Extractives from the Amaryllidaceae:

Brunsvigia radulosa and Cyrtanthus breviflorus

By Jonathan Chetty

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requirements for the degree of
Master of Science
in the
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To my friend Christopher Lee Padachy,
who was tragically killed on 07/10/00

All 325 paragraphs, 6299 lines, 30 963 words and 192 981 characters is for you, Bro'

When wisdom enters your heart
And knowledge is pleasant to your soul
Discretion will preserve you
Understanding will keep you
Proverbs 2:10-11

PREFACE

The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, from February 2000 to February 2001 under the supervision of Prof. D. A. Mulholland and Dr. N. Crouch.

This study represents original work by the author and has not been submitted in any other form to another university. Where use was made of the work of others, it has been duly acknowledged in the text.

Signed:

J. Chetty
BSc (HONS) (UDW)

I hereby certify that the above statement is correct.

signed:

Professor D. A. Mulholland
Ph.D. (Natal)

Signed:

Doctor N. Crouch
Ph.D.

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I am indebted to Prof. C. B. Rogers, who taught me that the chemistry of alcohol was better than the alcohol itself.

God is Great !

List of Abbreviations

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

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

DEPT – distortionless enhancement by polarization transfer

brs – broad singlet

COSY – Correlated nuclear magnetic resonance spectroscopy

CNS – central nervous system

d – doublet

DMAPP – dimethylallyl pyrophosphate

FPP – farnesyl pyrophosphate

GPP – geranyl pyrophosphate

HETCOR – heteronuclear shift correlation nuclear magnetic resonance

HIV – human immunodeficiency virus

HMBC – heteronuclear multiple bond coherence

HRMS – high resolution mass spectroscopy

HSQC – heteronuclear multiple quantum coherence

Hz – hertz

IPP – isopentenyl pyrophosphate

LSD – lysergic acid diethylamide

m – multiplet

NADP – nicotinamide adenine dinucleotide phosphate

NOESY – nuclear Overhauser effect spectroscopy

PP – pyrophosphate

ppm – parts per million

s – singlet

STD – sexually transmitted disease

t – triplet

TLC – thin layer chromatography

TOCSY – total correlation spectroscopy

UV – ultra violet

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ABSTRACT

Extractives from the Amaryllidaceae family were studied in this work. Plants of the monocotyledonous family Amaryllidaceae are widely distributed. These plants occur abundantly and natively in the tropics and are very prominent in South Africa. Subterranean bulbs with thick fleshy scales or rhizomes are characteristic of this family. This family is known to be rich in alkaloids. Specific alkaloids, not found in other plants, are consistently found in the family. In South Africa, bulbs of the Amaryllidaceae family have, for at least 200 years, been used as traditional medicine.

Two species, *Cyrtanthus breviflorus* and *Brunsvigia radulosa* were examined. Both species are protected by the South African Flora Protection Act, 2000. These species had not been fully investigated previously. *Brunsvigia radulosa* Herb. was obtained from Mpumalanga and *Cyrtanthus breviflorus* Harv. from Kwazulu-Natal.

Cyrtanthus breviflorus, commonly known as Wild Crocus, is found along the coastal regions from the Eastern Cape to Kenya. The bulb of this plant is edible. The plant is used traditionally to treat intestinal worms (round and tape worms) and as love and protective charms. The ethanol extract of this plant yielded four alkaloids, haemanthamine, crinamine hydrochloride, tazettine and lycorine. The hexane extract yielded the known triterpenoids: lupeol, lupenone, glochidone, 3β,27-dihydroxylup-20(29)-ene and betulinaldehyde. Triterpenoids have not been previously isolated from *Cyrtanthus*.

Brunsvigia radulosa can be found in grassland areas from north-western Cape to the northern parts of South Africa. The hard bulbs of this plant are used to seal clay pots. This species is used in traditional medicine to straighten bones of children and to treat barrenness in women as well as facilitating an easy birth. The ethanol extract of this plant was rich in crinamine. Other alkaloids obtained included lycorine, 1-O-acetyl-lycorine, crinine, hamayne and anhydrolycorinium chloride.

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CHAPTER 1

An introduction to Amaryllidaceae alkaloids

1.1 AN INTRODUCTION TO THE ALKALOIDS

The term alkaloid is usually applied to basic, nitrogen-containing compounds of plant origin. Alkaloids have complex molecular structures, and they manifest significant pharmacological activity [1].

Alkaloids have diverse and important physiological effects on both humans as well as animals. Well known alkaloids include ephedrine (**1**), nicotine (**2**), morphine (**3**), quinine (**4**) and strychnine (**5**) [1,2].

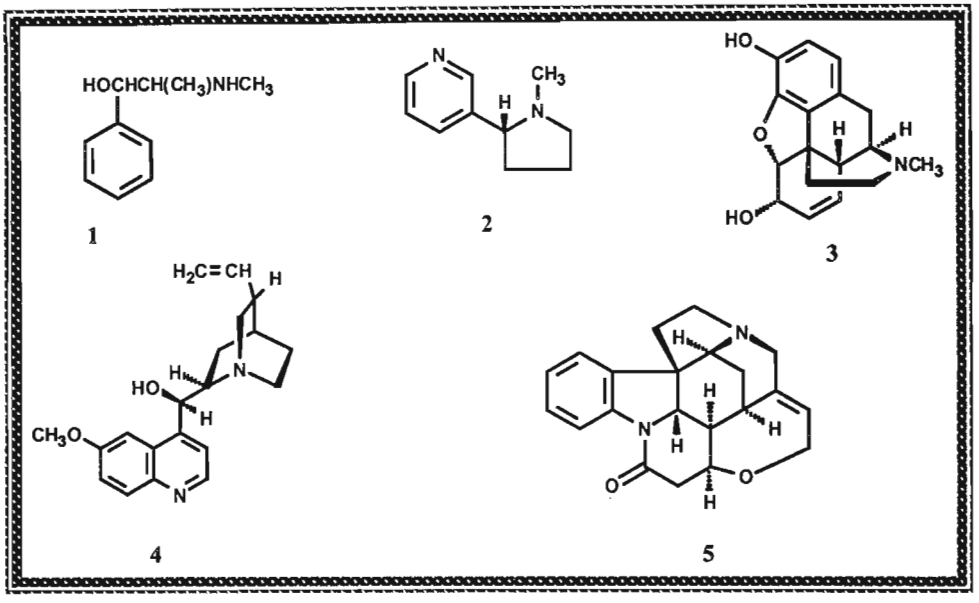


Figure 1.1. Well known alkaloids [1,2]

Alkaloids are found commonly in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species [3]. In general, a given species contains only a few types of alkaloid, though both the Opium poppy (*Papaver somniferum*) and the Ergot fungus (*Claviceps*) each contain about 30 different types. Certain plant families are particularly rich in alkaloids; all plants of the poppy family (Papaveraceae) are thought to contain them. The Ranunculaceae (buttercups), Solanaceae (nightshades), and Amaryllidaceae (Amaryllis) are other prominent alkaloid-containing families [3]. A few alkaloids have been found in animal species, such as the New World beaver (*Castor canadensis*) and poison-dart frogs (*Phyllobates*) [2,3].

The function of alkaloids in plants is not fully understood. It has been suggested that they are simply waste products of plants' metabolic processes, but evidence suggests that they may serve specific biological functions [2]. In some plants, the concentration of alkaloids increases just prior to seed formation and then drops off when the seed is ripe. This suggests that alkaloids may play a role in seed formation. Alkaloids may also protect some plants from destruction by certain insect species [1,3].

The chemical structures of alkaloids are extremely variable. Generally an alkaloid contains at least one nitrogen atom in an amine-type structure. This or another nitrogen atom can be active as a base in acid-base reactions. The name alkaloid ("alkali-like") was originally applied to the substances because they react with acids to form salts [1,2,3,4,5]. Most alkaloids have one or more of their nitrogen atoms as part of a ring of atoms. Alkaloid names generally end in the suffix -ine, a reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste [2,3].

Interest in the alkaloids stems from the wide variety of physiological effects, both wanted and unwanted, they produce in humans and other animals. Their use dates back to ancient civilizations, but scientific study of these compounds had to await the growth of organic chemistry as their structures are quite complex. The first alkaloid to be isolated and crystallized was the potent active constituent of the opium poppy, morphine, in 1805 [1,3].

The medicinal properties of alkaloids are quite diverse. Morphine **(3)** is a powerful narcotic used for the relief of pain, but its addictive properties limit its usefulness. Codeine, the methyl ether derivative of morphine found in the opium poppy, is an excellent analgesic that is not as addictive [3]. Certain alkaloids act as cardiac or respiratory stimulants. Quinidine, which is obtained from plants of the genus *Cinchona,* is used to treat arrhythmias, or irregular rhythms of the heartbeat. Many alkaloids affect respiration, but in a complicated manner such that severe respiratory depression may follow stimulation. Ergonovine, from the fungus *Claviceps purpurea*, and ephedrine (**1**), from *Ephedra* species, can act as blood-vessel constrictors [3].

Many alkaloids possess local anaesthetic properties. Cocaine, from *Erythroxylum coca*, is a very potent local anaesthetic. Quinine (**4**) is a powerful anti-malarial agent. It was formerly the drug of choice for treating this disease, though it has been replaced by less toxic and more effective synthetic drugs [4]. The alkaloid tubocurarine is the active ingredient in the South American arrow poison, curare, obtained from *Chondrodendron tomentosum*, and is used as a muscle relaxant in surgery. Two alkaloids, vincristine and vinblastine, from *Vinca rosea*, are widely used as chemotherapeutic agents in the treatment of many types of cancer [3,4].

Nicotine (**2**) obtained from the tobacco plant *Nicotiana tabacum* is the principal alkaloid and chief addictive ingredient of the tobacco smoked in cigarettes, cigars, and pipes [3,4]. Some alkaloids are illicit drugs and poisons. These include the hallucinogenic drugs from *Anhalonium* species and psilocybin from *Psilocybe mexicana*. Synthetic derivatives of the alkaloids morphine and lysergic acid produce heroin and LSD, respectively. The alkaloid coniine is the active component of the poison hemlock (*Conium maculatum*) [3,4]. Strychnine (**5**), from *Strychnos* species, is another powerful poison and was once used to control rodents [2,3,4].

Recently, two anti-HIV alkaloids nitidine (**6**) and magnoflorine (**7**) were found. Nitidine inhibited human lymphoblastoid cell killing by HIV-1 and magnoflorine showed anti-HIV activity with partial cytoprotection [6,7].

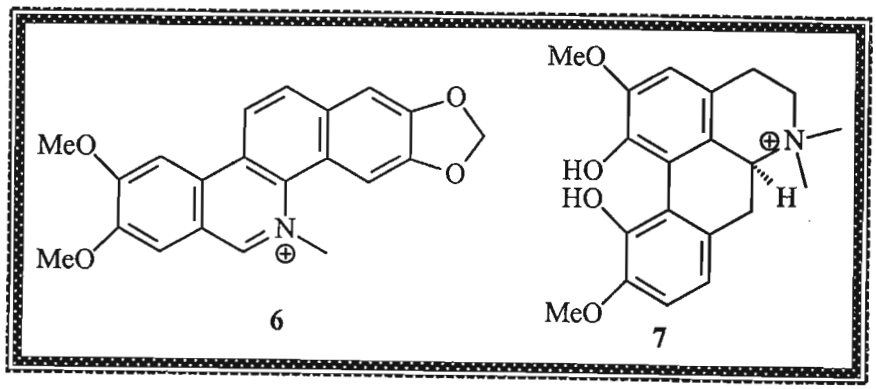


Figure 1.2: Recently discovered anti-HIV alkaloids [6,7]

1.2 THE AMARYLLIDACEAE ALKALOIDS

1.2.1 INTRODUCTION

The Amaryllidaceae is the family of Amaryllis and related plants. Members of the giant group Lilianae, the amaryllids are mostly bulbous plants, although some have a rhizome. The flowers are bisexual and quite regular; they characteristically appear in umbels at the top of a leafless stalk, the scape. The leaves are all produced from the base of the plant; most species are deciduous, but *Clivia* is evergreen [8].

The family Amaryllidaceae is monocotyledonous and is fairly well distributed. These plants occur abundantly in the tropics and are quite prominent in southern Africa, where the family is most diverse (18 genera; *ca.* 280 species) [9]. The Amaryllidaceae alkaloids are representative of a well established group of isoquinoline alkaloids [9]. These alkaloids, which are not found in other plants, are consistently found in the Amaryllidaceae family [10]. The high water content of the bulbs in the family limits the total alkaloid content to a maximum of 1-2 percent [10,11].

Bulbs of the Amaryllidaceae have, for several decades, been used as traditional medicine. Although sometimes whole plants are used, frequently it occurs that either a cold or warm infusion of bulbs is ingested orally or applied to a designated area. Some examples of traditional medicinal usage of some species of Amaryllidaceae in South Africa is described below in table 1 [12].

Table 1.1: Medicinal usage of some South African Amaryllidaceae [12]

SPECIES	MEDICINAL PROPERTIES AND USAGE
Amaryllis belladonna	Treatment of cancer, antispasmodic action
Boophane disticha	Used for chest pains and circumcision dressing
Brunsvigia species	Treat infertility, renal and liver complaints
Clivia miniata	Treat urinary infections, facilitates childbirth
Crinum species	Treatment of STDs, rheumatism, varicose veins
Cyrtanthus species	Cures stomach ailments, treatment of leprosy
Gethyllis linearis	Treatment of colic, flatulence and indigestion
Heamanthus species	Used to heal ulcers, febrile colds and asthma

Some Amaryllidaceae alkaloids also exhibit a toxic and a hallucinogenic effect. In smaller doses symptoms such as excessive salivation, emesis and diarrhoea are evident. At higher doses the Amaryllidaceae alkaloids can cause CNS excitation or depression, and may even be fatal. Consequently, the Amaryllidaceae alkaloids have been used as a source of poison [10,12].

It is well known that *Boophane disticha* can cause profound hallucinations. It is used in Basuto initiation ceremonies: consumption of a prepared alkaloidal concoction leads to intoxication which symbolizes the inhabitation of the spirit of manhood in the boys [12].

1.2.2 CLASSIFICATION OF THE AMARYLLIDACEAE ALKALOIDS

Alkaloids are often classified on the basis of their chemical structure. For example, those alkaloids that contain an indole ring system are known as indole alkaloids. On this basis, the principal classes of alkaloids are the pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, and the terpenoid and steroidal alkaloids. Alternatively, alkaloids can be classified according to the biological system in which they occur. For example, the opium alkaloids occur in the opium poppy (*Papaver somniferum*) [1,3]. This dual classification system has been, successfully, in use for a very long time [3].

The Amaryllidaceae family produces the class of isoquinoline alkaloids that are biosynthetically derived from the amino acids tyrosine and phenylalanine [9,11]. Almost all Amaryllidaceae alkaloids can be considered to be derived from a nucleus of fifteen carbon atoms comprised of two fragments, an aromatic C_6-C_1 unit and a C_6-C_2 moiety [11]. Variations in the degree of oxygenation, hydrogenation and substitution within these fragments accounts for the numerous alkaloids that have been isolated. The Amaryllidaceae alkaloids can be classified into eight different types [13].

Table 1.2: The different Amaryllidaceae alkaloid types [13]

TYPE OF ALKALOID	EXAMPLES
1. Lycorine	Lycorine, caranine, sternbergine
2. Homolycorine	Hippeastrine, neronine, masonine
3. Galanthamine	Galanthamine, sanguinine, childanthine
4. 5,10b-Ethanophenanthridine	Crinine, crinamine, haemanthamine
5. [2]Benzopyrano[3,4c]indole	Tazettine, pretazettine
6. Montanine	Montanine, brunsvigine, coccinine
7. Isocarbostyril/narciclasine	Narciclasine, kalbreclasine
8. Miscellaneous	Ismine, cherylline, belladine

Since only alkaloids of the 5,10b-ethanophenanthridine, [2]benzopyrano[3,4c]indole and lycorine type have been isolated in this investigation, only these will be discussed in some detail. Brief mention of the other groups will be made.

1. The lycorine type alkaloids.

Lycorine is the most common isoquinoline alkaloid and is easily isolated because of its relative insolubility. The lycorine type alkaloids consist of four rings and usually a methylenedioxy substituent. The isoquinoline skeleton is formed by ring A and ring B. Ring C is attached to the isoquinoline skeleton at C-11b and C-11c. Ring D is attached to the nitrogen atom and to C-3a.

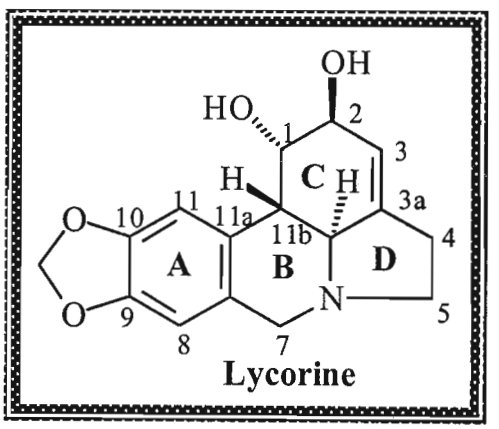


Figure 1.3: An alkaloid of the lycorine type

Variations in the substitution pattern in rings A, C and D in lycorine, has led to the class of alkaloids known as the lycorine type alkaloids. Interconversion of these alkaloids can be achieved by simple hydrolytic, oxidative and reductive reactions.

2. The homolycorine type alkaloids.

This type of alkaloids are characterized by an oxygen-containing heterogeneous B ring and a five membered nitrogen containing D ring. Ring B constitutes a lactone and the nitrogen atom in ring D is always methylated. Variations in the substitution pattern of ring A and ring C leads to a series of homolycorine type alkaloids.

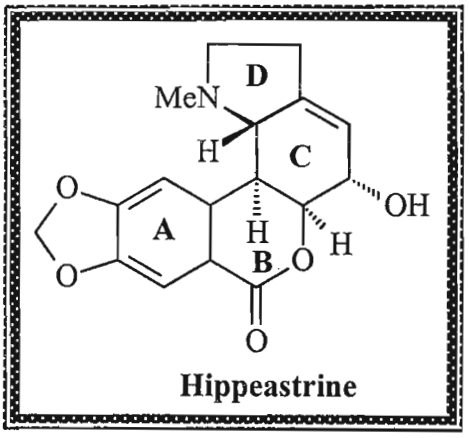


Figure 1.4: An alkaloid of the homolycorine type.

3. The galanthamine type alkaloids.

These type of alkaloids have a haemanthamine type skeleton with an open B ring. These alkaloids are unique in that ring A and ring C are joined by an ether linkage. These alkaloids also posses an α-orientated ethane nitrogen bridge. Ring C contains a single double bond and an allylic hydroxyl group.

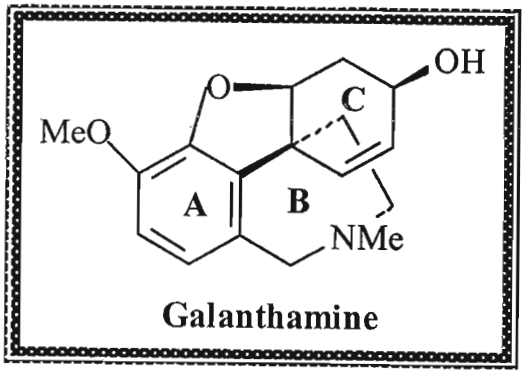


Figure 1.5: An alkaloid of the galanthamine type.

4. The 5,10b-ethanophenanthridine type alkaloids.

Alkaloids derived from this ring system are very common in the Amaryllidaceae. The 5,10b-ethanophenanthridine type alkaloids are further subdivided into the crinine type alkaloids and the haemanthamine type alkaloids.

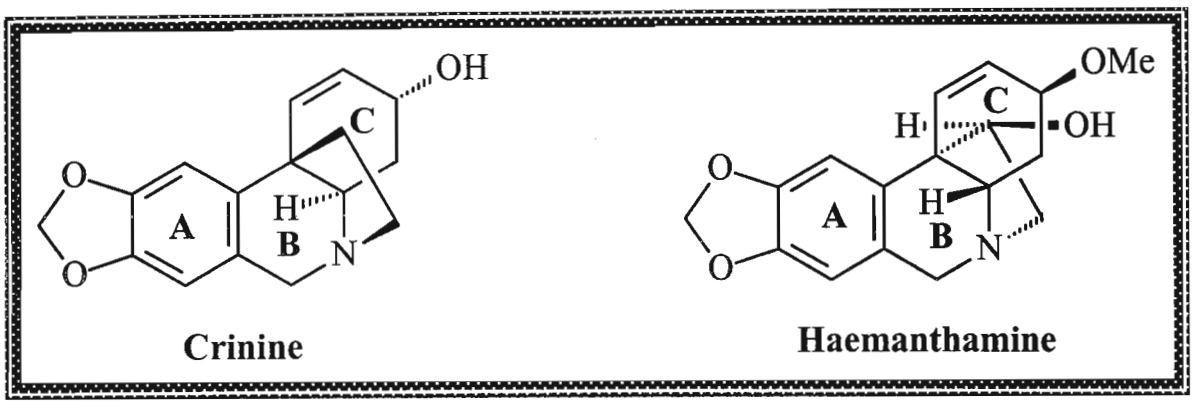


Figure 1.6: Alkaloids of the 5,10b-ethanophenanthridine type

The distinction between the crinine and the haemanthamine type alkaloids lies in the orientation of the 5-10b ethano bridge. In the former type of alkaloids, the bridge is in a β-orientation, while in the latter, it is in an α-position. Hydroxylations, hydrogenation, methylation, acetylation and inversion of the stereochemical configurations of substituents in crinine and haemanthamine result in the numerous 5,10b-ethanophenanthridine type alkaloids.

5. The [2]benzopyrano[3,4c]indole type of alkaloids.

[2]Benzopyrano[3,4c]indole type alkaloids have four rings. Ring A is aromatic with a methylenedioxy substituent. Ring B has a heterocyclic oxygen atom. Ring C is a six membered ring with a allylic methoxyl group. Ring D has a heterocyclic nitrogen atom and this nitrogen atom is methylated. Tazettine may be an artefact, which comes from pretazettine [13].

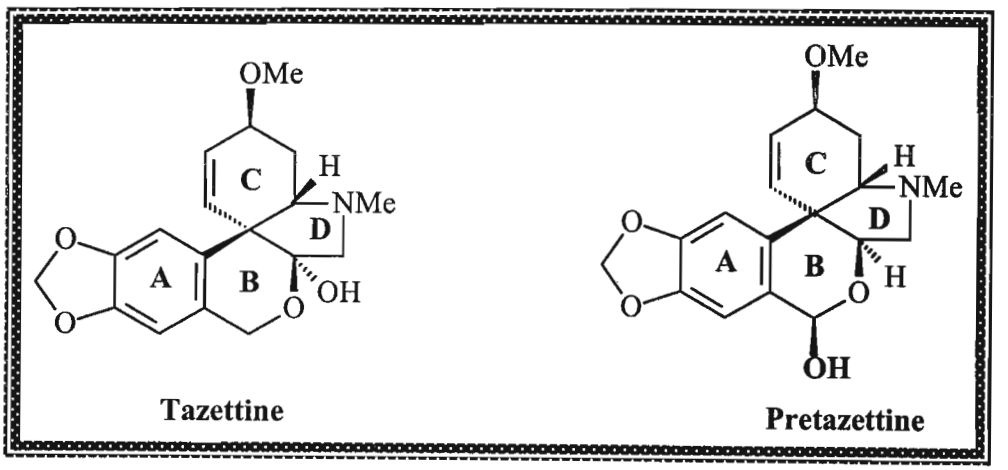


Figure 1.7: Alkaloids of the [2]benzopyrano[3,4c]indole type.

6. The montanine type alkaloids.

The alkaloids of this group are distinguished by their seven membered nitrogen containing B ring. The α or β nitrogen bridge across the B ring is also distinctive in these type of compounds.

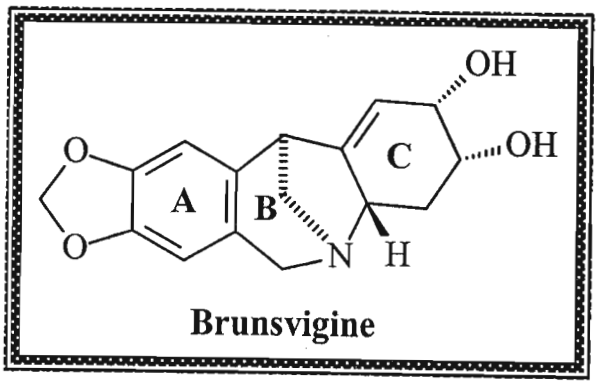


Figure 1.8: An alkaloid of the montanine type

7. The isocarbostyril/narciclasine type alkaloids.

This group of alkaloids has only three rings and usually a methylenedioxy substituent on ring A. These alkaloids can be distinguished by an amide group in ring B. Variations arise from the different substituents on ring C.

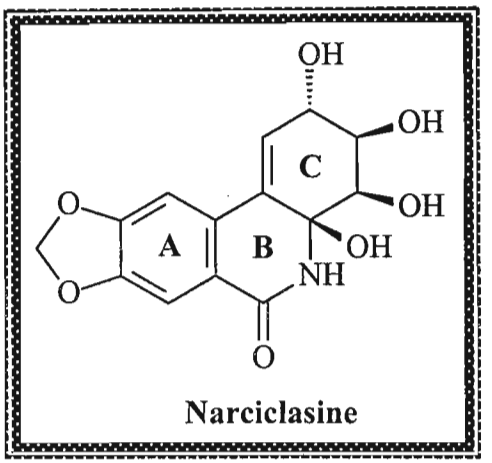


Figure 1.9: An alkaloid of the isocarbostyril/narciclasine type

8. Miscellaneous type alkaloids.

Alkaloids which cannot be grouped on the basis of the parent ring systems of groups one to seven, are placed here. These compounds are considered as being either trapped biosynthetic intermediates, rearrangement products related to known alkaloids or possible artefacts [13].

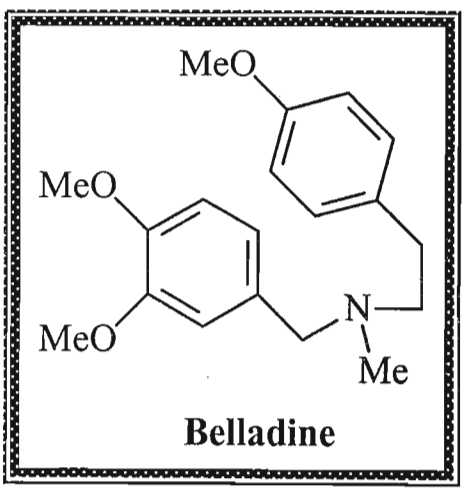


Figure 1.10: An alkaloid of the miscellaneous type.

1.2.3 BIOSYNTHESIS OF AMARYLLIDACEAE ALKALOIDS

Two important organic reactions play a vital role in the biosynthesis of alkaloids [14]. The first reaction is the Mannich reaction. This reaction involves the condensation of an amine, an aldehyde and a carbanion .

