# A chemical investigation of a KwaZulu-Natal Medicinal plant, *Momordica foetida* Schum. & Sond.

by

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Submitted in partial fulfillment of the requirements for the degree of

**MASTER OF SCIENCE**

in the

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**University of Natal, Durban.**

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# Declaration

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

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

Signed: _____

V.Sewram

I hereby certify that the above statement is correct.

Signed: _____

Professor R. Osborne

Signed: _____

Doctor D.A. Mulholland

# Acknowledgements

I would like to express my appreciation to my supervisor, Professor R. Osborne, and co-supervisor, Doctor D.A. Mulholland, for their invaluable assistance, guidance and support during the course of this project.

In addition I wish to thank:

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Dr P. Boshoff of the Cape Technikon for running the mass spectra.
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Mr B. Parel for his assistance in the organic research laboratory throughout the year.

I thankfully acknowledge the FRD and the University of Natal for financial assistance.

# Abstract

*Momordica foetida* Schum. & Sond. (Cucurbitaceae), locally known as *iNtshungu,* is widely used by the Zulu people of Natal-KwaZulu for the treatment of a variety of ailments.

The dried leaves leaves and stems of this plant was subjected to soxhlet extraction by refluxing with hexane, chloroform and methanol successively. Thin layer chromatography of the chloroform extract of the leaves revealed a multiplicity of compounds. The chloroform extract was further partitioned with sodium hydroxide resulting in an organic and aqueous phase. The organic phase, containing extract A, afforded two compounds, viz. compounds 1 and 2. The basic aqueous fraction was neutralised and re-extracted with chloroform to give extract B, affording five compounds, viz. compounds 3,4,5,6 and 7.

Structural elucidation was accomplished by techniques such as $^{1}H$ and $^{13}C$ NMR spectroscopy, HETCOR, COSY, FTIR and High Resolution Mass Spectrometry.

Compounds 1 and 2 were identified as cucurbitane triterpenoids known as momordicines which had been previously discovered in the related species of this plant, *Momordica charantia* L. The remaining five compounds were identified as novel compounds, although natural derivatives of compounds 6 and 7 had been isolated previously from *Momordica charantia* L. Compounds 3-7 were each isolated as an epimeric mixture but it was possible to select the resonances corresponding to the major epimer. These five epimers were respectively identified as 5,19-epoxy-19(*R*)-hydroxy-25-methoxy-5β-

cucurbita-6,23-diene-3β-ol **[102]**, 5,19-epoxy-19(*R*),25-dihydroxy-5β-cucurbita-6,23-diene-3β-ol **[103]**, 5,19-epoxy-19(*R*)-methoxy-25-hydroxy-5β-cucrbita-6,23-diene-3β-ol **[104]**, 5,19-epoxy-25-methoxy-5β-cucurbita-6,23-diene-3β-ol **[105]** and 5,19-epoxy-19(*R*),25-dimethoxy-5β-cucurbita-6,23-diene-3β-ol **[106]**.

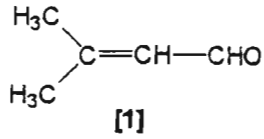
Appropriate reactions were performed, where possible, on the compounds isolated in order to confirm their identity.

# Table of Contents

# Abbreviations

| | |
|---|---|
| OAc | O-Acetate |
| ax | axial |
| bs | broad singlet |
| bt | broad triplet |
| *c* | concentration |
| $^{13}$C NMR | Carbon-13 nuclear magnetic resonance |
| COSY | Correlated spectroscopy |
| d | doublet |
| dd | doublet doublet |
| DEPT | Distortionless enhancement by polarization transfer |
| dt | doublet of triplets |
| gem | geminal |
| $^{1}$H NMR | Proton nuclear magnetic resonance |
| HETCOR | Heteronuclear chemical shift correlation |
| Hz | Hertz |
| m | multiplet |
| Me | Methyl |
| OMe | Methoxy |
| q | quartet |
| s | singlet |
| t | triplet |
| t.l.c. | thin layer chromatography |
| TMS | Tetramethylsilane |

# Structures refered to in thesis for easy reference



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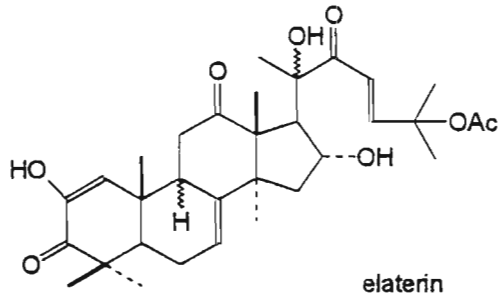
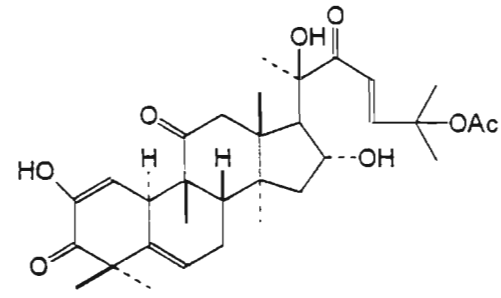


elaterin
(Cucurbitacin E)

[2a]
old structure



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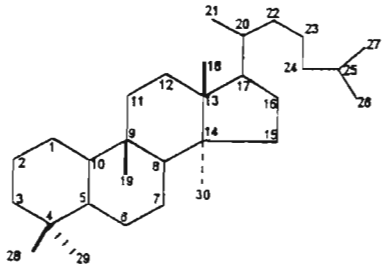

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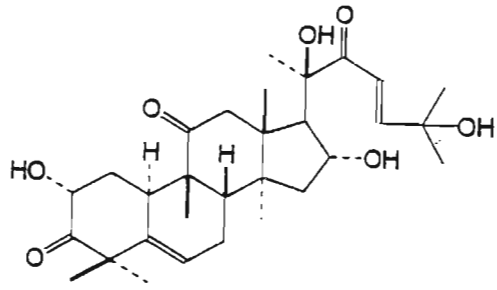

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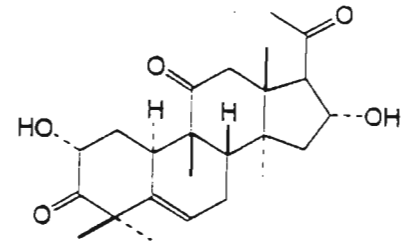

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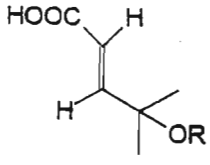

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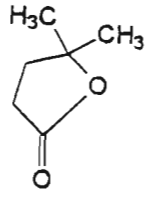

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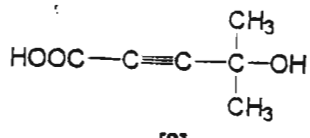

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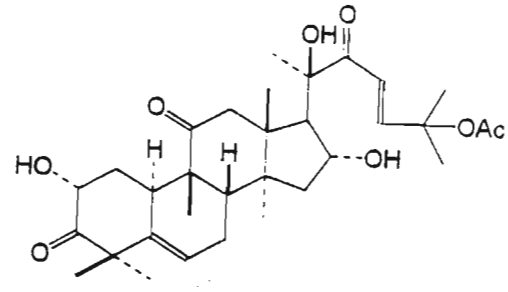

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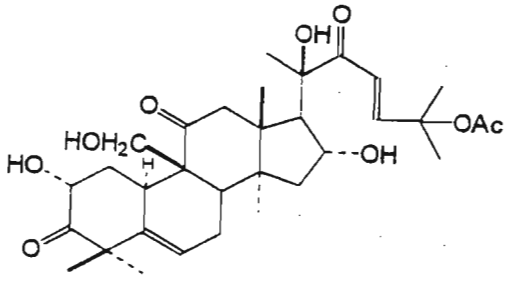

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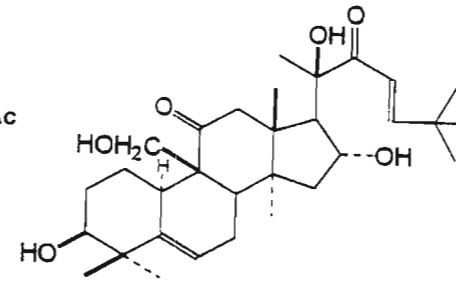

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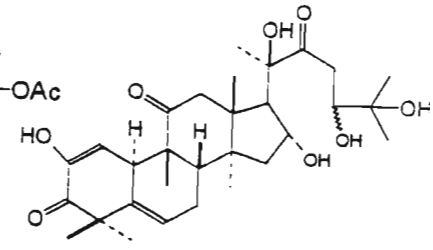

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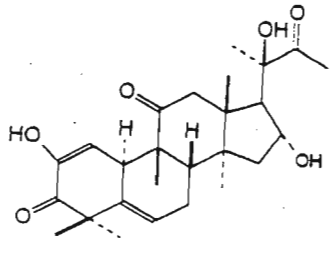

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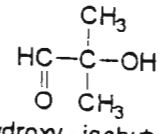

2 - Hydroxy - isobutyraldehyde
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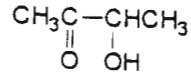

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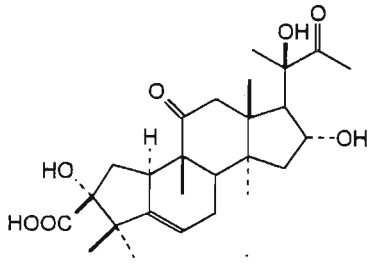

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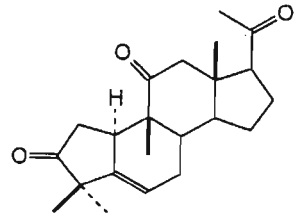

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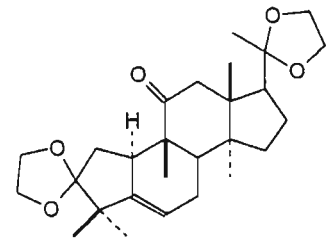

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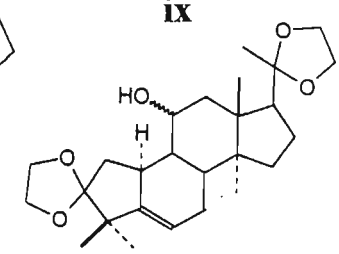

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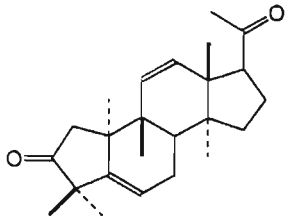

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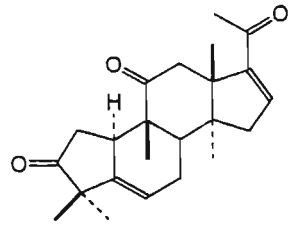

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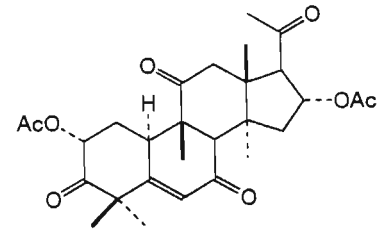

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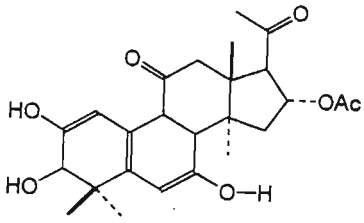

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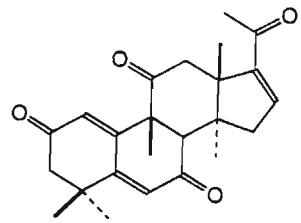

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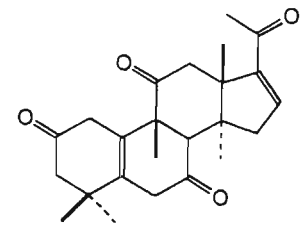

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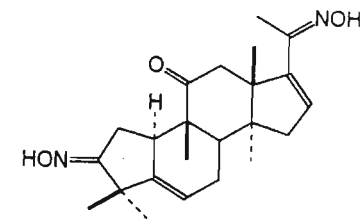

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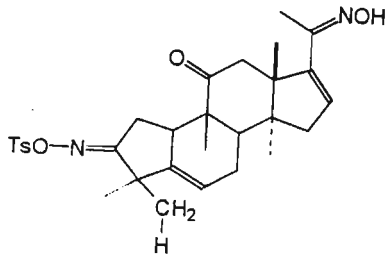

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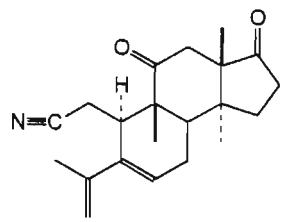

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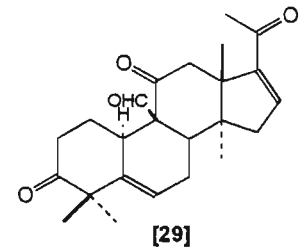

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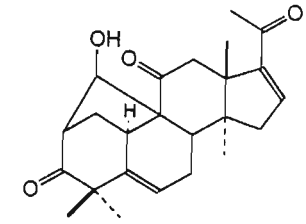

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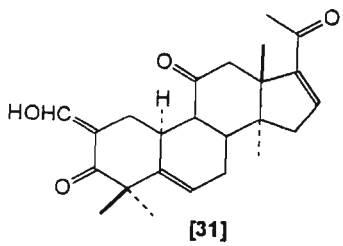

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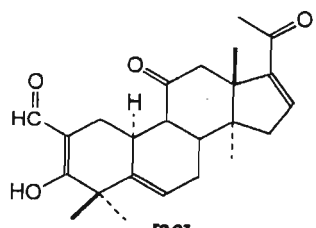

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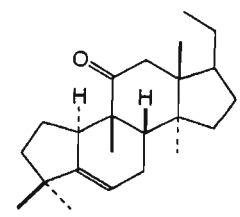

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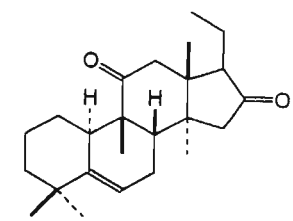

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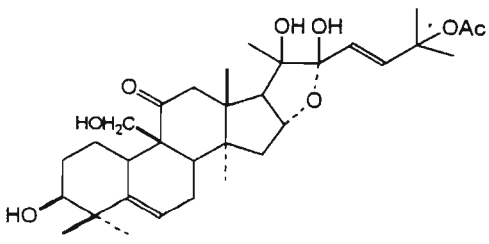

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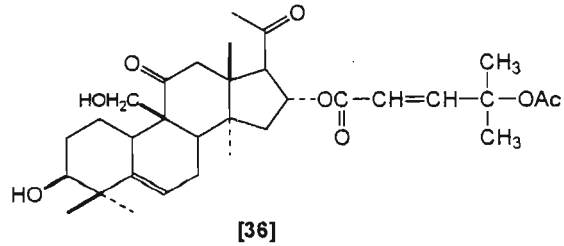

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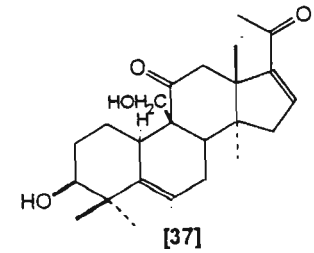

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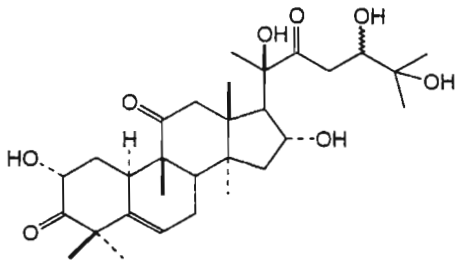

Cucurbitacins G and H

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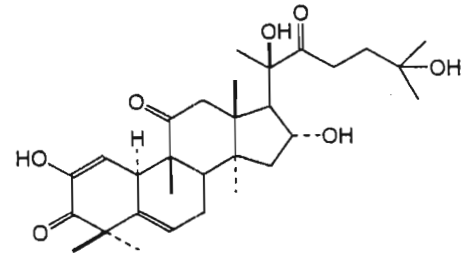
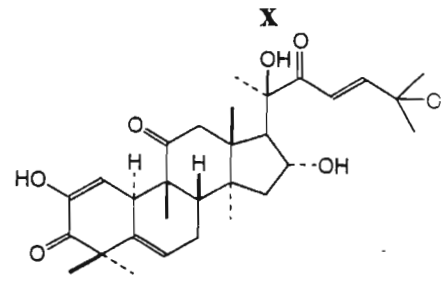


Cucurbitacin L

**[39]**



Cucurbitacin I

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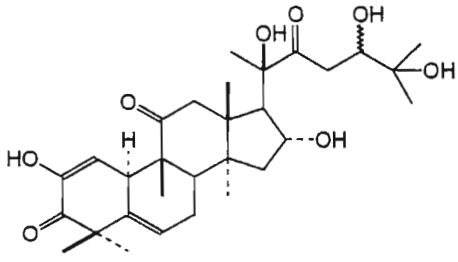

Cucurbitacins J and K

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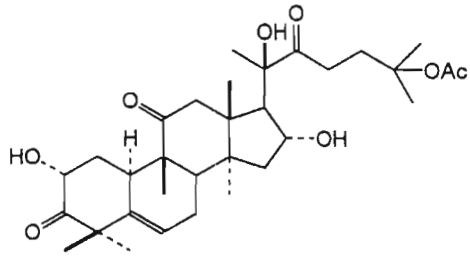

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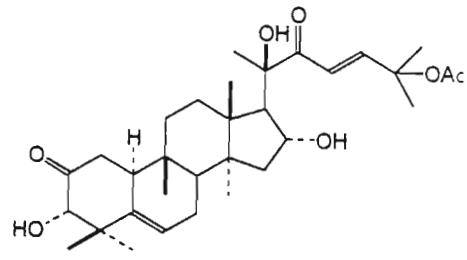
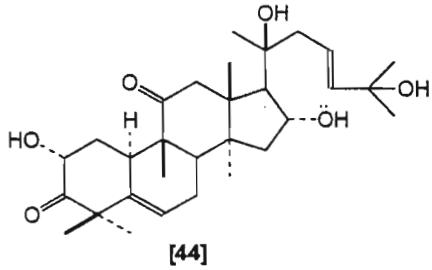
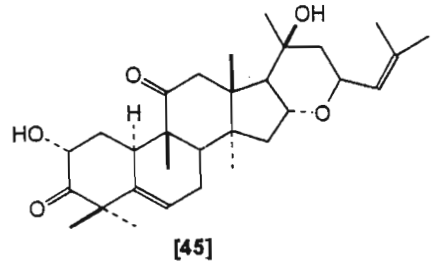


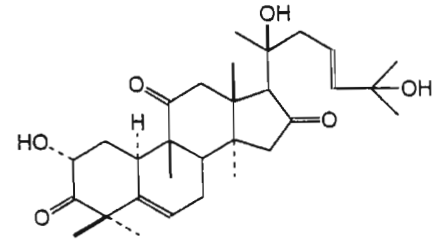
**[43]** Isocucurbitacin B



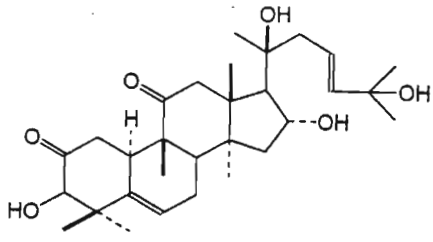
**[44]**



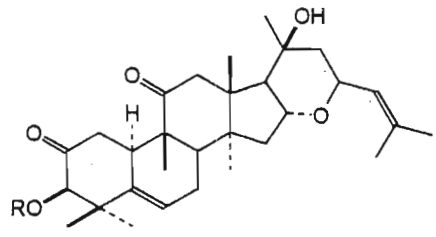
**[45]**



**[46]**

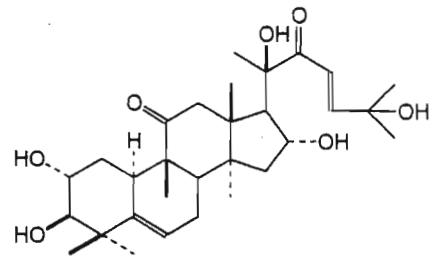


**[47]**



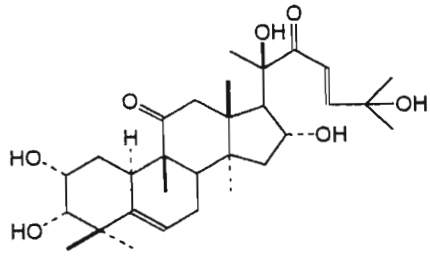
**[48a]** R = H

**[48b]** R = Ac



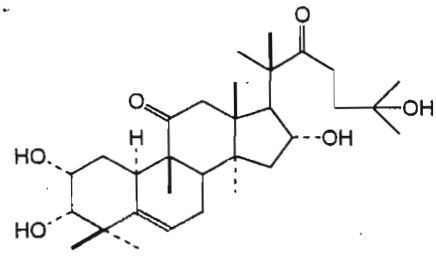
Cucurbitacin F

**[49]**



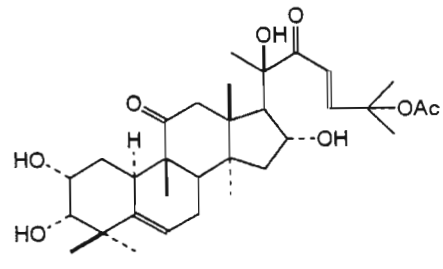
Cucurbitacin O

**[50]**



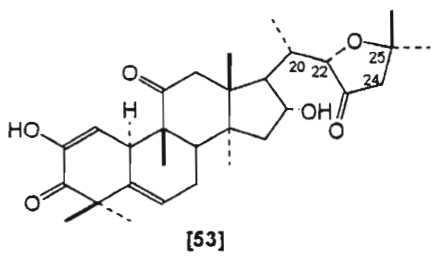
Cucurbitacin P

**[51]**

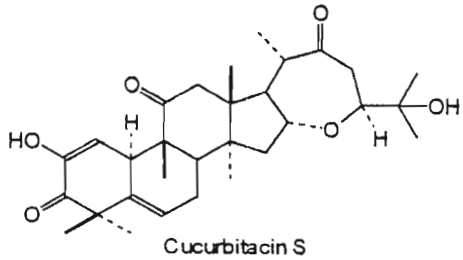


Cucurbitacin Q

**[52]**

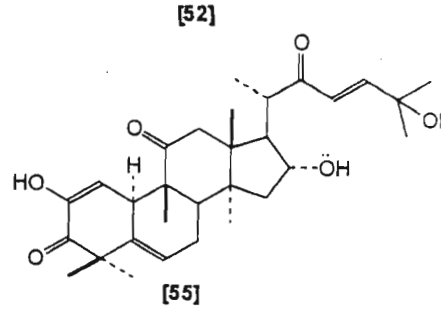


**[53]**

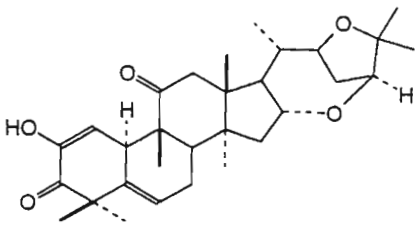


Cucurbitacin S

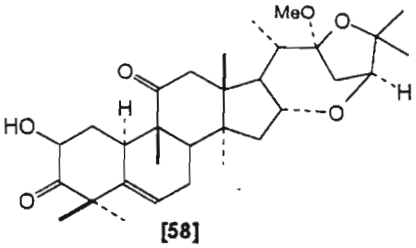
**[54]**



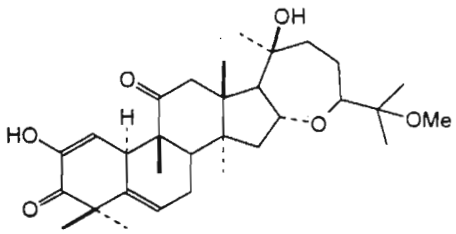
**[55]**



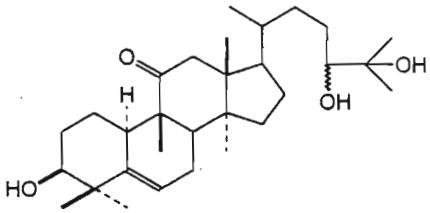
[57]



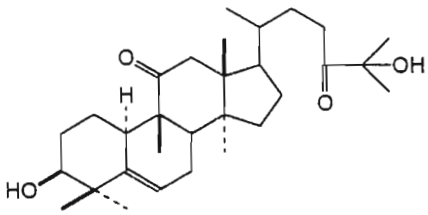
[58]



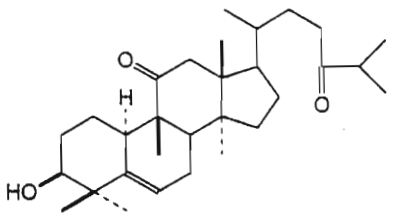
[59] Cucurbitacin T



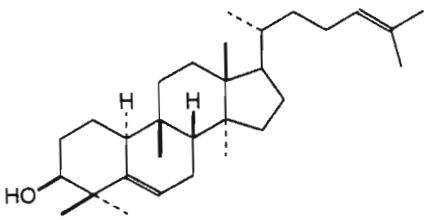
[60] Bryodulcosigenin



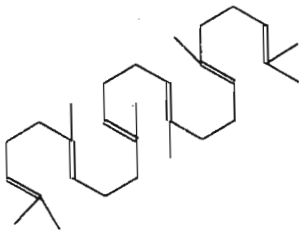
[61] Bryosigenin



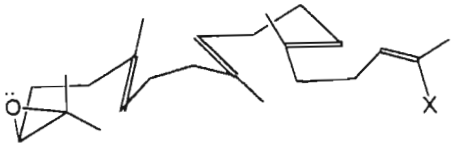
[62] Bryogenin



[63] 10α-cucurbita-5,24-dien-3β-ol

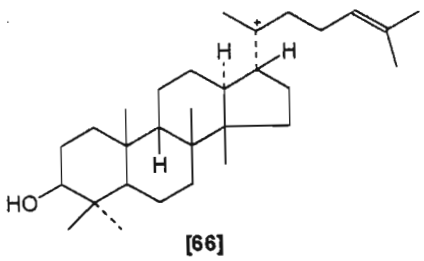


[64] Squalene

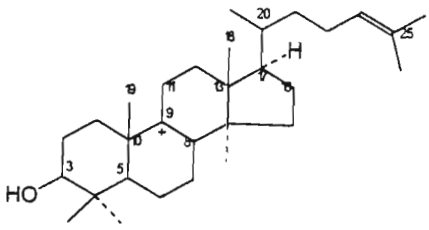


[65]

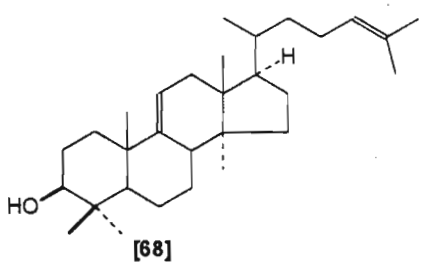
X = —$CH_2$—$CH_2$—CH=C(Me)(Me)



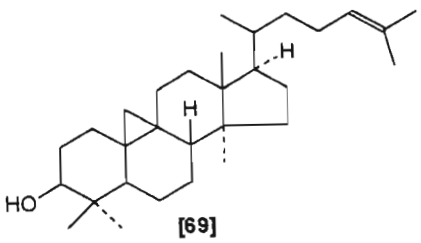
[66]



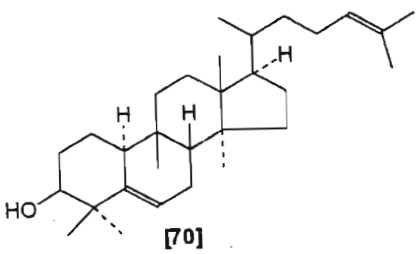
[67]



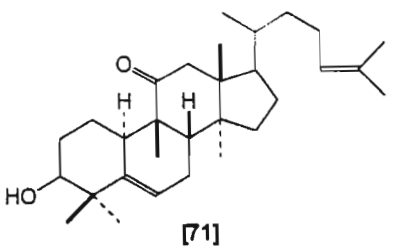
[68]



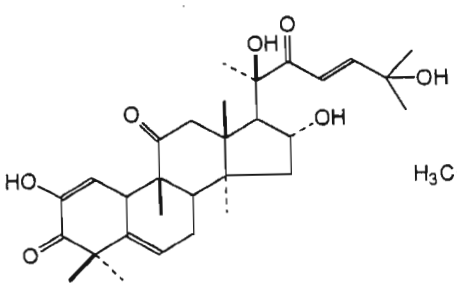
[69]



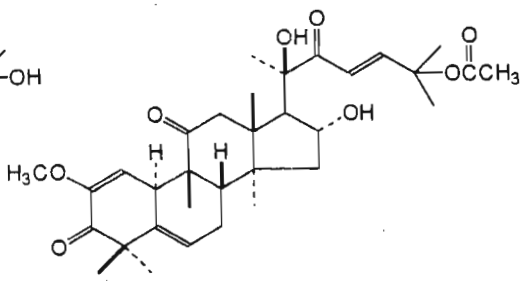
[70]



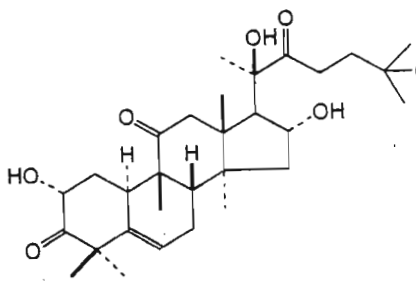
[71]



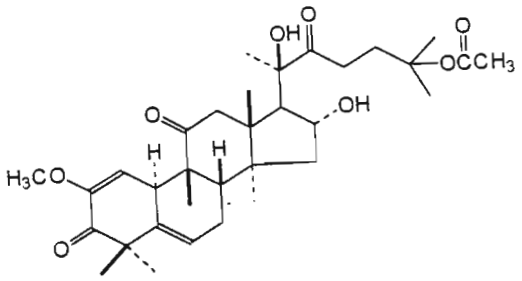
[75] Elatericin B



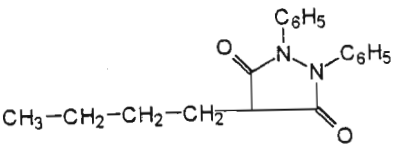
[76] (EME)



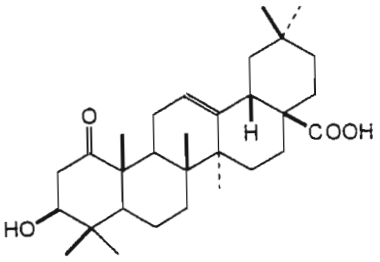
[77] Dihydroelatericin A



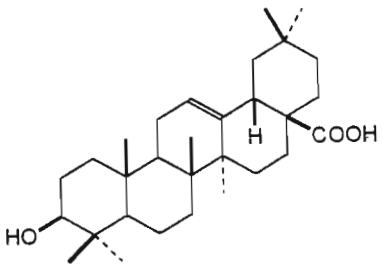
[78] (DEME)



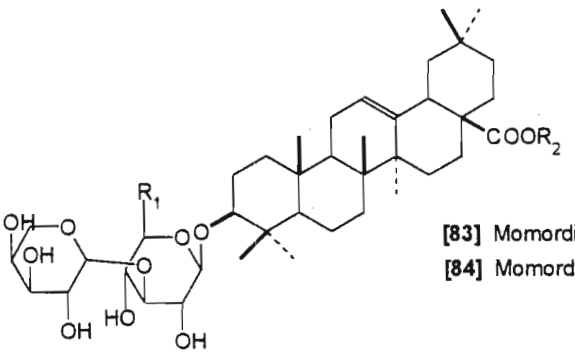
[80] Phenylbutazone



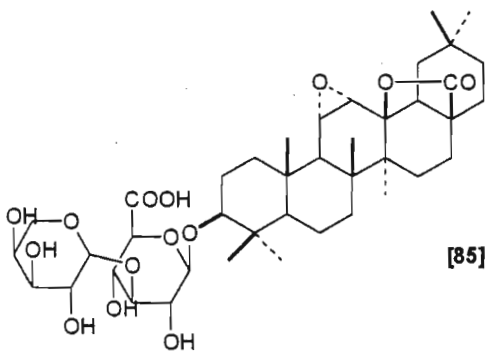
[81] Momordic acid



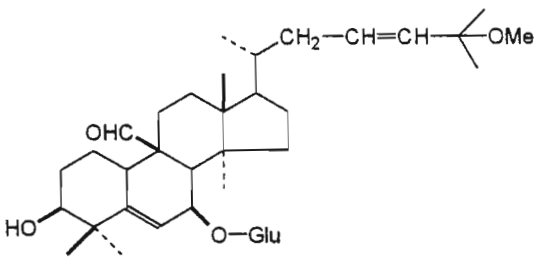
[82] oleanolic acid



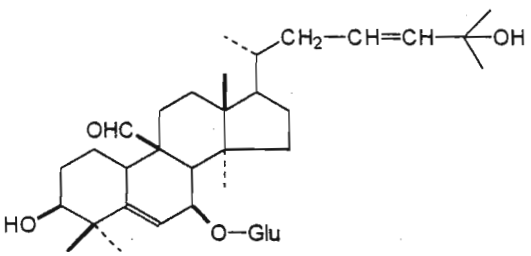
[83] Momordin I $R_1$ = COOH
[84] Momordin II $R_2$ = H



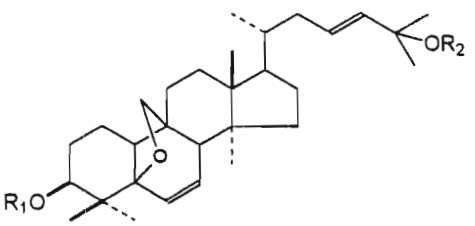
[85] Momordin III



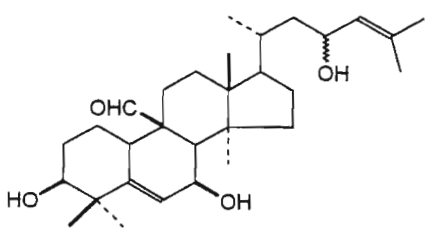
[86] Momordicoside K



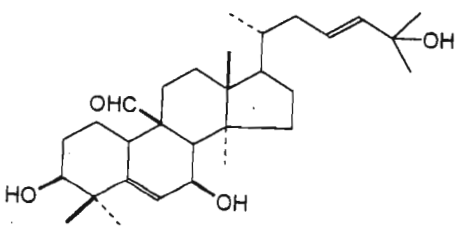
[87] Momordicoside L



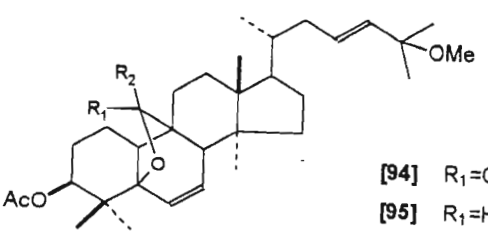
[88] $F_1$: $R_1$=β-D-glucopyranosyl, $R_2$=$CH_3$
[89] $F_2$: $R_1$=β-D-allopyranosyl, $R_2$=H
[90] G: $R_1$=β-D-allopyranosyl, $R_2$=$CH_3$
[91] I: $R_1$=β-D-glucopyranosyl, $R_2$=H



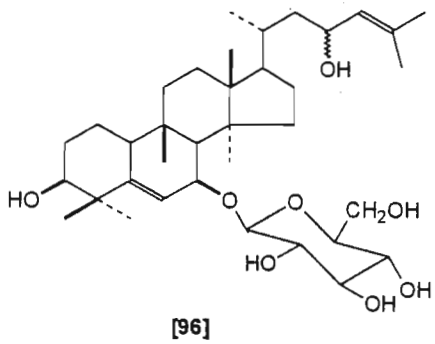
[92]



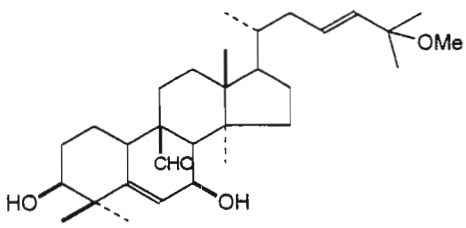
[93]



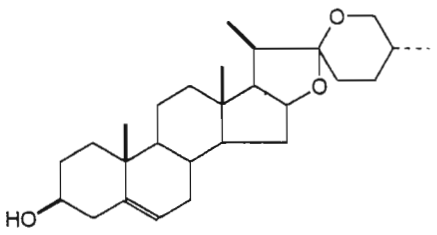
[94] $R_1$=OMe, $R_2$=H
[95] $R_1$=H, $R_2$=OMe



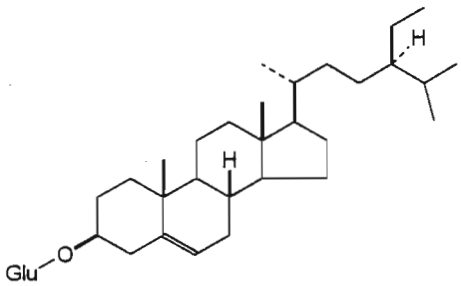
[96]



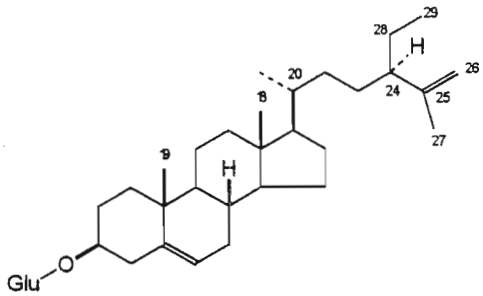
[97]



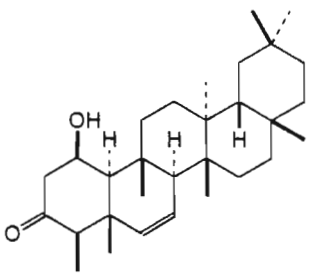
[98] Diosgenin



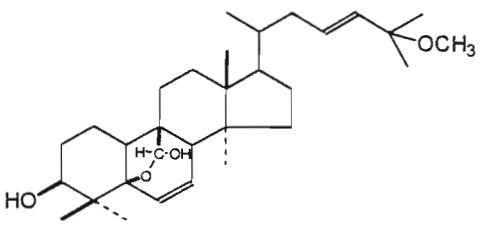
[99] β-sitosterol glucoside



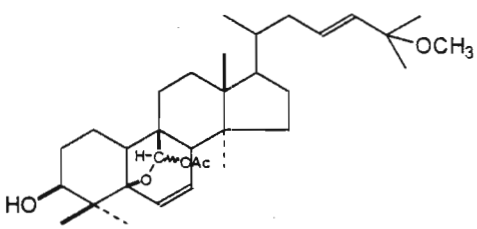
[100]5,25-stigmastadien-3β-ol glucoside



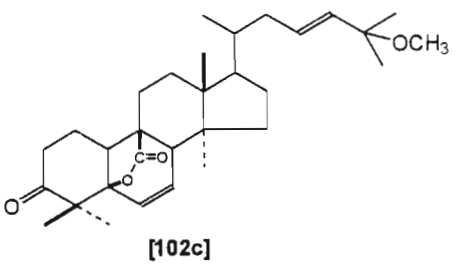
[101] 1β-hydroxyfriedel-6(7)-en-3-one



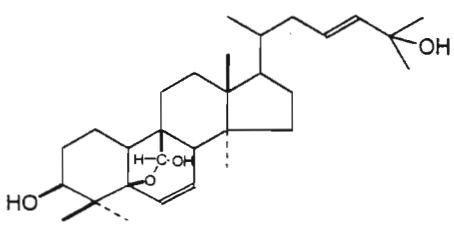
[102]



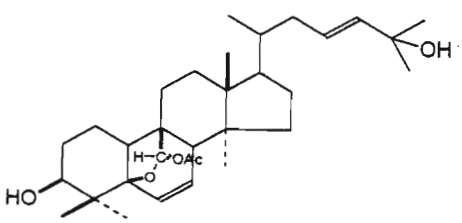
[102a] and [102b]



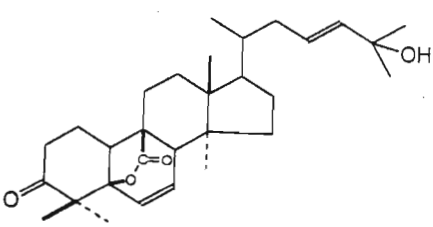
[102c]



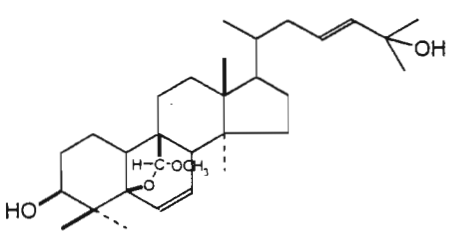
[103]



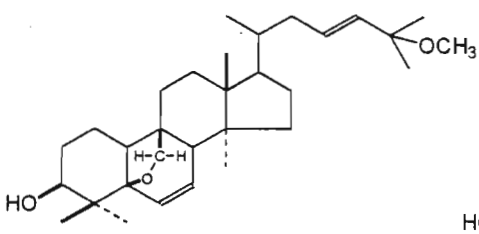
[103a] and [103b]



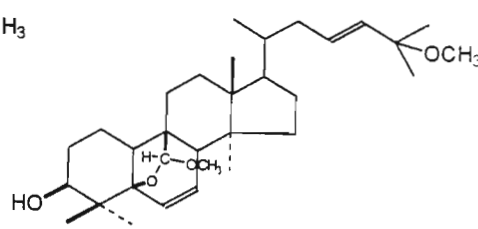
[103c]



[104]



[105]



[106]

# Preface

Despite the dramatic advances made in orthodox medicine over the past 100 years, there has been an increasing interest in alternative systems[1]. About 80% of the world's population relies on herbal medicines and governments of third-world countries, unable to sustain a complete coverage with western type drugs, have encouraged the rational development of traditional treatments[2]. The development and application of scientific methology to validate the medicinal and document the toxicological properties of traditional drugs is an important requirement for improving the quality of traditional medical practices.

The plant kingdom constitutes an abundant source of new chemical products which may be important due to their biological properties and in particular because of their potential use in medicine[3]. Traditional medicine in general is still poorly explored in terms of therapeutic potential or clinical evaluation. The extensive practice of traditional medicine in developing countries and the rapidly growing demand for basic and alternative therapeutic substances give importance to research and development in the field of traditional drugs[4]. An additional motivation is found in the practical necessity to integrate the potential of traditional medicine into current practices of modern health care. In this respect there are a number of facts to be considered.

Firstly, there is the daily reality of empirical practices of traditional medical systems among many millions of people in developing countries[2]. These medical provisions cover a tremendous demand for health care,

both preventive and curative in nature. Secondly, traditional medical practices are well adopted in the respective cultural societies[5], and thirdly, when not free of charge, the cost of these practices and drugs are, in general, within the financial means of the people.

The continent of Africa provides an extremely diverse range of plants that, over the centuries, has found use in curing a wide variety of ailments. Furthermore, traditional healers are acceptable, accessible and greatly utilised by African people and hence play an important role in tribal communities[6]. Unlike the orthodox practitioners, *inyangas* and *sangomas* never view man as separate from the earth but as an integral part of it. Man's interelationship with his enviroment is therefore incorporated into their healing philosophy. It is this holistic approach, routed in religion and cultural methodology, that makes the *sangomas* an important component in modern health care today.

# CHAPTER 1. The Cucurbitaceae and the cucurbitacins

## 1.1. The Cucurbitaceae

The plant family Cucurbitaceae, the gourds, comprise about 100 genera and 850 species, primarily of pantropical and subtropical distributions with range extensions into the temperate northern and southern hemispheres[7]. None of the genera is particularly large, the larger including *Cayaponia* (70 spp.), *Melothria* (60 spp), *Gurania* (49 spp), *Cyclanthera* (40 spp), *Trichosanthes* (25 spp) and *Sicyos* (35 spp). In southern Africa, the family is represented by 18 genera and 76 species[8]. Representatives of the genera *Zehneria*, *Kedrostis*, *Acanthoicyos*, *Momordica*, *Citrullus*, *Cucumis*, *Trochomeria*, *Lagenaria*, *Corallocarpus*, *Peponium* and *Coccinia* are widely distributed throughout Africa while species of *Dactyliandra* and *Cyclantheropsis* are confined to Namibia. Species of *Gerrardanthus*, *Oreosyce*, *Mukia*, *Ctenolepis* and *Cucumella* have a scattered distribution in the Cape, Transvaal, KwaZulu-Natal and Botswana.

The gourd family is distinguished by characters which include a prostrate or scandent growth habit, tendril-bearing herbaceous stems, unisexual flowers with an inferior ovary, with generally three parietal placentae almost filling the locule and the unisexual modifications of the androecium[9]. The Cucurbitaceae was subdivided by Pax (1889)[7] into five

subfamilies (Fevilleae, Melothrieae, Cucurbiteae, Sicyoideae, and Cyclanthereae).

Economically, the family is important as a source of food and ornamentation. Prominent among the food items are pumpkin and squash (*Cucurbita*); cucumber, gherkin and maskmellon (*Cucumis*); and watermelon and citron (*Citrullus*)[10]. Species of about 16 other genera are grown as ornamentals, mostly as gourds, including the Chinese watermelon (*Benincasa*), ivy gourd (*Coccinea*), squirting cucumber (*Ecballium*), calabash gourd (*Lagenaria*), dishcloth gourd (*Luffa*), balsam apple (*Momordica*) and the snake gourd (*Trichosanthes*)[7].

The potent physiological activity of plants belonging to the Cucurbitaceae has been known since antiquity. They were feared on account of their high toxicity and yet valued because of the medicinal properties ascribed to them, as illustrated by examples of plants found in southern Africa (Table 1). Pharmaceutical preparations, which are still used in modern medical practice, include colocynth from *Citrullus colocynthis*, bryonia from species of *Bryonia* and elaterium from *Ecballium elaterium*[10]. Bitterness occurs sporadically in cultivated cucurbits, sometimes presenting a real danger to human health[11]. It appears that the amount of bitter principles in the Cucurbitaceae is controlled by a combination of genetic, enviromental and developmental factors[12].

Interest in these substances was promoted by the discovery at the National Cancer Institute, Bethesda, USA, that some compounds possess anti-tumor activity[13]. These bitter principles, occurring very widely in the

Cucurbitaceae, have been named cucurbitacins followed by various letters according to the chronology of isolation, although other names have sometimes been used. Following the elucidation of their structures, cucurbitacins have been found also in the Primulaceae[14] and Rosaceae[15] and thus seem to have a wider distribution in the plant kingdom than first thought.

**Table 1.** Ethnomedical properties of some Cucurbitaceae plants found in southern Africa

| Botanical name | Common name | Locality | Ethnomedical properties |
|---|---|---|---|
| *Colocynthis citrullus* O. Ktze (*Citrullus vulgaris* Schrad.) | wild gourd watermelon | Cape Province Namibia | remedy for arthritic pain, ophthalmia, asthma, bronchitis[16] |
| *Cucumis africanus* L.f. (*C. hookeri* Naud.) | wild cucumber jelly melon *uhufafa*, *umaselwana* (Zulu) *uthangazana* (Xhosa) *mukake* (Venda) | Transvaal Swaziland | used as an emetic and purgative[17]. Indians in Natal use the boiled leaves as a poultrice[10]. |
| *Cucumis hirsutus* Sond. | wild cucumber | Zimbabwe Transvaal | used for constipation abdominal pains, venereal diseases[18] |
| *Acanthosicyos naudiniana* Sond. (*Citrullus naudinianus* (Sond.) Hook f.) | herero cucumber wild melon *mokapana* (Tswana) | Namibia Botswana, OFS, Transvaal, KwaZulu-Natal, Cape Province | thirst quencher[10], used as a remedy for hypoglycaemia[19] |
| *Cucumis myriocarpus* Naud. | bitter apple wild cucumber *isendelenja* (Zulu) *monyaku* (Sotho) | Botswana, KwaZulu-Natal, OFS, Lesotho, Transvaal, E. Cape | used as a purgative, emetic and applied to boils[10,19] |
| *Lagenaria siceraria* (Molina) Standl. (*L. vulgaris* Ser.) | bottle gourd calabash white pumpkin *amabhanga/ ikhomane* (Zulu) *iselwa* (Xhosa) *seho* (Sotho) *tshikumbu* (Venda) | Transvaal | used as pectorol, purgative and anthelmintic[10] |
| *Cucurbita pepo* L. convar. | pumpkin, squash marrow *ithanga* (Xhosa) *umliba/amathana* (Zulu) | cultivated | used as a remedy for hypertrophy of the prostrate gland as well as a diuretic and demulcent[10]. cure for pneumonia |

## 1.2. The cucurbitacins

### 1.2.1. Introduction

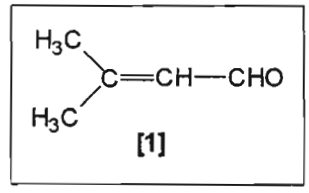
Studies of the cucurbitacin group of compounds, first undertaken by Enslin (1957)[20], have given much information on their structures. Despite this, the molecular formula of elaterin and other cucurbitacins remained uncertain for many years, until Rivett and Enslin[21] assigned tentative formulae (Table 2) for the crystalline cucurbitacins on the basis of X-ray studies. Several of these formulae have been revised successfully as discussed in this text.

**Table 2.** Tentative and revised formulae of crystalline cucurbitacins

| Crystalline cucurbitacins | Tentative formulae | Revised formulae |
|---|---|---|
| A | $C_{32}H_{46}O_4$ | $C_{32}H_{46}O_9$ |
| B | $C_{32}H_{48}O_8$ | $C_{32}H_{46}O_8$ |
| C | $C_{32}H_{50}O_8$ | $C_{32}H_{48}O_8$ |
| D | $C_{30}H_{46}O_7.{}^1/_2H_2O$ | $C_{30}H_{44}O_7$ |
| E | $C_{32}H_{44}O_8$ | |
| F | $C_{30}H_{48}O_7$ | $C_{30}H_{46}O_7$ |
| G | $C_{30}H_{52}O_9$ | $C_{30}H_{46}O_8$ |
| I | $C_{30}H_{44}O_8$ | $C_{30}H_{42}O_7$ |
| J | $C_{30}H_{44}O_8$ | |
| L | $C_{30}H_{46}O_7.{}^1/_2H_2O$ | $C_{30}H_{44}O_7$ |

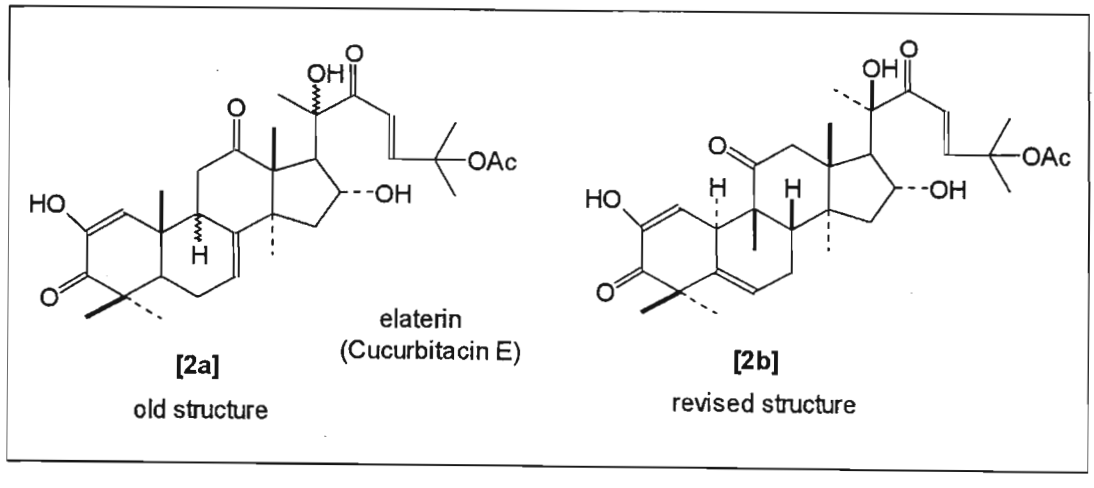
Rivett and Enslin also stipulated that all bitter principles contain two or more alcoholic hydroxyl groups and cucurbitacins A,B,C and E contain

one acetoxy group. The presence of several keto groups is a further characteristic structural feature of these substances. In cucurbitacins A,B,C,D,E,F and I, one of the keto groups is α,β-unsaturated. Cucurbitacins B,D,E,G,I,J,K and L contain a diosphenol (α-diketone) grouping which can combine with glucose or other monosaccharides to form naturally occurring enolglycosides[12]. The formation of senecio aldehyde **[1]** on oxidation of cucurbitacins A and C with chromic acid threw light on the nature of the side chains present.




**[1]**

The isolation of 1,2,8-trimethylphenanthrene from the dehydrogenation product of reduced cucurbitacin A indicated a possible structural relationship to the tetracyclic triterpenes[22]. Elaterin (cucurbitacin E), isolated from *Ecballium elaterium* and *Citrullus colocynthis* was the first cucurbitacin to have its structure fully elucidated and was initially assigned structure **[2a]**[23].




**[2a]**
old structure

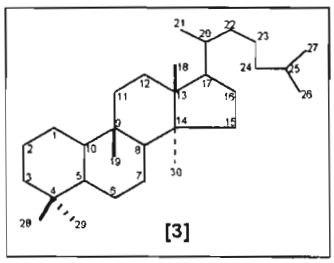
**[2b]**
revised structure

In 1960, in a communication dealing with the NMR spectrum of a compound containing a diosphenol chromophore, Noller and co-workers[24] observed that the olefinic proton involved in the ring A diosphenol system appeared as a doublet δ5,97 (J=3Hz), an observation which required the presence of an adjacent proton at C-10.

One year later Lavie et al.[25] (1961) proposed structure **[2b]**, consistent with the NMR observations and provided chemical and spectroscopic evidence for the location of a methyl group at C-9 instead of C-10. The cucurbitacins were thus the first representatives of tetracyclic triterpenoids with a C-9 methyl group. The carbon atom of this methyl group by convention on cucurbitane compounds is numbered as C-19. Final assignment of the ring C carbonyl to C-11 was given in 1962 by the same group[26].

At least 100 plant species in 30 genera and at least 3 families have been shown to contain cucurbitacins. It is thought that the cucurbitacins were selected for evolutionary processes in order to protect the Cucurbitaceae against attack by invertebrate and vertebrate herbivores[27].

The cucurbitacins are a biologically important group of highly oxygenated tetracyclic triterpenoids having a unique 19(10→9β) *abeo*-10α-lanostane (cucurbitane) skeleton **[3]** and a wide range of biological activities[28,29].




[3]

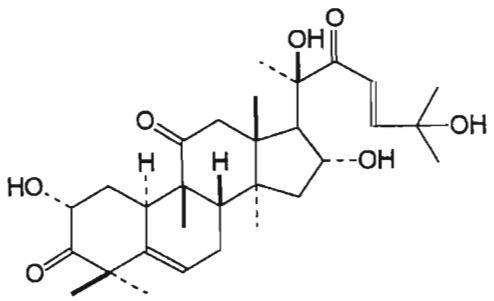
A number of cucurbitacins occur in the form of glycosides, and some of them lack the C-11 carbonyl function[30]. Chemically, cucurbitacins are classified according to the functionalities in ring A and C, side chain modifications, as well as stereochemical considerations.

## 1.2.2. Structure determination of cucurbitacins

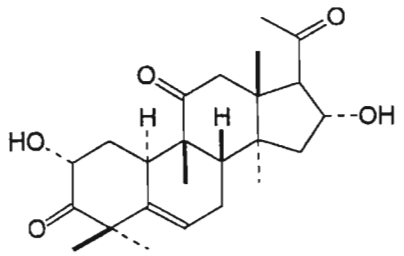
### 1.2.2.1. The side chain

The identification of the eight-carbon atom side chain of the cucurbitacins and its substitution pattern was based on reactions given in Scheme 1a and 1b. Following different cleavage reactions, the major fragment of the molecule afforded different compounds depending on the starting material and the reaction conditions.

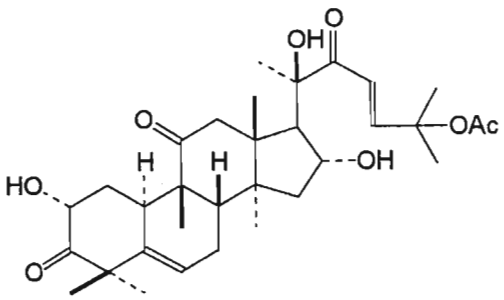
Treatment of elatericin A **[4]** with periodic acid induced cleavage of the C-20, C-22 bond with formation of a methyl ketone **[5]** and of *trans*-4-hydroxy-4-methyl-pent-2-enoic acid **[6a]**. Hydrogenation of **[6a]** afforded isocaprolactone **[7]** which was obtained in turn by hydrogenation of the acetylenic acid **[8]**[31,32]. A similar oxidation performed on cucurbitacin B **[9]** afforded the corresponding acetate **[6b]** identified by hydrolysis to the original acid **[6a]**[33]. Similar results were obtained with cucurbitacin A **[10]**; C **[11]**; E **[2b]** and their acetates, thus confirming that the tertiary hydroxyl (or acetate) is at the end of the side chain in all these compounds[33,34].



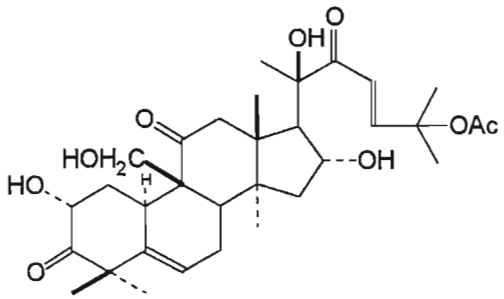
**[4]** Elatericin A



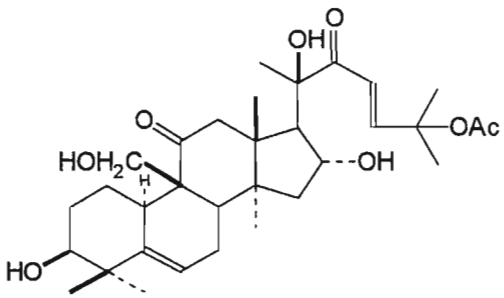
**[5]** Methyl ketone of Elatericin A



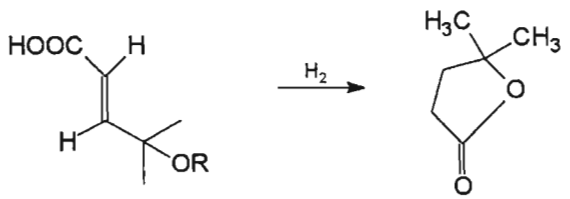
**[9]** Cucurbitacin B



**[10]** Cucurbitacin A



**[11]** Cucurbitacin C



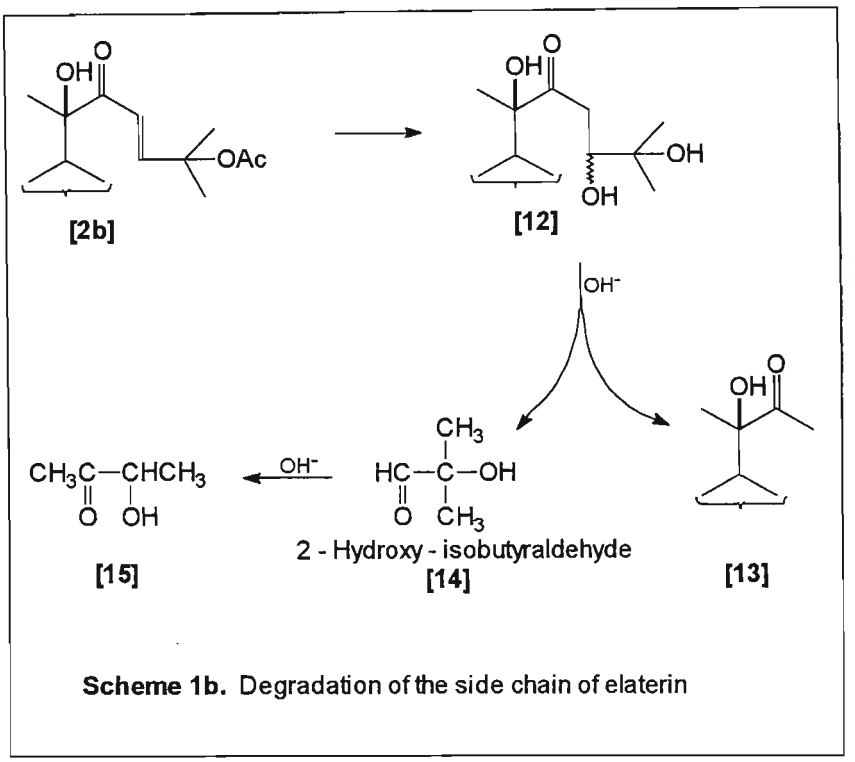
**[6a]** R = H
**[6b]** R = Ac

**[7]**

**[8]**

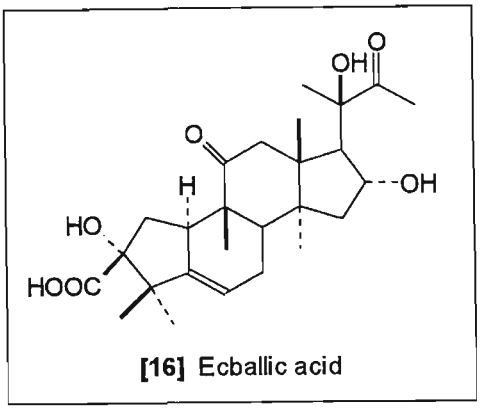
**Scheme 1a.** Degradation products of the side chain of elaterin

Further information on the nature of the side chain was obtained by alkali treatment of elaterin **[2b]** (Scheme 1b). This reaction, which resulted in the isolation of ecballic acid **[16]** (partial structure **[13]**) and acetoin **[15]**[20,35], was brought about by cleavage of the C-23, C-24 bond, and could be rationalised by the sequence **[12]**→**[14]**→**[15]**.




**Scheme 1b.** Degradation of the side chain of elaterin

The first step of this reaction was the hydration of the $\Delta^{23}$ bond to a mixture of 24-OH isomers **[12]**[36,37]. The retro-aldol cleavage of the 24-hydroxy-22-one moiety present in **[12]** gave 2-hydroxy-isobutyraldehyde **[14]** which, under the alkali conditions of the reaction, underwent an acyloin rearrangement to give acetoin **[15]**. This was confirmed by alkaline treatment of a synthetic sample of **[14]**[37]. Further proof of the structure of the side chain was provided by ozonolysis of elatericin A **[4]**, cucurbitacins A **[10]**, C **[11]** and E **[2b]** [37,20].



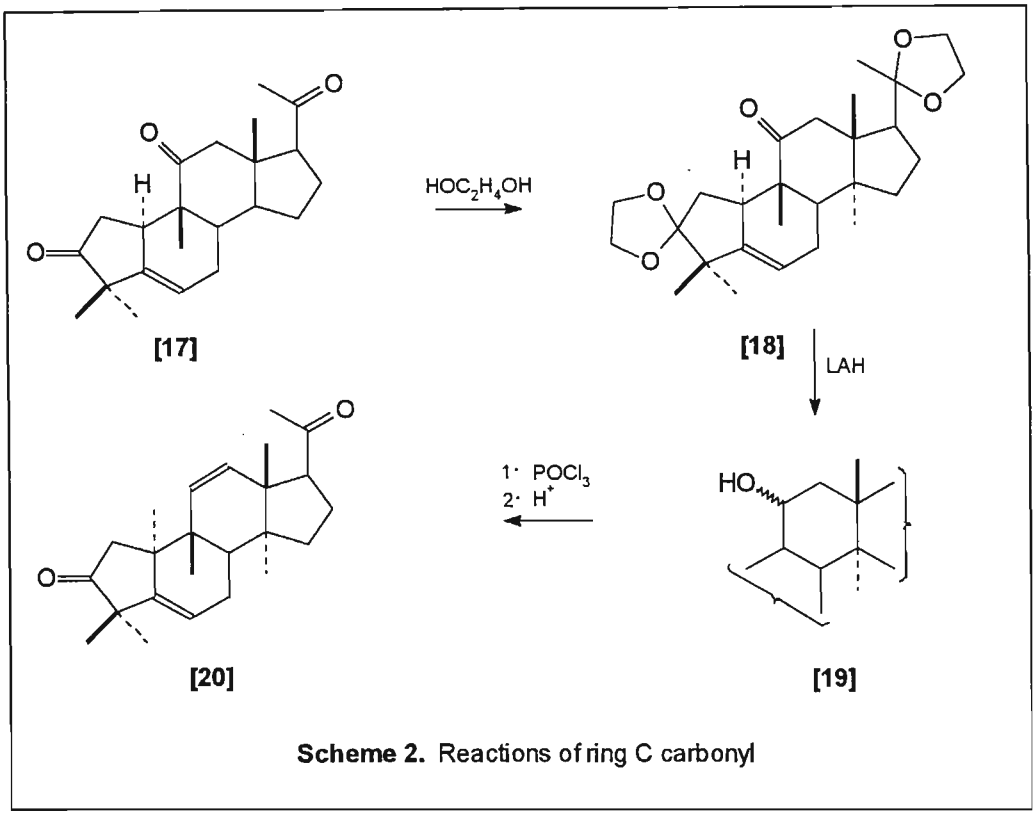

**[16]** Ecballic acid

## 1.2.2.2. The ring A substituents

Ring A substituents comprise either the α-hydroxyketones **[4]**, **[9]**, characterised by the formation of a formazan with triphenyltetrazolium chloride, or the diosphenols **[2b]** which give a deep green colour with ferric chloride[38].

