# EXTRACTIVES FROM THE MELIACEAE AND MELIANTHACEAE, AND INVESTIGATIONS INTO ENAMINE CHEMISTRY

by

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Submitted in partial fulfilment of the requirements

for the degree of

### DOCTOR OF PHILOSOPHY

in the

Department of Chemistry and Applied Chemistry,

University of Natal, Durban, South Africa

1997

TO MY WIFE, POPPY

# PART *A*

## EXTRACTIVES FROM THE
## MELIACEAE AND MELIANTHACEAE

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# PREFACE

The experimental work described in this thesis (Part A) was carried out in the Department of Chemistry and Applied Chemistry, University of Natal, Durban, under the supervision of Professor D. A. Mulholland.

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

SIGNED: _____

T. V. Monkhe, M.Sc.

I hereby certify that the above statement is correct.

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University of Natal

Durban

# ACKNOWLEDGEMENTS

# ABBREVIATIONS

| | |
|---|---|
| Ac- | acetate |
| br- | broad resonance |
| br s- | broad singlet |
| br m- | broad multiplet |
| $c$- | concentration |
| $^{13}C$ NMR- | carbon-13 nuclear resonance spectroscopy |
| COSY- | correlated nuclear resonance spectroscopy |
| d- | doublet |
| dd- | doublet of doublets |
| DEPT- | distortionless enhancement by polarisation transfer |
| dt- | doublet of triplets |
| $^{1}H$ NMR- | proton ($^{1}H$) nuclear resonance spectroscopy |
| HETCOR- | heteronuclear shift correlation nuclear resonance spectroscopy |
| Hz- | hertz |
| FTIR- | Fourier transformed infrared spectroscopy |
| m- | multiplet |
| Me- | methyl |
| ppm- | parts per million |
| q- | quartet |
| s- | singlet |
| t- | triplet |
| Tig- | tiglate |

# NOMENCLATURE

The numbering and stereochemistry of carbon atoms in the diterpenoid and triterpenoid compounds described in this text are depicted below.
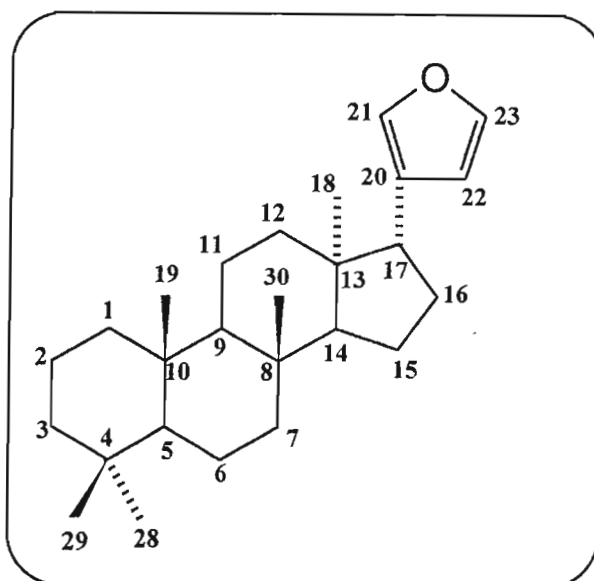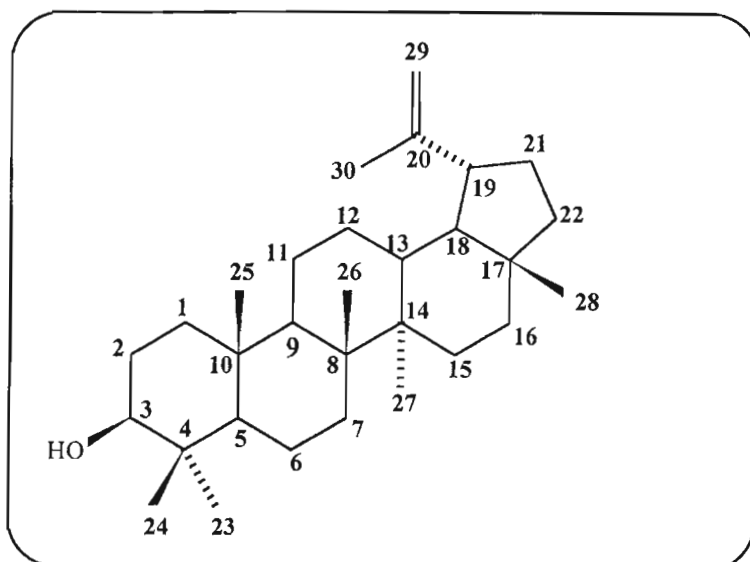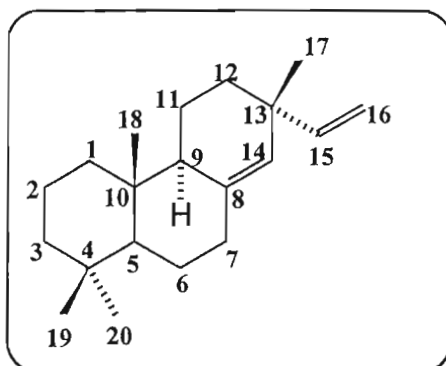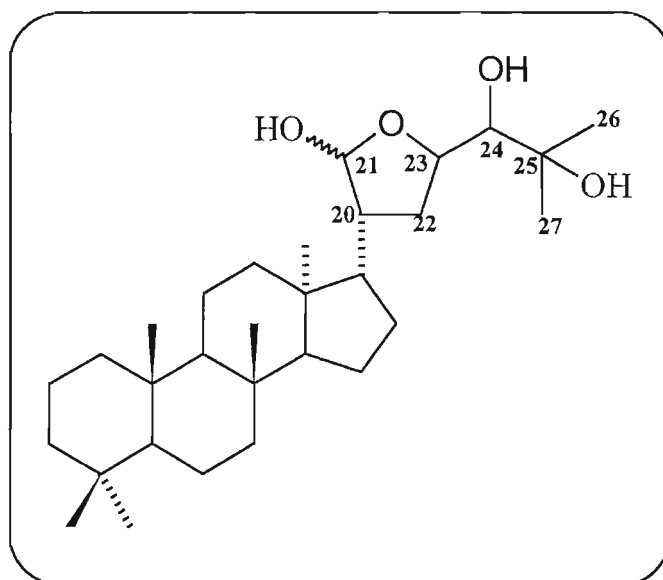
In all limonoid structures in this thesis, H-5 and H-9 are α-orientated and H-17 is β-orientated.

# ABSTRACT

Part A of this thesis is an account of the extractives isolated from one member of the Melianthaceae and two members of the Meliaceae. Plants belonging to these families are known to produce compounds with medicinal applications. Crude extracts from these plants are known to be widely used in traditional medicine. Structural elucidation was facilitated by the use of infrared, mass and nuclear magnetic resonance spectroscopic techniques.

Three 20(29)-lupene type compounds (two known and one knew) were isolated from the bark of *Bersama swinnyi* (Melianthaceae); oleanolic acid was isolated from the leaves.

The Meliaceae family is only represented by one species, *Dysoxylum spectabile,* in New Zealand. Two diterpenoids and two limonoids were isolated from the bark.

The extracts of the rootbark and stembark of the hitherto uninvestigated species *Turraea holstii* (Gurke), supplied by Professor H. S. Rajab, Moi University, Kenya, were examined. Eight limonoids and a protolimonoid were isolated from the rootbark. A limonoid and a protolimonoid were isolated from the stembark. Some of the compounds isolated were found in both extracts. Four of the eight limonoids isolated were of the neotrichilenone type.

The spectra for the compounds discussed in this text are presented in a separate book.

# CHAPTER 1

## TERPENOIDS - A BRIEF REVIEW

### Index

## 1.1. <u>Introduction</u>

The class of compounds called terpenoids comprise compounds derived from a common biosynthetic pathway based on melavonate as parent[1]. The important subgroup of steroids is sometimes singled out as a class in its own right.

Terpenoids are typically found in higher plants, mosses, liverworts, algae and lichens, although some are of insect or microbial origin. Most steroids are isolated from animal sources. Terpenoids have been known as ingredients of flavours, soaps, perfumes, drugs and pigments. Members of this class have been implicated in fields as diverse as mammalian sex hormones, pheromones, plant hormones and plant taxonomy.
The most commonly encountered forms of terpenoids are the monoterpenoids ($C_{10}$), sesquiterpenoids ($C_{15}$), diterpenoids ($C_{20}$), triterpenoids ($C_{30}$) and tetraterpenoids ($C_{40}$).

In 1953 Ruzicka[2] put forward his "Biogenetic Isoprene Rule" which postulated that the terpenoids were formed by the head-to-tail linkage of isoprene (1) units. This rule was later reformulated to include different types of terpenoids derived from a single parent compound unique to that class : geraniol (2) ($C_{10}$), farnesol (3) ($C_{15}$), geranylgeraniol (4) ($C_{20}$), squalene (5) ($C_{30}$), formed by the tail-to-tail or head-to-tail cyclisation and/or rearrangement. The biogenetic isoprene rule was later re-examined to include a variety of other compounds.

(1) isoprene  (2) geraniol  (3) farnesol

(4) geranylgeraniol

(5) squalene
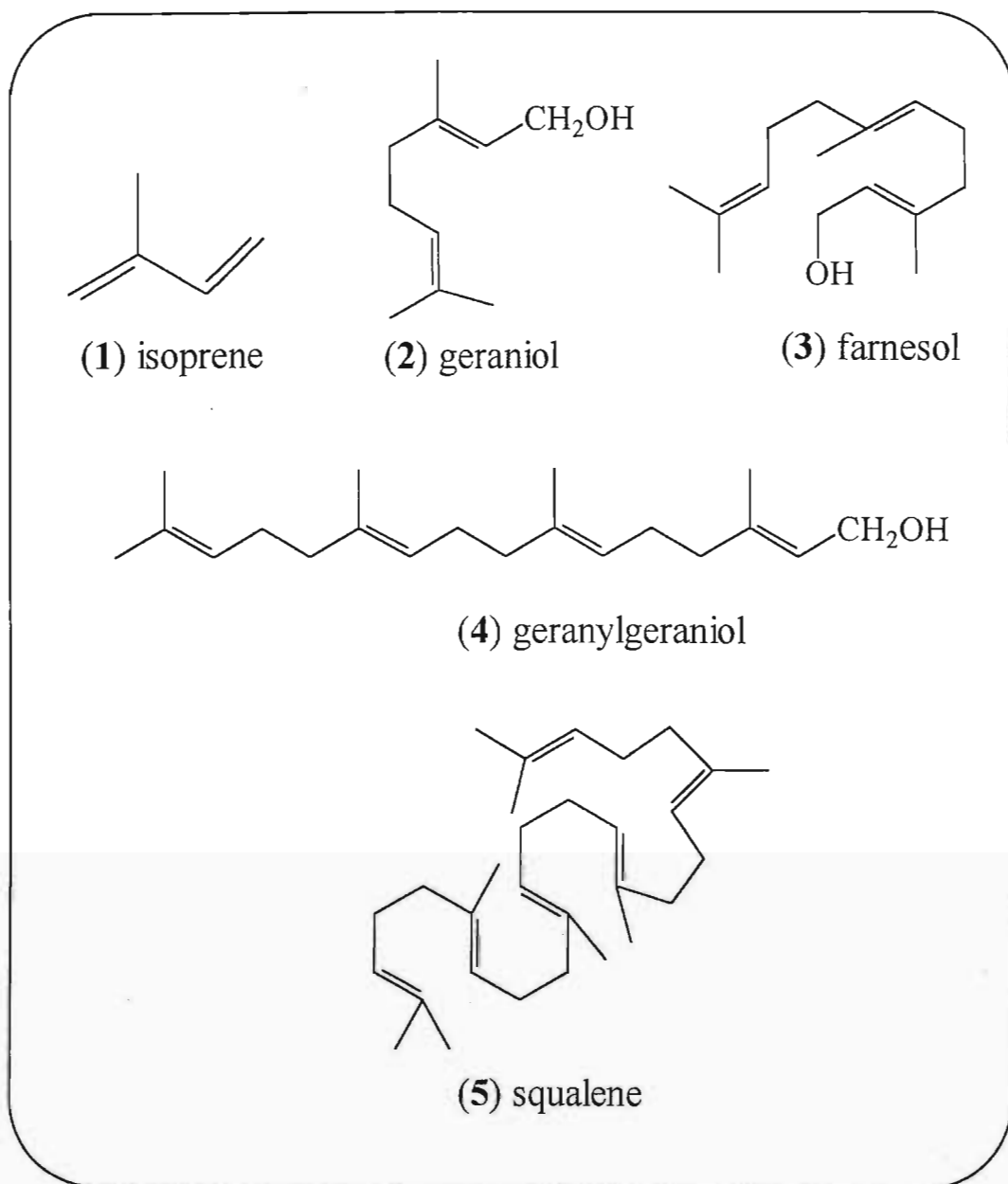
The subsequent multitude of structural and skeletal types within each class is derived from simple functionalisations, cyclisations and rearrangements of the parent compound and its derivatives.

## 1.2. Diterpenoids

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

Of the 170 carbon frameworks known for diterpenoids at present, eight (figure 1) of these account for some fifty percent of the known diterpenoids.



cembrane

labdane

clerodane

pimarane

abietane

kaurane

gibberellane

taxane

**FIGURE 1**
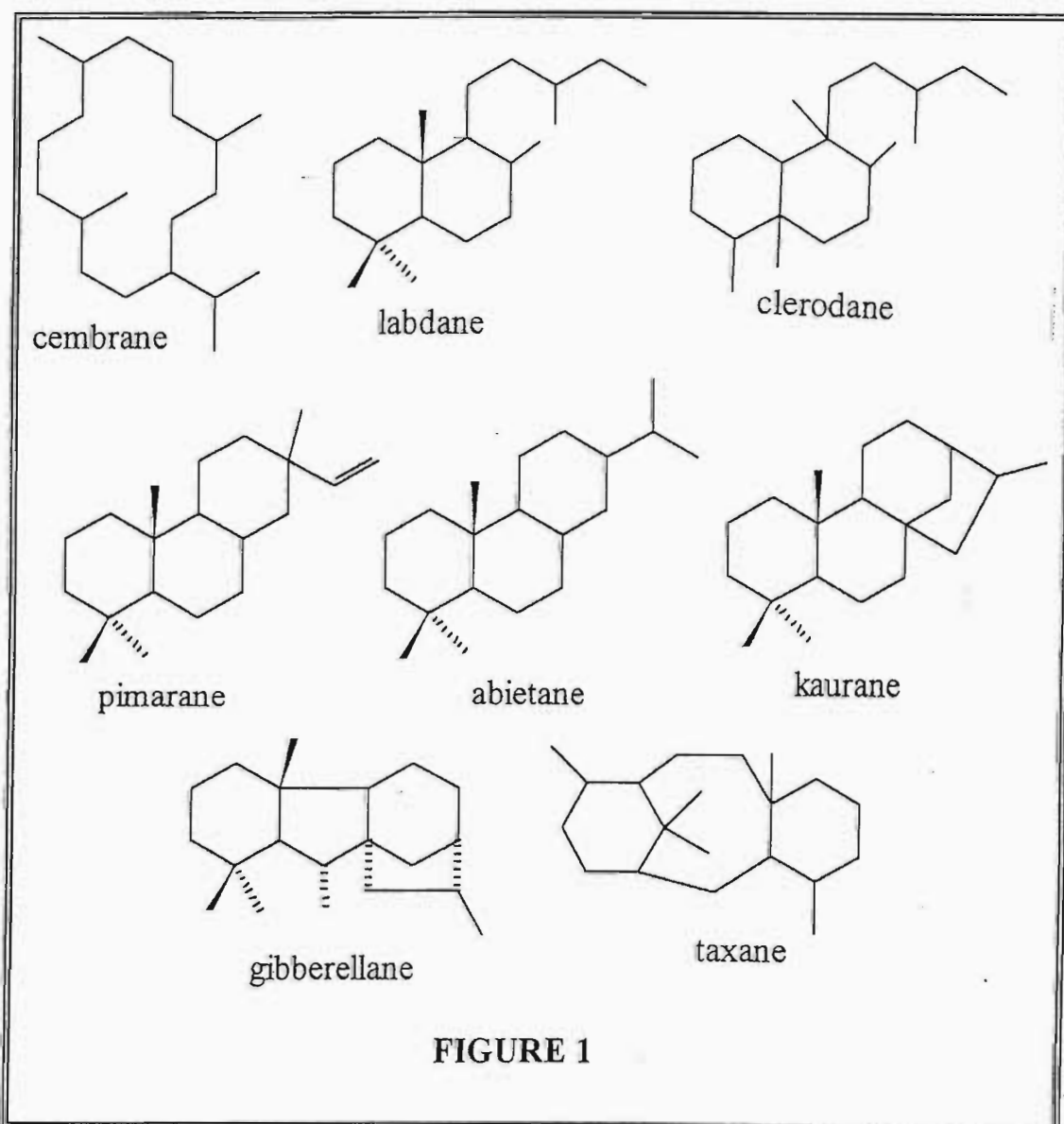
A number of classes of diterpenoids may be rationalised as arising by cyclisation of geranylgeraniol (**4**)[2,3] (Scheme 1).



**SCHEME 1**
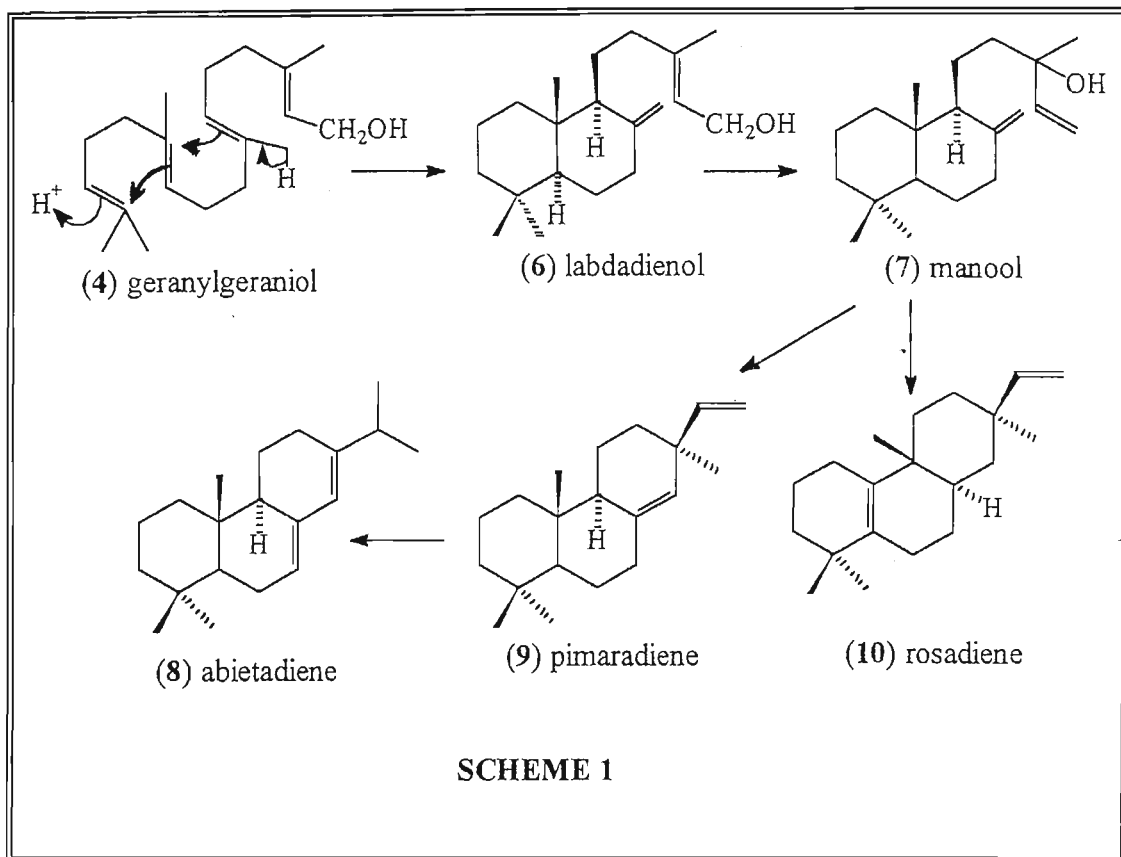
Unlike the triterpenoids and steroids, one of the characteristic features of the diterpenoids becomes apparent at the labdane stage, namely the formation of both normal (steroid-like) (**6**) and antipodal (**11**) A/B ring junctions.
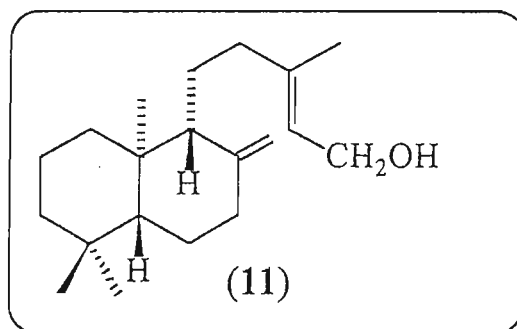


This may arise through different modes of coiling of the open - chain precursor on the cyclase enzyme surface.

Diterpenoids with a pimaradiene skeleton are quite widespread and include in their number pimaric acid (12), isopimaric acid (13), *ent*-isopimara-9(11),15-diene-19-oic acid (14) and sandaracopimaric acid (15).



(12) pimaric acid          (13) isopimaric acid

(14)          (15) sandaracopimaric acid
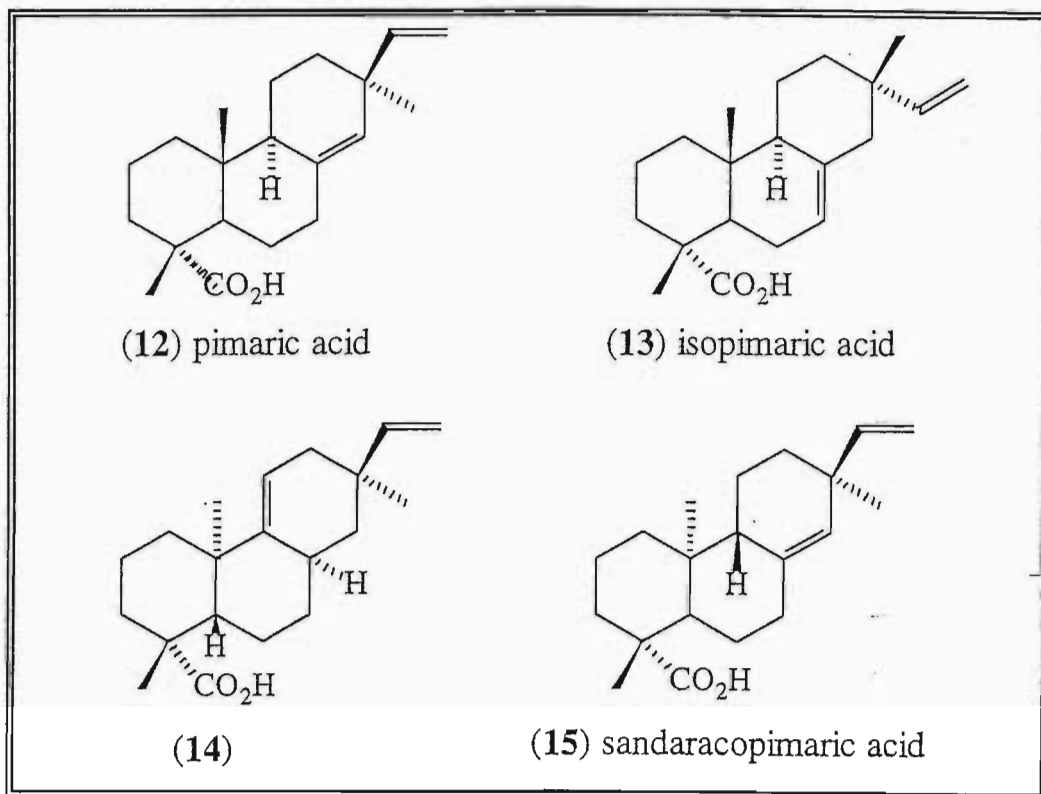
The tricyclic pimaradiene may act as the precursor in the formation of abietanes, cassanes and rosanes[4]. The tetracyclic diterpenoids were originally thought to arise by cyclisation of a suitably oriented pimaradiene to an intermediate non-classical carbocation (16)[5]. The ion might collapse to afford compounds of the kaurane, atisirene or the beyerene series or the pentacyclic trachylobane diterpenoids, as depicted in Scheme 2.

*ent*-beyerene

*ent*-atisirene

(16)

*ent*-trachylobane

*ent*-kaurene

**Scheme 2** : Formation of the beyerene, atisirene, kaurene or pentacyclic trachylobane diterpenoids
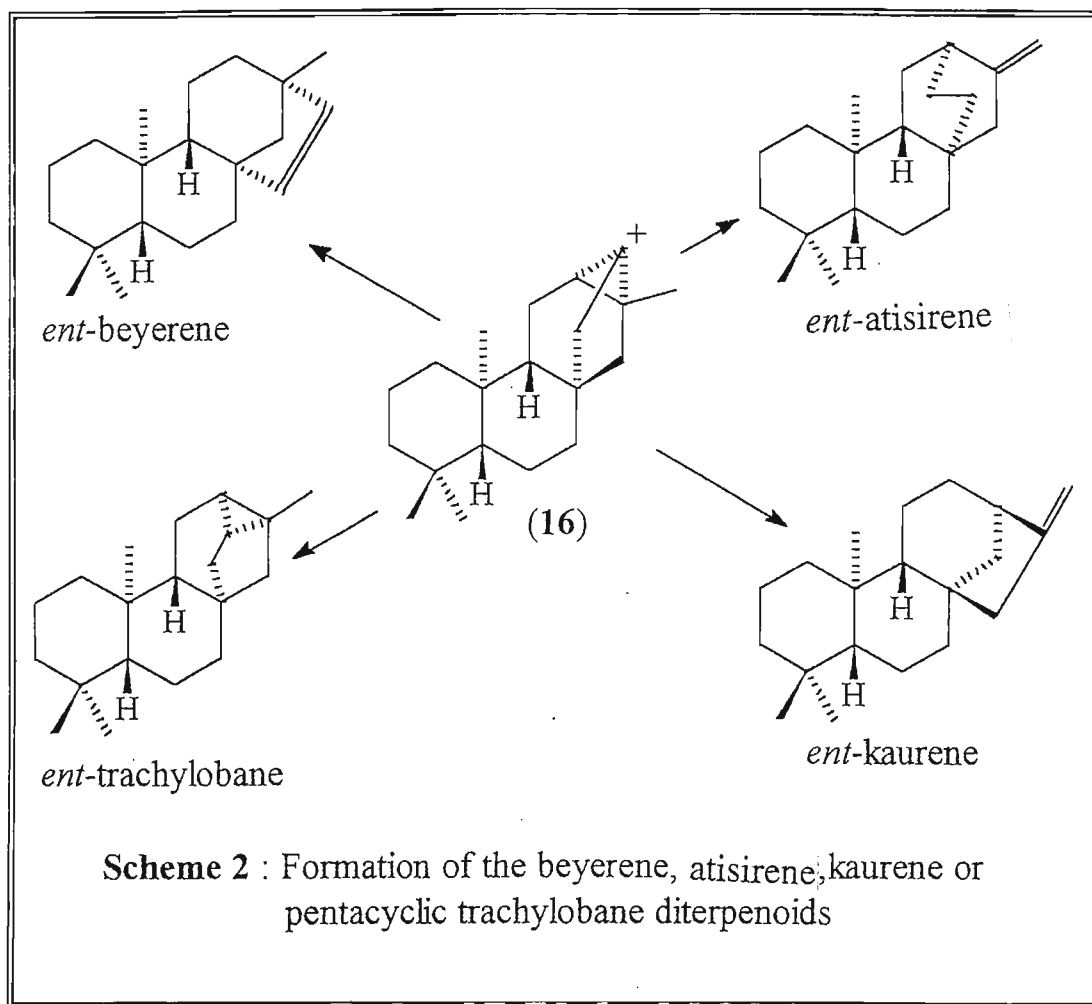
## 1.3. Triterpenoids

The triterpenoids form a large diverse group of naturally occurring compounds which are distributed throughout the plant kingdom. Lanosterol (**17**) belongs to a small but important group of triterpenoids of animal origin.

In general , triterpenoids are derived from the cyclisation of squalene (**5**) or the 3S isomer of squalene epoxide (**18**).

(**17**) lanosterol   (**18**) squalene epoxide
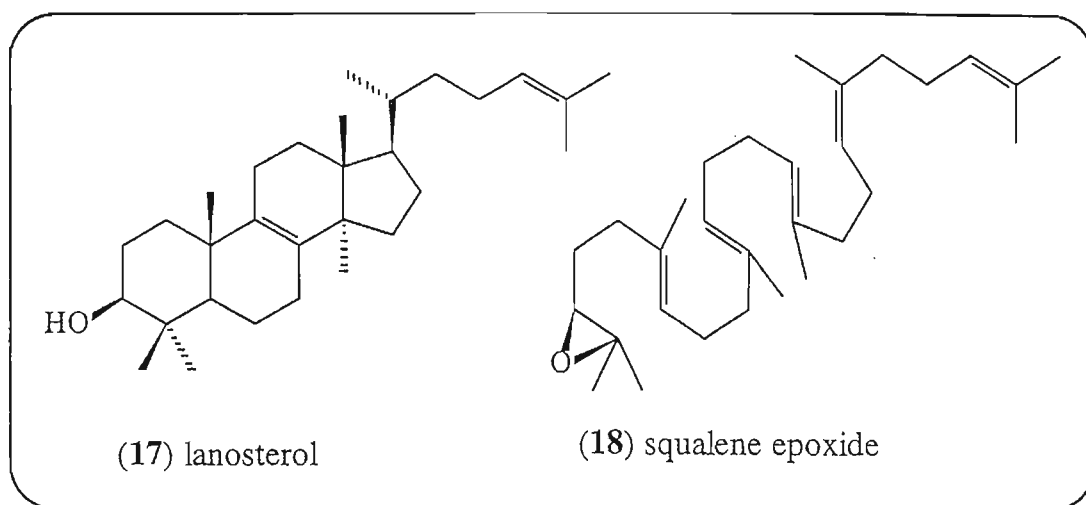
In accordance with the Biogenetic Isoprene Rule, the various skeletal types of tetracyclic and pentacyclic terpenoids are formed according to the conformation adopted by squalene or its epoxide at an enzyme surface prior to cyclization[2,6]. Depending on the cationic intermediates formed, various classes of terpenoids may arise[7]. The initially formed cation may undergo a series of 1,2-hydride and methyl migrations.

Cyclisation of squalene epoxide in the chair-boat-chair-boat leads directly to the formation of protosterol (**19**) via a bridged cation (**20**) [or a spirocation (**21**)], and cationic intermediate (**22**), or by a sequence of conversions to lanosterol (**17**), cycloartenol (**23**) and the cucurbitacins [cucurbitacin A (**24**)][8] as depicted in scheme 3.

(19) protosterol

(23) cycloartenol

(24) cucurbitacin A
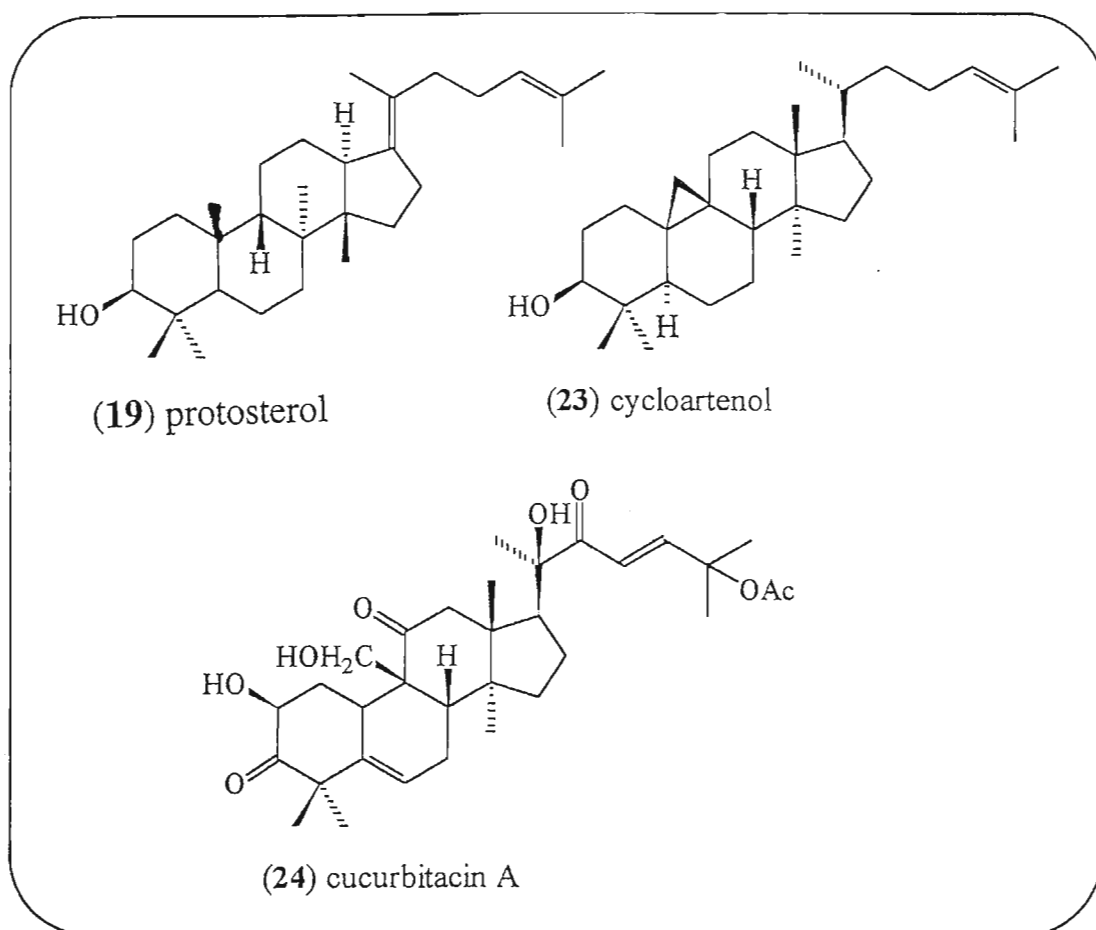
Cyclisation of squalene epoxide in the chair-chair-chair-boat conformation leads directly to the formation of the dammarenediols (25) via a cationic intermediate (26), or euphol (27) and tirucallol (28) via a sequence of 1,2-hydride and methyl shifts[9], as shown in scheme 4.
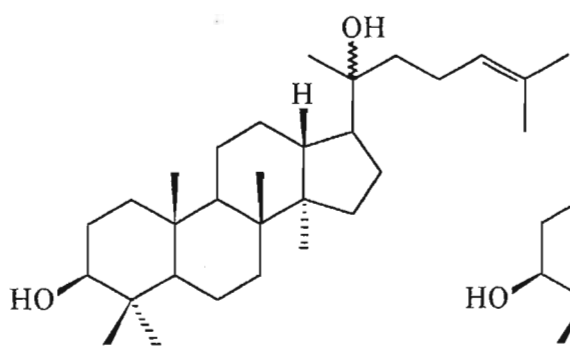
(**19**) dammarenediol

(**27**) euphol (20βH)
(**28**) tirucallol (20αH)

(**29**) lupeol

(18) squalene epoxide

(20) bridged cation

(21) spirocation

(22) cationic intermediate

(17) lanosterol
(23) cycloartenol
(24) cucurbitacin A

(19) protosterol

**Scheme 3** : Chair-boat-chair-boat cyclisation of squalene epoxide

**(18)** squalene epoxide

**(26)** cationic intermediate

**(27)** euphol
**(28)** tirucallol

**(25)** dammarenediols

**(29)** lupeol

**(32)** β-amyrin

**Scheme 4** : Chair-chair-chair-boat cyclisation of squalene epoxide
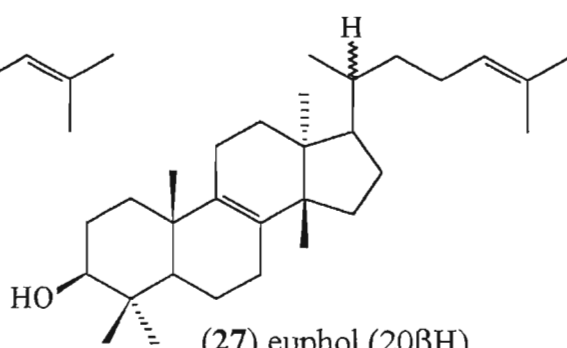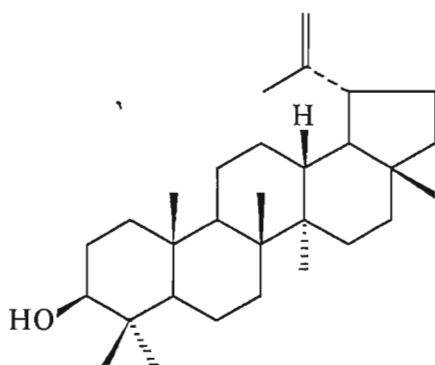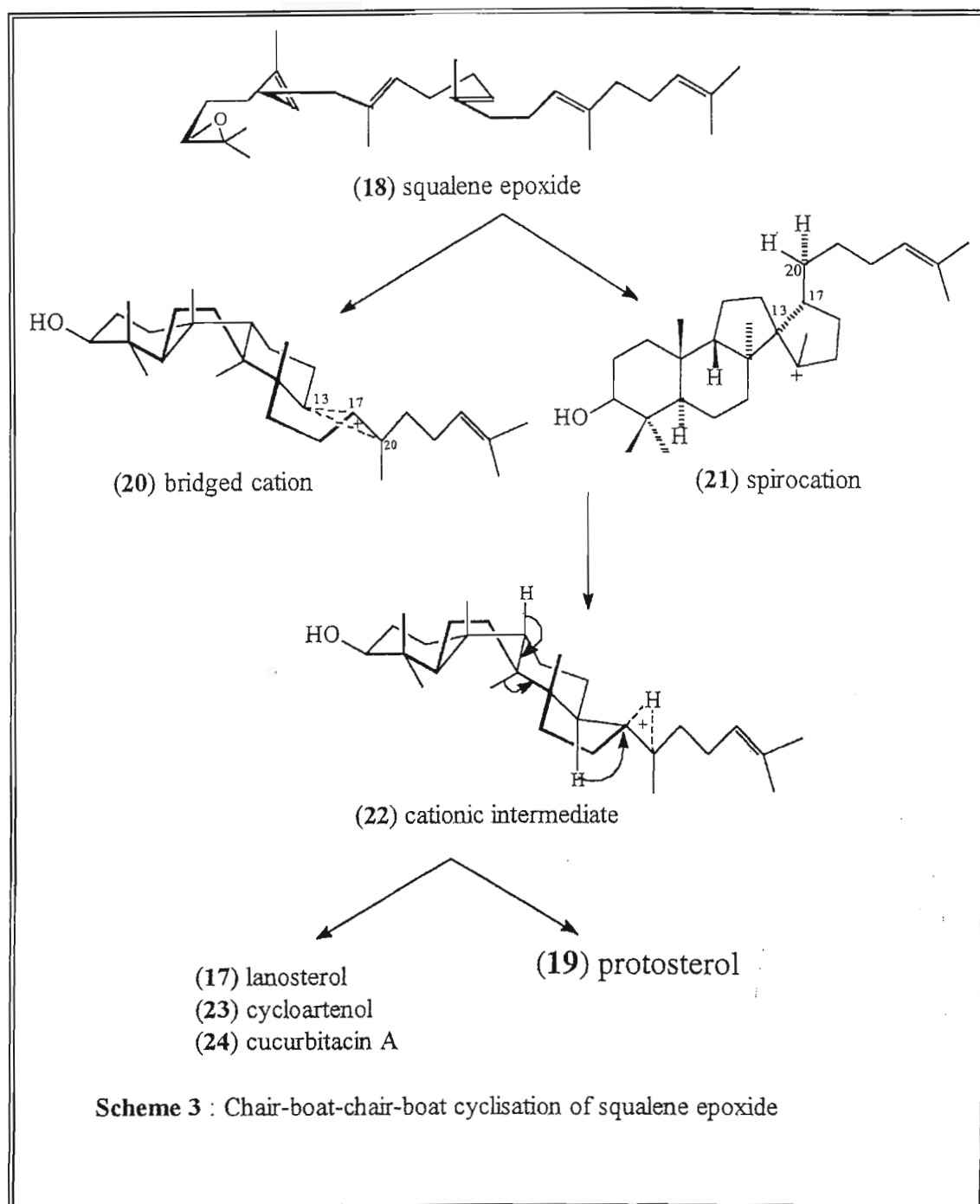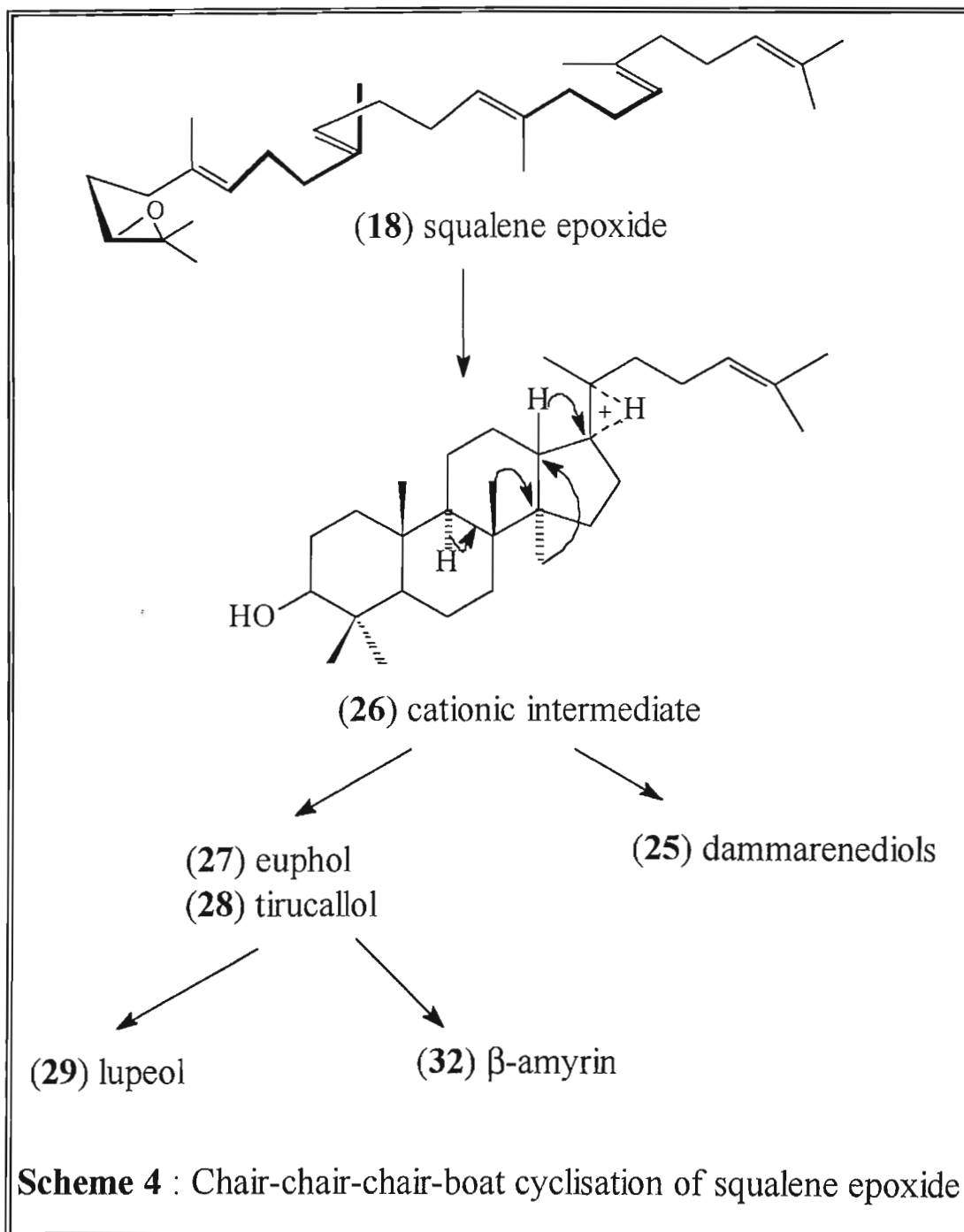
Euphol **(27)** and tirucallol **(28)** which only differ in their stereochemistry at C-20 form an integral part of limonoid biosynthesis as is discussed in section 1.4.2.

The cationic intermediate **(26)** may also lead to the pentacyclic triterpenes such as lupeol **(29)** of the lupane group. The 1,2-hydride and methyl

migrations lead to the formation of germanicol (**30**), δ-amyrin (**31**), β-amyrin (olean-12-en-3β-ol) (**32**), teraxerol (**33**) and other related compounds.



(**30**) germanicol

(**31**) δ-amyrin

(**32**) β-amyrin

(**33**) taraxerol
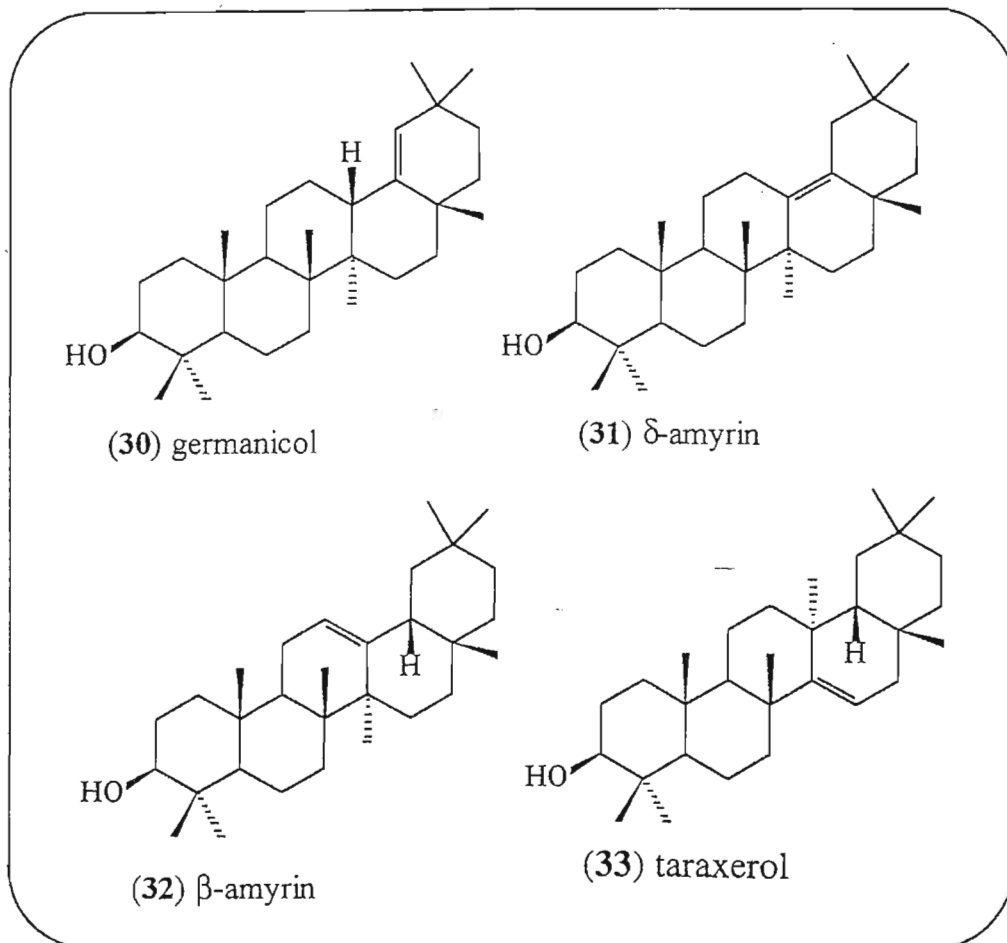
Cyclisation of squalene via other conformations is also possible. The all-chair conformation leads to the formation of diplotene (**34**) and tetrahymanol (**35**)[10].



(**34**) diplotene

(**35**) tetrahymanol
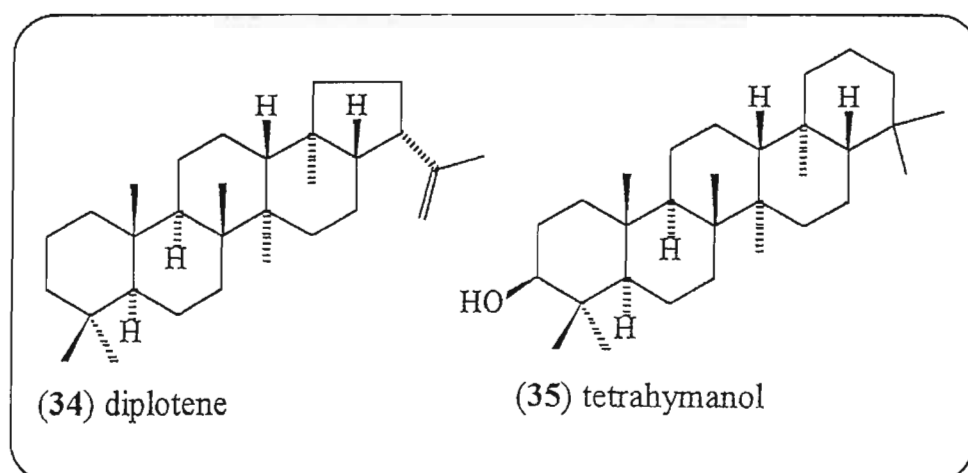
The chair-chair-chair-chair-boat conformation results in the formation of moretenol (36)[11] and chair-boat-chair-chair-boat cyclisation affords arborinol (37)[12].



(36) moretenol

(37) aborinol
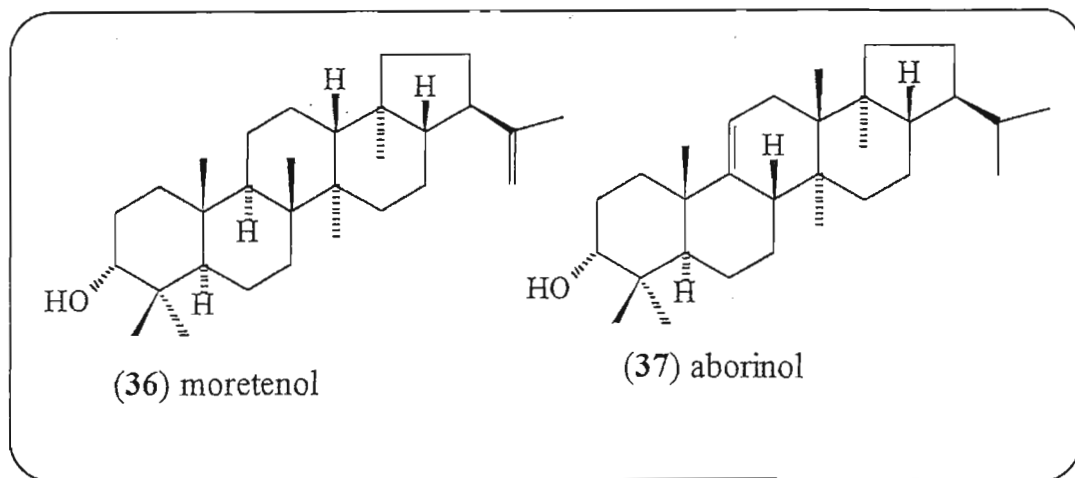
Cyclisation of squalene, or more probably its bis-epoxide from both ends affords onocerin (38). Further cyclisation leads to the pentacyclic serratane group, typified by serratenediol (39)[13].



(38) onocerin

(39) 14-serratene-3,21-diol
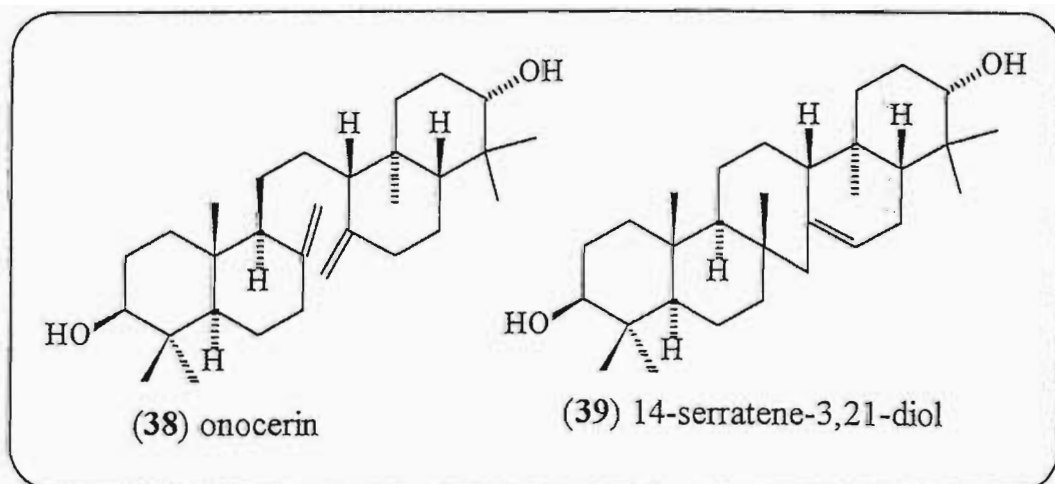
## 1.4. Limonoids

### 1.4.1. Introduction

The study of the limonoid chemistry of the Meliaceae began in the 1950's with the isolation of gedunin (**40**) from the West African timber tree *Entandrophragma angolense*[14]. The proof of the structure of gedunin was dependent on that of limonin (**41**)[15], an extractive of the genus *Citrus* (Rutaceae), which had recently been published. The common name limonoid is derived from that of limonin.



**(40)** gedunin          **(41)** limonin
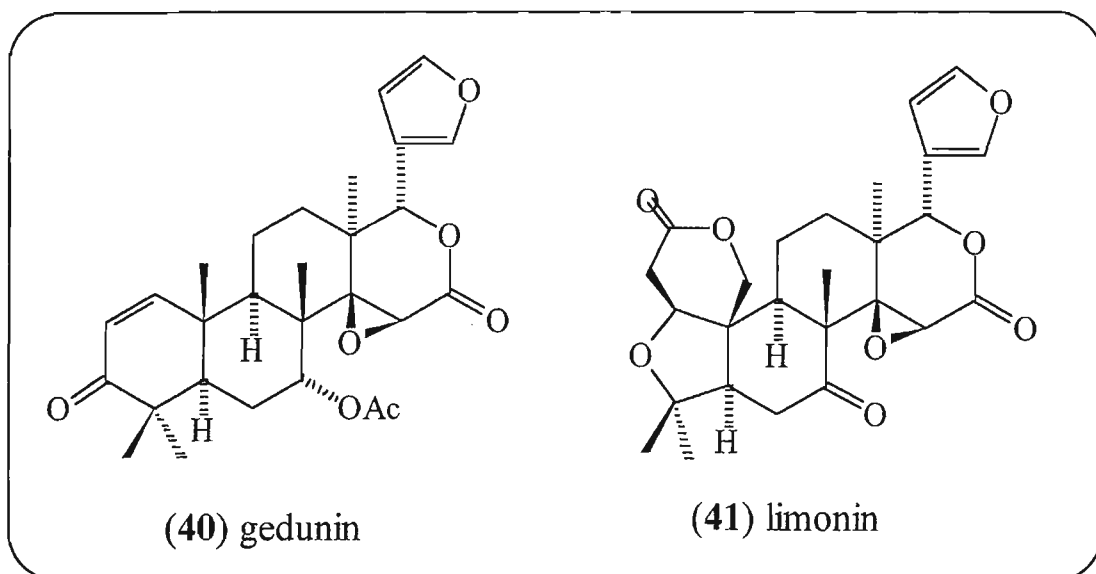
Over 300 limonoids have been isolated, several of which occur as different esters in different plants.

Although the thrust of research into these compounds has focussed on Meliaceous species, members of the Rutaceae, Cneoraceae and Simaroubaceae have also been found to possess limonoids.

A limonoid is defined as a compound with a C-22 nucleus and a β-substituted furan ring at the C-17α position. A number of structural

modifications of the C-22 tetracyclic nucleus lead to a large variety of limonoids. Classification of the known limonoids is based largely on the extent of oxidation of the tetracyclic nucleus[16,17].

Several limonoids occur as different esters in different plants, and mixed esters of the same compound are often present in the same plant extract. Separation of these compounds is sometimes difficult and is further complicated by isomerisation occurring during extraction and isolation[17].

## 1.4.2. Classification and biosynthesis of limonoids

Limonoids have been classified by Taylor[17] into ten distinct groups (Table 1) according to which of the four carbocyclic rings have been oxidised. Limonoids are thought to be derived from the euphane-tirucallane group of triterpenes by a series of oxidative and molecular rearrangements. Most of the reactions can be reproduced under laboratory conditions. The oxidations are either epoxidations of double bonds or Baeyer-Villiger attacks on ketones via a biological per-acid equivalent, presumably consisting of a peroxidase. The rearrangements are, in contrast, very ready and spontaneous. Oxidised intact triterpenes, which by their biological occurrence and oxidation pattern appear to be biochemical precursors of limonoids, are called protolomonoids.

It was originally suggested[15] that limonin (41) might arise from a compound with the apo-euphol structure (43) which could be biosynthesised from either euphol (27) or tirucallol (28) or perhaps the $\Delta^7$ isomer of euphol, butyrospermol (42). The apo-euphol rearrangement involves migration of

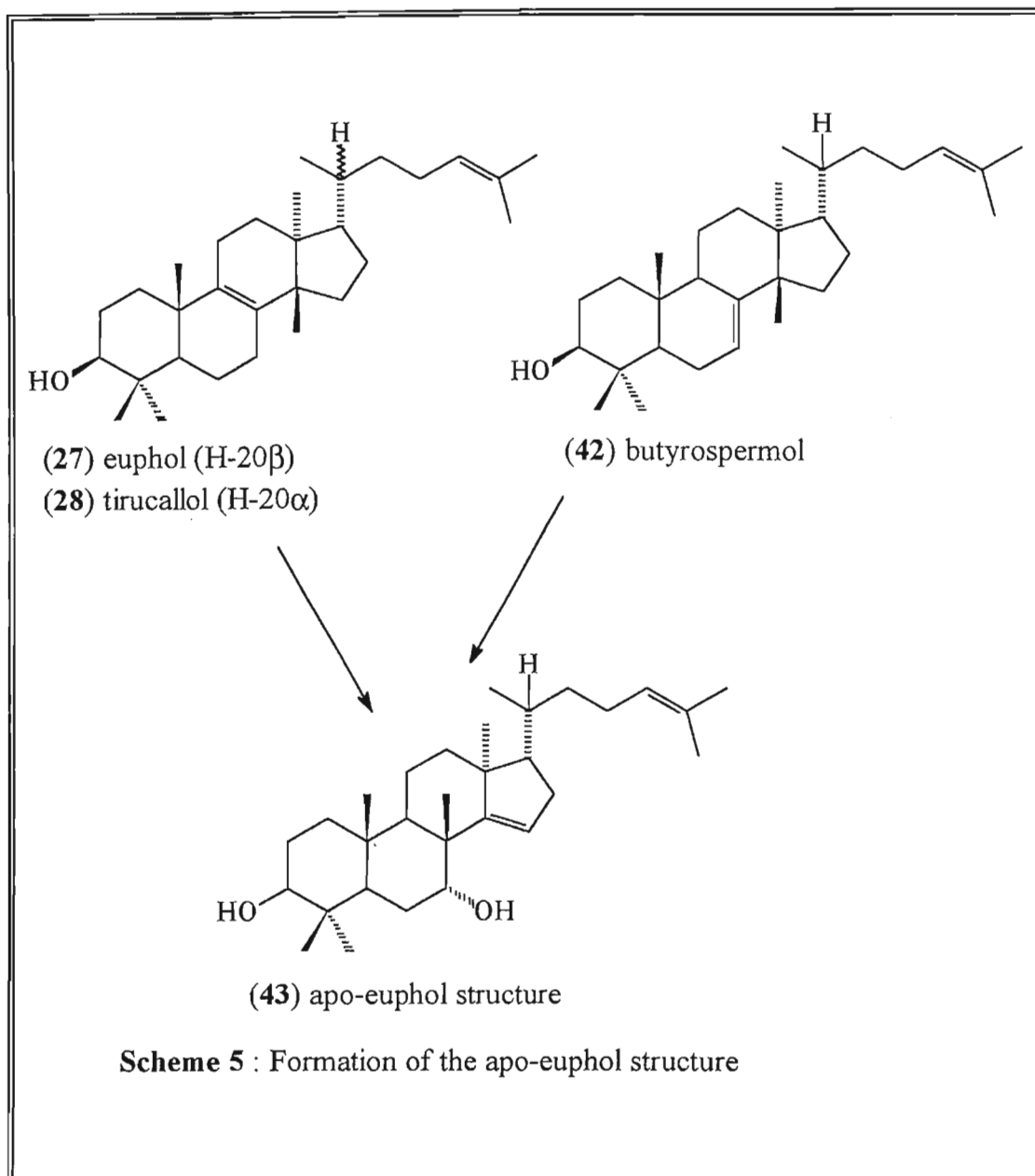the C-14 methyl group to C-8 and formation of a $\Delta^{14}$ double bond (Scheme 5).



**(27)** euphol (H-20β)
**(28)** tirucallol (H-20α)

**(42)** butyrospermol

**(43)** apo-euphol structure

**Scheme 5** : Formation of the apo-euphol structure

**Table 1: Categorization of the Meliaceous limonoids and protolimonoids**

| GROUP | EXAMPLE | RING A | RING B | RING C | RING D | SIDE CHAIN |
|---|---|---|---|---|---|---|
| I | turraeanthin (45) | intact | intact | intact | intact | intact |
| II | havanensin (61) | intact | intact | intact | intact | furan |
| III | gedunin (40) | intact | intact | intact | lactone | furan |
| IVa | andirobin (66) | intact | opened | intact | lactone | furan |
| IVb | mexicanolide (68) | intact | opened and cyclised | intact | lactone | furan |
| IVc | phragmalin (69) | bridged | opened and cyclised | intact | lactone | furan |
| V | methyl ivorensate (71) | lactone | opened | intact | lactone | furan |
| VI | obacunol (73) | lactone | intact | intact | lactone | furan |
| VII | nimbin (79) | intact | intact | lactone or opened | intact | furan or further oxidised |
| VIII | toonafolin (82) | intact | lactone | intact | intact | furan |
| IX | evodulone (84) | lactone | intact | intact | intact | furan |
| X | prieurianin (85) | lactone | opened | intact | intact | furan |
|  |  |  |  |  |  |  |

Oxidation of the side chain with the eventual loss of four carbon atoms results in the formation of the furan ring (Scheme 6).



euphol (H-20β)
tirucallol (H-20α)

protolimonoid

limonoid

**Scheme 6** : Formation of the furan ring
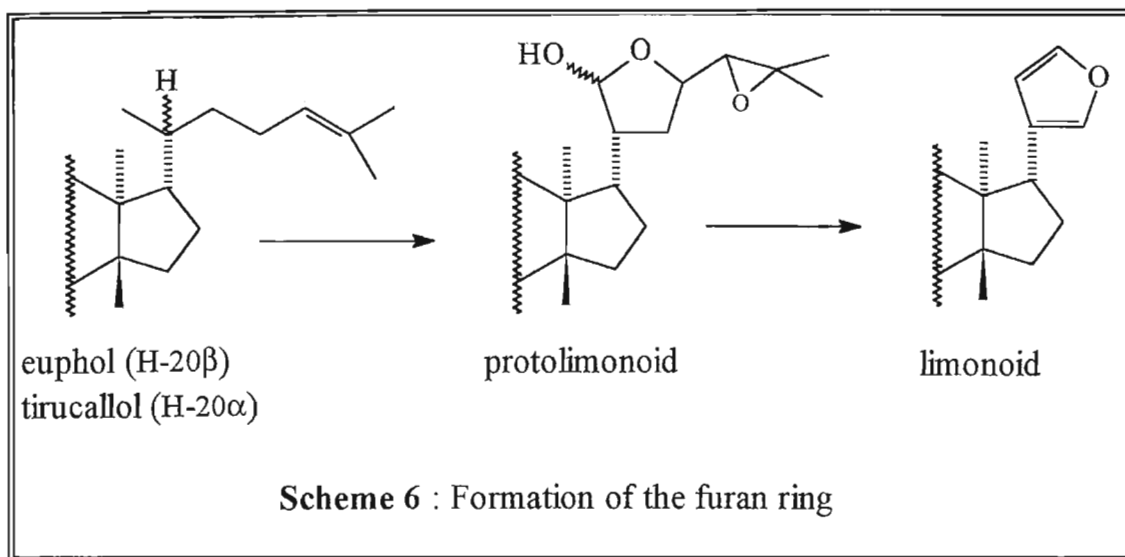
Though there is no direct evidence supporting certain stages of the biosynthetic pathway involving radioactive tracer inorporation, the processes involved are  sufficiently well supported by circumstantial evidence[18]. However, a number of laboratory simulations have provided substantive evidence for some of the related processes. Radioactive labelled precursors were utilised only in *Citrus* plants[19] and in *Azadirachta indica* leaves[20].

Formation of the apo-euphol structure may also arise from squalene via the dammarene cation (**44**), as depicted in Scheme 7.

The isolation of turraeanthin (**45**) from *Turraeanthus africanus*[21], gave a further clue as to the identity of the limonoid precursor. Turraeanthin (**45**) possesses a $\Delta^7$ double bond and a side chain more oxidised than tirucallol. This suggested that the hypothetical triterpene precursor could be the $\Delta^7$ isomer of tirucallol, tirucalla-7,24-dien-3β-ol (**46**)

**(45)** turraeanthin

**(46)** tirucalla-7,24-dien-3β-ol



**(5)** squalene

**(44)** dammarene cation

**(43)** apo-euphol structure

**Scheme 7** : Formation of the apo-euphol structure
via the dammarene ion
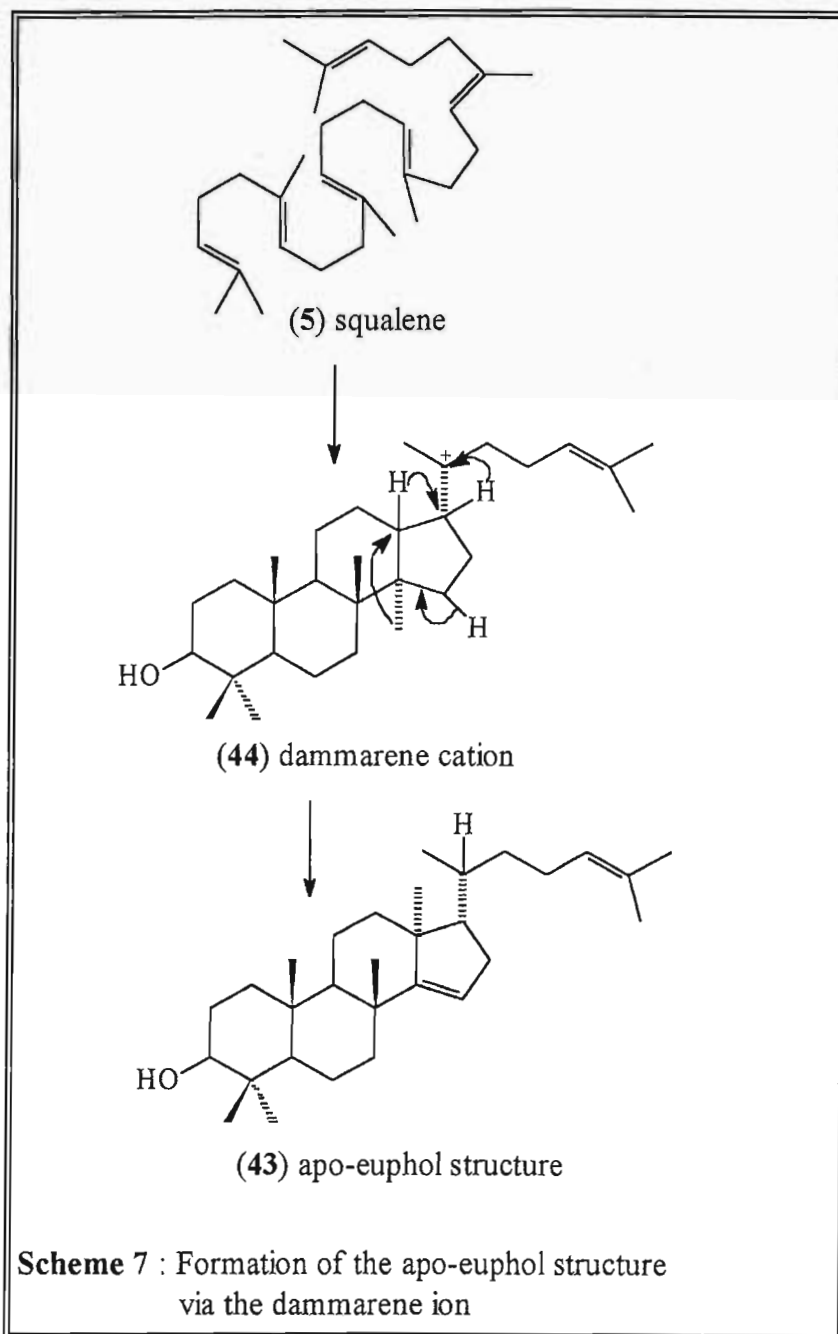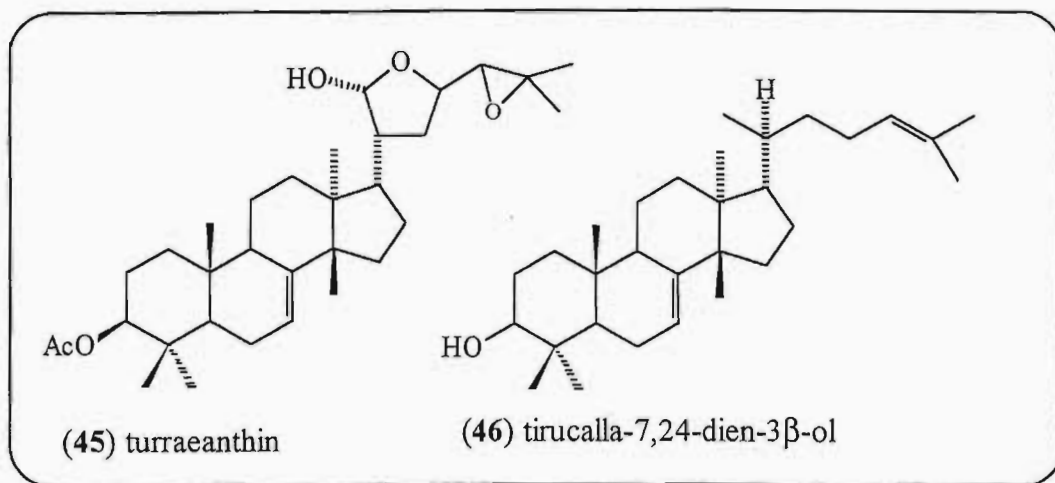
Although the triterpene precursor has not been found naturally, two related acids, acetyl-$\Delta^7$-elemolic acid (**47**), and acetyl-$\Delta^8$-elemolic acid (**48**), have been isolated.



(**47**) acetyl-$\Delta^7$-elemolic acid

(**48**) acetyl-$\Delta^8$-elemolic acid
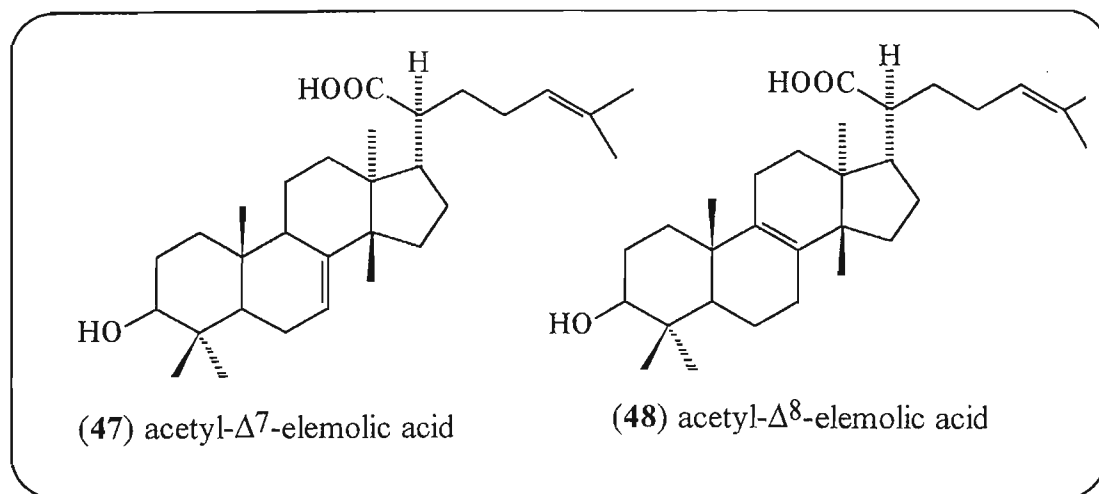
A mechanism has been suggested[22] by which tirucalla-7,24-dien-3β-ol (**46**) may be transformed to the apo-euphol structure. It was noted that in all the known limonoids having a C-7 hydroxyl group, the configuration is 7α. This suggested that the key step in the biosynthesis of the apo-euphol structure proceeded via the formation of the 7α,8α-epoxide, followed by the opening of the oxide ring and subsequent rearrangement to the 7α-hydroxy apo-euphol structure, as depicted in Scheme 8.

**Scheme 8** : Formation of the apo-euphol structure
via tirucalla-7,24-dien-3β-ol
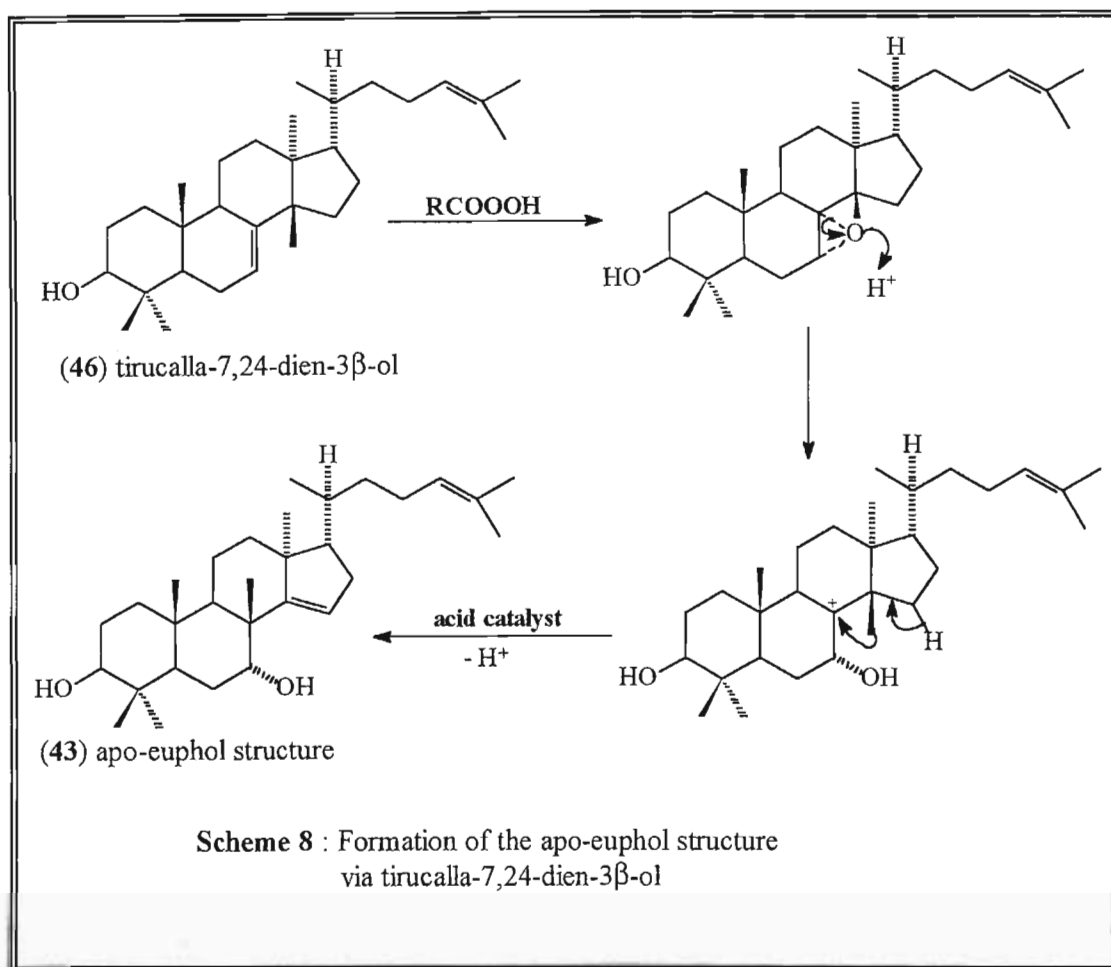
The above transformation has been performed on the 7α,8α-epoxide of

methyl acetyl-Δ[7]-dihydroelemolate **(49)** which was converted to the

7α-hydroxy apo-euphol derivative **(50)** (Scheme 9a) by the action of boron

trifluoride - etherate[22]. Lawrie *et al.*[23] demonstrated the above transformation

by converting dihydrobutyrospermol acetate **(51)** to

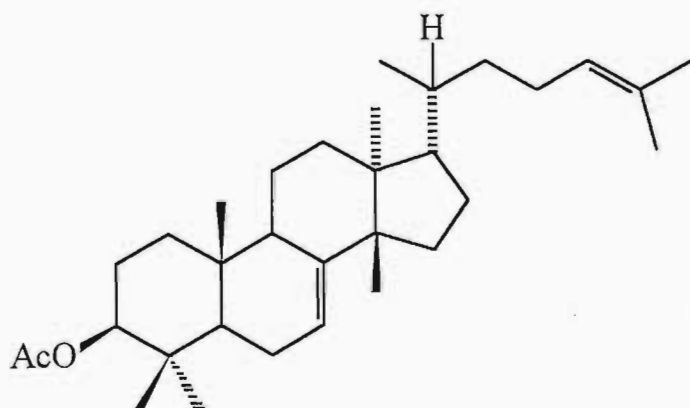7-oxo-apo-euph-14-en-3β-yl acetate **(52)** (Scheme 9b).
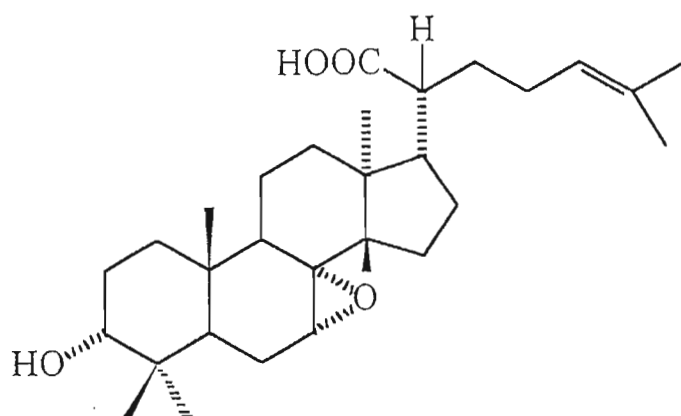
(**49**) 7α,8α-epoxymethylacetyl-dihydroelemolate
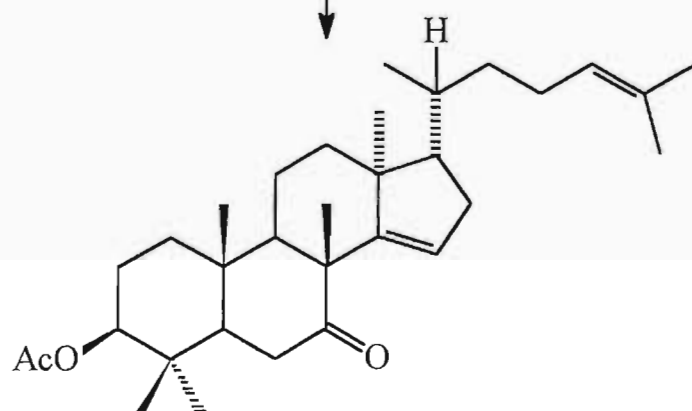
BF$_3$/ether
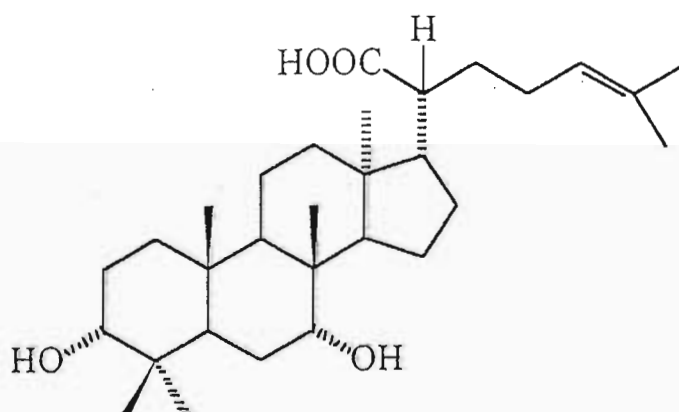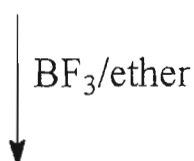
(**50**) 7α-hydroxy apo-euphol derivative

**Scheme 9a** : Laboratory synthesis of the apo-euphol structure

**(51)** dihydrobutyrospermol acetate

**(52)** 7-oxo-apo-euph-14-en-3β-yl acetate

**Scheme 9b** : Laboratory synthesis of the apo-euphol structure

## 1.4.2.1 Group I. Protolimonoids

Oxidised intact triterpenoids, which by their biological occurrence and oxidation pattern appear to be biological precursors of the limonoids, are called protolimonoids. This group of compounds includes members whose triterpenoid side chain is intact but usually highly oxidised and often cyclised to form an ether ring.

Two groups of protolimonoids have been identified. The first, like euphol (27), have a methyl group at C-14β and a $\Delta^7$ double bond, and include such compounds as turraeanthin (45), entandrophragma triol (53), bourjotinolone A (54) and sapelin B (55).



(45) turraeanthin

(53) entandrophragma triol

(54) bourjotinolone A

(55) sapelin B
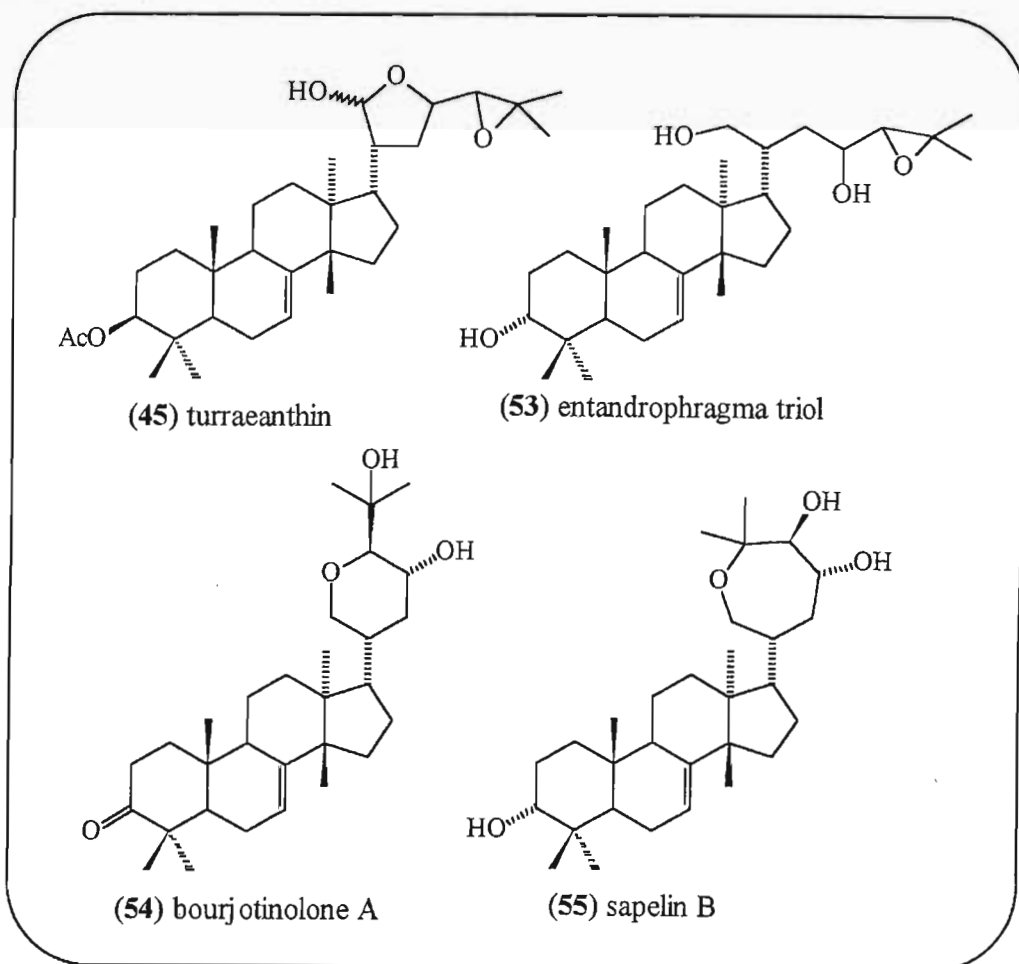
In the second group, the so-called apo-group, compounds have a methyl group at C-8, a $\Delta^{14}$ double bond and a 7α– hydroxy group. These

compounds include grandifoliolenone (**56**). Glabretal (**57**) occupies an intermediate position between the two groups as it has a 7α– hydroxy group and the 8β-methyl group, but the $\Delta^{14}$ double bond is replaced by a 13,14 - cyclopropane ring.



(**56**) grandifoliolenone          (**57**) glabretal
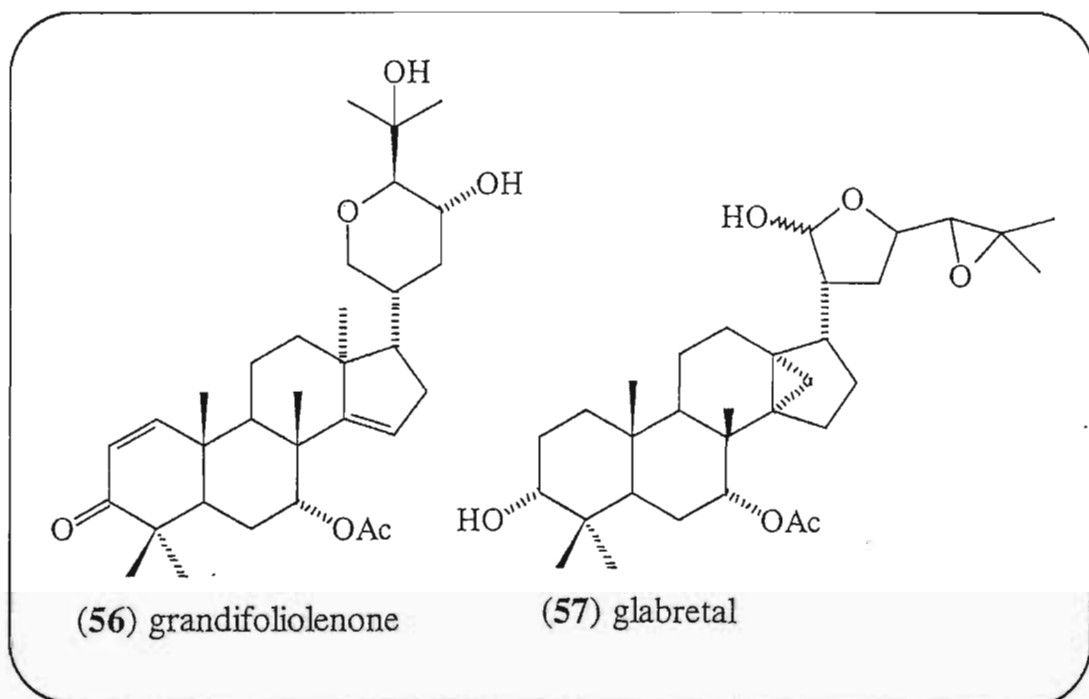
## 1.4.2.2 Group II : Havenensin group

The havanensin group consists of compounds with a furan side chain, *e.g.* azadirone, in which all four rings of the tetracyclic nucleus are intact.



(**58**) azadirone
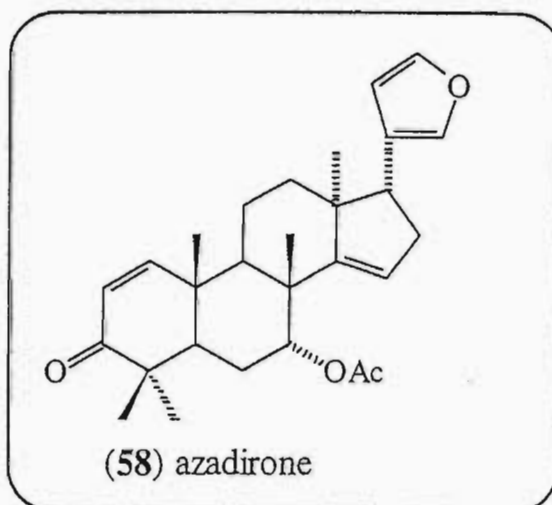
A mechanism has been proposed for the formation of the furan ring from the tirucallol side chain via the turraeanthin intermediate[21]. It was proposed that the eight carbon side chain is oxidised in stages to produce an aldehyde group at C-21, a hydroxy group at C-23 and a 24,25 - epoxide in place of the double bond. The subsequent cyclisation affords a turraeanthin side chain (Scheme 11).



**Scheme 11 :** Oxidation of a side chain
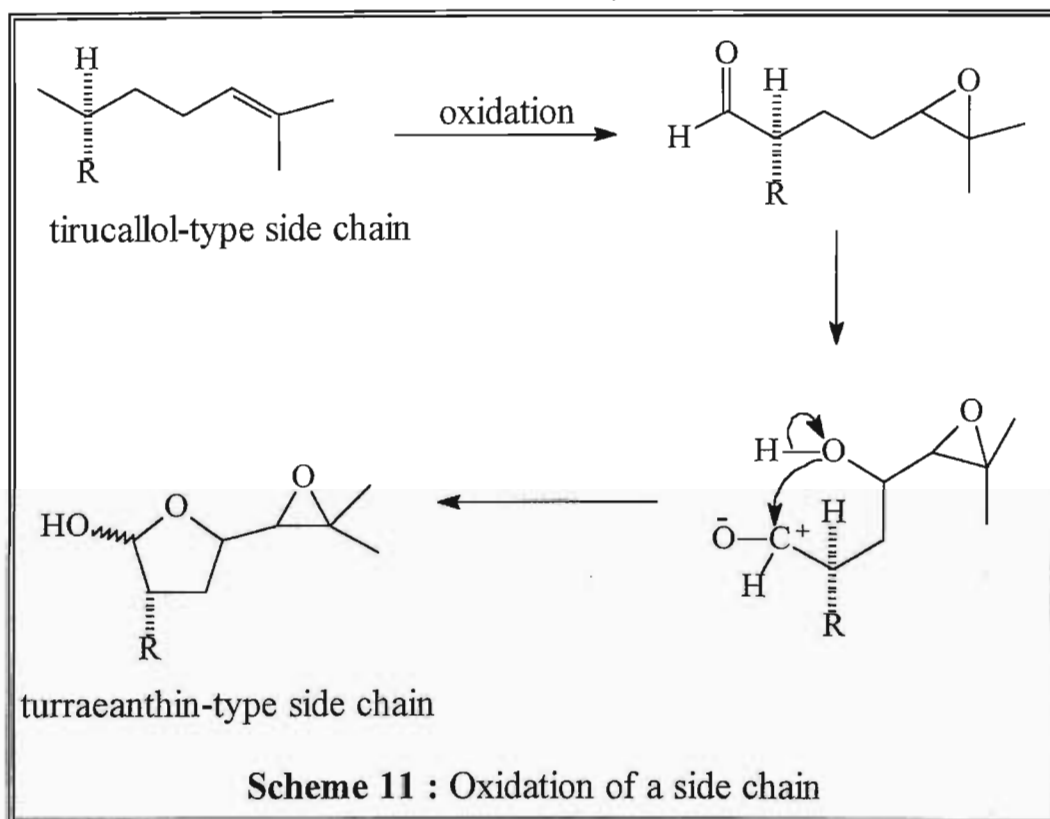
The formation of the furan ring from the turraeanthin-type side chain is thought to involve oxidation to give a keto group at C-24 which forms either by the rearrangement of the epoxide or by the formation of a diol from the epoxide. Baeyer-Villiger oxidative cleavage of the C-23,24 bond generates the dihydrofuran ring by loss of four carbon atoms. The subsequent dehydration yields a β-substituted furan ring, as shown in Scheme 12a. Support for this proposed pathway has been derived from the chemical conversion of the turraeanthin side chain to a furan ring[24,25].

**Scheme 12a : Furan ring formation**
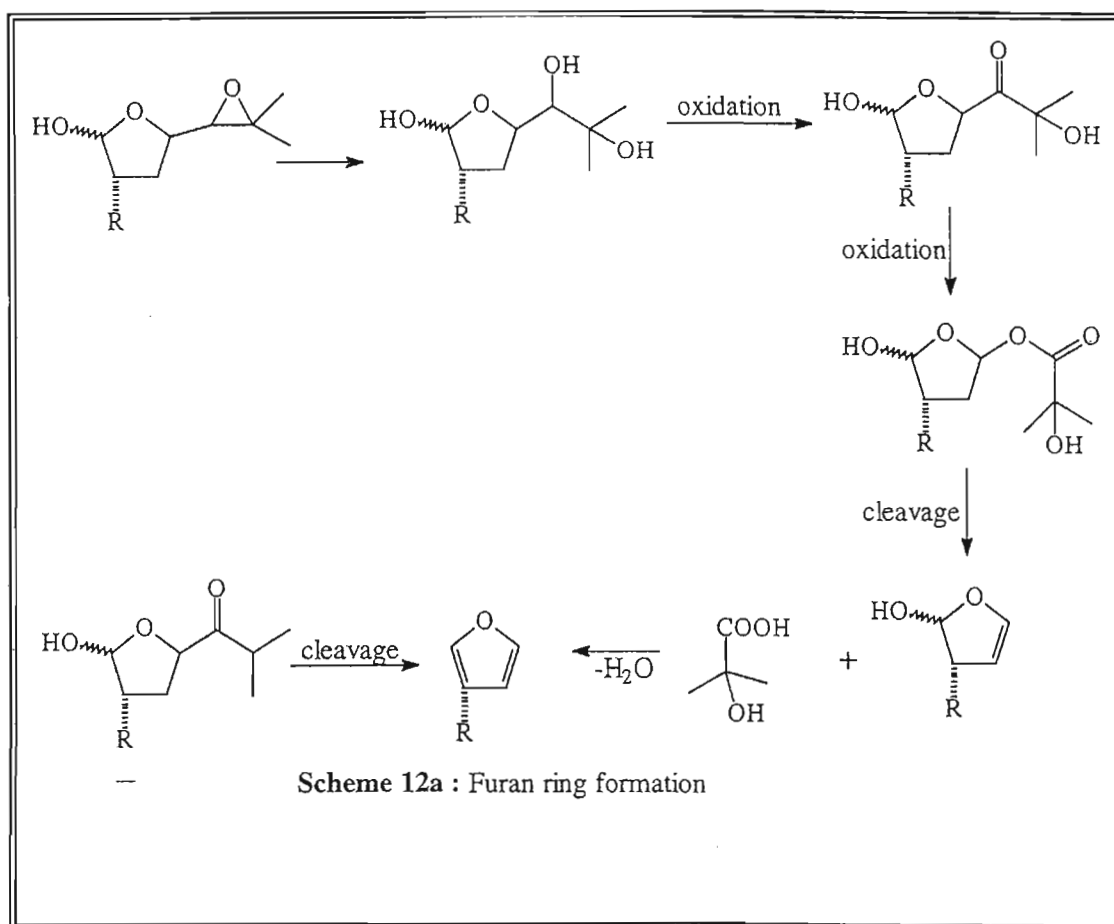
Turraeanthin (**45**), treated with sodium metaperiodate in aqueous dioxan containing a trace of perchloric acid gave a product which was mainly the labile cyclic hemi-acetal (**59**). Treatment of this with toluene-*p*-sulphonic acid in benzene gave the β-substituted furan ring (**60**) (Scheme 12b)[24,26].

No direct evidence exists to suggest that the furan ring formation is preceded by the apo-euphol rearrangement which has been observed both in compounds with hydrocarbon or oxidised side chain. However, the isolation of grandifoliolenone-type compounds in which the apo-euphol rearrangement has been completed and which lack the furan ring, and the lack of compounds with both $\Delta^7$ double bond and the furan ring, suggested that the apo-euphol rearrangement precedes the formation of the furan ring.

**Scheme 12b :** Furan ring formation
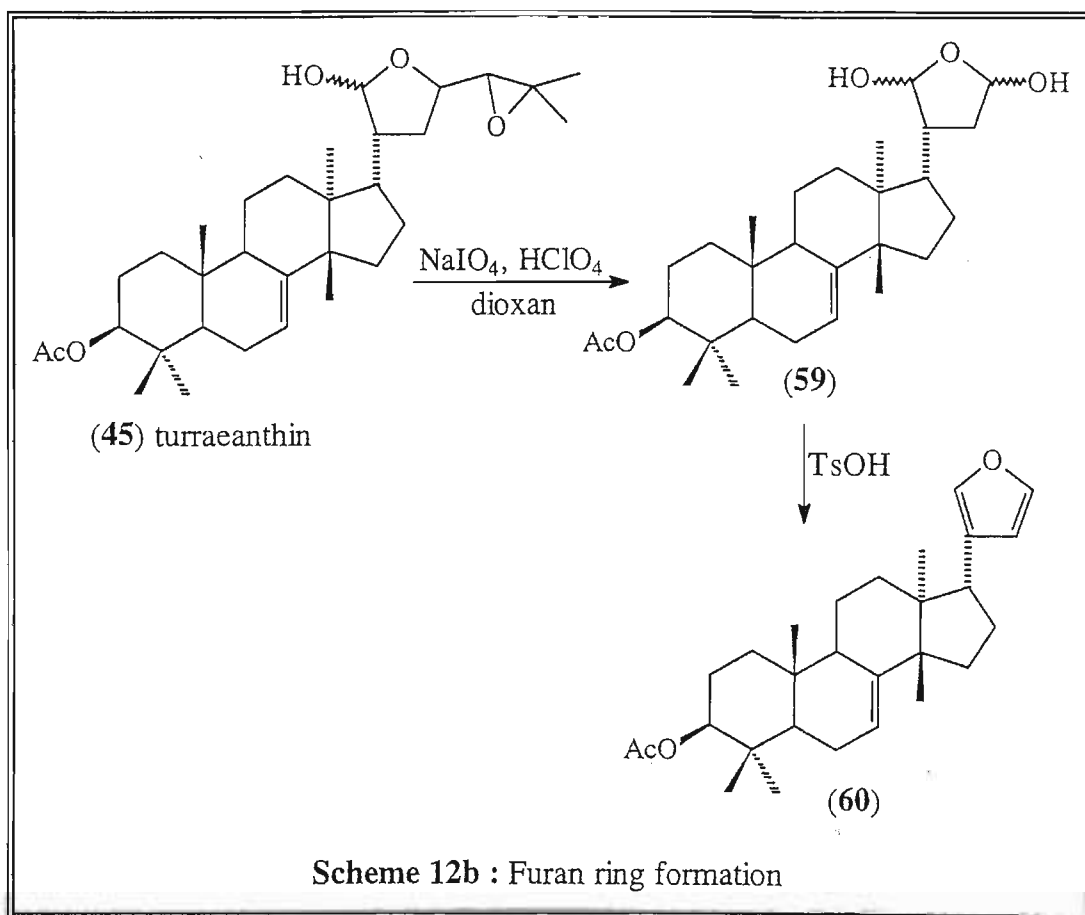
This group consists of two kinds of compounds, the first being of comparatively simple structure e.g. azaridone (**58**) and havanensin (**61**), the second consisting of more complex compounds such as heudelottins C (**62**), E (**63**) and F (**64**)[27].