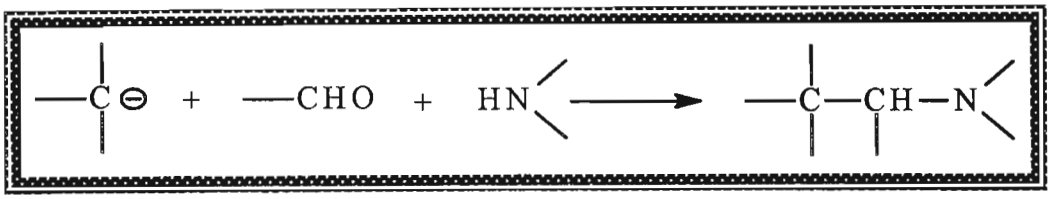
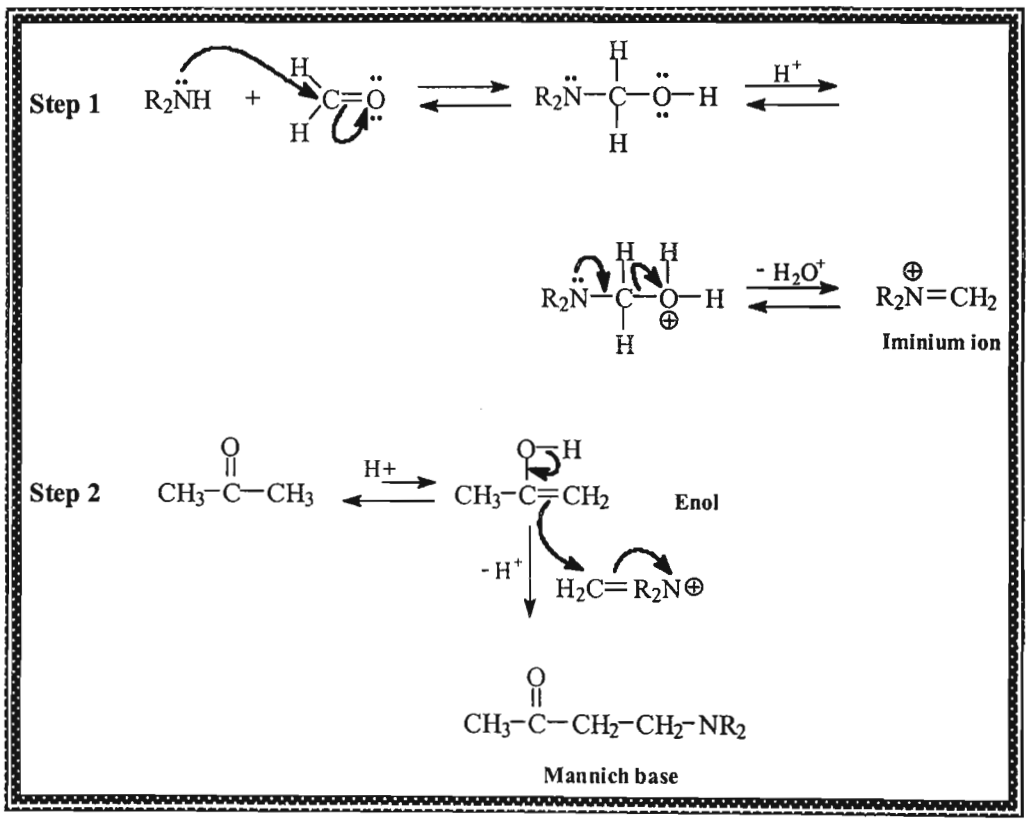


Figure 1.11: The Mannich reaction.

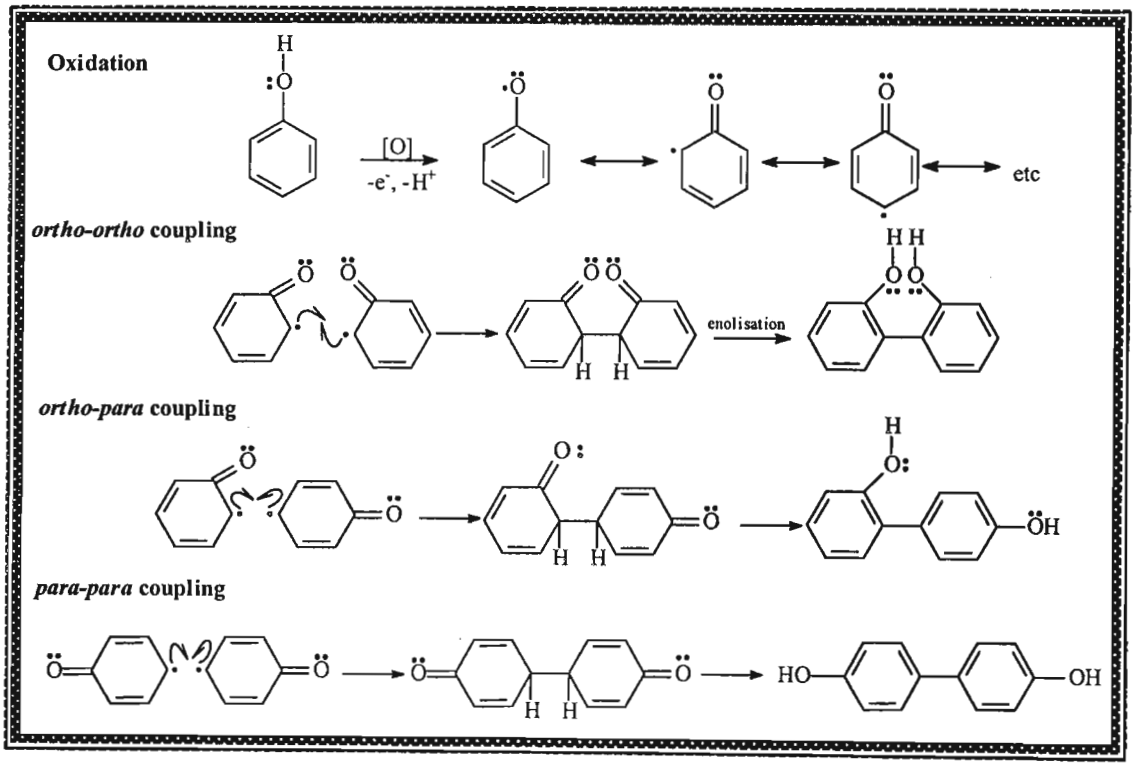
Initially (**step1**) formaldehyde reacts with a secondary amine to yield an iminium ion and subsequently (**step 2**) the iminium ion reacts with the enol form of an active hydrogen compound to form a Mannich base (**scheme 1.1**) [14].



Scheme 1.1: The mechanism of the Mannich reaction using acetone [14].

The second reaction is the oxidative coupling of phenols. This reaction is of particular importance in the biosynthesis of Amaryllidaceae alkaloids. The oxidative coupling of phenols is a free radical process that is catalyzed by enzymes [14].

Loss of an electron and a proton from phenol leads to a resonance-stabilized free radical. Two free radicals can then undergo coupling in several ways **(scheme 1.2)** [14]. Usually oxidative coupling occurs intramolecularly **(figure 1.13)**.



Scheme 1.2: Phenolic oxidative coupling [14].

In 1957 Barton and Cohen suggested that the Amaryllidaceae alkaloids were all related to an intermediate which is now known as norbelladine [15,16,17]. Tracer experiments have proved this to be correct [16,17]. The norbelladine structure arises from tyrosine and phenylalanine, which confer the C_6-C-C and the C_6-C components respectively [17,18,19,20].

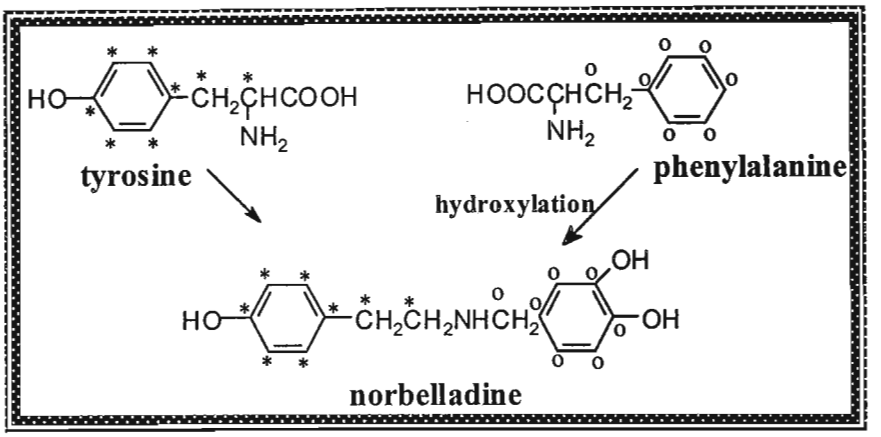
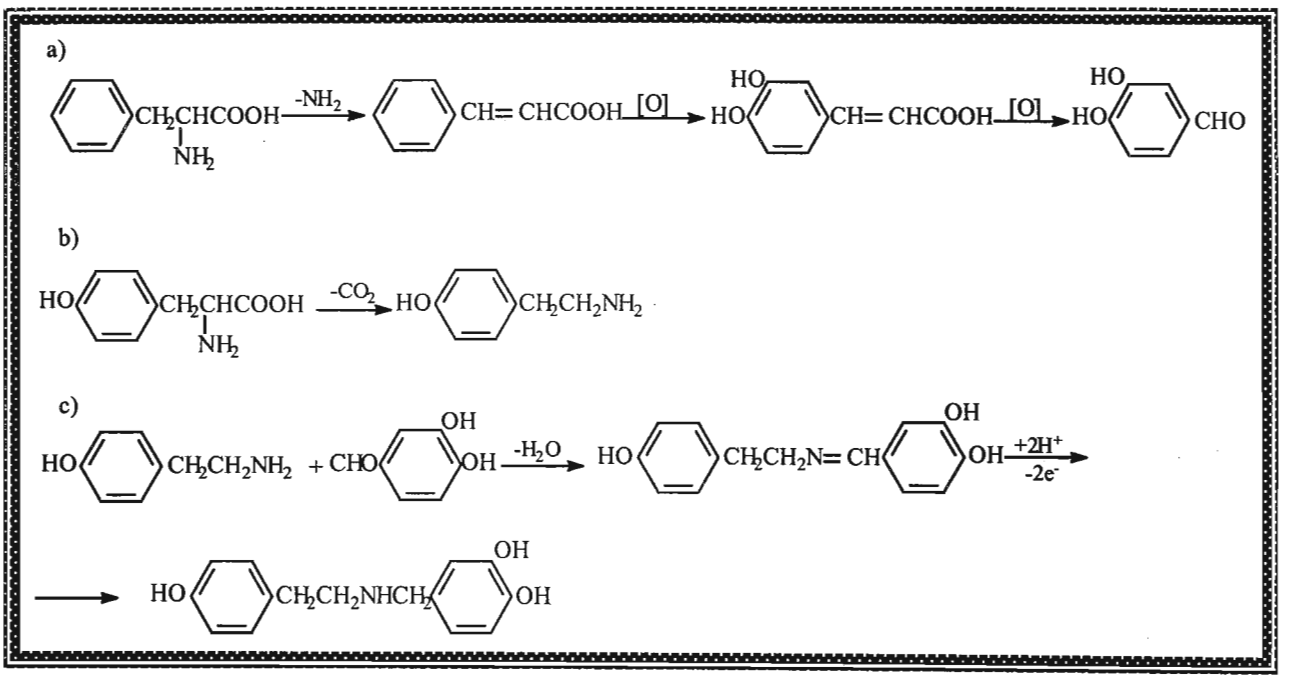


Figure 1.12: Norbelladine and its structural precursors.

In some plants, norbelladine may readily arise from phenylalanine by the hydroxylation of derived metabolites such as cinnamic acid **(scheme 1.3)** [20].



Scheme 1.3: Norbelladine from phenylalanine [20].

Barton and Cohen, were the first authors to propose a mechanism for the union of pathways, leading to the various types of alkaloid structures encountered in the Amaryllidaceae family [20]. They postulated the formation of three basic skeletons (**figure 1.13**); each arising by an alternate mode of coupling of their common precursor, norbelladine. All three pathways are now known to be correct and can be easily demonstrated through tracer investigations [15,16,20].

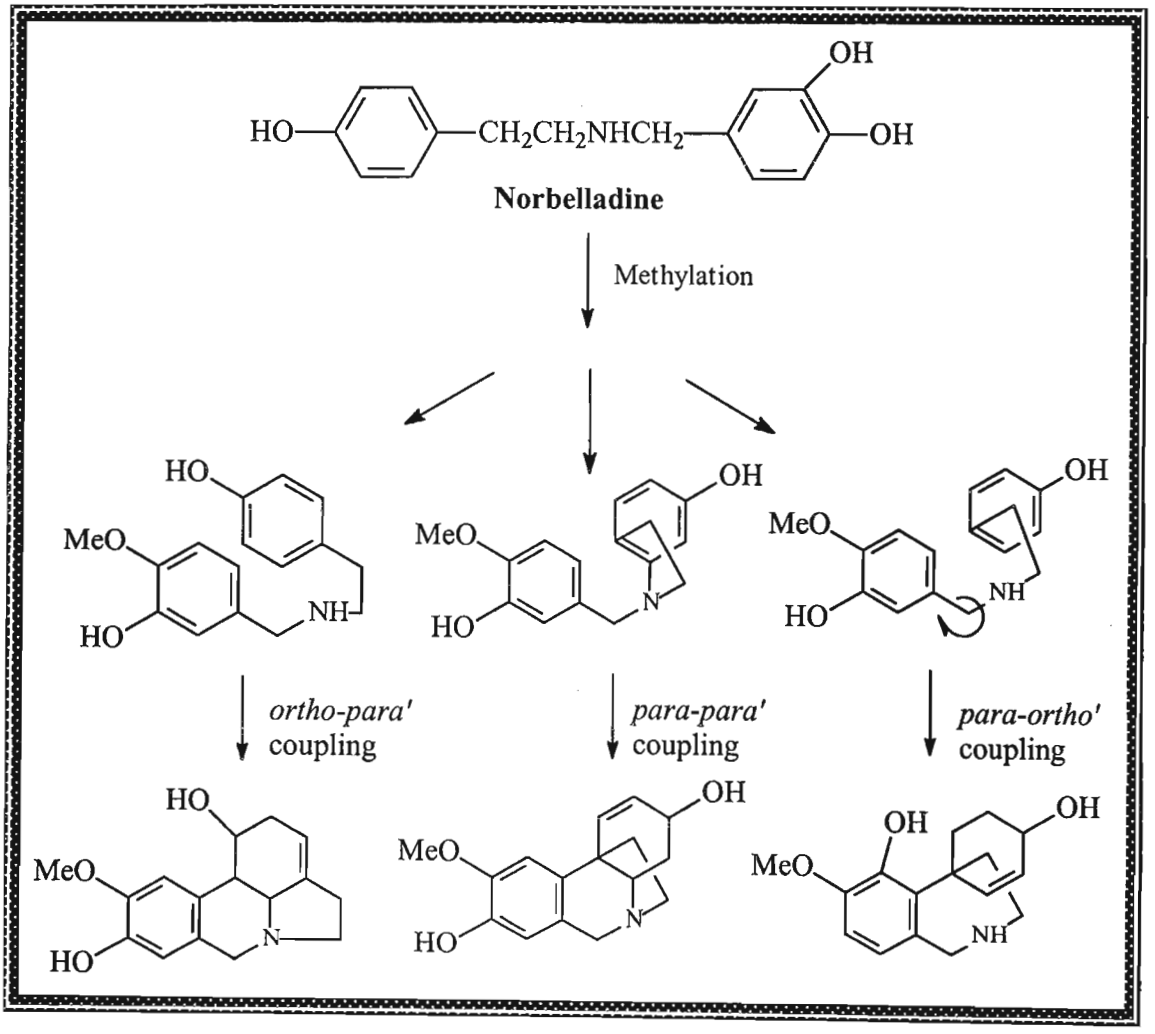
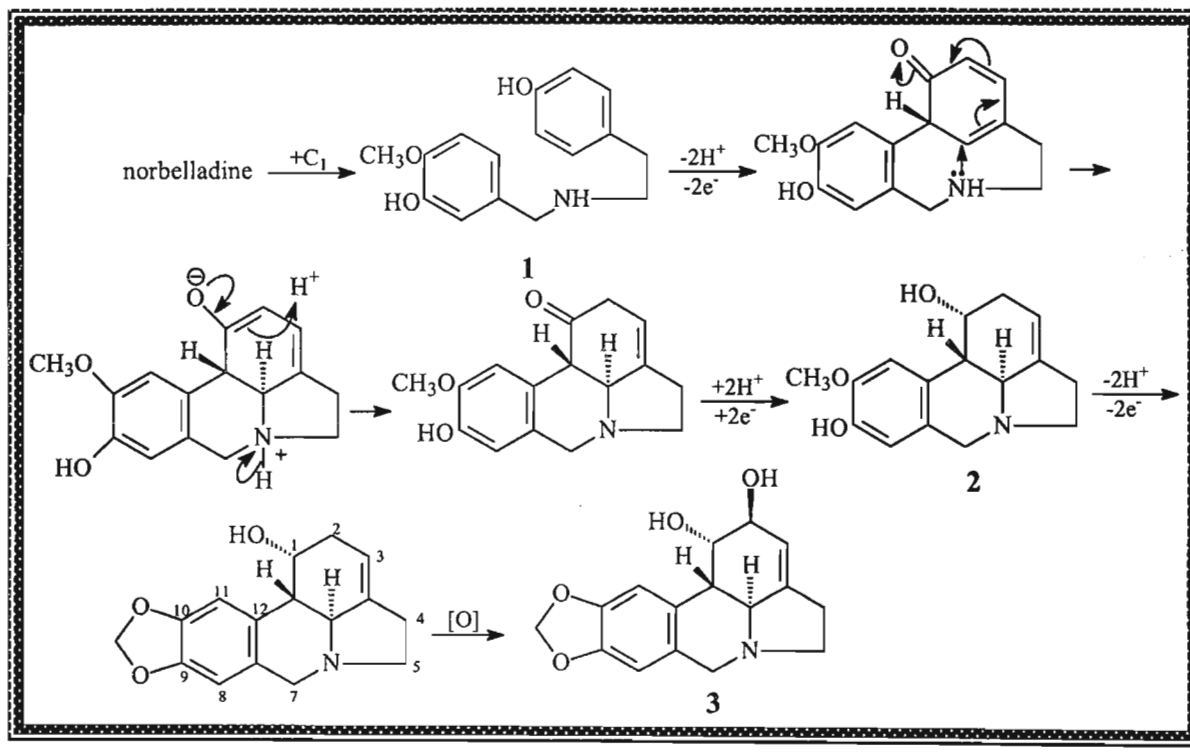


Figure 1.13: The three pathways leading to the Amaryllidaceae alkaloids [20].

Since, only alkaloids of the lycorine, crinine, haemanthamine and tazettine type were isolated in this investigation, only their biosynthesis will be discussed here in detail.

The biosynthesis of lycorine.

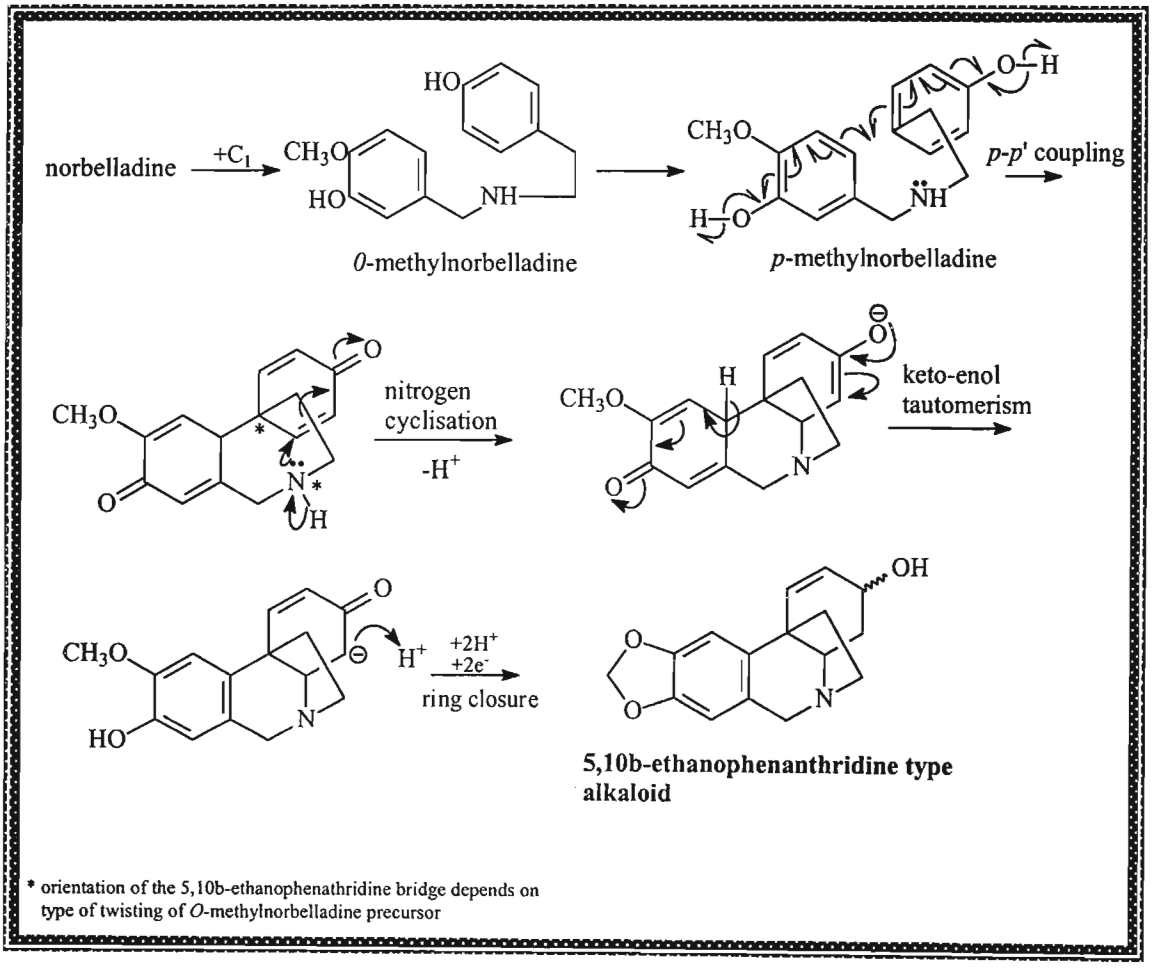
Lycorine type alkaloids arise from norbelladine (**scheme 1.4)** [20]. Norbelladine is first methylated to *O*-methylnorbelladine (**1**) and this is then followed by *ortho-para* oxidative coupling. The pathway involves the formation of a carbon - nitrogen bond, which occurs by the addition of the nitrogen atom to a dienone system. This leads to the formation of norpluvine (**2**). The methylenedioxy group is formed by the ring closure between the hydroxyl group and the adjacent methoxyl group in norpluvine (**2**). Finally hydroxylation at C-2 yields lycorine (**3**). It can be proven by tritium labeling, that the hydroxylation at C-2 proceeds so as to yield a β-orientated hydroxy group [19].



Scheme 1.4: The biosynthesis of lycorine [20].

The biosynthesis of 5,10b-ethanophenanthridine type alkaloids.

As in the biosynthesis of lycorine, *O*-methylnorbelladine is the precursor for the biosynthesis of 5,10b-ethanophenathridine type alkaloids (**scheme 1.5**) [17,20,21]. The difference here is that an alternate mode of coupling can be observed i.e. *para-para* instead of *ortho-para* oxidative coupling. The basic framework is completed by the nitrogen adding to the dienone system as was the case for lycorine biosynthesis. The resulting molecule has an ethane bridge between the nitrogen atom (position 5) and C-10b.

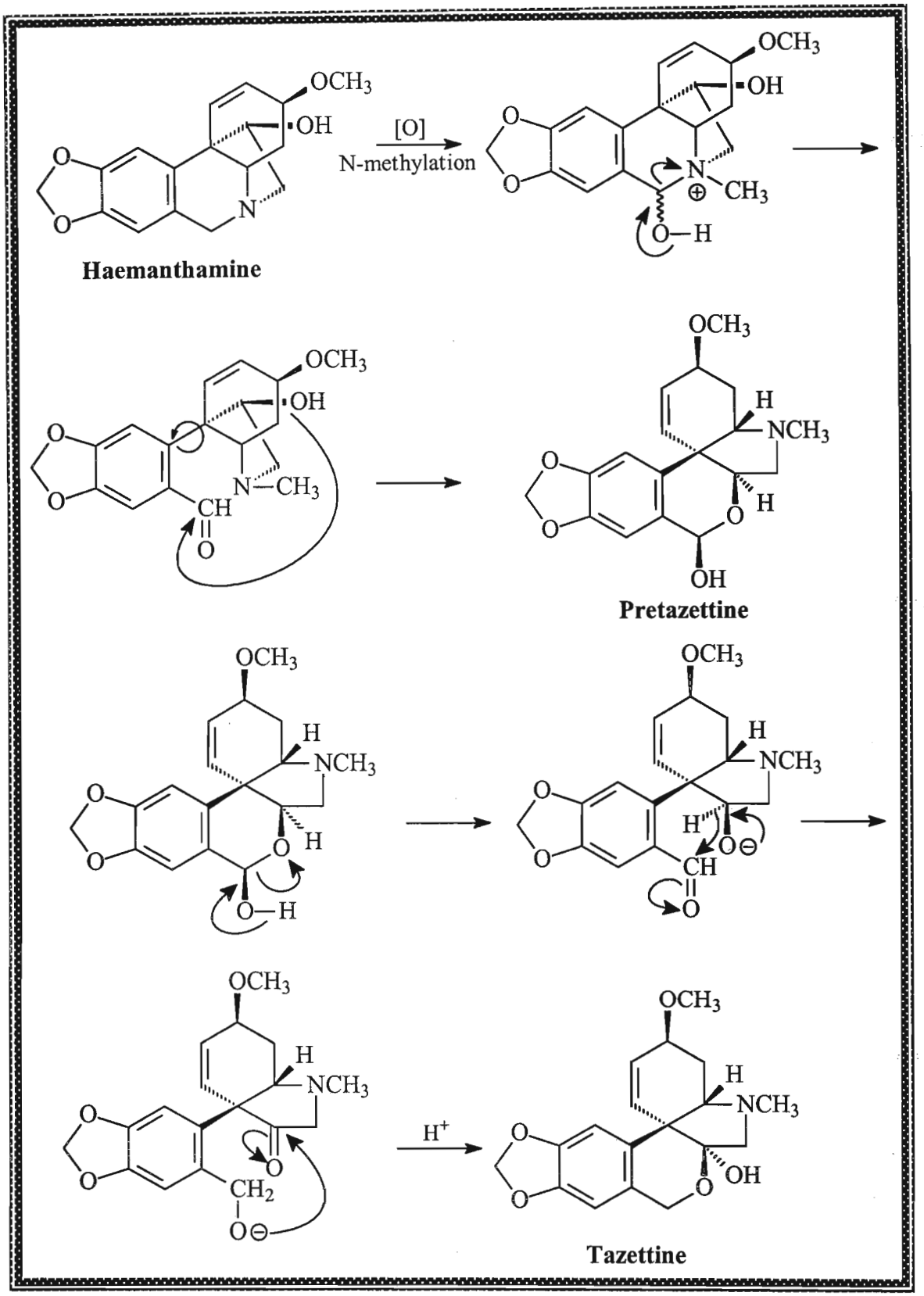


Scheme 1.5: The biosynthesis of crinine and haemanthamine type alkaloids [17,20,21].

The ethane bridge can have either an α or a β orientation depending on the manner in which *O*-methylnorbelladine twists. Consequently, two different series of alkaloids arise within the 5,10b-ethanophenathridine type alkaloids. *O*-methylnorbelladine twisting which leads to compounds with the β orientated ethane bridge constitute the crinine series of alkaloids, whilst those with the α orientated 5,10b ethanoic bridge are referred to as the haemanthamine type alkaloids. Hydroxylation at C-11 of these compounds is difficult to explain as this site lacks any formal activation. However such examples are not infrequent in secondary plant metabolism [20].

The biosynthesis of tazettine.

Haemanthamine is converted *via* the alkaloid haemanthidine into pretazettine (**scheme 1.6**). Pretazettine then undergoes rearrangement, under mildly basic conditions, to give tazettine. Tazettine is considered, by some, to be an artefact, arising from the true alkaloid, pretazettine [13,22]. The conversion of pretazettine into tazettine can be rationalized as an internal crossed-Cannizzaro reaction [20].



Scheme 1.6: The biosynthesis of tazettine from haemanthamine [20]

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CHAPTER 2

Extractives from *Brunsvigia radulosa*

2.1 A REVIEW OF PREVIOUS WORK

The genus *Brunsvigia* (Amaryllidaceae) is one of ten genera of the tribe Amaryllideae [1,2]. *Brunsvigia* is distributed over both summer and winter rainfall regions [3]. It grows profoundly in the winter rainfall area of South Africa [1,2]. The characteristics of *Brunsvigia* include large bulbs, prostrate or widely spreading leaves, large inflorescence, round flowers on large stalks and curved stamens. There are approximately sixteen species in South Africa [4].

Decoctions of the bulbs of *Brunsvigia grandiflora* are used by the Zulu as an oral treatment for coughs and colds and as enemas for renal and liver complaints [5]. The outer skin of the bulb of this plant is used by the Xhosa as a circumcision wound dressing and it is reported to promote very rapid healing [5,6]. The bulbs of *Brunsvigia minor* are also used as purgatives. In addition to this, bulb infusions are used for abdominal ailments [6,7].

