THE CHEMICAL INVESTIGATION OF *LEDEBOURIA ZEBRINA* AND *SCILLA NATALENSIS*

by

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in the

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TO MY DEAR PARENTS

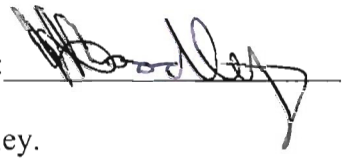
"If a man empties his purse into his head, no man can take it away from him. An investment in knowledge always pays the best interest."

Benjamin Franklin

PREFACE

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of Natal, Durban, under the supervision of Professor D.A. Mulholland and Dr. N. Crouch.

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

SIGNED: 

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சமரஞானதயாளா

(குரு வாக்கியம்)

ஓம் சகல சௌக்கியாதிசௌபாக்கியங்களோ
நம்பினோருக்குத் தற்பரமும் ஆனந்த ஞானவுருவே நீயே
துணை, கருணாகரக் கடவுளே நீயே துணை.

சமரச ஞான தயாளா, சமரச சீவதாரா
சமரச ஞானச் செல்வா, சமரசானந்தாகாப்பாய்

சபை வாக்கியம்.

ஓம் பிரியதாயகி பிரணவரூபா காப்பாய் தினமே
ஓம் சரஸ்வதிசமரசஞானந்தினி காப்பாய் தினமே
ஓம் சீதேவி வியாபகானந்தினி காப்பாய் தினமே
ஓம் உமாதேவி சிவசுதிரானந்தினி காப்பாய் தினமே
ஓம் மகேஸ்வரி மகேஸ்வரானந்தினி காப்பாய் தினமே
ஓம் மனோன்மணி சதாசிவானந்தினி காப்பாய தினமே
ஓம் நம: சிவாய வாழ்க, ஓம் நம: சிவாய வாழ்க,
ஓம் நம: சிவாய வாழ்க!

ABBREVIATIONS

Ac	acetate
br	broad resonance
br s	broad singlet
m	multiplet
c	concentration
^{1}H NMR	proton (^{1}H) nuclear magnetic resonance spectroscopy
^{13}C NMR	carbon-13 nuclear magnetic resonance spectroscopy
COSY	correlated spectroscopy
DEPT	distortionless enhancement by polarisation transfer
HMBC	heteronuclear multiple bond correlation
HSQC	heteronuclear multiple quantum coherence
NOESY	nuclear Overhauser effect spectroscopy
ROESY	Rotating frame nuclear Overhauser effect
FTIR	Fourier transformed infrared spectroscopy
HRMS	high resolution mass spectrometry
d	doublet
dd	double of doublets
dt	doublet of triplets
Hz	hertz
Me	methyl
ppm	parts per million
q	quartet
s	singlet
t	triplet
cm	centimeters
nm	nanometers
FTIR/I.R	infra red
UV	Ultra violet
rpm	revolutions per minute
m.p	melting point
t.l.c	thin layer chromatography
GC/MS	gas chromotography/mass spectrometry

Acetyl CoA	acetyl coenzyme A
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ax	axial
CNS	central nervous sytem
Malonyl CoA	malonyl coenzyme A
NIH	National Insitute of Health
Pi	inorganic phosphate
IPP	isoprene pyrophosphate
FPP	farnesyl pyrophosphate
GPP	geranyl pyrophosphate
DMAPP	dimethylallyl pyrophosphate
PAL	phenylalanine ammonia lyase
C4H	Cinnamate-4-hydroxylase
PPE	phosphodiesterase

List of Figures

List of Tables

List of Schemes

ABSTRACT

Ledebouria zebrina and *Scilla natalensis* Planch were the two species investigated in this work.

Ledebouria zebrina belongs to the Hyacinthaceae (Liliacea *sensu lato*) and to this date, the chemical composition of this species has not been investigated. Members of this family are found in southern Africa. The *Ledebouria* genus was formerly classified as part of the *Scilla* genus from which a large member of naturally occuring oxygen heterocycles known as homoisoflavanones have been isolated. In this work the bulbs of *L. zebrina* were investigated and five compounds were isolated. Three compounds were of the homoisoflavanone type while the remaining two belong to the eucosterol type triterpernoids.

Scilla natalensis Planch also belongs to the Hyacinthaceae family. Previous chemical investigations of the bulbs of this plant yield two homoisoflavanones of the 3-benzyl-4-chromanone type. Members of this family are mostly found in the Eastern parts of the country, ranging from the Eastern Cape to Mpumalanga province including Lesotho and Swaziland. The bulbs of this plant were investigated and this yielded ten compounds.

The structures of the isolated compounds were elucidated using spectroscopic methods.

TABLE OF CONTENTS

Chapter 3: Extractives from *Ledebouria zebrina*

Chapter 4: Extractives from *Scilla natalensis*

Chapter 1: Introduction

1.1 General

Since ancient times people have used medicinal plants. In China, medicinal plants have long enjoyed a prominent role in healthcare services. Chinese traditional medicine has a history extending back 4000 years with the *Yellow Emperor's Classic of Internal Medicine* considered to be the world's oldest extant medical book.[1] Medicinal plants in India have been collected in the wild and cultivated for millennia. The *Rig Veda*, written in India between the 4800-1600 BC is the earliest record in India of the use of trees, shrubs, herbs and grass combinations for curing aliments.[1] The ancient kingdoms and empires of Africa, such as Ethiopia, Nubia and Somalia had extensive codified healing recipes.[2] The history of healing arts in Africa can be traced back to ca. 3200 BC, when the *Ebers Papyrus*, one of the oldest medical literatures, listed several recipes used by ancient Egyptian healers.[2]

There is no clear distinction in traditional African medicine as to when a herb ceases to be a health food and when it becomes a medicine. In traditional African medicine, many food plants are used for therapeutic purposes, and medicines are not viewed as "necessary poisons". This is in contrast to Western orthodox medicine in which many drugs are seen as poisons, which in low doses may cure diseases. African plants such as the calabar bean (*Physostigma venenosum*) **(Figure 1.1)**, *Strophanthus*, Kola, the Madagascan periwinkle (*Catharanthus roseus*) and the Devils Claw (*Harpagophytum procumbens*) all have made it onto the pharmaceutical market. Something that is interesting to note is that there are African varieties of several "official" drugs. The African *Rauvolfia vomitoria*, for example, has a higher content of the antihypertensive alkaloid reserpine than the better-known Indian species. One of the best known African medicinal plants is the calabar bean. It contains alkaloids, which are used in the treatment of glaucoma and can prevent blindness.

Figure 1.1: Illustration of *Physostigma venenosum*, Photo:Erik Gotfredsen, website: http://www.liberherbarum.com/Pn0677ko.htm

Although there is a large number of research publications available on the constituents and biological activity of medicinal plants from Africa, the development of therapeutic agents from African medicinal plants has remained a somewhat neglected subject. The study of African medicinal plants also has not been taken as seriously and documented as fully as in other traditional societies, such as Indian and Chinese. Our knowledge of African medicinal plants is accordingly relatively limited. The little information available is often fragmented and incomplete following erosion of African healing cultures.

China has a rich heritage of medicinal plants. Traditional medicine is nowhere used more extensively than in China. Medicinal plants are important to the Chinese economy and they comprise a 40 percent share of the national medicine market.[1] The majority of China's factory-processed drugs are of plant origin. In fact, medicinal plant preparations are almost as important as synthetic drugs, such as antibiotics.

The most famous among Chinese traditional medicines is ginseng **(Figure 1.2)** from the plant *Panax ginseng.*[4] The ancient Chinese used ginseng to balance the body's "positive and negative energy" and, if it was taken regularly it was said to prolong life. Clinical

tests have demonstrated that it has therapeutic values in relieving stress, improving memory and concentration.[4]

Figure 1.2: *Panax ginseng* from which ginseng is extracted, no photographer given, website: http://quickchange.com/ginsengstore/

In India the majority of the population has used ancient practices known as Ayurvedic and Unain. However, the trained physicians mostly ignore these practises. This is truly unfortunate for much more could be learned and understood if the physicians and ancient practitioners worked together. An example is in the new evidence of lipid permeability of skin which supplies a logical scientific basis for the use of the traditional Ayurvedic techniques of oil and milk herbal massages and partial baths for headaches, rheumatism and eye ailments.[3]

One of the most famous amongst the plants of India is the neem tree (*Azadirachta indica*) **(Figure 1.3)**. For centuries people have cleaned their teeth with the neem twigs and smeared skin disorders with neem-leaf juices. The tree has been used to relieve so many different pains, fevers, infections and other complaints that it has been called the "village pharmacy".[5]

Figure 1.3: *Azadirachta indica* used by Indians for centuries, no photographer given, website: http://www.neemfoundation.org/intro.htm

Plants continue to be important sources for new drugs. The podophyllum resin of the mayapple (*Podophyllum peltatum*) **(Figure 1.4)** has long been used as a purgative, but the discovery of the cytotoxic properties of podophyllotoxin has subsequently led to the development of a commercially important drug. Although podophyllotoxin was found to be unsuitable as an anticancer agent because of its toxic side effects, its semi-synthetic derivative etoposide, which is manufactured from the resin of podophyllum, has been reported to be useful in the chemotherapeutic treatment of refractory testicular carcinomas, non-lymphocytic leukemias and non-Hodgkin's lymphomas, usually in combination with other anticancer drugs.[6]

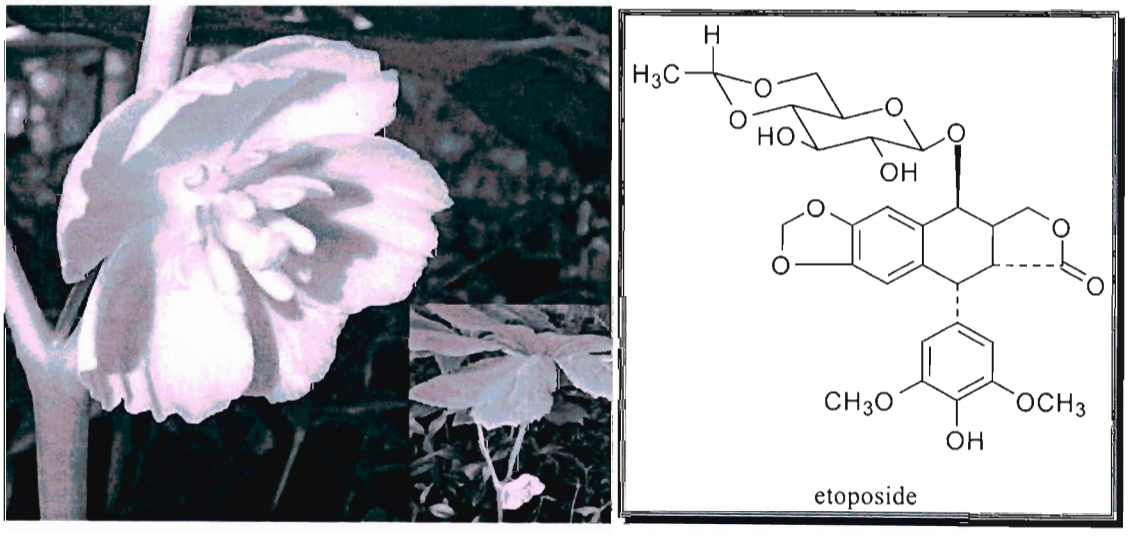

Figure 1.4: Structure of etopside (right) a derivative of natural podophyllum resin which is extracted from *Podophyllum peltatum* (left), Photo: Daniel Reed, website: www.2bnthewild.com

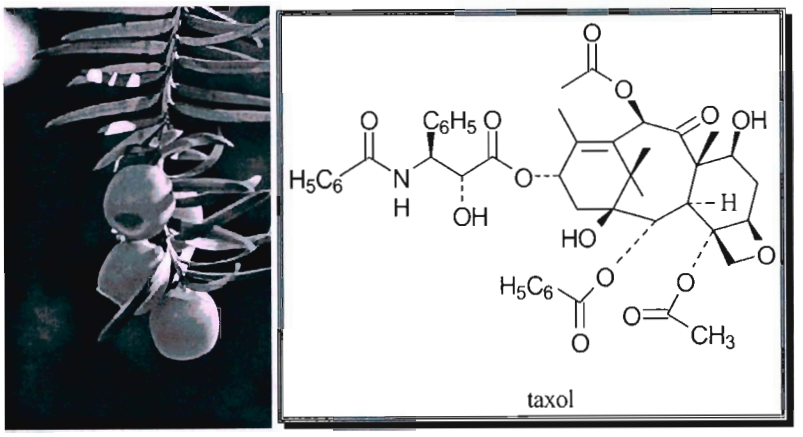

Figure 1.5: Structure of taxol (right) which is extracted from *Taxus brevifolia* (left), Photo: John Worrall, website: http://www.science.ubc.ca/~frst111/cgi-bin/db_gallery/expand.cgi?search=611

Taxol is another potent anticancer natural product. Taxol was isolated from the bark of the pacific yew (*Taxus brevifolia*) **(Figure 1.5)**. It is active against a number of leukemias and solid tumours of the breast, ovary and lungs in humans. The current problem with taxol is the difficulty in obtaining the drug. The amount of taxol in the yew bark is low and it takes 100 years to grow the tree, which only yields enough taxol to treat one patient. Also although it has been produced by total synthesis this is not a commercially viable method of producing it. Currently a semi-synthetic form of taxol is being produced. Recently a semi-synthetic derivative (Taxotere®), which comes from the needles of the yew tree (a renewable resource), has been approved by the Food and Drug Administration (FDA) for the treatment of breast and lung cancer. Other plant-derived compounds that have aided in the fight against cancer are compounds such as vincristine and vinblastine of the Madagascan periwinkle. Vinblastine is used for the treatment of Hodgkin's disease, breast cancer and testicular carcinoma.[8] Vincristine also from the same plant showed a different spectrum of activity and toxicity.[8] However, it was found to be neurotoxic and is now used in combination with other drugs in cancer chemotherapy.[8]

One of the worlds most used drugs is aspirin **(Figure 1.6)**. It is a derivative of the naturally-occurring salicin originally derived from willows (*Salix* spp.). It is used universally as a pain reliever.

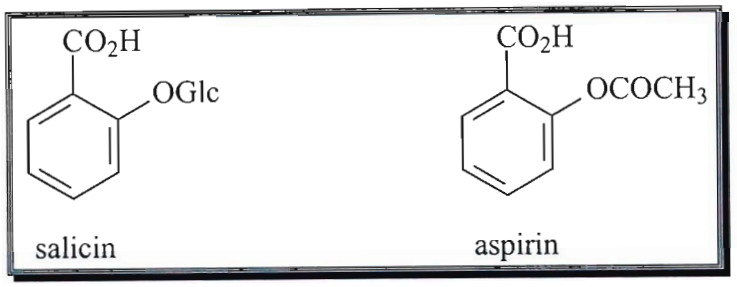

Figure 1.6: Structure of salicin and its acetylated form, aspirin

A new plant-derived drug is galanthamine **(Figure 1.7)** which was first isolated from *Galantus nivalis* but has now been found in many Amaryllidaceae species. It has recently been approved (February 2001) by the FDA for the treatment of Alzheimer's disease.

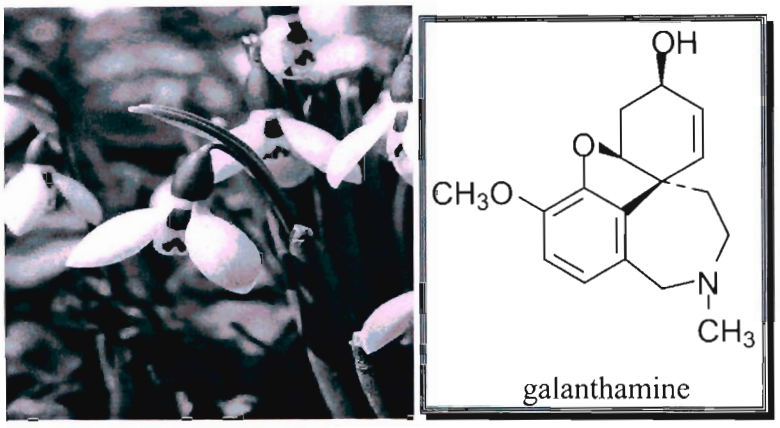

Figure 1.7: Structure of galanthamine (right) which was originally extracted from the bulbs of *Galantus nivalis*, Photo: John Worrall, website: http://www.hort.net/gallery/view/amy/galni40/

Plants have also played a major part in the production of substances from which contraceptives are manufactured. Plants such as soybeans, yams and sisal all have made a great contribution towards stabilizing the world population. They provided the basic

compounds from which steroidal compounds, now cheaply available as oral contraceptives, are synthesized. A good example is diosgenin **(Figure 1.8)**, which is extracted from yams. This is the starting compound in steroid hormone synthesis.

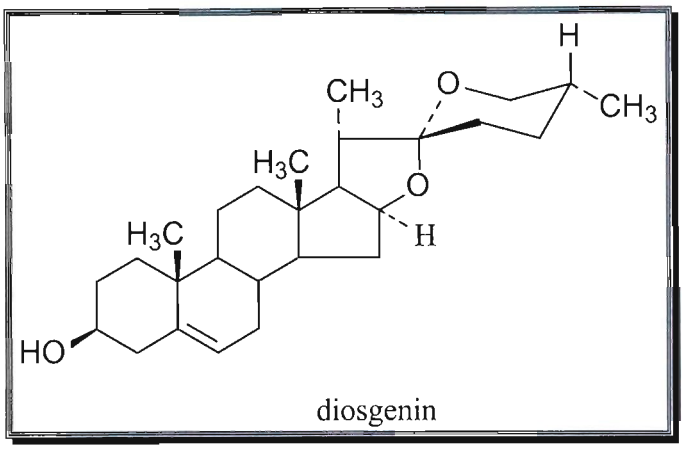

Figure 1.8: Structure of diosgenin which is used in steroid hormonal synthesis

Most spices, teas and other beverages such as coffee and cocoa owe their individual properties (flavours and aromas) to the pharmacologically active plant metabolites that they contain.[7] Most of them contain the purine alkaloids, caffeine, theobromine and theophylline. These are found naturally in the leaves, seeds and fruits of more than sixty plants. They may also be produced synthetically. Caffeine **(Figure 1.9)** is extracted mainly from tea leaves, coffee beans and coca beans. Caffeine is used to stimulate the central nervous system, cardiac muscles and respiratory system. It is mainly used to delay fatigue.

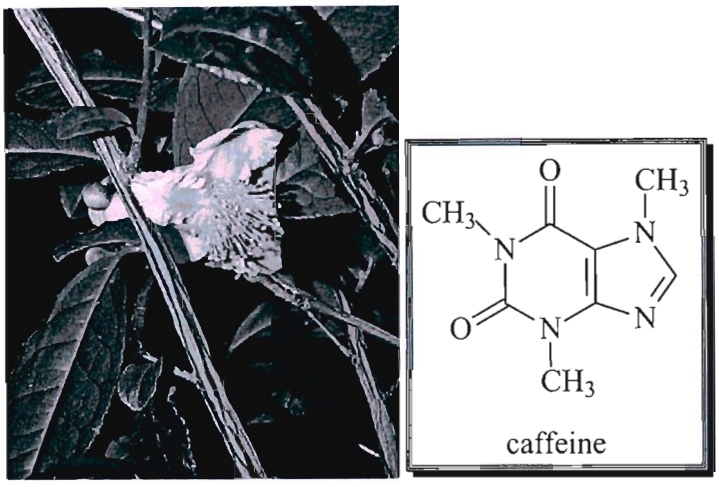

Figure 1.9: Structure of caffeine (right) which is extracted mainly from *Camellia sinensis* (left) photographer not given, website: http://www.plantoftheweek.org/week046.shtml

One plant that had a mixed effect on mankind is *Papaver somniferum* **(Figure 1.10)**, the opium poppy. It is the oldest known intoxicating plant in human history.[9] It was used in India, Taiwan and China where the desire for tranquility and sweet dreams led to numerous poisonings.[9] More than twenty alkaloids have been extracted from the opium poppy.[9] The most important ones are morphine, which is an important analgesic, codeine, which is a common ingredient of cough medicines and papaverine which is used in relieving the pain of stomach-ache.[9] Morphine and codeine were also the model from which synthetic analgesic drugs such as meperidine (Demerol), pentazocine (Talwin) and propoxyphene (Darvon) were synthesized.[9] Morphine has played another big, yet evil, part in human history: from it heroin is produced, an addictive poison that has led to many deaths.

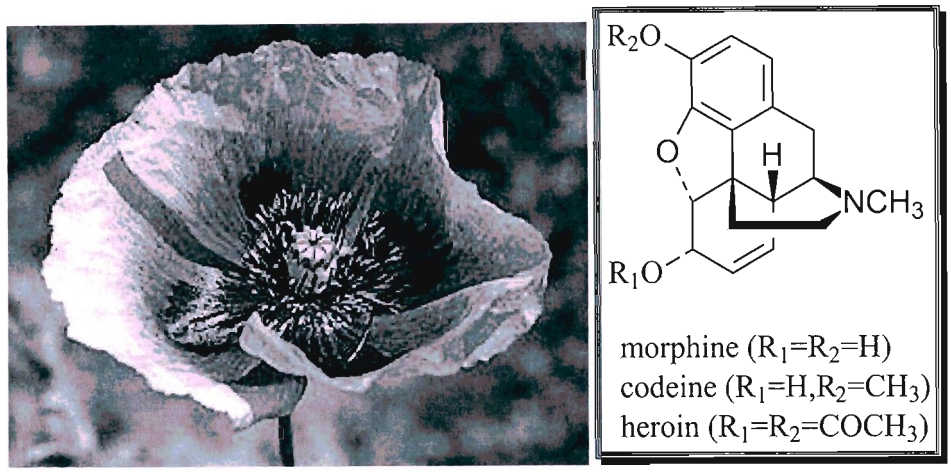

Figure 1.10: Structure of various alkaloids (right) isolated and also derived from *Papaver somniferum* (left), no photographer given, website: http://library.thinkquest.org/C007974/1_1pop.htm

Extracts of plants have been used as insecticides by humans since before the time of the ancient Romans, a practice that continues to the present. There are over two thousand species of plant known to have insecticidal properties.[6] The use of insecticidal plants is prevalent in the developing countries, where plants grown locally are cheaper for subsistence farmers than are synthetic chemical pesticides. A commonly-used natural pesticide is pyrethrin extracted from *Chrysanthemum cinerariaefolium*. Pyrethrins are used as a constituent of insect sprays for household use and as a post-harvest insecticide.

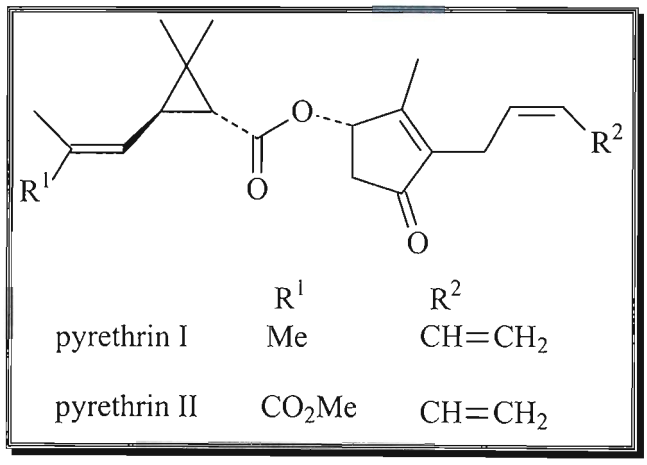

Figure 1.11: Structure of pyrethrins I and II which are used in insecticides

Plant natural products have long been, and will continue to be, important sources and models for drugs, insecticides and spices. Natural products often serve as chemical models for the design and synthesis of new drug entities. This is therefore a field of research that encourages one to study traditional practices of indigenous populations before they are lost, while at the same time obtaining invaluable information that could benefit mankind.

1.2 References

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Chapter 2: The Classification and Biosynthesis of Homoisoflavanones and Triterpenoids

2.1 Introduction

The Family Hyacinthaceae, formerly part of the Liliaceae, *sensu lato*, is richly represented in southern Africa. It comprises approximately sixty-seven genera and nine hundred species worldwide of which twenty-seven genera and three hundred and sixty-eight taxa are located in southern Africa.[40,41] The other major region of Hyacinthaceae diversity is from the Mediterranean to South West Asia.[42]

Within southern Africa, the Hyacinthaceae are widespread and well adapted to the fluctuating moist-arid climate, often withering down to bulbs during seasonal dry periods. In this study the phytochemistry of the bulbs of the plants were studied.

Although great difficulties in phylogenetically systematizing the genera have been acknowledged, a recent arrangement has been proposed.[40-42] Of the five sub-families delimited by Speta, three occur in southern Africa. These are the Hyacinthoideae, Urgineoideae and the Ornithogaloideae. In this study *Ledebouria zebrina* and *Scilla natalensis* were studied. Both belong to the sub-family Hyacinthoideae.

The two major classes of compounds isolated during this work were homoisoflavanones and eucosterol type nortriterpenoids. The biosynthesis of these compounds will be discussed in this chapter.

2.2 The Classification of Homoisoflavanones

The numbering of the basic homoisoflavanone skeleton is similar to that of all simple flavonoid compounds and is shown below **(Figure 2.1)** using a 3-benzyl-4-chromanone type **(1)** and a scillascillin type **(2)** as examples.

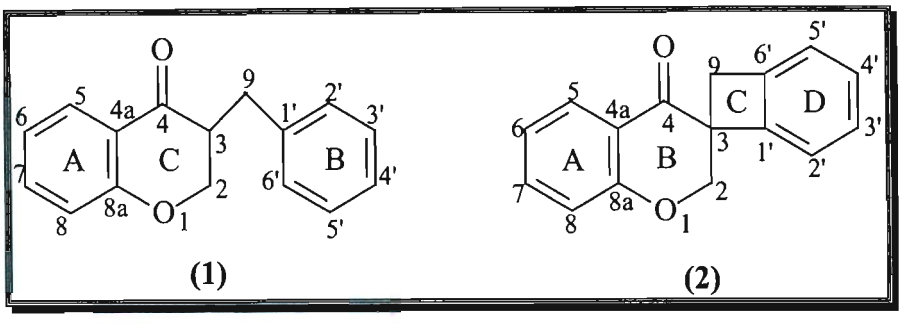


Figure 2.1: The numbering system for homoisoflavanones

Homoisoflavanones belong to a small family of natural products whose first member was isolated from the bulbs of *Eucomis bicolor* Bak.[1] They are restricted in their distribution, having been previously reported to occur mainly in taxa of the Hyacinthaceae.[2-10] However, these homoisoflavanones have also been reported to occur outside this family. Intricatin and intricatinol are two homoisoflavanones isolated from the Fabaceae Family **(Section 2.6)**. Within the Hyacinthaceae, members of the genus *Scilla*, *Eucomis* and *Muscari* have been dominant with regard to the production of homoisoflavanones.[2-5]

Homoisoflavanones, which are more commonly referred to as homoisoflavonoids, belong to a class of naturally occurring oxygen heterocycles.[11] The term homoisoflavonoid is actually a misnomer since these compounds don't undergo the characteristic 2,3-aryl migration of the C_6-C_3-C_6 moiety that is common in the biosynthesis of isoflavonoids.[10] Also, they have a sixteen-carbon skeleton instead of a fifteen-carbon skeleton characteristic of isoflavonoids.[13] The correct systematic name is 3-benzyl-4-chromanone. The spectroscopic behavior of the homoisoflavanones resembles very closely that of flavonoids.[14] This sixteen carbon skeleton bears either a

chromanone, chromone or chromane moiety to which is attached, in most cases, a benzyl or benzylidene group at the 3-position.[16] The homoisoflavanones can generally be classified into three types based on structural features:[11,14,16]

2.2.1 The 3-benzyl-4-chromanone or dihydroeucomin type

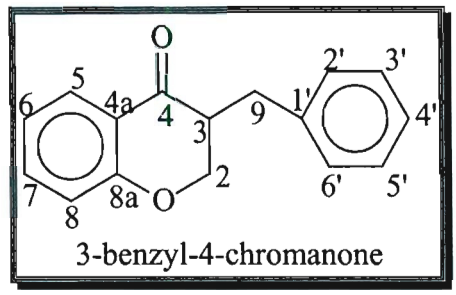


Figure 2.2: A homoisoflavanone of the 3-benzyl-4-chromanone type

This type of homoisoflavanone **(Figure 2.2)** shows a very characteristic coupling pattern in the ^{1}H NMR spectrum due to the 2H-2, H-3, 2H-9 proton coupled system. A pair of double doublets within the chemical shift range δ 4.10-4.32 ppm [AB of ABX, 2H-2], one multiplet in the range δ 2.72-2.87 ppm [H-3] and two double doublets in the range δ 2.65-2.73 ppm and δ 3.10-3.26 ppm [2H-9] are indicative of this arrangement.

2.2.2 The 3-benzyl-3-hydroxy-4-chromanone or eucomol type

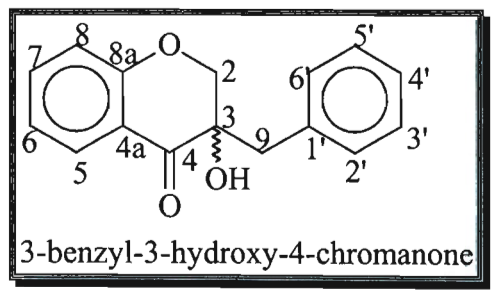


Figure 2.3: A homoisoflavanone of the 3-benzyl-3-hydroxy-4-chromanone type

These compounds **(Figure 2.3)** typically possess a hydroxy substituent at C-3. The characteristic feature in the ^{1}H NMR spectrum of this type of homoisoflavanone is the presence of two AB systems, one for the C-2 protons at δ 4.20-4.36 ppm and δ 4.12-4.17 ppm and another at δ 3.10-3.20 ppm and δ 2.65-2.75 ppm for the protons at C-9.[13]

2.2.3. The 3-benzylidene-4-chromanone or eucomin type

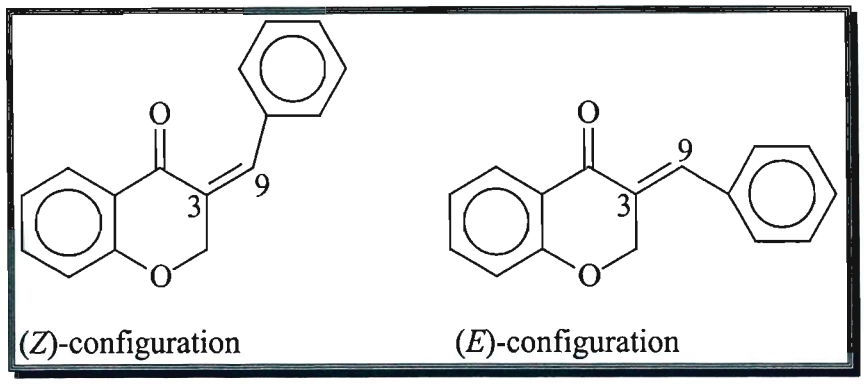


Figure 2.4: A homoisoflavanone of the 3-benzylidene-4-chromanone type

These compounds **(Figure 2.4)** are characterized by a 3,9-double bond and can be in the (*Z*)- or the (*E*)-configuration. The characteristic features in the ^{1}H NMR spectrum are the presence of an AB-system for 2H-2 and a singlet for H-9. The pair of doublets of 2H-2 are found to resonate at approximately δ 5.2-5.5 ppm while the resonance of H-9 depends on the configuration. The (*Z*)-configuration places the proton at C-9 away from the anisotropic region of the carbonyl group and causes the vinyl group proton to resonate at approximately δ 5.5 ppm. In the (*E*)-configuration the proton at C-9 proton is in the anisotropic region of the carbonyl group and causes it to resonate at δ 7.6-7.9 ppm.[13]

Besides these three basic structural types, a number of unusual compounds which contain a fourth ring **(Figure 2.5)**, have been isolated.[11,16] These compounds are thought to be biogenetically related to homoisoflavanones and are classified with this

group.[10,11,14] Scillascillin type compounds possess a unique 3-spiro-cyclobutane ring sytem.[14,16] The C-4 carbonyl group is retained in scillascillin-type compounds but is absent in brazilin and hematoxylin where a cyclopentane C-ring is observed. The biosynthetic origin of these more complex compounds has been investigated and it has been postulated that they are derived directly from a 3-benzyl-3-hydroxy-4-chromanone precursor.[10]

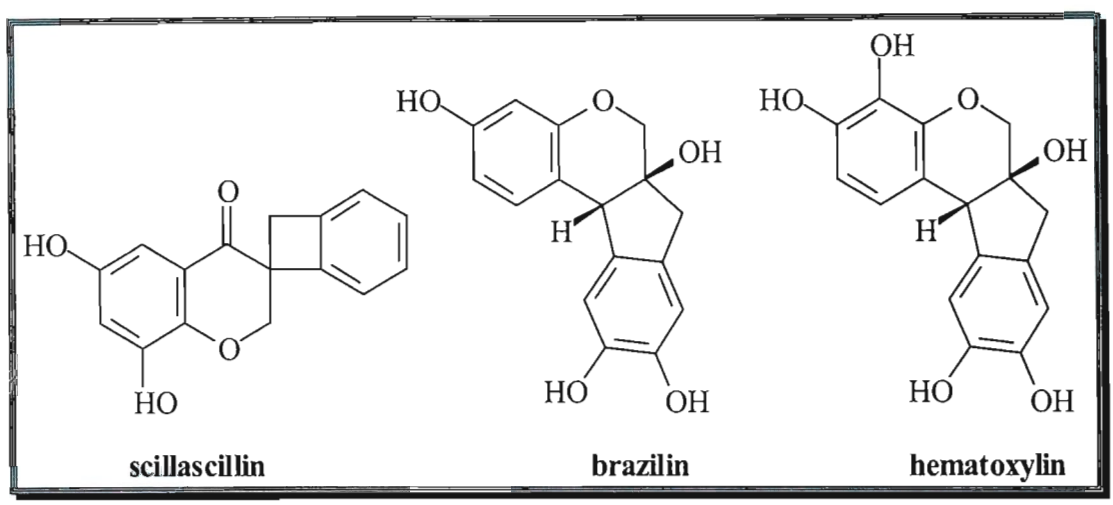
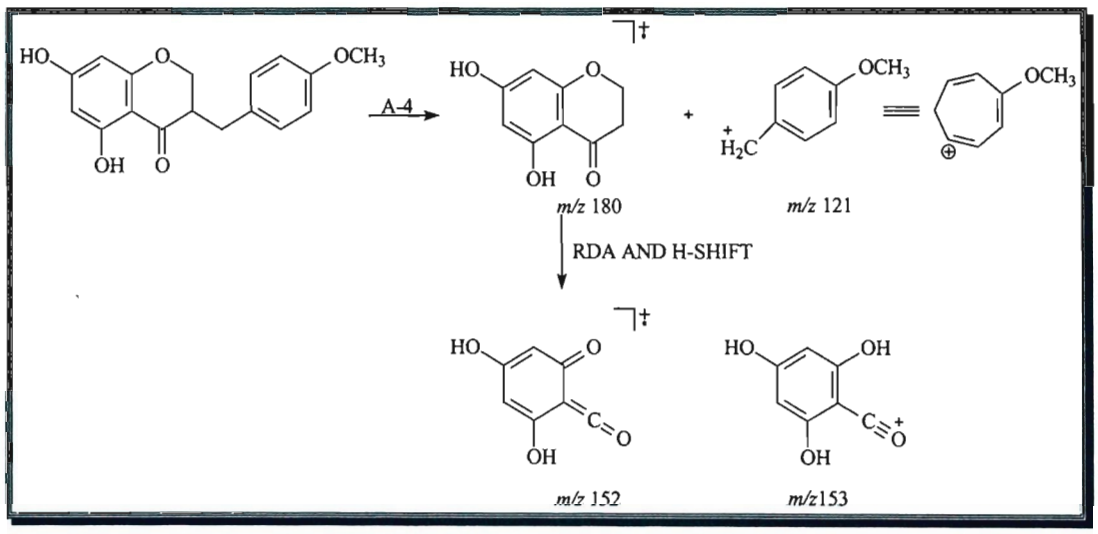


Figure 2.5: Homoisoflavanones of the scillascillin, brazilin and hematoxylin types

The ^{1}H NMR spectrum of homoisoflavanones shows also a number of characteristic features besides the splitting pattern of the H-2, H-9 and H-3 protons which are important in the structural elucidation of these compounds. The signals between δ 6.50-7.20-ppm generally indicate the substitution pattern on the B-ring. This is either an ABX or an AA'BB' system. The substituents present are attached to C-4' (AA'BB' system) or C-3' and C-4' (ABX system). The proton resonances of C-1', C-5' and C-6' all appear as double doublets. In most cases the *para* coupling is not seen.

The region between δ 5.80-6.30 ppm indicates the substituent pattern on the A-ring. There is usually only one or two proton resonances in this region which are normally due to H-6 and/or H-8. The position of the proton is normally assigned through the NOESY spectrum which shows NOESY correlations with the substituents at C-5 and C-7.

The mass spectrum is another important tool used in the determination of the structure of the compound. The first step is cleavage of the C-3, C-9 bond in what is known as an A-4 type cleavage. In dihydroeucomin this leads to fragments at *m/z* 180 and *m/z* 121 as shown in **scheme 2.1**. In most cases the tropylium ion gives rise to the base peak of the spectrum. One of the fragments formed may then undergo a *retro*-Diels Alder (RDA) reaction. In dihydroeucomin the chromanone fragment ion at *m/z* 180 may eliminate water, CO or undergo a RDA cleavage to give a fragment ion at *m/z* 152 or due to a hydrogen shift, *m/z* 153. The major pathway is the RDA cleavage and H-shift. The minor pathway is usually the subsequent loss of water, CO and methyl from the molecular ion.



Scheme 2.1: Fragmentation pattern of homoisoflavanones

2.3 The Biosynthesis of Homoisoflavanones

Phenolic compounds can be biosynthesized *via* the shikimate pathway or polyketide pathway.[17] Often compounds like flavonoids and homoisoflavanones are of mixed biosynthetic origin. In homoisoflavanones, the A-ring is polyketide derived while the B-ring is shikimate derived **(Figure 2.6)**.[23-25]

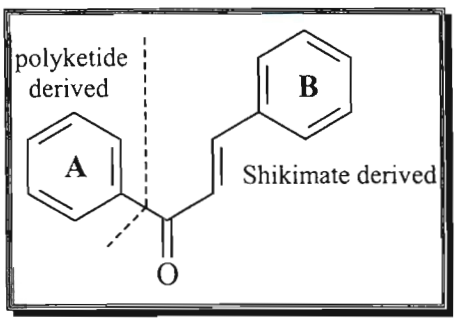


Figure 2.6: The origin of the A and B rings

The first step in the biosynthetic pathway leading to homoisoflavanones involves the formation of a chalcone. Since chalcones are known to be direct precursors of homoisoflavanones, the biosynthesis of chalcones is essentially a part of the biogenetic route to homoisoflavanones.[10,13,14] The precursors to chalcones are three malonyl CoA units and *p*-coumaroyl CoA.[23-25]

Malonyl-CoA is synthesized from acetyl-CoA and CO_2.[23-25] The reaction, which is shown below is catalysed by Acetyl-CoA carboxylase in the presence of adenosine 5'-triphosphate (ATP) and Mg^{2+}.[23-25]

$$CH_3CO^-SCoA + HCO_3^- + ATP \longrightarrow HOOC\text{-}CH_2\text{-}CO\text{-}SCoA + ADP + Pi$$

p-Coumaroyl CoA, the second precursor in chalcone formation, is derived from the shikimate pathway. This pathway is responsible for the production of the hydroxy cinnamic ester, from which the B-ring is derived.[23-25] The precursors of the shikimic

acid pathway, erythrose-4-phosphate and phosphoenolpyruvate, are primary metabolites of carbohydrate metabolism.[23-25]

The first step in the formation of the precursor is the deamination of phenylalanine, which is catalyzed by the enzyme phenylalanine ammonia lyase (PAL).[23-25] PAL catalyses the *anti*-elimination of ammonia and the (*pro*-3s)-proton from L-phenyl-alanine to yields *trans*-cinnamic acid.[23-25] Cinnamate-4-hydroxylase (C4H) catalyses the introduction of a hydroxy group into position 4 of the *trans*-cinnamic acid.[23-25] This reaction requires NADPH and molecular oxygen and exhibits properties characteristic for plant P450 enzymes.[23-25] This reaction mechanism involves a H-shift. The proton in the 4-position is oxidised to a hydroxy group, being moved itself to the 3-position. This shift was established by trituim-labelling experiments performed at the National Institute of Health at Bethesda, Washington D.C. and is called the NIH shift.[23-25] **(Figure 2.7)** This conversion is shown in **Scheme 2.2**.

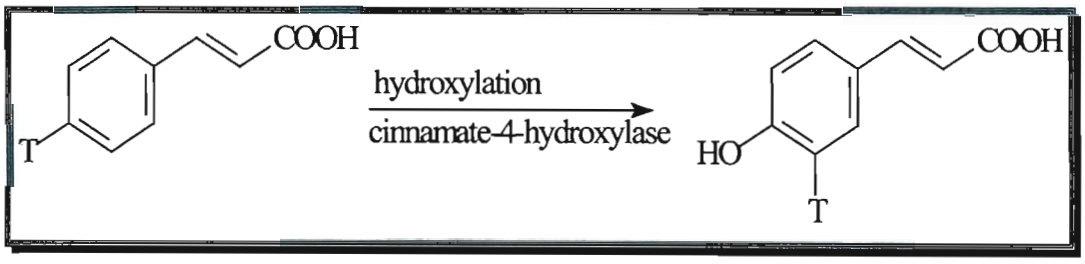
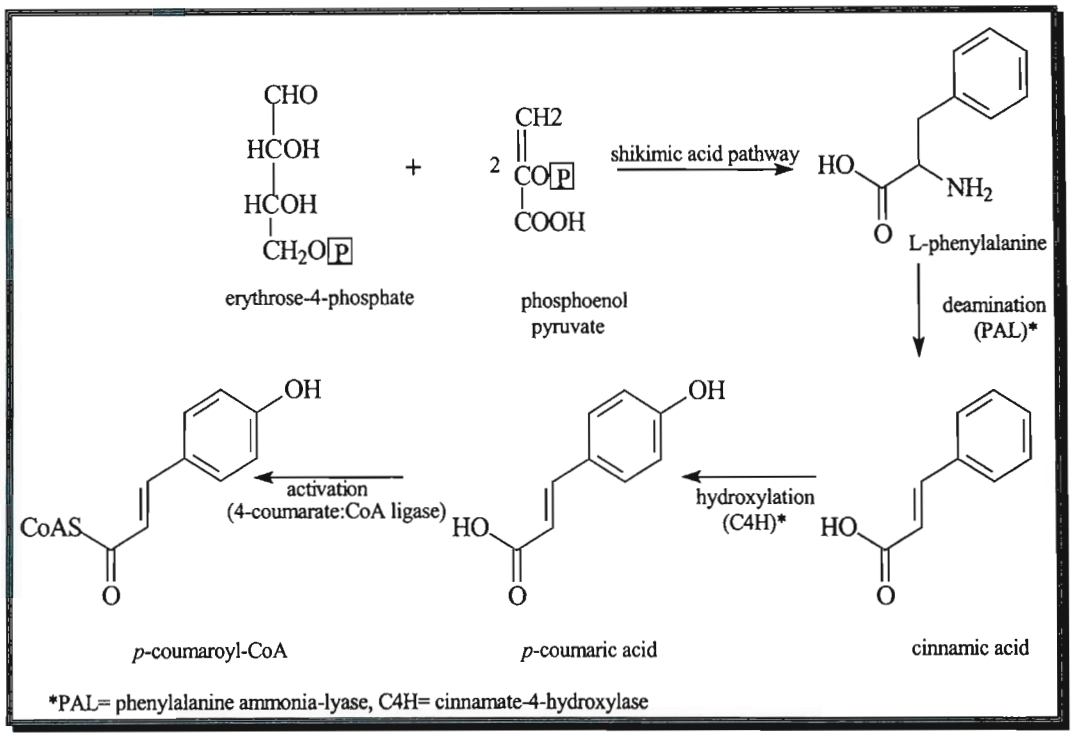


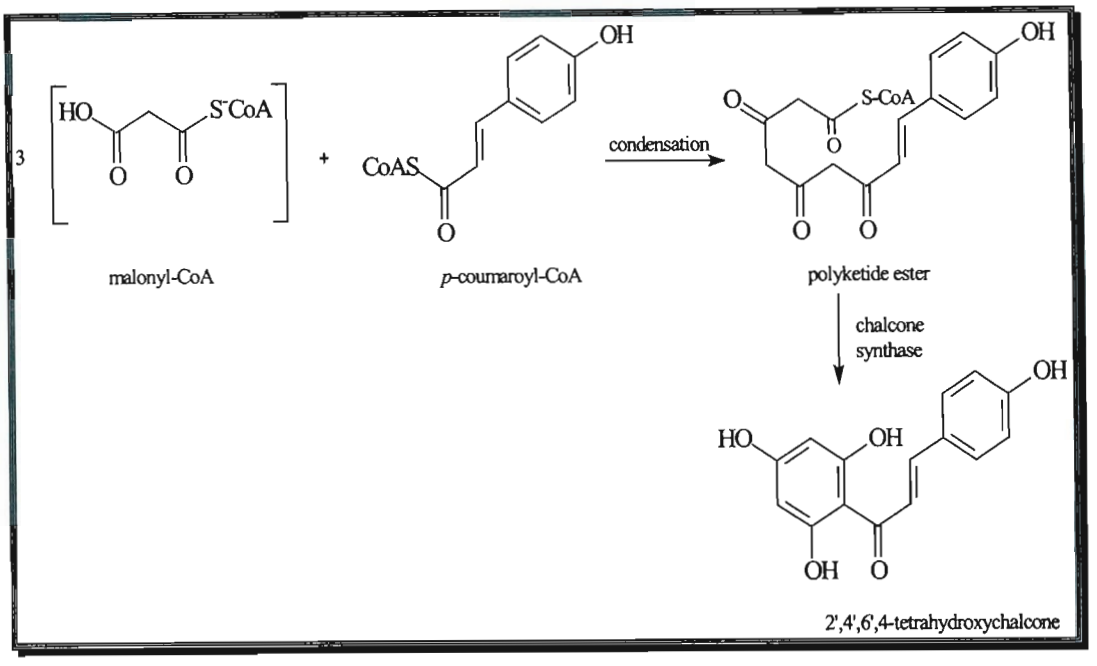
Figure 2.7: The NIH shift for the hydroxylation of cinnamic acid[23-25]

The *p*-coumaric acid is activated by the formation of a CoA ester, which is catalysed by 4-coumarate: CoA ligase.[23-25] This reaction which requires ATP and Mg^{2+} as cofactors, proceeds *via* an acyl-AMP intermediate, characterizing the enzyme as a synthase.[23-25]



Scheme 2.2: The biosynthesis of the intermediate *p*-coumaroyl CoA[23-25]

The formation of the chalcone is common to all classes of flavonoids and is thought to be the most important step in flavonoid biosynthesis.[23-25] The formation of the chalcone is catalysed by the enzyme chalcone synthase.[24,25] Since chalcone is the central intermediate for all flavonoids, chalcone synthase can be regarded as the key enzyme in flavonoid biosynthesis.[23-25] The overall reaction consists of three successive condensation steps with "acetate units" being derived from malonyl-CoA.[23-25] The result is the elongation of the aliphatic side chain of 4-coumarate by six carbon atoms **(Scheme 2.3)**.[23] The enzyme chalcone synthase then catalyzes the cyclization to give the aromatic ring A.[23]



Scheme 2.3: The biosynthesis of the 2',4',6',4-tetrahydroxychalcone[23-25]

In many biosynthetic pathways, S-substituted methionine is often the source of an additional carbon atom. Tetrahydroxychalcone is methylated with S-adenosylmethionine and is converted to the 2'-methoxy-4',6',4-trihydroxychalcone. This reaction is catalyzed by methyl transferase **(Figure 2.8)**.

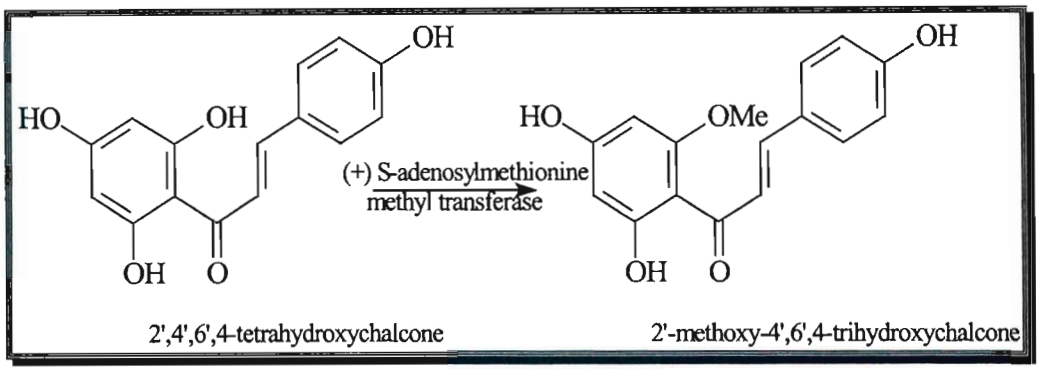
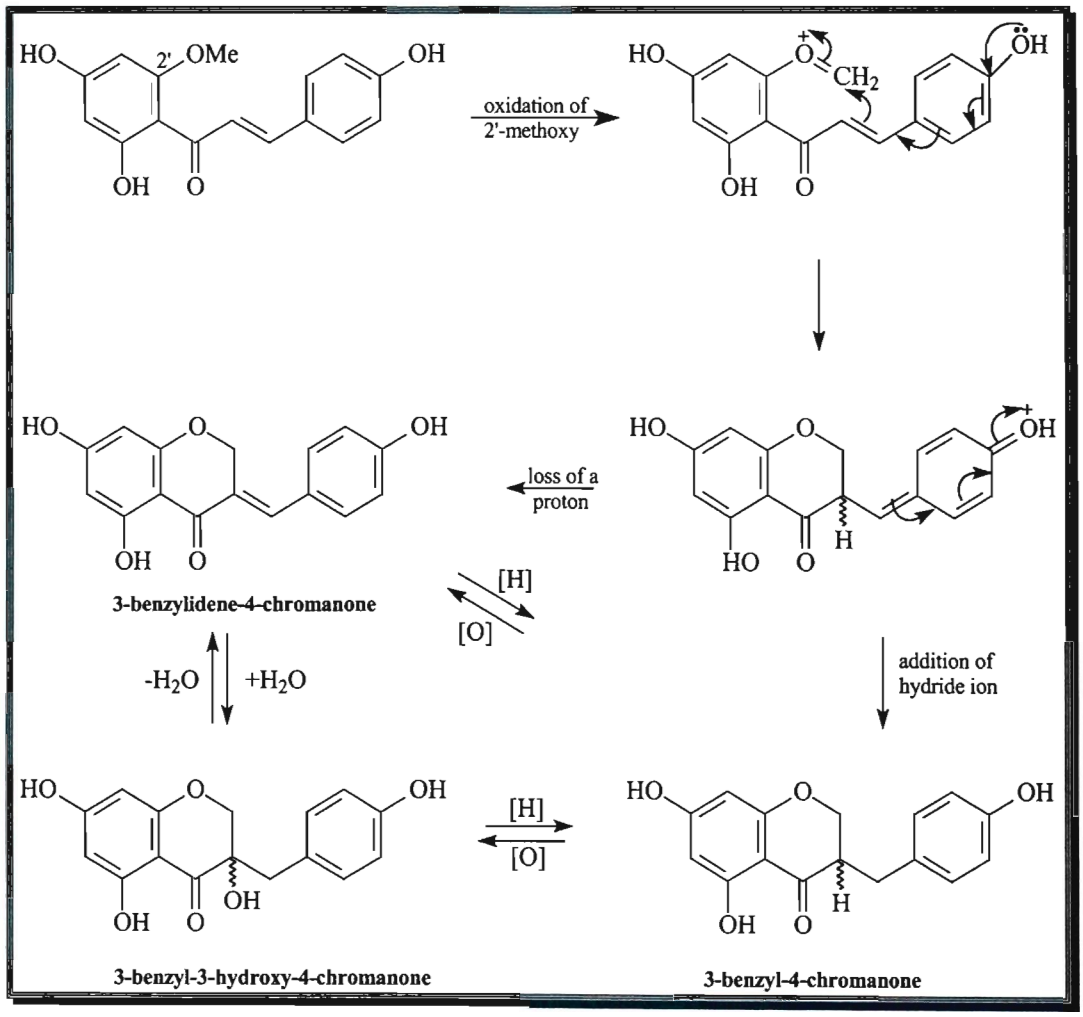


Figure 2.8: The formation of the 2'-methoxy-4',6',4-trihydroxychalcone[23-25]

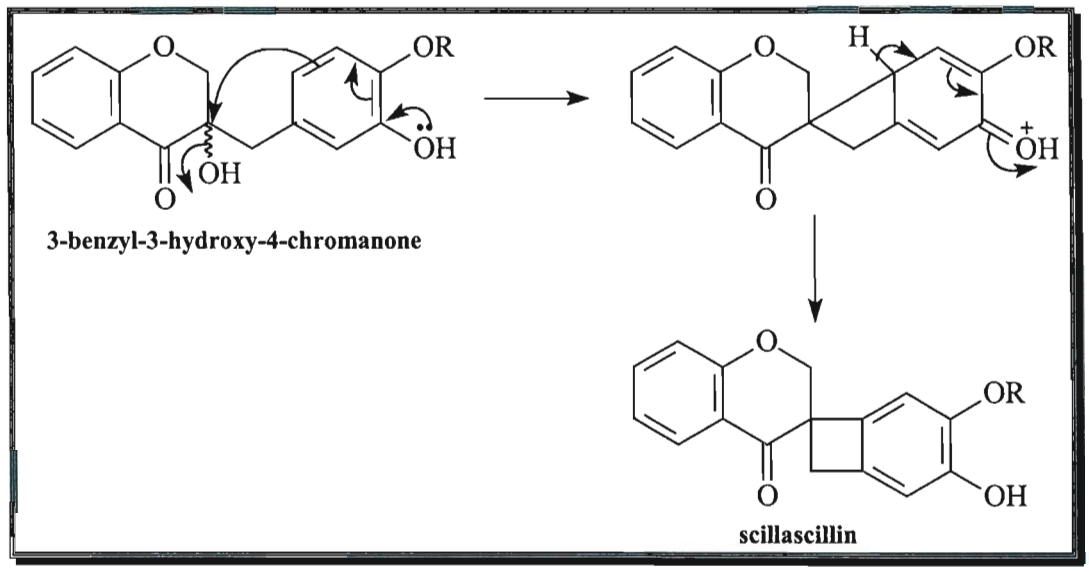
A scheme for the conversion of chalcones to homoisoflavanones has been proposed by Dewick[9] whereby the 2'-methoxy group is oxidised and then undergoes a subsequent cyclisation which produces the three basic types of homoisoflavanones. The addition of a hydride ion produces the 3-benzyl-4-chromanone type while the loss

of a proton leads to the formation of the 3-benzylidene-4-chromanones.[9] Hydration or oxidation at the C-3 position of a 3-benzyl-4-chromanone leads to a 3-benzyl-3-hydroxy-4-chromanone. These proposals are illustrated in **Scheme 2.4**.



Scheme 2.4: The proposed biosynthetic routes to basic homoisoflavanones[9]

Scillascillin type compounds which have a 3-spirocyclobutenyl ring are derived from a more complex mechanism. The precursor to scillascillin-type homoisoflavanones is thought to be the 3-benzyl-3-hydroxy-4-chromanone homoisoflavanones **(Scheme 2.5)**.



Scheme 2.5: The proposed biosynthetic route to scillascillin type homoisoflavanones[9]

2.4 The Triterpenoids of the Hyacinthaceae

The diverse, widespread and large family of natural products constructed from five carbon units (isoprene units) and comprising compounds with a C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30} or C_{40} skeleton are termed terpenoids.[17] These compounds are found in all parts of higher plants and also occur in mosses, liverworts, algae and lichens. The term "triterpenoid" refers to C_{30} compounds that are derived from squalene.[18] They have been known and investigated for over 100 years.

Spirocyclic nortriterpenoids have been isolated from members of the Hyacinthaceae. These compounds have a basic lanosterol triterpenoid skeleton. Eucosterol and 16β-hydroxyeucosterol are the two main types of nortriterpenoids isolated from various Hyacinthaceae species.[19] The phytochemical investigation of *Veltheimia bracteata* and *Eucomis bicolor* has also produced a number of lanosterol oligosaccharides that are closely related to eucosterol.[20,21] The variation in these compounds is due to the differing degrees of oxygenation in the aglycone and the different combination of sugars present.

The basic sterol numbering according to the IUPAC-IUB Rules for Sterol Nomenclature of 1967 is given in **Figure 2.9a**. However in 1989 there was a major change in the carbon numbering system **(Figure 2.9b)**. In this work the numbering system of IUPAC-IUB (1989) will be used although most of the literature on these compounds still uses the old numbering system.

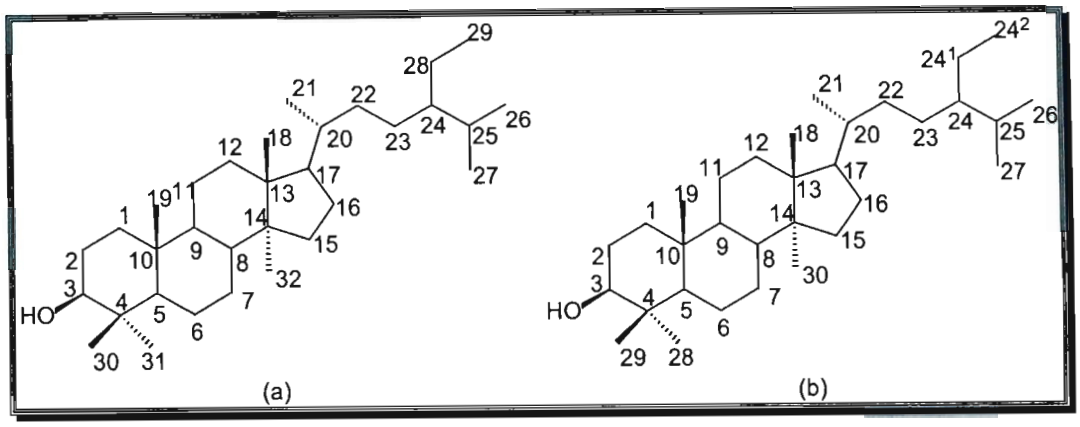


Figure 2.9: Basic structure of a sterol with carbon numbering. (a) Numbering according to the IUPAC-IUB (1976) recommendations. (b) Numbering according to the IUPAC-IUB (1989) recommendations

2.4.1 Eucosterol type nortriterpenoids:

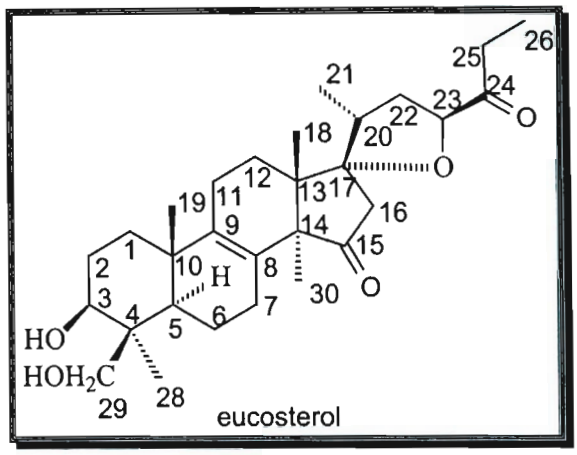


Figure 2.10: The structure of eucosterol

The majority of triterpenoids isolated in this work were found to be derivatives of eucosterol **(Figure 2.10)**. The key features in the ^{1}H and ^{13}C NMR spectra of eucosterol type compounds will be discussed here and will serve as the standard from which all the structures of the derivatives of eucosterol were determined in this work. The ranges of the NMR resonances for these types of compounds are given below. The exact values for eucosterol are given in brackets.

The ^{1}H NMR spectrum of eucosterol-type compounds shows a pair of doublets at δ 3.30-3.50 (3.38 in eucosterol) and 4.0-4.20 ppm (4.23 ppm) for the methylene protons of the CH_2OH group (C-29) and a multiplet for the proton at C-3 at δ 3.40-3.60 ppm (3.47 ppm). The methine proton at C-23 occurs as a triplet at approximately δ 4.64-4.80 ppm (4.67 ppm).

There are six methyl groups in the molecule. The 3H-26 methyl group protons resonate as a triplet at approximately δ 1.05-1.12 ppm (1.09 ppm). The 3H-21 methyl group protons resonate as a doublet at approximately δ 1.10-1.20 ppm (1.15 ppm). The 3H-18 and 3H-19 methyl group proton singlets resonate at approximately δ 0.9-1.05 ppm (0.96 and 0.98 ppm respectively), while the methyl proton singlet resonances of 3H-28 and 3H-30 resonate at approximately δ 1.05-1.30 (1.28 ppm) and 1.27-1.41 ppm (1.41 ppm) respectively. The ^{13}C NMR spectrum shows two carbonyl resonances at approximately δ 215 and 211 ppm for C-15 and C-24 respectively (215.1 ppm and 211.7 ppm in eucosterol). The fully substituted C-O resonance of C-17 occurs relatively downfield at approximately δ 91-100 ppm (99.2 ppm). The presence of an ether linkage in eucosterol is shown by the presence of six carbons bonded to oxygen atoms in the ^{13}C NMR spectrum whilst the molecular formula has only five oxygen atoms. This ether linkage is placed between C-17 and C-23, and leads to the formation of a heterocyclic ring E. This can be proven by the fact that on acetylation, the 3β, 29-diacetate is formed indicating only two free hydroxy groups.

The ^{13}C NMR spectrum also shows the presence of two fully substituted alkene carbon resonances at approximately δ 133.0-135.2 (133.1 ppm) and 133.1-137.0 ppm (136.6 ppm). This double bond is placed between C-8 and C-9 and this can be proved through the HMBC spectrum. This accounts for one of the eight double bond equivalents expected in eucosterol, two more are assigned to the keto groups and the five remaining double bonds equivalents are attributed to the pentacyclic ring structure, including the heterocyclic ring E.

The stereochemistry of the molecule can be confirmed through the NOESY spectrum. The H-20 resonance gives positive NOESY correlations with the 3H-18, 3H-21, H-22β and H-25. From this it may be concluded that the stereochemistry of H-20 is β or *R*. The H-23 resonance shows correlations with H-22α and 3H-21. This indicates that H-23 is α or *S*. The H-5α resonance shows correlations to H-3 and 3H-28. From this it may be concluded that H-3 and 3H-28 (the unoxidised methyl group) are α. The resonance ascribed to H-5 shows no correlation with H-19 which confirms that H-19 is β as expected. The 3H-21 and 3H-18 resonances shows a correlation with each other, as a result of the C-17, C-20 bond being β. The 3H-18 resonance shows no correlation with 3H-30 resonance as expected.

The eucosterol derivatives isolated will be compared against this standard.

2.5 The Biosynthesis of Triterpenoids

Terpenoids are built up from isoprene units.

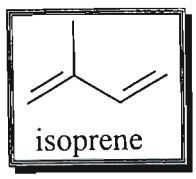


Figure 2.11: The isoprene unit, which is the basic building block of terpenoids

Isoprene **(Figure 2.11)** was first isolated more than one hundred years ago and isoprene pyrophosphate (IPP) was found to be an essential component in the biosynthesis of cell metabolites, e.g. geraniol **(Figure 2.12)** which is made up of two isoprene units.[27]

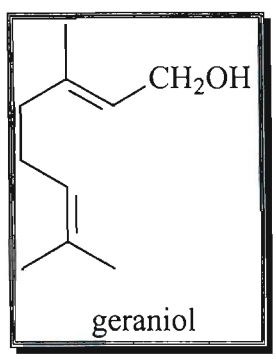
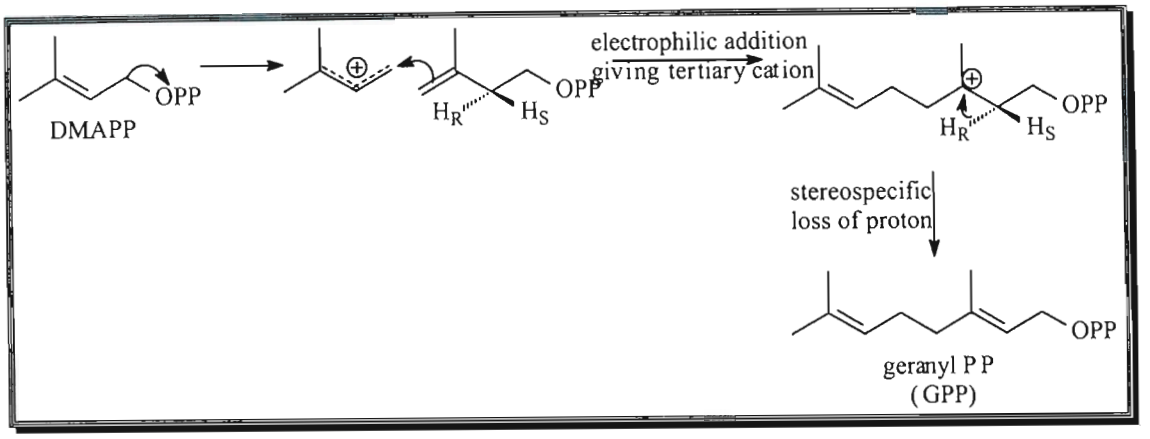


Figure 2.12: Geraniol, a monoterpenoid formed from two isoprene units

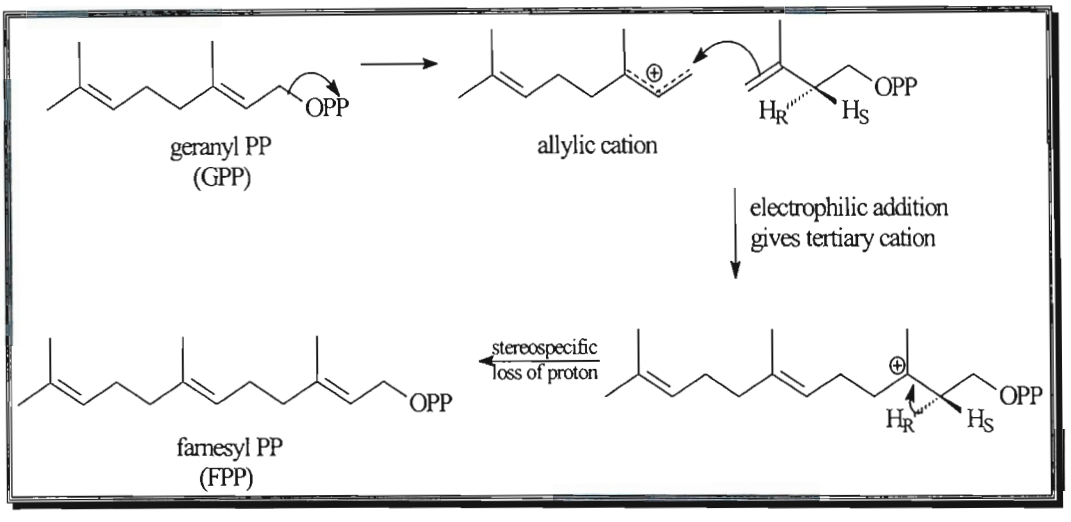
The triterpenoids are considered to be derived from an acyclic precursor which is cyclised and further elaborated to give different classes of compounds.[27] This acyclic precursor was found to be hexa-ene all-*trans* squalene and according to the Biogenetic Isoprene Rule, different types of triterpenoids are formed according to the conformation that squalene epoxide adopts, presumably at an enzyme surface, prior to cyclisation.[28,29] The precursor to squalene is two molecules of farnesyl pyrophosphate (FPP).[30] FPP is synthesised from the reaction of geranyl pyrophosphate (GPP) and IPP. The prenyl-tranferase reaction results in the formation of GPP. GPP is

synthesised from dimethylallyl pyrophosphate (DMAPP) and isopentyl pyrophosphate (IPP) **(Scheme 2.6)**.[30] This reaction is catalysed by the enzyme prenyl transferase.[30] The reaction involves the ionisation of DMAPP to the allylic cation, addition to the double bond of IPP, followed by a stereospecific loss of a proton.[30]



Scheme 2.6: The formation of geranyl pyrophosphate[9]

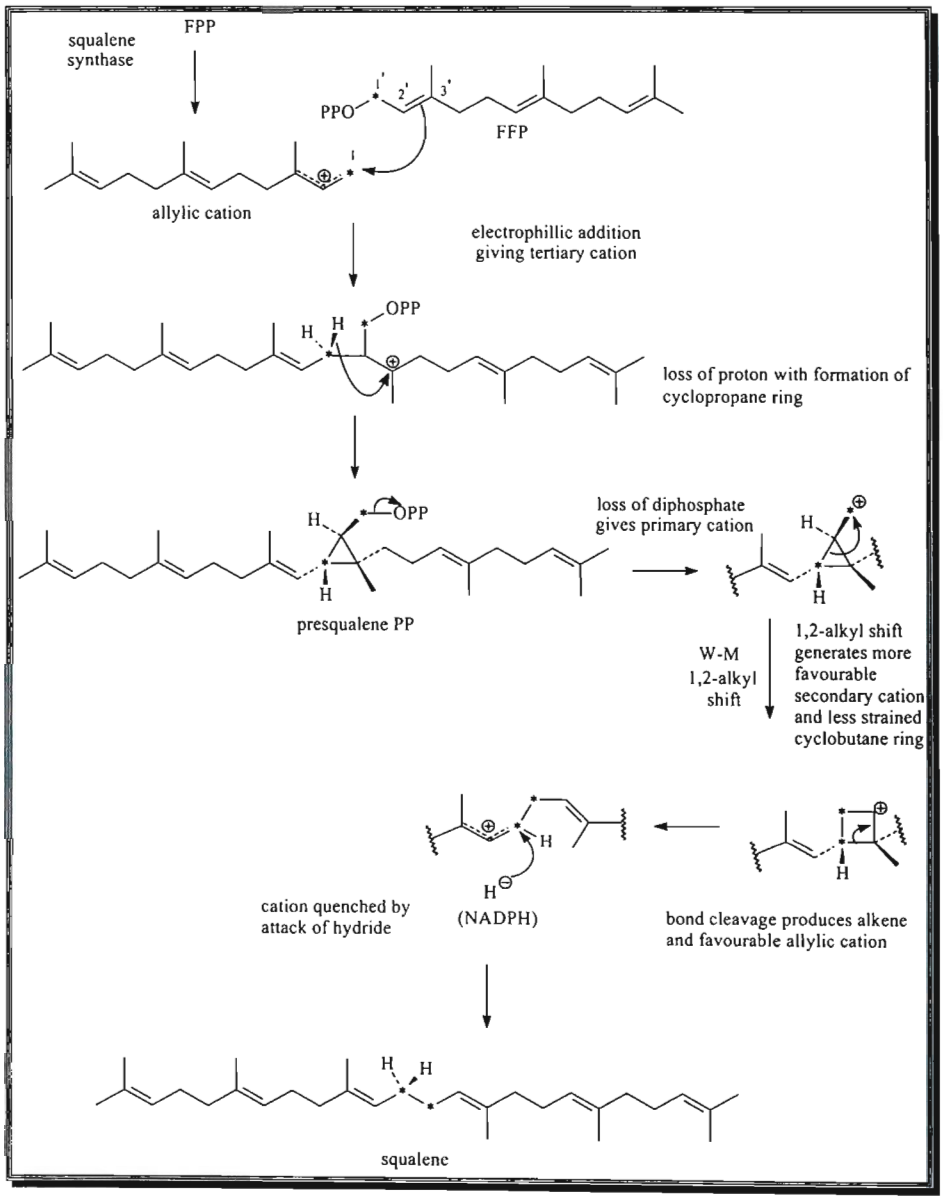
Geranyl pyrophosphate can react with another molecule of isopentyl pyrophosphate to form FPP, a fifteen-carbon compound **(Scheme 2.7)**. This reaction proceeds in an analogous manner to that of GPP.



