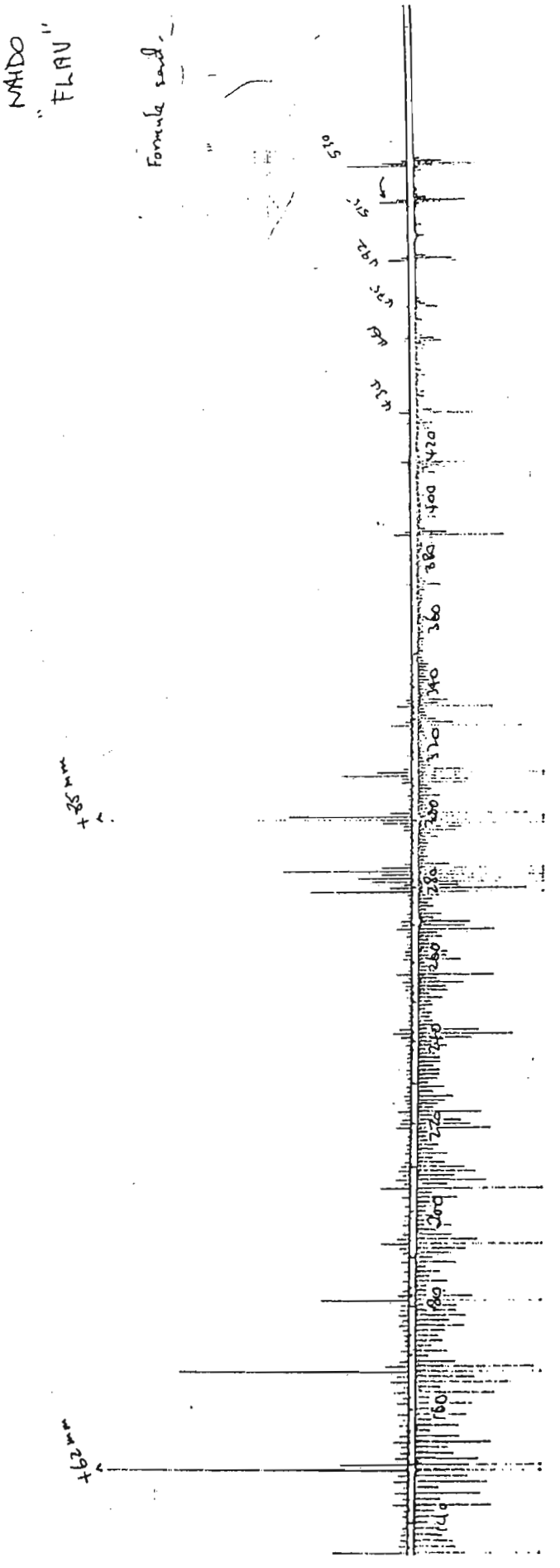


Extractives from *Dysoxylum pettigrewianum, Dysoxylum muelleri and Aglaia sapindina*