(**61**) havanensin
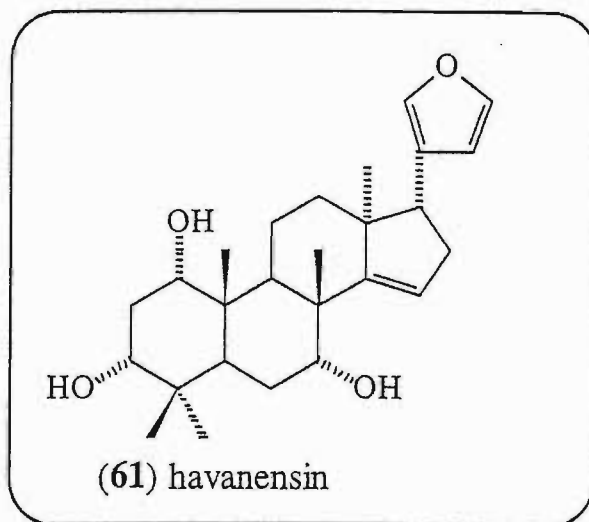
The heudelottin compounds are the simplest examples of the 11β-formyloxy-12α-(2-hydroxy-3-methylvaleryloxy) system common in the group X limonoids where rings A and B are modified.



(62) heudelottin C

(63) heudelottin E

(64) heudelottin F
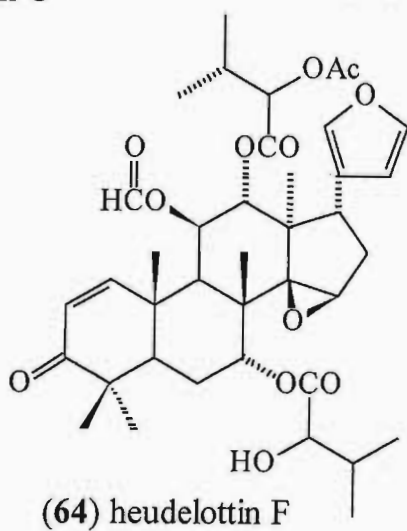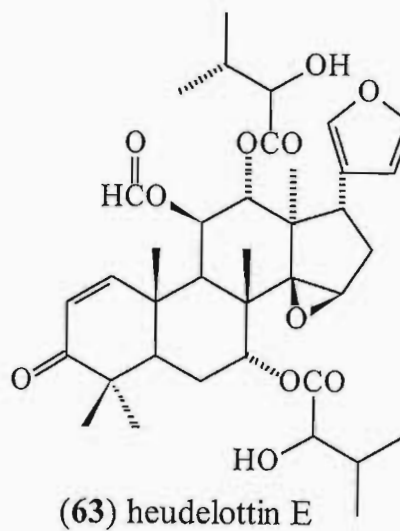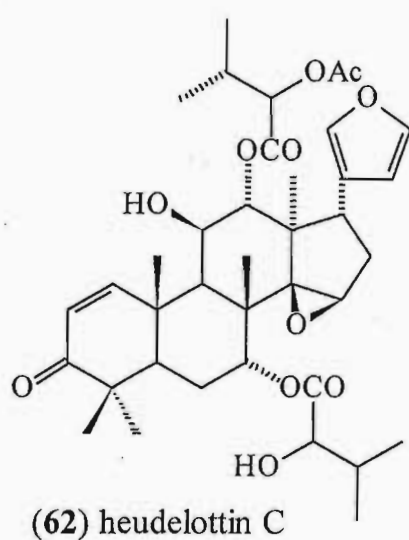
## 1.4.2.3 Group III : Gedunin group

Members of this group have rings A, B and C carbocyclic and ring D lactonised. They include such compounds as gedunin (**40**), and khivorin (**65**). Photogedunin (**66**), isolated from *Cedrela odorata*[28], is a gedunin derivative where the furan ring has been oxidised photochemically to give a 4-hydroxy-2,3-unsaturated γ-lactone. The biosynthesis of the lactone ring D in gedunin is depicted in Scheme 13.



(**65**) khivorin      (**66**) photogedunin



R' = H or Ac

**Scheme 13** : Oxygenation of ring D
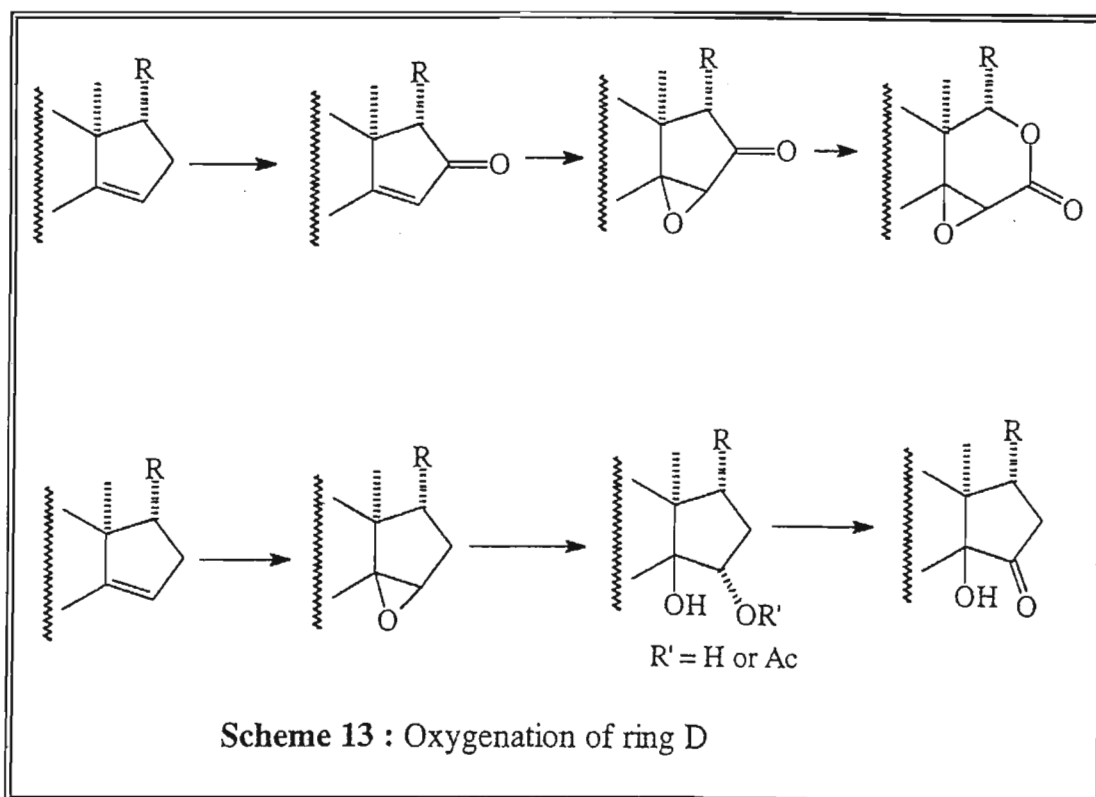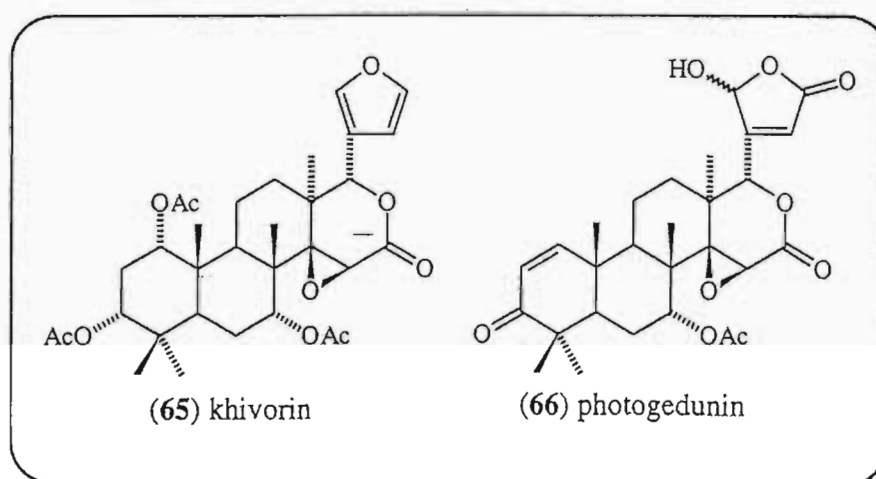
The first step involves the allylic oxidation of the carbocyclic ring to give a cyclopentenone ring followed by epoxide formation and further oxidation to generate the α,β-epoxido-δ–lactone. Alternatively, modification of ring D involves oxidation of the $\Delta^{14}$ double bond to give 14,15-oxide compounds and 14-hydroxy-15-ketone compounds, as in the heudelottins (**62, 63, 64**), in which ring D remains carbocyclic[29,30].

The hypothetical sequences in Scheme 13 are supported by laboratory synthetic work, and the fact that the possible intermediates have been isolated[29].

## 1.4.2.4 Group IV: Andirobin group

Members of this group have rings A and C intact, ring D lactonised and ring B cleaved, as in andirobin (**66**). Three subgroups are distinguishable within this group. The first includes limonoids related to andirobin (**66**) which may include compounds such as methyl angolensate (**67**) which contain a 1,14-oxide linkage.



(66) andirobin

(67) methyl angolensate
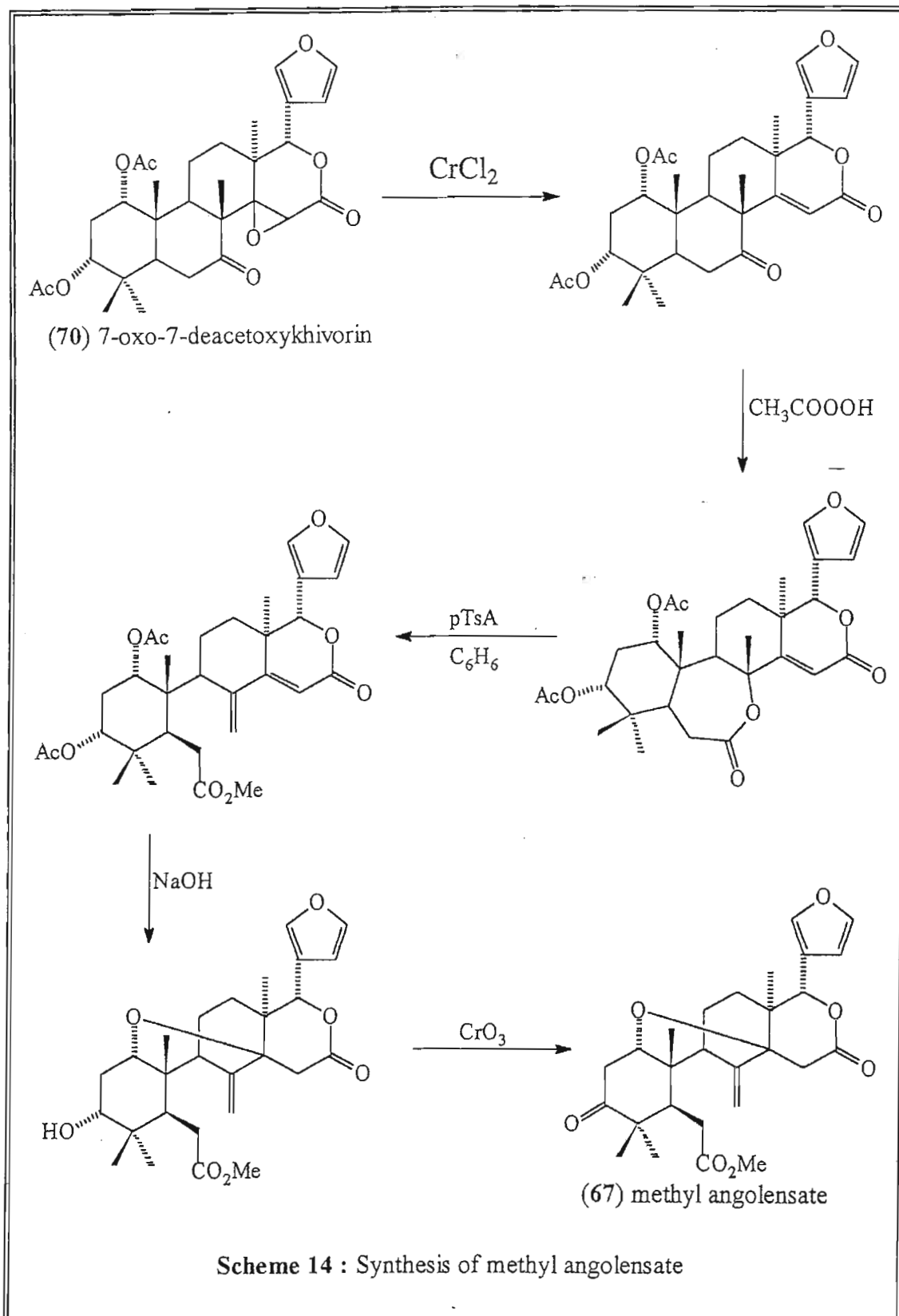
(68) mexicanolide
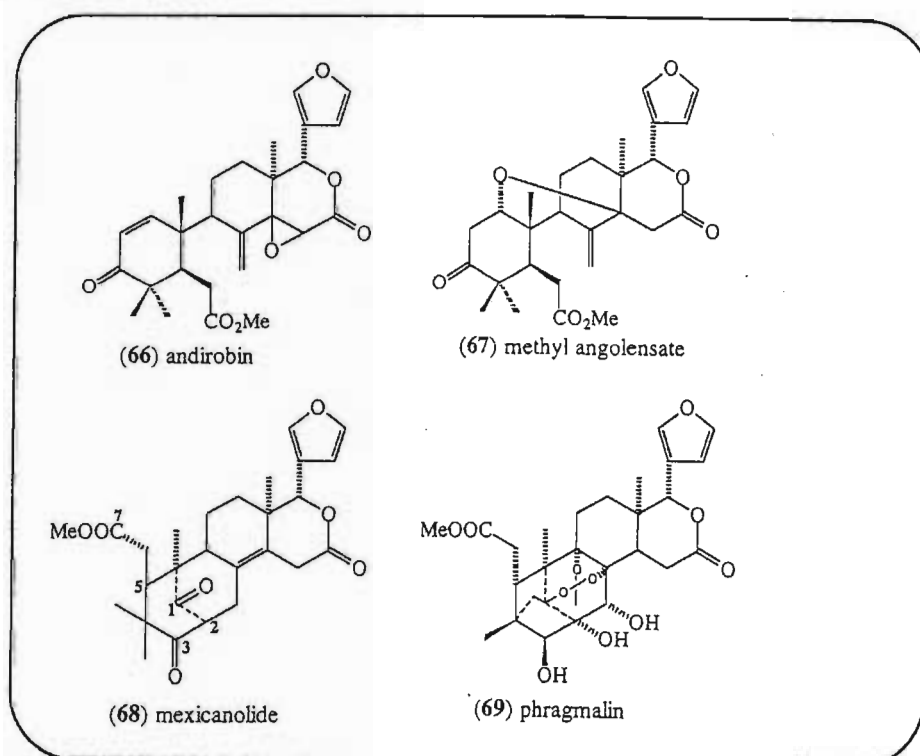
(69) phragmalin

The second includes those in which recyclisation of ring B has occurred to give a bridged ring system such as in mexicanolide (**68**). The third includes compounds such as phragmalin (**69**) which are characterised by ortho ester formation.
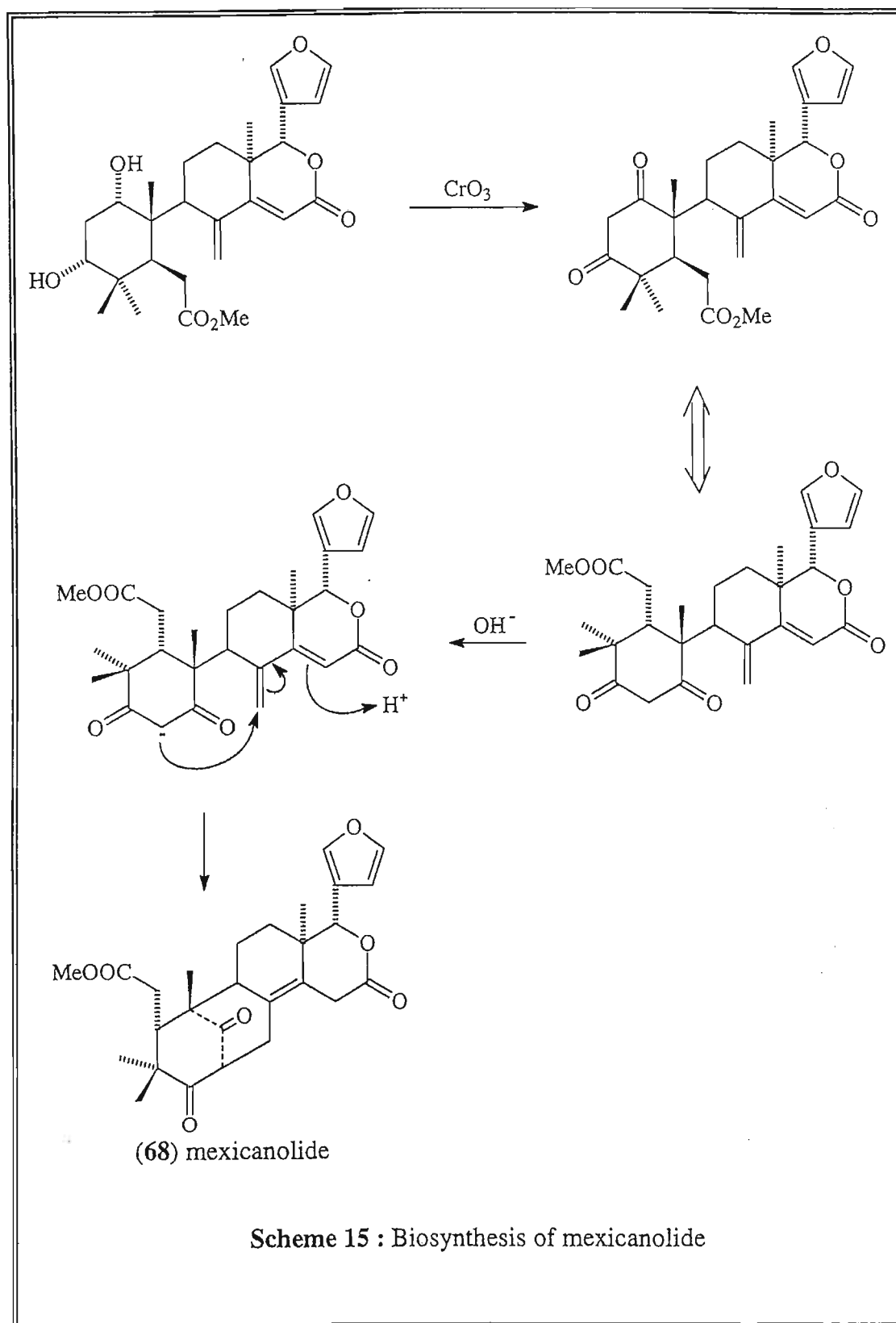
The Group IV compounds are presumed to have been biosynthetically derived from the gedunin group. In the methyl angolensate - type compounds, following opening of ring B, the α–hydroxy group at C-1 adds to the α,β-unsaturated lactone system (Michael addition), to form a 1,14-bridge, as shown in Scheme 14. Partial synthesis of methyl angolensate (**67**) was successfully accomplished utilising a Baeyer-Villiger oxidation (Scheme 14)[31].
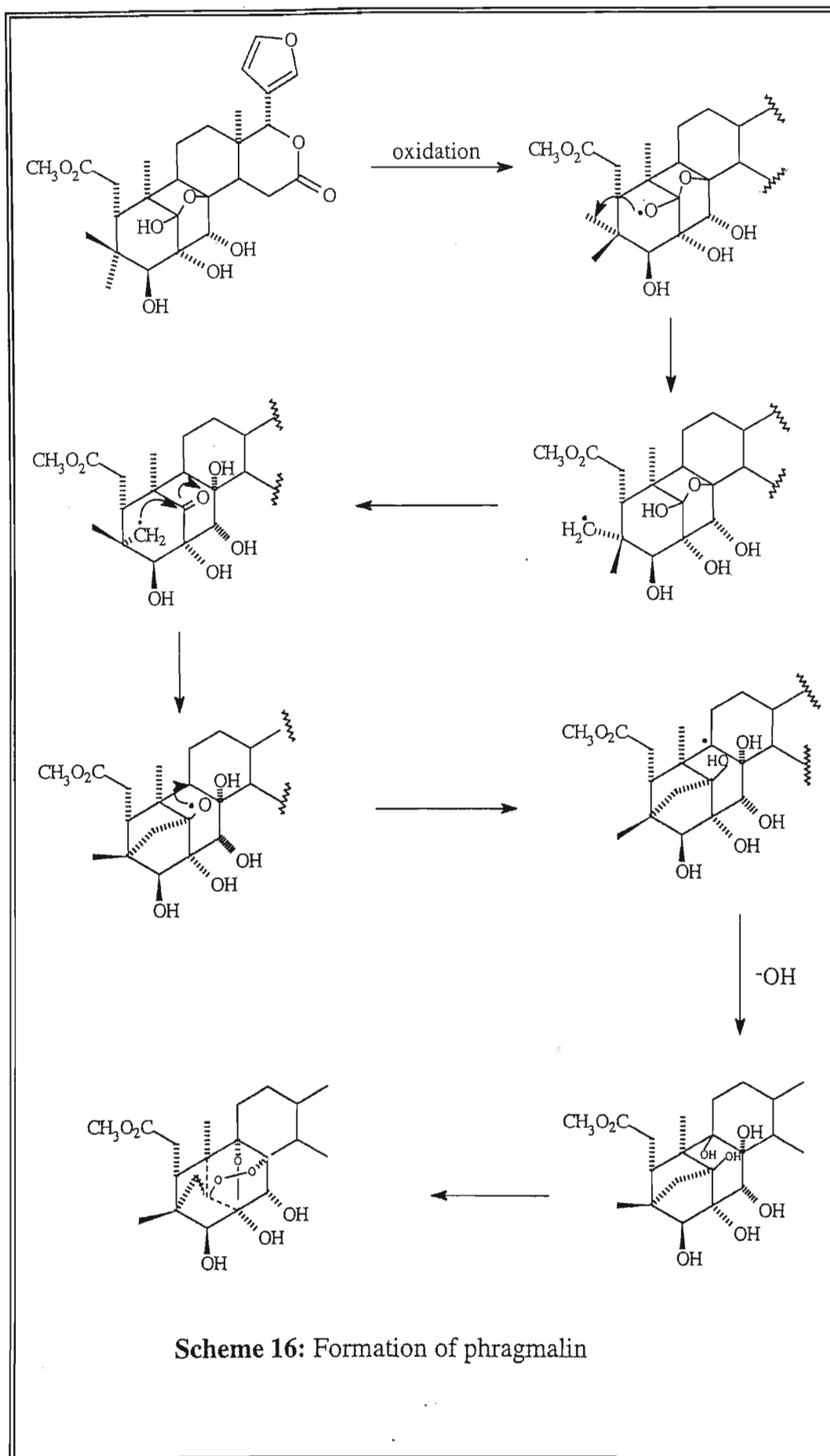
In the mexicanolide (**68**) subgroup (Group IVb), the opening of ring B and oxidation of the C-1 and C-3 hydroxy groups is followed by rotation about the C-9,10 bond and Michael-type addition of C-2 to the 8,30 - double bond[32] (Scheme 15). The biosynthesis of mexicanolide isomers with 8,30- and 14,15 - double bonds is not yet known. Laboratory synthesis has only afforded the 8,14 - isomer.

The phragmalin (**69**) subgroup (Group IVc) is derived from the gedunin group via mexicanolide (**68**). These limonoids have the C-29 methyl group oxidised and the C-1 ketone reduced, hence the transformation is merely an isomerisation. It has been suggested[33,34] the precursor is a ketal of the *Xylocarpus* type which is converted into an oxygen radical which oxidises C-29 to a radical, as shown in Scheme 16. The C-29 radical is presumed to attack the keto group at C-1, formed from the ketal, giving a second oxygen

radical which may finally oxidise C-29. Since C-8 oxidation is necessary to produce the original ketal, Scheme 16 explains why phragmalin derivatives are always oxidised at C-8 and C-9.



**Scheme 14** : Synthesis of methyl angolensate

**Scheme 15 :** Biosynthesis of mexicanolide

**Scheme 16:** Formation of phragmalin

### 1.4.2.5. Group V : Methyl ivorensate group

These compounds have both rings A and D lactonised, ring B opened and ring C carbocyclic. Methyl ivorensate (**71**) was first isolated in small amounts from *Khaya ivorensis*[35]. This compound is the first representative of this small group, and was synthesised by the oxidation of methyl angolensate (**67**) with perbenzoic acid[36]. Methyl ivorensate has also been isolated in the course of this work.



(**71**) methyl ivorensate
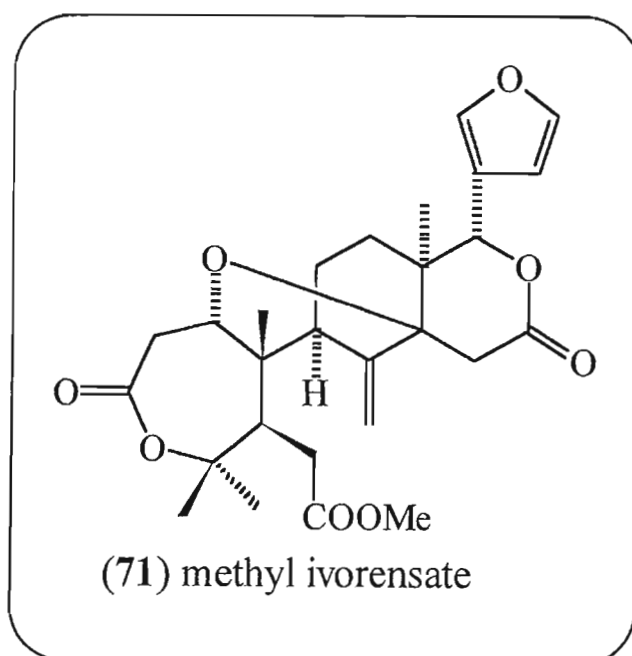
The probable biosynthetic pathway involves a multistep oxidation of the gedunin precursor as shown in Scheme 17.

(71) methyl ivorensate

**Scheme 17 :** Biosynthesis of methyl ivorensate
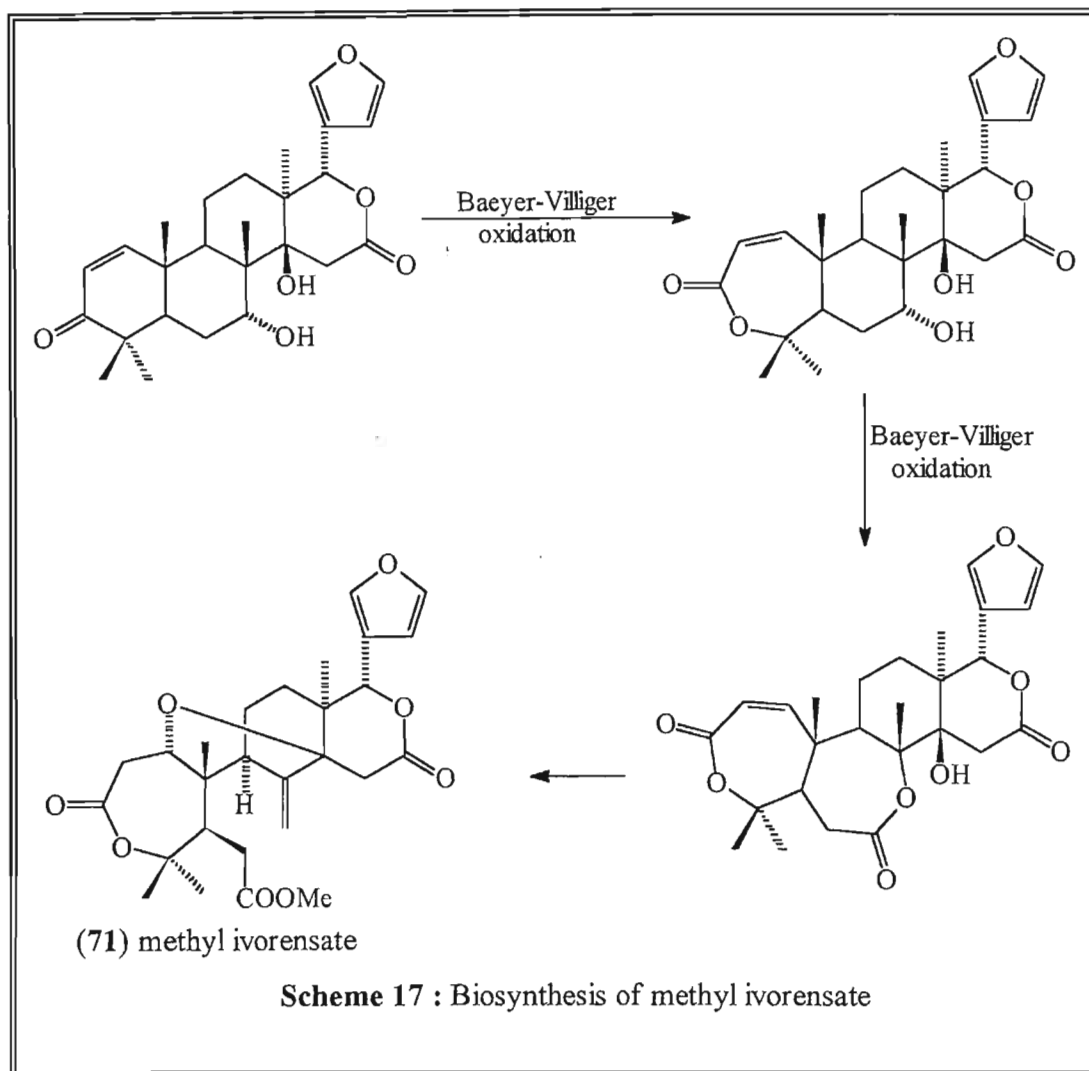
## 1.4.2.6 Group VI : Obacunol group

Limonoids of this group are distinguished by having both rings A and D lactonised, and rings B and C carbocyclic. This group of compounds to which limonin (41) belongs is characteristic of the Rutaceae and rare in the Meliaceae. Glycosides (74, 75, 76) of limonin (41), nomilin (72) and obacunol (73) respectively, were isolated from grapefruit seeds (*Citrus*, Rutaceae)[37]. In these compounds, ring D may be opened and a sugar moiety may be attached at C-17.
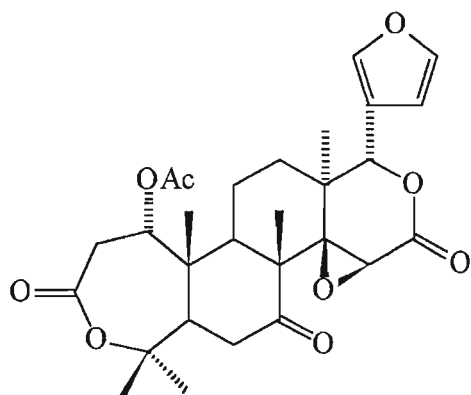
(72) nomilin
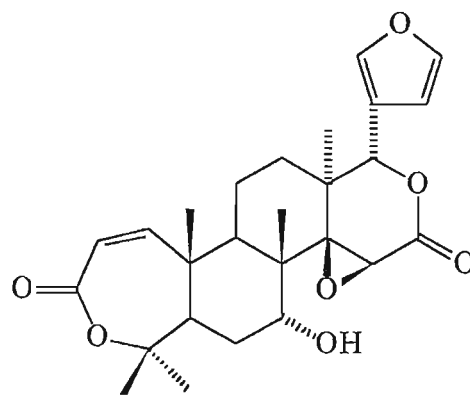
(73) obacunol
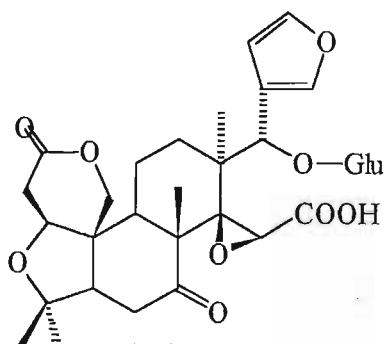


(74)

(75)

(76)

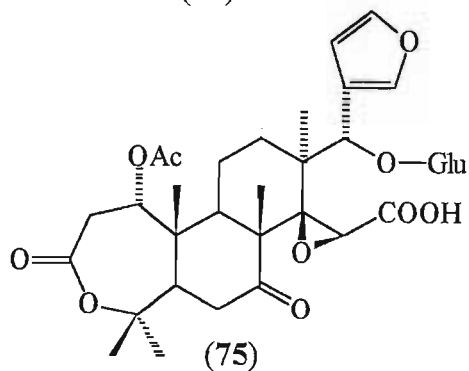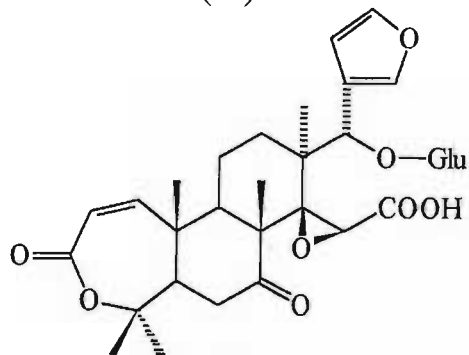Bitterness due to limonoids in certain citrus juices is one of the major problems of the world-wide citrus industry. This problem has led to the study of the biosynthesis of limonoids known to occur in *Citrus*. Analysis of commercial citrus juices indicated that orange juice contains the highest amount of limonoid glucosides (250 to 430 ppm) followed by grapefruit juice (140 to 230 ppm) and lemon juice (76 to 93 ppm)[38]. Limonin-17-β–D- glucopyranoside (74) is the major limonoid glucoside occurring in commercial juices[39].

In their study, Herman and Hasegawa[40], demonstrated the conversion of nomilin (72) and obacunone (77) to obacunoate (78) and further to limonin (41) using a radioisotope tracer technique (Scheme 18).



(72) nomilin

(41) limonin

**Scheme 18 : Biosynthesis of limonin**
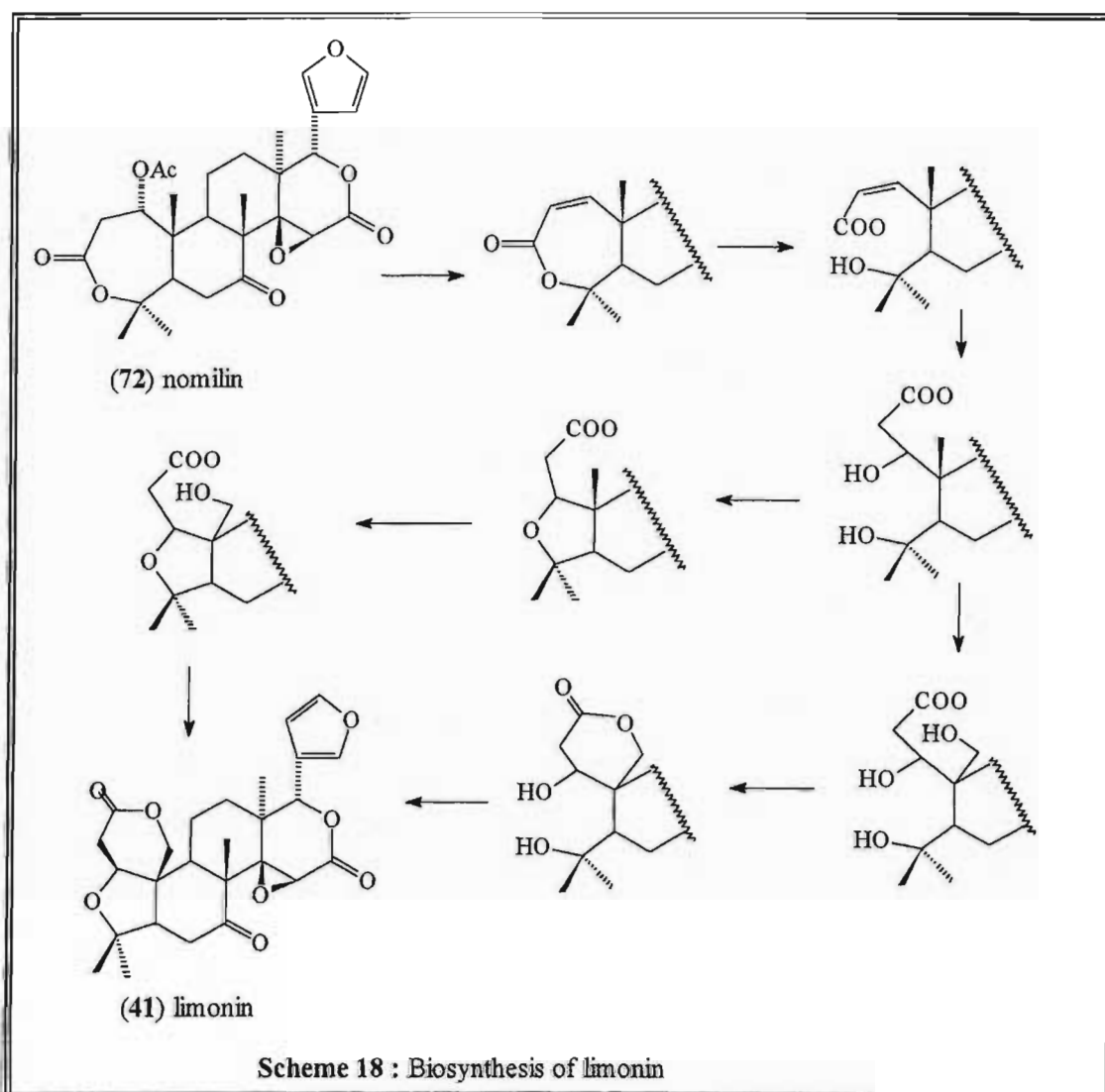
## 1.4.2.7. Group VII : Nimbin group

The compounds of this group have rings A, B and D intact, with ring C opened, as typified by nimbin (**79**). The biosynthesis of group VII limonoids has received considerable interest since they appear to exhibit a wide range of biological properties. These compounds appear to arise from a precursor related to deoxyhavenensin (**80**) by a Baeyer-Villiger cleavage of ring C as shown in Scheme 19. Feeding experiments in the leaves of *Azadirachta indica* showed that the $\Delta^8$-isomers of euphol (**27**), tirucallol (**28**) and butyrospermol (**42**) were more efficiently utilised than the $\Delta^7$- isomers in the biosynthesis of nimbolide (**81**)[20].

There are however several opinions as to what mechanism is involved in the opening of ring C by C-12,13 bond cleavage[41,42,43].



(**79**) nimbin

(**81**) nimbolide



(**80**) deoxyhavanensin

**Scheme 19** : Formation of nimbin-type limonoids
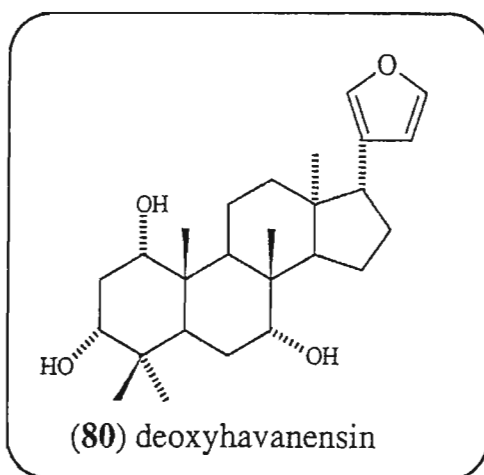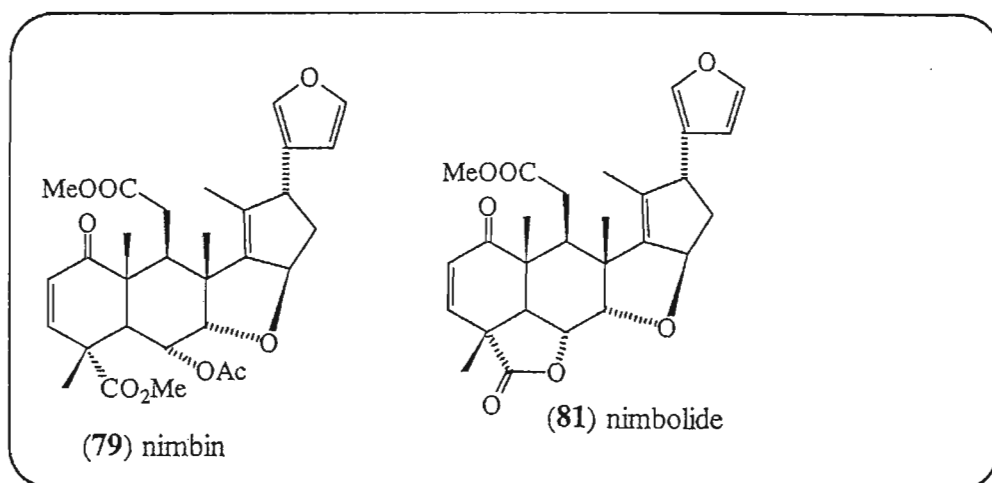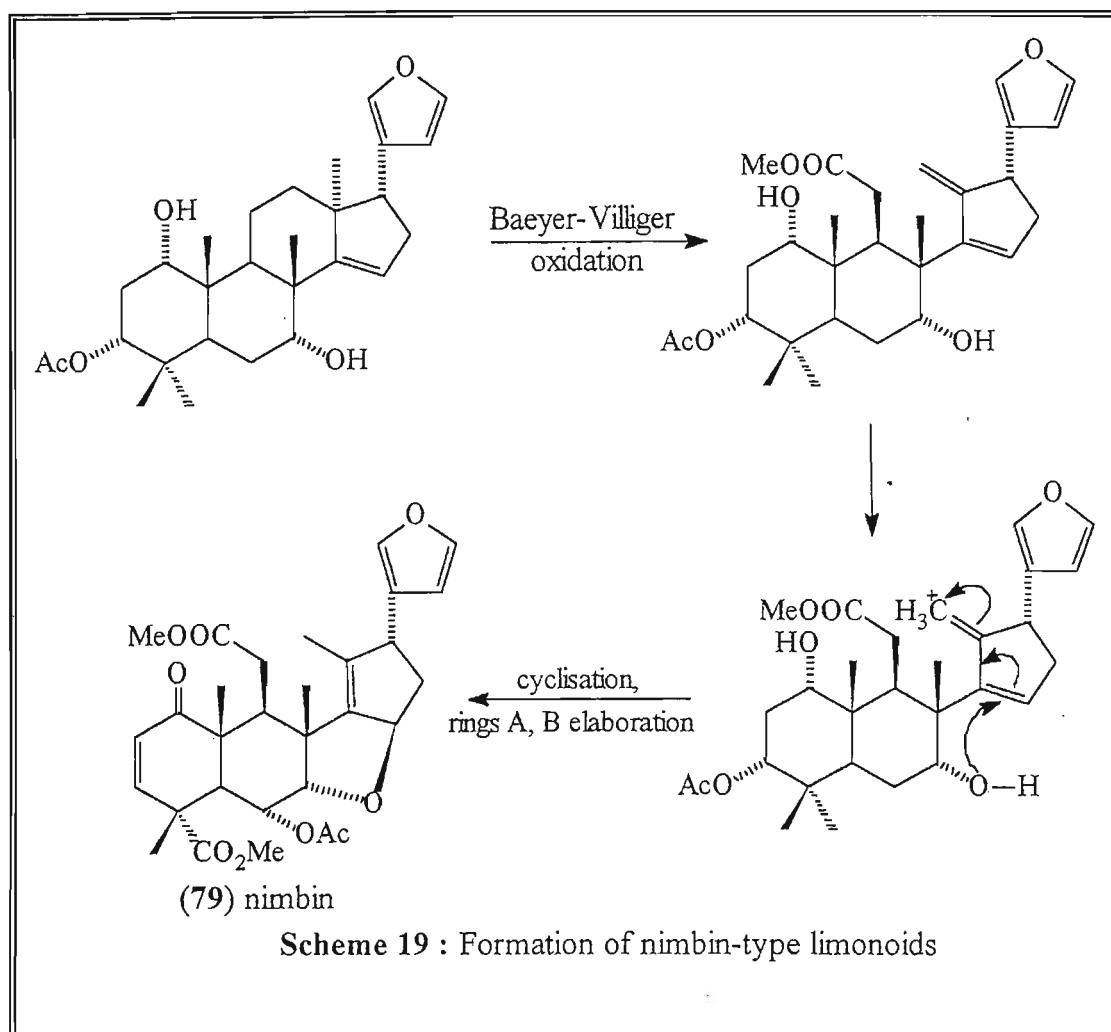
## 1.4.2.8 Group VIII : Toonafolin group

These limonoids have rings A, C and D intact with ring B usually opened. Toonafolin (**82**) and toonacilin (**83**) from *Toona ciliata*[44,45] are typical representatives of this group.

**(82)** toonafolin

**(83)** toonacilin
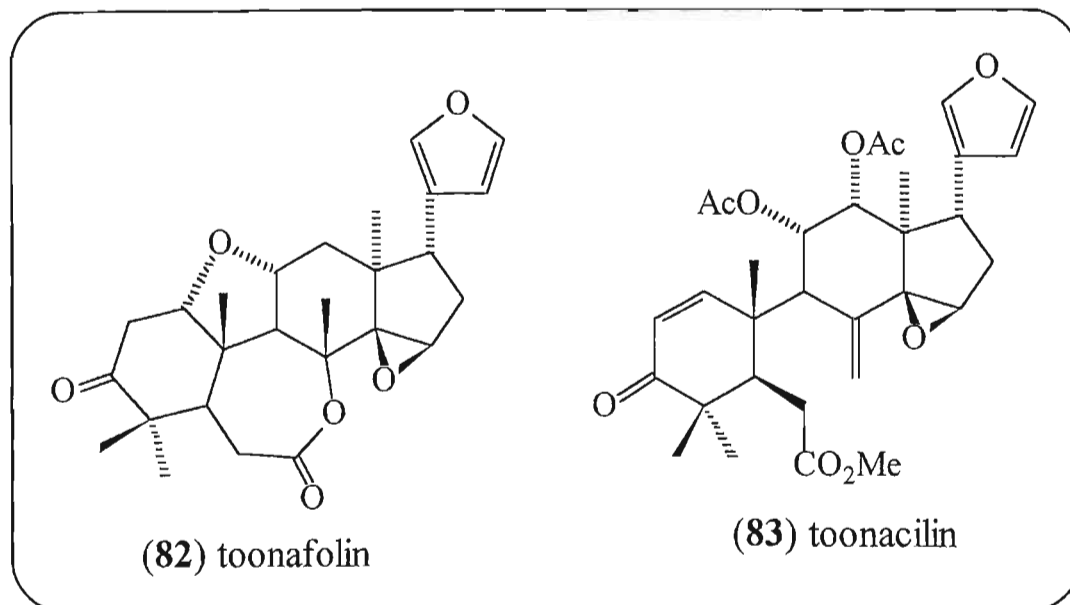
### 1.4.2.9 Group IX : Evodulone group

Members of this group have rings B and C intact, and rings A and D oxidised e.g. evodulone **(84)**. These compounds are generally considered to be biosynthetic precursors of the group X limonoids[46].



**(84)** evodulone
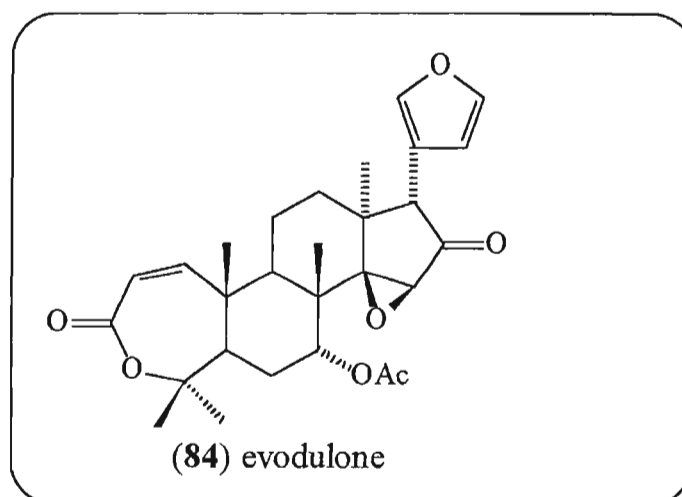
These compounds are formed from the havanesin-type compounds by a Baeyer-Villiger oxidation of ring A, as depicted in Scheme 20.

**Scheme 20 :** Formation of evodulone-type compounds
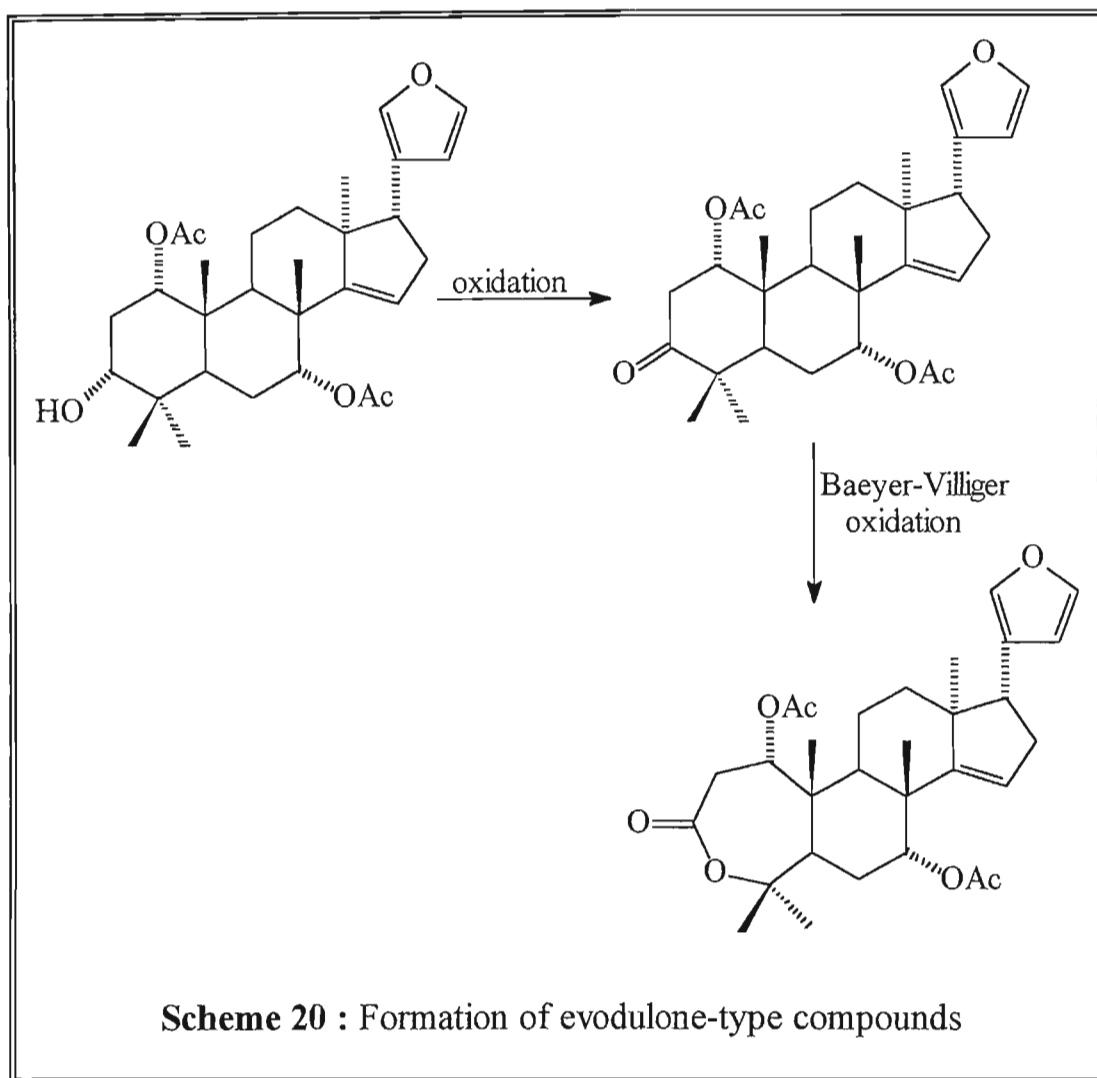
### 1.4.2.10. Group X : Prieurianin group

Members of this group are complex , highly oxidised compounds with ring C and D carbocyclic, ring B opened and ring A lactonised. The structures of these compounds resemble those of the evodulone group e.g. prieurianin (**85**) and dregeanin (**86**).

Group X limonoids may be derived from the Group IX limonoids by the opening of ring B which is assumed to proceed by Baeyer-Villiger oxidation (Scheme 21).

(85) prieurianin

(86) dregeanin



Group IX limonoid

hydrolysis
oxidation

Baeyer-Villiger
oxidation
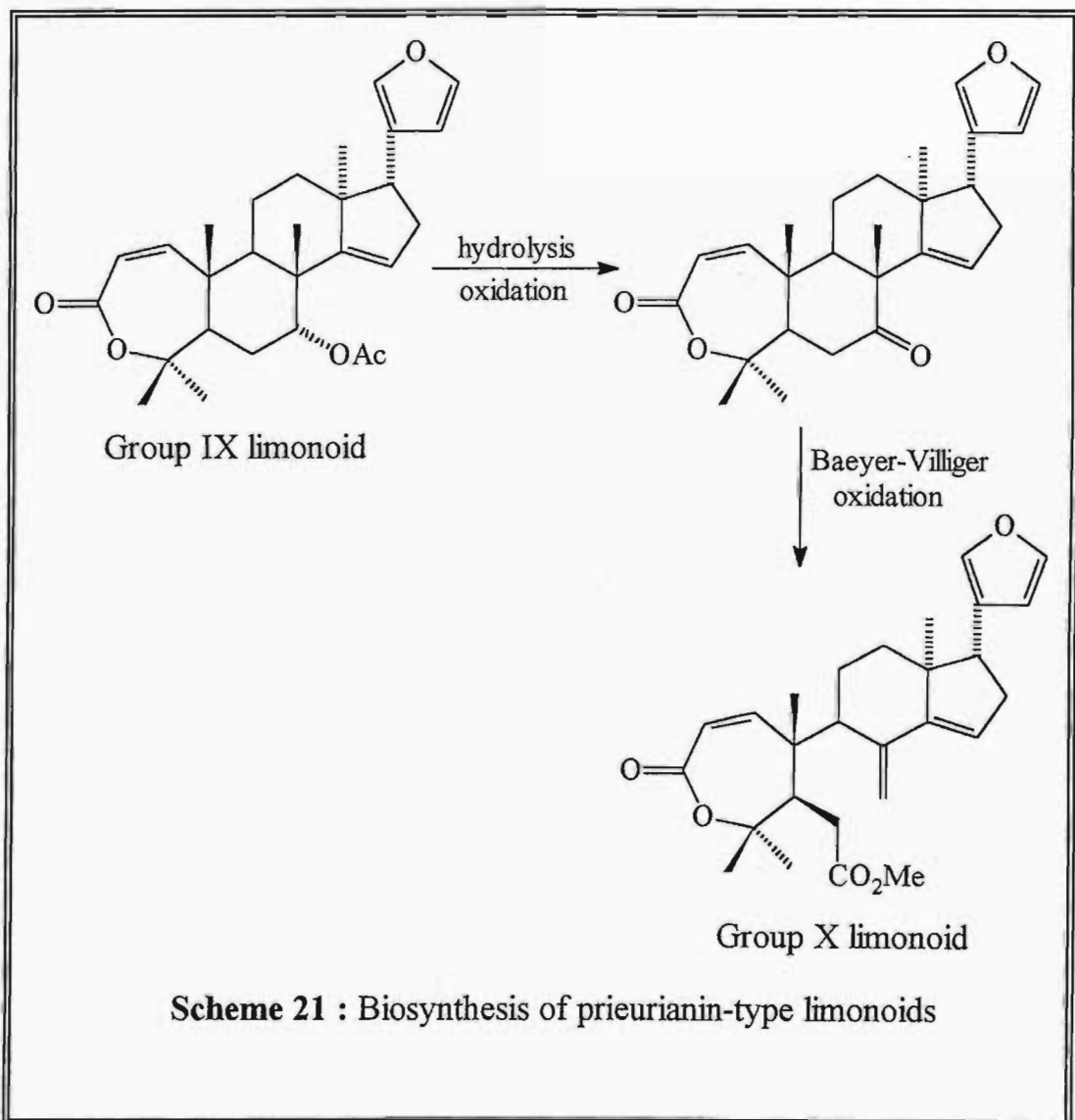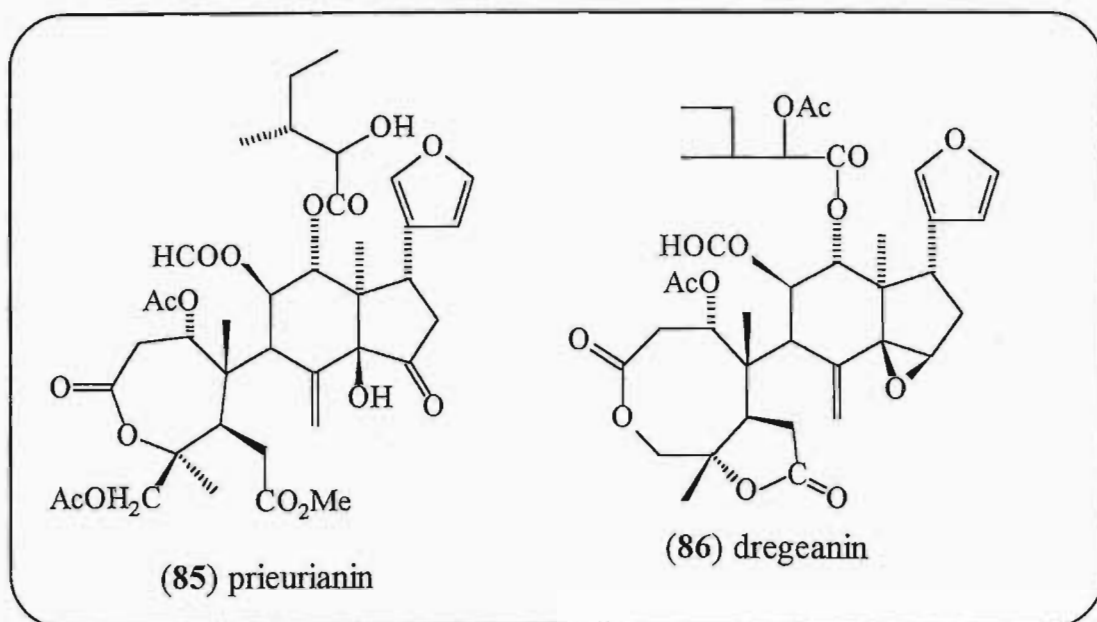
Group X limonoid

**Scheme 21** : Biosynthesis of prieurianin-type limonoids

The hypothetical routes leading to the formation of the different groups of limonoids discussed in the preceeding sections are depicted in Scheme 22.

Protolimonoid
e.g. turraeanthin (45)

1. apo-change
2. side chain oxidation

Group II
e.g. havanensin (61)

peroxidation of

ring D          ring C          ring B          ring A

Group III          Group VII          Group VIII          Group IX
e.g. gedunin (40)   e.g. nimbin (79)   e.g. toonafolin (82)   e.g. evodulone (84)

ring B                              peroxidation of

opened          opened and cyclised          ring D          ring B

Group IVa          Group IVb          Group VI          Group X
e.g. andirobin (66)   e.g. mexicanolide (68)   e.g. obacunol (73)   e.g. prieurianin (85)

peroxidation of ring A          further oxidation

Group V          Group IVc
e.g. methyl ivorensate (71)   e.g. phragmalin (69)

Scheme 22: Hypothetical routes in limonoid biosynthesis

## 1.5. Biological activity of limonoids

Many limonoids are available in large quantities, the timber of some species may yield 1% of an isolated limonoid. A single tree of *Entandrophragma angolense* may contain more than 100 kg of gedunin (**40**)[17]. The biological advantage for some plants of producing such large quantities of limonoids may be that many limonoids are active as insect antifeedants[47]. However most limonoids are not directly insecticidal.
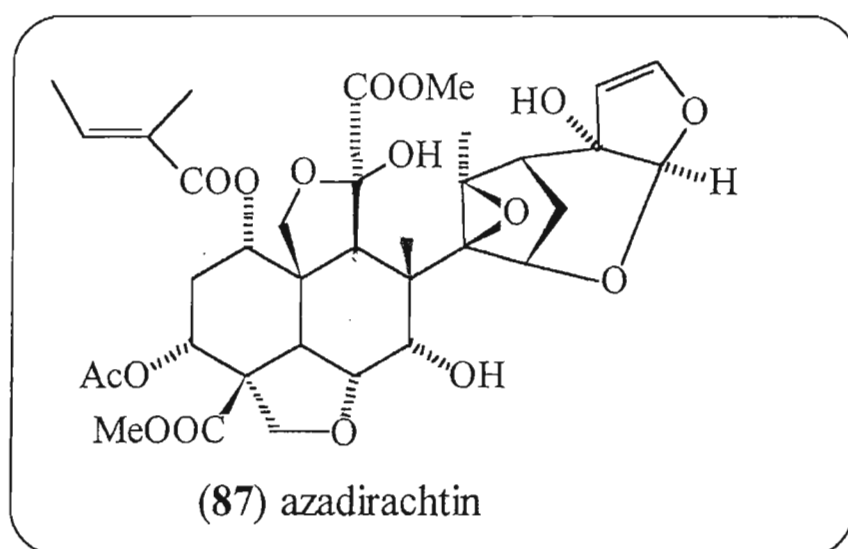


(**87**) azadirachtin

Azadirachtin (**87**), an insect - antifeedant, is known to affect over 200 species of insects[48,49].

Limonoids appear to have a wide range of biological activities, including insect-antifeedant and growth regulating properties, antifungal, bactericidal, antileukaemic properties and a variety of other medicinal effects in animals and humans[50]. Information on the biological activities of over 70 other limonoids is also available[51,52,53].

Group II limonoids with 14,15-epoxy D ring and a 19,28 lactol bridge e.g. sendanin (**88**) were found to be the most active anti-cancer agents.

Compounds with the epoxide and a 3-oxo-1-ene A ring system e.g. anthothecol (**89**) were somewhat less active and reduction of the 1,2 - double bond eliminated the activity.



**(88)** sendanin        **(89)** anthothecol
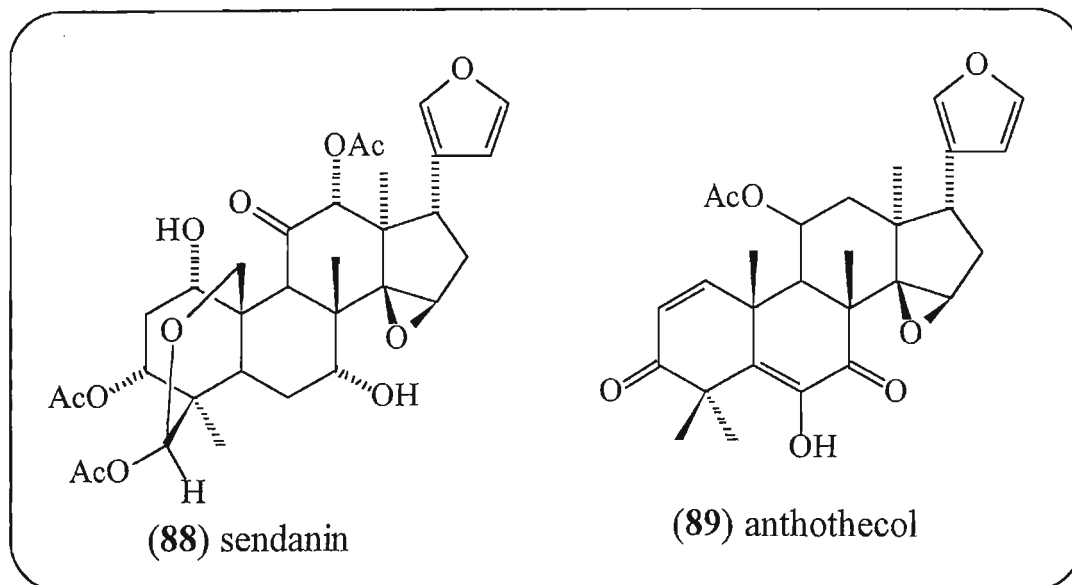
Group VII compounds such as prieurianin (**85**) and group I compounds were found to be weakly active. Other classes of limonoids were inactive[54].

*Citrus* limonoids, particularly nomilin (**72**), obacunone (**77**) and inchangin (**90**), have been found to induce the detoxifying enzyme glutathione.



**(90)** inchangin
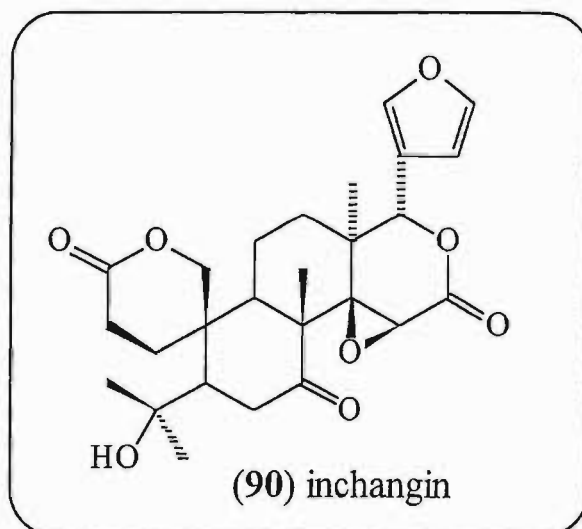
S-transferase (GST) which may reduce the carcinogenic activity of chemical carcinogens by facilitating rapid excretion[55]. It appears that the furan ring moiety is the critical site for enzyme - inducing activity[56]. Platelet-activating factor (PAF) is a fundamental mediator of mammalian cell function which is thought to play a significant role in a variety of pathophysiological states inducing acute allergy, inflammation, asthma, gastrointestinal ulceration and toxic shock. Six limonoid compounds, including swietemahonin G (**91**) and 3-O-acetylswietenolide (**92**), isolated from the ether extract of the seeds of *Swietenia mahogani* (Meliaceae), were found to inhibit aggregation of platelets[57].



(**91**) swietemahonin G            (**92**) 3-O-acetylswietenolide
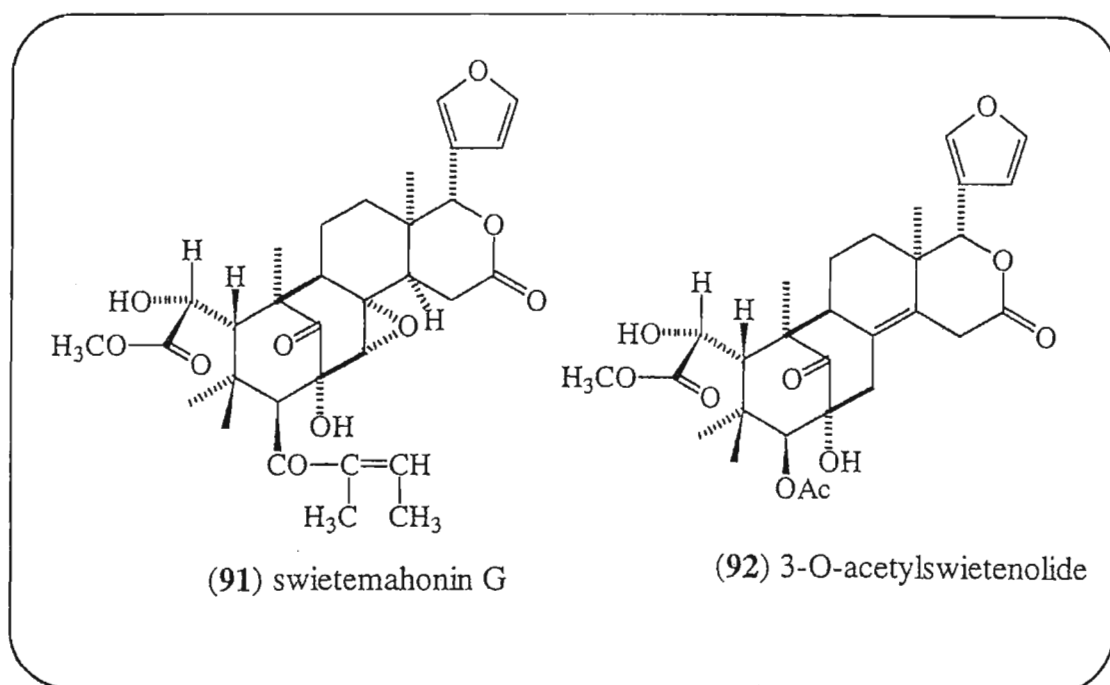
Cell adhesion processes play significant roles in pathological conditions, such as chronic inflammation, cancer metastases and viral infections. A series of seco-limonoids, such as (**93**), with uncommon hemi ortho ester A rings, isolated from the root of *Trichilia rubra*, were found to be potent inhibitors of LFA-1:ICAM-1 mediated cell adhesion[58].
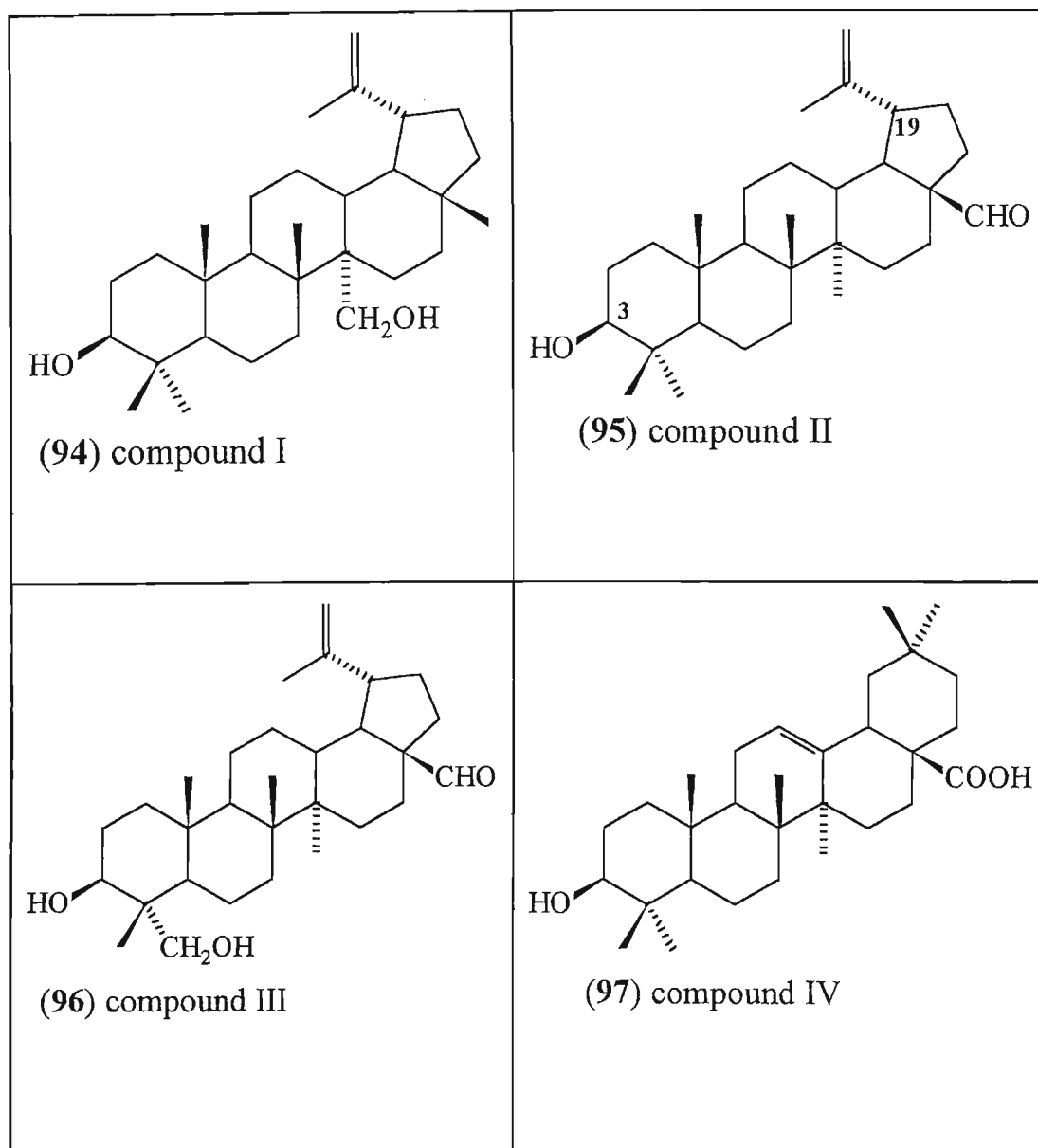
(93)
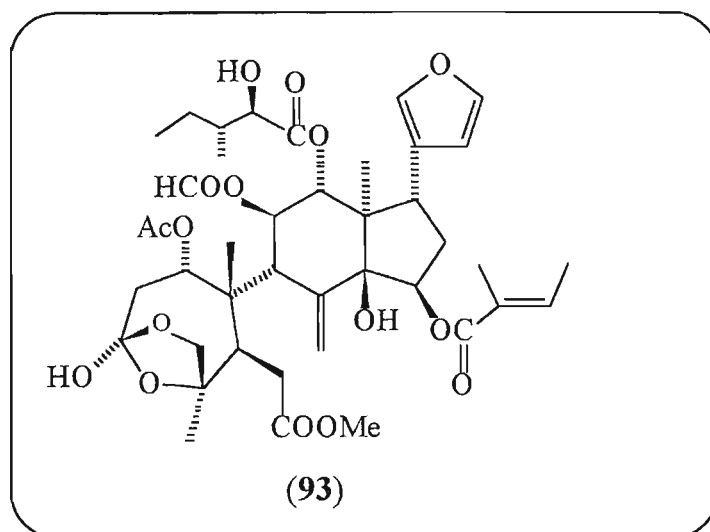
# CHAPTER 2

## EXTRACTIVES FROM *BERSAMA SWINNYI*

### <u>Index</u>

**(94)** compound I

**(95)** compound II

**(96)** compound III

**(97)** compound IV

## 2.1 Introduction

*Bersama swinnyi* Phill. (Melianthaceae), (Coastal White Ash), found in forests, on forest margins, on sandstone outcrops in the Transkei and southern KwaZulu-Natal (South Africa), is one of the most commonly used medicinal plants in KwaZulu-Natal[59]. Parts of this tree have been regarded as poisonous, but the bark which is said to be both bitter and burning is used in African medicine for barrenness, impotence, menstrual pain and leprosy. Other African species of *Bersama*, such as *Bersama abyssinica*, which has a wide distribution in Africa, occurring from Zimbabwe to Ethiopia, have yielded bufadienolides such as bersillogenin (**91**)[60], bersaldegenin-1,3,5,-orthoacetate (**92**)[61] and abyssinin (**93**)[62].



(**91**) bersillogenin

(**92**) bersaldegenin-1,3,5,-orthoacetate
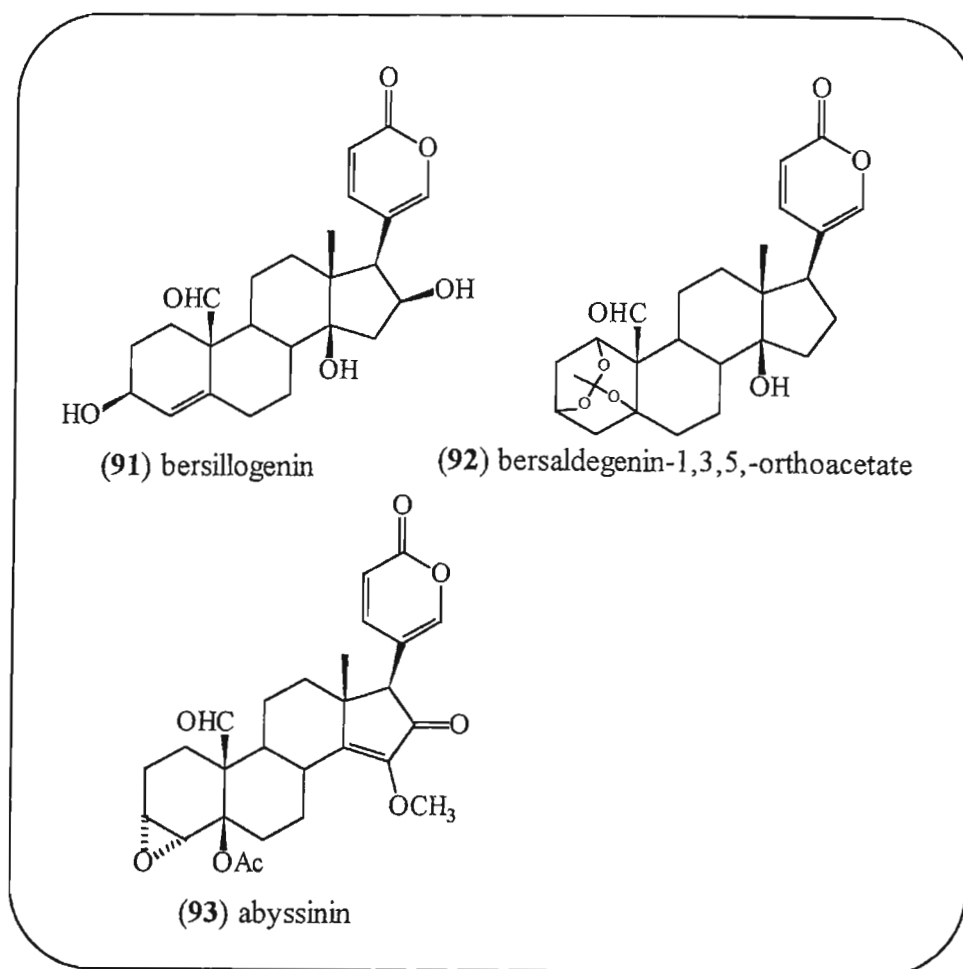
(**93**) abyssinin

The alcoholic extracts of *Bersama abyssinica* (stems and fruit) have shown tumour inhibitory activity[61,63]. The leaves have been shown to contain highly toxic bufadienolide aglycones[64]. Crude bark and leaf extracts of *Bersama yangambiensis* have been tested on guinea - pigs and were found to be toxic[65].

The work on the South African species has been stimulated by the reported anti - tumour activity of extracts from other African *Bersama* species and the fact that over exploitation of *B. swinnyi* for commercial purposes has led to its becoming rare in the wild. However, no bufadienolides were found from the bark and leaves of the South African *Bersama swinnyi*. Instead, two known compounds, compound I (**94**) and II (**95**) (lup-20(29)-ene-3β,27-diol and betulinaldehyde) and one new betulin - type compound, compound III (**96**) (23-hydroxy betulinaldehyde) were isolated from the chloroform extract of the bark. The hexane extracts of the bark and leaves yielded compound IV (**97**) (oleanolic acid). The aldehyde oxidation product of betulinaldehyde, betulinic acid, has recently been found to be uniquely effective against a line of human melanoma[66]. Oleanolic acid, like lupeol, has been proposed for development as an antiarthritic and antiinflamatory agent[67].

## 2.2 <u>Results and Discussion</u>

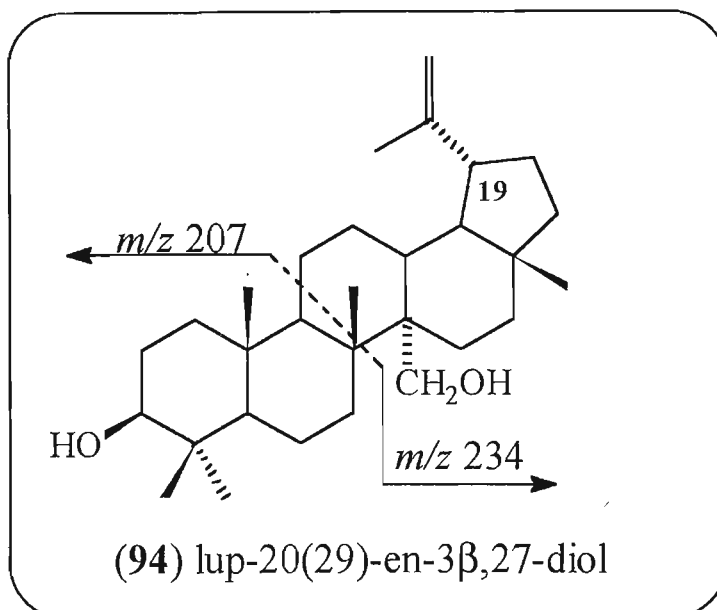### 2.2.1 <u>Structure elucidation of compound I (94)</u>

Compound I was isolated from the chloroform extract of *Bersama swinnyi* bark. The high resolution mass spectrum showed a molecular ion $[M]^+$ at *m/z* 442.3827, in agreement with the molecular formula $C_{30}H_{50}O_2$ (req. 442.3811). The IR spectrum of compound I showed absorption bands at 3450 $cm^{-1}$ (O-H stretching), 2942 $cm^{-1}$ and 2871 $cm^{-1}$ (saturated C-H stretchings) and 1690 $cm^{-1}$ (isolated C=C stretching).

The $^1H$ NMR spectrum showed a vinylic methyl proton resonance at δ 1.66 (3H, s) and five other methyl proton resonances at δ 0.74, δ 0.80, δ 0.94, δ 0,96, δ 1.00 (each 3H, s). Two proton resonances at δ 4.55 and δ 4.66 (each 1H, d, $J_{gem}$ = 2.0 Hz) (ABq) due to two non-equivalent geminal protons of a terminal methylene group, and the vinyl methyl group indicated the presence of a side-chain isopropenyl group which belongs to the lup-20(29)-ene and not the hop-22(29)-ene skeleton, which would give a broadened singlet[68].

Two coupled proton resonances δ 3.77 and δ 3.31 (each 1H, d, $J$ = 10.7Hz), and the proton resonance δ 3.16 (1H, dd, $J$ = 5.2Hz, $J$ = 10.9Hz) indicated the presence of $CH_2OH$ and CHOH groups respectively. The HETCOR spectrum showed that these proton resonances corresponded to the carbon resonances at δ 60.5 (t) and δ 79.0 (d) respectively. The presence of the two hydroxy groups was confirmed by the mass spectrum which showed peaks

at $m/z$ 411[$M^+$- $CH_2OH$] and $m/z$ 424 [$M^+$- $H_2O$] respectively. The chemical shift and the coupling constant of the proton resonance at $\delta$ 3.16 indicated a 3$\beta$ equatorial hydroxy group. On acetylation compound I afforded a diacetate (compound Ia) and the proton resonances arising from the $CH_2OAc$ group now occurred downfield at $\delta$ 4.23 and $\delta$ 3.83 respectively, and the proton resonance arising from the CHOAc group occurred at $\delta$ 4.40.

The mass spectrum of compound I indicated that the $CH_2OH$ group was at either C-27 or C-28 by a fragment at $m/z$ 234 ($C_{16}H_{26}O$) comprising rings D and E and part of ring C and a fragment at $m/z$ 203 [234 - $CH_2OH$][69]. The C-28 is ruled out as the ^{1}H NMR spectrum of compound I is not identical with that of betulin[70]. Whereas the ^{1}H NMR spectrum of betulin shows an H-19$\beta$ resonance at $\delta$ 2.99 (1H, m), that of compound I showed a proton resonance at $\delta$ 2.35 (1H, m, H-19$\beta$). Hence compound I is lup-20(29)-ene-3$\beta$,27-diol (94). The location of the axially oriented $CH_2OH$ group was further confirmed by NOE experiments on the diacetate (compound Ia). Irradiation of 3H-30 ($\delta$ 1.66) gave a positive NOE for H-27a ($\delta$ 4.23). A positive NOE was also observed for H-29a ($\delta$ 4.66). ^{13}C NMR resonances were compared with those reported for the 3$\alpha$-hydroxy isomer[71] as only ^{1}H NMR data is available in the literature for the 3$\beta$ isomer. This compound has been reported previously from *Lithocarpus cornea*[72].

**(94)** lup-20(29)-en-3β,27-diol

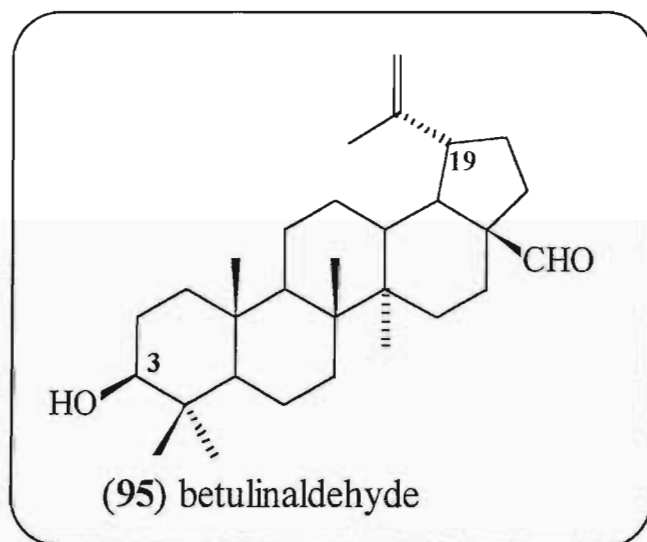## 2.2.2 Structure elucidation of compound II (95)

The high resolution mass spectrum of compound II showed a molecular ion $[M]^+$ at $m/z$ 440.3648 corresponding to the molecular formula $C_{30}H_{48}O_2$ (req. 440.3652) which indicated seven double bond equivalents. The IR spectrum showed absorption bands at 3427 cm$^{-1}$ (O-H stretching), 2941 cm$^{-1}$ and 2868 cm$^{-1}$ (saturated C-H stretchings), 1711 cm$^{-1}$ (saturated C=O stretching) and 757 cm$^{-1}$ (olefinic C-H deformation).

The $^1$H and $^{13}$C NMR spectra of compound II were similar to those of compound I. However, the $^1$H NMR spectrum of compound II showed a proton resonance at δ9.66 (1H, s) which corresponded in the HETCOR spectrum to a carbon resonance δ 206.71 (d) indicative of an aldehyde group and lacked the CH₂OH resonances.

The side-chain isopropenyl group gave proton resonances at δ 4.74 and δ 4.61 (each 1H, d, $J$ = 2.0Hz, ABq) corresponding in the HETCOR spectrum

to a carbon resonance at δ 110.2 (t), and a vinyl methyl proton resonance at

δ 1.67 (3H, s) corresponding to a carbon resonance at δ 19.0 (q). The proton

resonance δ 3.16 (1H, dd, $J = 5.3Hz$, $J = 10.9Hz$) indicated an equatorial 3β

hydroxyl group and the proton resonance at δ 2.84 (1H, dt, $J = 6.0Hz$, $J =$

11.1Hz) was ascribable to H-19β.

A literature[73] survey indicated that compound II was betulinaldehyde (**95**).

This was confirmed by comparison of literature and experimental ${}^1$H NMR[74]

and ${}^{13}$C NMR[75] data.



(**95**) betulinaldehyde

### 2.2.3 Structure elucidation of compound III (96)

Compound III was isolated from the chloroform extract of *B. swinnyi*.
Difficulty was however experienced in purifying this compound and as such
only a small quantity of the pure compound was obtained.
${}^1$H NMR, COSY and ${}^{13}$C NMR spectra obtained were very similar to those
of compound II (betulinaldehyde (**95**)). Compound III decomposed before
the IR, DEPT and mass spectra were obtained.

The $^1$H NMR spectrum showed proton resonances at $\delta$ 9.65 (1H, s, H-28), $\delta$ 2.84 (1H, dt, $J = 5.6$Hz, $J = 11.0$Hz, H-19$\beta$), and a pair of resonances at $\delta$ 3.39 and $\delta$ 3.70 (each 1H, d, $J = 10.4$Hz) ascribable to a -CH$_2$-O group. The chemical shift and half-width of a proton resonance at $\delta$ 3.60 (1H, m, $W_{1/2}= 7.5$Hz) indicated the presence of a $\beta$-equatorial hydroxy group at C-3 as in betulinaldehyde. Two broad resonances, each integrating to one proton, ascribable to two hydroxy group protons were observed at $\delta$ 2.40 and $\delta$ 2.17. These disappeared on addition of D$_2$O.

The side-chain isopropenyl group gave proton resonances at $\delta$ 4.74 and $\delta$ 4.60 (each 1H, d, $J = 2.0$Hz, ABq), and a vinyl methyl proton resonance at $\delta$ 1.67 (3H, s).

Whereas compound II (betulinaldehyde, **95**) showed six methyl proton resonances, compound III showed only five at $\delta$ 1.67, $\delta$ 0.95, $\delta$ 0.90, $\delta$ 0.85 and $\delta$ 0.84 (each 3H, s) suggesting that one of the methyl groups had been oxidised into a -CH$_2$OH group. The $^{13}$C NMR spectrum showed methyl carbon resonances at $\delta$ 19.0, $\delta$ 16.5, $\delta$ 15.9, $\delta$ 14.3 and $\delta$ 11.2. The first four resonances were ascribable to C-30, C-26, C-25 and C-27 respectively, in agreement with those observed for betulinaldehyde (**95**) (Table 2, Appendix). This suggested that either C-23 or C-24 was oxidised.

In a 3$\beta$,23-diol such as 23-hydroxyprimulagenin A (**98**), the primary carbinol protons resonate at $\delta$ 3.40 and $\delta$ 3.65 while in a 3$\beta$,24 diol they appear at $\delta$ 3.75 and $\delta$ 4.15[76]. In compound III they occur $\delta$ 3.39 and $\delta$ 3.70. Thus it was concluded that compound III was 23-hydroxy betulinaldehyde (lup-20(29)-ene-3$\beta$,23-diol-28-al).

This is the first reported isolation of this compound. The $^{13}$C NMR resonances (Table 2) were assigned by comparison with those of betulinaldehyde and 23-hydroxyprimulagenin A (**98**)[77].



(**96**) 23-hydroxybetulinaldehyde     (**98**) 23-hydroxyprimulagenin A
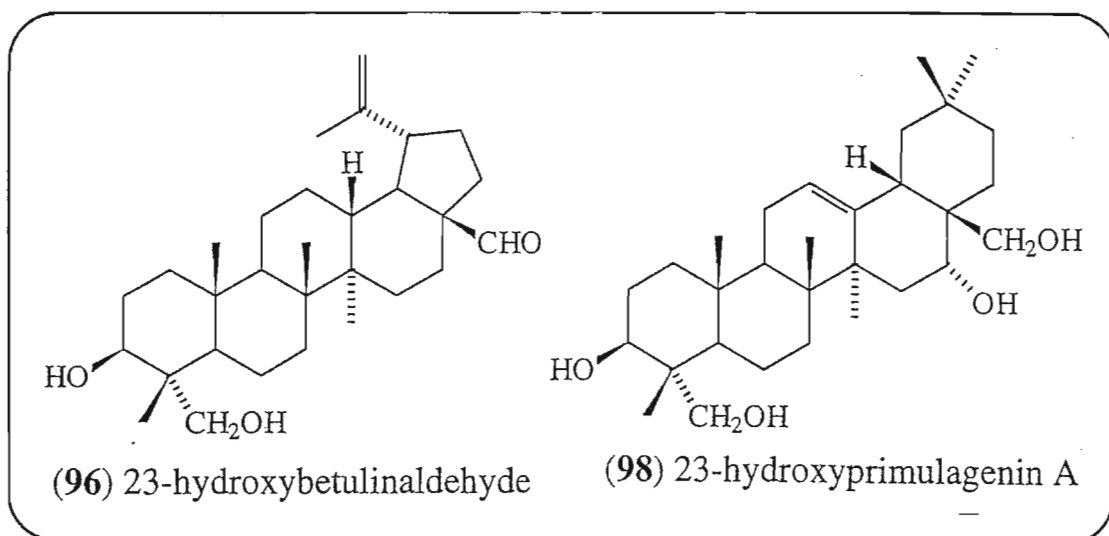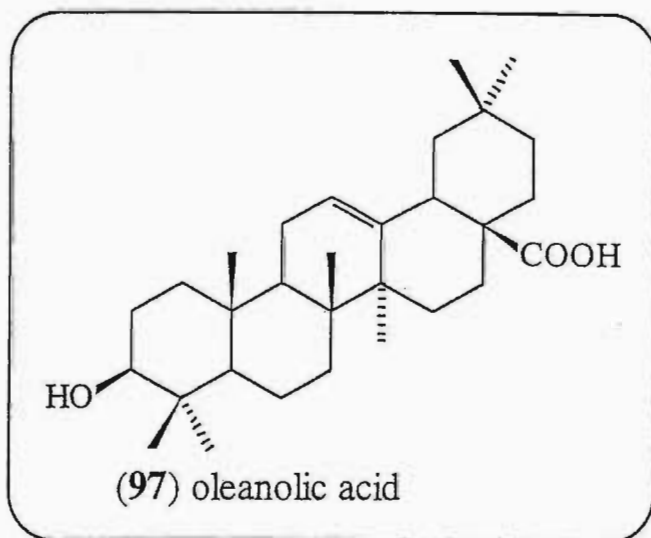
## 2.2.4 Structure elucidation of compound IV (97)

The high resolution mass spectrum of compound IV showed a molecular ion [M]$^+$ at *m/z* 456.3578 indicating a molecular formula $C_{30}H_{48}O_3$ (req. 456.3603). The IR spectrum showed absorption bands at 3450 cm$^{-1}$ (O-H stretching), 2942 cm$^{-1}$, 2869 cm$^{-1}$ (saturated C-H stretchings), and 1643 cm$^{-1}$ (C=C stretching)

The $^1$H NMR spectrum showed seven methyl proton resonances at δ 0.72, δ 0.75, δ 0.88, δ 0.89, δ 0.91, δ 0.96 and δ 1.10 (each 3H, s). The proton resonance at δ 5.26 (1H, m) indicated a trisubstituted double bond. The proton resonance at δ 3.20 (1H, dd, *J* = 4.9Hz, *J* = 10.3Hz) indicated a 3β-equatorial hydroxy group.

The $^{13}C$ NMR spectrum showed a carbon resonances at $\delta$ 182.90 (s, COOH group), $\delta$ 143.57 (s) and $\delta$ 122.63 (d) indicating a HC=C group, and $\delta$ 79.03 (d) a CHOH group.

The molecular formula $C_{30}H_{48}O_3$ indicated seven double bond equivalents. The presence of one alkene double bond and one carboxylic acid group indicated the presence of five rings. The presence in the $^{13}C$ NMR spectrum of six quatrenary carbon resonances and seven methyl resonances with one methyl group converted to a COOH group suggested an oleanane-type compound. The carbon resonances $\delta$ 122.63 (d) and $\delta$ 143.57 (s) of the olefinic carbon atoms were characteristic of the $\Delta^{12}$ double bond in olean-12-enes[78].

The olean-12-ene structure of compound IV was further suggested by the presence in the mass spectrum of a peak at $m/z$ 248 ($[C_{16}H_{24}O_2]^+$) due to a reverse Diels Alder fragmentation. The structure of compound IV was confirmed by comparison of the melting point and NMR data reported for oleanolic acid (97)[79].