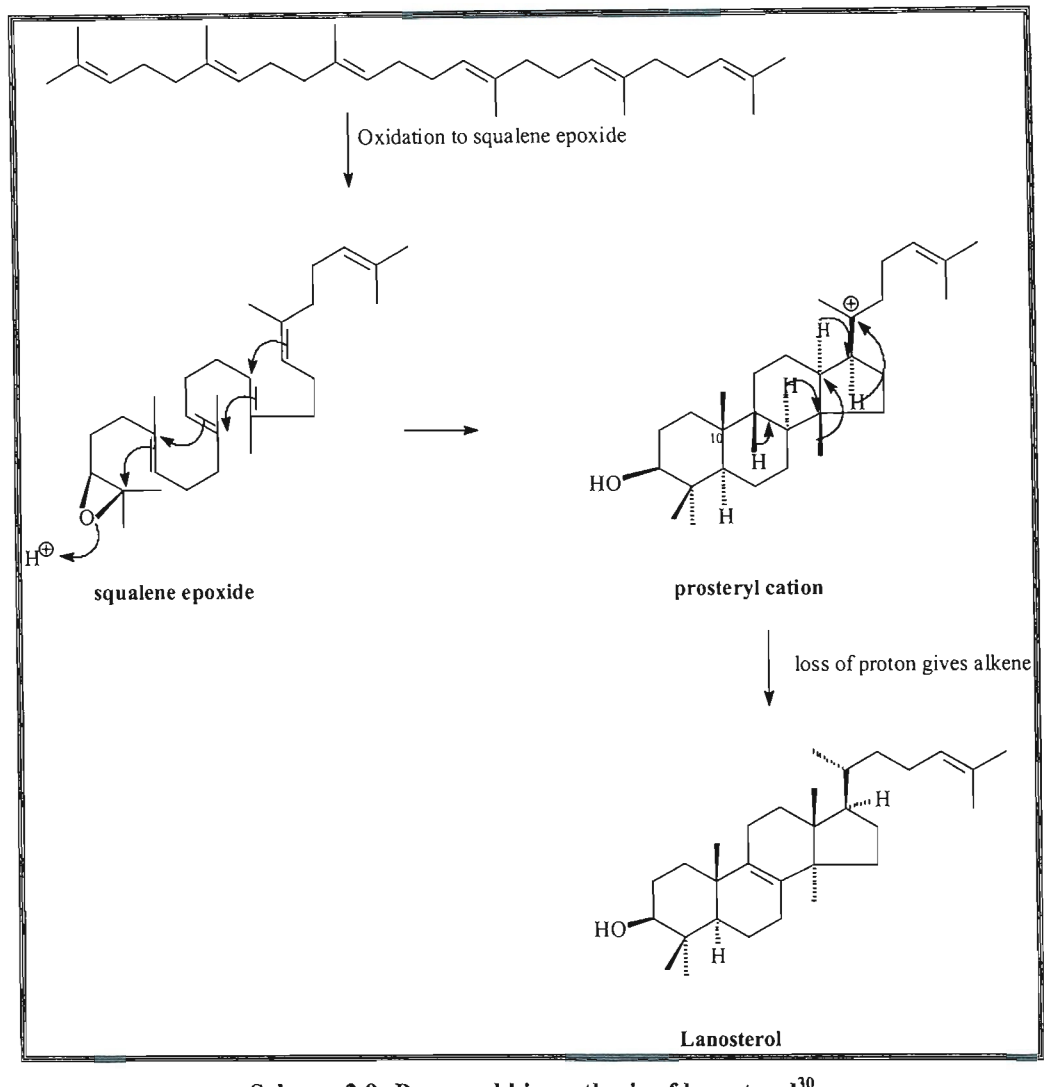
Scheme 2.7: The formation of farnesyl pyrophosphate[9]

Two molecules of FPP are joined tail to tail to yield squalene **(Scheme 2.8)**.[30] A proton from a C-1 position of one molecule of FPP is lost and a proton from NADPH is inserted.[30] The mechanism involves the addition of the 2,3-double bond of FPP onto the farnesyl carbocation similar to the chain extension of IPP. The tertiary

carbocation formed then loses a proton, which results in the formation of the cyclopropane ring, forming presqualene PP.[30] The loss of pyrophosphate from presqualene results in the formation of an unstable primary carbocation.[30] This carbocation then undergoes a Wagner-Meerwein rearrangement, which results in ring expansion and the formation of a more favourable secondary carbocation.[30] This is then followed by bond cleavage of the cyclobutane ring, producing a favourable allylic carbocation.[30] Squalene is then generated by quenching with a hydride ion from NADPH.[30]



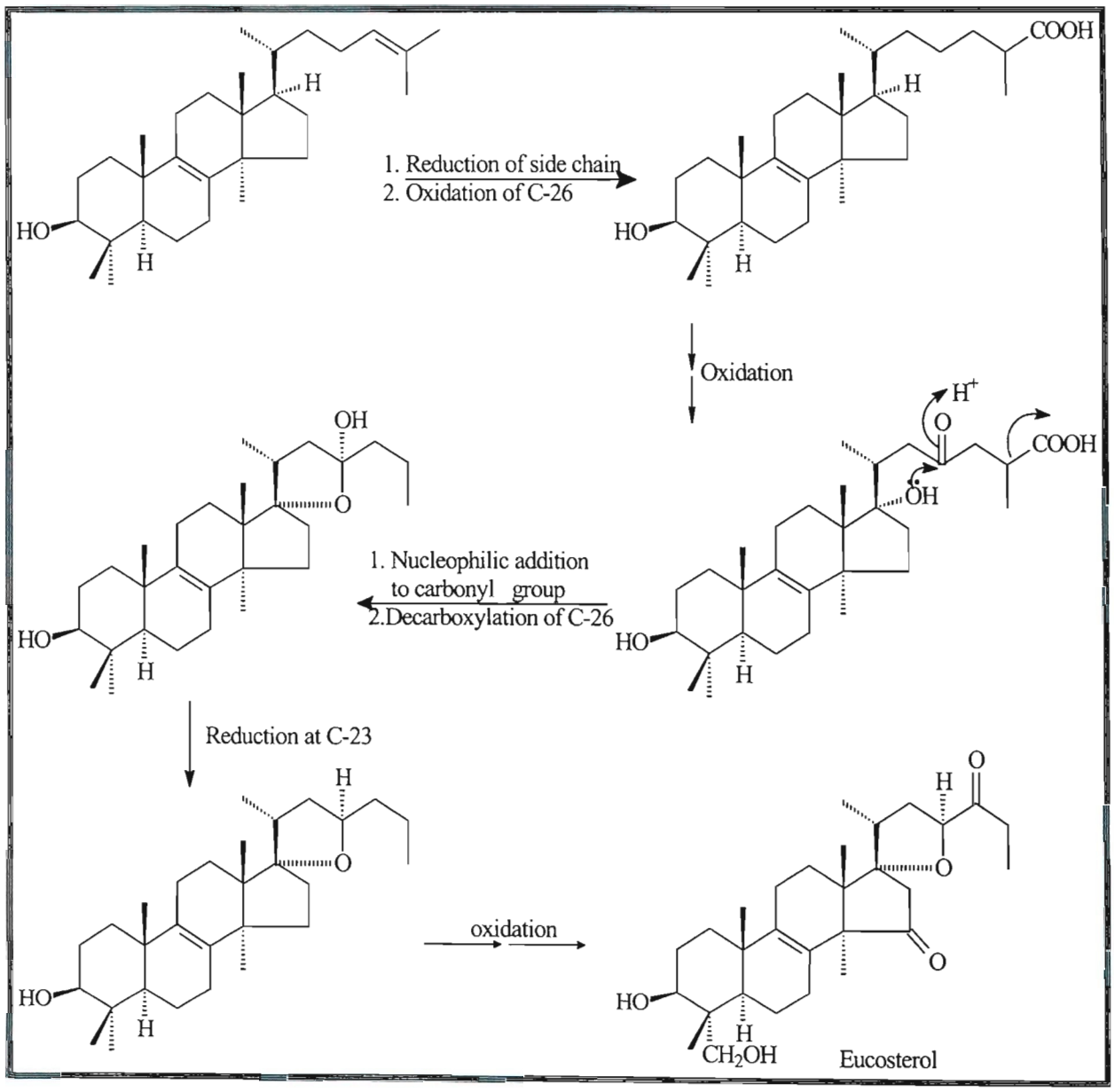
Scheme 2.8: The formation of squalene[30]



Scheme 2.9: Proposed biosynthesis of lanosterol[30]

Cyclization of squalene occurs through the intermediate squalene 2,3-oxide **(Scheme 2.9)**.[30] This reaction is catalysed by a flavoprotein requiring O_2 and NADPH as cofactors.[30] The protonation of the epoxide group allows the opening of the epoxide ring and the generation of the preferred tertiary carbocation, suitably placed to allow electrophilic addition to a double bond.[30] This process continues, generating a new carbocation after each ring formation, until a tertiary protosteryl cation is formed.[30] The loss of a proton results in the formation of the double bond and subsequent proton migrations result in the formation of lanosterol.[30]

Nortriterpenoids are thought to be produced from the basic lanosterol skeleton. A proposed biosynthetic pathway is illustrated below **(Scheme 2.10)** for the formation of eucosterol type nortriterpenoids.



Scheme 2.10: Proposed biosynthesis of eucosterol

2.6 The Biological Activity of Homoisoflavanones

Homoisoflavanones are known to be concentrated in the waxy, scale-like layers of bulbs.[14] In this work, the homoisoflavanones were indeed isolated from bulb extracts. Homoisoflavanones are known to possess anti-inflammatory, anti-mutagenic, anti-bacterial and analgesic properties.[31]

Two well known homoisoflavanones, intracatin and intricatinol **(Figure 2.13)**, isolated from the roots of *Hoffmanseggia intricata* (Fabaceae), have shown antimutagenic properties.[32†] Both showed varying inhibition of mutagenicity in *Salmonella typhimurium* of the carcinogens 2-amino-anthracene, acetylaminofluorine and ethyl methanesulphonate.[32] It was also shown that intricatinol, which possessed two hydroxy groups, was more broadly active than intricatin, which possesses one hydroxy group.[32] This led to the postulation that increased potency could be associated with the presence of extra hydroxy groups in ring A.[32]

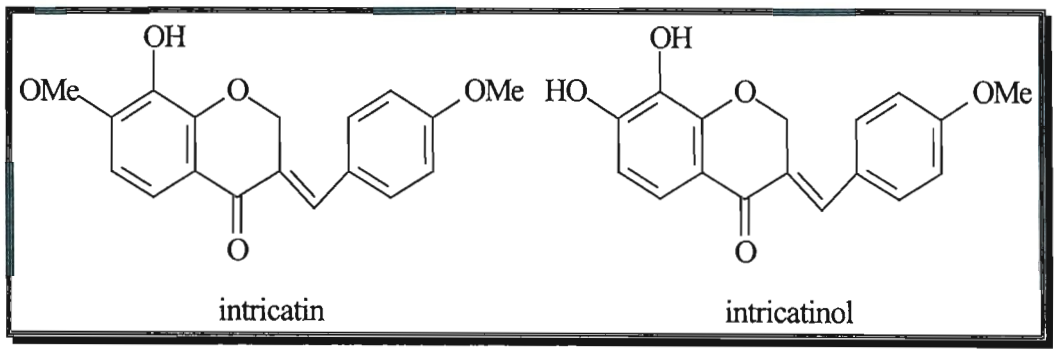


Figure 2.13 Intricatin and intricatinol

The anti-inflammatory properties of bulbs within the Hyacinthceae have been widely investigated.[33] The crude extracts from the bulbs of *Muscari comosum* were tested for anti-inflammatory properties, by using the inhibition of croton oil-induced dermatitis in the mouse ear.[33] Homoisoflavanone-rich fractions were shown to inhibit mouse

† Note that these homoisoflavanones from the Fabaceae have different ring A and B oxidation patterns compared to that of the Hyacinthaceae.

dermatitis.[33] The inhibitory effect obtained was comparable to that of the potent anti-inflammatory drug, indomethacin.[33]

Two homoisoflavanones isolated from *Veltheimia bracteata* (syn. V. *viridifolia*) (Hyacinthceae) were tested against mixtures of phosphodiesterase (PPE) isozymes.[34] The compound, 5-hydroxy-6,7,8-trimethoxy-3-(4'-hydroxybenzyl)-4-chromanone was reported to possess a weak selective profile for PDE IV and PDE V.[34] The study also investigated the *in vitro* activity of the homoisoflavanone on leukotriene synthesis.[34] A weak activity was reported for this system. The study also investigated the bronchiospasmoloytic *in vivo* activity of the homoisoflavanone on the respiration and on the cardiac system of guinea pigs.[34] The results obtained indicated that the compound induced a slight increase in the expiratory flow and a pronounced increase in the respiratory rate in the test subjects.[34] In addition, a moderated bronchiospasmolysis was detected in conjuction with a decrease in heart rate.[34] The conclusions were that the homoiosflavanone had a weak and especially short biological activity in the *in vivo* test systems.[34]

2.7 The Biological Activity of Triterpenoids

The wide occurrence and the structural diversity of triterpenoids has long attracted attention for the evaluation of their biological activities.

The relation between chemical structures and anticancer activity of some pentacyclic and tetracyclic triterpenoids has been studied.[35] The anticancer effects were tested against human cancer cell lines.[35] Epimandiol, a pentacyclic triterpenoid was found to be cytotoxic against HEC-1-A, CAMA-1, ME-180, u-87MG, CALAU-1 and SK-OV-3 cell lines.[35] Maniladiol, the 16β-epimer, exhibited cytotoxicity against ME-180 and CAMA-1, whereas sophorradiol, which lacks a 16-hydroxy group, was cytotoxic only against ME-180.[35] This led them to the conclusion that the presence of a 16α-hydroxy group is important for the appearance of cytotoxicity of 12-oleanenes. Plumeric acid, a nortriterpene acid and its methyl ester isolated from the leaves of *Plumeric acutifolia* showed antitumor activity.[35] The free acid at a concentration 25 $\mu g.ml^{-1}$ was 100% effective in inhibiting *Yoshida sarcoma* cells *in vitro*.[35]

Bioassay-directed fractionation of the cytotoxic antileukemic extracts of *Prunella vulgaris*, *Psychotria serpens* and *Hyptis capitata* led to the isolation of ursolic acid as one of the active principles.[36] Ursolic acid showed significant cytotoxicity in the lymphocytic leukemia cells P-388 and L-1210 as well as the human lung carcinoma cell A-549 cell line.[36] It also demonstrated marginal cytotoxicity in the KB and human colon (HCT-8) and mammary (MCF-7) tumor cells.[36] Further investigations on the cytotoxic fraction from the plant *Hyptis capitata* led to the isolation of two new and three known triterpenoids.[36] Hyptatic A and 2α-hydroxyursolic acid demonstrated significant *in vitro* cytotoxicity in human colon HCT-8 tumour cells.[36]

The anti-inflammatory activity of some triterpenoid derivatives of the oleanane series was examined on the arachidonic (AA)-induced ear edema in mice.[37] Of the

compounds examined the dihemiphthalate derivatives showed strong inhibition of ear edema on both topical and oral administration. Topical ID_{50} values were approximately the same as nordihydroguaiaretic acid. Given topically these compounds were also capable of inhibiting PGE_2 and LTC_4 formation at an early stage of AA-induced ear edema. The most effective time for the topical administration of the compounds against ear edema was found to be 0-30 minutes before AA application. This is different from dexamethasone, which requires a time lag for reaction.[37] Pfaffic acid, a hexacyclic noriterterpene isolated from *Pfaffia peniculata* also showed high inhibitory effects on the growth of cultured tumour cells, such as melanoma (b-16), Hela (s-3) and Lewis lung carcinoma cells.[38]

No previous work has been done on the biological activity of eucosterol type nortriterpenoids. This may be because of the very few occasions that they have been previously isolated. These compounds could be quite interesting to test because of the spirocyclic tetrahydrofuranoid ring.

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Chapter 3: Extractives from *Ledebouria zebrina*

3.1 Introduction

Ledebouria zebrina (Bak.) S. Venter belongs to the Hyacinthaceae (Liliaceae *sensu lato*) Family, and is a member of the Hyacinthoideae subfamily. Members of this family are widely distributed, but are particularly well represented in southern Africa and in the region stretching from the Mediterranean to south-western Asia.[1] There are approximately thirty species of *Ledebouria* in Africa and Asia, fifteen of which are common in South Africa.[2] *Ledebouria* was formerly included in the genus *Scilla* but has now been independently classified.

Ledebouria zebrina **(Figure 3.1)** is distributed in South Africa throughout Mpumalanga and Kwazulu Natal.[3] It usually grows in moist, shaded conditions often associated with forest vegetation. The bulbs have very characteristic purplish-brown outer scales which are membranous.[3]

Plants of the *Ledebouria* genus are extensively used by traditional healers in Kwazulu-Natal, particularly in enemas and as purgatives for both humans and cattle.[4] Although actual uses for *Ledebouria zebrina* have not been recorded, this species has recently been identified in the Warwick Triangle ethnomedicinal plant market (*Crouch 838*, NH). Other members of *Ledebouria* are used by traditional healers.[4] *Ledebouria revoluta* is used by the Sotho people as a charm to drive away lightning and for the treatment of lumbago. *Ledebouria cooperi* is also used by the Sotho women during pregnancy and boys during circumcision rites.[4] *Ledebouria ovatifolia* is used for the treatment of gastro-enteritis and in purgatives for adults.

Figure 3.1: ***Ledebouria zebrina*** **growing near Blyde Nature Reserve, Mpumalanga Photo: N.Crouch**

This is the first reported phytochemical study of this plant. Both the dichloromethane and methanol extracts of this plant were worked on. This led to the isolation of five compounds **(Figure 3.2)**, three homoisoflavanones and two nortriterpenoids, all isolated from the dichloromethane extract. The methanol extract was difficult to work with because the compounds present came out in mixtures that were very difficult to separate. Of the five compounds isolated, three have not been reported previously. The compounds isolated were 5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(I)**, 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(II)**, (22*R*,23*S*)-17α,23-epoxy-3β, 22, 29-trihydroxy-27-nor-lanost-8-en-24-one **(III)**, 5,7-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(IV)** and (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one **(V)**.

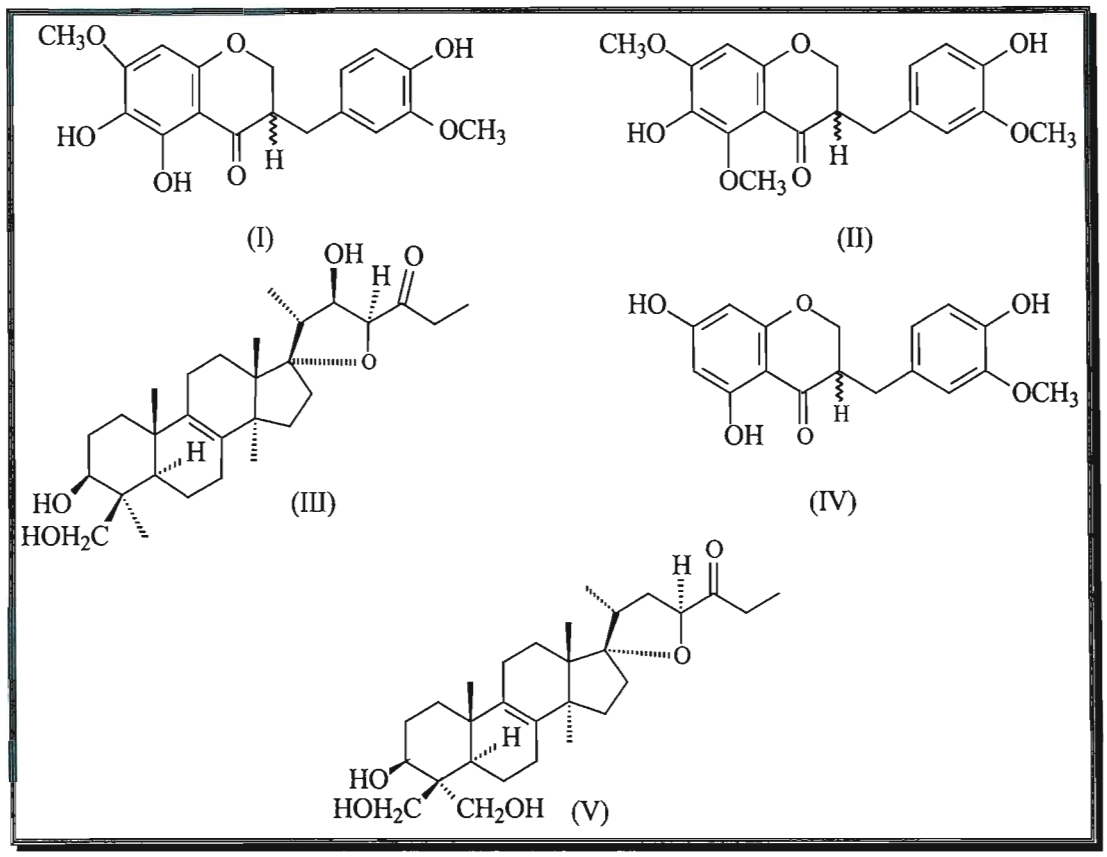


Figure 3.2: Compounds isolated from *Ledebouria zebrina*

3.2 Results and Discussion

3.2.1 Structural elucidation of compound I, 5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina A)

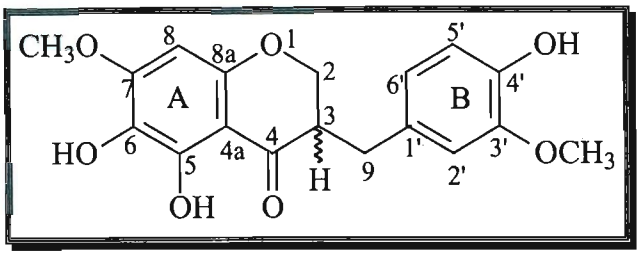


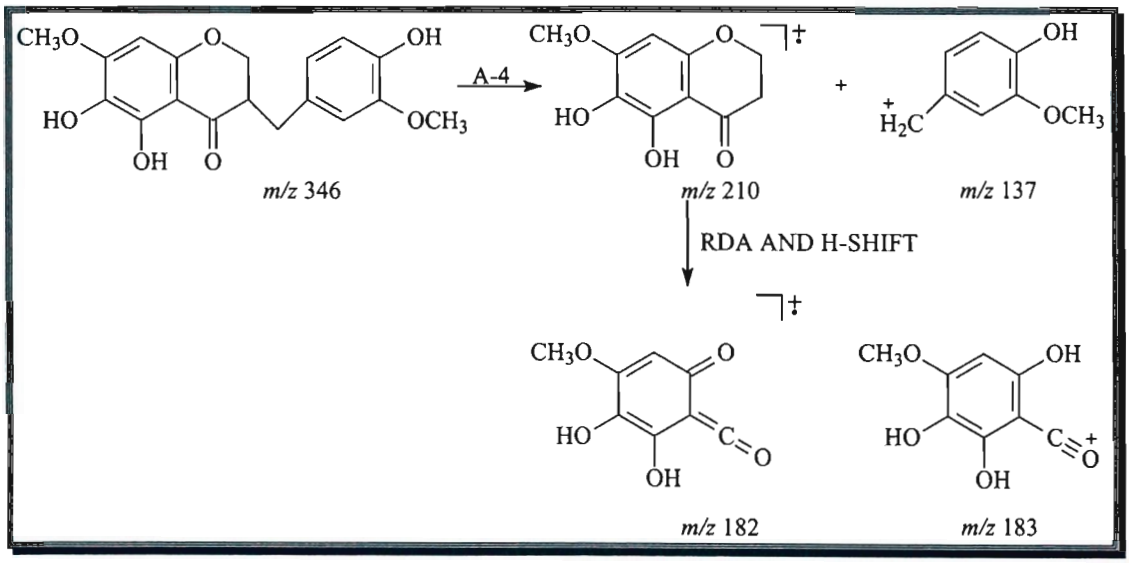
Figure 3.3: Structure of zebrina A

Zebrina A **(Figure 3.3)**, was isolated as an orange gum. This compound was found to have a molar mass of 346.10575 $g.mol^{-1}$ corresponding to a molecular formula of $C_{18}H_{18}O_7$. From the molecular formula, a double bond equivalence of 10 was deduced.

The proton NMR spectrum of zebrina A **[spectrum 1]** showed the characteristic splitting pattern of the 2H-2, H-3 and 2H-9 protons. This was indicative of a 3-benzyl-4-chromanone-type of homoisoflavanone. Each of the H-2 protons was split by each other and the H-3 proton and this resulted in a pair of double doublets at δ 4.10 ppm (7.0, 11.5 Hz) and δ 4.25 ppm (4.0, 11.5 Hz) assignable to the two H-2 protons. The same splitting pattern was displayed by the interaction of H-3 and the two H-9 protons and the double doublet resonances of the H-9 protons were found at δ 3.15 ppm (4.0, 13.7 Hz) and δ 2.67 ppm (10.4, 13.7 Hz). The H-3 proton was found to occur as a multiplet at δ 2.79 ppm. The proton NMR spectrum also showed the presence of two methoxy group proton resonances at δ 3.87 ppm and δ 3.90 ppm.

The appearance of an ABX system in the proton NMR spectrum with resonances at δ 6.71 (d, 2.0 Hz, H-2') ppm, δ 6.83 ppm (d, 8.2 Hz, H-5') and δ 6.69 ppm (dd, 8.2, 2.0 Hz, H-6') was indicative of the presence of three non-equivalent protons on the B ring and

this also indicated that the B ring was 1', 3', 4'-trisubstituted. One methoxy group and one hydroxy group were attached to this ring as shown by the presence of a base peak of *m/z* 137 in the mass spectrum **[spectrum 8]** which corresponds to a hydroxy-methoxybenzyl / tropylium ion **(Scheme 3.1)**. The substitution pattern on this ring was deduced from the NOESY spectrum **[spectrum 7]**. The NOESY spectrum showed a correlation between the methoxy group proton signal at δ 3.87 ppm and the H-2' resonance at δ 6.71 ppm. Thus the methoxy group was placed at C-3'. The H-6' and H-2' resonances showed NOESY correlations with H-2a and H-2b. The assignment of H-2', H-5' and H-6' was confirmed by coupling constants of 8.2 and 2.0 Hz, which are due to *ortho* coupling between H-5' and H-6' and *meta* coupling between H-6' and H-2'. The hydroxy group was placed at the remaining position (C-3').



Scheme 3.1: Fragmentation pattern of zebrina A[9]

The substitution pattern on the A-ring could be deduced from the mass, ^{1}H NMR and UV spectra. The appearance of a singlet integrating to one proton at δ 6.00 ppm (usually due to H-6 or H-8) in the proton NMR spectrum indicated that there was only one unsubsituted position on the A ring. The existence of a peak at *m/z* 210 in the mass spectrum was evidence of a dihydroxy-methoxychromanone fragment ion **(Scheme 3.1)**.[9] This implied that the A-ring contained two hydroxy and one methoxy groups. The

methoxy group was assigned to the C-7 position due to no bathochromic shift in the UV spectrum **[spectrum 10]** when NaOAc was added.[5,6] One hydroxy group was assigned to the C-5 position due a bathochromic shift of 27 nm in the UV spectrum **[spectrum 11]** with $AlCl_3$.[5,6] The downfield signal due to a hydroxy group proton (δ 11.78) was assigned to the proton of the strongly hydrogen-bonded 5-hydroxy group.[7] This was confirmed by the downfield shift of the carbonyl group (δ 198.7) that becomes deshielded due to the chelating effects with the hydroxy group at C-5. Placement of the second hydroxy group was difficult since it could be placed at positions C-6 or C-8. Since no NOESY correlation could be seen between the hydroxy group proton at C-5 and this proton, which would have indicated that the proton was at C-6 instead, the second OH group was placed at position C-6 and the proton at C-8.

The structure was confirmed using the HMBC spectrum **[spectrum 5]**. The HMBC, COSY and NOESY data are provided in **Table 3.1.**

The infrared data **[spectrum 12]** supported the postulated homoisoflavanone structure for zebrina A. Peaks were observed at 3460 cm^{-1} (O-H stretching), 2927 cm^{-1} (aliphatic C-H stretching), 1653 cm^{-1} (C=O stretching), 1514 and 1444 cm^{-1} (aromatic C=C stretching).

A literature search for the compound indicated that it was novel and it was named zebrina A (5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone).

Acetylation of zebrina A, yield a triacetate derivative (zebrina acetate), confirming the presence of three hydroxy groups in the original structure.

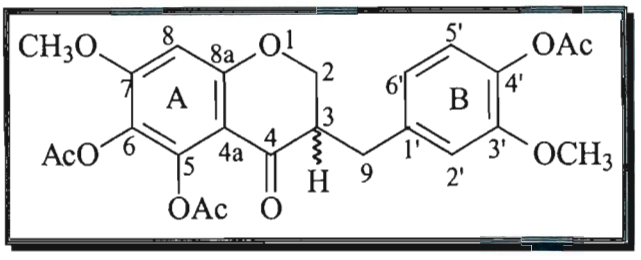


Figure 3.4 Structure of zebrina acetate

The ^{1}H NMR spectrum **[spectrum 1a]** of zebrina acetate **[Figure 3.4]** had a three-proton singlet and a six-proton singlet at δ 2.28 and 2.29 ppm respectively which was indicative of three acetate methyl groups. The singlet proton for ring A was shifted upfield to δ 6.40 ppm. The H-5' resonance was also shifted upfield to δ 6.96 ppm. The ^{13}C NMR spectrum **[spectrum 2a]** showed additional resonances for the acetate carbonyls (δ 168.2, 168.5 and 169.1 ppm) and methyl resonances (δ 20.2, 20.7 and 20.8 ppm) compared to zebrina A. The infrared spectrum **[spectrum 12a]** showed no O-H stretching confirming that all the hydroxy groups were converted to acetate groups but an extra carbonyl stretch band at 1765 cm^{-1} attributable to the extra ester carbonyl groups.

Table 3.1: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
2	H-2a-4.25 (dd,4.0,11.5) H-2b-4.10 (dd,7.0,11.5)	69.3	H-9a,b;H-3	H-2b;H-3 H-2a;H-3	H-3;H-2b H-6';H-3;H-2a;H-9b
3	2.80 (m)	47.1	H-9a,b;H-2 b	H2a,b; H-9a,b	H-2a,b;H-9a,b;H-2'
4		198.7	H-9 a,b;H-2 a, b		
4a		102.4	H-8		
5		146.6	7-OMe		
6		128.7			
7		154.6	7-OCH_3;H-8		
8	6.00 (s)	91.0	7-OCH_3	7-OCH_3	7-OCH_3
8a		156.0	H-2a;b;H-8		
9	H-9a-3.15 (dd,4.0,13.7) H-9b-2.67 (dd,10.4,13.7)	32.5	H-2a,b;H-2'	H-9a;H-3; H-9b;H-3	H-9b;H-3;H-2' H-9a;H-3;H-2';H-2b
1'		129.6	H-9a,b;H-5';H-3		
2'	6.71 (d,2.0 Hz)	111.4	H-9a,b	3'-OMe	H-9a,b; 3'-OCH_3;H-3
3'		148.1	H-2a,b;H-5'; OMe-3'		
4'		144.4	H-2';H-5';H-6'		
5'	6.83 (d,8.2 Hz)	114.4	H-6'	H-6'	
6'	6.69 (dd,8.2, 2.0 Hz)	121.9	H-9a,b;H-3;H-2'	H-5'	H-3; H-2b
7-OCH_3	3.90 (s)	56.3		H-8	H-8
3'-OCH_3	3.87 (s)	55.9		H-2'	H-2'
5-OH	11.78 (s)				

3.2.2 Structural Elucidation of Compound II, 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina B)

This compound was isolated as an amorphous gum and was found to be novel.

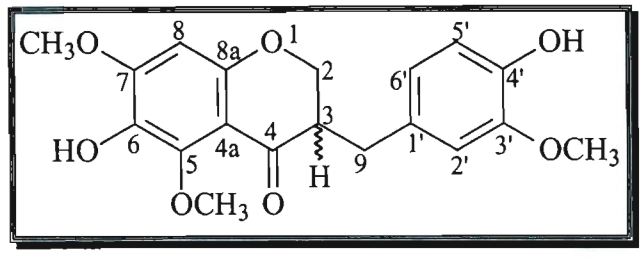
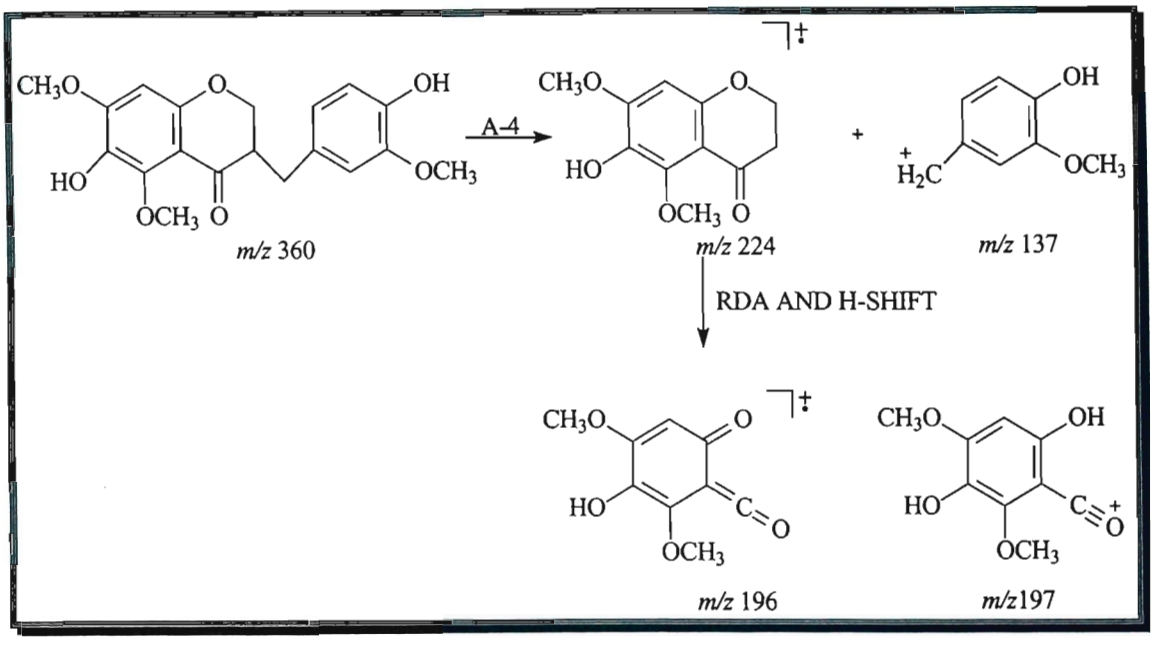


Figure 3.5: Structure of zebrina B

Zebrina B **(Figure 3.5)** also belongs to the 3-benzyl-4-chromanone type of homoisoflavanones. The resonances observed for 2H-2, H-3 and 2H-9 in the ^{1}H NMR spectrum **[spectrum 13]** were similar to those of zebrina A.

Inspection of the mass spectrum **[spectrum 20]** revealed a parent ion at, *m/z* 360.12137 g.mol^{-1}, which corresponded to a molecular formula of $C_{19}H_{20}O_7$, and was consistent with the proposed homoisoflavanone structure. Intense signals were observed at *m/z* 137 and 224 due to A-4 fragmentation. The fragment at *m/z* 224 suggested that ring A had one hydroxy and two methoxy substituents and the hydroxy-methoxybenzyl / tropylium ion at *m/z* 137 similarly suggested that the B ring had one hydroxy and one methoxy group as substituents. The signal at *m/z* 197 is typically formed *via* the retro-Diels Alder cleavage of the chromanone moiety.[9]



Scheme 3.2: Fragmentation pattern of zebrina B[9]

The infra-red data **[spectrum 24]** supported the homoisoflavanone structure for zebrina B. Peaks were observed at 3430 cm^{-1} (O-H stretching), 2930 cm^{-1} (aliphatic C-H stretching), 1619 cm^{-1} (C=O stretching), and 1521 cm^{-1} (aromatic C=C stretching).

The aromatic region of the ^1H NMR spectrum confirmed the substitution patterns indicated by the mass spectrum. The aromatic ABX system with resonances at δ 6.68 (dd, 8.0, 1.9 Hz, H-6'), δ 6.72 (d, 1.9 Hz, H-2') and δ 6.82 (d, 8.0 Hz, H-5') ppm, confirmed the substitution pattern of the B ring and was similar to that of zebrina A. The NOESY spectrum **[spectrum 19]** showed a correlation between a methoxy group proton resonance and the proton at C-2'. Thus, the methoxy group was placed at C-3' and the hydroxy group at C-4'. The remaining aromatic signal in the spectrum was a singlet resonance (integrating to one proton) at δ 6.26 ppm. This confirmed the type of substituents on the A ring, that had been suggested by the mass spectrum. One of the methoxy groups was assigned to the C-5 position due to the lack of a bathochromic shift in the UV spectrum **[spectrum 23]** with $AlCl_3$.[5,6] The other methoxy group was assigned to the C-7 position due to no bathochromic shift in the UV spectrum **[spectrum 22]** when

NaOAc was added.[5,6] Placement of the hydroxy group was done through the HMBC spectrum [**spectrum 17**]. The resonance ascribed to C-8a (δ 157.1 ppm) shows a HMBC correlation with the aromatic proton at δ 6.26 ppm and this is more likely to be a ^{2}J correlation with the proton at C-8 than a ^{4}J correlation with the proton at C-6. The hydroxy group is therefore placed at C-6.

These assignments were confirmed by the HMBC, NOESY and COSY spectra and are listed in **Table 3.2**. This is the first report of this structure and it was called zebrina B (6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone.

Table 3.2: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	^{1}H (ppm)	^{13}C (ppm)	HMBC→C	COSY	NOESY
2	H-2a-4.01 (dd,7.1,11.4) H-2b-4.25 (dd,3.9,11.4)	69.1		H-2b;H-3 H-2a;H-3	H-3;H-6';H-2b H-2a;H-3
3	2.71 (m)	48.5	H-9a,b	H-2a,b;H-9a,b	H-2a,b;H-2'; H-9b
4		191.5	H-2a,b; H-9a,b;H-8		
4a		108.0	H-8		
5		146.1	5-OCH_3		
6		133.8	H-8		
7		153.8	H-8;7-OCH_3		
8	6.26 (s)	95.9		7-OCH_3	7-OCH_3
8a		157.1	H-2a,b;H-8		
9	H-9a-.63(dd,10.6,13.4) H-9b-3.14 (dd,3.7,13.4)	32.6		H-9b;H-3 H-9a;H-3	H-9b;H-2' H-9a;H-3;H-2'
1'		130.2	H-9a,b		
2'	6.72 (d,1.9)	121.9	H-6';H-9a,b		3'-OCH_3; H-9a,b;H-3
3'		146.6	H-5';3'-OCH_3		
4'		144.3	H-6'		
5'	6.82 (d,8.0)	114.3		H-6'	H-6'
6'	6.68 (dd,8.0,1.9)	111.4	H-9a,b	H-5'	H-5';H-2a
3'-OCH_3	3.86 (s)	55.9			H-2'
5- OCH_3	3.92 (s)	61.7			
7- OCH_3	3.90 (s)	56.3		H-8	H-8

3.2.3 Structural Elucidation of Compound III, (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one (22β-hydroxy-15-deoxoeucosterol)

This was the third compound isolated during the course of this work. It was isolated as white crystals with a melting point between 233-236°C.

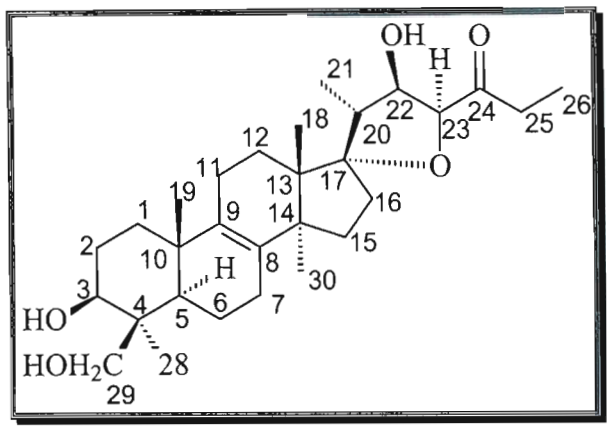


Figure 3.6: Structure of 22β-hydroxy-15-deoxoeucosterol

The compound **(Figure 3.6)** isolated was a derivative of 15-deoxo-eucosterol-type nortriterpenoids. The mass spectrum **[spectrum 31]** did not show a molecular ion peak but showed an M^+-H_2O peak at *m/z* 456 g.mol^{-1}. The molecular formula was found to be $C_{29}H_{46}O_5$-from the mass spectrum in conjunction with the NMR spectra. From the molecular formula a double bond equivalence of seven was deduced.

The proton NMR spectrum **[spectrum 25]** showed the presence of the same number of methyl group proton resonances as found in eucosterol (six methyl groups). The spectrum showed an extra doublet at δ 4.26 ppm, which corresponded to a proton attached to the same carbon as an oxygen. This resonance showed a COSY **[spectrum 29]** correlation with H-23. This implied that a secondary hydroxy group was present at C-22. This resonance also showed a NOESY correlation with H-23 and 3H-21 and this implied that H-22 was α and the hydroxy group was β. This assignment was confirmed through the

HMBC spectrum **[spectrum 28]**, which showed a ^{3}J correlation from C-17 to H-22. This compound was found to lack the keto group at C-15 present in eucosterol. This was determined through the HMBC spectrum. The HMBC spectrum showed a ^{3}J correlation from the only carbonyl group (δ 213.9 ppm) present to the methyl group proton triplet assigned to 3H-26 (δ 1.06 ppm) indicating that the carbonyl group was at C-24 and not at C-15. The rest of the structure was similar to eucosterol. The HMBC, COSY and NOESY data that were used to confirm the structure are shown in **Table 3.4**.

The infrared spectrum **[spectrum 32]** showed a broad signal at 3420 cm^{-1}, which accounted for the presence of hydroxy group stretching, a sharp peak at 1720 cm^{-1} which indicated the presence of a ketone carbonyl group. A strong peak at 2933 cm^{-1} indicated the presence of C-H stretching.

Finally a literature search for the compound was done. It was found to be (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one a compound previously isolated from *Scilla scilloides*.[10] In order to confirm the structure the carbon data were compared with those from literature. The reference compound, however, was run in deuterated pyridine while compound **III** was run in deuterated methanol. To confirm that the NMR data was identical, compound **III** was re-run in deuterated pyridine **[spectrum 33]** to get a better comparison with literature values. The ^{13}C NMR data of compound III and those of the reference compound **(Table 3.3)** corresponded very well. From this it was concluded the compound III was (22*R*,23*S*)-17α,23-epoxy-3β, 22, 29-trihydroxy-27-nor-lanost-8-en-24-one.

Acetylation of compound III, yield a triacetate derivative (IIIA), confirming the presence of three hydroxy group in the original structure.

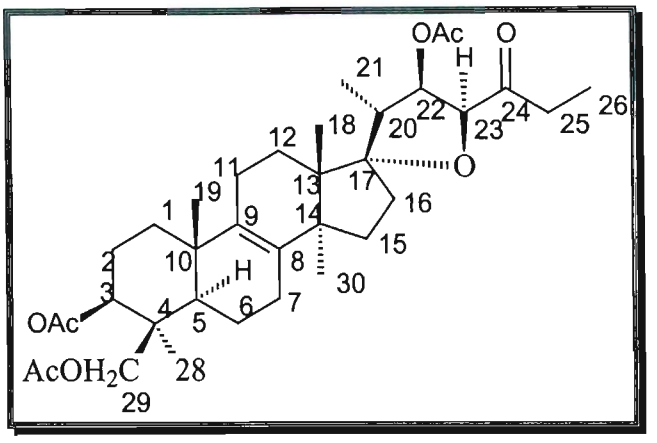


Figure 3.7: Structure of Compound IIIA

The ^{1}H NMR spectrum **[spectrum 25a]** of IIIA **[Figure 3.7]** had an extra three three-proton singlets at δ 1.96, 2.01 and 2.03 ppm, which indicated three acetate methyl groups. The H-3 and H-22 resonances were shifted downfield to δ 4.55 and 5.12 ppm respectively. The H-29 protons were shifted downfield to δ 4.16 and 4.32 ppm. The ^{13}C NMR spectrum **[spectrum 26a]** showed additional resonances for the acetate carbonyl carbons (δ 169.9, 170.6 and 171.1 ppm) and methyl resonances (δ 21.4, 21.3 and 21.2 ppm) compared to compound III. The infrared spectrum **[spectrum 32a]** showed no O-H stretching confirming that all the hydroxy groups were converted to acetate groups.

Table 3.3: Comparison of ^{13}C NMR data for compound III with (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one[10] (C_5D_5N)

CARBON	^{13}C NMR data for Compound III (ppm)	^{13}C NMR Literature data[10] (ppm)
1	35.8	35.9
2	29.0	29.0
3	80.0	79.9
4	43.1	43.1
5	52.3	52.3
6	18.9	19.0
7	26.8	26.9
8	134.8	134.5
9	135.2	135.2
10	37.1	37.1
11	21.1	21.2
12	25.4	25.4
13	49.9	50.0
14	50.3	50.3
15	32.6	32.6
16	40.4	40.4
17	97.5	97.3
18	19.5	19.6
19	20.0	20.4
20	51.6	51.6
21	16.1	16.2
22	80.7	80.6
23	88.0	87.9
24	211.1	210.6
25	33.7	33.7
26	7.5	7.6
28	23.3	23.3
29	64.4	64.3
30	26.6	26.6

Table 3.4: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one (CD_3OD)

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	H-1 α-1.81 (m) H-1-β-1.83 (m)	36.8	3H-19		H-5
2	1.75 (m) (2H)	29.0	H-1	H-3	
3	#	81.4	3H-28	H-2	
4		43.7	3H-28;H-29		
5	1.22 (m)	52.9	3H-19;3H-28	H-6	H-1
6	1.51 (m) (H-2)	19.7	3H-28	H-5	
7	2.11 (m) (2H)	27.8			
8		136.7	3H-19		
9		135.9	3H-30		
10		38.1	3H-19		
11	2.14 (m) (2H)	21.9		H-12	3H-18;3H-30;H-12
12	1.53 (m) (2H)	26.4	3H-18	H-11	3H-21;H-20;3H-18
13		51.2	3H-18;3H-30		
14		51.3	3H-18;3H-30		
15	H-15α-1.40 (m) H-15β-1.74 (m)	33.2	3H-30	H-16α,β H-16α,β	3H-30 3H-18
16	H-16α-2.42 (m) H-16β-2.15 (m)	41.0		H-15α,β H-15α,β	 3H-18
17		98.7	3H-18;3H-21; H-22		
18	1.00 (s)	20.1			3H-21;H-20;H-12;H-16β
19	1.04 (s)	20.2	H-5		H-1;H-11;H-12
20	2.30 (m)	53.1	3H-21	3H-21	3H-21;3H-18;H-20
21	1.10 (d)*	16.3	H-22	H-20	H-12;3H-18
22	4.26 (d,4.9)	81.9	3H-21	H-23	H-23;3H-21
23	4.64 (d,5.9)	88.5	H-22;H-20	H-22	H-22;3H-21
24		213.9	H-23;3H-26; H-25		
25	2.56 (q,7.3)	34.4	3H-26	3H-26	H-25
26	1.06 (t)*	7.4	H-25	H-25	H-26
28	1.25 (s)	23.1	H-29a	H-5;H-1	
29	H-29a-3.41(d,11.3) H-29b-4.20(d,12.0)	65.2	3H-28;H-5	H-29b H-29a	H-19;H-29b H-29a
30	1.29 (s)	26.6			H-15α;H-20

#-peak obscured by solvent peak

*-peaks obscured, *J* values could not be determined

3.2.4 Structural elucidation of compound IV, 5,7-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina C)

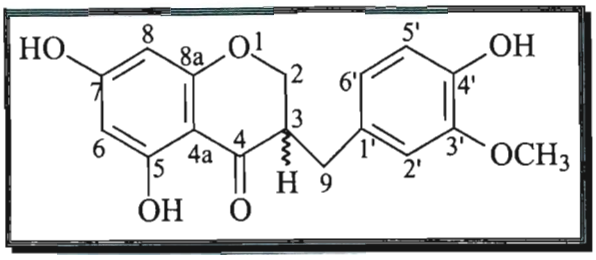


Figure 3.8: Structure of zebrina C

Zebrina C **(Figure 3.8)** was isolated as a yellow gum and was found to be novel. This compound was found to have a molar mass of 316.09462 $g.mol^{-1}$ corresponding to a molecular formula of $C_{17}H_{16}O_6$. From the molecular formula, a double bond equivalence of 10 was deduced. This compound also belonged to the 3-benzyl-4-chromanone type of homoisoflavanones. The proton NMR spectrum **[spectrum 34]** of compound **IV** showed the characteristic splitting pattern of the 2H-2, H-3 and 2H-9 as was seen in zebrina A and B.

The appearance of an ABX system in the ^1H NMR spectrum with resonances at δ 6.78 ppm (d, 8.2 Hz, H-5'), δ 6.79 ppm (d, J =2.0Hz, H-2') and δ 6.69 ppm (dd, J=8.2, 2.0Hz, H-6') was similarly to that of zebrina A and B indicative of the presence of three non-equivalent protons on the B ring. A methoxy group and a hydroxy group were attached to this ring as shown by the presence of a base peak of *m/z* 137 in the mass spectrum **[spectrum 41]** which corresponds to a hydroxy-methoxybenzyl / tropylium ion **(Scheme 3.2)**.

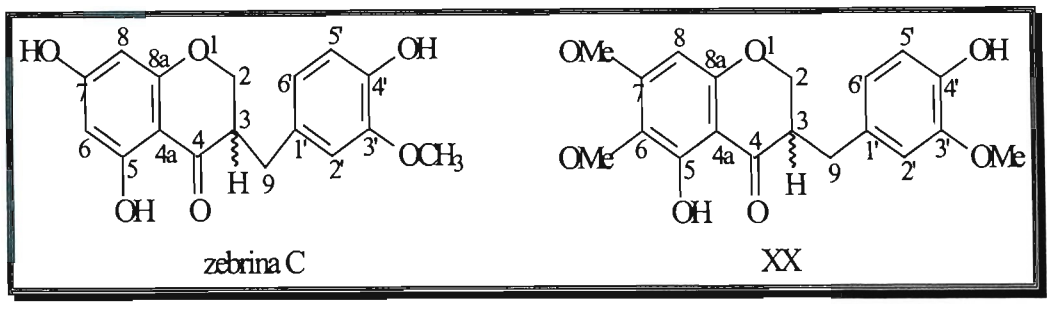
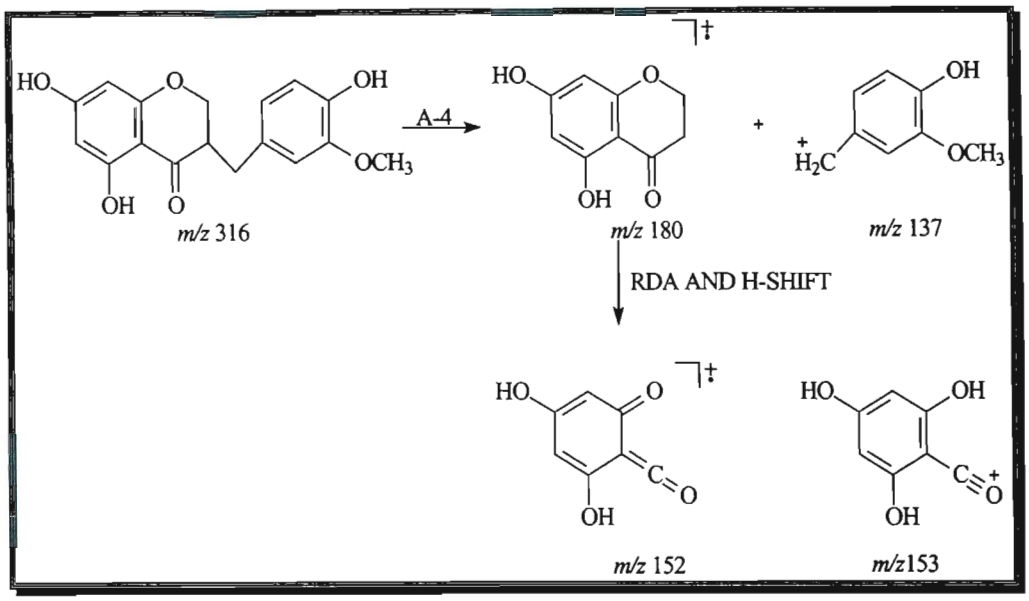


Figure 3.9: Comparision of the B ring in zebrina C and 5-hydroxy-6,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (XX)

The substitution pattern of ring B in zebrina C could not be determined through the NOESY spectrum **[spectrum 40]** as was done in zebrina A and B because the proton resonances of H-5' and H-2' overlapped. The substitution pattern on this ring was therefore deduced by comparing chemical shift values of zebrina C and 5-hydroxy-6,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(Figure 3.9, Table 3.5)**.[11] In this comparison particular emphasis was placed on the resonances of C-1', C-2', C-3', C-4', C-5', C-6'. The chemical shift values compared quite favourable which led us to assign the resonance at δ 145.4 ppm to C-3' and that at δ 145.7 ppm to C-4'. The HMBC spectrum **[spectrum 38]** showed a ^{3}J correlation from C-3' to the methoxy group. The methoxy group was placed at C-3' and the hydroxy group at C-4'. To confirm this substitution pattern a ROESY spectrum **[spectrum 40a]** was run but this also proved to be inconclusive, since both resonances where too close together.



Scheme 3.3: Fragmentation pattern of zebrina C[9]

The substitution pattern of the A-ring could be deduced from the mass, ^1H and UV spectra. The existence of a peak at *m/z* 180 in the mass spectrum was evidence of a dihydroxychromanone fragment ion **(Scheme 3.3)**. This implied that the A-ring contained two hydroxy groups. One hydroxy group was assigned to the C-5 position due a bathochromic shift of 29 nm in the UV spectrum **[spectrum 44]** with $AlCl_3$.[5,6] The downfield signal due to a hydroxyl group proton (δ 11.78 ppm) was assigned to the strongly hydrogen-bonded 5-hydroxy group.[3] This was confirmed by the downfield shift of the carbonyl group that is deshielded due to the chelating effects with the hydroxy group.[1,2] The second hydroxy group was placed at C-7 due to a bathochromic shift of 21 nm in the UV spectrum **[spectrum 43]** with NaOAc.[5,6] The appearance of two *meta*-coupled doublets in the ^1H NMR spectrum, each integrating to one proton, at δ 5.89 and 5.96 ppm were assigned to H-6 and H-8 respectively. The HMBC, COSY and NOESY data are presented in **Table 3.6**.

The infrared data **[spectrum 45]** supported the proposed homoisoflavanone structure for zebrina C. Peaks were observed at 3379 cm^{-1} (O-H stretching), 2921 cm^{-1} (aliphatic C-H stretching), 1636cm^{-1} (C=O stretching), 1522 and 1448 cm^{-1} (aromatic C=C stretching).

A literature search for the compound indicated that it was novel and it was named zebrina C (5,7-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone).

Table 3.5: Comparison of the chemical shift values of ring B, of zebrina C with 5-hydroxy-6,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone[11] ($CDCl_3$)

Carbon	^{13}C NMR data for zebrina C (ppm)	^{13}C NMR Literature data [11] (ppm)
C-1'	131.0	131.1
C-2'	115.1	115.3
C-3'	145.4	145.6
C-4'	145.7	145.8
C-5'	110.8	111.0
C-6'	120.6	120.8

Table 3.6: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 5,7-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
2	H-2a-4.25 (dd,4.2,11.5) H-2b-4.10 (dd,7.1,11.5)	69.0	H-9a,b	H-2b;H-3 H-2a;H-3	H-3;H-2b H-3;H-2b
3	2.79 (m)	46.6	H-9a,b; H-2a, b	H-2a,b; H-9a,b	H-2a,b;H-9a,b;H-2'; H-6'
4		197.9	H-9a,b;H-3		
4a		102.6	H-6;H-8; 5-OH		
5		164.7	H-5		
6	5.96 (d,2.2)	96.6	H-8;5-OH		
7		164.3	H-6;H-8		
8	5.89 (d,2.2)	95.0	H-6		
8a		163.2	H-2a,b;H-8		
9	H-9a-2.63 (dd,10.4,3.3) H-9b-3.13 (dd,4.4,13.9)	31.9	H-2 a,b;H-6'	H-3;H-9b H-9a;H-3	H-2';H-3;H-9b;H-6' H-2';H-3;H-9b;H-6'
1'		131.0	H-9a,b		
2'	6.79 (d,2.0)	115.1	H-9a,b;H-6'		3'-OCH_3;H-9a,b;H-3
3'		145.4			
4'		145.7			
5'	6.78 (d,8.2)	110.8		H-6'	H-6'
6'	6.69 (dd,8.2,2.0)	120.6	H-9a,b;H-2'	H-5'	H-9a,b;H-3;H-5'
3'-OCH_3	3.86 (s)	56.0			H-2'
5-OH	12.11 (s)				

3.2.5 Structural Elucidation of V, (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one

This compound was isolated as an orange gum.

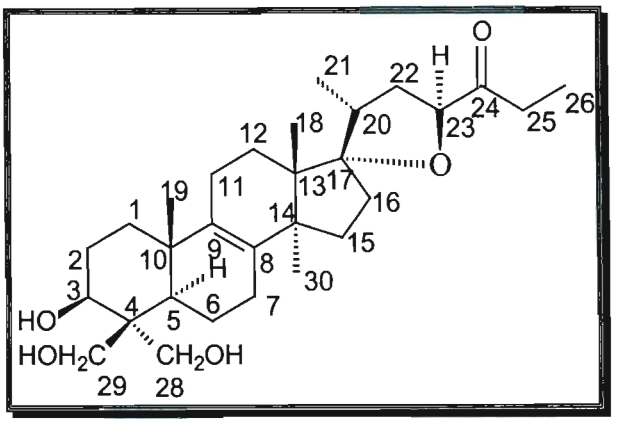


Figure 3.10: Structure of Compound V

The mass spectrum **[spectrum 53]** showed a molecular ion peak at *m/z* 474.33398 g. mol^{-1} which corresponded to a molecular formula of $C_{29}H_{46}O_5$. From this a double bond equivalence of seven was deduced. This compound was found to be another derivative of eucosterol type nortriterpenoids **(Figure 3.8)**.

The ^1H NMR spectrum **[spectrum 46]** showed the presence of five methyl groups in the molecule compared with six found in eucosterol. The methyl group protons of 3H-26 and 3H-21 were not easily recognized in this spectrum because they were superimposed at δ 1.04 ppm. This was illustrated through the HSQC spectrum **[spectrum 49]**, which showed two methyl group carbons correlating to the resonance at δ 1.04 ppm. The HMBC spectrum **[spectrum 50]** showed ^3J correlations from both C-17 and C-24 to the resonance δ 1.04 ppm indicating the presence of the 3H-26 and 3H-21 proton resonances at δ 1.04 ppm. The remaining four methyl group protons in eucosterol, the standard compound, all appear as singlets but there were only three methyl group singlets in compound **V**. The one absent in compound **V** was again determined through the HMBC

spectrum. The HMBC spectrum showed a ^{3}J correlation from C-17 to the methyl group proton singlet at δ 0.86 ppm. This methyl group proton resonance was assigned to 3H-18. The HMBC spectrum also showed a ^{3}J correlation from C-9 to a methyl group proton singlet at δ 0.92 ppm. This was assigned to the methyl group protons at C-19. The HMBC spectrum further showed a ^{3}J correlation from C-8 to the remaining methyl group proton singlet at δ 1.19 ppm. This was assigned to the methyl group protons at C-30. This implied that no methyl groups occurred at C-4.

The ^{13}C NMR spectrum **[spectrum 47]** did not show the ketone resonance for C-15 found in eucosterol: instead it showed the presence of an extra methylene group carbon for C-15. The ^{1}H NMR spectrum showed the presence of an extra oxygenated methylene group (δ 3.75, 4.13 ppm) compared to eucosterol. This primary hydroxy group was placed at C-28. This placement was confirmed through the HMBC spectrum. The HMBC spectrum showed ^{3}J correlations from C-3 to the usual C-29 hydroxy methyl group (δ 3.73, 4.13 ppm, ea d, 11.2 Hz) and this second hydroxy methyl group (δ 3.73, 4.33 ppm, ea. d, 11.5 Hz). This indicated that both methyl groups at C-28 and C-29 had been oxidised to alcohols. **Table 3.8** shows the HMBC, COSY and NOESY data for Compound **V**.

The infrared data **[spectrum 54]** supported the proposed structure. Peaks were observed at 3437 cm^{-1} (O-H stretching) 2929 cm^{-1} (aliphatic C-H stretching) 2853 cm^{-1} (C-H stretching) and 1731 cm^{-1} (C=O stretching).

Finally a literature search of the compound was undertaken. It was found to be (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one, a compound previously isolated from *Muscari comosum* (Hyacinthaceae).[12] A comparison was made with the ^{13}C NMR data with the compound from literature. **Table 3.7** shows the carbon comparison of compound **V** and literature values.[12] The carbon data compares favorably. Compound **V**

was therefore deduced to be (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one.

Table 3.7: Comparison of ^{13}C NMR data of compound V with (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one[12] ($CDCl_3$)

CARBON	^{13}C NMR data for Compound V (ppm)	^{13}C NMR Literature data[12] (ppm)
1	35.15	35.3
2	27.33	27.5
3	77.62	77.86
4	45.82	45.96
5	46.99	47.29
6	18.59	18.72
7	26.09	26.24
8	133.85	134.14
9	135.18	134.48
10	36.59	36.50
11	20.69	20.78
12	24.77	24.91
13	48.50	48.69
14	50.43	50.55
15	31.62	31.70
16	39.55	39.70
17	97.03	97.18
18	19.43	19.51
19	19.13	19.23
20	43.48	43.63
21	17.11	17.18
22	36.73	36.77
23	81.39	81.52
24	213.64	213.63
25	32.21	32.30
26	7.35	7.39
28	70.85	71.27
29	63.62	63.81
30	25.80	25.91

Table 3.8: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-nor-lanost-8-en-24-one ($CDCl_3$)

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	1.72 (m) (2H)	35.2	3H-19		3H-19
2	1.76 (m)(2H)	27.3	H-1	H-3	3H-19
3	3.72 (m)	77.6	H-28;H-29	H-2	H-28
4		45.8	H-5		
5	1.25 (m)	47.0	3H-19;H-28;H-29	H-6	H-7
6	1.46 (m) (2H)	18.6		H-5	3H-19
7	1.98 (m) (2H)	26.1			H-5
8		133.9	3H-30		
9		135.2	3H-19		
10		36.7	3H-19;H-5		
11	H-11α-2.12 (m) H-11β-1.94 (m)	20.7		H-12α,β H-12α,β	 3H-19,H-12α,β
12	H-12α-1.38 (m) H-12β-2.18 (m)	24.8	3H-18	H-11α,β H-11α,β	 3H-21;3H-18
13		48.5	3H-18;3H-30		
14		50.4	3H-18;3H-30		
15	H-15α-1.60 (m) H-15β-1.32 (m)	31.6	3H-30	H-16α,β H-16α,β	3H-30 H-16β
16	H-16α-2.15 (m) H-16β-1.60 (m)	39.6		H-15α,β H-15α,β	 H-20,3H-21,3H-18,H-15β
17		97.0	3H-18;3H-21; H-22;H-22		
18	0.86 (s)	19.4	3H-21		3H-21;H-20;H-12β;H-16β
19	0.92 (s)	19.1			H-29b;H-6;H-1;H-7
20	2.14	43.5	3H-21	3H-21	3H-18;H-16β
21	1.04 (d)*	17.1	H-22	H-20	3H-18;H-23;H-12β
22	1.78	36.7	3H-21	H-23	H-23;3H-21,
23	4.64 (dd,7.3,10.4)	81.4		H-22	H-22;3H-21;H-25
24		213.6	3H-26		
25	2.53 (q, 7.33)	32.2	3H-26	3H-26	H-23;H-26
26	1.04 (t)*	7.4	H-25	H-25	H-25a
28	28a-3.75 (d, 11.2) 28b-4.13 (d,11.2)	70.9	H-5;H-29a	H-28; H-28b	H-28a,b
29	29a-3.75 (d,11.5) 29b-4.33 (d,11.5)	63.6	3H-28;H-5;H-3	H-29a; H-29b	3H-19;H-29a,b
30	1.19 (s)	25.8	3H-18		H-15α;H-16β

*-peaks obscured, *J* values could not be determined

3.3 Test for Anti-fungal Properties

Homoisoflavanones are often cited in the literature (**Section 2.6**) as having anti-fungal properties.[4,13-15] Accordingly a simple test for the antifungal activity was performed on zebrina A, zebrina B and compound III. *Fusarium moliniforme* was chosen as the fungus since it is a very common strain found to infect seeds and developing plants.

The three compounds were spotted on a TLC plate and incubated for 7 days after application of the prepared fungal spray. The assay was then stained with Tinopal solution and viewed under UV light at 254 nm.

Zebrina A and B did not show anti-fungal activity, as a substantial amount of fungal mycelia was evident on and around the applied compounds. Compound III, the eucosterol type-nortriterpenoid, showed the most promising results with no fungal growth within 8 mm of the point of application.

Further tests are still to be carried out in a collaborating laboratory.

3.4 Foreword to Experimental

3.4.1 Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

Nuclear magnetic resonance spectroscopy was carried out on either a 400 MHz Varian UNITY-INOVA spectrophotometer or a 300MHz Gemini spectrophotometer. The spectra of compound **III** were run at a temperature of 60°C in deuterated methanol (CD_3OD). All other spectra were recorded at room temperature in either deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated pyridine (C_5D_5N) or deuterated dimethylsulphoxide (DMSO). The chemical shifts were all recorded in ppm relative to TMS. The ROESY spectrum was run on a 500 MHz Varian UNITY-INOVA spectrophotometer by Mr Martin Watson from the University of Natal, Pietermaritizburg.

3.4.2 Infrared Spectroscopy (I.R. Spectroscopy)

The infra-red spectra were recorded using a Nicolet Impact 400D Fourier-Transform Infra-Red (Ft-IR) spectrometer. The crystalline compounds were analysed using KBr discs and the non-crystalline samples were dissolved in dichloromethane and analysed on a sodium chloride window. The spectra were calibrated against an air background.

3.4.3 Ultraviolet Absorption Spectroscopy (U.V. Spectroscopy)

The ultra-violet absorption spectra were obtained on a Varian DMS 300 UV-visible spectrometer. The solvent in which the spectra were recorded was dichloromethane. The NaOAc and $AlCl_3$ solutions used for bathochromic shifts were prepared by dissolving 0.5g of each salt (anhydrous) in 100 ml volumetric flasks with methanol.

3.4.4 Melting Points

Melting points for crystalline compounds isolated were determined on an Ernst Leitz Weltzlar melting point apparatus and are uncorrected.

3.4.5 Mass Spectrometry

High resolution mass spectra were recorded for all novel compounds on a Kratos 9/50 HRMS instrument. The mass spectrometry was performed by Dr. P. Boshoff at the Cape Technikon. All other mass spectra were recorded on an Agilent MS 5973 instrument connected to a GC 6890 by Mr B. Parel.

3.4.6. Optical Rotations

Optical rotations were measured at room temperature in chloroform using either a Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (4x200 mm) unjacketed flow tube or a Perkin Elmer 241 Polarimeter with a 10 cm flow tube.

3.4.7 General Chromatography

The isolation process involved column and thin layer chromatographic techniques. In column chromatography, different sized columns were used ranging from 2-8 cm in diameter depending on the amount of sample available and the purification stage. Separation of crude extracts was generally carried out on a column using Merck Art. 9385 silica gel. Final purifications were found to be most successful when use was made of open 0.75 cm diameter Pasteur pipettes as columns. All separations were carried out under gravity. Both the column and thin layer techniques made use of varying ratios of dichloromethane, ethyl acetate and hexane. Thin layer chromatography was carried out on 0.2 mm silica gel, aluminium-backed plates (Merck Art. 5554). The plates were first viewed under UV and then developed using an anisaldehyde: conc. H_2SO_4: methanol [1:2:97] spray reagent. The plates were first analysed under UV light (366 nm) and then heated.

3.4.8 Preparative Thin Layer Chromatography (PTLC)

Compounds which were visible under UV light were isolated using this technique. The aluminium-backed plates (Merck Art. 5554) were lined with the extract sample 1 cm from the bottom of the plate. The plates were loaded by dipping a capillary tube in the extract solution and allowing it to run onto the silica gel by touching it to the plates. The plates were then developed in a chromatography tank and the compound of interest was detected using UV light. The marked portion was cut out and boiled in methanol and dichloromethane.

3.4.9 Acetylation Procedure

Pyridine (1 ml) and acetic anhydride (1 ml) were added to the sample (20 mg) in a round-bottomed flask. The sample was left to stand for 48 hours. Methanol (5 ml) was then added to the sample to react with the excess acetic anhydride, and toluene (4x10 ml) was added successively to remove pyridine. After each addition, the solvent was evaporated off on a rotovapor. Thereafter, methanol (5x10 ml) was added to remove the toluene. The sample was then spotted on a TLC plate to see whether the reaction had gone to completion or needed to be purified.

3.4.10 Anti-Fungal Assay

Fusarium moliniforme was isolated from *Trichila dregeana* seeds and subcultured on PDA (PDA (25g), Bactoagar (15g), NaCl (30g) and distilled water (900 ml) in sealed petri dishes. The fungus was incubated for 20 days. A plastic ice-cream container was sterilized with 100% ethanol and allowed to dry. The three compounds of interest were dissolved in dichloromethane and spotted onto a TLC plate (1.5 cm apart) using capillary tubes. The plate was left to stand until the solvent had completely evaporated.

A spore suspension was prepared by flooding the petri dishes with 500 ml of nutrient solution [glucose and mineral solution (1:10)].

Mineral solution: KH_2PO_4 (7 g), $NaHPO_4.H_2O$ (3 g), KNO_3 (4 g), $MgSO_4.7H_2O$ (1 g), NaCl (1 g) and tap water (1000 ml)

Glucose solution: 30% (m/v) glucose solution

The nutrient solution was poured into a spray bottle and sprayed evenly over the TLC plate. This was placed in the ice-cream container along with moist, sterile paper and sealed. The chamber was incubated at 25°C for 7 days.

The plate was subsequently sprayed with Tinopal solution in phophate buffer (as described below) at pH of 8 and viewed under a UV light at 254 nm.

Tinopal solution: Na_2HPO_4 (7.1 g) was dissolved in distilled water (500 ml) to create a 0.1M solution. NaH_2PO_4 (1.56 g) was dissolved in distilled water (100 ml) to create a 0.1M solution. The Mettler Toledo MP 230 pH meter was calibrated using pH 4 and pH 7 standards. The Na_2HPO_4 solution, containing a magnetic bead, was placed on a magnetic stirrer and the pH electrode was placed in the solution. The Na_2HPO_4 solution was added dropwise until a pH of 8 was obtained. A volume of 300 ml of this buffer was decanted into a spray bottle, to which 0.15 g of Tinopal UNPA-GX ($C_{40}H_{44}N_{12}O_{10}S_2$) was added.

3.5 Experimental

Dr Neil Crouch of the National Botanical Institute collected the plant material from a swamp in the Blyde Nature Reserve, in Mpumalanga and a voucher specimen was retained at the Natal Herbarium (N. Crouch 860 NH). The part of the plant investigated was the bulbs. The bulbs (4.495 kg) were chopped into small pieces, air dried for approximately 48 hours and then extracted successively with dichloromethane (mass of extract-25.23g) and methanol (mass of extract-369.93g) by agitation on a Labcon Mechanical shaker at 140rpm. Extraction with each solvent was carried out for approximately 72 hours. The extracts obtained were then filtered and the solvent was removed using a BUCHI rotavapor. General chromatographic techniques were employed **(Section 3.4)** and this led to the isolation of five compounds: 5,8-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(I)** (32 mg), 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(II)** (16 mg), (22*R*,23*S*)-17α,23-epoxy-3β, 22, 29-trihydroxy-27-nor-lanost-8-en-24-one **(III)** (46mg), 5,7- dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **(IV)** (13 mg) and (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one **(V)** (50 mg).