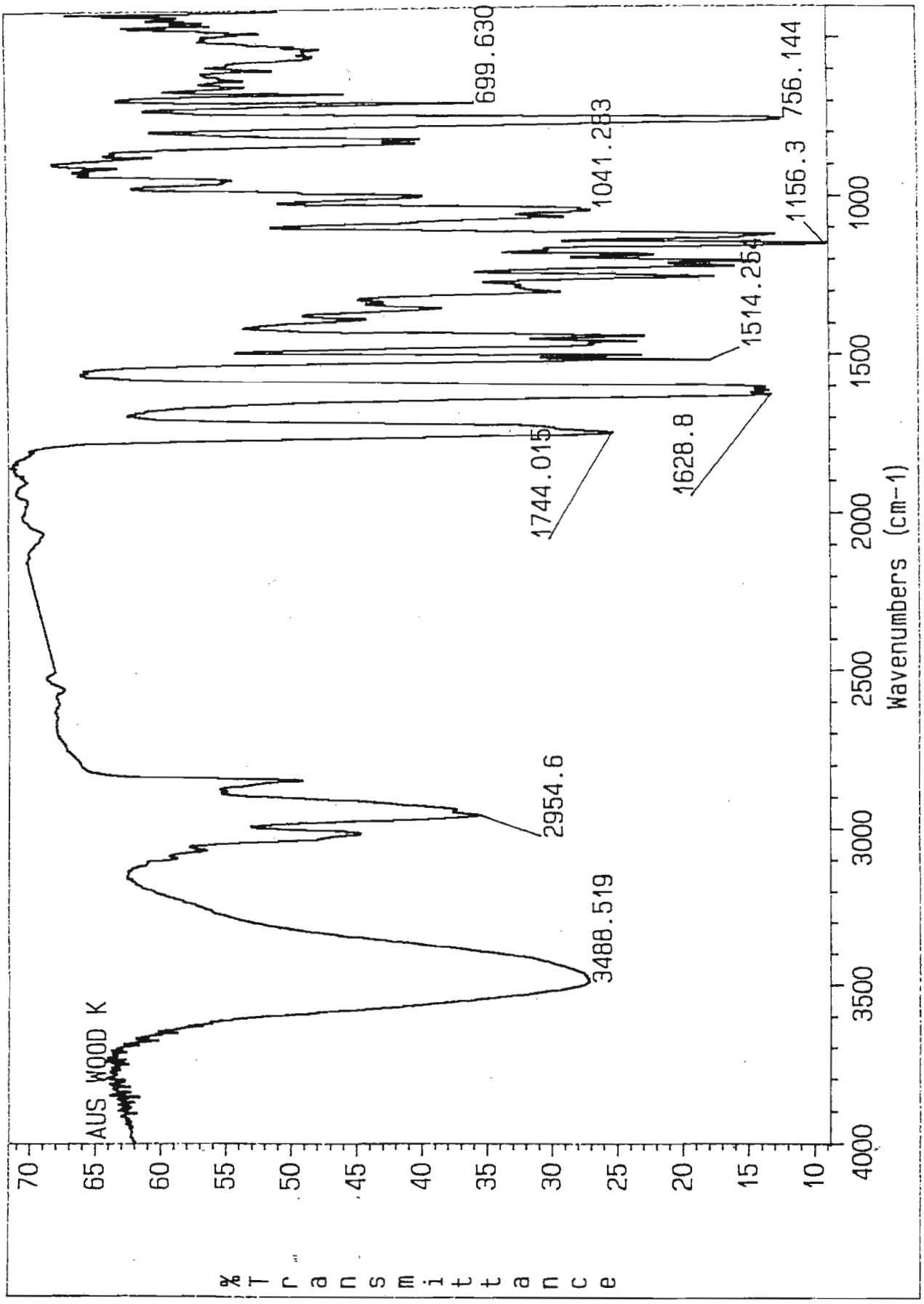
Compound 4-F



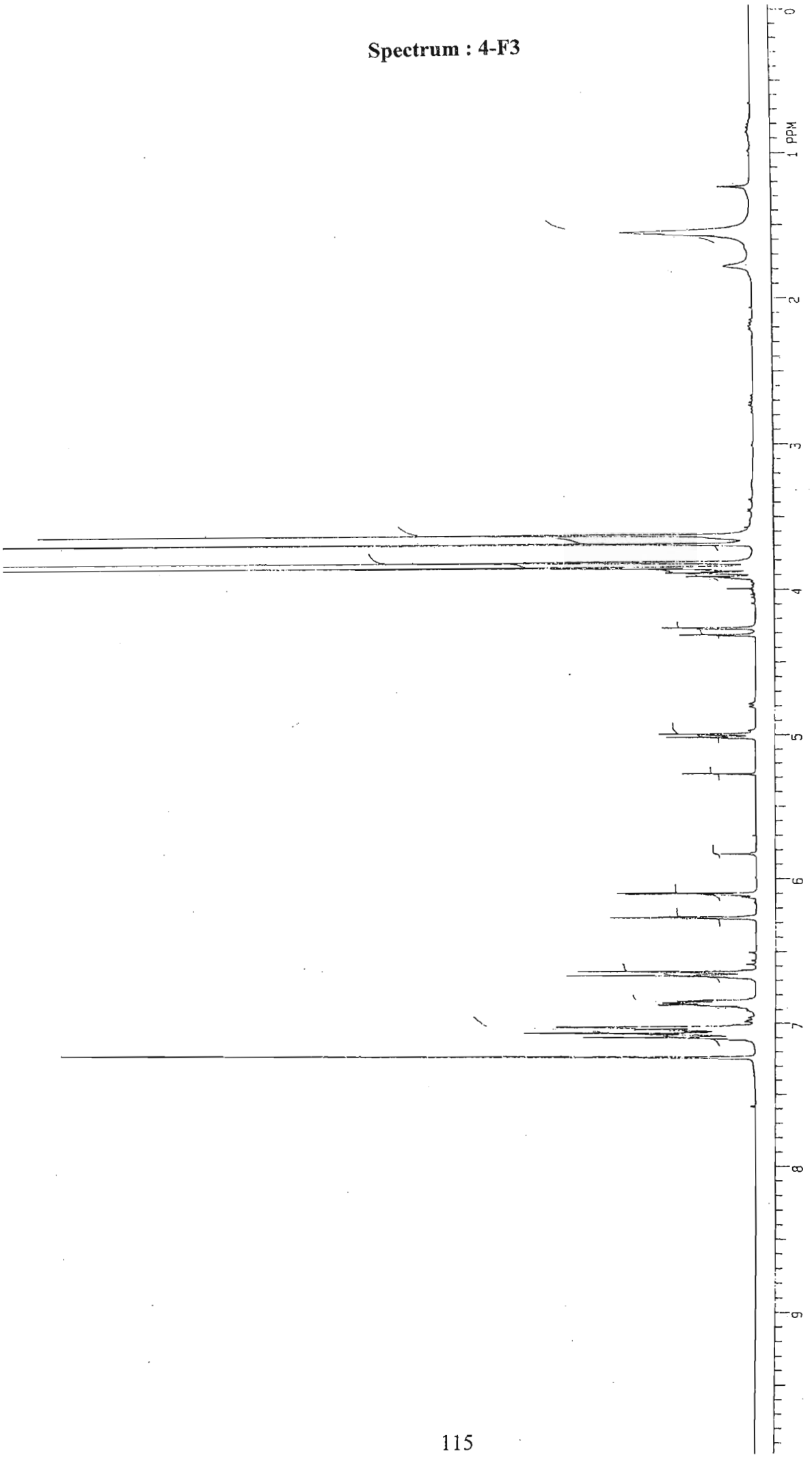
Spectrum : 4-F1



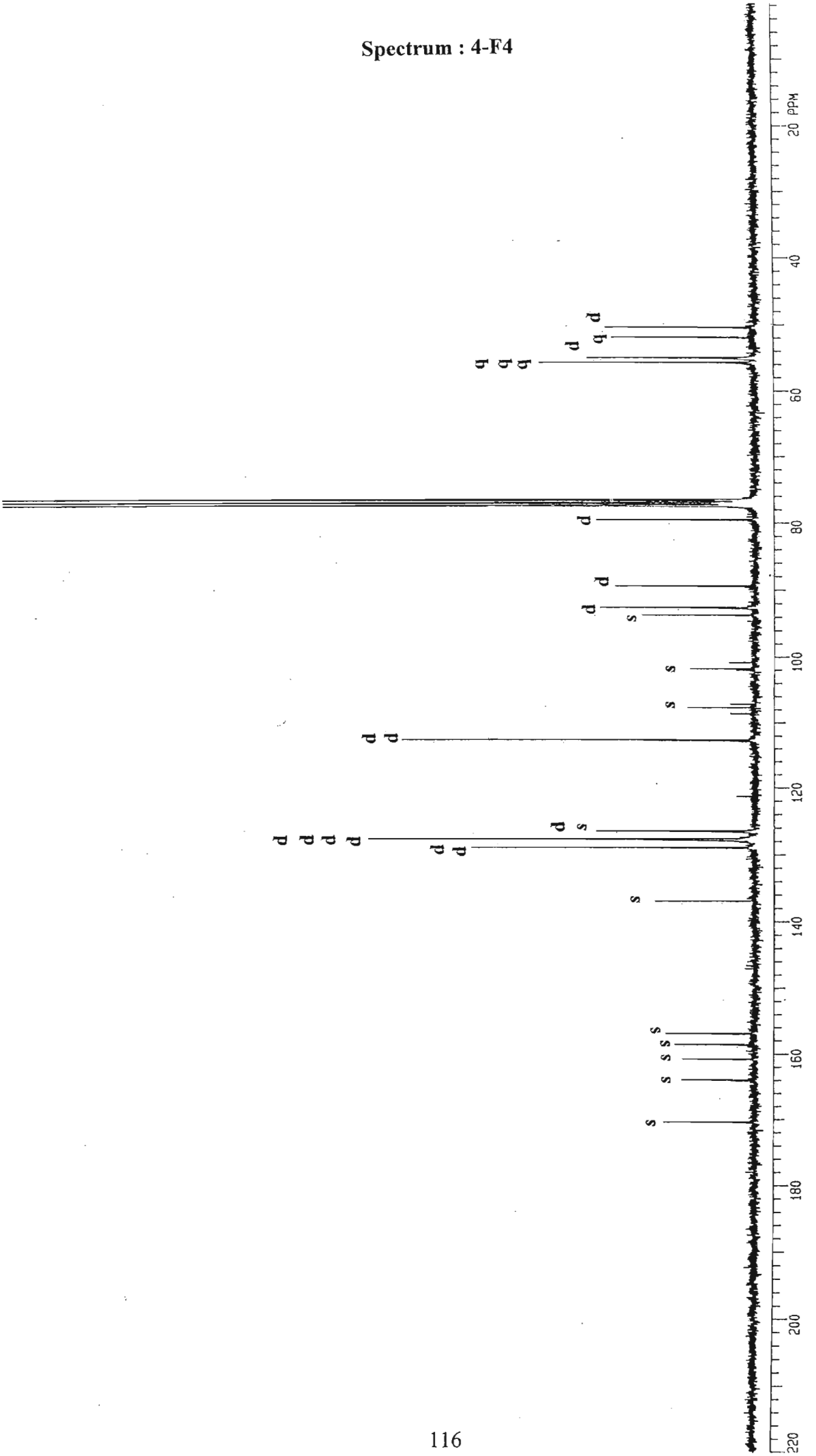
Spectrum : 4-F2