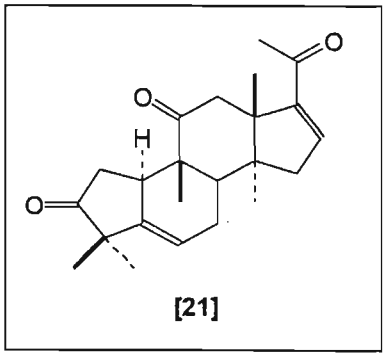
## 1.2.2.3. The 19-methyl group and ring C carbonyl

The placing of the 19-methyl group at C-9 and of the ring C carbonyl at C-11 was based on a series of independent experiments performed on derivatives of elatericin A **[4]**[39,40] and cucurbitacin A **[10]**[41] (Scheme 2). The triketone **[17]** yielded only a bisethylene-ketal **[18]** thus revealing the hindered nature of one of the ketones. Lithium aluminium hydride reduction to **[19]** followed by dehydration produced a compound with a double bond in ring C. The NMR spectrum of the diketone **[20]** obtained after hydrolysis of the ketal groups exhibited an AB pattern for the two vinylic *cis* protons (H-11 and H-12) thus indicating that no vicinal proton was present at C-9 to induce additional coupling of the H-11[39].



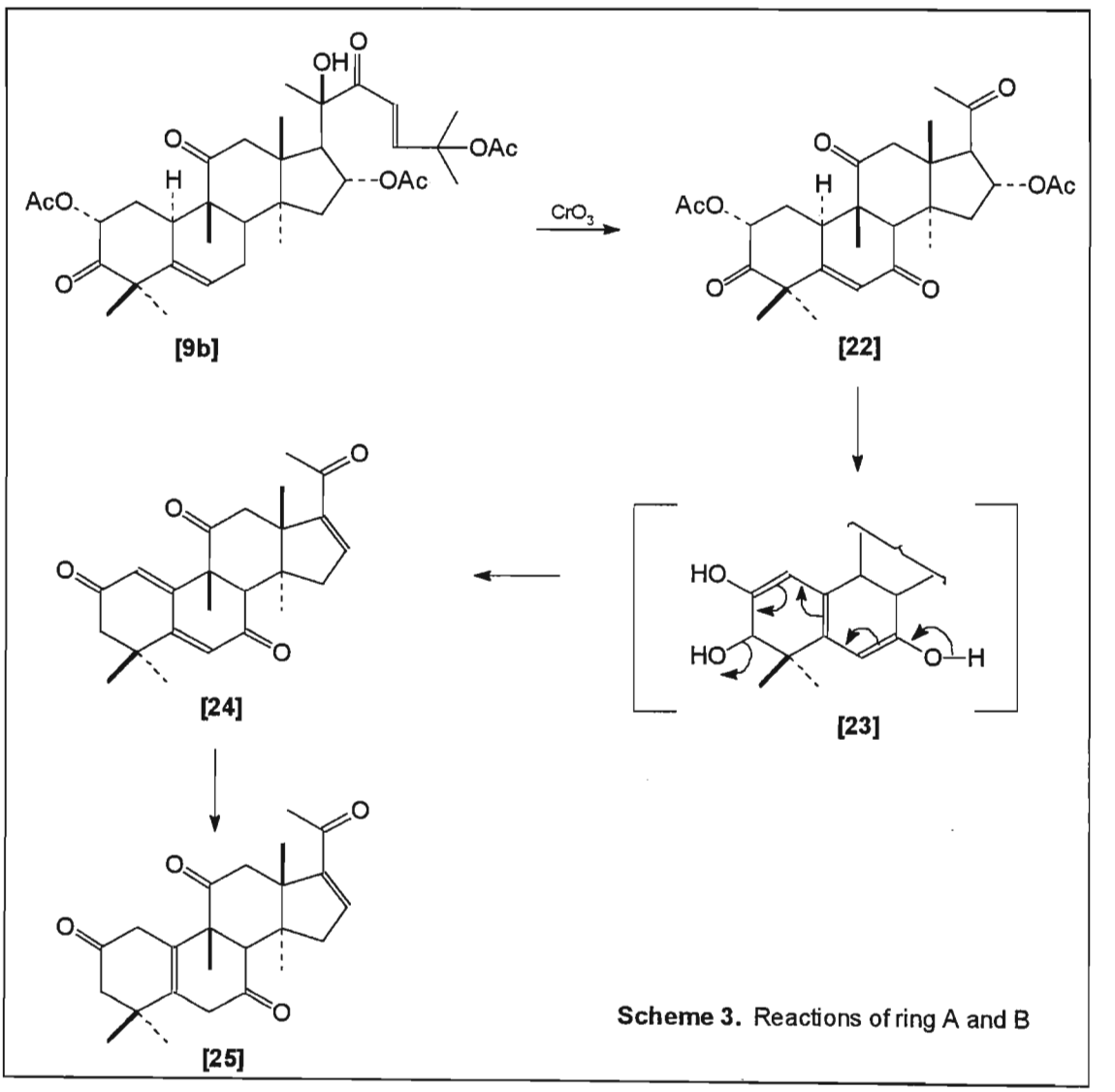

**Scheme 2.** Reactions of ring C carbonyl

The formation of a C-11, C-12 double bond in **[20]** left two possibilities for the ring C ketone, either at C-11 or at C-12. Its assignment to C-11 was based on UV and IR spectra of **[21]**($\lambda_{max}$ 240 nm and $\upsilon_{max}$ 1666 $cm^{-1}$)[39,40].



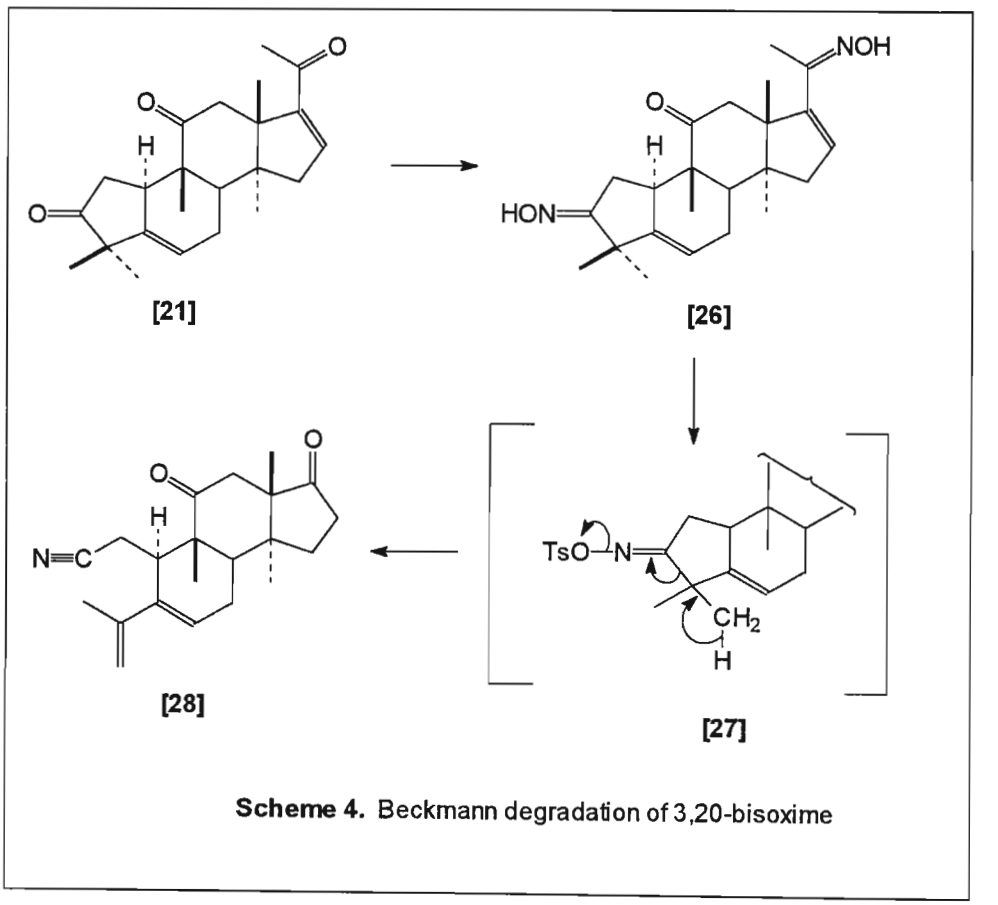
## 1.2.2.4. The ring B double bond and 16-hydroxy group

Reactions which permitted assignment of the ring B double bond to the 5,6-position also established the absence of a methyl group at C-10, a characteristic feature of the cucurbitane skeleton. Oxidation of cucurbitacin B acetate **[9b]** with $CrO_3$ afforded "cucurbitone B" **[22]**[42] (Scheme 3) which rearranged with methanolic HCl to the conjugated dienedione **[24]**. The mechanism proposed for the formation of the latter is based on the intermediate enol **[23]**. The dienedione **[24]** was reduced to the β,γ-unsaturated compound **[25]** when treated with zinc in acetic acid[41].




**Scheme 3.** Reactions of ring A and B

The relationship between the 5,6-double bond and the *gem*-dimethyl group in ring A was demonstrated by means of the nitrile **[28]**, formed as a result of the Beckmann rearrangement of the 3,20-bisoxime **[26]**[39] (Scheme 4). Whereas the 20-oxime, in the presence of p-toluene-sulfonyl chloride, afforded the expected enamine, leading to the 17-ketone, the 3-oxime underwent an abnormal rearrangement **[27]** which resulted in cleavage of ring A to **[28]**. A UV band of the latter was characteristic of a conjugated diene; the nature of this chromophore was confirmed by the appropriate NMR signals for the C-28 terminal methylenic protons.

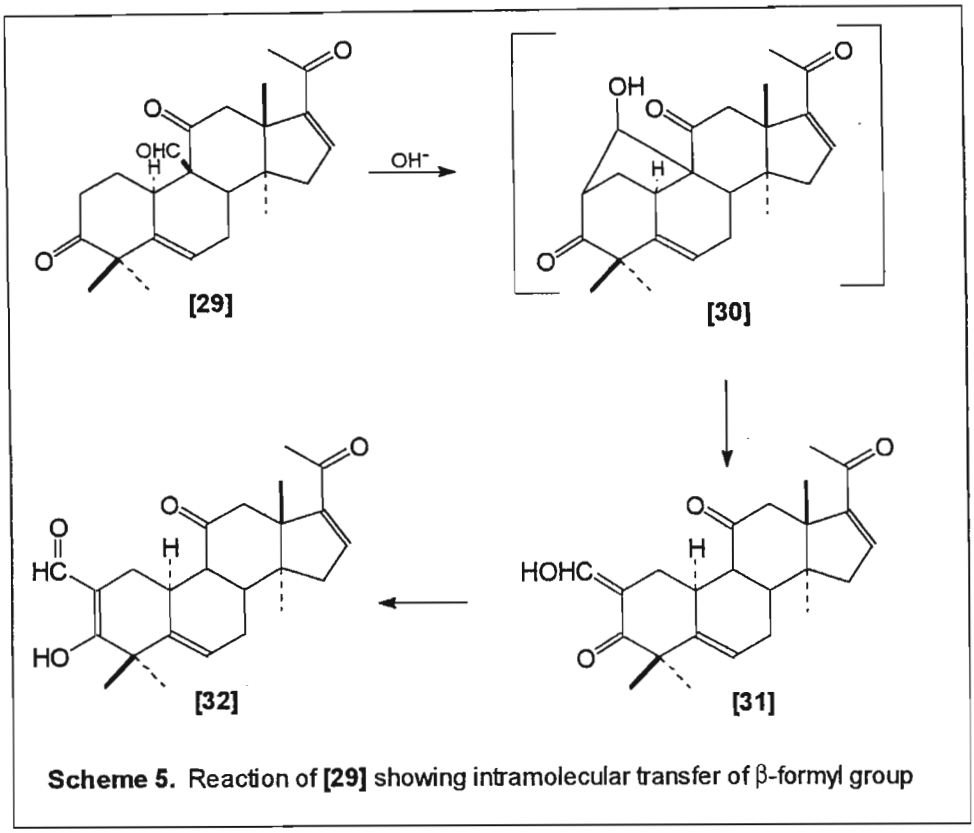



**Scheme 4.** Beckmann degradation of 3,20-bisoxime

The location of the hydroxy group at C-16 was based on reactions performed on different degradation products obtained from several of the cucurbitacins analysed[38,43,44].

## 1.2.3. Stereochemistry of the Cucurbitacins

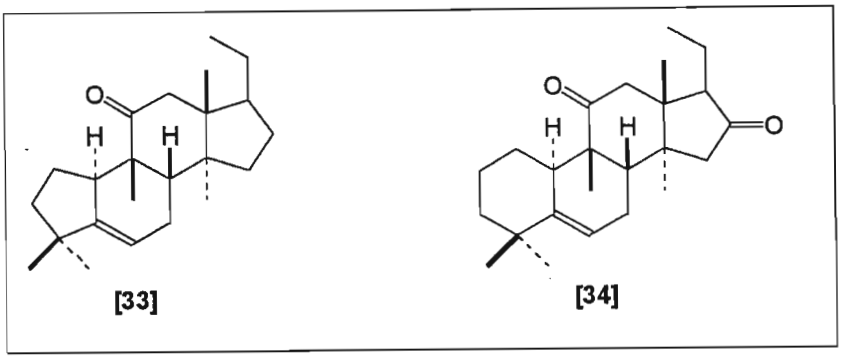
In the presence of ethanolic potassium hydroxide at room temperature, the triketoaldehyde **[29]** (a derivative of cucurbitacin C) afforded two isomeric enolic compounds **[31]** and **[32]** (Scheme 5). This conversion which was formulated as an intramolecular transfer of a β-formyl group implied the presence of two keto groups. The readily enolisable 3-ketone enabled the anionic 2-carbon atom to attack the formyl group with formation of the intermediate aldol **[30]**. By cleavage of the C-9, C-19 bond in the latter, the formyl group is transferred to C-2. As expected, the reaction did not take place with the 3-hydroxy analogue of **[29]**. The transfer of the formyl group ([29]→[30]→[31]) greatly contributed to the elucidation of the stereochemistry of rings A/B and B/C. This reaction could only take place if the B/C ring fusion is *cis* with the formyl group at position 9 and consequently the hindered ketone at C-11. Furthermore, the configuration of H-10 had to be opposite to that of the 9-formyl group[41].




**Scheme 5.** Reaction of **[29]** showing intramolecular transfer of β-formyl group

As stated earlier, the cucurbitacins possess the 19-methyl group at position C-9 and a hydrogen at position C-10; moreover, these two substituents had to be in a *trans* relationship, in agreement with the sequence [29] [30] [31] (Scheme 5), i.e. 10α-H, 9β-$CH_3$ and 8β-H. An independent demonstration for the α-orientation of H-10 was further based on optical rotatory dispersion (ORD) measurements[40].

The B/C and C/D ring fusion was also studied using ORD measurements of the monoketone **[33]** and 16-ketone derivative **[34]** respectively. The compounds were found to be orientated as follows: 8β, 9β, 13β, 14α. Hence a lanostane - type fusion was indicated[23,40].



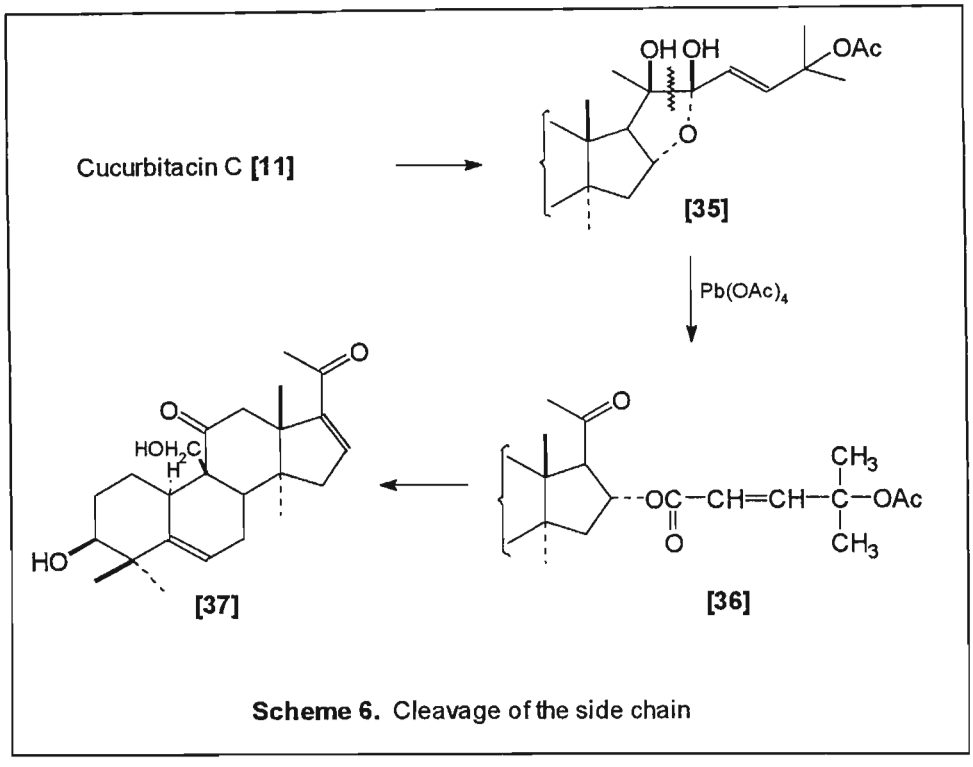

[33] [34]

The orientation of the side chain at C-17 was studied using the Cotton effect[40]. Based on these results, the orientation was found to be β.

To establish the stereochemistry of the 16-OH, the molecular rotation shift resulting from its acetylation was compared with that observed for similar groups in steroids, leading to the conclusion that this group was α-orientated.

## 1.2.4. Cleavage of the side chain

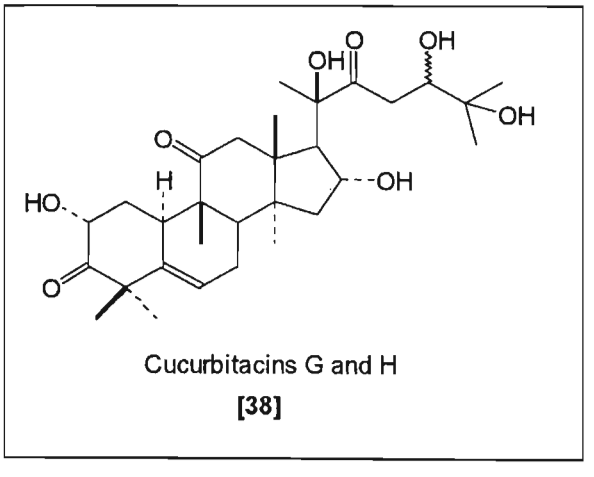
An interesting aspect of the chemistry of the cucurbitacins is the opportunity for intramolecular attack on the 22-one by the 16α-OH with the formation of a "pseudo-glycol" **[35]**. The formation of the 16-ester **[36]** following lead tetraacetate cleavage of **[11]** has been interpreted as taking place through cleavage of C-20, C-22 bond in **[35]**[45]. Under alkaline conditions the 16-ester **[36]** was removed and the product dehydrated to the corresponding $\Delta^{16}$-20-one **[37]** (Scheme 6).




**Scheme 6.** Cleavage of the side chain

## 1.2.5. Cucurbitacins G and H

These two isomers **[38]** have been isolated from roots of *Cucumis hirsutus* Sond. in which they occur together with cucurbitacins B and D (elatericin A)[46]. They are both α-hydroxy ketones (positive triphenyltetrazolium chloride test) and do not display the characteristic 230 nm band for the α,β-unsaturated carbonyl of the side chain.



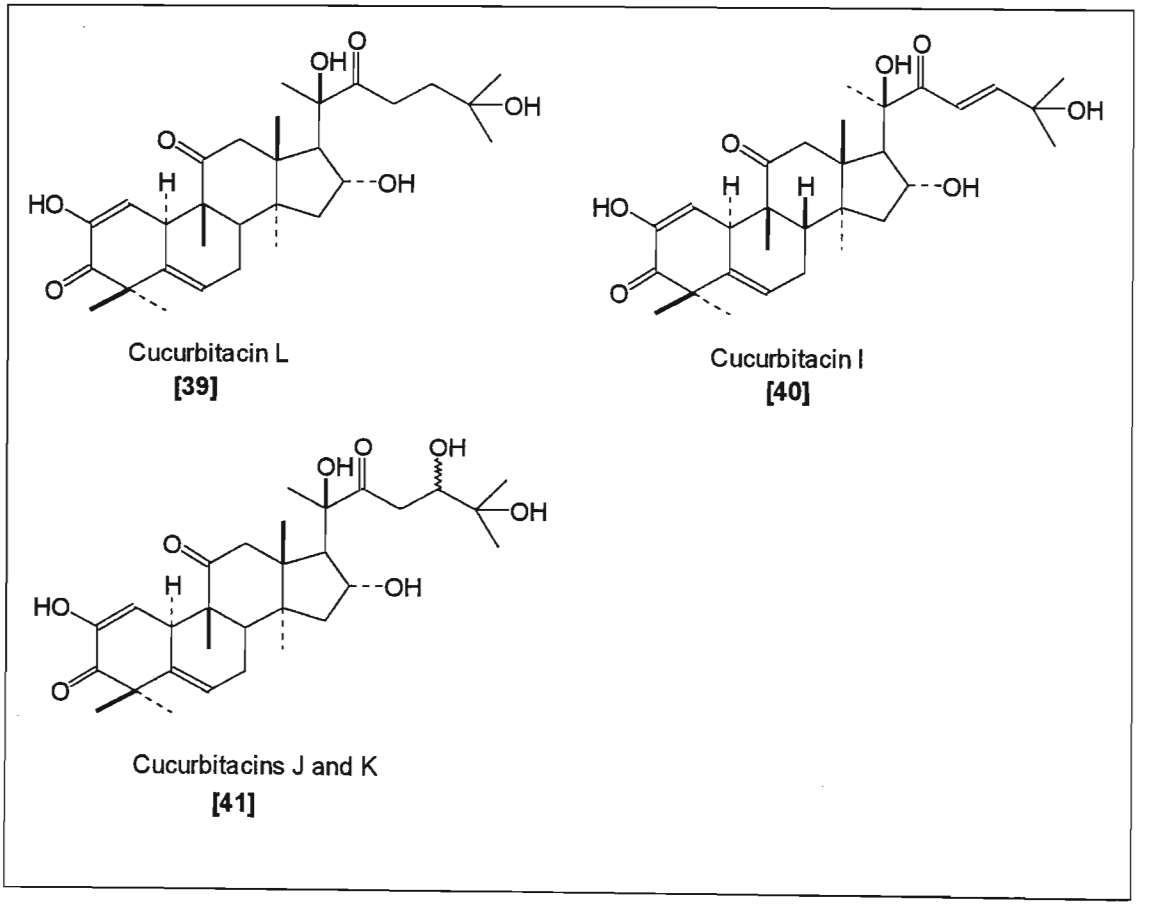

Cucurbitacins G and H

**[38]**

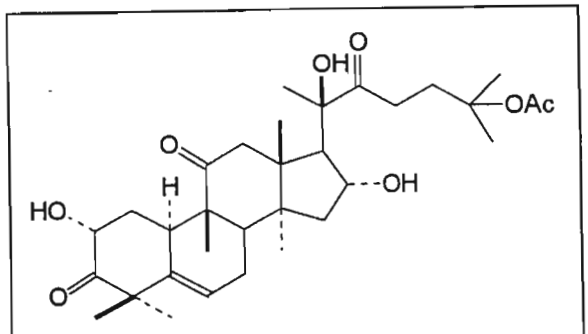
## 1.2.6. Cucurbitacins J, K, and L

Cucurbitacins J, K and L, which possess a diosphenol grouping in ring A, occur as bitter glycosides in the roots of *Citrullus ecirrhosus* Cogn. and have been isolated by enzymatic hydrolysis[47]. They have also been obtained directly as the free aglycones from the roots of *Bryonia dioica* Jack.[48], in which they occur probably as glycosides but with the glycosidic bond cleaved by the enzyme elaterase in the plant.

Cucurbitacin L **[39]** occurs in *Citrullus colocynthis* (L.) Schrad. together with elaterin **[2b]** and cucurbitacins I **[40]** as the corresponding glycosides[49]. Cucurbitacins J and K **[41]** are isomeric at C-24.



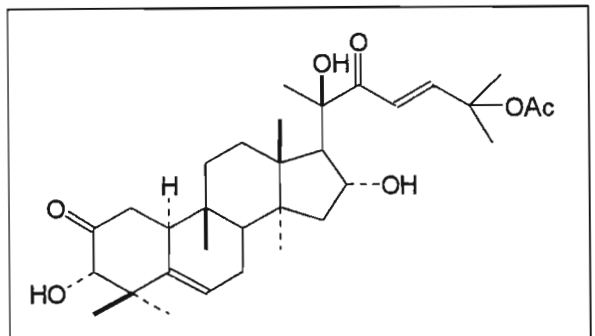
## 1.2.7. Dihydrocucurbitacin B




**[42]** Dihydrocucurbitacin B

This compound **[42]** was isolated from *Marah oreganus* H., where it occurs together with other cucurbitacins[50]. The structure was established by comparison with an authentic sample prepared by the catalytic hydrogenation of cucurbitacin B **[9]**[51].

## 1.2.8. Isocucurbitacin B



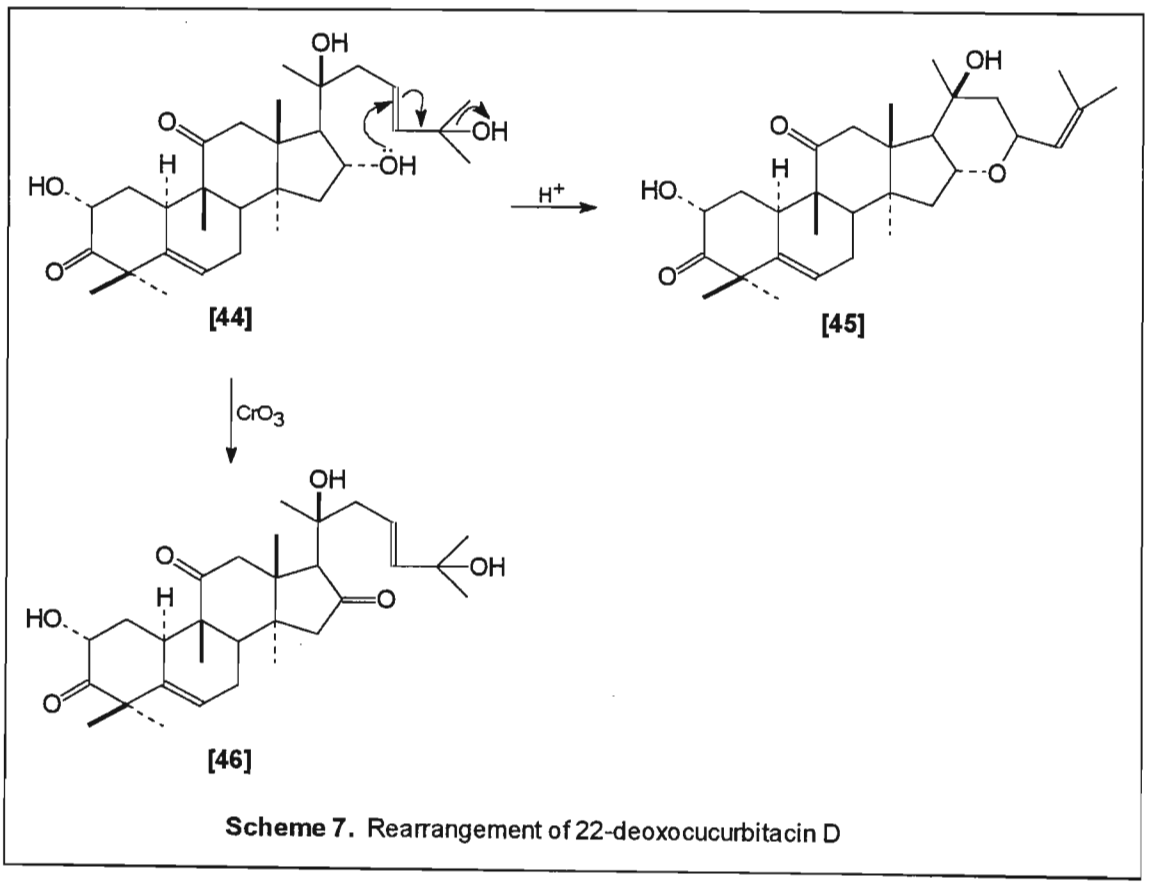

**[43]** Isocucurbitacin B

This compound was first isolated from *Luffa echinata* Roxb.[52] and erroneously assigned a 3-keto-2(axial)-hydroxy structure. Subsequently, the same compound was isolated from *Marah oreganus* and assigned structure **[43]**[53] on the basis of reduction experiments carried out with diosphenol - containing systems.

## 1.2.9. 22-deoxocucurbitacin D and 22-deoxoisocucurbitacin D

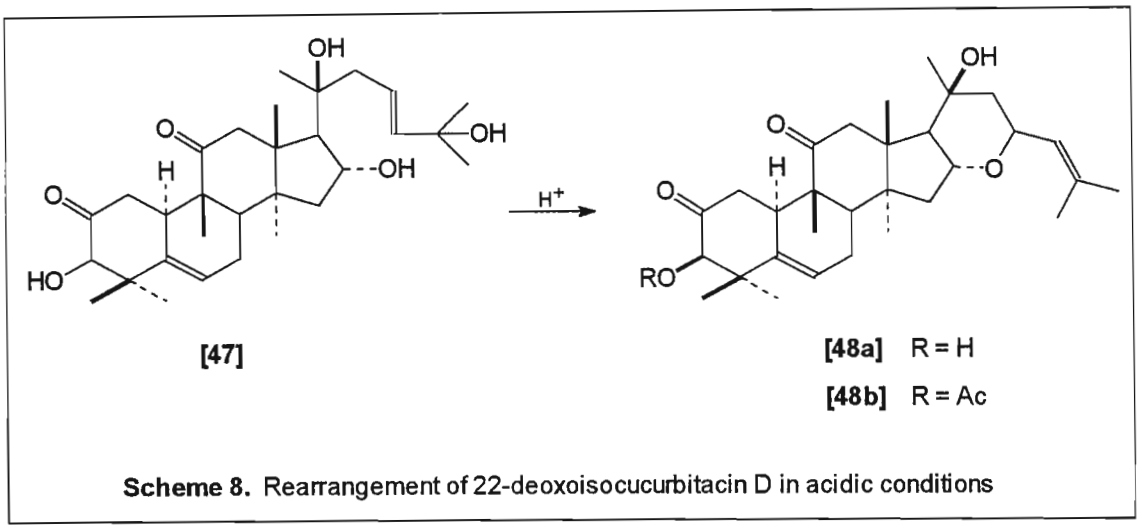
The two cucurbitacins **[44]** and **[47]** were isolated together from a hybrid obtained by the crossbreeding of two varieties of *Lagenaria siceraria* Standl.[54]. Upon treatment with dilute acid, **[44]** underwent a rearrangement involving the elimination of the tertiary 25-hydroxyl and formation of a C-16, C-23 bond in a six membered ring ether **[45]**.

The involvement of the C-16 hydroxy group was demonstrated by oxidising **[44]** to the corresponding 16-one **[46]** thereby preventing the formation of **[45]**.




**Scheme 7.** Rearrangement of 22-deoxocucurbitacin D

22-deoxocucurbitacin D **[47]**, when treated with dilute acid, behaved similarly to **[44]** and gave the anhydro-derivative **[48a]** (Scheme 8). Acetylation of the latter afforded a monoacetate **[48b]** which displayed a singlet for H-3 in the $^1$H NMR spectrum indicating a 2-keto-3-hydroxy system.




**[47]**

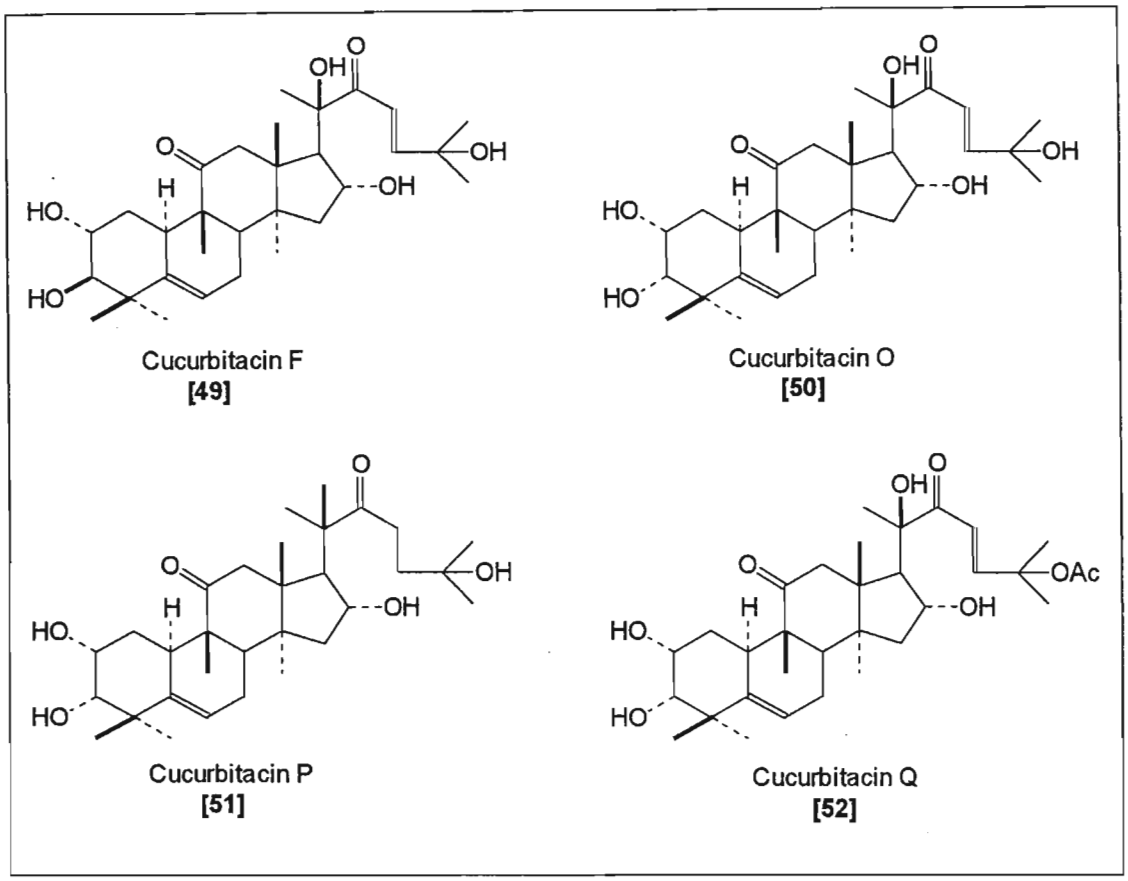
**[48a]** R = H

**[48b]** R = Ac

**Scheme 8.** Rearrangement of 22-deoxoisocucurbitacin D in acidic conditions

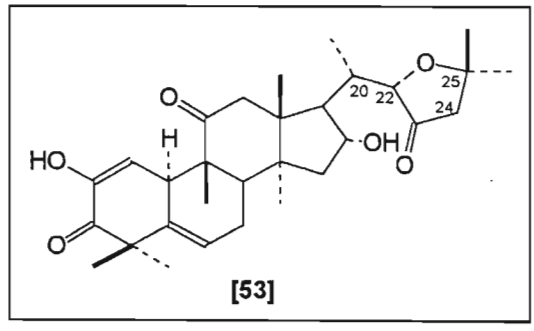
## 1.2.10. Cucurbitacins F, O, P and Q

The distinguishing feature of these cucurbitacins is the presence of a 2,3-diol system in ring A. They all occur in nature as free aglycones. Cucurbitacin F **[49]** was isolated from the leaves of *Cucumis dinteri* Cogn.[55] and was later found in *Cyaponia angustiloba* Cogn.[56], whereas the other three compounds ( i.e. cucurbitacin O **[50]**, cucurbitacin P **[51]**, cucurbitacin Q **[52]**) were isolated from *Brandegea bigelovii* Cogn[57].

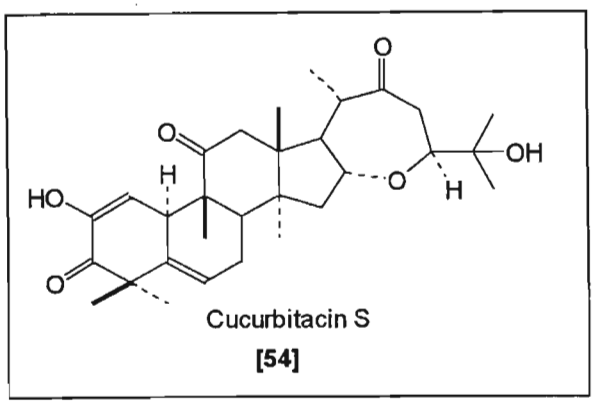



## 1.2.11. Cucurbitacin S

This compound was isolated from *Bryonia dioica* Jacq.[58] and was initially assigned structure **[53]** on the basis of chemical and spectral evidence.



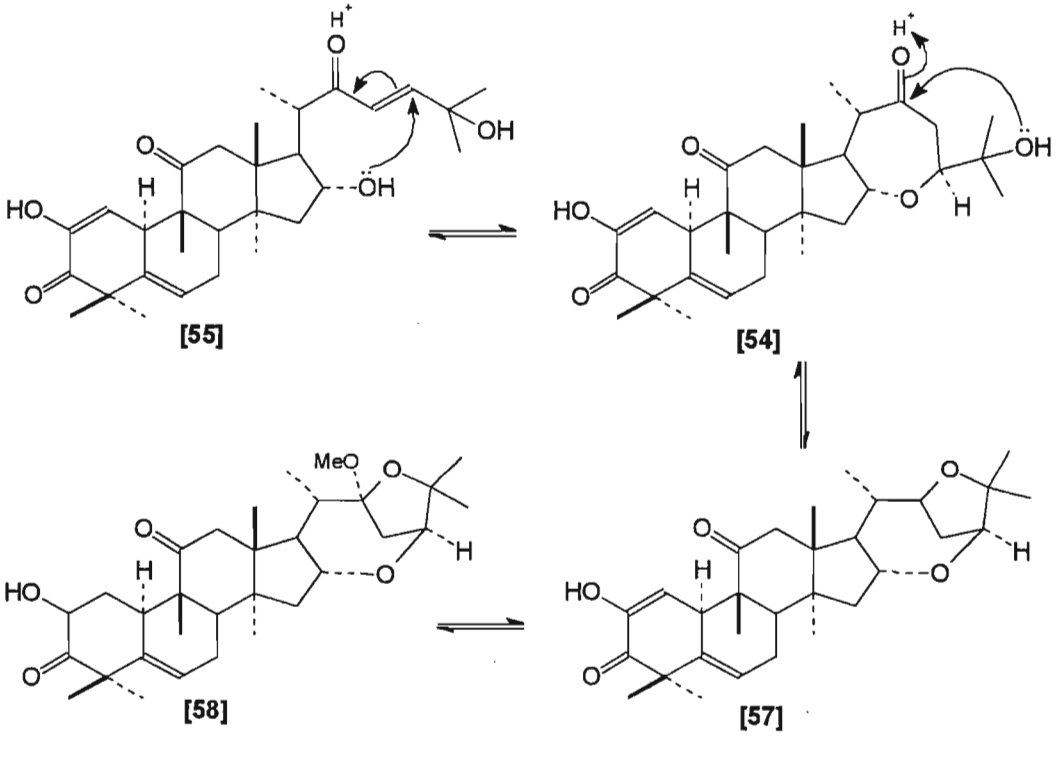

However, the structure was later revised to **[54]**[59].




Cucurbitacin S

**[54]**

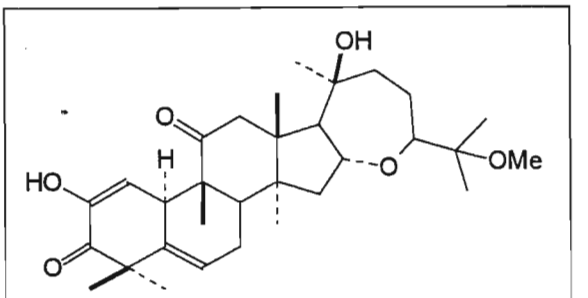
It was suggested that cucurbitacin S could not have been a natural compound[59]. A plausible hypothetical natural compound could be **[55]**, which may serve as a parent for cucurbitacin S by cyclization and acetal formation at C-22 as shown in Scheme 9.




**Scheme 9.** Suggested preparation of Cucurbitacin S and other artefacts

## 1.2.12. Cucurbitacin T

This compound **[59]** was isolated from fruits of *Colocynthis vulgaris* Schrad.[60] together with cucurbitacin I **[40]** and J **[41]**.



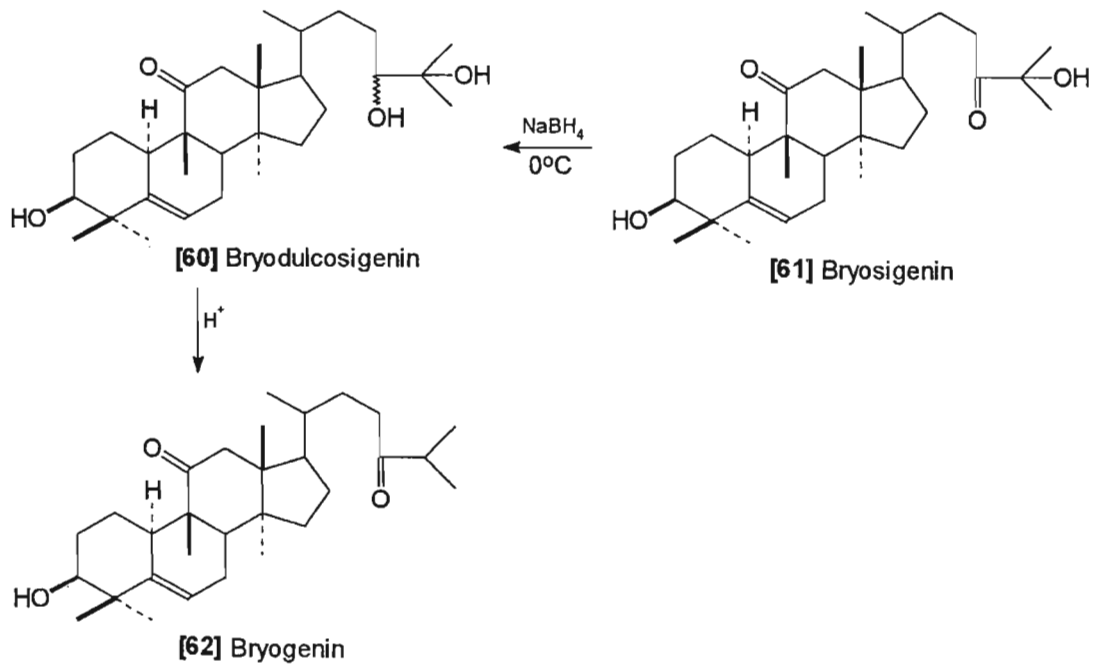

**[59]** Cucurbitacin T

It was assumed that [59] was an artefact formed from **[40]** during acid treatment of the methanolic extract. However, the artefactual origin of **[59]** was ruled out as the treatment of **[40]** with 1M sulphuric acid in methanol did not afford even trace amounts of **[59]**[60].

## 1.2.13. Bryodulcosigenin, bryosigenin, bryogenin

These compounds exist in nature as glycosides and have been grouped together according to their substitution pattern. They all possess an axial hydroxy group at position 3 and lack the C-16 hydroxyl group. The eight carbon atom side chain is at a lower oxidation level than in other cucurbitacins.

Bryodulcosigenin **[60]** has been isolated from the roots of *Bryonia dioica* Jack. following enzymatic hydrolysis of the glycoside bryodulcoside[61,62]. Bryosigenin **[61]** has been obtained as a minor component from the above mixture. The relationship between **[60]** and **[61]** was established by converting the latter to the former using sodium borohydride at ice bath temperature for selective reduction of the 24 - ketone (Scheme 10).



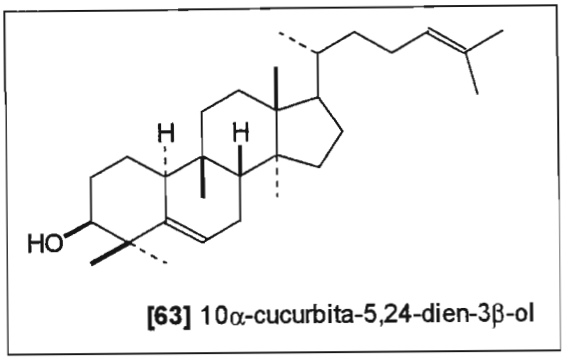

**Scheme 10.** Relationship between cucurbitacins from *Bryonia dioica* Jack.

Hydrolysis of **[60]** yielded bryogenin **[62]** suggesting that **[62]** does not occur in nature but is the product of the dehydration of the secondary-tertiary glycol system present in the side chain of **[60]**[63].

# 1.3. Biosynthesis of cucurbitacins

## 1.3.1. Introduction

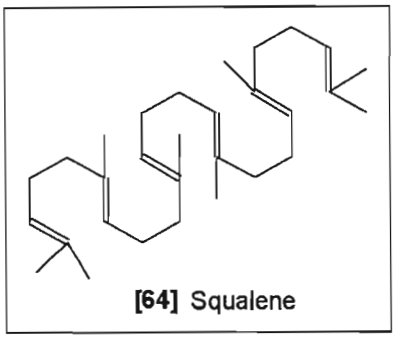
It has been shown that 10α-cucurbita-5,24-dien-3β-ol **[63]** represents the parent compound of cucurbitacins[64].




**[63]** 10α-cucurbita-5,24-dien-3β-ol

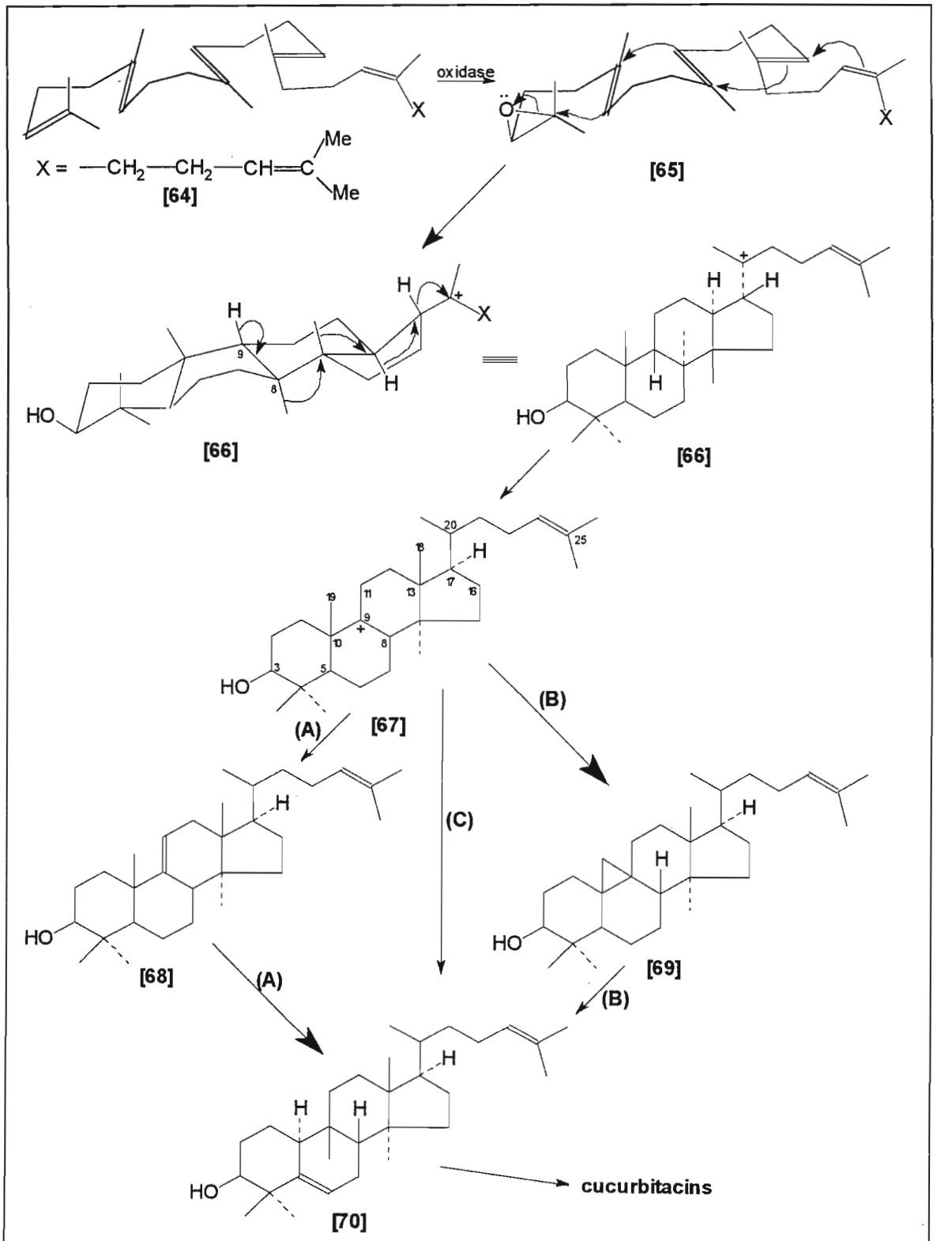
## 1.3.2. Cyclization of squalene-2,3-epoxide to [63]

Triterpenoids were found to originate biogenetically from the 30 atom compound squalene **[64]**, a tail -to- tail condensate of farnesol, which is a sesquiterpene alcohol.



**[64]** Squalene

By obtaining labelled 10α-cucurbita-5,24-dien-3β-ol from [3-$^{3}$H] squalene-2,3-epoxide incubated with microsomes of *Cucurbita maxima* Duch. ex Lam. seedlings, Balliano and co-workers[65] were able to propose a route for the biosynthesis of cucurbitacins (Scheme 11).




**Scheme 11.** Proposed route for the biosynthesis of cucurbitacins

They concluded that squalene-2,3-epoxide **[65]**, folded in a chair-boat-chair-boat confirmation, cyclized to give the carbocation **[66]**. This is then stabilised by methyl and hydrogen migrations (H-17→H-20; H-13→H-17; Me-14→Me-13; Me-8→Me-14; H-9→H-8) yielding a lanostane C-9 carbonium ion **[67]**, which is bonded to the enzyme by a suitable nucleophilic group. Withdrawal of the enzyme could then allow the further migration of the C-10 methyl group to C-9, according to the rules of Wagner-Meerwein rearrangements. The subsequent hydrogen migration from C-5 to C-10 and elimination of the C-6 proton by a basic site of the enzyme will give 10α-cucurbita-5,24-dien-3β-ol **[70]** (route C), the parent compound of cucurbitacins.

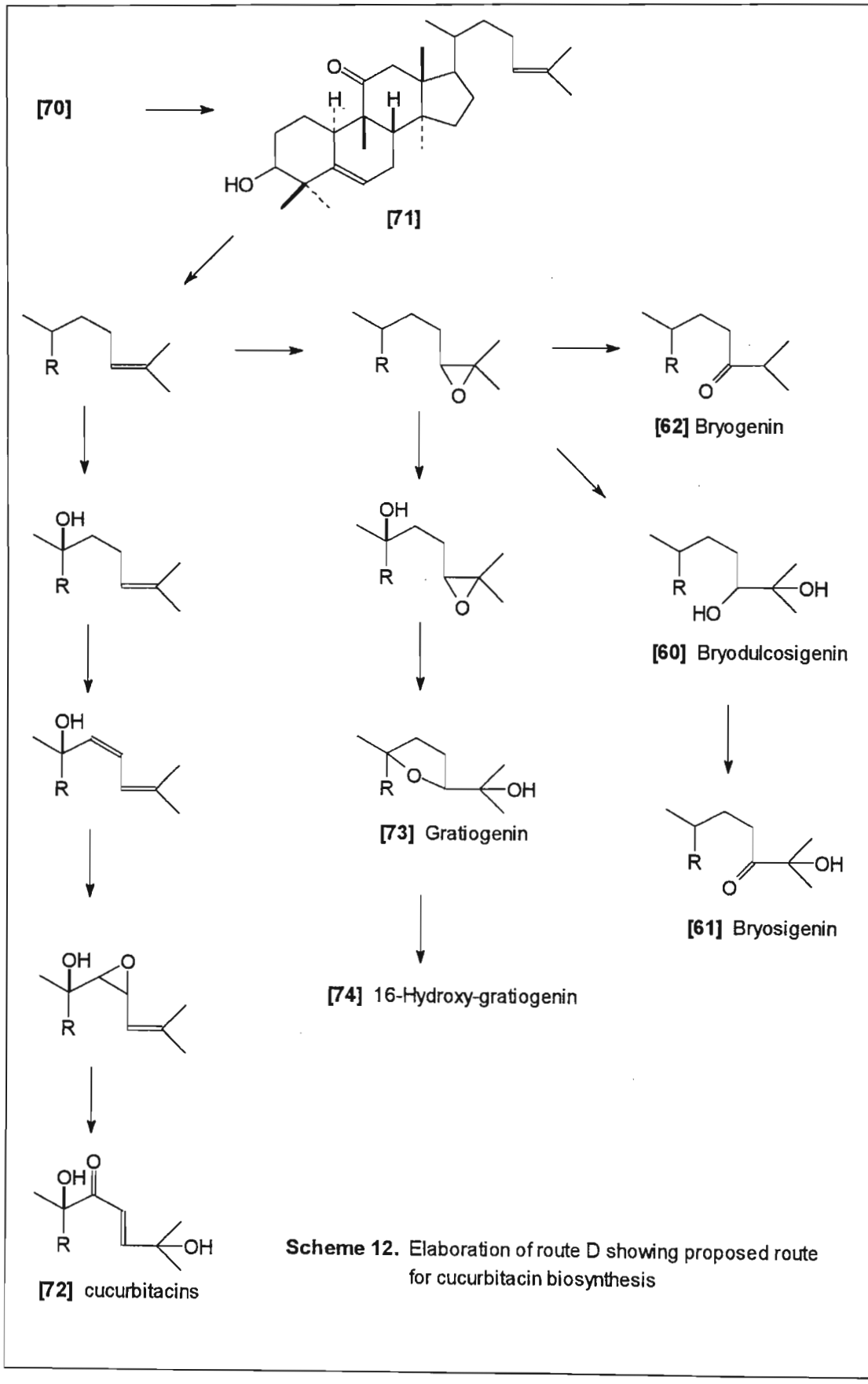
Alternatively, loss of the C-11 protons from **[67]** (route A) could give parkeol **[68]**. The migration of the C-10 methyl to C-9, induced by an electron deficiency at C-9, followed by hydrogen migration (H-5→H-10) and elimination of a C-6 proton, will give the cucurbitane skeleton **[70]**. Since parkeol was not found in *Bryonia dioica* Jack.[66], *Cucurbita maxima* Duch. ex Lam.[45] nor in *Cucumis sativis* L.[8], even in trace amounts, route A was considered to be hypothetical.

The next possibilty could include loss of a proton from the C-19 methyl group (route B) with closure of the 9β,19-cyclopropane ring to give cycloartenol **[69]**, the supposed general precursor of phytosterols[67]. Such an intermediate as **[69]**, by opening of the 9β, 19-cyclopropane ring in concert with hydrogen migration and proton elimination, could lead to **[70]**. However, the opening of the cyclopropane ring in **[69]** is inhibited

by the presence of the 4β-methyl group[32] thereby decreasing the possibility of route B.

The results obtained by Balliano et al. lead to the conclusion that the biosynthesis of cucurbitane triterpenoids from squalene-2,3-epoxide occurs through direct cyclization by an enzyme very similar to squalene-2,3-epoxide-cycloartenol synthetase without the intermediacy of lanostane-type triterpenoids such as cycloartenol **[69]** or parkeol **[68]**.

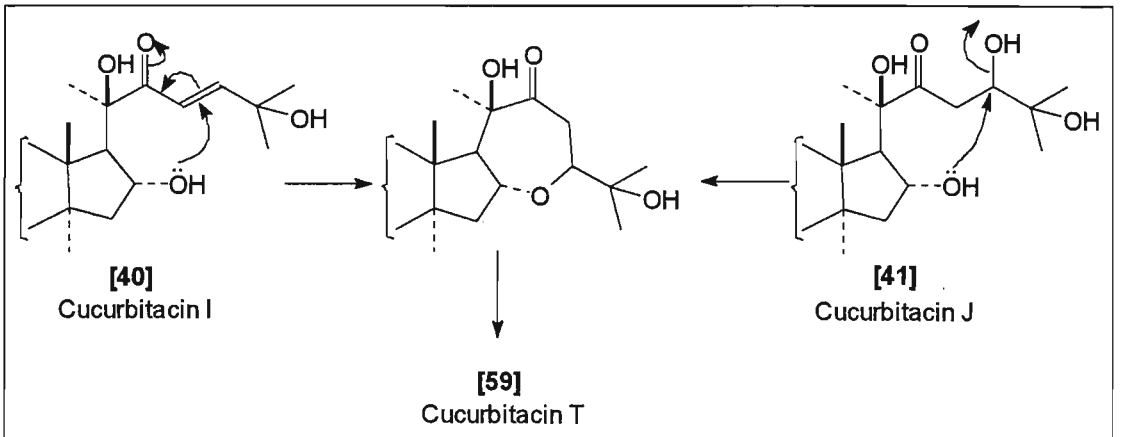
An elaboration of route D can be seen in Scheme 12. The side chain of **[71]** is transformed to produce different end products.




**Scheme 12.** Elaboration of route D showing proposed route for cucurbitacin biosynthesis

## 1.3.3. Cucurbitacin T

Biosynthetically, cucurbitacin T **[59]** could originate from either cucurbitacin I **[40]** or J **[41]** as shown in Scheme 13. The co-occurrence of these three cucurbitacins in *Citrullus vulgaris* Schrad. is of biosynthetic significance[60].




**Scheme 13.** Biosynthesis of Cucurbitacin T

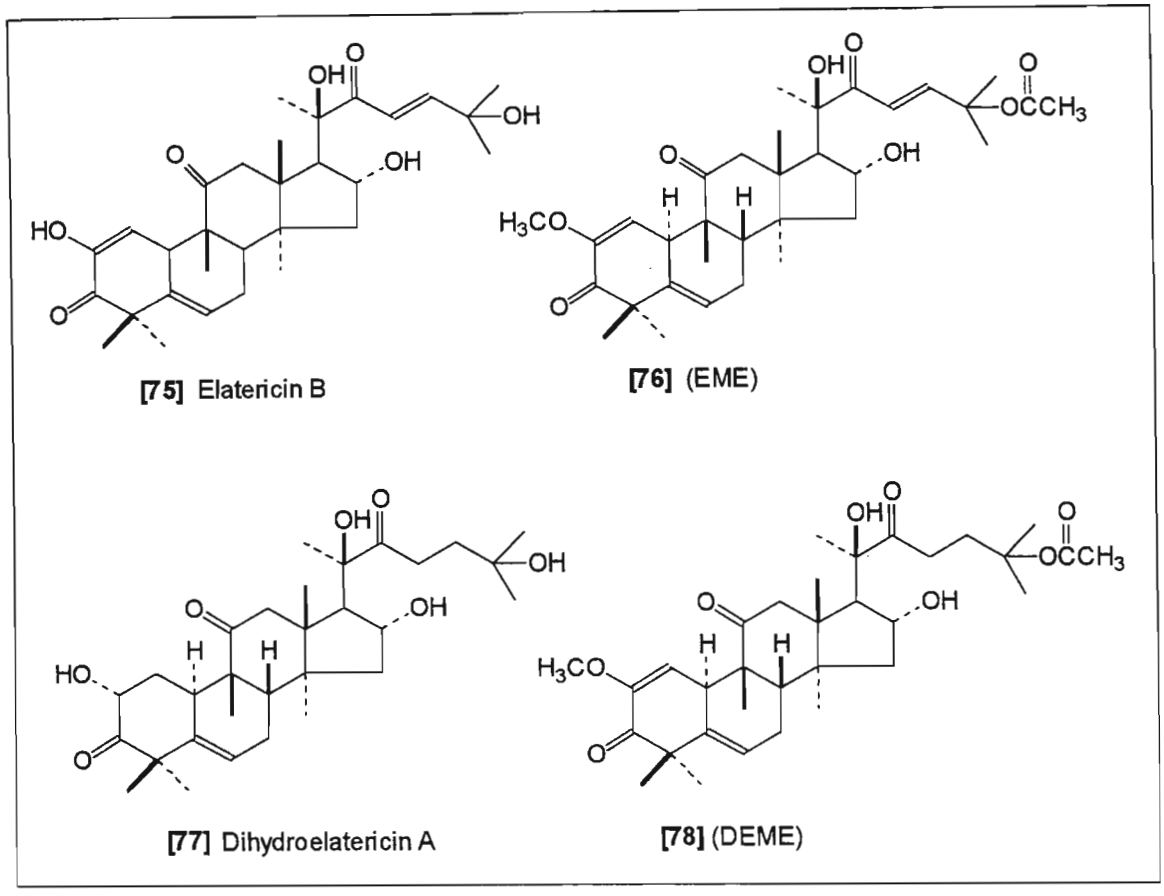
## 1.4. Biological activity of cucurbitacins

Cucurbitacins were reported to exhibit high cytotoxicity against tumor cells. Studies on the antitumor effect of cucurbitacins undertaken by Gitter and co workers[68] showed that elatericin A **[4]**, elatericin B**[75]** and elaterin **[2]** isolated from *Ecballium elaterium* L. and *Citrullus colocynthis* O. Ktze. produced a moderate inhibition of growth of Sarcoma 180, Sarcoma Black and Ehrlich ascites carcinoma. Sarcoma 180 proved to be the most sensitive tumor. These observations support the ethnomedical claims of these plants as a treatment for cancer.

Further studies undertaken by Gallily et al.[69] have shown that elaterin methyl ether (EME) **[76]** is the most active antitumor compound of the cucurbitacin derivatives studied. Dihydroelaterin A **[77]** and dihydroelaterin methyl ether (DEME) were the least effective.

It could therefore be concluded that the methylation of the enolic hydroxyl group of elaterin enhances its antitumor activity. The cytotoxic and antitumor activities are also dependent on the existence of a double bond in the side chain of these compounds since hydrogenation of the $\Delta^{23,24}$ double bond was accompanied by a significant decrease in cytotoxicity of the resultant dihydrocucurbitacin and DEME. Furthermore, acetylation of the C16α-OH group of cucurbitacin B produced fabacein[70] with marked

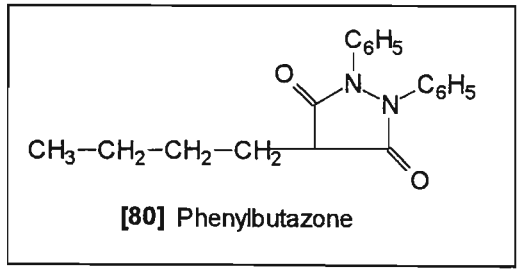
diminution of cytotoxicity, indicating that the α,β-unsaturated ketone system is activated by hydrogen bonding between the C16α-OH group and C-22 carbonyl group.




Shohat et al.[71] studied the combined effect of cucurbitacins and X-ray treatment on transplanted tumors in mice and found that the tumor growth-inhibitory effect of the combined treatments was more pronounced at all levels than that obtained by applying the corresponding doses of X-rays or cucurbitacins separately.

In 1981, Witkowski and Konopa[72] studied the mode of cytotoxic action of cucurbitacins and found that it involved their binding with glucocorticoid receptors of sensitive cells.

The alcoholic extracts of *Cucumis trigonus* Roxb. exhibited a marked analgesic effect when tested using pethidine hydrochloride as a standard drug[73]. In addition, the extract showed a significant anti-inflammatory activity against both exudative and proliferative manifestations of inflammation and the activity compared very favourably with the non-steroidal anti-inflammatory agent, phenylbutazone **[80]**.




**[80]** Phenylbutazone

Preliminary chemical tests of the extract revealed the presence of glycosides and tannins which may be reponsible for the actions reported.

Yesilada et al.[74] isolated cucurbitacin B **[9]** from the fruit juice of *Ecballium elaterium* L. and found this compound to have anti-inflammatory principles.

Studies are presently being undertaken to determine whether the seed extracts of different Cucurbitaceae plants invariably possess protein synthesis inhibiting (i.e. ribosome inactivating protein-like) activity[75]. The ribosome inactivating proteins have been gaining importance because of their interesting and potentially useful biological activities which comprise cytotoxic/antitumor[76], anti-human immunodeficiency virus[77], immunosuppressive, abortifacient, and protein synthesis inhibiting activities[78].

# Chapter 2. The Genus *Momordica*

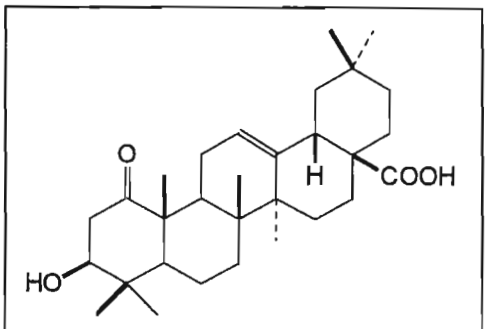
## 2.1. Introduction

*Momordica* L., a genus restricted naturally to the Old World, is widely distributed in Africa. Several species of the genus are thought to have been introduced to the New World with the slave trade from Africa to Brazil[53]. Bird dispersal of the seeds of wild or domesticated forms may account for the spread of the species within continents. The plants are perennial herbaceous climbers with simple to hairy stems. The flowers and fruit vary with different species[19]. Three species, viz. *Momordica cochininensis* Spreng., *Momordica charantia* L. and *Momordica foetida* Schum. & Sond. are widely used medicinally. The latter species is the least investigated.