Rock paintings of *Brunsvigia radulosa* have been found in the Caledon River Valley area, in Western Lesotho [8]. The significance of this arises from the fact that plants were often painted to express their magical and / or medicinal properties as opposed to their food value [9]. It is believed that *Brunsvigia radulosa* is a plant which can induce psychoactive states when it is consumed [8]. A decoction of *Brunsvigia radulosa* is used by the Southern Sotho as a remedy for barrenness [7]. It is also known that excessive ingestion can cause necrodegenerative hepatitis [5]. The pharmacological activity of the plant extract of *Brunsvigia radulosa* was shown to be effective against the murine P-388 lymphocytic leukemia *in vivo* [1,10].

The chemical examination of the bulbs of *Brunsvigia radulosa* has revealed the presence of alkaloids [7,54]. The main alkaloid was brunsvigine **(5)**. It was observed that summer bulbs yield brunsvigine (**5**), brunsvinine (structure unknown) and crinamine (**8**) whereas autumn bulbs yield brunsvigine (**5**), crinamine (**8**) and lycorine (**1**) [7]. **Table 2.1** shows the *Brunsvigia* species examined and the alkaloids identified from them.

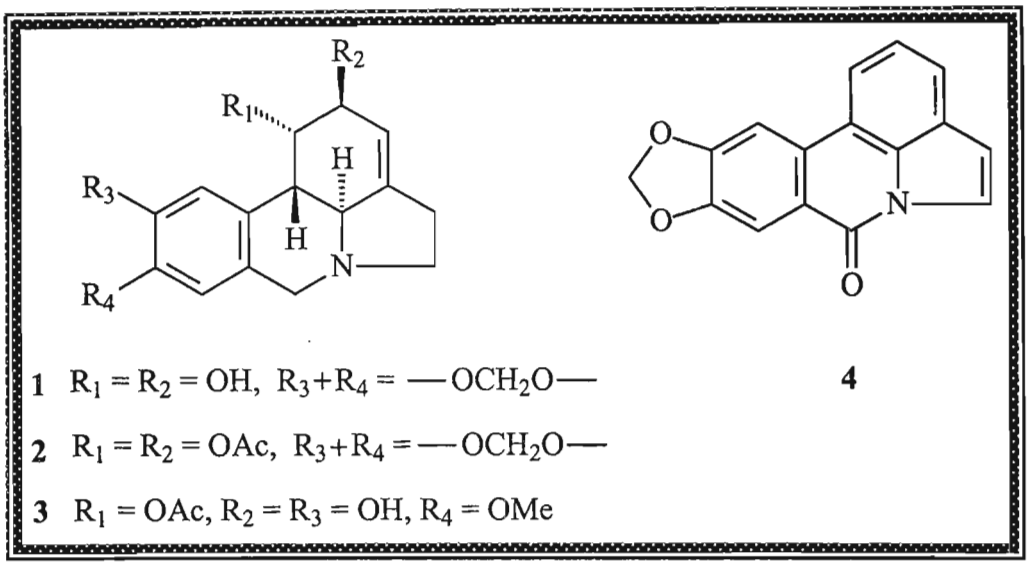
Table 2.1: Previously identified alkaloids from *Brunsvigia*

Brunsvigia Species examined	Alkaloids identified	Reference
B. bosmaniae	galanthamine (**6**)	50
B. comptonii	galanthamine (**6**)	50
B. cooperi	brunsvigine (**5**)	51
	crinamine (**8**)	51
	lycorine (**1**)	51
B. gregaria	crinamidine (**23**)	52
	crinine (**13**)	52
	flexinine (**21**)	52
	galanthamine (**6**)	50
	lycoramine (**7**)	52
	lycorine (**1**)	52
	undulatine (**24**)	52
B. josephinae	brunsbelline (**17**)	36
	buphanidrine (**16**)	36
	buphanisine (**14**)	36
	crinamine (**8**)	43
	crinine (**13**)	36
	galanthamine (**6**)	50
	hamayne (**10**)	43
	3-*O*-acetylhamayne (**11**)	43
	hippadine (**4**)	36
	joesphinine (**21**)	43
	sternbergine (**3**)	43
	undulatine (**24**)	36
B. litoralis	crinine (**13**)	5
	galanthamine (**6**)	50
	lycorine (**1**)	5
	diacetyllycorine (**2**)	5

Brunsvigia Species examined	Alkaloids identified	Reference
B. orientalis	1-epibowdensine (**18**)	39
	1-epideactylbowdensine (**19**)	39
	1-epidemethoxy-bowdensine (**20**)	39
	buphanidrine (**16**)	39
	epibuphanisine (**15**)	39
	buphanisine (**14**)	39
	crinamidine (**23**)	39
	crinamine (**8**)	39
	6-hydroxycrinamine (**9**)	39
	crinine (**13**)	39
	lycorine (**1**)	39
	undulatine (**24**)	39
B. radulosa	brunsvigine (**5**)	7
	galanthamine (**6**)	50
	lycorine (**1**)	7
	crinamine (**8**)	7

The bulb of an unidentified South African species of *Brunsvigia* is reported to contain a haemolytic sapogenin [7]. The alkaloids identified from *Brunsvigia* can be classified, to date, into four distinct alkaloid types: the lycorine type, the haemanthamine type, the montanine type and the galanthamine type.

The lycorine type



1 $R_1 = R_2 = OH$, $R_3+R_4 =$ —OCH_2O—

2 $R_1 = R_2 = OAc$, $R_3+R_4 =$ —OCH_2O—

3 $R_1 = OAc$, $R_2 = R_3 = OH$, $R_4 = OMe$

4

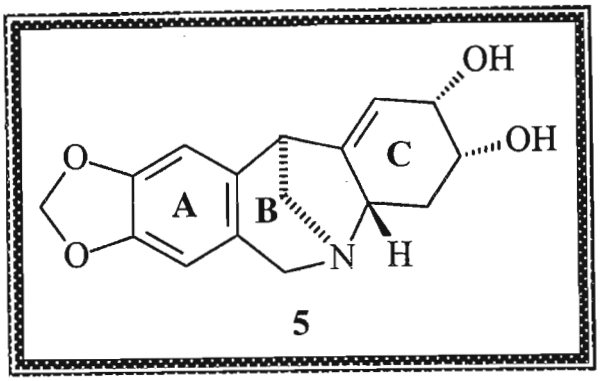
Figure 2.1: Previously isolated lycorine type compounds from *Brunsvigia.*

Lycorine (**1**) is a widely distributed alkaloid in the Amaryllidaceae family and it has been isolated from over thirty genera. There has been substantial interest in the synthesis of lycorine and its derivatives [11]. Among the phenanthridine alkaloids, lycorine (**1**) is considered to be the most important because of its biological activity [12]. The bioactivity of lycorine and lycorine type alkaloids, previously isolated from South African species of *Brunsvigia*, are given in **table 2.2**.

Table 2.2: The bioactivity of lycorine type alkaloids from *Brunsvigia*.

Alkaloid	Activity	Reference
Lycorine (1)	Inhibition of growth and cell division in higher plants and algae.	13
	Inhibits cyanide – resistant respiration and peroxidase enhancement.	13, 14
	Inhibits ascorbic acid biosynthesis *in vivo*.	13, 15
	Active against RNA and DNA viruses. Inhibits the growth of Herpes simplex type I, poliomyelitis, coxsackie, Semliki Forest and measles virus.	16, 17,18,19
	Weak protozoicide.	13, 16
	Increases contractility and rate of isolated perfused heart. Effects are mediated by stimulation of β - adrenergic receptors.	20
	Moderate anti – tumoural activity.	16, 20
	Inhibitor of HeLa cells growth.	21
	Decreases the growth of viruses by inhibiting protein synthesis.	17, 18, 22
	Respiratory stimulant.	16
1,2-Di-*O*-acetyllycorine (2)	The acetylation of lycorine causes either reduced activity or total loss of activity.	13
Sternbergine (3)	Inhibits ascorbic acid biosynthesis, but to a markedly lesser extent than lycorine.	13
Hippadine (4)	Acts on the germ cells in their earlier stages of spermatocytogenesis. Reversibly inhibits fertility in male rats.	23

The montanine type

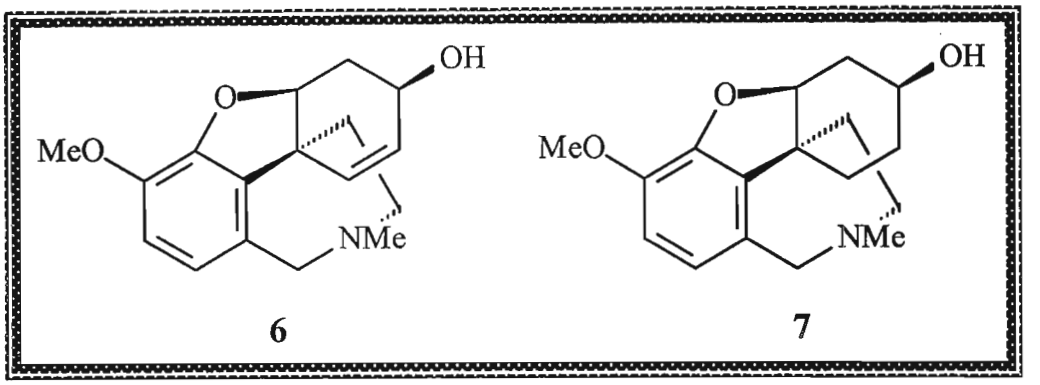


5

Figure 2.2: Previously isolated montanine type alkaloid from *Brunsvigia.*

Only one alkaloid of the montanine type has been isolated from *Brunsvigia*. Brunsvigine (**5**) has been isolated, together with brunsvinnine, crinamine and lycorine from *Brunsvigia cooperi* [19].

The galanthamine type

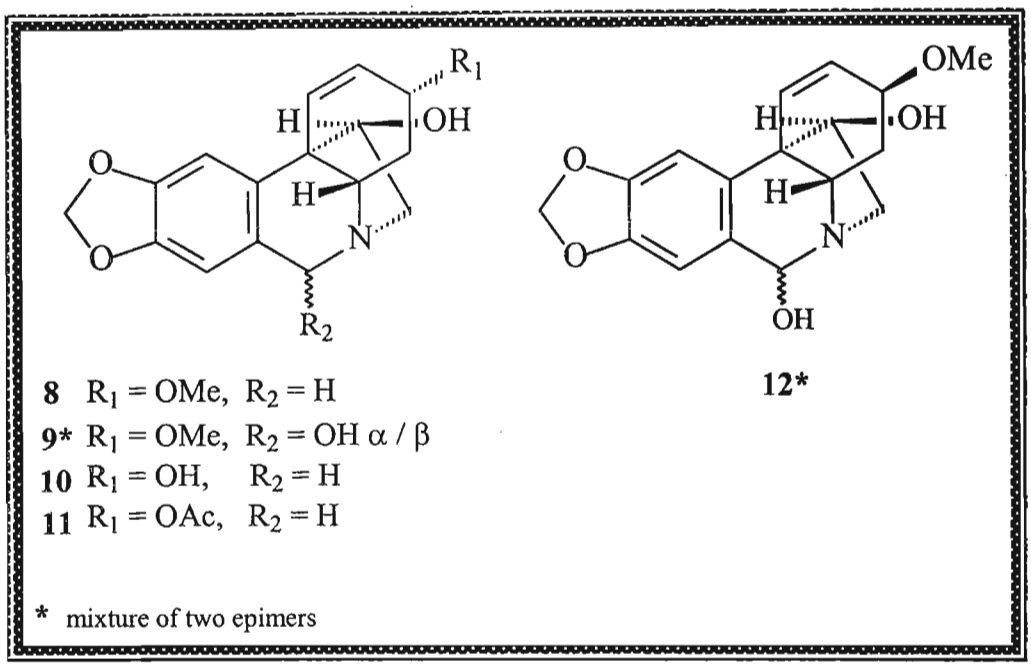


6 7

Figure 2.3: Previously isolated galanthamine type alkaloids from *Brunsvigia.*

Galanthamine (**6**) and lycoramine (**7**) have been isolated from *Brunsvigia* species. Galanthamine is used for its anticholinesterase activity in the treatment of disturbances in the peripheral sympathetic synaptic transmission [24]. It is also used in pharmaceutical drugs for the treatment of alcoholism and Alzheimer's disease [16]. Lycoramine produces acute poisoning of the cardiovascular, neuromuscular and central nervous system [25].

The haemanthamine type



8 R_1 = OMe, R_2 = H
9* R_1 = OMe, R_2 = OH α / β
10 R_1 = OH, R_2 = H
11 R_1 = OAc, R_2 = H

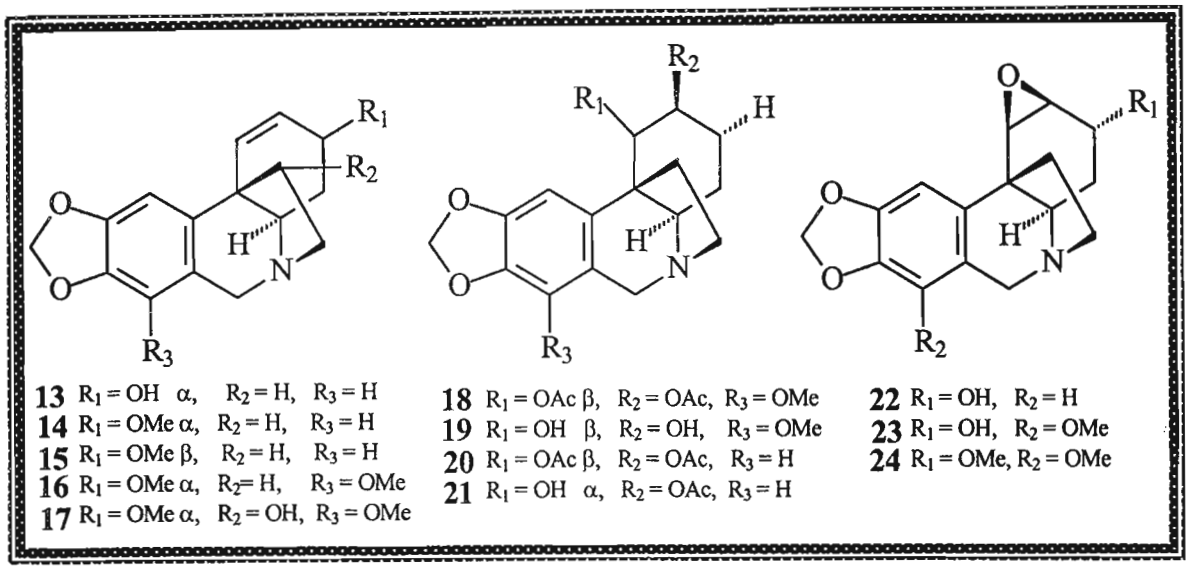
12*

* mixture of two epimers

Figure 2.4: Previously isolated haemanthamine type alkaloid from *Brunsvigia.*

Crinamine (**8**), is a powerful transient hypotensive agent in dogs. It shows respiratory depressant activity [16,19]. Crinamine has also shown strong cytotoxic and moderate antimalarial activity [26]. 6-Hydroxycrinamine (**9**) is isolated as a mixture of isomers. Hamayne (**10**) and 3-*O*-hamayne (**11**) are well known alkaloids in the Amaryllidaceae family. Haemanthidine (**12**) is active against A-431, KB, Lu1, Me12 and ZR-75-1 cell lines. It has shown significant activity against LnCaP and HT cell lines [27].

The crinine type



13 R_1 = OH α, R_2 = H, R_3 = H
14 R_1 = OMe α, R_2 = H, R_3 = H
15 R_1 = OMe β, R_2 = H, R_3 = H
16 R_1 = OMe α, R_2 = H, R_3 = OMe
17 R_1 = OMe α, R_2 = OH, R_3 = OMe

18 R_1 = OAc β, R_2 = OAc, R_3 = OMe
19 R_1 = OH β, R_2 = OH, R_3 = OMe
20 R_1 = OAc β, R_2 = OAc, R_3 = H
21 R_1 = OH α, R_2 = OAc, R_3 = H

22 R_1 = OH, R_2 = H
23 R_1 = OH, R_2 = OMe
24 R_1 = OMe, R_2 = OMe

Figure 2.5: Previously isolated crinine type alkaloids from *Brunsvigia*

Crinine (**13**), buphanisine (**14**), epibuphanisine (**15**), buphanidrine (**16**), brunsbelline (**17**), 1-epibowdensine (**18**), 1-epideacetylbowdensine (**19**), 1-epidemethoxy-bowdensine (**20**), josephinine (**21**), flexinine (**22**), crinamidine (**23**) and undulatine (**24**) have been isolated from *Brunsvigia* (**Table 2.1**).

Crinine shows weak analgesic activity in mice and is a tachycardiac agent in dogs [16]. All of the above alkaloids exhibit some analgesic activity and this is attributed to their resemblance to morphine and codeine skeletons [28]. The above alkaloids, however, are quite toxic.

2.2 Results and Discussion

In a previous investigation of *Brunsvigia radulosa* only four alkaloids, brunsvigine (**5**), galanthamine (**6**), lycorine (**1**) and crinamine (**8**), have been isolated [7]. *Brunsvigia radulosa* was thought to posses more alkaloids than reported and hence further investigation was required. Of particular importance was the potent inhibitory activity of *Brunsvigia radulosa* against the P-388 lymphocytic leukemia. This investigation was carried out to isolate novel alkaloids that might manifest significant bioactivity and thus be medicinally beneficial. The ethanol extract of the bulbs yielded six alkaloids, lycorine (**I**), 1-*O*-acetyllycorine (**II**), crinine (**IV**), crinamine (**V**), hamayne (**VI**) and anhydrolycorinium chloride (**VII**), after separation by column chromatography over silica gel. Lycorine (**I**) was acetylated to yield 1,2-di-*O*-acetyllycorine (**III**).

Four alkaloids which were not reported before from *Brunsvigia radulosa*, are reported here, including the potent antineoplastic agent anhydrolycorinum chloride (**VII**).

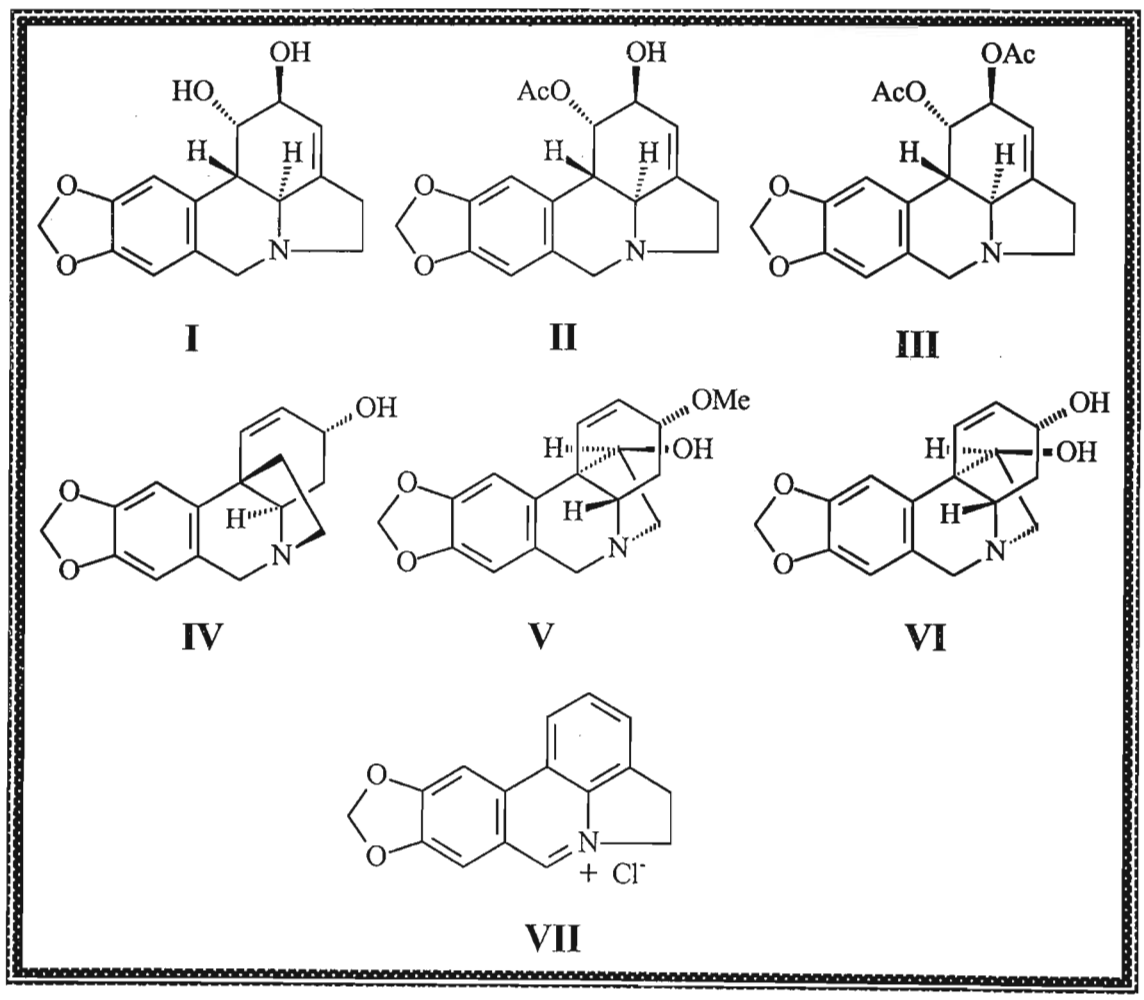


Figure 2.6: Compounds isolated from *Brunsvigia radulosa*

2.2.1 The Structural elucidation of compound I

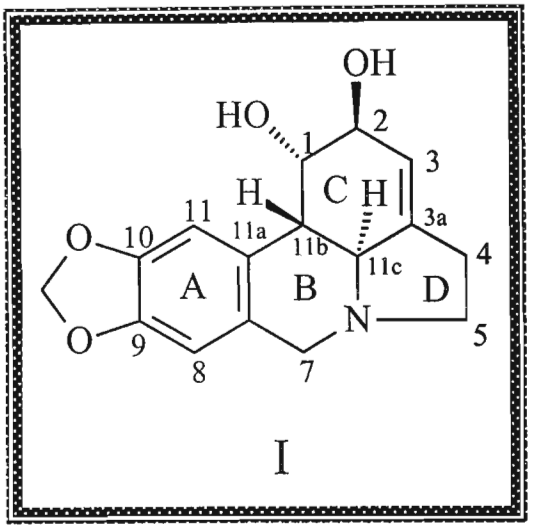
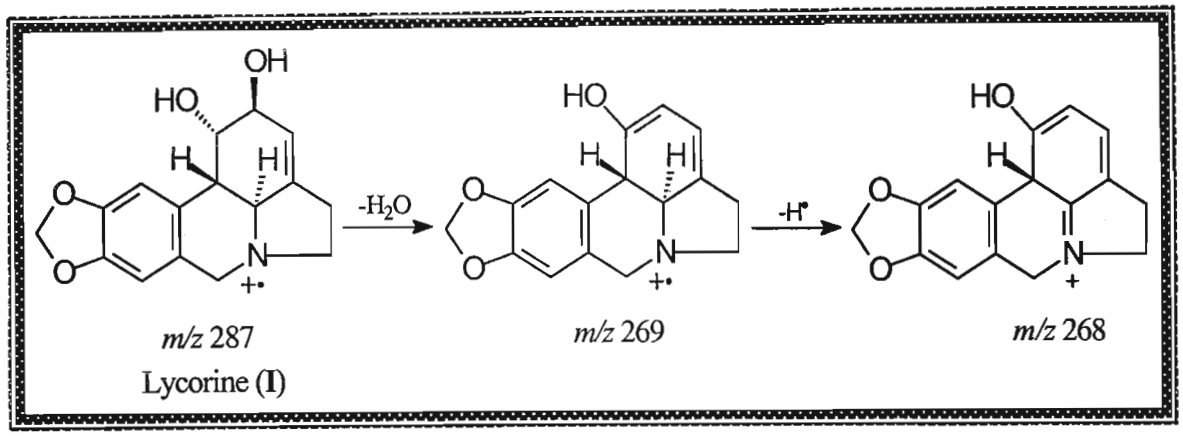


Figure 2.7: COMPOUND I: lycorine

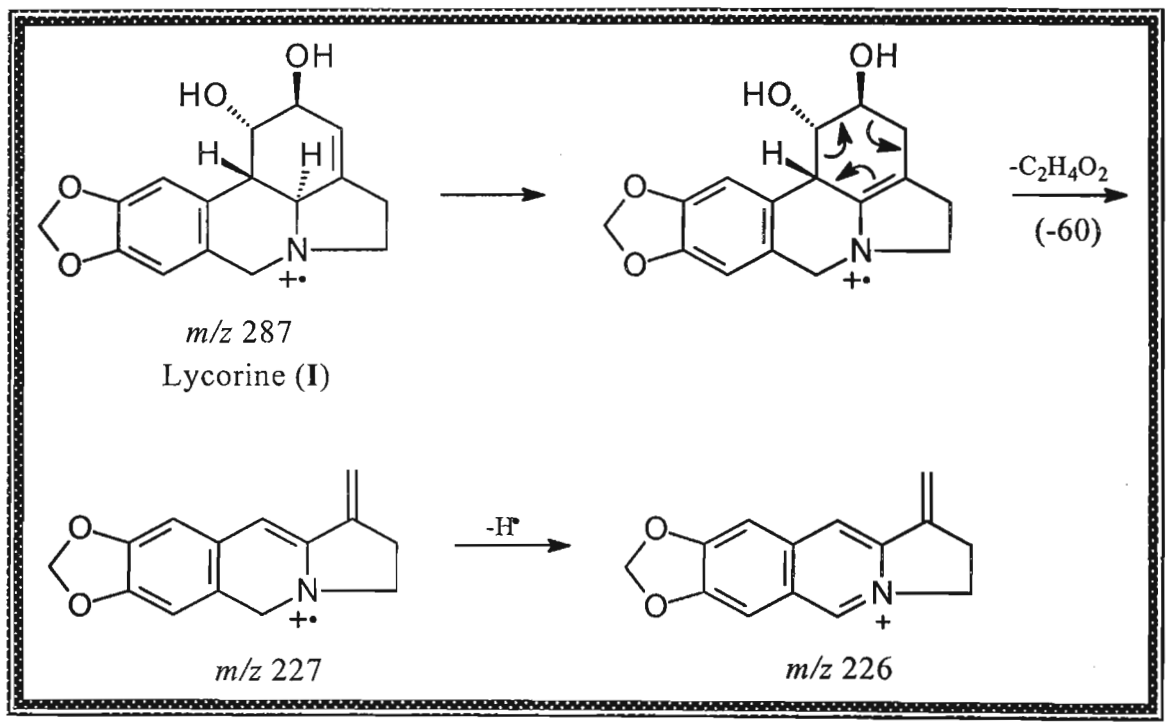
Lycorine (**I**) was isolated as a white crystalline material. Lycorine has been isolated previously from *Brunsvigia radulosa* [7]. Lycorine is the most widespread alkaloid of the Amaryllidaceae [16]. It exhibits extensive bioactivity (see preceding chapter). Spectra for lycorine are given on pages 153-157 in appendix A.

HRMS showed an intense molecular ion peak at *m/z* 287.1449, which corresponds to the molecular formula $C_{16}H_{17}NO_4$. Twin base peaks occur at *m/z* 227 (M-60) and *m/z* 226 (M-61). The only other fragment of reasonable intensity occurs at *m/z* 268 (M-19). This peak is due to the loss of water as well as a hydrogen atom (**scheme 2.1**). The ease of the loss of water from the molecular ion has been found to be greatly dependent on the stereochemistry of the C-2 hydroxyl group i.e. a C-2α hydroxyl group is more easily lost than a C-2β hydroxyl group [29]. Thus in the mass spectrum of lycorine (**I**), the relative intensity of this peak is low, while in 2-epilycorine it is the base peak [29].



Scheme 2.1: Mass fragmentation pattern of lycorine (I) [29]

The twin base peaks at *m/z* 227 (M-60) and *m/z* 226 (M-61) were shown to result from the loss of carbon atoms C-1 and C-2 and their substituents, by a reverse Diels Alder fragmentation (**scheme 2.2**) [29]. This fragmentation pattern is common to all 3, 3a – unsaturated lycorine type alkaloids.