3.5.1 Physical data for Compound I

Name: **5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina A)**

Yield: 32 mg, 20 mg was used for the acetylation

Physical Description: orange gum

Optical rotation: $[\alpha]_D$: -60° (c=0.01)

Mass **[spectrum 8]**: $[M^+]$ at *m/z* 346.10575, $C_{18}H_{18}O_7$ requires 346.105253 $g.mol^{-1}$

EIMS: *m/z* 346.10575 (42.5%)

m/z 210 05345 (47.5%)

m/z 183 (7%)

m/z 137 (100%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 12]**: 3460 (O-H stretching), 2927 (C-H stretching), 2868 (C-H stretching), 1653 (C=O stretching), 1514and 1444 (C=C stretching)

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum 9]**: 356.0 (5.04)

291.0 (5.80)†

207.0 (6.0)

with $AlCl_3$ **[spectrum 11]**: 318.0

with NaOAc **[spectrum 10]**: 291.0

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 1]**: **Table 3.1**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 2]**: **Table 3.1**

† This peak shifts on addition of $AlCl_3$ in the presence of a hydroxy group at C-5 and on addition of NaOAc in the presence of a hydroxy group at C-7

3.5.2 Physical data for Compound II

Name: **6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina B)**

Yield: 16 mg

Physical Description: yellowish-orange gum

Optical rotation: $[\alpha]_D$: +41.7° (c=0.012)

Mass **[spectrum 20]**: $[M^+]$ at 360.12137, $C_{19}H_{20}O_7$ requires 360.120903 $g.mol^{-1}$

EIMS: *m/z* 360.12137 (100%)

m/z 224.06841 (63.3%)

m/z 223.05594 (40.3%)

m/z 137.06032 (82.9%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 24]**: 3430 (O-H stretching), 2930 (C-H stretching), 2852 (C-H stretching), 1619 (C=O stretching) and 1521 (C=C stretching)

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum 21]**: 339.0 (5.4)

279.0 (5.81)

209.0 (6.1)

with $AlCl_3$ **[spectrum 23]**: 279.0

with NaOAc **[spectrum 22]**: 279.0

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 13]**: **Table 3.2**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 14]**: **Table 3.2**

3.5.3 Physical data for Compound III

Name: **(22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one (22β-hydroxy-15-deoxoeucosterol)**

Yield: 46 mg from *Ledebouria zebrina*, 20 mg from *Scilla natalensis*, 20 mg used for acetylation

Physical Description: white crystals

Melting point: 233-238°C (Literature value: 235-239 °C)[10]

Optical Rotation: $[\alpha]_D$: -18° (c=0.096) (Literature: $[\alpha]_D$:-36.7° (c=0.22))[10]

[†]Mass **[spectrum 31]**: $[M^+]$ not seen, $[M-H_2O]^+$ at 456 g.mol^{-1}

EIMS: *m/z* 456 (9%)

m/z 315 (75%)

m/z 69 (100%)

m/z 57 (75%)

Infrared: ν_{max}^{KBr} cm^{-1} **[spectrum 32]**: 3420 (O-H stretching), 2933 (C-H stretching), 2877 (C-H stretching) and 1720 (C=O stretching)

^{1}H NMR: δ_H (ppm) CD_3OD **[spectrum 25]**: **Table 3.4**

^{13}C NMR: δ_c (ppm) C_5D_5N **[spectrum 26]**: **Table 3.3**

^{13}C NMR: δ_c (ppm) CD_3OD **[spectrum 33]**: **Table 3.4**

[†] High resolution mass spectra were only obtained for novel compounds

3.5.4 Physical data for Compound IV

Name: **5,7-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone (zebrina C)**

Optical Rotation: sample was too dilute

Yield: 13 mg

Physical Description: orange gum

Optical rotation: $[\alpha]_D$: -98.2° (c=0.009)

Mass **[spectrum 41]**: $[M^+]$ at *m/z* 316.09462, $C_{17}H_{16}O_6$ requires 316.094688 $g.mol^{-1}$

EIMS: *m/z* 316.09462 (42%)

m/z 179.03435 (6%)

m/z 137.06054 (100%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 45]**: 3380 (O-H stretching), 2922 (C-H stretching), 1637 (C=O stretching) and 1522 (C=C stretching)

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum 42]**: 289.0 (5.5)

206.0 (5.8)

with $AlCl_3$ **[spectrum 43]**: 311.0

with NaOAc **[spectrum 44]**: 325.0

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 34]**: **Table 3.6**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 35]**: **Table 3.6**

3.5.5 Physical data for Compound V

Name: **(23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one**

Yield: 50 mg from *Ledebouria zebrina*, 12 mg from *Scilla natalensis*

Physical Description: orange-brown gum

Optical Rotation: $[\alpha]_D$: -22.7° (c=0.088) (Literature: $[\alpha]_D$:-27.0° (c=0.5))[12]

Mass **[spectrum 53]**: $[M^+]$ at *m/z* 474.33398, $C_{29}H_{46}O_5$ requires 474.334525 $g.mol^{-1}$

EIMS: *m/z* 474.33398 (51%)

m/z 459.30952 (27%)

m/z 456.32257 (8%)

m/z 417.29715 (25%)

m/z 57.03385 (100%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 54]**: 3437 (O-H stretching), 2929 (C-H stretching), 2853 (C-H stretching) and 1731 (C=O stretching)

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 46]**: **Table 3.8**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 47]**: **Table 3.8**

3.6 References

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Chapter 4: Extractives from *Scilla natalensis*

4.1 Introduction

Scilla natalensis Planch. **(Figure 4.1)** belongs to the Hyacinthaceae Family. Plants belonging to this genus are widely distributed throughout Europe, Africa and Asia. There are approximately eight species worldwide, of which six are found in southern Africa.[1] To the Zulu, *S. natalensis* is known as *inguduza*, which means "searching the body for the cause of the ailment".[2]

Scilla natalensis is a summer-growing plant, possessing medium to large-sized bulbs (10-15 cm in diameter), which are mostly exposed above the soil surface.[1] The bulbs are covered with brown membranous leaf scales. The leaves are uniformly green and hairy at times and are about 14 cm long and 4 cm broad at the base.[3] The inflorescence, whose length reaches 35 cm, is erect and rigid and bears deep blue and occasionally white flowers.[4,5] *S. natalensis* is endemic to the eastern parts of southern Africa, occurring throughout most of the Eastern Cape, Lesotho, KwaZulu-Natal, the Free State, Swaziland and in Mpumalanga along the escarpment.[6]

Scilla natalensis has, for many years, been used by various ethnic groups to treat a range of ailments.[1] It is the most popular item in the muthi markets around South Africa and it is becoming a less common sight in the wild.[1] It was recently listed as vulnerable.[7] It is used by different tribes for the treatment of different aliments. For example, the Zulu use decoctions from it as ingredients in infusions taken to facilitate labour at birth.[8] The Sotho eat the cooked bulbs with food as an aperient, include bulb decoctions in enemas to treat "internal tumours" and rub powdered bulbs into scarifications over sprains and fractions.[9,10] The Tswana rub the powered bulbs into the back joints and other parts with the belief that it makes them strong and resilient to witchcraft.[10,11] The Swati apply a lotion prepared by boiling the macerated bulbs in water to boils and veld sores.[10]

Figure 4.1: *Scilla natalensis* growing near Blyde Nature Reserve, Mpumalanga Photo: N.Crouch

Previous phytochemical investigations of *Scilla natalensis* have resulted in the isolation of two homoisoflavanones (**VI** and **VII**).[3] The dichloromethane extract only was investigated in this work. This led to the isolation of ten compounds **(Figure 4.2)**. Three of the compounds isolated have not been reported previously. Two compounds, (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one (**III**) and (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one (**V**) were previously isolated from *Ledebouria zebrina* in this work. Other compounds isolated were 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (**VI**), 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone (**VII**), (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene (**VIII**), (22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene (**IX**), (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (**X**), 5,7-dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*][1,3]benzodioxol]-4-one (**XI**), (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (**XII**), (22*R*,23*S*)-17α,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione (**XIII**).

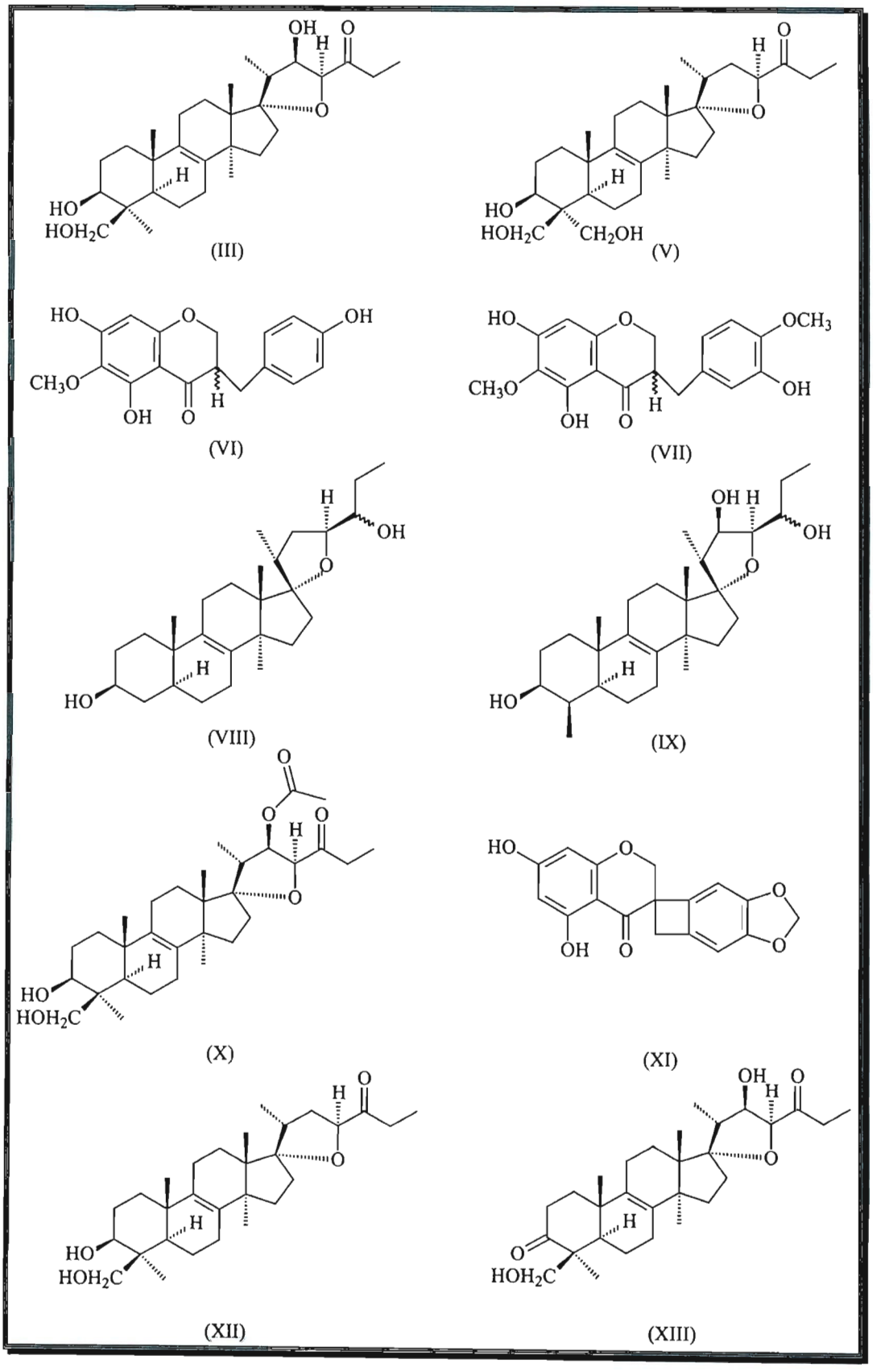

Figure 4.2: Compounds isolated from *Scilla natalensis*

4.2 Results and Discussion

4.2.1 Structural Elucidation of Compound VI, 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (3,9-dihydroeucomnalin)

This compound was previously isolated from the same plant.[3] It was isolated as an orange gum.

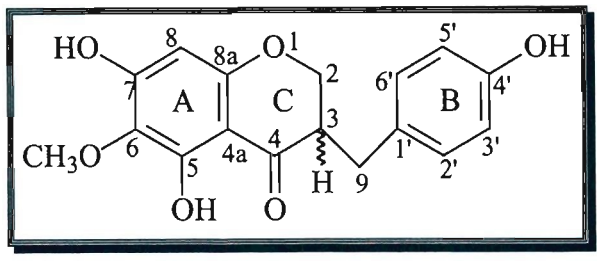

Figure 4.3: Structure of 3,9-dihydroeucomnalin

Compound **VI (Figure 4.3)** was found to have a molar mass of 316 $g.mol^{-1}$ corresponding to the molecular formula $C_{17}H_{16}O_6$. A double bond equivalence of 10 was deduced. This compound also belongs to the 3 benzyl-4-chromanone type of homoisoflavanones.

The ^1H NMR spectrum **[spectrum 55]** showed the presence of one methoxy group (δ 3.76 ppm). The appearance in the mass spectrum **[spectrum 57]** of peaks at *m/z* 209, 183 and 182 corresponding to the A-4 and RDA fragments **(Scheme 2.1)** gave an indication that two hydroxy groups and one methoxy group were attached to ring A. One hydroxy group was assigned to the C-5 position due to a bathochromic shift of 25 nm seen in the UV spectrum **[spectrum 60]** when $AlCl_3$ was added.[12,13] This was confirmed by the downfield shift of the C-4 carbonyl group (δ 199.6 ppm). The second hydroxy group was assigned to C-7 due to a bathochromic shift of 40 nm in the UV spectrum **[spectrum 59]** when NaOAc was added.[12,13] The methoxy group was placed at C-6 by comparison against the literature chemical shift data for this compound.[3] The ^1H NMR spectrum also showed a pair of doublets at δ 7.11 and δ

6.79 ppm, which indicated that the ring B was a *para*-disubstituted benzene ring. This was confirmed by a base peak of *m/z* 107 in the mass spectrum corresponding to a hydroxybenzyl / tropylium ion. The hydroxy group was assigned to C-4′.

A comparison was made of the ^{1}H and ^{13}C NMR data of compound **VI** and data from literature. Both the ^{13}C and ^{1}H NMR data were similar confirming that compound **VI** was, in fact, 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone.

Table 4.1: Comparison of ^{1}H NMR data of compound VI with 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone[3] (CD_3OD)

Proton (s)	^{1}H NMR data for Compound VI (ppm)	^{1}H NMR Literature data[3] (ppm)
2H-2	4.22 (dd,7.0,11.4) 4.35 (dd,4.3,11.4)	4.10 (dd,7.2,11.4)† 4.20 (dd,4.2,11.4)†
H-3	2.75 (m)	2.83 (m)
H-8	5.97 (s)	5.94 (s)
2H-9	2.79 (dd,13.7,10.1) 3.13 (dd,4.4,13.7)	2.67 (dd,13.5,10.6) 3.13 (dd,4.2,13.5)
H-2'/H-6'	7.11 (d,8.4)	7.09 (d,8.4)
H-3'/H-5'	6.79 (d,8.4)	6.78 (d,8.4)
6-OCH3	3.81 (s)	3.81 (s)

† Original spectra were analysed and showed that these double doublets were inaccurately reported by previous student

Table 4.2: Comparison of ^{13}C NMR data of compounds VI with 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone[3] (CD_3OD)

Carbon	^{13}C NMR data for Compound VI (ppm)	^{13}C NMR Literature data[3] (ppm)
2	70.6	70.3
3	49.9	#
4	199.6	200.2
4a	102.9	103.1
5	156.0	156.80
6	130.1	130.2
7	161.1	160.5*
8	97.0	96.0
8a	161.1	160.1*
9	33.1	33.0
1'	130.1	130.2
2'	131.2	131.2
3'	116.4	116.4
4'	157.3	157.4
5'	116.4	116.4
6'	131.2	131.2
6-OCH_3	61.5	61.0

#-obscured by solvent peak

*-Comparison with original spectrum in laboratory showed the compound was not as pure, resulting in the presence of more than one peak in this region and the wrong peaks could have been assigned

4.2.2 Structural elucidation of Compound VII, 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone

This compound was previously isolated from the same plant.[3] It was isolated as a yellow gum.

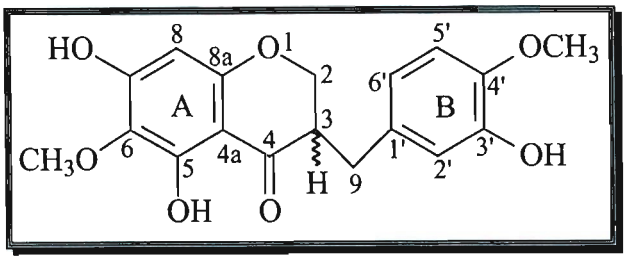

Figure 4.4: Stucture of Compound VII

Compound **VII (Figure 4.4)** was found to have a molar mass of 346 $g.mol^{-1}$ corresponding to a molecular formula of $C_{18}H_{18}O_7$. A double bond equivalence of ten was deduced. This compound also belongs to the 3-benzyl-4-chromanone-type of homoisoflavanones.

The ^1H NMR spectrum **[spectrum 62]** showed that compound **VII** had two methoxy groups (δ 3.86, 3.76 ppm). Substitution in ring A was deduced from the mass and ^1H NMR spectra. Although the two fragments due to RDA fragmentation could not be detected in the mass spectrum **[spectrum 64]** the presence of a low intensity peak at *m/z* 209 corresponding to a dihydoxy-methoxychromanone fragment ion was testimony to the existence of a ring A with two hydroxy and one methoxy group. The appearance of a singlet integrating to one proton at δ 5.97 ppm (normally H-6/8) in the ^1H NMR was also an indication that there was only one unsubsituted position in ring A. The fact that the chemical shift was upfield of 6.0 ppm provided evidence that a hydroxy group occurred at C-7.[14,15] This was confirmed by a bathochromic shift of 40 nm with NaOAc. A hydroxy group also occurred at C-5 and this was indicated by a bathochromic shift of 24 nm with $AlCl_3$. The methoxy group was placed at position C-6 by comparison of the chemical shift data.[3] Ring B was also found to be trisubstituted as revealed by the appearance of an aromatic ABX system in the ^1H

NMR spectrum. A base peak at *m/z* 137 in the mass spectrum corresponding to a hydroxy-methoxybenzyl ion indicated that ring B contained one hydroxy and one methoxy substituent. The methoxy group was assigned to C-4' due to a NOESY correlation with H-5' and the hydroxy group was placed at C-3'.

A comparison was made of the ^{1}H and ^{13}C NMR data of compound **VII** and that from the literature. Both the ^{1}H and ^{13}C NMR data were similar and it was concluded that compound **VII** was, in fact, 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)-4-chromanone.

Table 4.3: Comparison of ^{1}H NMR data of compound VII with 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone[3] (CD_3OD)

Proton (s)	^{1}H NMR data for Compound VII (ppm)	^{1}H NMR Literature data[3] (ppm)
2H-2	4.22 (dd,7.1,11.5) 4.38 (dd,4.4,11.3)	4.10 (dd,7.2,11.4)† 4.26 (dd,4.2,11.4)†
H-3	2.87 (m)	2.85 (m)
H-8	5.97 (s)	5.95 (s)
2H-9	2.68 (dd,10.2,13.5) 3.13 (dd,4.5,13.5)	2.62 (dd,10.2,13.5) 3.11 (dd,4.5,13.5)
H-2'	6.75 (d,2.1)	6.75 (d,2.1)
H-5'	6.88 (d,8.2)	6.89 (d,8.1)
H-6'	6.70 (dd,2.1, 8.2)	6.70 (dd,2.1, 8.1)
6-OCH_3	3.76 (s)	3.81 (s)
4'-OCH_3	3.86 (s)	3.86 (s)

† Original spectra were analysed and showed that these double doublets were incorrectly reported

Table 4.4: Comparison of ^{13}C NMR data of compound VII with 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone[3] (CD_3OD)

Carbon	^{13}C NMR data for Compound VII (ppm)	^{13}C NMR Literature data[3] (ppm)
2	70.6	70.3
3	#	#
4	200.1	200.1
4a	*	103.0
5	155.7	156.8
6	*	130.5
7	161.1	160.8
8	97.0	96.0
8a	*	160.1
9	33.2	33.2
1′	132.3	132.3
2′	117.1	117.1
3′	147.7	147.9
4′	147.7	147.7
5′	112.9	113.0
6′	121.4	121.4
3'-OCH_3	61.1	61.0
4'-OCH_3	56.5	56.6

#-obscured by solvent peak
*-sample was too weak to decipher these peaks

4.2.3 Structural elucidation of Compound VIII, (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene (natalensis A)

This compound was isolated as a white powder and the structure has not been previously reported.

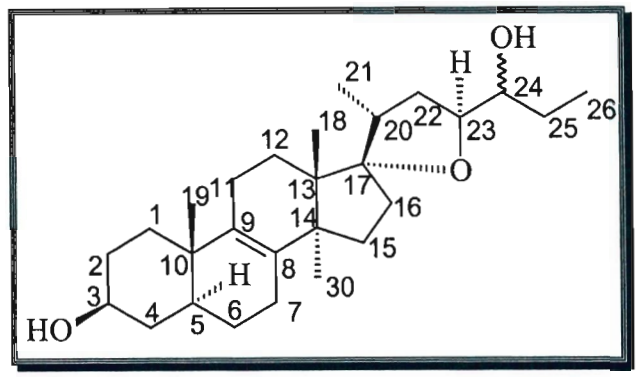

Figure 4.5: Stucture of natalensis A

The mass spectrum **[spectrum 76]** showed a molecular ion peak at *m/z* 416.32916 g.mol^{-1} which corresponded to a molecular formula of $C_{27}H_{44}O_3$. The molecular formula indicated that two further carbon atoms had been lost from the eucosterol skeleton. From the molecular formula, a double bond equivalence of six was deduced. This compound was found to be a derivative of eucosterol type nortriterpenoids **(Figure 4.5)**.

The ^{1}H NMR spectrum **[spectrum 69]** showed the presence of five methyl groups in the molecule instead of the six found in eucosterol. The methyl group protons of 3H-26 and 3H-21 were easily recognized to be present by the presence of a triplet at δ 0.99 ppm for 3H-26 protons and the presence of a doublet at δ 1.04 ppm for the 3H-21 protons. The remaining methyl group protons all appear as singlets and the one absent was determined through the HMBC spectrum **[spectrum 73]**. The HMBC spectrum shows ^3J and ^2J correlations from C-13 to two methyl group proton resonances at δ 1.11 ppm and δ 0.91 ppm. These methyl group protons were assigned to C-30 and C-18 respectively. The HMBC spectrum also showed a ^3J correlation

from C-9 to a methyl group proton at δ 0.92 ppm. This could only be the methyl group protons of C-19. This implied that the methyl group at C-4 was missing. The ^{1}H NMR spectrum did not show the primary hydroxy group protons at C-29 found in eucosterol. This indicated that at C-4 the C-28 methyl group and the C-29 primary hydroxy group were both absent.

The ^{13}C NMR spectrum **[spectrum 70]** did not show the presence of the carbonyl groups at C-24 and C-15 found in eucosterol. Instead it showed the presence of an extra secondary hydroxy group methine proton (δ 78.9 ppm). The ^{1}H NMR resonance (δ 3.35 ppm) showed a COSY **[spectrum 74]** correlation with the H-23 resonance. This meant that the hydroxy group was either at position C-22 or C-24. The HMBC spectrum showed a ^{3}J correlation from C-26 to this resonance, indicating that it was at C-24. The stereochemistry of the hydroxy group on C-24 has not been determined due to the small amount of material available. The remaining HMBC, COSY and NOESY correlations are shown on **Table 4.5**

The infrared data **[spectrum 77]** supported the proposed structure. Peaks were observed at 3396 cm^{-1} (O-H stretching) 2928 cm^{-1}, (aliphatic C-H stretching) and 2864 cm^{-1} (C-H stretching).

A literature for the compound indicated that it was novel and was named natalensis A (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene).

Table 4.5: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene ($CDCl_3$)

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	1.92 (m) (2H)	21.4			
2#*	H-2α-1.30 (m) H-2β-1.62 (m)	38.3		H-3	H-3 H-3;H-4β
3	3.60 (m)	71.2		H-2	H-4α;H-2
4#*	H-4α-1.15 (m) H-4β-1.72 (m)	35.0			H-3 H-2β
5	1.32 (m)	40.4	3H-19	H-6α,β	H-6α
6	H-6α-1.96 (m) H-6β-1.75 (m)	41.0		H-5 H-5	H-5
7§*	2.06 (m)	21.4			
8		135.2	3H-30		
9		133.8	3H-19		
10		35.6	3H-19		
11	H-11α-1.82 (m) H-11β-1.40 (m)	31.6		H-11β;H-12α;β H-11α;H-12α,β	H-12α;H-11β H-12β;H-11α;3H-19
12	H-12α-2.08 (m) H-12β-2.20 (m)	24.8	3H-18	H-11α;β H-11α,β	3H-30 3H-21;3H-18;H-11β
13		50.6	3H-18;3H-30		
14		48.7	3H-18;3H-30		
15	H-15α (d,1.31) H-15β (d,1.65)	31.7	3H-30		3H-30 3H-18
16§*	1.15 (m) 1.72 (m)	35.0			
17		95.6	3H-21		
18	0.91 (s)	19.1			H-20;H-15β;H-12β; 3H-21
19	0.92 (s)	17.1			H-11
20	2.18 (m)	44.7	3H-21	3H-21;H-22α,β	3H-21;3H-18; H-22α,β;H-25
21	1.04 (d, 6.8)	17.6	H-22	H-20	H-23;H-12β; H-22α;3H-18
22	H-22α-1.51 (dd, 6.0, 6.2) H-22β-1.66**	36.4	3H-21	H-23;H-20 H-23;H-20	3H-21;H-23;H-20 H-24;H-20
23	3.82 (m)	80.9	H-20;H-24;H-25	H-22α,β;H-24	3H-21;H-22α
24	3.35 (m)	78.9	3H-26;H-25	H-23;H-25	3H-26;H-25;H-22β
25	1.40(q, 7.3)	25.6	3H-26	3H-26	H-24
26	0.99 (t, 7.3)	9.9	H-24;H-25	H-25	H-24;H-25
30	1.11 (s)	26.5	H-15		H-15α;H-12α

#-Interchangeable

§-Interchangeable

*-COSY spectrum too complex in this part of the spectrum to assign these resonances

**- Coupling constant could not be worked out superimposed with other peaks

4.2.4 Structural elucidation of compound IX, (22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene (natalensis B)

This compound was isolated a yellowish-white gum and was found to be novel **(Figure 4.5)**.

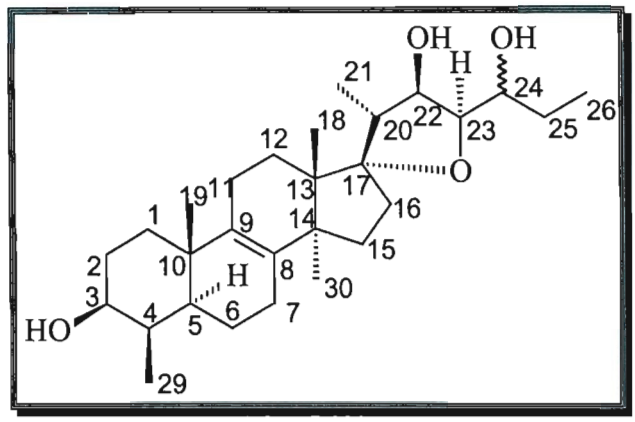

Figure 4.6: Structure of natalensis B

The mass spectrum **[spectrum 85]** showed a molecular ion peak at *m/z* 446.33844 g.mol^{-1}, which corresponds to a molecular formula of $C_{28}H_{46}O_4$. This indicated that one carbon atom had been lost from the eucosterol-type structure. From the molecular formula, a double bond equivalence of six was deduced.

The ^{1}H NMR spectrum **[spectrum 78]** of this compound was very similar to that of natalensis A. The primary hydroxy group at C-29, and the carbonyl groups at C-24 and C-15 were all missing from this structure. This compound, however, showed the presence of a 3H-29 methyl proton resonance (δ 1.13 ppm) in the ^{1}H NMR spectrum which now appeared as a doublet. The stereochemistry was determined through the NOESY spectrum **[spectrum 84]**. The NOESY spectrum showed NOESY correlations between 3H-29 and 3H-19 and between H-4 and H-5α. This implied that 3H-29 was β.

Using the COSY spectrum **[spectrum 83]** the 3H-21 methyl group proton resonance (δ 0.99 ppm) was seen to be coupled to H-20 which was, in turn, coupled to an oxygenated methine resonance (H-22) at δ 4.13 ppm. This showed coupling to H-23 (δ 4.01 ppm) and a further oxygenated proton resonance at δ 4.07 ppm which was assigned to H-24. The H-24 resonance was, in turn, coupled to 2H-25 which was further coupled to the 3H-26 (δ 1.05 ppm) triplet. The corresponding ^{13}C NMR resonances **[spectrum 79]** occurred at δ16.5, 53.2, 81.2, 83.0, 73.6, 27.9 and 10.9 ppm for 3H-21, H-20, H-22, H-23, H-24, H-25, 3H-26 respectively. The H-22 resonance showed a NOESY correlation with the α hydrogen H-23, indicating that the hydroxy group at C-22 was β. The stereochemistry of the hydroxy group on C-24 is still to be determined, but this requires the re-collection of more plant material and the isolation of of larger amounts of this compound for the preparation of Mosher esters. The remaining HMBC, COSY and NOESY correlations are shown on **Table 4.6**.

The infrared data **[spectrum 86]** supported the proposed structure. Peaks were observed at 3393 cm^{-1} (O-H stretching), 2924 cm^{-1} (aliphatic C-H stretching) and 2853 cm^{-1} (C-H stretching).

A literature search for the compound indicated that it was novel and it was named natalensis B ((22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene).

Table 4.6: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene (C_5D_5N)

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	H-1α-1.05 (m) H-1β-1.65 (m)	35.5	3H-19	H-2β;H-3	3H-19;3H-29;H-2β
2	H-2α1.72 (m) H-2β1.93 (m)	32.6		H-3	3H-19;H-3
3	3.16 (m)	75.6	3H-29;H-2,a,b; H-4	H-2;H-4	H-2β;3H-19;3H-29
4	1.41 (m)	39.9	3H-29;H-2;H-3,	H-3-	H-5
5	#	47.5	3H-19;3H-29; H-4;H-2		H-4
6**	H-6α-1.93 (m) H-6β-2.06 (m)	21.8		H-7α,β	H-7β
7**	H-7α-1.16 (m) H-7β-1.67 (m)	21.2		H-7β H-7β	3H-19;H-6β
8		135.7	3H-30		
9		134.1	3H-19		
10		36.6	3H-19;H-2a,b		
11	H-11α-2.06 (m) H-11β-1.92 (m)	25.7			3H-18
12	1.42 (m) (2H)	25.6	3H-18		3H-18, 3H-21
13		50.3	3H-30;H-20		
14		50.1	3H-18;H-16		
15	H-15α-1.30 (m) H-15β-1.55 (m)	32.2	3H-30;H-16	H-16α,β H-16α,β	3H-18
16	H-16α-2.68 (m) H-16β-2.28 (m)	41.2	H-20	H-15α,β H-15α,β	3H-30 H-20
17		95.3	3H-21;H-23;H- 15;H-22,;H-18		
18	0.88 (s)	19.6	H-16		H-20;H-15;H-21;H- 11β;H-12
19	0.90 (s)	18.4			3H-29;H-3;H-6
20	2.39 (m)	53.2	3H-21	3H-21; H-22	3H-21;3H-18;H- 16β
21	O.99 (d,7.1)	16.5	H-22;H-20	H-20	H-22;H-23;H- 20;3H-18;H-12
22*	4.13	81.2	3H-21;H-20	H-23;H-22	H-23;3H-18
23*	4.01	83.0	H-22,H-20; H-25;	H-22	H-22;H-25;3H-21
24*	4.07	73.6	H-25;3H-26;	H-25	H-25;H-26
25	1.75 (q, 7.3)	27.9	H-23;3H-26;	3H-26;H-24	H-23;H-24;3H-26
26	1.05 (t,7.3)	10.9		H-25	H-24;H-25
29	1.13(d,6.2)	15.8	H-3;H-4;H-5		H-3;3H-19;H-1β
30	1.33	26.9			H-16α

*-peaks obscured, *J* values could not be determined

**- carbon values interchangeable

#-obscured by 3H-18, 3H-19

4.2.5 Structural elucidation of Compound X, (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (22β-acetoxy-15-deoxoeucosterol)

This was the tenth compound isolated during the course of this work. It was isolated as white crystals with a melting point of 180°C.

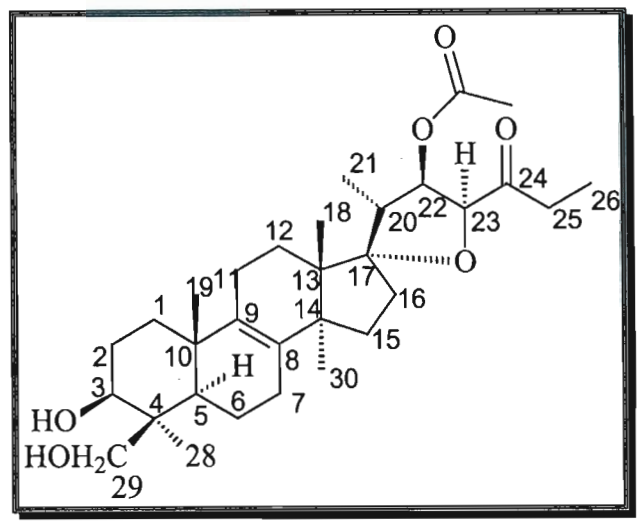

Figure 4.7: Structure of 22β-acetoxy-15-deoxoeucosterol

The mass spectrum **[spectrum 93]** did not show a molecular ion peak but showed an $[M^+-CH_3COOH]$ fragment at *m/z* of 456. The molecular formula was found to be of $C_{31}H_{48}O_6$ from the mass spectrum in conjunction with the NMR spectra and a double bond equivalence of 8 was deduced. The compound isolated also belongs to the class of compounds known as the eucosterol-type triterpenoids **(Figure 4.7)**.

The ^1H NMR spectrum **[spectrum 87]** of this compound was very similar to that of eucosterol and to that of compound **IV**. It showed the presence of an extra methyl group proton resonance, which was an acetoxy methyl group proton resonance (δ 1.99 ppm). The HMBC spectrum **[spectrum 90]** showed a ^2J correlation from the carbonyl carbon of the acetate to this methyl group proton resonance and a ^3J correlation to the methine proton resonance of C-22. The acetate group was therefore placed at C-22. The H-22 resonance showed a NOESY correlation with H-23α, indicating that the

acetoxy group at C-22 was β. The remaining HMBC, NOESY and COSY data are shown in **Table 4.8.**

The infrared data **[spectrum 94]** supported the postulated structure. Peaks were observed at 3427 cm^{-1} (O-H stretching), 2917 cm^{-1} (aliphatic C-H stretching), 2852 cm^{-1} (aliphatic C-H stretching) and 1734 cm^{-1} (C=O stretching).

Finally a literature search for the compound was undertaken. It was found to be (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,22-dihydroxy-27-nor-lanost-8-en-24-one a compound previously isolated from *Veltheimia bracteata* (syn. *V. viridifolia)* and *Ledebouria cooperi.*[16,17] A comparison was made with the NMR data of the compounds from literature. However one was run in pyridine, the other in deuterated methanol and compound **X** in deuterated chloroform. In order to get a better comparison, compound **X,** was re-run in deuterated methanol as no deuterated pyridine was available **[spectrum 95]**, **Table 4.7** shows a comparison of ^{13}C NMR data compound **X** and literature.[16] The data compared quite favourable. Compound **X** was therefore deduced to be be (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,22-dihydroxy-27-nor-lanost-8-en-24-one.

Table 4.7: Comparison of ^{13}C NMR data for compound X with *(22R,23S)*-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one[16] (CD_3OD)

Carbon	^{13}C NMR data for Compound X (ppm)	^{13}C NMR Literature data[16] (ppm)
1	36.7	36.3
2	29.0	28.6
3	81.1	80.7
4	43.6	43.2
5	51.3	50.9
6	19.7	19.3
7	26.6	26.2
8	136.4	136
9	135.8	135.4
10	38.0	37.6
11	21.8	21.4
12	26.1	25.7
13	50.5	50.1
14	50.9	50.5
15	33.2	32.8
16	40.9	40.5
17	98.6	98.2
18	19.8	19.4
19	20.2	19.8
20	#	49.3
21	15.7	15.3
22	83.2	82.8
23	85.8	85.4
24	211.5	211.1
25	34.2	33.8
26	7.5	7.1
30	23.1	22.7
31	65.2	64.8
30	27.7	27.3
CH_3COO^-	20.9	20.5
CH_3COO^-	171.5	171.1

#-obscured by solvent signal

TABLE 4.8: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one ($CDCl_3$)

Carbon	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	H-1α-1.18 (m) H-1β-1.72 (m)	35.2	3H-19;H-2	H-1	H-3 3H-19;H-2
2	1.76 (m) (2H)	28.2		H-3;H-1α,β	H-1α
3	3.41 (m)	80.7	H-3	H-2	H-1α
4		42.7	H-30;H-5		
5	2.55 (m)	51.1	3H-19;3H-28	H-6	
6	H-6α-1.74 (m) H-6β-1.40 (m)	18.2	H-5		H-7;3H-28 3H-19
7	2.04 (m) (2H)	26.4	H-5;H-6		
8		134.8	H-30;H-7		
9		134.2	3H-19;H-1		
10		36.7	3H-19;H-5;H-1		
11	2.05 (m) (2H)	20.7		H-12α,β	3H-18
12	H-12α-2.16 (m) H-12-β-1.41 (m)	24.8	3H-18	H-11 H-11	3H-30;H-12β 3H-18;3H-21; H-12α
13		50.0	3H-30;H-12;H-16		
14		49.6	3H-18;3H-30;H-16;H-15		
15	H-15α-1.38 (m) H-15β-1.64 (m)	32.0	3H-30;H-20	H-16α,β H-16α,β	3H-18
16	H-16α-2.32 (m) H-16β-1.84 (m)	39.9	H-20;H-15	H-15α,β H-15α,β	H-20
17		97.4	3H-18;3H-21;H-22;H-23;CH_3COO		
18	0.95 (s)	19.2	H-12		H-20;H-21;H-16a;H-12β;H-11
19	0.99 (s)	19.8	H-5		H-29
20	2.38 (m)	49.1	3H-21	H-20	3H-18;H-22;3H-21;H-16β
21	1.07 (d,7.1)	15.3	H-20;H-22	3H-21	H-22;H-23;H-20;3H-18;H-12β
22	5.12 (d,5.1)	82.0	H-20;3H-21; H-23	H-23	H-23;3H-21;H-20
23	4.72 (d,5.1)	84.6	H-20	H-22	H-22;3H-21;H-25
24		210.0	3H-26;H-25;H-23		
25	2.50 (q,7.1)	33.3	3H-26	H-25	H-23;3H-26
26	1.04 (t,7.1)	7.1	H-25	3H-26	H-25;3H-18
28	1.25 (s)	22.2	H-5;H-6		H-3;H-6β
29	3.30 (d,11.0) 4.20 (d,11.2)	64.3	3H-28	H-29a H-29b	3H-19
30	1.30 (s)	25.8	H-15		
$\underline{C}H_3COO$	1.99 (s)	21.0			
$CH_3\underline{C}OO$		169.9	$C\underline{H}_3COO$;H-22		

4.2.6 Structural Elucidation of Compound XI, 5,7-dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*][1,3]benzodioxol]-4-one (scillascillin)

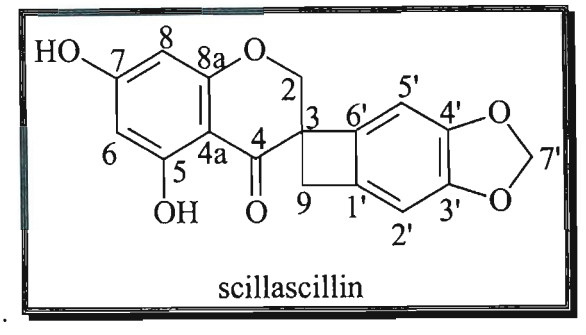

Figure 4.8: Structure of scillascillin

This compound **(Figure 4.8)** was isolated as an amorphous material.

The ^{1}H NMR spectrum **[spectrum 96]** showed the presence of two sets of doublets for the methylene protons at C-2 and C-9. The proton at C-3 is absent and this is typical of scillascillinoid-type compounds. The 2H-2 proton resonances occur downfield at δ 4.41 *(*d, 11.1 Hz) and 4.46 *(*d, 11.1 Hz) ppm because of the presence of an adjacent oxygen atom. The H-9 protons were found to occur at δ 2.92 *(*d, 13.5 Hz) and 3.42 *(*d, 13.5 Hz) ppm. The ^{1}H NMR spectrum also showed the presence of an extra methylene group. The methylene carbon was found to resonate at δ 100.2 ppm in the ^{13}C NMR **[spectrum 97]**. It was assigned to the carbon of a methylene dioxy group.

The presence of a pair of doublets at δ 6.52 and 6.64 (J=<1Hz) each integrating to one proton were indicative of protons *para* to each other and this implied a 1', 3', 4', 6'-tetra-substituted ring B. The HMBC spectrum **[spectrum 99]** showed a ^{3}J correlation from C-3' and C-4' to the proton resonance of the methylene dioxy group (2H-7'). This group was placed between C-3' and C-4'.

The substitution pattern in ring A was determined through UV and ^{1}H NMR spectra. One hydroxy group was assigned to the C-5 position due a bathochromic shift of 27 nm in the UV spectrum **[spectrum 104]** with $AlCl_3$.[12,13] This was confirmed by the downfield shift of the carbonyl group (δ 196.0 ppm) that becomes deshielded due to the chelating effects with the hydroxy group.[12,13] The second hydroxy group was placed at C-7 due to a bathochromic shift of 21 nm **[spectrum 103]** when NaOAc was added. [12,13] This assignment was confirmed by a coupling constant of 2.2 Hz, which was due to *meta* coupling between H-6 and H-8.

A literature search for the compound resulted in it being found to be scillascillin itself, previously isolated from *Scilla scilloides* and *Muscari neglectum*.[18,19] The ^{1}H (**Table 4.9**) and ^{13}C (**Table 4.10**) NMR data were compared to literature.[18] There were certain discrepancies and this was attributed to the fact that a mixture of solvents were used since scillascillin was only partial soluble in chloroform so methanol was also added, while the literature sample was run only in CD_3OD.

Table 4.9: Comparison of ^{1}H NMR data of compound XI ($CDCl_3$ + CD_3OD) with 5,7-dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*][1,3]benzodioxol]-4-one[18] (CD_3OD)

Proton	^{1}H NMR data for Compound XI (ppm)	^{1}H NMR Literature data[18] (ppm)
2-H	4.41(d, 11.1 Hz) 4.46(d, 11.1 Hz)	4.50† 4.53†
9-H	2.92 (d, 13.5 Hz) 3.42 (d,13.5 Hz)	2.97 3.45
6-H	5.90	5.90
8-H	5.90	5.92
2'-H	6.52	6.56
5'-H	6.64	6.73
O-CH_2-O	5.81	5.82

† Coupling constants not given in literature

Table 4.10: Comparison of ^{13}C NMR data of compound XI (CD_3OD+ $CDCl_3$) with 5,7-dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*] [1,3]benzodioxol]-4-one[18] (CD_3OD)

Carbon	^1H NMR data for Compound XI (ppm)	^1H NMR Literature data[18] (ppm)
2	73.3	75.7
3	53.2	56.6
4	196.0	197.7
4a	101.4	102.5
5	163.2	165.9
6	96.8	97.4
7	166.6	168.7
8	95.3	96.1
8a	164.1	165.0
9	35.0	35.7
1'	135.3	136.2
2'	103.9	106.5
3'	148.3	149.6
4'	147.0	148.9
5'	105.7	104.7
6'	134.1	137.4
O-CH_2-O	100.2	101.2

4.2.7 Structural Elucidation of Compound XII, (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (15-deoxoeucosterol)

This compound was isolated as a yellow gum. It was also found to be a derivative of eucosterol-type nortriterpenoids.

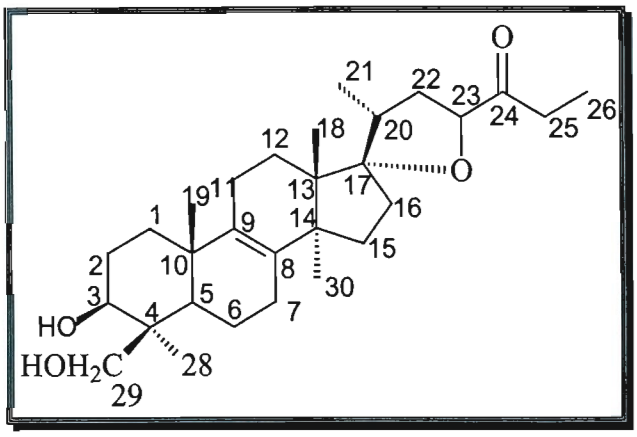

Figure 4.9: Structure of 15-deoxoeucosterol

This compound **(Figure 4.9)** showed the same number of methyl group protons as eucosterol (six methyl groups). The C-O region of the ^{13}C NMR spectrum **[spectrum 106]** showed the methylene carbons C-29 (δ 64.2 ppm) and methine carbon at C-3 (δ 80.7 ppm) and the fully substituted C-17 and C-21. All of this was similar to eucosterol. However, the ^{13}C NMR spectrum only showed the presence of one carbonyl group. This carbonyl group was assigned to C-24. The HMBC **[spectrum 108]** showed a ^{3}J correlation from the carbonyl group to the triplet methyl resonance protons of 3H-26. This confirmed that the carbonyl group was at C-24.

Finally a literature search of the compound was undertaken. The compound was found to be (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one a compound previously isolated from *Scilla scilloides*.[20] In order to confirm the structure, the carbon data was compared with that of literature. The reference compound, however, was run in pyridine while compound **XII** was run in deuterated chloroform. However an attempt to get a ^{13}C NMR spectrum of compound **XII** in

pyridine failed as the compound had decomposed. **Table 4.11** shows the carbon data in different solvents and **Table 4.12** shows the **HMBC**, **COSY** and **NOESY** data.

Table 4.11: Comparison of ^{13}C data of Compound XII ($CDCl_3$) with (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one[20] (C_5D_5N)

Carbon	^{13}CNMR data for Compound XII (ppm)	^{13}C NMR Literature data[20] (ppm)
1	35.3	35.7
2	28.2	28.9
3	80.7	79.8
4	42.8	43.1
5	51.1	51.5
6	18.2	18.9
7	26.4	26.8
8	134.0	134.4
9	135.1	134.9
10	36.8	37.0
11	20.7	21
12	24.8	25.2
13	48.6	48.7
14	50.5	50.7
15	31.7	31.9
16	39.6	39.6
17	97.0	96.8
18	19.1	19.2
19	19.9	20.0
20	43.5	43.6
21	17.1	17.2
22	36.7	36.8
23	81.5	81.4
24	213.6	211.8
25	32.2	32.2
26	7.4	7.7
27	22.7	23.3
28	64.4	64.2
29	25.8	26.2

Table 4.12: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one ($CDCl_3$)

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H†	COSY	NOESY
1	1.17 (m) (2H)	35.3	3H-19	H-2	3H-19
2	1.75 (m) (2H)	28.2		H-3;H-29	H-19;H-29
3	3.45 (m)	80.7	H-1	H-3	3H-28
4		42.8	3H-28		
5	1.15	51.1	3H-19;3H-28	H-6α,β	
6	H-6α-1.73 (m) H-6β-1.36 (m)	18.2		H-5;H-7 H-5;H-7	H-28 H-7;3H-19
7	2.00 (m) (2H)	26.4			H-6β;3H-19
8		134.0	3H-30		
9		135.1	3H-19		
10		36.8	3H-19;H-5		
11	1.99 (m) (2H)	22.7		H-12	3H-19;H-12
12	1.98 (m) (2H)	24.8	3H-18	H-11	3H-18;H-11
13		48.6	3H-18;3H-30		
14		50.5	3H-18;3H-30		
15	H-15α-1.34 (m) H-15β-1.60 (m)	31.7	3H-18;3H-30	H-16α,β;H-15β H-16α,β;H-15α	3H-30 3H-18
16	H-16α-1.98 (m) H-16β-1.62 (m)	39.6	3H-18	H-15α,β; H-15α,β	3H-18;H-20
17		97.0	3H-18;3H-21		
18	0.87 (s)	19.1	H-12		H-20;H-16β;H-15β;H-12β; 3H-21
19	0.92 (s)	19.9	H-5		H-29b;H-11;H-6β
20	2.15	43.5	3H-21	3H-21;H-22α,β	H-16β;3H-21;3H-18;H-22β;
21*	1.04	17.1	H-22	H-20	H-23;H-20;H-22α; 3H-18
22*	H-22α-1.81 (m) H-22β-1.92 (m)	36.7	3H-21	H-23 H-23	H-23;H-22α;3H-21 H-20
23	4.51 (q,7.3)	81.5	H-20	H-22α,β	H-22α;H-25;3H-26
24		213.6	3H-26;H-25;H-22		
25	2.54 (q,7.3)	32.2	3H-26	3H-26	3H-26
26*	1.06	7.4	H-25	H-25	H-25
28	1.21	25.8			H-3;H-6α
29	H-29a-3.32 (d,10.8) H-29b-4,22 (d,11.2)	64.4	H-28	H-29b H-29a	H-29b 3H-19;H-29a
30	1.23	22.2	H-15		H-15α

*- coupling constant could not be determined because of poor reolution and it being superimposed

† The HMBC correlations have been tentatively assigned because no expansion could be obtained-problems with NMR spectrometer

4.2.8 Structural Elucidation of Compound XIII, (22*R*,23*S*)-17α,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione

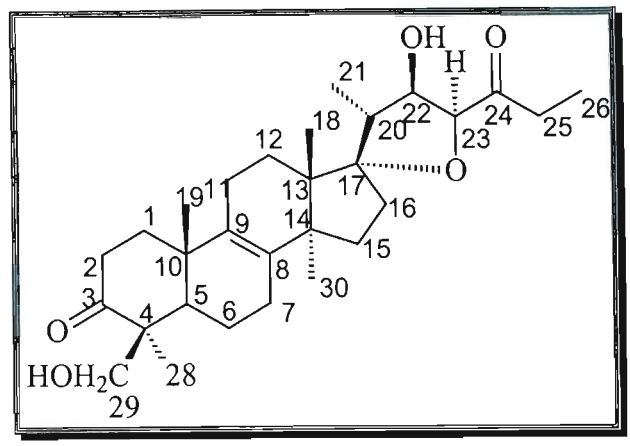

Figure 4.10: Structure of natalensis C

Natalensis C was isolated as white crystals with a melting point between 88-90°C. It was found to be a derivative of eucosterol type nortriterpenoids. The mass spectrum **[spectrum 111]** showed a molecular ion peak at *m/z* 472.31953 g.mol^{-1}, which corresponded to a molecular formula of $C_{29}H_{44}O_5$.

The ^{13}C NMR spectrum **[spectrum 112]** showed the presence of two carbonyl groups. The carbonyl carbon resonance at δ 210.4 ppm had a ^3J correlation, in the HMBC spectrum **[spectrum 114],** with the methyl group triplet resonance of 3H-26 indicating it was at C-24. The second carbonyl carbon resonance at δ 214.5 ppm had ^3J correlations with the methyl group proton resonance of 3H-28 and with the oxygenated methylene proton resonance of 2H-29, indicating the second carbonyl resonance was at C-3. The C-O region of the ^{13}C NMR spectrum showed the presence of an extra methine proton (δ 79.3 ppm). The HMBC spectrum showed ^3J correlations from C-17 and C-21 to the oxygenated methine proton resonance indicating the resonance was at C-22. This assignment was confirmed through the COSY **[spectrum 115]** correlation with the H-23 resonance, indicating it to be at C-22. A NOESY **[spectrum 116]** correlation occurred between the resonance of H-23α and the

oxygenated methine proton resonance, indicating the proton to be α and the hydroxy group to be β.

A literature search for the compound was done and it was found to be novel. It was named natalensis C. **Table 4.13** shows the HMBC, COSY and NOESY data.

Table 4.13: ^{1}H, ^{13}C, HMBC, COSY and NOESY data (22*R*,23*S*)-17α,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione

	^{1}H (ppm)	^{13}C (ppm)	HMBC→H	COSY	NOESY
1	1.93 (m) (2H)	20.7			3H-19
2	1.32 (m)(2H)	36.6			
3		214.5	H-28;29		
4		54.4	H-28;H-29		
5	1.50	52.9			
6	H-6α-1.63 (m) H-6β-1.53 (m)	19.1			3H-28 3H-19
7	1.98 (m) (2H)	26.0			
8		#	3H-30		
9		133.6	3H-19		
10		36.8	3H-19		
11	H-11α-2.78 (m) H-11β-2.21 (m)	35.2		H-12α,β	3H-19
12	H-12α-1.36 (m) H-12β-2.30 (m)	24.8	3H-18	H-11α H-11α	3H-18;3H-21
13		49.6	3H-18		
14		49.3	3H-30		
15	H-15α-1.34 (m) H-15β-1.59 (m)	32.0	3H-30	H-15β H-15α	3H-18;
16	H-16α-2.21(m) H-16β-2.23(m)	33.0	3H-21	H-16β H-16α	H-16β H-16α;3H-21
17		96.7	3H-18;3H-21;H-22		
18	0.84 (s)	18.9			H-15β;3H-21;H-20
19	1.13 (s)	19.2			H-6β;H-29;H-11β
20	2.35 (m)	51.6	3H-21	3H-20	3H-21;H-22;3H-18
21*	0.94	15.6	H-22	H-20	3H-21;H-22;3H-18
22	4.42	79.3	3H-21;H-20;H-23	H-23	H-23,3H-21
23	4.74 (d)	87.2	H-20	H-22	H-22
24		210.4	3H-26		
25	2.51 (d,7.3) 2.61 (d,7.5)	33.0	3H-26	H-26	3H-26
26*	0.96	7.1	H-25	H-25	H-25
28	1.27	19.9	H-29		3H-19;H-29;H-6α
29	3.63 (d,11.0) 4.24 (d,11.0)	64.4	3H-28	H-29a,b	
H-30	1.40	26.0			

#- undersolvent

*- peaks obscured, *J* could not be determined

4.2.9 Compounds re-isolated from *Scilla natalensis*

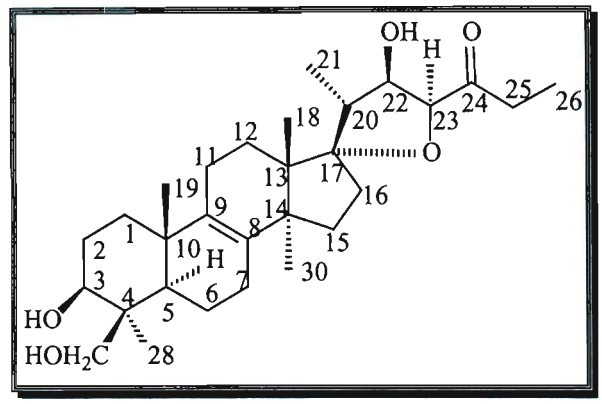

Figure 4.11: Structure of Compound III

Compound III which was isolated from *Ledebouria zebrina* was re-isolated from *Scilla natalensis*.

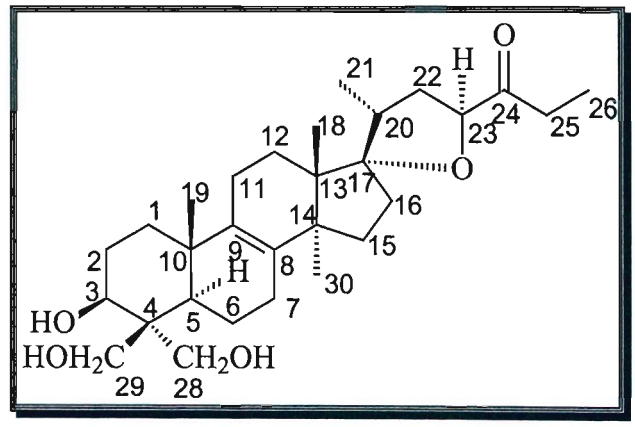

Figure 4.12: Structure of Compound V

Compound **V** which was isolated from *Ledebouria zebrina* was re-isolated from *Scilla natalensis*

4.3 Experimental

Dr Neil Crouch of the National Botanical Institute collected the plant material from a swamp in the Blyde Nature Reserve and a voucher specimen was retained at the Natal Herbarium (*N. Crouch 855 NH*). The part of the plant investigated was the bulbs. The bulbs (3.08 kg) were chopped into small pieces, air dried for approximately 48 hours and then extracted successively with dichloromethane (mass of extract 10.65 g) and methanol (mass of extract 150.6 g) by agitation on a Labcon mechanical shaker at 140 rpm. Extraction with each solvent was carried out for approximately 72 hours. The extracts obtained were then filtered and the solvent was removed using a BUCHI rotavapor. The dichloromethane extract has only been worked on to date. General chromatographic techniques were employed (**Section 3.4**) and this led to the isolation of ten compounds: compounds, (22*R*,23*S*)-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one **(III)** (20 mg), (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one **(V)** (12 mg), 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)-4-chromanone **(VI)** (12 mg), 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)-4-chromanone **(VII)** (10 mg), (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene **(VIII)** (20 mg), (22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene **(IX)** (30 mg), *(*22*R*,23*S)*-22-acetoxy-17α,23-epoxy-3β,22,29-trihydroxy-27-nor-lanost-8-en-24-one **(X)** (15 mg), with 5,7-dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*][1,3]benzodioxol]-4-one **(XI)** (17 mg), (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one **(XII)** (15 mg), (22*R*,23*S*)-17α,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione **(XIII)** (30 mg).

4.3.1 Physical data for Compound VI

Name: **5,7 dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone**

Yield: 12 mg

Physical Description: orange gum

Optical rotation: sample was too dilute

Mass **[spectrum 57]**: $[M^+]$ at *m/z* 316, $C_{17}H_{16}O_6$ requires 316.094688 $g.mol^{-1}$

EIMS: *m/z* 316

m/z 209

m/z 183

m/z 182

m/z 107

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 61]**: 3375 (O-H- stretching), 2924 (C-H stretching), 2855 (C-H stretching), 1634 (C=O stretching), 1506 & 1450 (C=C stretching)

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum 58]**: 291.0 (5.6)

214.0 (5.8)

with $AlCl_3$ **[spectrum 60]**: 316.0

with NaOAc **[spectrum 59]**: 331.0

^1H NMR: δ_H (ppm) CD_3OD **[spectrum 55]**: **Table 4.1**

^{13}C NMR: δ_c (ppm) CD_3OD **[spectrum 56]**: **Table 4.2**

4.3.2 Physical data of Compound VII

Name: **5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone**

Yield: 10 mg

Physical Description: yellow gum

Optical rotation: sample was too dilute

Mass **[spectrum 64]**: $[M^+]$ at *m/z* 346, $C_{18}H_{18}O_7$ requires 346.105253 $g.mol^{-1}$

EIMS: *m/z* 346

m/z 209

m/z 137

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 68]**: 2926 (C-H stretching), 2856 (C-H stretching), 1733 (C=O stretching) and 1474 (C=C stretching)

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum 65]**: 291.0 (5.6)

205.0(5.8)

with $AlCl_3$ **[spectrum 66]**: 315.0

with NaOAc **[spectrum 67]**: 331.0

^1H NMR: δ_H (ppm) CD_3OD **[spectrum 62]**: **Table 4.3**

^{13}C NMR: δ_c (ppm) CD_3OD **[spectrum 63]**: **Table 4.4**

4.3.3 Physical data for Compound VIII

Name: **(23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene (natalensis A)**

Physical Description: yellow gum

Yield: 20 mg

Optical Rotation: $[\alpha]_D$: +20.4° (c=0.054 g/100ml)

Mass **[spectrum 76]**: $[M^+]$ at *m/z* 416.32916, $C_{27}H_{44}O_3$ requires 416.329046 $g.mol^{-1}$

EIMS: *m/z* 416.32916 (6%)
m/z 401.29875 (6%)
m/z 357.2722 (6%)
m/z 247.20661 (60%)
m/z 169.11988 (100%)
m/z 57.08823 (42%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 77]**: 3396 (O-H stretching), 2928 (C-H stretching) and 2864 (C-H stretching)

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 69]**: **Table 4.5**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 70]**: **Table 4.5**

4.3.4 Physical data for Compound IX

Name: **(22*R*,23*S*)-17α, 23-epoxy-3β, 22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene (natalenis B)**

Physical Description: yellowish-white gum

Yield: 30 mg

Optical Rotation: $[\alpha]_D$: +8.1° (c=0.08g/100 ml)

Mass **[spectrum 85]**: $[M^+]$ at *m/z* 446, $C_{28}H_{46}O_5$ requires 446.339610 $g.mol^{-1}$

EIMS: *m/z* 446.33844(5%)

m/z 431.31547(8%)

m/z 261.22223 (100%)

m/z 57.03376(12%)

Infrared: ν_{max}^{NaCl} cm^{-1} **[spectrum 86]**: 3393 (O-H stretching), 2924 (C-H stretching) and 2853 (C-H stretching)

^1H NMR: δ_H (ppm) C_5D_5N **[spectrum 78]**: **Table 4.6**

^{13}C NMR: δ_c (ppm) C_5D_5N **[spectrum 79]**: **Table 4.6**

4.3.5 Physical data for Compound X

Name: **(22*R*,23*S*)-22-acetoxy-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (22β-acetoxy-15-deoxoeucosterol)**

Physical Description: white crystalline solid

Yield: 15 mg

Melting Point: 180°C (Literature: 176°C)[17]

Optical Rotation: $[\alpha]_D$: -15° (c=0.09) (Literature: $[\alpha]_D$-17.0°)[17]

Mass **[spectrum 93]**: $[M^+]$ not seen, $[M\text{-}CH_3COOH]^+$ at 456 $g.mol^{-1}$

EIMS: *m/z* 456
m/z 315
m/z 289
m/z 159
m/z 119

Infrared: ν_{max}^{KBr} cm^{-1} **[spectrum 94]**: 3427 (O-H stretching), 2917 (C-H stretching) 2852 (C-H stretching) and 1734 (C=O stretching)

^1H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 87]**: **Table 4.7**

^{13}C NMR: δ_c (ppm) CD_3OD **[spectrum 88]**: **Table 4.8**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 95]**: **Table 4.7**

4.3.6 Physical data of Compound XI

Name: **5,7-Dihydroxyspiro[2*H*-1-benzopyran-3-(4*H*),5'(6'*H*)-cyclobuta[*f*][1,3] benzodioxol]-4-one' (scillascillin)**

Yield: 17 mg

Physical Description: orange gum

Optical rotation: Vial brokewith sample in it. The amount recovered was too little to get optical rotation

Mass: [M^+]: Vial broke with sample in it. The amount recovered was too little too get mass spectrum

Infrared: ν_{max}^{NaCl} **:** Vial broke with sample in it. The amount recovered was too little to get an IR spectrum

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε) **[spectrum102]**: 289.0 (5.5)
206.0 (5.8)
with $AlCl_3$ **[spectrum 104]**: 311.0
with NaOAc **[spectrum103]**: 328.0

^{1}H NMR: δ_H (ppm) CD_3OD+ $CDCl_3$ **[spectrum 96]**: **Table 4.9**

^{13}C NMR: δ_c (ppm) CD_3OD+ $CDCl_3$ **[spectrum 97]**: **Table 4.10**

4.3.7 Physical data for Compound XII

Name: **(23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-nor-lanost-8-en-24-one (15-deoxoeucosterol)**

Yield: 15 mg

Physical Description: yellow gum

Optical Rotation: Sample decomposed

Mass: [M^+] Sample decomposed

Infrared: Sample decomposed

^{1}H NMR: δ_H (ppm) $CDCl_3$ **[spectrum 105]**: **Table 4.12**

^{13}C NMR: δ_c (ppm) $CDCl_3$ **[spectrum 106]**: **Table 4.11, 4.12**

4.3.8 Physical data for Compound XIII

Name: **(22*R*,23*S*)-17α,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione (natalensis C)**

Physical Description: white crystals

Melting Point: 88-91°C

Yield: 30 mg

Optical Rotation: Waiting for sample to return from HRMS analysis

Mass **[spectrum 117]**: $[M^+]$ at *m/z* 472.31953, $C_{29}H_{44}O_5$ requires 472.318875 $g.mol^{-1}$

EIMS: *m/z* 472.31953(1%)
m/z 457.29733(4.5%.)
m/z 442.30776(9.7%)
m/z 257.19093(100%)
m/z 57.03431(62.8%)

Infrared: ν_{max}^{KBr} cm^{-1} **[spectrum 118]**: 3439 (O-H stretching), 2949 (C-H stretching) and 1701 (C=O stretching)

^1H NMR: δ_H (ppm) CD_3OD **[spectrum 111]**: **Table 4.13**

^{13}C NMR: δ_c (ppm) CD_3OD **[spectrum 112]**: **Table 4.12**

4.4 References

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Chapter 5: Conclusion

5.1 Discussion

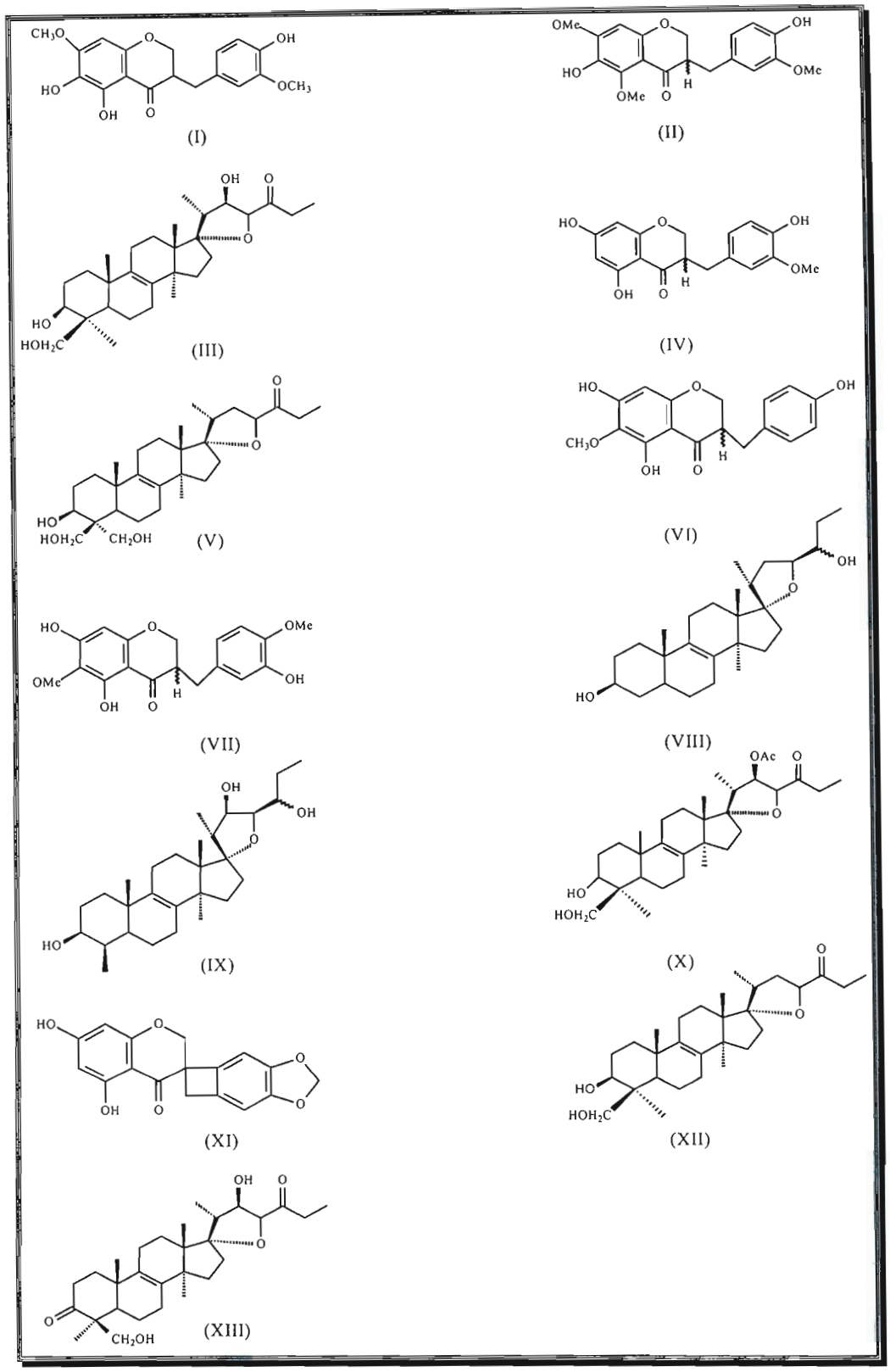
The findings contained in this work have proven to be quite interesting. The investigation of members of the Hyacinthaceae family has provided two types of compounds, homoisoflavanones and eucosterol type nortriterpenoids. The isolation of homoisoflavanones was not surprising given the well-documented occurrence of these compounds in this family. However, the different eucosterol type compounds isolated were quite interesting in that previously only a small number (ten) of these eucosterol type nortritepenoids have been reported to occur in the Hyacinthaceae and of those reported seven contain a pentasaccharide attached to C-3. In this study seven eucosterol type compounds were isolated of which three were novel and two were reported to occur for the first time in the Hyacinthaceae.

Ledebouria zebrina yielded three homoisoflavanones of the 3-benzyl-4-chromanone type and two eucosterol type nortriterpenoids. All three of the homoisoflavanones were novel, while one of the eucosterol type compounds was reported to occur in the Hyacinthaceae for the first time. *Scilla natalensis* yielded ten compounds of which two homoisoflavanones were of the 3-benzyl-4-chromanone type and one was a scillascillin-type. Seven eucosterol type nortriterpenoids were isolated of which three were novel and one was reported to occur in the Hyacinthaceae. The structures of the various compounds isolated are shown in **Scheme 5.1.**

The initial antifungal activity of the eucosterol type nortriterpenoid is quite interesting. No previous biological activity for these types of compounds has been reported. Further tests of these eucosterol type compounds are to be carried out in the future by collaborating laboratories

Future work will be to investigate the methanol extract of *Scilla natalensis*.

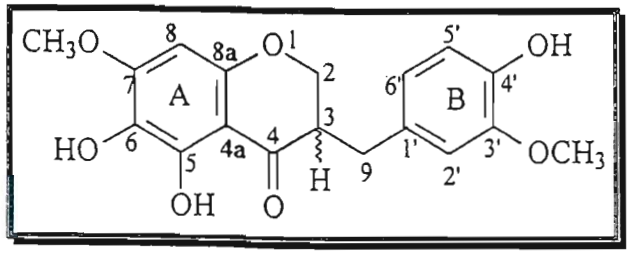
Finally the field of natural product research is proving to be a dynamic one. There is a constant need to find new drugs for the diseases that plague man. Southern Africa has well over 25 000 species of higher plants and the Cape Floral Kingdom has nearly 9 000 species making it the most diverse temperate flora on earth. With South Africa's remarkable biodiversity and cultural diversity, it is not surprising to find that approximately 4 000 species of plants are used as medicines, and of these, some 350 species are commonly used and traded.