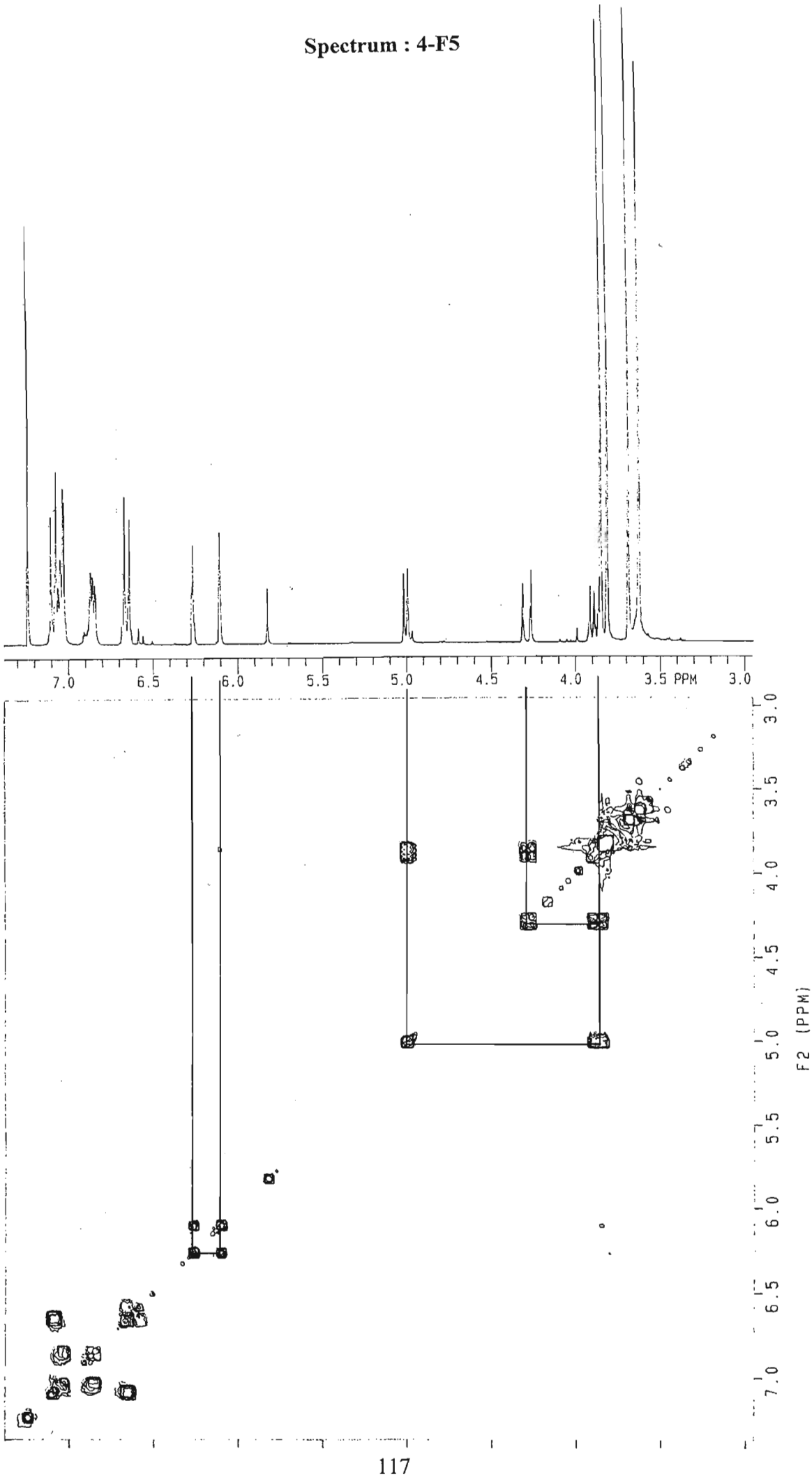
Spectrum : 4-F3



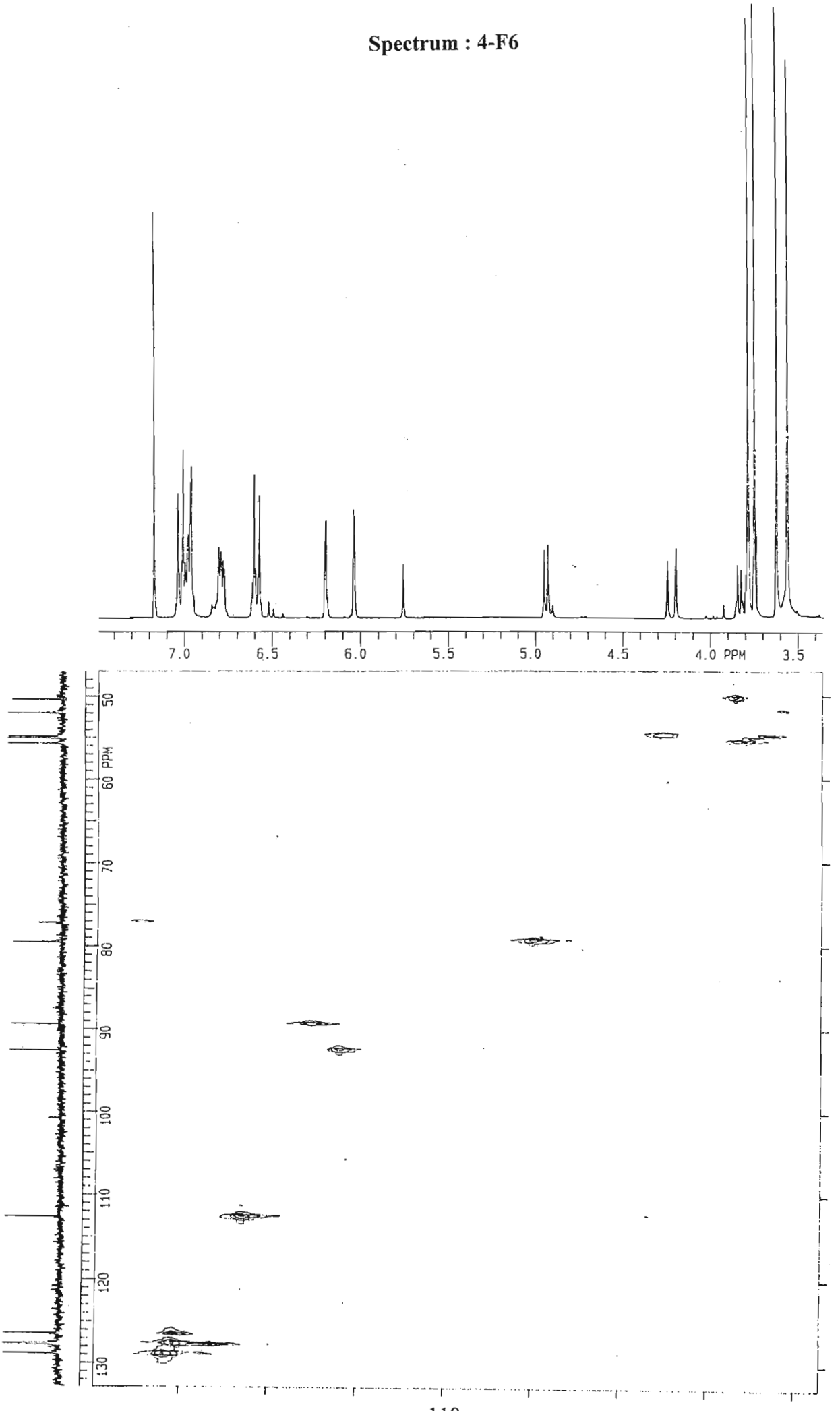
Spectrum : 4-F4



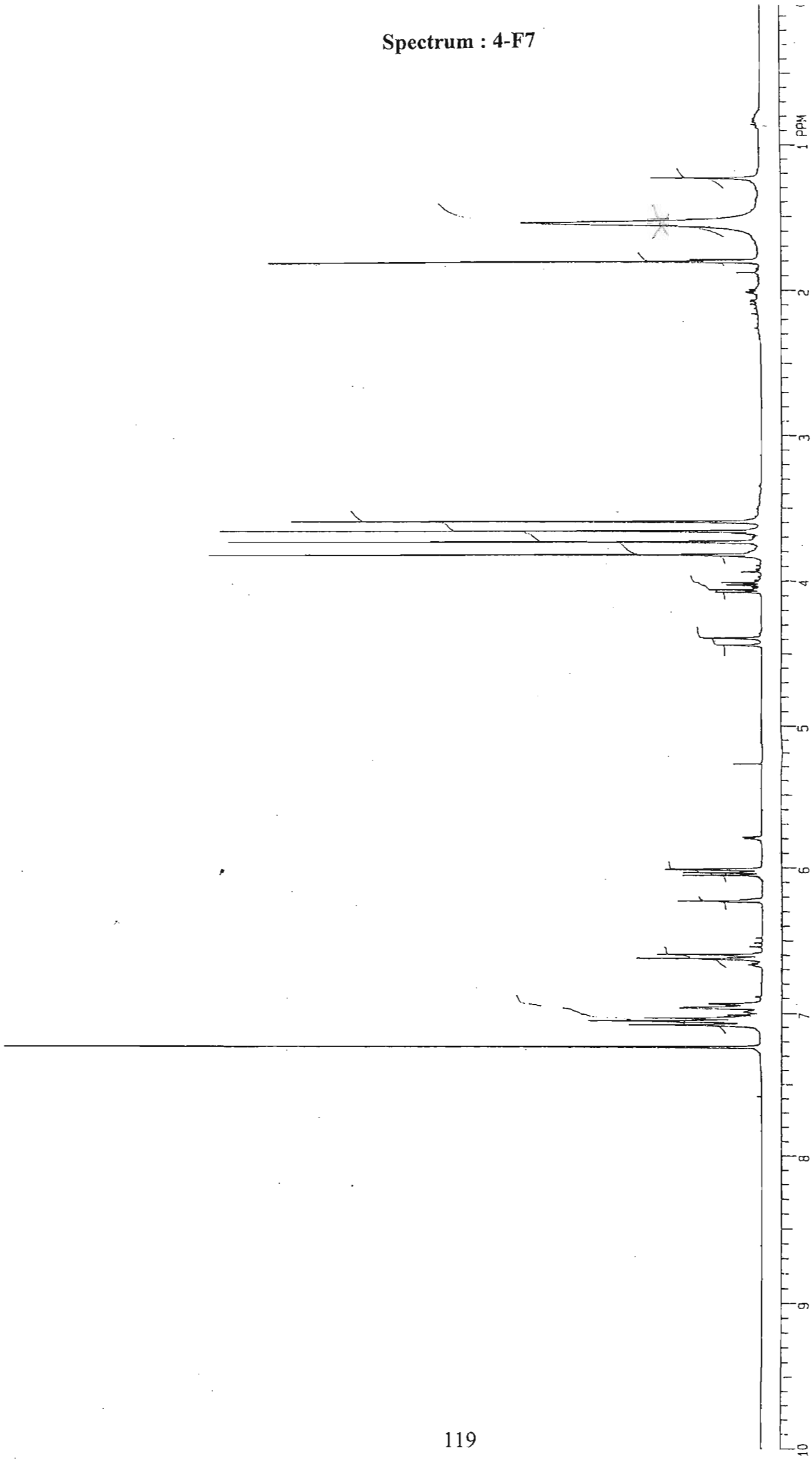
Spectrum : 4-F5



Spectrum : 4-F6