Scheme 2.2: Mass fragmentation pattern of lycorine (I) [29]

The infra red spectrum showed a band at ν_{max} 3330 (O - H stretching) indicative of hydroxyl groups. Sharp bands were also observed at 1044 cm^{-1} and 1004 cm^{-1} (C - O stretching). Bands at 1505 cm^{-1} and 1498 cm^{-1} (aromatic C = C stretching) suggested an aromatic ring. Bands observed at 1274 cm^{-1} and 1241 cm^{-1} (C – N stretching) are characteristic for C – N bonds. The band at 945 cm^{-1} (C – O – C stretching) is indicative of the methylenedioxy group.

The ^1H NMR spectrum showed two singlets at δ_H 6.91 and δ_H 6.67, integrating to one proton each, for the *para*-oriented aromatic protons. According to Ghosal's review, in the lycorine type alkaloids, the H-11 signal always appears at a lower field than the corresponding H-8 signal [30]. The H-11 proton being near in space to the C-ring, experiences a diamagnetic shift from the unsaturation in that ring [30]. Hence, the signal at δ_H 6.91 was assigned to H-11 and the signal at δ_H 6.67 was assigned to H-8. The singlet at δ_H 5.94 integrates to two protons and was assigned to the two methylenedioxy protons. The broad singlet observed in the olefinic region of the spectrum, at δ_H 5.59, was assigned to H-3. The resonances at δ_H 4.48 and δ_H 4.20, each integrating to one proton, were assigned to H-1 and H-2 respectively. The two benzylic protons, H-7α and H-7β, were observed resonating at δ_H 3.57 (d) and δ_H 4.15 (d) respectively. β-protons at position 5 and 7 are deshielded more than their α - homologues due to the effect of the *cis* – lone pair of electrons of the nitrogen atom [39,40]. The multiplet at δ_H 3.38 and the doublet doublet at 2.48 (J=17.52 Hz, 8.69 Hz) were assigned to H-5β and H-5α respectively and this was consistent with literature. The resonance ascribed to H-11b was observed as a multiplet at δ_H 2.70 and the resonance attributed to H-11c appeared as a doublet at δ_H 2.93 (J=11.20 Hz). The multiplet at δ_H 2.65 was assigned to the two H-4 protons left. All assignments were consistent with literature [32]. Two dimensional spectra for lycorine could not be obtained due to its relative insolubility in common organic solvents. Furthermore lycorine is a relatively well known alkaloid and it is easily identified from its ^1H and ^{13}C NMR spectra.

Table 2.3: ^{1}H NMR data for compound I and literature data for lycorine

(*J* given in Hz in parentheses)

	^{1}H NMR data for COMPOUND I	^{1}H NMR literature data for LYCORINE [15]	^{1}H NMR literature data for LYCORINE [32]
Proton No.	$^{\otimes}$**shift / δ_H ppm**	$^{\otimes\otimes}$ **shift / δ_H ppm**	$^{\otimes}$ **shift / δ_H ppm**
H-1	4.48 s	4.58 dd (1.1, 2.2)	4.50 s
H-2	4.20 brs	4.26 brs	4.20 brs
H-3	5.59 brs	5.77 brs	5.58 brs
H-4	2.65 m	2.88 m	2.65 m
2H-5	2.48 dd (17.52, 8.69) 3.38 m	3.49 m 3.75 m	2.48 dd (17.58, 8.72) 3.38 m
H-7α	3.57 d (14.22)	4.19 d (14.0)	3.60 d (14.28)
H-7β	4.15 d (14.22)	4.48 d (14.0)	4.16 d (14.28)
H-8	6.67 s	6.80 s	6.67 s
H-11	6.91 s	6.98 s	6.91 s
H-11b	2.70 m	2.99 dd (11.8, 2.2)	2.70 m
H-11c	2.93 d (11.20)	3.95 d (11.8)	2.93 d (11.36)
OCH_2O	5.94 s	5.95 s	5.94 s

$^{\otimes}$ ^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum measured in CD_3OD – CD_3COOD (3:1), 270 MHz.

The ^{13}C NMR data observed for compound **I** was consistent with that reported for lycorine in the literature (**table 2.4**). Sixteen signals were observed in the ^{13}C NMR spectrum. Nine signals were observed in the low-field region (> 90 ppm) and these corresponded to the aromatic carbons, the olefinic carbons and the methylene carbon of the methylenedioxy group. The high-field region contained the seven signals corresponding to the saturated carbon resonances of lycorine.

Table 2.4: ^{13}C NMR data for compound I and literature data for lycorine

	^{13}C NMR data for COMPOUND I	^{13}C NMR data for LYCORINE [26]	^{13}C NMR data for LYCORINE [31]
Carbon No.	**$^{\otimes}$ shift / δ_C ppm**	**$^{\otimes\otimes}$ shift / δ_C ppm**	**$^{\otimes}$ shift / δ_C ppm**
C-1	73.1 CH	70.2 CH	73.1 CH
C-2	71.9 CH	71.7 CH	71.9 CH
C-3	119.1 CH	118.5 CH	119.2 CH
C-3a	143.6 C	141.7 C	143.6 C
C-4	29.2 CH_2	28.1 CH_2	29.3 CH_2
C-5	54.5 CH_2	53.3 CH_2	54.7 CH_2
C-7	57.5 CH_2	56.7 CH_2	57.7 CH_2
C-7a	130.4 C	129.6 C	130.4 C
C-8	108.2 CH	107.0 CH	108.2 CH
C-9	148.2 C	145.2 C	148.2 C
C-10	147.7 C	145.7 C	147.7 C
C-11	106.0 CH	105.1 CH	106.0 CH
C-11a	129.7 C	129.6 C	129.7 C
C-11b	41.3 CH	40.2 CH	41.3 CH
C-11c	62.5 CH	60.8 CH	62.5 CH
O$\underline{C}H_2$O	102.2 CH_2	100.6 CH_2	102.3 CH_2

$^{\otimes}$ ^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum measured in DMSO-*d6*, 75.6 MHz.

2.2.2 The structural elucidation of compound II

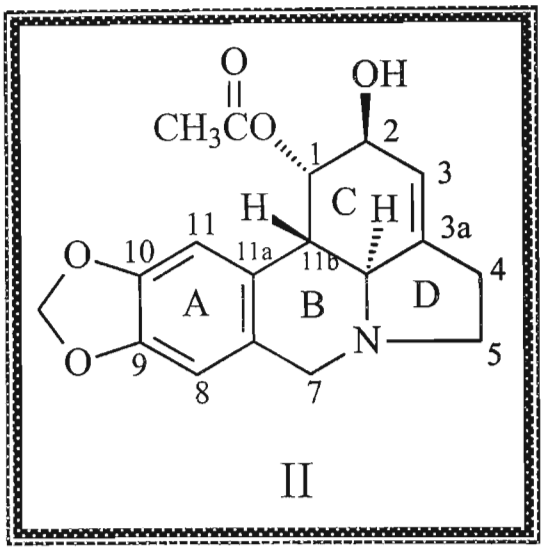


Figure 2.8: COMPOUND II: 1-*O*-acetyllycorine

1-*O*-Acetyllycorine (**II**) was isolated as a white crystalline material. 1-*O*-Acetyllycorine was identified on the basis of its physical and spectroscopic data (**table 2.5** and **table 2.6**). This is the first report of 1-*O*-acetyllycorine in *Brunsvigia radulosa*. Spectra for 1-*O*-acetyllycorine (**II**) are given on pages 158-164 in appendix A.

A molecular ion peak at *m/z* 329.1272 was observed in the mass spectrum of 1-*O*-acetyllycorine and this corresponded to a molecular formulae of $C_{18}H_{19}NO_5$. The mass spectrum contained intense twin peaks at *m/z* 227 (M – 102) and *m/z* 226 (M – 103). These peaks were also observed in the spectrum of lycorine (**I**) and are common to all 3,3a-unsaturated lycorine type alkaloids. As was the case for lycorine, these peaks can be attributed to the loss of C-1 and C-2, together with their substituents (**scheme 2.2**). In addition to the different molecular ions present, the mass spectrum of lycorine differs from that of 1-*O*-acetyllycorine (**II**), in that a peak corresponding to the loss of water (**scheme 2.1**) is not observed in the latter compound. This is consistent with the literature of acetyl derivatives of lycorine [29].

The infra red spectrum showed bands at ν_{max} 945 cm^{-1} (C – O – C) indicating the presence of the methylenedioxy group, 1241 cm^{-1} (C –N stretching) indicating C – N bonds, 1485 cm^{-1} (aromatic C = C stretching) indicative of an aromatic ring, 1051 cm^{-1} and 1004 cm^{-1} (C – O stretching), 1748 cm^{-1} (C = O stretching) indicative of the acetate group and the band at ν_{max} 3440 cm^{-1} (O – H stretching) confirming the presence of an hydroxyl group.

Examination of the ^1H NMR spectrum showed a marked similarity to that of lycorine (**I**). The two singlets at δ_H 6.67 (H-8) and δ_H 6.76 (H-11) and the singlet at δ_H 5.94, which integrated to two protons, indicated the presence of an unaltered ring A and dioxolane ring system. In the same way, the appearance of the two doublets of an AB system at δ_H 3.60 (J=14.28 Hz, a typical value for geminal coupling) and δ_H 4.16 (J=14.28 Hz) and the broad singlet of an olefinic proton at δ_H 5.58 were consistent with the presence of lycorine – like B and C rings. Furthermore, the resonances for H-4 and H-5 were consistent with those of lycorine (**I**) with respect to both the chemical shift and multiplicity.

The difference between the ^1H NMR spectrum of compound **II** and that of lycorine (**I**) was that the former spectrum showed a methyl proton resonance at δ_H 1.93. In addition to this, the broad singlet that appeared at δ_H 4.48 and attributed to H-1 in lycorine, now appeared at δ_H 5.73 in the ^1H NMR spectrum of compound **II**. This was indicative of the hydroxyl group on C-1 being acetylated.

The ^{13}C NMR spectrum of compound **II** showed eighteen signals. The low-field region (>90 ppm) contained the signals of the carbonyl group, the olefinic and aromatic carbons as well as the carbon of the methylenedioxy group. Eight signals corresponding to saturated carbon resonances were found in the high field region.

The ^1H and ^{13}C NMR resonances were assigned by comparison to literature data and were confirmed by 2D NMR techniques (**table 2.5** and **2.6**)

Table 2.5: ^{1}H NMR data for compound II and literature data for 1-*O*-Acetyllycorine [31], (*J* given in Hz in parentheses)

	^{1}H NMR data for compound II	**^{1}H NMR data for 1-*O*- acetyllycorine [31]**
Proton No.	**$^{\otimes}$ shift / δ_H ppm**	**$^{\otimes\otimes}$ shift / δ_H ppm**
H-1	5.73 s	5.50 m
H-2	4.19 brs	4.13 m
H-3	5.58 brs	5.56 m
H-4	2.68 m	2.59 m
H-5	2.50 dd (17.60, 8,80) 3.33 m	2.35 brq (18.50, 8.50) 3.32 ddd (18.50, 8.5, 50.20)
H-7α	3.60 d (14.30)	3.48 d (14.0)
H-7β	4.16 d (14.30)	4.14 d (14.0)
H-8	6.67 s	6.60 s
H-11	6.76 s	6.54 s
H-11b	2.90 brs	2.73 d (10.30)
H-11c	2.90 brs	2.84 d (10.30)
OCH_2O	5.94 s	5.89 s
$OCOCH_3$	1.93 s	1.91 s

$^{\otimes}$ ^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum measured in $CDCl_3$, 270 MHz.

Table 2.6: ^{13}C NMR data for Compound II and literature data for 1-*O*-acetyllycorine [31]

	^{13}C NMR data for compound II	^{13}C NMR data for 1-*O*- acetyllycorine [31]
Carbon No.	⊗ **shift / δ_C ppm**	⊗ **shift / δ_C ppm**
C-1	73.4 CH	73.4 CH
C-2	70.3 CH	70.3 CH
C-3	119.0 CH	119.0 CH
C-3a	143.8 C	143.8 C
C-4	29.3 CH_2	29.3 CH_2
C-5	54.6 CH_2	54.6 CH_2
C-7	57.6 CH_2	57.6 CH_2
C-7a	130.4 C	130.4 C
C-8	108.4 CH	108.4 CH
C-9	148.1 C	148.1 C
C-10	147.9 C	147.9 C
C-11	105.7 CH	105.7 CH
C-11a	128.3 C	128.3 C
C-11b	40.1 CH	40.1 CH
C-11c	62.9 CH	62.9 CH
OCOCH_3	20.8 CH_3	20.8 CH_3
OCOCH_3	172.2 C	172.2 C
OCH_2O	102.4 CH_2	102.4 CH_2

⊗ ^{13}C NMR spectrum measured in CD_3OD, 75 MHz

2.2.3 The preparation and structural confirmation of compound III

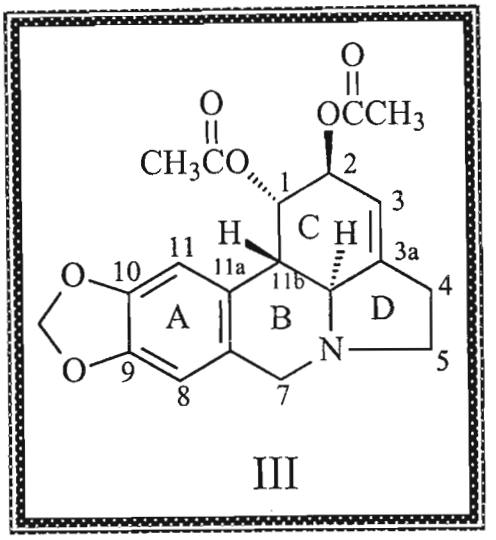


Figure 2.9: COMPOUND III: 1,2-di-*O*-acetyllycorine

Lycorine (**I**) was acetylated to yield compound **III**. Compound **III** was recrystallized from Et_2O – MeOH to give colourless platelets. The physical and spectral (IR, ^1H NMR, ^{13}C NMR, MS) properties observed for compound **III**, were consistent with those reported for 1,2-di-*O*-acetyllycorine [29,33]. Spectra for compound **III** are given on pages 165-171 in appendix A.

The mass spectrum of 1,2-di-*O*-acetyllycorine (**III**), showed a molecular ion peak at *m/z* 371 ($C_{20}H_{21}NO_6$) and significant peaks at *m/z* 311, 252, 251, 250, 227, 226, 149, 97 and 43. The peaks at *m/z* 227 and 226 were in agreement with the fragmentation scheme proposed for lycorine type alkaloids (**scheme 2.2**).

The infra red spectrum of compound **III** was similar to that of 1-*O*-acetyllycorine.

The ^1H and the ^{13}C NMR spectra of **III** were similar to those of 1-*O*-acetyllycorine (**II**). The ^1H NMR spectrum showed an additional methyl resonance at δ_H 2.12. Furthermore, the H-2 signal in **II** appeared at δ_H 4.19 (brs) but in **III** it appeared at δ_H 5.29 (brs). This suggested that the C-2 hydroxy group had been acetylated. Thus the acetylation of lycorine causes the two methine protons, H-1 and H-2, to shift downfield corresponding to $\Delta\delta_H$ 1.30 and $\Delta\delta_H$ 1.09 respectively. These findings are consistent with literature [15].

Table 2.7: Comparison of ^{1}H NMR data for lycorine (I), 1-*O*-acetyllycorine (II) and 1,2-di-*O*-acetyllycorine (III) (*J* given in Hz in parentheses)

	^{1}H NMR data for compound I	^{1}H NMR data for compound II	^{1}H NMR data for compound III
Proton No.	$^{\otimes}$**shift / δ_H ppm**	$^{\otimes}$ **shift / δ_H ppm**	$^{\otimes}$ **shift / δ_H ppm**
H-1	4.48 s	5.73 s	5.78 s
H-2	4.20 brs	4.19 brs	5.29 brs
H-3	5.59 brs	5.58 brs	5.56 brs
H-4	2.60 m	2.68 m	2.70 m
H-5	2.48 dd (17.52, 8.69) 3.38 m	2.50 dd (17.60, 8,80) 3.33 m	2.52 dd (17.70, 8.90) 3.40 m
H-7α	3.57 d (14.22)	3.60 d (14.30)	3.59 d (14.29)
H-7β	4.15 d (14.22)	4.16 d (14.30)	4.18 d (14.19)
H-8	6.67 s	6.67 s	6.68 s
H-11	6.91 s	6.76 s	6.77 s
H-11b	2.70 m	2.90 brs	2.91 s
H-11c	2.93 d (11.20)	2.90 brs	2.91 s
$OC\underline{H}_2O$	5.94 s	5.94 s	5.94 s
$OCOC\underline{H}_3-1$	----------	1.93 s	1.95 s
$OCOC\underline{H}_3-2$	----------	----------	2.12 s

$^{\otimes}$ ^{1}H NMR spectrum measured in CD_3OD, 300 MHz.

The acetylation of an allylic hydroxyl group may cause β carbons to be shielded and γ carbons to be deshielded [1,34]. A comparison of **II** with **III**, shows that in **III** the C-1 (β) and C-3 (β) resonances have been shielded by $\Delta\delta_C$ 2.8 and $\Delta\delta_C$ 3.7 ppm respectively. Furthermore C-3a and C-11b in **III**, the γ carbons, have been deshielded by $\Delta\delta_C$ 3.1 and $\Delta\delta_C$ 0.2 ppm respectively. This evidence confirms the acetylation of the allylic hydroxyl group.

Table 2.8: Comparison of ^{13}C NMR data for lycorine (I), 1-*O*-acetyllycorine (II) and 1,2-di-*O*-acetyllycorine (III)

	^{13}C NMR data for COMPOUND I	^{13}C NMR data for COMPOUND II	^{13}C NMR data for COMPOUND III
Carbon No.	⊗ **shift / δ_C ppm**	⊗ **shift / δ_C ppm**	⊗ **shift / δ_C ppm**
C-1	73.1 CH	73.4 CH	70.6 CH
C-2	71.9 CH	70.3 CH	72.0 CH
C-3	119.1 CH	119.0 CH	115.3 CH
C-3a	143.6 C	143.8 C	146.9 C
C-4	29.2 CH_2	29.3 CH_2	29.3 CH_2
C-5	54.5 CH_2	54.6 CH_2	54.5 CH_2
C-7	57.5 CH_2	57.6 CH_2	57.6 CH_2
C-7a	130.4 C	130.4 C	130.4 C
C-8	108.2 CH	108.4 CH	108.3 CH
C-9	148.2 C	148.1 C	148.1 C
C-10	147.7 C	147.9 C	146.9 C
C-11	106.0 CH	105.7 CH	105.9 CH
C-11a	129.7 C	128.3 C	127.5 C
C-11b	41.3 CH	40.1 CH	41.5 CH
C-11c	62.5 CH	62.9 CH	62.6 CH
$O\underline{C}H_2O$	102.2 CH_2	102.4 CH_2	102.4 CH_2
$OCO\underline{C}H_3-1$	----------	20.8 C	20.6 CH_3
$O\underline{C}OCH_3-1$	----------	172.2 C	171.6 C
$OCO\underline{C}H_3-2$	----------	----------	20.9 CH_3
$O\underline{C}OCH_3-2$	----------	----------	171.4 C

⊗ ^{13}C NMR spectrum measured in CD_3OD, 75 MHz.

2.2.4 The structural elucidation of compound IV

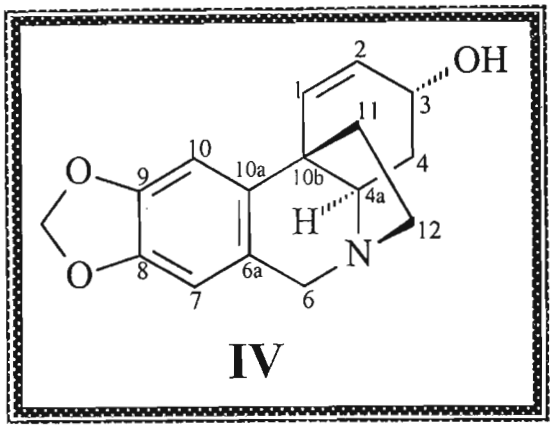


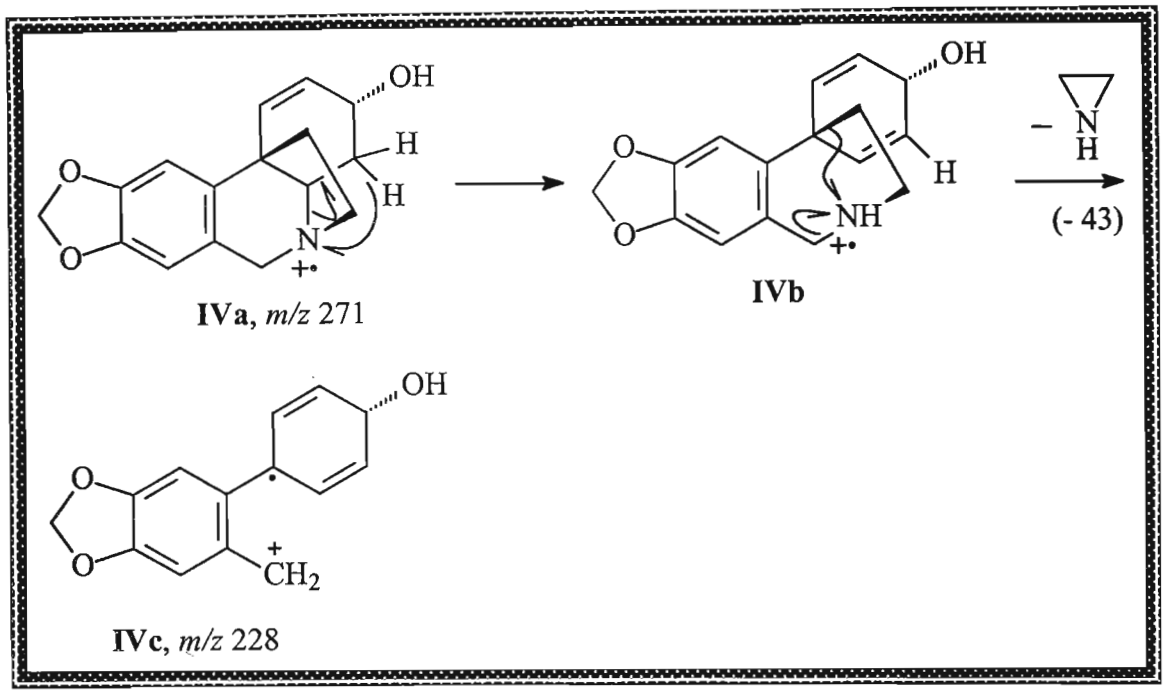
Figure 2.10: COMPOUND IV: crinine

Compound **IV** ($C_{16}H_{17}NO_3$) was recrystallized from MeOH to yield colourless needles with a melting point of 207 – 208 °C. Compound **IV** responded to Dragendorff reagent, was insoluble in dilute aqueous NaOH and gave a negative $FeCl_3$ test. Spectra for compound **IV** are given on pages 172-179 in appendix A.

The base peak in the mass spectrum of compound **IV** corresponded to the molecular ion (*m/z* 271). The mass spectrum of compound **IV** displayed minor peaks corresponding to fragments of *m/z* 256 (M – 15), 254 (M – 17), 242 (M – 29) and 240 (M – 31). The first ion of appreciable abundance in the spectrum, occurred at *m/z* 228. High resolution mass spectrometry has shown this peak to be of the composition 65% $C_{14}H_{12}O_3^+$ and 35% $C_{14}H_{14}NO_2^+$ (**table 2.9**) [35].

Table 2.9: HRMS measurements of some ions of crinine (IV) [35]

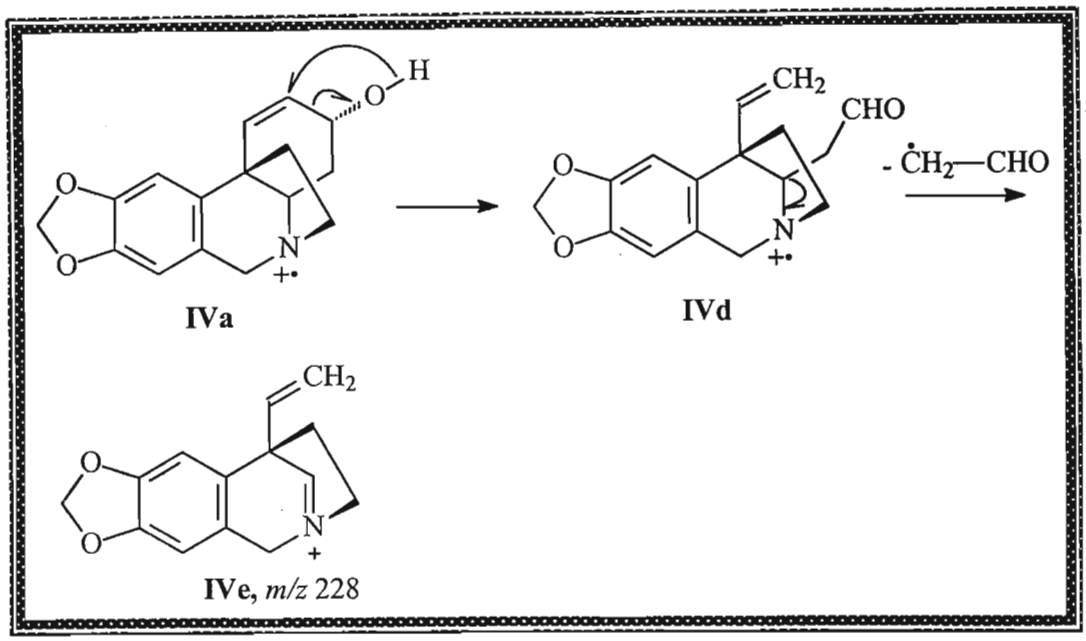
m/z	COMPOUND	CALCULATED	OBSERVED
228	$C_{14}H_{12}O_3$ (65 %)	228.0790	228.0786
228	$C_{14}H_{14}NO_2$ (35 %)	228.1029	228.1025
216	$C_{13}H_{12}O_3$	216.0786	216.0786
199	$C_{13}H_{11}O_2$	199.0759	199.0759
187	$C_{12}H_{11}O_2$	187.0762	187.0759
173	$C_{11}H_9O_2$	173.0601	173.0602
172	$C_{11}H_8O_2$	172.0526	172.0524



Scheme 2.3: A suggested mechanism for the formation of the major fragment at *m/z* 228 [35]

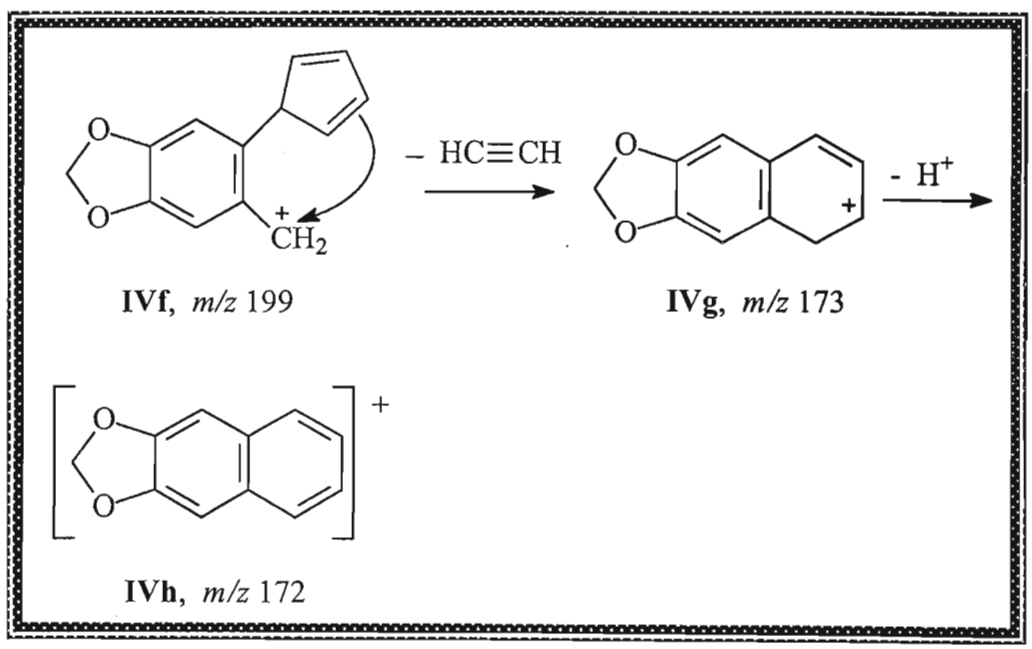
Fragmentation of the molecular ion (**IVa**) of crinine to yield **IVb**, followed by elimination of a neutral amine fragment would generate the species **IVc**, *m/z* 228. **Scheme 2.3** provides a possible mechanism which accounts for the origin of the major component of mass 228 in the spectrum of crinine.

