THE CHEMICAL INVESTIGATION OF THE AMARYLLIDACEAE AND HYACINTHACEAE

by

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TO MY FAMILY

"Do not go where the path may lead, but go where there is no path, and leave a trail."

Unknown

PREFACE

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of KwaZulu-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.

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To my family: This work is dedicated especially for you. You have always been there to support me from a very young age and without the encouragement and support of you I with not have been able to reach what seemed like an impossible dream. To mum and dad thank you for all the sacrifices you have made over the years without this I will not be the person I am today. To my sisters, Thiloshni and Keshni, thank you for the encouragement and for all the financial support your have rendered to me over the years it has finally come to an end. To the special lady in my life thank you for understanding and supporting me during the last months of the write up.

ABBREVIATIONS

Ac	acetate
br	broad resonance
brs	broad singlet
brd	broad doublet
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 single quantum coherence
NOESY	nuclear Overhauser effect spectroscopy
FTIR	Fourier transformer 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
I.R	infra red
UV	Ultra violet
rpm	revolutions per minute
m.p	melting point
t.l.c	thin layer chromatography
GC/MS	gas chromatography/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
ref	reference
TCA	time course assay
lit	literature
EDTA	ethylenediaminetetraacetic acid
SRB	sulforhodamine B
NSAID	Non-steroidal anti-inflammatory drug
min	minute
calc.	calculated
glu	glucose
rha	rhamnose

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ABSTRACT

This work is an account of investigations into the chemistry of members of the Amaryllidaceae and Hyacinthaceae families.

The plants of the family Amaryllidaceae are a large group comprising over sixty genera and more than a thousand species. They are widely distributed, but are found more richly in the tropics, with a particularly high density in South Africa, with smaller centers of diversity in Andean South America and the Mediterranean. Amaryllidaceae plants have been extensively used by local traditional healers and have been reported to have numerous pharmacological uses. The alkaloids isolated from this family are a group of isoquinoline alkaloids found exclusively in this family. Plants belonging to two Amaryllidaceae genera were investigated phytochemically, one from each of the sub-tribes Crinineae and Amaryllidineae were investigated phytochemically. *Brunsvigia natalensis* is used in local traditional medicine to "straighten bones of children", treat barrenness in women and ease childbirth. This is the first phytochemical investigation of *Brunsvigia natalensis*, and yielded two new alkaloids, a new ceramide type compound and a known flavanoid. A comparative phytochemical investigation was carried out on the bulbs and seeds of *Crinum stuhlmanni*, which resulted in a number of different alkaloids being isolated from the seeds and bulbs of this plant.

The southern African Hyacinthaceae is a large and chemically morphologically diverse group of plants. This family comprises approximately sixty-seven genera and nine hundred species worldwide, of which twenty-seven genera and three hundred and sixty – eight species are found locally. There are five sub-families of which three occur in southern Africa. The chemical constituents of this family can be divided into four classes, namely homoisoflavanones, steroidal compounds, bufadienolides and miscellaneous compounds. These plants are used in local traditional medicine for treating ailments such as hangovers, rheumatic fever, sprains and even cancer. The phytochemistry of three Hyacinthaceae plants was studied. The phytochemical investigation of *Drimia macrocentra* and *Urginea riparia* yielded a novel bufadienolide glycoside. These glycosides are quite unusual with the glycone attached to the aglycone at C-2 and C-3 and this has only been reported only once before in this family. The phytochemical investigation of *Ledebouria revoluta* yielded a number of homoisoflavanones. These homoisoflavanones have been shown to have anti-inflammatory activity and all of the compounds isolated in this work have been screened for this activity.

Structural elucidation was carried out using spectroscopic methods such as NMR, MS, UV and IR.

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Chapter 6: Extractives from *Ledebouria asperifolia*

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Chapter 1: A Brief History of Alkaloids Used by Man

1.1 General

The use of alkaloid-containing plant extracts as potions, medicines and poisons can be traced back to almost the start of civilisation.[1] From the time Socrates drank the cup of hemlock which contained the poisonous coniine, alkaloids have attracted enormous attention.[2] Other famous examples include Cleopatra's use of atropine-containing extracts of Egyptian henbane (*Hyoscyamus muticus* Linn.) to dilate her pupils and thereby make her appear more alluring. Medieval European women utilized extracts of the deadly nightshade, *Atropa belladonna* Linn., for the same purpose, hence the epithet *belladonna* meaning 'pretty lady'.[1] In total, approximately 13,000 plant species are known to have been used medicinally throughout the world.[1] Approximately 25% of contemporary materia medica is derived from plants and used either as pure compounds or models for synthetic drugs.[1]

Atropine **(Figure 1. 1)**, which belongs to the group of tropane alkaloids, is the racemic form of hyoscyamine, which comes from *Atropa belladonna*. It is used to dilate the pupils of the eyes.[3] It is further used as a CNS stimulant and as a treatment for nerve gas poisoning.[3] It was the model for the synthetic drug tropicamide **(Figure 1. 1)**, which is also used to dilate the pupils and as a diagnostic tool in the detection of Alzheimer's disease.[1]

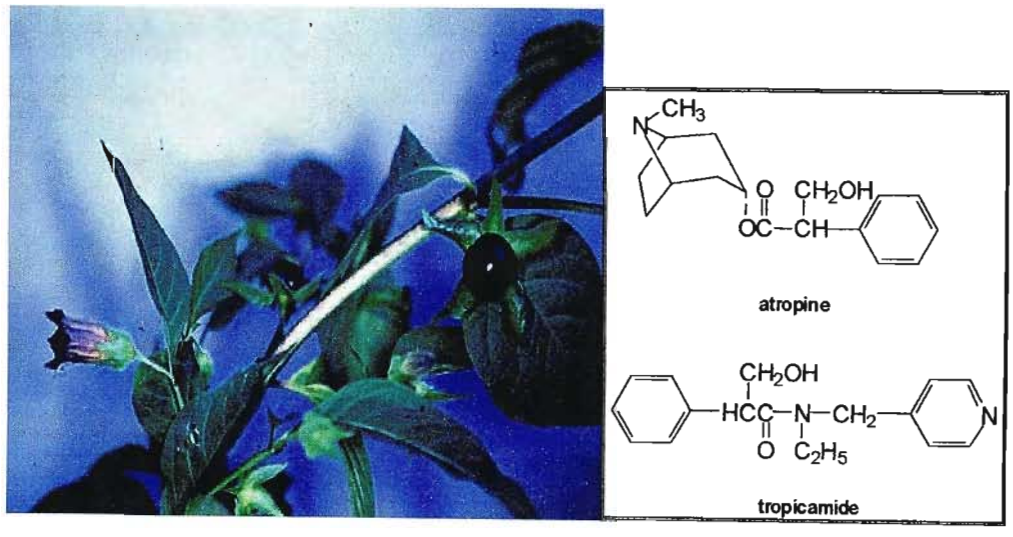

Figure 1. 1 Structures of atropine and tropicamide (right). Atropine was originally extracted from *Atropa belladonna* (left), photographer: Alice B. Russell, website: http://www.ces.ncsu.edu/depts/hort/consumer/poison/images/AtropBe3.htm

Quinine **(Figure 1. 2)**, isolated originally from *Cinchona succirubra* Pav. ex Klotzch, has been the principal antimalarial drug for over two centuries. The beneficial effects of cinchona bark were first discovered in South America in the 1630s.[4] Without this antimalarial drug the

exploration of Dutch and British explorers into Africa and India might have failed. Quinine has been a model for a number of synthetic antimalarial drugs produced: chloroquine, primaquine and mefloquine.[3]

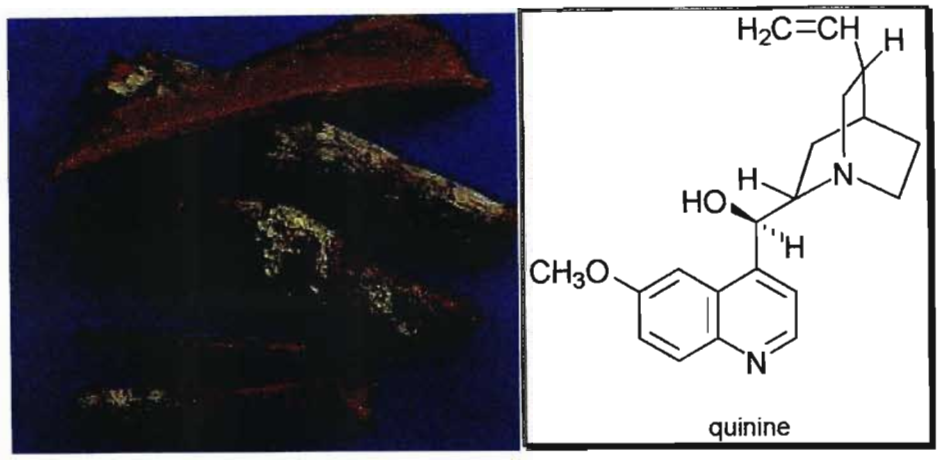

Figure 1. 2 Structure of quinine (right), which was originally extracted from *Cinchona succirubra* (left), photographer not given, website: http://www.cocura.de/P328.html

Another class of pharmacologically-important alkaloids are the ergot alkaloids. These alkaloids are isolated from the dried sclerotium of the fungus *Claviceps purpurea*.[3,5] This group of alkaloids is responsible for ergotism. Ergotism results in alimentary upsets (e.g. diarrhoea, abdominal pains and vomiting), circulatory change (e.g. coldness of hands and feet due to decrease in diameter of blood vessels) and neurological symptoms (e.g. headaches, convulsions, psychotic disturbances and hallucinations).[3] In 1926, at least 11,000 cases of ergotism occurred in Russia.[5] However, the alkaloids derived from ergot have now assumed new importance for their pharmacological properties. Two examples of important ergot alkaloids are ergonovine and methysergide **(Figure 1. 3)**. Ergonovine has potent uterine contraction activity and is used in treating postpartum haemorrhages.[5] It is also used during the final stages of labour.[3] Methysergide is used as a cranial vasodilator in the treatment of migraine headaches.[5] Other ergot alkaloids have been used to treat sexual disorders. The most famous of this type of alkaloids, however, is lysergic acid diethylamide, LSD, which is a derivative of lysergic acid. It is a powerful hallucinogen. Although it is not addictive, it can cause schizophrenia and there is danger of serious physical accidents to the patient.[3]

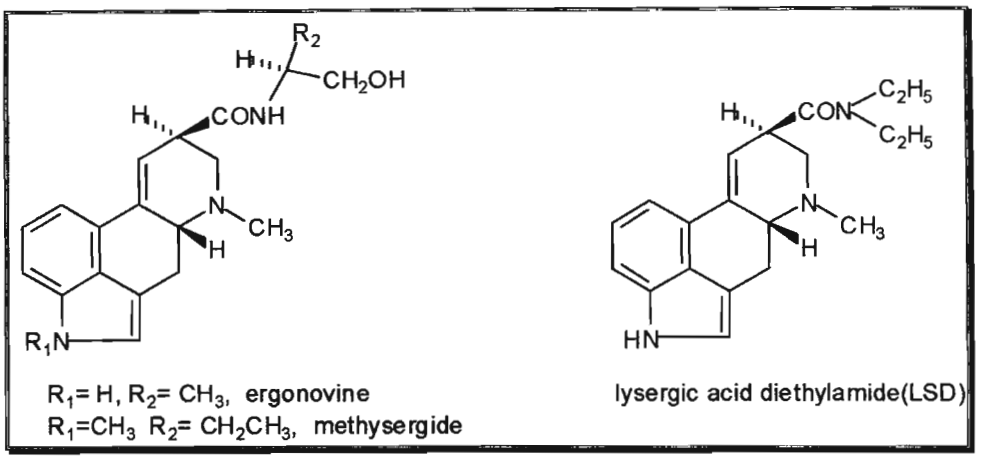

Figure 1. 3 Structures of some ergot alkaloids

Rauvolfia, a crude powered mixture of the dried rhizome and roots of *Rauvolfia serpentina* (L.) Kurz **(Figure 1. 4)**, is used in India as an antidote to snake-bite, fever, vomiting and to treat insanity.[3] Clinical tests in the late 1940s showed it to be effective for the control of hypertension.[3] It was employed in treating high blood pressure and to relieve anxiety and restlessness and was thus important in initiating the tranquillizer era.[3] This crude extract of rauvolfia contained mainly the two alkaloids reserpine and deserpidine. Both these alkaloids have been used as antihypertensives and mild tranquillizers in their pure form.[3] However, the use of reserpine alone leads to severe depression in the patients; accordingly, crude rauvolfia is used instead.[3]

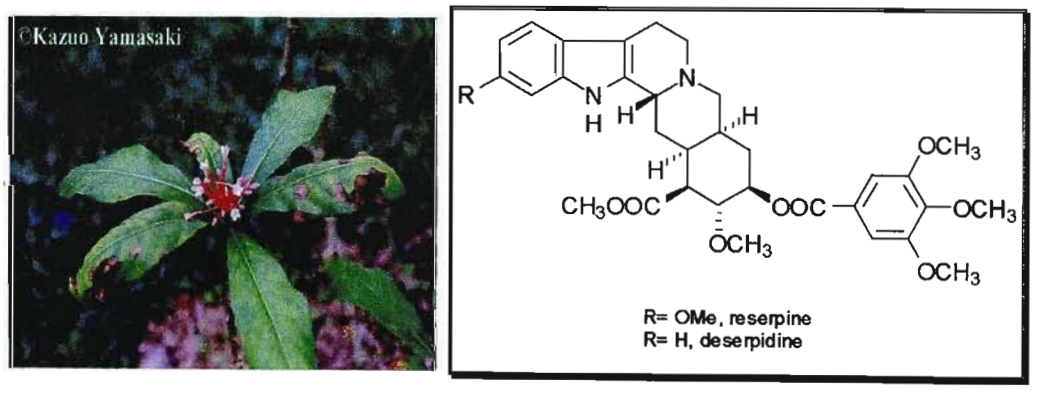

Figure 1. 4 Structures of reserpine and deserpidine (right), which are extracted mainly from *Rauvolfia serpentina* (left) photographer: Kazuo Yamasaki, website: http://pharm1.pharmazie.uni-greifswald.de/systematik/7_bilder/yamasaki/Ravolfia.jpg

Alkaloid-derived compounds that have aided in the fight against cancer are compounds such as vincristine and vinblastine from *Catharanthus roseus* G.Don. (Madagascan Periwinkle) **(Figure 1. 5)**. Vinblastine is used for the treatment of Hodgkin's disease, breast cancer and testicular carcinoma.[3] Vincristine, also from the same plant, shows a different spectrum of

activity and toxicity.[3] However, it has been found to be neurotoxic and is now used in combination with other drugs in cancer chemotherapy.[3]

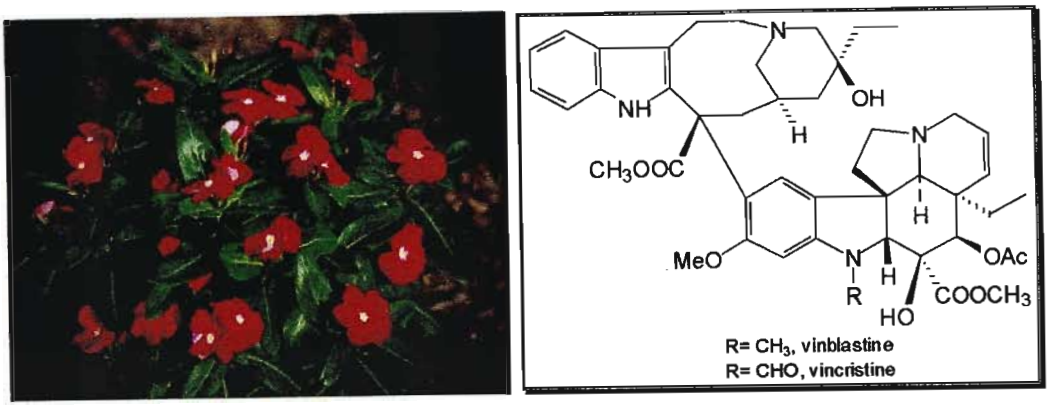

Figure 1. 5 Structures of vinblastine and vincristine (right), which are extracted from *Catharanthus roseus* (left), photographer Victory Cranberry, website: http://www.ces.ncsu.edu/depts/hort/consumer/factsheets/annuals/catharanthus_roseus.html

Another anticancer alkaloid is colchicine, a three ring membered amide, isolated from *Colchicum autumnale* Linn. **(Figure 1. 6)**. Colchicine is also used to reduce the inflammation and pain of gout. It is used in the study of genetics due to its capacity to induce polyploidy.[6] Pre-treatment of seeds with colchicine leads to various mutations of plants and it is therefore used for selective purposes in agriculture.[6]

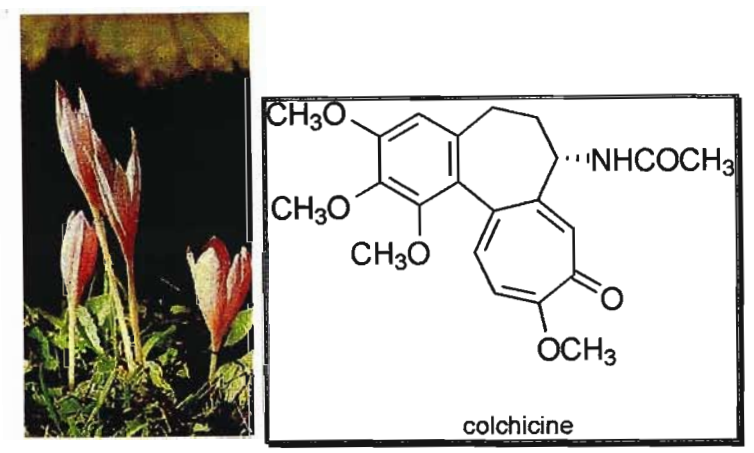

Figure 1. 6 Structure of colchicine (right), which was originally extracted from *Colchicum autumnale* (left), photographer not given on website: http://library.thinkquest.org/C007974/1_1aut.htm

Curare was used by the South American Indians as an arrow poison. Curare was made by using the bark and stems of either *Chondrodendron tomentosum* Ruiz & Pav. or *Strychnos toxifera* Schomb. ex Benth. The active ingredient in *Chondrodendron tomentosum* was found to be the isoquinoline alkaloid tubocurarine.[3] Tubocurarine **(Figure 1. 7)** is used as a muscle relaxant in surgical operations, reducing the need for anaesthesia.[3] Furthermore, it has also

been used to relax the heart muscles during open heart surgery and to treat spastic paralysis.[3] However, due to its limited availability, a number of synthetic analogues are now used.

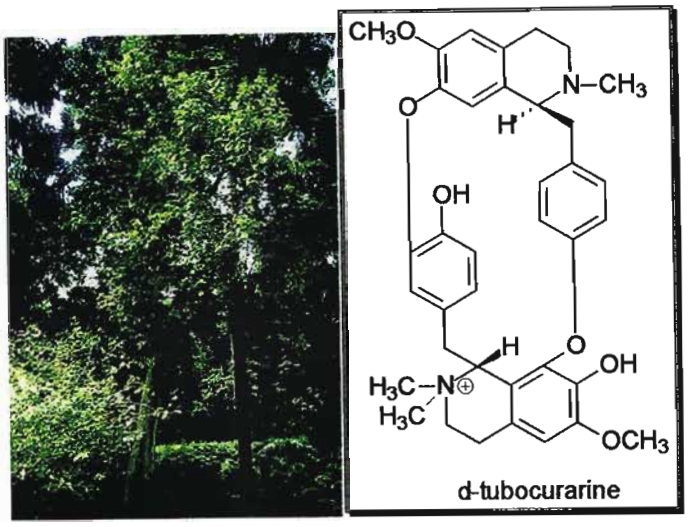

Figure 1. 7 Structure of *d*-tubocurarine (right), and *Strychnos toxifero* (left) which is used in the preparation of curare, photographer: Thomas Schoepke, website: http://library.thinkquest.org/C007974/1_3stry.htm

A new plant-derived drug is galanthamine, which was first isolated from *Galanthus nivalis* Falk **(Figure 1. 8),** 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. It is not a cure for Alzheimer's but rather slows the rate of cognitive decline.

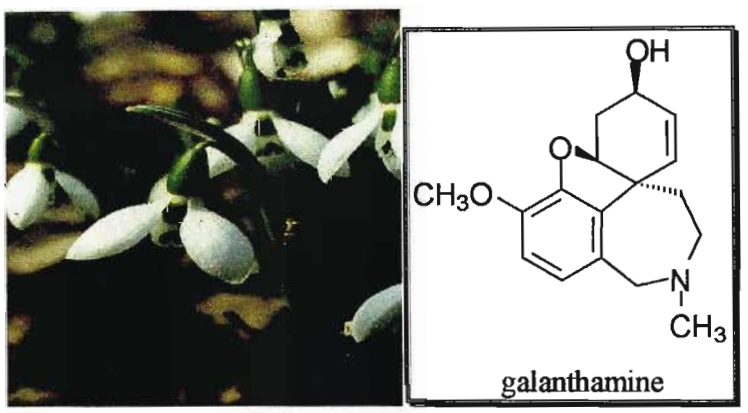

Figure 1. 8 Structure of galanthamine (right), which was originally extracted from the bulbs of *Galanthus nivalis* (left), photographer: John Worrall, website: http://www.hort.net/gallery/view/amy/galni40/

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.[3]

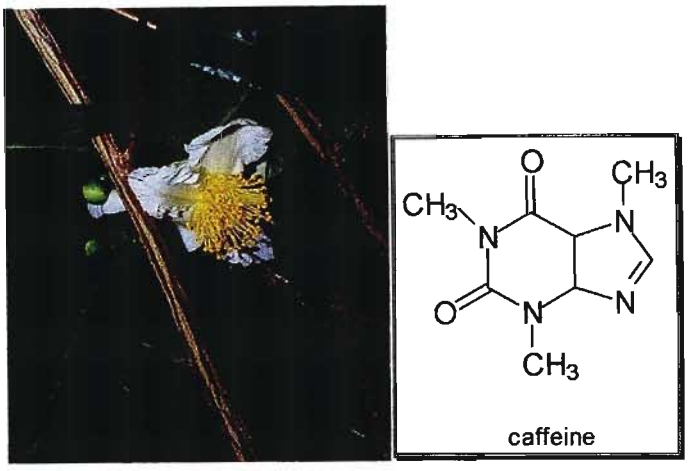

Figure 1. 9 Structure of caffeine (right), which is extracted mainly from *Camellia sinensis* (left) photographer not given, website: http://www.plantoftheweek.org/week046.shtml

Cocaine **(Figure 1. 10)** is isolated from the leaves of the plant *Erythroxylum coca* T.Plowman (coca plant). In the late 1800s cocaine was used for the treatment of a variety of illnesses including alcohol and morphine addiction. However, many of the patients became addicted to cocaine. Cocaine was also originally used in the Coca Cola soft drink; it was however removed in 1906 because of its addictive properties. Cocaine is still used medically as an anaesthetic for topical application.[3] It is used as a constituent in Brompton's cocktail, which is given to control pain in terminally ill patients.[3] It has been a model for several synthetic drugs. An example is lignocaine (lidocaine), which is one of the mostly widely used anaesthetics.[3] Another derivative of cocaine is crack, a highly addictive drug, which is processed from cocaine hydrochloride to a free base for smoking. It is a central nervous stimulant that gives the user a feeling of pleasure and a so-called "high". It has caused numerous deaths worldwide.

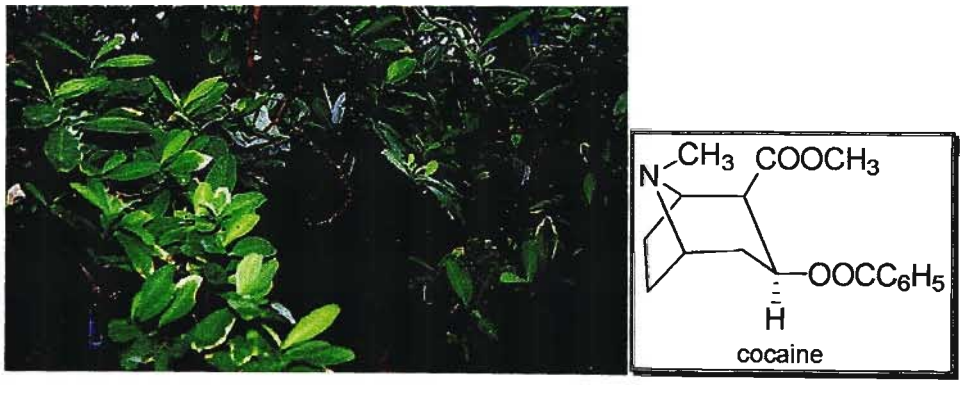

Figure 1. 10 Structure of cocaine (right), which is extracted mainly from *Erythroxylum coca* (left), photographer Thomas Schöpke, website: http://library.thinkquest.org/C007974/1_2coc.htm

Another alkaloid stimulant is nicotine. Nicotine is produced by the plant *Nicotiana tabacum* Linn. **(Figure 1. 11)** that is cultivated widely for tobacco smoking. Nicotine in small doses can act as a respiratory stimulant, however in large doses it causes respiratory depression.[3] Despite the links between smoking and cancer the smoking habit continues worldwide. Nicotine is toxic to man due to its effect on the nervous system. Studies suggest that nicotine can improve memory by stimulating the transmission of nerve impulses and this finding may account for the lower incidence of Alzheimer's disease in smokers.[3] However, any health benefits of smoking are more than outweighed by the increased risk of heart, lung and respiratory diseases. Nicotine is also used as an insecticide and fumigant. To date, no insect has evolved resistance to nicotine.[10]

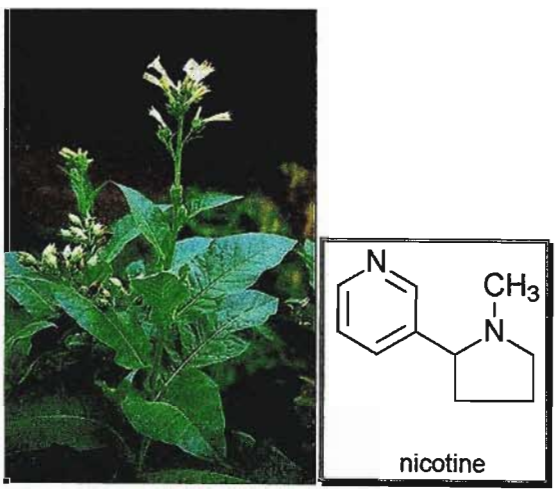

Figure 1. 11 Structure of nicotine (right), which is extracted mainly from *Nicotiana tabacum* (left), photographer not given, website: http://library.thinkquest.org/C007974/1_1tob.htm

Ephedra, extracted mainly from *Ephedra sinica* Stapf **(Figure 1. 12)**, is used worldwide as a dietary supplement. It has been used for the treatment of asthma and also as a natural decongestant and antihistamine. Ephedra contains mainly the two alkaloids ephedrine and pseudoephedrine. Ephedrine is a sympathomimetic amine with effects similar to adrenaline.[3] The effects have a longer duration than adrenaline. It is also used as a bronchodilator, giving relief to asthma, and as a nasal decongestant.[3] Pseudoephedrine is used in cough and cold preparations and as a decongestant.[3]

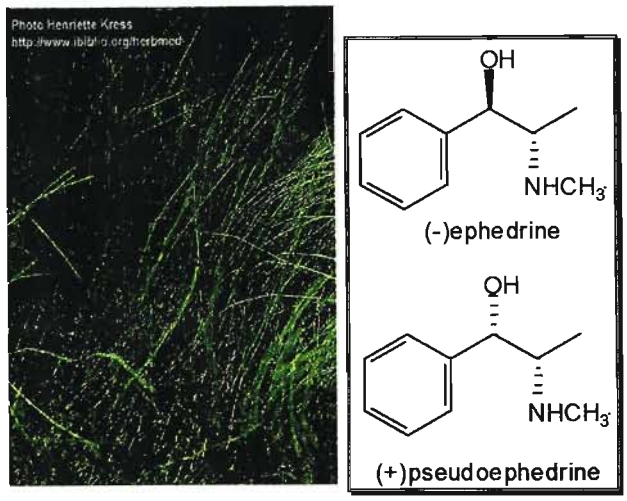

Figure 1. 12 Structures of ephedrine and pseudroephrine (right), which are extracted mainly from *Ephedra sinica* (left), photographer: Henriette Kress, website: http://ibiblio.org/herbmed/pictures/p05/pages/ephedra-sinica.htm

One plant that had a mixed effect on mankind is *Papaver somniferum* Linn. **(Figure 1. 13)**, the opium poppy. It is the oldest known intoxicating plant in human history.[9] It was originally used mainly in India 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 as a pain reliever.[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 problematic part in recent human history: from it heroin is produced, an addictive poison that has led to many deaths.

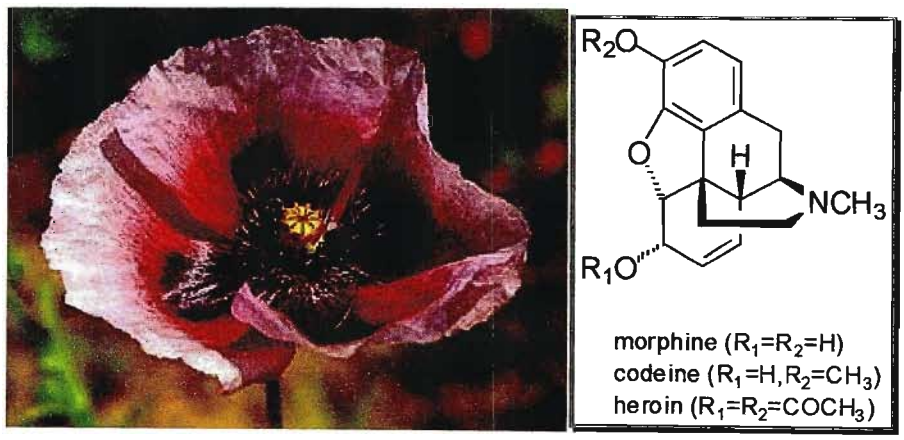

Figure 1. 13 Structures of various alkaloids (right), isolated and derivatised from *Papaver somniferum* (left), no photographer given, website: http://library.thinkquest.org/C007974/1_1pop.htm

Alkaloids have long been, and will continue to be, important sources and models for drugs, insecticides and spices. Alkaloids, if used correctly, can be extremely beneficial to man; their abuse may be fatal.

1.2 References

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Chapter 2: The Classification and Biosynthesis of Alkaloids

2.1 Introduction

Alkaloids are a group of nitrogen-containing bases.[1] They are natural products of non-peptide origin.[2] Their basic character is reflected in the name, alkaloid, derived from alkaline which means basic. In the early eighteenth century Serturner isolated one of the first alkaloids, morphine, from the opium poppy. He referred to it as " vegetable alkali" as he recognized it to have basic properties. In 1818, however, W. Meisner proposed that such "vegetable alkalis" should be called alkaloids and this became accepted and today the word generally denotes a basic, physiologically active nitrogen heterocycle of some complexity obtained from plant sources.[3]

Despite the fact that all nitrogenous metabolites are classified as alkaloids, they can be subdivided into three distinct groups according to their molecular structures and biosynthetic origins. The **true alkaloids** are those compounds in which nitrogen forms part of a heterocyclic ring system and are biosynthesized from amino acids. **Pseudoalkaloids** are also characterized by nitrogenous heterocyclic ring systems but these are not biosynthetically derived from amino acids. The third group contains physiologically active amines, which are classified as **protoalkaloids**.[4, 5]

Due to the significant pharmacological activities and proven medicinal value of a large number of alkaloids, the chemical investigation of these compounds has received a great deal of attention and has been closely associated with the development of potential therapeutic agents.[4,]

In this work, alkaloids from the Amaryllidaceae family have been investigated. This family compromises over sixty genera and more than a thousand species worldwide.[6-9] It is found mainly in southern Africa and to a lesser extent in South America and the Mediterranean.[8, 9]

Within southern Africa, the amaryllids are found throughout the three major areas of the country, from the savannah in the north to the tropical environment of the east and the winter rainfall areas in the south west.[9] Of the nine tribes, two are present in South Africa, the Amaryllideae and the Haemantheae. The Amaryllideae can be further subdivided into two monophyletic sub-tribes: The Crinineae (*Boophane*, *Crinum*, *Ammocharis* and *Cybistetes*) and the Amaryllidineae (*Amaryllis*, *Nerine*, *Brunsvigia*, *Crossyne*, *Hessea*, *Strumaria* and *Carpolyza*). The Haemantheae contains six genera: *Scadoxus*, *Cyrtanthus*, *Haemanthus*, *Clivia*, *Gethyllis* and *Apodolirion*.[9]

In this study, *Crinum stuhlmannii* Baker and *Brunsvigia natalensis* Baker were studied.

2.2 The Classification of Amaryllidaceae Alkaloids

The nomenclature of alkaloids has not been systematised. This is due mainly to the complexity and variety of the compounds involved. Classification of alkaloids is therefore problematic with some authors preferring a classification based on chemical structures (e.g. pyridine, tropane, pyrrolizidine alkaloids), while others base theirs on plant genera. The Amaryllidaceae alkaloids use both systems in their classification.

Some of the general characteristics of the Amaryllidaceae alkaloids are:[11]

1. A fundamental ring system composed of a C_6-C_1 and a *N*-C_2-C_6 building block, derived from L-phenylalanine (L-Phe) and L-tyrosine (L-Tyr), respectively.
2. Weak bases (pK_a 6-9).
3. Contain usually one nitrogen atom which is secondary, tertiary or even quaternary and the carbon content varies from 16 to 20 atoms.

The alkaloids of this group can be classified into nine skeletal homogeneous subgroups:[10] lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine, galanthamine and a miscellaneous subgroup. The alkaloids in all eight groups have a six membered aromatic A ring. The lycorine, homolycorine, crinine, narciclasine and galanthamine type alkaloids also exhibit some degree of similarity in that they have identical points of fusion for the A, B and C rings. The tazettine series also has six membered B and C rings; however, the points of fusion between these rings are significantly different. Alkaloids from the montanine series deviate from the above trend and typically display a seven membered B ring and a six membered C ring. Those alkaloids grouped into the miscellaneous class do not conform to any of the other classes and very little homogeneity is observed.

2.2.1 The Lycorine Type Alkaloids

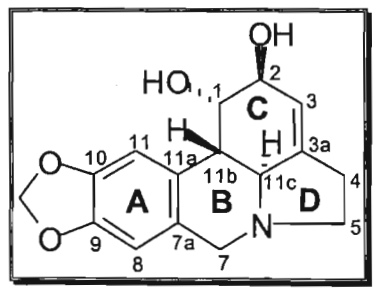


Figure 2. 1 Lycorine, an alkaloid of the lycorine type

Lycorine is the most common alkaloid of this group.[10] The lycorine type alkaloids have an additional five membered heterogeneous D ring together with the A, B and C rings and, in

many cases, the C and / or D rings contain some degree of unsaturation and oxygenation. The general characteristics of the ^{1}H NMR spectra are:[11]

1. Two singlets for the *para*-oriented disubstituted aromatic protons, H-8 and H-11, in the range δ_H 6.5-7.2 ppm.
2. A unique olefinic proton ~ δ_H 5.5 ppm (H-3).
3. Two doublets as an AB system corresponding to the benzylic protons at C-7.
4. The deshielding observed for the β protons at C-7 and C-5 in relation to their α-homologues is due to the effect of the *cis*-lone pair of the nitrogen atom.

2.2.2 The Homolycorine Type Alkaloids

The homolycorine type alkaloids differ from the lycorine type alkaloids in that the heteroatom in ring B is oxygen instead of nitrogen. Ring B constitutes a lactone and the nitrogen atom in ring D is always methylated. This group also includes lactone, hemiacetal or cyclic ether-containing (unusual) alkaloids.

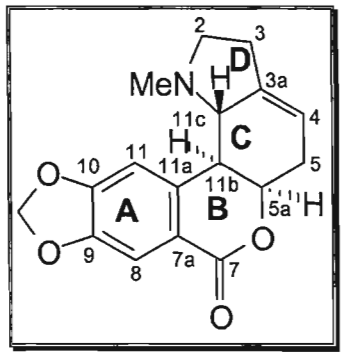


Figure 2. 2 Masonine, an alkaloid of the homolycorine type

The general characteristics of the ^{1}H NMR spectra are:[11]

1. Two singlets for the *para*-aromatic protons, H-8 and H-11. In ring B lactone alkaloids, one can differentiate between the H-8 and H-11 protons due to the deshielding of H-8 by the *peri*-carbonyl group (C-7).
2. In ring B hemiacetal alkaloids, the substituent at C-7 is always α and the benzylic proton H-7 is β and appears as a singlet between δ_H 5-6 ppm, depending on the substituent at C-7.
3. H-4 in the C ring is generally vinylic and is found around δ_H 5.5 ppm.
4. Between δ_H 2.0-2.2 ppm there is a singlet corresponding to the *N*-methyl group proton resonance.
5. If a substituent occurs at C-5 it is always α.
6. The H-2α proton is more deshielded than H-2β due to the *cis* lone pair of the nitrogen atom.

2.2.3 Crinine - Haemanthamine Types

Both of these alkaloid types contain a 5,10b-ethanophenanthridine bridge. These ring systems are very common to Amaryllidaceae alkaloids with variations in position, type and configuration of oxygenated substituents and the presence of alkaloids with enantiomeric basic nuclei.[10] In the crinine type alkaloids the 5,10b-ethano bridge (ring D) is situated in a β orientation. This is the distinction between crinine alkaloids and haemanthamine alkaloids in which the 5,10b-ethano bridge is oriented in an α orientation.

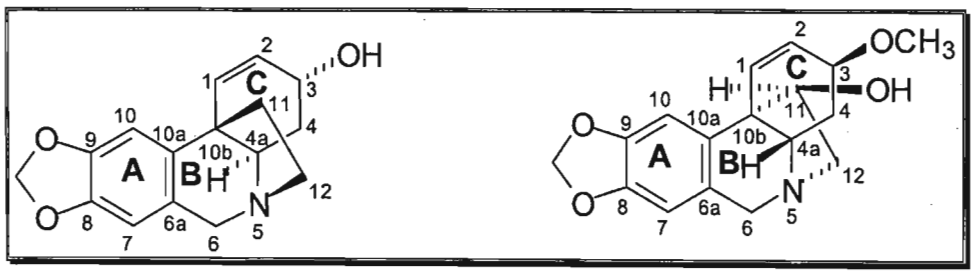


Figure 2. 3 Crinine and haemanthamine, alkaloids of the crinine and haemanthamine types

The general characteristics of the ^{1}H NMR spectra are:[11]

1. Two singlets for the *para*-oriented aromatic protons in the range δ_H 6.4-7.0 ppm.
2. When $CDCl_3$ is used as a solvent, the magnitude of the coupling constant between each olefinic proton (H-1 and H-2) and H-3 gives information about the configuration of the substituent at C-3.
3. A large coupling constant between H-4α and H-4a ($J_{4\alpha,4a}$~13 Hz) occurs due to their *trans*-diaxial position and is characteristic of the haemanthamine series.
4. An AB system corresponding to the benzylic protons at C-6.

2.2.4 Pre-tazettine Type Alkaloids

This type of alkaloid has a [2]benzopyrano[3,4]indole skeleton.[10] It contains four rings, an aromatic A ring, a six membered B ring containing an oxygen atom, a unique six membered ring C and a nitrogen-containing five-membered D ring with the nitrogen being methylated.

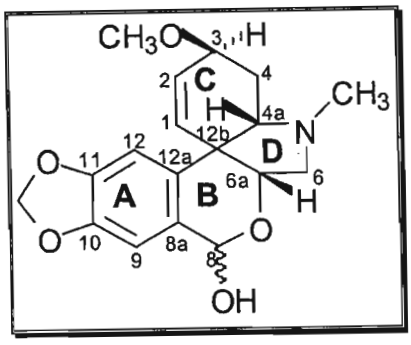


Figure 2. 4 An alkaloid of the pre-tazettine type

The general characteristics of the ^{1}H NMR spectra:[11]

1. A *N*-methyl group proton resonance at δ_H 2.4-2.5 ppm is used to distinguish this type from the haemanthamine type alkaloids.
2. The methylenedioxy group (δ_H 5.9-6.0 ppm) is always present.

2.2.5 Galanthamine Type Alkaloids

This class has a similar skeleton to crinine / haemanthamine types but has an open B ring, and a methyl group attached to the nitrogen atom. These alkaloids are unique in that the A ring and the C ring are joined by an ether linkage. They contain an α-orientated ethane nitrogen bridge.

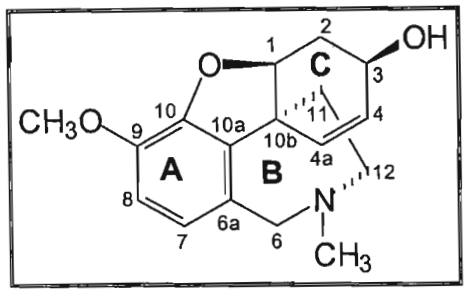


Figure 2. 5 Lycoramine, an alkaloid of the galanthamine type

The general characteristics of the ^{1}H NMR spectra are:[11]

1. Two doublets for the two *ortho*-oriented disubstituted aromatic protons, with a coupling constant of $J_{7,8}$~8 Hz.
2. The stereochemistry of the substituent at C-3 is determined by considering the coupling constants of the olefinic protons H-4 and H-4a. If a coupling constant of $J_{3,4}$ ~5 Hz is observed the substituent is pseudoaxial while a coupling constant of ~0 Hz indicates the substituent at C-3 is pseudoequatorial.
3. A pair of doublets is observed for the AB system of the benzylic protons at C-6.
4. The H-1 proton resonance is deshielded by the presence of a 1,10-ether bridge.

5. The presence of an *N*-methyl group is common but occasionally *N*-formyl or *N*-acetyl derivatives have been reported.

2.2.6 Montanine Type Alkaloids

This class is characterized by having a seven membered nitrogen containing B ring with a methylene bridge extending from the nitrogen atom to C-11 on the other side of the ring forming a six membered and five membered ring.

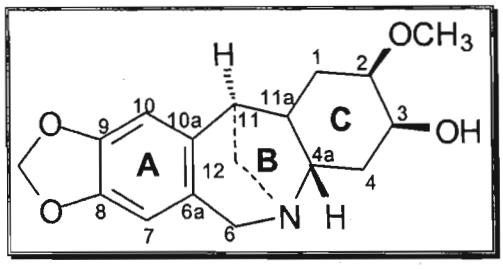


Figure 2. 6 Coccinine, an alkaloid of the montanine type

The general characteristics of the ^{1}H NMR spectra are:[11]

1. A pair of doublets for the *para* orientated protons (H-7 and H-10) with a coupling constant of *J*~1Hz.
2. The H-1 olefinic resonance at ~ δ_H 5.5 ppm.
3. The deshielded pair of doublets (*J*=16Hz) characteristic of the C-6 methylene protons.
4. Two triplets of doublets typical of the H-4 methylene protons at C-4.

2.2.7 Narciclasine Type Alkaloids

These contain only three rings, an aromatic A ring, an amide containing B ring and a C ring which is extensively oxygenated.

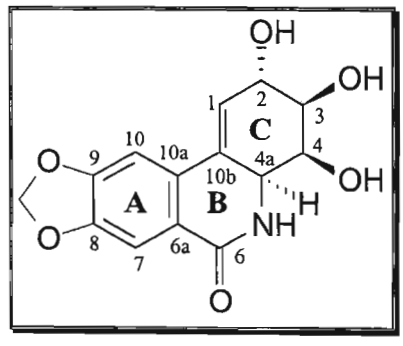


Figure 2. 7 Narciclasine, an alkaloid of the narciclasine type

The general characteristics of the ^{1}H NMR spectra are:[11]

1. A pair of doublets (*J*~1 Hz) for the *para* orientated protons (H-7 and H-10).
2. The H-4a proton occurs at ~δ_H 4.4 ppm.
3. The C-ring is normally highly oxygenated.

2.3 The Biosynthesis of Amaryllidaceae Alkaloids

The Amaryllidaceae alkaloids are biosynthetically derived from two amino acids, making them members of the true class of isoquinoline alkaloids.[4,15] The amino acid, phenylalanine, gives rise to the C_6-C_1 unit from which the aromatic ring A and the benzylic carbon are derived. The second amino acid, tyrosine, gives rise to the C_6-C_2 unit from which the aliphatic C ring is derived. It is also responsible for the provision of the nitrogen atom. Research has shown that although tyrosine and phenylalanine are closely related, they are not interchangeable in the biosynthesis.

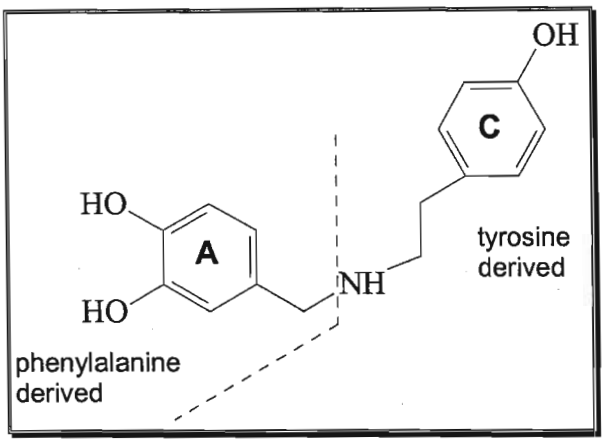
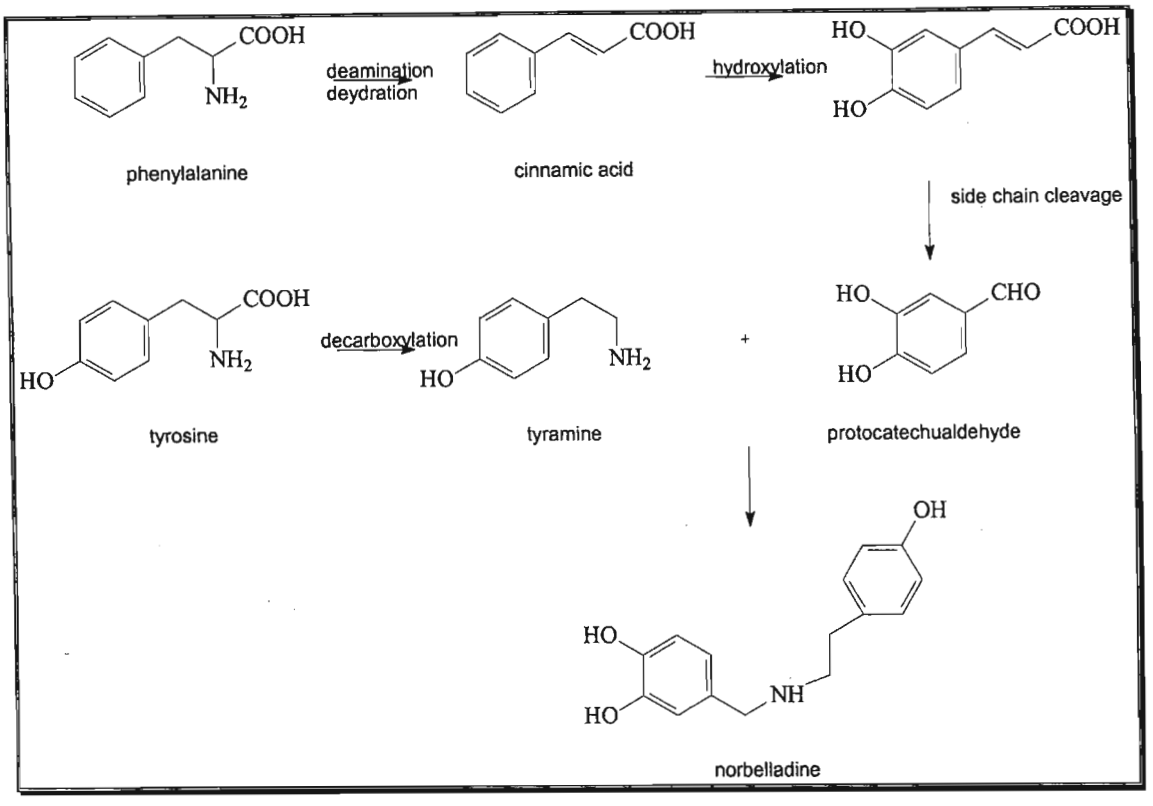


Figure 2. 8 The origin of the A and C rings

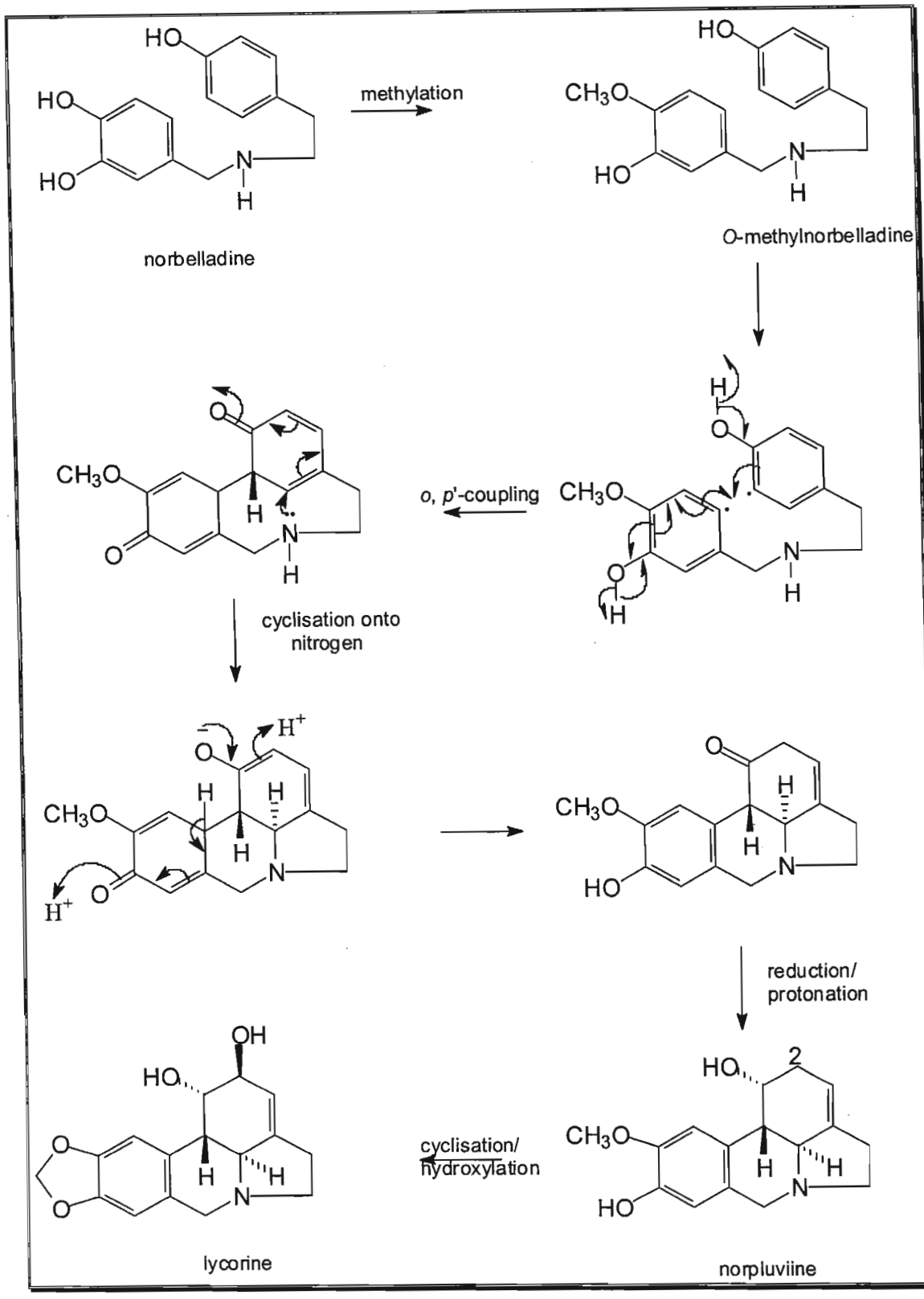
The skeletal structures of all of the Amaryllidaceae alkaloids are related to an intermediate known as norbelladine. This precursor, norbelladine, is formed by the condensation of tyramine and protocatechualdehyde. Tyramine is derived from the decarboxylation of tyrosine *via* a decarboxylation enzyme (**Scheme 2. 1**). Protocatechualdehyde is derived from phenylalanine *via* cinnamic acid.[4,5,12] The first step in the production of protocatechualdehyde involves the deamination of phenylalanine by the enzyme phenylalanine ammonia lyase (PAL), to give *trans*-cinnamic acid. Hydroxylation of cinnamic acid results in the formation of caffeic acid. Cleavage of the side chain of caffeic acid results in the formation of protocatechualdehyde.[10]



Scheme 2. 1 The biosynthesis of norbelladine[4]

The hypothesis of Barton and Cohen, that these alkaloids could arise from different modes of phenol oxidative coupling of norbelladine and its derivatives, has been of central importance in biosynthetic studies.[4,5,13] This hypothesis has been proven true by tracer studies.[10] Hydroxy groups *ortho* and/or *para* to sites of new bond formation between aromatic rings are essential for biosynthetic schemes of this type.[12] The precursor *O*-methylnorbelladine can be twisted in different ways so that various modes of oxidative phenolic coupling are possible.[4,5,12,15]

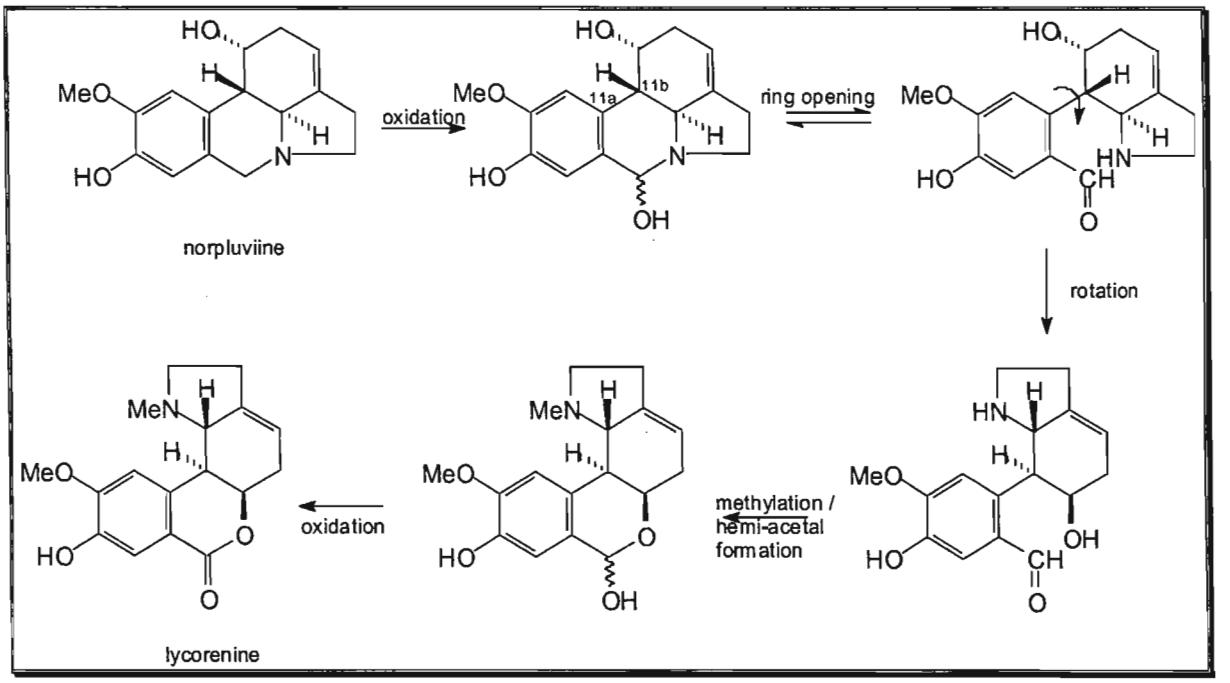
The biosynthetic pathway for lycorine type alkaloids postulates that norbelladine is the precursor.[4,5,12,15] Norbelladine is first methylated to produce *O*-methylnorbelladine. This is followed by *o-p'* phenolic coupling and cyclisation onto nitrogen to produce norpluviine (**Scheme 2. 2**).[4,5] The hydroxy and methoxy groups on norpluviine combine to form a methylenedioxy group. The final step in the formation of lycorine is the hydroxylation of C-2. It has been proven by tritium labeling that the configuration of the hydroxy group at C-2 is β.[11]



Scheme 2. 2 The biosynthesis of lycorine type alkaloids [4,11,12,14,15]

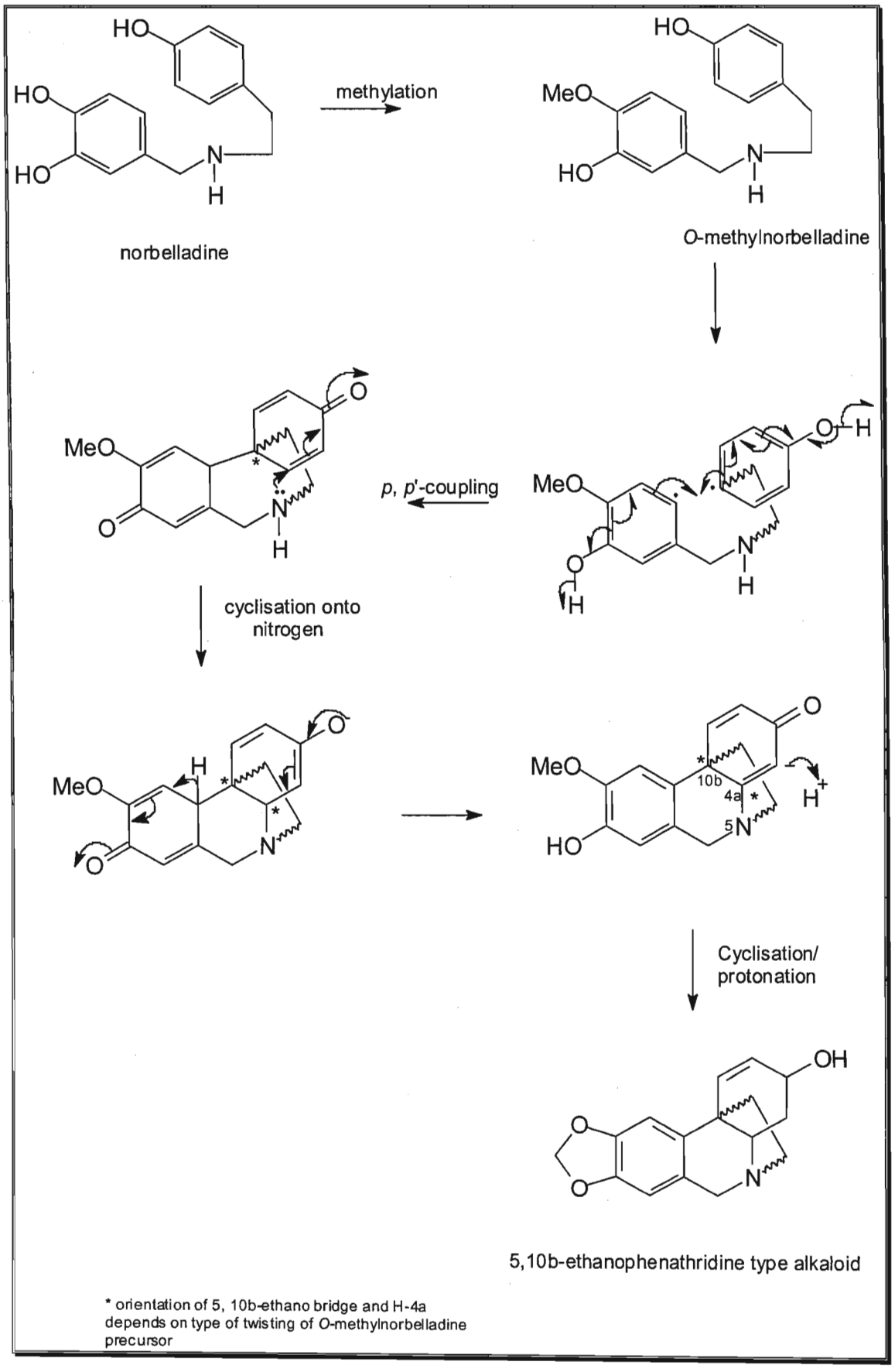
As in the biosynthesis of lycorine, *O*-methylnorbelladine is the precursor for homolycorine type alkaloids. The difference here is that there is a different mode of oxidative coupling: instead of *ortho-para* you now have *para-para* (**Scheme 2. 3**).[11] Norpluviine undergoes

benzylic oxidation at position 7, which is followed by ring opening to produce an amino aldehyde.[14] Rotation about the C-11a-C-11b bond is then followed by hemiacetal formation, oxidation and methylation of the nitrogen atom to produce lycorenine.[11,16]



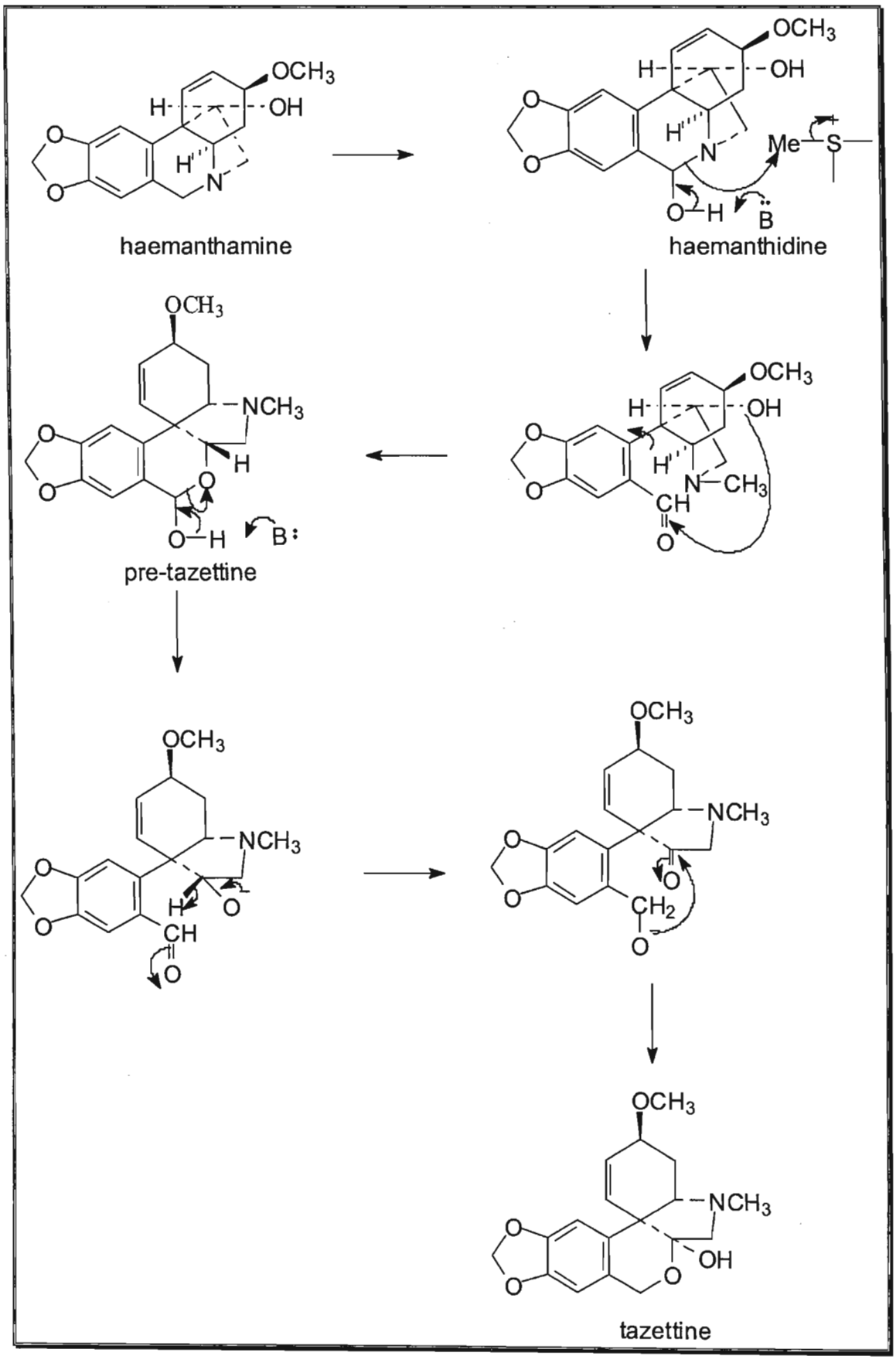
Scheme 2. 3 The biosynthesis of homolycorine type alkaloids [4,11,14,15]

Crinane type alkaloids are also derived *via* phenol oxidative coupling. However the *O*-methylnorbelladine precursor is twisted in such a way that *p-p'* oxidative coupling between the two aromatic rings takes place (**Scheme 2. 4**).[4,5,15] The resulting molecule has an ethanoic bridge between the nitrogen atom at position 5 and C-10b, hence the nomenclature 5,10b-ethanophenanthridine alkaloids. The bridge formed can be α or β and this gives rise to the two different series of alkaloids. The haemanthamine type alkaloids typically have an α 5,10b-ethanobridge and crinine type alkaloids, conversely, exhibit a β orientated bridge. Tracer studies done to determine whether these two types of alkaloids can be interconverted *in vivo* have shown that this is not possible.[16] Haemanthamine, itself, has a hydroxy group at C-11, which has been shown to arise by hydroxylation with normal retention of configuration.[12]



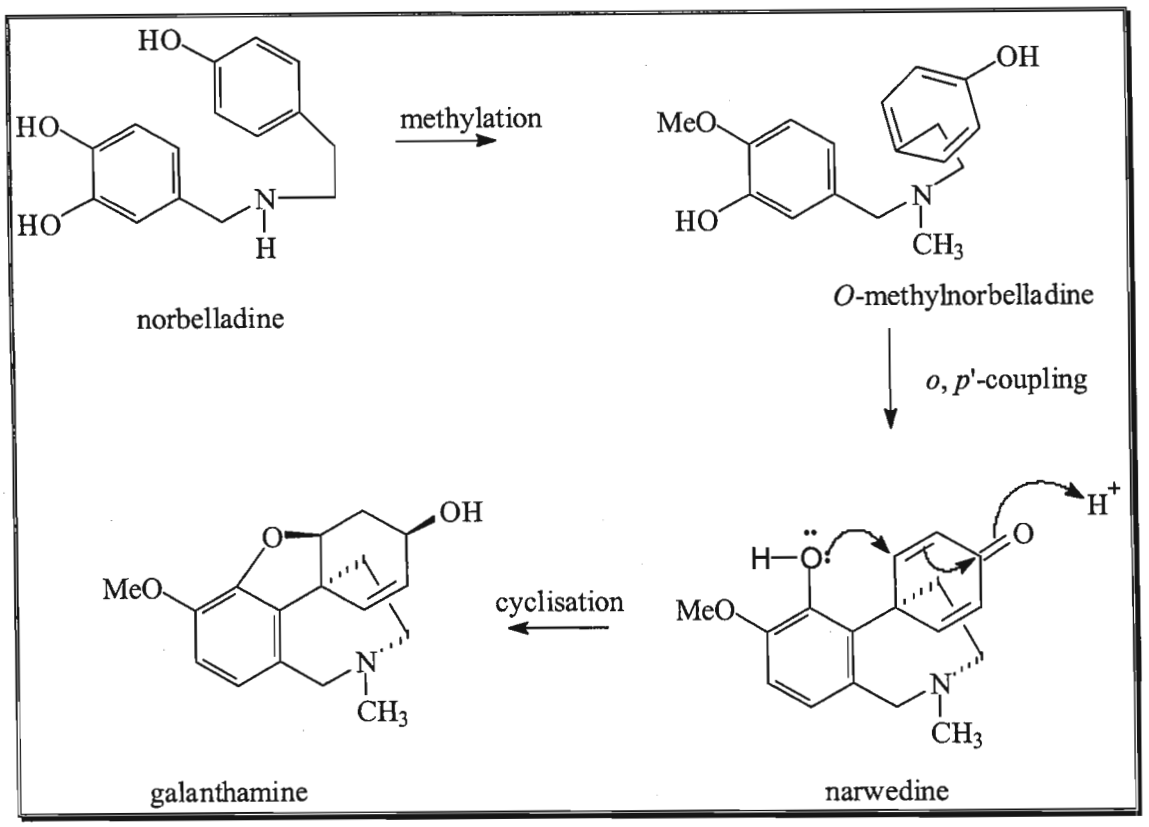
Scheme 2. 4 The biosynthesis of crinane/5,10b-ethanophenanthridine type alkaloids [10,13]

The biosynthesis of pre-tazettine occurs by a process analogous to the conversion of norpluviine into lycorenine (**Scheme 2. 5**). In this case haemanthamine is converted *via* haemanthidine into pre-tazettine. Under mildly basic conditions pre-tazettine is converted to tazettine. Tazettine was for a long time considered to be a true alkaloid, however it has been shown to be a artefact, arising from the true alkaloid pre-tazettine.[17,21] This conversion is thought to be an internal crossed Cannizzaro reaction.[17]



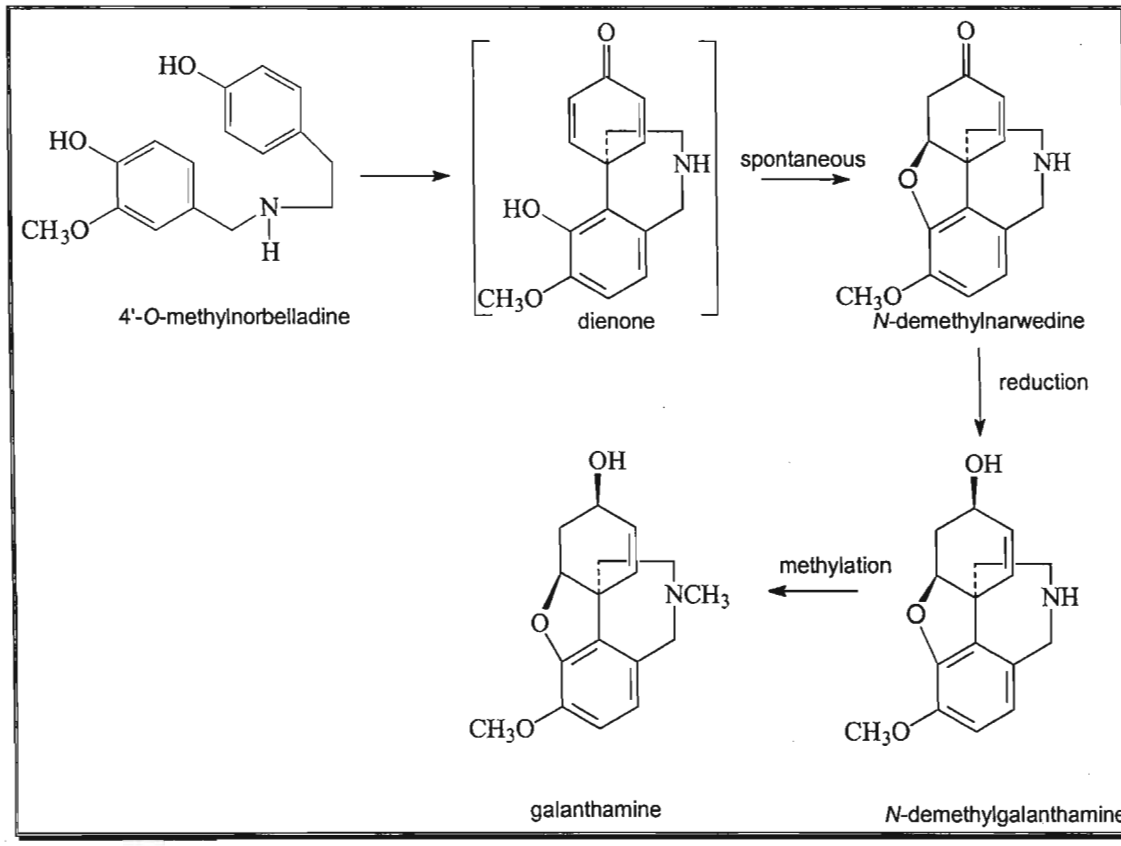
Scheme 2. 5 The biosynthesis of tazettine type alkaloids [17]

The pathway to galanthamine involves *para-ortho'* oxidative coupling similar to that found in other Amaryllidaceae type alkaloids. Initial tracer studies done on the King Daffodil showed that methylation of norbelladine should occur before *ortho-para'* coupling, with narwedine the direct precursor to galanthamine (**Scheme 2. 6**).[10,11,17]



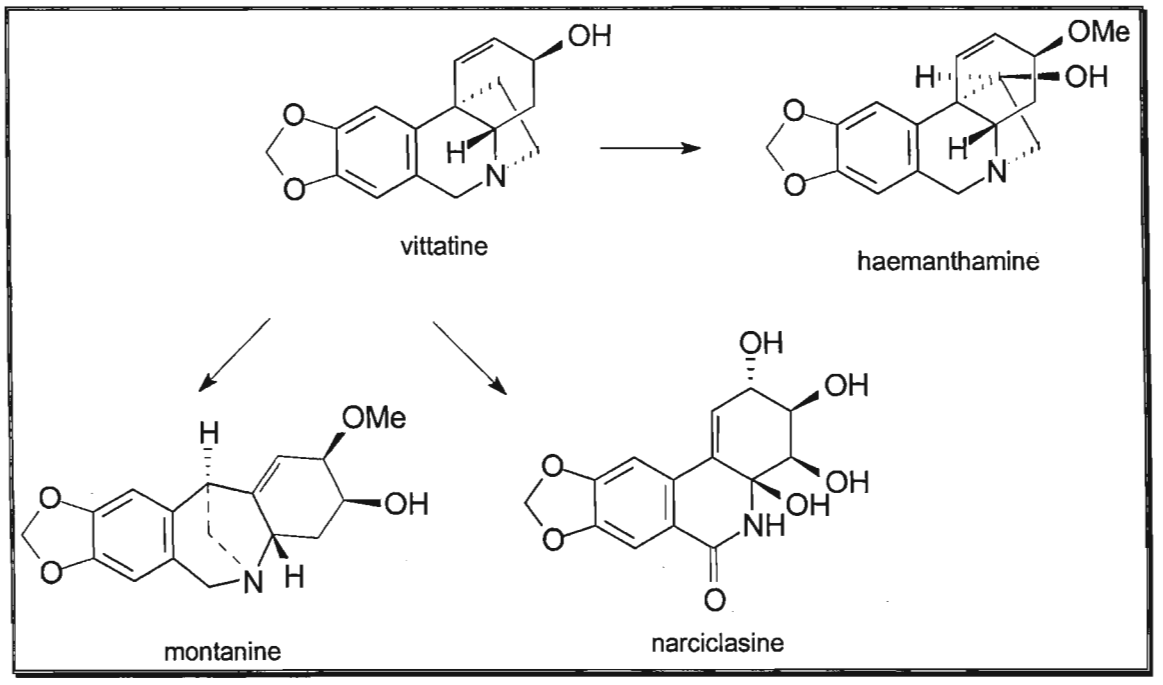
Scheme 2. 6 The biosynthesis of galanthamine type alkaloids [10,11,17]

However, recent reports have postulated a new biosynthetic pathway to galanthamine (**Scheme 2. 7**).[20] Radioactive as well as ^{13}C-labelled 4'-*O*-methylnorbelladine was fed to *Leucojum aestivum* Linn. and radioactive galanthamine and *N*-demethylgalanthamine were produced. This biosynthesis is thought to proceed by phenolic coupling of 4'-*O*-methlynorbelladine to yield an unstable dienone that spontaneously rearranges, without enzyme catalysis, to form *N*-demethylnarwedine.[20] This alkaloid is then enzymically and stereoselectively reduced to galanthamine.[20] According to this study, methylation of the nitrogen atom occurs after the phenol coupling, narwedine is not the direct precursor to galanthamine, and narwedine and galanthamine could possibly exist in equilibrium.[20]



Scheme 2. 7 Proposed biosynthesis of galanthamine [20]

Montanine type alkaloids are biosynthetically derived *via* the *para-para'* oxidative coupling of the norpluviine precursor. These alkaloids have a similar intermediate to the haemanthamine type alkaloids but not haemanthamine itself, as tracer experiments have shown.[16,18] Tritium labeled haemanthamine was fed into *Haemanthus coccineus* Linn., but no radioactive montanine type alkaloids were produced indicating that haemanthamine is not a precursor.[16] Researchers therefore speculated that the C-3 methoxy substituent is a point of interference in the *in vivo* rearrangement to the montanine nucleus.[16] However, tritium labeled vittatine was incorporated into both montanine and haemanthamine type alkaloids from *Rhodophiala bifida* Traub. which confirms the suggestion that a C-3 methoxy group hinders this arrangement (**Scheme 2. 8**).[16] The study also showed the specific radioactivity of vittatine-derived haemanthamine was substantially higher than that of montanine.[16] This indicates that 11-hydroxyvittatine is converted more efficiently to haemanthamine which requires only the methylation of the hydroxy group at C-3 while the formation of montanine involves a rearrangement of the vittatine ring system in addition to the methylation of the oxygen at C-2.[16]



Scheme 2. 8 The biosynthetic relationship between haemanthamine, montanine and narciclasine

The biosynthesis of narciclasine type alkaloids is thought to proceed either *via* *O*-methylnorbelladine with *para-para* oxidative coupling of the *O*-methylnorbelladine.[11,19] It has an haemanthamine type intermediate. In feeding experiments on *Pancratium maritimum* Linn., tritium labeled vittatine was incorporated into narciclasine, demonstrating it to be an intermediate in the biosynthesis.[19] In another feeding experiment on *Narcissus pseudonarcissus* Linn., tritium labeled norpluviine was injected and radioactive labelled narciclasine was produced, however the conversion was low.[14] It has been suggested, therefore, that since narciclasine type alkaloids are structurally similar to both haemanthamine and lycorine, these compounds could be derived *via* both biosynthetic routes. Despite this, the vittatine to narciclasine conversion is still significantly more efficient.[14]

2.4 The Biological Activity of Amaryllidaceae Alkaloids

The wide occurrence and the structural diversity of Amaryllidaceae alkaloids has long attracted attention for their biological activity. As early as the fourth century BC, oil from *Narcissus poeticus* Huds. was used in cancer treatment by the Greek physician Hippocrates of Cos (the "Father of Medicine").[22] A large number of Amaryllidaceae plants have been used in folk medicine.

Bioassay-guided fractionation was employed on the alkaloids from *Crinum amabile* Donn. to determine the biological activity.[23] Compounds 1-5 were evaluated for antimalarial and cytoxic potential.[2]

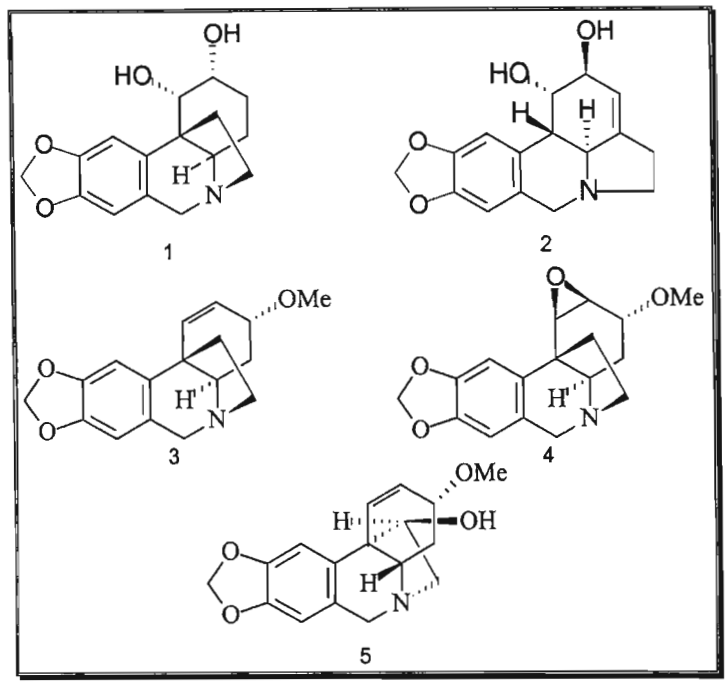


Figure 2. 9 Alkaloids isolated from *Crinum amabile*[23]

(-)Augustine (4) was found to be the most active alkaloid, showing a significant cytotoxic response to human breast cancer, lung cancer, colon cancer, fibrosarcoma, melanoma, oral epidermoid carcinoma, murine lymphoid neoplasma, epidermoid carcinoma, hormone-dependent human prostatic cancer, hormone-dependent breast cancer and human glioblastoma.[23] Compounds 1 and 3 were not active. However, crinamine (5), differing in its absolute configuration from 1 and 3, demonstrated strong cytotoxic results. Lycorine showed good results in all the cancer lines tested. Crinamine (5), lycorine (2) and (-) augustine all showed moderate anti-malarial activity but lycorine and augustine showed low selectivity indices compared to the control compounds used.[23] Lycorine has also shown further biological activity including inhibition of growth and cell division in higher plants and algae, inhibition of cyanide resistant respiration and of peroxidase enhancement.[24,25,26] Further studies have shown the A, B and methylenedioxy rings have little effect on the inhibitory effect

of the lycorine molecule. However electron donor groups on the C ring at C-1 and C-2 and a β configuration of the D ring result in increased inhibition.[27]

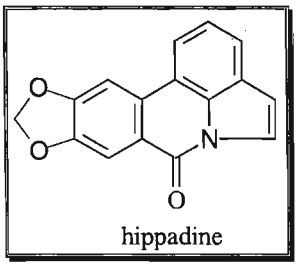


Figure 2. 10 Structure of hippadine

Hippadine, a phenanthridone type alkaloid, has been shown to reversibly inhibit fertility in male rats.[28] Rats treated with hippadine showed a 83.3 % fertility loss. However, 80% of the rats regained fertility between 8 and 10 weeks while the remaining 20% remained sterile for up to 12 weeks. Initially the onset of infertility was delayed with the loss of DNA and an increase in concentration of protein. This together with the reversibility of the damage to the germinal activity, suggestted that the alkaloid possibly acted on the germ cells in their earlier stages of spermatocytogenesis.[28] These observations suggest that hippadine exerts its effects at the genetic level and may prove to be a useful agent in fertility control.[28]

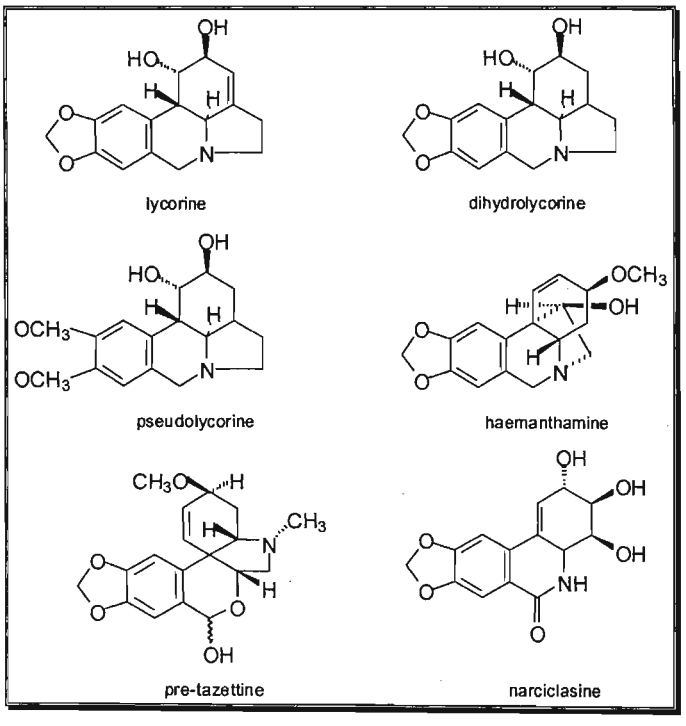


Figure 2. 11 Structures of different alkaloids tested on animal cell growth

The effects of eighteen alkaloids from various Amaryllidaceae bulbs were tested on (a) animal cell growth (b) DNA, RNA, and protein synthesis by intact cells and (c) protein synthesis in

cell-free systems.[29] Six of the eighteen alkaloids halted HeLa cell growth at 10^{-1} mM or lower concentrations. At their growth inhibitory concentration, these six compounds block protein synthesis in ascites cells and stabilise HeLa cell polysomes *in vivo*. EMC RNA-directed cell free polypeptide synthesis is also inhibited by these six compounds. It is therefore thought that they halt protein synthesis in eukaryotic cells by inhibiting the peptide bond formation step. Narciclasine was found to be the most potent of the six inhibitors and it is also known to have antitumor and antimitotic effects.[30]

It is due to these various pharmacological activities that this family has become the focus of many pharmacological and phytochemical studies.

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Chapter 3: Extractives from *Brunsvigia natalensis*

3.1 Introduction

Brunsvigia natalensis Baker belongs to the Amaryllidaceae family. This family comprises nine tribes worldwide, of which two are found in southern Africa, the Amaryllideae and Haemantheae. The genus *Brunsvigia* Heist. has been placed in the Amaryllideae.[1]

Brunsvigia natalensis is also known as the Natal Candelabra Flower, or Kanderlaarblom.[2] It is found in grassland and mainly marshy areas. The Zulu name for this plant is umbhola, a reference to the rotten smell around the bulbs.[2] It is distributed in southern Africa mainly in the mountainous highlands of KwaZulu-Natal, Mpumalanga and Swaziland.[2] The subglobose bulbs are approximately 5 cm in diameter.[3] It is used in traditional medicine to 'straighten bones in children'.[2] .

Figure 3. 1 *Brunsvigia natalensis*, Photo: Erich van Wyk

This is the first reported phytochemical study of this plant. The dichloromethane and methanol extracts of this plant were investigated. This led to the isolation of four compounds, three alkaloids and a flavanoid **(Figure 3. 2)**. Of the four compounds isolated, three have not been reported previously. The compounds isolated were nivanine A (compound **I**), nivanine B (compound **II**), 7-hydroxyflavan (compound **III**) and brunsceramide (compound **IV**).

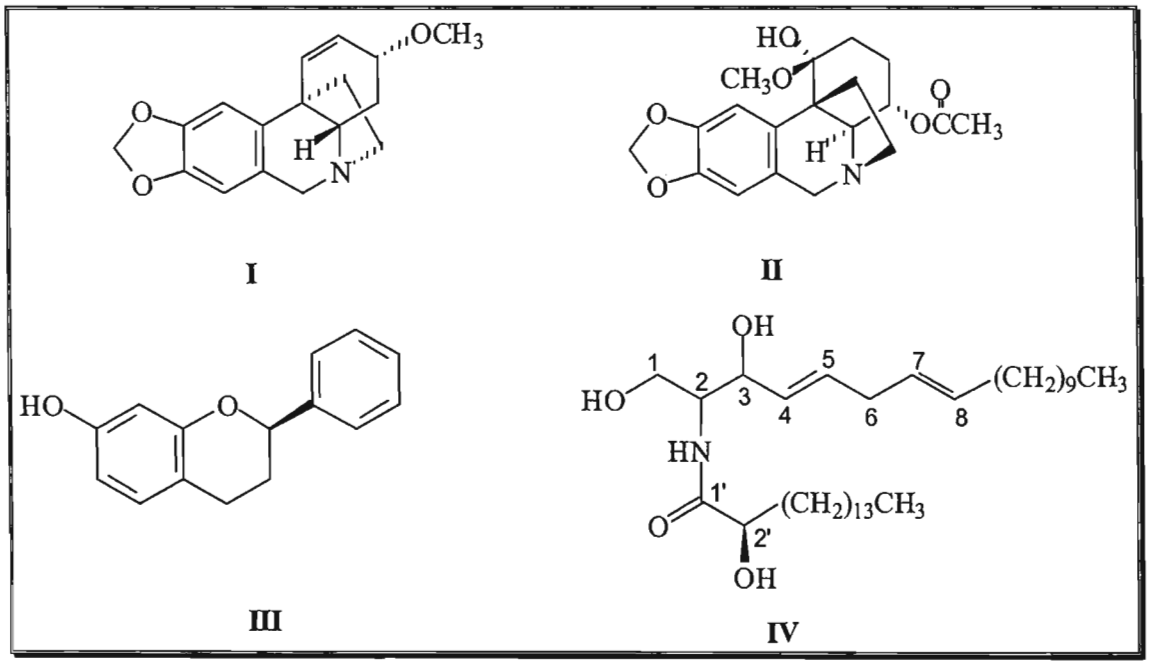


Figure 3. 2 Compounds isolated from *Brunsvigia natalensis*

3.2 Results and Discussion

3.2.1 Structural Elucidation of Compound I, 11-Dehydroxycrinamine (nivanine A) (spectra 1.a-i)

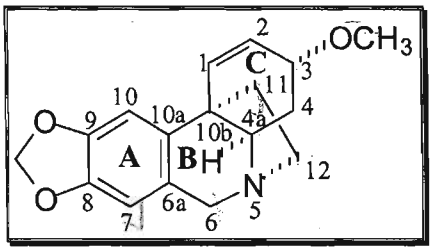


Figure 3. 3 Structure of compound I, nivanine A

Compound **I (Figure 3. 3)**, was isolated as an orange amorphous material. This compound was found to have a molar mass of 285.13782 g. mol^{-1} corresponding to a molecular formula of $C_{17}H_{19}NO_3$. It was found to belong to the 5,10b-ethanophenanthridine type of alkaloids. The structure of this compound was identified using NMR spectroscopy together with the mass spectrum.

The substitution pattern on the A ring was confirmed through the presence of two *para* orientated aromatic protons (δ_H 6.45 and 6.77) and a two proton resonance at δ_H 5.87, which is typical of protons of a methylenedioxy group, in the ^1H NMR spectrum. Other characteristic features observed in the ^1H NMR spectrum included a doublet resonance (δ_H 5.78, 10.3 Hz) and double doublet resonance (δ_H 6.40, 2.2 and 10.3 Hz) in the olefinic region, assigned to H-2 and H-1 of a 1, 2-double bond, and a methoxy group proton resonance at δ_H 3.38. Another typical alkaloid resonance was a double doublet at δ_H 3.20 (dd, 3.7 and 13.2 Hz) assigned to the H-4a resonance.[4-6] The C-4a (δ_C 66.55), together with C-7 (δ_C 106.90) resonances showed a ^3J HMBC correlation to the methylene protons at δ_H 3.75 (d, 16.9 Hz) and 4.36 (16.9 Hz), which were assigned to 2H-6. The HMBC spectrum showed ^3J correlations from C-6 (δ_C 61.95) and C-4a (δ_C 66.55) to the resonances at δ_H 2.88 (m) and 3.41 (m), which were seen to correspond in the HSQC spectrum to a methylene carbon resonance at δ_C 53.15. This was assigned to C-12, one of the two bridging carbons. The COSY spectrum shows correlations between the 2H-12 resonances (at δ_H 2.88 (m) and 3.41 (m)) and the methylene proton resonances at δ_H 2.07 (m) and 2.11 (m) assigned to 2H-11, which were seen to correspond in the HSQC spectrum to the methylene carbon resonance at δ_C 44.62 ascribed to C-11.

Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. In this technique the shape, amplitude and sign of the CD spectrum can be used to determine

configuration and conformational aspects of optically active compounds.[7] The sign of the CD peak generally relates to the configuration of the molecule. Consequently, comparison with compounds of known stereochemistry can be used to determine the stereochemistry of unknown compounds. This technique is useful in determining the stereochemistry of the ethanobridge in crinane compounds since the configuration at the optically active benzylic carbon C-10b determines the shape and sign of the CD spectrum. For the α-5,10b-ethanophenanthridine series, a minimum or a negative cotton effect is observed at between 245-250 nm and a maximum or positive cotton effect at 285-290 nm in the CD spectrum. This is in direct contrast to crinane alkaloids with a β-5,10b-ethano bridge where a maximum is generally observed between 245-250 nm and a minimum between 285-290 nm in the CD spectrum.[4-6] A positive Cotton effect for compound I was observed at 279 nm followed by a negative effect at 249 nm, which indicated the bridge was of the α configuration. A ^3J correlation seen in the HMBC spectrum from C-3 (δ_C 76.81) to the methoxy group proton resonance at δ_H 3.38, indicated the methoxy substituent to be present at C-3. This methoxy substituent at C-3 can be on the same side (*cis*) or on the opposite side (*trans*) of the 5,10b-ethano bridge and this can be determined from the splitting pattern of the H-2 resonance. The H-1 and H-2 atoms are the protons of a double bond and are planar **(Figure 3. 4)** with a dihedral angle of approximately 0° and they split each other into a doublet with coupling constants of 10 Hz. If the methoxy substituent at C-3 and 5,10b-ethano bridge are on the same side, then the dihedral angle between H-2 and H-3 is approximately 90° (giving a $J_{2,3}$ of approximately zero) and the H-2 resonance remains as a doublet. However, if the methoxy substituent and the ethano bridge are on opposite sides, the dihedral angle is approximately 30°, which results in the H-2 resonance being split into a double doublet with coupling constants of 10 ($J_{1,2}$) and 5 ($J_{2,3}$) Hz. Thus a H-2 resonance split into a doublet indicates a *cis* relationship between the methoxy substituent and the bridge and if the H-2 resonance is split into a double doublet, this indicates a *trans* relationship between substituent and the ethano bridge.[4-6] The H-2 resonance was found to be a doublet, indicating a *cis* relationship between the methoxy substituent at C-3 and the 5,10-ethano bridge.

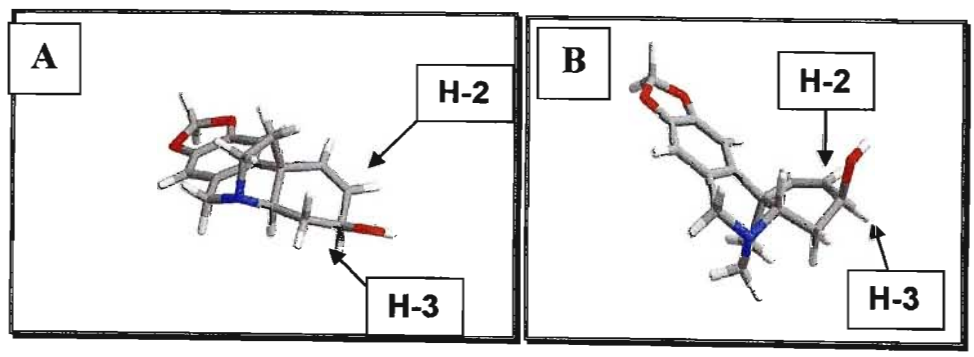
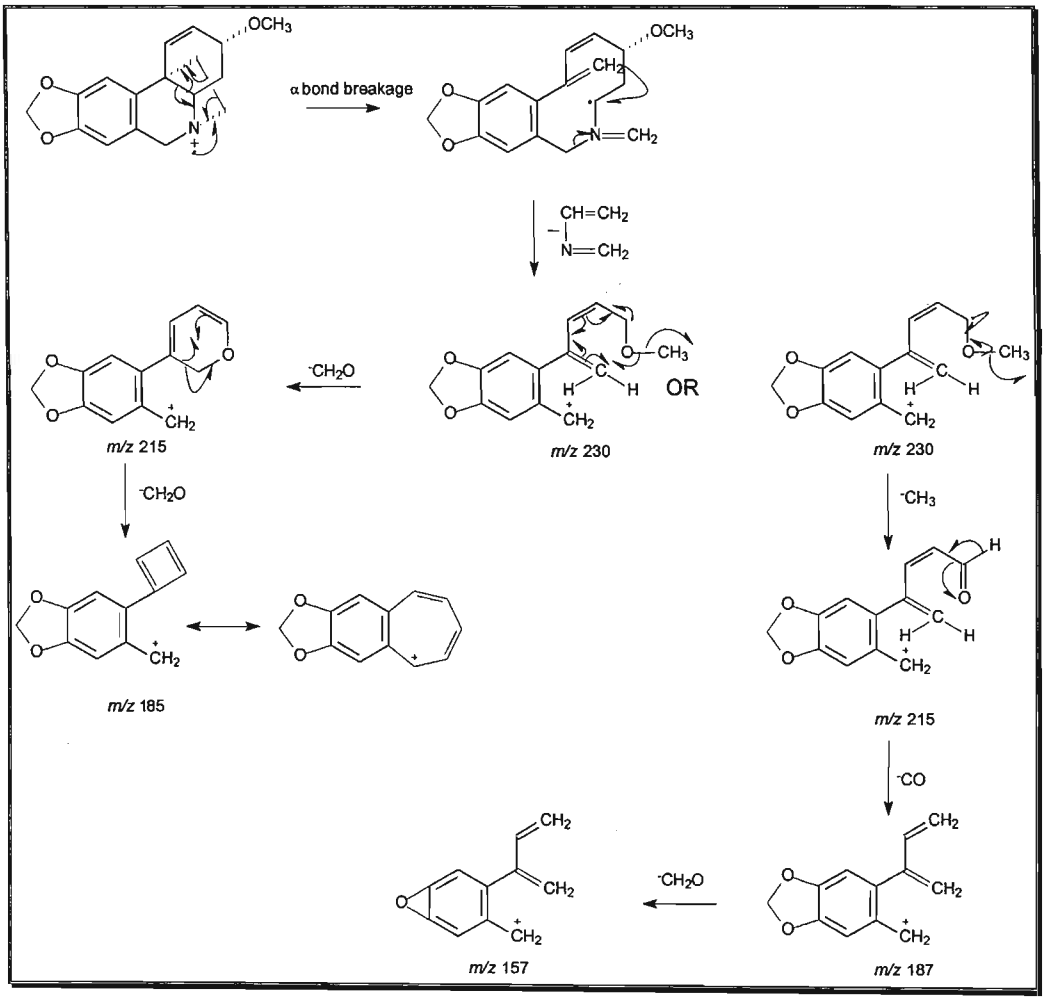


Figure 3. 4 Molecular model showing the different dihedral angles between H-2 and H-3. (a) shows a dihedral angle of 90 degrees between H-2 and H-3; (b) shows a dihedral angle of approximately 30 degrees between H-2 and H-3.

A proposed mass spectral fragmentation pattern is shown in **Scheme 3. 1**. The loss of a fragment of 55 mass units from the parent ion can result from the loss of a CH_2CHNCH_2 fragment, leaving a fragment ion at *m/z* 230 as shown in the **Scheme 3. 1**.[8] This fragment ion at *m/z* 230 can lose a methyl group (*m/z* 215) and, subsequently, can lose carbon monoxide to give the fragment ion at *m/z* 187.[8] Further fragmentation could involve the loss of formaldehyde from the methylene dioxy group, which results in a fragment ion at *m/z* 157. The alternative fragmentation route for the ion at *m/z* 230 is the successive loss of $2xCH_2O$ fragments resulting in ions at *m/z* 215 and 185.[8]



Scheme 3. 1 Fragmentation pattern of compound I[8]

The infrared data supported the postulated alkaloid structure for compound **I**. Peaks were observed at 3384 cm^{-1} (O-H stretching), 2922 cm^{-1} (aliphatic C-H stretching), 1492 cm^{-1} (aromatic C=C stretching) and 1239 (C-N stretching).

A literature search indicated that this structure has not been reported previously. It was found to be the epimeric of epibuphanisine which has previously been isolated from *Ammocharis coranica* (Ker-Gawl.) Herb., *Brunsvigia josephinae* (Red.) Ker-Gawl and *Boophane flava* W.F.Barker ex Snijman.[6,9,10] However, it was different in the orientation of the 5,10b-ethano bridge and the stereochemistry at C-3. In nivanine A the bridge and substituent were both in the α configuration compared to epibuphanisine which has the bridge and stereochemistry at C-3 to be both in the β configuration.

Table 3.1: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for nivanine A ($CDCl_3$)

CARBON	δ_H	δ_c	δ_c*	HMBC (C→H)	COSY	NOESY
1	6.40 (dd, 10.3,2.2 Hz)	129.05	131.9	11_{exo}	2	2,10
2	5.78 (d, 10.3)	128.81	125.8	4β	1	1;3;3-OCH_3
3	3.97 (m)	76.81	72.2	1;3-OCH_3; 4α,β	4α,β	2;4a;4β;3-OCH_3
4α	1.53 (ddd, 11.0,11.7,11,7)	30.82	29.0	2; 4a	3,4a, 4β	4β;11_{exo};3-OCH_3
4β	2.19 (m)				3,4a,4α	3;4a;4α
4a	3.20 (dd, 3.7, 13.2 Hz)	66.55	63.4	1;4α,β;6α;12_{endo};$11_{exo,endo}$	4α,β	3;4β;6β
6α	3.75 (d, 16.9 Hz)	61.95	61.6	7;$12_{exo, endo}$	6β	6β;7,12_{exo}
6β	4.36 (d, 16.9 Hz)				6α	4a;6α;7
6a		125.62	124.3	6α,β;10		
7	6.46 (s)	106.90	107.0	6α,β; 10		6α,β
8		146.19[a]	146.1	OCH_2O; 7;10		
9		145.79[a]	146.5	OCH_2O ;7;10		
10	6.77 (s)	102.77	103.1	7		1
10a		138.45	137.5	1;4a;6α,β;7;$11_{exo, endo}$		
10b		44.80[b]	44.6	2;4α,β;10;$11_{exo, endo}$		
11_{exo}	2.07 (m)	44.62[b]	43.4	1;4a;6β;12_{exo}	$12_{exo, endo}$	4α;11_{endo},12_{exo}
11_{endo}	2.11 (m)				$12_{exo, endo}$	11_{exo};12_{endo}
12_{endo}	2.88 (m)	53.15	53.2	4a; 6α,β; $11_{exo, endo}$	$11_{exo, endo}$;12_{exo}	6α;12_{exo}
12_{exo}	3.41 (m)				$11_{exo, endo}$;12_{endo}	11_{exo};12_{endo}
-OCH_2O-	5.87 (dd, 5.5,4.4 Hz)	100.76	101.0			
3-OCH_3	3.38 (s)	55.91	56.7			2;3;4αβ

* Literature spectra of epibuphanisine run in $CDCl_3$.[6]

[a] ^{13}C NMR resonance values interchangeable.

[b] ^{13}C NMR resonance values interchangeable.

[c] ^{13}C NMR resonance values interchangeable.

3.2.2 Structural Elucidation of Compound II, 4α–acetyl-2-deacetyl-1β–methoxyjosephinine (nivanine B) (Spectra 2.a-i)

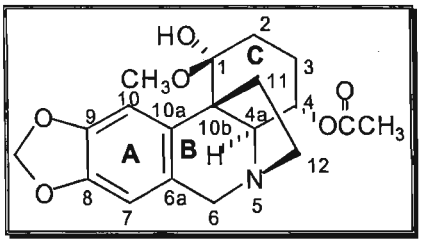


Figure 3. 5 Structure of compound II, nivanine B

Compound **II (Figure 3. 5)** was isolated as a white amorphous material. This compound was found to have a molar mass of 361 g. mol^{-1}[Ŧ] suggesting a molecular formula of $C_{19}H_{23}NO_6$ and also belongs to the 5,10b-ethanophenanthridine group of alkaloids. The structure was determined using NMR spectroscopy and was found to be a new alkaloid named nivanine B.

The substitution pattern on the A ring was the same as the previous compound with two *para* orientated aromatic protons (δ_H 7.24 and 6.53) and a two proton resonance at approximately δ_H 5.86, in the ^1H NMR spectrum, which is typical of protons of a methylenedioxy group (2H). Other features observed in the ^1H NMR spectrum included a methoxy group proton resonance at δ_H 3.95 and an acetoxy group proton resonance at δ_H 2.09. A typical resonance for the 5,10b-ethanophenanthridine alkaloids is the AB splitting pattern for the H-6 methylene protons which were observed as a pair of doublets (d, 16.9 Hz) at δ_H 3.76 and δ_H 3.93.[4-6] The C-6 resonance showed a ^3J HMBC correlation to the resonance at δ_H 4.46 (d, 10.2 Hz) assigned to H-4a.

The COSY spectrum was used to assign the protons of ring C. The H-4a (δ_H 4.46) resonance showed a correlation in the COSY spectrum to a resonance at δ_H 4.98, which was assigned to H-4. This resonance showed a corresponding carbon resonance in the HSQC spectrum at δ_C 75.86 indicating an oxygenated methine carbon to be present. The carbonyl carbon resonance of the acetate group (δ_c 170.99) showed a ^3J HMBC correlation also to this resonance at δ_H 4.98. This implied that C-4 had an acetate group attached to it. The resonance at δ_H 4.98, in turn, showed a correlation in the COSY spectrum to two protons of a methylene group (δ_H 1.88 and 2.01) which were assigned to 2H-3. These resonances, in turn, showed correlations to the protons of another methylene group (δ_H 1.70 and 1.99) which were assigned as 2H-2. The carbon resonances for ring A and B were assigned by comparison to the previous compound together with the HMBC

[Ŧ] HRMS is currently being done.

and COSY spectra. This left the acetal carbon resonance at δ_C 93.84 to be assigned and the only position unassigned was C-1. This resonance showed a ^3J HMBC correlation to the H-3 resonance at δ_H 2.01 and was thus confirmed to be C-1. The methoxy proton group resonance (δ_H 3.95) showed a correlation in the NOESY spectrum to the H-10 resonance and was thus assigned the β orientation. The other substituent on C-1 was assigned to be a hydroxy group by mass difference from the mass spectrum, and also because of the chemical shift of the δ_C of 93.48 ppm. Circular dichroism spectroscopy was used to determine the absolute configuration of the 5, 10b-ethano bridge. A positive Cotton effect was observed at 284 nm followed by a further positive effect at 247 nm, which indicated the bridge was of the β configuration since negative Cotton effects are typically observed at 243 nm for the α orientated series.[7, 15]

The infrared data supported the postulated alkaloid structure for compound **II**. Peaks were observed at 3437 cm^{-1} (O-H stretching), 2949 cm^{-1} (aliphatic C-H stretching), 1727 (C=O stretching), 1472 cm^{-1} (aromatic C=C stretching) and 1262 cm^{-1} (C-N stretching).

A literature search for the compound showed that it has not been described previously and was named nivanine B. **Table 3.2** shows the ^1H and ^{13}C NMR resonances together with the HMBC, COSY and NOESY NMR data.

Table 3.2: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for nivanine B ($CDCl_3$)

CARBON	δ_H	δ_c	HMBC (C→H)	COSY	NOESY
1		93.48	3		
2α	1.70 (m)	27.63		3α,β	2β
2β	1.99 (m)			3α,β	2α;3β
3α	1.88 (m)	26.07	2	4;2α,β	3β;4a
3β	2.01(m)			4;2α,β	3α;4
4	4.98 (m)	74.77	4a	4a, 3α,β	3β;4a
4a	4.46 (d, 10.2 Hz)	75.86	4	4	3α;4
6α	3.76 (d, 16.9 Hz)	52.03	4a;7	6β	6β;12_{endo}
6β	3.93 (d, 16.9 Hz)*			6α	6α
6a		139.62	6α,β;10		
7	7.53 (s)	102.45			4;4a
8		148.77	OCH_2O; 7;10		
9		133.59	OCH_2O ;7;10		
10	7.24 (s)	100.67			1-OCH_3
10a		135.86	4a;6α,β;11β		
10b		52.52	10		
11_{exo}	2.41 (m)	38.46	4a	$12_{exo,\ endo}$	11_{endo}; 4-$OCOCH_3$
11_{endo}	2.07 (m)			$12_{exo,\ endo}$	11_{exo};12_{endo}
12_{endo}	2.72 (m)	53.69	6α,β	$11_{exo,\ endo}$;12_{exo}	12_{exo}
12_{exo}	3.55 (m)			$11_{exo,\ endo}$;12_{endo}	12_{endo}
- OCH_2O-	5.86 (s)	100.67			
3-OCH_3	3.95 (s)	59.11			10
4-$OCOCH_3$	2.09 (s)	21.33			11_{exo}
4-$OCOCH_3$		170.99	4;4-$OCOCH_3$		

* Coupling constant could not be determined as resonances overlap each other. However it was assumed to have the same coupling constant as 6 α.

3.2.3 Structural Elucidation of Compound III, 7-Hydroxyflavan (spectra 3.a-k)

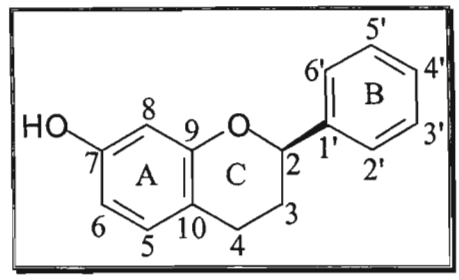


Figure 3. 6 Structure of compound III, 7-hydroxyflavan

Compound **III (Figure 3. 6)** was isolated as an orange amorphous material. It was found to have a molar mass of 226 g. mol^{-1}, corresponding to a molecular formula of $C_{15}H_{14}O_2$.[†] The structure of this compound was deduced based on the NMR data as being a flavanoid type of compound. The ^{1}H NMR spectrum together with the COSY spectrum showed coupled proton resonances at δ_H 5.04 (dd 2.5, 10.0 Hz), δ_H 2.16 (m) and 2.12 (m)) and δ_H 2.71 (m) and 2.89 (m) assigned to H-2, 2H-3 and 2H-4 of a chromane moiety. The presence of resonances at δ_H 7.41 (2H) (m), δ_H 7.38 (2H) (m) and δ_H 7.33 (m) was indicative of a monosubstituted benzene ring, linked to the chromane moiety at C-2. This linkage of the benzene ring at C-2 was confirmed on the basis of a ^3J HMBC correlation from C-2 (δ_C 77.80) to the H-2' and H-6' (δ_H 7.41) resonances.

Circular dichroism spectroscopy was used to determine the absolute configuration of the stereogenic centre at C-2. A negative Cotton effect was observed at 282 nm and 242 nm, which indicated the stereogenic centre to have the 2*R* absolute configuration.[16,17]

The structure of ring A remained to be determined. The presence of an aromatic AB system in the ^{1}H NMR spectrum, with resonances at δ_H 6.93 (d, 7.9 Hz), δ_H 6.32 (dd, 7.9, 2.5 Hz) and δ_H 6.28 (d, 2.5 Hz) was indicative of the presence of three non-equivalent protons on the A ring and that the A ring was tri-substituted. Two of the three substituents were linkages between the chromane ring C to the aromatic ring A. That the remaining substituent was an hydroxyl group was determined both from the molecular formula and the disappearance of the broad resonance at δ_H 5.41 ascribed to the free hydroxyl group proton resonance.[a] The position of this substituent was determined using the HMBC, COSY and NOESY spectra. The C-4 resonance showed a ^3J HMBC correlation to the aromatic resonance at δ_H 6.93 (d, 7.9 Hz) which was assigned to H-5. The COSY spectrum showed a correlation between H-5 and the aromatic proton resonance at δ_H

[†] HRMS experiments were not done on known compounds due to financial restraints.
[a] The ^{1}H NMR spectrum was reacquired after adding CD_3OD.

6.32 (dd, 7.9, 2.5 Hz) this was assigned to H-6. The H-6 resonance showed a correlation in the NOESY spectrum to the broad resonance at δ_H 5.41. This, in turn, showed a correlation in the NOESY spectrum to the remaining aromatic resonance at δ_H 6.28 (d, 2.5 Hz) assigned to H-8. This implied that the hydroxyl group was present at C-7.

A literature search for the compound indicated that it was similar to 7-hydroxyflavan, previously isolated from the bulbs of *Narcissus pseudonarcissus* L (Amaryllidaceae) and is an anti-fungal agent.[11] It was different from the known compound from literature at the chiral centre in compound III it was found to be 2*R* instead of 2*S* found in literature. The ^{13}C NMR data are not provided in the literature. **Table 4.3** shows the ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 7-hydroxyflavan.

Table 3.3: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 7-hydroxyflavan ($CDCl_3$)

CARBON	δ_H	δ_c	HMBC (C→H)	COSY	NOESY
2	5.04 (dd,2.4,10.0 Hz)	77.80	3α,β;4α,β;2'/6'	3α,β	3α,β;4β;2';6'
3α	2.12 (m)	29.96	2;4α,β	2;4α,β	2;3β,4α;2';6'
3β	2.16 (m)				2;3α;4α,β;2';6'
4α	2.71 (m)	24.27	2;3α,β;5	3α,β	3α,β;4β;5
4β	2.89 (m)				2;3α,β;4α;5
5	6.93 (d, 7.9 Hz)	130.49	4α,β	6	4α,β;6
6	6.32 (dd, 2.5, 7.9 Hz)	103.12	4β;5;8	5	5;7-OH
7	-	155.67[a]			
8	6.28 (d, 2.5 Hz)	108.0	4β;6		7-OH
9		155.79[a]			
10		114.06	3α,β;4α,β;8		
1'		141.52	2;3α;3';5'		
2'	7.41 (m)*	125.95	2;4';6'	3'	2;3α,β
3'	7.38 (m)*	128.46	5'	2';4'	
4'	7.33 (m)*	127.80	2';6'	3';5'	
5'	7.38 (m)*	128.40	3'	4';6'	
6'	7.41 (m)*	125.95	2;2';4'	5'	2;3α,β
7-0H	5.41				6;8

* Coupling constant could not be determined as resonances overlap each other.

[a] Carbon value interchangeable.

3.2.4 Structural Elucidation of Compound IV, (2*S*,3*S*,4*E*,7*E*]-1,3-dihydroxy-2-[(2'*R*)-2'-hydroxyhexadecanoylamino]-4,7-octadediene (brunsceramide) (spectra 4.a-j)

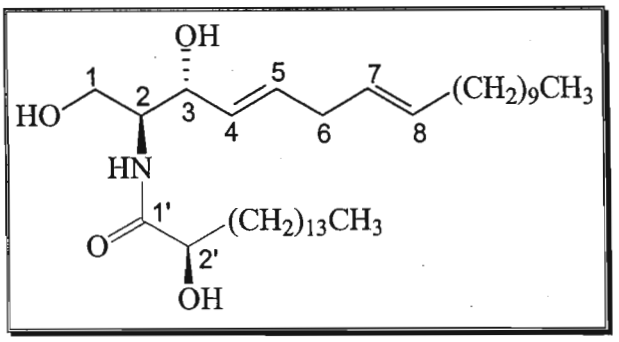


Figure 3. 7 Structure of compound IV, brunsceramide

Compound **IV (Figure 3. 7)** was isolated as an orange amorphous material. The highest peak in the mass spectrum occurred at *m/z* 533 and the odd number suggested the presence of a nitrogen atom. The presence of a carbonyl group carbon resonance at δ_C 175.1 in the ^{13}C NMR spectrum and peaks at 3363 and 1643 cm^{-1} in the IR spectrum, indicated the possible presence of an amide. The presence of a secondary amide was further suggested by a correlation in the HMBC spectrum between the carbonyl resonance and a one proton doublet resonance at δ_H 7.19 (d, 7.7 Hz) which disappeared on re-running the ^1H NMR spectrum in deuterated methanol, indicating a NH group.

The COSY spectrum was useful in determining the type of compound present. The *N*-H resonance showed coupling in the COSY spectrum to a one proton multiplet at δ_H 3.93 (H-2) which was seen to be coupled to two non - equivalent oxymethylene protons at δ_H 3.88 (m) and 3.72 (brd, 7.9 Hz, 2H-1) and an oxymethine proton resonance at δ_H 4.27 (m, H-3). The oxymethine proton resonance showed further coupling to an alkene proton resonance at δ_H 5.51(dd, 6.3, 15.4 Hz, H-4) which was further coupled to a second alkene proton at 5.78 (brd, 15.4 Hz, H-5). This proton of the second alkene group proton was seen to be coupled to protons of a methylene group at δ_H 2.10 (brs, 2H-6). In turn these methylene group protons were seen to be coupled to the proton of a second double bond at δ_H 5.32 (m) (H-7). The large coupling constants of 15.4 Hz between the olefinic protons of H-4 and H-5 and the coupling constant of approximately 13 Hz between H-7 andH-8 (although this was difficult to determine accurately due to the H-7/H-8 resonances being very close to each other) indicated that both double bonds were of the *E* configuration. .This led to the following partial structure being suggested from the NMR data **(Figure 3.8)**.

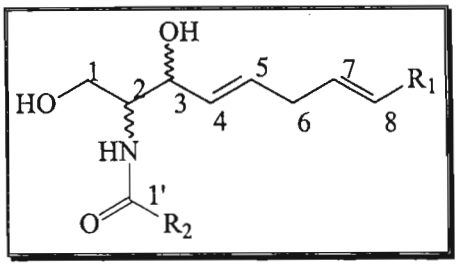


Figure 3. 8 Partial structure of compound IV based on NMR data

A literature search based on the above partial structure **(Figure 3.8)** indicated the compound to be a ceramide type. Ceramides contain a C-18 sphingosine chain base and hence the following partial structure could be tentatively deduced **(Figure 3.9)**.

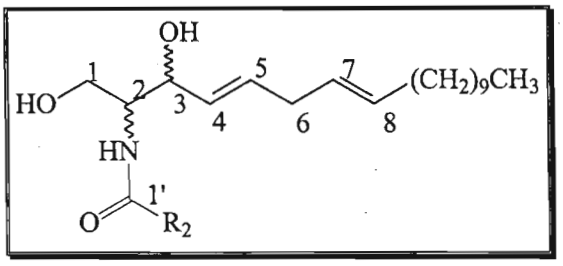


Figure 3. 9 Partial structure of compound IV including sphingosine chain base

Lee *et al.* have undertaken extensive investigations on the fragmentation patterns in the mass spectra of ceramides.[20] Their studies indicate the presence of two major peaks in the mass spectrum of ceramides. The fragments are shown as A and B on the structure below **(Figure 3.10)**. These two fragments must differ in mass by 17 mass units.

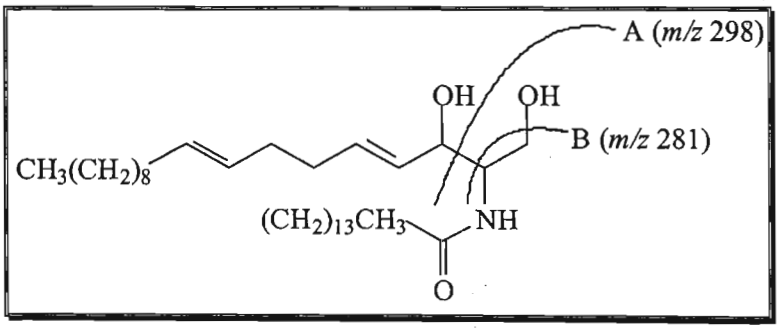


Figure 3. 10 Typical fragmentation pattern of a ceramide [20,21]

Peaks differing in 17 mass units were found at *m/z* 314 and *m/z* 297 in the mass spectrum of compound IV. If one takes off the mass of 70 required for the fragment below **(Figure 3.11)** from 297 a mass of 227 remains.

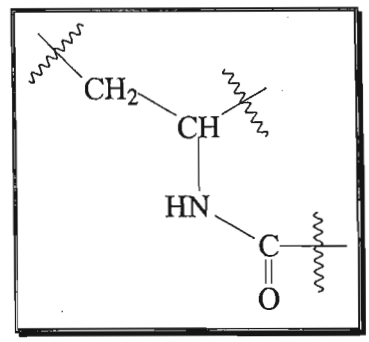


Figure 3. 11 Showing the known partial structure of compound IV

This corresponds to a molecular formula of $C_{15}H_{31}O$ indicating one of the carbons of the lower chain to have a hydroxy group attached. The presence of a hydroxy group on this chain was further indicated by a unassigned double doublet at δ_H 4.10 (dd, 3.8, 8.1 Hz) in the ^1H NMR spectrum which showed coupling to only one methylene group in the COSY spectrum, so it was placed at C-2'. Thus the overall proposed structure is shown below **(Figure 3.12)**.

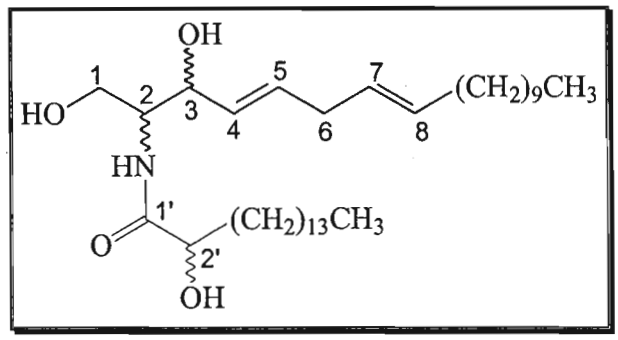


Figure 3. 12 Proposed structure of compound IV

In order to confirm the proposed structure the mass spectrum was re-examined again. The proposed structure requires a molecular ion peak at *m/z* 551. The highest peak in the mass spectrum occurred at *m/z* 533, indicating the loss of a unit of water.

A literature search for these type of compounds showed that three related compounds, two with a C-2' hydroxy group, have been found previously in higher plants, from *Physalis philadelphica* (Solanaceae) **(Figure 3.13)**.[22]

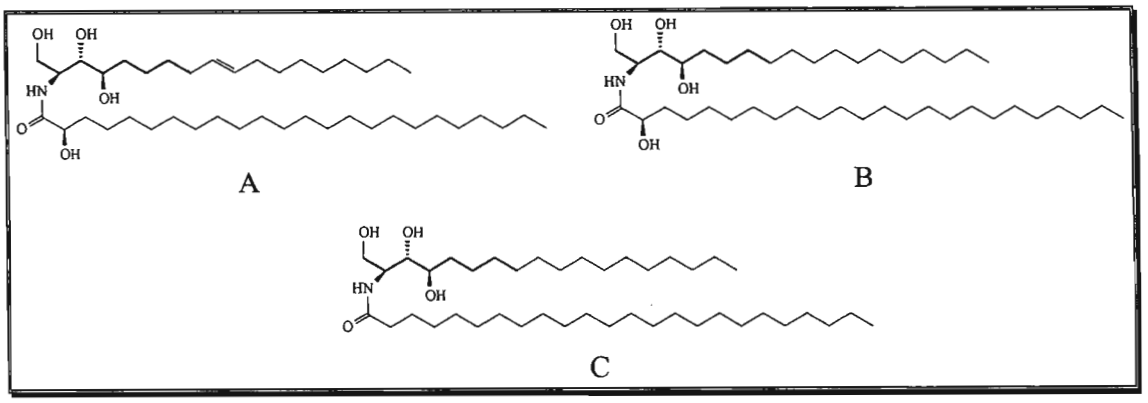


Figure 3. 13 Ceramides isolated from *Physalis philadelphica* [22]

In the EIMS of the three compounds, the molecular ion peak is very small, occurring at a relative abundance of 2% (A), 3% (B) and 0.8% (C) respectively. It is therefore likely that the molecular ion was not detected in the EIMS of compound **IV**.

The stereochemistry of the three chiral centres, C-2, C-3 and C-3', could not be determined due to insufficient amounts of the compound remaining for the preparation of Mosher esters. However, from literature studies, compounds isolated previously all had the 2*S*, 3*S*, 2'*R* stereochemistry. [22,28]. Re-isolation of brunsceramide is required to confirm the stereochemistry.

Ceramides have been isolated previously from sponges, [23-25] sea stars, [26-27] gorgonians, [21] and green algae.[28] To the best of our knowledge this is the first report of the isolation of ceramides from a plant of the genus *Brunsvigia* or the family Amaryllidaceae. The HMBC, COSY and NOESY data are shown for the key functional groups in **Table 3.4**

Table 3.4: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for brunsceramide ($CDCl_3$)

CARBON	δ_H	δ_c	HMBC (C→H)	COSY	NOESY
1a	3.88 (m)	62.1	3	1b;2	1b;2
1b	3.72 (brd, 7.9)			1a;2	1a;2
2	3.93 (m)	54.3	3	1a,b;*N*-H;3	1a,b;2
3	4.27 (m)	74.2	1;4;5	2;4	2;4;5
4	5.51 (dd, 6.3,15.4)	128.9	6	3;5	3;6
5	5.78 (d, 15,4)	133.7	6	4;6	3;6
6	2.10 (brs)	26.7	7;8	5;7	4;6;7
7	5.32 (m)*	128.4	6;9	6	6
8	5.38 (dd, 6.3,15.1)*	130.9	6;9	9	9
9	2.00	27.3	7;8	8;10	8
1'		175.1	*N*-H		
2'	4.10 (3.8,8.1,4.3)	72.4		3'	
3'a	1.54 (m)	34.74		2';3'b	3'b
3'b	1.64 (m)			2';3'a	3'a
N-H	7.19 (d, 7.7 Hz)			2	

* Difficult to determine *J* due to peak overlapping.

3.3 Foreword to Experimental

3.3.1 Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

Nuclear magnetic resonance spectroscopy was carried out on either a 400 MHz Varian UNITY-INOVA spectrophotometer or a 300 MHz Gemini spectrophotometer. All spectra were recorded at room temperature in either deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), or deuterated pyridine (C_5D_5N). The chemical shifts were all recorded in ppm relative to TMS.

3.3.2 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.

3.3.3 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 methanol. The NaOAc and $AlCl_3$ solutions used for bathochromic shifts were prepared by dissolving 0.5 g of each salt (anhydrous) in 100 ml volumetric flasks with methanol.

3.3.4 Melting Points

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

3.3.5 Mass Spectrometry

All low-resolution mass spectra were recorded on an Agilent MS 5973 instrument connected to a GC 6890 by Mr B. Parel or myself. High-resolution mass spectra were recorded on a Micromass VG 70 SEQ operated at 8kV in CI mode by Mr T. van der Merwe.

3.3.6 Optical Rotations

Optical rotations were recorded at room temperature in methanol on a Perkin Elmer polarimeter (Model 341).

3.3.7 Circular dichroism

CD spectra were recorded using a Jasco J700 spectro-polarimeter at the Department de Products Naturals, Facultat de Pharmacia, at the University of Barcelona, Spain and at the Department of Chemistry at the University of the Free State.

3.3.8 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 for the alkaloids and Sephadex for the glycosides. All separations were carried out under gravity. Both the column and thin layer techniques made use of varying ratios of dichloromethane, ethyl acetate, hexane and methanol. Sephadex columns were eluted using methanol as the solvent. 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. The alkaloid compounds were sprayed with Dragendorff reagent: basic bismuth nitrate (1.7 g) dissolved in a mixture of acetic acid (20 ml) and water (80 ml) (solution a); potassium iodide (16 g) in water (40 ml) (solution b); a and b are mixed 1: 1 (v/v) (stock solution - may be kept for several months in a refrigerator); for spraying: stock solution (1 ml) is mixed with acetic acid (2 ml) and water (10 ml). Colour reaction: orange-brown spots on a yellow background for alkaloids.

3.3.9 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, boiled in methanol and dichloromethane and filtered to remove the silica gel from the filtrate containing the compound of interest.

3.3.10 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 under reduced pressure. 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.

3.3.11 Anti-Cancer Screening

CSIR Biochemtek in Pretoria carried out this screening under the supervision of Dr V. Maharaj. The three cell lines utilised were MCF7 (breast), TK10 (renal) and UACC62 (melanoma). The method employed was obtained from the National Cancer Institute webpage.[12]

The cell lines are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. Cells are inoculated into 96-well flat bottom, polystyrene plates in 180 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines.[1] Each cell line is plated on duplicate plates. After cell inoculation, the microtiter plates are incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.[12]

After 24 hours, the plates of each cell line are fixed *in situ* with time course assay (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. Compounds are diluted in complete media with 0.1% Gentamicin sulfate and dispensed into wells in a volume of 20 µl to yield a test concentration of 100 µg/ml. NSC 123127 (Adriamycin) is used as the standard and is included into each plate.[12]

Cells are fixed *in situ* by the addition of cold TCA (final concentration 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, plates washed five times with tap water and air-dried. SRB at a 0.4 % (w/v) in 1 % acetic acid is added to each well and the plates are incubated for 10 minutes at room temperature.[12] Unbound dye is removed by washing six times

with 1 % acetic acid and the plates are air-dried. Bound sulforhodamine B (SRB) is solubilized with 10 mM trizma base and the absorbance is measured at a wavelength of 515 mm.[1]

3.3.12 Inflammatory and Microsomal Screening

Anti-inflammatory screening was carried out by a fellow member of the group, Karen du Toit, in Prof. J. Van Staden's laboratory at the University of Kwa-Zulu Natal in Pietermaritzburg. The method used was extracted from Jager *et al.* [13] The procedure was carried out at 4°C. Sheep seminal vesicles were homogenised in 150 ml 0.1 M K-Pi, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 4000 x *g* for 15 min; the supernatant was further centrifuged at 17 000 x g for 10 min. The microsomes were isolated by centrifugation at 100 000 x *g* for 1 hour. The microsomal pellet was resuspended in 0.1 M K-Pi, pH 7.4 and adjusted to 10 mg protein/ml. Aliquots were stored at -70°C. Protein determinations were performed with the Bio-Rad Protein assay kit.

The bioassays were performed according to the method of White and Glassman.[14] Ten microlitres (0.3 μg protein) of sheep seminal vesicle microsomes and 50 μl f co-factor solution (L-adrenalin and reduced glutathione, 0.3 mg/ml each in 0.1 M Tris buffer, pH 8.2) were preincubated in an ice bath for 15 min. Twenty microlitres of solvent, test solution or standard solution (20 μl of aqueous solutions; 2.5 μl of ethanolic solutions + 17.5 μl water; 2.5 μl of a 8 x 10^{-4} M ethanolic indomethacin solution + 17.5 μl water) and 20 μl [^{14}C]arachidonic acid (16 Ci/mole, 30 mM) were added and the assay mixture incubated at 37°C for 10 min. the reaction was terminated by adding 10 μl 2 N HCl. A blank was kept in the ice bath. After incubation 5 μl of a 0.2 mg/ml carrier solution of unlabelled prostaglandins (PGE_2: PGF2 1:1) was added. The prostaglandins were separated from unmetabolised arachidonic acid by column chromatography into scintillation vials. After mixing with scintillation solution the radioactivity was counted. The experiments were carried out in duplicate.

3.4 Experimental

Brunsvigia natalensis Baker was collected near Belfast, Mpumalanga, South Africa and a voucher specimen retained (*N. Crouch 865*, NH).

The bulbs (4.2 kg) were dried, chopped into smaller pieces, and extracted successively with dichloromethane and methanol by agitation on a Labcon Mechanical shaker at 140 rpm. The extracts obtained were then filtered and the solvent removed under reduced pressure. A dichloromethane extract (32.71 g) and methanol extract (100.9 g) were thus obtained. ^{1}H NMR

analysis of the crude methanol extracts revealed nothing of interest and the extract was not investigated further.

The dichloromethane extract was loaded onto a 5 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-27; 10% ethyl acetate in dichloromethane fractions 28-51; 20% ethyl acetate in dichloromethane fractions 52-80; 40% ethyl acetate in dichloromethane fractions 81-91; 80% ethyl acetate in dichloromethane fractions 92-110; 100% ethyl acetate fractions 117-139, 1% methanol in dichloromethane fractions 140-165; 2% methanol in dichloromethane fractions 166-242; 5% methanol in dichloromethane fractions 243-269; 10% methanol in dichloromethane fractions 270-293; 20% methanol in dichloromethane fractions 294-321). Purification of fractions 215-232 using a 2% methanol in dichloromethane solvent system afforded compound **I** at a yield of 0.8% (39.0 mg) and compound **II**, at a yield of 0.2% (10.3 mg). Purification of fraction 11-16 using a 5% ethyl acetate in dichloromethane solvent system afforded a partially pure compound **III**. Preparative TLC using a 10% ethyl acetate afforded pure compound **III**, at a yield of 1.3% (67.2 mg). Purification of fractions 166-186 using a 5% methanol in dichloromethane solvent system afforded compound **IV** at a yield of 0.4% (20 mg).