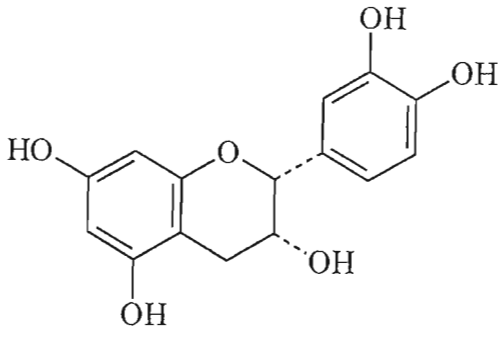


Spectrum : 4-F7

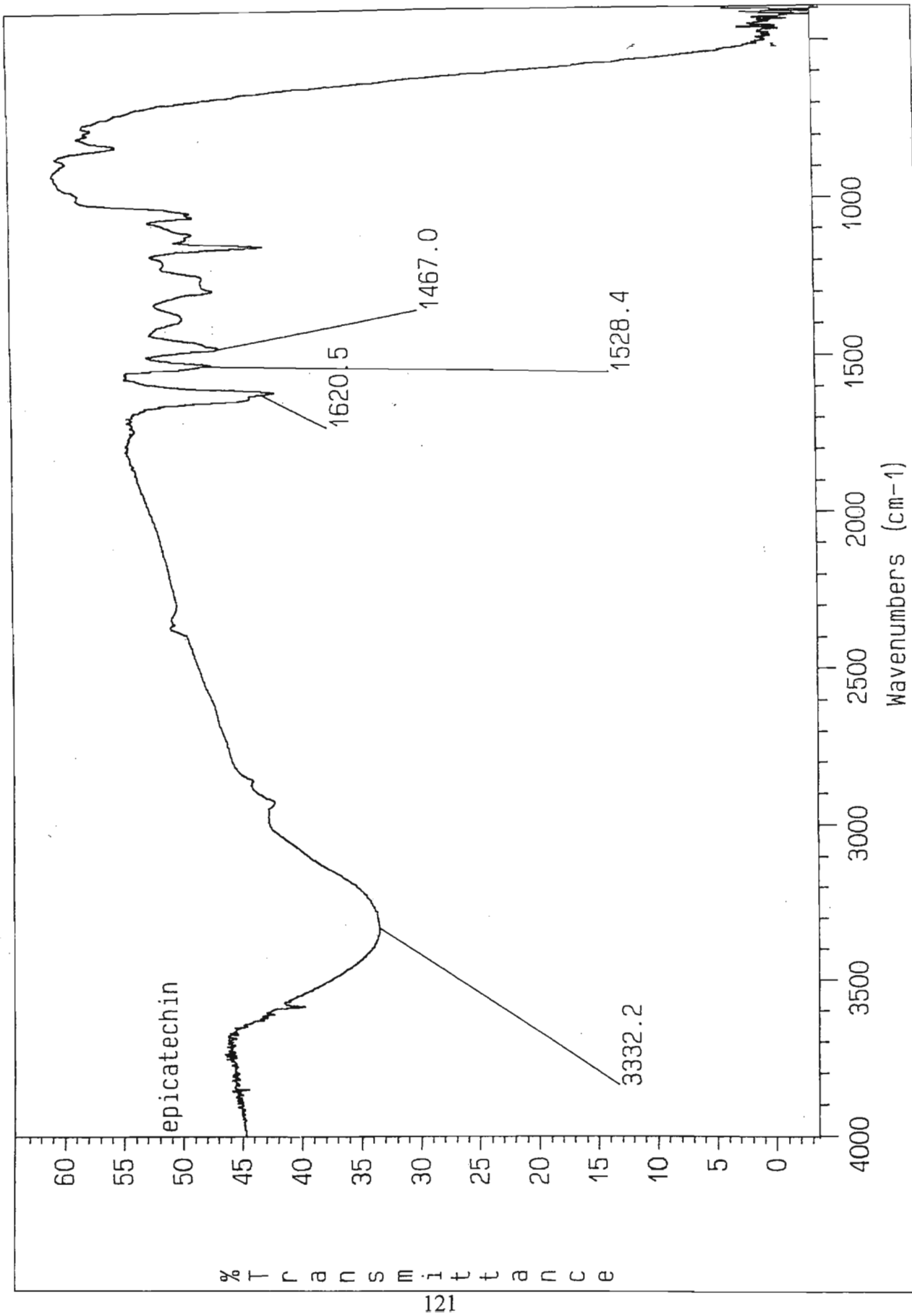


Extractives from *Trichilia dregeana*

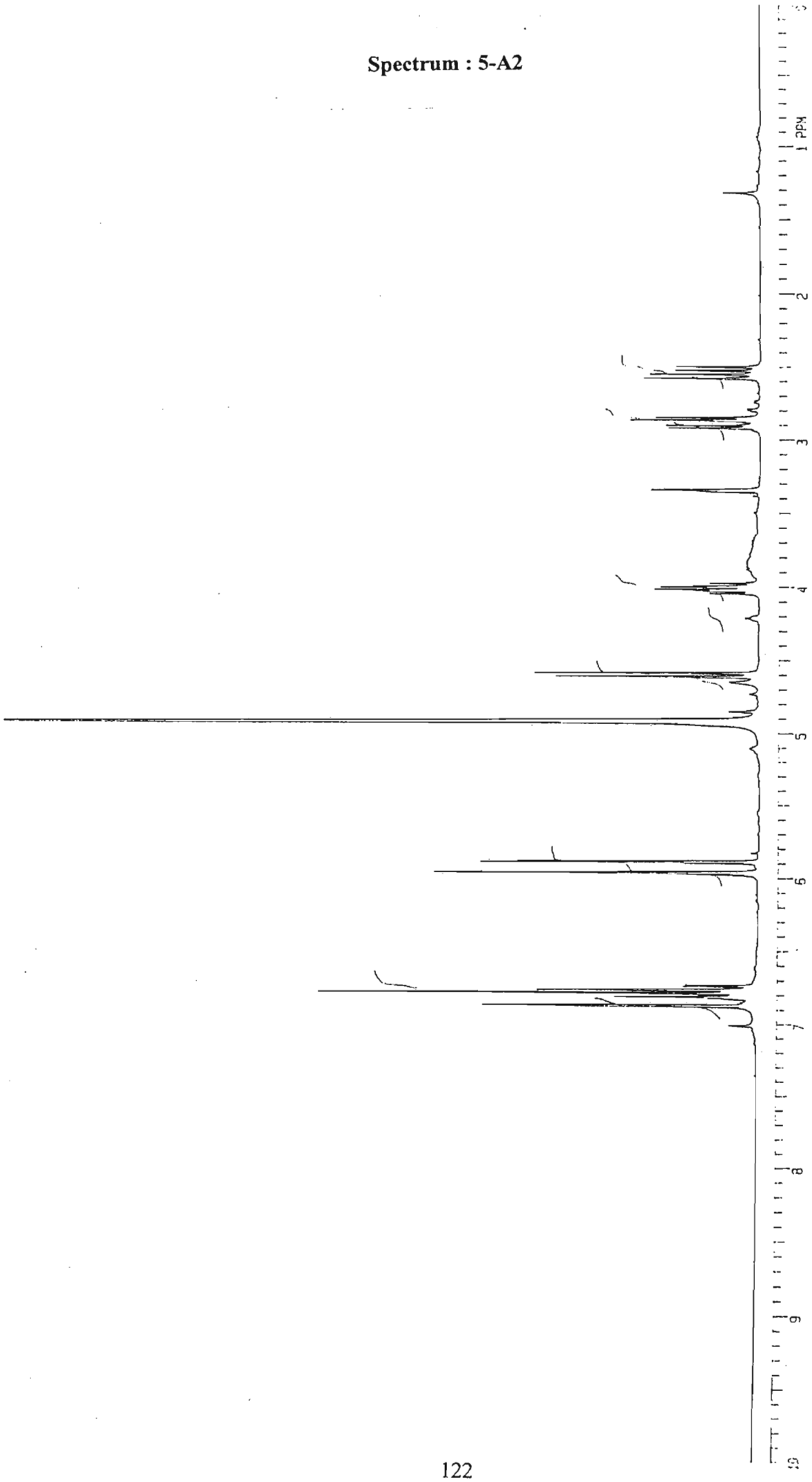