A mechanism for the formation of the minor fragment at *m/z* 228 ($C_{14}H_{14}NO_2^+$) in the mass spectrum of crinine involves the formation of the species **IVd**, from the molecular ion **Va**. The subsequent elimination of $\bullet CH_2CHO$ would then produce the minor fragment at *m/z* 228 (**scheme 2.4**) [35].



Scheme 2.4: A suggested mechanism for the formation of the minor fragment at *m/z* 228 [35]

A prominent ion of mass 199 (M – 272) in the mass spectrum of crinine corresponds to $C_{13}H_{11}O_2^+$ (**table 2.9**). The origin of this ion is considered to be the expulsion of a formyl radical from the species **IVc** to yield **IVf** (*m/z* 199) [35]. Loss of acetylene from **IVf** yields **IVg** (*m/z* 173) (**scheme 2.5**).



Scheme 2.5: Mechanism for the formation of fragments at *m/z* 173 and 172 [35]

Fragment IVg, then loses a hydrogen atom to yield the oxygenated naphthalene ion **(IVh)**. All three schemes provide plausible pathways which are consistent with the results of high resolution mass spectrometry (**table 2.8**) [35].

Compound **IV** displayed UV absorptions at λ 291 nm and λ 230 nm in methanol. This is consistent with the UV spectra of 5,10b-ethanophenanthridine type alkaloids having a methylenedioxy aryl chromophore and an isolated double bond [30].

The infra red spectrum exhibited absorption frequencies at ν_{max} 3350 cm^{-1} (O – H stretching) and 1036 cm^{-1} (C – O stretching). Peaks were also observed at ν_{max} 2928 cm^{-1} (aliphatic C – H stretching), 1496 cm^{-1} (aromatic C = C stretching), 1238 cm^{-1} (C – N stretching) and a peak at ν_{max} 941 cm^{-1} (C – O – C stretching) which is indicative of a methylenedioxy group.

The ^{1}H and ^{13}C NMR resonances were in agreement with previously published data [36]. 2-D NMR techniques (COSY and HETCOR) were used for the assignment of the ^{1}H NMR and part of the ^{13}C NMR spectra.

The ^{1}H NMR spectra of compound **IV** exhibited methylenedioxy proton doublets and aromatic proton singlets in the downfield region. The superimposed methylenedioxy resonances were located at δ_H 5.89. The chemical shifts of the aromatic hydrogens at C-7 and C-10 gave typical signals of aryl hydrogens adjacent to oxygen functions [37]. The singlet resonances at δ_H 6.83 and δ_H 6.47 were thus assigned to the aromatic H-10 and H-7 protons respectively. These assignments were based on Haugwitz's proposal that the H-10 proton signal occurs as a sharp singlet and is located at a consistently lower field than the H-7 proton signal [37]. Furthermore, Haugwitz *et al.* found that the latter signal was slightly shorter and broader than the former, indicative of weak splitting, presumably from the coupling with one or both of the benzylic hydrogen atoms [37,38].

The benzylic protons (H-6α and H-6β) were clearly differentiated as an AB-system with a geminal coupling constant of 16.9 Hz. This coupling was clearly visible in the COSY spectrum. The doublets, at δ_H 3.81 and δ_H 4.43, were assigned to H-6β and H-6α respectively. H-6α was assigned to the resonance at the lower field due to its *cis*-relation with the nitrogen lone pair [39,40].

H-1, H-2 and H-3 of compound **IV** gave rise to an ABX pattern in the ^{1}H NMR spectrum. The chemical shifts and the coupling constants can be determined by direct measurement and first order considerations [37]. The olefinic spectral region showed two resonances. The doublet located at the lower end of the olefinic region is a one – proton doublet ($J_{1,2}$ = 10.0 Hz) which was assigned to H-1. The remaining doublet doublet resulting from H-2 coupling with H-1 ($J_{1,2}$ = 10.0 Hz) and with H-3 ($J_{2,3}$ = 5.4 Hz). The resonance for H-3 occurred as a multiplet at δ_H 4.35.

The magnitude of the coupling constants between each olefinic proton (H-1 and H-2) and H-3 gives information about the configuration of the C-3 substituent. The hydrogen atoms, H-1 and H-2 have a dihedral angle of approximately O° (from molecular models). They split each other into doublets of coupling constant 10 Hz. If the oxygen substituent at C-3 and the 5,10b ethane bridge are on the same side of the molecule, then the dihedral angle between H-2 and H-3 is approximately 90° and the H-2 resonance remains as a doublet of 10 Hz. However, if the oxygen substituent and the ethane bridge are on opposite sides, then the dihedral angle between H-2 and H-3 is approximately 30° and the H-2 doublet is further split into a double doublet of $J_{1,2}$ = 10 Hz and $J_{2,3}$ = 5 Hz. Thus a H-2 resonance which is split into a doublet of 10 Hz indicates a *cis* relationship between the oxygen substituent at C-3 and the 5,10b ethane bridge while a H-2 resonance split into a doublet doublet of 10 Hz and 5 Hz suggests a *trans* relationship [1,36,37,40].

The subsequent inspection of the ^{1}H NMR spectrum of compound **IV** revealed a *trans* orientation between the ethane bridge and the oxygen substituent on C-3 as the H-2 resonance is split into a doublet doublet ($J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 5.4 Hz).

The location of the aliphatic hydroxyl group is derived from the splitting pattern of the H-2 signal group. Only in the case of a hydroxyl group being substituted in C-3, which is biosynthetically facile, can the observed doublet of doublets be explained. H-2 couples with H-3 and with H-1 to yield the observed multiplicity.

The C-4, C-11 and C-12 methylene protons and the C-4a methine proton were completely assigned by means of 2-D COSY and HETCOR experiments. H-3 is seen to be coupled to H-1, H-2, H-4α and H-4β in the COSY spectrum. Since H-1 and H-2 have been assigned (olefinic resonances), the remaining two resonances (δ_H 1.74 and 1.98) can be assigned to the H-4 protons. These resonances also showed coupling in the HETCOR spectrum to a methylene carbon resonance. The large coupling (J = 13.8 Hz), which is due to their *trans* diaxial configuration allowed the doublet of doublets at δ_H 3.38 and the triplet of doublets at δ_H 1.74 to be assigned to H-4a and H-4β, respectively. The doublet at δ_H 1.98 was thus assigned to H-4α.

Four resonances, two for H-11 and H-12 each remained. Of these, the two lower field resonances were assigned to H-12 because of their proximity to the ring nitrogen atom. The H-12 protons were observed as a broad doublet at δ_H 3.30 Hz and a multiplet at δ_H 2.93. The low field signal was attributed to H-12$_{exo}$ because of its co-planarity with the nitrogen lone pair [39,40]. The remaining two resonances were assigned to H-11$_{endo}$ and H=11$_{exo}$ by comparison with the literature [36].

Table 2.10: ^{1}H NMR data for compound IV and literature data for crinine [36]

(*J* given in Hz in parentheses)

	^{1}H NMR data for compound IV	**^{1}H NMR data for CRININE [36]**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes}$shift / δ_H ppm**
H-1	6.53 d (10.0)	6.59 d (10.0)
H-2	5.96 dd (5.4, 10.0)	5.96 dd (5.4, 10.0)
H-3	4.35 m	4.33 m
H-4α	1.98 brd (13.8)	2.06 brd (13.5)
H-4β	1.74 ddd (4.2, 13.8, 13.8)	1.74 ddd (4.2, 13.5, 13.5)
H-4a	3.38 dd (3.8, 13.8)	3.42 dd (3.9, 13.5)
H-6α	4.43 d (16.9)	4.41 d (16.8)
H-6β	3.81 d (16.9)	3.78 d (16.8)
H-7	6.47 s	6.48 s
H-10	6.83 s	6.85 s
H-11$_{exo}$	1.86 m	1.94 ddd (6.3, 10.8, 12.5)
H-11$_{endo}$	2.20 ddd (4.0, 9.0, 13.2)	2.19 ddd (3.7, 8.7, 12.5)
H-12$_{exo}$	3.30 d (12.6)	3.36 brd (12.8)
H-12$_{endo}$	2.93 m	2.91 ddd (6.3, 8.7, 12.8)
OC$\underline{H_2}$O	5.89 brs	5.89d - 5.91d (1.2)

$^{\otimes}$ ^{1}H NMR spectrum measured in $CDCl_3$, 300 MHz.

Analysis of the ^{13}C NMR and DEPT spectra revealed the presence of five methylene carbons (δ_C 100.8, 61.5, 53.1, 43.6, 32.3), six methine resonances (δ_C 13.7, 128.0, 106.9, 102.9, 63.3, 62.9) and five quaternary carbon resonances. The COSY, DEPT and HETCOR spectra were used to fully assign all protonated carbons. The resonance at δ_C 44.3 was assigned to the aliphatic quaternary carbon. The remaining four aromatic quaternary carbon resonances (C-6a, C-8, C-9, C-10a) were assigned by comparison to literature [32].

Table 2.11: ^{13}C **NMR data for Compound IV and literature data for crinine** [32]

	^{13}C NMR data for COMPOUND IV	^{13}C NMR data for crinine [32]
Carbon No.	**$^{\otimes}$ shift / δ_C ppm**	**$^{\otimes\otimes}$ shift / δ_C ppm**
C-1	131.7 CH	131.0 CH
C-2	127.8 CH	128.0 CH
C-3	63.7 CH	63.3 CH
C-4	32.6 CH_2	32.3 CH_2
C-4a	62.8 CH	62.9 CH
C-6	62.0 CH_2	61.5 CH_2
C-6a	125.9 C	125.0 C
C-7	106.9 CH	106.9 CH
C-8	145.8 C	145.8 C
C-9	146.2 C	146.3 C
C-10	102.9 CH	102.9 CH
C-10a	138.1 C	137.8 C
C-10b	44.3 C	44.3 C
C-11	43.9 CH_2	43.6 CH_2
C12	53.4 CH_2	53.1 CH_2
O$\underline{C}H_2$O	100.8 CH_2	100.8 CH_2

$^{\otimes}$ ^{13}C NMR spectrum measured in $CDCl_3$, 75 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum measured in $CDCl_3$, 50 MHz.

2.2.5 The structural elucidation of compound V and VI.

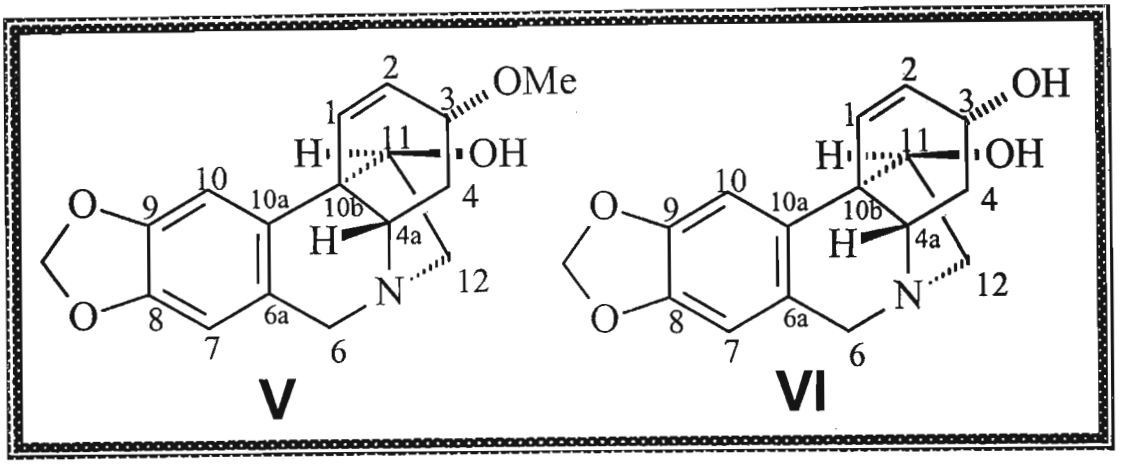


Figure 2.11: COMPOUND V: crinamine and COMPOUND VI: hamayne

Compound **V** was isolated as white crystalline material. Compound **V** was found to be the major alkaloid in *Brunsvigia radulosa*. The physical and spectral data of **V** were comparable with those of crinamine. Crinamine has been found previously in *Brunsvigia radulosa* [7], *Brunsvigia orientalis* [39], *Brunsvigia josephinae* [1] and many other crinum species [16]. Spectra for compound **V** are given on pages 180-189 in appendix A.

The mass spectrum of compound **V** showed a very weak molecular ion peak (< 1%) at *m/z* 301, which excludes the haemanthamine series. Its fragmentation pattern was consistent with crinamine and is characterized by a *cis* configuration between the C-3 substituent group and the 5,10b-ethano bridge, together with a substituent in the bridge and a double bond in ring C [43]. The presence of a hydroxyl group at C-11 governs almost entirely the fragmentation pattern of this kind of alkaloid due to the ability of the hydroxy proton to rearrange [43].

The weak molecular ion peak of **V** can be attributed to its facile loss of methanol (**figure 2.12**). The loss of methanol to give a peak at *m/z* 269 (M-32), is highly favoured when both the ethane bridge and the methoxyl group at the C-3 position are on the same side of the molecule [39]. The driving force for the elimination of methanol in crinamine is the release of steric strain originating from the proximity of the methoxyl group and the two-carbon bridge [35].

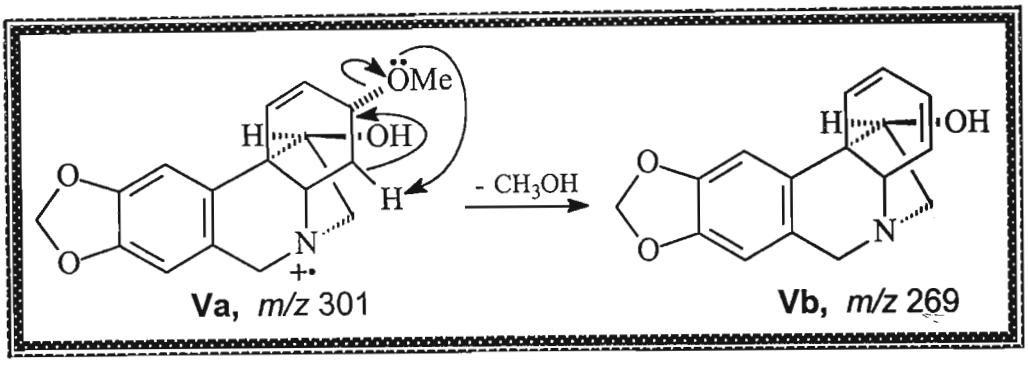
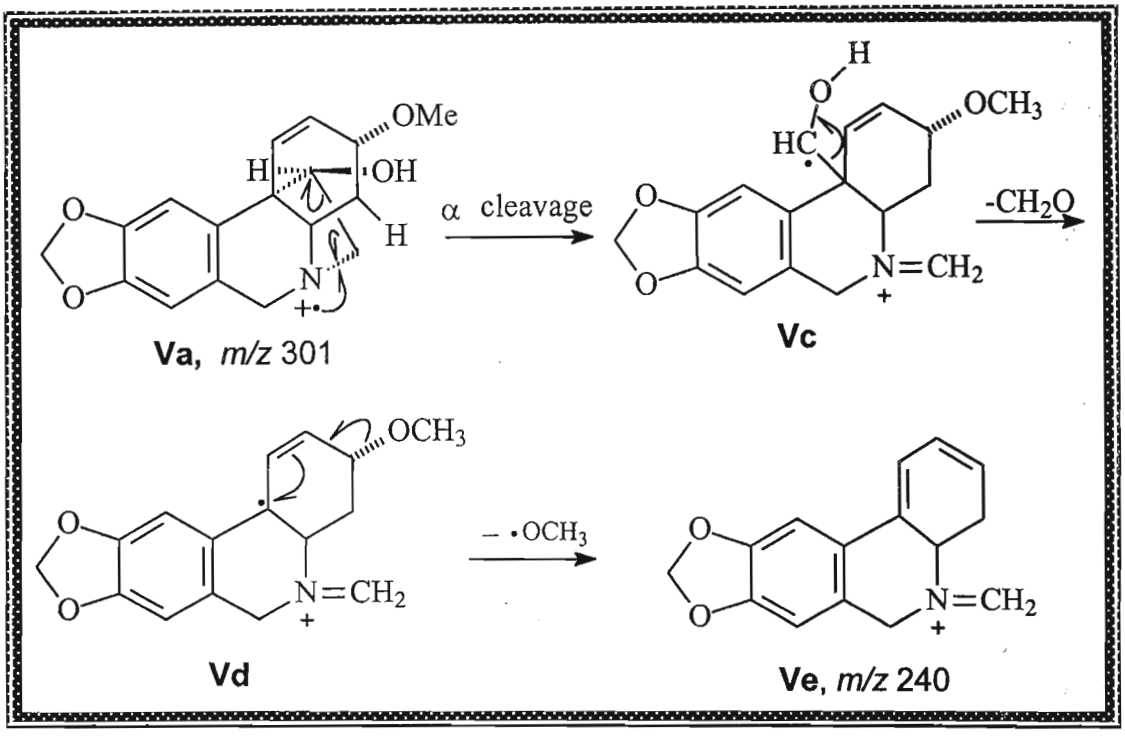


Figure 2.12: Mechanism for the formation of the base peak in compound V

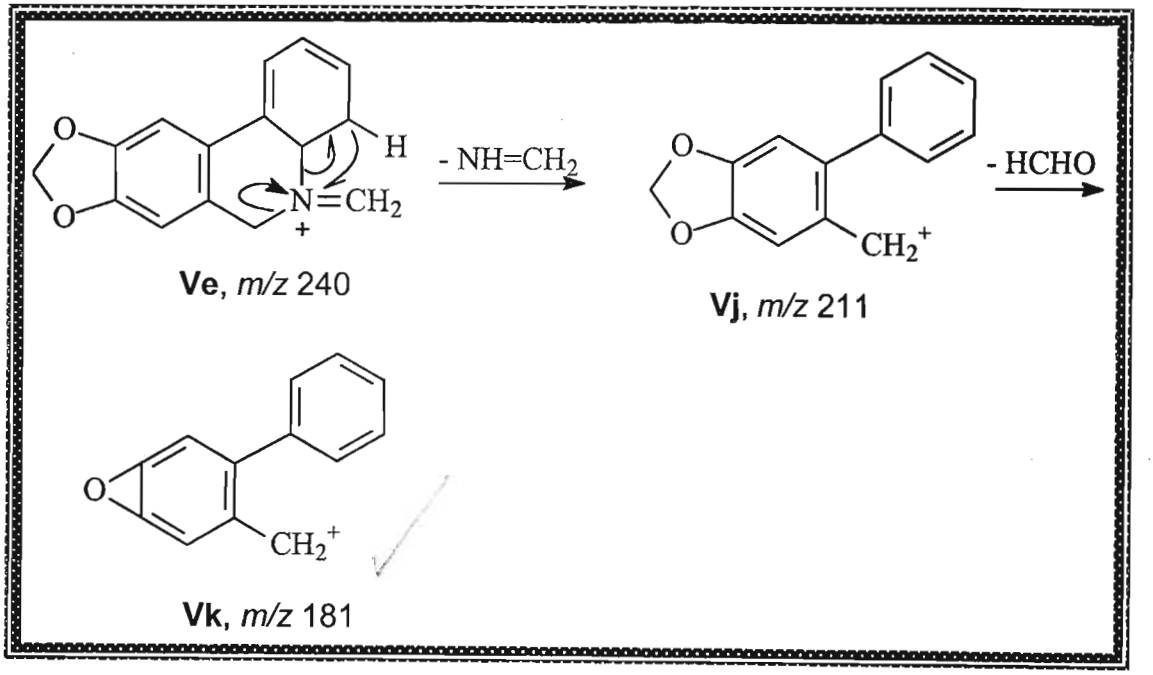
The mechanism for the formation of the base peak, *m/z* 269, is shown above **(figure 2.12**.



Scheme 2.6: The mass spectrometeric fragmentation pattern of compound V [35]

The species **Va** undergoes an α - cleavage to yield **Vc**. The subsequent loss of CH_2O from **Vc** results in the formation of **Vd**. **Vd** then losses a methoxyl radical, resulting in the species **Ve**, which is the *m/z* 240 fragment observed in the mass spectrum.

High resolution mass spectrometry has also established the composition of the peak at *m/z* 211 in the spectrum of crinamine to be $C_{14}H_{11}O_2^+$ [35]. The origin of this species can be shown to result from the loss of the neutral fragment $NH{=}CH_2$ from the ion **Ve** (*m/z* 240) resulting in the formation of **Vj** (*m/z* 211). **Vj** can lose 30 mass units by the ejection of formaldehyde from the methylenedioxy group. The product of this decomposition can be represented by **Vk** (*m/z* 211).



Scheme 2.7: The mass spectometeric fragmentation pattern of compound V [35]

The UV spectra of Amaryllidaceae alkaloids, taken in a neutral solvent (e.g. methanol or ethanol), provides information regarding the type of the ring system (e.g. lycorine/ lycorenine/5,10b-ethanophenathridine/pyrrolophenanthridone) [30]. The UV spectrum of compound **V** was consistent with that of a 5,10b-ethanophenathridine type alkaloid. Alkaloids with a methylenedioxy aryl chromophore and an isolated double bond, exhibit a maximum near λ240 nm and another maximum around λ280-290 nm [30]. Compound **V** exhibited a absorbance around λ_{max} 291.2 nm and a shoulder at λ_{max} 240.5 nm in methanol.

The infra red spectrum of compound **V** was consistent with that of crinamine [26]. The infra red spectrum showed bands at V_{max} 3400 cm^{-1} (O – H stretching) and 1044 cm^{-1} (C – O stretching), 1630 cm^{-1} and 1492 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1241 cm^{-1} (C – N stretching) indicative of carbon nitrogen bonds and 945 cm^{-1} (C – O – C stretching), suggestive of the presence of a methylenedioxy group.

The ^1H NMR spectrum of compound **V**, recorded in deuterated methanol, exhibited three singlets at δ_H 6.89, 6.56 and 5.91. These resonances were assigned to the aromatic protons, H-10 and H-7, and to the protons of the methylenedioxy group respectively. The assignment of these resonances was consistent with literature [30,37]. The two olefinic resonances of compound **V** were observed as a doublet at δ_H 6.11 (J = 10.32 Hz) and a doublet of doublets at δ_H 6.31 (J = 2.13, 10.44 Hz). These resonances were assigned to H-2 and H-1 respectively. The NOESY spectrum showed a NOESY correlation between H-1 and H-10, confirming their assignments.

The COSY spectrum showed strong coupling between H-1 and H-2. Furthermore, H-1 could be seen coupling to a resonance at δ_H 4.10 Hz. This multiplet was assigned to H-3. The NOESY spectrum showed a NOESY correlation between H-2 and H-3, thus confirming their assignments. The absence of coupling between H-2 and H-3 (the dihedral angle between H-2 and H-3 is approximately 90°), an allylic coupling between the vinylic H-1 and the allylic H-3 and the NOESY correlation between H-3 and H-4a, were indicative of a *cis* – relationship between the C-3 substituent and the 5,10b ethane bridge. This was confirmed by the mass spectrum of **V**, which showed the facile loss of methanol, which is typical for compounds having both the ethane bridge and the methoxyl group, at the C-3 position, on the same side of the molecule [39].

The NOESY spectrum showed a correlation between the H-3 proton and the protons of the methoxyl group. This allowed the methoxyl group to be assigned to C-3 as opposed to C-11. The COSY spectrum showed H-3 additionally coupling to the two protons on C-4, at δ_H 2.08 and δ_H 2.15. These resonances were ascribed to H-4α and H-4β respectively. The HETCOR spectrum confirmed that these protons were geminal. The NOESY correlation between H-4α and the methoxyl group protons confirmed the stereochemical assignments of the H-4 protons. The COSY spectrum also showed the H-4 protons coupling to a resonance at δ_H 3.28. This signal was assigned to H-4a. NOESY correlations between the two H-4 protons and H-3, as well as the NOESY correlation between H-4a and the two H-4 protons suggested that their assignments were correct. The H-6 protons appeared as a pair of doublets at δ_H 3.73 and δ_H 4.33. These resonances were seen to be coupled in the COSY spectrum. The coupling constant of 16 Hz is typical for such geminal protons. The HETCOR spectrum confirmed that the protons were geminal. The lower field resonance was assigned to H-6β due to its *cis* – relation with the nitrogen lone pair [39,40]. The NOESY interaction between H-6β and H-4a confirmed their assignments.

The doublet of doublets at δ_H 4.00 was assigned to H-11_{endo}. The HETCOR spectrum showed a correlation between this assigned resonance and a methine carbon at δ_C 81.0. The H-11 proton was assigned to the *endo*-position due to its NOESY

interaction with the H-10 proton. The deshielding effect on H-11 in relation to the reported data for alkaloids with no bridge substituent in this series [39,41], and the NOESY interaction between H-10 and H-11 were consistent with a C-11 hydroxyl substituent at the *exo*-position. The remaining resonances at δ_H 3.50 and δ_H 3.23 were assigned to H-12_{exo} and H-12_{endo} respectively. The HETCOR spectrum showed a correlation between these resonances and a methyl carbon at δ_C 63.6. The strong NOESY interaction between H-11_{endo} and H-12_{endo} suggested that their assignments were correct.

Table 2.12: ^{1}H NMR data for compound V and literature data for crinamine [26] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound V	**^{1}H NMR lit. data for crinamine [26]**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**
H-1	6.31 dd (10.44, 2.13)	6.23 br s
H-2	6.11 d (10.32)	
H-3	4.10 m	3.98 m
H-4α	2.08 m	2.08 m
H-4β	2.15 dd (13.06, 10.44)	
H-4a	3.28 dd (13.25, 3.30)	3.22 dd (13.2, 3.2)
H-6α	3.73 d (16.91)	3.67 d (16.9)
H-6β	4.33 d (16.61)	4.29 d (16,9)
H-7	6.56 s	6.46 s
H-10	6.89 s	6.78 s
H-11_{endo}	4.00 dd (6.17, 2.75)	3.92 m
H-12_{exo}	3.23 dd (13.73, 3.45)	3.40 m
H-12_{endo}	3.50 dd (13.73, 6.16)	
OCH_3	3.42 s	3.38 s
OCH_2O	5.91 s	5.87 s

$^{\otimes}$ ^{1}H NMR spectrum recorded in CD_3OD, 300 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 300 MHz.

Compound **VI** was isolated as white crystalline material with a melting point of 180 °C. Its physical and spectral properties were consistent with those of hamayne. Hamayne has been previously isolated from *Brunsvigia josephinae* [43]. Spectra for compound **VI** are given on pages 190-197 in appendix A.

The mass spectrum of compound **VI** showed a weak molecular ion peak at *m/z* 287 which corresponded to the formula $C_{16}H_{17}NO_4$. The base peak of compound **VI** occurred at *m/z* 269 ($M^+ - 18$) and was attributed to the loss of water. The mass spectrum of compound **VI** closely matched that of crinamine (**V**). Comparison of the molecular ion peaks suggested that compound **VI** was methylated. The peaks at *m/z* 240, 211 and 181 were accounted for in the same way as those of crinamine (**V**) **(scheme 2.7 and 2.8)**.

The infra red spectrum of **VI** showed bands at ν_{max} 3400 cm^{-1} (O – H stretching), 1044 and 1037 cm^{-1} (C – O stretching), 1478 cm^{-1} (aromatic C = C stretching), 1235 cm^{-1} (C – N stretching) and 945 cm^{-1} (C – O – C stretching), indicative of the methylenedioxy group.