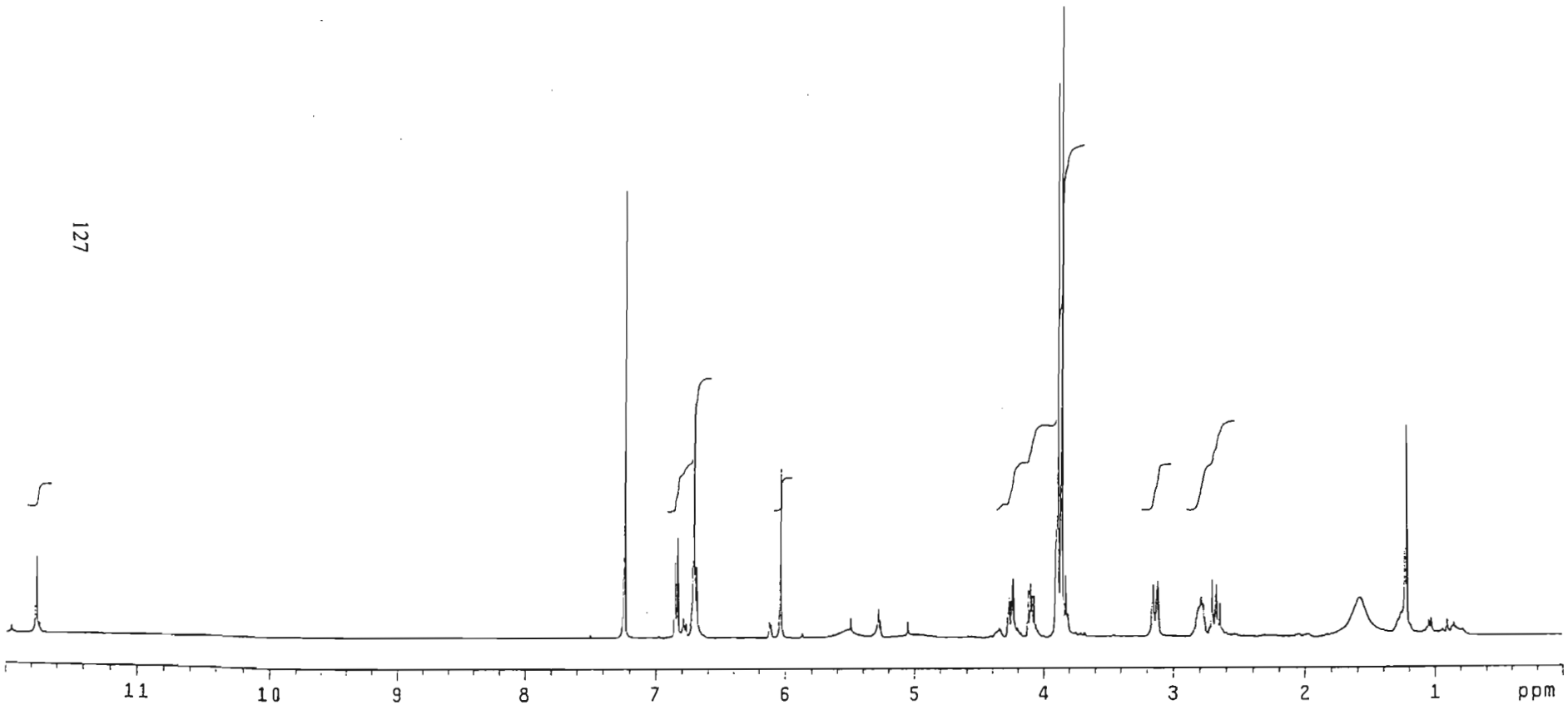


Scheme 5. 1 Compound isolated from *Ledebouria zebrina*(I-V) and *Scilla natalensis* (III,V-XIII)

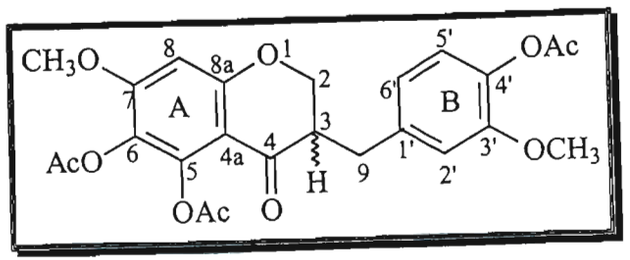
Appendix A

List of Spectra

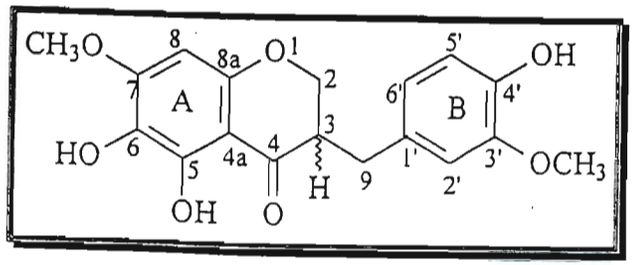




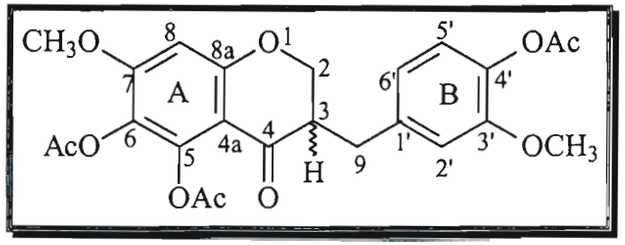
Spectrum 1: ^{1}H NMR spectrum of Compound (I) ($CDCl_3$)



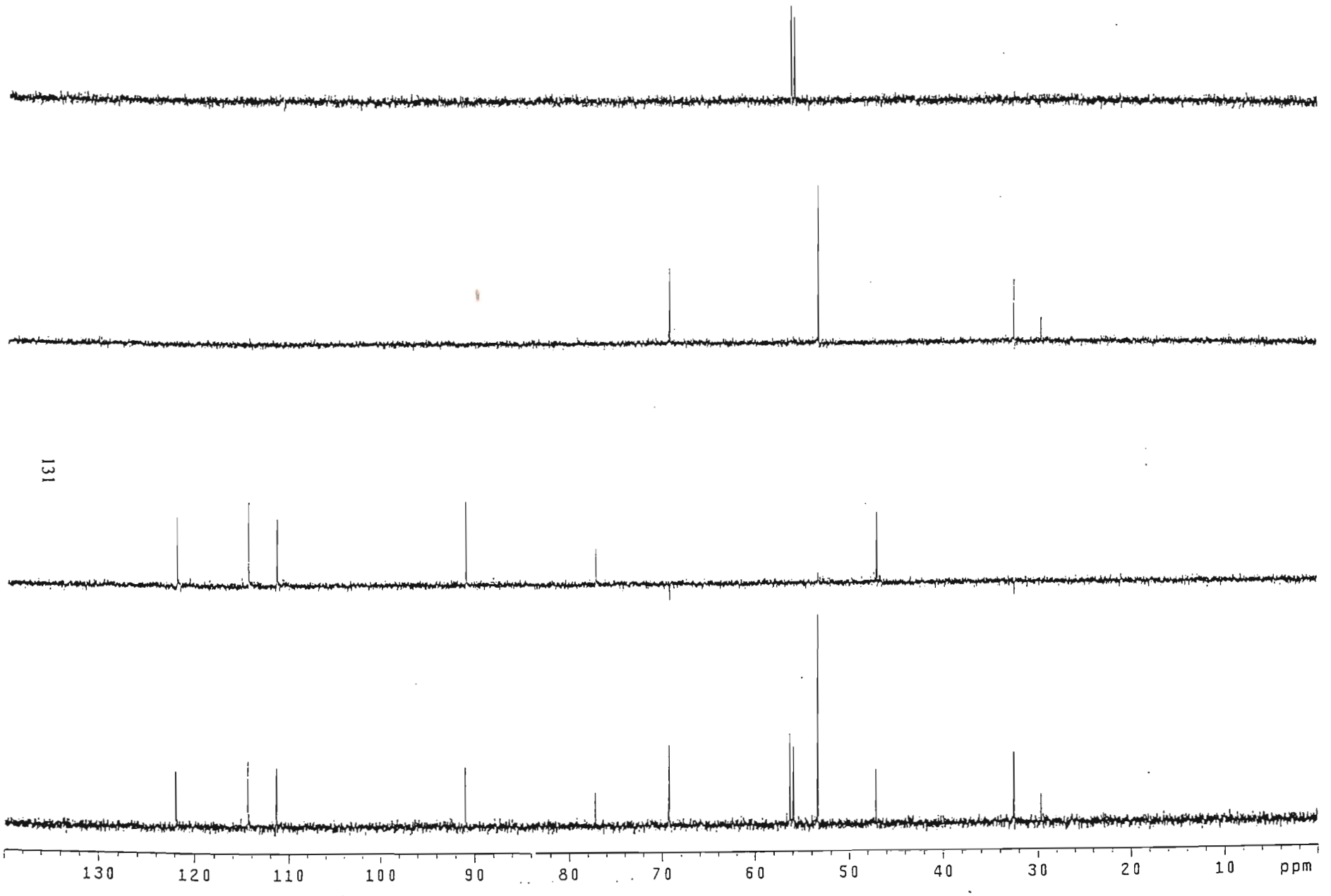
Spectrum 1a: ^{1}H NMR spectrum of Compound (I) acetate ($CDCl_3$)



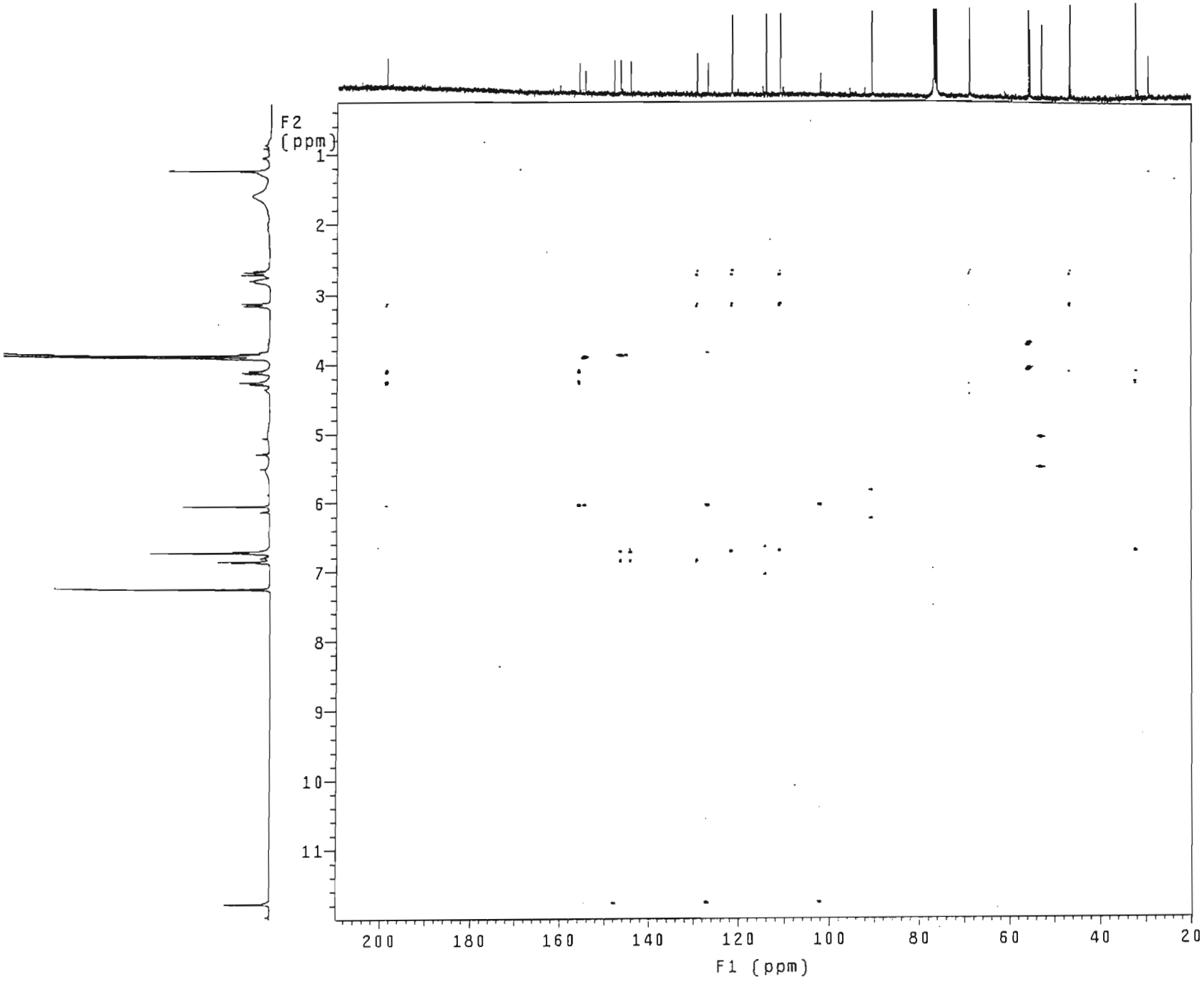
Spectrum 2: ^{13}C NMR spectrum of Compound (I) ($CDCl_3$)



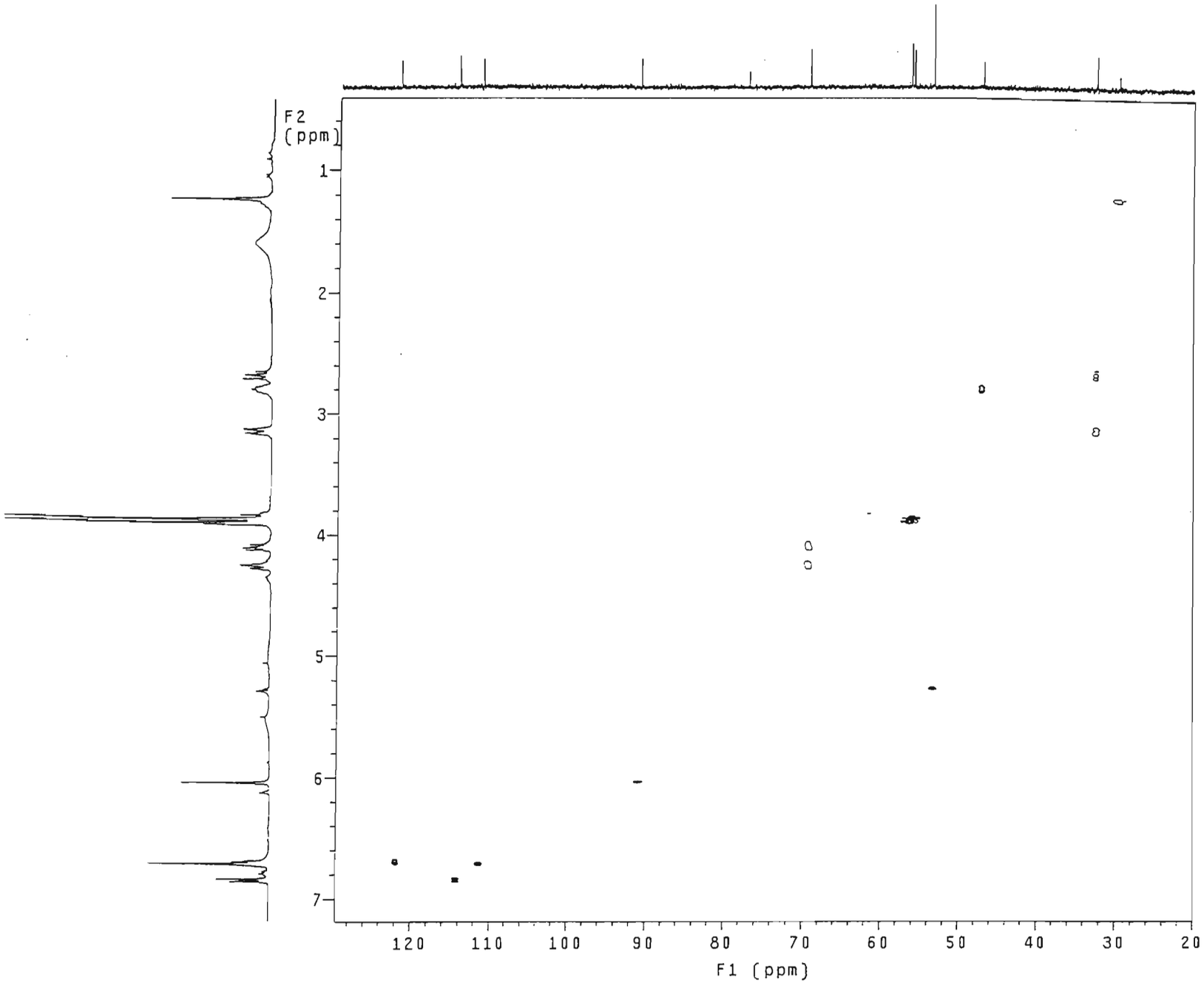
Spectrum 2a: ^{13}C NMR spectrum of Compound (I) acetate ($CDCl_3$)



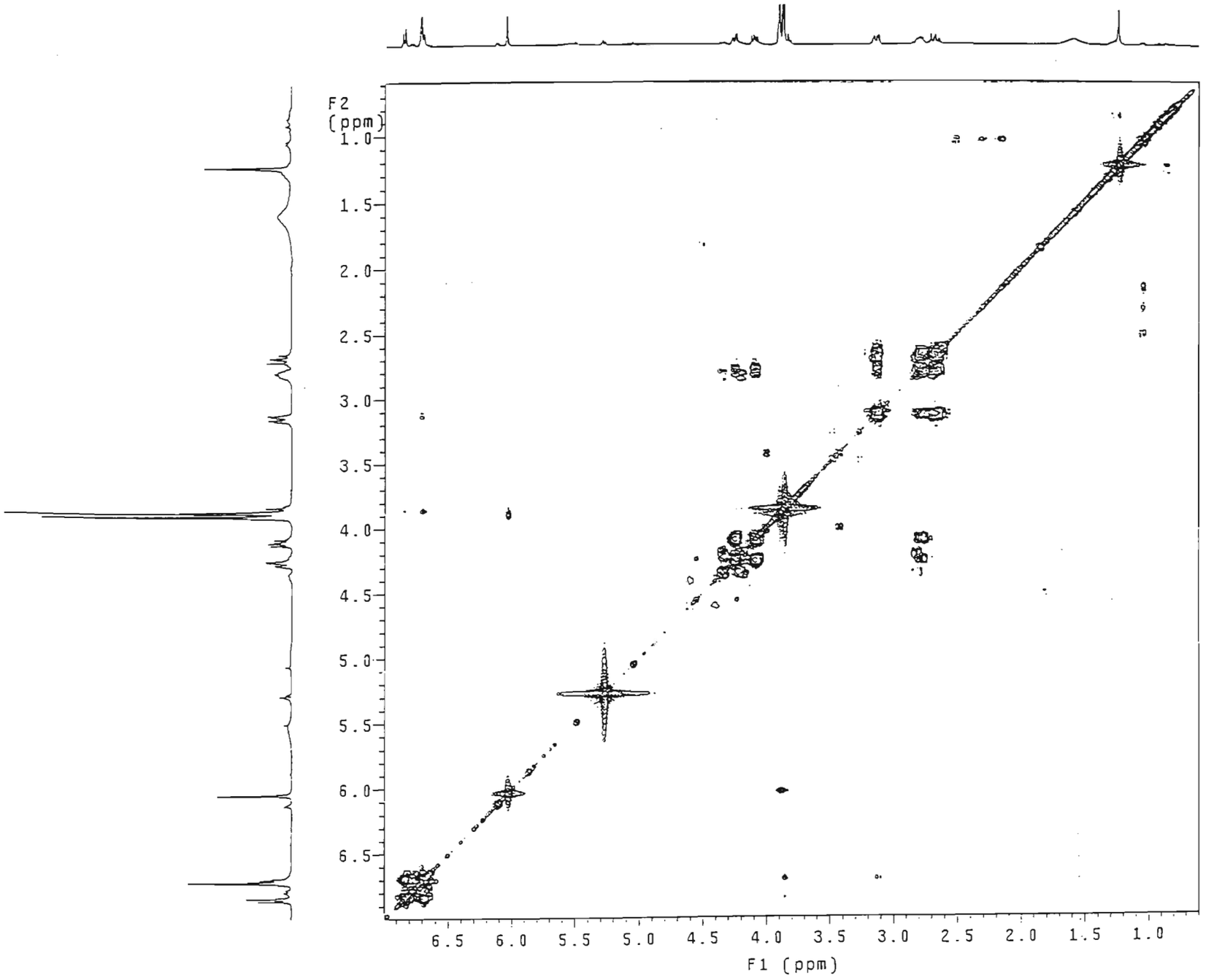
Spectrum 3: ADEPT spectrum of Compound (I) ($CDCl_3$)



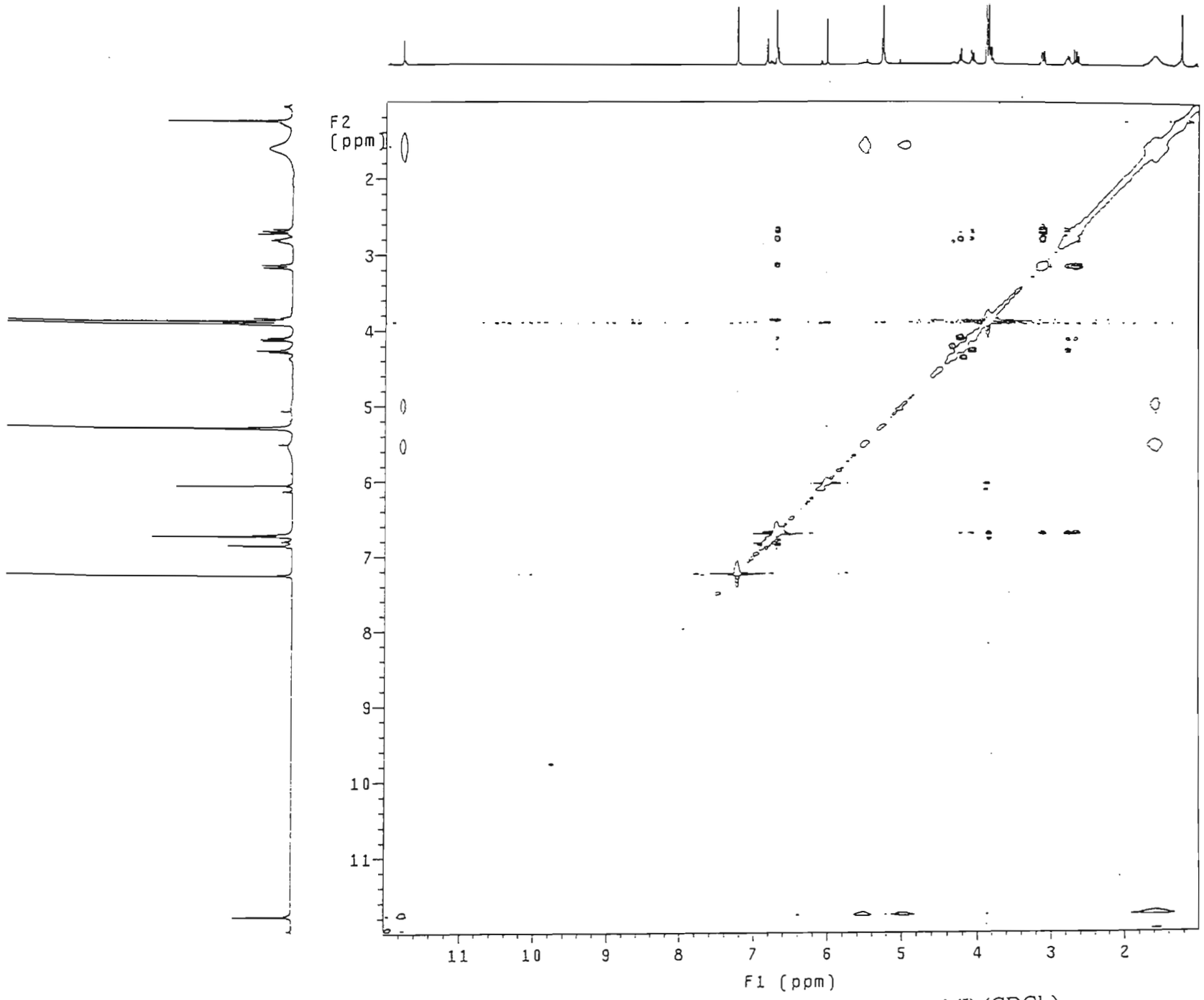
Spectrum 5: HMBC spectrum of Compound (I) ($CDCl_3$)



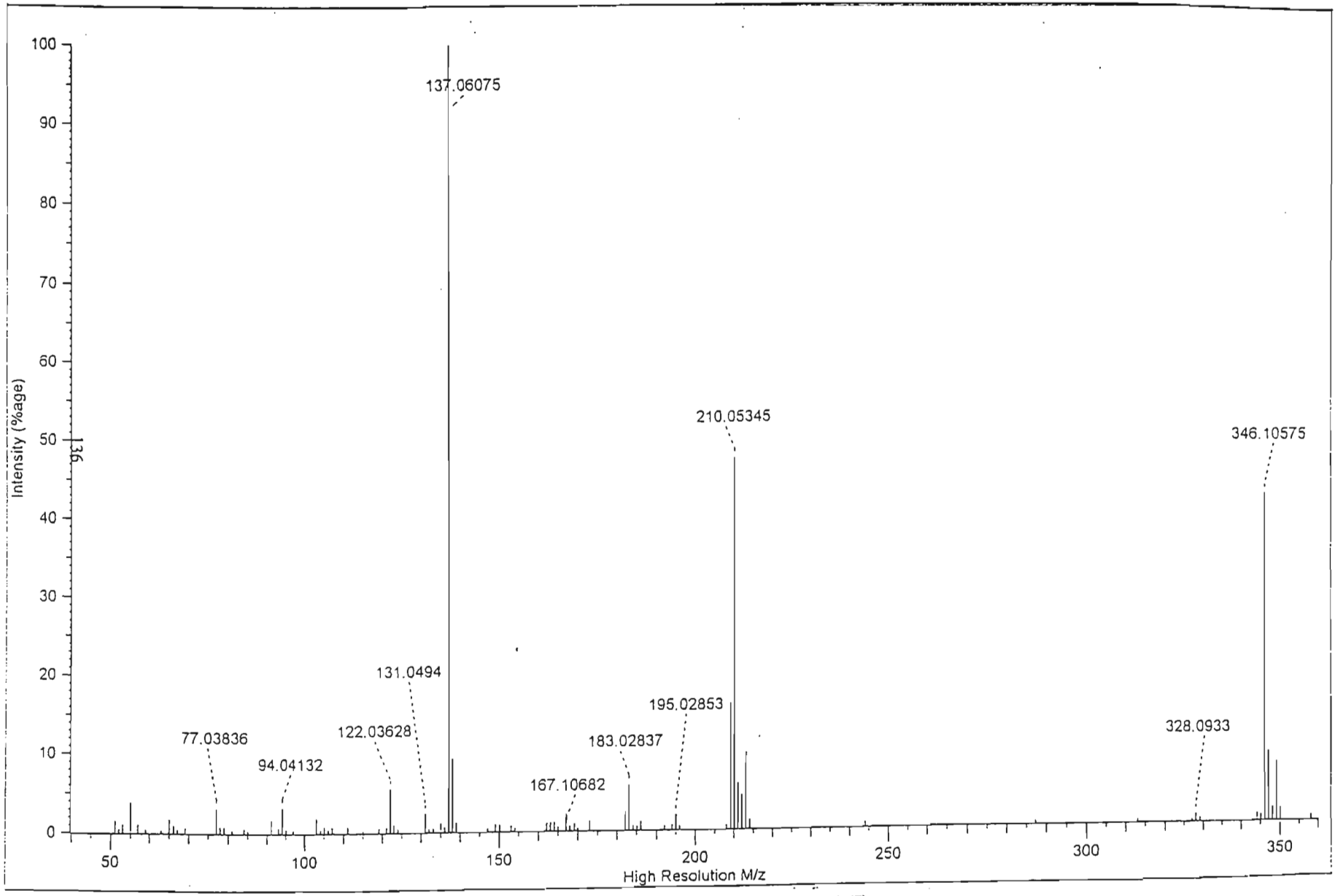
Spectrum 4: HSQC spectrum of Compound (I) ($CDCl_3$)



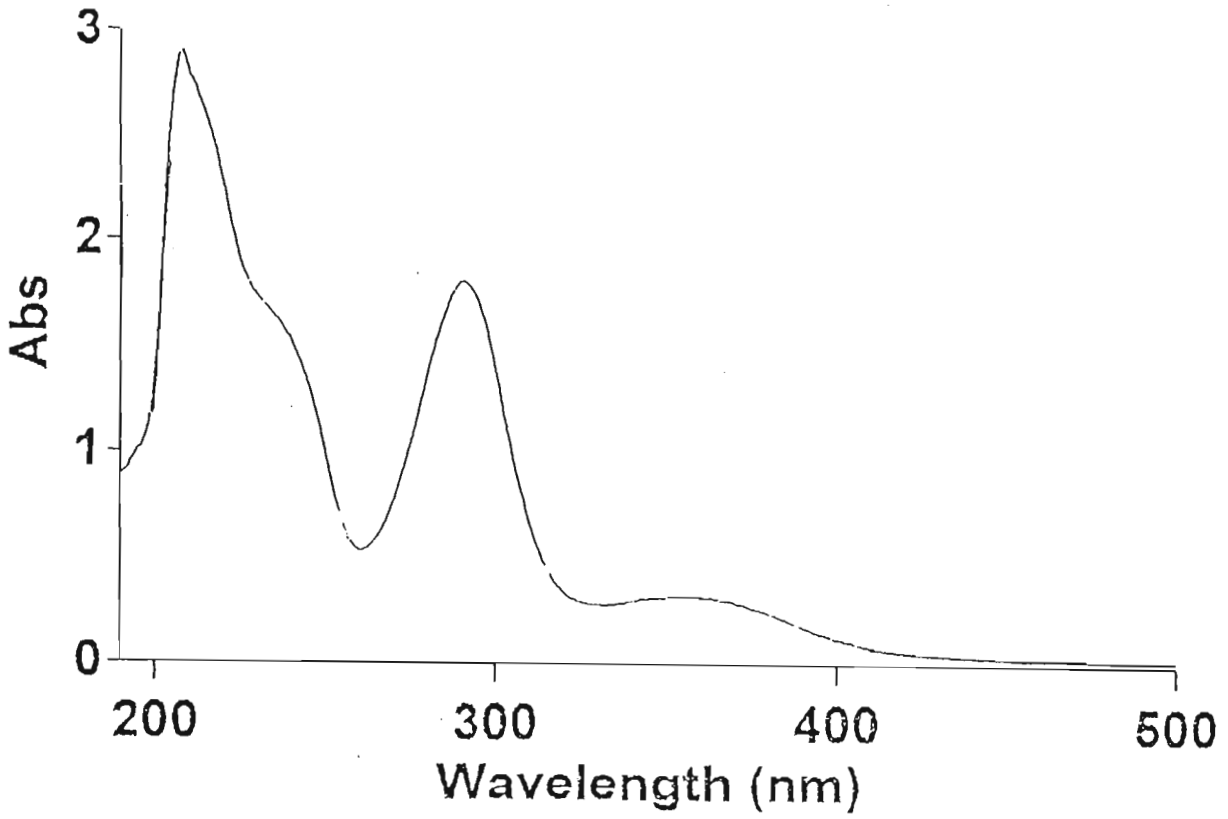
Spectrum 6: COSY spectrum of Compound (I) ($CDCl_3$)



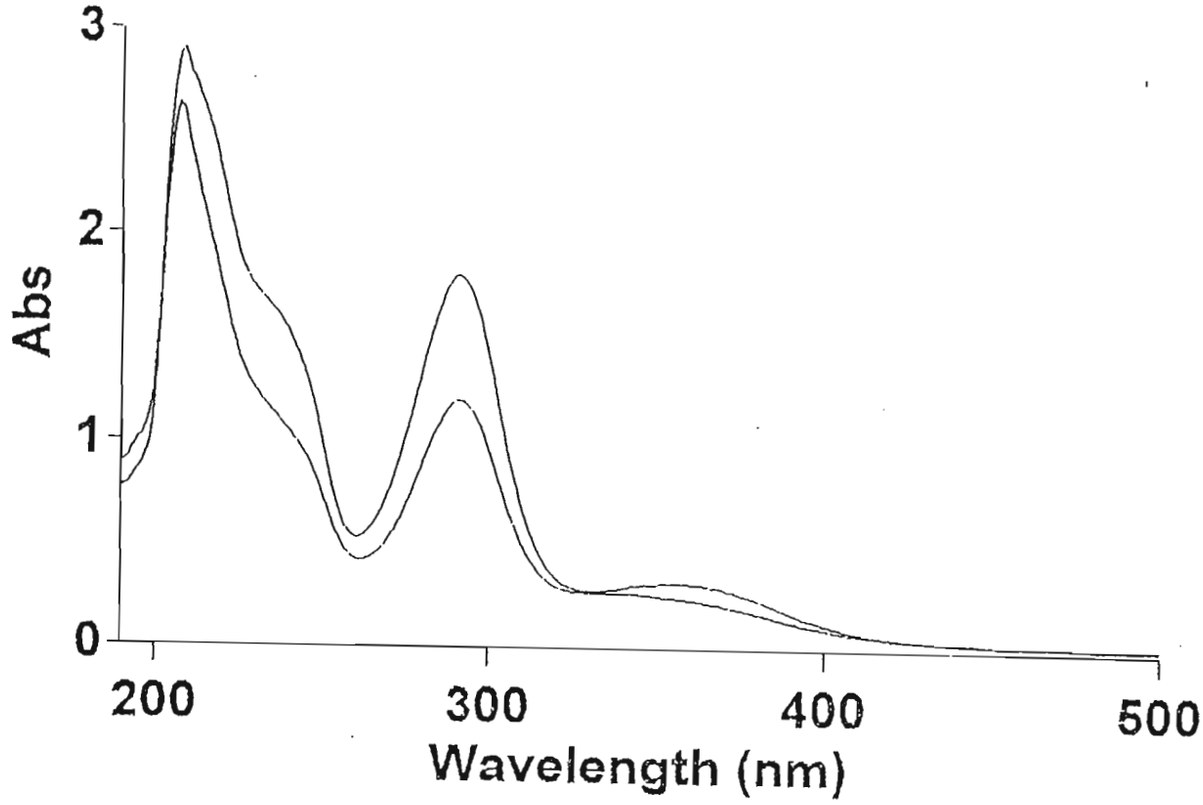
Spectrum 7: NOESY spectrum of Compound (I) ($CDCl_3$)



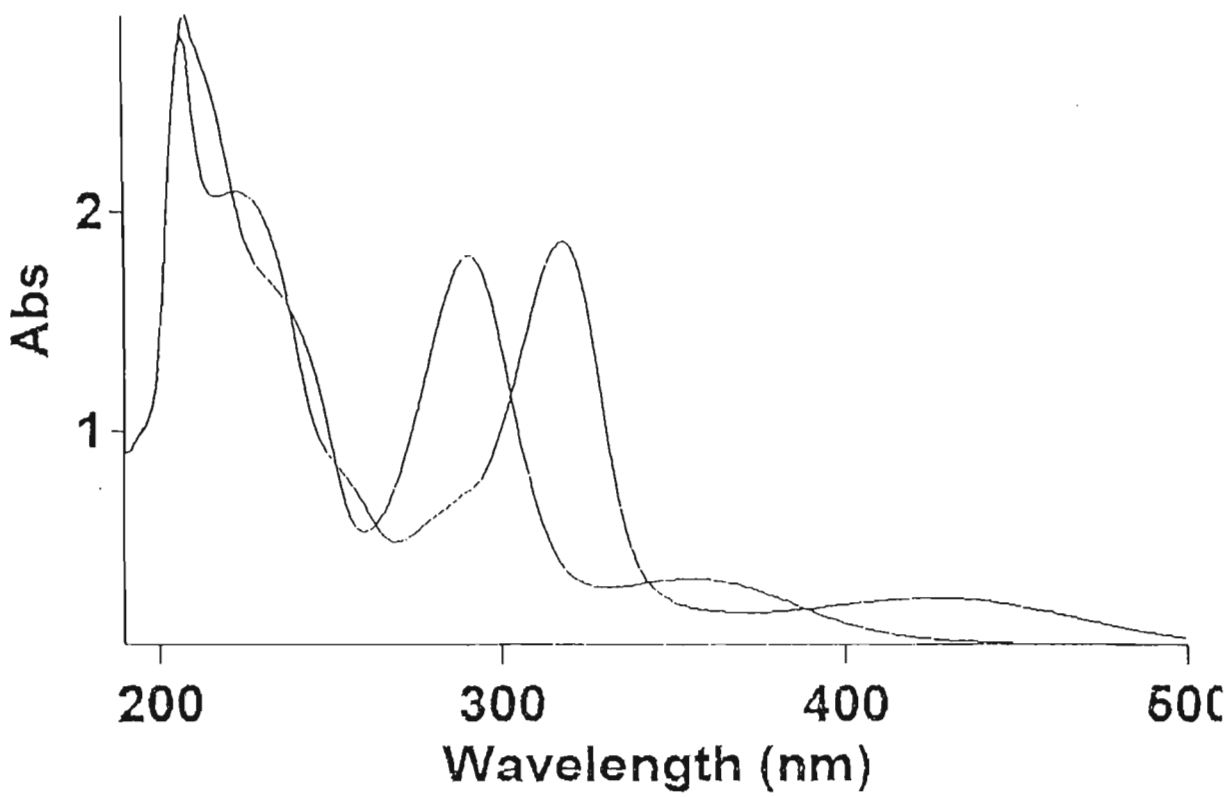
Spectrum 8: Mass spectrum of Compound (I)



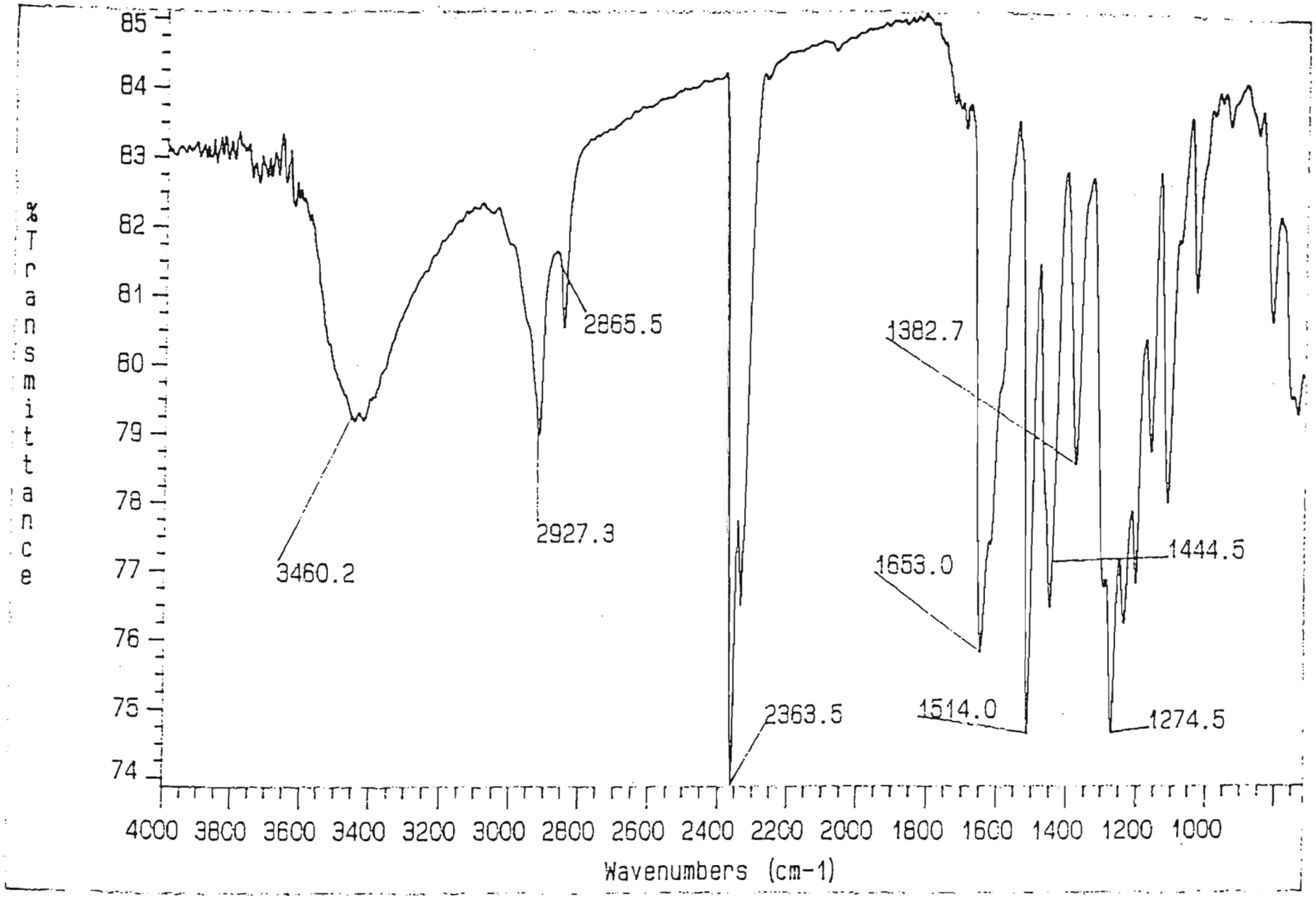
Spectrum 9: UV spectrum of Compound (I)



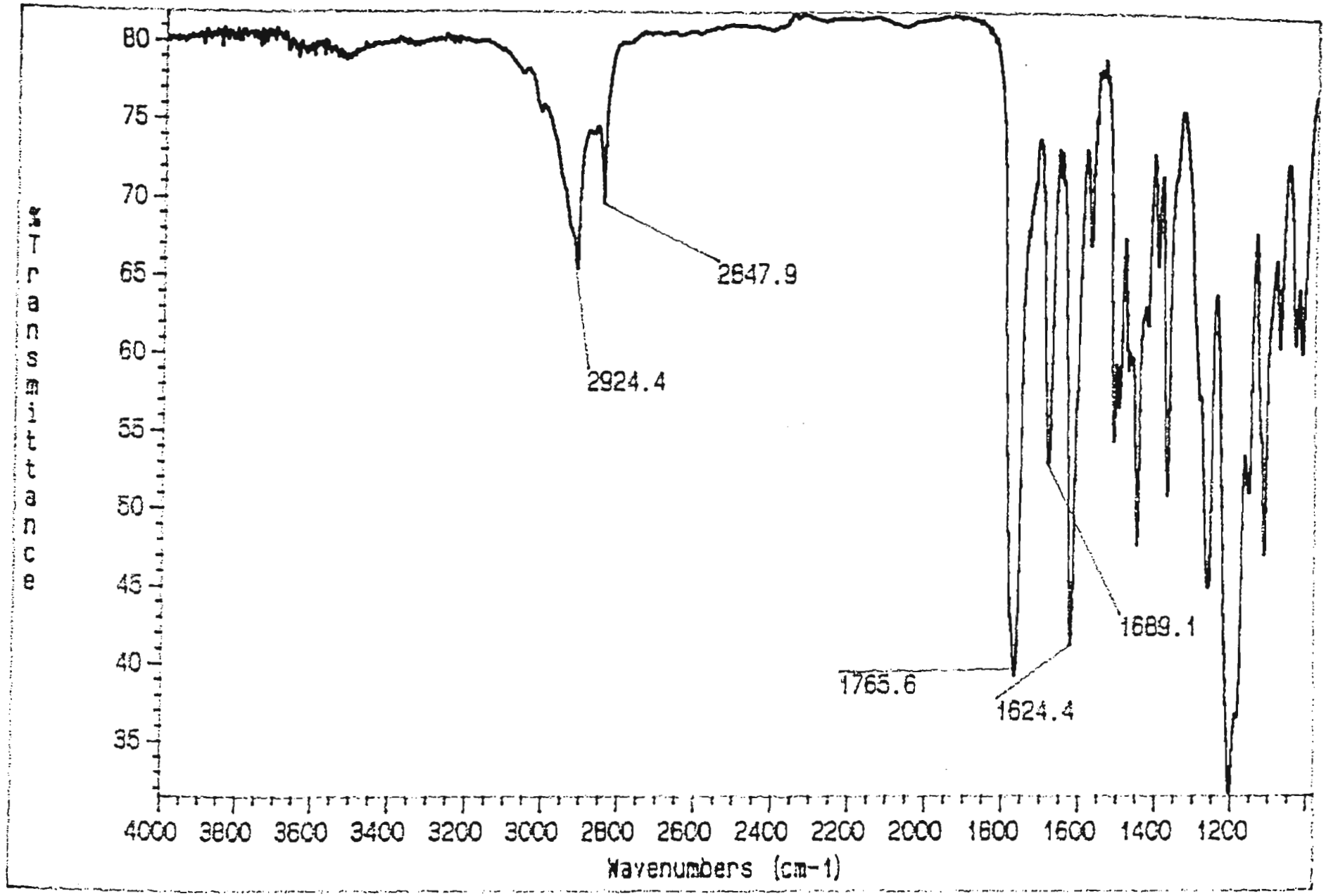
Spectrum 10: UV spectrum of Compound (l) with NaOAc



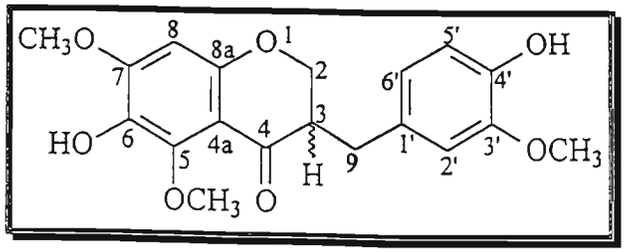
Spectrum 11: UV spectrum of Compound (I) with $AlCl_3$

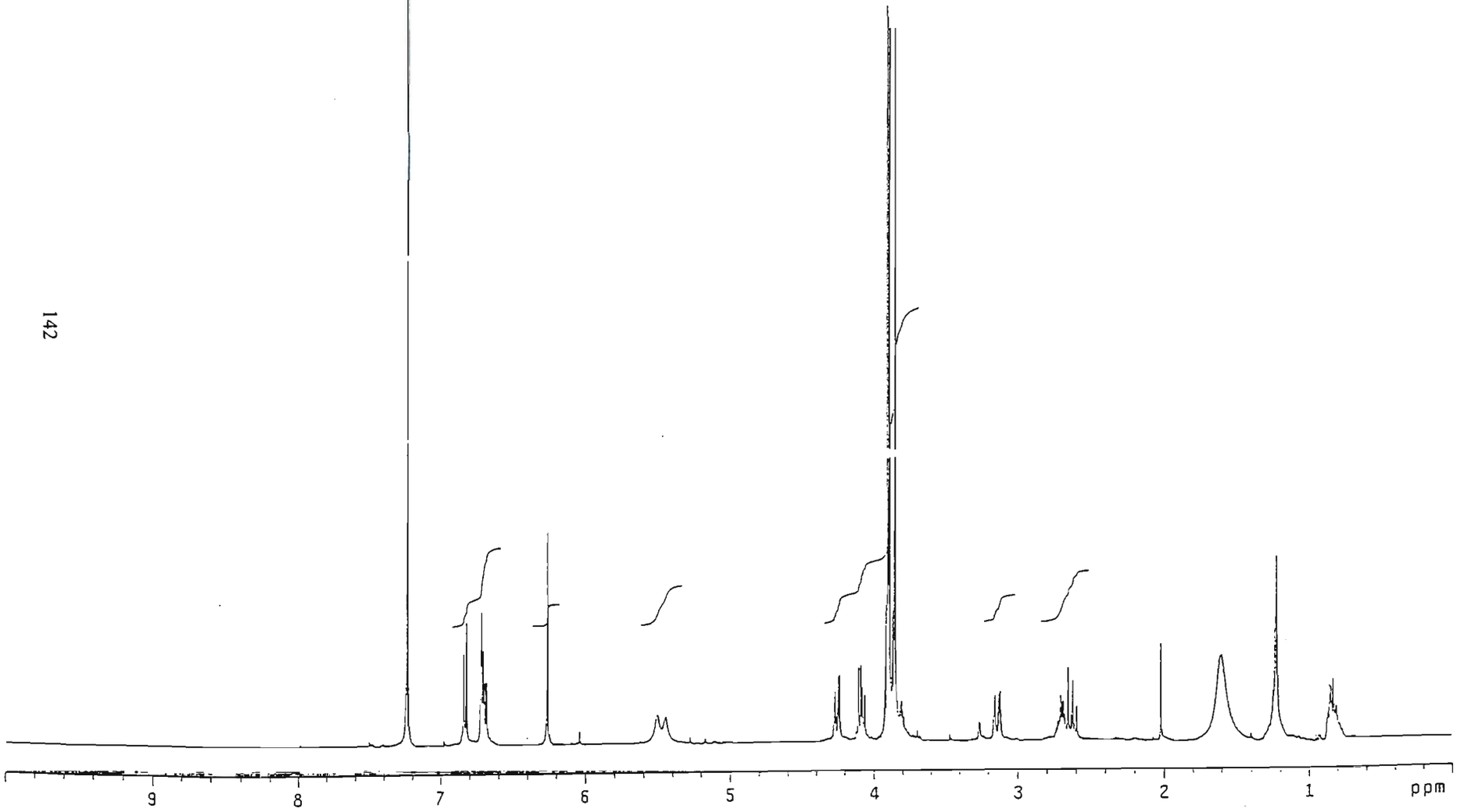


Spectrum 12: IR spectrum of Compound (I)

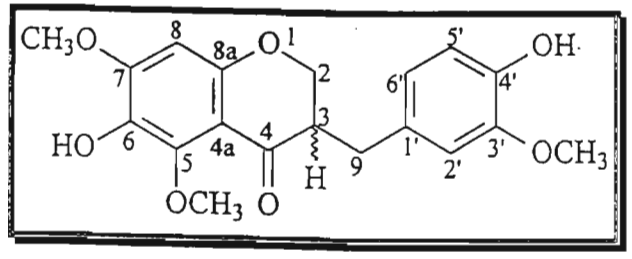


Spectrum 12a: IR spectrum of Compound (I) acetate

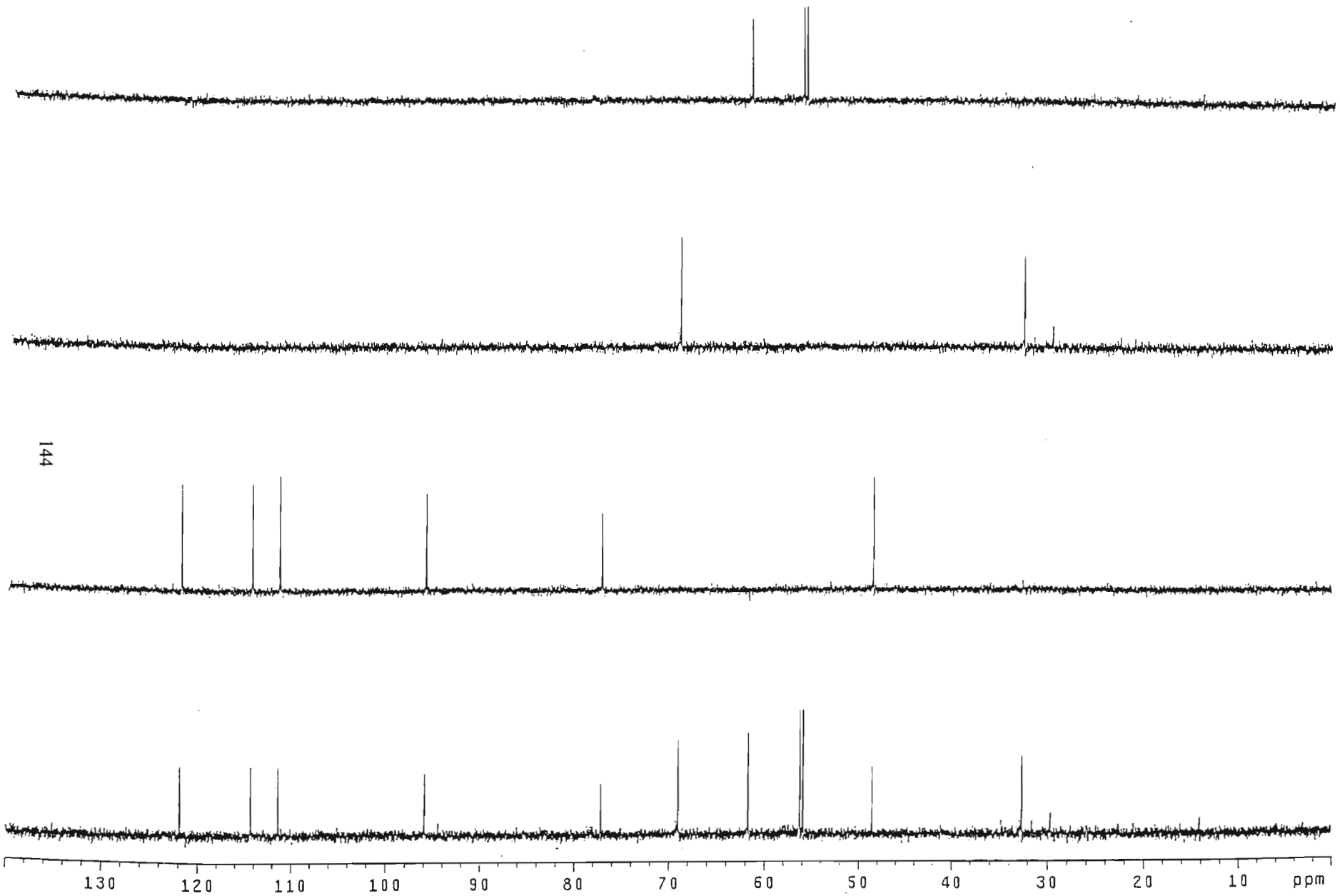




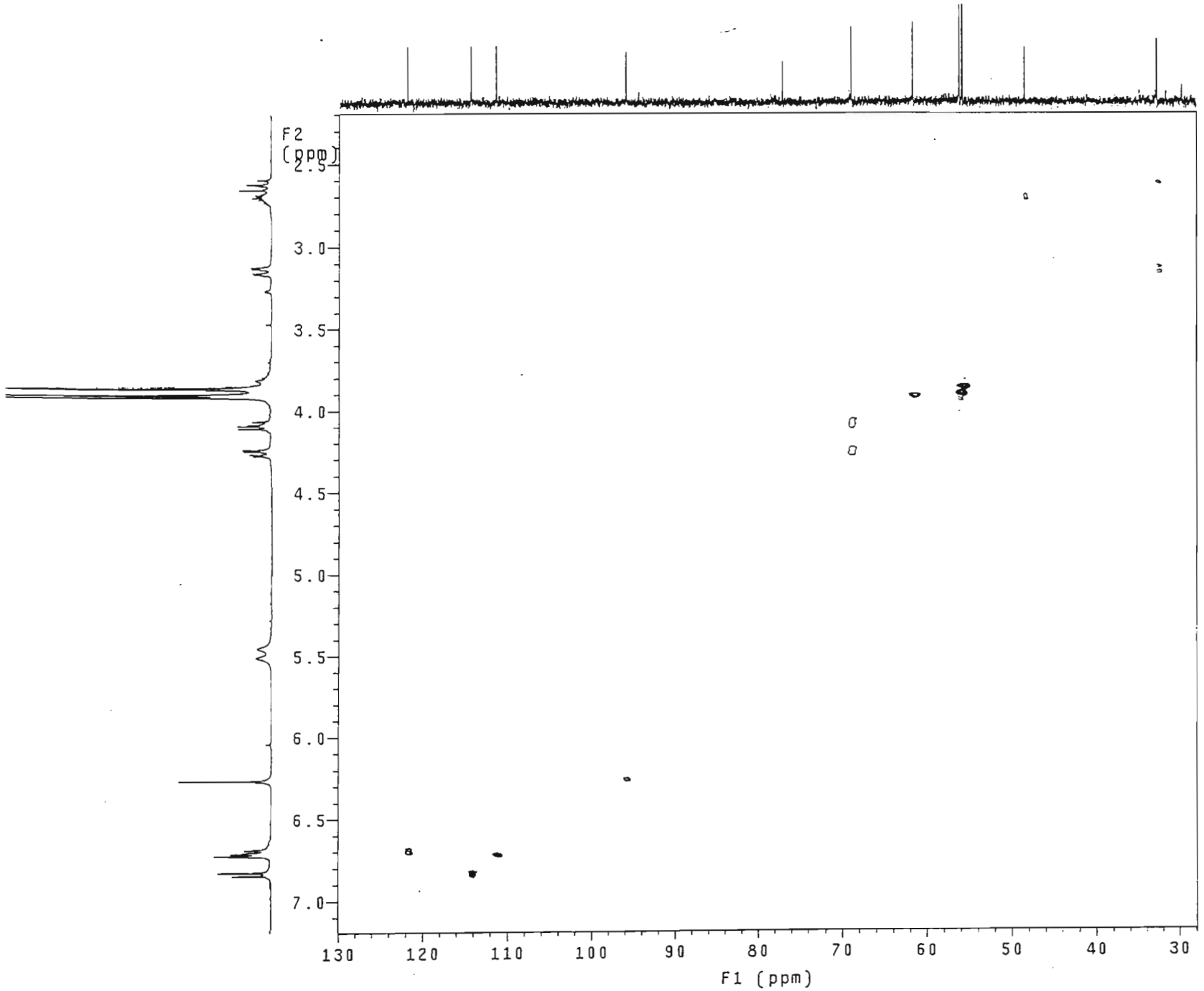
Spectrum 13: ^{1}H NMR spectrum of Compound (II) ($CDCl_3$)



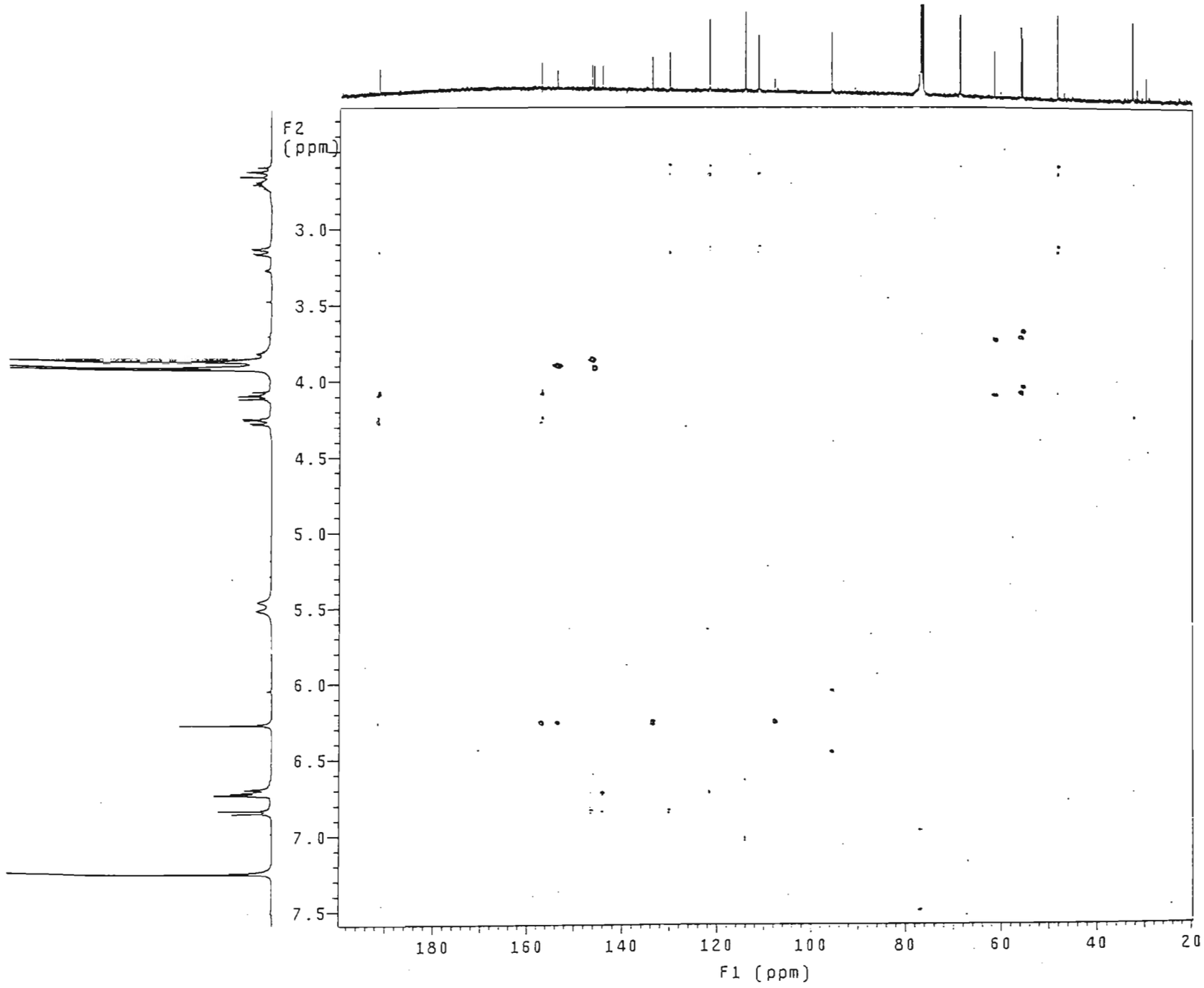
Spectrum 14: ^{13}C NMR spectrum of Compound (II) ($CDCl_3$)



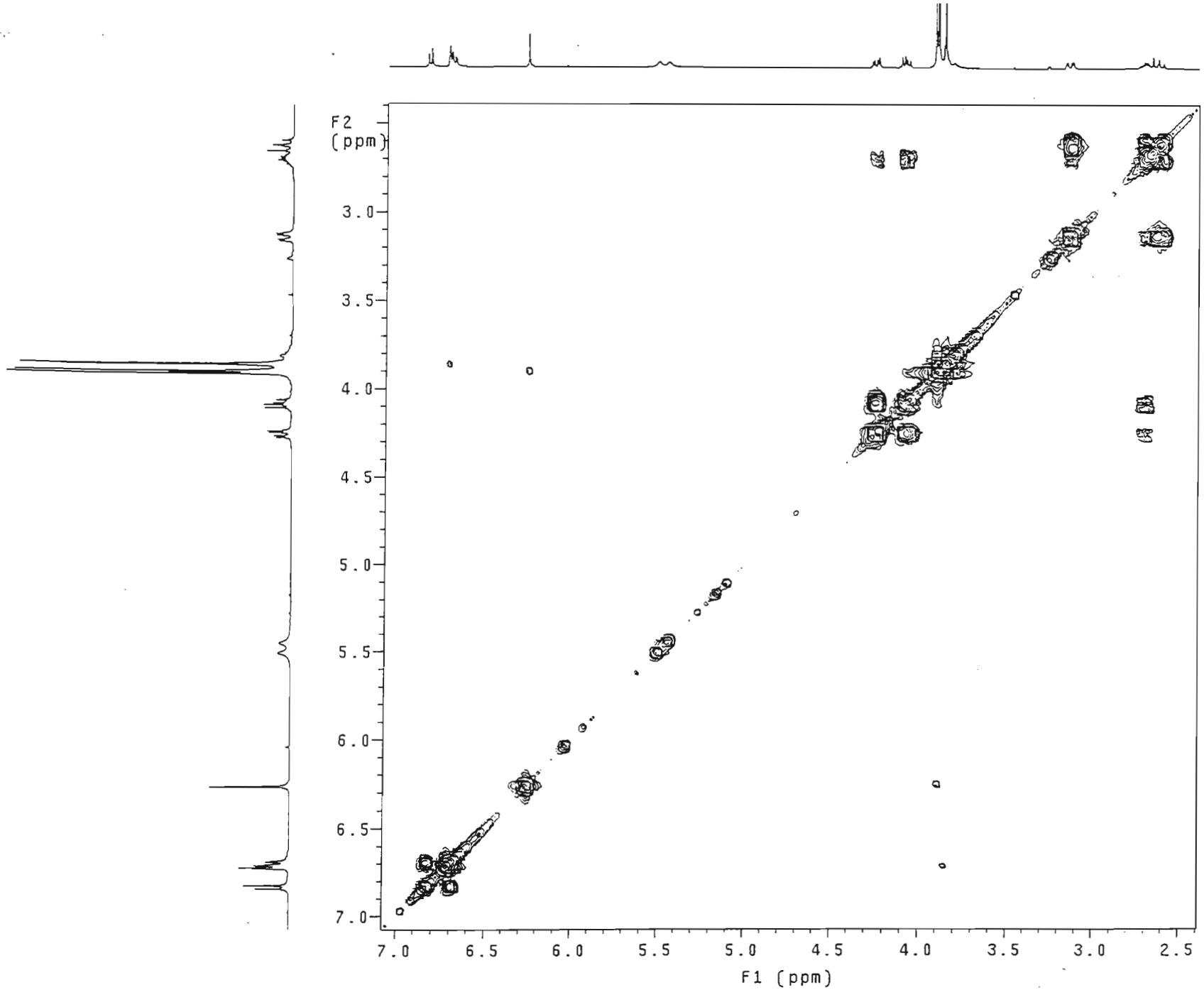
Spectrum 15: ADEPT spectrum of Compound (II) ($CDCl_3$)



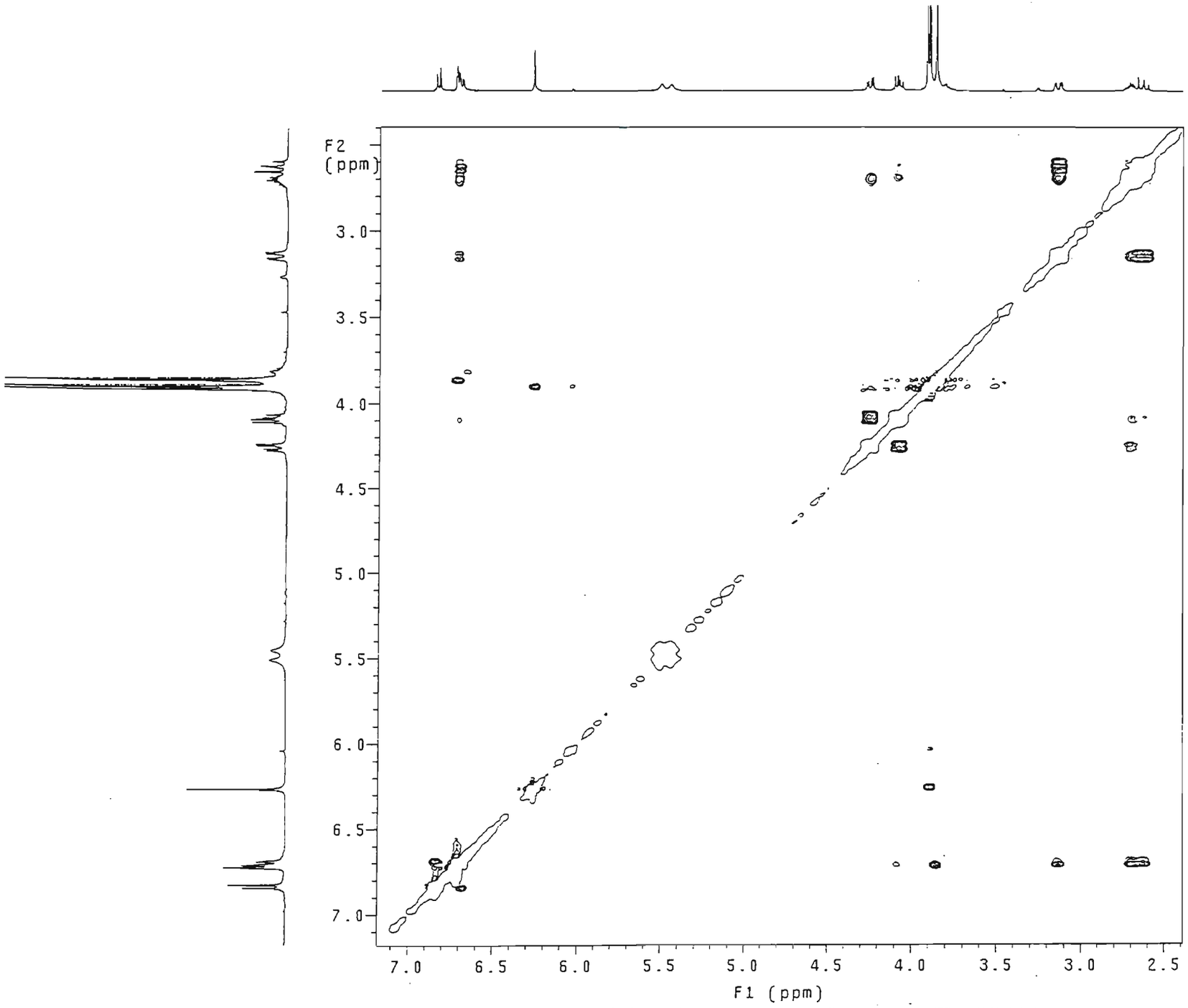
Spectrum 16: HSQC spectrum of Compound (II) ($CDCl_3$)



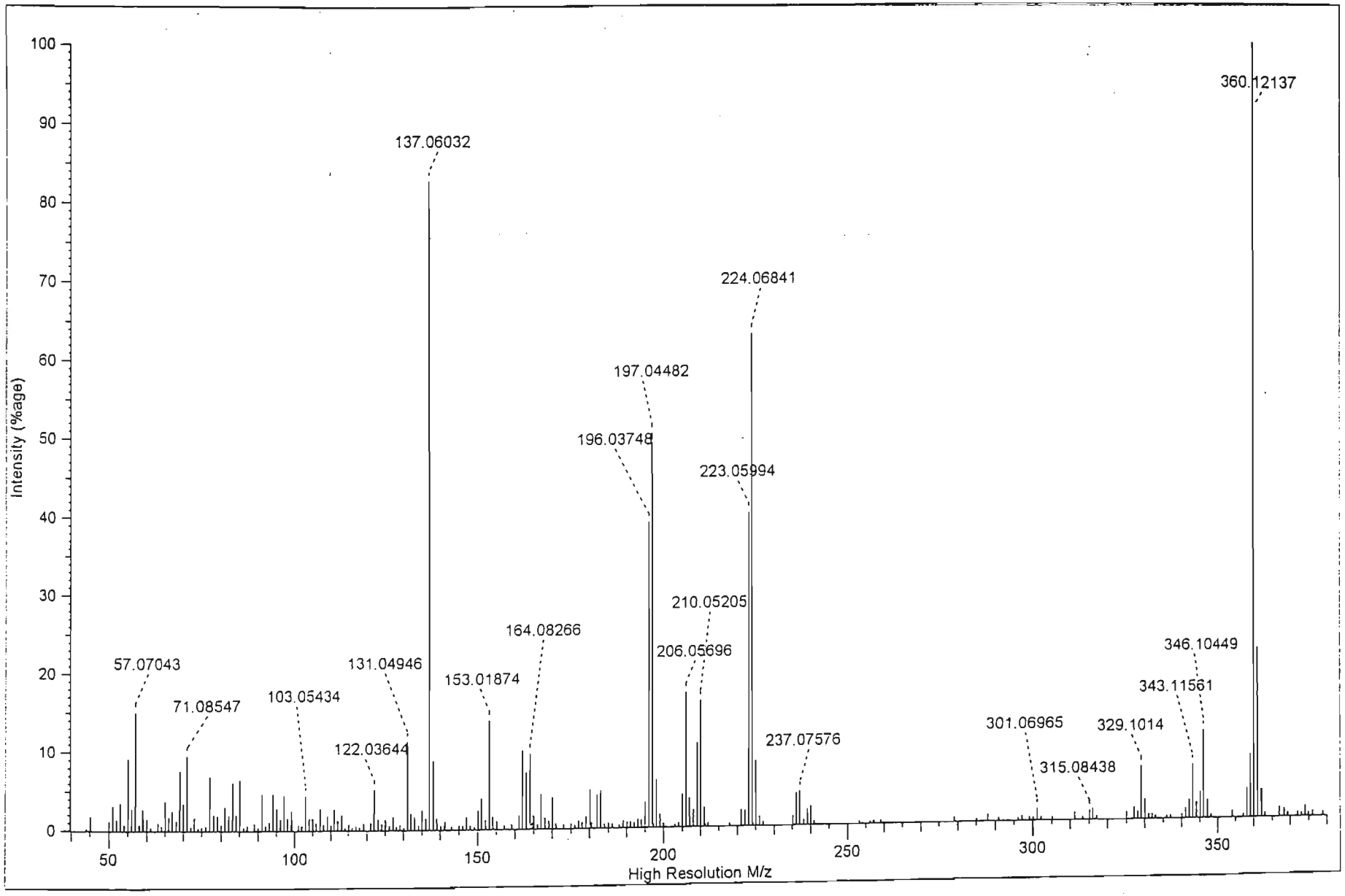
Spectrum 17: HMBC spectrum of Compound (II) ($CDCl_3$)



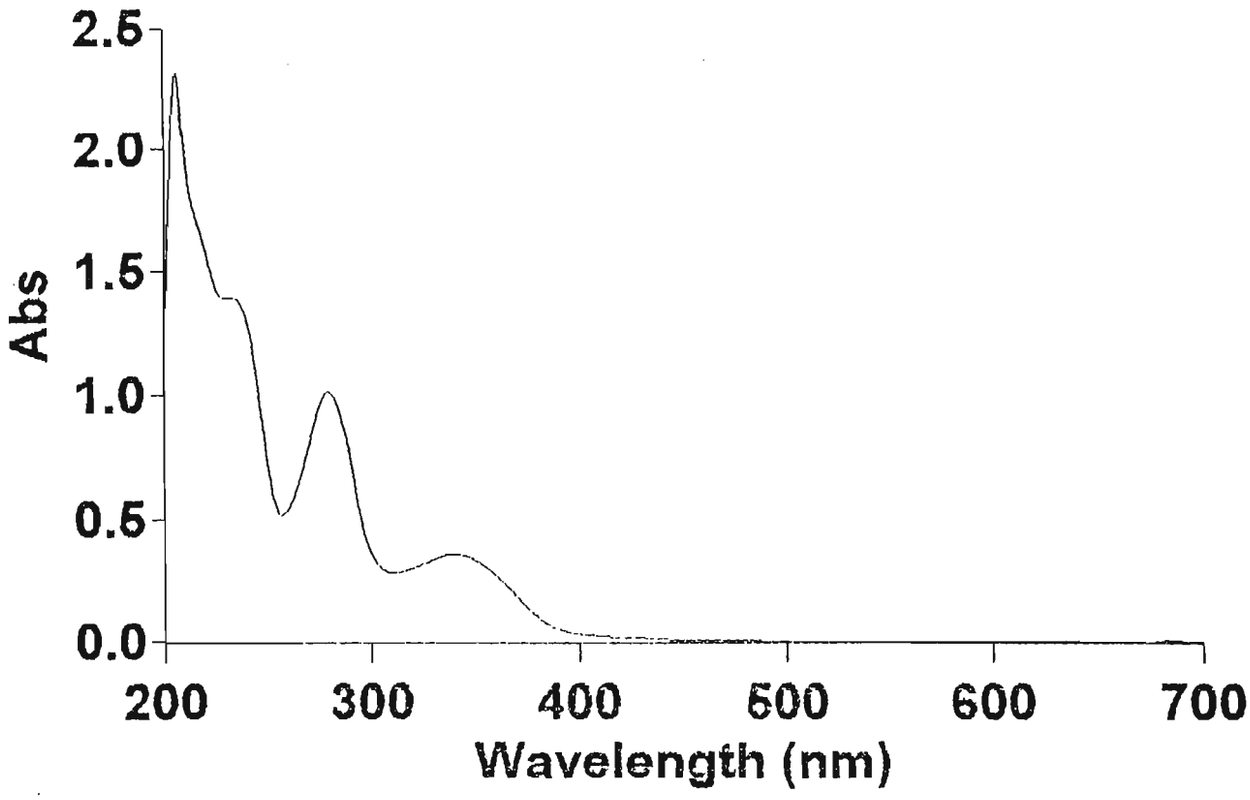
Spectrum 18: COSY spectrum of Compound (II) ($CDCl_3$)



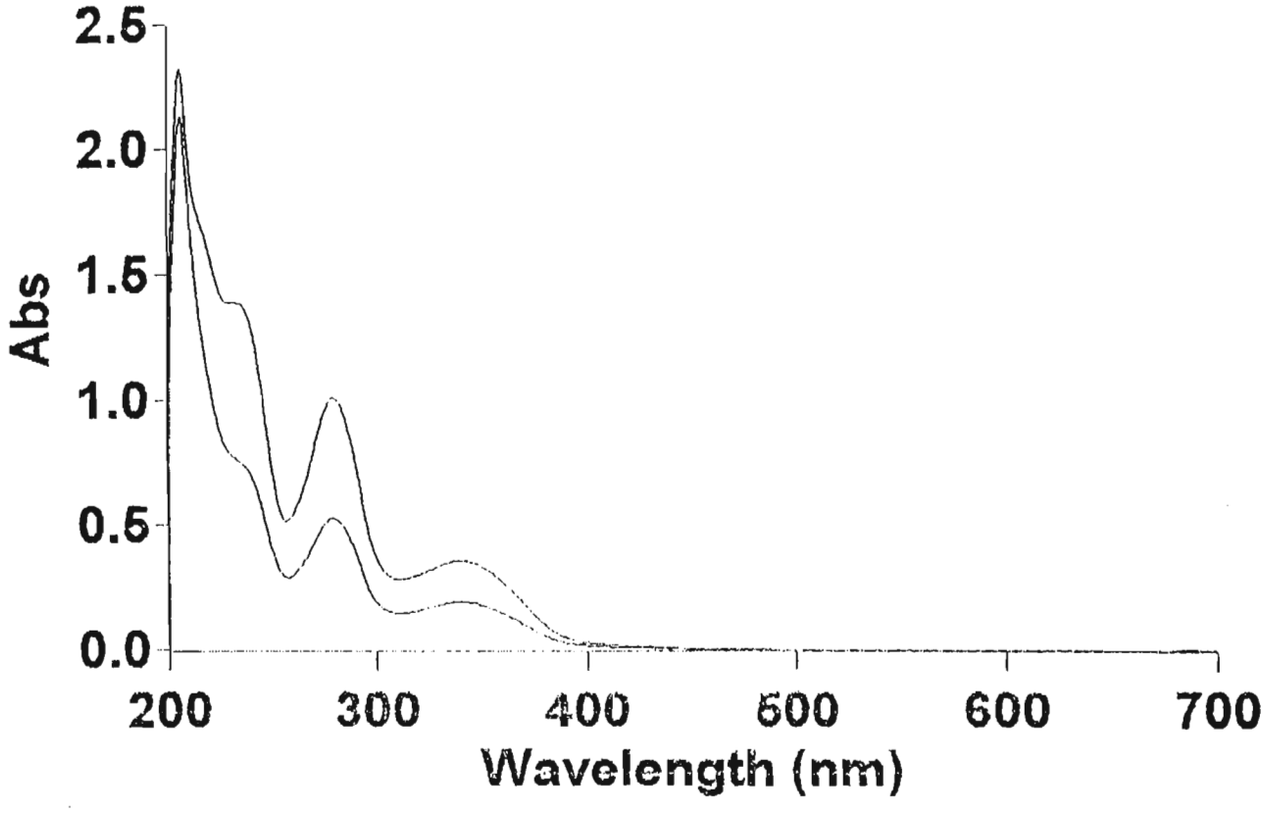
Spectrum 19: NOESY spectrum of Compound (II) ($CDCl_3$)