Compound 5-A



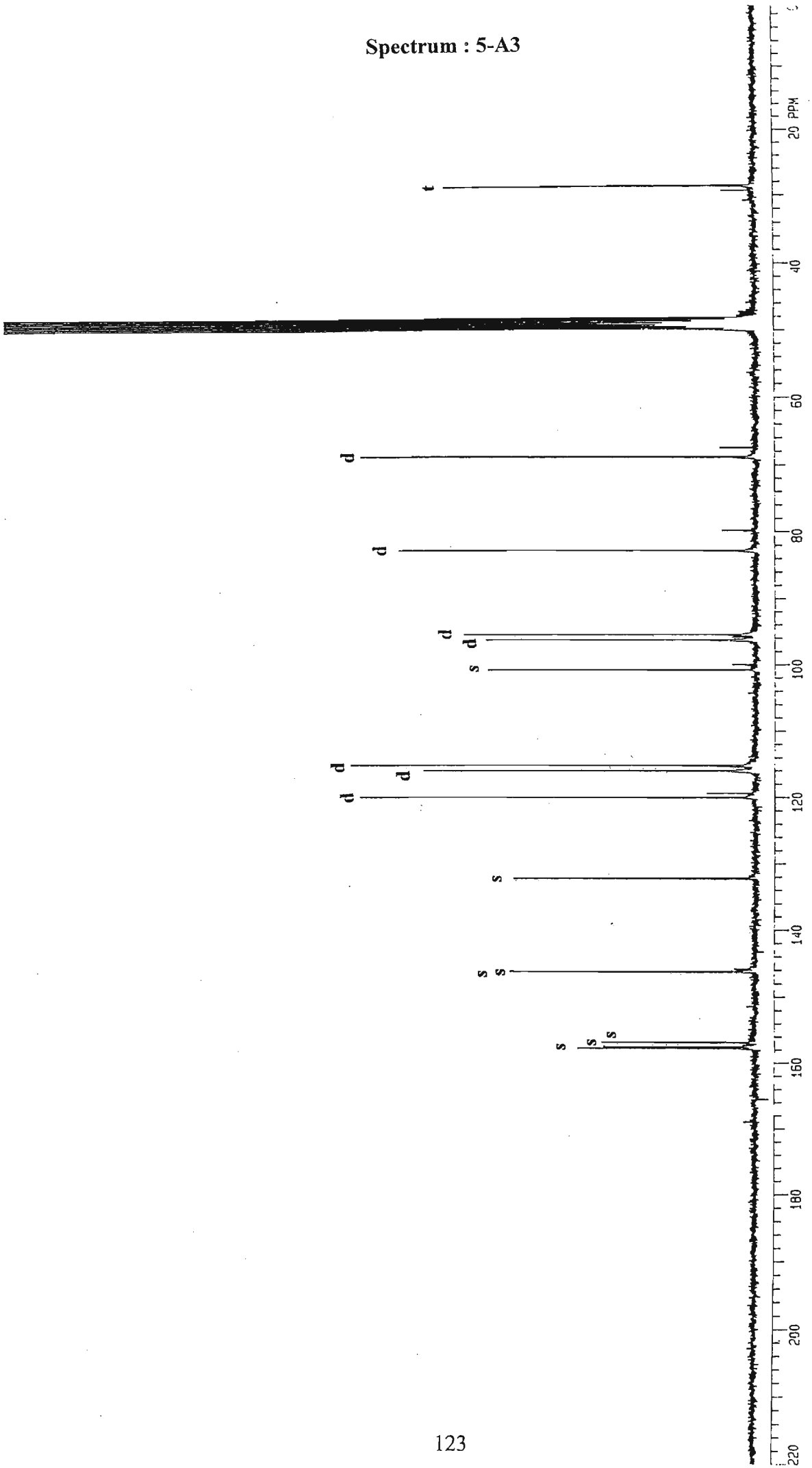
Spectrum : 5-A1



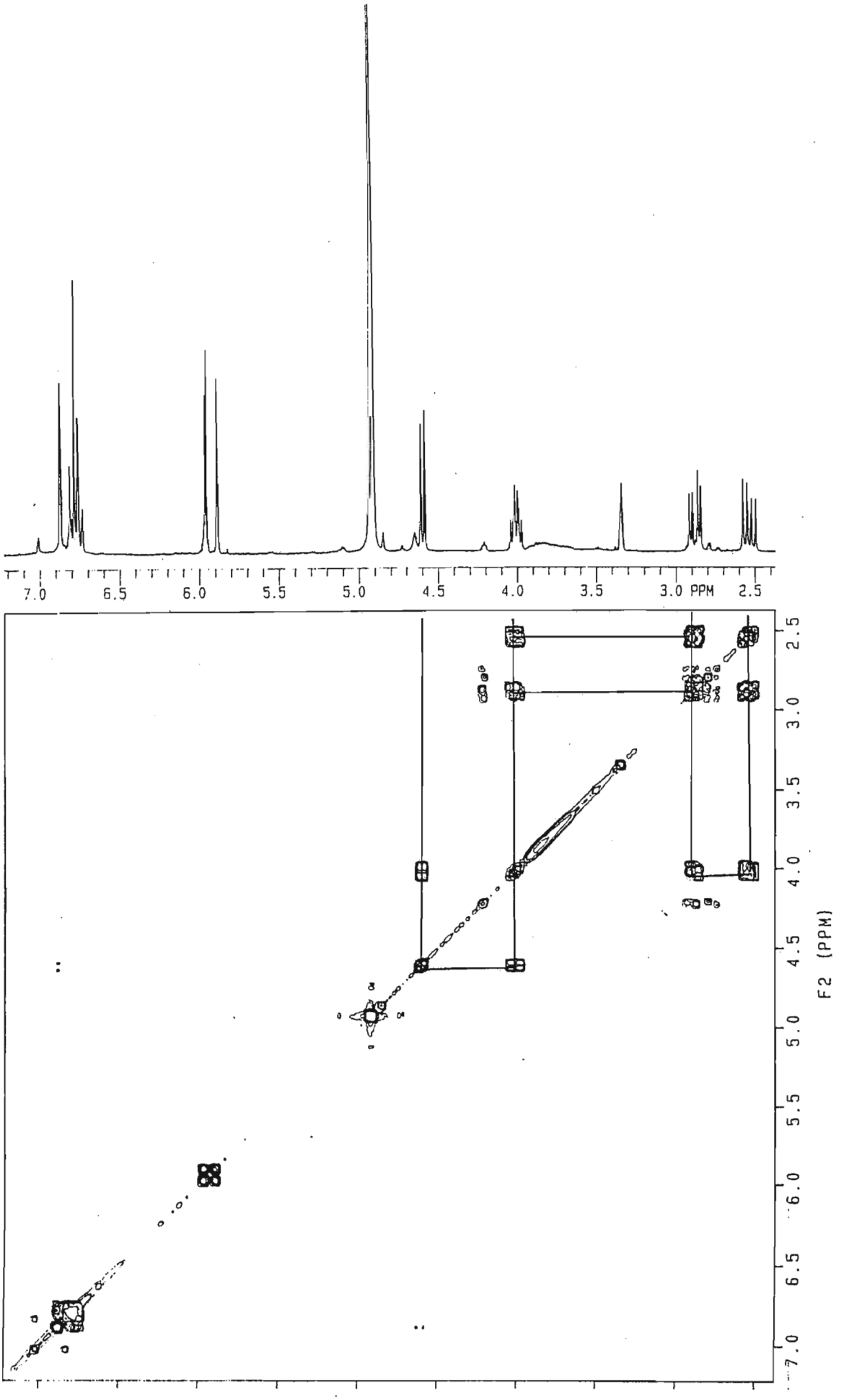
Spectrum : 5-A2



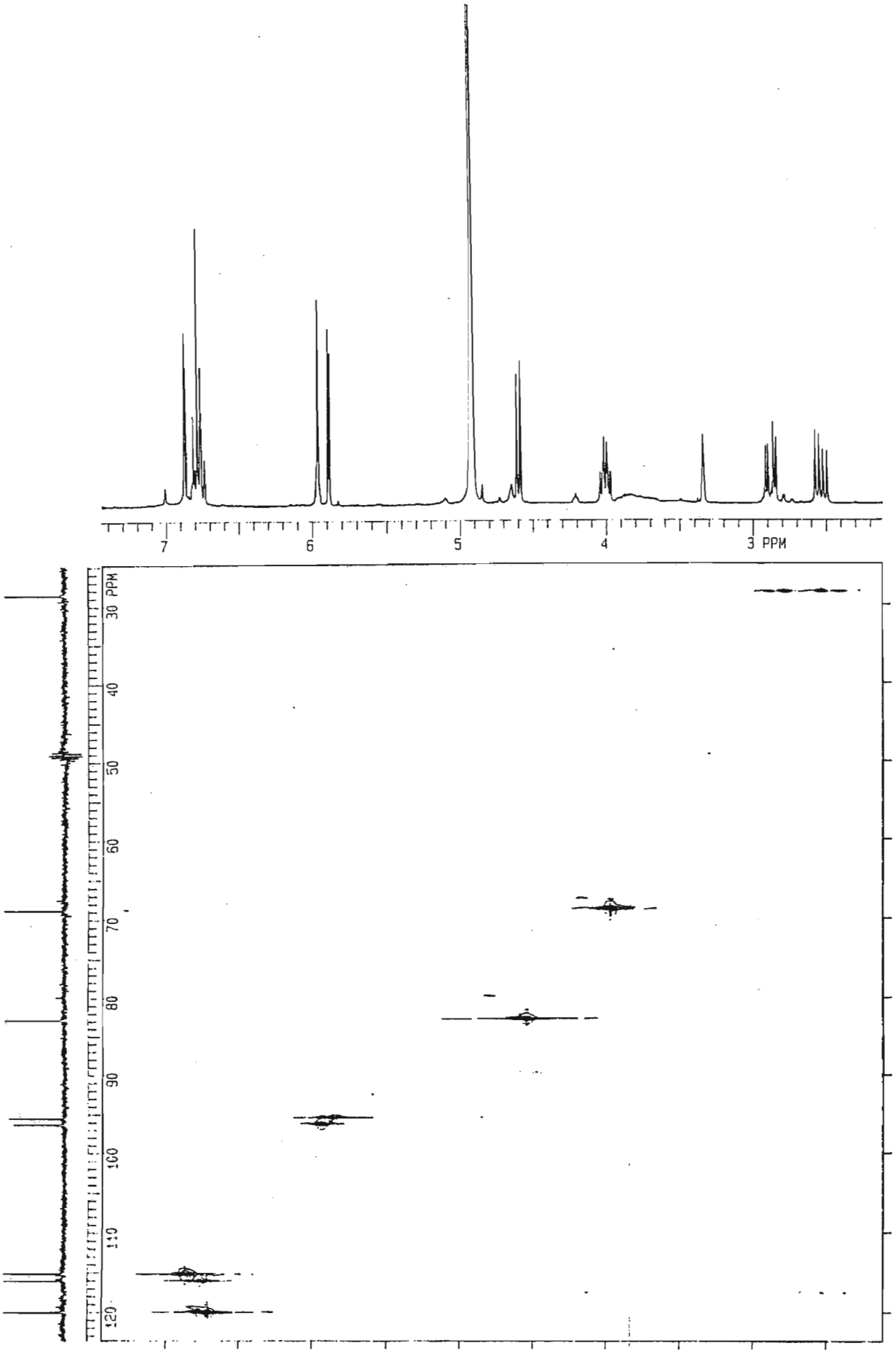
Spectrum : 5-A3