3.4.1 Physical data for Compound I

Name: **nivanine A, 11-Dehydroxycrinamine**
Yield: 39.0 mg
Physical description: orange amorphous material
Optical rotation: +68.91 (c, 0.534 in methanol)
Mass spectrum: HRMS: [M^+] at *m/z* 285.13782, $C_{17}H_{19}NO_3$ requires 285.136494 g. mol^{-1}
EIMS *m/z*: 285, 270, 230, 215, 198, 128, 115, 68
CD nm: 486, 467, 441, 418, 388, 372, 346, 323, 303, 279, 250, 213
Infrared: ν_{max}^{NaCl} cm^{-1}: 3384 (O-H stretching), 2922 (C-H stretching), 1492 (aromatic C=C stretching), 1239 (C-N stretching) and 1039 (C-O stretching)
^1H NMR: δ_H (ppm) $CDCl_3$: **Table 3.1**
^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 3.1**

3.4.2 Physical data for Compound II

Name: **nivanine B, 4α–acetyl-2-deacetyl-1β–methoxyjosephinine**
Yield: 10.3 mg
Physical description: white amorphous material

Optical rotation: +19.84 (c, 0.126 in methanol)

Mass spectrum: GC-MS: [M^+] at *m/z* 361, $C_{16}H_{14}O_5$ requires 361.152538 g. mol^{-1}

EIMS *m/z*: 361, 331, 317, 288, 234, 204, 191, 133, 115, 43, 103, 89, 77, 43

CD nm: 470, 460, 450, 441, 428, 411, 387, 343, 308, 285, 248

Infrared: ν_{max}^{NaCl} cm^{-1}: 3437 (O-H stretching), 2949 (C-H stretching), 1727 (C=O stretching), 1472 (aromatic C=C stretching) and 1262 (C-N stretching)

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 3.2**

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

3.4.3 Physical data for Compound III

Name: **7-hydroxyflavan**

Yield: 67.2 mg

Physical description: orange-brown amorphous material

Mass spectrum: GC-MS: [M^+] at 226 g. mol^{-1} requires a mass of 226.099380 g. mol^{-1}

EIMS *m/z*: 226, 197, 165, 135, 104, 71, 51

CD nm: 293, 286, 282, 274, 265, 261, 255, 251, 242, 238, 234, 227, 223

Infrared: ν_{max}^{NaCl} cm^{-1}: 3380 (O-H stretching), 2926 (C-H stretching), 1627 (C=O stretching), 1503, 1414 (aromatic C=C stretching), 1305 (C-N stretching) and 1155 (C-O stretching)

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 3.3**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 3.3**

3.4.4 Physical data for Compound IV

Name: **brunsceramide,(2*S*,3*S*,4*E*,7*E*]-1,3-dihydroxy-2-[(2'*R*)-2'-hydroxyhexadecanoylamino] -4,7-octadediene**

Yield: 20 mg

Physical description: brownish-yellow amorphous material

Optical rotation: -1.95 (*c*, 0.128 in methanol)

Mass spectrum: [M^+] not found, $C_{34}H_{65}NO_4$ requires 551.491360 g. mol^{-1}

EIMS *m/z*: 533 [M^+-H_2O], 336, 314, 297, 266, 149, 109, 83, 60

Infrared: ν_{max}^{NaCl} cm^{-1}: 3362 (O-H stretching), 2921, 2854 (C-H stretching)

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 3.4**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 3.4**

3.5 Reference

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Chapter 4: A Comparative Study of the Bulbs and Seeds of *Crinum stuhlmannii*

4.1 Introduction

Crinum stuhlmannii Baker (syn. *C. delagoense* I.Verd.) belongs to the Amaryllidaceae family. This family has nine tribes of which two, the Amaryllideae and Haemantheae, occur in southern Africa. The genus *Crinum* L., with its twenty-three regional species, has been placed in the Amaryllideae.[1]

Crinum stuhlmannii **(Figure 4. 1)** is known in Zulu as umnduze, and in English as the Candy-striped Crinum.[2,3] The original name 'delagoense' comes from Delagoa Bay which was thought to be the closest civilized point to the site of its discovery.[4] It is distributed in southern Africa from northern KwaZulu-Natal through to coastal Mozambique.[3] It usually occurs in grassland, bushveld and on sandy soils. The bulbs may reach 220 mm in diameter.[3] It is used in traditional medicine to treat urinary tract problems.[2] Unspecified parts are used medicinally for the treatment of cattle.[2] All members of the *Crinum* genus are considered capable of causing dermatitis.[2]

Figure 4. 1 *Crinum stuhlmannii*, Photo: Neil Crouch

This present study was undertaken as a previous one by Nair *et al.*[5] reported on the constituents of plants collected from a region (Natal Midlands) not likely to accomodate *Crinum delagoense*. As the botanical voucher proved untraceable, an attempt was made to verify the earlier report. Six alkaloids were isolated in the previous study as shown in **Figure 4. 2.**[5]

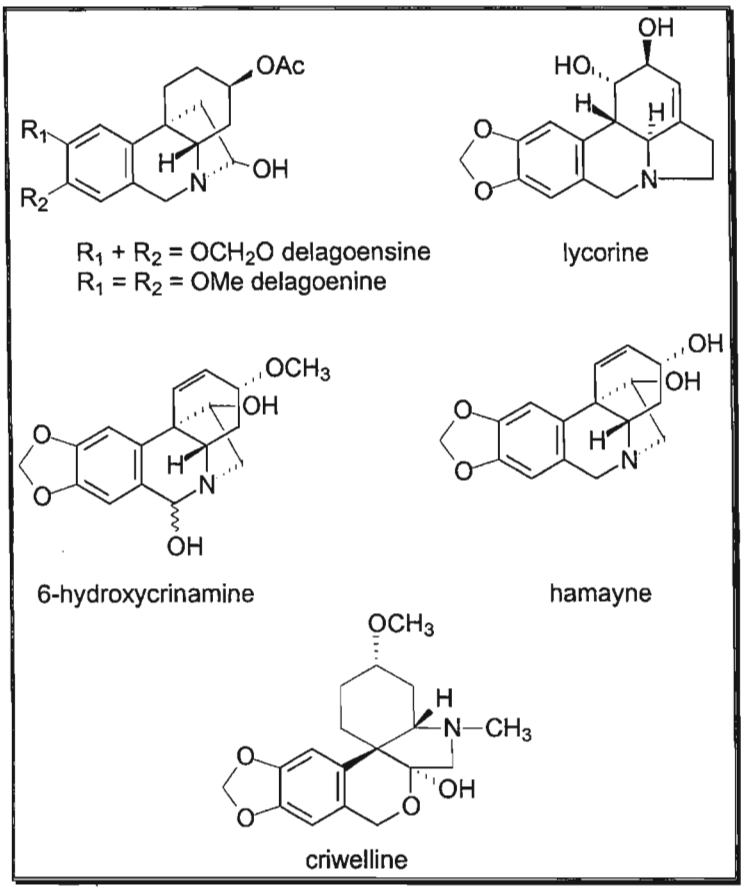


Figure 4. 2 Compounds isolated previously from *Crinum stuhlmannii*[5]

Secondly, a comparative study of the bulbs and seeds was carried out to identify organ-specific production of chemical constituents. This led to the isolation of eleven alkaloids **(Figure 4. 3)**. The compounds isolated were lycorine (compound **V**), 8,9-methylenedioxophenanthridine (compound **VI**), 6-hydroxycrinamine (compound **VII**), 6-ethoxycrinamine (compound **VIII**), haemanthamine (compound **IX**), hamayne (compound **X**), stuhlmanine A (compound **XI**), stuhlmanine B and C (compound **XIIA** and **XIIB**), *N*-methyldelagoenine (compound **XIII**), and stuhlmanine D (compound **XIV**).

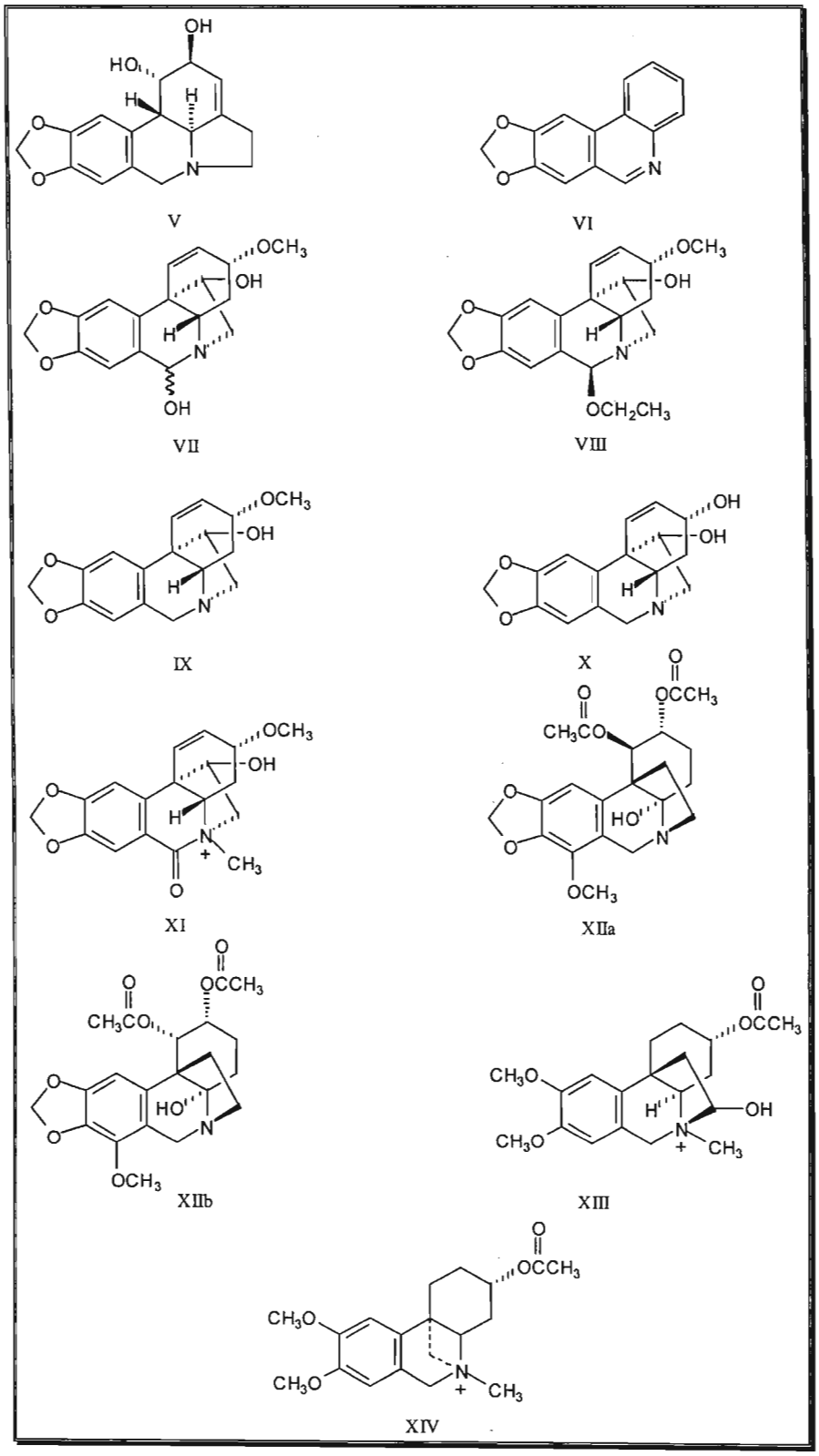


Figure 4. 3 Compounds isolated, in this study, from *Crinum stuhlmannii*

4.2 Results and Discussion

4.2.1 Structural Elucidation of Compound V, Lycorine (spectra 5.a-h)

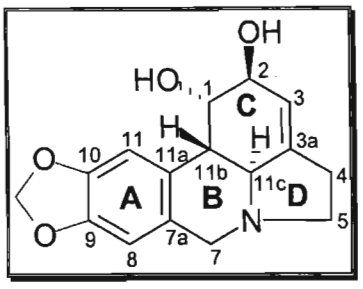


Figure 4. 4 Structure of compound V, lycorine

Compound **V** **(Figure 4. 4)** was isolated as a white crystalline material with a melting point of 244-247°C. This compound was found to have a molar mass of 287 g. mol^{-1} corresponding to a molecular formula of $C_{16}H_{17}NO_4$.

Compound **V** was identified based on its NMR data as lycorine. It is insoluble in most organic solvents, which makes it difficult when one compares NMR data, as the NMR data tends to be reported in the literature in different solvent mixtures. The substitution pattern on the A ring was confirmed in the ^1H NMR spectrum by the presence of two *para* orientated aromatic protons and a two proton resonance at δ_H 5.75, which is typical of protons of a methylenedioxy group. Other characteristic features observed in the ^1H NMR spectrum included a broad singlet in the olefinic region assigned to H-3 (δ_H 5.80), the pair of doublets for the 2H-7 methylene protons ($\delta_{H7\alpha}$ 3.30, $\delta_{H7\beta}$ 3.98, 14.0 Hz). The multiplet at δ_H 3.11 and the double doublet at δ_H 2.08 (dd, 8.4, 17.6 Hz) were attributed to the two H-5 proton resonances. The resonance for the 2H-4 protons appeared at δ_H 2.41, superimposed as a multiplet. The resonances ascribed to H-1 and H-2 were found at δ_H 5.09 and 4.82 respectively, with corresponding carbon resonances at δ_C 73.43 and 72.22 respectively indicating the presence of oxygen substituents which were determined to be hydroxy substituents from the molecular formula. The H-11b resonance was found to occur as a doublet at δ_H 3.23 (d, 10.6 Hz). The resonance ascribed to H-11c appeared as a multiplet at δ_H 3.19. The H-11b resonance has a β configuration on biosynthetic grounds and it showed a correlation in the NOESY spectrum with the H-1 resonance indicating the orientation of the H-1 atom to be β. The HMBC, COSY and NOESY data are provided in **Table 4.1**.

The infrared data supported the postulated alkaloid structure for compound **V**. Peaks were observed at 3333 cm^{-1} (O-H stretching), 2957 cm^{-1} (aliphatic C-H stretching), 1056 cm^{-1} (C-O stretching).

A literature search for the compound indicated that it was lycorine, a common alkaloid previously isolated from *Crinum augustum*, *Crinum amabile* and *Crinum stuhlmannii* and numerous other members of the Amaryllidaceae family.[5-8] Comparison of the ^{13}C NMR spectra **(Table 4.1)** proved to be quite difficult as they are often run in different solvents.

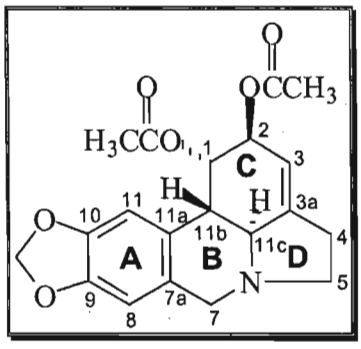


Figure 4. 5 Structure of lycorine di-acetate (VA)

Acetylation of lycorine (**V**) afforded the di-acetate (**VA**). The ^{1}H NMR spectrum of the di-acetate showed the downfield shift of H-1 from δ_H 5.09 to 5.72. The H-2 resonance also shifted downfield from δ_H 4.82 to 5.23. This confirmed that acetylation occurred at both positions 1 and 2 of ring C. Further evidence of the formation of the di-acetate was the two singlet methyl resonances at δ_H 1.93 and δ_H 2.06. Comparison of the ^{13}C NMR data of the acetate to that of 1,2-di-O-acetyllycorine (**Table 4.2**) however did show discrepancies. [16]

Table 4.1: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for lycorine (C_5D_5N)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C* (ppm)	HMBC (C→H)	COSY	NOESY
1	5.09 (s)	73.43	70.21		2;11b	2;11;11b
2	4.82 (brs)	72.22	71.72	1	3	1;3
3	5.80 (brs)	119.56	118.48	1	2;4α,β	2;4α,β
3a		143.01	141.68	4α,β;5β,11b;11c		
4α,β	2.41 (m)	28.99	28.13	5α	3;5α,β	3
5α	2.08 (dd, 8.4,17.6 Hz)	54.15	53.31	7α	4α,β;5β	4α,β;5β;7α
5β	3.11 (m)[c]				4α,β;5α	4α,β;5α;7β
7α	3.30 (d, 14.0 Hz)	57.62	56.73	5α,8	7β	5α;7β;11c
7β	3.98 (d, 14.0 Hz)				7α	7α;8
7a		130.72[b]	129.69	8;11		
8	6.52 (s)	107.55	107.01	7α,β;11		7β
9		146.67[a]	145.20	8;11		
10		146.19[a]	145.65	8;11		
11	7.10 (s)	106.07	105.06	8		1
11a		130.54[b]	129.57	8;11		
11b	3.23 (d, 10.6 Hz)	41.91	40.18	1;11	1	1
11c	3.19 (m)[c]	62.03	60.83	1;5β;7β;11b		7α
OCH_2O	5.75 (dd, 7.3, 1.3 Hz)	101.13	100.57			

[a] ^{13}C NMR resonance values interchangeable.

[b] ^{13}C NMR resonance values interchangeable.

[c] Resonance obscured; *J* values could not be determined.

* Literature spectra run in DMSO-d_6.[7]

Table 4.2: Comparison of the NMR data for diacetylycorine ($CDCl_3$) with literature

Carbon	δ_C (ppm)	δ_C* (ppm)
1	69.24	70.6
2	70.87	72.0
3	113.84	115.3
3a	146.07[a]	146.9
4α,β	28.66	29.3
5α	53.61	54.5
5β		
7α	56.87	57.6
7β		
7a	129.38	130.4
8	107.31	108.3
9	146.44[a]	148.1
10	146.31[a]	146.9
11	105.05	105.9
11a	126.55	127.4
11b	40.46	41.4
11c	61.21	62.6
OCH_2O	100.97	102.4
1-OCO$\underline{CH_3}$[b]	20.93	20.6
2-OCO$\underline{CH_3}$[b]	170.01	171.6
1-O$\underline{CO}CH_3$[c]	21.14	20.9
2- O$\underline{CO}CH_3$[c]	169.78	171.4

[a] ^{13}C NMR resonance values interchangeable.

[b] ^{13}C NMR resonance values interchangeable.

[c] ^{13}C NMR resonance values interchangeable.

* Literature spectra run in $CDCl_3$.[16]

4.2.2. Structural Elucidation of Compound VI, 8,9-Methylenedioxophenanthridine (Trisphaeridine) (spectra 6.a-h)

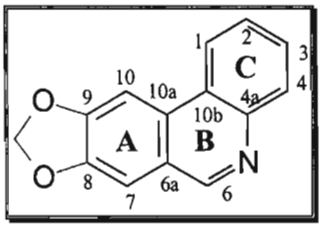


Figure 4. 6 Structure of compound VI, trisphaeridine

This compound **(Figure 4.5)** was isolated as fine yellow needle-like crystals with a melting point of 138-140°C. Compound **VI** was found to have a molar mass of 223 g. mol^{-1} corresponding to a molecular formula of $C_{14}H_9NO_2$. This compound was identified using the mass and NMR spectra.

The NMR spectra of compound **VI** proved to be quite interesting with peaks observed only downfield of 6 ppm. The substitution pattern on the A ring was confirmed through the presence of two *para* orientated aromatic protons (δ_H 7.34, H-7; δ_H 7.92, H-10) and a resonance at δ_H 6.17 (2H), for protons of the methylenedioxy group, in the ^{1}H NMR spectrum. Other features observed in the ^{1}H NMR spectrum were four coupled aromatic proton signals, which included a pair of double doublets for H-1 (δ_H 8.38, dd, 1.3, 8.0 Hz) and H-4 (δ_H 8.18, dd, 1.5, 8.2 Hz) and multiplets for the H-2 (δ_H 7.63) and H-3 (δ_H 7.68) aromatic ring C protons. The remaining signal at δ_H 9.09 in the proton NMR spectrum was found to a be an iminic proton singlet which corresponded to a peak at δ_C 151.25 in the ^{13}C NMR spectrum this was assigned to H-6 and hence a double bond was placed between C-6 and the nitrogen atom. The HMBC, COSY and NOESY data are provided in **Table 4.2**.

A literature search for the compound indicated that it was 8,9-methylenedioxophenanthridine **(VI)** (trisphaeridine), previously isolated from *Lapierdra martinezii* (Amaryllidaceae).[9] The ^{13}C NMR data is not provided in the literature, however, the ^{1}H NMR data was given and is shown in **Table 4.3**. The ^{1}H NMR data obtained proved to be close to literature values.

Table 4.3: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for, 8,9-methylenedioxophenanthridine ($CDCl_3$)

Carbon	δ_H (ppm)	δ_{H^*} (ppm)	δ_C (ppm)	HMBC (C→H)	COSY	NOESY
1	8.38 (dd, 1.3, 8.0 Hz)	8.36	122.04	3	2	2;10
2	7.63 (m)	7.61	126.94	4	1;3	3;1
3	7.68 (m)	7.67	128.27	1	2;4	2,4
4	8.18 (dd, 1.5, 8.2)	8.11	129.48	2	3	3
4a			~130[a]	1		
6	9.09 (s)	9.06	151.25	7		7
6a			122.88	6,10		
7	7.34 (s)	7.32	105.65	6		6
8			~152[a,b]	7;10;OCH_2O		
9			148.37[b]	7;10;OCH_2O		
10	7.92 (s)	7.89	100.02			1
10a	-		130.63	6;7		
10b	-		124.30	10		
OCH_2O	6.17 (s)	6.15	102.05			

[a] Exact carbon values could not be determined as resonances were very weak and could only be detected through HMBC correlations to these peaks.

[b] ^{13}C NMR resonance values interchangeable.

* Literature spectra run in $CDCl_3$.[9]

4.2.3 Structural Elucidation of Compound VII, 6-Hydroxycrinamine (spectra 7.a-j)

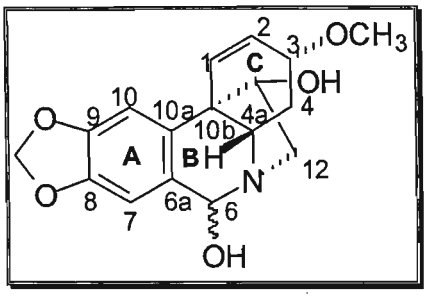


Figure 4. 7 Structure of compound VII, 6-hydroxycrinamine

Compound **VII** (**Figure 4. 7**), was isolated as white crystals with a melting point of 142-144°C. This compound was found to have a molar mass of 317 g. mol^{-1} corresponding to a molecular formula of $C_{17}H_{19}NO_5$. It was identified on the basis of the NMR data and found to be a 5,10b-ethanophenanthridine type of alkaloid, which was isolated as an inseparable mixture of the two C-6 epimers. This was indicated by the pairing of some of the peaks in the ^{13}C NMR spectrum. Assignment of resonances for the major epimer has been done only, as the minor epimer was present in very low amounts and resonances were very hard to assign. With the aid of the NOESY spectrum it was determined that the major epimer contained the 6β-OH group and the minor, the 6α-OH group.

Resonances in the ^1H NMR spectrum due to two *para* orientated aromatic protons (δ_H 6.75 (s), H-7), δ_H 6.84 (s), H-10) and a two proton resonance at δ_H 5.89, which is typical of protons of a methylenedioxy group, confirmed the A ring to have only two unsubstituted positions. Other characteristic features observed in the ^1H NMR spectrum included a double doublet resonance (δ_H 6.28, 2.2 and 10.3 Hz) and doublet resonance (δ_H 6.09, 10.3 Hz) in the olefinic region assigned to H-1 and H-2 respectively of a 1,2-double bond and a methoxy group singlet proton resonance at δ_H 3.39. The absence of an AB splitting pattern for the methylene proton at about 4 ppm, which is seen for all C-6 unsaturated crinane alkaloids confirmed the substituted nature of C-6.[10] The HMBC spectrum showed ^3J correlations from C-7 and C-10a to a resonance at δ_H 4.96, which was seen in the HSQC spectrum to correspond to a carbon resonance at δ_C 88.94 and, hence, a hydroxy group was placed at C-6. Another typical resonance was a double doublet at δ_H 3.56 (dd, 4.0 and 13.6 Hz) which was assigned to the H-4a resonance.[10] The HMBC spectrum showed ^3J correlations from C-6 and C-4a to the resonances at δ_H 3.15 (dd, 3.6, 14.3 Hz) and 3.43 (dd, 3.3, 6.2 Hz), which corresponded to a methylene carbon resonance of δ_C 58.92, in the HSQC spectrum. This resonance was assigned to C-12, one of the two carbons of the bridge. The downfield shift in the resonance can be attributed to the adjacent nitrogen atom.

The second bridging carbon was identified through a correlation in the COSY spectrum between the H-11 (δ_H 3.85) and 2H-12 (δ_H 3.15 and 3.43) resonances. The corresponding oxymethine C-11 carbon resonance at δ_C 79.42, was assigned to C-11.

Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. A positive Cotton effect was observed at 276 nm followed by a negative Cotton effect at 251 nm, which indicated the bridge was of the α configuration since negative Cotton effects are typically observed at 250 nm for the α orientated series.[10-12,17-18] An HMBC ^3J correlation from C-3, which occurred at δ_C 77.87, to the methoxy group proton resonance at δ_H 3.39 indicated the methoxy substituent to be present at C-3. The orientation of the methoxy group was determined to be α, since the H-2 resonance was split into a doublet (see section 3.2.1 for explanation).[10-12]

The orientation of the bridge substituents was determined using the NOESY spectrum. A correlation between the H-10 resonance and the H-11 resonances indicated H-11 to be *endo* and the hydroxy substituent on C-11 to be *exo*. A further correlation in the NOESY spectrum from the H-11 *endo* resonance to the H-12 resonance at δ_H 3.43 indicated it to be *endo*. The H-12 *endo* resonance showed a correlation in the NOESY spectrum to the H-6 resonance and this could only have been H-6α with the hydroxy group at C-6β. This implied the major epimer isolated contained the hydroxy group at C-6β.

The infra-red data supported the alkaloid structure for compound **VII**. Peaks were observed at 3411 cm^{-1} (O-H stretching), 2893 cm^{-1} and 2821 (aliphatic C-H stretching), 1488 cm^{-1} (aromatic C=C stretching), 1248 (C-N stretching) and 1042 cm^{-1} (C-O stretching). These assignments were confirmed by the HMBC, NOESY and COSY spectra and are listed in **Table 4.4**.

A literature search for the compound identified it as 6-hydroxycrinamine previously isolated from *Brunsvigia orientalis* and also the from the bulbs of *Crinum delagoense* as reported by Nair *et al.*[5,13] However there was one major discrepancy in current assignments compared to literature. The resonances earlier assigned to H-1, C-1 and H–2, C-2 [13] are opposite to what is here assigned to compound **VII**. The assignment of H-1, in compound **VII**, was confirmed through an HMBC correlation to C-3 and a correlation in the NOESY spectrum to H-10. The assignment of H-2 was confirmed through a correlation in the NOESY spectrum to the H-3 resonance and methoxy group proton resonance. The literature assignment requires correction.

Table 4.4: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 6-hydroxycrinamine (CD_3OD)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C* (ppm)	δ_C** (ppm)	HMBC (C→H)	COSY	NOESY
1	6.28 (dd, 2.2, 10.3 Hz)	125.75	136.4	136.2	2;3	2	2;10
2	6.09 (d, 10.3 Hz)	134.37	123.0	123.2	1;3;4β	1	1;3;3-OCH_3
3	4.07 (m)	77.87	75.9	75.6	1;3-OCH_3;4α,β	4α,β	2;4β;4a
4α	2.12 (m)	30.41	29.4	29.4	2;4a	3;4α,β;4a	4β
4β	2.00 (m)					3;4a;4α	3;4a;4α,12_{exo}
4a	3.56 (dd, 4.0,13.6 Hz)	61.80	59.5	64.8	1;4α,β;6;12_{endo}	4α,β	3;4β
6	4.96 (s)	88.94	88.0	85.5	4a;7;$12_{exo, endo}$		7;12_{endo}
6a		129.22	127.3	128.8	6;10		
7	6.75 (s)	110.29	109.5	108.3	6;10		6
8[a]		149.24	146.5	146.7	7;10;OCH_2O		
9[a]		147.69	147.8	147.5	7;10;OCH_2O		
10	6.84 (s)	103.77	102.8	102.7			1;11
10a		138.52	135.8	134.6	4a;6;7;11		
10b		51.81	50.4	50.8	2;4α,β;10;12_{endo}		
11_{endo}	3.85 (m)	79.61	78.1	79.0	4a;12_{exo}	$12_{exo, endo}$	10;12_{endo}
12_{exo}	3.15 (dd, 3.6, 14.3 Hz)	58.92	57.7	51.8	4a;6	11;12_{endo}	$12_{exo, endo}$
12_{endo}	3.43 (3.3, 6.2 Hz)					11;12_{exo}	6;11;12_{exo}
OCH_2O	5.89 (s)	102.43	101.1	101.1			
3-OCH_3	3.39 (s)	55.81	55.9	55.9	3		2

* Literature spectra run in $CDCl_3$ with H-6α.[13]

** Literature spectra run in $CDCl_3$ with H-6β.[13]

[a] ^{13}C NMR resonance values interchangeable.

4.2.4 Structural Elucidation of Compound VIII, 6β-Ethoxycrinamine (spectra 8.a-j)

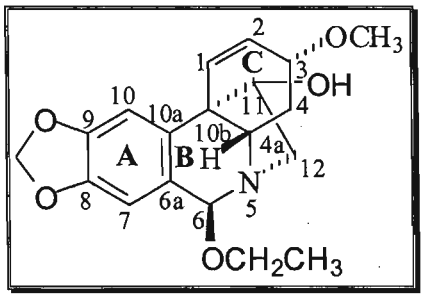


Figure 4. 8 Structure of compound VIII, 6β-ethoxycrinamine

Compound **VIII (Figure 4. 8)**, was isolated as a brownish amorphous material. The CI mass spectrum showed a $[M+H]^+$ peak at *m/z* 346 g. mol^{-1}. Chemical ionisation mass spectra show peaks at one mass unit higher than those expected in electron impact. It was identified on the basis of the NMR data and found to be a 5,10b-ethanophenanthridine type of alkaloid. This compound was found to be closely related to 6-hydroxycrinamine with the presence of an ethoxy group at C-6 instead of an hydroxyl group as found in 6-hydroxycrinamine.

The absence of an AB splitting pattern for the methylene proton at about 4 ppm, which is seen for all C-6 unsaturated crinane alkaloids confirmed the substituted nature of C-6.[10] The HMBC spectrum showed a ^3J correlations from C-7 and C-10a to a resonance at δ_H 4.51 (s), which corresponded to a carbon resonance of δ_C 94.27, in the HSQC spectrum, and indicated the presence of an oxygen-containing substituent at C-6. The HMBC spectrum showed a ^3J HMBC correlation from the methylene carbon at δ_C 64.17 to H-6 (δ_H 4.51). This carbon resonance corresponds to a pair of coupled proton resonances at δ_H 3.65 (dd, 2.4, 7.1) and 4.01 (m) in the HSQC spectrum. The COSY spectrum shows correlation from the two methylene proton resonances at δ_H 3.65 and 4.01 to the methyl group proton resonance at δ_H 1.24 (t, 7.1), implying the presence of an ethoxy group at C-6.

The HMBC spectrum showed ^3J correlations from C-6 and C-4a to a 2 proton resonance at δ_H 3.27 (d, 5.9 Hz), which corresponded to a methylene carbon resonance of δ_C 58.47, in the HSQC spectrum. This resonance was assigned to C-12, one of the two carbons of the bridge. The downfield shift in the resonance can be attributed to the adjacent nitrogen atom. The second bridging carbon was identified through a correlation in the COSY spectrum between the H-12 (δ_H 3.27) and H-11 (δ_H 3.85, t, 4.8) resonances. The corresponding oxymethine resonance was found to occur at δ_C 78.45, and was assigned to C-11.

Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. A positive Cotton effect was observed at 289 nm, which indicated the bridge was of the α configuration since positive Cotton effects are typically observed at 280 nm for the α orientated series.[10-12,17-18] An HMBC ^{3}J correlation from C-3, which occurred at δ_C 76.10 to the methoxy group proton resonance at δ_H 3.39 indicated the methoxy substituent to be present at C-3. The H-1 and H-2 proton resonances overlapped. From literature, if H-2 is split into a doublet we should have a coupling constant of 10 Hz and if is split into a double doublet the coupling constants are 10 and 5 Hz the assumption was made that if it was a double doublet we would have seen the larger coupling constant however this was not the case and we assumed that H-2 was split into a doublet only.[10-12] The orientation of the methoxy group was therefore determined to be α.[10-12]

The remaining assignments were completed by comparison to the previous compound (6-hydroxycrinamine (compound **VII**)). The compound isolated is believed to be an artefact of the extraction process, and could have possible formed when ethanol (a standard solvent used for the extraction of alkaloids) was used during the extraction procedure.

The orientation of the bridge substituents was determined using the NOESY spectrum. A correlation between the H-10 resonance and the H-11 resonance indicated H-11 to be *endo* and the hydroxy substituent on C-11 to be *exo*. The H-12 resonance showed a correlation in the NOESY spectrum to the H-6 resonance as this can only be H-6α, the ethoxy group is at C-6β. This is very similar to compound **VII**. The major epimer of compound **VII** has the H-6 also in the α orientation. This suggests that the β-OH form is the more stable form of 6-hydroxycrinamine.

The infra-red data [spectrum] supported the alkaloid structure for compound **VIII**. Peaks were observed at 3361 cm^{-1} (O-H stretching), 2925 cm^{-1} (aliphatic C-H stretching), 1479 cm^{-1} (aromatic C=C stretching), 1248 cm^{-1} (C-N stretching) and 1033 cm^{-1} (C-O stretching).

A literature search for the compound confirmed it not to have been isolated previously and it was named 6β-ethoxycrinamine. The ^{1}H, ^{13}C, HMBC, NOESY and COSY data are listed in **Table 4.5**.

Table 4.5: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 6β-ethoxycrinamine ($CDCl_3$)

Carbon	δ_H (ppm)	δ_C (ppm)	HMBC (C→H)	COSY	NOESY
1	6.20 (m)[a]	123.43	3;4;11		10,11
2	6.21 (m)[a]	136.43			3,3-OCH_3
3	4.03(m)	76.10	1;2;3-OCH_3;4α,β	4α,β	4a;3-OCH_3; 4α,β
4α,β	2.03 ddd (1.8,9.2,8.8)	29.67	2	2;3;4a	3;3-OCH_3;4a; 12α,β
4a	3.52 (t, 8.8, 9.2)	59.95	1';4α,β;6,12	4	3; 4α,β
6	4.51 (s)	94.27	7;12		7; 6a,b-$O\underline{CH_2}CH_3$;12
6a		126.97	10		
7	6.70 (s)	109.51	6		6
8		147.82[a]	OCH_2O;7;10		
9		146.31[a]	OCH_2O;7;10		
10	6.72 (s)	102.69	-		1;11
10a		136.43	6;71		
10b		50.35	10;1;2		
11	3.85 (t, 4.8)	78.45		12	1;10;12
12	3.27 (d,5.9)	58.47	6;11	11	6;11; 4α,β;
-OCH_2O-	5.88 (dd, 12.8)	101.04			
3-OCH_3	3.39 (s)	55.86	3		2;3; 4α,β
6-$O\underline{CH_2}CH_3$	3.65 (m)	64.17	6, 6-$OCH_2\underline{CH_3}$	6-$O\underline{CH_2}CH_3$. 6-$OCH_2\underline{CH_3}$	6;6-$O\underline{CH_2}CH_3$.;6-$OCH_2\underline{CH_3}$
6-$O\underline{CH_2}CH_3$	4.01 (7.1, 2,4)			6-$O\underline{CH_2}CH_3$.;6-$OCH_2\underline{CH_3}$	6;6-$O\underline{CH_2}CH_3$.;6-$OCH_2\underline{CH_3}$
6-$OCH_2\underline{CH_3}$	1.24 (7.1)	15.45		6-$O\underline{CH_2}CH_3$	6-$O\underline{CH_2}CH_3$

[a] Resonance superimposed *J* values could not be determined, 2nd order spectrum.

4.2.5 Structural Elucidation of Compound IX, Haemanthamine (spectra 9.a-i)

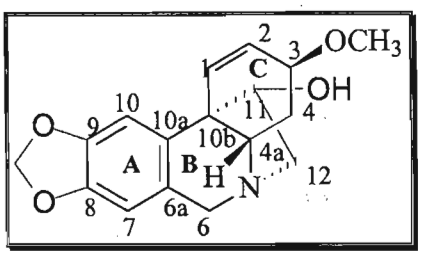


Figure 4. 9 Structure of compound IX, haemanthamine

Compound IX (**Figure 4. 9**) was isolated as a yellow amorphous material and identified through comparison with literature NMR data to be haemanthamine. It was found to have a molar mass of 301 g. mol^{-1} corresponding to a molecular formula of $C_{17}H_{19}NO_4$ and was also a 5,10b-ethano bridge type of alkaloid.

The substitution pattern on the A ring was similar to the previous compounds isolated with the presence of only two *para* orientated aromatic protons (δ_H 6.52 and 6.89) and a resonance at δ_H 5.86 (2H), which is typical of a methylenedioxy group, in the ^1H NMR spectrum. Other characteristic features observed in the ^1H NMR spectrum included: the AB splitting of the methylene protons of H-6 at δ_H 3.76 (d, 16.7 Hz) and 4.28 (d, 16.7 Hz),[10] the H-4a resonance at δ_H 3.30 (m) and a doublet at δ_H 6.44 (d, 10.1 Hz) and a double doublet at 6.23 (dd, 4.9, 10.1 Hz) in the olefinic region assigned to H-1 and H-2 of a 1, 2-double bond. The double doublet resonance for H-2 implied a *trans* relationship between the 5,10b-ethano bridge and the substituent at C-3.[10-12] The C-3 resonance (δ_C 72.98) showed a ^3J HMBC correlation to the methoxy proton group resonance at δ_H 3.33 and this indicated the methoxy group was attached at C-3.

Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. A positive Cotton effect was observed at 271 nm followed by a negative Cotton effect at 254 nm, which indicated the bridge was of the α configuration since negative Cotton effects are typically observed at 250 nm for the α orientated series.[10-12,17-18] Due to a *trans* relationship between the bridge and the methoxy group proton, the orientation of the methoxy group attached at C-3 is β.[10-12]

The C-6 resonance showed a ^3J HMBC correlation to the methylene protons at δ_H 3.14 (dd, 3.2, 13.9 Hz) and 3.44 (dd, 7.0, 13.9 Hz) assigned to 2H-12. The 2H-12 proton resonances showed a correlation in the COSY spectrum to the resonance at δ_H 3.94 (m), which had a corresponding

carbon, seen in the HSQC spectrum at δ_C 79.72 indicating the presence of an oxygen substituent on C-11 and this was deduced to be an hydroxyl substituent from the molecular formula. A correlation in the NOESY spectrum between the H-10 resonance and the H-11 resonance indicated H-11 to be *endo* and the hydroxy substituent on C-11 to be *exo*. A further correlation in the NOESY spectrum from the H-11 *endo* resonance to the H-12 resonance at δ_H 3.44 indicated it to be H-12 *endo*.

The IR data supported the postulated structure with peaks observed at 2928 cm^{-1} (aliphatic C-H stretching), 1438 cm^{-1} (aromatic C=C stretching), 1239 cm^{-1} (C-N stretching) and 1037 cm^{-1} (C-O stretching).

A literature search for the compound identified it as haemanthamine isolated from *Scadoxus puniceus* (*Haemanthus puniceus* L.) and numerous other Amaryllidaceae taxa.[8,14] The ^{1}H, ^{13}C, HMBC, COSY and NOESY data are shown in **Table 4.6**.

Table 4.6: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for hamaenthamine (CD_3OD)

Carbon	δ_H	δ_C	δ_C*	HMBC (C→H)	COSY	NOESY
1	6.44 (d, 10.1 Hz)	127.98	127.3	2;3	2	2;10;11
2	6.23 (dd, 4.9,10.1 Hz)	129.39	131.6	1;3;4β	1;3	1;3;3-OCH_3
3	3.85 (m)	72.98	72.7	1;2;4α,β;4a;3-OCH_3	2;4α,β	2;4α,β;3-OCH_3
4α	2.14 (ddd, 4.2, 9.2, 13.5 Hz)	27.78	28.1	2;4a;3;3-OCH_3	3;4a;4β	3;4β;12_{exo}
4β	1.97 (ddd, 4.4, 9.0, 13.4 Hz)				3;4a;4α	3;4a;4α
4a	3.30 (m)	67.90	62.6	1;3;4α,β;12_{endo}	4α,β	4β;6β
6α	3.76 (d, 16.7 Hz)	60.36	61.2	4a;7; $12_{exo, endo}$	6β;7	6β,7;12_{endo}
6β	4.28 (d, 16.7 Hz)				6α;7	4a;6α;7
6a		125.21	126.5	6α,β;10		
7	6.52 (s)	106.66	106.7	6α,β;10	6α,β	6α,β
8		147.00[a]	146.3	6α,β;7;10;OCH_2O		
9		146.52[b]	146.0	7;10;OCH_2O		
10	6.89 (s)	103.11	103.2	6α,β;7		1;11
10a		135.52	135.2	1;4a;6α,β;7;11		
10b		50.34	49.9	1;2;4α,β;4a;6α,β;10; 12_{endo}		
11	3.94 (m)	79.72	80.0	4a;6α,β;12_{exo}	$12_{exo, end}$	1;10;12_{endo}
12_{endo}	3.44 (dd, 7.0, 13.9 Hz)	62.57	63.5	4a;6α,β	11; 12_{endo}	6α;11;12_{exo}
12_{exo}	3.14 (dd,3.2, 13.9 Hz)				11;12_{exo}	4α;12_{exo}
-OCH_2O-	5.86 (s)	55.61	100.7			
3-OCH_3	3.33 (s)	55.61	56.5	3;4α		2

* Literature spectra run in $CDCl_3$.[14]

[a] ^{13}C NMR resonance values interchangeable.

4.2.6 Structural Elucidation of Compound X, Hamayne (spectra 10.a-i)

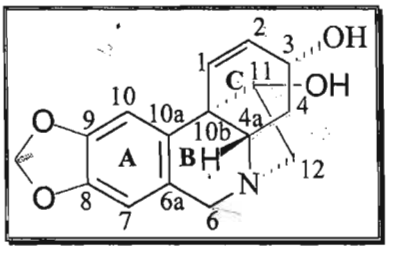


Figure 4. 10 Structure of compound X, hamayne

Compound **X** (**Figure 4. 10**) was isolated as a brownish amorphous material and identified by comparison against literature data to be hamayne, a compound previously isolated from the bulbs of *Crinum delagoense*.[5]

The substitution pattern on the A ring was similar to the previous compounds isolated with the presence of only two *para* orientated aromatic protons (δ_H 6.61 and 6.05) and a resonance at δ_H 5.91 (2H), which is typical of protons of a methylenedioxy group, in the ^{1}H NMR spectrum. Other characteristic features observed in the ^{1}H NMR spectrum included resonances in the olefenic region (δ_H, 6.22, dd, 10.3, 2.2 Hz; 6.05 d, 10.3 Hz assigned to H-1 and H-2 of a 1,2-double bond. The doublet resonance for H-2 implied a *cis* relationship between the 5,10b-ethano bridge and the hydroxy substituent at C-3.[10-12, 17-18]

Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. A positive Cotton effect was observed at 294 nm followed by a negative Cotton effect at 253 nm, which indicated the bridge was of the α configuration since negative Cotton effects are typically observed at 250 nm for the α orientated series.[10-12,17-18.] Due to a *cis* relationship between bridge and the hydroxy group the orientation of the hydroxy group attached at C-3 is α.[10-12,17-18]

The C-6 resonance showed a ^3J HMBC correlation to the methylene protons at δ_H 3.45 (dd, 3.3, 13.9 Hz) and 3.73 (dd, 6.2, 13.9 Hz) assigned to 2H-12. The 2H-12 protons showed a correlation in the COSY spectrum to the resonance at δ_H 4.03 (m), which had a corresponding carbon resonance, seen in the HSQC spectrum, at δ_C 79.74 indicating the presence of an oxygen substituent at C-11 and this was deduced to be an hydroxyl substituent from the molecular formula. A correlation in the NOESY spectrum between the H-10 resonance and the H-11 resonance indicated H-11 to be *endo* and the hydroxy substituent on C-11 to be *exo*. A further correlation in the NOESY spectrum from the H-11 *endo* resonance to the H-12 resonance at δ_H 3.45 indicated it to be H-12 *endo*.

The infrared spectrum supported the postulated structure with peaks at 3349 cm^{-1} (O-H stretching), 2922 cm^{-1} and 2853 (C-H stretching), 1610 cm^{-1}, 1465 cm^{-1} (aromatic C=C stretching) and 1265 cm^{-1} (C-N stretching).

A literature search for the compound identified it as hamayne, first isolated from *Crinum bulbispermum* (Burm.f) Milne-Redh. & Schweick. in 1984.[15] It has more recently been isolated from *Ammocharis coranica* and *Crinum stuhlmannii*.[5,16] The ^{1}H, ^{13}C, HMBC, COSY and NOESY data are shown in **Table 4.7**.

Table 4.7: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for hamayne (CD_3OD)

Carbon	δ_H	δ_C	δ_C*	HMBC (C→H)	COSY	NOESY
1	6.22 (dd, 10.3, 2.2 Hz)	123.58	124.0		2	2;10
2	6.05 (d, 10.3 Hz)	137.50	137.4		1	1;3
3	4.36 (m)	67.85[a]	67.7	1;4α	4α,β	2;4a;4β
4α	2.23 (m)	33.26	33.5		3;4a;4β	4β;12_{exo}
4β	2.11 (m)				3;4a;4α	3;4a;4α
4a	3.58 (dd, 3.7,13.6 Hz)	67.24[a]	65.6	1;4α,β;6α;11	4α,β	3;4β;6β
6α	4.01 (d, 16.1 Hz)	60.27	60.7	7;$12_{exo,\ endo}$	6β	6β,7
6β	4.49 (d, 16.1 Hz)				6α	4a;6α;7
6a		123.58	124.7	6α,β;10		
7	6.61 (s)	107.95	107.9	6α		6α,β
8		148.86[b]	148.1	7;OCH_2O		
9		148.29[b]	148.6	10;OCH_2O		
10	6.92 (s)	104.55	104.4			1
10a		135.77	136.4	6α,β;7		
10b		51.64	51.6	4α,β;10		
11	4.03 (m)	79.74	80.2		$12_{exo,\ endo}$	12_{endo}
12_{endo}	3.45 (dd, 3.3,13.6 Hz)	62.19	63.2	6α,β	11;12_{endo}	4α;12_{endo}
12_{exo}	3.73 (dd,6.2,13.9 Hz)				11;12_{exo}	11;12_{exo}
-OCH_2O-	5.91	102.62	102.5			

[a] ^{13}C NMR resonance values interchangeable.

[b] ^{13}C NMR resonance values interchangeable.

* Literature spectra run in CD_3OD. [16]

4.2.7 Structural Elucidation of Compound XI, *N*-methyl-3-*epi*-crin-6-amide (stuhlmanine A) (spectra 11.a-j)

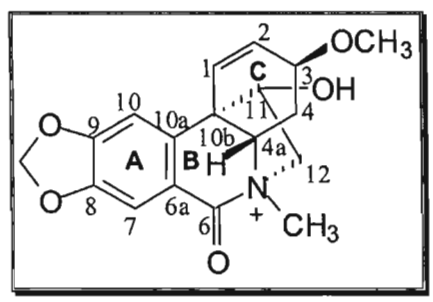


Figure 4. 11 Structure of compound XI, stuhlmanine A

Compound **XI (Figure 4. 11)** was isolated as a brownish amorphous material. The mass spectrum showed a molecular ion peak at 330.13415g. mol^{-1} which corresponded to a molecular formula of $C_{18}H_{20}NO_5$. It was found to be a new alkaloid that belongs to the 5,10b-ethano bridge type of alkaloids.

The substitution pattern on the A ring was similar to the previous compounds isolated with the presence of only two *para* orientated aromatic protons (δ_H 7.42 and 6.84) and a two proton broad singlet resonance at δ_H 6.05, which is typical of a methylenedioxy group in the ^1H NMR spectrum. However, in comparison with the H-7 resonance in previous alkaloids of this type that have been isolated, these resonances are much further downfield.

Other characteristic features observed in the ^1H NMR spectrum included resonances δ_H 5.70, 1.3, 10.3 Hz, H-1, (δ_H 6.09, H-2) in the olefinic region assigned to protons of a 1,2-double bond and a methoxy group proton resonance at δ_H 3.42. The COSY spectrum showed correlations between the H-2 resonance and a resonance at δ_H 3.91 (m), assigned to H-3. The H-3 resonance, in turn, showed correlations to the 2H-4 resonances ($\delta_{H4\alpha}$ 2.36, m, $\delta_{H4\beta}$ 2.44, brd 16.0 Hz) which, in turn, showed correlations to the H-4a resonance (δ_H 3.33, m). The resonance assigned to H-3 showed a corresponding carbon resonance at δ_C 72.12 in the HSQC spectrum, which showed a ^3J HMBC correlation to the methoxy group proton resonance (δ_H 3.42) implying the methoxy group to be present at C-3. The orientation of the methoxy group was determined with the aid of CD spectra and the H-2 resonance. Circular dichroism was used to determine the absolute configuration of the 5,10b-ethano bridge. A positive Cotton effect was observed at 279 nm followed by a negative Cotton effect at 258 nm, which indicated the bridge was of the α configuration.[10-12,18-19] The resonance for H-2 was obscured by the methylenedioxy group proton resonance. However, on expanding this region, a doublet with a coupling constant of 5 Hz was measured. From literature,

if H-2 is split into a doublet only a coupling constant of 10 Hz would be observed; if split into a double doublet the coupling constants are 10 and 5 Hz the assumption was made that part of the H-2 resonance was obscured.[10-12] This double doublet indicates a *trans* relationship to the bridge and hence the methoxy group is β.[10-12]

The methylene group carbon resonance at δ_C 53.83 was assigned to C-12, the first of the two bridging carbons. The second bridging carbon was identified through a correlation in the COSY spectrum between the H-11 and the 2H-12 proton resonances. The H-11 resonance occurred at δ_H 4.73 (dd, 3.4, 7.7 Hz), and corresponded to a oxymethine carbon resonance at δ_C 81.25, ascribed to C-11 from the molecular formula. The absence of an AB splitting pattern for the methylene proton at about 4 ppm, which is seen for all C-6 unsaturated crinane alkaloids, confirmed the substituted nature of C-6.[10] The signal in the ^{13}C NMR spectrum at δ_C 167.17 indicating the presence of a carbonyl group, showed a ^3J HMBC correlation in the HMBC spectrum to the H-7 resonance. This implied the presence of a carbonyl group at C-6. The IR confirmed the presence of an amide carbonyl group with a peak at 1724 cm^{-1}. The remaining signal in the ^{13}C NMR spectrum was a methyl carbon resonance at δ_C 42.64. This resonance showed a HMBC correlation to the H-12 resonance and the only possible position it could be placed was attached to the nitrogen atom. This implied that a *N*-methyl group was present and could explain why the C-4a resonance was a short broad peak and the methyl group was so far downfield in the ^1H NMR spectrum. Other IR resonances were 3373 cm^{-1} (O-H stretching), 2925 and 2854 cm^{-1} (C-H stretching) and 1274 cm^{-1} (C-N stretching).

A literature search confirmed the novelty of the compound as a natural product and it was named stuhlmanine A. The HMBC, COSY and NOESY data are shown in **Table 4.8.**

Table 4.8: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for stuhlmanine A (CD_3OD)

Carbon	δ_H	δ_C	HMBC (C→H)	COSY	NOESY
1	5.70 (dd, 10.3, 1.3 Hz)	128.30	3	2	2;10
2	6.09 (m)[b]	129.54	3	1;3	1;3; 3-OCH_3
3	3.91(m)	72.12	3-OCH_3	2;4α,β	2;4α;3-OCH_3
4α	2.36 (m)	28.78	2	3;4a	3;4β;10
4β	2.44 (brd, 16.0 Hz)			3,4a	4α
4a	3.33 (m)[b]	64.27	*N*-CH_3	4α,β	N-CH_3
6		167.17	7;10		
6a		119.51	10		
7	7.42 (s)	111.36	10		
8		154.40[a]	7;10;OCH_2O		
9		148.94[a]	7;10; OCH_2O		
10	6.84 (s)	104.89			1;4a;4α
10a		142.86	7		
10b		46.88	10		
11	4.73 (dd, 3.4, 7.7 Hz)	81.25	4a	12a,b	12a
12a	2.91 (m)	53.83	N-CH_3	11;12b	11;12b;N-CH_3
12b	3.22 (m)			11;12a	12a
-OCH_2O-	6.05 (m)	103.94			
3-OCH_3	3.42 (s)	56.98	3		2;3
N-CH_3	2.58 (s)	42.64	12b		4a;12a

[a] ^{13}C NMR resonance values interchangeable.

[b] resonances obscured-could not determine coupling constant.

4.2.8 Structural Elucidation of Compound XIIA and B, 4a-hydroxy-1,2-di-*epi*-bowdensine (stuhlmanine B) and 4a-hydroxy-2-*epi*-bowdensine (stuhlmanine C) (spectra 12.a-j)

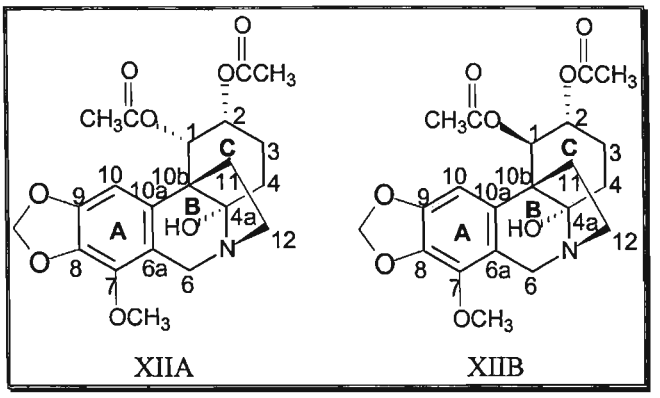


Figure 4. 12 Structure of compound XIIA andXIIB, stuhlmanine B and C

Compound **XII** (**Figure 4. 12**) was isolated as a yellow amorphous material. It showed one spot on tlc analysis. The mass spectrum showed a molecular ion peak at *m/z* 419.15897 corresponding to a molecular formula of $C_{21}H_{25}NO_8$. However the, ^1H and ^{13}C NMR spectra showed distinctive doubling of some resonances and it was found to be a mixture of two inseparable epimers at C-1. Both epimers were found to be new alkaloids of the 5,10b-ethano bridge type of alkaloids and were named stuhlmanines B and C.

The structural determination of one epimer is discussed first. By similar arguments, the signals for the second epimer were assigned. The signals due to each epimer were distinguished on the basis of size of the resonances and the integration. The appearance of only one singlet at δ_H 6.72 and the methylenedioxy group proton resonance at δ_H 5.88 indicated only one unsubstituted position on the A ring thus differentiating this compound from compounds previously isolated from this species. The presence of the AB splitting pattern for the 2H-6 methylene protons at δ_H 3.83 and 4.08 indicated H-6 was unsubstituted.[10] The C-6 (δ_C 52.76) resonance showed a ^3J HMBC correlation to the methylene protons at δ_H 2.72 (m) and 3.54 (m) ascribable to 2H-12. The 2H-12 resonances showed correlations in the COSY spectrum to the methlyene protons at δ_H 1.69 (m) and 2.16 (m), which were assigned to 2H-11.

The proton NMR spectrum also showed the presence of two acetate group proton signals and one methoxy group proton signal belonging to this compound. The H-6 resonance at δ_H 3.83 (m) showed a correlation in the NOESY spectrum to the methoxy group proton resonance at δ_H 3.99 (s) indicating its presence at C-7 and identifying H-10 as the unsubstituted position on ring A.

The quaternary carbon resonance at δ_C 91.93 pm showed ^3J HMBC correlations to H-6 (δ_H 3.83) and H-12 (δ_H 1.69) and was assigned to C-4a. By mass difference C-4a was determined to have a hydroxy group attached. Further HMBC correlations was seen from C-4a, together with the carbonyl resonance, of one which of the two acetates, to a resonance at δ_H 6.23, which can be either to H-3 or H-1. The NOESY spectrum showed a correlation from H-10 (δ_H 6.72) to the resonance at δ_H 6.23 enabling this resonance to be assigned to H-1. This resonance, in turn, showed a correlation in the COSY spectrum to the resonance at δ_H 4.99 enabling this resonance to be assigned to H-2. Similarly, the carbonyl carbon resonance of the remaining acetate group showed a ^3J HMBC correlation to thus H-2 resonance, placing the second acetate group at C-2. The stereochemistry of the acetate at C-2 was assigned to be in the axial position due to a very small coupling constant. The COSY spectrum was used to complete the assignment of the C ring. The H-2 resonance (δ_H 4.99) showed correlations to the methylene protons at δ_H 1.72 (m) and 2.14 (m) which were assigned to 2H-3. The 2H-3 resonances, in turn, showed correlations to resonances at δ_H 1.98 (m) and 2.18 (m), which were assigned to 2H-4.

Circular dichroism spectroscopy was used to determine the absolute configuration of the 5, 10b-ethano bridge. A positive Cotton effect was observed at 275 nm followed by a further positive effect at 253 nm, which indicated the bridge was of the β configuration since positive Cotton effects are typically observed at approximately 250 nm for the β orientated series.[10-12,17,18]

A similar set of reasoning lead to the assignment of the structure of the second epimer. The only difference noted was in the NOESY spectrum. The H-10 resonance showed a correlation to the acetoxy group proton resonance attached to C-1, which was absent in the assignment of stuhlmanine B. With the aid of a molecular model it could be seen that the acetoxy group orientation was β.

A literature search for the compounds indicated that they have both been isolated for the first time and they were named stuhlmanine B and stuhlmanine C. The HMBC, COSY and NOESY data are shown in **Table 4.9** and **4.10** for both epimers.

Table 4.9: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for stuhlmanine B ($CDCl_3$)

Carbon	δ_H	δ_C	HMBC (C→H)	COSY	NOESY
1	6.23 (d, 2.7 Hz)	71.33		2	2;10
2	4.99 (m)	69.65	1,4α,β	1;3α,β	1;3α;4α;10; 2-$COCH_3$
3α	1.72 (m)	22.34	1	2;4α,β	2;4α
3β	2.14 (m)			2;4α,β	3β; 2-$COCH_3$
4α	1.98 (m)	26.09		3α,β	2;3α;4β
4β	2.18 (m)			3α,β	4α;2-$COCH_3$, 11_{exo}
4a		91.93	1;6α		
6α	4.08 (d, 17.9 Hz)	52.76	12_{exo}	6β	4α;6β;2-$COCH_3$
6β	3.83 (d, 17.9 Hz)			6α	6α;12_{exo};7-OCH_3
6a		115.97	6α,β;10		
7		140.11	OCH_3;10;6α,β		
8		149.63	OCH_2O;10		
9		134.0	OCH_2O;10		
10	6.72 (s)	98.05			1;2
10a		134.45	6α,β;11β		
10b		49.60	1;4;10		
11_{exo}	1.69 (m)	34.64		11_{exo};$12_{exo,endo}$	12_{exo};2-$OCOCH_3$
11_{endo}	2.16 (m)			11_{endo};$12_{exo,endo}$	12_{endo}
12_{exo}	2.72 (m)	52.88	6α,β	$11_{exo,endo}$;12_{exo}	6β;12_{exo}
12_{endo}	3.54 (m)			$11_{exo,endo}$;12_{endo}	11_{endo};12_{endo}
-OCH_2O-*	5.88 (s)	101.02			
OCH_3*	3.99 (s)	59.148			6β
1-$OCOCH_3$	2.09 (s)	21.18			2-$COCH_3$
2-$OCOCH_3$*	1.97 (s)	20.88			2;3β ;11_{exo}; 6α;1-$COCH_3$
1-$OCOCH_3$*		169.84	1;1- $OCOCH_3$		
2- $OCOCH_3$		170.32	2- $OCOCH_3$		

* Proton and carbon values interchangeable with corresponding resonances between epimers.

Table 4.10: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for stuhlmanine C ($CDCl_3$)

Carbon	δ_H	δ_C	HMBC (C→H)	COSY	NOESY
1	5.32 (d, 4.5 Hz)	72.46		2	2;10;1- $OCO\underline{CH_3}$
2	5.60 (brs)	68.22	1		1;3α;2-$OCO\underline{CH_3}$
3	1.87 (m)	24.55		2;4	3β;2
	2.16 (m)			2;4	
4α;β	1.95 (m)	27.50	3β	3α,β	
4a		93.12	6α		
6α	4.42 (d, 17.9 Hz)	52.33		6α	6β
6β	3.83 (d, 17.9 Hz)			6β	6β;7-OCH_3
6a		115.18	6α,β;10		
7		139.89	6α;7-OCH_3;10		
8		149.08	OCH_2O;10		
9		133.82	OCH_2O;10		
10	6.16 (s)	98,87			1; 1-$OCO\underline{CH_3}$
10a		135.30	6α,β;1		
10b		48.85	1;10		
11_{exo}	2.06 (m)	35.51	1	$11\beta;12_{exo,\ endo}$	
11_{endo}	2.99 (m)			11α;12α,β	1-$OCO\underline{CH_3}$
12_{exo}	2.92 (m)	49.88	6α,β;10	$11_{exo,\ endo}$;12_{endo}	12_{endo}
12_{endo}	3.52 (m)			$11_{exo,\ endo}$ 12_{exo}	12_{exo}
-OCH_2O-*	5.84 (s)	100.85			
OCH_3*	3.97 (s)	59.186			6β
1-$OCO\underline{CH_3}$	2.07 (s)	21.15			1;10;11_{endo}
2-$OCO\underline{CH_3}$*	2.20 (s)	21.05			1;2;3α,β
1-$O\underline{CO}CH_3$*		170.08	1;1-$OCO\underline{CH_3}$		
2- $O\underline{CO}CH_3$		169.78	2-$OCO\underline{CH_3}$		

*Proton and carbon values interchangeable with corresponding resonances between epimers

4.2.9 Structural Elucidation of Compound for XIII, *N*-Methyldelagoenine (spectra 13.a-i)

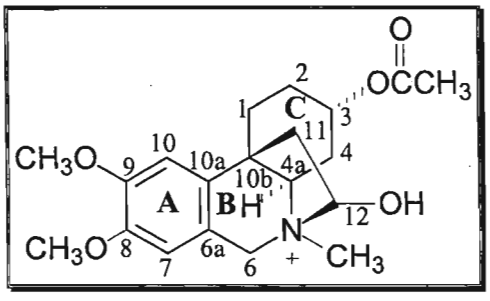


Figure 4. 13 Structure of compound XIII, *N*-methyldelagoenine

Compound **XIII (Figure 4. 13)** was isolated as an orange amorphous material. The mass spectrum showed a molecular ion peak at *m/z* 362.20451 g. mol^{-1} corresponding to a molecular formula of $C_{20}H_{28}NO_5$. It was found to be a new alkaloid that belonged to the 5,10b-ethano bridge type of alkaloids and was found to the *N*-methyl derivative of delagoenine.

The substitution pattern on the A ring was similar to the previous compounds isolated with the presence of only two *para* orientated aromatic protons (δ_H 6.63 and 6.64). However, the methylenedioxy group at C-8 and C-9 was absent. The C-8 and C-9 resonances showed ^3J HMBC correlations to two methoxy group proton resonances and these were placed at C-8 and C-9. A characteristic feature in the ^{1}H NMR spectrum of the 5,10b-ethano bridge type alkaloid is the AB splitting pattern of the H-6 protons which occurred at δ_H 4.59, (d, 15.6 Hz) and δ_H 4.71 (d, 15.6 Hz) [10] and the H-4a resonance at δ_H 3.65 (dd, 5.2, 12.4 Hz). The C-4a and C-6 resonances showed a ^3J HMBC correlation to the methyl group proton resonance at δ_H 3.08 (s). This methyl group was therefore assigned to be a *N*-methyl group. Further correlations from C-6 were seen to a resonance at δ_H 5.98 (brd, 5.9 Hz) assigned to H-12. The H-12 resonance showed a correlation in the COSY spectrum to the methylene protons at δ_H 2.55 (dd, 6.7, 13.5 Hz) and 2.19 (brs) which were assigned to 2H-11.

Assignment of the ring C protons was done with the aid of the COSY spectrum. The methylene group proton resonances at δ_H 2.29 (brd, 13.5 Hz) and 1.92 (m) showed correlations to H-4a (δ_H 3.65) and were assigned to 2H-4. The methylene group proton resonances showed further correlations to the resonance at δ_H 5.18 (brs), assigned to H-3, which, in turn, showed correlations to the two H-2 methylene protons (δ_H 1.78 (brd, 14.1 Hz) and 1.92 (m)), which showed further correlations to the 2H-1 (δ_H 2.21 (m)) resonance. The acetoxy group was placed at C-3.

Circular dichroism spectroscopy was used to determine the absolute configuration of the 5, 10b-ethano bridge. A positive Cotton effect was observed at 259 nm and a negative Cotton effect at 280 nm, which indicated the bridge was of the β configuration since negative Cotton effects are typically observed at 280 nm for the β orientated series.[10-12,17,18]

The infrared spectrum showed a carbonyl group with a peak at 1734 cm^{-1}. Other IR resonances occurred at 2956 cm^{-1} and 2848 cm^{-1} (C-H stretching), 1515 cm^{-1} (aromatic C=C stretching) and 1262 cm^{-1} (C-N stretching).

A literature search for the compound identified it has being the *N*-methyl derivative of the known delagoenine which was previous isolated from *C. stuhlmannii*.[5] The HMBC, COSY and NOESY data are shown in **Table 4.11** together with the ^{13}C NMR data of *N*-methyldelagoenine.

Table 4.11: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for *N*-methyldelagoenine ($CDCl_3$)

Carbon	δ_H	δ_C	δ_C^*	HMBC (C→H)	COSY	NOESY
1	2.21 (m)[a]	23.86[b]	23.3	2α,β;11α,β	2α,β	10
2a	1.78 (brd, 14.1 Hz)	23.77[b]	24.7		1;3	2b;3
2b	1.92 (m)[a]				1;3	2a;3
3	5.18 (brs)	67.48	69.8		2;4α,β	2a,b;4β
4α	2.29 (brd, 13.5 Hz)	26.65	30.3	4a	3;4a	3;4a;4β
4β	1.92 (m)[a]				3.4a	3;4β
4a	3.65 (dd, 5.2, 12,4 Hz)	68.17	62.5	4α,β;6α,β;*N*-CH_3	4α,β	3;4α
6α	4.59 (d, 15.6 Hz)	64.11	56.9	4a;7;*N*-CH_3	6β	4a;6β;7
6β	4.71 (d, 15.6 Hz)				6α	6α;7
6a		118.38	121.4	6α,β;10;*N*-CH_3		
7	6.64 (s)[d]	108.66[d]	108.9	6α,β		6α,β;8-OCH_3
8		148.78[c]	148.2	6α;8-OCH_3;10		
9		148.57[c]	148.1	7;9-OCH_3		
10	6.63 (s)[d]	105.74[d]	106.2			1;11_{endo};9-OCH_3
10a	—	134.45	139.0	4a;6α,β;7;11α,β		
10b		42.28	44.3	4α,β; 4a; 10		
11_{exo}	2.55 (dd,6.7,13.5 Hz)	44.10	46.2	4a;6α;12	11_{endo};12	11_{endo}
11_{endo}	2.19 (m)				11_{exo};12	10;11_{exo}
12	5.98 (d, 5.9 Hz)	~109[f]	93.8	11α,4a;*N*-CH_3	$11_{exo,endo}$	
8-OCH_3*	3.75 (s)[e]	56.00[e]	56.0			7
9-OCH_3*	3.79 (s)[e]	55.90[e]	56.0			10
3-CO$\underline{CH_3}$	1.93 (s)	21.07	21.2			
3-$\underline{CO}CH_3$		170.07	170.2	3-CO$\underline{CH_3}$		
N-CH_3	3.08 (s)	41.66		6α		

* Carbon value for delagoenine in $CDCl_3$.[5]

[a] Resonances obscured. Accordingly, coupling constants could not be determined.

[b,c,d,e] ^{1}H and ^{13}C NMR resonance values interchangeable.

[f] Exact value could not be determined as resonance was weak.

4.2.10 Structural Elucidation of Compound for XIV, Stuhlmanine D (spectra 14.a-j)

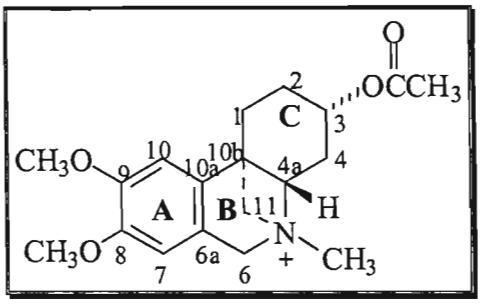


Figure 4. 14 Structure of compound XIV, stuhlmanine D

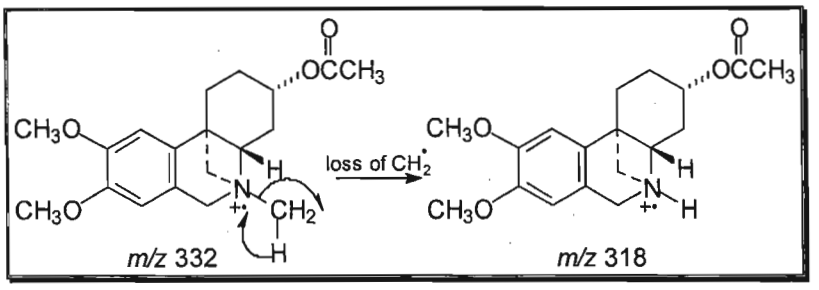
Compound **XIV (Figure 4. 14)** was isolated as an orange amorphous compound and was found to be a novel structure. It was found to have a 5,10b-methano bridge instead of the 5,10b-ethano bridge, and this is the first reported occurrence of this type of bridge in the crinamine and haemanthamine type alkaloids. The GC-LC-MS gave a highest peak at 332 g. mol^{-1}. However, the expected [M^+] peak at *m/z* 332.186184 for $C_{19}H_{26}NO_3$ (suggested by the NMR spectra) could not be observed in the CI HRMS, which did, however show the [$M-CH_2$]$^+$ peak at *m/z* 318.17176 (calc. for [$C_{18}H_{24}NO_4$]$^+$ 318.170533).

The substitution pattern on the A ring was the same as the previous compound (compound **XIII**) isolated with only two *para* orientated aromatic protons (δ_H 6.52 and 6.77) and methoxy groups at C-8 and C-9. The characteristic AB splitting pattern resonances of the 2H-6 protons which occurred at δ_H 3.84 (m) and δ_H 4.02 (d, 15.1 Hz) [10] and the H-4a resonance at δ_H 2.88 (dd, 4.2, 13.2 Hz) were present in this molecule. This compound also had an *N*-methyl group (δ_H 2.45 (s). The C-4a and C-6 resonances showed a ^3J HMBC correlation to the methyl group proton resonance at δ_H 2.45 (s) confirming its presence.

Assignment of ring C was done with the aid of the COSY spectrum. The H-4a (δ_H 2.88) resonance showed correlations to the methylene group proton resonances at δ_H 1.75 (d, 2.2, 12.2 Hz) and 2.09 (m) ascribable to 2H-4. These methylene group proton resonances showed further correlations to the resonance at δ_H 5.22 (brs) assigned to H-3, which, in turn, showed correlations to the methylene group protons of H-2 (δ_H 1.81 (m) and 1.93 (dd, 1.7, 9.6 Hz)), which further showed correlations to 2H-1 (δ_H 1.81 (m) and 2.13 (m)). The acetoxy group was placed at C-3. The carbon resonances for rings A, B and C were assigned using the HMBC, COSY and NOESY spectra and this left one unassigned carbon resonance at δ_C 47.78. The C-10a resonance showed a ^3J HMBC correlation to the methylene protons of this unassigned carbon, at δ_H 2.18

(dd, 2.2, 14.1 Hz) and δ_H 2.74 (dd, 6.3, 14.1 Hz), and were thus assigned to 2H-11. The appearance of a double doublet resonance for H-11 could be explained by it undergoing W coupling to both H-1 and N-C<u>H</u>$_3$. The protons corresponded to the carbon resonance at δ_C 47.78 in the HSQC spectrum. This resonance, in turn, showed a ^3J HMBC correlation to the resonances at 2H-1 confirming the assignment.

The mass spectrum supported the proposed structure. The base peak at *m/z* 258 could result from the loss of a CH_2 fragment (**Scheme 4. 1**) and subsequent loss of a unit of acetic acid (60 mass units)



Scheme 4. 1 Fragmentation pattern of compound XIV

Circular dichroism spectroscopy was used to determine the absolute configuration of the 5, 10b-ethano bridge. A positive Cotton effect was observed at 292 nm and a negative cotton effect at 247 nm, which indicated the bridge was of the α configuration since negative Cotton effects are typically observed at approximately 250 nm for the α orientated series.[10-12,17,18] Although the bridge was not an ethano bridge the stereochemistry of the bridge was still assigned by comparison, in a manner similar to that used in the haemanthamine and crinane series.[10-12] The stereochemistry of H-4a was therefore assigned to be β. This H-4a resonance showed a correlation in the NOESY spectrum to the H-3 proton resonance, implying H-3 to be β. Therefore the acetoxy group substituent on C-3 was α.

The infra-red spectrum showed a carbonyl group with a peak at 1724 cm^{-1}. Other IR resonances were 3373 cm^{-1} (O-H stretching), 2925 and 2854 cm^{-1} (C-H stretching) and 1274 cm^{-1}(C-N stretching).

A literature search for the compound indicated that it was isolated for the first time and it was named stulhmanine D. The HMBC, COSY and NOESY data are shown in **Table 4.12.**

Table 4.12: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for stulhmanine D ($CDCl_3$)

CARBON	δ_H	δ_C	HMBC (C→H)	COSY	NOESY
1α	2.13 (m)[a]	27.63		1β;2α,β	1α;10
1β	1.81 (m)[c]			1α;2α,β	1β;2α;4a;4*β*
2α	1.81 (m)[a]	24.77		1α,β;3	2*β*;3
2β	1.93 (dd, 1.7, 9.6 Hz)			1α,β;3	1*β*;2*α*
3	5.22 (brs)	68.48	1*α*	2α,β;4α,β	1*β*;2β;4β
4α	1.75 (dd, 2.2, 12.2 Hz)	28.00[b]		3;4a	3;4*β*;11_{exo}
4β	2.09 (m)			3;4a	1*β*;3;4*α*;4a;*N*-CH_3
4a	2.88 (dd, 4.2, 13.2 Hz)	~61[d]	1*α*;11_{exo};*N*-CH_3	4α,β	1α;6*β*;4*β*
6α	4.02 (d, 15.1 Hz)	60.98	7;*N*-CH_3	6β	6*β*;7
6β	3.84 (d, 15.1 Hz)			6α	4a;6*α*
6a		~124[d]	10		
7	6.52 (s)	108.46	-		6*α*;8-OCH_3
8		148.38	10;8-OCH_3		
9		148.24	7; 9-OCH_3		
10	6.77 (s)	107.02	-		1*α*;11_{exo} ;9-OCH_3
10a		134.11	7,$11_{exo,\ endo}$		
10b		42.86	10;1α,β;$11_{exo,\ endo}$		
11_{endo}	2.18 (dd, 2.2, 14.1 Hz)	47.78	1*β*	11β	10
11_{exo}	2.74 (dd, 6.3, 14.1 Hz)			11α	4*α*
N-CH_3	2.45 (s)	39.50			4β
8-OCH_3	3.84 (s)	56.05[b]			7
9-OCH_3	3.84 (s)	55.92[b]			10
3-CO$\underline{CH_3}$	1.99 (s)	21.29			
3-$\underline{CO}CH_3$		170.16	3-CO$\underline{CH_3}$		

[a] Obscured.

[b] ^{13}C NMR resonance values interchangeable.

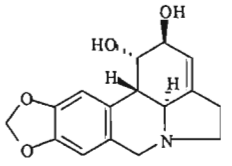
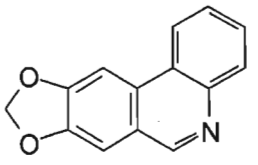
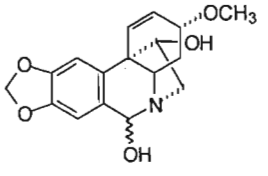
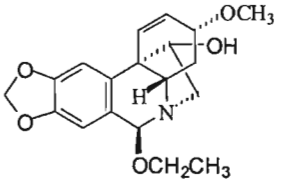
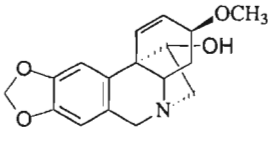
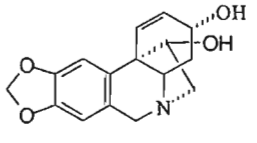
[c] Resonances overlap.

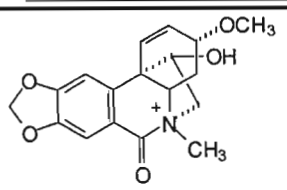
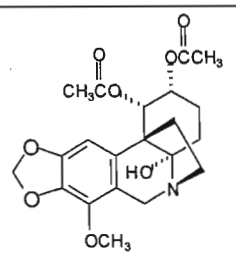
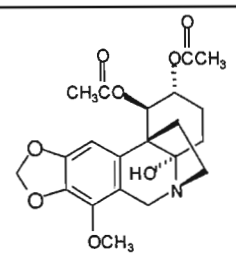
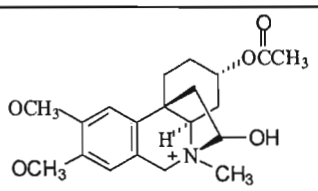
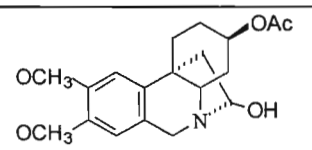
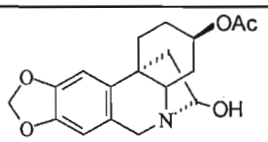
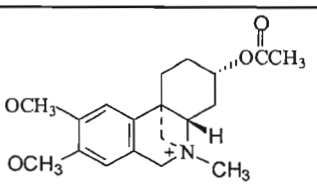
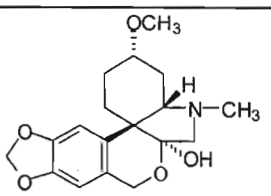
[d] Exact carbon values could not be determined as resonances were of low intensity/broadened.

4.3 Comparison of Bulbs and Seed Chemistry of *Crinum stuhlmannii*

The different compounds isolated from the bulbs and seeds of *Crinum stuhlmannii* during this and the previous study (under the name *Crinum delagoense*)[5] are shown in **Table 4.13**.

Table 4.13: The different chemical constituents isolated from the seeds and bulbs of *Crinum stuhlmannii*

Structures of Compounds isolated from *Crinum stuhlmannii*	Source and Report		
	Bulbs-current	**Bulbs-previous[5]**	**Seeds-current**
	+	+	-
	+	-	-
	+	+	+
	+	-	-
	-	-	+
	-	+	+

	+	-	-
	+	-	-
	+	-	-
	+	-	-
	-	+	-
	-	+	-
	+	-	-
	-	+	-

6-Hydroxycrinamine was the only common chemical constituent isolated from the seeds, and both studies of the bulbs. Lycorine was the only other common constituent found in both bulbs studied. However, Nair *et al.*[5] isolated a novel alkaloid named delagoenine in their study of the bulbs whilst in the current study the *N*-methyl derivative of delagoenine (Compound **XIII**) was found. This indicates that the bulbs studied previously were possibly *C. stuhlmannii* although the production of delagoenine and its derivatives may yet prove not to be restricted to the genus *Crinum*. Hamayne which was isolated in the previous study by Nair *et al.*[5] was isolated from the seeds of *C. stuhlmannii*. The only common constituent isolated in current studies of the bulbs and seeds of *C. stuhlmannii* was 6-hydroxy crinamine and this was isolated in fairly large yields from both bulbs and seeds. None of the novel alkaloids (stuhlmanine A, B, C, D or *N*-methyldelagoenine) were isolated from both seeds and bulbs which indicate that these chemical constituents may be organ-specific.

4.4 Experimental

The plant material of *Crinum stuhlmannii* Baker was collected in Maputaland near Kosi Bay and a voucher specimen was retained (*N. Crouch 953*, NH). The parts of the plant investigated were the bulbs and seeds. General chromatographic techniques were employed **(Chapter 3)**.

The seeds (1.1 kg) were dried, chopped into smaller pieces and extracted with ethanol by agitation on a Labcon Mechanical shaker at 140 rpm. The extract obtained was then filtered and the solvent was removed under reduced pressure 8.2 g of ethanol extract was obtained. The extract was dissolved in water and lowered to a pH of 2 where it was extracted with 3 x 200ml with chloroform. This was called the acidic extract (4.6 g). The pH of the original extract was raised to a pH of 10 and again extracted with 3 x 200 mL chloroform. This was called the basic extract (1.2 g). Both the acidic and basic extracts were investigated.

The acidic extract of the seeds was loaded onto a 5 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-34; 1% methanol in dichloromethane fractions 35-61; 2% methanol in dichloromethane fractions 61-74; 5% methanol in dichloromethane fractions 75-99; 10% methanol in dichloromethane fractions 100-116; 20% methanol in dichloromethane fractions 117-129). Purification of fraction 39 using a 100% dichloromethane solvent system afforded compound **VII** at a yield of 0.6% (23 mg). Purification of fractions 82-83 using a 2% methanol in dichloromethane solvent system afforded compound **IX** at a yield of 0.2% (12.3 mg). Purification of fractions 75-79 using 2% methanol in dichloromethane afforded compound **X** at a yield of 0.1% (7.3 mg).

The basic extract of the seeds was loaded onto a 2 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-18; 1% methanol in dichloromethane fractions 19-31; 5% methanol in dichloromethane fractions 32-53; 10% methanol in dichloromethane fractions 54-73; 20% methanol in dichloromethane fractions 74-91). Although TLC analysis indicated interesting spots, the constituents present were in very low amounts and no further work could be done.

The bulbs (3.5 kg) were dried, chopped into smaller pieces and extracted with ethanol by agitation on a Labcon Mechanical shaker at 140 rpm. The extract obtained was then filtered and the solvent was removed under reduced pressure. 37.27 g of ethanol extract was obtained. The extract was dissolved in water and lowered to a pH of 2 where it was extracted with 3 x 200ml with chloroform. This was called the acidic extract (mass = 4.5 g). The pH of the original extract was raised to a pH of 10 and again extracted with 3 x 200 mL chloroform. This was called the basic extract (3.3 g). Both the acidic and basic extracts were investigated.

The acidic extract of the bulbs was loaded onto a 5 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-50; 1% methanol in dichloromethane fractions 51-70; 2% methanol in dichloromethane fractions 71-97; 5% methanol in dichloromethane fractions 98-127; 10% methanol in dichloromethane fractions 128-160; 20% methanol in dichloromethane fractions 161-174). The acidic extract was dissolved in chloroform and filtered. This afforded compound **V** at a yield of 3% (120.7 mg). Fractions 22-27 were combined and loaded onto a 1 cm column and eluted with a 100% dichloromethane solvent system to afford compound **VI** at a yield of 0.2% (10.3 mg). Fractions 83-84 were combined and purified using a 2% methanol in dichloromethane solvent system. This afforded compound **VIII**, at a yield of 0.3% (12.3 mg). Purification of fractions 100-110 using 2% methanol in dichloromethane afforded compound **XII** at a yield of 0.2% (10.7 mg). Purification of fractions 120-127 using a 5% methanol in dichloromethane solvent system afforded compound **XIII** at a yield of 0.4% (15.6 mg).

The basic extract of the bulbs was loaded onto a 2 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-23; 1% methanol in dichloromethane fractions 24-43; 2% methanol in dichloromethane fractions 44-66; 5% methanol in dichloromethane fractions 67-110; 10% methanol in dichloromethane fractions 111-140; 20% methanol in dichloromethane fractions 141-179). Compound **VII** was obtained in pure form from fractions 74-75 at a yield of 1% (44 mg). Purification of fraction 119-121 using a 10% methanol in dichloromethane solvent system afforded compound **XI**, at a yield

of 0.2% (12.2 mg). Purification of fraction 98 using a 5% methanol in dichloromethane solvent system afforded compound **XIV**, at a yield of 0.4% (20 mg).

4.4.1 Physical data for Compound V

Name: **lycorine**

Yield: 120.7 mg

Physical description: white crystalline

Melting Point: 244-247°C (lit. 251-254°C)[12]

Optical rotation: -60.32 (0.202 g in 100 ml methanol) (lit. -62° (methanol, 0.1))[7]

Mass: GC-MS: [M^+] at 287, $C_{16}H_{17}NO_4$ requires 287.115758 g. mol^{-1}

EIMS: 287, 268, 250, 227, 226, 147

Infrared: ν_{max}^{NaCl} cm^{-1}: 3350 (O-H stretching), 2926 (C-H stretching), 2852 (C-H stretching), 1603 and 1480 (aromatic methylenedioxy)

^1H NMR: δ_H (ppm) C_5D_5N: **Table 4.1**

^{13}C NMR: δ_c (ppm) C_5D_5N: **Table 4.1**

4.4.2 Physical data for Compound VI

Name: **8,9-methylenedioxophenanthridine**

Yield: 10.3 mg

Physical description: yellow needle-like crystals

Melting Point: 138-140°C (Lit. 138-139°C)[9]

Optical rotation: +6.25 (*c*, 0.104 methanol) (value not given in lit.)[9]

Mass spectrum: GC-MS: [M^+] at 223, $C_{14}H_9NO_2$ requires 223.63329 g. mol^{-1}

EIMS m/z: 223, 222, 164, 138, 137, 111

Infrared: ν_{max}^{NaCl} cm^{-1}: 1477 (aromatic C=C stretching) and 1262 (C-N stretching)

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 4.3**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 4.3**

4.4.3 Physical data for Compound VII

Name: **6-hydroxycrinamine**

Yield: 67 mg (44 mg from bulbs and 23 mg from seeds)

Physical description: white crystals

Melting Point: 142-144°C (lit. 150-152°C)[10]

Optical Rotation: +94.50 (c, 0.300 in methanol) (lit. 40° (*c*, 0.5 in methanol))[10]

Mass spectrum: GC-MS: $[M^+]$ 317, at $C_{17}H_{19}NO_5$ requires 317.126323 g. mol^{-1}

EIMS *m/z*: 317, 271, 270, 211, 181, 153

CD nm: 492, 477, 447, 430, 410, 372, 360, 345, 329, 302, 277, 252, 230, 217

Infrared: ν_{max}^{NaCl} cm^{-1}: 3410 (O-H stretching), 2893 and 2821 (C-H stretching), 1488 (aromatic C=C stretching), 1248 (C-N stretching) and 1042 (C-O stretching).

^1H NMR: δ_H (ppm) CD_3OD: **Table 4.4**

^{13}C NMR: δ_c (ppm) CD_3OD: **Table 4.4**

4.4.4 Physical data for Compound VIII

Name: **6β-ethoxycrinamine**

Yield: 12.3 mg

Physical description: brown amorphous material

Optical rotation: +16.89 (*c*, 0.074 g in methanol)

Mass spectrum: CIMS: $[M^+]$ not seen, $C_{19}H_{23}NO_5$ requires 345.15623 g. mol^{-1}

EIMS *m/z*: 346 $[M+H]^+$, 328, 314, 300, 268, 254, 240, 224, 149, 123, 109, 97, 83.

CD nm: 489, 467, 456, 443, 409, 375, 330, 290, 224

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

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 4.5**

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

4.4.5 Physical data for Compound IX

Name: **hamaenthamine**

Yield: 12.3 mg

Physical description: yellow amorphous material

Optical rotation: +48.00 (*c*, 0.06 methanol)

Mass spectrum: GC-MS: $[M^+]$ at *m/z* 301, $C_{17}H_{19}NO_4$ requires 301.131408 g. mol^{-1}

EIMS: 272, 240, 181, 153, 128, 115

CD nm: 469, 453, 431, 388, 359, 343, 326, 308, 291, 272, 254, 236, 212

Infrared: ν_{max}^{NaCl} cm^{-1}: 2928 (C-H stretching), 1438 (aromatic C=C stretching), 1239 (C-N stretching)

^1H NMR: δ_H (ppm) CD_3OD: **Table 4.6**

^{13}C NMR: δ_c (ppm) CD_3OD: **Table 4.6**

4.4.6 Physical data for Compound X

Name: **hamayne**

Yield: 7.3 mg

Physical description: brown amorphous material

Optical rotation: the yield of sample was too low to obtain a stable reading

Mass spectrum: GC-MS: $[M^+]$ at *m/z* 287, $C_{16}H_{17}NO_4$ requires 287.115758 g. mol^{-1}

EIMS *m/z*: 287, 215, 185, 149, 115

CD nm: 491, 462, 443, 429, 405, 374, 337, 294, 264, 239, 217

Infrared: ν_{max}^{NaCl} cm^{-1}: 3349 cm^{-1} (O-H stretching), 2922 cm^{-1} (C-H stretching), 1565 cm^{-1} (C=C stretching) and 1265 cm^{-1} (C-N stretching).

^1H NMR: δ_H (ppm) CD_3OD: **Table 4.7**

^{13}C NMR: δ_c (ppm) CD_3OD: **Table 4.7**

4.4.7 Physical data for Compound XI

Name: **stuhlmanine A, *N*-methyl-3-*epi*-crin-6-amide**

Yield: 12.2 mg

Physical description: brown amorphous gum

Optical rotation: -7.80 (*c*, 0.186 in methanol)

Mass spectrum: HRMS: $[M^+]$ at *m/z* 330.13415, $C_{18}H_{20}NO_5$ requires 330.134148 g. mol^{-1}

EIMS *m/z*: 330, 329, 299, 279, 256, 216, 149, 129, 107

CD: 476, 454, 414, 350, 319, 279, 258, 234, 217

Infrared: ν_{max}^{NaCl} cm^{-1}: 3373 (O-H stretching), 2923 and 2855 (C-H stretching), 1727 (C=O stretching), 1479 (aromatic C=C stretching) and 1281 (C-N stretching).

^{1}H NMR: δ_H (ppm) CD_3OD: **Table 4.8**

^{13}C NMR: δ_c (ppm) CD_3OD: **Table 4.8**

4.4.8 Physical data for Compound XIIA and XIIB

Name: **stuhlmanines B, 4a-hydroxy-1,2-di-*epi*-bowdensine; stuhlmanine C, 4a-hydroxy-2-*epi*-bowdensine**

Yield: 10.7 mg

Physical description: yellow amorphous material

Optical rotation: +36.36 (c, 0.11g in methanol)

Mass spectrum: HRMS: [M^+] at *m/z* 419.15897, $C_{21}H_{25}NO_8$ requires 419.158017 g. mol^{-1}

EIMS *m/z*: 419, 359, 316, 291, 273, 216, 204

CD nm: 447, 432, 400, 374, 342, 303, 276, 254, 235, 225

Infrared: ν_{max}^{NaCl} cm^{-1}: 3380 (O-H stretching), 2941 (C-H stretching), 1740 (C=O stretching), 1482 (aromatic C=C stretching) and 1260 (C-N stretching)

^{1}H NMR: δ_H (ppm) $CDCl_3$: **Tables 4.9 and 4.10**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Tables 4.9 and 4.10**

4.4.9 Physical data for Compound XIII

Name: ***N*-Methyldelagoenine**

Yield: 15.6 mg

Physical description: orange amorphous material

Optical rotation: +93.09 (*c,* 0.362 in methanol)

Mass spectrum: HRMS: [M^+] at *m/z* 362.20451, $C_{20}H_{28}NO_5$ requires 362.196748 g. mol^{-1}

EIMS *m/z*: 362, 333, 318, 308, 274, 258, 242, 218, 179, 130. 129. 68, 35.

CD nm: 488, 456, 405, 372, 338, 319, 299, 280, 259

Infrared: ν_{max}^{NaCl} cm^{-1}: 2956 and 2848 (C-H stretching), 1734 (C=O stretching), 1515 (C=C stretching) and 1262 (C-N stretching).

^{1}H NMR: δ_H (ppm) $CDCl_3$: **Table 4.11**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 4.11**

4.4.10 Physical data for Compound XIV

Name: **stuhlmanine D**

Yield: 15.6 mg

Physical description: orange amorphous material

Optical rotation: -55.51 (c, 0.118 in methanol)

Mass spectrum: HRMS: [M^+] not found, $C_{19}H_{26}NO_4$ requires 332.186184 g. mol^{-1}

EIMS *m/z*: 318 $[M\text{-}CH_2]^+$, 258, 218, 179, 43

CD nm: 479, 286, 451, 404, 373, 336, 319, 292, 269, 248, 227

Infrared: ν_{max}^{NaCl} cm^{-1}: 2946 (C-H stretching), 1729 (C=O stretching), 1516 (C=C stretching) and 1255 (C-N stretching).

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 4.12**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 4.12**

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Chapter 5: The Classification and Biosynthesis of Homoisoflavanones and Bufadienolides

5.1 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.[1,2] The other major region of Hyacinthaceae diversity stretches from the Mediterranean to South West Asia.[3]

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 was studied.

Although great difficulties in phylogenetically systematizing the genera have been acknowledged, a recent arrangement has been proposed.[1-3] 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 revoluta* (L.*f.)* Jessop and *Drimia macrocentra* (Baker) Jessop (Urgineoideae) were studied.

The two major classes of compounds isolated from this family during this work were homoisoflavanones and bufadienolides. The biosynthesis of these compounds will be discussed in this chapter.

5.2 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 5. 1)** using a 3-benzyl-4-chromanone type **(1)** and a scillascillin type **(2)** as examples.

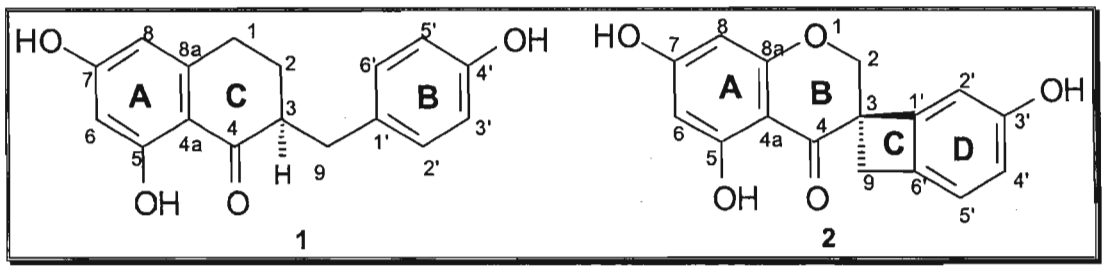


Figure 5. 1 The numbering system for homoisoflavanones

Homoisoflavanones belong to a small family of natural products whose first member was isolated from the bulbs of *Eucomis bicolor* Bak.[4] They are restricted in their distribution, having been previously reported to occur mainly in taxa of the Hyacinthaceae.[5-13] However, these homoisoflavanones have also been reported to occur outside this family. Intricatin and intricatinol are two homoisoflavanones isolated from the Fabaceae family **(Section 5.6)**. Until recently homoisoflavanones were thought to be present in only the Hyacinthoideae of the Hyacinthaceae.[5-8] However, they have very recently been reported from the Ornithogaloideae.[50]

Homoisoflavanones, which are more commonly referred to as homoisoflavonoids, belong to a class of naturally occurring oxygen heterocycles.[14] The term homoisoflavonoid is actually incorrect since these compounds do not undergo the characteristic 2,3-aryl migration of the C_6-C_3-C_6 moiety that is common in the biosynthesis of isoflavonoids.[13] Also, they have a sixteen-carbon skeleton instead of a fifteen-carbon skeleton characteristic of isoflavonoids.[15] The correct systematic name is 3-benzyl-4-chromanones. The spectroscopic behavior of the homoisoflavanones resembles very closely that of flavonoids.[16] 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.[18] The homoisoflavanones can generally be classified into three types based on structural features:[14,16,18]

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

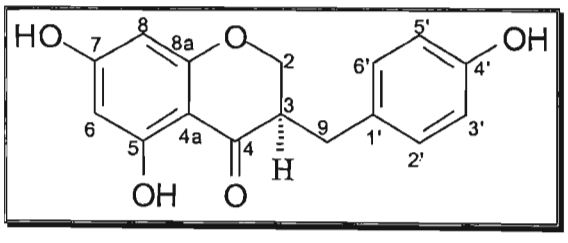


Figure 5. 2 A homoisoflavanone of the 3-benzyl-4-chromanone type

This type of homoisoflavanone **(Figure 5. 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_H 4.1-4.3 ppm [AB of ABX, 2H-2], one multiplet in the

range δ_H 2.7-2.9 ppm [H-3][†] and two double doublets in the range δ_H 2.6-2.7 ppm and δ_H 3.1-3.3 ppm [2H-9] are indicative of this arrangement.

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

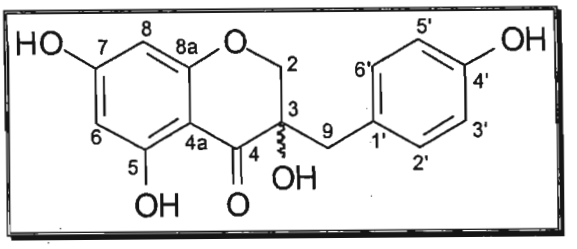


Figure 5. 3 A homoisoflavanone of the 3-benzyl-3-hydroxy-4-chromanone type

These compounds **(Figure 5. 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_H 4.20-4.4 ppm and δ_H 4.1-4.2 ppm and another at δ_H 3.1-3.2 ppm and δ_H 2.7-2.8 ppm for the protons at C-9.[15]

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

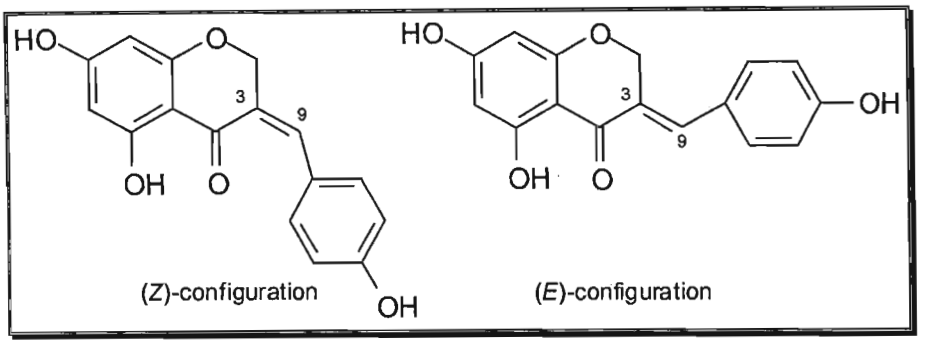


Figure 5. 4 A homoisoflavanone of the 3-benzylidene-4-chromanone type

These compounds **(Figure 5. 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 the 2H-2 resonances and a singlet for the H-9 resonance. The pair of doublets for 2H-2 are found to resonate at approximately δ_H 5.2-5.5 ppm while the position of the H-9 resonance 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_H 5.5 ppm. In the (*E*)-configuration the proton at C-9 is in the anisotropic region of the carbonyl group and causes it to resonate at δ_H 7.6-7.9 ppm.[15]

[†] The absolute stereochemistry can be determined using CD spectra and is discussed in Chapter 6

Besides these three basic structural types, a number of unusual compounds which contain a fourth ring **(Figure 5. 5)**, have been isolated.[14,18] These compounds are thought to be biogenetically related to homoisoflavanones and are classified with this group.[13,14,16] Scillascillin type compounds possess a unique 3-spiro-cyclobutane ring sytem.[16,18] 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.[13]

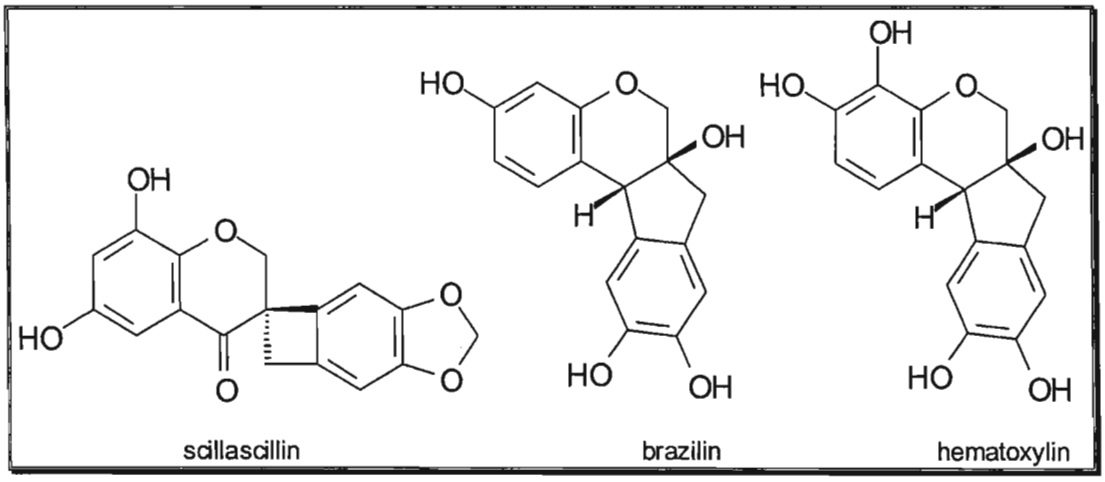


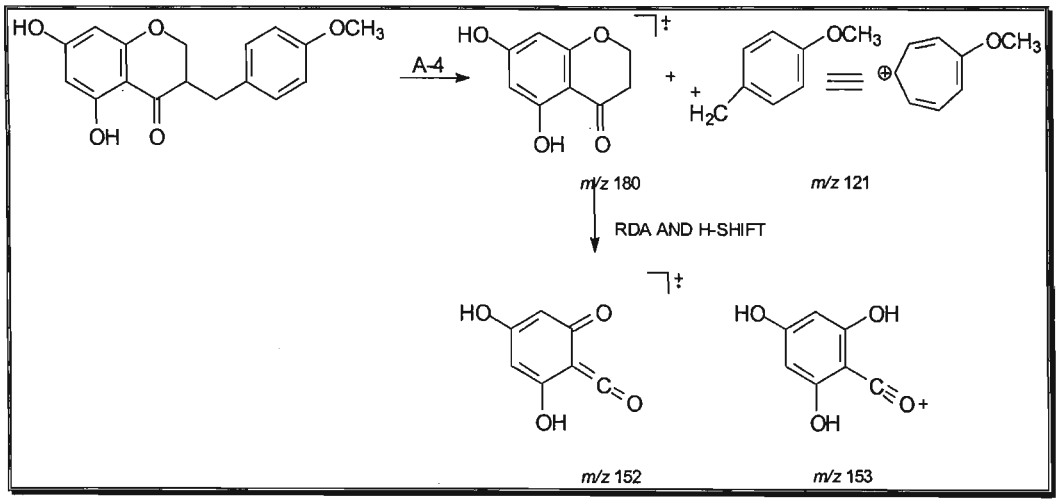
Figure 5. 5 Homoisoflavanones of the scillascillin, brazilin and hematoxylin types

The ^{1}H NMR spectra of homoisoflavanones also show 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_H 6.50-7.20 ppm generally indicate the substitution pattern on the B-ring. This is usually either an ABX or an AA'BB' system. The substituents present are attached to C-4' (AA'BB' system) giving a pair of doublets, each integrating to two protons, or C-3' and C-4' (ABX system) where the proton resonances of C-1', C-5' and C-6' all appear as double doublets.

The region between δ_H 5.80-6.30 ppm indicates the substitution pattern on the A-ring. There are usually only one or two proton resonances in this region which are due to H-6 and/or H-8. The position of the protons is normally assigned by using the NOESY spectrum.

The UV spectrum is another tool one can use in determining the substitution pattern of the A ring. When $AlCl_3$ is added to a homoisoflavanone containing a hydroxy group at C-5, one observes a bathochromic shift in the UV spectrum.[5,7] Similarly if NaOAc is added to a homoisoflavanone containing a hydroxy group at C-7, there is a bathochromic shift in the UV spectrum.[5,7]

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.[16] In dihydroeucomin this leads to fragment ions at *m/z* 180 and *m/z* 121 as shown in **Scheme 5. 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.[16] 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.[16] The major pathway is the RDA cleavage and H-shift.[16] The minor pathway is usually the subsequent loss of water, CO and methyl from the molecular ion.[16]



Scheme 5. 1 Fragmentation pattern of homoisoflavanones[16]

5.3 The Biosynthesis of Homoisoflavanones

Phenolic compounds can be biosynthesized *via* the shikimate pathway or polyketide pathway.[19] 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 5. 6)**.[20-22]

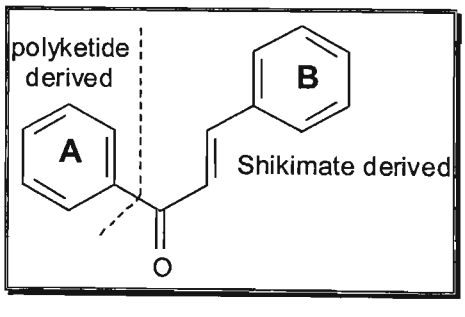


Figure 5. 6 The origin of the A and B rings

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.[13,15,16] The precursors to chalcones are three malonyl CoA units and *p*-coumaroyl CoA.[20-22]

Malonyl-CoA is synthesized from acetyl-CoA and carbon dioxide.[20-22] The reaction, which is shown below is catalysed by acetyl-CoA carboxylase in the presence of adenosine 5'-triphosphate (ATP) and Mg^{2+}.[20-22]

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.[20-22] The precursors of the shikimic acid pathway, erythrose-4-phosphate and phosphoenolpyruvate, are primary metabolites of carbohydrate metabolism.[20-22]

The first step in the formation of the precursor is the deamination of phenylalanine **(Scheme 5. 2)**, which is catalyzed by the enzyme phenylalanine ammonia lyase (PAL).[20-22] PAL catalyses the *anti*-elimination of ammonia and the (pro-3s)-proton from L-phenylalanine to yield *trans*-cinnamic acid.[20-22] Cinnamate-4-hydroxylase (C4H) catalyses the introduction of a hydroxy group at position 4 of the *trans*-cinnamic acid.[20-22] This reaction requires NADPH and molecular oxygen and exhibits properties characteristic of those catalysed by plant P450 enzymes.[20-22] This reaction mechanism involves a H-shift. The proton at 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.[20-22] **(Figure 5.7)**

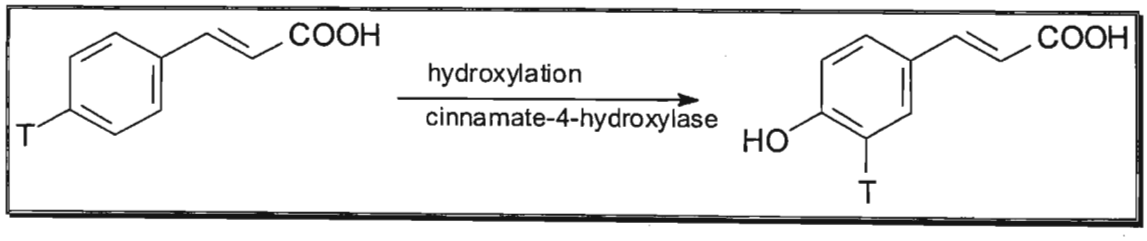
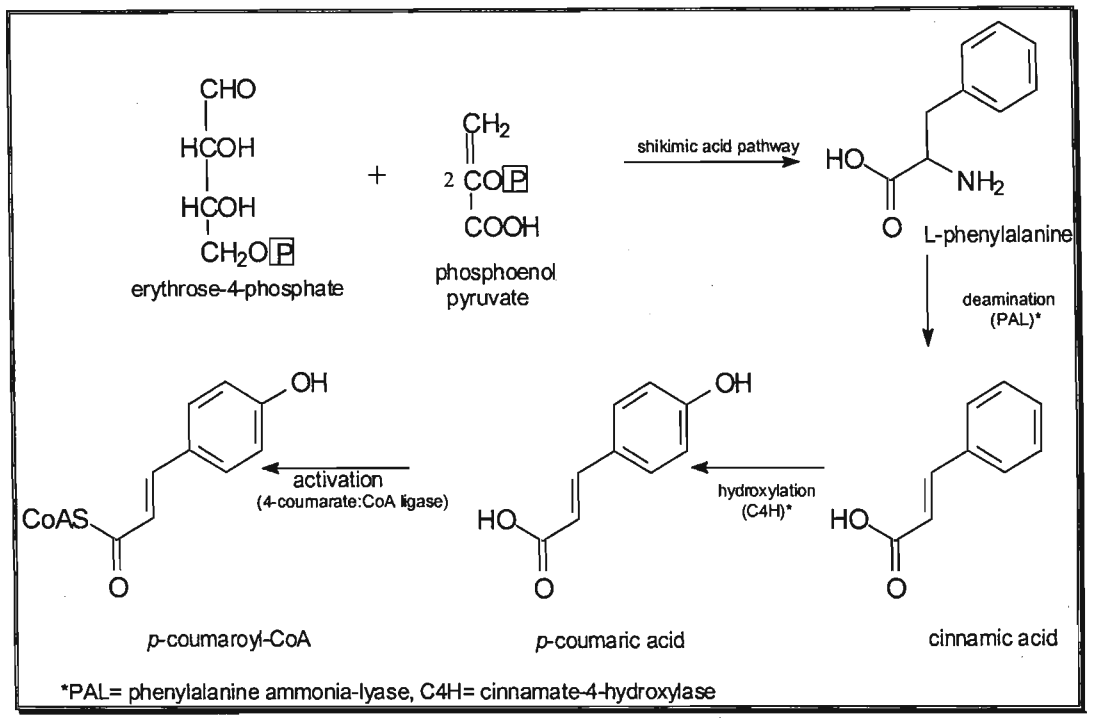


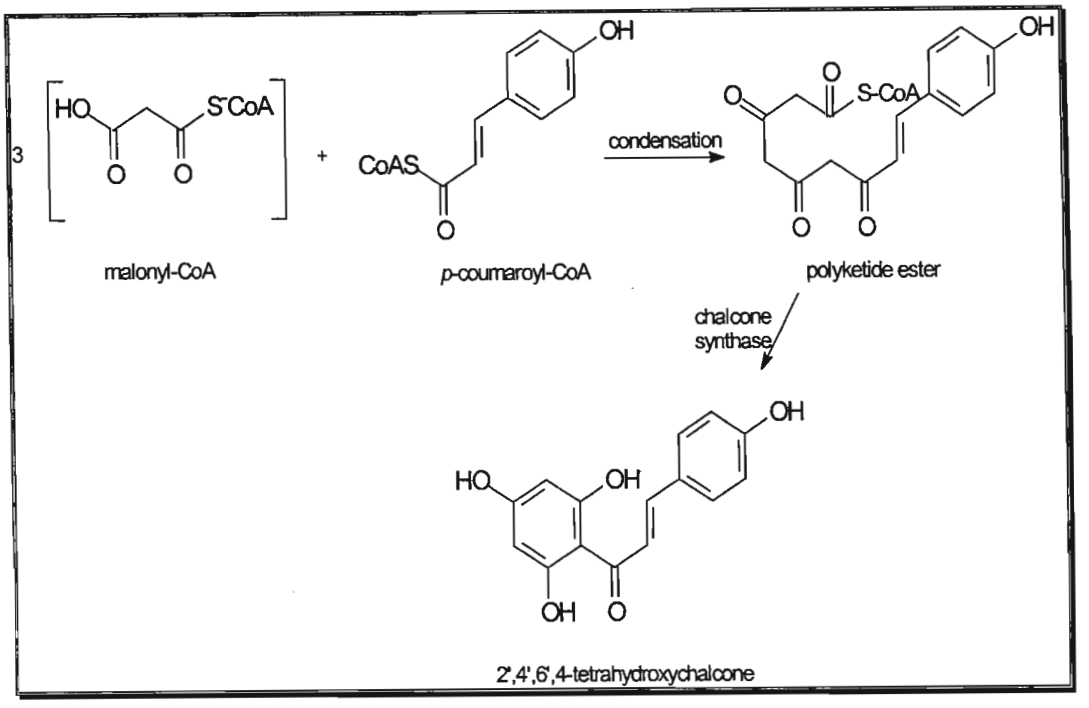
Figure 5. 7 The NIH shift for the hydroxylation of cinnamic acid[20-22]

The *p*-coumaric acid is activated by the formation of a CoA ester, which is catalysed by 4-coumarate: CoA ligase.[20-22] This reaction which requires ATP and Mg^{2+} as cofactors, proceeds *via* an acyl-AMP intermediate, characterizing the enzyme as a synthase.[20-22]



Scheme 5. 2 The biosynthesis of the intermediate p-coumaroyl CoA[20-22]

The formation of the chalcone is common to all classes of flavonoids and is thought to be the most important step in flavonoid biosynthesis.[20-22] The formation of the chalcone is catalysed by the enzyme chalcone synthase.[21,22] Since the chalcone is the central intermediate for all flavonoids, chalcone synthase can be regarded as the key enzyme in flavonoid biosynthesis.[20-22] The overall reaction consists of three successive condensation steps with "acetate units" being derived from malonyl-CoA.[20-22] The result is the elongation of the aliphatic side chain of 4-coumarate by six carbon atoms **(Scheme 5. 3)**.[20] The enzyme chalcone synthase then catalyzes the cyclization to give the aromatic ring A.[20]



Scheme 5. 3 The biosynthesis of the 2',4',6',4-tetrahydroxychalcone[20-22]

In many biosynthetic pathways, S-substituted methionine is often the source of an additional carbon atom. 2',4',6',4-Tetrahydroxychalcone is methylated with S-adenosylmethionine and is converted to the 2'-methoxy-4',6',4-trihydroxychalcone. This reaction is catalyzed by a methyl transferase **(Figure 5. 8)**.[20-22]

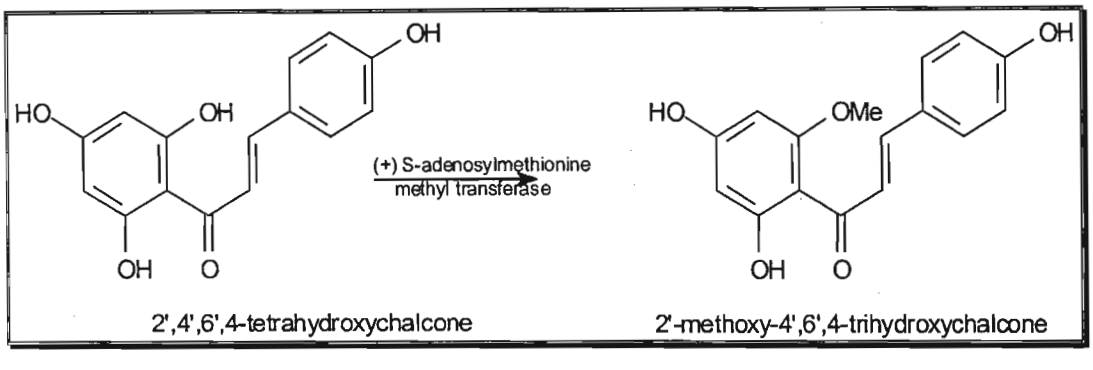
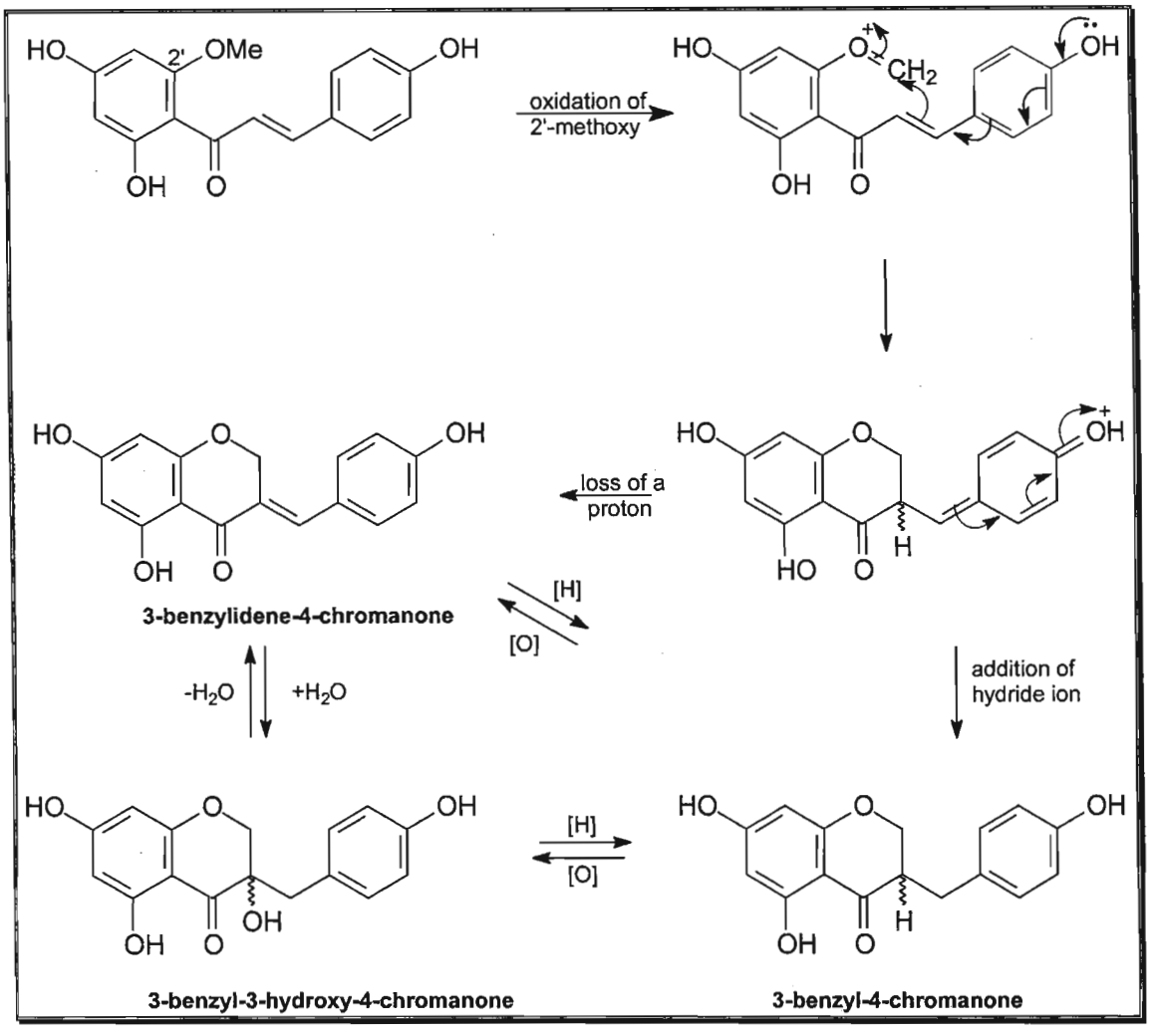


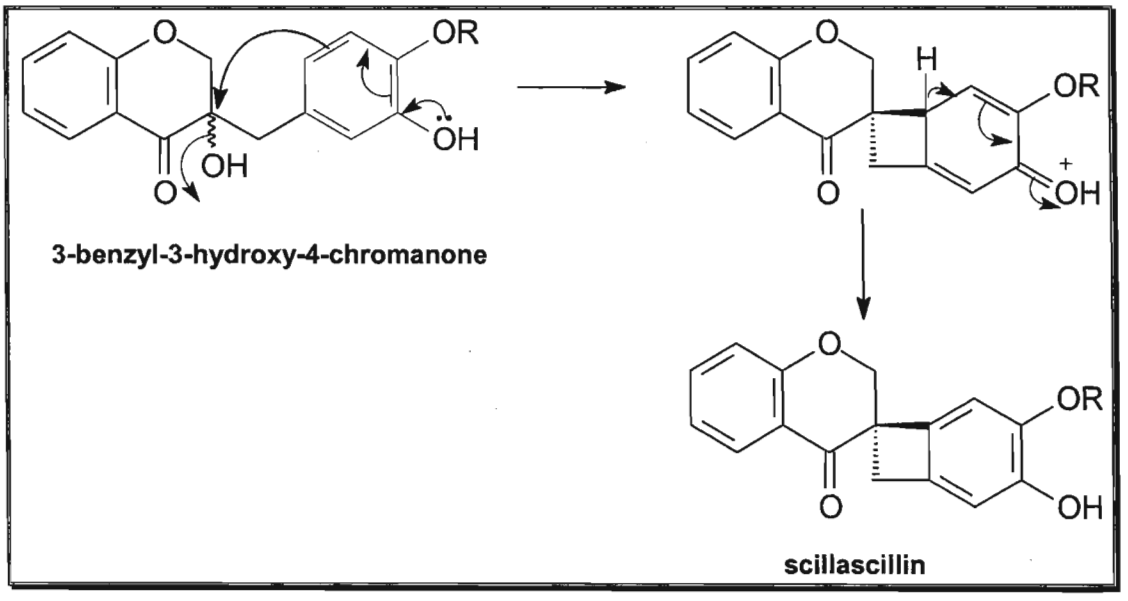
Figure 5. 8 The formation of 2'-methoxy-4',6',4-trihydroxychalcone[20-22]

A scheme for the conversion of chalcones to homoisoflavanones has been proposed by Dewick[12] 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.[12] 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 5. 4.**



Scheme 5. 4 The proposed biosynthetic routes to basic homoisoflavanones[12]

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 5. 5)**.



Scheme 5. 5 The proposed biosynthetic route to scillascillin type homoisoflavanones[12]

5.4 The Classification of Bufadienolides

The numbering system used for bufadienolides is shown below (**Figure 5. 9**).

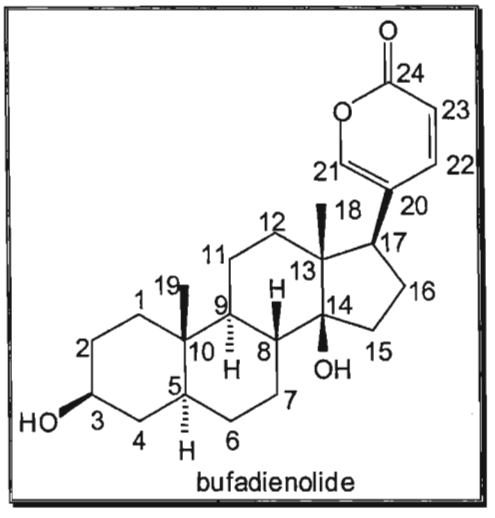


Figure 5. 9 The numbering system for bufadienolides

Bufadienolides are one of the two types of cardiac glycosides found in the Hyacinthaceae, with the other being the cardenolides. The group at C-17 defines the class of cardiac glycoside (**Figure 5. 10**). The cardenolides have a butyrolactone ring while the bufadienolides have an α-pyrone ring.

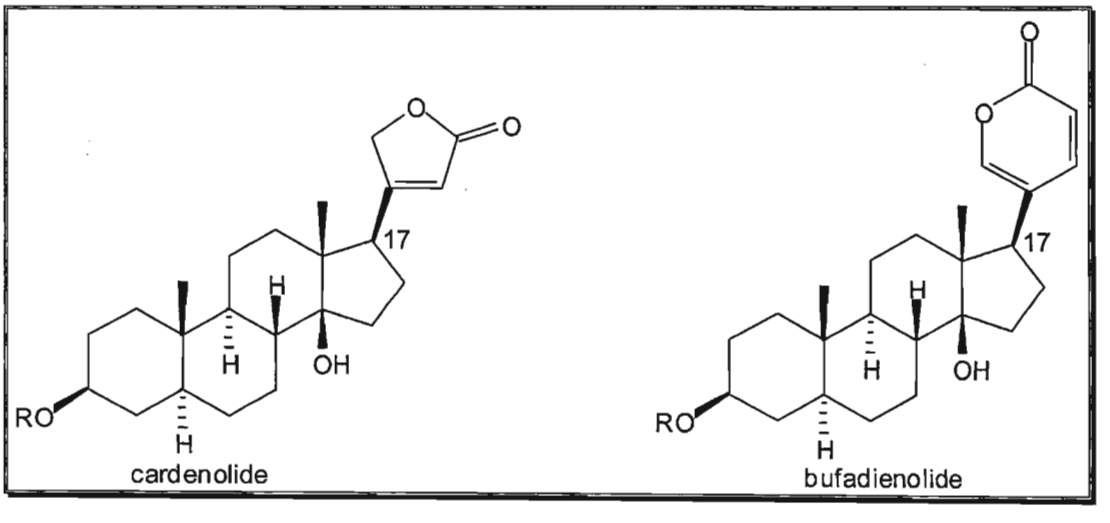


Figure 5. 10 The structural difference between a cardenolide and bufadienolide

Bufadienolides were first isolated from the skin of the toad (*Bufa* spp.), from which they derived their name. In the Hyacinthaceae, bufadienolides have been shown to occur predominantly in the subfamily Urgineoideae.

Some of the characteristic features of cardiac glycosides are:

1. The presence of predominantly *cis*-fusion of the A/B and C/D rings[†].
2. The presence of a 14β-hydroxy group.
3. The presence of an unsaturated lactone at C-17β.
4. A sugar moiety often occurs at C-3β.

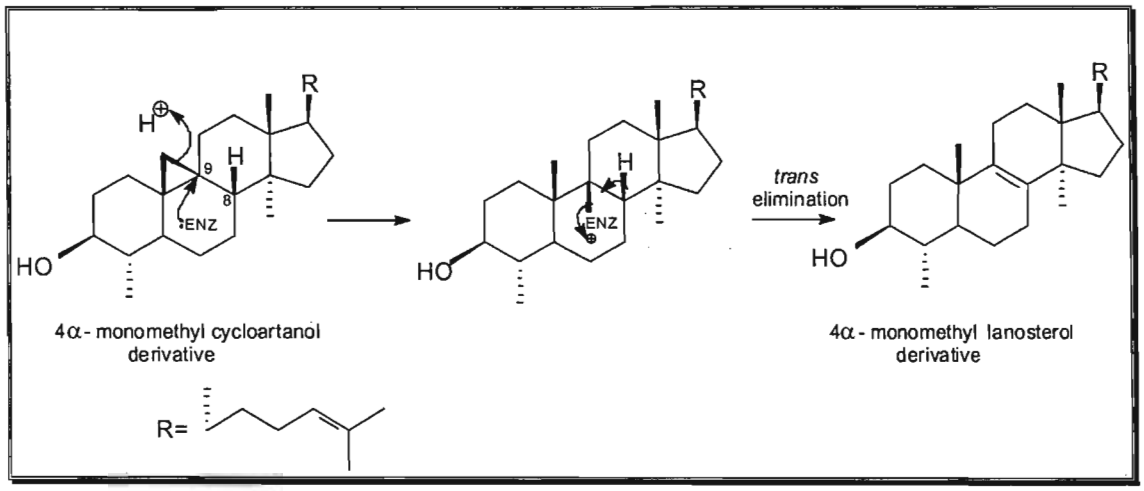
The ^{1}H NMR spectrum of a bufadienolide is very characteristic with the lactone ring proton resonances of H-21, H-22 and H-23 occurring at δ_H 7.35-7.45, δ_H 7.90-8.05 and δ_H 6.20-6.30 ppm respectively and only two methyl group proton resonances present. If a sugar molecule is attached to the steroidal structure one usually finds the anomeric proton resonance between δ_H 4.4-5.1 ppm.