The ^1H NMR spectrum of compound **V** was similar to that of crinamine except for the absence of a methoxyl group proton resonance. The spectrum recorded in CD_3OD, exhibited three singlets at δ_H 6.60, 6.92 and 5.93, for the aromatic protons, H-10 and H-7, and for the protons of the methylenedioxy group respectively. The olefinic protons, H-1 and H-2, were observed as a doublet of doublets at δ_H 6.25 and a doublet at δ_H 6.09 respectively. H-1 is seen to be coupled with H-2 and H-3 ($J_{1,2}$ = 10.38, $J_{1,3}$ = 2.26). H-2 is seen to be coupled only with H-1 ($J_{1,2}$ = 10.38), as the dihedral angle between H-2 and H-3 is approximately 90° (see **page 49**). The multiplicities of H-1 and H-2 were in agreement with the *cis* – relationship between the C-3 substituent and the 5,10b – ethano bridge [43]. Two doublets for the AB system of the C-6 protons were observed at δ_H 3.95 and δ_H 4.47. These signals were assigned to H-6α and to H-6β respectively on the basis of their proximity to the nitrogen lone pair [39].

Weak coupling between H-1 and H-3 observed in the COSY spectrum, allowed the latter proton to be assigned. H-3 was observed resonating at δ_H 4.37 as a multiplet.

The H-3 resonance also exhibited strong coupling with the multiplets at δ_H 2.11and δ_H 2.20. These resonances were attributed to the protons on C-4. The two resonances also correlated with the C-4 resonance at δ_C 33.5 in the HETCOR spectrum, thus confirming their assignments. The H-4 resonances were also observed to be coupled to the H-4a resonance in the COSY spectrum. The doublet of doublets at δ_H 3.48 (J = 13.62, 4.40 Hz) was accordingly assigned to H-4a. The H-11 proton resonance appeared as a one proton multiplet at δ_H 4.05. This resonance was also seen to be coupled to the resonances at δ_H 3.38 and δ_H 3.66. From the HETCOR spectrum it could be inferred that these resonances were for geminal protons. The doublet of doublets at δ_H 3.66 was assigned to H-12_{endo} on the basis of its proximity to the nitrogen lone pair [39].

Table 2.13: ^{1}H NMR data for compound VI and literature data for hamayne [43] (J given in Hz in parenthesis)

	^{1}H NMR data for compound VI	**^{1}H NMR lit. data for hamayne** [43]
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**
H-1	6.25 dd (10.38, 2.26)	6.19 s
H-2	6.09 d (10.38)	
H-3	4.37 m	4.35 m
H-4α	2.11 m	2.10 m
H-4β	2.20 dd (13.43, 10.44)	
H-4a	3.48 dd (13.62, 4.40)	3.25 dd (13.50, 4.50)
H-6α	3.95 d (16.48)	3.65 d (16.00)
H-6β	4.47 d (16.48)	4.30 d (16.00)
H-7	6.60 s	6.47 s
H-10	6.92 s	6.81 s
H-11_{endo}	4.05 m	4.00 m
H-12_{exo}	3.38 dd (13.73, 3.42)	3.35 m
H-12_{endo}	3.66 dd (13.73, 6.96)	
OCH_2O	5.93 s	5.90 s

$^{\otimes}$ ^{1}H NMR spectrum recorded in CD_3OD, 300 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 200 MHz.

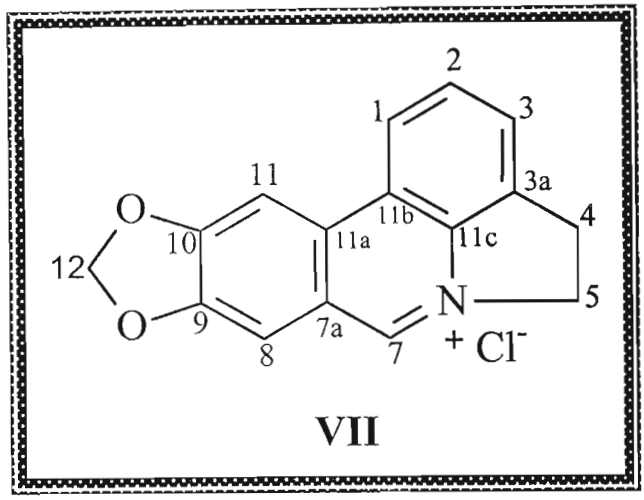
Table 2.14: ^{13}C NMR data of compound V and compound VI and literature data for crinamine and hamayne

	^{13}C NMR data of COMPOUND V	^{13}C NMR lit. data of crinamine [26]	^{13}C NMR data of COMPOUND VI	^{13}C NMR lit. data of hamayne [43]
Carbon No.	$^{\otimes}$ **shift/δ_C ppm**	$^{\otimes\otimes}$**shift/δ_C ppm**	$^{\otimes}$ **shift/δ_C ppm**	$^{\otimes\otimes}$**shift/δ_C ppm**
C-1	125.8 CH	123.60 CH	124.0 CH	122.9 CH
C-2	134.2 CH	135.99 CH	137.4 CH	137.4 CH
C-3	77.6 CH	76.02 CH	67.7 CH	67.0 CH
C-4	30.6 CH_2	30.14 CH_2	33.5 CH_2	33.2 CH_2
C-4a	67.4 CH	66.10 CH	67.9 CH	65.6 CH
C-6	61.5 CH_2	63.44 CH_2	60.7 CH_2	63.0 CH_2
C-6a	126.6 C	126.55 C	124.7 C	125.2 C
C-7	107.8 CH	106.87 CH	107.9 CH	106.8 CH
C-8	147.8 C	146.22 C	148.1 C	146.3 C
C-9	148.2 C	146.50 C	148.6 C	146.8 C
C-10	104.3 CH	103.18 CH	104.4 CH	103.3 CH
C-10a	137.2 C	135.32 C	136.4 C	135.4 C
C-10b	51.7 C	50.26 C	51.6 C	49.8 C
C-11	81.0 CH	79.99 CH	80.2 CH	79.5 CH
C12	63.6 CH_2	61.18 CH_2	63.2 CH_2	60.5 CH_2
O$\underline{C}H_3$	55.8 CH_3	55.80 CH_3	----------	----------
O$\underline{C}H_2$O	102.2 CH_2	100.87 CH_2	102.5 CH_2	101.0 CH_2

$^{\otimes}$ ^{13}C NMR spectrum recorded in CD_3OD, 75 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 50 MHz.

2.2.6 The structural elucidation of compound VII



VII

Figure. 2.13: COMPOUND VII : anhydrolycorinium chloride

Compound **VII** was isolated as pale yellow needles (24 mg). Its solubility behaviour, high decomposition point (>280 °C) and colour were consistent with a quaternary ammonium halide type of system [45]. In addition to this, an aqueous neutral solution of compound **VII** gave a strong test for chloride ions with $AgNO_3$. Spectral data, particularly UV and the ^1H NMR spectrum obtained for the chloride salt, were consistent with a phenanthridinium structure. Spectra for compound **VII** are given on pages 198-208 in appendix A.

The physical and spectral (IR, UV, ^1H NMR, ^{13}C NMR and MS) properties observed for compound **VII** were consistent with those reported for anhydrolycorinium chloride [45,46,47]. The non–chiral anhydrolycorinium chloride was first isolated from *Amaryllis belladonna* and its structure elucidated by Pettit *et al.* in 1984 [45]. Compound **VII** responded to Dragendorff reagent and a blue flourescence could be seen when a TLC plate, containing **VII**, was exposed to UV light.

Compound **VII** exhibited three high intensity UV maxima at λ 258, 268, 279 nm and a maximum at λ 340 nm was also observed in methanol. The UV maxima of compound **VII** were consistent with those of anhydrolycorinium chloride [45,47].

The low resolution mass spectrum of **VII** did not show a molecular ion peak. The anhydrolycorinium cation ($C_{16}H_{12}NO_2^+$) requires 250.0836 mass units. The base peak in the mass spectrum of **VII** occurred at *m/z* 248 and could be attributed to the loss of two hydrogen atoms and the subsequent aromatization of the five membered nitrogen ring. The minor peak at *m/z* 218 is due to the loss of formaldehyde from the dioxolane ring (**scheme 2.8**). No mass spectrometric details were given in Pettit's paper on anhydrolycorinium chloride [45].

The infra red spectrum of compound **VII** was consistent with that of anhydrolycorinium chloride. Peaks were observed at ν_{max} 3402 (due to water of crystallization), 1486, 1271, 1039 and 917 cm^{-1} (C – O – C stretching).

The ^1H NMR spectrum of compound **VII** suggested a lycorine type structure having some structural modifications on the B and C rings. The ^1H NMR spectrum showed a pair of triplets corresponding to two protons each at δ_H 3.85 and δ_H 5.27. The resonance at δ_H 5.27 was assigned to H-5 due to its proximity to the nitrogen atom. The signal at δ_H 3.85 was thus assigned to H-4. Vicinal coupling between the two triplets was observed in the COSY spectrum. In addition to this, coupling between H-5 and H-7 is observed in the COSY spectrum, thereby confirming the assignments of the methylene protons. The most downfield resonance (δ_H 9.64) in the ^1H NMR spectrum was assigned to the iminic proton H-7. The downfield chemical shift of H-7 is due to the nitrogen quaternization or iminium salt effect [48,49].

The NOESY spectrum showed a correlation between H-7 and a sharp singlet at δ_H 7.79 (**figure 2.14**). This singlet was assigned to H-8. The other sharp singlet in the spectrum at δ_H 8.32 was assigned to H-11. These assignments are consistent with Ghosal's proposal for the *para*–oriented protons of ring A [30]. The doublet at δ_H 8.54 was attributed to H-1. The observed multiplicity, together with its spatial proximity to H-11, as observed in the NOESY spectrum, confirmed this assignment. The resonance assigned to H-1 can be seen to be coupled to the H-2 resonance in the COSY spectrum. The correlated peak at δ_H 7.94 was accordingly assigned to H-2.

The multiplicity of H-2 (triplet) confirms its assignment. The peak at δ_H 7.86 was assigned to H-3. H-3 can be seen to be coupled to H-2 in the COSY spectrum. The NOESY correlation between H-3 and H-4 confirmed their assignments **(fig. 2.14)**. NOESY correlations were also observed between the two H-4 and the two H-5 protons. Finally, the intense singlet, integrating for two protons, at δ_H 6.39 was assigned to the geminal H-12 protons of the methylenedioxy group. This singlet was observed to be coupled to a methylene carbon at δ_C 104.5 in the HSQC spectrum.

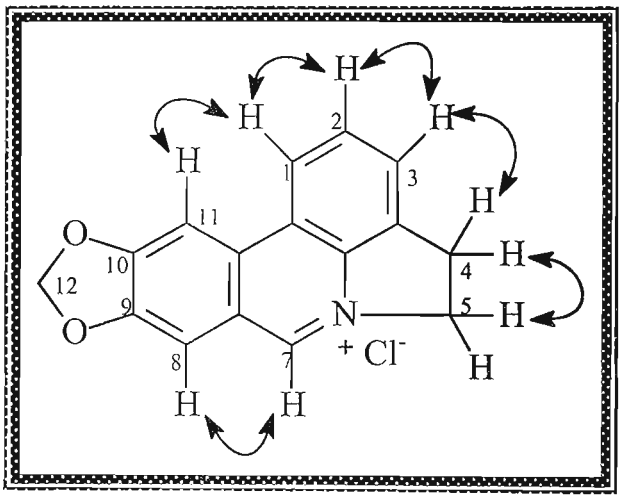


FIGURE 2.14: The NOESY interactions observed for anhydrolycorinium chloride

Table 2.15: ^{1}H NMR data for compound VII and literature data for anhydrolycorinium chloride (*J* given in Hz in parentheses)

	^{1}H NMR data for compound VII	^{1}H NMR Lit. data for anhydrolycorinium chloride [45]	^{1}H NMR Lit. data for anhydrolycorinium chloride [46]
Proton No.	$^{\otimes}$**Shift / δ_H ppm**	$^{\otimes\otimes}$**shift / δ_H ppm**	$^{\otimes\otimes\otimes}$**shift / δ_H ppm**
H-1	8.54 d (8.2)	7.80 m	8.22 d (8.4)
H-2	7.94 t (7.1)		7.84 t (7.3)
H-3	7.86 d (7.0)		7.77 d (7.4)
2H-4	3.85 t (6.6)	3.65 t (6.0)	3.72 t (6.6)
2H-5	5.27 t (6.6)	5.07 t (6.0)	5.13 t (6.6)
H-7	9.64 s	9.26 s	9.33 s
H-8	7.79 s	7.62 s	7.58 s
H-11	8.32 s	7.68 s	7.95 s
2H-12	6.39 s	6.38 s	6.33 s

$^{\otimes}$ ^{1}H NMR measured in CD_3OD, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR measured in D_2O, 100 MHz.

$^{\otimes\otimes\otimes}$ ^{1}H NMR measured in D_2O, 500 MHz.

The ^{13}C NMR spectrum of compound **VII** contained sixteen carbon signals. Two of the carbon signals corresponding to C-3a and C-11c overlapped each other. A DEPT spectrum revealed the presence of seven quartenary carbons, six methine carbons and three methylene carbons. The ^{13}C NMR spectrum of compound **VII** was consistent with that of literature [45,46,47]. The literature for anhydrolycorinium chloride contains either unassigned or tentative assignments with regard to the ^{13}C NMR data [45,47]. Several ^{13}C NMR spectral assignments corresponding to the pair C-1/C-2 and also the quarternary carbons, have been changed in accordance with HMQC and HMBC data. The unequivocal carbon assignments are given in **table 2.16**.

Table 2.16: ^{13}C NMR and HMBC data for compound VII

	^{13}C NMR data for compound VII		HMBC
Carbon No.	**$^{\otimes}$shift / δ_C ppm**		
C-1	120.5	CH	H-3, H-2
C-2	131.5	CH	----------
C-3	126.1	CH	H-1
C-3a	136.9	C	2H-5, H-2, 2H-4
C-4	27.5	CH_2	H-5
C-5	55.8	CH_2	H-4
C-7	145.1	CH	H-8
C-7a	123.1	C	H-7, H-11
C-8	107.4	CH	H-7
C-9	157.4	C	H-8, H-11, H-12
C-10	151.1	C	H-8, H-11, H-12
C-11	101.2	CH	----------
C-11a	133.7	C	H-1, H-7, H-8
C-11b	124.1	C	H-2, H-11
C-11c	136.9	C	H-1, H-3, H-4, H-7
C-12	104.5	CH_2	----------

$^{\otimes}$ ^{13}C NMR spectrum measured in CD_3OD, 100 MHz.

2.3 CONCLUSION

The bulbs of *Brunsvigia radulosa* were investigated. The ethanol extract of the bulbs yielded six alkaloids, lycorine (**I**), 1-*O*-acetyllycorine (**II**), crinine (**IV**), crinamine (**V**), hamayne (**VI**) and anhydrolycorinium chloride (**VII**), after separation by column chromatography over silica gel. Lycorine (**I**) was acetylated to yield 1,2-di-acetyllycorine (**III**). Compounds **II**, **IV**, **VI** and **VII** have not been isolated previously from *Brunsvigia radulosa*.

Lycorine (**I**) is an ubiquitous Amaryllidaceae alkaloid with significant bioactivity. Its biological activities include the inhibition of growth and cell division in higher plants and algae [13], inhibition of cyanide–resistant respiration [13,14], inhibition of amino acid biosynthesis *in vivo*, as well as being active against RNA and DNA viruses [16-19]. Crinine (**IV**) shows weak analgesic activity in mice and is a tachycardiac agent in dogs [16]. Crinamine (**V**), is a powerful transient hypotensive agent in dogs. It shows respiratory depressant activity [16,19]. Crinamine has also shown strong cytotoxic and moderate antimalarial activity [26].

In 1980, Charlson, showed that an extract of *Brunsvigia radulosa* increases the life span of P-388 leukaemic mice [10]. Sixty-four to sixty-nine percent life extension has been reported [10,11]. In 1984, Pettit *et al.* isolated anhydrolycorinium chloride (**VII**) from *Amaryllis belladonna* and showed it to be a potent antineoplastic agent with strong inhibitory activity against murine P-388 lymphocytic leukemia [45]. The work presented here suggests that anhydrolycorinium chloride (**VII**) could be the principal antineoplastic component in *Brunsvigia radulosa*.

2.4 Foreword to experimental

Nuclear magnetic resonance spectroscopy (NMR)

NMR spectrometry was recorded using a 300 MHz Varian Gemini spectrometer and a Varian Unity Inova 400 Mhz spectrometer. The spectra were obtained using the solvents deuterated methanol or deuterated chloroform. Each spectrum was referenced against the central line of the deuteriochloroform signal at δ_C 77.0, the deuteriochloroform singlet at δ_H 7.24 ppm, the deuteriomethanol signal at δ_C 49.0 ppm or the deuteriomethanol signal at δ_H 3.34 ppm.

Infra red spectroscopy (IR)

Infra red spectra were recorded using a Nicolet Impact 400 D spectrometer which was calibrated against an air background. Either KBr disks or a NaCl window was used .

Melting points

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

Optical rotations

Optical rotations were measured at room temperature in methanol or chloroform using an Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (4 x 200 nm) unjacketed flow tube.

Mass spectroscopy

GC/MS spectra were recorded using either a Finnigan 1020 GC/MS spectrometer at the Cape Technikon or an Agilent 6890 GC system with a 5973N Mass Selective instrument at the University of Natal – Durban.

Ultraviolet absorption spectrometry

A Varian DMS 300 UV-visible spectrometer was used to obtain UV spectra. Methanol was used as the solvent in all cases.

Thin layer chromatography (TLC)

TLC was used to monitor the column chromatographic process using silica gel (O.2 mm thick) on aluminium backed plates (Merck Art.5554) which contained a fluorescent indicator (F254). Plates were first analyzed under UV light and then developed either with *p*-anisaldehyde spray reagent or freshly prepared Dragendorff reagent. The *p*-anisaldehyde reagent was made up by mixing *p*-anisaldehyde, concentrated sulphuric acid and methanol in the ratio 1:2:97. TLC plates were developed with an heat gun. Dragendorff reagent was prepared by dissolving bismuth (III) nitrate (1.7 g) and tartaric acid (20 g) in 80 ml of water to form solution A. Potassium iodide (16 g) was dissolved in 40 ml water to form solution B. Equal potions of solution A and B were mixed to form a stock solution which was stored in the freezer. Shortly before use, a solution of tartaric acid (10 g) in 50 ml of water was added to 10 ml of the stock solution. TLC plates with alkaloids present on them, showed a dark orange colour when sprayed with Dragendorff reagent.

Acetylation

Lycorine (**I**), was acetylated. Lycorine (20 mg) was placed in a round bottom flask with pyridine (1 cm^3) and acetic anhydride (1 cm^3). The flask was heated on a steam bath for 10 minutes and then left at room temperature for approximately 48 hours. Thereafter, excess acetic anhydride was removed by the addition of methanol (3 x 10 ml). This was followed by the removal of pyridine by adding toluene (3 x 10 ml). After each addition, the solvent was evaporated off on the Rotavapor. Traces of toluene was then removed by the addition of excess methanol. The sample was then spotted on a TLC plate to test for the completion of the reaction.

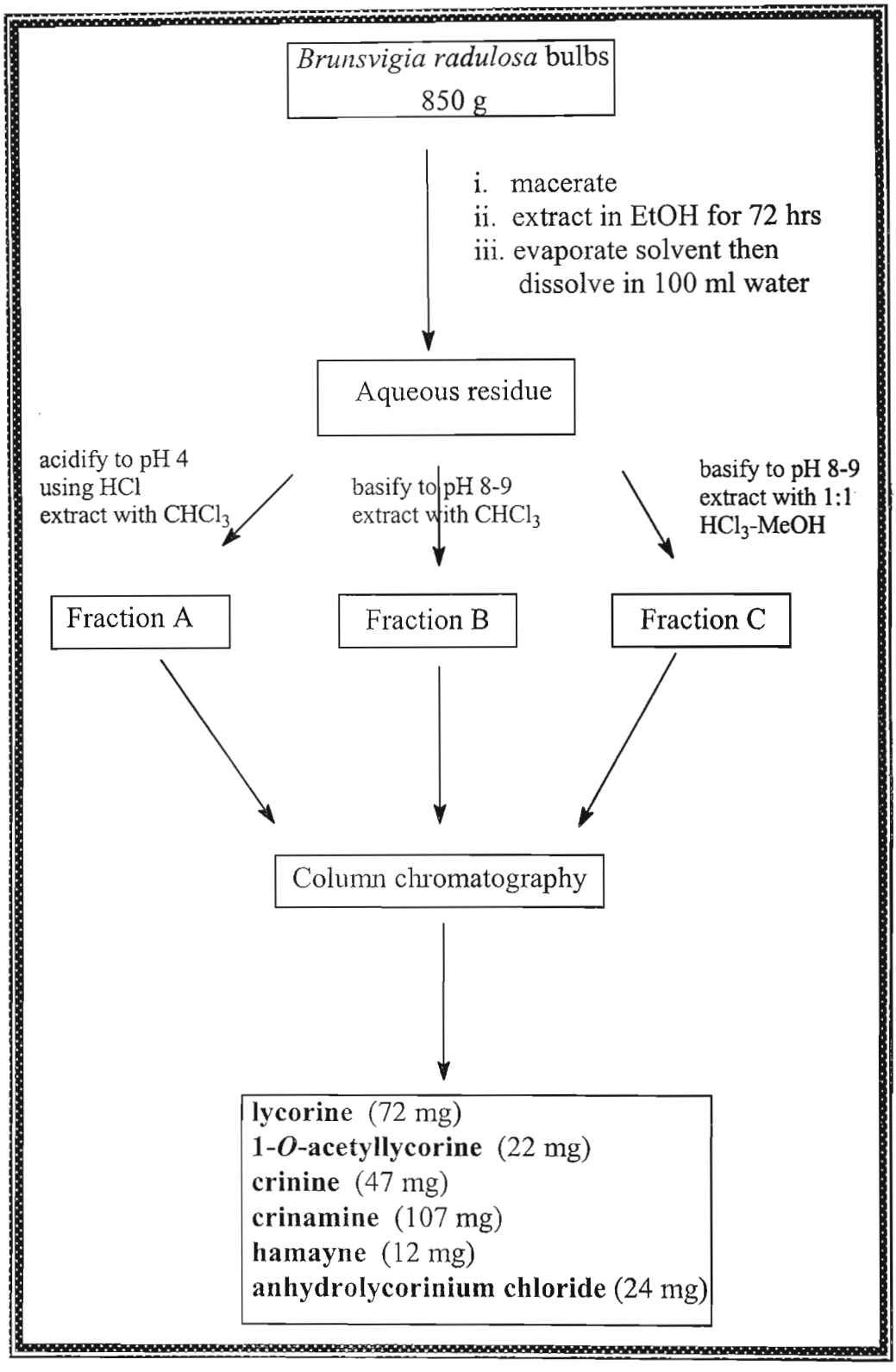
Column Chromatography

Different size columns ranging from 1-3 cm in diameter were used in the crude isolation procedure. Final purifications were carried out on an open, 0.75 cm diameter pasteur pipette column. Merck 9385 silica gel was used as the solid phase and elution was allowed to proceed by gravity. Solvents used in the chromatographic process included hexane, methylene chloride, ethyl acetate and methanol.

2.5 Experimental

Brunsvigia radulosa Herb. was obtained from Buffelskloof, Mpumalanga and identified by Dr N. Crouch. A voucher specimen (*Crouch 799*) was deposited at the Natal Herbarium. The leaves were removed and the dried bulbs (850 g) were finely cut and extracted with 99% ethanol on a Labcon shaker for 96 hours. The extract was evaporated under reduced pressure. The viscous extract was dissolved in water (100ml) and acidified to pH 4. Neutral material was removed with ether and discarded. The acid solution was then extracted with chloroform, to provide extract A. Basifying the solution to pH 8-9 and then extracting it with chloroform, gave extract B. Finally, chloroform – methanol (1:1) extraction of the basic solution gave extract C. TLC analysis suggested that all three extracts were similar. Thus A, B and C (3.2 g) were combined and subjected to column chromatography.

Chromatographic separation of the combined extracts using a methylene chloride – methanol step gradient (19:1, 18:2, 16:4, 12:8, 10:10, 8:12, 4:16, 0:20) as the eluant and collecting approximately 30 x 35 ml fractions for each step, gave lycorine **(I)**, which crystallized out of fractions 53-61, 1-*O*-acetyllycorine **(II)**, present in fractions 22-27, crinine **(IV)**, present in fractions 14-17, crinamine **(V)**, found in fractions 34-40, hamayne **(VI)**, present in fractions 49-54 and anhydrolycorinium chloride **(VII)**, present in fractions 73-77.