Spectrum : 5-A4

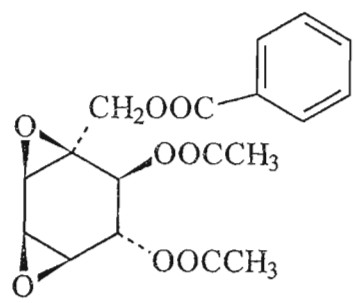


Spectrum : 5-A5

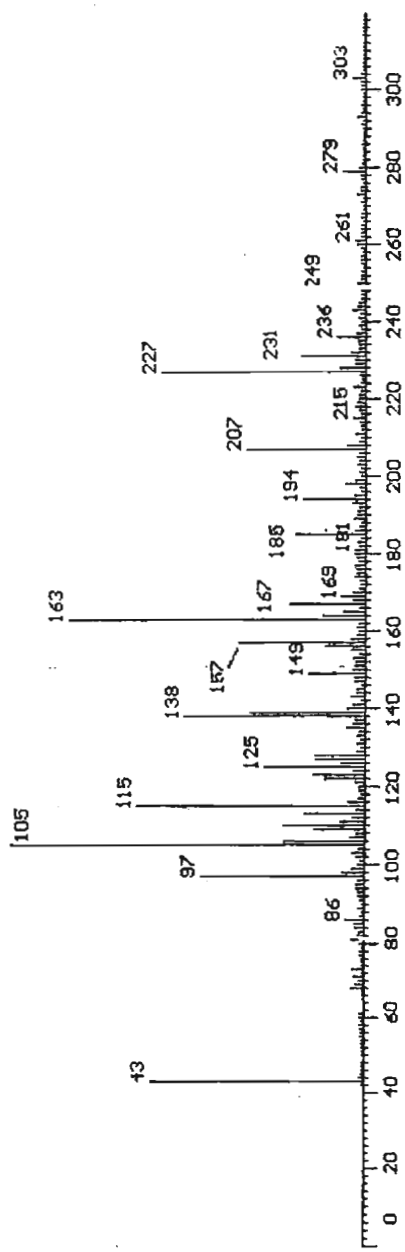


Extractives from *Monanthotaxis caffra*