5.5 The Biosynthesis of Bufadienolides

Bufadienolides are thought to arise from the manipulation of cholesterol in animals. In plants and algae, cycloartenol is the precursor.[23]

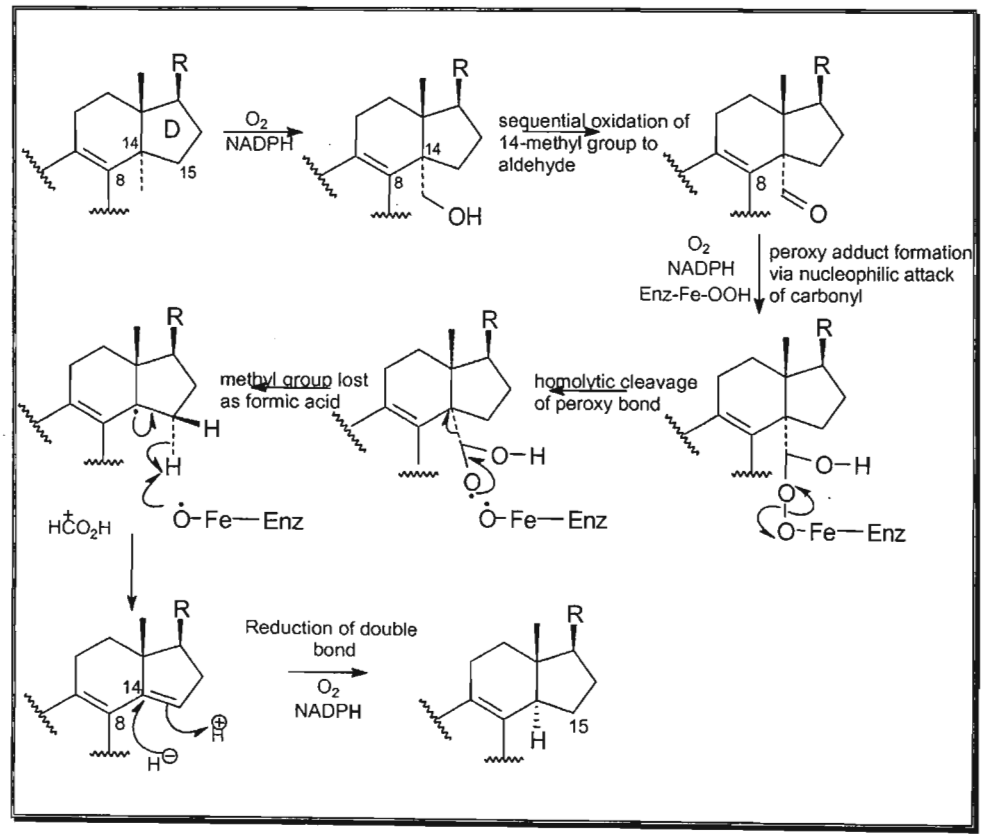
The first step in the conversion of cycloartenol to cholesterol is the opening of the cyclopropane ring **(Scheme 5. 6)** and the formation of a lanosterol derivative. It has been suggested that a nucleophilic group from an enzyme attacks C-9, thereby opening the ring, with a proton incorporated from water into the methylene group of the cyclopropane ring.[23] The stereochemistry at C-8 (H-β) is unfavorable for a concerted mechanism involving loss of H-8 with cyclopropane ring opening. The double bond between C-8 and C-9 is formed by a *trans*-elimination to form a lanosterol derivative.[23]

[†] Physodine C and D isolated from *Drimia physodes* (Jacq.) Jessop have *trans* fused A/B and C/ D rings[43]



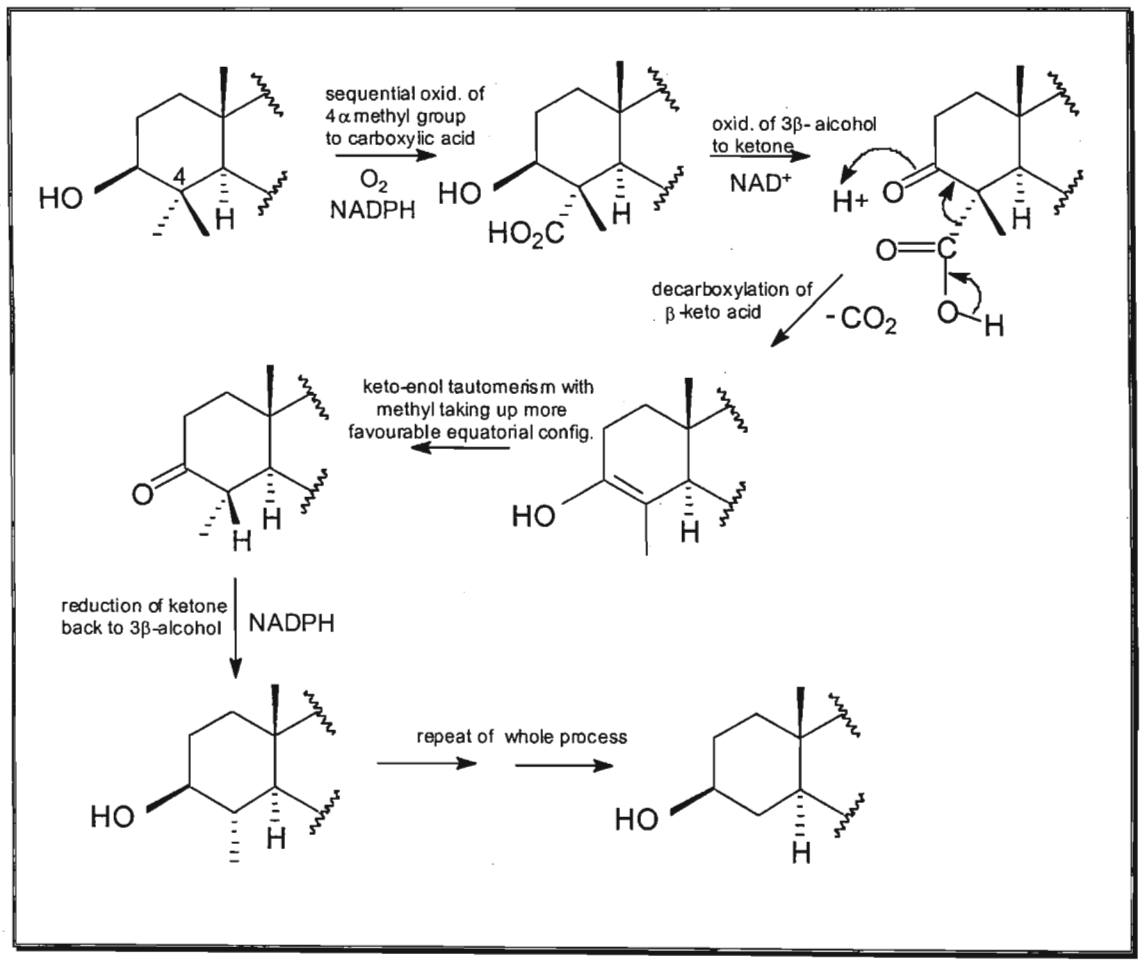
Scheme 5. 6 The opening of the cyclopropane ring[23]

The C-14 methyl group is lost next as formic acid **(Scheme 5. 7).**[23] This reaction is catalysed by a cytochrome P-450 mono-oxygenase. Two oxidation reactions give the 14α-formyl derivatives, with the loss of the formyl group thought to occur *via* a Baeyer-Villiger type oxidation. The 14,15-double bond is then reduced by NADPH with the H-15 proton being derived from water.[23]



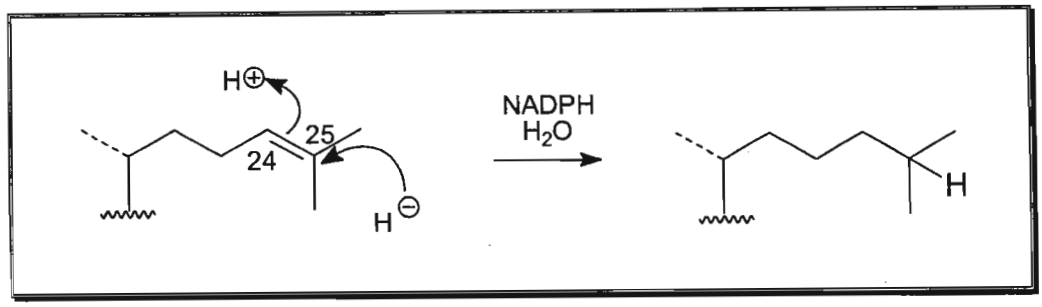
Scheme 5. 7 Showing loss of the methyl group of C-14 [23]

The next step is the sequential loss of the two methyl groups **(Scheme 5. 8)** at C-4 *via* a decarboxylation mechanism. This occurs by the sequential oxidation of the 4α-methyl group to a carboxyl group and the 3-hydroxyl group to a ketone, thereby generating a β-keto acid.[23] This is followed by the decarboxylation of the β-keto acid. Keto-enol tautomerism occurs with the remaining methyl group taking up the more favoured equatorial configuration. The ketone (C-3) is then reduced back to the 3β-alcohol, after which the process repeats itself.[23]



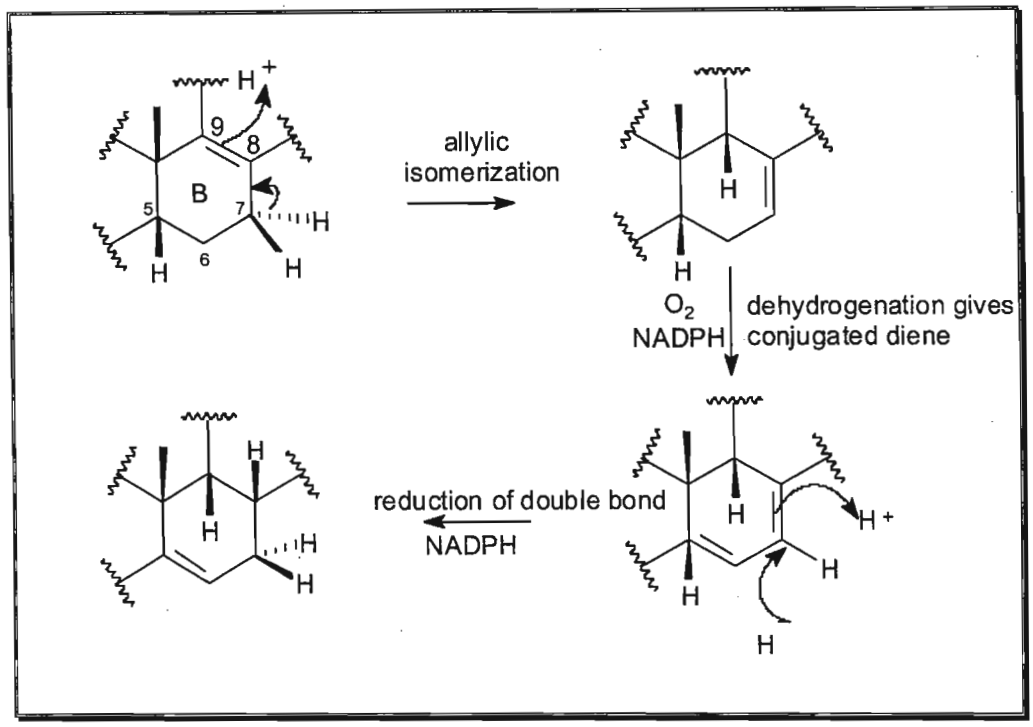
Scheme 5. 8 Showing loss of the two methyl groups of C-4 [23]

A NADPH-dependent reductase reduces the Δ^{24} double bond **(Scheme 5. 9)**. The hydride from the coenzyme is added at C-25, and the H-24 is derived from water.[23]



Scheme 5. 9 Loss of C-24 double bond [23]

The final step in the formation of cholesterol involves the migration of the Δ^8 double bond to Δ^5 *via* Δ^7 and the $\Delta^{5,7}$ diene **(Scheme 5. 10).**[23] This sequence involves an allylic isomerization with the protons at C-8 and C-9 being generated from water and that at C-7 from NADPH.[23]

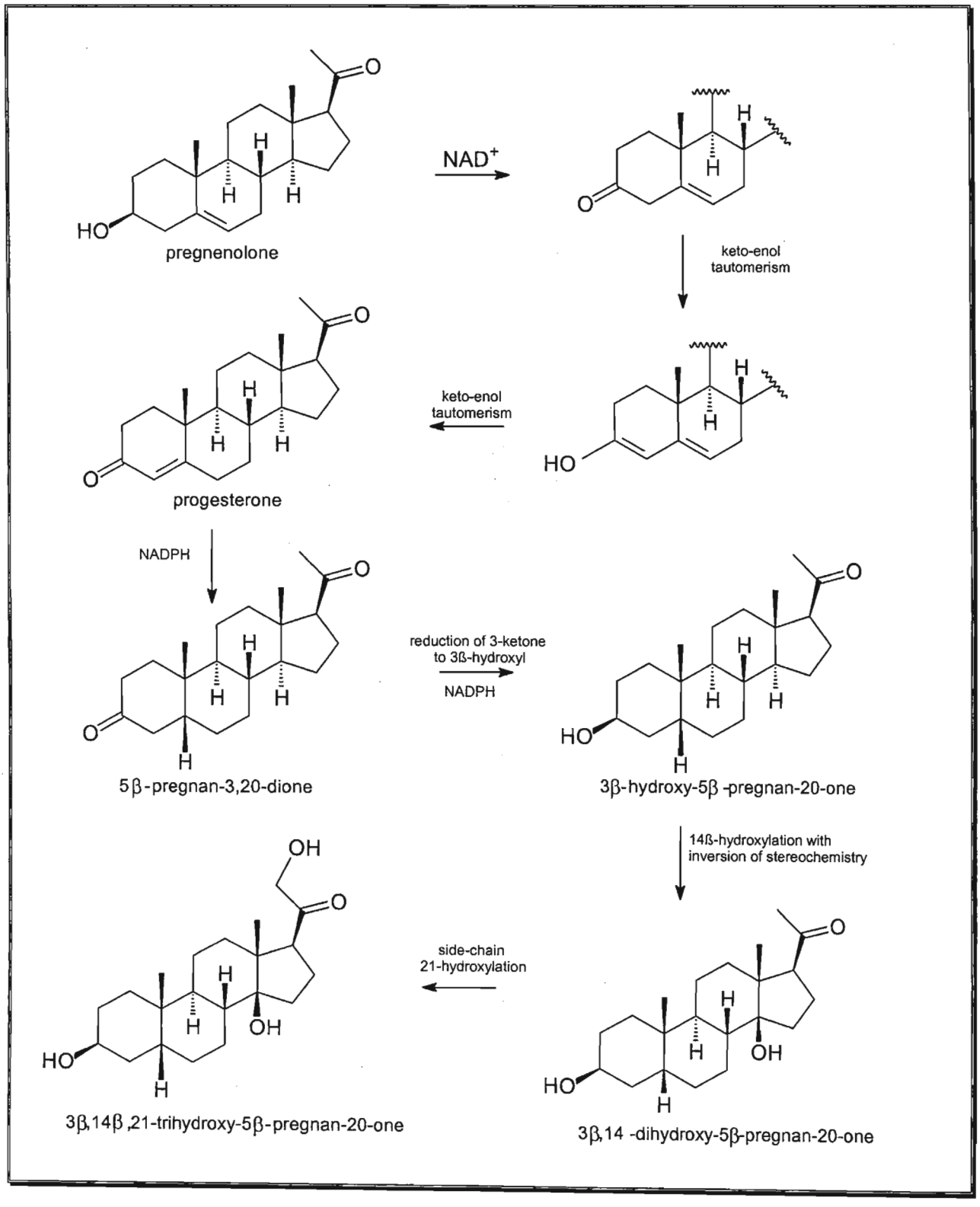


Scheme 5. 10 Allylic isomerization of double bond [23]

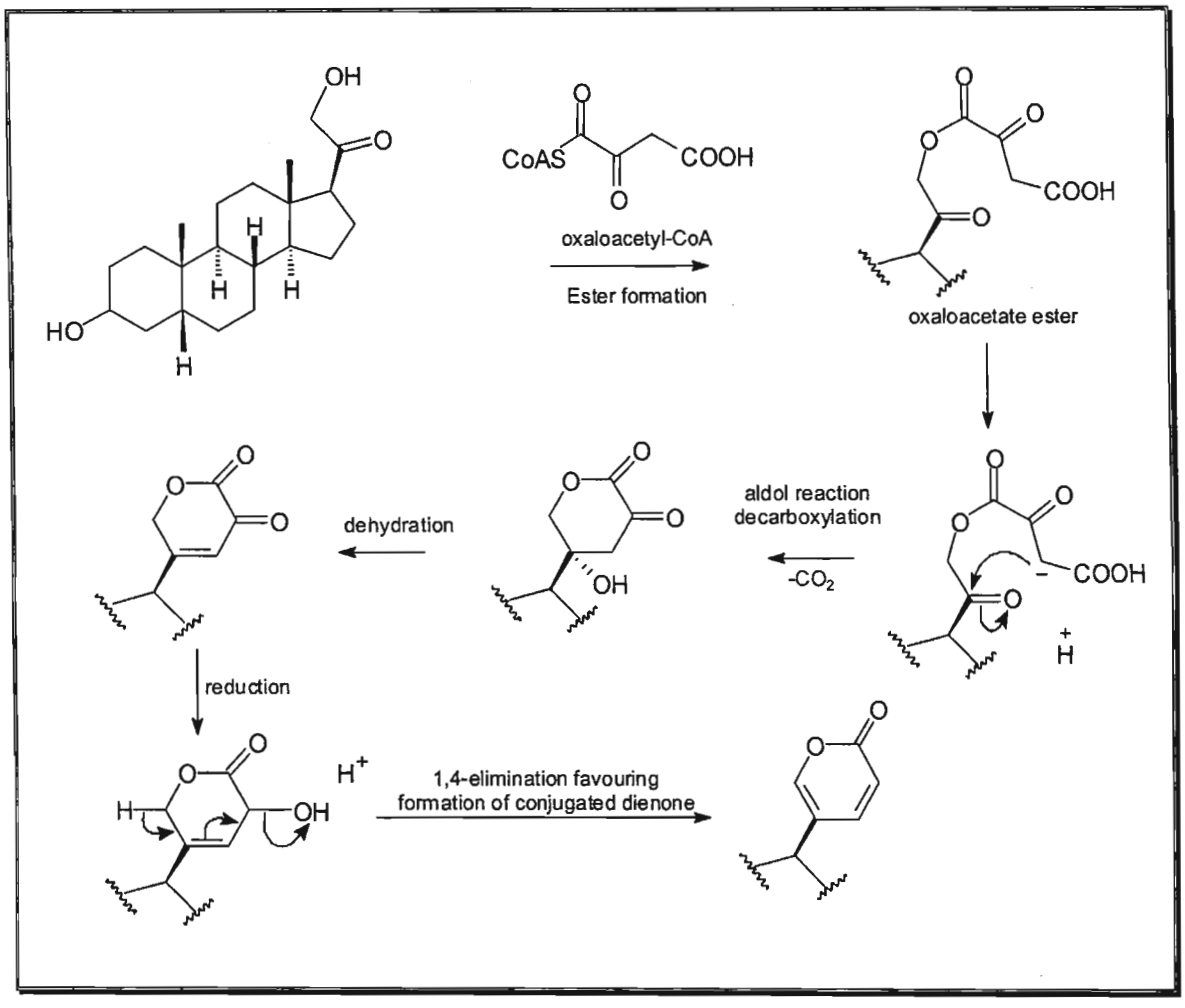
The side chain of cholesterol is cleaved to form a two-carbon acetyl group. This shortening is achieved by successive oxidation, hydration and cleavage of the C-20/22 bond to give pregnenolone which is, in turn, oxidized to give progesterone. Reduction of the Δ^4 double bond gives the *cis* fused A/B system, 5β-pregnan-3,20-dione.[23] Further reduction of the C-3 ketone gives a β-hydroxy group **(Scheme 5. 11).** Hydroxylation occurs at C-14 with inversion of the stereochemistry.[23] This is very peculiar as normally hydroxylation by mono-oxygenase occurs with retention of configuration.[23] This is followed by hydroxylation of the side chain at C-21.[23]

In bufadienolides, the formation of the lactone ring **(Scheme 5. 12)** then occurs. This ring is created by the incorporation of the three carbons from oxaloacetate *via* an aldol addition

process, with the fourth carbon being lost by decarboxylation.[23] The last step is the ketone formation which involves dehydration, reduction and a 1,4-elimination giving the dienone.[23]



Scheme 5. 11 Conversion of pregnenolone to 3β-hydroxy-5β-pregnan-20-one [23]



Scheme 5. 12 Proposed formation of bufadienolide lactone ring [23]

5.6 The Biological Activity of Homoisoflavanones

Homoisoflavanones are known to be concentrated in the waxy, scale-like layers of bulbs.[16] In this work, the homoisoflavanones were isolated from bulb extracts. Homoisoflavanones are known to possess anti-inflammatory, anti-mutagenic, anti-bacterial and analgesic properties.[24]

Two well known homoisoflavanones, intracatin and intricatinol **(Figure 5. 11)**, isolated from the roots of *Hoffmanseggia intricata* T. S. Brandeg. (Fabaceae), have shown antimutagenic properties, with both showing varying inhibition of mutagenicity in *Salmonella typhimurium* of the carcinogens 2-amino - anthracene, acetylaminofluorine and ethyl methanesulphonate.[25†] It was also shown that intricatinol, which possesses two hydroxy groups, was more broadly active than intricatin, which possesses one hydroxy group.[25] This led to the postulation that increased potency could be associated with the presence of extra hydroxy groups on ring A.[25]

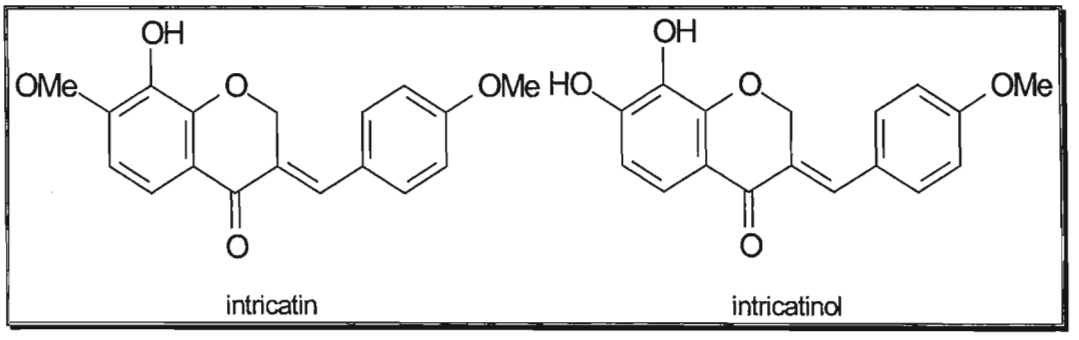


Figure 5. 11 Intricatin and intricatinol

The anti-inflammatory properties of bulbs within the Hyacinthaceae have been widely investigated.[26] The crude extracts of the bulbs of *Muscari comosum* Mill. (Hyacinthaceae) were tested for anti-inflammatory properties, by using the inhibition of croton oil-induced dermatitis in the mouse ear.[26] Homoisoflavanone-rich fractions were shown to inhibit mouse dermatitis.[26] The inhibitory effect obtained was comparable to that of the potent anti-inflammatory drug, indomethacin.[26]

Two homoisoflavanones isolated from *Veltheimia bracteata* Harv. ex Baker (syn. *V. viridifolia* Jacq.) (Hyacinthaceae; Hyacinthoideae) were tested against mixtures of phosphodiesterase (PPE) isozymes.[27] 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.[27] The study also investigated the *in vitro* activity of this homoisoflavanone on leukotriene synthesis.[27] A weak activity was reported for this system. The study also investigated the bronchiospasmolytic *in vivo* activity of the homoisoflavanone on the respiration and on the

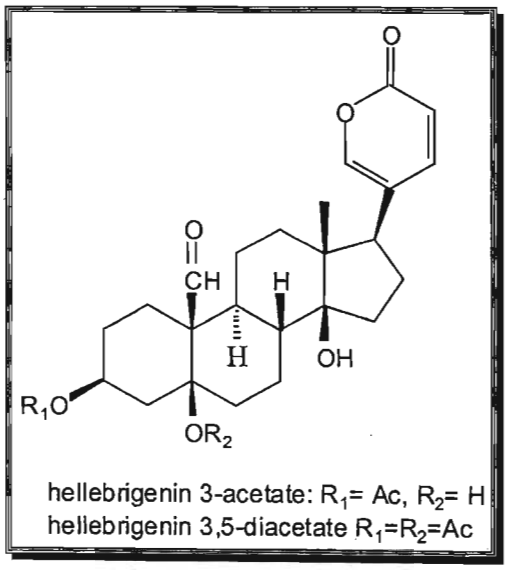
† Note that these homoisoflavanones from the Fabaceae have different ring A oxidation patterns to the homoisoflavanones isolated from the Hyacinthaceae

cardiac system of guinea pigs.[27] 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.[27] In addition, a moderated bronchiospasmolysis was detected in conjunction with a decrease in heart rate.[27] The conclusions were that the homoisoflavanone had a weak and especially short biological activity in the *in vivo* test systems.[27]

5.7 The Biological Activity of Bufadienolides

The cardiac glycosides are an important class of naturally occurring drugs with actions that are both beneficial and toxic. Bufadienolides act as a blood pressure stimulant and also show antineoplastic and respiratory activity.[53]

Pharmacological studies have shown that the bufadienolides' activity lies with the aglycone part of the molecule.[53] However the sugar moiety plays an important role in the kinetics of action. In general, cardiac glycosides with more lipophilic character are absorbed faster and have longer duration of action and this is determined by sugar residues and the number of hydroxyl groups on the aglycone part of the glycoside.[53] The stereochemistry of the steroidal nucleus also plays an important role. Structures with an A/B *cis* ring fusion have been shown to be more active than those that contain an A/B *trans* system and a similar sort of effect is observed for the C/D ring system.[53] The lactone ring alone, when not attached to the steroid structure, is not active; therefore one is led to believe that the activity lies in the steroid skeleton. However the lactone plays an important role in receptor binding as saturation of the lactone ring dramatically reduces the biological activity.[53]



hellebrigenin 3-acetate: R_1= Ac, R_2= H
hellebrigenin 3,5-diacetate R_1=R_2=Ac

Figure 5. 12 Two bufadienolides isolated from *Bersama abyssinica*[28]

Hellebrigenin 3-acetate and hellebrigenin 3,5-diacetate **(Figure 5. 12)** isolated from the stem bark of *Bersama abyssinica* Fresen. (Melianthaceae) were shown to exhibit inhibitory activity

against human carcinoma of the nasopharynx in cell culture (KB).[28] Both compounds were also tested for *in vivo* activity against the Walker intramuscular carcinoma 256 in rats. Hellebriginin 3-actetate showed significant activity and was the first cardiotonic steroid to show activity against an *in vivo* tumour system.[28] Further studies on the fruit of *Bersama abyssinica*, yielded three novel bufadienolides, all of which have shown *in vitro* inhibitory activity against cells derived from human carcinoma of the nasopharynx.[29]

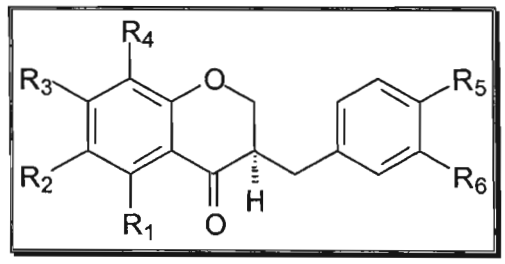
Work done on the eggs of the toad, *Bufa marinus*, yielded five novel bufadienolides. All five compounds showed inhibitory Na^+, K^+ and ATPase enzymatic activity.[30]

Drimia maritima (L.) W.T.Stearn, from Europe, contains principally the bufadienolide scillaren A and proscillaridin A, although is not used as a cardiac glycoside, it is used for its expectorant action in preparations such as Gee's linctus.[23] This species is also known to contain bufadienolides such as glucoscilliroside and scilliroside, which have been used as a rodenticide.[23] Its no longer used as it is considered to be an inhumane way of killing rats.[23]

5.8 Homisoflavanones Isolated from Hyacinthaceae

A literature search was undertaken to see which homoisoflavanones have been isolated previously from the southern African Hyacinthaceae. The following tables list the various homoisoflavanones isolated. The table has been divided (5.8.1-5.8.3) according to the three different classes of homoisoflavanones.

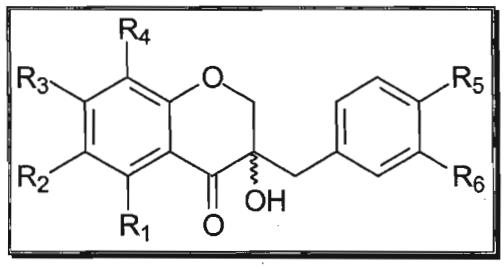
5.8.1 The 3-benzyl-4-chromanone type of homoisoflavanones



Name	R_1	R_2	R_3	R_4	R_5	R_6	Plant Source	Ref
1. 3,9-dihydro-eucomin	OH	H	OH	H	OCH_3	H	*Eucomis bicolor* Baker *Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D.	16,31 32 33
2. 7-*O*-methyl-3,9-dihydro-eucomin	OH	H	OCH_3	H	OCH_3	H	*Eucomis bicolor* Baker *Schizocarphus nervosus* (Burch.) Van der Merwe	31 39
3. 3,9-dihydropunctatin	OH	H	OH	OCH_3	OH	H	*Eucomis comosa* (Houtt.) Wehrh. *Eucomis pallidiflora* Baker	34 55
4. 7-*O*-methyl-3,9-dihydropunctatin	OH	H	OCH_3	OCH_3	OH	H	*Ledebouria cooperi* (Hook.f.) Jessop *Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D. *Drimiopsis maculata* Lindl. & Paxt.	35 32 33 42
5. 4'-*O*-methyl-3,9-dihydropunctatin	OH	H	OH	OCH_3	OCH_3	H	*Eucomis comosa* (Houtt.) Wehrh.	16,34
6. 4'-demethyl-5-*O*-methyl-3,9-dihydro-eucomin	OCH_3	H	OH	H	OH	H	*Eucomis comosa* (Houtt.) Wehrh. *Eucomis montana* Compton	16,34 32
7. 3,9-dihydro-autumnalin	OH	OCH_3	OH	H	OH	H	*Eucomis autumnalis* (Mill.) Chitt. *Merwilla dracomontana* (Hilliard & Burtt) Speta *Merwilla plumbea* (Lindl.) Speta *Schizocarphus nervosus* (Burch.) Van der Merwe *Ledebouria cooperi* (Hook.f.) Jessop	35 37 37 38 35
8. 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	OCH_3	OH	H	OCH_3	H	*Merwilla dracomontana* (Hilliard & Burtt) Speta	37
9. 5,7-dihydroxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone	OH	H	OH	H	OCH_3	OH	*Scilla kraussii* Baker *Schizocarphus nervosus* (Burch.) Van der Merwe	37 38 33

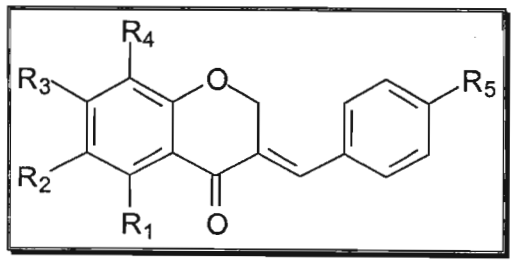
							Resnova humifusa (Baker) U. & D.M.-D.	
10. 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone	OH	OCH_3	OH	H	OCH_3	OH	*Merwilla plumbea (Lindl.)* Speta *Spetae lachenaliiflora* Wetschnig & Pfosser	37 35
11. 5,7-dimethoxy-3-(4'-methoxybenzyl)-4-chromanone	OCH_3	H	OCH_3	H	OCH_3	H	*Schizocarphus nervosus* (Burch.) Van der Merwe	38,39
12. 5-hydroxy-7-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone	OH	H	OCH_3	H	OCH_3	OH	*Schizocarphus nervosus* (Burch.) Van der Merwe	39
13. 5-hydroxy-6,7,8-trimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	OCH_3	OCH_3	OCH_3	OH	H	*Veltheimia bracteata* Harv. ex. Baker	27
14. 5,8-dihydroxy-6,7-dimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	OCH_3	OCH_3	OH	OH	H	*Veltheimia bracteata* Harv. ex. Baker	27
15. 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	H	OH	H	OH	H	*Ledebouria ovatifolia* (Baker) Jessop *Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D. *Drimiopsis maculata* Lindl. & Paxt.	40 32 33 42
16. 5 hydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OH	H	OCH_3	H	OH	OCH_3	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
17. 5-hydroxy-6,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OH	OCH_3	OCH_3	H	OH	OCH_3	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
18. 5,7-dihydroxy-3-(3',4'-dimethoxybenzyl)-4-chromanone	OH	H	OH	H	OCH_3	OCH_3	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
19. 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OCH_3	OH	OCH_3	H	OH	H	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
20. 5,6,7-trimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OCH_3	OCH_3	OCH_3	H	OH	H	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
21. 8 hydroxy-5,7-dimethoxy-3-(4'-methoxybenzyl)-4-chromanone	OCH_3	H	OCH_3	OH	OCH_3	H	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
22. 5,7-dimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OCH_3	H	OCH_3	H	OH	H	*Schizocarphus nervosus* (Burch.) Van der Merwe *Drimiopsis burkei* Baker	38 42
23. 5,6-dihydroxy-7-methoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OH	OH	OCH_3	H	OH	OCH_3	*Scilla zebrina* Baker	41
24. 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OCH_3	OH	OCH_3	H	OH	OCH_3	*Scilla zebrina* Baker	41,50
25. 5,7- dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OH	H	OH	H	OH	OCH_3	*Scilla zebrina* Baker *Drimia delagoensis* (Baker) Jessop	41 42
26. 7-hydroxy-5,6-dimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OCH_3	OCH_3	OH	H	OH	H	*Resnova humifusa* (Baker) U. & D.M.-D.	33
27. 5,6-dihydroxy-7-methoxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	OH	OCH_3	H	OH	H	*Drimiopsis maculata* Lindl. & Paxt.	42
28. 6-hydroxy-5,7-dimethoxy-3-(4'-hydroxy)-4-chromanone	OCH_3	OH	OCH_3	H	OH	H	*Scilla zebrina* Baker	50
29. 5-hydroxy-7-methoxy-3-(4'-hydroxy)-4-chromanone	OH	H	OCH_3	H	OH	H	*Lachenalia rubida* Jacq.	55

5.8.2 The 3-benzyl-3-hydroxy-4-chromanone type of homoisoflavanones



Name	R_1	R_2	R_3	R_4	R_5	R_6	Plant Source	Ref
30. eucomol	OH	H	OH	H	OCH_3	H	*Eucomis bicolor* Baker *Merwilla dracomontana* (Hilliard & Burtt) Speta *Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D.	4 37 32 33
31. 7-O-methyleucomol	OH	H	OCH_3	H	OCH_3	H	*Eucomis bicolor* Baker *Eucomis montana* Compton *Ornithogalum longibracteatum* Jacq.†	4 32 50
32. 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	H	OH	H	OH	H	*Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D.	32 33
33. 3,7-dihydroxy-5,6-dimethoxy-3-(4'-hydroxybenzyl)-4-chromanone	OCH_3	OCH_3	OH	H	OH	H	*Eucomis montana* Compton	32
34. 3,5-dihydroxy-3-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone	OH	H	OCH_3	H	OH	OCH_3	*Albuca fastigiata* Dryand†	42

5.8.3 The 3-benzylidene-4-chromanone type of homoisoflavanones

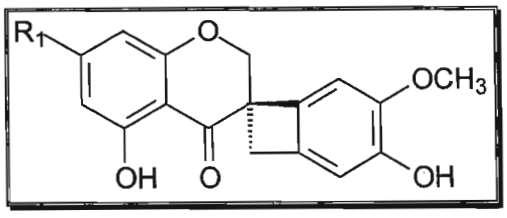


Name	R_1	R_2	R_3	R_4	R_5	Plant Source	Ref
35. eucomin	OH	H	OH	H	OCH_3	*Eucomis bicolor* Baker *Eucomis montana* Compton	4 32
36. punctatin	OH	H	OH	OCH_3	OH	*Eucomis comosa* (Houtt.) Wehrh.	34
37. 4'-O-methyl-punctatin	OH	H	OH	OCH_3	OCH_3	*Eucomis autumnalis* (Mill.) Chitt. *Eucomis comosa* (Houtt.) Wehrh.	36 34

† Subfamily Ornithogaloideae

38. 4'-dimethyl-eucomin	OH	H	OH	H	OH	*Eucomis comosa* (Houtt.) Wehrh. *Schizocarphus nervosus* (Burch.) Van der Merwe	34 38,39
39. 7-O-methyl-eucomin	OCH_3	H	OH	H	OCH_3	*Eucomis bicolor* Baker	31
40. autumnalin	OH	OCH_3	OH	H	OH	*Eucomis autumnalis* (Mill.) Chitt. *Schizocarphus nervosus* (Burch.) Van der Merwe	36 38
41. 5,7-dihydroxy-6-methoxy-3-(4'-methoxybenzylidene)-4-chromanone	OH	OCH_3	OH	H	OCH_3	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
42. 5-hydroxy-7-methoxy-3-(4'-hydroxybenzylidene)-4-chromanone	OH	H	OCH_3	H	OH	*Schizocarphus nervosus* (Burch.) Van der Merwe	38
43. 5,6,7-trihydroxy-8-methoxy-3-(4'-hydroxybenzylidene)-4-chromanone	OH	OH	OH	OCH_3	OH	*Eucomis pallidiflora* Baker	55

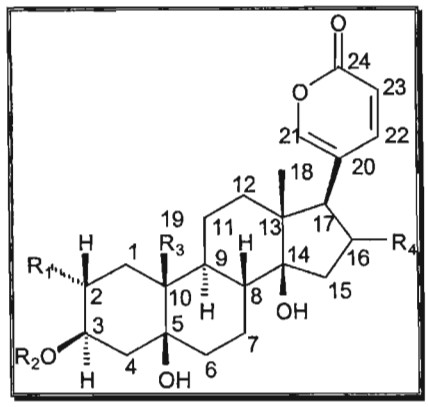
5.8.4 The Scillascillin type of homoisoflavanones



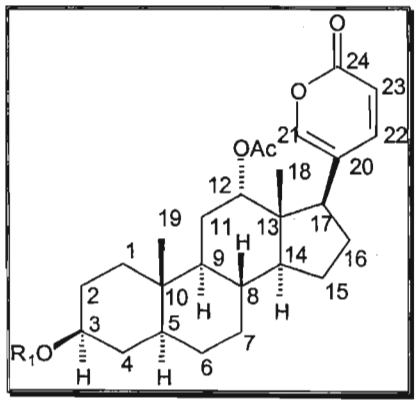
Name	R_1	Plant Source	Ref
44. 3',5-dihydroxy-4',7-dimethoxyspiro{2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one	OCH_3	*Drimiopsis* maculata Lindl. & Paxt. *Eucomis humilis* Baker	42 35
45. 3',5,7-trihydroxy-4'-methoxyspiro{2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien}-4-one	OH	*Drimiopsis maculata* Lindl. & Paxt. *Eucomis montana* Compton *Resnova humifusa* (Baker) U. & D.M.-D. *Eucomis humilis* Baker	42 32 33 35

5.9 Bufadienolides isolated from Hyacinthaceae

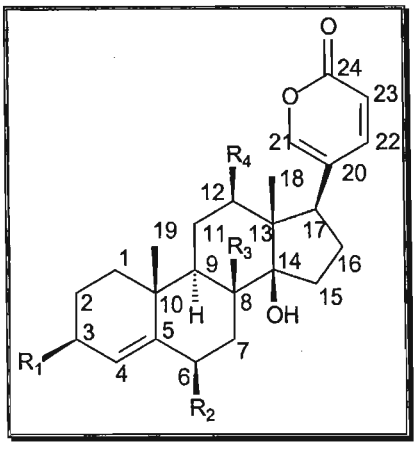
A similar literature search, as conducted for the homoisoflavanones, was done for the bufadienolides from the Hyacinthaceae. The different bufadienolides have been divided into six major parent skeletal groups which incorporates all of the compounds.



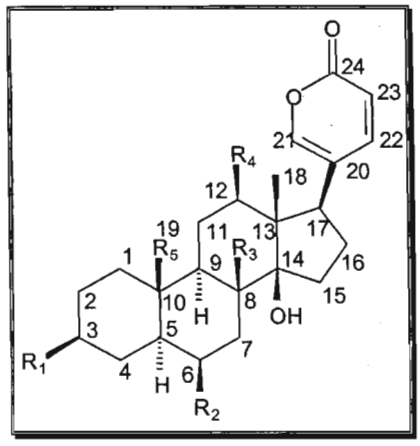
Name	R_1	R_2	R_3	R_4	Plant Source	Ref
1. physodine A	H	β-D-digit	CHO	H	*Drimia physodes* (Jacq.) Jessop	43
2. physodine B	OAc	β-D-glc-(1→4)-O-(2-O-acetyl-α-L-thev)	CHO	H	*Drimia physodes* (Jacq.) Jessop	43
3. hellebrigenin	H	H	CHO	H	*Drimia altissima* (L.f.) Ker Gawl. *Drimia depressa* (Baker) Jessop	44 45
4. hellebrigenin 3-O-β-D-glucopyranoside	H	β-D-glc	CHO	H	*Drimia altissima* (L.f.) Ker Gawl. *Drimia depressa* (Baker) Jessop	44 45
5. hellebrigenol 3-O-β-D-glucopyranoside	H	β-D-glc	CH_2OH	H	*Drimia depressa* (Baker) Jessop	45
6. bovoruboside	H	α-L-thev	CHO	=O	*Drimia depressa* (Baker) Jessop *Bowiea volublis* Harv. ex. Hook.f.	46 51
7. bovokryptoside	H	α-L-thev	CHO	βOH	*Drimia depressa* (Baker) Jessop *Bowiea volublis* Harv. ex. Hook.f.	46 51



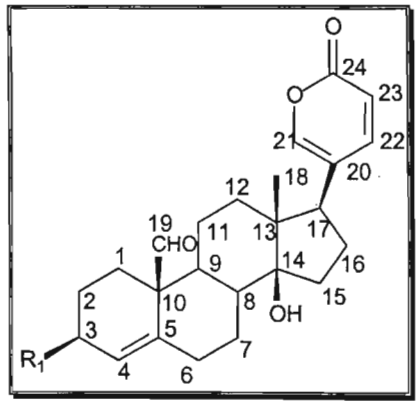
Name	R_1	Plant Source	Ref
8. physodine C	β-D-xyl-(1→4)-O-α-L-rha	*Drimia physodes* (Jacq.) Jessop.	43
9. physodine D	β-D-xyl-(1→3)-O-(4'-O-acetyl-α-L-rha)	*Drimia physodes* (Jacq.) Jessop	43



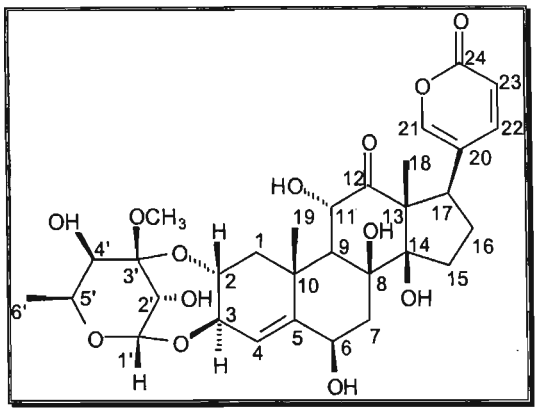
Name	R_1	R_2	R_3	R_4	Plant Source	Ref
10. scillirosidin	OH	OAc	OH	H	*Drimia sanguinea* (Schinz) Jessop	47
11. desacetylscillirosidin	OH	OH	OH	H	*Drimia sanguinea* (Schinz) Jessop	47
12. 12β-hydroxyscillirosidin	OH	OAc	OH	OH	*Drimia sanguinea* (Schinz) Jessop *Drimia robusta* Baker	47 35
10. scillirosidin-12-one	OH	OAc	OH	=O	*Drimia robusta* Baker	35
13. 12β-hydroxy-desacetyl-scillirosidin	OH	OH	OH	OH	*Drimia sanguinea* (Schinz) Jessop	47
14. 12β-hydroxyscilliroside	O-β-D-glc	OAc	OH	OH	*Drimia sanguinea* (Schinz) Jessop	47
15. 12β-hydroxyscillirosidin-3-one	=O	OAc	OH	OH	*Drimia sanguinea* (Schinz) Jessop	47
16. 12β-hydroxyscillirubrosidin-3-one	=O	H	OH	OH	*Drimia sanguinea* (Schinz) Jessop	47
17. scillaren A	O-β-D-glc-(1→4)-α-L-rha	H	H	H	*Drimia sanguinea* (Schinz) Jessop *Drimia indica* (Roxb.) Jessop	48 49
18. scilliphaeoside	O-α-L-rha	H	H	OH	*Drimia indica* (Roxb.) Jessop	49
19. urginin	O–α-L-rha-(1→3)-[β-D-glc-(1→4)]-α-L-rha	H	H	H	*Drimia altissima* (L.f.) Ker Gawl.	50,54
20. proscillaridin A	O-α-L-rha	H	H	H	*Drimia indica* (Roxb.) Jessop *Drimia robusta* Baker	49 35
21. 14β-hydroxybufa-4,20,22-trienolide 3β-O-{α-L-rhamnopyranosyl-[(1→4)-β-D-glucopyranoside]-(1→3)-α-L-rhamnopyranoside	O-α-L-rha-[(1→4)-β-D-glc]-(1→3)-rha	H	H	H	*Drimia altissima* (L.f.) Ker Gawl.	44



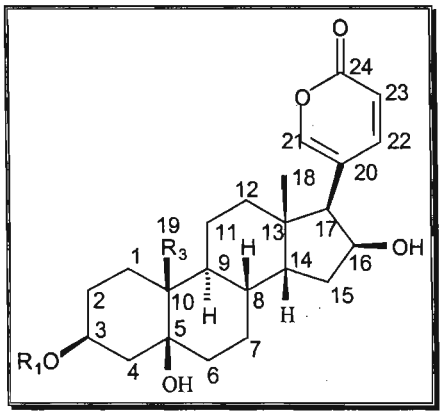
Name	R_1	R_2	R_3	R_4	R_5	Plant Source	Ref
22. 5α-4,5-dihydro-12β-hydroxy-scillirosidin	OH	OAc	OH	OH	CH_3	*Drimia sanguinea* (Schinz) Jessop	47
23. 5α-4,5-dihydroxy-scillaren A	O-β-D-glc-(1→4)-α-L-rha	H	H	H	CH_3	*Drimia sanguinea* (Schinz) Jessop	48
24. bovogenin A	OH	H	H	H	CHO	*Bowiea volubilis* Harv. ex. Hook.f.	51
25. bovoside A*	O-(6'-deoxy-3'-O-methyl)-β-D-glc	H	H	H	CHO	*Bowiea volubilis* Harv. ex. Hook.f.	51



Name	R_1	Plant Source	Ref
26. scilliglaucosidin/nabogenin	OH	*Drimia altissima* (L.f.) Ker Gawl. *Bowiea volubilis* Harv. ex. Hook.f.	44 51
27. scilliglaucosidin-3-one	=O	*Drimia altissima* (L.f.) Ker Gawl.	44
28. scilliglaucosidin 3β-O-α-L-rhamnopyranoside	O-α-L-rha	*Drimia altissima* (L.f.) Ker Gawl.	44
29. scilliglaucosidin 3β-O-β-D-glucopyranoside	O-β-D-glc	*Drimia altissima* (L.f.) Ker Gawl.	44



Name	Plant Source	Ref
30. rubellin	*Drimia calcarata* (Baker) Stedje	52



Name	R_1	Plant Source	Ref
31. 5β-3β,16β-dihydroxybufa-20,22-dienolide	H	*Drimia depressa* (Baker) Jessop	55
32. 5β-16β-dihydroxybufa-20,22-dienolide 3β-O-β-D-galactoside	O-β-D-gal	*Drimia depressa* (Baker) Jessop	55

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Chapter 6: Extractives from *Ledebouria revoluta*

6.1 Introduction

Ledebouria revoluta (L.f.) Jessop belongs to the subfamily Hyacinthoideae of the Hyacinthaceae[1]. 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 forty-six species of *Ledebouria* Roth in Africa and Asia, thirty-eight of which are native to South Africa.[2] *Ledebouria* was formerly included in the genus *Scilla* L. but has now been independently placed.

Ledebouria revoluta **(Figure 6. 1)** is distributed through much of the eastern half of South Africa.[3] It occurs in areas of woodland and grassland vegetation.[3] The leaves are polymorphic in shape, fleshiness and markings.[3] Flower colour varies from green tinged with pink, to a dark pinkish purple with the flowers being sometimes scented.[3] The bulbs have a purplish-brown outer scale and are membranous.[3]

Plants of *Ledebouria* are extensively used by traditional healers in KwaZulu-Natal, particularly in enemas and as purgatives for both humans and cattle.[4] *Ledebouria revoluta* is used by the Sotho as a charm to drive away lightning, and for the treatment of lumbago.[4] Other members of the genus used traditionally include *Ledebouria cooperi* (Hook.f.) Jessop used by the Sotho women during pregnancy, and by boys during circumcision rites.[4] *Ledebouria ovatifolia* (Baker) Jessop is used for the treatment of gastro-enteritis and in purgatives for adults.[4]

Figure 6. 1 ***Ledebouria revoluta*****, Photo: N.Crouch**

This is the first reported phytochemical study of this plant. Both the dichloromethane and methanol extracts of this plant were investigated. This led to the isolation of four homoisoflavanones **(Figure 6. 2)**. The compounds isolated were 5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone (compound **XV**), 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone (compound **XVI**), 7,8-dimethoxy-5-hydroxy-3-(4'-hydroxymethoxybenzyl)-4-chromanone (compound **XVII**) and 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (compound **XVIII**).

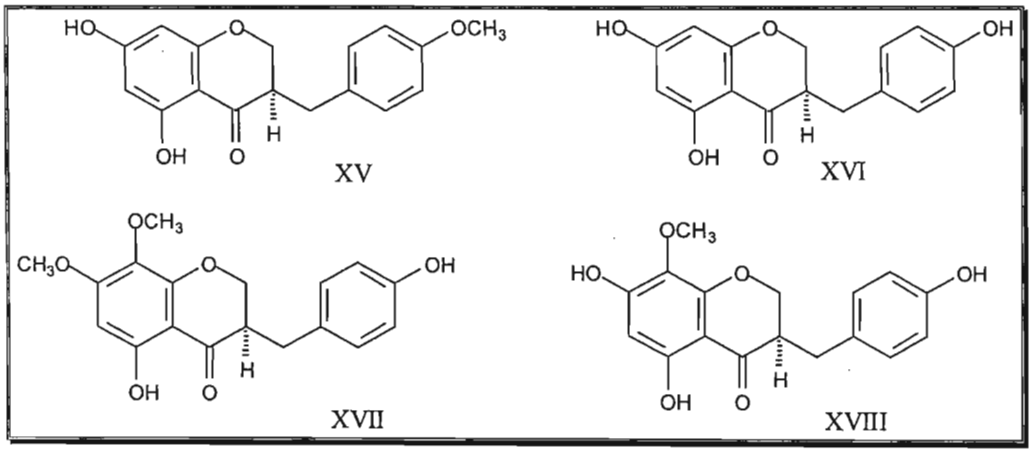


Figure 6. 2 Compounds isolated from *Ledebouria revoluta*

6.2 Results and Discussion

6.2.1 Structural Elucidation of Compound XV, 5,7-Dihydroxy-3-(4'-methoxybenzyl)-4-chromanone (3,9-dihydro-eucomin) (spectra 15.a-k)

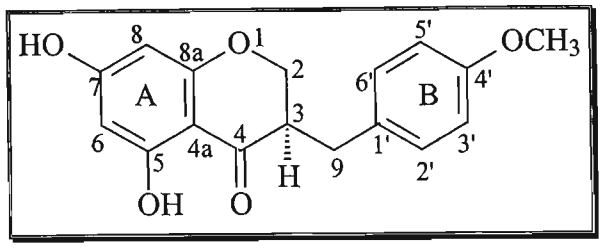
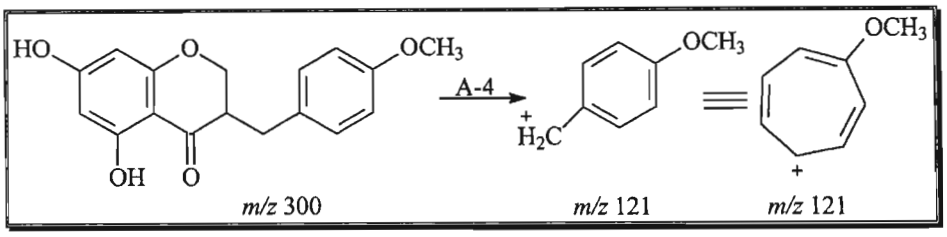


Figure 6. 3 Structure of compound XV, 3,9-dihydro-eucomin

Compound **XV** **(Figure 6. 3)**, was isolated as white needle-like crystals with a melting point of 152-154°C. This compound was found to have a molar mass of 300 g. mol^{-1} corresponding to a molecular formula of $C_{17}H_{16}O_5$. From the molecular formula, a double bond equivalence of ten was deduced.

The proton NMR spectrum of compound **XV** 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_H 4.11 (6.8, 11.5 Hz) and δ_H 4.25 (4.3, 11.5 Hz) assigned 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_H 3.15 (4.3, 13.7 Hz) and δ_H 2.70 (10.7, 13.7 Hz). The H-3 proton was found to occur as a multiplet at δ_H 2.78. The proton NMR spectrum also showed the presence of one methoxy group proton resonance at δ_H 3.78.

The appearance of an AA'BB' system in the proton NMR spectrum with resonances at δ_H 6.85 (d, 8.6 Hz, H-3', H-5') and δ_H 7.14 (d, 8.6 Hz, H-2', H-6') was indicative of the presence of two pairs of equivalent protons on the B ring and this also indicated that the B ring was 1',4'-disubstituted. The methoxy group was attached to this ring because of the presence of a base peak of *m/z* 121 in the mass spectrum which corresponds to a methoxybenzyl / methoxytropylium ion **(Scheme 6. 1)**. The substitution pattern on this ring was confirmed using the NOESY spectrum. The NOESY spectrum showed a correlation between the methoxy group proton signal at δ_H 3.78 and the H-3' and H-5' resonance at δ_H 6.85. Thus the methoxy group was placed at C-4' on ring B.



Scheme 6. 1 Fragmentation pattern of compound XV[5]

The substitution pattern on the A-ring could be deduced from the mass, ^{1}H NMR and UV spectra. The appearance of a pair of doublets each integrating to one proton at δ_H 5.97 (d, 2.1 Hz) and 5.90 (d, 2.1 Hz) due to H-6 and H-8 (as predetermined on biosynthetic grounds) in the proton NMR spectrum indicated that there were two unsubsituted position on the A ring. The loss of *m/z* 121 mass units in the mass spectrum indicates that the methoxy group is on ring B and because of the molecular formula ring A must have 2 hydroxy groups. One hydroxy group was assigned to the C-7 position due to a bathochromic shift of 40 nm in the UV spectrum when NaOAc was added.[6,7] The other hydroxy group was assigned to the C-5 position due a bathochromic shift of 24 nm in the UV spectrum with $AlCl_3$.[6,7] The downfield signal due to a hydroxy group proton (δ_H 12.12 (s)) was assigned to the proton of the strongly hydrogen-bonded 5-hydroxy group.[8] This was confirmed by the downfield shift of the carbonyl group (δ_C 198.0) that becomes deshielded due to the chelating effects with the hydroxy group at C-5.[8] The structure was confirmed using the HMBC spectrum. The HMBC, COSY and NOESY data are provided in **Table 6.2**.

The infrared data supported the postulated homoisoflavanone structure for compound **XV**. 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).

Table 6.1 CD data for the 3-benzyl-4-chromanone type of homoisoflavones isolated.

Sample code	λ_{ext}, nm	[θ]
Compound **XV**	331.6	-3.8
	293.3	-13.6
	284.6	-17.5
	252.6	+8.7
Compound **XVI**	330.8	-2.4
	290.1	-12/8
	284.0	-11.8
	256.3	+5.8
Compound **XVII**	347.8	-2.9
	293.6	-9.1
	284.6	-7.5
	260.4	+4.5
Compound **XVIII**	372.0	-4.6
	298.2	-10.2
	289.2	-12.3
	264.7	+6.5

The absolute configuration at C-3 for this compound could be reported as circular dichromism (CD) experiments were carried out by Prof. D. Ferreira at the University of Mississippi on all four homoisoflavanones isolated (**Table 6.1**). The negative Cotton effects between 284 and 298 nm for the four compounds indicated a 3R absolute configuration for all four compounds.[9] This *R* configuration gives H-3 as α.

A literature search for the compound indicated that it was 5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone isolated previously from *Eucomis bicolor* Baker and *Resnova humifusa* (Baker) U. & D.M.-D. (syn. *Drimiopis maxima* Baker).[10,11] Comparison of the ^{13}C NMR data **(Table 6.2)** proved them to be very close indeed and compound XV was identified as 5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone.

Table 6.2: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C* (ppm)	HMBC (C→H)	COSY	NOESY
2a	4.11 (dd, 6.8, 11.5 Hz)	68.95	68.94	3;9a,b	2b;3	2b;3
2b	4.25 (dd, 4.3, 11.5 Hz)				2a;3	2a;3
3	2.78 (m)	46.82	46.82	2a;9a,b	2a,b;9a,b	2a,b;9a,b;2'
4		197.95	197.93	2a,b;3;9 a,b		
4a		102.66	102.65	5-OH;6;8		
5		164.69	164.68	6		
6	5.97 (d, 2.1 Hz)	96.61	96.60	5-OH;7-OH;8		5-OH;7-OH
7		164.28	164.26	8		
8	5.90 (d, 2.1 Hz)	95.01	94.99	6		7-OH
8a		163.19	163.17	2a;b;6;8		
9a	2.70 (dd, 10.7, 13.7 Hz)	31.97	31.95	2a,b;3	3;9a	3;9b;2'/6'
9b	3.15 (dd, 4.3, 13.7 Hz)				3;9b	3;9a;2'/6'
1'		129.74	130.06			
2'/6'	7.14 (d, 8.6 Hz)	130.14	130.10	3;9a,b;3'/5'	3'/5'	2a;9a;3'/5'
3'/5'	6.85 (d, 8.6 Hz)	114.13	114.12		2'/6'	2'/6';4'-OCH_3
4'		158.44	158.43	4'-OCH_3; 3'/5';2'/6'		
5-OH	12.12 (s)					6
7-OH	5.79 (s)					6;8
4'-OCH_3	3.78 (s)	55.29	55.27			3'/5'

*Literature values obtained in $CDCl_3$.[11]

6.2.2. Structural Elucidation of Compound XVI, 5,7-Dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone (spectra 16.a-h)

This compound was isolated as white needle-like crystals.

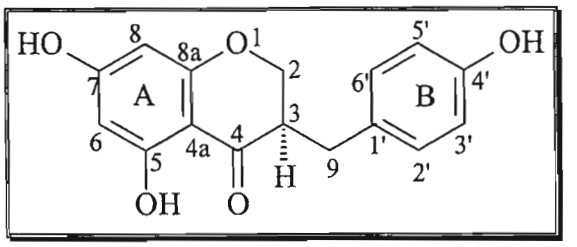
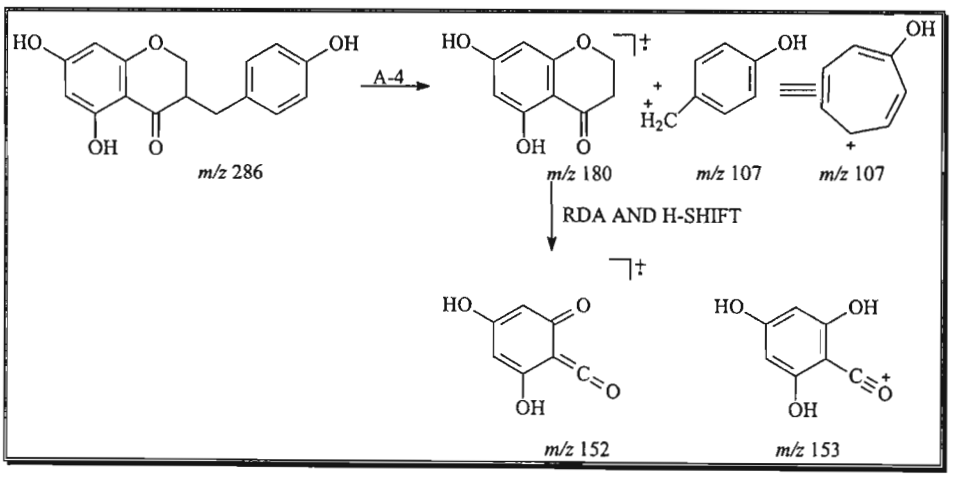


Figure 6. 4 Structure of compound XVI, 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone

This compound **(Figure 6. 4)** 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 were similar to those of compound **XVI**.

Inspection of the mass spectrum revealed a parent ion at, *m/z* 286, which corresponded to a molecular formula of $C_{16}H_{14}O_5$, and was consistent with the proposed homoisoflavanone structure. Strong fragment ions were observed at *m/z* 107 and 180 due to A-4 fragmentation. The fragment ion at *m/z* 180 indicates that ring A had two hydroxy groups and the hydroxybenzyl / tropylium ion at *m/z* 107 similarly suggested that the B ring had one hydroxy group as a substituent. The fragment ion at *m/z* 153 is formed *via* the retro-Diels Alder cleavage of the chromanone moiety is shown in **Scheme 6.2**.[5]



Scheme 6. 2 Fragmentation pattern of compound XVI[5]

The infra-red data supported the homoisoflavanone structure for compound XVI. 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 pattern indicated by the mass spectrum. The aromatic AA'BB' system with resonances at δ_H 6.99 (d, 8.6 Hz, H-2',H-6'), δ_H 6.72 (d, 8.6 Hz, H-3',H-5'), confirmed the substitution pattern of the B ring and was found to be similar to that of compound **XV**. The ^1H NMR spectrum showed no methoxy group proton resonances to be present. However, instead of an methoxy group at C-4', one finds the presence of a hydroxy group. The remaining aromatic signals in the spectrum were a pair of doublet resonances (integrating to one proton each) at δ_H 5.84 (d, 2.1 Hz) and 5.90 (d, 2.1 Hz). This confirmed the type of substituents on the A ring that had been suggested by the mass spectrum. The substitution pattern was the same as for compound **XV**. One of the hydroxy groups was assigned to the C-5 position due to a bathochromic shift (40 nm) in the UV spectrum with $AlCl_3$.[6,7] The other hydroxy group was assigned to the C-7 position due to a bathochromic shift (25 nm) in the UV spectrum when NaOAc was added.[6,7]

These assignments were confirmed by the HMBC, NOESY and COSY spectra and are listed in **Table 6.3**. This compound was found to differ from compound **XV** only in the substitution of the B-ring. Instead of a methoxy group, a hydroxy group was found present. A literature search for the compound indicated that it was 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone previously isolated from *Muscari comosum* (L.) Mill.[12,13]

Table 6.3: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C^* (ppm)	HMBC (C→H)	COSY	NOESY
2a	4.02 (dd, 6.8, 11.5 Hz)	68.67	70.1	3;9a,b	2b;3	2b;3;2'/6'
2b	4.17 (dd, 4.3, 11.5 Hz)				2a;3	2a;3;2'/6'
3	2.68 (m)	46.73	48.2	2a,b;9a,b	2a,b;9a,b	2a,b;9a,2'/6
4		197.90	199.4	2a,b;3;8;9a,b;		
4a		101.79	102.8	6;8		
5		163.01	165.8	6		
6	5.90 (d, 2.1 Hz)	96.31	97.1	8		
7		166.3	168.2	6;8		
8	5.84 (d, 2.1 Hz)	95.11	95.9	6		
8a		162.96	164.7	2a;b;8		
9a	2.59 (dd, 10.7, 13.7 Hz)	31.98	32.9	2a,b;3;	3;9b	2a,b;3;9b;2'/6'
9b	3.06 (dd, 4.3, 13.7 Hz)				3;9a	9a;2'/6'
1'		128.83	130.2	3;9a,b;3'/5'		
2'/6'	6.99 (d, 8.6 Hz)	130.03	131.2	9a,b	3'/5'	2a,b;3;9a,b
3'/5'	6.72 (d, 8.6 Hz)	115.40	116.4	2'/6'	2'/6'	2'/6'
4'		155.2	157.2	3'/5';2'/6'		

* Literature values obtained in CD_3OD.[13]

6.2.3 Structural Elucidation of Compound XVII, 7,8-Dimethoxy-5-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone (spectra 17.a-h)

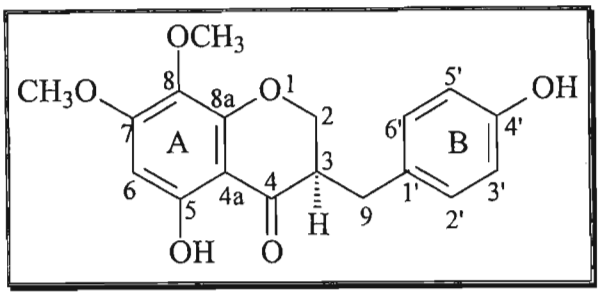
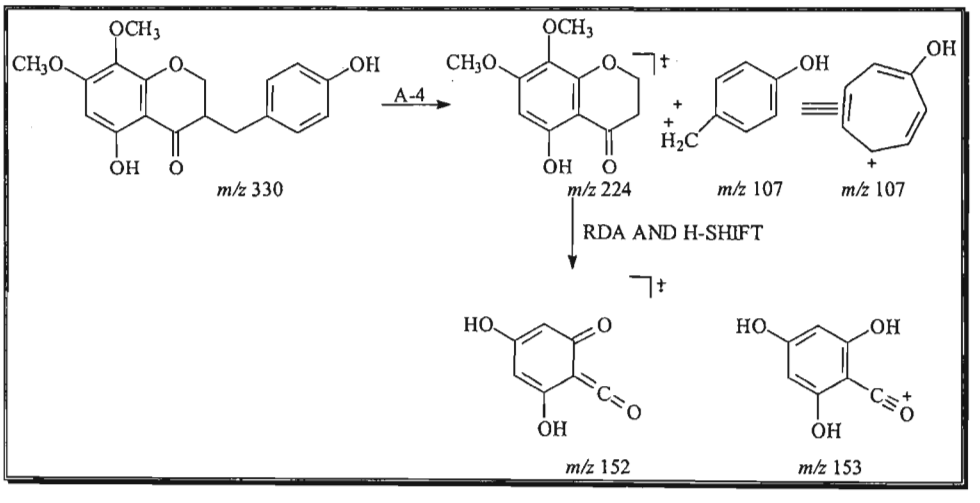


Figure 6. 5 Structure of compound XVII, 7,8-dimethoxy-5-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone

This compound **(Figure 6. 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 were similar to those of compound **XV** and **XVI**. The proton NMR spectrum also showed the presence of two methoxy group proton resonances at δ_H 3.76 and δ_H 3.86.

Inspection of the mass spectrum revealed a parent ion at, *m/z* 330, which corresponded to a molecular formula of $C_{18}H_{18}O_6$, and was consistent with the proposed homoisoflavanone structure. Strong fragment ions were observed at *m/z* 224 and 107 due to A-4 fragmentation. The fragment ion at *m/z* 224 indicates that ring A had two methoxy groups and one hydroxyl group and the hydroxybenzyl / tropylium ion at *m/z* 107 similarly suggested that the B ring had one hydroxy group as a substituent. The fragment ion at *m/z* 153 is formed *via* the *retro*-Diels Alder cleavage of the chromanone moiety is shown in **Scheme 6.3**.[5]



Scheme 6. 3 Fragmentation pattern of compound XVII[5]

The appearance of an AA'BB' system in the proton NMR spectrum with resonances at δ_H 6.76 (d, 8.6 Hz, H-3', H-5') and δ_H 7.07 (d, 8.6 Hz, H-2', H-6') was indicative of the presence of two pairs of equivalent protons on the B ring and this also indicated that the B ring was 1', 4'-disubstituted. The mass spectrum showed a peak at *m/z* 107 corresponding to a hydroxybenzyl fragment indicating the possible presence of a hydroxy group at C-4'.

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_H 6.08 (s) (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* 224 in the mass spectrum was evidence of a dimethoxy-hydroxychromanone fragment ion.[5] This implied that the A-ring contained two methoxy groups and one hydroxy group. One hydroxy group was assigned to the C-5 position due a bathochromic shift of 27 nm in the UV spectrum with $AlCl_3$.[6,7] The downfield signal due to a hydroxy group proton (δ_H 12.03 (s)) was assigned to the proton of the strongly hydrogen-bonded 5-hydroxy group.[8] This was confirmed by the downfield shift of the carbonyl group (δ_C 198.3) that becomes deshielded due to the chelating effects with the hydroxy group at C-5.[8] When NaOAc was added there was no shift in the UV spectrum and this indicated that no hydroxy group was found at C-7 instead a methoxy group (δ_H 3.86) was assigned to C-7. The remaining methoxy group was assigned to H-8. This assignment was done due to a NOESY correlation existing between the 5-OH group proton (δ_H 12.03) and the singlet proton (δ_H 6.08), thus indicating that the proton had to be at C-6 and the methoxy group at C-8. The structure was confirmed using the HMBC spectrum. The HMBC, COSY and NOESY data are provided in **Table 6.4**.

The infrared spectrum supported the postulated homoisoflavanone structure. 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 7,8-dimethoxy-5-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone chromanone previously isolated from *Ledebouria cooperi* (Hook.f) Jessop.[14]

Table 6.4: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 7,8-dimethoxy-5-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_{C^*} (ppm)	HMBC (C→H)	COSY	NOESY
2a	4.17 (dd, 6.8, 11.5 Hz)	69.16	70.4	9a,b	2b;3	3;2b;2'/6'
2b	4.31 (dd, 3.9, 11.5 Hz)				2a;3	3;2a;
3	2.77 (m)	46.86	48.2	2a;9a,b	2a,b;9a,b	2a,b;2'/6'
4		198.32	199.6	2a,b;3;9 a,b;6;5-OH		
4a		102.22	102.9	5-OH;6		
5		160.24	161.1	5-OH;6		
6	6.08 (s)	92.99	93.4		7-OCH_3	7-OCH_3;5-OH
7		161.30	162.3	7-OCH_3;5-OH;6		
8		129.32	129.6	7-OCH_3;	7-OCH_3	
8a		153.59	154.8	2a;b;6		
9a	2.68 (dd, 10.3, 13.7 Hz)	31.94	32.6	2a,b;3;2'/6'	9a;3;	9b;2'/6'
9b	3.13 (dd, 4.3, 13.7 Hz)				9b;3	9a;2'/6'
1'		129.32	129.6	3,9a,b;3'/5'		
2'/6'	7.07 (d, 8.6 Hz)	130.22	130.8	9a,b;3'/5'	3'/5'	2a;3;9a,b;3'/5'
3'/5'	6.76 (d, 8.6 Hz)	115.55	116.0	2'/6',4'-OCH_3	2'/6'	2'/6'
4'		154.71	156.9	3'/5';2'/6'		
5-OH	12.03 (s)	-				6
7-OCH_3	3.86 (s)	56.17	56.3		6	6
8-OCH_3	3.76 (s)	61.32	61.1			

*Literature values obtained in CD_3OD.[14]

6.2.4 Structural Elucidation of Compound XVIII, 5,7-Dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone (spectra 18.a-h)

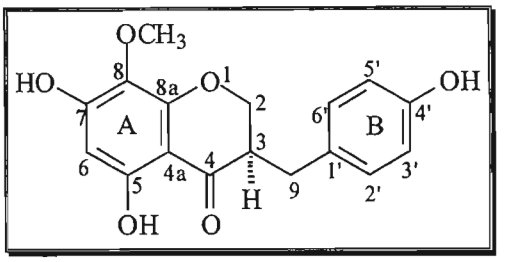
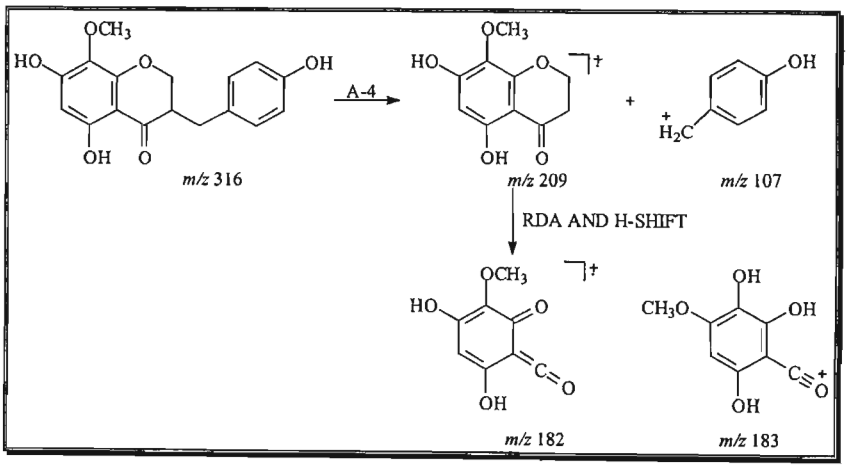


Figure 6. 6 Structure of compound XVIII, 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone

Compound **XVIII (Figure 6. 6)**, was isolated as a yellow amorphous material. This compound was found to have a molar mass of 316 g. mol^{-1} corresponding to a molecular formula of $C_{17}H_{16}O_6$. From the molecular formula, a double bond equivalence of ten was deduced. This compound also belongs to the 3-benzyl-4-chromanone type of homoisoflavanones. The resonances observed for 2H-2, H-3 and 2H-9 in the ^1H NMR spectrum were similar to those of compounds **XV**, **XVI** and **XVII**. The proton NMR spectrum also showed the presence of one methoxy group proton resonance at δ_H 3.91.

The appearance of an AA'BB' system in the proton NMR spectrum with resonances at δ_H 6.77 (dd, 8.6, 1.7 Hz, H-3', H-5') and δ_H 7.01 (dd, 8.1,1.7 Hz, H-2', H-6') was indicative of the presence of two pairs of equivalent protons on the B ring and this also indicated that the B ring was 1', 4'-disubstituted. The mass spectrum **(Scheme 6. 4)** showed a peak at *m/z* 107 corresponding to a hydroxybenzyl fragment indicating the possible presence of a hydroxy group at C-4'.



Scheme 6. 4 Fragmentation pattern of compound XVIII[5]

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_H 6.12 (s) (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* 209 in the mass spectrum was evidence of a dihydroxymethoxychromanone fragment ion.[5] This implied that the A-ring contained two hydroxy groups and one methoxy group. One hydroxy group was assigned to the C-5 position due a bathochromic shift of 27 nm in the UV spectrum with $AlCl_3$.[6,7] The downfield signal due to a hydroxy group proton (δ_H 12.03 (s)) was assigned to the proton of the strongly hydrogen-bonded 5-hydroxy group.[8] This was confirmed by the downfield shift of the carbonyl group (δ_C 198.3) that becomes deshielded due to the chelating effects with the hydroxy group at C-5.[8] The hydroxy group was assingned to H-7 due to a bathachromic shift of 36 nm in the UV spectrum with NaOAc. The methoxy group was assigned to H-8. This assignment was done due to a NOESY correlation existing between the 5-OH group proton (δ_H 12.03) and the singlet proton (δ_H 6.08), thus indicating that the proton had to be at C-6 and the methoxy group at C-8. The structure was confirmed using the HMBC spectrum. The HMBC, COSY and NOESY data are provided in **Table 6.5**.

The infrared data supported the postulated homoisoflavanone structure. 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 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone previously isolated from *Eucomis comosa* (Houtt.) Wehrh. and *Eucomis pallidiflora* Baker.[15,16] Comparison of the carbon data proved them to be very close indeed.[16]

Table 6.5: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone ($CDCl_3$)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C^* (ppm)	HMBC (C→H)	COSY	NOESY
2a	4.20 (dd, 6.4, 11.5 Hz)	69.41	69.4	9a,b	2b;3	3;2b;9a;2'/6'
2b	4.33 (dd, 3.9, 11.5 Hz)				2a	3;2a;2'/6'
3	2.81 (m)	46.77	46.8	9a	2a	3;9a;2'/6'
4		197.70	197.7	2a,b		
4a		a	102.4	5-OH;6		
5		157.71	157.7	6		
6	6.12 (s)	95.87	95.9	5-OH		5-OH
7		160.25	160.2	5-OH;6		
8		a	127.6	8-OCH_3		
8a		152.99	153.0	2a		
9a	2.72 (dd, 10.3, 13.2 Hz)	31.91	31.9	2'/6'	9b	2b;3;9b;2'/6'
9b	3.13 (dd, 4.3, 13.2 Hz)				9a	2a;3;9a;2'/6'
1'		129.79	129.8	3;3'/5'		
2'/6'	7.09 (d, 8.6 Hz)	130.29	130.3	9a,b	3';5'	3;2a,b;9a,b;3'/5'
3'/5'	6.77 (dd, 8.6 Hz)	115.58	115.6	2'/6'	2';6'	2'/6'
4'		154.47	154.4	3'/5';2'/6'		
5-OH	11.74 (s)	-				6
8-OCH_3	3.91 (s)	61.51	61.5			

* Literature values obtained in $CDCl_3$.[16]
[a] Resonance was too weak to be determined.

6.3 Experimental

Material of *Ledebouria revoluta* (L.f.) Jessop was collected from Long Tom Pass, Mpumalanga, and a voucher retained (*N. Crouch 853*, NH).

The bulbs (mass 0.6 kg) were dried, chopped into smaller pieces and extracted successively with dichloromethane and methanol by agitation on a Labcon Mechanical shaker at 140 rpm. The extracts obtained were then filtered and the solvent removed under reduced pressure, to give a dichloromethane extract (5.00 g) and a methanol extract (15.00 g). ^{1}H NMR analysis of the crude methanol extracts showed nothing of interest and the extract was not investigated further.

The dichloromethane extract was loaded onto a 5 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions at a time (100% dichloromethane fractions 1-20; 5% ethyl acetate in dichloromethane fractions 21-40; 10% ethyl acetate in dichloromethane fractions 41-60; 20% ethyl acetate in dichloromethane fractions 61-80; 40% ethyl acetate in dichloromethane fractions 81-100; 100% ethyl acetate in dichloromethane fractions 101-120). Purification of fractions 7-12 using a 20% ethyl acetate in hexane solvent system afforded compound **XV** at a yield of 0.3% (15.7 mg). Purification of fractions 39-43 using a 30% ethyl acetate in dichloromethane solvent system afforded compounds **XVI** at a yield of 0.7% (32.5 mg) and compound **XVIII**, at a yield of 0.3% (15.0 mg). Purification of fractions 82-89 using a 40% ethyl acetate in hexane solvent system afforded compound **XVII** at a yield of 1% (50.3 mg).

6.3.1 Physical data for Compound XV

Name: **5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone**
Yield: 15.7 mg
Physical description: white needle-like crystals
Melting Point: 152-154°C (lit. 161-163)[10,11]
Optical rotation: -2.50 (*c*, 0.16 in methanol) (value not given in literature)
Mass spectrum: GC-MS: [M^+] at *m/z* 300, $C_{17}H_{16}O_5$ requires 300.09974 g. mol^{-1}
EIMS *m/z*: 300, 121
Infrared: ν_{max}^{NaCl} cm^{-1}: 3460 (O-H stretching), 2927 (C-H stretching), 2868 (C-H stretching), 1653 (C=O stretching), 1514 and 1444 (C=C stretching)
UV: $\lambda_{max}^{MeCl_2}$ nm (log ε): 205.0 (5.8), 291.0 (5.6)
with $AlCl_3$: 315.0
with NaOAc: 331.0

^{1}H NMR: δ_H (ppm) $CDCl_3$: **Table 6.2**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 6.2**

6.3.2 Physical data for Compound XVI

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

Yield: 32.5 mg

Physical description: white needle-like crystals

Melting point: 97-100°C (lit. 103-104°C)[12,13]

Optical rotation: -12.38 (c, 0.214 in methanol) (lit. -34°, *c* 0.4 in methanol)[12,13]

Mass spectrum: GC-MS: [M^+] at *m/z* 286, $C_{16}H_{14}O_5$ requires 286.084124 g. mol^{-1}

EIMS *m/z*: 286,180, 171, 153, 133, 107

Infrared: ν_{max}^{NaCl} cm^{-1}: 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): 214.0 (5.8), 291.0 (5.6)

with $AlCl_3$: 331.0

with NaOAc: 316.0

^{1}H NMR: δ_H (ppm) $CDCl_3$: **Table 6.3**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 6.3**

6.3.3 Physical data for Compound XVII

Name: **7,8-dimethoxy-5-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone**

Yield: 50.3 mg

Physical description: white, amorphous material

Melting point: 121-123°C

Optical Rotation: -19.94 (c, 0.178 in methanol) (lit. -15°, *c* 0.4 in methanol)[14]

Mass spectrum: HRMS: [M^+] at *m/z* 330.11133, $C_{18}H_{18}O_6$ requires 330.110339 g. mol^{-1}

EIMS *m/z*: 330, 209, 181, 107

Infrared: ν_{max}^{NaCl} cm^{-1}: 3460 (O-H stretching), 2927 (C-H stretching), 1653 (C=O stretching) and 1514 and 1414 (aromatic C=C stretching).

UV: $\lambda_{max}^{MeCl_2}$ nm (log ε): 356.0 (5.2), 291.0 (5.8)

with $AlCl_3$: 318.0

with NaOAc: 291.0

^{1}H NMR: δ_H (ppm) $CDCl_3$: **Table 6.4**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 6.4**

6.2.4 Physical data for Compound XVIII

Name: **5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone**

Yield: 15.0 mg

Physical description: yellow amorphous material

Optical rotation: -7.796 (c, 0.186 g in methanol) (lit. -37(dioxin))[17]

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

EIMS *m/z*: 316, 210, 209, 183, 107

Infrared: ν_{max}^{NaCl} cm^{-1}: 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): 289.0 (5.5), 206.0 (5.8)

with $AlCl_3$: 311.0

with NaOAc]: 325.0

^1H NMR: δ_H (ppm) $CDCl_3$: **Table 6.5**

^{13}C NMR: δ_c (ppm) $CDCl_3$: **Table 6.5**

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Chapter 7: Extractives from *Drimia macrocentra* and *Urginea riparia*

7.1 Introduction

Drimia macrocentra (Baker) Jessop (syn. *Urginea macrocentra* Baker) of the Hyacinthaceae family (Liliaceae *sensu lato*) has been placed in the subfamily Urgineoideae. This plant is commonly called the Natal Slangkop as the leaf/inflorescence resembles an elevated snake's head. Although for many years its ingestion has been blamed as a cause of mortality in stock,[1,2] no phytochemical analyses have been reported for this species.

Drimia macrocentra **(Figure 7.1)** is distributed in South Africa along the coast and uplands of KwaZulu-Natal, extending as far south as the Transkei of the Eastern Cape.[1] It grows in moist or marshy ground at altitudes up to 2685 m.[1] The bulbs may attain a diameter of 5 cm, and are an ovoid shape. The bulbs have very characteristic white outer scales that are fleshy and brittle.[1]

This plant is known to the Zulu as ujobo and the bulb is highly esteemed as an anthelmintic agent.[1]

Urginea riparia Baker is a poorly understood bulbous species found both in the Midlands and coastal regions of KwaZulu-Natal and the Transkei of the Eastern Cape. It frequents rocky, exposed stream and riverside habitats (as suggested by its specific epithet), growing usually in clumped colonies with its bulbs submerged and leaves erect and emergent. Its relation to other urgineoids of the Hyacinthaceae remains to be properly elucidated, for at present its closest relative appears to be *Drimia calcarata* (Baker) Stedje (syn. *Urginea calcarata* (Baker) Hilliard & Burtt) under which it has been synonymised (as syn. *Drimia modesta* (Baker) Jessop).[3]

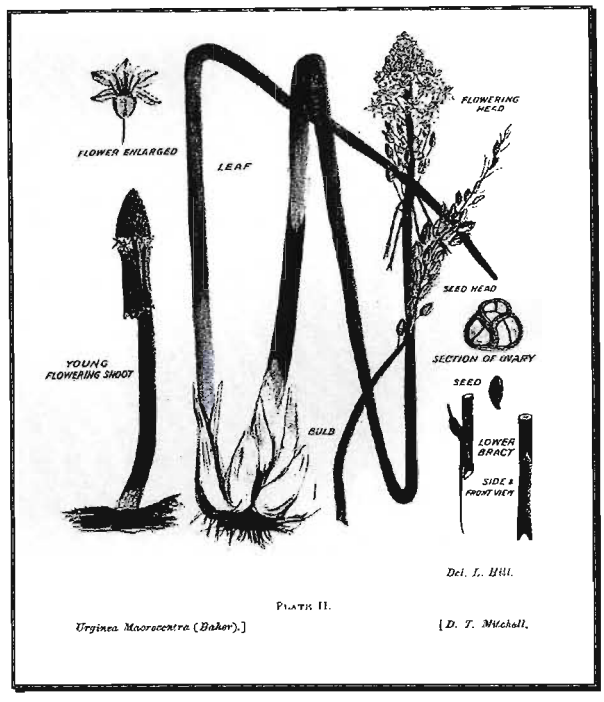

Figure 7. 1 Illustration of *Drimia macrocentra from* Mitchell[1]

This is the first reported phytochemical study on both *Drimia macrocentra* and *Urginea riparia*. The dichloromethane and methanol extracts of the plant bulbs were investigated. This led to several bufadienolide - rich fractions in very low concentrations however only two bufadienolides were isolated **(Figure 7. 2)**. Of the compounds isolated, one (**XX**) has not been reported previously. The compounds isolated were rubellin (compound **XIX**, from *Drimia macrocentra*) and riparian (compound **XX**, from *Urginea riparia*).

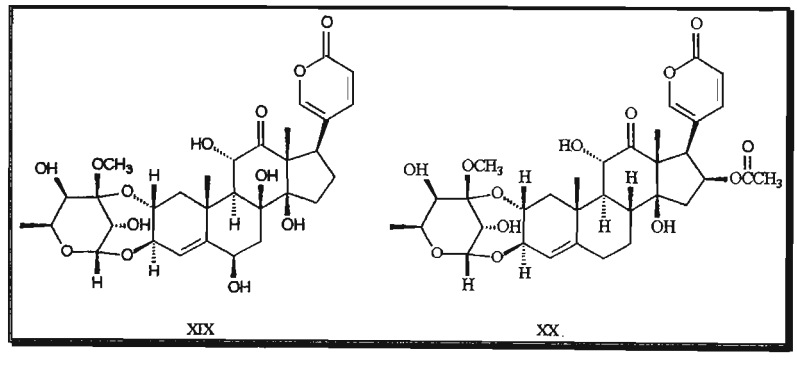

Figure 7. 2 Compounds isolated from *Drimia macrocentra* and *Urginea riparia*

7.2 Results and Discussion

7.2.1 Structural Elucidation of Compound XIX, Rubellin (spectra 19.a-i)

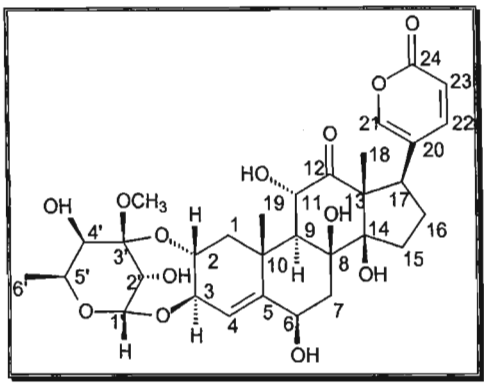

Figure 7. 3 Structure of compound XIX, rubellin

Rubellin (compound **XIX**) was isolated as the major constituent from the methanol extract of *Drimia macrocentra*. The structure proposed below from the NMR spectra suggested a molar mass of 620 g. mol^{-1}. However the molecular ion peak was not seen in the FAB mass spectrum but the highest peak at *m/z* 586[T] was seen suggesting the loss of two hydroxy groups.

The ^{1}H NMR spectrum showed the characteristic resonances at δ_H 7.54 (d, 2.6 Hz) (H-21), δ_H 7.92 (dd, 2.6, 9.7 Hz), H-22) and δ_H 6.34 (d, 9.7 Hz, H-23), which, together with the presence of the two methyl group proton resonances at δ_H 1.09 (s, 3H-18) and δ_H 1.72 (s, 3H-19), indicated the presence of a bufadienolide. The C-21 and C-22 resonances showed ^{3}J HMBC correlations to the resonance at δ_H 4.08 (t, 8.8 Hz) assigned to H-17. The C-17 resonance, in turn, showed a ^{3}J HMBC correlation to the methyl resonance at δ_H 1.09 (s) assigned to 3H-18. The resonance at δ_C 86.69 was ascribed to C-14 on the basis of a ^{3}J HMBC correlation with the 3H-18 resonance and a hydroxy group was placed at this position by comparison with literature.[4]

The ^{13}C NMR spectrum showed the presence of two carbonyl resonances at δ_C 164.64 and δ_C 214.95. The resonance at δ_C 164.46 was assigned to the carbonyl group of the lactone ring (C-24) as it showed HMBC correlations to the H-21, H-22 and H-23 resonances. The remaining carbonyl resonance was assigned to C-12 due to a ^{3}J HMBC correlation with the 3H-18 resonance. A further HMBC correlation from C-12 to the resonance at δ_H 5.21 (d, 12.3 Hz) led to it being assigned to H-11. The corresponding carbon resonance in the HSQC spectrum was at δ_C 73.58 which indicated the presence of a oxygen substituent and a hydroxy group was placed at this position by comparison with literature.[4] The H-11 resonance

[T] FAB HRMS of compound is currently being completed to confirm the molecular ion

showed a correlation in the COSY spectrum to the resonance at δ_H 1.65 (d, 12.3 Hz) which was assigned to H-9. The C-9 resonance at δ_C 54.48 showed a ^3J HMBC correlation to the second methyl group proton resonance (δ_H 1.72 (s)) assigned to 3H-19. The resonance at δ_C 41.35 showed HMBC correlations to 3H-19 and H-11 resonances and was assigned to C-10. A further HMBC correlation were seen between C-10 and to the resonance at δ_H 5.47 (d, 1.8 Hz), which was seen to correspond to a carbon resonance at δ_C 126.98 in the HSQC spectrum which was assigned to C-4 and enabled the placement of the double bond between C-4 and C-5. The H-4 resonance showed a correlation in the COSY spectrum to the resonance at δ_H 5.47 (d, 1.8 Hz), which had a corresponding carbon resonance at δ_C 79.87 and was assigned to H-3. A further correlation in the COSY spectrum between the H-3 resonance and a resonance at δ_H 5.22 (brs) with a corresponding carbon resonance at δ_H 72.39 in the HSQC spectrum indicated a further oxygen substituent at C-2. The resonance at δ_c 75.03 was assigned to C-6 on the basis of a ^3J HMBC correlation to H-4 and a hydroxy group was placed at C-6. The stereochemistry of the hydroxy group at C-6 was determined using the NOESY spectrum: the absence of a NOESY correlation between the 3H-19 and H-6 resonances implied H-6 was not β. The fully substituted carbon resonance at δ_C 78.98 was ascribed to C-8 on the basis of a ^3J HMBC correlation with H-6 and a hydroxy group was placed at C-8.

The stereochemistry at C-11 was determined through the NOESY spectrum. A correlation in the NOESY spectrum between both the 3H-18 and the 3H-19 methyl group proton signals and the H-11 resonance indicated H-11 was in a β position. Similarly a correlation in the NOESY spectrum between the 3H-19 methyl group proton singlet resonance and the H-1 resonance at δ_H 2.86 allowed for the assignment of a β orientation to this proton and hence assignment of the α orientation to the other H-1 resonance at δ_H 1.40.

The glycone part of the bufadienolide glycoside was found to be a carbohydrate moiety doubly linked to the aglycone at the C-2 and C-3 positions. The NOESY spectrum showed this linkage to be α at C-2 and β at C-3. The H-1β resonance showed a positive correlation to the H-2 proton and it was assigned the β configuration. The H-2β resonance showed no correlation in the NOESY spectrum to the H-3 proton hence H-3 was assigned the α configuration. The hemiacetal carbon resonance at δ_C 98.86 was assigned to C-1', the acetal (C-3') carbon resonance occurred at δ_C 100.21. A methoxy group was attached to C-3' as shown by a ^3J correlation between C-3' and the methoxy group proton resonance. The resonance at δ_C 70.08 was assigned to C-2' on the basis of a ^2J correlation to H-1' and further ^2J HMBC correlations between C-1' and C-3' and the H-2' proton. The C-2' resonance showed a further ^3J correlation to a resonance at δ_H 3.67 (d, 1.7 Hz) this was assigned to H-4'. The corresponding carbon resonance in the HSQC spectrum was at δ_C 71.80 implying the

presence of an oxygen substituent. This substituent was determined to be an hydroxy group by comparison with literature. In turn, C-4' showed a ^{3}J correlation to a methyl group resonance at δ_H 1.23 (d, 6.2 Hz), which was assigned to H-6'. The remaining resonance in the ^{13}C NMR spectrum was at δ_C 74.83 implying the presence of an oxygen substituent. This was assigned to C-5'. The HMBC together with by comparison with literature determined C-5' and C-1' to be linked *via* an ether linkage.[4] The stereochemistry of the various substituents of the carbohydrate moiety was again determined using the NOESY spectrum in conjunction with a molecular model. No correlation in the NOESY spectrum were seen between H-3α and H-1' indicating the orientation of H-1' to be β. A positive correlation in the NOESY spectrum between H-1'β and H-2' indicated the orientation of H-2' to be β. The H-2' resonance showed a correlation with the 3'-methoxy group proton resonance indicating the orientation of the methoxy group to be β. This implied the H-3' which was also attached to C-3' was α oriented. The H-3' resonance showed a correlation in the NOESY spectrum to H-5' indicating it to be α. This resonance, in turn, showed a correlation to H-4'.

The IR spectrum supported the postulated structure. In the IR spectrum peaks were observed at 3420 cm^{-1} (O-H stretching), 2923 cm^{-1} (aliphatic C-H stretching), 1708 cm^{-1} (C=O stretching). A literature search for the compound indicated it to be rubellin isolated previously from *Drimia modesta* (Baker) Jessop (as *Urginea rubella* Baker).[4] This bufadienolide glycoside was previously isolated in 1949 but its structure was only elucidated in 1988.[4] Comparison of the NMR data proved to be difficult as they were run in different solvents and one did see discrepancies. The assignments in literature was done without the aid of 2D experiments and will require correcting. **Table 7.1** shows the ^{1}H and ^{13}C NMR data together with the HMBC, COSY and NOESY NMR data.

Table 7.1: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for rubellin (CD_3OD)

Carbon	δ_H (ppm)	δ_C (ppm)	δ_C* (ppm)	HMBC (C→H)	COSY	NOESY
1α	1.40 (d, 13.5 Hz)	47.75	47.33	9;19	1β;2	1β;9
1β	2.86 (dd, 3.5,13.5 Hz)				1α	1α;2;19
2	5.22 (brs)	72.39	74.99	1α;3;4	1β;3	1β;19
3	4.55 (dd, 1.8,8.1 Hz)	79.87	73.78	1α,β	2;4	4;5'
4	5.47 (d, 1.8 Hz)	126.98	127.56	2;6	3	3;6
5		146.52	145.16	6;19		
6	4.50 (m)	75.03	71.88	4;7α	7α,β	4;7,β
7α	1.54 (dd, 4.6, 7.4 Hz)	38.67	40.94		6;7α	6;7β
7β	2.54 (dd, 4.6, 14.3 Hz)				6;7β	6,7α
8		78.98	78.72	6;7;9		
9	1.65 (d, 12.3 Hz)	54.48	48.37	11;19	11	1α
10		41.35	38.51	1α;4;9;11;19		
11	5.21 (brs)	73.58	72.94	9	9	18;19
12		214.95	214.73	9;11;17;18		
13		63.84	63.15	16α,β;17;18		
14		86.69	86.26	16α,β;17;18		
15α	1.74 (m)	35.14	34.78		15β;16α,β	16α
15β	1.40 (m)				15α;16α,β	16β
16α	2.00 (m)	29.66	[b]	17	15α,β;16β;17	15α;16β;17
16β	1.76 (dd, 3.5,8.4 Hz)				15 α,β;16α;17	15β;16α;22
17	4.08 (t, 8.8 Hz)	42.99	42.64	18;21	16α,β	16α;18;21;22
18	1.09 (s)	20.57	18.00			11;17;21;22
19	1.72 (s)	24.12	24.14	19		1β;11
20		123.01	121.43	17;21;23		
21	7.54 (d, 2.6 Hz)	151.34	150.87	17;22	22	17;18
22	7.92 (dd, 2.6,9.7 Hz)	148.95	147.32	17;21	21;23	16β;17;18;23
23	6.34 (d, 9.7 Hz)	115.99	115.92		23	22
24		164.46	161.72	21;22;23		
1'	5.16 (d, 4.8 Hz)	98.86	98.23	2'	2'	2'
2'	4.39 (d, 4.8 Hz)	70.08	79.23	1';4'	1'	1'
3'		100.21	99.90	1';2';4;3'-OCH_3		5'
4'	3.67 (d, 1.7 Hz)	71.80	70.15	6'	5';6'	5';6'
5'	4.66 (dd, 1.7, 6.3 Hz)	74.83	71.80	1';6'	6'	3;4';6'
6'	1.23 (d, 6.3 Hz)	17.94	20.44	5';6'	5'	4';5'
OCH_3	3.34 (s)	[a]	[b]			

[a] Peak obscured by solvent.

[b] Carbon value not given in literature.; * Literature sample run in d_6-acetone.[4]

7.2.2 Structural Elucidation of Compound XX, 16β-Acetoxy-6β,12β-deoxyrubellin (riparian) (spectra 20.a-l)

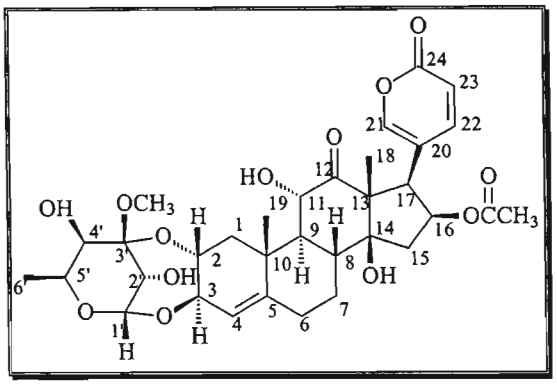

Figure 7. 4 Structure of compound XX, riparian

Compound **XX** was isolated as white crystals from the methanol extract of *Urginea riparia*. The structure derived from the NMR spectra above required a molecular formula of $C_{33}H_{42}O_{13}$. However the FAB MS showed a highest peak at *m/z* 604[Ŧ] corresponding to the loss of CH_2CO fragment. The loss of 42 mass units can occur for acetate groups.[5] This compound was found to be closely related to rubellin with a acetoxy group occurring at C-16β, and lack of hydroxy groups at C-6β and C-8β which were present in rubellin.

The ^1H NMR spectrum showed the characteristic resonances of the lactone ring of the bufadienolide skeleton (H-21, 7.56 (brs), H-22, 8.35 dd (9.9, 2.6 Hz) and H-23, 6.23 d (9.9 Hz). The NMR spectra of compound **XX** were found to be similar to those rubllein from *Drimia macrocentra*. However, the tertiary hydroxy group at C-8, and the secondary hydroxy group at C-6 were missing from this compound. This compound instead showed the presence of a acetoxy group at C-16. This deduction was made through a correlation seen in the COSY spectrum between the H-17α resonance and the resonance at 5.44 (m) which was assigned to H-16. The carbonyl carbon resonance of the acetate group also showed a ^3J correlation to the H-16 resonance and the acetate group was placed at C-16. This deduction was further confirmed with the aid of the HMBC spectrum, which showed a ^3J correlation from the C-14 resonance to the H-16 proton resonance at δ_H 5.44 (m). The stereochemistry was determined by use of the NOESY spectrum and a model. A positive correlation was seen in the NOESY spectrum between the H-17α resonance and the H-16 resonance implying H-16 was α. A correlation in the COSY spectrum between the H-9 resonance at δ_H 1.44 (m) and the resonance at δ_H 2.23 (m) led to the assignment of H-8. A correlation in the NOESY spectrum between the 3H-19 and the H-11 resonances indicated that H-11 was in the β orientation. A correlation in the NOESY spectrum between the H-11β and the H-8

[Ŧ] FAB HRMS is currently being done to confirm molecular formula.

resonances indicated that H-8 was in the β orientation. The aglycone was assigned to be a carbohydrate moiety as in the previous compound (compound **XIX**).

The infrared spectrum and ultraviolet spectrum confirmed the postulated structure. Peaks were observed at 3418 cm^{-1} (O-H stretching), 2935 cm^{-1} (aliphatic C-H stretching) and 1706 cm^{-1} (C=O stretching).

A literature search for the compound was undertaken and it was found to be a novel structure. It was named **riparian**. **Table 7.2** showed the HMBC, COSY and NOESY data.

Table 7.2: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for riparian (C_5D_5N)

Carbon	δ_H (ppm)	δ_C (ppm)	HMBC (C→H)	COSY	NOESY
1α	1.92 (d, 13.4 Hz)	45.54	19	1β;2	1β;11
1β	3.18 (d, 13.4 Hz)			1α;2	1α;19
2	5.66 (brs)	72.53	4	1α,β;3	19
3	4.85 (d, 7.7 Hz)	79.17	1';2	1;2;4	4
4	5.41 (s)	122.56		3	3
5		144.42	19		
6	2.41 (d, 11.7 Hz)	28.92		7	7
7	1.12 (m)	29.93		6;8	6;8
8	2.23 (m)	41.07	19	7;9	7;11
9	1.44 (m)[a]	53.95	11;19	8;11	
10		41.28	1;4;19		
11	4.81 (d, 11.7 Hz)	74.81	9	9	8;19
12		213.40	11;17;18		
13		63.90	15;17;18		
14		84.37	15;16;17;18		
15	1.98 (m)	40.34	16	16	16
16	5.44 (m)	73.55	15;17	15;17	15;17
17	4.67 (d, 8.8 Hz)	47.19	15;18	16	16;18;21
18	1.09 (s)	17.73			17;21;22
19	1.22 (s)	19.90	9;19		1β;2;11
20		116.38	17;21;23		
21	7.56 (brs)	152.94	17	22	17;18
22	8.35 (dd, 9.9, 2.6 Hz)	[b]	17;21	21;23	18;23
23	6.23 (d, 9.9 Hz)	113.37		22	22
24		161.63	21;23		
1'	5.71 (d, 4.8 Hz)	98.81	2'	2'; 3	2'
2'	5.21 (d, 4.8 Hz)	69.75	1';4'	1'	1'
3'		100.33	1';2';4';3'-OCH_3		
4'	4.01 (s)	71.66	6'	5'	5';6';3'-OCH_3
5'	4.97 (d, 6.3 Hz)	73.90	6';1'	4';6'	4';6'
6'	1.46 (d, 6.3 Hz)	18.44		5'	4';5'
3'-OCH_3	3.47 (s)	48.29			4'
16-$\underline{C}OCH_3$		169.78	16;16-$CO\underline{C}H_3$		
16-$CO\underline{C}H_3$	1.58 (s)	20.60			

[a] Peak obscured.
[b] Under solvent peak.

7.3 Experimental

The plant material of *Urginea riparia* Baker was collected from Lupitana Gorge in the Transkei and a voucher specimen retained (*Crouch, Styles and Van den Bergh 921*, NU).

The bulbs (3 kg) were dried, chopped into smaller pieces and extracted successively with dichloromethane and methanol by agitation on a Labcon Mechanical shaker at 140 rpm for 24 hours. The extracts obtained were then filtered and the solvent removed under reduced pressure to yield a dichloromethane extract (1.20 g) and methanol extract (15.00 g). Analysis of the crude methanol extract resulted in no compounds being isolated from this extract.

The dichloromethane extract was loaded onto a 2 cm diameter column and eluted with a step gradient solvent system collecting 100 ml fractions (100% dichloromethane fractions 1-3; 10% ethyl acetate in dichloromethane fractions 4-17; 20% ethyl acetate in dichloromethane fractions 18-25; 50% ethyl acetate in dichloromethane fractions 26-40; 100% ethyl acetate in dichloromethane fractions 41-51). Purification of fractions 26-40 using a 20% ethyl acetate in dichloromethane solvent system afforded partially pure compound **XX**. Final purification was obtained using a Sephadex column eluted with 100% methanol to afford compound **XX** at a yield of 1.1% (13 mg).

Drimia macrocentra (Baker) Jessop (syn. *Urginea macrocentra* Baker) was collected at Bushman's Nek, in the KwaZulu-Natal Drakensberg, and a voucher specimen retained a (*N. Crouch*, NH 938).

The bulbs (0.65 kg) were dried, chopped into smaller pieces and extracted successively with dichloromethane and methanol by agitation on a Labcon Mechanical shaker at 140 rpm. The extracts obtained were then filtered and the solvent removed under reduced pressure. A dichloromethane extract (3.00 g) and methanol extract (27.00 g) were obtained. TLC analysis of the dichloromethane and methanol extracts showed that they were very similar and they were combined.

The extract was loaded onto a 5 cm diameter column and eluted with a step gradient solvent system collecting 50 ml fractions (100% dichloromethane fractions 1-3; 5% ethyl acetate in dichloromethane fractions 4-14; 10% ethyl acetate in dichloromethane fractions 15-27; 50% ethyl acetate in dichloromethane fractions 28-63; 5% methanol in dichloromethane fractions 64-83; 10% methanol in dichloromethane fractions 84-107). Purification of fractions 64-83 using a 2% methanol in dichloromethane solvent system afforded partially pure compound **XIX**. Final purification was obtained using a Sephadex column eluted with 100% methanol and afforded compound **XIX** at a yield of 0.3% (15 mg).

7.3.1 Physical data for Compound XIX

Name: **rubellin**

Yield: 15 mg

Physical description: yellow gum

Optical rotation: sample was too little to obtain a stable reading.

Mass spectrum: $[M^+]$ not seen, $C_{31}H_{40}O_{13}$ requires 620.246982 g. mol^{-1}

EIMS *m/z*: 586, 530, 458, 373, 191, 149, 129, 109

Infrared: ν_{max}^{NaCl} cm^{-1}: 3420 (O-H stretching), 2923 (C-H stretching), 1708 (C=O stretching)

^1H NMR: δ_H (ppm) CD_3OD: **Table 7.1**

^{13}C NMR: δ_c (ppm) CD_3OD: **Table 7.1**

7.3.2 Physical data for Compound XX

Name: **riparian**

Yield: 13 mg

Physical description: brownish gum

Optical rotation: +18.18 (c, 0.022 in methanol)

Mass spectrum: $[M^+]$ not seen, $C_{33}H_{42}O_{13}$ requires 646.262542 g. mol^{-1}

EIMS: 604 $[M^+-CH_2CO]^+$, 430, 390, 191, 149, 136, 123

Infrared: ν_{max}^{NaCl} cm^{-1}: 3418 (O-H stretching), 2935 (C-H stretching), 1706 (C=O stretching)

^1H NMR: δ_H (ppm) C_5D_5N: **Table 7.2**

^{13}C NMR: δ_c (ppm) C_5D_5N: **Table 7.2**

7.4 References

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Chapter 8: Screening Results and Conclusion

The various compounds isolated in the current study were screened in the cancer, anti-oxidant and inflammatory cell line screens. These cell lines were chosen due to their availability at the time of the study.

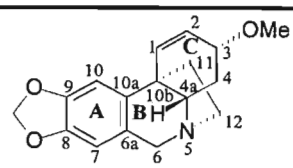
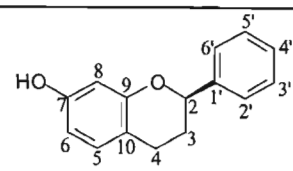
8.1 Anti-cancer Screening

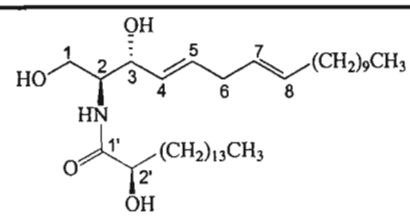
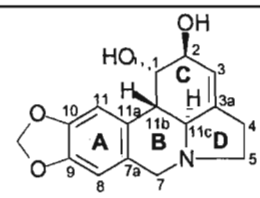
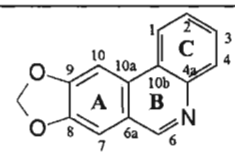
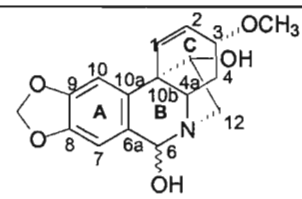
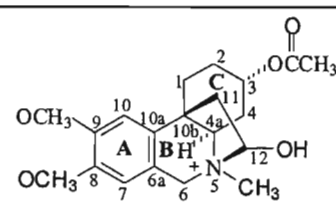
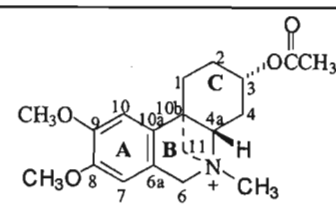
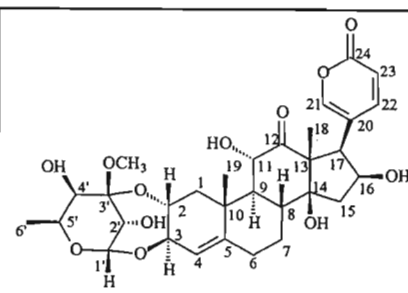
The anti-cancer bioassays were undertaken at the CSIR Bio/Chemtek laboratories in Pretoria under the supervision of Dr. V. Maharaj.

The three cell line prescreening method, which was developed by the National Cancer Institute (USA), was used in testing compounds isolated for anticancer activity. The three cell line prescreen identifies a large proportion of the compounds that would be inactive in multi-dose 60 cell line screening.[1] Computer modeling indicates that approximately 50% of compounds can be eliminated by this prescreen without a significant decrease in the ability to identify active agents, while increasing the throughput and efficiency of the main cancer screen with limited loss of information.[1] The three cell lines utilized were MCF7 (breast), TK10 (renal) and UACC62 (melanoma).

These results are presented as dose-response curves for each sub panel (melanoma, breast, renal). The dose-response curve is a plot of the PG (percent growth) against the concentration for each cell line. The response parameters GI_{50} (50% growth inhibition), TGI (total growth inhibition) and LC_{50} (lethal concentration killing 50%), are interpolated values from these graphs representing the concentrations at which PG is +50, 0 and -50 respectively. **Table 8.1** gives the TGI values for compounds tested.

Table 8.1. Cancer Screening Results

		Structure	Total Growth Inhibition (ppm)
Brunsvigia natalensis	I		Inactive in all three cell lines
	III		~55 (melanoma, breast) ~60 (renal)

	IV		Inactive in all three cell lines
Crinum stuhlmannii	V		<<6.25 (melanoma) ~ 39 (renal) 100 (breast)
	VI		Inactive in all three cell lines
	VII		20 (melanoma) Inactive in breast and renal cell lines
	XIII		100 (breast, melanoma) Inactive in renal cell line
	XIV		Inactive in all three cell lines
Urginea riparia	XX		<<<3.5 (renal) <<<7 (breast) << 10.5 (melanoma)

Compounds **I**, **IV**, **VI**, **XIV** **(Figure 8. 1-4)** were all inactive in the three cell lines tested.

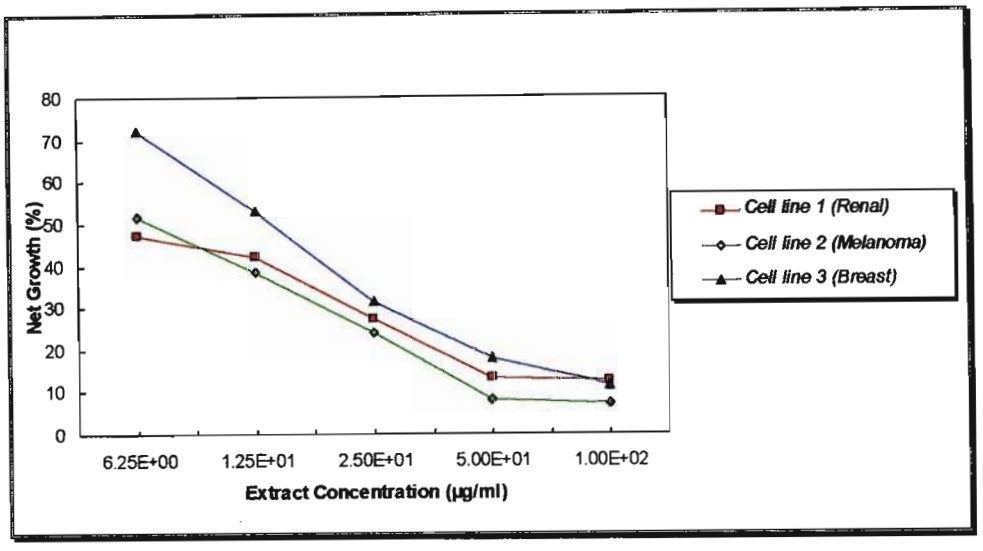


Figure 8. 1 Dose-response curves of compound I

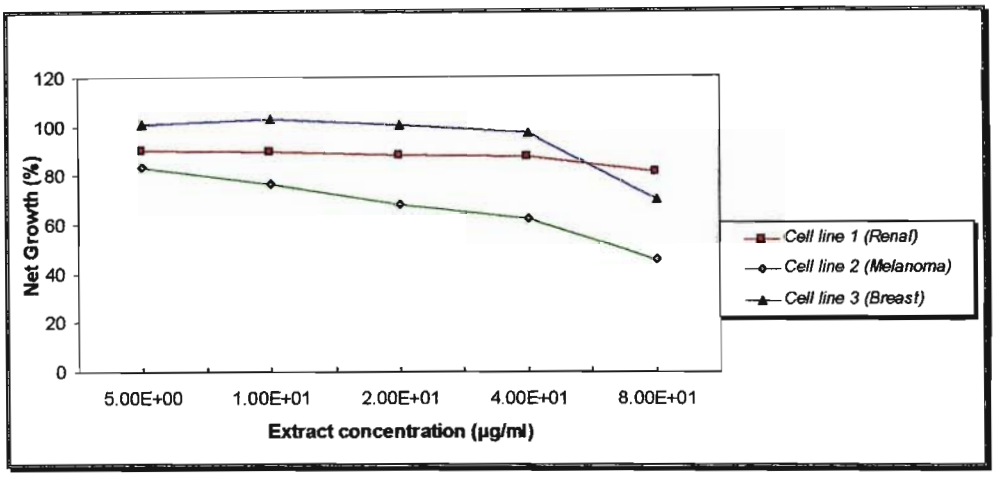


Figure 8. 2 Dose-response curves of compound IV

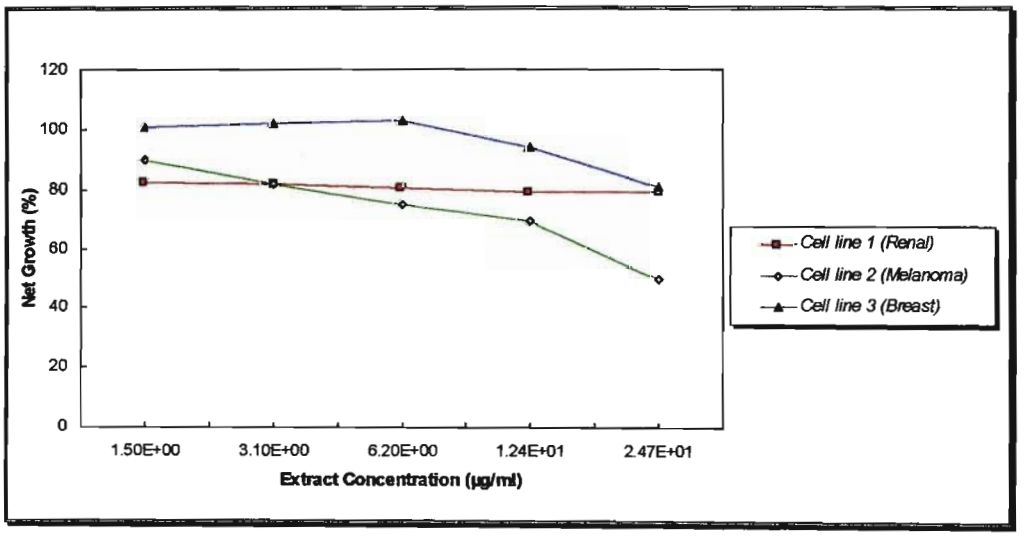


Figure 8. 3 Dose-response curve of compound VI

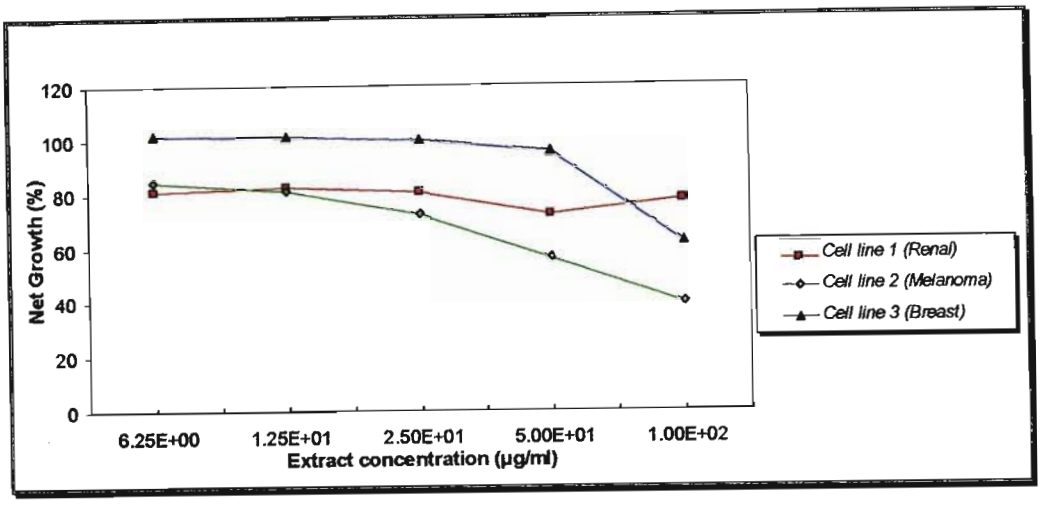


Figure 8. 4 Dose-response curves of compound XIV

Compound **III (Figure 8. 5)** showed TGI values of approximately 55 ppm in the melanoma and breast cell lines and approximately 60 ppm in the renal cell lines. These results indicate that compound III has weak anti-cancer activity.

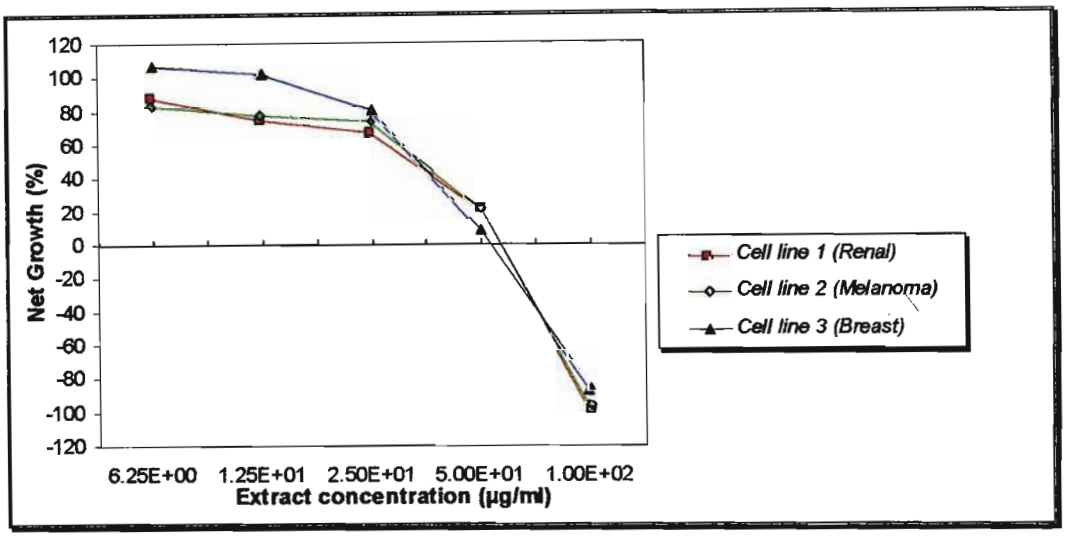


Figure 8. 5 Dose-response curves of compound III

Compound **V (Figure 8. 6)** showed TGI values of < 6.25 ppm in the melanoma cell line and approximately 39 ppm and 100 ppm activity in the renal and breast cell lines respectively. These results indicate moderate to weak anti-cancer activity. The different graphical profiles of the three different cancer cell lines against compound **V** indicate the possibility of compound **V** being non - cytotoxic. All cells being killed at very similar concentration values and at very low concentrations is indicative of cytotoxicity.

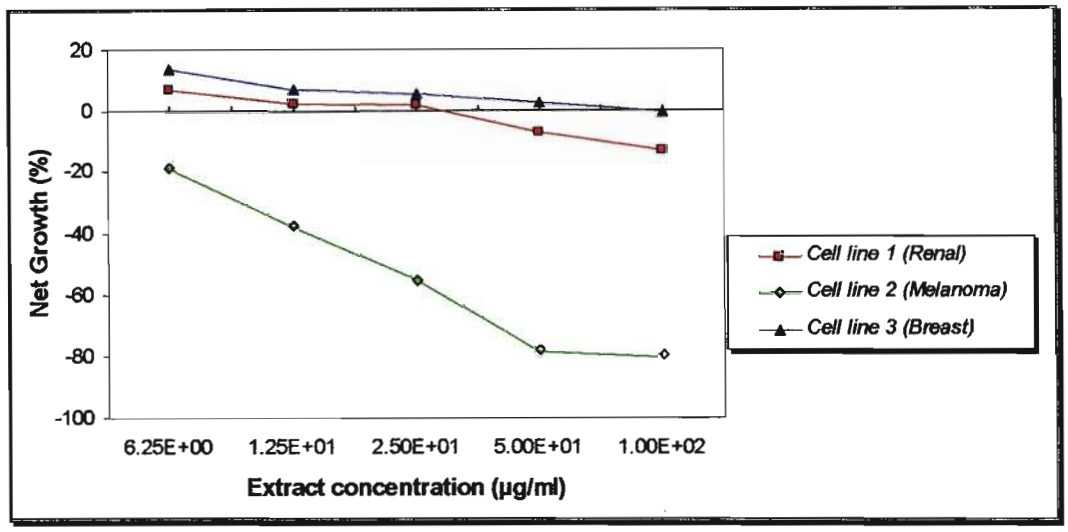


Figure 8. 6 Dose-response curves of compound V

Compound **VII (Figure 8. 7)** showed TGI values of 20 ppm in the melanoma cell line only and was inactive in the breast and renal cell lines. These results indicate very weak anti-cancer activity.

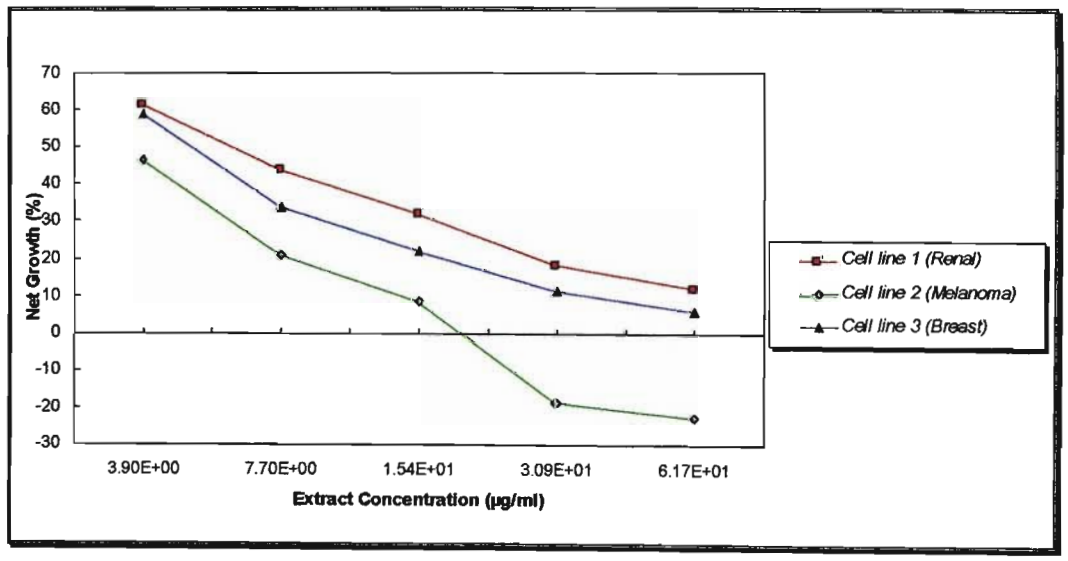


Figure 8. 7 Dose-response curves of compound VII

Compound **XIII (Figure 8. 8)** showed TGI values of 100 ppm in the breast and melanoma cell lines and was inactive in the renal cell line. These results indicate very weak anti-cancer activity.

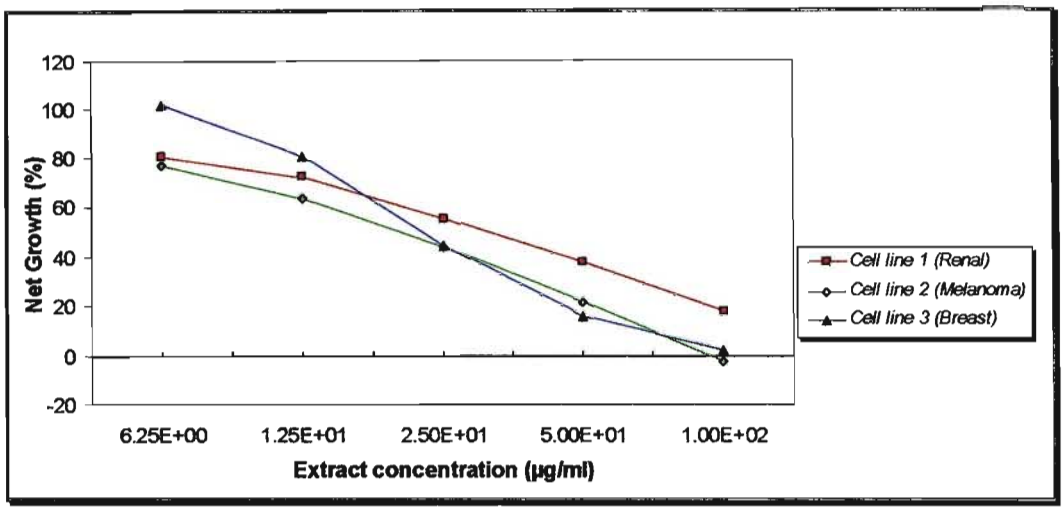


Figure 8. 8 Dose-response curves for compound XIII

Compound **XX (Figure 8. 9)** showed TGI values of <3.5 ppm in the renal, <7 ppm in the breast and <10.5 ppm in the melanoma cell line. These results indicate moderate anti-cancer activity. The different graphical profiles of the three cell lines against compound **XX** indicate the possibility of its being non-cytotoxic. All cells being killed at very similar concentration values and at very low concentrations is indicative of cytotoxicity. However bufadienolide compounds are known to be cytotoxic and separate cytotoxicity tests need to be performed on this compound.

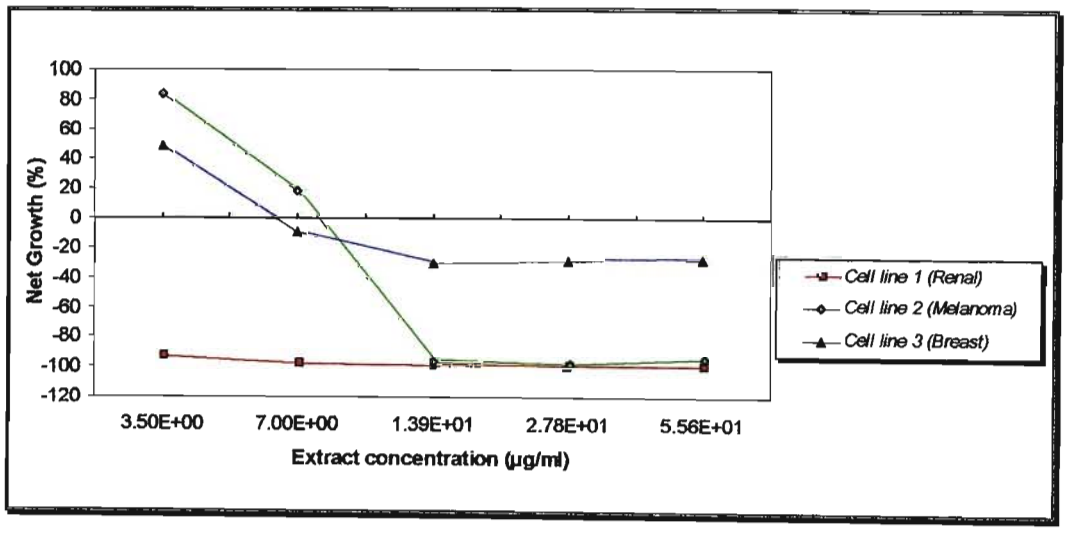


Figure 8. 9 Dose-response curves of compound XX

8.2 Anti-oxidant Screening

The anti-oxidant bioassays were carried out at the CSIR Bio/Chemtek laboratories in Pretoria under the supervision of Dr V. Maharaj.

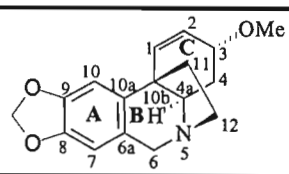
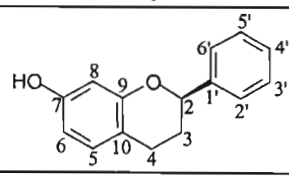
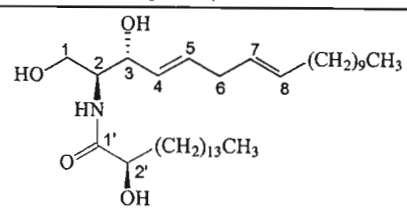
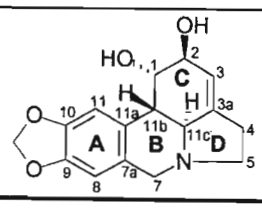
The DPPH (2,2-diphenyl-1-picrylhydrazyl radical) method was used to determine the antioxidant activity. This test has become widely used since its publication in 2000.[2] It involves the measurement of the disappearance of DPPH• into the reduced form (DPPH-H):

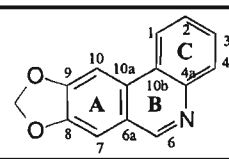
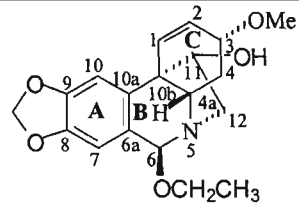
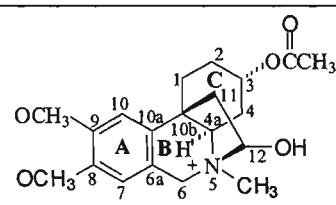
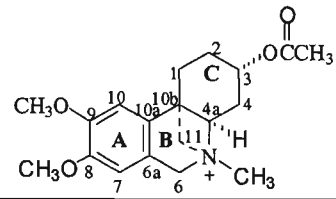
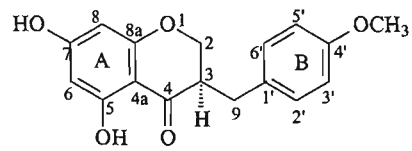
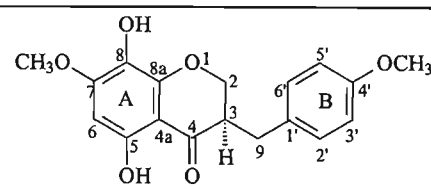
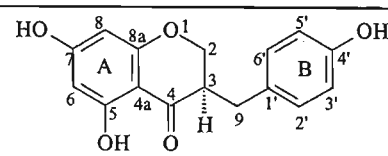
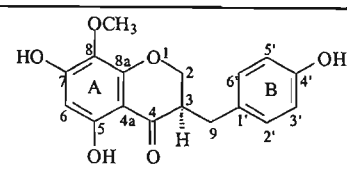
$$DPPH^{\bullet}\ (purple) + (AH)_n \longrightarrow DPPH\text{-}H\ (yellow) + (A^{\bullet})_n$$

Usually the colour change from purple to yellow is evaluated by measuring the disappearance of the purple colour at 515 nm but it can also be recorded by monitoring the appearance of the yellow product.

Each sample (35 µl, 100 ppm) was added to a well in an ELISA plate, which contains 315 µl of 90 µM DPPH dissolved in methanol. The plate was covered in aluminum foil and left to stand at room temperature for 1 hour. The radical scavenging capacities of the samples were determined by using a 340 ATC ELISA Plate Reader to measure the disappearance of DPPH at 515 nm.

Table 8.2. Anti-oxidant Assay Results using DPPH

Source		Structure	% Decolourisation of DPPH at 100 ppm
Brunsvigia natalensis	I		19.48
	III		13.12
	IV		8.98
	V		23.42

Crinum stuhlmanni	VI		17.67
	VIII		19.75
	XIII		6.77
	XIV		20.62
Ledebouria revoluta	XV		11.24
	XVII		9.61
	XVI		11.43
	XVIII		97.46

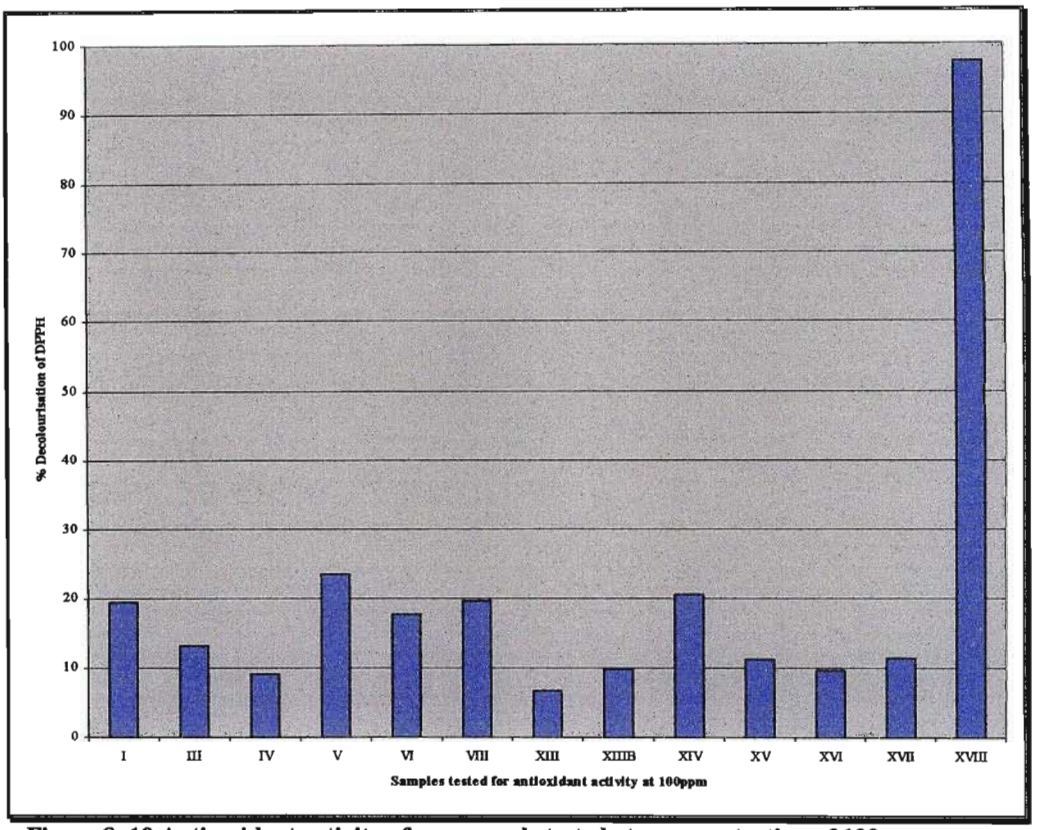


Figure 8. 10 Anti-oxidant activity of compounds tested at a concentration of 100 ppm

The anti-oxidant activity results were found to be low **(Table 8.2 and Figure 8. 10)** at 100 ppm, except for compound **XVIII** which showed a 97.48% decolourisation of the DPPH radical. Further tests, varying the concentration of compound **XVIII** (5,7-dihydroxy-8-methoxy-3-(4'-hydroxybenzyl)-4-chromanone) were conducted.

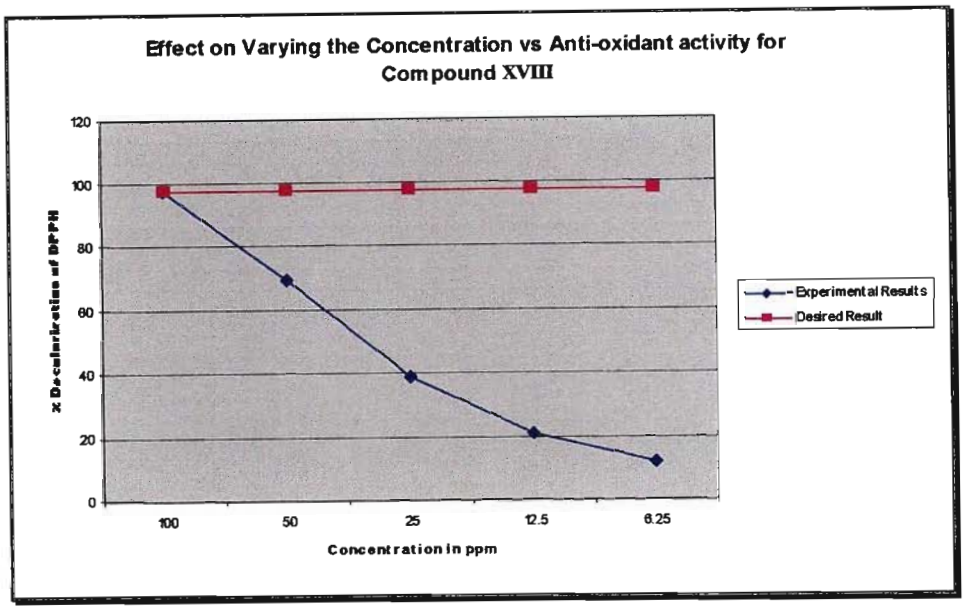


Figure 8. 11 Dose-response curve of compound XVIII in antioxidant assay

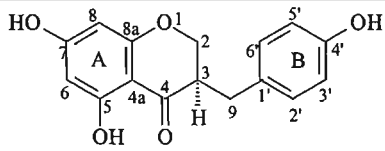
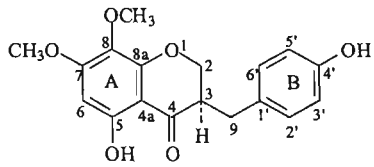
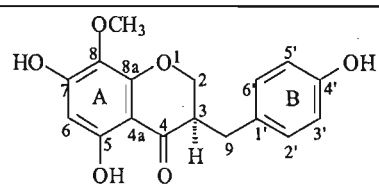
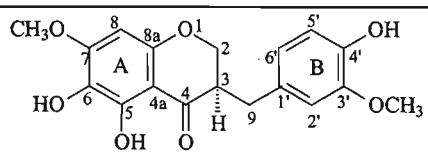
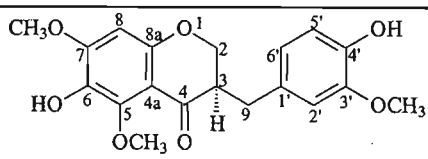
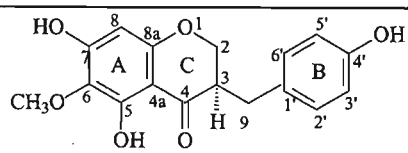
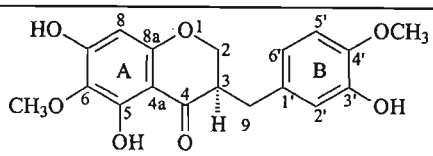
As the concentration of the compound **(Figure 8. 11)** is decreased the anti-oxidant activity decreases. A more desirable result would have seen the anti-oxidant activity remain high at lower concentrations for compound **XVIII**. This was not observed **(Figure 8. 11)**, and further tested were not undertaken.

8.3 Anti-Inflammatory Screening

The anti-inflammatory activity screening involved the use of microsomal cells and the enzymes, cyclooxygenase (COX-1 and COX-2). Enzyme assays are especially useful in evaluating the effects of traditional remedies for inflammation and pain related to the production of prostaglandins.[4] COX-1 and COX-2 are the enzymes responsible, in the arachidonic acid cascade, for the formation of prostaglandins. Non-steroidal, anti-inflammatory drugs (NSAIDS) act by inhibiting the activity of COX. The COX assay is an example of a mechanism-based assay that uses enzymes to detect inhibitors of inflammation. This bioassay tests for the presence of NSAIDS by measuring the degree of inhibition of COX enzyme activity. The percentage inhibition is measured against indomethacin as a positive control.

The COX-1 and 2 bioassays as well as the microsomal cell tests were carried out by a colleague, Karen du Toit at the University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Prof. J. van Staden.

Table 8.3. Anti-Inflammatory Assay Results for Microsomal cells, COX-1 and COX-2

		Structure	%Inhibition		
			Microsomal cells	COX-1	COX-2
Ledebouria revoluta	XVI		b	53	14
	XVII		b	29	a
	XVIII		b	29	a
Ledebouria zebrina[†]	XXIII		70	31	2.5
	XXIV		46	a	a
	XXV		68	11.5	14
	XXVI		70	19	12

[a] no inhibition
[b] tests were not done on this compound

[†] Compounds were isolated from this plant during my M.Sc. work.[6]

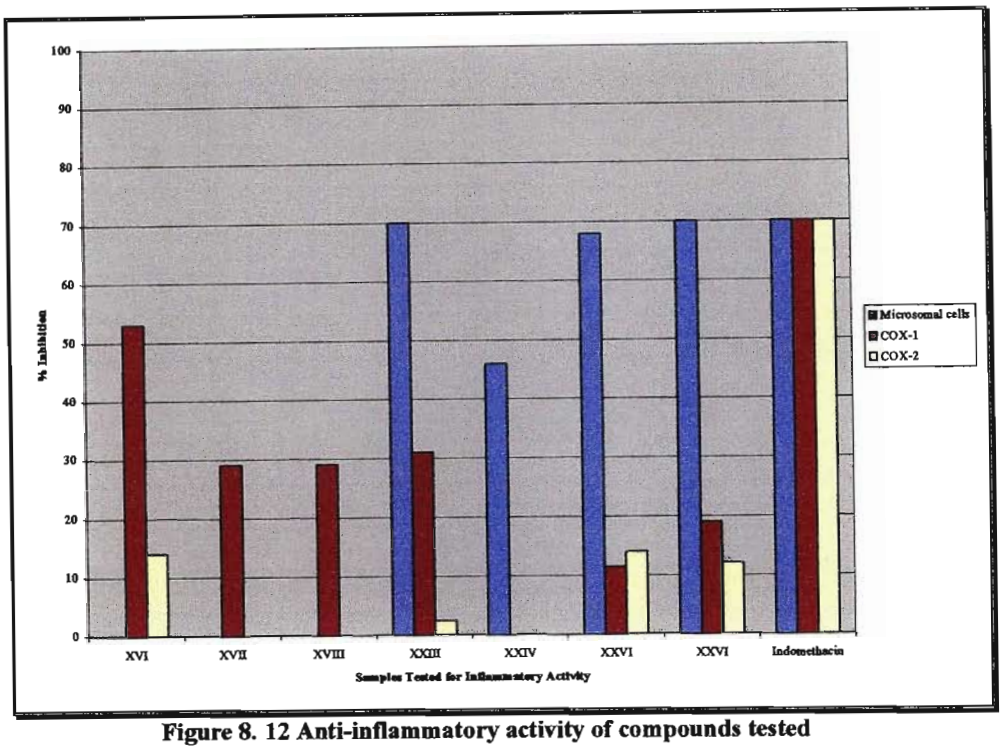


Figure 8. 12 Anti-inflammatory activity of compounds tested

The results for the microsomal cell tests were good **(Figure 8. 12)**. However the results for COX-1 and COX-2 inhibition were low compared to the control. The control used was indomethacin, which had an inhibition of 70%. By changing the functional group at C-5 from a hydroxy group (compound **XXIII**) to a methoxy group (compound **XXIV**) the percentage inhibition dropped by 24% for the microsomal cell and the activity in the COX-1 assay decreased from 31% to 0% activity for **XXIV**. Compound **XVI**, which had three hydroxy substituents on the structure, showed good inhibition of COX-1.

8.4 Conclusion

Twenty one compounds were isolated during this study, ten compounds were isolated for the first time and four plants (possibly five) were also studied phytochemically for the first time.

Two representatives of the Amaryllidaceae were investigated. The phytochemistry of *Brunsvigia natalensis* was studied for the first time and yielded four compounds, two novel alkaloids, one flavanoid and a novel ceramide type of compound **(Figure 8. 13)**. This is the first reported occurrence of a ceramide type compound in the Amaryllidaceae family.