Scheme 2.8: Extraction of *Brunsvigia radulosa*

2.5.1 PHYSICAL DATA FOR COMPOUND I

NAME: lycorine

Yield: 72 mg

Melting point: 248 °C (lit. 250 °C [26])

Optical rotation: $[\alpha]_D$ = -63° (c=1.5, EtOH); lit = -62° (c=0.1, EtOH) [5]

UV: λ_{max} (MeOH), (log ε), 210 (4.22), 237 (3.43), 292 (3.43)

Mass: $[M^+]$ at *m/z* 287.1156, $C_{16}H_{17}NO_4$ requires 287.1156.
EIMS: *m/z* (rel. int.): 287 (57.34) $[M^+]$, 286 (37.36), $[M^+ - H]$, 270 (12.35) $[M^+ - OH]$, 269 (11.07) $[M^+ - H_2O]$, 268 (31.44) $[M^+ - H_2O - H]$, 250 (23.19), 227 (80.82) $[M^+ - CHOHCHOH]$, 226 (100.00) $[M^+ - CHOHCHOH - H]$, 147 (7.78), 111 (8.01)

Infra red: ν_{max} (cm^{-1}): 3330, 3410 (O – H stretching), 1505, 1498, 1274, 1241, 1044, 1004, 945 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 2.48 (1H, dd, H-5, *J* = 17.52, 8.69), 2.65 (2H, m, H-4), 2.70 (1H, m, H-11b), 2.93 (1H, d, H-11c, *J* = 11.20), 3.38 (1H, m, H-5), 3.57 (1H, d, H-7α, *J* = 14.22), 4.15 (1H, d, H-7β, *J* = 14.22), 4.20 (1H, brs, H-2), 4.48 (1H, s, H-1), 5.59 (1H, brs, H-3), 5.94 (2H, s, $OC\underline{H}_2O$), 6.67 (1H, s, H-8), 6.91 (1H, s, H-11)

^{13}C NMR: (CD_3OD): 73.1 (CH, C-1), 71.9 (CH, C-2), 119.1 (CH, C-3), 143.6 (C, C-3a), 29.2 (CH_2, C-4), 54.5 (CH_2, C-5), 57.5 (CH_2, C-7), 130.4 (C, C-7a), 108.2 (CH, C-8), 148.2 (C, C-9), 147.7 (C, C-10), 106.0 (CH, C-11), 129.7 (C, C-11a), 41.3 (CH, C-11b), 62.5 (CH, C-11c), 102.2 (CH_2, $O\underline{C}H_2O$)

2.5.2 PHYSICAL DATA FOR COMPOUND II

NAME: 1-*O*-Acetyllycorine

Yield: 22mg

Melting point: 217 °C (lit. 217-219 °C [5])

Optical rotation: $[\alpha]_D$ = -88° (c=0.5, MeOH); lit = -106° (c=0.5, $CHCl_3$) [5]

Mass: $[M^+]$ at *m/z* 329.1272, $C_{18}H_{19}NO_5$ requires 329.1263
EIMS: *m/z* (rel. int.): 329 (61.4) $[M^+]$, 315 (38.24), 270 (13.39) $[M^+ - CH_3COO]$, 269 (14.88) $[M^+ - CH_3COOH]$, 268 (14.31) $[M^+ - CH_3COOH - H]$, 254 (34.02) 252 (18.47) $[M^+ - CH_3COOH - OH]$, 250 (25.24), $[M^+ - CH_3COOH - H_2O - H]$, 228 (38.73), 227 (83.63) $[M^+ - CHOC(O)CH_3 - CHOH]$, 226 (100) $[M^+ - CHOC(O)CH_3 - CHOH - H]$, 149 (29.09), 43 (35.61)

Infra red: ν_{max} (cm^{-1}): 3440, (O – H stretching), 1748, (C = O stretching), 1485, 1241, 1051, 1004, 945 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 2.51 (1H, dd, H-5, *J* = 17.6, 8.8), 2.68 (2H, m, H-4), 2.90 (1H, brs, H-11b), 2.90 (1H, brs, H-11c), 3.33 (1H, m, H-5), 3.61 (1H, d, H-7α, *J* = 14.3), 4.17 (1H, d, H-7β, *J* = 14.3), 4.19 (1H, brs, H-2), 5.73 (1H, s, H-1), 5.58 (1H, brs, H-3), 5.94 (2H, s, $OC\underline{H}_2O$), 6.67 (1H, s, H-8), 6.76 (1H, s, H-11), 1.93 (3H, s, $OCOC\underline{H}_3$)

^{13}C NMR: (CD_3OD): 73.4 (CH, C-1), 70.3 (CH, C-2), 119.0 (CH, C-3), 143.8 (C, C-3a), 29.3 (CH_2, C-4), 54.6 (CH_2, C-5), 57.6 (CH_2, C-7), 130.4 (C, C-7a), 108.4 (CH, C-8), 148.1 (C, C-9), 147.9 (C, C-10), 105.7 (CH, C-11), 128.3 (C, C-11a), 40.1 (CH, C-11b), 62.9 (CH, C-11c), 102.4 (CH_2, $O\underline{C}H_2O$), 20.8 (CH_3, $OCO\underline{C}H_3$), 172.2 (C, $O\underline{C}OCH_3$)

2.5.3 PHYSICAL DATA FOR COMPOUND III

NAME: 1,2-di-*O*-acetyllycorine

Melting point: 205 °C (lit. 208-210 °C [5])

Optical rotation: $[\alpha]_D$ = -61° (c=1.0, MeOH); lit = -22° (c=0.9, $CHCl_3$) [16]

Mass: $[M^+]$ at *m/z* 371.1360, $C_{20}H_{21}NO_6$ requires 371.1368
EIMS: *m/z* (rel. int.): 371 (20.37) $[M^+]$, 311 (12.24) $[M^+ - CH_3COOH]$, 252 (100) $[M^+ - CH_3COOH - CH_3COO]$, 251 (14.02) $[M^+ - 2XCH_3COOH]$, 250 (46.41) $[M^+ - 2XCH_3COOH - H]$, 227 (18.24) $[M^+ - 2XCHOC(O)CH_3]$, 226 (31.17) $[M^+ - 2XCHOC(O)CH_3 - H]$, 149 (5.99), 97 (9.56), 43 (18.24)

Infra red: ν_{max} (cm^{-1}): 1742, (C = O stretching), 1492 (aromatic C=C stretching), 1248 (C – N stretching), 1044, 1004, 945 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 1.95 (3H, s, $OCOC\underline{H}_3$-1), 2.12 (3H, s, $OCOC\underline{H}_3$-2), 2.52 (1H, dd, H-5, *J* = 17.70, 8.90), 2.71 (2H, m, H-4), 2.91 (2H, s, H-11b, H-11c), 3.40 (1H, m, H-5), 3.59 (1H, d, H-7α, *J* = 14,29), 5.29 (1H, brs, H-2), 5.56 (1H, brs, H-3), 5.78 (1H, s, H-1), 5.94 (2H, s, OCH_2O), 6.68 (1H, s, H-8), 6.77 (1H, s, H-11)

^{13}C NMR: (CD_3OD): 70.6 (CH, C-1), 72.0 (CH, C-2), 115.3 (CH, C-3), 146.9 (C, C-3a), 29.3 (CH_2, C-4), 54.5 (CH_2, C-5), 57.6 (CH_2, C-7), 130.4 (C, C-7a), 108.3 (CH, C-8), 148.1 (C, C-9), 146.9 (C, C-10), 105.9 (CH, C-11), 127.5 (C, C-11a), 41.5 (CH, C-11b), 62.6 (CH, C-11c), 102.4 (CH_2, $O\underline{C}H_2O$), 20.6 (CH_3, $OCO\underline{C}H_3$ – 1), 171.6 (C, $O\underline{C}OCH_3$ – 1), 20.9 (CH_3, $OCO\underline{C}H_3$ – 2), 171.4 (C, $O\underline{C}OCH_3$ – 2)

2.5.4 PHYSICAL DATA FOR COMPOUND IV

NAME: crinine

Yield: 47 mg

Melting point: 207 – 208 °C (lit. 208 – 210 °C [5])

Optical rotation: $[\alpha]_D$ = -16° (c=1.0, MeOH); lit = -9° (c=0.6, EtOH) [5]

UV: λ_{max} (MeOH), (log ε), 210 (3.89), 291 (3.15), 230 (3.01)

Mass: $[M^+]$ at *m/z* 271, $C_{16}H_{17}NO_3$ requires 271
EIMS: *m/z* (rel. int.): 271 (100) $[M^+]$, 254 (7.78) $[M^+ - OH]$, 240 (20.21), 228 (20.21) $[M^+ - CH_2CH_2NH]$, 199 (86.58) $[M^+ - CH_2CH_2NH - HCHO]$, 173 (27.46) $[M^+ - CH_2CH_2NH - HCHO - C_2H_2]$, 141 (18.08), 115 (36.64), 98 (2.11), 77 (18.10), 56 (29.34), 39 (11.29)

Infra red: ν_{max} (cm^{-1}): 3350, (O – H stretching), 2928, (aliphatic C – H stretching), 1496 (aromatic C=C stretching), 1238 (C – N stretching), 1036 (C – O stretching), 941 (C – O – C stretching)

^{1}H NMR: ($CDCl_3$): 1.74 (1H, ddd, H-4β, *J* = 4.2, 13.8, 13.8), 1.86 (1H, m, H-11_{exo}), 1.98 (1H, brd, H-4α, *J* = 13.8), 2.20 (1H, ddd, H-11_{endo}, *J* = 4.0, 9.0, 13.2), 2.93 (1H, m, H-12_{endo}), 3.30 (1H, d, H-12_{exo}, *J* = 12.6), 3.38 (1H, dd, H-4a, *J* = 3.8, 13.8), 3.81 (1H, d, H-6β, *J* = 16.9), 4.35 (1H, m, H-3), 4.43 (1H, d, H-6α, *J* = 16.9), 5.89 (2H, d, $OC\underline{H_2}O$, *J* = 2.2), 5.96 (1H, dd, H-2, *J*= 5.4, 10.0), 6.47 (1H, s, H-7), 6.53 (1H, d, H-1, *J* = 10.0), 6.83 (1H, s, H-10)

^{13}C NMR: ($CDCl_3$): 131.7 (CH, C-1), 127.8 (CH, C-2), 63.7 (CH, C-3), 32.6 (CH_2, C-4), 62.8 (CH, C-4a), 62.0 (CH_2, C-6), 125.9 (C, C-6a), 106.9 (CH, C-7), 145.8 (C, C-8), 146.2 (C, C-9), 102.9 (CH, C-10), 138.1 (C, C-10a), 44.3 (C, C-10b), 43.9 (CH_2, C-11), 53.4 (CH_2, C-12), 100.8 (CH_2, $O\underline{C}H_2O$)

2.5.5 PHYSICAL DATA FOR COMPOUND V

NAME: crinamine

Yield: 107 mg

Melting point: 190 °C (lit. 190 – 192 °C [5])

Optical rotation: $[\alpha]_D$ = +166° (c=1.0, MeOH); lit = +180° (c=0.55, $CHCl_3$) [5]

UV: λ_{max} (MeOH), (log ε), 210 (3.89), 240.5 (3.44), 291.2 (3.59)

Mass: HRMS: $[M^+]$ at *m/z* 301.1298, $C_{17}H_{19}NO_4$ requires 301.1313

EIMS: *m/z* (rel. int.): 301 (<1) $[M^+]$, 270 (37.03) $[M^+ - OCH_3]$, 269 (100.00) $[M^+ - CH_3OH]$, 268 (28.49) $[M^+ - CH_3OH - H]$, 252 (17.49), 250 (11.08), 240 (28.20) $[M^+ - CHOH - OCH_3]$, 226 (32.41) $[M^+ - OCH_3 - CHOHCH_2]$, 211 (13.80) $[C_{14}H_{11}O_2]^+$, 181 (26.10) $[C_{13}H_{19}O]^+$, 149 (7.47), 115 (6.05) 60 (12.45), 45 (11.88), 43 (15.69)

Infra red: ν_{max} (cm^{-1}): 3400, (O – H stretching), 1630, 1492 (aromatic C=C stretching), 1241 (C – N stretching), 1044 (C – O stretching), 945 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 2.08 (1H, m, H-4α), 2.15 (1H, dd, H-4β, *J* = 13.06, 10.44), 3.23 (1H, dd, H-12_{exo}, *J* = 13.73, 3.45) 3.28 (1H, dd, H-4a, *J* = 13.25, 3.30), 3.42 (3H, s, $OC\underline{H}_3$), 3.50 (1H, dd, H-12_{endo}, *J* = 13.73, 6.16), 3.73 (1H, d, H-6α, *J* = 16.91), 4.00 (1H, dd, H-11_{endo}, *J* = 6.17, 2.75), 4.10 (1H, m, H-3),4.33 (1H, d, H-6β, *J* = 16.61), 5.91 (2H, s, $OC\underline{H}_2O$), 6.11 (1H, d, H-2, J = 10.32),6.31 (1H, dd, H-1, *J* = 10.44, 2.13), 6.56 (1H, s, H-7), 6.89 (1H, s, H-10)

^{13}C NMR: (CD_3OD): 125.8 (CH, C-1), 134.2 (CH, C-2), 77.6 (CH, C-3), 30.6 (CH_2, C-4), 67.4 (CH, C-4a), 61.5 (CH_2, C-6), 126.6 (C, C-6a), 107.8 (CH, C-7), 147.8 (C, C-8), 148.2 (C, C-9), 104.3 (CH, C-10), 137.2 (C, C-10a), 51.7 (C, C-10b), 81.0 (CH, C-11), 63.6 (CH_2, C-12), 55.8 (CH_3, $O\underline{C}H_3$), 102.2 (CH_2, $O\underline{C}H_2O$)

2.5.6 PHYSICAL DATA FOR COMPOUND VI

NAME: hamayne

Yield: 12 mg

Melting point: 80 °C (lit. 82 – 84 °C [5])

Optical rotation: $[\alpha]_D$ = +80° (c=0.5, MeOH); lit = +79° (c=0.52, EtOH) [5]

UV: λ_{max} (MeOH), (log ε), 211 (4.24.), 241.5 (3.58), 290.7 (3.59)

Mass: EIMS: *m/z* (rel. int.): 287 (<1) $[M^+]$ $(C_{16}H_{17}NO_4)^+$, 270 (19.03) $[M^+ - OH]$, 269 (100.00) $[M^+ - H_2O]$, 268 (29.11) $[M^+ - H_2O - H]$, 240 (28.94), $[M^+ - CHOH - OH]$, 211 (14.83) $[C_{14}H_{11}O_2]^+$, 181 (36.97) $[C_{13}H_{19}O]^+$, 149 (16.19), 115 (8.24) 57 (7.40), 43 (8.13)

Infra red: ν_{max} (cm^{-1}): 3400, (O – H stretching), 1478 (aromatic C=C stretching), 1235 (C – N stretching), 1044 (C – O stretching), 1037 (C – O stretching), 945 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 2.11 (1H, m, H-4α), 2.20 (1H, dd, H-4β, *J* = 13.43, 10.44), 3.38 (1H, dd, H-12_{exo}, *J* = 13.73, 3.42), 3.48 (1H, dd, H-4a, *J* = 13.62, 4.40), 3.66 (1H, dd, H-12_{endo}, *J* = 13.73, 6.96), 3.95 (1H, d, H-6α, *J* = 16.48), 4.05 (1H, m, H-11_{endo}), 4.37 (1H, m, H-3), 4.47 (1H, d, H-6β, *J* = 16.48), 5.93 (2H, s, O$C\underline{H}_2$O), 6.09 (1H,d, H-2, *J* = 10.31), 6.25 (1H, dd, H-1, *J* = 10.38, 2.26), 6.60 (1H, s, H-7), 6.92 (1H, s, H-10)

^{13}C NMR: (CD_3OD): 122.9 (CH, C-1), 137.4 (CH, C-2), 67.0 (CH, C-3), 33.2 (CH_2, C-4), 65.6 (CH, C-4a), 63.0 (CH_2, C-6), 125.2 (C, C-6a), 106.8 (CH, C-7), 146.3 (C, C-8), 146.8 (C, C-9), 103.3 (CH, C-10), 135.4 (C, C-10a), 49.8 (C, C-10b), 79.5 (C, C-11), 60.5 (CH_2, C-12), 101.0 (CH_2, O$C\underline{H}_2$O)

2.5.7 PHYSICAL DATA FOR COMPOUND VII

NAME: anhydrolycorinium chloride

Yield: 24 mg

Melting point: 280 °C decomposes (lit. 280 – 285 °C [45])

UV: λ_{max} (MeOH), (log ε), 258 (3.47), 268 (3.18), 279 (4.45), 340 (4.33)

Mass: EIMS: 250 (8.27) [M^+], 249 (61.09) [M^+ - H], 248 (100) [M^+ - 2H], 190 (18.92), 163 (9.01), 123 (9.89), 95 (18.17), 63 (3.11)

Infra red: ν_{max} (cm^{-1}): 3402 (due to water of crystallization), 1486, 1271, 1039 and 917 cm^{-1} (C – O – C stretching)

^{1}H NMR: (CD_3OD): 8.54 (1H, d, H-1, J = 8.2), 7.94 (1H, t, H-2, J = 7.1), 7.86 (1H, d, H-3, J = 7.0), 3.85 (2H, t, H-4, J = 6.6), 5.27 (2H, t, H-5, J = 6.6), 9.64 (1H, s, H-7), 7.79 (1H, s, H-8), 8.32 (1H, s, H-11), 6.39 (2H, s, H-12)

^{13}C NMR: (CD_3OD): 120.5 (CH, C-1), 131.5 (CH, C-2), 126.1 (CH, C-3), 136.9 (C, C-3a), 27.5 (CH_2, C-4), 55.8 (CH_2, C-5), 145.1 (CH, C-7), 123.1 (C, C-7a), 107.4 (CH, C-8), 157.4 (C, C-9), 151.1 (C, C-10), 101.2 (CH, C-11), 133.7 (C, C-11a), 124.1 (C, C-11b), 136.89 (C, C-11c), 104.52 (CH_2, C-12)

2.6 References

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CHAPTER 3

An introduction to triterpenoids

3.1 Introduction to terpenoids

Terpenes are a class of compounds that can be isolated from plants. Oxygen – containing terpenes are referred to as terpenoids [1,2]. Terpenoids are typically found in all parts of a plant i.e. seeds, flowers, foliage, roots and wood of higher plants. They also occur in mosses, liverworts, algae and lichens and some are of insect or microbial origin [3]. The terpenoids form a large and structurally diverse family of natural products derived from C_5 isoprene units [4]. The terpenoids can therefore be conveniently classified according to the number of isoprene units in the carbon skeleton (**table 3.1**) [3].

Table 3.1: Classes of terpenoids [3]

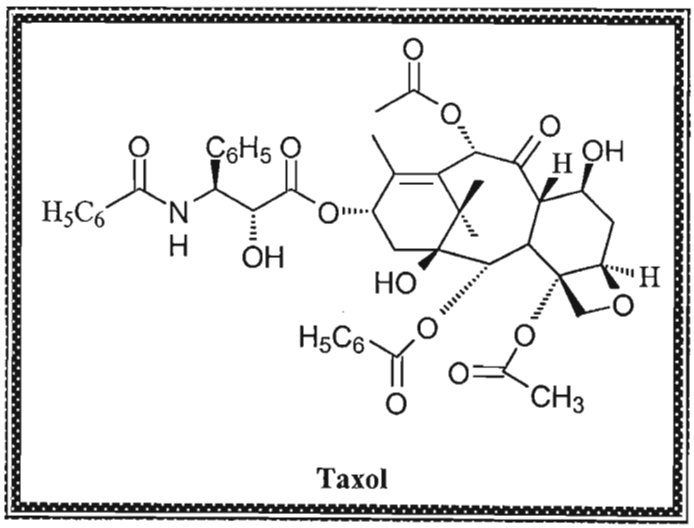
No. of isoprene units	Name	occurrence in plants
1	hemiterpenoids (C_5)	emissions, oils
2	monoterpenoids (C_{10})	oils, petals
3	sesquiterpenoids (C_{15})	oils, petals, resins
4	diterpenoids (C_{20})	oil, resins, heart-wood
5	sesterterpnoids (C_{25})	oil, resins, heart-wood
6	triterpenoids (C_{30})	oil, resins, heart-wood, leaf wax
8	tetraterpenoids (C_{40})	all green tissue, roots, petals
n	polyisoprenoid ($C_{45\text{-}100000}$)	latex, leaf wax

There are more than 22 000 known triterpenoid structures [5]. Each of which originates from isopentenyl pyrophosphate (IPP). The study of terpenoids is made more complex because the functions of most of the terpenoids present in plants are not known. Some of these compounds may at one time have served as defensive agents against predators, such as insects, that no longer exist. It is also possible that some terpenoids are chemical by–products of biochemical transformations in plants that are not as well tuned as those in mammals [5].

Over the past sixty years, terpenoids have provided the substrate for numerous studies on the fundamentals of mechanistic organic chemistry, for example on the nature of carbocations and their rearrangements, on the relationships between structure and colour and on the Woodward – Hoffmann rules for correlation of orbital symmetries during organic reactions. Commercially, triterpenoids have been used in the manufacture of perfumes, soaps, flavourants, colourants, disinfectants, detergents and in many pharmaceuticals [3].

Lanosterol is a biologically important triterpenoid compound. It is the biosynthetic precursor of cholesterol. Lanosterol cannot be broken into isoprene units because migration of methyl groups occurs in its biochemical precursor. Cholesterol is derived from lanosterol by a process that removes three methyl groups. Because cholesterol contains only twenty-seven carbons, it is not a triterpenoid and is properly referred to as a trinortriterpenoid [5].

A number of terpenoids, with very complex structures, have been shown to have potent activity in inhibiting cancerous cell division. Often, these compounds are too toxic to be of value in chemotherapy. Taxol is a well known exception, which is effectively used as a chemical agent for suppressing the growth of hard tumors. Taxol is found only in very small amounts in the bark of the pacific yew tree. Three trees are required to isolate sufficient taxol for the treatment of one patient [5].

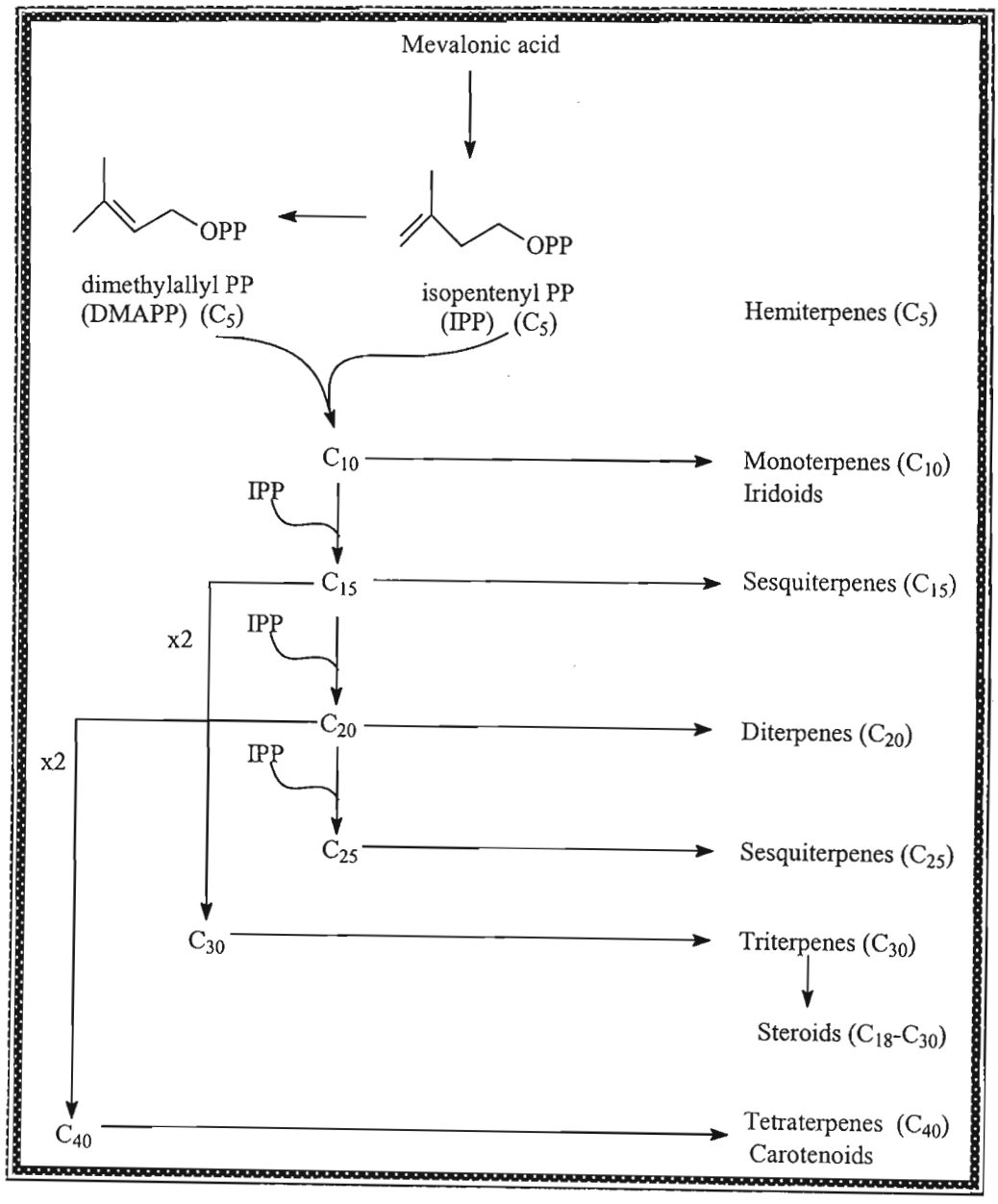


Taxol

Figure 3.1: The structure of taxol

3.2 Biosynthesis of terpenoids

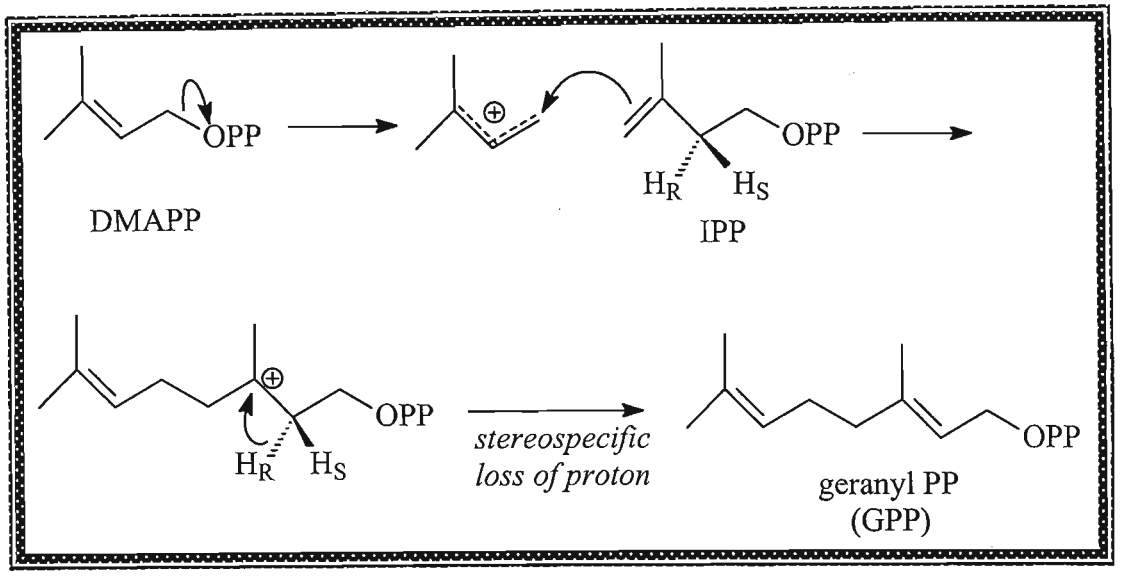
Terpenoids are derived from isoprene units. Isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are the reactive intermediates in the pathways leading to more complex terpenoid structures. These isoprene units combine to yield several classes of terpenoids, as illustrated in **scheme 3.1** [4]. Isoprene units are derived from mevalonic acid *via* acetate metabolism [4].



Scheme 3.1: The biosynthesis of terpenoids

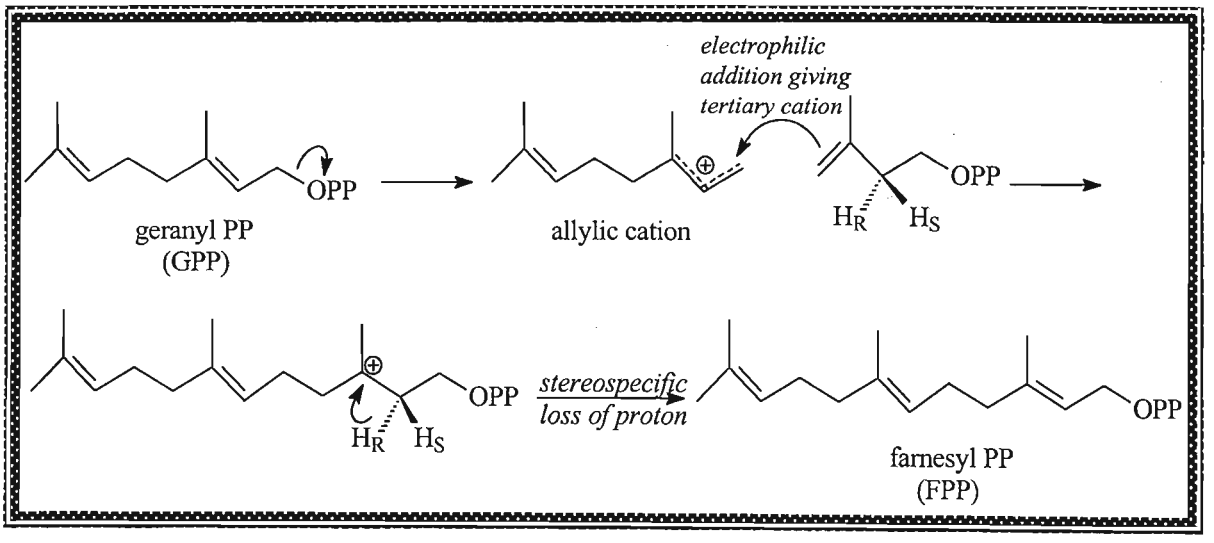
Since only triterpenoids have been isolated in this work, only their biosynthesis will be considered.

3.2.1 Biosynthesis of triterpenoids



Scheme 3.2: The formation of geranyl pyrophosphate

Combination of DMAPP and IPP via the enzyme prenyl transferase yields geranyl pyrophosphate (GPP) (**scheme 3.2**). The mechanism involves ionization of DMAPP to the allylic carbocation. This carbocation is then added to the double bond of IPP. The loss of a proton yields GPP. Stereochemically, the proton lost (H_R) is the same as that lost on the isomerization of IPP to DMAPP [4].