### 2.1.1. *Momordica cochininensis*

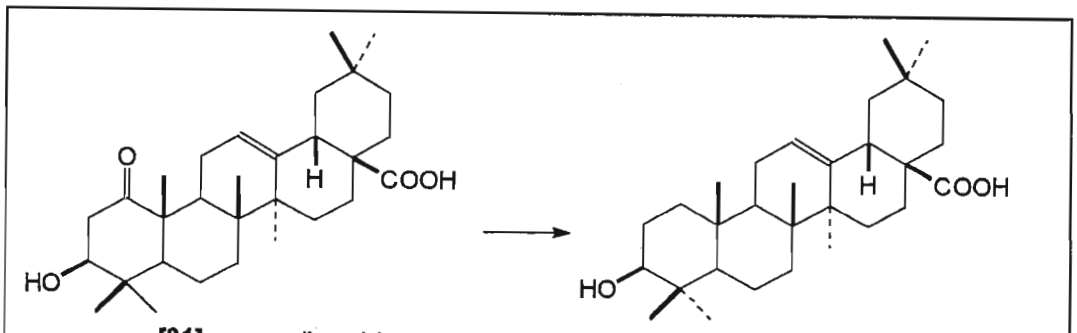
*Momordica cochinchinensis* Spreng. is a perennial climber occurring from China to the Moluccas and has been used in traditional medicines in East and Southern Asia[79]. The seeds are considered to have cooling properties and are used as a remedy for fluxes, liver and spleen disorders, hemorrhoids, bruises and swelling[80]. The Chinese call the crude drug *mubiesi* and the Japanese call it *mokubesi*[81]. The roots of this plant were also used therapeutically in Japan as an effective antitussive expectorant[82].

Investigations into the chemical constituents of this plant have revealed the presence of pentacyclic triterpenoids and their glycosides. Studies carried out by Murakani and colleagues (1966)[81] on the seed of this plant resulted in the isolation of momordic acid **[81]**, the first example of a triterpenic acid having a 1-oxo group in the oleanane series.




**[81]** Momordic acid

A Wolff-Kishner reduction (Scheme 14) converted momordic acid into a compound **[82]**, $C_{30}H_{48}O_3$, which was subsequently identified as oleanolic acid. This confirmed that **[81]** was the 1-oxo-derivative of oleanolic acid.

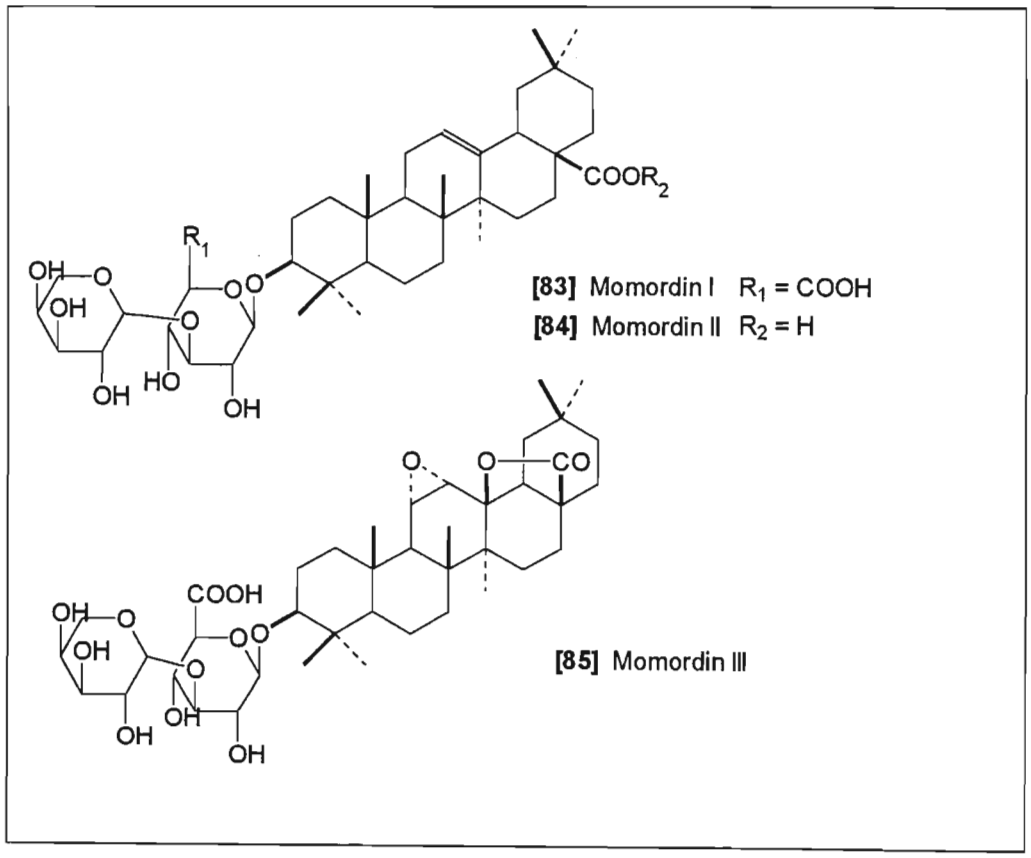



**[81]** momordic acid

**[82]** oleanolic acid

**Scheme 14.** Wolff - Kishner reduction of momordic acid

Further studies undertaken by Masayo et al. (1985)[80], resulted in the isolation and structure elucidation of three saponins named momordins I **[83]**, II **[84]**, III **[85]**. Their structures were determined on the basis of chemical and spectral evidence as oleanolic acid 3-O-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranoside **[83]**; 28-O-β-D-glucopyranoside of momordin I **[84]** and 3β-hydroxy-11α,12α-epoxy-olean-28,13-olide-3-O-α-L arabinopyranosyl (1→3)-β-D-glucurono-pyranoside **[85]**.



## 2.1.2. Biological activity

Ng and co-workers (1986)[83] reported the presence of saponins with hemolytic activity in the tubers of *Momordica cochininensis* Spreng.. This hemolytic activity was due to steryl glycosides. This hemolytic fraction contained proteins as judged by its high absorbance at 280 nm and by its SDS - polyacrylamide gel electrophoretic and immuno-electrophoretic profiles.

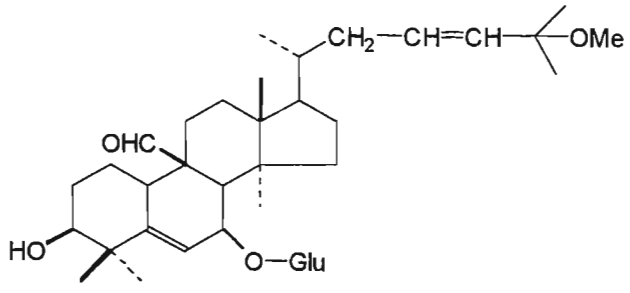
In the same year, Reza-ul-Jalil and associates[84] isolated two glycosides containing oleanolic acid as the aglycone. One glycoside, named MG-1, contained glucose and the other, MG-2, contained glucose and arabinose. Both these glycosides showed hypoglycaemic activities in streptozotocin induced diabetic rats at a dose of 25 mg/kg. MG-1 was found to be more effective than MG-2. This difference in potency could be attributed to their difference in polarity, the latter being more polar and hence less permeable through biological membrane. These glycosides however lowered haemoglobin content and produced focal necrosis in the liver.

In 1987 Yeung et al.[85] isolated an abortifacient protein from the root tubers of *Momordica cochininensis* Spreng. which they designated as momorcochin. This was found to be the least basic abortifacient protein.

## 2.2.1. *Momordica charantia*

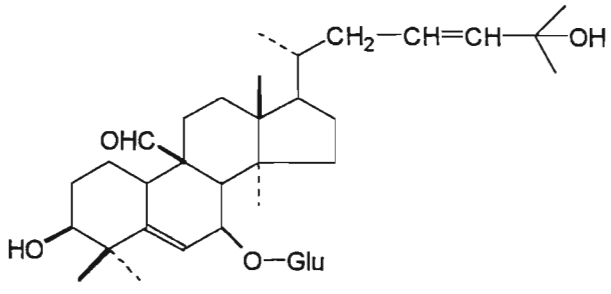
*Momordica charantia* L., the African cucumber or balsam pear[86], is widely distributed in West Africa, India and Japan. The leaves contain antibacterial and insecticidal principles[10]. In Japan, the plant is used in a remedy for skin ailments, headache and constipation[87] and as an antidiabetic agent[88].

A number of cucurbitane triterpenoids named momordicosides and momordicines have been isolated from the fruits and leaves of this plant. Momordicosides K **[86]** and L **[87]**, isolated as bitter principles from immature fruits of *M. charantia* L., have been characterised as unique cucurbitacins having a formyl group and a 7-O-β-D-glucopyranosyl moiety[89].




**[86]** Momordicoside K

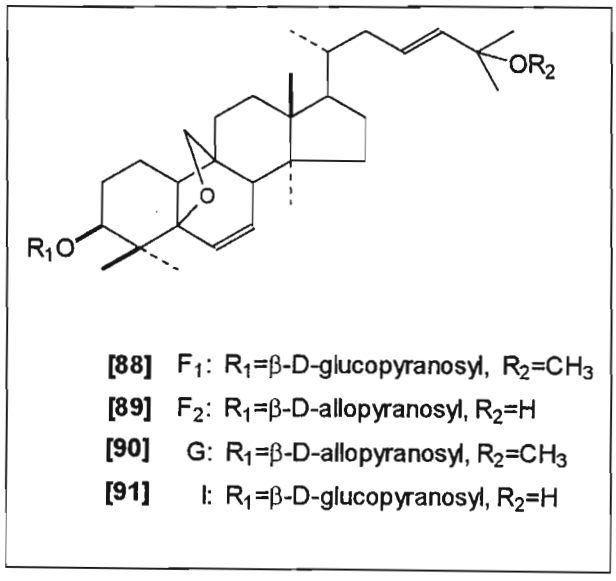
7-O-β-D-glucopyranoside of 3β,7β-dihydroxy-25-O-methyl-cucurbita-5,23-dien-19-al




**[87]** Momordicoside L

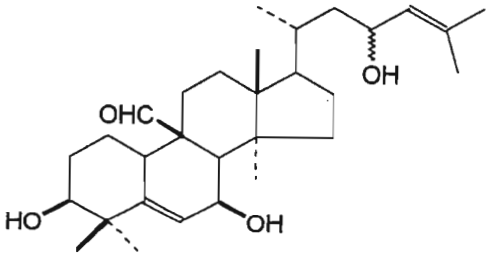
7-O-β-D-glucopyranoside of 3β,7β-25-trihydroxy-cucurbita-5,23-dien-19-al

In 1982, investigations undertaken by Okabe et al.[90] resulted in the isolation of four non-bitter cucurbitacins designated momordicosides $F_1$ **[88]**, $F_2$ **[89]**, G **[90]** and I **[91]**. These compounds were isolated in crystalline form from the methanol extract of fresh immature fruits.

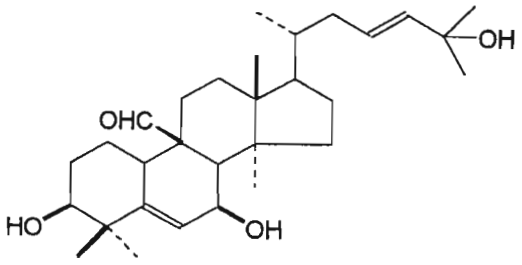



**[88]** $F_1$: $R_1$=β-D-glucopyranosyl, $R_2$=$CH_3$
**[89]** $F_2$: $R_1$=β-D-allopyranosyl, $R_2$=H
**[90]** G: $R_1$=β-D-allopyranosyl, $R_2$=$CH_3$
**[91]** I: $R_1$=β-D-glucopyranosyl, $R_2$=H

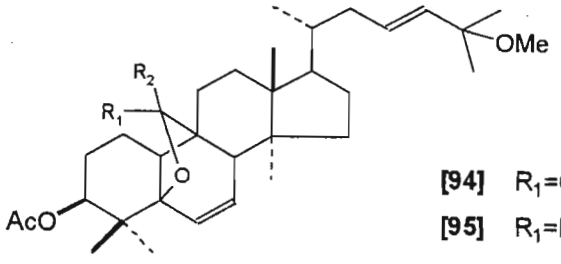
Continued investigations into the chemical constituents of this plant revealed the presence of compounds **[92]**, **[93]**, **[94]** and **[95]**[91].




**[92]**

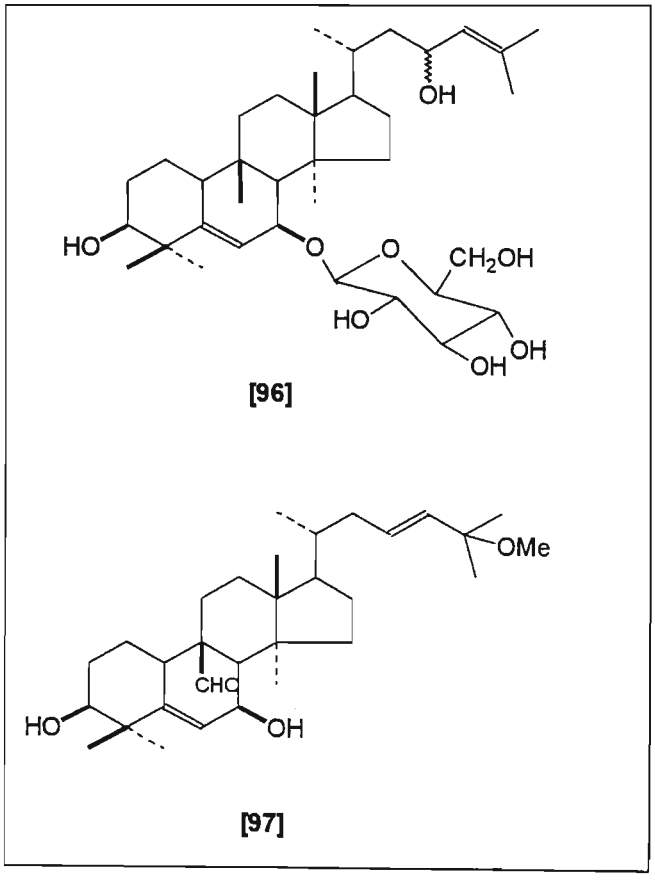



**[93]**




**[94]** $R_1$=OMe, $R_2$=H
**[95]** $R_1$=H, $R_2$=OMe

In 1990, studies carried out by Fatope et al.[87] resulted in the isolation of two new compounds **[96]** and **[97]** (cucurbitane triterpenoids) from the chloroform extract of the leaves. Compound **[93]** was also isolated and complete carbon peak assignments were given.



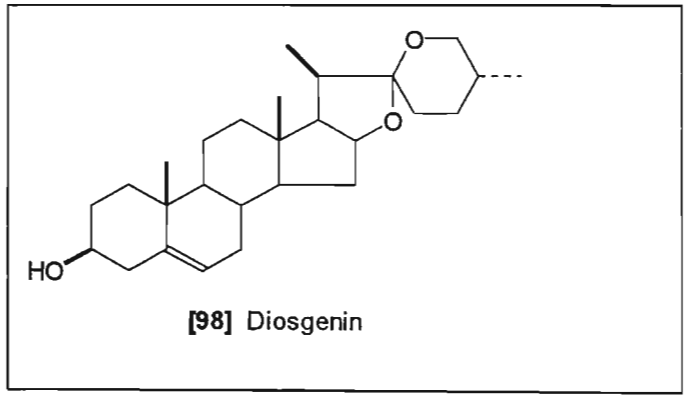

**[96]**

**[97]**

## 2.2.2. Biological activity

Studies by Lotlikar and Rao[88] on the pharmacology of the hypoglycaemic principle isolated from *Momordica charantia* L. led to the conclusion that charantin was not the only compound responsible for the hypoglycaemic activity present in the fruits of this species.

Clinical trials in patients with diabetes mellitus were carried out by Baldwa and co-workers[92] after extraction and purification of vegetable insulin. Although the onset of action was found to be similar to standard zinc crystalline insulin, the peak effects were significantly different. Khanna and Mohan[93] were able to extract an abortifacient factor present in this crude extract and one of the constituents isolated was diosgenin **[98]**.




**[98]** Diosgenin

A steryl glycoside fraction was isolated from the seeds by Ng and co-workers[94] and this was found to exhibit an inhibitory action on lipid metabolism *in vitro*.

## 2.3.1. *Momordica foetida*

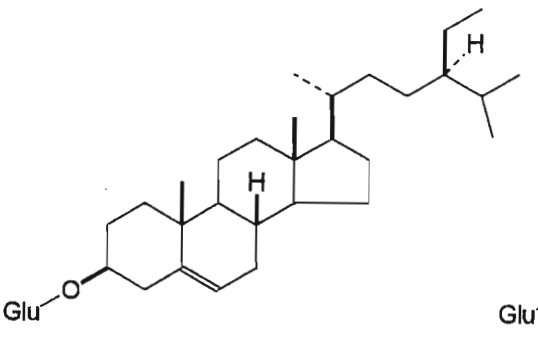
*Momordica foetida* Schum. & Sond., locally known by the Zulu people as *iNtshungu*, is a dioecious or occasionally monoecious perennial herbaceous climber with stout, smooth or sometimes hairy stems up to 5 metres long, marked with linear dark spots[19]. The leaves are simple, often deeply lobed, extripulate, alternate and frequently very hairy. The flowers are actinomorphic and unisexual and may be large and showy. The fruit is usually a fleshy berry and is edible[9].

This plant is found in Africa south of the Sahara. In southern Africa, it occurs in the Cape Province, KwaZulu-Natal, Transvaal and Swaziland.

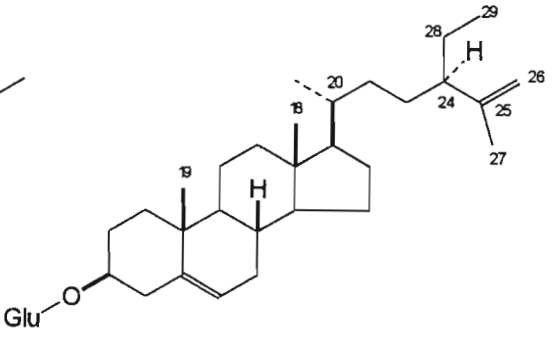
The Zulus drink a decoction of the root or leaf for the treatment of boils[10]. According to Bryant[10], the Zulu people take an infusion of the runner as a sedative for irritable stomach. The leaf is a Chagga earache remedy[95] and in tropical Africa is used for roundworm[96]. The Ganda of Uganda take an infusion of the leaf and root as an abortifacient and ecbolic[97]. In Tanzania, the fruit pulp is regarded as poisonous to weevils, moths and ants and is used as a repellant[98]. This plant is used in KwaZulu-Natal as a remedy for hypertension and is also recognised for its antidiabetic properties[99].

This plant is said to contain alkaloids although no substantiative proof has been presented[100]. Previous studies on this plant resulted in the isolation and identifiaction of 3 compounds, viz. β-sitosterol glucoside **[99]**; 5,25-stigmastadiene-3β-ol glucoside **[100]** and 1β-hydroxyfriedel-6(7)-ene-3-one **[101]**[101,102].

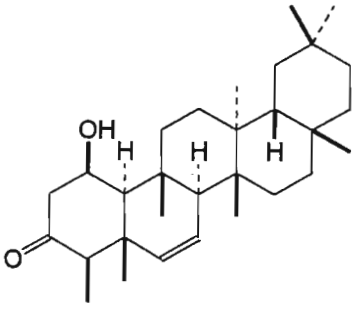
Compounds **[99]** and **[100]** were found to co-exist as a chromatographically homogenous product named foetidin[101]. Compound **[101]** appeared to be the first member of the naturally occuring friedelane series having a double bond at position 6.




**[99]** β-sitosterol glucoside

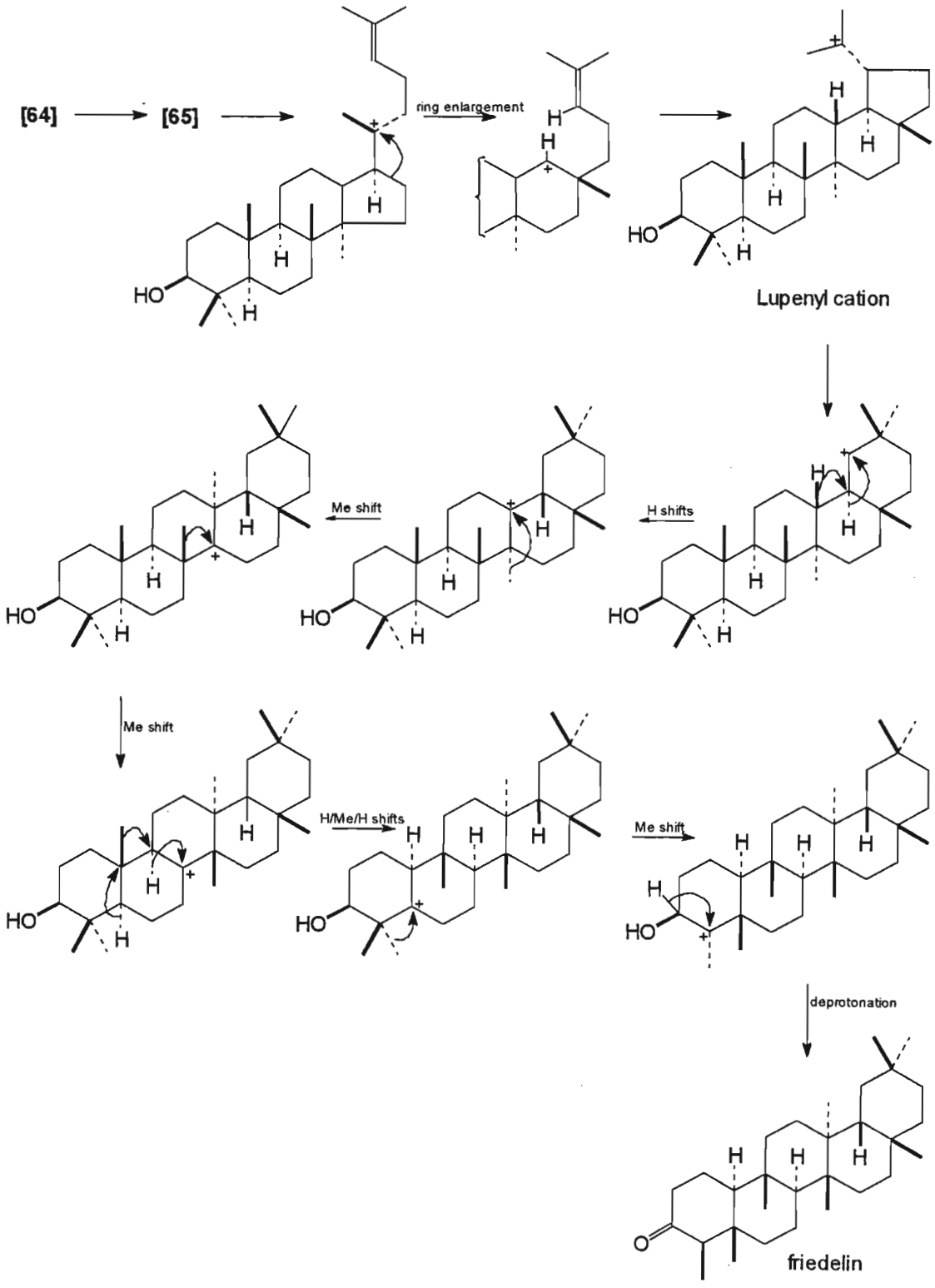



**[100]**5,25-stigmastadien-3β-ol glucoside




**[101]** 1β-hydroxyfriedel-6(7)-en-3-one

The friedelane nucleus could have formed by a series of backbone rearrangements as illustrated in Scheme 14.




**Scheme 14.** Backbone rearrangement of pentacyclic triterpenoids showing the formation of the friedelin nucleus

## 2.3.2. Biological activity

Marquis and co-workers[103] studied the effect of foetidin on blood glucose level of albino rats. It was found that foetidin had no significant antidiabetic property when administered to alloxan treated rats but it was capable of reducing the blood sugar level in fasting rats. These results did not seem very promising.

# CHAPTER 3. Results and Discussion

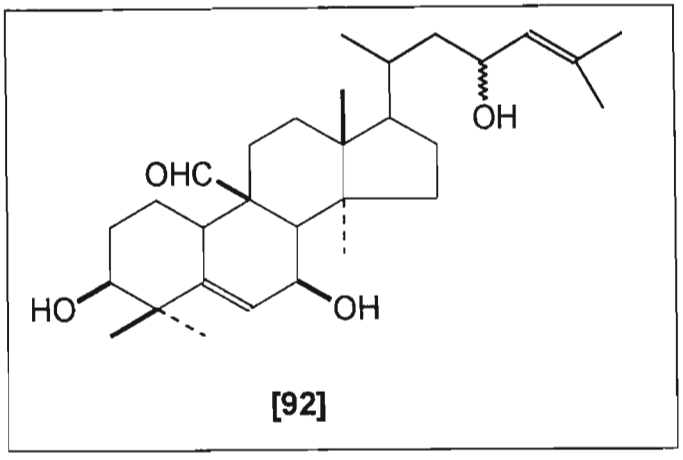
*Momordica foetida* Schum. & Sond. which is grown widely in Durban and the surrounding areas was readily obtained. Fresh plant material was purchased at the Umlazi herbal market and the leaves were separated from the vine before being air dried. The dried leaves were powdered and soxhlet extracted by refluxing with hexane, chloroform and methanol successively. The chloroform extract of the leaves was chosen for investigation. Further partitioning of the chloroform extract with 1M NaOH resulted in two extracts (Experimental 4.7.), viz, extracts A and B.

Extract A yielded two compounds, **[92]** and **[93]** identified as cucurbitane triterpenoids which had been isolated previously from the related species, *Momordica charantia* L.

Extract B yielded five compounds labelled compound **[102]**, **[103]**, **[104]**, **[105]** and **[106]**. All of the five compounds were identified as novel compounds although the natural derivatives of compounds **[105]** and **[106]** have been isolated previously from *Momordica charantia* L.[90,91]. Compound **[105]** was isolated previously as the glycoside[90] while compound **[106]** as the acetate[91].

## 3.1. Extractives from extract A

### 3.1.1. Compound 1 [92]




[92]

High resolution mass spectroscopy indicated that the compound had a molar mass of 472.3551 $g.mol^{-1}$ (calculated 472.3552 $g.mol^{-1}$), consistent with a molecular formula $C_{30}H_{48}O_4$. The mass spectrum (spectrum 1a) displayed peaks at *m/z* 454 ($M^+$-$H_2O$) and 436 ($M^+$-$2H_2O$), due to ready dehydration of the compound, showing that at least two hydroxyl groups were present, and at *m/z* 443 ($M^+$-CHO) due to α-cleavage of the aldehyde group. Application of the double bond equivalence formula indicated a double bond equivalence of seven. A positive Lieberman-Burchard test confirmed this compound to be a triterpenoid which was isolated as an amorphous powder.

The infrared spectrum (spectrum 1b) displayed a strong band at 3404 $cm^{-1}$ indicating the presence of a hydroxyl group. Differentiation between primary, secondary and tertiary alcohols is possible in many cases from the position of the C-O stretching bands. In this spectrum,

the strongest C-O band at 1084 $cm^{-1}$ suggested a secondary hydroxy group. The band at 1666 $cm^{-1}$ indicated the presence of unsaturation. The strong bands at 2947 $cm^{-1}$ and 2879 $cm^{-1}$ arose due to the presence of alkyl groups and the presence of methyl groups was shown by the complexity of bands between 1467 $cm^{-1}$ and 1379 $cm^{-1}$. The strong sharp band at 1709 $cm^{-1}$ indicated the presence of an aldehyde group. This was further confirmed by the positive results obtained from the Tollen's test, i.e. a silver mirror formed on the walls of the test tube due to oxidation of the aldehyde to a carboxylate salt by the silver ion complexed with ammonia (Tollen's reagent). The two weak bands between 2820 $cm^{-1}$ and 2740 $cm^{-1}$ generally ascribed to the C-H stretching vibrations of an aldehyde group were not visible due to the presence of strong bands due to alkyl groups in the same region of the spectrum.

The $^{13}C$ NMR spectrum (spectrum 1d) confirmed the presence of thirty carbon atoms. The multiplicity assignments which where made from the DEPT spectrum (spectrum 1e) revealed the presence of seven quartets, seven triplets and ten doublets. The remaining six carbon resonances were assigned as singlets. The resonances at δ41.4, 49.9, 45.4 and δ47.6 indicated four quaternary carbons while the resonances at δ65.9, 76.1 and δ66.2 were due to three oxygen-bearing carbons. This was confirmed in the $^{1}H$ NMR spectrum (spectrum 1c) by resonances at δ4.44 (1H, bt, $J_{23,24}$=8.65Hz, H-23); δ 3.95 (1H, bs, $W_{1/2}$=8.00Hz, H-7α) and δ3.54 (1H, bs, $W_{1/2}$=7.61Hz, H-3α) respectively. The width at half peak heights for H-7 and H-3 were 8.00Hz and 7.61Hz respectively, indicating α orientation in each case, hence the hydroxy groups had to

be β-substituted. The molecular model indicated the 3β-OH to be axial when ring A assumed the chair conformation.

The $^{1}H$ NMR spectrum also showed the presence of a one-proton singlet at δ9.69 ascribable to an aldehyde proton. This was supported by the carbon resonance at δ207.8 (d). The nature of the carbon skeleton was revealed by the presence of four tertiary methyl group proton resonances at δ1.22, 1.03, 0.89 and δ0.73 (each 3H, s) and one secondary methyl group proton resonance at δ0.96 (3H, d) while the downfield shift of a further two methyl groups (δ1.66 and δ1.68, each 3H, s) suggested that these were due to vinylic methyl group protons. The presence of two one-proton doublets at δ5.87 and δ5.17 together with resonances at δ145.5 (s), 123.9 (d), 129.9 (d) and δ133.9 (s) in the $^{13}C$-NMR spectrum revealed the presence of two double bonds. The 2D $^{1}H$-$^{1}H$ COSY spectrum (spectrum f) revealed that these proton resonances were not coupled indicating that both the double bonds were tri-substituted.

The COSY spectrum (spectrum f) also revealed that the aldehyde proton was not coupled. This meant that the aldehyde group was attached to a quaternary carbon. The biosynthetic pathway of cucurbitacins suggested that C-9 was the most likely quaternary carbon to which this functional group could be attached. The orientation of the aldehyde group was considered to be β on the basis of cucurbitacin biosynthesis.

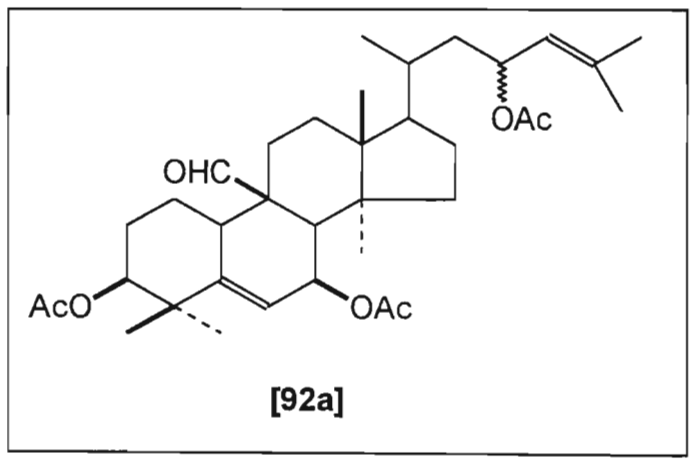
The expanded COSY spectrum (spectrum g) revealed coupling between a pair of one-proton doublets at δ5.87 and a broad singlet resonating at δ3.95. These peaks were assigned to H-6 and H-7α respectively. The

H-6 signal was also long range coupled to a resonance at δ2.52 ascribable to H-10 due to long range coupling to H-6 across the 5,6 double bond. The H-6 signal was split into a doublet by H-7 and thereafter further split by H-10 ($J_{6,7}$=5.13Hz, $J_{6,10}$=1.92Hz) resulting in a double doublet. The H-10 signal resonated as a multiplet due to splitting induced by H-6 as well as the two protons attached to C-1. From the 2D $^{1}H$-$^{13}C$ HETCOR spectrum (spectrum h), a doublet at δ36.6 could be assigned to C-10.

The COSY spectrum also confirmed coupling between H-24 (δ5.17, $J_{23,24}$=8.65Hz) and H-23 (δ4.44, $J_{23,24}$=8.65Hz). The H-24 doublet was also seen to be long-range coupled to the two vinylic methyl groups at δ1.68 and δ1.66 respectively. The two methyl group proton resonances were assigned 3H-26 and 3H-27 respectively and correlated with resonances at δ18.1 (q) and 25.7 (q) in the HETCOR spectrum. H-23 was coupled to a multiplet at δ1.61 assigned to H-22 and this signal correlated with a carbon signal at δ44.4 (t).

The doublets at δ128.9 (d) and 123.9 (d) were assigned to C-6 and C-24 by means of the HETCOR spectrum. The three doublets in the C-O region at δ76.1, 66.2 and δ65.9 correlated with proton resonances at δ3.54, 3.95 and δ4.44 which were ascribed to H-3α, H-7α and H-23 respectively. The HETCOR spectrum also revealed that the secondary methyl group proton resonance at δ0.96 (d) corresponded to a carbon resonance at δ18.8 (q). The signals between δ2.30 and δ2.24 occurred as a result of hydroxy protons since after the addition of $D_2O$ to the sample (spectrum i), these signals disappeared.

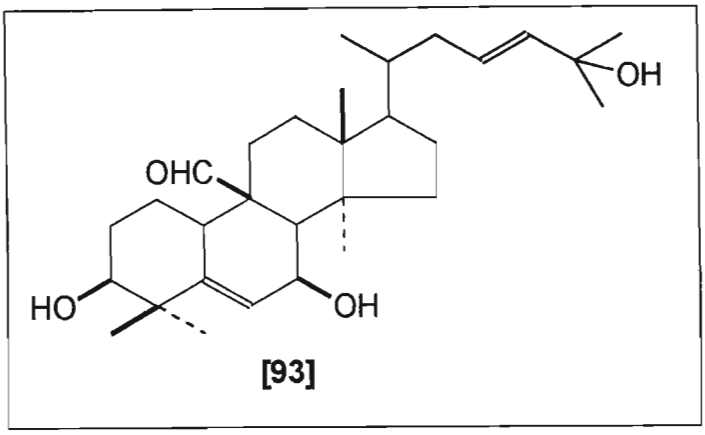
Acetylation of compound 1 **[92]** yielded a triacetate **[92a]** (spectrum j). This was revealed in the $^1$H NMR spectrum by the presence of three, three-proton singlets at δ1.99, δ2.00 and δ2.01 ascribable to the three proton resonances of each of the acetate methyl groups. The peaks ascribed to H-23, H-7α and H-3α were shifted downfield to δ5.55, 5.17 and 4.79 respectively on acetylation. This downfield shift occurred due to deshielding of the protons induced by the acetate carbonyl group. Acetylation of this compound confirmed the presence of three secondary hydroxy groups and the fact that H-23, H-7 and H-3 shifted downfield confirmed that the placement of the hydroxy groups in these positions was correct.




**[92a]**

On the basis of all the spectral evidence obtained and the similarities between our data to that published in literature[91], compound 1 was assigned structure **[92]**.

### 3.1.2. Compound 2 [93]




[93]

High resolution mass spectrometric analysis (spectrum 2a) of this compound showed that the the $M^+$ peak occurred at *m/z* 472.3552 (calculated 472.3552), correct for the formula $C_{30}H_{48}O_4$ as in compound 1. The mass spectrum also displayed peaks at *m/z* 454 ($M^+$-$H_2O$), 436 ($M^+$-$2H_2O$) due to ready dehydration of the compound showing that at least two hydroxyl groups were present and at *m/z* 443 due to α-cleavage of an aldehyde functional group. The molecular formula indicated a double bond equivalence of seven. A positive Liebermann-Burchard test suggested that the compound was a triterpenoid.

The infrared spectrum (spectrum 2b) displayed strong sharp bands at 3431 $cm^{-1}$ and 3273 $cm^{-1}$ due to OH stretching vibrations. This was further confirmed by C-O stretching vibrations at 1134 $cm^{-1}$ and 1089 $cm^{-1}$ suggesting that at least one tertiary and one secondary hydroxyl group was present. The band at 3067 $cm^{-1}$ indicated the presence of unsaturation. The strong bands at 2955 $cm^{-1}$ and 2877 $cm^{-1}$ arose due to the presence of alkyl groups and the presence of methyl groups was shown by the complexity of bands between 1465 $cm^{-1}$ and 1375 $cm^{-1}$.

The sharp band at 1701 $cm^{-1}$ was due to the carbonyl stretching vibrations of an aldehyde group. The presence of an aldehyde was further confirmed by the positive results obtained from the Tollen's test. The two weak bands between 2820 $cm^{-1}$ and 2740 $cm^{-1}$ generally ascribed to the C-H stretching vibrations of an aldehyde were not visible due to the presence of strong bands due to alkyl groups in the same region of the spectrum.

The $^{1}H$ NMR spectrum of this compound (spectrum 2c) was similar to that of compound 1 except for the absence of the H-23 triplet at δ4.44 and lack of the downfield shifted methyl groups indicating that no vinylic methyl groups were present.

The $^{13}C$ NMR spectrum (spectrum 2d) confirmed the presence of 30 carbon atoms. The DEPT spectrum (spectrum 2e) revealed seven quartets, seven triplets and ten doublets. The remaining six carbon resonances were assigned as singlets. The resonances at δ145.5 (s), 139.6 (d), 125.1 (d) and δ123.9 (d) indicated that there were two alkene double bonds in the molecule, one of which was trisubstituted and the other disubstituted. The resonances at δ76.1 (d), 66.3 (d) and δ70.7 (s) occurred due to the presence of two secondary and one tertiary carbinyl group respectively.

The $^{1}H$ NMR spectrum showed a resonance at δ9.61 (1H, s) indicating the presence of an aldehyde group which was further confirmed by a doublet at δ207.8 in the $^{13}C$ NMR spectrum. This aldehyde functional group was attached to C-9 with β orientation for similar reasons as

discussed for compound 1. The $^1$H NMR spectrum also revealed the presence of seven methyl groups at δ0.73, 0.87, 1.03, 1.22 (3H, s, 4x$CH_3$), 0.89 (3H, d, $J$=6.0Hz) and δ1.29 (6H, s, 2x$CH_3$). The spectrum also displayed a resonance at δ5.87 (1H, dd, $J_{6,7}$=5.13Hz, $J_{6,10}$=1.92Hz) ascribable to H-6 and was coupled to H-7α (1H, δ3.95, bs, $W_{1/2}$=8.00Hz) and H-10 (1H, δ2.52, m) as deduced from the 2D $^1$H-$^1$H COSY spectrum (spectrum 2f). The resonance at δ5.56 (m) integrated to two protons and correlated with carbon signals at δ125.1 (d) and δ139.6 (d) in the HETCOR spectrum (spectrum 2g). These signals were ascribed to C-23 and C-24 respectively. Thus the proton signals for H-23 and H-24 were superimposed at δ5.56.

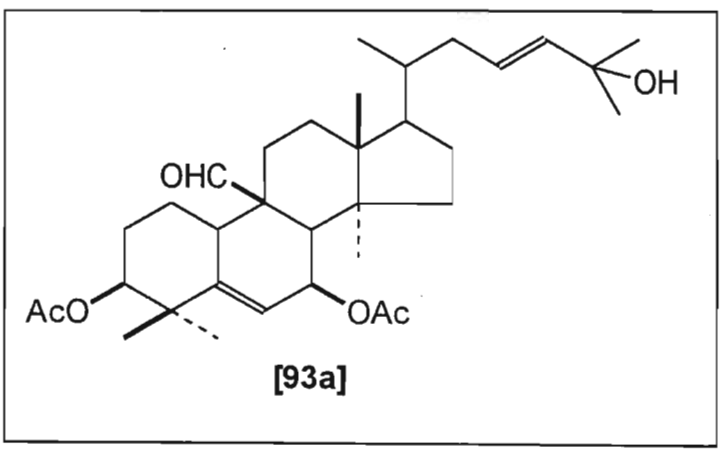
In the COSY spectrum, coupling was also observed between the resonance at δ5.56 and a multiplet at δ1.69. This multiplet was assigned to 2H-22, the corresponding C-22 signal was seen to occur at δ39.0 (t) in the HETCOR spectrum. H-10 was also coupled to a multiplet signal at δ1.82 which correlated with a carbon signal at δ27.4 (t) ascribable to C-1.

The broad signal resonating at δ3.55 ($W_{1/2}$=8.25Hz) was ascribed to the equatorial H-3α. This resonance was coupled to a multiplet at δ1.71 which corresponded to a carbon signal at δ28.9 (t). This signal was assigned to C-2. The carbon signal at δ123.9 (d) correlated with the proton resonace at δ5.87 which had previously been assigned to H-6. The two doublets in the C-O region at δ76.1 and δ66.3 were ascribed to H-3α and H-7α respectively from the HETCOR spectrum.

The $^1H$ NMR spectrum displayed a sharp singlet at δ1.29 (6H) which correlated with carbon resonances at δ29.8 (q) and 29.9 (q). The six proton signal was ascribed to the 3H-26 and 3H-27 protons. Since the disubstituted double bond was placed between C-23 and C-24, C-25 had to have either a hydrogen or an oxygen atom present. As the 3H-26 and 3H-27 resonances appeared as a singlet, C-25 had to have an oxygen atom attached to it. This possibility was further supported by the carbon resonance at δ70.7 (s) leading to the conclusion that a tertiary hydroxy group was at C-25. The signal at δ2.38 was due to a hydroxy proton since addition of $D_2O$ to the sample resulted in the disappearance of this signal (spectrum 2h).

This compound was identified as 3β,7β,25-trihydroxycucurbita-5,23-diene-19-al which was previously isolated from the leaves of *Momordica charantia* L. by Fatope et al.[87] The δ values for the carbon signals were not identical since their NMR spectra were obtained in deuteropyridine whereas our spectra were obtained in deuterochloroform. The rest of the peak assignments are listed in tables 3 and 5 and are in good agreement with those of compound 1 **[92]**.

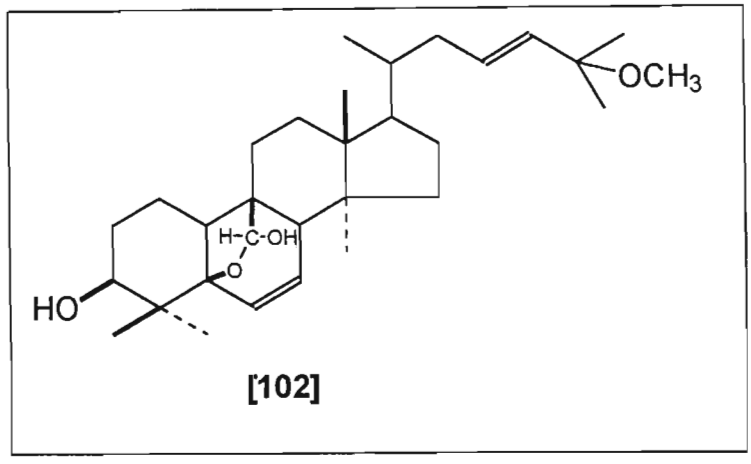
Acetylation of this compound with acetic anhydride/pyridine resulted in the formation of the diacetate **[93a]** (spectrum 2i) as seen by the two sharp resonances at δ2.01 and δ2.00 due to acetate methyl group protons, thus confirming the presence of only two secondary hydroxy groups. The H-7α and H-3α resonances were shifted downfield to δ5.19 and 4.79 respectively due to deshielding induced by the acetate carbonyl groups.




**[93a]**

## 3.2. Extractives from extract B

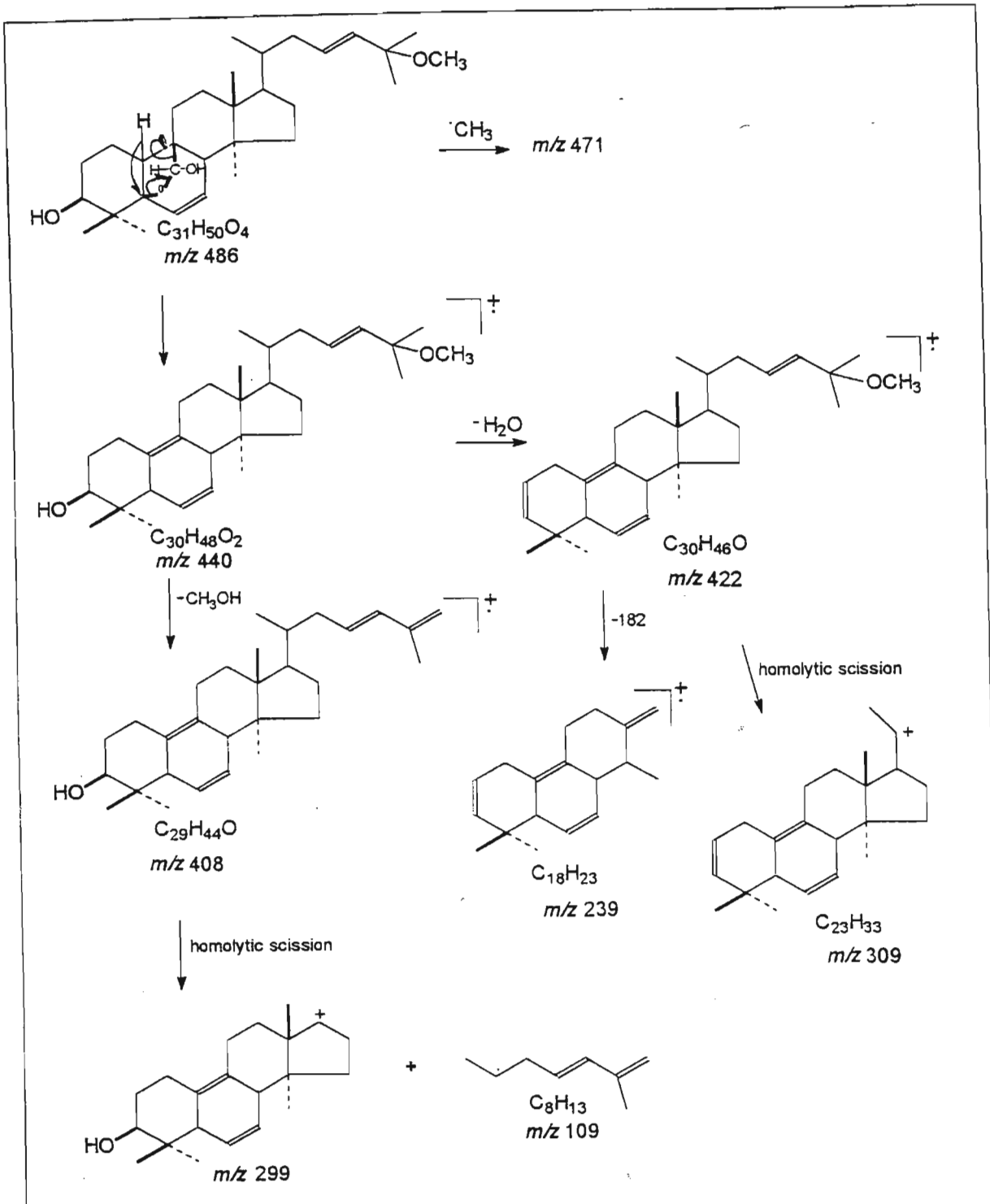
### 3.2.1. Compound 3 [102]




[102]

This compound was obtained in larger quantities than the other four compounds from this extract. Hence it was decided to discuss the structure elucidation of this compound first with respect to extract B since frequent references are made to this compound during the structure elucidation of the other compounds.

High resolution mass spectroscopy indicated the compound to have a molar mass of 486.3725 $g.mol^{-1}$ (calculated 486.3709 $g.mol^{-1}$), correct for the molecular formula $C_{31}H_{50}O_4$. The mass spectrum (spectrum 3a) displayed peaks at *m/z* 471 ($M^+$-$CH_3$), 440 ($M^+$-$CO_2H_2$), 422 ($M^+$-$CO_2H_2$-$H_2O$) and 408 ($M^+$-$CH_3OH$). A proposed fragmentation scheme for the molecule is illustrated in Scheme 15. All proposed structures were supported by high resolution mass measurements.




**Scheme 15. Proposed fragmentation of Compound 3**
**All structures were supported by high resolution mass measurements**

The infrared sprectrum (spectrum 3b) displayed a broad band at 3292 $cm^{-1}$ indicating the presence of a hydroxy group. The band at 3024 $cm^{-1}$ indicated the presence of unsaturation and this was confirmed by the band at 1649 $cm^{-1}$. The bands between 2949 $cm^{-1}$ and 2877 $cm^{-1}$ were due to alkyl groups and the presence of methyl groups was shown by the sharp band at 1375 $cm^{-1}$. The band at 1080 $cm^{-1}$ indicated that a secondary hydroxy group was present while the peak at 1118 $cm^{-1}$ due to the asymmetric C-O-C stretching vibration suggested that an ether linkage was present.

The $^{13}C$ NMR spectrum (spectrum 3d) displayed strong resonances for 31 carbons although other minor signals suggested the presence of an impurity. Multiplicity assignments made from the DEPT spectrum (spectrum 3e) indicated eight quartets, seven triplets and ten doublets. The remaining six carbon resonances were assigned as singlets. The four resonances at δ136.8 (d), 132.7 (d), 132.4 (d) and δ128.3 (d) were due to olefinic carbons and indicated the presence of two disubstituted double bonds. Unlike compounds 1 and 2, this compound lacked the $\Delta^{5,6}$ double bond. The resonances at δ48.5 (s), 48.0 (s), 45.1 (s) and δ37.2 (s) were due to quaternary carbon atoms and their peak assignments were made with comparison to those of compounds 1 and 2. The resonances δ105.4 (d), 86.6 (s), 76.1 (d), 74.9 (s) and δ50.3 (q) were due to oxygen-bearing carbons.

The $^{1}H$ NMR spectrum (spectrum 3c) displayed a resonance at δ6.06 (1H, dd) and this was seen to be coupled in the COSY spectrum (spectrum 3f) to a double doublet resonating at δ5.65 (1H, dd). These

resonances correlated with carbon resonances at δ132.7 (d) and 132.4 (d) in the HETCOR spectrum (spectrum 3g). The resonances at δ6.06 and 5.65 were ascribed to H-6 and H-7 respectively based on comparison with chemical shifts of **[88]** and **[90]**[90] and the first double bond was placed between C-6 and C-7. H-6 was split into a doublet by H-7 ($J_{6,7}$=9.76Hz) followed by further splitting by H-8 ($J_{6,8}$=2.30Hz) due to long-range coupling across the $\Delta^{6,7}$ double bond . This was confirmed by the COSY spectrum where H-6 was seen to be long-range coupled to a broad singlet at δ2.81 ascribable to H-8. This resonance at δ2.81 correlated with a carbon resonance at δ41.4 (d) in the HETCOR spectrum. The H-7 signal was also split by H-6 ($J_{6,7}$=9.76Hz) into a doublet followed by further splitting induced by H-8 ($J_{7,8}$=3.72Hz) as observed in the COSY spectrum, to yield a double doublet. The facts that both $^{13}$C NMR resonances (δ132.7 and δ132.4) were doublets and the long range coupling between H-6 and H-8 rules out the possibility of a biosynthetically anticipated 5,6-double bond.

The COSY spectrum also revealed coupling between a multiplet at δ5.42 and a singlet at δ5.40 with each signal integrating to one proton. These resonances were ascribed to H-23 and H-24 respectively and corresponded to carbon resonances at δ128.3 (d) and 136.6 (d) in the HETCOR spectrum. Hence the second double bond was placed between C-23 and C-24. The H-23 multiplet was also seen to be coupled to a broad multiplet at δ1.76 (2H) ascribable to 2H-22. This signal correlated with a carbon resonance at δ27.1 (t).

The $^1$H NMR spectrum also revealed the presence of a one-proton doublet at δ5.11 (1H, d, *J*=8.1Hz), the corresponding carbon resonance could be seen to occur at δ105.4 (d) in the HETCOR spectrum. This is the correct chemical shift for hemiacetal and acetal carbons[104] hence it was concluded that this peak was due to a carbon atom joined to two oxygen atoms. The COSY spectrum displayed coupling between the doublet at δ5.11 and a one-proton doublet at δ2.71. Upon addition of $D_2O$ to the sample, the resonance at δ2.71 disappeared and the doublet at δ5.11 collapsed into a singlet (spectrum h). This confirmed that the resonance at δ105.4 (d) was due to a hemiacetal carbon atom. On the basis of this observation, it was concluded that the doublet at δ2.71 was due to a hydroxy proton and the hemiacetal proton was coupled to this hydroxy proton thereby appearing as a doublet. This was further supported by the fact that the hydroxy proton resonance had no corresponding carbon resonance in the HETCOR spectrum.

The carbon resonance at δ76.1 (d) correlated with the one-proton multiplet signal at δ3.38 ascribable to H-3α. The H-3α signal appeared as a multiplet due to splitting induced by the H-2 axial and equatorial protons as well as the hydroxy proton. This was confirmed in the COSY spectrum by observing the H-3 proton coupled to a one-proton doublet signal at δ3.75 which collapsed upon addition of $D_2O$ (spectrum 3h). The H-3α signal was also coupled to a multiplet at δ1.65 ascribable to 2H-2. This signal correlated with a carbon resonance at δ23.1 (t). H-3 was assumed to be α orientated with a β-substituted hydroxy group as with compounds 1 and 2 due to the parent compound of cucurbitacins **[63]** having a β-substituted hydroxy group. The resonance at δ2.24 (1H,

t, $J$=9.19Hz) was observed to have a carbon resonance at δ40.6 (d) and was ascribable to H-10. The COSY spectrum also revealed coupling between H-10 and a multiplet signal at δ1.41 ascribable to 2H-1. This signal correlated with a carbon resonance at δ17.3 (t) in the HETCOR spectrum.

Hemiacetals are products of addition of alcohols to aldehydes and since compounds 1 and 2 each possess an aldehyde group attached to C-9, it was appropriate to attach the hemiacetal carbon to C-9 as well, and the ether linkage between C-19 and C-5. The C-19 signal resonated at δ105.4 (d) and C-5 at δ86.6 (s) in the $^{13}C$ NMR spectrum. The 5,9-hemiacetal ring was β-substituted following cucurbitacin stereochemistry. Furthermore, there was no NOE observed between H-19 and H-8 (spectrum 3i) suggesting that this compound was the (*R*)-epimer.

The proton and carbon spectra displayed a few minor peaks which were initially attributed to an impurity, however now that the presence of a hemiacetal ring has been confirmed, the minor peaks could be attributed to the (*S*)-epimer. The hemiacetal ring opens and closes in solution and an equilibrium is eventually established. This phenomenon makes it impossible to purify a single epimer, nevertheless the most stable epimer is always present in larger quantities. As the two epimers were present in different concentrations, it was possible to select the resonances corresponding to the major epimer[106].

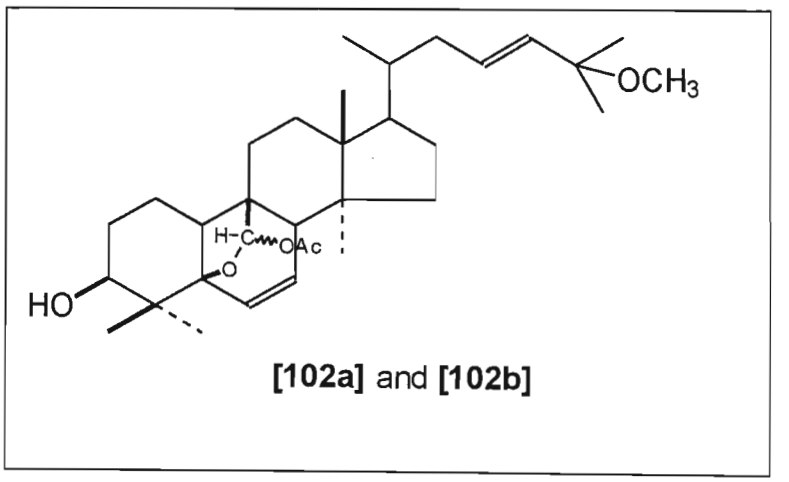
The singlet at δ3.13 (3H) was due to a methoxy group and this correlated with a quartet at δ50.3 in the HETCOR spectrum. The methoxy group was placed at C-25 since it was established that H-19 was coupled to a hydroxy proton. The resonance for C-25 occured at δ74.9 (s).

The $^1$H NMR spectrum also indicated the presence of seven methyl groups as deduced from the integrated signals. The methyl resonances at δ0.83, 0.85, 0.87, 0.89 and δ1.19 correlated with carbon resonances at δ14.7 (q), 18.7 (q), 20.5 (q), 23.9 (q) and δ19.7 (q). The signal at δ1.23 (6H, s) correlated with resonances at δ26.2 (q) and 25.8 (q) ascribable to C-26 and C-27 respectively. From the HETCOR it was observed that C-26 resonated further downfield than C-27 due to the influence of H-24.

The rest of the peak assignments were made accordingly with comparison with those of compounds 1 and 2, and are listed in tables 4 and 6.

Acetylation of compound 3 **[102]** with acetic anhydride/pyridine at room temperature yielded two colourless amorphous compounds **[102a]** and **[102b]**. The $^1$H NMR spectra of these two products (spectrum 3j and 3k) indicated that these were the two isomers of compound 3 acetate, compound 3 being isomeric at C-19. Both these spectra displayed a resonance at δ2.08 (3H, s) indicating monoacetate formation. The H-3 resonances in both the spectra were unaffected, indicating that C-3 hydroxy group did not acetylate. A simple Dreiding model made it clear as to why the C-3 hydroxy group was unaffected. The model indicated that the 5,19-β-epoxy ring and the C-3 β-substituted hydroxy group were in close proximity to each other. A hydrogen bond could exist between

the C-3 hydroxy group and the ether oxygen thereby preventing the C-3 hydroxy group from acetylating. In spectrum 3j and 3k, the H-19 doublets had shifted downfield and appeared as singlets at δ5.99 superimposed on the H-6 doublet and δ5.82 respectively due to deshielding induced by the acetate carbonyl groups. The two epimers were now separable since, upon acetylation, the hemiacetal ring becomes fixed making it unable to open and close in solution. The two epimers thus displayed different retention times on column chromatography.

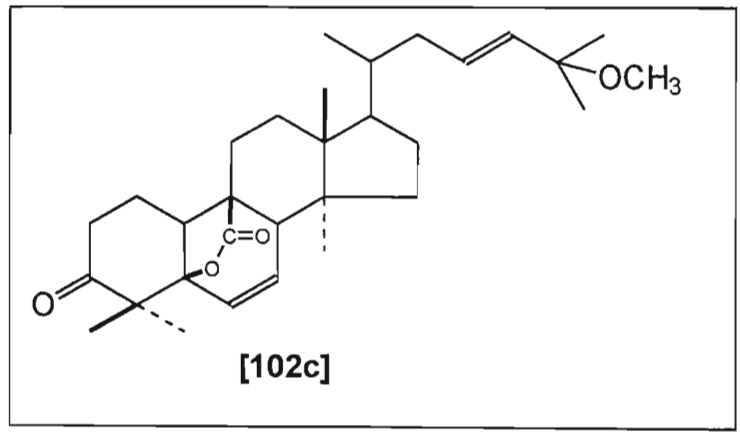



**[102a]** and **[102b]**

An attempt was made to convert the hemiacetal ring into a five membered ring lactone by oxidation. Due to the sensitivity of hemiacetals towards acids, the Jones' oxidation procedure was not followed. Instead a more gentle procedure known as Sarett's oxidation[105] was followed.

Treatment of compound 3 **[102]** with pyridine-$CrO_3$ complex yielded a colourless crystalline material **[102c]**. The $^1H$ NMR spectrum (spectrum 3l) revealed that the doublets at δ5.11 and δ2.71 had disappeared. Furthermore, the H-3α multiplet and the doublet at δ3.75 due to the

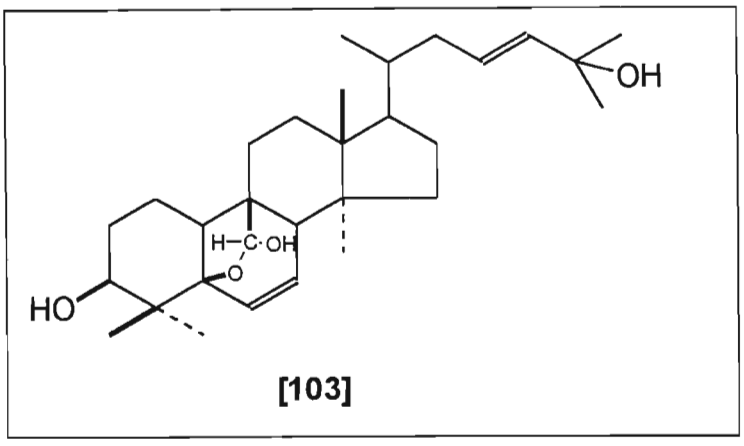
hydroxy proton had also disappeared. The $^{13}C$ NMR spectrum (spectrum 3m) revealed the loss of the hemiacetal carbon resonance at δ105.4 (d) and the appearance of a lactone carbonyl carbon resonance at δ181.0. The carbon resonance ascribed to C-3 at δ76.1(d) had also disappeared. The carbon resonance ascribable to the keto group was not distinguished due to the low concentration of the sample however the $^{1}H$ NMR spectrum provided confirmation of the presence of the C-3 keto group. This confirmation was obtained by the appearance of a triplet of doublets at δ2.71 attributable to the axial C-2 proton, the chemical shift and splitting pattern of which is characteristic of the proton when a keto group is present at C-3[106].




**[102c]**

Compound 3 was identified as 5,19-epoxy-19(*R*)-hydroxy-25-methoxy-5β-cucurbita-6,23-diene-3β-ol **[102]** and has not been isolated previously.

### 3.2.2. Compound 4 [103]




[103]

High resolution mass spectroscopy (spectrum 4a) indicated a molecular mass of 426.3498 g.mol$^{-1}$ correct for the molecular formula $C_{29}H_{46}O_2$. However, the $^1H$ and $^{13}C$ NMR spectra indicated that this compound was similar to compound 3 suggesting the molecular ion may have been too weak to be detected. Examination of the $^{13}C$ NMR spectrum revealed the presence of 30 carbon atoms. As seen for compound 3 the loss of $CH_2O_2$ occurs quite readily to give a formula of $C_{29}H_{46}O_2$. Hence the correct molecular formula was suggested be $C_{30}H_{48}O_4$. The peak at *m/z* 426 may have been due to the loss of the hemiacetal fragment $CH_2O_2$ (46).

The infrared spectrum (spectrum 4b) displayed a broad band at 3365 cm$^{-1}$ due to O-H stretching vibrations. The bands at 2945 cm$^{-1}$ and 2877 cm$^{-1}$ indicated the presence of alkyl groups and the band at 1377 cm$^{-1}$ indicated that methyl groups were present. The band at 1082 cm$^{-1}$ was due to C-O stretching vibrations while the presence of unsaturation was indicated by a band at 1651 cm$^{-1}$. The band at 1116 cm$^{-1}$ indicated that an ether linkage was present.

The $^{1}H$ NMR spectrum (spectrum 4c) was similar to that of compound 3 except for the absence of the methoxy group proton signal at δ3.13 (3H, s) which was placed C-25 in the previous compound. There were no downfield shifted methyl group proton signals thereby ruling out the possibility of a $\Delta^{24,25}$ double bond.

The $^{13}C$ NMR spectrum (spectrum 4d) again showed the sample was a mixture but resonances due to the 30 carbon atoms of the major epimer could be identified. The DEPT spectrum (spectrum 4e) revealed the presence of seven quartets, seven triplets and ten doublets. The remaining six carbon signals were assigned as singlets. The resonances at δ139.6 (d), 132.7 (d), 132.4 (d) and δ125.2 (d), indicated that two disubstituted double bonds were present while those at δ105.4 (d), 86.6 (s), 76.0 (s) and δ70.7 (s) were due to oxygen-bearing carbons.

As in compound 3 proton resonances at δ6.08 (1H, dd) and δ5.66 (1H, dd) were assigned to H-6 and H-7 respectively. H-6 was split into a doublet by H-7 ($J_{6,7}$=9.78Hz) and thereafter further split by long range coupling to H-8 ($J_{6,8}$=2.30Hz) resulting in a double doublet. H-7 was also split into a doublet by H-6 followed by further splitting by H-8 resulting in a double doublet ($J_{6,7}$=9,78Hz; $J_{7,8}$=3.72Hz). A multiplet at δ1.78 ascribable to 2H-22 as in compound 3 was seen coupled in the COSY spectrum (spectrum 4f) to a multiplet at δ5.58 integrated to two protons. On this basis, it was deduced that the resonance at δ5.58 was due to H-23 and H-24 with the signals superimposed.

The doublet at δ5.10 ($J$=7.80Hz) was characteristic of a hemiacetal proton being coupled to the hydroxy group proton. This was confirmed in the COSY spectrum by observing the hemiacetal proton resonance coupled to a doublet at δ2.62 which in the proton spectrum disappeared upon addition of $D_2O$ (spectrum 4g) and thereby collapsing the hemiacetal doublet signal into a singlet. Another doublet at δ3.74 also disappeared on addition of $D_2O$ and this signal was initially seen coupled to H-3α which occured as a multiplet at δ3.40.

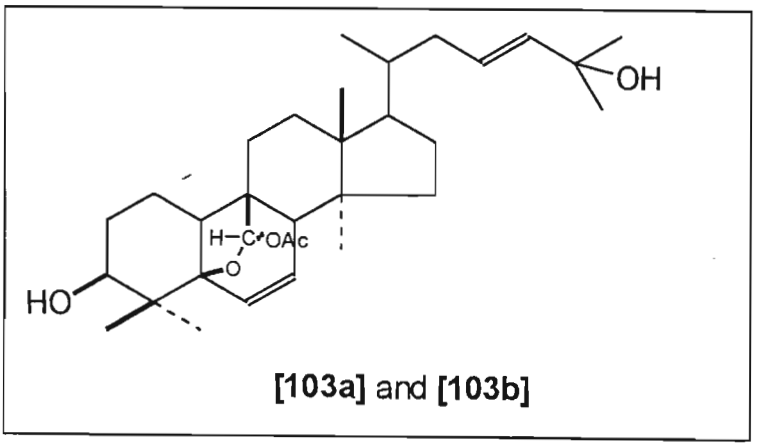
The $^{13}C$ NMR spectrum displayed a carbon resonance at δ86.6 (s) as in compound 3 indicating that this oxygen bearing carbon was located at C-5. Hence the ether linkage was established between C-5 and the hemiacetal carbon (C-19). Furthermore H-6 was only seen to be coupled to H-7 hence the singlet at δ86.6 was assigned to C-5.