(97) oleanolic acid

# CHAPTER 3

## EXTRACTIVES FROM *DYSOXYLUM SPECTABILE*
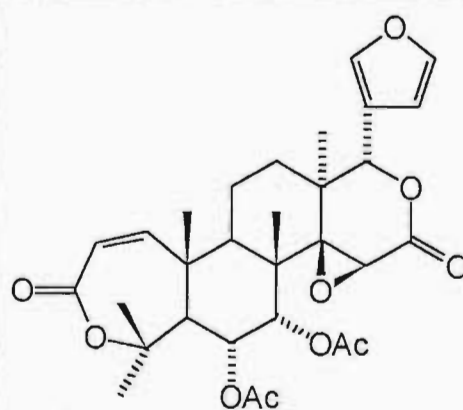
### Index
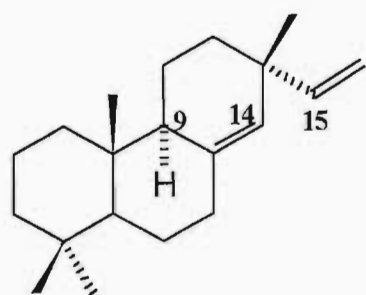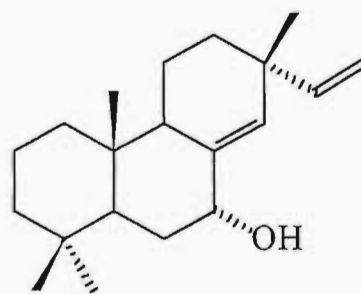
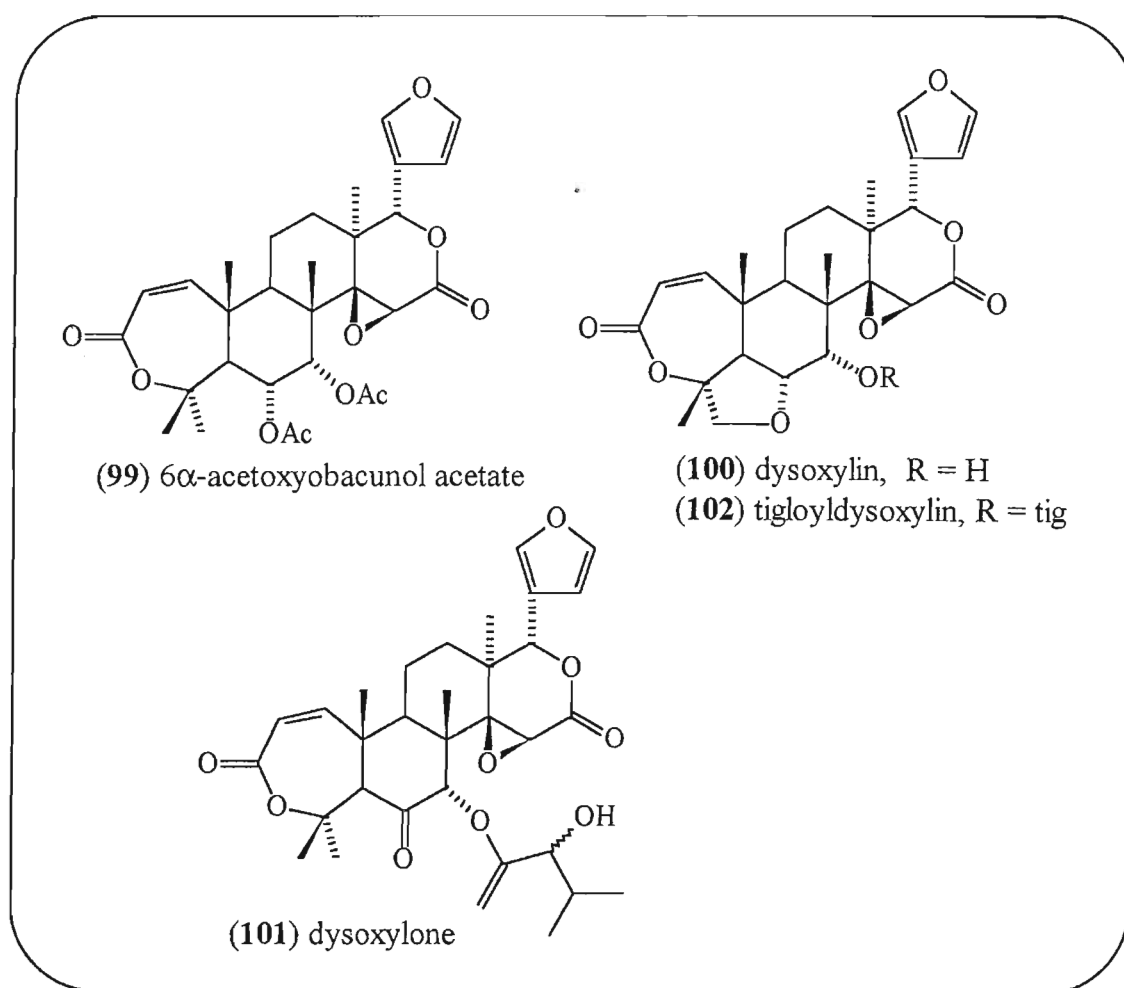(103) compound V



(104) compound VI
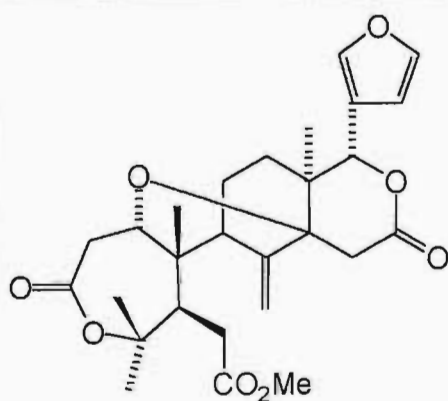


(105) compound VII



(99) compound VIII

## 3.1 Introduction

The genus *Dysoxylum* of the Meliaceae family is known for the interesting variety of compounds it produces. The only limonoids reported previously from *Dysoxylum* species were 6α-acetoxyobacunol acetate (**99**) from *Dysoxylum muelleri*[80], 6a-acetoxyobacunol acetate (**99**) and the related limonoids dysoxylin (**100**), dysoxylone (**101**) and tigloyldysoxylin (**102**) from *Dysoxylum richii*[81], and the simple limonoids with unoxidised rings A-D from *Dysoxylum binectariferum*[82].



(**99**) 6α-acetoxyobacunol acetate

(**100**) dysoxylin, R = H
(**102**) tigloyldysoxylin, R = tig

(**101**) dysoxylone

The Meliaceae family is only represented by one species *Dysoxylum spectabile* in New Zealand. The hexane extract of the bark of *Dysoxylum*

*spectabile*, collected in New Zealand by Professor D. A. H. Taylor, yielded four compounds (V, VI, VII, VIII).

The first two compounds (V, VI) were diterpenoids, the known sandaracopimaradiene (**103**) and 7α-hydroxysandaracopimaradiene (**104**). Compounds VII and VIII were identified as methyl ivorensate (**105**) and 6α-acetoxyobacunol acetate (**99**).

This is the third isolation of 6α-acetoxyobacunol acetate (**99**) from the *Dysoxylum* genus. Methyl ivorensate (**105**) is not common and has only been found previously in the *Khaya* genus[35]. The genus *Khaya* is part of the Swietenieae tribe of the subfamily Swietenioideae. It is curious that this uncommon limonoid has been found only in two such distant genera of the Meliaceae family.

Several interesting minor limonoid constituents were also present but not in sufficient quantities for purification or identification. An examination of the ^{1}H NMR spectrum of the mixture of minor components indicated that limonoids with an α,β-unsaturated ring A lactone, an opened ring B, a formate group and ring D lactone were present.

## 3.2 Results and discussion

### 3.2.1 Structure elucidation of compound V (103)

The high resolution mass spectrum of compound V gave a molecular ion [M]$^+$ at $m/z$ 272.2490 in agreement with the molecular formula $C_{20}H_{32}$ (req. 270.2504) which indicated five double bond equivalents. The IR spectrum showed absorption bands at 2924 cm$^{-1}$ and 2850 cm$^{-1}$ (saturated C-H stretchings), 1463 cm$^{-1}$ (C-H deformation) and 758 cm$^{-1}$ (olefinic C-H deformation).

The $^1$H NMR spectrum showed olefinic proton resonances at δ 5.76 (1H, dd, $J$ = 10.6Hz, $J$ = 17.5Hz), δ 4.88 (1H, dd, $J$ = 17.5Hz, $J$ = 1.5Hz) and δ 4.86 (1H, dd, $J$ = 10.6Hz, $J$ = 1.5Hz) ascribable to an ABC system of a vinyl group carried by a quartenary carbon. Another olefinic proton resonance occurred at δ 5.19 (1H, br s, $W_{1/2}$= 5.0Hz). These characteristic data led to the proposal of a diterpene of the pimaradiene type[83].

The $^{13}$C NMR spectrum supported a pimaradiene skeleton because of the similarity between the spectrum of compound V and the $^{13}$C NMR data reported in a systematic analysis of diterpenic compounds[84,85]. Comparison of the olefinic carbon resonances of compound V with those of pimaradienic (A), sandaracopimaradienic (B), and isopimaradienic (C) systems[85a] showed the compound to be sandaracopimaradiene (99). This comparison permitted the determination of the stereochemistry at C-13 and the localisation of the olefinic double bond at the 8,14 position.

(A)   (B)   (C)
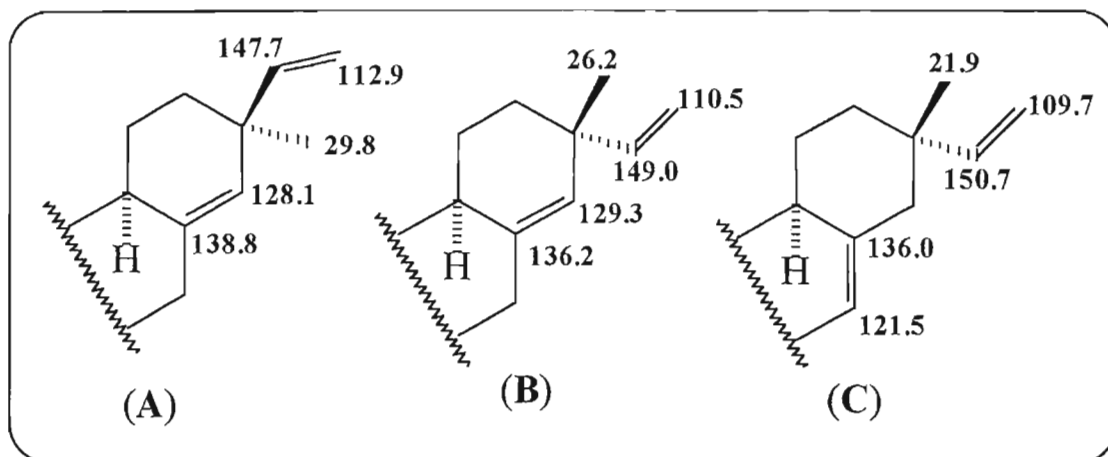
The $^{13}$C NMR spectrum showed olefinic carbon resonances at δ 149.2 (d), δ 137.3 (s), δ 128.5 (d) and δ 110.0 (t) ascribable to C-15, C-8, C-14 and C-16 respectively. The C-17 methyl carbon resonance occurred at δ 26.0 (q). The $^{1}$H NMR spectrum showed four methyl proton singlets at δ 0.78, δ 0.83, δ 0.86 and δ 1.02 in agreement with those reported[83] for H-19, H-18, H-20 and H-17 respectively for sandaracopimara-8(14),15-diene (103). This compound has been reported previously from *Xylia dolabriformis*[86b].
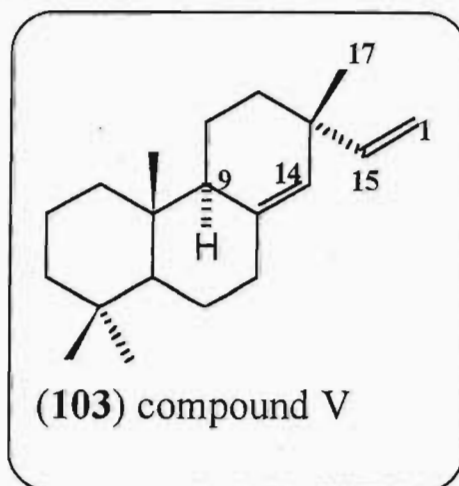


(103) compound V

### 3.2.2 Structure elucidation of compound VI (104)

High resolution of the $[M]^+$ molecular ion gave $m/z$ 288.2459 indicating a molecular formula $C_{20}H_{32}O$ (req. 288.2453). The IR spectrum showed absorption bands at 3427 $cm^{-1}$ (O-H stretching), 2925 $cm^{-1}$ and 2867 $cm^{-1}$ (saturated C-H stretchings), 1247 $cm^{-1}$ (C-O stretching), 910 $cm^{-1}$ and 760 $cm^{-1}$ (olefinic C-H deformations).

The NMR spectra of compound VI were very similar to those of compound V. The presence of a hydroxy group was indicated by a **CH**-OH proton resonance at δ 4.17 (1H, br m, $W_{1/2}$= 4.3Hz). The HETCOR spectrum showed that this proton resonance corresponded with the carbon resonance at δ 73.4 (d). The position and stereochemistry of the hydroxy group remained to be determined. Comparison of the spectral data with those of certain hydroxylated pimaradienes[85,86] indicated that the C-7 position was hydroxylated.

The $^{13}C$ NMR chemical shifts of compound V (**103**, sandaracopimaradiene) fitted well with the data of compound VI except for C-5, C-6, C-7, C-8, C-9 and C-14 carbons. The determination of the C-7 stereochemistry originated from the large γ-shielding effects ($^{13}C$ NMR) at C-5 and C-9, which indicated the axial character[87]. The deshielding effects at C-6 and C-8 supported the placing of the hydroxy group at C-7α. The well established $^{13}C$ NMR shift increments for a hydroxy substituent[87d] allowed a similar conclusion.

The $^1$H NMR data reported[86e] for 7α-hydroxysandaracopimara-8(14),15 -diene (104) fitted well with the data for compound VI. The COSY spectrum showed long-range coupling between H-14 (δ 5.49) and H-9α (δ 2.10). Acetylation of compound V afforded a monoacetate (104a). The H-7β resonance was observed at δ 5.30 (1H, t, $J$ = 2.8Hz) and the H-14 proton resonance had moved downfield from δ 5.19 in compound VI to δ 5.62 (1H, br s) in the monoacetate. This compound has been reported previously from *Zexmenia phyllocephala*[86e].



(104) compound VI, R = H
(104a) R = Ac
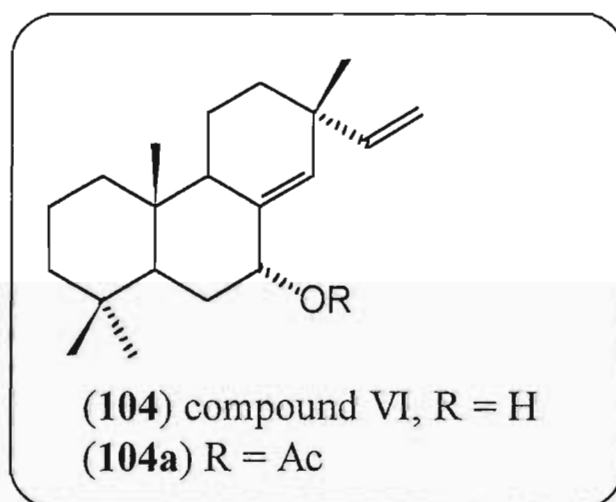
### 3.2.3. Structure elucidation of compound VII (105)

The high resolution mass spectrum of compound VII showed the molecular ion peak at *m/z* 486.2238 corresponding to the molecular formula $C_{27}H_{34}O_8$ ( req. 486.2251). IR absorption bands occurred at 2958 cm$^{-1}$ ( C-H stretching), 1732 cm$^{-1}$ (C=O stretchings).

Compound VII was shown to be a limonoid by the presence of the C-17 β-substituted furan ring proton resonances at δ 7.45 (H-21), δ 7.35 (H-23)

and δ 6.38 (H-22), and the corresponding carbon resonances at δ 142.7 (d, C-21), δ 141.0 (d, C-23) and δ 110.0 (d, C-22). C-20 occurred at δ 120.7(s).

The $^1$H NMR spectrum showed four tertiary methyl group proton resonances at δ 0.85, δ 0.98, δ 1.35 and δ 1.58 (each s, 3H), a carbomethoxy group proton resonance at δ 3.70 (s, 3H), and proton resonances at δ 5.14(1H, s) and δ 4.90 (1H, s) ascribable to a C=CH$_2$ (an exocyclic) double bond. .

The $^{13}$C NMR spectrum showed two lactone carbonyl resonances at δ 169.6 (s) and δ 170.0 (s), an ester carbonyl resonance at δ 173.2 (s), a carbomethoxy carbon resonance at δ 52.3 (q) and olefinic double bond carbon resonances at δ 145.7(s) and δ 111.9 (t).

These features indicated the nature of the carbon skeleton : ring B was opened with a carbomethoxy group at C-7, and a $\Delta^{8,30}$ double bond. The presence of two lactone rings suggested that the compound could be methyl ivorensate (105). This was confirmed by the comparison of the NMR data with that reported for methyl ivorensate[88,89] (Table 2, Appendix).
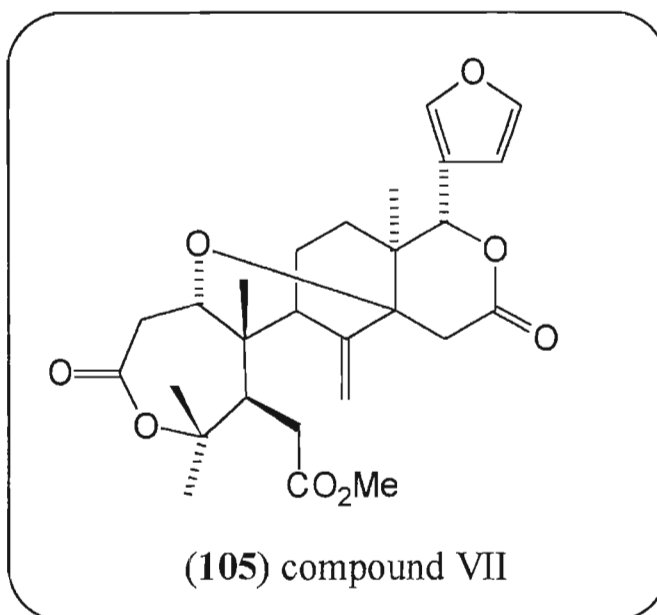
**(105)** compound VII

### 3.2.3 <u>Structure elucidation of compound VIII (99)</u>

Only a small quantity of pure compound VIII was obtained insufficient for the $^{13}$C NMR, DEPT, COSY and HETCOR spectra. The structure of compound VIII was worked out solely from the $^1$H NMR spectrum. The IR spectrum showed intense broad absorption bands at 1747 cm$^{-1}$ ($\alpha,\beta$-unsaturated lactone carbonyl stretching) and 1705 cm$^{-1}$ (C=O stretching).

The $^1$H NMR spectrum of compound VIII had resonances at $\delta$ 7.39 (2H) and $\delta$ 6.31 (1H) which are characteristic of the furan ring protons H-21, H-23 and H-22 respectively, typical of limonoid compounds.

The resonances at $\delta$ 5.58 (1H, s) and $\delta$ 3.59 (1H, s) correspond to H-17$\beta$ and H-15$\alpha$ of a ring D lactone with a 14,15-epoxide, as in gedunin. The proton resonances at $\delta$ 6.55 (1H, d, $J = 11.6$Hz) and $\delta$ 5.93 (1H, d, $J = 11.6$Hz) are

characteristic of H-1 and H-2 in a seven membered α,β-unsaturated ring A lactone.

The ${}^1$H NMR spectrum also showed two acetate methyl group proton resonances at δ 1.98 (3H, s) and δ 2.12 (3H, s), and the two corresponding oxygen-related methine protons at δ 5.15 (1H, dd, $J$ = 10Hz, $J$ = 5Hz) and δ 4.90(1H, d, $J$ = 5Hz). The five methyl groups were indicated by the proton resonances at δ 1.23, δ 1.24, δ 1.41, δ 1.44 and δ 1.53 (each 3H, s). The structural arrangement with rings A and D lactonized and rings B and C intact suggested an obacunol-type limonoid.

A literature[73] search indicated that Compound VIII was 6a-acetoxy-obacunol acetate (99). This was confirmed by comparison of ${}^1$H NMR data with that reported for 6a-acetoxyobacunol acetate[81] (99) (Table 2).



(99) compound VIII
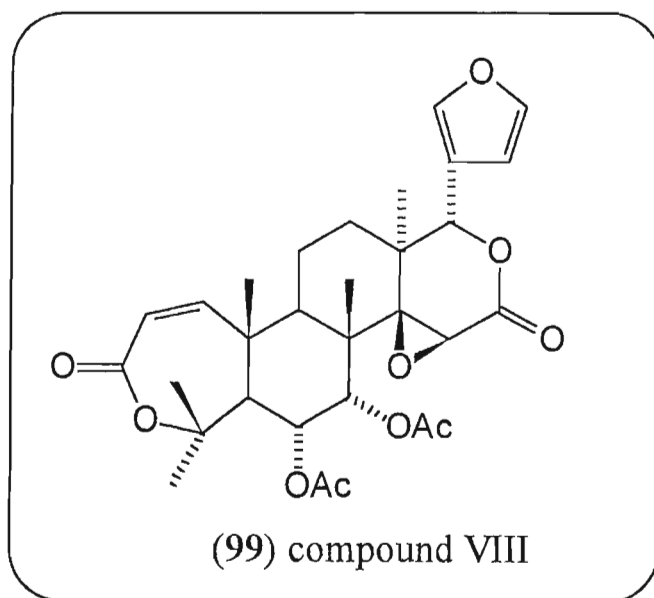
# CHAPTER 4

## EXTRACTIVES FROM *TURRAEA HOLSTII*

### Index

**(113)** compound IX

**(83)** compound X

**(114)** compound XI

**(118)** compound XII

**(119)** compound XIII

**(120)** compound XIV

**(121)** compound XV

**(122)** compound XVI

**(124)** compound XVII

**(125)** compound XVIII

## 4.1 Introduction

The genus *Turraea* (Meliaceae) is comprised of a group of 60 - 70 species of shrubs and trees widespread in Africa and the Indian Ocean islands. This genus belongs to the Turraeae tribe , subfamily Meliodeae, where it is accompanied by a number of smaller genera, and by the genus *Nymania*. *Nymania* is controversial as it is superficially unlike other Meliaceae, but was found to contain prieurianin (**85**)[90], a characteristic complex limonoid of the genera *Trichilia* and *Guarea*. The isolation of prieurianin (**85**) and nymania-1 (**106**), characteristic limonoids of *Nymania capensis*, from *Turraea obtusifolia* provided evidence of a chemotaxonomic link between the genera *Nymania* and *Turraea*[91,92].

The seeds of *Turraea floribunda* have produced limonoids with 11a,12a-substitution[93]. The rootbark and stembark on the other hand, produce limonoids with 11b,12a-substitution[91,94,95], very similar to havanensin (**61**), heudelottin (**62,63,64**) and hirtin (**107**), which are typical limonoids of the genus *Trichilia*, representing intermediates or byproducts on the route to the more characteristic prieurianin compounds of the *Trichilia* genus.

The hexane extract of the stem of *Turraea nilotica* gave three protolimonoids, but no limonoids[96]. However, the methanolic extract of the rootbark afforded one limonoid, nilotin (**108**)[97]. The methanolic extract of the rootbark of *Turraea robusta* has afforded a protolimonoid, turranolide (**109**), and limonoids such as mzikonone (**110**) and nimbolinin B (**111**)[98]. *Turraea mombasa* produces prieurianin-type limonoids[99], whereas *Turraea*

*villosa* produced, villosterol (**112**) a pregnane steroid possessing *cis*-fused A and B rings[100].
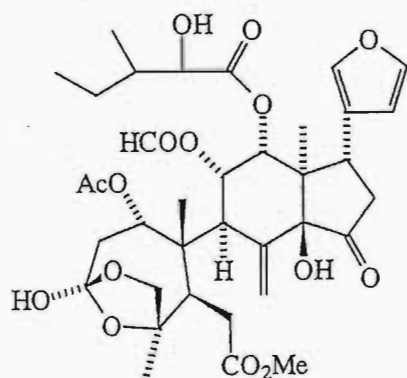


(**106**) nymania-1
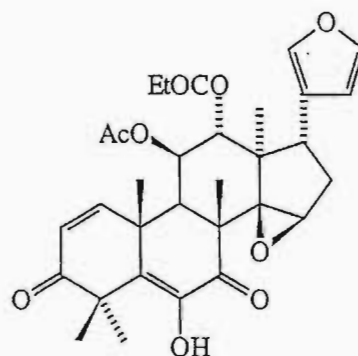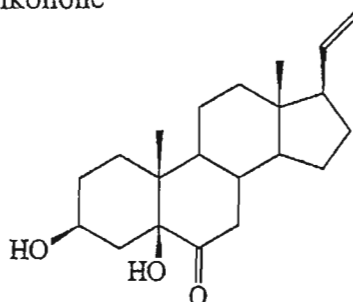
(**107**) hirtin
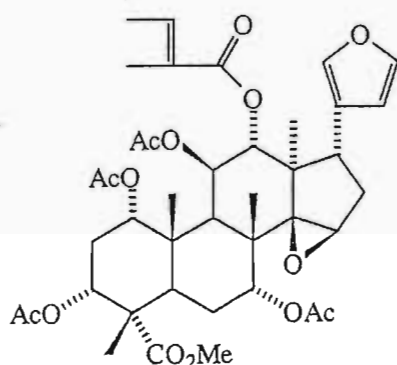
(**108**) nilotin
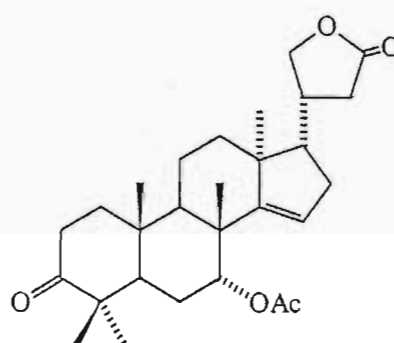
(**109**) turranolide
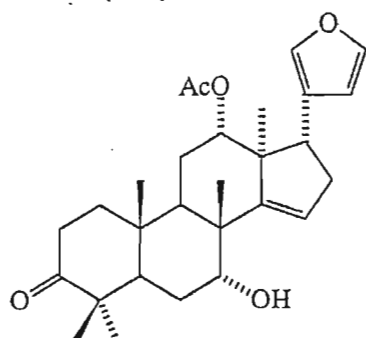
(**110**) mzikonone
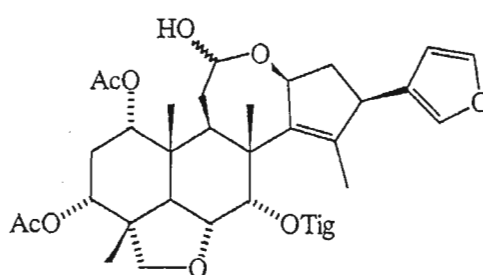
(**111**) nimbolinin B

(**112**) villosterol

The methanolic extracts of the rootbark and stembark of the hitherto uninvestigated species *Turraea holstii* (Gurke), supplied by Professor H. S. Rajab, Moi University, Kenya, were examined. Eight limonoids (compounds IX - XIV and XVI) and a protolimonoid (compound XVII) were isolated from the rootbark. A limonoid (compound XV) and a protolimonoid (compound XVIII) were isolated from the stembark. Some of the compounds isolated were found in both extracts.

## 4.2 Results and Discussion

### 4.2.1 Structure elucidation of compound IX (113)

High resolution of the $M^+$ signal gave the mass of 512.2780 g/mol, thus suggesting the formula $C_{30}H_{40}O_7$ (calculated 512.2772 g/mol). Compound IX was shown to be a limonoid by the presence of β-substituted furan ring proton resonances at δ 7.35 (H-23), δ 7.24 (H-21) and δ 6.26 (H-22) and the corresponding carbon resonances at δ 142.6 (d, C-23), δ 139.7 (d, C-21) and δ 111.1 (d, C-22). C-20 occurred at δ 124.5 (s).

A proton resonance at δ 5.59 (br m, 1H) suggested H-15 of a $\Delta^{14}$ double bond. This H-15 resonance corresponded to a C-15 carbon resonance at δ 120.7 (d). The COSY spectrum showed that the H-15 signal was coupled to two signals at δ 2.41 (ddd, $J = 3.4$Hz, $J = 7.5$Hz, $J = 16$Hz) and δ 2.55 (1H, m) both corresponding in the HETCOR spectrum to a carbon signal at δ 34.3 (t, C-16). The two H-16α and H-16β signals were seen in the COSY

spectrum to be coupled to a proton signal at δ 2.82 (1H, dd, $J = 7.4$Hz, $J = 10.7$Hz) corresponding to a methine carbon signal at δ 51.5 (d, C-17). The $^1$H NMR spectrum showed only four tertiary methyl group proton resonances at δ 1.18, δ1.09, δ0.96 and δ0.83 (each s, 3H). The presence of two acetate groups was indicated by two acetate methyl proton resonances at δ 2.01 and δ 1.98 (each s, 3H) and the corresponding CH-O methine proton resonances at δ 4.90 (1H, t, $J = 2.8$Hz) and δ 4.66 (1H, t, $J = 2.8$Hz). The two proton resonances were each coupled to a proton resonance at δ 2.09 (2H, m). This is typical of ring A with α–oriented acetate groups at C-1 and C-3. The corresponding C-1, C-2 and C-3 carbon resonances were shown in the HETCOR spectrum to occur at δ 72.24 (d), δ 27.67 (t) and δ 71.74 (d) respectively. A -$CH_2$-O proton signal at δ 3.57 (br m, 2H) corresponding in the HETCOR spectrum to a carbon resonance at δ 77.90 (t) suggested that one of the methyl groups had been oxidised to a $CH_2$-O group.

A CH-O methine proton resonance at δ 4.17 (1H, d, $J = 3.1$Hz) indicated the presence of a hydroxy group which was placed at C-7α as an oxygen functional group was needed at C-7α because of the limonoid biosynthesis. The COSY spectrum showed that the H-7β resonanace was coupled to a resonance at δ 4.13 (1H, dd, $J = 3.1$Hz, 12.2Hz) ascribable to H-6 which in turn was coupled to a resonance at δ 2.66 (1H, d, $J = 12.2$Hz) ascribable to H-5. The H-6 chemical shift of δ 4.13 (d) and the fact that the H-7β resonance was a doublet suggested an oxygen atom at C-6. This oxygen atom was thought to be α because the $J_{5,6}$ coupling constant was 12.2Hz

indicative of a *trans* H-5, H-6 configuration[101,102]. The coupling constant of 3.1Hz indicates a *cis* configuration of H-6 and H-7β protons.

The molecular formula $C_{30}H_{40}O_7$ indicated eleven double bond equivalents. Since rings A to D, the furan ring, $\Delta^{14}$ double bond and two acetate groups accounted for ten double bond equivalents, an ether linkage between C-28 and C-6 was assumed, giving structure (113).

The NMR data of compound IX was identical to that of 1,3-diacetylvilasinin (113) which has been isolated previously from the seed oil of *Azadirachta indica*[103].



(113)
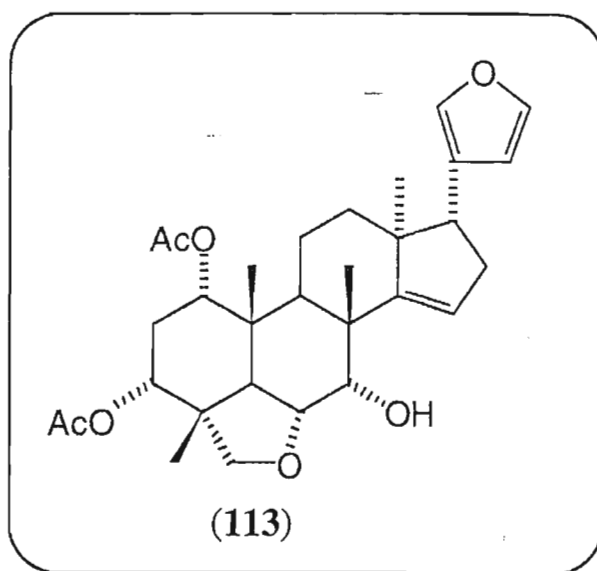
## 4.2.2 Structure elucidation of compound X (83)

The high resolution mass spectrum gave $M^+$ at *m/z* 554.2500 indicating a molecular formula $C_{31}H_{38}O_9$ (calculated 554.2514). The loss of two acetate groups was indicted by fragment peaks at *m/z* 494 and *m/z* 434 in the mass spectrum. The infrared spectrum had absorption bands at 1748 cm$^{-1}$ (ester

carbonyl stretching), 1679 cm$^{-1}$ ($\alpha,\beta$-unsaturated six membered ring ketone stretching) and 1237 cm$^{-1}$ (C-O stretching).

The $^1$H NMR spectrum had resonances at $\delta$ 7.26 , $\delta$ 7.09 and $\delta$ 6.12 which are characteristic of the $\beta$-substituted furan ring protons H-23, H-21 and H-22 respectively, typical of limonoid compounds. The corresponding furan ring carbon resonances were evident at $\delta$ 142.5 (d, C-23), $\delta$ 140.3 (d, C-21) and $\delta$ 111.2 (d, C-22) in the $^{13}$C NMR spectrum. C-20 occurred at $\delta$ 122.2 (s).

A carbomethoxy group proton resonance at $\delta$ 3.64 (3H, s) and exocyclic methylene proton resonances at $\delta$ 5.18 (1H, br s) and $\delta$ 5.28 (1H, br s) indicated that compound X had ring B opened. The exocyclic nature of the double bond was further indicated by carbon resonances at $\delta$ 136.8 (s) and $\delta$ 120.8 (t).

The HETCOR spectrum showed that a proton resonance at $\delta$ 3.85 (1H, br s) corresponded to a carbon resonance at $\delta$ 59.58 (d). This confirmed the presence of an epoxide ring which was placed at the 14,15 position. The H-17 proton resonance was evident at $\delta$ 3.02 (1H, dd, $J = 7.0$Hz, $J = 10.7$Hz).

The presence of an isolated $\alpha,\beta$-unsaturated system in ring A was indicated in the $^1$H NMR spectrum by a pair of doublets at $\delta$ 7.41 (1H, d, J = 10.5Hz) and $\delta$ 6.13 (1H, d, J = 10.5Hz) ascribable to H-1 and H-2 respectively and the corresponding carbon resonances at $\delta$ 152.3 (d) and $\delta$ 125.7 (d) ascribable to C-1 and C-2 respectively. C-3 occurred at $\delta$ 203.9 (s)

The presence of two acetate groups was indicated by two acetate methyl proton resonances at δ 1.67 (3H, s) and δ 1.87 (3H, s), and carbon resonances of the acetate carbonyl groups (δ 169.7 and δ170.1, each s) and acetate methyl groups (δ 20.6 and δ 21.3, each q). A 12α acetate group was indicated by the upfield shift of one of the acetate methyl proton resonance (δ 1.67). This upfield shift is due to the shielding effect of the furan ring in ring B opened limonoids. The C-12α acetate methyl shows no such effect in limonoids with intact ring B[104]. The proton resonance at δ 5.68 (1H, d, *J* = 10.8Hz) was ascribable to H-12β and the carbon resonance at δ 75.2 (d) to C-12. The H-12β resonance was seen to be coupled to a signal at δ 5.50 (1H, dd, *J* = 10.8Hz, 7.2Hz) ascribable to H-11 which in turn was coupled to a proton resonance at δ 2.97 (1H, d, *J* = 7.2Hz) ascribable to H-9α. In ring B cleaved compounds, like toonacilin (83)[44], ring C can be chair-like and thus for an 11α, 12α-dioxygenated system the coupling constants for H-9, H-11 and H-11, H-12 are small (4.0 -4.4)[44]. In order to accommodate the large coupling constants observed (7.2Hz and 10.8Hz, respectively) for these protons in compound X, the 11-acetoxy group must be β. These coupling constants agree with those of the prieurianin-type compounds[99]. Thus structure (83a) was assigned to compound X. This compound, 11-epitoonacilin, has not been isolated previously.



(83a)
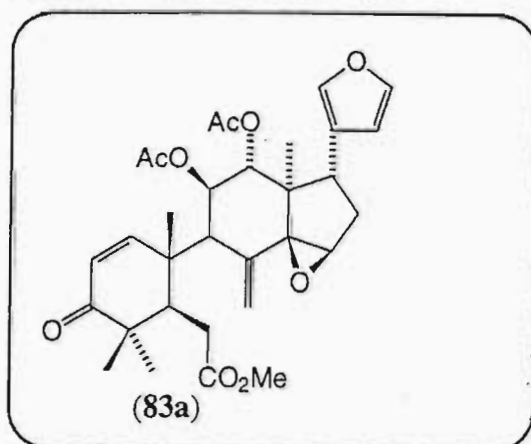
### 4.2.3 Structure elucidation of compound XI (114)

High resolution of the $M^+$ signal gave a mass of 538.2207 g/mol correct for the molecular formula $C_{30}H_{34}O_9$ (calculated 538.2202 g/mol). The loss of an acetic acid molecule was indicated by a fragment peak at *m/z* 478 in the mass spectrum. The infrared spectrum had absorption bands at 3408 cm$^{-1}$ (O-H stretching), 1749 cm$^{-1}$ (ester carbonyl stretching), 1685 cm$^{-1}$ ($\alpha,\beta$-unsaturated six membered ring ketone stretching), 1628 cm$^{-1}$ (intramolecularly H-bonded ketone)[105] and 1238 cm$^{-1}$ (C-O stretching). Compound XI was shown to be a limonoid by the presence of the $\beta$-substituted furan ring proton resonances at $\delta$ 7.29 (H-23), $\delta$ 7.10 (H-21) and $\delta$ 6.07 (H–22) and the corresponding carbon resonances at $\delta$ 142.8 (d, C-23), $\delta$ 140.4 (d, C-21) and $\delta$ 111.0 (d, C-22). C–20 occurred at $\delta$ 121.7(s).

The $^1$H NMR spectrum also showed resonances at $\delta$ 6.91 (1H, d, $J$ = 10.0Hz) and $\delta$ 6.13 (1H, d, J = 10.0Hz) ascribable to H-1 and H-2 respectively of a ring A enone system. The corresponding carbon resonances occurred at $\delta$ 150.1 (d) and $\delta$ 127.8 (d) and were ascribable to C-1 and C-2 respectively. C-3 was evident at $\delta$ 202.9 (s).
Disappearance of the proton resonance that occurred in the $^1$H NMR spectrum at $\delta$ 6.45 (1H, s) on deuteration suggested a diosphenol, as found in hirtin (107). The carbon resonances at $\delta$ 134.1 (s), $\delta$ 140.9 (s) and $\delta$ 196.9 (s) were attributed to C-5, C-6 and C-7 respectively.
The presence of a 14,15-epoxide group was confirmed by the proton resonance at $\delta$ 3.89 (1H, s) ascribable to H-15$\alpha$. The corresponding carbon

resonance was evident in the $^{13}$C NMR spectrum at $\delta$ 55.1 (d, C-15). C-14 occurred at $\delta$ 77.2 (s).

The presence of two acetate groups was indicated by the acetate methyl proton resonances at $\delta$ 1.94 (3H, s) and $\delta$ 2.15 (3H, s) and the corresponding methine proton resonances at $\delta$ 5.19 (1H, br s) and $\delta$ 5.36 (1H, br s). These two methine resonances were seen in the COSY spectrum to be coupled . The proton resonance at $\delta$ 5.36 (1H, br s) was in turn coupled to a resonance at $\delta$ 2.92 (1H, br s). The two acetates were placed at C-11 and C-12. The stereochemistry was ascertained from the very small coupling constants ($J_{9,11} = J_{11,12} \sim 0$Hz), the dihedral angles H-9$\alpha$:H-11$\alpha$ and H-11$\alpha$:H-12$\beta$ being close to $90^0$. This coupling was consistent with that found in hirtin (**107**)[105]. The COSY spectrum also showed long-range coupling between H-9$\alpha$ and H-12$\beta$.

The COSY spectrum showed that the proton resonance at $\delta$ 2.90 (1H, dd, $J = 7.0$Hz, $J = 11.3$Hz) ascribable to H-17 was coupled to the two H-16 resonances at $\delta$ 2.29 (1H, dd, $J = 7.0$Hz, $J = 13.5$Hz) and $\delta$ 1.97 (1H, dd, $J = 11.3$Hz, $J = 13.5$Hz). The $^1$H NMR spectrum also showed five methyl proton resonances at $\delta$ 0.77, $\delta$ 1.27, $\delta$ 1.35, $\delta$ 1.48 and $\delta$ 1.55 (each 3H, s). The corresponding carbon resonances were shown in the HETCOR spectrum at $\delta$ 15.6 (q), $\delta$ 24.7 (q), $\delta$ 22.6 (q), $\delta$ 21.1 (q) and $\delta$ 26.8 (q). Thus structure (**114**) was assigned to compound XI. This compound, 11$\beta$,12$\alpha$-diacetoxycedrelone, has not been isolated previously.

(114)
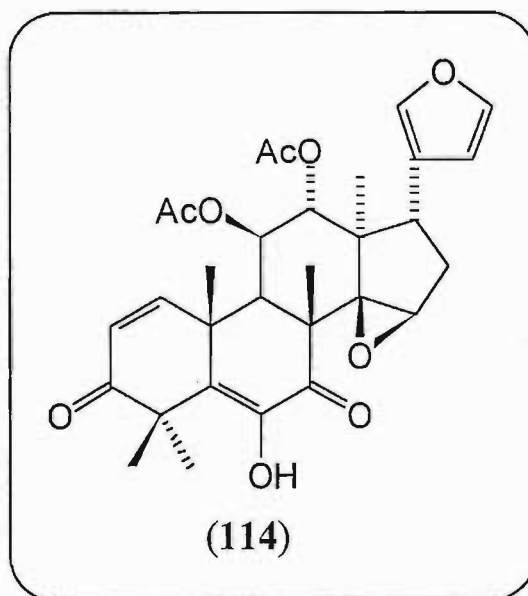
### 4.2.4 Structure elucidation of compound XII (118)

The high resolution mass spectrum gave M⁺ at *m/z* 468.2518 correct for the molecular formula $C_{28}H_{36}O_6$ (calculated 468.2512). The loss of an acetic acid molecule was indicated by a fragment peak at *m/z* 408 in the mass spectrum. The infrared spectrum showed absorption bands at 3468 cm⁻¹ (O-H stretching), 1728 cm⁻¹ (saturated C=O stretching), 1666 cm⁻¹ (α,β-unsaturated six membered ring ketone stretching), and 1249 and 1031 cm⁻¹ (C-O stretchings).

The ¹H NMR signals were assigned by comparison of the chemical shifts of compound XIV with those of 7-acetylneotrichilenone (115)[106], mzikonone (116)[107] and (117)[108].

The ¹H NMR spectrum showed resonances at δ 7.39, δ 7.34 and δ 6.30 typical of the β-substituted furan ring protons of limonoids ascribable to H-23, H-21 and H-22 protons respectively. The HETCOR spectrum indicated that the corresponding carbon resonances occurred at δ 143.3 (d,

C-23), δ 140.3 (d, C-21) and δ 110.6 (d, C-22). The C-20 resonance was evident at δ 122.4.

The presence of an α,β-unsaturated ketone in ring A was indicated in the $^1$H NMR spectrum by a pair of doublets at δ 6.89 (d, $J$ = 10.1Hz) and δ 5.83 (d, $J$ = 10.1Hz) ascribable to H-1 and H-2 respectively. The corresponding enone carbon resonances occurred in the $^{13}$C NMR spectrum at δ 157.1 (d, C-1) and δ 126.1 (d, C-2). The resonance at δ 203.8 (s) was ascribable to C-3.

A proton resonance at δ 2.90 (1H, s) was attributed to H-14α in ring D with a carbonyl group at C-15 as in (**117**)[108]. The C-14 and C-15 carbon resonances were evident in the $^{13}$C NMR spectrum at δ 60.5 (d) and δ 219.7 (s) respectively.

The proton resonance at δ 3.46 (1H, t, $J$ = 10.0Hz) ascribable to H-17β was seen to be coupled in the COSY spectrum to a resonance at δ 2.53 ( 2H, d, $J$ = 10.0Hz) ascribable to 2H-16. The corresponding C-17 and C-16 carbon resonances were observed in the HETCOR spectrum at δ 38.2 (d, C-17) and δ 43.0 (t, C-16) respectively.

The presence of an acetate group was indicated by the acetate methyl proton resonance at δ 2.00 (3H, s) and the carbon resonances of the acetate carbonyl group (δ 170.3, s) and the acetate methyl group (δ 21.2, s). The broad proton resonance at δ 3.90 (1H) with $W_{1/2}$ = 7.6Hz ascribable to a CH-O proton indicated the presence of a axial hydroxy group. The corresponding carbon resonance occurred at δ 69.8 (d). The proton

resonance in the $^1$H NMR spectrum at $\delta$ 5.18 (1H, t, $J$ = 3.4Hz) was assigned to the proton on the same carbon atom as the acetate group. The HETCOR spectrum indicated that the corresponding carbon resonance occurred at $\delta$ 72.4 (d).

Biosynthetically an oxygen atom is needed at C-7. When a hydroxy group occurs at C-7$\alpha$, H-14 occurs at $\delta$ 2.8[108]. When an acetate group is present , H-14 shifts to about $\delta$ 2.5[106]. The H-14 resonance occurred at $\delta$ 2.90, therefore the hydroxy group was placed at C-7$\alpha$. In the neotrichilenone-type compounds, C-11 and C-12 are the only places where an acetoxy group can be introduced to give a multiplet. Substitution at C-12 results in a triplet as in mzikonone (**116**); substitution at C-11 should result in a more complex multiplet. The acetate group was therefore placed at C-12. The stereochemistry at C-12 was confirmed by NOE experiments. Irradiation of H-12 ($\delta$ 5.18) gave a positive NOE for H-17 and H-22 ($\delta$ 6.30). This indicated that the acetate group was $\alpha$-orientated. Hence structure (**118**) was assigned to compound XII. This compound has not been isolated previously.
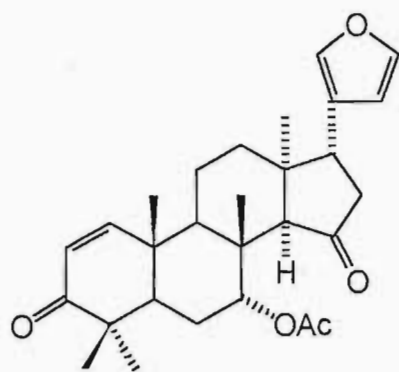
(115) 7-acetylneotrichilenone
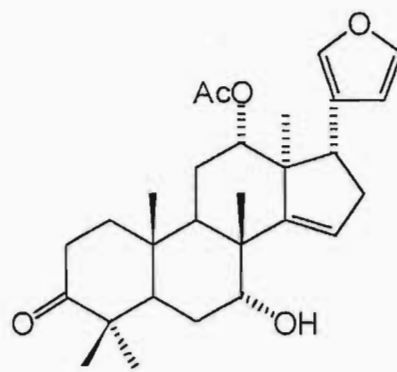
(116) mzikonone
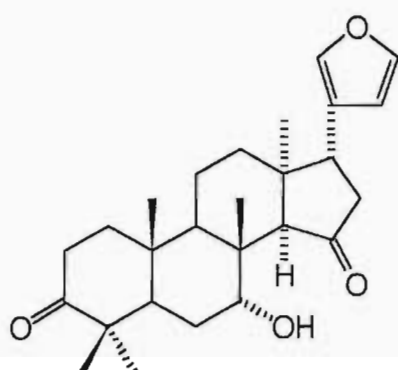
(117) 1,2-dihydroneotrichilenone
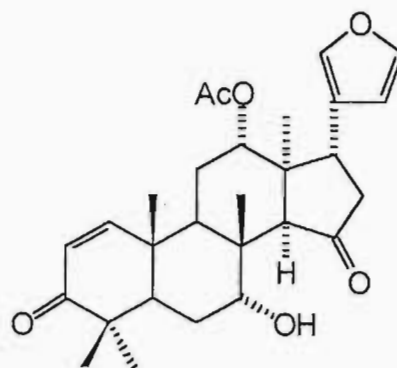
(118) 12α-acetoxyneotrichilenone

## 4.2.5 <u>Structure elucidation of compound XIII (119)</u>

The high resolution mass spectrum of compound XIII showed a molecular ion $M^+$ at $m/z$ 512.2784 correct for the molecular formula $C_{30}H_{40}O_7$ (calculated 512.2772). The loss of an acetic acid molecule was indicated by a fragment peak at $m/z$ 452.

The $^1H$ NMR spectrum of compound XIII was very similar to that of compound XII. The pair of doublets at δ 6.89 (H-1) and δ 5.83 (H-2) in the

$^1$H NMR spectrum of compound XII was, however, absent in the $^1$H NMR spectrum of compound XIII.

The presence of two acetate groups was indicated by two acetate methyl proton resonances at δ 2.03 (3H, s) and δ 2.11 (3H, s) and the corresponding methine proton resonances at δ 4.93 (1H, br m, $W_{1/2}$= 5.8Hz) and δ 5.12 (1H, t, $J$ = 3.2Hz) ascribable to H-7β and H-12β respectively.

The H-14 proton resonance had shifted upfield from δ 2.90 (compound XIV) to δ 2.52 in compound XIII due to the shielding effect of the 7α-acetate group[106]. Hence the structure (119) was assigned to compound XIII. This compound has not been isolated previously.



(119)
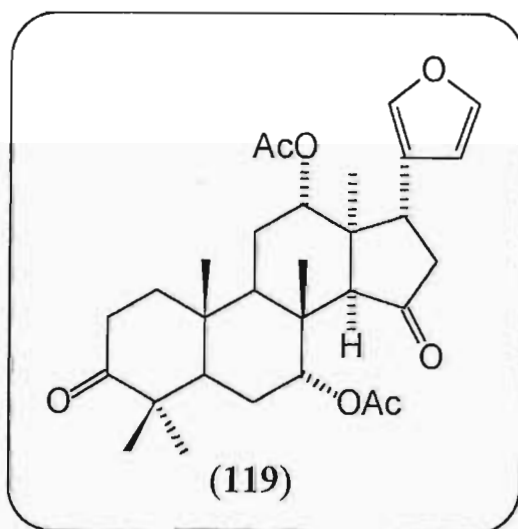
The β-substituted furan ring was indicated by the proton resonances in the $^1$H NMR at δ 7.38 (H-23), δ 7.29 (H-21) and δ 6.28 (H-22). The furan ring carbon resonances occurred in the $^{13}$C NMR spectrum at δ 143.38 (d, C-23), δ 140.3 (d, C-21), δ 122.4 (s, C-20) and δ 110.6 (d, C-22).

The C-3 and C-15 carbon resonances were evident at δ 216.4 (s) and δ 217.7 (s) respectively.

## 4.2.6 Structure elucidation of compound XIV (120)

Compound XIV was obtained in very small quantities but quite pure. As such only a proton spectrum was obtained. The ¹H NMR spectrum of compound XIV was very similar to those of compounds XII (118) and XIII (119) and the structure (120) of compound XIV was easily worked out by comparison with (118) and (119).



(120)
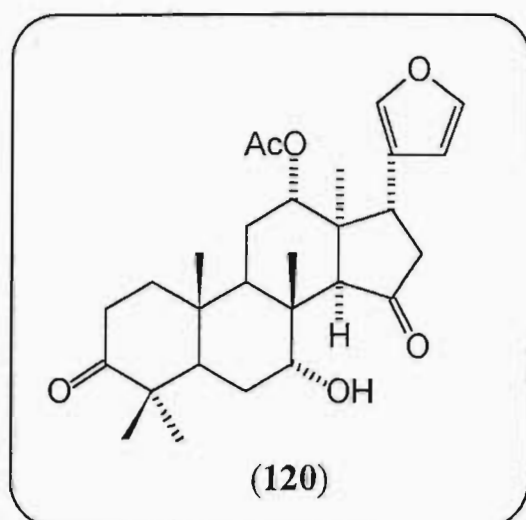
The β-substituted furan ring proton resonances occurred in the ¹H NMR spectrum at δ 7.38 (H-23), δ 7.29 (H-21) and δ 6.29 (H-22). The acetate methyl group proton signal was evident at δ 1.97 (3H, s). The corresponding methine proton signal occurred at δ 5.13 (1H, t, $J$ = 3.4Hz). The CH-OH proton resonance occurred at δ 3.87 (1H, br s). The chemical shift (δ 2.87) of the H-14 proton signal in the ¹H NMR spectrum indicated the 7α position of the hydroxy group. The acetate group was then placed at C-12α. The compound was acetylated and the ¹H NMR spectrum was identical with that of compound XIII (119).

### 4.2.7 <u>Structure elucidation of compound XV (121)</u>

Compound XV was obtained in very small quantities. The $^1$H NMR spectrum was very similar to those of compounds XII (114) and XIII (115), and the structure (121) of compound XV was determined by comparison with these compounds.

The $^1$H NMR spectrum showed resonances at δ 7.38 (1H, d, $J$ = 1.7 Hz), δ 7.11 (1H, s) and δ 6.15 (1H, d, $J$ = 1.7 Hz) typical of the H-23, H-21 and H-22 protons of the β-substituted furan ring of limonoids. The corresponding carbon resonances were shown in the HETCOR spectrum to occur at δ 143.4 (d, C-23), δ 139.9 (d, C-21) and δ 110.6 (d, C-22). The C-20 carbon resonance occurred in the $^{13}$C NMR spectrum at δ 122.8 (s).

The presence of two acetate groups was indicated by the acetate methyl proton resonances at δ 2.09 (3H, s) and δ 2.18 (3H, s) and the two methine CH-OAc proton resonances at δ 4.90 (1H, br m) and δ 5.45 (1H, br s). A hydroxy group was indicated by the CH-OH proton resonace at δ 3.83 (1H, br m) and the corresponding carbon resonance at δ 68.9 (d). An acetate group was placed at the C-7α position as the H-14 proton resonance occurred at δ 2.58 (1H, s) (c.f. compound XIII (119)) rather than at δ 2.90 (compound XII (118)). The methine proton resonance at δ 4.90 was therefore ascribable to H-7β.

The COSY spectrum showed that the CH-OH methine proton resonance at δ 3.83 was coupled to the CH-OAc methine proton at δ 5.45, which in turn was coupled to a methine proton resonance at δ 1.92 (1H, br s). These

resonances were respectively assigned to H-12, H-11 and H-9α. The H-9α

proton signal (δ 1.92) was also seen to be long-range coupled to the H-12

resonance (δ 5.45). The stereochemistry was ascertained from the very small

coupling constants, $J_{9,11} = J_{11,12} \sim 0$ Hz, the dihedral angles H-9α:H-11α and

H-11α:H-12β being close to $90^0$. This coupling was consistent with that

observed in compound XI (**114**).

The carbonyl carbon resonances at δ 216.2 (s) and δ 217.9 (s) were

ascribable to C-3 and C-16 respectively. The proton resonance at δ 3.88

(1H, t, $J = 10.0$ Hz) ascribable to H-17 was seen in the COSY spectrum to

be coupled to a 2H-16 resonance at δ 2.50 (2H, m), which was

superimposed on the 2H-2 proton resonance.

Acetylation of compound XV afforded a triacetate (**121a**). The three acetate

groups were indicated in the $^1$H NMR spectrum by the acetate methyl proton

resonances at δ 2.06, δ 2.10 and δ 2.17 (each 3H, s). The H-12β resonance

was observed at δ 5.19 (1H, d, $J_{11,12} = 3.3$ Hz) in the triacetate. The proton

resonances at δ 3.94 (1H, t, $J = 10.0$ Hz) and δ 2.57 (1H, s) were ascribable

to H-17 and H-14 respectively.



(**121**) R = H
(**121a**) R = Ac
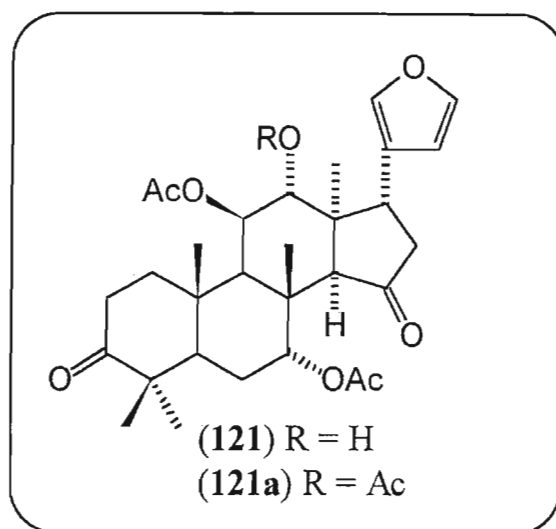
## 4.2.8 Structure elucidation of compound XVI (122)

The high resolution mass spectrum of compound XVI gave M$^+$ at $m/z$ 640.3233 correct for the molecular formula $C_{36}H_{48}O_{10}$ (calculated 640.3244)The infrared spectrum of compound XVI showed absorption bands at 1747 cm$^{-1}$ and 1736 cm$^{-1}$ (C=O stretchings), 1658 cm$^{-1}$ (C=C stretching), 1265 cm$^{-1}$, 1241 cm$^{-1}$ and 1060 cm$^{-1}$ (C-O stretchings) and 756 cm$^{-1}$ (olefinic C-H deformation).

The $^1$H NMR spectrum had proton resonances at δ 7.26 (1H, d, $J = 1.5$Hz), δ 7.22 (1H, s) and δ 6.35 (1H, d, $J = 1.5$Hz) whch are characteristic of the β-substituted furan ring protons H-23, H-21 and H-22 of limonoids. The corresponding furan ring carbon resonances occurred in the $^{13}$C NMR spectrum at δ 142.8 (d, C-23), δ 138.9 (d, C-21) and δ 110.4 (d, C-22). C-20 occurred at δ 129.3 (s).

The presence of a tiglate ester group was indicated by an alkene proton resonance at δ 6.97 (1H, q, $J = 7.2$Hz) and a methyl proton resonance at δ 1.81 (3H, d, $J = 7.2$Hz). Two acetate groups were indicated by the acetate methyl proton resonances at δ 1.98 (s) and δ 1.88 (s). Two methine proton resonances at δ 4.92 (1H, t, $J = 2.6$Hz) and δ 4.71 (1H, t, $J = 2.6$Hz), both seen in the COSY spectrum to be coupled to a proton resonance at δ 2.18 (2H, m), were indicative of ring A with α-oriented ester groups at C-1 and C-3. The HETCOR spectrum showed the corresponding carbon resonances at δ 71.6 (d) and δ 70.9 (d). The C-2 carbon resonance was seen to occur in the $^{13}$C NMR spectrum at δ 27.5 (t). The -CH-O proton resonance at δ 5.70

(1H, d, $J = 2.8$Hz) ascribable to H-7 was seen in the COSY spectrum to be coupled to another -CH-O proton resonance at δ 4.05 (1H, dd, $J = 12.8$Hz, $J = 2.8$Hz) ascribable to H-6 which in turn was coupled to a proton resonance at δ 2.77 (1H, d, $J = 12.8$Hz) ascribable to H-5. The $J_{5,6}$=12.8Hz and $J_{6,7}$= 2.8Hz coupling constants indicated a *trans* H-5, H-6 configuration and a *cis* configuration of H-6 and H-7. The COSY spectrum showed that the -CH₂-O proton resonance at δ 3.47 (2H, m) ascribable to 2H-28 was long-range coupled to a methyl proton resonance at δ 1.15 (3H, s) ascribable to 3H-29. The lack of a hydroxy absorption band in the infrared spectrum indicated an ether linkage between C-28 and C-6.

The presence of tetra-substituted double bond, as indicated by the carbon resonances at δ 140.6 (s) and δ 142.9 (s), and the presence of a deshielded methyl [δ 1.71 (3H, s)] indicated a ring C seco limonoid. The acetal carbon resonance at δ 98.0 (d) was ascribable to C-12. The corresponding H-12 proton resonance occured in the $^1$H NMR spectrum at δ 4.58 (1H, br m). These spectral properties were very similar to those reported for nimbolinin B (**111**)[98,109] except for the presence of a methoxy group [δ 3.04 (3H, s)] which had replaced the C-12 hydroxy group in nimbolinin B. Hence structure (**122**) was assigned to compound XVI.

The COSY spectrum showed the H-15 proton resonance at δ 4.88 (1H, d, $J = 7.8$Hz) to be coupled to a proton resonance at δ 2.35 (1H, m) ascribable to H-16α, and the H-17 proton resonance at δ 3.30 (1H, d, $J = 8.3$Hz) to be coupled to a proton resonance at δ 1.57 (1H, m) ascribable to H-16β.

An attempt to establish the stereochemistry at C-12 by NOE experiments failed owing to the weakness of the sample.

Ekong *et al.*[110] suggested that such compounds could be formed from intact limonoids such as (123), possibly *via* a hydroxy-aldehyde intermediate. The hydroxy-aldehyde in turn forms nimbolinin B as an internal hemiacetal (111).



(122) R = Me
(111) nimbolinin B, R = H

(123)

hydroxy-aldehyde intermediate

(111) nimbolinin B

**Scheme 23: Possible formation of nimbolinin B**
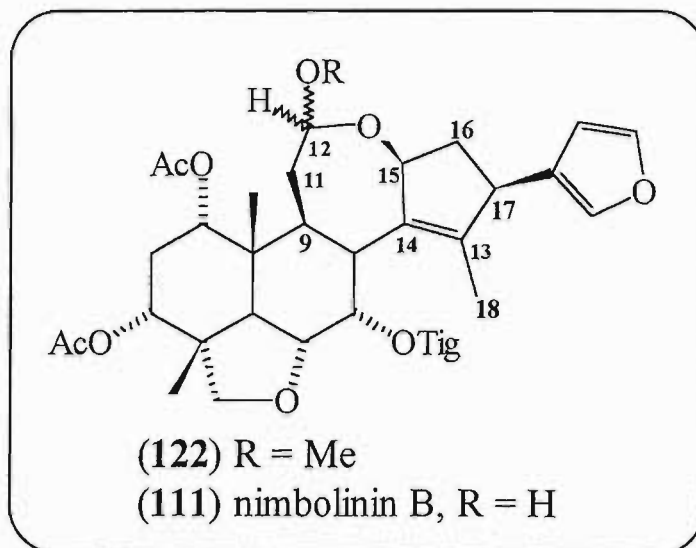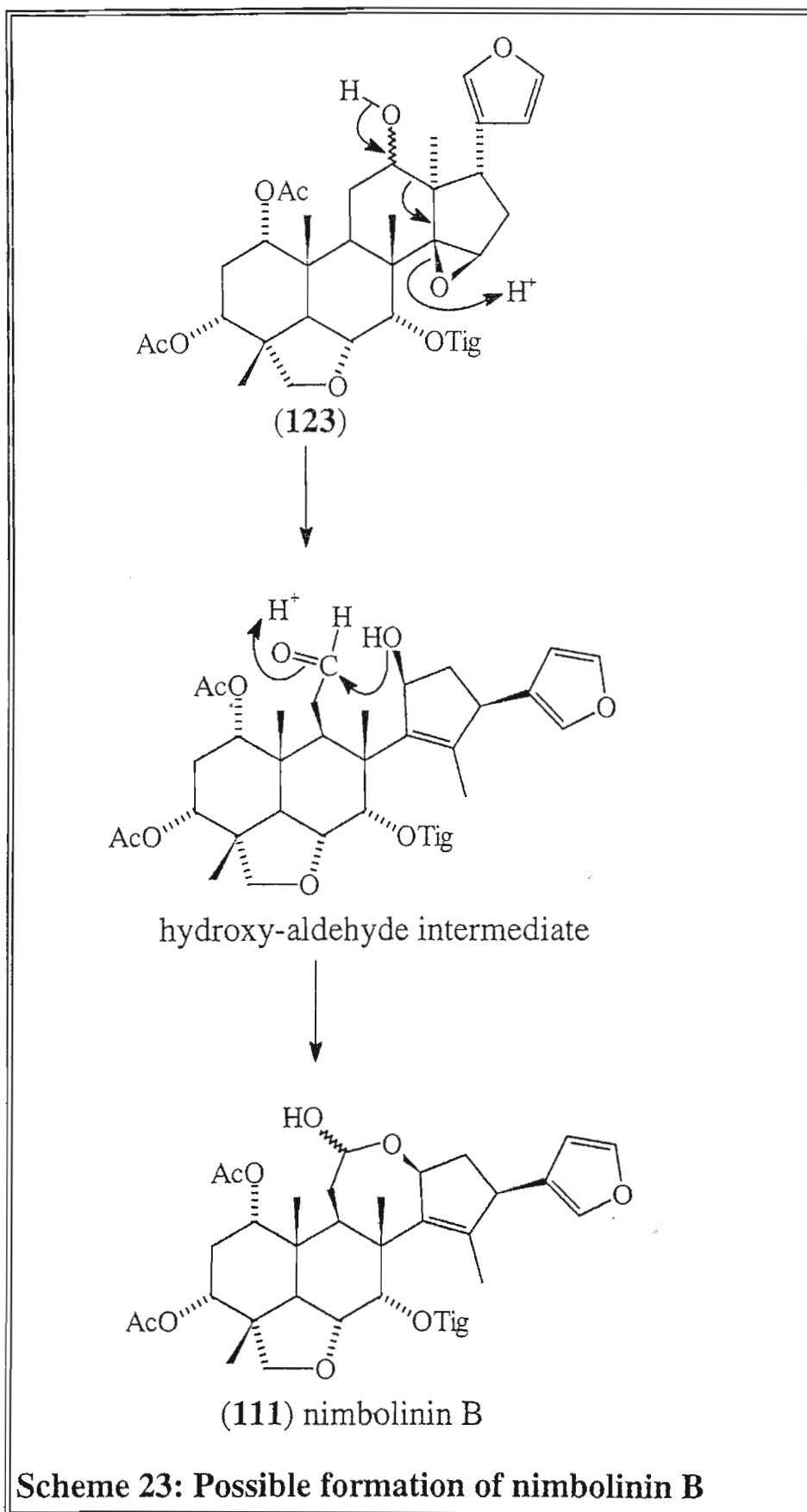
### 4.2.9. Stucture elucidation of compound XVII (124)

Relatively large amounts (492 mg) of this compound were isolated from the root bark and the stem bark of *Turraea holstii*. Structure (124) was assigned to compound XVII and the reasons for this structure are outlined below. Several reactions carried out were useful in the structure elucidation of compound XVII. Acetylation, Sarret's oxidation and Jones' oxidation afforded compounds XVIIa (124a), XVIIb (124b) and XVIIc (124c) respectively.The mass spectrum of compound XVII showed that the highest peak occurred at *m/z* 498.3353 correct for the ion $C_{31}H_{46}O_5$ (calculated 498.3345). However analysis of the NMR data indicated that this was the $[M^+ - 32]$ peak indicating a loss of a methanol molecule. This further suggested a true molecular formula $C_{32}H_{50}O_6$ (530 g/mol). This molecular formula indicated eight double bond equivalents. The loss of a second methanol molecule was indicated by a fragment peak at *m/z* 466 in the mass spectrum.



(124) R = H
(124a) R = Ac

(124b) R = O, R' = OH, H
(124c) R = R' = O
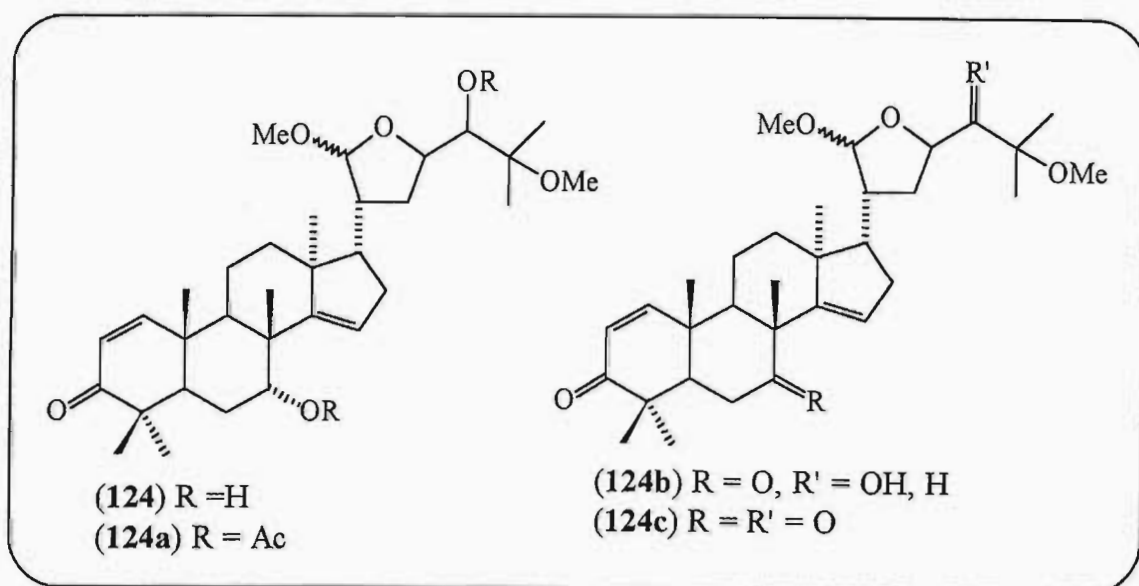
The infrared spectrum showed absorption bands at 3467 cm$^{-1}$ (O-H stretching), 1677cm$^{-1}$ ($\alpha,\beta$-unsaturated six membered ring ketone stretching), and 1091 cm$^{-1}$ and 1045 cm$^{-1}$ (C-O stretchings).

The presence of an $\alpha,\beta$-unsaturated ketone in ring A was indicated in the $^1$H NMR spectrum by a pair of doublets at $\delta$ 7.11 (1H, d, $J$ = 10.2Hz) and $\delta$ 5.80 (1H, d, $J$ = 10.2Hz) ascribable to H-1 and H-2 respectively. The carbon resonances associated with this enone system were observed in the $^{13}$C NMR spectrum at $\delta$ 204.1 (s), $\delta$ 158.2 (d) and $\delta$ 125.5 (d) ascribable to C-3, C-1 and C-2 respectively.

The presence of a trisubstituted double bond was indicated by resonances at $\delta$ 161.6 (s) and $\delta$ 119.6 (d) in the $^{13}$C NMR spectrum. The HETCOR spectrum showed that the $\delta$ 119.6 doublet correlated with a proton resonance at $\delta$ 5.46 (1H, dd, $J$ = 1.6Hz, $J$ = 3.5Hz) which was ascribable to H-15.

Two secondary hydroxy groups were present as indicated by the two methine proton resonances at $\delta$ 3.96 (1H, br m, W$_{1/2}$ = 6.6Hz) and $\delta$ 3.34 (1H, d, $J$ = 6.7Hz).

Biosynthetically an oxygen functional group is needed at position C-7$\alpha$ and the proton resonance at $\delta$ 3.96 was tentatively assigned to H-7$\beta$ since the chemical shift and half-width were typical for this proton when a hydroxy group is present at C-7$\alpha$. Acetylation of compound XVII resulted in the shift in the $^1$H NMR spectrum of H-7$\beta$ from $\delta$ 3.96 to $\delta$ 5.22. This H-7$\beta$ proton resonance was superimposed with the H-15 proton resonance which

had also shifted on acetylation of the compound from δ 5.46 in compound

XVII (124) to δ 5.22 in compound XVIIa (124a). This confirmed the

placing of the hydroxy group at C-7α as the chemical shift of H-15 is

affected by substitution at C-7α. The COSY spectrum showed that the H-7β

proton resonance (δ 3.96) was coupled to a proton resonance at δ 1.75-1.85

(2H, m) ascribable to 2H-6 which in turn was coupled to a proton resonance

at δ 2.38 (1H, dd, $J$ = 4.0Hz, $J$ = 11.3Hz) ascribable to H-5.

The structure of the compound thus far was :
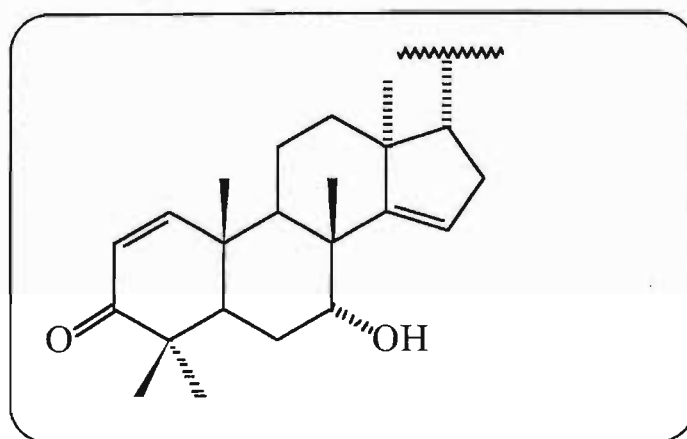


The above accounted for $C_{22}H_{31}O_2$ of the total $C_{32}H_{50}O_6$. This meant that the

side chain consisted of $C_{10}H_{19}O_4$. The above structure also accounted for

seven of the eight double bond equivalents. Since all the double bonds were

accounted for, the side chain therefore had one ring. The proposed structure

of the side chain was determined by a study of the $^1H$ NMR, $^{13}C$ NMR and

mass spectra.

The $^1H$ NMR spectrum indicated the presence of two methoxy groups at

δ 3.21 (3H, s) and δ 3.34 (3H, s) and seven tertiary methyl groups at

δ 1.03 (3H, s), δ 1.07 (3H, s), δ 1.10 (3H, s), δ 1.13 (3H, s), δ 1.14 (6H, s)

and δ 1.22 (3H, s). The above structure accounted for only five tertiary

methyl groups. Besides the doublet at δ 71.5 corresponding to C-7, the

^{13}C NMR spectrum also showed three extra C-O resonances at δ 75.1 (d), δ 76.2 (d) and δ 77.2 (s). The carbon resonance at δ109.7 (d) indicated the presence of a O-CH-O group and was ascribable to C-21. The HETCOR spectrum correlated this signal to a proton signal at δ 4.79 (1H, d, $J$ = 3.6Hz, H-21). The HETCOR spectrum showed that the doublet at δ 75.1 was correlated to a proton signal at δ 4.20 (1H, m, H-23) which in turn was shown in the COSY spectrum to be coupled to two proton resonances (2H-22) in the region δ 1.5 - δ 2.0 and the CH-O proton resonance at δ 3.34 (1H, m, H-24). The structure of the side chain was worked out on the basis of the above data. The COSY spectrum showed that the C-25 methoxy proton resonance at δ 3.21 was long-range coupled to tertiary methyl proton resonances at δ 1.14 and δ 1.22 ascribable to 3H-26 and 3H-27.

The fragment peaks in the mass spectrum at $m/z$ 326 and $m/z$ 395 corresponding to fragment **a** ($C_{22}H_{30}O_2$) and fragment **b** ($C_{26}H_{35}O_3$) respectively, confirmed the structure (**124**).

Oxidation (Sarret's) yielded compound XVIIb (**124b**), where only the 7a-hydroxy group was oxidised. The ^{1}H NMR spectrum of compound XVIIb showed that the H-15 proton resonance had shifted downfield from δ 5.46 in the original compound to δ 5.92 in compound XVIIb. The H-5 proton resonance had also shifted downfield from δ 2.38 (1H, dd, $J$ = 4.0Hz, $J$ = 11.3Hz) to δ 2.85 (1H, t, $J$ = 4.4Hz). The ^{13}C NMR spectrum showed the carbon resonances at δ 46.1 (d, C-5), δ 24.2 (t, C-6), δ 44.8 (s, C-8) and δ 36.7 (d, C-9) in compound XVII had all shifted downfield to δ 52.5 (d), δ 36.2 (t), δ 52.6 (s) and δ 44.8 (d) respectively. C-7 occurred at δ 209.5 (s).

Jones' oxidation resulted in compound XVIIc (**124c**), where both the C-7 and C-24 hydroxy groups were oxidised. The $^1$H NMR spectrum of compound XVIIc showed that the H-5 and H-15 proton resonances were in the same positions as in compound XVIIb. However the H-21 and H-23 proton resonances had both shifted from $\delta$ 4.79 and $\delta$ 4.21 in the original compound to $\delta$ 4.96 (1H, d, $J = 2.7$Hz) and $\delta$ 5.03 (1H, dd, $J = 5.3$Hz, $J = 10.1$Hz) respectively. The $^{13}$C NMR spectrum of compound XVIIc showed carbon resonances in the carbonyl region at $\delta$ 203.6 (s), $\delta$ 209.6 (s) and $\delta$ 211.7 (s) ascribable to C-3, C-7 and C-24 respectively. The carbon resonances at $\delta$ 75.1 (d, C-23) and $\delta$ 77.2 (s, C-25) in compound XVII had shifted downfield to $\delta$ 76.9 (d) and $\delta$ 81.7 (s) respectively.
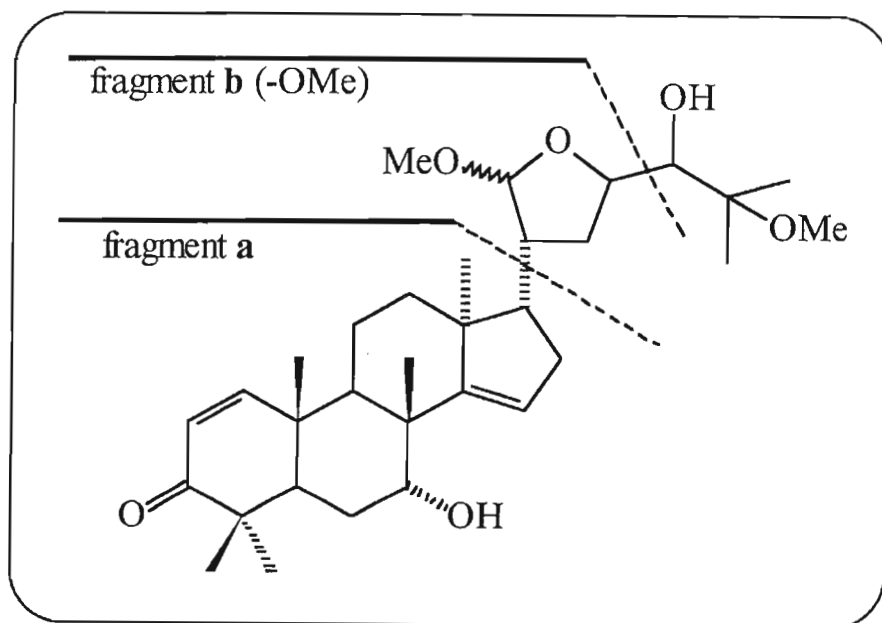
#### 4.2.10. Structure elucidation of compound XVIII (125)

High resolution of the highest peak in the mass spectrum of compound XVIII gave $m/z$ 466.3081 correct for the formula $C_{30}H_{42}O_4$ (calculated 466.3083). Analysis of the NMR data indicated that this was the $[M^+-32-18]$ peak indicating the loss of methanol and water molecules. This further indicated a molecular formula $C_{31}H_{48}O_6$ (516 g/mol) Fragment peaks at $m/z$ 326 and $m/z$ 395 were observed, and these corresponded, as in compound XVII (124), to fragment a ($C_{22}H_{30}O_2$) and fragment b ($C_{26}H_{35}O_3$) respectively.

The $^1H$ NMR spectrum of compound XVIII was very similar to that of compound XVII (124). The H-1,H-2, H-7β and H-15 proton resonances were observed at δ 7.11 (1H, d, $J = 10.2Hz$), δ 5.80 (1H, d, $J = 10.2Hz$), δ 3.96 (1H, t, $J = 2.6Hz$) and δ 5.46 (1H, dd, $J = 3.4Hz$, $J = 5.5Hz$) respectively. A broad singlet at δ 3.51 (1H) ascribable to H-24, was seen in the COSY spectrum to be coupled to a broad resonance at δ 2.80 (OH) which disappeared on addition of $D_2O$.

The proton resonance at δ 3.34 (3H, s) indicated the presence of a methoxy group. Comparison of $^1H$ NMR data for compound XVIII with that of compound XVII indicated that this methoxy group should be placed at C-21. Since the C-25 carbon resonance in compound XVIII occurred at δ 72.3 (s) whereas in compound XVII (124) was observed at δ 77.2 (s), a hydroxy group was therefore placed at C-25 in compound XVIII, hence the structure (125).

fragment **b** (-OH)

fragment **a**

(125)
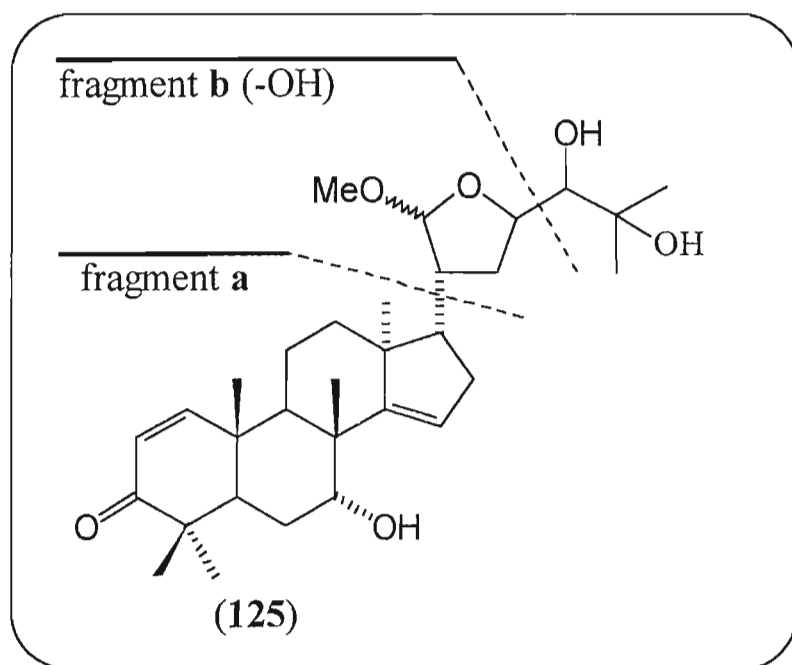
# CHAPTER 5

## EXPERIMENTAL

## <u>INDEX</u>

## 5.1 <u>General</u>

### 5.1.1 <u>Proton ($^1$H) Nuclear Magnetic Resonance Spectroscopy</u>

$^1$H NMR spectra were recorded at room temperature on a Varian Gemini 300 MHz spectrometer using deuteriochloroform (CDCl$_3$) as a solvent. Chemical shifts were recorded relative to the chloroform singlet at δ 7.24.

### 5.1.2 <u>Carbon ($^{13}$C) Nuclear Magnetic Resonance Spectroscopy</u>

$^{13}$C NMR spectra wre recorded at room temperature on a Varian Gemini 300 MHz spectrometer at 75.4 MHz. The spectra were recorded with proton noise decoupling and chemical shifts were assigned relative to the central line of the CDCl$_3$ triplet at δ 77.09.

### 5.1.3 <u>Infrared Spectroscopy</u>

Fourier transform infrared spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer using KBr disks, or NaCl cells with chloroform as solvent.

### 5.1.4 <u>Melting Point Determination</u>

Melting points wre determined on a Kofler micro hotstage melting point apparatus and are uncorrected. Compounds for melting point determination were first recrystallised form chloroform or methanol.

### 5.1.5 Optical Rotations

Optical rotations were recorded at room temperature in chloroform solution on an Optical Activity Ltd Type AA-5 polarimeter.

### 5.1.6 Mass Spectrometry

High resolution masses and mass spectra were recorded by Dr. P. Boshoff at the Cape Technikon.

### 5.1.7 Chromatography

#### 5.1.7.1. Thin Layer Chromatography (T.L.C.)

Analytical T.L.C. was performed using 0.2 mm thick aluminium-backed silica gel 60 sheets (Merck Art. 5553), employing one of the following solvent systems in the appropriate ratios :

$CH_2Cl_2$ : EtOAc

$CH_2Cl_2$ : EtOAc : hexane

$CH_2Cl_2$ : hexane

EtOAc : hexane

All solvents were analytical grade or redistilled before use.

The spots on the T.L.C. plates were visualised by spraying with anisaldehyde spray reagent, containing anisaldehyde:conc. sulphuric acid: methanol in the ratio of 1.25:2.5:96.25. Coloured spots formed after heating the sprayed plates at 110°C for several minutes.

### 5.1.7.2. <u>Column chromatography</u>

Three different types of columns were employed. Initial separation was perfomed using a 6 or 8 cm diameter glass column packed with silica gel 60 (0.040-0.053 mm particle size, 230-400 mesh ASTM, Merck Art. 9385), in which elution proceeded by gravity. Flash chromatography was performed in a glass column (1.5 and 2.5 cm diameter) packed with silica gel 60 (same as in the initial column). For the final purification of of some compounds, columns made of 0.75 cm diameter pasteur pipette packed with silica gel as above. The above mentioned solvent systems were also applicable to the column separations.

### 5.1.8 <u>Extraction of plant material</u>

Air-dried bark samples were ground in a coffee grinder before extraction. Extraction of the *Bersama* and *Dysoxylum* samples was performed using a soxhlet apparatus, successively with hexane , chloroform and methanol for 24 hours each. Solvents were removed on a rotavapor and the resulting gum was chromatographed on gravity columns, and further purification was achieved by repeated column chromatography. The methanolic extracts of the root bark and the stem bark of *Turraea holstii* were obtained from Prof Rajab of Moi University, Kenya. The extraction procedure provided by Prof. Rajab is as follows:

Both the root bark and the stem bark were air-dried and grounded to a fine powder. These were allowed to stand in 2 litres of methanol at room temperature for one week. The extracts were decanted and the residual pulp

similarly extracted a second time. The combined extracts were evaporated under vacuum resulting in a gum.

## 5.2 Extractives from *Bersama Swinnyi*

The stem and leaves of *Bersama swinnyi* were collected from the Vernon Crookes Nature Reserve. 177 g of ground bark and 445 g of ground leaves were used in the hexane (section 5.1.7.3) which yielded 2.3 g and 9.2 g of hexane extracts respectively. Chloroform extraction of the bark yielded 1.8 g of extract. Chromatographic separation yielded compounds I -IV. The hexane extracts of the bark and leaves yielded compound IV (**97**). The chloroform extract of the bark yielded compounds I (**94**), II (**95**) and III (**96**).

### 5.2.1 Physical data of compound I (94)

*20(29)-Lupene-3β,27-diol*

Yield : 320 mg

Melting point : 215-217°C  (lit. value 214-215°C)*

Infrared Spectrum:

$\nu_{max}$(KBr) : 3450 cm$^{-1}$ (O-H stretching), 2942 cm$^{-1}$ and 2871 cm$^{-1}$ (>CH$_2$, CH$_3$ stretchigs), 1690 cm$^{-1}$ (C=C stretching)

Mass Spectrum:

EIMS *m/z* 442.3827 ([M]$^+$ ,C$_{30}$H$_{50}$O$_2$, req. 442.3811)

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* **all literature values here and further on are taken from the *Dict. Nat. Prod.*[73] unless otherwise stated**

Optical Rotation:

$[\alpha]_D = +66.6^0$ (c,0.48 in CH₃Cl), (lit. value $+67^0$)

¹³C NMR :

Table 2 (page 126, Appendix)

¹H NMR :

δ(ppm): 4.66 (1H, d, $J = 2.0$ Hz, H-29a), 4.55 (1H, d, $J = 2.0$ Hz, H-29b), 3.77 (1H, d, $J = 10.7$ Hz, H-27a), 3.31 (1H, d, $J = 10.7$ Hz, H-27b), 3.16 (1H, dd, J = 5.2 Hz and 11.0 Hz, H-3α), 2.35 (1H, m, H-19), 1.66 (3H, s, H-30), 1.00 (3H, s, H-26), 0.96 (3H, s, H-23), 0.94 (3H, s, H-25), 0.80 (3H, s, H-28), 0.74 (3H, s, H-24)

### 5.2.1.1 Acetylation of compound I (94)

Acetic anhydride (5ml) was added to a magnetically stirred solution of compound I (55mg) in pyridine (5ml). The mixture was warmed briefly on a steam bath and left to stand overnight. Methanol was added and the solvent removed under reduced pressure. Addition of toluene (3x10ml) and evaporation under reduced pressure removed the remaining traces of pyridine. Methanol (3x10ml) was added to remove the traces of toluene from the mixture. T.l.c. showed that all the compound had been acetylated. Column chromatography was used to purify the product.

Yield: 43.5 mg

Melting Point: 250-252°C (lit. value 249-250°C)[71]

¹H NMR :

δ(ppm): 4.66 (1H, d, $J = 2.0$ Hz, H-29a), 4.57 (1H, d, $J = 2.0$ Hz, H-29b), 4.40 (1H, dd, $J = 5.7$ Hz, $J = 10.4$ Hz, H-3α), 4.23 (1H, d, $J = 11.0$ Hz, H-27a), 3.83 (1H, d, $J = 11.0$ Hz, H-27b), 2.43 (1H, m, H-19),

1.66 (3H, s, H-30), 1.01 (3H, s, H-28), 0.95 (3H, s, H-26), 0.82 (6H, s, H-23 and H-24), 0.81 (3H, s, H-25)

### 5.2.2 Physical data of compound II (95)

*3β-Hydroxy-20(29)-lupen-28-al (betulinaldehyde)*

Yield: 335 mg

Melting Point: 192-194$^0$C (lit. value 192-193$^0$C)

Optical Rotation: $[\alpha]_D$ = +19.6$^0$ (c, 1.3 in CH$_3$Cl), (lit. value +19.2$^0$)

Infrared Spectrum:

$\nu_{max}$ (NaCl): 3427 cm$^{-1}$ (O-H stretching), 2941 cm$^{-1}$ and 2868 cm$^{-1}$

(>CH$_2$, CH$_3$ stretchings), 1711 cm$^{-1}$ (C=O stretching), 757 cm$^{-1}$ (olefinic

C-H deformation)

Mass Spectrum:

EIMS *m/z* 440.3648 ([M]$^+$, C$_{30}$H$_{48}$O$_2$, req. 440.3652)

$^{13}$C NMR:

Table 2 (page 126, Appendix)

$^1$H NMR:

δ(ppm): 9.66 (1H, s, H-28), 4.74 (1H, d, *J* = 2.0 Hz, H-29a), 4.61 (1H, d, *J* = 2.0 Hz, H-29b), 3.16 (1H, dd, *J* = 5.3 Hz, *J* = 11.0Hz, H-3a), 2.84 (1H, td, *J* = 6.0 Hz, *J* = 11.0 Hz, H-19), 1.67 (3H, s, H-30), 0.95 (3H, s, H-24), 0.94 (3H, s, H-23), 0.89 (3H, s, H-26), 0.80 (3H, s, H-25), 0.73 (3H, s, H-27)

### 5.2.3 <u>Physical data of compound III</u> (96)

*3β,23-Dihydroxy-20(29)-lupen-28-al (23-hydroxybetulinaldehyde)*

Yield: 12.3 mg

$^{13}$C NMR:

Table 2 (page 126, Appendix)

$^{1}$H NMR:

δ(ppm): 9.65 (1H, s, H-28), 4.74 (1H, d, $J$ = 2.0 Hz, H-29a), 4.60 (1H, d, $J$ = 2.0 Hz, H-29b), 3.70 and 3.39 (each 1H, d, $J$ = 10.4 Hz, 2H-23), 3.60 (1H, br m, H-3α), 2.84 (1H, td, $J$ = 5.6 Hz, $J$ = 11.0 Hz, H-19), 2.40 and 2.17 (each 1H, br s, OH), 1.67 (3H, s, H-30), 0.95 (3H, s, H-27), 0.90 (3H, s, H-26), 0.85 and 0.84 (each 3H, s, H-24 and H-25)

### 5.2.4 <u>Physical data of compound IV</u> (97)

*3β-Hydroxy-12-oleanen-28-oic acid, (oleanolic acid)*

Yield:760 mg

Melting Point: 307-309$^{0}$C (lit. value 306-308$^{0}$C)

Optical Rotation:

$[\alpha]_D$ = +79.5$^{0}$ (c, 1.0 in CH$_3$Cl), (lit. value +79.5$^{0}$)

Infrared Spectrum:

$v_{max}$ (KBr): 3450 cm$^{-1}$ (O-H stretching), 2942 and 2869 cm$^{-1}$ (>CH$_2$, CH$_3$ stretchings), 1643 cm$^{-1}$ (C=C stretching)

Mass Spectrum:

EIMS *m/z* 456.3578 ([M]$^+$, C$_{30}$H$_{48}$O$_3$, req. 456.3603)

$^{13}$C NMR:

Table 2 (page 126, Appendix)

¹H NMR:

δ(ppm): 5.26 (1H, m, H-12), 3.20 (1H, dd, J = 4.9 Hz and 10.3 Hz,

H-3α), 2.80 (1H, dd, J = 4.0 Hz and 13.4 Hz, H-18), 1.10, 0.96, 0.91,

0.89, 0.88, 0.75, 0.72 (each 3H, s, CH₃)

## 5.3. Extractives from *Dysoxylum spectabile*

The *D. spectabile* sample was collected and identified by Peter Tijsen,

Wellington Botanical Gardens.

63.4 g of ground bark of *Dysoxylum spectabile* was extracted according to

5.1.7.3 resulting in 3.4 g and 2.7 g of hexane and chloroform extracts.

Chromatographic separation of the extracts yielded compounds V-VIII. The

hexane extract yielded the oily compounds V (**99**) and VI (**100**) and the

chloroform extract yielded compounds VII (**101**) and VIII (**102**).

### 5.3.1 Physical data of compound V (103)

*8(14),15-sandaracopimaradiene*

Yield: 127 mg

Melting point: amorphous

Optical Rotation:

$[\alpha]_D = -16.7^0$ (c, 0.600 in CHCl₃) (lit. value -12.4⁰)

Infrared Spectrum:

$v_{max}$ (NaCl): 2924 cm⁻¹ and 2850 cm⁻¹ (>CH₂, CH₃ stretchings), 758 cm⁻¹

(olefinic C-H deformation)

Mass Spectrum (spectrum E-2, page ++)

EIMS *m/z* 272.2490 ([M]⁺, C₂₀H₃₂, req. 270.2504)

$^{13}$C NMR:

Table 3 (page 127, Appendix)

$^{1}$H NMR:

δ(ppm): 5.76 (1H, dd, $J$ = 10.6 Hz, $J$ = 17.5 Hz, H-15), 5.19 (1H, br s, $W_{1/2}$= 5.0Hz, H-14), 4.86 (1H, dd, $J$ = 1.5 Hz, $J$ = 10.6 Hz, H-16a), 4.88 (1H, dd, $J$ = 1.5 Hz, $J$ = 17.5 Hz, H-16b), 1.02 (3H, s, 3H-17), 0.86 (3H, s, 3H-20), 0.83 (3H, s, 3H-18), 0.78 (3H, s, 3H-20)


### 5.3.2 Physical data of compound VI (104)

*7α–Hydroxysandaracopimara-8(14),15-diene*

Yield: 97 mg

Melting point: amorphous

Optical Rotation:

$[α]_D$ = -37$^0$ (c, 0.60 in CHCl$_3$), (lit. value -69.1$^0$, c2.37)

Infrared Spectrum:

$ν_{max}$ (NaCl): 3427 cm$^{-1}$ (O-H stretching), 2925 cm$^{-1}$ and 2867 cm$^{-1}$ (saturated C-H stretchings)

Mass Spectrum:

EIMS *m/z* 288.2459 ([M]$^+$, C$_{20}$H$_{32}$O, req. 288.2453)

$^{13}$C NMR:

Table 3 (page 127, Appendix)

$^{1}$H NMR:

δ(ppm): 5.76 (1H, dd, $J$ = 10.6 Hz, $J$ = 17.4 Hz, H-15), 5.49 (1H, br s, H-14), 4.92 (1H, dd, $J$ = 1.4 Hz, $J$ = 17.4 Hz, H-16b), 4.90 (1H, dd, $J$ = 1.4 Hz, $J$ = 10.6 Hz, H-16a), 4.17 (1H, t, $J$ = 2.7 Hz, H-7β), 1.02 (3H, s, 3H-17), 0.88, 0.83, 0.75 (each 3H, s, CH$_3$).

### 5.3.2.1 Acetylation of compound VI

Acetylation of compound VI (20 mg) was carried out using acetic anhydride in pyridine at room temperature, as described in 5.2.1.1

Yield: 17 mg

$^1$H NMR:

δ(ppm): 5.73 (1H, dd, $J$ = 10.3Hz, $J$ = 17.9Hz, H-15), 5.62 (1H, br s, H-14), 5.30 (1H, t, $J$ = 2.8Hz, H-7β), 4.88 (1H, dd, $J$ = 10.3Hz, $J$ = 1.5Hz, H-16a), 4.88 (1H, dd, $J$ = 17.9Hz, $J$ = 1.5Hz, H-16b), 1.99 (3H, s, OCOCH₃), 1.02 (3H, s, 3H-17), 0.82 (6H, s, 2xCH₃), 0.78 (3H, s, CH₃)

### 5.3.3 Physical data of compound VII (105)

*Methyl ivorensate*

Yield: 64.5 mg

Melting Point: 278-280$^0$C (lit. value 279-281$^0$C)

Optical Rotation:

$[\alpha]_D$ = -98.0$^0$ (c, 1.1 in CH₃Cl), (lit. value -97.5$^0$)

Infrared Spectrum:

$v_{max}$ (NaCl): 2958 cm$^{-1}$ (saturated C-H stretching), 1732 (lactone carbonyl stretching)

Mass Spectrum:

EIMS $m/z$ 486.2238 ([M]$^+$, C₂₇H₃₄O₈, req. 486.2251)

$^{13}$C NMR:

Table 3 (page 127, Appendix)

¹H NMR:

δ(ppm): 7.45 (1H, s, H-21), 7.35 (1H, d, $J$ = 1.7 Hz, H-23), 6.38 (1H, d, $J$ = 1.7 Hz, H-22), 5.68 (1H, s, H-17), 5.14 (1H, s, H-30a), 4.90 (1H, s, H-30b), 3.70 (3H, s, CO₂Me), 3.35 (1H, dd, $J_{1,2a}$ = 2.5 Hz, $J_{1,2b}$ = 5.8 Hz, H-1), 3.10 (1H, dd, $J_{1,2a}$ = 2.5 Hz, $J_{2a,2b}$ = 15.2 Hz, H-2a), 2.91 (1H, d, $J_{15a,15b}$ = 18.3 Hz, H-15a), 4.87 (1H, dd, $J_{1,2b}$ = 5.8 Hz, $J_{2a,2b}$ = 15.2 Hz, H-2a), 2.53 (1H, d, $J_{15a,15b}$ = 18.3 Hz, H-15b), 1.58, 1.35, 0.98, 0.85 (each 3H, s, 4 x CH₃)

### 5.3.4 Physical data of compound VIII (99)

*6α-acetoxyobacunol acetate*

Yield: < 7 mg

Infrared Spectrum:

$\nu_{max}$ (NaCl): 1747 cm⁻¹ and 1705 cm⁻¹ (ester and lactone carbonyl stretchings), 1384 cm⁻¹ (C(CH₃)₂ stretching), 1233 cm⁻¹ (C-O stretching)

¹H NMR:

δ(ppm): 7.39 (2H, br m, H-21, H-23), 6.55 (1H, d, $J$ = 11.6 Hz, H-1), 6.31 (1H, d, $J$ = 1.5 Hz, H-22), 5.93 (1H, d, $J$ = 11.6 Hz, H-2), 5.58 (1H, s, H-17), 5.14 (1H, dd, $J_{6,7}$ = 2.6 Hz, $J_{5,6}$ = 12.3 Hz, H-6β), 4.90 (1H, d, $J_{6,7}$ = 2.6 Hz, H-7β), 3.60 (1H, s, H-15), 2.63 (1H, d, $J_{5,6}$ = 12.3 Hz, H-5), 2.55 (1H, dd, $J$ = 5.8 Hz, 12.0 Hz, H-9), 2.12, 1.98 (each 3H, s, -O-COCH₃), 1.53, 1.44, 1.41, 1.24, 1.23 (each 3H, s, CH₃)

## 5.4. Extractives from *Turraea holstii*

The plant material was collected in the Wesu area, Taita Taveta District, Coast Province, Kenya. The plant was identified by Mr G. Mwachala of the National Museums of Kenya Herbarium and a voucher specimen was deposited in the same department.

The ground root bark (360 g) and stembark (296 g) of *Turraea holstii* were extracted with methanol (section 5.1.7.3) resulting in 19.1 g and 17.9 g respectively of crude methanol extracts. Each extract was partitioned between methylene chloride and methanol-water mixture. A methanol:water (70:30) mixture was added to each extract. The mixture was then extracted with methylene chloride (3x 100 ml). The organic fractions were combined and evaporated under reduced pressure to afford 7.8 g and 5.7 g of rootbark and stembark extracts. Chromatographic separation of the rootbark extract yielded compounds IX, X, XI, XIII, XIV, XVI and XVII. Separation of the stembark extract yielded compounds X, XI, XII, XV and XVIII.