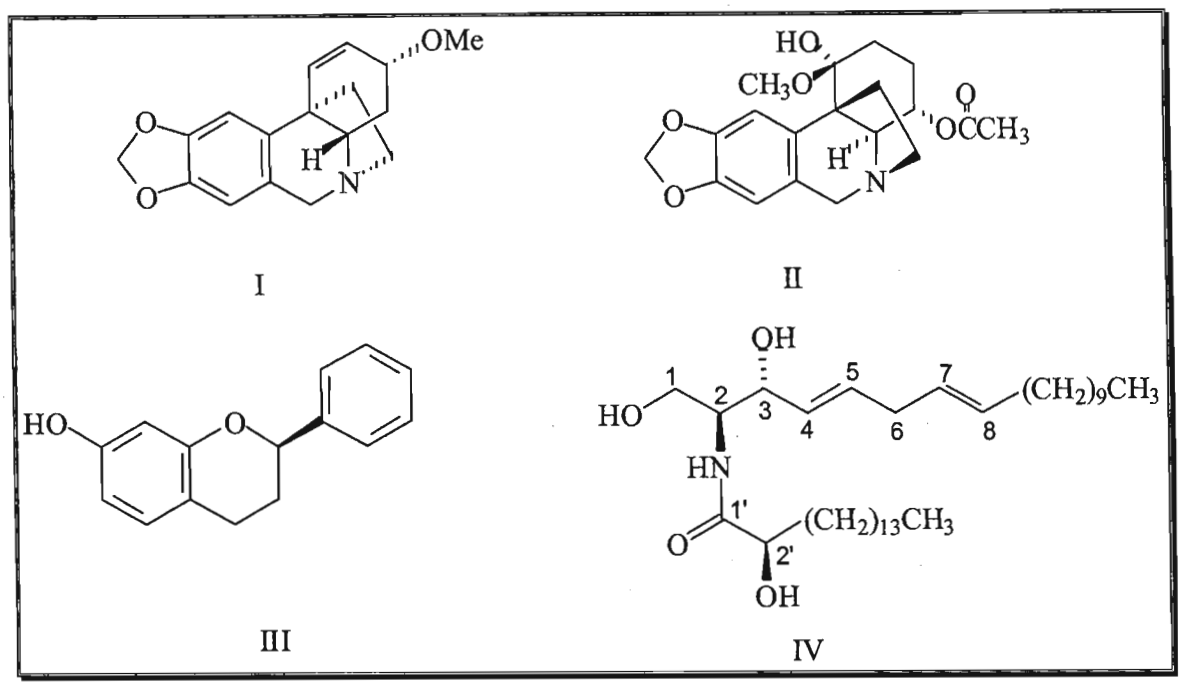


Figure 8. 13 Compounds isolated from *Brunsvigia natalensis*

The study on *Crinum stuhlmannii* was undertaken as a previous one by Nair *et al.*[5] reported on material collected from a region (Natal Midlands) not likely to fall within the natural distribution of this species. As the botanical voucher proved untraceable, an attempt was made to verify the earlier report. Secondly, a comparative study of the bulbs and seeds was carried out to identify organ-specific production of chemical constituents in an amaryllid. This led to the isolation of eleven alkaloids **(V-XIV) (Figure 8. 14)**. 6-Hydroxycrinamine and lycorine were the only two constituents common to the current and earlier study. In the previous study Nair *et al.*[5] isolated a novel alkaloid named delagoenine in their study of the bulbs, which at that stage was the only reported crinane type alkaloid possess a hydroxy group at C-12. In the current study the *N*-methyl derivative of delagoenine (Compound **XIII**) was isolated which leads one to conclude that possibly the bulbs studied in the previous study were in fact the same. In the present study, the only constituent common to bulbs and seeds of *C. stuhlmannii* was 6-hydroxycrinamine and this was isolated in fairly large yields from both sources. Notably, none of the novel alkaloids (stuhlmanine A, B, C, D or *N*-methyl delagoenine), were isolated from both sources, indicating that these constituents are organ-specific.

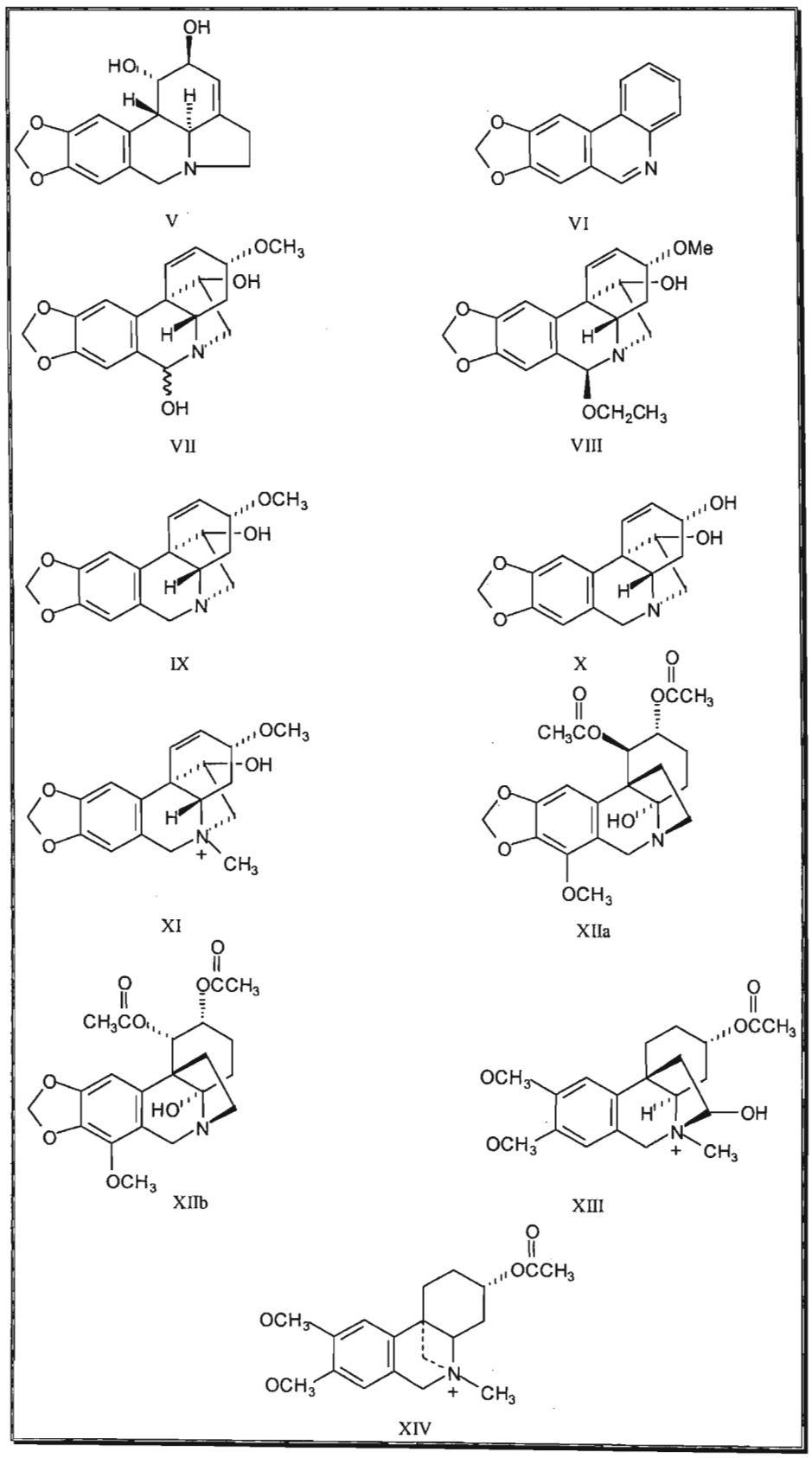


Figure 8. 14 Compounds isolated from *Crinum stuhlmannii*

Representatives of two of the southern African Hyacinthaceae sub-families were investigated in this work. *Ledebouria revoluta* (Hyacinthoideae) yielded four known homisoflavonoids **(Figure 8. 15)**.

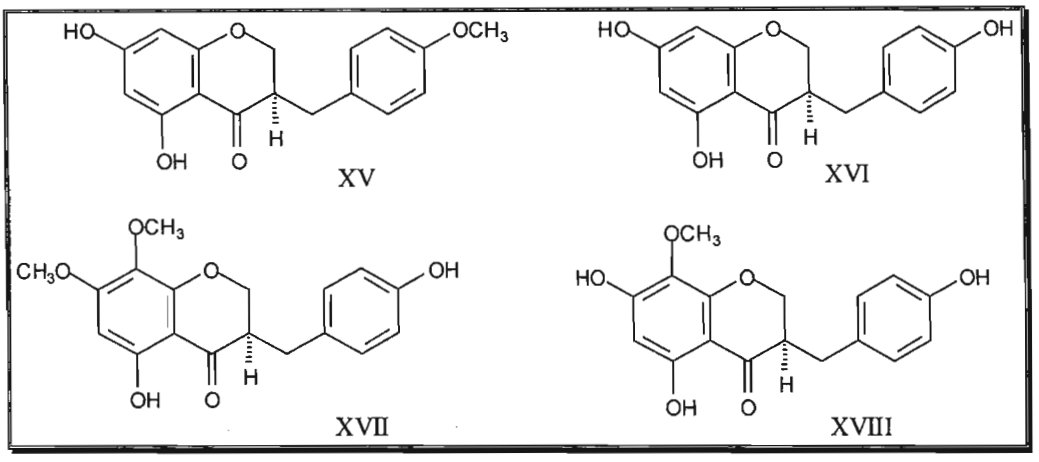


Figure 8. 15 Compounds isolated from *Ledebouria revoluta*

Drimia macrocentra and *Urginea riparia* (Urgineoideae) yielded one bufadienolide glycoside **(Figure 8. 16)** each, with the unusual attachment at two positions between the glycone and agylcone.

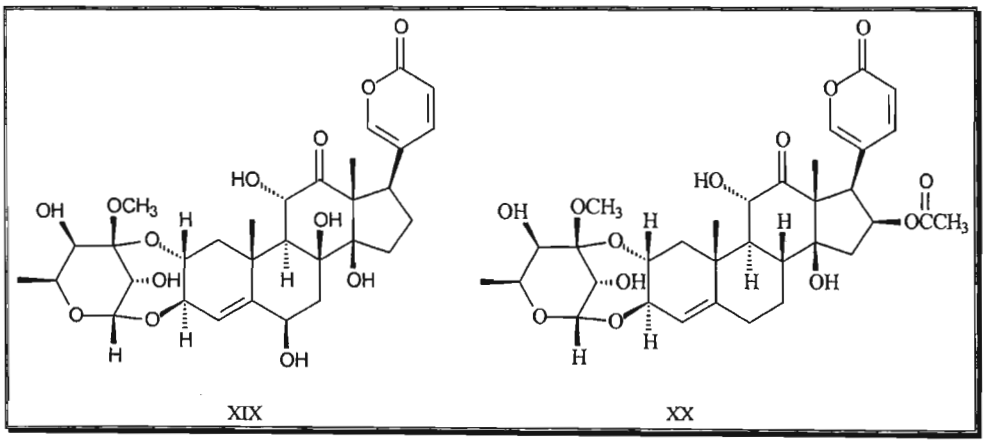


Figure 8. 16 Compounds isolated from *Drimia macrocentra* and *Urginea riparia*

The compounds isolated were also tested for activity in cancer, anti-oxidant and anti-inflammatory cell lines. None showed significant activity warranting further analysis.

The study of natural product chemistry however is still motivated by the potential discovery of new drugs that could be developed for use in clinical medicine.

8.5 References

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6. Moodley, N., 2002. The chemical investigation of *Ledebouria zebrina* and *Scilla natalensis*. MSc. dissertation. University of Natal, Durban, S.A., pp. 42-80.

APPENDIX A

LIST OF SPECTRA

Compound I:

Compound II:

Compound III:

Compound IV:

Compound V:

Compound VI:

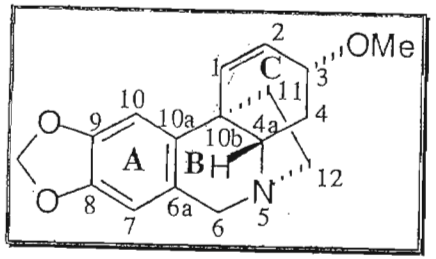
Compound VII:

Compound VIII:

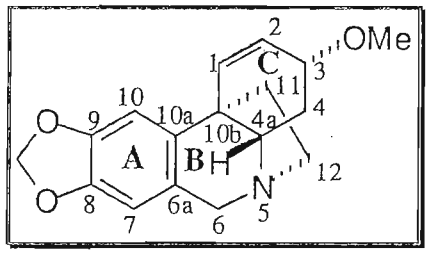
Compound IX:

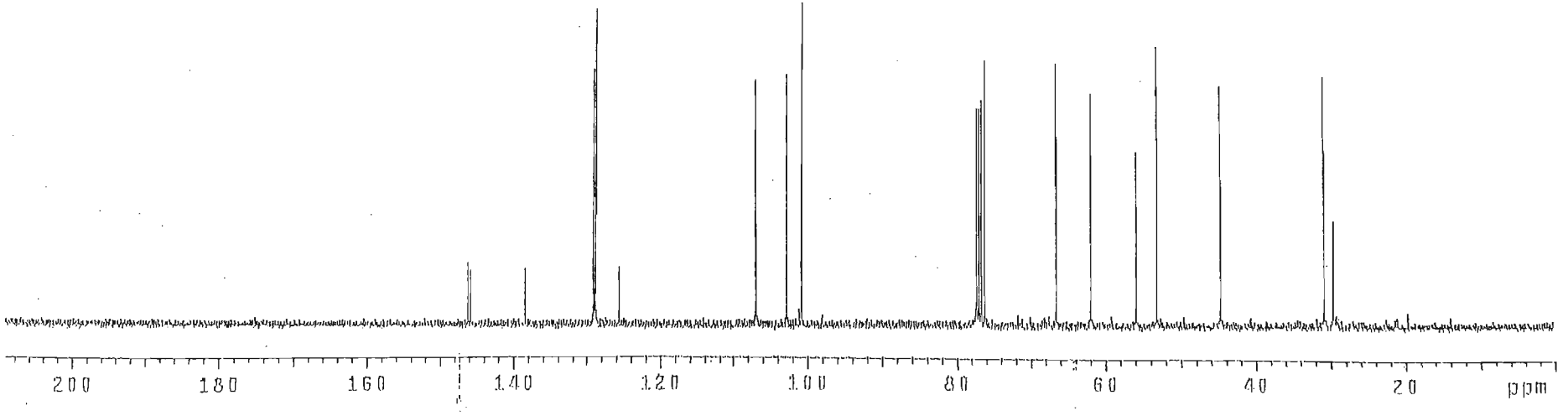
Compound X:

Compound XX:

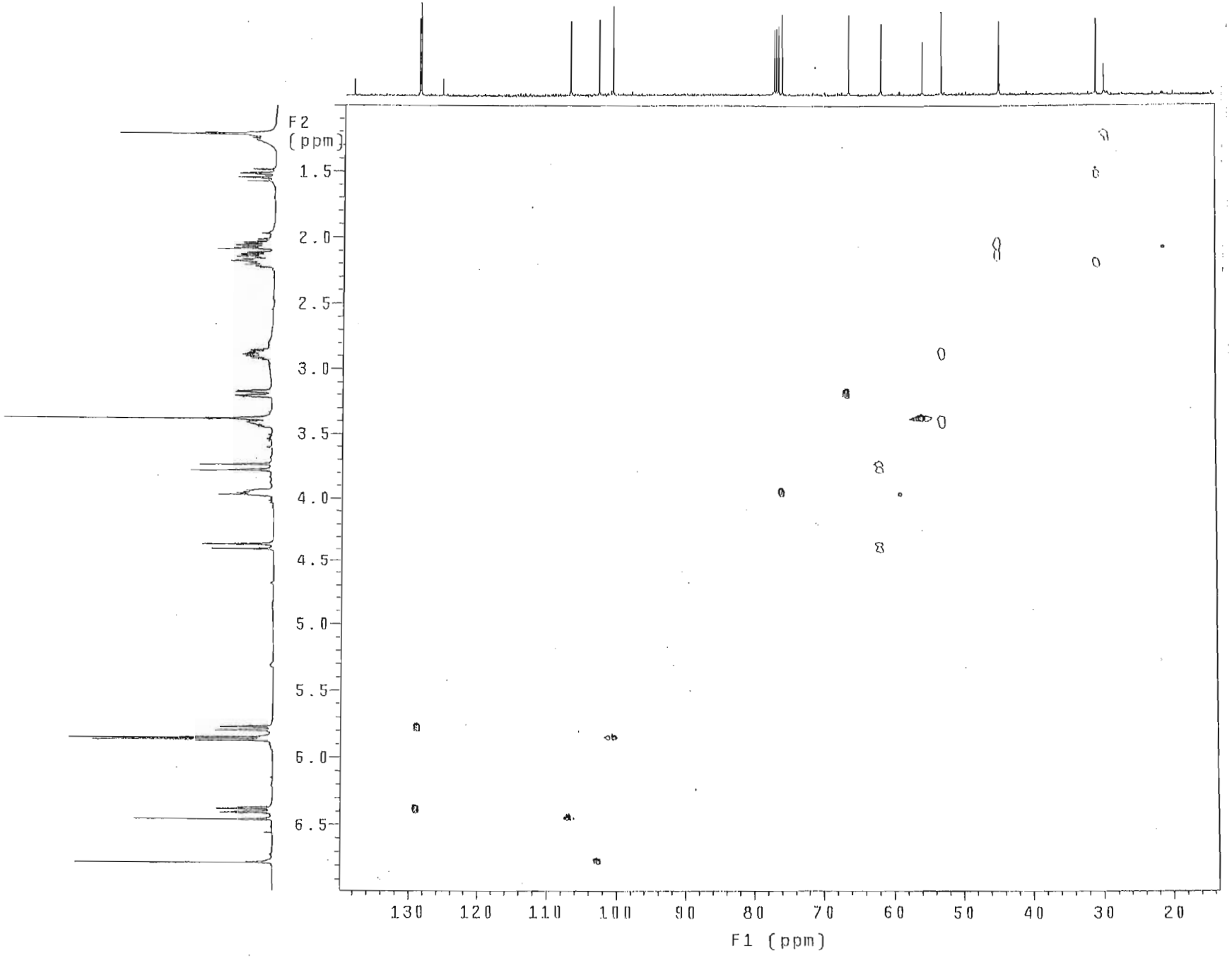


SPECTRUM I.a: ^{1}H NMR spectrum of compound I ($CDCl_3$)

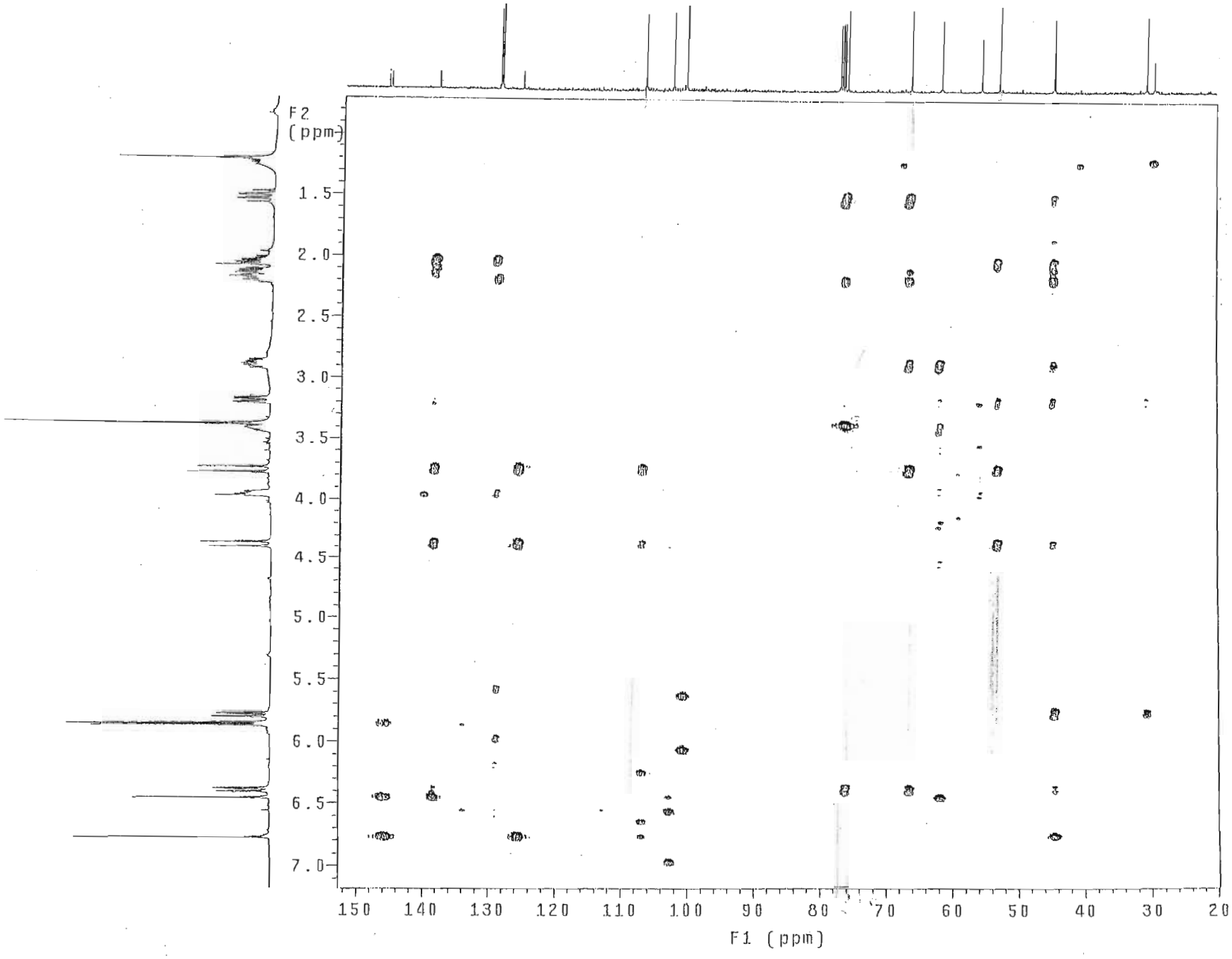




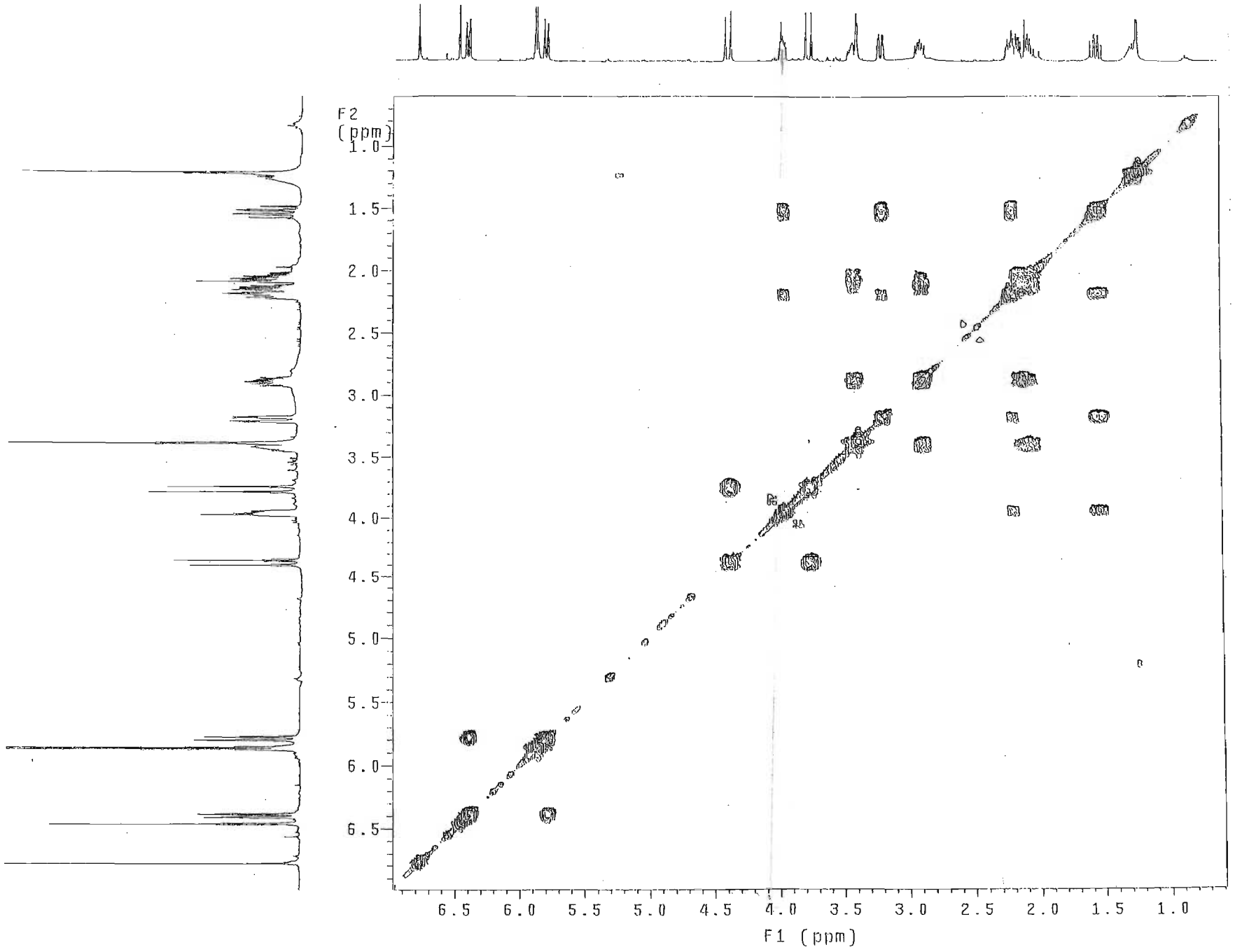
SPECTRUM I.b: ^{13}C NMR spectrum of compound I ($CDCl_3$)



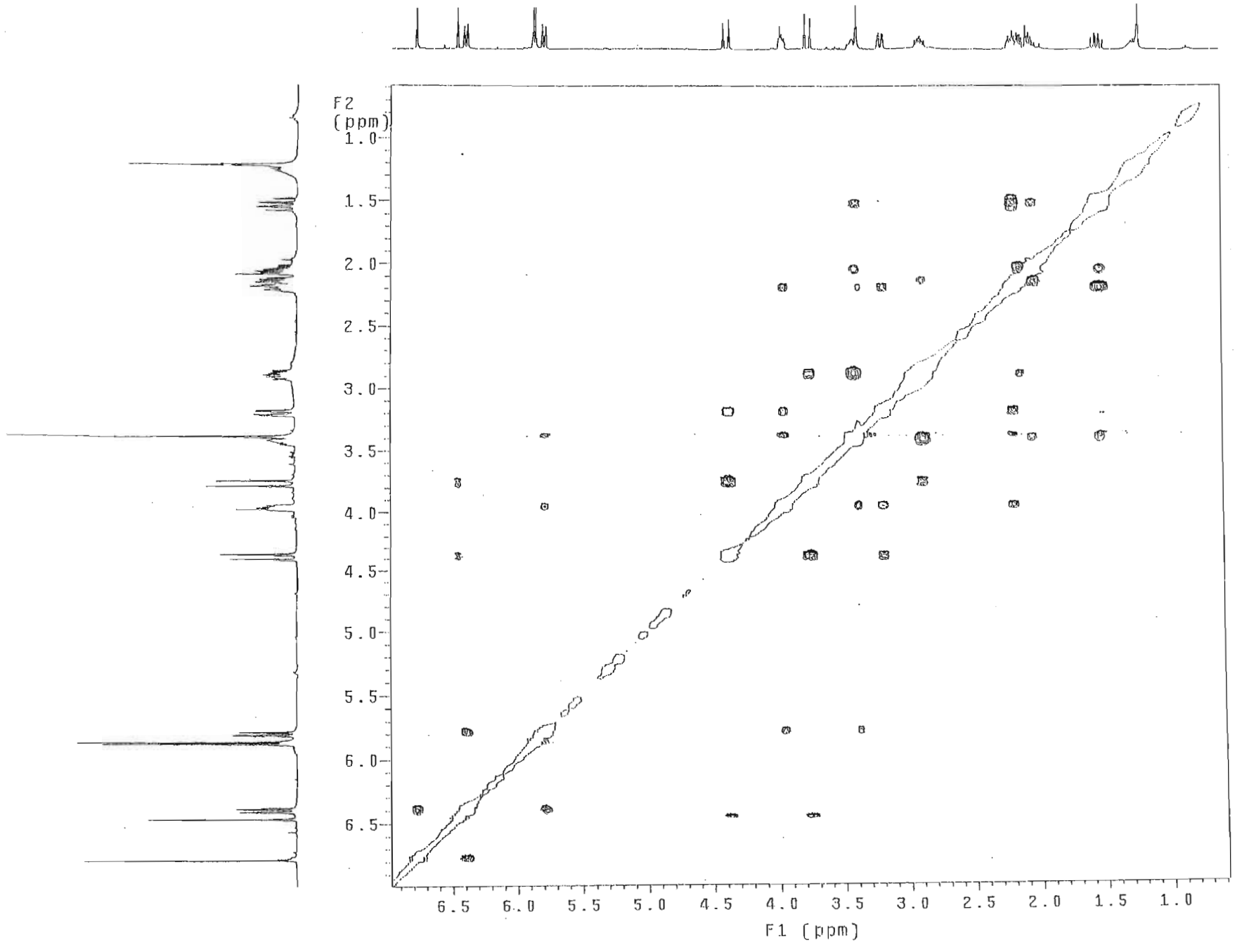
SPECTRUM 1.c: HSQC spectrum of compound I ($CDCl_3$)



SPECTRUM 1.d: HMBC spectrum of compound I ($CDCl_3$)



SPECTRUM 1.e: COSY spectrum of compound I ($CDCl_3$)



SPECTRUM 1.f: NOESY spectrum of compound I ($CDCl_3$)

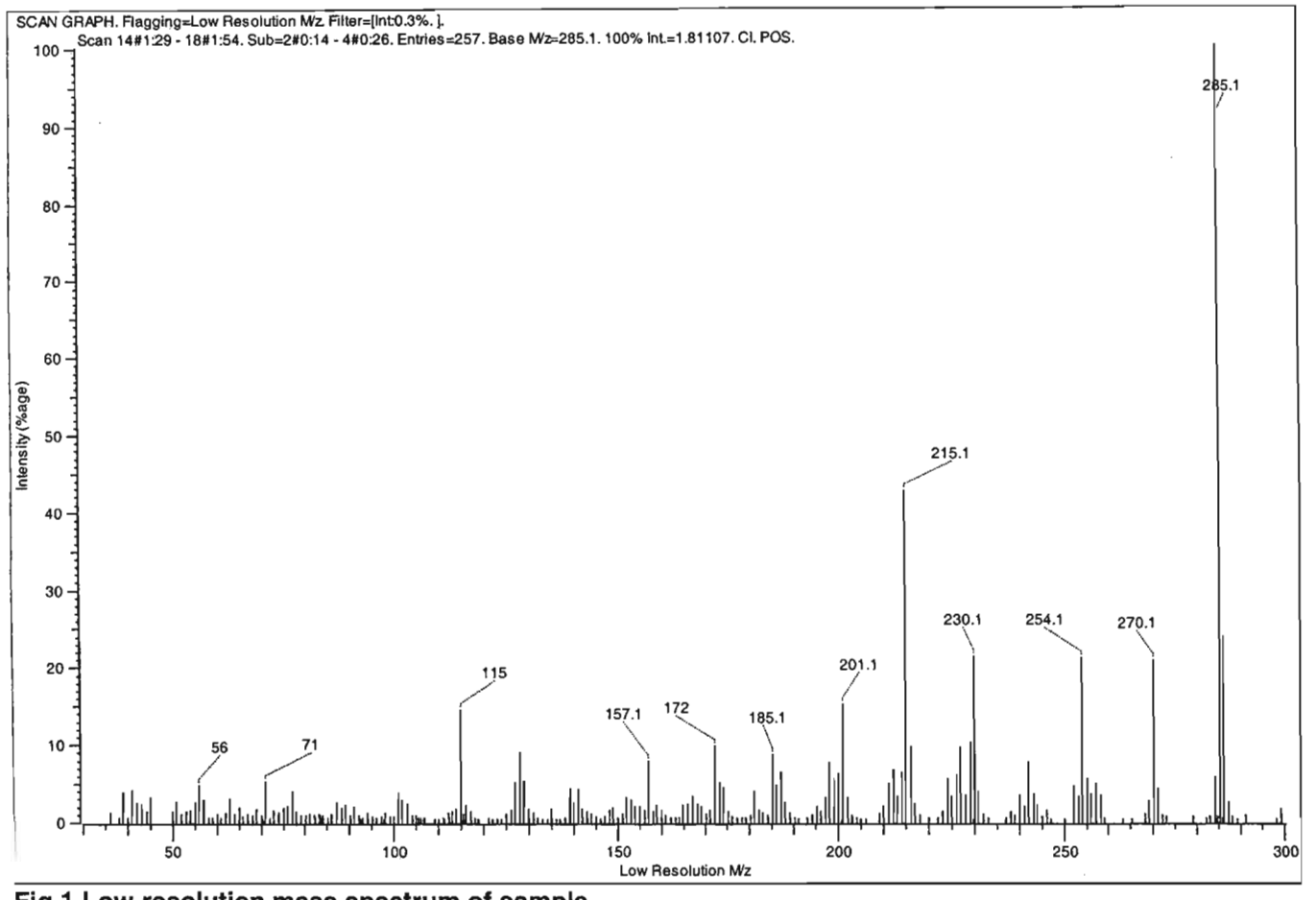
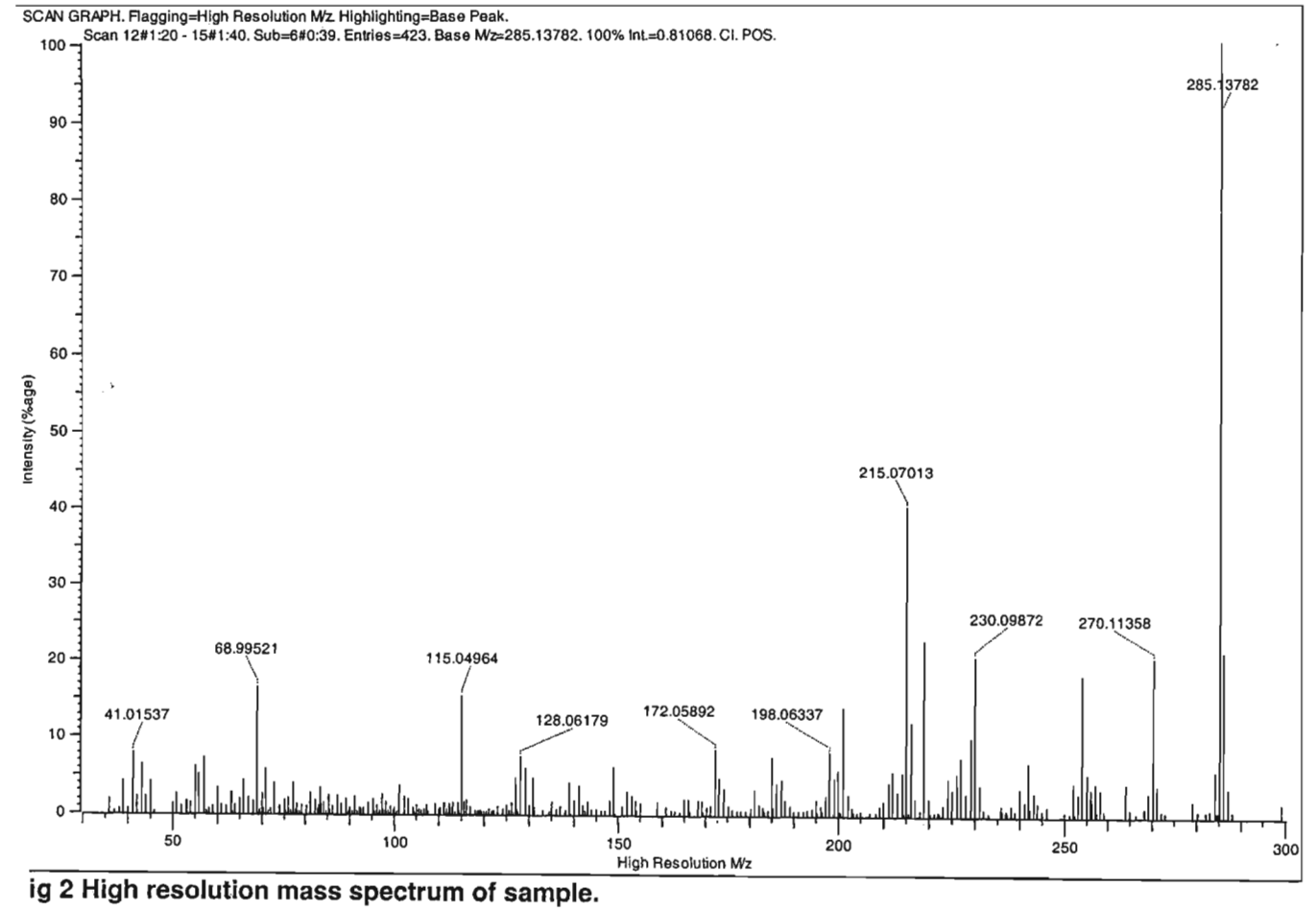
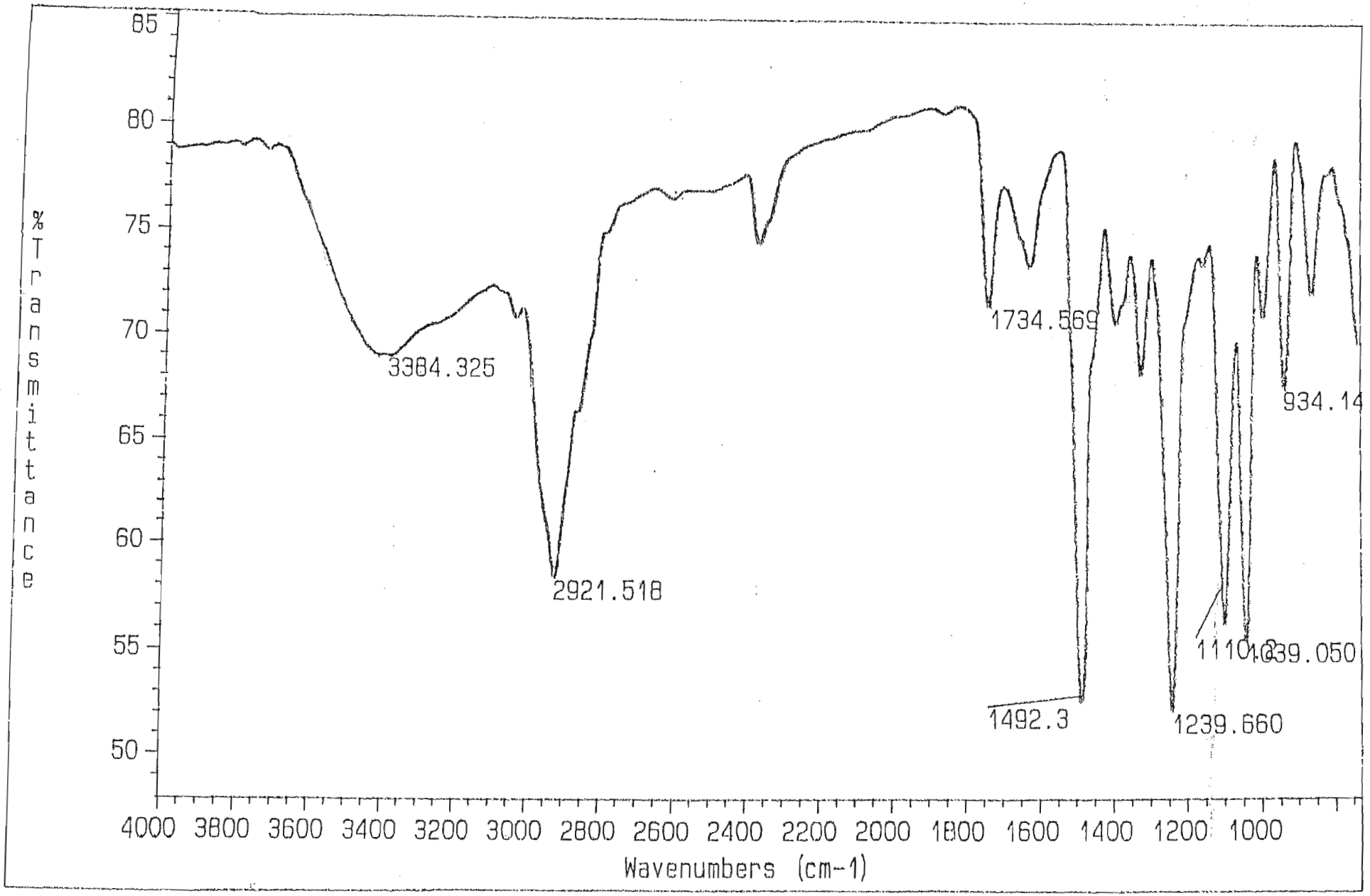


Fig 1 Low resolution mass spectrum of sample.

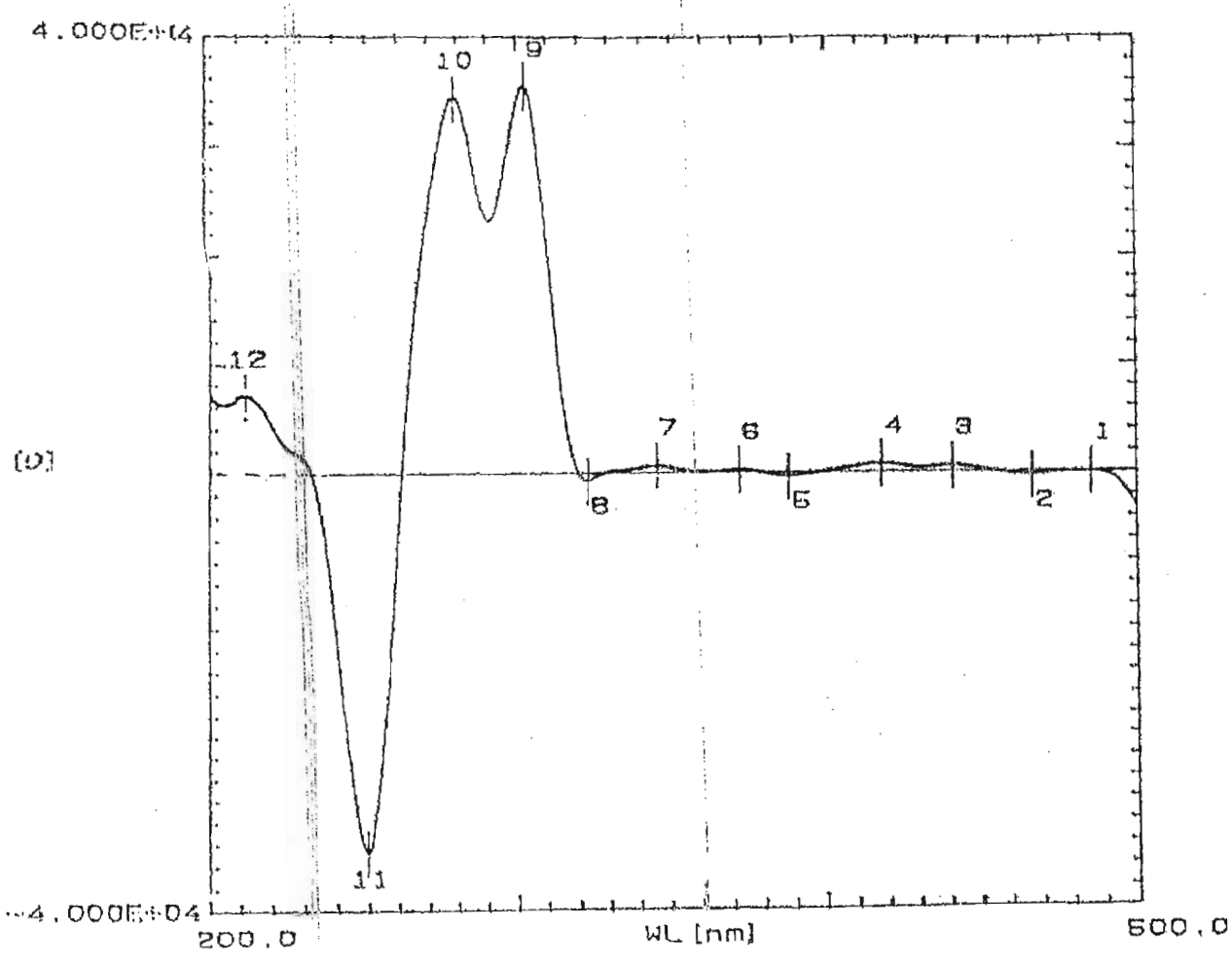


ig 2 High resolution mass spectrum of sample.

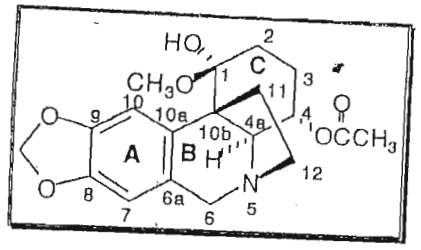
Spectrum 1.g: Mass spectra of compound I



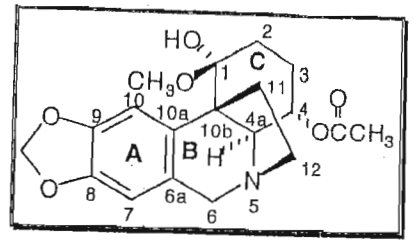
SPECTRUM 1.h: Infrared spectrum of compound I



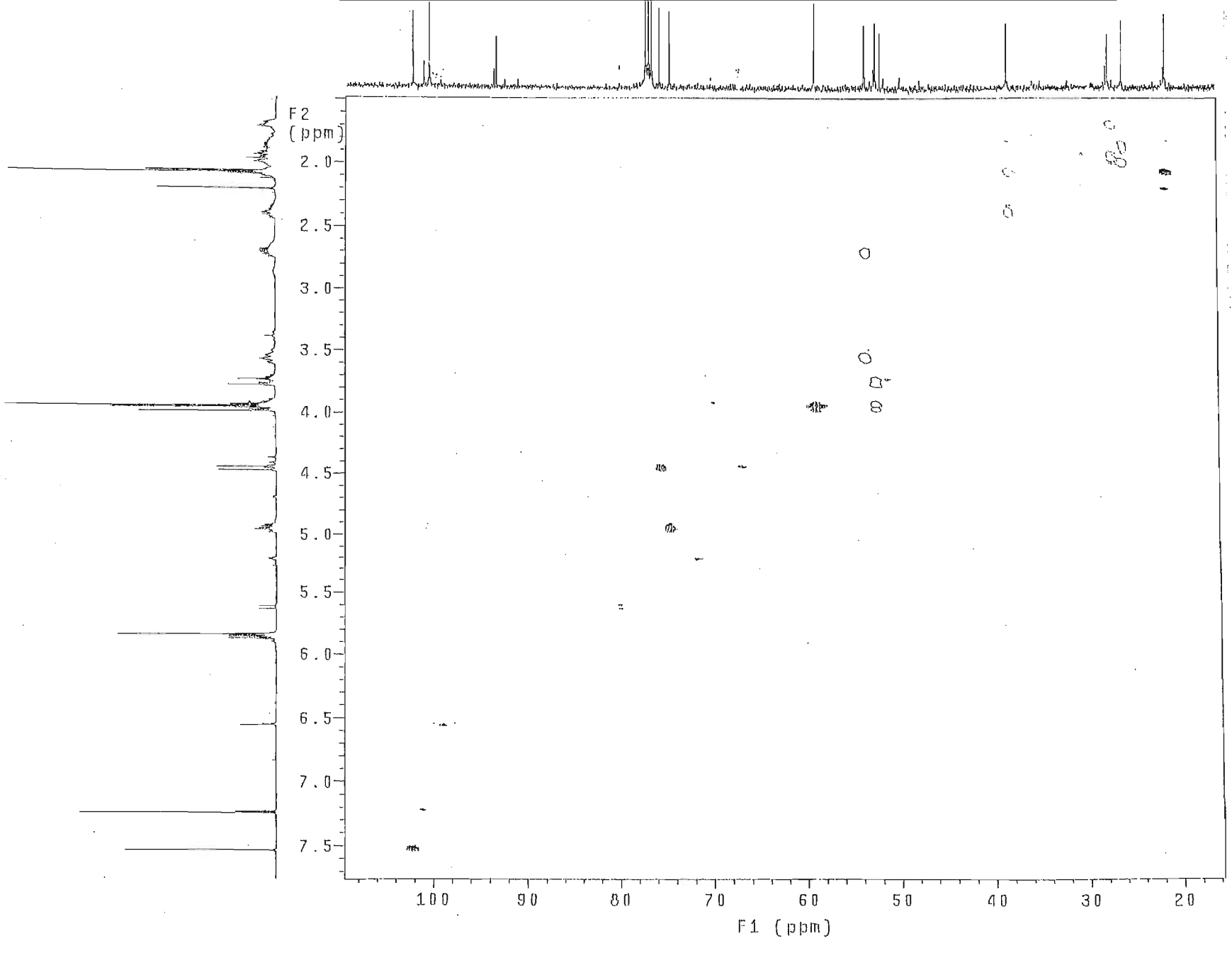
SPECTRUM 1.i: CD spectrum of compound I



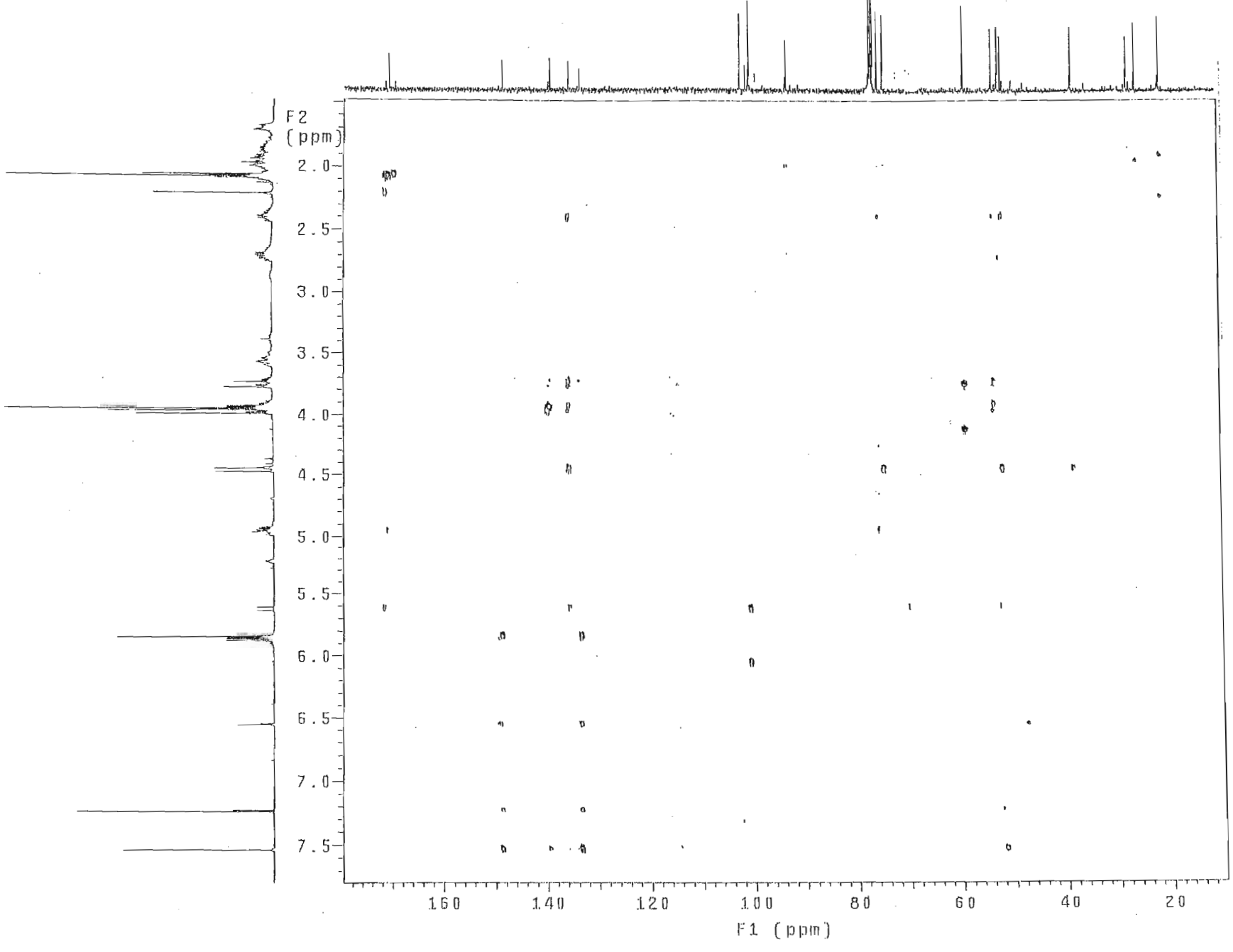
SPECTRUM 2.a: ^{1}H NMR spectrum of compound II ($CDCl_3$)



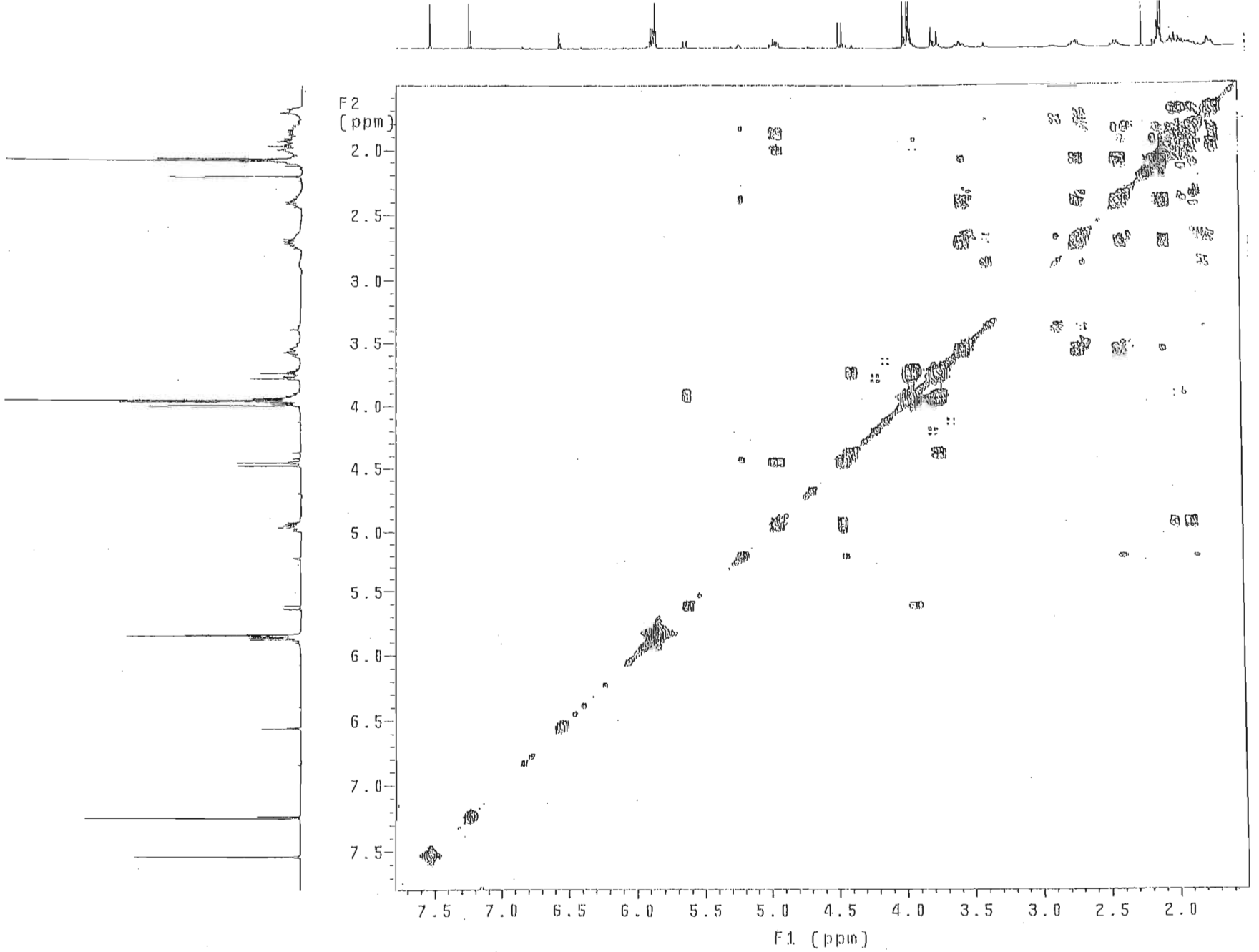
SPECTRUM 2.b: ^{13}C NMR spectrum of compound II ($CDCl_3$)



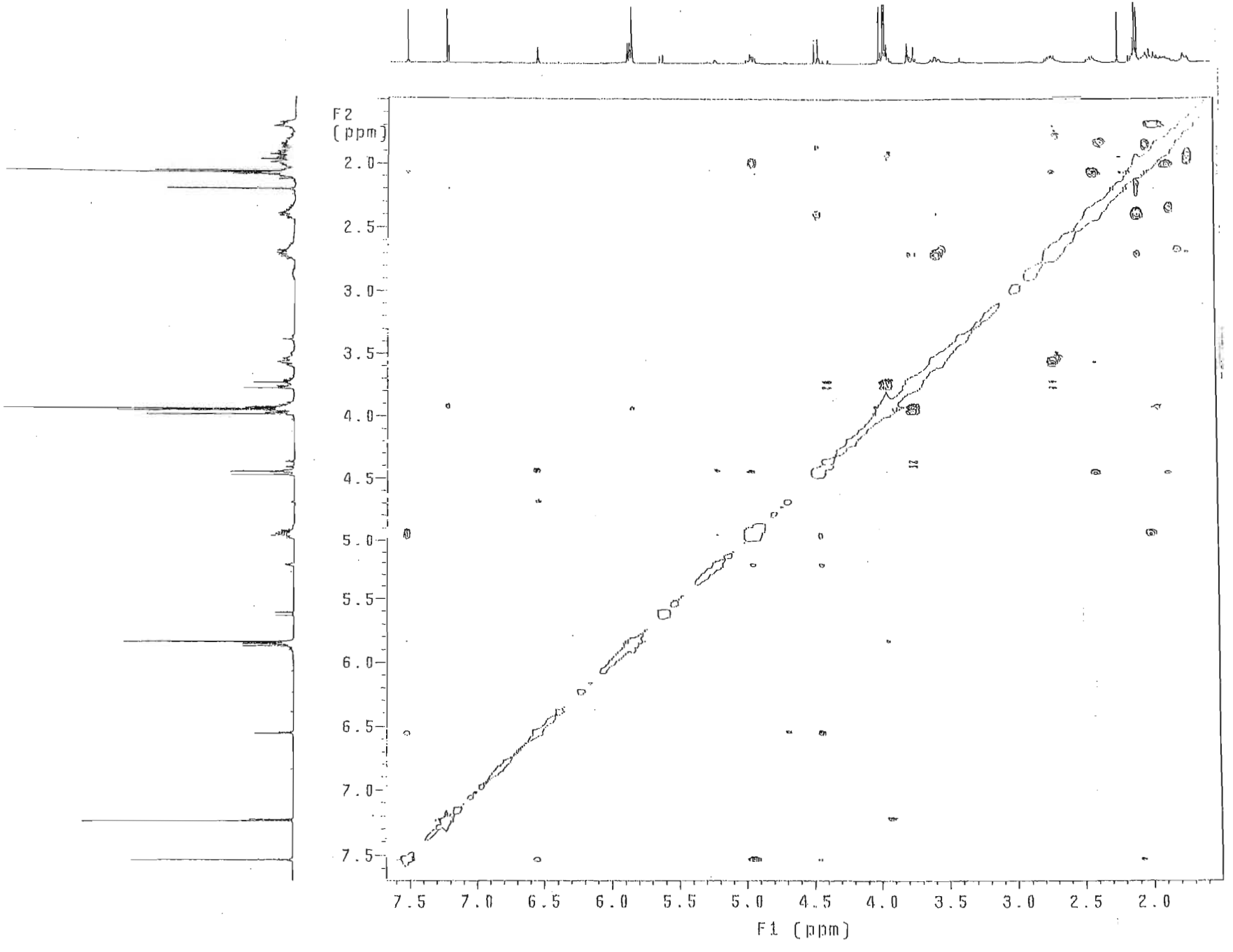
SPECTRUM 2.c: HSQC spectrum of compound II ($CDCl_3$)



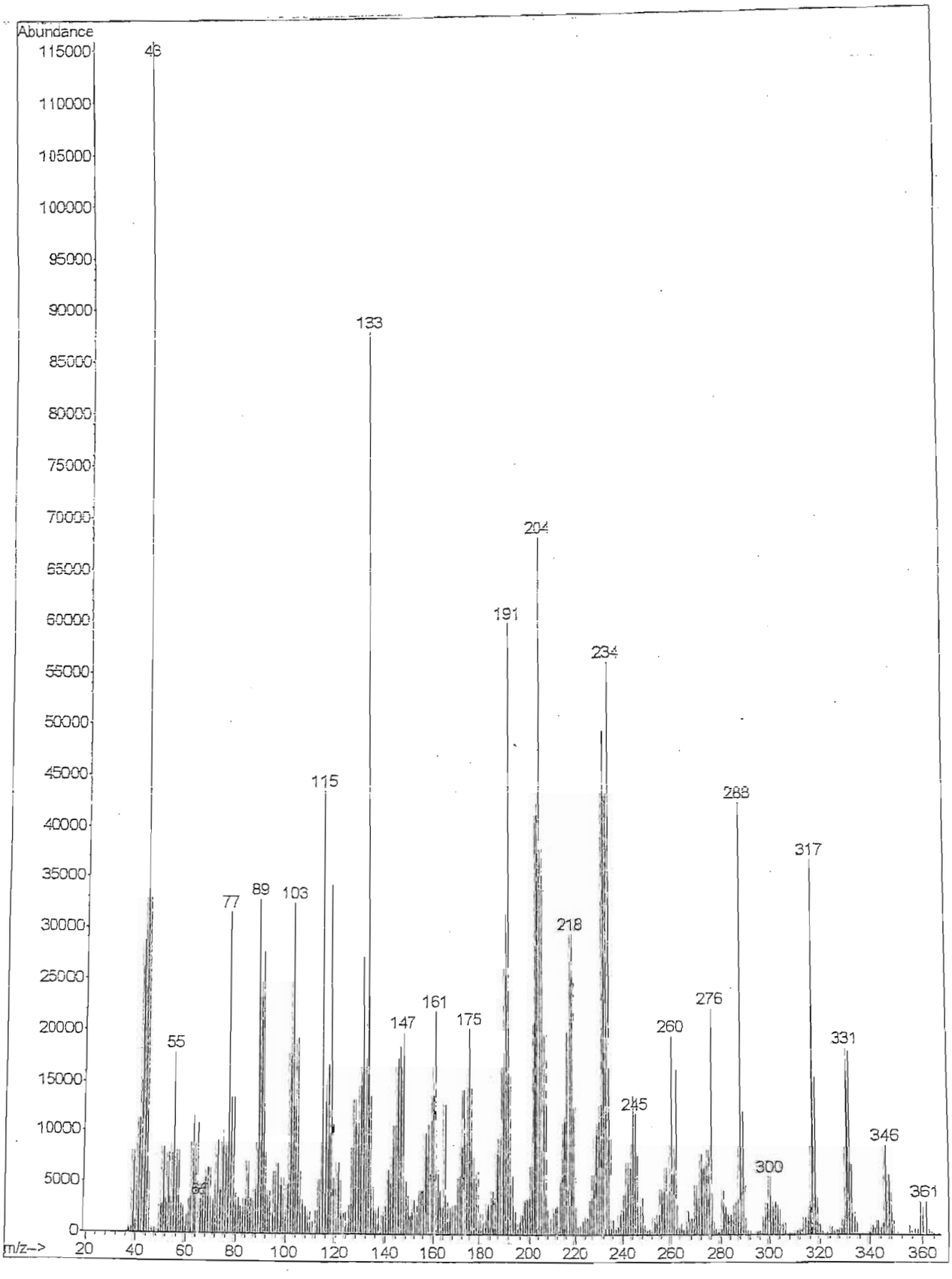
SPECTRUM 2.d: HMBC spectrum of compound II ($CDCl_3$)



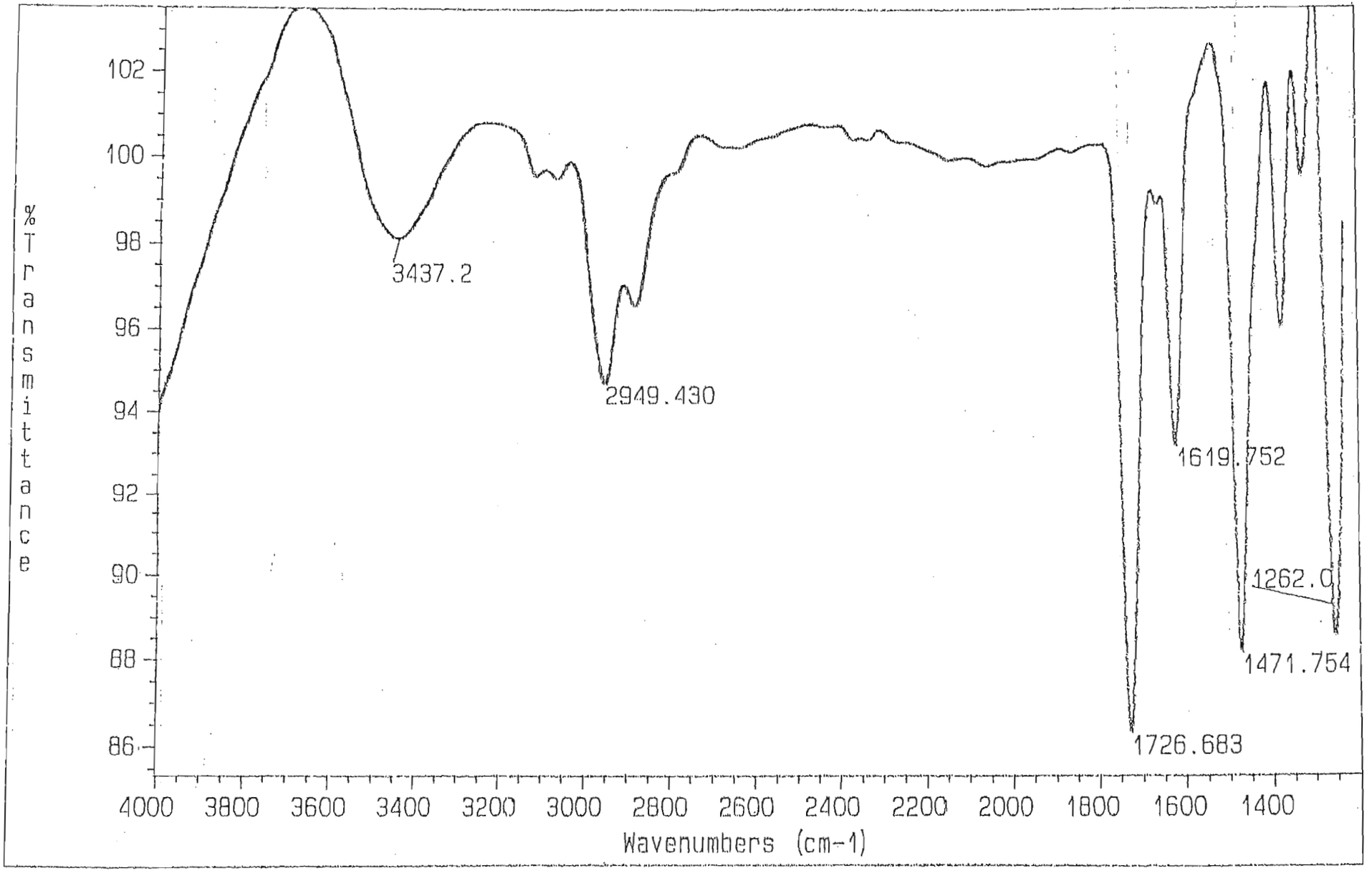
SPECTRUM 2.e: COSY spectrum of compound II ($CDCl_3$)



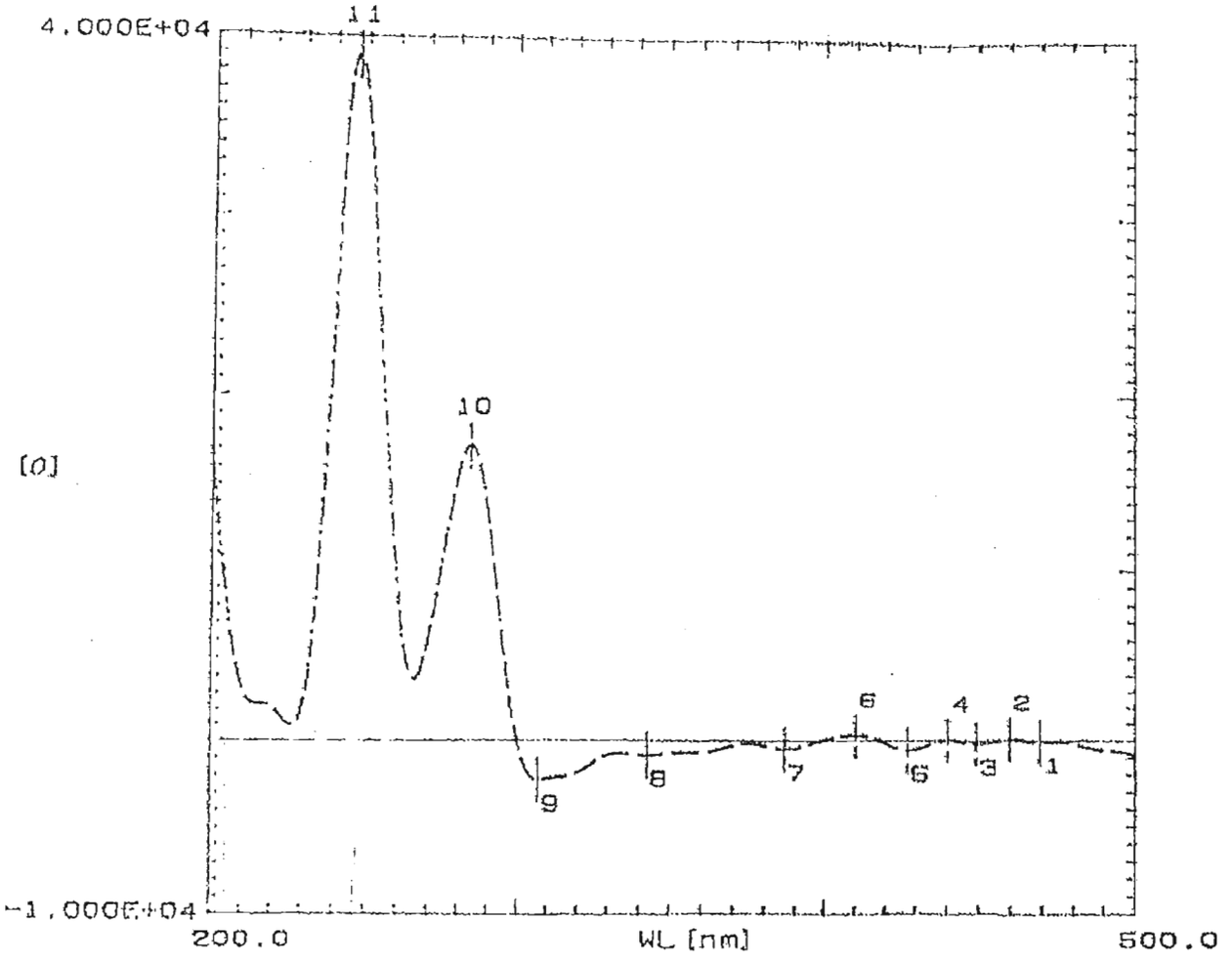
SPECTRUM 2.f: NOESY spectrum of compound II ($CDCl_3$)



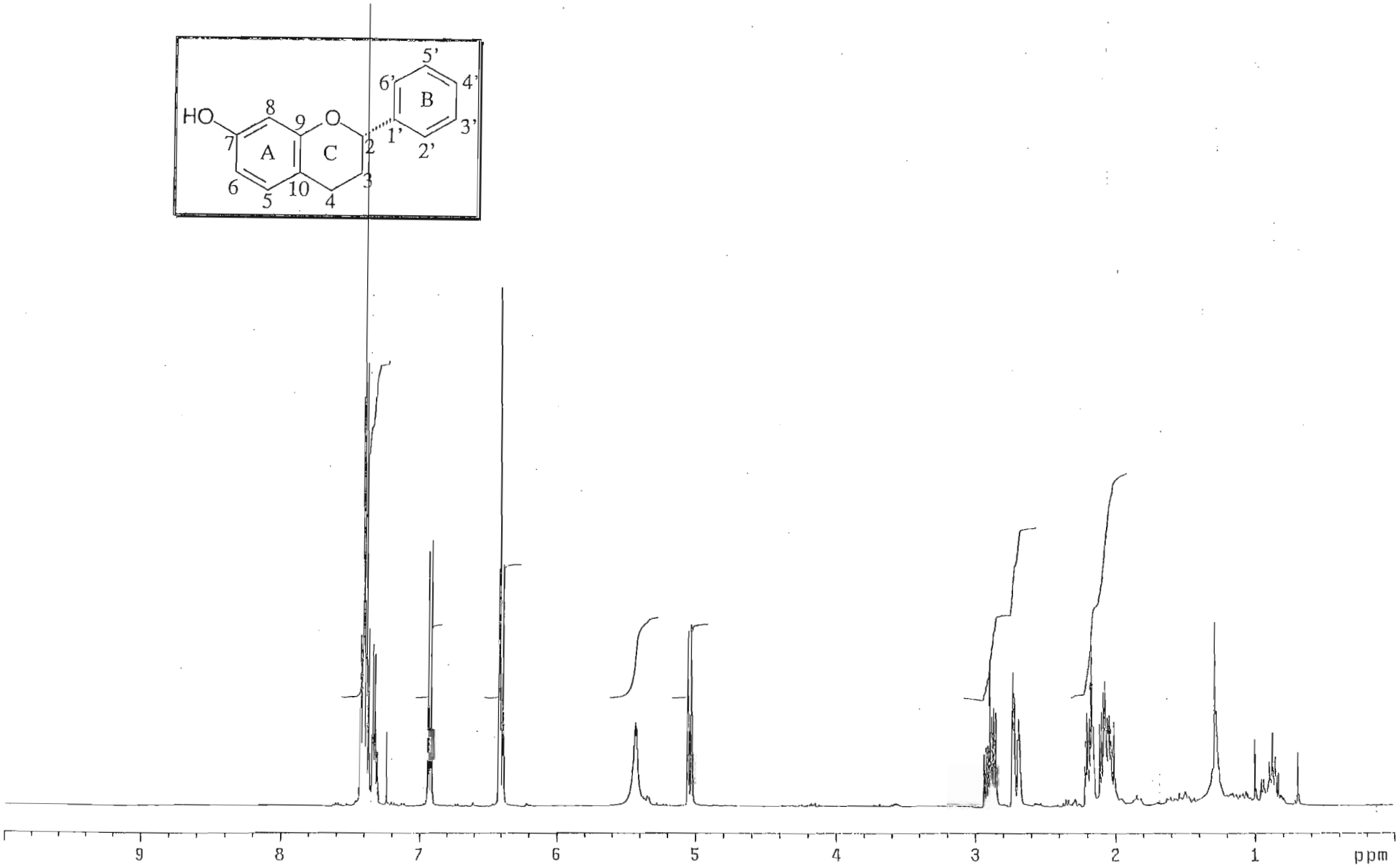
SPECTRUM 2.g: Mass spectrum of compound II



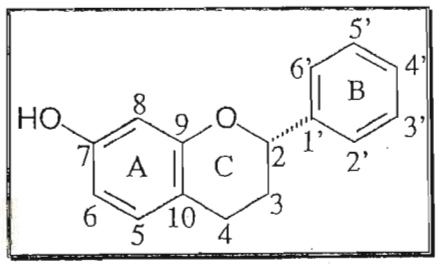
SPECTRUM 2.h: Infrared spectrum of compound II

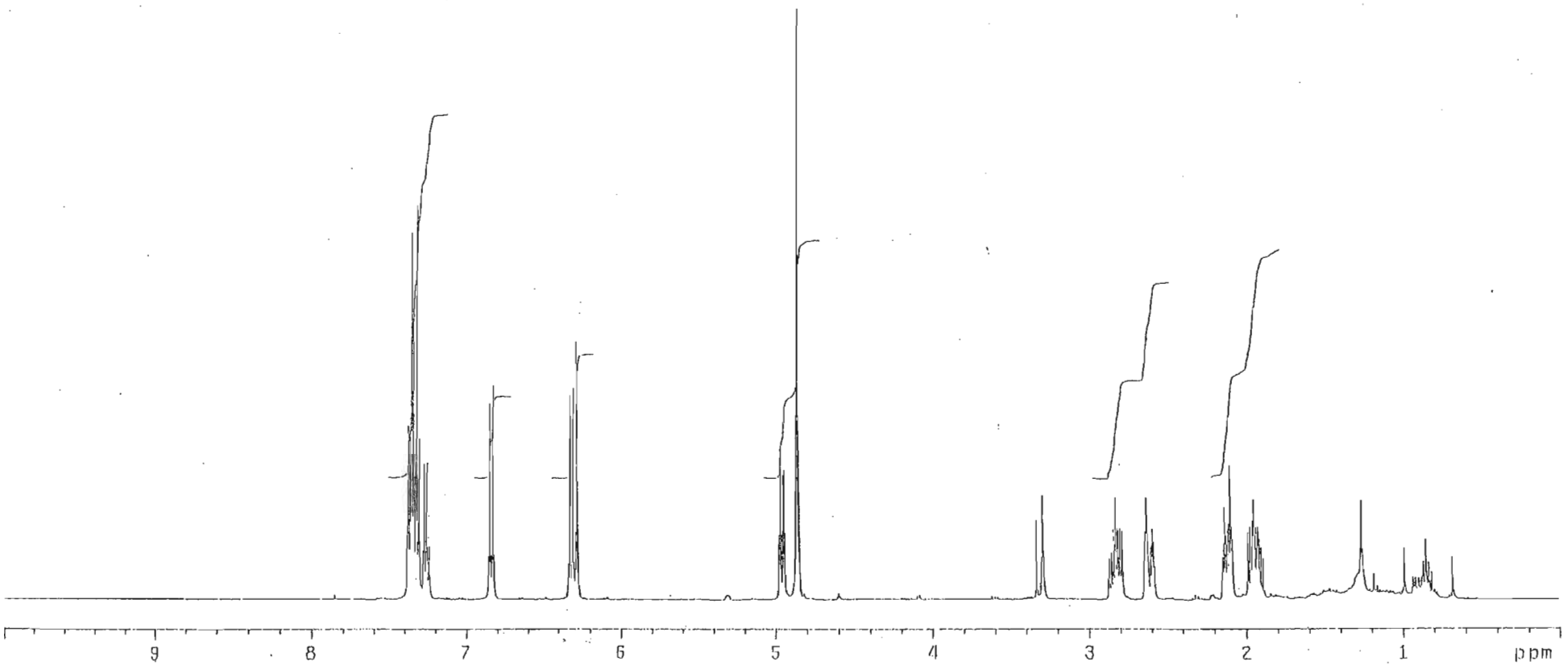


SPECTRUM 2.i: CD spectrum of compound II

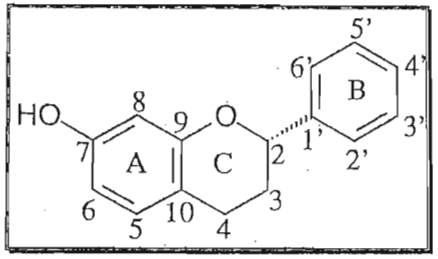


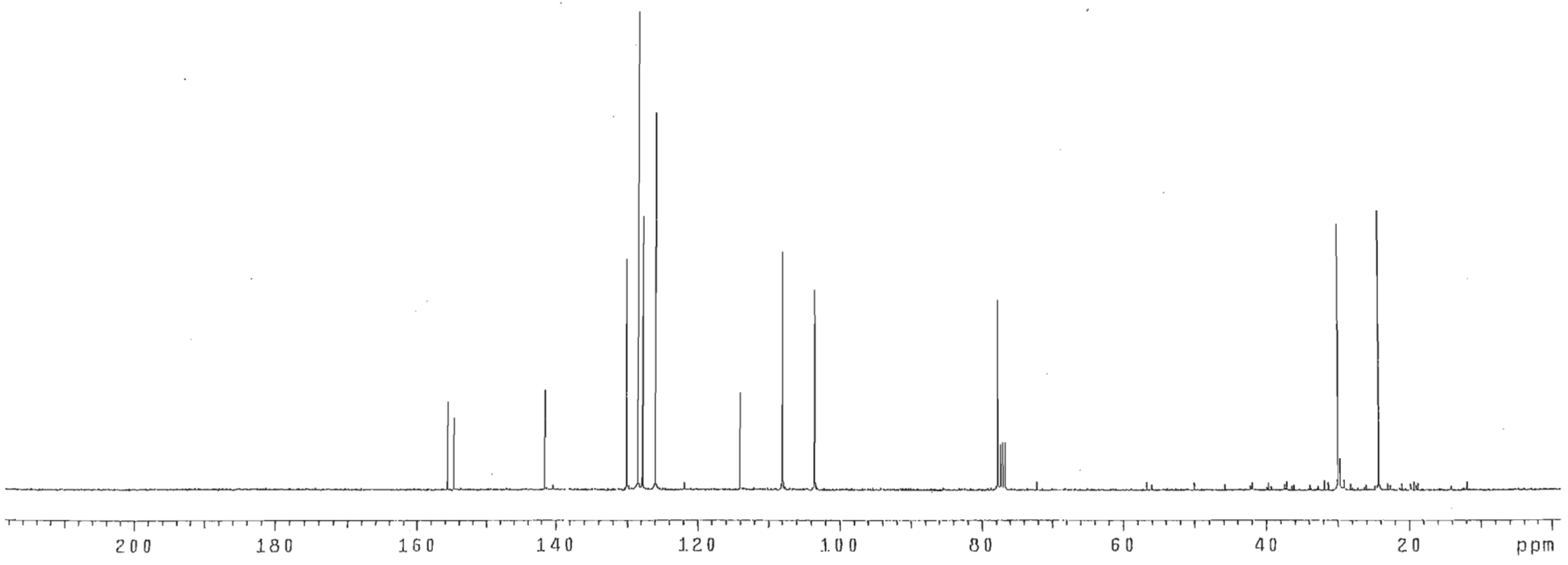
SPECTRUM 3.a: ^{1}H NMR spectrum of compound III ($CDCl_3$)



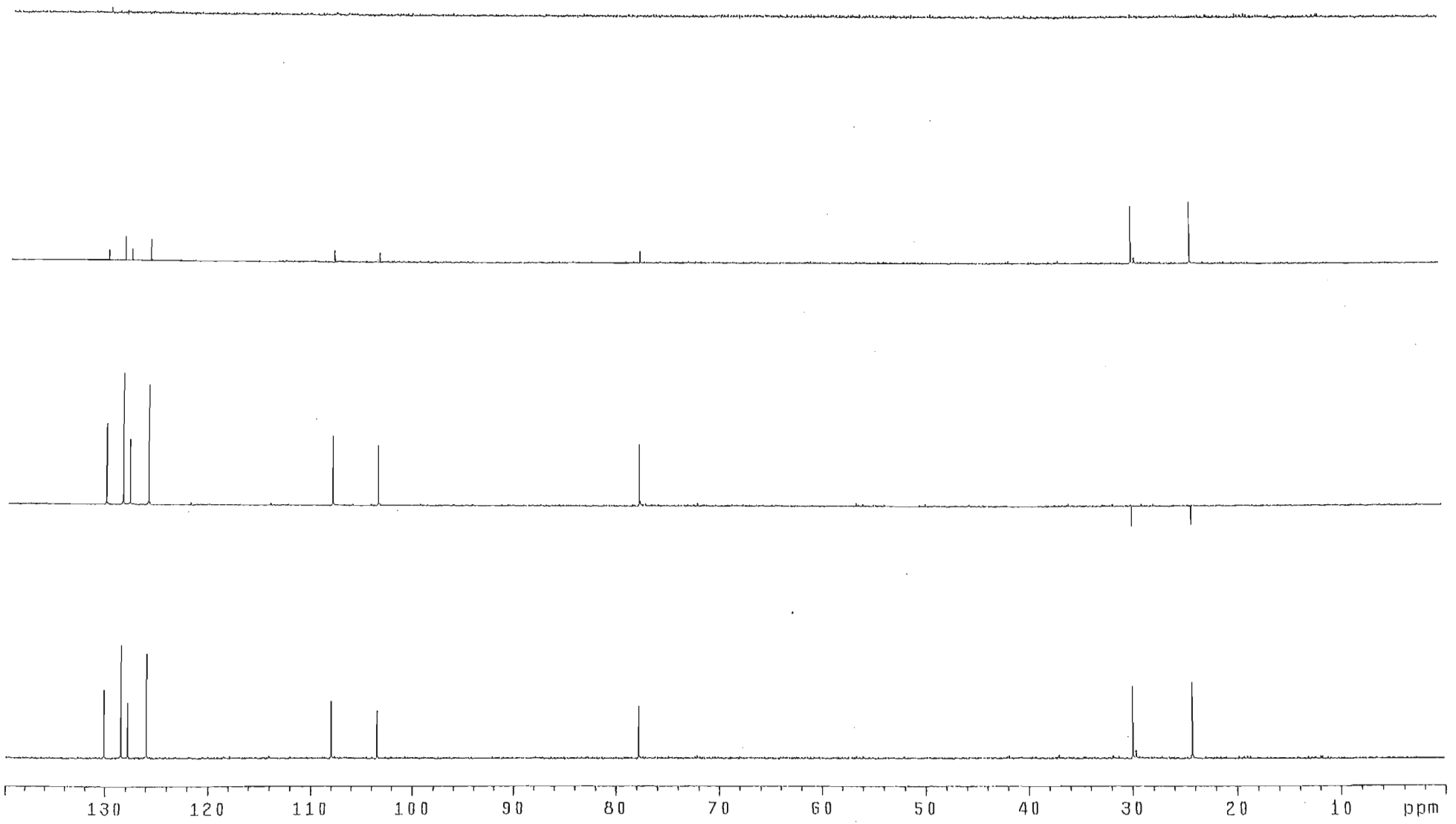


SPECTRUM 3.b: ^{1}H NMR spectrum of compound III (CD_3OD)

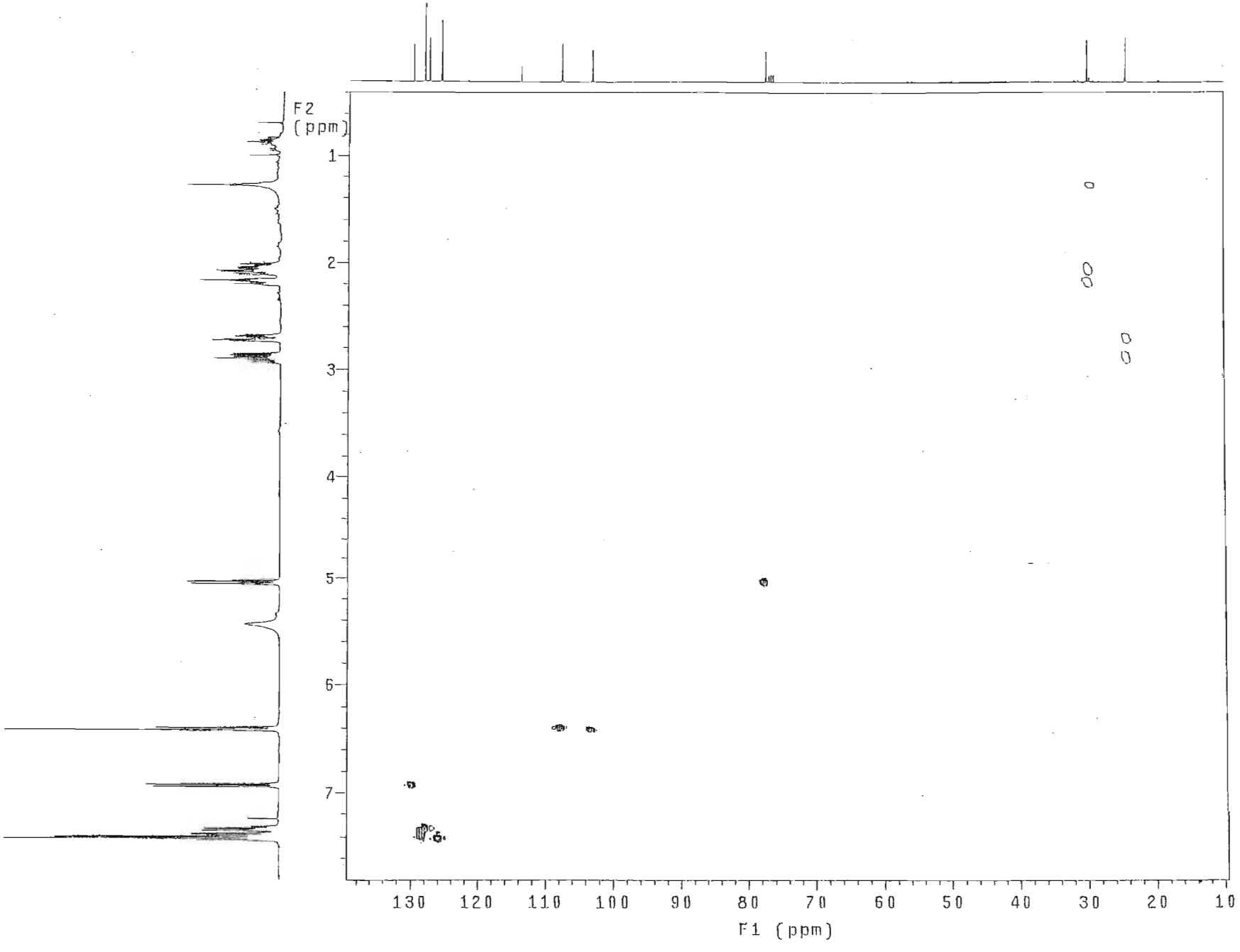




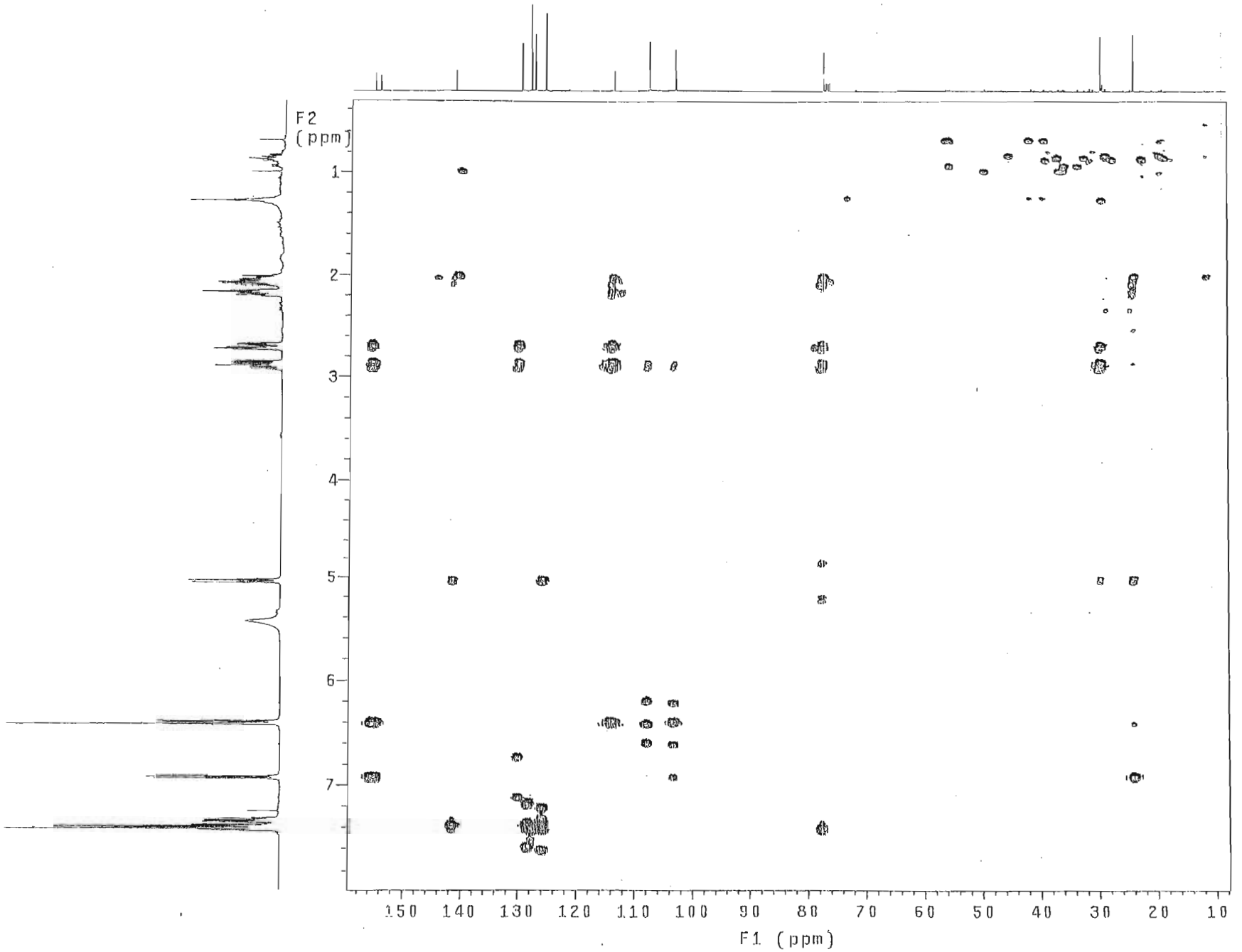
SPECTRUM 3.c: ^{13}C NMR spectrum of compound III ($CDCl_3$)



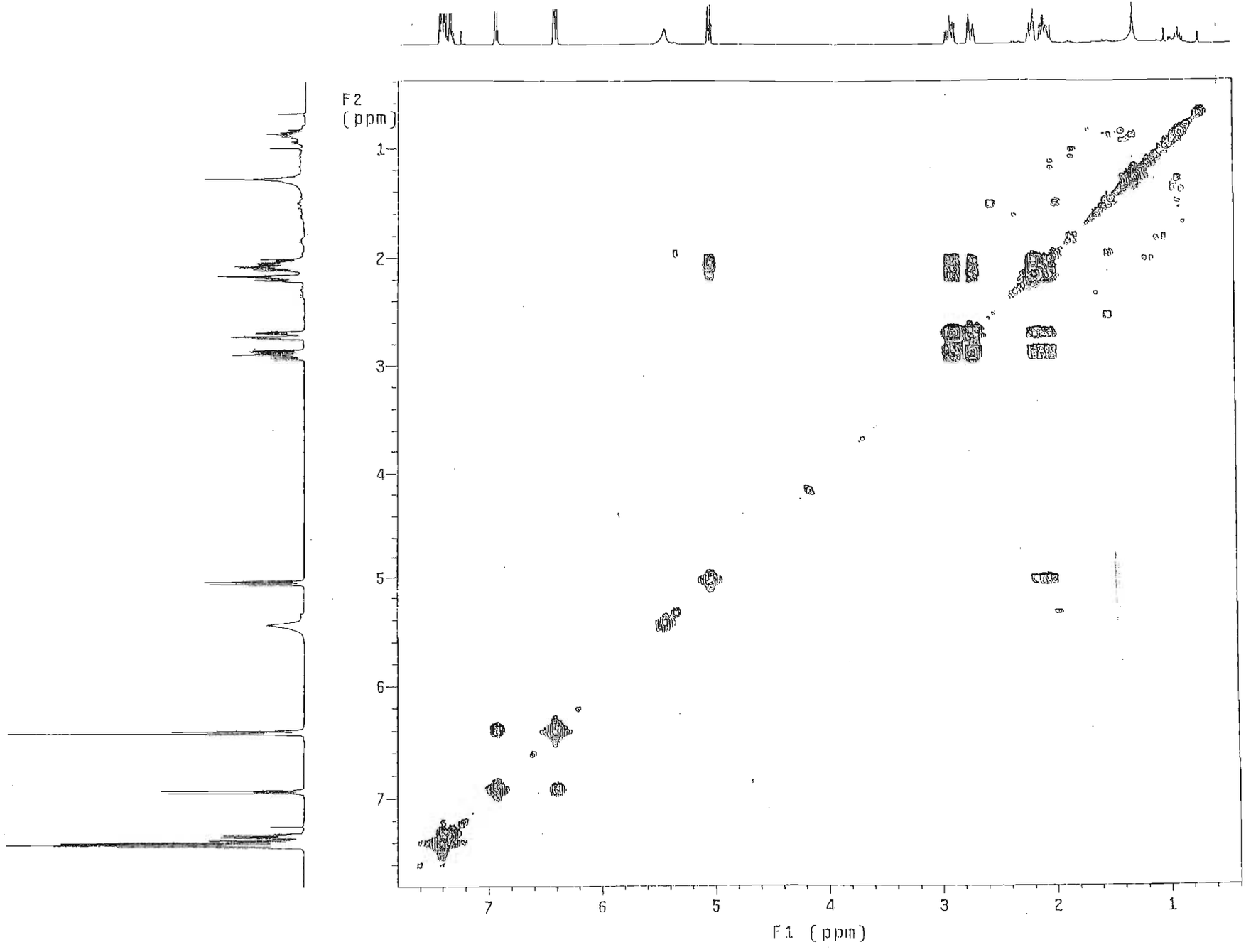
SPECTRUM 3.d: ADEPT spectrum of compound III ($CDCl_3$)



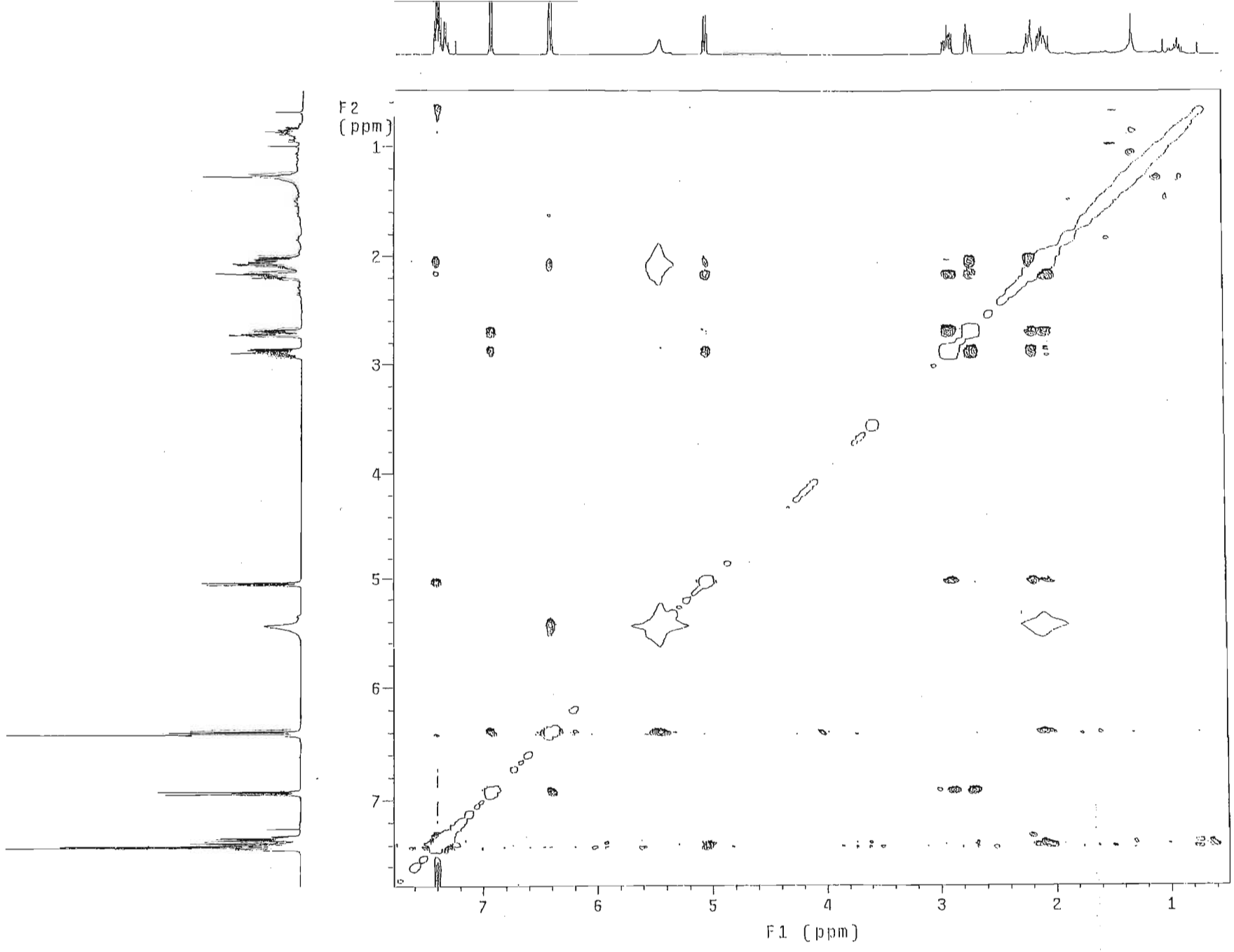
SPECTRUM 3.e: HSQC spectrum of compound III ($CDCl_3$)



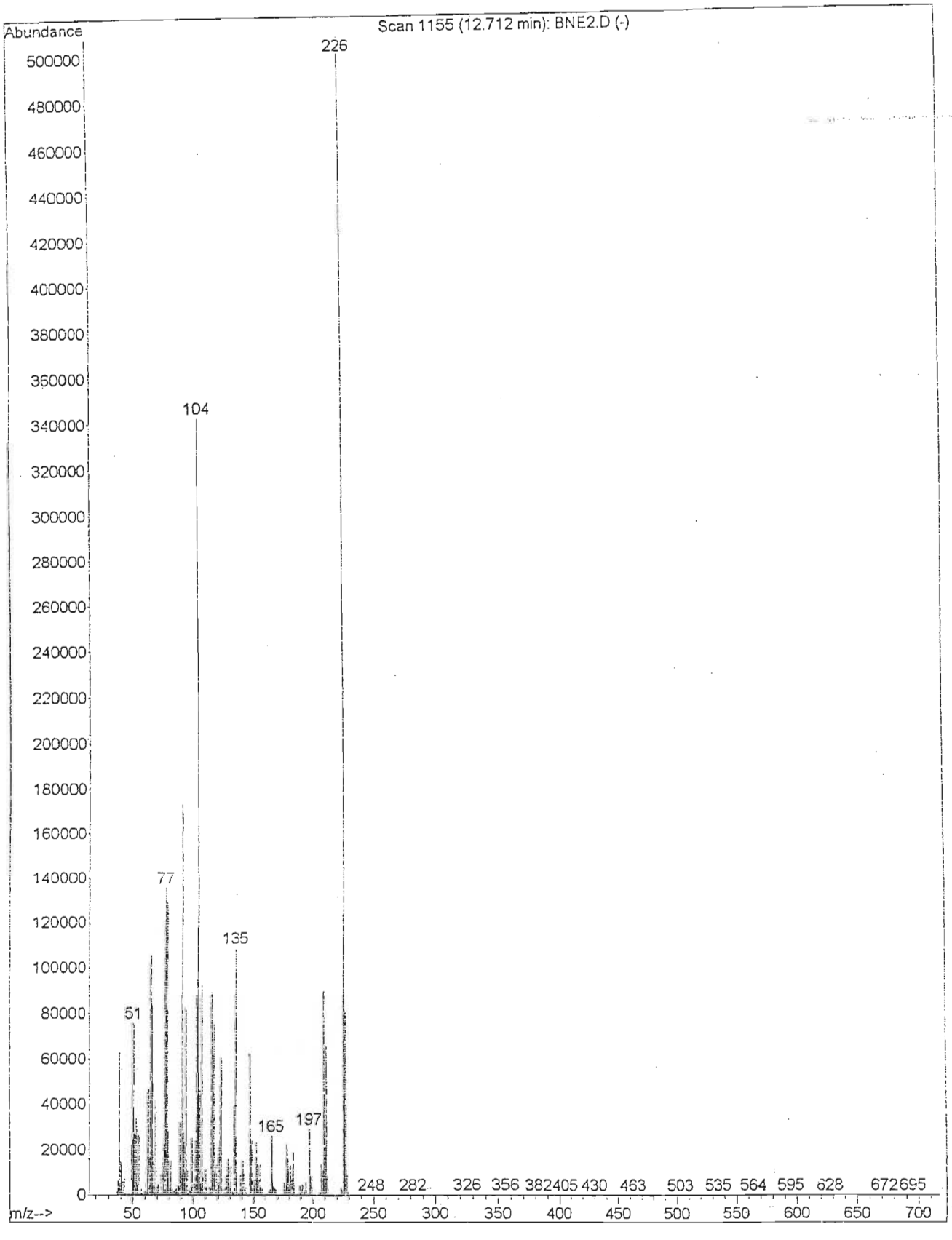
SPECTRUM 3.f: HMBC spectrum of compound III ($CDCl_3$)



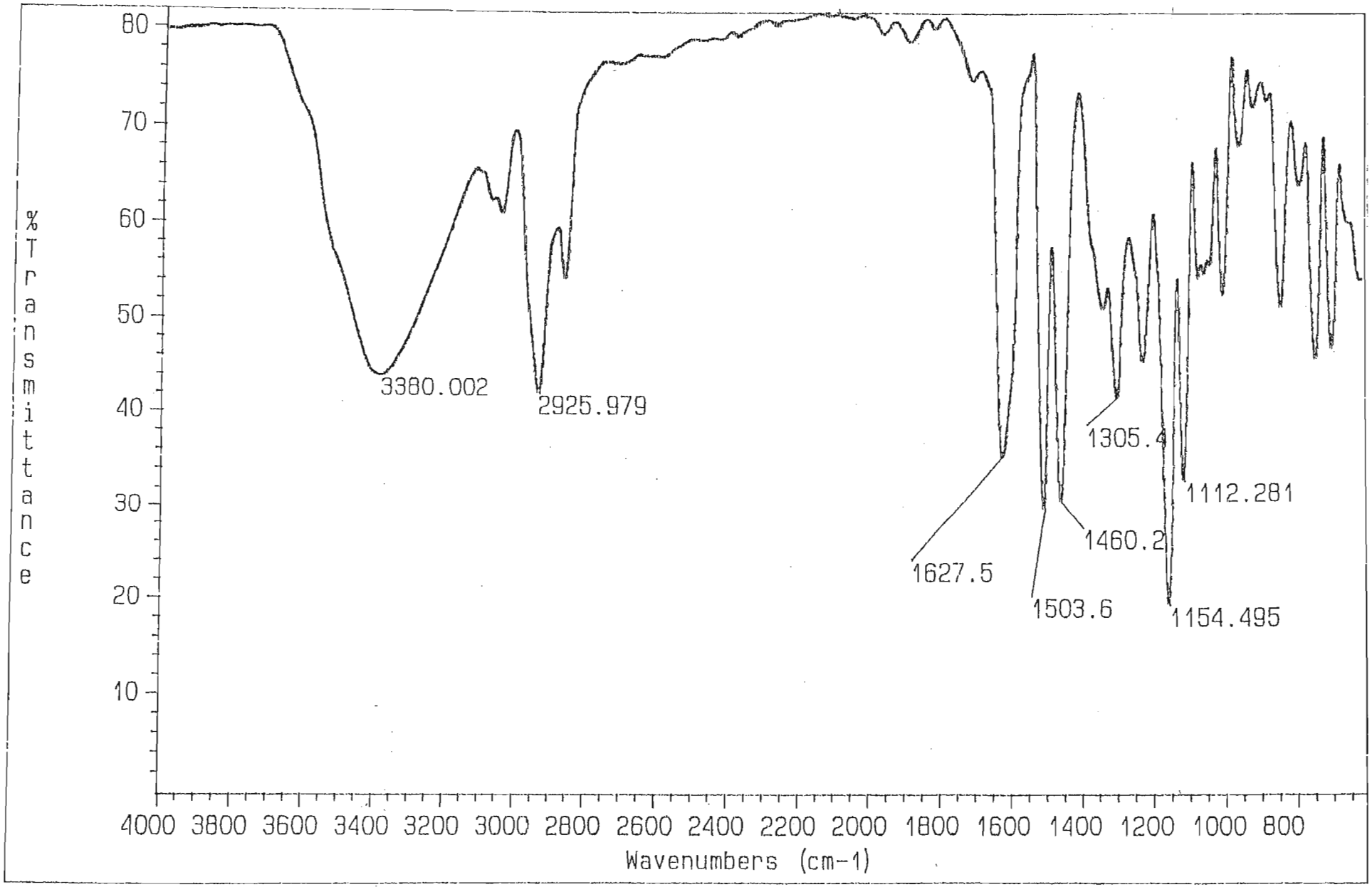
SPECTRUM 3.g: COSY spectrum of compound III ($CDCl_3$)



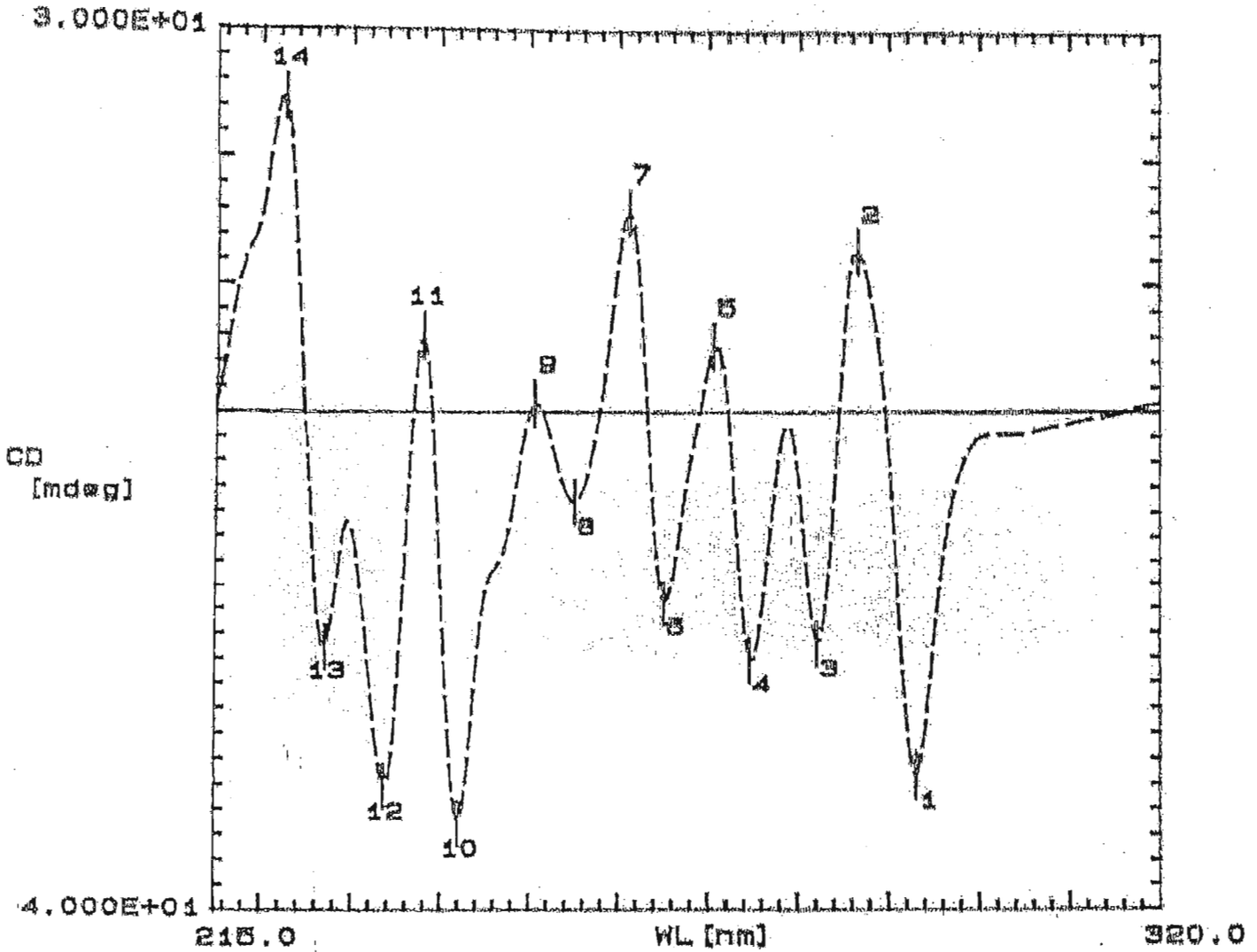
SPECTRUM 3.h: NOESY spectrum of compound III ($CDCl_3$)



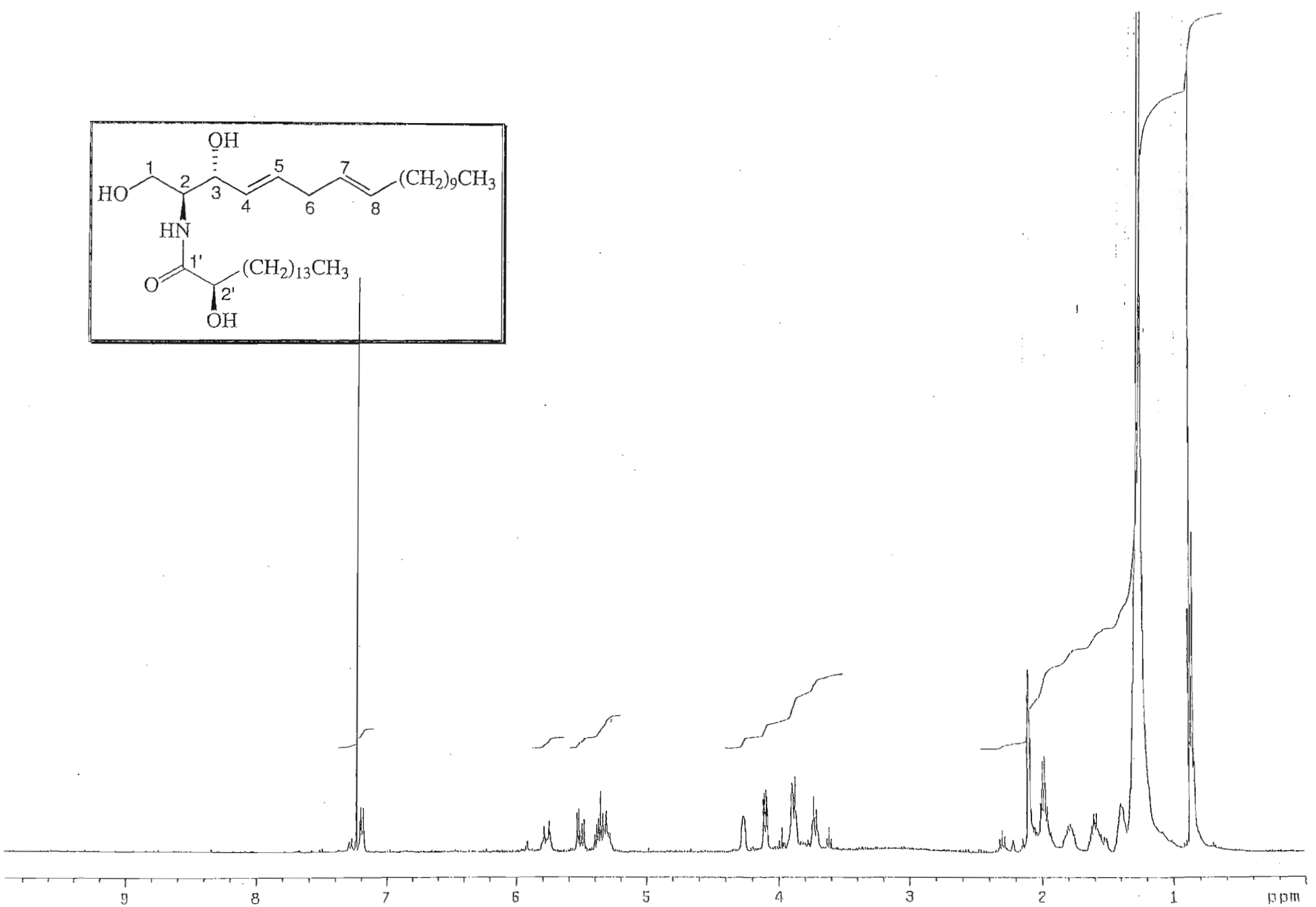
SPECTRUM 3.i: Mass spectrum of compound III



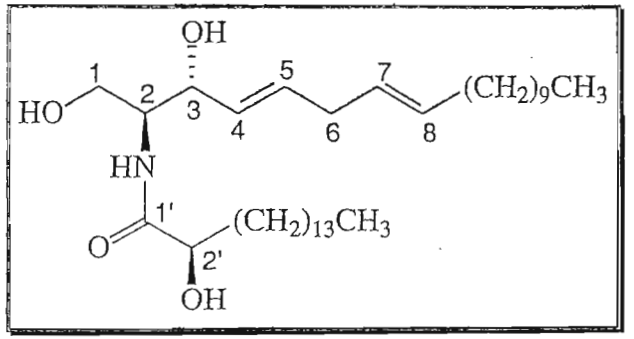
SPECTRUM 3.j: Infrared spectrum of compound III



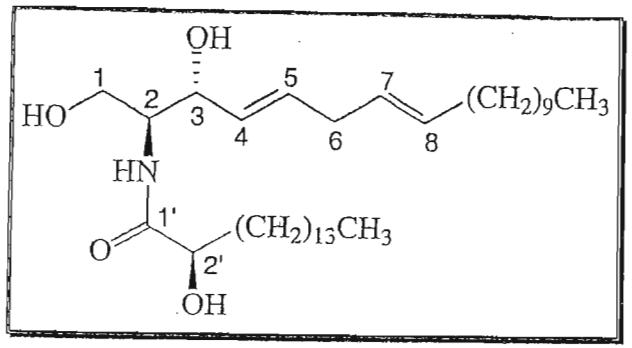
SPECTRUM 3.k: CD spectrum of compound III

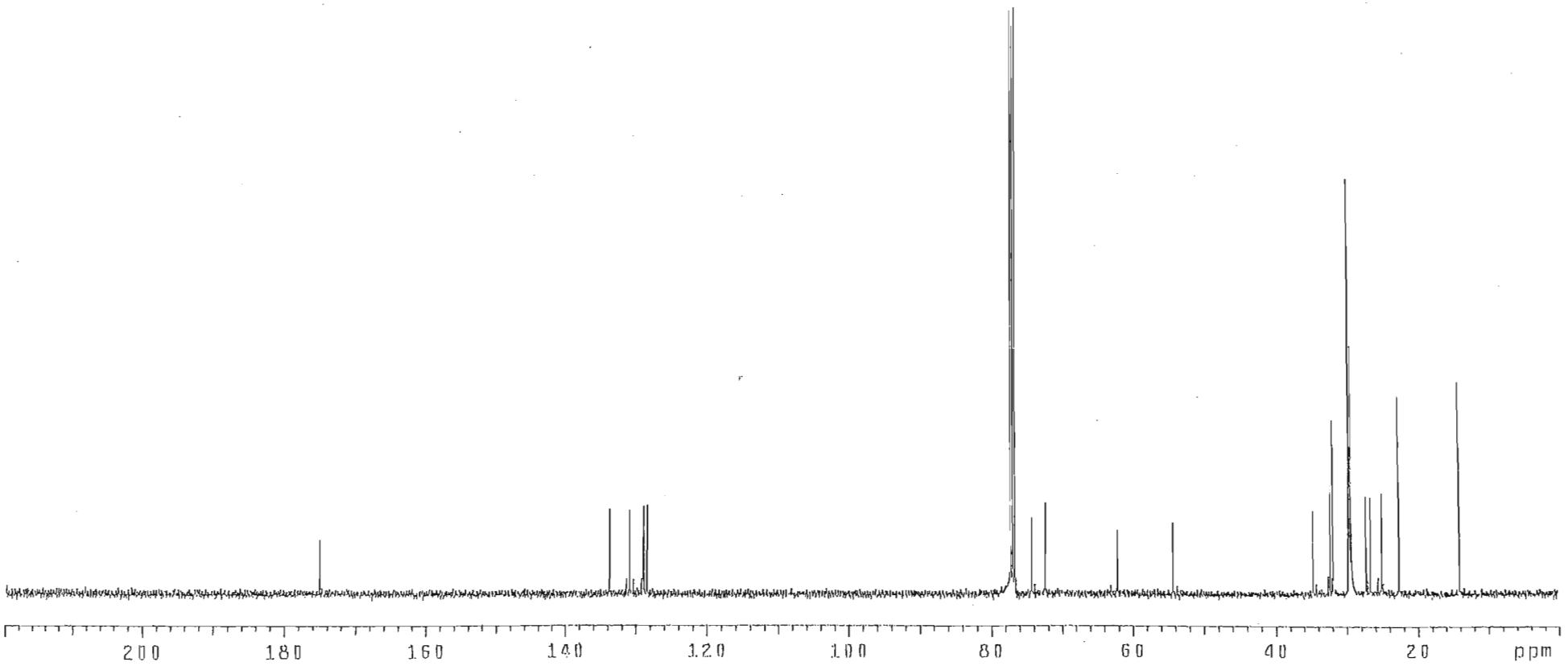


SPECTRUM 4.a: ^{1}H NMR spectrum of compound IV ($CDCl_3$)

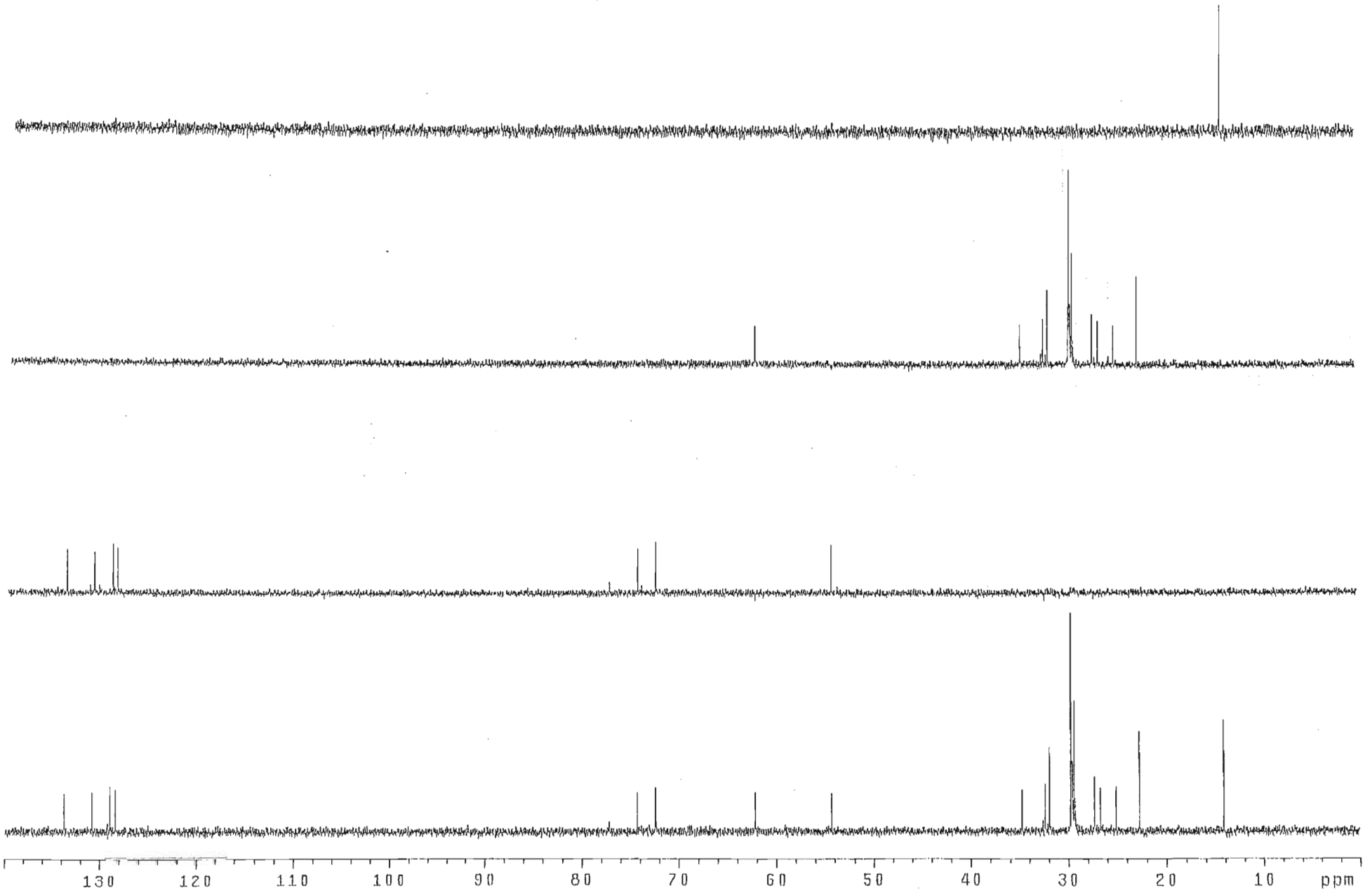


SPECTRUM 4.b: ^{1}H NMR spectrum of compound IV (CD_3OD)

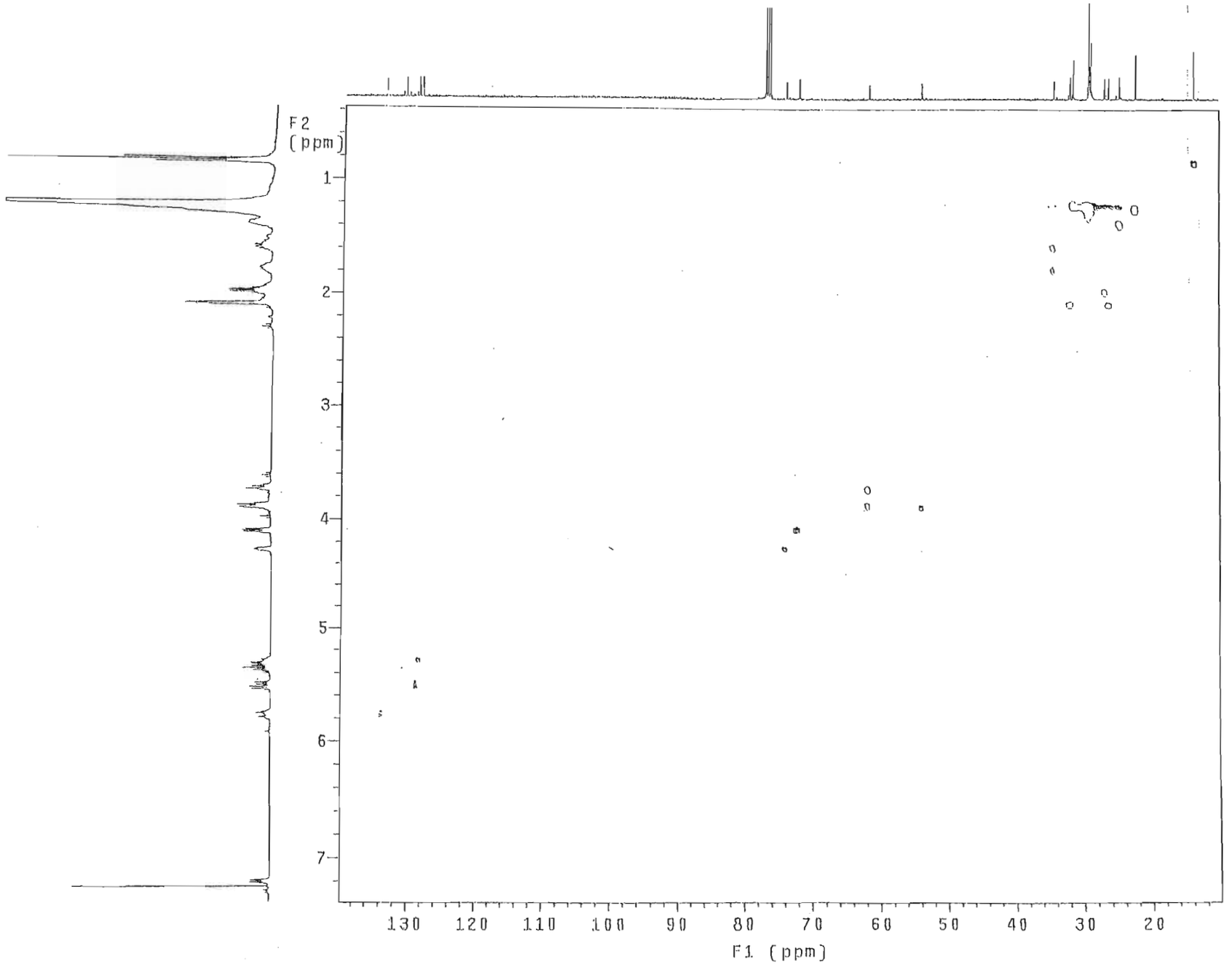




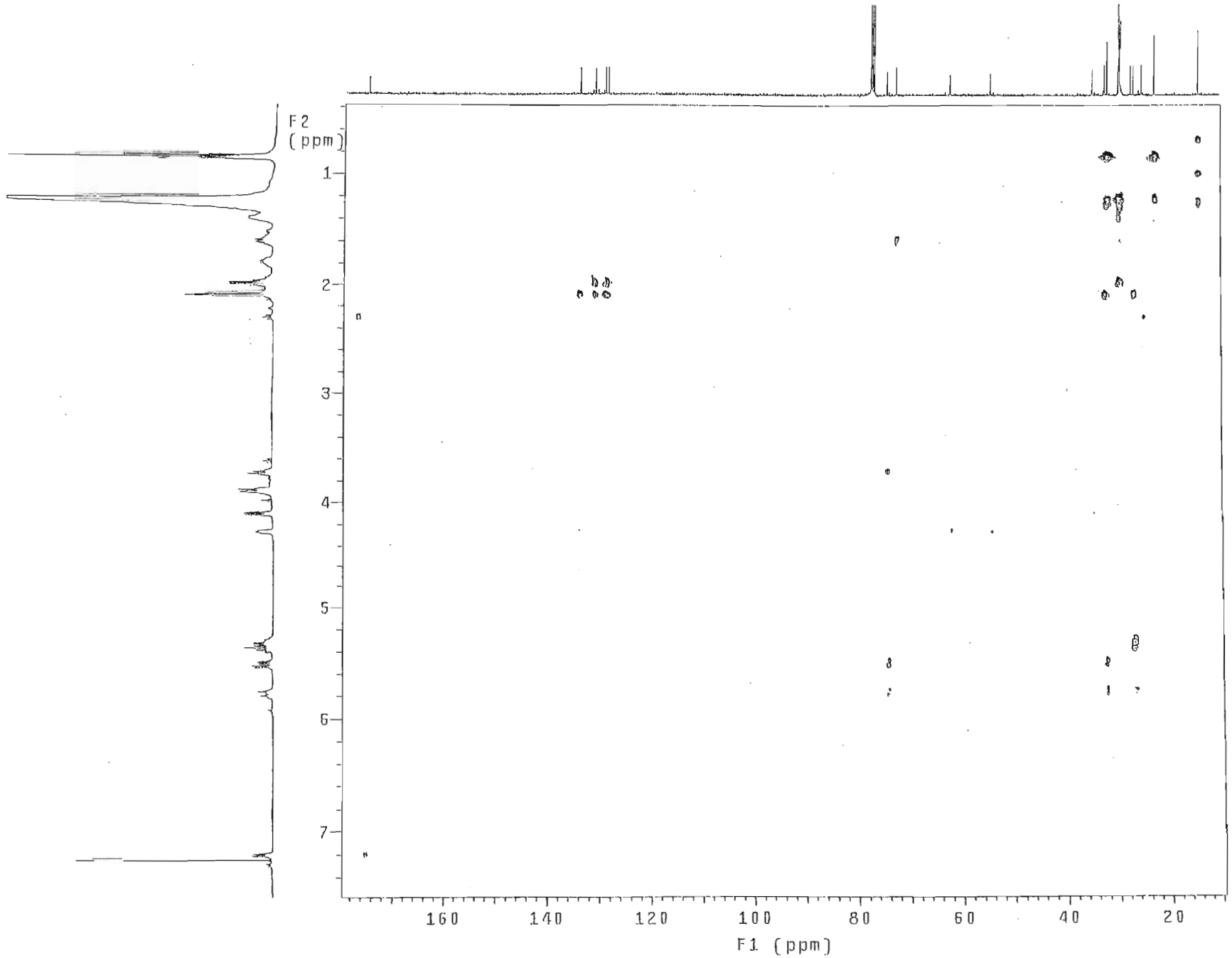
SPECTRUM 4.c: ^{13}C NMR spectrum of compound IV ($CDCl_3$)



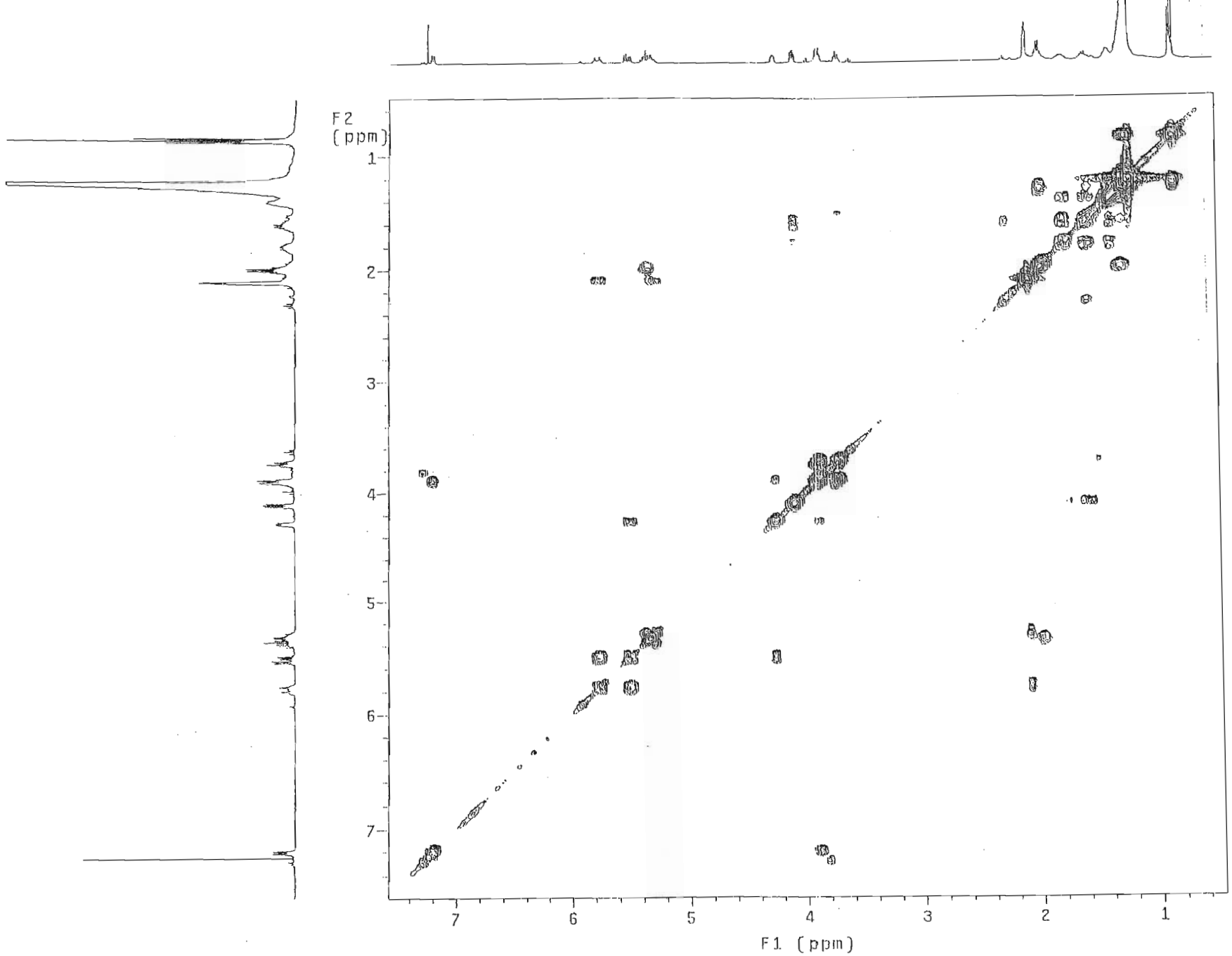
SPECTRUM 4.d: ADEPT spectrum of compound IV ($CDCl_3$)