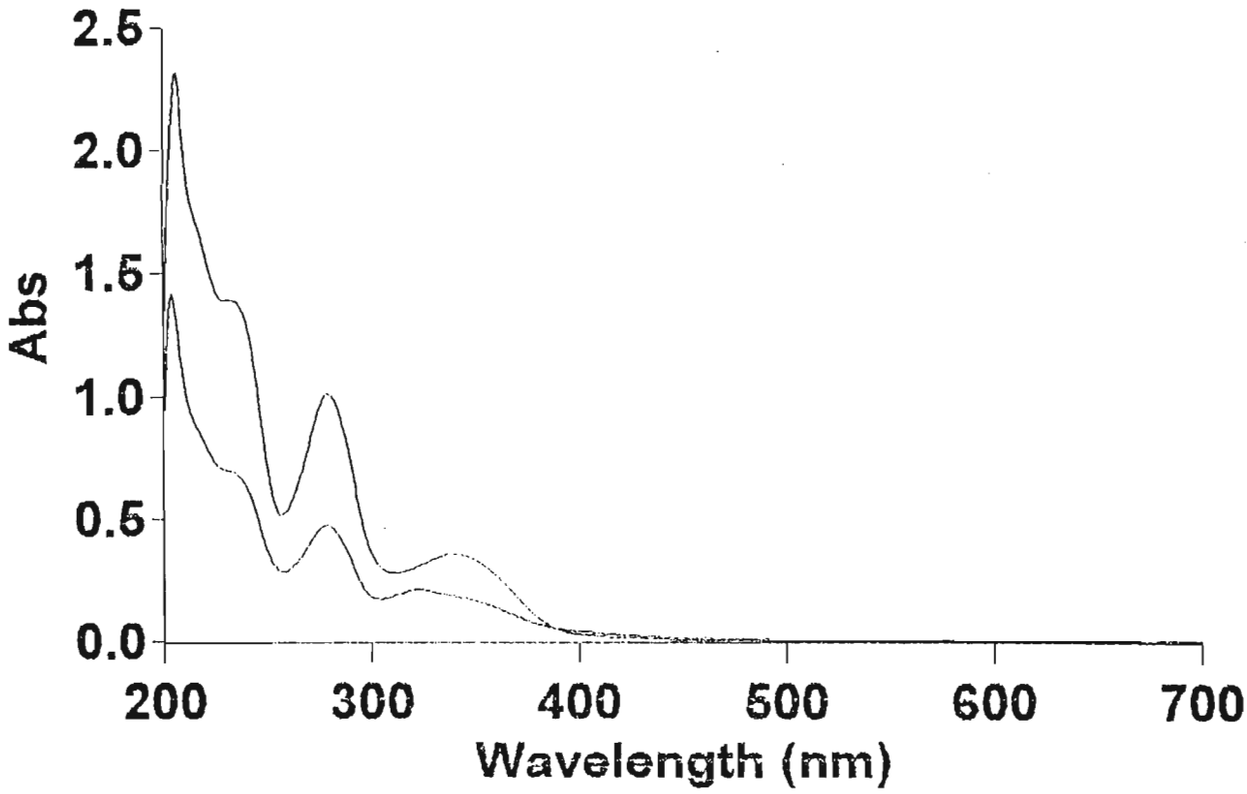
Spectrum 20: Mass spectrum of Compound (II)



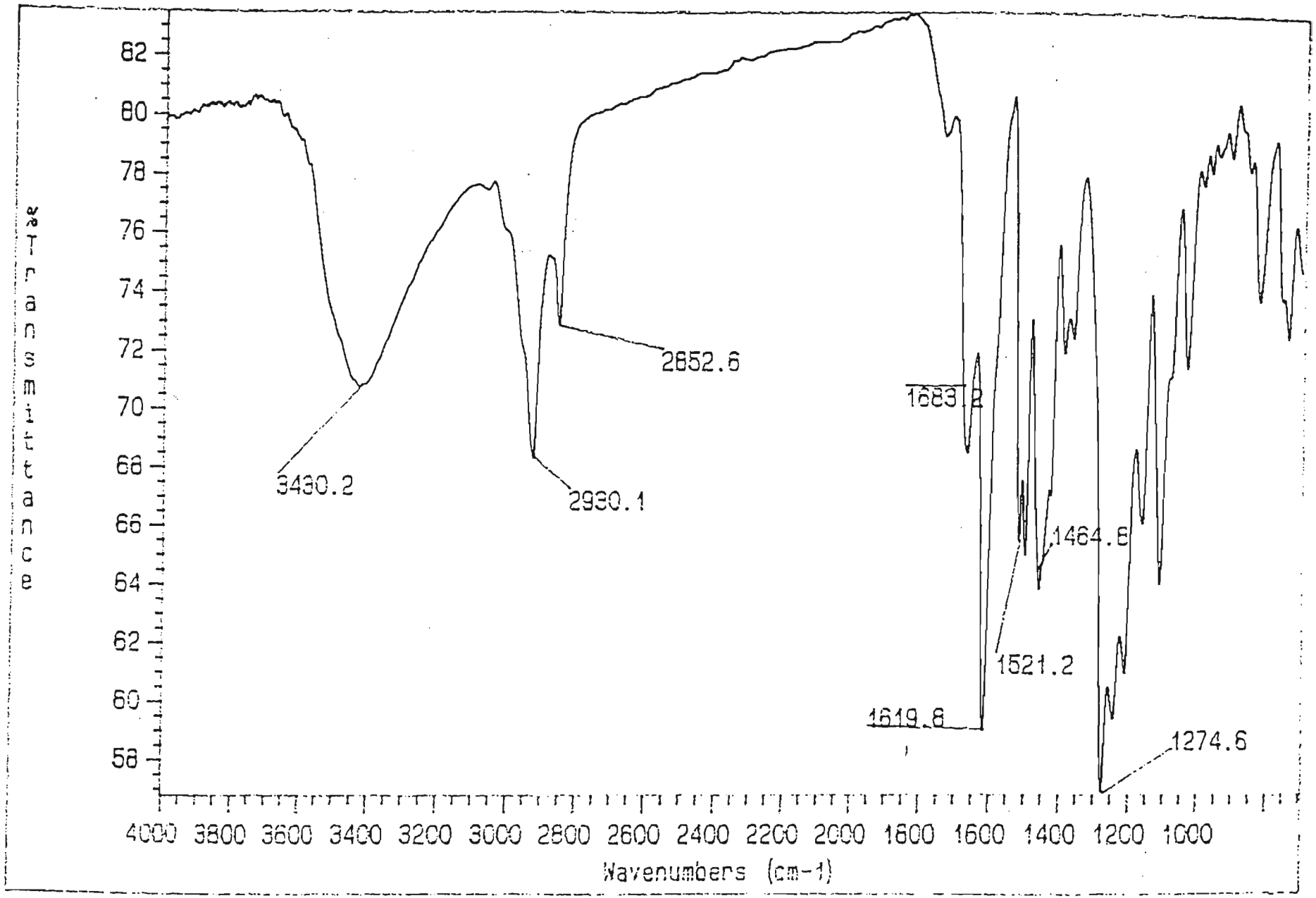
Spectrum 21: UV spectrum of Compound (II)



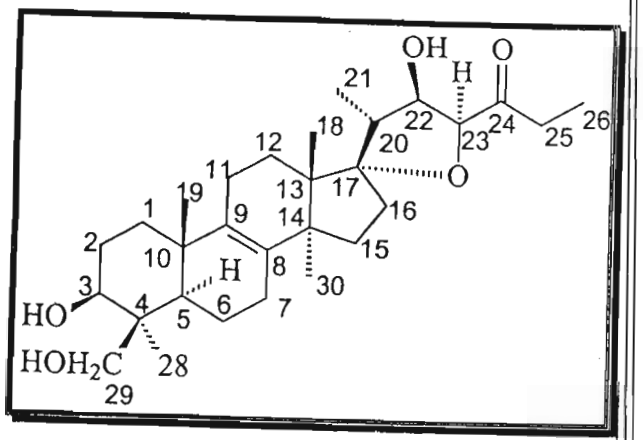
Spectrum 22: UV spectrum of Compound (II) with NaOAc

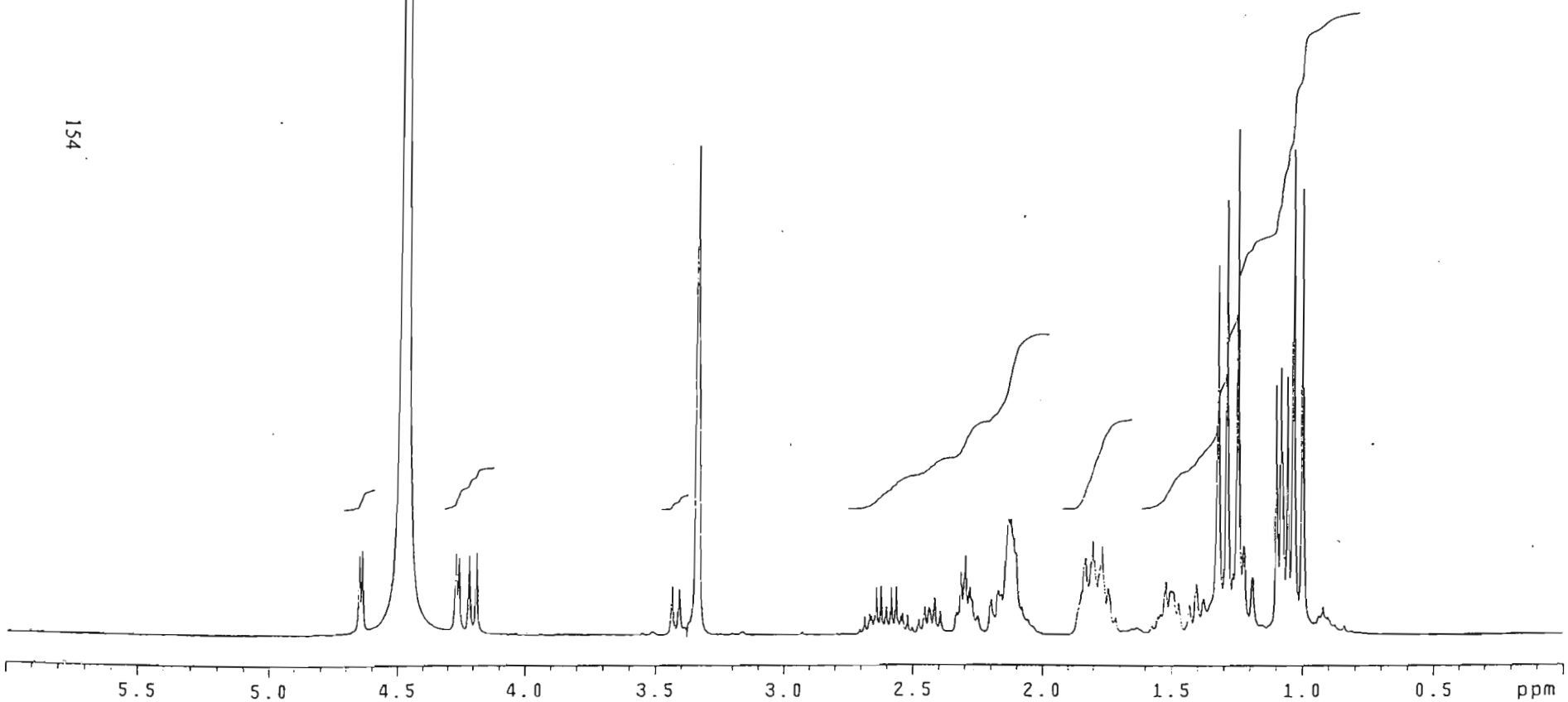


Spectrum 23: UV spectrum of Compound (II) with $AlCl_3$

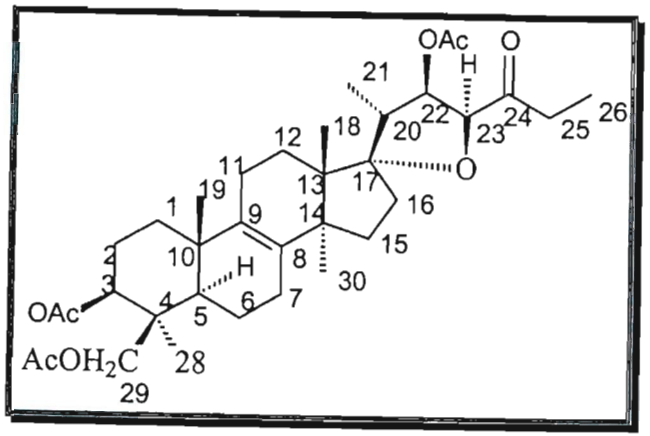


Spectrum 24: IR spectrum of Compound (II)

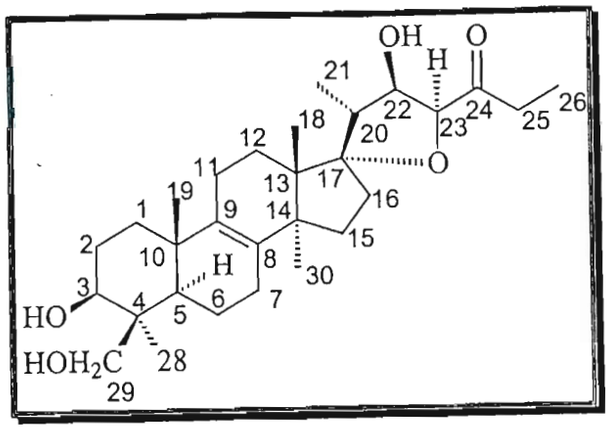




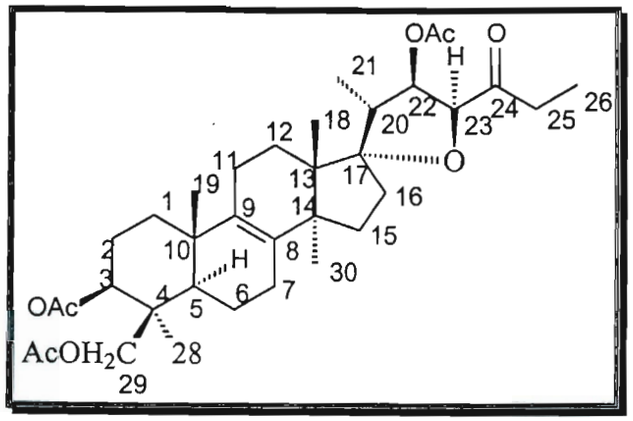
Spectrum 25: ^1H NMR spectrum of Compound (III) (CD_3OD)



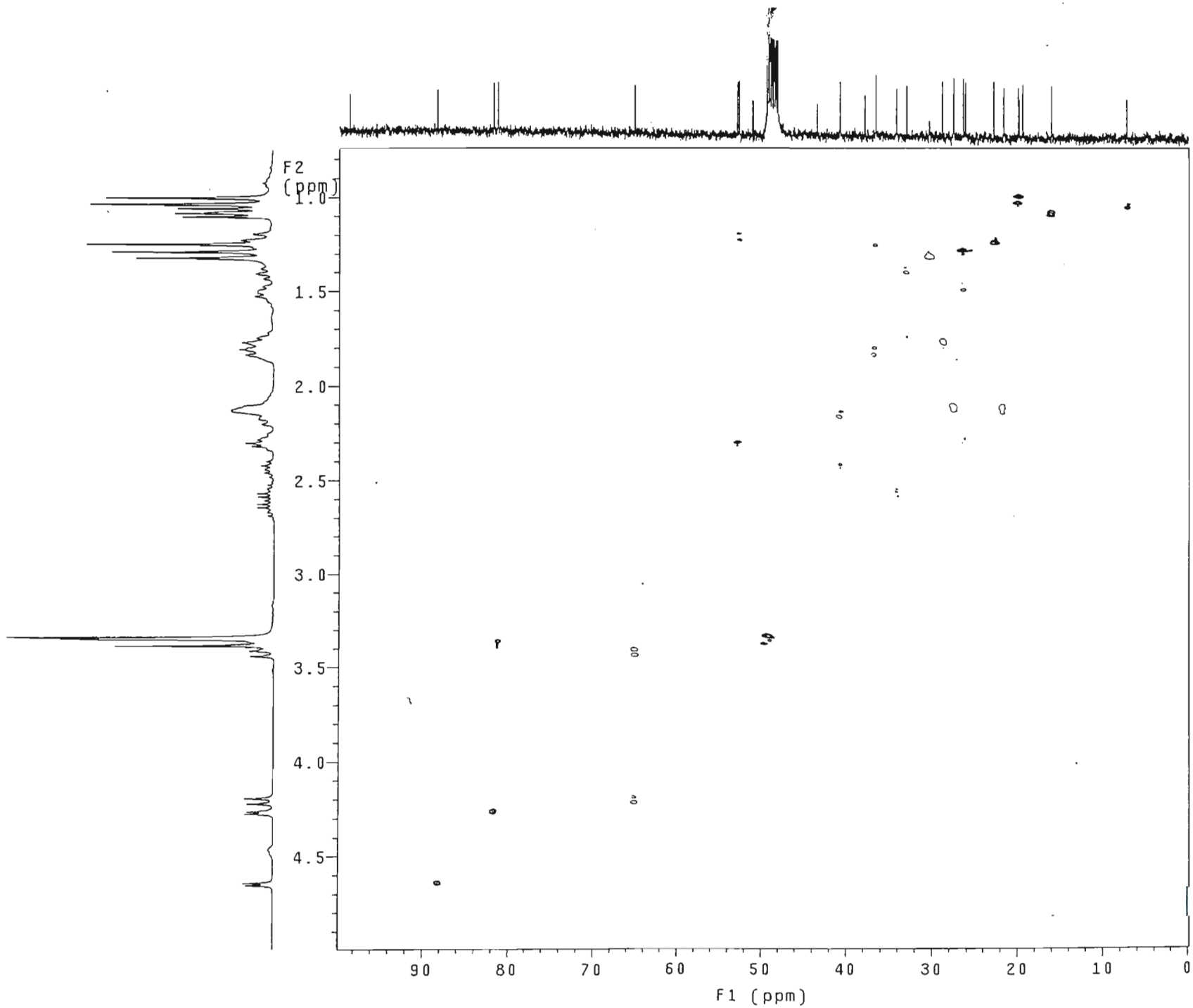
Spectrum 25a: ^{1}H NMR spectrum of Compound (III) acetate ($CDCl_3$)



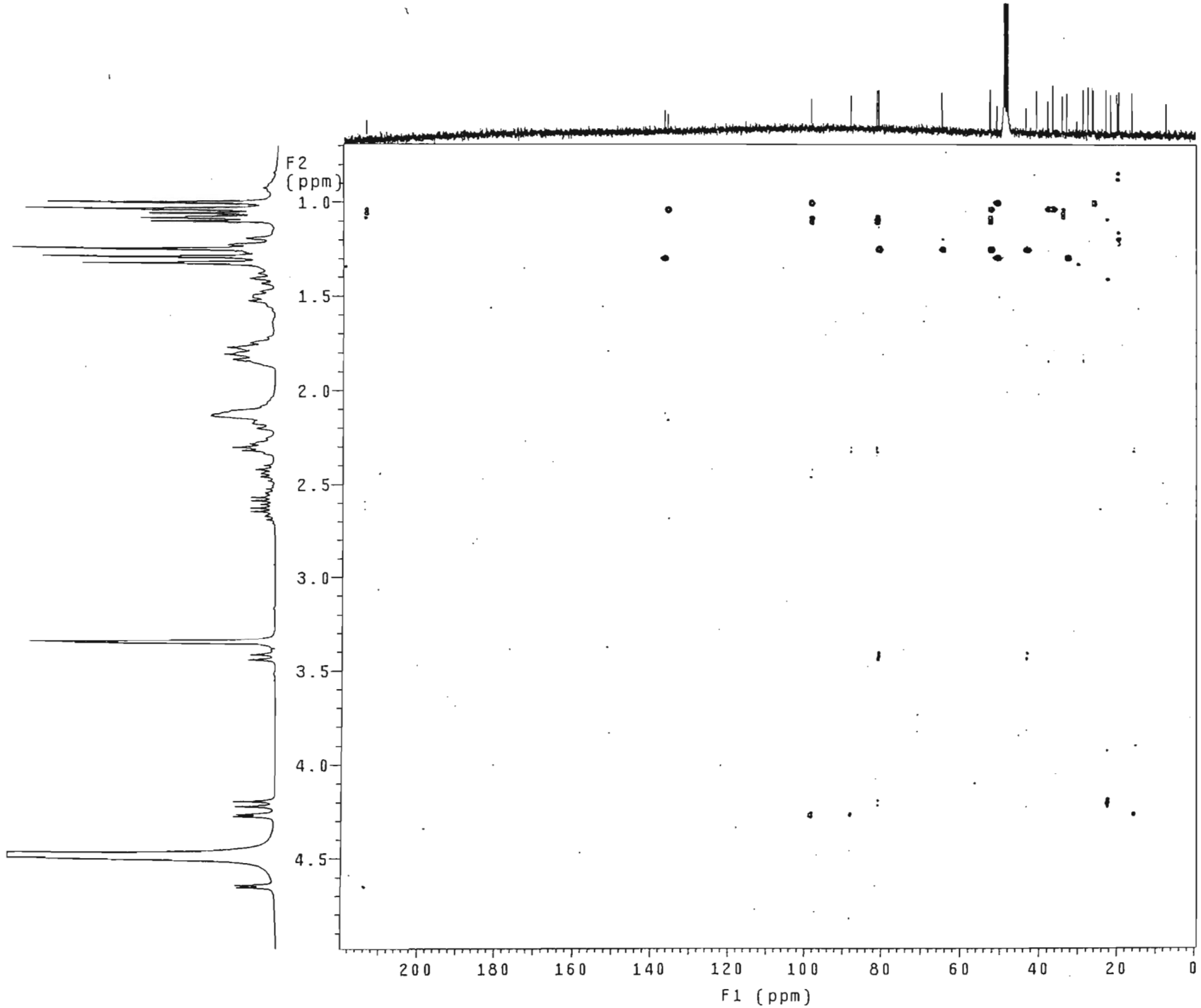
Spectrum 26: ^{13}C NMR



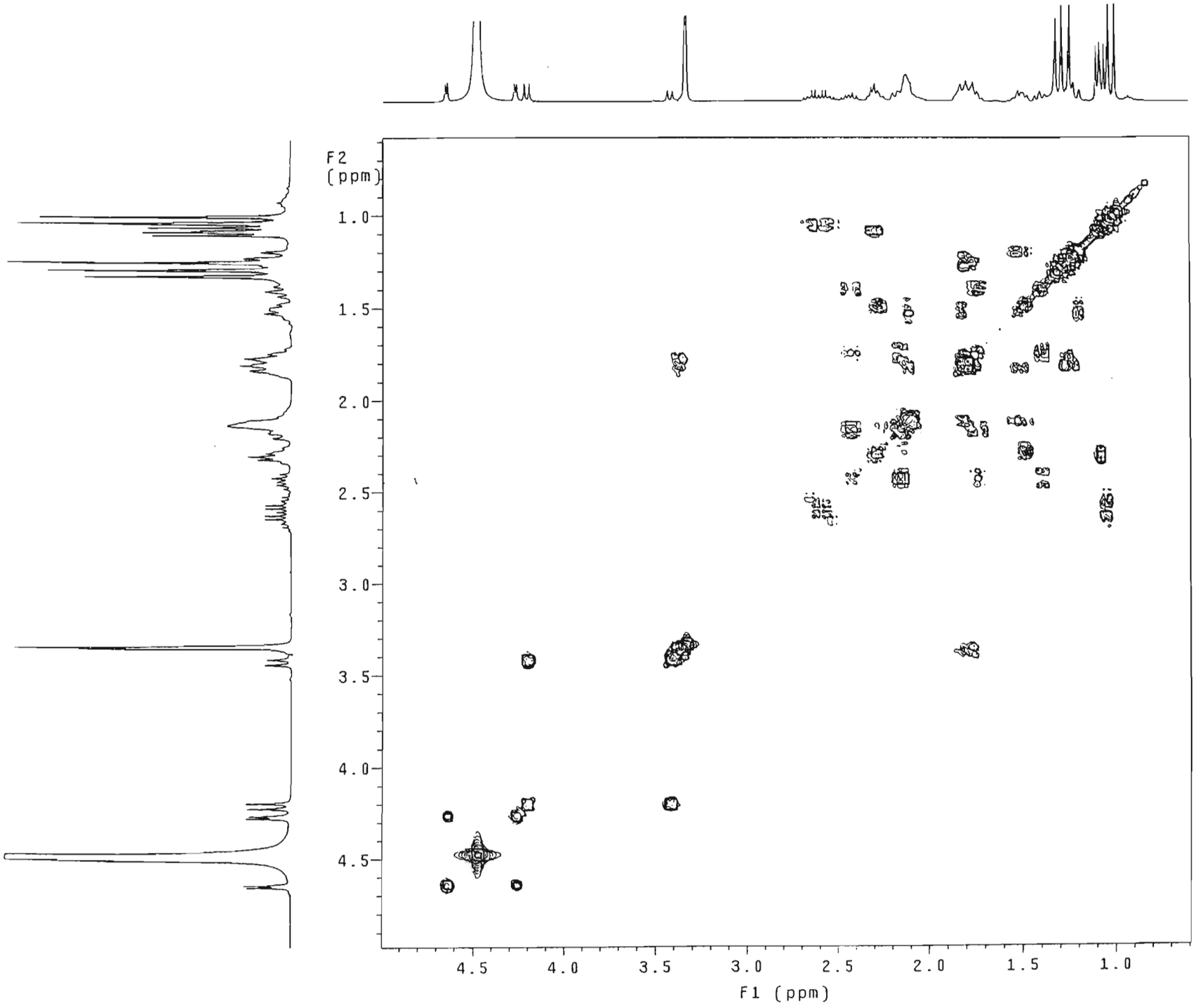
Spectrum 26a: ^{13}C NMR spectrum of Compound (III) acetate ($CDCl_3$)



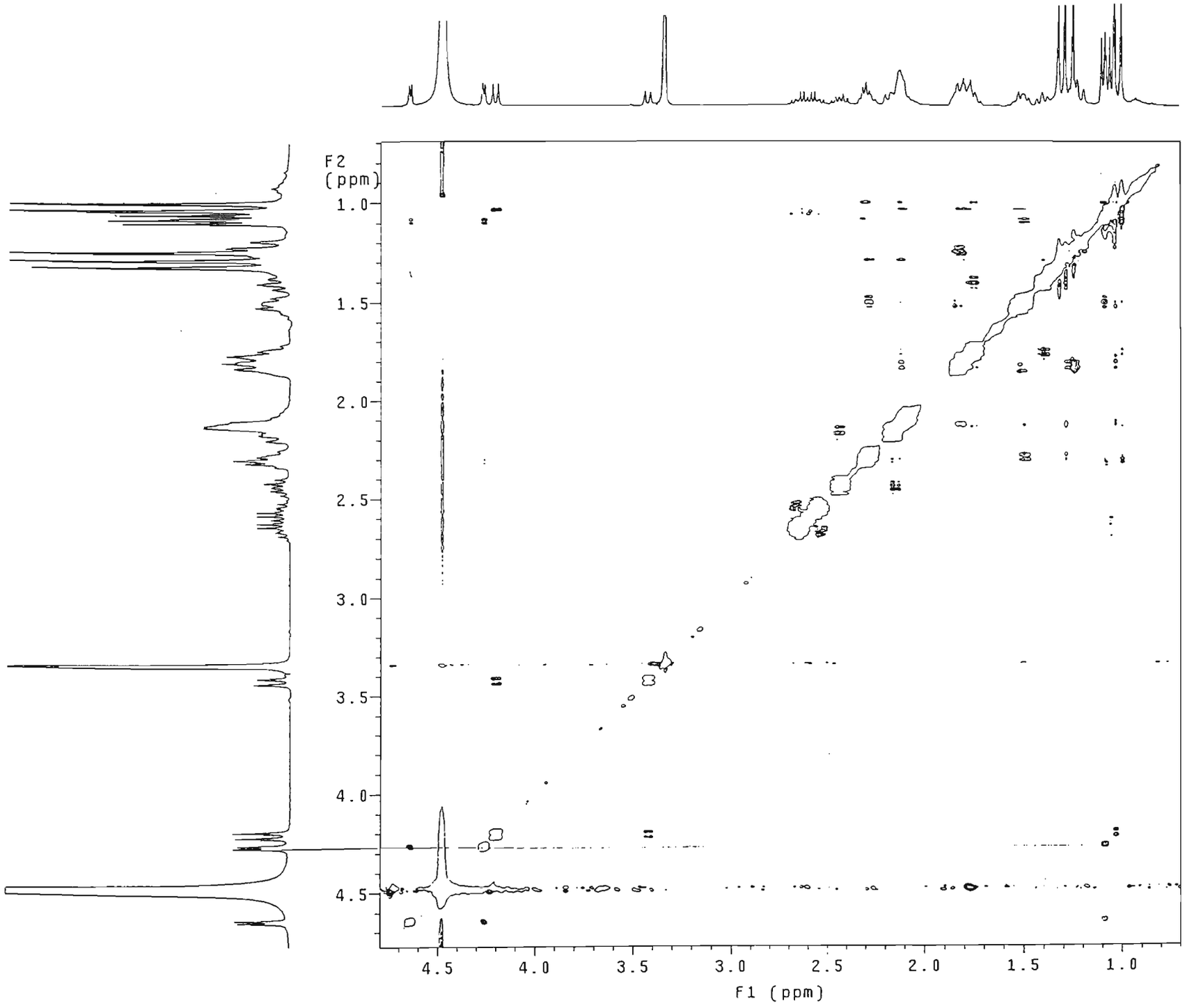
Spectrum 27: HSQC spectrum of Compound (III) (CD_3OD)



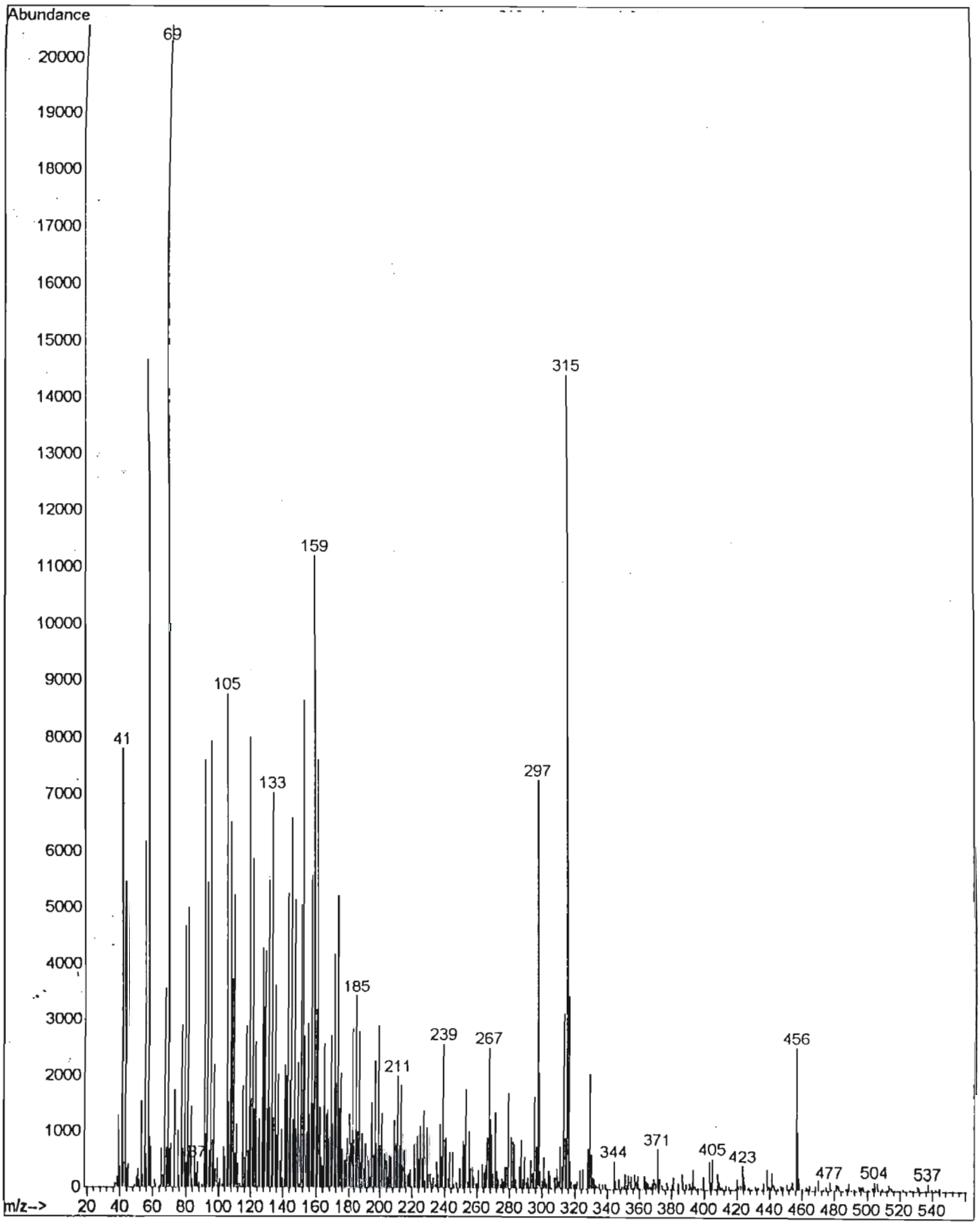
Spectrum 28: HMBC spectrum of Compound (III) (CD_3OD)



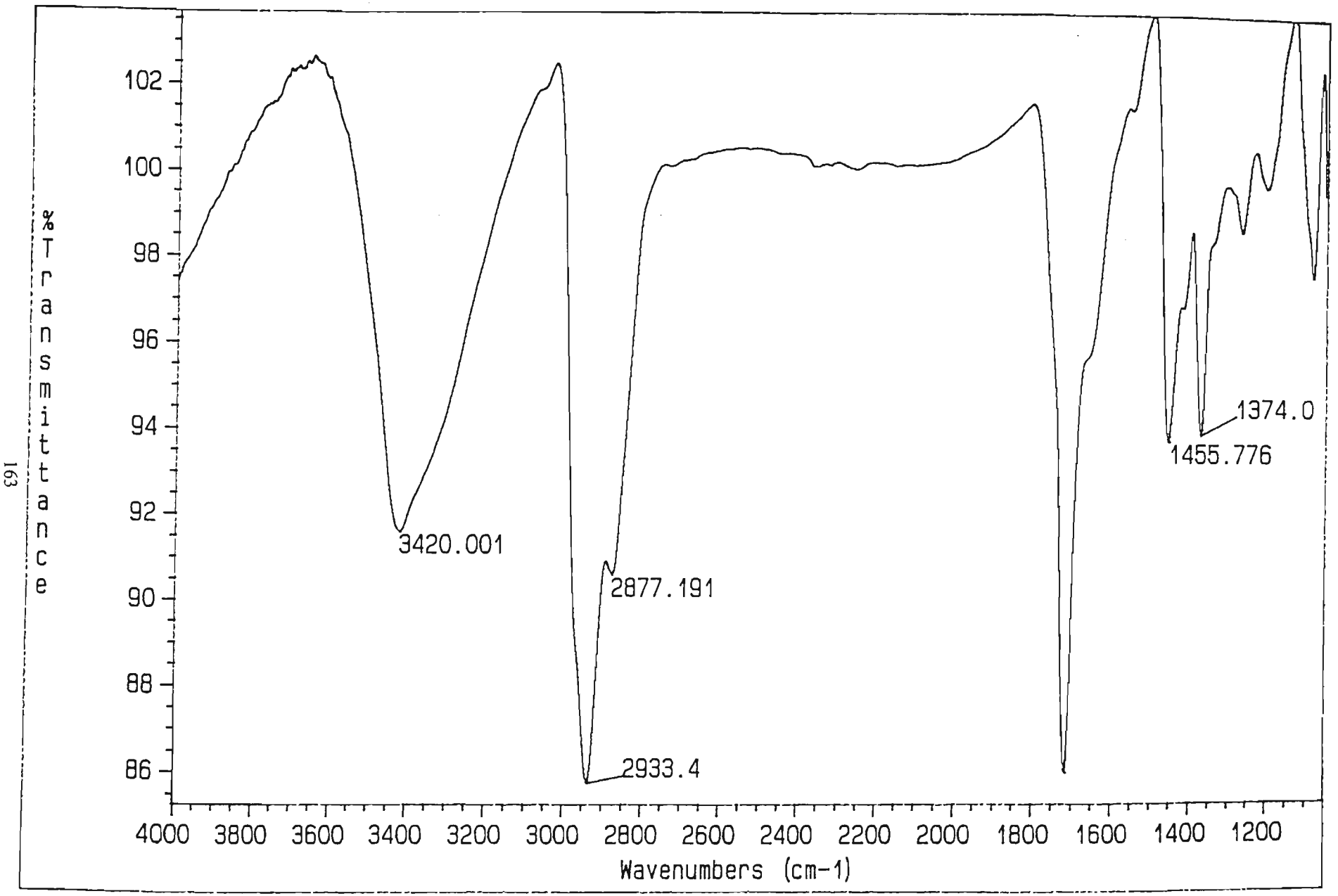
Spectrum 29: COSY spectrum of Compound (III) (CD_3OD)



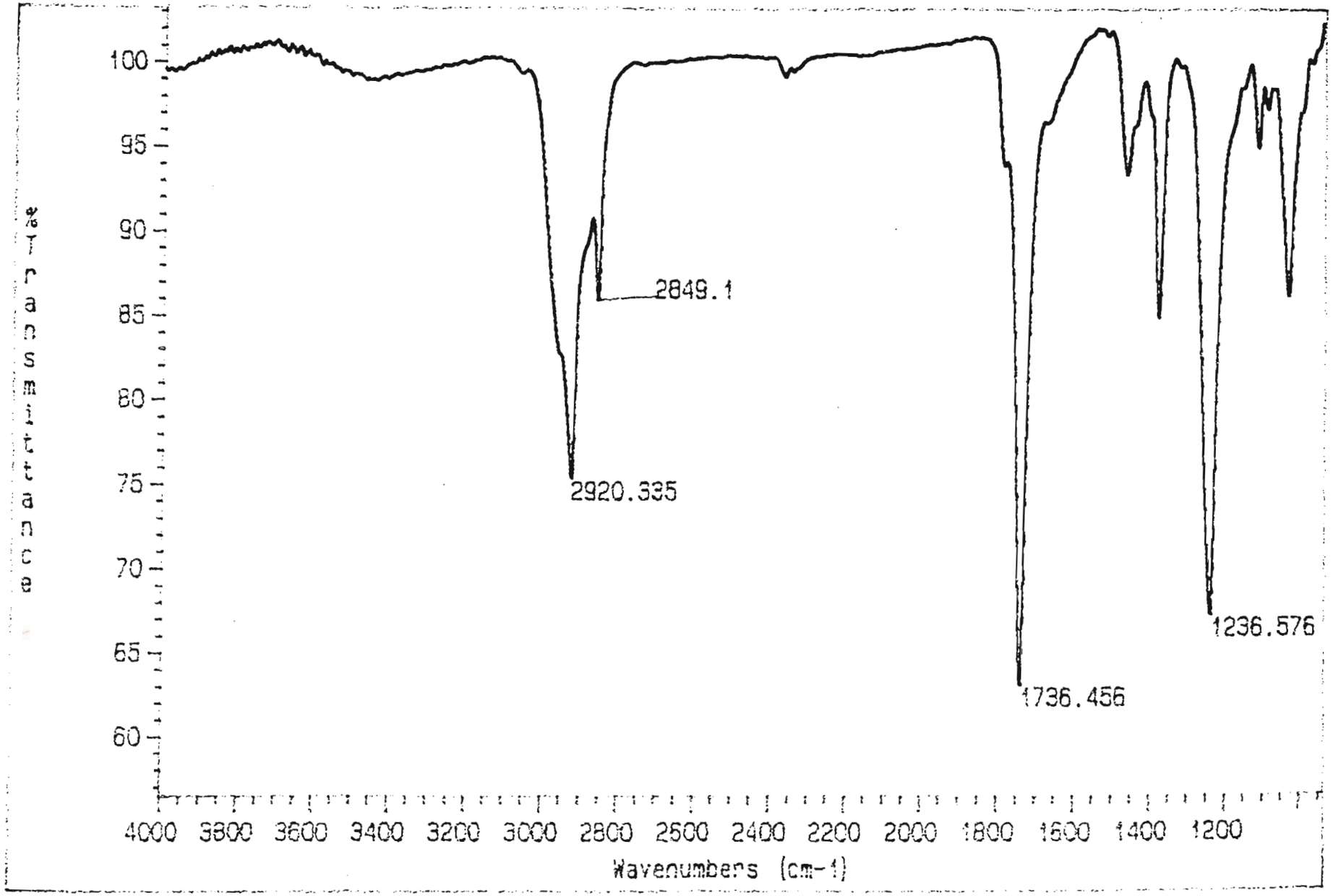
Spectrum 30: NOESY spectrum of Compound (III) (CD3OD)



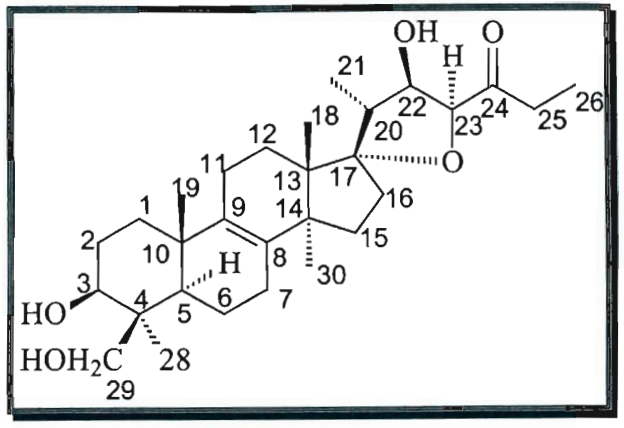
Spectrum 31: Mass spectrum of Compound (III)



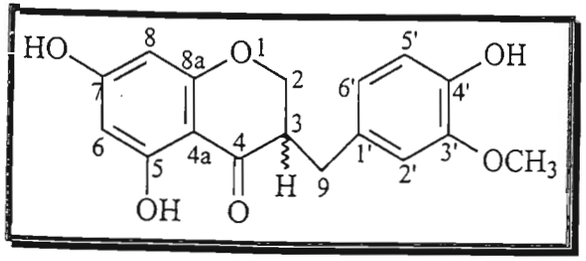
Spectrum 32: IR spectrum of Compound (III)



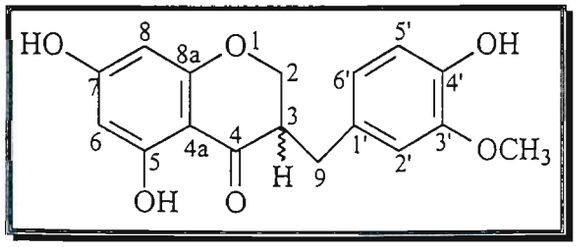
Spectrum 32a: IR spectrum of Compound (III) acetate



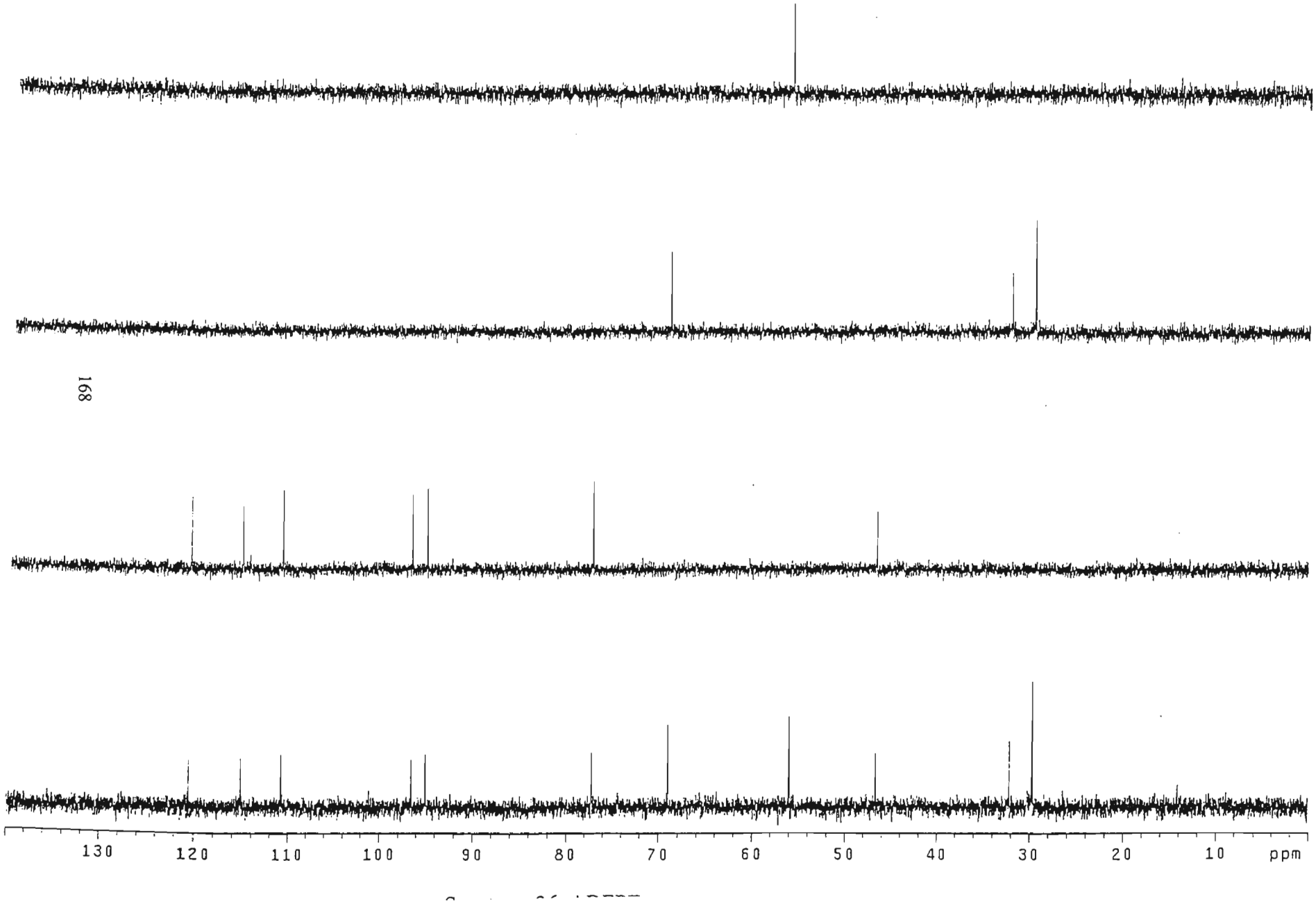
Spectrum 33: ^{13}C NMR spectrum of Compound (III) (C_5D_5N)

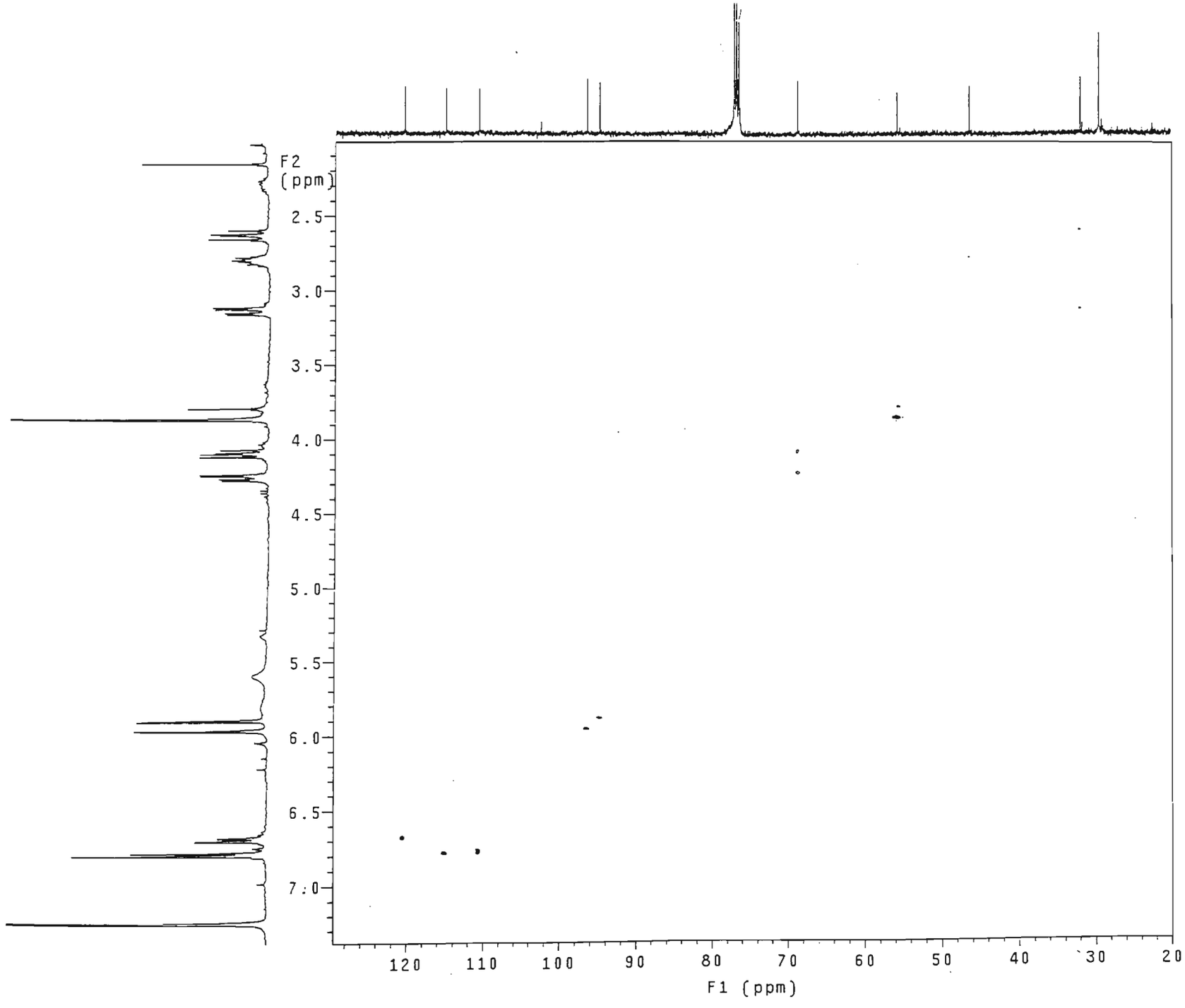


Spectrum 34: ^{1}H NMR spectrum of C

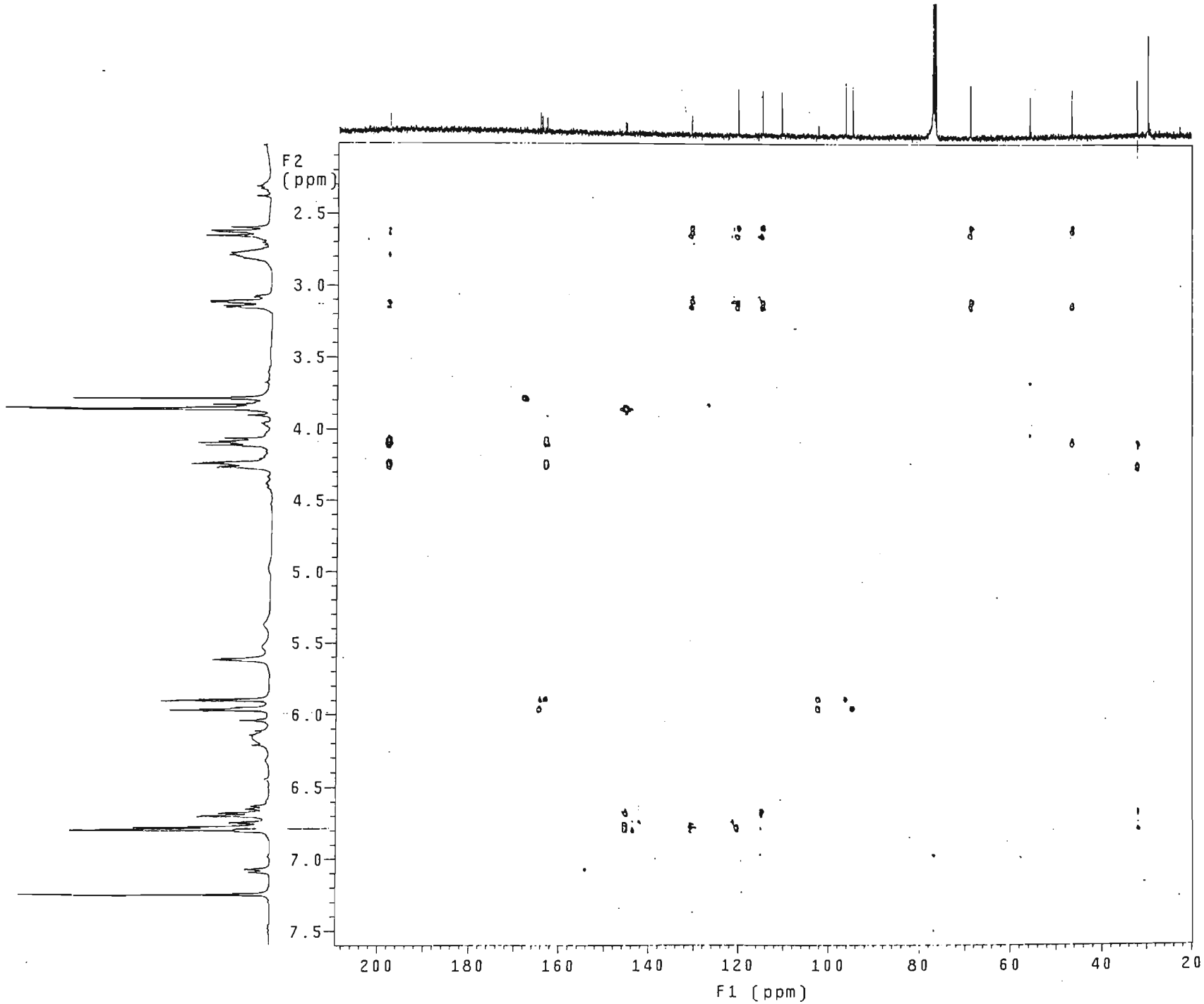


Spectrum 35: ^{13}C NMR spectr... of C...

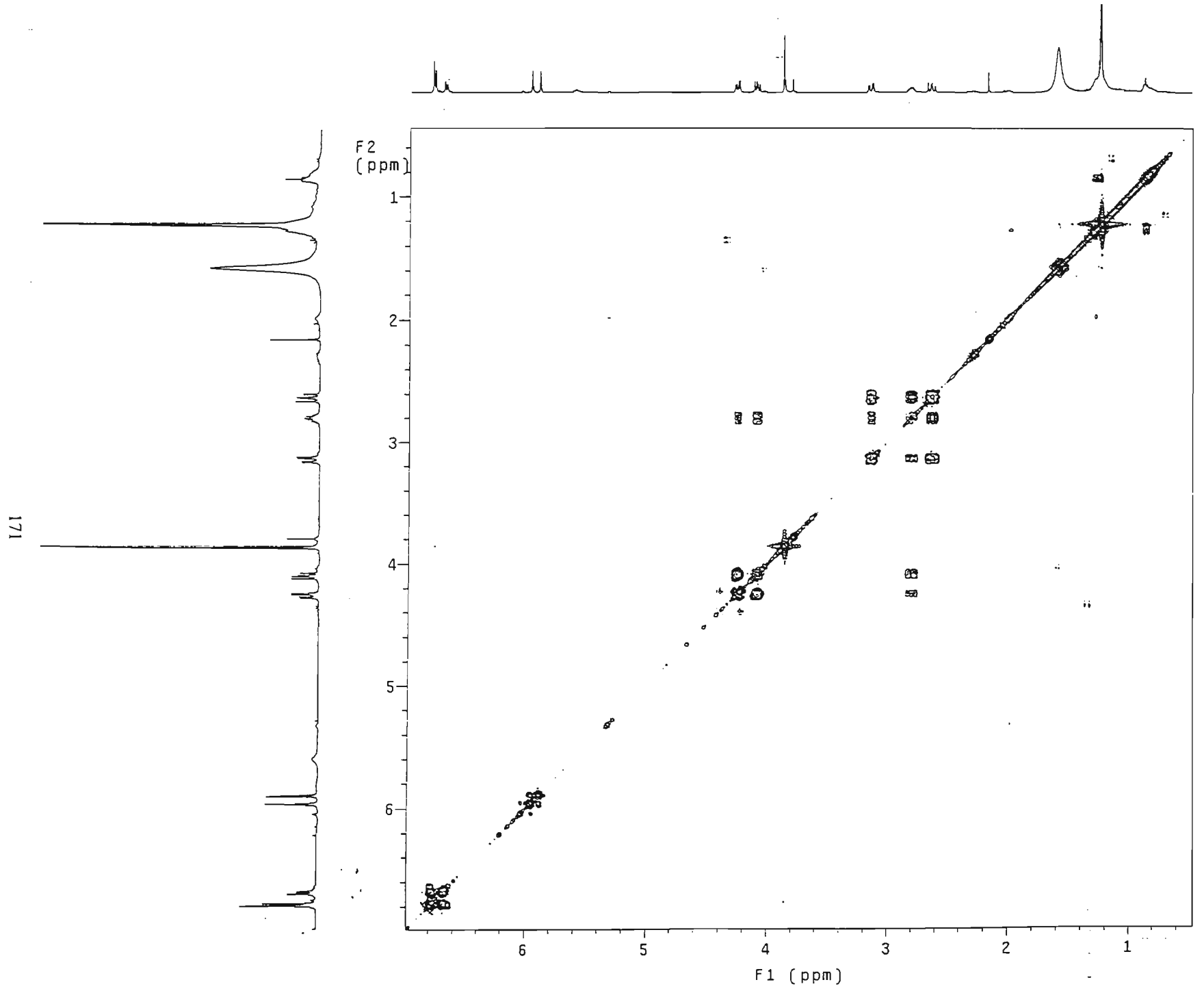

168
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



Spectrum 37: HSQC spectrum of Compound (IV) ($CDCl_3$)



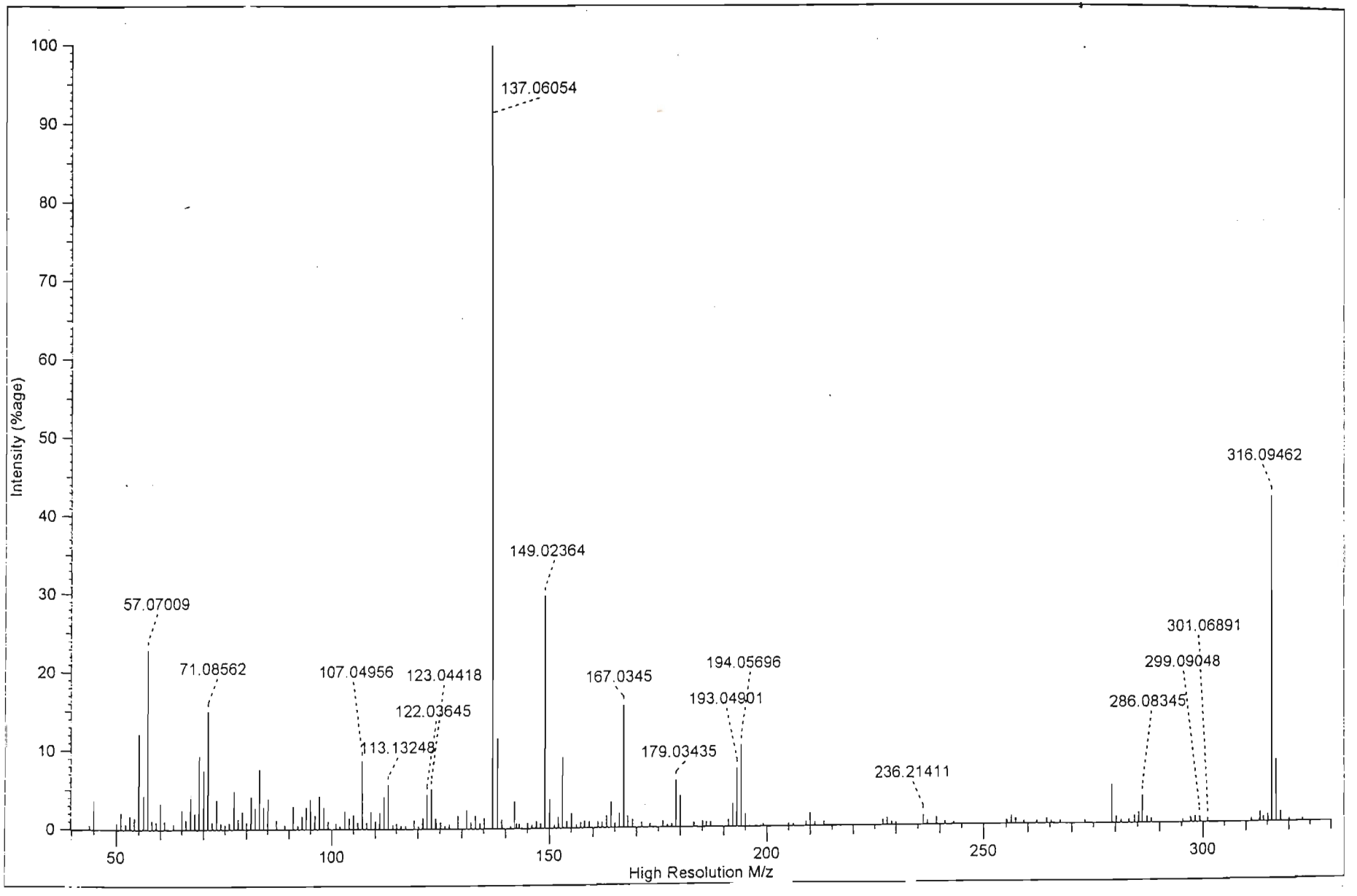
Spectrum 38: HMBC spectrum of Compound (IV) ($CDCl_3$)



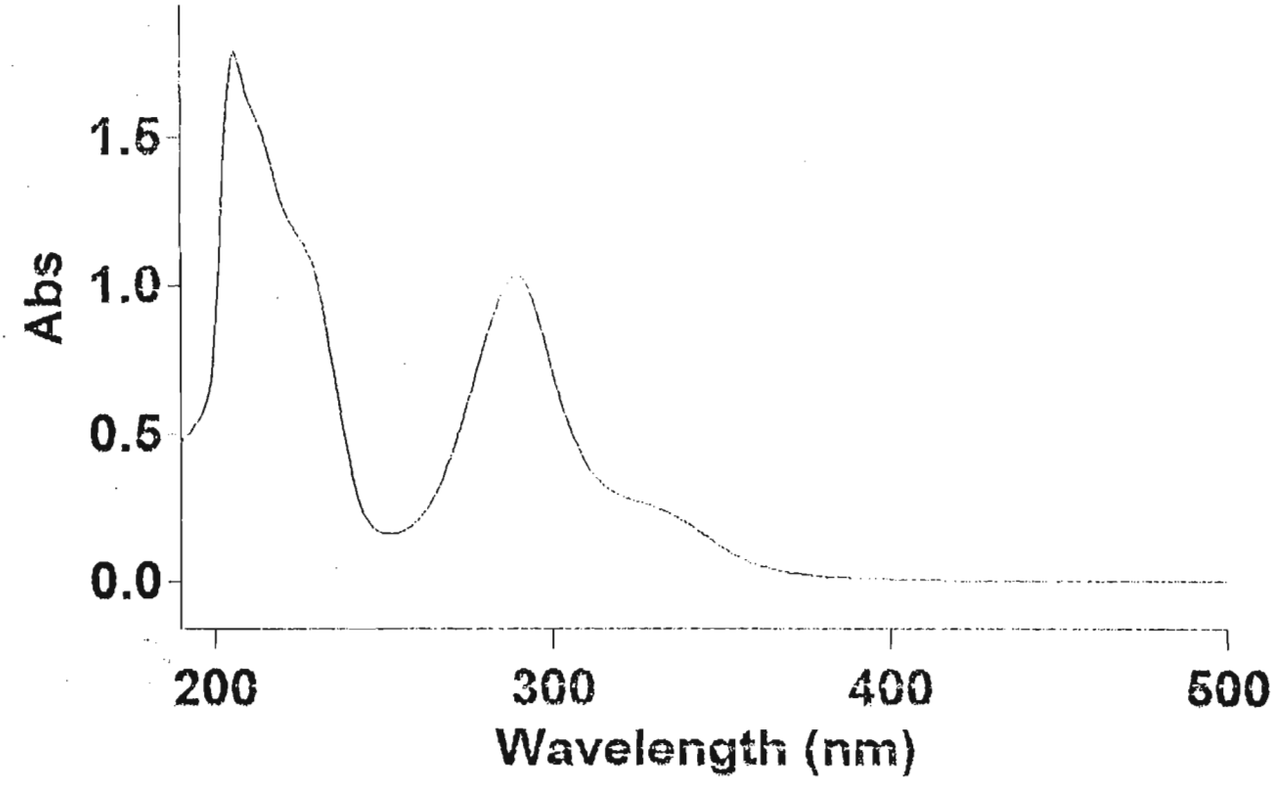
Spectrum 39: COSY spectrum of Compound (IV) ($CDCl_3$)

Spectrum 40: NOESY spectrum of Compound (IV) ($CDCl_3$)

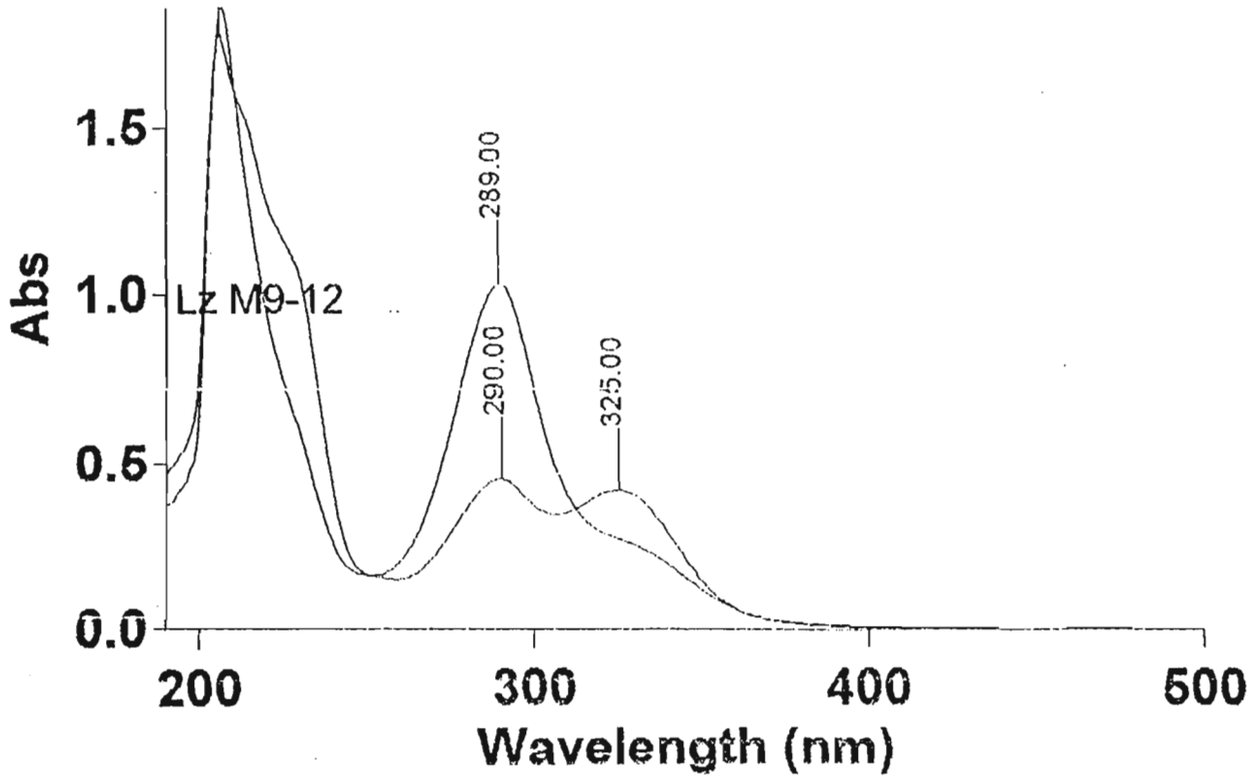
Figure 10 – 1D ROESY spectrum of Compound (IV) (CDCl3)



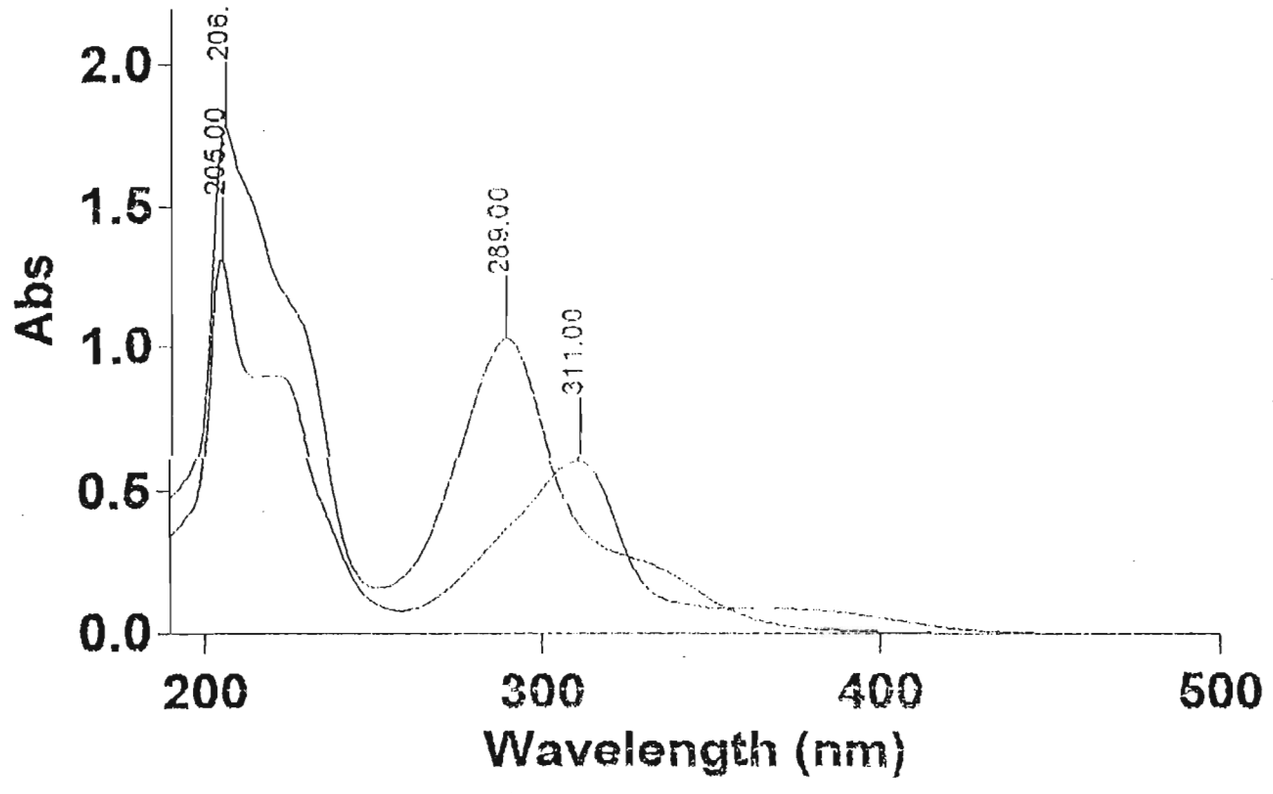
Spectrum 41: Mass spectrum of Compound (IV)



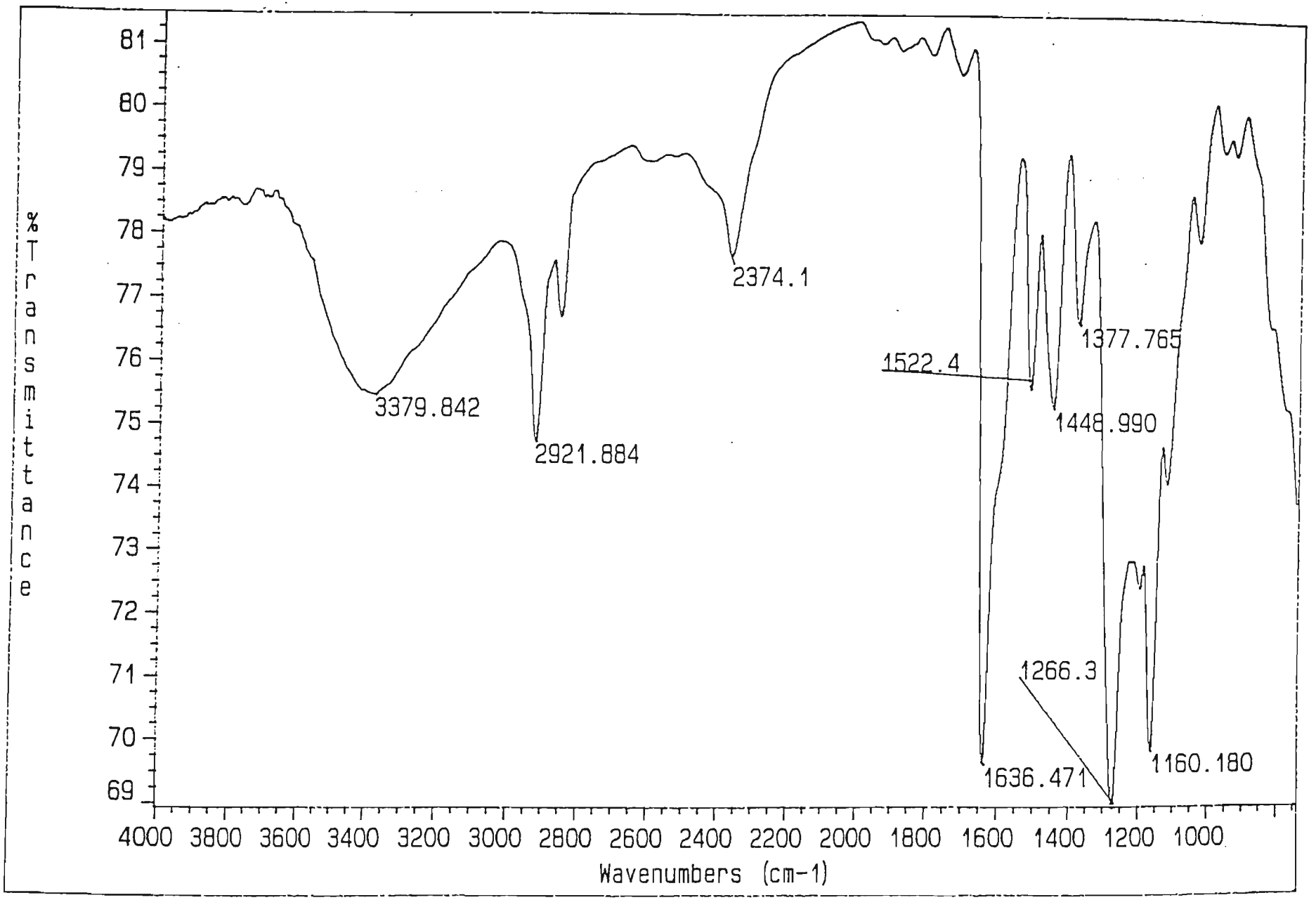
Spectrum 42: UV spectrum of Compound (IV)