### 5.4.1 Physical data of compound IX (113)

*1,3-Diacetylvilasinin*

Yield: 84.3 mg

Melting Point: 127-129$^0$C (lit. value 157-158$^0$C), (lit. value 128-131$^0$C)[111]

Optical Rotation:

$[\alpha]_D$= -6.3$^0$ (c, 0.97 in CH$_3$Cl), (lit. value -6.5$^0$)

Mass Spectrum:

EIMS *m/z* 512.2780([M]$^+$, C$_{30}$H$_{40}$O$_7$, req. 512.2772), 452 ([M-60]$^+$, loss of CH$_3$COOH), 392 ([M-60-60]$^+$, loss of 2x CH$_3$COOH)

$^{13}$C NMR:

Table 4 (page 128, Appendix)

**$^1$H NMR:**

δ(ppm): 7.35 (1H, d, $J$ = 1.8Hz, H-23), 7.24 (1H, s, H-21), 6.26 (1H, d, $J$ = 1.8Hz, H-22), 5.59 (1H, dd, $J_{15,16\beta}$= 1.7Hz, $J_{15,16\alpha}$= 3.6Hz, H-15), 4.90 (1H, t, $J$ = 2.8Hz, H-3β), 4.66 (1H, t, $J$ = 2.8Hz, H-1β), 4.17 (1H, d, $J_{6,7}$= 3.1Hz, H-7β), 4.13 (1H, dd, $J_{6,7}$= 3.1Hz, $J_{5,6}$= 12.2Hz, H-6β), 3.57 (2H, br m, 2H-28), 2.82 (1H, dd, $J_{17,16\alpha}$= 7.4Hz, $J_{17,16\beta}$= 10.7Hz, H-17), 2.66 (1H, d, $J_{5,6}$= 12.2Hz, H-5), 2.55 (2H, m, H-9, H-16β), 2.41 (1H, ddd, $J_{15,16\alpha}$= 3.6Hz, $J_{17,16\alpha}$= 7.4Hz, $J_{16\alpha,16\beta}$= 16.0Hz, H-16α), 2.09 (2H, m, 2H-2), 2.01, 1.98 (each 3H, s, -O-COCH$_3$), 1.18, 1.09, 0.96, 0.83 (each 3H, s, CH$_3$)

### 5.4.2 Physical data of compound X (83)

*Toonacilin*

Yield: 75 mg

Melting Point: 119-121$^0$C (lit. value 118-119$^0$C)

Optical Rotation:

$[\alpha]_D$ = +68.5$^0$ (c, 0.897 in CHCl$_3$), (lit. value +69$^0$)

Infrared Spectrum:

$v_{max}$ (NaCl): 2935 cm$^{-1}$ (-CH$_3$, >CH$_2$ stretching), 1748 cm$^{-1}$ (ester carbonyl stretching), 1679cm$^{-1}$ (α,β-unsaturated carbonyl stretching), 1237 cm$^{-1}$ (C-O stretching)

Mass Spectrum:

EIMS *m/z* 554.2500 ([M]$^+$, C$_{31}$H$_{38}$O$_7$, req. 554.2514), 494 ([M-60]$^+$, loss of CH$_3$COOH), 434 ([M-60-60]$^+$, loss of 2x CH$_3$COOH)

**$^{13}$C NMR:**

Table 4 (page 128, Appendix)

¹H NMR:

δ(ppm): 7.41 (1H, d, $J$=10.5Hz, H-1), 7.26 (1H, d, $J$=1.8Hz, H-23),7.10 (1H, s, H-21), 6.13 (1H, d, $J$=10.5Hz, H-2), 6.12 (1H, d, $J$=1.8Hz, H-22), 5.68 (1H, d, $J_{11,12}$=10.8Hz, H-12β), 5.50 (1H, dd, $J_{9,11}$=7.2Hz, $J_{11,12}$=10.8Hz, H-11β), 5.28 (1H, br s, H-30a), 5.18 (1H, br s, H-30b), 3.85 (1H, br s, H-15), 3.64 (3H, s, OC-O-CH₃), 3.02 (1H, dd, $J$=7.0Hz, $J$=10.7Hz, H-17), 2.95 (1H, d, $J_{9,11}$=7.2Hz, H-9), 1.87, 1.67 (each 3H, s, O-COCH₃), 1.06, 0.95, 0.94, 0.88 (each, 3H, s, CH₃)

### 5.4.4 Physical data of compound XI (114)

*11β,12α-Diacetoxycedrelone*

Yield: 83.0 mg

Melting Point: 137-139⁰C

Infrared Spectrum:

ν$_{max}$ (NaCl): 3408 cm⁻¹ (O-H stretching), 1749 cm⁻¹ (ester carbonyl stretching), 1685 cm⁻¹ (α,β-unsaturated carbonyl stretching), 1628 cm⁻¹ (intramolecularly H-bonded ketone), 1238 cm⁻¹ (C-O stretching)

Mass Spectrum:

EIMS $m/z$ 538.2207 ([M]⁺, C₃₀H₃₄O₉, req. 538.2202), 478 ([M-60]⁺, loss of CH₃COOH)

¹³C NMR:

Table 4 (page 128, Appendix)

¹H NMR:

δ(ppm): 7.29 (1H, d, $J$=1.7Hz, H-23), 7.10 (1H, s, H-21), 6.91 (1H, d, $J$=10.0Hz, H-1), 6.45 (1H, s, OH), 6.13 (1H, d, $J$=10.0Hz, H-2), 5.36 (1H, br s, H-11a), 5.19 (1H, br s, H-12b), 3.89 (1H, br s, H-15), 2.90

(1H, dd, $J$=7.0Hz, $J$=11.3Hz, H-17), 2.29 (1H, dd, $J$=7.0Hz, $J$=13.5Hz, H-16b), 1.97 (1H, dd, $J$=11.3Hz, $J$=13.5Hz, H-16a), 2.15, 1.94 (each 3H, s, -O-COCH₃), 1.55, 1.48, 1.35, 1.27, 0.77 (each 3H, s, CH₃)

### 5.4.5 Physical data of compound XII (118)

*12α-acetoxyneotrichilenone*

Yield: 79 mg

Melting Point: 126-128°C

Optical Rotation:

$[\alpha]_D$ = +37.3⁰ (c, 0.134 in CHCl₃)

Infrared Spectrum:

$v_{max}$ (NaCl): 3468 cm⁻¹ (O-H stretching), 2969 cm⁻¹ (saturated C-H stretching), 1728 cm⁻¹ (ester carbonyl stretching), 1666 cm⁻¹ (α,β-unsaturated carbonyl stretching), 1249 cm⁻¹ (C-O stretching)

Mass Spectrum :

EIMS $m/z$ 468.2518 ([M]⁺, C₂₈H₃₆O₆, req. 46.2512), 408 ([M-60]⁺, loss of CH₃COOH)

¹³C NMR:

Table 4 (page 128, Appendix)

¹H NMR:

δ(ppm): 7.39 (1H, d, $J$=1.6Hz, H-23), 7.34 (1H, s, H-21), 6.89 (1H, d, $J$=10.1Hz, H-1), 6.30 (1H, d, $J$=1.6Hz, H-22), 5.83 (1H, d, $J$=10.1Hz, H-2), 5.18 (1H, t, $J$=3.4Hz, H-12β), 3.90 (1H, br s, $W_{1/2}$=7.6Hz, H-7β), 3.46 (1H, t, $J$=10.0Hz, H-17), 2.90 (1H, s, H-14), 2.53 (2H, br d, $J$=10,0Hz, 2H-16), 2.00 (3H, s, -O-COCH₃), 1.13 (3H, s, CH₃), 1.07 (9H, s, 3x CH₃), 0.79 (3H, s, CH₃)

### 5.4.6 Physical data of compound XIII (119)

*12α-Acetoxy-7-Acetyl-1,2-dihydroneotrichilenone*

Yield: 55.6 mg

Melting Point: 107-109°C

Optical Rotation:

$[\alpha]_D = +21.6^0$ (c, 0.12 in $CH_3Cl$)

Infrared Spectrum:

$\nu_{max}$ (NaCl): 2966 cm$^{-1}$ (-CH$_3$, >CH$_2$ stretching), 1743 cm$^{-1}$, 1738 cm$^{-1}$ (ester carbonyl stretchings), 1251 cm$^{-1}$ (C-O stretching)

Mass Spectrum:

EIMS *m/z* 512.2784 ([M]$^+$, $C_{30}H_{40}O_7$, req. 512.2772), 452 ([M-60]$^+$, loss of $CH_3COOH$, 392 ([M-60-60]$^+$, loss of 2x $CH_3COOH$)

$^{13}C$ NMR:

Table 5 (page 129, Appendix)

$^1H$ NMR:

δ(ppm): 7.38 (1H,d, *J*=1.8Hz, H-23), 7.29 (1H, s, H-21), 6.28 (1H, d, *J*=1.8Hz, H-22), 5.12 (1H, t, *J*=3.2Hz, H-12β), 4.93 (1H, br m, $W_{1/2}$=5.8Hz, H-7β), 3.42 (1H, t, *J*=10.0Hz, H-17), 2.52 (1H, s, H-14), 2.4-2.6 (4H, m, 2H-16, H-9,H-5), 2.11, 2.03 (each 3H, s, -O-COCH$_3$), 1.11 (3H, s, CH$_3$), 0.99 (9H, s, 3xCH$_3$), 0.76 (3H, s, CH$_3$)

### 5.4.7 Physical data of compound XIV (120)

*12α-Acetoxy-1,2-dihydroneotrichilenone*

Yield: 11.5 mg

¹H NMR:

δ(ppm): 7.38 (1H,d, $J$=1.7Hz, H-23), 7.29 (1H, s, H-21), 6.29 (1H, d,

$J$=1.7Hz, H-22), 5.13 (1H, t, $J$=3.4Hz, H-12β), 3.87 (1H, br m,

$W_{1/2}$=7.6Hz, H-7β), 3.45 (1H, t, $J$=10.0Hz, H-17), 2.87 (1H, s, H-14),

2.52 (2H, br d, $J$=10.0Hz, H-17) 1.99 (3H, s, -O-COCH₃),1.09, 1.04,

0.94, 0.78 (each 3H, s, CH₃)

### 5.4.7.1 Acetylation of compound XIV

Compound XIV was acetylated in the usual manner and yielded an

amorphous gum (compound XIVa). The ¹H NMR spectrum of compound

XIVa was the same as that of compound XIII.

¹H NMR:

(cf compound XIII)

### 5.4.8 Physical data of compound XV (121)

*11β-Acetoxy-7-Acetyl-12α-hydroxy-1,2-dihydroneotrichilenone*

Yield: 53.7 mg

Melting Point: 131-133°C

¹³C NMR:

Table 5 (page 129, Appendix)

¹H NMR:

δ(ppm): 7.38 (1H, d, $J$=1.7Hz, H-23), 7.11 (1H, s, H-21), 6.15 (1H, d,

$J$=1.7Hz, H-22), 5.45 (1H, br m, H-11α), 4.90 (1H, br m, H-7β), 3.88

(1H, t, $J$=10.0Hz, H-17), 3.83 (1H, br s, H-12β), 2.58 (1H, s, H-14),

2.51 (4H, m, 2H-16, 2H-2), 2.18, 2.09 (each 3H, s, -O-COCH3), 1.92 (1H, br s, H-9), 1.39, 1.12, 1.01, 0.98, 0.83 (each 3H, s, CH₃)

### 5.4.8.1 Acetylation of compound XV

Acetylation of compound XV (13 mg) was carried out using acetic anhydride in pyridine at room temperature, as described in section 7.2.1.1

Yield: 10 mg

¹H NMR:

δ(ppm): 7.39 (1H, d, *J*=1.7Hz, H-23), 7.20 (1H, s, H-21), 6.23 (1H, d, *J*=1.7Hz, H-22), 5.46 (1H, br m, H-11α), 5.19 (1H, d, *J*=3.3Hz, H-12β), 4.92 (1H, br m, H-7β), 3.94 (1H, t, *J*=10.0Hz, H-17), 2.57 (1H, s, H-14), 2.17, 2.10, 2.06 (each 3H, s, O-COCH₃), 1.41, 1.13, 1.02, 0.99, 0.72 (each 3H, s, CH₃)

### 5.4.9 Physical data of compound XVI (122)

*12-O-Methylnimbolinin B*

Yield: 97mg

Melting Point: 121-123°C

Optical Rotation:

[α]_D= -62.5⁰ (c, 0.28 in CH₃Cl)

Infrared Spectrum:

ν_max(NaCl): 1747 cm⁻¹ and 1736 cm⁻¹ (ester carbonyl stretchings), 1658 cm⁻¹ (C=C stretching), 1265 cm⁻¹, 1241 cm⁻¹ and 1060 cm⁻¹ (C-O stretchings), 756 cm⁻¹ (olefinic C-H deformation)

¹³C NMR:

Table 5 (page 129, Appendix)

**¹H NMR:**

δ(ppm): 7.26 (1H, d, $J$=1.5Hz, H-23), 7.22 (1H, s, H-21), 6.97 (1H, qq, $J$=1.5Hz, $J$=7.2Hz, H-3'), 6.35 (1H, d, $J$=1.5Hz, H-22), 5.70 (1H, d, $J$=2.8Hz, H-7β), 4.92 (1H, t, $J$=2.6Hz, H-3b), 4.88 (1H, d, $J$=7.8Hz, H-15), 4.71 (1H, t, $J$=2.6Hz, H-1b), 4.58 (1H, br m, H-12), 4.05 (1H, dd, $J$=2.8Hz, $J$=12.8Hz, H-6b), 3.47 (2H, d, $J$= 8.9Hz, 2H-28), 3.30 (1H, d, $J$=8.3Hz, H-17), 3.15 (1H, br d, $J$=9.8Hz, H-9), 3.04 (3H, s, -O-Me), 2.77 (1H, d, $J$=12.8Hz, H-5), 2.35 (1H, m, H-16a), 2.16-2.19 (2H, m, 2H-2), 1.98 (6H, s, 3H-5', -O-COCH₃), 1.88 (3H, s, -O-COCH3), 1.81 (3H, d, $J$=7.2Hz, 3H-4'), 1.71 (3H, s, 3H-18), 1.69 (1H, m, H-11b), 1.61 (1H, m, H-11a), 1.57 (1H, m, H-16b), 1.40 (3H, s, 3H-30), 1.15 (3H, s, 3H-29), 0.96 (3H, s, 3H-19)

### 5.4.10 Physical data of compound XVII (124)

Yield: 492 mg

Melting Point: 125-127°C

Optical Rotation:

$[\alpha]_D$= -30.1 (c, 1.1 in CH₃Cl)

Infrared Spectrum:

$\nu_{max}$(NaCl): 3467 cm⁻¹ (O-H stretching), 1677 cm⁻¹ (α,β-unsaturated six membered ring ketone stretching), 1091 cm⁻¹ and 1045 cm⁻¹ (C-O stretchings)

Mass Spectrum:

EIMS $m/z$ 498.3353 ([M-32]⁺, C31H46O5, req. 498.3345, loss of CH₃OH), 466 ([M-32-32]⁺, loss of 2x CH₃OH), 395 (C₂₆H₃₅O₃), 326 (C₂₂H₃₀O₂)

$^{13}$C NMR:

Table 6 (page 131, Appendix)

$^1$H NMR:

δ(ppm): 7.11 (1H, d, *J* = 10.2Hz, H-1), 5.80 (1H, d, *J* = 10.2Hz, H-2), 5.46 (1H, dd, *J* = 1.6Hz, *J* = 3.5Hz, H-15), 4.79 (1H, d, *J* = 3.6Hz, H-21), 4.21 (1H, m, H-23), 3.96 (1H, br m, $W_{1/2}$= 6.6Hz, H-7β), 3.34 (1H, d, *J* = 6.7Hz, H-24), 3.34 (3H, s, O-CH₃), 3.21 (3H, s, O-CH₃), 2.56 (1H, d, *J* = 6.7Hz, 24-OH), 2.38 (1H, dd, *J* = 4.0Hz, *J* = 11.3Hz, H-5), 1.22 (3H, s, CH₃), 1.14 (6H, s, 2x CH₃), 1.13, 1.10, 1.07, 1.03 (each 3H, s, CH₃)

### 5.4.10.1 Acetylation of compound XVII

Acetylation of compound XVII (20 mg) was carried out using acetic anhydride in pyridine at room temperature, as described in section 7.2.1.1. Compound XVIIa was obtained.

Yield: 16.5 mg

$^{13}$C NMR:

Table 6 (page 131, Appendix)

$^1$H NMR:

δ(ppm): 7.14 (1H, d, *J* = 10.3Hz, H-1), 5.82 (1H, d, *J* = 10.3Hz, H-2), 5.21 (2H, m, H-7β, H-15), 4.99 (1H, d, *J* = 3.4Hz, H-24), 4.77 (1H, d, *J* = 3.5Hz, H-21), 4.18 (1H, m, H-23), 3.34 (3H, s, O-CH₃), 3.18 (3H, s, O-CH₃), 2.11 (3H, s, -OCOCH₃), 1.92 (3H, s, -OCOCH₃), 1.23, 1.20, 1.15, 1.14 (each 3H, s, CH₃), 1.05 (6H, s, 2x CH₃), 1.01 (3H, s, CH₃)

## 5.4.10.2 Sarret oxidation of compound XVII

Chromium trioxide (250mg) was added to a magnetically stirred mixture of pyridine (10ml) and methylene chloride (10ml). The flask was fitted with a calcium chloride drying tube and stirring was continued for 15 minutes. A solution of compound XVII (40mg) in methylene cholride was added. the mixture was stirred for 3 hours at room temperature. The mixture was poured into water (15ml) and the aqueous solution was extracted with ether (3x 15ml) and the organic fractions were combined. Compound XVIIb was obtained.

Yield: 27mg

$^{13}$C NMR:

Table 6 (page 131, Appendix)

$^1$H NMR:

$\delta$(ppm): 7.12 (1H, d, $J = 10.3$Hz, H-1), 5.92 (1H, dd, $J = 1.8$Hz, $J = 3.5$Hz, H15), 5.87 (1H, d, $J = 10.3$Hz, H-2), 4.79 (1H, d, $J = 3.7$Hz, H-21), 4.17 (1H, m, H-23), 3.33 (1H, d, $J = 7.6$Hz, H-24), 3.33 (3H, s, OMe), 3.20 (3H, s, OMe), 2.85 (1H, t, $J = 4.4$Hz, H-5), 1.35, 1.32, 1.21 (each 3H, s, CH$_3$), 1.12 (6H, s, 2xCH$_3$), 1.08, 1.00 (each 3H, s, CH$_3$)

## 5.4.10.3 Jones oxidation of compound XVII

40 mg of compound XVII was dissolved in 10 ml of acetone and Jones' reagent (2ml) added to the mixture which was stirred for 1 hour at room temperature and then extracted with chloroform. Evaporation of the chloroform yielded a white crystalline compound (compound XVIIc)

Yield: 29 mg

Melting Point:

$^{13}$C NMR:

Table 6 (page 131, Appendix)

$^1$H NMR:

δ(ppm): 7.12 (1H, d, $J$ = 10.3Hz, H-1), 5.88 (1H, d, $J$ = 10.3Hz, H-2), 5.89 (1H m, H-15), 5.03 (1H, dd, $J$ = 5.3Hz, $J$ = 10.1Hz, H-23), 4.96 (1H, d, $J$ = 2.7Hz, H-21), 3.36 (3H, s, OMe), 3.23 (3H, s, OMe), 2.85 (1H, t, $J$ = 14.4Hz, H-5), 2.33-2.40 (3H, m, H-6a, H-22a, H-20), 2.03-2.16 (3H, m, H-6b, 2H-16), 1.36 (3H, s, CH$_3$), 1.32 (6H, s, 2xCH$_3$), 1.30, 1.13, 1.09, 1.01 (each 3H, s, CH$_3$)

## 5.4.11 Physical data of compound XVIII (125)

Yield:

Melting Point: 131-133$^0$C

Optical Rotation:

$[\alpha]_D$= -30.1 (c, 1.1 in CH$_3$Cl)

Mass Spectrum:

EIMS $m/z$ 466.3081 ([M-32-18]$^+$, loss of CH$_3$OH and H$_2$O), 395 (C$_{26}$H$_{35}$O$_3$), 326 (C$_{22}$H$_{30}$O$_2$)

$^{13}$C NMR:

Table 6 (page 131, Appendix)

$^1$H NMR:

δ(ppm): 7.11 (1H, d, $J$ = 10.2Hz, H-1), 5.80 (1H, d, $J$ = 10.2Hz, H-2), 5.46 (1H, dd, $J$ = 1.6Hz, $J$ = 3.5Hz, H-15), 4.80 (1H, d, $J$ = 3.7Hz, H-21), 4.42 (1H, dd, $J$ = 4.7Hz, $J$ = 5.8Hz, H-23), 3.96 (1H, t, $J$ = 2.6Hz, H-7β), 3.51 (1H, br m, H-24), 3.34 (3H, s, O-CH$_3$), 2.80 (1H, br m, 24-OH), 2.38 (1H, dd, $J$ = 4.2Hz, $J$ = 11.0Hz, H-5), 1.63, 1.54 (each 3H, s, CH$_3$), 1.14 (6H, s, 2x CH$_3$), 1.10, 1.07, 1.04 (each 3H, s, CH$_3$)

# APPENDIX

## <u>Tables</u>

## Table 2
### $^{13}$C NMR data of compounds I, II, III and IV
(Chemical shifts in δ(ppm), and multiplicities are included in brackets)

| carbon | compound I | compound II | compound III | compound IV | 23-Hydroxyprimulagenin A |
|--------|-----------|-------------|--------------|-------------|----------------------------|
| 1  | 38.7 (t)   | 38.7 (t)   | 38.4 (t)   | 38.4 (t)   | 38.4 (t)   |
| 2  | 27.4 (t)[a]| 27.4 (t)   | 27.0 (t)   | 27.2 (t)   | 26.4 (t)   |
| 3  | 79.0 (d)   | 79.0 (d)   | 77.2 (d)   | 77.2 (d)   | 76.7 (d)   |
| 4  | 38.9 (s)   | 38.9 (s)   | 41.9 (s)   | 38.7 (s)   | 41.9 (s)   |
| 5  | 55.3 (d)   | 55.3 (d)   | 49.9 (d)   | 55.2 (d)   | 49.9 (d)   |
| 6  | 18.3 (t)   | 18.3 (t)   | 18.4 (t)   | 18.3 (t)   | 18.5 (t)   |
| 7  | 34.2 (t)[b]| 34.3 (t)   | 34.1 (t)   | 32.6 (t)   | 32.6 (t)   |
| 8  | 40.9 (s)   | 40.8 (s)   | 40.8 (s)   | 39.3 (s)   | 40.0 (s)   |
| 9  | 50.4 (d)   | 50.5 (d)   | 50.5 (d)   | 47.6 (d)   | 47.0 (d)   |
| 10 | 37.2 (s)   | 37.2 (s)   | 37.1 (s)   | 37.1 (s)   | 36.9 (s)   |
| 11 | 20.8 (t)   | 20.8 (t)   | 20.7 (t)   | 22.9 (t)   | 23.4 (t)   |
| 12 | 25.2 (t)   | 25.5 (t)   | 25.5 (t)   | 122.6 (d)  | 122.6 (d)  |
| 13 | 37.3 (d)   | 38.7 (d)   | 38.6 (d)   | 143.6 (s)  | 143.1 (s)  |
| 14 | 42.7 (s)   | 42.6 (s)   | 42.6 (s)   | 41.6 (s)   | 41.6 (s)   |
| 15 | 27.0 (t)[a]| 29.3 (t)   | 29.2 (t)   | 27.7 (t)   | 34.7 (t)   |
| 16 | 29.2 (t)[c]| 28.8 (t)   | 28.8 (t)   | 23.4 (t)   | 74.9 (d)   |
| 17 | 47.8 (s)   | 59.3 (s)   | 59.3 (s)   | 46.5 (s)   | 40.6 (s)   |
| 18 | 47.8 (d)   | 48.1 (d)   | 48.0 (d)   | 41.0 (d)   | 42.8 (s)   |
| 19 | 48.8 (d)   | 47.5 (d)   | 47.5 (d)   | 45.9 (t)   | 47.0 (t)   |
| 20 | 150.5 (s)  | 149.7 (s)  | 149.7 (s)  | 30.7 (s)   | 30.4 (s)   |
| 21 | 29.7 (t)[c]| 29.9 (t)   | 29.9 (t)   | 33.8 (t)   | 35.4 (t)   |
| 22 | 34.0 (t)[b]| 33.2 (t)   | 3302 (t)   | 32.4 (t)   | 26.8 (t)   |
| 23 | 28.0 (q)   | 28.0 (q)   | 71.9 (t)   | 28.1 (q)   | 71.9 (t)   |
| 24 | 15.4 (q)   | 15.3 (q)   | 11.2 (q)   | 15.5 (q)   | 11.5 (q)   |
| 25 | 16.1 (q)[d]| 15.9 (q)   | 15.9 (q)   | 15.3 (q)   | 16.1 (q)   |
| 26 | 16.0 (q)   | 16.1 (q)   | 16.5 (q)   | 17.1 (q)   | 17.2 (q)   |
| 27 | 60.5 (t)   | 14.3 (q)   | 14.3 (q)   | 25.9 (q)   | 27.3 (q)   |
| 28 | 14.8 (q)   | 206.7 (d)  | 206.7 (d)  | 182.9 (s)  | 70.8 (t)   |
| 29 | 109.7 (t)  | 110.2 (t)  | 110.2 (t)  | 33.1 (q)   | 32.8 (q)   |
| 30 | 19.1 (q)   | 19.0 (q)   | 19.0 (q)   | 23.6 (q)   | 25.5 (q)   |

[a-d] Values in a vertical column may be interchanged.

## Table 3
## $^{13}$C NMR data of compounds V, VI, VII and VIII
(Chemical shifts in δ(ppm), and multiplicities are included in brackets)

| carbon | compound V | compound VI | compound VII |
|--------|-----------|-------------|--------------|
| 1 | 39.4 (t) | 42.1 (t) | 73.4 (d) |
| 2 | 19.1 (t) | 19.0 (t) | 41.7 (t) |
| 3 | 42.2 (t) | 46.1 (t) | 169.6 (s) |
| 4 | 33.3 (s) | 32.9 (s) | 83.8 (s) |
| 5 | 54.8 (d) | 47.0 (d) | 51.8 (d) |
| 6 | 22.6 (t) | 29.3 (t) | 34.1 (t) |
| 7 | 36.0 (t) | 73.3 (d) | 173.2 (s) |
| 8 | 137.3 (s) | 139.6 (s) | 145.7 (s) |
| 9 | 50.7 (d) | 46.1 (d) | 43.4 (d) |
| 10 | 38.3 (s)[a] | 38.4 (s)[a] | 47.6 (s) |
| 11 | 18.8 (t) | 18.3 (t) | 24.0 (t) |
| 12 | 34.6 (t) | 34.3 (t) | 32.0 (t) |
| 13 | 37.4 (s)[a] | 37.4 (s)[a] | 41.7 (s) |
| 14 | 128.5 (d) | 133.9 (d) | 81.3 (s) |
| 15 | 149.2 (d) | 148.3 (d) | 35.7 (t) |
| 16 | 110.0 (t) | 110.6 (t) | 170.0 (s) |
| 17 | 26.0 (q) | 25.6 (q) | 79.3 (d) |
| 18 | 33.8 (q) | 33.4 (q) | 13.8 (q) |
| 19 | 22.1 (q) | 22.0 (q) | 22.6 (q)[a] |
| 20 | 15.0 (q) | 14.2 (q) | 120.6 (s) |
| 21 | | | 142.7 (d)[b] |
| 22 | | | 110.0 (d) |
| 23 | | | 141.0 (d)[b] |
| 28 | | | 28.8 (q)[a] |
| 29 | | | 22.1 (q)[a] |
| 30 | | | 111.9 (t) |
| COOMe | | | 52.3 (q) |

[a,b] Values in a vertical column may be interchanged.

## Table 4
## $^{13}$C NMR data of compounds IX, X, XI and XII
**(Chemical shifts in δ(ppm), and multiplicities are included in brackets)**

| carbon | compound IX | compound X | compound XI | compound XII |
|---|---|---|---|---|
| 1 | 72.2 (d) | 152.3 (d) | 150.1 (d) | 157.1 (d) |
| 2 | 27.2 (t) | 125.7 (d) | 127.8 (d) | 126.1 (d) |
| 3 | 71.7 (d) | 203.9 (s) | 202.9 (s) | 204.8 (s) |
| 4 | 42.3 (s) | 46.2 (s) | 53.4 (s)[a] | 45.6 (s)[a] |
| 5 | 39.6 (d) | 45.1 (d) | 134.1 (s) | 38.8 (d) |
| 6 | 72.8 (d) | 31.3 (t) | 140.9 (s) | 25.4 (t) |
| 7 | 74.0 (d) | 174.2 (s) | 196.9 (s) | 69.8 (d) |
| 8 | 45.8 (s) | 136.8 (s) | 45.5 (s)[a] | 39.0 (s)[a] |
| 9 | 33.6 (d) | 52.9 (d) | 42.3 (d)[b] | 43.8 (d) |
| 10 | 39.2 (s) | 45.2 (s) | 48.4 (s)[a] | 44.2 (s)[a] |
| 11 | 15.2 (t) | 71.3 (d) | 72.5 (d) | 23.5 (t) |
| 12 | 32.9 (t) | 75.2 (d) | 78.7 (d) | 72.4 (d) |
| 13 | 47.4 (t) | 42.0 (s) | 40.0 (s)[a] | 42.2 (s)[a] |
| 14 | 159.9 (s) | 77.3 (s) | 77.2 (s) | 60.5 (d) |
| 15 | 120.7 (d) | 59.6 (d) | 15.1 (d) | 219.7 (s) |
| 16 | 34.3 (t) | 33.5 (t) | 32.0 (t) | 43.0 (t) |
| 17 | 51.5 (d) | 37.8 (d) | 41.6 (d)[b] | 38.2 (d) |
| 18 | 21.2 (q) | 13.6 (q) | 21.1 (q)[c] | 21.3 (q)[b] |
| 19 | 26.2 (q) | 21.3 (q) | 26.8 (q)[c] | 21.4 (q)[b] |
| 20 | 124.5 (s) | 122.2 (s) | 121.7 (s) | 122.4 (s) |
| 21 | 139.7 (d) | 140.3 (d) | 140.4 (d) | 140.3 (d) |
| 22 | 111.1 (d) | 111.2 (d) | 111.0 (d) | 110.6 (d) |
| 23 | 142.6 (d) | 142.5 (d) | 142.8 (d) | 143.3 (d) |
| 28 | 77.9 (t) | 23.0 (q)[a] | 24.7 (q)[c] | 27.4 (q)[b] |
| 29 | 15.4 (q) | 22.7 (q)[a] | 22.6 (q)[c] | 18.6 (q)[b] |
| 30 | 19.5 (q) | 120.8 (t) | 15.6 (q)[c] | 19.1 (q)[b] |
| OCOCH₃ | 170.3 (s) | 170.1 (s) | 169.3 (s) | 170.3 (s) |
| " | 170.0 (s) | 169.7 (s) | 168.9 (s) | - |
| OCOCH₃ | 21.1 (q) | 20.6 (q) | 20.9 (q) | 21.2 (q) |
| " | 21.2 (q) | 20.6 (q) | 21.2 (q) | - |
| COOCH₃ | - | 52.1 (q) | - | - |

[a,b] Values in a vertical column may be interchanged

## Table 5
## $^{13}$C NMR data of compounds XIII, XV and XVI
(Chemical shifts in δ(ppm), and multiplicities are included in brackets)

| carbon | compound XIII | compound XV | compound XVI |
|---|---|---|---|
| 1 | | 38.7 (t) | 71.6 (d) |
| 2 | | 33.5 (t) | 27.5 (t) |
| 3 | | 216.2 (s) | 70.9 (d) |
| 4 | | 39.3 (s)[a] | 42.4 (s) |
| 5 | | 47.1 (d) | 40.1 (d) |
| 6 | | 22.9 (t) | 72.1 (d) |
| 7 | | 74.4 (d) | 75.0 (d) |
| 8 | | 45.2 (s)[a] | 45.1 (s) |
| 9 | | 44.5 (d) | 35.8 (d) |
| 10 | | 37.2 (s)[a] | 40.7 (s) |
| 11 | | 73.8 (d) | 38.1 (t) |
| 12 | | 68.9 (d) | 98.0 (d) |
| 13 | | 46.4 (s)[a] | 140.6 (s) |
| 14 | | 58.1 (d) | 142.9 (s) |
| 15 | | 217.9 (s) | 76.5 (d) |
| 16 | | 42.5 (t) | 31.4 (t) |
| 17 | | 37.0 (d) | 46.6 (d) |
| 20 | | 122.8 (s) | 129.3 (s) |
| 21 | | 139.9 (d) | 138.9 (d) |
| 22 | | 110.6 (d) | 110.4 (d) |
| 23 | | 143.4 (d) | 142.8 (d) |
| OCOCH$_3$ | | 170.0 (s) | |
| " | | 169.4 (s) | |
| CH$_3$ | | 33.5 (q) | |
| " | | 26.5 (q) | |
| " | | 21.9 (q) | |
| " | | 21.3 (q) | |
| " | | 20.9 (q) | |
| " | | 19.6 (q) | |
| " | | 17.7 (q) | |

[a] Values in a vertical column may be interchanged.

## Table 6
### $^{13}$C NMR data of compounds XVII, XVIIa, XVIIb, XVIIc and XVIII
(Chemical shifts in δ(ppm), and multiplicities are included in brackets)

| carbon | XVII | XVIIa | XVIIb | XVIIc | XVIII |
|--------|------|-------|-------|-------|-------|
| 1 | 158.2 (d) | 158.2 (d) | 156.5 (d) | 156.5 (d) | 161.6 (d) |
| 2 | 125.5 (d) | 125.5 (d) | 126.0 (d) | 126.0 (d) | 125.5 (d) |
| 3 | 205.2 (s) | 204.6 (s) | 203.5 (s) | 203.6 (s) | 205.1 (s) |
| 4 | 44.2 (s)$^a$ | 42.7 (s)$^a$ | 44.7 (s)$^a$ | 44.7 (s)$^a$ | 44.8 (s)$^a$ |
| 5 | 46.1 (d) | 46.2 (d) | 52.5 (d) | 52.6 (d) | 45.9 (d) |
| 6 | 24.2 (t) | 23.8 (t) | 36.2 (t)$^b$ | 36.2 (t)$^b$ | 24.2 (t) |
| 7 | 71.5 (d) | 74.4 (d) | 209.5 (s) | 209.6 (s) | 71.5 (d) |
| 8 | 44.8 (s)$^a$ | 44.2 (s)$^a$ | 52.6 (s)$^a$ | 52.6 (s)$^a$ | 44.2 (s)$^a$ |
| 9 | 36.7 (d) | 38.3 (d) | 44.8 (d) | 44.8 (d) | 36.7 (d) |
| 10 | 40.2 (s) | 39.9 (s)$^a$ | 39.6 (s)$^a$ | 39.6 (s)$^a$ | 40.2 (s)$^a$ |
| 11 | 16.3 (t) | 16.5 (t) | 17.3 (t) | 17.4 (t) | 16.3 (t) |
| 12 | 34.8 (t)$^b$ | 34.8 (t)$^b$ | 33.8 (t) | 34.1 (t) | 32.5 (t)$^b$ |
| 13 | 46.9 (s) | 46.8 (s) | 47.4 (s)$^a$ | 47.4 (s)$^a$ | 46.9 (s) |
| 14 | 161.6 (s) | 159.2 (s) | 152.8 (s) | 152.7 (s) | 161.6 (s) |
| 15 | 119.6 (d) | 118.6 (d) | 126.0 (d) | 126.0 (d) | 119.6 (d) |
| 16 | 35.5 (t) | 35.1 (t) | 35.5 (t)$^b$ | 35.8 (t)$^b$ | 35.8 (t) |
| 17 | 57.8 (d) | 57.8 (d) | 57.9 (d) | 57.1 (d) | 57.7 (d) |
| 20 | 44.5 (d) | 46.1 (d) | 44.7 (d) | 47.1 (d) | 44.5 (d) |
| 21 | 109.2 (d) | 108.4 (d) | 109.1 (d) | 108.9 (d) | 109.5 (d) |
| 22 | 32.5 (t)$^b$ | 33.2 (t)$^b$ | 35.3 (t)$^b$ | 35.4 (t)$^b$ | 34.7 (t)$^b$ |
| 23 | 75.1 (d) | 74.9 (d) | 75.1 (d) | 76.9 (d) | 74.6 (d) |
| 24 | 76.2 (d) | 75.4 (d) | 76.3. (d) | 211.7. (s) | 77.9 (d) |
| 25 | 77.2 (s) | 76.2 (s) | 77.2 (s) | 81.7 (s) | 72.3 (s) |
| -CH₃ | 27.5 (q) | 27.3 (q) | 27.9 (q) | 27.8 (q) | 30.0 (q) |
| " | 27.1 (q) | 27.0 (q) | 26.8 (q) | 26.7 (q) | 27.5 (q) |
| " | 21.6 (q) | 22.6 (q) | 21.6 (q) | 22.5 (q) | 27.1 (q) |
| " | 21.5 (q) | 21.5 (q) | 21.2 (q) | 22.2 (q) | 27.0 (q) |
| " | 20.1 (q) | 21.3 (q) | 21.0 (q) | 21.4 (q) | 21.5 (q) |
| " | 19.6 (q) | 20.0 (q) | 20.1 (q) | 21.0 (q) | 19.6 (q) |
| " | 18.9 (q) | 19.0 (q) | 18.4 (q) | 18.5 (q) | 18.9 (q) |
| -OCH₃ | 55.5 (q) | 55.4 (q) | 55.5 (q) | 55.6 (q) | 55.6 (q) |
| " | 49.2 (q) | 49.5 (q) | 49.2 (q) | 51.0 (q) | - |
| -OCOCH₃ | - | 170.8 (s) | - | - | - |

| " | - | 170.2 (s) | - | - | - |
|---|---|---|---|---|---|
| -OCOCH₃ | - | 21.2 (q) | - | - | - |
| " | - | 21.1 (q) | - | - | - |
|  |  |  |  |  |  |

[a,b] Values in a vertical column may be interchanged.

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# PART *B*

## INVESTIGATIONS INTO

## ENAMINE CHEMISTRY

# TABLE OF CONTENTS

# ACKNOWLEDGEMENTS

# ABBREVIATIONS

| | |
|---|---|
| Ac- | acetate |
| br- | broad resonance |
| br s- | broad singlet |
| br m- | broad multiplet |
| $c$- | concentration |
| $^{13}$C NMR- | carbon-13 nuclear resonance spectroscopy |
| COSY- | correlated nuclear resonance spectroscopy |
| d- | doublet |
| dd- | doublet of doublets |
| DEPT- | distortionless enhancement by polarisation transfer |
| dt- | doublet of triplets |
| $^{1}$H NMR- | proton ($^{1}$H) nuclear resonance spectroscopy |
| HETCOR- | heteronuclear shift correlation nuclear resonance spectroscopy |
| Hz- | hertz |
| FTIR- | Fourier transformed infrared spectroscopy |
| m- | multiplet |
| Me- | methyl |
| ppm- | parts per million |
| q- | quartet |
| s- | singlet |
| t- | triplet |
| Tig- | tiglate |

# CHAPTER 1

## ENAMINE CHEMISTRY- A BRIEF REVIEW

### Index

# 1. Introduction

The term "enamine" was first introduced by Wittig and Blumenthal[1] in 1927 to indicate an unsaturated amine structure **(1)** analogous to an enolic form **(2)** of a ketone.
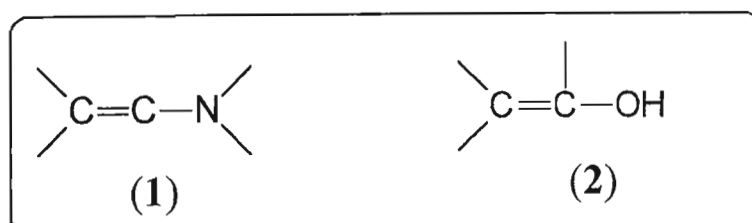


The simplest enamine of a carbonyl compound was prepared in 1921 by Meyer and Hopf[2] who made N,N-dimethylvinylamine **(4)** (the enamine of acetaldehyde) by pyrolysis of choline **(3)**.
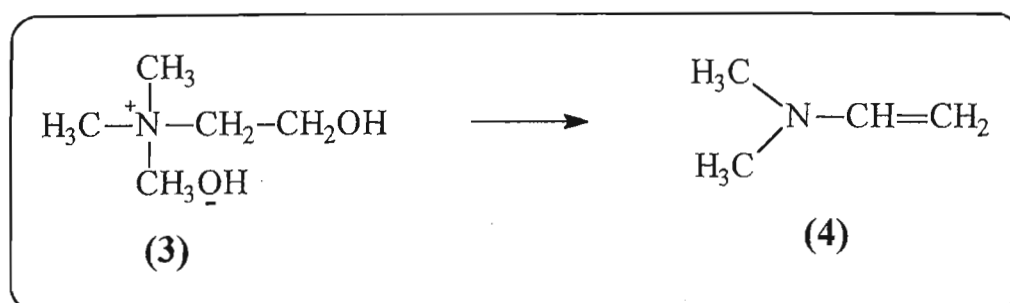


This is not a general method and it remained for Mannich and Davidson[3] to provide the synthesis which, with some modification, is still the one used today: viz. the reaction of an aldehyde or ketone with a secondary amine in the presence of a dehydrating agent. Opitz and co-workers[4] have shown that if the nitrogen is primary or secondary then the imine (Schiff base) tautomer **(5)** is the most favourable, unless stabilized by further conjugation with, usually, a carbon-carbon or carbon-oxygen double bond.
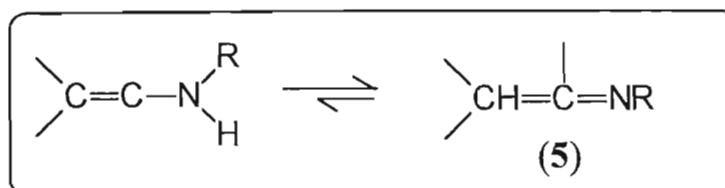
$$>\!\!C\!\!=\!\!C\!\!-\!\!N\overset{R}{\underset{H}{\diagup}} \quad \rightleftharpoons \quad >\!\!CH\!\!=\!\!C\!\!=\!\!NR$$

(5)

The orbital interaction between the nitrogen lone pair and the π-electrons of the double bond results in another canonical form (6) of the enamine.

$$>\!\!\overset{..}{N}\!\!-\!\!\underset{\alpha}{C}\!\!=\!\!\underset{\beta}{C}\!\!< \quad \longleftrightarrow \quad >\!\!\overset{+}{N}\!\!=\!\!\underset{\alpha}{C}\!\!-\!\!\underset{\beta}{\overset{-}{C}}\!\!<$$

(6)
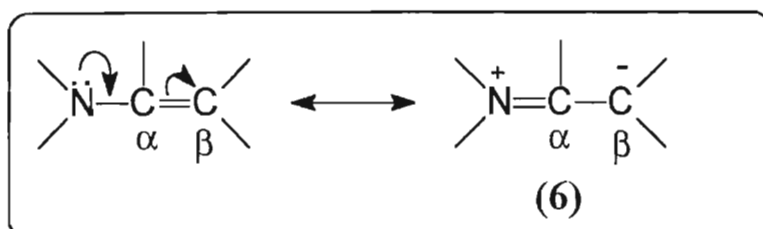
This polarization has been clearly demonstrated by the nuclear magnetic resonance spectra of the various cyclohexanone enamines[5,6]. The proton attached to the β-carbon atom is markedly shielded and consequently appears upfield ($\delta 4.1 - 4.6$) compared with the olefinic proton signal of cyclohexene ($\delta 5.6$). The increased density at the β-carbon atom results in the electrophilic attack occurring at the β-carbon atom of the enamine to give an iminium cation (7) or, to a varying extent, at the nitrogen atom to give the enammonium cation(8)

$$>\!\!\overset{-}{C}\!\!-\!\!C\!\!=\!\!\overset{+}{N}\!\!< \quad \xrightarrow{E^+} \quad >\!\!\overset{E}{\underset{|}{C}}\!\!-\!\!C\!\!=\!\!\overset{+}{N}\!\!<$$

(7)

$$>\!\!C\!\!=\!\!C\!\!-\!\!N\!\!< \quad \xrightarrow{E^+} \quad >\!\!C\!\!=\!\!C\!\!-\!\!\overset{+}{N}\!\!\overset{E}{\diagup}$$

(8)
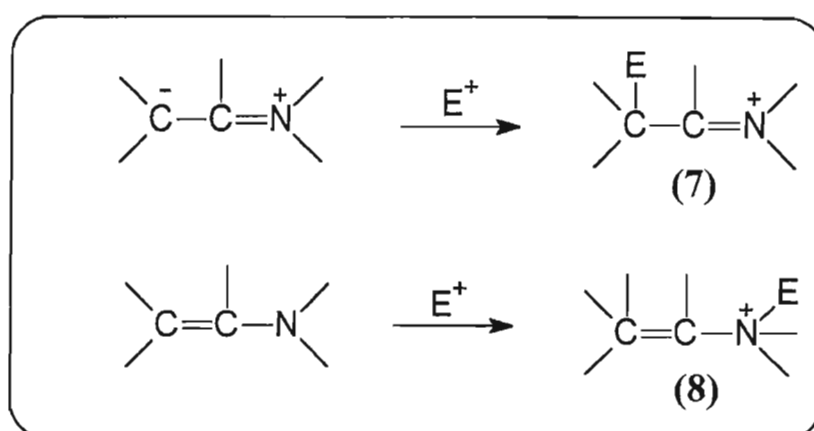
Enamine reactivity had been known since 1883 when first Collie[7] and then Benary[8] and later Robinson[9] described the C-alkylation or acylation of aminocrotonic esters, but it is without doubt the pioneering work of Gilbert

Stork which made the preparation and reaction of enamines to receive appreciable attention. Reviews[10, 11] of enamines and their chemistry have been published. To date, the most comprehensive reviews have been those of Hickmott[12, 13, 14].

The realization of the full potential of enamines as reactive intermediates in organic synthesis really only came to light after Stork *et al.*[15] published their report on enamines derived from aldehydes and ketones. The Stork alkylation or acylation, as these reaction have become known[16], refer to the C-alkylation or acylation of a carbonyl carbon *via* an enamine intermediate. Further publications by Stork *et al.* covered the factors affecting structure and reactivity[17], spectroscopic data, preparations, tosylation of enamines[18], the synthesis of bridged bicyclic compounds and ring enlargement[19], heterocyclic synthesis[20, 21], natural product synthesis[22,23] and the formation and reaction of metallo-enamines[24].

The major advantages of the enamine reaction over other carbon-carbon bond forming reactions in complex syntheses, is that it offers a mild and relatively reliable method by which mono-alkylation or acylation can be achieved without the production of O-substituted and to some extent di-substituted products.

Research has shown the Stork reaction to be critically dependent on the conditions under which the reaction is carried out. The pathway of the reaction can be affected by changes in the solvent, amine moiety in the enamine, temperature, molar proportions of reagents, etc. Because such changes produce such a diversity of products, Hickmott[12] has proposed an extension to the definition of the Stork reaction to include "conversion of an

aldehyde or ketone into a C-alkylated, acylated, carbocyclic or heterocyclic derivative by reaction of an electrophile with an enamine intermediate".

## 1.2 Structure and Reactivity

Mixtures of structurally isomeric enamines are usually obtained from unsymmetrical ketones such as 2-alkylcyclanones and branched chain acyclic ketones. Johnson *et al.*[25] have shown that these isomers undergo rapid acid catalysed equilibrium, but no thermal or base catalysed equilibration was observed, even after a week in pyrrolidine at $80^0$.

The isomer distribution varies with the amine used. The pyrrolidine enamines of 2-methyl-cyclohexanones exist as <10% in the more substituted form **(9t)**, whereas morpholine enamines occur from 30-65% in the more substituted form **(9t)**[25]. Optically active (+)-methylpiperidine gave only the more substituted enamine **(9t)**. Similar differences exist between pyrrolidine and morpholine enamines of 2-alkoxycyclohexanones and 3-alkoxy-*trans*-decal-2-ones[26].

**SCHEME 1**
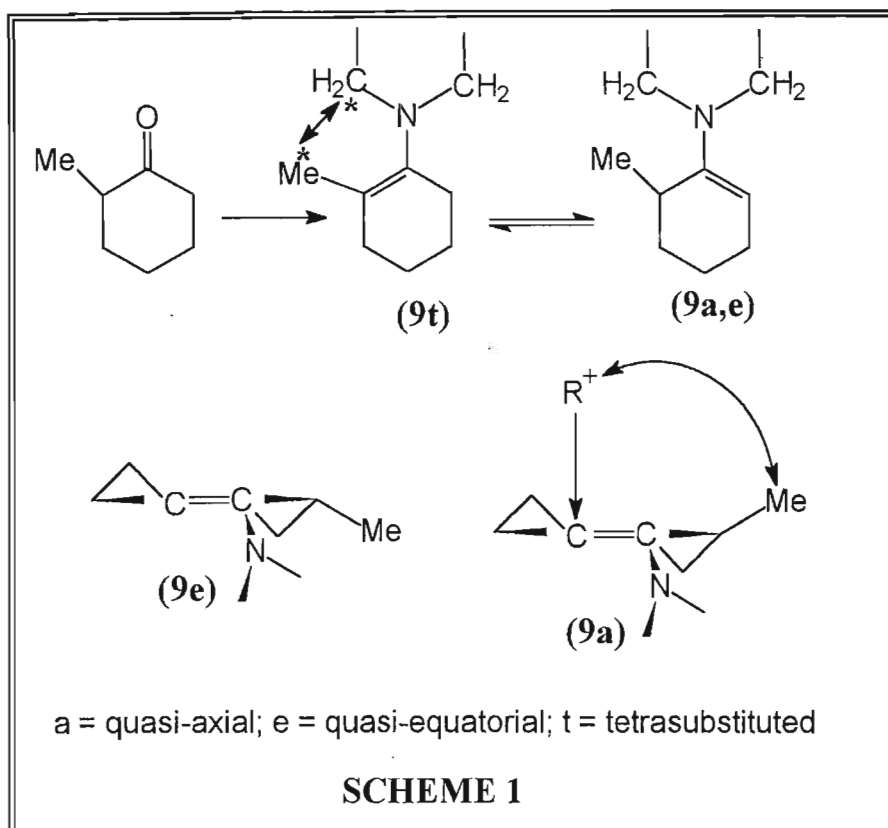
The isomer containing the tetrasubstituted double bond **(9t)** would be destabilised by severe steric interaction ($A^{1,3}$ strain)[27] between the methyl and amine -methylene groups if these were coplanar. In the ground state these steric interactions could be reduced by rotation about the N-C ($sp^2$) bond. However, such a rotation would reduce the orbital interaction between the nitrogen lone pair and the $\pi$ electrons of the double bond. Clearly a balance between these two conflicting requirements must exist in order to minimize the energy of **(9t)**. The less substituted form of the enamine **(9a,e)** can exist in two possible conformations in which the methyl substituent can either be quasi-axially **(9a)** or quasi-equatorially **(9e)** oriented. Isomer **(9e)** is destabilized by less severe allylic interactions ($A^{1,2}$ strain)[27]. Thus the most stable isomer is **(9a)**.

This results in significant consequences. $\alpha,\alpha$-Disubstitution of ketones *via* their enamines is not usually observed since for maximum orbital interaction

between the nitrogen lone pair and the $\pi$-electrons of the double bond, the methylene group of the amine moiety and the methyl group of the ketone (starred groups in **(9t)**) must become coplanar[27]. A product-like transition state would be destabilized by the increasing $A^{1,3}$-interactions. The net result is that further alkylation or acylation therefore takes place at the less substituted $\alpha'$-position of the ketone. However, the role of substitution is reduced because of the developing 1,3-diaxial interactions, as shown in **(9a)**, or the developing steric interactions associated with a twist or boat conformation if electrophilic attack occurs from the equatorial direction. Hickmott considers axial attack of **(9e)** to be a higher energy process (than axial attack of **(9a)**) owing to developing $A^{1,3}$-interactions in the transition state. Available evidence suggests that equatorial attack is also less favourable.

A further consequence of allylic strain is that, when an equatorial 2-substituent cannot be converted into the axial conformer by ring-flipping, as with *cis*-4-t-butyl-2-methylcyclohexanone, then epimerisation to the *trans* isomer occurs. This gives a method for the conversion of a more stable *cis*-diequatorial 2,4-disubstituted cyclohexanone to the less stable *trans*-isomer[25]. Allylic strain also accounts for the fact that the enamines of 3-methylcyclohexanones exist mainly as isomer **(11)** in Scheme 2[29].

Ax = axial attack of electrophile R+
Eq = equatorial attack

**SCHEME 2**
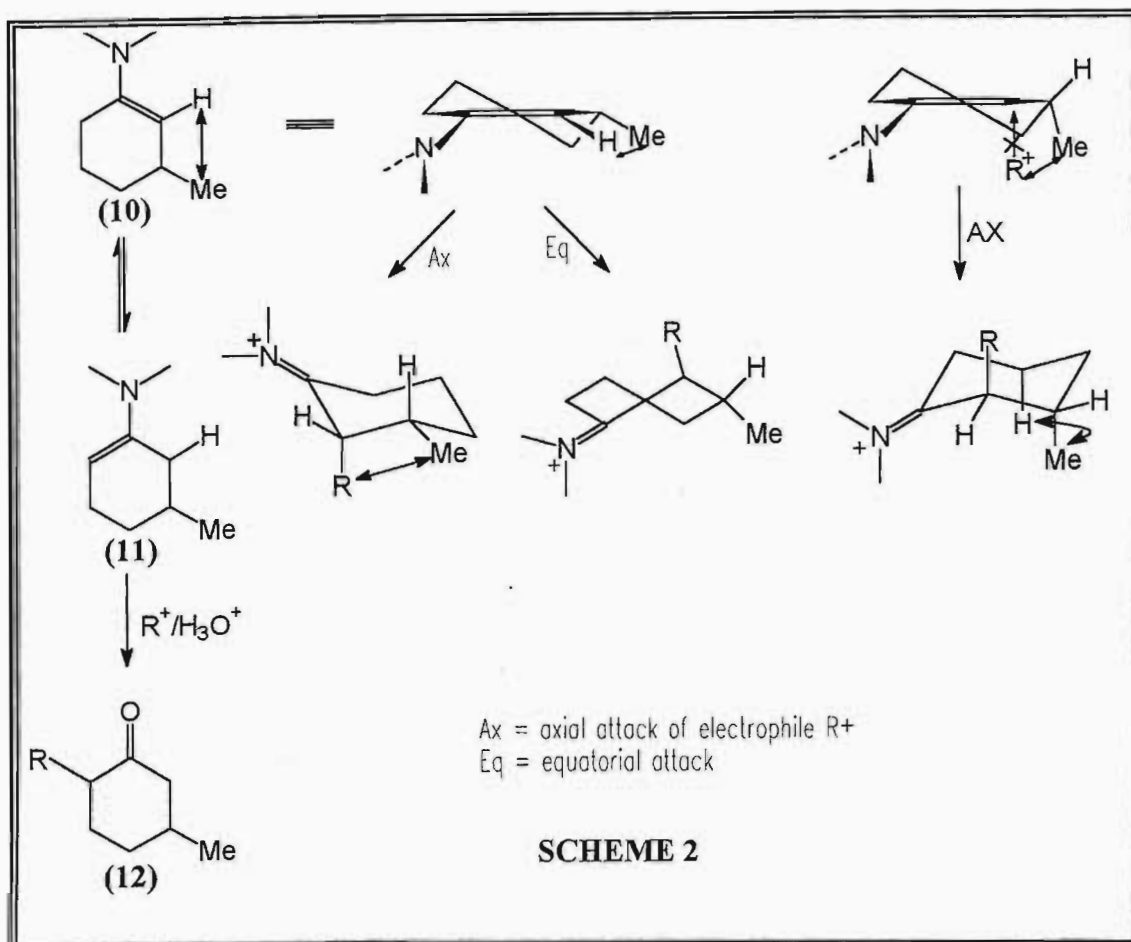
Provided that the activation energy of the reaction is larger than the barrier to isomer interconversion it follows from the Curtin Hammet principle[30], that the product distribution must reflect transition state energies rather than the ground state isomer population.

Alkylation[31], acylation[32] and halogenation[33] of the enamine mixture (10), (11) has been shown to give the 2-substituted-5-methylcyclohexanone as the major product. This clearly demonstrates the rapid equilibrium between the enamine isomers. In the case of product-like transition states, formation of 2-substituted-3-methylcyclohexanones will be inhibited by developing steric interactions as shown in Scheme 2.

Acyclic enamines show the same trend. Pocar *et al.*[34] have demonstrated that the less substituted enamine is the more reactive isomer and that interconversion of enamine isomers may or may not occur during a reaction depending upon the reagent and experimental conditions used.

The major factors which affect the reactivity of an enamine are the amine moiety and the degree of substitution at the α- and β-positions. Reactivity at C-β is increased by α-alkyl substituents owing to increased electron density at C-α by hyperconjugative and inductive effects, provided that steric interactions do not prevent or reduce the lone pair interactions[35]. Conversely, the steric and electronic effects of β-substituents decrease the reactivity at C-β owing to the decreased electron density at this position. The order of reactivity is therefore normally

$$R_2NC(R) = CH_2 > R_2NC(R) = CHR > R_2NCH = CHR > R_2NCH = CR_2$$

Cyclic and acyclic ketone enamines are therefore more readily C-alkylated than aldehyde enamines. In the case of enamines from cyclic ketones, spectroscopic evidence suggests that reactivity may vary with ring size in the order $5>12>8>6>7$[6].

## 1.3 Imines

Spectroscopic studies [36, 37] of imine-enamine tautomerism have shown that, unless the enamine is further stabilized by conjugation with an unsaturated system,[36, 38, 39, 40] the equilibrium is usually almost completely in favour of the imine form. With few exceptions, such as the t-butylamine imine of

cyclohexanone ($\delta_{=CH}$ 4.6), signals due to the enamine tautomer cannot be observed in the proton NMR spectra of imines. The existence of this tautomerism has been proved by the fact that the enamine form reacts with a variety electrophilic reagents at the β-position of the enamine (α-position to the original carbonyl function).

Pfau and Ribieri[41] reported the production of three C-alkylated products (15)-(17) in the reaction of N-isopropylidene isopropylamine (13) with dimethyl maleate.



**SCHEME 3**
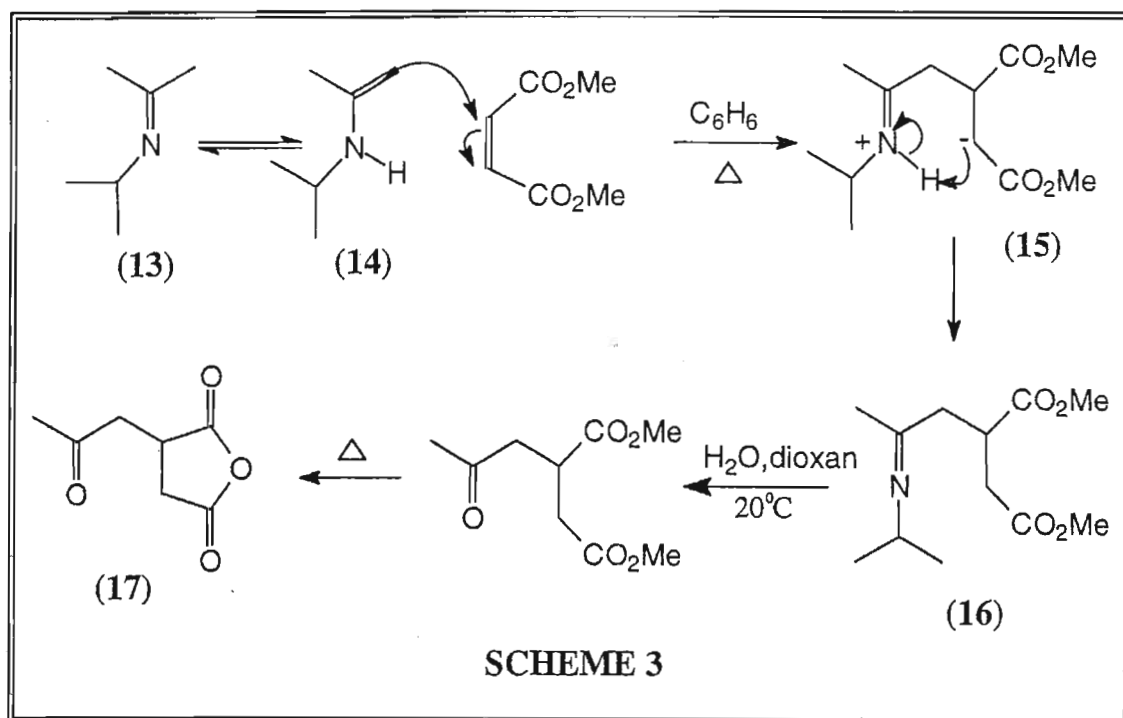
In methanol, no olefinic signals were observed in the proton NMR spectrum of (13). However, two signals at δ 1.94(3H) and δ 2.01(3H), corresponding to the two magnetically non-equivalent methyl groups attached to the imine double bond, were observed. In deuterated methanol, these signals disappeared rapidly. This means that although the imine form (13) predominated, the six hydrogens rapidly exchange *via* the enamine form

(14). This observation is extremely useful as it provides an *in situ* preparation of the enamine of acetone.

## 1.4 Regioselectivity

Atta-ur-Rahman *et al.*[42] claimed that only N-alkylation of N-isopropylidenecyclohexylamine (18) occurred with methyl acrylate (19). Pfau *et al.*[43] have shown that in fact several reactions occur, particularly C-alkylation yielding (20), (21), (24), (25), and that no N-alkylation occurs whatsoever (scheme 4).

R = cyclohexyl, R₁ = H, R₂ = Me

**SCHEME 4**
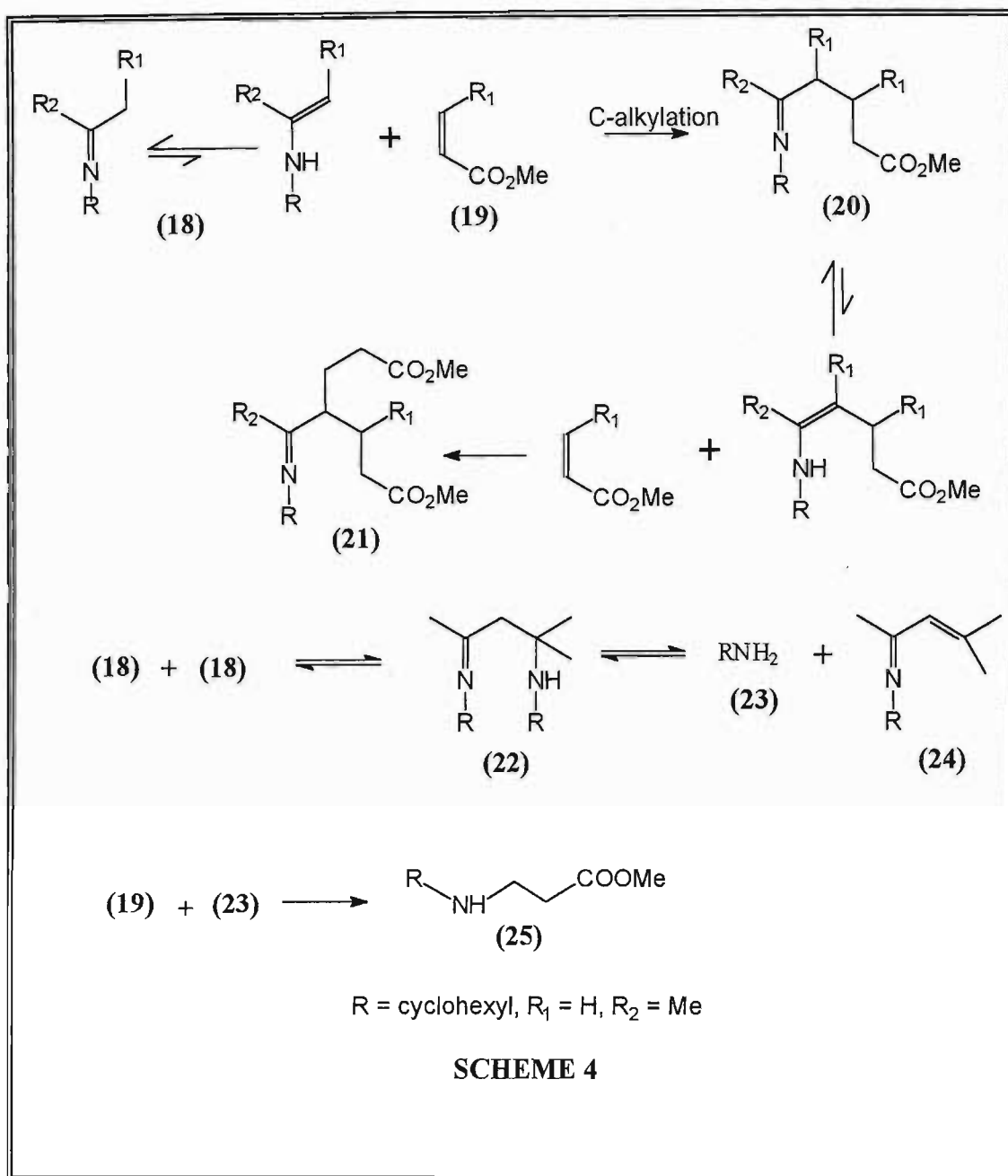
The α,α-bisalkylated product **(21)** was produced in 86% yield. This result is in contrast to the reaction of tertiary enamines derived from unsymmetrical ketones[12]. Both Hickmott *et al.*[44] and Pfau *et al.*[45] have shown that alkylation of imines of 2-methylcyclohexanone with electrophilic alkenes occurs at the more substituted position (C-2) of the derived secondary enamine tautomer to give α,α-disubstituted cyclic ketones preferentially on hydrolysis.

The underlying hypothesis was that the imine of 2-methylcyclohexanone (27) would be in equilibrium mainly with the more substituted secondary enamine (26) rather than the less substituted double bond isomer (28). The reason for this is that enamine (26) is stabilized over enamine (28) by the hyperconjugative interaction of the methyl group without incurring allylic destabilization between the methyl group and the α-methylene of the amine substituent (R).

Furthermore since there is no $A^{1,3}$ strain present in the imine (29), produced by the alkylation of enamine (26), and minimal $A^{1,3}$ strain in the transition state leading to it, it was predicted that alkylation would give mainly the 2,2-disubstituted cyclohexanone (31) on hydrolysis rather than the 2,6-disubstituted cyclohexanone (32)[44].

Hickmott and Brookes[46] reported their investigation into the alkylation of benzylamine or n-propylamine imines of acyclic ketones, butanone, pentan-2-one, pentan-3-one, 3-methylbutanone, 2-methylpentanone and 4-methylpentanone. The electrophilic alkenes used were acrylonitrile, methylacrylate and phenylvinylsulphone. The reaction was found to be sensitive to steric effects and as a result only mono-alkylation occurred. However, the reaction was found to be not as highly regioselective as the corresponding alkylation of the 2-substituted cyclohexanone imines.

Reagents : (i) $CH_2=CH-Z$ $(-H^+)$ ; (ii) $H_2O$ ; $Z = CO_2Me$, CN, $SO_2Ph$

**SCHEME 5**
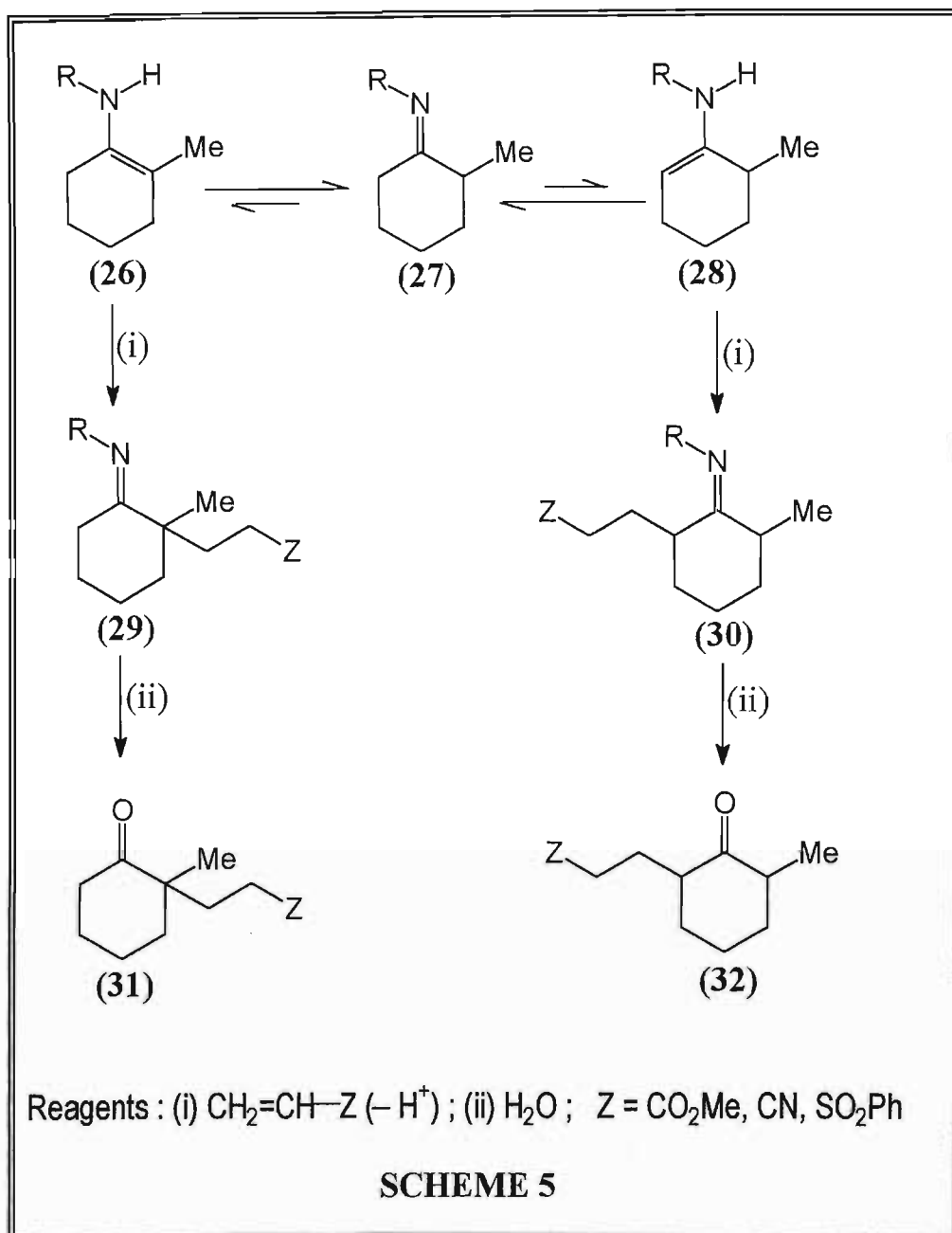
In the case of unsymmetrical acyclic imines, the regioselectivity of the reaction depended on the substituents present in the imine and, to a lesser extent, the alkylating agent. The reaction varied from 100% attack at the more substituted α-position to 70% attack at the less substituted α'-position depending upon the steric hindrance present and the stabilization of the competing secondary enamine tautomers.

Hickmott *et al.*[47] have reported a one-step synthesis (Scheme 6) of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]-octan-5-one (**35**) from acyclic precursors.
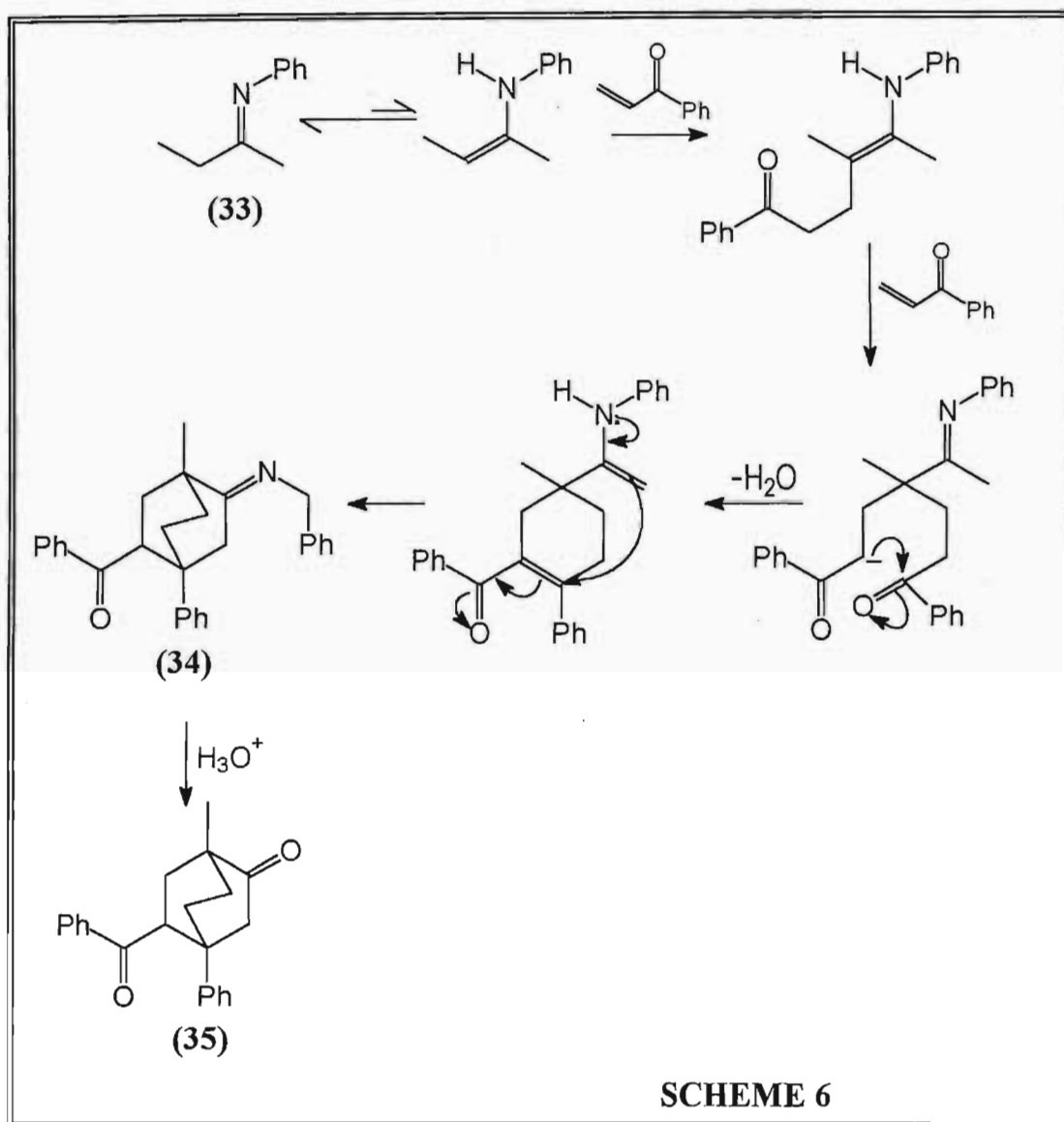


**SCHEME 6**

Two equivalents of phenyl vinyl ketone react three times with butanone imine (**33**), once at C-1 and twice at C-2. Four different carbon-carbon bonds are formed sequentially in this reaction (Scheme 6). As far as the authors are aware this reaction constitutes the first one-step synthesis of a bridged bicyclic system from acyclic precursors. This work has now been continued in this work in an attempt to prepare azatwistane derivatives from imine (**34**).

## 1.5 Dienamines

Dienamines are $\alpha,\beta$-$\gamma,\delta$-unsaturated amines and are usually prepared from $\alpha,\beta$- or $\beta,\gamma$-unsaturated ketones and secondary amines, under dehydrating conditions similar to those used in the preparation of simple enamines[48, 49].

Herr and Heyl[49] in 1953 reported the preparation of steroidal dienamines, removing the water formed by azeotropic distillation. However, one of the earlier preparations of a dienamine, was by Bowden *et al*[50] in 1946. They prepared 1-diethylaminobutadiene from crotonaldehyde and diethylamine at -10$^0$C in the presence of anhydrous potassium carbonate.

Condensation of secondary amines with $\alpha,\beta$-unsaturated ketones in the presence of p-toluenesulphonic acid is slower than that with corresponding saturated ketones. As observed for simple enamines, the rate of the reaction depends on the ketone and the amine used. Pyrrolidine, being more reactive than morpholine, requires a shorter reaction time, approximately 24 hours, compared to 1-6 days for morpholine dienamines. Satisfactory yields may be obtained by the azeotropic removal of water formed using a solvent such as benzene or toluene and a Dean and Stark head. Better yields are usually obtained when the condensate is passed, for an additional period, over molecular sieves[51, 52].

Depending on the conditions under which dienamines are prepared, they may exist as either cross-conjugated, non-conjugated or linear conjugated double bond isomers. Frequently, a mixture of isomers is obtained. For example, dienamines derived from $\Delta^{1,8a}$-2-octalones exist as mixtures of

mainly exocyclic (36) (60-100%; R' = H; R",R"'= H,Me) together with the linear endocyclic diene (37) (15-40%). The presence of a substituent (R') at C-3 quasi-axially oriented in order to reduce $A^{1,2}$ strain[53], reduces the proportion of the exocyclic isomer (36) owing to 1,3 diaxial interactions but the proportion is increased by an 8-methyl substituent due to hyperconjugative stabilization[54].



(36)  (37)  (38)
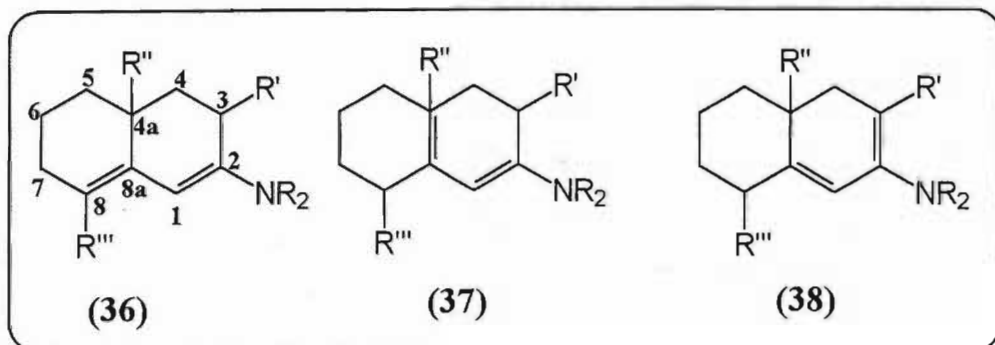
Contrary to previous reports[55, 56], Firrell observed that none of the cross-conjugated isomer (38) was present in the mixture and it was suggested that steric effects were responsible for this[54]. The exocyclic isomer is also favoured by the smaller deviation from coplanarity of the double bond system thus resulting in increased mesomeric stabilization. The proportion of the exocyclic isomer is increased when the dienamine is derived from pyrrolidine, since orbital interaction of the nitrogen lone pair would tend to enhance the mesomeric effect[57]. The chemical shifts and isomer distribution for the dienamines derived from $\Delta^{1,8a}$-2-octalones as reported by Firrell are shown in Table 1.

Like simple enamines, the reactions of dienamines are often critically dependent on the experimental conditions employed, the pathway of the reaction being influenced by changes in solvent, amine moiety, temperature and catalysts. The stereoselectivity and regioselectivity of dienamine

reactions may also be altered by changes in experimental conditions leading to a diversity of products.

## Table 1 : Dienamine preparations
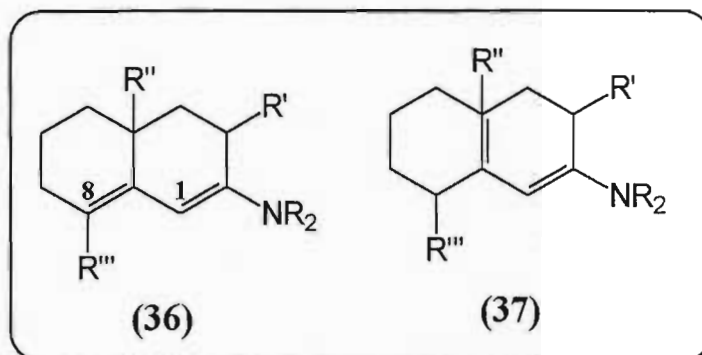


| KETONE | AMINE | (36) | | | (37) | |
|---|---|---|---|---|---|---|
| | | $H_8$ | $H_1$ | % | $H_1$ | % |
| $\Delta^{1,8a}$-2-octalone (R',R'',R'''= H) | M | 5.22 | 5.14 | 60 | 4.64 | 40 |
| $\Delta^{1,8a}$-2-octalone (R',R'',R'''= H) | P | 5.13 | 4.88 | 70 | 4.33 | 30 |
| 3-Me-$\Delta^{1,8a}$-2-octalone (R'',R'''= H;R' = Me) | M | 5.28 | 5.17 | 45 | 4.68 | 55 |
| 3-Me-$\Delta^{1,8a}$-2-octalone (R'',R'''= H;R' = Me) | P | 5.13 | 4.82 | 47 | 4.26 | 53 |
| 8-Me-$\Delta^{1,8a}$-2-octalone (R',R''= H;R''' = Me) | M | - | 5.48 | 85 | 4.83 | 15 |
| 8-Me-$\Delta^{1,8a}$-2-octalone (R',R''= H;R''' = Me) | P | - | 5.03 | 100 | | |
| 4a-Me-$\Delta^{1,8a}$-2-octalone (R',R'''= H;R'' = Me) | M | 5.24 | 5.16 | 100 | | |
| 4a-Me-$\Delta^{1,8a}$-2-octalone (R',R'''= H;R'' = Me) | P | 5.07 | 4.82 | 100 | | |

M = Morpholine     P = Pyrrolidine

It has been shown that alkylation of dienamines derived from α,β-unsaturated ketones with ethyl α-bromoacetate[58a], methyl iodide[59] and 1,3-dichlorobut-2-ene[58g] resulted in preferential reaction at the β-position. Examples in the literature of reaction at the β-position are relatively numerous, particularly the reaction of dienamines with alkylating agents[58,59]. In addition to the fact that the electron density is higher at the β-position, Stork[59] attributed the preference for β- over γ-alkylation of the dienamine to the lowering of the transition state energy by release of the halide counter anion in close proximity to the positively charged iminium ion. This is illustrated in Scheme 7.



**SCHEME 7**
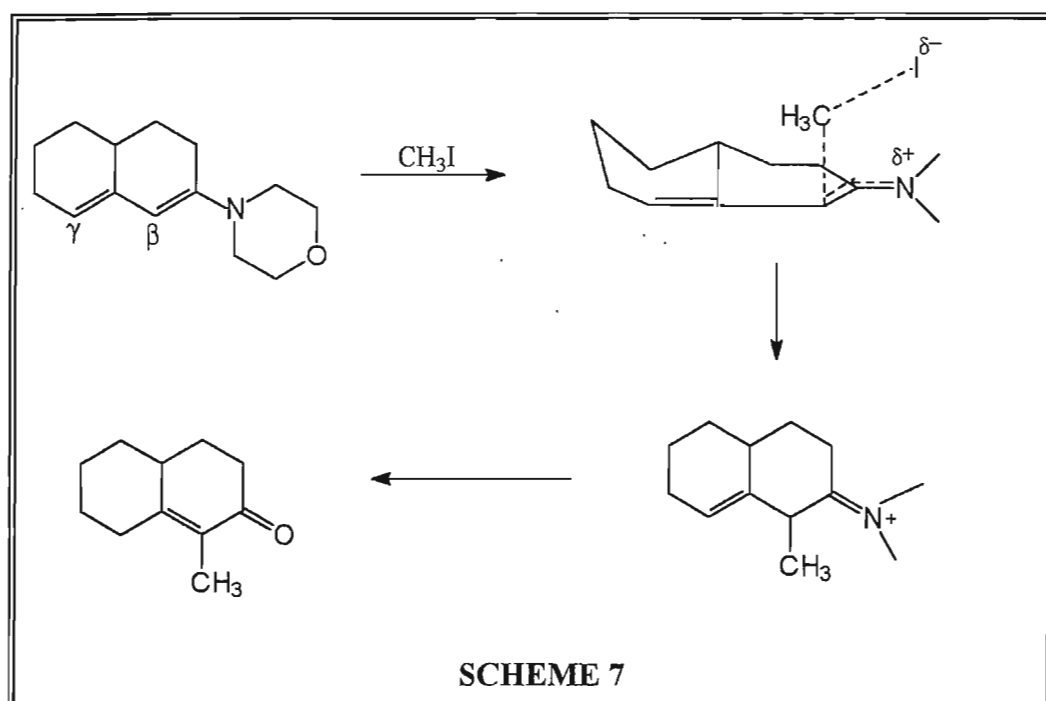
It has been shown that N-alkylation is favoured by low temperatures. Depending on the nucleophilic strength of the counter ion, the N-alkylated product could revert to starting materials at elevated temperatures leading to direct C-alkylation[60]. As mentioned earlier, the course of the reaction of dienamines with allylic halides depends on both the amine component and the allylic halide. The reaction of crotyl and cinnamyl bromides with the

pyrrolidine dienamine **(39)** gives predominantly or exclusively the products of direct C-alkylation **[(39)--(43)--(44)]**. The morpholine and piperidine dienamines react with crotyl chloride giving mainly **(45)**, and its $\Delta^5$-double bond isomer, whereas cinnamyl bromide give **(46)** (R'' = H) and **(46)** (R''= $CH_2CH=CHPh$) *via* double suprafacial [3,3] sigmatropic rearrangements **[(40)--(41)--(42)--(46)** (R'' = H)]. Deprotonation and repetition of this process gives **(46)** (R'' = $CH_2CH=CHPh$) (Scheme 8).

**SCHEME 8**
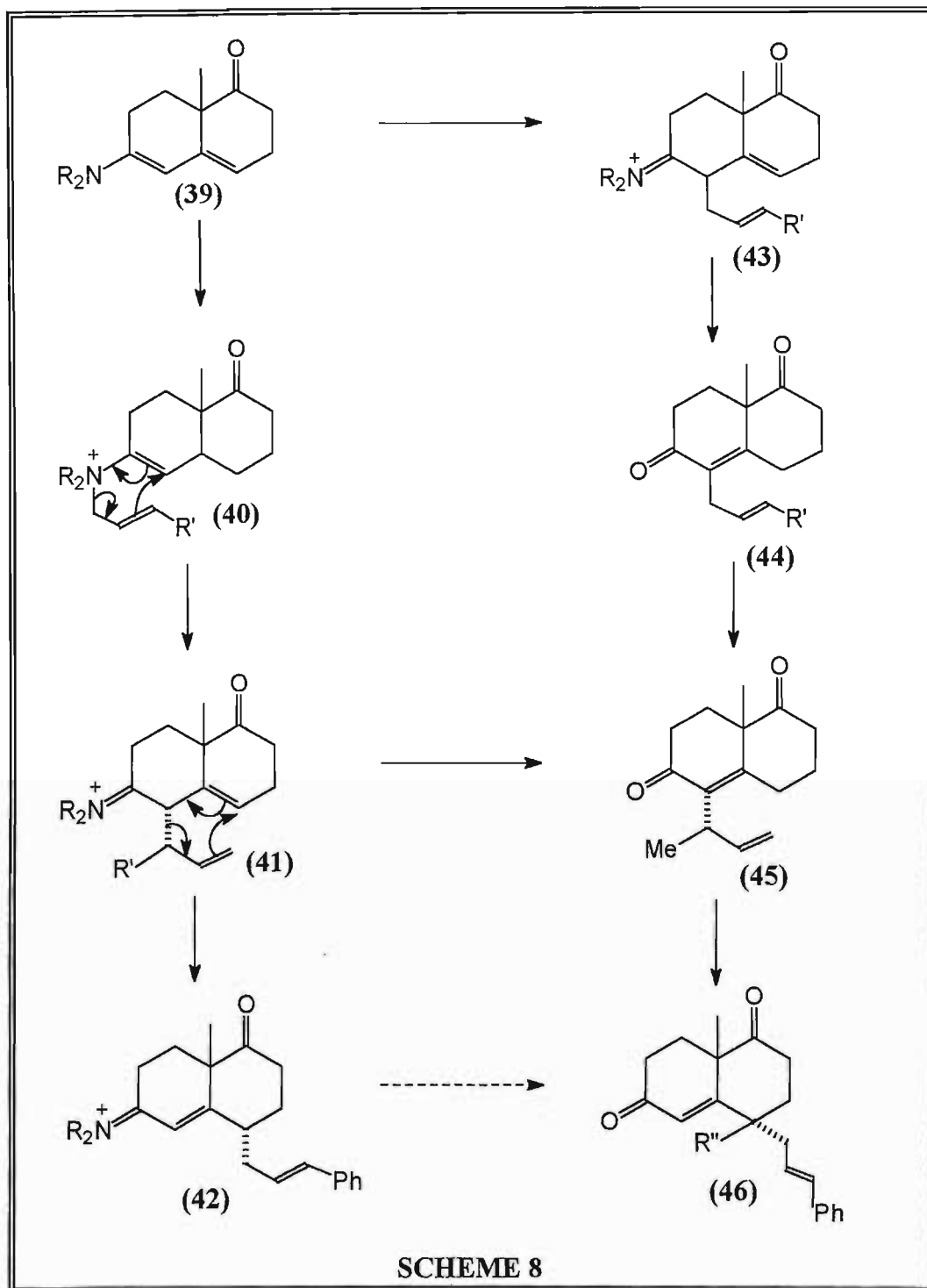
Similarly, the methylation and benzylation of the pyrrolidine dienamine of 3-methyl-$\Delta^{1,8a}$-2-octalone [a mixture of **(36)** and **(37)** (R", R''' = H; R' = Me)] gives only products derived from β-alkylation of the dienamine in both protic and aprotic solvents. However, the reaction with acrylonitrile and methyl acrylate has been shown to be solvent dependent[61] (Scheme 9).
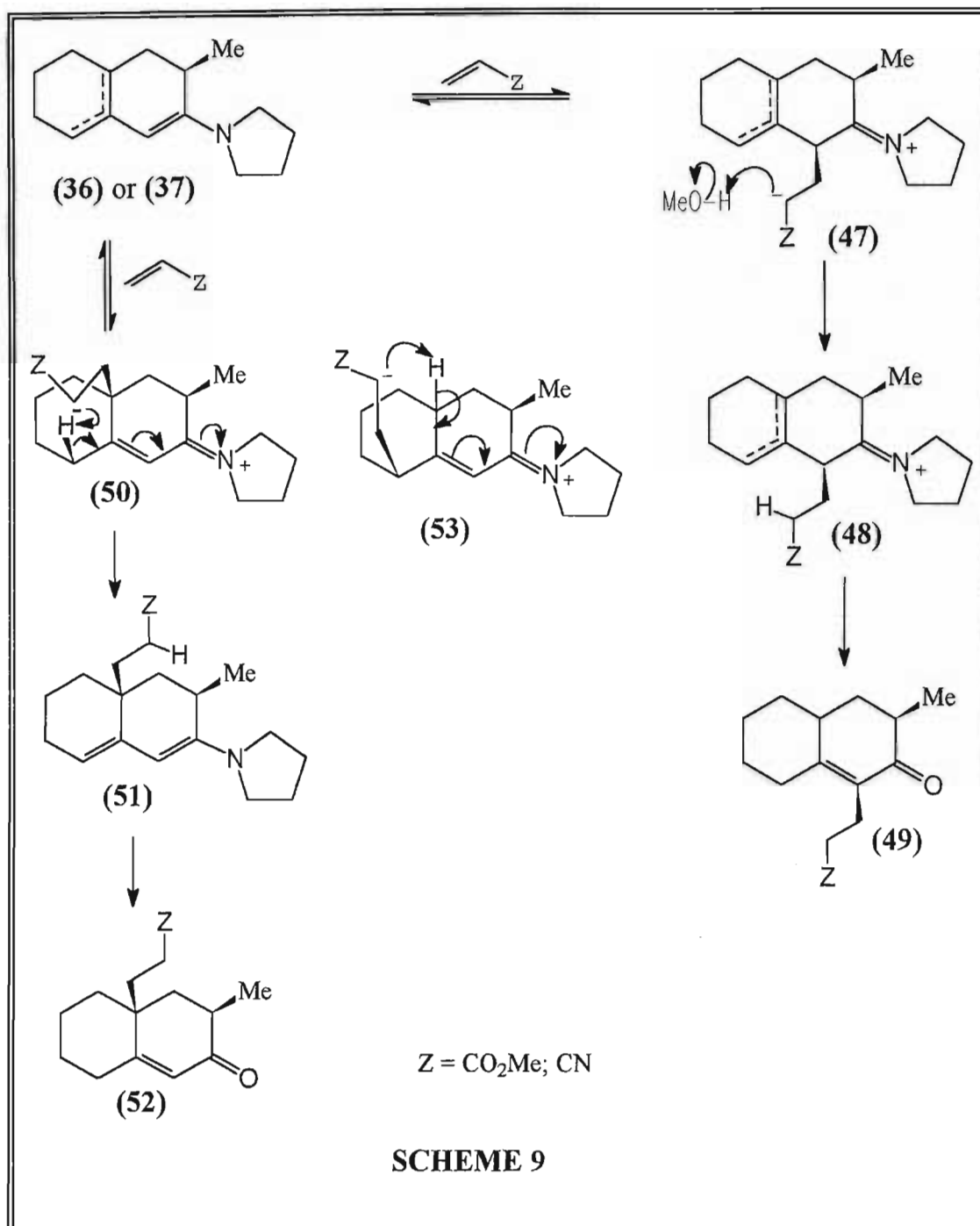
**SCHEME 9**

In protic solvents, alkylation occurred at C-1 of the dienamine (β-position) to give (49) on hydrolysis, whereas in aprotic solvents, alkylation occurred at C-4a (α-position) to give (52) on hydrolysis (Scheme 9).

The explanation offered for these results was based on the following principles:

i.     The methyl group in (36) or (37) will be quasi-axial, rather than quasi-equatorial in order to minimize $A^{1,2}$-strain[27];

ii.    reaction of an electrophilic alkene, such as methyl acrylate with an enamine involves the reversible formation of a zwitterionic intermediate[12];

iii.   formation of this zwitterionic intermediate may be rendered irreversible by subsequent protonation of the anionic centre, either by a protic solvent (methanol) or by transfer of an axial hydrogen, activated by the iminium group, to the anionic centre *via* a cyclic six-membered transition state[12], and

iv.    reaction at the β-position of a dienamine (i.e. C-1 in (36) or (37)) is a lower energy process than reaction at the δ-position (C-4a or C-8).

It was proposed that the zwitterionic intermediate (47) was formed initially by axial attack *syn* to the methyl group, in both protic and aprotic solvents. However, in protic solvents (such as methanol), this process may be rendered irreversible by protonation of the carbanionic centre by the solvent, thus leading to the iminium salt (48). Subsequent regeneration of the corresponding substituted dienamine and hydrolysis then gives the C-1 alkylated octalone (49).

It was proposed that the formation of (47) in aprotic solvents (such as dioxane or acetonitrile) was reversible and did not lead to product formation. The reason for this was that there is no acidic axial proton at C-3 (or C-1) which can be transferred to the carbanionic centre of the zwitterion. The equatorial hydrogens at these positions are less acidic than axial

hydrogen since the C-H bond orbitals are orthogonal to those of the iminium group. Consequently, elimination of methyl acrylate or acrylonitrile was the preferred mode of reaction for (47) in aprotic solvents. Reversion of (47) to starting dienamine therefore allowed thermodynamically favoured alkylation at C-4a or C-8 to compete with kinetically favoured alkylation at C-1. Alkylation at C-4a or C-8 can be rendered irreversible by stereoelectronically favoured transfer of an activated axial proton, vinylogous to the iminium group, from C-8 or C-4a respectively *via* a cyclic six-membered transition state as depicted in structures (50) and (53) respectively. The former gave dienamine (51) and hence the 4a-alkylated actalone (52) on hydrolysis.

It was noted that the formation of zwitterion (50) rather than (53) was surprising. Alkylation at C-8 would not be subjected to any 1,3-diaxial destabilization with the 3-methyl group, as would alkylation at C-4a. Furthermore, the conjugated double-bond system in an endocyclic dienamine is not coplanar[9] as it is in an exocyclic dienamine, so that the $\pi$-electron density would be predicted to be greater at C-8 than at C-4a. Formation of (50) was presumably favoured by the somewhat closer proximity of the negative charge to the 2-iminium group in the transition state leading to (50). Support for the above explanation was provided by two further observations. Firstly, alkylation of dienamine (36) (R' = R" = H; R'" = Me) (Scheme 10), in which the 3-methyl group was replaced by one at C-8, gave only the 2-alkylated product (56) on hydrolysis, in both protic and aprotic solvents. Secondly, formation of the kinetically favoured zwitterion (54) would be rendered irreversible by stereoelectronically favoured transfer

of the activated axial 3-proton to the carbanionic centre of the zwitterion

leading to dienamine (55) and the β-alkylated product (56) on hydrolysis.



**SCHEME 10**
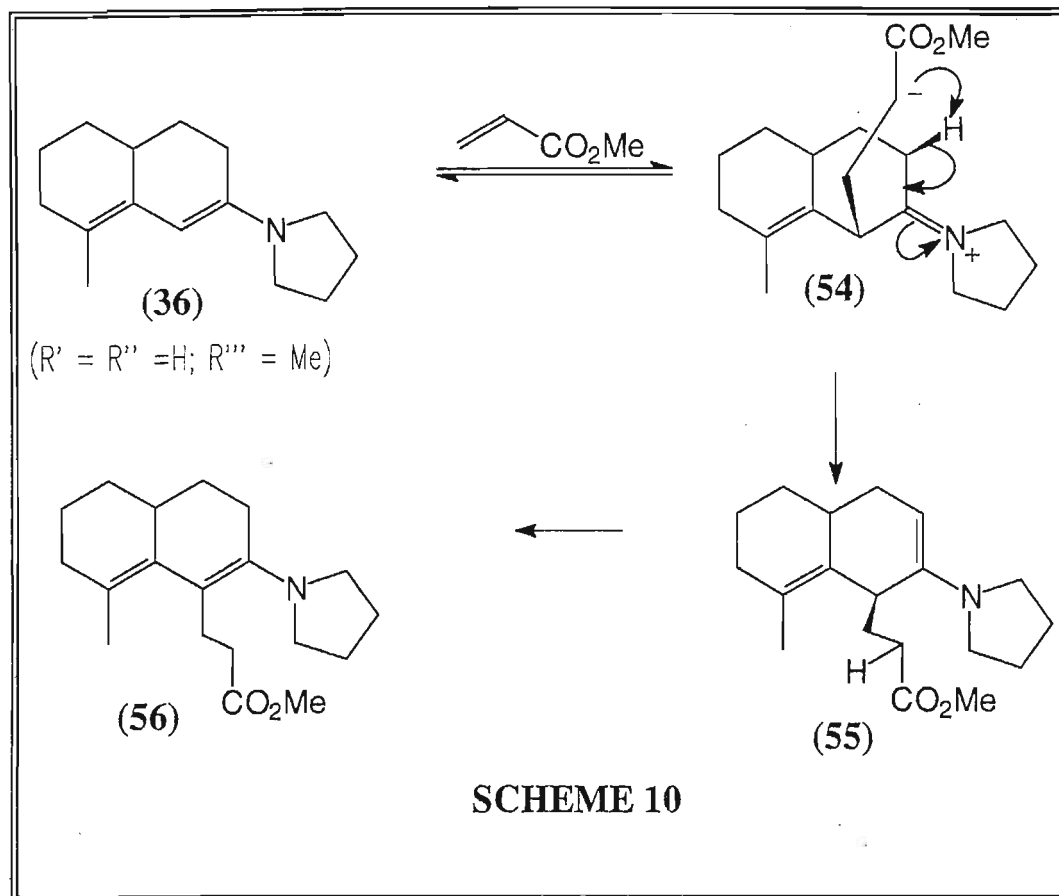
Section 2.2 of this work discusses the reinvestigation of the reaction of

pyrrolidine dienamine (39) of 4a-methyl-5-oxo-$\Delta^{1,8a}$-2-octalone with methyl

vinyl ketone.