The resonances ascribable to 3H-26 and 3H-27 both occured at δ1.29 as a singlet as in compound 3 thereby ruling out the possibility of a hydrogen or double bond at C-25. C-25 was therefore established as an oxygen bearing carbon that resonated at δ70.7 (s). Since the proton and carbon spectra lacked evidence of a methoxy group, it was appropriate to replace the methoxy group in the previous compound with a tertiary hydroxy group.

The rest of the carbon and proton resonances were in agreement with those of compound 3 and are listed in tables 4 and 6 respectively. Due to the similarities of these spectra with those of compound 3, it was not

considered necessary to obtain a HETCOR spectrum. As in Compound 3, this compound was assumed to be the (*R*)-epimer.

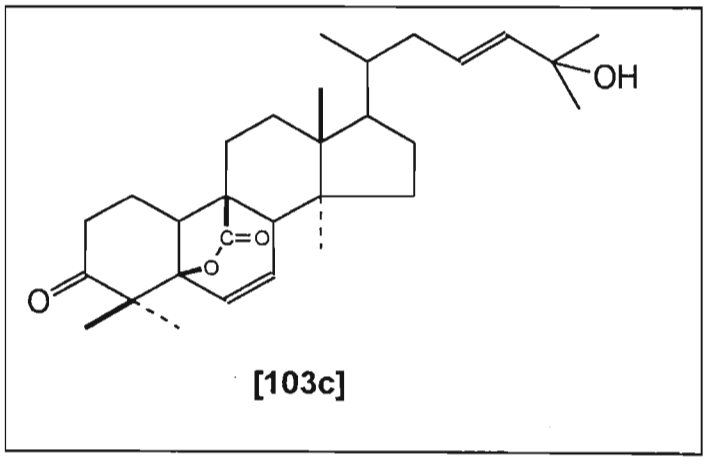
Acetylation of compound 4 yielded two compounds **[103a]** and **[103b]** as monoacetates (spectra 4h and 4j) as observed by the single acetate methyl group proton resonances in both spectra. This was expected since upon acetylation the hemiacetal ring became fixed and the two epimers were separable. It was observed as before that H-3 had not shifted downfield however the signal due to the hemiacetal proton had shifted to δ5.80 and δ5.98 respectively in spectra 4f and 4g and the H-19 doublets had collapsed into singlets. Furthermore, the doublet at δ2.62 due to the hydroxy group had also disappeared. Based on the evidence obtained, it can be concluded that hydroxy group at C-19 acetylated whereas the C-3 β-substituted hydroxy group did not acetylate due to hydrogen bonding between C-3 OH and the ether oxygen. This was seen for compound 3 when a Dreiding model was built to explain the phenomenon. $^{13}C$ NMR spectra for compounds **[103a]** and **[103b]** (spectra 4i and 4k) were obtained in order to distinguish between the two epimers and a model was built to try and account for the small differences in the $^{1}H$ NMR spectra however no logical conclusions could be drawn. In the $^{13}C$ NMR spectrum of **[103b]**, the acetate group




**[103a]** and **[103b]**

carbonyl carbon occured at δ170 (s) but the corresponding peak in **[103a]** could not be discerned due to the small amount of sample available.

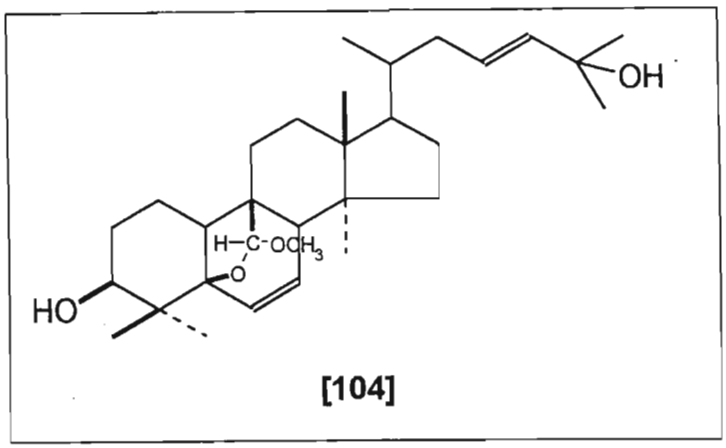
Treatment of compound 4 **[103]** with pyridine-$CrO_3$ complex yielded a colourless material **[103c]**. The $^1H$ NMR spectrum (spectrum 4l) revealed that the doublets at δ5.11 and δ2.71 had disappeared. Furthermore, the H-3α multiplet and the doublet at δ3.75 due to the hydroxy proton had also disappeared. The appearance of a triplet of doublets at δ2.71 attributable to the axial C-2 proton was noted, the chemical shift and splitting pattern of which is characteristic of the proton when a keto group is present at C-3[106]. All this suggested that compound **[103]** had been oxidised. The infrared spectrum (spectrum 4m) displayed characteristic absorption bands at 1749 $cm^{-1}$ due to the stretching vibrations of a lactone carbonyl and at 1726$cm^{-1}$ indicating the presence of a saturated ketone. A $^{13}C$ NMR spectrum could not be obtained since the sample was extremely weak.




**[103c]**

Compound 4 has been identified as 5,19-epoxy-19(*R*),25-dihydroxy-5β-cucurbita-6,23-diene-3β-ol and is the second novel compound to be isolated in this work from this plant.

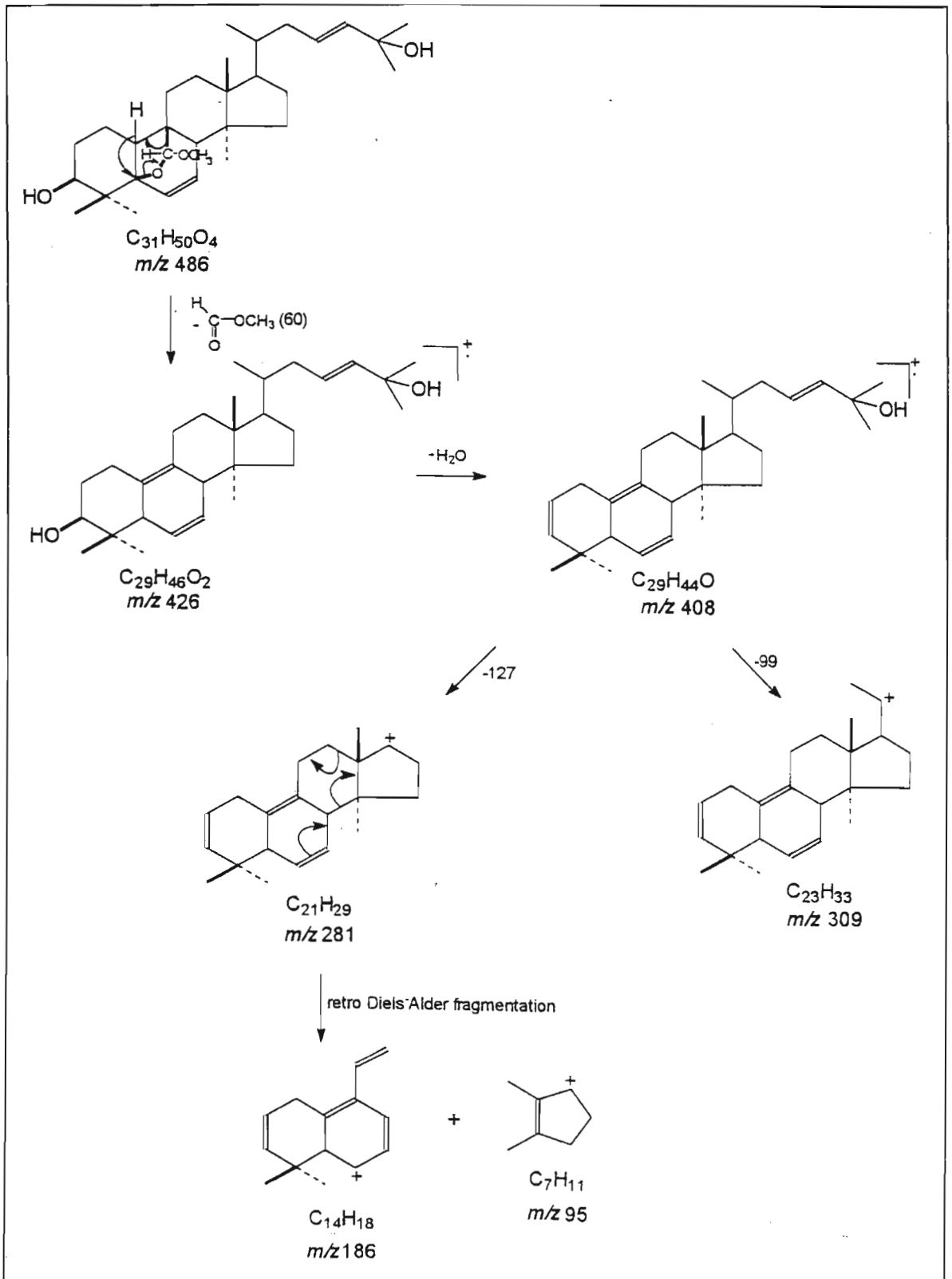
### 3.2.3. Compound 5 [104]




[104]

This compound was isolated in very small quantities and as a result, much caution was exercised during final purification. A Pasteur pipette packed with silica gel was used as a mini column through which the compound was passed.

High resolution mass spectroscopy indicated the compound had a molar mass of 486.3725 $g.mol^{-1}$ (calculated 486.3709 $g.mol^{-1}$) correct for the molecular formula $C_{31}H_{50}O_4$. The mass spectrum (spectrum 5a) displayed peaks at *m/z* 426 ($M^+$-$C_2H_4O_2$) and 408 ($M^+$-$C_2H_4O_2$-$H_2O$). A proposed fragmentation scheme for the molecule is illustrated in Scheme 16 and all proposed structures were supported by high resolution mass measurements.




**Scheme 16. Proposed fragmentation of Compound 5**
**All structures were supported by high resolution mass measurements**

The infrared spectrum (spectrum 5b) displayed strong absorption bands at 3499 $cm^{-1}$ and 3456 $cm^{-1}$ due to OH stretching vibrations. The minor band at 3028 $cm^{-1}$ together with the band at 1651 $cm^{-1}$ indicated unsaturation in the molecule. The prominent bands at 2951 $cm^{-1}$ and 2877 $cm^{-1}$ were due to alkyl groups and this was confirmed by the band at 1460 $cm^{-1}$. The sharp band at 1377 $cm^{-1}$ indicated the presence of methyl groups. The bands at 1157 $cm^{-1}$ and 1082 $cm^{-1}$ were due to C-O stretching vibrations in alcohol groups while the asymmetric C-O-C stretching vibration at 1114 $cm^{-1}$ suggested that an ether linkage was present.

The $^{1}H$ NMR spectrum (spectrum 5c) displayed resonances at δ6.00 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz) and 5.50 (1H, dd, $J_{6,7}$=9.78Hz, $J_{7,8}$=3.72Hz), as in compounds 3 and 4, ascribable to H-6 and H-7 respectively. A broad resonance at δ5.57 integrated to two protons and as with compound 4, this signal was ascribed to superimposed H-23 and H-24 resonances.

The $^{13}C$ NMR spectrum (spectrum 5d) displayed strong resonances for 31 carbons. The DEPT spectrum (spectrum 5e) indicated eight quartets, seven triplets and ten doublets. The remaining six resonances were assigned as singlets. The carbon spectrum indicated the presence of four alkene carbons at δ139.5 (d), 133.0 (d), 130.5 (d) and δ125.3 (d), five oxygen-bearing carbons at δ114.7 (d), 85.1 (s), 76.2 (d), 70.7 (s) and δ57.3 (q) and four quaternary carbons at δ48.9 (s), 47.9 (s), 45.1 (s) and δ37.1 (s). The fact that the alkene carbon resonances were all doublets indicated that the two double bonds were disubstituted.

The resonances at δ133.0 (d) and 130.5 (d), correlated with proton resonances at δ6.00 and 5.50 respectively in the HETCOR spectrum (spectrum 5f) and were thus ascribed to C-6 and C-7 respectively. The resonances δ139.5 (d) and 125.3 (d) corresponded to the superimposed H-23 and H-24 signals at δ5.57 and were assigned to C-23 and C-24. Upon comparison of these resonances with those of compound 4, the signal at δ139.5 (d) was assigned to C-24 and that at δ125.3 (d), assigned to C-23.

The carbon spectrum also displayed a signal at δ114.7 (d) as with compounds 3 and 4. This carbon signal correlated with a proton resonance at δ4.40 (1H, s) in the HETCOR spectrum and was assigned to C-19. Unlike compounds 3 and 4, H-19 resonated as a singlet suggesting that there was no hydroxy group at C-19.

The $^{1}$H NMR spectrum displayed a strong singlet at δ3.37 (3H) characteristic of methoxy group protons. This signal corresponded with a carbon resonance at δ57.3 (q) in the HETCOR spectrum. The COSY spectrum (spectrum 5g) revealed long range coupling between H-19 and the methoxy group protons indicating that the methoxy group had replaced the hydroxy group at C-19 as opposed to compounds 3 and 4. These findings lead to the conclusion that compound 5 was an acetal.

The oxygen bearing carbon resonance at δ85.1 (s) was assigned to C-5 and as with the previous two compounds, the carbon atom attached to two oxygen atoms occured at C-19.

The C-3 signal occured at δ76.2 (d) and correlated with a broad singlet at δ3.41 ascribed to H-3α. The COSY spectrum indicated coupling between H-3α and a doublet at δ3.70 which collapsed upon addition of $D_2O$ to the sample (spectrum 5h). This indicated that the resonance at δ3.70 (d) was due to the C-3β hydroxy proton. The hydroxy group was assumed to be β-substituted as in compound 3 and 4.

In the carbon spectrum, the singlet at δ70.7 was assigned to C-25 bearing a tertiary hydroxy group as in compound 4.

The COSY spectrum displayed coupling between H-6 and H-7 resonances. The H-6 signal was also long-range coupled to a broad singlet at δ2.13 (1H) which correlated with a carbon resonance at δ49.8 (d). This singlet in the proton spectrum was assigned to H-8 for similar reasons as discussed for the previous two compounds. The sharp signal at δ1.29 (6H) correlated with carbon signals at δ30.0 (q) and δ29.9 (q) which were ascribed to C-26 and C-27 respectively.

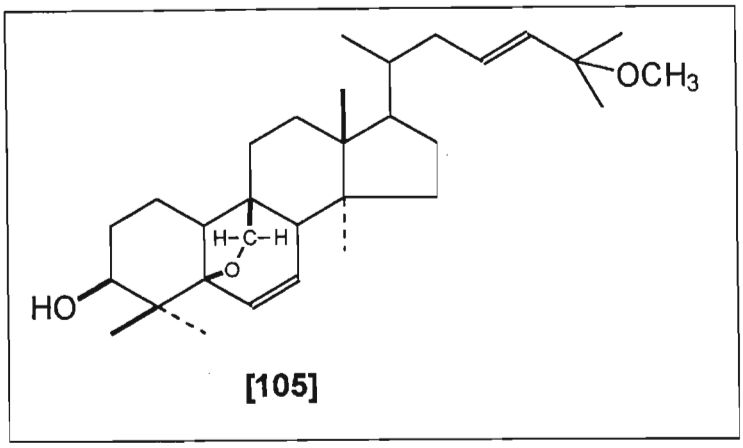
The remainder of the peaks were assigned in a similar manner to those of compounds 3 and 4. The assignments were in good agreement with the previous two compounds and are detailed in tables 4 and 6.

The proton and carbon spectra displayed minor resonances that were due to the (*S*)-epimer present in small quantities as explained for compound 3.

The standard acetylation procedure was followed but from the proton spectrum (spectrum 5i), it was observed that H-3 signal did not shift downfield as expected and no acetate methyl group proton signal appeared. Hence it was concluded that the C-3 hydroxy group did not acetylate due to the proposed hydrogen bonding between the C-3β hydroxy group and the ether oxygen between C-5 and C-19. The hydroxy group at C-25 did not acetylate under the reaction conditions.

Compound 5 was identified as 5,19-epoxy-19(*R*)-methoxy-25-hydroxy-5β-cucurbita-6,23-diene-3β-ol and was assigned structure **[104]**.

### 3.2.5. Compound 6 [105]




[105]

Compound 6 had a mass of 470.3409 g.mol$^{-1}$ consistent with a molecular formula $C_{31}H_{50}O_3$. The mass spectrum (spectrum 6a) displayed peaks at *m/z* 455 ($M^+$-$CH_3$), 440 ($M^+$-$CH_2O$) and 422 ($M^+$-$CH_2O$-$H_2O$). The loss of water indicated the presence of a hydroxy group. The fragmentation profile was similar to that observed for the previous compounds indicating structural similarities in the triterpene nucleus.

Infrared analysis (spectrum 6b) showed an absorption band at 3472 cm$^{-1}$ indicating the presence of a hydoxy group. The bands at 3020 cm$^{-1}$ and 1683 cm$^{-1}$ were indicative of unsaturation in the molecule. The bands at 2943 cm$^{-1}$ and 2883 cm$^{-1}$ are due to alkyl groups, while the presence of methyl groups was indicated by the sharp band at 1379 cm$^{-1}$. The infrared spectrum also indicated the asymmetric C-O-C stretching vibration at 1138 cm$^{-1}$ suggesting that an ether linkage was present.

The $^{1}H$ NMR spectrum (spectrum 6c) had four distinctive signals at δ6.00 (1H, dd, $J_{6,7}$=9.78Hz; $J_{6,8}$=2.30Hz, H-6), δ5.60 (1H, dd, $J_{7,8}$=3.72Hz; $J_{6,7}$=9.78Hz, H-7), δ5.47 (1H, m, H-23), δ5.40 (1H, s, H-24) indicating the presence of $\Delta^{6,7}$ and $\Delta^{23,24}$ double bonds.

The $^{13}C$ NMR spectrum (spectrum 6d) displayed resonances for 31 carbons, eight of which were quartets, eight were triplets and nine were doublets as indicated by the DEPT spectrum (spectrum 6e). The remaining six carbon resonances were assigned as singlets. Unlike the carbon spectra of the previous compounds, this spectrum showed no hemiacetal carbon resonance. Instead, there was a signal at δ79.8 (t) which correlated with proton resonances at δ3.65 and δ3.49 in the HETCOR spectrum (spectrum 6f). This indicated that the protons were non-equivalent and evidence of geminal coupling was inferred from the fact that both these protons resonated as doublets ($J_{gem}$=8.40Hz) and were also seen coupled in the COSY spectrum (spectrum 6g).

The COSY spectrum also displayed coupling between H-6 and H-7 as well as between H-23 and H-24. The corresponding carbon resonances of these protons occured at δ131.8 (d), 131.5 (d), 128.3 (d) and δ136.8 (d) respectively. The H-23 multiplet was also coupled to a multiplet at δ1.75 which correlated with a carbon resonance at δ27.3 (t) ascribable to C-22. The carbon resonance at δ76.1 (d), typical of C-3, correlated with a broad resonance at δ3.31 (1H, $W_{1/2}$=6.8Hz) ascribable to H-3α indicating the presence of a β-substituted hydroxy group. The COSY spectrum revealed coupling between H-3 and a doublet at δ3.97. This

doublet disappeared on addition of $D_2O$ (spectrum 7g) and was hence ascribed to the C-3 hydroxy group.

The resonance at δ3.13 (3H, s), corresponding to a carbon signal at δ50.3 (q), indicated that a methoxy group was present. On comparison of the carbon spectrum with that of compound 3, it was logical to place the methoxy group at C-25 since the chemical shift for this carbon was identical in both compounds. Thus far the following structural features were identified:

a) the presence of a C-3 β-substituted hydroxy group,

b) the presence of $\Delta^{6,7}$ and $\Delta^{23,24}$ double bonds and

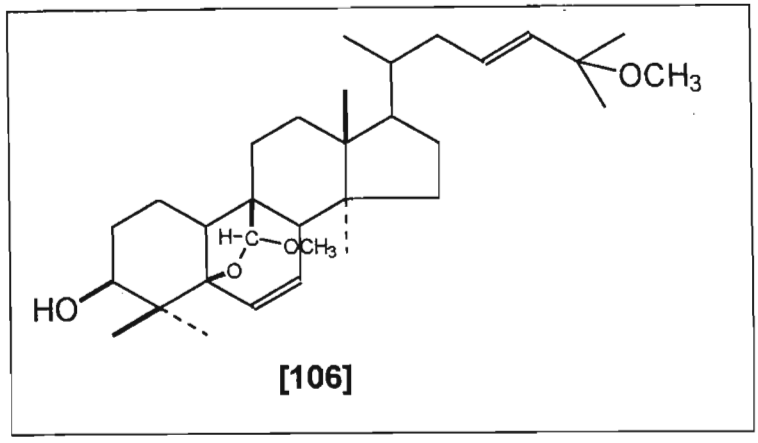
c) a C-25 methoxy group

No evidence of a hemiacetal ring was observed. However, the IR spectrum did indicate the presence of an ether linkage. It was appropriate to place this ether linkage between C-5 and C-19 as in the case of the previous compounds. The triplet at δ79.8 was hence assigned to C-19.

This compound was previously isolated from immature fruits of *Momordica charantia* L. as the glycosides, momordicosides F1 **[88]** and G **[90]**[90]. The aglycone was obtained from enzymatic hydrolysis using crude hesperidinase[90]. The reported NMR data of the aglycone were in good agreement with the data reported for our compound, indicating that the structure was correct.

Hence compound 6 was identified as 5,19-epoxy-25-methoxy-5β-cucurbita-6,23-diene-3β-ol and assigned structure **[105]**.

### 3.2.5. Compound 7 [106]




[106]

High resolution mass spectroscopy indicated that the compound had a molar mass of 500.3865 $g.mol^{-1}$, consistent with a molecular formula $C_{32}H_{52}O_4$. The mass spectrum (spectrum 7a) displayed peaks at *m/z* 440 ($M^+$-$C_2H_4O_2$), 375, 309, 172 and 109.

The infrared spectrum (spectrum 7b) displayed a band at 3516 $cm^{-1}$ due to OH stretching vibrations. The strong bands at 2926 $cm^{-1}$ and 2876 $cm^{-1}$ indicated the presence of alkyl groups while the complexity of bands between 1460 $cm^{-1}$ and 1377 $cm^{-1}$ confirmed the presence of methyl groups. The band at 1114 $cm^{-1}$ was due to the asymmetric C-O-C stretching vibration indicating the presence of an ether.

The $^1H$ NMR spectrum displayed proton resonances at δ3.37 (3H, s) and δ3.13 (3H, s) characteristic of two methoxy groups, a broad signal at δ3.48, ascribable to H-3α, that was slightly superimposed by the methoxy group proton signal and a sharp resonance at δ4.40 (1H, s),

characteristic of a hemiacetal proton as in compound 5. The H-6 signal at δ6.07 (1H, dd, $J_{6,7}$=9.78Hz; $J_{6,8}$=2.30Hz) and the H-24 signal were also quite distinct. However, a multiplet integrating to two protons occurred at δ5.50 (m). The COSY spectrum (spectrum 7d) revealed that both H-6 and H-24 were coupled to this multiplet. It was therefore concluded that this resonance was due to the superimposed resonances of H-7 and H-23. Furthermore, coupling was also noticed between H-22 (δ1.72, m) and the H-23 signal.

A total 32 carbon signals was evident in the carbon spectrum (spectrum 7e), the multiplicities (spectrum 7f) and chemical shifts of which were similar to those observed in compound 3. However, a second quartet was observed at δ57.3 (q). This chemical shift was similar to that observed for the methoxy group carbon in compound 5, suggesting that the second methoxy group was attached to the hemiacetal group carbon.

The COSY spectrum also revealed coupling between H-3α and the β-substituted hydroxy group, a common feature in the compounds discussed. There was also long range coupling observed between H-6 and a broad signal at δ2.24 ascribable to H-8

There was no HETCOR spectrum obtained for this compound since the above data and reasoning indicated that this compound was the 19-methoxy analogue of compound 3.

The two epimers of this compound were previously isolated from the leaves and vines of *Momordica charantia* L. presumably as the natural acetates[91], but no nmr peak assignments were given by the authors.

This compound was identified as 5,19-epoxy-19(*R*),25-dimethoxy-5β-cucurbita-6,23-diene-3β-ol and was assigned structure **[106]**.

# CHAPTER 4. Experimental

## 4.1. Spectroscopic techniques

### 4.1.1. $^{1}H$ and $^{13}C$ NMR spectroscopy

All $^{1}H$ NMR, $^{13}C$ NMR and 2-dimensional NMR spectra were recorded at room temperature on a Varian Gemini 300 MHz spectrometer. The solvent used was deuteriochloroform ($CDCl_3$), and all δ values were relative to TMS. A singlet at δ1.52 occured in the proton spectra for most of the compounds and was due to a solvent impurity which is indicated with an X in the relevant spectra.

### 4.1.2. Infrared Spectroscopy

The samples were prepared in KBr discs and infrared spectra recorded on a 2020 Galaxy series FTIR spectrophotometer coupled with a Spectra Tech Advanced Analytical Microscope. The data was acquired using the Mattson software (copyrighted 1989).

### 4.1.3. High Resolution Mass spectrometry

High resolution mass spectra were recorded at the Cape Technikon on a Kratos High Resolution MS 9/50 mass spectrometer by Dr P. Boshoff. The voltage of the ion source was maintained at 70 eV.

## 4.2. Melting point

Melting points were determined on a Kofler micro hotstage melting point apparatus and are uncorrected.

## 4.3. Optical rotations

Optical rotations were recorded at 25,5 °C in chloroform solution on a Perkin-Elmer 241 polarimeter. A sodium lamp was used at a wavelength of 589 nm. The concentrations of the sample solutions are expressed in g/100ml.

## 4.4. Chromatography

### 4.4.1. Column chromatography

Column chromatography was performed repeatedly in order to separate and purify the compounds present in the extracts. The length and diameter of the columns as well as the particle size of the stationary phase had to be varied in order to optimise resolution of the compounds.

Initially, use was made of a glass column packed with silica gel 60 (0,2-0,5 mm particle size, 35-20 mesh ASTM, Merck Art 7734), with gravity elution. This was followed by the use of gravity columns packed with silica gel 60 (0,040-0,053 mm particle size, 230-400 mesh ASTM, Merck Art.9385). In certain cases, flash chromatography was found useful for the speedy separation of mixtures. The latter technique involved the use of pressure in order to elute the various fractions from

the column. The flow rate was maintained at approximately 60 ml/min for flash chromatography.

### 4.4.2. Thin Layer Chromatography (TLC)

Thin layer chromatography was conducted on the crude extracts and the various fractions collected, using pre-coated 0,2 mm thick aluminium-backed silica gel 60 (Merck Art 5553) TLC plate. The spots on the plates were visualised by spraying with a reagent comprising anisaldehyde , sulphuric acid (conc) and methanol in the ratio 1,25 : 2,5 : 96,25. Coloured spots were observed after heating the plates with a heatgun.

## 4.5. Liebermann-Burchard test

This test was conducted for the identification of compounds containing a triterpene nucleus. The Libermann-Burchard reagent was prepared by adding 9.5 ml acetic anhydride to 0.5 ml sulphuric acid (conc). An equal volume of this reagent was then added to a solution of the compound under investigation, in chloroform. The formation of an intense blue colour confirmed the presence of a triterpene.

## 4.6. Tollen's test

This test was performed to confirm the presence of an aldehyde. One drop of 10% aqueous sodium hydroxide was added to 1 ml of 5% aqueous silver nitrate in a test tube. The tube was shaken followed by

the addition of a dilute solution of ammonium hydroxide with shaking until the precipitate of silver hydroxide just dissolved. Approximately 5 mg of the compound under investigation was added to the reagent and the solution heated for 5 min in a water bath. The formation of a silver mirror on the walls of the test tube confirmed the presence of an aldehyde.

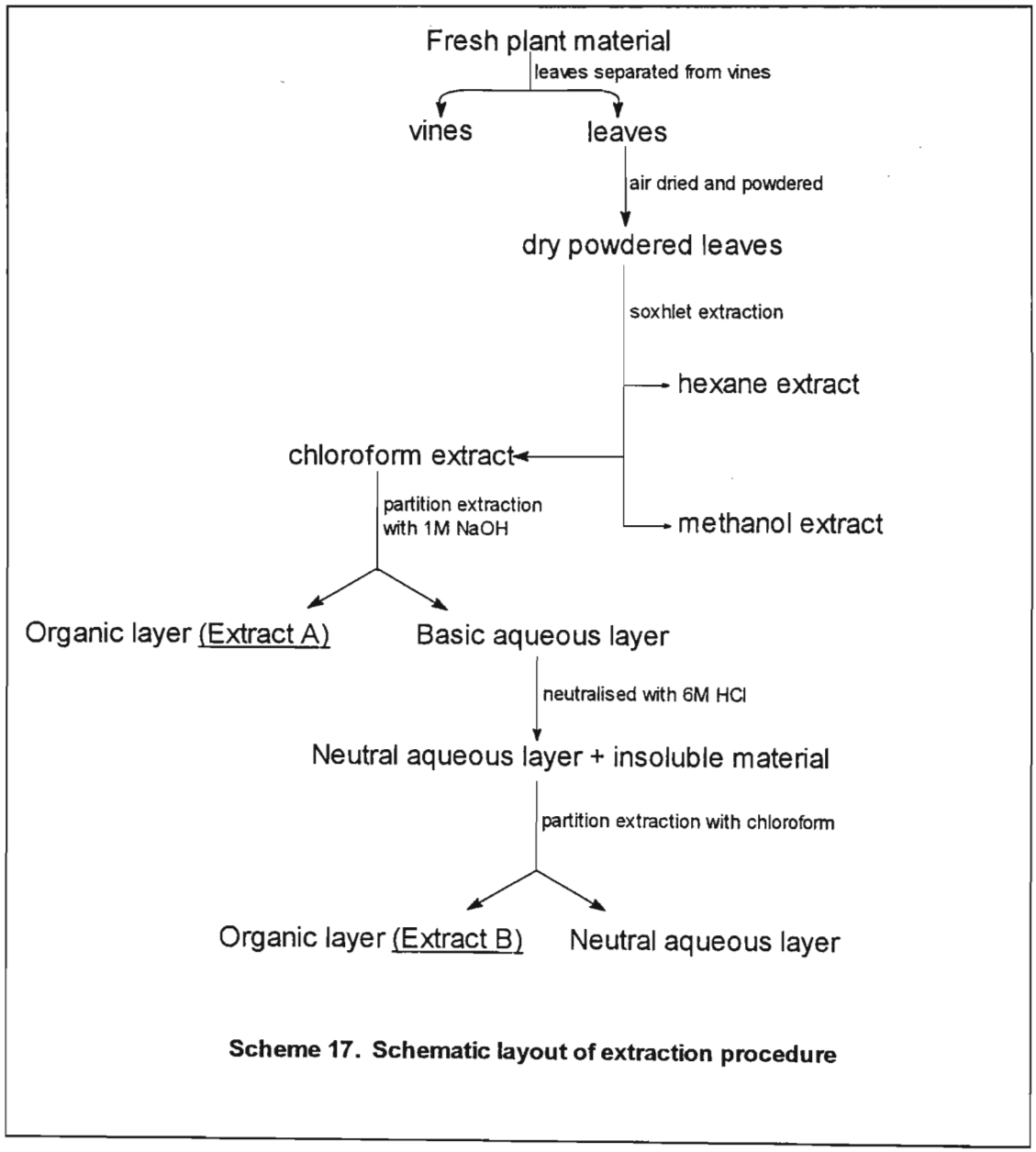
## 4.7. Preparation and extraction of plant material

Fresh plant material of *Momordica foetida* Schum. & Sond. was purchased at the Umlazi herbal market during December 1993 and its identification authenticated by comparison with a reference specimen at the Natal Herbarium.

The leaves were carefully separated from the vines and air dried for one week. The leaves were thereafter ground to powder in a coffee grinder. The powdered leaves (440 grams) were then extracted in a soxhlet apparatus by refluxing successively with hexane, chloroform and methanol. The hexane and methanol extracts were reserved for possible future investigations. The chloroform extract was concentrated under vacuum and thereafter extracted with 1M NaOH. This resulted in an organic and an aqueous phase. The organic phase, containing extract A, afforded two compounds viz. compounds 1 and 2.

The basic aqueous fraction was neutralised to litmus paper with 6M HCl and re-extracted with chloroform (3 x 250 ml). The combined extracts were dried over $Na_2SO_4$, filtered and concentrated under vacuum. This

extract, extract B (1.39 grams), afforded five compounds viz. compounds 3, 4, 5, 6, and 7. The extraction procedure is outlined in Scheme 17.




**Scheme 17. Schematic layout of extraction procedure**

## 4.8 Acetylation of compounds 1, 2, 3, 4, 5 and 6

A quantity of 2 mg of each compound was each dissolved in 1 ml pyridine and the solution gently warmed on a steam bath. A 1 ml aliquot of acetic anhydride was then added to each solution, which was stirred and left to stand overnight at room temperature. Thereafter 10 ml methanol was added to hydrolyse the residual anhydride. Two 10 ml portions of toluene was then added to the mixture and subsequently removed under reduced pressure in order to remove traces of pyridine. The residual toluene was removed under reduced pressure by the addition of two 10 ml aliquots of methanol.

## 4.9 Oxidation of compounds 3 and 4

The compounds were oxidised using the chromium trioxide-pyridine complex (Sarret oxidation)[105].

The complex was prepared by gently adding 1 g chromic anhydride to 10 ml reagent grade pyridine. The first phase consisted of slow dissolution of the anhydride without formation of the complex, however, after about one-third of the chromic anhydride had been added and mostly dissolved, the yellow complex began to precipitate. At the end of the addition a slurry of the complex remained.

A quantity of 2 mg of compounds 3 and 4 in 2 ml pyridine were each added to 2 ml quantities of the slurry. The reaction flask was stoppered,

the contents mixed thoroughly and allowed to stand at room temperature overnight. The reaction mixture was poured into water and extracted with 3 x 50 ml portions of benzene-ether (1:1). The organic layer was concentrated under reduced pressure and the resulting product purified by column chromatography.

## 4.10. Extractives from Extract A

Column chromatography of extract A (5.39 grams) resulted in the isolation of two compounds.

### 4.10.1. Compound 1 [92]

(3β,7β,23ξ-trihydroxy-cucurbita-5,24-diene-19-al)

Mobile phase: 50% chloroform, 50% ethyl acetate

$R_f$ value: 0.25

Description: white amorphous powder

Yield: 70mg

Mass: $[M]^+$ at *m/z* 472.3551 ($C_{30}H_{48}O_4$ requires 472.3552)

Mass Spectrum: (Spectrum 1a)

EIMS *m/z* 472, 454, 436, 426, 408, 389, 309

IR spectrum: (Spectrum 1b)

$\upsilon_{max}$ (KBr): 3404 $cm^{-1}$ (OH)

2947 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1709 $cm^{-1}$ (CHO)

1666 $cm^{-1}$ (C=C)

1467 $cm^{-1}$ (C-H deformations)

1379 $cm^{-1}$ ($>C(CH_3)_2$)

Optical rotation: $[\alpha]_D = +73.1°$ ($CHCl_3$, *c* 0.160) (*lit.* +81.3°)[91]

melting point: 123-125 °C (*lit.* 125-128 °C)[91]

$^1$H NMR: (300MHz, Spectrum 1c)

δ 9.69 (1H, s, CHO), 5.87 (1H,d, $J_{6,7}$=5.13 Hz, $J_{6,10}$=1.92Hz, H-6), 5.17 (1H, d, $J_{24,23}$=5.17 Hz, H-24), 4.44 (1H, bt, $J_{23,24}$=5.17Hz, H-23), 3.95 (1H, bs, $W_{1/2}$=8.00Hz, H-7α), 3.54 (1H, b,$W_{1/2}$=7.61Hz, H-3α), 2.52 (1H, m, H-10), 2.07 (1H, m, H-17), 1.81 (2H, m, H-1), 1.75 (2H, m, H-2), 1.71 (1H, m, H-20), 1.68 (3H, s, 3H-27), 1.66 (3H, s, 3H-26), 1.65 (2H, m, H-12), 1.61 (2H, m, H-22), 1.45 (2H, m, H-16), 1.38 (2H, m, H-15), 1.22, 1.03, 0.96, 0.73, (each 3H, s, 4x$CH_3$), 0.89 (3H, d, 3H-21).

$^{13}C$ NMR: (75MHz, Spectrum 1d)

(Table 3 for assignments)

δ 207.8 (d), 145.5 (s), 133.9 (s), 128.9 (d), 123.9 (d), 76.1 (d), 66.3 (d), 65.9 (d), 50.77 (d), 49.9 (s), 47.6 (s), 47.5 (d), 45.4 (s), 44.4 (t), 41.4 (s), 36.6 (d), 34.6 (t), 32.6 (d), 29.1 (t), 28.4 (t), 27.7 (t), 27.2 (q), 25.7 (q), 25.4 (q), 23.5 (t), 21.2 (t), 18.8 (q), 18.1 (q), 17.9 (q), 14.8 (q).

### 4.10.1.1. Acetylation of compound 1

A quantity of 2mg of compound 1 was dissolved in pyridine and the acetylation procedure carried out as described in 4.8. to yield a colourless amorphous substance **[92a]**.

$^{1}H$ NMR data of **[92a]** (300MHz, spectrum 1j)

δ9.80 (1H, s, CHO), 5.84 (1H, dd, $J_{6,7}$=5.13Hz, $J_{6,10}$=1.92Hz, H-6), 5.57 (1H, H-23), 5.17 (1H, d, $J_{24,23}$=5.40Hz, H-24), 5.07 (1H, d, H-7α), 4.79 (1H, bs, $W_{1/2}$=6.8Hz, H-3α), 2.52 (1H, m, H-10), 1.99, 1.99, 1.97 (3H, s, 3xO-C-$OCH_3$), 1.70 (3H, s, 3H-27), 1.67 (3H, s, 3H-26), 1.23, 1.09, 0.83, 0.78 (each 3H, s, 4x$CH_3$), 0.92 (3H, d, $J$=5.7Hz, 3H-21).

### 4.10.2. Compound 2 [93]

(3β,7β,25-trihydroxycucurbita-5,23-dien-19-al)

Mobile phase: 40% ethyl acetate, 60% chloroform

$R_f$ value: 0.30

Description: white powder

Yield: 20mg

Mass: $[M]^+$ at *m/z* 472.3552 ($C_{30}H_{48}O_4$ requires 472.3552)

Mass Spectrum: (Spectrum 2a)

EIMS m/z 472,454, 436, 426, 408, 389, 309

IR spectrum: (Spectrum 2b)

$\upsilon_{max}$ (KBr): 3273 $cm^{-1}$, 3431 cm-1 (OH)

2955 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1701 $cm^{-1}$ (CHO)

1465 $cm^{-1}$ (C-H deformations)

1375 $cm^{-1}$ ($>C(CH_3)_2$)

1134 $cm^{-1}$ (C-O stretching)

melting point: 188-191 °C (*lit* 190-192 °C)[91]

$^{1}$H NMR: (300MHz, Spectrum 2c)

δ 9.61 (1H, s, CHO), 5.87 (1H, dd, $J_{6,7}$=5.13Hz; $J_{6,10}$=1.92Hz, H-6), 5.56 (2H, m, H-23; H-24), 3.95 (1H, bs, $W_{1/2}$=8.00Hz, H-7α), 3.55 (1H, bs, $W_{1/2}$=7.61Hz, H-3α), 2.52 (1H, m, H-10), 1.82 (2H, m, H-1), 1.71 (2H, m, H-2), 1.69 (2H, m, H-22), 1.43 (1H, m, H-20), 1.30 (2H, m, H-15), 1.29 (6H, s, 3H-26; 3H-27), 1.22, 1.03, 0.89, 0.87, 0.73 (each 3H, s, 5x$CH_3$).

$^{13}$C NMR: (75 MHz, Spectrum 2d)
(Table 3 for assignments)

δ 207.8 (d), 145.5 (s), 139.6 (d), 125.1 (d), 123.9 (d), 76.1 (d), 70.7 (s), 66.3 (d), 49.9 (d), 49.9 (s), 47.5 (d), 47.5 (s), 45.3 (s), 41.4 (s), 39.0 (t), 36.6 (d), 36.1 (d), 34.7 (t), 29.9 (q), 29.8 (q), 28.9 (t), 28.4 (t), 27.4 (t), 27.2 (q), 25.3 (q), 23.5 (t), 21.2 (t), 18.7 (q), 17.9 (q), 14.9 (q).

### 4.10.2.1. Acetylation of Compound 2

A quantity of 2mg of compound 2 was acetylated following the procedure outlined in 4.8. to yield a colourless amorphous substance **[93a]**.

$^1$H NMR data of **[93a]** (300MHz, Spectrum 2i)

δ 9.81 (1H, s, CHO), 5.85 (1H, dd, $J_{6,7}$=5.13Hz, $J_{6,10}$=1.92Hz, H-6), 5.56 (2H, m, H-23; H-24), 5.18 (1H, d, $J_{6,7}$=5.13, H-7α), 4.80 (1H, bs, $W_{1/2}$=7.2Hz, H-3α), 2.53 (1H, m, H-10), 2.01, 2.00 (each 3H, s, 3xO-$COCH_3$), 1.28 (6H, s, 3H-26, 3H-27), 1.13, 1.10, 0.84, 0.78 (3H, s, 4x$CH_3$), 0.87 (3H, d, $J$=6.0Hz, 3H-21).

## 4.11. Extractives from Extract B

Extract B (1.39 grams) was separated by means of column chromatography and so obtained five compounds.

### 4.11.1. Compound 3 [102]

(5,19-epoxy-19(*R*)-hydroxy-25-methoxy-5β-cucurbita-6,23-diene-3β-ol)

Mobile phase: 40% hexane, 30% methylene chloride, 30% ethyl acetate

$R_f$ value: 0.22

Description: Colourless needles

Yield: 12.3 mg

Mass: $[M]^+$ at *m/z* 486.3725 ($C_{31}H_{50}O_4$ requires 486.3709)

Mass Spectrum: (Spectrum 3a)

EIMS *m/z* 486, 471, 440, 422, 408, 309, 239, 109

IR Spectrum: (Spectrum 3b)

$\upsilon_{max}$ (KBr): 3292 $cm^{-1}$ (OH)

2949 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1649 $cm^{-1}$ (C=C)

1375 $cm^{-1}$ ($>C(CH_3)_2$)

1080 $cm^{-1}$ (C-O stretching)

Optical rotation: $[\alpha]_D$ = -55.9° ($CHCl_3$, c 0.102)

Melting point: 182-184°C (Recrystallised from a mixture of hexane:diethyl ether (1:2))

$^1$H NMR: (300MHz, Spectrum 3c)

δ 6.06 (1H, dd, $J_{6,7}$=9.78Hz; $J_{6,8}$=2.30Hz, H-6), 5.65 (1H, dd, $J_{7,8}$=3.72Hz; $J_{6,7}$=9.78Hz, H-7), 5.42 (1H, m, H-23), 5.40 (1H, s, H-24), 5.11 (1H, d, $J$=7.98Hz, H-19), 3.75 (d, OH), 3.38 (1H, m, H-3), 3.13 (3H, s, $OCH_3$), 2.82 (1H, bs, H-8), 2.71 (d, OH), 2.45 (1H, bt, $J$=9.19Hz, H-10), 1.94 (2H, m, H-16), 1.82 (2H, m, H-12), 1.76 (2H, m, H-22), 1.65 (2H, m, H-1), 1.40 (1H, m, H-17), 1.38 (2H, t, $J$=11.58Hz, H-15), 1.23 (6H, s, 3H-26; 3H-27), 1.19 (3H, s, 3H-21), 0.89, 0.87, 0.85, 0.83 (each 3H, s, 4x$CH_3$).

$^{13}C$ NMR: (75 MHz, Spectrum 3d)

(Table 4 for assignments)

δ 136.8 (d), 132.7 (d), 132.4 (d), 128.3 (d), 105.4 (d), 86.6 (s), 76.1 (d), 74.9 (s), 50.3 (q), 49.9 (d), 48.5 (s), 48.0 (s), 45.1 (s), 41.4 (d), 40.6 (d), 39.4 (t), 37.2 (s), 36.1 (d), 33.5 (t), 30.5 (t), 27.9 (t), 27.1 (t), 26.2 (q), 25.8 (q), 20.5 (q), 23.1 (t), 23.9 (q), 19.7 (q), 18.7 (q), 17.3 (t), 14.7 (q).

#### 4.11.1.1. Acetylation compound 3

A quantity of 2mg of compound 3 was acetylated in the usual manner to yield the two epimers **[102a]** and **[102b]**.

$^{1}H$ NMR data (300MHz, Spectrum 3k) Acetate 1

δ 6.08 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz, H-6), 5.82 (1H, s, H-19), 5.53 (1H, dd, $J_{7,8}$=3.72Hz, $J_{6,7}$=9.78Hz, H-7), 5.48 (1H, m, H-23), 5.42 (1H, s, H-24), 3.39 (1H, m, H-3α), 3.13 (3H, s, $OCH_3$), 2.40 (1H, bs, H-10), 2.07 (3H, s, O-C-$OCH_3$), 1.23 (6H, s, 3H-26, 3H-27), 1.20 (3H, s, 3H-21), 0.89, 0.87, 0.85, 0.83 (each 3H, s, 4x$CH_3$).

$^1$H NMR data (300MHz, Spectrum 3j) Acetate 2

δ 5.99 (2H, m, H-6, H-19), 5.65 (1H, dd, *J*=9.78Hz, H-7), 5.48 (1H, m, H-23), 5.40 (1H, s, H-24), 3.46 (1H, m, H-3α), 3.13 (3H, s, OCH3), 2.80 (1H, bs, H-10), 2.07 (3H, s, O-C-$OCH_3$), 1.23 (6H, s, 3H-26, 3H-27), 1.20 (3H, s, 3H-21), 0.89, 0.87, 0.85, 0.83 (each 3H, s, 4x$CH_3$).

### 4.11.1.2. Oxidation of compound 3

A quantity of 2mg of the compound was oxidised following the procedure outlined in 4.9. to yield **[102c]**.

$^1$H NMR data of **[102c]** (300MHz, Spectrum 3l)

δ 6.18 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz, H-6), 5.80 (1H, dd, $J_{7,8}$=3.72Hz, $J_{6,7}$=9.78Hz, H-7), 5.48 (1H, m, H-23), 5.42 (1H, s, H-24), 3.13 (3H, s, $OCH_3$), 2.71 (1H, td, H-$2_{ax}$), 1.24 (6H, s, 3H-26, 3H-27), 1.20, 1.17, 0.92, 0.91. 0.90 (each 3H, s, 5x$CH_3$).

$^{13}$C NMR data of **[102c]** (75MHz, Spectrum 3m)

δ 180.7 (s), 137.0 (d), 134.3(d), 130.3 (d), 128.1 (d), 88.0 (s), 74.8 (s), 51.1 (d), 50.3 (q), 50.2 (s), 47.8 (s), 47.7 (s), 45.1 (d), 44.5 (d), 39.9 (t), 39.3 (s), 36.1 (d), 35.2 (t), 33.2 (t), 29.8 (t), 27.4 (t), 26.2 (q), 25.8 (q), 25.2 (q), 23.4 (t), 21.8 (q), 19.3 (q), 18.6 (q), 16.9 (t), 14.6 (q).

### 4.11.2. Compound 4 [103]

(5,19-epoxy-19(*R*),25-dihydroxy-5β-cucurbita-6,23-diene-3β-ol)

Mobile phase: 23% hexane, 34% methylene chloride, 43% ethyl acetate

Rf value : 0.25

Description: colourless gum

Yield: 8.4 mg

Mass: $[M\text{-}CH_2O_2]^+ = 426.3498$ ($C_{29}H_{46}O_2$ requires 426.3498)

Mass Spectrum: (Spectrum 4a)

EIMS *m/z* 426, 408, 393, 365, 339, 309

IR Spectrum: (Spectrum 4b)

$\upsilon_{max}$ (KBr): 3365 $cm^{-1}$ (OH)

2945 $cm^{-1}$, 2877 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1651 $cm^{-1}$ (C=C)

1460 $cm^{-1}$ (C-H deformations)

1377 $cm^{-1}$ ($>C(CH_3)_2$)

1082 $cm^{-1}$ (C-O stretching)

Optical rotation: $[\alpha]_D = -93.2^\circ$ ($CHCl_3$, *c* 0.278)

$^{1}$H NMR: (300MHz, Spectrum 4c)

δ 6.06 (1H, dd, $J_{6,7}$=9.80Hz, $J_{6,8}$=2.30Hz, H-6), 5.65 (1H, dd, $J_{6,7}$=9.80Hz, $J_{7,8}$=3.72Hz, H-7), 5.58 (2H, m, H-23, H-24), 5.10 (1H, d, $J$=7.80Hz, H-19), 3.40 (1H, m, H-3α), 2.82 (1H, m, H-8), 2.45 (1H, bt, $J$=2.19Hz, H-10), 1.92 (2H, m, H-16), 1.78 (2H, m, H-22), 1.68 (2H, m, H-2), 1.61 (2H, m, H-11), 1.50 (1H, m, H-20), 1.47 (2H, m, H-1), 1.35 (2H, m, H-15), 1.30 (1H, m, H-17), 1.29 (6H, s, 3H-26, 3H-27), 1.20 (3H, d, $J$=5.70Hz, 3H-21), 0.87, 0.86, 0.85, 0.83 (each 3H, s, 4x$CH_3$).

$^{13}$C NMR: (75MHz, Spectrum 4d)

(Table 4 for assignments)

δ 139.6 (d), 132.7 (d), 132.4 (d), 125.2 (d), 105.4 (d), 86.6 (s), 76.0 (d), 70.7 (s), 50.0 (d), 48.5 (s), 48.0 (s), 45.1 (s), 41.4 (d), 40.6 (d), 39.1 (t), 38.0 (t), 36.2 (d), 33.5 (t), 30.5 (t), 30.0 (t), 29.8 (q), 27.9 (t), 27.1 (q), 23.9 (q), 23.1 (t), 20.4 (q), 19.7 (q), 18.6 (q), 17.3 (t), 14.7 (q).

### 4.11.2.1. Acetylation of compound 4

A quantity of 2mg of compound 4 was acetylated in the manner described in 4.8 to yield two monoacetates **[103a]** and **[103b]** (Spectrum 4h and 4i).

$^1$H NMR data (300MHz, Spectrum 4h) Acetate 1

δ 6.06 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz, H-6), 5.63 (1H, m, H-7), 5.82 (1H, s, H-19), 5.58 (2H, m, H-23, H-24), 3.37 (1H, m, H-3α), 2.80 (1H, m, H-8), 2.06 (3H, s, O-C-$OCH_3$), 1.29 (6H, s, 3H-26, 3H-27), 1.20, 0.87, 0.86, 0.85, 0.83 (each 3H, s, 5x$CH_3$).

$^1$H NMR data (300MHz, Spectrum 4i) Acetate 2

δ 6.00 (2H, m, H-6, H-19), 5.63 (1H, dd, $J_{6,7}$=9.80Hz, $J_{7,8}$=3.72Hz, H-7), 5.58 (2H, m, H-23, H-24), 3.38 (1H, m, H-3α), 2.79 (1H, m, H-8), 2.45 (1H, m, H-10), 2.09 (3H, s, O-C-$OCH_3$), 1.29 (6H, s, 3H-26, 3H-27), 1.23, 1.18, 0.87, 0.85, 0.80 (each 3H, s, 5x$CH_3$).

### 4.11.2.2. Oxidation of compound 4

A quantity of 2mg of the compound was oxidised following the procedure outlined in 4.9. to yield **[103c]**.

$^1$H NMR data of **[103c]** (300MHz, Spectrum 4l)

δ 6.18 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6.8}$=2.30Hz, H-6), 5.78 (1H, dd, $J_{6,7}$=9.78Hz, $J_{7,8}$=3.72Hz, H-7), 5.58 (2H, m, H-23, H-24), 2.71 (1H, td, H-$2_{ax}$), 1.29 (6H, s, 3H-26, 3H-27), 1.20, 1.17, 0.92, 0.90, 0.88 (each 3H, s, 5x$CH_3$).

### 4.9.3. Compound 5 [104]

(5,19-epoxy-19(*R*)-methoxy-25-hydroxy-5β-cucrbita-6,23-diene-3β-ol)

Mobile phase: 47% hexane, 32% methylene chloride, 21% ethyl acetate

Rf value: 0.27

Description: white amorphous powder

Yield: 4.90 mg

Mass: $[M]^+$ at *m/z* 486.3725 ($C_{31}H_{50}O_4$ requires 486.3709)

Mass Spectrum: (Spectrum 5a)
EIMS m/z 486, 426, 408, 309, 281, 186, 95

IR Spectrum: (Spectrum 5b)

$\upsilon_{max}$ (KBr): 3455 $cm^{-1}$ (OH)
2951 $cm^{-1}$ ($-CH_3$, $>CH_2$)
1651 $cm^{-1}$ (C=C)
1460 $cm^{-1}$ (C-H deformations)
1377 $cm^{-1}$ ($>C(CH_3)_2$)
1157 $cm^{-1}$ (C-O stretching)

Optical rotation: $[\alpha]D = -52.8°$ ($CHCl_3$, *c* 0.036)

melting point: 102-104°C

$^1$H NMR: (300MHz, Spectrum 5c)

δ 6.10 (1H, dd, $J_{6,7}$=9.71Hz, $J_{6,8}$=2.30Hz, H-6), 5.57 (2H, bs, H-23; H-24), 5.50 (1H, dd, $J_{6,7}$=9.71Hz, $J_{7,8}$=3.72Hz, H-7), 4.40 (1H, s, H-19), 3.70 (d, OH), 3.41 (1H, bs, H-3α), 3.37 (3H, s, $OCH_3$), 2.29 (1H, m, H-10), 2.13 (1H, bs, H-8), 1.92 (2H, m, H-16), 1.72 (2H, m, H-22), 1.65 (2H, m, H-12), 1.57 (2H, m, H-2), 1.52 (2H, m, H-11), 1.48 (1H, m, H-20), 1.41 (1H, m, H-17), 1.39 (2H, m, H-15), 1.34 (2H, m, H-1), 1.29 (6H, s, 3H-26; 3H-27), 1.23 (3H, s, 3H-21), 0.88, 0.86, 0.83, 0.84 (each 3H, s, 4x$CH_3$).

$^{13}$C NMR: (75MHz, Spectrum 5d)
(Table 4 for assignments)

δ 139.5 (d), 133.0 (d), 130.5 (d), 125.3 (d), 114.7 (d), 85.1 (s), 76.2 (d), 70.7 (s), 57.3 (q), 50.1 (d), 49.8 (d), 48.9 (s), 47.9 (s), 45.1 (s), 39.1 (t), 37.9 (d), 37.1 (s), 36.2 (d), 33.5 (t), 30.4 (t), 29.9 (q), 30.0 (q), 27.8 (t), 27.1 (t), 24.4 (q), 21.4 (t), 20.6 (q), 20.5 (q), 18.6 (q), 16.5 (t), 15.0 (q).

### 4.9.4. Compound 6 [105]

(5,19-epoxy-25-methoxy-5β-cucurbita-6,23-diene-3β-ol)

Mobile phase: 53% hexane, 35% methylene chloride, 12% ethyl acetate

Rf value: 0.20

Description: white powder

Yield: 4.80 mg

Mass: $[M]^+$ at *m/z* 470.3632 ($C_{31}H_{50}O_3$ requires 470.3409)

Mass Spectrum: (Spectrum 6a)

EIMS m/z 470, 455, 440, 422, 309

IR Spectrum: (Spectrum 6b)

$\upsilon_{max}$ (KBr): 3472 $cm^{-1}$ (OH)

2943 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1446 $cm^{-1}$ (C-H deformations)

1379 $cm^{-1}$ ($>C(CH_3)_2$)

1080 $cm^{-1}$ (C-O stretching)

Melting point: 139-141°C

$^{1}$H NMR: (300MHz, Spectrum 6c)

δ 6.00 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz, H-6), 5.60 (1H, dd, $J_{6,7}$=9.78Hz, $J_{7,8}$=3.72Hz, H-7), 5.47 (1H, m, H-23), 5.40 (1H, s, H-24), 3.65, 3.49 (1H, dd, $J_{gem}$=8.40Hz, H-19), 3.31 (1H, m, H-3α), 3.13 (3H, s, OMe), 2.32 (1H, m, H-8), 2.18 (1H, m, H-10), 1.75 (2H, m, H-22), 1.61 (2H, m, H-2), 1.51 (2H, m, H-11), 1.48 (1H, m, H-17), 1.32 (2H, m, H-1), 1.31 (2H, m, H-15), 1.23 (6H, s, 3H-26, 3H-27), 1.18, 0.89, 0.87, 0.85, 0.83 (each 3H, s, 5x$CH_3$).

$^{13}$C NMR: (75 MHz, Spectrum 6d)
(Table 4 for assignments)

δ 136.8 (d), 131.8 (d), 131.5 (d), 128.3 (d), 87.5 (s), 79.8 (t), 76.1 (d), 74.8 (s), 52.0 (d), 50.3 (q), 49.9 (d), 48.6 (s), 45.5 (s), 45.2 (s), 39.4 (t), 38.8 (d), 37.2 (s), 36.1 (d), 33.2 (t), 30.8 (t), 27.9 (t), 27.3 (t), 26.1 (q), 25.8 (q), 24.5 (q), 23.6 (t), 20.5 (q), 20.0 (q), 18.7 (q), 17.6 (t), 14.9 (q).

### 4.9.5. Compound 7 [106]

(5,19-epoxy-19(*R*),25-dimethoxy-5β-cucurbita-6,23-diene-3β-ol)

Mobile phase: 54% hexane, 36% methylene chloride, 10% ethyl acetate

Rf value: 0.25

Description: colourless gum

Yield: 6.5 mg

Mass: $[M]^+$ at *m/z* 500.3874 ($C_{32}H_{52}O_4$ requires 500.3865)

Mass Spectrum: (Spectrum 7a)

EIMS m/z 500, 440, 375, 309, 172, 109

IR Spectrum: (Spectrum 7b)

$\upsilon_{max}$ (KBr): 3516 $cm^{-1}$ (OH)

2926 $cm^{-1}$ , 2876 $cm^{-1}$ ($-CH_3$, $>CH_2$)

1377 $cm^{-1}$ ($C(CH_3)_2$)

1109 $cm^{-1}$ (C-O-C stretching)

1070 $cm^{-1}$ (C-O stretching)

Optical rotation: $[\alpha]_D = -37.1°$ ($CHCl_3$, *c* 0.054)

$^{1}$H NMR: (300MHz, Spectrum 7c)

δ 6.07 (1H, dd, $J_{6,7}$=9.78Hz, $J_{6,8}$=2.30Hz), 5.50 (1H, m, H-7), 5.45 (1H, m, H-23), 5.40 (1H, s, H-24), 4.40 (1H, s, H-19), 3.37, 3.13 (each 3H, s, 2xOMe), 3.48 (1H, m, H-3α), 2.24 (1H, m, H-8), 2.15 (1H, m, H-10), 1.72 (2H, m, H-22), 1.59 (1H, m, H-20), 1.45 (2H, m, H-1), 1.23 (6H, s, 3H-26, 3H-27), 0.90, 0.88, 0.87, 0.86, 0.83 (3H, s, 5x$CH_3$).

$^{13}$C NMR: (75MHz, Spectrum 7e)

δ 133.5 (d), 130.5 (d), 128.4 (d), 114.7 (d), 85.1 (s), 76.2 (d), 74.9 (s), 57.3 (q), 50.3 (q), 50.0 (d), 49.8 (d), 48.9 (s), 48.0 (s), 45.1 (s), 39.4 (t), 37.9 (d), 37.1 (s), 36.8 (d), 36.1 (d), 33.5 (t), 30.4 (t), 27.8 (t), 27.1 (t), 26.1 (q), 25.8 (q), 24.4 (q), 21.4 (t), 20.6 (q), 19.9 (q), 18.7 (q), 16,5 (t), 15.0 (q).

# Appendix 1

Table 3. $^{13}C$ NMR data for compounds 1 and 2 (75MHz, $CDCl_3$, TMS as internal standard) All chemical shift values are expressed in ppm (δ)

| Carbon number | Compound 1 | Compound 2 |
|---|---|---|
| 1 | 27.7 (t) | 27.4 (t) |
| 2 | 28.4 (t) | 28.4 (t) |
| 3 | 76.1 (d) | 76.1 (d) |
| 4 | 41.4 (s) | 41.4 (s) |
| 5 | 145.5 (s) | 145.5 (s) |
| 6 | 123.9 (d) | 123.9 (d) |
| 7 | 66.2 (d) | 66.3 (d) |
| 8 | 50.8 (d) | 49.9 (d) |
| 9 | 49.9 (s) | 49.9 (s) |
| 10 | 36.6 (d) | 36.6 (d) |
| 11 | 23.5 (t) | 23.5 (t) |
| 12 | 29.1 (t) | 28.9 (t) |
| 13 | 45.4 (s) | 45.3 (s) |
| 14 | 47.6 (s) | 47.5 (s) |
| 15 | 34.6 (t) | 34.7 (t) |
| 16 | 21.2 (t) | 21.2 (t) |
| 17 | 47.4 (d) | 47.5 (d) |
| 18 | $14.8^{I}$ (q) | $14.9^{I}$ (q) |
| 19 | 207.8 (d) | 207.8 (d) |
| 20 | 32.6 (d) | 36.1 (d) |
| 21 | 18.8 (q) | 18.7 (q) |
| 22 | 44.4 (t) | 39.0 (t) |
| 23 | 65.9 (d) | $125.1^{II}$ (d) |
| 24 | 128.9 (d) | $139.6^{II}$ (d) |
| 25 | 133.9 (s) | 70.7 (s) |
| 26 | 18.1 (q) | 29.8 (q) |
| 27 | 25.7 (q) | 29.9 (q) |
| 28 | $25.3^{I}$ (q) | $27.1^{I}$ (q) |
| 29 | $27.2^{I}$ (q) | $25.3^{I}$ (q) |
| 30 | $17.9^{I}$ (q) | $17.9^{I}$ (q) |

NOTE: Assignments denoted $^{I}$ and $^{II}$ within any vertical column can be interchanged.

Table 4. $^{13}C$ NMR data for compounds 3, 4, 5, 6 and 7 (75MHz, $CDCl_3$, TMS as internal standard) All chemical shift values are expressed in ppm (δ)

| Carbon number | Compound 3 | Compound 4 | Compound 5 | Compound 6 | Compound 7 |
|---|---|---|---|---|---|
| 1 | 17.3 (t) | 17.3 (t) | 16.5 (t) | 17.6 (t) | 16.5 (t) |
| 2 | 23.1 (t) | 23.1 (t) | 21.4 (t) | 23.6 (t) | 21.4 (t) |
| 3 | 76.1 (d) | 76.0 (d) | 76.2 (d) | 76.1 (d) | 76.2 (d) |
| 4 | 45.1 (s) | 45.1 (s) | 45.1 (s) | 45.2 (s) | 45.1 (s) |
| 5 | 86.6 (s) | 86.6 (s) | 85.1 (s) | 87.5 (s) | 85.1 (s) |
| 6 | 132.7 (d) | 132.7 (d) | 33.0 (d) | 131.8 (d) | 133.5 (d) |
| 7 | 132.4 (d) | 132.4 (d) | 30.5 (d) | 131.5 (d) | 130.5 (d) |
| 8 | 41.4 (d) | 40.6 (d) | 49.8 (d) | 52.0 (d) | 49.8 (d) |
| 9 | 48.0 (s) | 48.0 (s) | 48.9 (s) | 45.5 (s) | 48.9 (s) |
| 10 | 40.6 (d) | 41.4 (d) | 37.9 (d) | 38.8 (d) | 36.1 (d) |
| 11 | 30.5 (t) | 30.5 (t) | 30.4 (t) | 30.8 (t) | 30.4 (t) |
| 12 | 39.4 (t) | 39.1 (t) | 39.1 (t) | 39.4 (t) | 39.4 (t) |
| 13 | 37.2 (s) | 38.0 (s) | 37.1 (s) | 37.2 (s) | 37.1 (s) |
| 14 | 48.5 (s) | 48.5 (s) | 47.9 (s) | 48.6 (s) | 48.0 (s) |
| 15 | 33.5 (t) | 33.5 (t) | 33.5 (t) | 33.2 (t) | 33.5 (t) |
| 16 | 27.9 (t) | 27.9 (t) | 27.8 (t) | 27.9 (t) | 27.8 (t) |
| 17 | 49.4 (d) | 50.0 (d) | 50.1 (d) | 49.9 (d) | 50.1(d) |
| 18 | $14.7^{I}$ (q) | $14.7^{I}$ (q) | $15.0^{I}$ (q) | 14.9I (q) | $15.0^{I}$ (q) |
| 19 | 105.4 (d) | 105.4 (d) | 114.7 (d) | 79.8 (t) | 114.7 (d) |
| 20 | 36.1 (d) | 36.2 (d) | 36.2 (d) | 36.1 (d) | 37.9 (d) |
| 21 | 18.7 (q) | 18.6 (q) | 18.6 (q) | 18.7 (q) | 18.7 (q) |
| 22 | 27.1 (t) | 30.0 (t) | 27.1 (t) | 27.3 (t) | 27.1 (t) |
| 23 | 128. 3 (d) | 125.2 (d) | 125.3 (d) | 128.3 (d) | 128.4 (d) |
| 24 | 136.8 (d) | 139.6 (d) | 139.5 (d) | 136.8 (d) | 136.8 (d) |
| 25 | 74.9 (s) | 70.7 (s) | 70.7 (s) | 74.8 (s) | 74.9 (s) |
| 26 | $26.2^{II}$ (q) | $29.8^{II}$ (q) | $30.0^{II}$ (q) | $26.1^{II}$ (q) | $26.1^{II}$ (q) |
| 27 | $25.8^{II}$ (q) | $27.1^{II}$ (q) | $29.9^{II}$ (q) | $25.8^{II}$ (q) | $25.8^{II}$ (q) |
| 28 | $20.5^{I}$ (q) | $23.9^{I}$ (q) | $20.6^{I}$ (q) | $20.5^{I}$ (q) | $20.6^{I}$ (q) |
| 29 | $23.9^{I}$ (q) | $20.4^{I}$ (q) | $24.4^{I}$ (q) | $24.5^{I}$ (q) | $24.4^{I}$ (q) |
| 30 | $19.7^{I}$ (q) | $19.7^{I}$ (q) | $20.5^{I}$ (q) | $20.0^{I}$ (q) | $19.9^{I}$ (q) |
| OMe | 50.3 (q) | | 57.3 (q) | 50.3 (q) | 57.3 (q) |
| OMe | | | | | 50.3 (q) |

NOTE: Assignments denoted $^{I}$ and $^{II}$ within any vertical column can be interchanged.