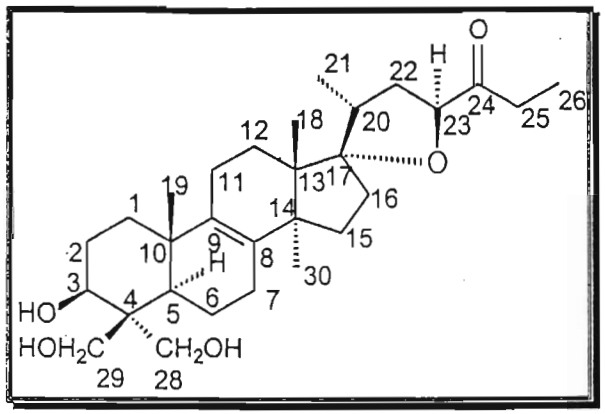
Spectrum 43: UV spectrum of Compound (IV) with NaOAc



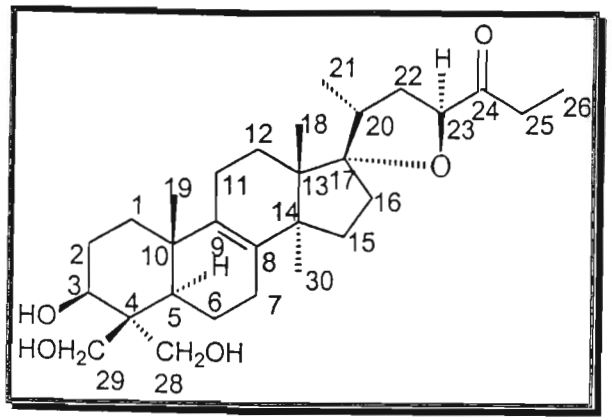
Spectrum 44: UV spectrum of Compound (IV) with $AlCl_3$

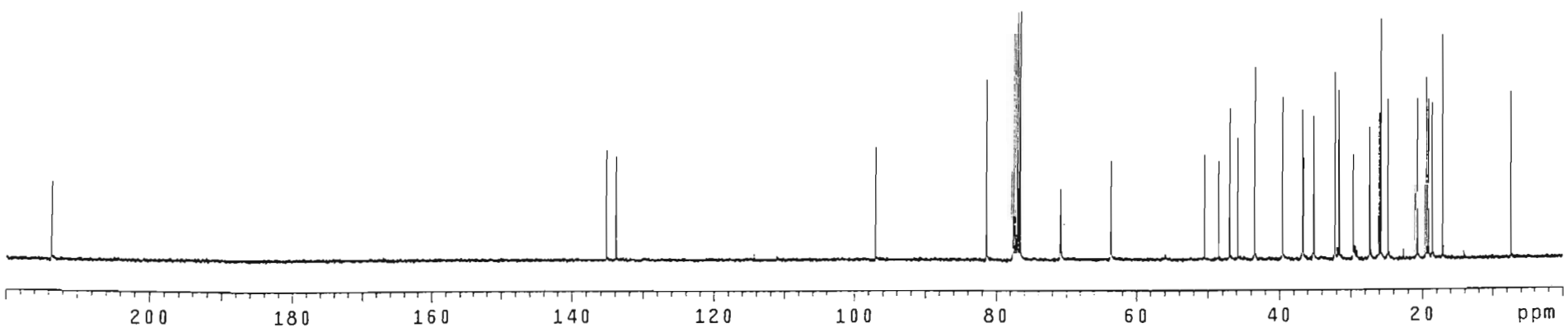


Spectrum 45: IR spectrum of Compound (IV)

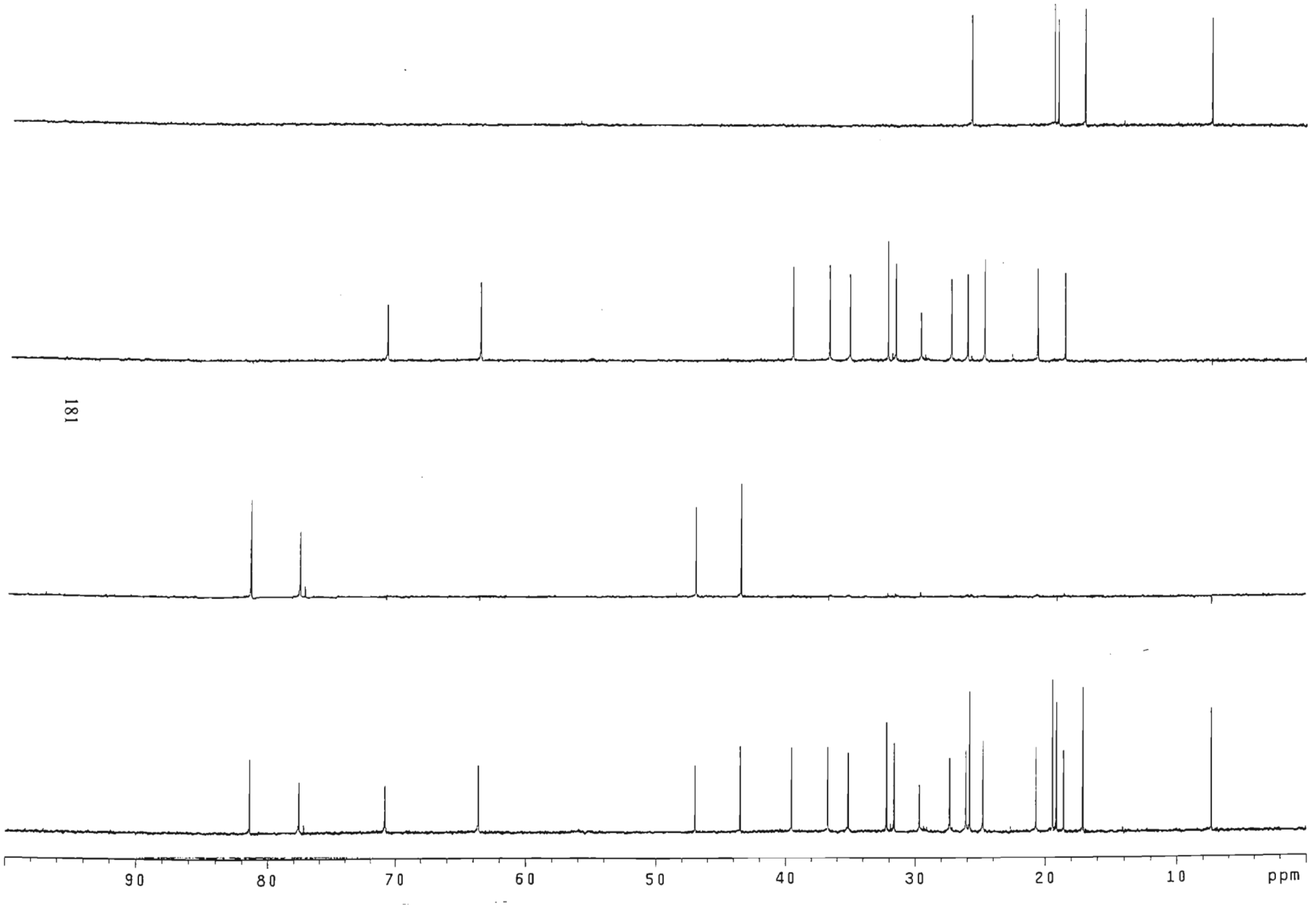


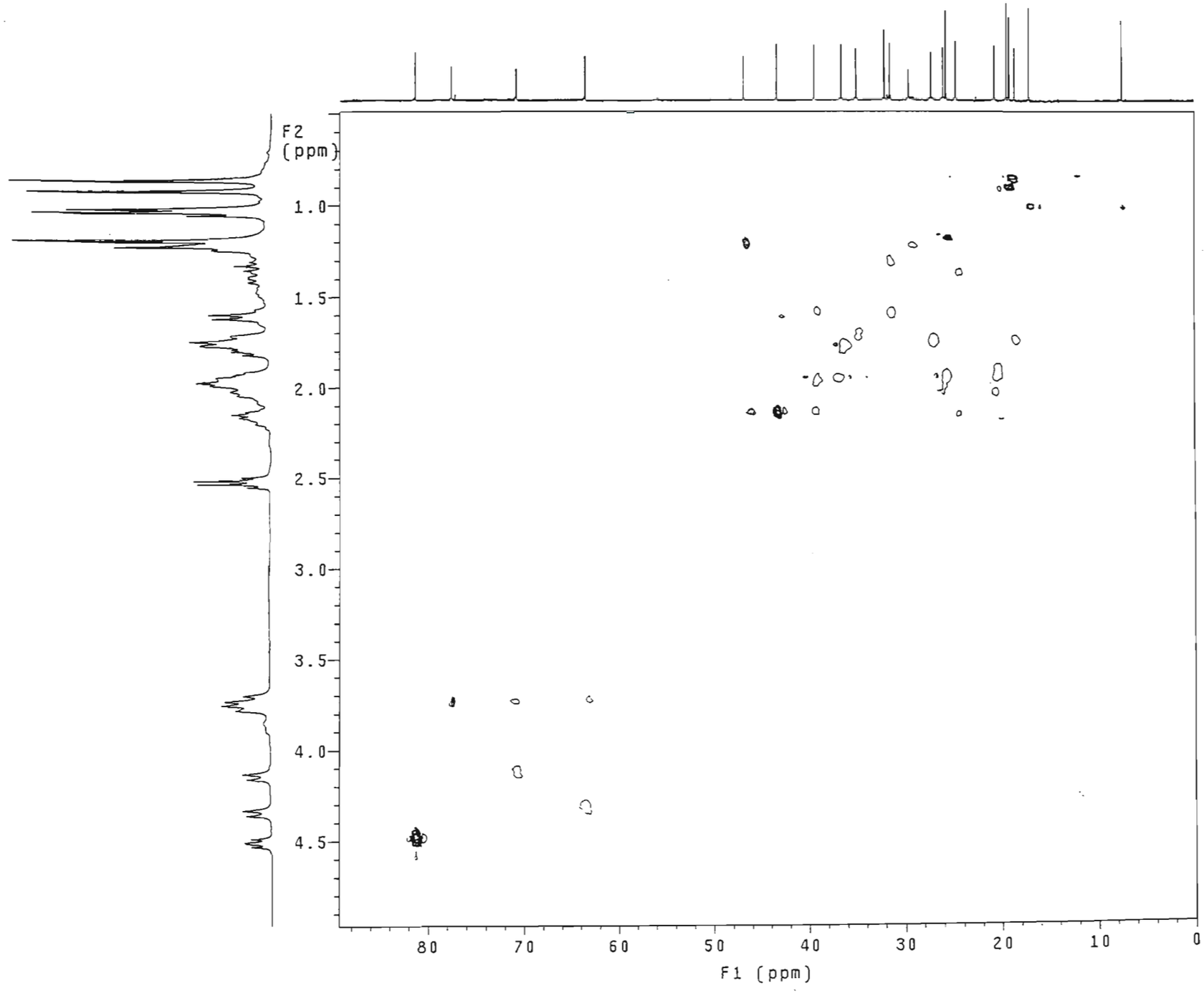
Spectrum 46: ^{1}H NMR spectrum of Compound (V) ($CDCl_3$)



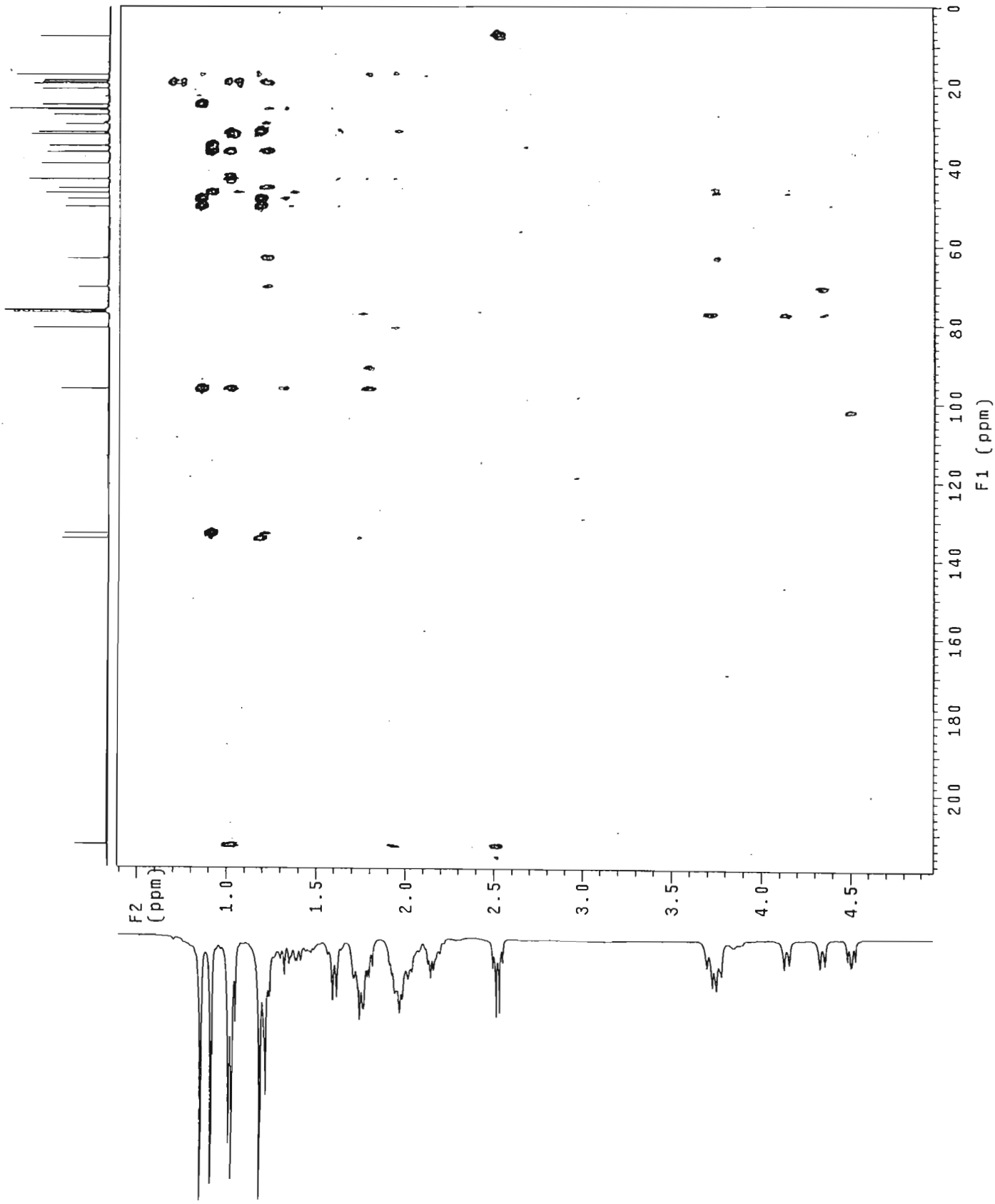


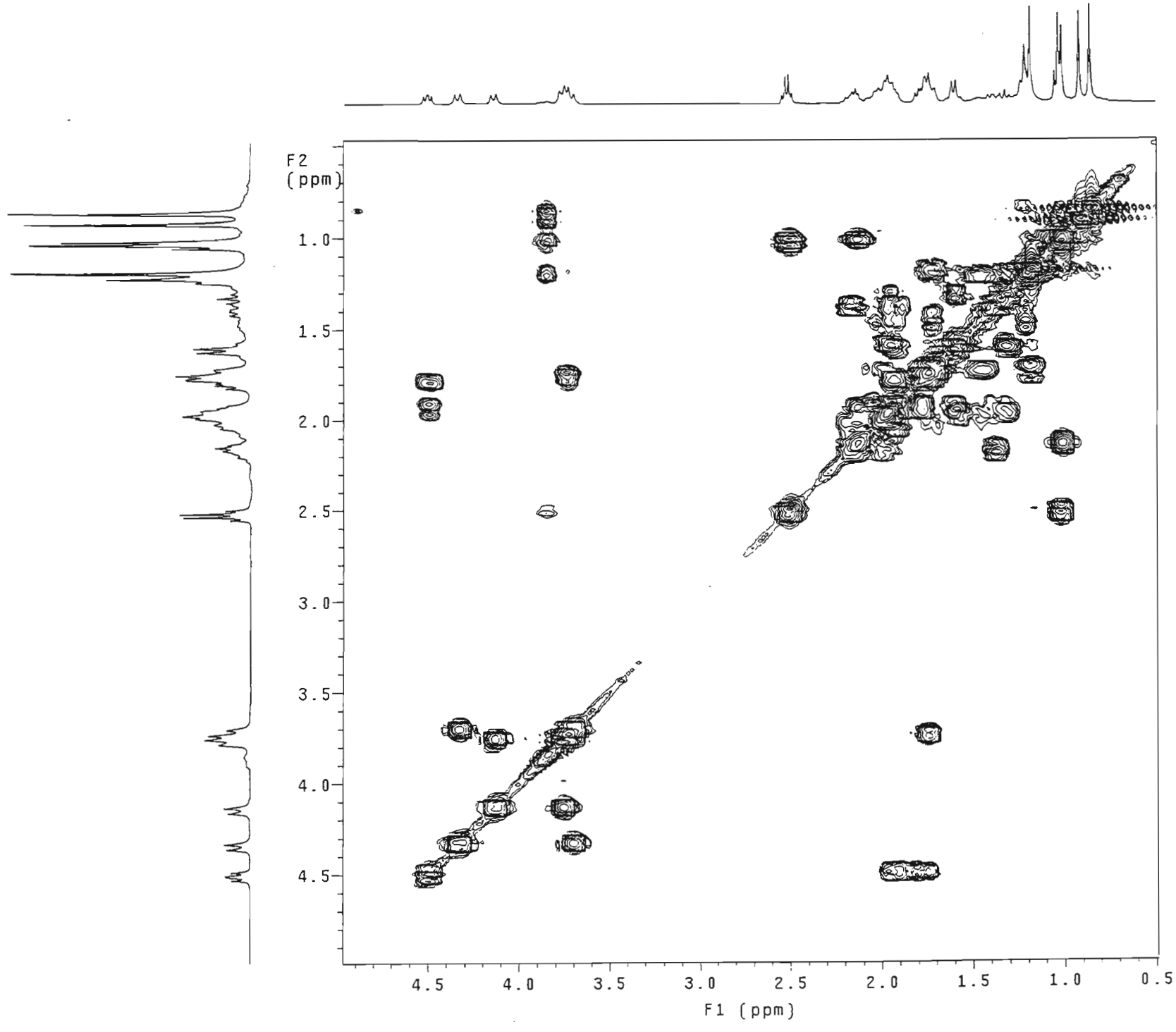
Spectrum 47: ^{13}C NMR spectrum of Compound (V) ($CDCl_3$)

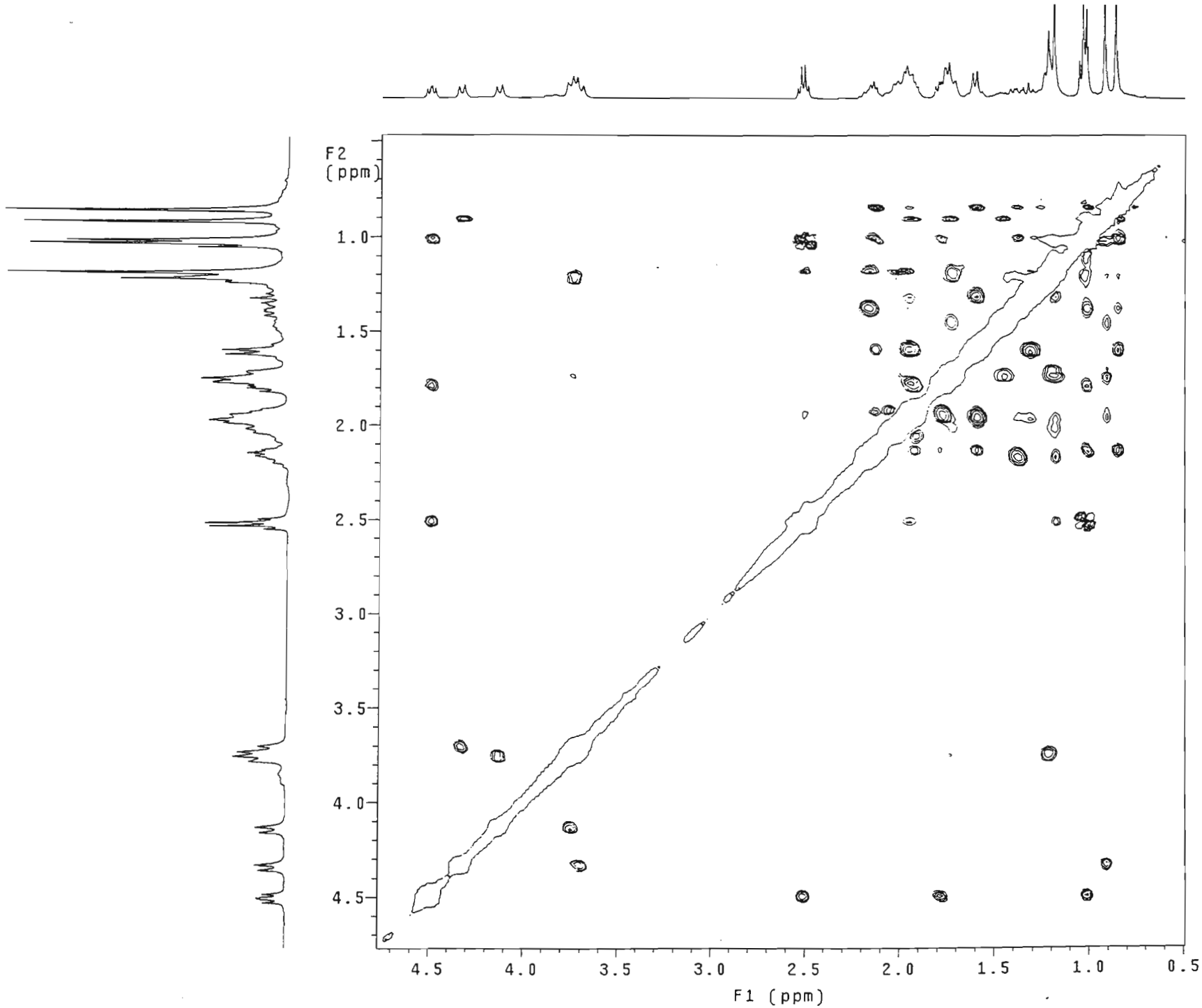

90
80
70
60
50
40
30
20
10
ppm

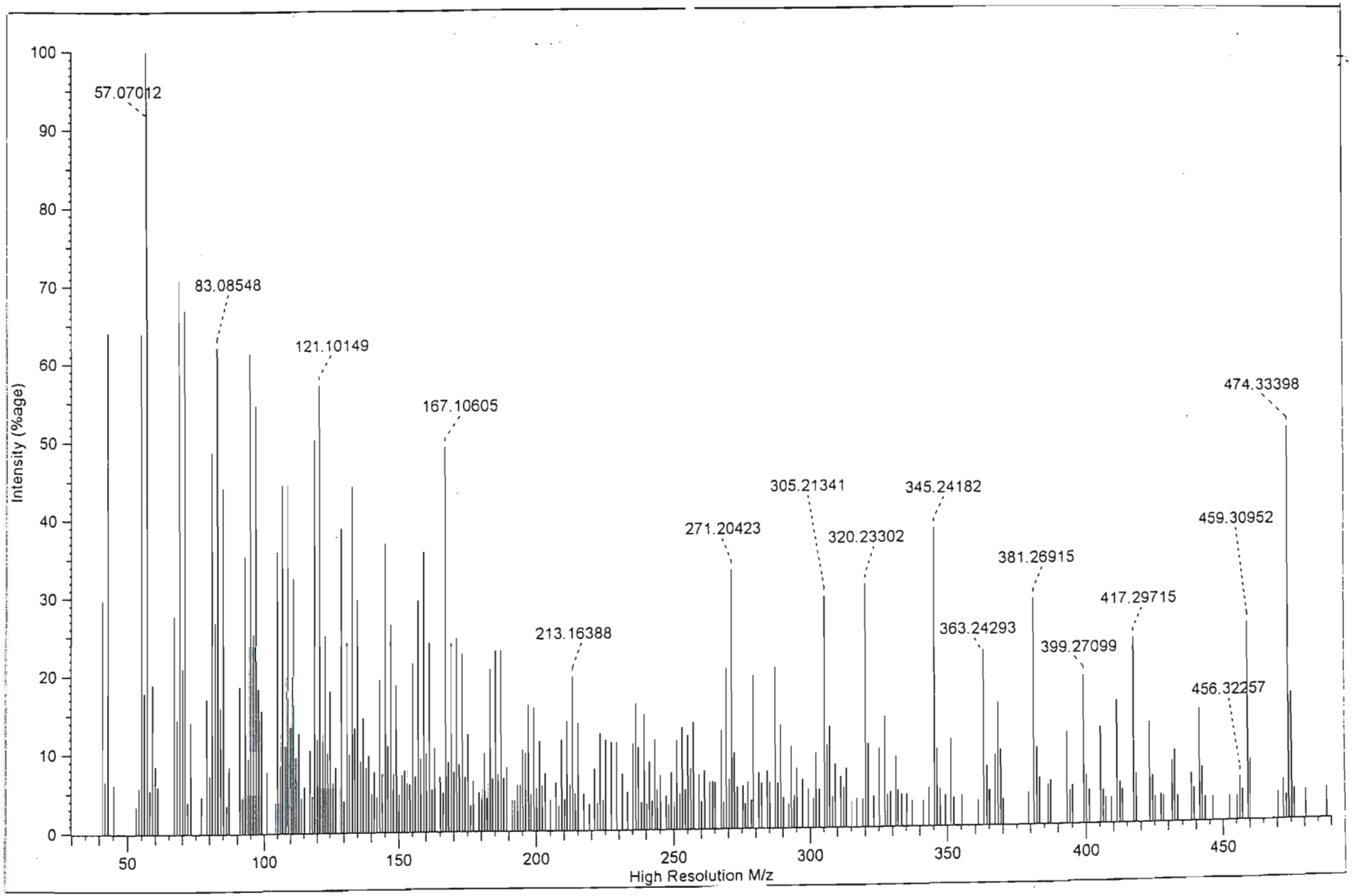


Spectrum 49: HSQC spectrum of Compound (V) ($CDCl_3$)

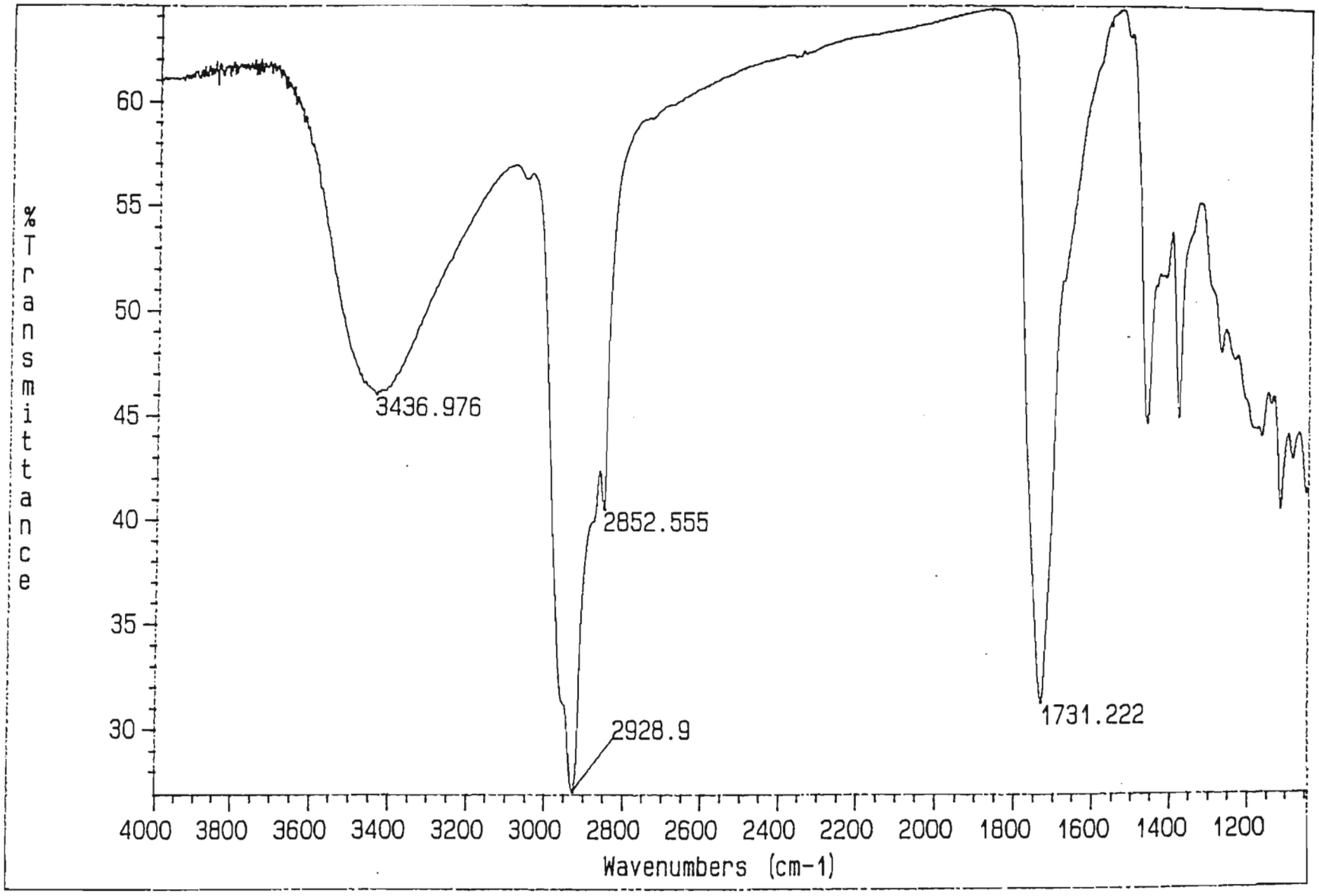

F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
F1 (ppm)
0
20
40
60
80
100
120
140
160
180
200


F2
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)

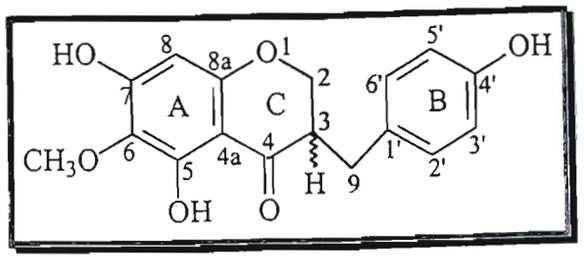

F2
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)



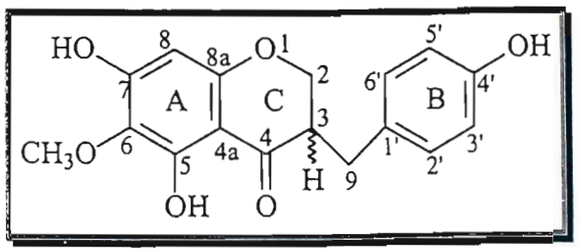
Spectrum 53: Mass spectrum of Compound (V)



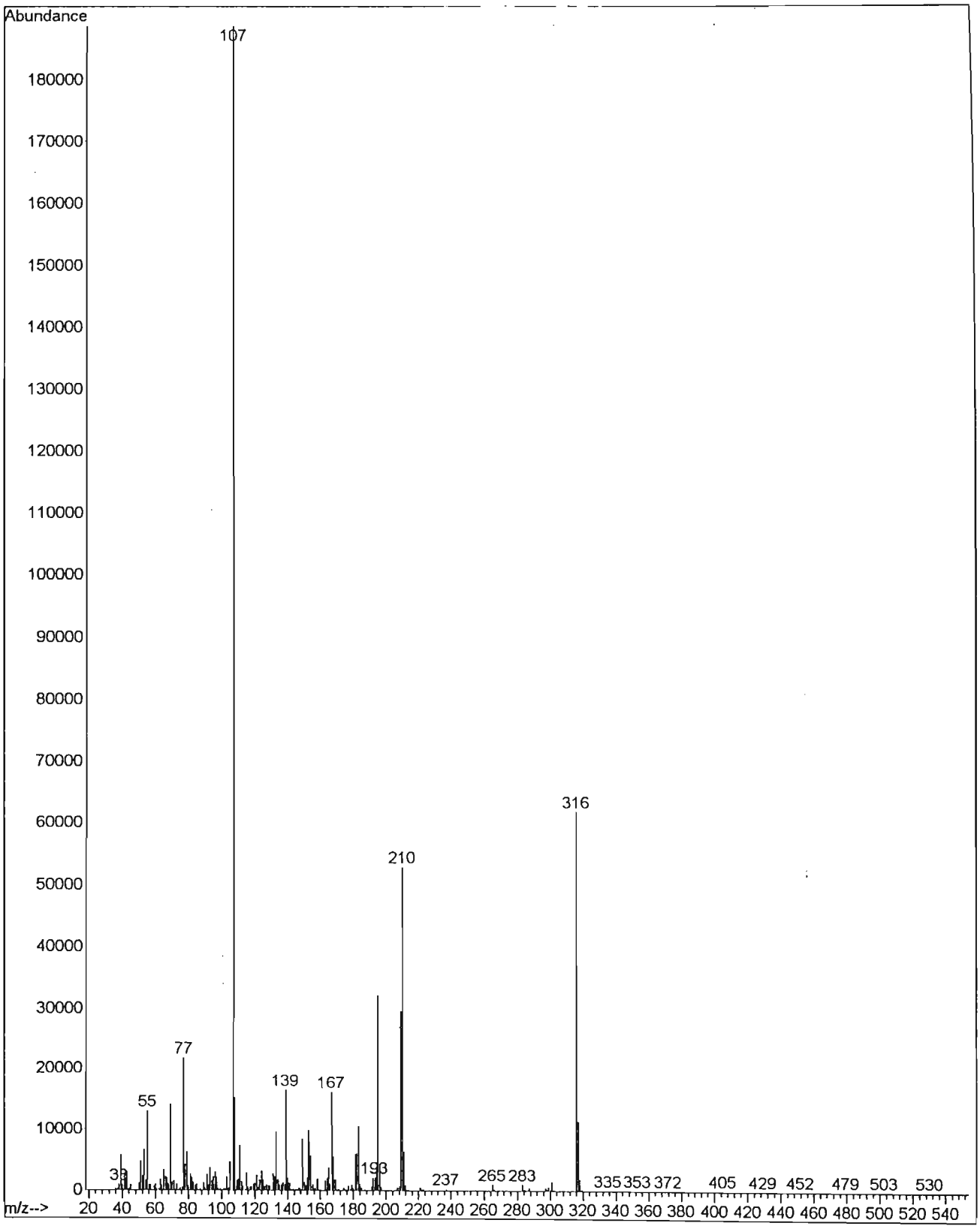
Spectrum 54: IR spectrum of Compound (V)



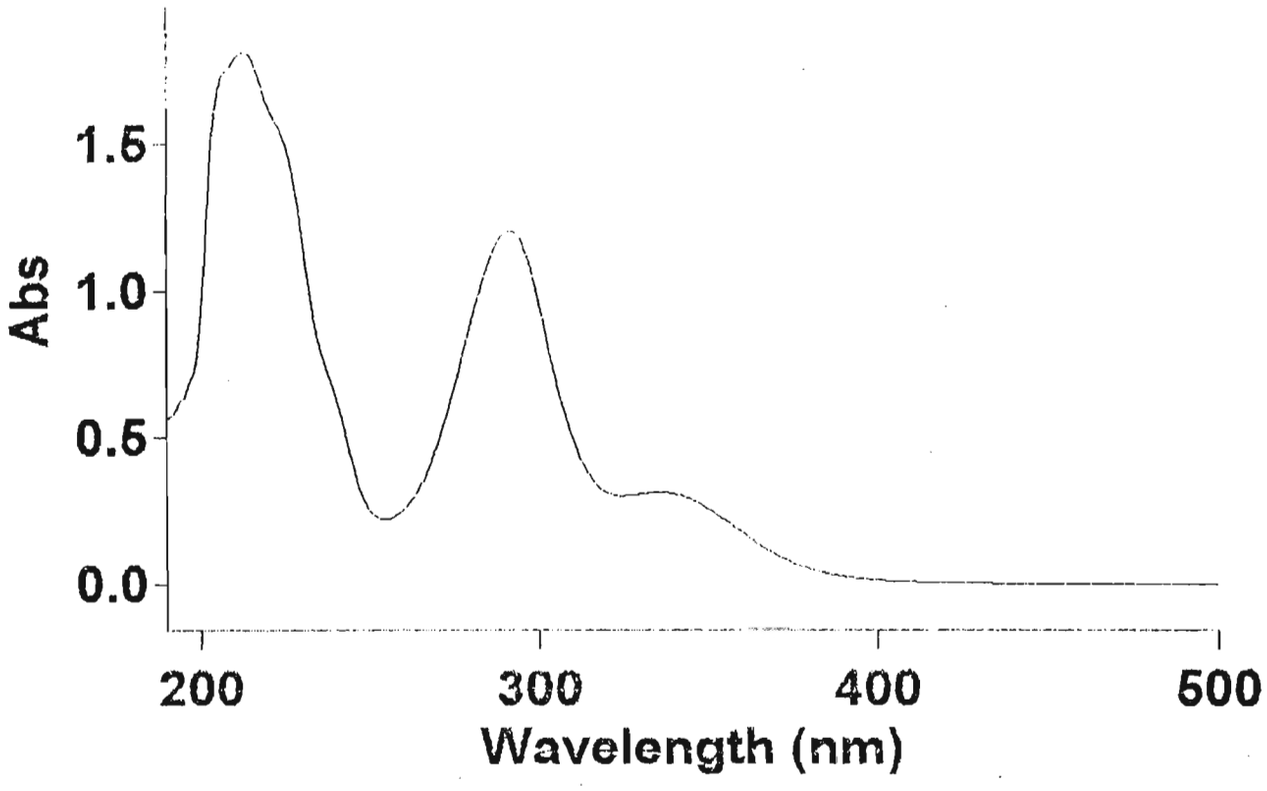
Spectrum 55: ^{1}H NMR spectrum of Compound (VI) (CD_3OD)



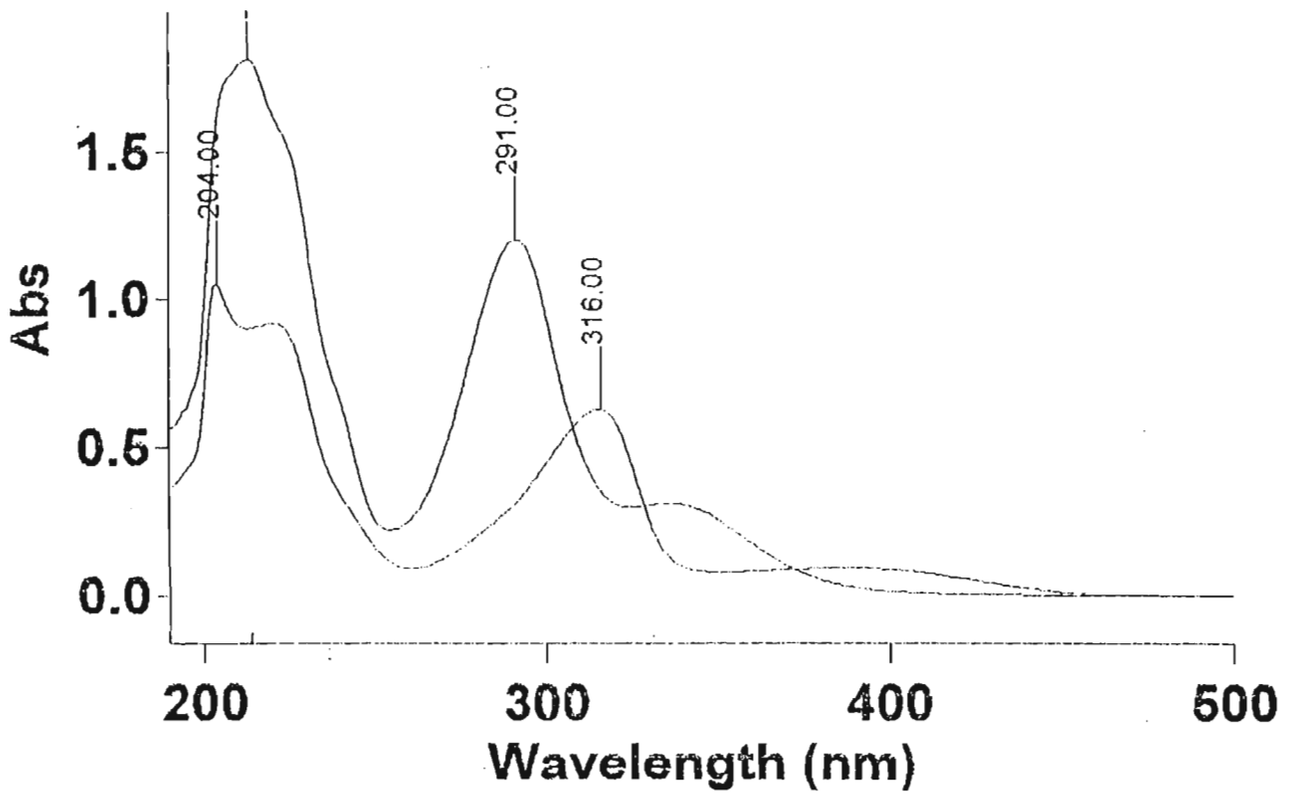
Spectrum 56: ^{13}C NMR spectrum of Compound (VI) (CD_3OD)



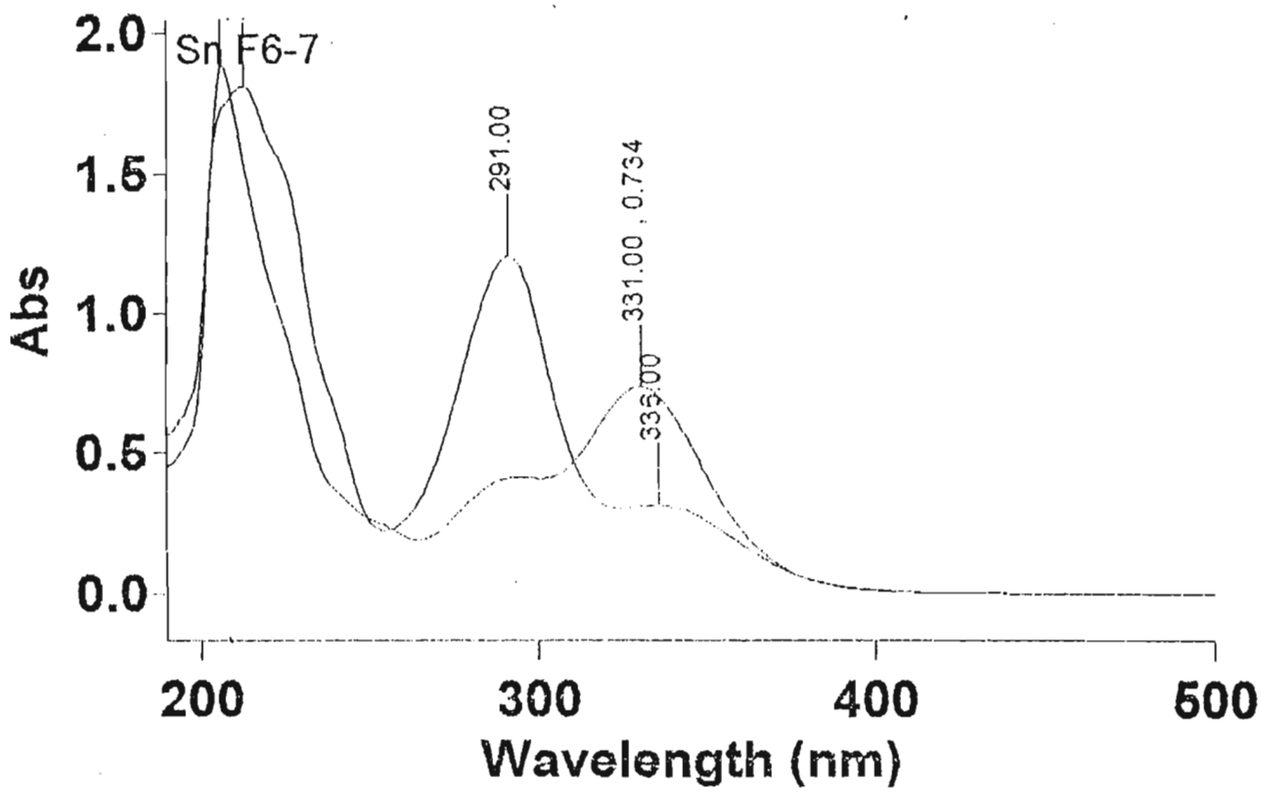
Spectrum 57: Mass spectrum of Compound (VI)



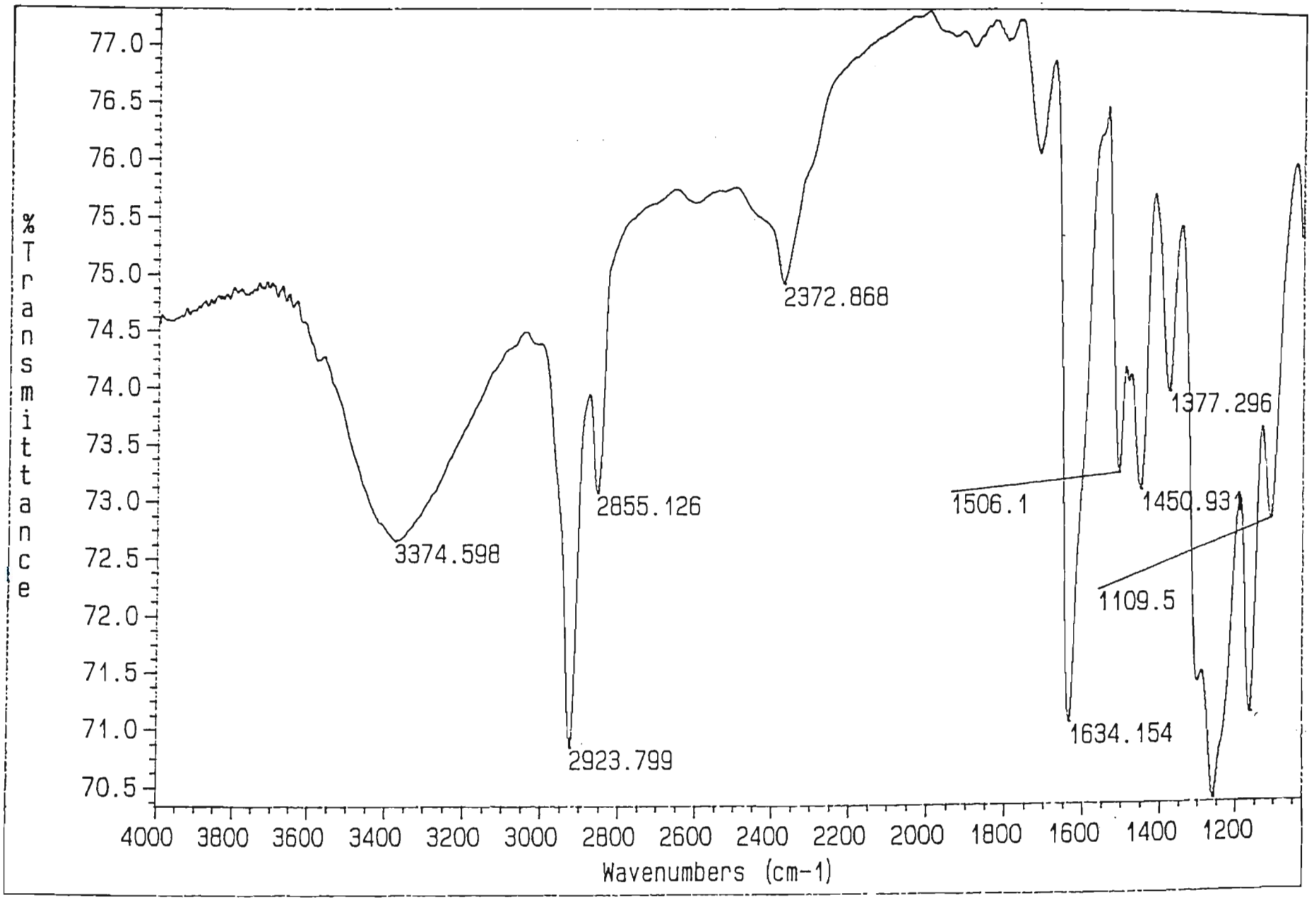
Spectrum 58: UV spectrum of Compound (VI)

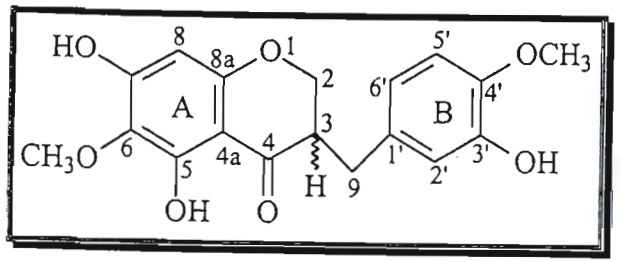


Spectrum 59: UV spectrum of Compound (VI) with NaOAc

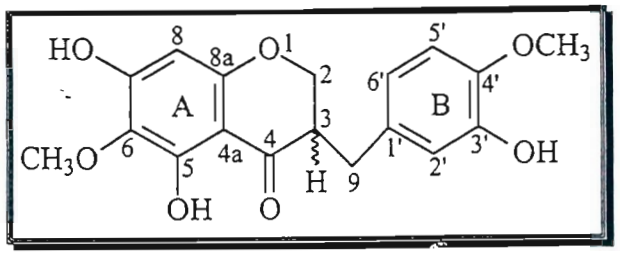


Spectrum 60: UV spectrum of Compound (VI) with $AlCl_3$


%Transmittance
77.0
76.5
76.0
75.5
75.0
74.5
74.0
73.5
73.0
72.5
72.0
71.5
71.0
70.5
3374.598
2923.799
2855.126
2372.868
1506.1
1634.154
1450.931
1377.296
1109.5
4000 3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200
Wavenumbers (cm-1)

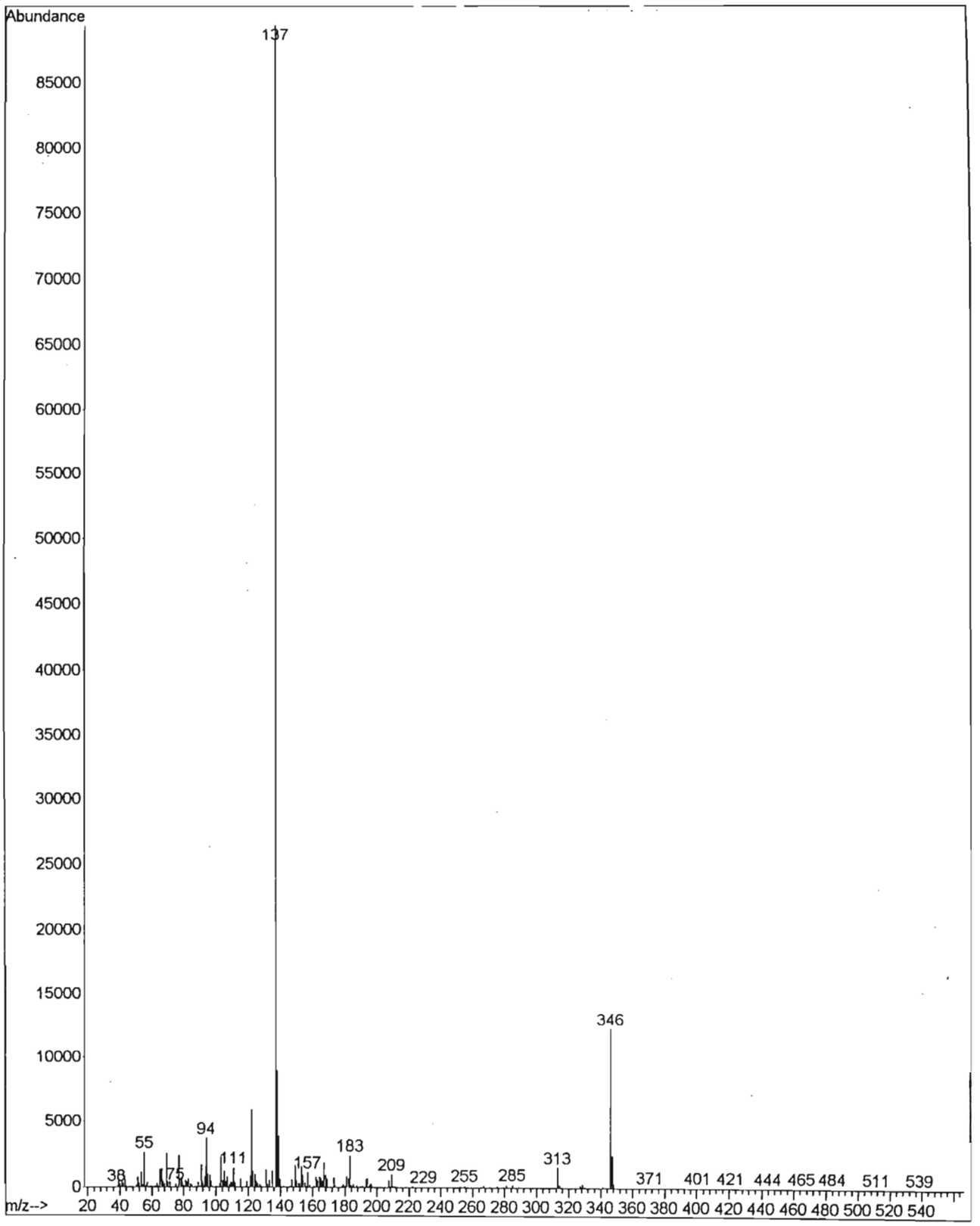


Spectrum 62: ^1H NMR spectrum of Compound (VII) (CD_3OD)

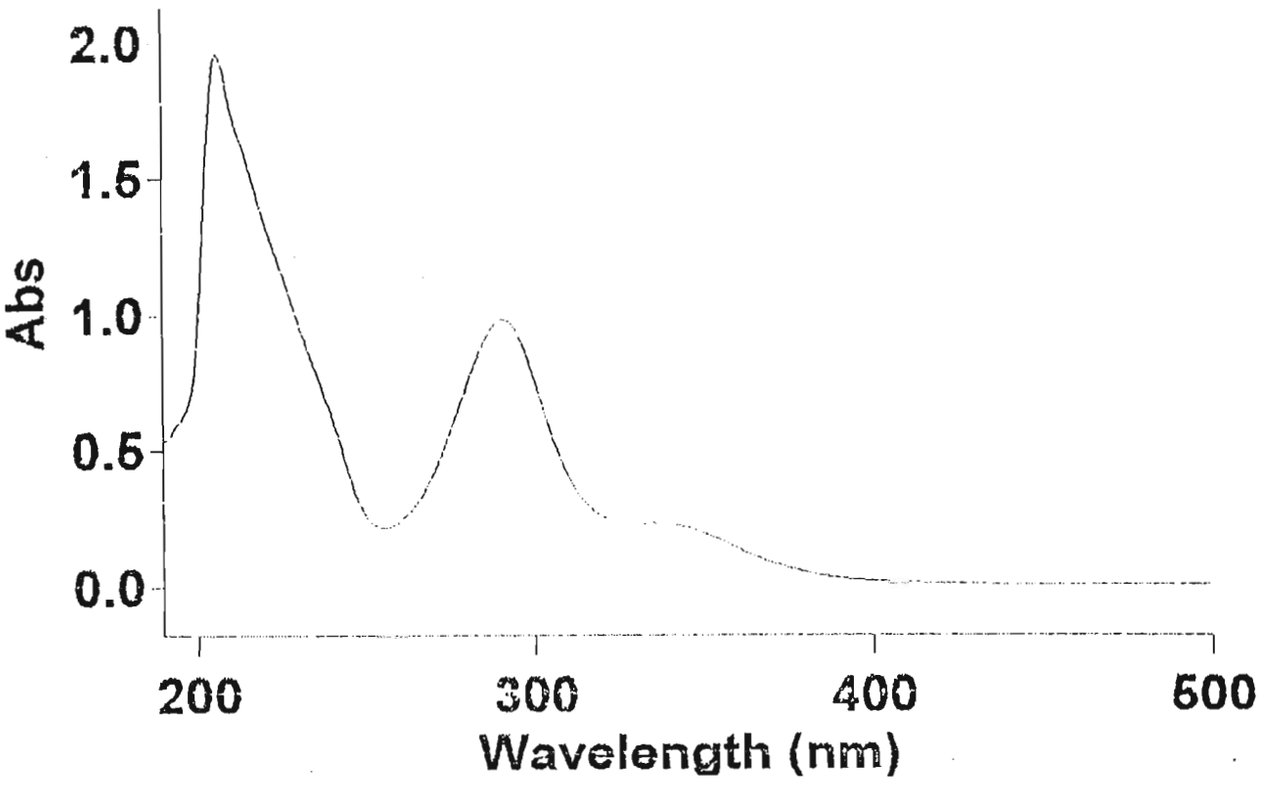

HO
8
8a
O
1
2
3
4
4a
5
6
CH_3O
OH
O
H
9
A
B
5'
6'
4'
3'
2'
1'
OCH_3
OH

196

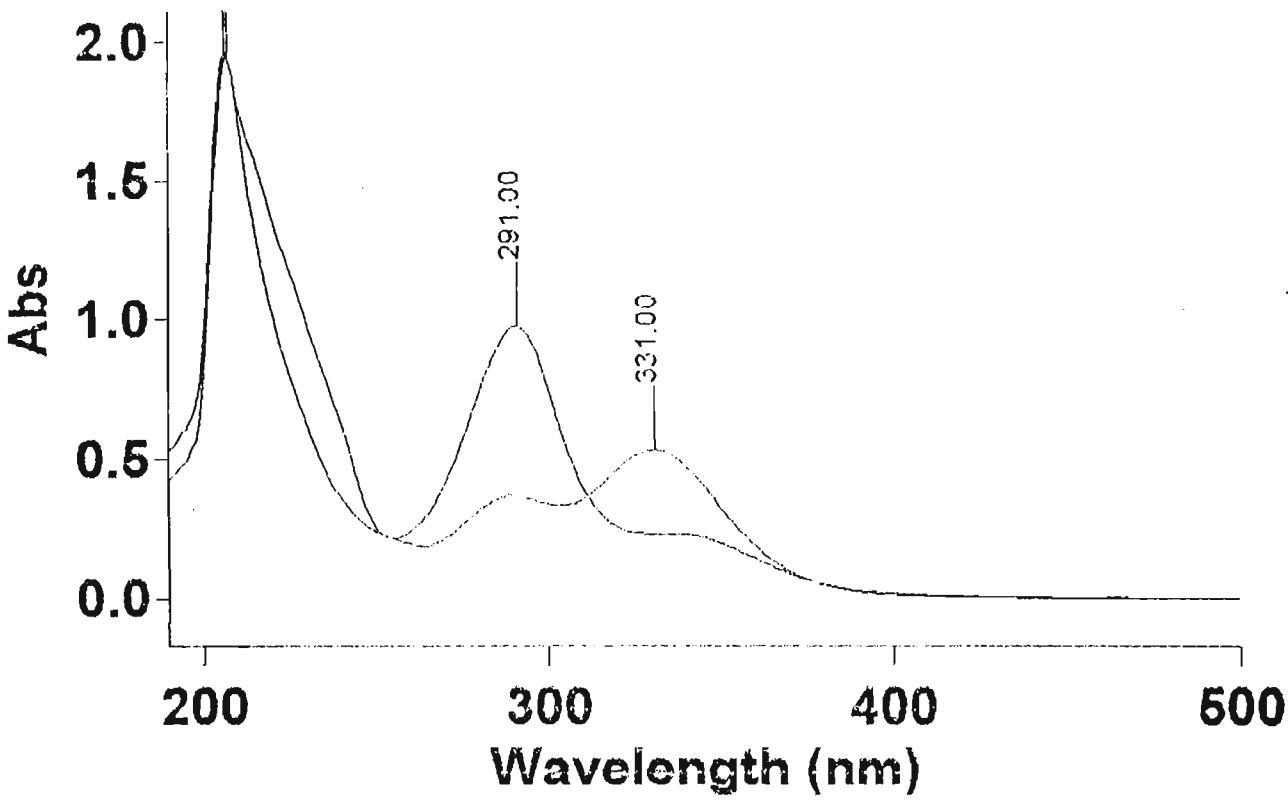
200
180
160
140
120
100
80
60
40
20
ppm



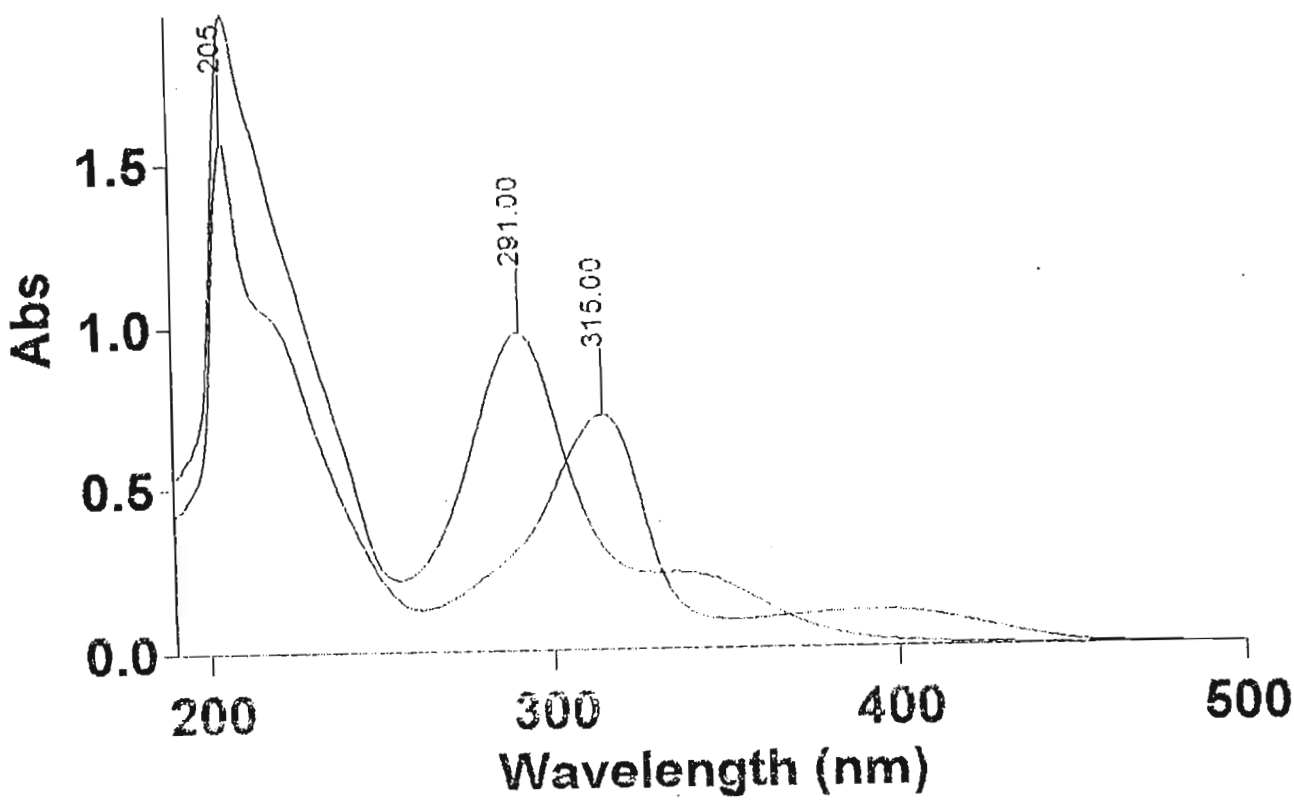
Spectrum 64: Mass spectrum of Compound (VII)



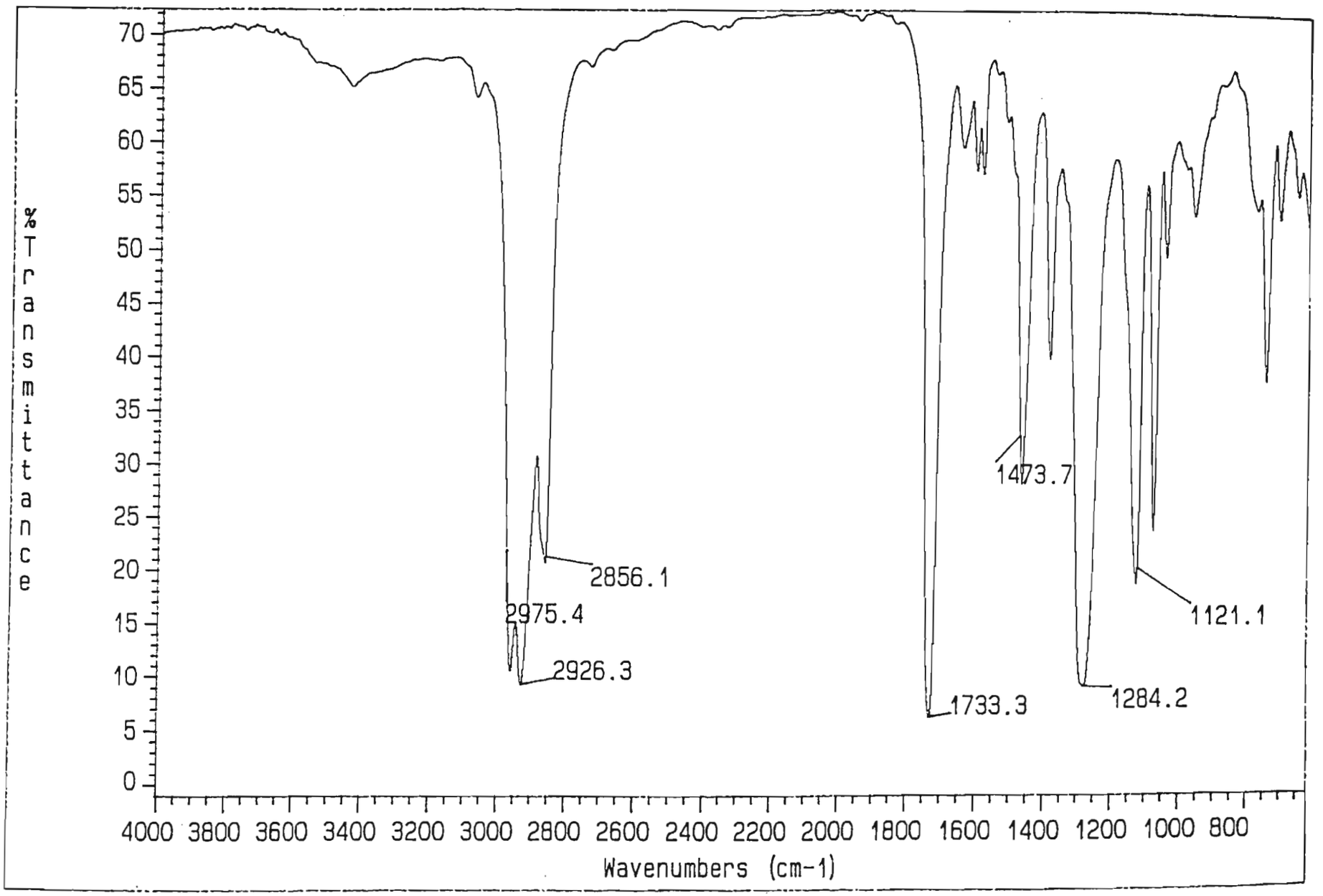
Spectrum 65: UV spectrum of Compound (VII)



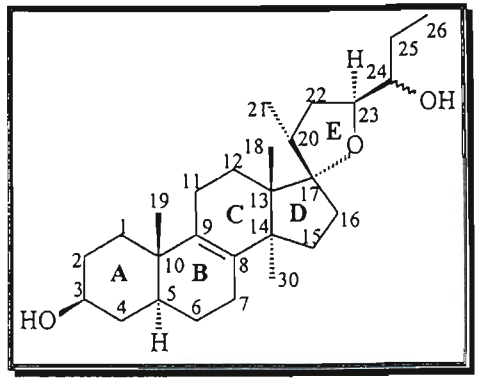
Spectrum 66: UV spectrum of Compound (VII) with NaOAc



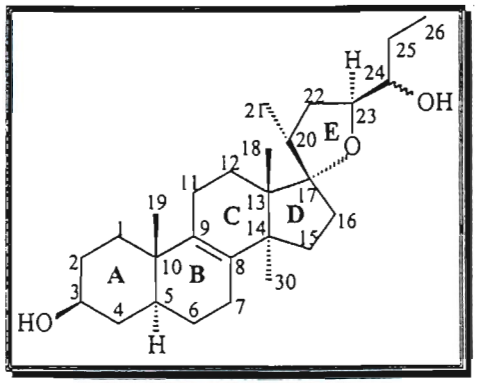
Spectrum 67: UV spectrum of Compound (VII) with $AlCl_3$



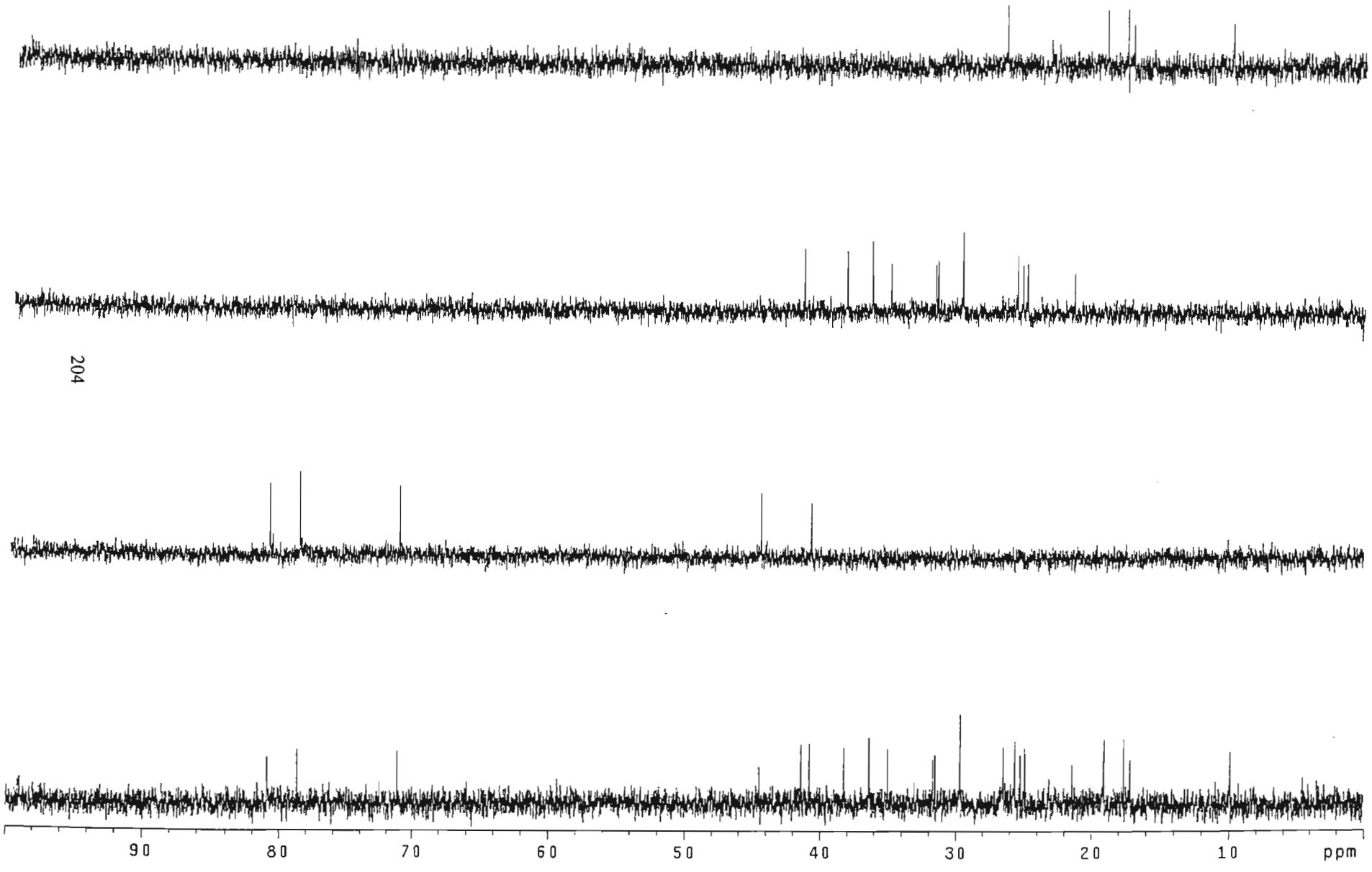
Spectrum 68: IR spectrum of Compound (VII)