# CHAPTER 2

## RESULTS AND DISCUSSION

### Index

## 2.1 Reductive amination of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one

### 2.1.1 Introduction

It has long been recognised that hydrocarbon moieties, be they aliphatic, cycloaliphatic, or aromatic in nature, promote the transport of drugs containing them across cell membranes and increase their affinity for lipophilic regions in receptor molecules.

As many drugs today are polyfunctionalized, we considered that the multifunctional 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2] octan-5-one (35) produced by Hickmott and Rae[47] was ideal for similar modification.

The objective of this investigation was to determine the possible utilisation of the carbonyl groups in the bicyclo[2.2.2]octanone for the introduction of pharmacophoric groups which might lead to this compound being biologically active. Several methods considered for the conversion of the bicyclo[2.2.2]octonone into an amino-derivative were either harsh (e.g. Leuckart reaction) and might lead to the opening of the ring system or suffer from being multistage processes. The preferred method therefore appeared to be reductive amination[62,63] of the carbonyl functions employing sodium cyanoborohydride as a very mild reducing agent.

Sodium cyanoborohydride reduces a wide variety of organic functional groups with remarkable selectivity. The reduction of aldehydes and ketones is pH dependent, the reaction proceeding readily at pH 3-4. Reaction of an aldehyde or ketone with ammonia, primary amine or secondary amine at

pH ~7 in the presence of BH$_3$CN$^-$ leads to primary secondary or tertiary amines respectively *via* reductive amination of the carbonyl group. Although pH 6-8 is optimum for reductive aminations, these reactions have been successfully exploited at pH's as low as 4 and as high as 10. The only requirement appears to be the presence of enough proton source to generate a positively charged C=N$^+$ moiety.[62]

The method used in this investigation is similar to that of Birch *et al.*[62] and Hickmott and Wood[64] in their preparation of amino-adamantanes. The proton source used was toluene-4-sulphonic acid instead of methanolic hydrogen chloride and the reaction mixture was heated under reflux.

### 2.1.2 Preparation of 5-benzyl-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane

Reductive amination of 2-benzoyl-4-methyl-1- phenylbicyclo[2.2.2]octan-5-one **(35)** using sodium cyanoborohydride and benzylamine in the presence of toluene-4-sulphonic acid was carried out in "super-dry" methanol under reflux for 20 hours followed by the usual hydrolytic workup. Purification using flash-column chromatography[64] gave a product which was identified from spectroscopic and analytical data. The infra-red spectrum showed the absence of carbonyl absorptions and thus indicated that the reaction had occured at both carbonyl groups. However elemental analysis showed roughly half the expected nitrogen content for the bis-aminated product. This, together with the mass spectral data ( M$^+$ 393), indicated the presence of one nitrogen atom and therefore pointed to the formation of a nitrogen bridge between the benzoyl and ring carbonyl groups. The azatwistane

structure **(56)** was therefore proposed and this structure was subsequently confirmed by 300 MHz NMR spectroscopy and a single crystal x-ray structure determination as 5-benzyl-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane **(56)**.



(56)
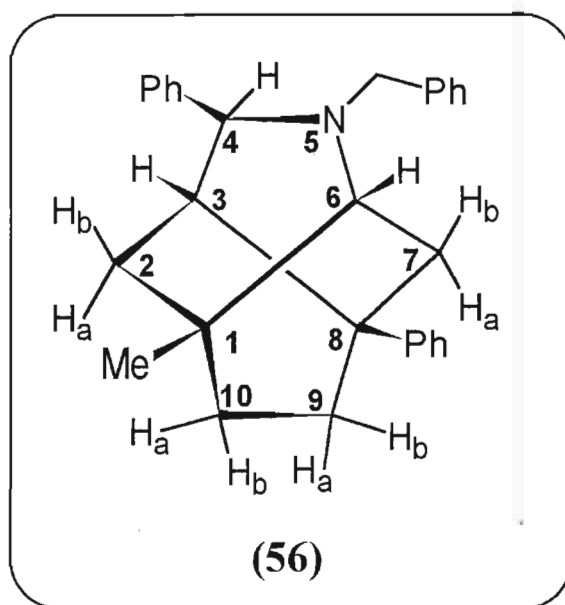
The $^1$H NMR and $^{13}$C NMR chemical shift assignments were made from the following observations and reasoning. The DEPT spectrum showed three high field methine carbon signals at δ 46.60, δ 57.79 and δ 64.47 ppm, and five methylene carbon signals at δ 28.68, δ 30.44, δ 32.53, δ 35.98, and δ 55.06 as required for structure **(56)**. The HETCOR spectrum showed that the proton attached to the low field methine carbon gave a <u>singlet</u> at δ$_H$ 3.55. This at first sight is surprising since there is no methine proton in the azatwistane structure **(56)** which does not have one or more protons on an adjacent carbon. However, examination of a molecular model shows that the tricyclic system is composed of distorted boat shaped rings, rather than true boat or twist configurations. As a consequence the dihedral angle between the methine proton at C-4 (i.e. H-4) and that at C-3 (i.e. H-3) is

close to 90⁰. It follows therefore, from the Karplus equation, that the coupling between these two protons is close to zero.

The low field proton signal at δ 3.55 is therefore assigned to H-4 and the carbon giving the signal at δ 64.47 is C-4. The other two methine protons gave doublets at δ 2.43 and δ 2.21 ppm (HETCOR). These are clearly attributable to H-3 and H-6, both of which have a methylene group adjacent to them. The molecular model again shows that the dihedral angles between each of these methine protons and <u>one</u> of the adjacent methylene protons is close to 90⁰. This means that both methine protons are effectively coupled to only one proton in the adjacent methylene groups, and therefore should give doublets, as is observed. Since H-6 is attached to the carbon *alpha* to the nitrogen, the lowest field proton signal (doublet) at δ 2.43 is assigned to H-6 and the carbon signal at δ 57.79 to C-6 (HETCOR). The proton signal at δ 2.21(1H, d) is accordingly assigned to H-3 and the carbon signal at δ 46.60 to C-3 (HETCOR).

The lowest field methylene carbon signal at δ 55.06 can clearly be attributed to the benzyl methylene group. The methylene protons are rendered non-equivalent by the assymetry of the ring system and give an AB quartet at δ 3.35 ($J = 13.7$ Hz) and δ 3.59 ($J = 13.7$ Hz).

The next lowest field methylene proton signal, a doublet of doublets, appears at δ 2.34. This integrates to one proton and is assigned to Hb-7 since the COSY spectrum shows (i) it is not coupled to H-6 (dihedral angle approximately 90⁰) and (ii) it is coupled to the same methylene proton (Ha-7) as H-6 and which is part of a multiplet of two overlaid proton signals

at δ 1.64-1.75. The HETCOR spectrum confirms both protons are attached to the same carbon (C-7) which gives a signal at δ 28.68. The molecular model indicates that the additional splitting manifested in the signal from Hb-7, since it does not arise from coupling with H-6 (dihydral angle approximately $90^0$), must arise by W-coupling with Ha-9. This is not quite a planar W but is presumably near enough planar to allow a weak long range coupling interaction to occur. The COSY spectrum therefore establishes the signal due to Ha-9 as a multiplet at δ 2.0.

The methine proton H-3 is vicinally coupled to the methylene proton Ha-2 which gives a signal comprising part of a multiplet at δ1.10-1.30. This proton is geminally coupled to Hb-2, which is not coupled to H-3 (dihedral angle approximately $90^0$), and which gives a signal as part of a multiplet at δ 1.64-1.75. The attached carbon (C-2) is assigned to the carbon signal at δ 30.44 (HETCOR).

The methylene proton Ha-9 is geminally coupled to the Hb-9 which gives a signal as part of the multiplet at δ 1.40-1.60 (COSY and HETCOR) and C-9 is therefore assigned to the signal at δ 35.98 (HETCOR). Proton Ha-9 is also vicinally coupled to Ha-10 and Hb-10 which give proton signals as part of the multiplets at δ 1.10-1.30 and δ 1.40-1.60 and thus establishes the carbon signal at δ 32.54 as arising from C-10 (HETCOR).

The quaternary carbons at C-1 and C-8 give carbon signals at δ 34.56 (s) and δ 39.74 (s) and the methyl carbon gives a resonance at δ 24.78 (q) corresponding in the HETCOR spectrum to a proton resonance at δ 0.91

(3H, s). The C-1 carbons of the three benzene rings give carbon resonances at δ 140.68, δ 143.46 and δ 148.64 (each s), and the remaining aromatic carbons give carbon resonances (doublets) at δ 126-130 corresponding in the HETCOR spectrum to proton resonances at δ 7.20-7.50. This completes the full carbon and proton chemical shift assignments for this product and the results are summarised below:

$^1$H-NMR (300 MHz; CDCl$_3$), δ(ppm)

| | |
|---|---|
| 0.91 | (s; 3H; CH$_3$) |
| 1.10-1.30 | (m; 2H; Ha-2; Ha-10) |
| 1.40-1.60 | (m; 2H Hb-9; Hb-10) |
| 1.64-1.75 | (m; 2H; Hb-2; Ha-7) |
| 2.00 | (m; 1H; Ha-9) |
| 2.21 | (d; 1H; J = 6.0 Hz; H-3) |
| 2.34 | (dd; 1H; J = 12.6; 2.7 Hz; Hb-7) |
| 2.43 | (d; 1H; J = 4.8 Hz; H-6) |
| 3.35 | (d; 1H; J = 13.7 Hz; C$\underline{H}$-Ph) |
| 3.55 | (s; 1H; H-4) |
| 3.59 | (d; 1H; J = 13.7 Hz; C$\underline{H}$-Ph) |
| 7.20-7.50 | (15H; 3 x Ph) |

$^{13}$C-NMR and DEPT Spectra (300 MHz; CDCl$_3$), δ(ppm)

| | | |
|---|---|---|
| 24.78 (q; CH$_3$) | 28.68 (t; C-7) | 30.44 (t; C-2) |
| 32.53 (t; C-10) | 34.56 (s; C-1) | 35.98 (t; C-9) |
| 39.74 (s; C-8) | 46.60 (d; C-3) | 55.06 (t; $\underline{C}$H$_2$-Ph) |

57.79 (d; C-6)          64.47 (d; C-4)

140.68, 143.46, 148.64 (3s, 3 quaternary C-1 Ph carbons)

126.36-129.49 (Phenyl $\underline{C}$H carbons)

The isomer ratio[isomer I : isomer II (67.5%:32.5%)] and the azatwistane yield (63%) indicate that the azatwistane is not formed solely from one isomer (isomer I), but isomer interconversion occurs *via* epimerisation of the benzoylated ring carbon (C-2) by enolysation and reprotonation, as shown in scheme 11.
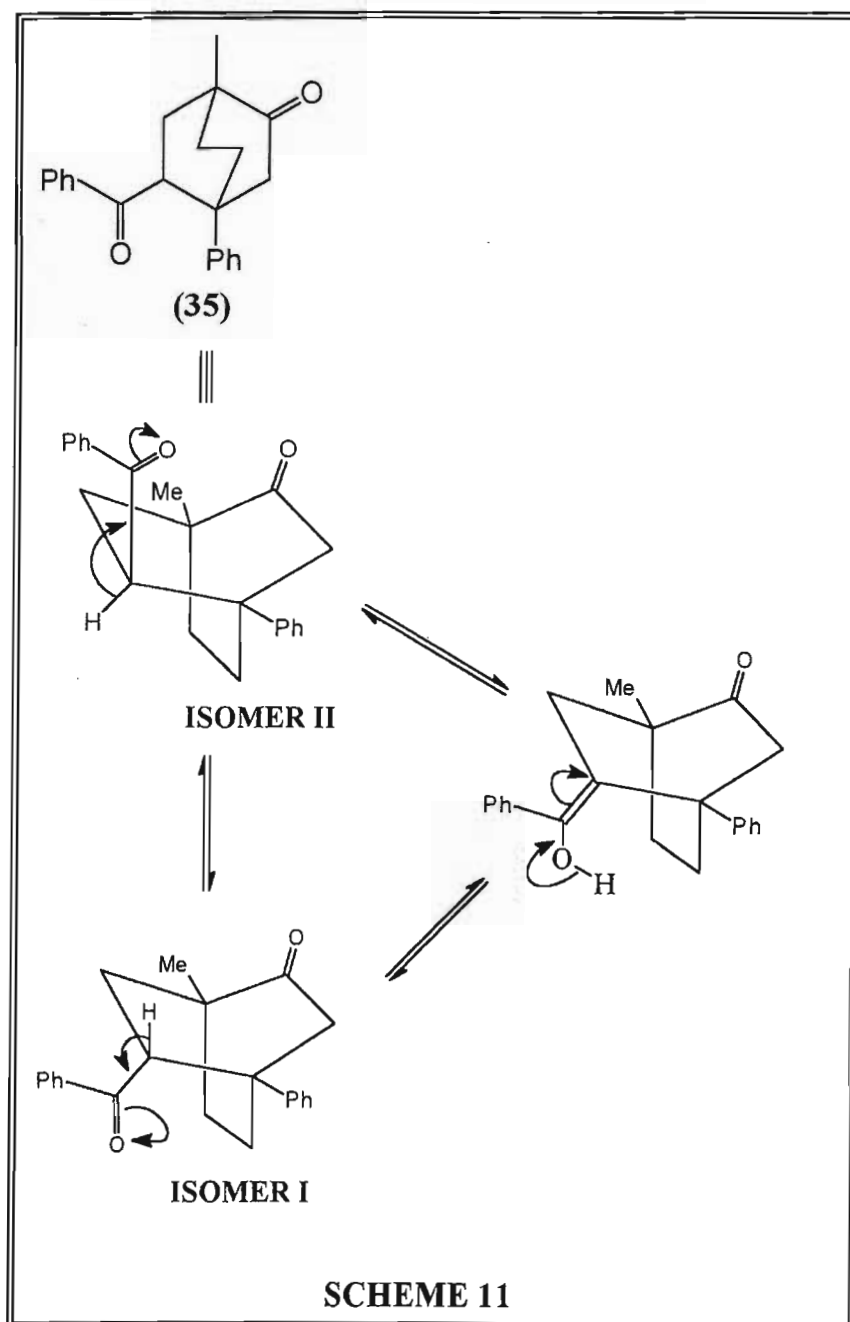


**SCHEME 11**

Scheme 12 shows the plausible sequence of events that lead to the formation of (56). As can be seen from the mechanism, benzylamine would be expected to react initially with the more reactive C-5 carbonyl group of (35) rather than the less reactive benzoyl carbonyl group.



SCHEME 12
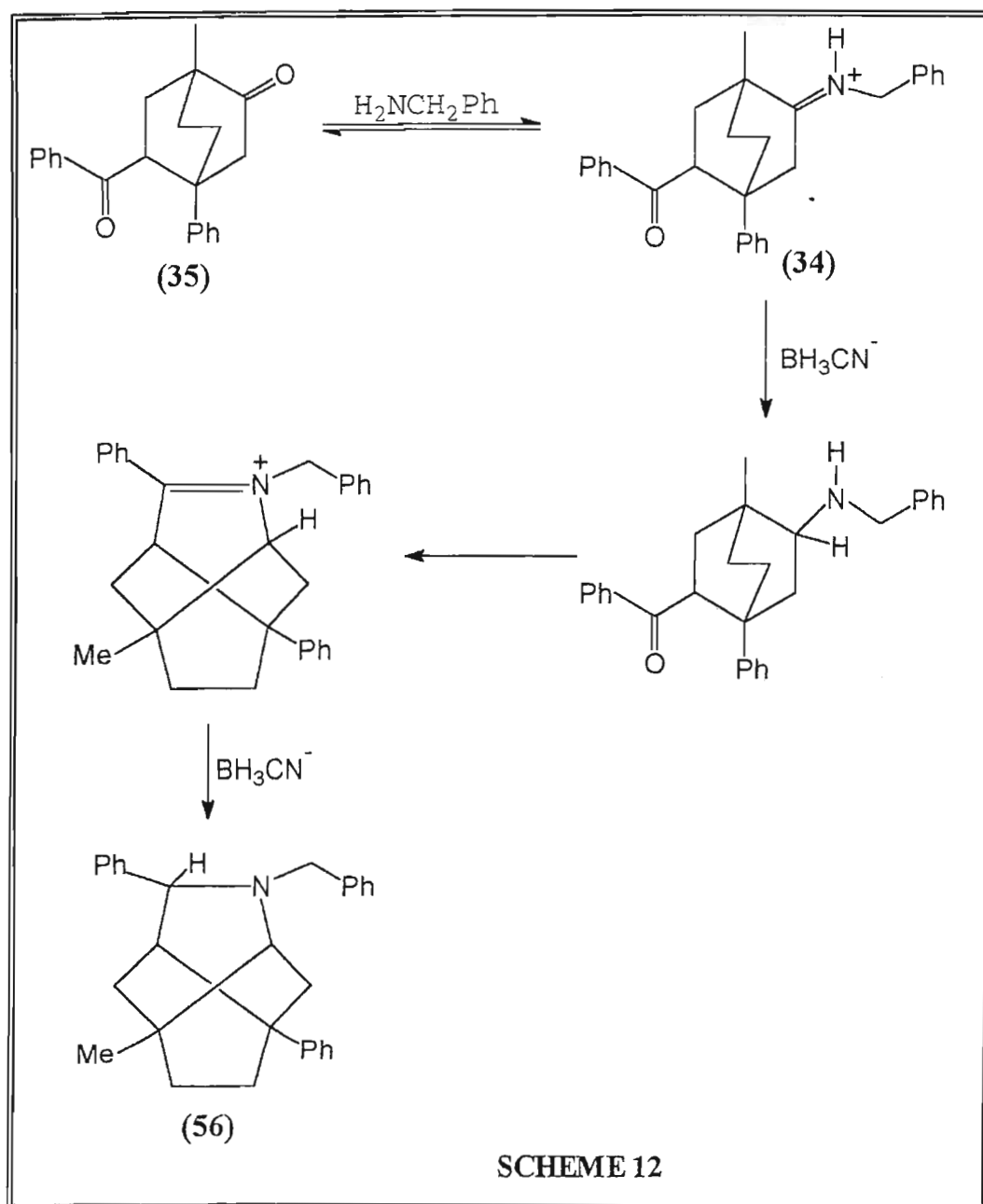
The single crystal x-ray structure determination confirmed the structure of (56).

# Crystal structure of (56)
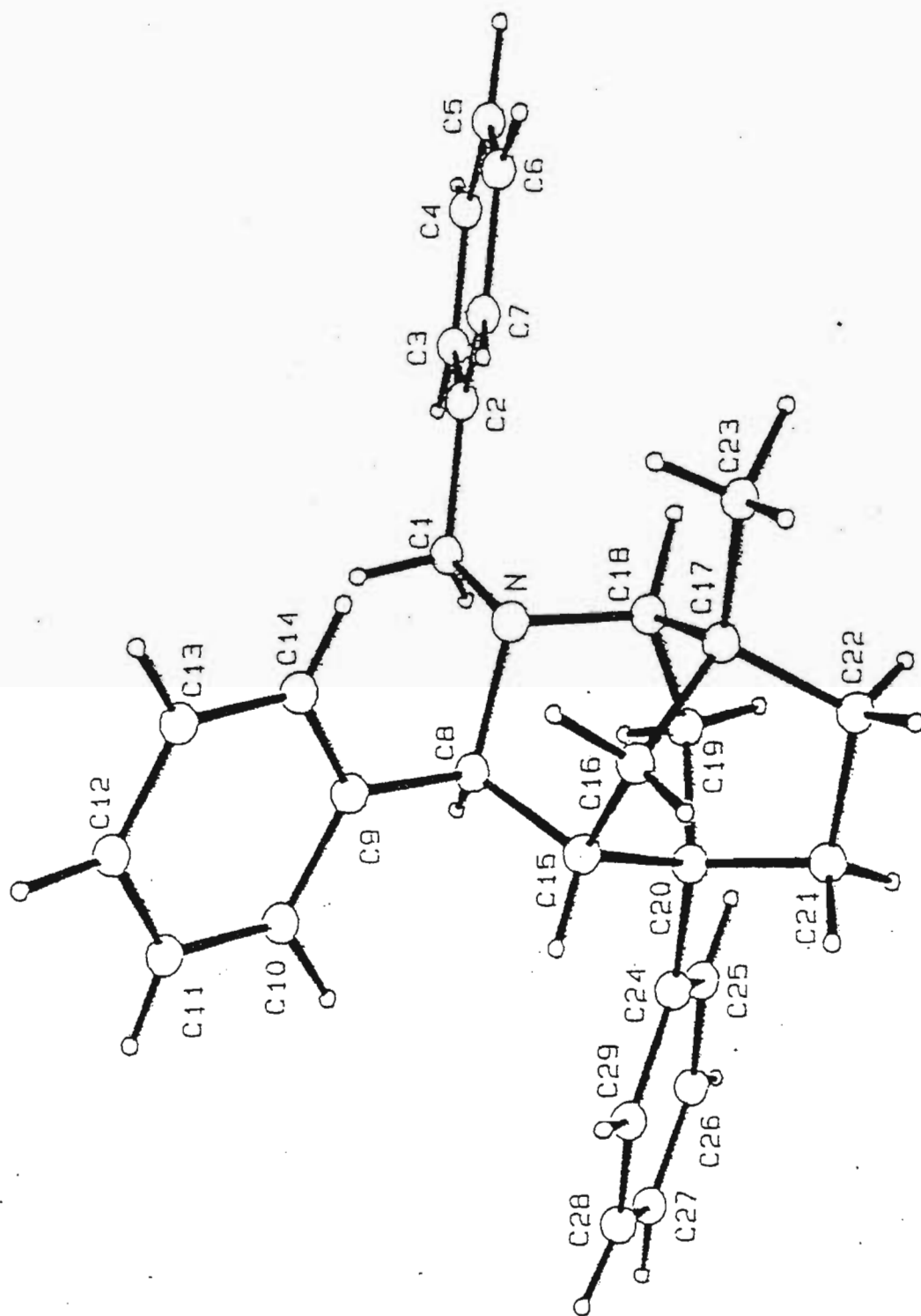
### 2.1.3 Attempted preparation of 1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane

This synthesis was attempted by treatment of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one (35) with sodium cyanoborohydride and ammonium acetate as the amine source in "super-dry" methanol. However microanalysis of the product obtained showed that the nitrogen content was far too high. This suggested that possibly the bis-amination product (57) had been formed,



(57)
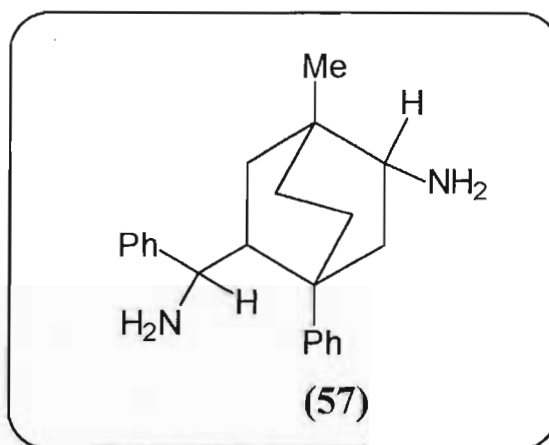
|  | C | H | N | M$^+$ |
|---|---|---|---|---|
| Required: | 82.45 | 8.81 | 8.74 | 320 |
| Experimental: | 82.20 | 7.38 | 8.30 | 328 |

However GC/MS indicated a molecular ion at m/e 328, and the infrared spectrum showed only one NH absorption at (KBr) 3300 cm$^{-1}$ whereas for an NH$_2$ group there should have been two absorptions arising from the symmetric and asymmetric stretching of the N-H bonds. The infrared spectrum also showed an absorption at 2250 cm$^{-1}$ indicative of the presence of a nitrile group. A broad but somewhat small absorption at 3400 cm$^{-1}$ suggested that maybe an amino-cyanohydrin (58) had been formed, viz:

(58)
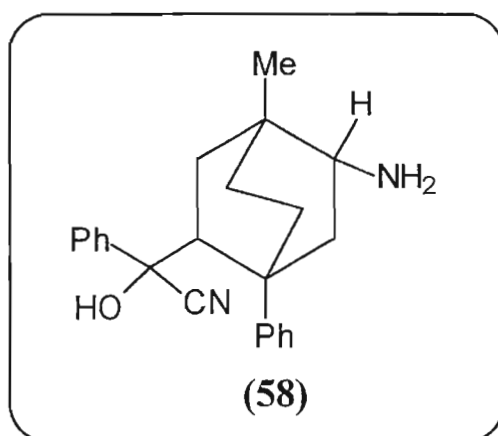
|  | C | H | N | M⁺ |
|---|---|---|---|---|
| Required: | 79.73 | 7.57 | 8.09 | 346 |
| Experimental: | 82.20 | 7.38 | 8.30 | 328 |

or, in view of the single NH stretching absorption, an iminocyanohydrin, viz:



(59)
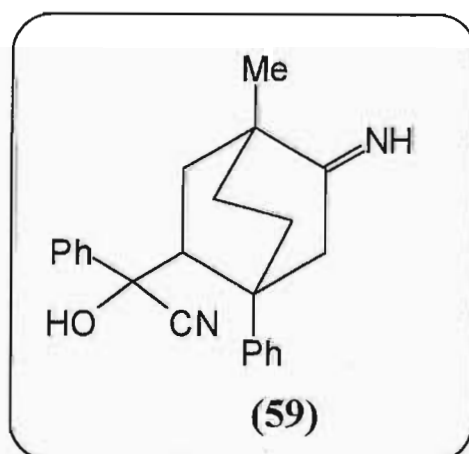
|  | C | H | N | M⁺ |
|---|---|---|---|---|
| Required: | 80.20 | 7.02 | 8.13 | 344 |
| Experimental: | 82.20 | 7.38 | 8.30 | 328 |

However both these structures were ruled out by the mass spectral data and by comparison of the NMR spectra with those of an authentic cyanohydrin

prepared from acetone. The singlet at $\delta_c 64.66$ in the $^{13}$C-NMR spectrum of the acetone cyanohydrin, attributed to the quaternary carbon to which the hydroxyl and nitrile groups are attached, was not observed in the $^{13}$C-NMR spectrum of the product formed.

Furthermore the only peak in the $^1$H-NMR spectrum which could conceivably be due to OH was shown by deuterium exchange not to be acidic. However the C-13 NMR spectrum did support the presence of a nitrile group in that there was a characteristic resonance at $\delta_c 120.75$ which could be attributed to the CN group. These observations therefore lead us to the conclusion that the product formed is 6-cyano-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane, viz:



(60)
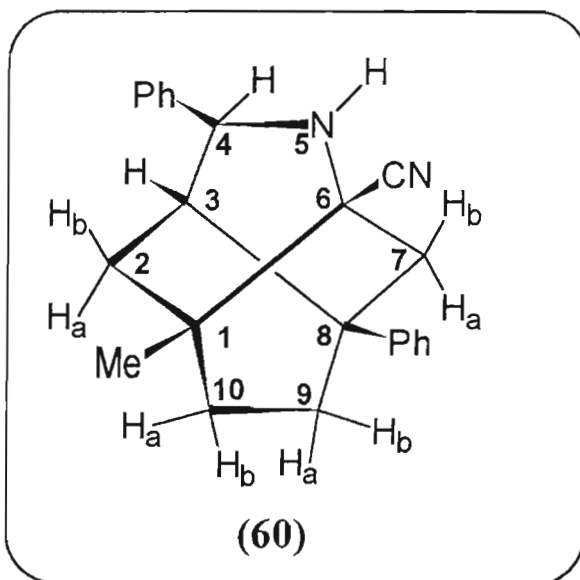
|  | C | H | N | M$^+$ |
|---|---|---|---|---|
| Required: | 84.10 | 7.37 | 8.50 | 328 |
| Experimental: | 82.20 | 7.38 | 8.30 | 328 |

This structure explains the presence of only one NH stretching absorption and the absence of an OH peak in the $^1$H-NMR spectrum. The DEPT spectrum showed the presence of two high field methine carbon signals at $\delta_C$43.55 and $\delta$54.57 ppm and four methylene carbon signals at $\delta_C$29.42, 30.63, 34.50 and $\delta$41.66 ppm, as required for the above structure. The HETCOR spectrum showed that the proton attached to the low field methine carbon gave a singlet at $\delta_H$ 4.21. By analogy with the reasoning already discussed for the assignment of spectral data for 5-benzyl-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane, this signal is therefore assigned to H-4 and the signal at $\delta_C$54.57 to C-4.

The other methine proton appeared as a doublet at $\delta_H$2.33 and is attributed to H-3, coupled with only one of the adjacent methylene protons (Ha-2) since the dihedral angle with Hb-2 is close to 90$^0$. The signal at $\delta_C$43.55 is therefore assigned to H-3 (HETCOR).

The low field doublet of doublets at $\delta_H$2,61 is assigned to Hb-7, geminal coupled to Ha-7 ($\delta_H$2.42, J=12.6 Hz) and W-coupled to Ha-9 ($\delta$2.10) (COSY); C-7 is therefore responsible for the signal at $\delta_C$41.66. The low field shift of this signal relative to the signal for C-7 ($\delta$28.68) in 5-benzyl-1-methyl-4,8-diphenyl-5- azatricyclo[4.4.0.0$^{3,8}$]decane can be attributed to the deshielding of an additional carbon *beta* to C-7 and therefore provides additional evidence for the nitrile substituent being at C-6. The HETCOR spectrum also showed the presence of three quaternary carbons at $\delta_C$37.65 and $\delta$38.46 (C-1 and C-8) and $\delta_C$55.64 (C-6) as required

for the proposed structure. Assignment of the remaining signals are summarised below.

$^1$H-NMR (300 MHz, CDCl$_3$), $\delta_H$(ppm)

| | |
|---|---|
| 1.25 | (s; 3H; CH$_3$) |
| 1.30-1.50 | (m; 2H; Ha-2; Ha-10) |
| 1.50-1.65 | (m; 2H; Hb-9; Hb-10) |
| 1.70-1.90 | (m; 1H; Hb-2) |
| 2.10 | (m; 1H; Ha-9) |
| 2.33 | (d; 1H; J=6.1 Hz; H-3) |
| 2.42 | (d; 1H; J=12.6 Hz; Ha-7) |
| 2.61 | (dd; 1H; J=12.6, 2.7 Hz; Hb-7) |
| 4.21 | (s; 1H; H-4) |
| 7.15-7.50 | (10H; 2Ph) |

$^{13}$C-NMR and DEPT (300 MHz; CDCl$_3$), $\delta_C$ (ppm)

| | | |
|---|---|---|
| 22.23 (q; CH$_3$) | 29.42 (t; C-10) | 30.63 (t; C-2) |
| 34.50 (t; C-9) | 37.65 (s; C-1) | 38.46 (s; C-8) |
| 41.66 (t; C-7) | 43.55 (d; C-3) | 54.57 (d; C-4) |
| 55.64 (s; C-6) | 120.75 (s; CN) | |

141.72, 145.63 (2 x S; 2 C-1 carbons of 2 Ph groups)

| | | |
|---|---|---|
| 125.79 (2 x d) | 126.44 (d) | 126.80 (2 x d) |
| 126.86 (d) | 128.61 (2 x d) | 128.86 (2 x d) |

X-ray crystal structural analysis (Appendix) confirmed the structure of (60).

The formation of 6-cyano-1-methyl-4,8-diphenyl-5-azatricyclo$[4.4.0.0^{3,8}]$ decane **(60)** was surprising since Birch *et al.*[62] did not observe any α-aminonitrile formation in their preparation of *endo*-norbornylamine *via* reductive amination of 2-norbornanone with ammonia at $25^0$C. The only rational explanation for the -cyanoamino product formation is that at $90^0$C, $NaBH_3CN$ generates a strong $CN^-$ nucleophile as an attacking species, although the $CN^-$ might not be completely free but solvated in methanol $(MeO-H....CN^-)$.

The mechanism proposed for the formation of **(60)** is depicted in Scheme 12. This mechanism is similar to the Strecker synthesis which involves the addition of HCN to C=O or C=N to give α-aminonitrile intermediates in the synthesis of amino acids[65] and sterically hindered amines.[66-69] Cyanohydrin or a Schiff base has been postulated as an intermediate without decisive evidence.[70] Ogata and Kawasaki[71], and later Stanley *et al.*[72] presented evidence for a cationic imine intermediate in α-aminonitrile formation. This supported an earlier report by Stewart and Li[73] that direct displacement of the hydroxyl group of the cyanohydrin was unlikely in the presence of amines.

SCHEME 13
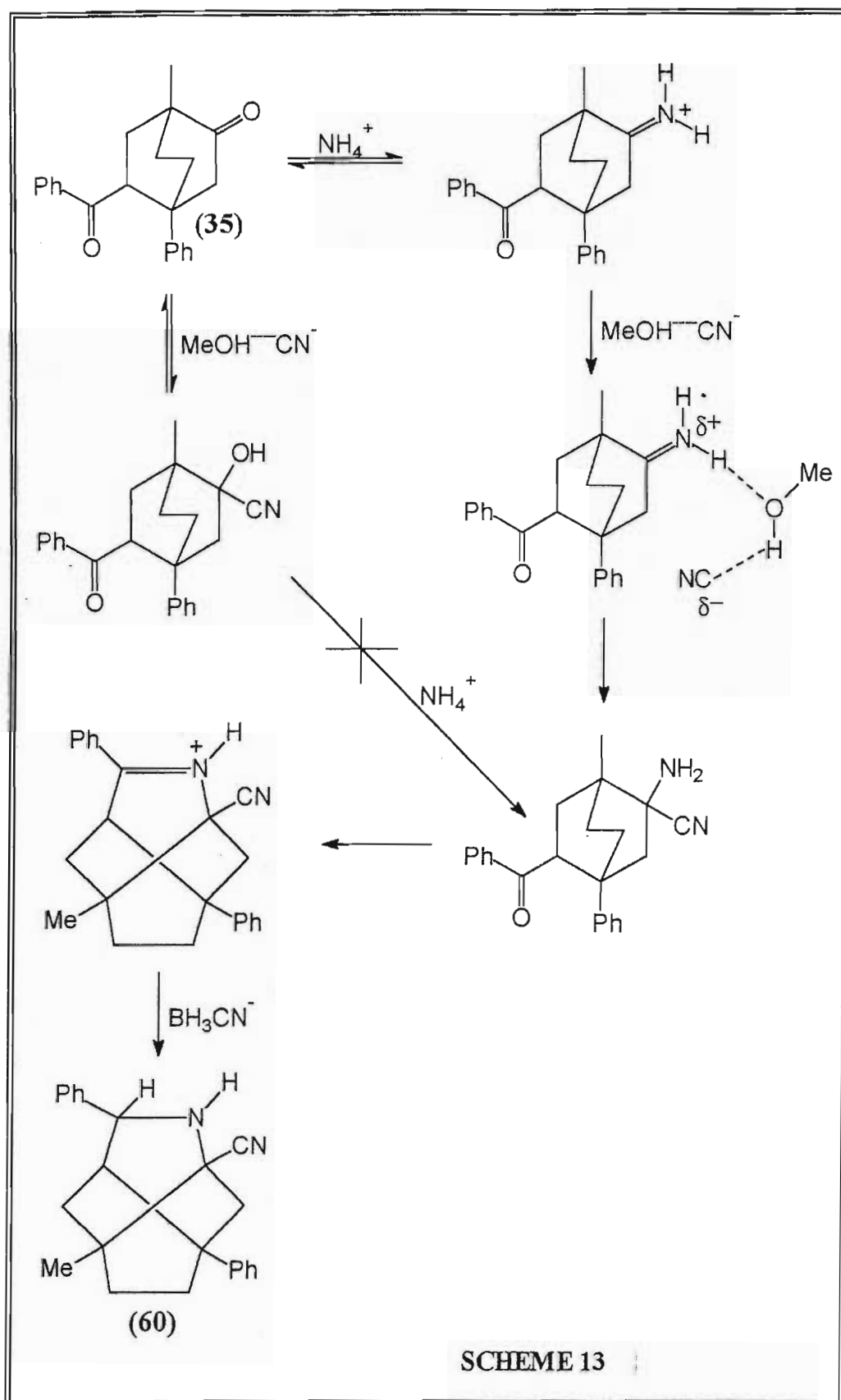
## 2.2 Reaction of MVK with the pyrrolidine dienamine of

## 4a-methyl-5-oxo-$\Delta^{1,8a}$-2-octalone

On the basis of the observations already mentioned in the Introduction, it would be expected that the reaction of methyl vinyl ketone with dienamines derived from $\Delta^{1,8a}$-2-octalones would show solvent dependent regioselectivity. The reaction of the pyrrolidine dienamine (39a) and (39b) of 4a-methyl-5-oxo-$\Delta^{1,8a}$-2-octalone with methyl vinyl ketone in toluene has recently been investigated.[74]

The three main components of the reaction mixture were identified as the [4+2] cycloaddition product (64), the aromatized β,δ-annulation product (62) previously reported by Pandit et al.[75] and (63) produced during the disproportionation process involved in the aromatization of the β,δ-annulation product (Scheme 14). The formation of the [4+2] cycloaddition product (64) was totally unexpected since it was derived from the cross-conjugated dienamine which according to $^1$H-nmr measurements was not present[74].

The reaction of methyl vinyl ketone with the pyrrolidine dienamine of 4a-methyl-5-oxo-$\Delta^{1,8a}$-2-octalone was repeated in an attempt to isolate compound (65) which could be obtained from (61) by a double bond rearrangement resulting in aromatization (Scheme 15).

The reaction was carried out in dry toluene under reflux for 45 hours followed by aqueous hydrolysis and the usual hydrolytic workup. The combined acid washings were basified (pH>10) and extracted with methylene chloride. GC-MS analysis of the crude mixture showed that it consisted largely of pyrrolidine.

**SCHEME 14**

(61)

H⁺

(65)

SCHEME 15
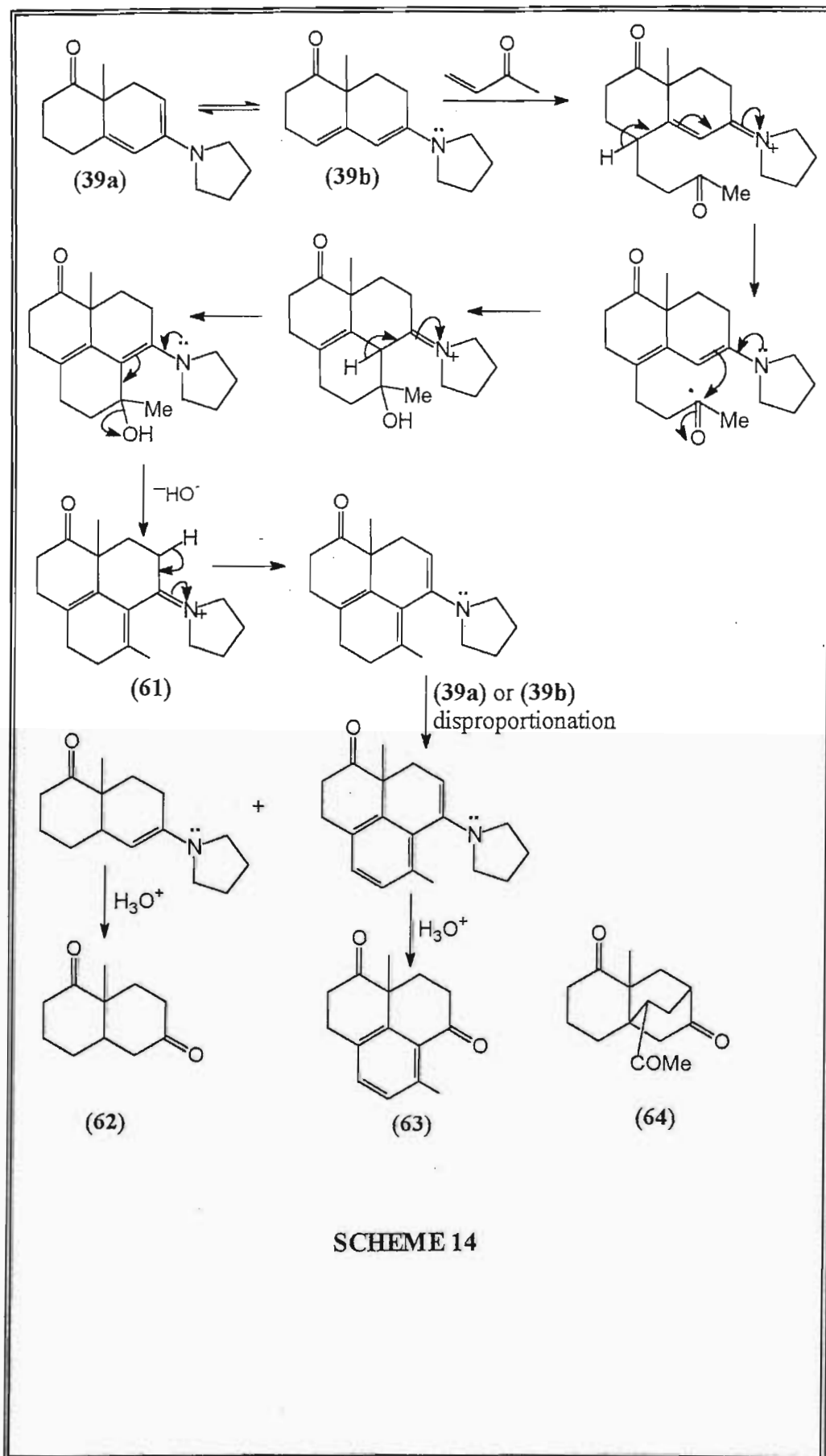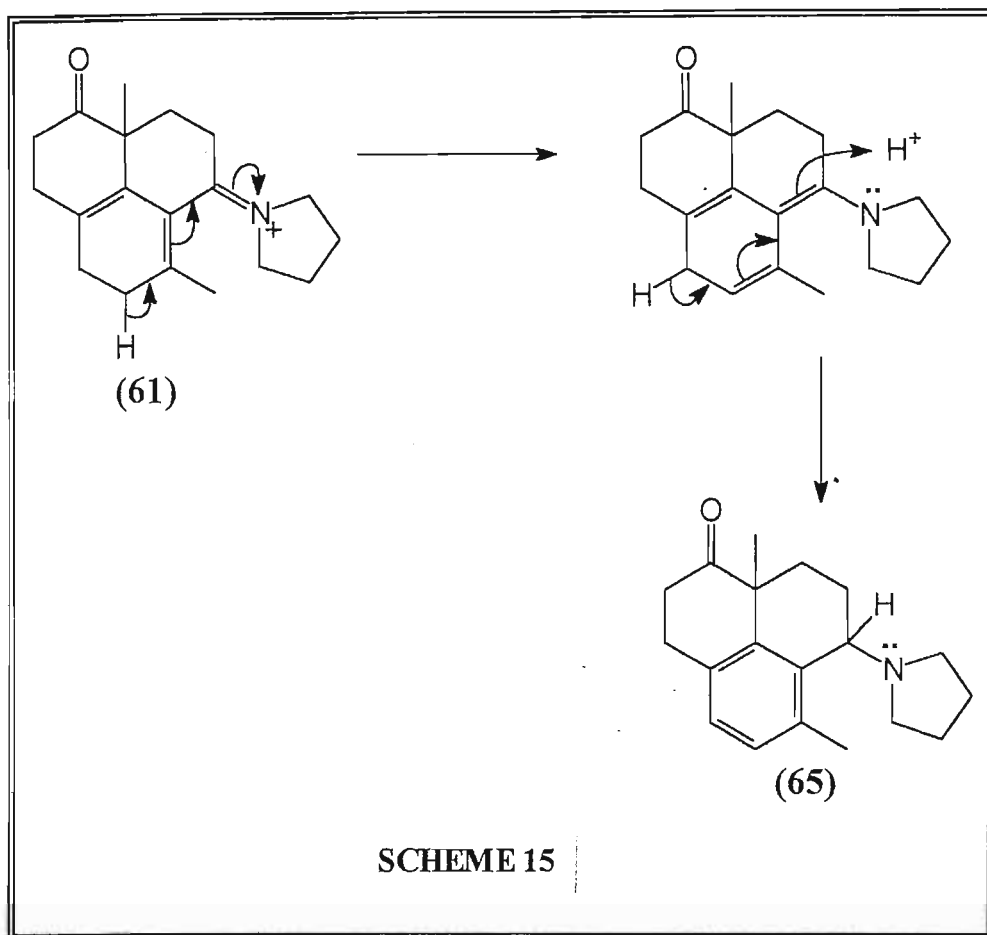
# CHAPTER 3

## EXPERIMENTAL

### Index

## 3.1 General

### 3.1.1 Nuclear Magnetic Resonance Spectroscopy

$^{13}C$ and $^{1}H$ Nuclear Magnetic Resonance spectra were recorded in deuteriochloroform solution with a 200 MHz or 300 MHz Gemini Spectrometer using the central line of the deuteriochloroform triplet at $\delta_C$77.09 ppm and the deuteriochloroform singlet at $\delta_H$7.24 ppm.

The following abbreviations were used when assigning the spectra:
s: singlet;   d: doublet;   q: quartet;   m: multiplet;   $J$: coupling constant (Hz);
dd: doublet of doublets.

### 3.1.2 Infra-Red Spectroscopy

The infra-red (IR) spectra were recorded on a Shimadzu IR-408 infra-red spectrophotometer and were calibrated against the 1601 $cm^{-1}$ peak of polystyrene film.  KBr was used as dispersing agent for solids.  The spectra of oils were recorded neat, as a thin film between two NaCl discs.

### 3.1.3 Gas Chromatography

The gas-liquid chromatography (g.l.c.) analyses were carried out using a Varian 3400 gas-liquid chromatography, using ultra-high purity nitrogen as carrier gas (flow rate: 24 ml/min), a 14.75 m glass capillary column (Phase: SPB1; I.D.-0.25 micros) and a flame ionization detector (FID).  The g.l.c. spectra were obtained at initial temperature: $140^0C$; initial hold time: 2 min.;

ramp rate: $16^0$C/min; final column temperature: $300^0$C; final hold time: 20 min. Experimental yields quoted are calculated from the masses of the crude reaction mixtures using g.l.c. percentage (based on integrated peak areas).

### 3.1.4 Gas Chromatography - Mass Spectroscopy (GC-MS)

The GC-MS spectra were recorded with a Finnigan 1020 automated spectrometer operating at 70 eV.

### 3.1.5 C/H/N Analysis

Micro-analyses were carried out by the Department of Chemistry of the Natal University in Pietermaritzburg.

### 3.1.6 General Chromatography

Analytical thin layer chromatography (TLC) was carried out on Merck:Art. 5553 aluminium-backed silica gel (0.2 mm) plates. The spots on the plates were visualised by spraying with the spray reagent comprising anisaldehyde : concentrated sulphuric acid : methanol (1.25:2.5:96.25). Coloured spots were formed after the plates had been heated.

All flash-column chromatography was carried out on Merck: Art. 938 silica gel. The solvent system was generally a mixture of hexane, methylene chloride and ethyl acetate (unless otherwise stated), the ratios of which were

chosen to give the desired compound(s) on $R_f$ value of approximately 0.35[76] when tested by TLC.

### 3.1.7 Melting Point Determination

Melting points were measured on a Kofler hot-stage melting point apparatus and are uncorrected.

### 3.1.8 X-Ray Structure Determination

X-ray structure determination was carried out by the x-ray Crystallographic Unit in the Department of Chemistry, University of Natal, Pietermaritzburg.

### 3.2 Purification and drying of solvents and reagents

The solvents were purified and dried by the following methods:
Methanol was dried following the method in Vogel[77]. Magnesium turnings, washed with ether and dried (2.6 g), iodine (0.26 g) and methanol (50 ml) were heated under reflux, the condenser being fitted with a drying tube. After all the magnesium had been consumed, methanol was then fractionally distilled (64.5$^0$C) with the exclusion of moisture and stored over molecular sieves (3A, BDH : Bead typed). This "super-dry" methanol was used in the reactions described in the experimental section.

Benzene was allowed to stand over anhydrous calcium chloride for 24 h., and then distilled onto molecular sieves (5A), the fraction: 78-80$^0$C being collected.

Toluene was dried by standing over anhydrous calcium chloride for 24 h., followed by distillation into a vessel containing molecular sieves (5A). Further drying prior to use was achieved by the addition of sodium wire.

Ether was dried by addition of sodium wire.

Methyl vinyl ketone was distilled under reduced pressure from quinol and allowed to stand over molecular sieves (4A) for 12 hours prior to use.

Phenyl vinyl ketone was prepared according to the method outlined in the experimental section and dried over molecular sieves (4A) for 12 hours prior to use.

Where molecular sieves were employed for drying purposes, between 50 and 70 g were added per litre of solvent or reagent. Activation of the sieves was achieved by heating in a muffle furnace at $350^0$C overnight and cooled in a desiccator.

## 3.3 Preparation of Phenyl Vinyl Ketone (PVK)

Acetophenone (22.18 g; 0.19 mol) dimethylamine hydrochloride (20.00 g; 0.25 mol) and paraformaldehyde (7.5 g; 84 mmol) were placed in a round-bottomed flask and then a mixture of conc. HCl (0.38 ml) in ethanol (95%; 80 ml) was added to the flask. When the addition had been completed the mixture was raised to the boil and heated under reflux for 2 hours. Acetone (150 ml) was added to the warm reaction mixture. On

cooling, crystals separated. These were filtered and dried (40-50°C) for 3 hours to give β-dimethylaminopropiophenone hydrochloride.

The β-dimethylaminopropiophenone hydrochloride (20.00 g; 0.13 mol) was steam distilled to give phenyl vinyl ketone which was extracted with dichloromethane (3 x 50 ml). The solution was dried over anhydrous magnesium sulphate to which quinol (0.01 g) had been added and then the solvent was removed on a rotatory evaporator to give the desired ketone (10.64 g; 62%).

The IR spectrum showed $v_{max}$(film) cm$^{-1}$

| 1610 | (C=C) |
| 1670 | (C=O) |



**PVK**
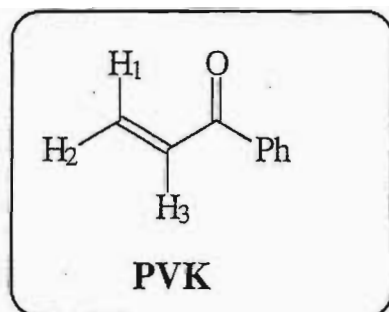
The $^1$H NMR spectrum (300 MHz; CDCl$_3$) showed δ(ppm)

| 5.80 | (dd; 1H; J = 2.0 Hz; J = 11.0 Hz; H$_1$) |
| 6.40 | (dd; 1H; J = 2.0 Hz; J = 17 Hz; H$_2$) |
| 7.13 | (dd; 1H; J = 11.0Hz; J = 17Hz; H$_3$) |
| 7.4-8.0 | (complex; 5H; Ph) |

### 3.4 Preparation of N-(2-Butylidine)Benzylamine

Butan-2-one (36.0 g; 0.50 mol), benzylamine (54.6 g; 0.51 mol) and toluene-4-sulphonic acid (0.6 g) were heated under reflux in benzene (100 ml), the water being removed azeotropically *via* a Dean and Stark water separator for 24 hours. The solvent was then removed on a rotatory evaporator, and the residue distilled under vacuum to give N-(2-butylidine)benzylamine (33) (51.51 g; 64%). BP 68-72$^0$C/0.20 mm Hg

The IR spectrum showed $\nu_{max}$(film) cm$^{-1}$

| 1660 | (C=N) |
| 1660 | (-Ph) |



(33) N-(2-Butylidine)benzylamine
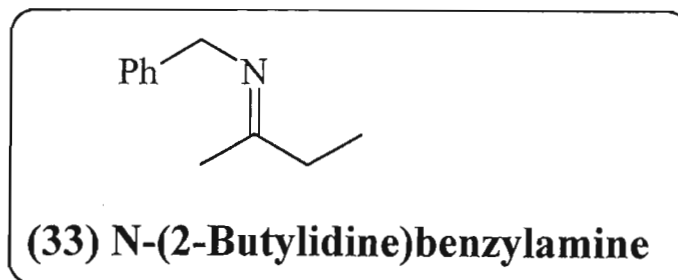
The $^1$H NMR spectrum (300 MHz; CDCl$_3$) showed $\delta$(ppm)

| 1.14 | (t; 3H; J = 7.4 Hz; C$\underline{H}_3$-CH$_2$) |
| 1.87 | (s; 3H; CH$_3$) |
| 2.33 | (m; 2H; CH$_3$-C$\underline{H}_2$) |
| 4.48 | (s; 2H; Ph-C$\underline{H}_2$-N) |
| 7.32 | (m; 5H; $\underline{Ph}$-CH$_2$-N) |

### 3.5 Preparation of

### 2-Benzoyl-4-Methyl-1-Phenylbicyclo[2.2.2]octan-5-one[48, 78]

N-(2-Butylidene)benzylamine (10.00 g; 0.062 mol), phenyl vinyl ketone (18.00 g; 0.14 mol) in "super-dry" methanol (200 ml), were heated under reflux for 6 hours in the presence of molecular sieves (4A). Water (10 ml) was added and the mixture heated under reflux for a further hour. The solvent was removed on a rotatory evaporator and the residue extracted with dichloromethane (2 x 100 ml), satd. aq. hydrochloric acid (2M; 2 x 100 ml), satd. sodium hydrogen carbonate (2 x 50 ml), water (2 x 50 ml) and satd. sodium chloride (50 ml) and finally dried over anhydrous magnesium sulphate. Removal of the solvents on a rotatory evaporator gave a brown oil (22.40 g). The crude product was shown by capillary g.l.c. to contain 47.10% of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one **(35)**. This corresponds to 10.55 g of the bicyclo[2.2.2]octanone in 22.40 g of crude product, and therefore a yield of 53.5%

A portion (5.00 g) of the crude product was purified by flash column chromatography [hexane:dichloromethane:ethyl acetate (60:30:20)] and gave 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one **(35)** as a mixture of two isomers (isomer I, 67.5% ; isomer II, 32.5%) . The 500 MHz $^1$H NMR and $^{13}$C NMR spectra have been assigned previously[48]. The 300 MHz $^1$H NMR and $^{13}$C NMR spectra have been included for comparison purposes.

(35) ISOMER I      (35) ISOMER II
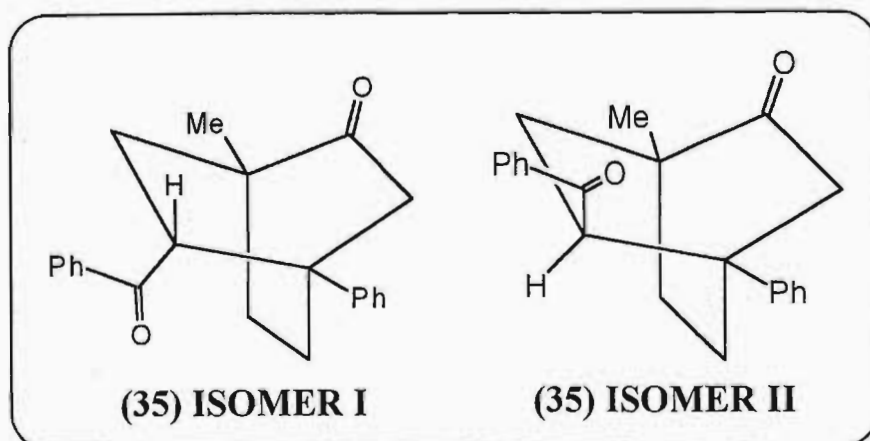
## 3.6 Reductive amination of

### 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one

### 3.6.1 Reductive Amination with Benzylamine

A mixture of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2]octan-5-one (5.00 g; 0.016 mol)[isomer I, 67.5% : isomer II, 32.5%], benzylamine (8.45 g; 0.079 mol) and sodium cyanoborohidride (1.00 g; 0.016 mol) in "super-dry" methanol (100 ml) was heated under reflux for 20 hours in the presence of molecular sieves (4A). The molecular sieves were filtered off. Concentrated HCl was added until pH<2, and the methanol was removed *in vacuo*. The residue was taken up in 10 ml of water and extracted with dichloromethane (3 x 50 ml). The aqueous solution was brought to pH>10 with solid potassium hydroxide, saturated with sodium chloride, and extracted with dichloromethane ( 3 x 50 ml). The combined extracts were dried (anhydrous magnesium sulphate) and evaporated *in vacuo* to give a brown oil (9.32 g) which was shown by capillary g.l.c. to contain 44.74% of product with $t_R$ = 25.02. This corresponds to 4.17 g of the pure product.

A portion (5.00 g) of the crude product was subjected to flash column chromatography using hexane, dichloromethane and ethyl acetate (60:30:10) as eluent and taking 35 fractions (40 ml). Fractions 3-6 were combined on the basis of t.l.c. and evaporated to give white crystals which proved to be 5-benzyl-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$] decane **(56)**.

Yield: 66% (based on g.l.c. percentage).

MP 144-146$^0$C

The GC-MS showed M$^+$:393.

The IR spectrum showed $v_{max}$(KBr) (cm$^{-1}$)

1355        (tertiary C-N stretching)
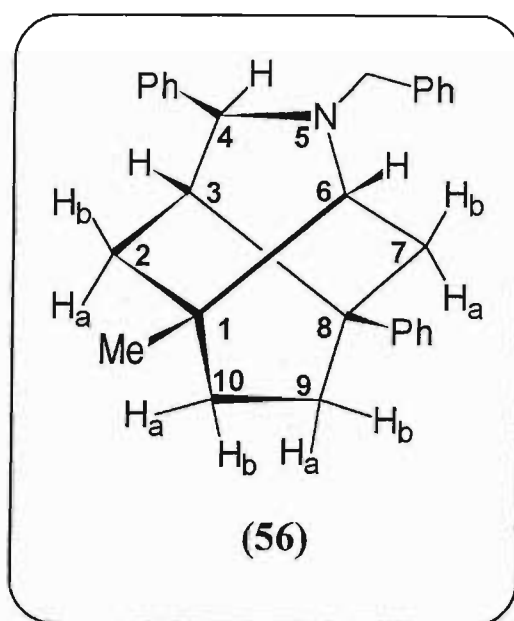


**(56)**

The $^1$H NMR spectrum (300 MHz, CDCl$_3$) showed δ(ppm)

| | |
|---|---|
| 0.91 | (s; 3H; CH$_3$) |
| 1.10-1.30 | (m; 2H; Ha-2; Ha-10) |
| 1.40-1.60 | (m; 2H; Hb-9; Hb-10) |
| 1.64-1.75 | (m; 2H; Hb-2; Ha-7) |
| 2.00 | (m; 1H; Ha-9) |
| 2.21 | (d; 1H; J = 6.0 Hz; H-3) |
| 2.34 | (dd; 1H; J = 12.6 Hz; J = 2.7 Hz; Hb-7) |
| 2.43 | (d; 1H; J = 4.8 Hz; H-6) |
| 3.35 | (d; 1H; J = 13.7 Hz; C$\underline{H}$-Ph) |
| 3.55 | (s; 1H; H-4) |
| 3.59 | (d; 1H; J = 13.7 Hz; C$\underline{H}$-Ph) |
| 7.20-7.50 | (15H; 3 x Ph) |

The $^{13}$C NMR and DEPT spectra (300 MHz; CDCl$_3$) showed δ(ppm)

| | | |
|---|---|---|
| 24.78 (q; CH$_3$) | 28.68 (t; C-7) | 30.44 (t; C-2) |
| 32.53 (t; C-10) | 34.56 (s; C-1) | 35.98 (t; C-9) |
| 39.74 (s; C-8) | 46.60 (d; C-3) | 55.06 (t; $\underline{C}$H$_2$-pH) |
| 57.79 (d; C-6) | 64.47 (d; C-4) | |

140.68; 143.46; 148.64 (3s; 3 quaternary Ph carbons)

126.36-129.49 (Phenyl $\underline{C}$H carbons)

C/H/N Analysis

|  | C | H | N |
|---|---|---|---|
| Required: | 88.50 | 7.94 | 3.56 |
| Experimental: | 88.39 | 8.19 | 3.51 |

## 3.6.2 Reductive Amination with ammonia

A mixture of 2-benzoyl-4-methyl-1-phenylbicyclo[2.2.2] octan-5-one (two isomers) (5.00 g; 0.016 mol), ammonium acetate (12.30 g; 0.16 mol), sodium cyanoborohydride (1.00 g; 0.016 mol) and toluene-4-sulphonic acid (0.3 g) in "super-dry" methanol (150 ml) was heated under reflux for 24 hours in the presence of molecular sieves. Work up in the same way gave a crude product (5.13 g) which was shown by capillary g.l.c. to be a multicomponent mixture containing 37.49% of product with $t_R = 15.66$. This corresponds to 1.93 g of the pure product.

A portion (4.00 g) of the crude product was subjected to flash column chromatography using hexane, dichloromethane and ethyl acetate [60:30:10] as eluent and taking 28 fractions. Fractions 1-3 were combined on the basis of t.l.c. and evaporated to give white crystals. These crystals proved to be 6-cyano-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$] decane **(60)**.

Yield: 36.8% (based on g.l.c. percentage).

MP 186-188$^0$C

The GC-MS showed M$^+$ 328

The IR spectrum showed $\nu_{max}$(KBr) (cm$^{-1}$)

3300            (NH)

2250            (CN)



**(60)**
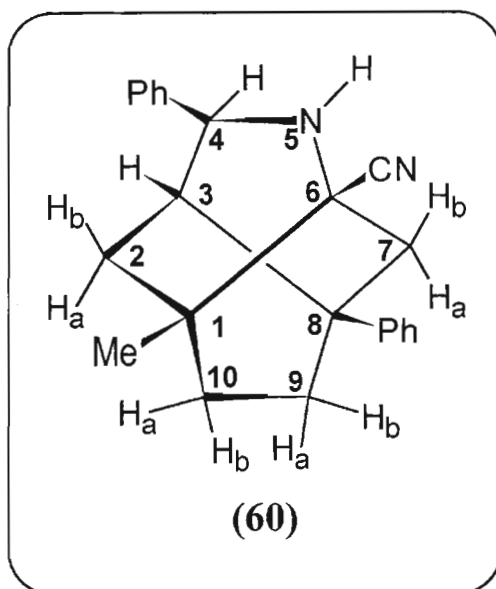
The $^1$H NMR spectrum (300 MHz; CDCl$_3$) showed δ(ppm)

1.25            (s; 3H; CH$_3$)

1.3-1.5         (m; 2H; Ha-2, Ha-10)

1.5-1.65        (m; 2H; Hb-9; Hb-10)

1.7-1.9         (m; 1H; Hb-2)

2.10            (m; 1H; Ha-9)

2.33            (d; 1H; J = 6.1 Hz; H-3)

2.42            (d; 1H; J = 12.6 Hz; Ha-7)

2.61            (dd; 1H; J = 12.6 Hz; J = 2.7 Hz; Hb-7)

4.21            (s; 1H; H-4)

7.15-7.50       (10H; 2 x Ph)

The $^{13}C$ NMR and DEPT spectra (300 Hz; CDCl$_3$) showed (ppm)

| | | |
|---|---|---|
| 22.23 (q; CH$_3$) | 29.42 (t; C-10) | 30.63 (t; C-2) |
| 34.50 (t; C-9) | 37.65* (s; C-1) | 38.46* (s; C-8) |
| 41.66 (t; C-7) | 43.55 (d; C-3) | 54.57 (d; C-4) |
| 55.64 (s; C-6) | 120.75 (s; CN) | |

141.72; 145.63; (2 x s; 2 quaternary phenyl carbons)

| | | |
|---|---|---|
| 125.79 (2 x d) | 126.44 (d) | 126.80 (2 x d) |
| 126.86 (d) | 128.61 (2 x d) | 128.86 (2 x d) |

*Interchangeable

C/H/N Analysis

| | C | H | N |
|---|---|---|---|
| Required: | 84.10 | 7.37 | 8.53 |
| Experimental: | 82.20 | 7.38 | 8.30 |

## 3.7 Preparation of 4a-Methyl-5-oxo-$\Delta^{1,8a}$-2-octalone

This was prepared by the literature method[79].

A solution of 2-methylcyclohexane-1,3-dione (25.20 g; 0.20 mol), methyl vinyl ketone (21.00 g; 0.30 mol) and potassium hydroxide (0.2 g) in methanol 100 ml was heated under reflux until the dione dissolved (3 hr). The solvent and excess methyl vinyl ketone were removed *in vacuo*. The intermediate 2-methyl-2-(3-oxobutyl)cyclohexane-1,3-dione was dissolved in benzene (100 ml) and a Dean and Stark head attached. Traces of water and methanol were removed by distillation of benzene (20 ml). The solution

was cooled well below the boiling point, pyrrolidine (1.5 ml) added and the mixture heated under reflux until no further liberation of water was observed (30 min). The water was removed and 50 ml of benzene distilled off. The reaction mixture was cooled to room temperature, diluted with ether and washed with water (40 ml) containing hydrochloric acid (6 ml; 10%) and finally with water (40 ml). The aqueous phases were extracted with ether (2 x 50 ml) and the combined ether layers washed with water (3 x 50 ml), chloride solution (50 ml) and dried over anhydrous magnesium sulphate. The solvents were removed *in vacuo* and the residue distilled under reduced pressure. The fraction distilling at 136-140°C/0.65 mmHg was collected (22.24 g), diluted with ether (5 ml) and left in the freezer overnight. The resulting crystals were collected and washed with hexane to give 4a-methyl-5-oxo-2-octalone (15.72 g; 44%), MP 48-50°C.



**4a-Methyl-5-oxo-$\Delta^{1,8a}$-2-octalone**
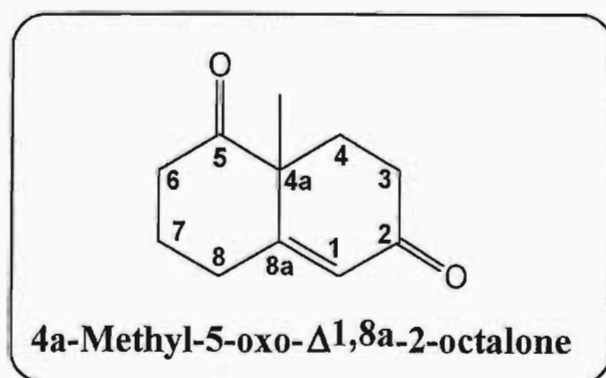
The IR spectrum showed $v_{max}$ (film) (cm$^{-1}$)

| | |
|---|---|
| 1620 | (C=C) |
| 1660 | (CO; $\alpha,\beta$-unsaturated) |
| 1710 | (CO) |

The $^1$H NMR spectrum (200 MHz; CDCl$_3$) showed $\delta$(ppm)

| | |
|---|---|
| 1.48 | (s; 3H' CH$_3$) |
| 1.6-1.9 | (m; 1H) |
| 2.0-2.3 | (m; 2H) |
| 2.4-2.6 | (m; 4H) |
| 2.7-2.9 | (m; 2H) |
| 5.85 | (s; 1H; H-1) |

The $^{13}$C NMR spectrum (200 Hz; CDCl$_3$) showed δ(ppm)

| | | |
|---|---|---|
| 21.90 (t) | 22.11 (q; CH$_3$) | 28.57 (t) |
| 30.69 (t) | 32.46 (t) | 36.55 (t) |
| 49.61 (s; C-4a) | 124.31 (d; C-1) | 166.17 (s; C-8a) |
| 197.73 (s; C-1) | 210.06 (s; C-5) | |

## 3.8 Pyrrolidine dienamine of 4a-Methyl-5-oxo-Δ$^{1,8a}$-2-octalone

A solution of 4a-methyl-5-oxo-$^{1,8a}$-2-octalone (12.00 g; 0.067 mol), pyrrolidine (13.00 g; 0.18 mol) and toluene-4-sulphonic acid (0.3 g) in toluene (120 ml) was heated under reflux for 24 h. using a Dean and Stark head followed by an additional 24 h. period of reflux over molecular sieves (4A). The volatiles were removed *in vacuo* and the residue distilled under reduced pressure to give the pyrrolidine dienamine of 4a-methyl-5-oxo-$^{1,8a}$-2-octalone (**39b**) (7.85 g; 51%), BP 170-172°C/1.0 mm Hg.

**39b**
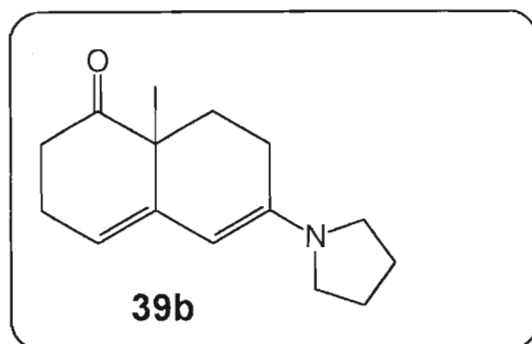
The IR spectrum showed $\nu_{max}$ (film) cm$^{-1}$

| 1600 and 1625 | (C=C) |
| 1705 | (CO) |

The $^1$H NMR spectrum (200 MHz; CDCl$_3$) showed δ(ppm)

| 1.13 | (s; 3H; CH$_3$) |
| 1.4-2.8 | (complex methylene/methine envelope) |
| 3.06 | (m; 4H; CH$_2$-N-CH$_2$) |
| 4.72 | (S; 1H; H-1) |
| 5.16 | (dd; 1H; J = 3.6, 4.8 Hz; H-8) |

$^1$H NMR measurements indicated the dienamine to be only in the exocyclic form **(39)**.

### 3.9 Reaction of MVK with the Pyrrolidine dienamine of
### 4a-Methyl-5-oxo-Δ$^{1,8a}$-2-octalone

Methyl vinyl ketone (1.60 g; 0.023 mol) was added dropwise under nitrogen to a stirred solution of the pyrrolidine dienamine of 4a-methyl-5-oxo-$^{1,8a}$-2-octalone (5.00 g; 0.022 mol) in dry toluene (100 ml), and heated under reflux for 45h. A positive pressure of nitrogen was

maintained throughout the reaction. The mixture was hydrolysed by heating under reflux for 5 h. with a buffer solution of anhydrous sodium acetate (5 g) and glacial acetic acid (10 ml) in water (10 ml). The volatiles were removed *in vacuo* and the residue extracted with methylene chloride (3 x 50 ml). The combined methylene chloride extracts were washed successively with 2N-hydrochloric acid (3 x 50 ml). The aqueous solution was basified (pH>10) with solid hydroxide and extracted with methylene chloride. The organic layer was dried (anhydrous $MgSO_4$), filtered and evaporated under reduced pressure. The GC-MS analysis showed the crude product to consist largely of pyrrolidine.

# APPENDIX

## A. CRYSTAL STRUCTURE DATA AND STEREOSCOPIC DRAWING FOR 5-BENZYL-1-METHYL-4,8-DIPHENYL-5-AZATRICYCLO[4.4.0.0$^{3,8}$]DECANE (56)[*]

| | |
|---|---|
| Formula | C$_{29}$H$_{31}$N |
| M | 393 |
| Space Group | I4 |
| R/A | 14.816 (4) |
| Z | 8 |
| Final R | 0.0438 (2487 reflections, 365 parameters) |

The carbon-carbon and carbon-nitrogen bond lengths are given in Table 2, the carbon-hydrogen bond lengths are given in Table 3 and the bond angles in Table 4.

[*]Ref : Field, J. S. and Ramasar, N.; Personal communication

The stereoscopic drawing of 5-benzyl-1-methyl-4,8-diphenyl-5-azatricyclo[4.4.0.0$^{3,8}$]decane (56)
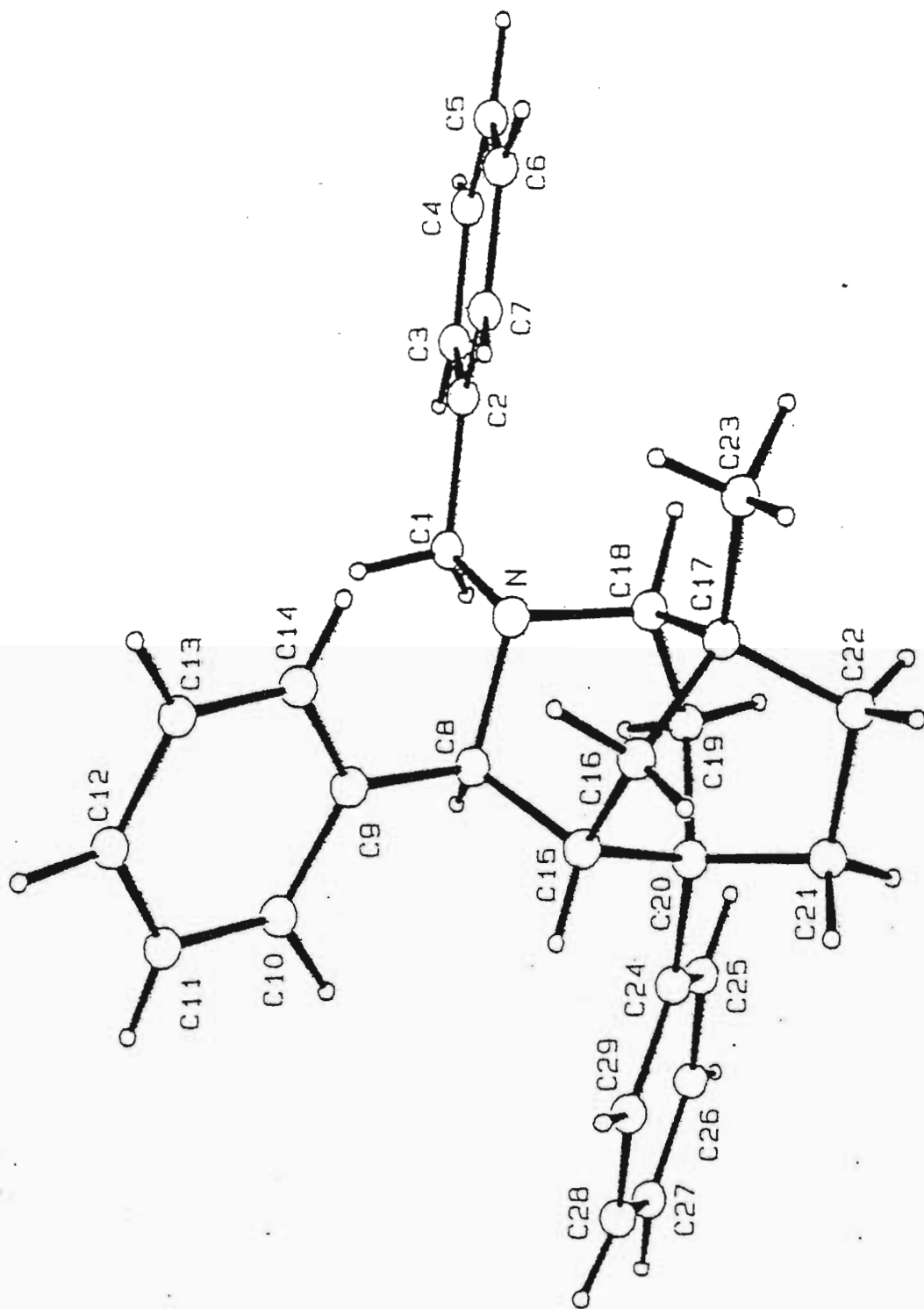
TABLE 2

| C-C AND C-N BOND LENGTHS (Å) | | | |
|---|---|---|---|
| | BOND LENGTHS (Å) | | BOND LENGTHS (Å) |
| N-C(1) | 1.462(4) | C(13)-C(14) | 1.387(5) |
| N-C(8) | 1.489(4) | C(15)-C(16) | 1.528(5) |
| N-C(18) | 1.484(4) | C(15)-C(20) | 1.568(4) |
| C(1)-C(2) | 1.516(5) | C(16)-C(17) | 1.534(5) |
| C(2)-C(3) | 1.394(5) | C(17)-C(18) | 1.576(5) |
| C(2)-C(7) | 1.368(5) | C(17)-C(22) | 1.546(5) |
| C(3)-C(4) | 1.387(5) | C(17)-C(23) | 1.524(5) |
| C(4)-C(5) | 1.361(6) | C(18)-C(19) | 1.525(5) |
| C(5)-C(6) | 1.367(6) | C(19)-C(20) | 1.544(5) |
| C(6)-C(7) | 1.392(5) | C(20)-C(21) | 1.555(5) |
| C(8)-C(9) | 1.522(4) | (C20)-C(24) | 1.517(5) |
| C(8)-C(15) | 1.540(4) | C(21)-C(22) | 1.545(6) |
| C(9)-C(10) | 1.399(5) | C(24)-C(25) | 1.392(5) |
| C(9)-C(14) | 1.374(5) | C(24)-C(29) | 1.383(5) |
| C(10)-C-11) | 1.382(6) | C(25)-C(26) | 1.370(6) |
| 11)-C(12)C( | 1.368(7) | C(26)-C(27) | 1.360(6) |
| C(12)-C(13) | 1.364(7) | C(27)-C(28) | 1.355(6) |
| | | C(28)-C(29) | 1.382(5) |

TABLE 3

| C-H BOND LENGTHS (Å) | | | |
|---|---|---|---|
| | BOND LENGTHS (Å) | | BOND LENGTHS (Å) |
| C(1)-H(1A) | 1.02(3) | C(18)-H(18) | 1.07(3) |
| C(1)-H(1B) | 1.01(3) | C(19)-H(19A) | 1.01(3) |
| C(3)-H(3) | 1.01(3) | C(19)-H(19B) | .94(3) |
| C(4)-H(4) | .95(3) | C(21)-H(21A) | 1.04(3) |
| C(5)-H(5) | 1.00(3) | C(21)-H(21B) | .96(3) |
| C(6)-H(6) | .92(3) | C(22)-H(22A) | .88(3) |
| C(7)-H(7) | 1.02(3) | C(22)-H(22B) | 1.06(3) |
| C(8)-H(8) | .99(3) | C(23)-H(23A) | .92(3) |
| C(10)-H(10) | 1.02(3) | C(23)-H23B) | 1.09(3) |
| C(11)-H(11) | .91(3) | C(23)-H23C) | 1.03(3) |
| C(12)-H(12) | 1.04(3) | C(25)-H(25) | .92(3) |
| C(13)-H(13) | .98(3) | C(26)-H(26) | .92(3) |
| C(14)-H(14) | .90(3) | C(27)-H(27) | .95(3) |
| C(15)-H(15) | .94(3) | C(28)-H(28) | .90(3) |
| C(16)-H(16A) | 1.09(3) | C(29)-H(29) | .95(3) |
| C(16)-H(16B) | .90(3) | | |

TABLE 4

| BOND ANGLES (⁰) | | | |
|---|---|---|---|
| | BOND ANGLES(⁰) | | BOND ANGLES (⁰) |
| C(1)-N-C-(8) | 109.7(2) | C(1)-N-C(18) | 113.1(3) |
| C(8)-N-C(18) | 108.4(2) | N-C(1)-C(2) | 114.7(3) |
| N-C(1)-H(1A) | 111(2) | C(2)-C(1)-H(1A) | 111(2) |
| N-C(1)-H(1B) | 109(2) | C(2)-C(1)-H(1B) · | 109(2) |
| H(1A)-C(1)-H(1B) | 101(3) | C(1)-C(2)-C(3) | 118.4(3) |
| C(1)-C(2)-C(7) | 122.3(3) | C(3)-C(2)-C(7) | 119.3(3) |
| C(2)-C(3)-C(4) | 120.0(4) | C(2)-C(3)-H(3) | 116(2) |
| C(4)-C(3)-H(3) | 124(2) | C(3)-C(4)-C(5) | 120.6(4) |
| C(3)-C(4)-H(4) | 115(2) | C(5)-C(4)-H(4) | 125(2) |
| C(4)-C(5)-C(6) | 119.3(4) | C(4)-C(5)-H(5) | 118(2) |
| C(6)-C(5)-H(5) | 123(2) | C(5)-C(6)-C(7) | 121.3(4) |
| C(5)-C(6)-H(6) | 123(2) | C(7)-C(6)-H(6) | 115(2) |
| C(2)-C(6)-C(67) | 119.5(4) | C(2)-C(7)-H(7) | 118(2) |
| C(6)-C(7)-H(7) | 122(2) | N-C(8)-C(9) | 111.7(3) |
| N-C(8)-C(15) | 106.3(2) | C(9)-C(8)-C(15) | 112.2(3) |
| N-C(8)-H(8) | 112(2) | C(9)-C(8)-H(8) | 105(2) |
| C(15)-C(8)-H(8) | 109(2) | C(8)-C(9)-C(10) | 118.1(3) |
| C(8)-C(9)-C(14) | 122.9(4) | C(10)-C(9)-C(14) | 119.0(4) |
| C(9)-C(10)-C(11) | 119.6(4) | C(9)-C(10)-H(10) | 117(2) |
| C(11)-C(10)-H(10) | 123(2) | C(10)-C(11)-C(12) | 120.5(4) |
| C(10)-C(11)-H(11) | 112(2) | C(12)-C(11)-H(11) | 128(2) |
| C(11)-C(12)-C(13) | 120.3(4) | C(11)-C(12)-H(12) | 116(2) |
| C(13)-C(12)-H(12) | 124(2) | C(12)-C(13)-C(14) | 120.0(5) |
| C(12)-C(13)-H(13) | 119(2) | C(14)-C(13)-H(13) | 121(2) |
| C(9)-C(14)-C(13) | 120.6(4) | C(9)-C(14)-H(14) | 118(2) |
| C(13)-C(14)-H(14) | 121(2) | C(8)-C(15)-C(16) | 106.6(3) |
| C(8)-C(15)-C(20) | 109.5(3) | C(16)-C(15)-C(20) | 109.0(3) |
| C(8)-C(15)-H(15) | 111(2) | C(16)-C(15)-H(15) | 109(2) |
| C(20)-C(15)-H(15) | 111(2) | C(15)-C(16)-C(17) | 105.2(3) |
| C(15)-C(16)-H(16A) | 112(2) | C(17)-C(16)-H(16A) | 110(2) |
| C(15)-C(16)-H(16B) | 116(2) | C(17)-C(16)-H(16B) | 111(2) |
| H(16A)-C(16)-H(16B) | 103(3) | C(16)-C(17)-C(18) | 105.5(3) |
| C(16)-C(17)-C(22) | 105.2(3) | C(18)-C(17)-C(22) | 108.9(3) |
| C(16)-C(17)-C(23) | 113.0(3) | C(18)-C(17)-C(23) | 112.2(3) |
| C(22)-C(17)-C(23) | 111.5(3) | N-C(18)-C(17) | 107.8(3) |

| BOND ANGLES ($^0$) | | | |
|---|---|---|---|
| | BOND ANGLES ($^0$) | | BOND ANGLES ($^0$) |
| N-C(18)-C(19) | 109.4(3) | C(17)-C(18)-C(19 | 108.2(3) |
| N-C(18)-H(18) | 110(2) | C(17)-C(18)-H(18) | 111(2) |
| C(19)-C(18)-H(18) | 111(2) | C(18)-C(19)-C(20) | 105.8(3) |
| C(18)-C(19)-H(19A) | 112(2) | C(20)-C(19)-H(19A) | 111(2) |
| C(18)-C(19)-H(19B) | 113(2) | C(20)-C(19)-H(19B) | 113(2) |
| H(19A)-C(19)-H(19B) | 101(3) | C(15)-C(20)-C(19). | 105.1(3) |
| C(15)-C(20)-C(21) | 109.2(3) | C(19)-C(20)-C(21) | 104.4(3) |
| C(15)-C(20)-C(24) | 113.4(3) | C(19)-C(20)-C(24) | 113.7(3) |
| C(21)-C(20)-C(24) | 110.6(3) | C(20)-C(21)-C(22) | 108.5(3) |
| C(20)-C(21)-H(21A) | 108(2) | C(22)-C(21)-H(21A) | 111(2) |
| C(20)-C(21)-H(21B) | 108(2) | C(22)-C(21)-H(21B) | 113(2) |
| H(21A)-C(21)-H(21B) | 109(3) | C(17)-C(22)-C(21) | 109.2(3) |
| C(17)-C(22)-H(22A) | 115(2) | C(21)-C(22)-H(22A) | 111(3) |
| C(17)-C(22)-H(22B) | 109(2) | C(21)-C(22)-H(22B) | 109(2) |
| H(22A)-C(22)-H(22B) | 105(3) | C(17)-C(23)-H(23A) | 109(2) |
| C(17)-C(23)-H(23B) | 118(2) | H(23A)-C(23)-H(23B) | 100(3) |
| C(17)-C(23)-H(23C) | 111(2) | H(23A)-C(23)-H(23C) | 106(3) |
| H(23B)-C(23)-H(23C) | 113(2) | C(20)-C(24)-C(25) | 121.8(3) |
| C(20)-C(24)-C(29) | 122.7(3) | C(25)-C(24)-C(29) | 115.5(4) |
| C(24)-C(25)-C(26) | 122.2(4) | C(24)-C(25)-H(25) | 117(2) |
| C(26)-C(25)-H(25) | 121(2) | C(25)-C(26)-C(27) | 121.4(4) |
| C(25)-C(26)-H(26) | 120(2) | C(27)-C(26)-H(26) | 118(2) |
| C(26)-C(27)-C(28) | 117.6(4) | C(26)-C(27)-H(27) | 122(2) |
| C(28)-C(27)-H(27) | 121(2) | C(27)-C(28)-C(29) | 122.0(4) |
| C(27)-C(28)-H(28) | 118(2) | C(29)-C(28)-H(28) | 120(2) |
| C(24)-C(29)-C(28) | 121.3(4) | C(24)-C(29)-H(29) | 116(2) |
| C(28)-C(29)-H(29) | 123(2) | | |

# B. CRYSTAL STRUCTURE DATA AND STEREOSCOPIC DRAWING FOR 6-CYANO-1-METHYL-4,8-DIPHENYL-5-AZATRICYCLO[4.4.0.0$^{3,8}$]DECANE (60)
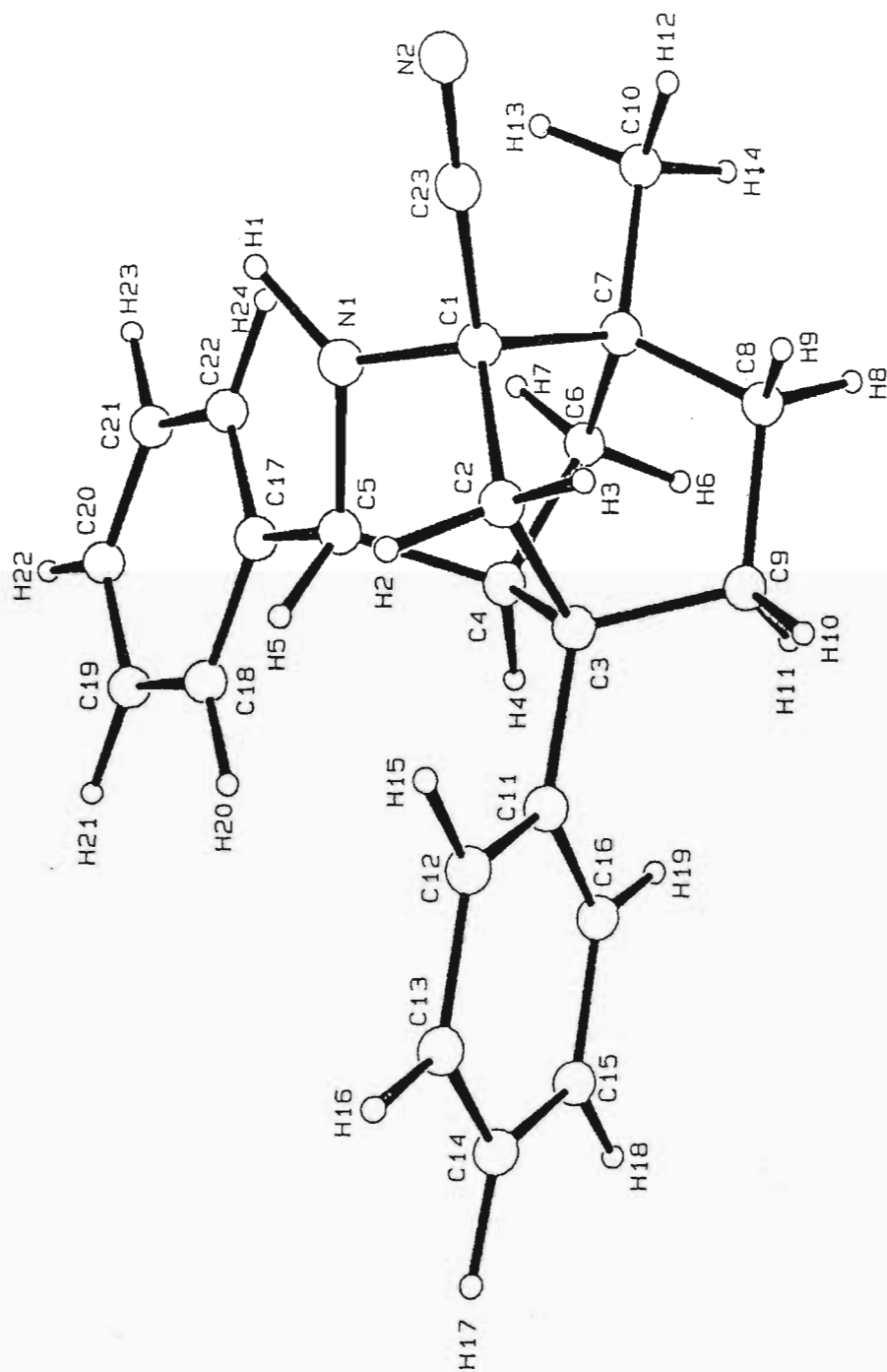
Table . FRACTIONAL COORDINATES ($\times 10^4$) AND ISOTROPIC THERMAL FACTORS ($Å^2$, $\times 10^3$) FOR

$C_{23}H_{24}N_2$

|        | x/a       | y/b       | z/c       | $U_{eq}$  |
|--------|-----------|-----------|-----------|-----------|
| N(1)   | 3693(1)   | 2103(1)   | 9163(1)   | 39        |
| H(1)   | 4401(1)   | 2443(1)   | 9610(1)   | 76(2)*    |
| N(2)   | 5439(2)   | −610(2)   | 9092(1)   | 56        |
| C(1)   | 3704(2)   | 935(2)    | 8568(1)   | 38        |
| C(2)   | 2579(2)   | 152(2)    | 8567(1)   | 41        |
| H(2)   | 2316(2)   | 99(2)     | 9213(1)   | 76(2)*    |
| H(3)   | 2662(2)   | −858(2)   | 8317(1)   | 76(2)*    |
| C(3)   | 1712(2)   | 986(2)    | 7967(1)   | 41        |
| C(4)   | 2092(2)   | 2507(2)   | 8088(1)   | 38        |
| H(4)   | 1385(2)   | 3166(2)   | 7882(1)   | 76(2)*    |
| C(5)   | 2542(1)   | 2729(2)   | 9047(1)   | 37        |
| H(5)   | 1962(1)   | 2262(2)   | 9452(1)   | 76(2)*    |
| C(6)   | 3096(2)   | 2774(2)   | 7561(1)   | 43        |
| H(6)   | 2793(2)   | 2987(2)   | 6893(1)   | 76(2)*    |
| H(7)   | 3596(2)   | 3617(2)   | 7832(1)   | 76(2)*    |
| C(7)   | 3817(2)   | 1478(2)   | 7627(1)   | 43        |
| C(8)   | 3204(2)   | 488(2)    | 6966(1)   | 53        |
| H(8)   | 3370(2)   | 770(2)    | 6318(1)   | 76(2)*    |
| H(9)   | 3518(2)   | −522(2)   | 7106(1)   | 76(2)*    |
| C(9)   | 1913(2)   | 528(2)    | 7038(1)   | 53        |
| H(10)  | 1550(2)   | −464(2)   | 6915(1)   | 76(2)*    |
| H(11)  | 1512(2)   | 1235(2)   | 6569(1)   | 76(2)*    |
| C(10)  | 5055(2)   | 1741(3)   | 7466(2)   | 59(1)     |
| H(12)  | 5535(2)   | 809(3)    | 7516(2)   | 76(2)*    |
| H(13)  | 5426(2)   | 2448(3)   | 7947(2)   | 76(2)*    |
| H(14)  | 5077(2)   | 2166(3)   | 6826(2)   | 76(2)*    |
| C(11)  | 478(2)    | 795(2)    | 8139(1)   | 46        |
| C(12)  | 147(2)    | −109(3)   | 8747(2)   | 61(1)     |
| H(15)  | 787(2)    | −725(3)   | 9109(2)   | 76(2)*    |
| C(13)  | −999(2)   | −226(3)   | 8888(2)   | 73(1)     |
| H(16)  | −1243(2)  | −932(3)   | 9367(2)   | 76(2)*    |
| C(14)  | −1822(2)  | 537(3)    | 8424(2)   | 73(1)     |
| H(17)  | −2699(2)  | 462(3)    | 8558(2)   | 76(2)*    |
| C(15)  | −1512(2)  | 1397(3)   | 7799(2)   | 83(1)     |

Table  . /Cont.

| | | | |
|---|---|---|---|
| H(18) | −2167(2) | 1976(3) | 7425(2) | 76(2)* |
| C(16) | −377(2) | 1539(3) | 7659(2) | 72(1) |
| H(19) | −143(2) | 2216(3) | 7161(2) | 76(2)* |
| C(17) | 2575(2) | 4202(2) | 9321(1) | 40 |
| C(18) | 1567(2) | 4814(2) | 9506(2) | 60(1) |
| H(20) | 783(2) | 4243(2) | 9436(2) | 76(2)* |
| C(19) | 1565(2) | 6152(3) | 9775(2) | 74(1) |
| H(21) | 779(2) | 6612(3) | 9923(2) | 76(2)* |
| C(20) | 2546(2) | 6890(2) | 9865(2) | 66(1) |
| H(22) | 2540(2) | 7928(2) | 10080(2) | 76(2)* |
| C(21) | 3556(2) | 6307(2) | 9671(2) | 63(1) |
| H(23) | 4332(2) | 6893(2) | 9730(2) | 76(2)* |
| C(22) | 3565(2) | 4962(2) | 9403(2) | 49 |
| H(24) | 4354(2) | 4512(2) | 9254(2) | 76(2)* |
| C(23) | 4688(2) | 69(2) | 8870(1) | 42 |

\* isotropic temperature factor.

$$U_{eq} = \tfrac{1}{3} \Sigma_i \Sigma_j U_{ij} a_i^* a_j^* (a_i \cdot a_j)$$

Table . ANISOTROPIC TEMPERATURE FACTORS ($\mathring{A}^2$, $\times 10^3$) FOR $C_{23}H_{24}N_2$

|  | U(11) | U(22) | U(33) | U(23) | U(13) | U(12) |
|---|---|---|---|---|---|---|
| N(1) | 36(1) | 35(1) | 45(1) | -1(1) | -4(1) | 4(1) |
| N(2) | 61(1) | 56(1) | 49(1) | 3(1) | 1(1) | 23(1) |
| C(1) | 41(1) | 32(1) | 39(1) | 4(1) | 0(1) | 6(1) |
| C(2) | 46(1) | 31(1) | 46(1) | 4(1) | -1(1) | -1(1) |
| C(3) | 43(1) | 36(1) | 42(1) | 1(1) | -4(1) | -2(1) |
| C(4) | 38(1) | 33(1) | 41(1) | 5(1) | -1(1) | 2(1) |
| C(5) | 36(1) | 31(1) | 42(1) | 2(1) | 2(1) | 1(1) |
| C(6) | 46(1) | 40(1) | 45(1) | 11(1) | 6(1) | 7(1) |
| C(7) | 47(1) | 41(1) | 43(1) | 8(1) | 9(1) | 9(1) |
| C(8) | 64(1) | 54(1) | 41(1) | -2(1) | 2(1) | 11(1) |
| C(9) | 64(1) | 51(1) | 42(1) | -4(1) | -5(1) | 5(1) |
| C(10) | 52(1) | 60(1) | 69(1) | 18(1) | 21(1) | 12(1) |
| C(11) | 43(1) | 40(1) | 52(1) | -5(1) | -4(1) | -6(1) |
| C(12) | 55(1) | 70(1) | 57(1) | 1(1) | -1(1) | -15(1) |
| C(13) | 62(1) | 91(2) | 67(2) | -9(1) | 8(1) | -33(1) |
| C(14) | 49(1) | 72(2) | 97(2) | -35(2) | 10(1) | -16(1) |
| C(15) | 45(1) | 62(2) | 137(3) | -2(2) | -9(1) | -3(1) |
| C(16) | 46(1) | 64(1) | 102(2) | 16(1) | -10(1) | -4(1) |
| C(17) | 43(1) | 35(1) | 42(1) | 2(1) | 3(1) | 2(1) |
| C(18) | 52(1) | 48(1) | 83(2) | 0(1) | 19(1) | 6(1) |
| C(19) | 78(2) | 52(1) | 99(2) | -3(1) | 34(1) | 19(1) |
| C(20) | 97(2) | 38(1) | 65(1) | -5(1) | 15(1) | 7(1) |
| C(21) | 72(1) | 42(1) | 73(2) | -4(1) | -2(1) | -7(1) |
| C(22) | 47(1) | 40(1) | 60(1) | -5(1) | 1(1) | -2(1) |
| C(23) | 46(1) | 39(1) | 42(1) | 3(1) | 2(1) | 6(1) |