Table 5. $^1$H NMR data for compounds 1 and 2 (300MHz, $CDCl_3$, TMS as internal standard) All chemical shift values are expressed in ppm (δ)

| Proton | Compound 1 | Compound 2 |
|---|---|---|
| 1 | 1.81 | 1.82 |
| 2 | 1.75 | 1.71 |
| 3 | 3.54 | 3.55 |
| 6 | 5.87 | 5.87 |
| 7 | 3.95 | 3.95 |
| 8 | 1.39 | |
| 10 | 2.52 | 2.52 |
| 11 | 1.54 | |
| 12 | 1.65 | |
| 15 | 1.38 | 1.30 |
| 16 | 1.45 | |
| 17 | 2.07 | |
| 3H-18 | 0.89[I] | 0.89[I] |
| 19 | 9.69 | 9.61 |
| 20 | 1.71 | 1.43 |
| 3H-21 | 0.96 | 0.87[I] |
| 22 | 1.61 | 1.69 |
| 23 | 4.44 | 5.56 |
| 24 | 5.17 | 5.56 |
| 3H-26 | 1.66 | 1.29 |
| 3H-27 | 1.68 | 1.29 |
| 3H-28 | 1.22[I] | 1.22[I] |
| 3H-29 | 1.03[I] | 1.03[I] |
| 3H-30 | 0.73[I] | 0.73[I] |

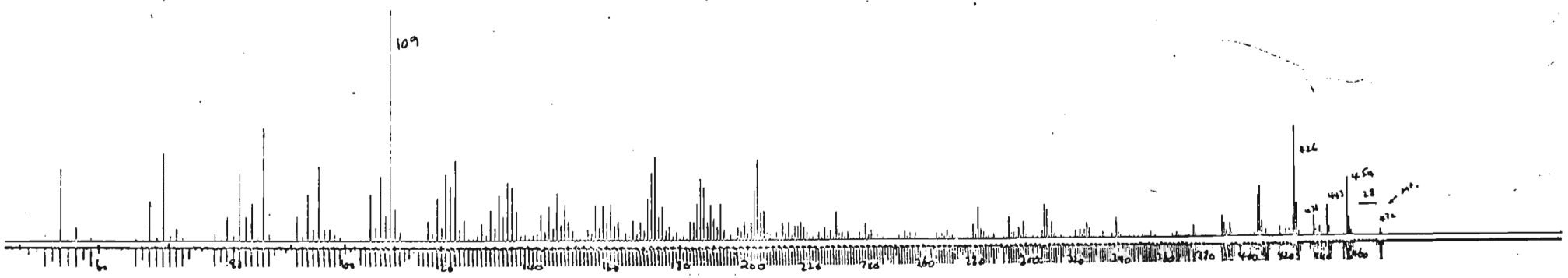
NOTE: Assignments denoted[I] within any vertical column can be interchanged

Table 6. $^{1}H$ NMR data for compounds 3,4,5,6, and 7 (300MHz, CDCl3, TMS as internal standard) All chemical shift values are expressed in ppm (δ)

| Proton | Compound 3 | Compound 4 | Compound 5 | Compound 6 | Compound 7 |
|---|---|---|---|---|---|
| 1 | 1.41 | 1.47 | 1.34 | 1.32 | 1.45 |
| 2 | 1.65 | 1.68 | 1.57 | 1.61 | 1.72 |
| 3 | 3.38 | 3.40 | 3.41 | 3.31 | 3.48 |
| 6 | 6.06 | 6.06 | 6.10 | 6.00 | 6.07 |
| 7 | 5.65 | 5.65 | 5.50 | 5.60 | 5.50 |
| 8 | 2.82 | 2.82 | 2.13 | 2.32 | 2.24 |
| 10 | 2.45 | 2.45 | 2.29 | 2.18 | 2.15 |
| 11 | 1.61 | 1.61 | 1.52 | 1.51 | |
| 12 | 1.82 | | 1.65 | | |
| 15 | 1.38 | 1.35 | 1.39 | 1.31 | |
| 16 | 1.94 | 1.92 | 1.92 | | |
| 17 | 1.40 | 1.30 | 1.41 | 1.38 | |
| 18 | $0.83^{I}$ | $0.83^{I}$ | $0.83^{I}$ | $0.83^{I}$ | $0.83^{I}$ |
| 19 | 5.11 | 5.10 | 4.40 | 3.65; 3.49 | 4.40 |
| 20 | 1.47 | 1.50 | 1.48 | 1.48 | 1.59 |
| 21 | 1.19 | 1.20 | 1.23 | 1.18 | $0.90^{I}$ |
| 22 | 1.76 | 1.78 | 1.72 | 1.75 | 1.72 |
| 23 | 5.42 | 5.58 | 5.57 | 5.47 | 5.45 |
| 24 | 5.40 | 5.58 | 5.57 | 5.40 | 5.40 |
| 26 | 1.23 | 1.29 | 1.29 | 1.23 | 1.23 |
| 27 | 1.23 | 1.29 | 1.29 | 1.23 | 1.23 |
| 28 | $0.89^{I}$ | $0.86^{I}$ | $0.87^{I}$ | $0.87^{I}$ | $0.87^{I}$ |
| 29 | $0.85^{I}$ | $0.85^{I}$ | $0.84^{I}$ | $0.85^{I}$ | $0.86^{I}$ |
| 30 | $0.87^{I}$ | $0.87^{I}$ | $0.88^{I}$ | $0.89^{I}$ | $0.88^{I}$ |
| OMe | 3.13 | | 3.37 | 3.13 | 3.37 |
| OMe | | | | | 3.13 |

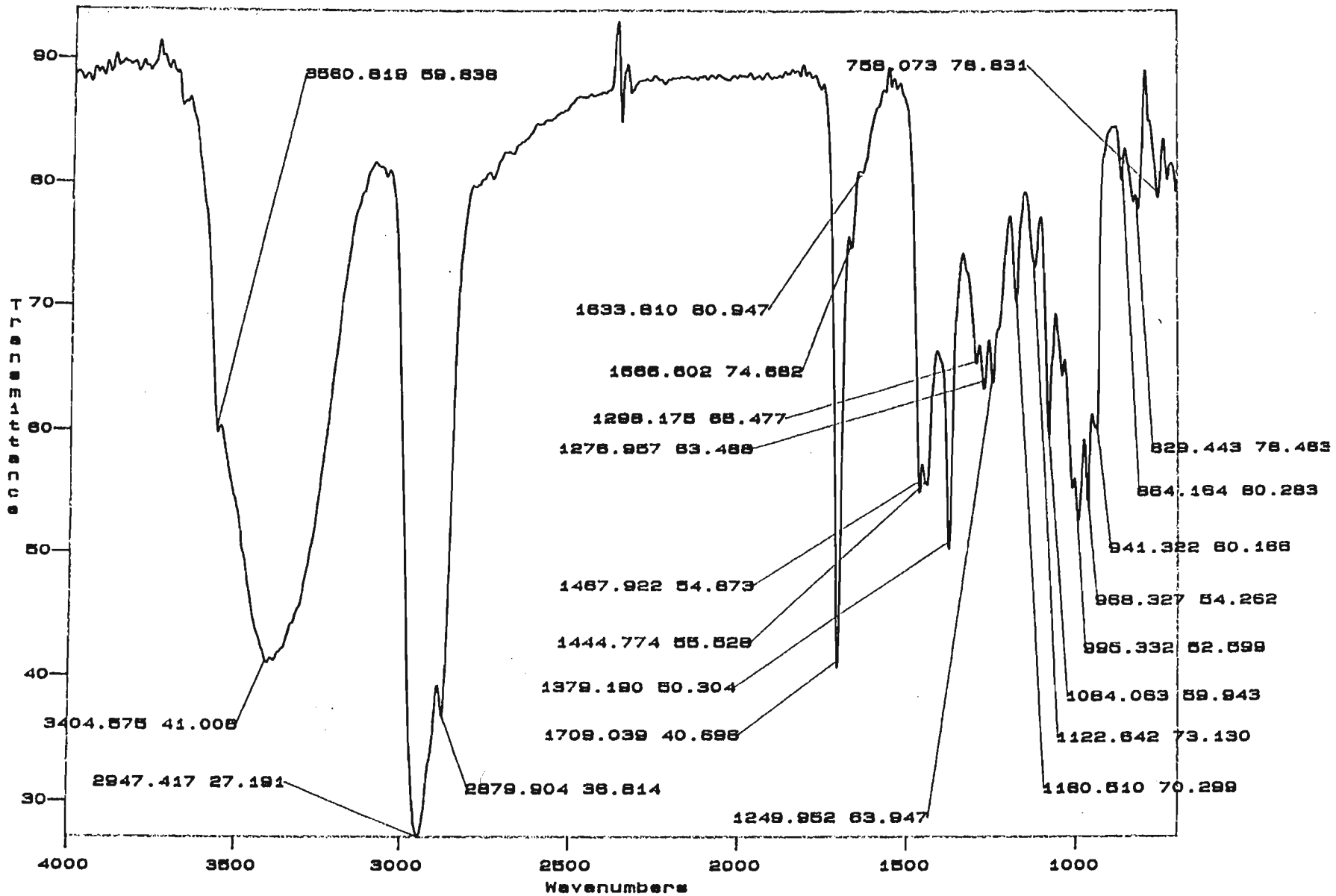
NOTE: All assignments denoted $^{I}$ in any vertical column can be interchanged

# APPENDIX 2

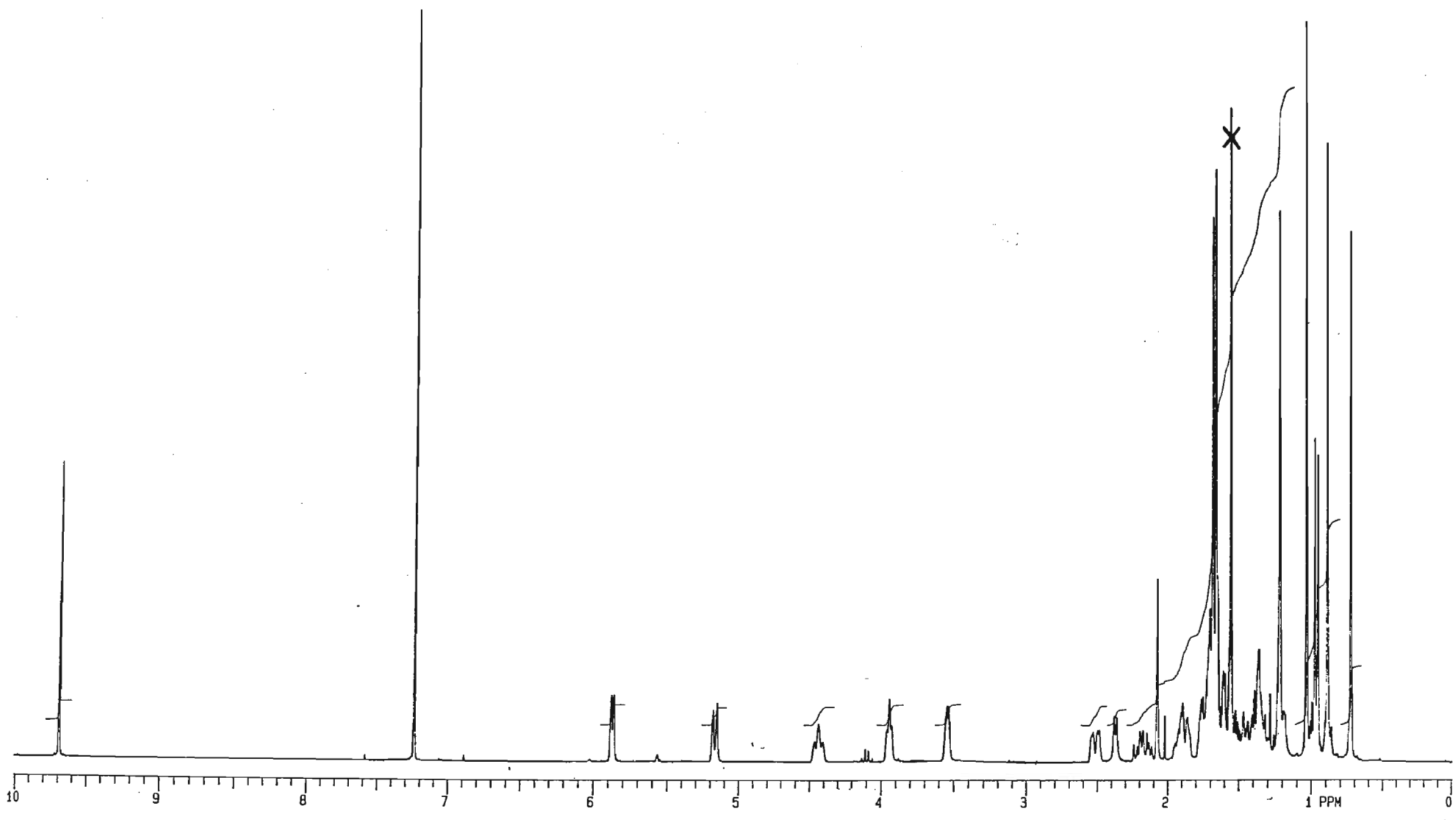





Spectrum 1a: Mass spectrum of **[92]**




Spectrum 1b: Infrared spectrum of [92]

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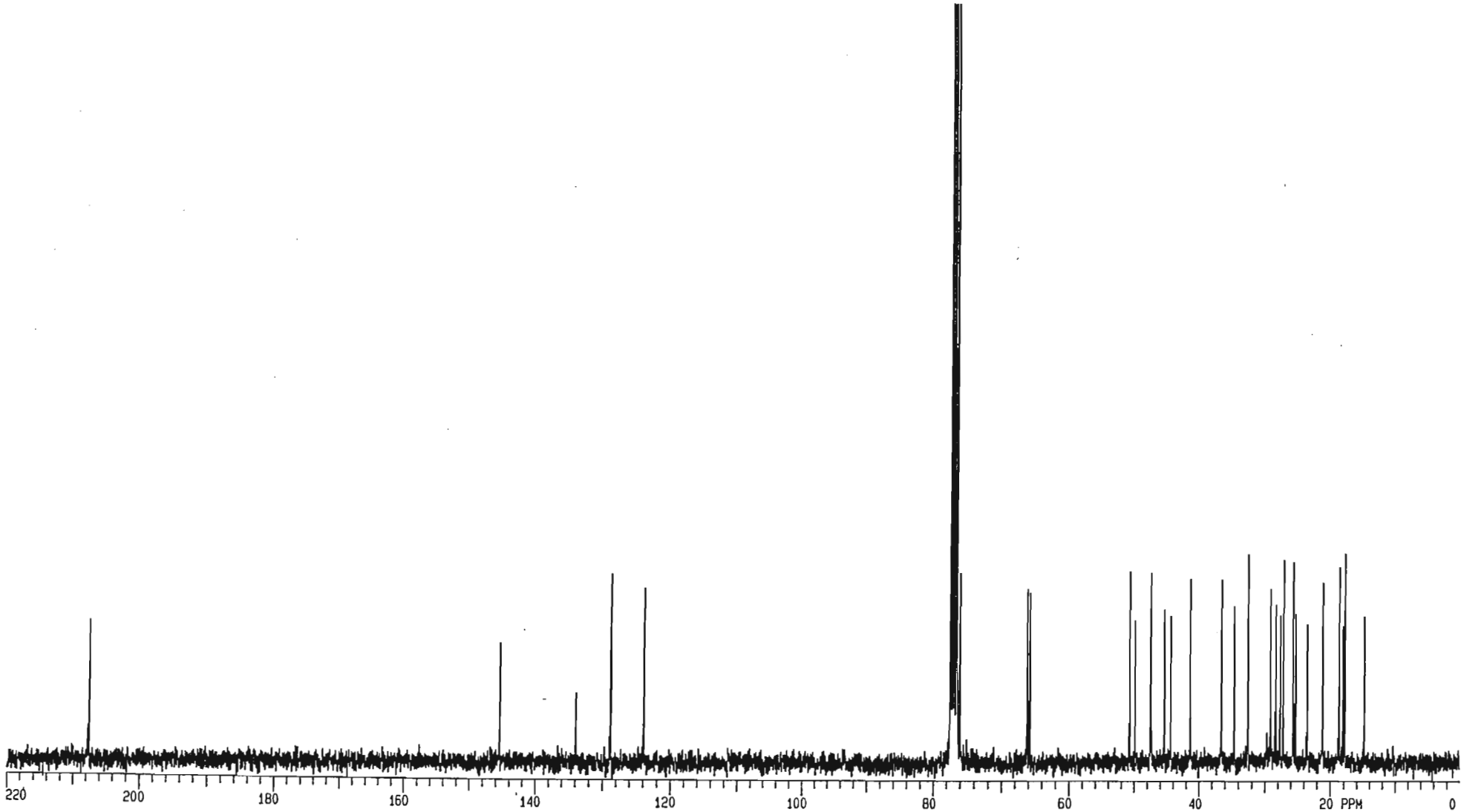
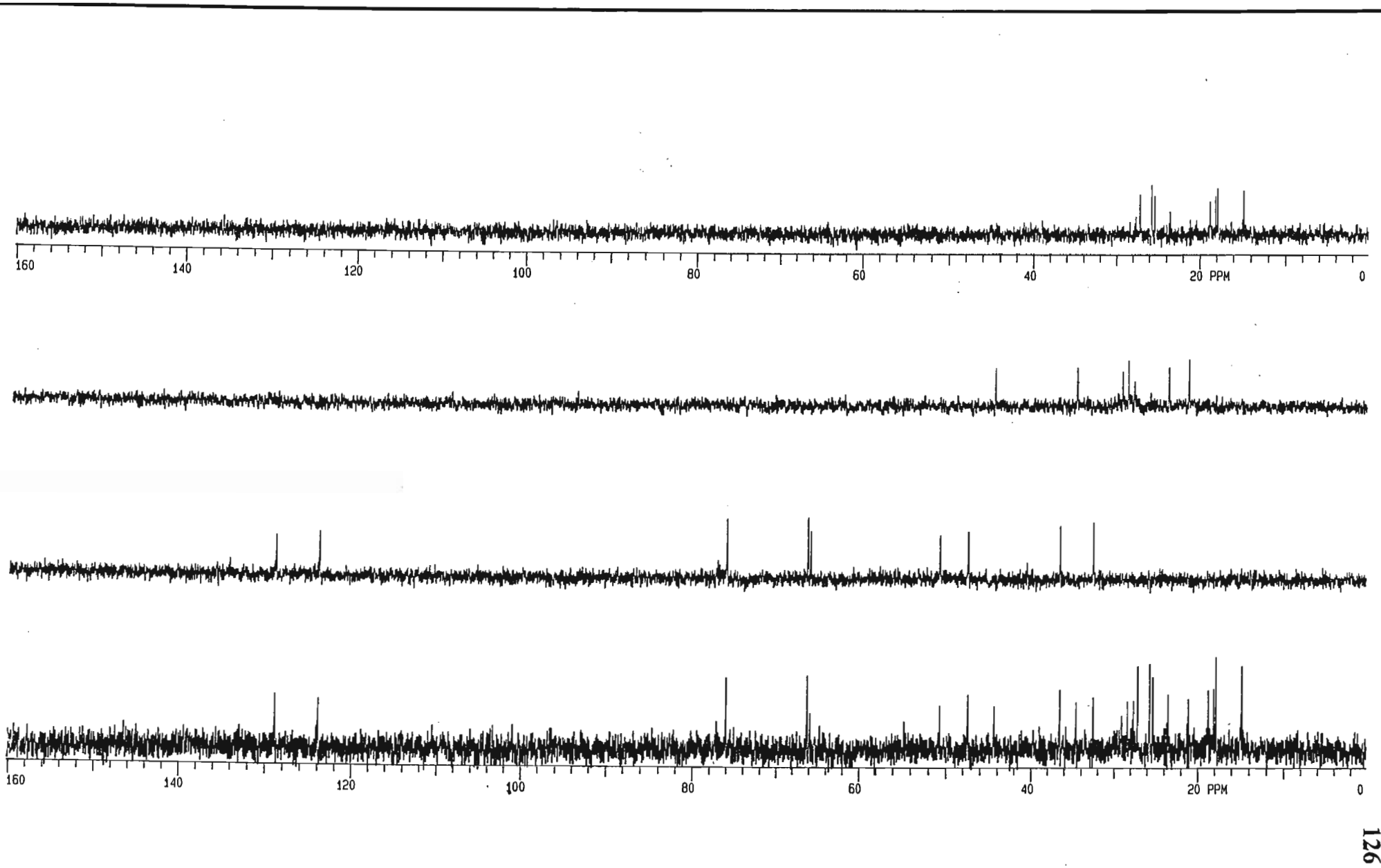
Spectrum 1c: $^{1}H$ NMR spectrum of **[92]**