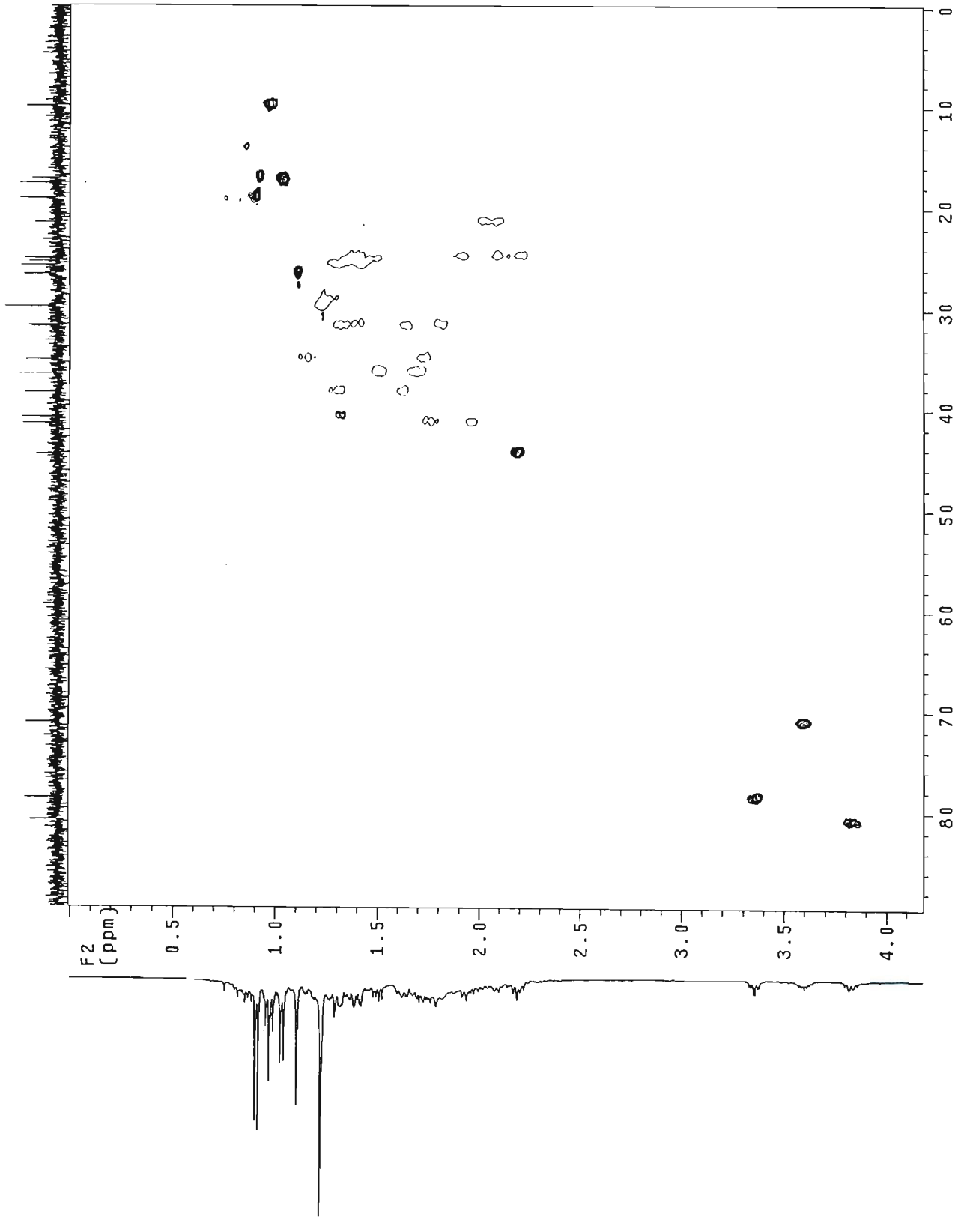


Spectrum 69: ^{1}H NMR spectrum of Compound (VIII) ($CDCl_3$)

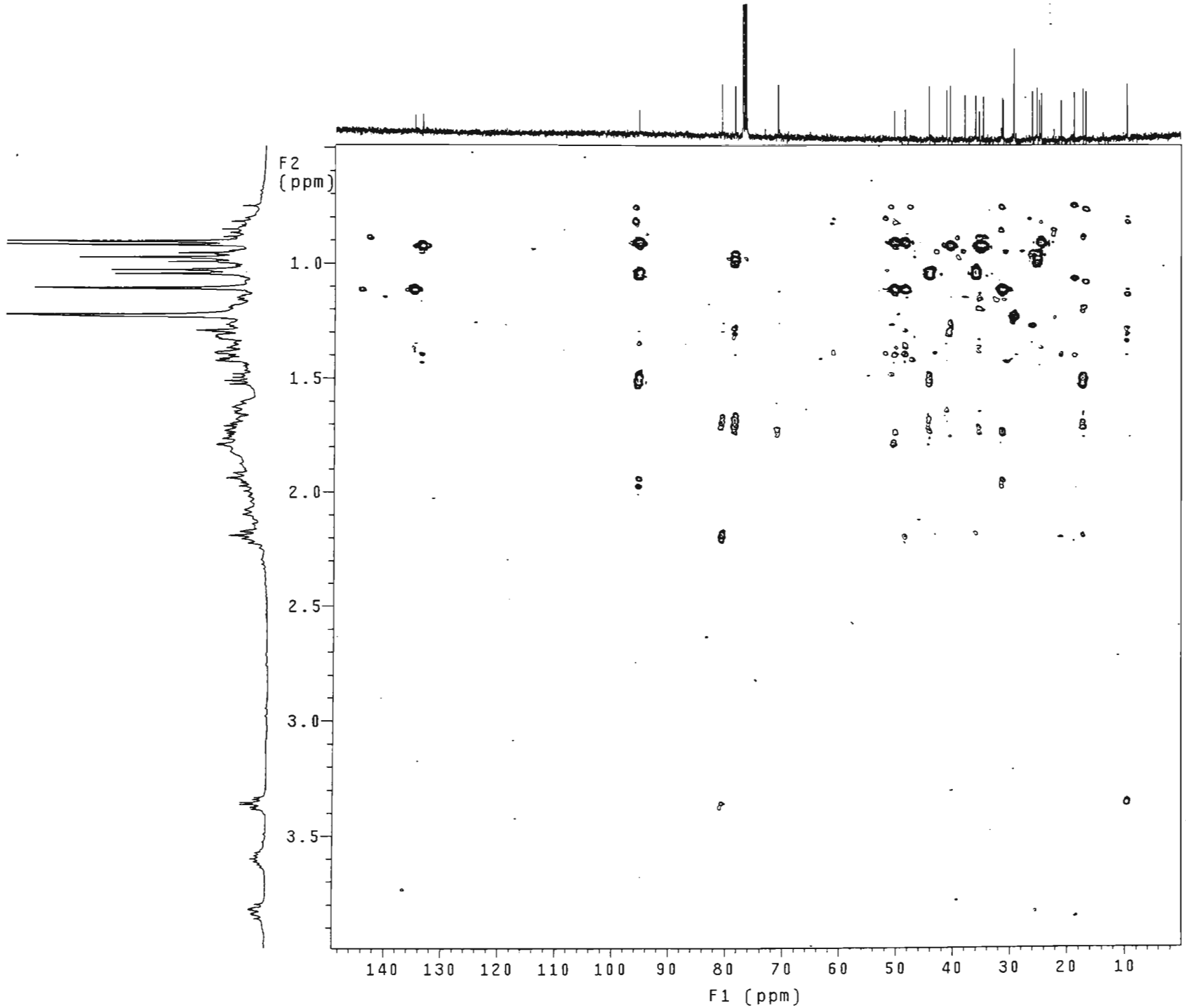


Spectra 70: ^{13}C NMR spectrum of Compound (VIII) ($CDCl_3$)

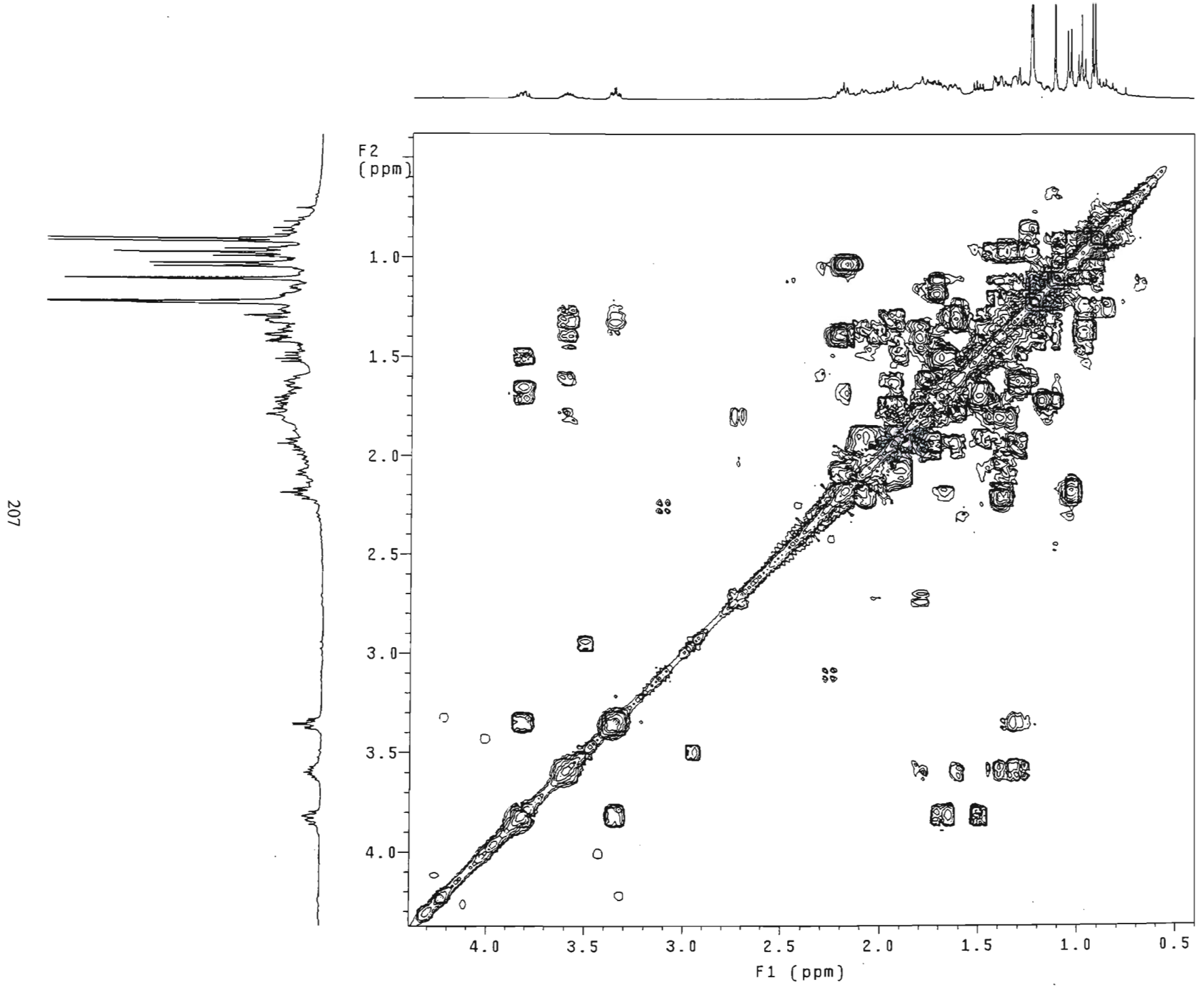

204
90
80
70
60
50
40
30
20
10
ppm



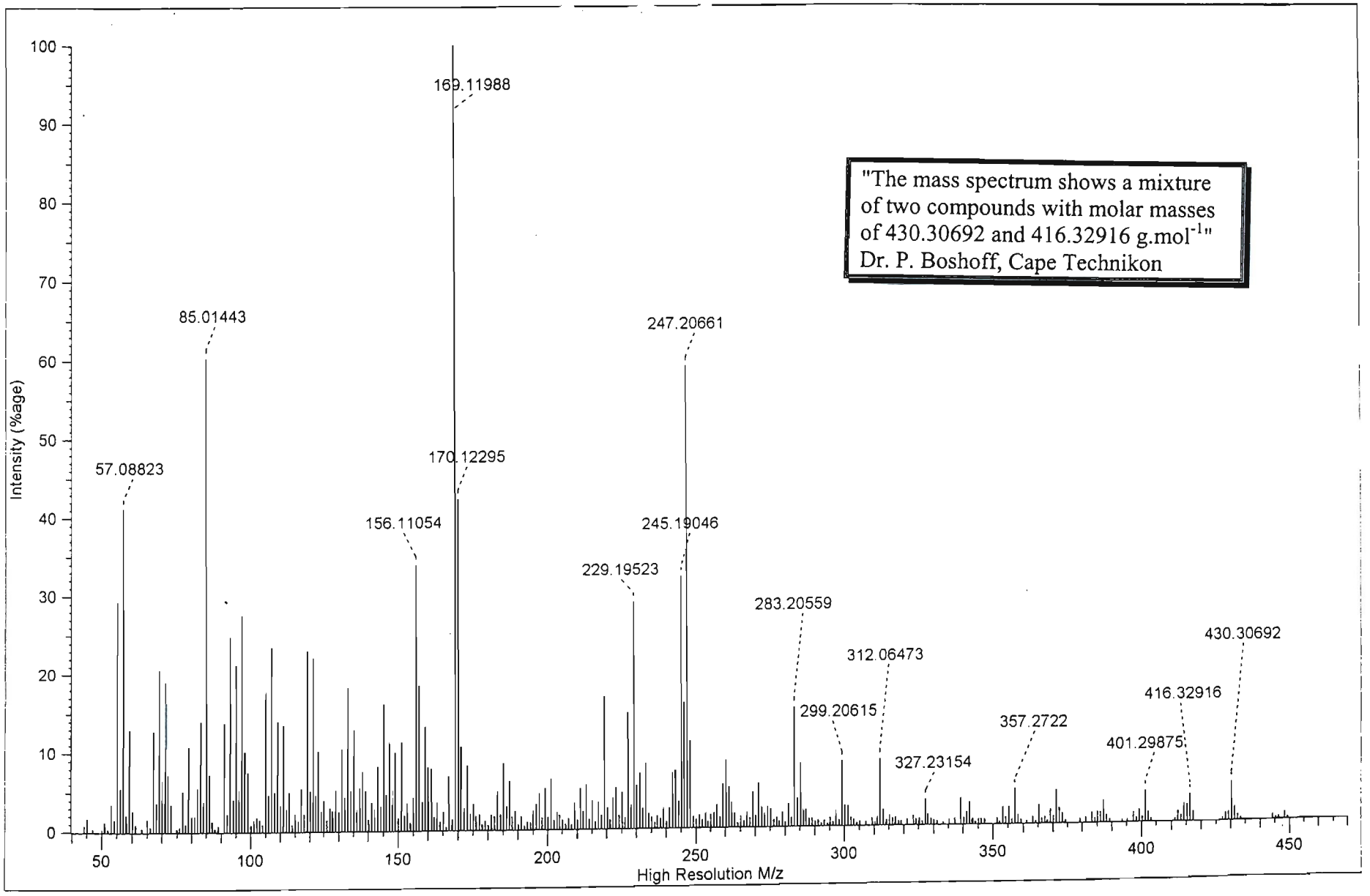
Spectrum 72: HSQC spectrum of Compound (VIII) ($CDCl_3$)



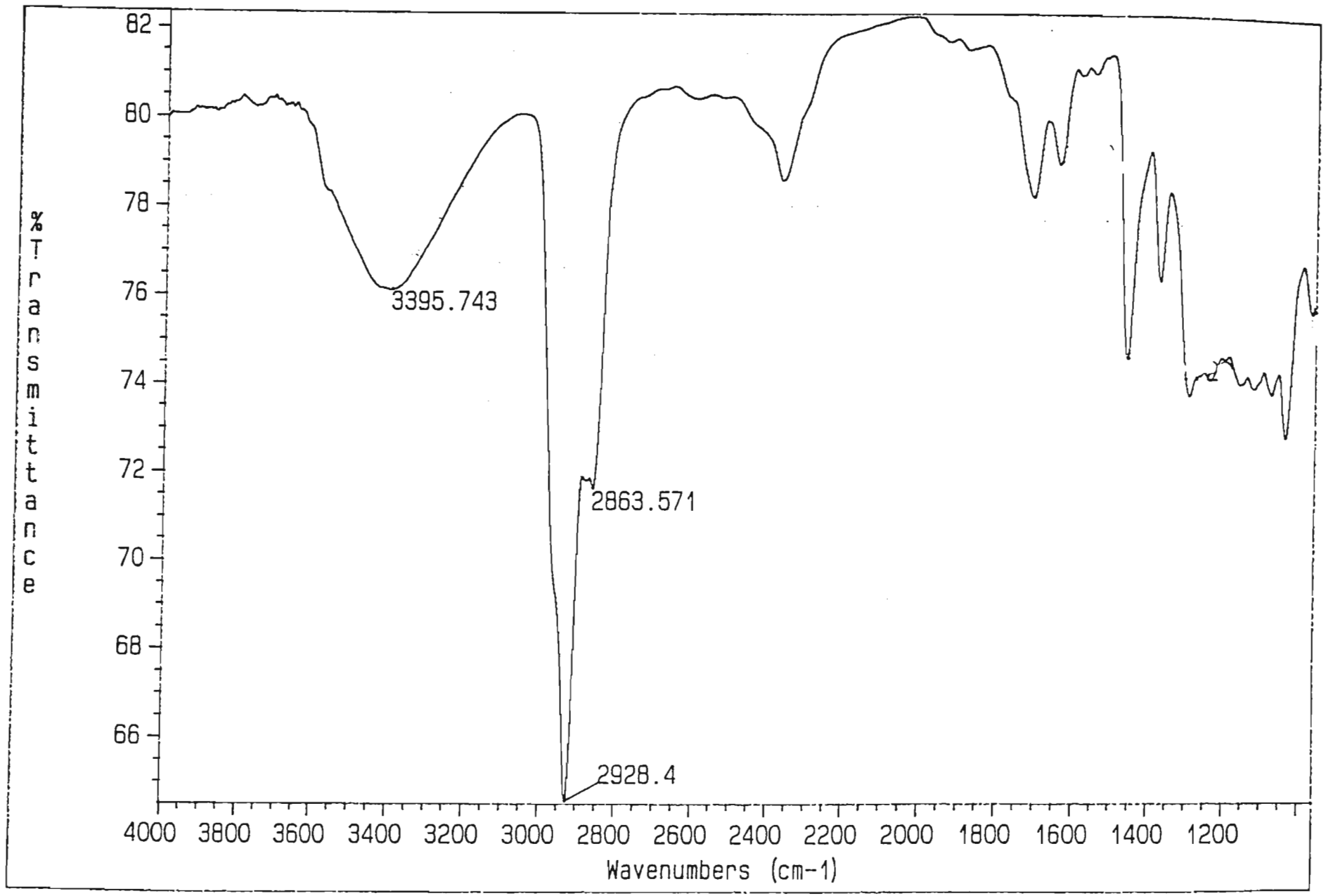
Spectrum 73. HMBC [illegible] of Compound (VIII) ($CDCl_3$)


F2
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)

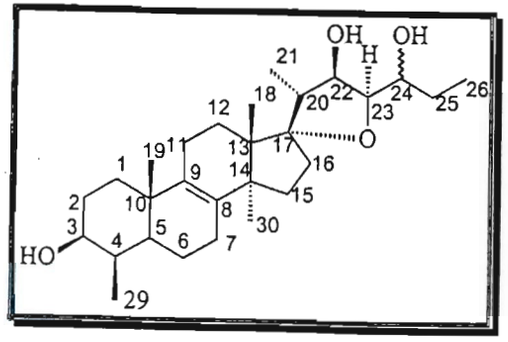
Spectrum 75: NOESY spectrum of Compound (XIII) (CDCl3)

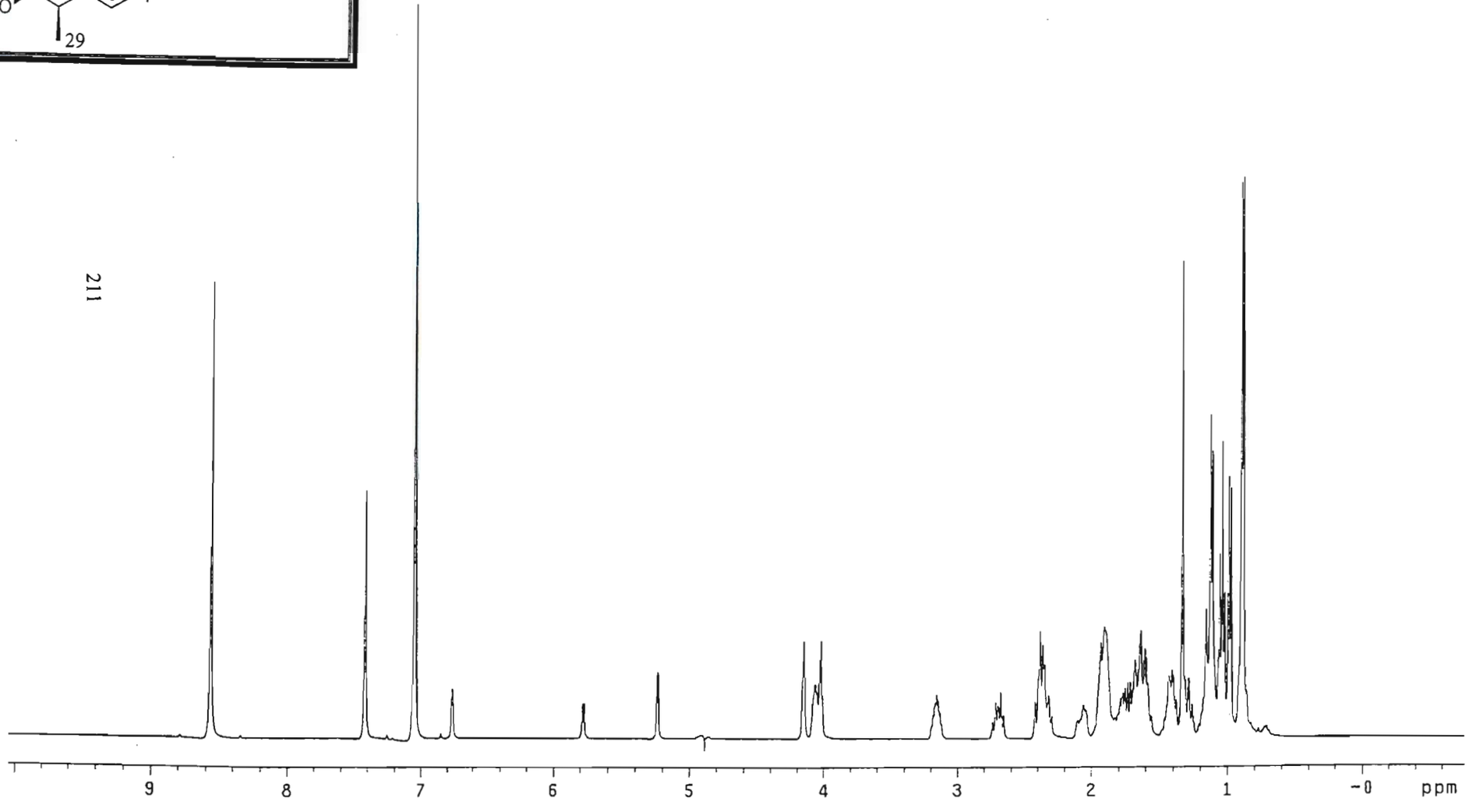


Spectrum 76: Mass spectrum of Compound (VIII)

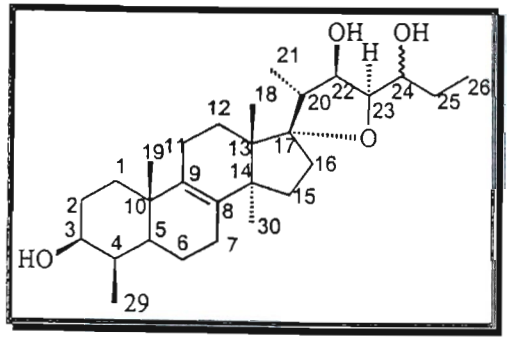


Spectrum 77: IR spectrum of Compound (VIII)

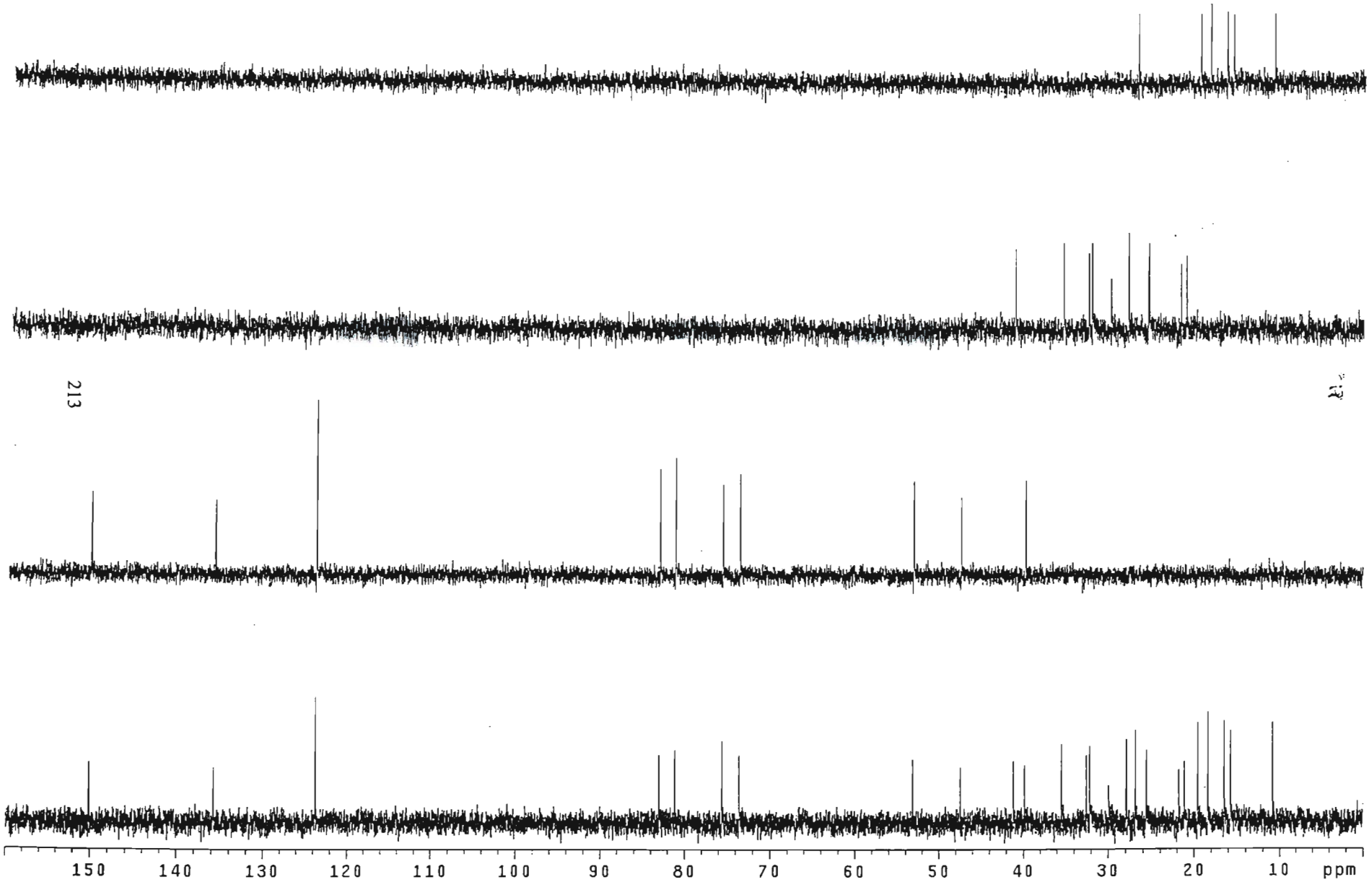




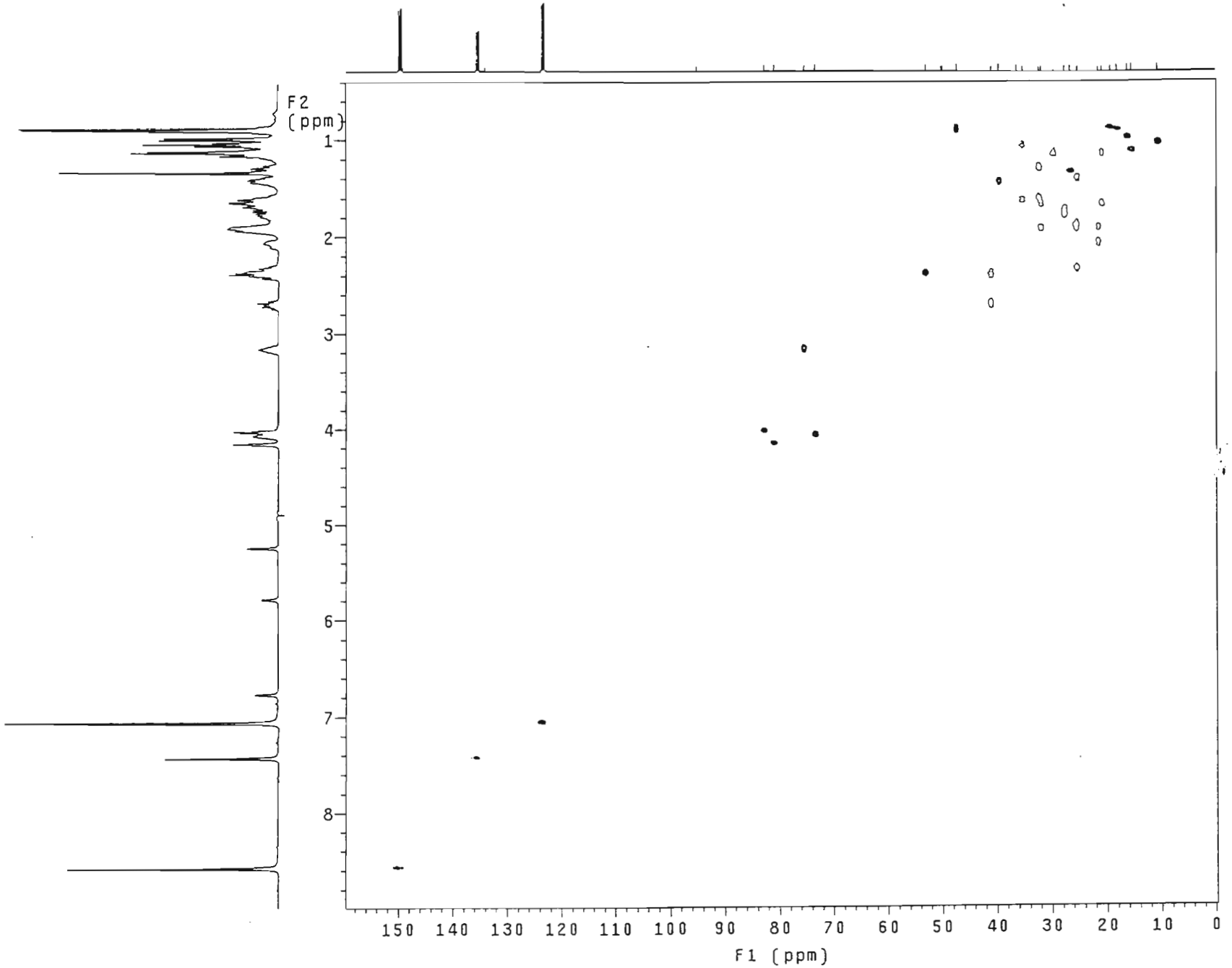
Spectrum 78: ^{1}H NMR spectrum of Compound (IX) (C_5D_5N)



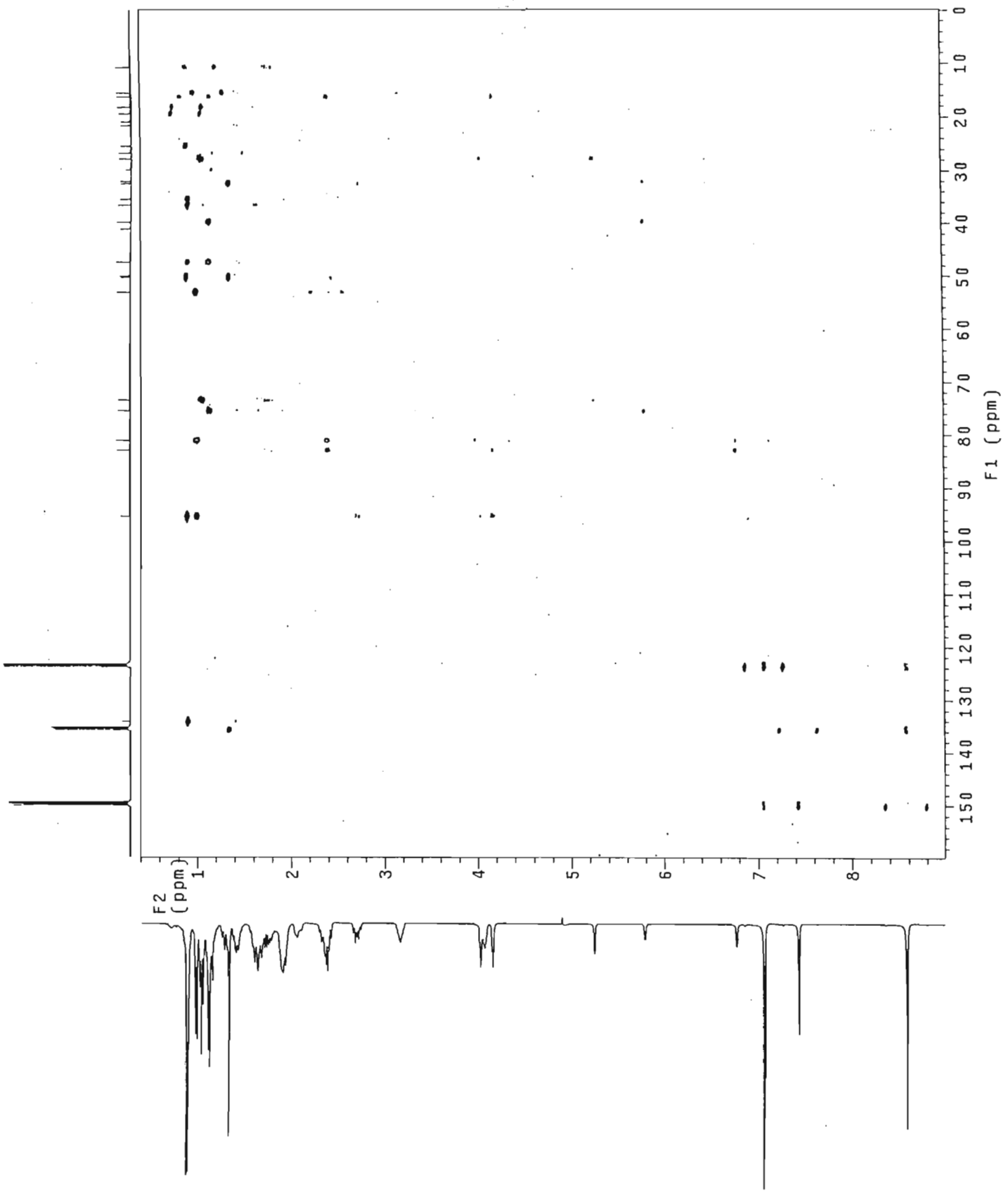
Spectrum 79: ^{13}C NMR spectrum of C



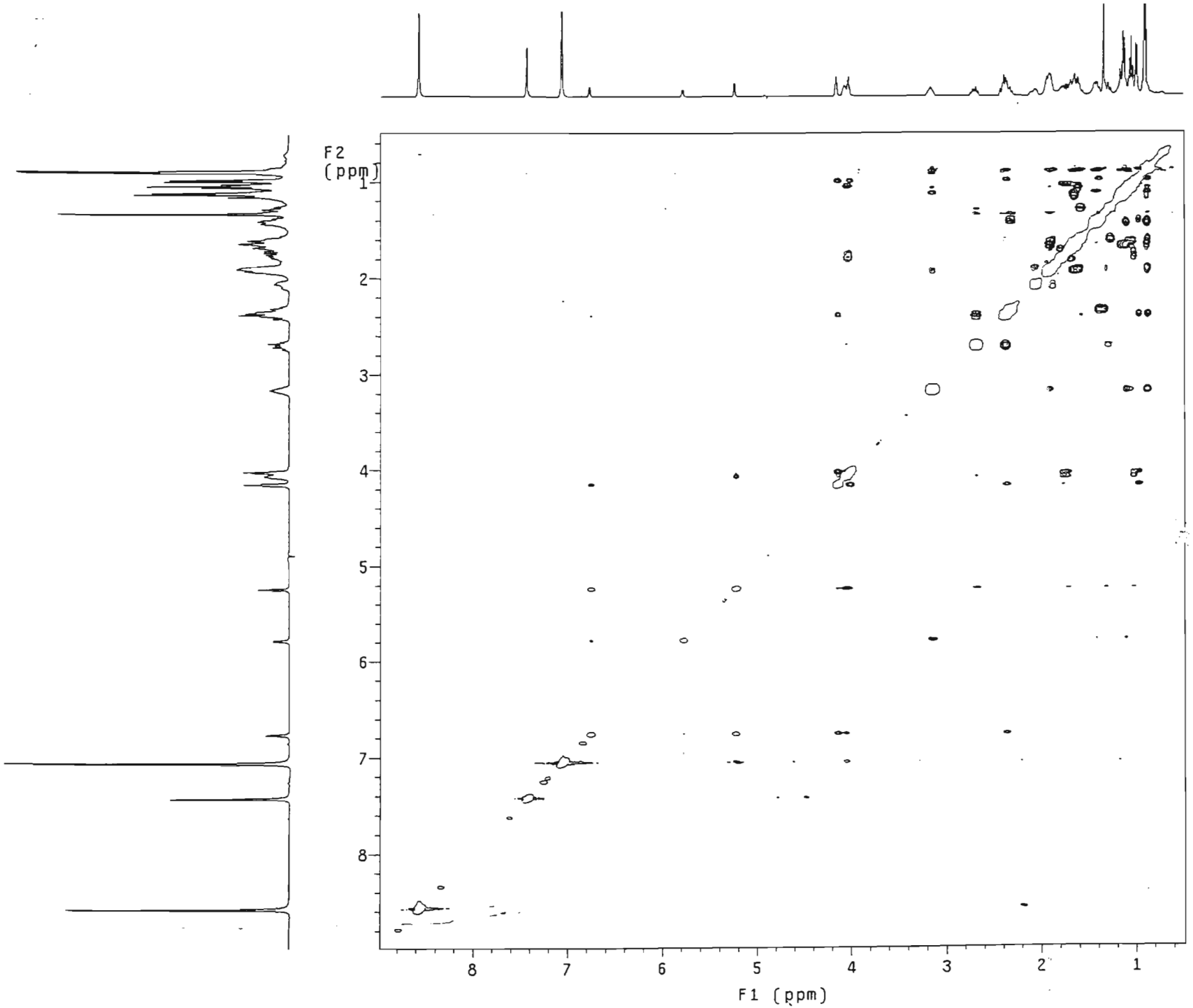
Spectrum 80: ADEPT spectrum of Compound (IX) (C D N)



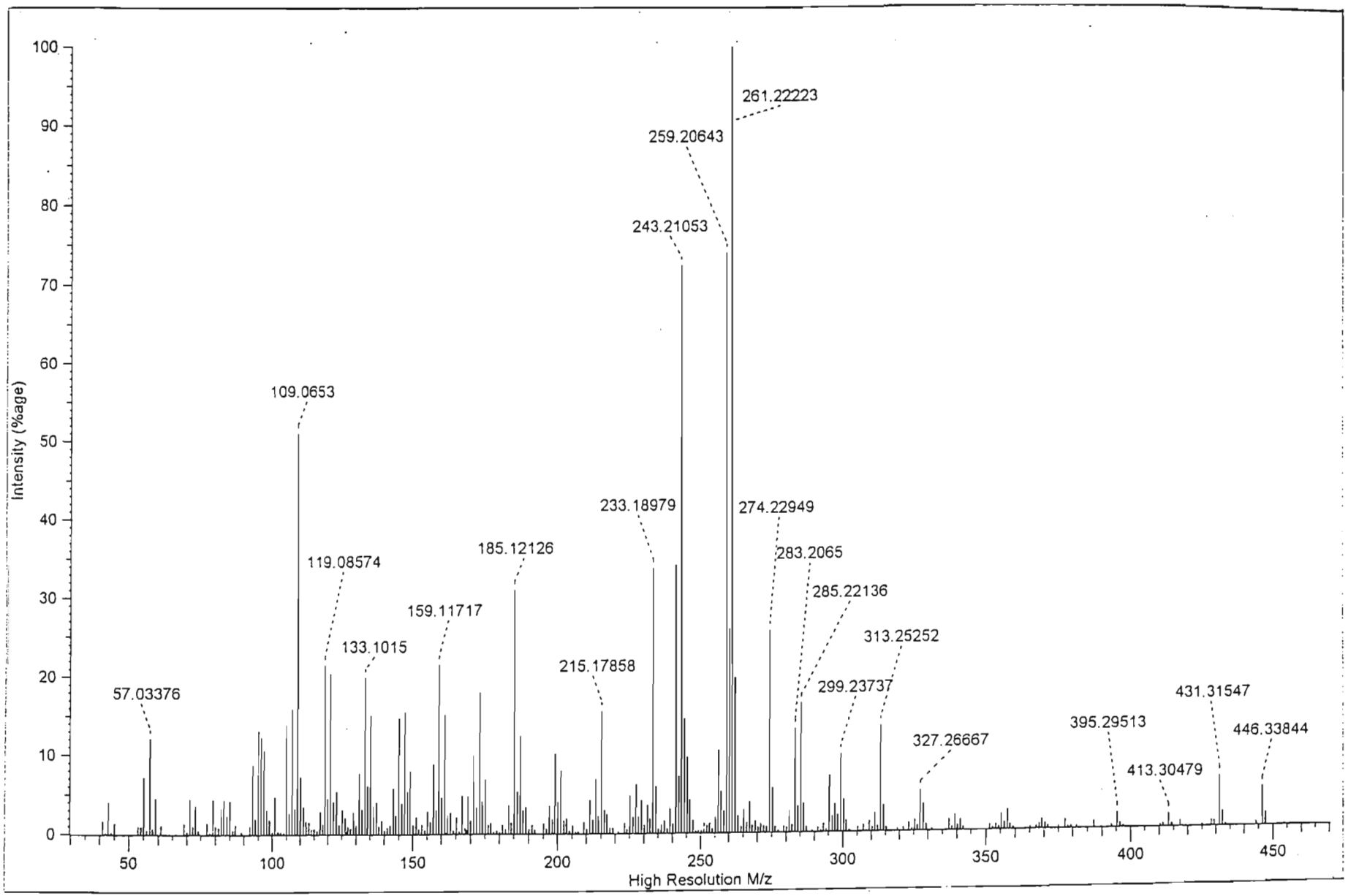
Spectrum 81: HSQC spectrum of C[illegible]


F2 (ppm)
F1 (ppm)

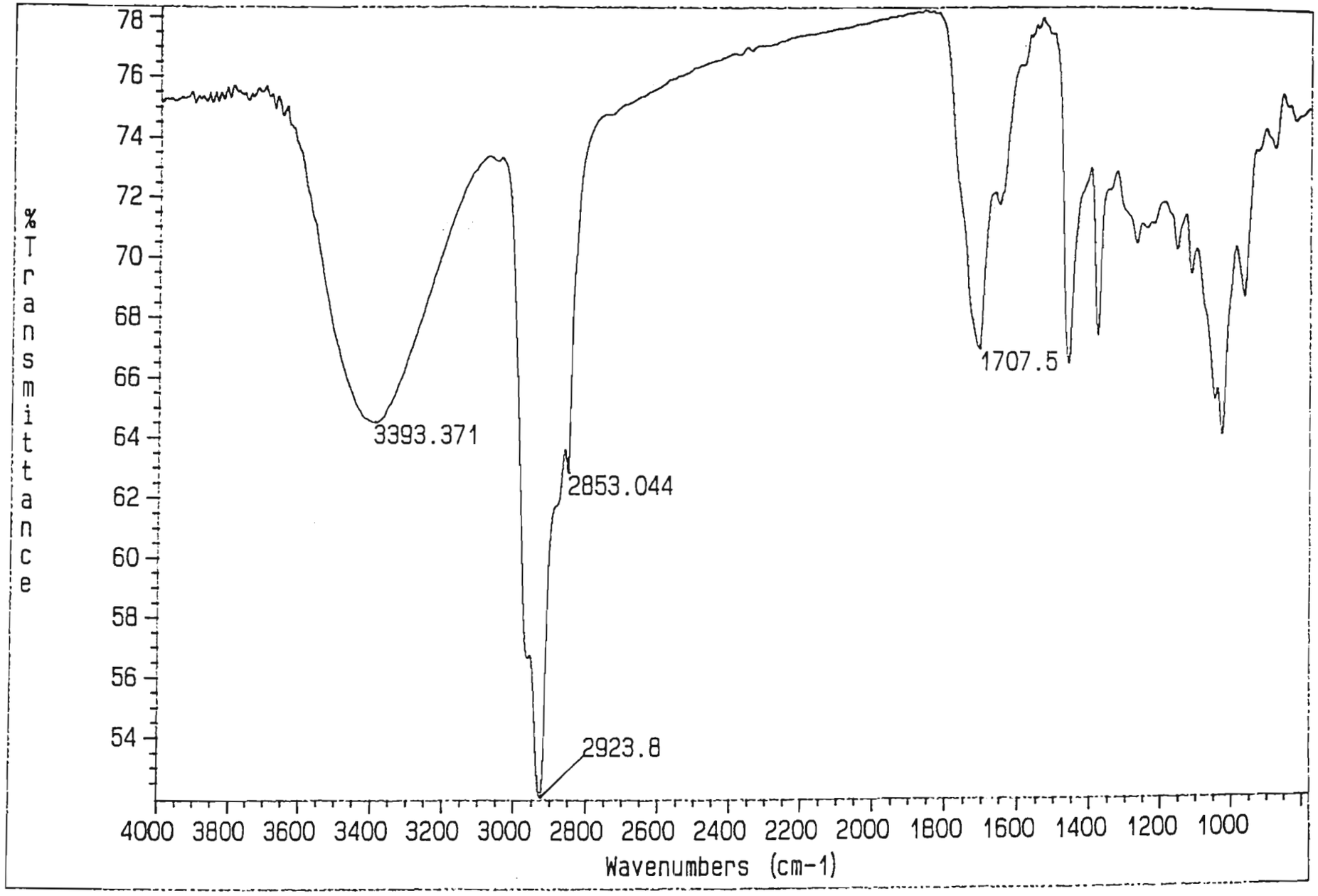
F2 (ppm)
F1 (ppm)



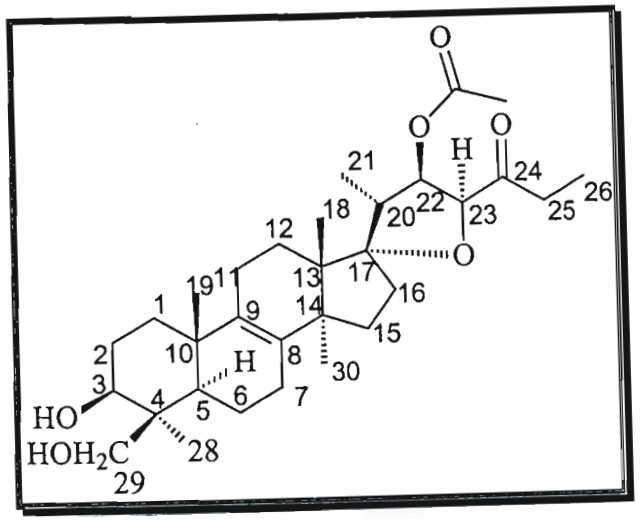
Spectrum 84: NOESY



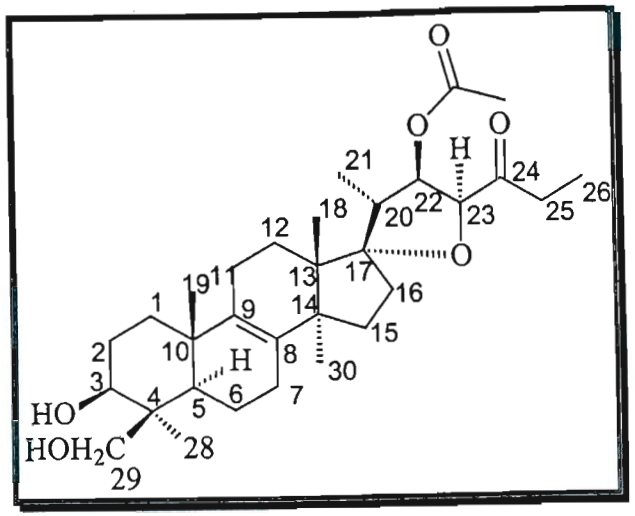
Spectrum 85: Mass spectrum of Compound (IX)

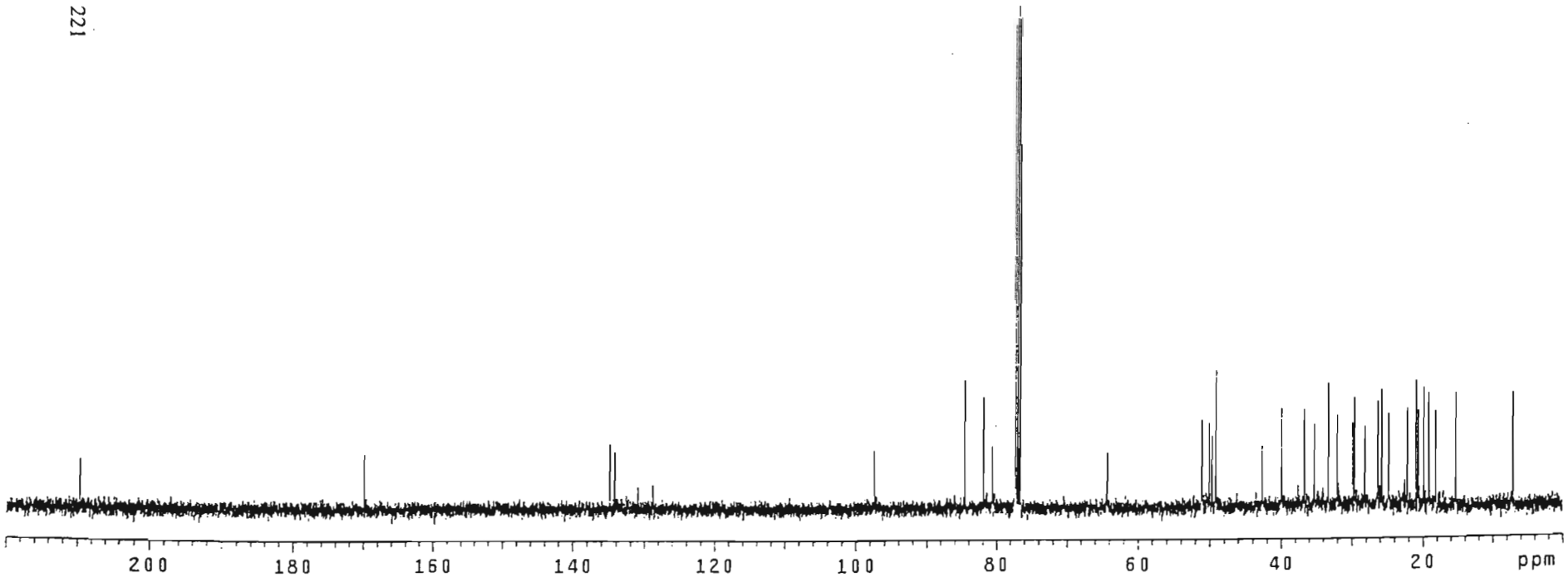


Spectrum 86: IR spectrum of Compound (IX)

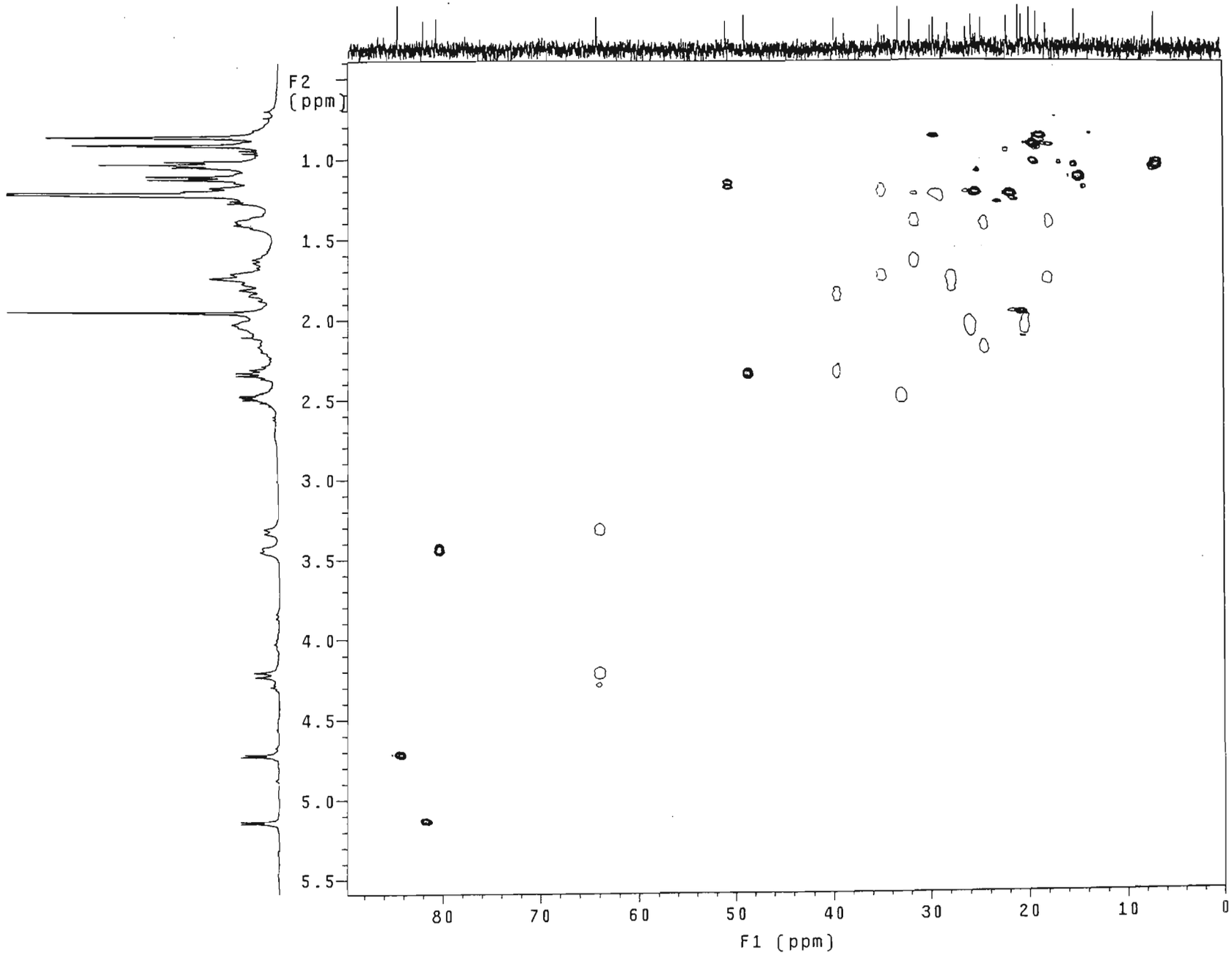


Spectrum 87: ^{1}H NMR spectrum of Compound (X) ($CDCl_3$)

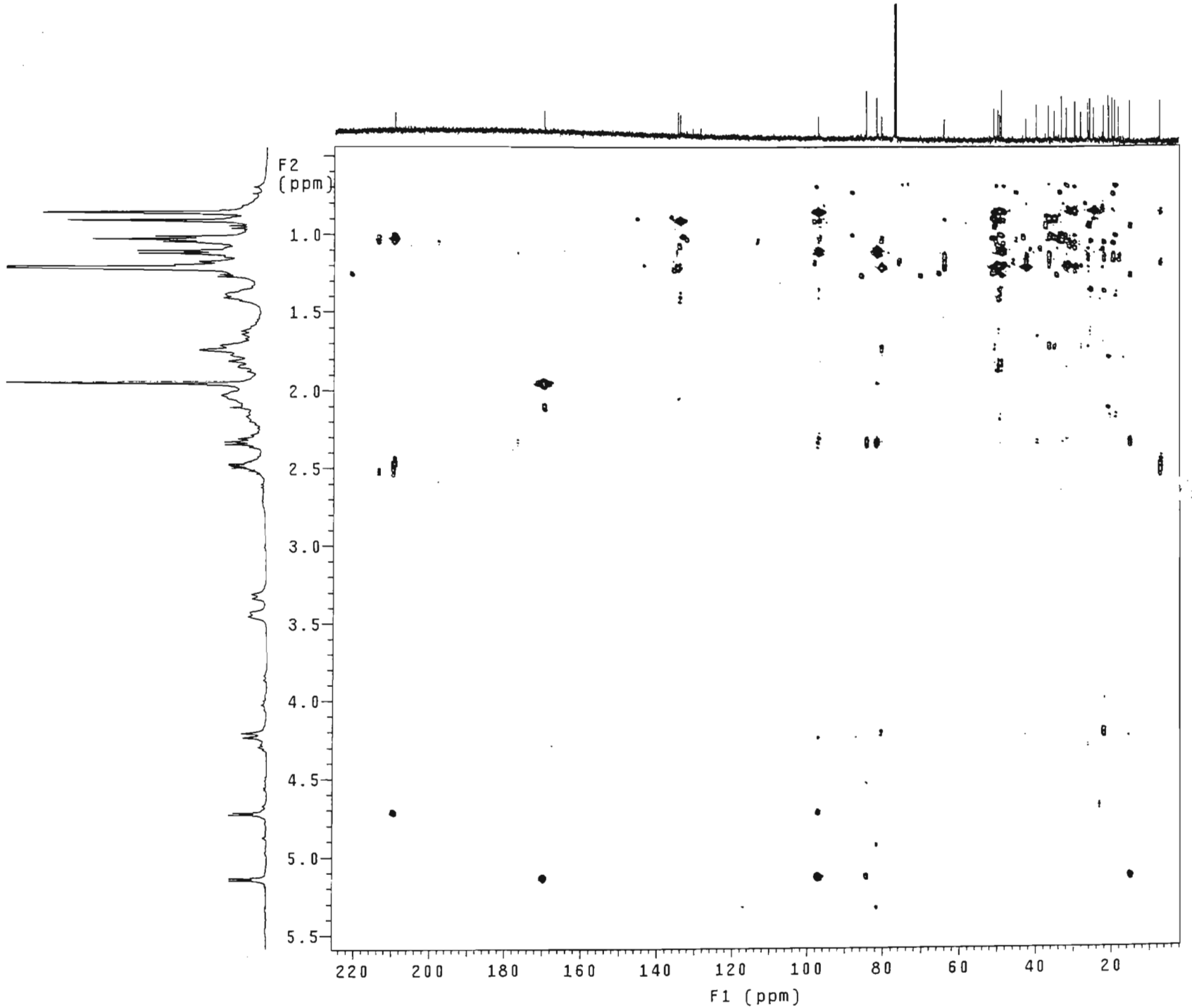




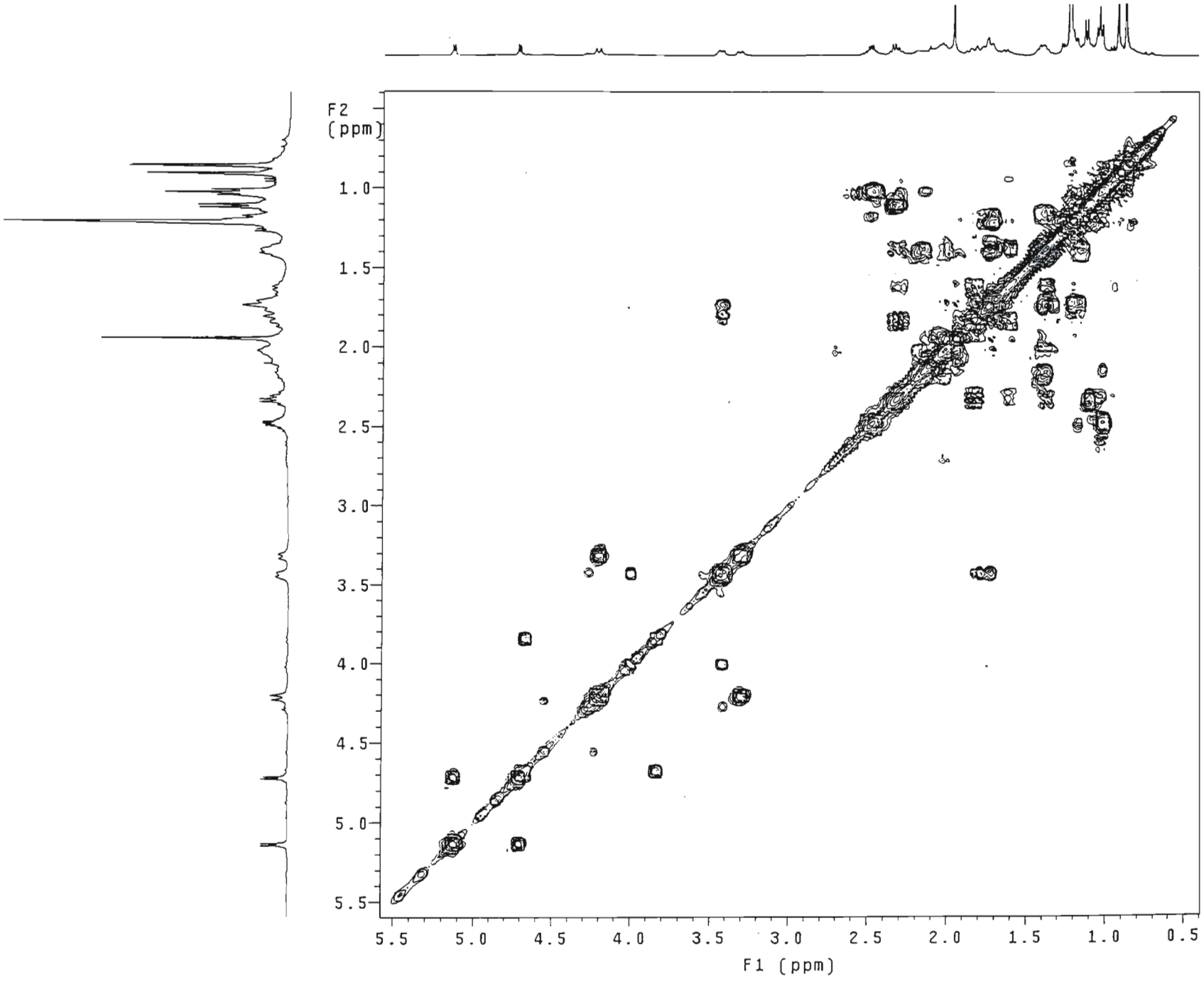
Spectrum 88: ^{13}C NMR spectrum of Compound (X) ($CDCl_3$)



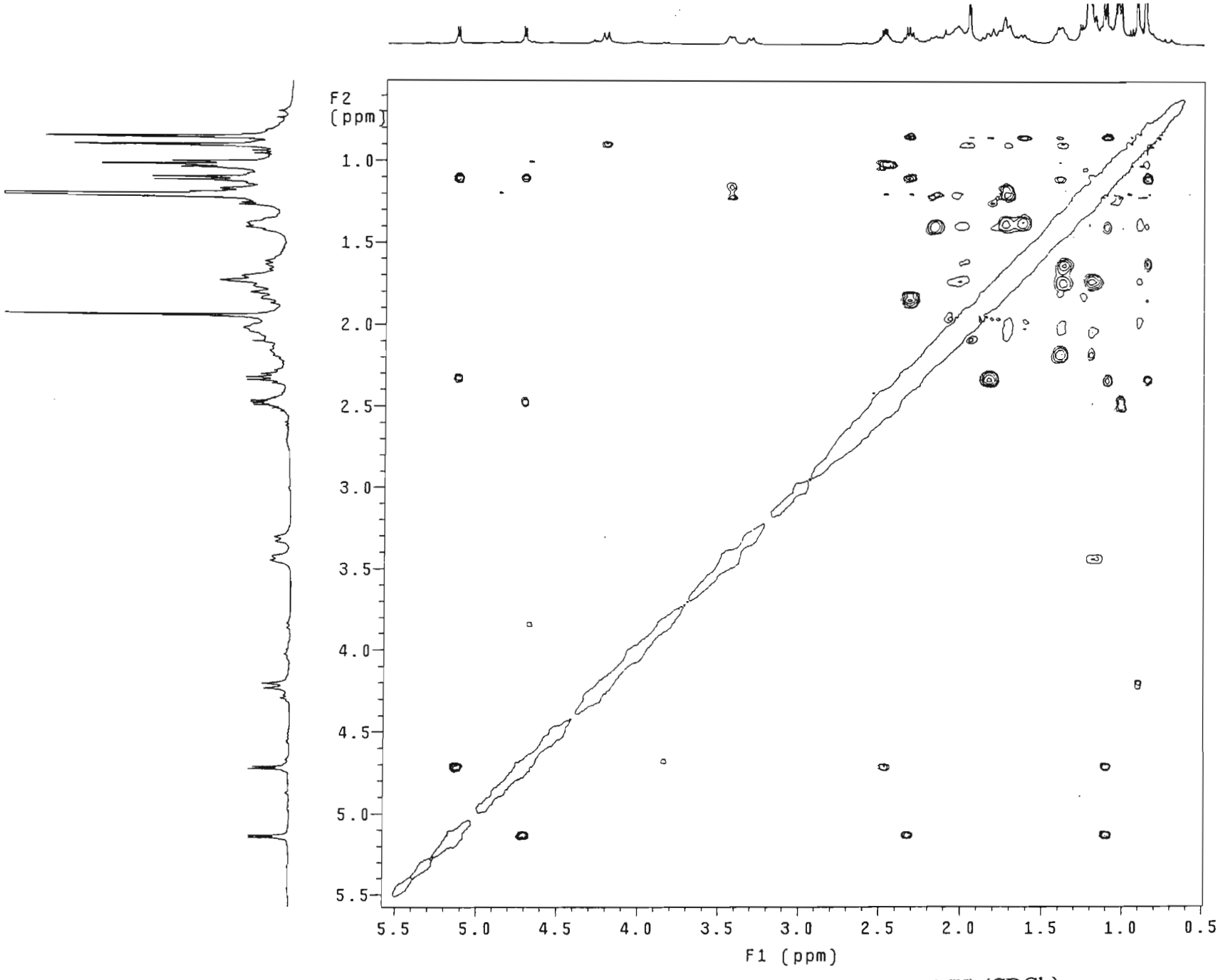
Spectrum 89: HSQC spectrum of Compound (X) ($CDCl_3$)



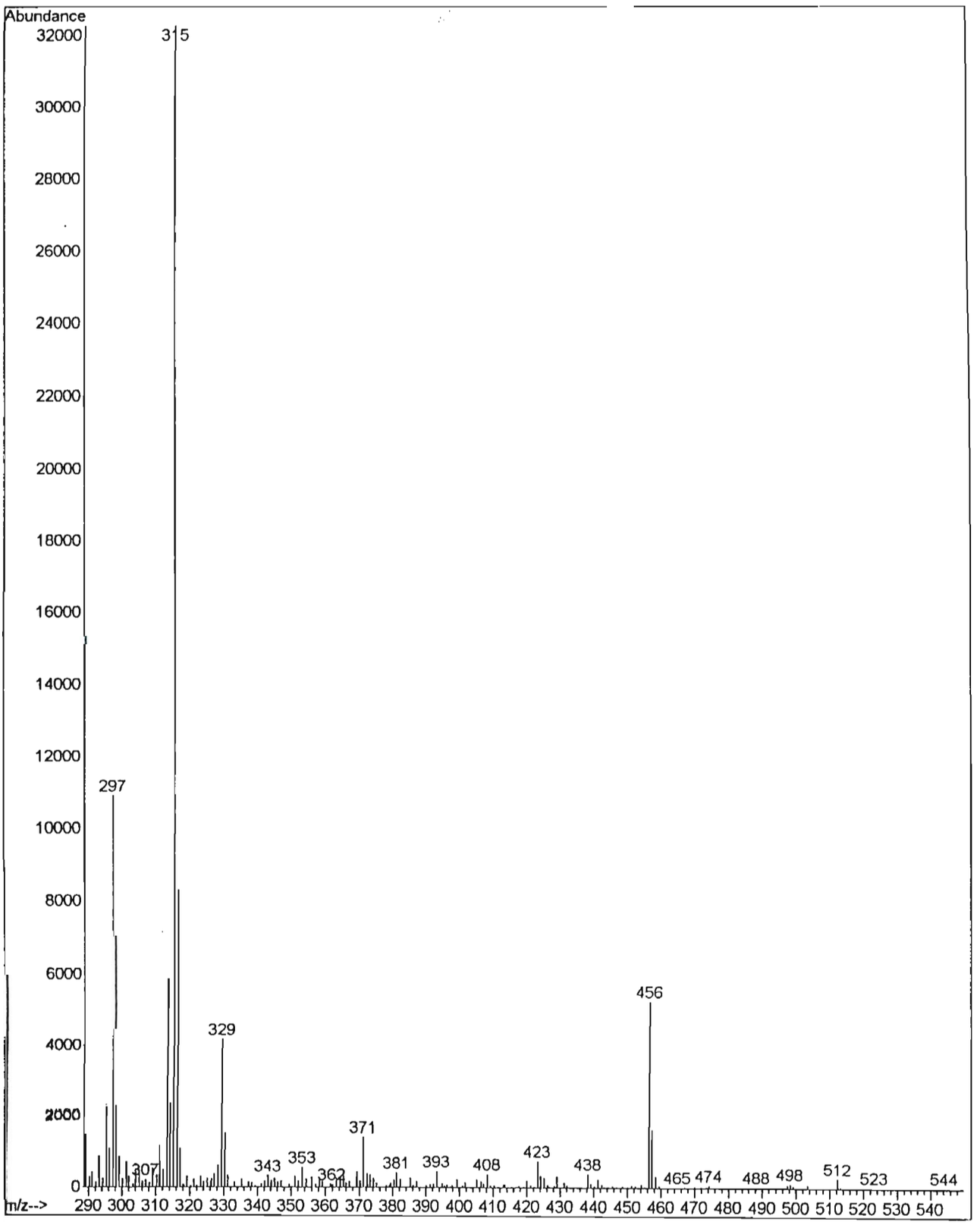
Spectrum 90: HMBC spectrum of Compound (X) ($CDCl_3$)



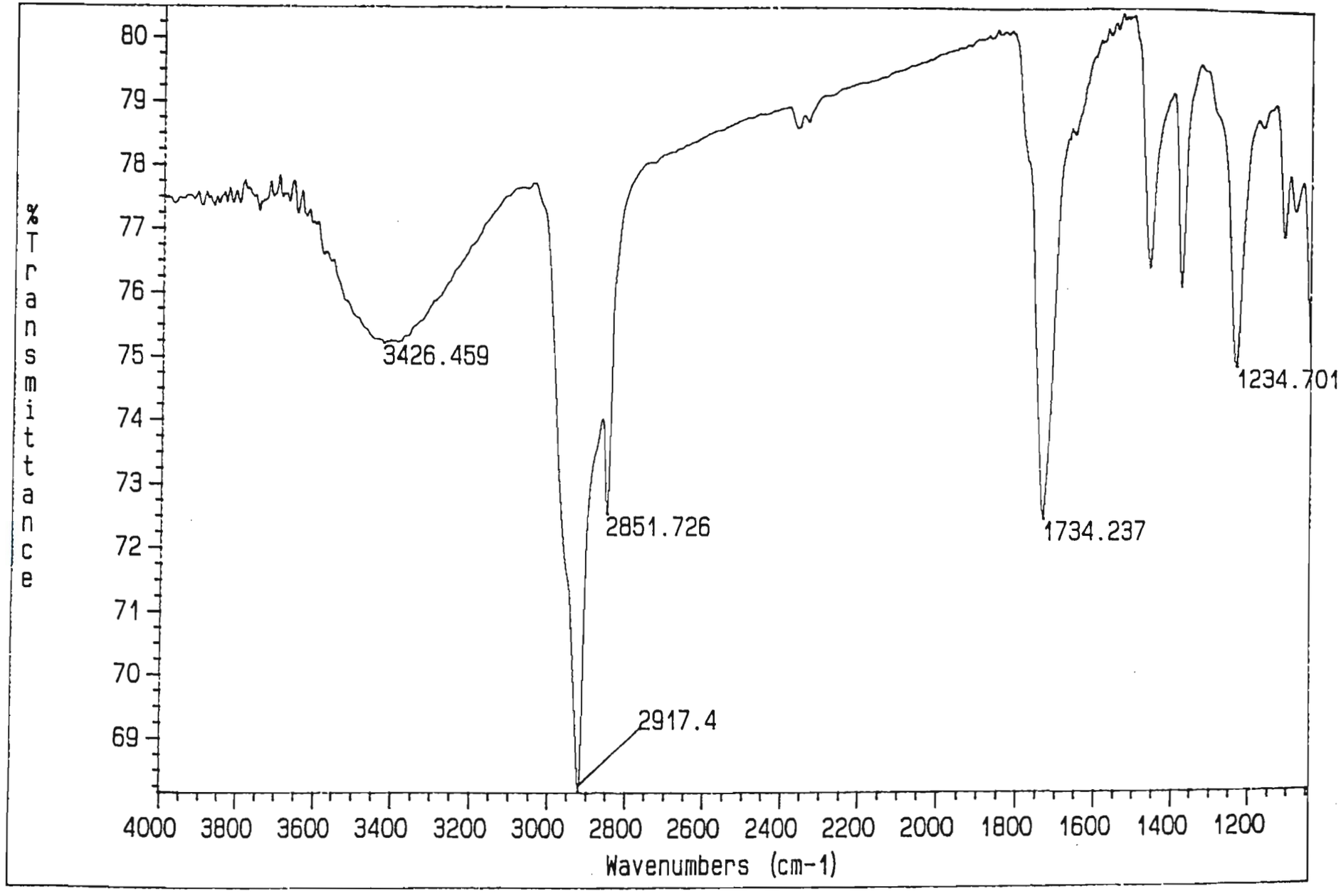
Spectrum 91: COSY spectrum of Compound (X) ($CDCl_3$)