Table . INTERATOMIC ANGLES (°) FOR

$C_{23}H_{24}N_2$

| | | | |
|---|---|---|---|
| H(1)-N(1)-C(1) | 125.8(1) | H(1)-N(1)-C(5) | 125.6(1) |
| C(1)-N(1)-C(5) | 108.6(1) | N(1)-C(1)-C(2) | 109.2(1) |
| N(1)-C(1)-C(7) | 108.6(1) | C(2)-C(1)-C(7) | 109.3(1) |
| N(1)-C(1)-C(23) | 108.3(1) | C(2)-C(1)-C(23) | 110.9(1) |
| C(7)-C(1)-C(23) | 110.5(1) | C(1)-C(2)-H(2) | 111.0(1) |
| C(1)-C(2)-H(3) | 110.8(1) | H(2)-C(2)-H(3) | -21.6(0) |
| C(1)-C(2)-C(3) | 104.3(1) | H(2)-C(2)-C(3) | 110.3(1) |
| H(3)-C(2)-C(3) | 111.0(1) | C(2)-C(3)-C(4) | 106.0(1) |
| C(2)-C(3)-C(9) | 104.2(2) | C(4)-C(3)-C(9) | 108.7(2) |
| C(2)-C(3)-C(11) | 114.3(1) | C(4)-C(3)-C(11) | 111.3(1) |
| C(9)-C(3)-C(11) | 111.8(1) | C(3)-C(4)-H(4) | 109.9(1) |
| C(3)-C(4)-C(5) | 108.4(1) | H(4)-C(4)-C(5) | 112.0(1) |
| C(3)-C(4)-C(6) | 109.1(1) | H(4)-C(4)-C(6) | 110.8(1) |
| C(5)-C(4)-C(6) | 106.5(1) | N(1)-C(5)-C(4) | 105.8(1) |
| N(1)-C(5)-H(5) | 112.3(1) | C(4)-C(5)-H(5) | 109.0(1) |
| N(1)-C(5)-C(17) | 111.8(1) | C(4)-C(5)-C(17) | 113.7(1) |
| H(5)-C(5)-C(17) | 104.4(1) | C(4)-C(6)-H(6) | 110.5(1) |
| C(4)-C(6)-H(7) | 110.3(1) | H(6)-C(6)-H(7) | -21.6(0) |
| C(4)-C(6)-C(7) | 106.0(1) | H(6)-C(6)-C(7) | 110.5(1) |
| H(7)-C(6)-C(7) | 110.1(1) | C(1)-C(7)-C(6) | 104.2(1) |
| C(1)-C(7)-C(8) | 108.6(2) | C(6)-C(7)-C(8) | 105.6(1) |
| C(1)-C(7)-C(10) | 112.7(1) | C(6)-C(7)-C(10) | 112.3(2) |
| C(8)-C(7)-C(10) | 112.8(2) | C(7)-C(8)-H(8) | 109.6(1) |
| C(7)-C(8)-H(9) | 109.2(1) | H(8)-C(8)-H(9) | -21.6(0) |
| C(7)-C(8)-C(9) | 109.5(2) | H(8)-C(8)-C(9) | 109.9(1) |
| H(9)-C(8)-C(9) | 109.3(1) | C(3)-C(9)-C(8) | 109.0(2) |
| C(3)-C(9)-H(10) | 109.4(1) | C(8)-C(9)-H(10) | 109.9(1) |
| C(3)-C(9)-H(11) | 109.6(1) | C(8)-C(9)-H(11) | 109.5(1) |
| H(10)-C(9)-H(11) | -21.6(0) | C(7)-C(10)-H(12) | 110.2(1) |
| C(7)-C(10)-H(13) | 108.7(1) | H(12)-C(10)-H(13) | -21.6(0) |
| C(7)-C(10)-H(14) | 109.5(1) | H(12)-C(10)-H(14) | -21.6(0) |
| H(13)-C(10)-H(14) | -21.6(0) | C(3)-C(11)-C(12) | 123.0(2) |
| C(3)-C(11)-C(16) | 119.4(2) | C(12)-C(11)-C(16) | 117.5(2) |
| C(11)-C(12)-H(15) | 119.0(1) | C(11)-C(12)-C(13) | 120.6(2) |
| H(15)-C(12)-C(13) | 120.4(2) | C(12)-C(13)-H(16) | 119.7(2) |

Table  . /Cont.

| | | | |
|---|---|---|---|
| C(12)-C(13)-C(14) | 121.0(2) | H(16)-C(13)-C(14) | 119.4(2) |
| C(13)-C(14)-H(17) | 120.1(2) | C(13)-C(14)-C(15) | 119.1(2) |
| H(17)-C(14)-C(15) | 120.9(2) | C(14)-C(15)-H(18) | 118.7(2) |
| C(14)-C(15)-C(16) | 120.6(3) | H(18)-C(15)-C(16) | 120.7(2) |
| C(11)-C(16)-C(15) | 121.2(3) | C(11)-C(16)-H(19) | 118.8(1) |
| C(15)-C(16)-H(19) | 120.0(2) | C(5)-C(17)-C(18) | 118.6(2) |
| C(5)-C(17)-C(22) | 123.0(2) | C(18)-C(17)-C(22) | 118.4(2) |
| C(17)-C(18)-H(20) | 119.6(1) | C(17)-C(18)-C(19) | 120.4(2) |
| H(20)-C(18)-C(19) | 120.0(1) | C(18)-C(19)-H(21) | 119.7(1) |
| C(18)-C(19)-C(20) | 120.8(2) | H(21)-C(19)-C(20) | 119.5(1) |
| C(19)-C(20)-H(22) | 120.5(1) | C(19)-C(20)-C(21) | 119.7(2) |
| H(22)-C(20)-C(21) | 119.8(1) | C(20)-C(21)-H(23) | 120.0(1) |
| C(20)-C(21)-C(22) | 119.8(2) | H(23)-C(21)-C(22) | 120.2(1) |
| C(17)-C(22)-C(21) | 120.8(2) | C(17)-C(22)-H(24) | 119.7(1) |
| C(21)-C(22)-H(24) | 119.4(1) | N(2)-C(23)-C(1) | 178.9(2) |

Table  . INTERATOMIC DISTANCES (Å) FOR

$C_{23}H_{24}N_2$

| | | | |
|---|---|---|---|
| N(1)-H(1) | 1.080(0) | N(1)-C(1) | 1.480(2) |
| N(1)-C(5) | 1.486(2) | N(2)-C(23) | 1.135(2) |
| C(1)-C(2) | 1.537(2) | C(1)-C(7) | 1.573(2) |
| C(1)-C(23) | 1.478(2) | C(2)-H(2) | 1.080(0) |
| C(2)-H(3) | 1.080(0) | C(2)-C(3) | 1.545(2) |
| C(3)-C(4) | 1.576(2) | C(3)-C(9) | 1.552(3) |
| C(3)-C(11) | 1.520(3) | C(4)-H(4) | 1.080(0) |
| C(4)-C(5) | 1.539(2) | C(4)-C(6) | 1.532(3) |
| C(5)-H(5) | 1.080(0) | C(5)-C(17) | 1.517(2) |
| C(6)-H(6) | 1.080(0) | C(6)-H(7) | 1.080(0) |
| C(6)-C(7) | 1.536(2) | C(7)-C(8) | 1.540(3) |
| C(7)-C(10) | 1.531(3) | C(8)-H(8) | 1.080(0) |
| C(8)-H(9) | 1.080(0) | C(8)-C(9) | 1.540(3) |
| C(9)-H(10) | 1.080(0) | C(9)-H(11) | 1.080(0) |
| C(10)-H(12) | 1.080(0) | C(10)-H(13) | 1.080(0) |
| C(10)-H(14) | 1.080(0) | C(11)-C(12) | 1.385(3) |
| C(11)-C(16) | 1.397(3) | C(12)-H(15) | 1.080(0) |
| C(12)-C(13) | 1.398(3) | C(13)-H(16) | 1.080(0) |
| C(13)-C(14) | 1.371(4) | C(14)-H(17) | 1.080(0) |
| C(14)-C(15) | 1.368(4) | C(15)-H(18) | 1.080(0) |
| C(15)-C(16) | 1.387(3) | C(16)-H(19) | 1.080(0) |
| C(17)-C(18) | 1.392(3) | C(17)-C(22) | 1.383(3) |
| C(18)-H(20) | 1.080(0) | C(18)-C(19) | 1.388(3) |
| C(19)-H(21) | 1.080(0) | C(19)-C(20) | 1.363(3) |
| C(20)-H(22) | 1.080(0) | C(20)-C(21) | 1.385(3) |
| C(21)-H(23) | 1.080(0) | C(21)-C(22) | 1.395(3) |
| C(22)-H(24) | 1.080(0) | | |

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# SPECTRA

# PART $\mathcal{A}$

## TABLE OF CONTENTS

# COMPOUND I (94)



**(94)** lup-20(29)-en-3β,27-diol

## Index

MON 96

MASS 24

NO INFO GIVEN :
ASSUME NO Nitrogen.
$\equiv O6$

3827
3827
442.3827 found.
442.3811 $\equiv C_{30}H_{50}O_2$
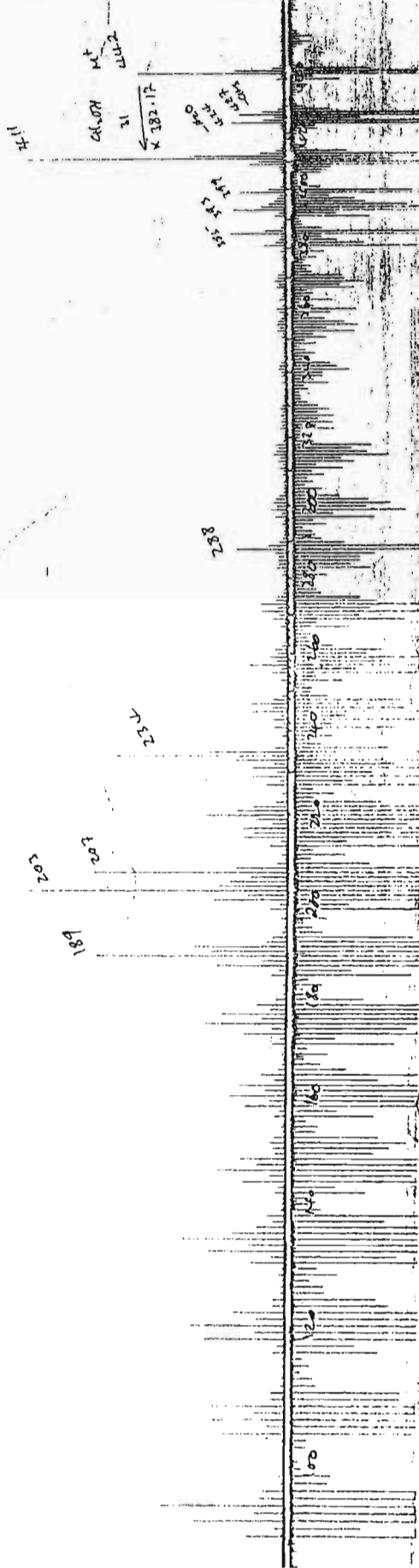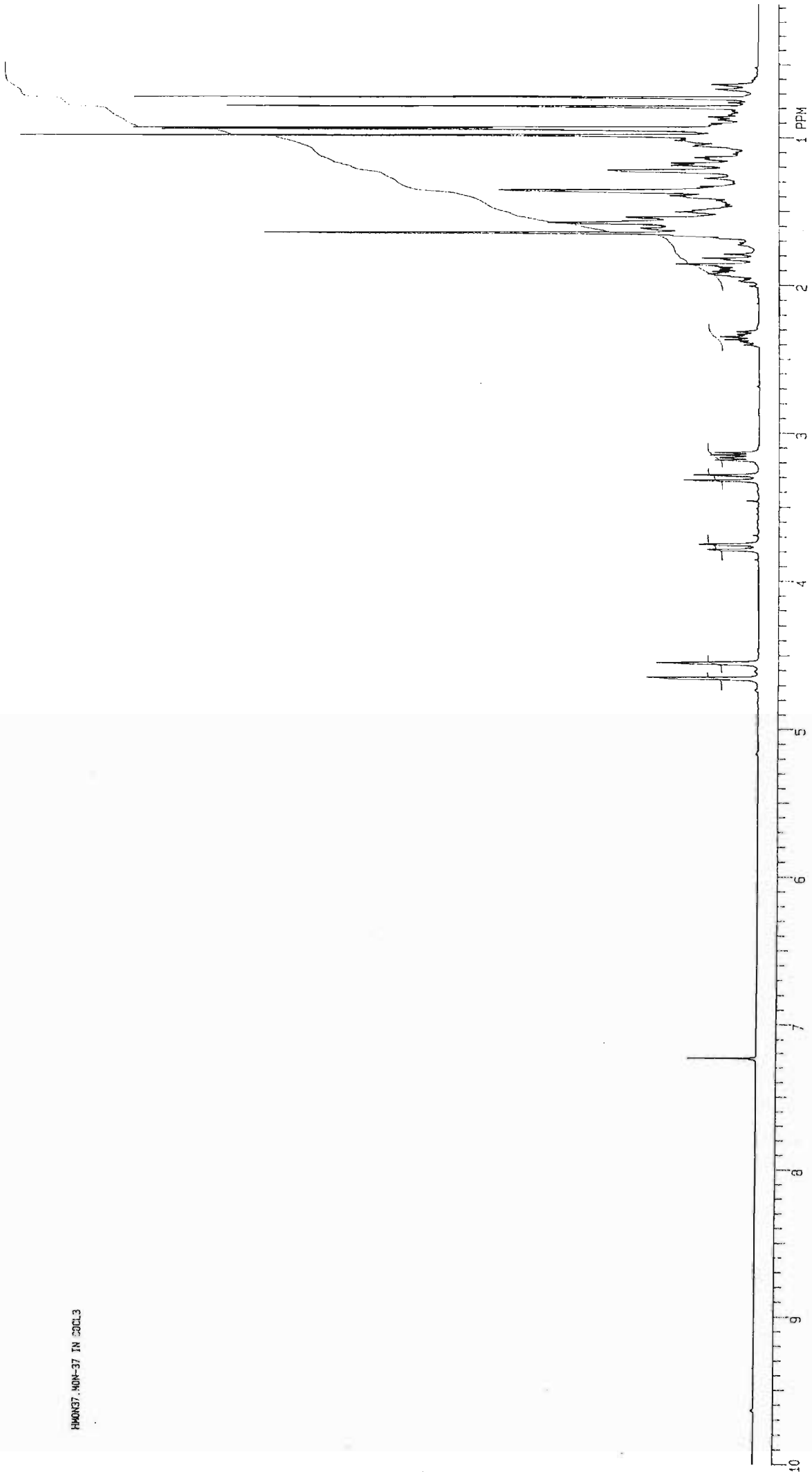
$\mathcal{A}$3

411

CH$_3$OH
M$^+$
442
$\times$ 182.17

288

189

207

207

$\mathcal{A}$ 4

HMQN37.H0N-37 IN CDCL3

CNQN37.M0N-37 IN CDCL3

20 PPM
40
60
80
100
120
140
160
180
200
220

DMDN37.MON-37 IN CDCL3

CH3

CH2

CH

CHn

20 PPM

40

60

80

100

120

140

𝒜 6
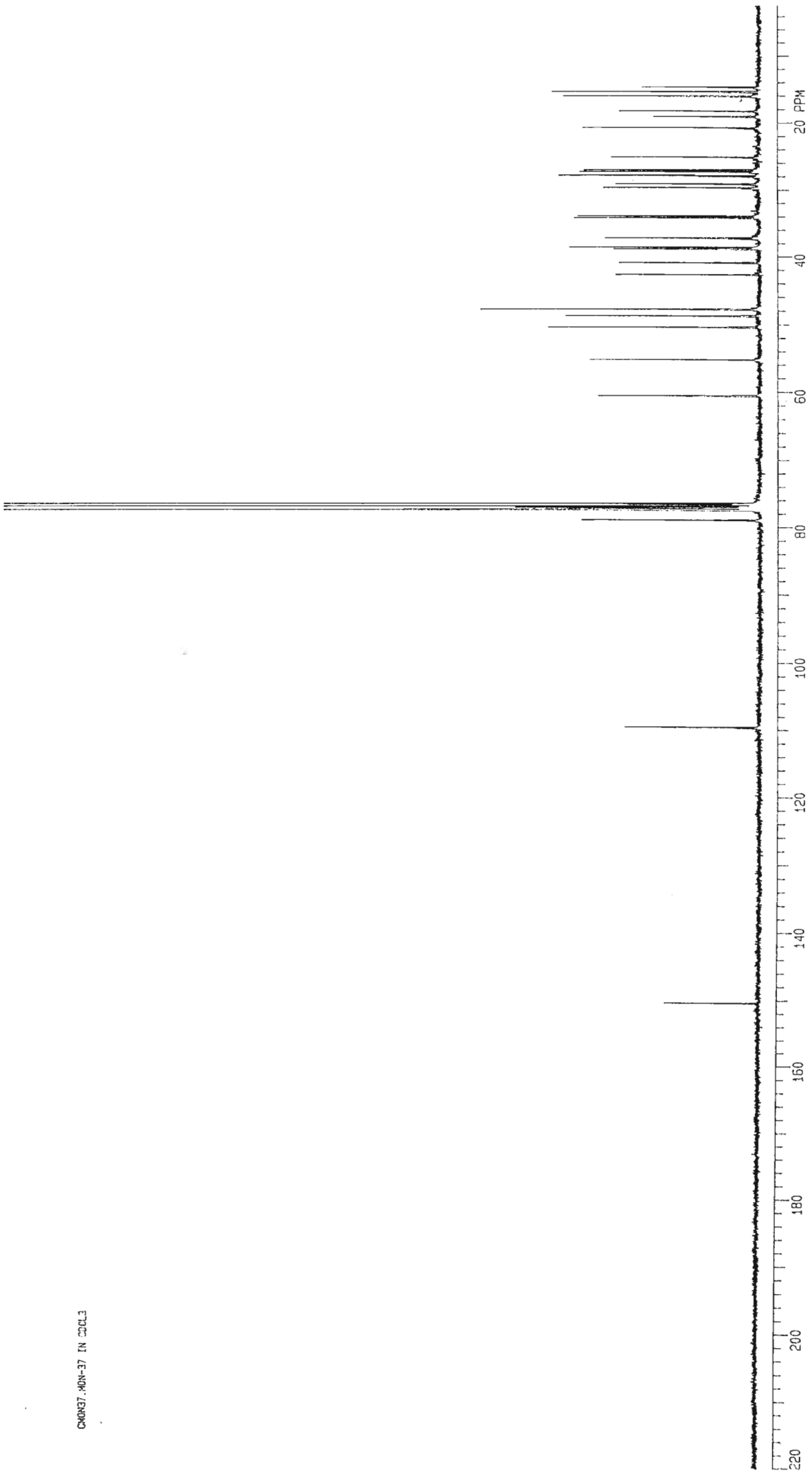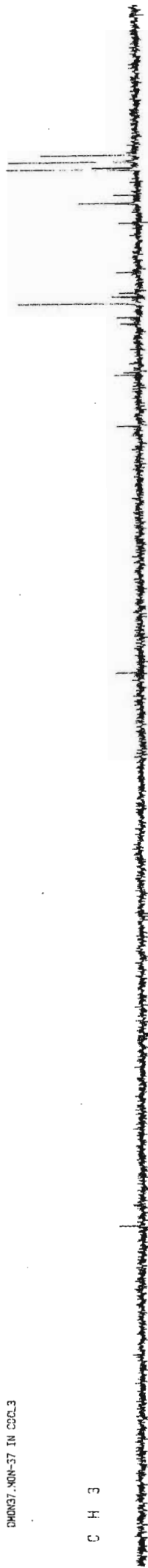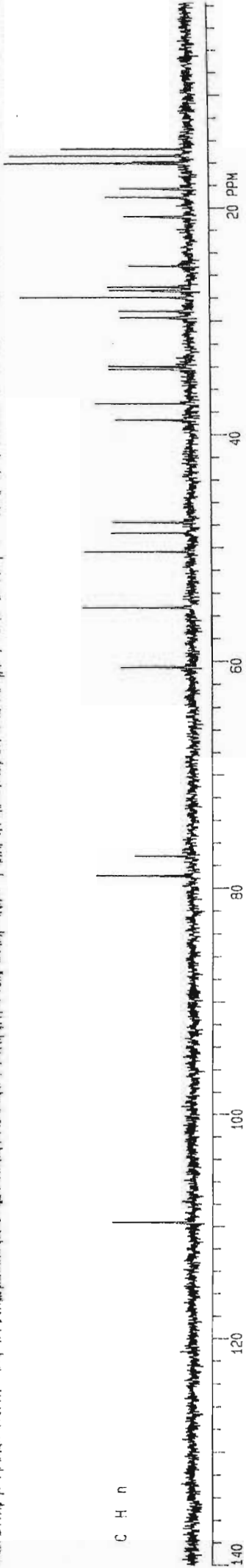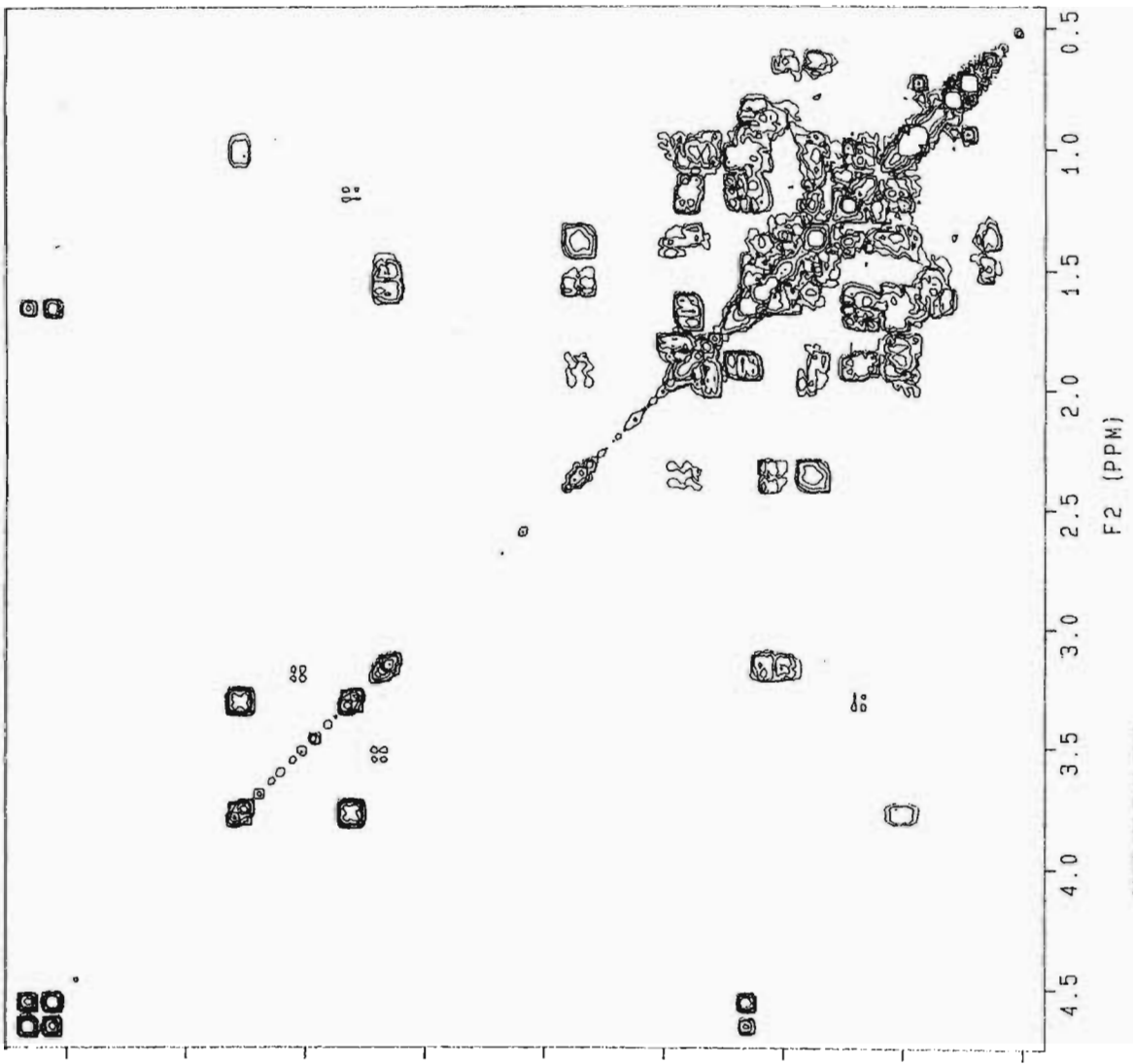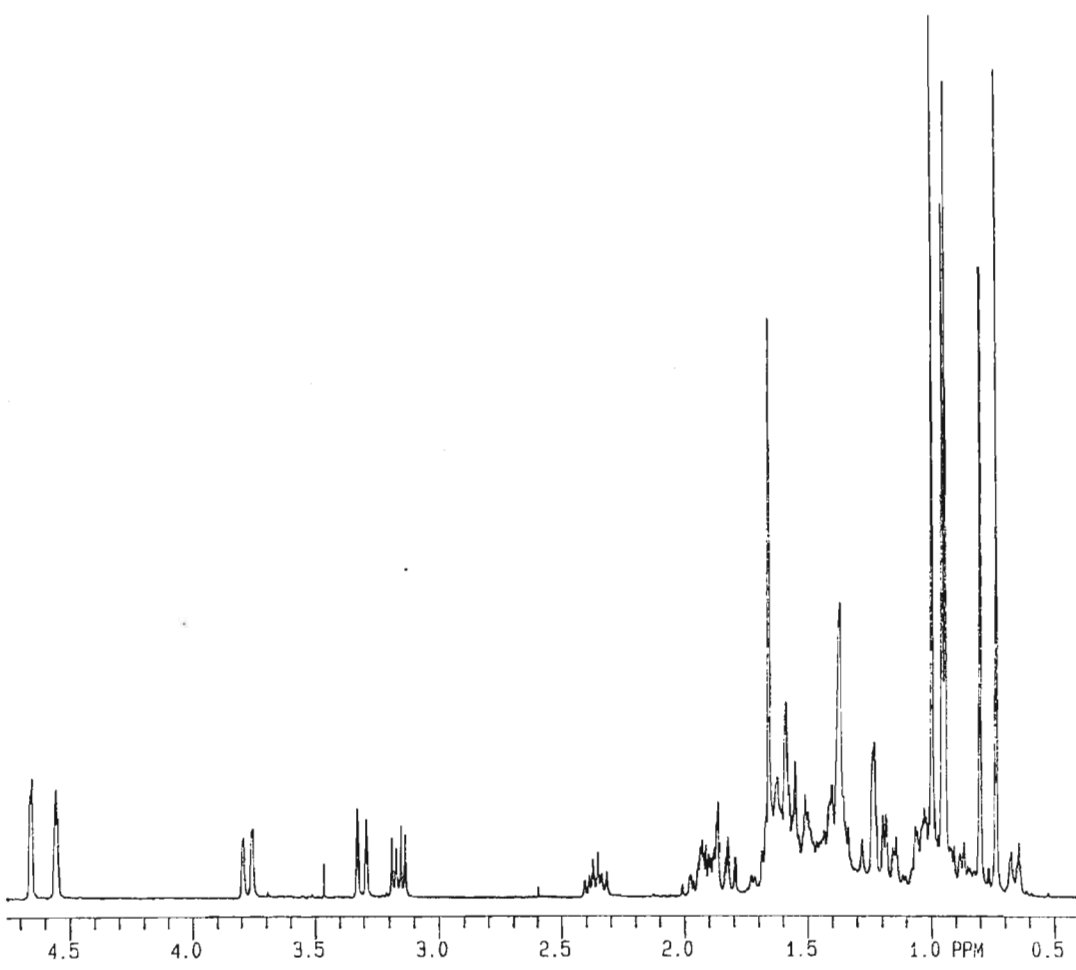
CYM037.MON-37 IN CDCL3
1H COSY-60

F2 (PPM)
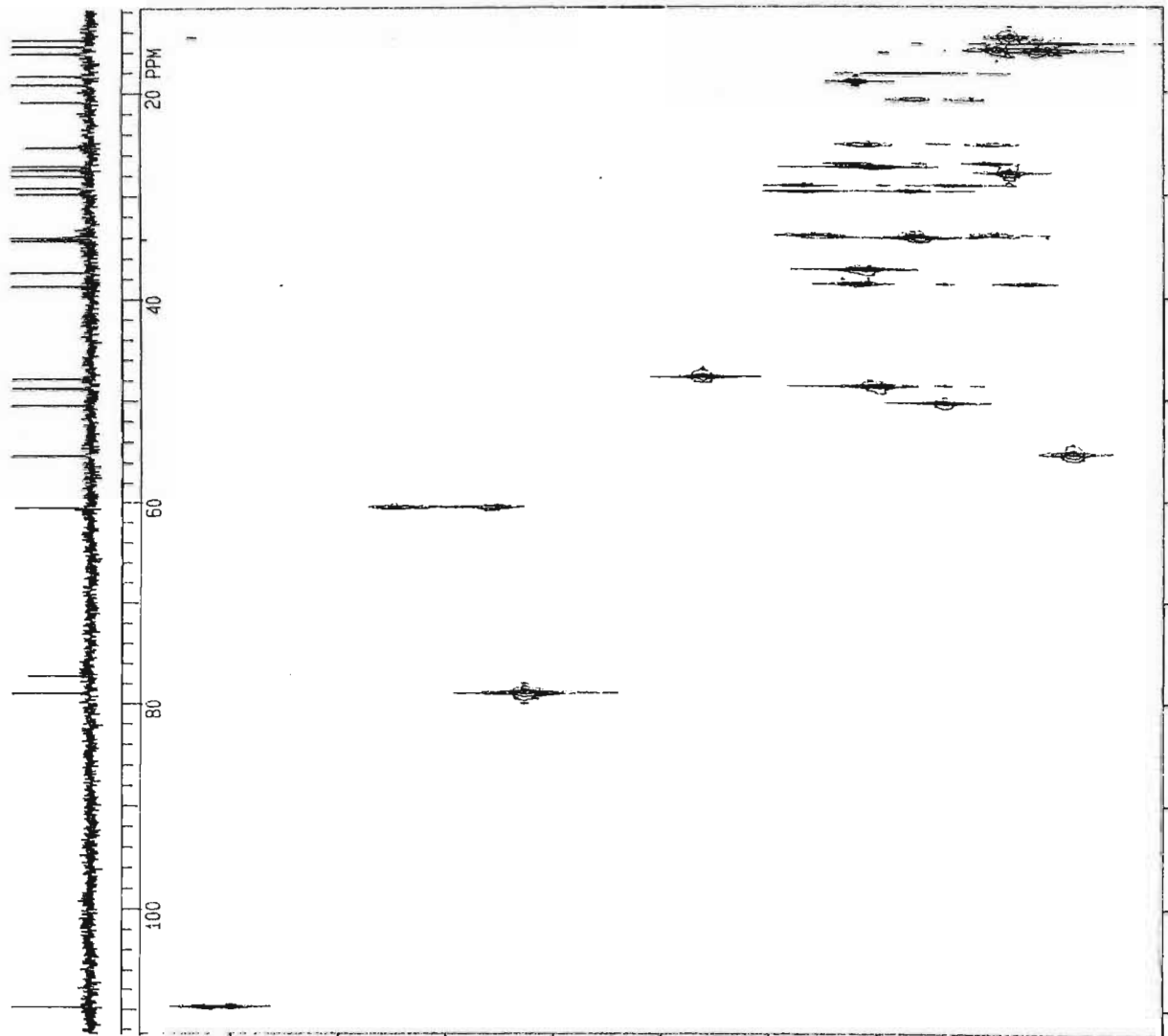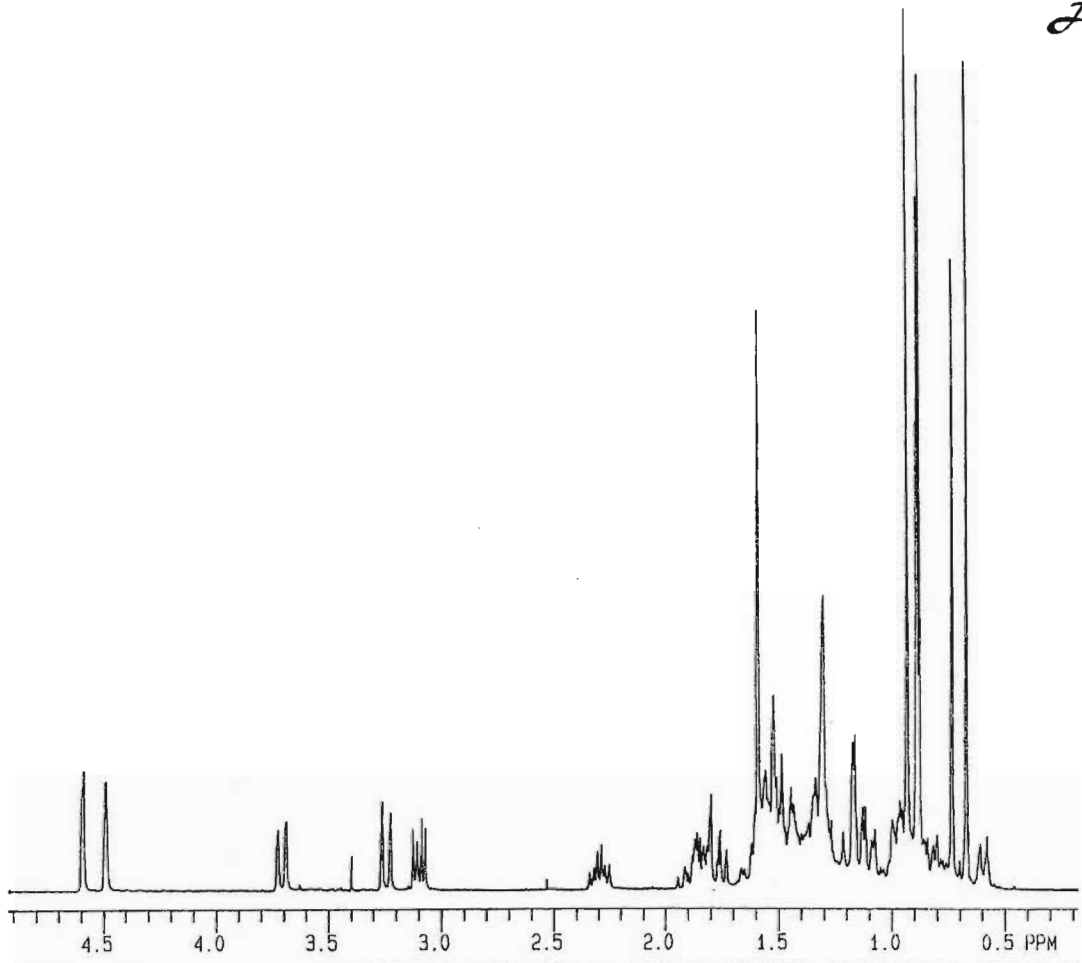
HCM037.MON-37 IN CDCL3
1H/13C HETCOR

# COMPOUND Ia (94a)
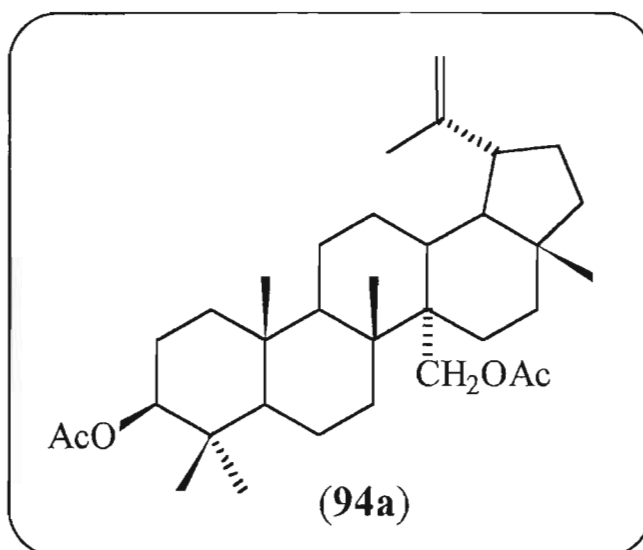


(94a)

## Index

# COMPOUND II (95)

𝒜 11

HMONG9.HON-69 IN CDCL3

CMON69.MON-69 IN CDCL3

𝒜 12

CMON69.MON-69 IN CDCL3

CH3

CH2

CH

CHn

*A* 13
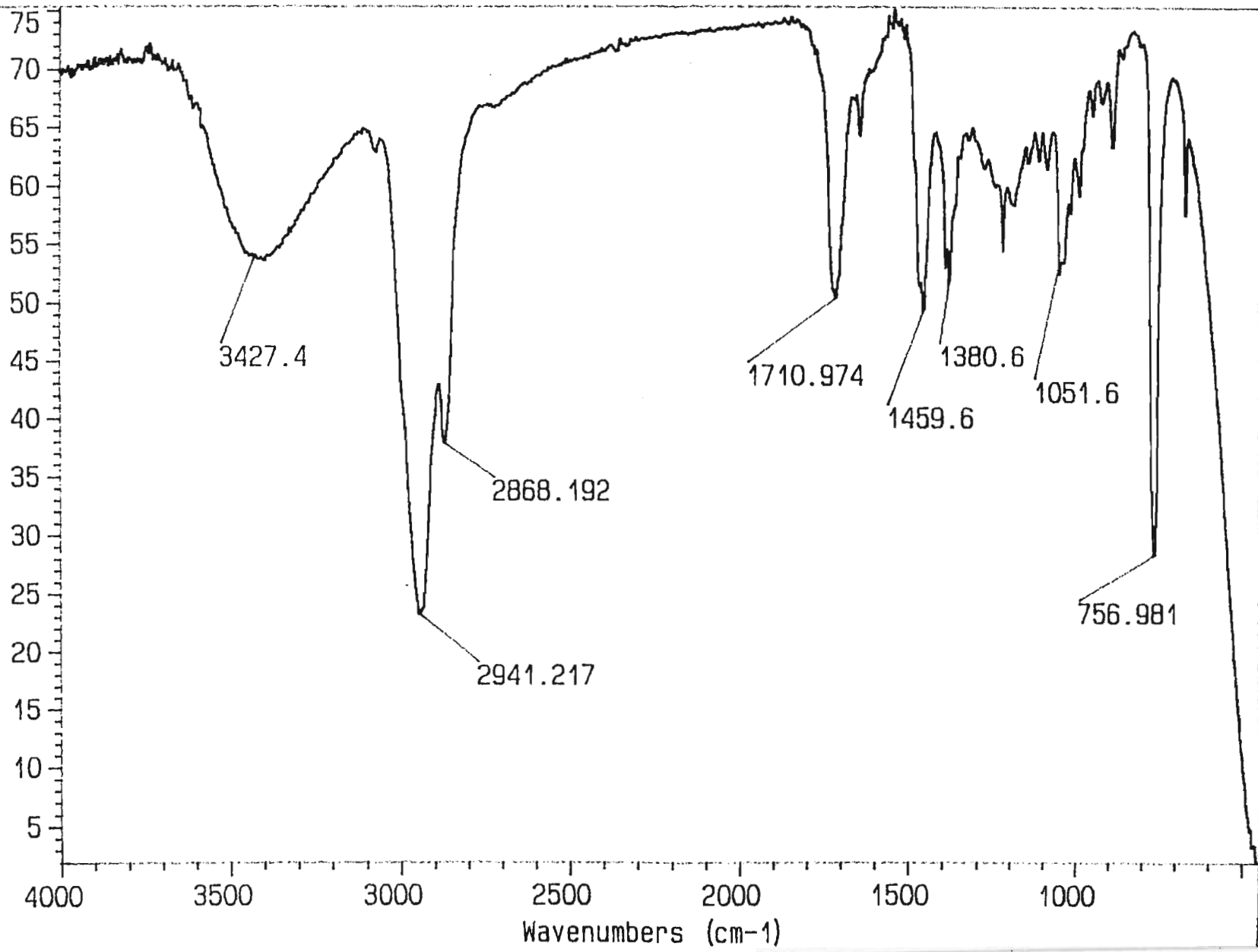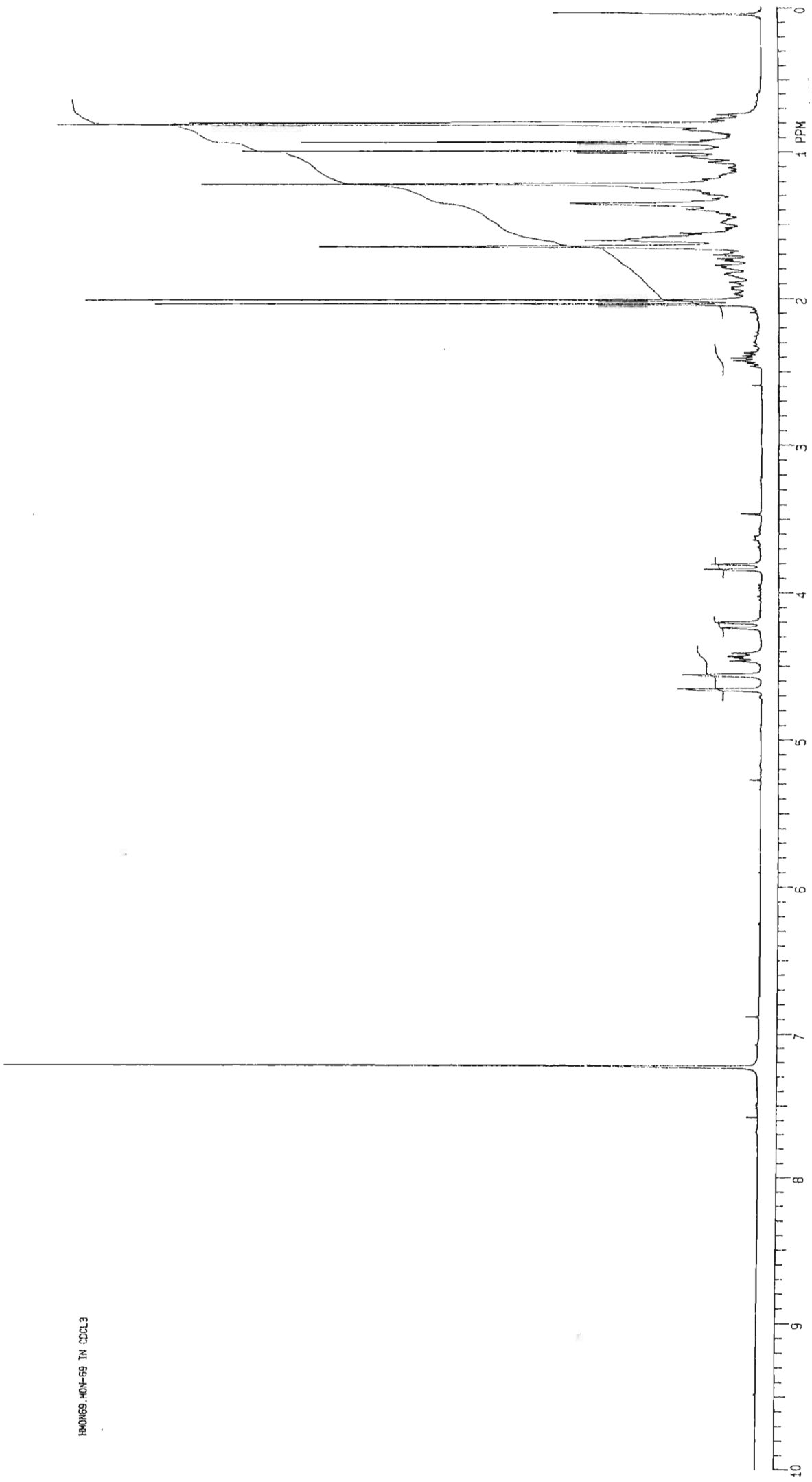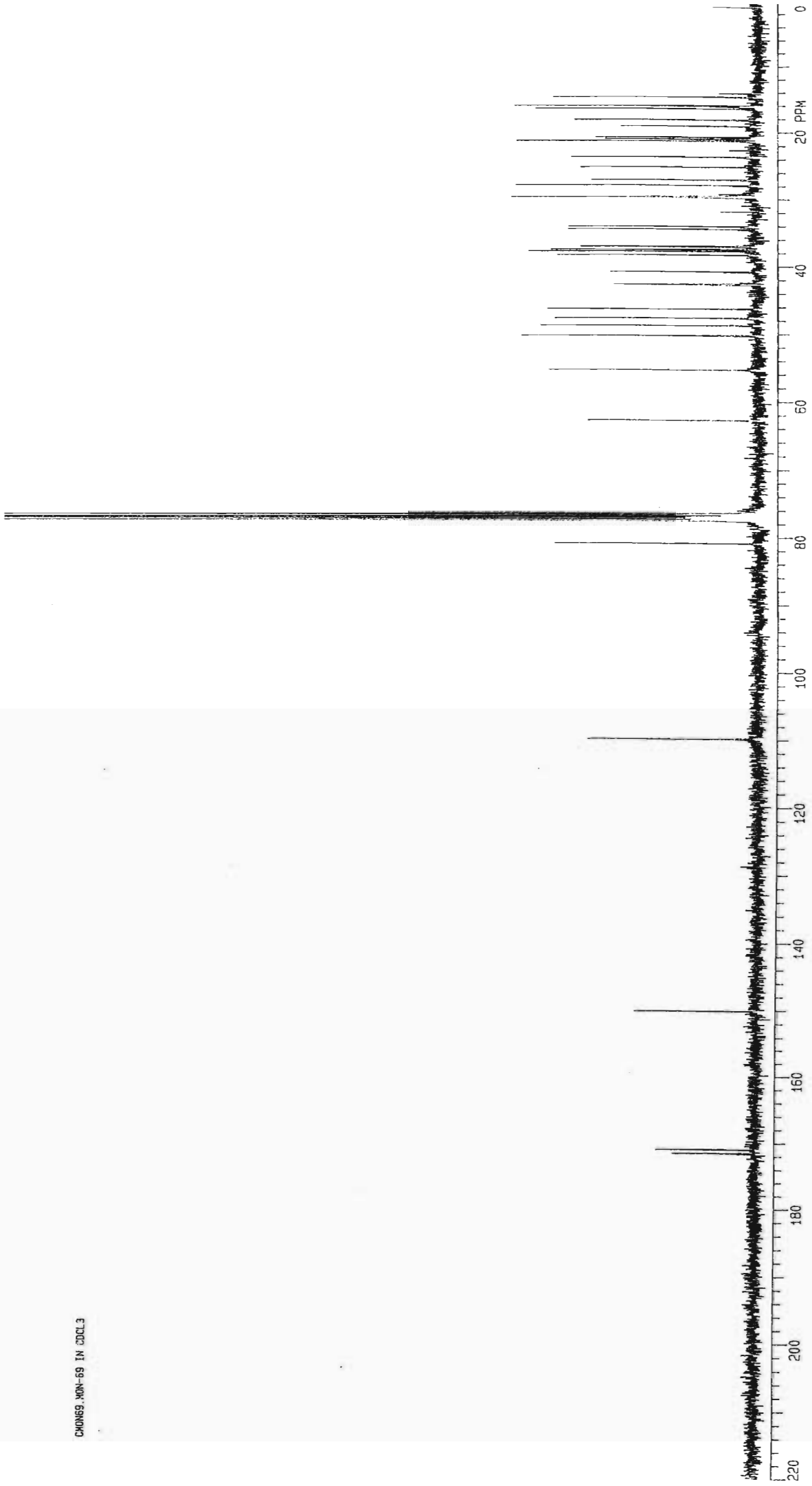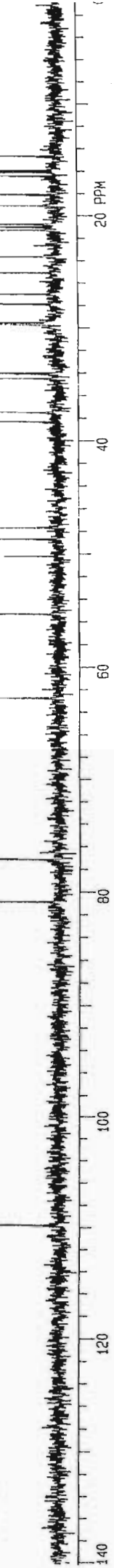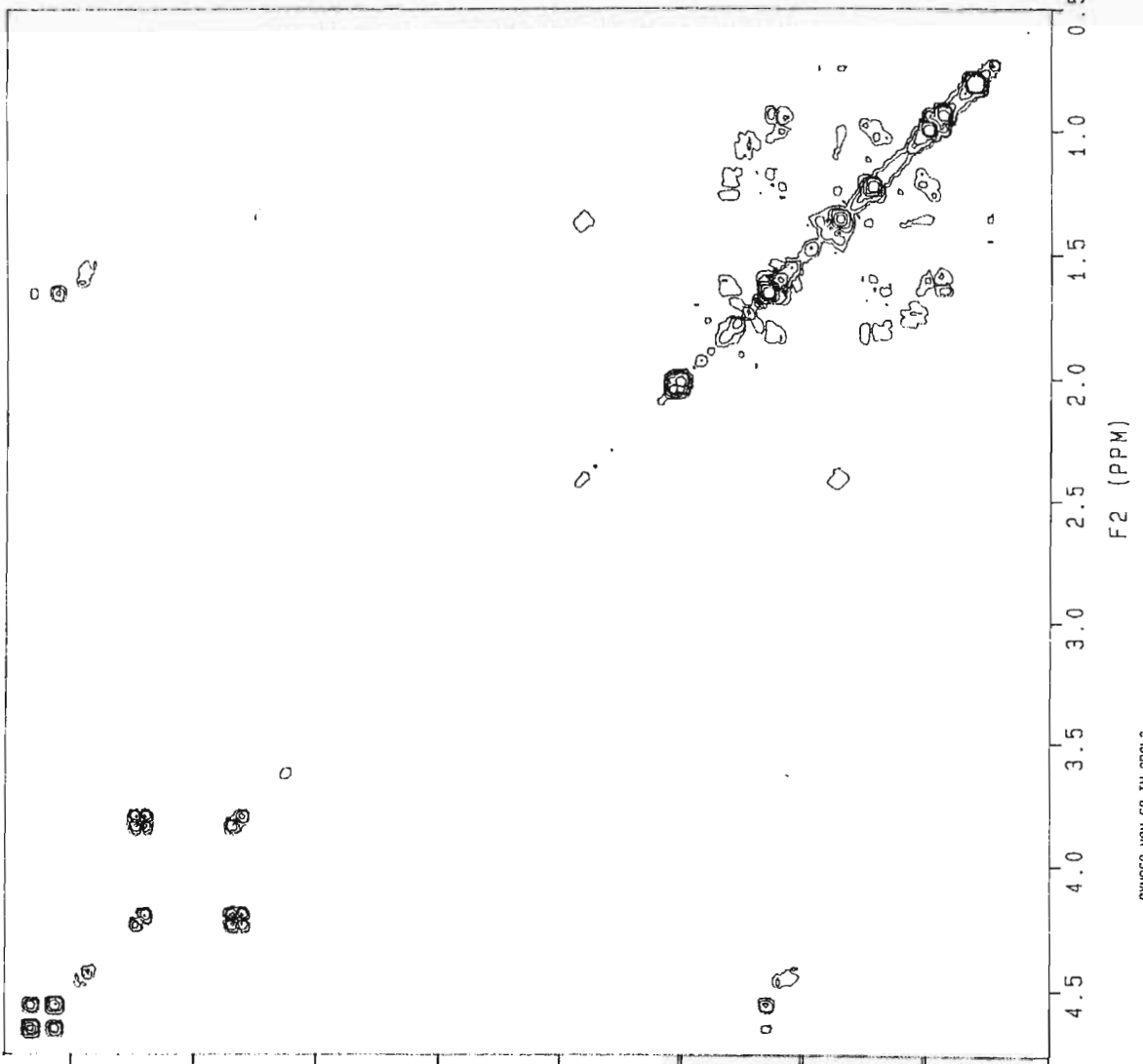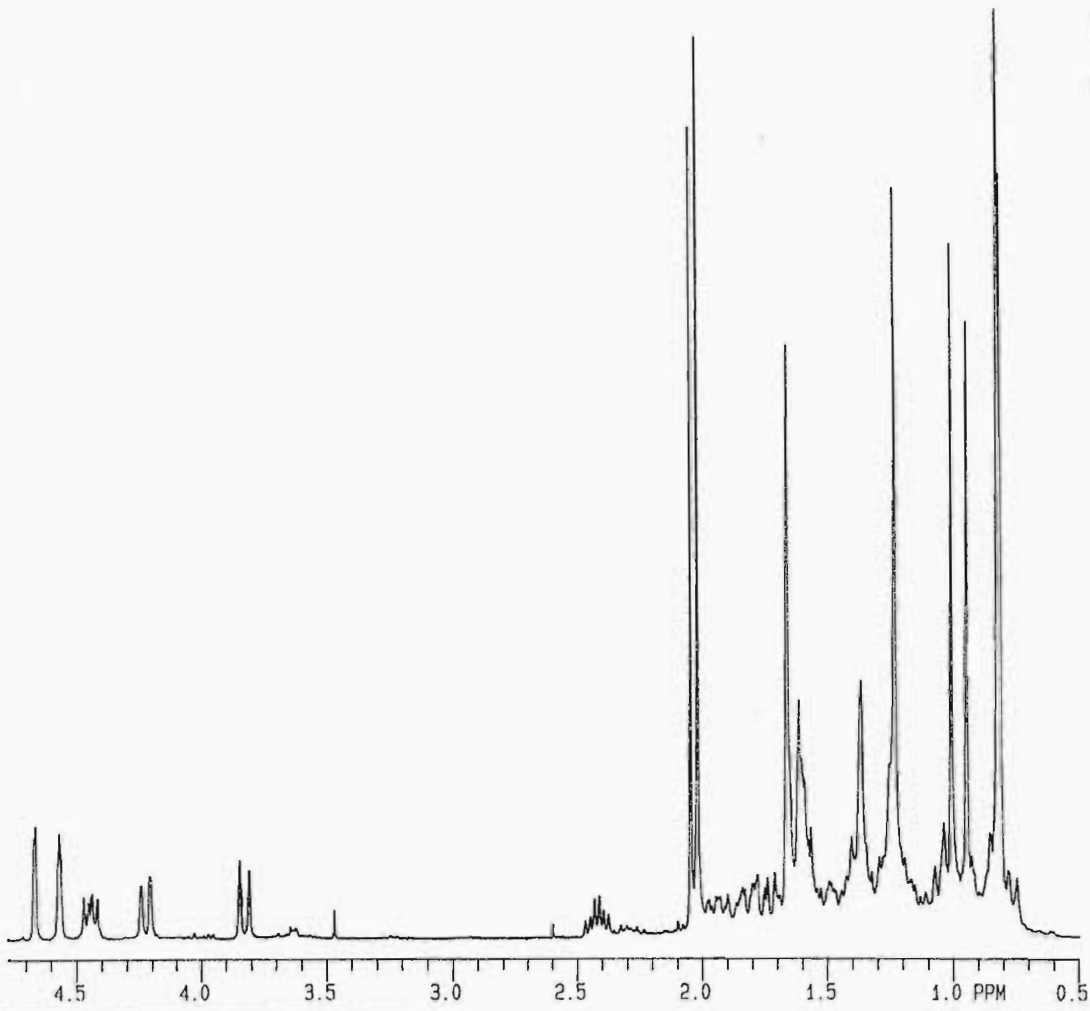
CYN069.MON-69 IN CDCL3
1H COSY-60

F2 (PPM)

HCM069.MON-69 IN CDCL3
1H/13C HETCOR

# COMPOUND II (95)



(95) betulinaldehyde
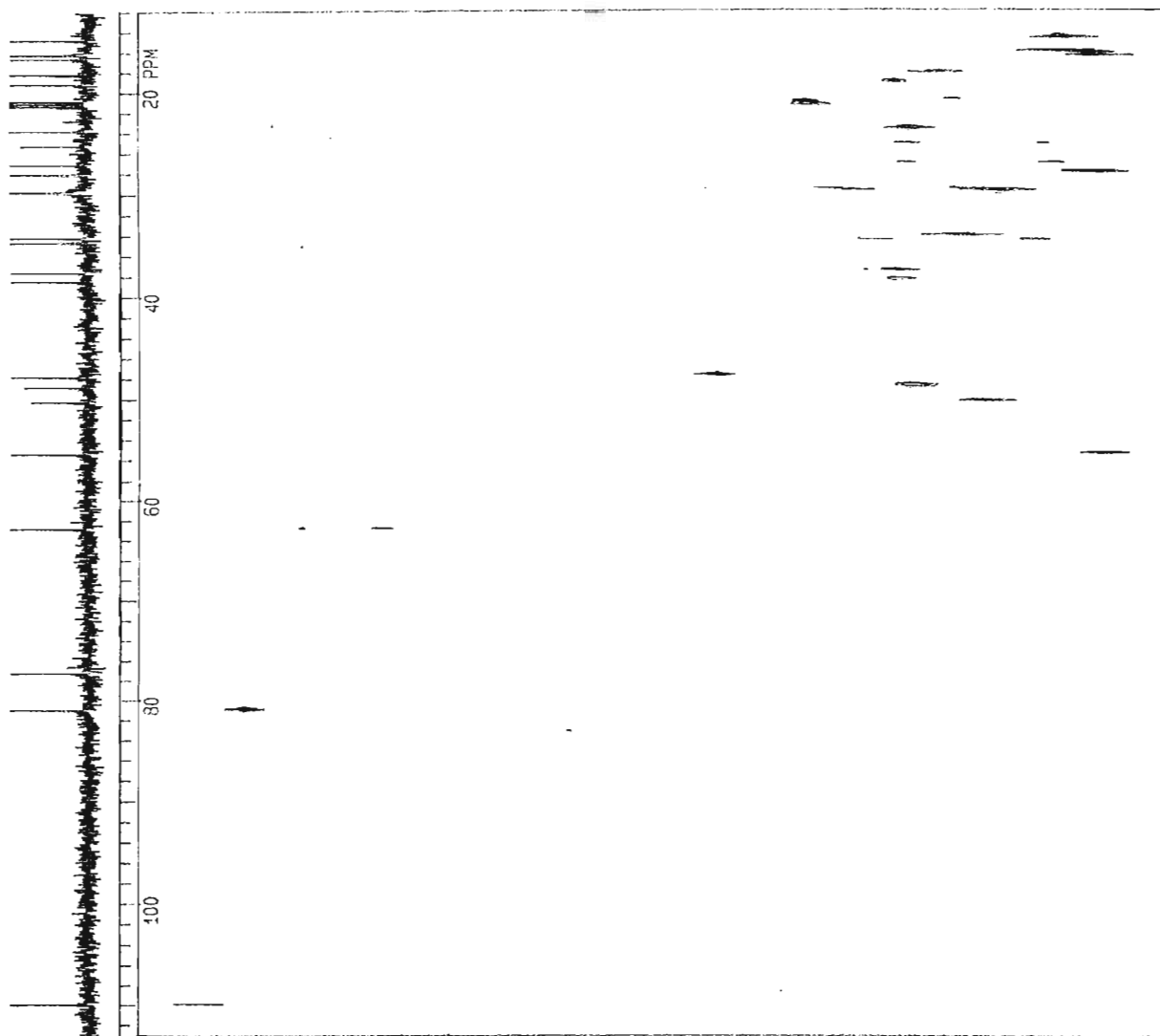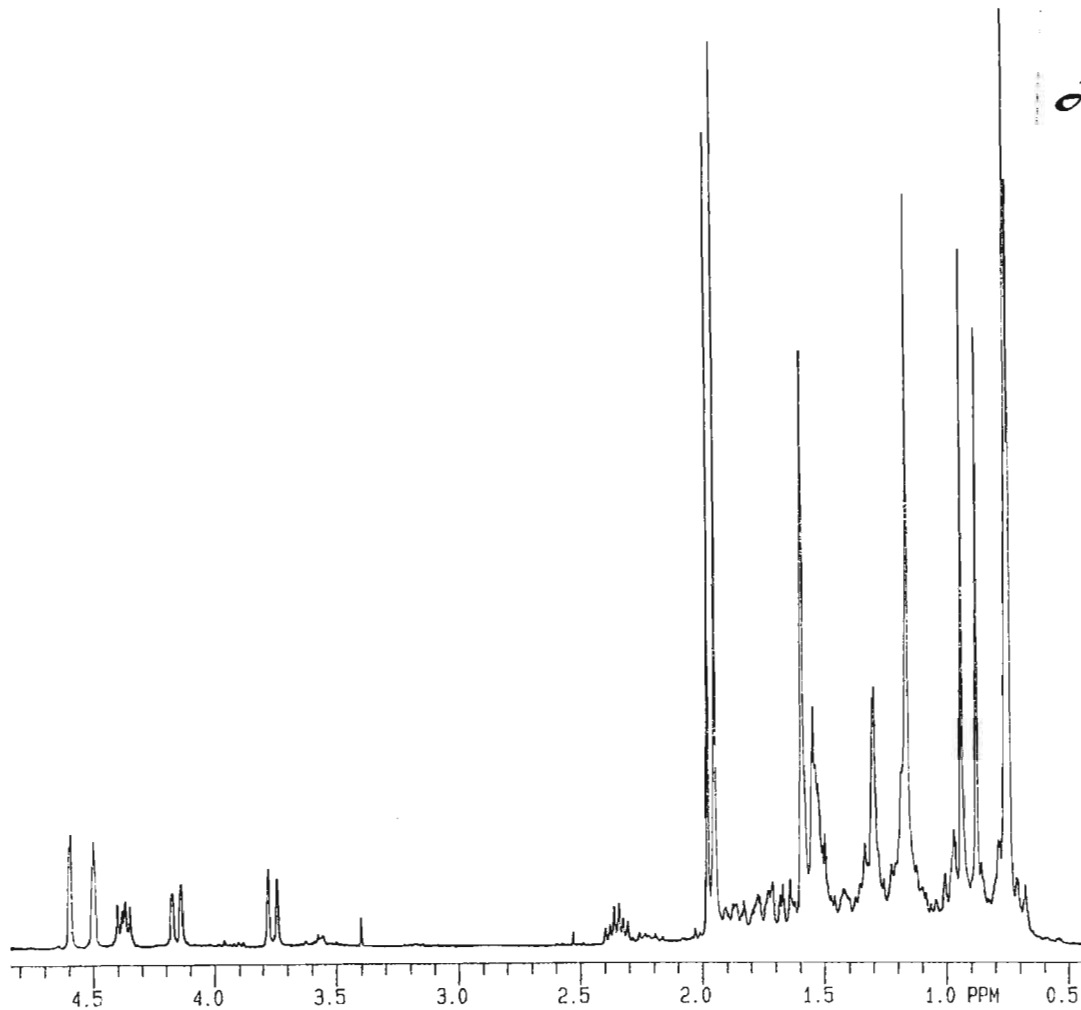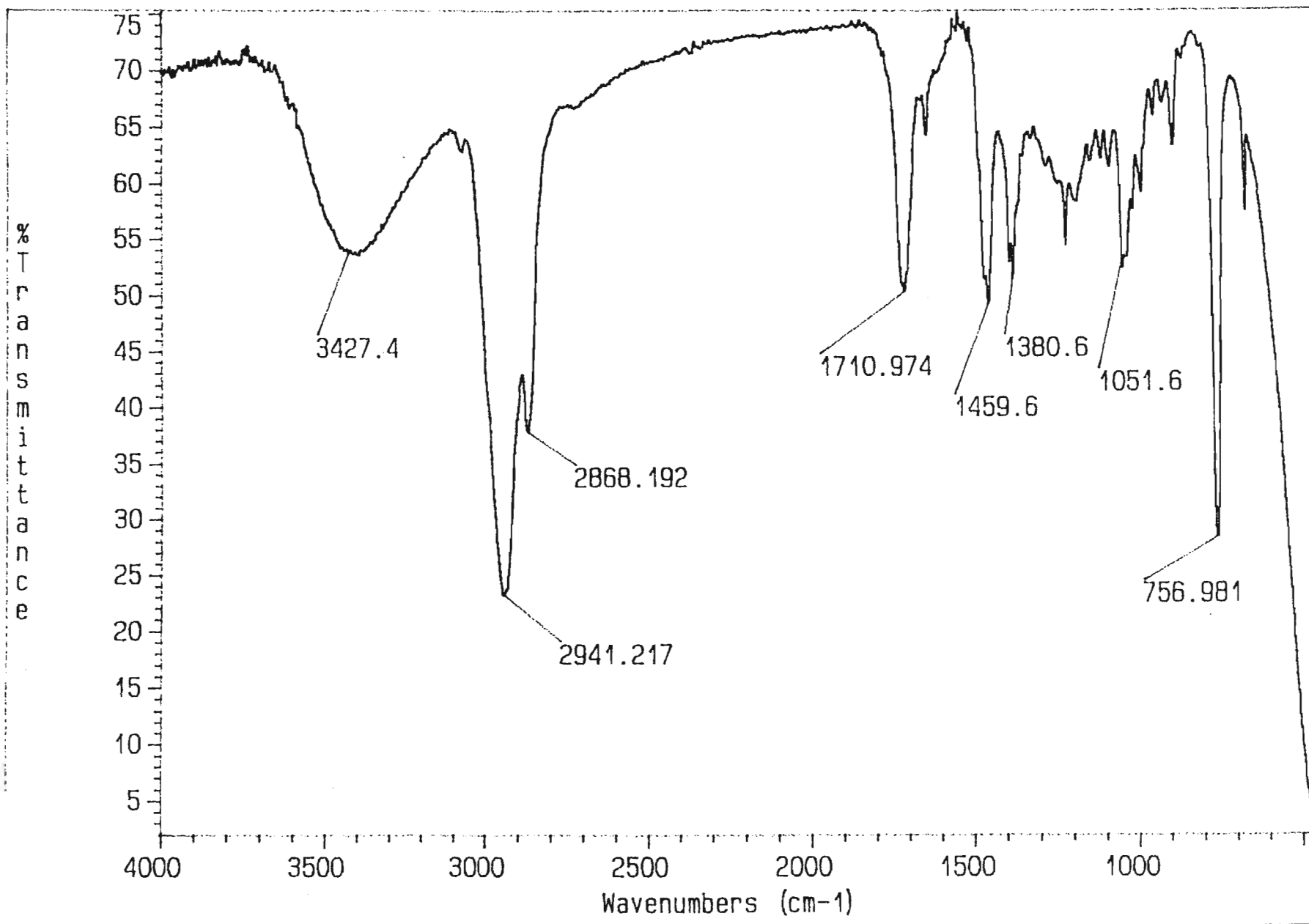
## Index

Mon 35

4410-3648
440 36 52
= $C_{30}H_{48}O_2$
as expected

$M^+$
440

411

207

HMON35.MON-35 IN CDCL3

A 19

A 20
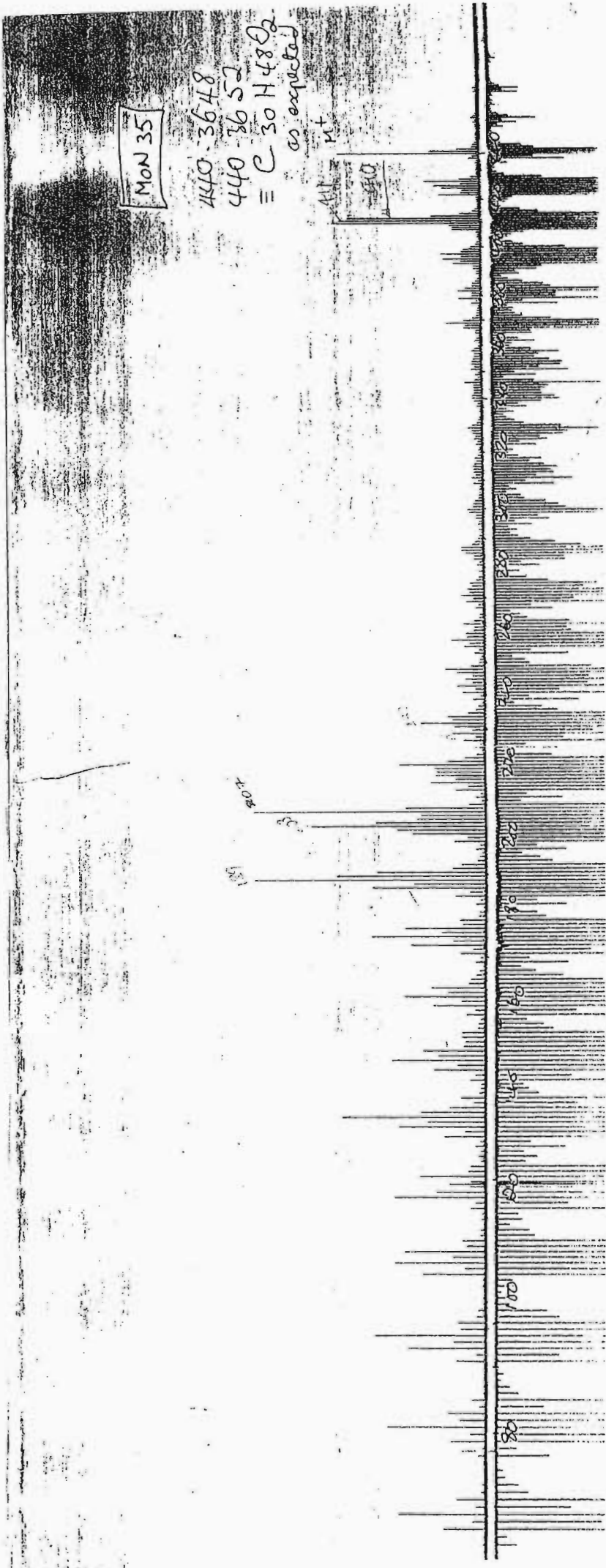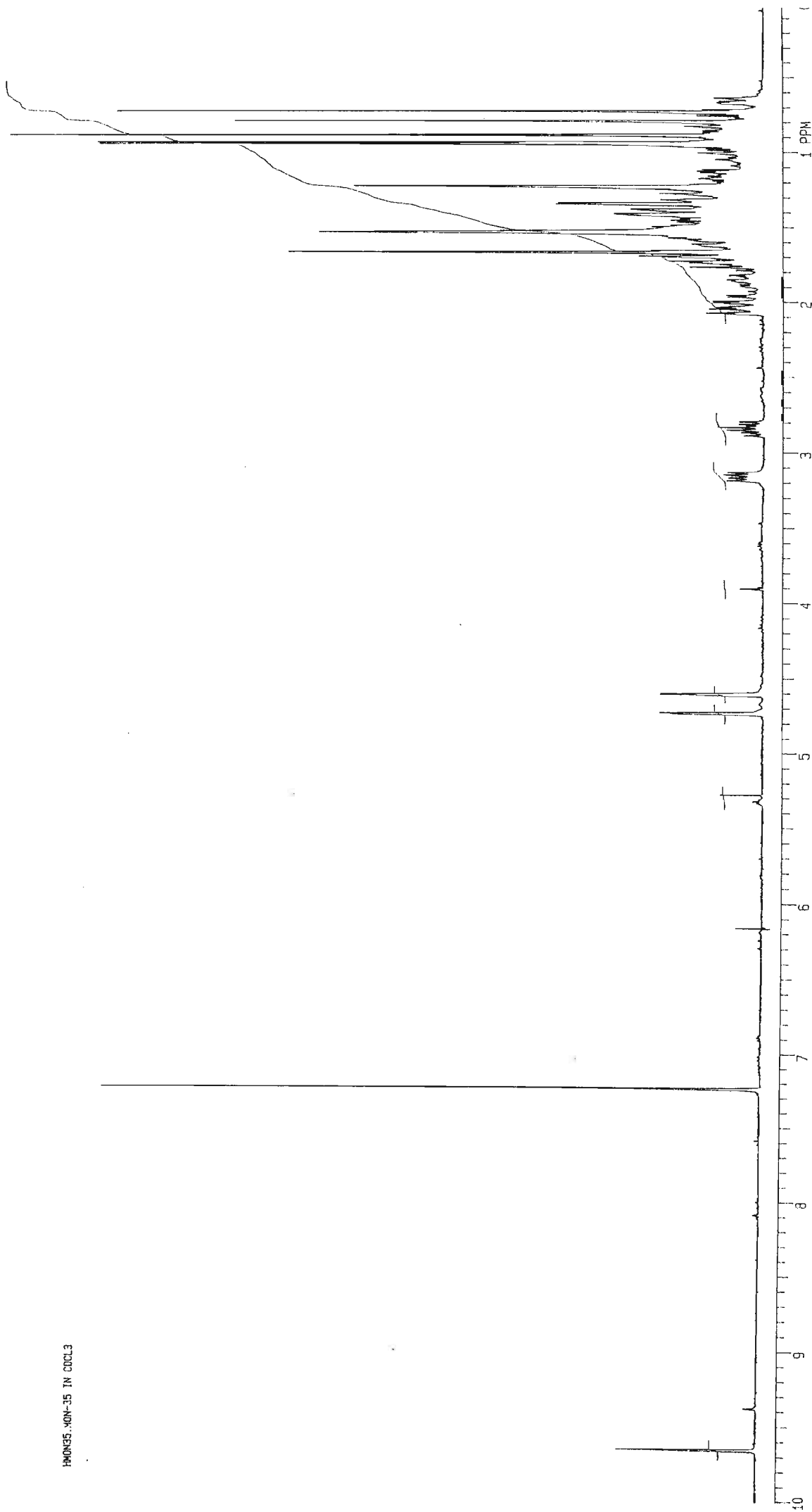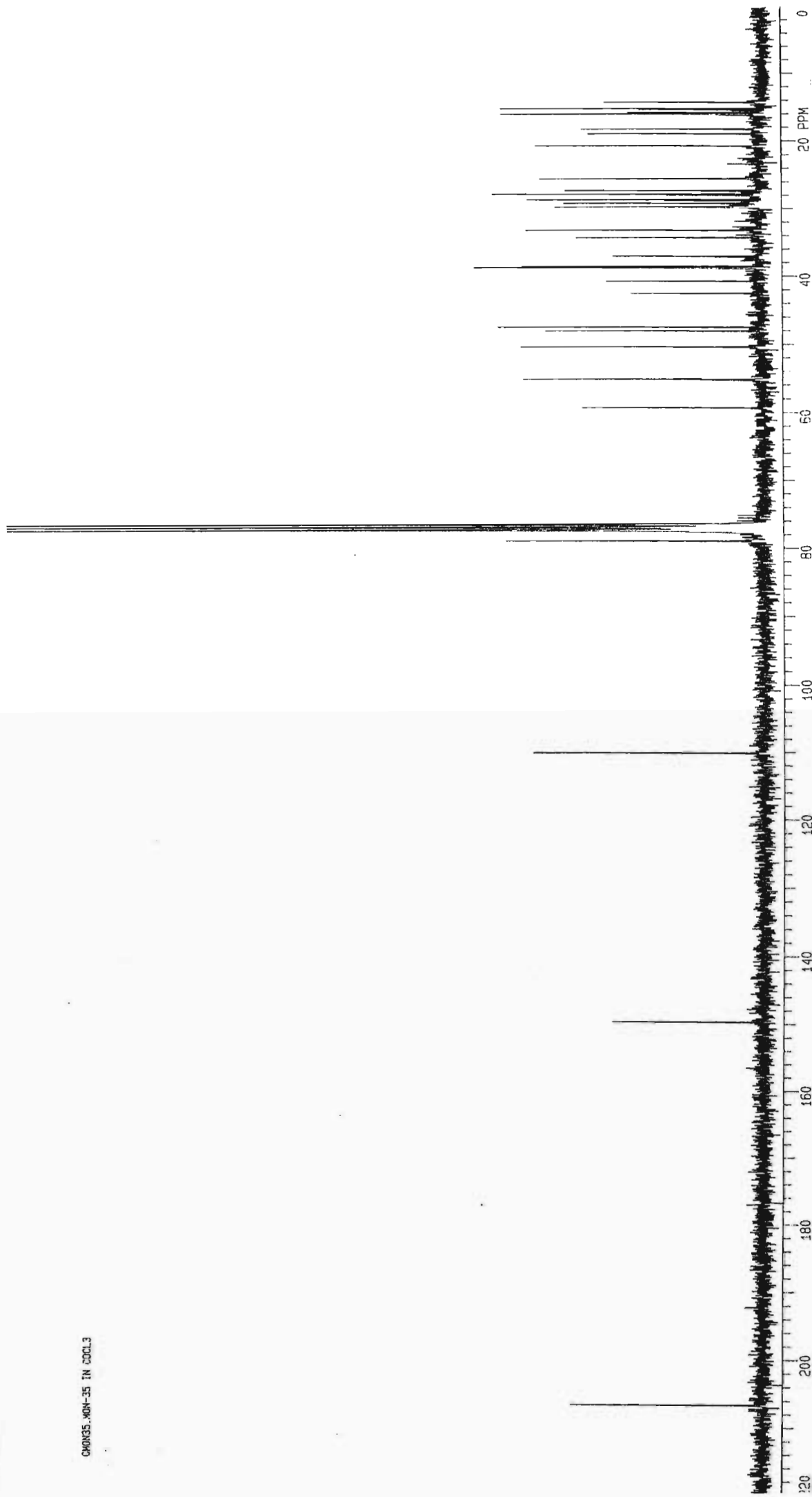
CHON35.M0N-35 IN CDCL3

DHON35.NON-35 IN CDCL3

C H 3

C H 2

C H

C H n

𝒜 21

20 PPM

40

60

80

100

120

140

160

A 22
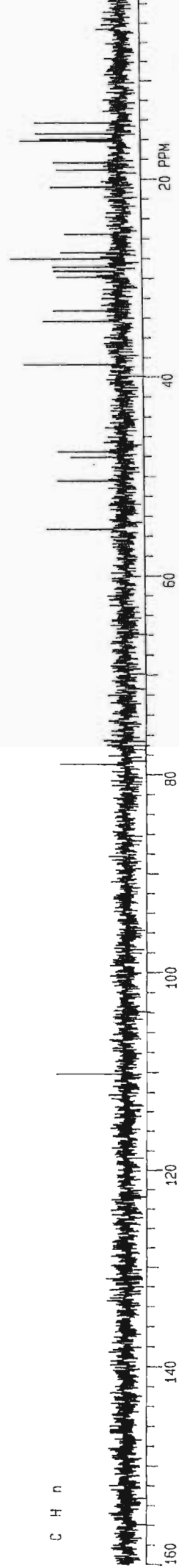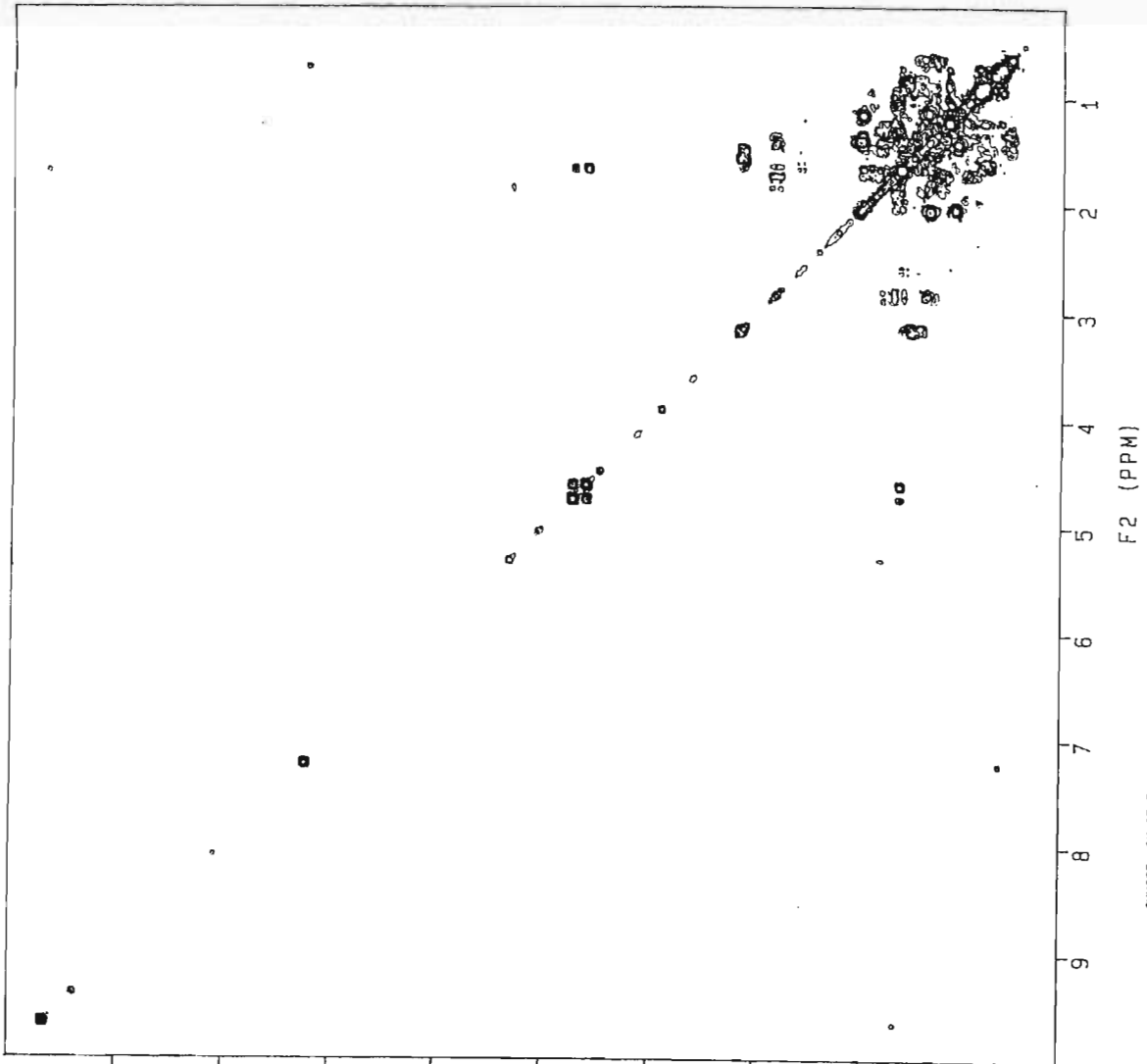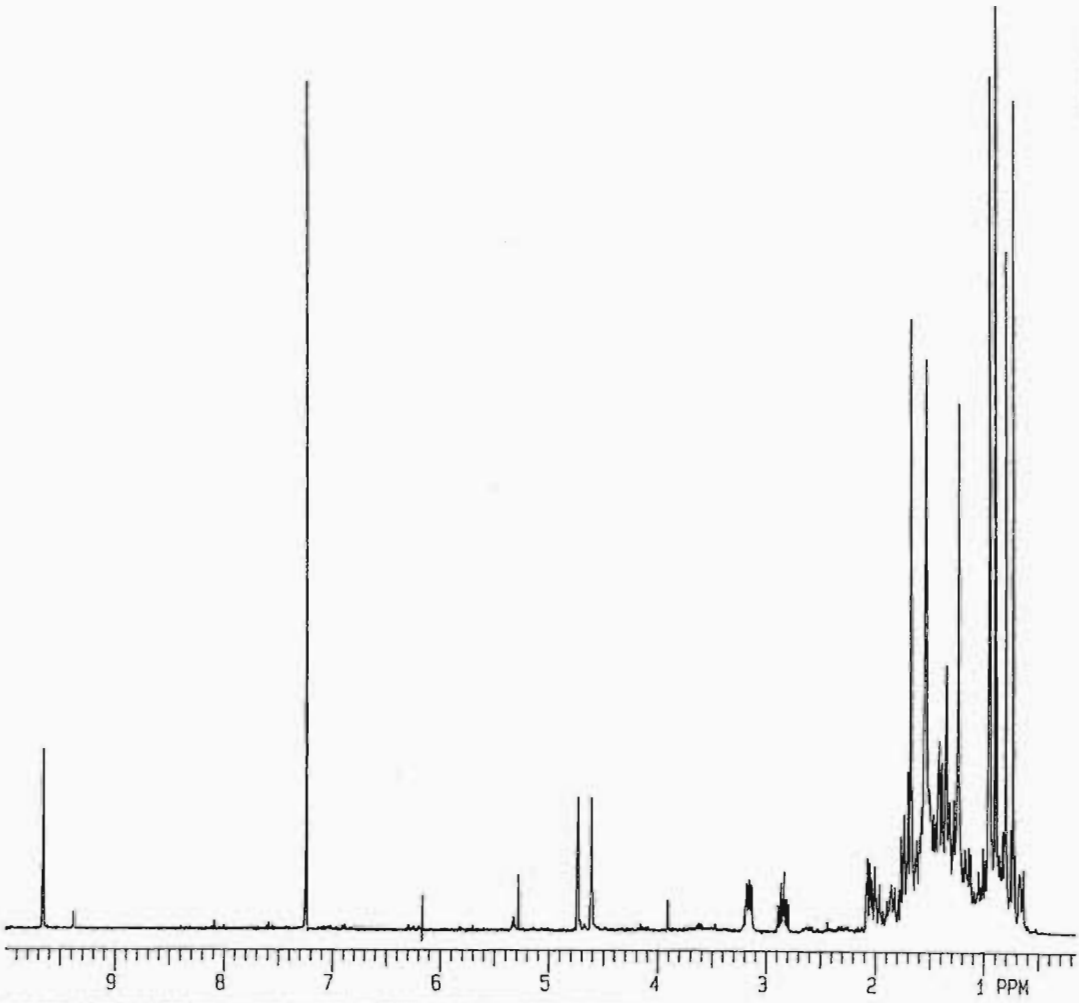
CYM035.MON-35 IN CDCL3
1H COSY-60

F2 (PPM)

HCN035.MON-35 IN CDCL3
1H/13C HETCOR

# COMPOUND III (96)



(96) 23-hydroxybetulinaldehyde

## Index

HKON33.HON-33 IN CDCL3

3.7190
3.7025
3.6828
3.6693
3.6439
3.6181
3.5926
3.5707
3.4095
3.3748
2.8881
2.8700
2.8511
2.8324
2.8149
2.7957

26

A 27

CHON33.MON-33 IN CDCL3

CYN033.HON-33 IN CDCL3
1H COSY-60

F2 (PPM)

# COMPOUND IV (97)



(97) oleanolic acid

## Index

Win 96

(Mon 3)

456.958
457.3603
$\equiv C_{30}H_{48}O_3.$

HHONH96.HON-96 IN CDCL3

A 33

CMON96.NON-96 IN CDCL3

DMONS6.HON-96 IN CDCL3

C H 3

C H 2

C H

C H n

160   140   120   100   80   60   40   20 PPM

𝒜 34

CYHN96.NON-96 IN CDCL3
1H COSY-60

F2 (PPM)

HCMN96.MON-96 IN CDCL3
1H/13C HETCOR

# COMPOUND V (103)



(103) compound V

## Index

N2BH2

T. Monkite

N2 BH2

272.2490
272.2504
$\equiv C_{20}H_{32}$

M+ 272

-CH3

137

137

HNZBH2.NZBH-2 IN CDCL3

*A* 40

CN2BH2.N2BH-2 IN CDCL3

𝒜 41

DNZBH2.NZBH-2 IN CDCL3

CH3

CH2

CH

CHn

A 42

160    140    120    100    80    60    40    20 PPM    0
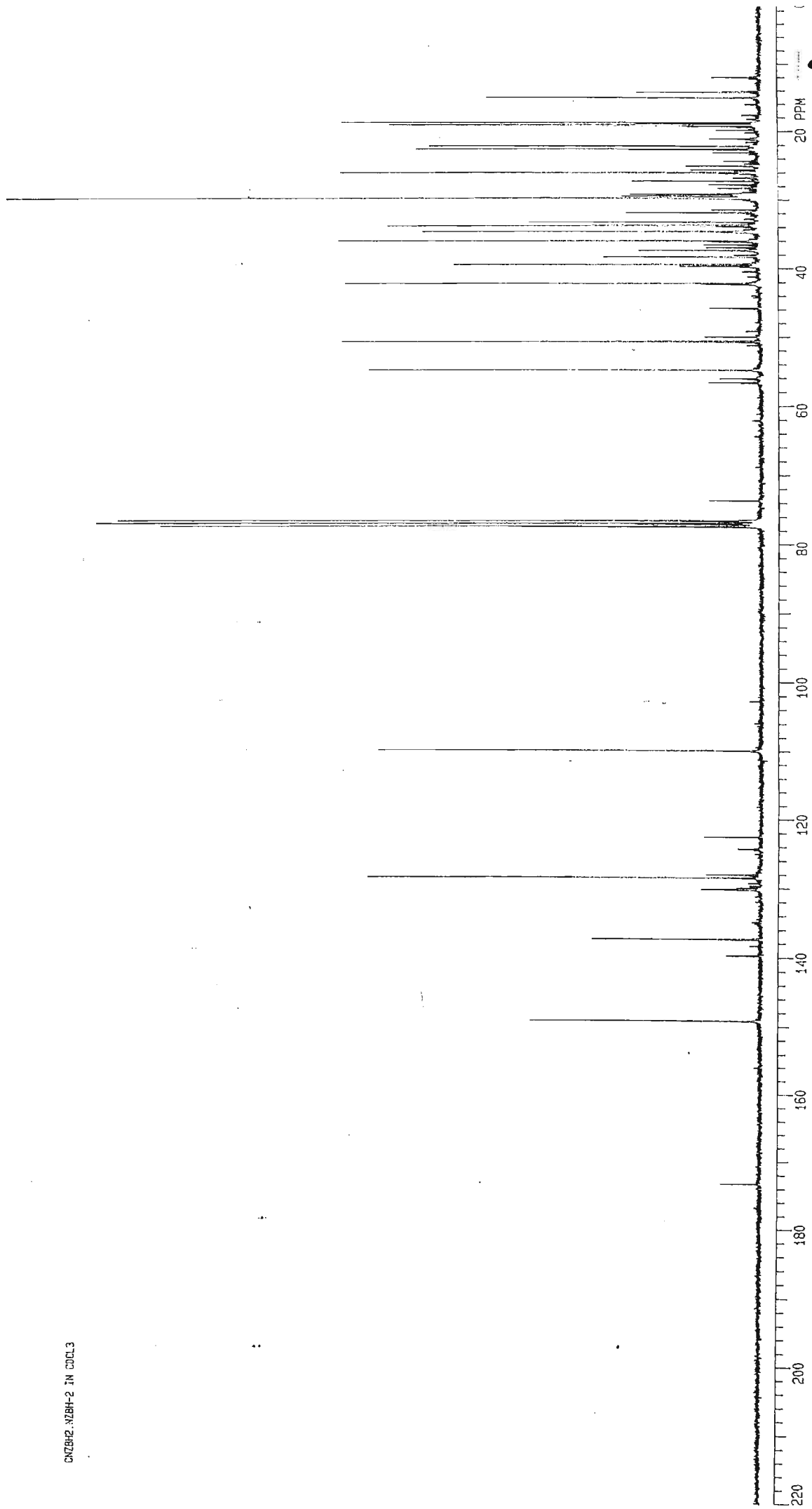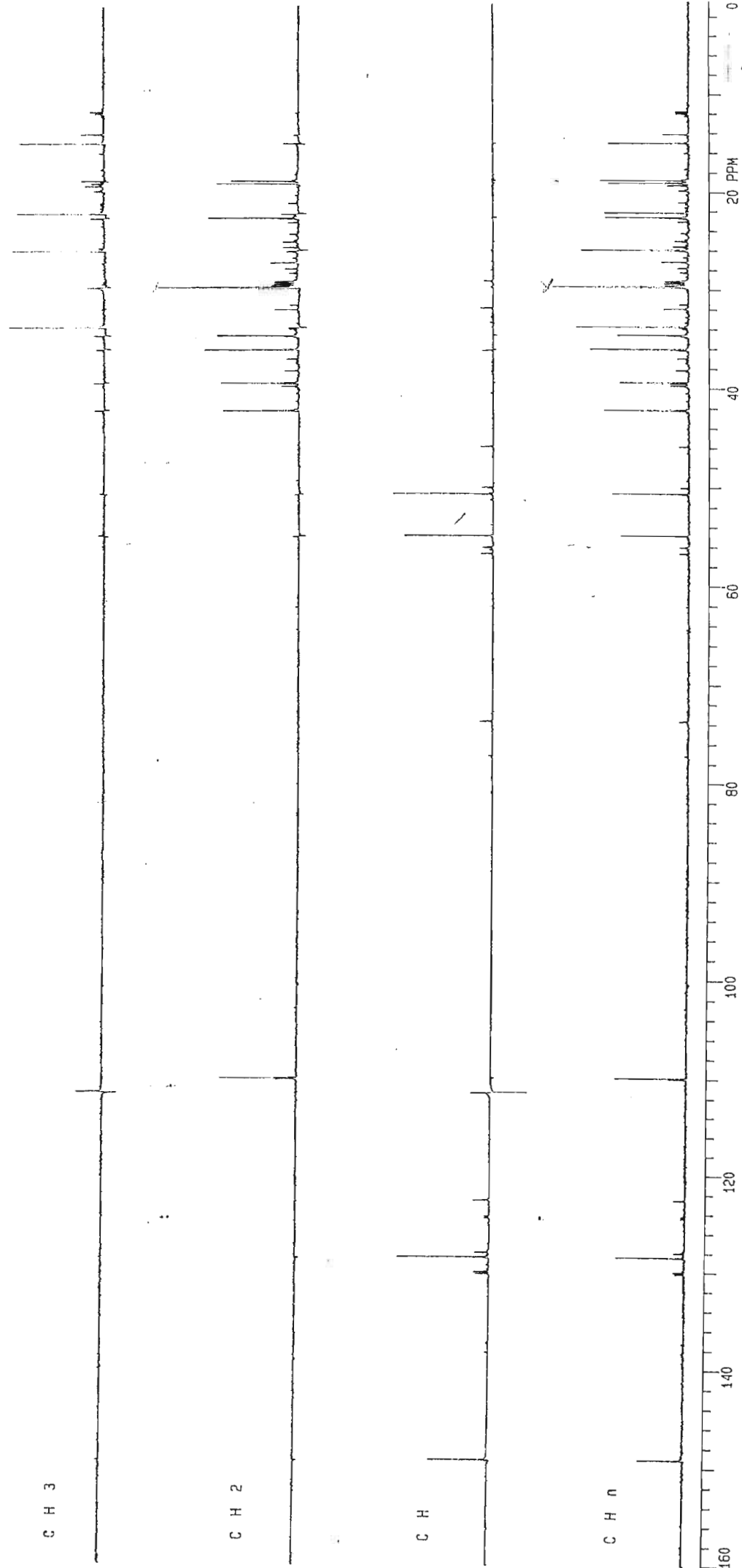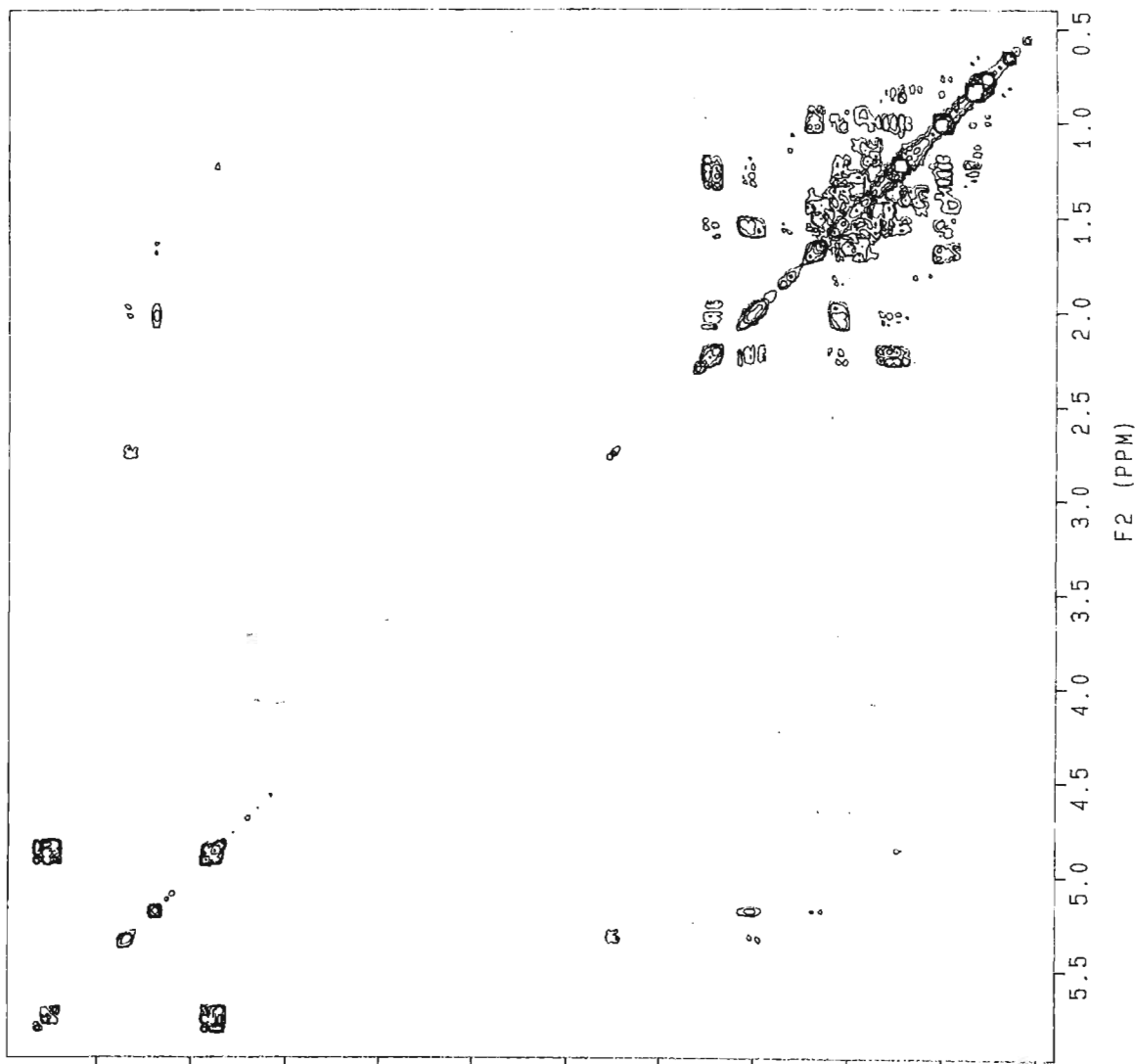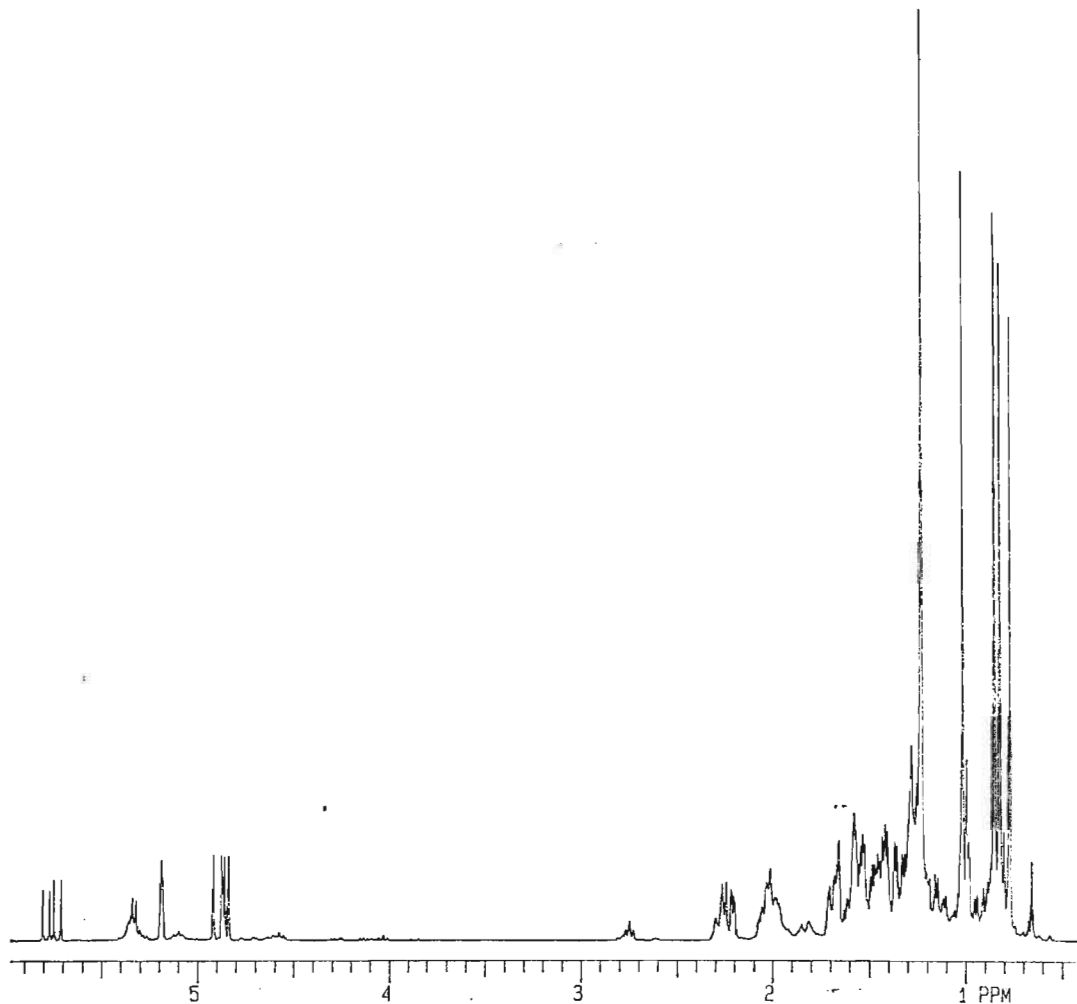
CYZBH2.N2BH-2 IN CDCL3
1H COSY-60