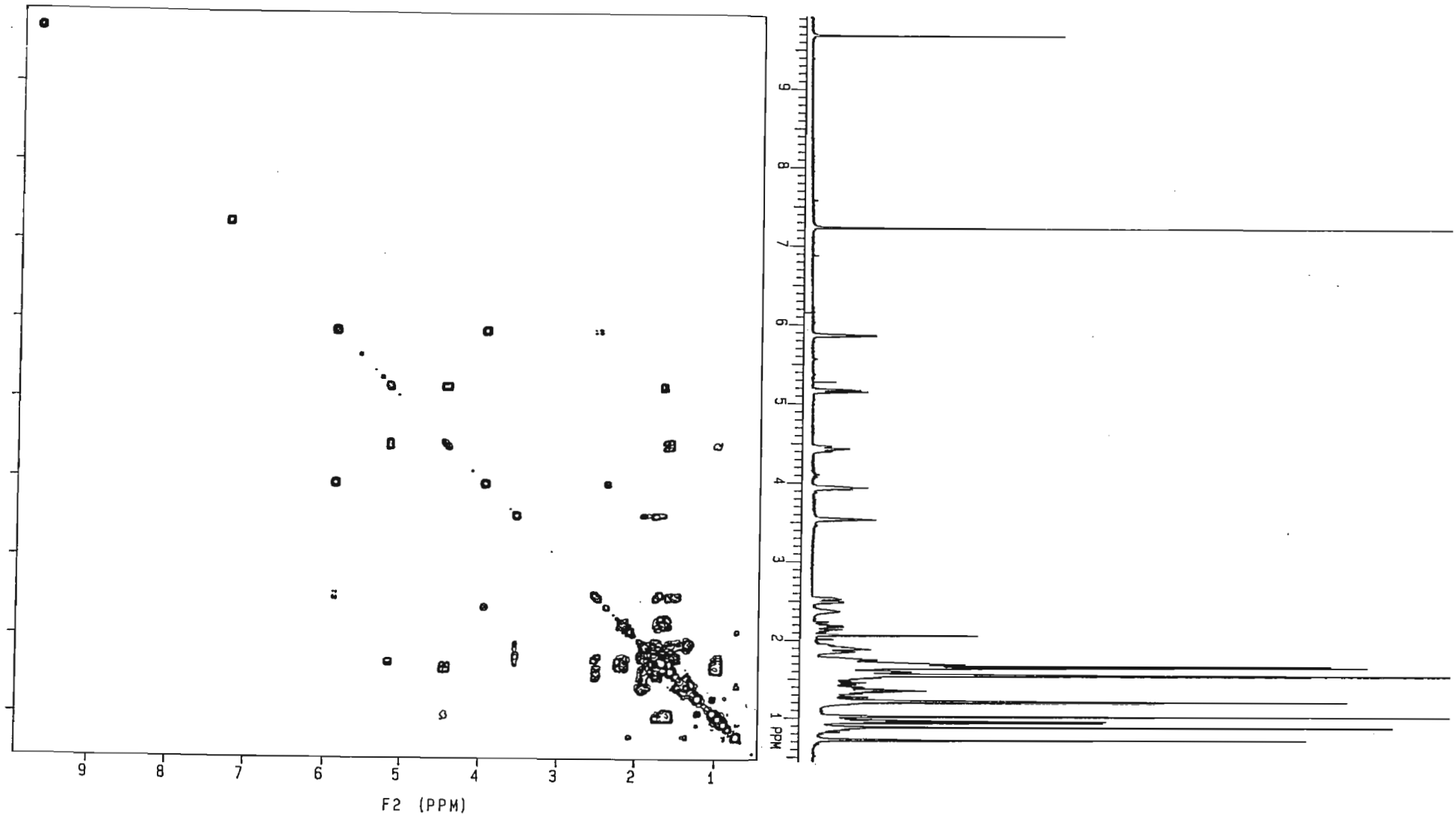



Spectrum 1d: $^{13}C$ NMR spectrum of **[92]**

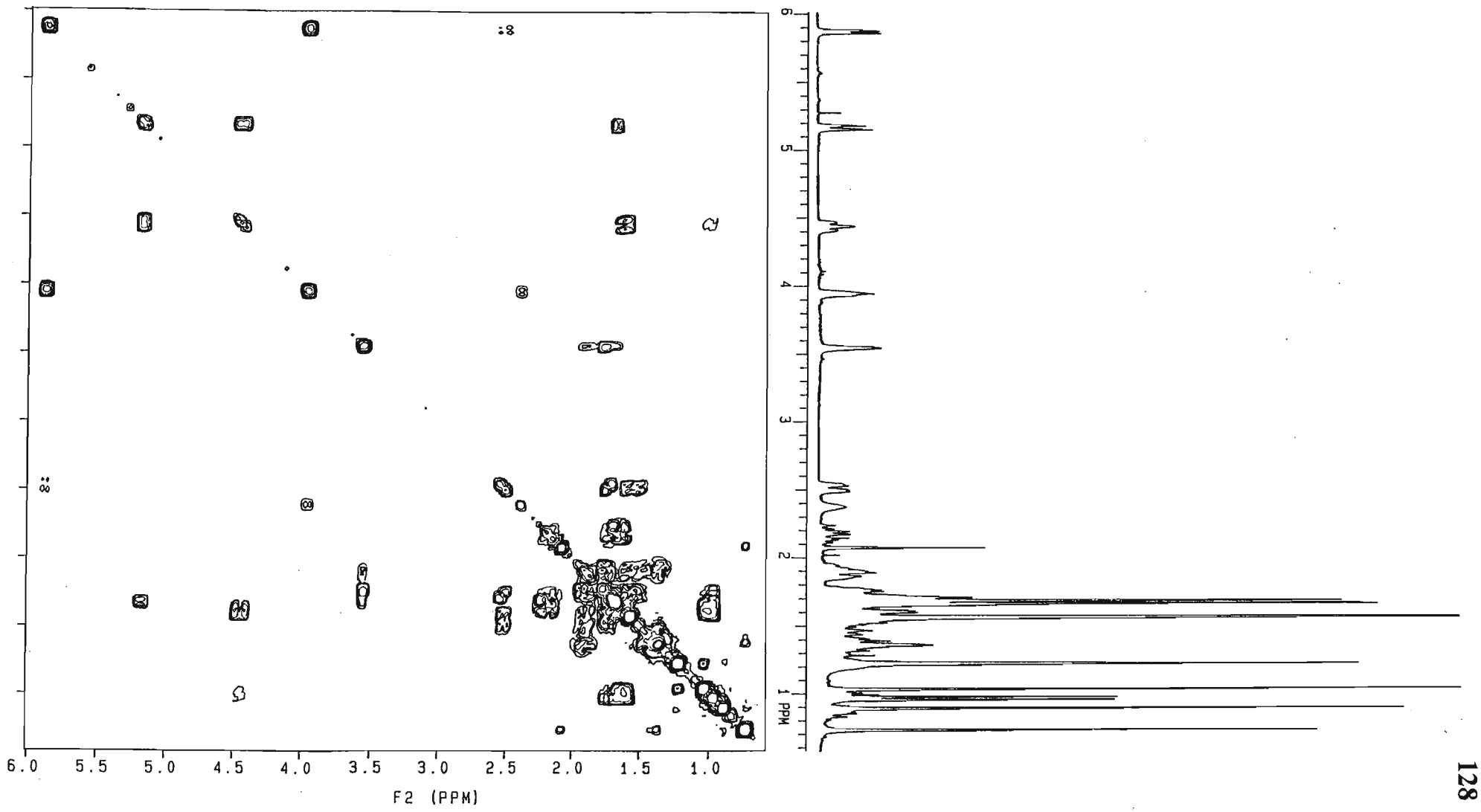




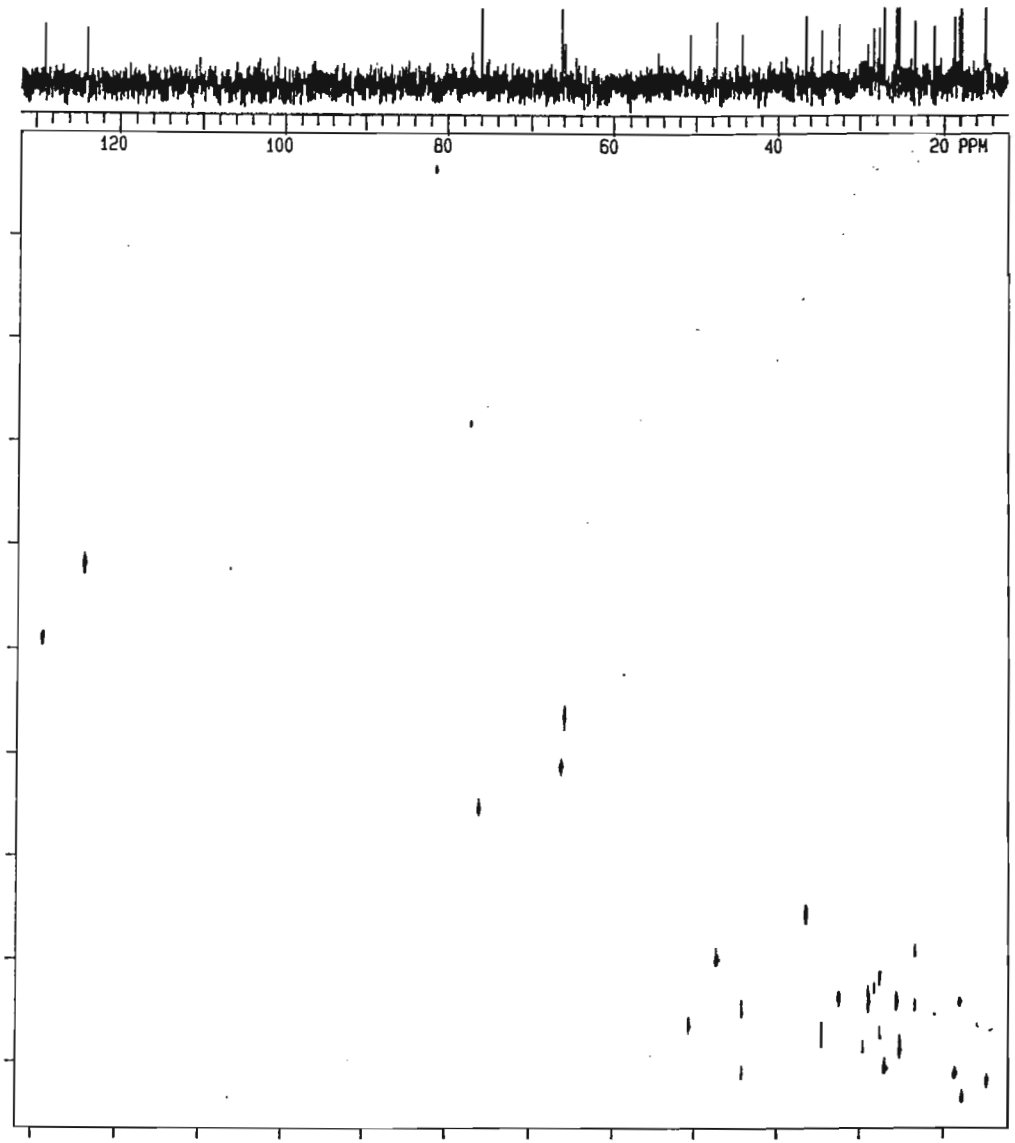

Spectrum 1e: DEPT spectrum of **[92]**

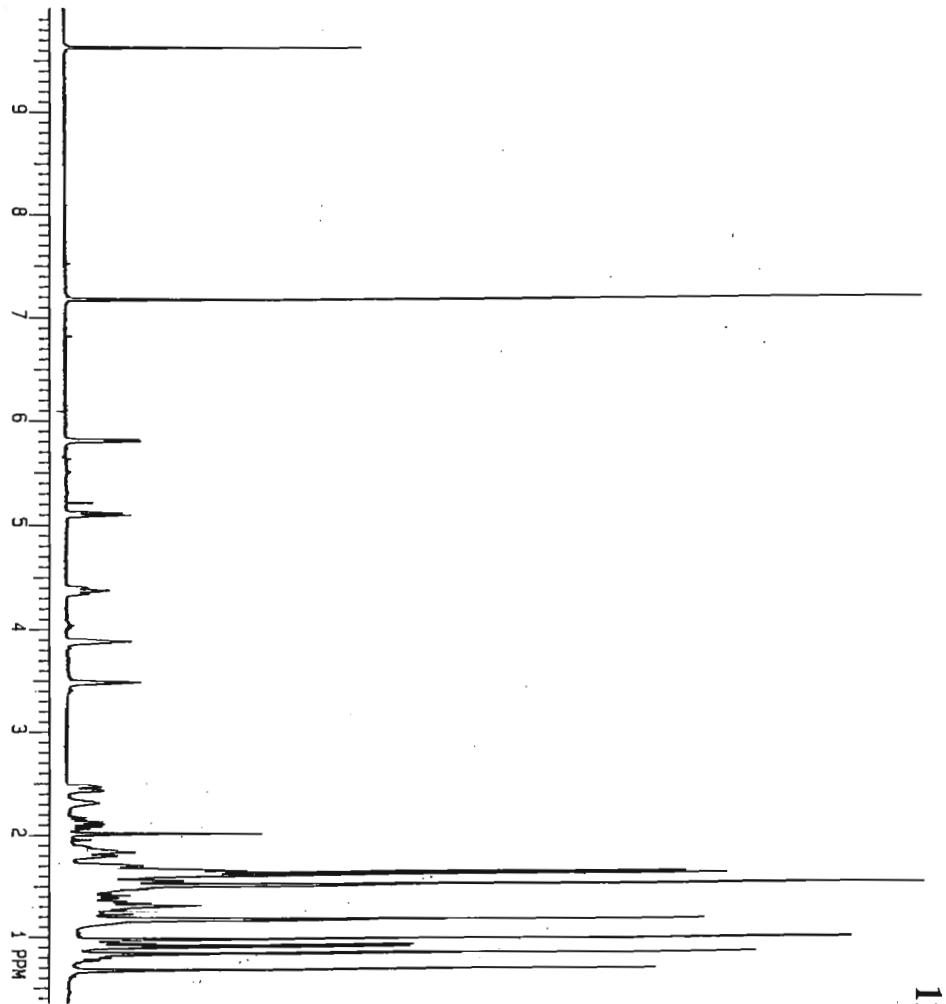



Spectrum 1f: COSY spectrum of **[92]**

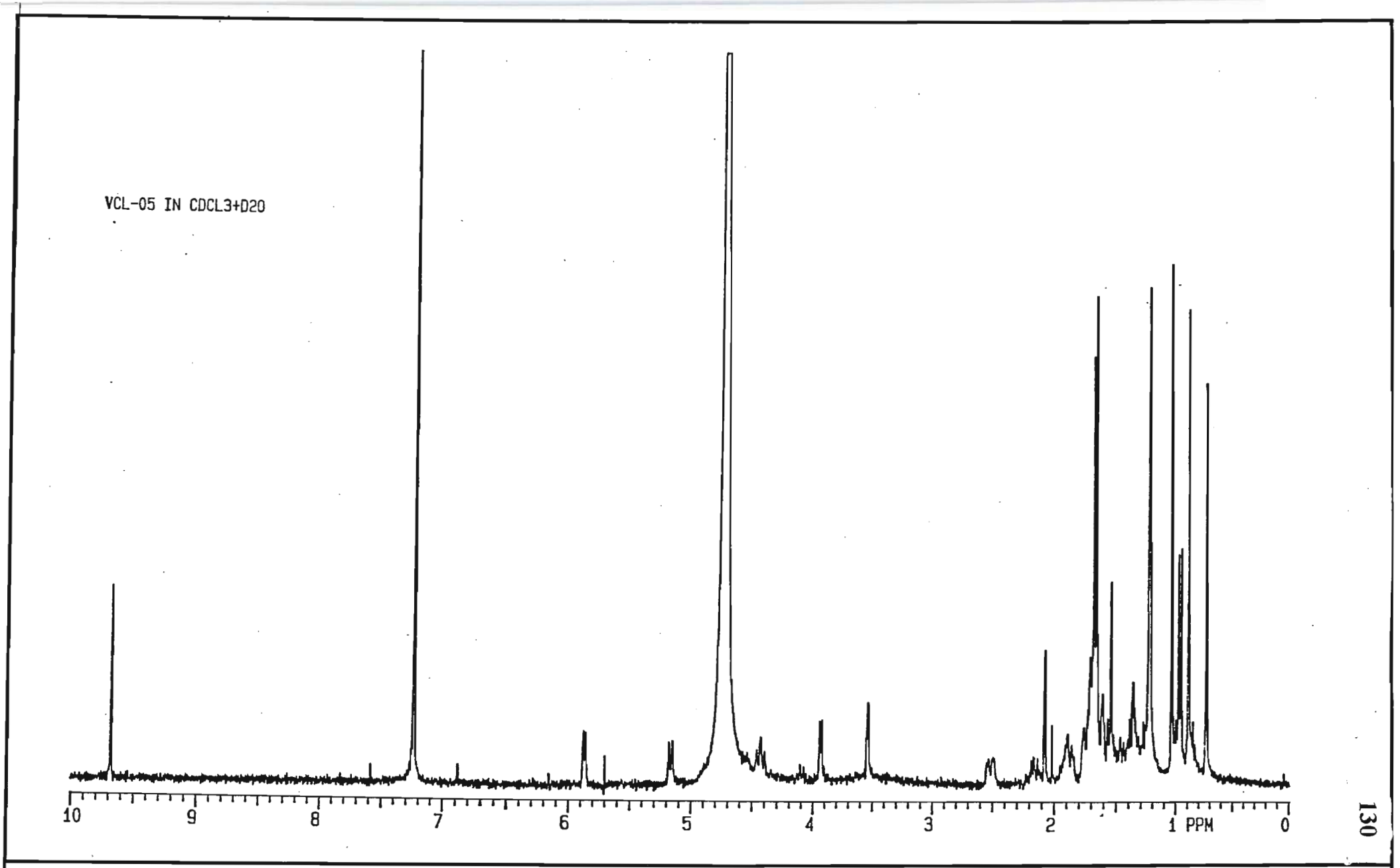



Spectrum 1g: Expanded COSY spectrum of **[92]**

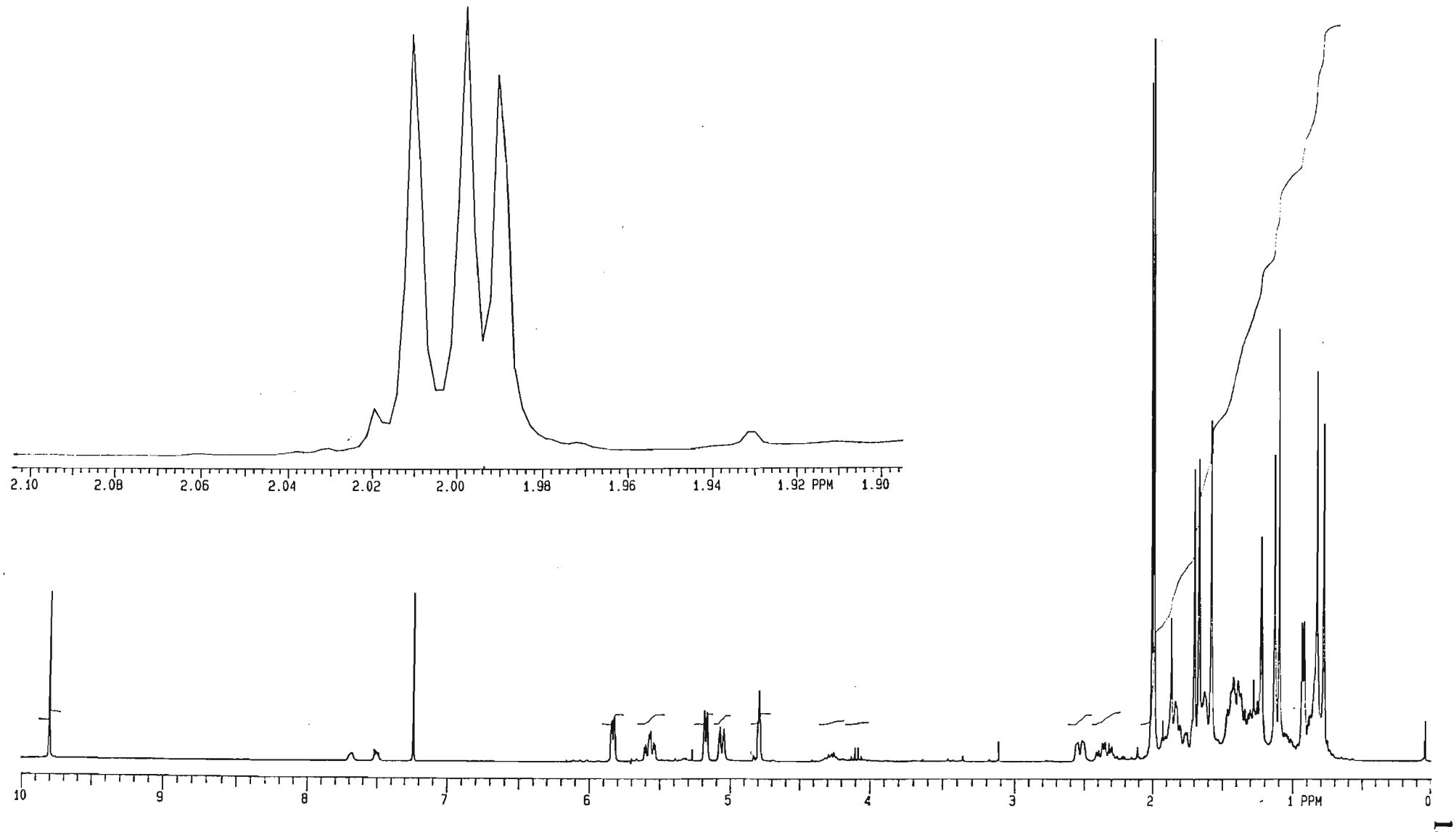



Spectrum 1h: HETCOR spectrum of **[92]**

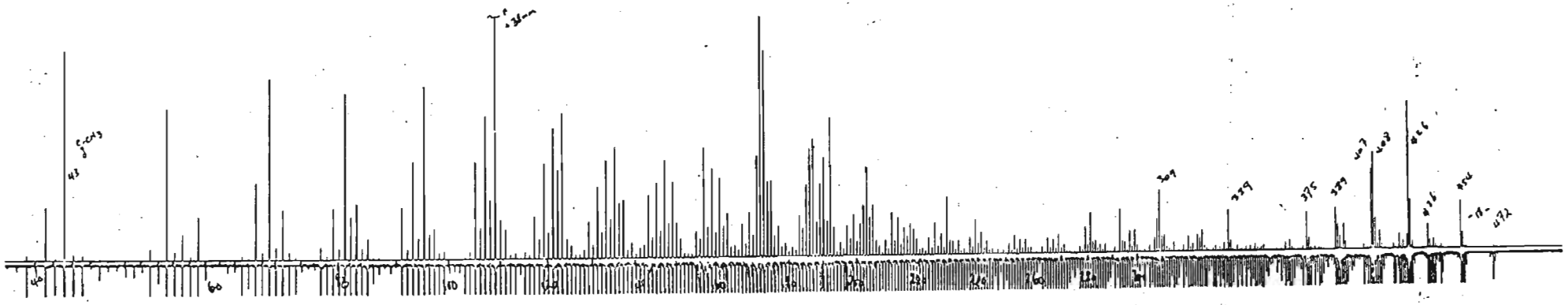



Spectrum 1i: $^{1}H$ NMR spectrum of **[92]** with $D_2O$



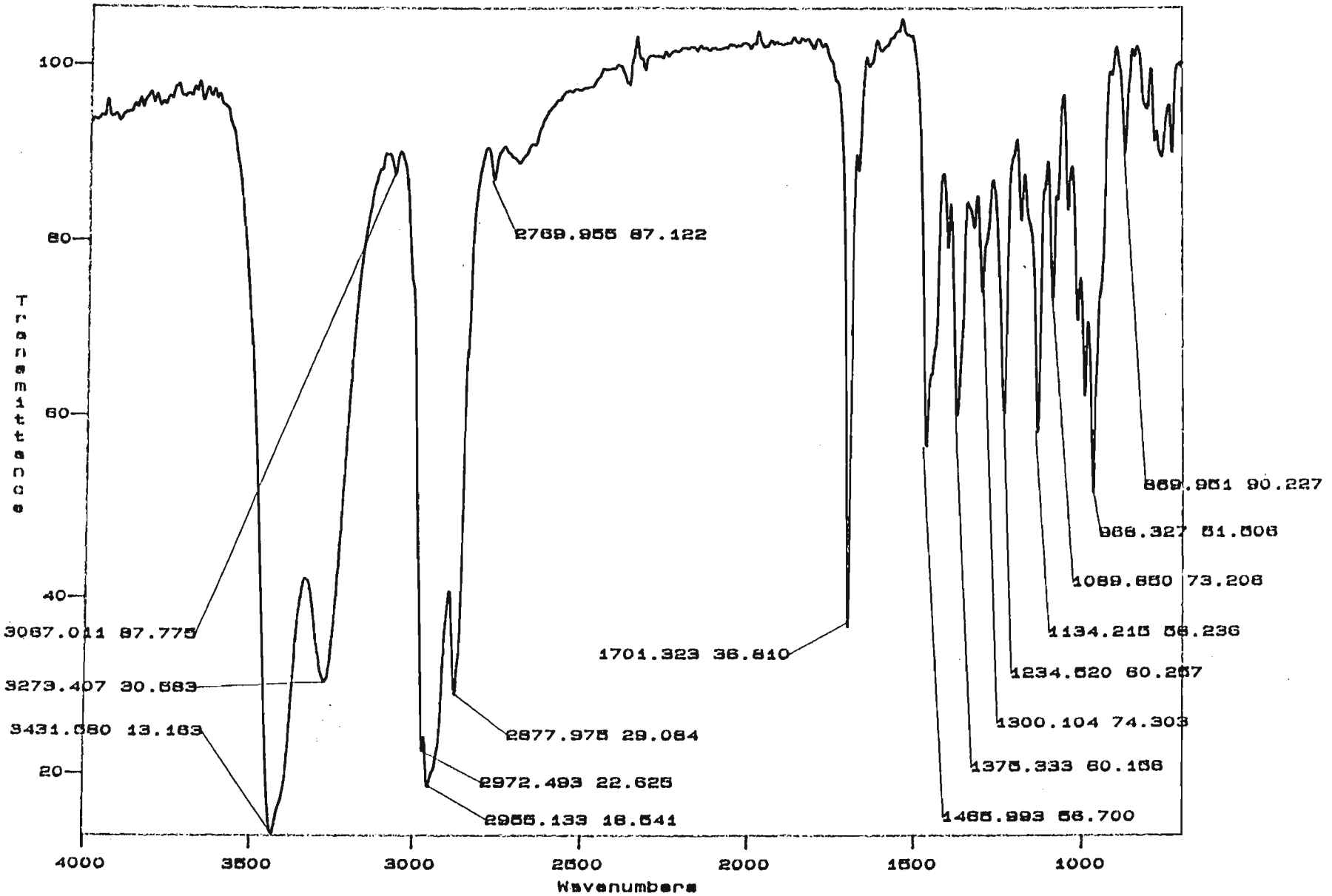



Spectrum 1j: $^{1}$H NMR spectrum of **[92a]**

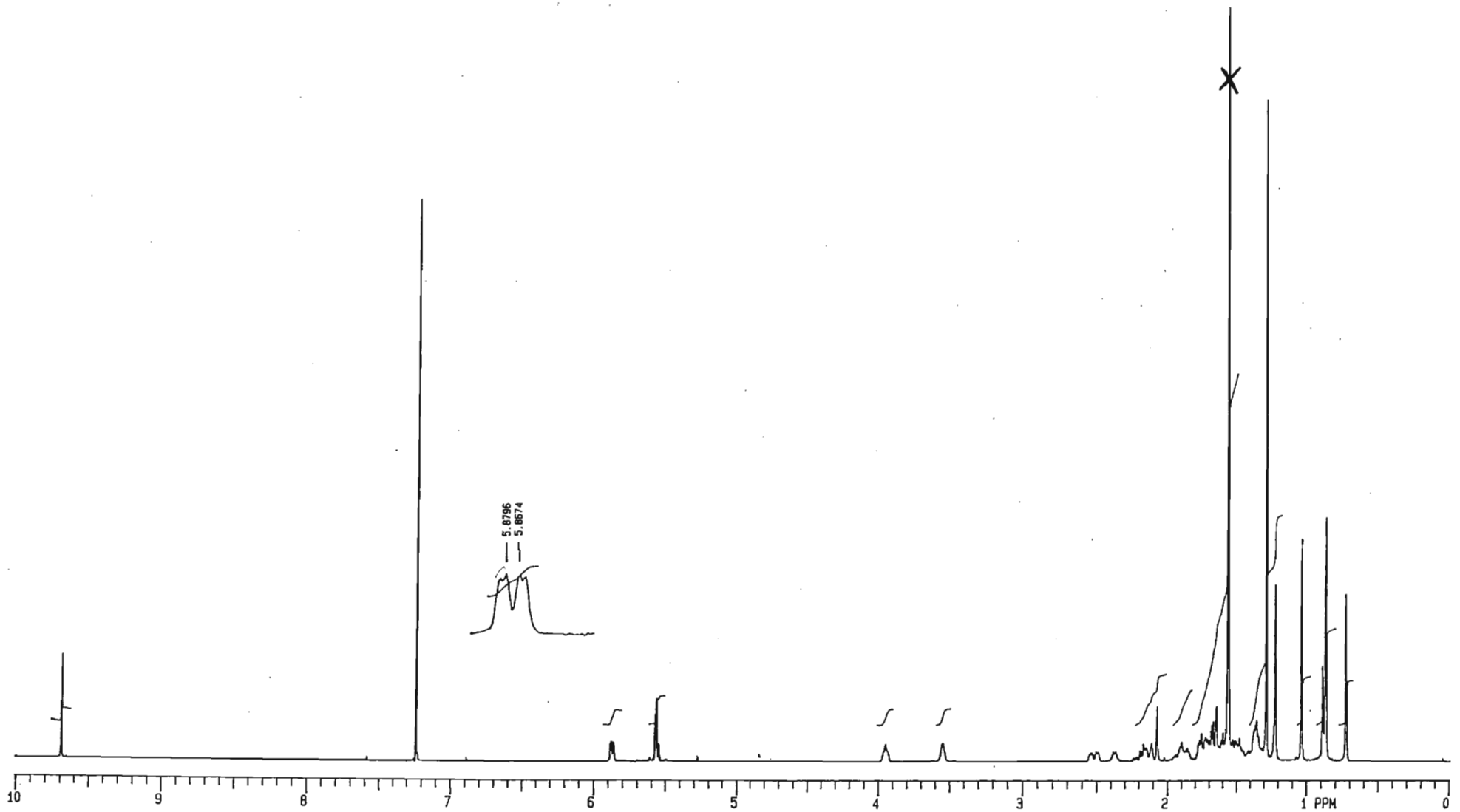




Spectrum 2a: Mass spectrum **[93]**

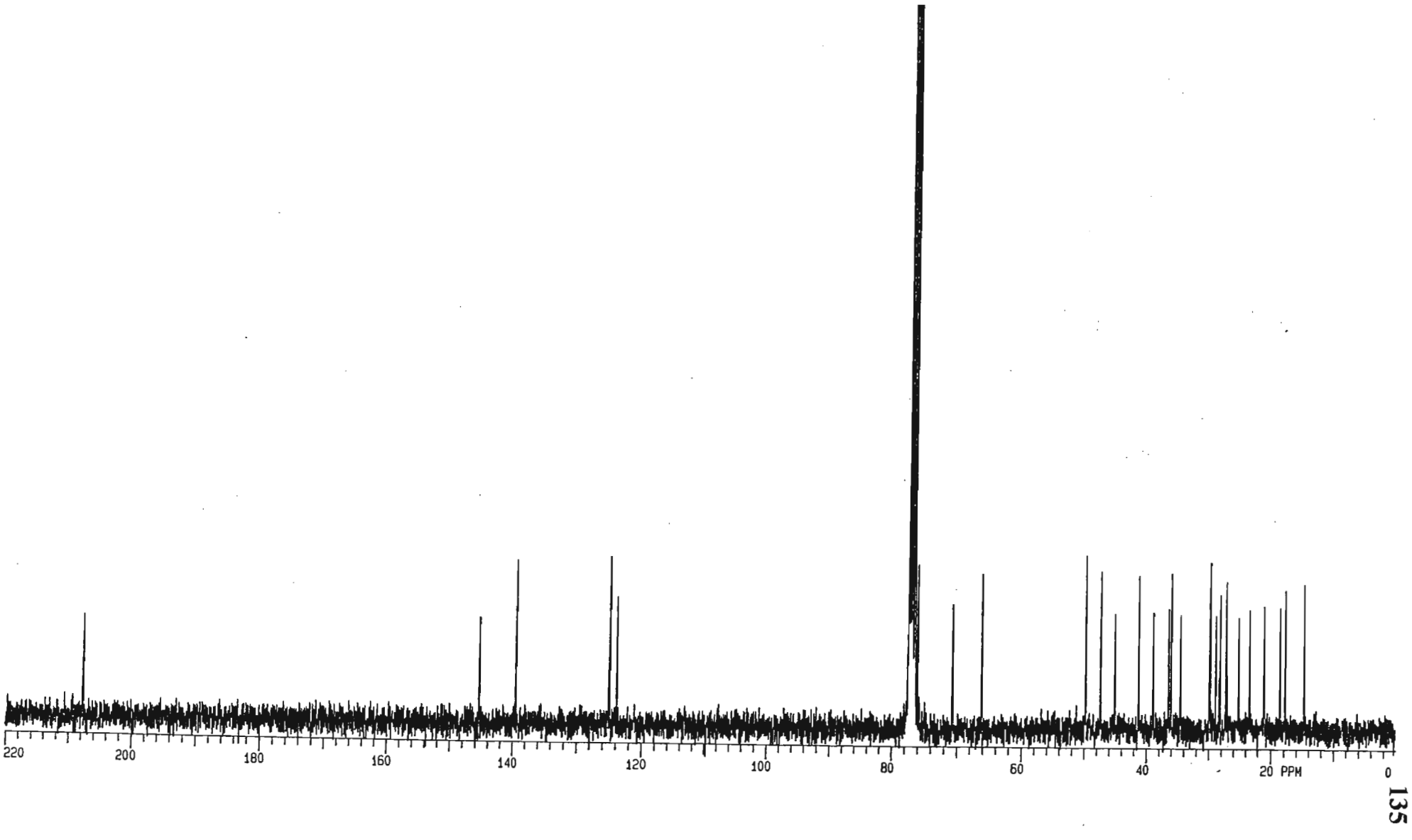



Spectrum 2b: Infrared spectrum of **[93]**



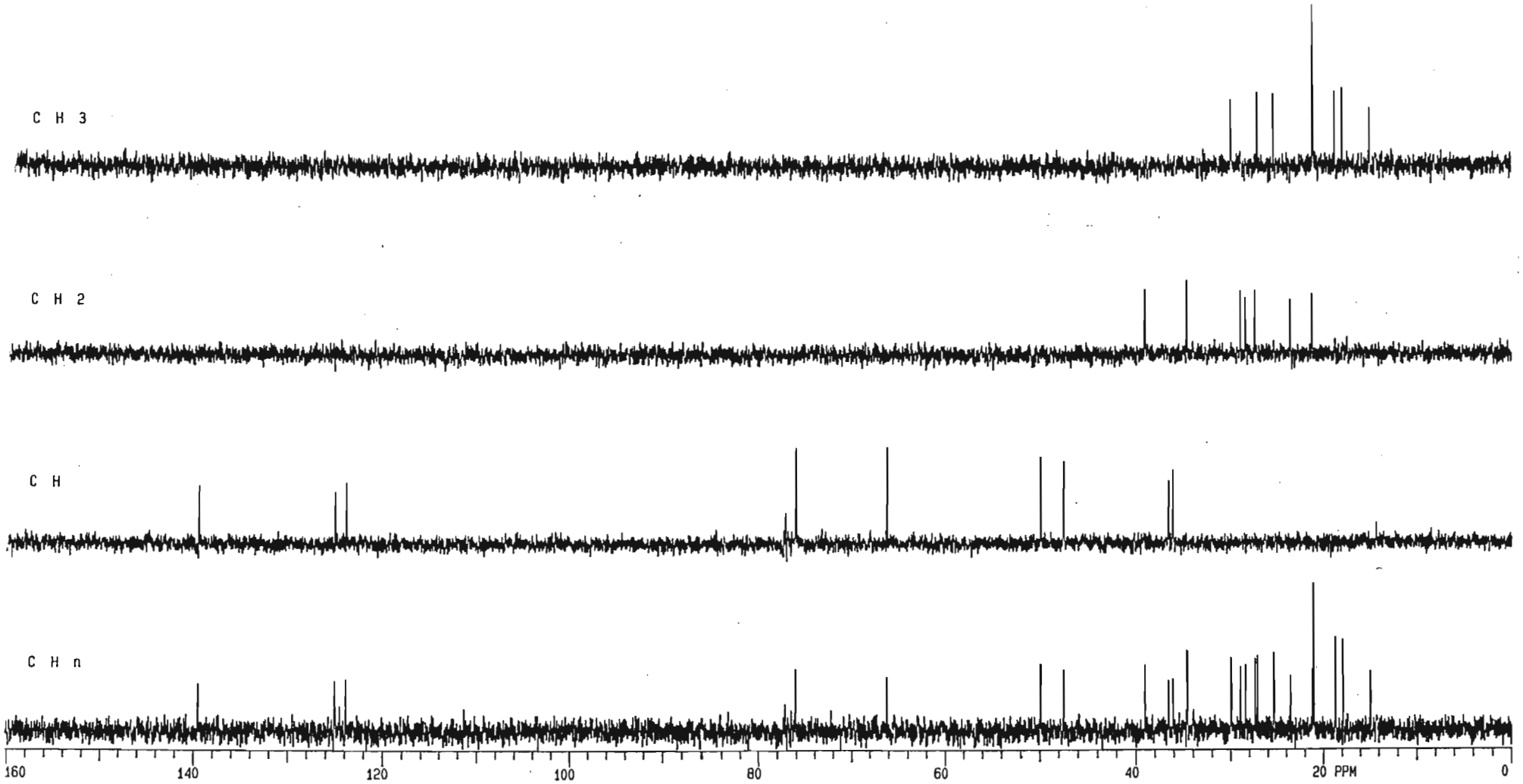



Spectrum 2c: $^{1}H$ NMR spectrum of **[93]**



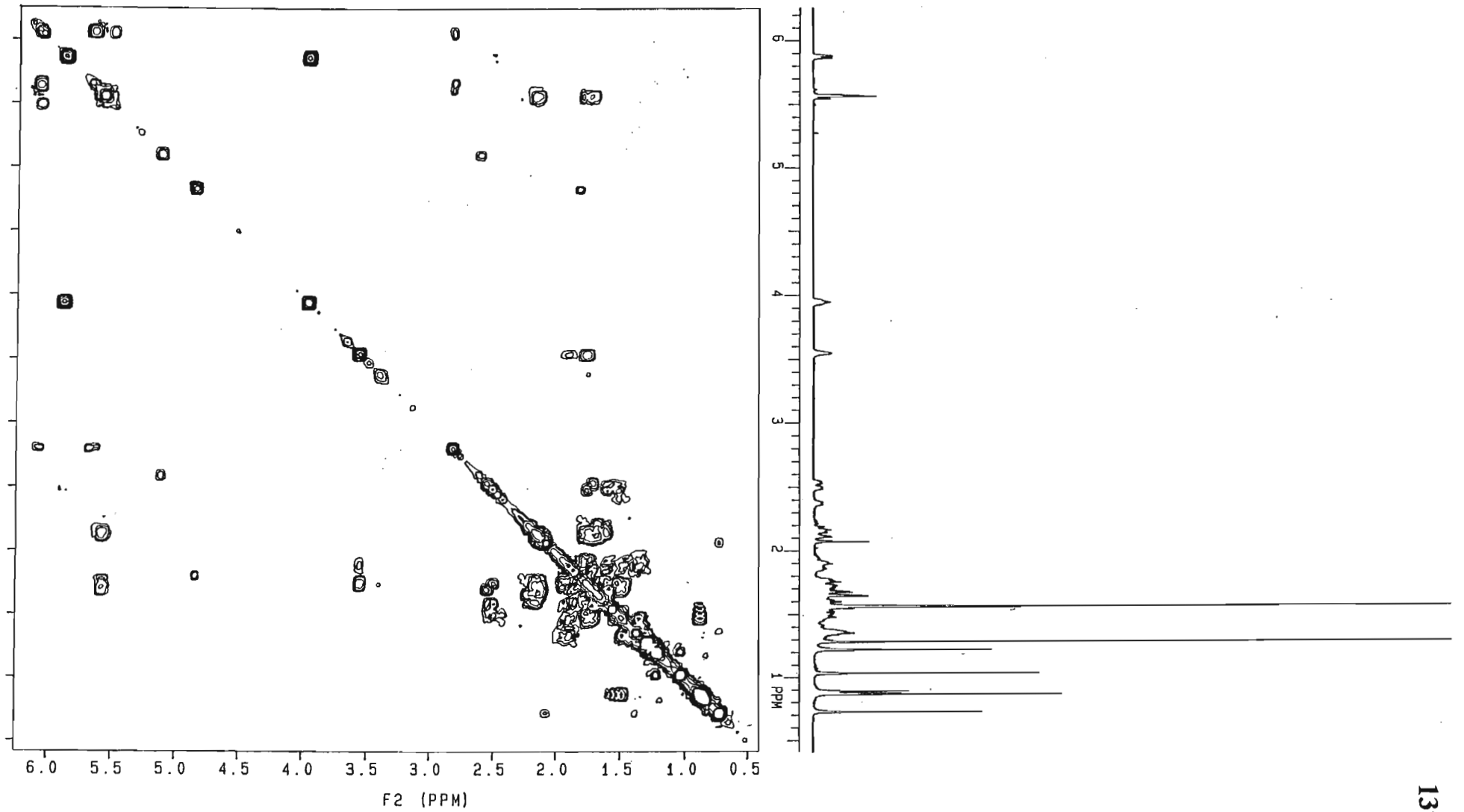



Spectrum 2d: $^{13}C$ NMR spectrum of **[93]**



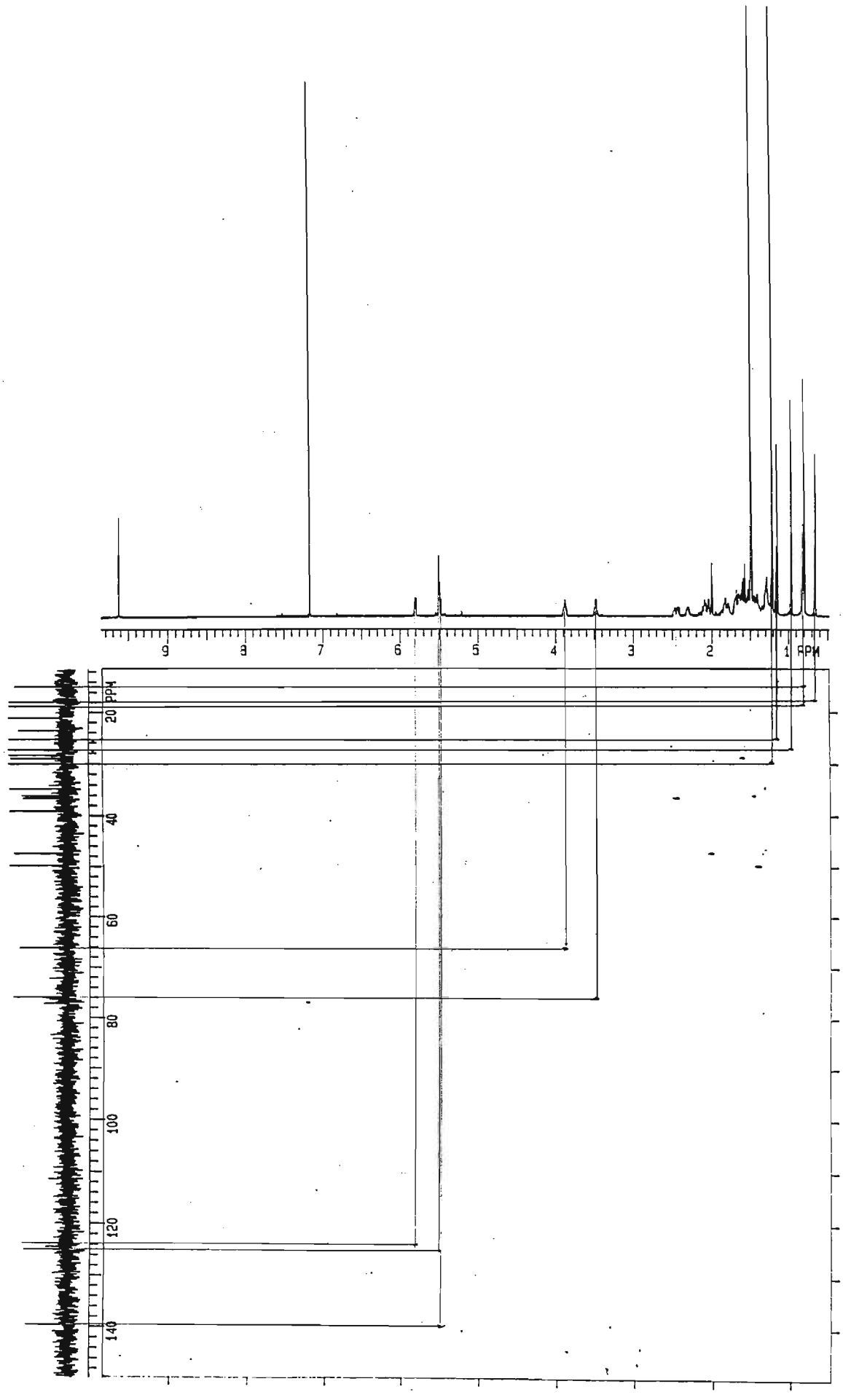



Spectrum 2e: DEPT spectrum of **[93]**

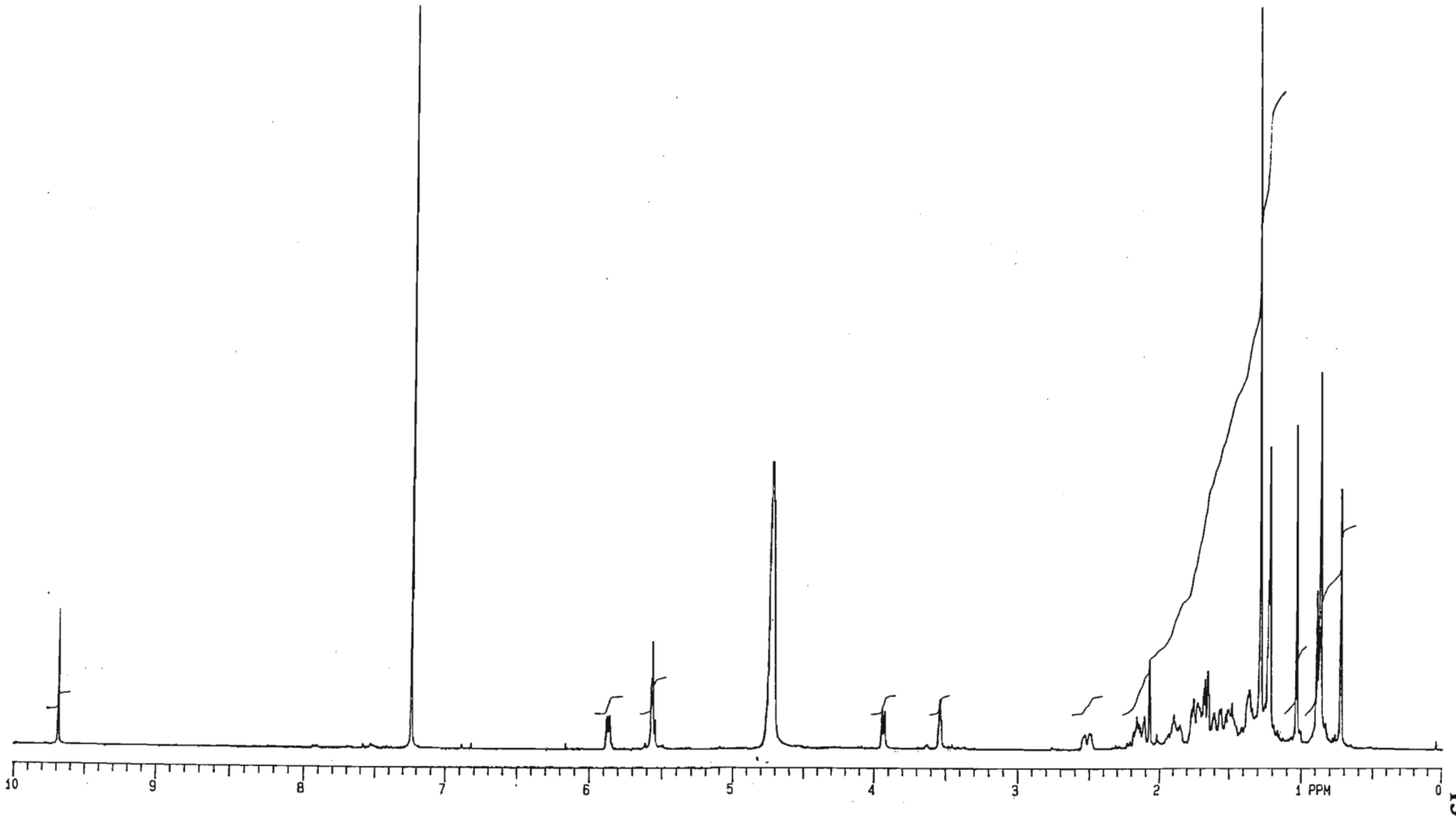




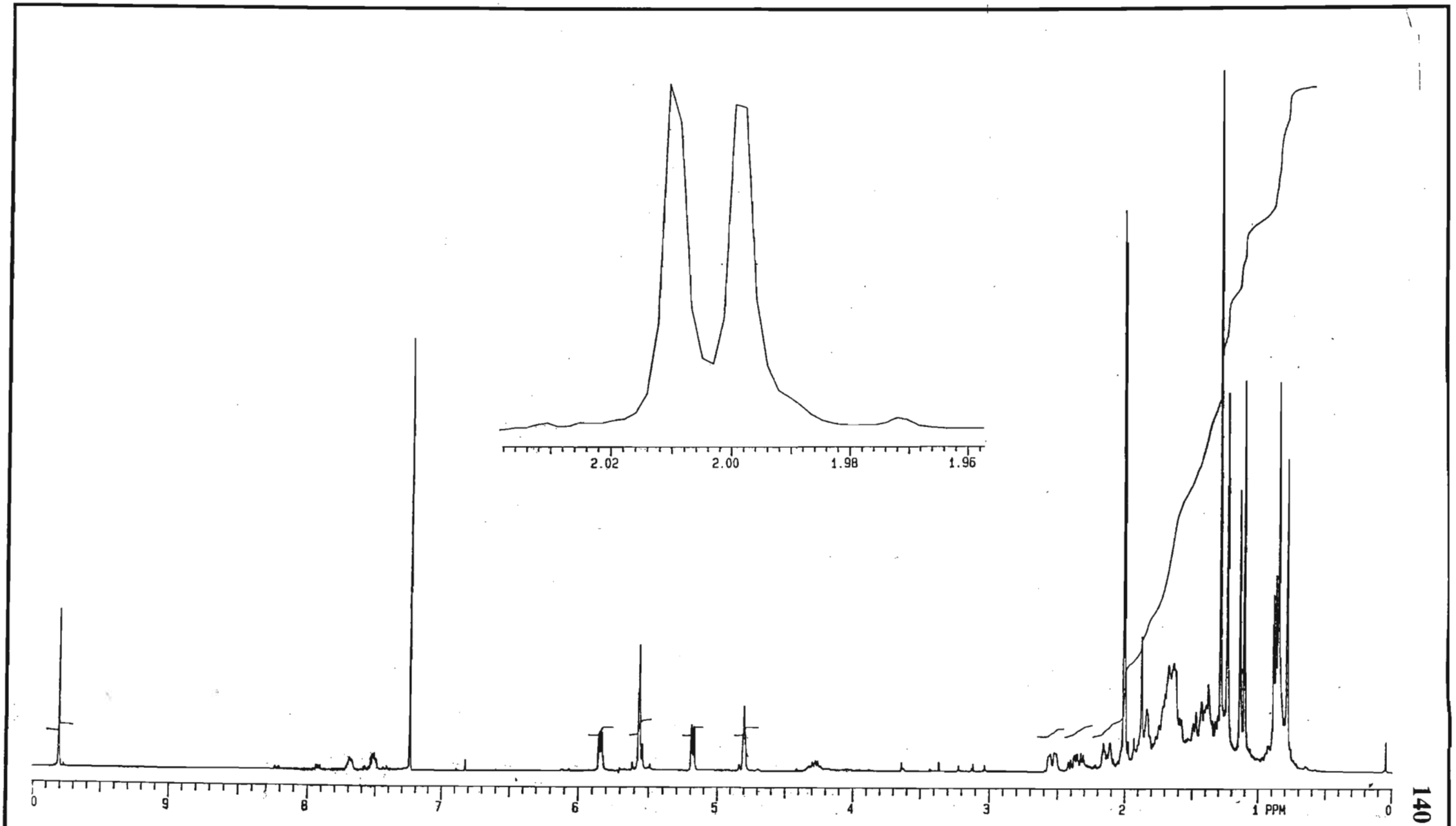

Spectrum 2f: COSY spectrum of **[93]**




Spectrum 2g: HETCOR spectrum of **[93]**

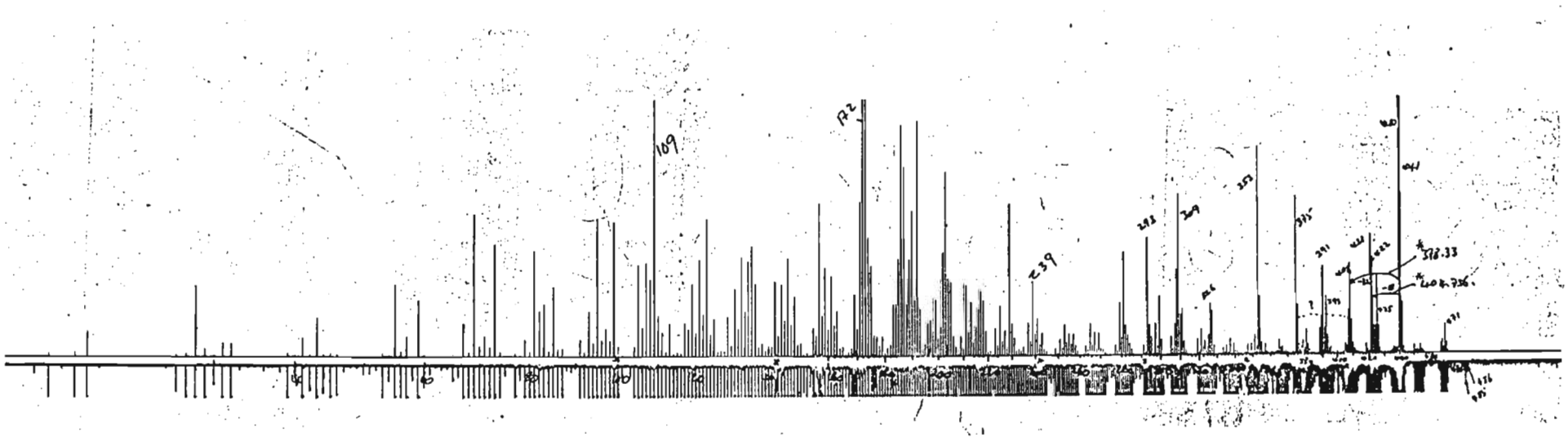



Spectrum 2h: $^{1}H$ NMR spectrum of **[93]** with $D_2O$



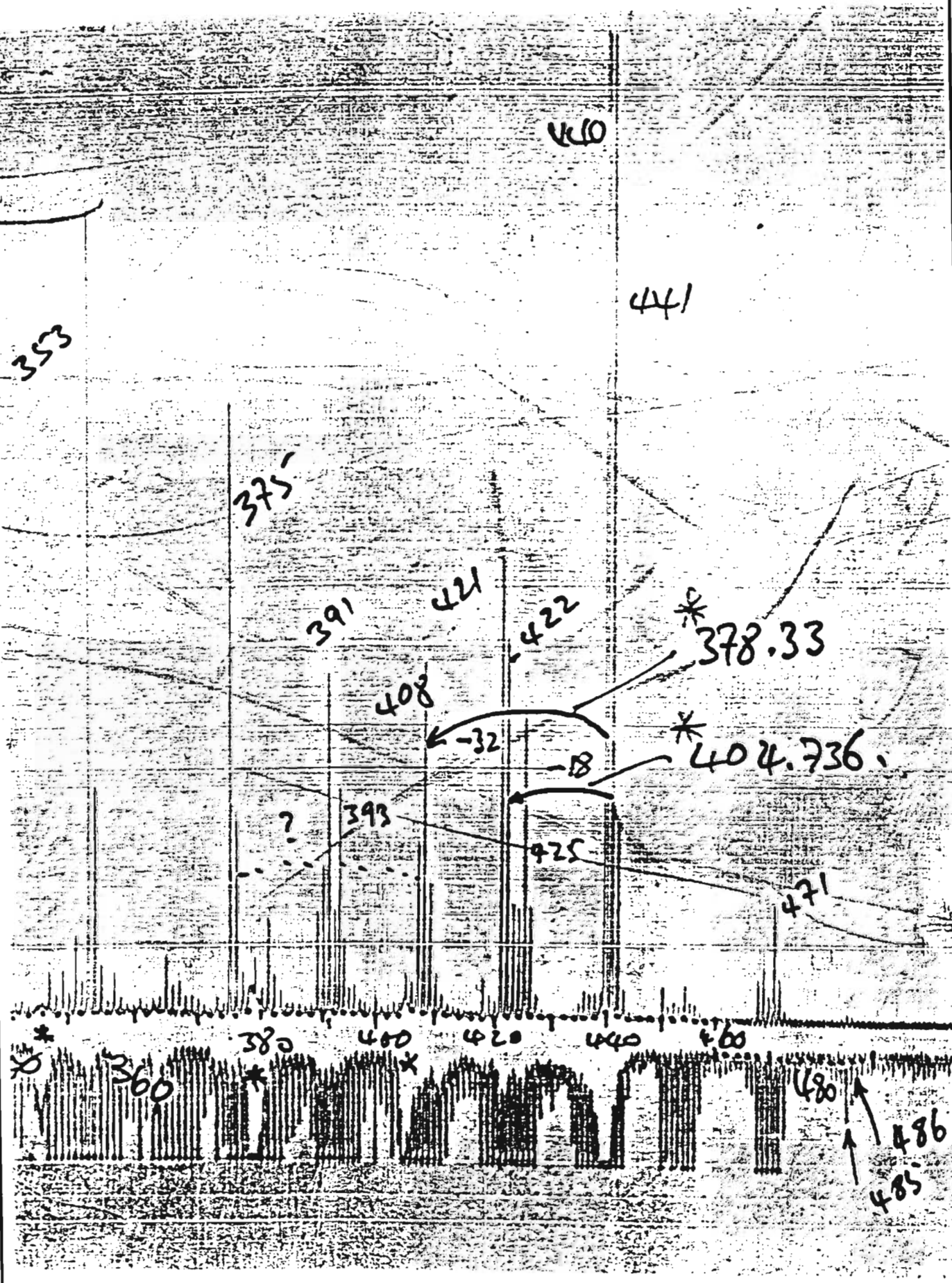



Spectrum 2i: $^{1}$H NMR spectrum of **[93a]**

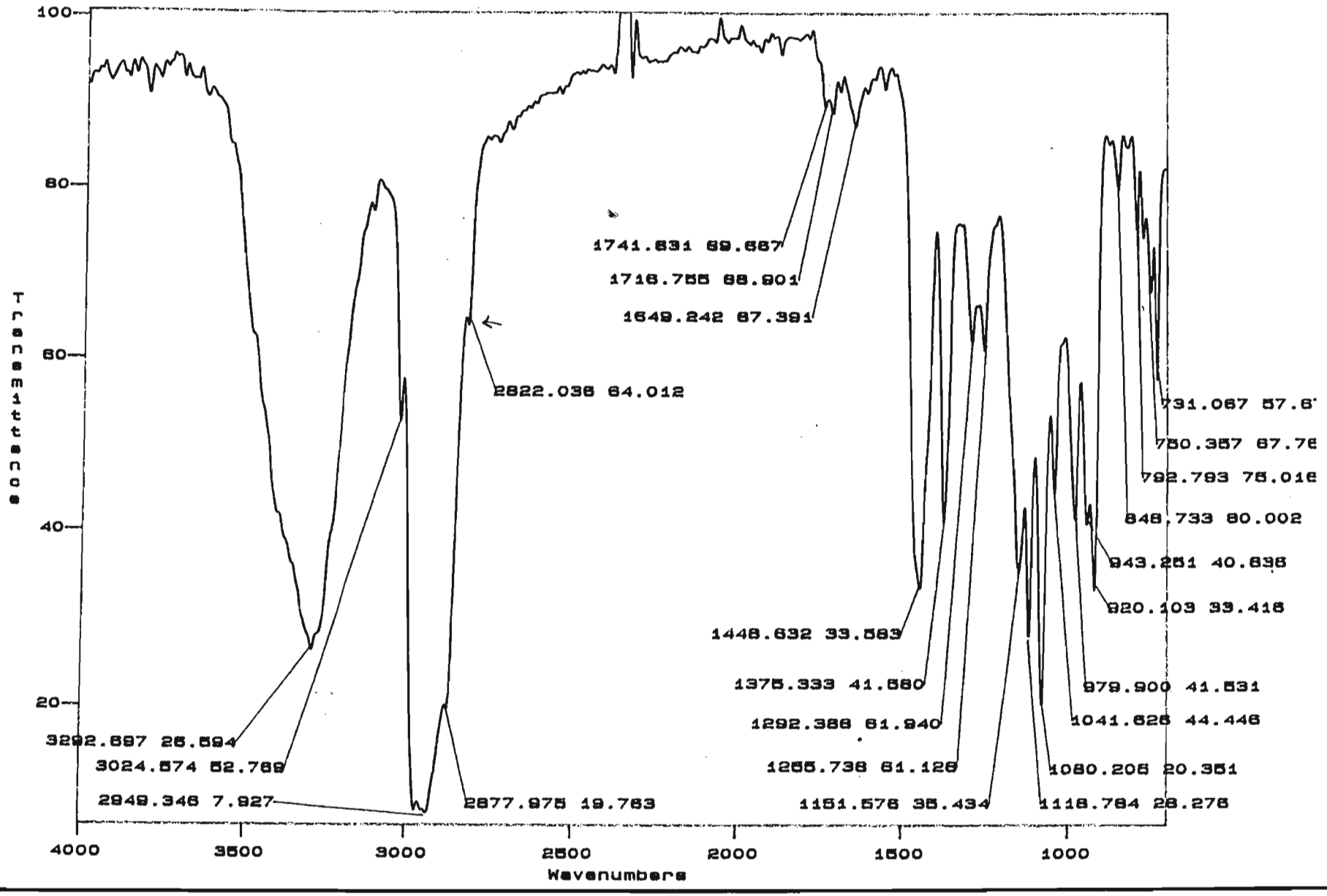




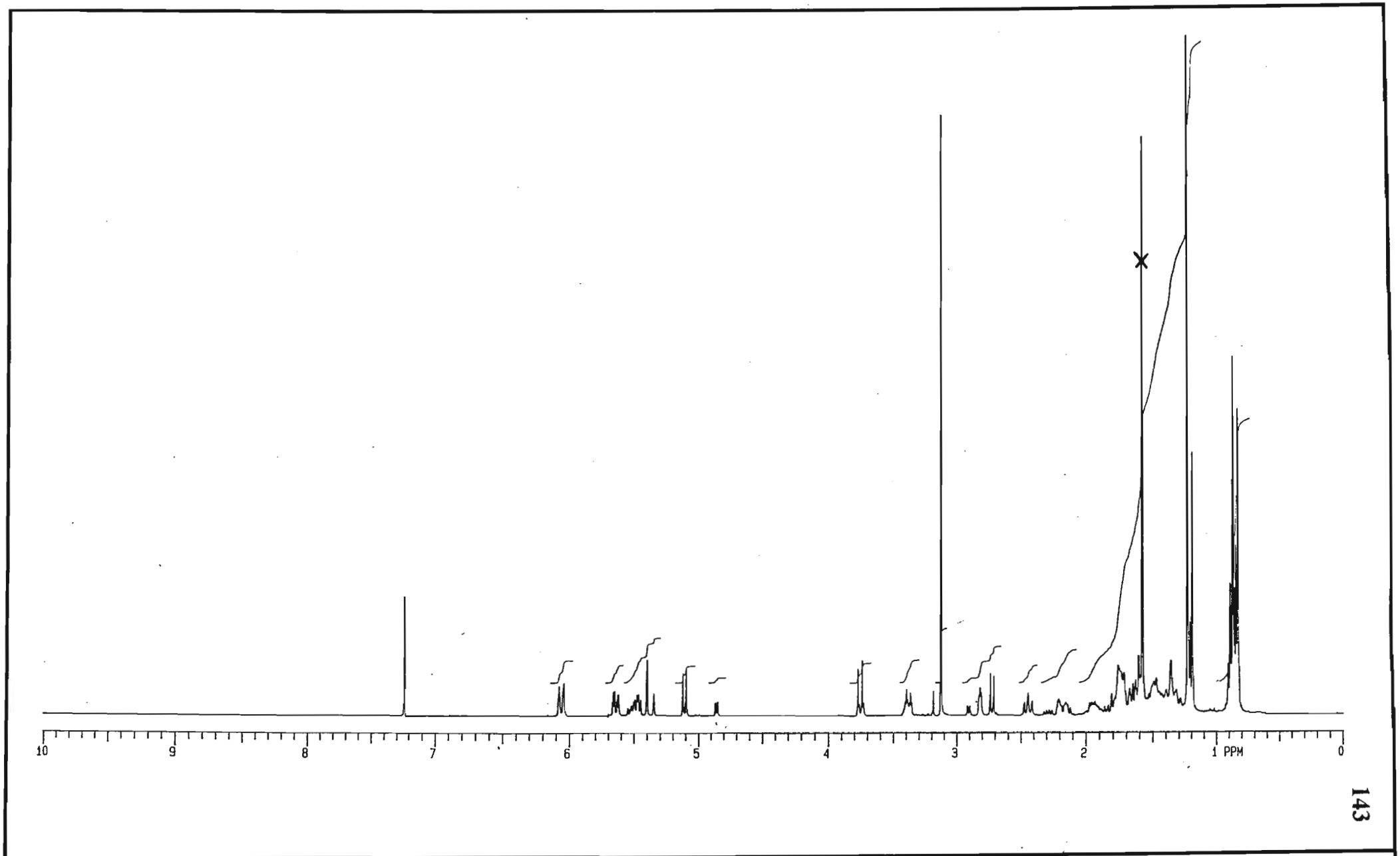

Spectrum 3a: Mass spectrum **[102]**




Enlarged mass spectrum of [102]

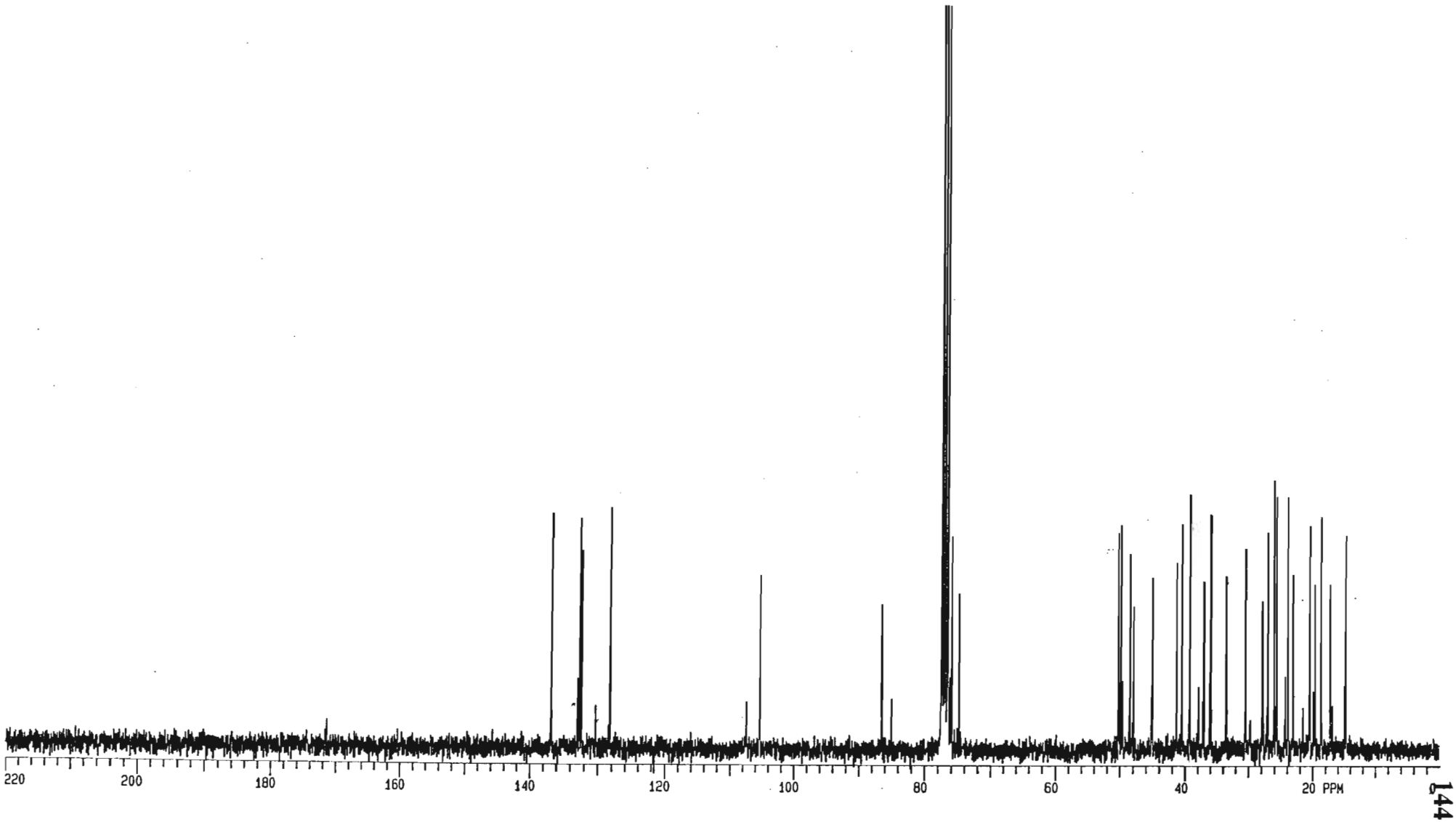



Spectrum 3b: Infrared spectrum of **[102]**

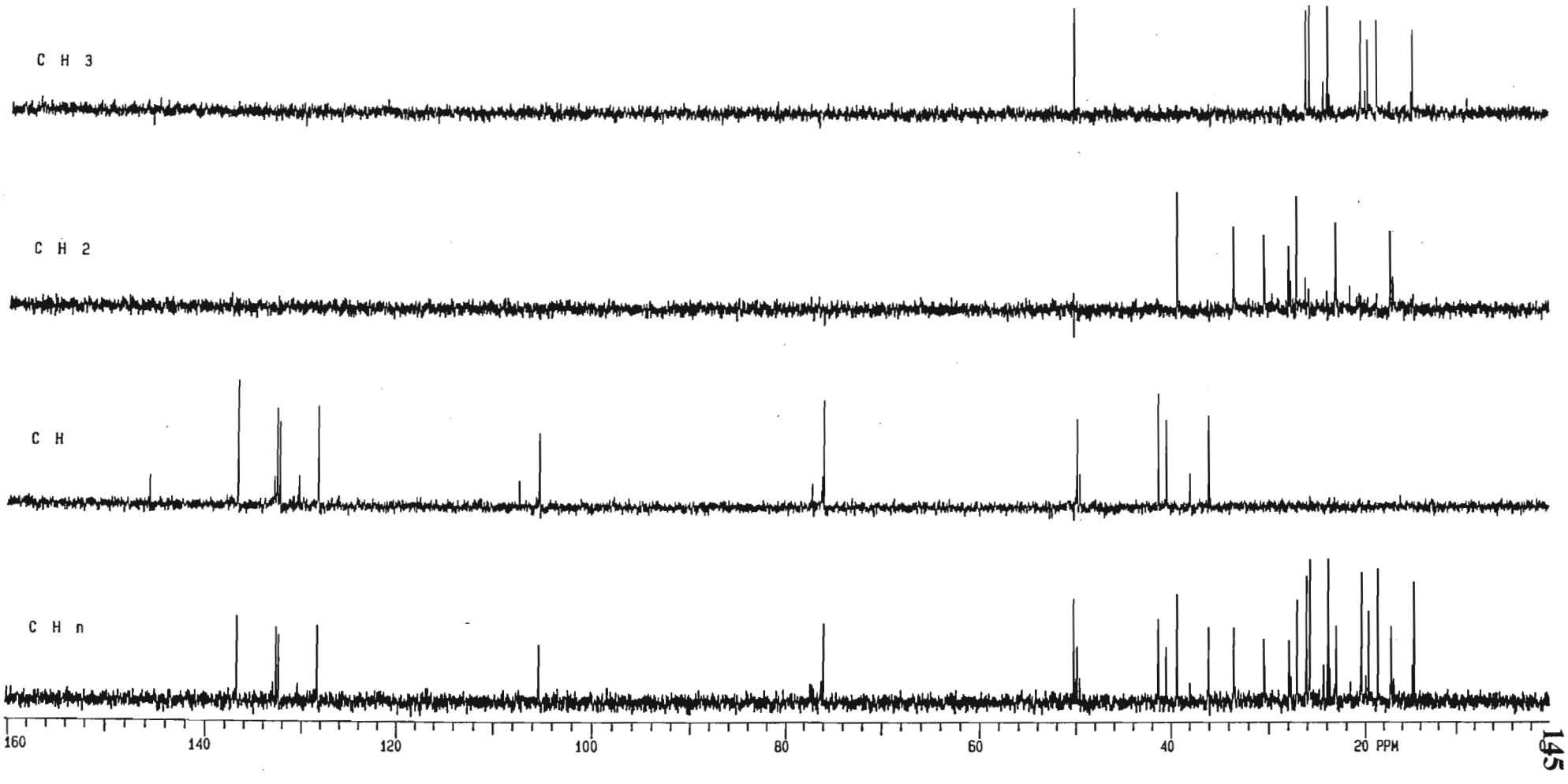





Spectrum 3c: $^{1}H$ NMR spectrum of **[102]**

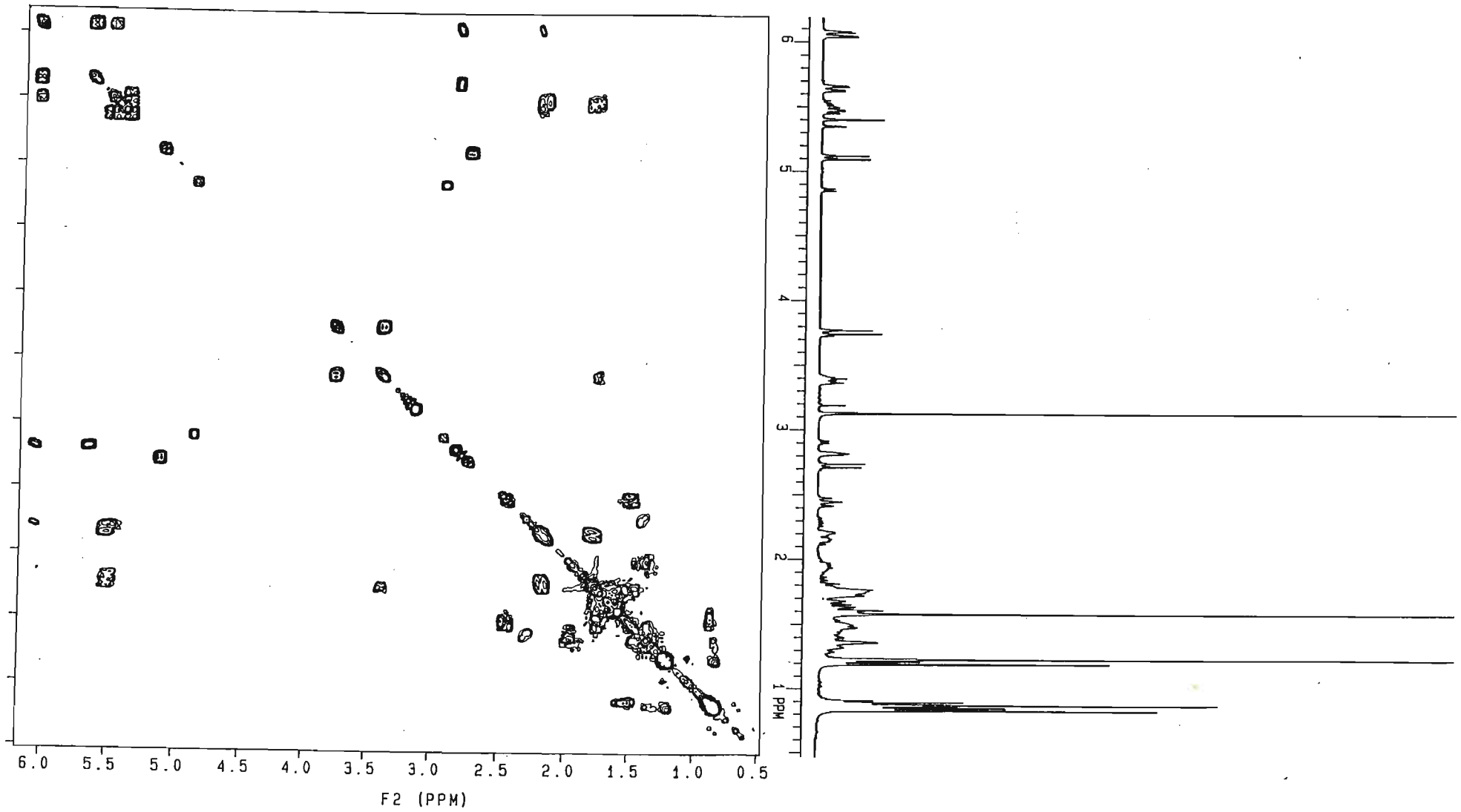



Spectrum 3d: $^{13}C$ NMR spectrum of **[102]**

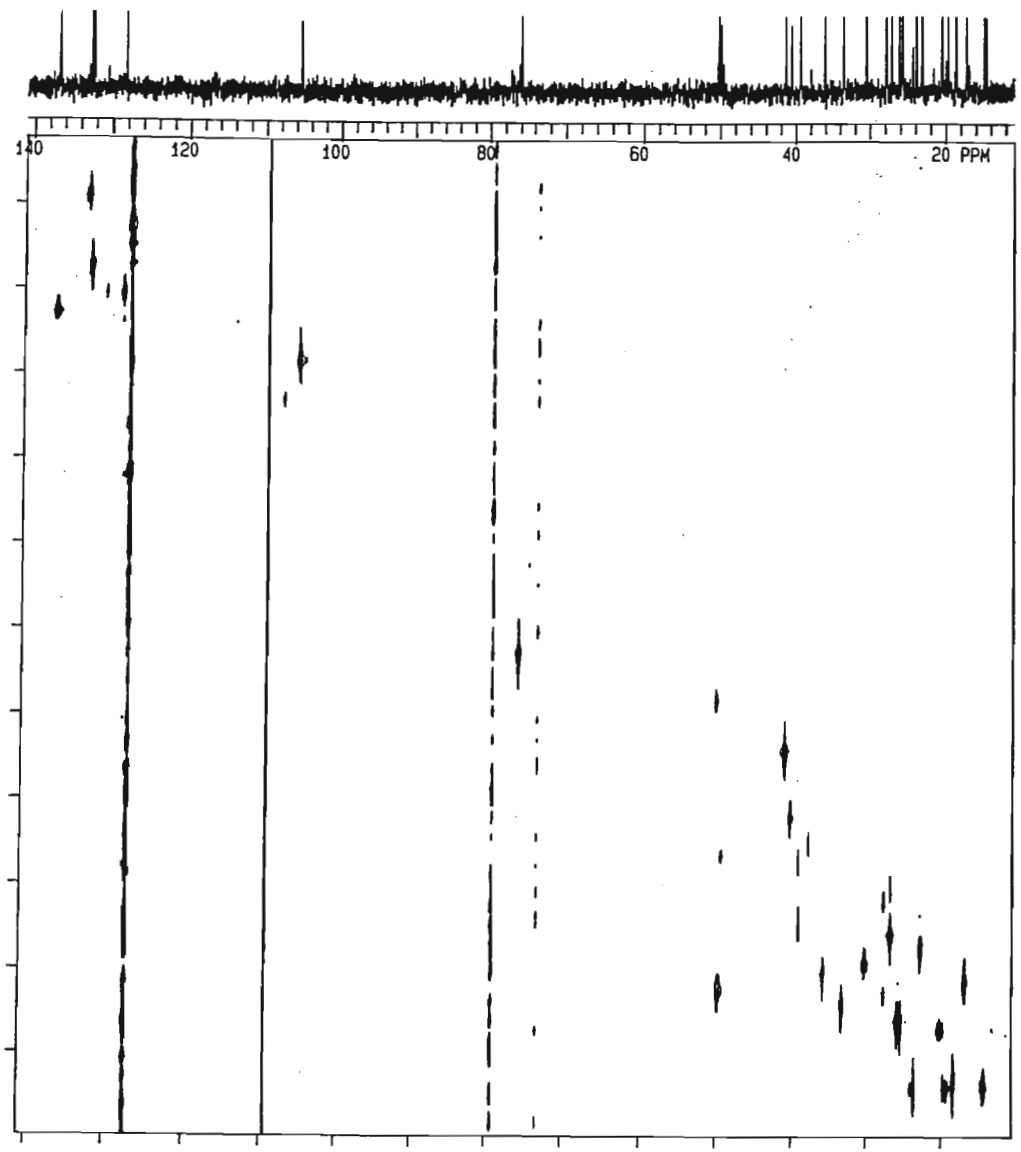


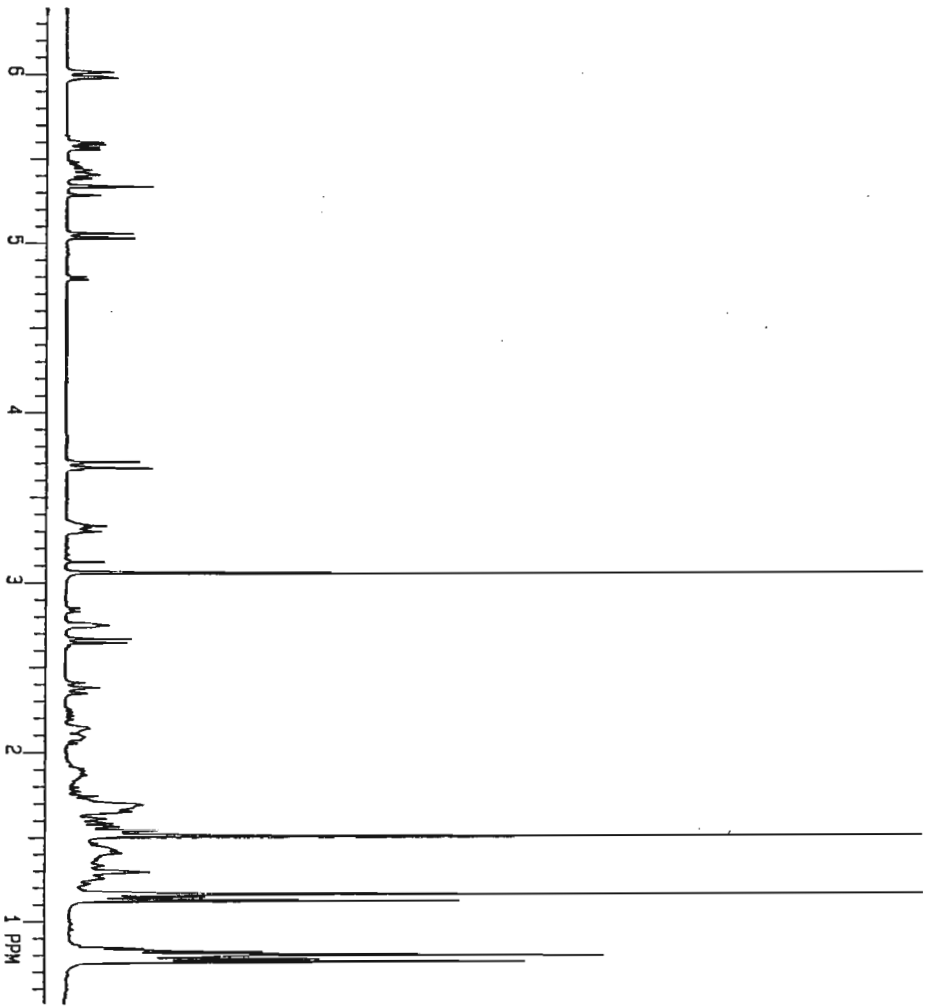



Spectrum 3e: DEPT spectrum of **[102]**




Spectrum 3f: COSY spectrum of **[102]**

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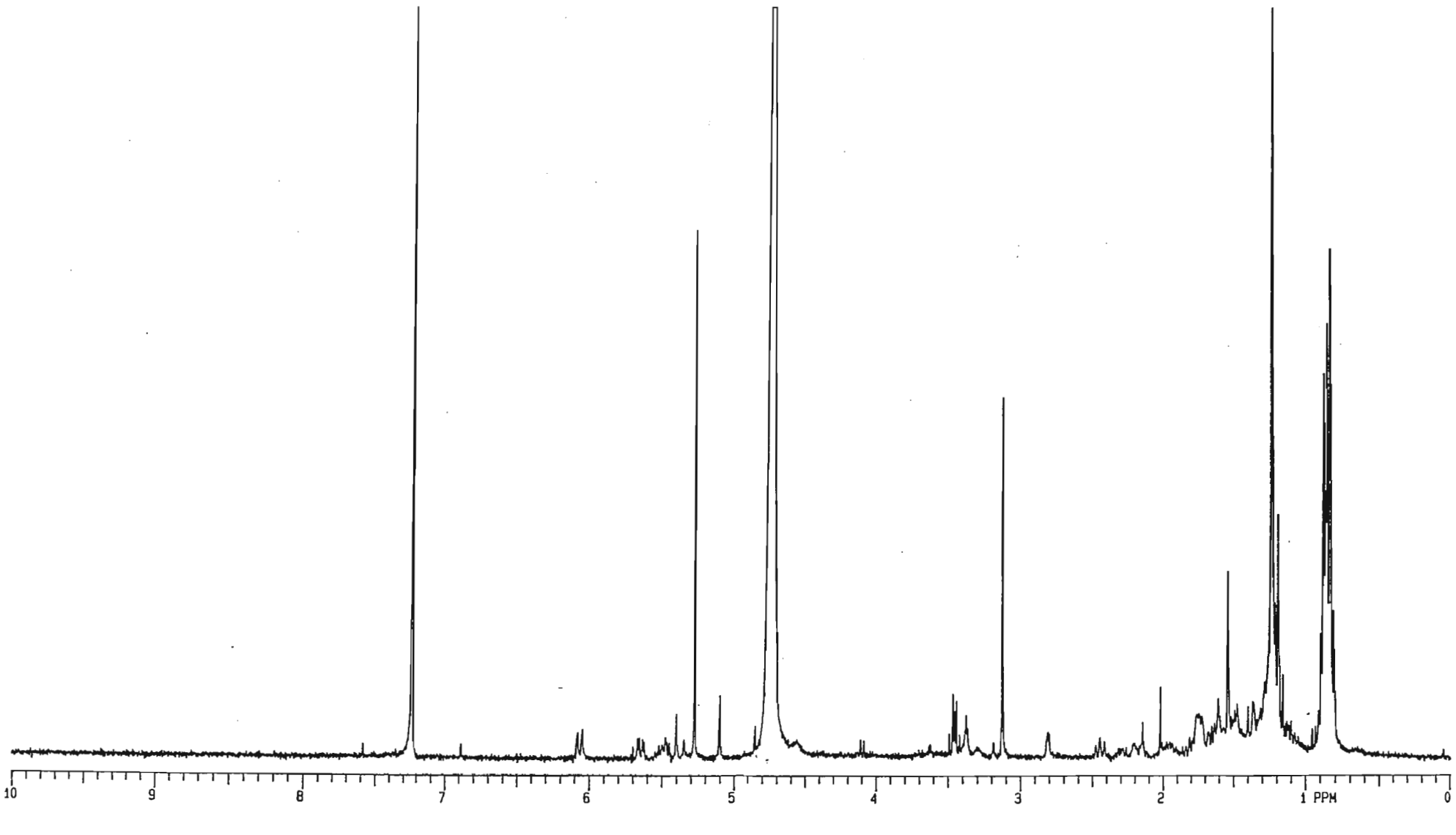
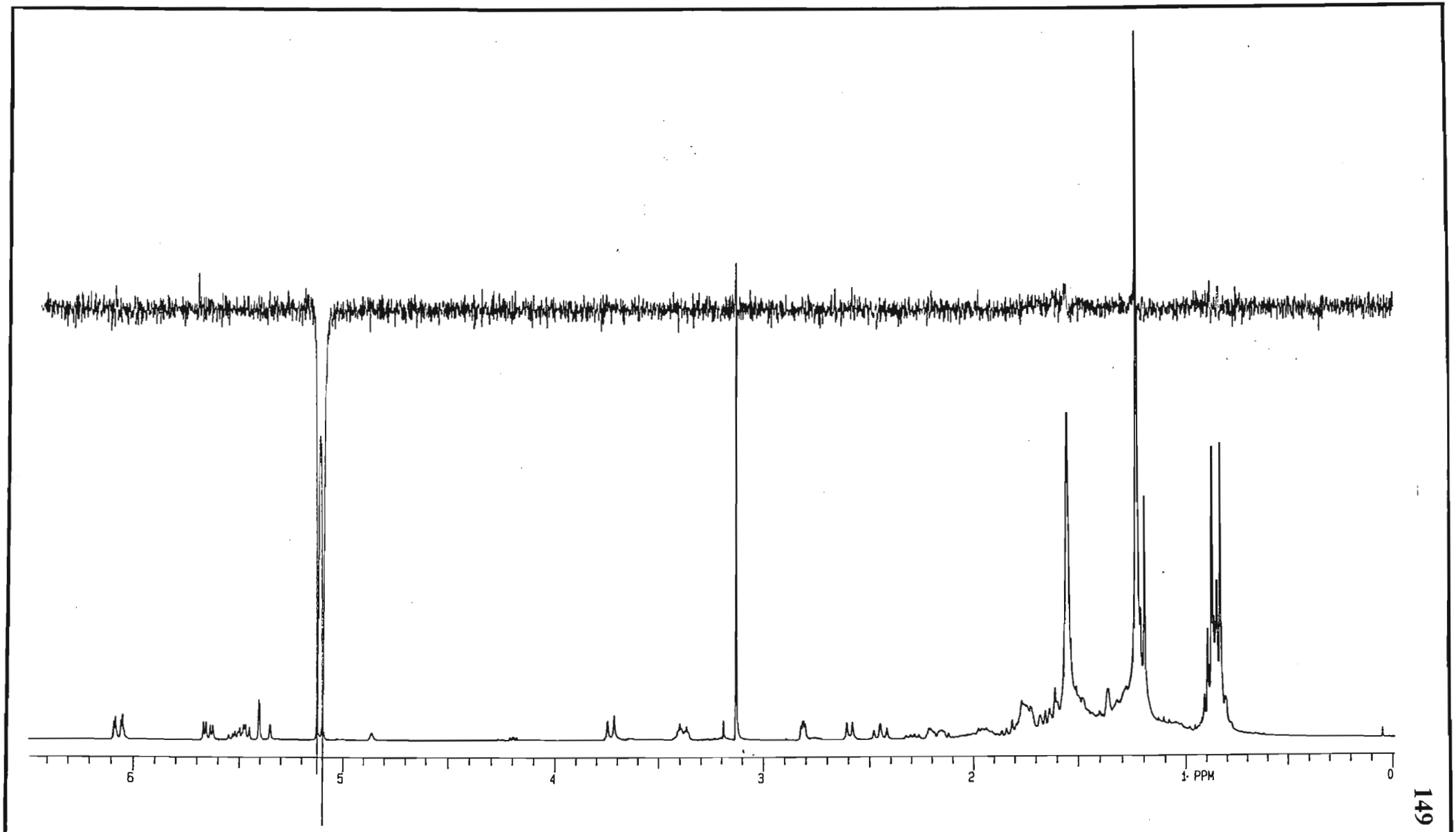
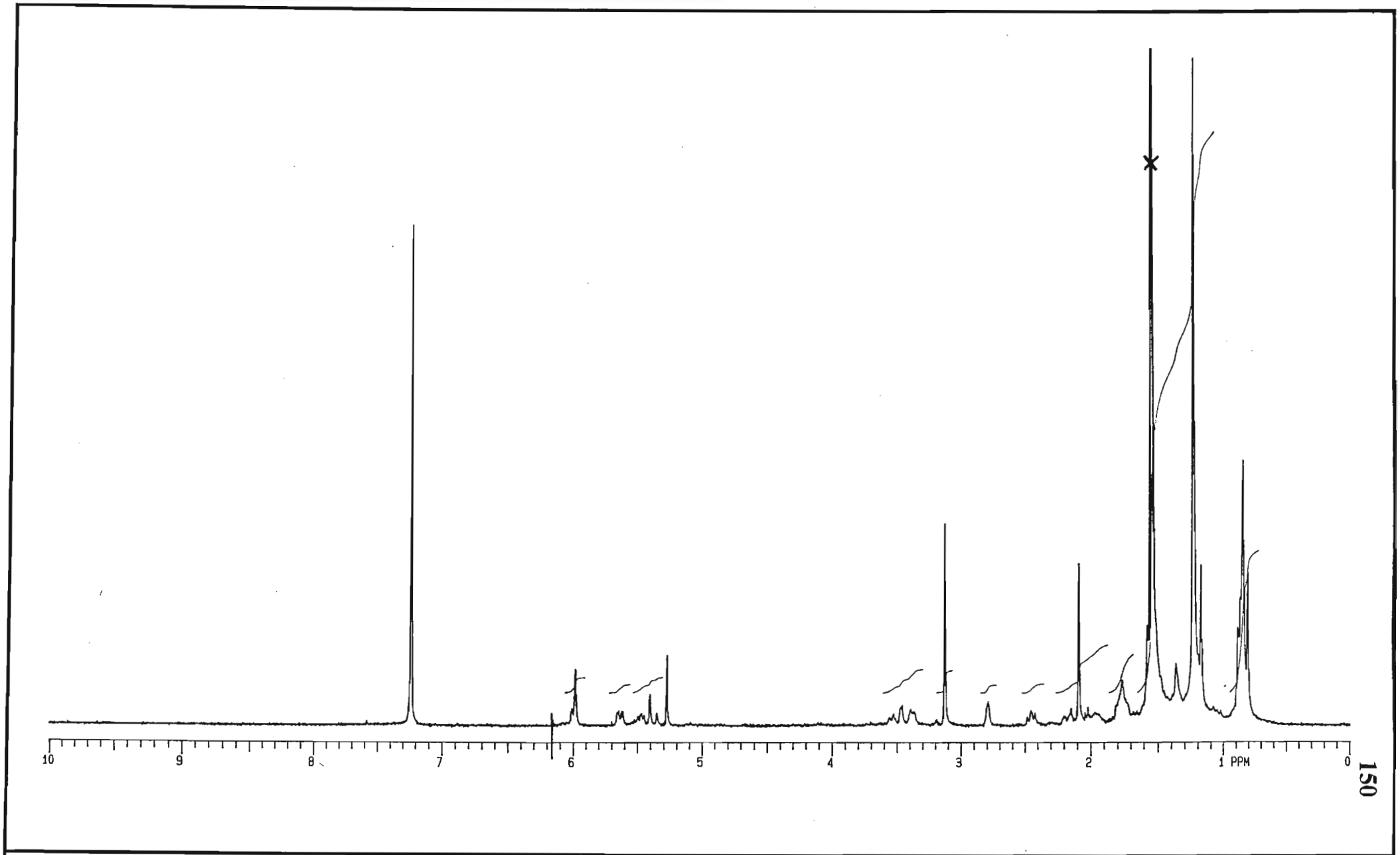
Spectrum 3g: HETCOR spectrum of **[102]**