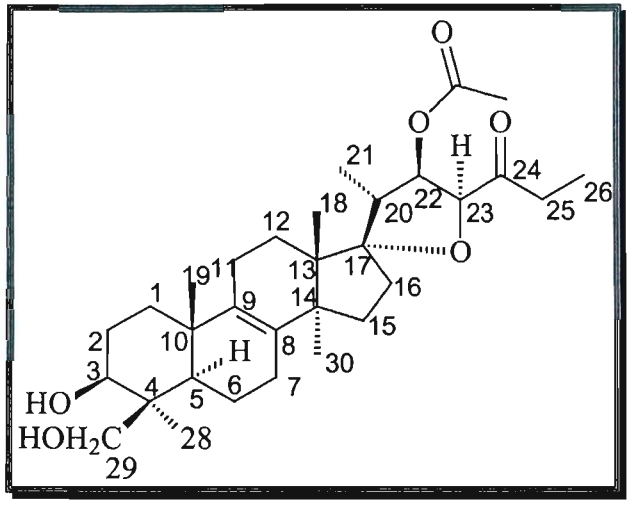
Spectrum 92: NOESY spectrum of Compound (X) ($CDCl_3$)



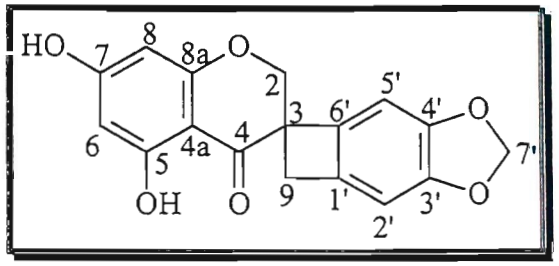
Spectrum 93: Mass spectrum of Compound (X)

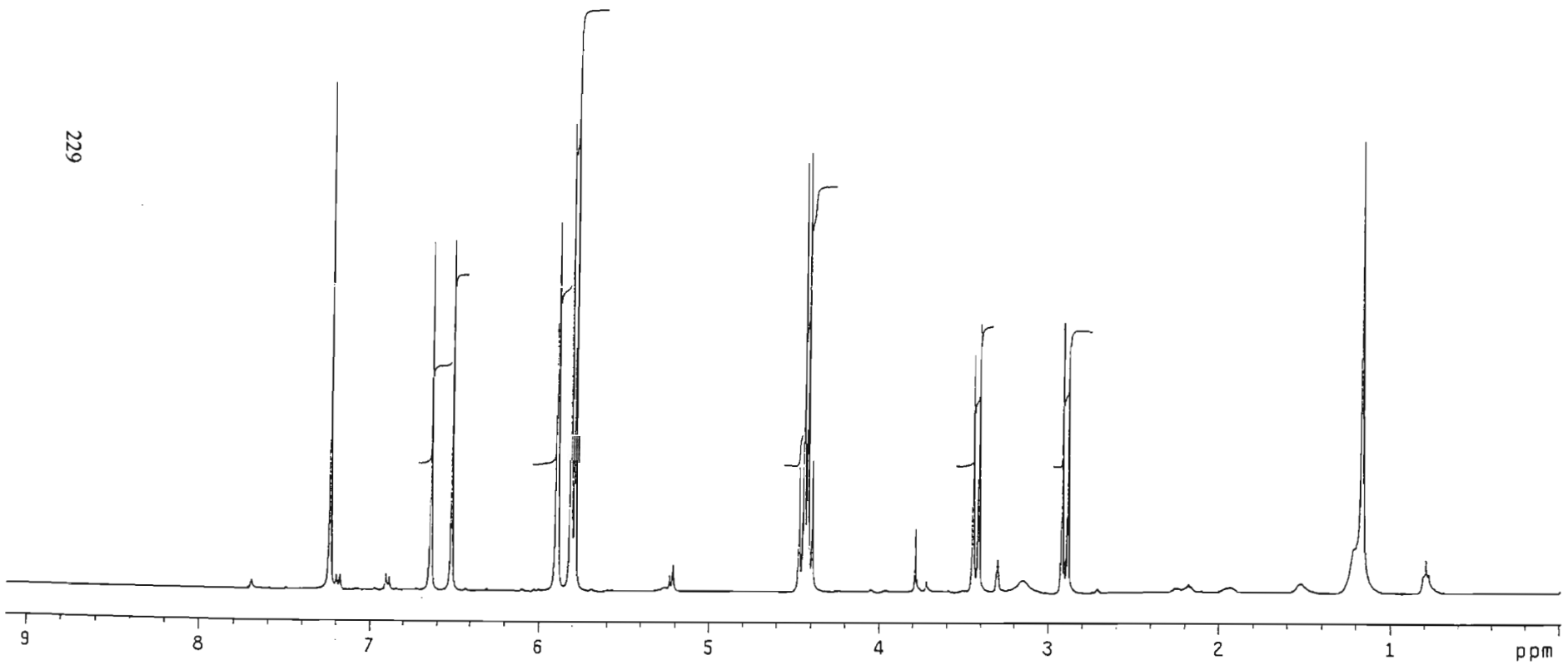


Spectrum 94: IR spectrum of Compound (X)

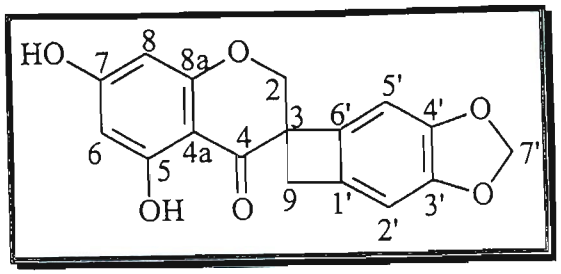


Spectrum 95: ^{13}C NMR spectrum of Compound (X) (CD_3OD)

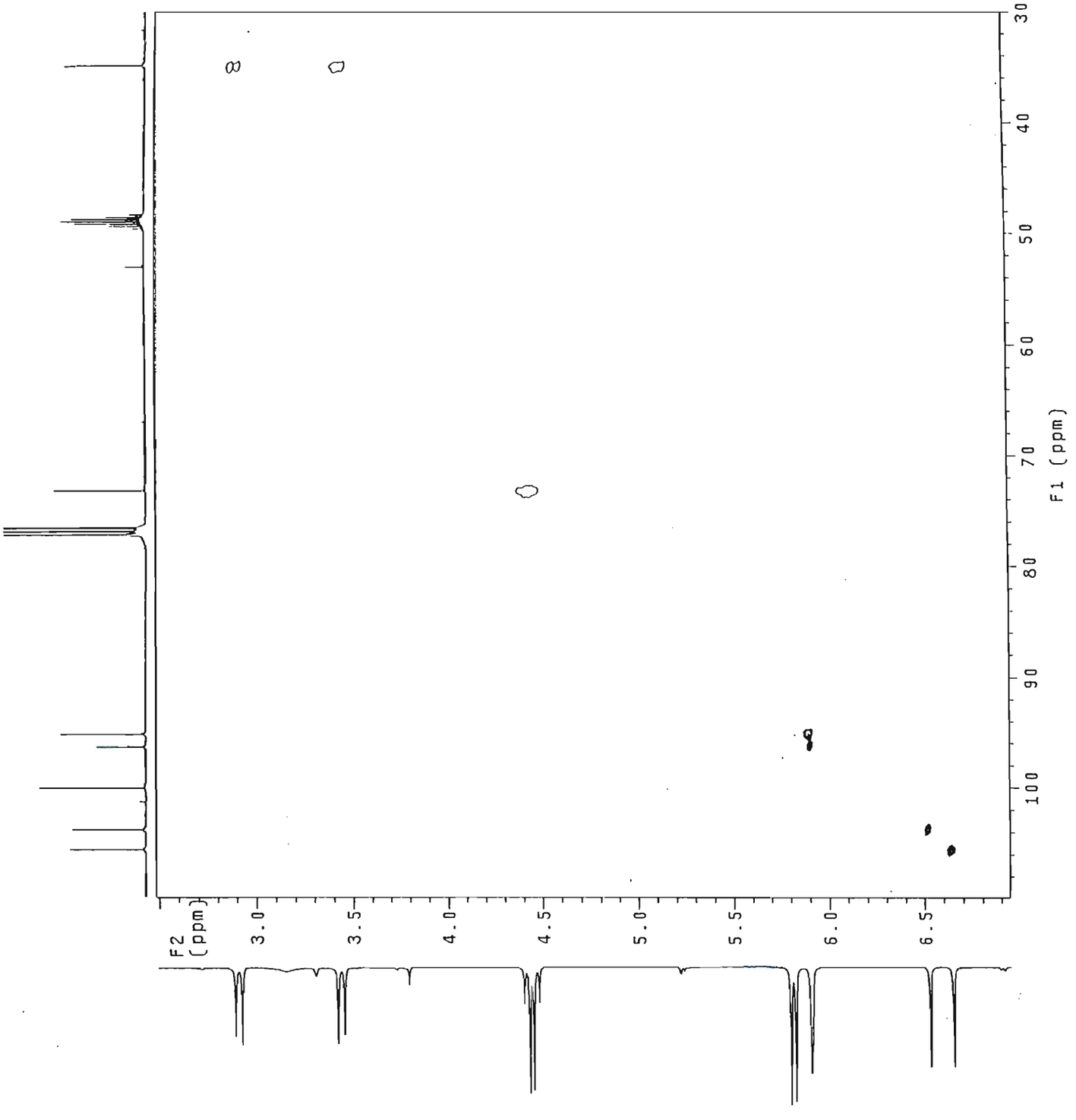


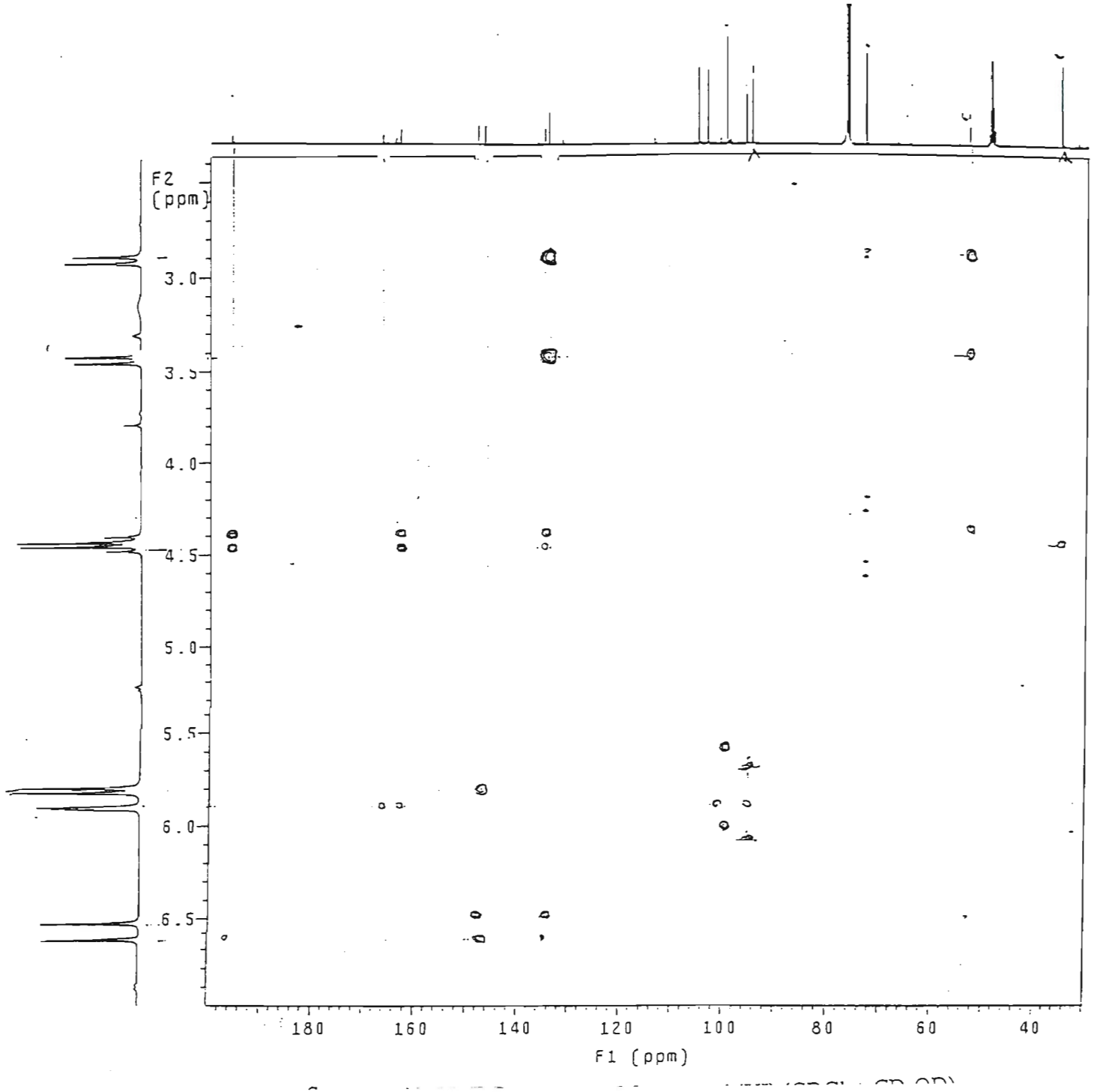


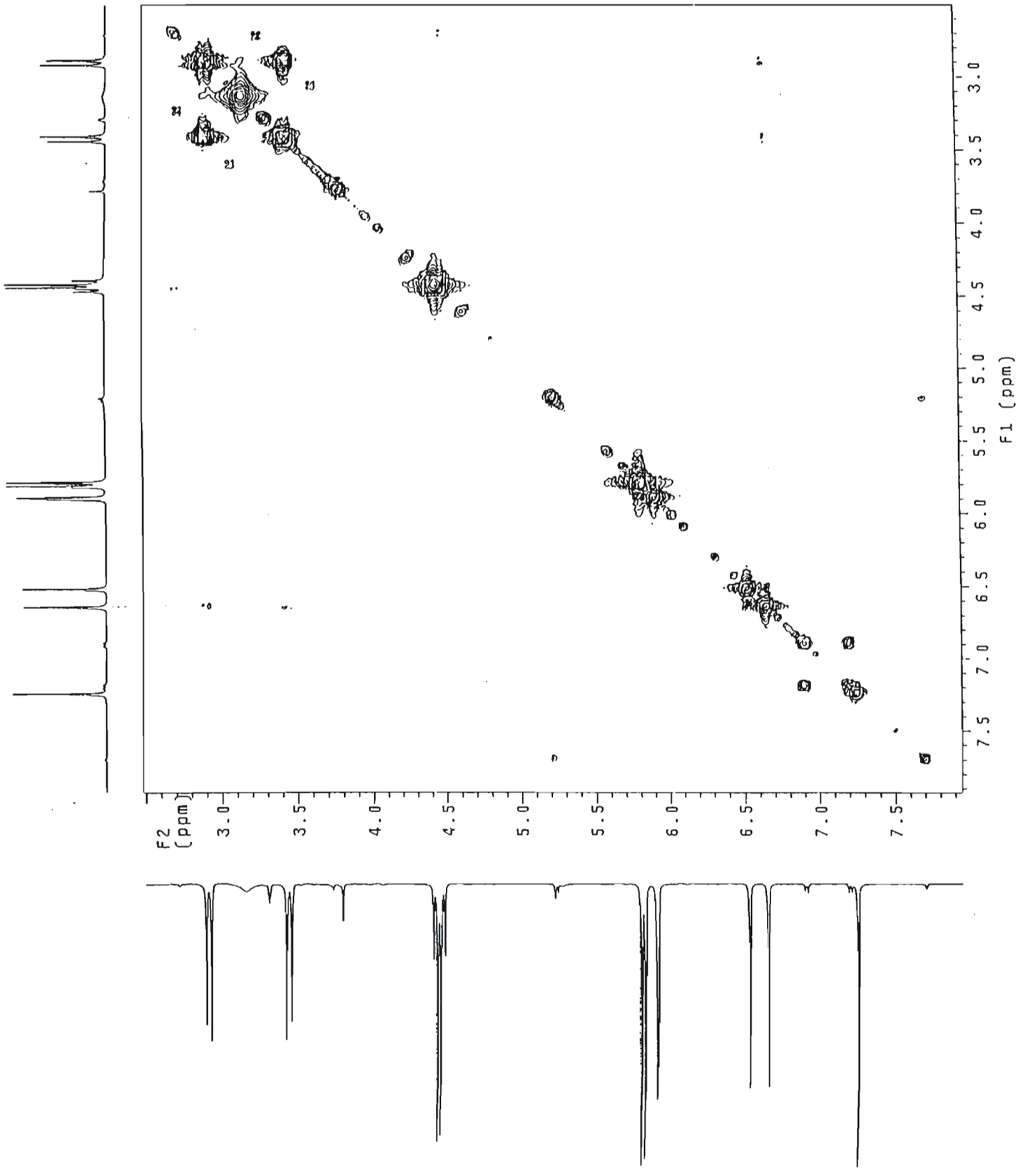
Spectrum 96: ^{1}H NMR spectrum of Compound (XI) ($CDCl_3 + CD_3OD$)

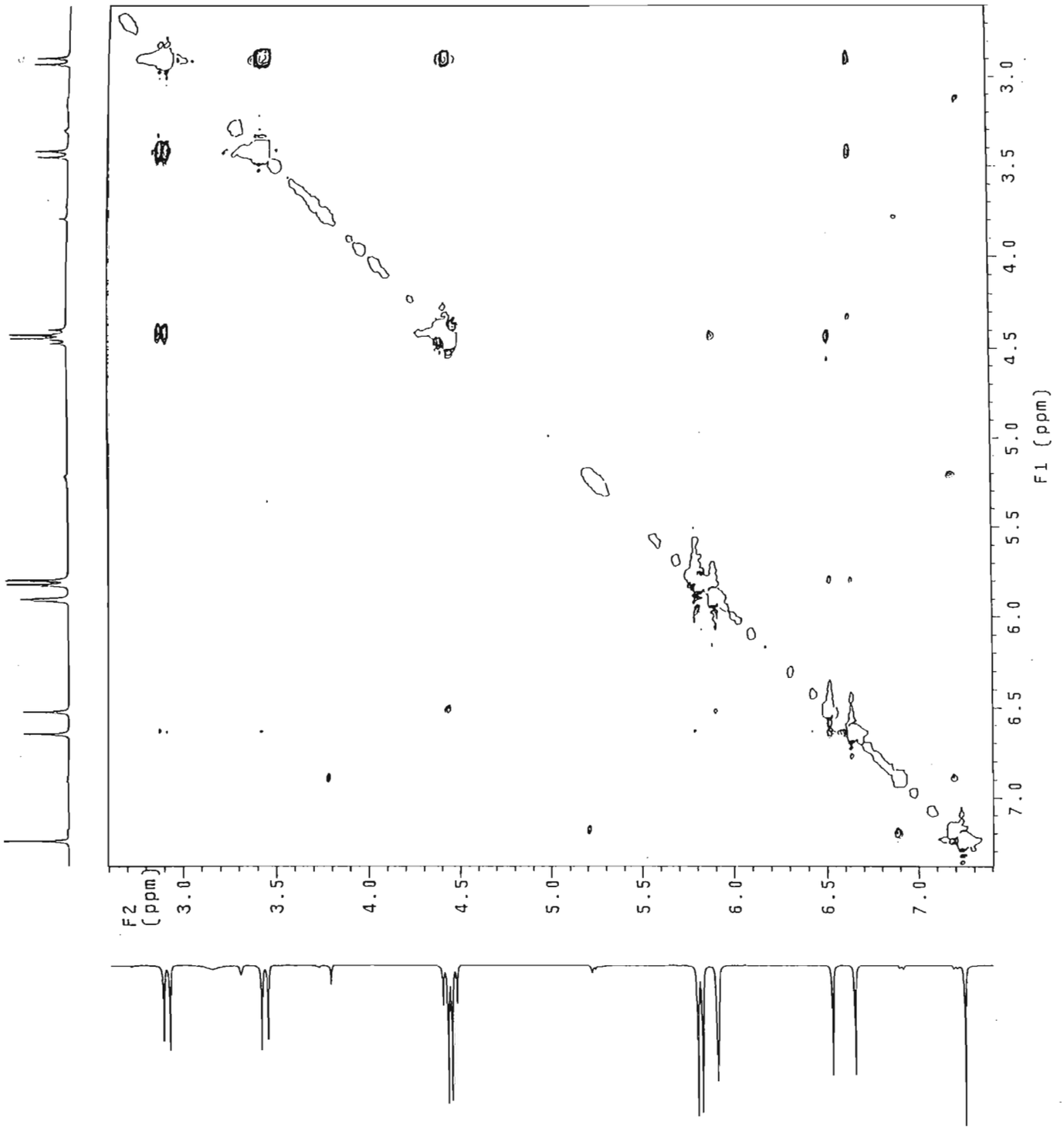


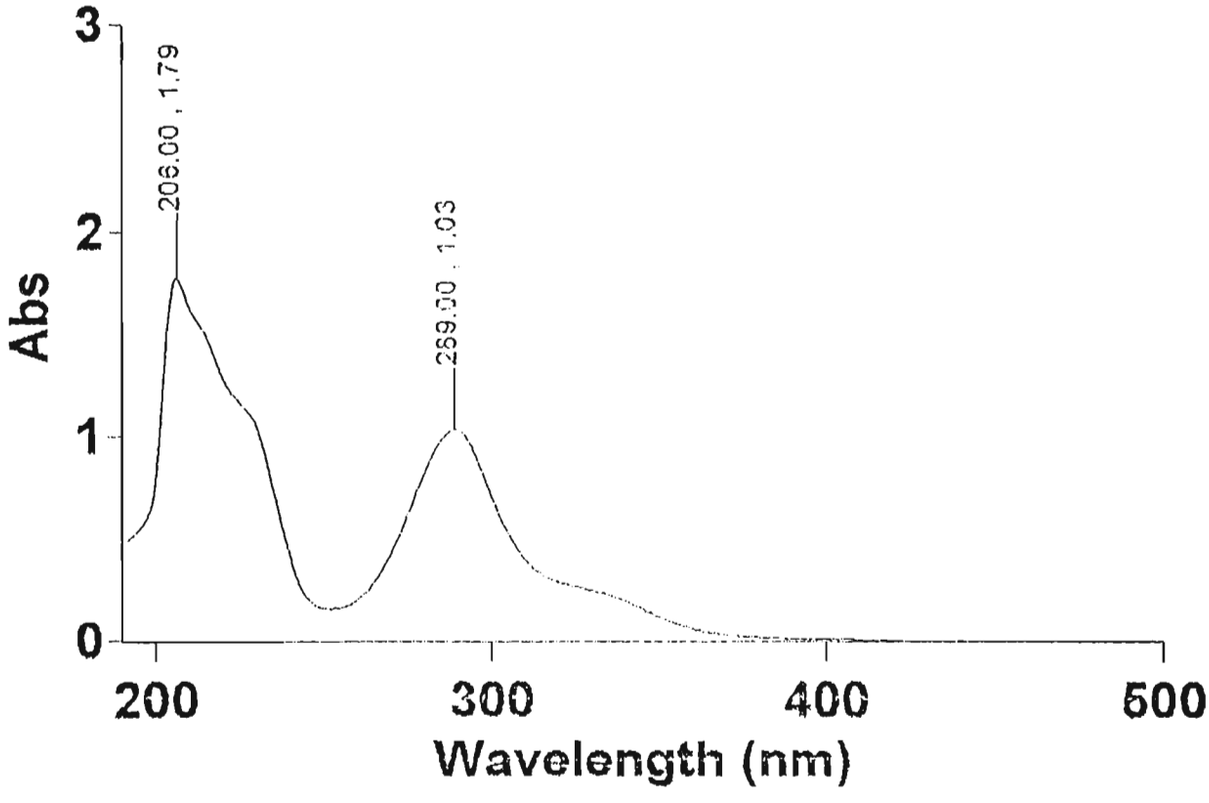
Spectrum 97: ^{13}C NMR spectrum of Compound (XI) ($CDCl_3$ + CD_3OD)


F2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
F1 (ppm)
30
40
50
60
70
80
90
100

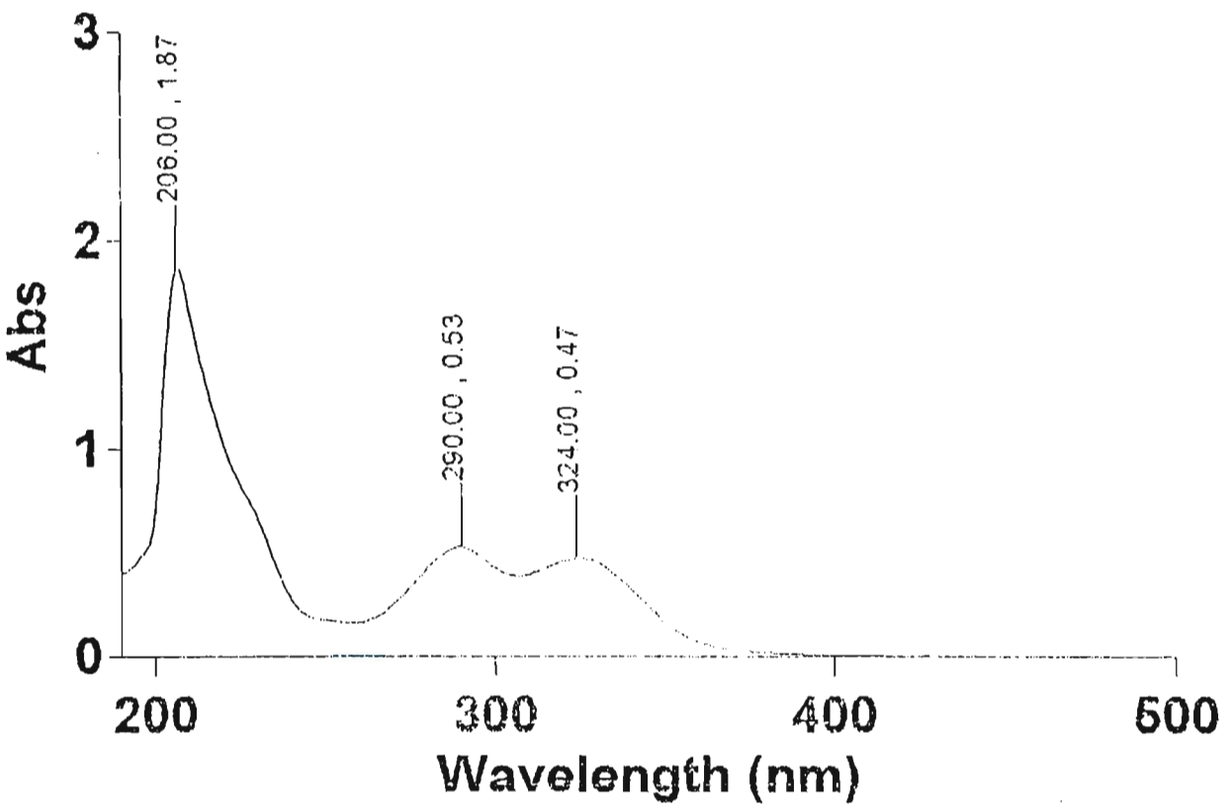

F2
(ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
180
160
140
120
100
80
60
40
F1 (ppm)


F2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
F1 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5

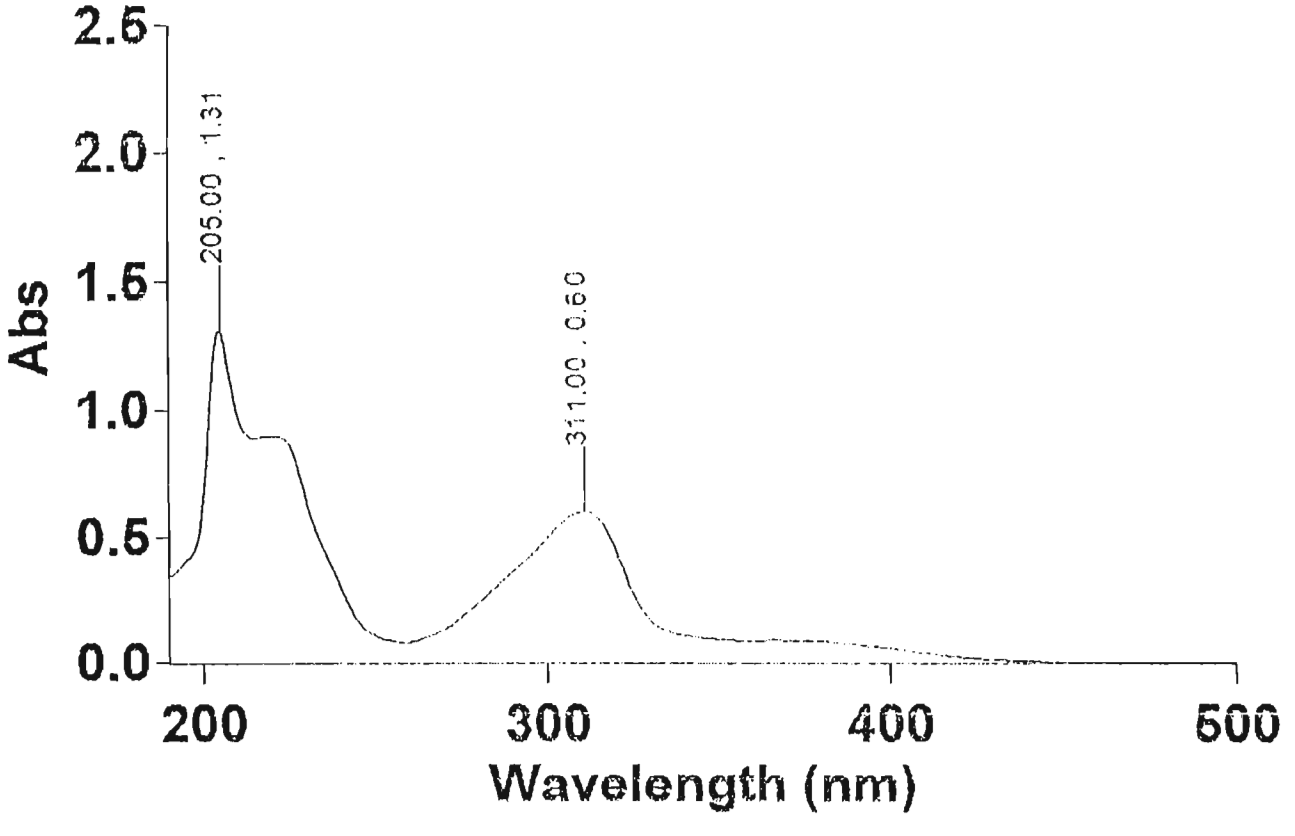

F2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
F1 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0



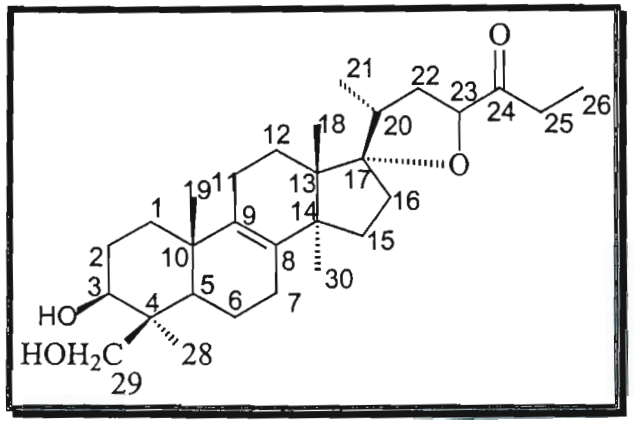
Spectrum 102: UV spectrum of Compound (XI)



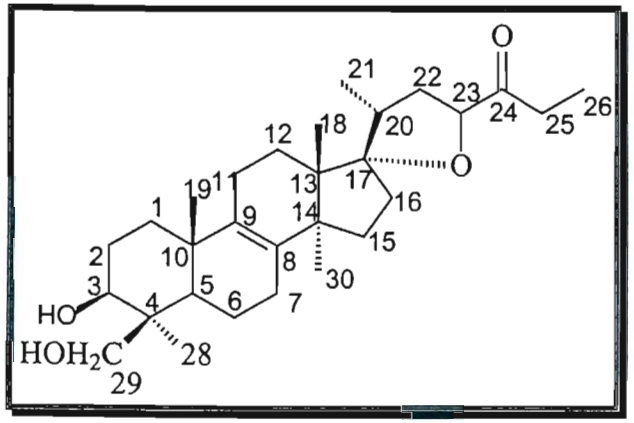
Spectrum 103: UV spectrum of Compound (XI) with NaOAc



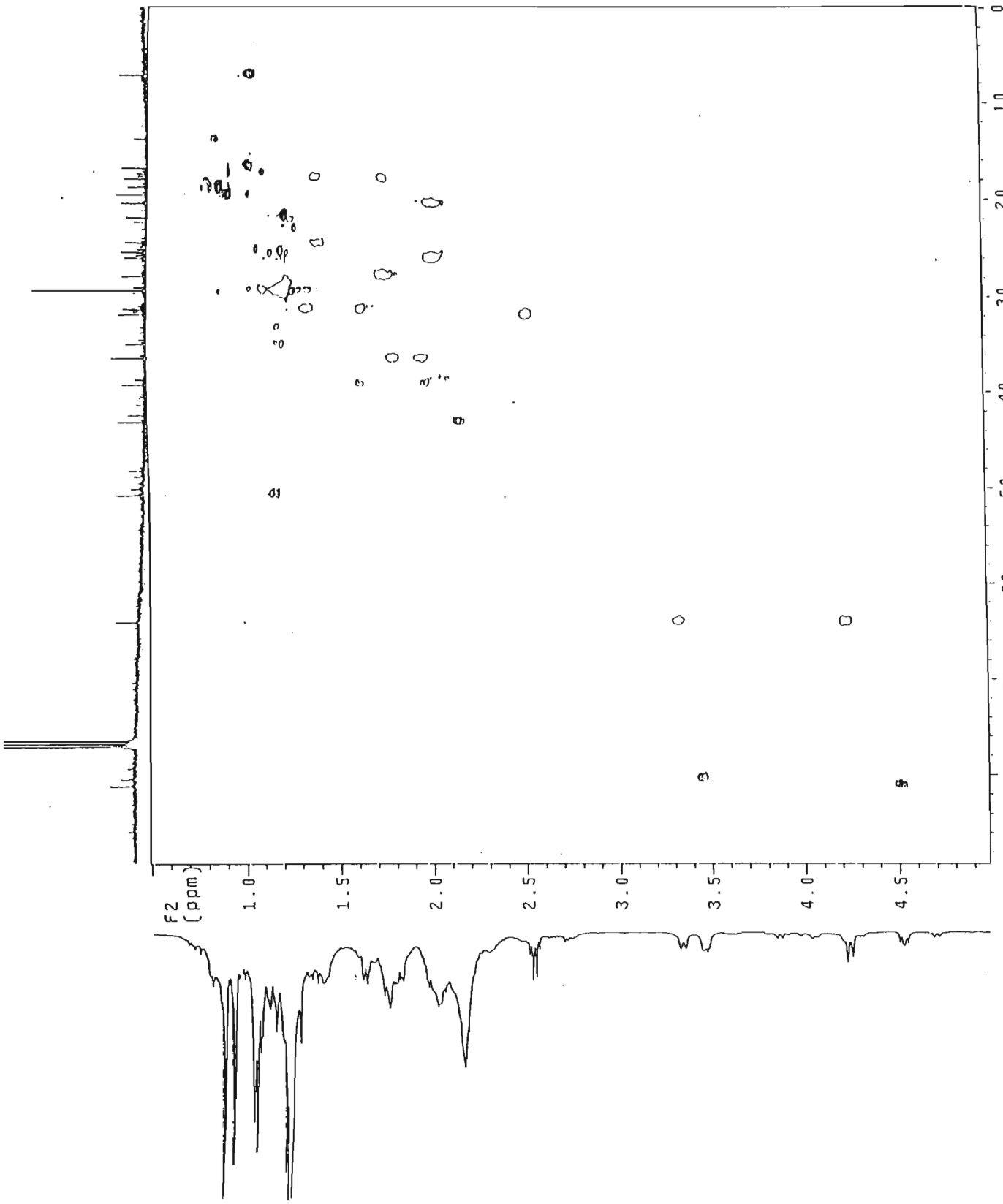
Spectrum 104: UV spectrum of Compound (XI) with $AlCl_3$

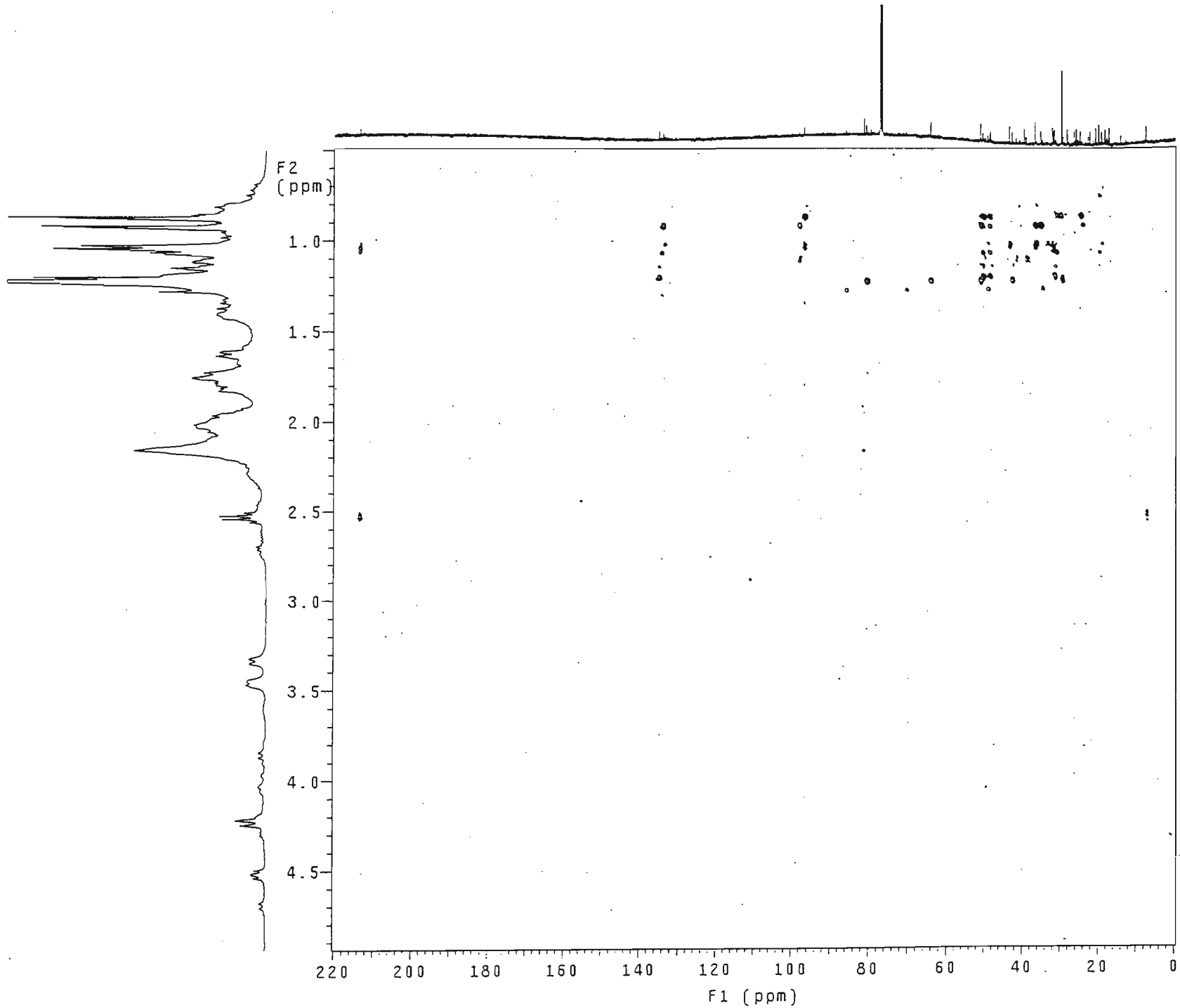


Spectrum 105: ^{1}H NMR spectrum of Compound (XII) ($CDCl_3$)

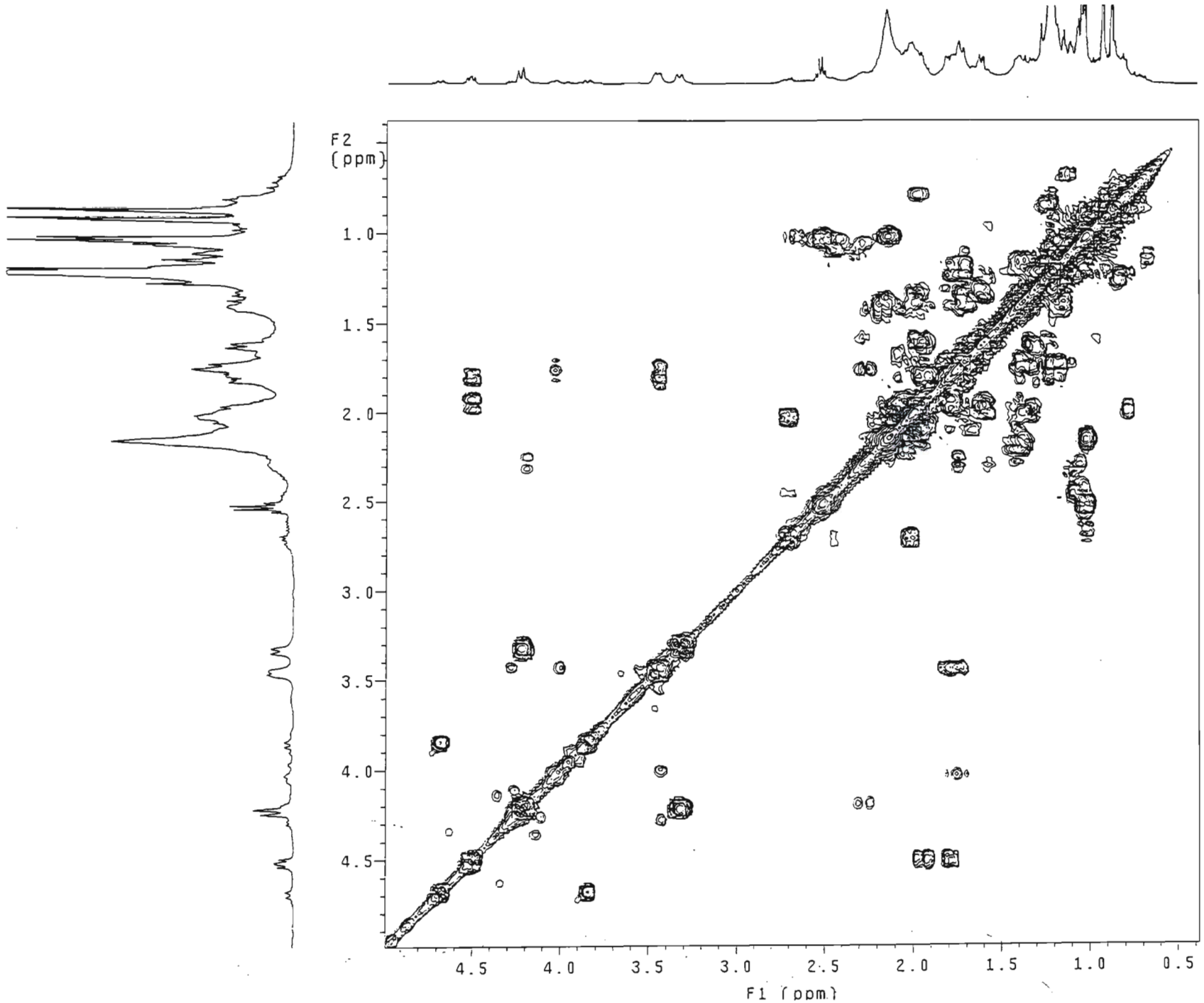

O
21
22
23
24
25
26
12
18
20
O
11
19
13
17
16
1
9
14
15
2
10
8
30
3
5
6
7
HO
4
28
HOH2C
29

200
180
160
140
120
100
80
60
40
20
ppm

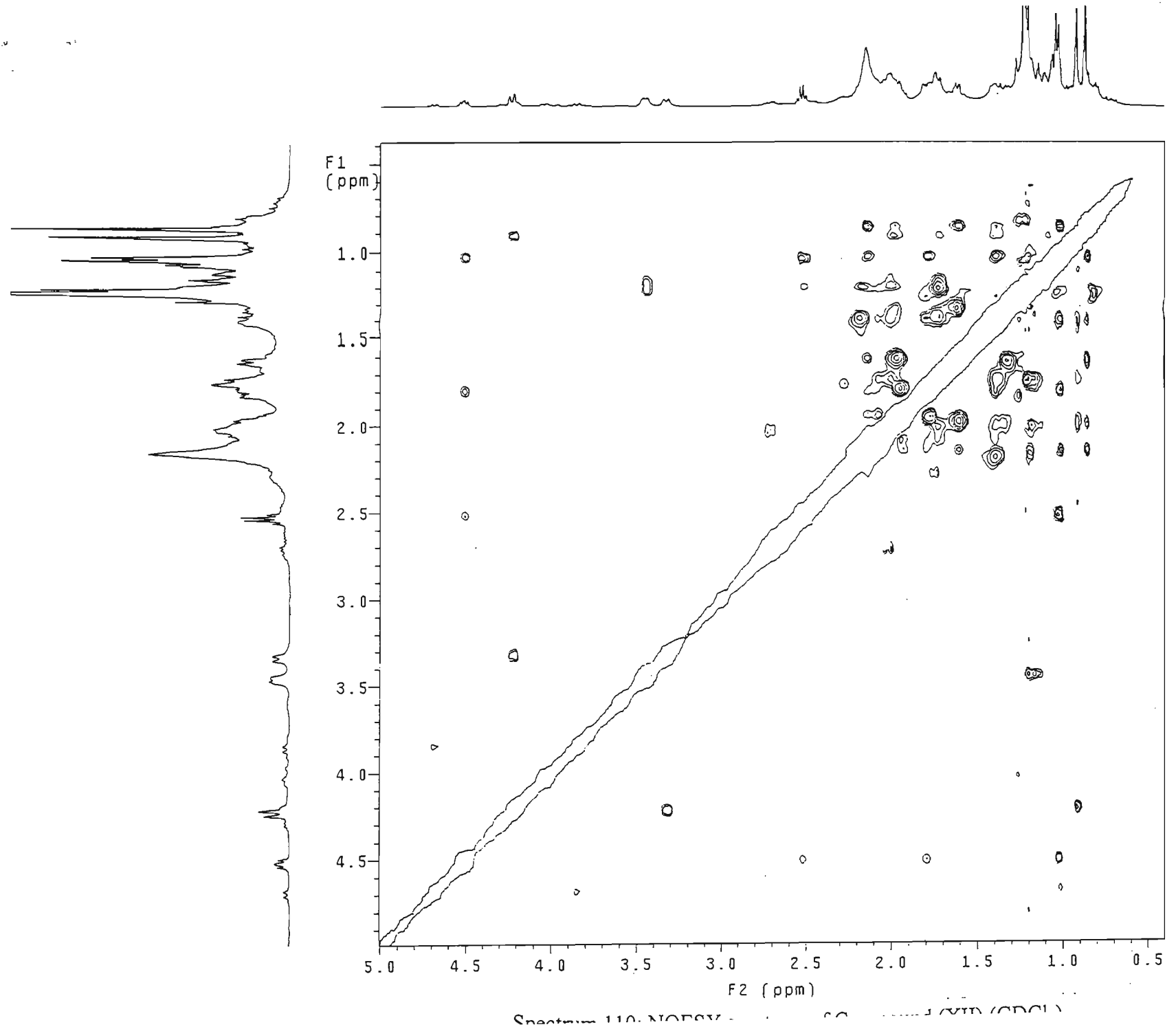

F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
F1 (ppm)
0
10
20
30
40
50
60
70
80

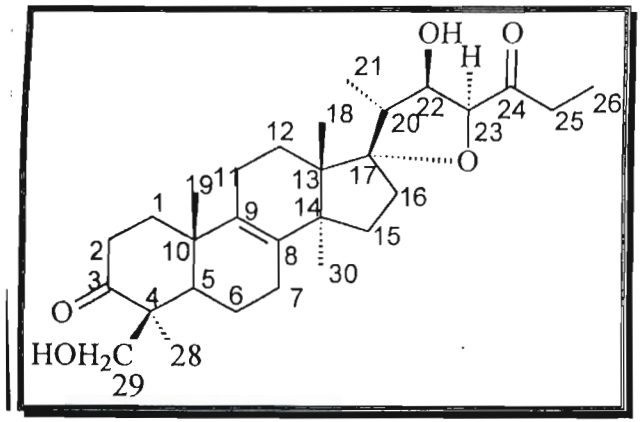


Spectrum 100 HMBC spectrum of Compound (XII) ($CDCl_3$)

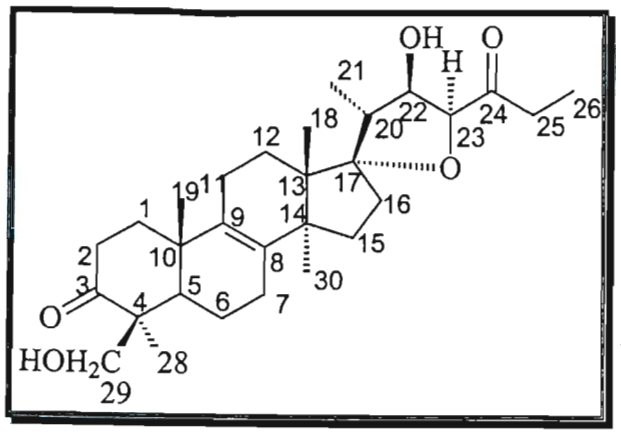


Spectrum 109: COSY spectrum of Compound (XII) ($CDCl_3$)


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

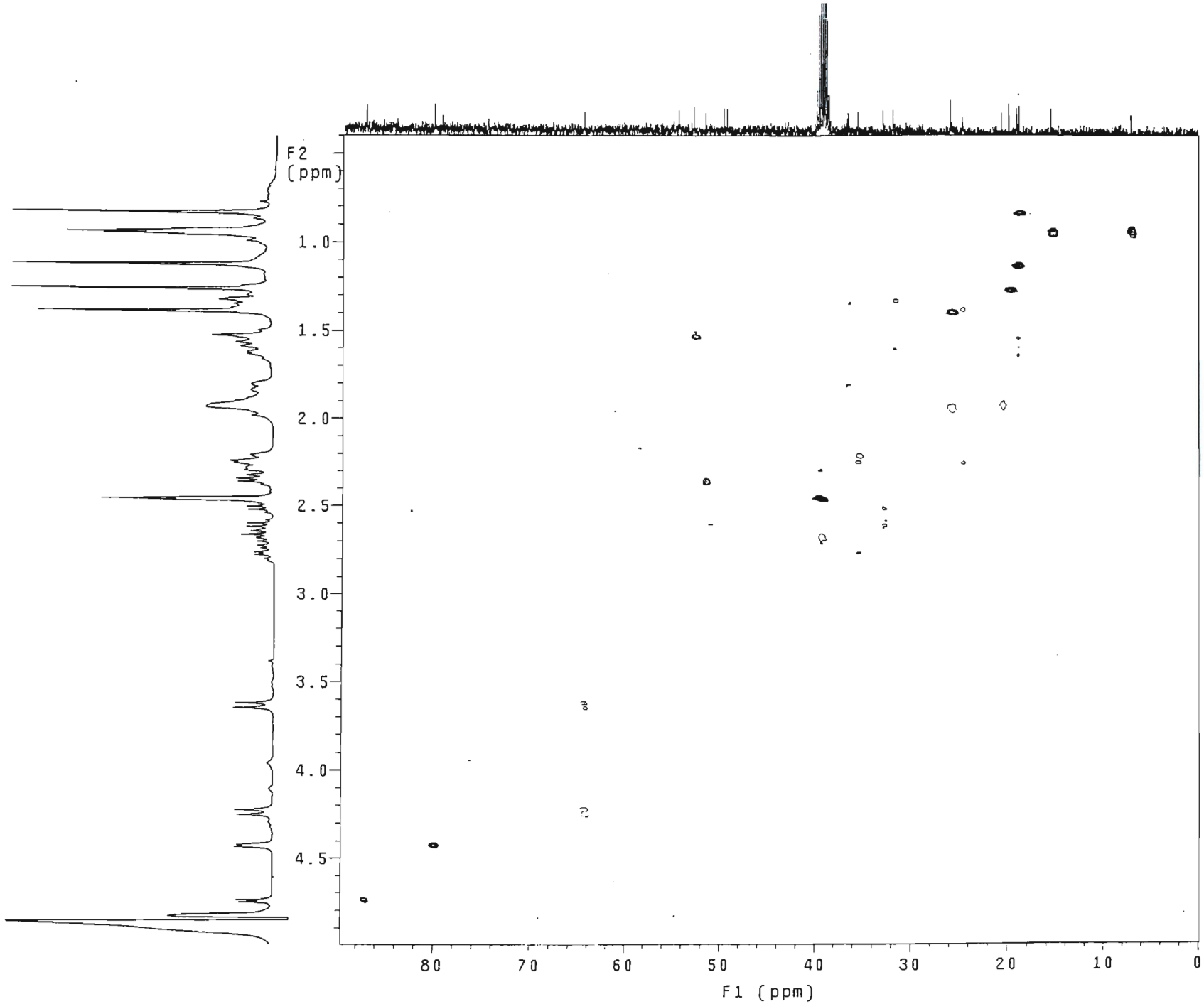


Spectrum 111: ^{1}H NMR spectrum of Compound (XIII) (C_5D_5N + DMSO)

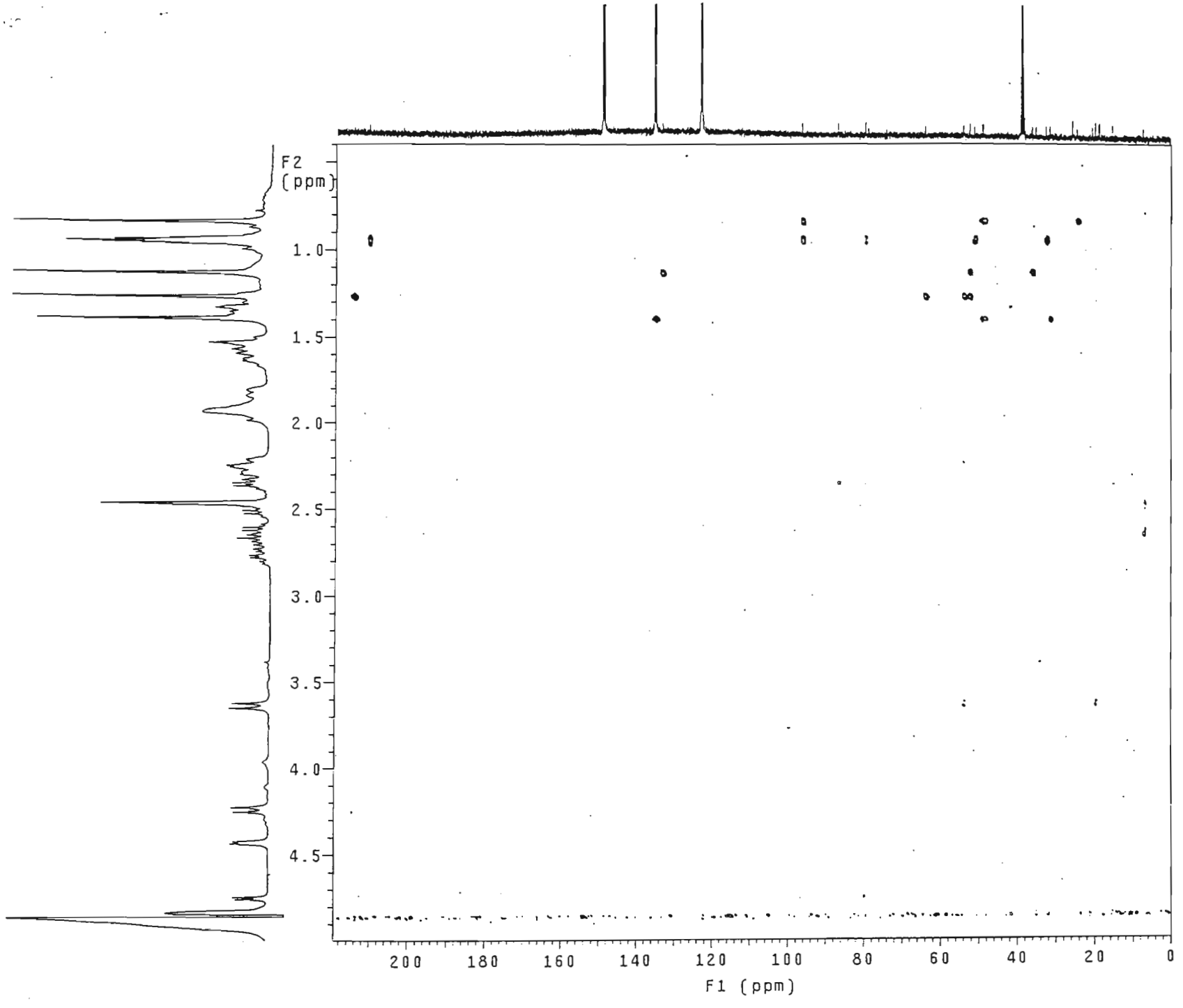

OH
H
O
21
18
20
22
24
25
26
12
23
O
19
11
13
17
16
1
9
14
15
2
10
8
30
3
5
6
7
O
4
28
HOH2C
29

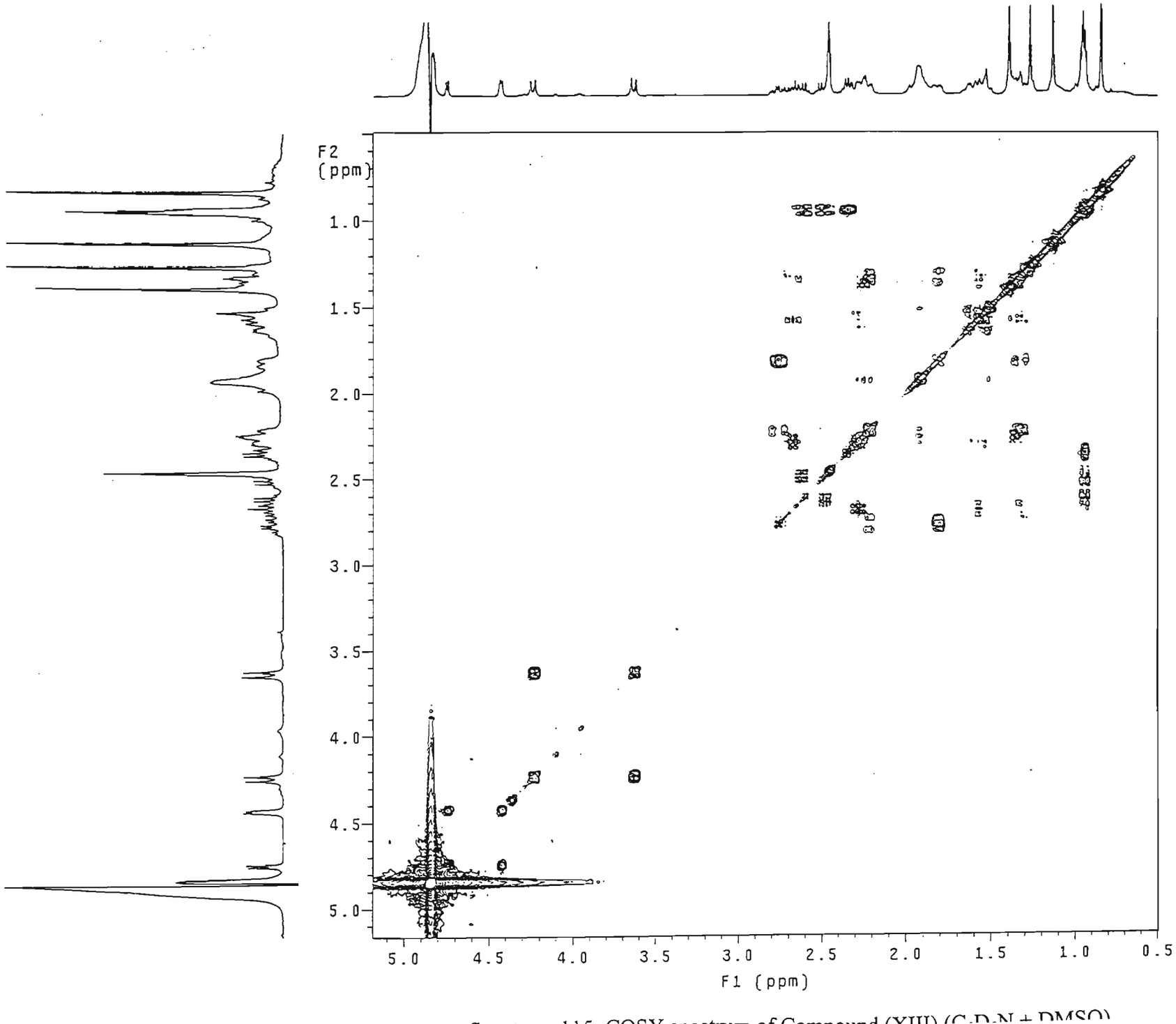
245

200
180
160
140
120
100
80
60
40
20
ppm

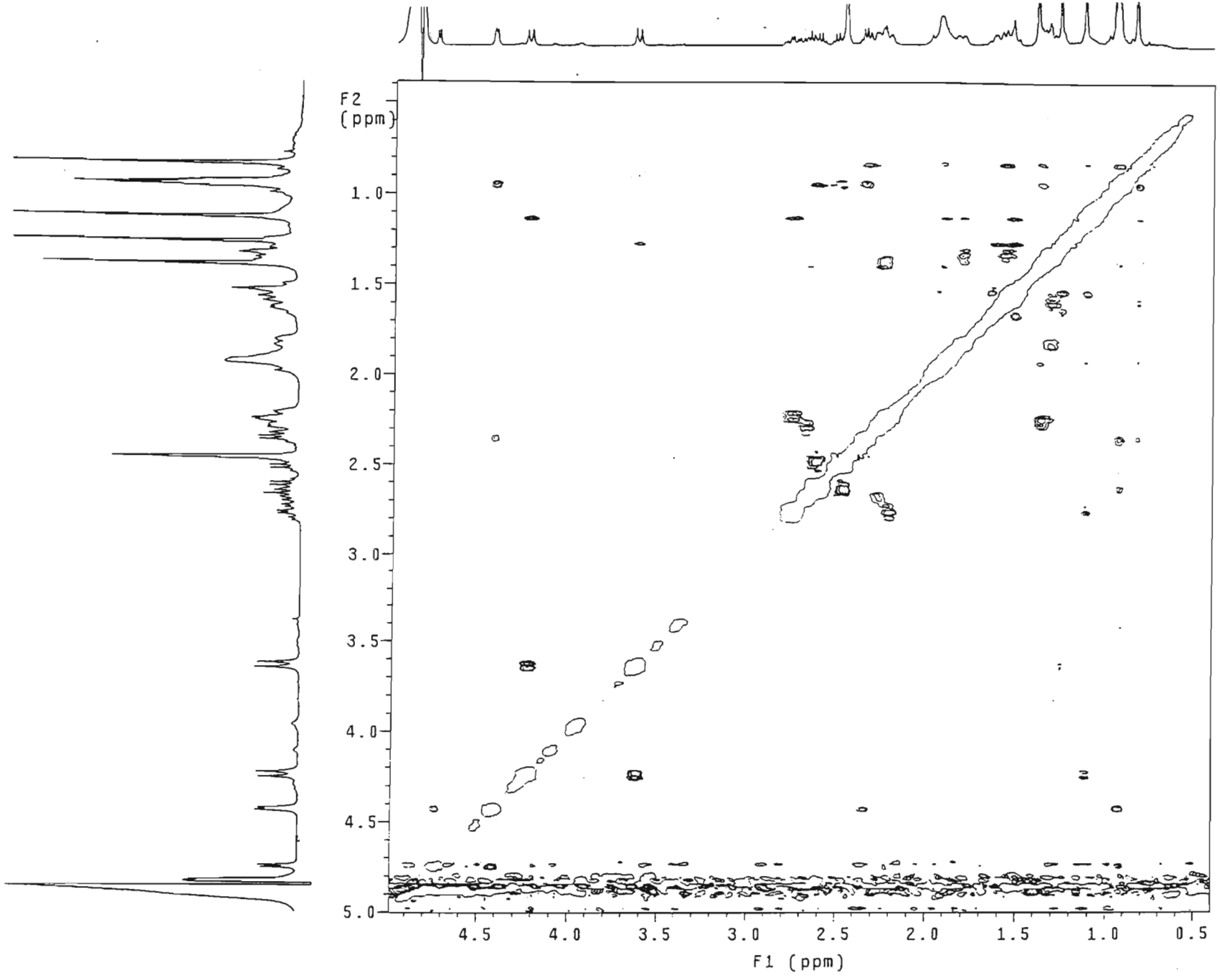


Spectrum 113: HSQC spectrum of Compound (XIII) (C_5D_5N + DMSO)

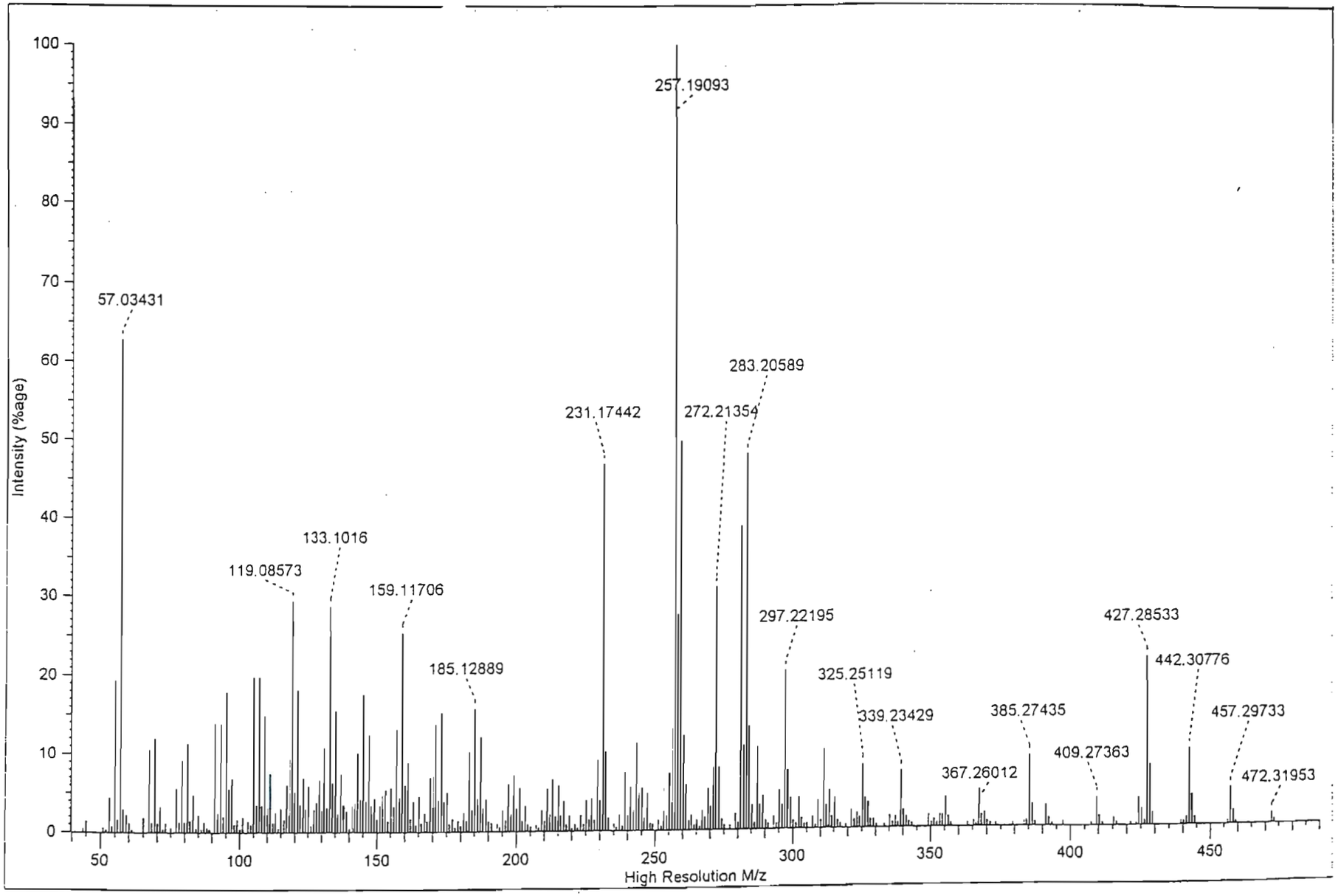

F2
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
200
180
160
140
120
100
80
60
40
20
0
F1 (ppm)



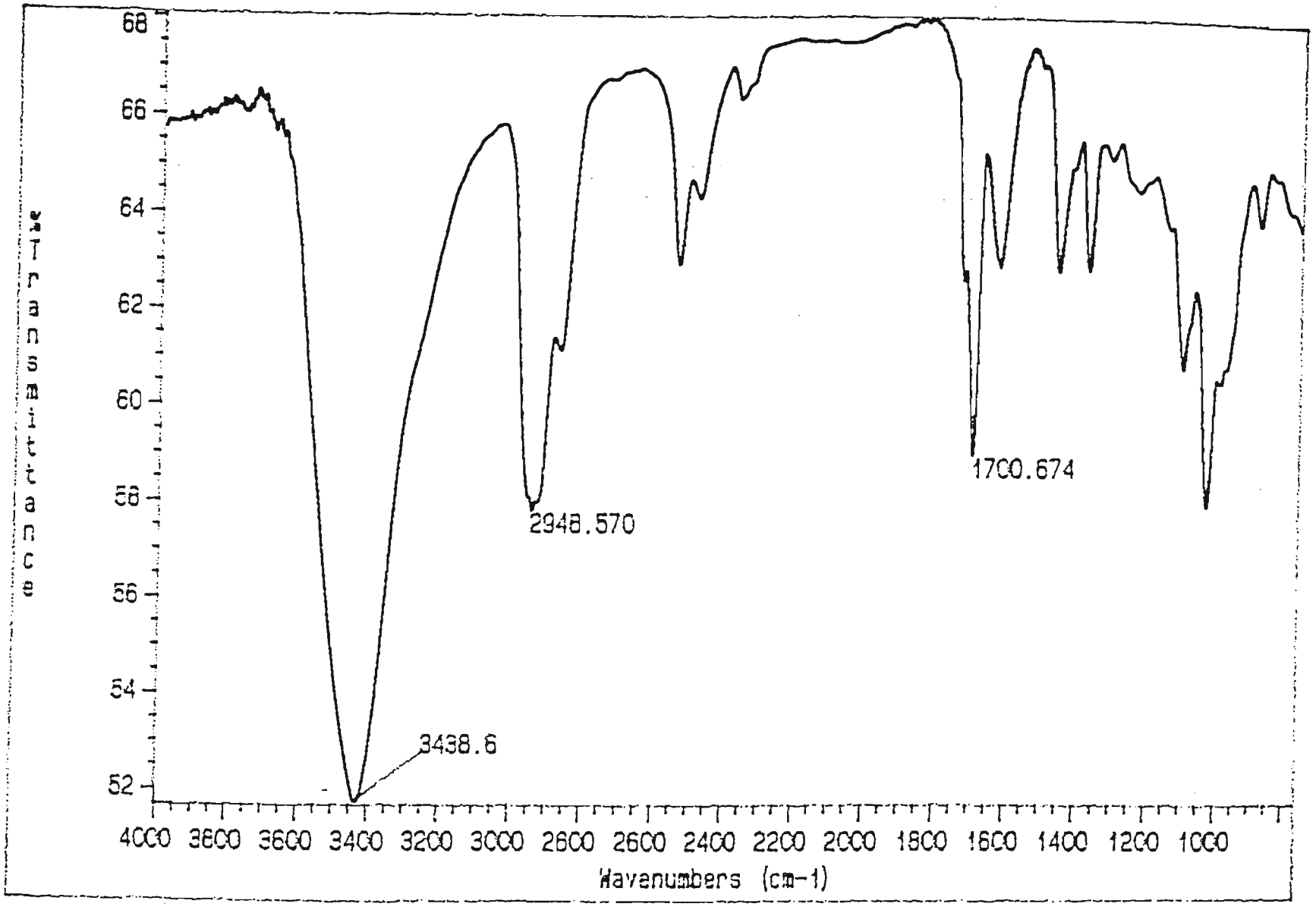
Spectrum 115: COSY spectrum of Compound (XIII) (C5D5N + DMSO)



Spectrum 116: NOESY spectrum of Compound (XIII) (C_5D_5N+ DMSO)



Spectrum 117: Mass spectrum of Compound (XIII)



Spectrum 118: IR spectrum of Compound (XIII)