F2 (PPM)

A 44
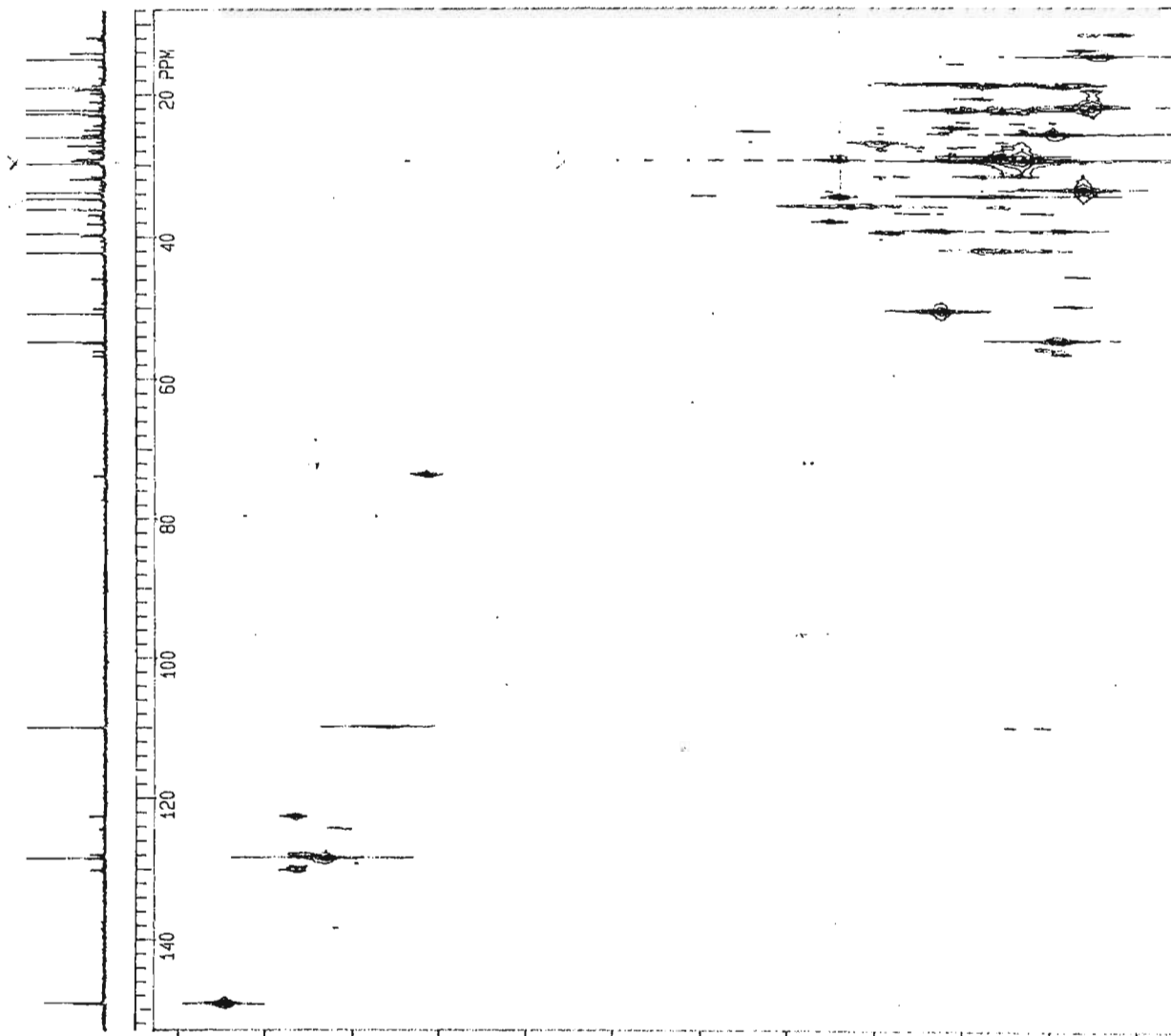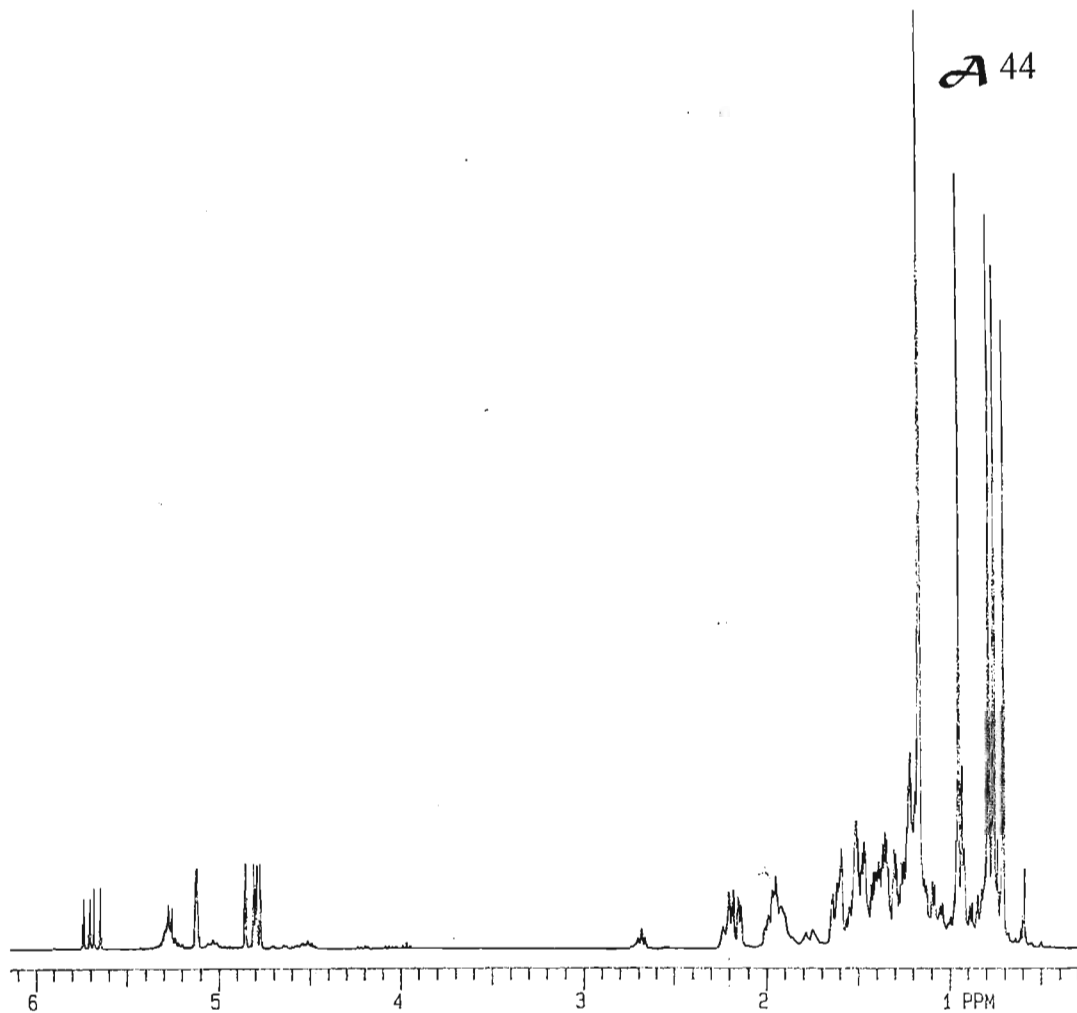
HC2BH2.NZBH-2 IN CDCL3
1H/13C HETCOR

A 45
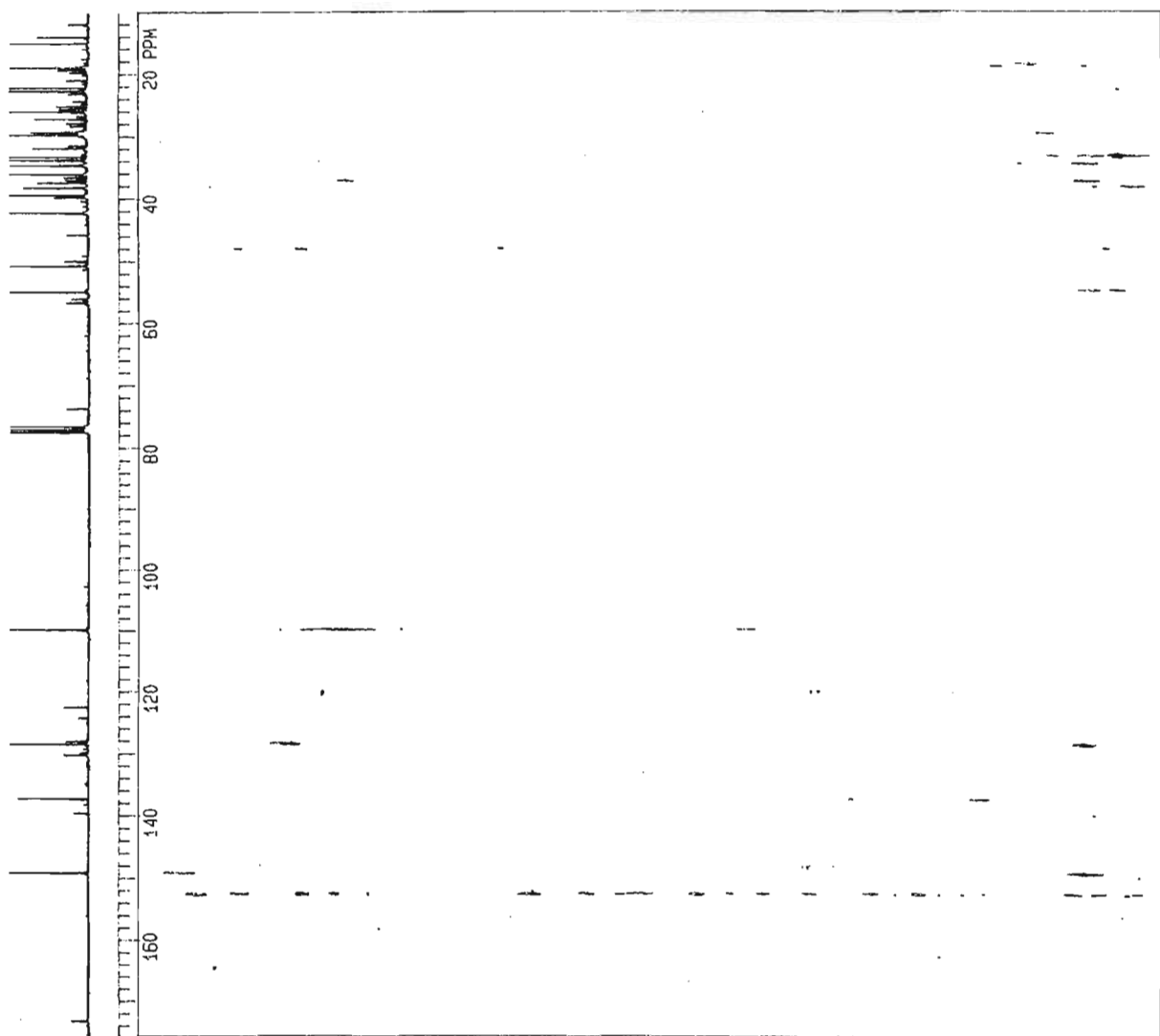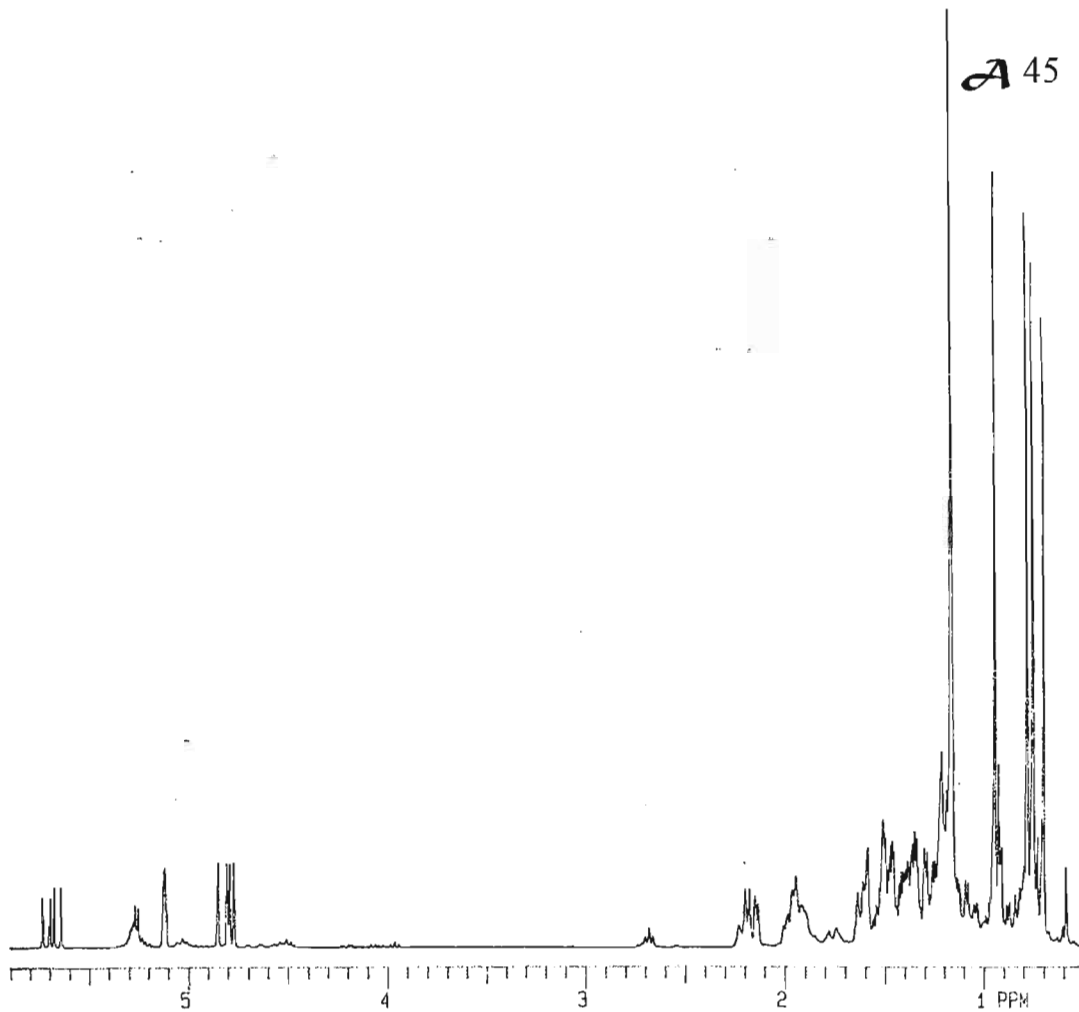
LHCBH2.N2BH-2 IN CDCL3
1H/13C DELAYED HETCOR

# COMPOUND VI (104)



(104) compound VI

## Index

$\mathcal{A}$ 47

M+
288

288.2458
288.2458
= $C_{20}H_{32}O$

255
43
$H_2O$
$CH_3$
270
280
260
240
220
200
180
160
140
120
100
80
60
40
20

HN2B15.N2BH-15 IN CDCL3

HN2B15.N2BH-15 IN CDCL3

*A* 50

5.4863

4.9443
4.9154
4.9109
4.9001
4.8802
4.8757

4.1801
4.1712

5.6  5.4  5.2  5.0  4.8  4.6  4.4  4.2 PPM

CRZB15.N2BH-15 IN CDCL3

*A* 51
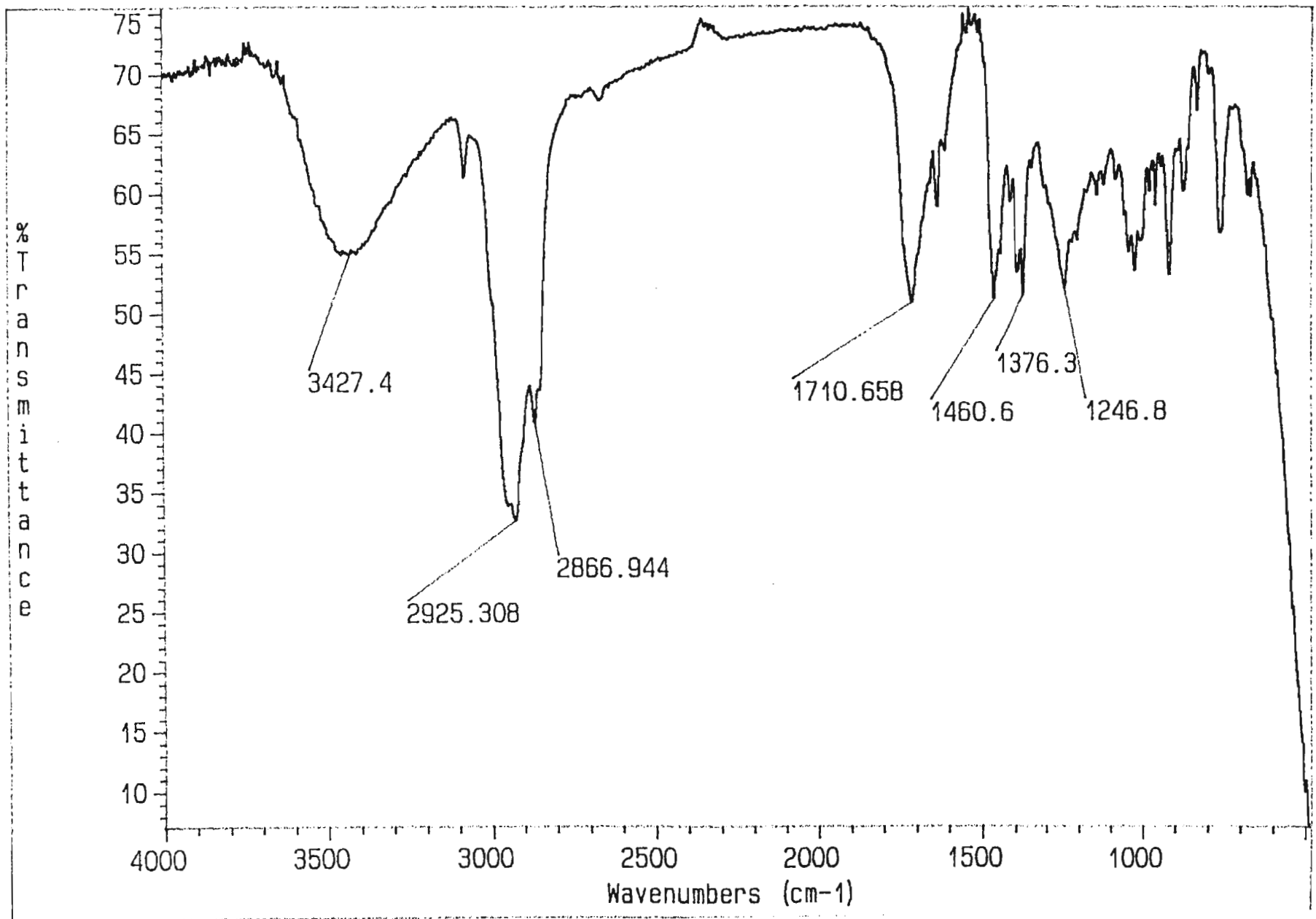
DNZB15.NZBH-15 IN CDCl3

CH3

CH2

CH

Cn

A 52

𝒜 53

CYNB15.NZBH-15 IN CDCL3
1H COSY-60

HCNB15.NZBH-15 IN CDCL3
1H/13C HETCOR

# COMPOUND VIa (104a)
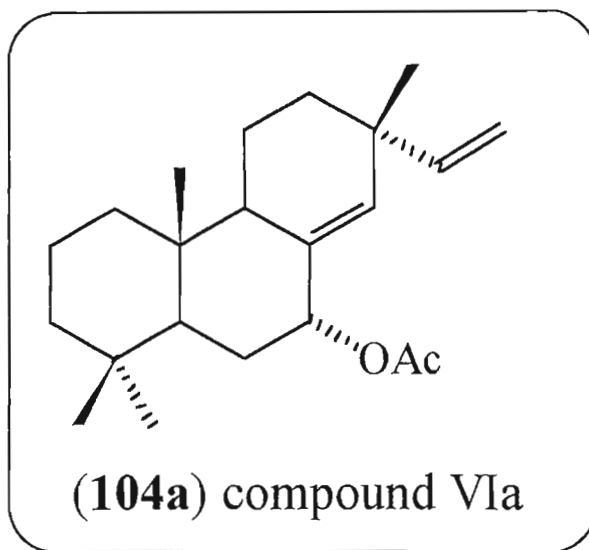


(104a) compound VIa

## Index

# COMPOUND VII (105)

*A* 56

# COMPOUND VII (105)
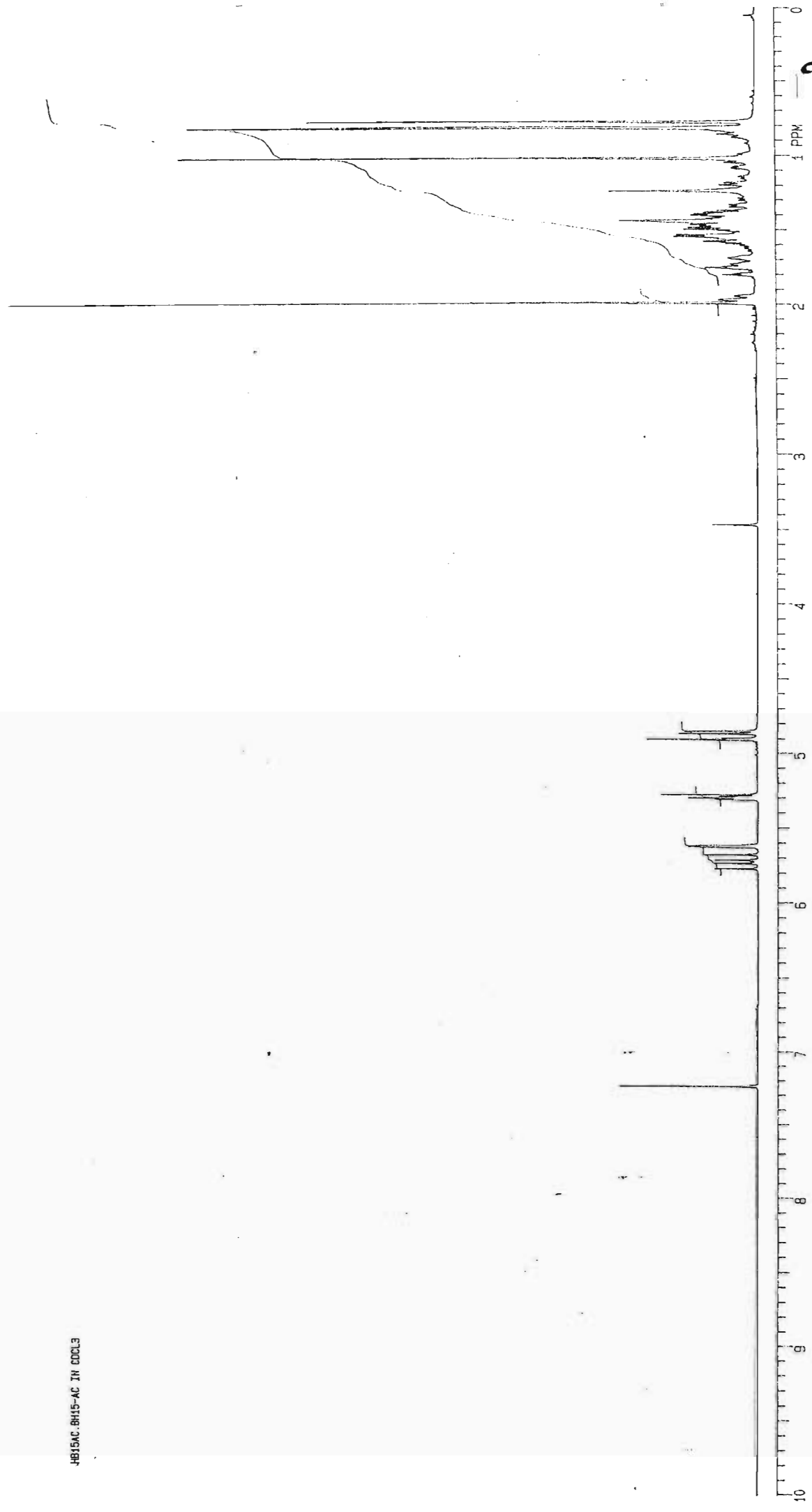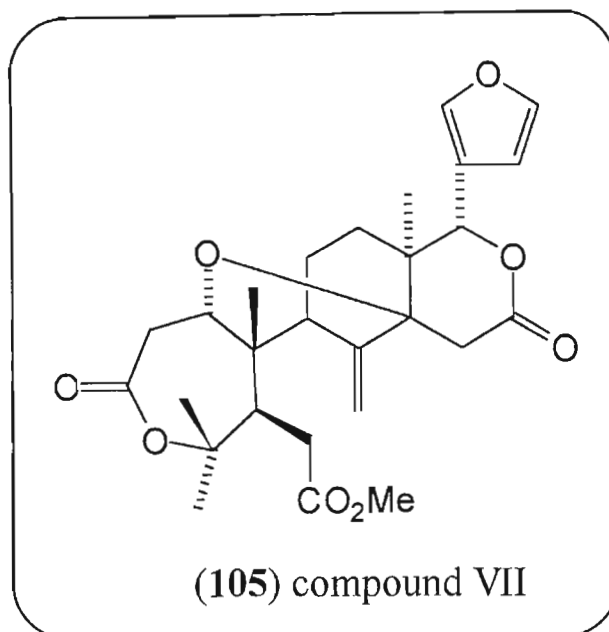


(105) compound VII

## Index
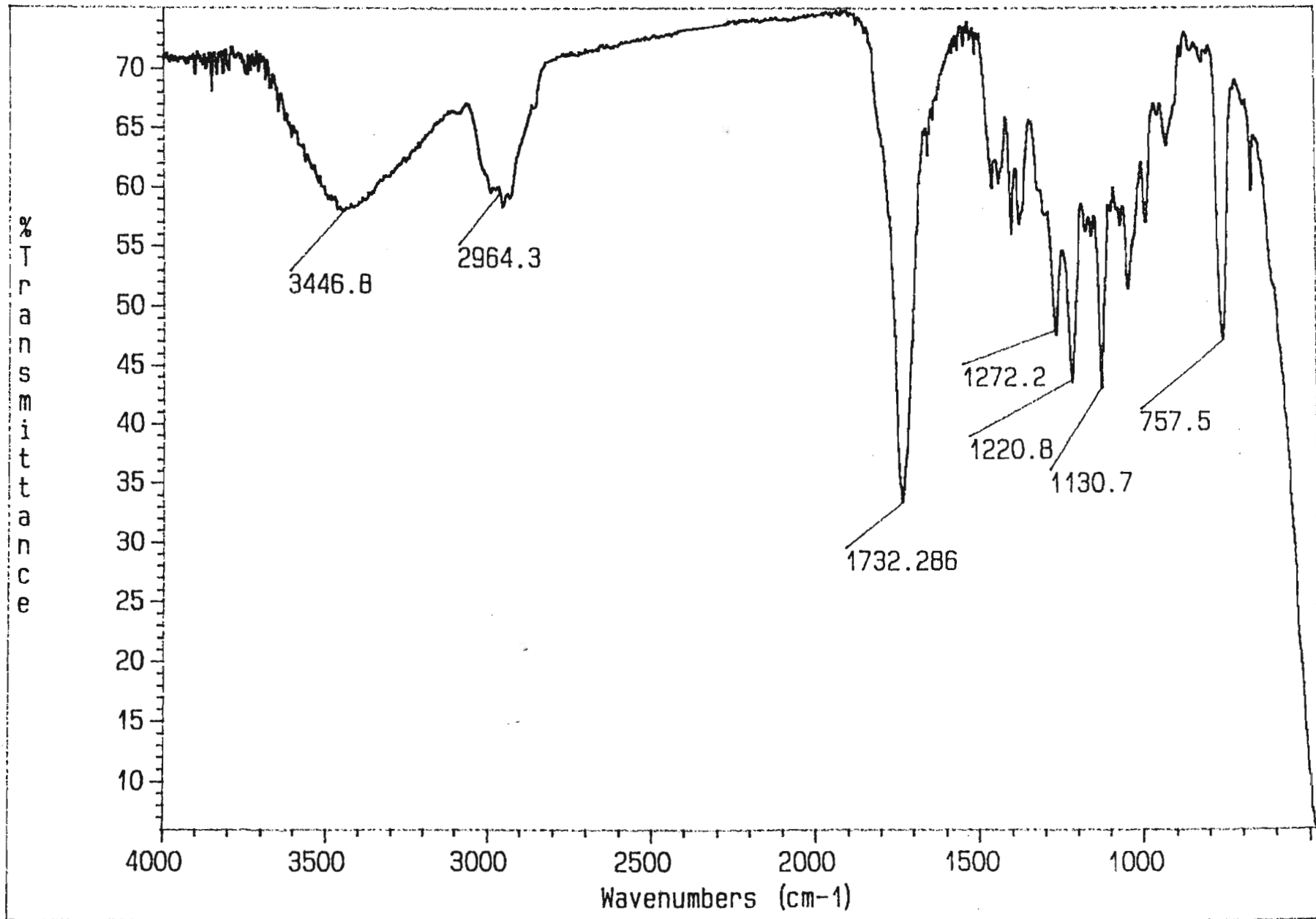
# COMPOUND VIII (99)

486.2238
486.2251
= $C_{27}H_{34}O_8$

486

HNZB10.NZBH-10 IN CDCL3

CNZB10.NZBH-10 IN CDCL3

CYNB10.NZBH-10 IN CDCL3
1H COSY-60

# COMPOUND VIII (99)



(99) compound VIII

## Index

A 66

HN2B11.N2BH-11 IN CDCL3

II N 213 H-8.

# COMPOUND IX (113)



(113)

## Index

A 69

HTHRH4. THRH-4 IH CDCL3

CTHBH4.THBH-4 IN CDCL3

*A* 71

DTHRH4. THRH-4 IN CDCL3

CH3

CH2

CH

CHn

𝒜 72

CYRH4.THRH-4 IN CDCL3
1H COSY-60

F2 (PPM)

HCRH4.THHH-4 IN CDCL3
1H/13C HETCOR

# COMPOUND X (83)
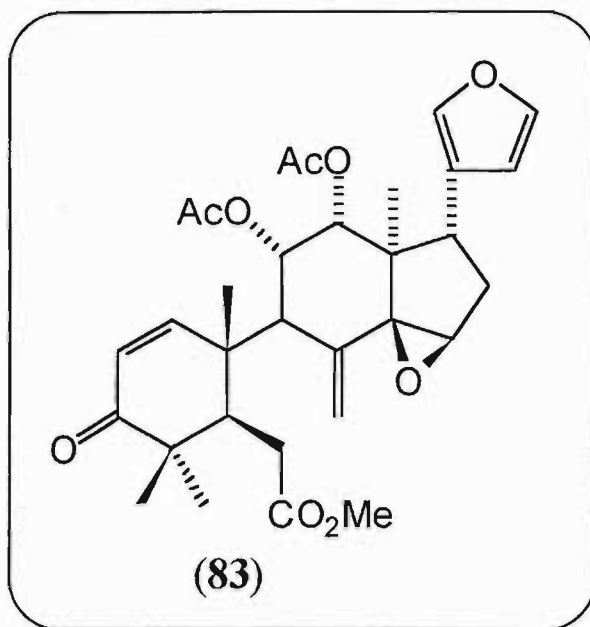


(83)
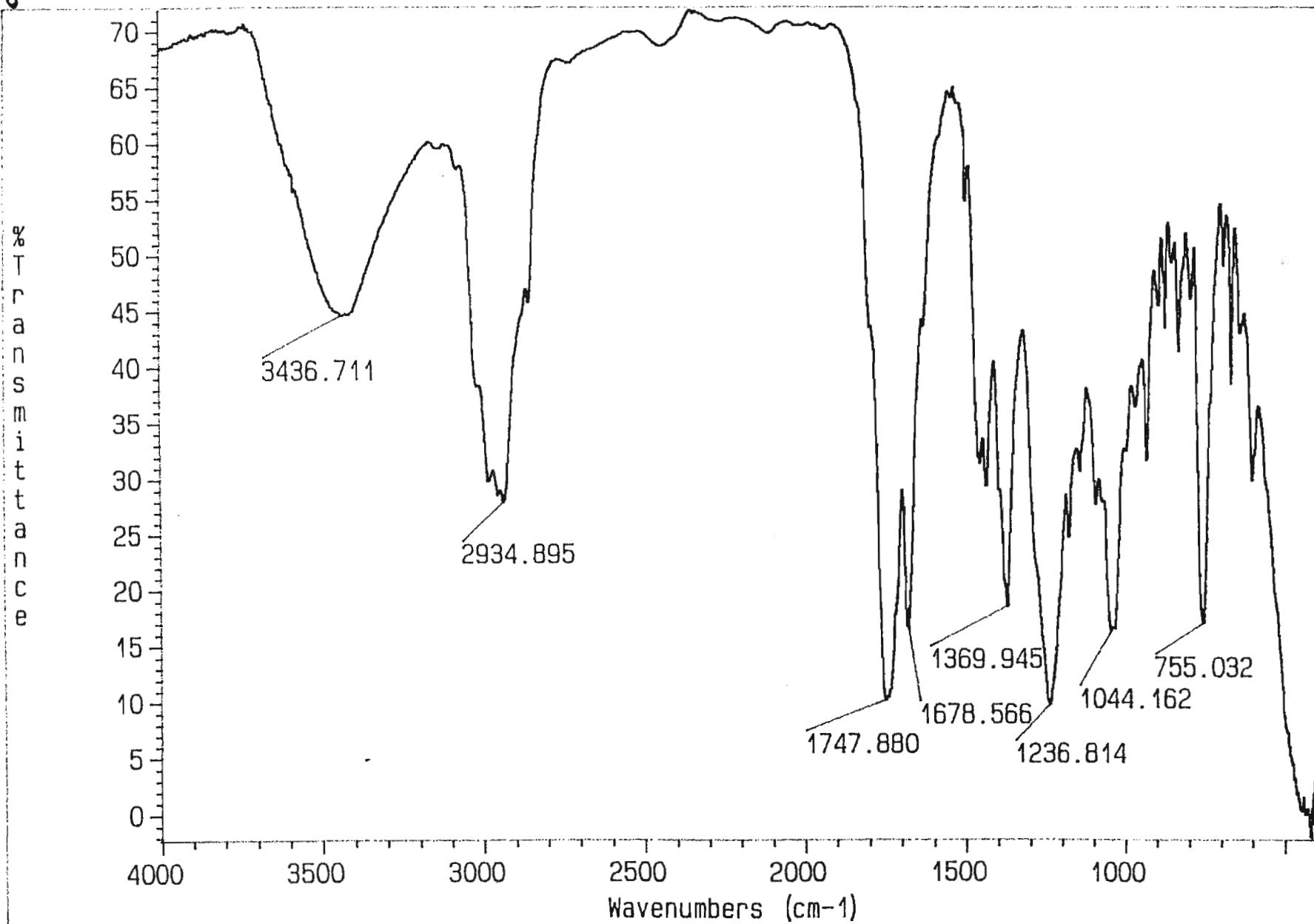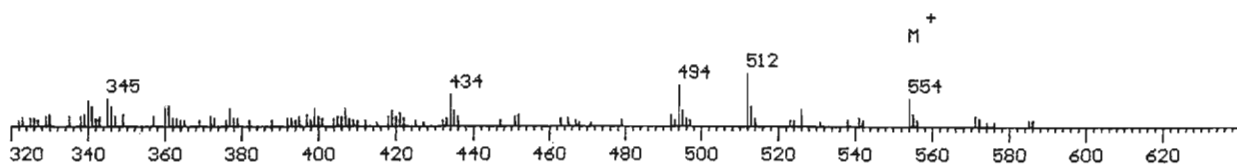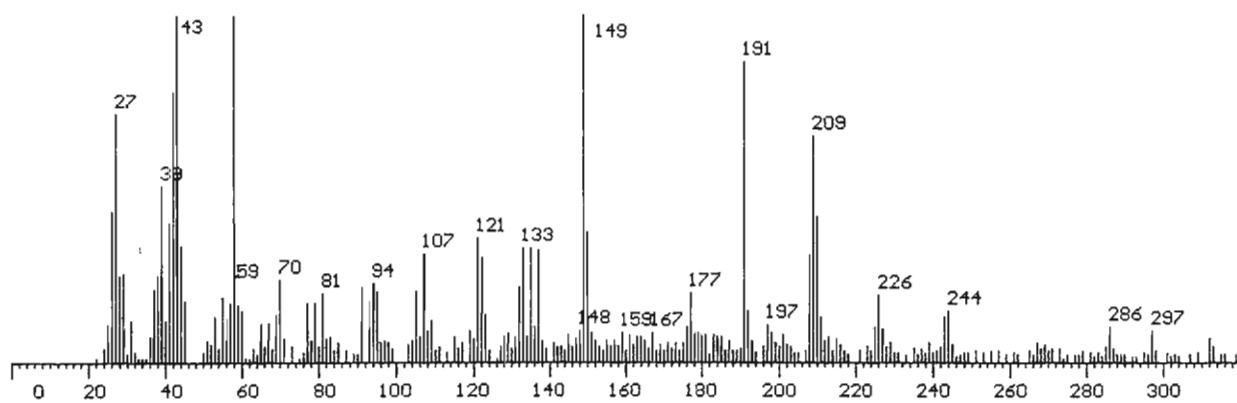
## Index

HTNMR12.THRH-12 IN CDCL3

*A* 78
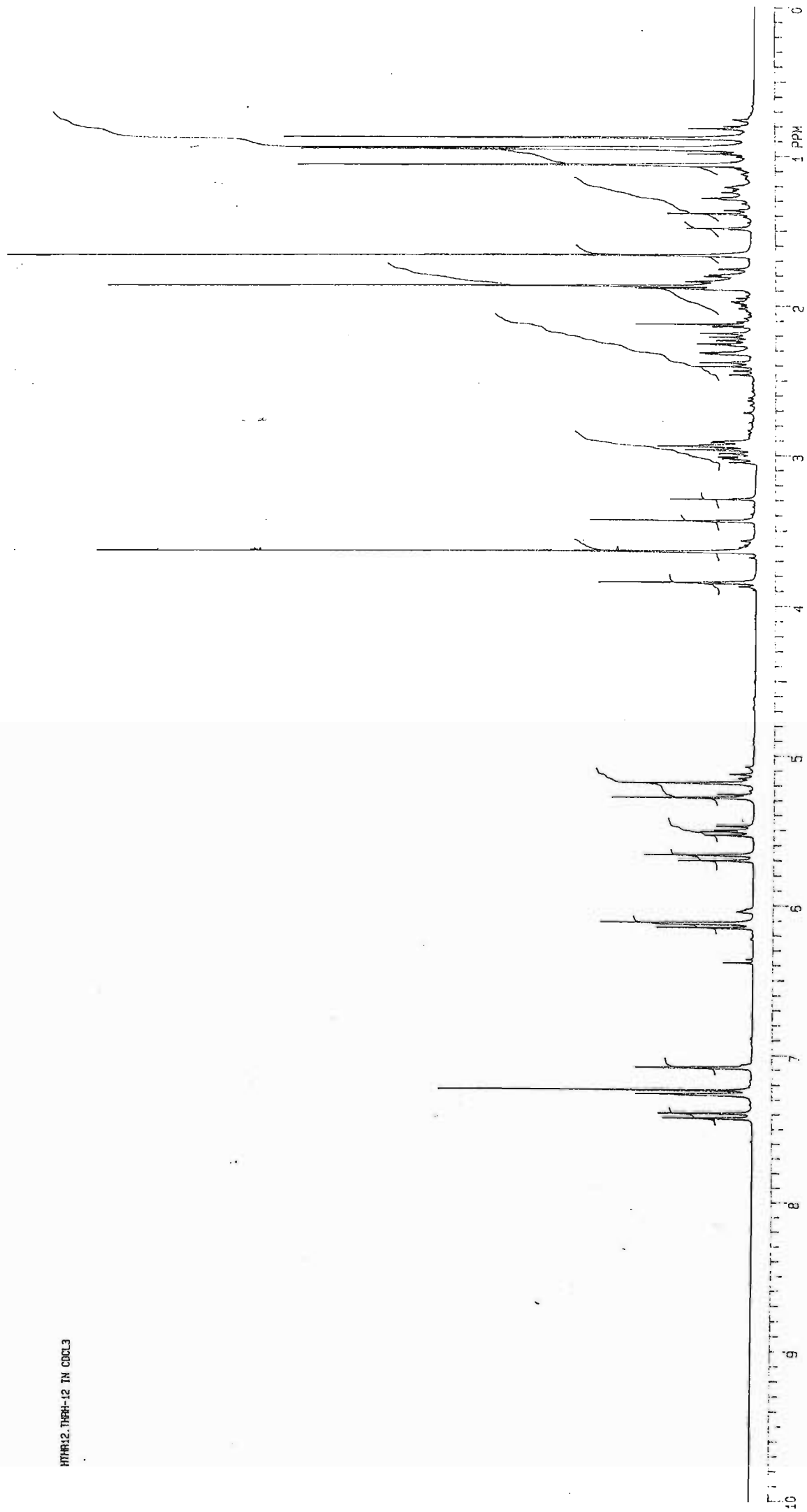
CTHR12.THRH-12 IN CDCl3

*A* 79

DTHR12.THRW-12 IN CDC.3
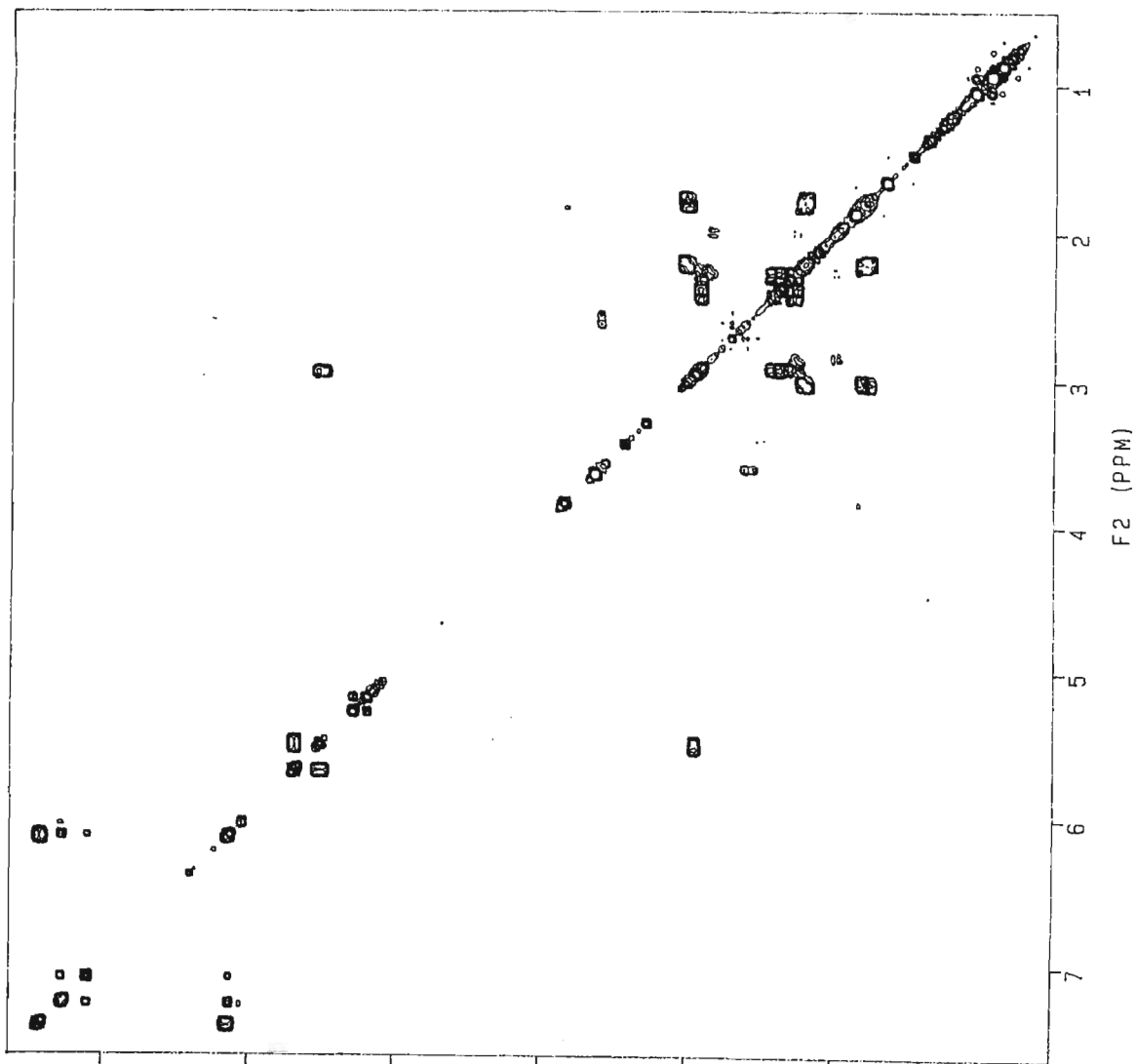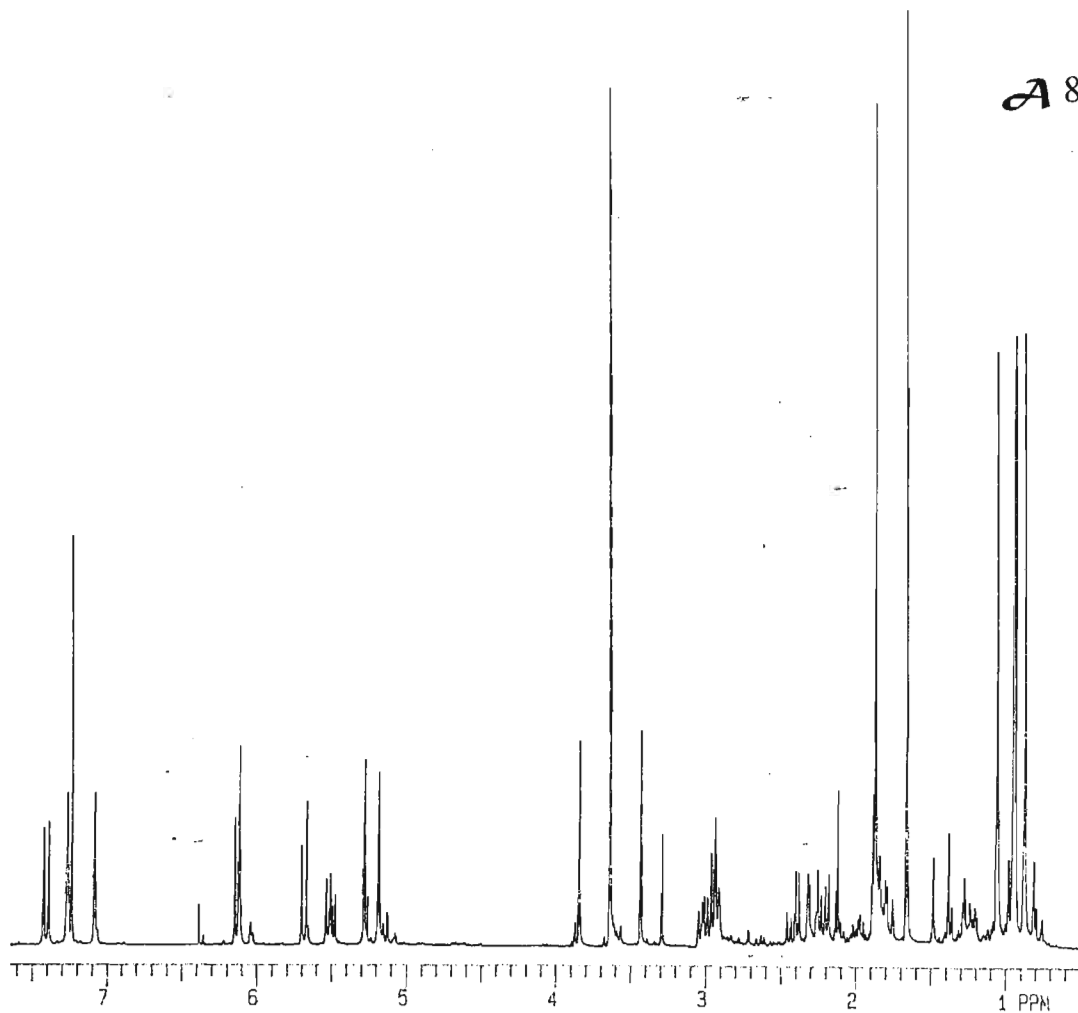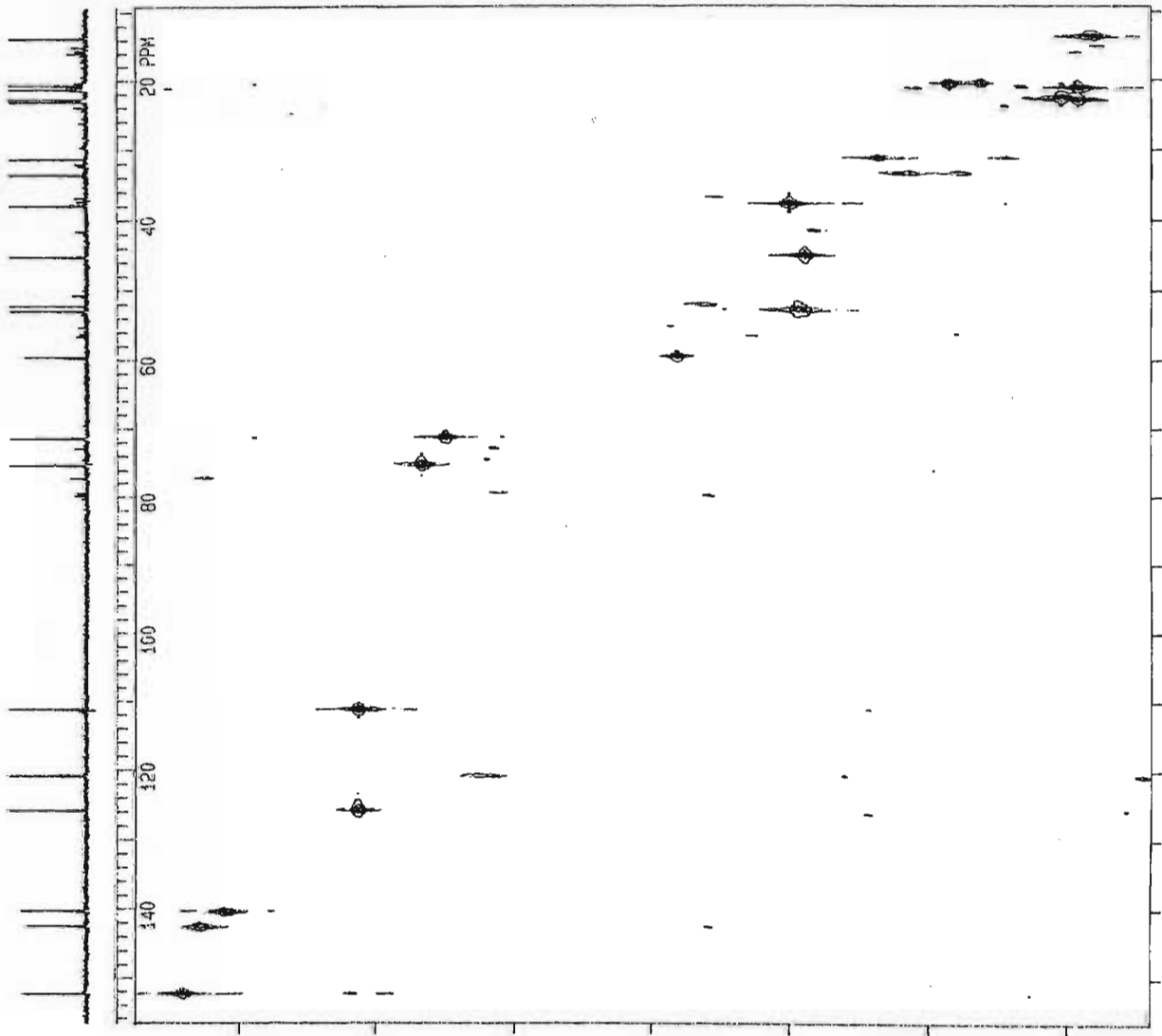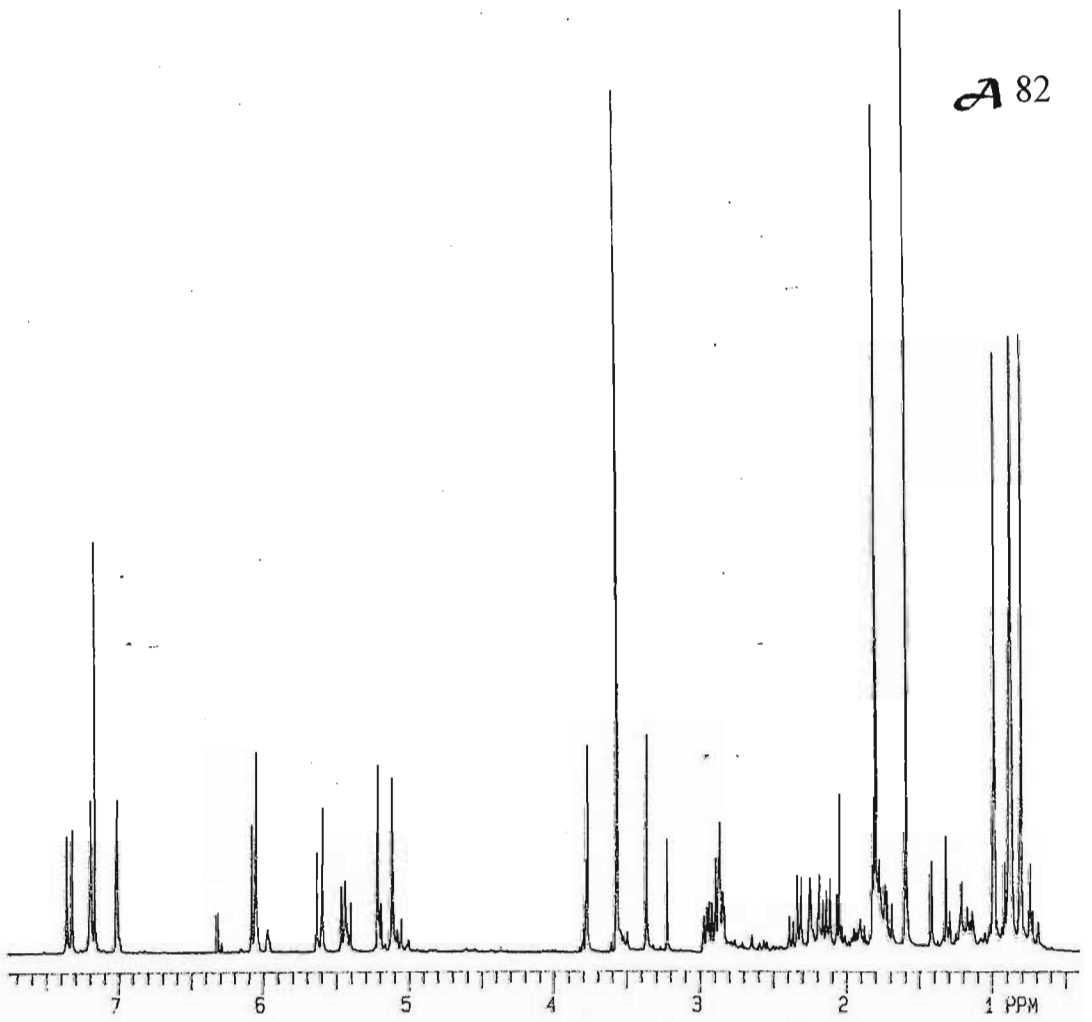
C H 3

C H 2

C H

C H

CYRH12.THRH-12 IN CDCL3
1H COSY-60

F2 (PPM)

HCRH12. THRH-12 IN CDCL3
1H/13C HETCOR

# COMPOUND XI (114)
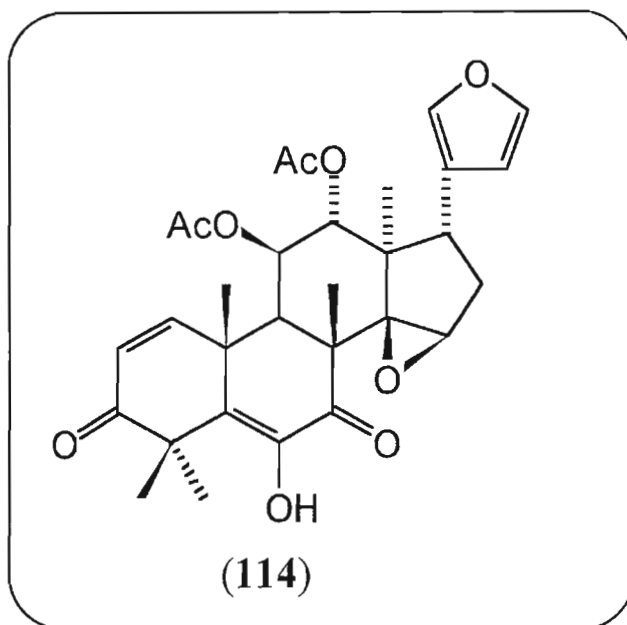


(114)

## Index

HTHSH1. THSH-1 IN CDCL3 = TMSC1

= THRC 3

A 86

A 87
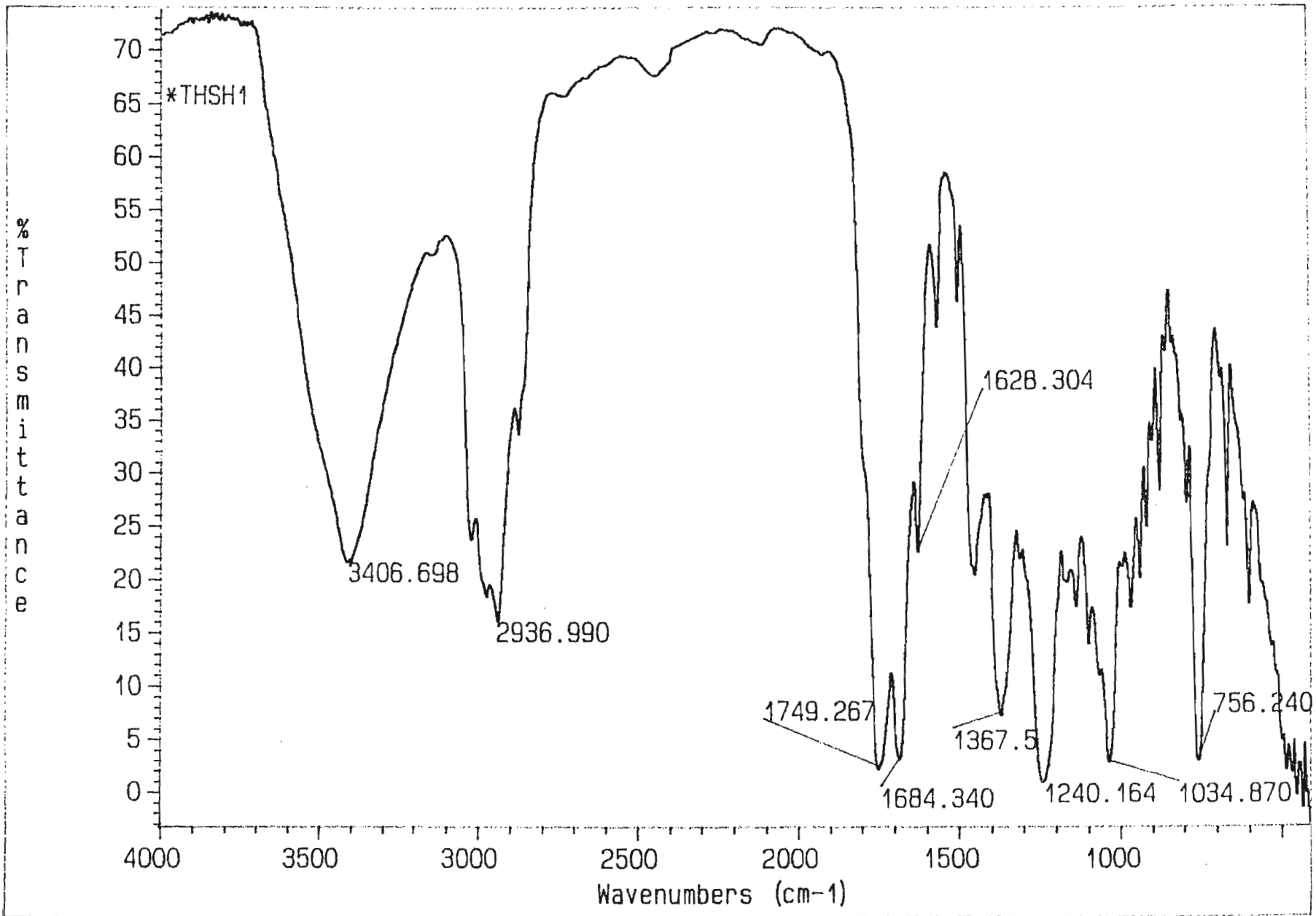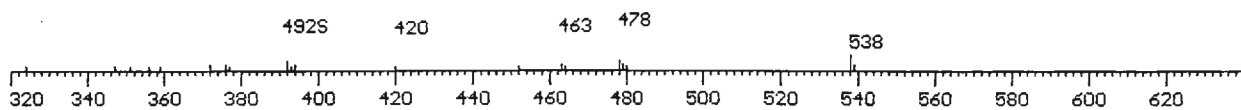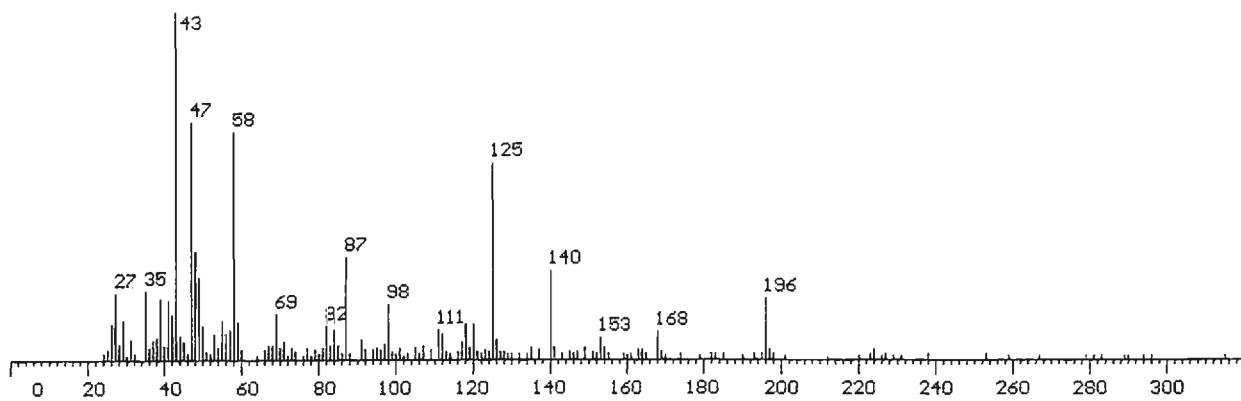
CTHRC3.THRC-3 IN CDCL3

DTHSH1.THSH-1 IN CDCL3

C H 3

C H 2

C H

C H n

A 88

160   140   120   100   80   60   40   20 PPM   0
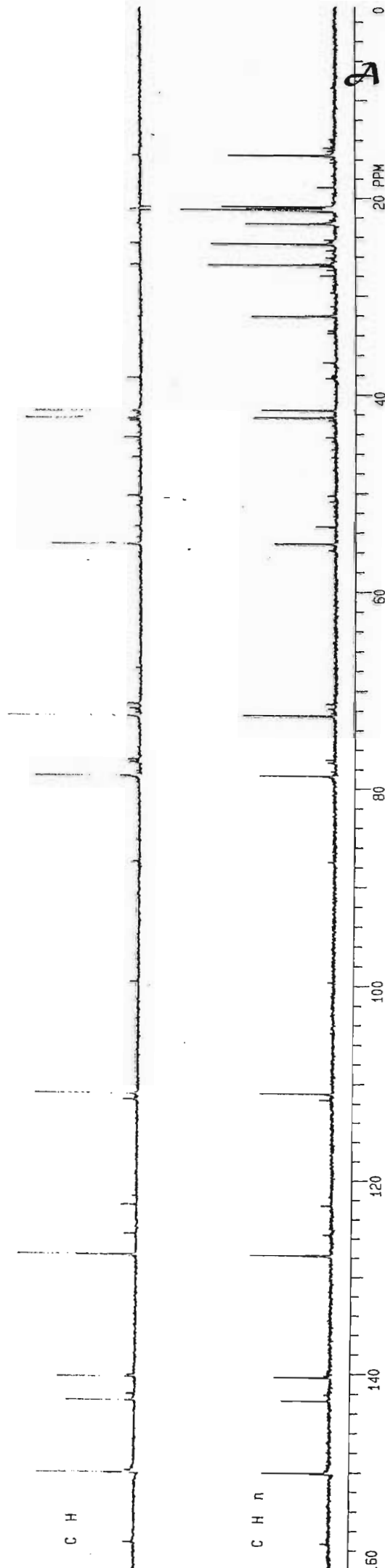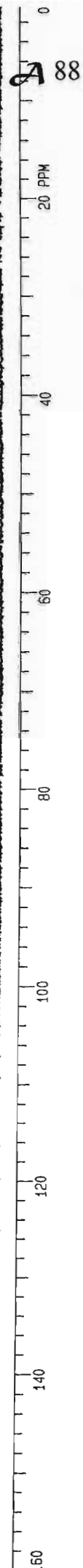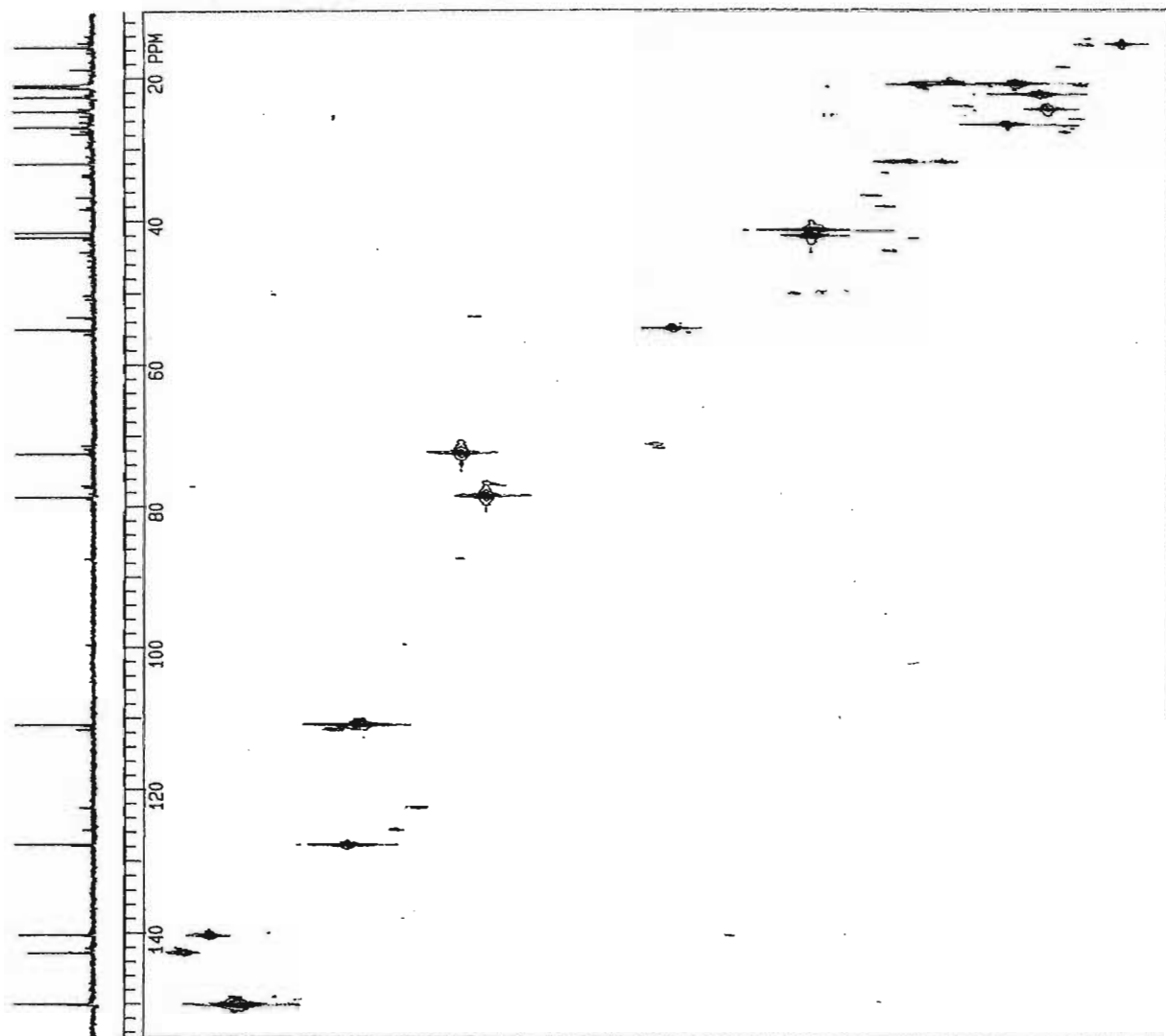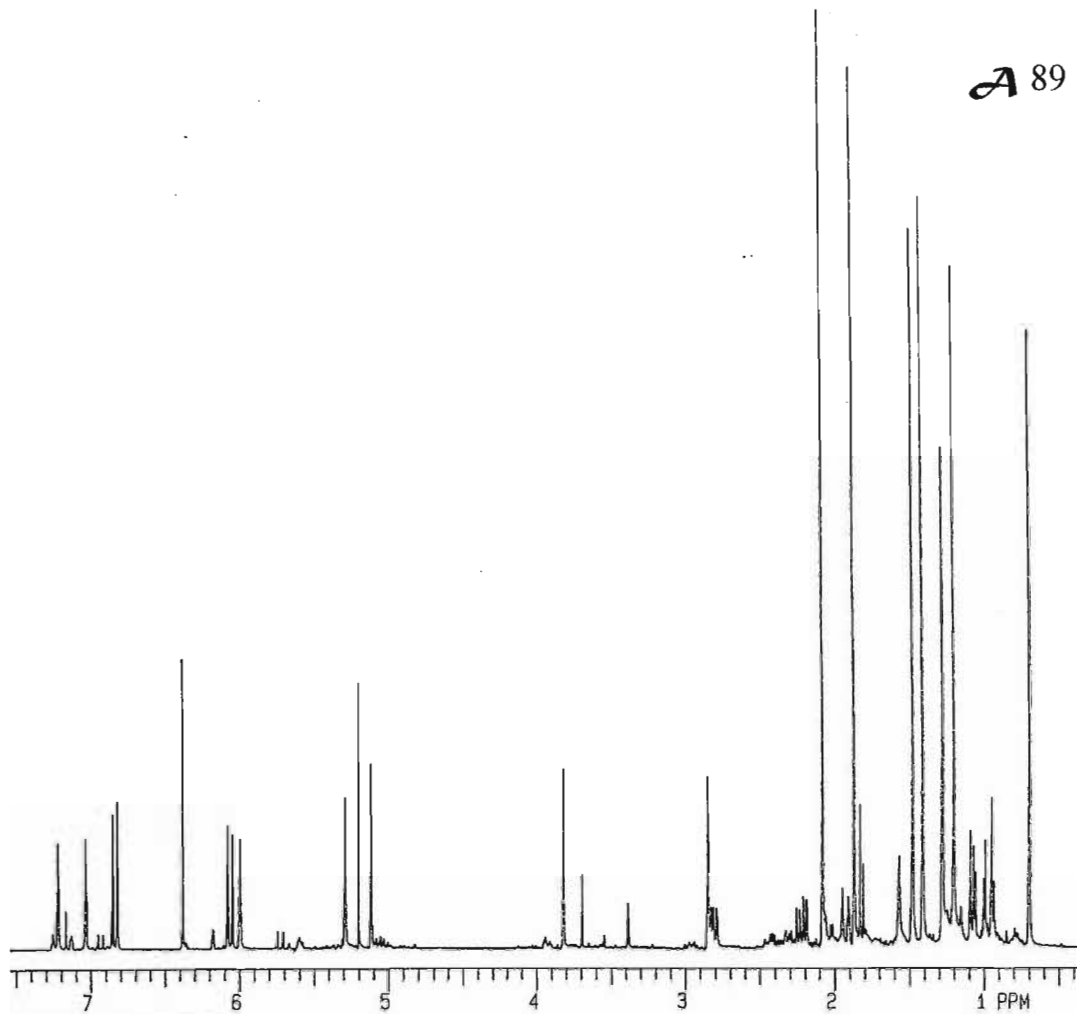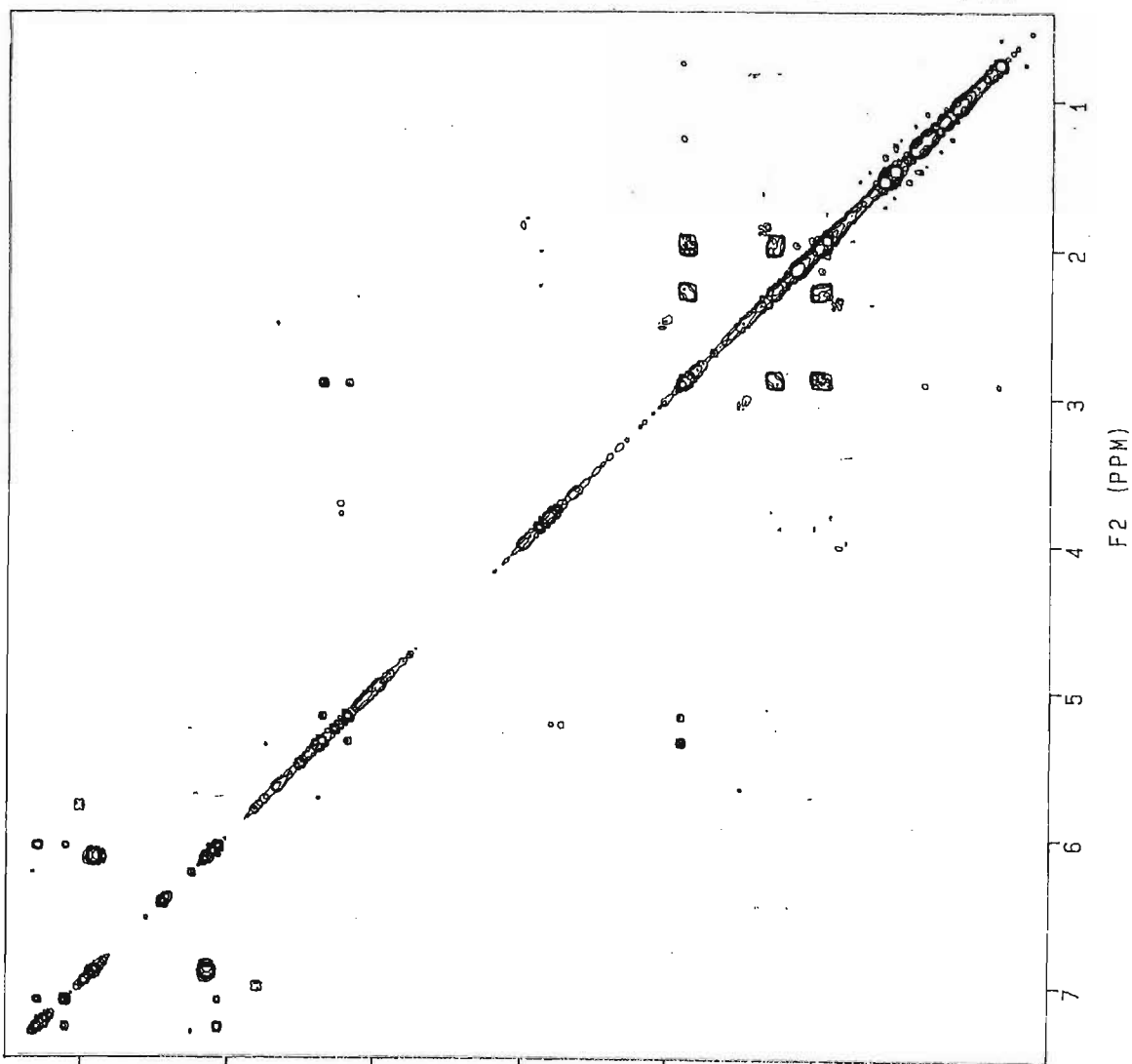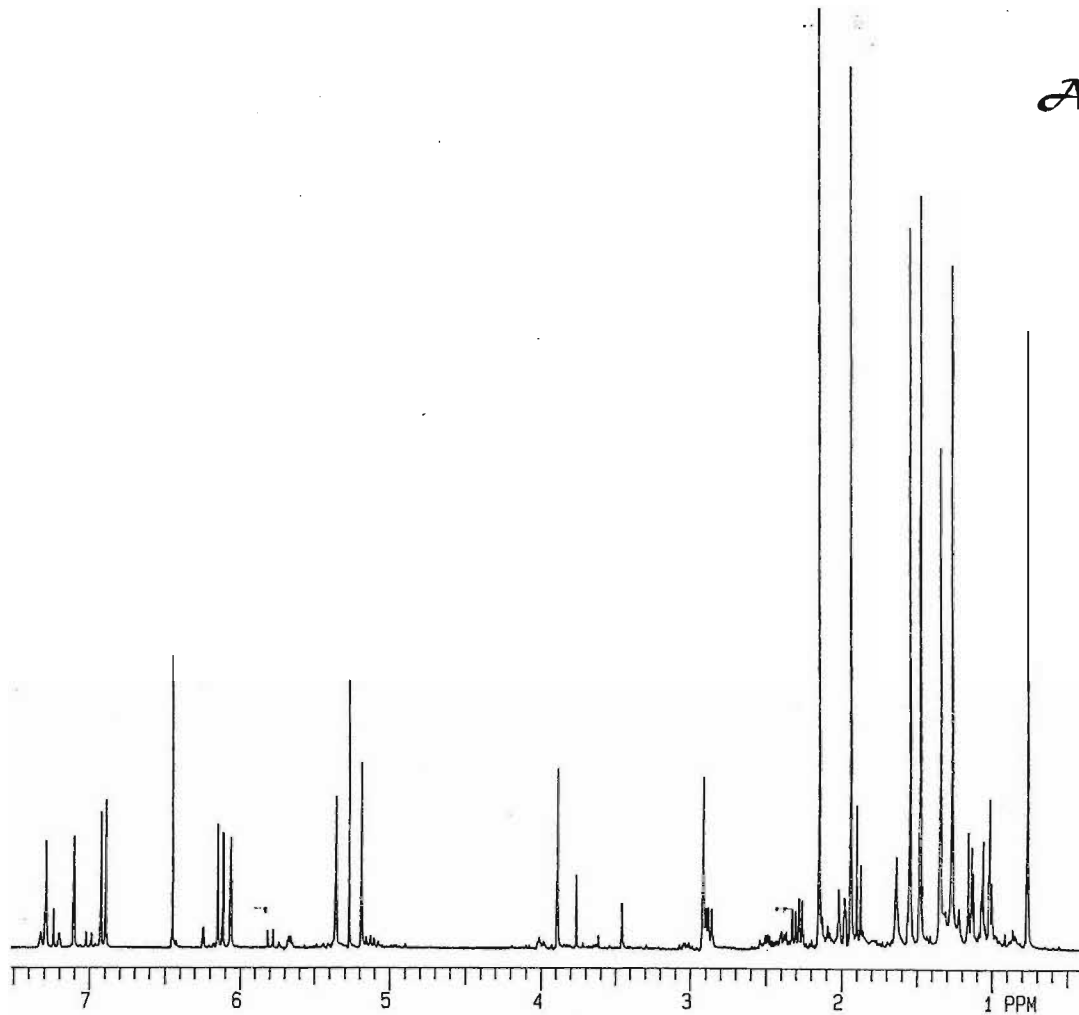
CYHSH1.THSH-1 IN CDCL3
1H COSY-60

F2 (PPM)

# COMPOUND XII (118)



**(118)** 12α-acetoxyneotrichilenone

## Index

HTHSC3.THSC-3 IN CDCL3

A 94

HTHSC3.1HSC-3 IN CDCL3

2.5501
2.5188
2.5119
2.3438
2.3359
2.2999
2.2922
2.1422
2.0391
2.0285
1.9958
1.9766
1.9652
1.9374
1.9292
1.8930
1.8851
1.8381
1.8279
1.7994
1.7895
1.7315
1.7217
1.7124
1.6839
1.6737
1.6462

95

A 96

CTHSC3.THSC-3 IN CDCL3

DTHSC3.THSC-3 IN CDCL3

A 97

CYHSC3.THSC-3 IN CDCL3
1H COSY-60

F2 (PPM)

HCHSC3.THSC-3 IN CDCL3
1H/13C HETCOR

# COMPOUND XIII (119)



(119)
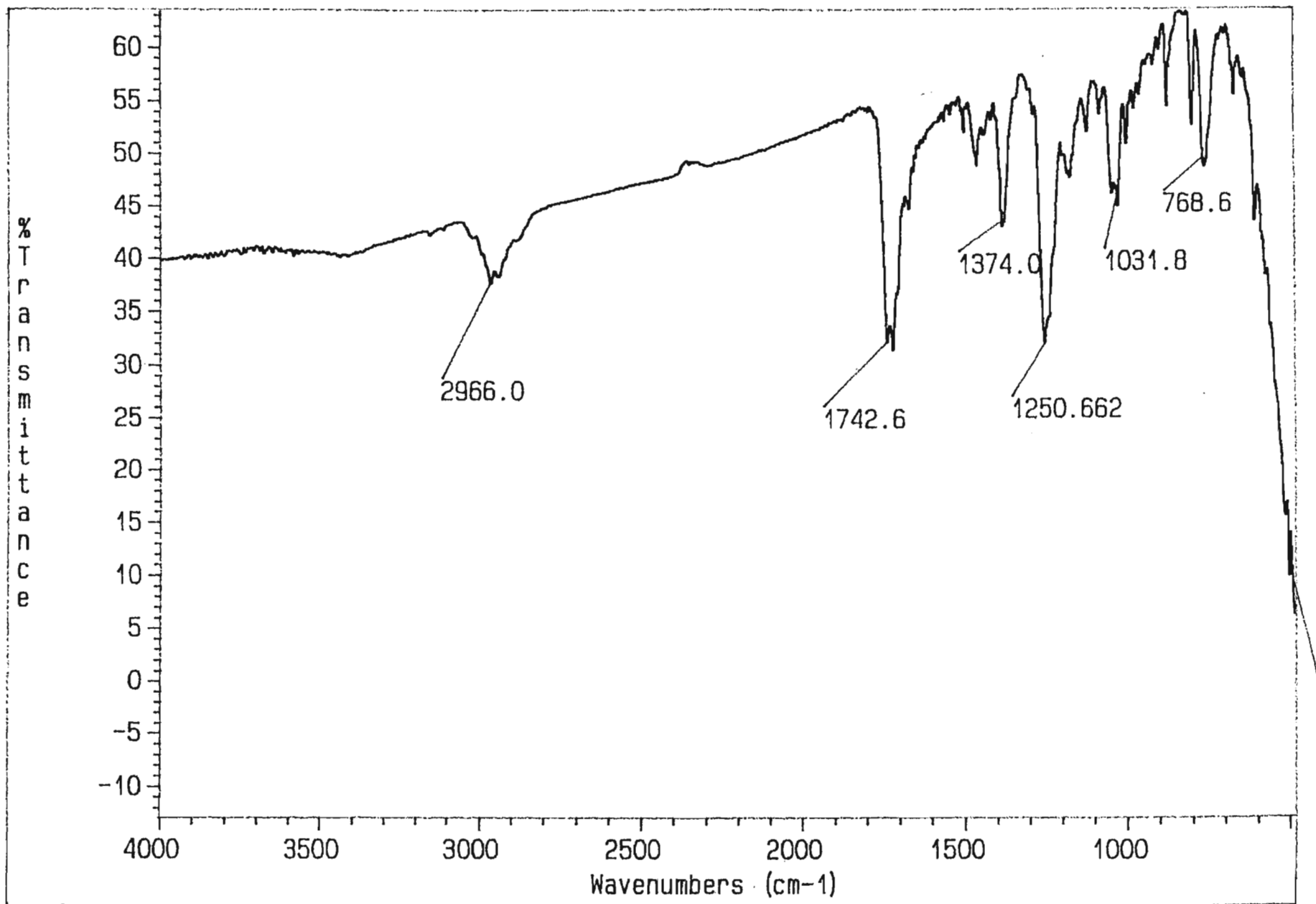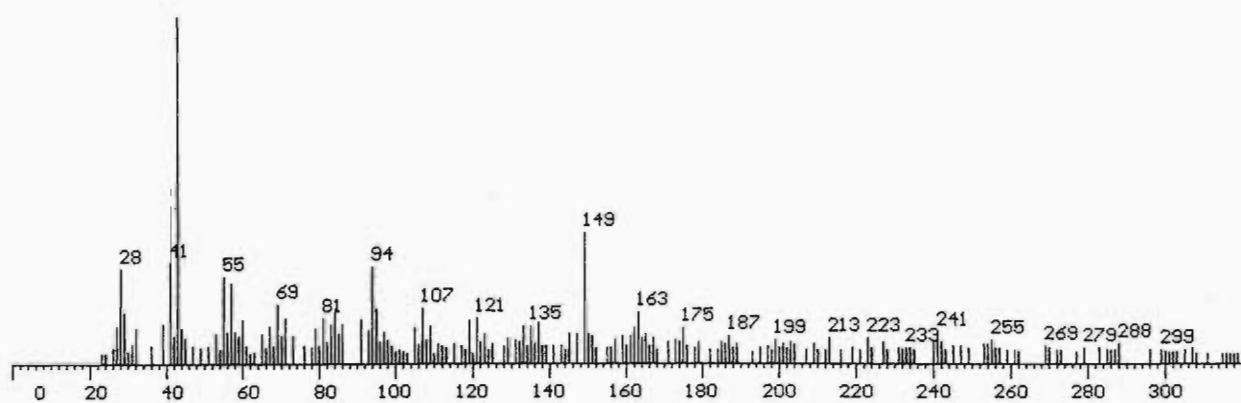
## Index

512.2784
512.2772
≡ C₃₀H₄₀O₇

A 103

HTHRC4.THRC-4 IN CDCL3

CTHRCJ.THRC-4 IN CDCL3

A 104
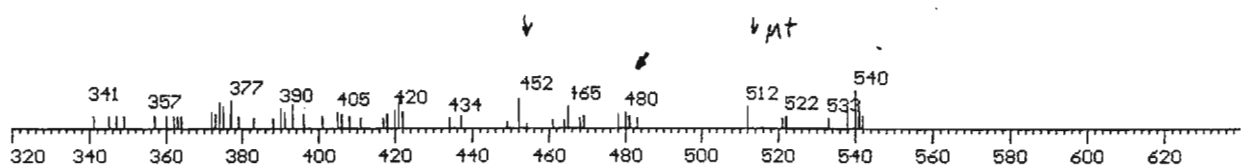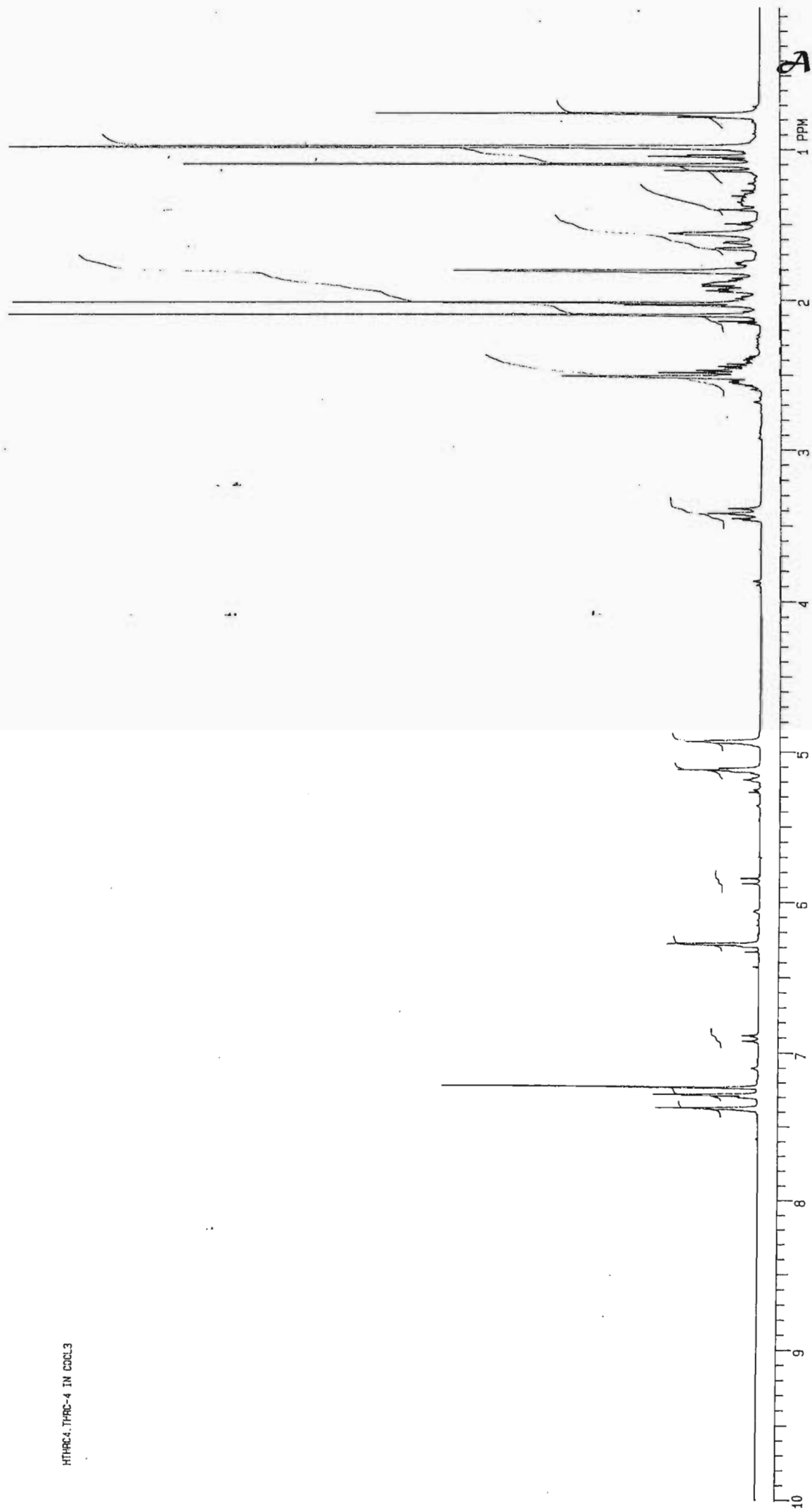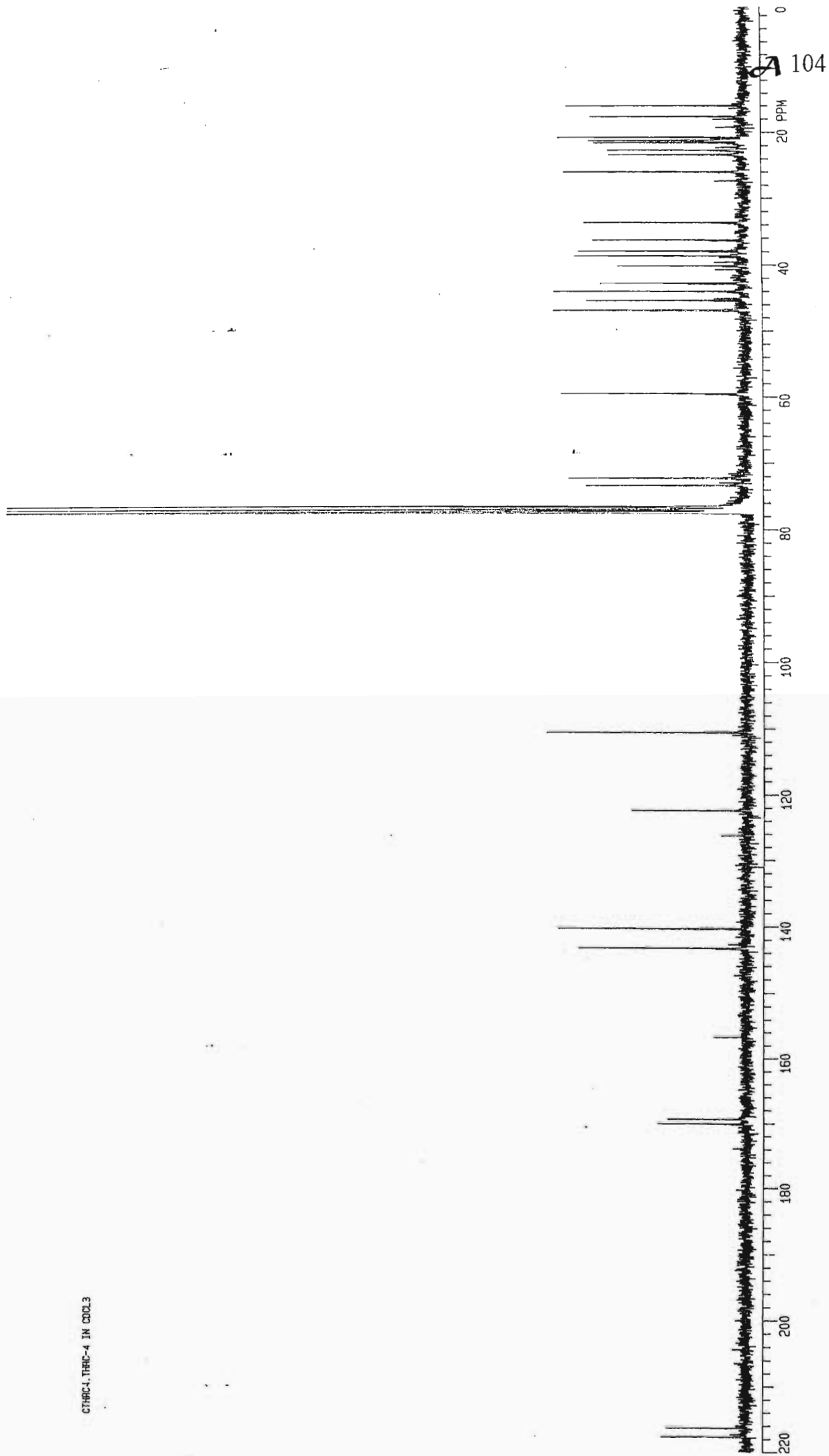
DTHRC4.THRC-4 IN CDCL3

CH3

CH2

CH

CHn

CYHRC4.THRC-4 IN CDCL3
1H COSY-60

F2 (PPM)

# COMPOUND XIV (120)



(120)