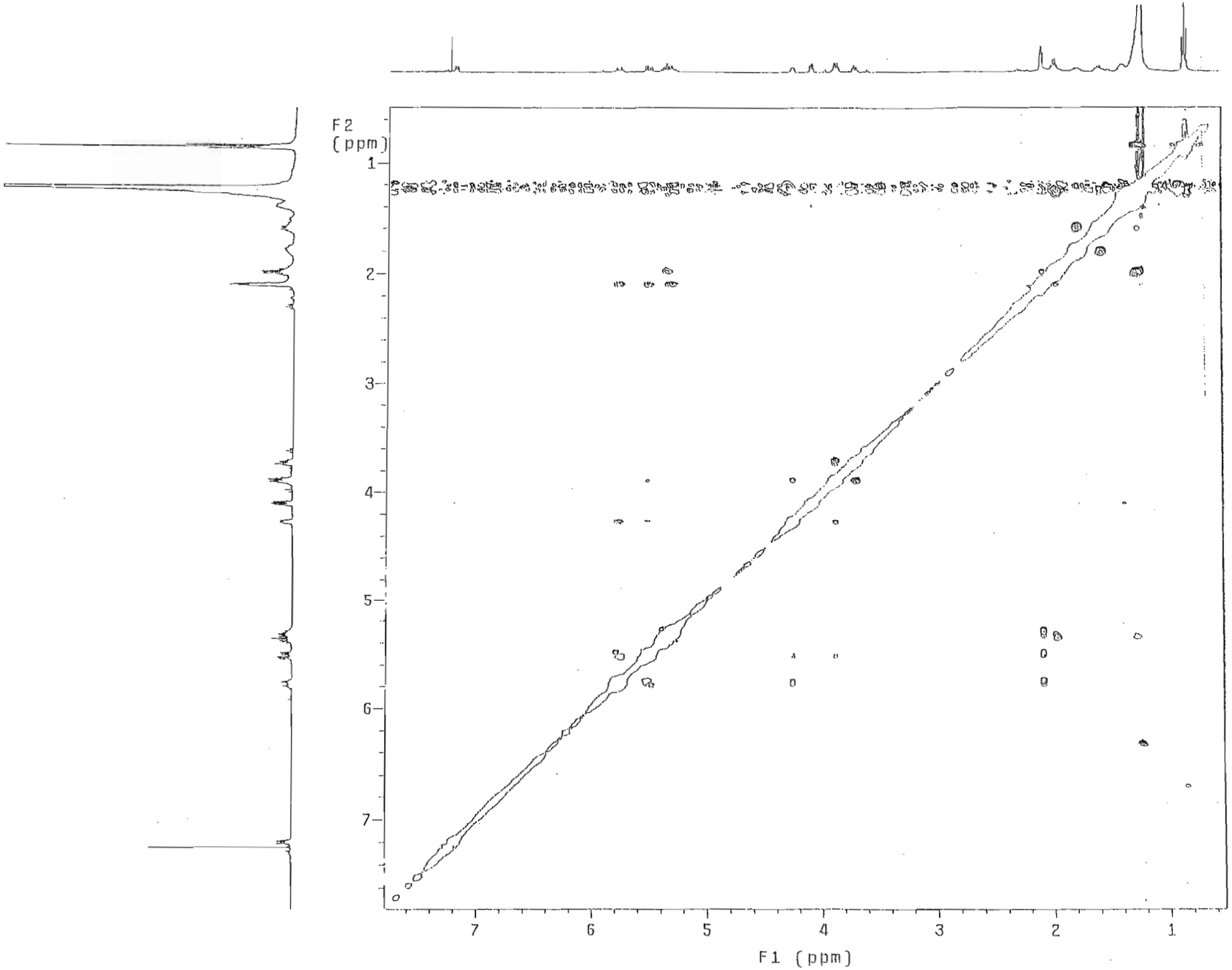
SPECTRUM 4.e: HSQC spectrum of compound IV ($CDCl_3$)



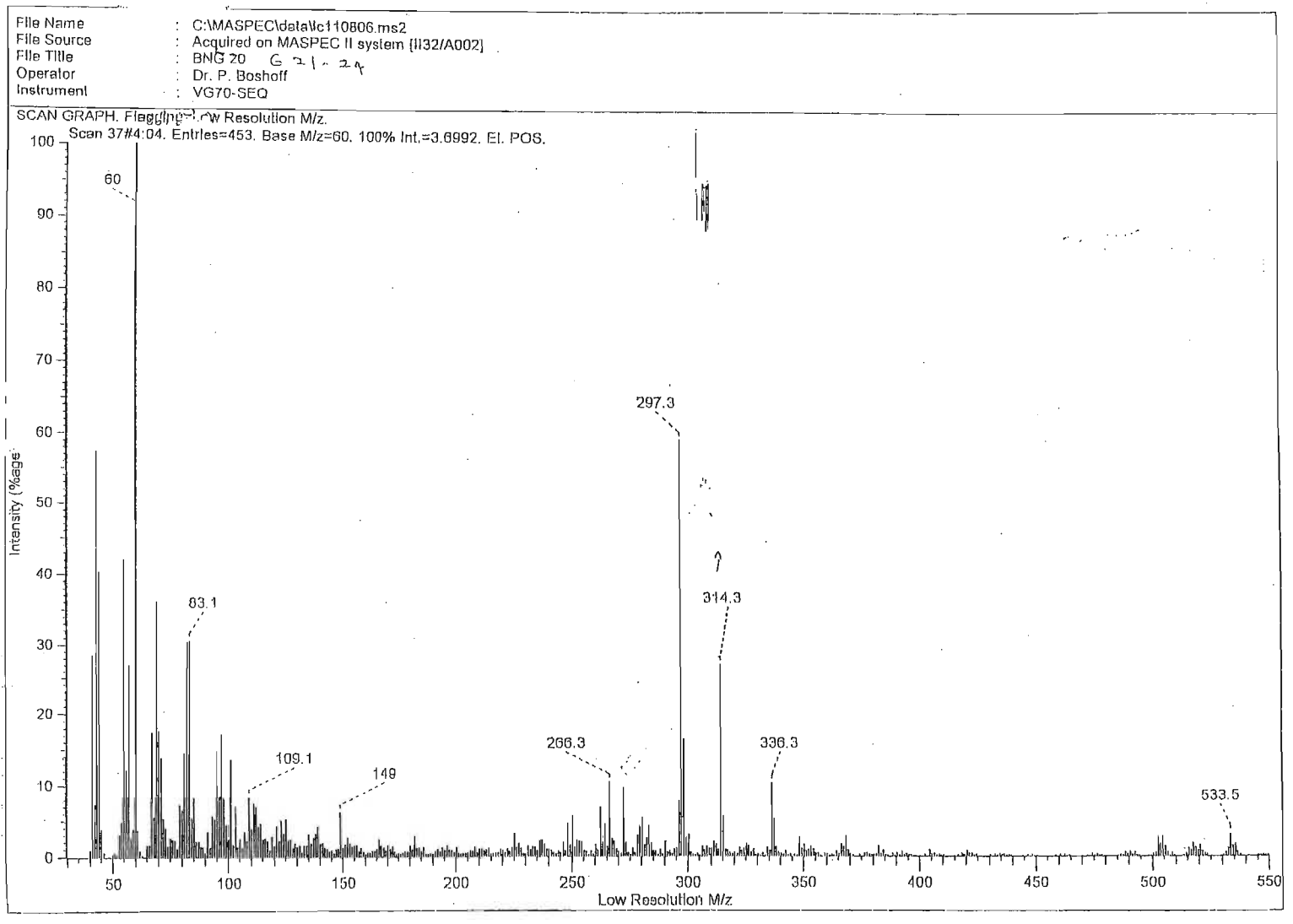
SPECTRUM 4.f: HMBC spectrum of compound IV ($CDCl_3$)



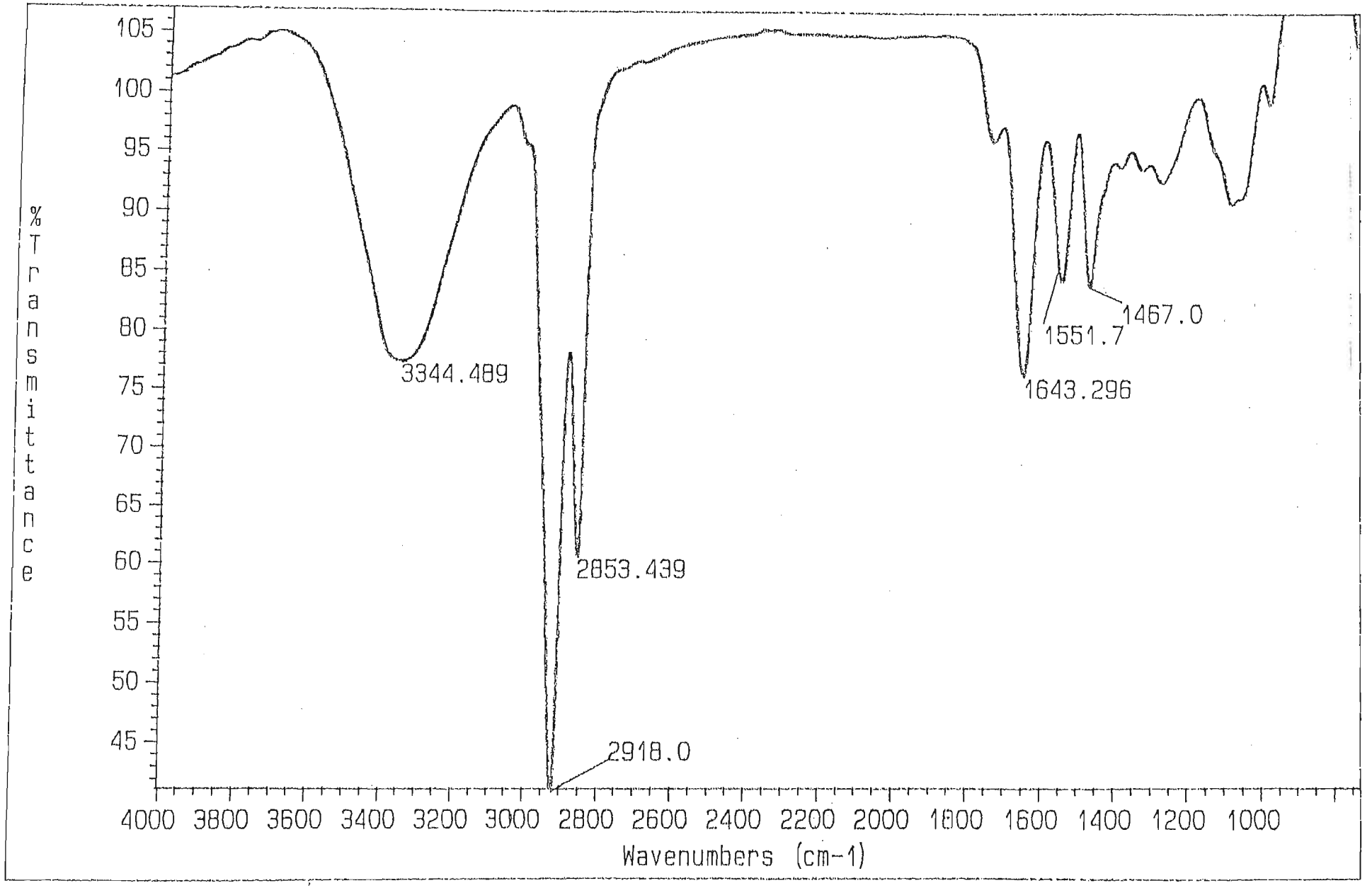
SPECTRUM 4.g: COSY spectrum of compound IV ($CDCl_3$)



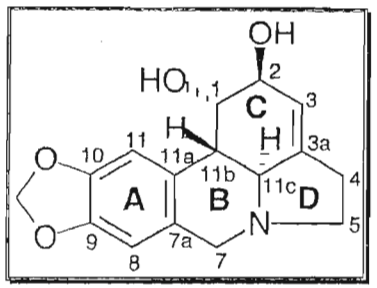
SPECTRUM 4.h: NOESY spectrum of compound IV ($CDCl_3$)



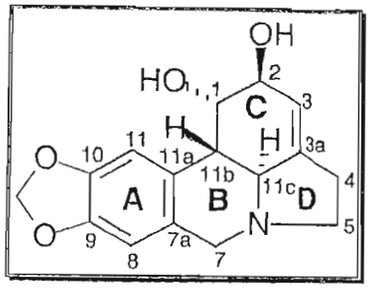
SPECTRUM 4.i: Mass spectrum of compound IV

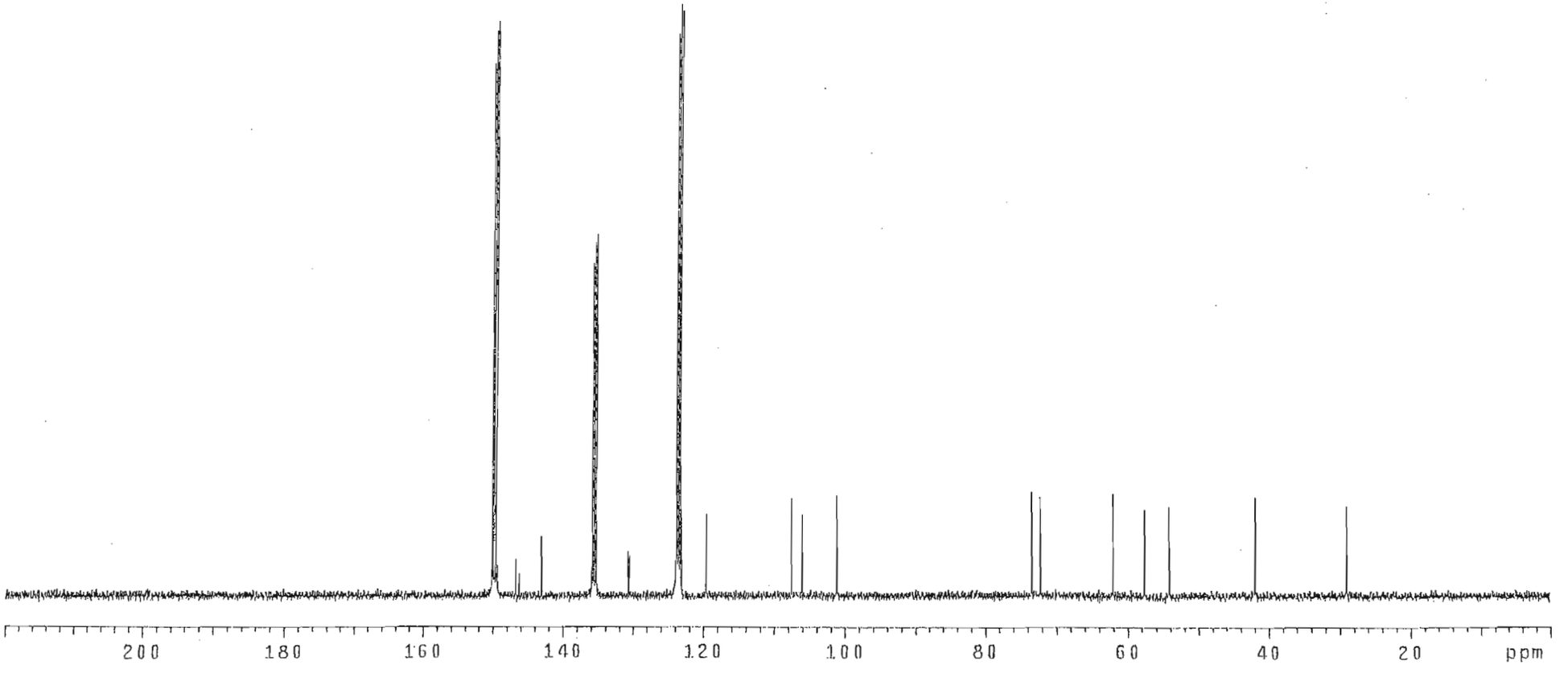


SPECTRUM 4.j: Infrared spectrum of compound IV

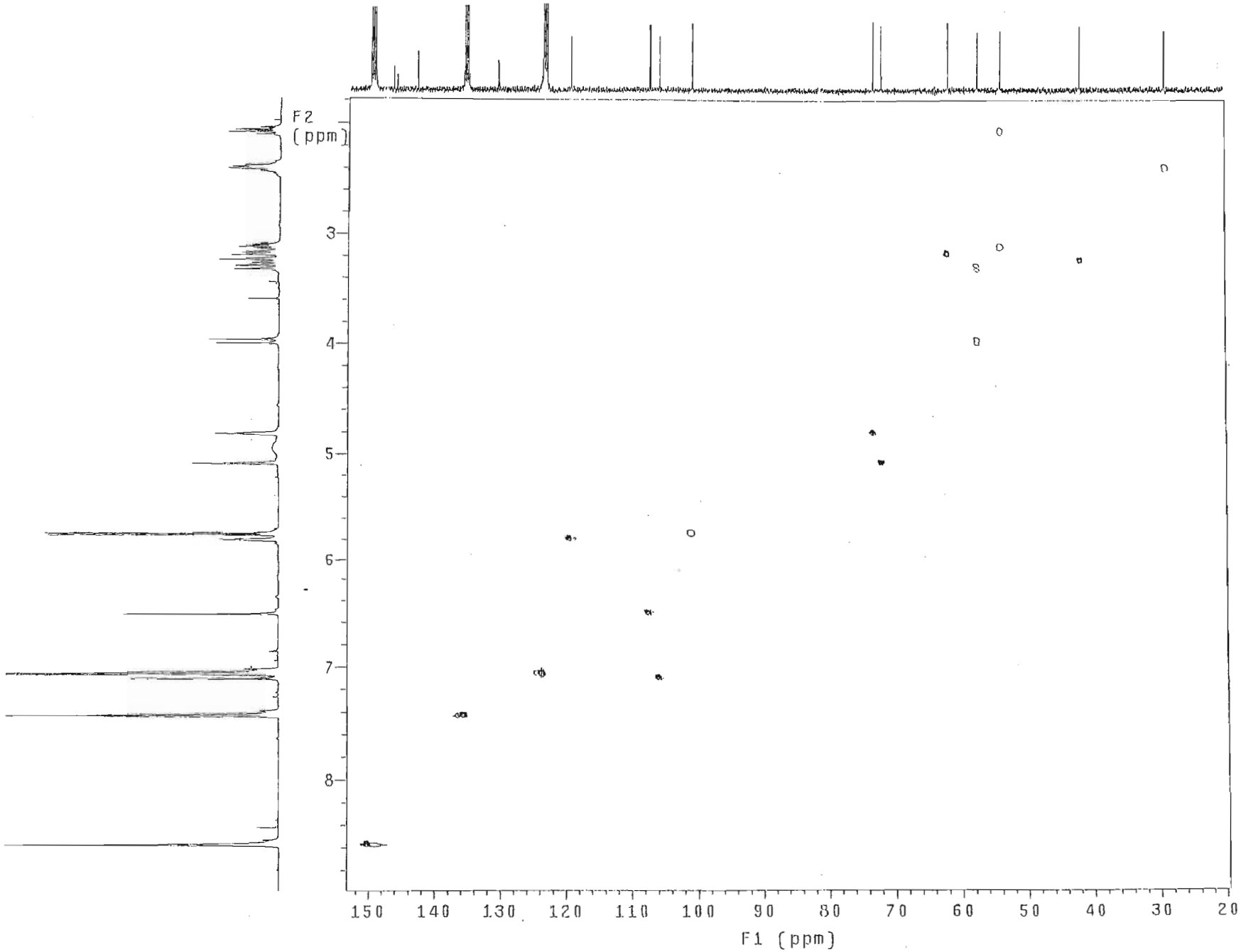


SPECTRUM 5.a: ^{1}H NMR spectrum of compound V (C_5D_5N)

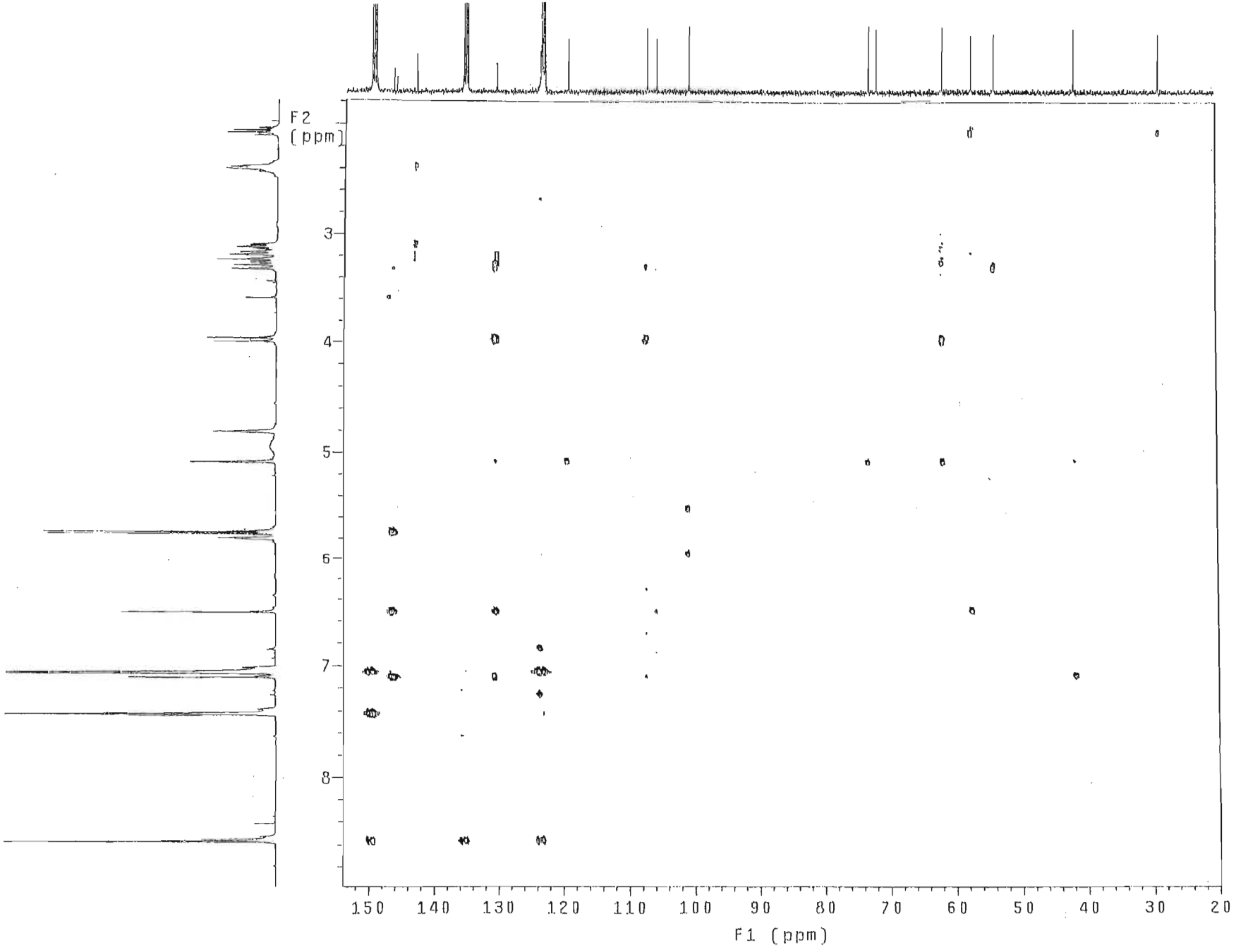




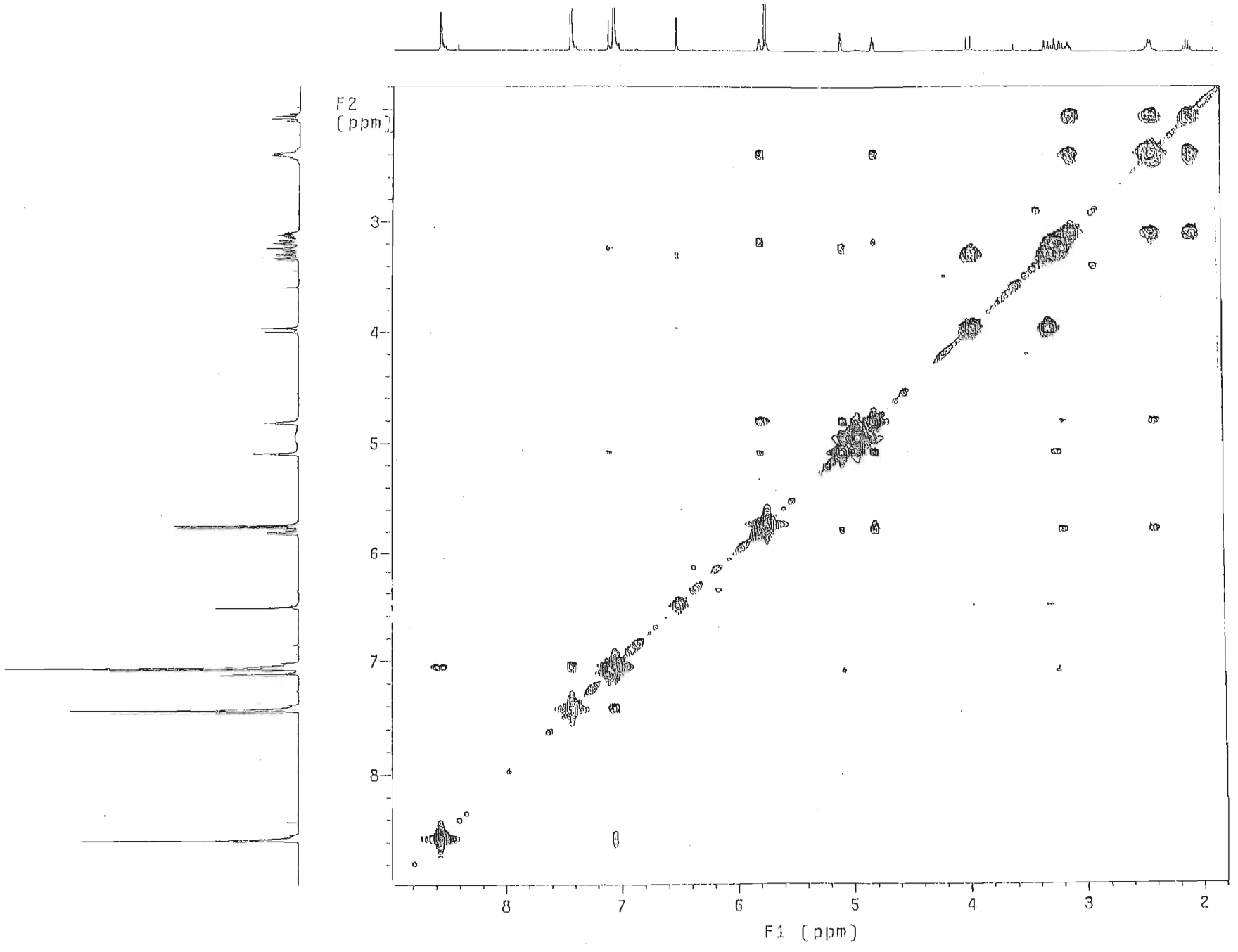
SPECTRUM 5.b: ^{13}C NMR spectrum of compound V (C_5D_5N)



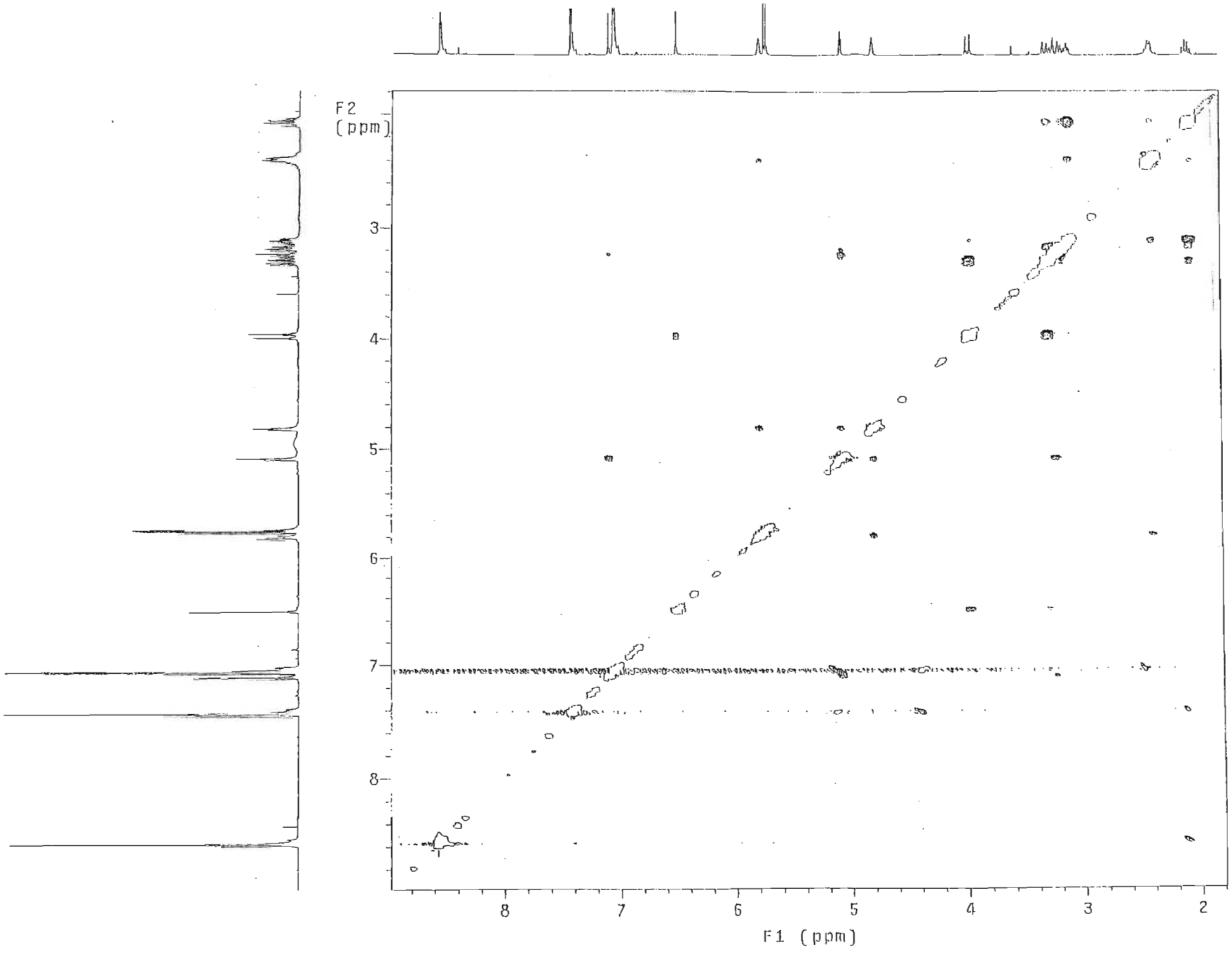
SPECTRUM 5.c: HSQC spectrum of compound V (C_5D_5N)



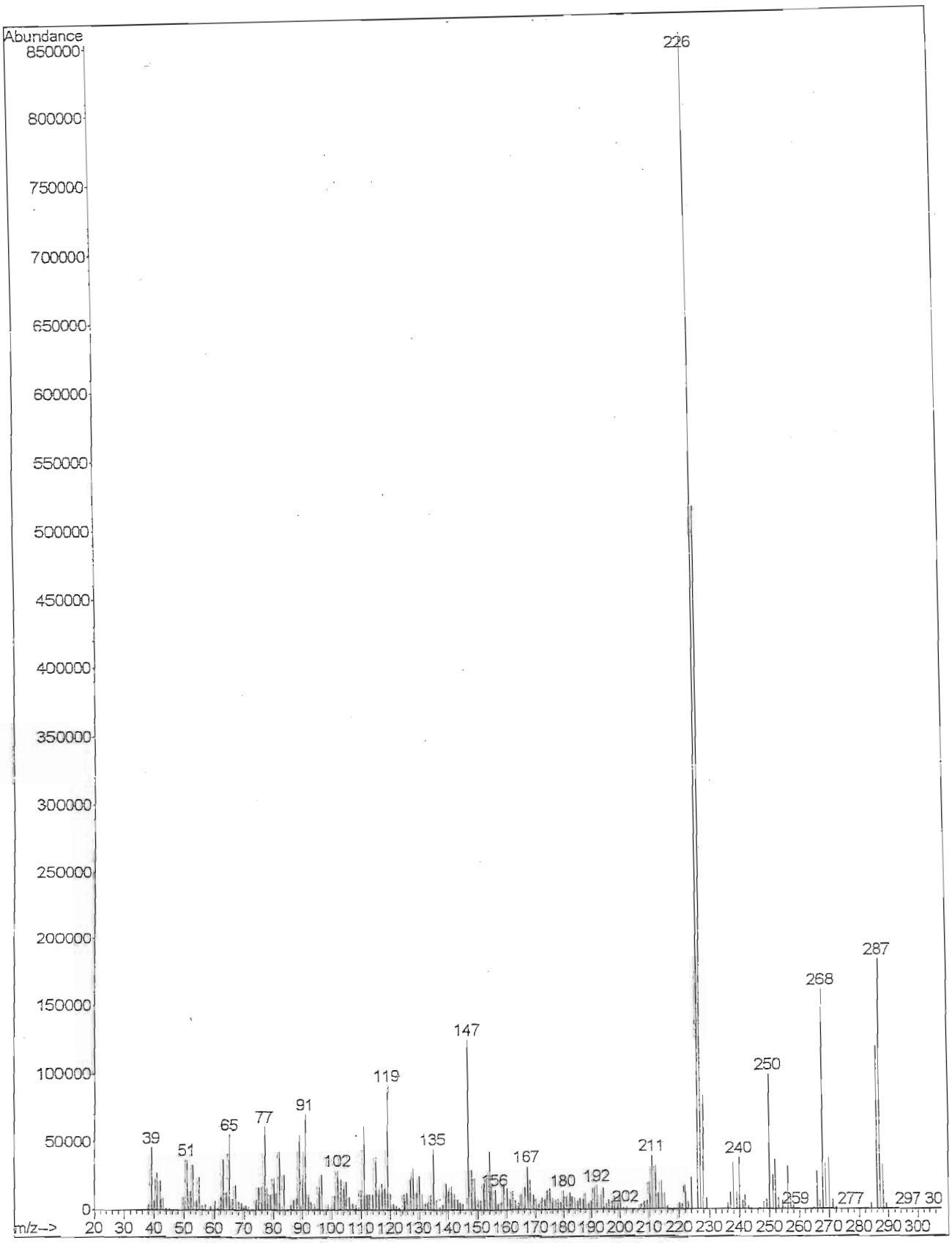
SPECTRUM 5.d: HMBC spectrum of compound V (C_5D_5N)



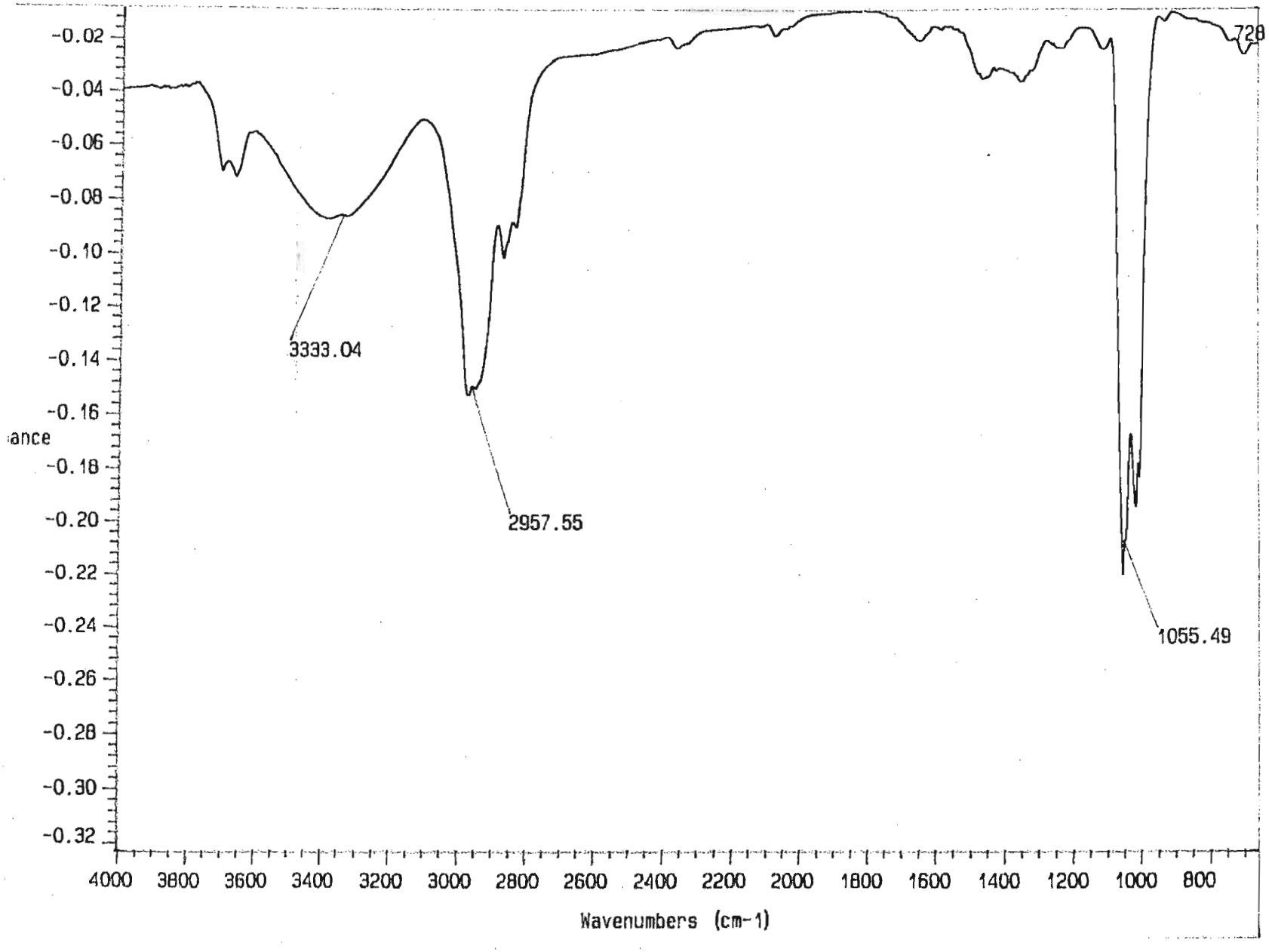
SPECTRUM 5.e: COSY spectrum of compound V (C_5D_5N)



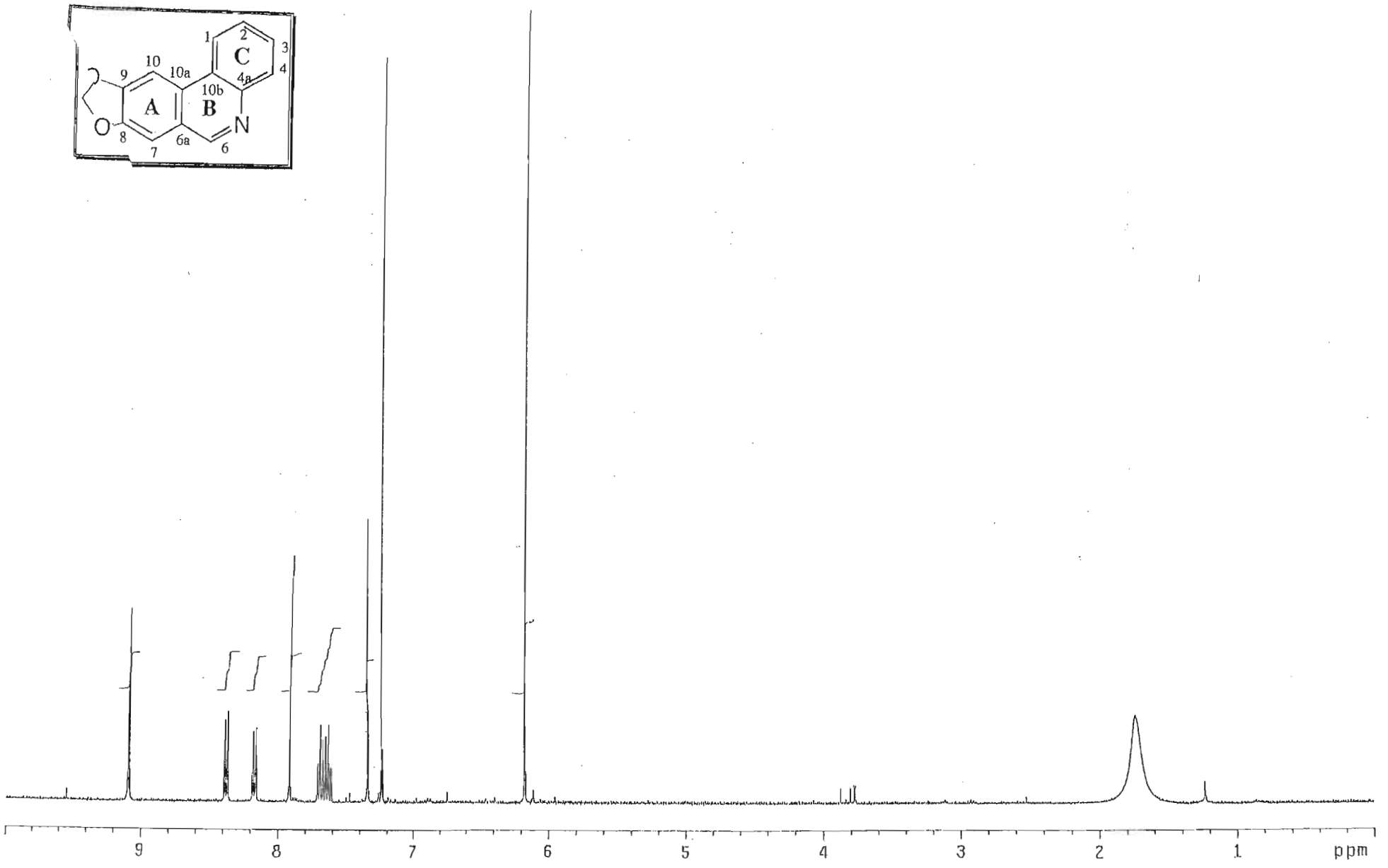
SPECTRUM 5.f: NOESY spectrum of compound V (C_5D_5N)



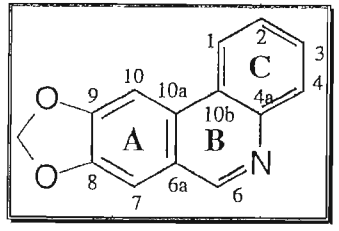
SPECTRUM 5.g: Mass spectrum of compound V



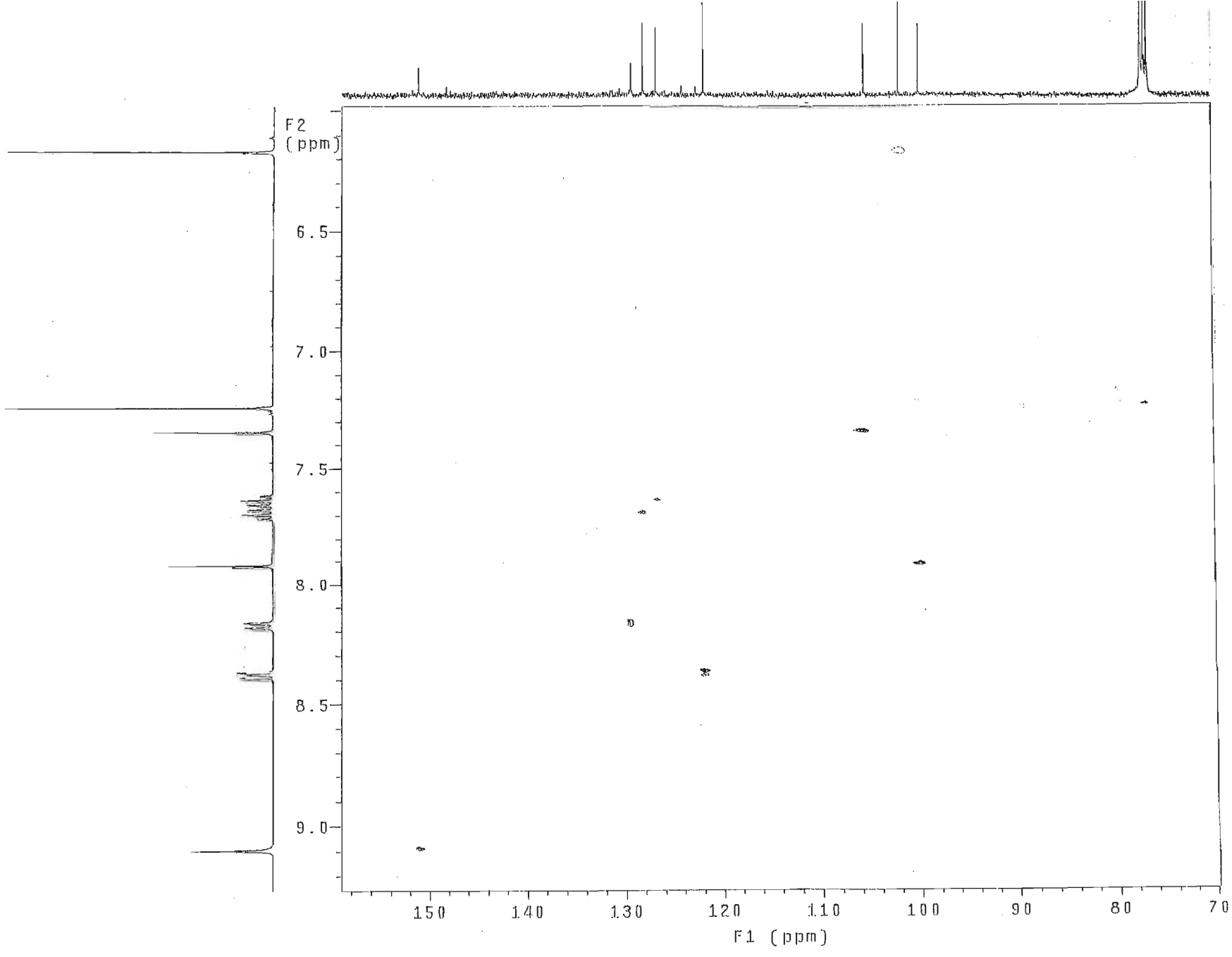
SPECTRUM 5.h: Infrared spectrum of compound V



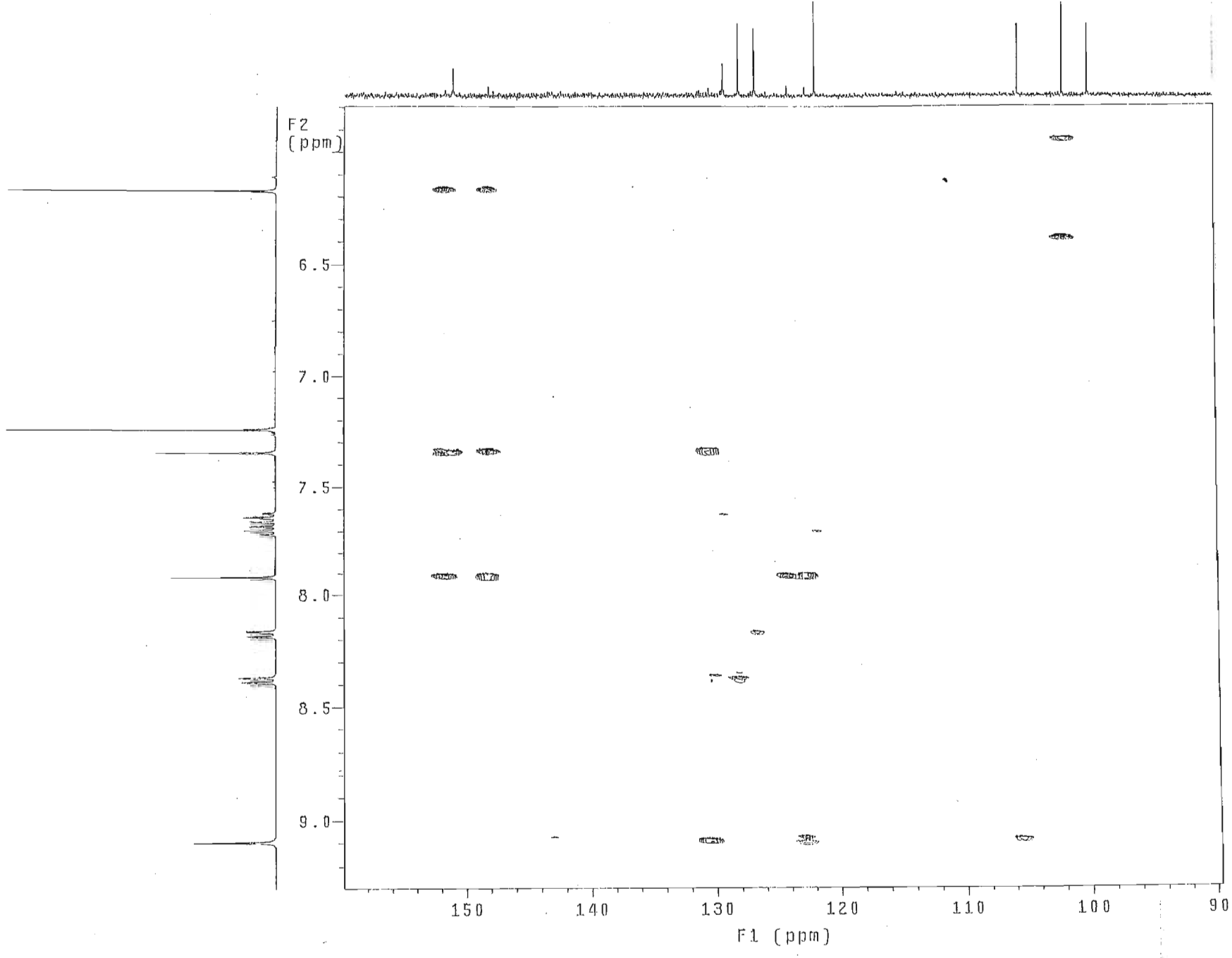
SPECTRUM 6.a: ^{1}H NMR spectrum of compound VI ($CDCl_3$)



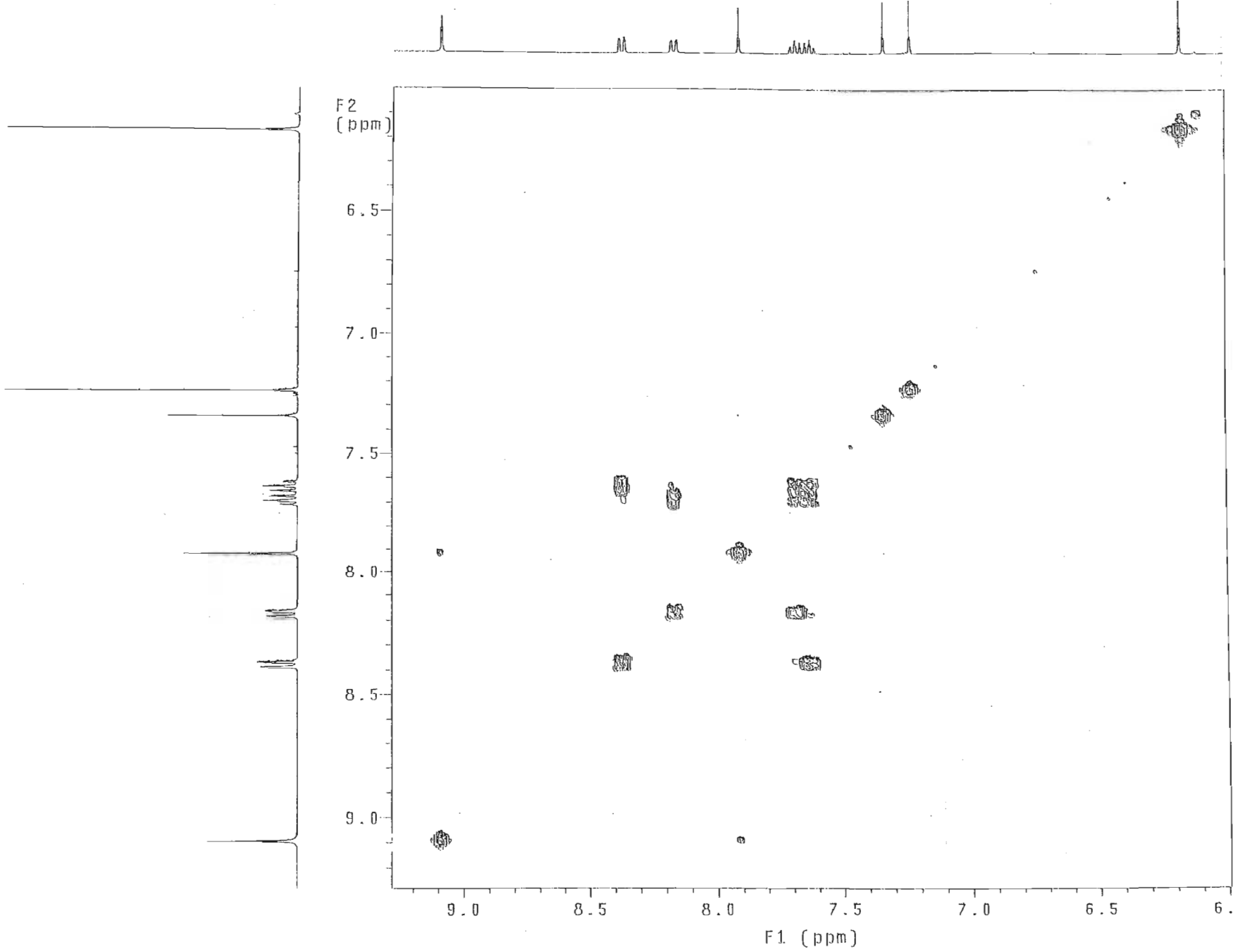
SPECTRUM 6.b: ^{13}C NMR spectrum of compound VI ($CDCl_3$)



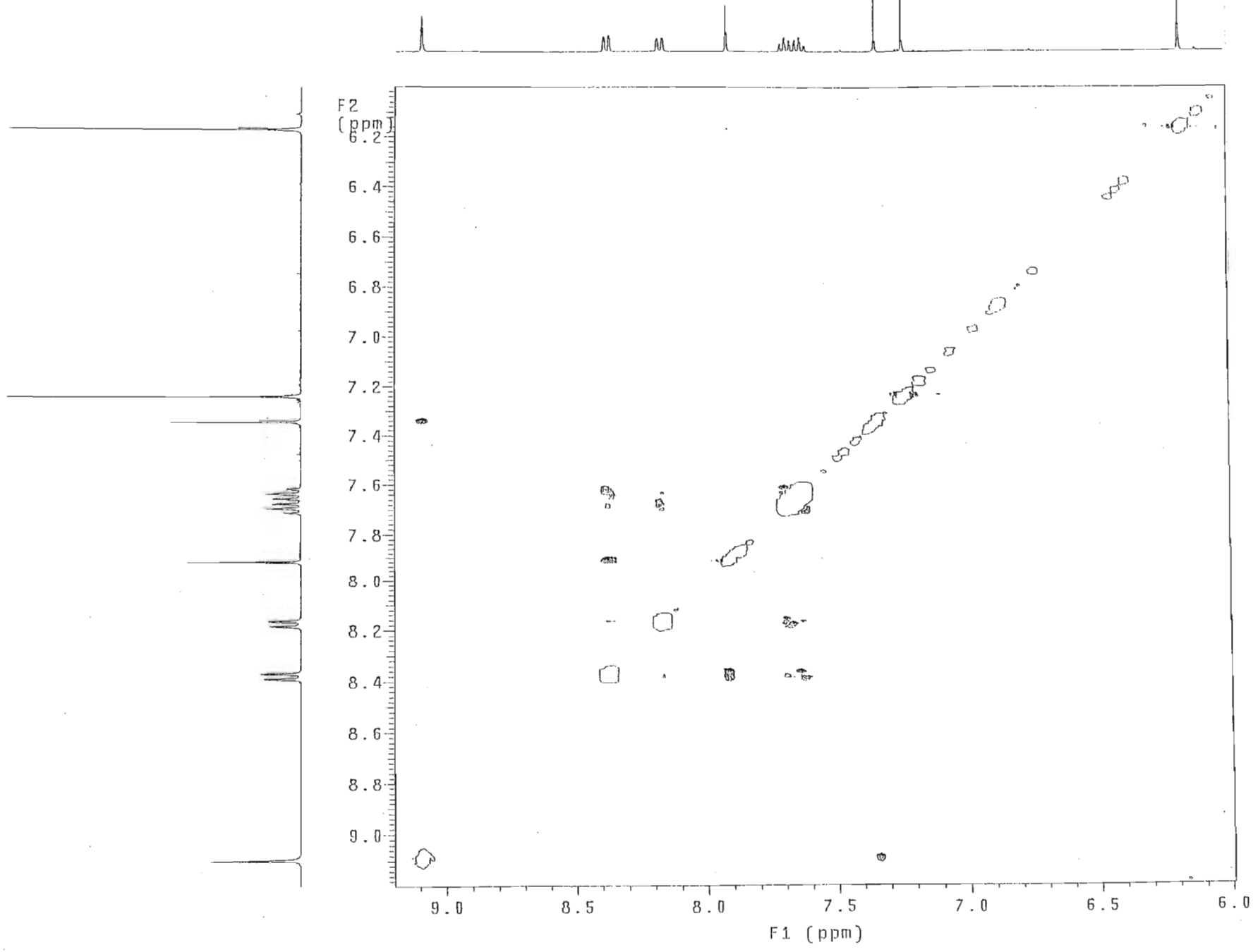
SPECTRUM 6.c: HSQC spectrum of compound VI ($CDCl_3$)



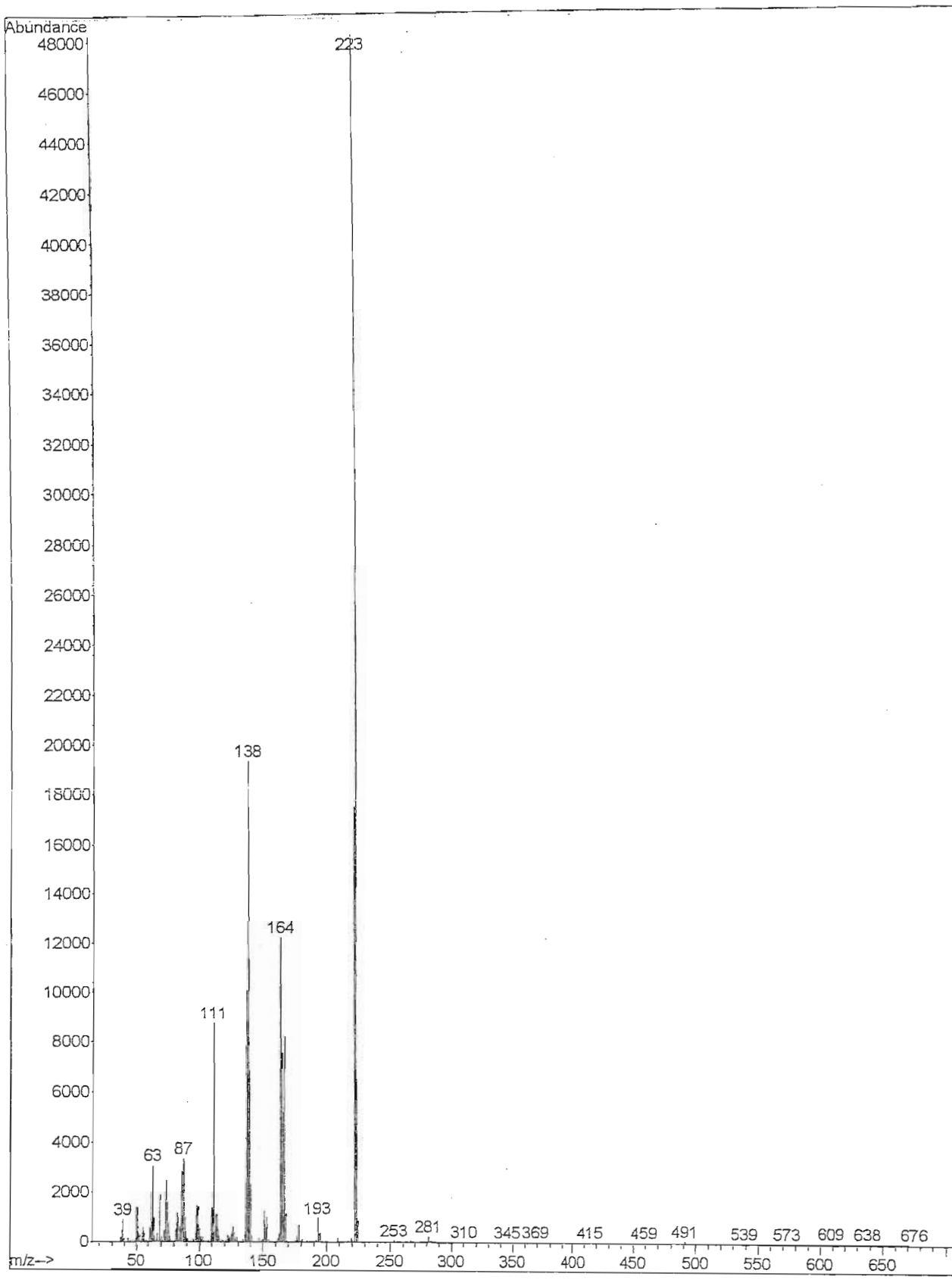
SPECTRUM 6.d: HMBC spectrum of compound VI ($CDCl_3$)



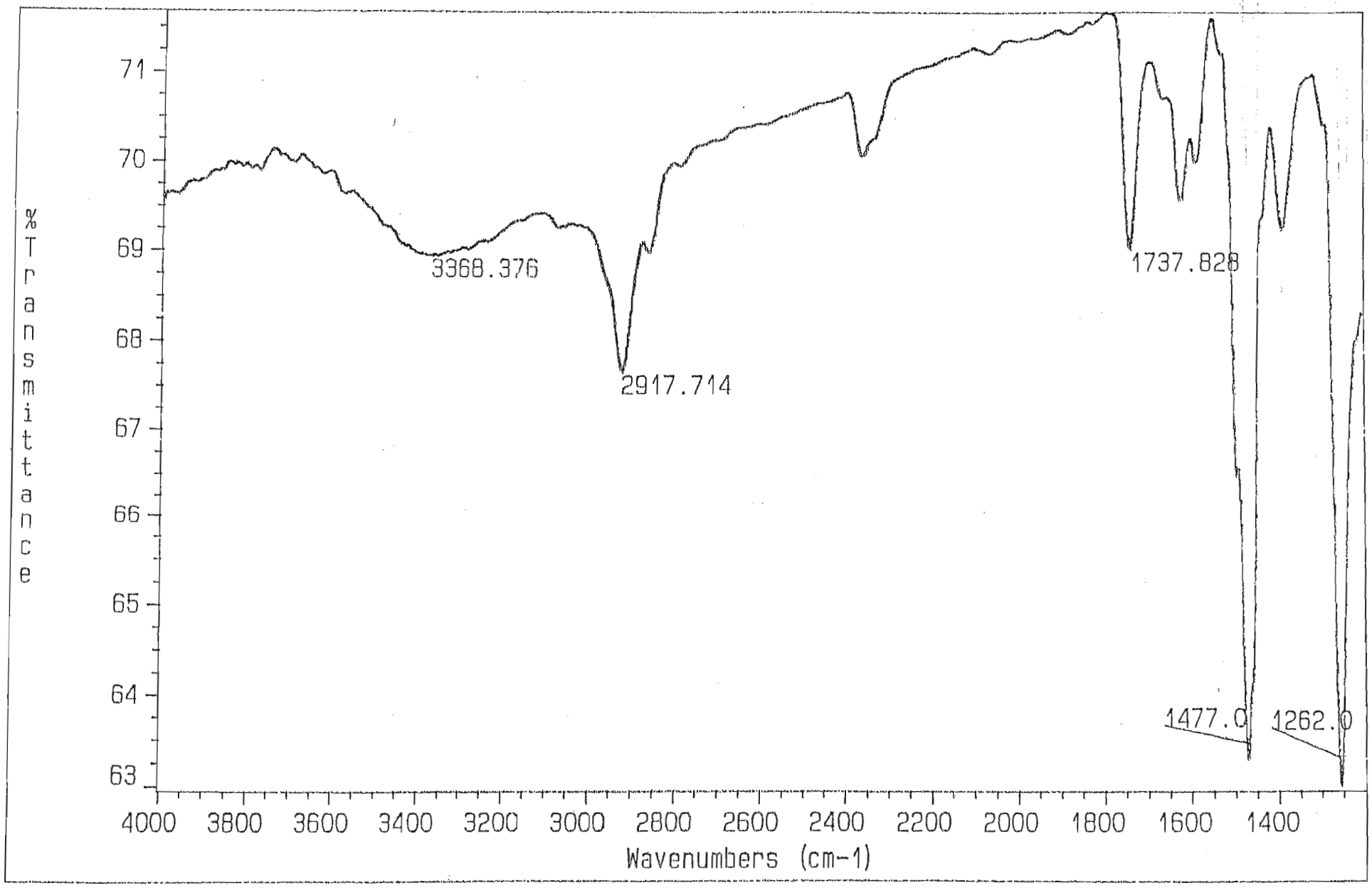
SPECTRUM 6.e: COSY spectrum of compound VI ($CDCl_3$)



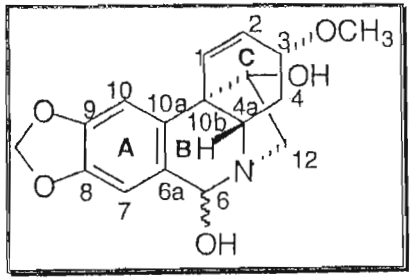
SPECTRUM 6.f: NOESY spectrum of compound VI ($CDCl_3$)



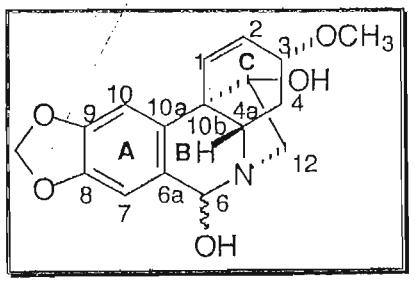
SPECTRUM 6.g: Mass spectrum of compound VI



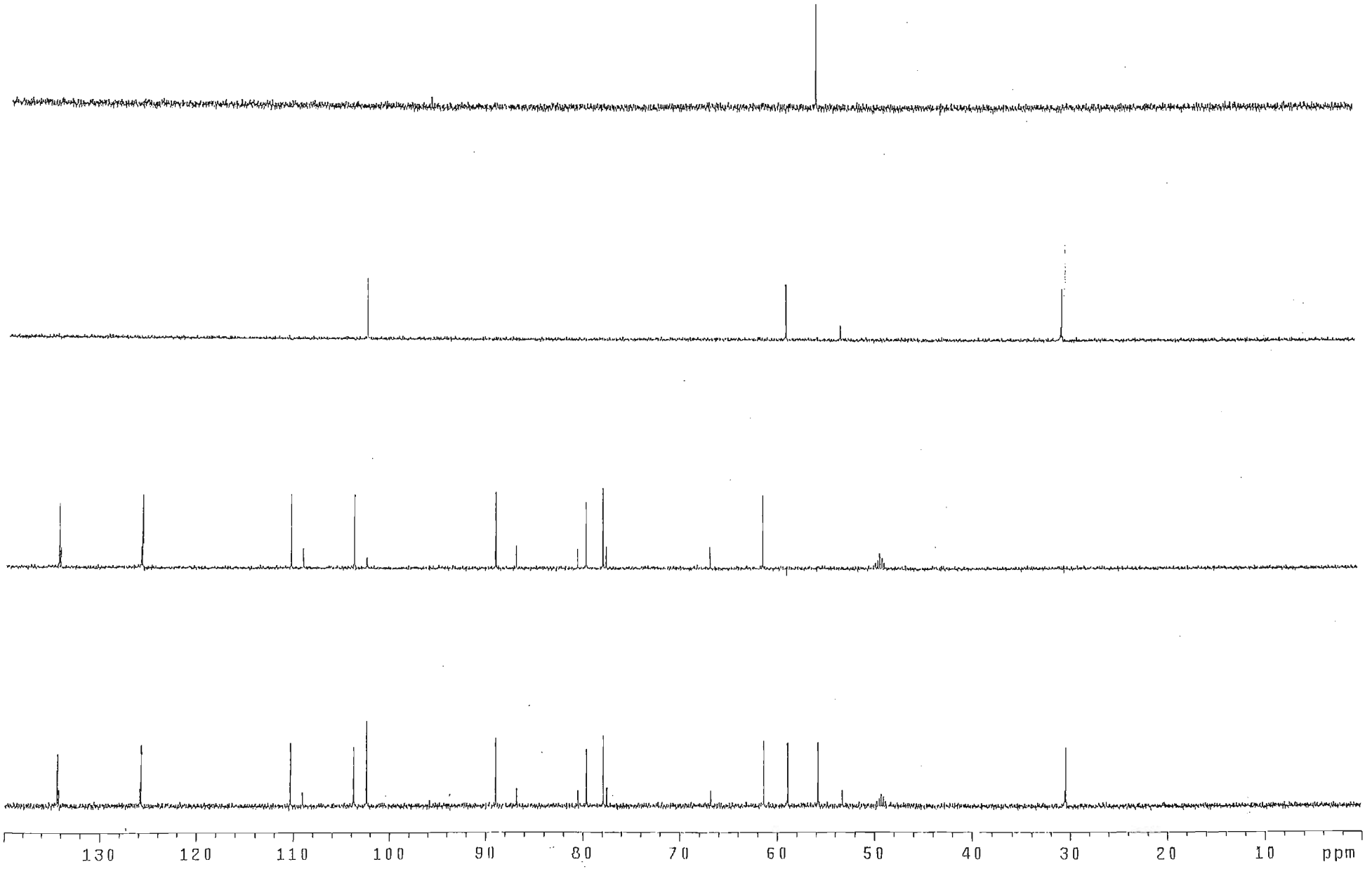
SPECTRUM 6.h: Infrared spectrum of compound VI



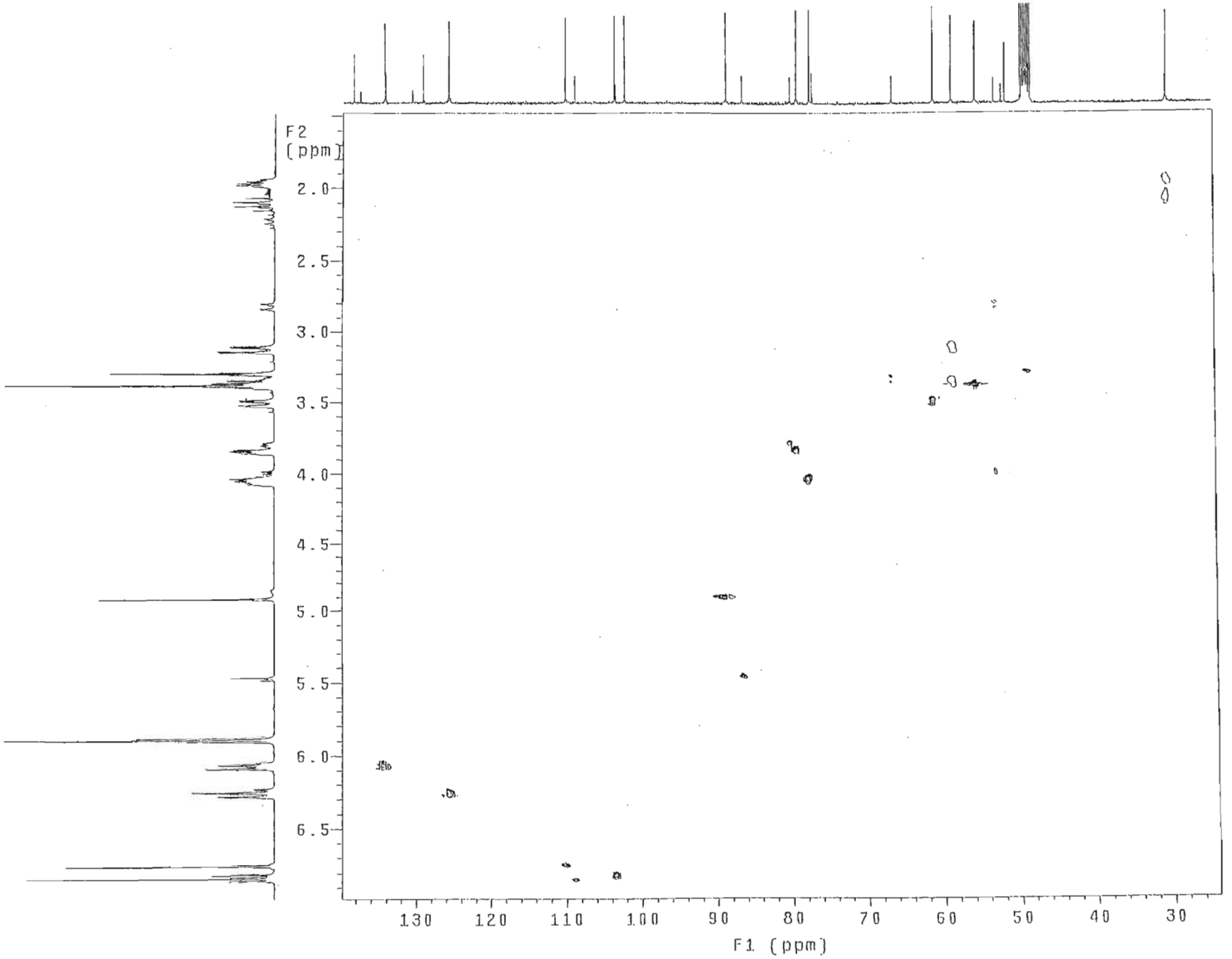
SPECTRUM 7.a: ^{1}H NMR spectrum of compound VII (CD_3OD)



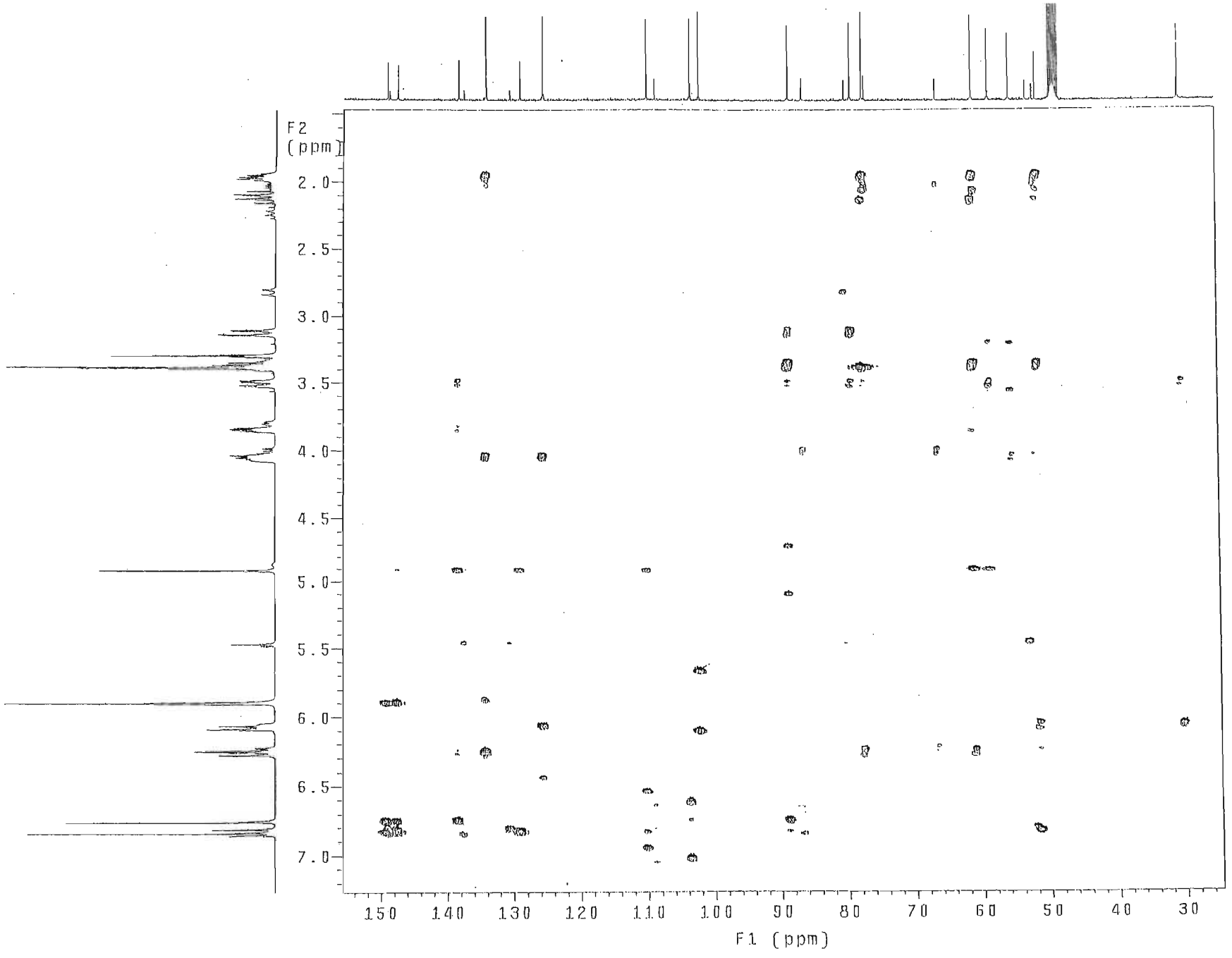
SPECTRUM 7.b: ^{13}C NMR spectrum of compound VII (CD_3OD)



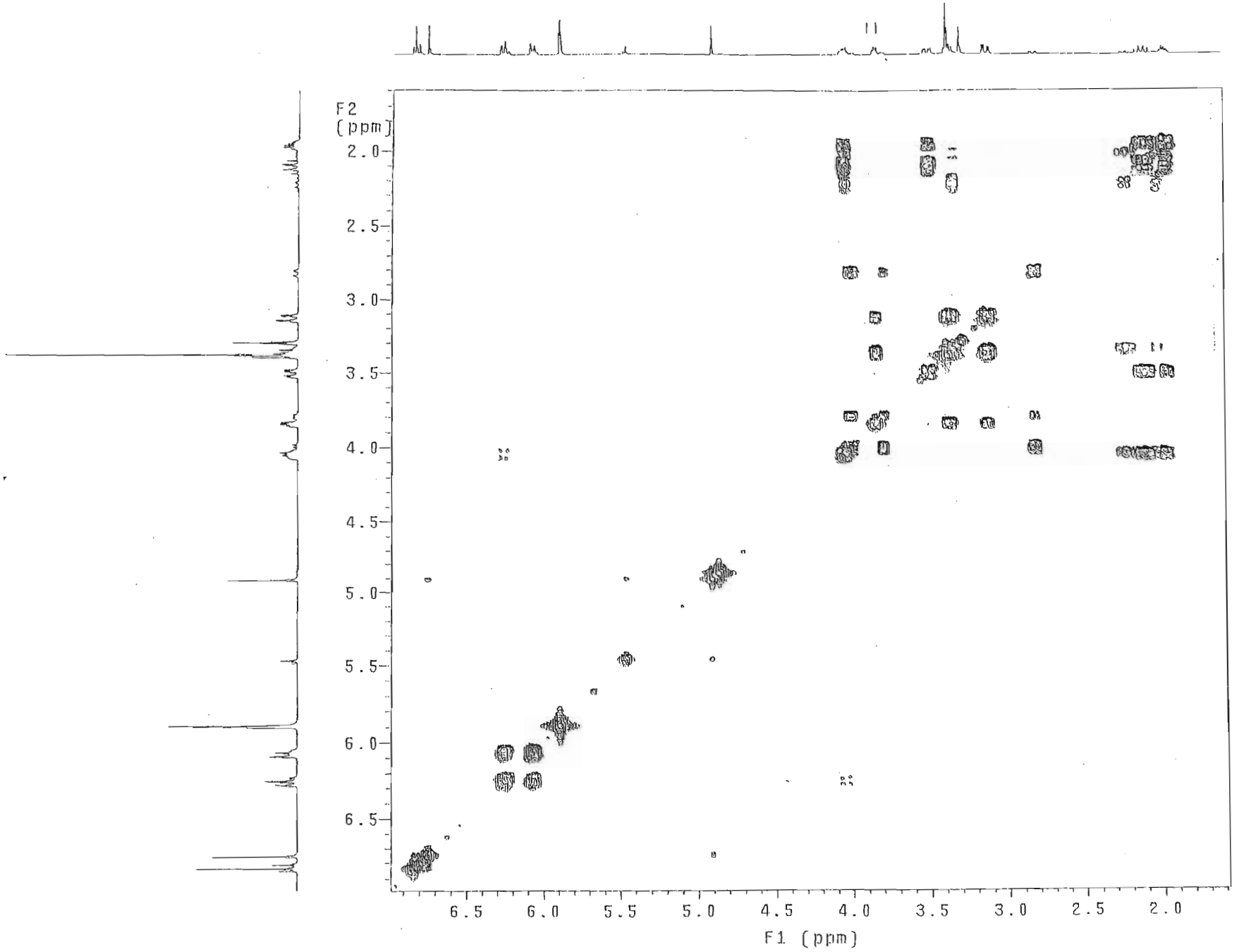
SPECTRUM 7.c: ADEPT spectrum of compound VII (CD_3OD)



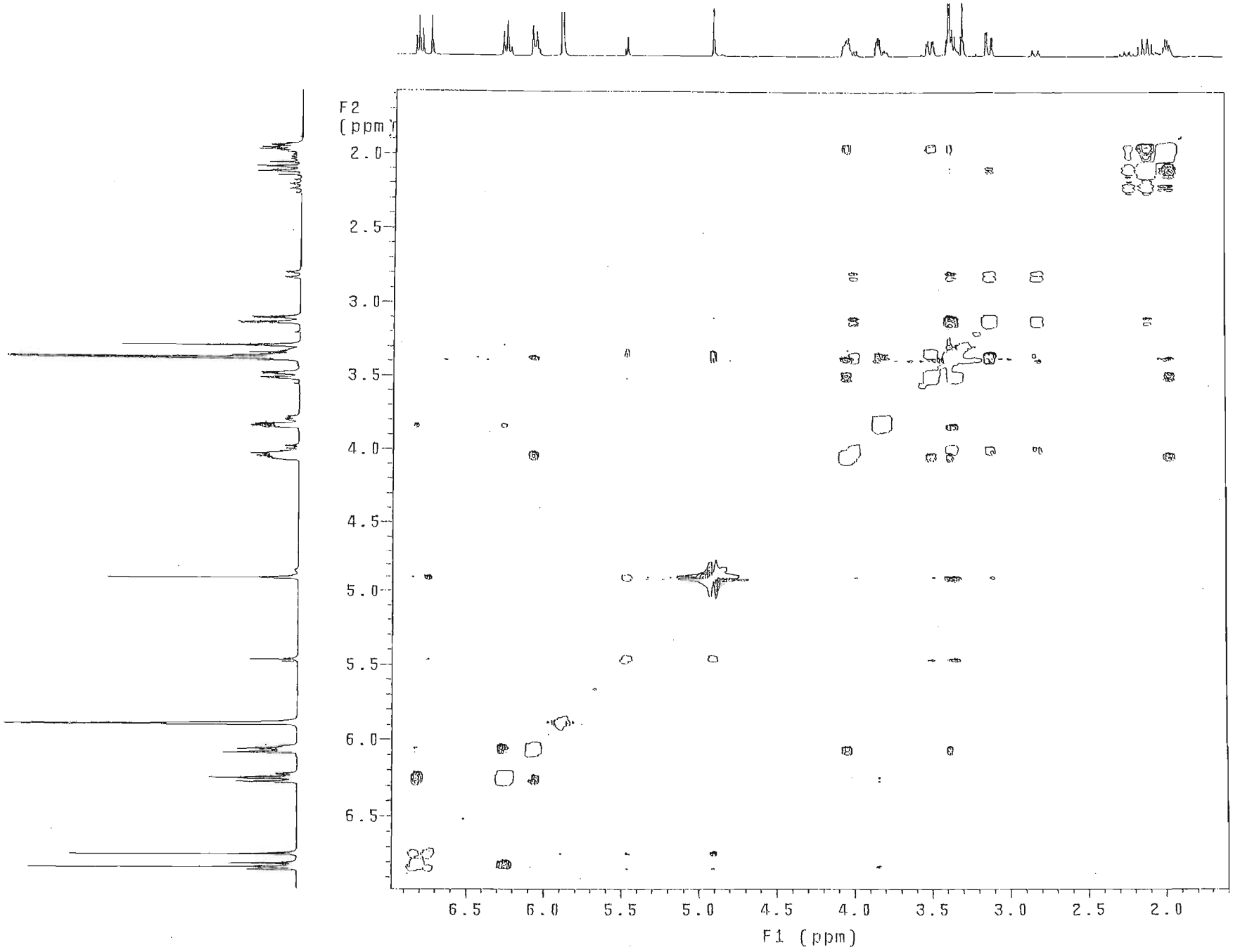
SPECTRUM 7.d: HSQC spectrum of compound VII (CD_3OD)



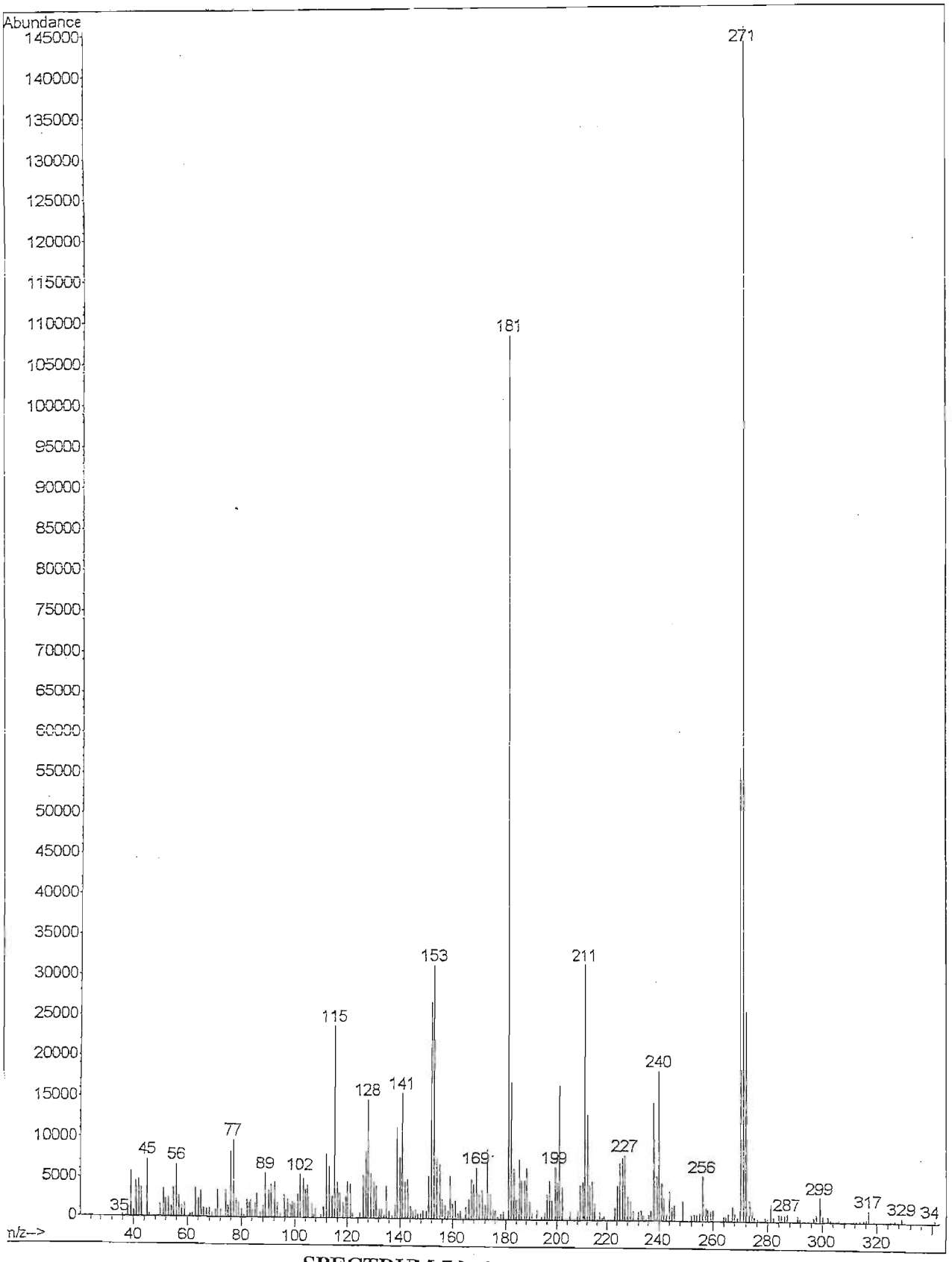
SPECTRUM 7.e: HMBC spectrum of compound VII (CD_3OD)



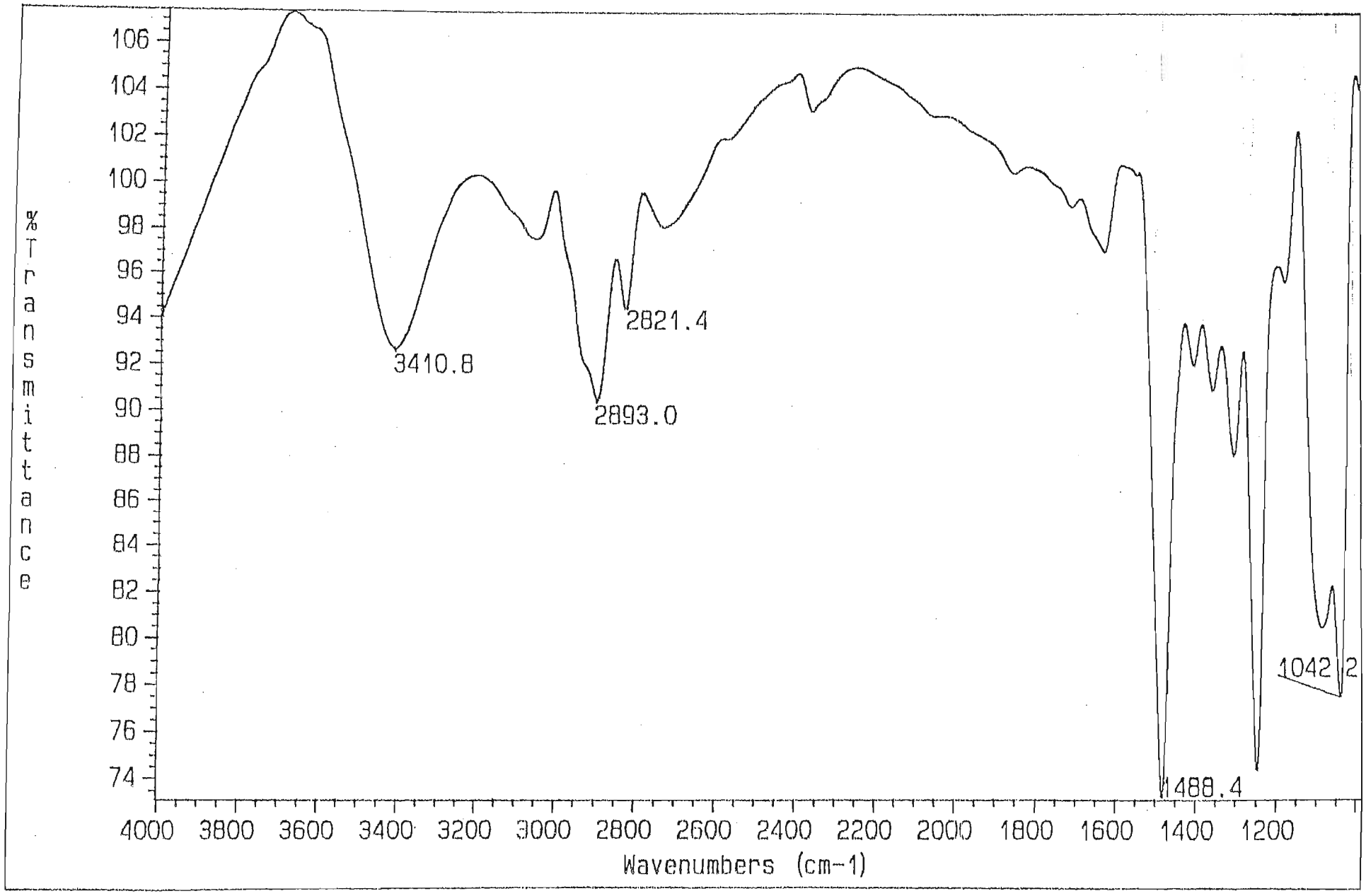
SPECTRUM 7.f: COSY spectrum of compound VII (CD_3OD)



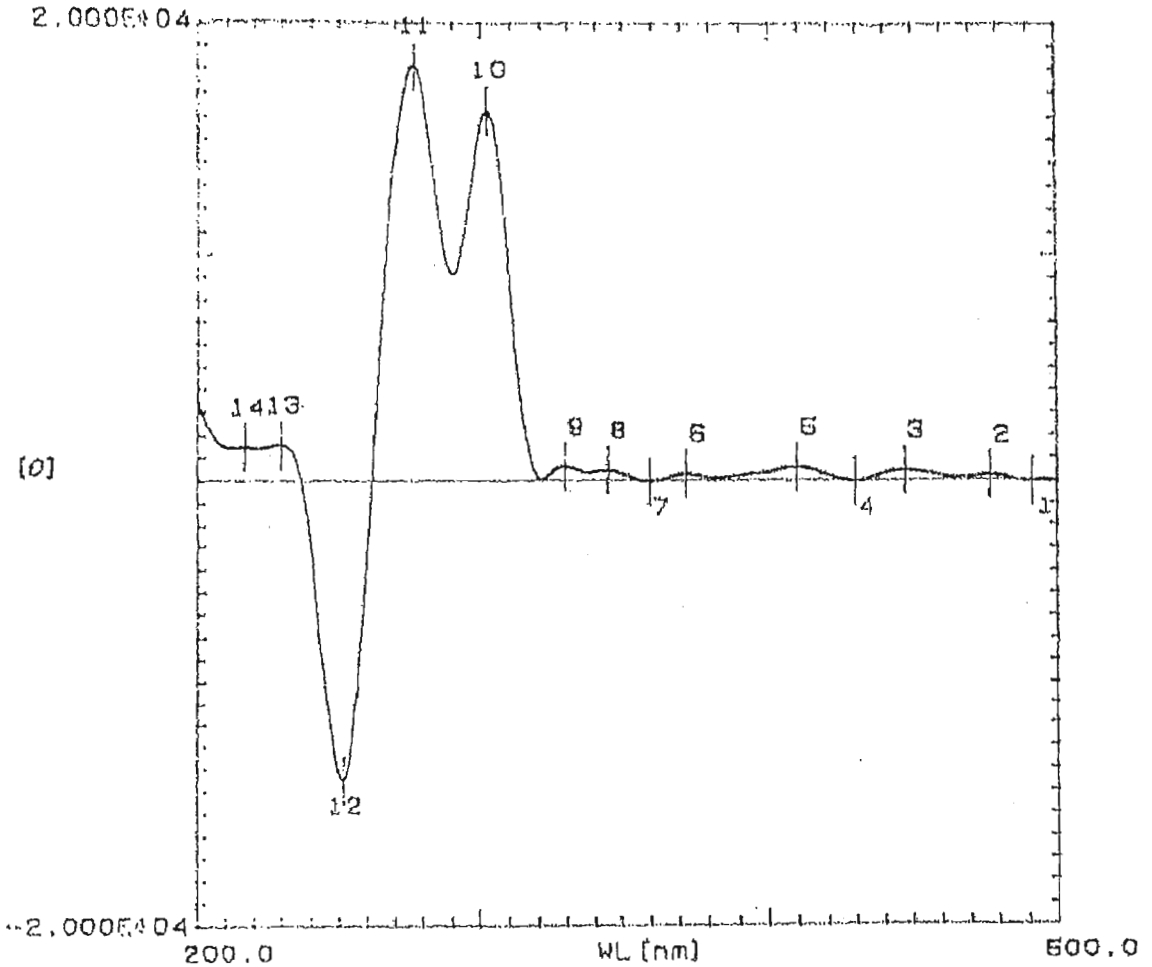
SPECTRUM 7.g: NOESY spectrum of compound VII (CD_3OD)



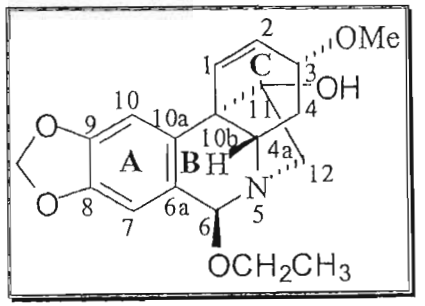
SPECTRUM 7.h: Mass spectrum of compound VII

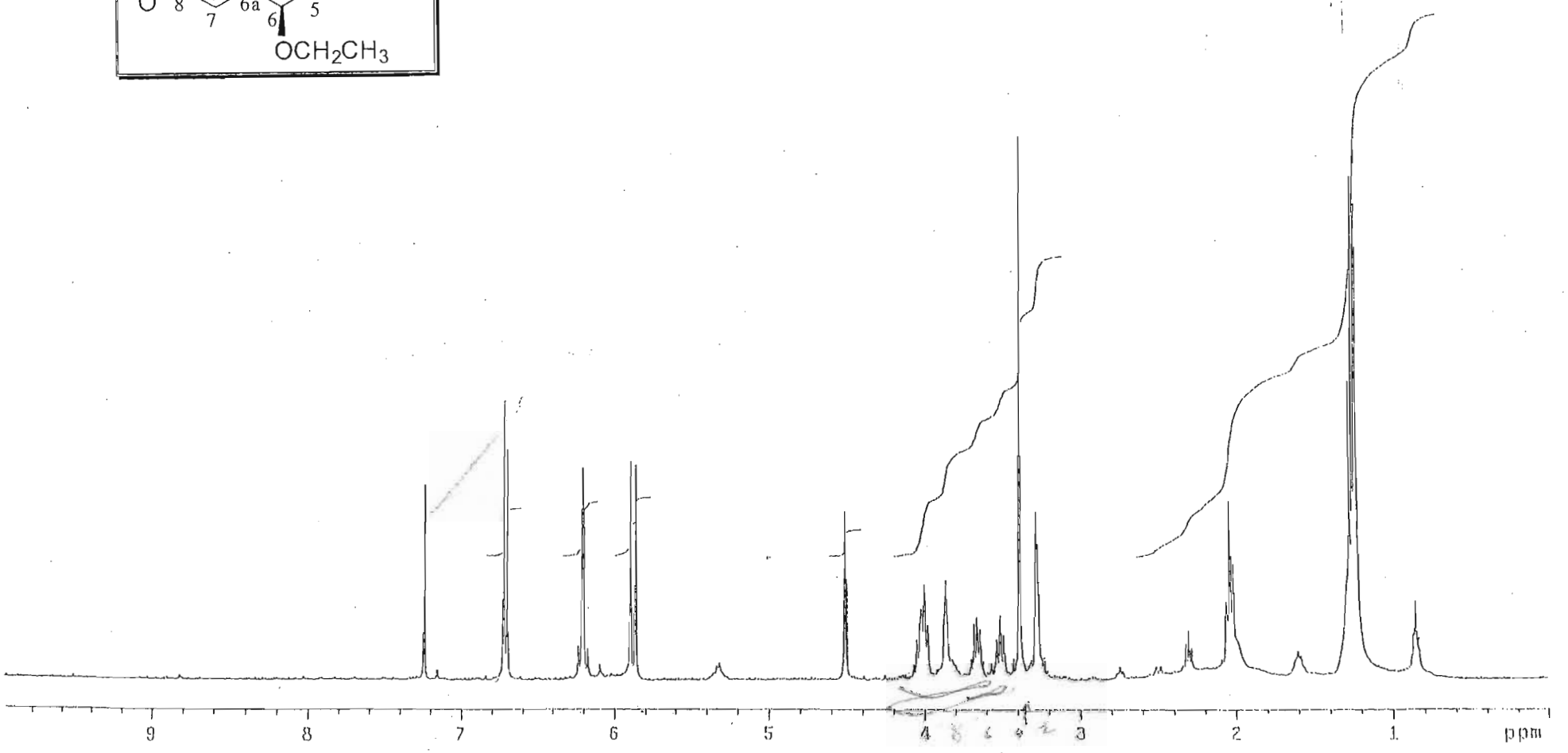


SPECTRUM 7.i: Infrared spectrum of compound VII

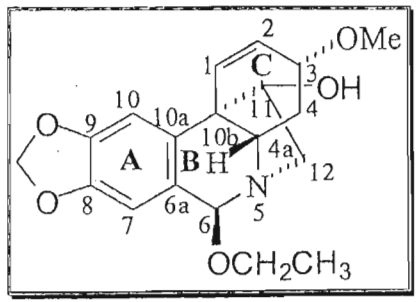


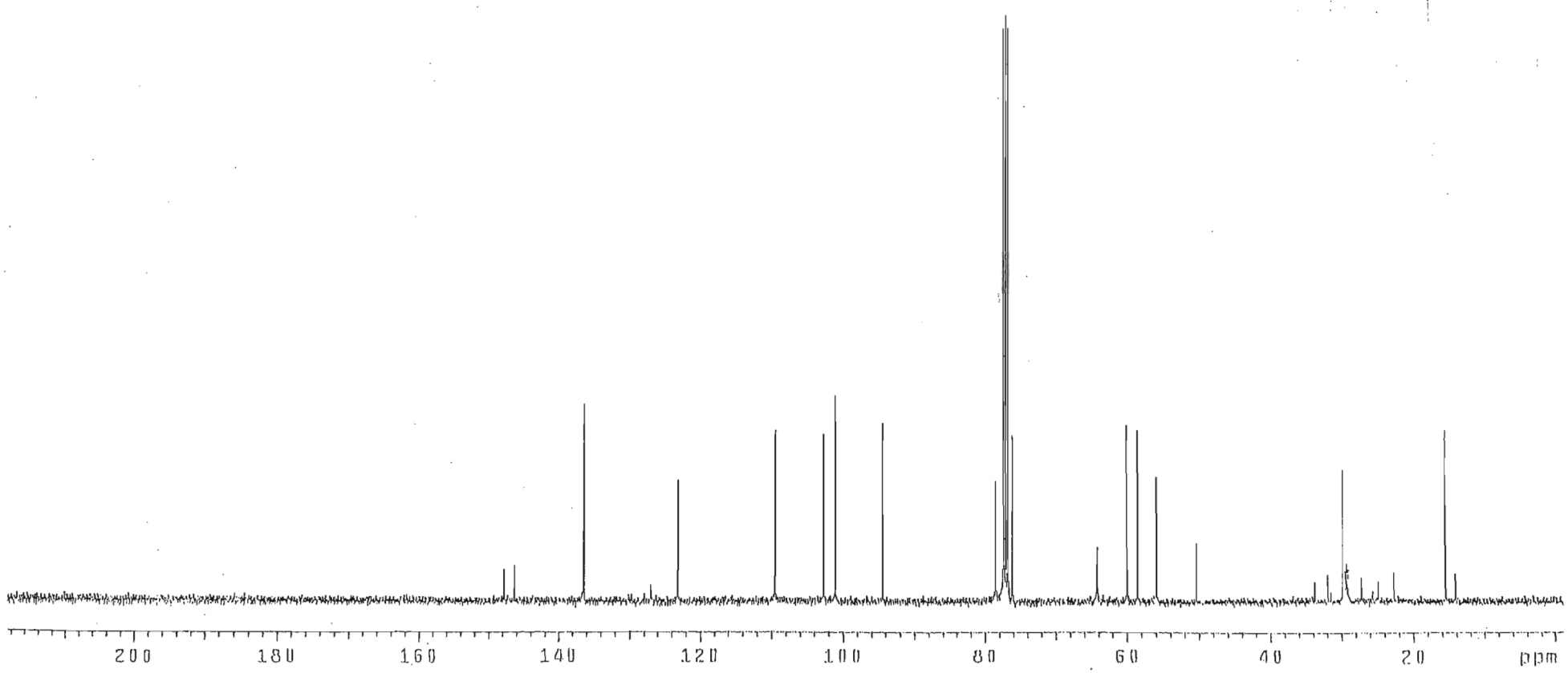
SPECTRUM 7.j: CD spectrum of compound VII



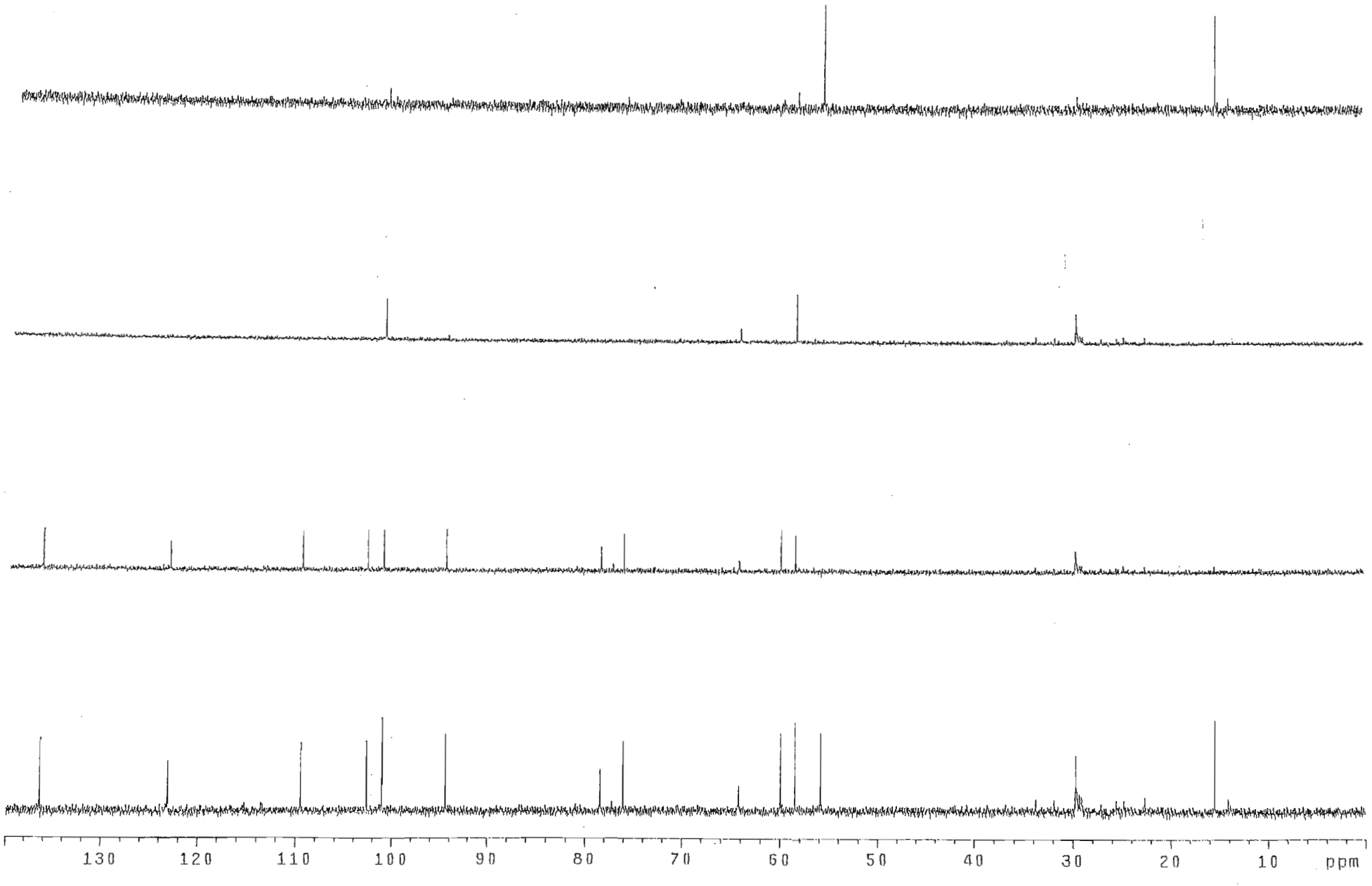


SPECTRUM 8.a: ^{1}H NMR spectrum of compound VIII ($CDCl_3$)

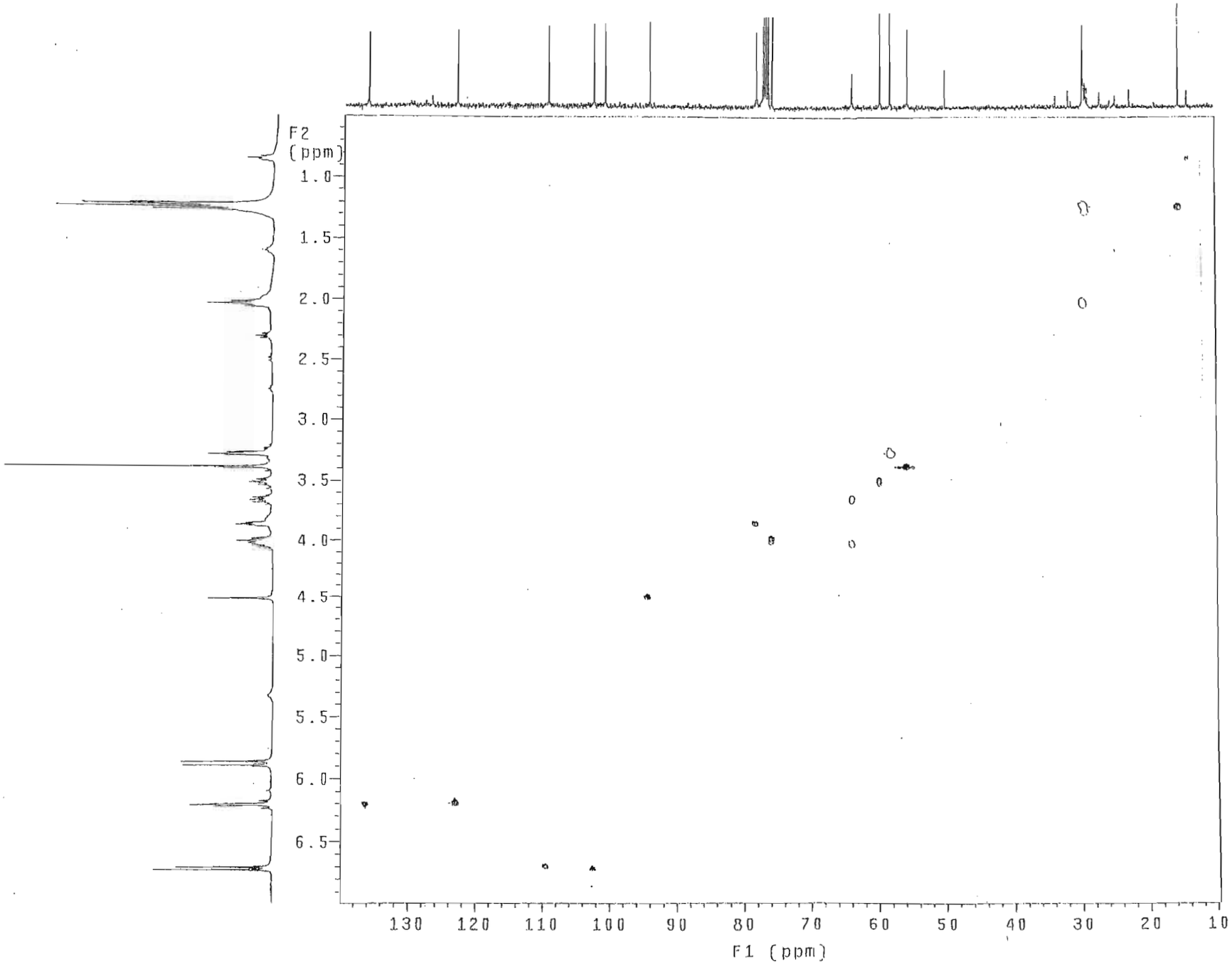




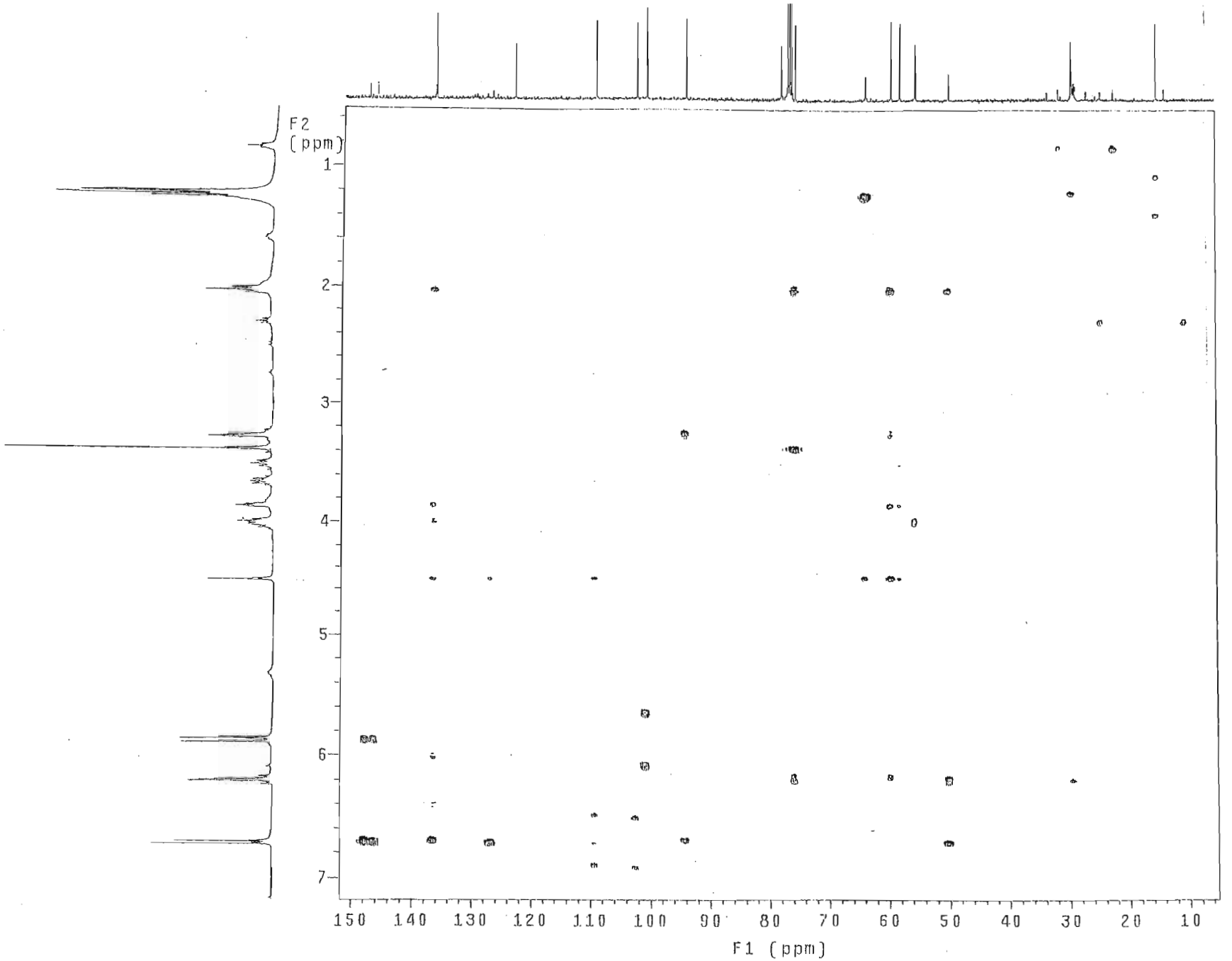
SPECTRUM 8.b: ^{13}C NMR spectrum of compound VIII ($CDCl_3$)



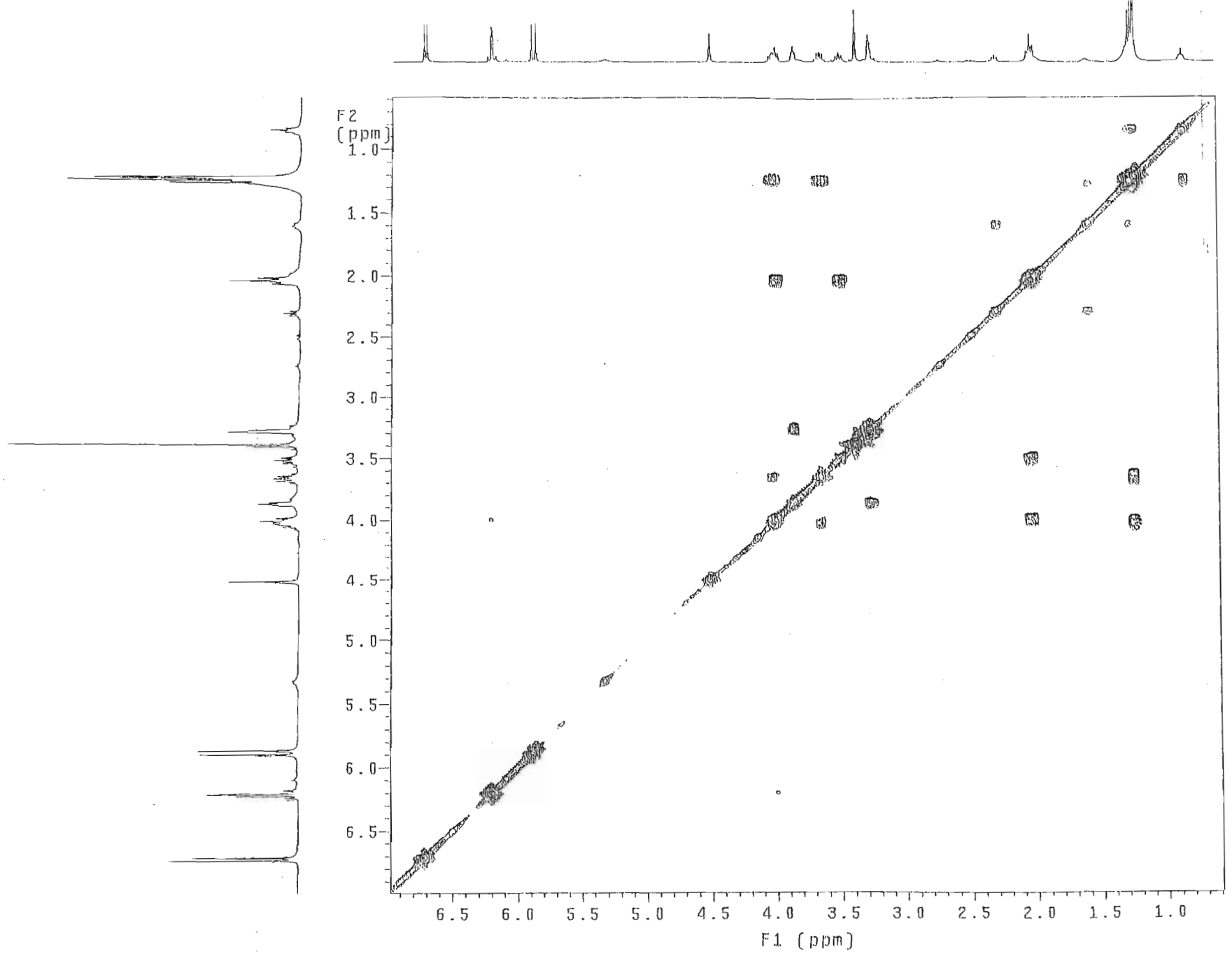
SPECTRUM 8.c: ADEPT spectrum of compound VIII ($CDCl_3$)



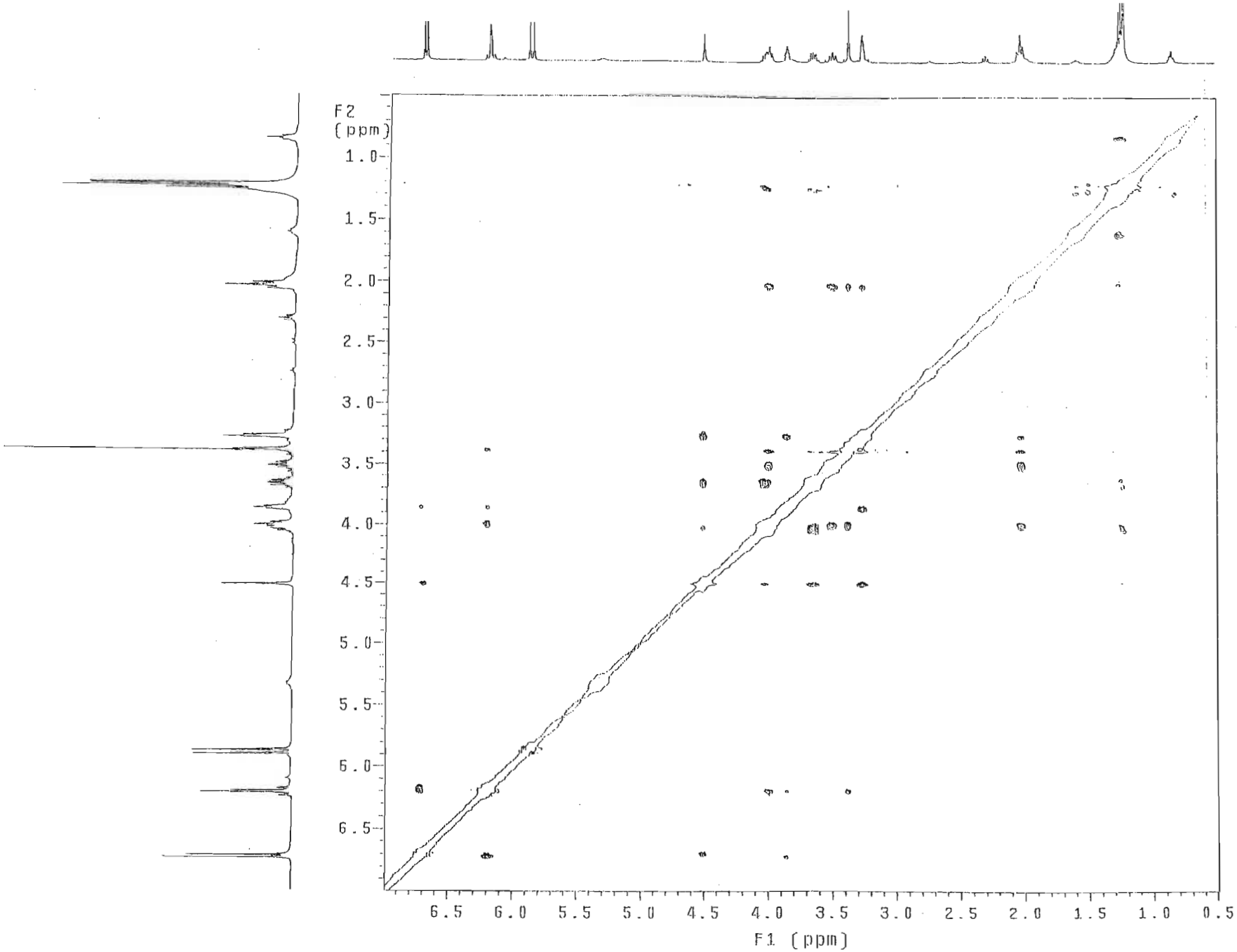
SPECTRUM 8.d: HSQC spectrum of compound VIII ($CDCl_3$)



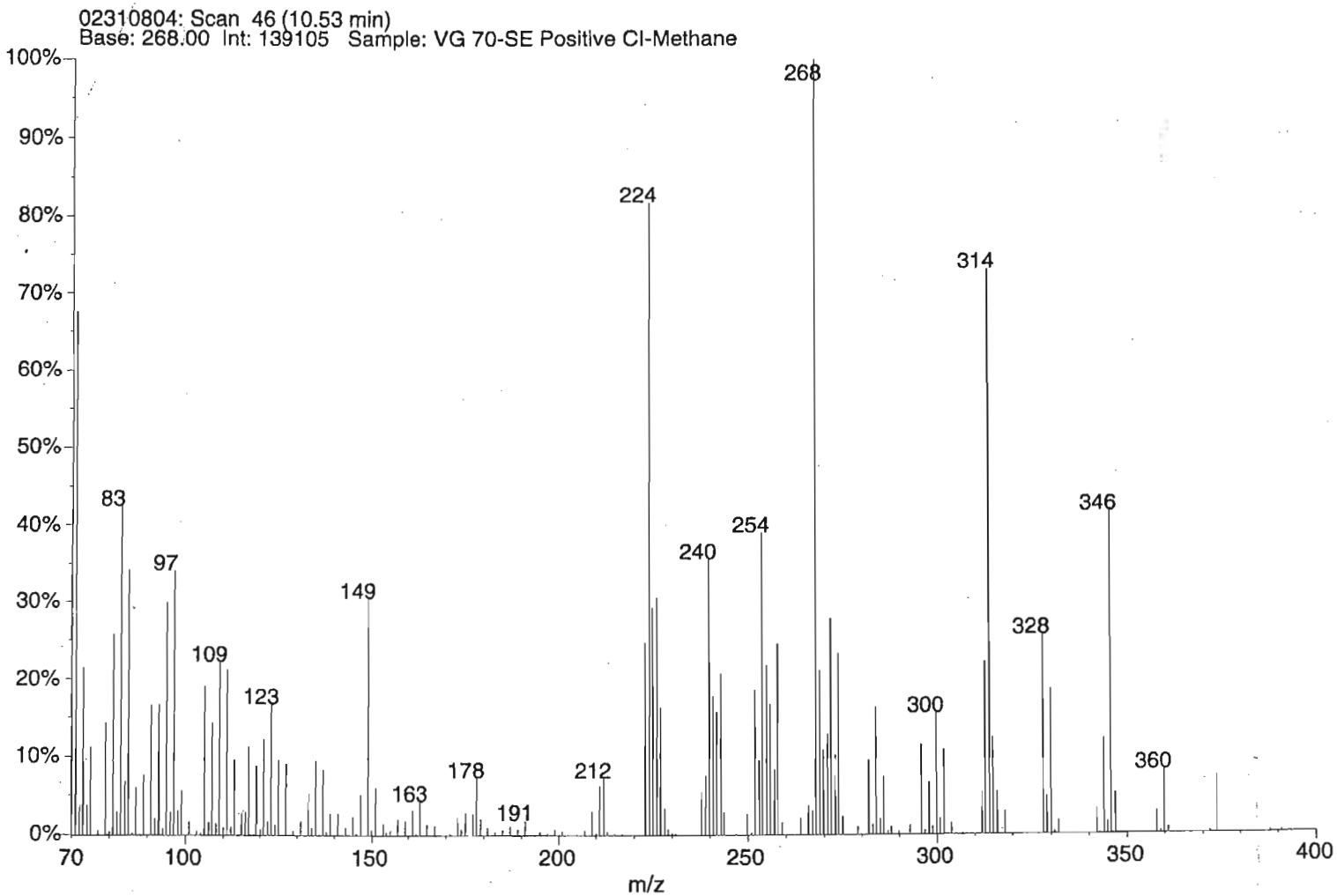
SPECTRUM 8.e: HMBC spectrum of compound VIII ($CDCl_3$)



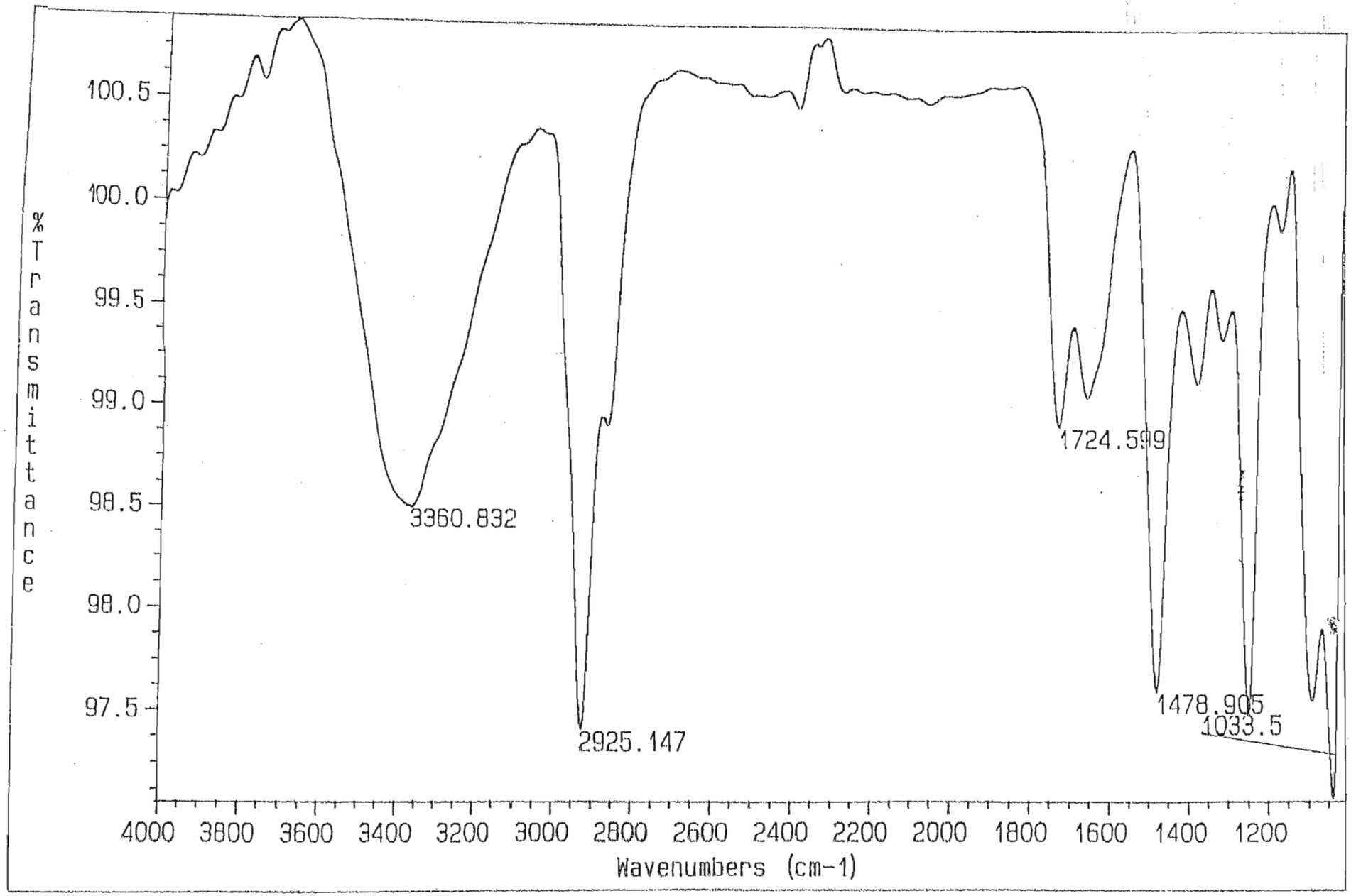
SPECTRUM 8.f: COSY spectrum of compound VIII ($CDCl_3$)



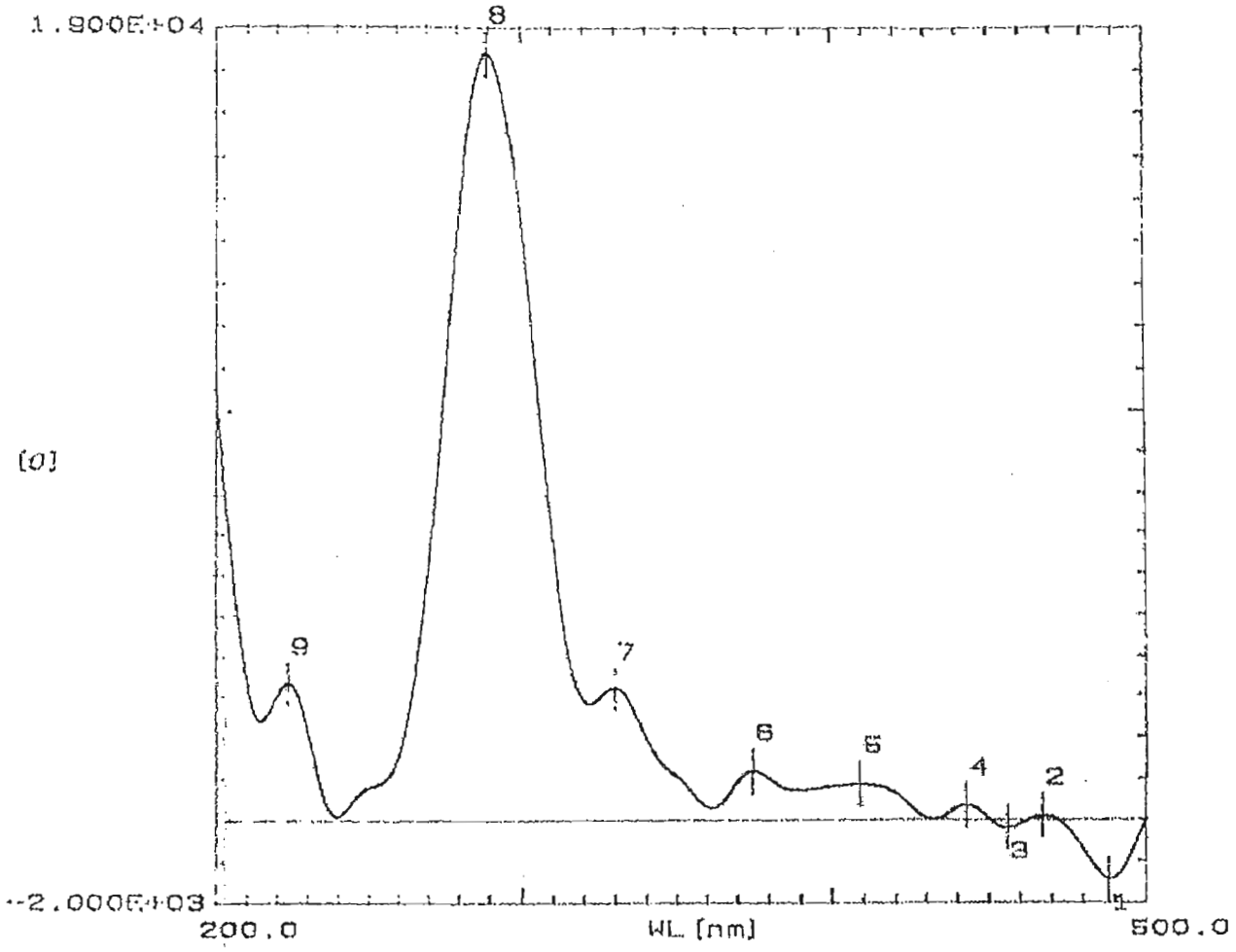
SPECTRUM 8.g: NOESY spectrum of compound VIII ($CDCl_3$)