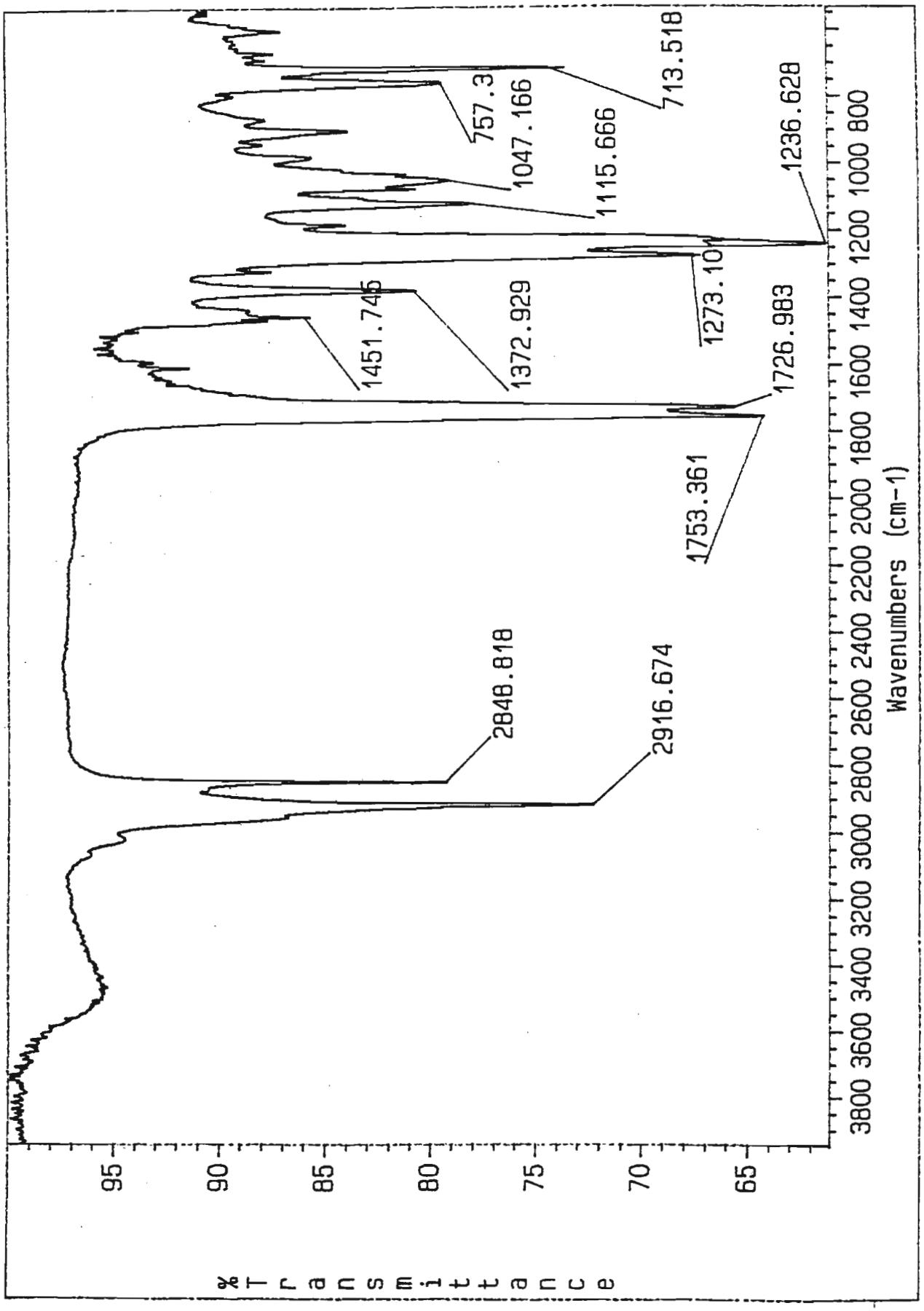
Compound 6-A



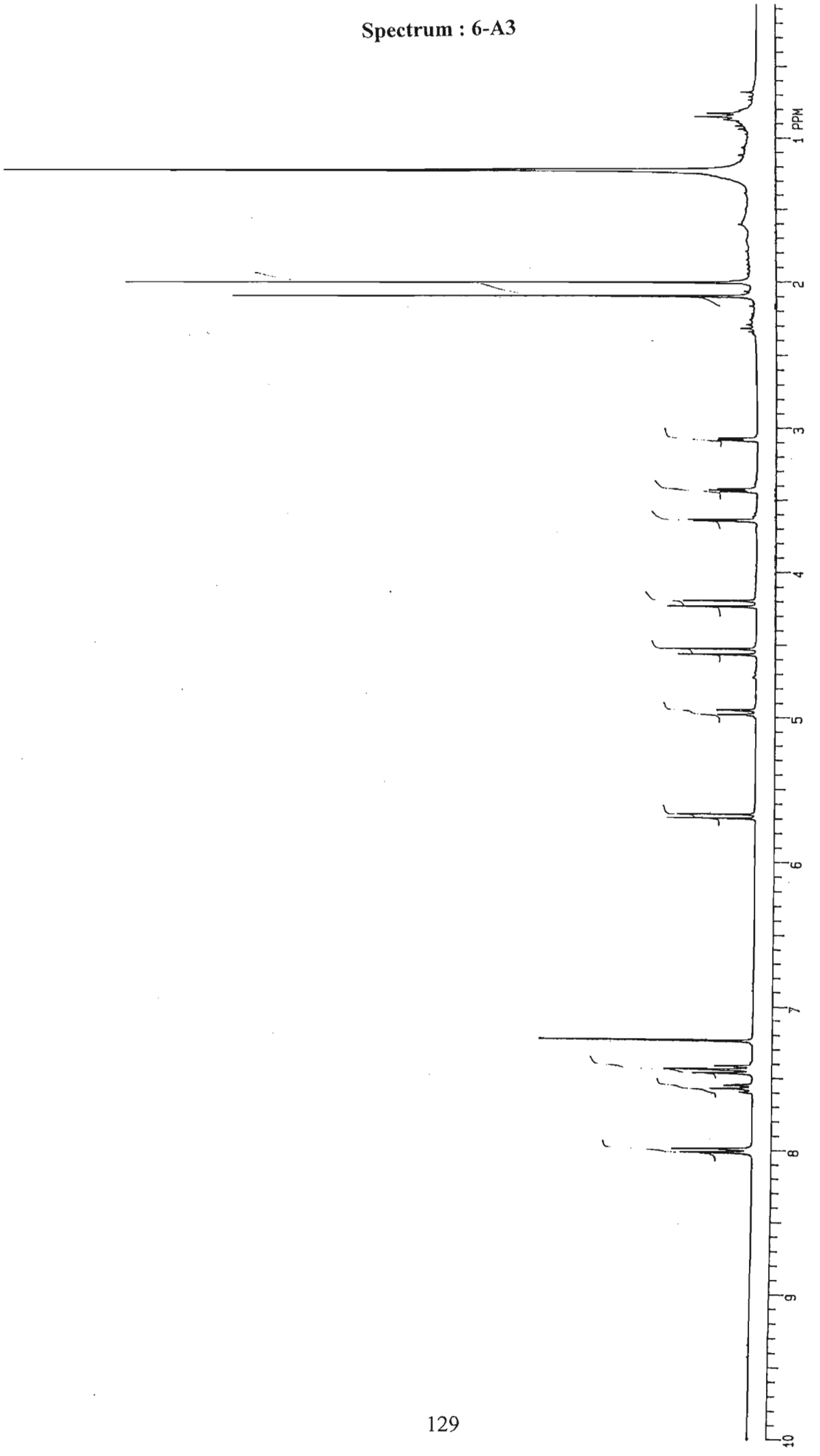
Spectrum : 6-A1



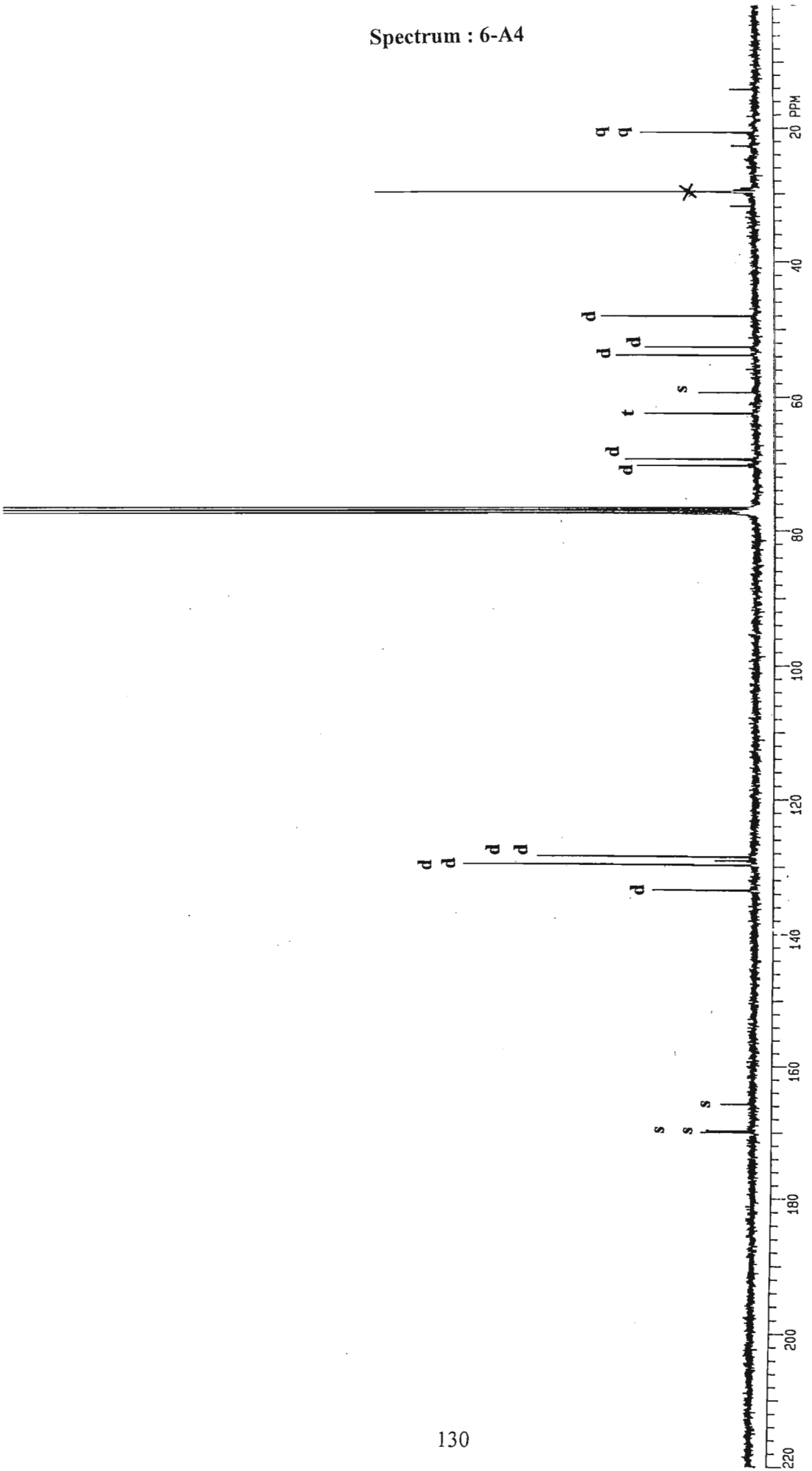
Spectrum : 6-A2



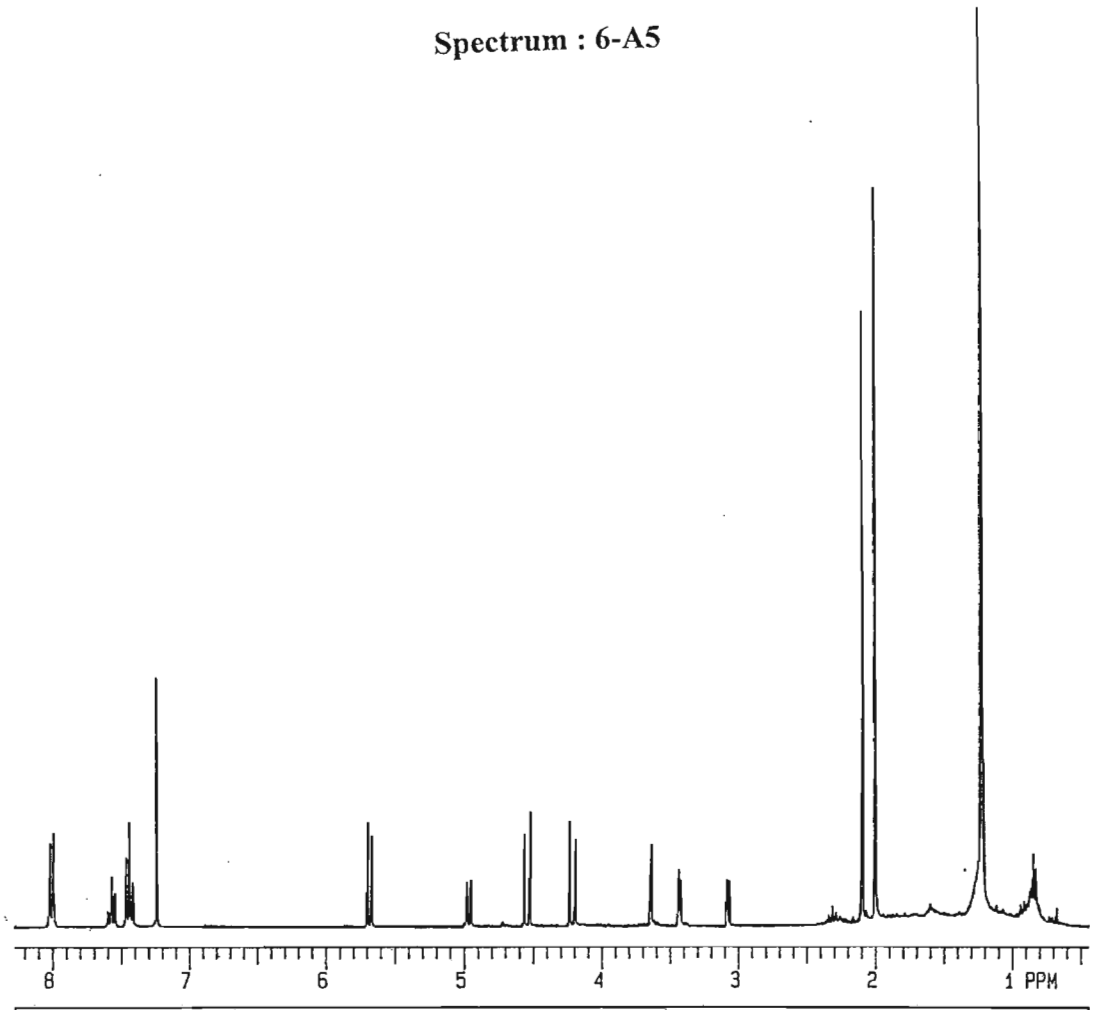