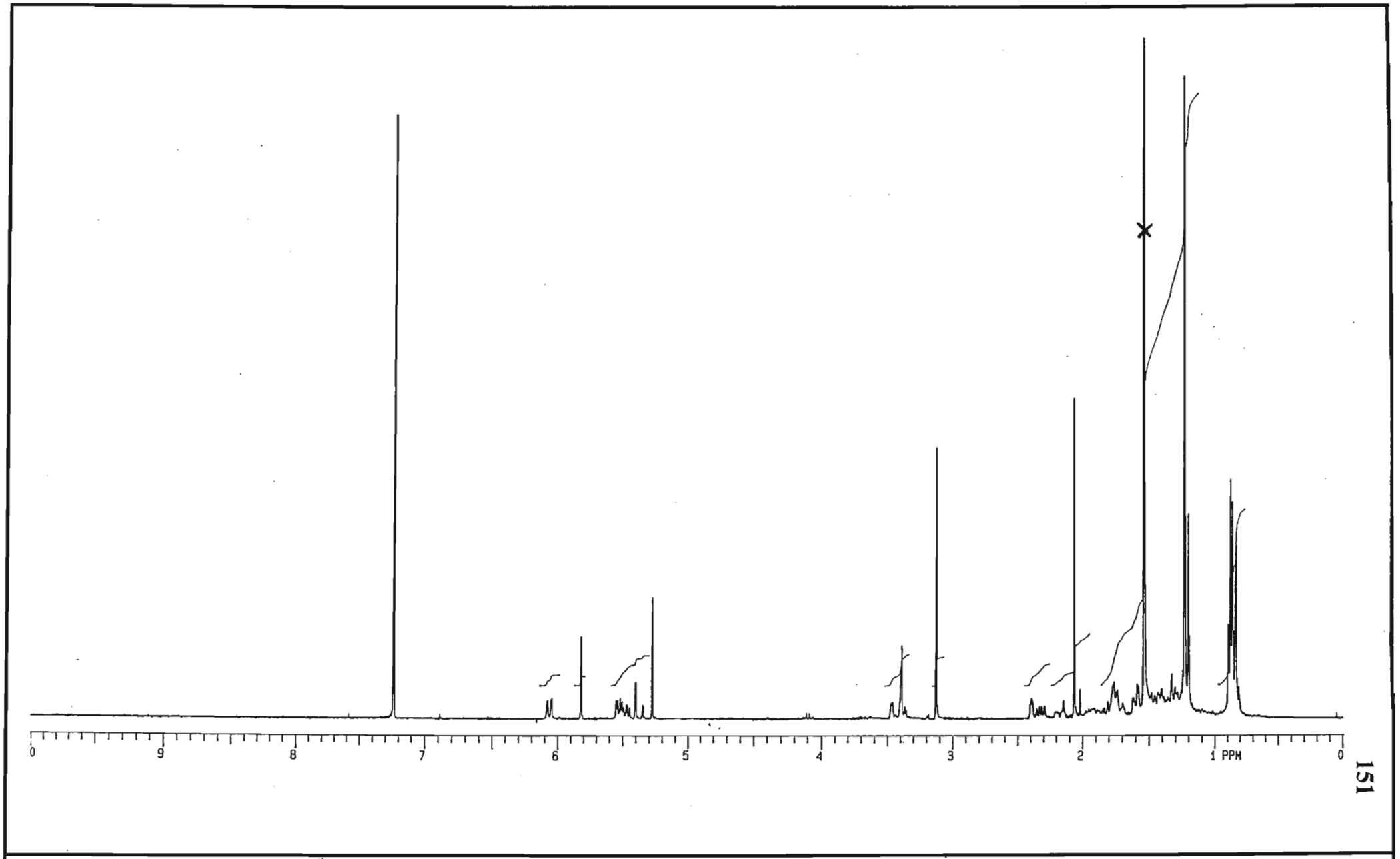

Spectrum 3h: $^{1}H$ NMR spectrum of **[102]** with $D_2O$




Spectrum 3i: NOE spectrum of **[102]**

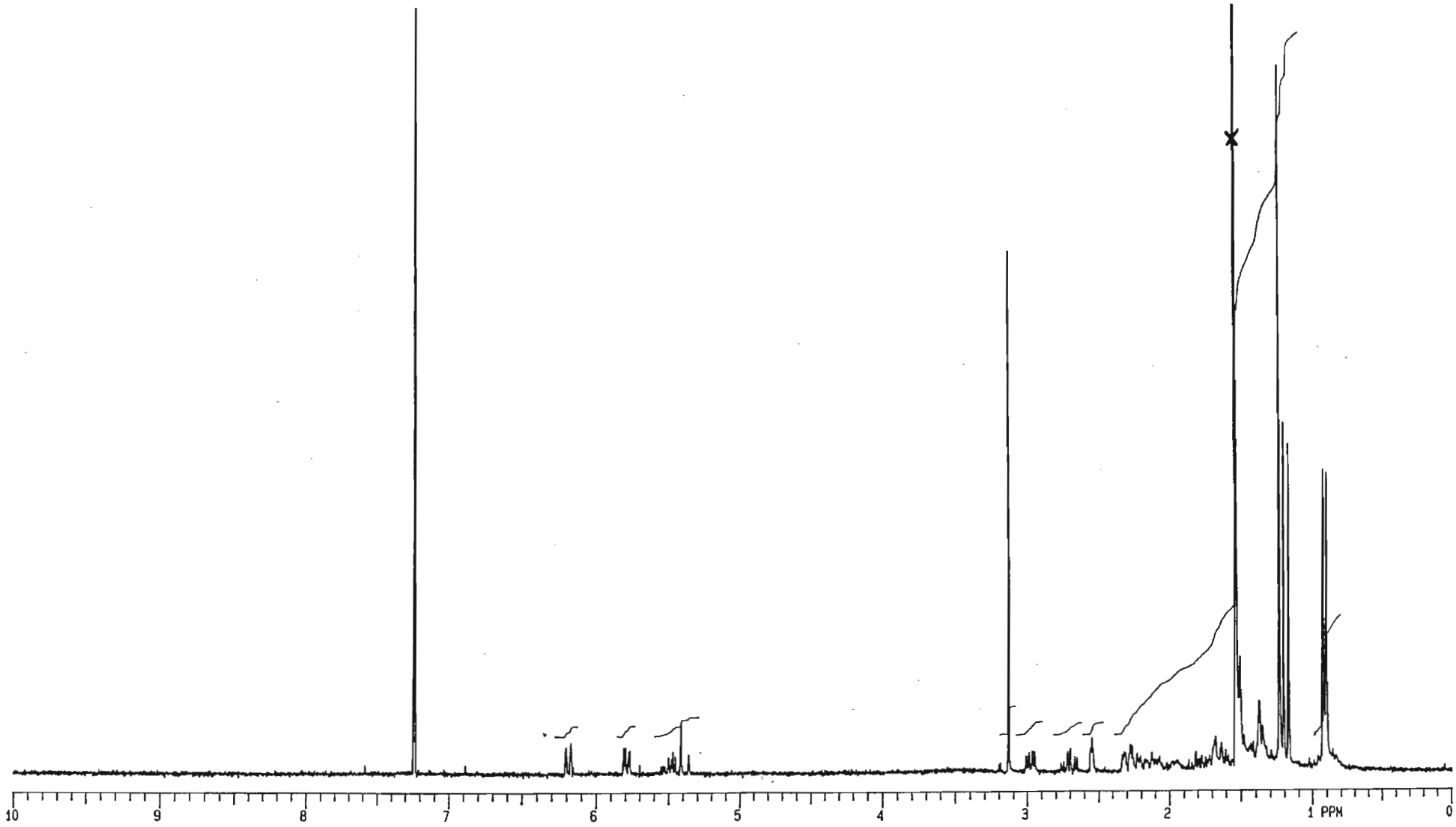



Spectrum 3j: $^{1}$H NMR spectrum of **[102a]**



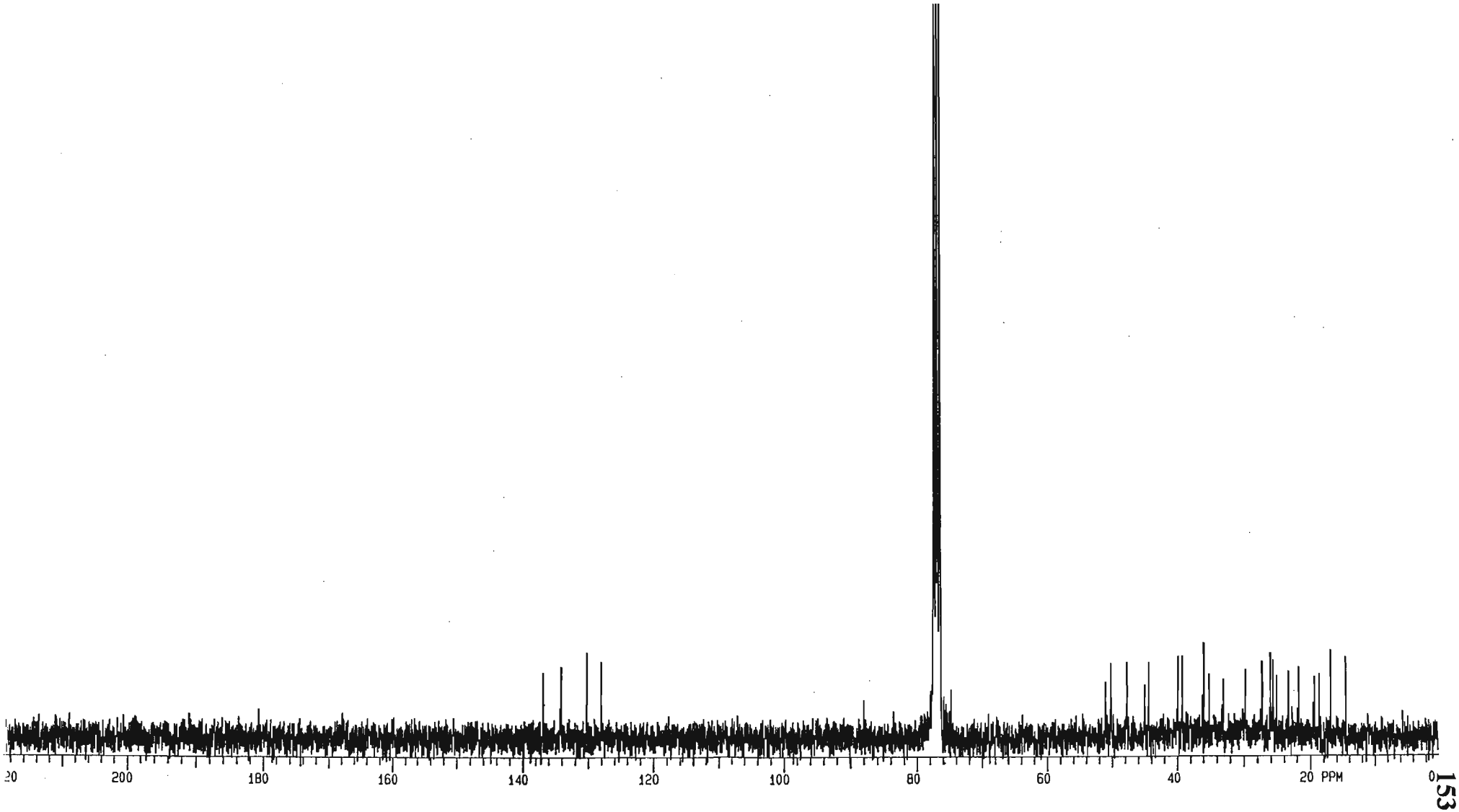



Spectrum 3k: $^{1}$H NMR spectrum of **[102b]**

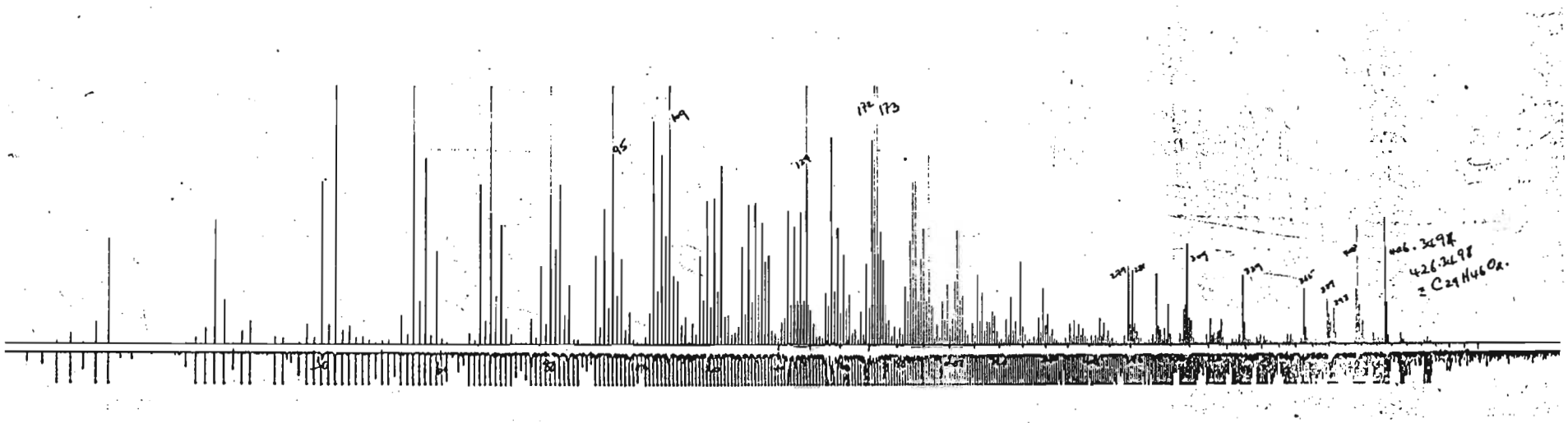




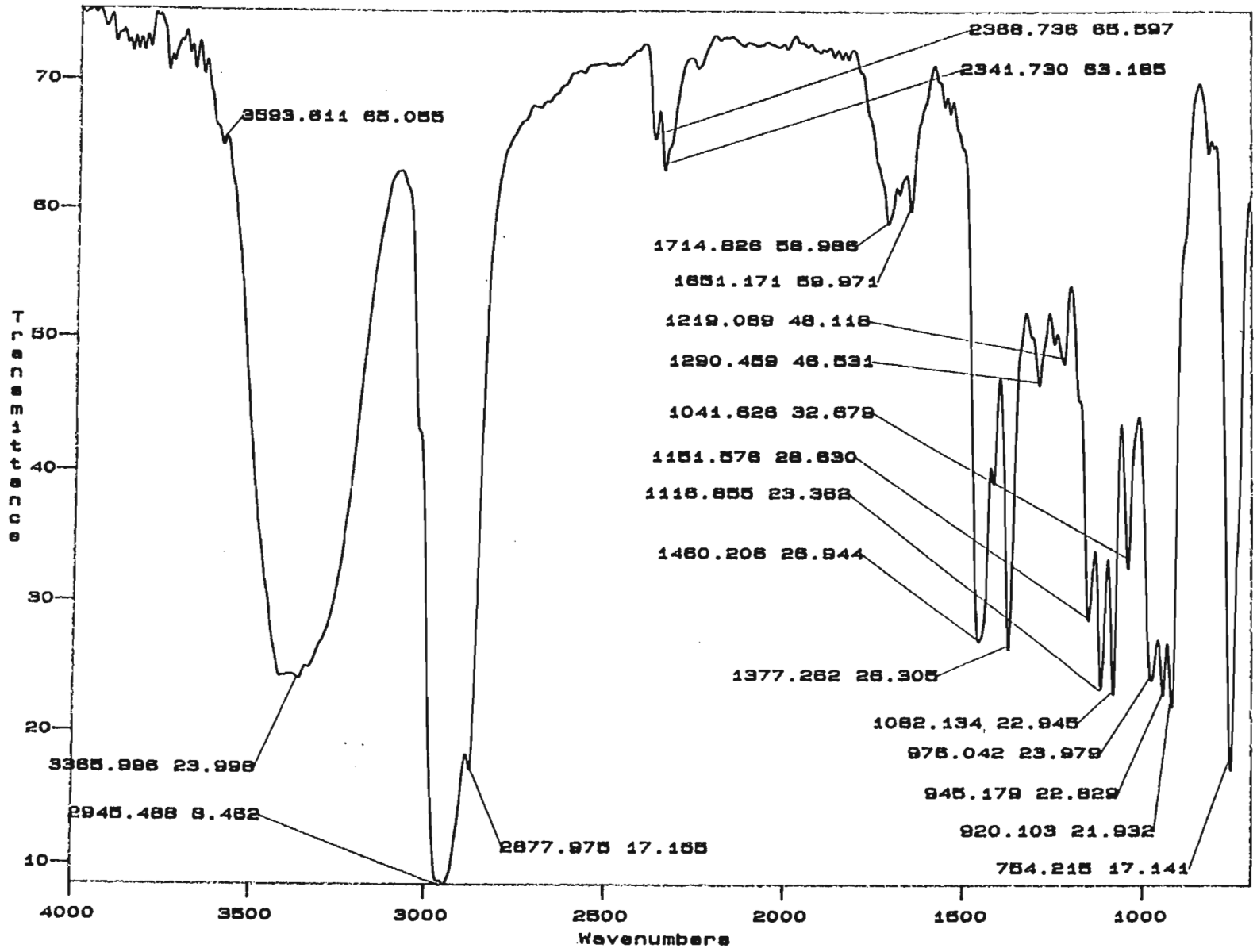

Spectrum 31: $^{1}H$ NMR spectrum of **[102c]**



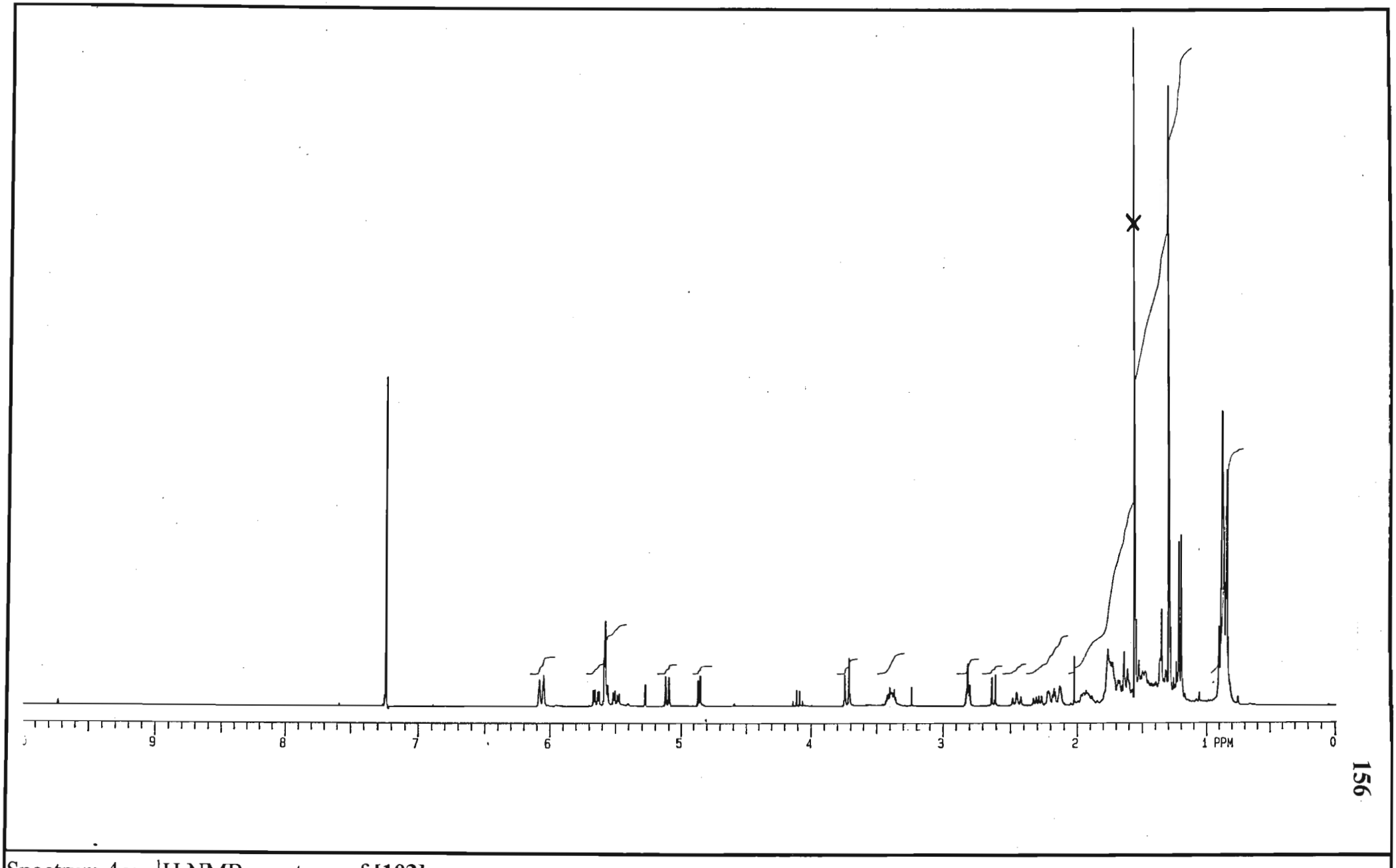

Spectrum 3m: $^{13}C$ NMR spectrum of **[102c]**




Spectrum 4a: Mass spectrum **[103]**

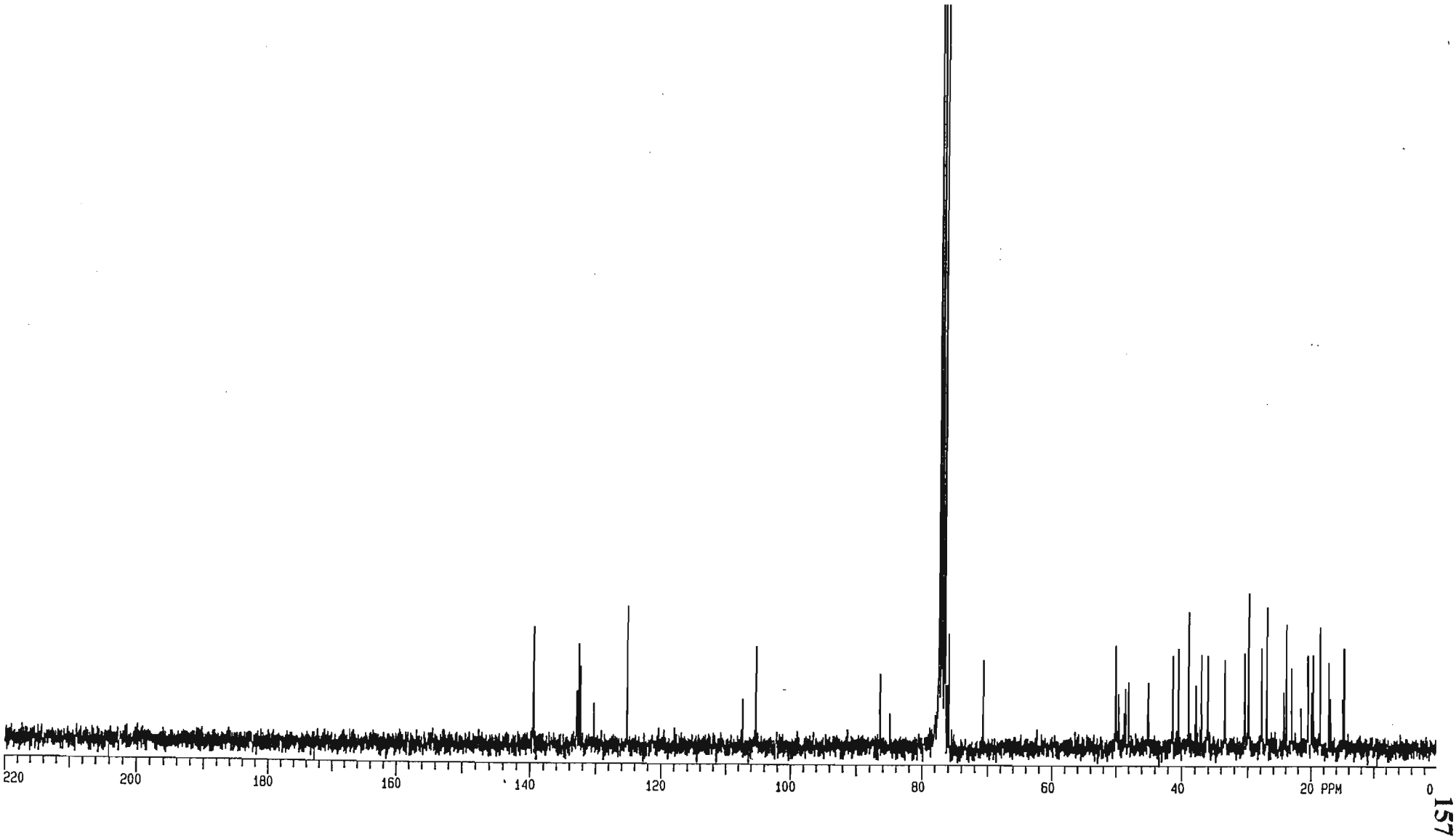



Spectrum 4b: Infrared spectrum of **[103]**

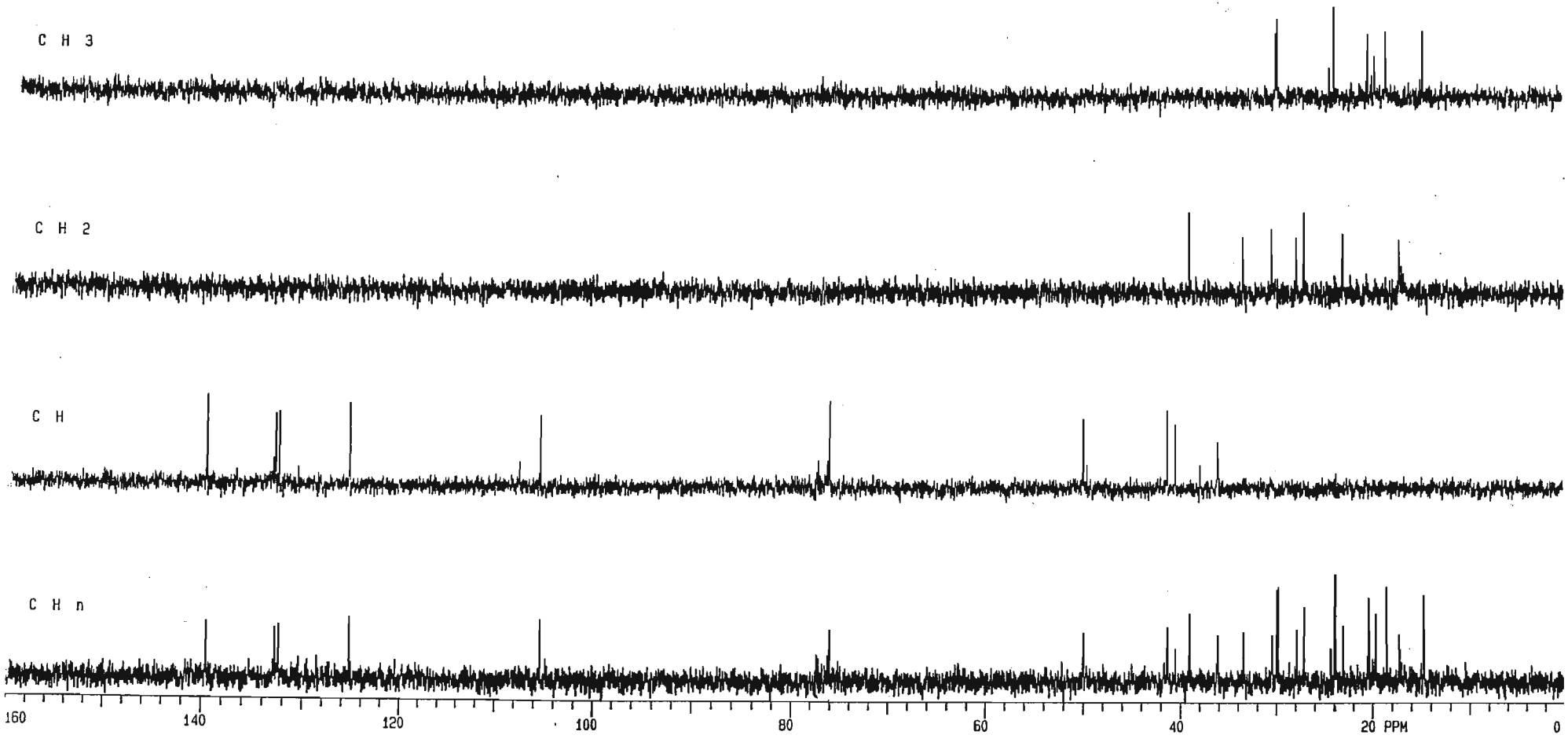




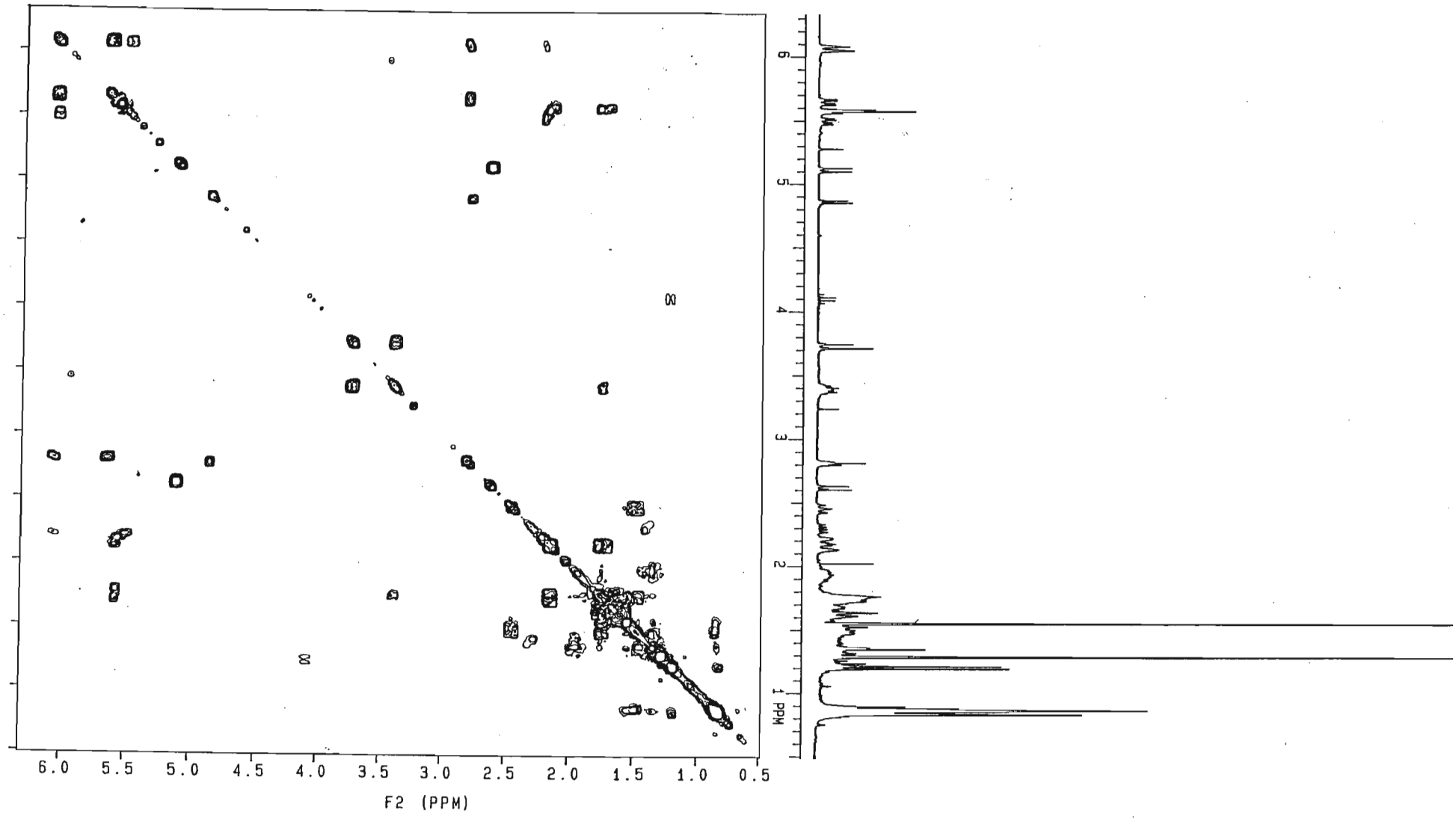

Spectrum 4c: $^{1}$H NMR spectrum of **[103]**



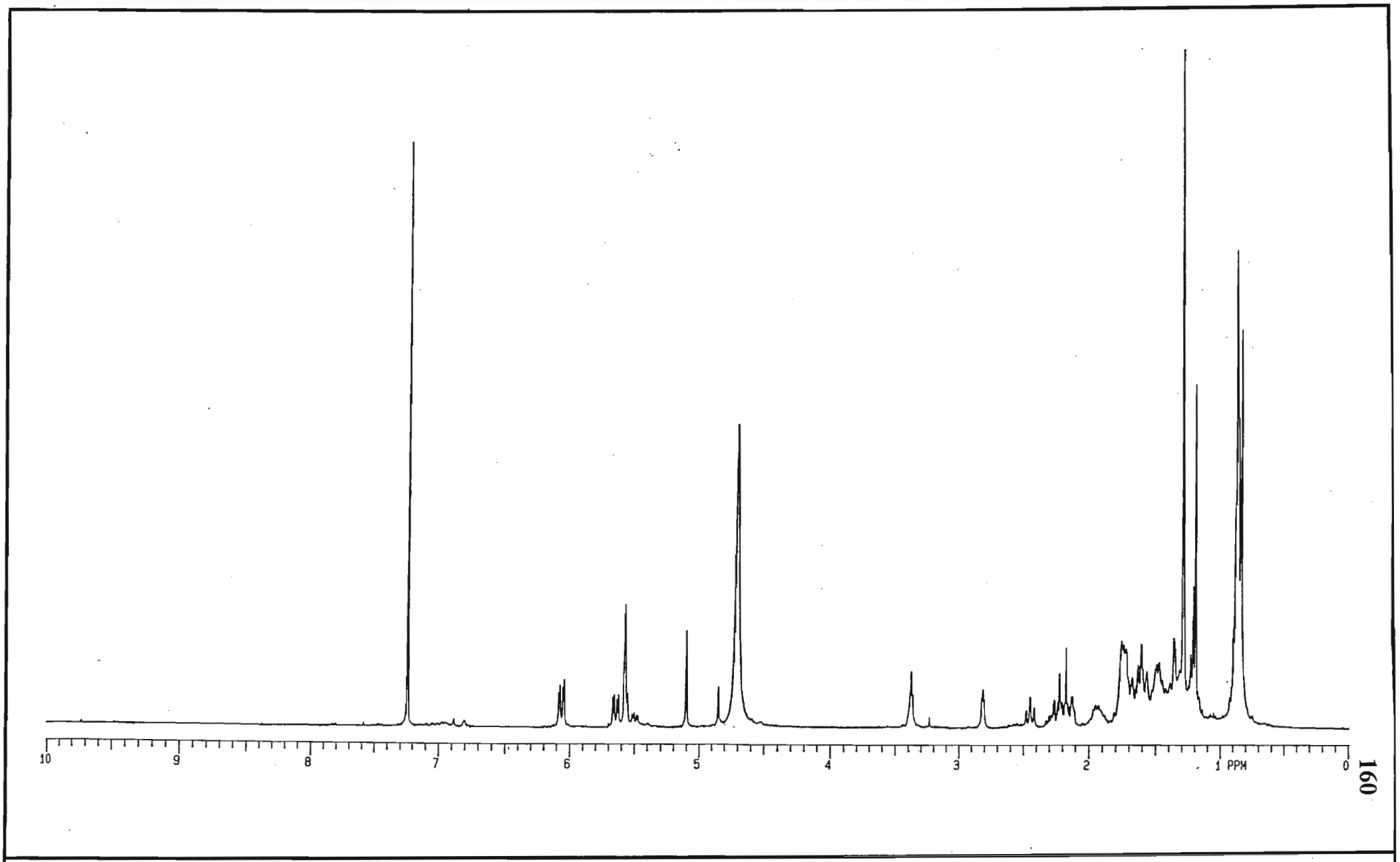

Spectrum 4d: $^{13}C$ NMR spectrum of **[103]**



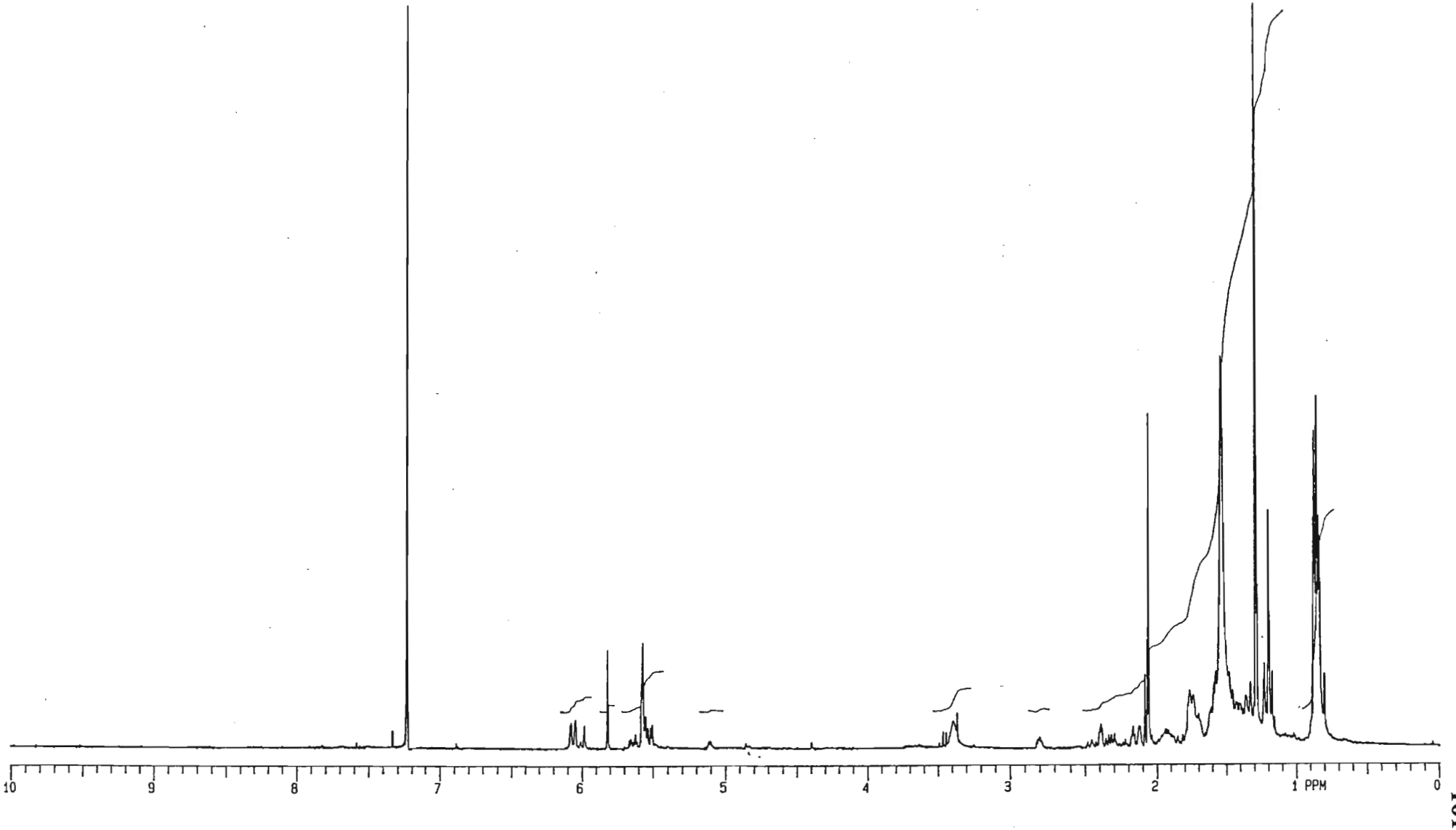

Spectrum 4e: DEPT spectrum of **[103]**




Spectrum 4f: COSY spectrum of **[103]**




Spectrum 4g: $^{1}H$ NMR spectrum of **[103]** with $D_2O$

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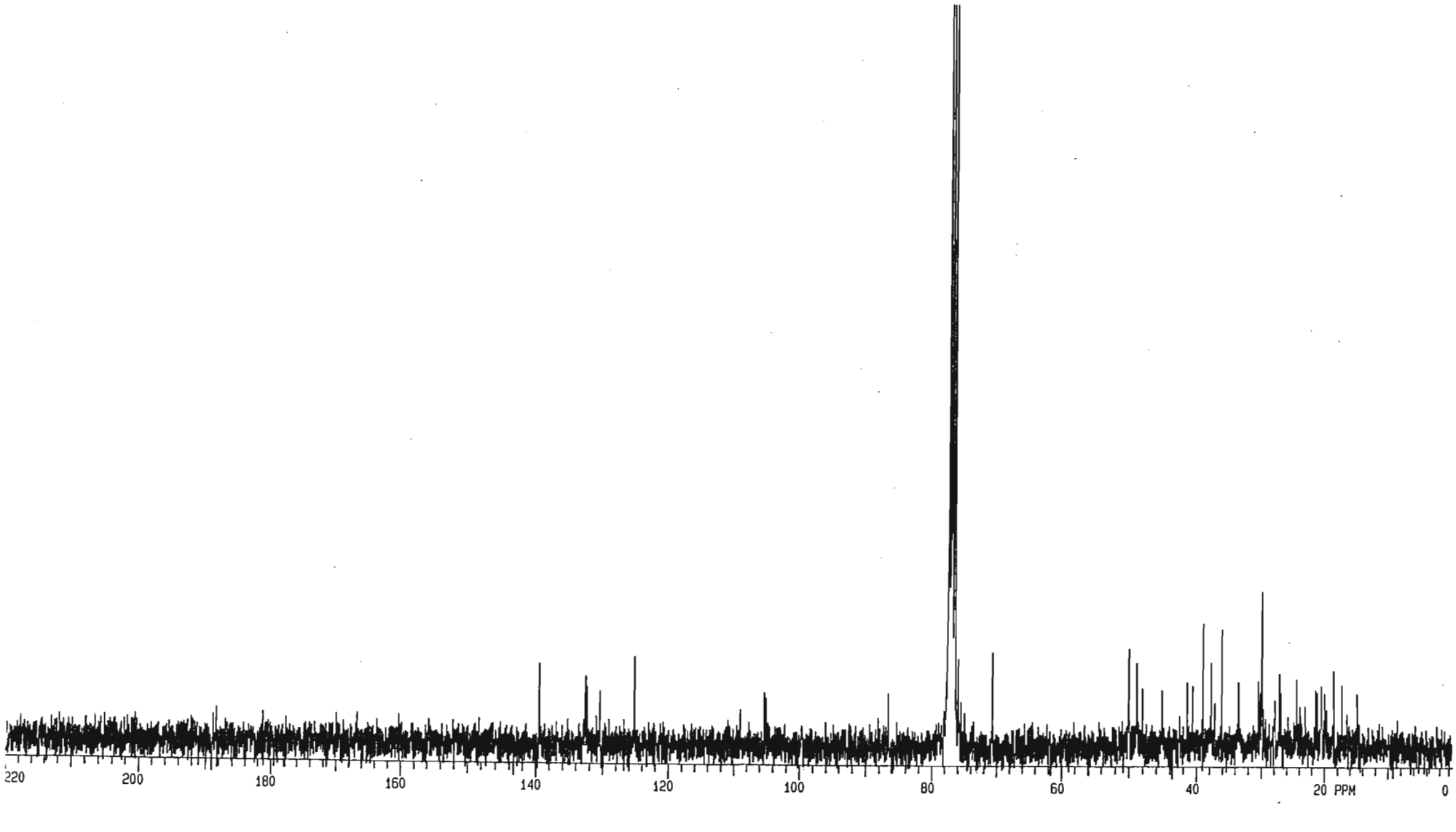
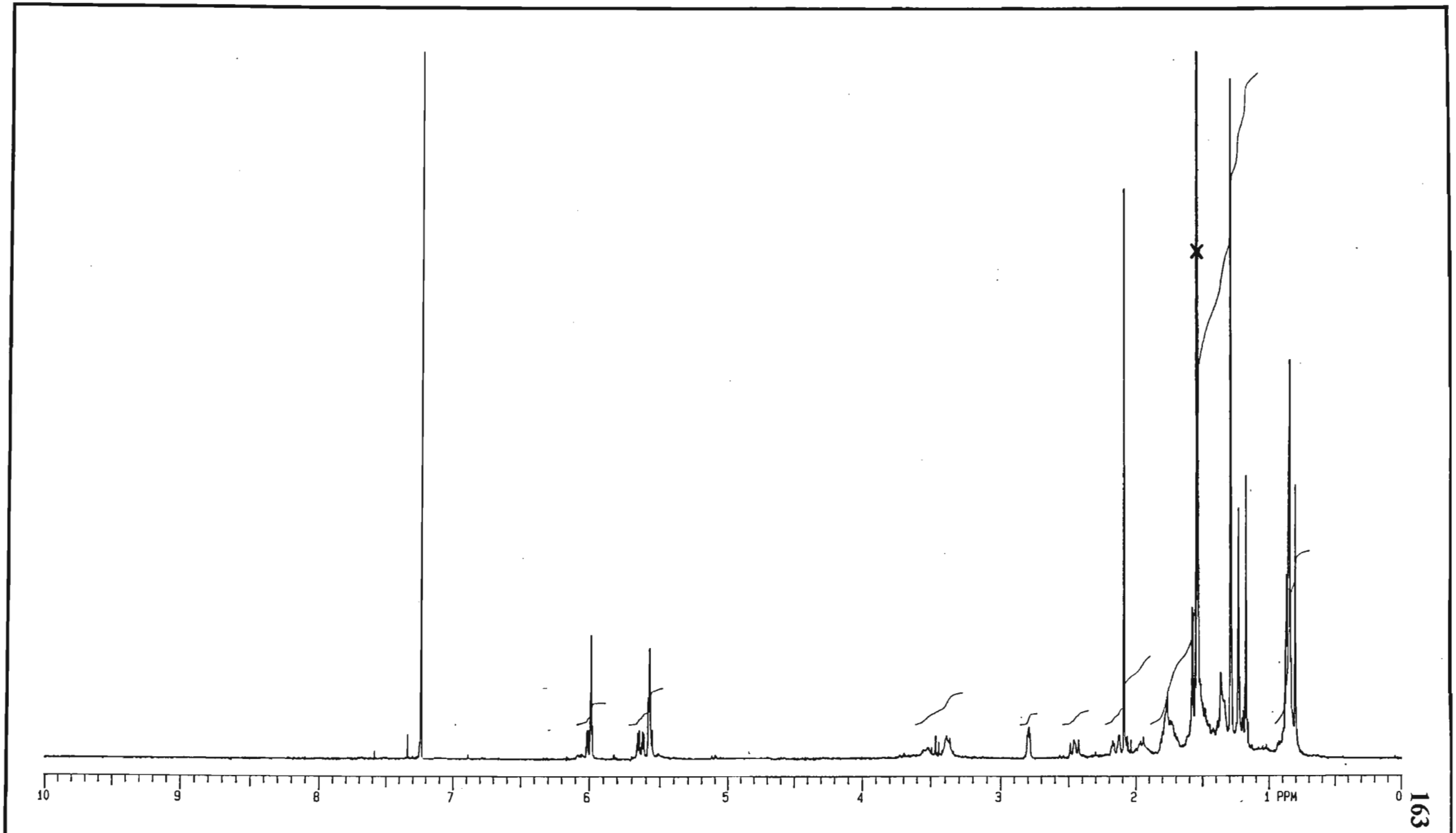
Spectrum 4h: $^{1}$H NMR spectrum of **[103a]**