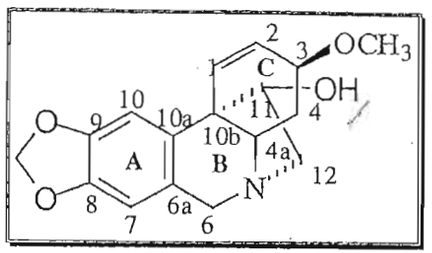
SPECTRUM 8.h: Mass spectrum of compound VIII



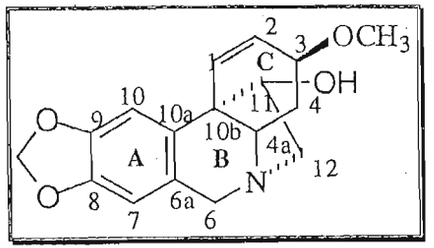
SPECTRUM 8.i: Infrared spectrum of compound VIII



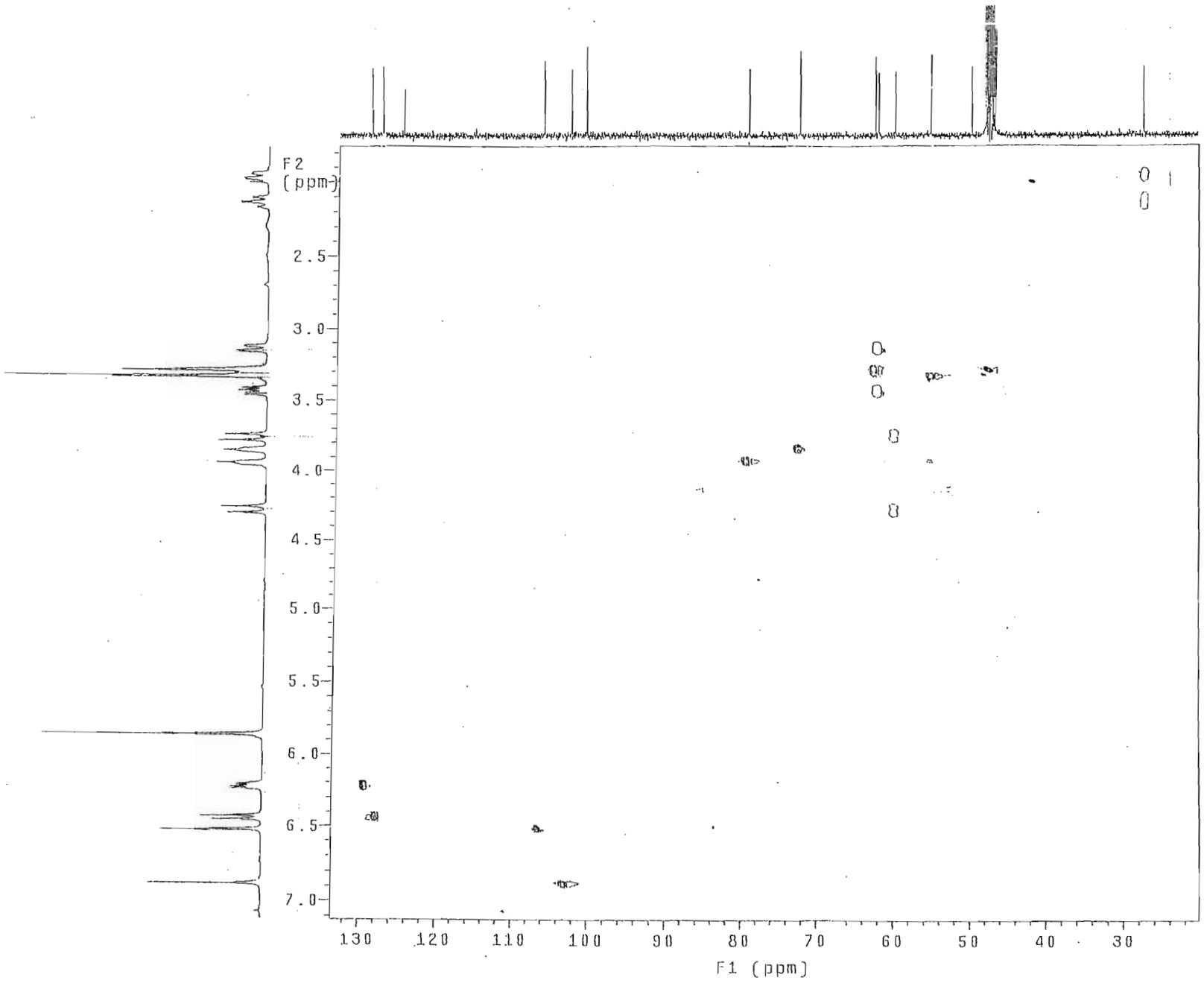
SPECTRUM 8.j: CD spectrum of compound VIII



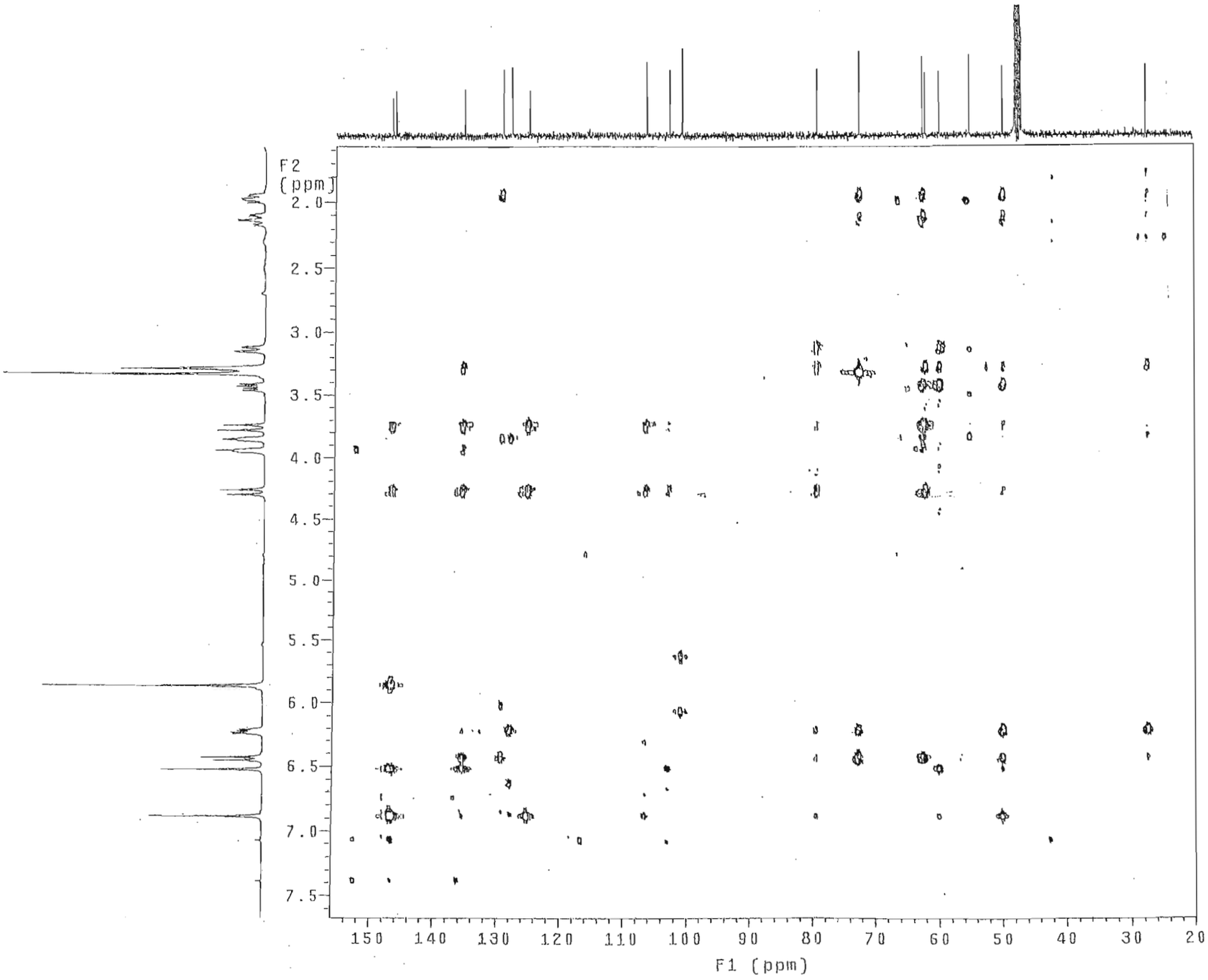
SPECTRUM 9.a: ^{1}H NMR spectrum of compound IX (CD_3OD)



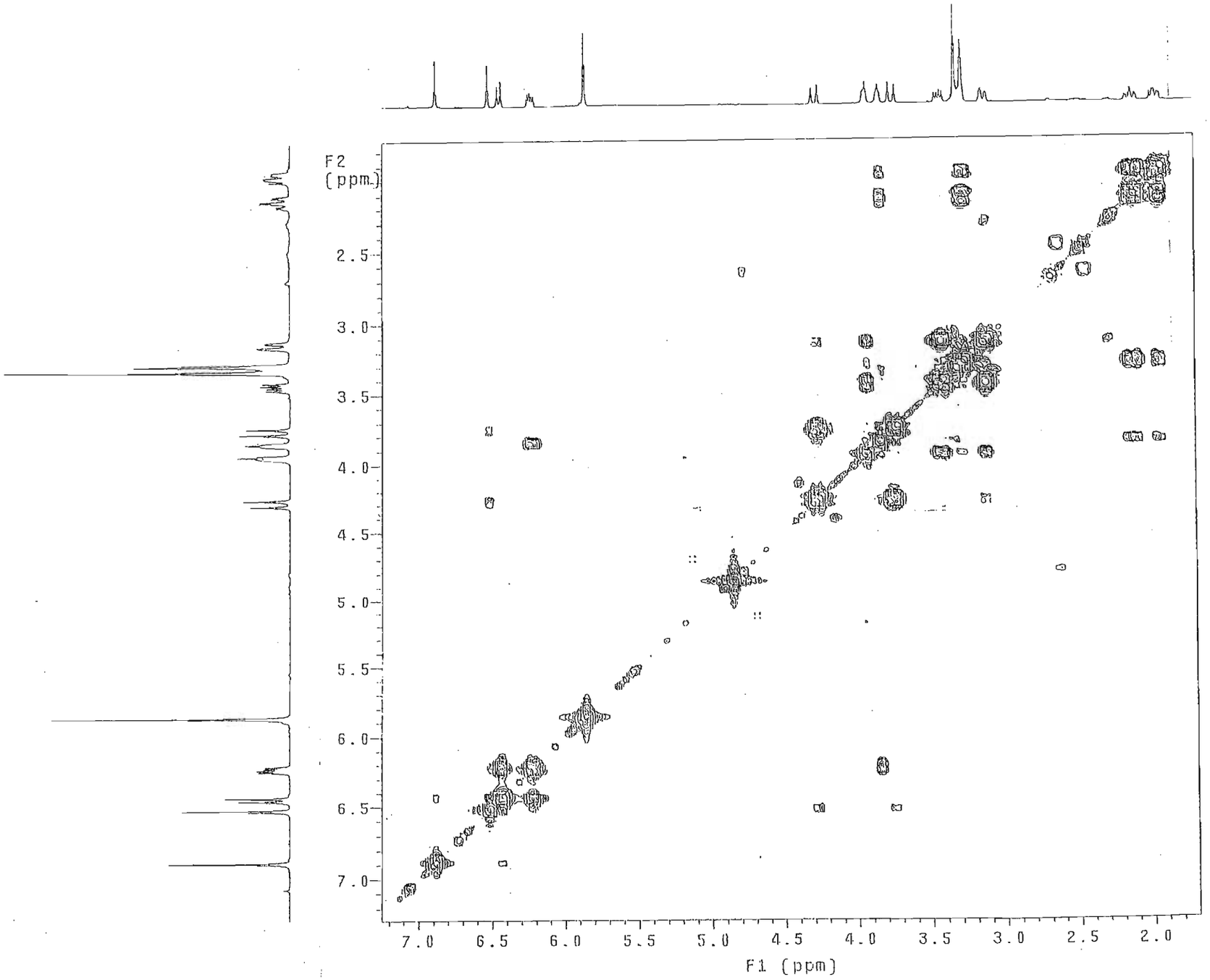
SPECTRUM 9.b: ^{13}C NMR spectrum of compound IX (CD_3OD)



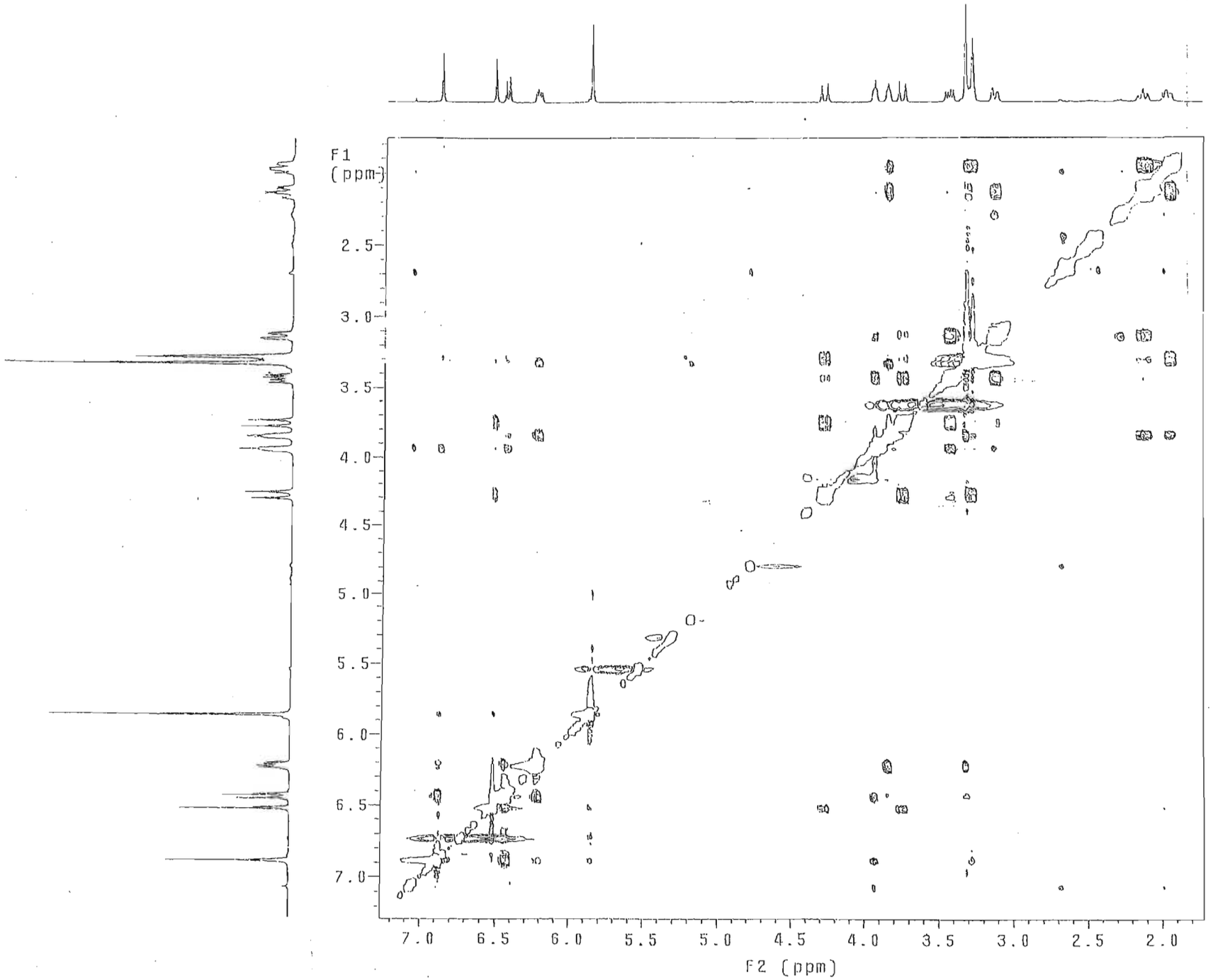
SPECTRUM 9.c: HSQC spectrum of compound IX (CD_3OD)



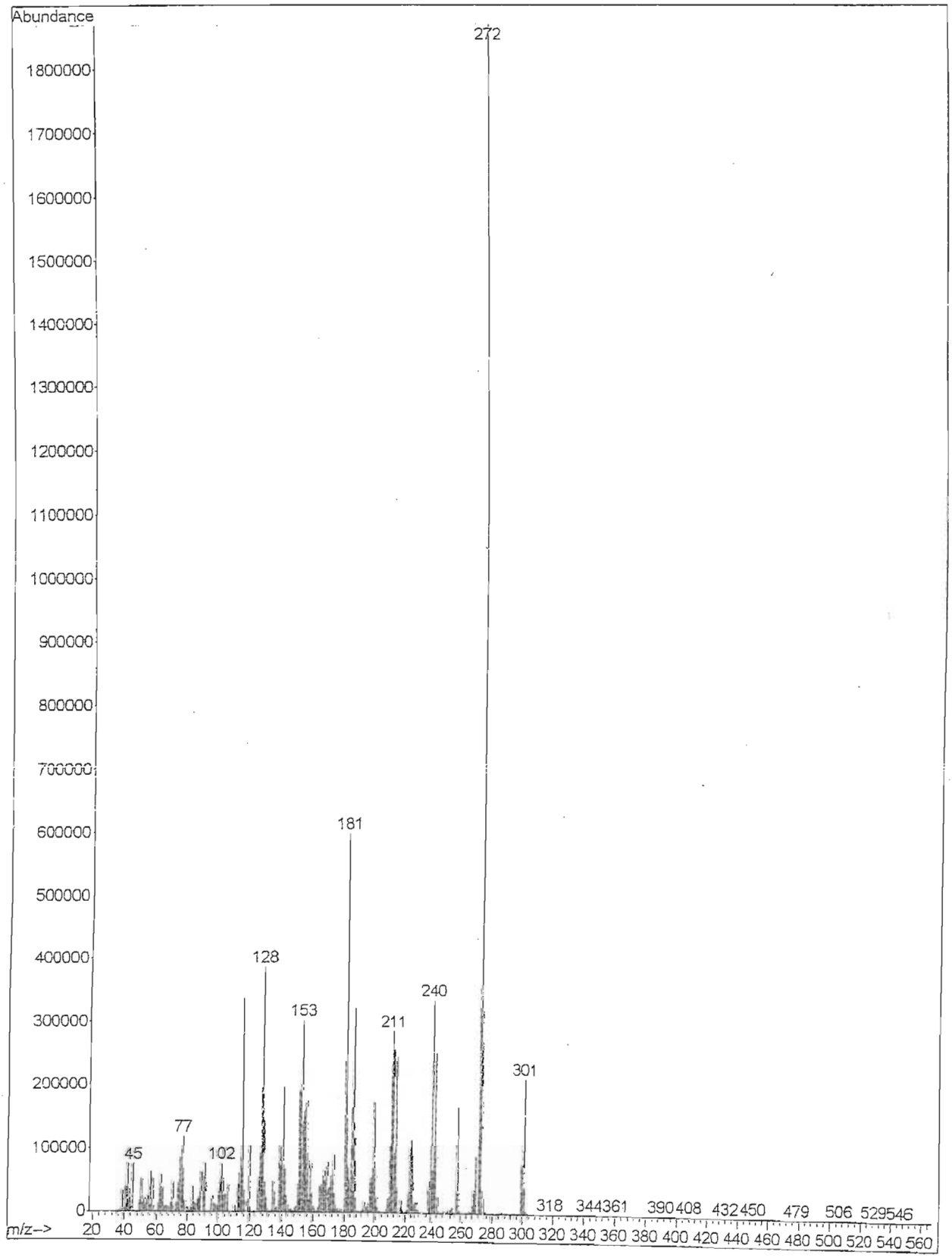
SPECTRUM 9.d: HMBC spectrum of compound IX (CD_3OD)



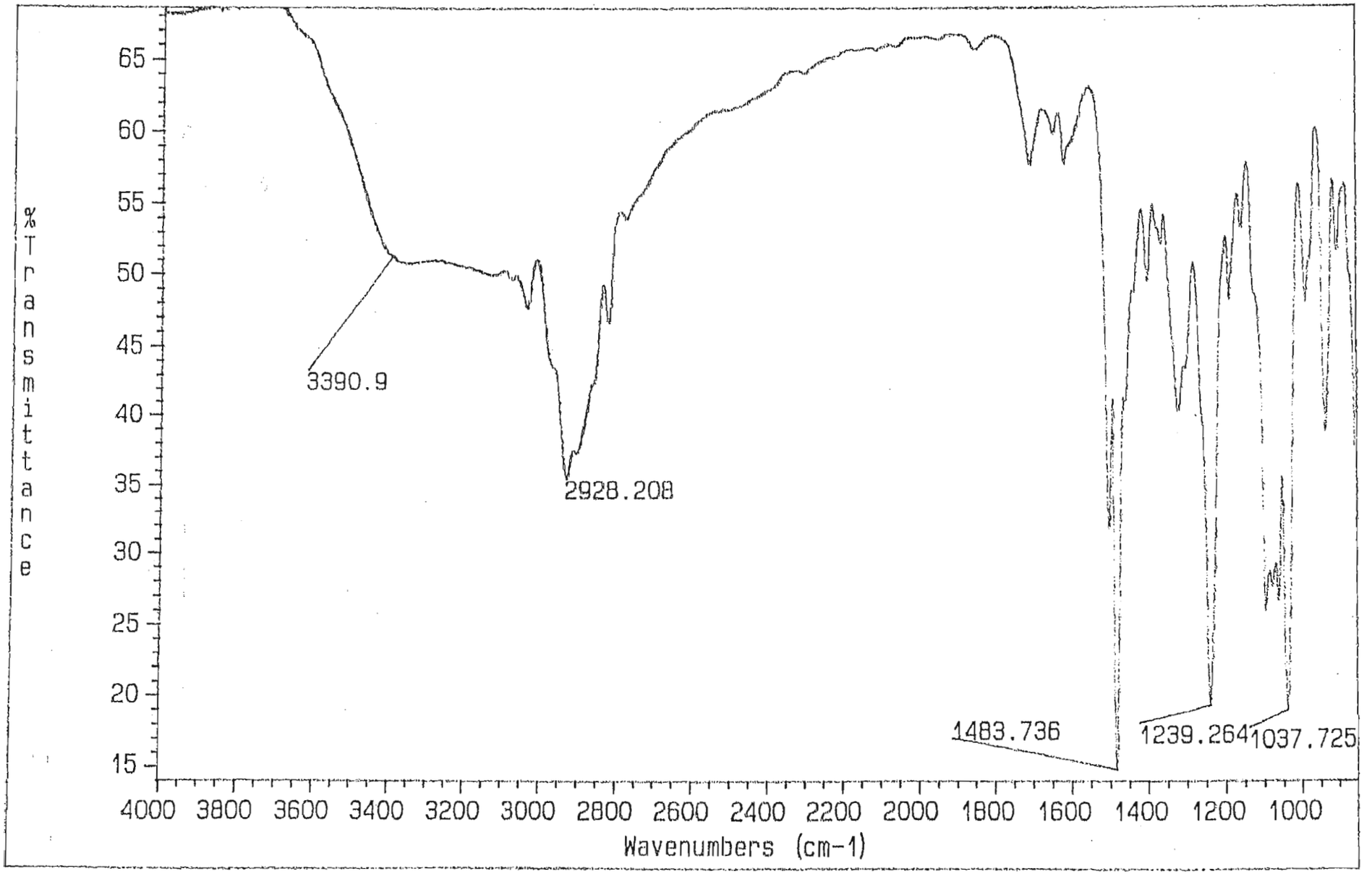
SPECTRUM 9.e: COSY spectrum of compound IX (CD_3OD)



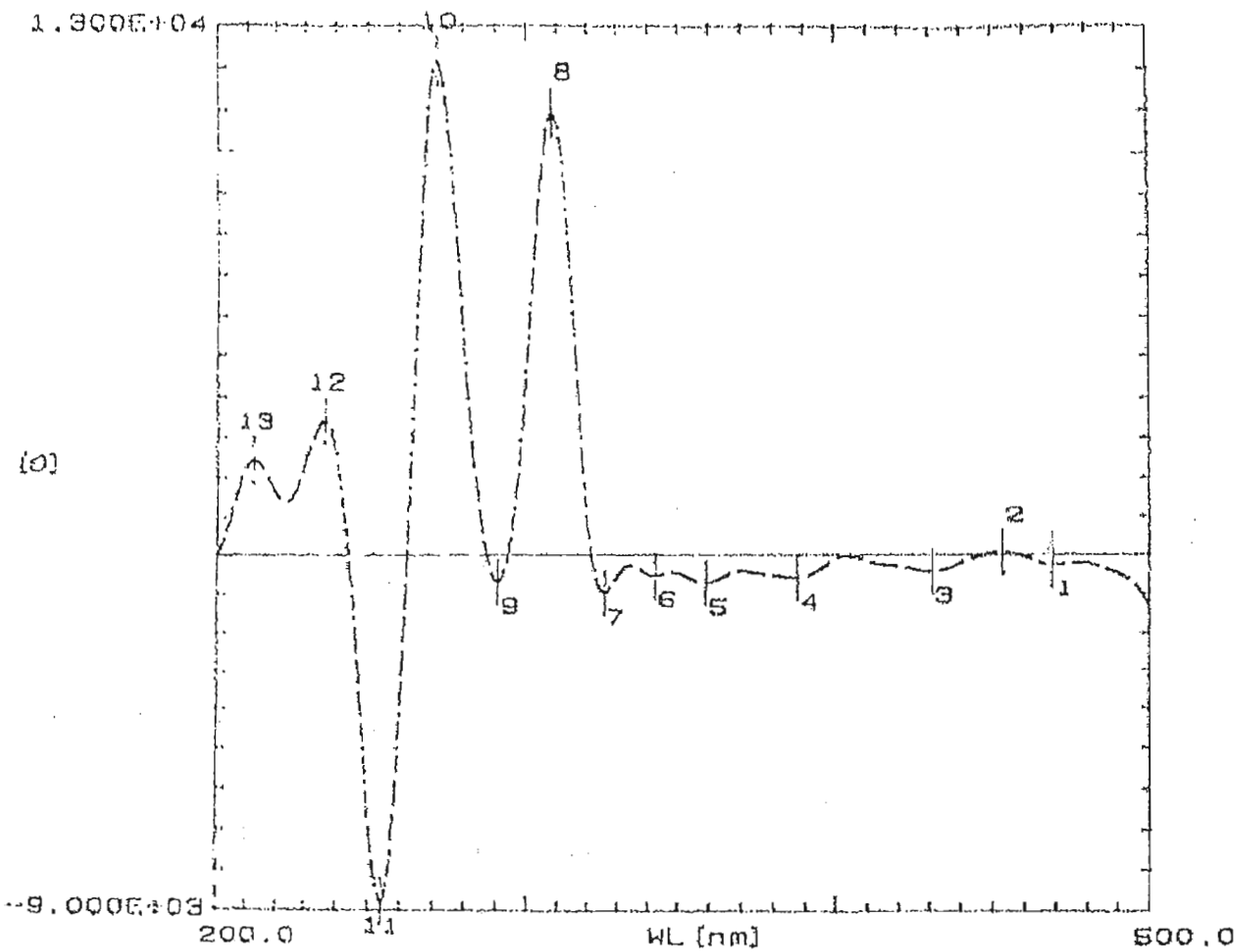
SPECTRUM 9.f: NOESY spectrum of compound IX (CD_3OD)



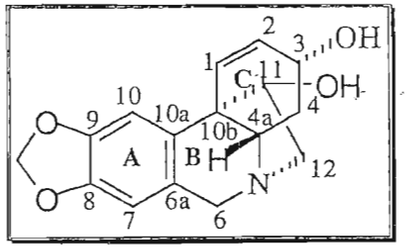
SPECTRUM 9.g: Mass spectrum of compound IX



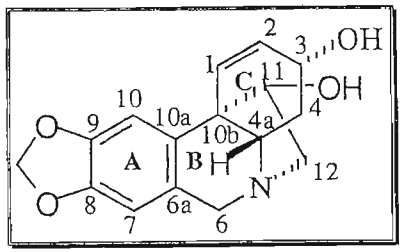
SPECTRUM 9.h: Infrared spectrum of compound IX



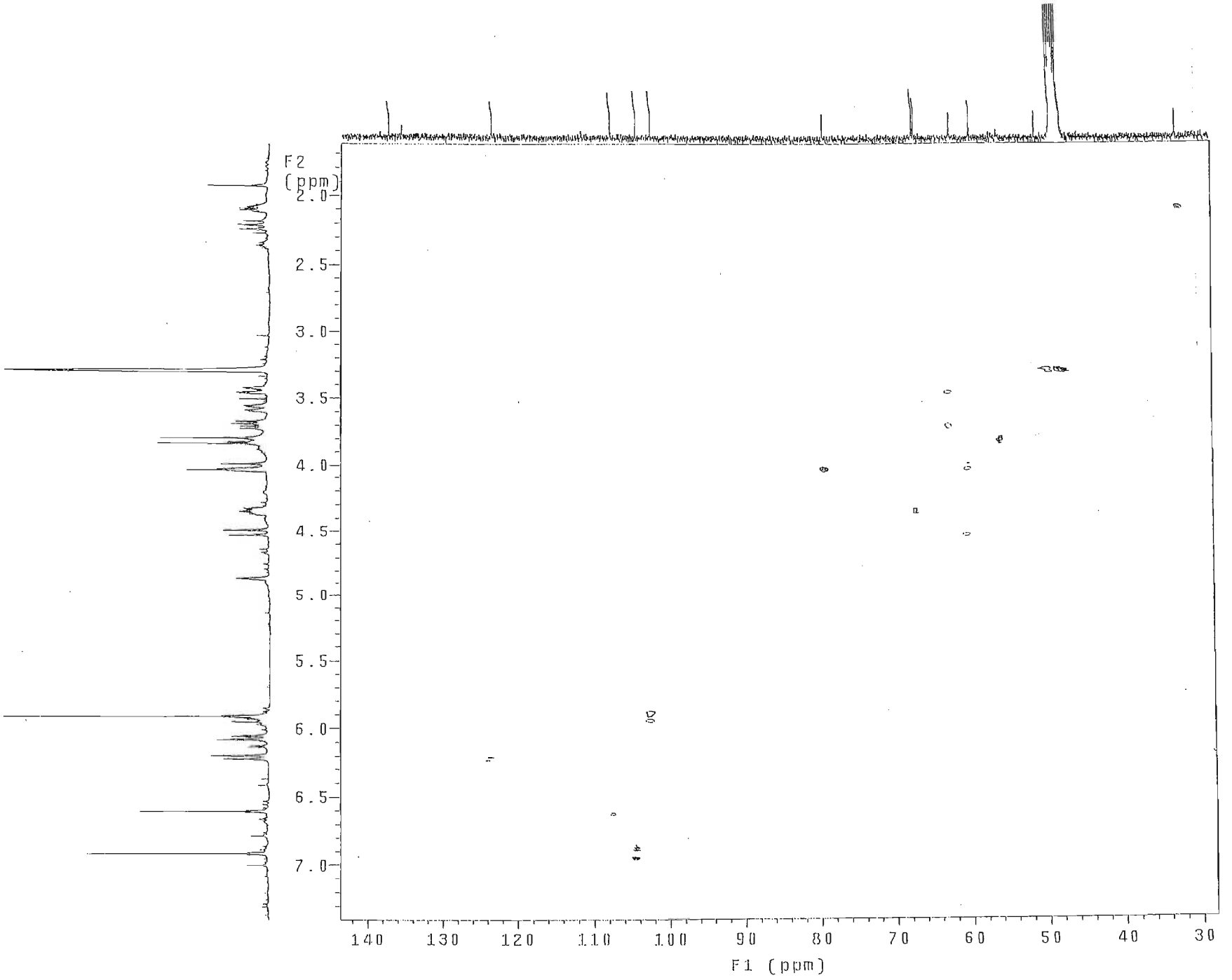
SPECTRUM 9.i: CD spectrum of compound IX



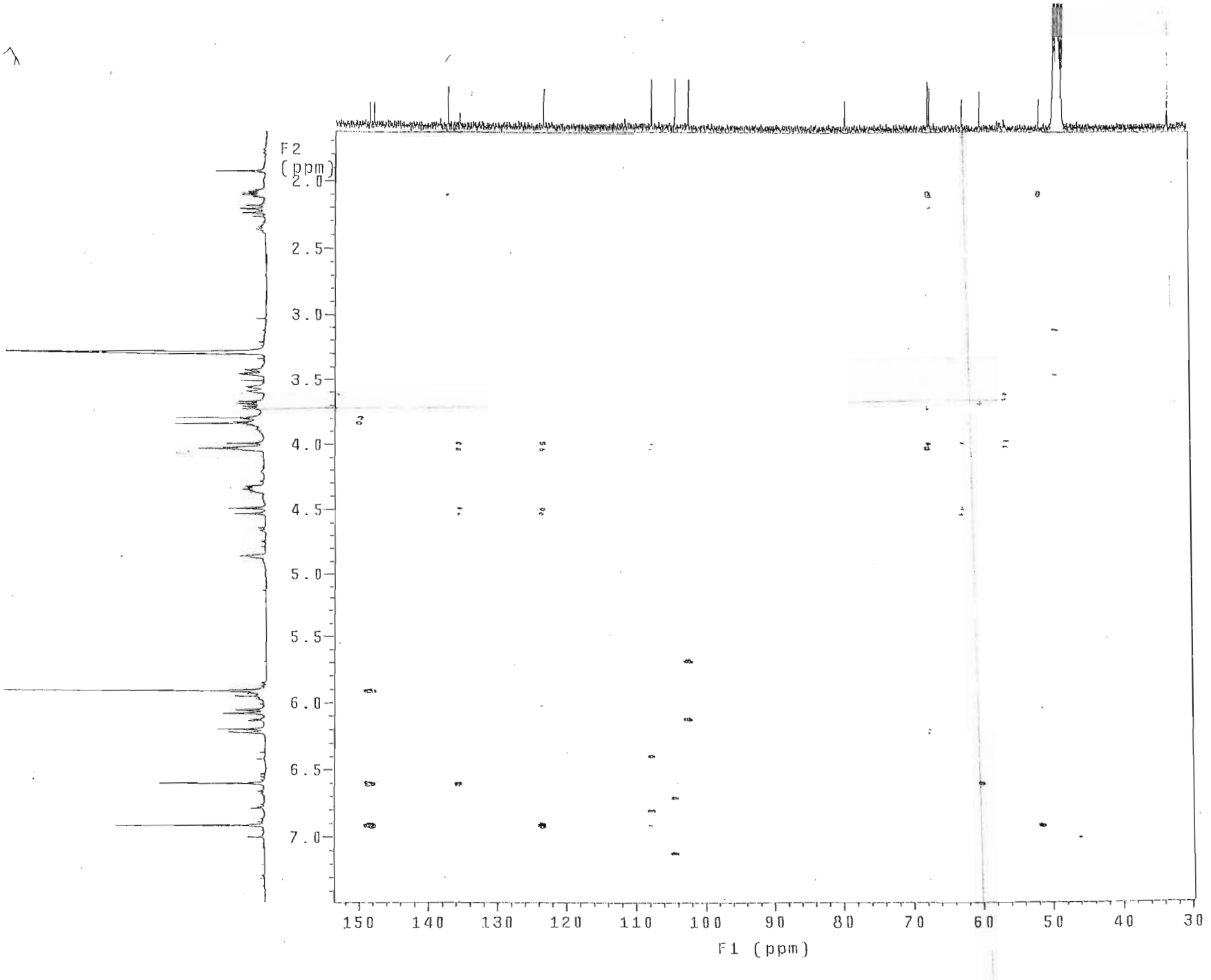
SPECTRUM 10.a: ^{1}H NMR spectrum of compound X (CD_3OD)



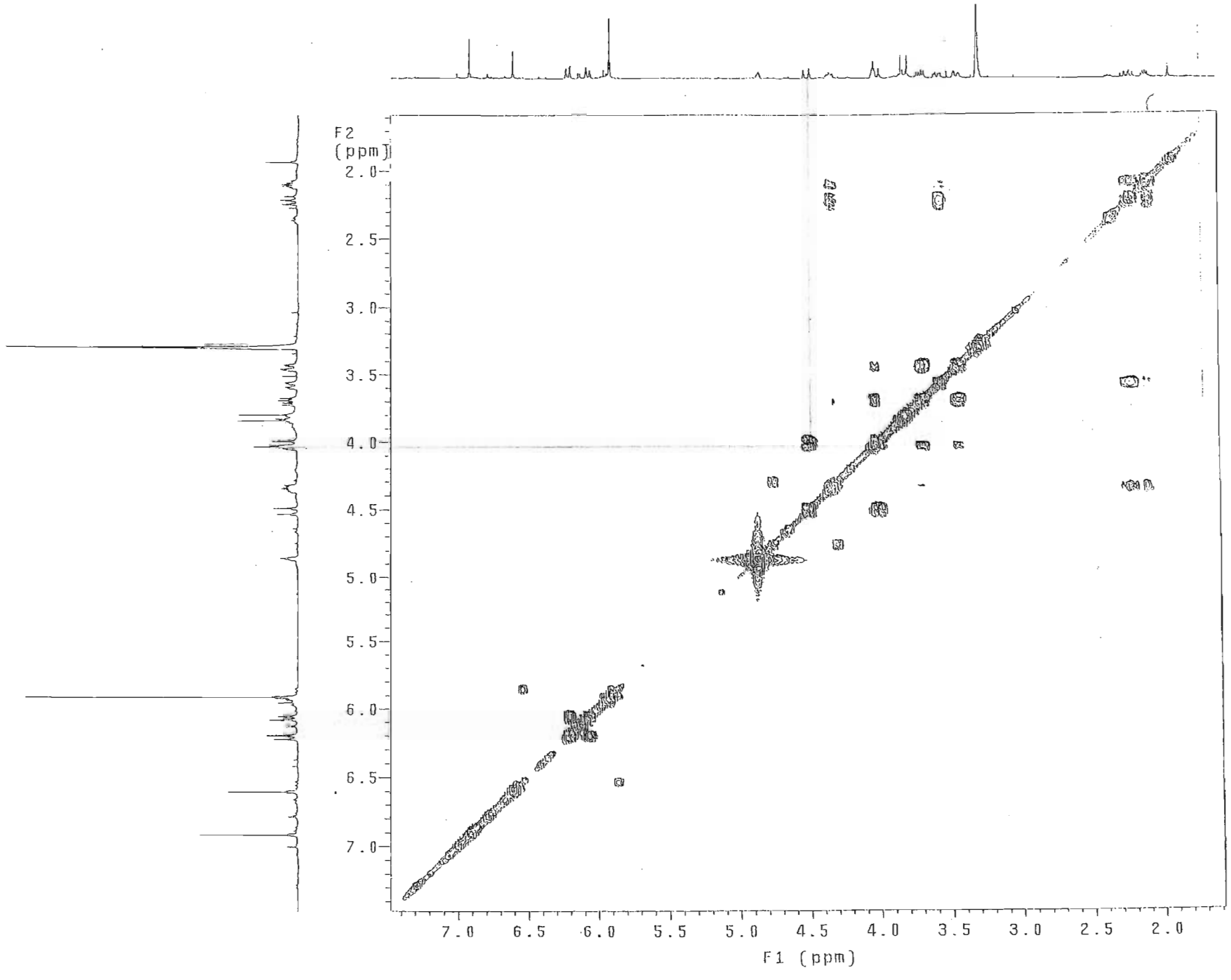
SPECTRUM 10.b: ^{13}C NMR spectrum of compound X (CD_3OD)



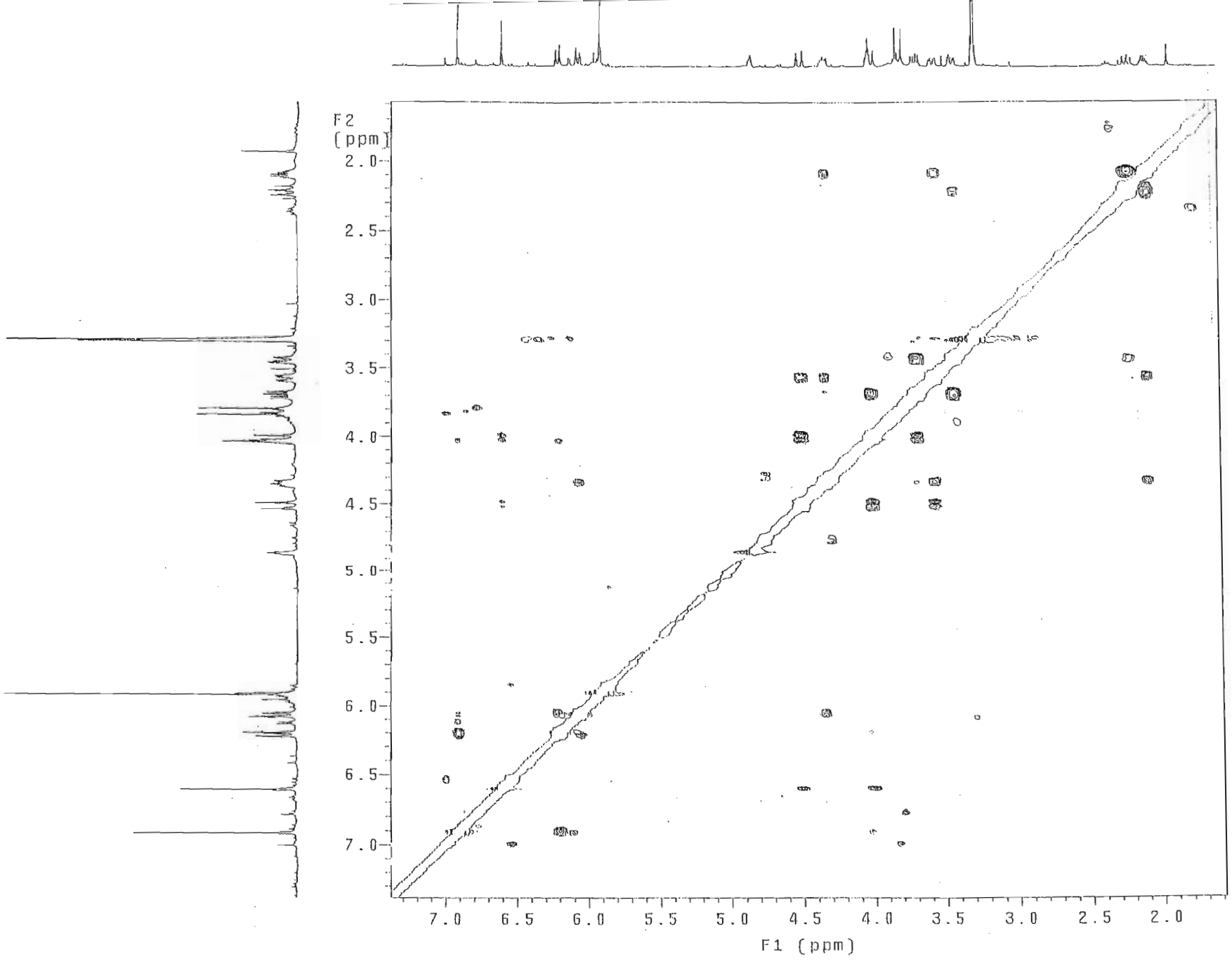
SPECTRUM 10.c: HSQC spectrum of compound X (CD_3OD)



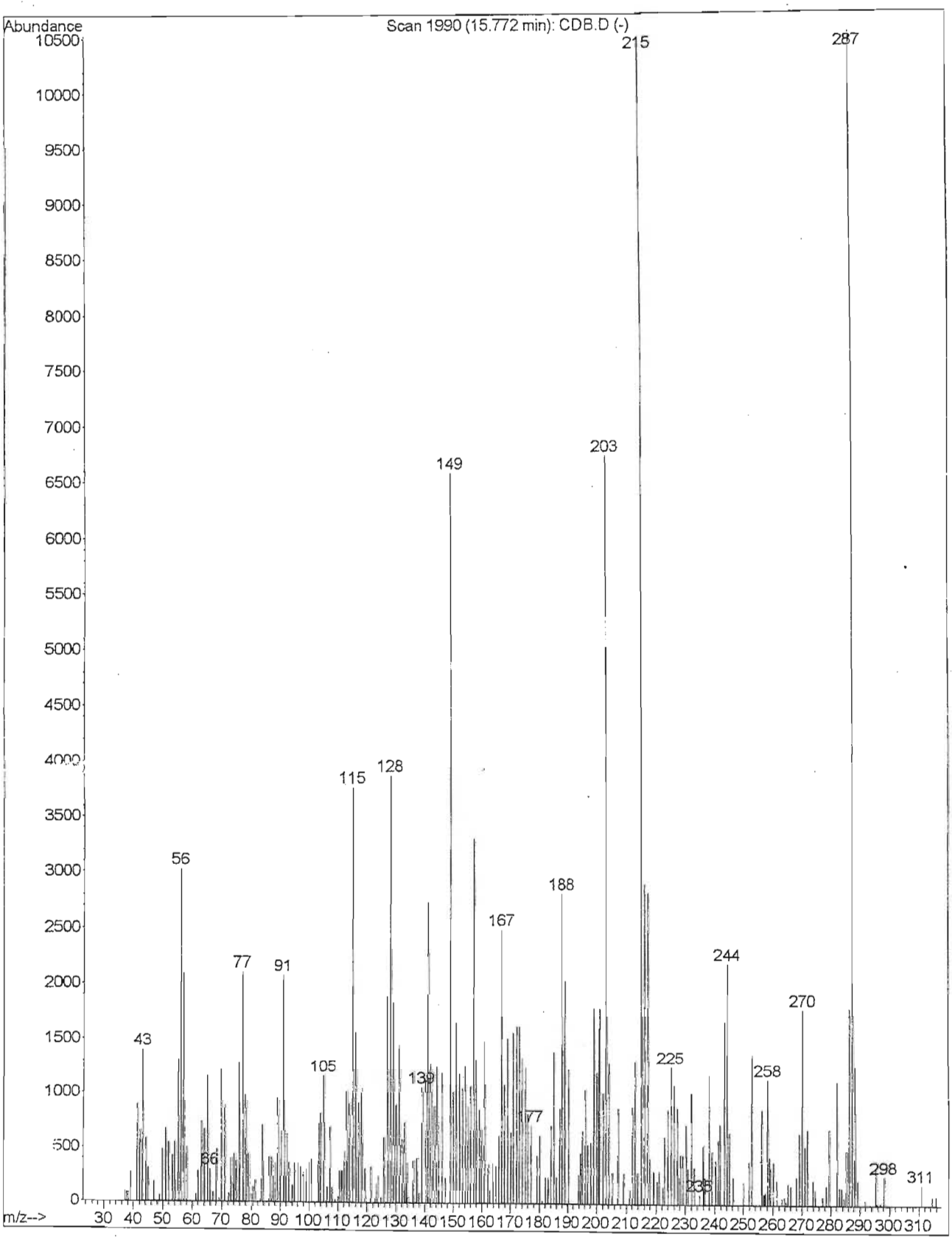
SPECTRUM 10.d: HMBC spectrum of compound X (CD_3OD)



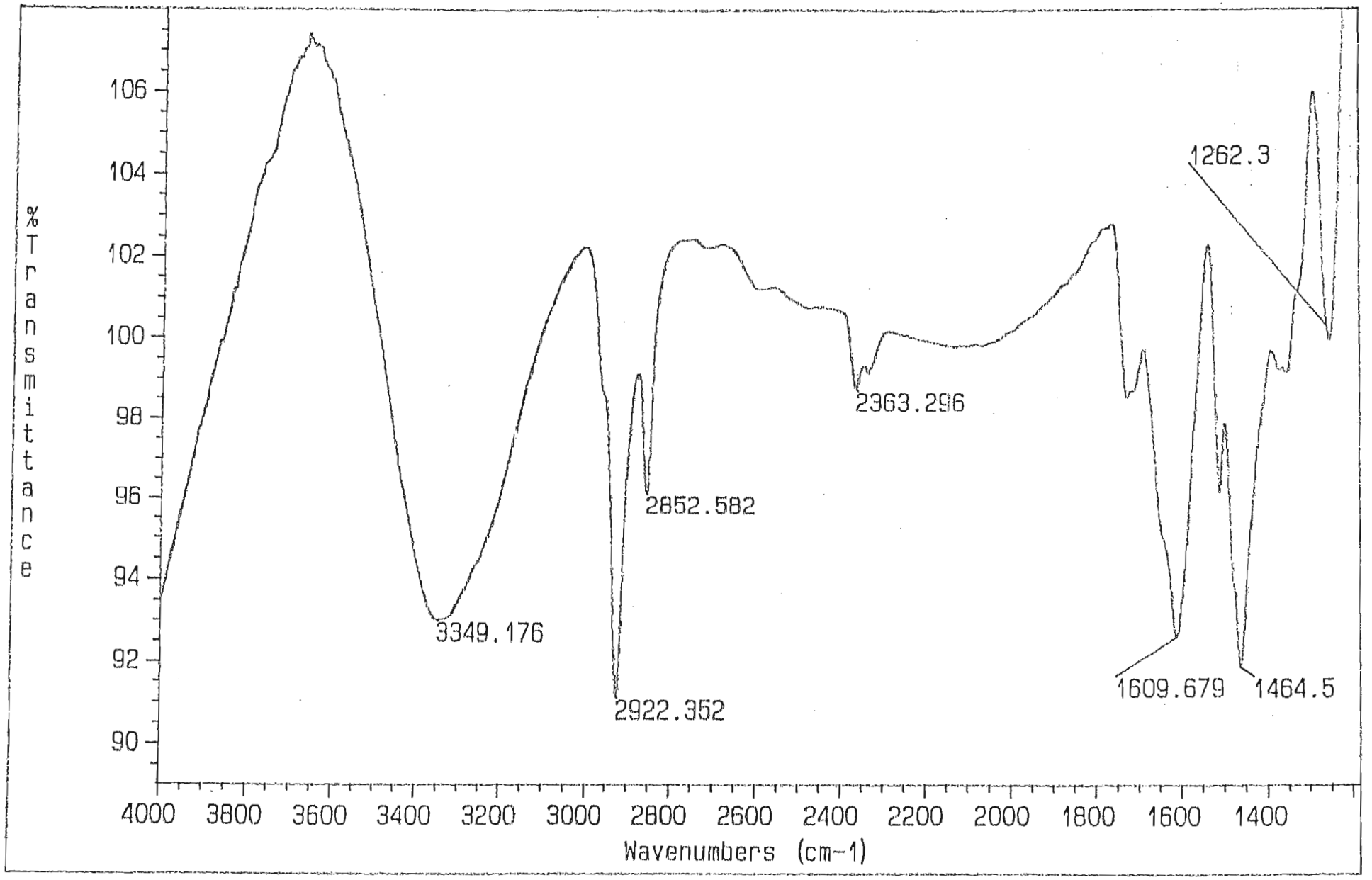
SPECTRUM 10.e: COSY spectrum of compound X (CD_3OD)



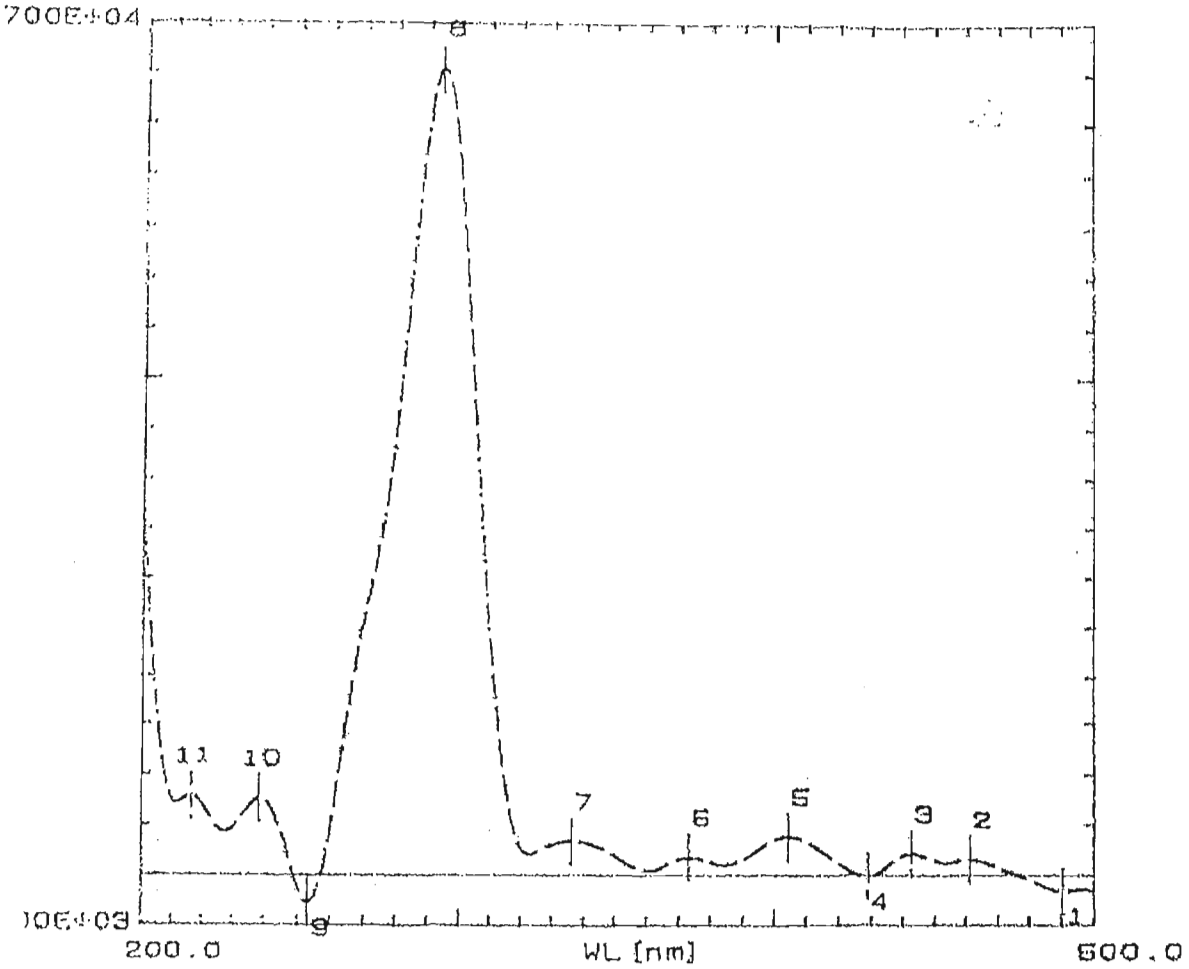
SPECTRUM 10.f: NOESY spectrum of compound X (CD_3OD)



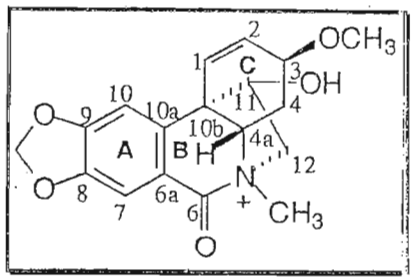
Spectrum 10.g: Mass spectra of compound X



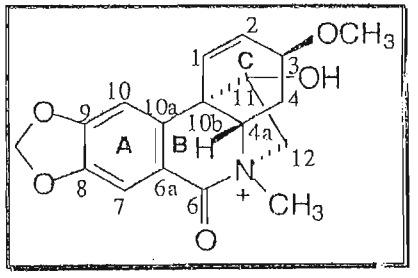
SPECTRUM 10.h: Infrared spectrum of compound X



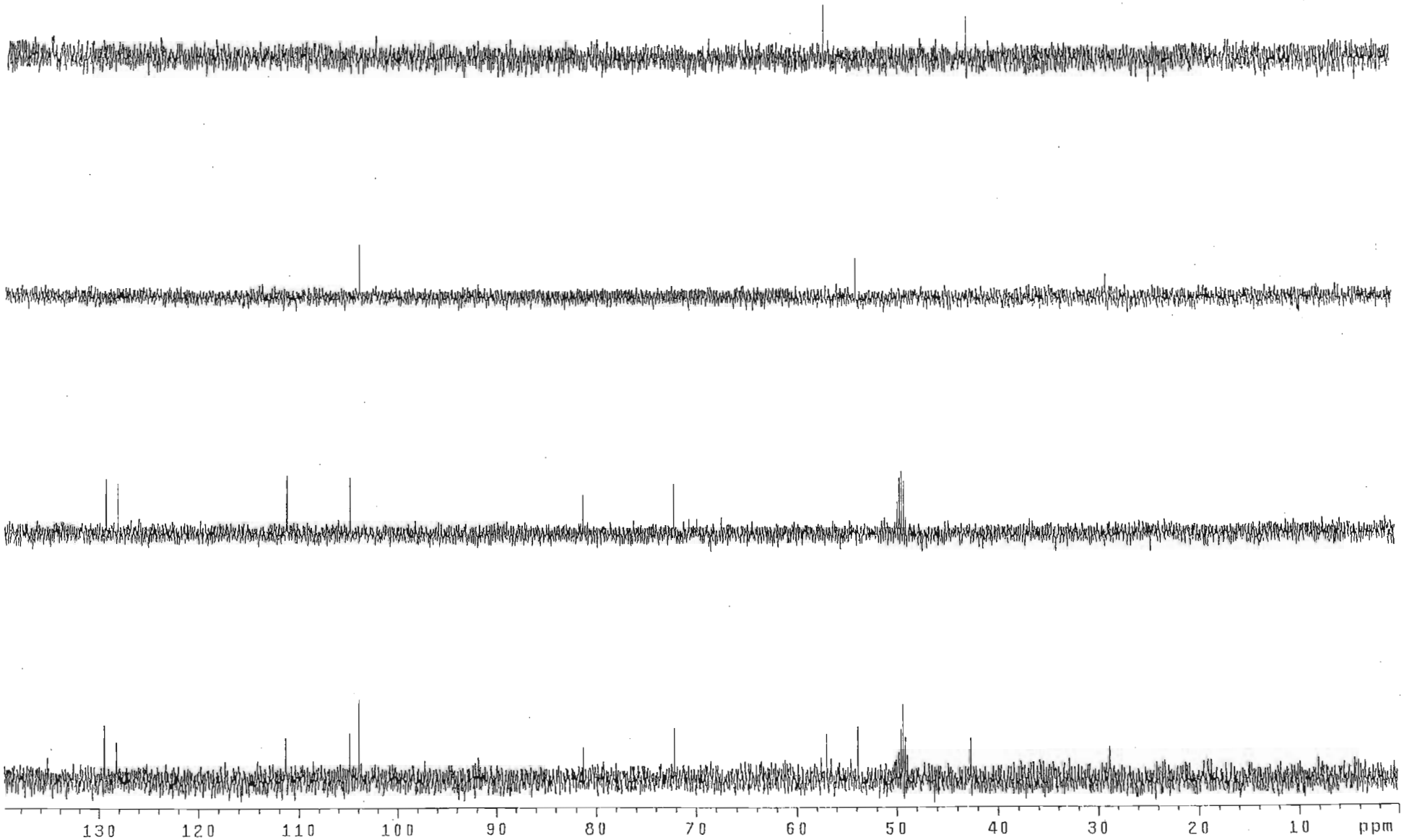
SPECTRUM 10.i: CD spectrum of compound X



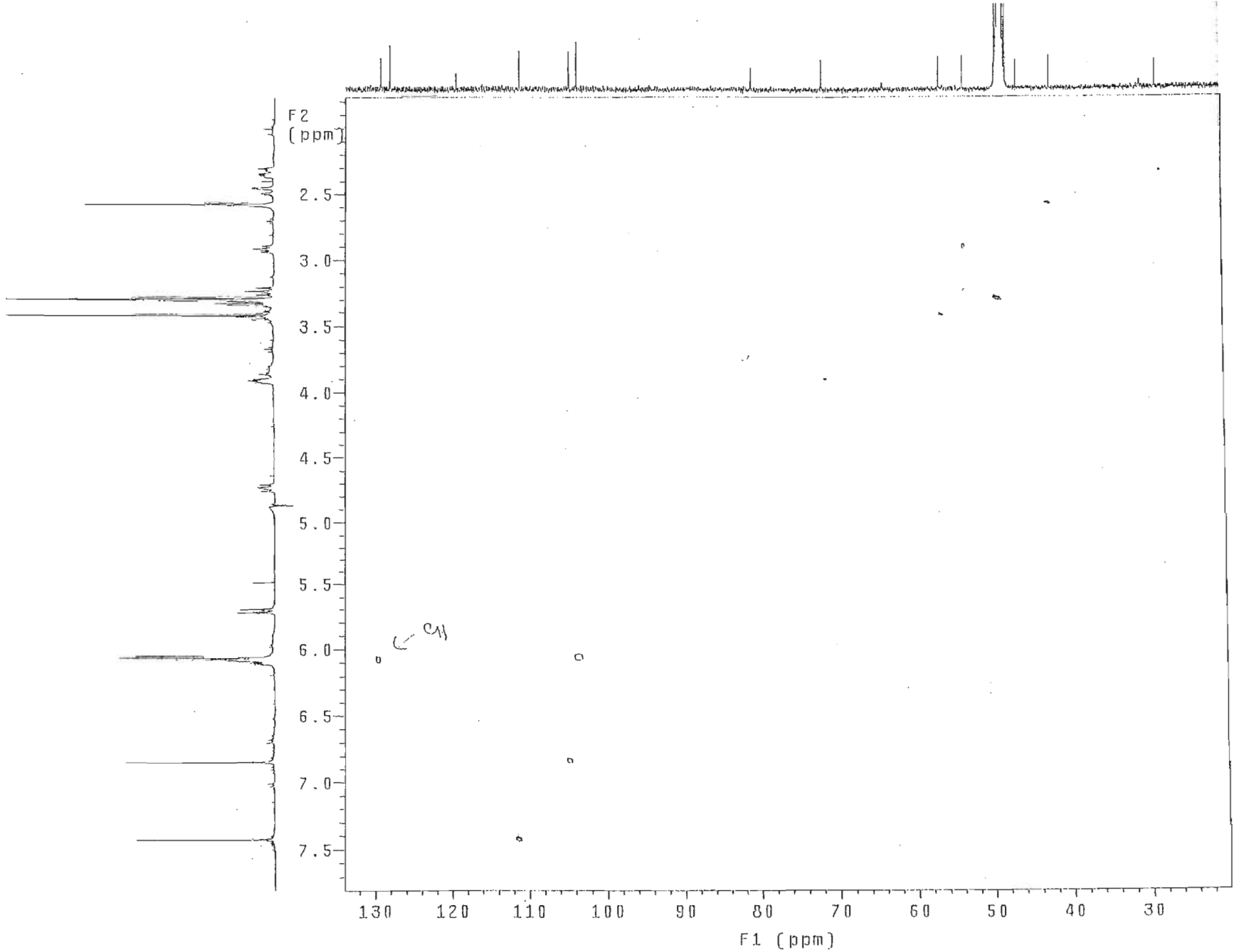
SPECTRUM 11.a: ^{1}H NMR spectrum of compound XI (CD_3OD)



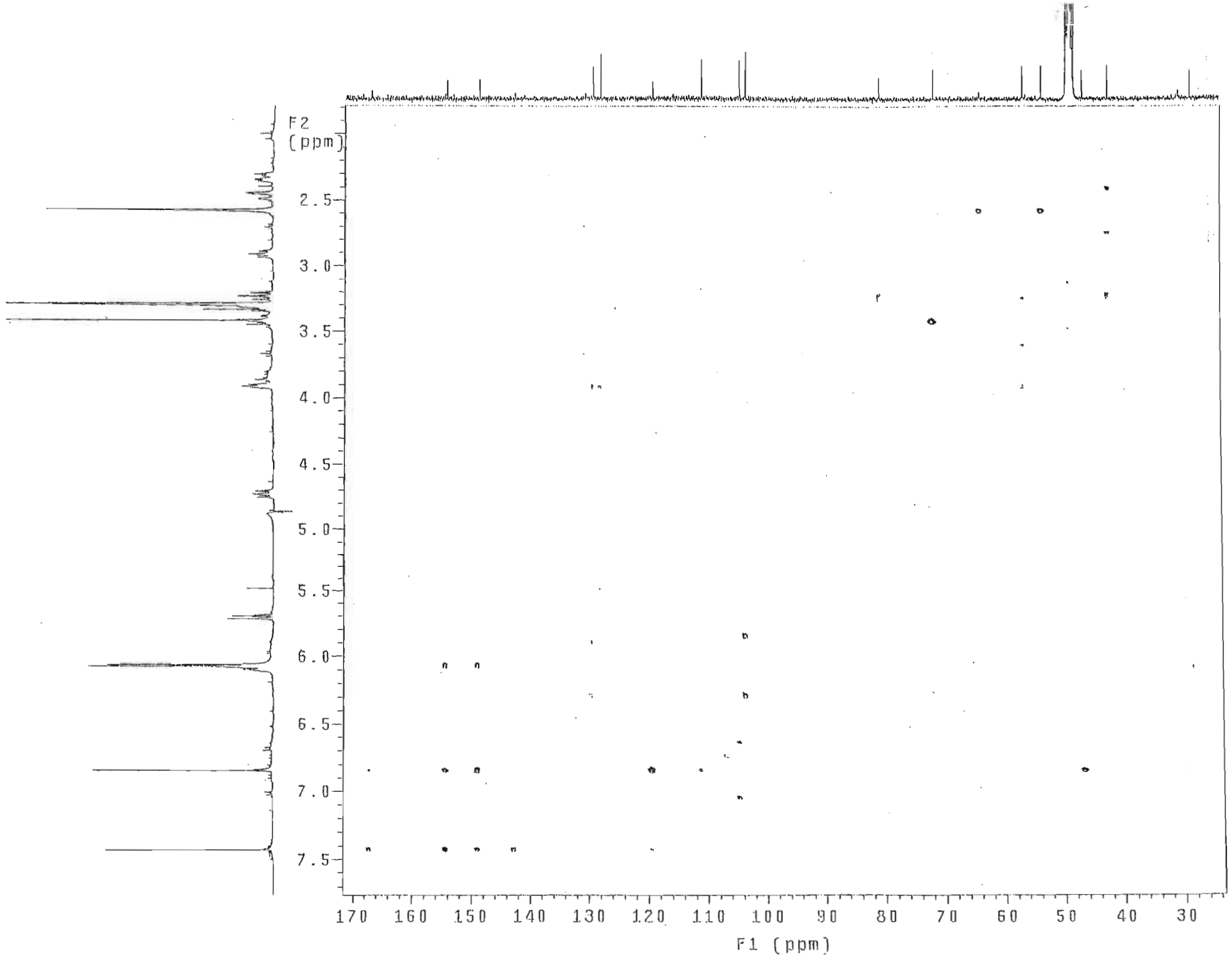
SPECTRUM 11.b: ^{13}C NMR spectrum of compound XI (CD_3OD)



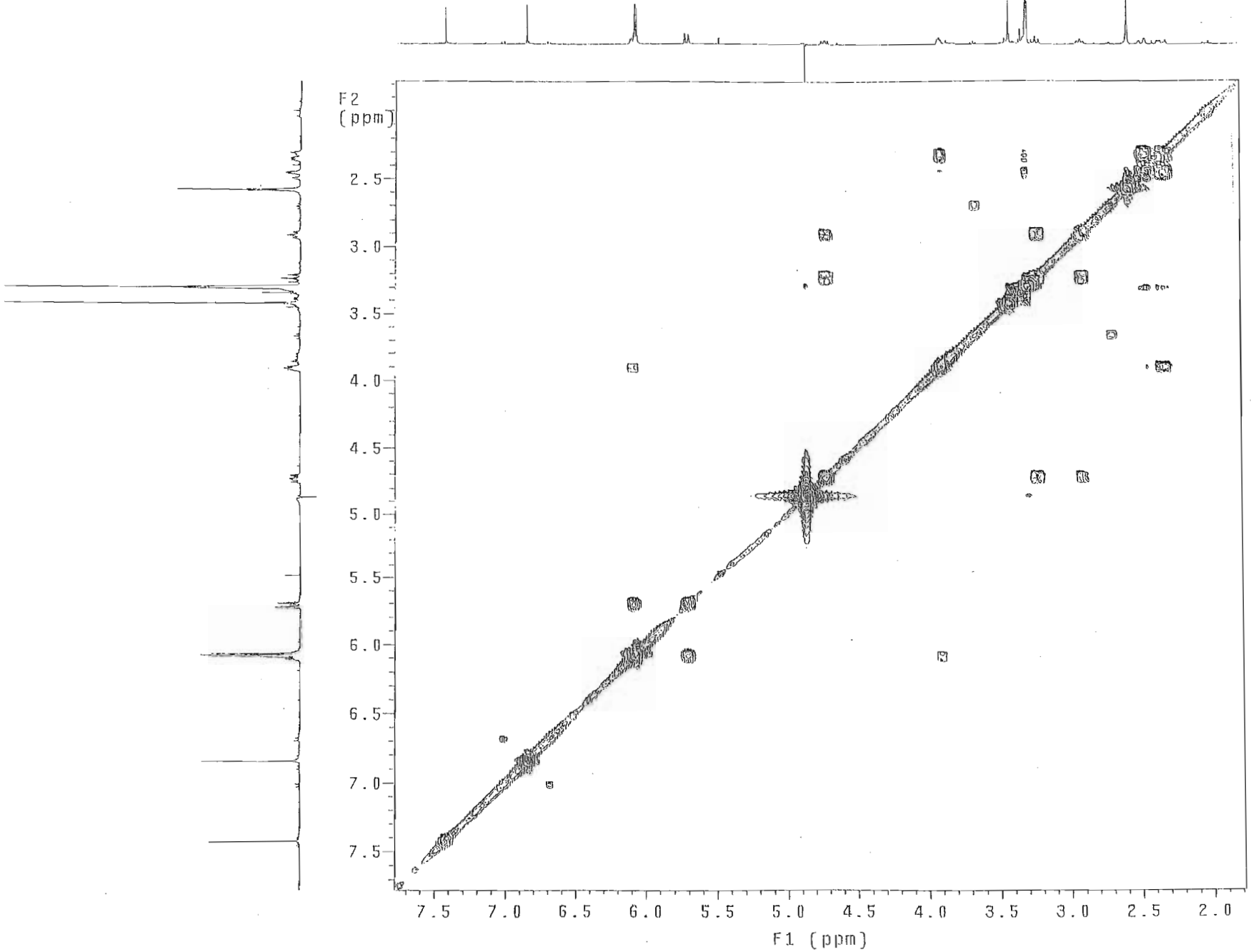
SPECTRUM 11.c: ADEPT spectrum of compound XI (CD_3OD)



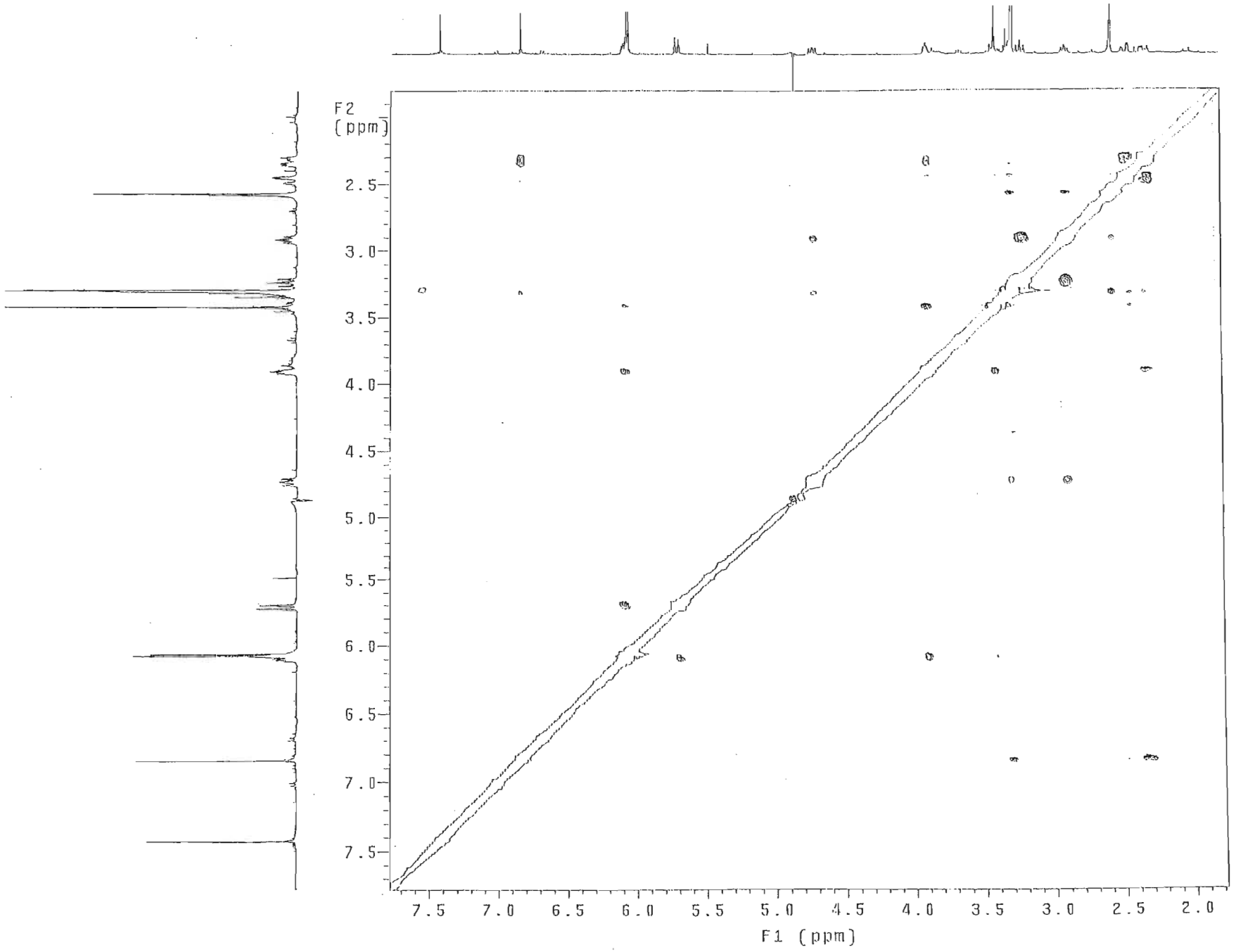
SPECTRUM 11.d: HSQC spectrum of compound XI (CD_3OD)



SPECTRUM 11.e: HMBC spectrum of compound XI (CD_3OD)



SPECTRUM 11.f: COSY spectrum of compound XI (CD_3OD)



SPECTRUM 11.g: NOESY spectrum of compound XI (CD_3OD)

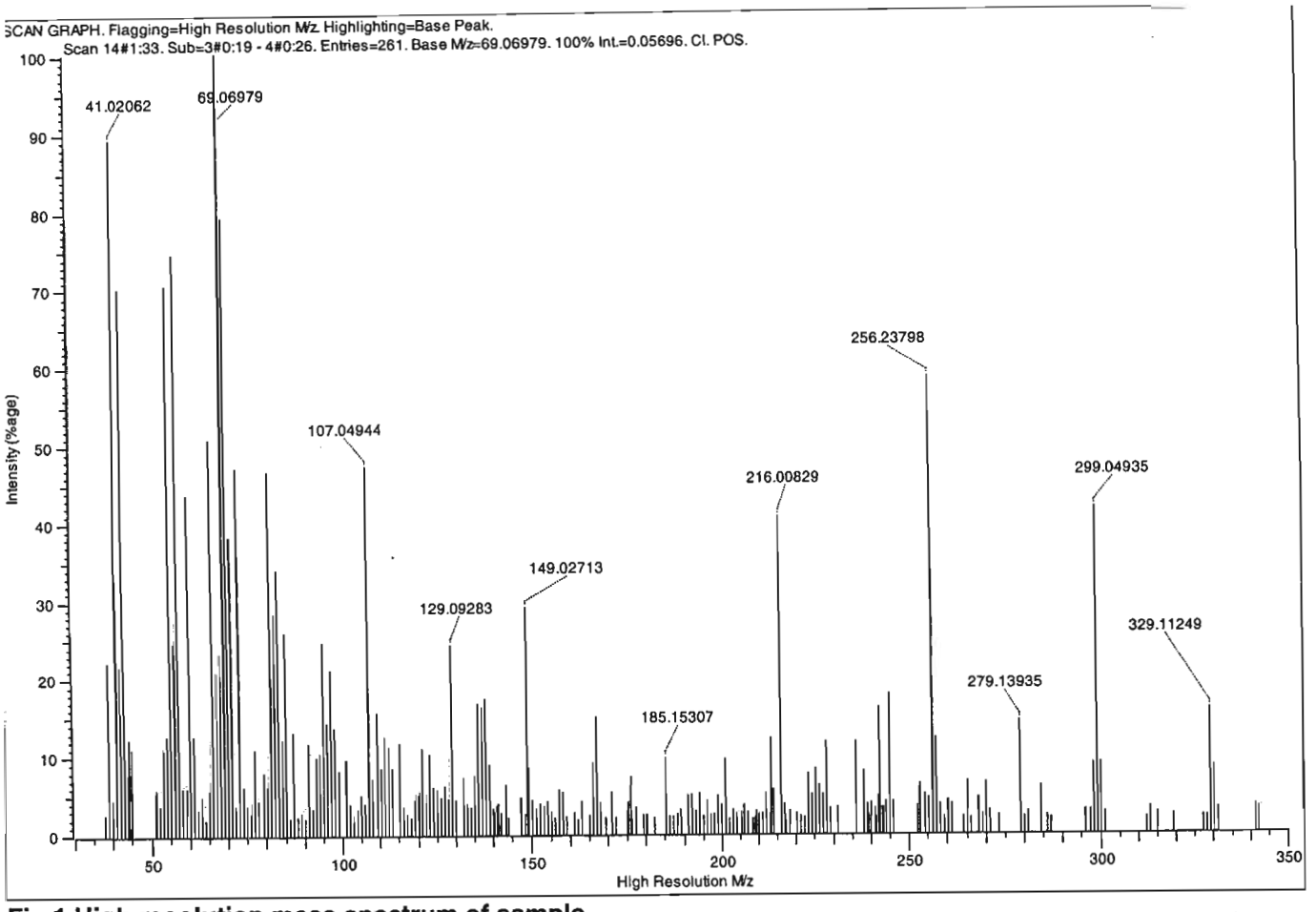


Fig 1 High resolution mass spectrum of sample.

ATOMIC COMPOSITION REPORT (MANUAL)

Selected isotopes:

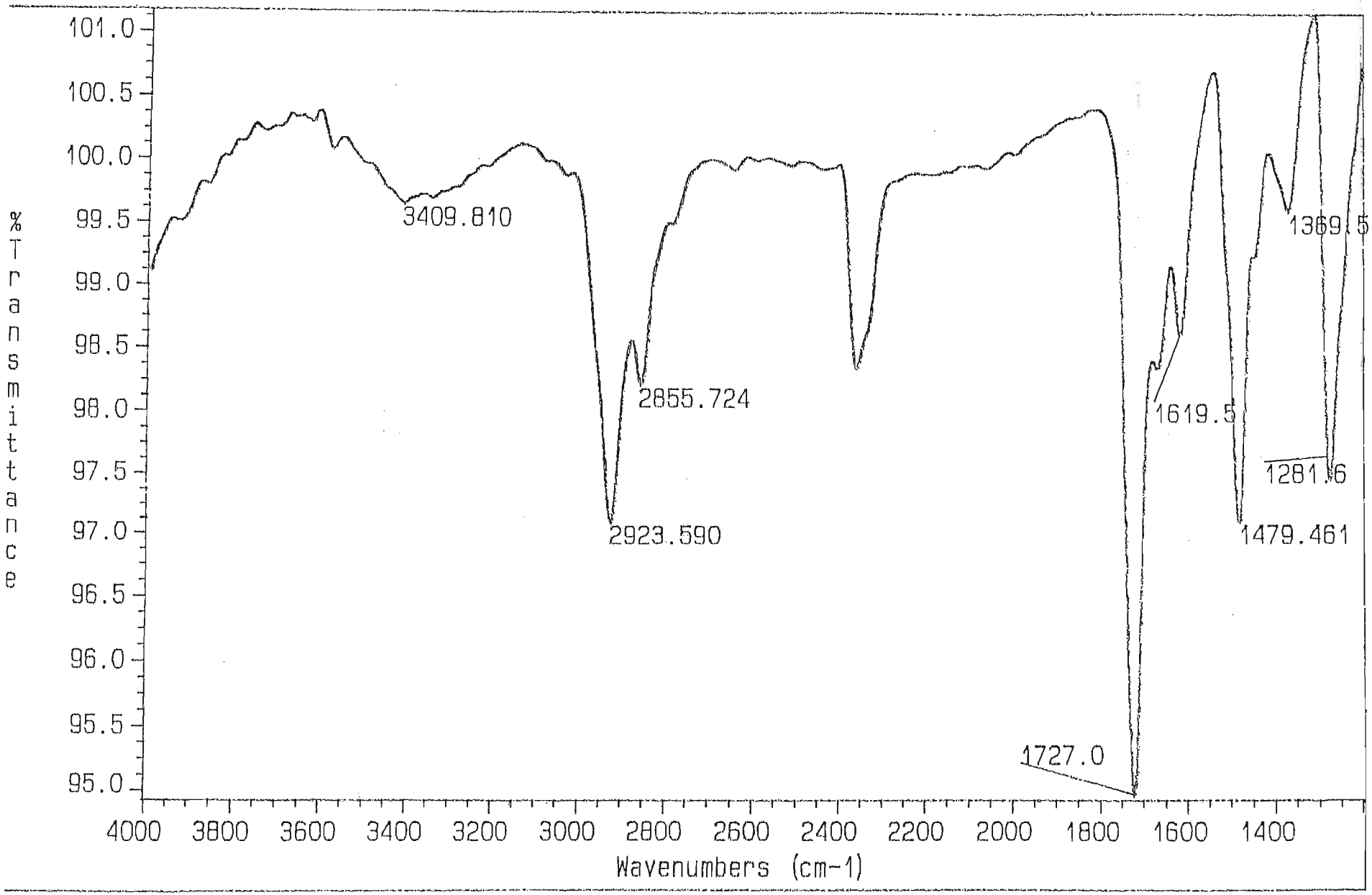
Symbol	Min	Max	V'cy	Name
H	0	30	1	Hydrogen-1
C	0	30	4	Carbon-12
O	0	7	2	Oxygen-16
N	0	1	3	Nitrogen-14

Allowable error = minimum of 20.0 ppm, 10.0 mmu.

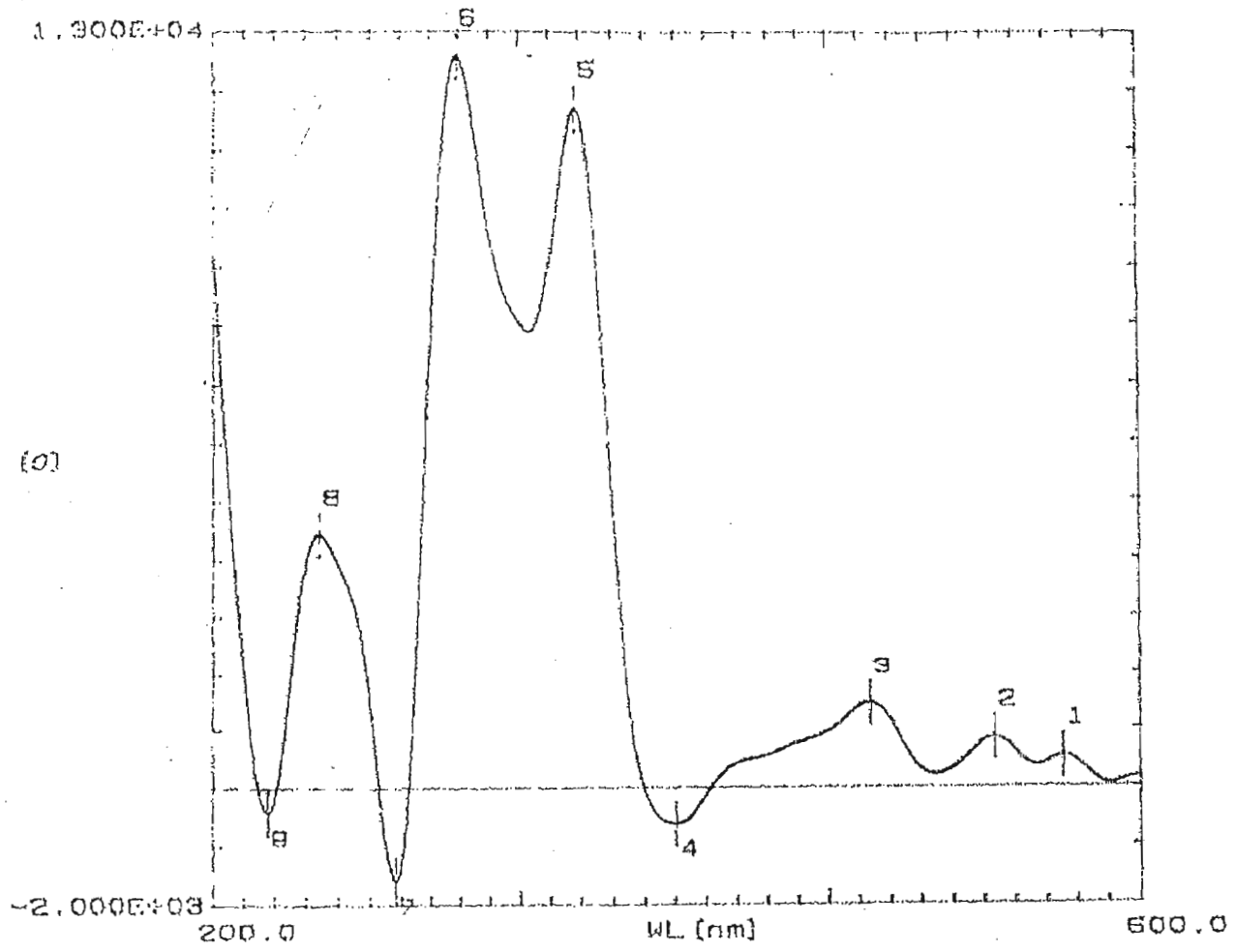
Mass	Calculated	ppm	mmu	Formula
329.11249	329.11777	16.0	5.3	H17.C22.O3
330.13421	330.13415	-0.2	-0.1	H20.C18.O5.N
	330.12827	-18.0	-5.9	H16.C25.N

***** End of Atomic Composition Report *****

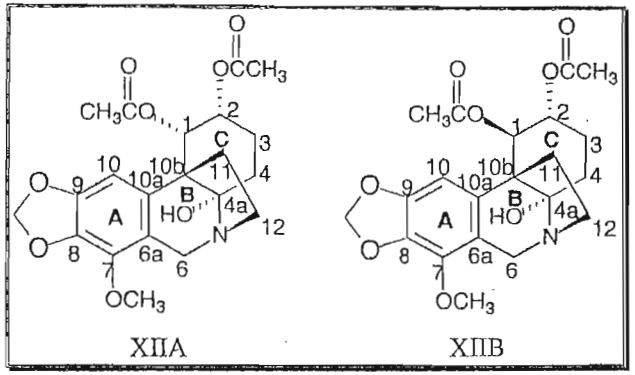
Spectrum 11.h: Mass spectra of compound XI



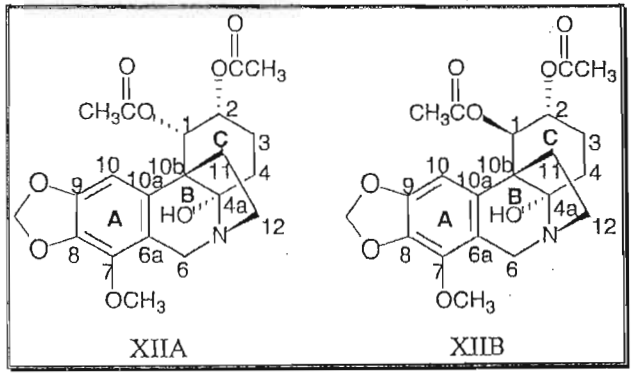
SPECTRUM 11.i: Infrared spectrum of compound XI



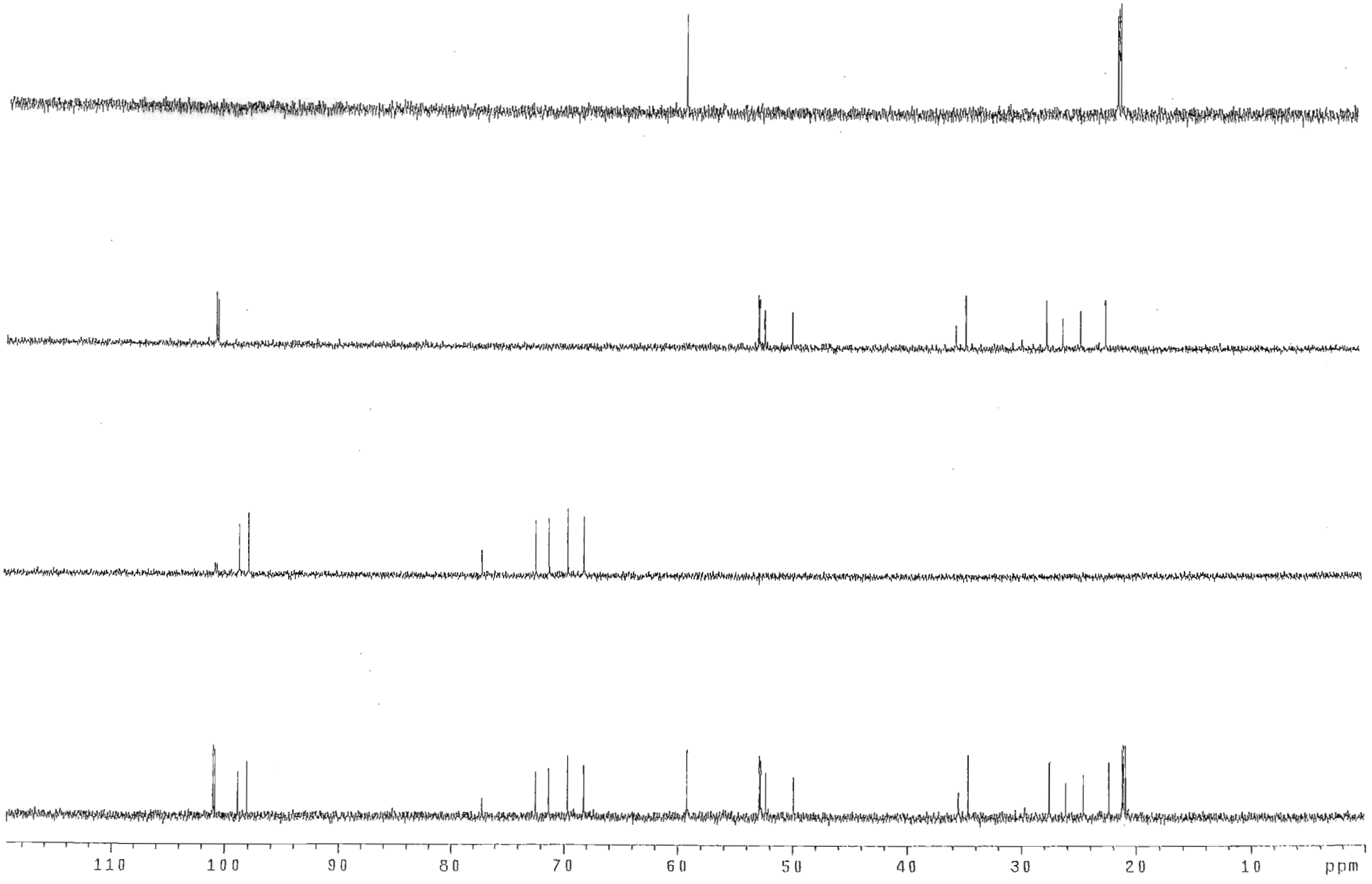
SPECTRUM 11.j: CD spectrum of compound XI



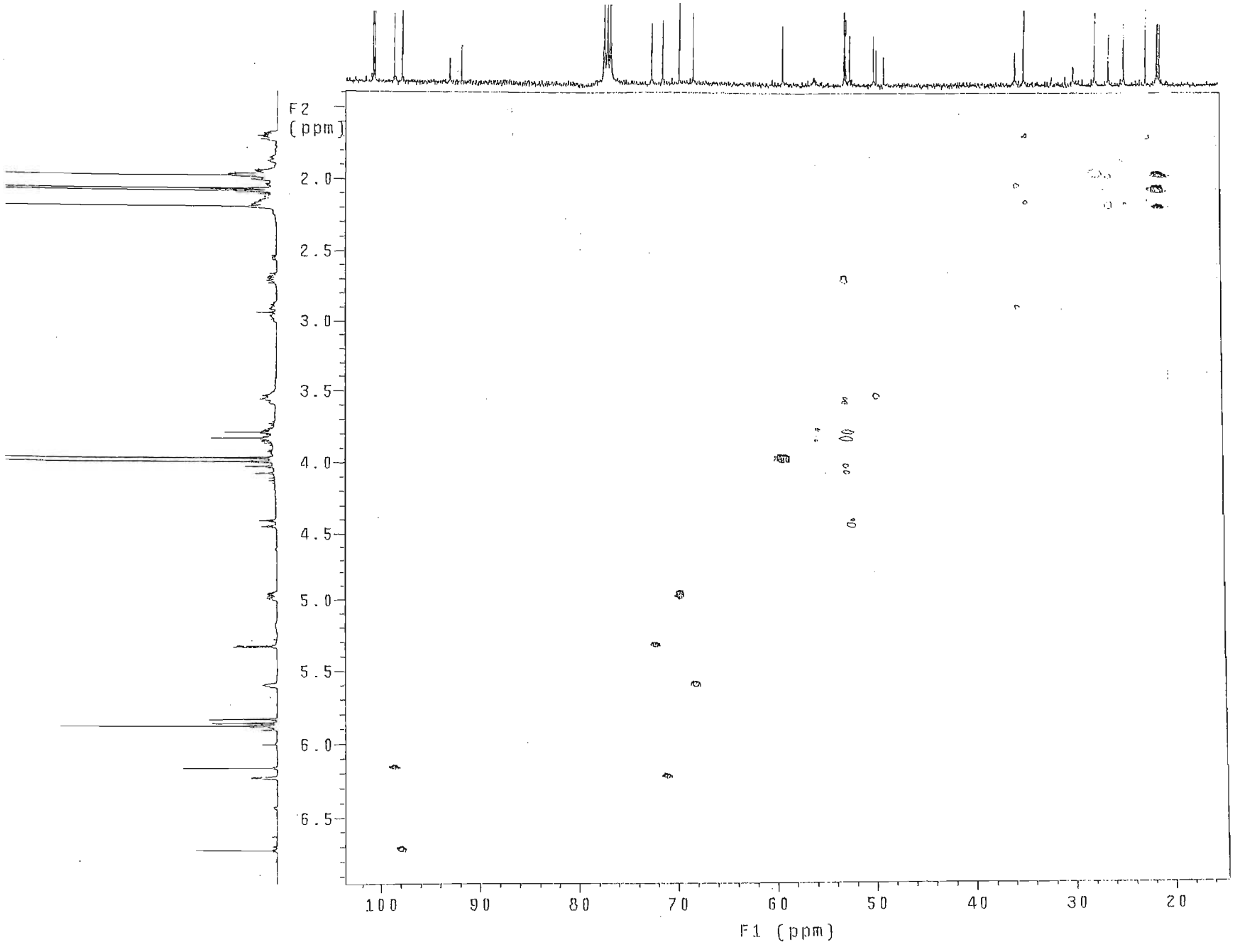
SPECTRUM 12.a: ^{1}H NMR spectrum of compound XII ($CDCl_3$)



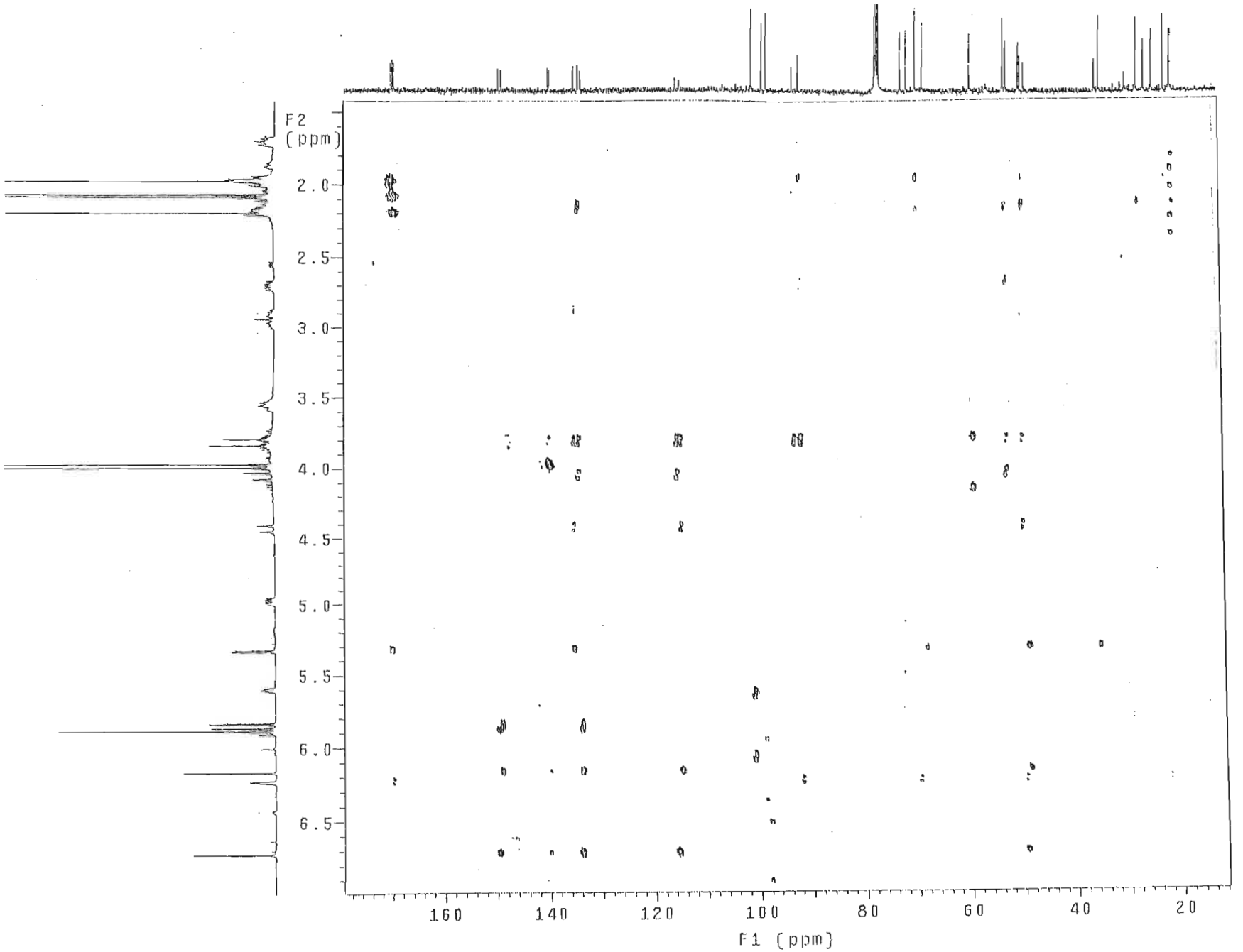
SPECTRUM 12.b: ^{13}C NMR spectrum of compound XII ($CDCl_3$)



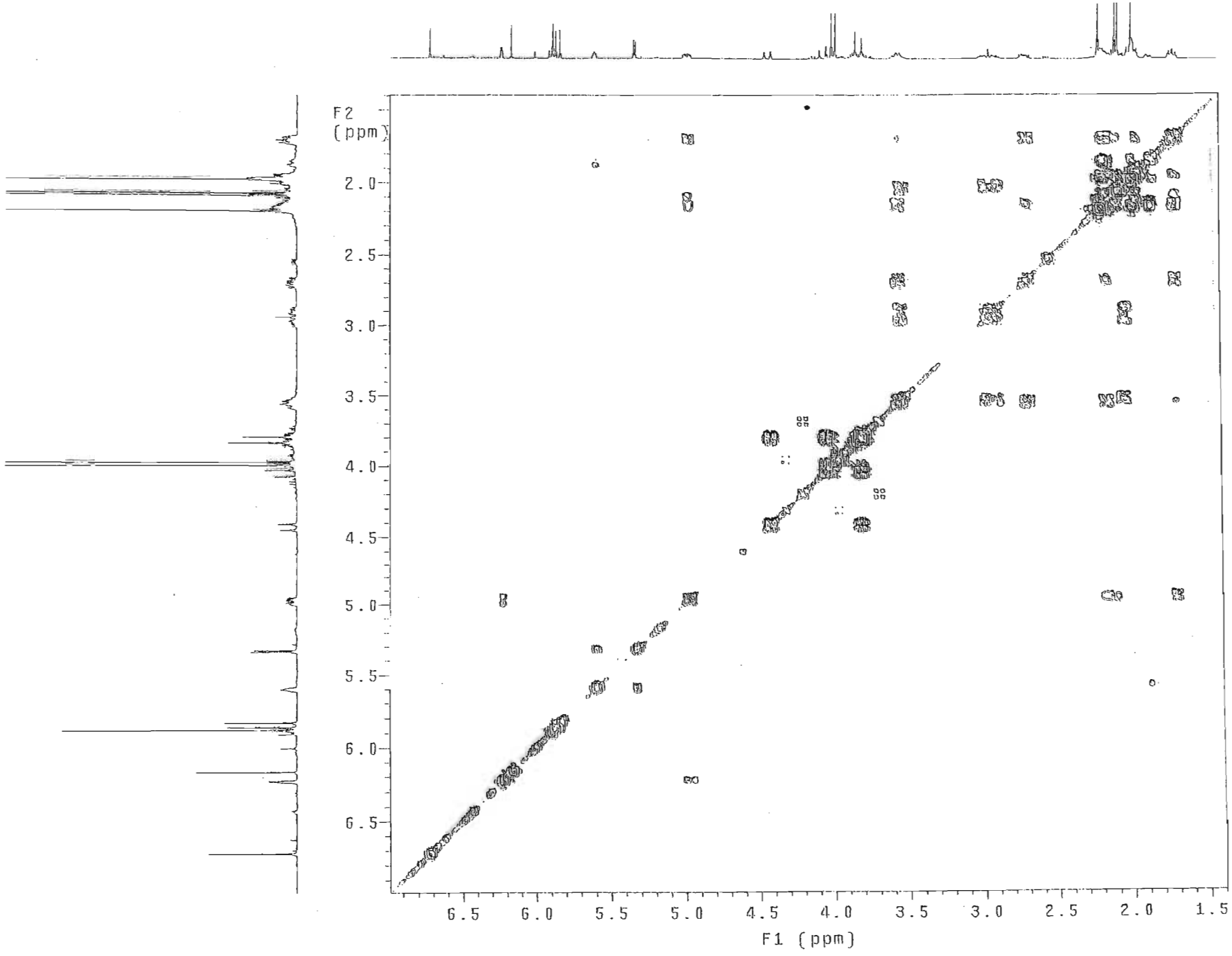
SPECTRUM 12.c: ADEPT spectrum of compound XII ($CDCl_3$)



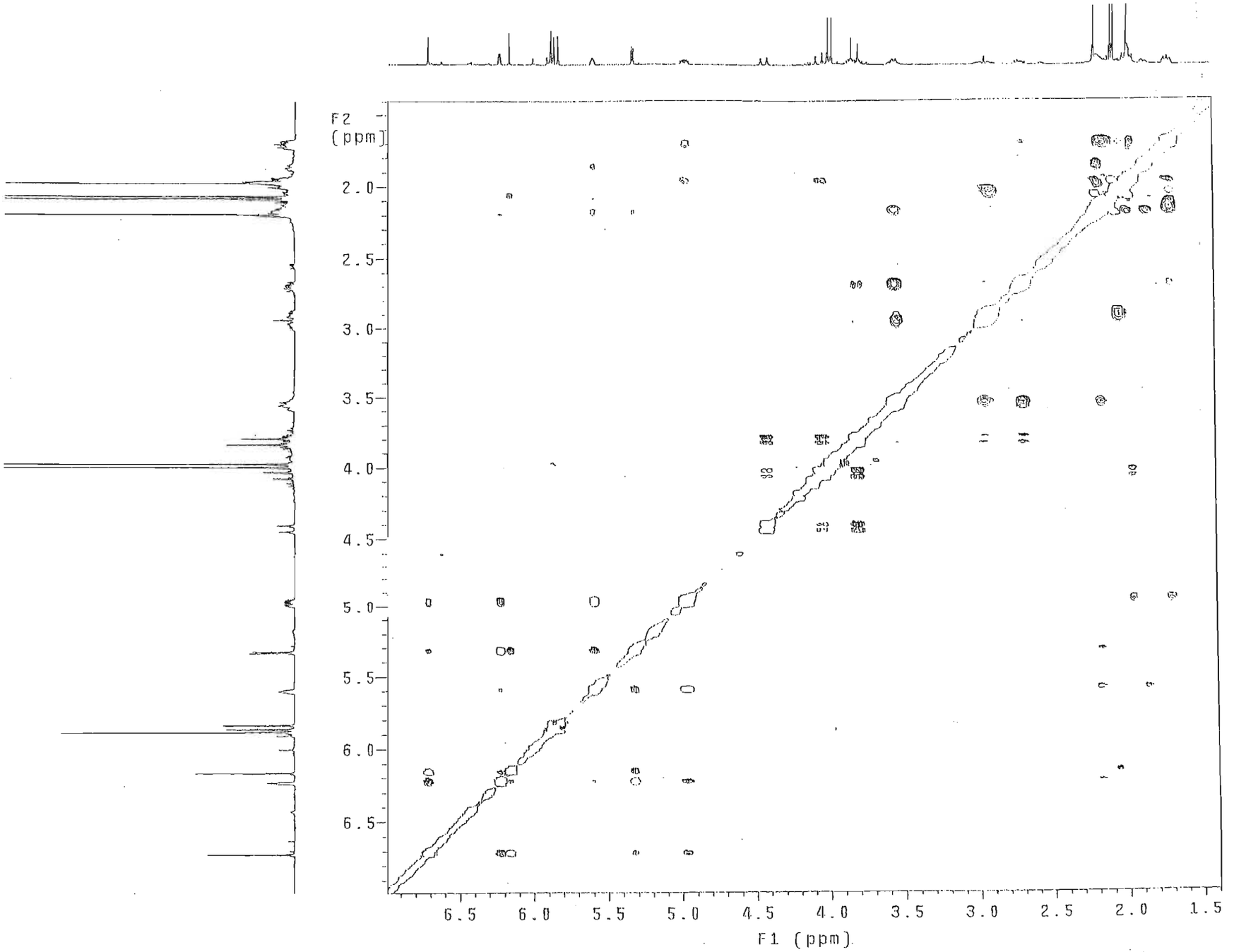
SPECTRUM 12.d: HSQC spectrum of compound XII ($CDCl_3$)



SPECTRUM 12.e: HMBC spectrum of compound XII ($CDCl_3$)



SPECTRUM 12.f: COSY spectrum of compound XII ($CDCl_3$)



SPECTRUM 12.g: NOESY spectrum of compound XII ($CDCl_3$)

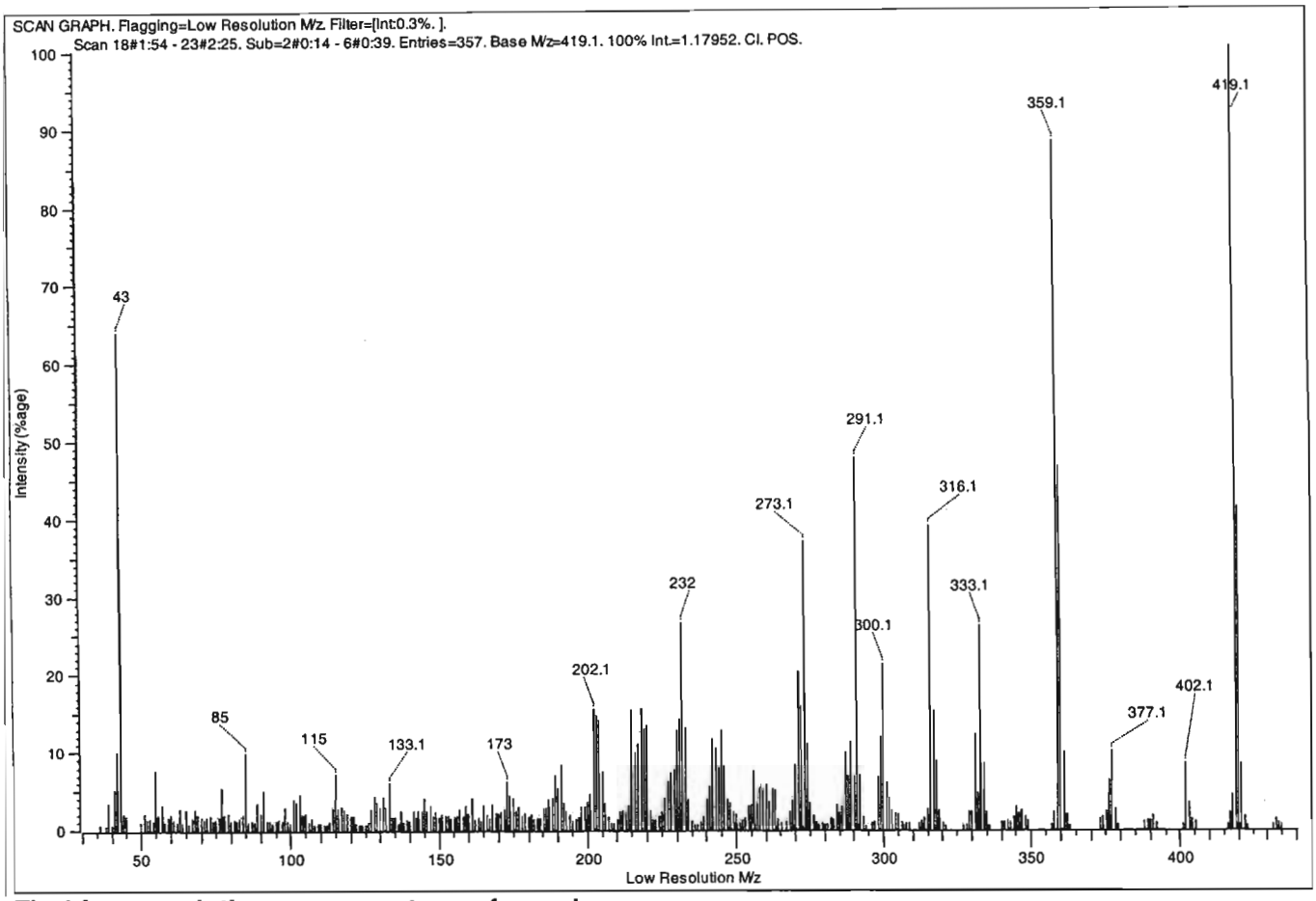


Fig 1 Low resolution mass spectrum of sample.

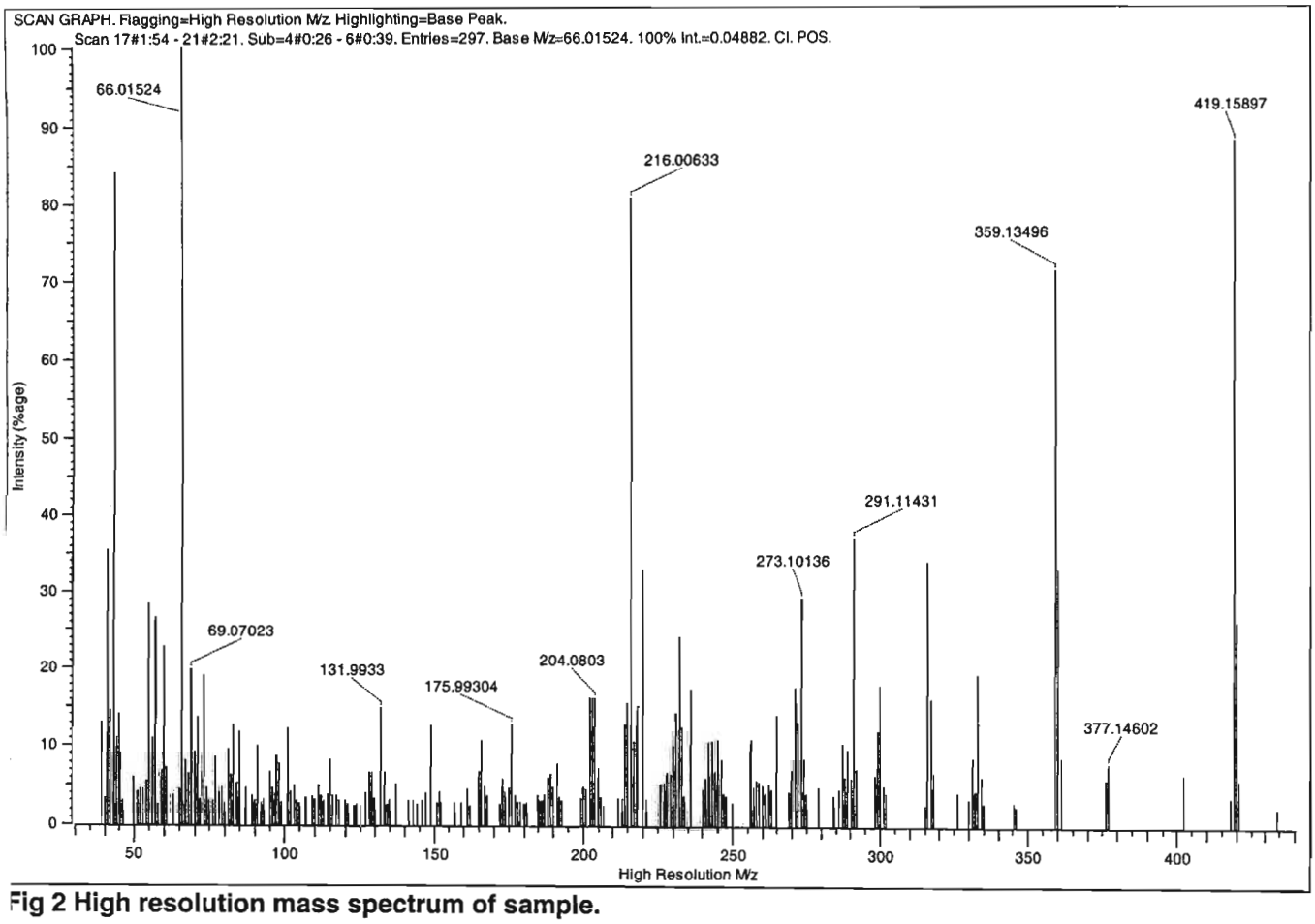
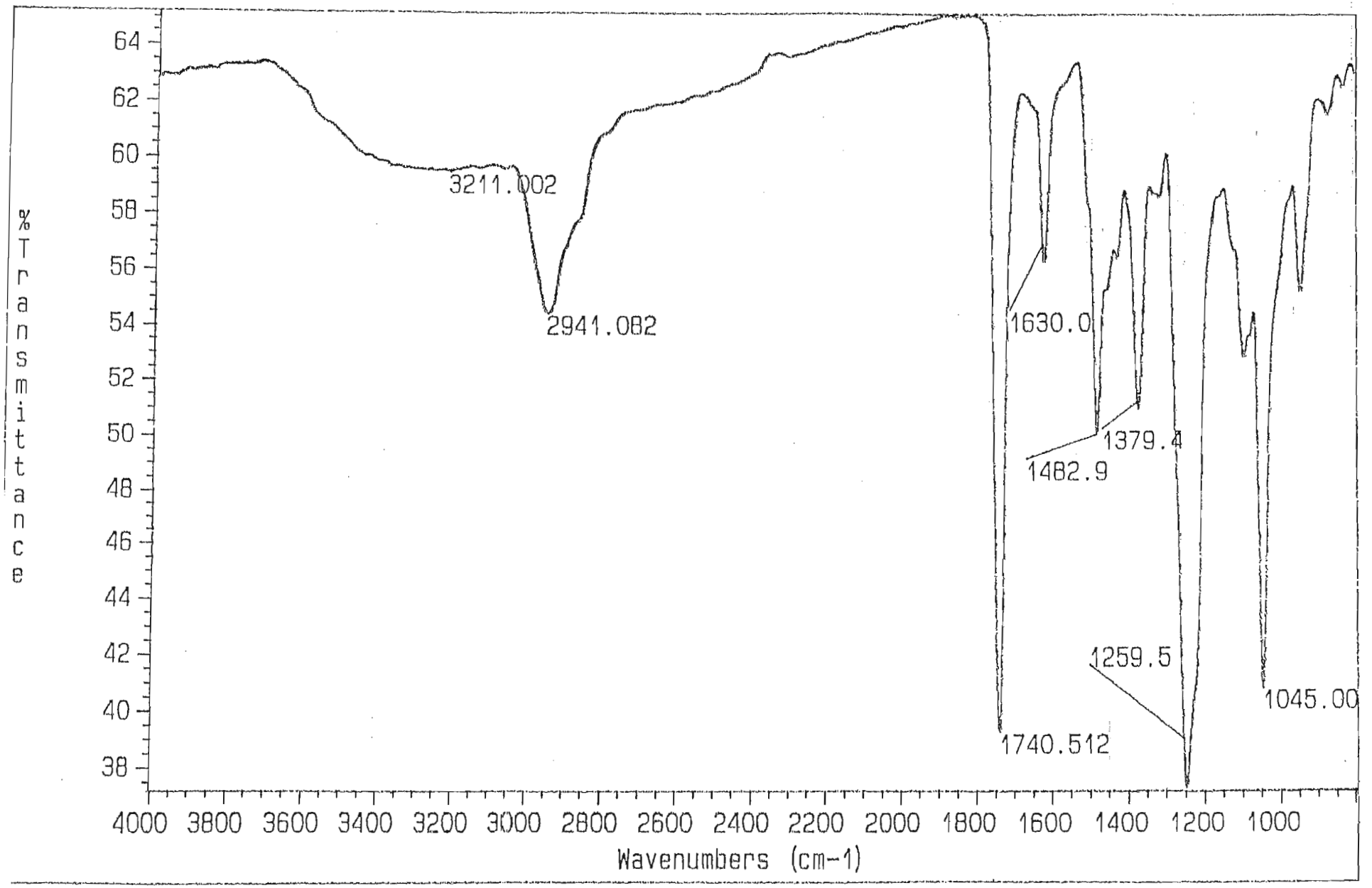
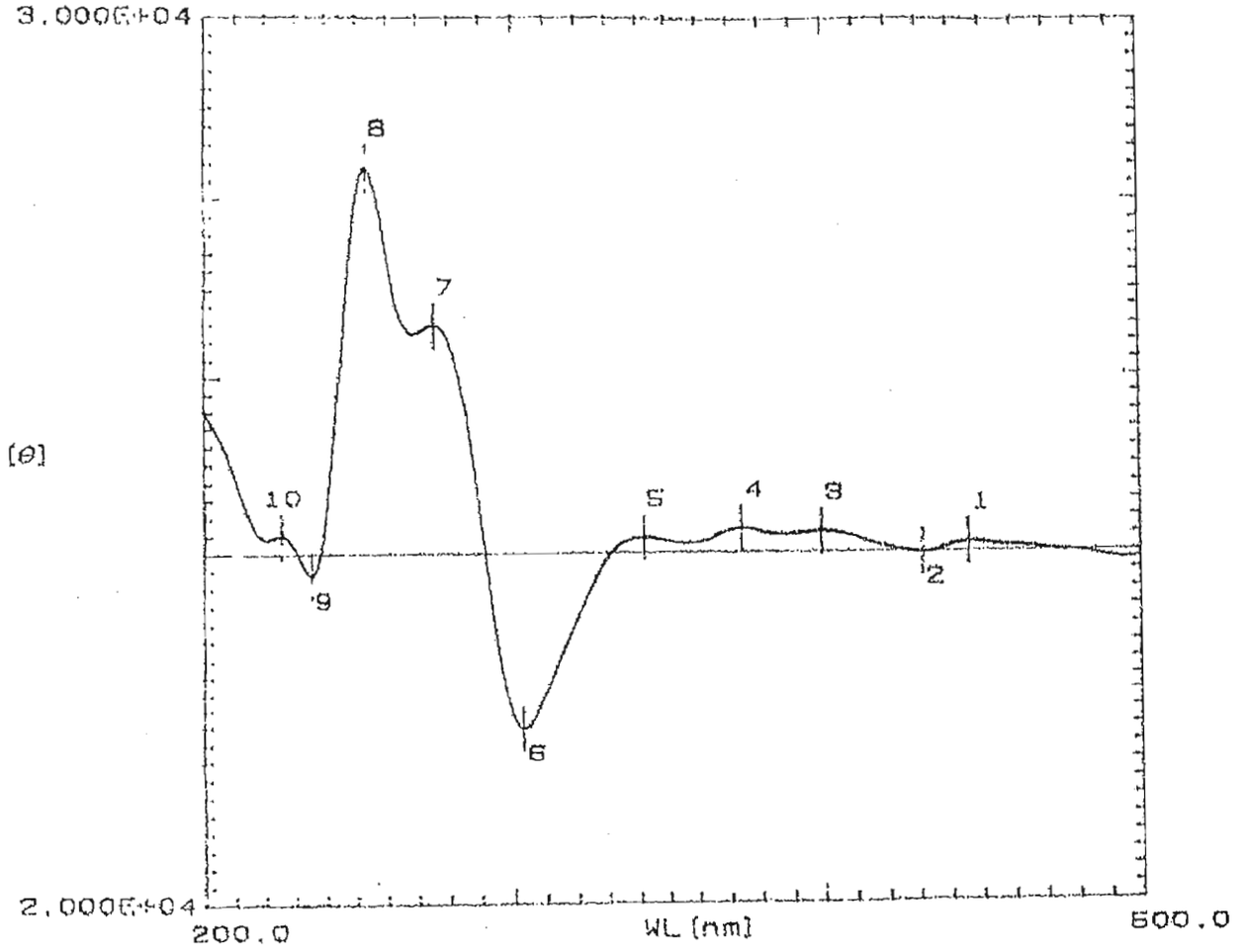


Fig 2 High resolution mass spectrum of sample.

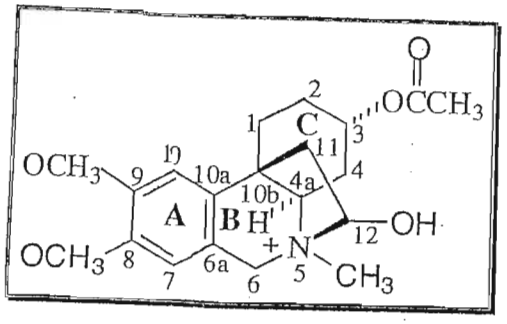
Spectrum 12.h: Mass spectra of compound XII



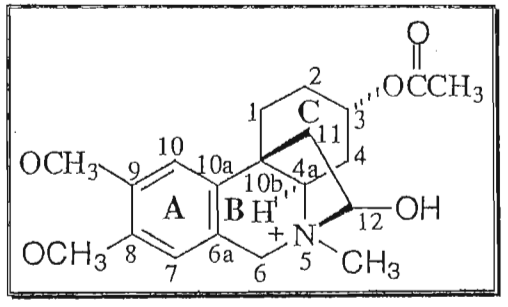
SPECTRUM 12.i: Infrared spectrum of compound XII

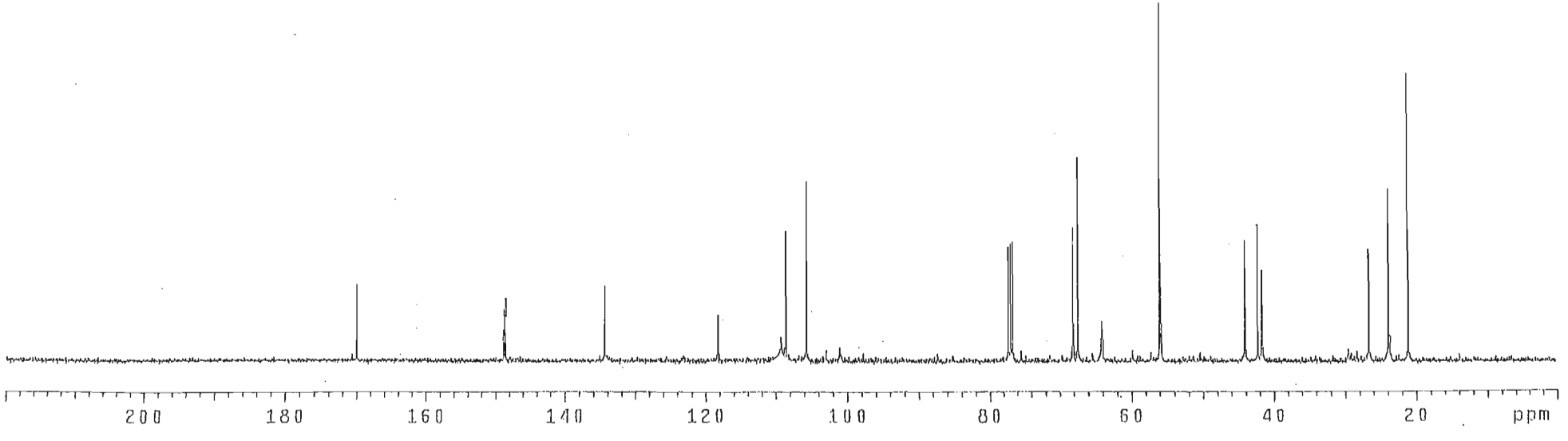


SPECTRUM 12.j: CD spectrum of compound XII

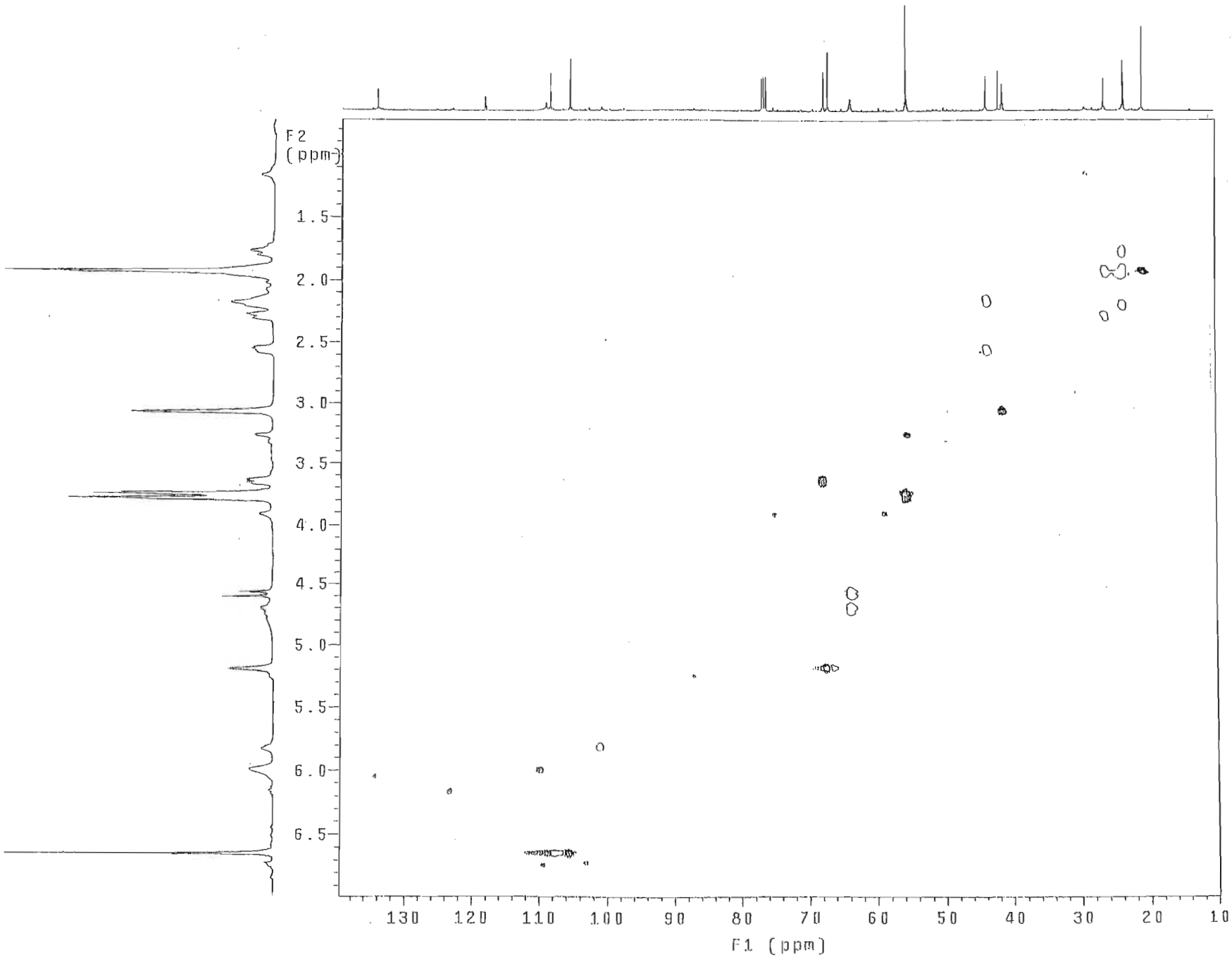


SPECTRUM 13.a: ^{1}H NMR spectrum of compound XIII ($CDCl_3$)

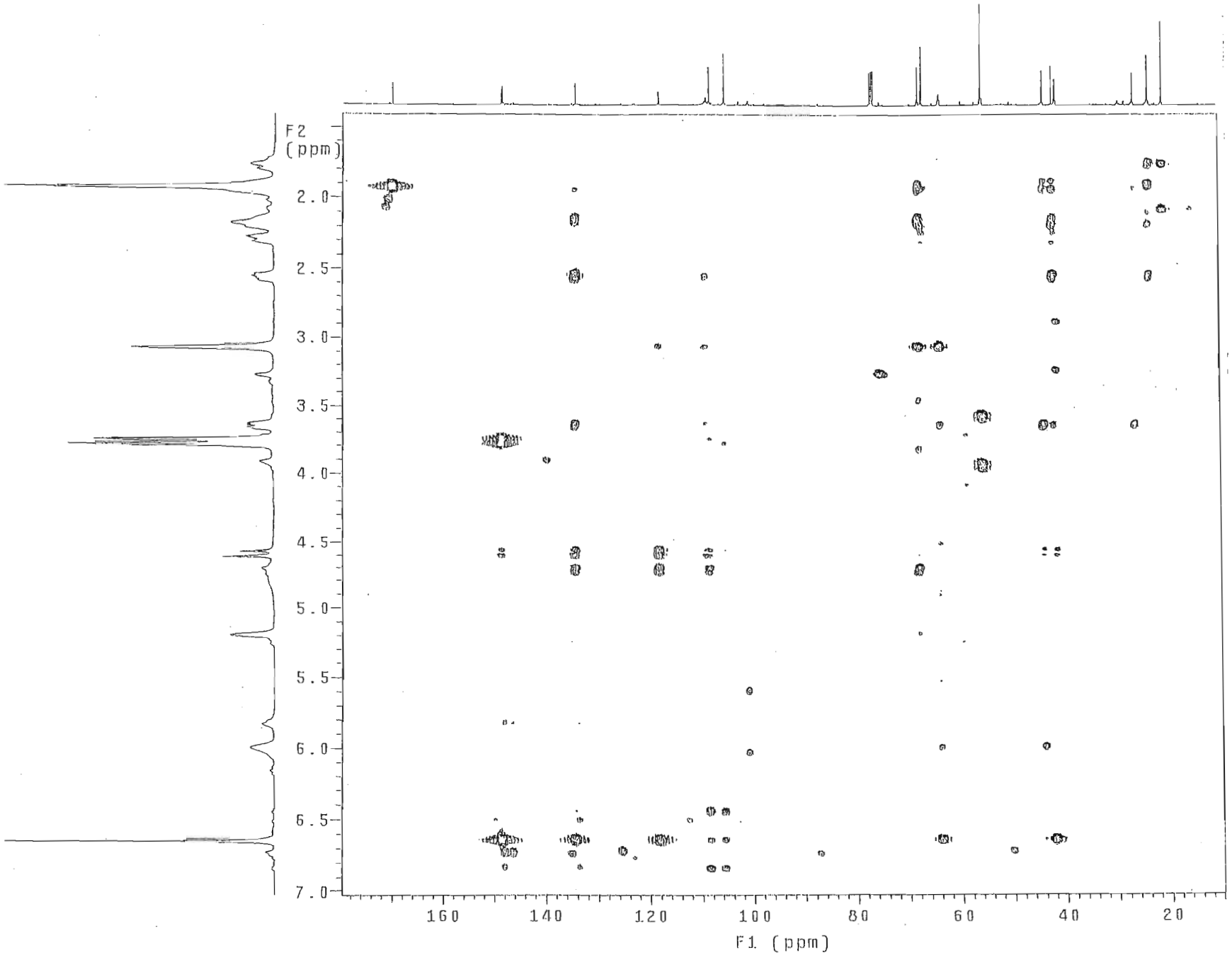




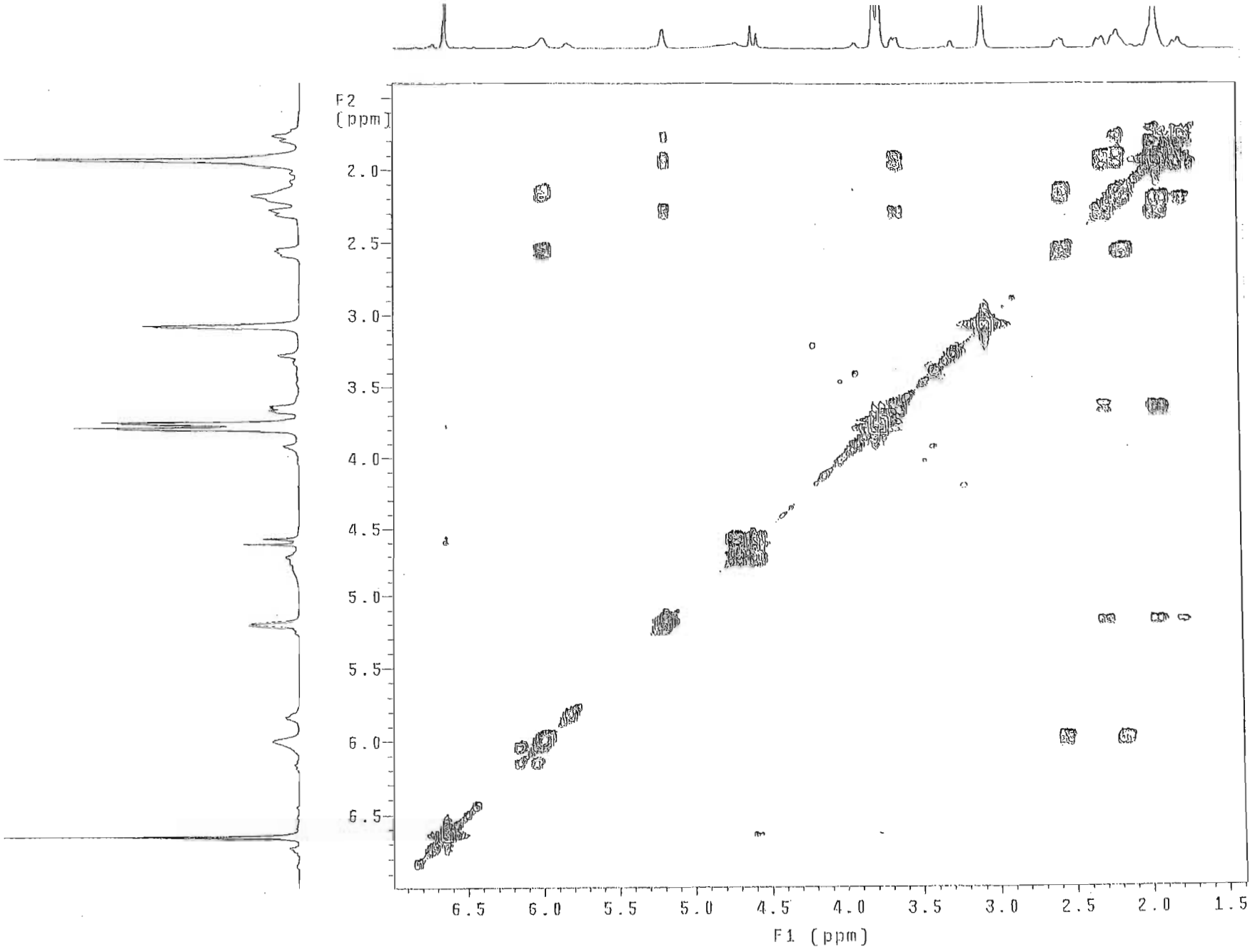
SPECTRUM 13.b: ^{13}C NMR spectrum of compound XIII ($CDCl_3$)



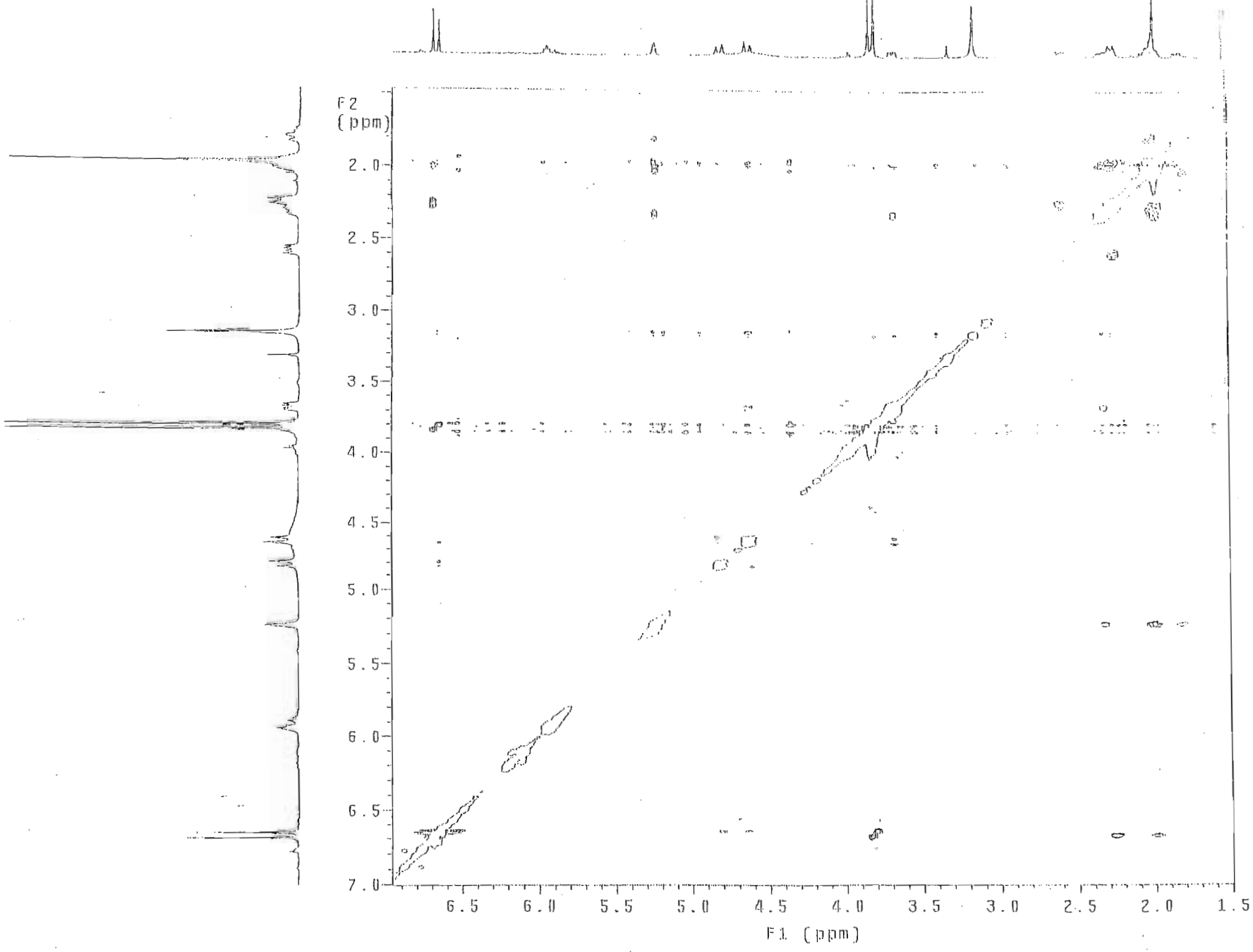
SPECTRUM 13.c: HSQC spectrum of compound XIII ($CDCl_3$)



SPECTRUM 13.d: HMBC spectrum of compound XIII ($CDCl_3$)



SPECTRUM 13.e: COSY spectrum of compound XIII ($CDCl_3$)



SPECTRUM 13.f: NOESY spectrum of compound XIII ($CDCl_3$)

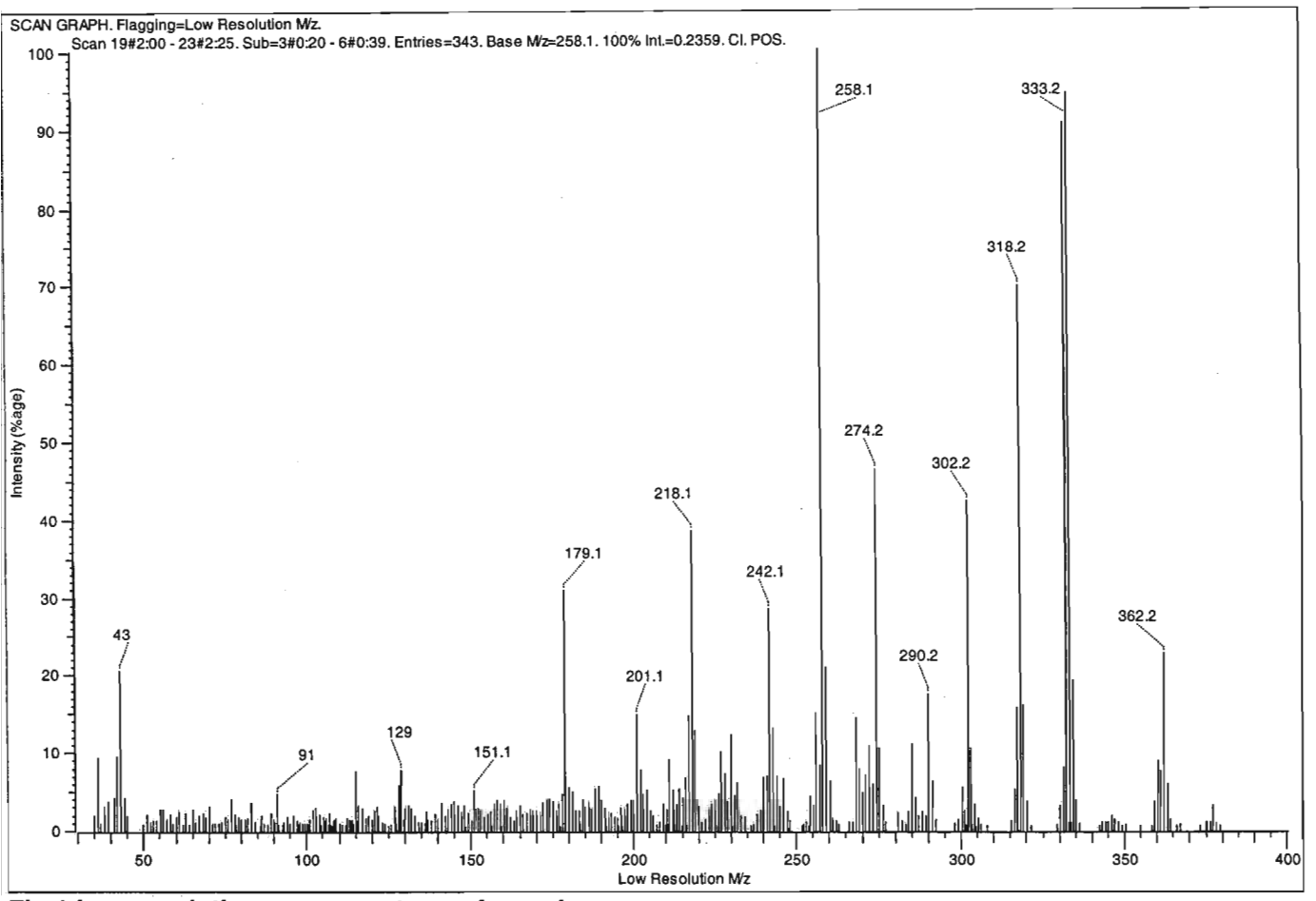


Fig 1 Low resolution mass spectrum of sample.