Spectrum : 6-A3

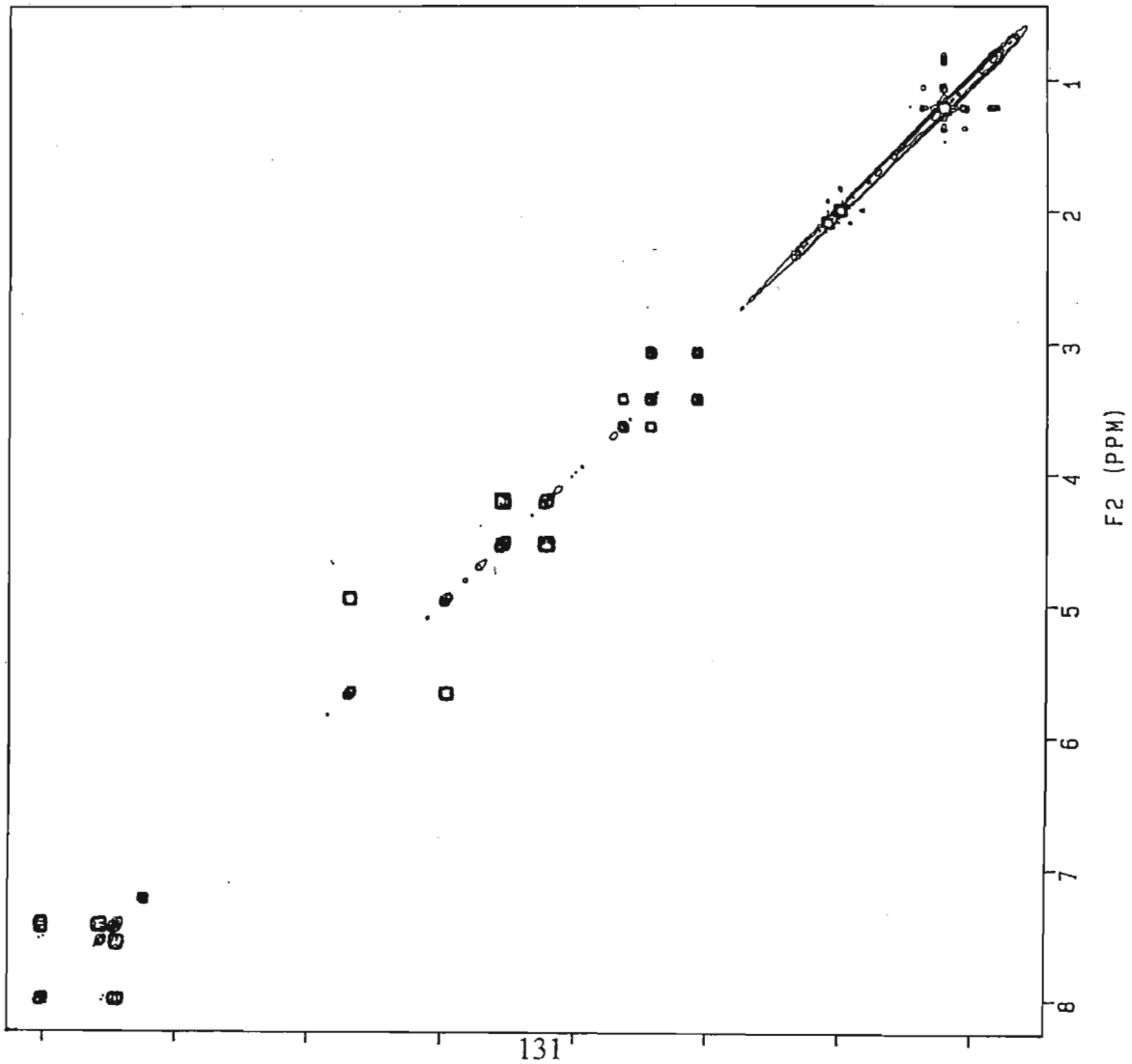


Spectrum : 6-A4

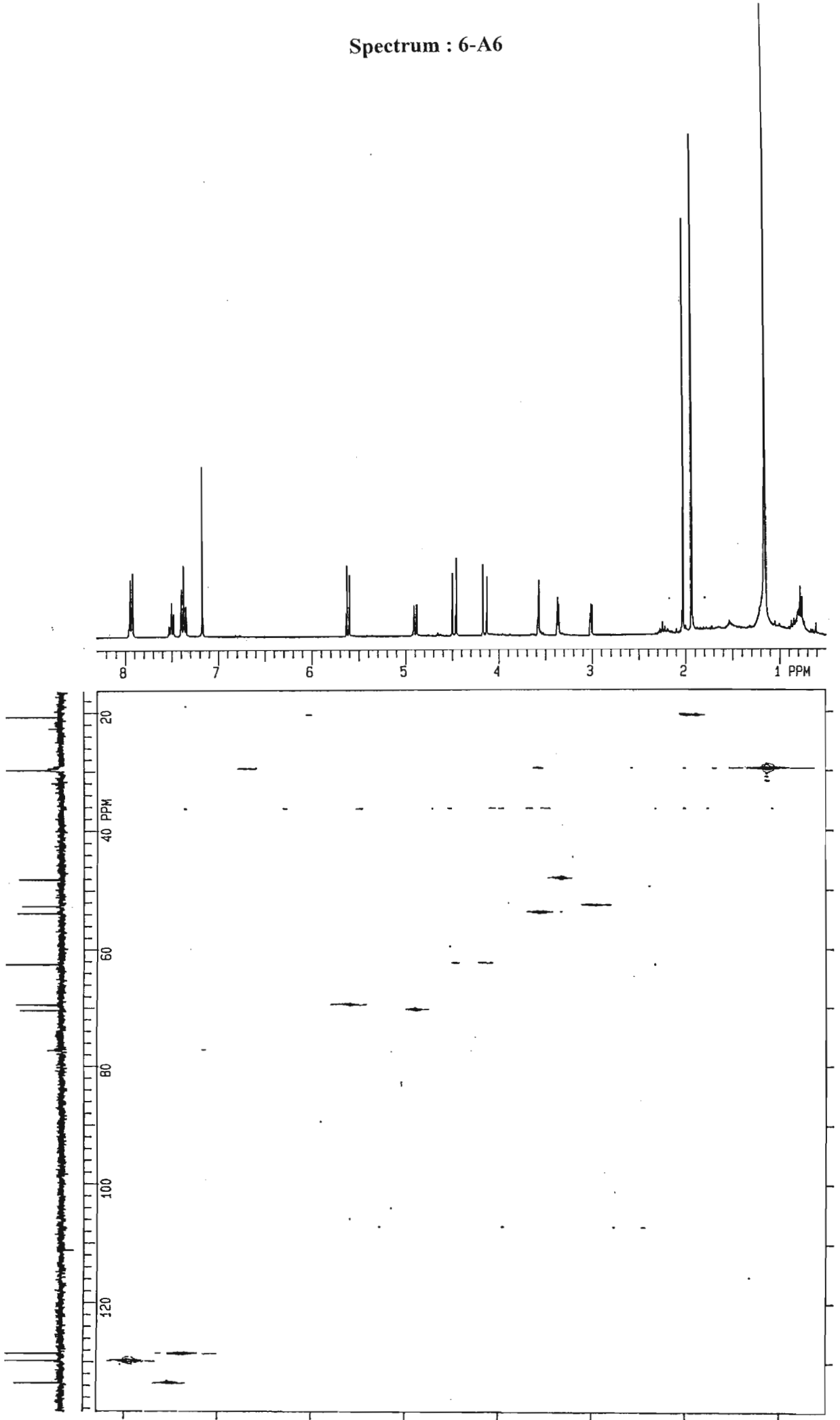


Spectrum : 6-A5





Spectrum : 6-A6



Spectrum : 6-A7