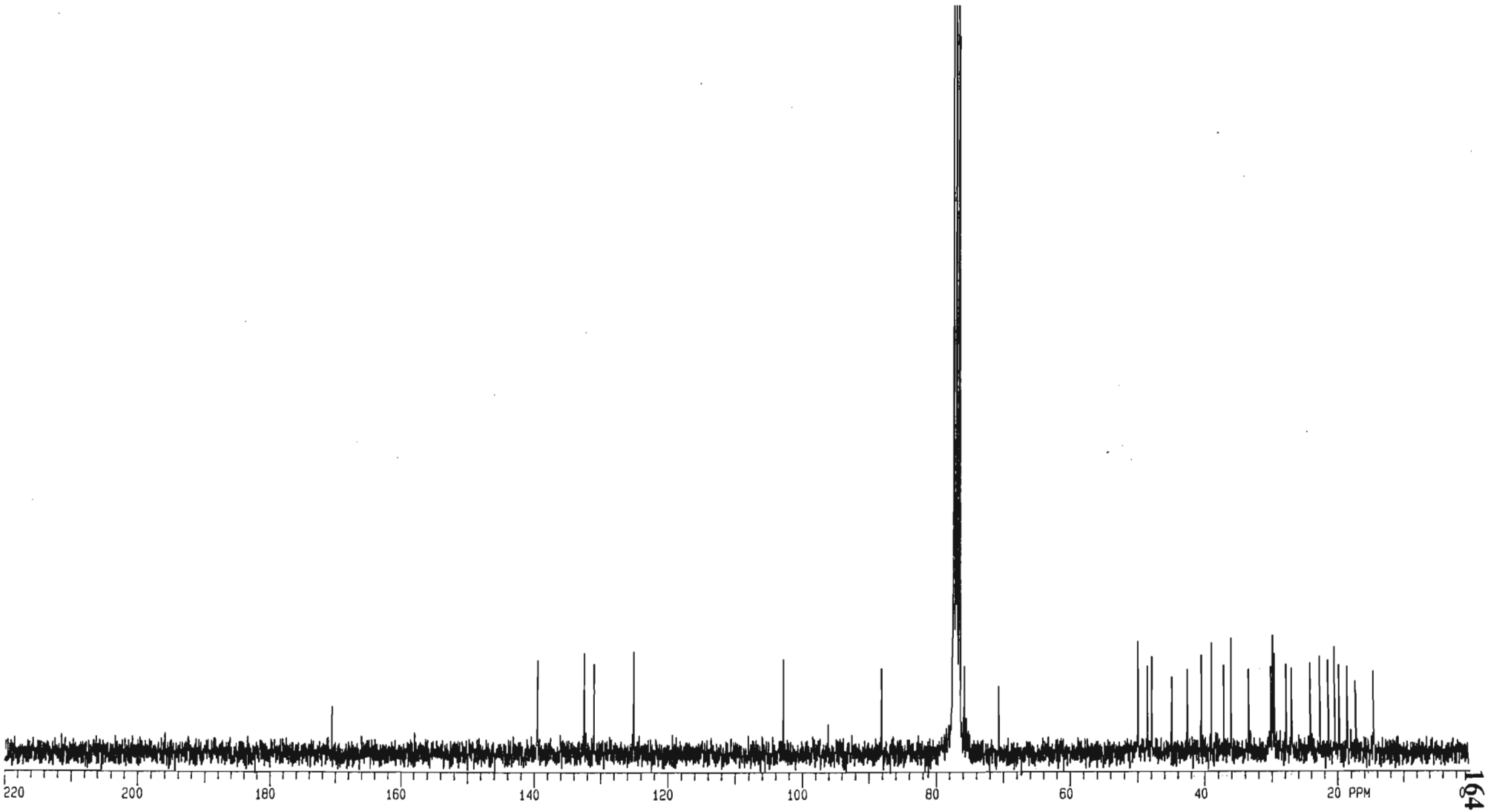



Spectrum 4i: $^{13}C$ NMR spectrum of **[103a]**

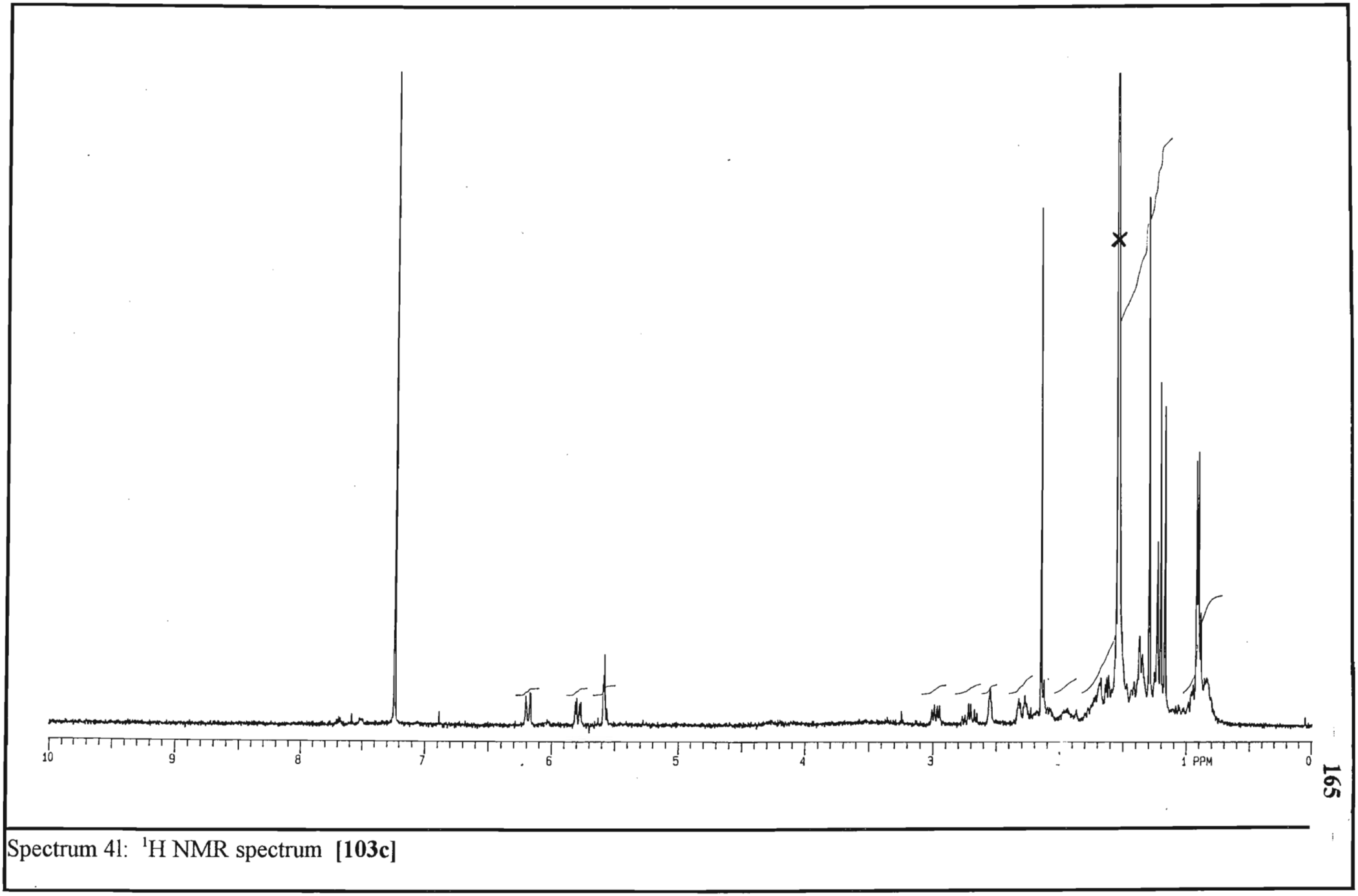




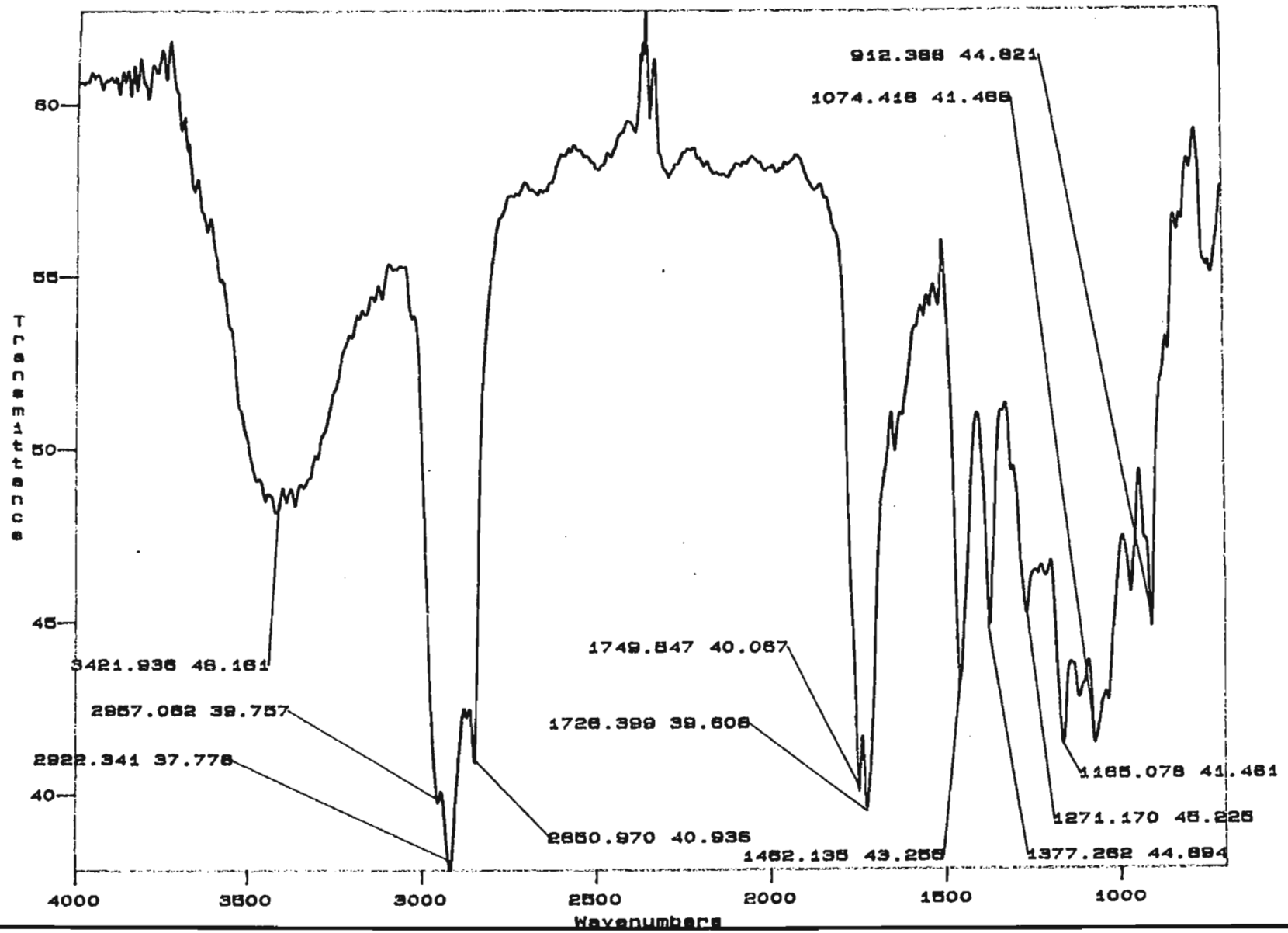

Spectrum 4j: $^{1}H$ NMR spectrum of **[103b]**



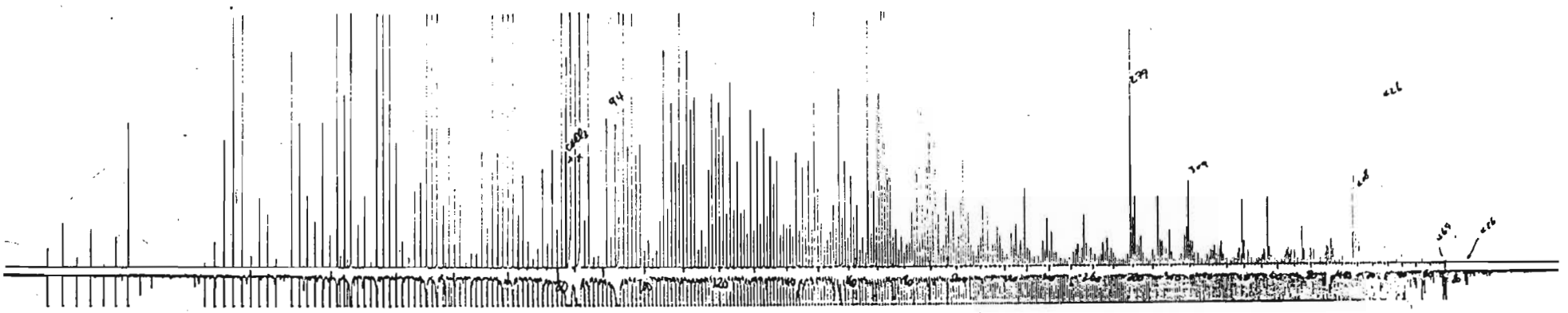

Spectrum 4k: $^{13}C$ NMR spectrum of **[103b]**




Spectrum 41: $^{1}H$ NMR spectrum **[103c]**

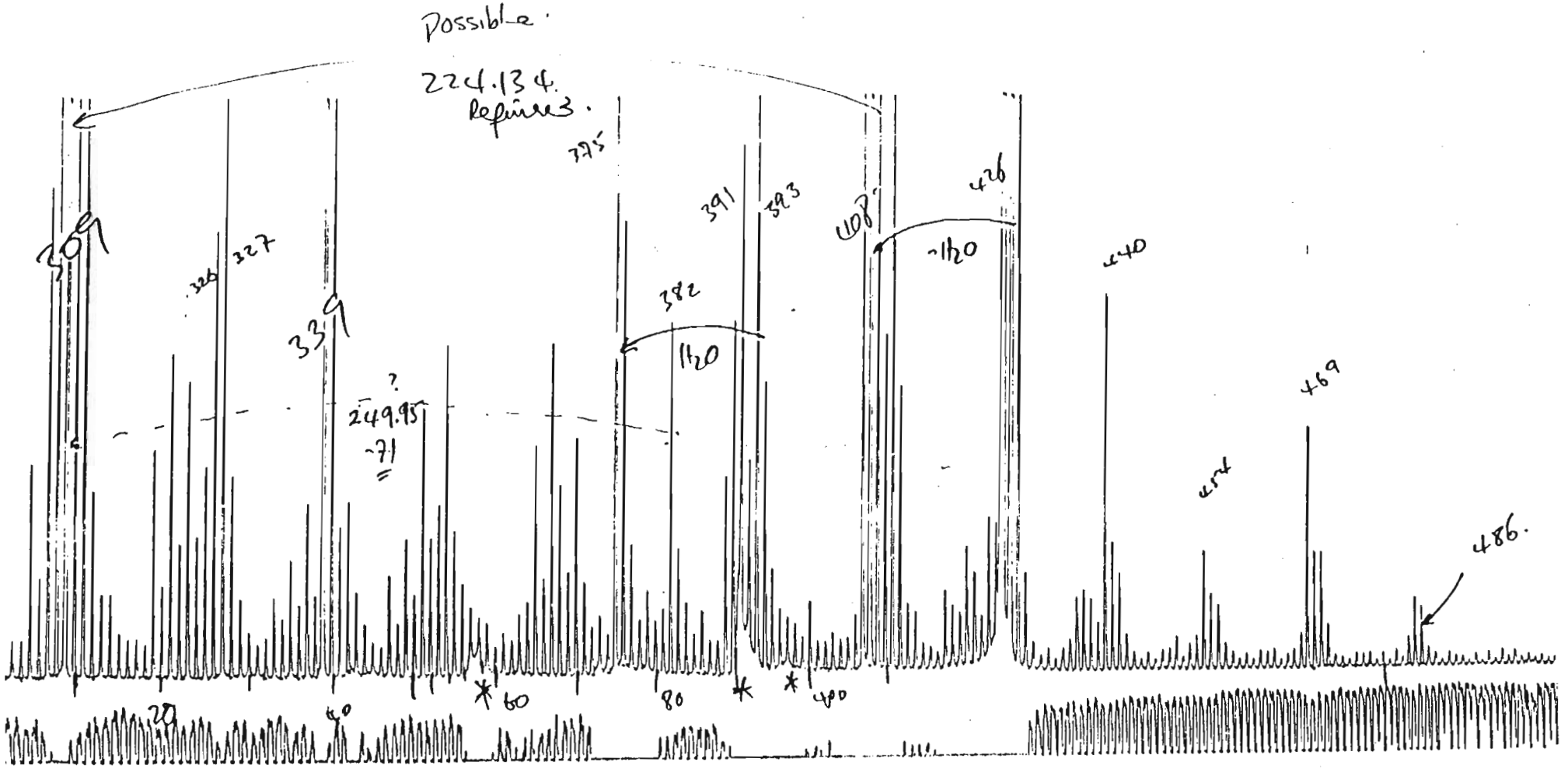



Spectrum 4m: Infrared spectrum of **[103c]**



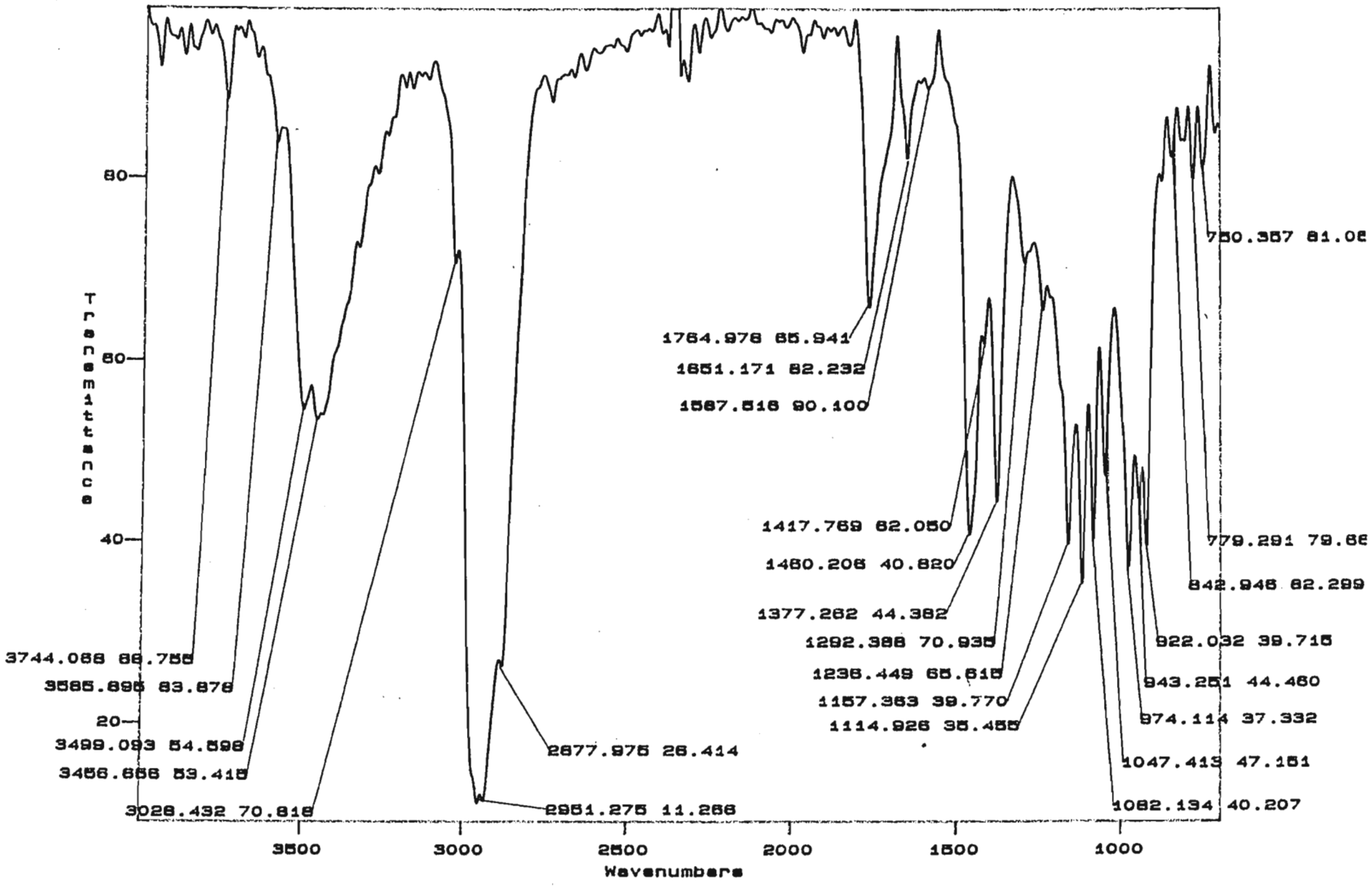


Spectrum 5a: Mass spectrum **[104]**

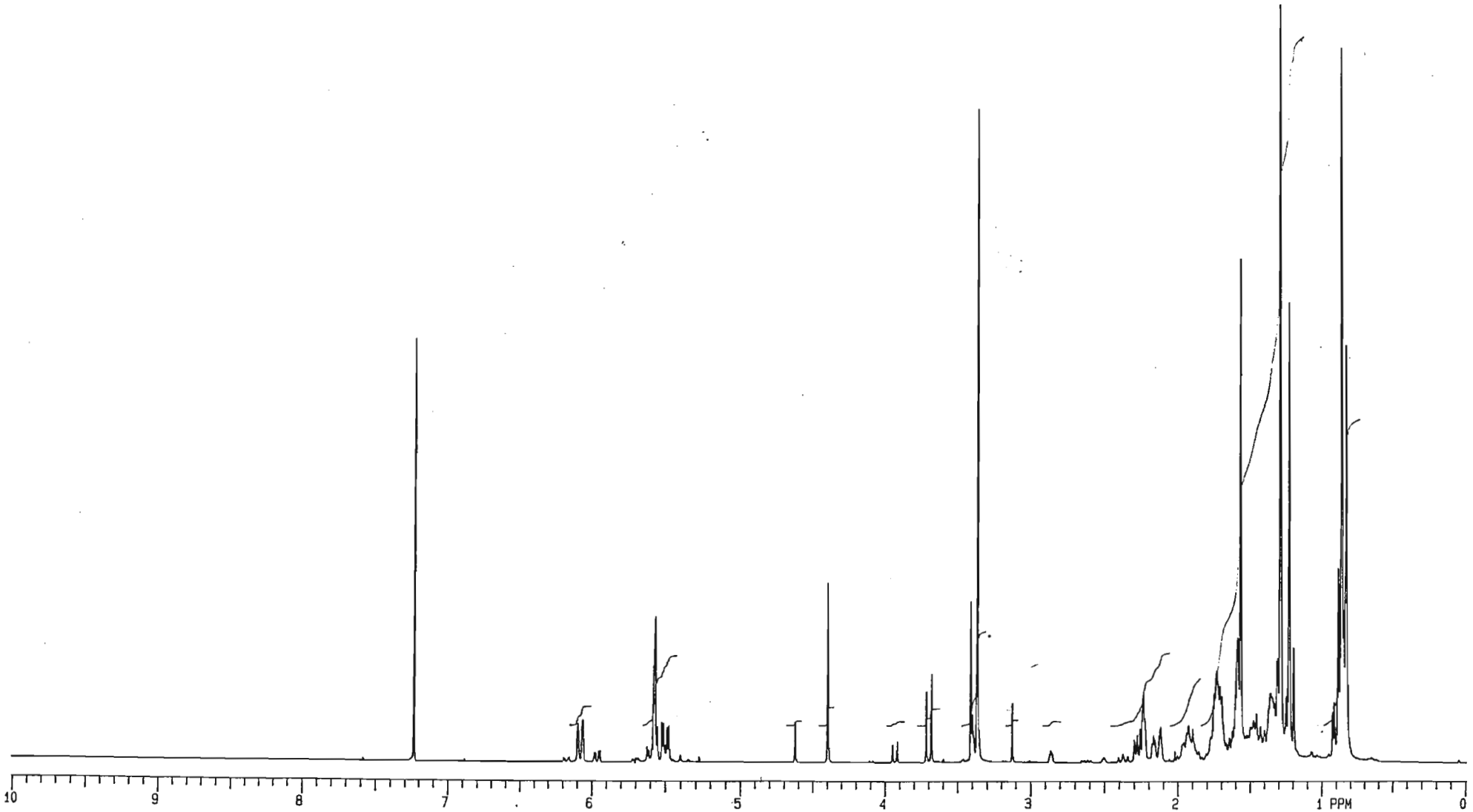



167a

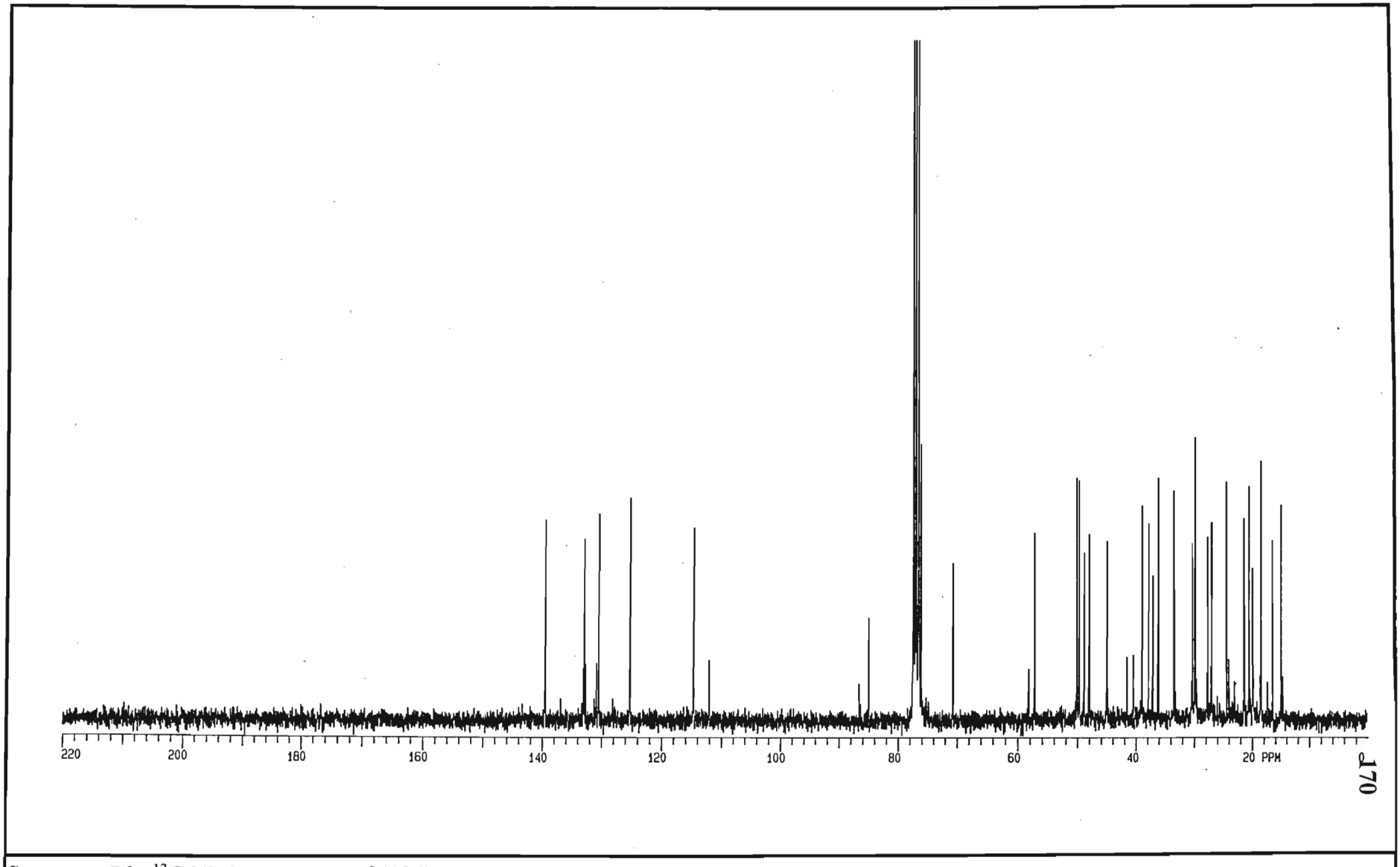
Enlarged mass spectrum of **[104]** |



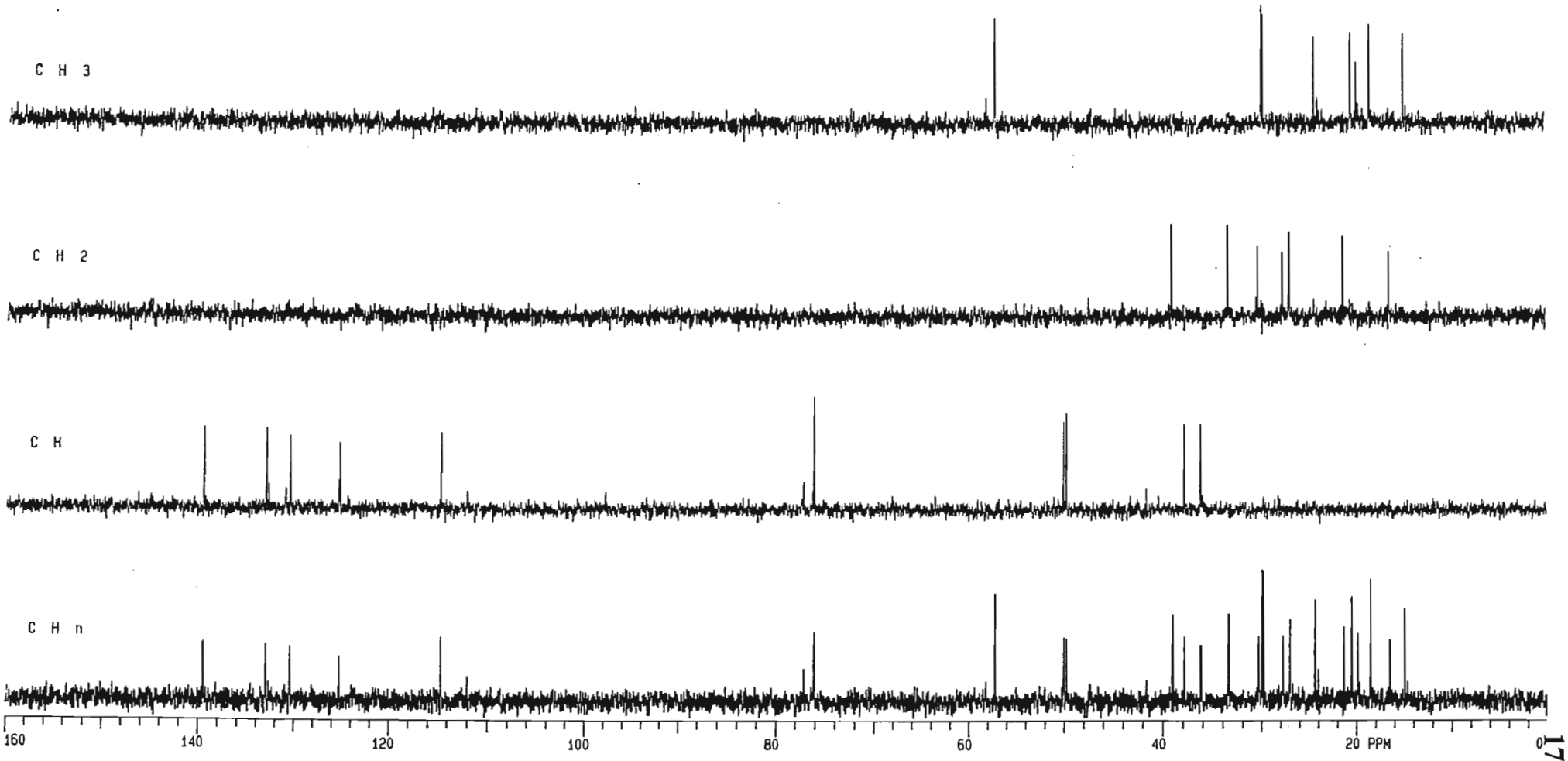

Spectrum 5b: Infrared spectrum of **[104]**



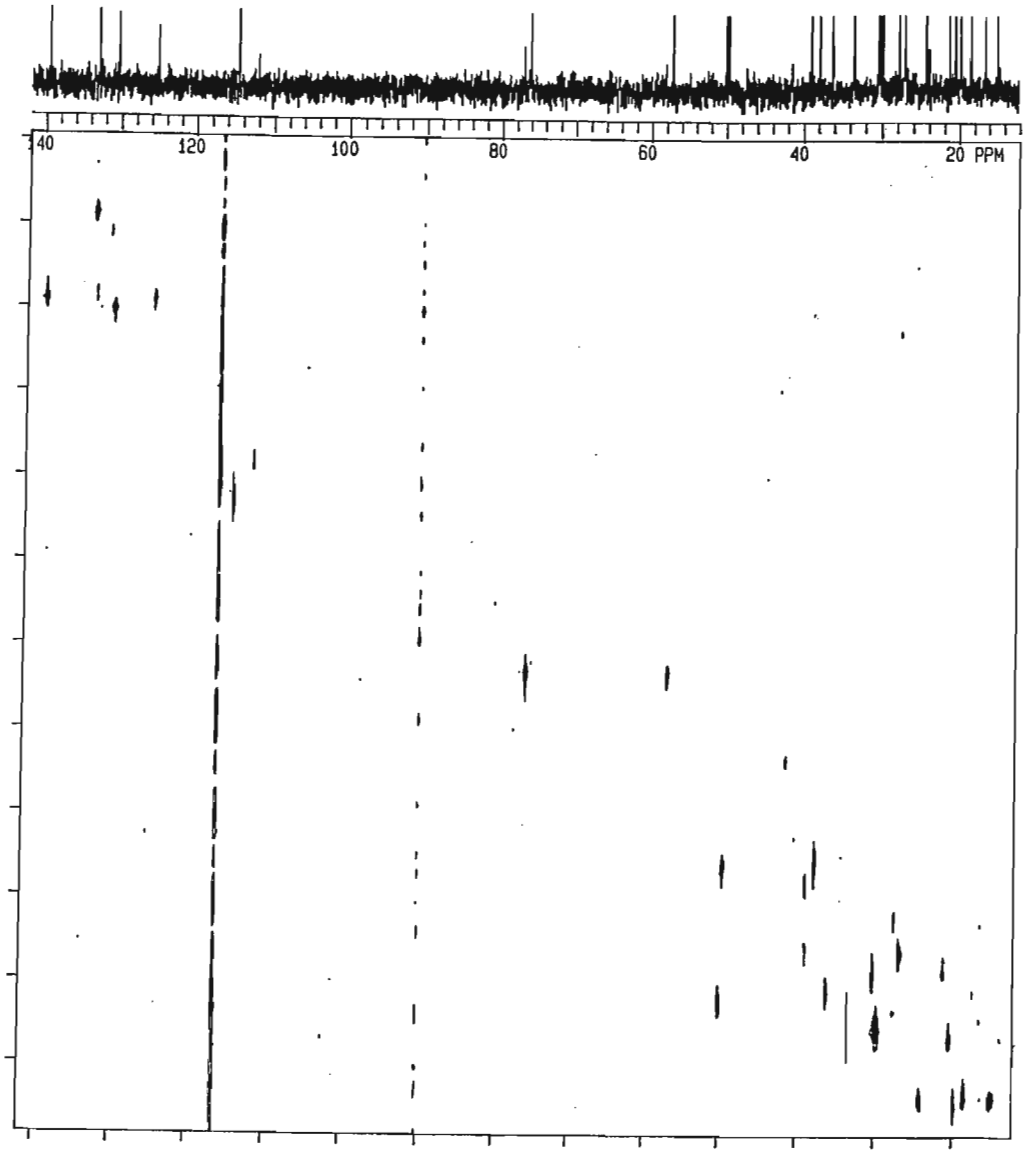

Spectrum 5c: $^{1}H$ NMR spectrum of **[104]**

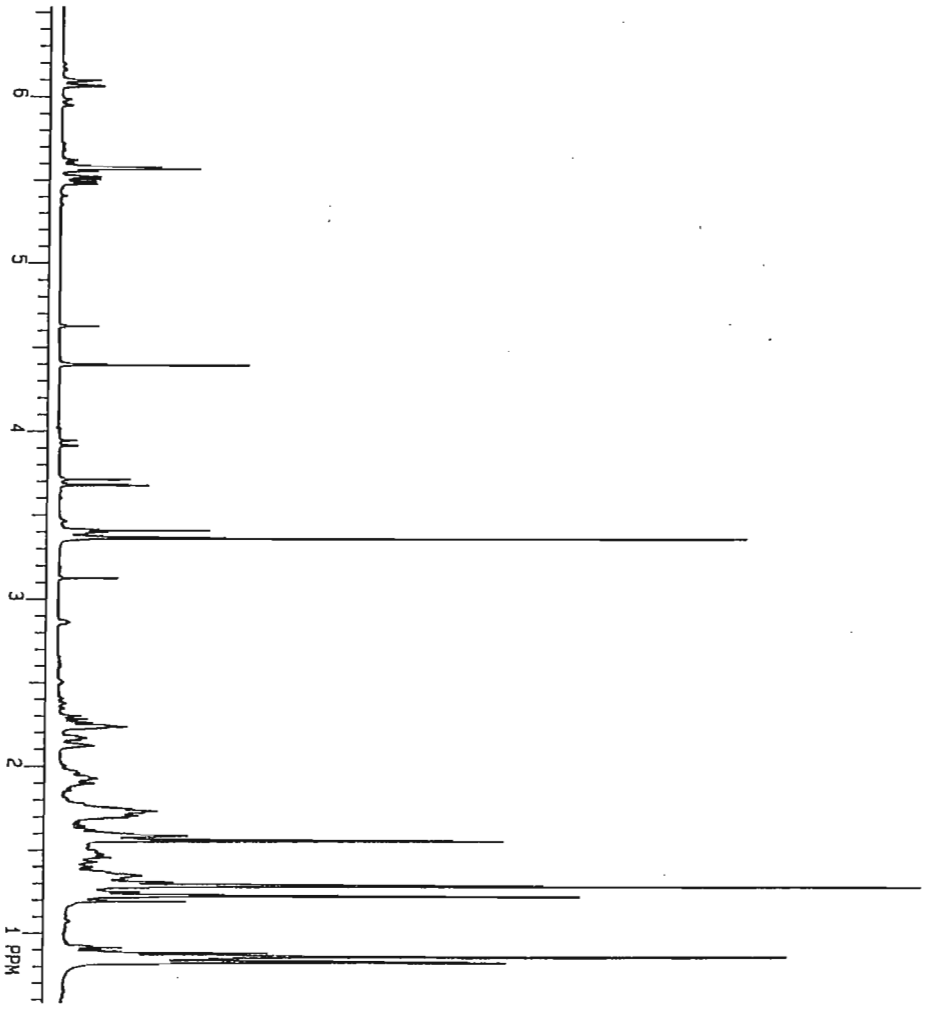



Spectrum 5d: $^{13}C$ NMR spectrum of **[104]**



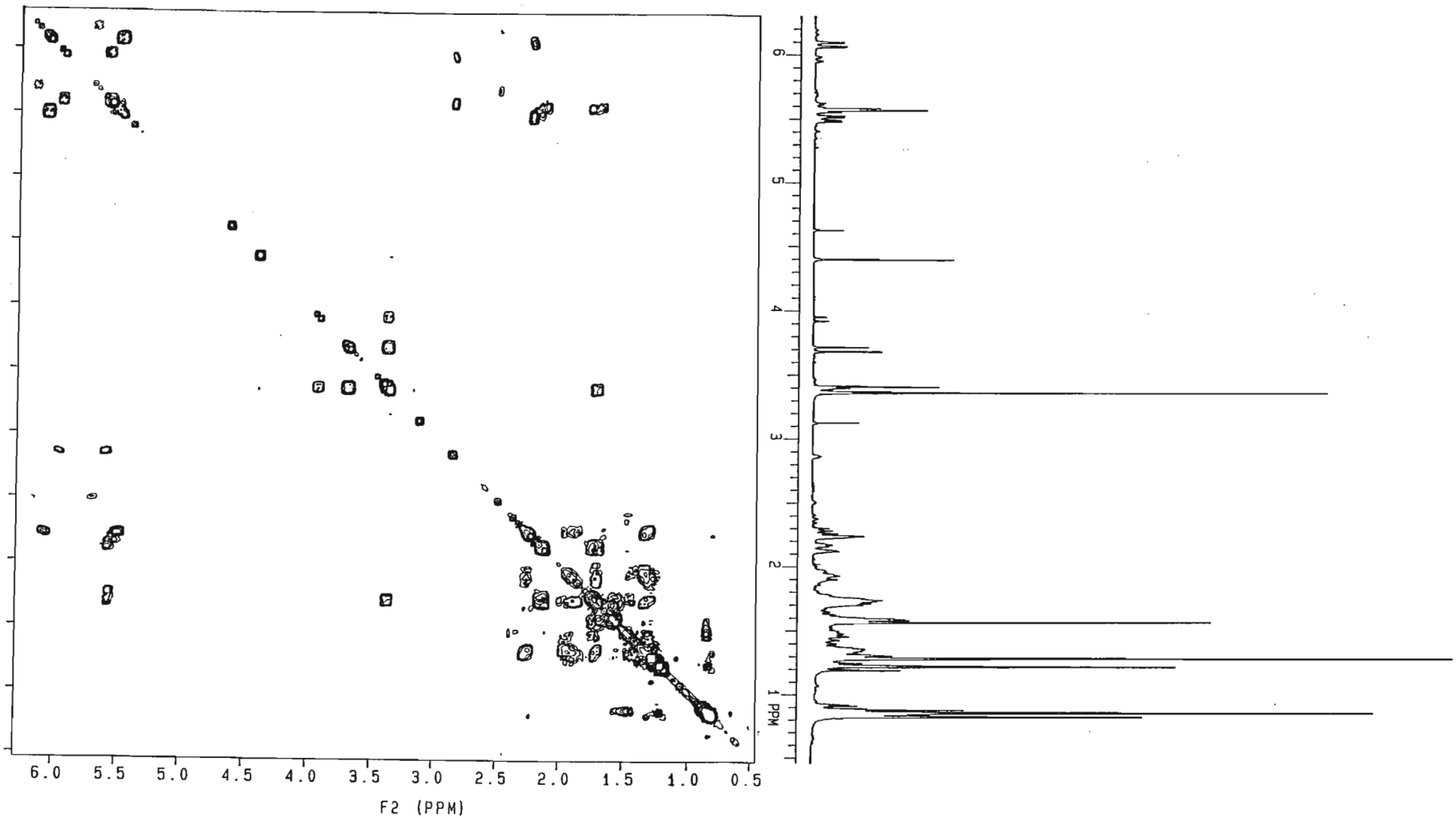

Spectrum 5e: DEPT spectrum of **[104]**



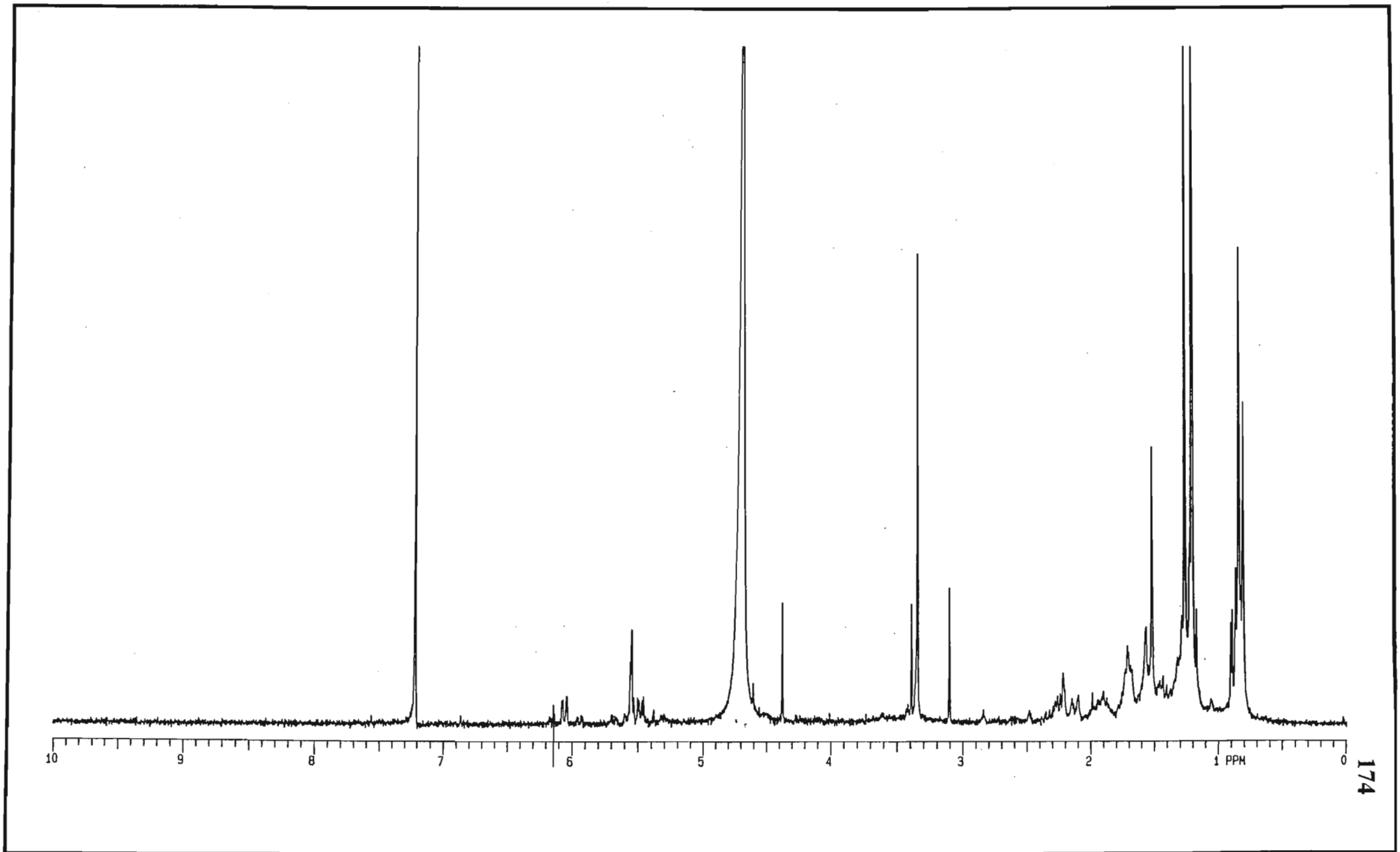





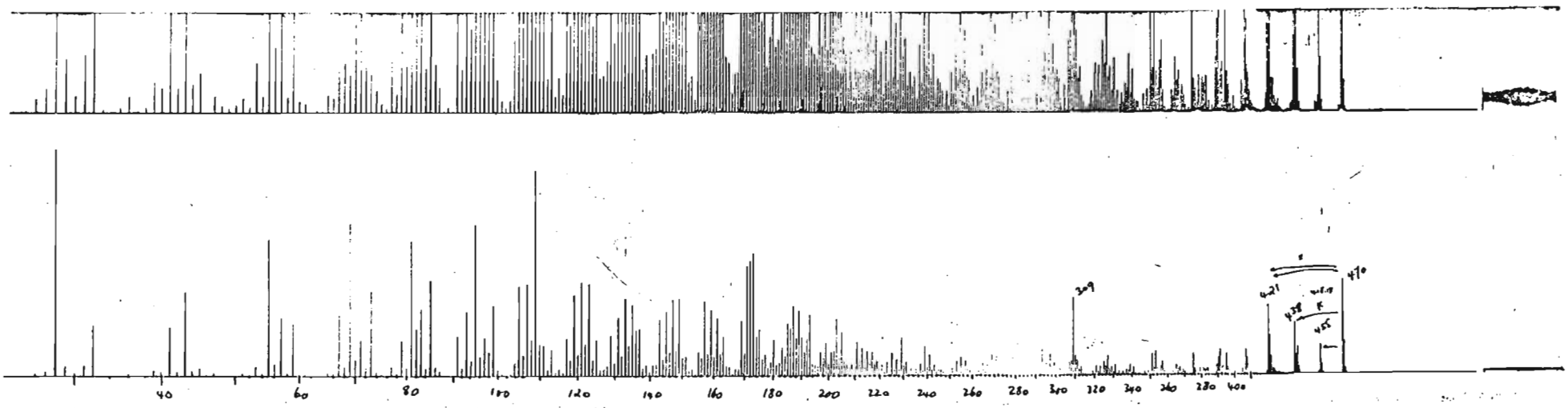

Spectrum 5f: HETCOR spectrum of **[104]**



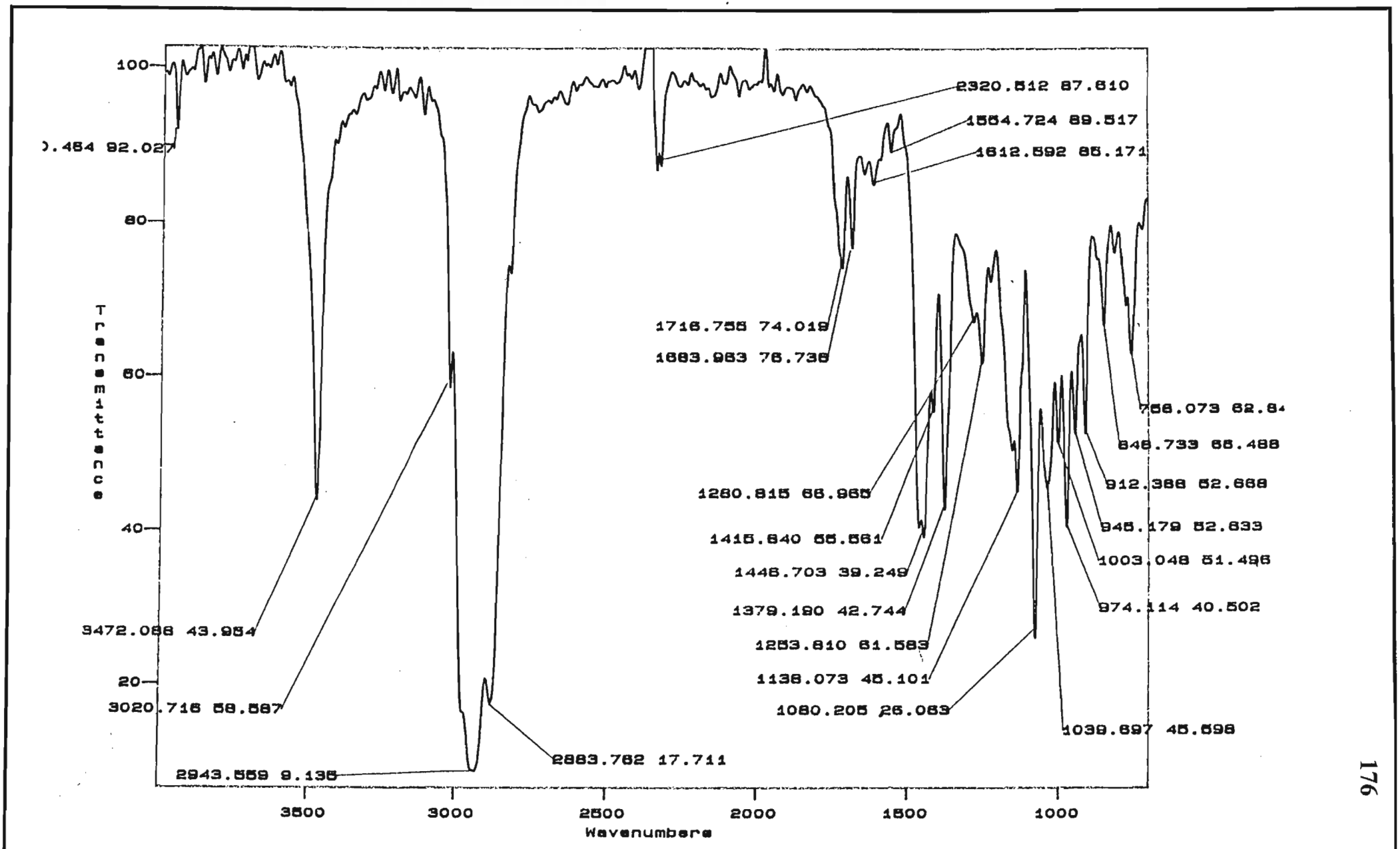

Spectrum 5g: COSY spectrum of **[104]**



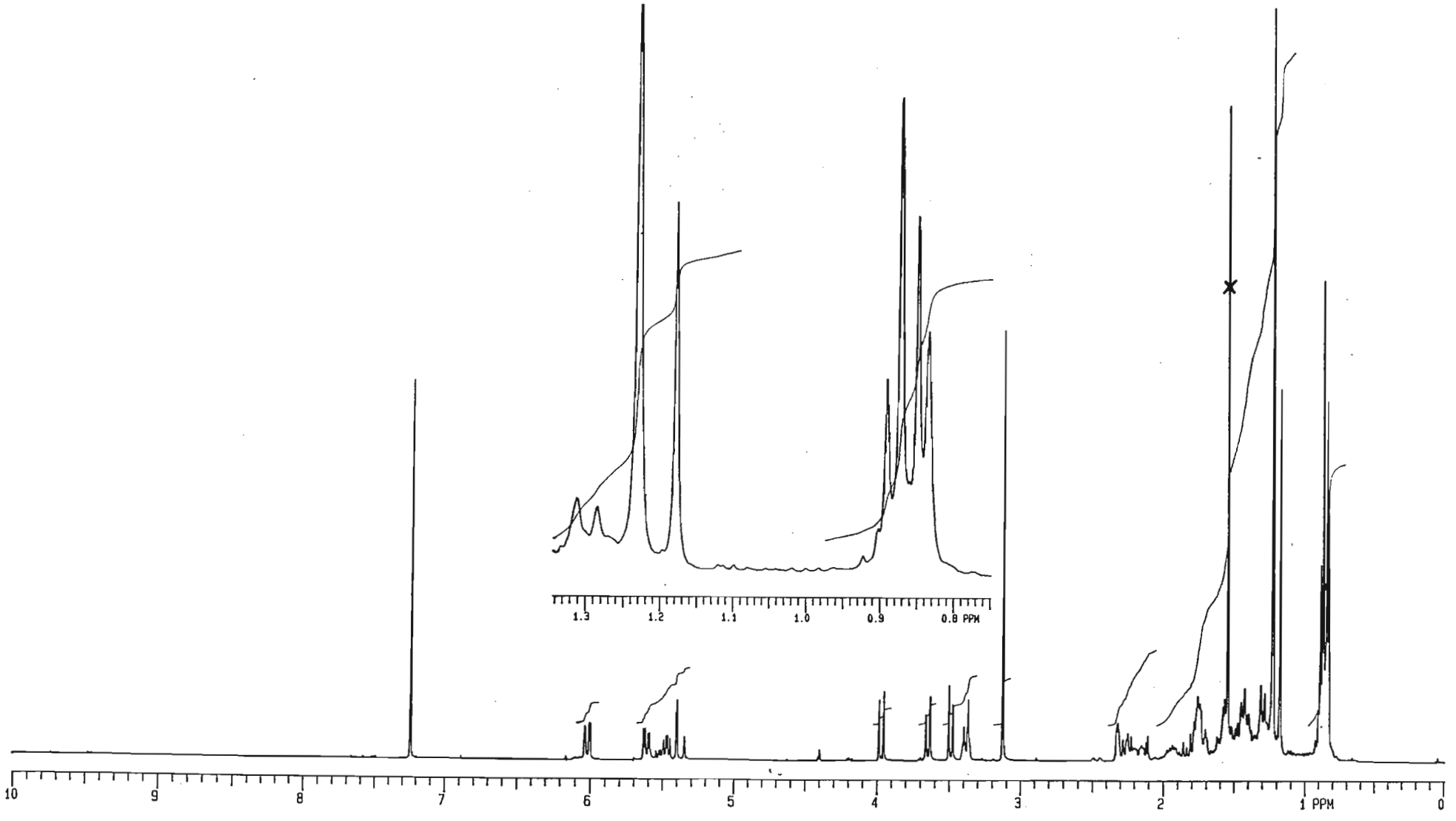

Spectrum 5h: $^{1}H$ NMR spectrum of **[104]** with $D_2O$



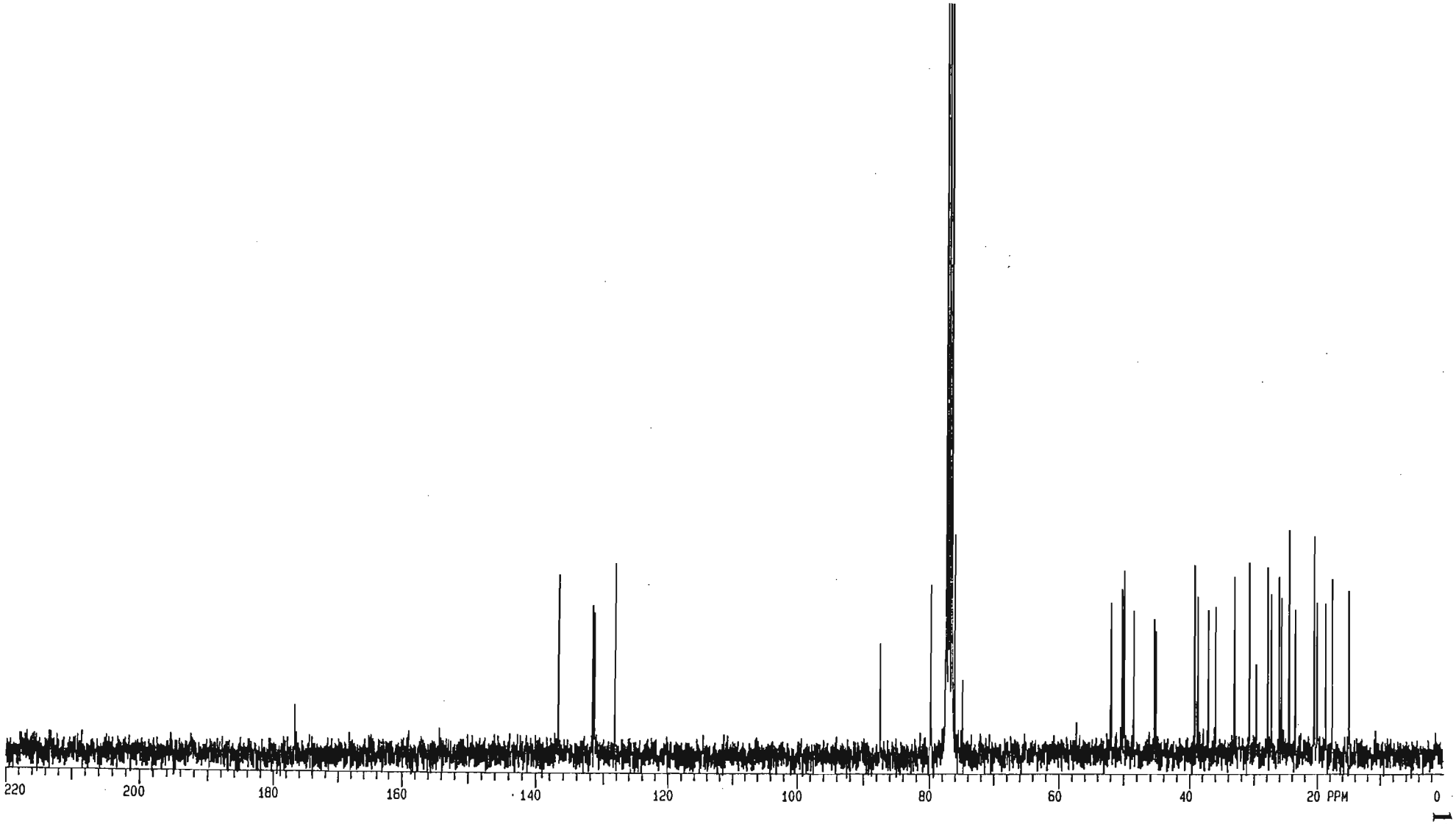

Spectrum 6a: Mass spectrum **[105]**




Spectrum 6b: Infrared spectrum of **[105]**

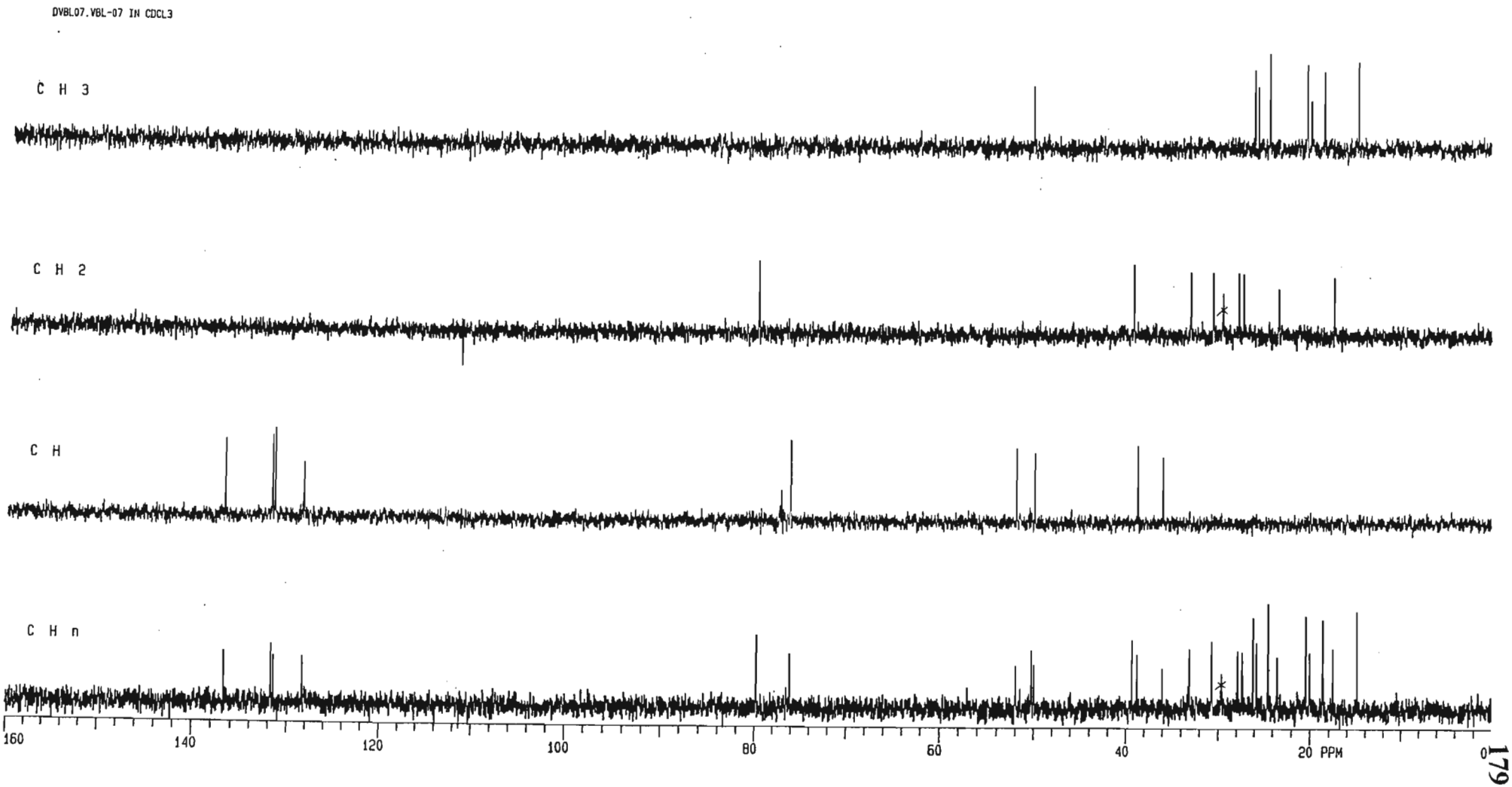



Spectrum 6c: $^{1}H$ NMR spectrum of **[105]**

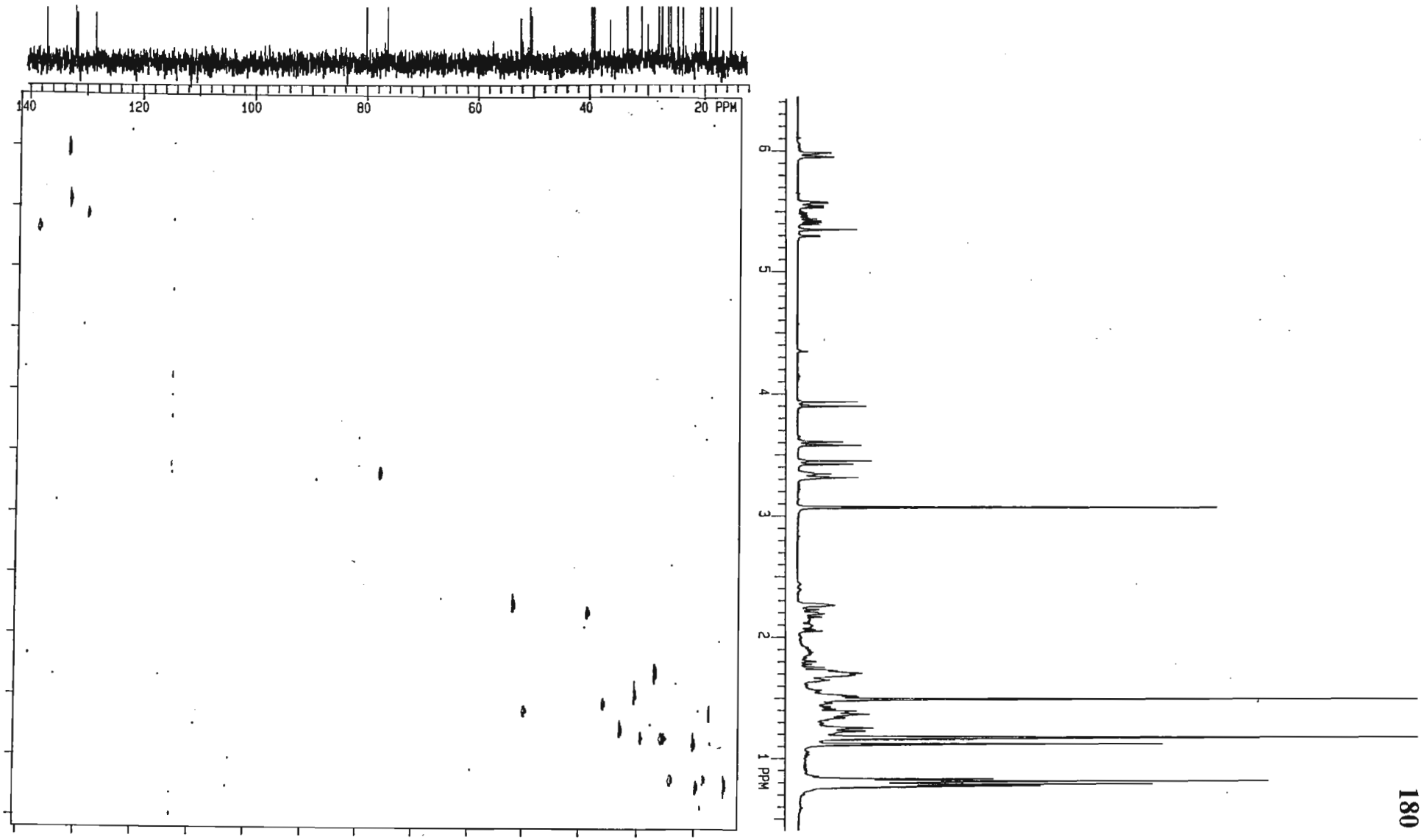





Spectrum 6d: $^{13}C$ NMR spectrum of **[105]**

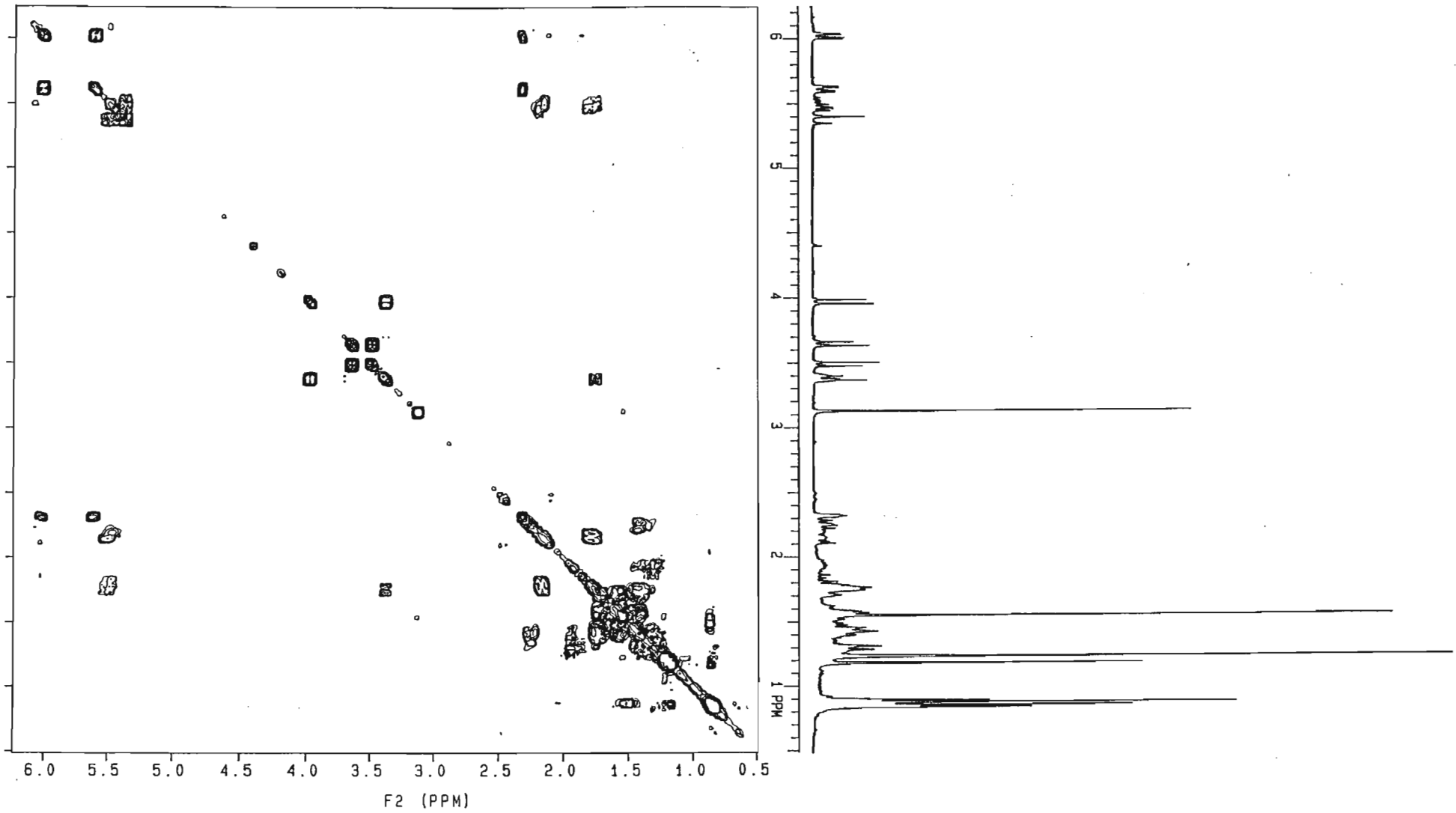



Spectrum 6e: DEPT spectrum of **[105]**

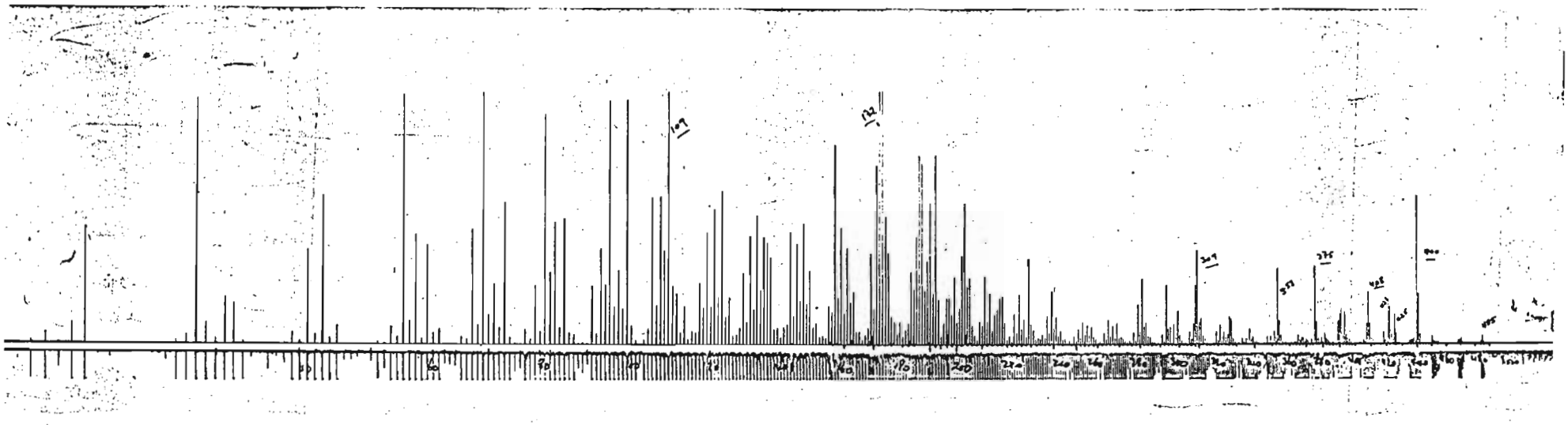





Spectrum 6f: HETCOR spectrum of **[105]**

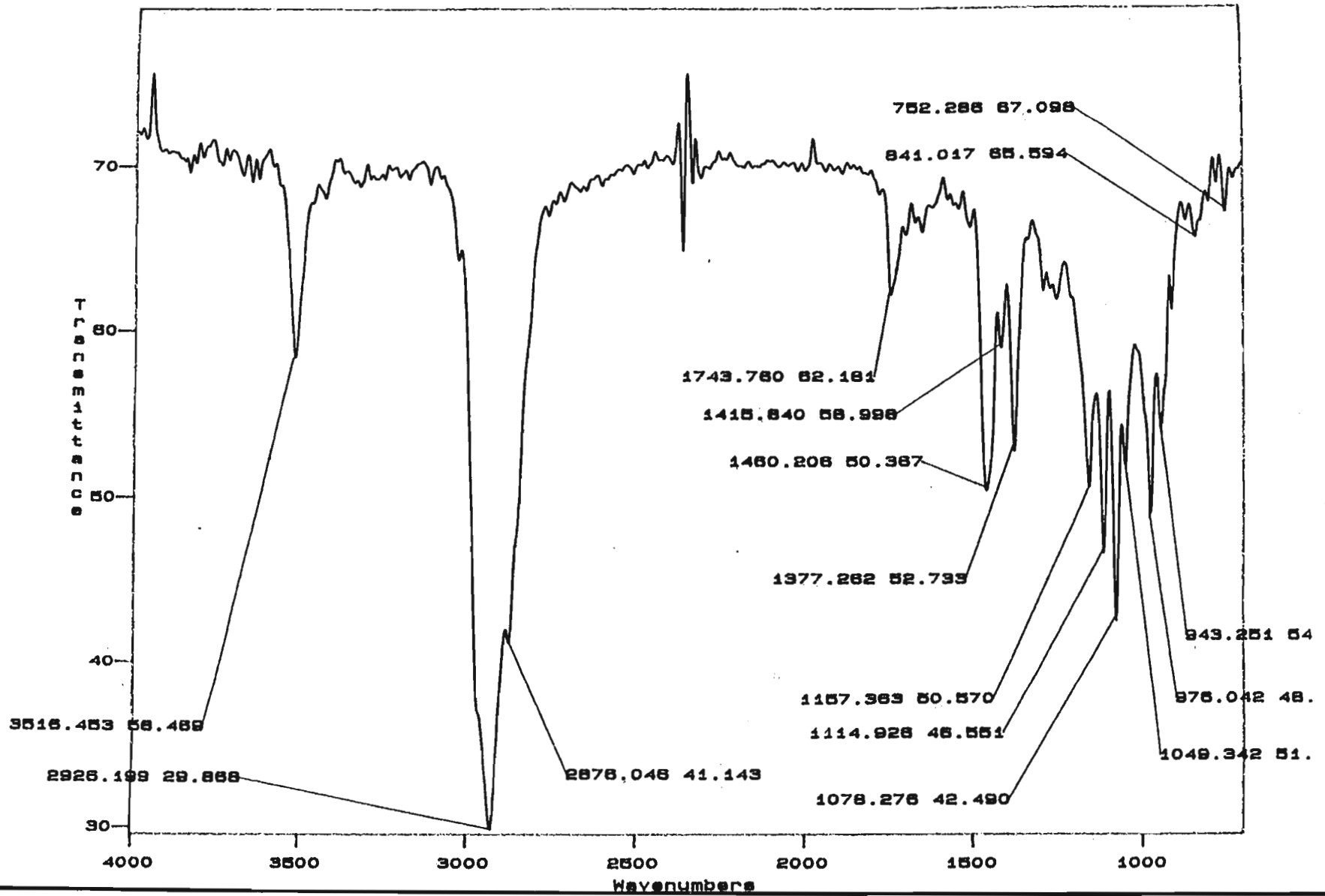



Spectrum 6g: COSY spectrum of **[105]**

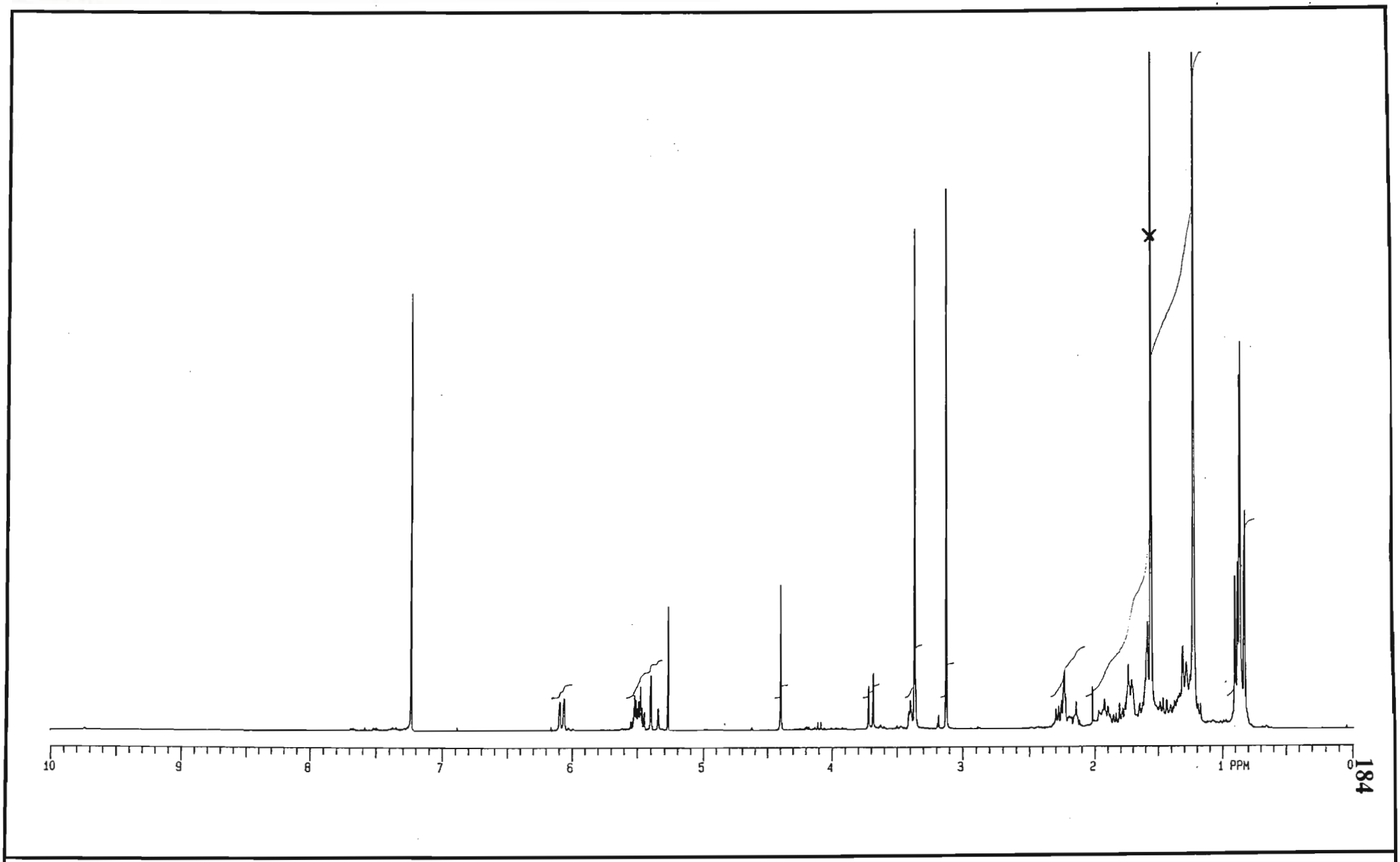




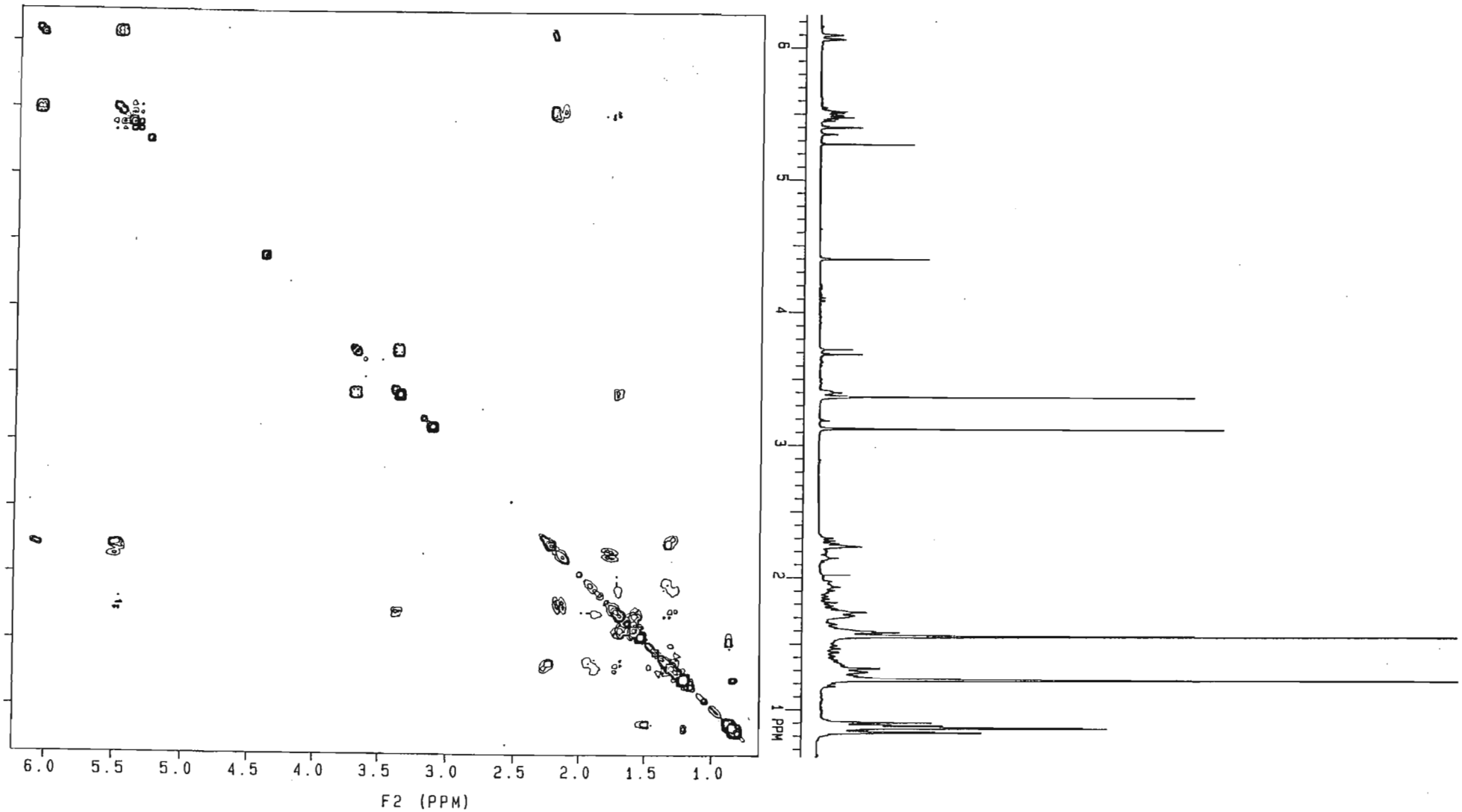
Spectrum 7a: Mass spectrum **[106]**




Spectrum 7b: Infrared spectrum of **[106]**

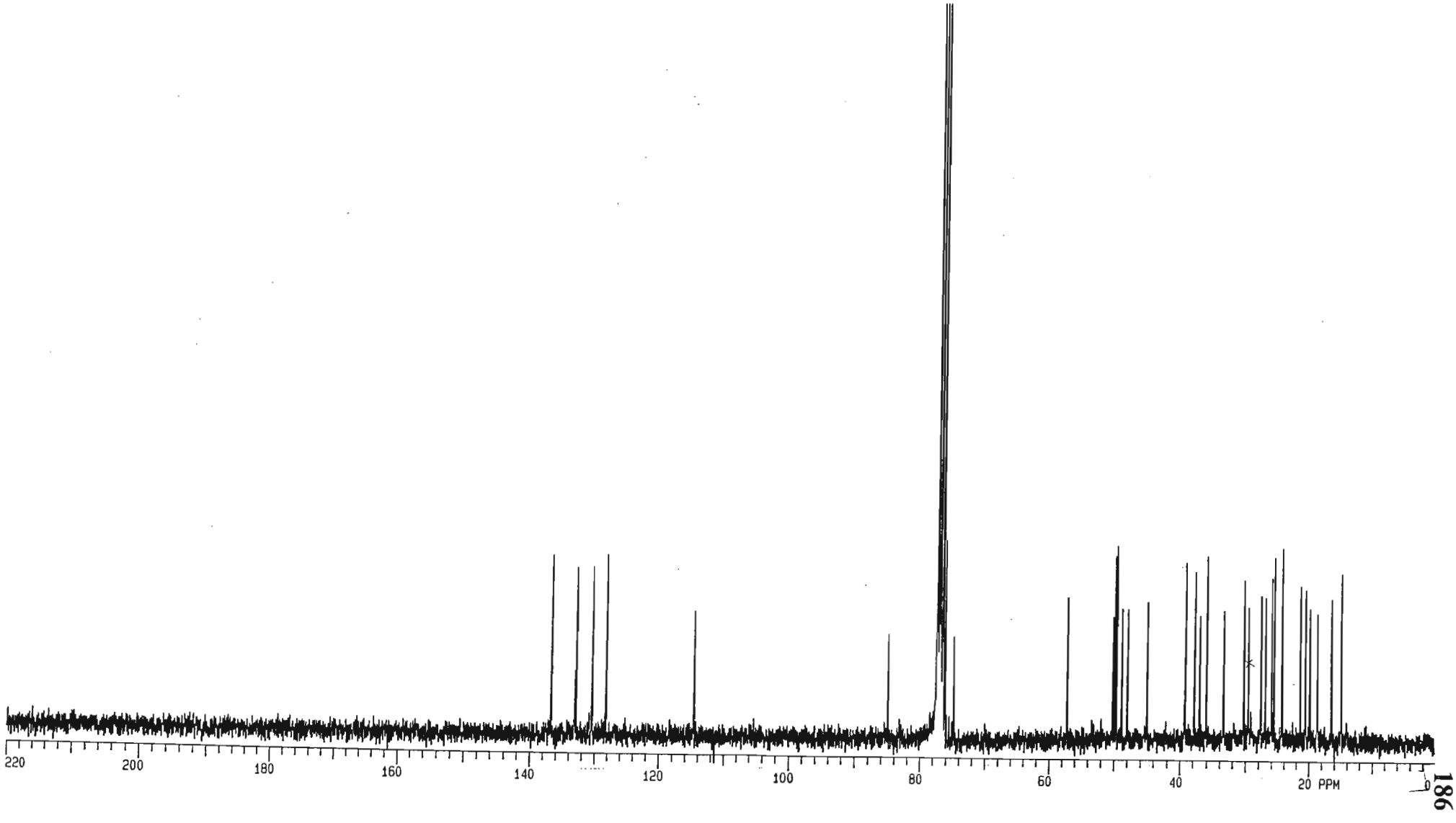



Spectrum 7c: $^{1}H$ NMR spectrum of **[106]**



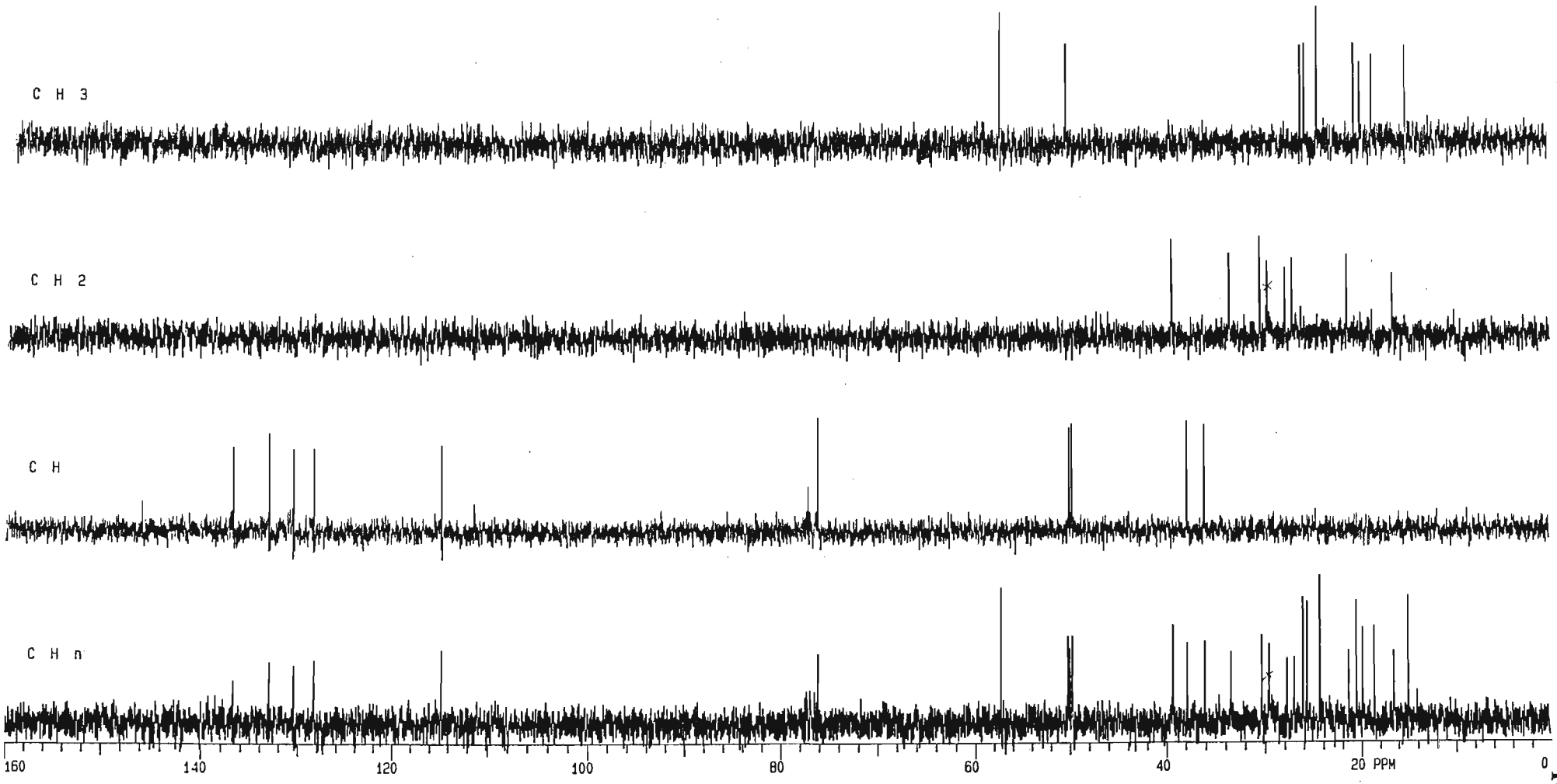



Spectrum 7d: COSY spectrum of **[106]**

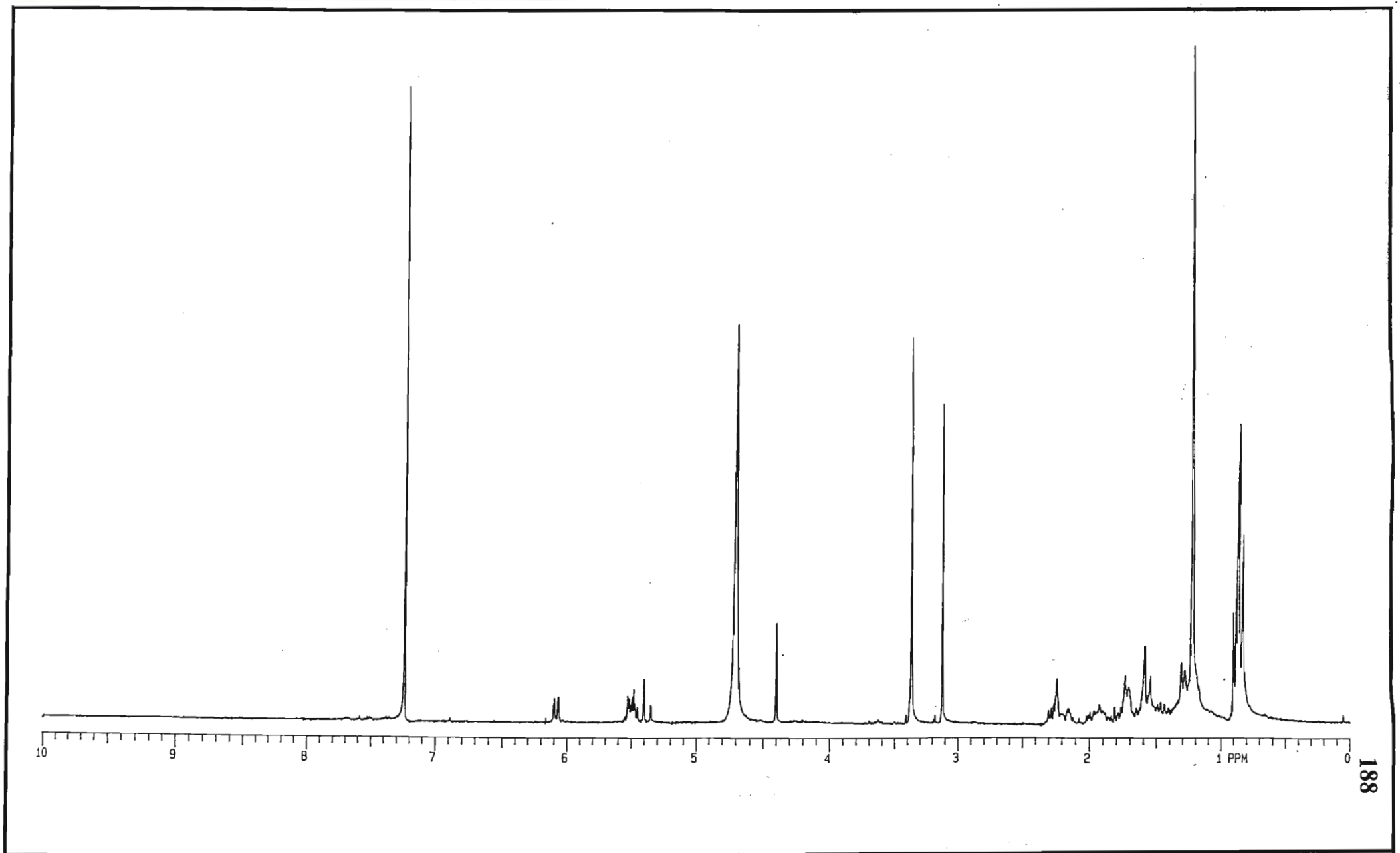





Spectrum 7e: $^{13}C$ NMR spectrum of **[106]**




Spectrum 7f: DEPT spectrum of **[106]**






Spectrum 7g: $^{1}H$ NMR spectrum of **[106]** with $D_2O$

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