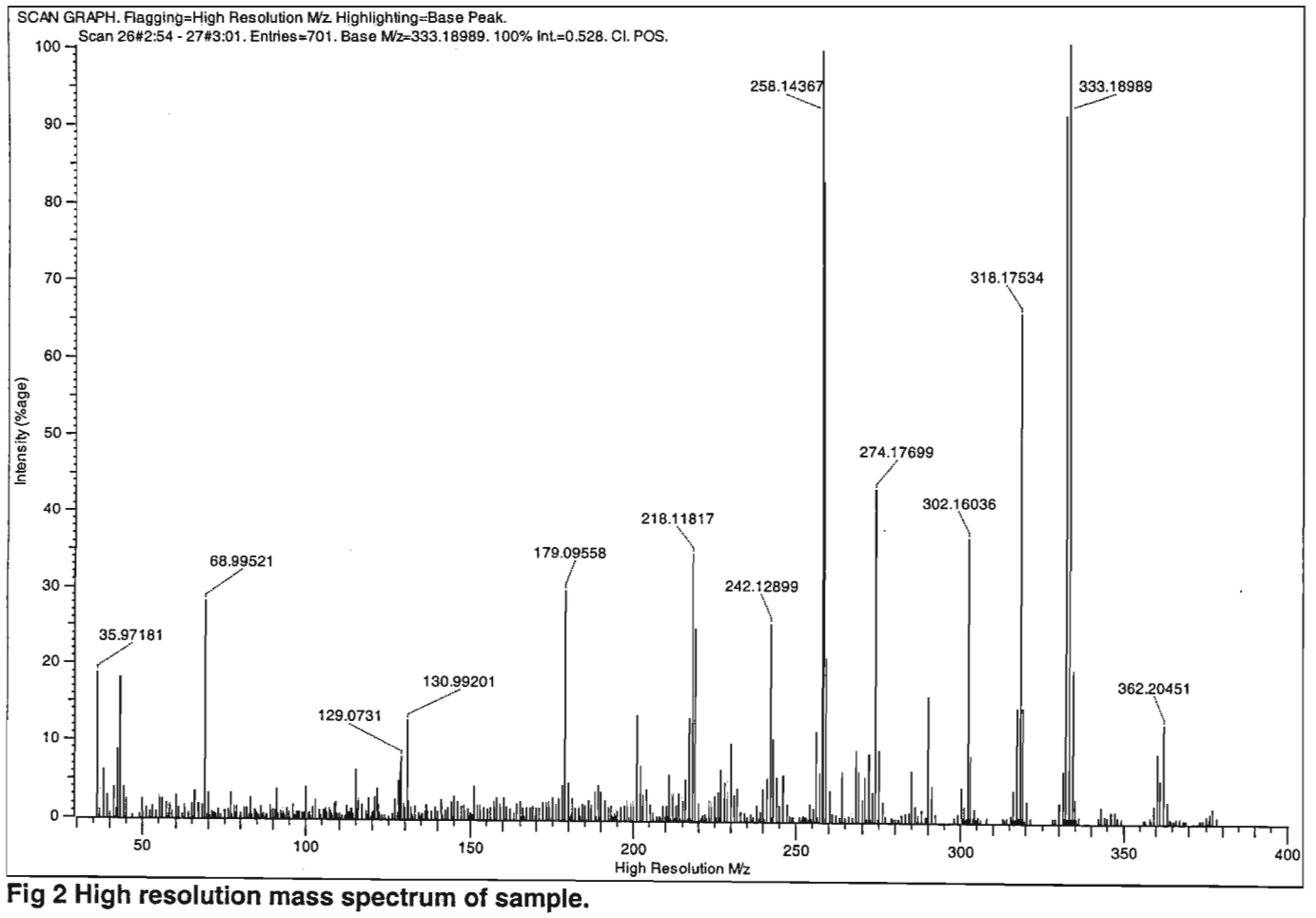
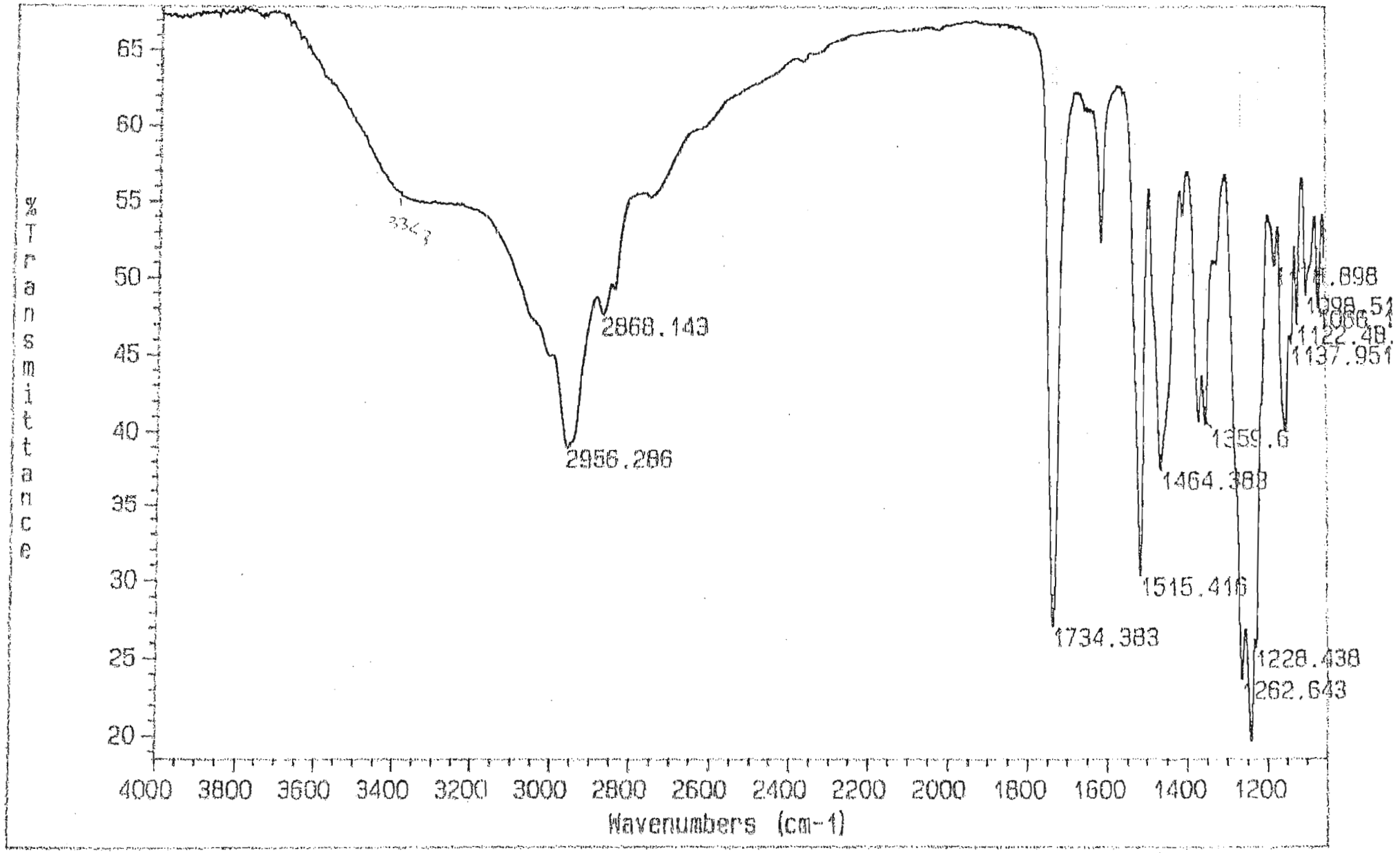
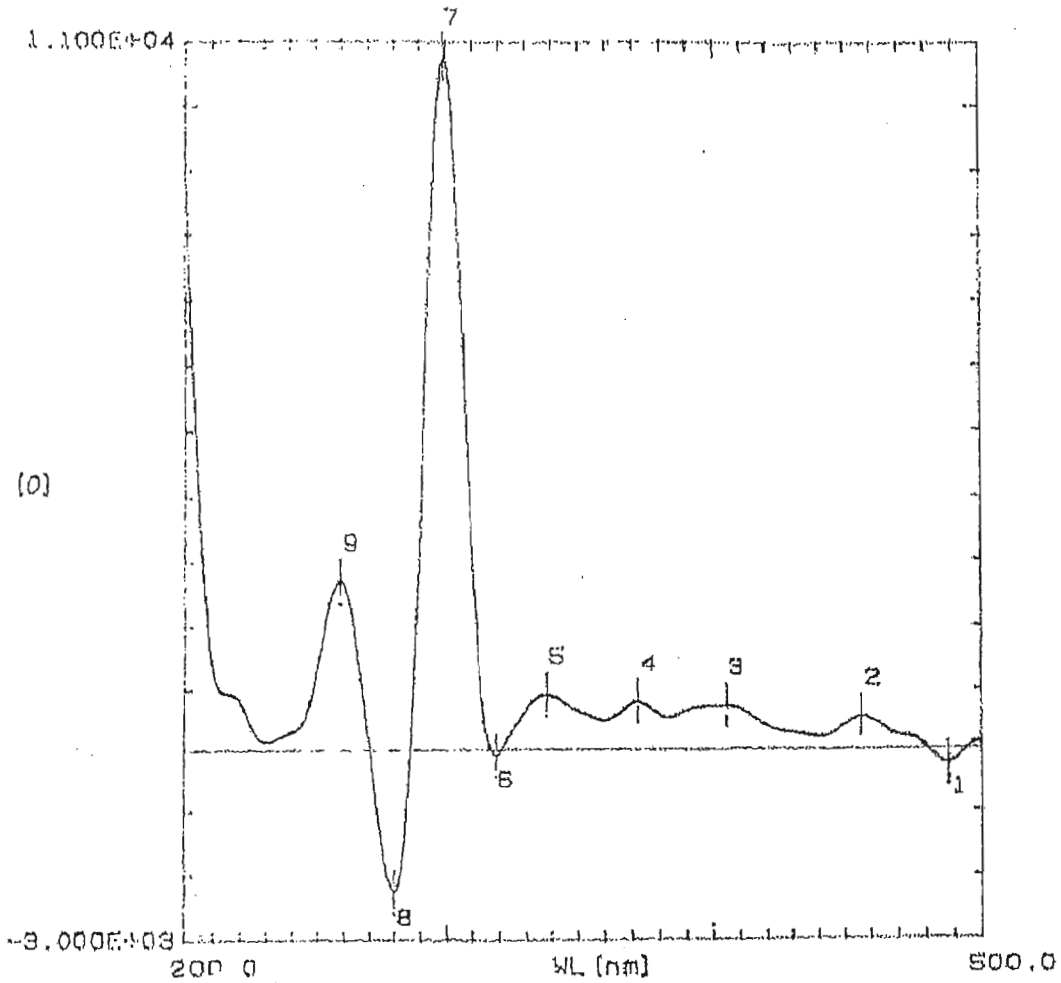


Fig 2 High resolution mass spectrum of sample.

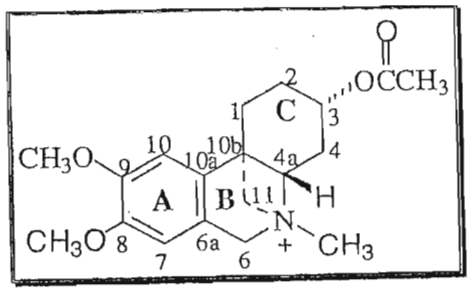
Spectrum 13.g: Mass spectra of compound XIII



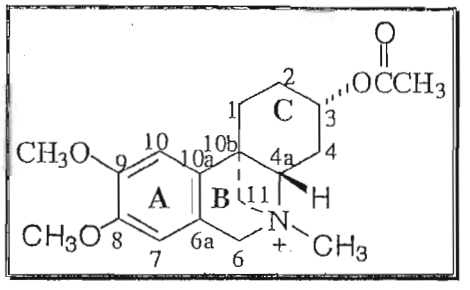
SPECTRUM 13.h: Infrared spectrum of compound XIII

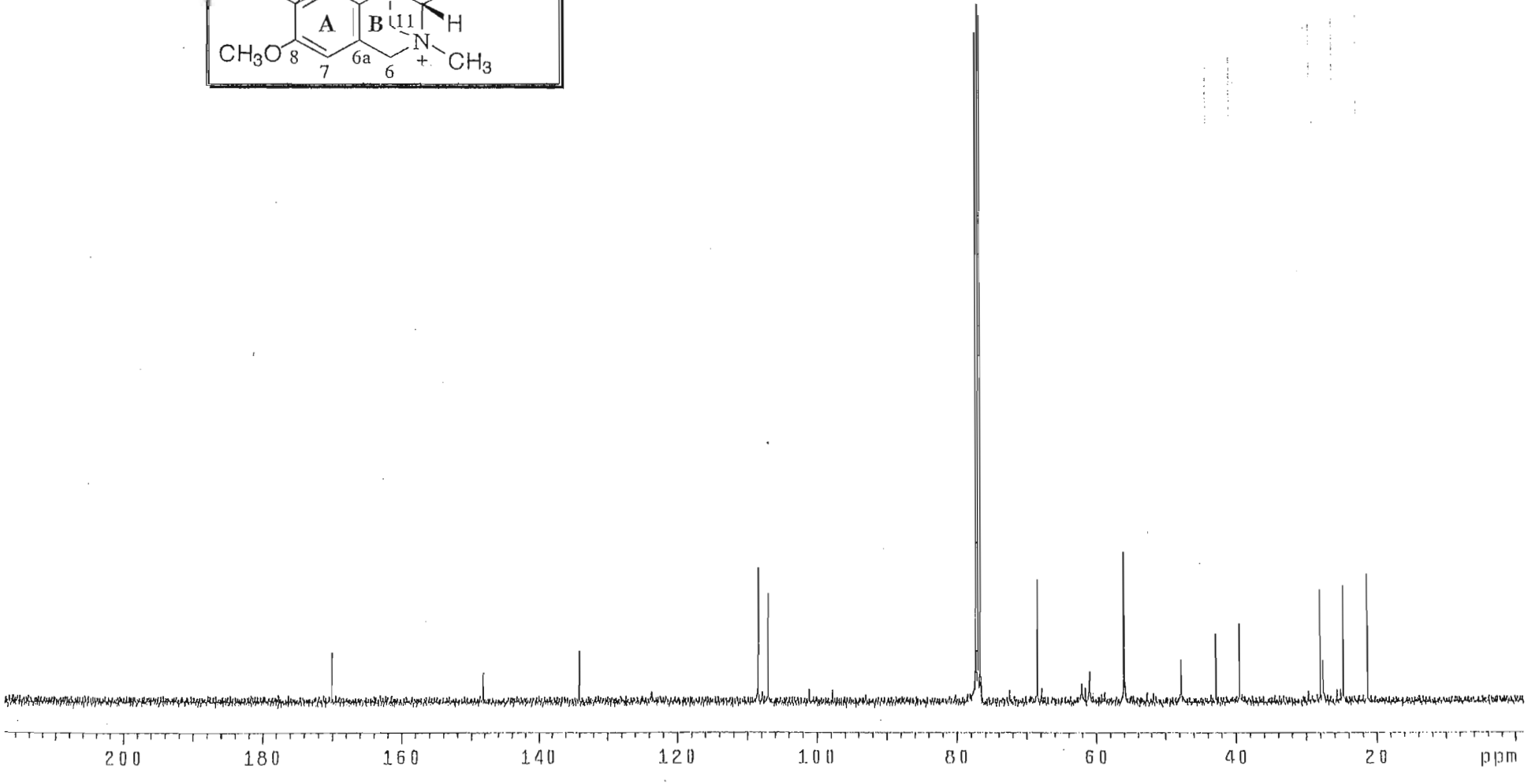


SPECTRUM 13.i: CD spectrum of compound XIII

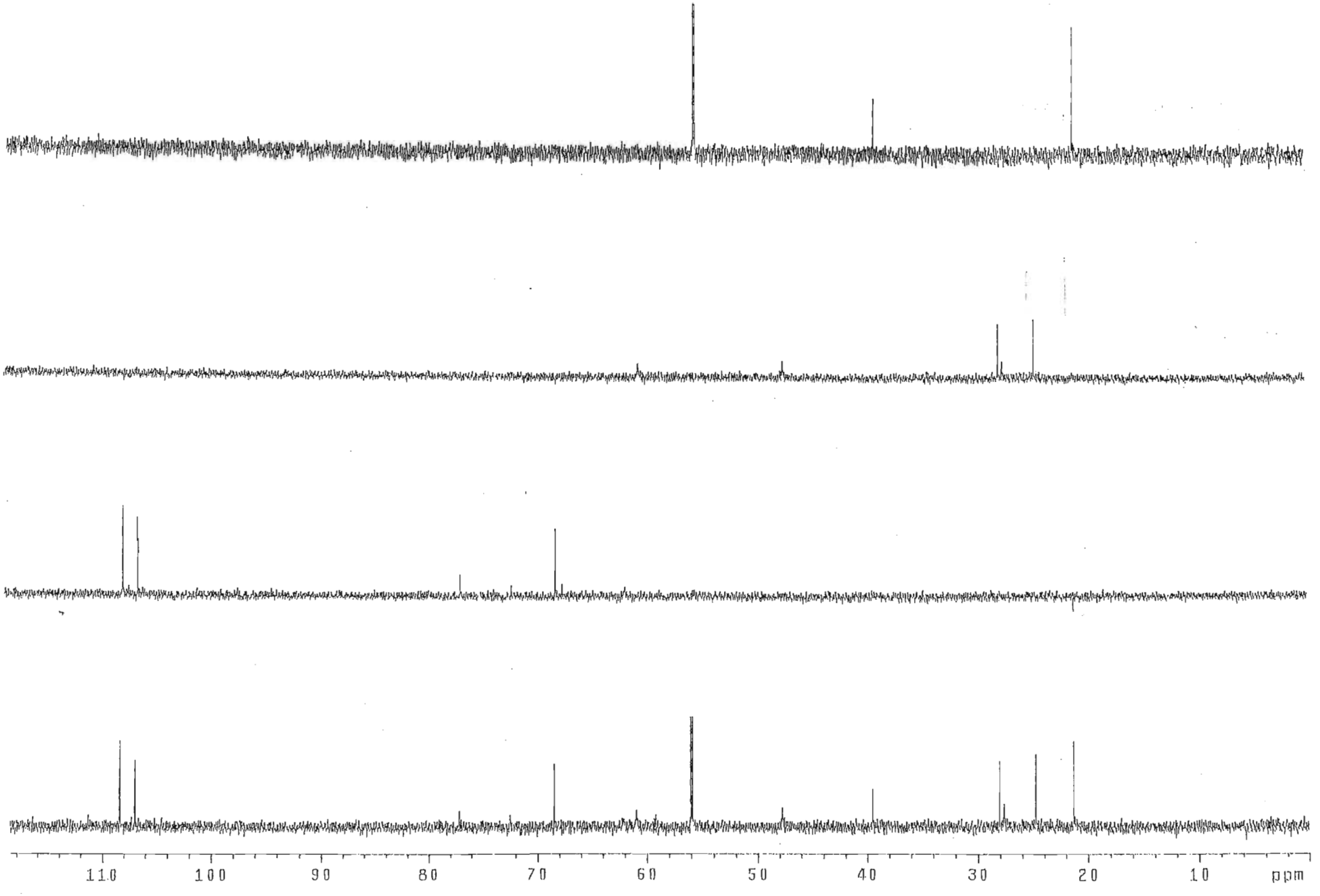


SPECTRUM 14.a: ^{1}H NMR spectrum of compound XIV ($CDCl_3$)

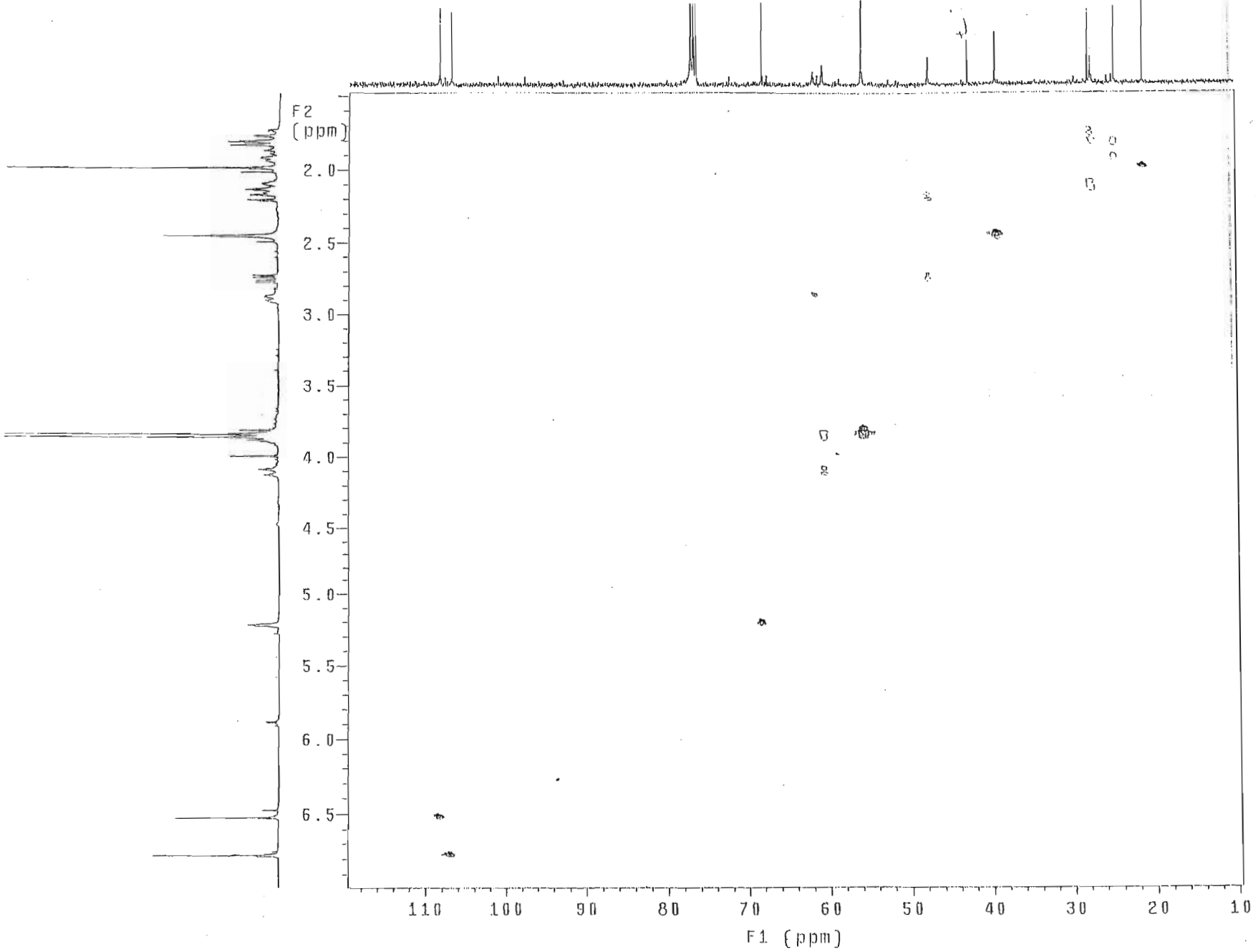




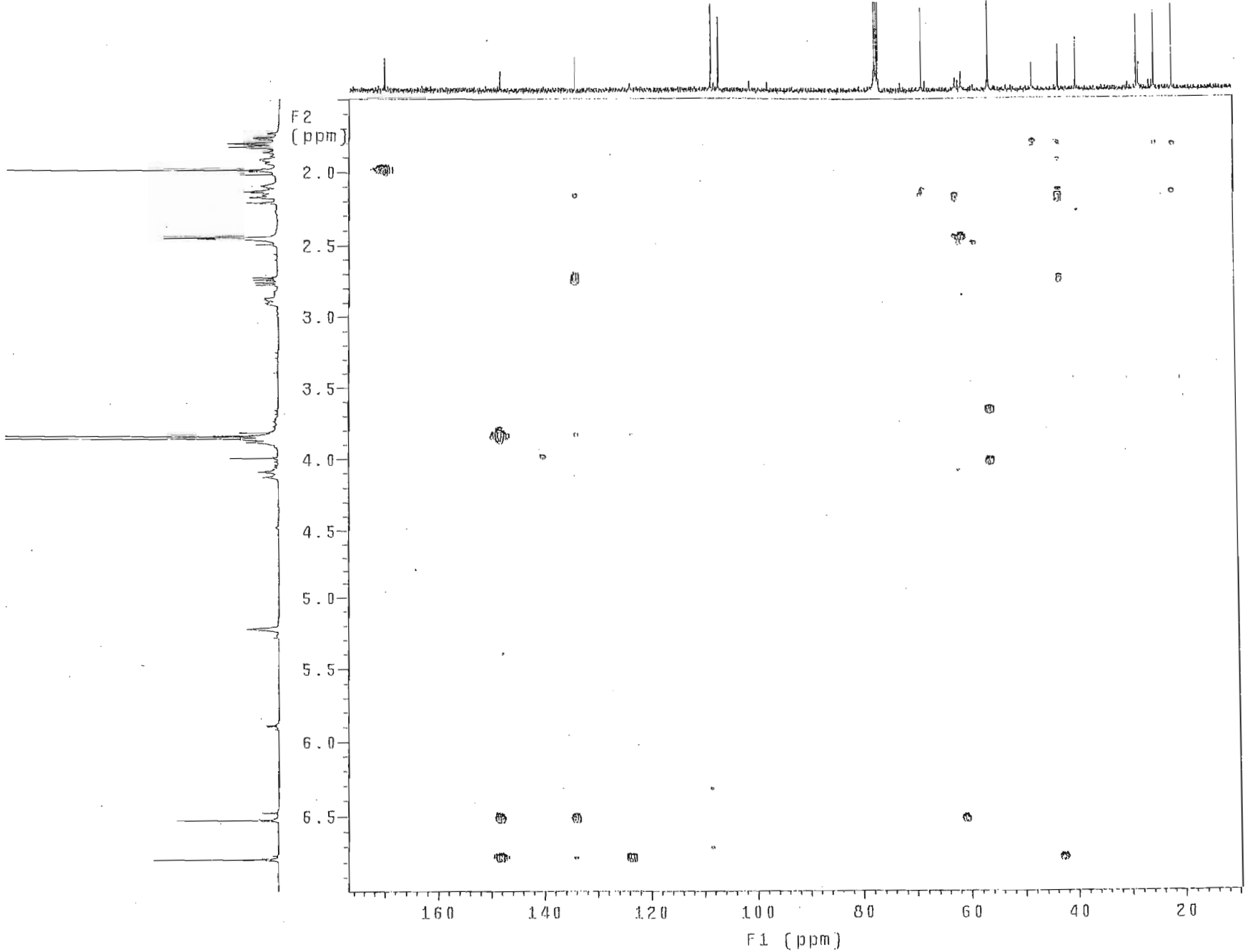
SPECTRUM 14.b: ^{13}C NMR spectrum of compound XIV ($CDCl_3$)



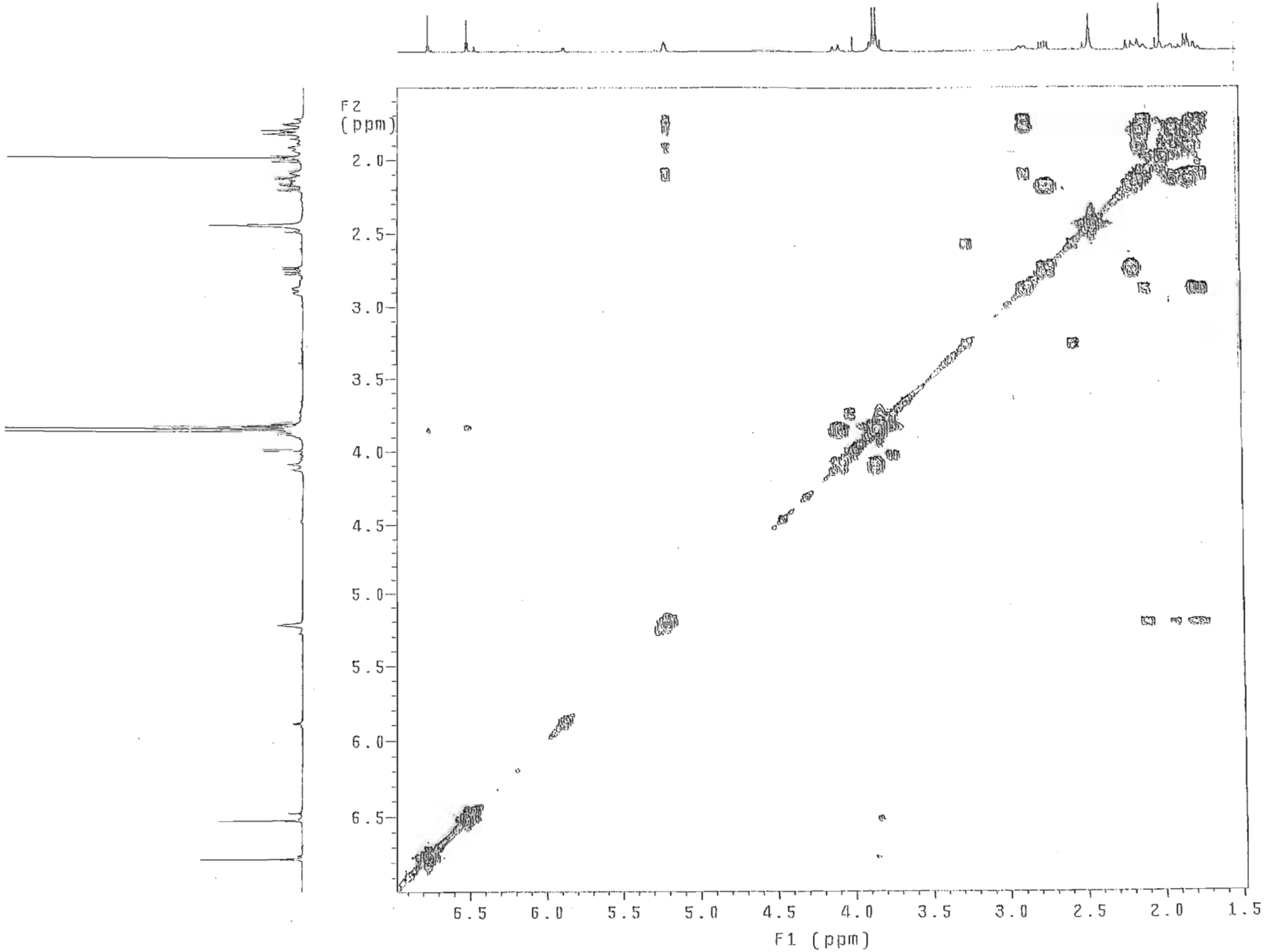
SPECTRUM 14.c: ADEPT spectrum of compound XIV ($CDCl_3$)



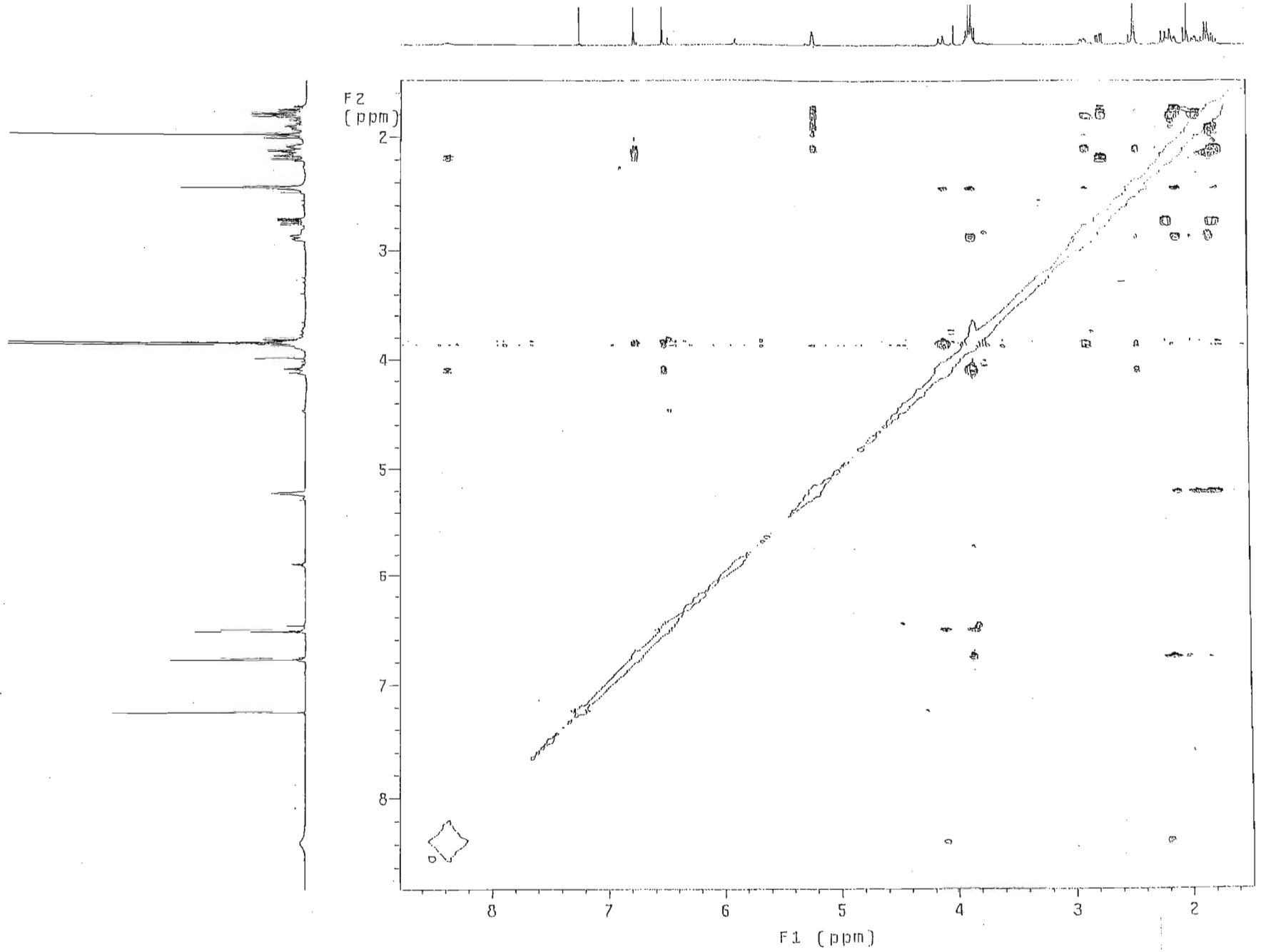
SPECTRUM 14.d: HSQC spectrum of compound XIV ($CDCl_3$)



SPECTRUM 14.e: HMBC spectrum of compound XIV ($CDCl_3$)



SPECTRUM 14.f: COSY spectrum of compound XIV ($CDCl_3$)



SPECTRUM 14.g: NOESY spectrum of compound XIV ($CDCl_3$)

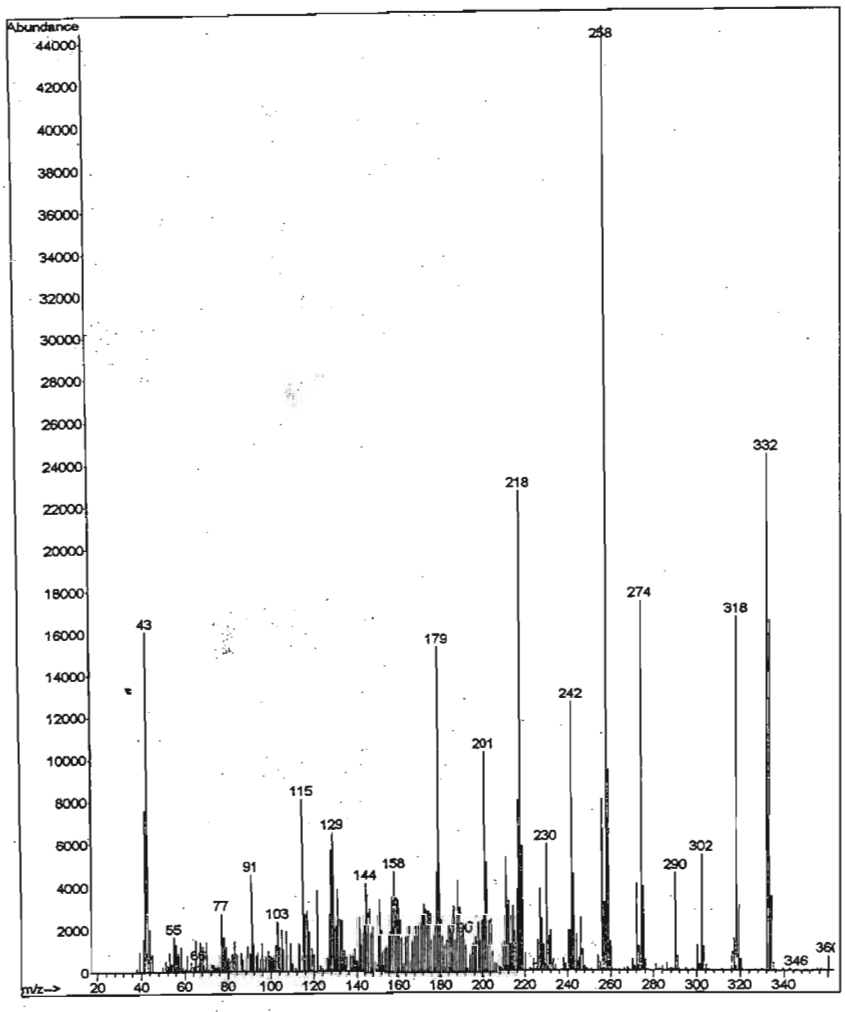


Fig 1 Low resolution mass spectrum of sample.

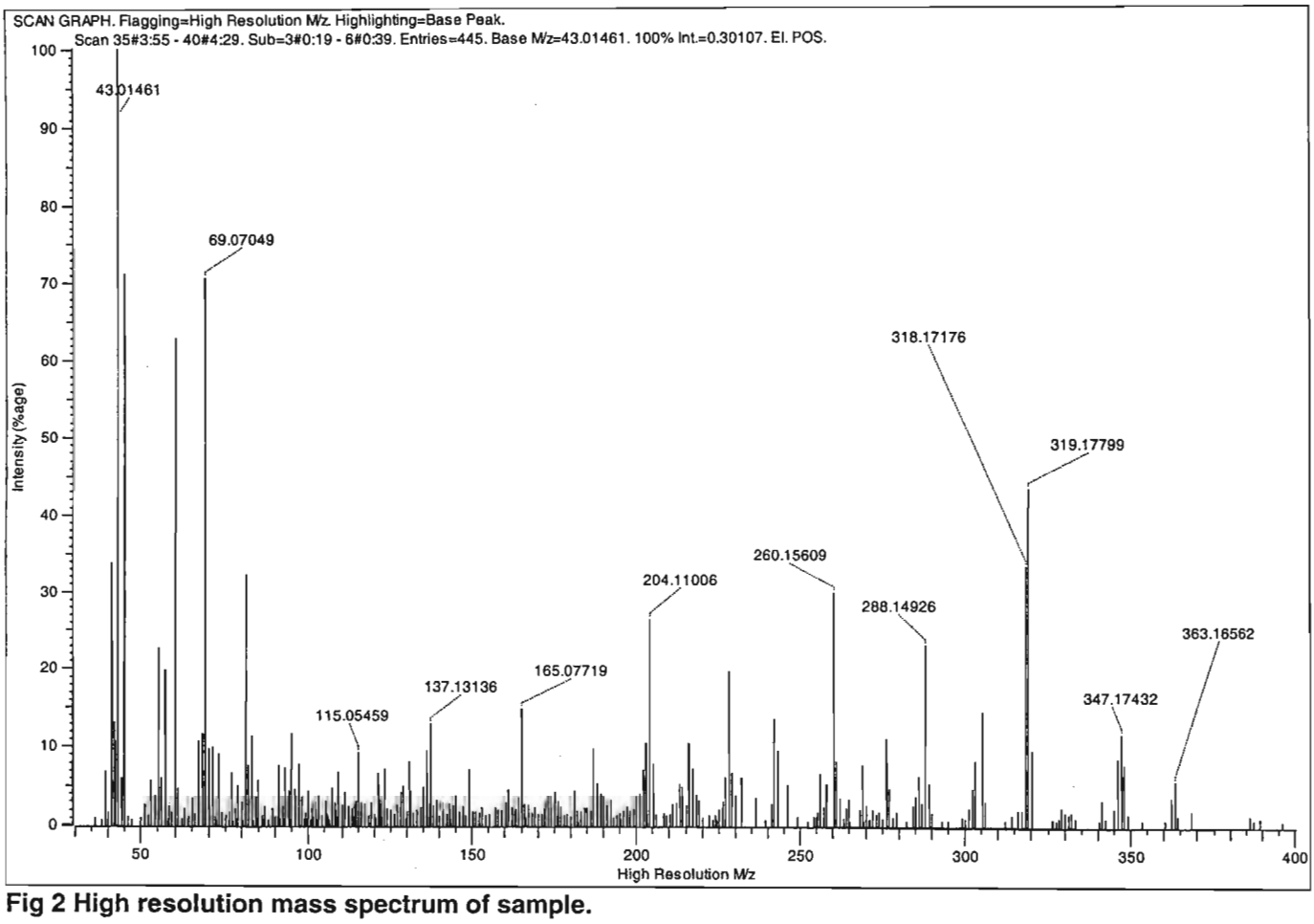
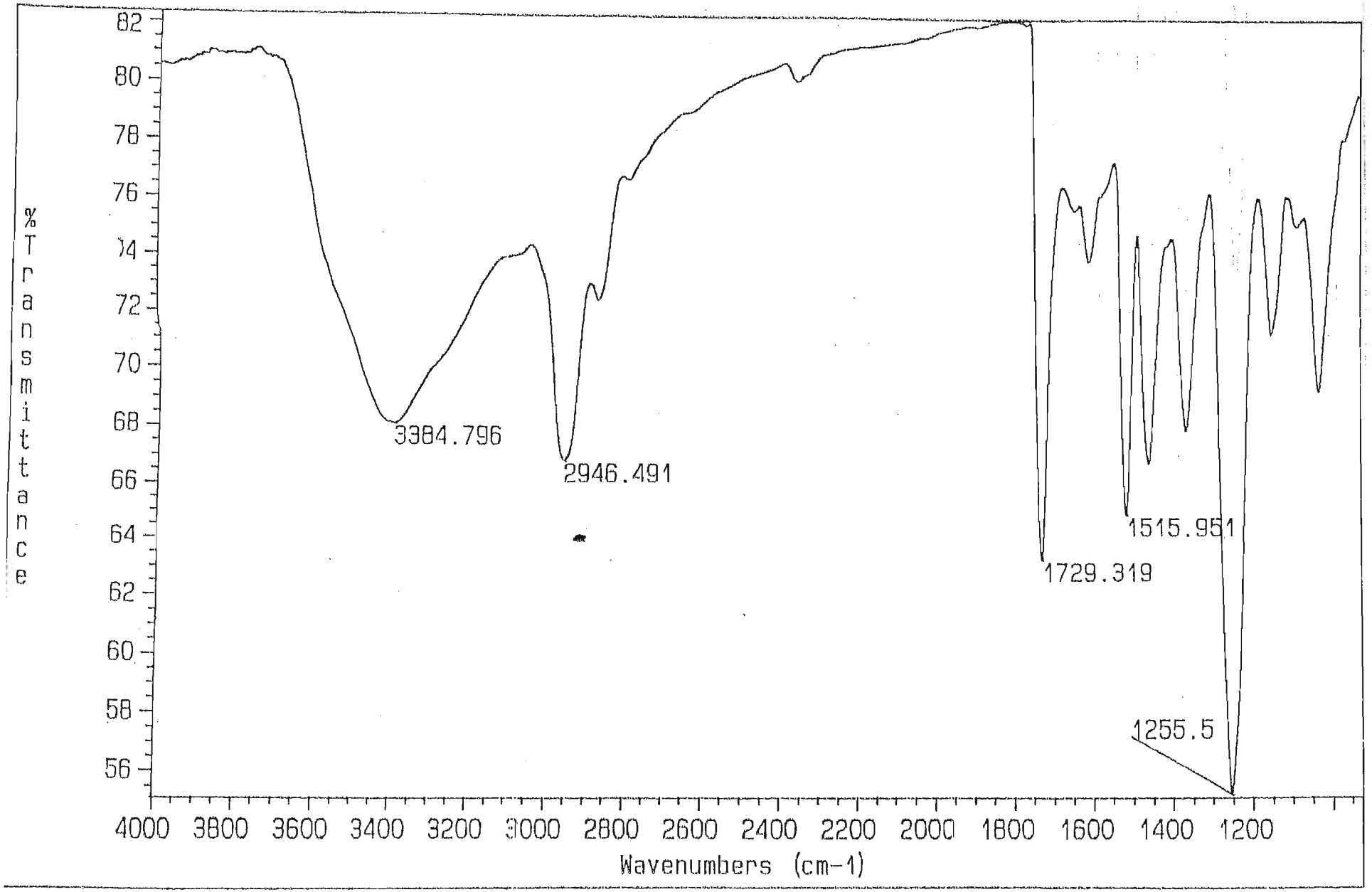
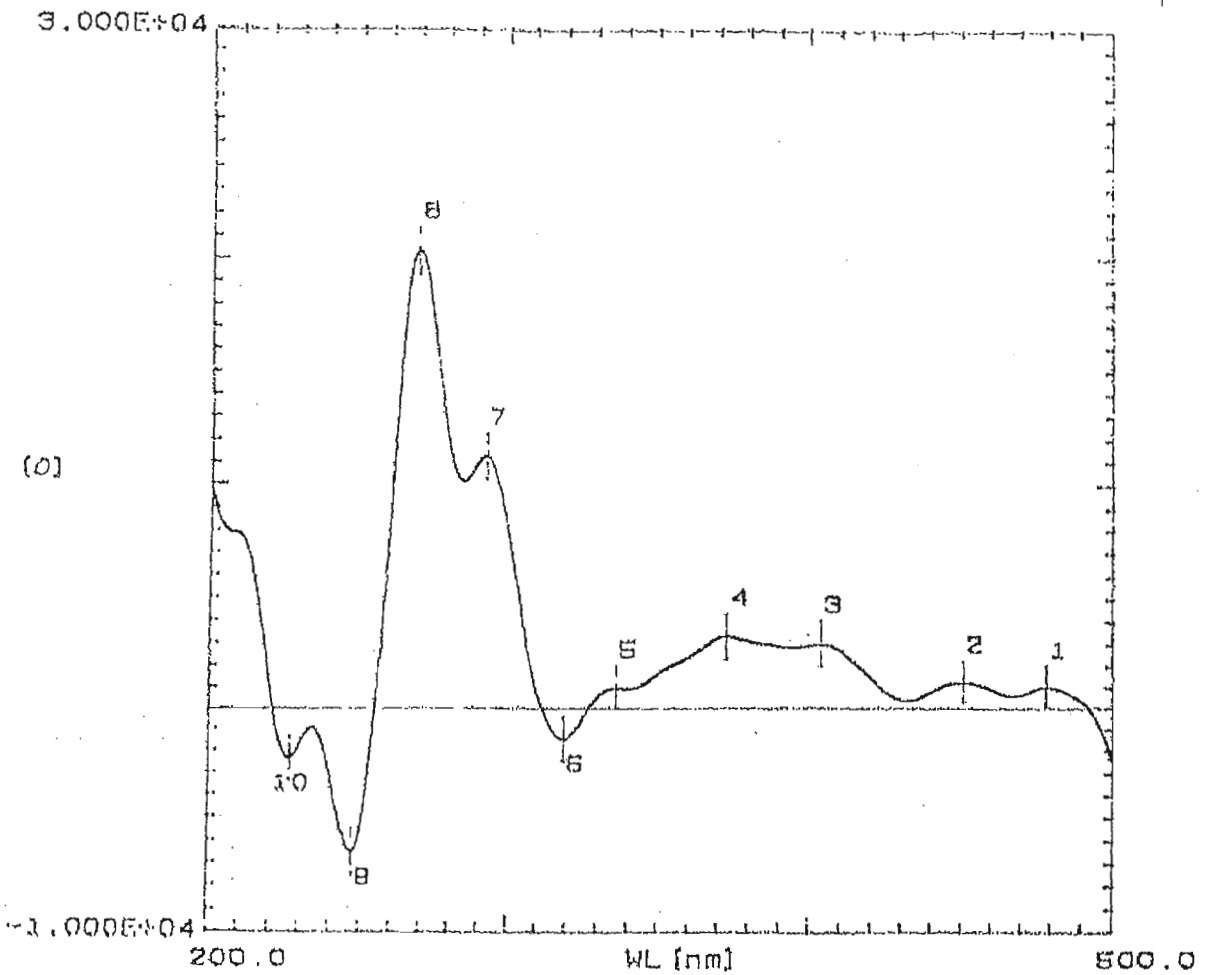


Fig 2 High resolution mass spectrum of sample.

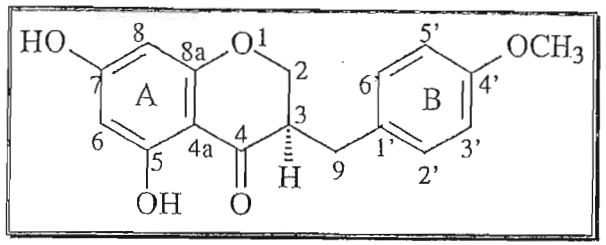
Spectrum 14.h: Mass spectra of compound XIV



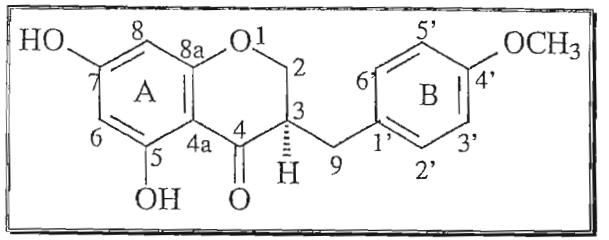
SPECTRUM 14.i: Infrared spectrum of compound XIV

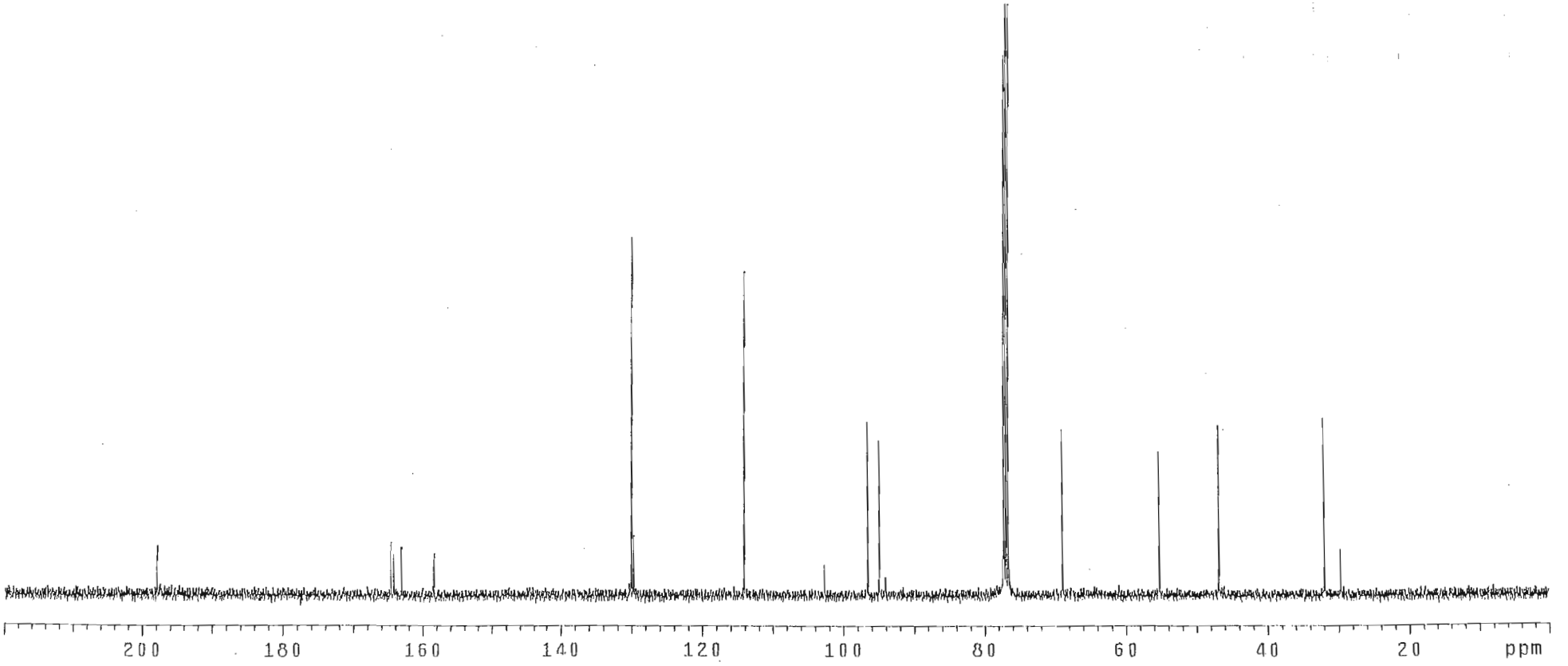


SPECTRUM 14.j: CD spectrum of compound XIV

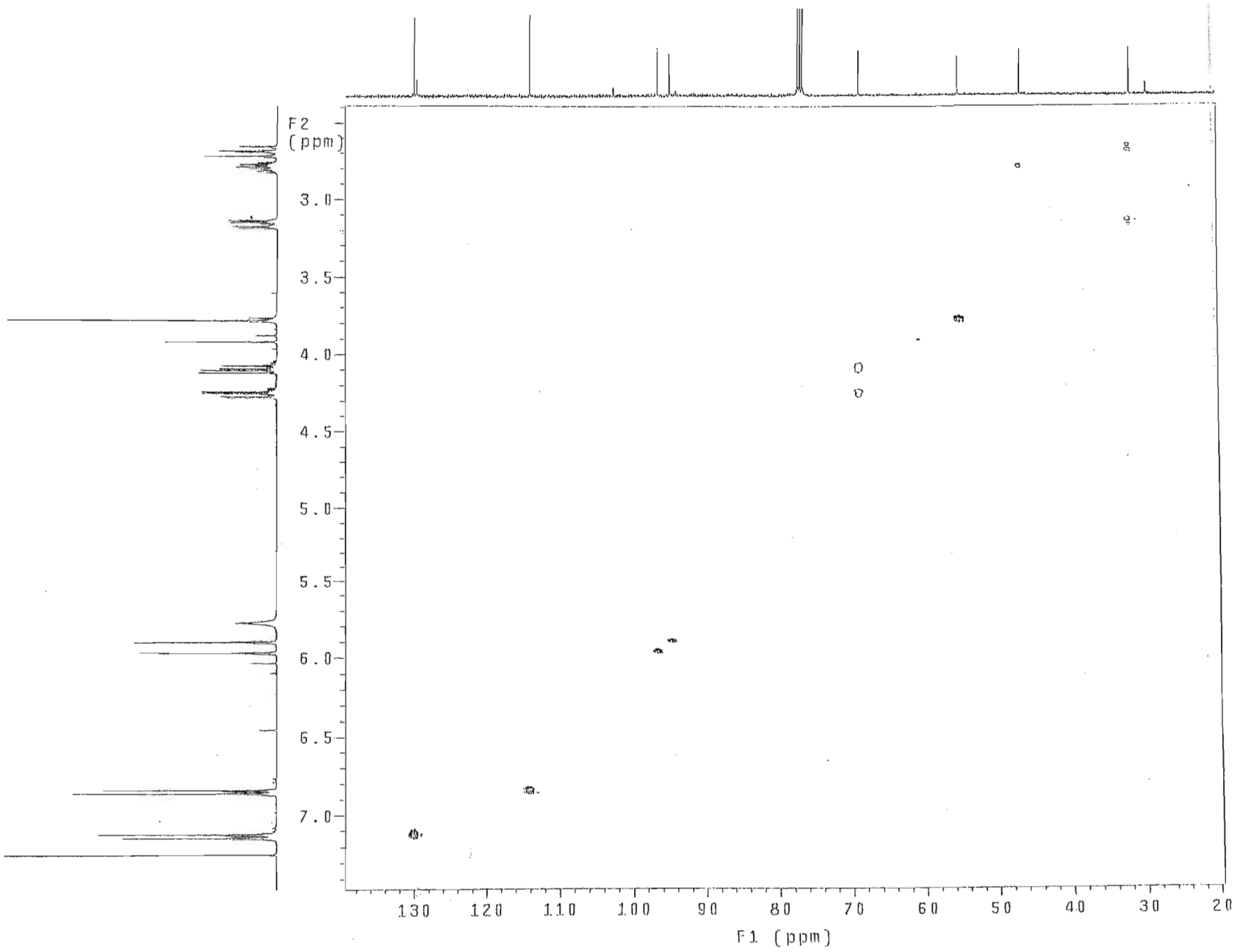


SPECTRUM 15.a: ^{1}H NMR spectrum of compound XV ($CDCl_3$)

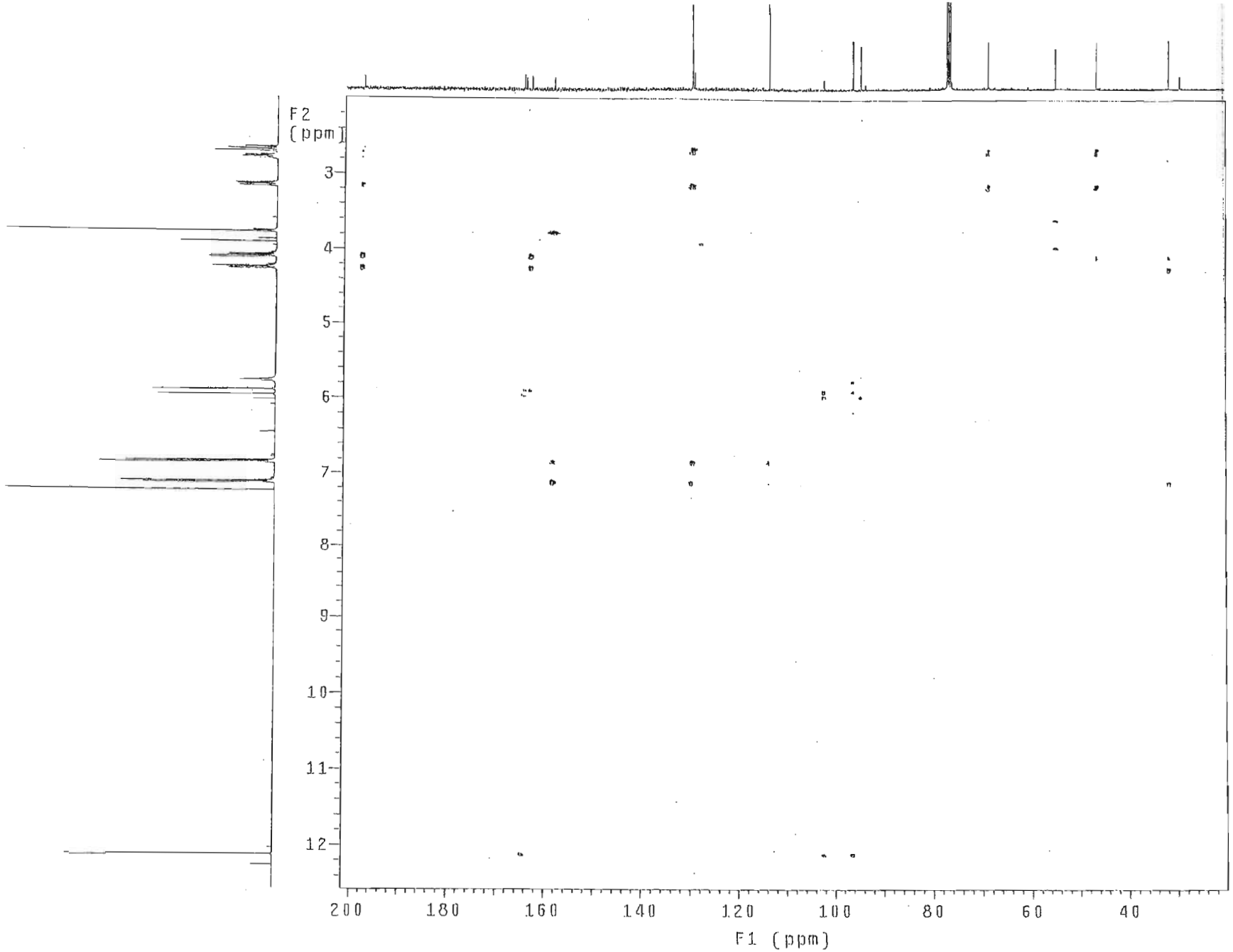




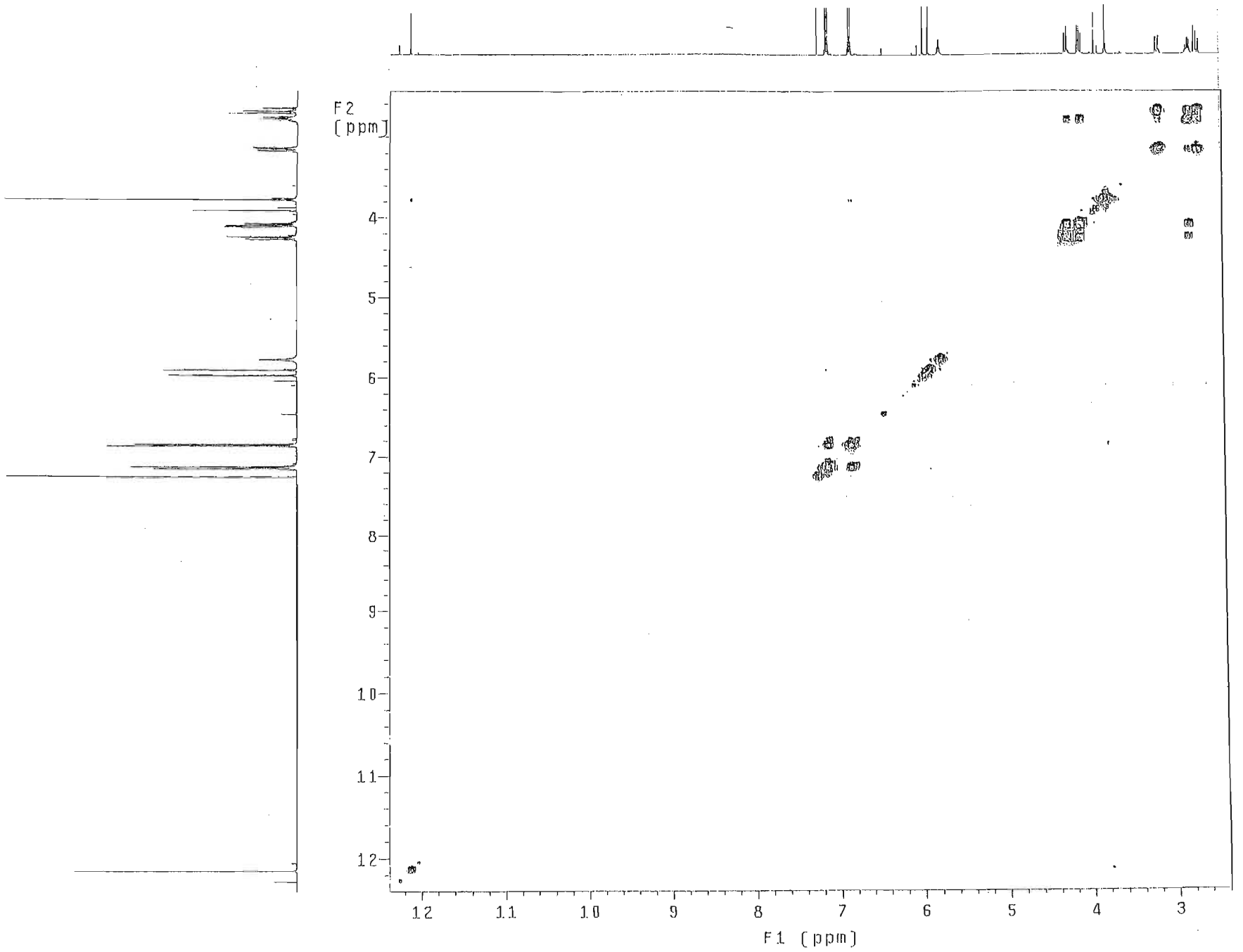
SPECTRUM 15.b: ^{13}C NMR spectrum of compound XV ($CDCl_3$)



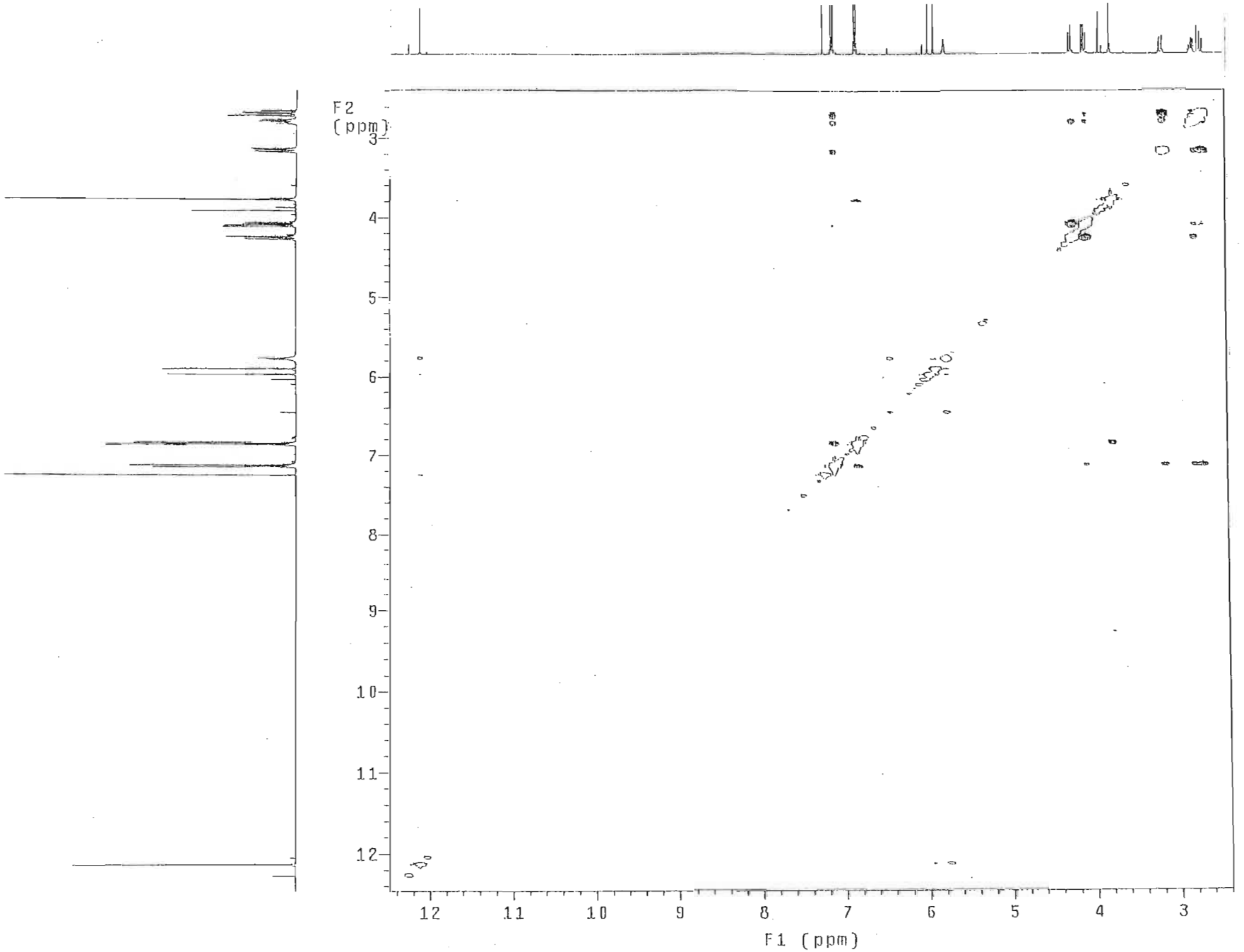
SPECTRUM 15.c: HSQC spectrum of compound XV ($CDCl_3$)



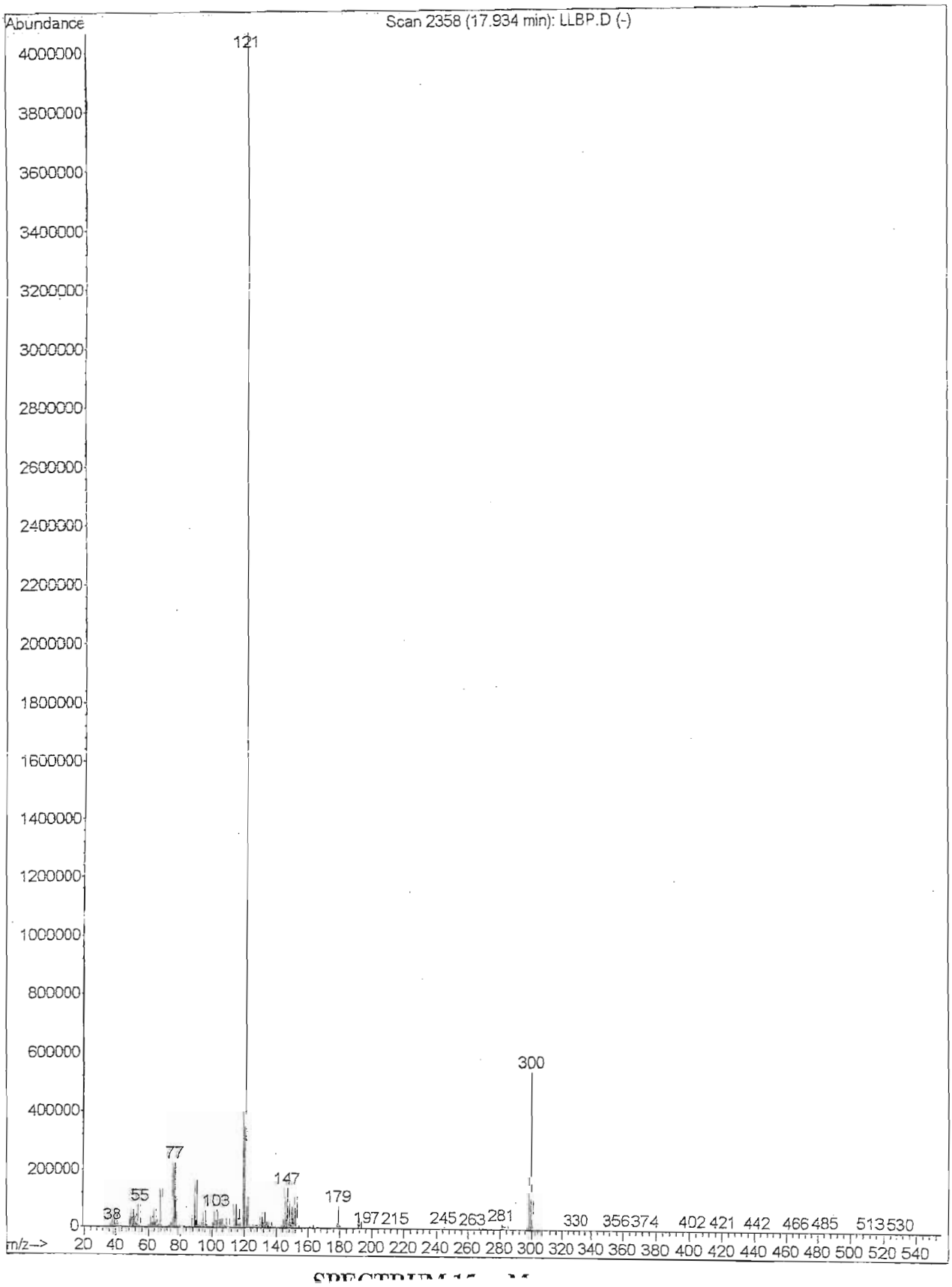
SPECTRUM 15.d: HMBC spectrum of compound XV ($CDCl_3$)



SPECTRUM 15.e: COSY spectrum of compound XV ($CDCl_3$)



SPECTRUM 15.f: NOESY spectrum of compound XV ($CDCl_3$)


Abundance
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300
77
147
55
103
179
38
m/z-->
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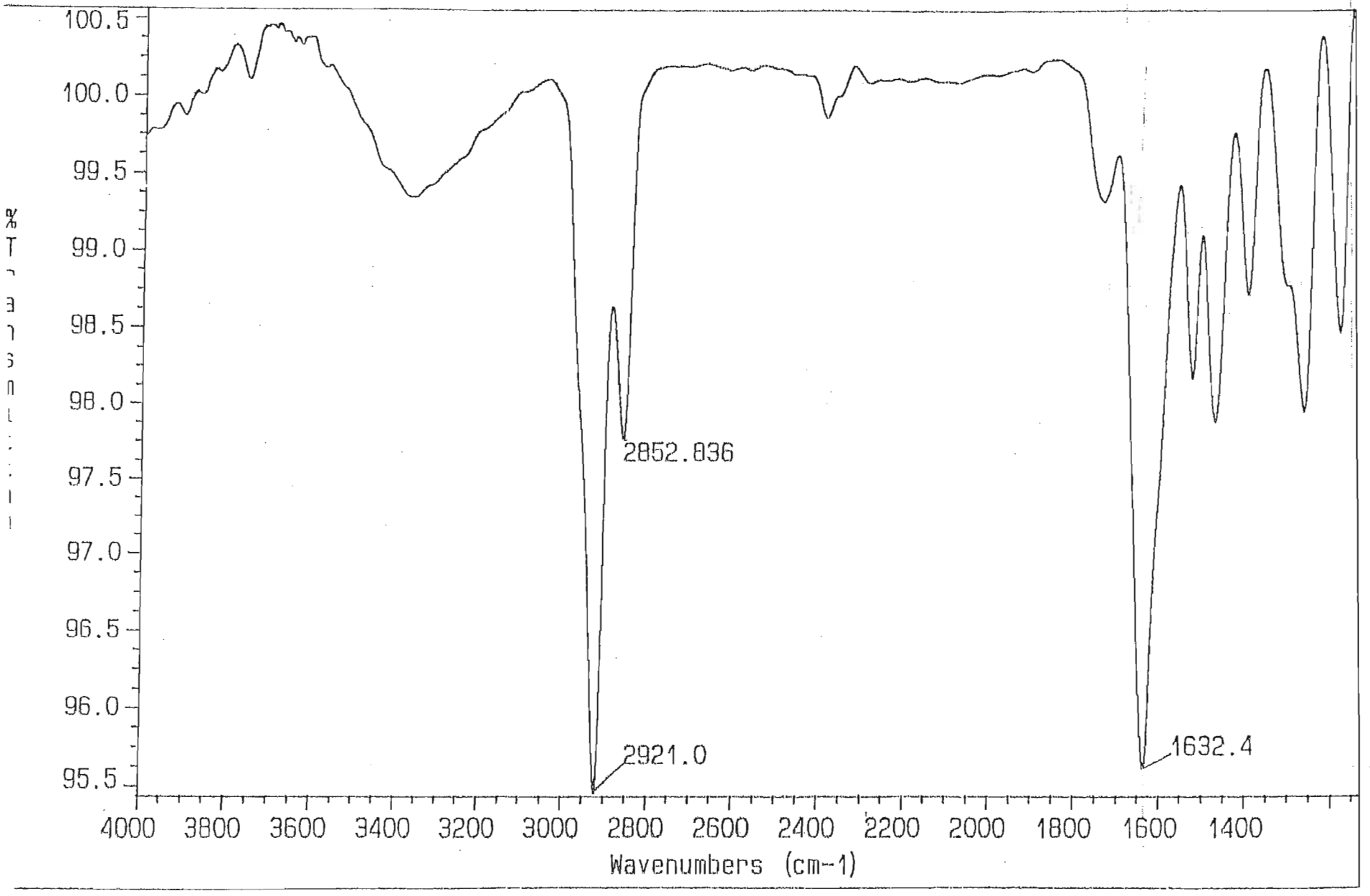
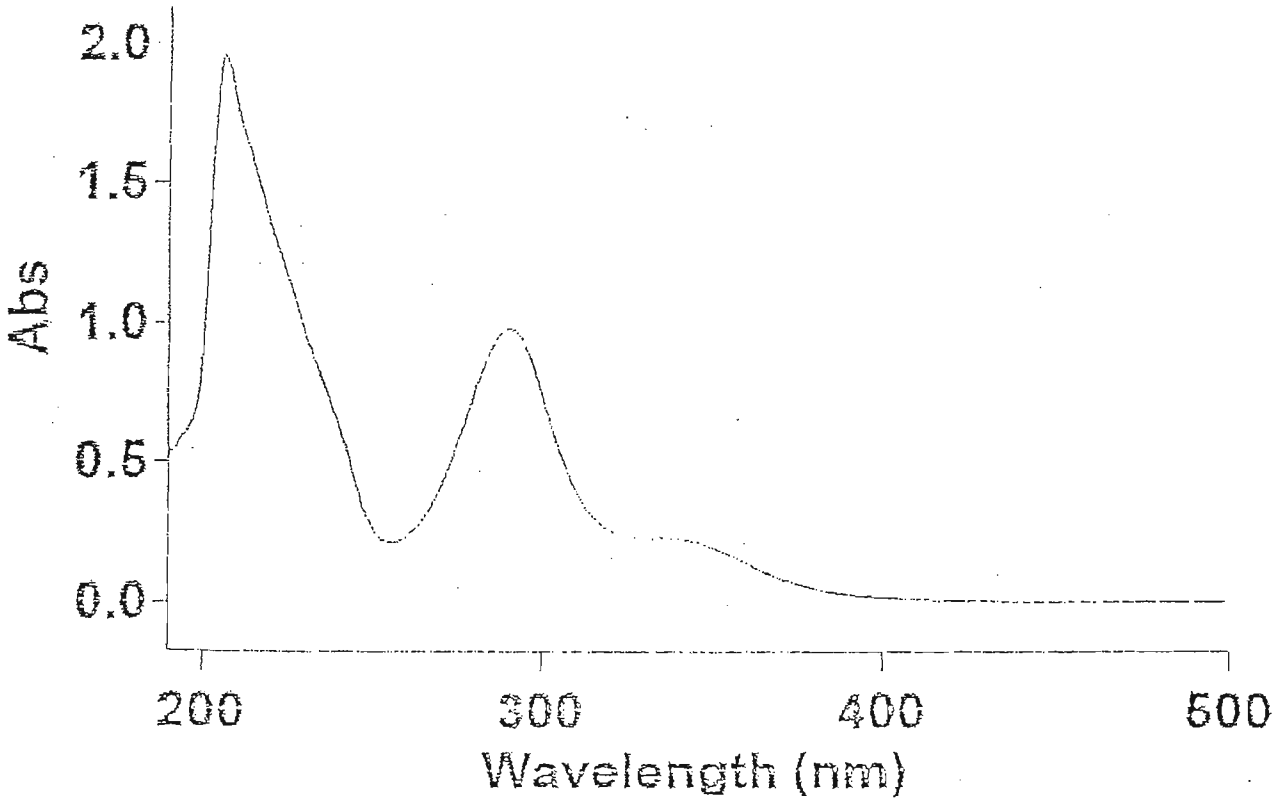
3800000
3600000
3400000
3200000
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540

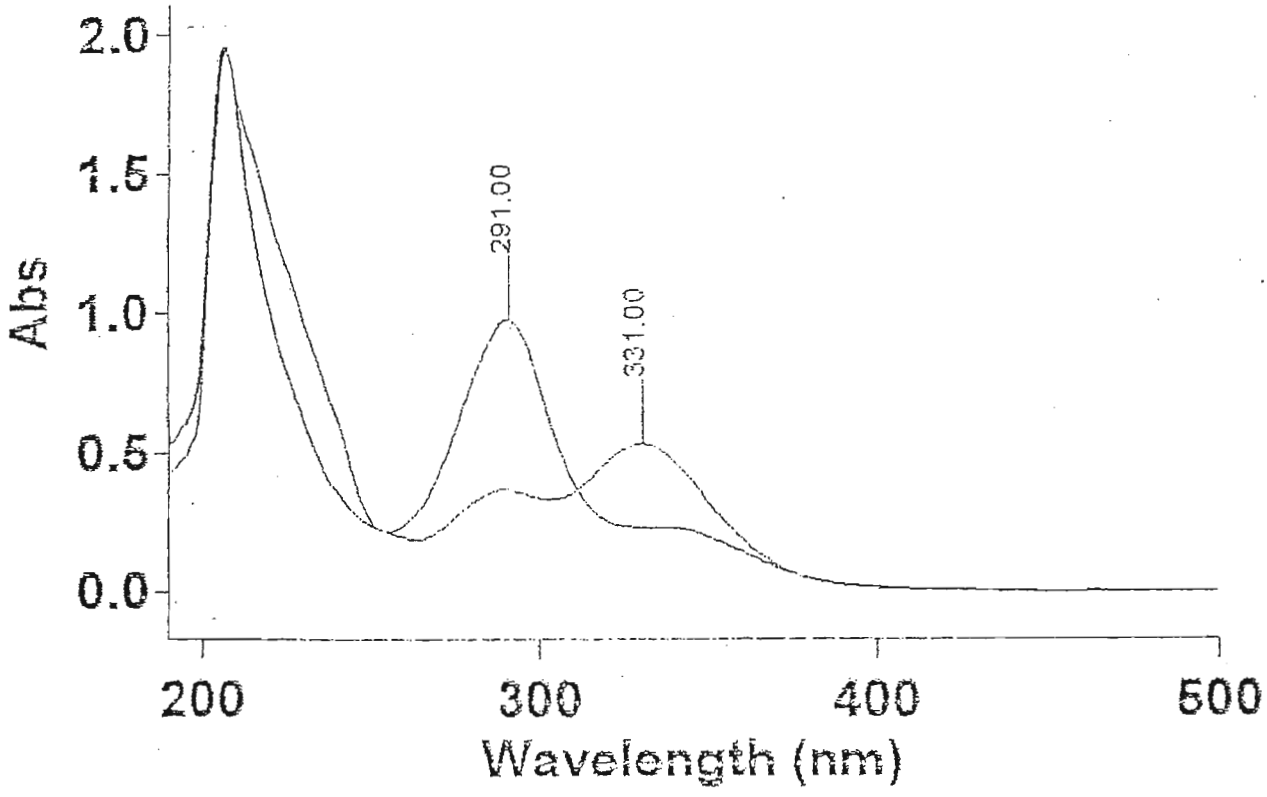
SPECTRUM 15



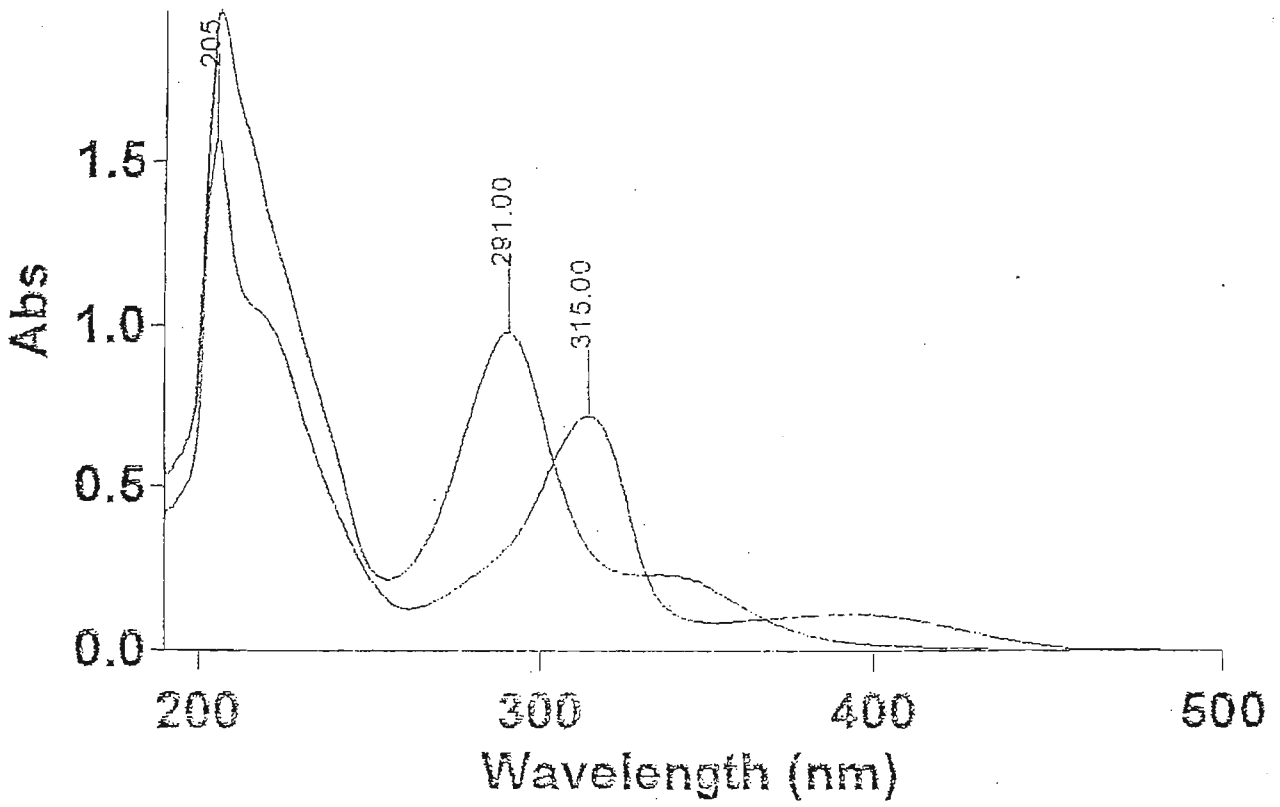
SPECTRUM 15.h: Infrared spectrum of compound XV



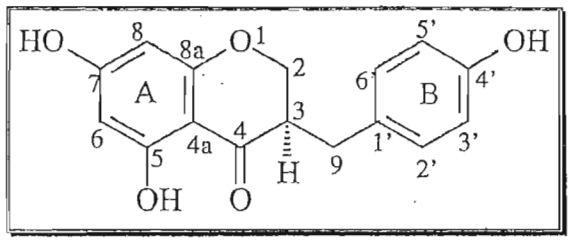
SPECTRUM 15.i.: UV spectrum of compound XV

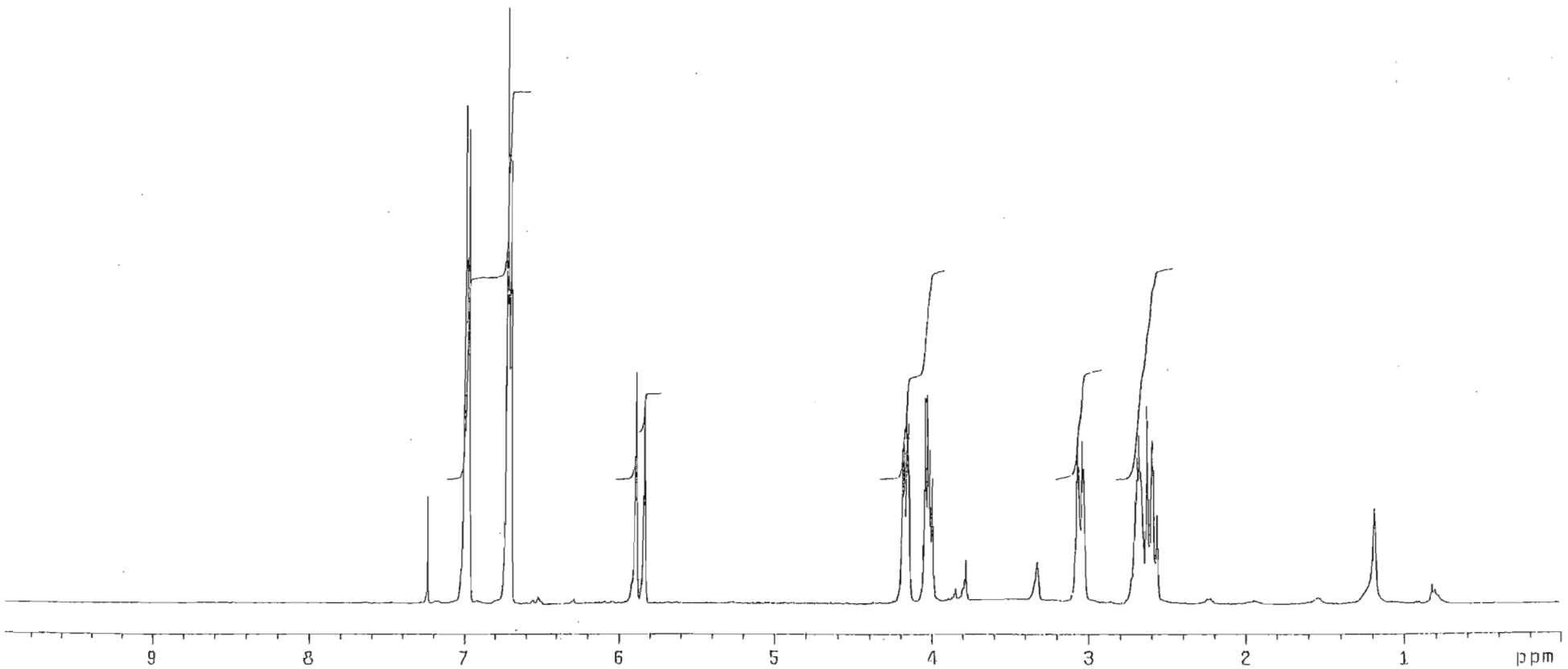


SPECTRUM 15.j.: UV spectrum of compound XV with NaoAc

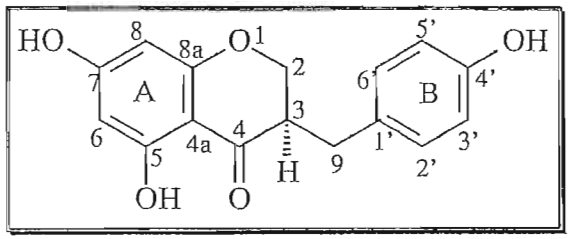


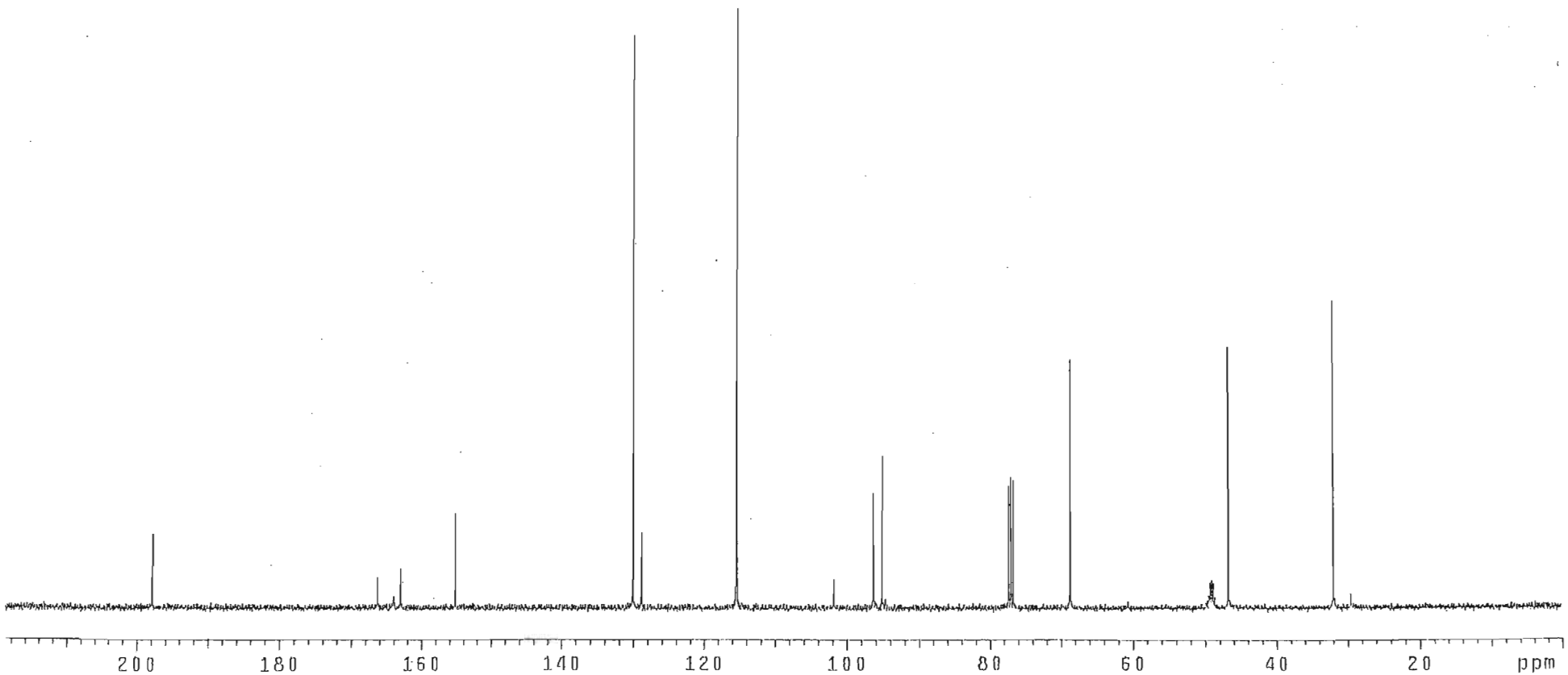
SPECTRUM 15.k.: UV spectrum of compound XV with $AlCl_3$



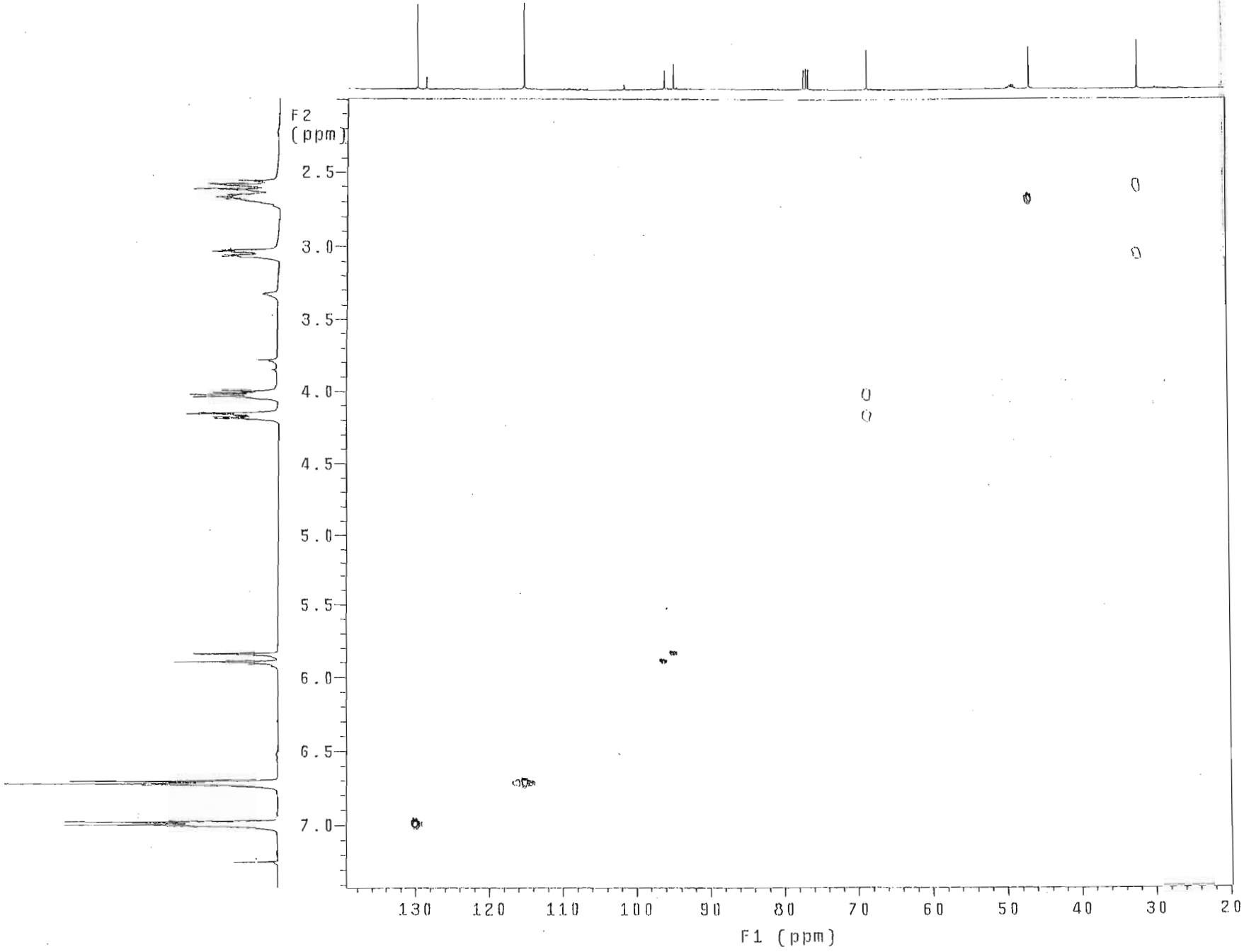


SPECTRUM 16.a: ^{1}H NMR spectrum of compound XVI ($CDCl_3$)

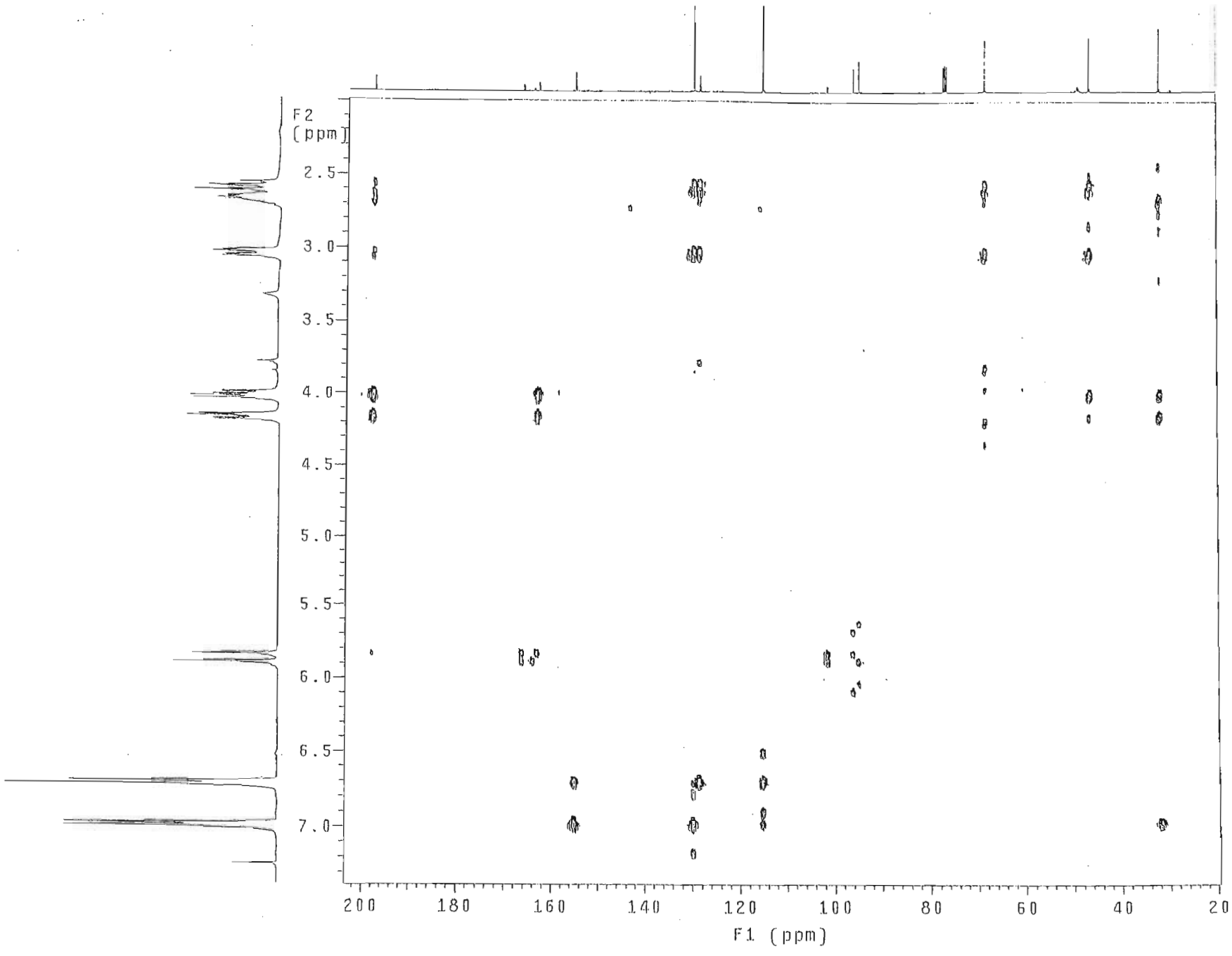




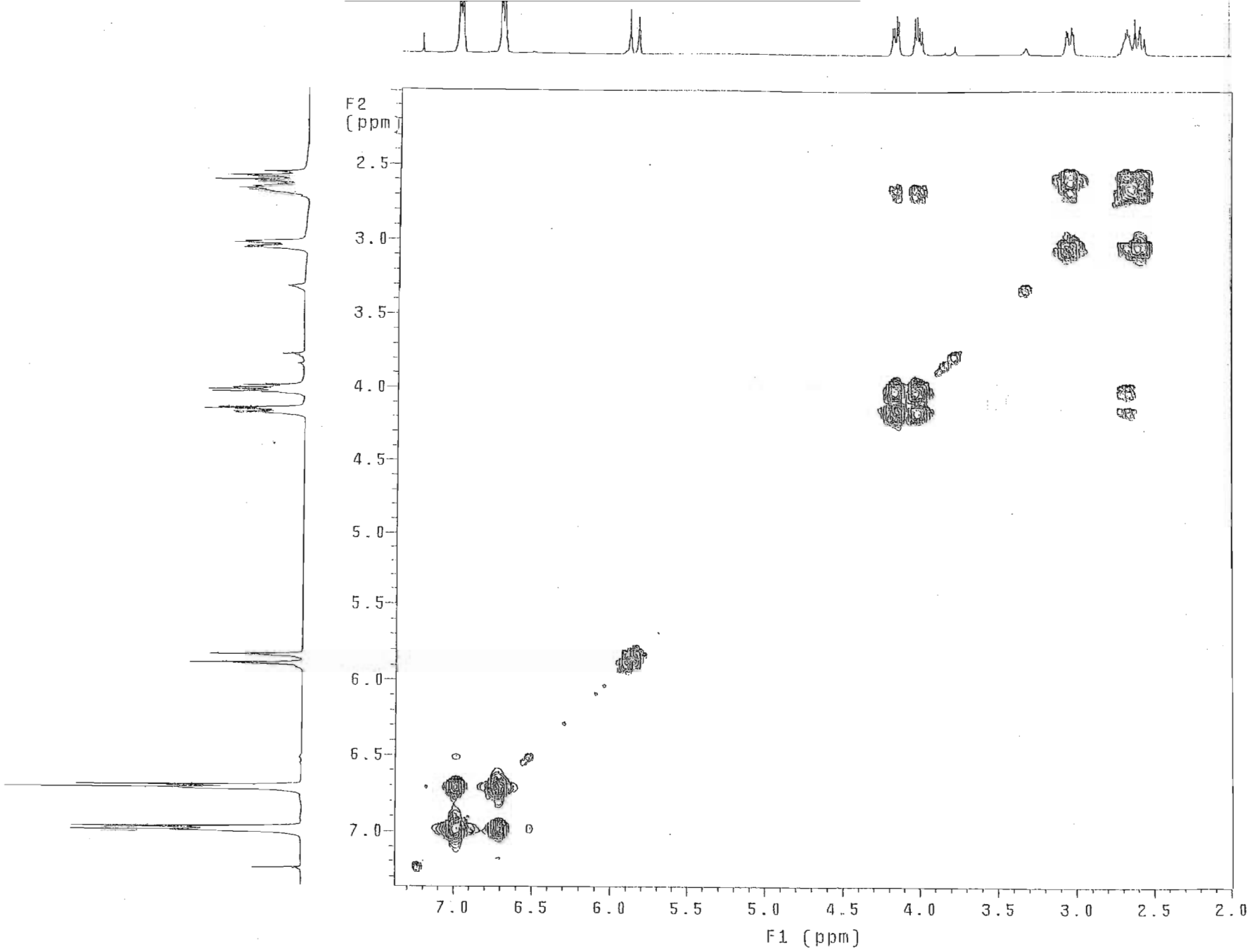
SPECTRUM 16.b: ^{13}C NMR spectrum of compound XVI ($CDCl_3$)



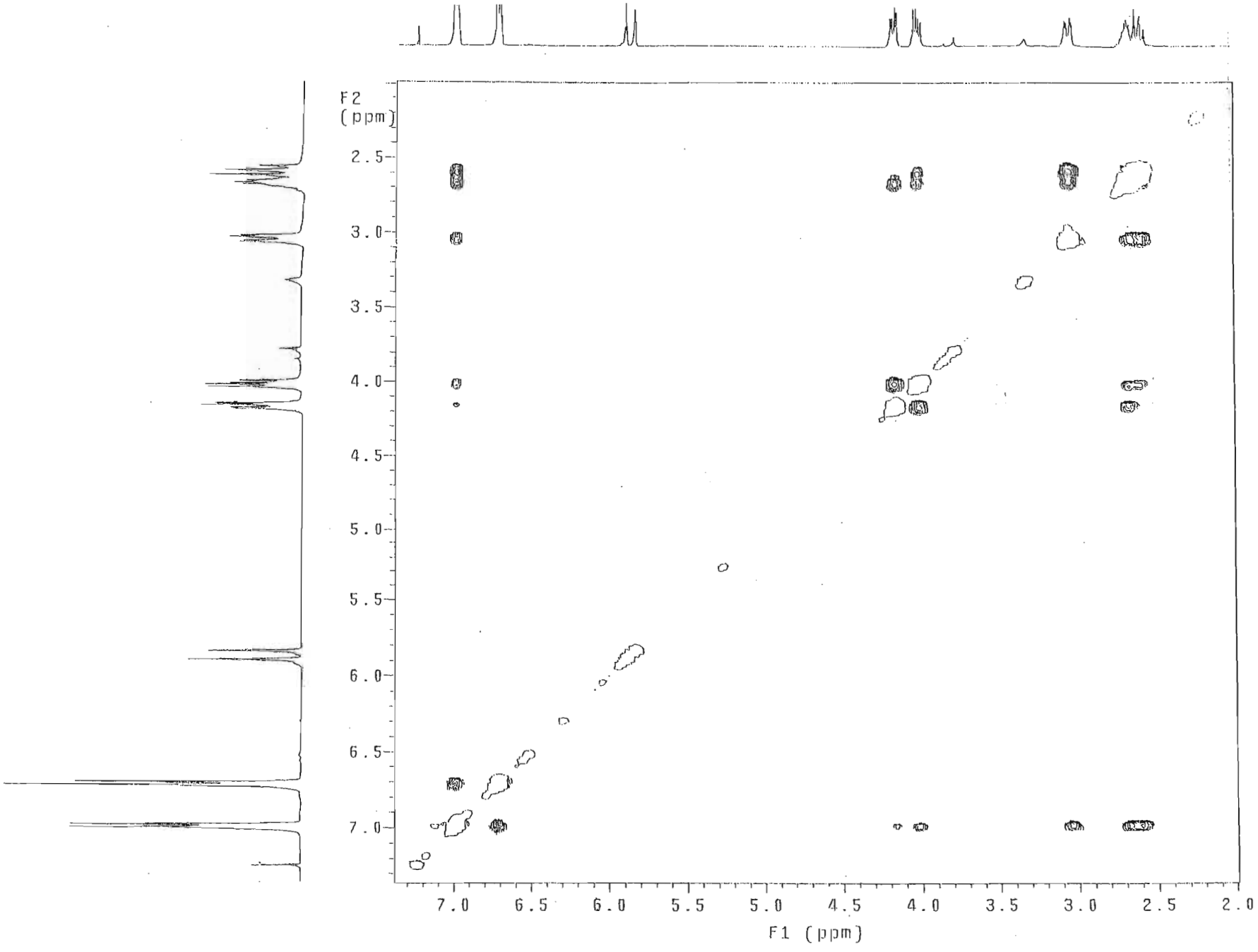
SPECTRUM 16.c: HSQC spectrum of compound XVI ($CDCl_3$)



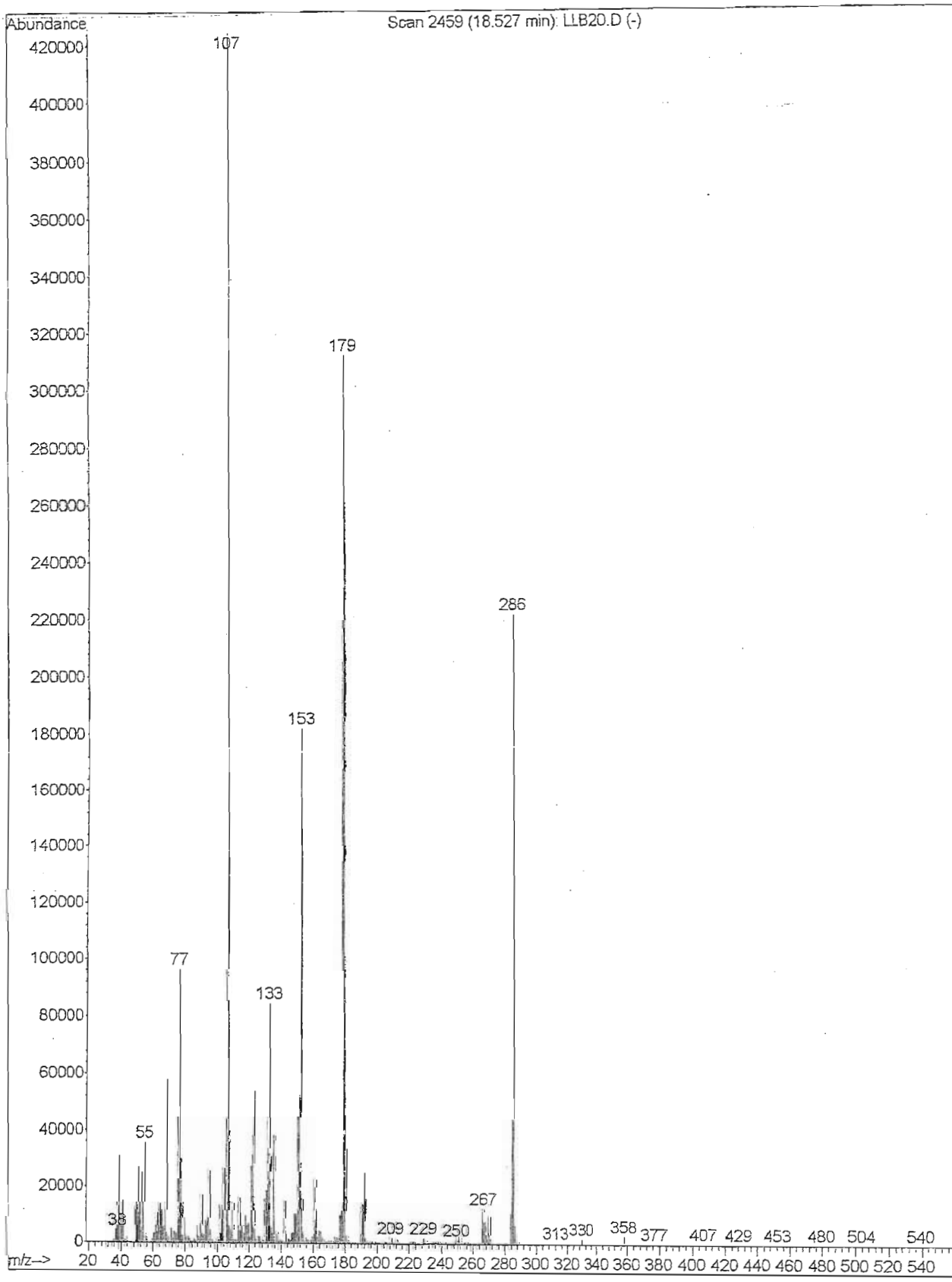
SPECTRUM 16.d: HMBC spectrum of compound XVI ($CDCl_3$)



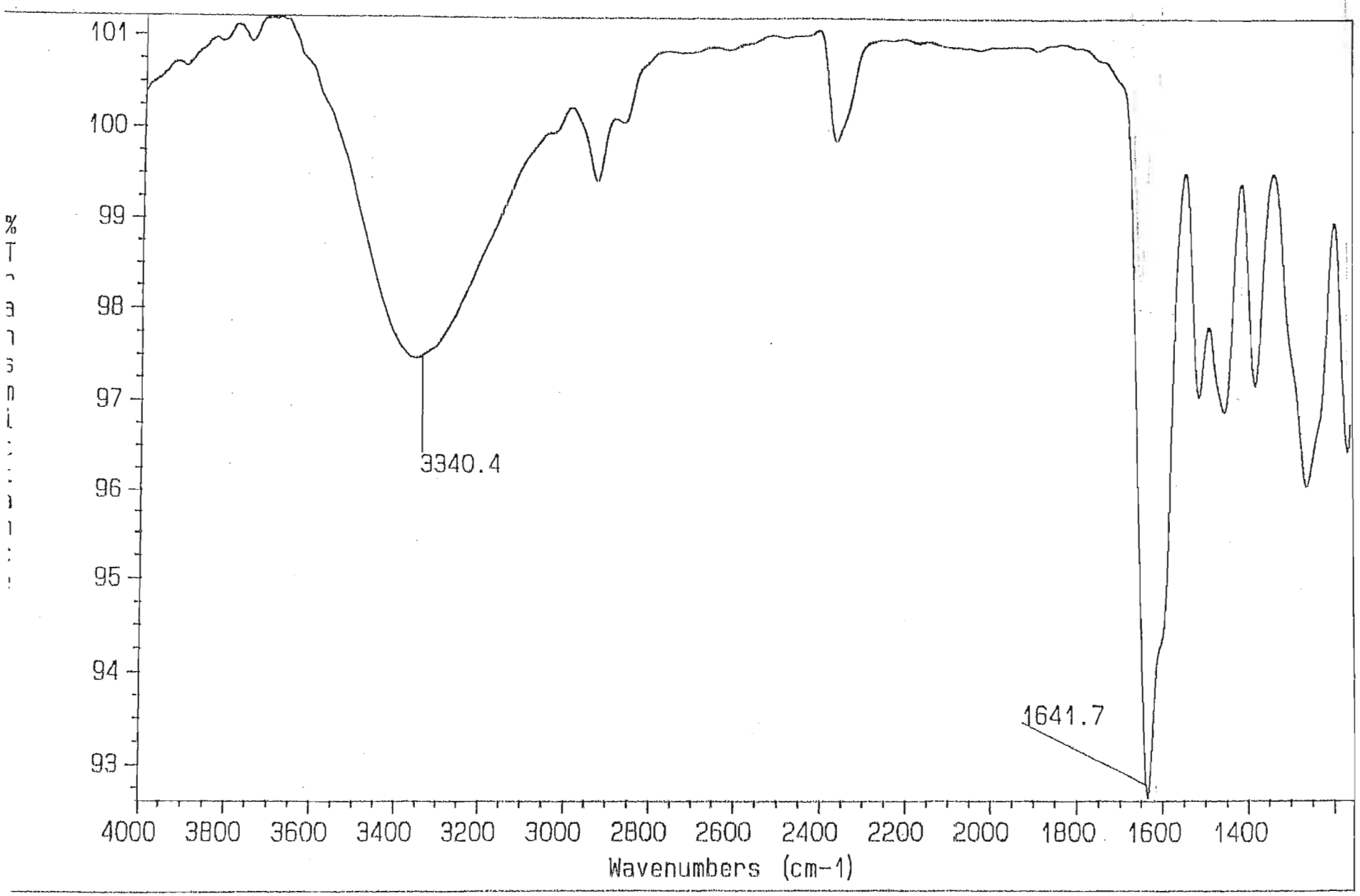
SPECTRUM 16.e: COSY spectrum of compound XVI ($CDCl_3$)



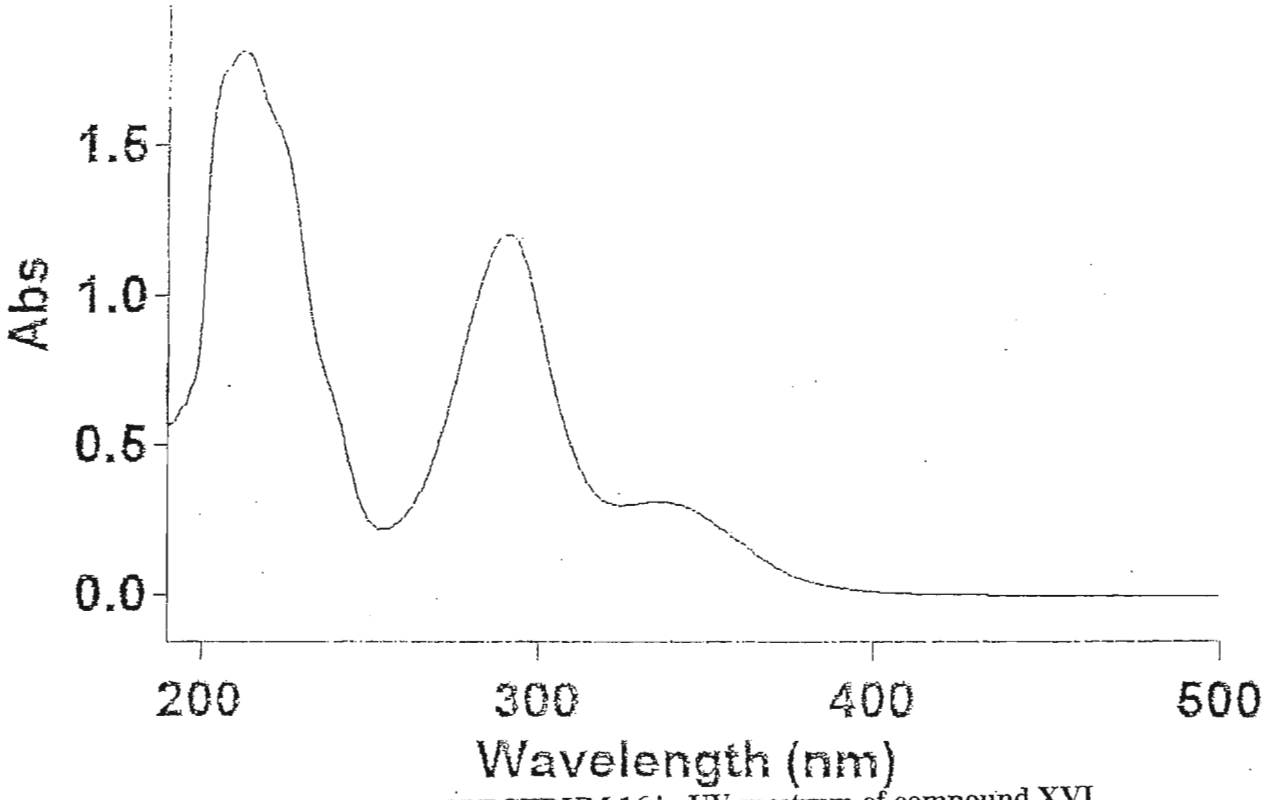
SPECTRUM 16.f: NOESY spectrum of compound XVI ($CDCl_3$)


Abundance
Scan 2459 (18.527 min): LLB20.D (-)
420000
400000
380000
360000
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
107
179
286
153
77
133
55
38
267
209 229 250
313 330 358 377 407 429 453 480 504 540
m/z-->
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540

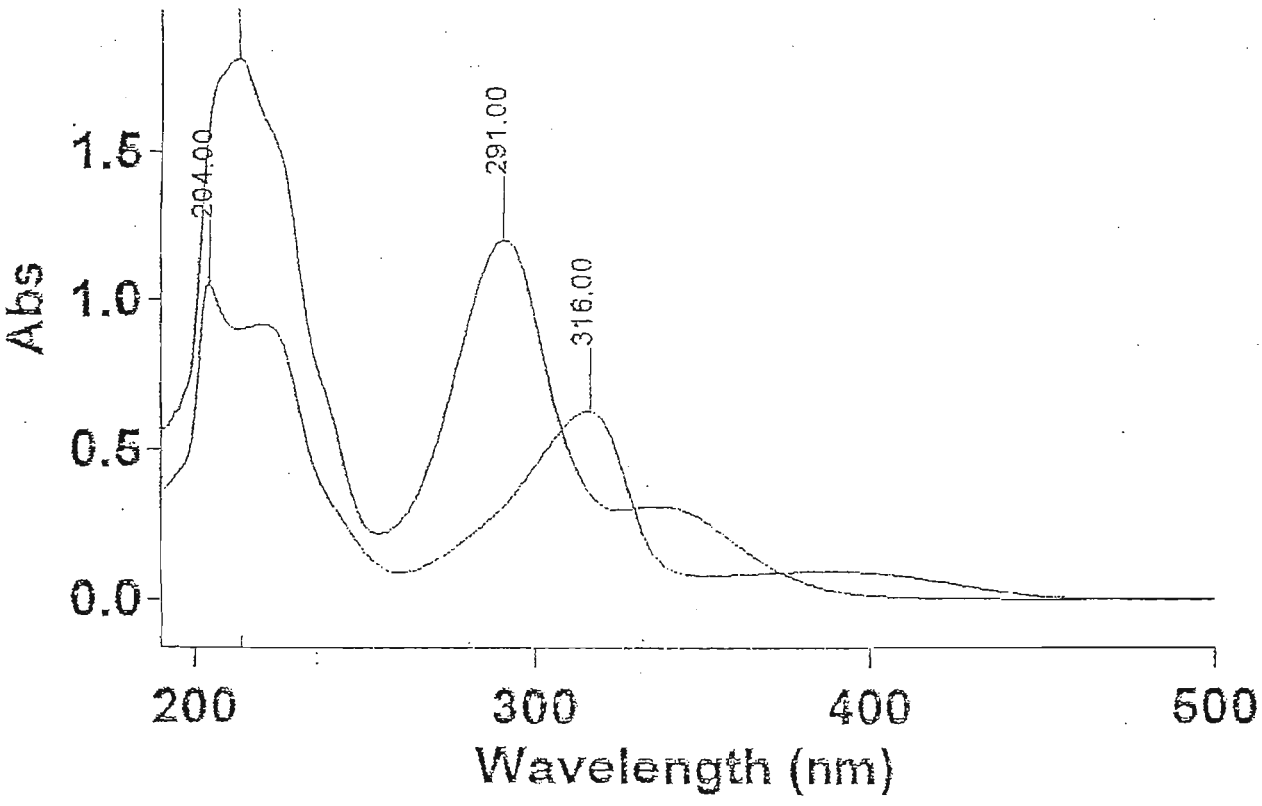
SPECTRUM 16



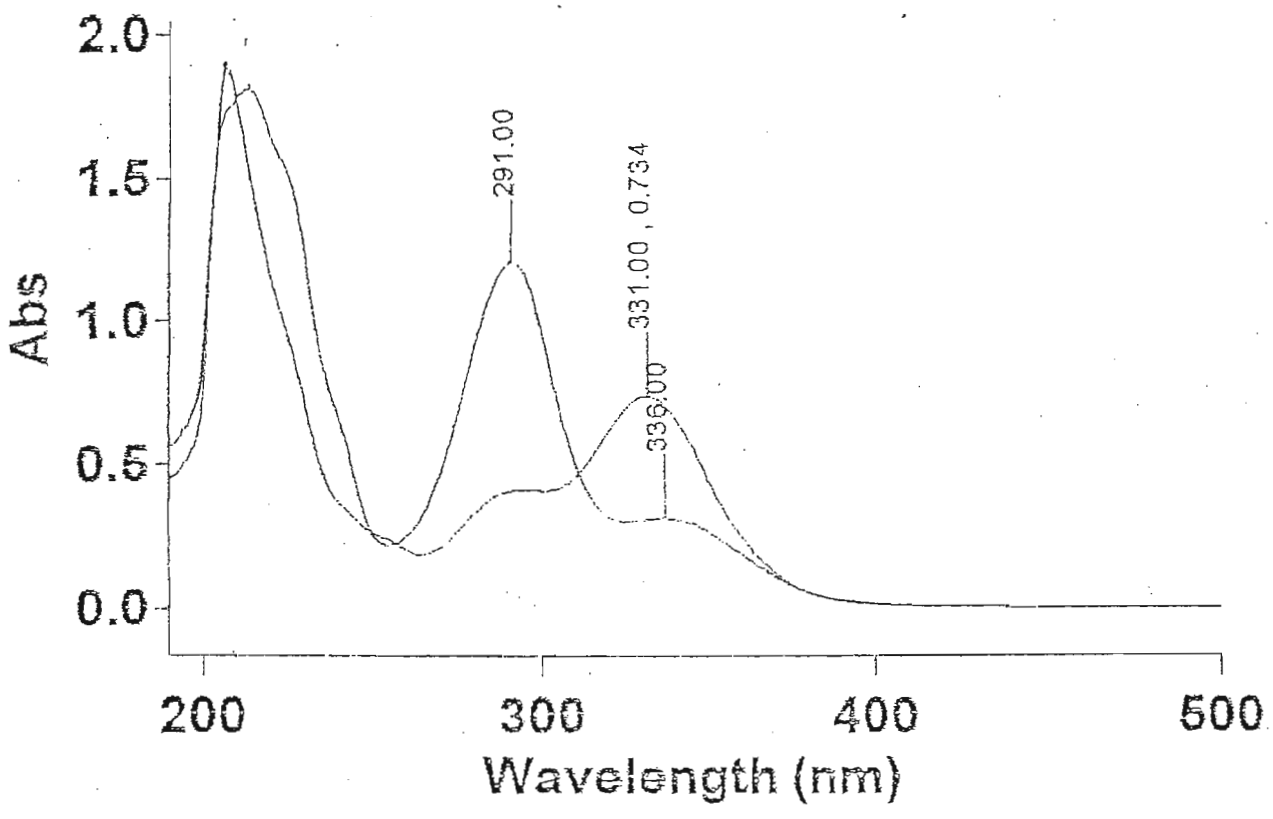
SPECTRUM 16.h: Infrared spectrum of compound XVI