## Index

108

HTHRC7.THRC-7 IN CDCL3

# COMPOUNDS XV (121), XVa (121a)
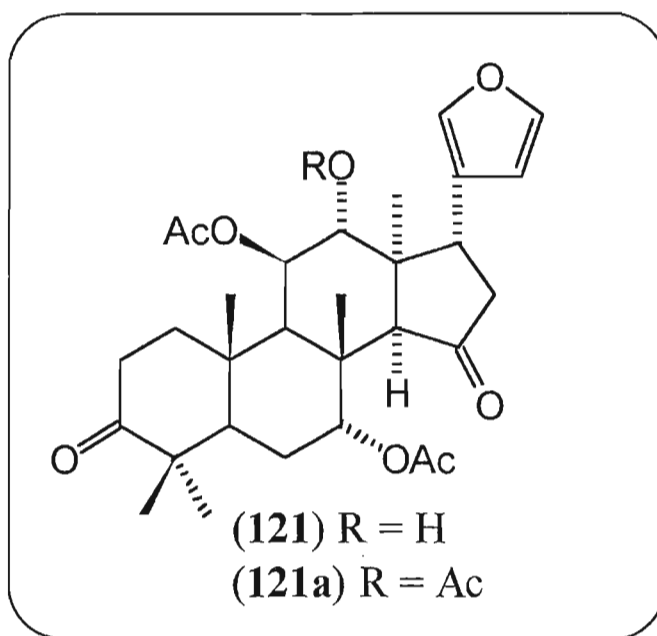


(121) R = H
(121a) R = Ac

## Index

A 110
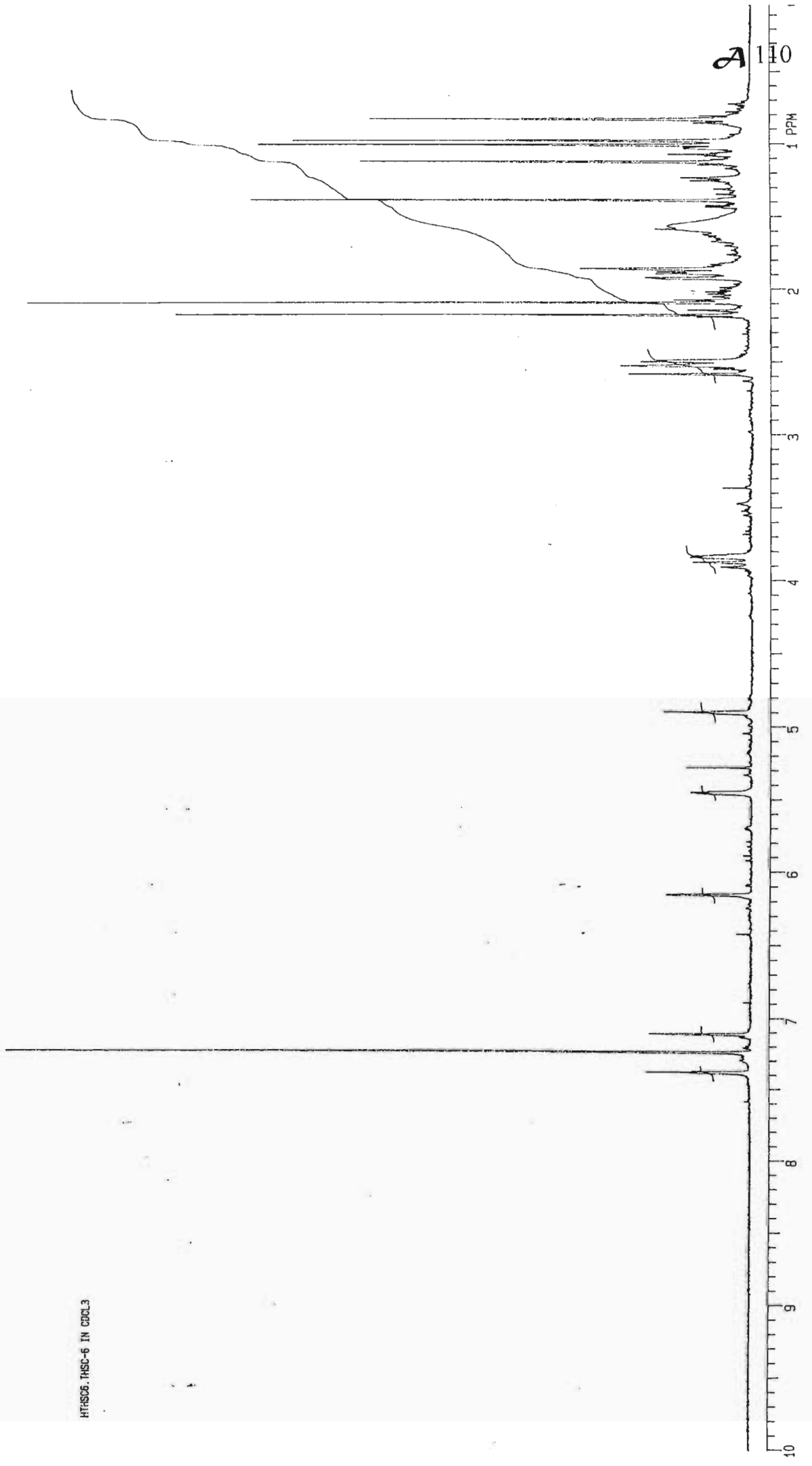
HTHSO6.THSC-6 IN CDCL3
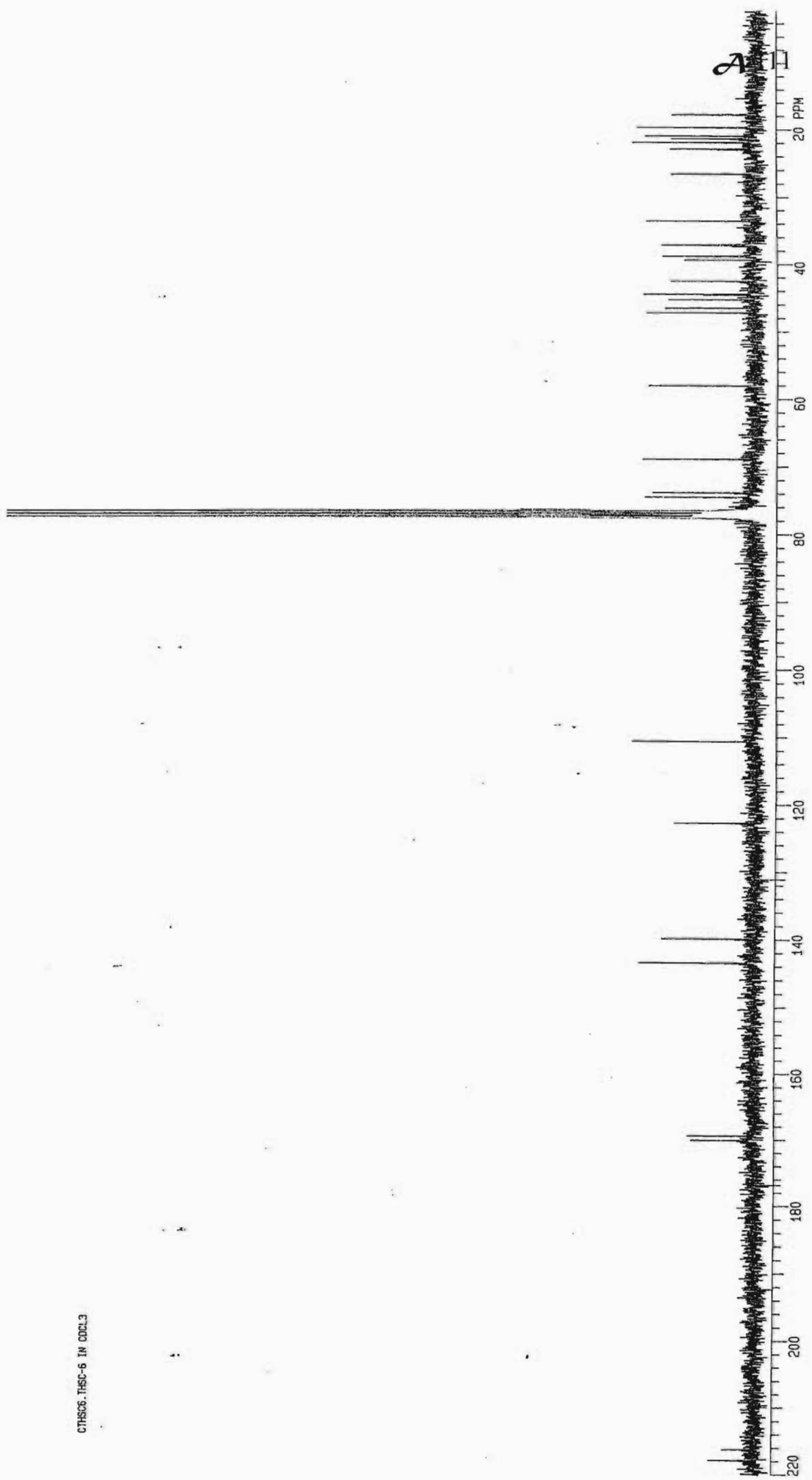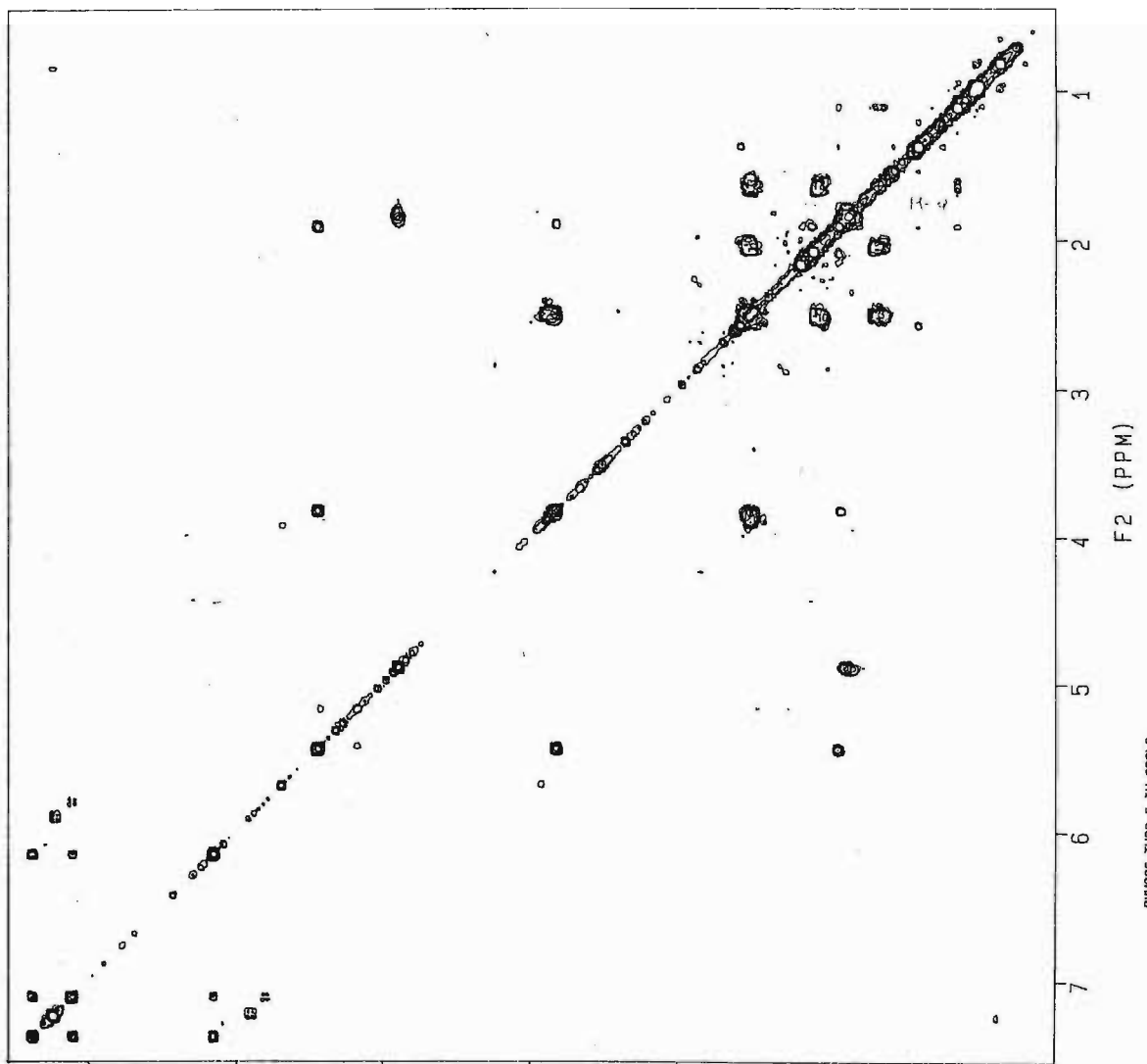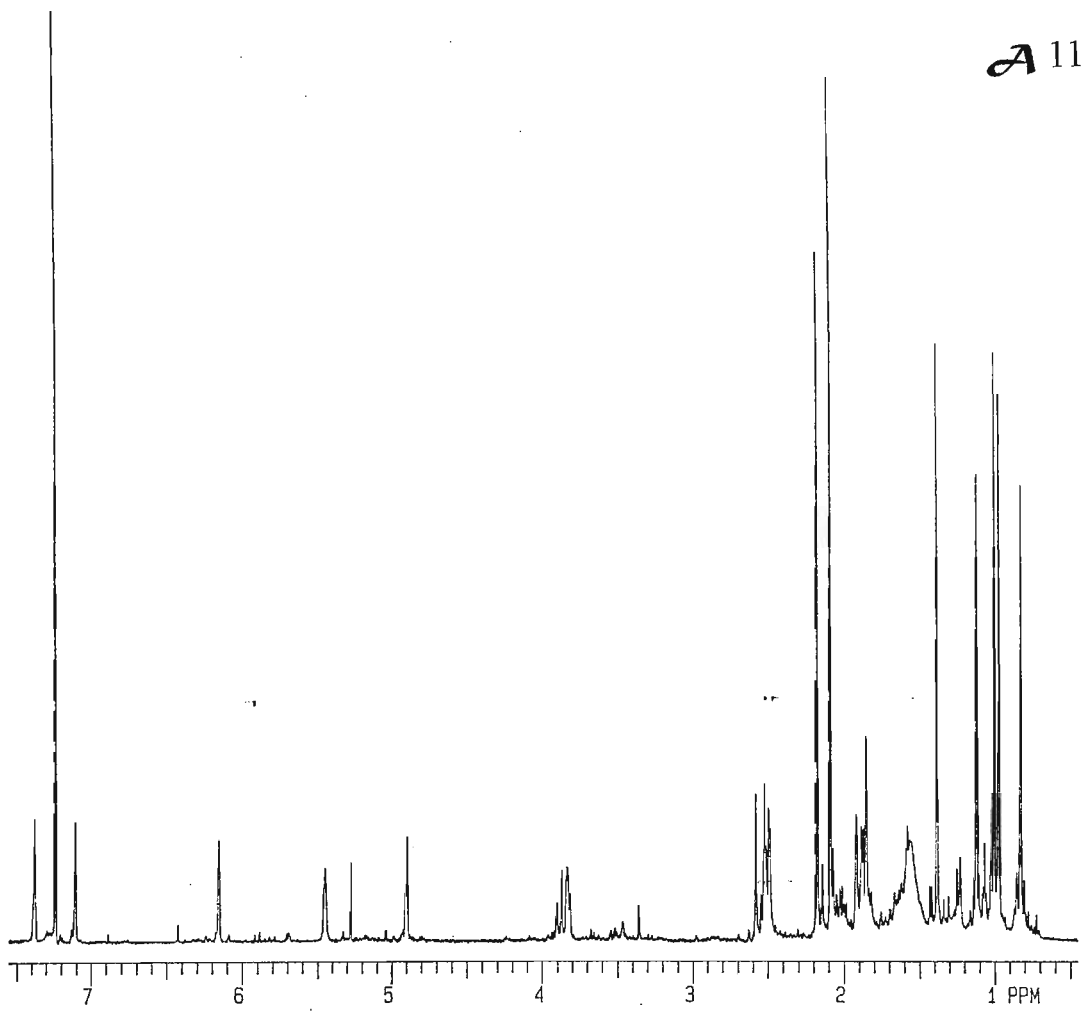
CTHSC6.THSC-6 IN CDCL3

CYHSC6.THSC-6 IN CDCL3
1H COSY-60

HCHSC6.THSC-6 IN CDCL3
1H/13C HETCOR

HSC6AC.SC6-AC IN CDCL3

# COMPOUND XVI (122)



(122)

## Index

$\mathcal{A}$ 117

*A* 118

CTHR18.THR4-18 IN CDCL3

OTHR18.TH0H-18 IN C0CL3

CH3

CH2

CH

CHn

A 119

CYTH18.THRN-18 IN CDCL3
1H COSY-60

F2 (PPM)

HCTH18. TARH-18 IN CDCl3
1H/13C HETCOR

# COMPOUND XVII (124)



(124)

## Index

HTFR19.THRH-19 IN CDCL3

2.5725
2.5503

2.4061
2.3923

2.3681
2.3548

2.2952

2.2165

2.1992
2.1837
2.1736
2.1589
2.1465
2.1325

2.1024

2.0696

2.0501
2.0454

1.9945
1.9809
1.9703
1.9551
1.9396
1.9300
1.9241
1.9146
1.9054

1.8641

1.8385

1.7791
1.7689
1.7545
1.7437
1.7309
1.7195

1.6957

1.6580

1.6224

1.5744

1.5478
1.5366

A 127

HTNA19.TNAN-19 IN CDCL3

CTHA19.THRH-19 IN CDCL3

DTHA19.THAH-19 IN CDCL3

CH3

CH2

CH

CHn

CYHR19.THRH-19 IN CDCL3
1H COSY-60

F2 (PPM)

*A* 131

# COMPOUND XVIIa (124a)



(124a)

## Index

1 PPM

HSH8AC.SH-8AC IN CDCL3

CSHBAC.SH8-AC IN CDCL3

A 134

DSHBAC.SH8-AC IN CDCL3

$\mathcal{A}$ 135

CYHBAC.SH8-AC IN CDCL3
1H CCSY-60

F2 (PPM)

HCHBAC.SHB-AC IN CDCL3
1H/13C HETCOR

# COMPOUND XVIIb (124b)



(124b)

## Index

A 139

HSHOXB.SHS-OXB IN CDCL3

140

20 PPM

40

60

80

100

120

140

160

180

200

220

CSHOX8.SHS-OX8 IN CDCL3

# COMPOUND XVIIc (124c)



(124c)

## Index

A 142

HSHOXJ.SH5-OXJ IN CDCL3

A 143

CSHDXJ.SH5-OXJ IN CDCL3

a-144

CYSOXJ.SH5-OXJ IN CDCL3
1H COSY-60

F2 (PPM)

HCSDXj.SH5-DXj IN CDCL3
1H/13C HETCOR

# COMPOUND XVIII (125)



(125)

## Index

𝒜 148

APPHSH3.THSH+3 1H CDCL3

4.8066
4.7941

4.4550
4.4385
4.4220
4.4037

4.4 PPM

2.4079
2.3937
2.3709
2.3573

2.3237

2.2413
2.2189
2.2010

2.1772
2.1589
2.1471

2.1081
2.0952

2.0684
2.0541
2.0440
2.0285
2.0139
2.0037
1.9872

1.9209
1.9044
1.8810
1.8727
1.8623
1.8523
1.8389
1.8348

1.7752

1.7396

1.7140
1.7030

1.6786
1.6696
1.6591

1.6336

1.6143
1.5940
1.5840
1.5588

1.5300

1.5038
1.4979

1.4 PPM

1.2837
1.2764

1.2282

1.2

149

150

20 PPM

40

60

80

100

120

140

160

180

200

220

CTHSH3.THSH-3 IN CDCL3

DTHSH3.THSH-3 IN CDCL3

A-151

CH3

CH2

CH

CHn

A 152

CYHSH3.THSH-3 IN CDCL3
1H COSY-60

F2 (PPM)

A 153

HCHSH3.THSH-3 IN CDCL3
1H/13C HETCOR

# PART ℬ

## TABLE OF CONTENTS

PVK

PVK

N-(2-butylidine)benzylamine

WAVENUMBER CM⁻¹

398 5

N-(2-butylidine)benzylamine

4a-methyl-5-oxo-$\Delta^{1,8a}$-octan-2-one

WAVENUMBER CM⁻¹

4a-methyl-5-oxo-Δ$^{1,8a}$-octan-2-one
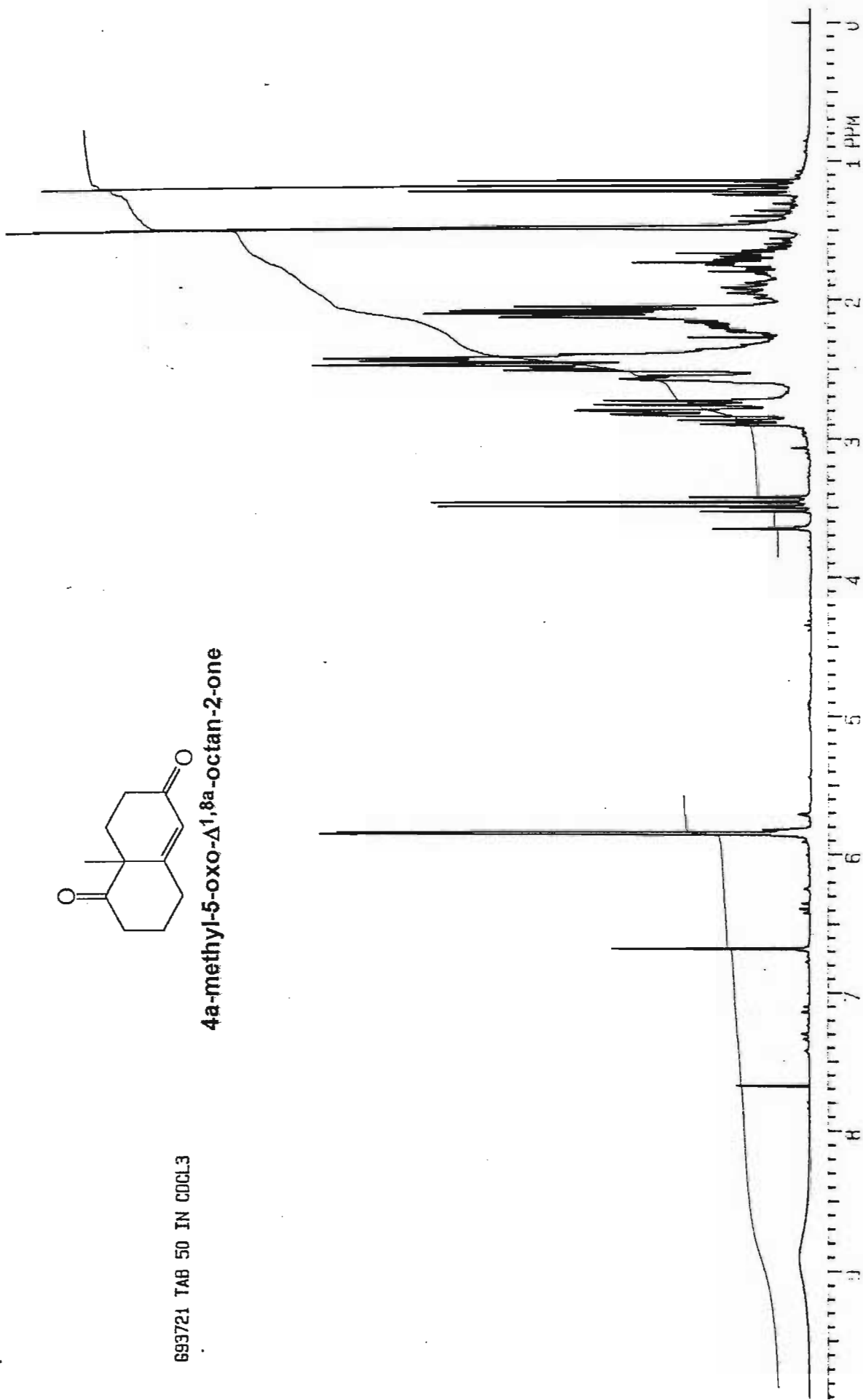
693721 TAB 50 IN CDCL3

4a-methyl-5-oxo-Δ$^{1,8a}$-octan-2-one

693721 TAB 50 IN CDCL3

39b
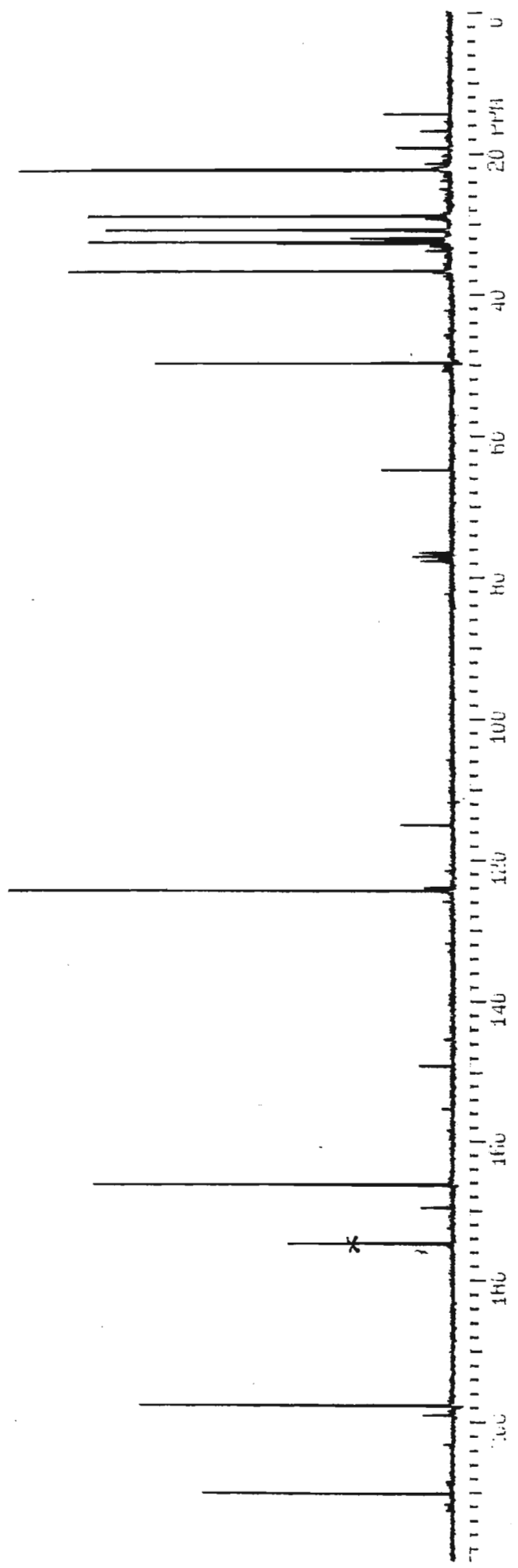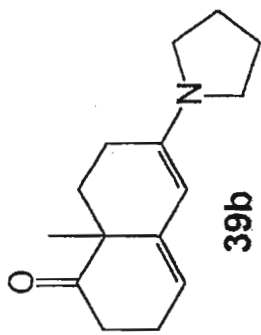
G93730 TAB 51 IN CDCL3

35 ( ISOMER 1)

$\mathcal{B}$ 11

35 ( ISOMER 1 )

35 (ISOMER 2)

1B64.TA6-64 IN CDCL3

B 13.
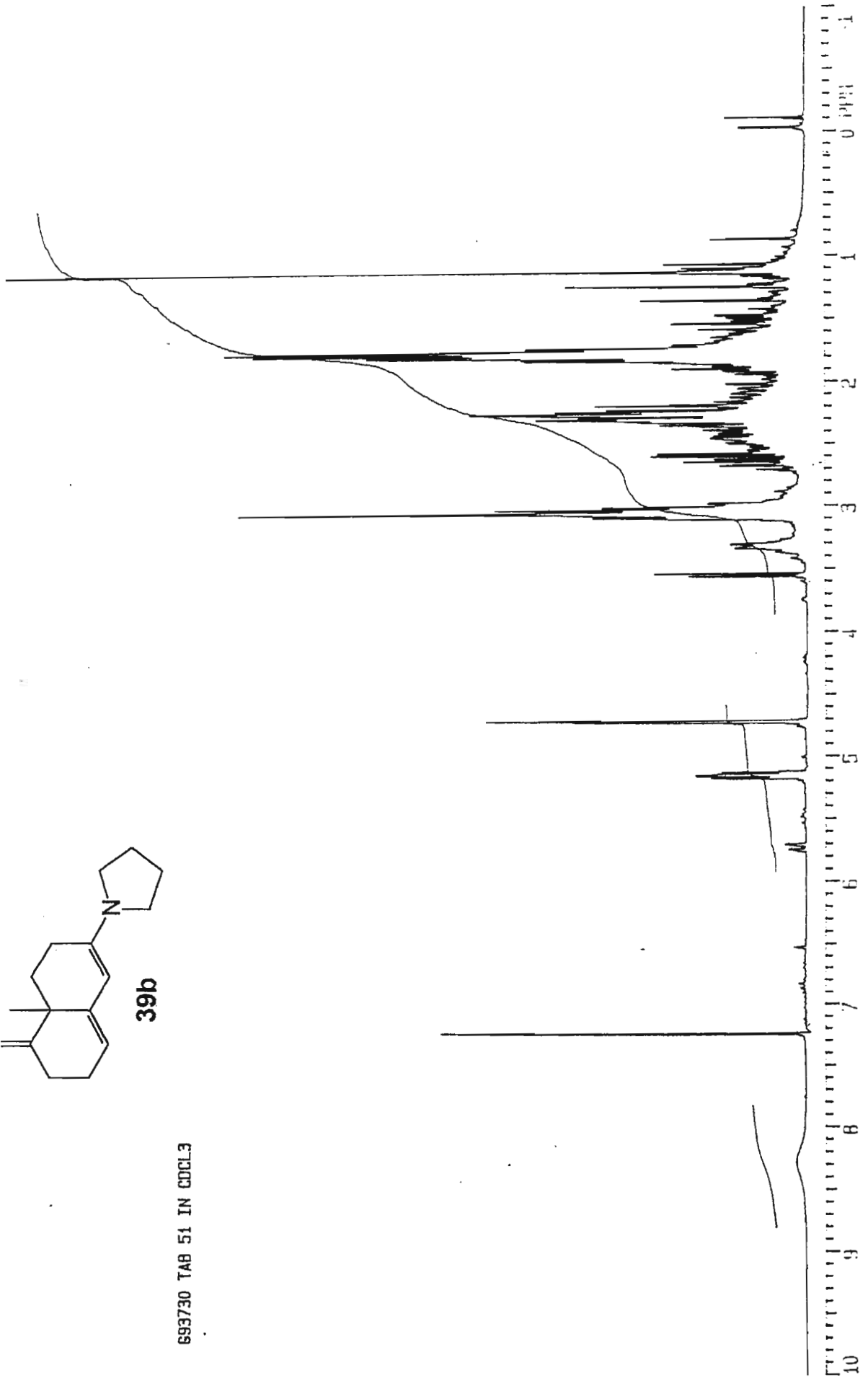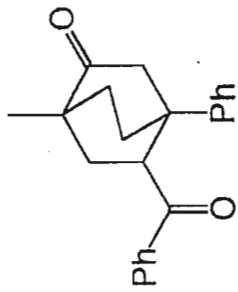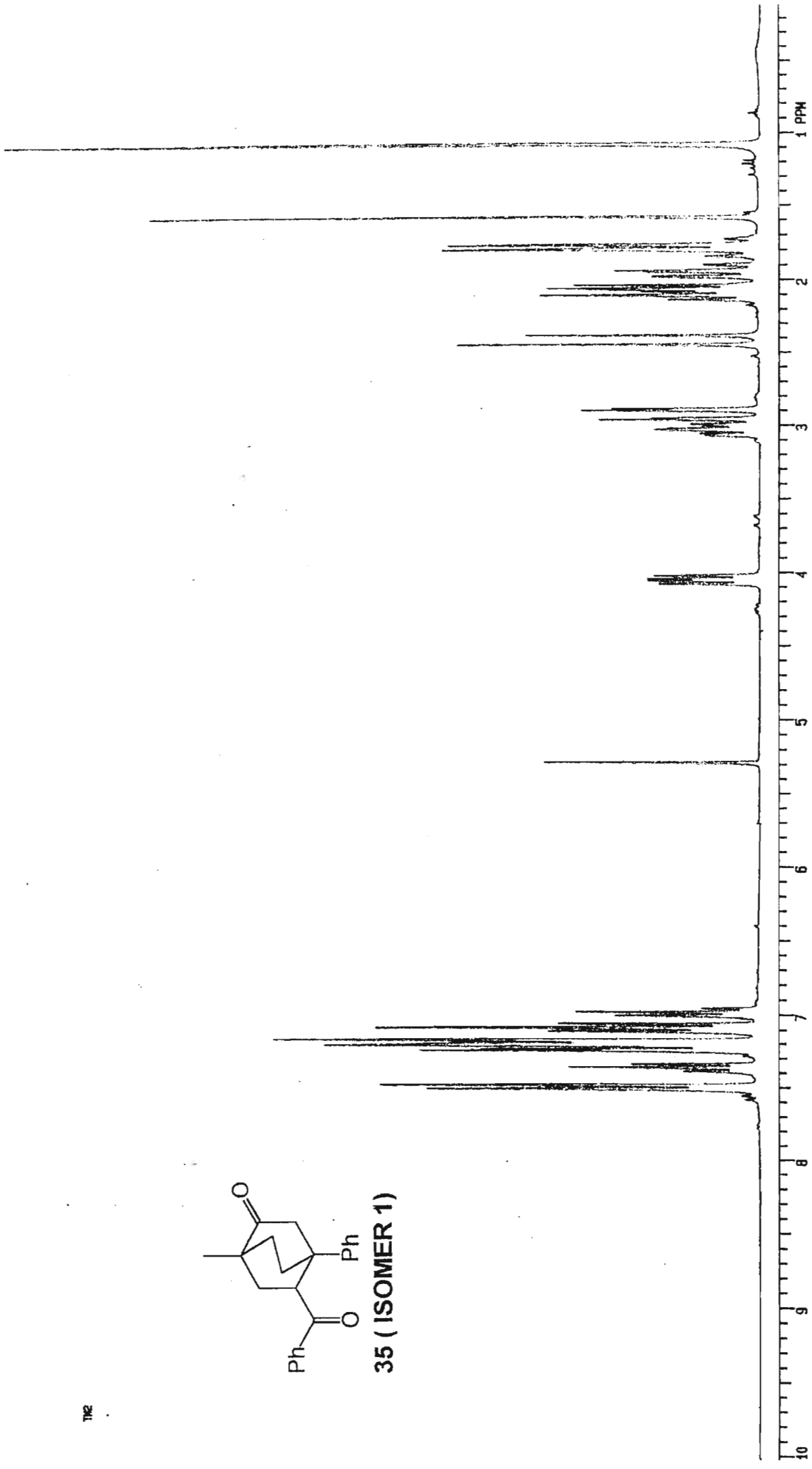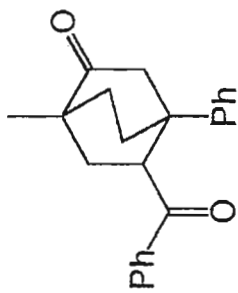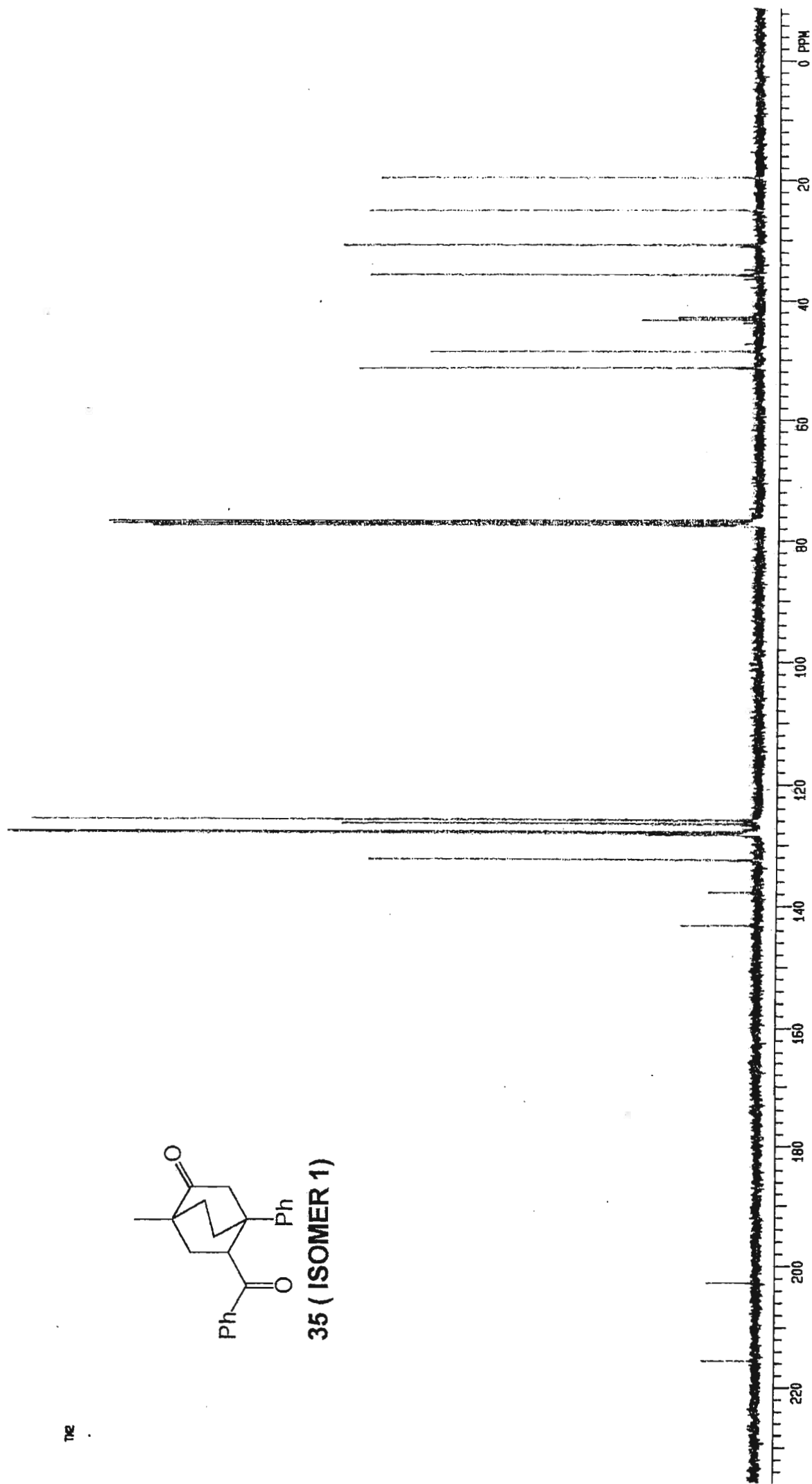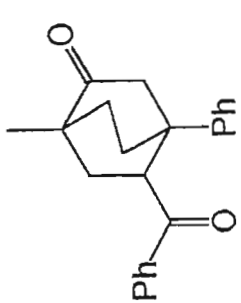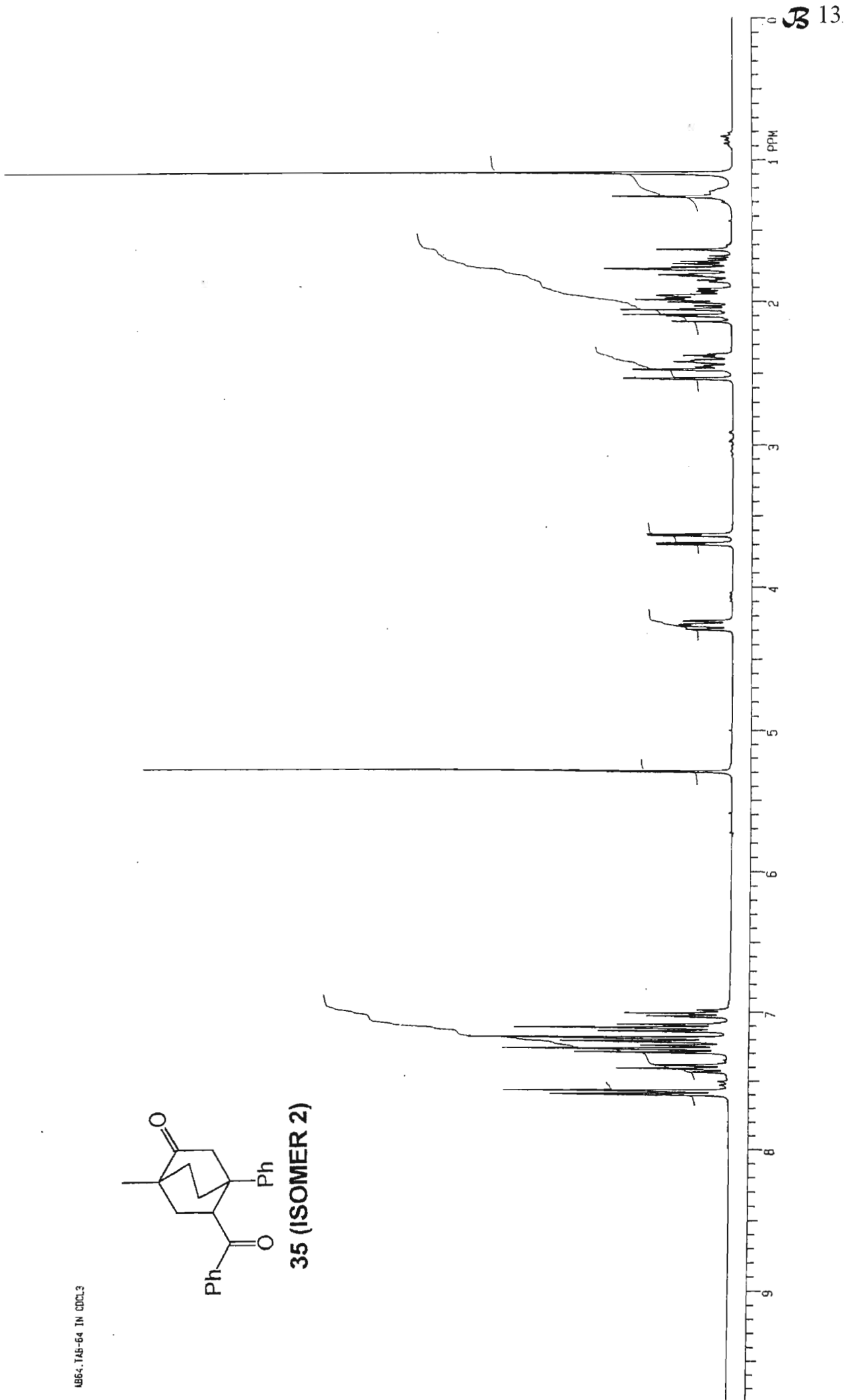
CTAB64.fid-64 IN CDCL3

35 (ISOMER 2)
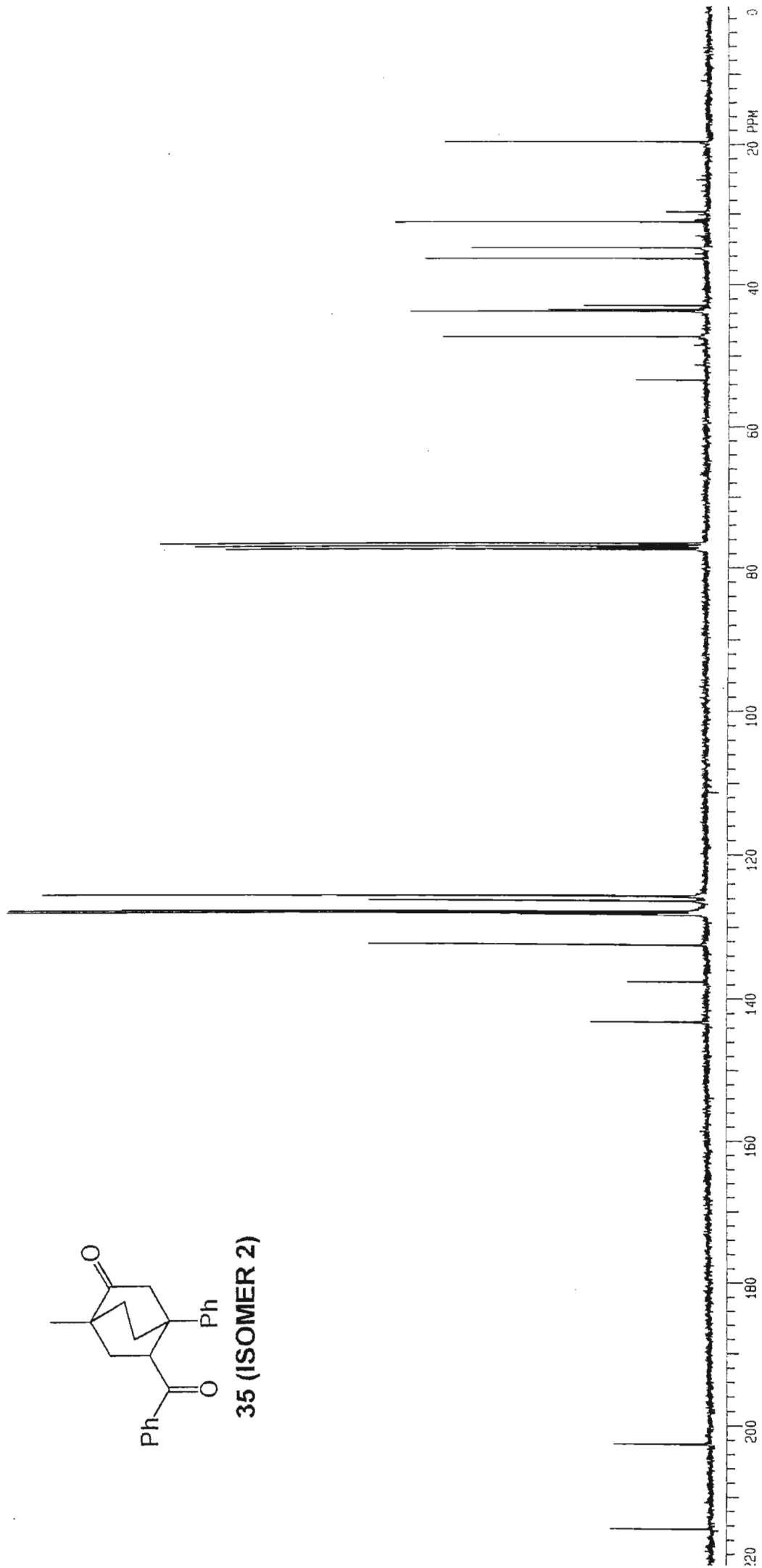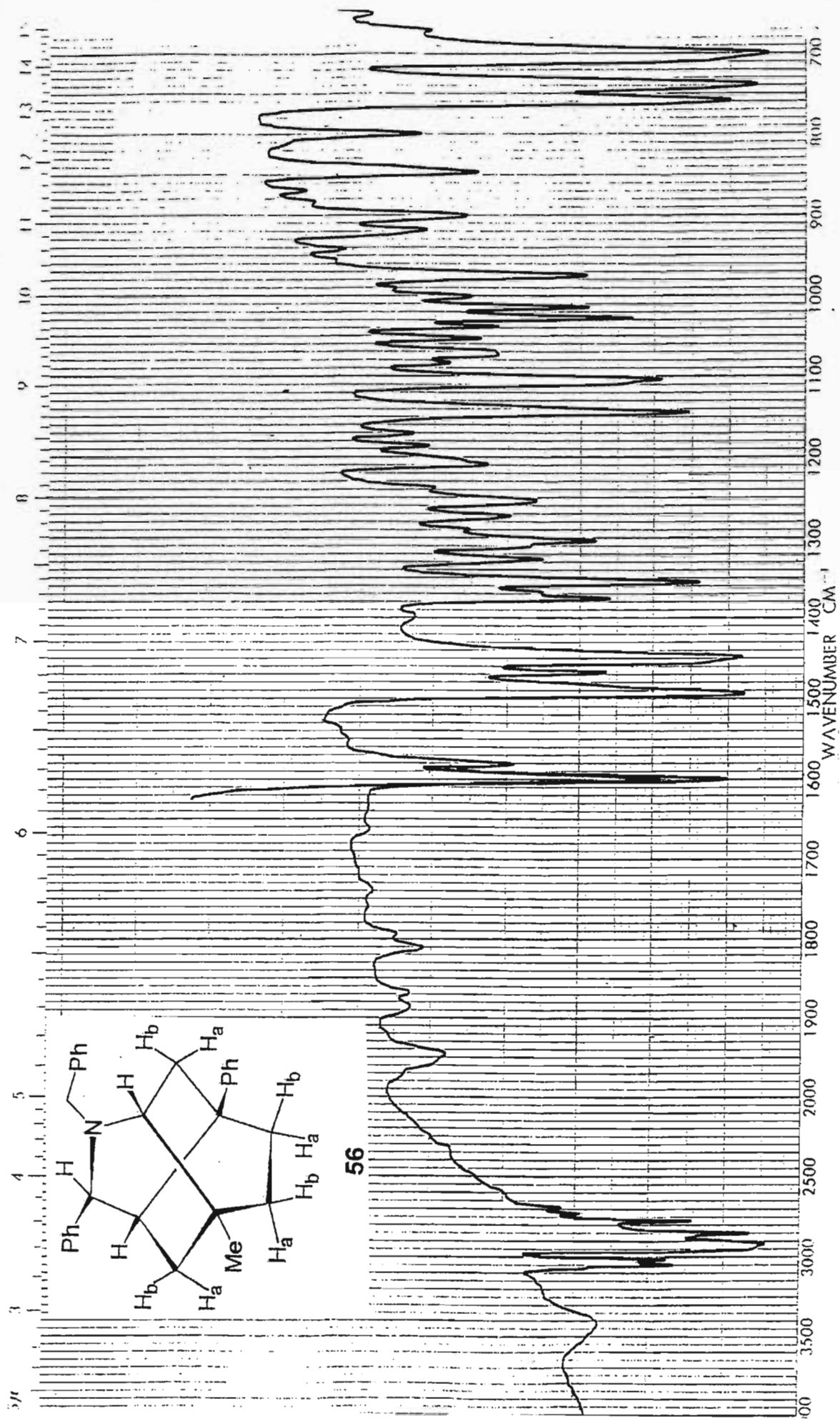
B 14

56

HTAB37.TAB-37 IN CDCL3
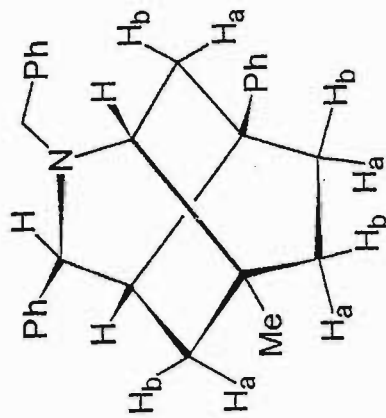
56
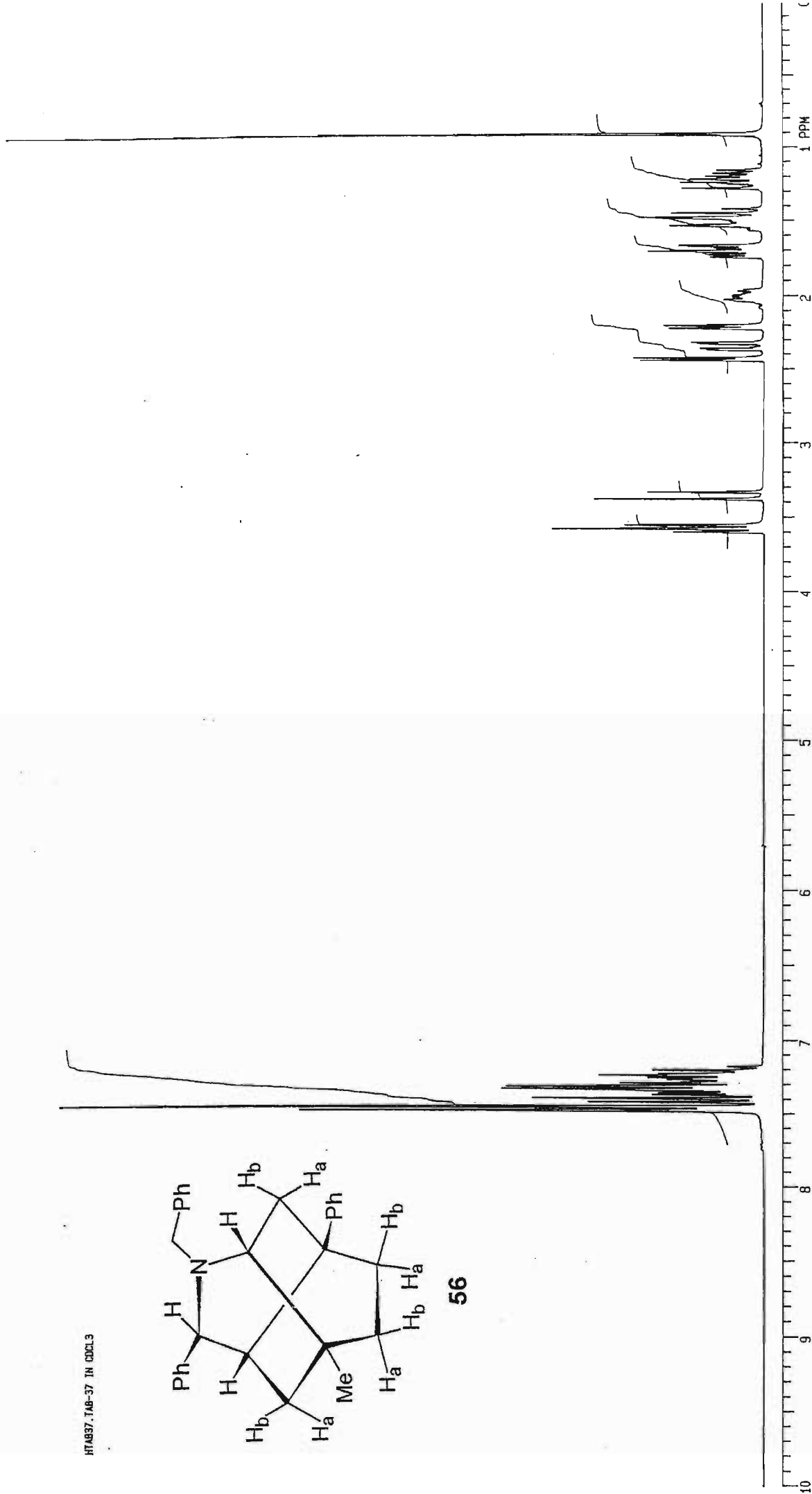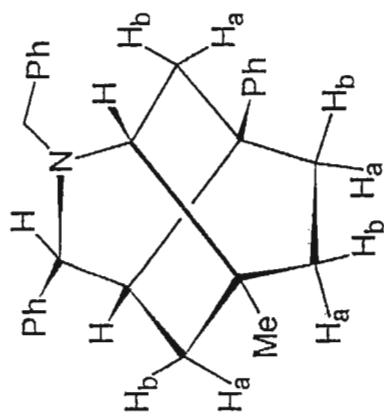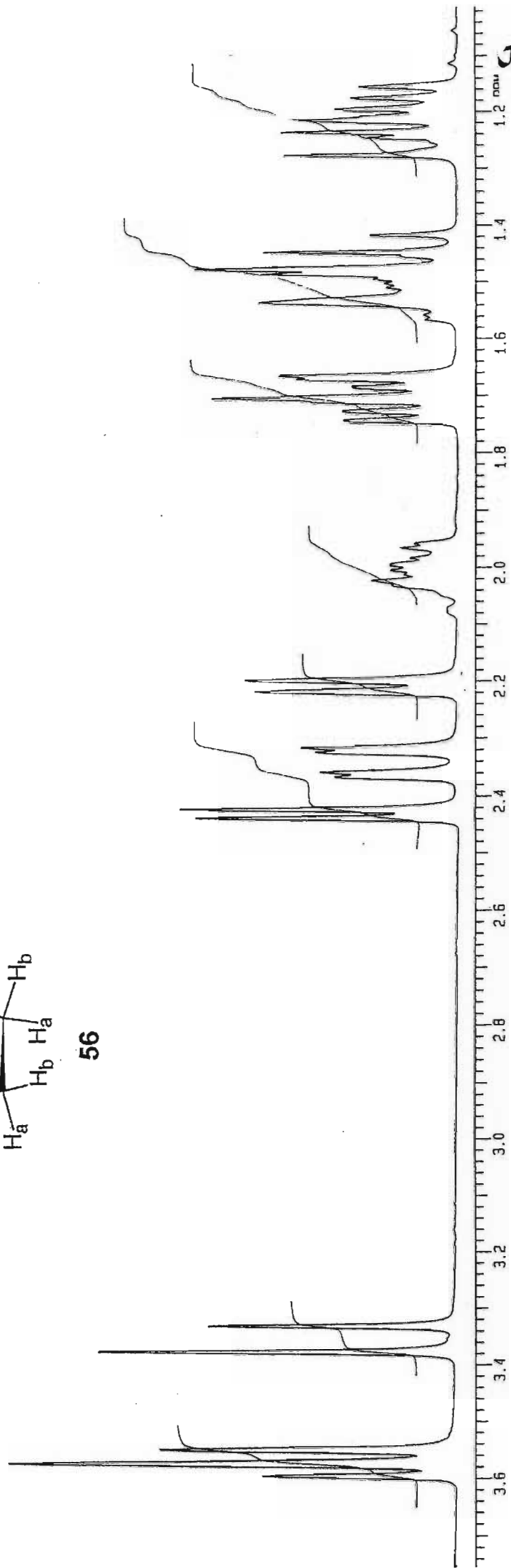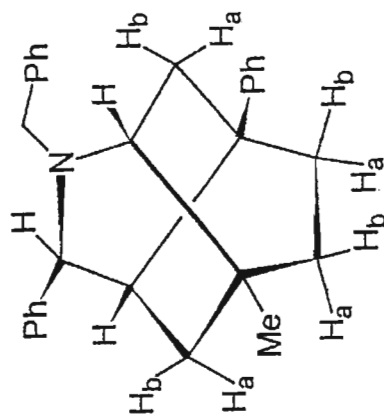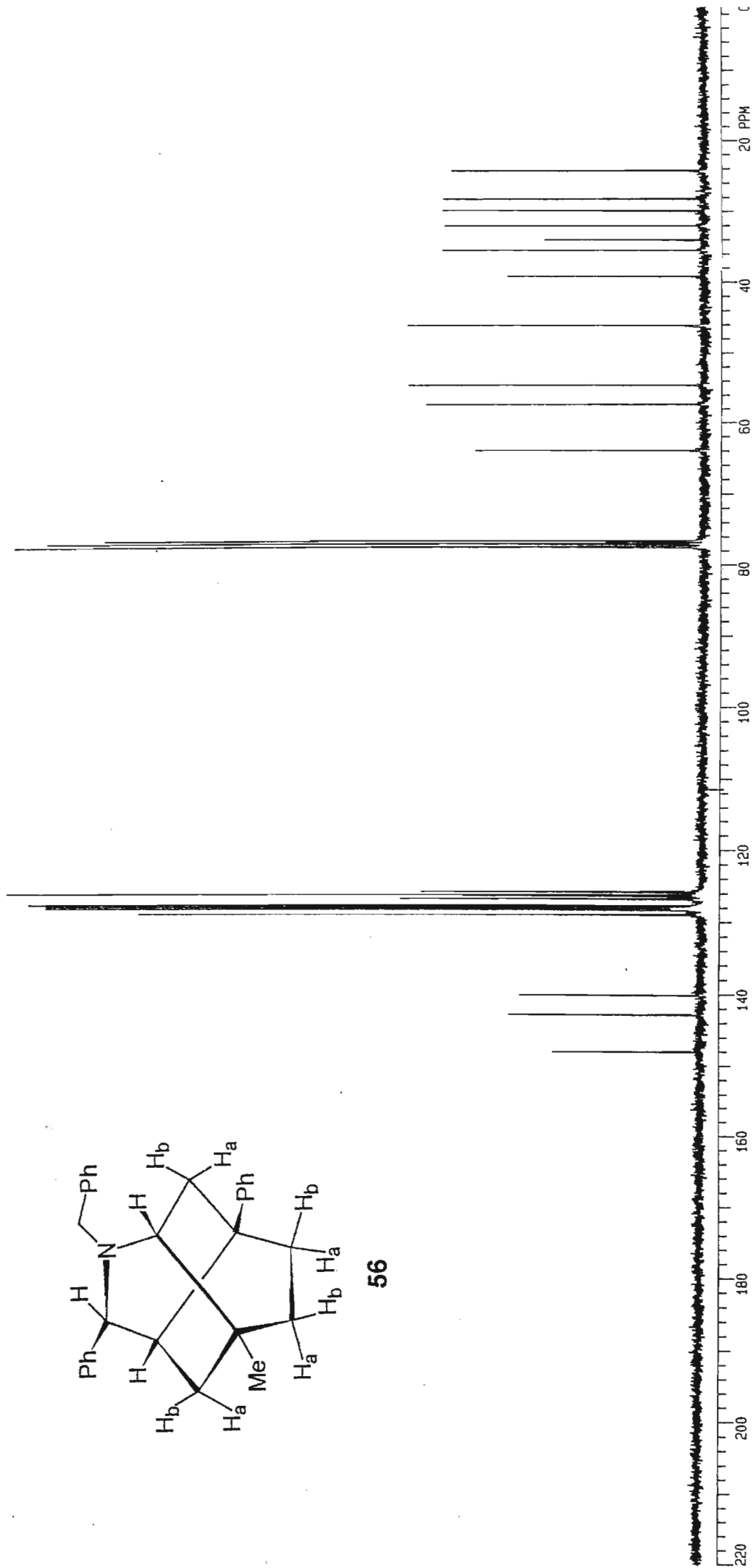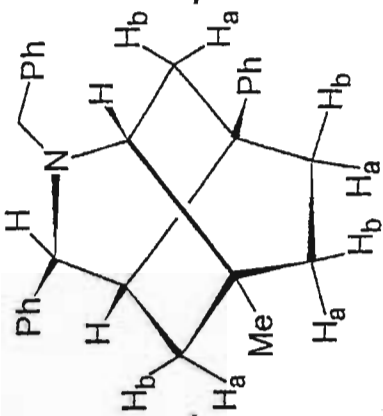
56

HT.AB37.TA8-37 IN CDCl3

56

$\mathcal{B}$ 18

DTAB37.TAB-37 IN CDCL3

C H 3

C H 2

C H

C H n

0

20 PPM

40

60

80

100

120

140

160

**56**

Ph

Hb

Ha

H

N

Ph

Hb

H

Ha

Ph

Me

Ha

Hb

Hb

Ha

56

F2 (PPM)

56

**56**

HTAB44.TAB-44 IN CDCL3

60

B 24

HTAB44.TAB-44 IN CDCL3

60

B 25

60

$\mathcal{B}$ 26.
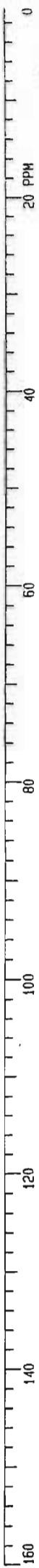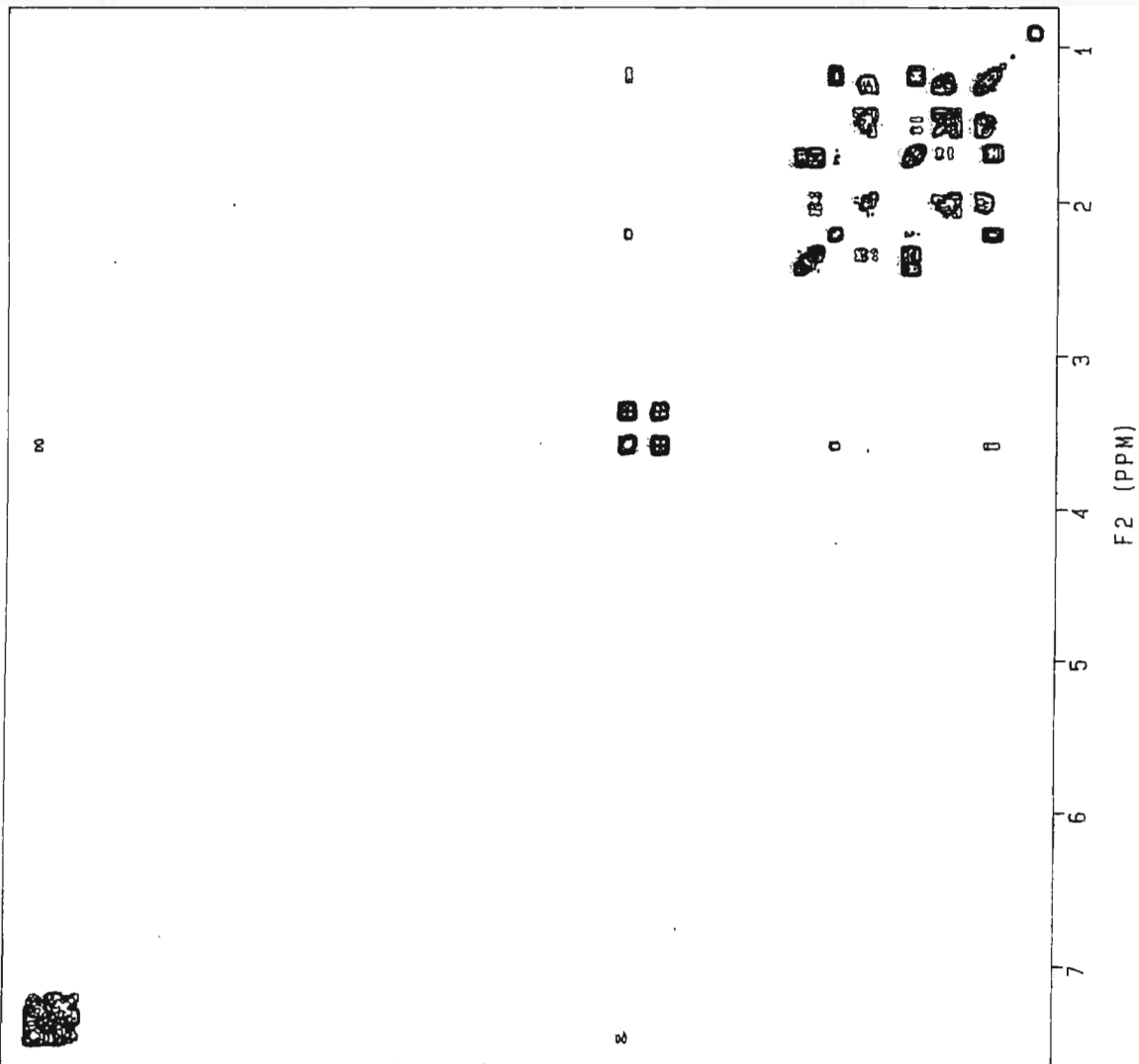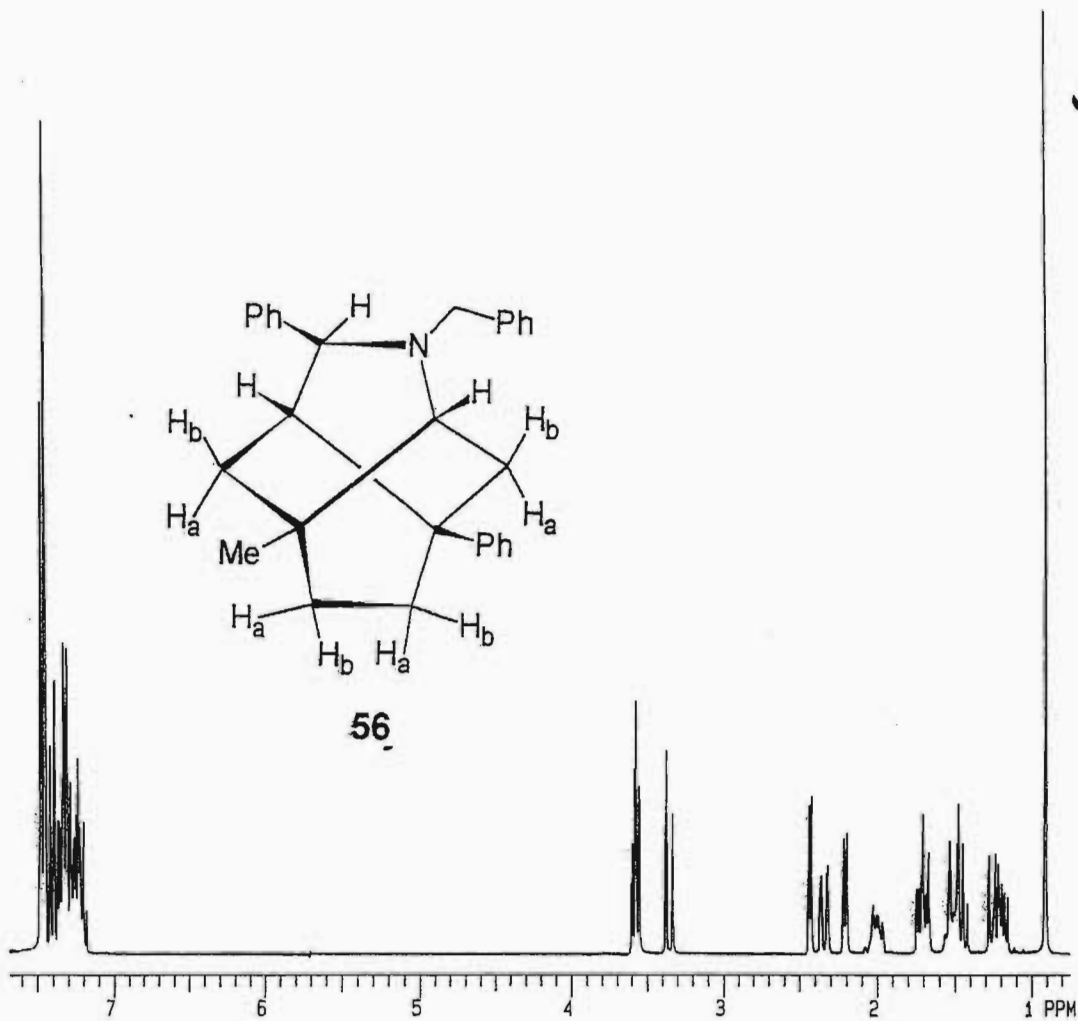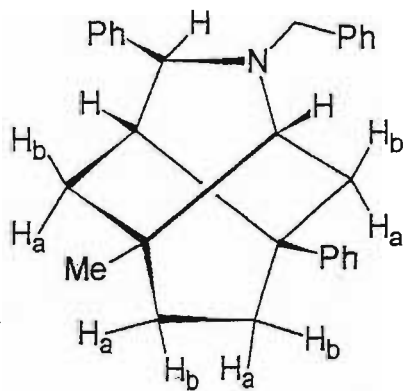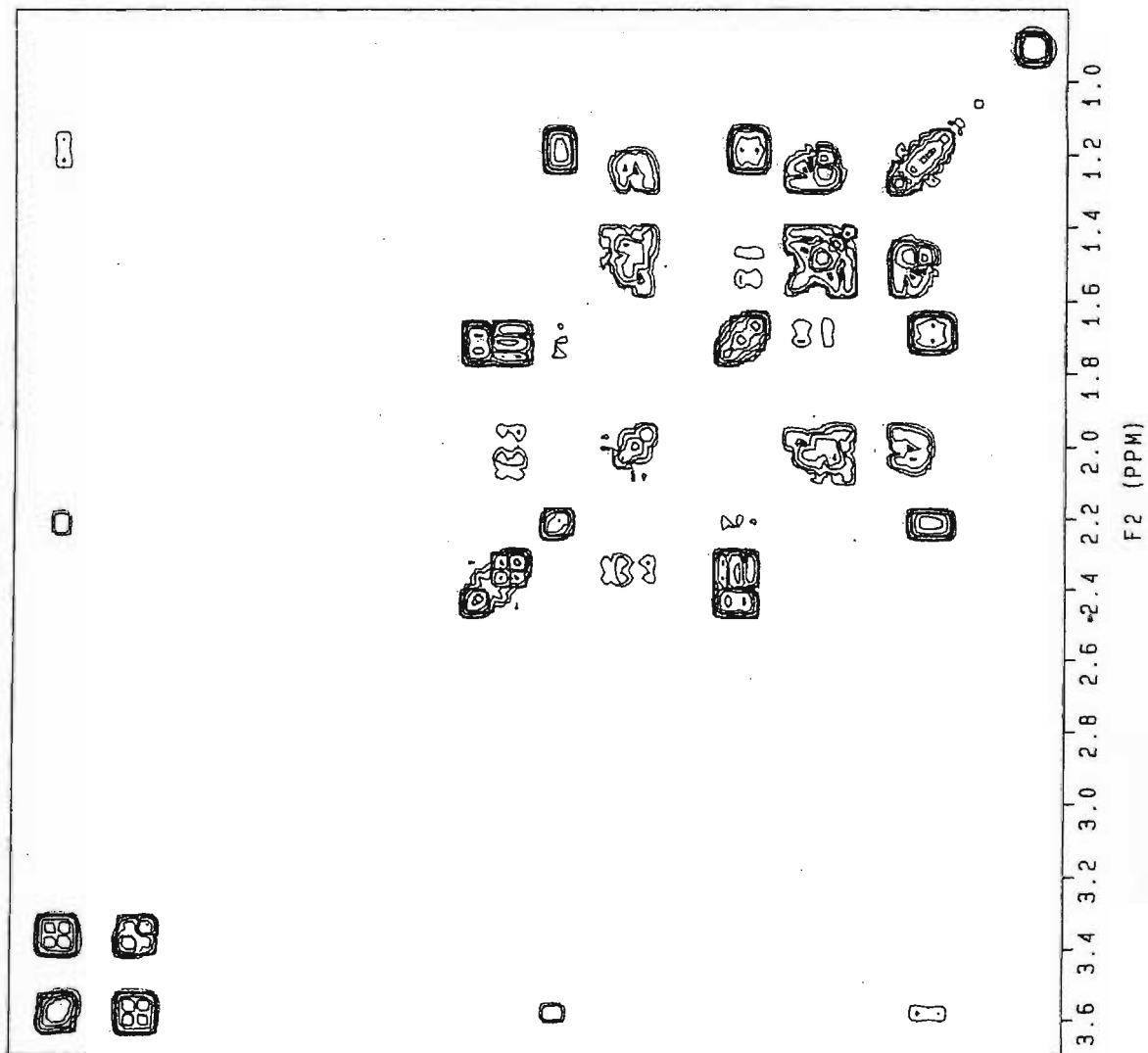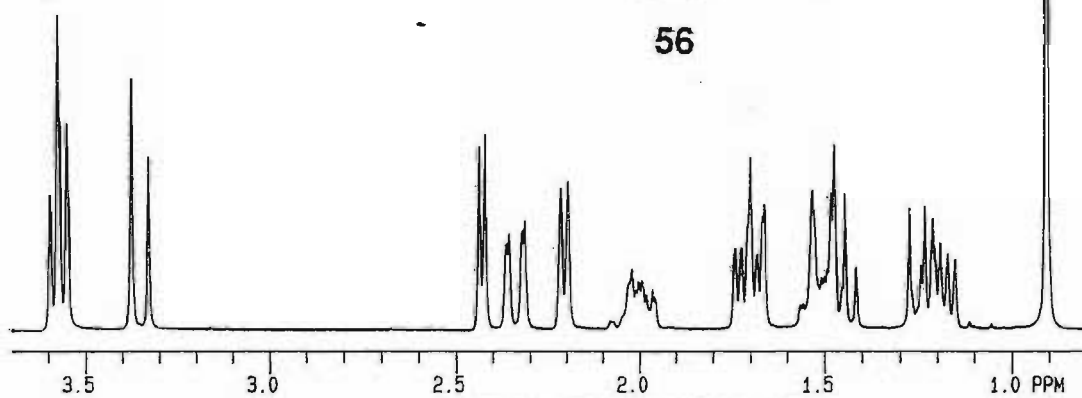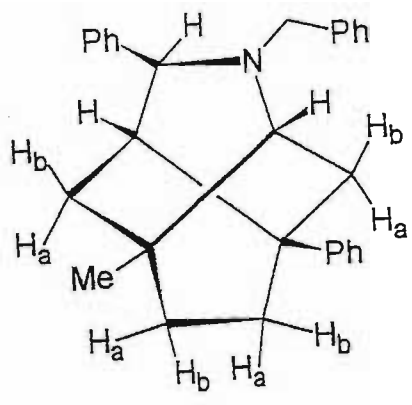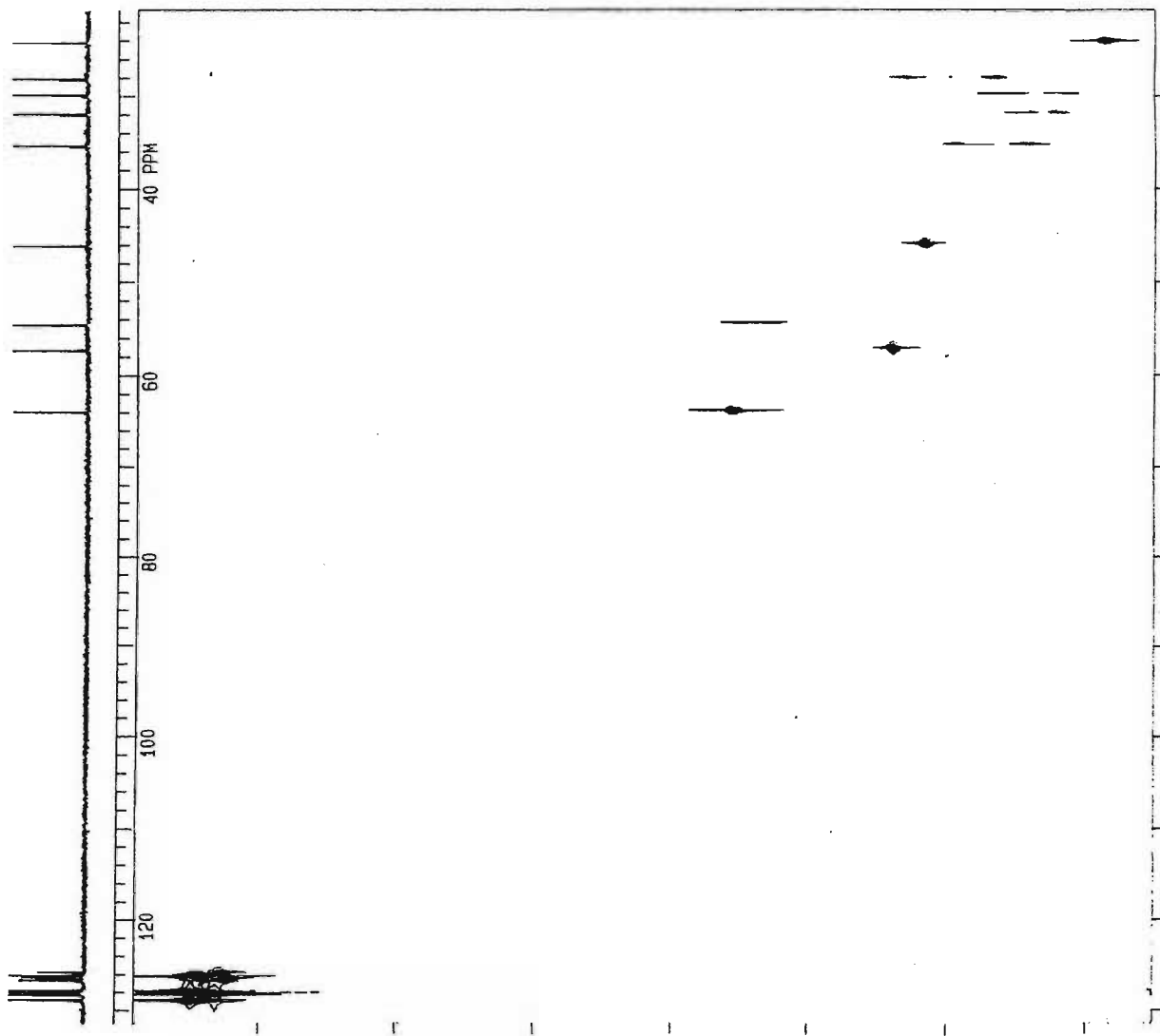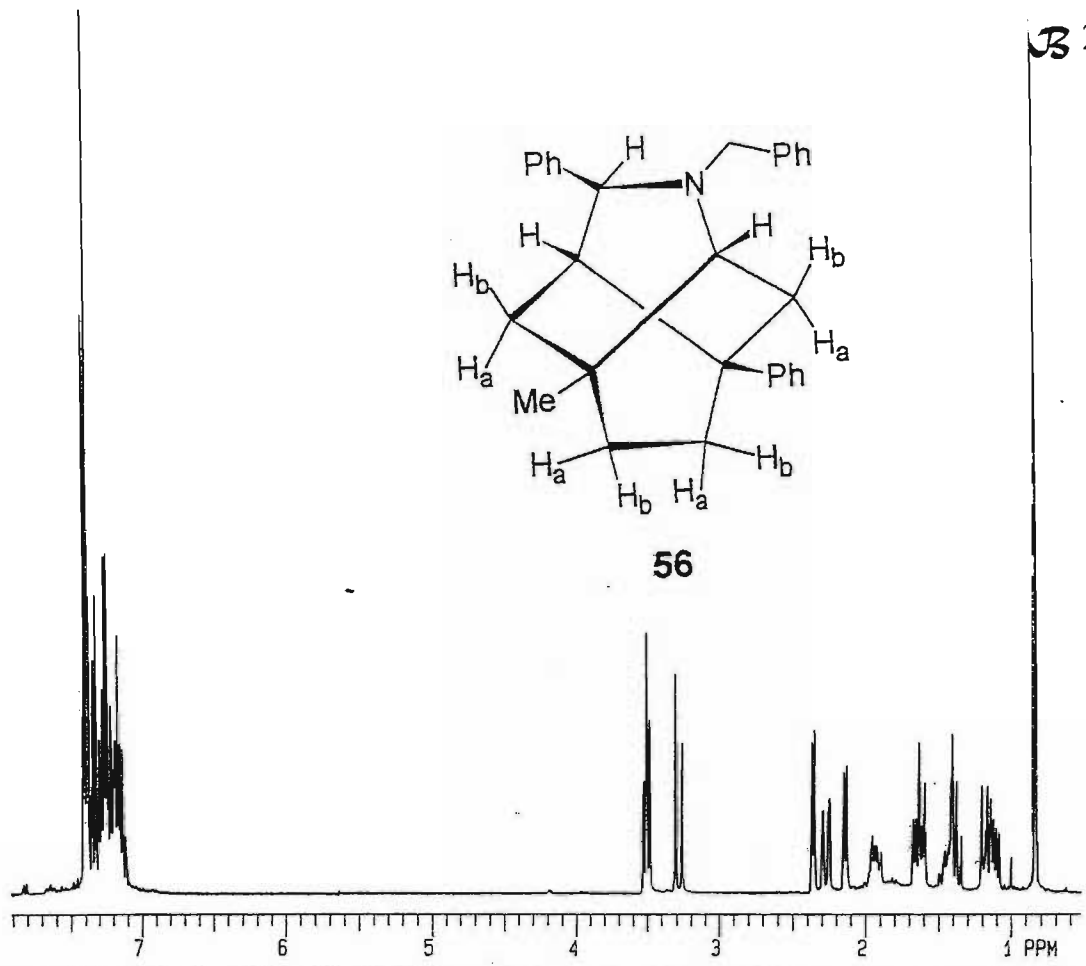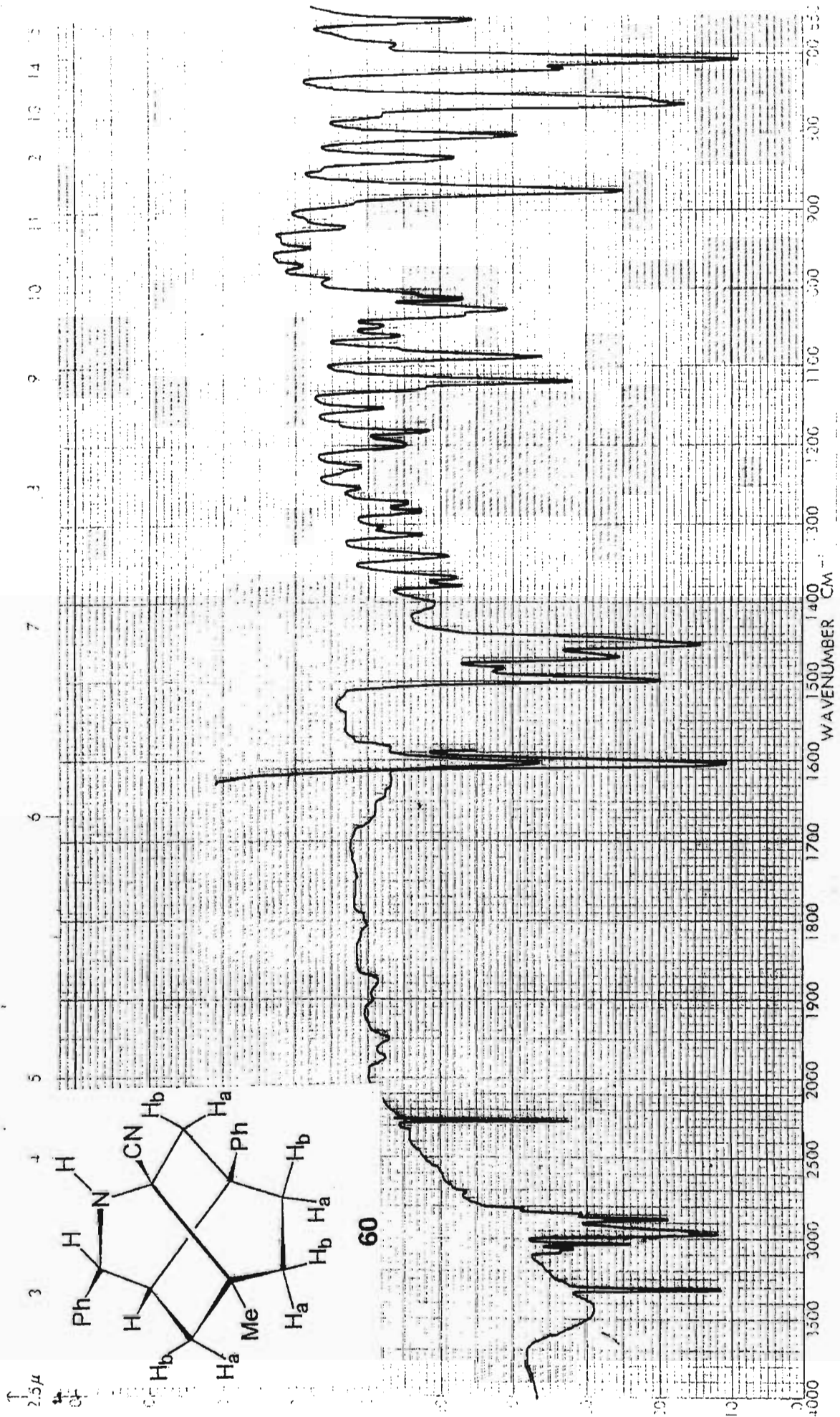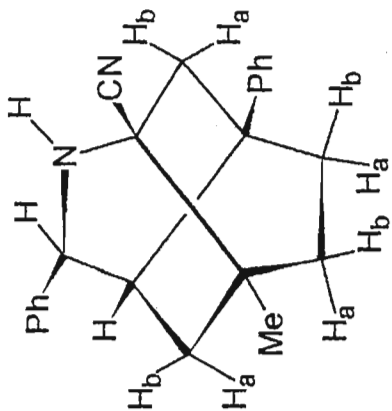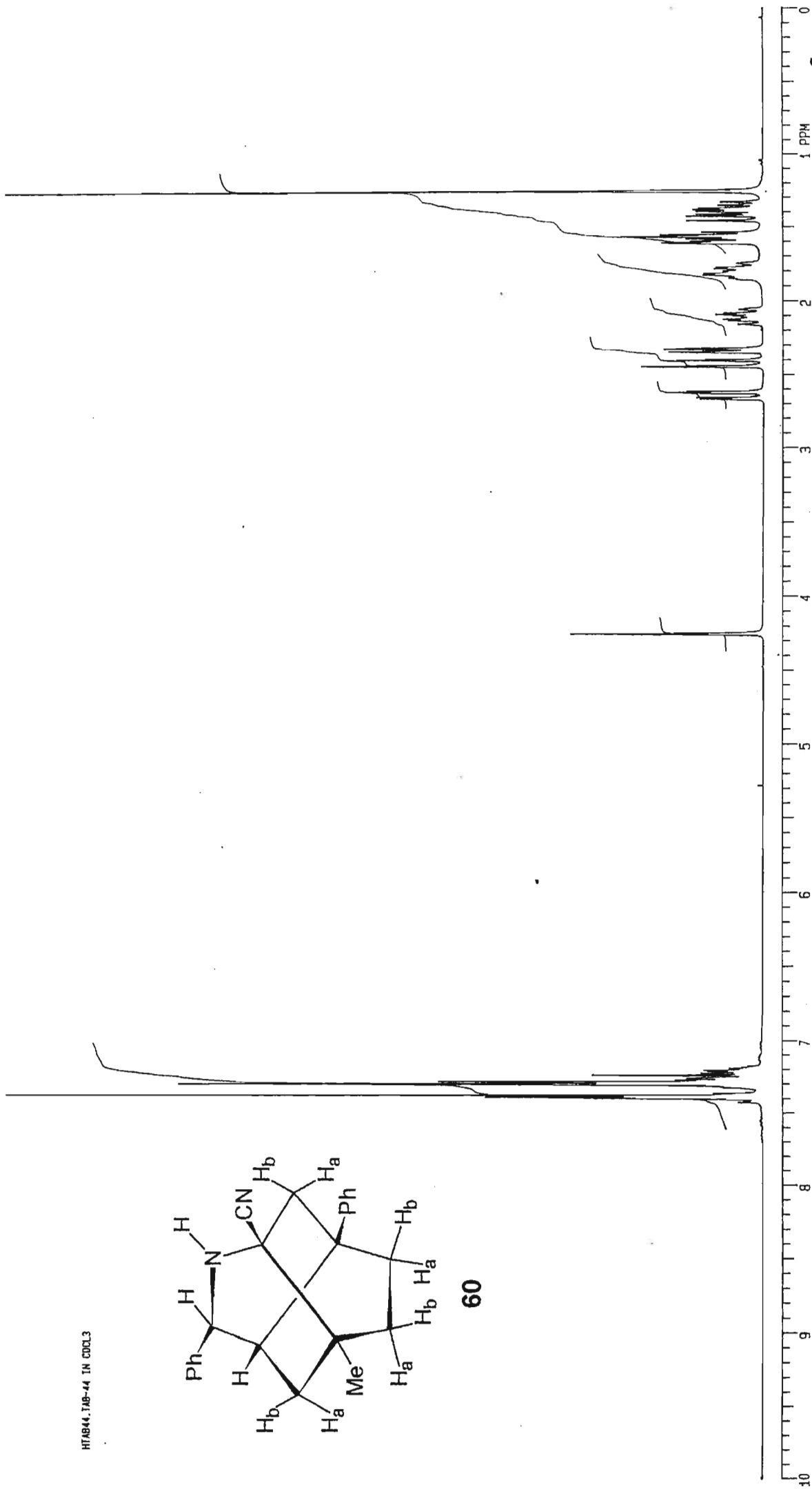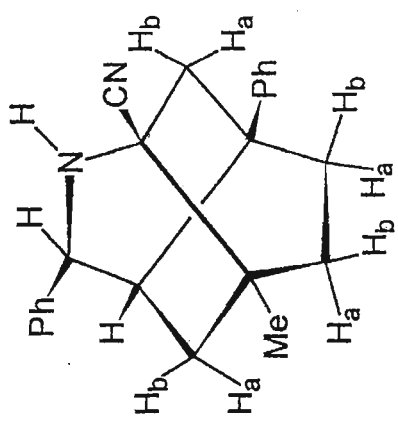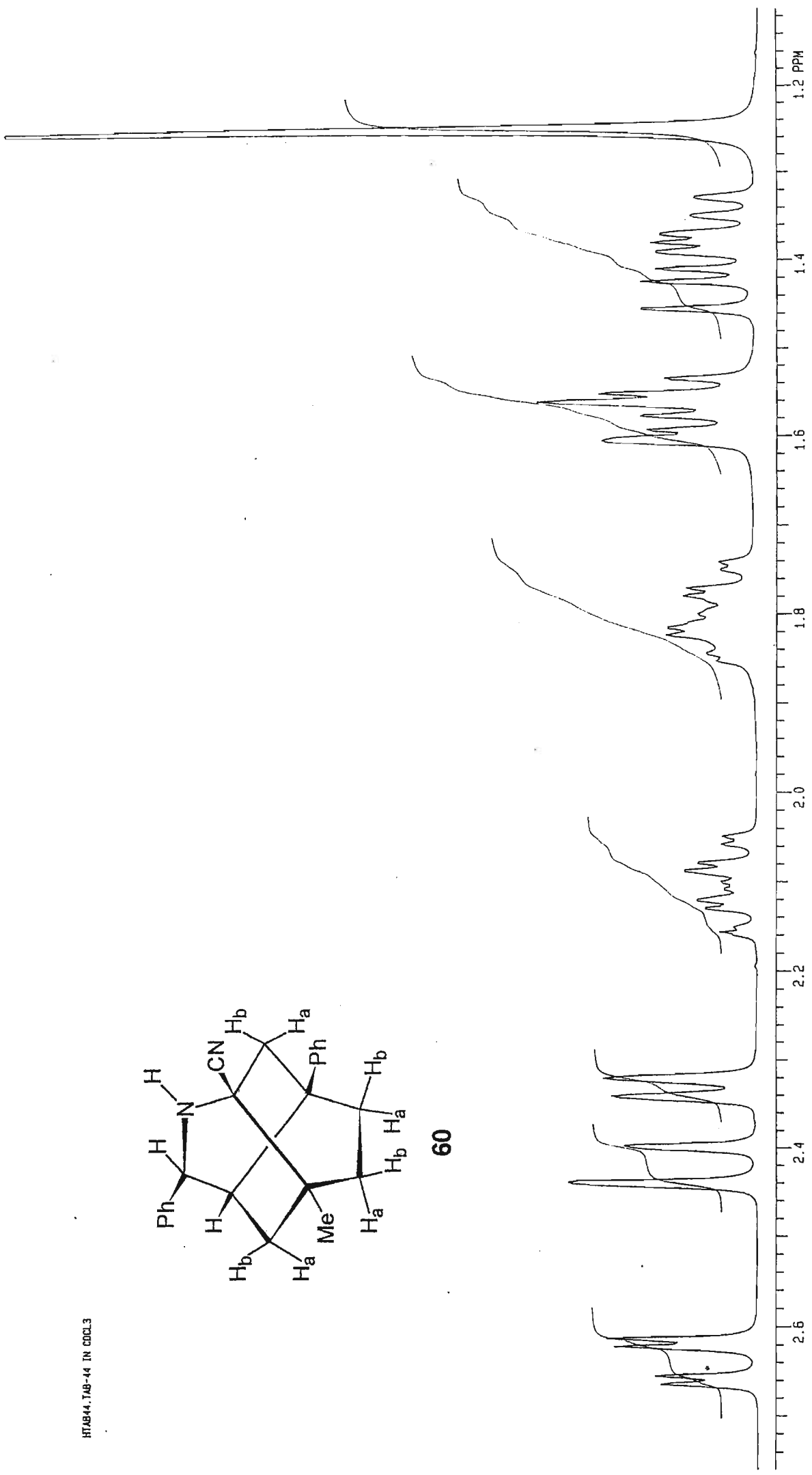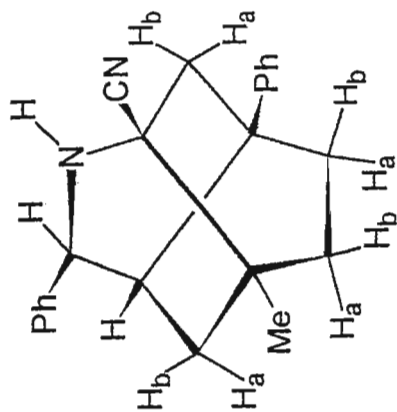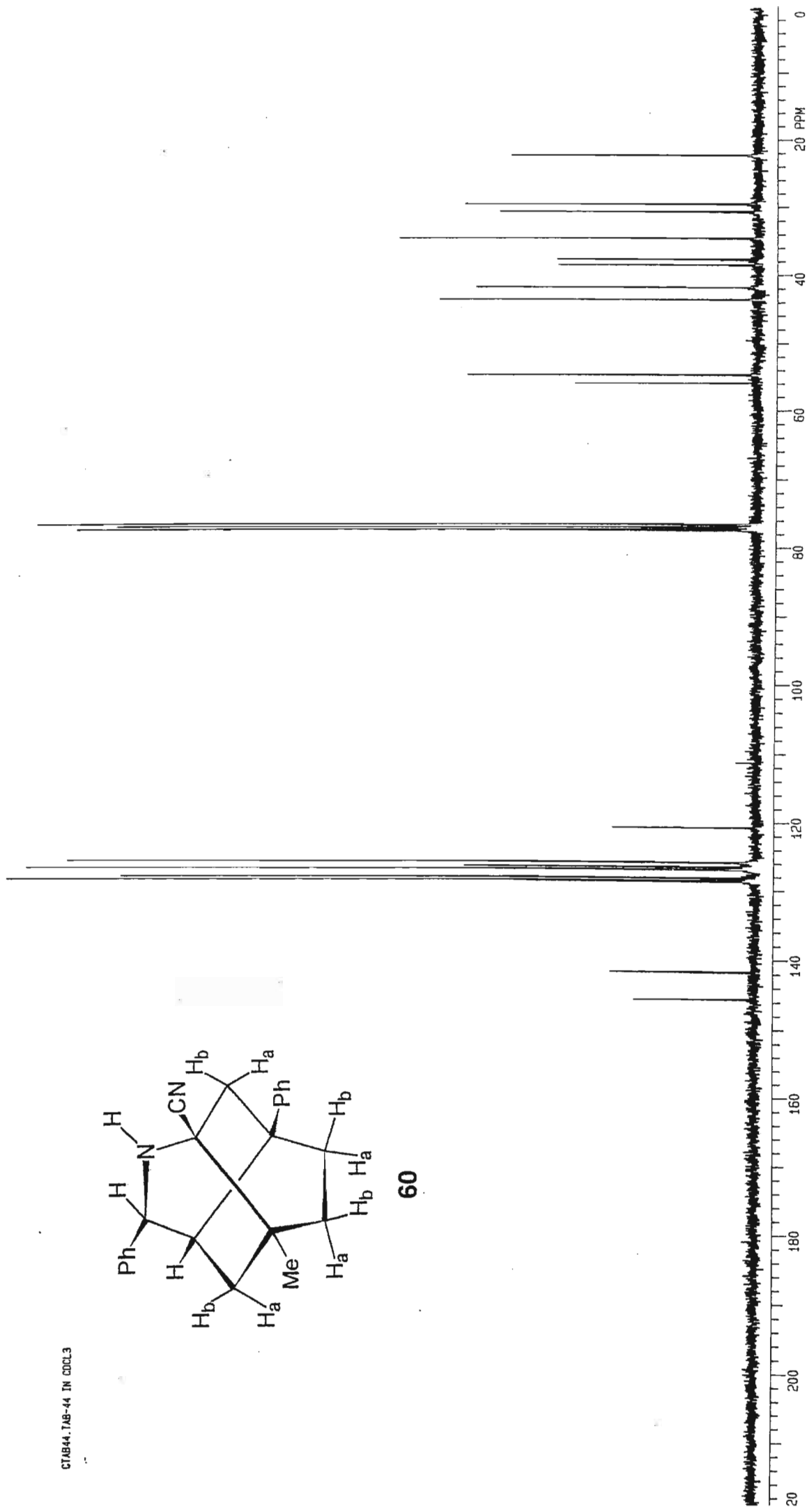
DTAB44.TAB-44 IN CDCL3

C H 3

C H 2

C H

C H n

60

20 PPM

40

60

80

100

120

140

160

B 27

60

60

60

**60**