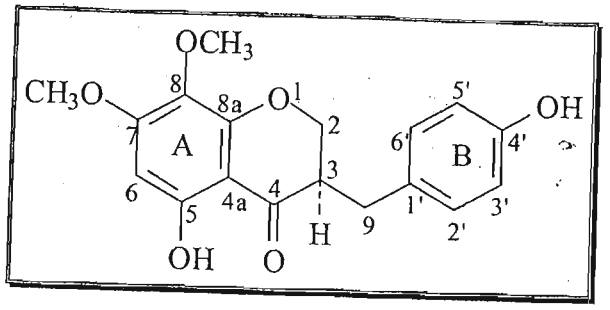
SPECTRUM 16.i.: UV spectrum of compound XVI



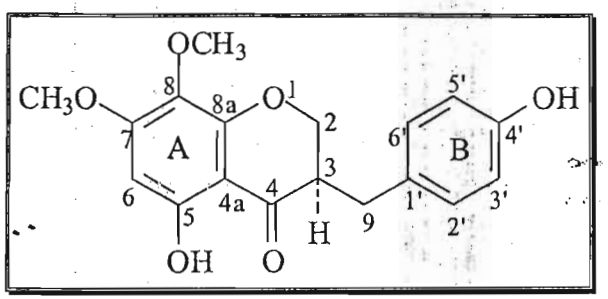
SPECTRUM 16.j.: UV spectrum of compound XVI with NaoAc

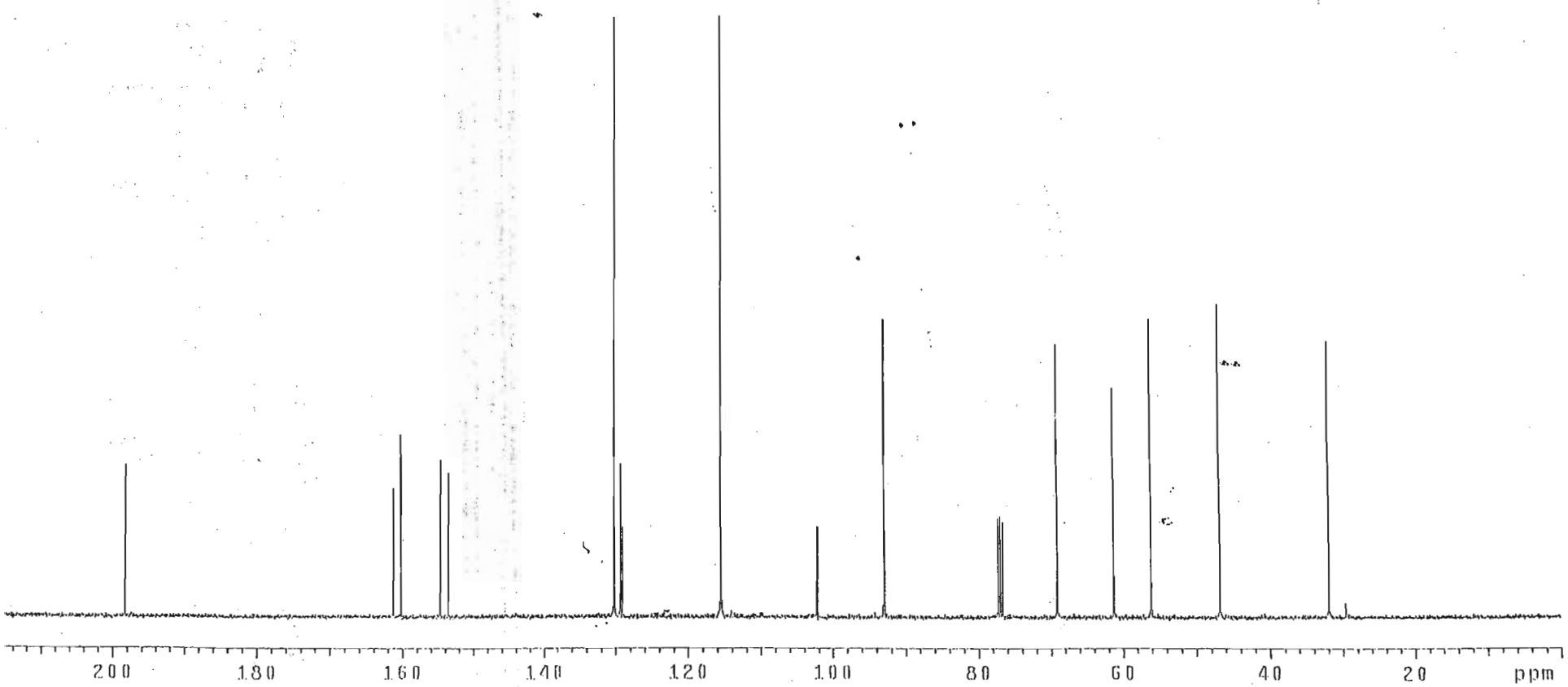


SPECTRUM 16.k.: UV spectrum of compound XVI with $AlCl_3$

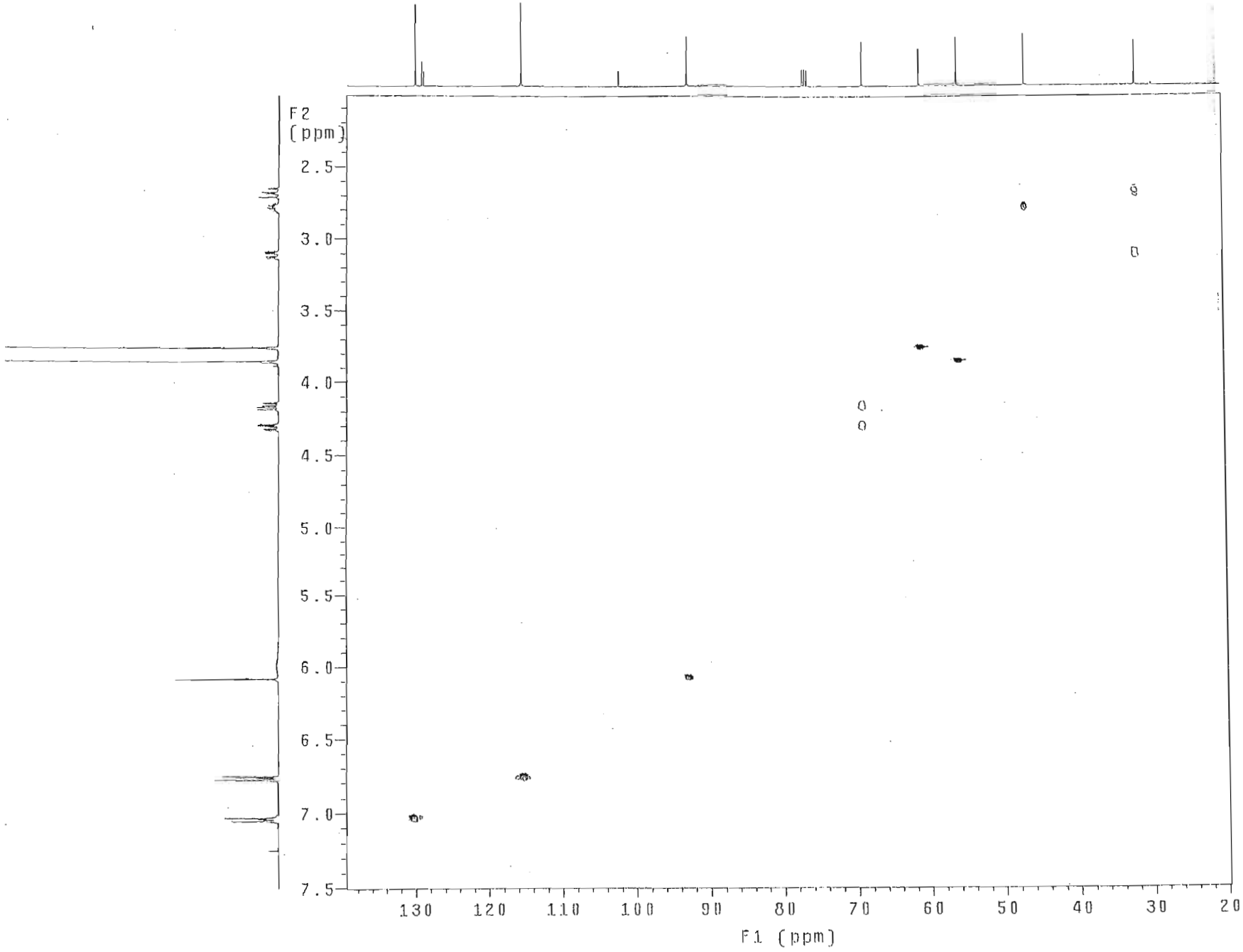


SPECTRUM 17.a: ^{1}H NMR spectrum of compound XVII ($CDCl_3$)

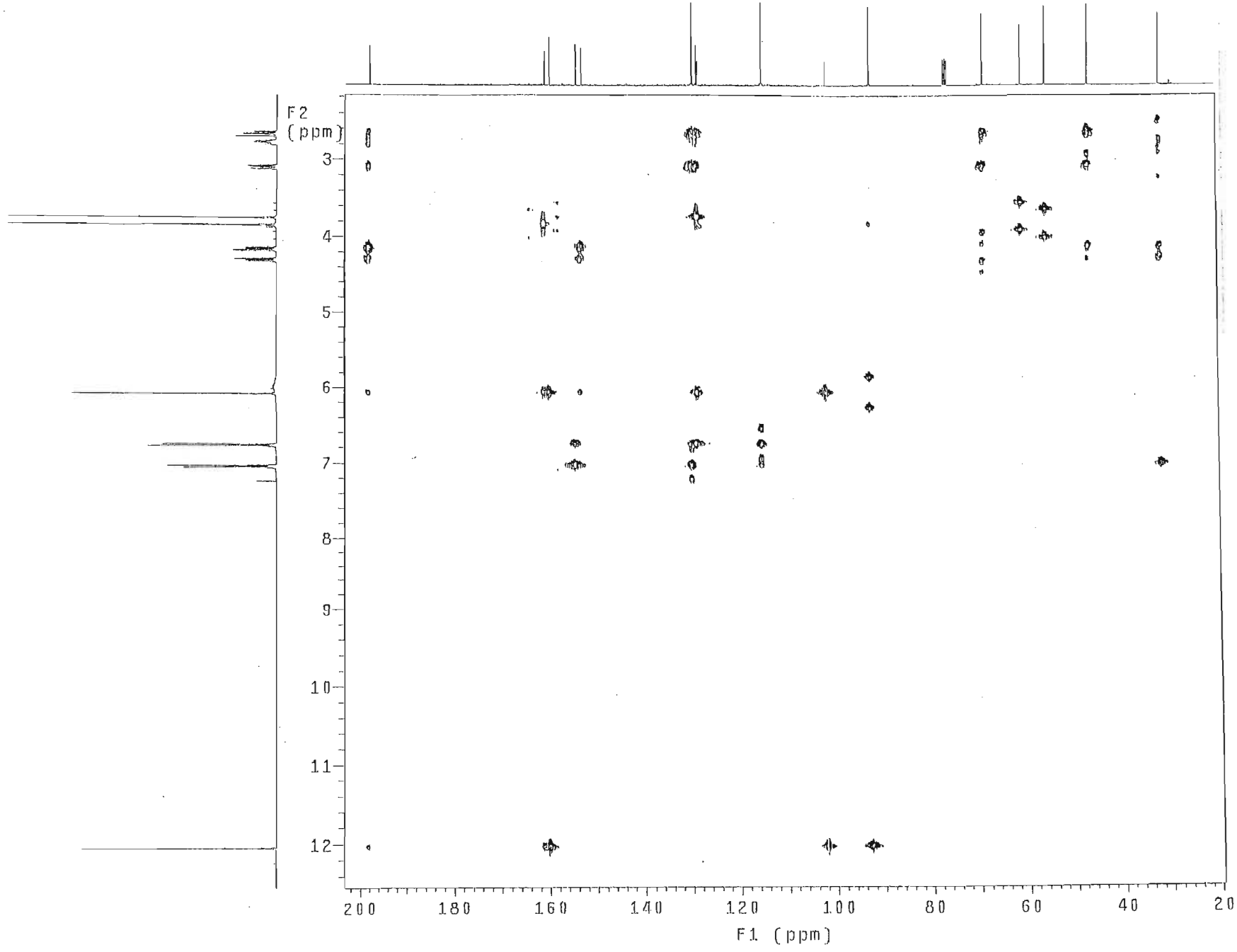




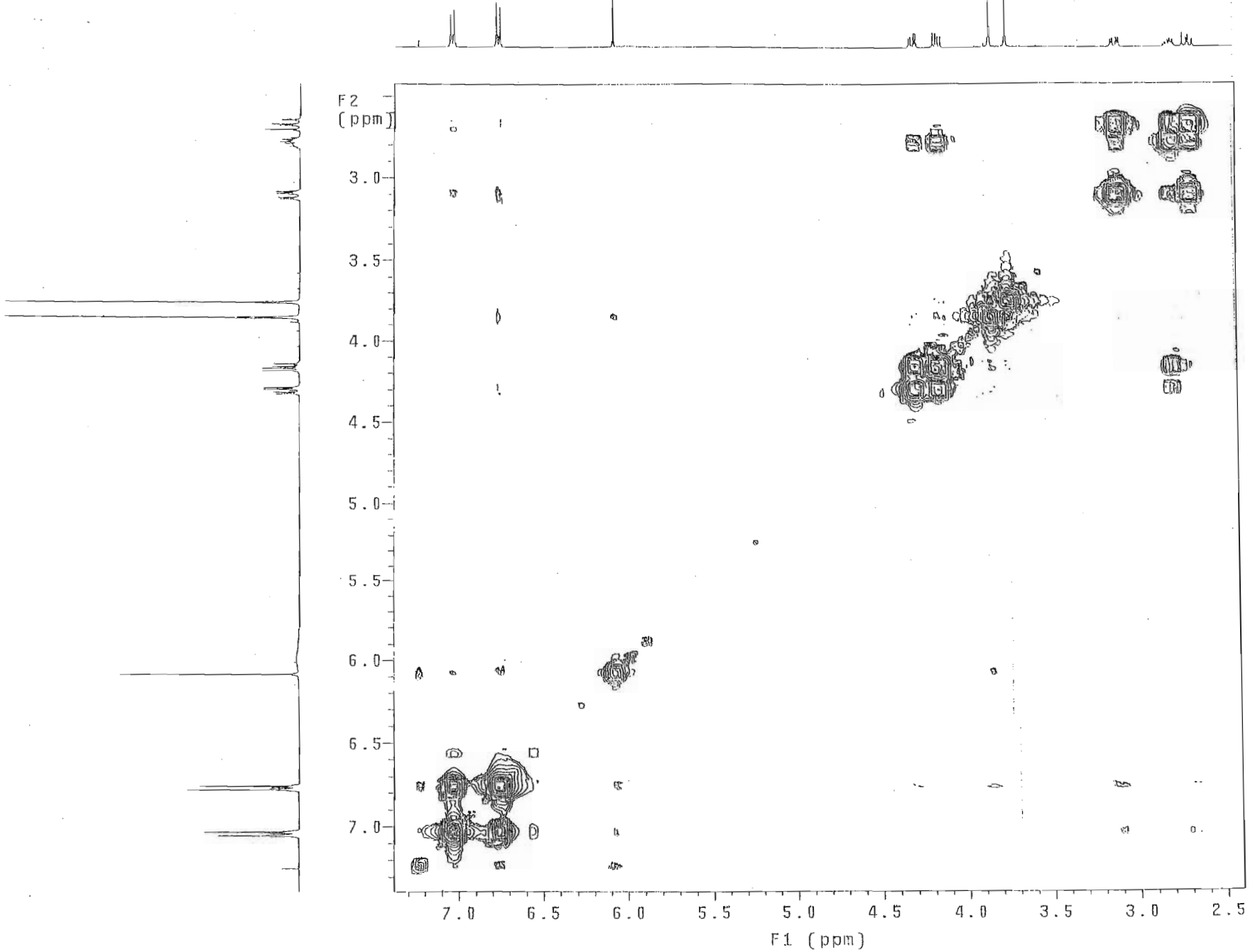
SPECTRUM 17.b: ^{13}C NMR spectrum of compound XVII ($CDCl_3$)



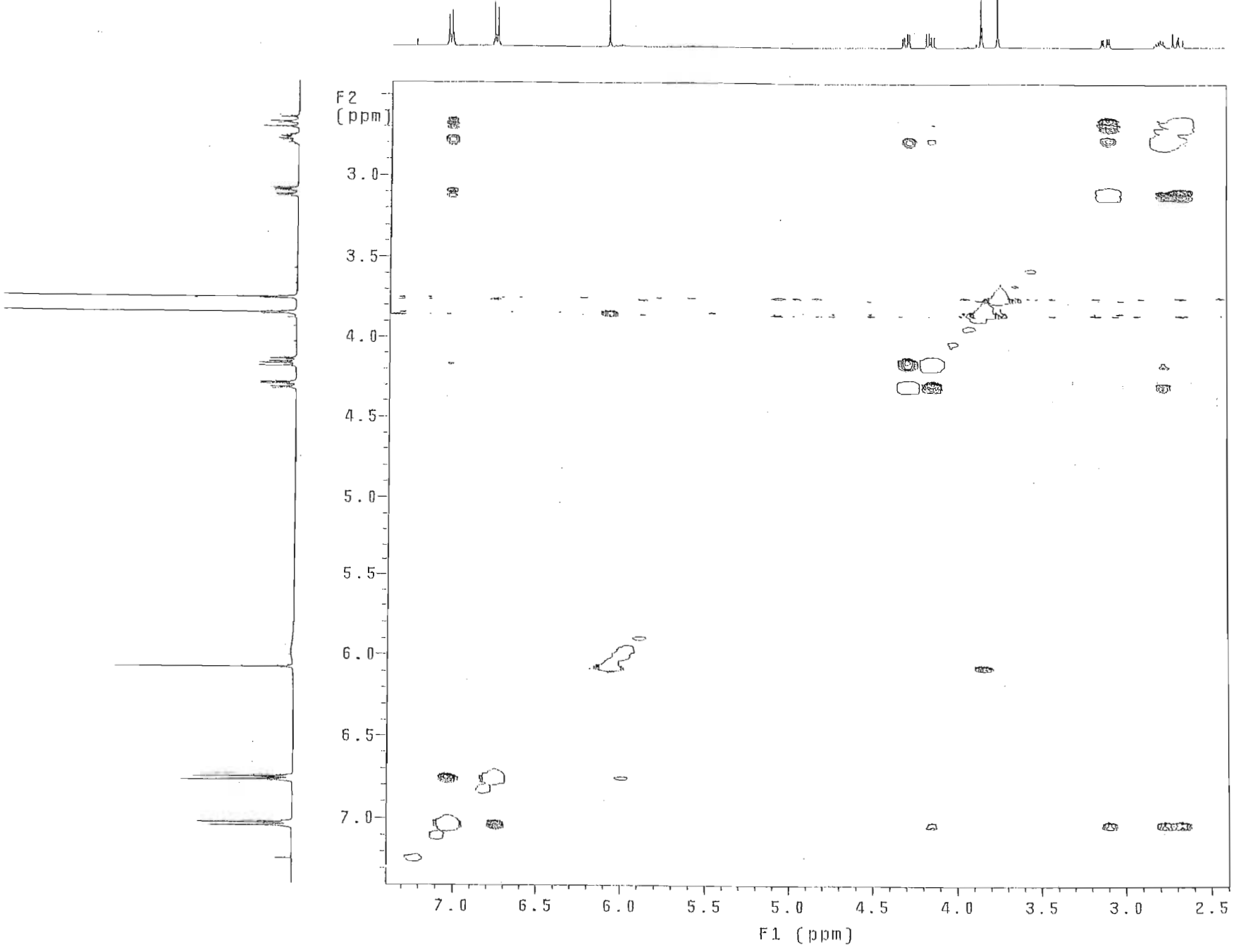
SPECTRUM 17.c: HSQC spectrum of compound XVII ($CDCl_3$)



SPECTRUM 17.d: HMBC spectrum of compound XVII ($CDCl_3$)



SPECTRUM 17.e: COSY spectrum of compound XVII ($CDCl_3$)



SPECTRUM 17.f: NOESY spectrum of compound XVII ($CDCl_3$)

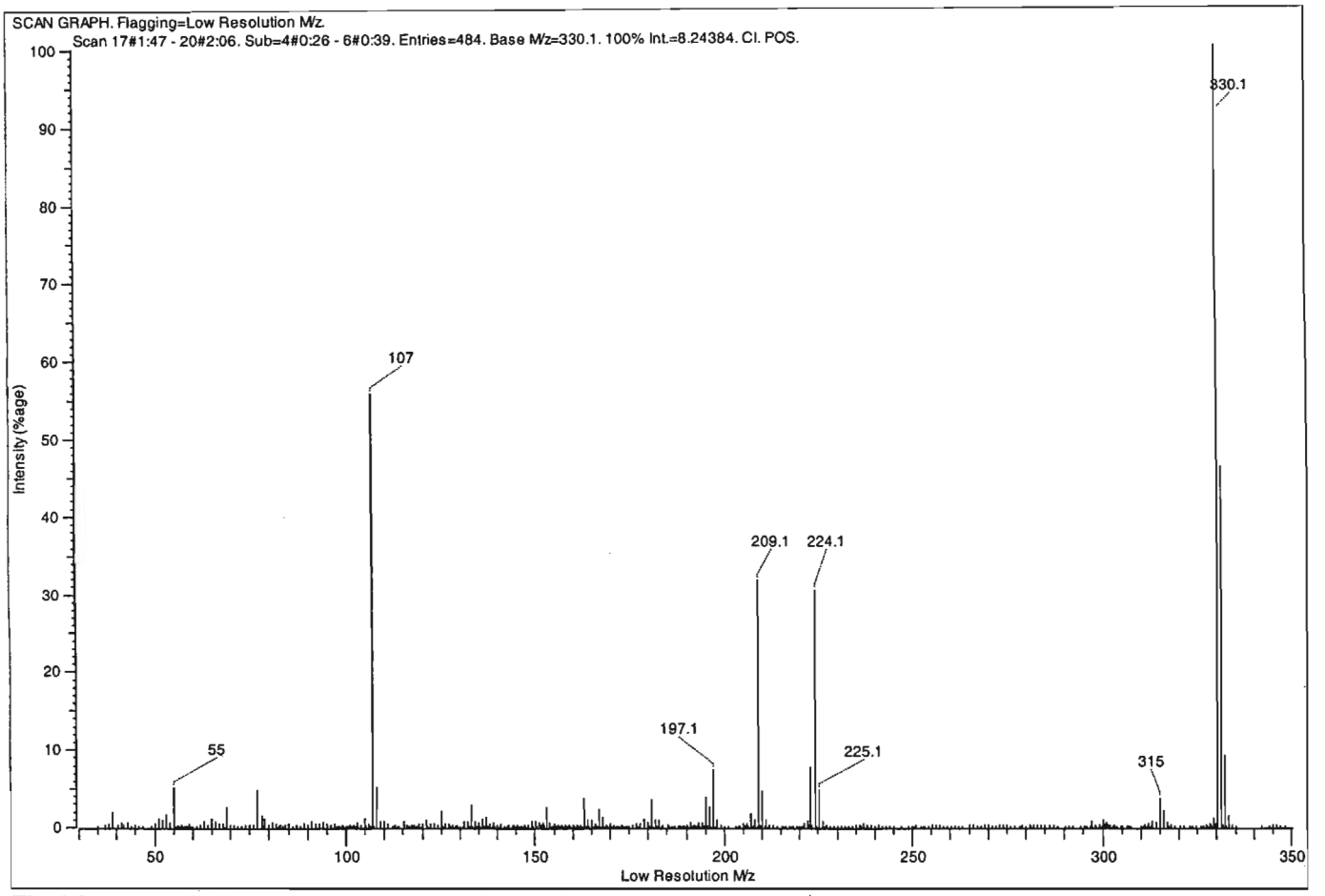


Fig 1 Low resolution mass spectrum of sample.

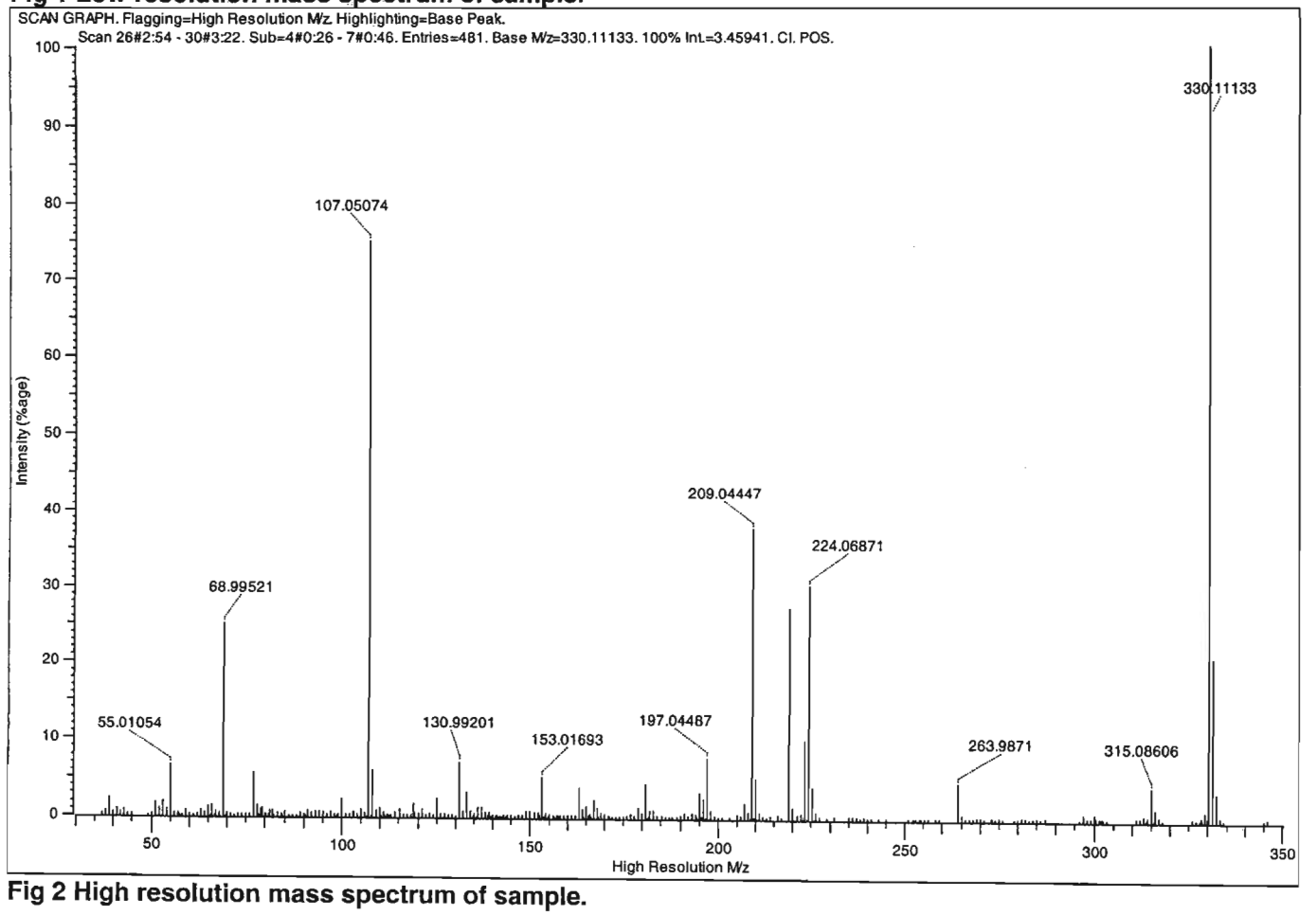
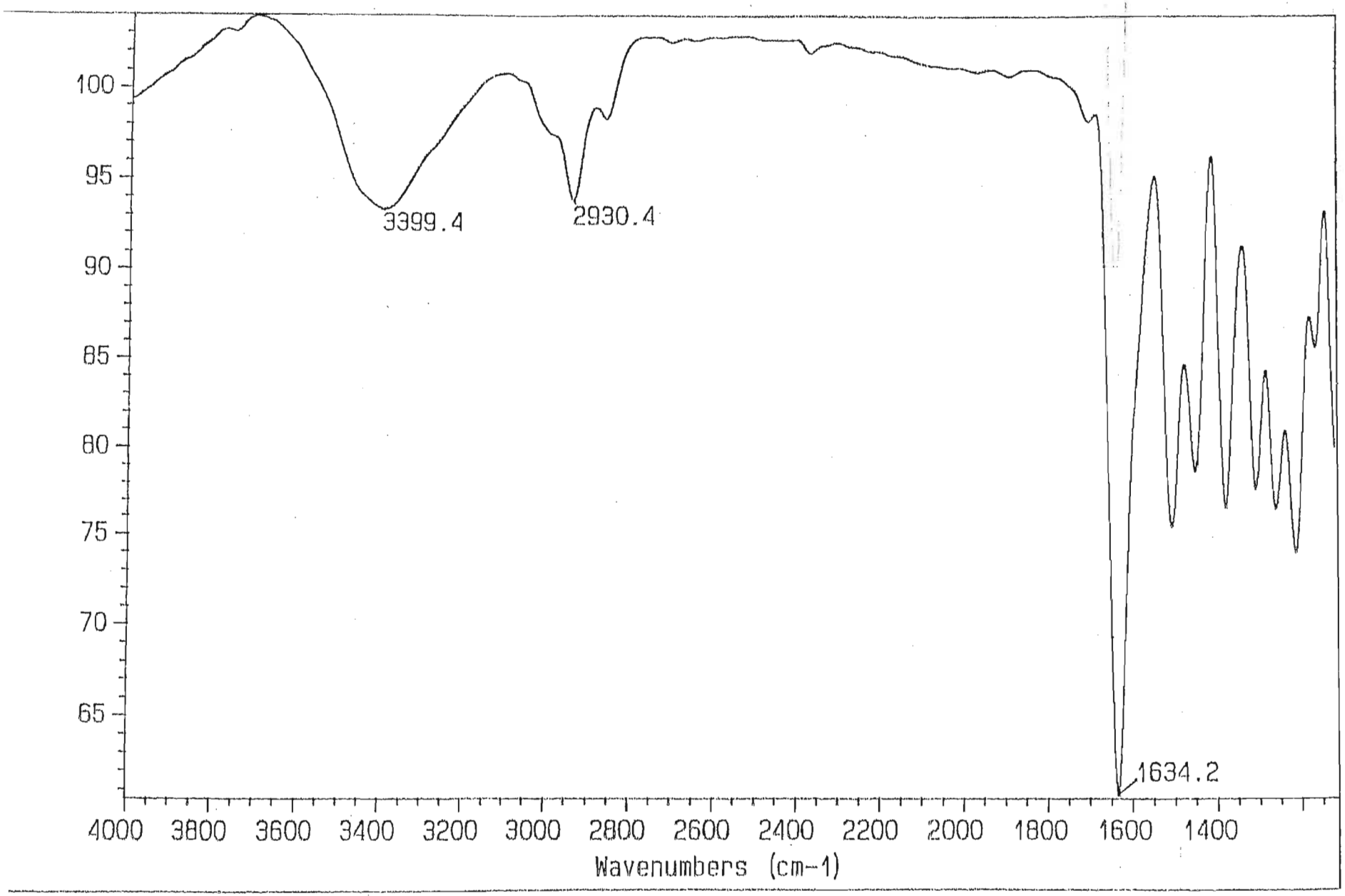
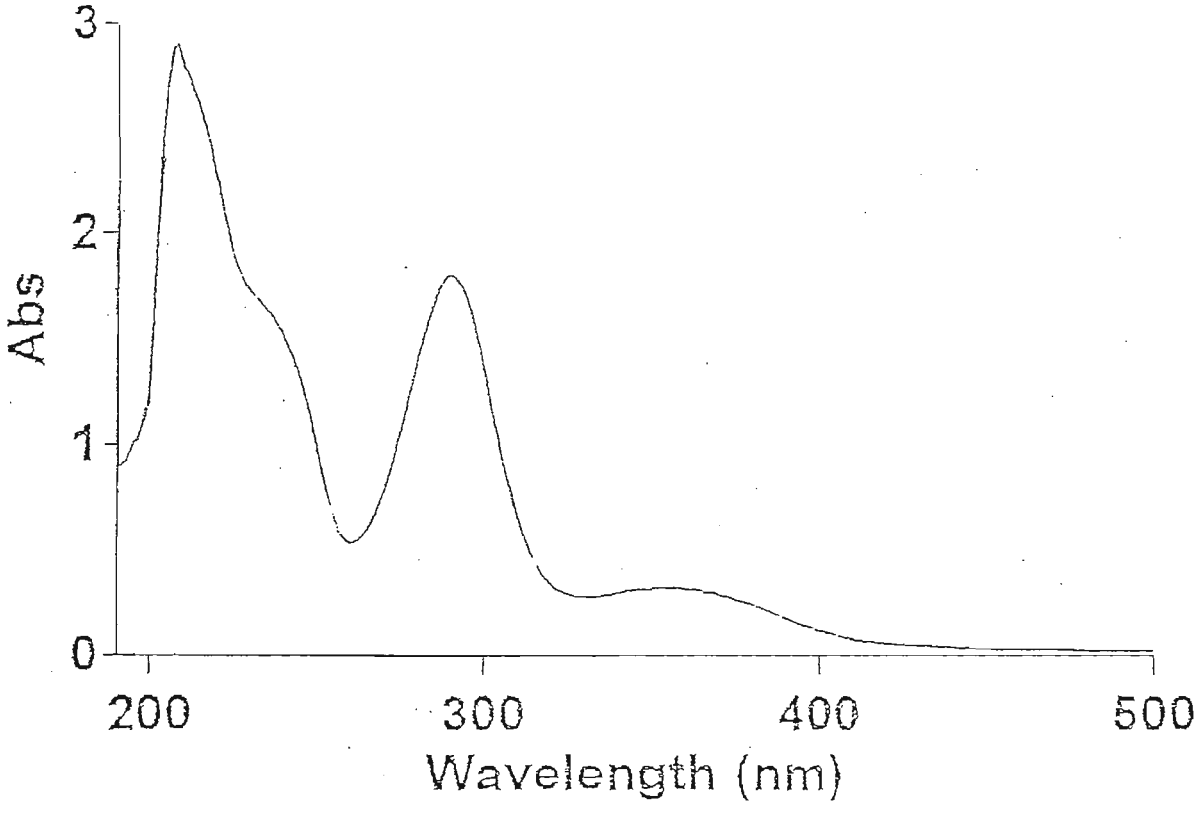


Fig 2 High resolution mass spectrum of sample.

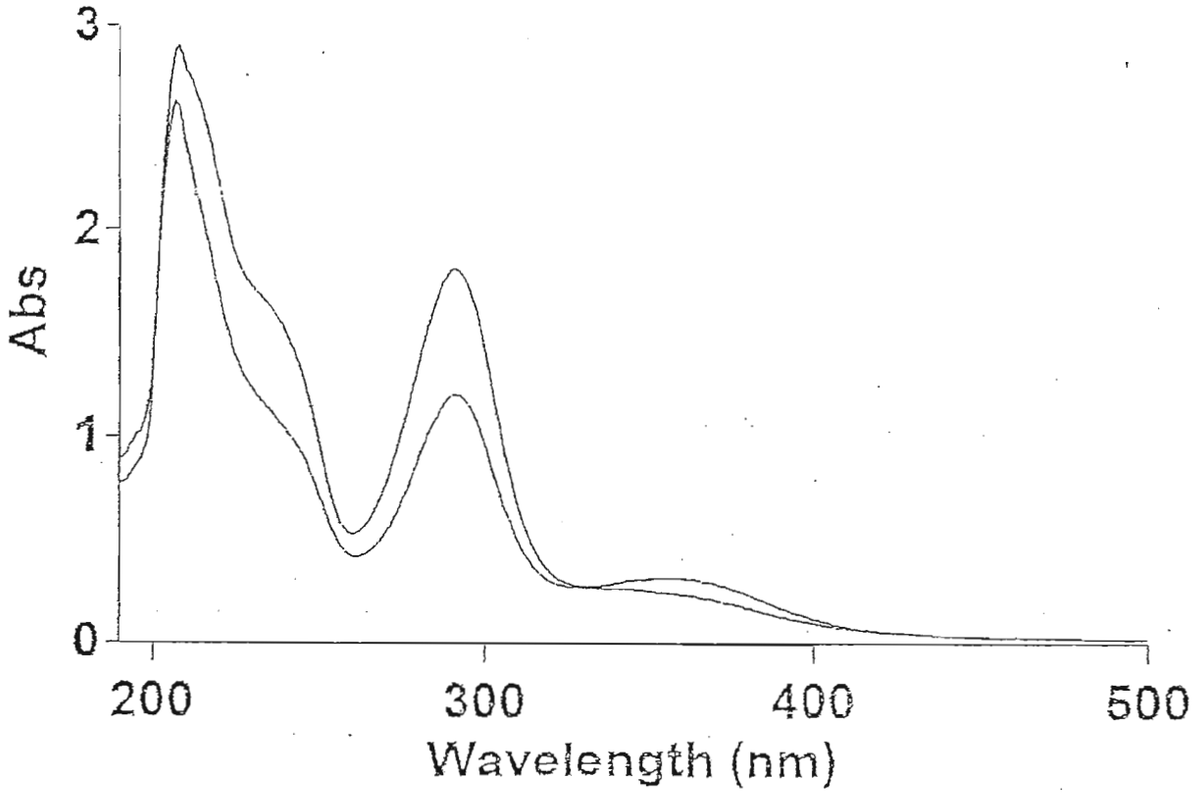
Spectrum 17.g: Mass spectra of compound XVII



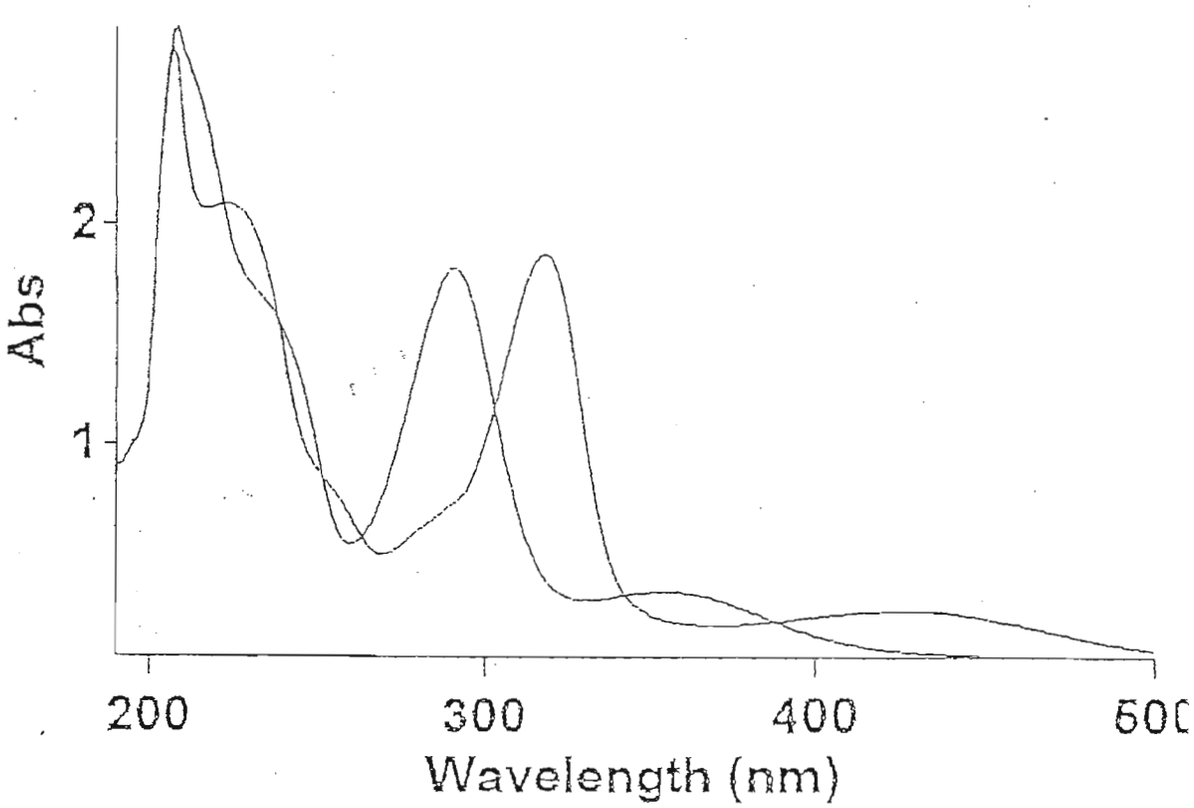
SPECTRUM 17.h: Infrared spectrum of compound XVII



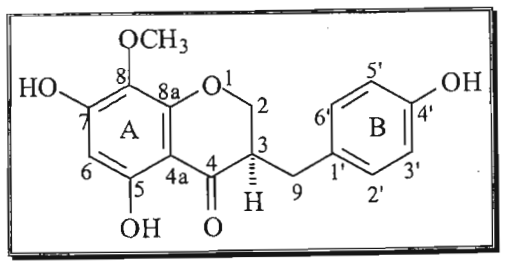
SPECTRUM 17.i.: UV spectrum of compound XVII



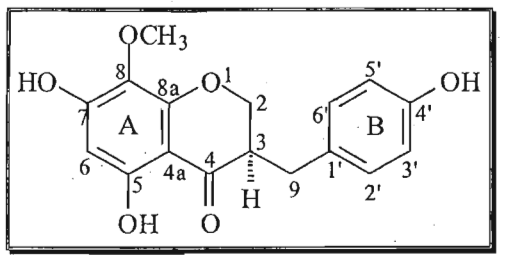
SPECTRUM 17.j.: UV spectrum of compound XVII with NaoAc

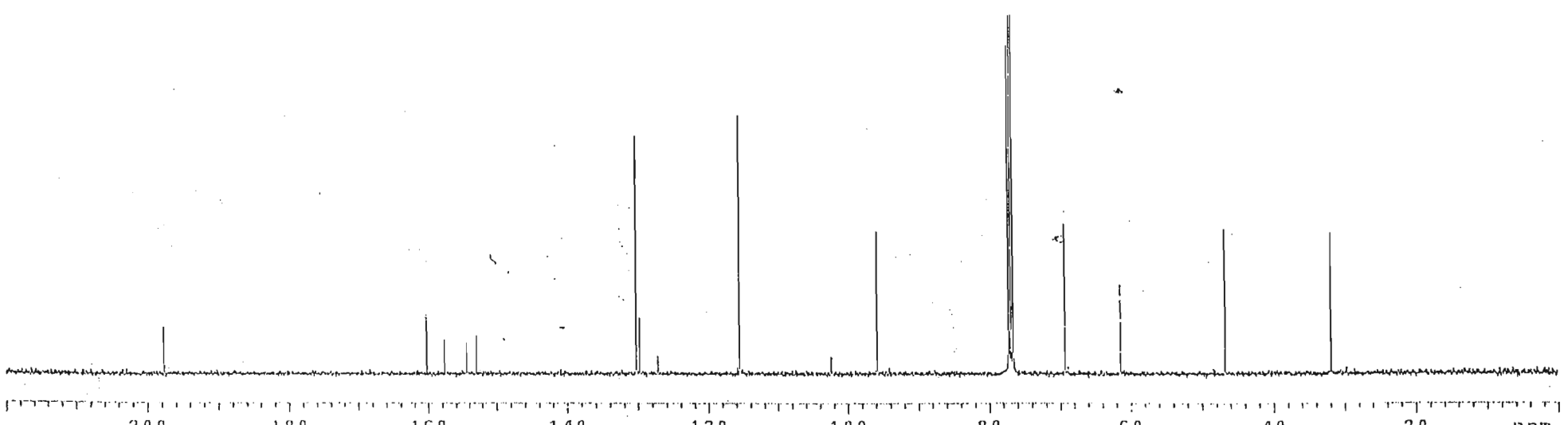


SPECTRUM 17.k.: UV spectrum of compound XVII with $AlCl_3$

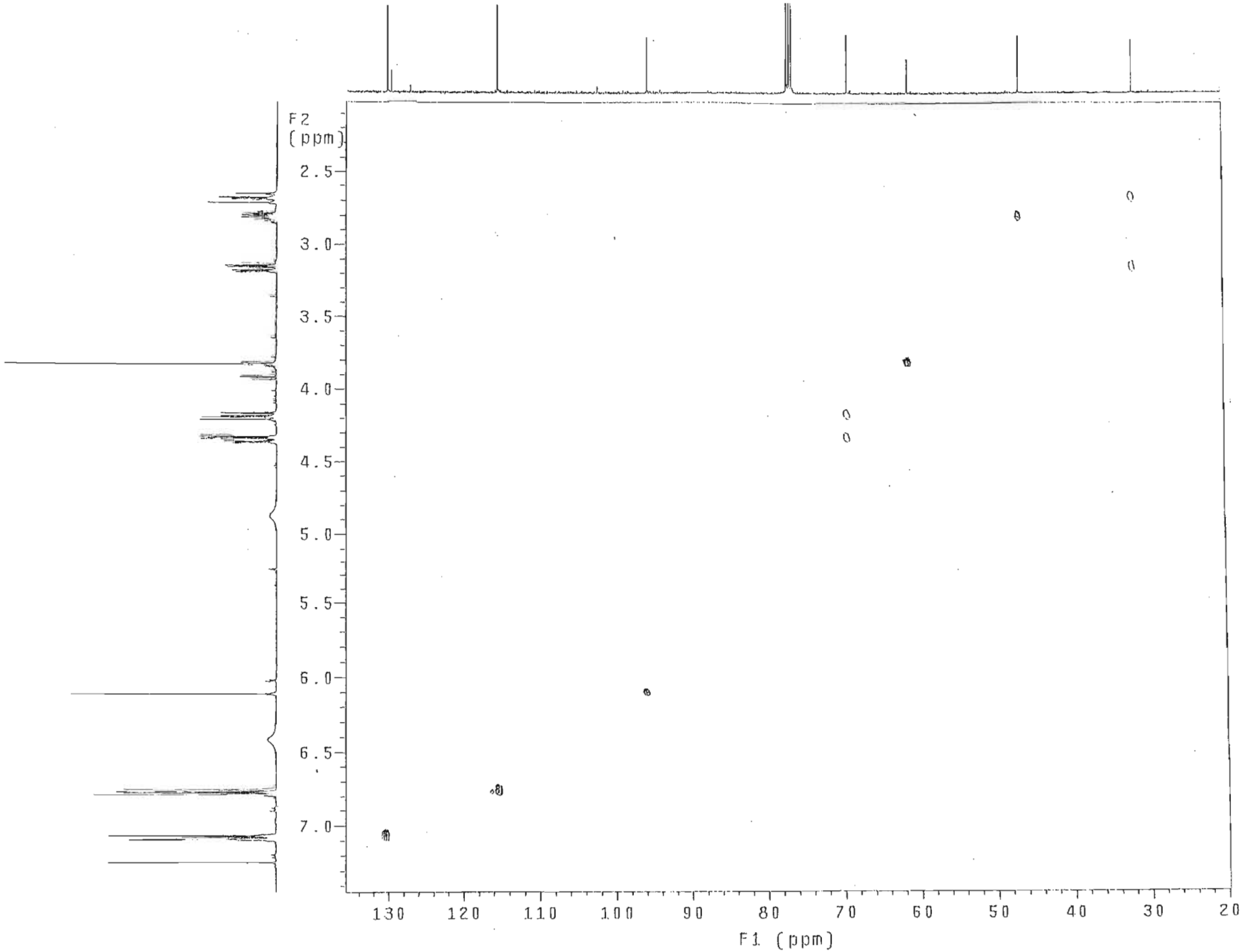


SPECTRUM 18.a: ^{1}H NMR spectrum of compound XVIII ($CDCl_3$)

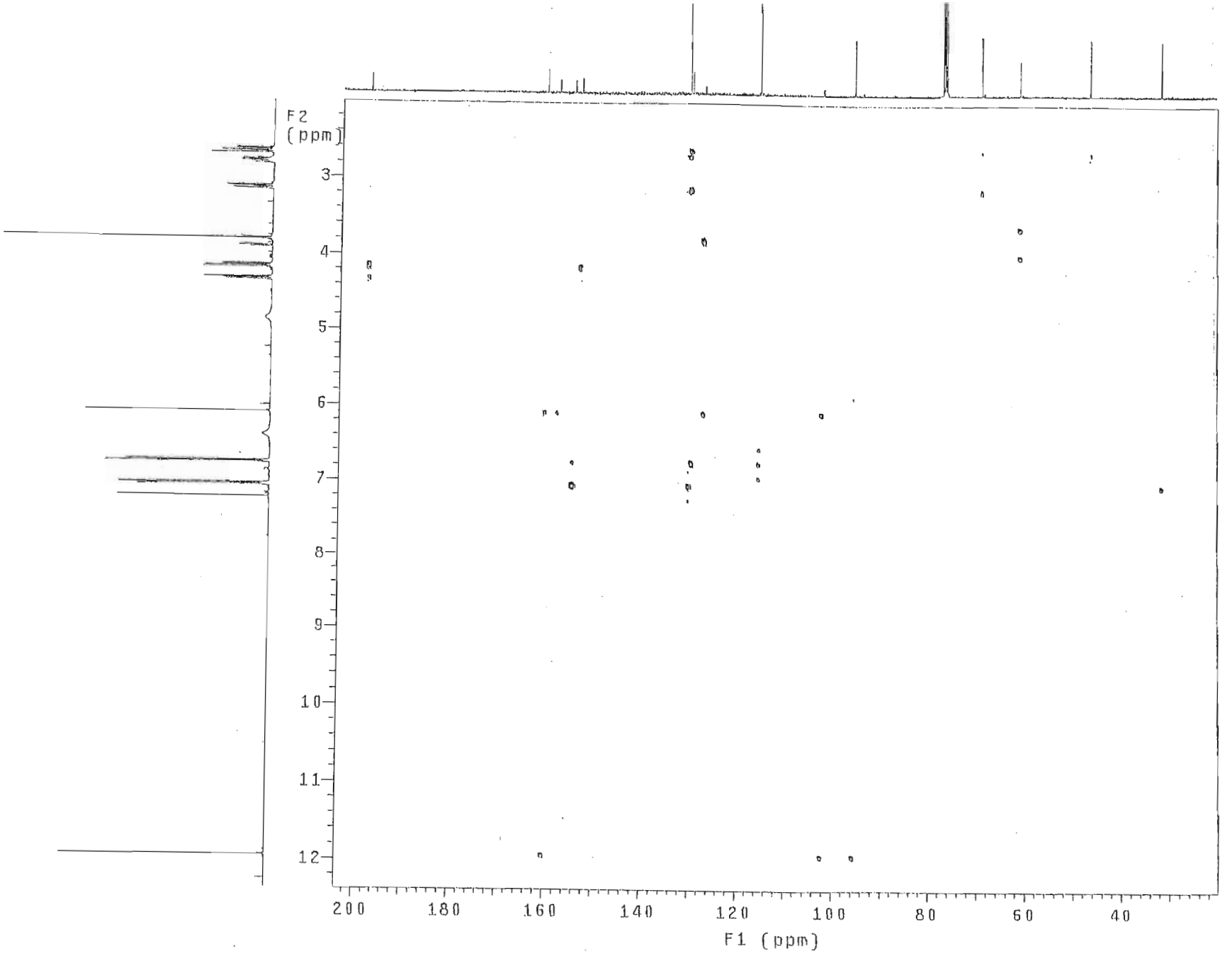




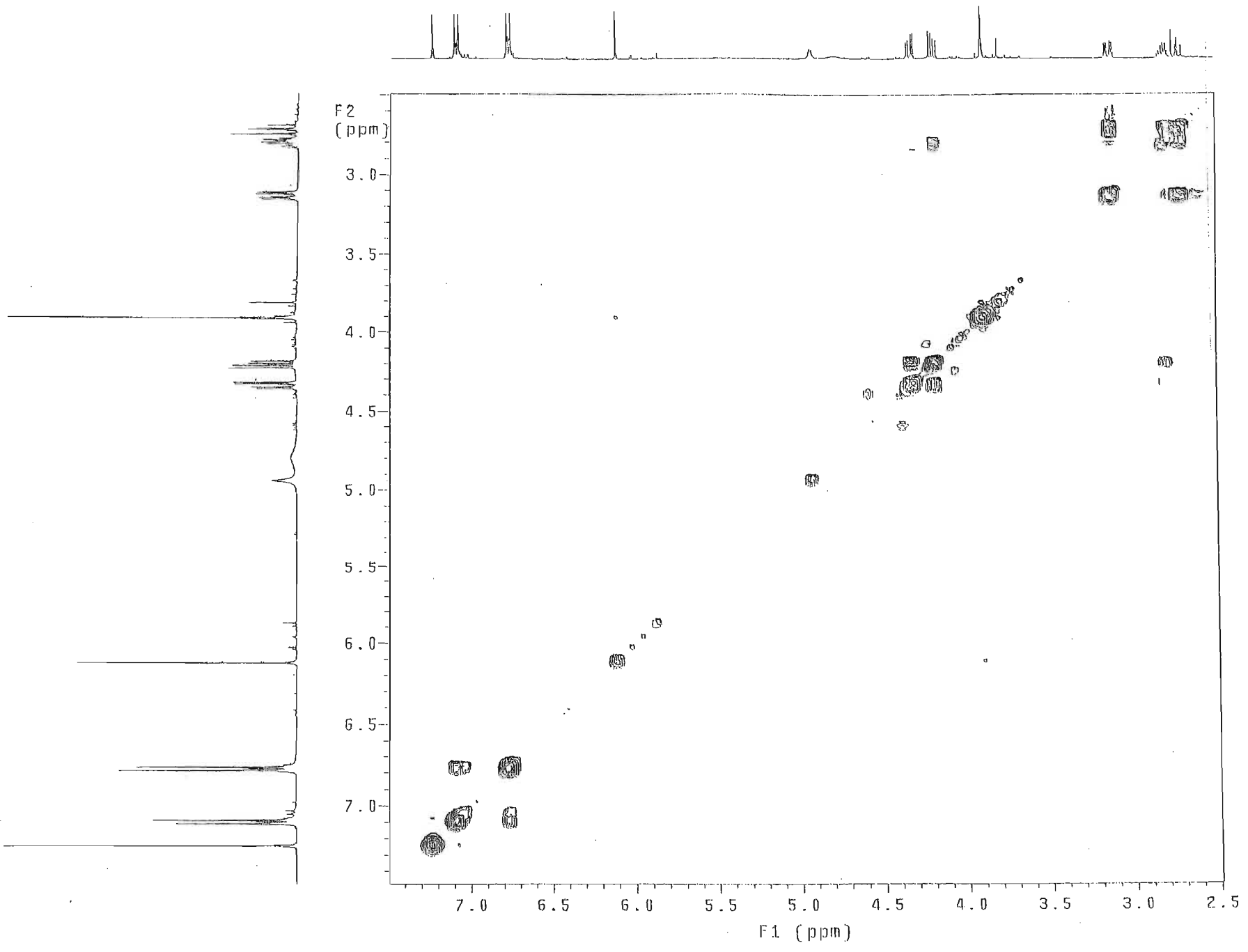
SPECTRUM 18.b: ^{13}C NMR spectrum of compound XVIII ($CDCl_3$)



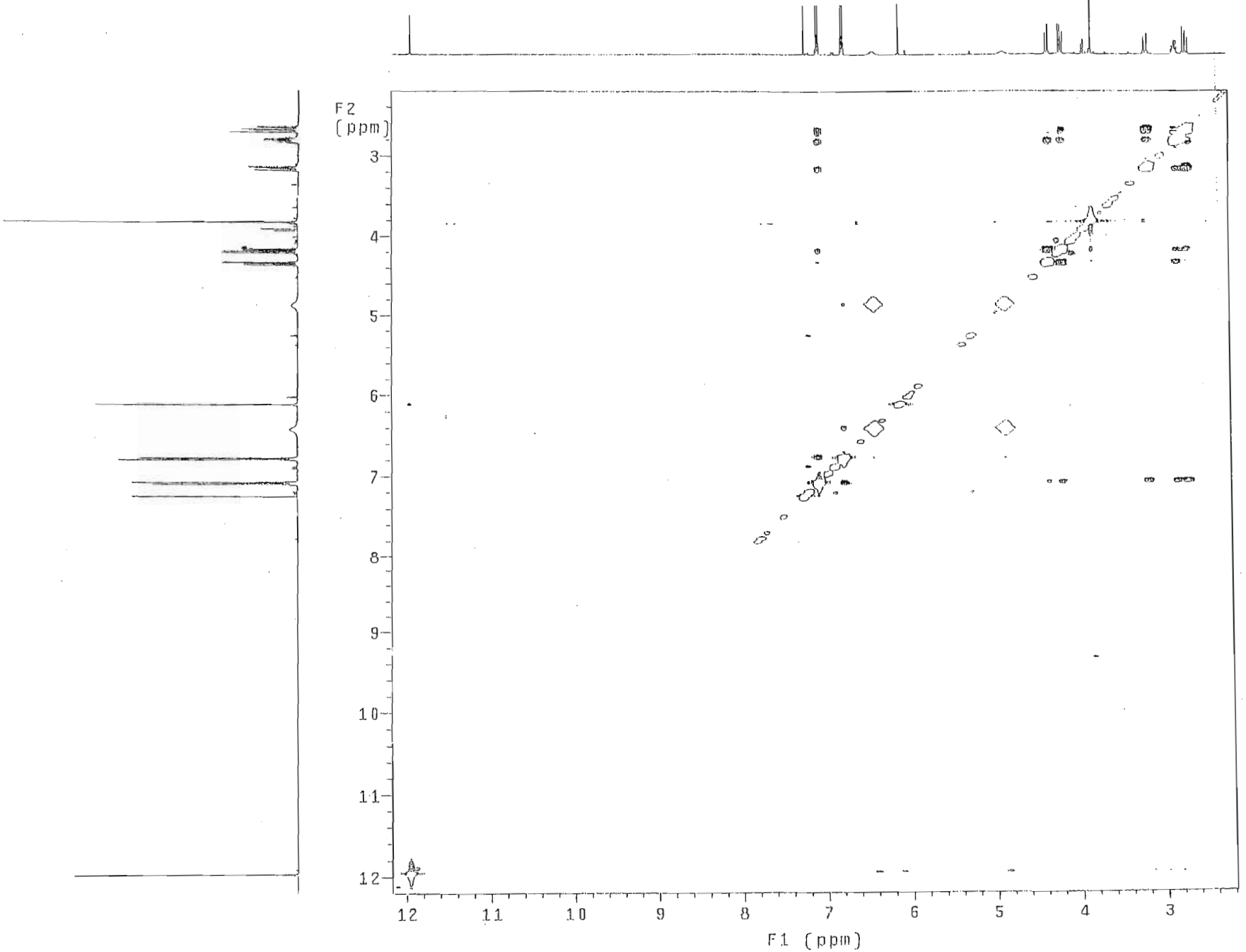
SPECTRUM 18.c: HSQC spectrum of compound XVIII ($CDCl_3$)



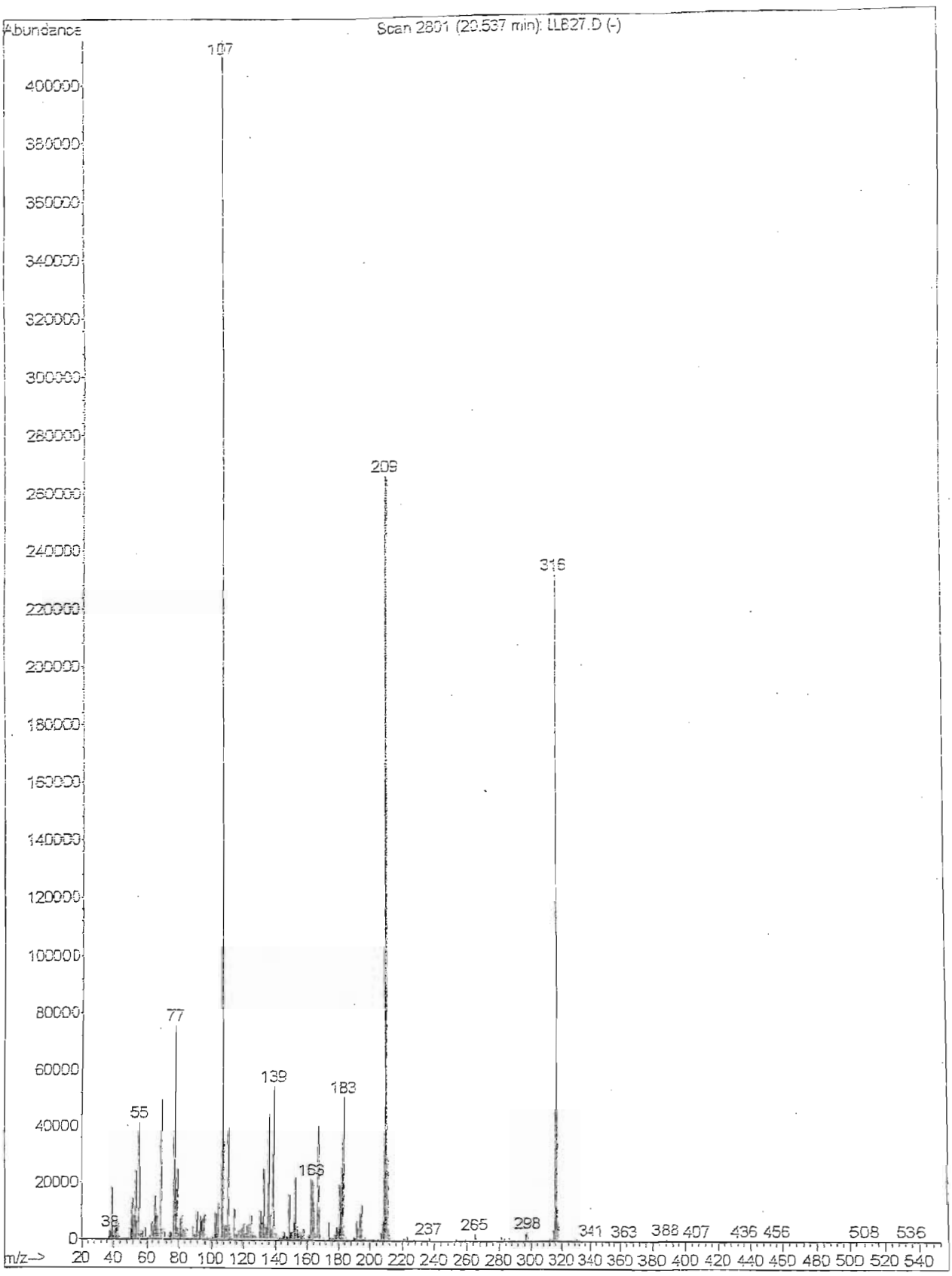
SPECTRUM 18.d: HMBC spectrum of compound XVIII ($CDCl_3$)



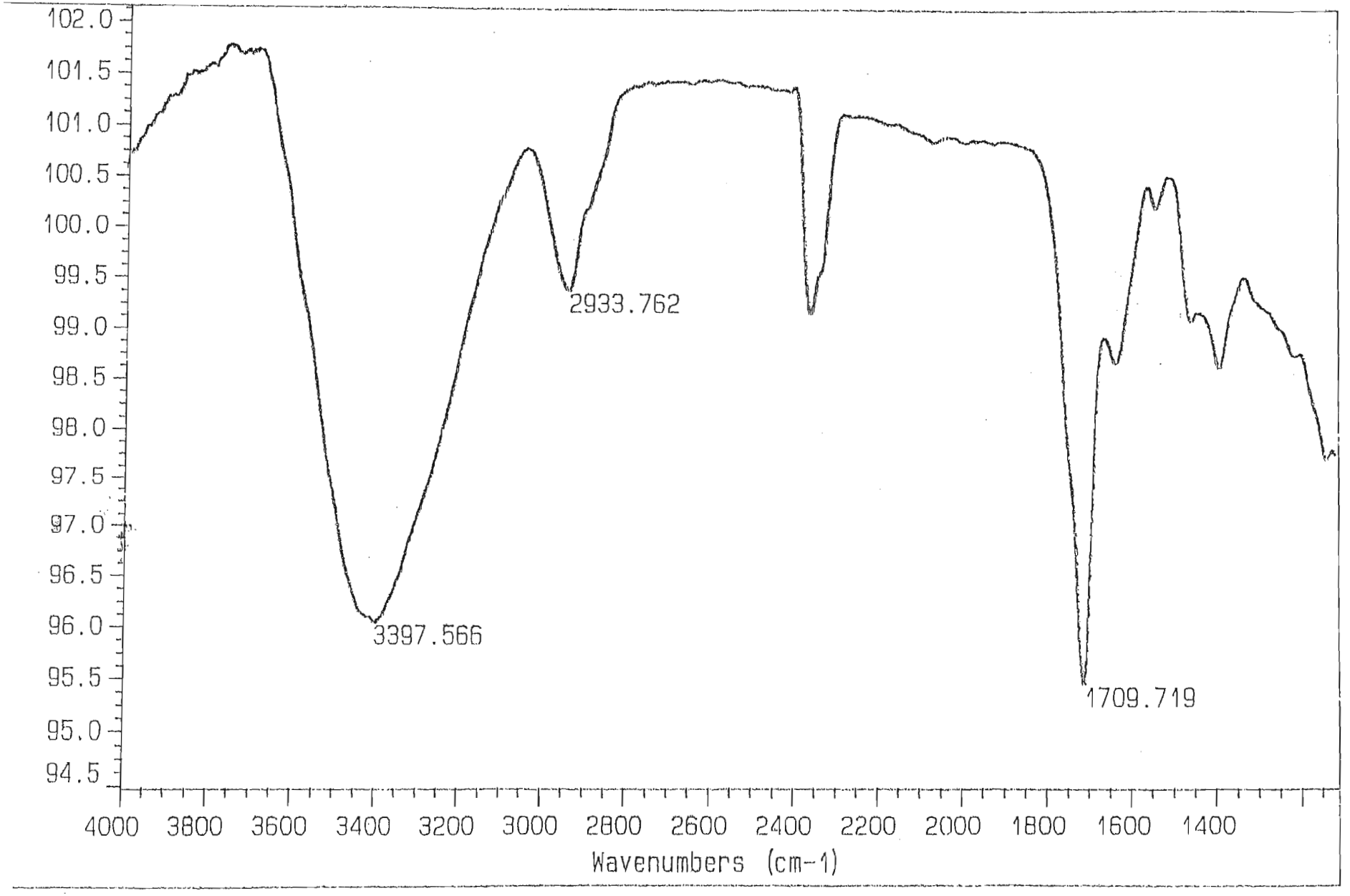
SPECTRUM 18.e: COSY spectrum of compound XVIII ($CDCl_3$)



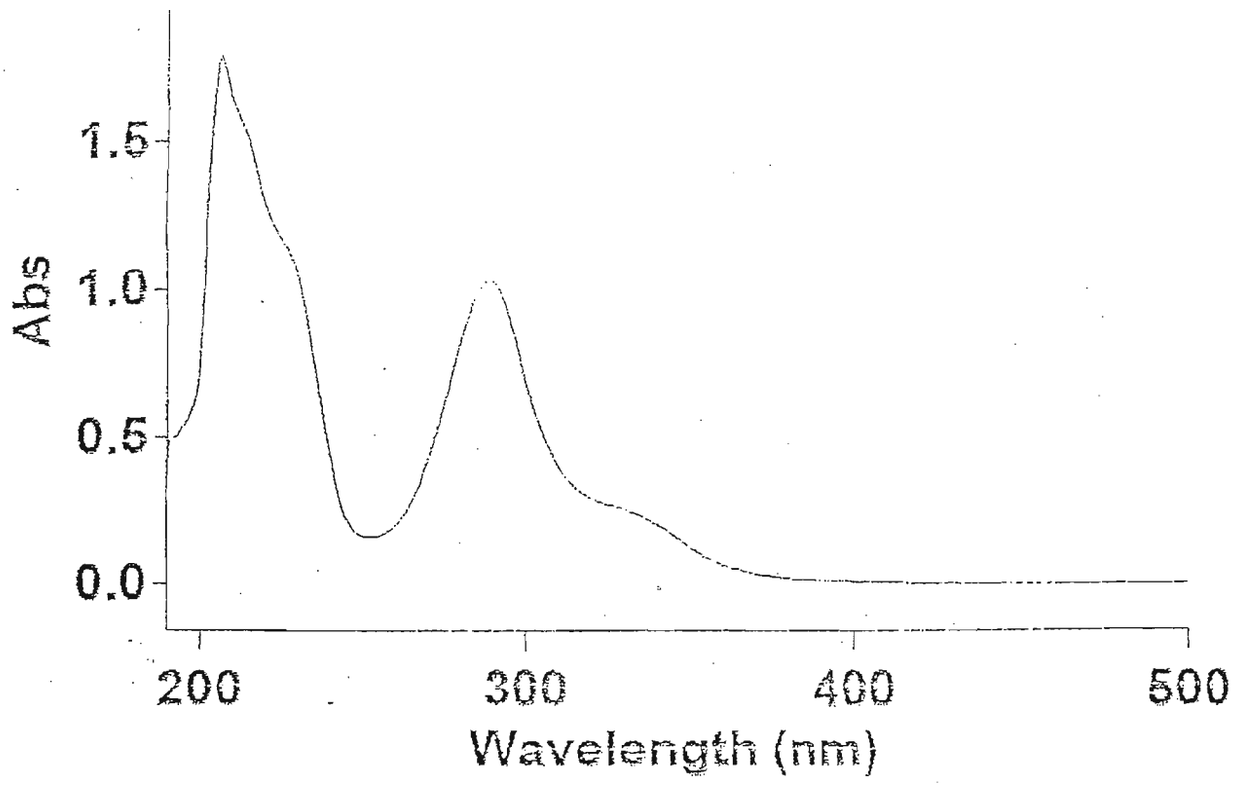
SPECTRUM 18.f: NOESY spectrum of compound XVIII ($CDCl_3$)



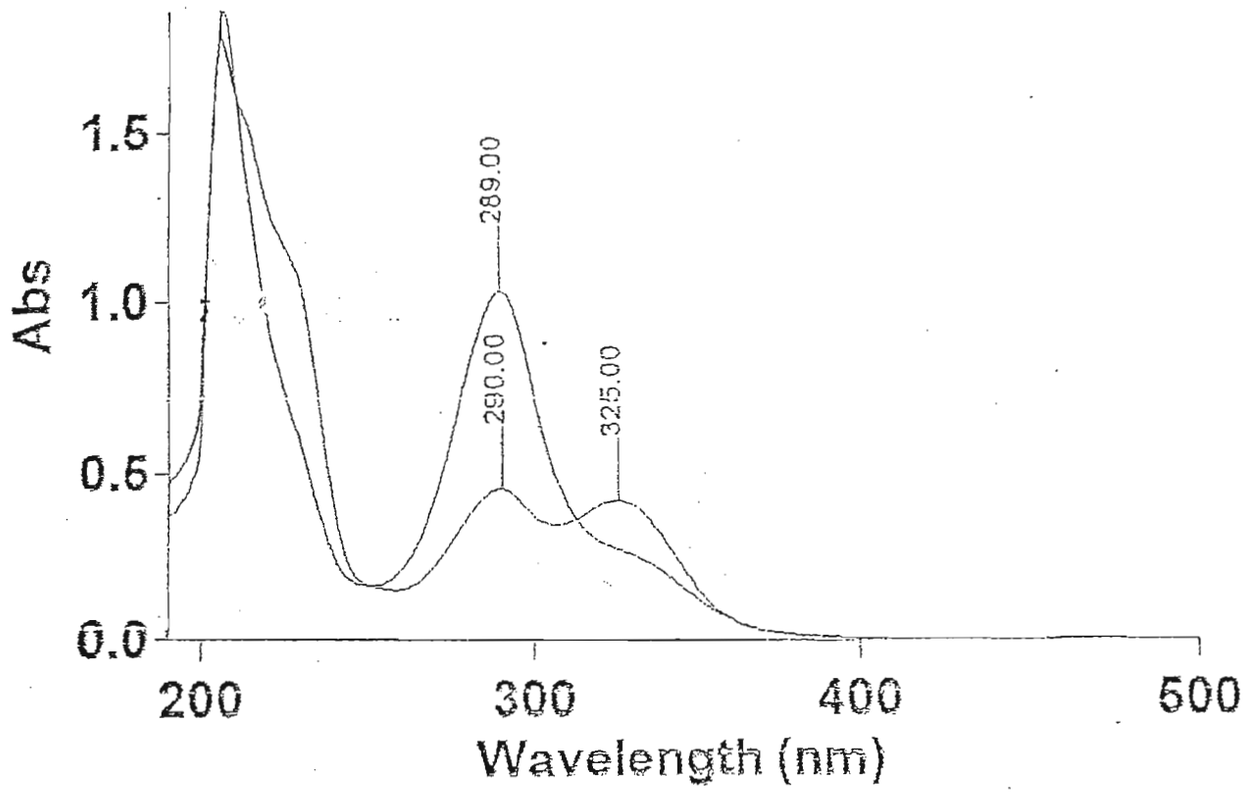
Spectrum 18.g: Mass spectra of compound XVIII



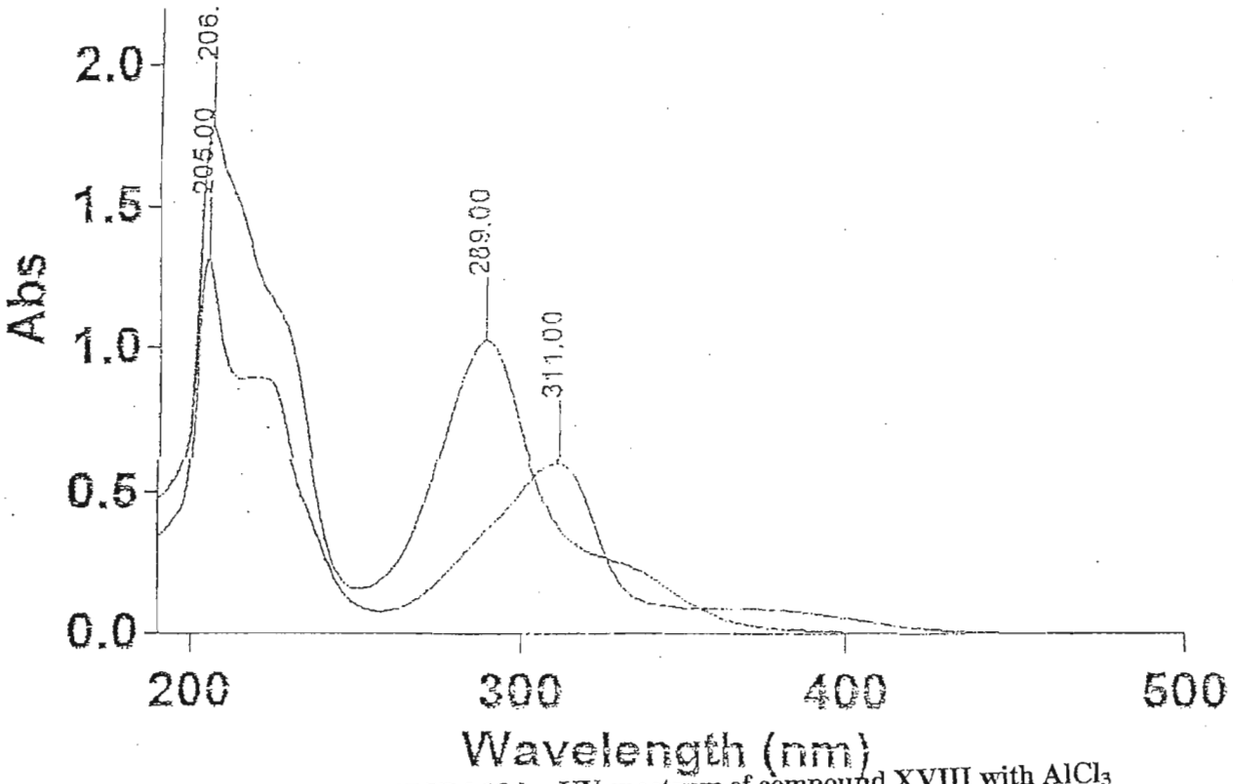
SPECTRUM 18.h: Infrared spectrum of compound XVIII



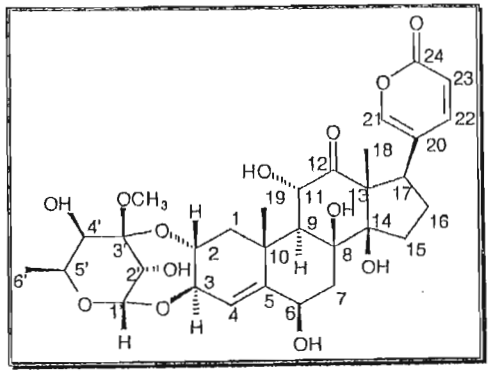
SPECTRUM 18.i.: UV spectrum of compound XVIII



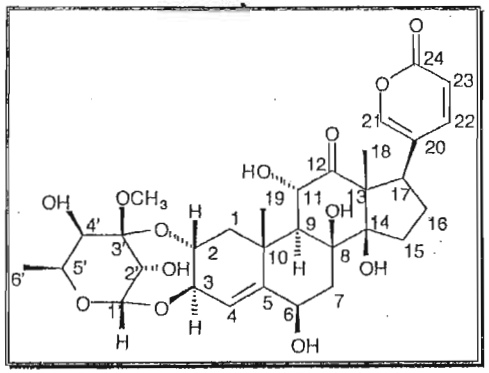
SPECTRUM 18.j.: UV spectrum of compound XVIII with NaoAc



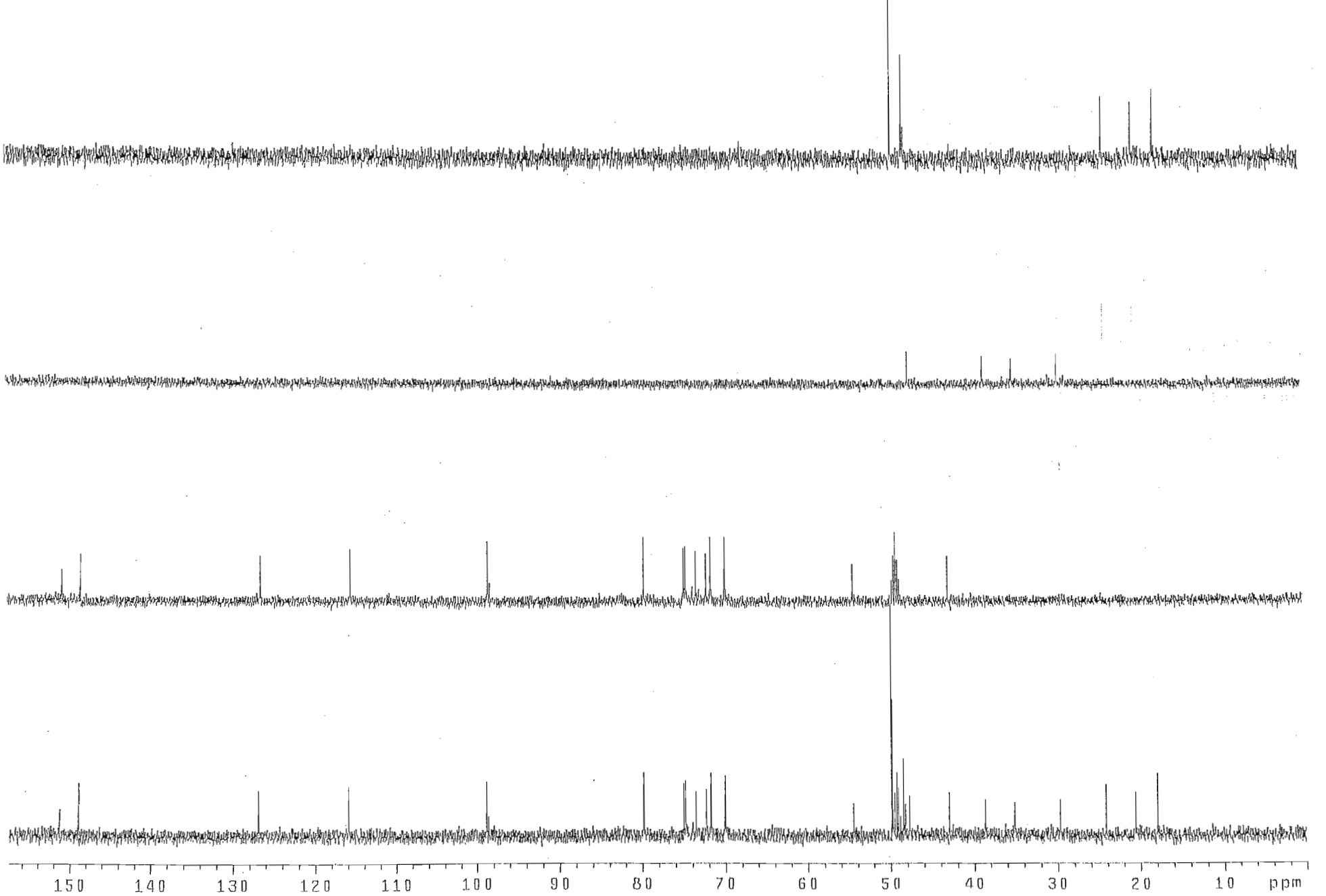
SPECTRUM 18.k.: UV spectrum of compound XVIII with $AlCl_3$



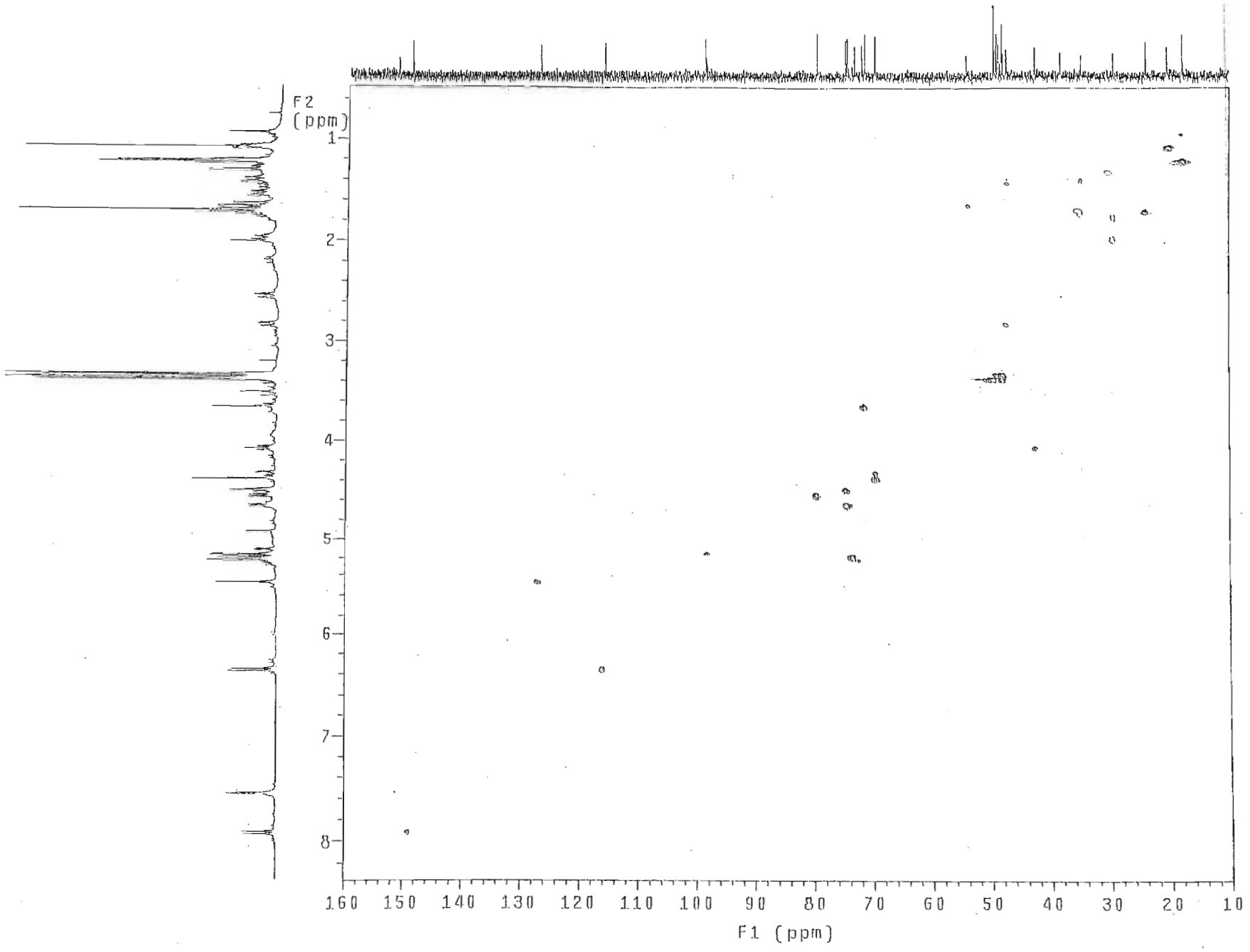
SPECTRUM 19.a: ^{1}H NMR spectrum of compound XIX (CD_3OD)



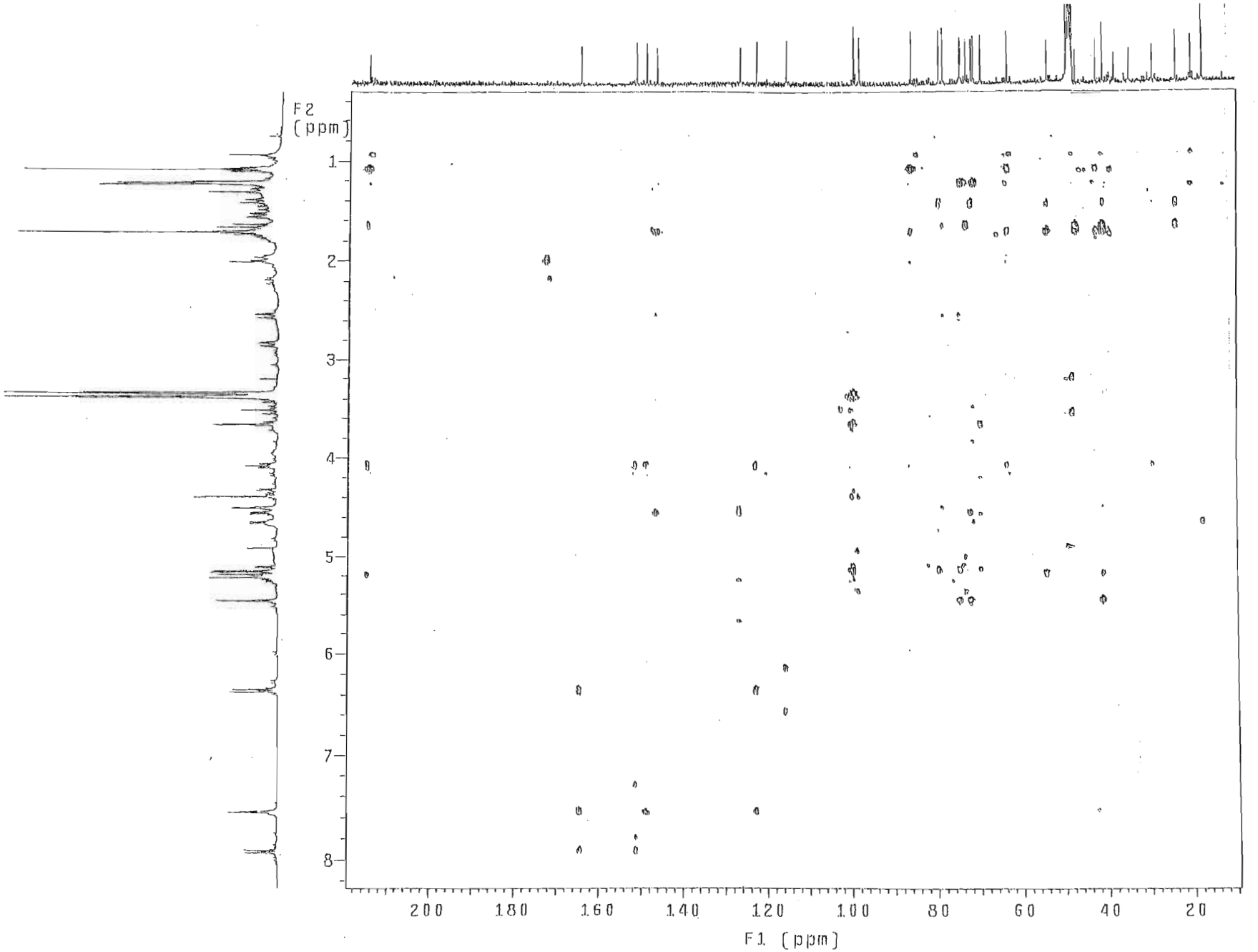
SPECTRUM 19.b: ^{13}C NMR spectrum of compound XIX (CD_3OD)



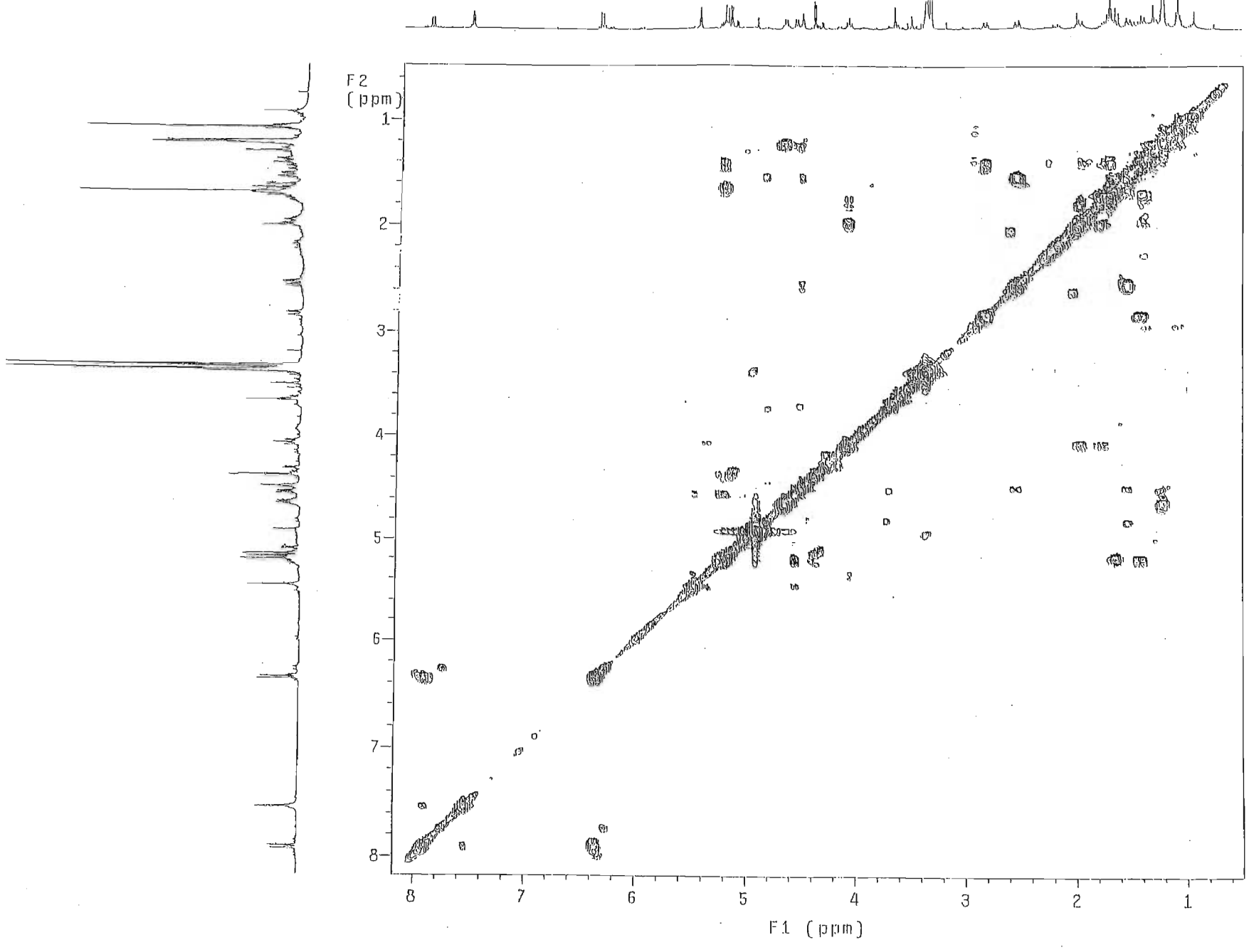
SPECTRUM 19.c: ADEPT spectrum of compound XIX (CD_3OD)



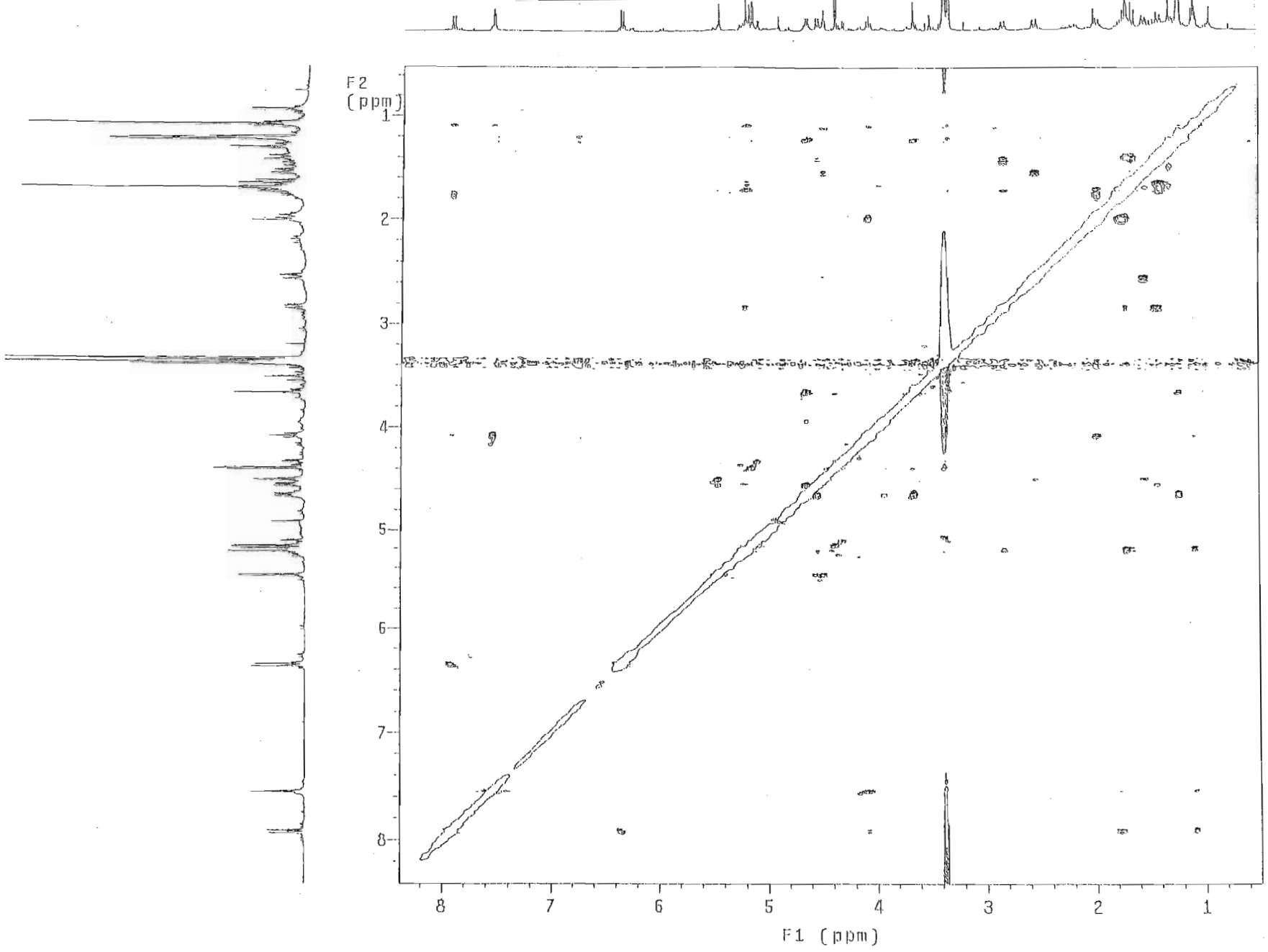
SPECTRUM 19.d: HSQC spectrum of compound XIX (CD_3OD)



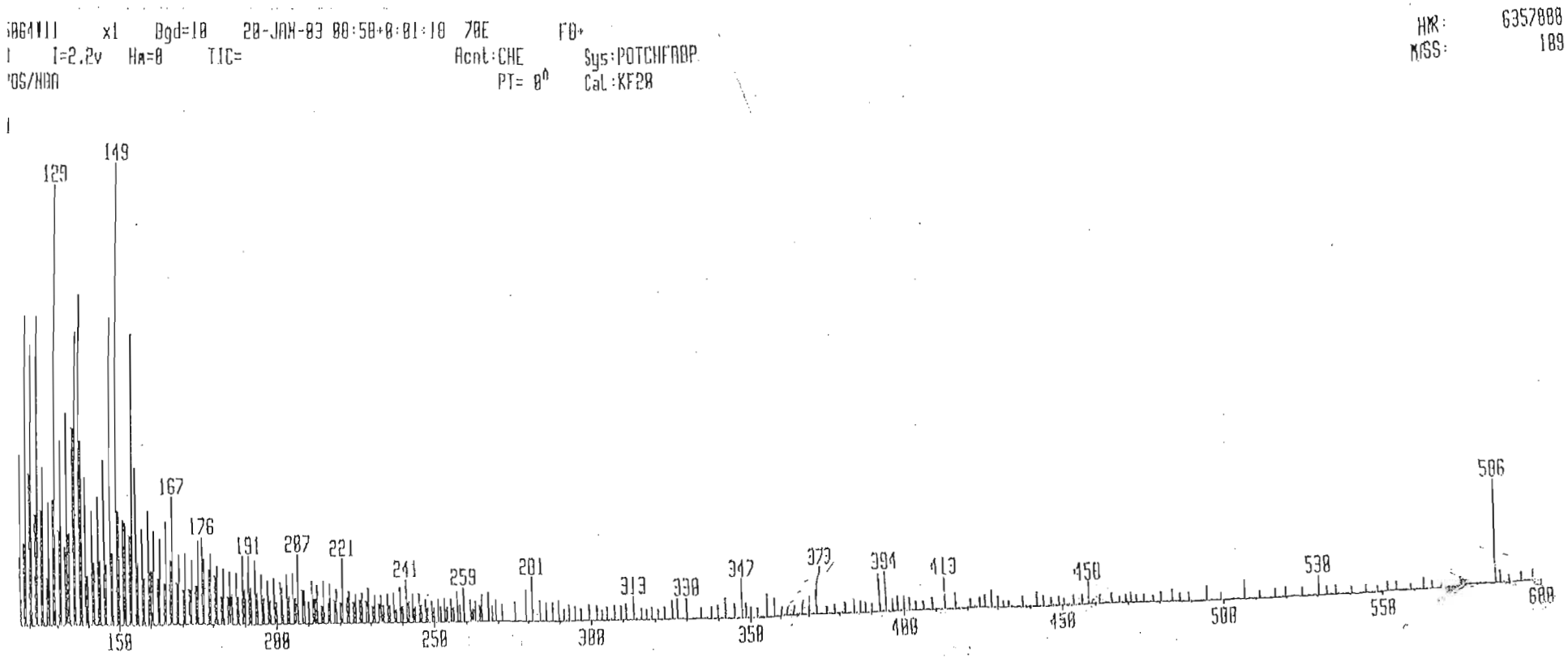
SPECTRUM 19.e: HMBC spectrum of compound XIX (CD_3OD)



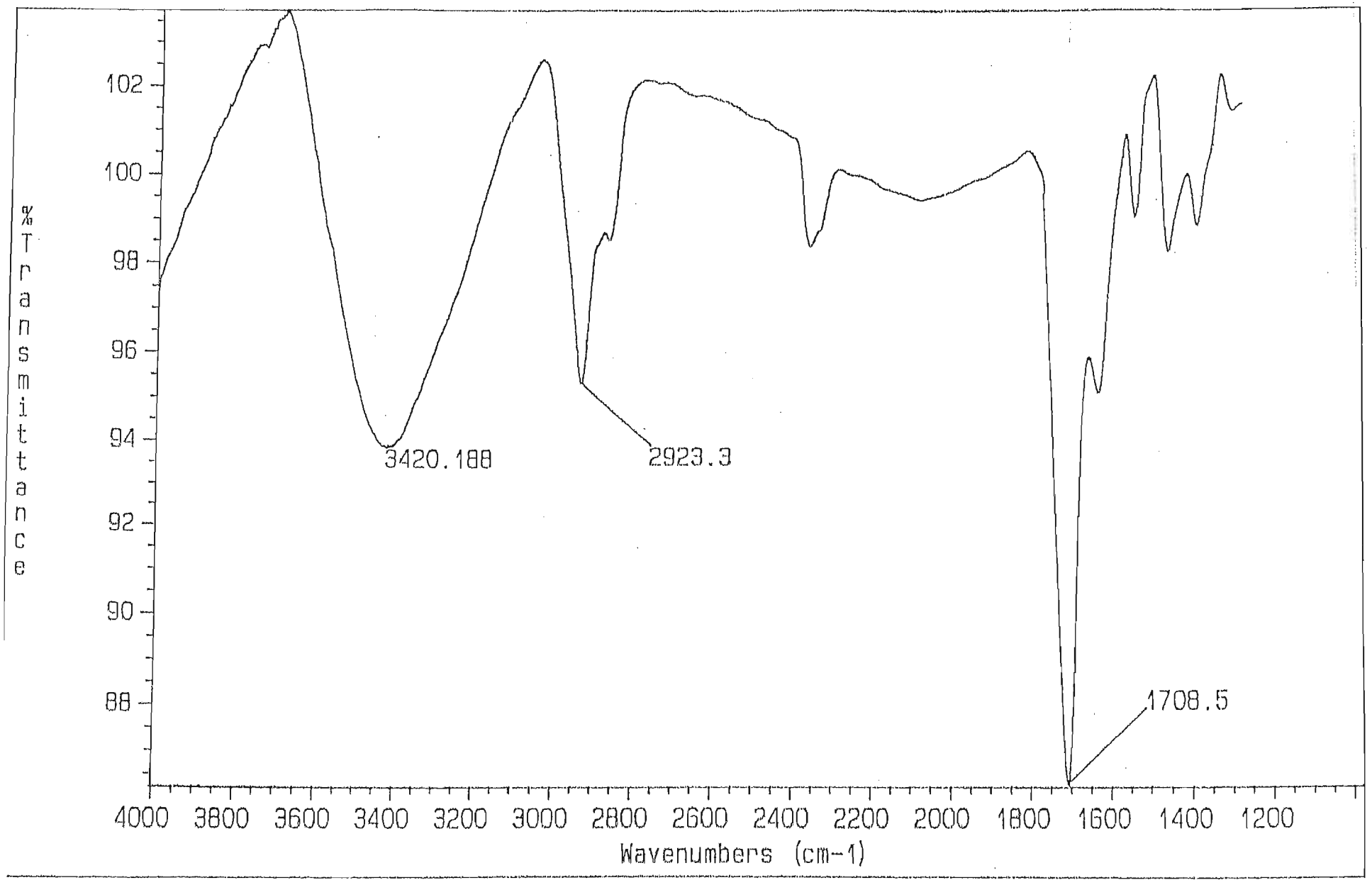
SPECTRUM 19.f: COSY spectrum of compound XIX (CD_3OD)



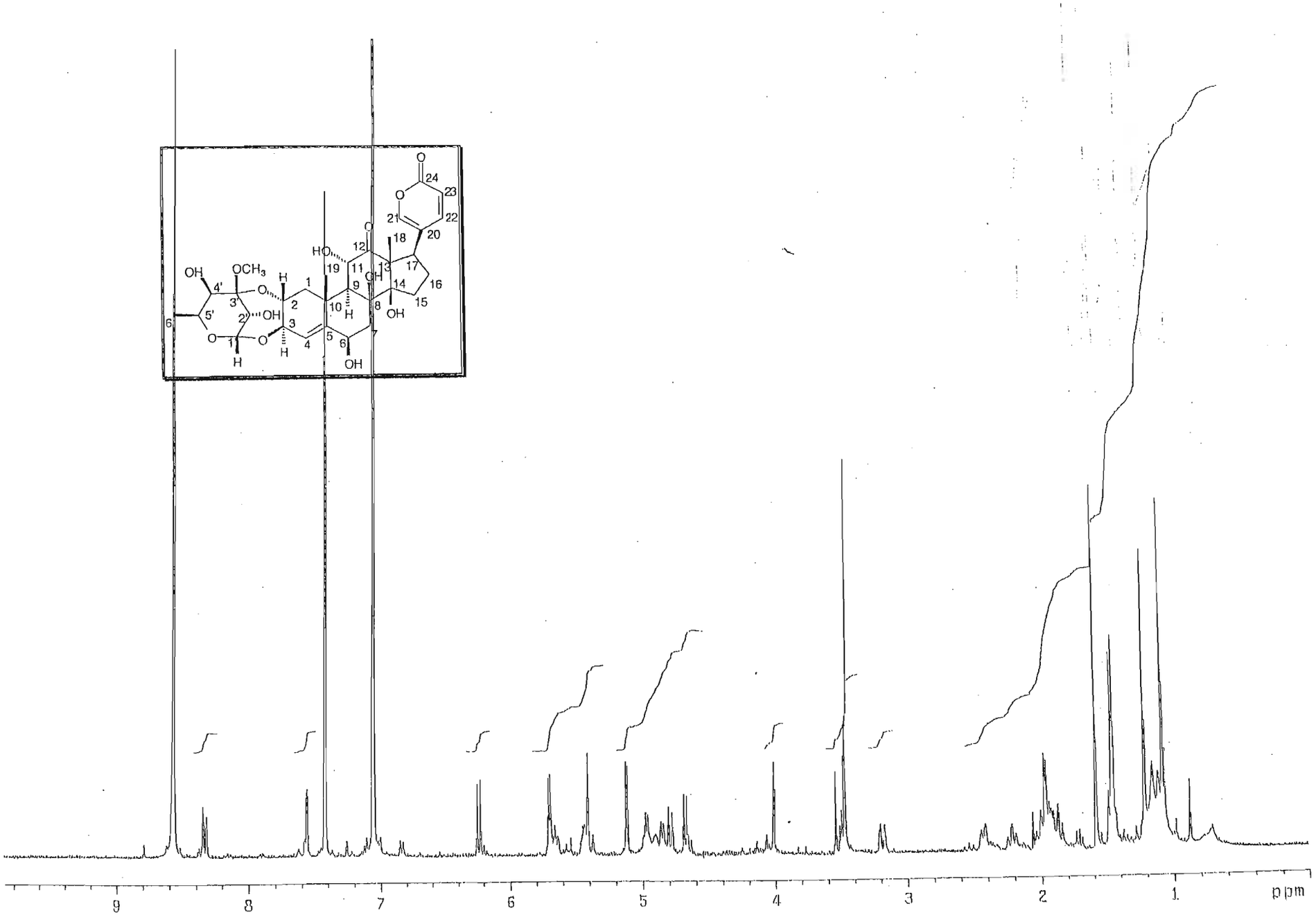
SPECTRUM 19.g: NOESY spectrum of compound XIX (CD_3OD)



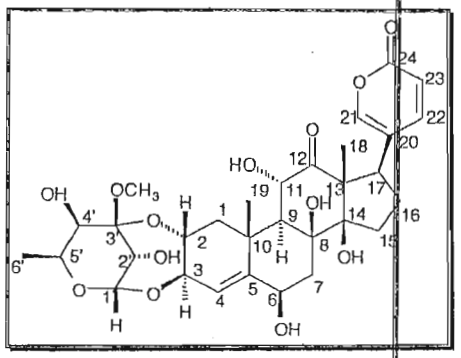
SPECTRUM 19.h: Mass spectrum of compound XIX



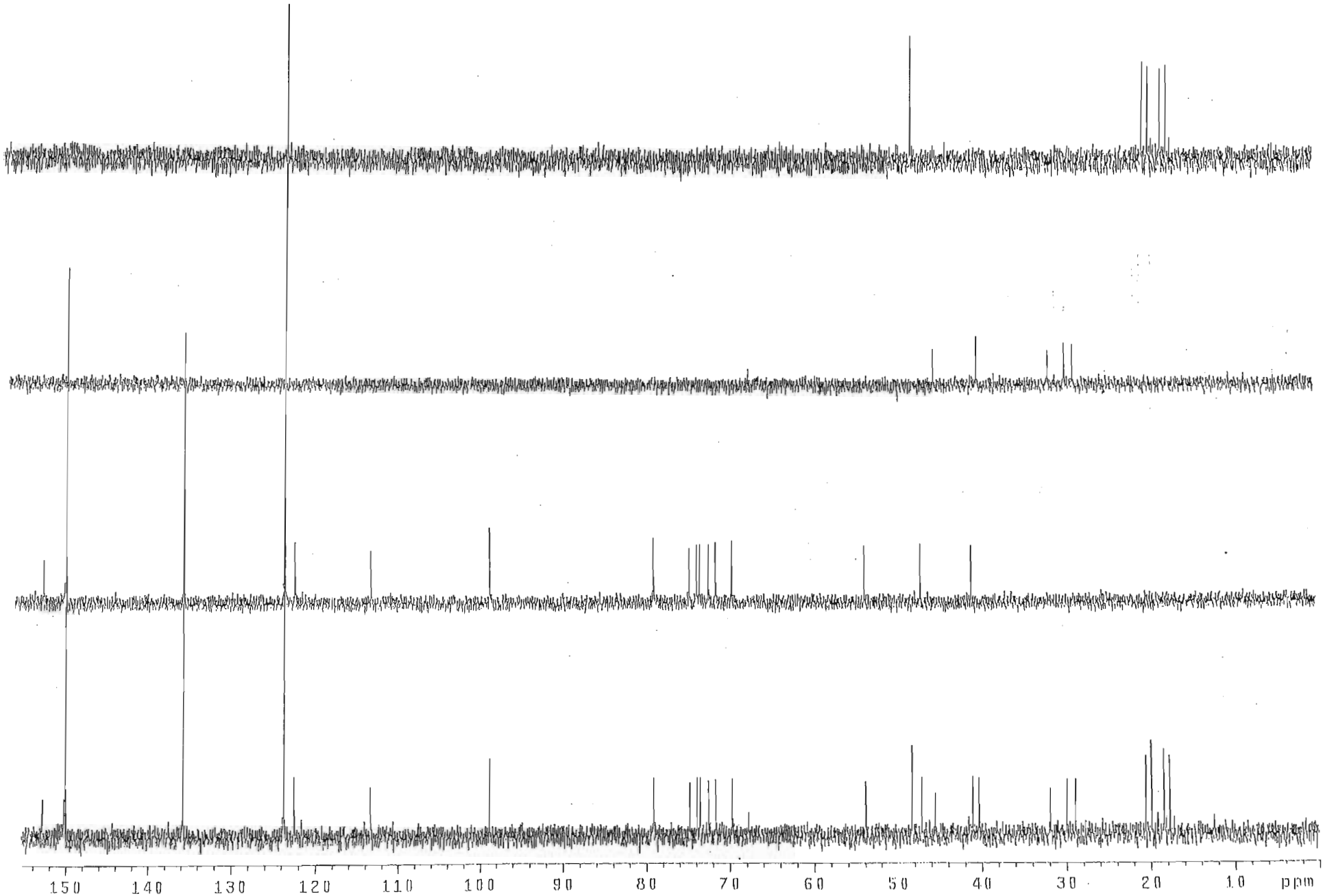
SPECTRUM 19.i: Infrared spectrum of compound XIX



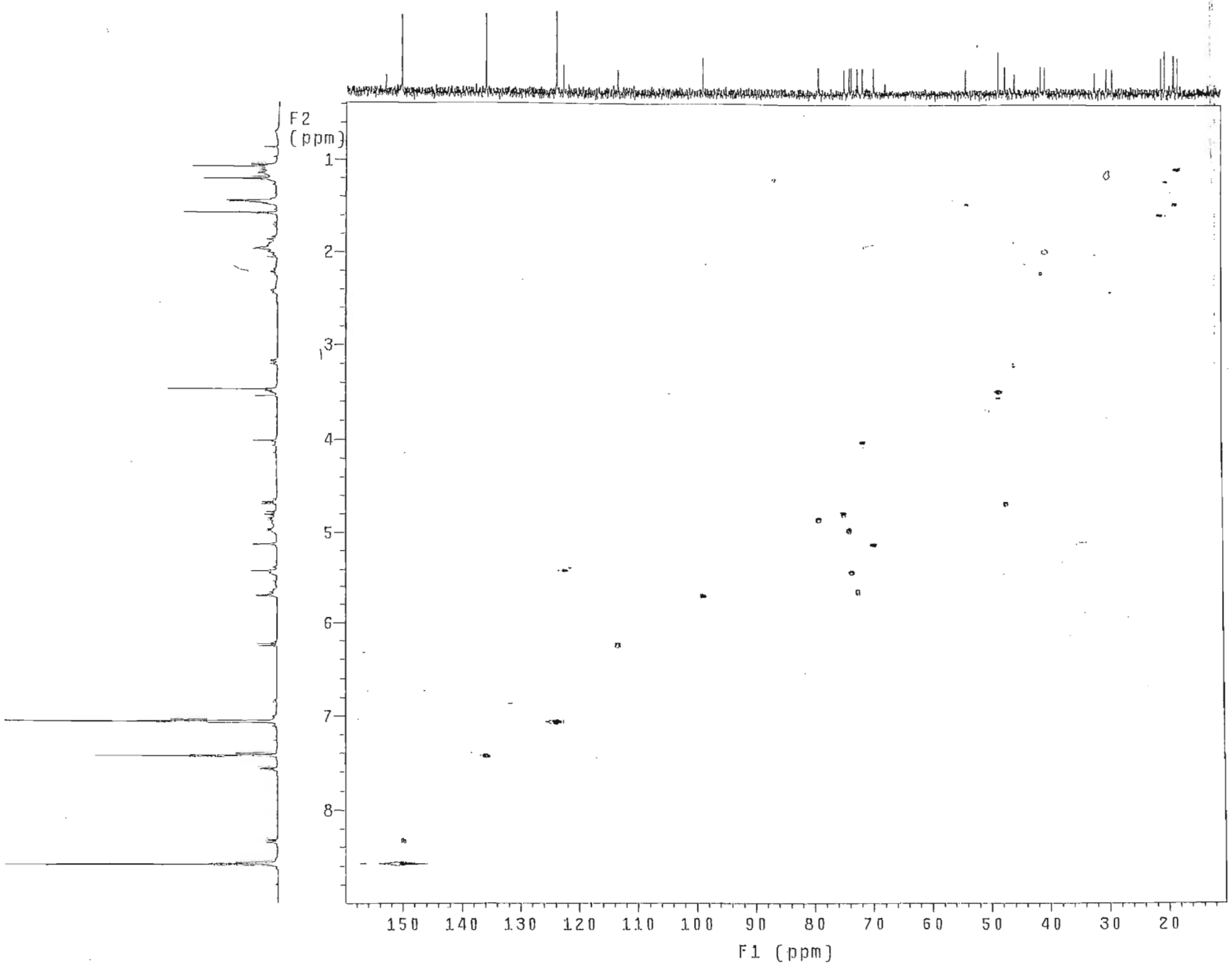
SPECTRUM 20.a: ^{1}H NMR spectrum of compound XX (C_5D_5N)



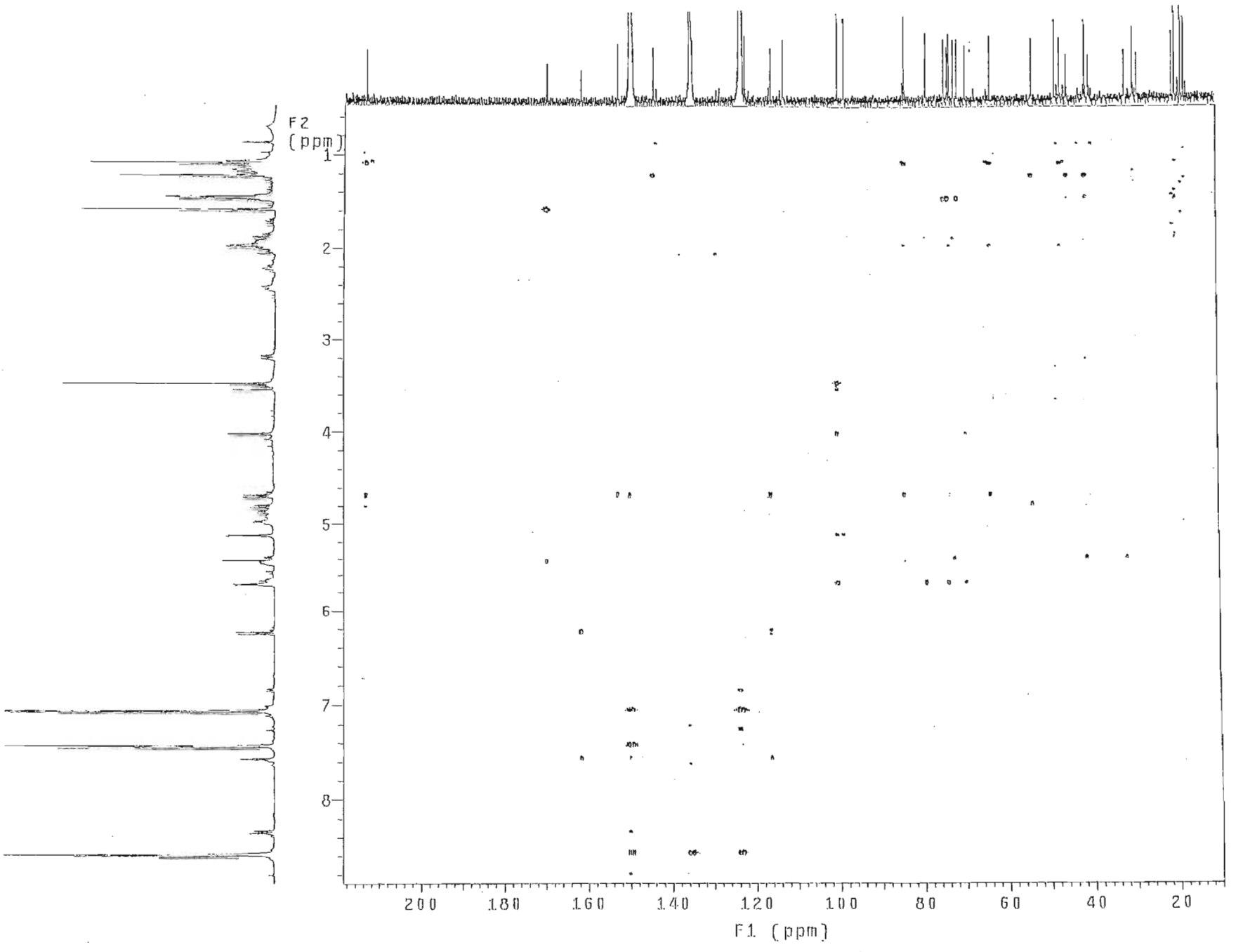
SPECTRUM 20.b: ^{13}C NMR spectrum of compound XX (C_5D_5N))



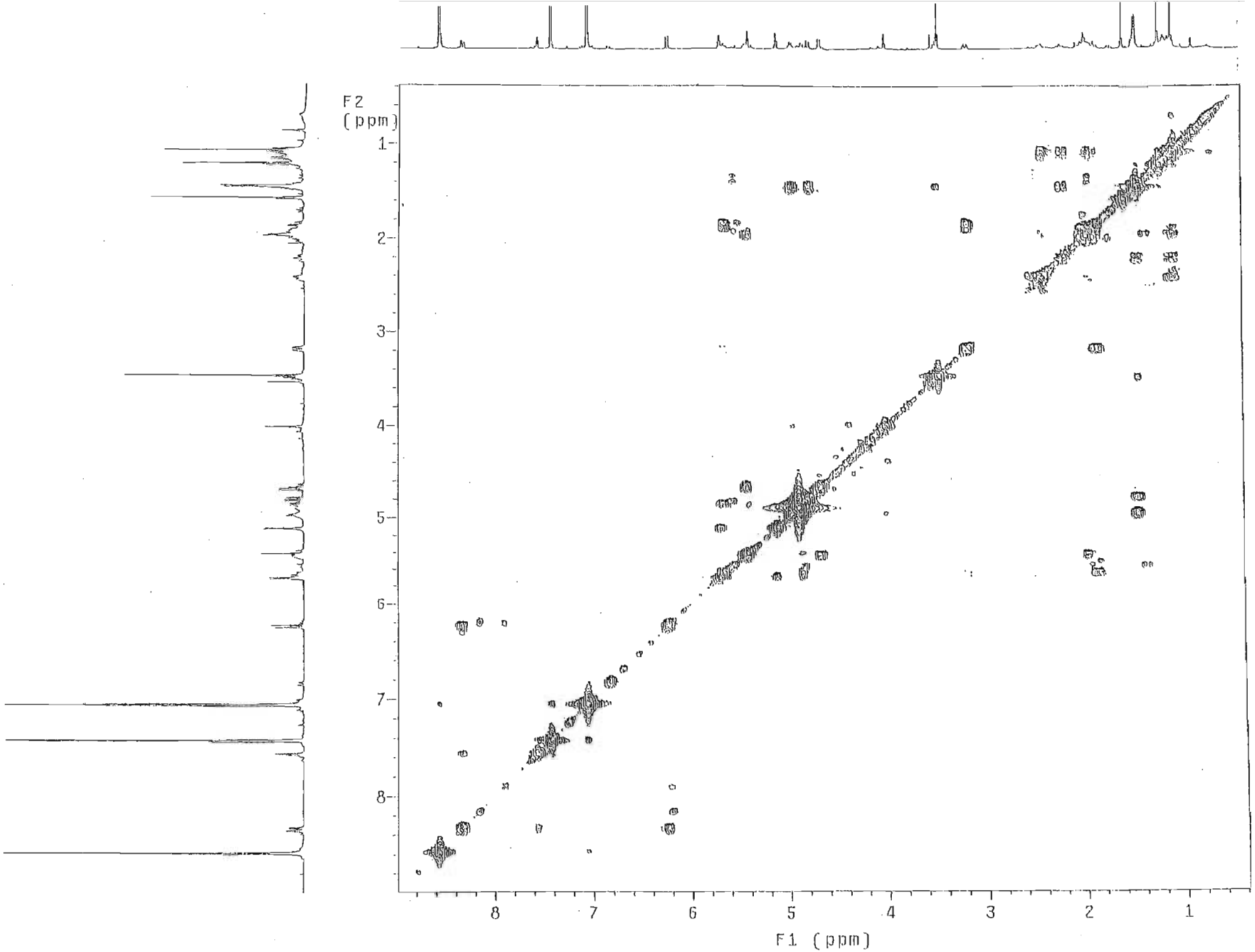
SPECTRUM 20.c: ADEPT spectrum of compound XX (C_5D_5N)



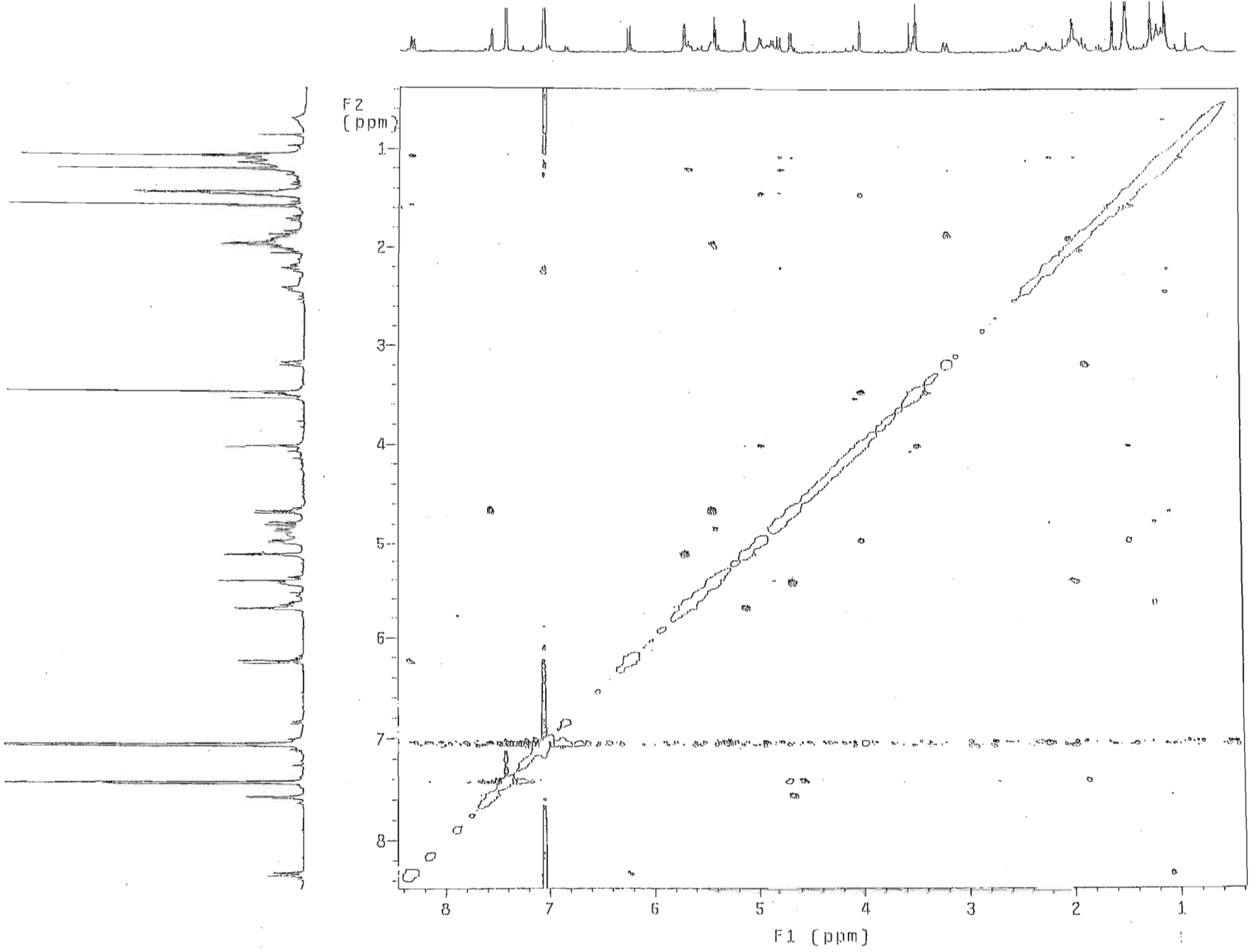
SPECTRUM 20.d: HSQC spectrum of compound XX (C_5D_5N)



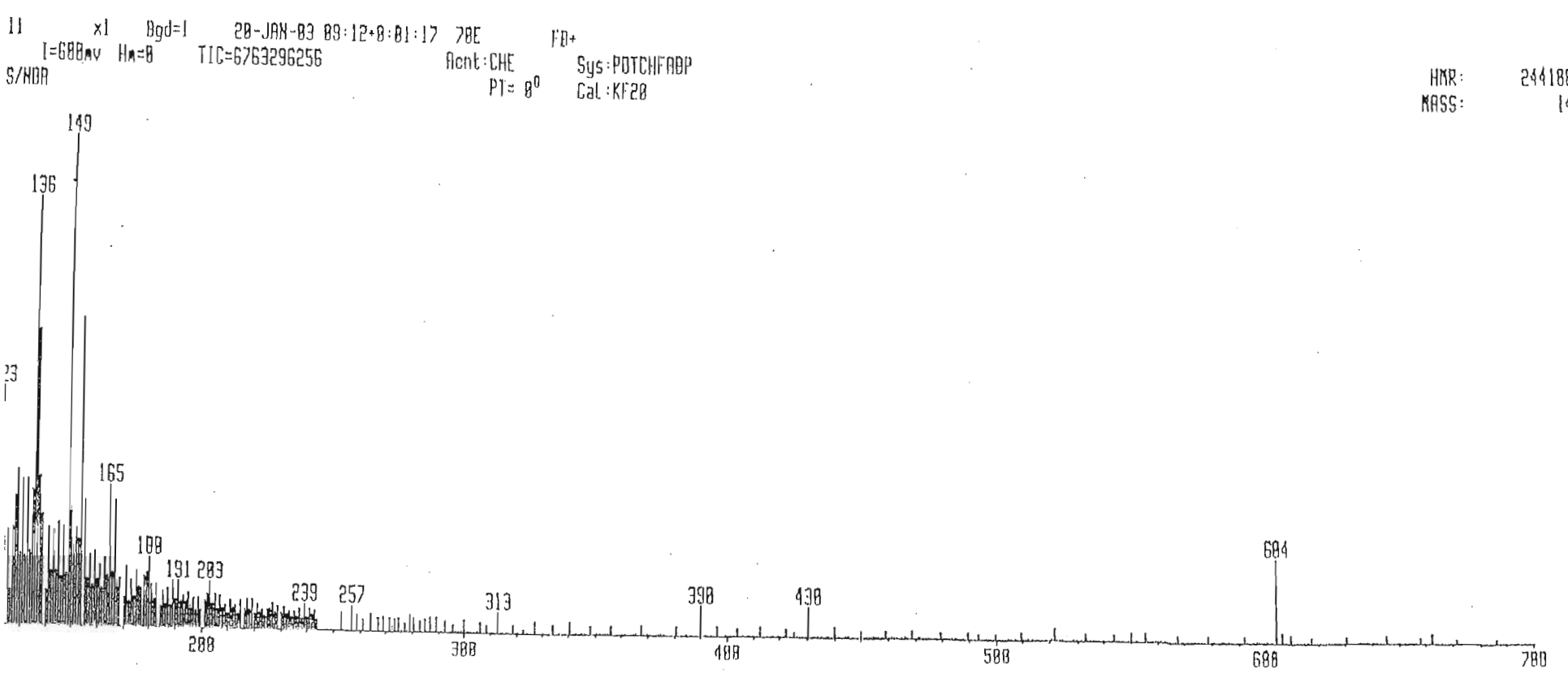
SPECTRUM 20.e: HMBC spectrum of compound XX (C_5D_5N)



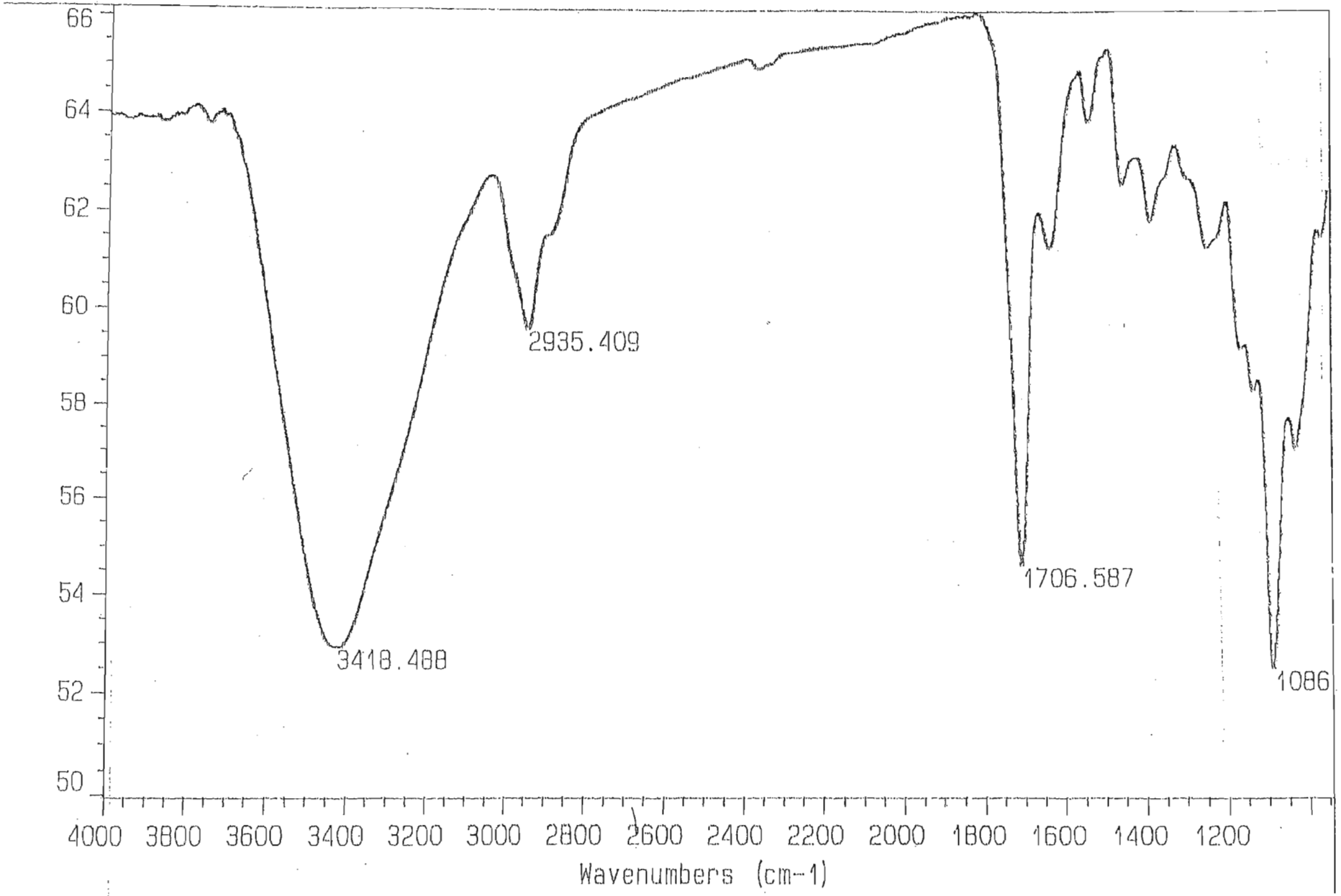
SPECTRUM 20.f: COSY spectrum of compound XX (C_5D_5N)



SPECTRUM 20.g: NOESY spectrum of compound XX (C_5D_5N)



SPECTRUM 20.h: Mass spectrum of compound XX



SPECTRUM 20.i: Infrared spectrum of compound XX