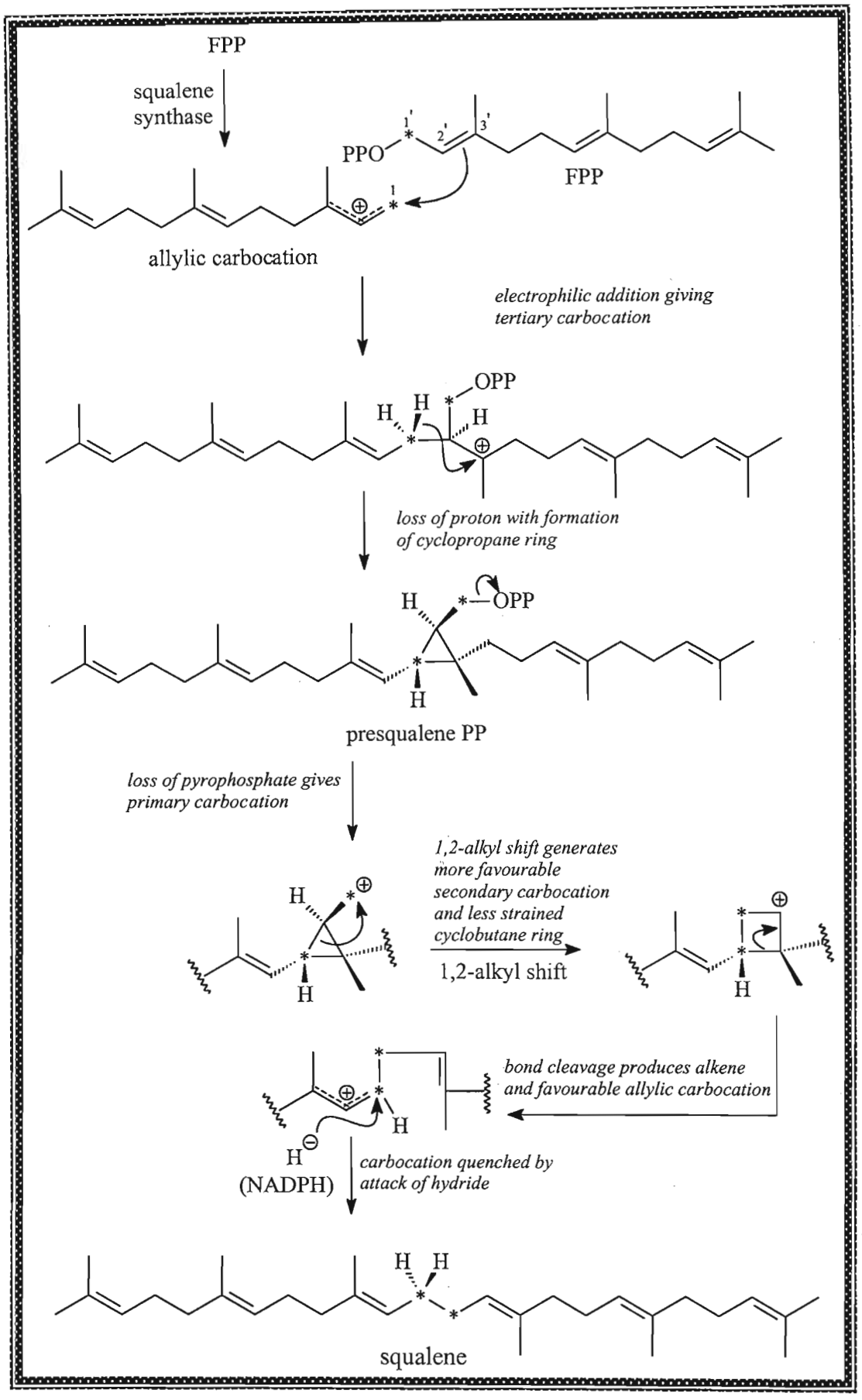


Scheme 3.3: The formation of farnesyl pyrophosphate

Geranyl pyrophosphate can react with another molecule of isopentenyl pyrophosphate to form farnesyl pyrophosphate (FPP), a fifteen carbon compound. The reaction proceeds in an analogous manner to that of GPP formation (**scheme 3.3**).

Two molecules of farnesyl PP are joined in a tail-to-tail fashion to yield the hydrocarbon squalene (**scheme 3.4**). A proton from a C-1 position of one molecule of FPP is lost, and a proton from NADPH is inserted. The mechanism involves the addition of the 2,3-double bond of FPP on to the farnesyl carbocation in an analogous manner to the chain extension using IPP. The resultant tertiary carbocation is discharged by the loss of a proton and formation of the cyclopropane ring, forming presqualene PP.

Loss of pyrophosphate from presqualene PP yields a primary carbocation. This carbocation then undergoes a Wagner-Meerwein rearrangement resulting in stability from ring expansion as well as from the generation of the more favourable secondary carbocation. This is the followed by bond cleavage in the cyclobutane ring, thus producing a favourable allylic carbocation. The generation of squalene is then completed by the supply of a hydride ion from NADPH [4].

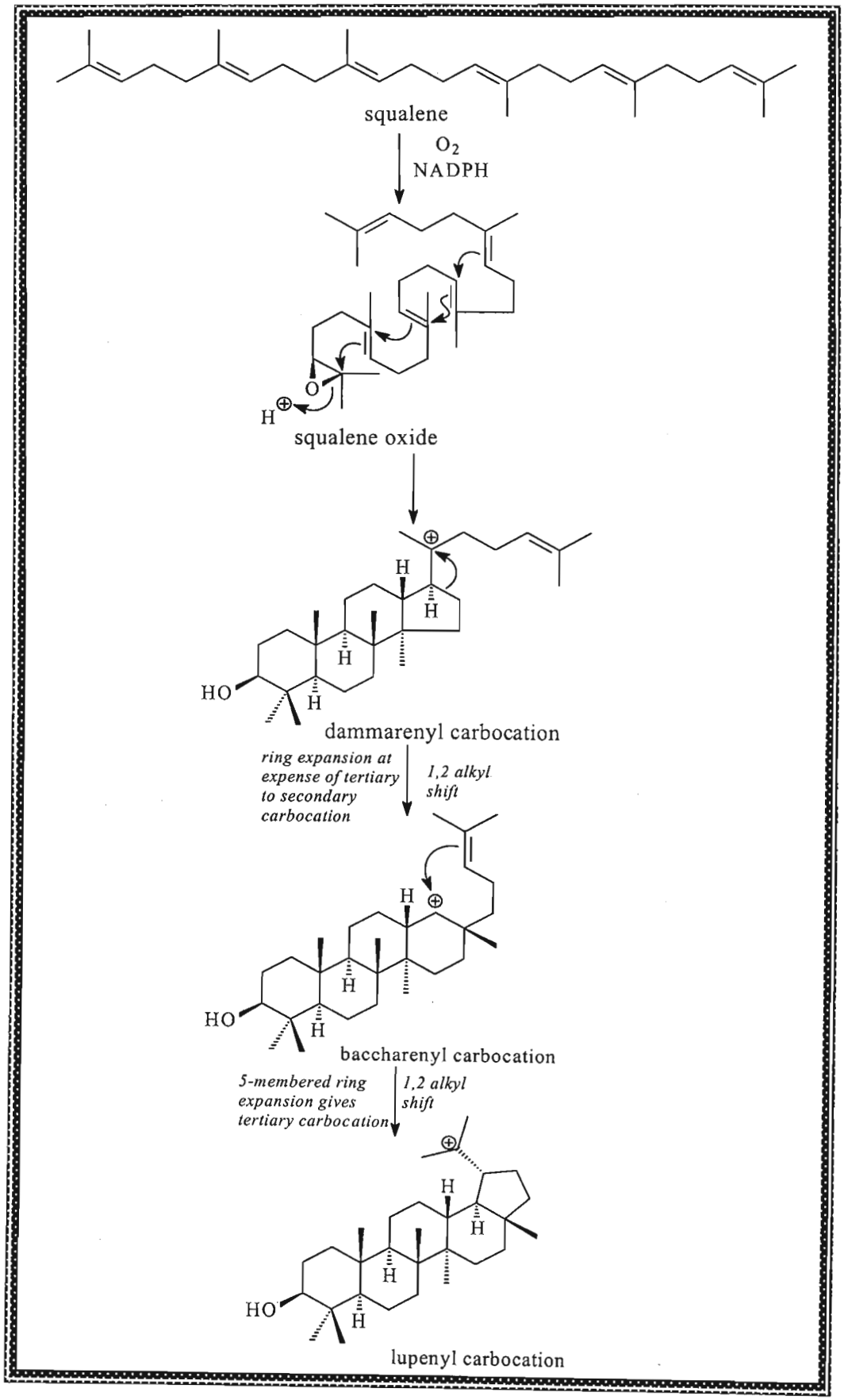


Scheme 3.4: The formation of squalene [4]

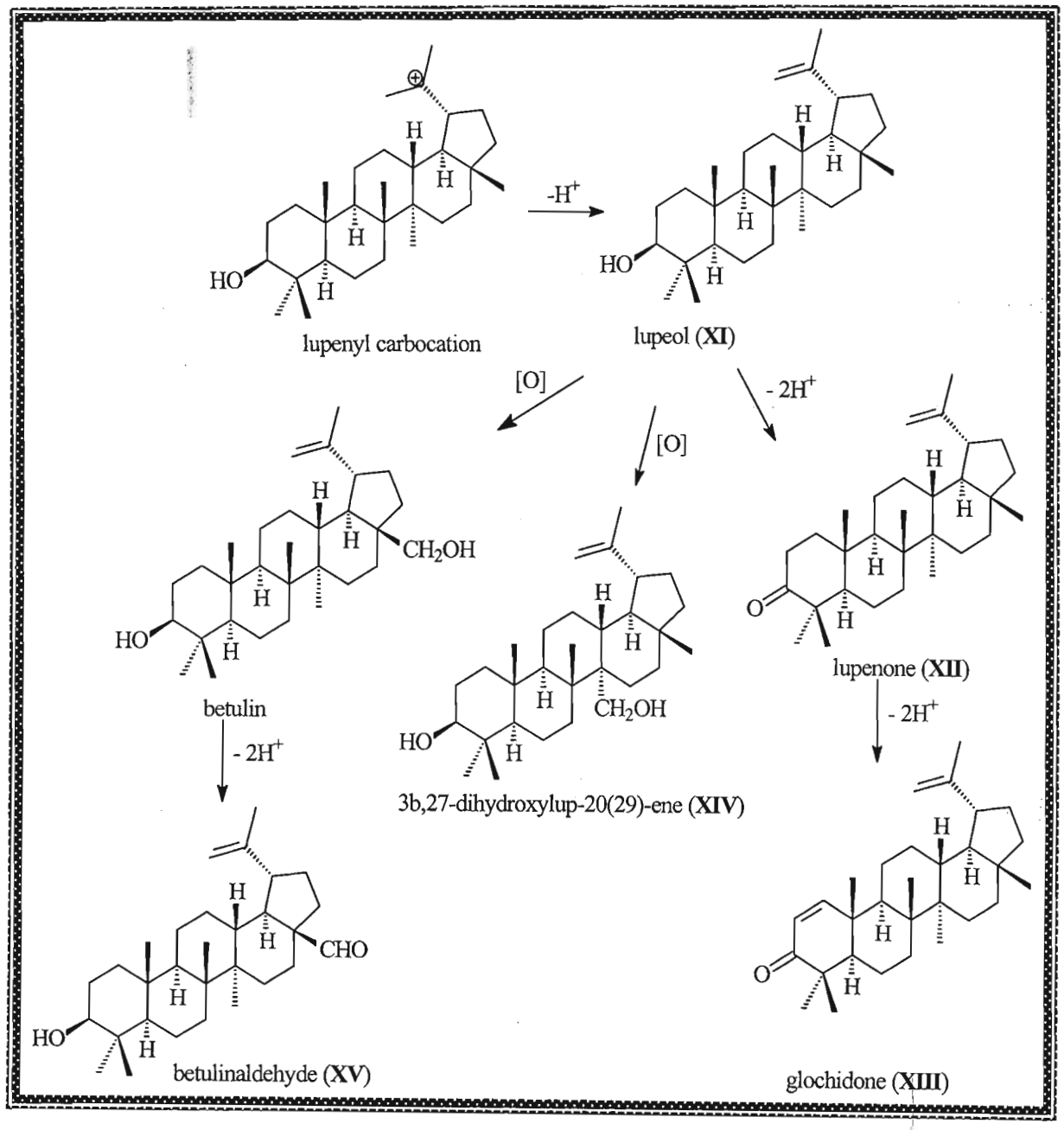
Cyclisation of squalene occurs through the intermediate squalene-2,3-oxide. It is produced in a reaction catalysed by a flavoprotein requiring oxygen and NADPH as cofactors. Protonation of the epoxide group, opens up the epoxide ring and in doing so generates the preferred tertiary carbocation. Electrophilic addition to a double bond then occurs and this results in the formation of a six member ring (**scheme 3.5**). This process continues, generating a new carbocation after each ring is formed, until a tertiary dammarenyl carbocation is reached. Wagner-Meerwein rearrangements then occur in the dammarenyl carbocation to yield the baccharenyl carbocation.

Although this involves the sacrifice of a tertiary for a secondary carbocation, the reaction proceeds in this way as some ring strain is relieved by the formation of a six member ring. A pentacyclic ring system can now be formed by cyclization on to the double bond, giving a new five member ring and the tertiary lupenyl carbocation. Enzymes are actively involved and facilitate each step in the biosynthesis [4].

Loss of a proton from the lupenyl carbocation gives lupeol (**XI**) (**scheme 3.6**). Lupeol can then be oxidised to yield lupenone (**XII**), which is further oxidised to yield glochidone (**XIII**). Lupeol can also be oxidised to yield 3β,27-dihydroxylup-20(29)-ene (**XIV**), or it can be oxidised to yield betulin, which is then further oxidised to the aldehyde (**XV**).



Scheme 3.5: The formation of the lupenyl carbocation [4]



Scheme 3.6: The formation of compounds XI, XII, XIII, XIV and XV from the lupenyl carbocation

3.3 References

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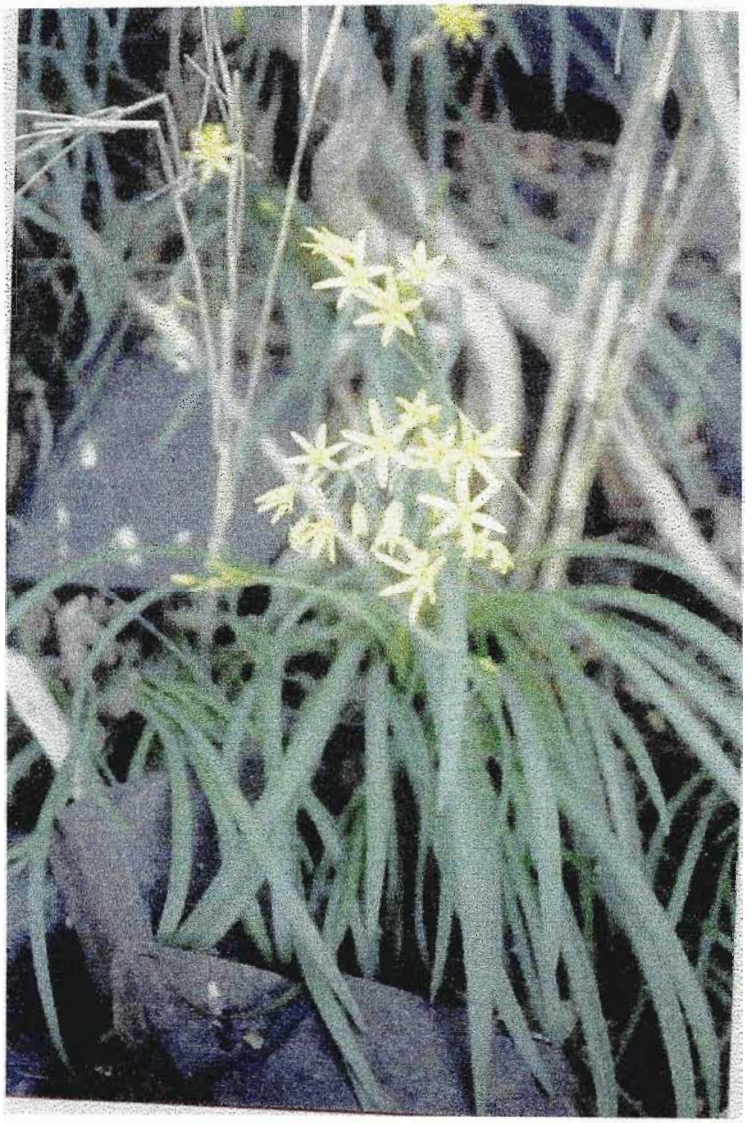
CHAPTER 4

Extractives from *Cyrtanthus breviflorus*



Cyrthantus breviflorus

4.1 A review of previous work

Plants of the family Amaryllidaceae are widely distributed in southern Africa [1]. The Amaryllidaceae plants occurring in southern Africa belong exclusively to the tribes Amaryllideae and Haemantheae, which are two of nine tribes in this family [1,2]. The tribe Haemantheae consists of the following six genera: *Cyrtanthus, Haemanthus, Clivia, Scadoxus, Gethyllis* and *Apodolirion* [1].

The word *Cyrtanthus* is derived from the Latin words *Kyrtos*, meaning curved, and from the word *anthos*, which refers to a flower. The genus *Cyrtanthus* is characterized by few narrow leaves, buried bulbs, one to few flowers in the inflorescence, flower tubes are narrow or trumpet-like and tepal lobes are shorter than the curved flower tubes [3]. *Cyrtanthus* is native to Africa and is found mainly in South Africa. There are approximately fifty known species to date [3].

Bulbs of many species of the Amaryllidaceae family have been used in traditional medicine in South Africa [1,4]. *Cyrtanthus* is no exception: *C. breviflorus, C. stenanthus, C. contractus, C. mackenii, C. tuckii* and *C. sanguineus* are all reportedly used by Zulu people as protective sprinkling charms against storms and evil spirits [1,5]. Bulb infusions of *C. sanguineus* are taken regularly from the fourth month of pregnancy to ensure an easy labour [5,6]. *C. sanguineus* is also a popular and beautiful garden plant, which has been in cultivation since 1846 [3]. *C. mackenii, C. brachyscyphus, C. obliquus and C. obrienii* are also popular garden plants [3]. Bulbs of several *Cyrtanthus* species such as *C. galpinii, C. stenanthus* and *C. tuckii* are edible [3]. The bulb of *C. obliquus* is one of the ingredients in a medicine for scrofula and in another for troublesome chronic coughs. A decoction of the bulb alone is a remedy for tubercular cough. Dried bulbs of *C. obliquus* are also made into a powder and then rubbed onto fractures to aid in their healing [4]. The bulb of *C. obliquus* is a South African remedy for cystitis and leprosy [1, 4]. Bulb infusions of this species are also used for stomach aches [1,7].

Cyrtanthus breviflorus Harv. is also known as Wild Crocus and Yellow Fire Lily. The word *breviflorus* refers to short flowers [6]. The Zulu name for *C. breviflorus* is "uvelabohleke" [3]. This plant is characterized by strap–shaped leaves which are upright, long and narrow, growing to 200 mm. Attractive, golden–yellow funnel–shaped flowers crown the tip of an erect 200 mm long peduncle. Flowering occurs in spring. Plants are often found flourishing after a grass burn fire [6]. The bulbs of *Cyrtanthus breviflorus* are very glutinous. *C. breviflorus* is often dormant during the winter months [6]. *C. breviflorus* can be found in moist or dry grassland regions along the coast from eastern Cape to Kenya [3]. *C. breviflorus* is a schedule two protected species in South Africa [8].

The bulbs of *C. breviflorus* are used for urinary and chest complaints and the roots below the bulb are used to treat broken limbs and sprains [6]. Bulb infusions are taken as love charm emetics and are also used as protective sprinkling charms [5]. Bulbs are used to treat roundworm and tapeworm [1,5]. *C. breviflorus* has been reported to be a dangerous plant, having been suspected of causing poisoning in cattle [1,5].

Seven *Cyrtanthus* species have been examined phytochemically and seven different alkaloids have been identified from them **(table 4.1)** [1]. The alkaloids from *Cyrtanthus* can be classified into five distinct types: the lycorine, the homolycorine, the haemanthamine, the narciclasine and the galanthamine type.

Table 4.1: Previously identified alkaloids from *Cyrtanthus*

Cyrtanthus species examined	Alkaloid found	Reference
C. brachyscyphus	galanthamine	1, 9
C. elatus	galanthamine	1, 10
	haemanthamine	1, 10
	haemanthidine	1, 10
	hippeastrine	1
	lycorine	1, 10
	narciclasine	1, 11
	parkamine	1
C. falcatus	galanthamine	1, 9
C. herrei	galanthamine	1, 9, 12
C. montanus	galanthamine	1, 9
C. obrienii	galanthamine	1, 9
C. pallidus	lycorine	1

The lycorine type alkaloids

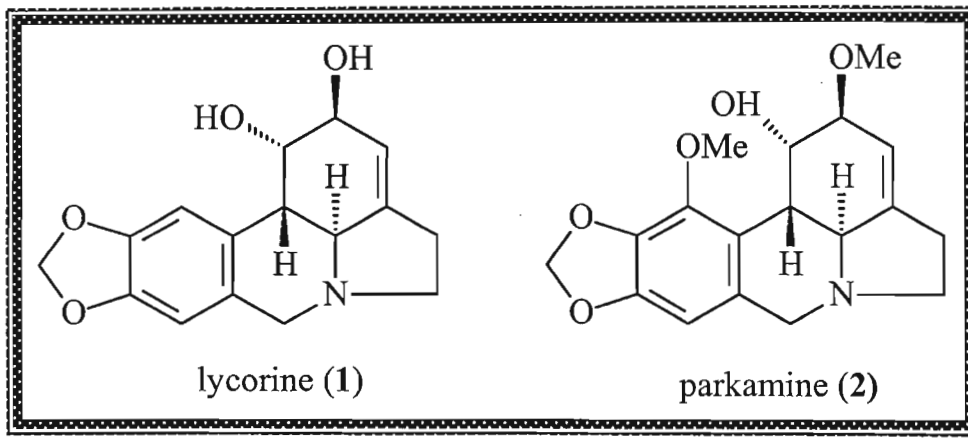


Figure 4.1: Previously isolated lycorine type alkaloids from *Cyrtanthus*

Lycorine (**1**) is an ubiquitous alkaloid in the Amaryllidaceae family. Its isolation is usually facile due to its relative insolubility in most organic solvents. Lycorine (**1**) usually precipitates out from crude extracts [13]. Parkamine (**2**) is closely related to **1**, except that methoxy groups are present at C-2 and C-11.

A homolycorine type alkaloid

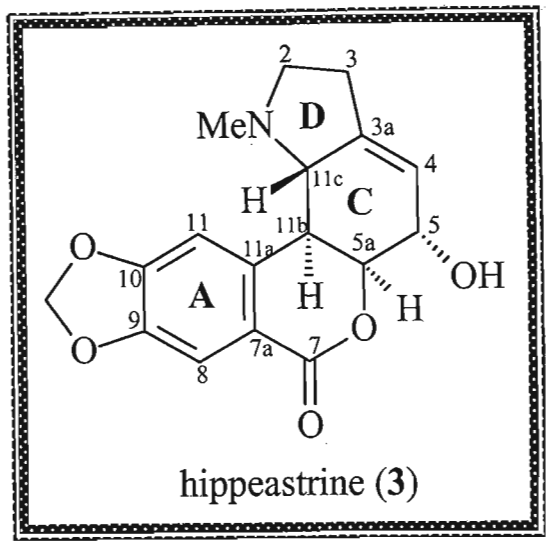


Figure 4.2: A previously isolated homolycorine type alkaloid from *Cyrtanthus*

Hippeastrine (**3**) has a characteristic 3a,4-double bond and an α hydroxyl group at C-5. The orientation of the hydrogen at C-5a is α to the C ring. The bioactivity of hippeastrine (**3**) is given in **table 4.2**.

The haemanthamine type alkaloids

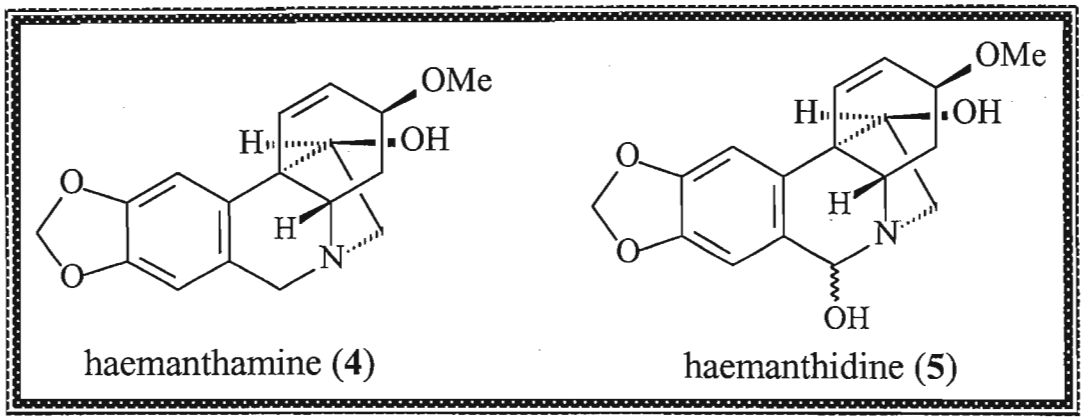


Figure 4.3: Previously isolated haemanthamine type alkaloids from *Cyrtanthus*

Haemanthamine (**4**) is commonly isolated from the family Amaryllidaceae [1]. It has a characteristic 1,2-double bond as well as a β-orientated methoxy group. The most distinguishing feature of this alkaloid is the 5,10b-ethane nitrogen bridge, which has an α orientation. Haemanthidine (**5**) is isolated as a mixture of the 6α and 6β hydroxyl isomers. The bioactivity of haemanthamine (**4**) and haemanthidine (**5**) is given in **table 4.2**.

A narciclasine type alkaloid

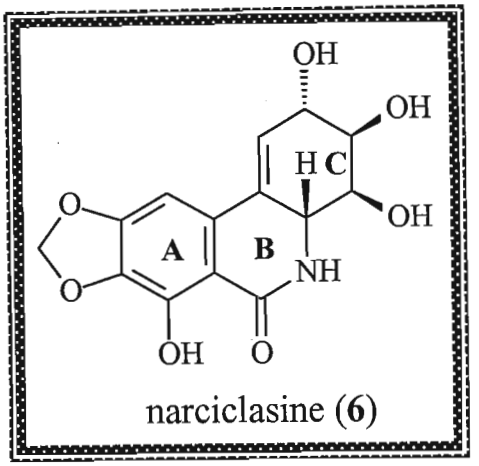


Figure 4.4: A previously isolated narciclasine type alkaloid from *Cyrtanthus*

Narciclasine (**6**) is distinguished by the presence of an amide group in ring B. The bioactivity of narciclasine (**6**) is given in **table 4.2**.

A galanthamine type alkaloid

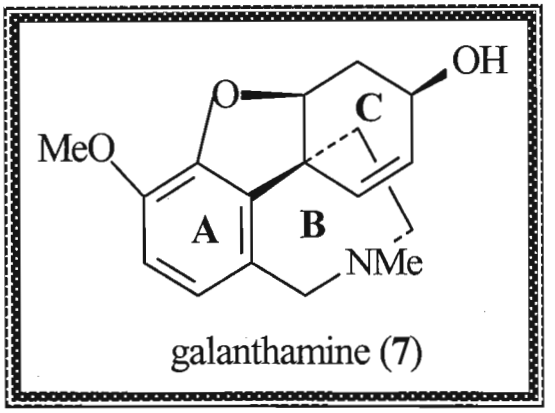


Figure 4.5: A previously isolated galanthamine type alkaloid from *Cyrtanthus*

Galanthamine (**7**) is unique in that ring A and ring C are joined by a 10,1 ether linkage. This alkaloid also possesses an α-orientated ethane nitrogen bridge. Ring C contains a single double bond and an allylic hydroxyl group. The bioactivity of galanthamine (**7**) is given in **table 4.2**.

Table 4.2: The bioactivity of alkaloids isolated from *Cyrtanthus* species

⊗Previously isolated alkaloids from *Cyrtanthus breviflorus*	Bioactivity	Reference
hippeastrine (3)	Active against *Herpes simple* type I virus.	14
	Significantly active against LNCaP and HT cell lines.	15
	Cytotoxic against non-cancerous LMTK cells.	16
	Weakly cytotoxic against Molt 4 lymphoid cells.	16
haemanthamine (4)	Cytotoxic against a variety of cultured cells (*in vitro*).	17
	Cytotoxic against fibroblastic LMTK cells.	16
	Inhibitor of HeLa cells.	18
	moderately cytotoxic against Molt 4 Lymphoid cells.	16
haemanthidine (5)	Active against A-431, KB, Lu1, Me12 and ZR-75-1 cell lines	15
	Significantly active against LNCaP and HT cell lines.	15
narciclasine (6)	Protein synthesis inhibitor.	19
	Antimitotic agent.	19
	Possesses antiviral props.	19
	Most active antineoplastic agent of the Amaryllidaceae alkaloids	19
galanthamine (7)	Potential treatment for Alzheimer's disease (currently under clinical trial)	20
	used for its anticholinesterase activity	21

⊗ for the bioactivity of the lycorine type alkaloids see **table 2.2.**

4.2 Results and discussion

Cyrtanthus breviflorus has not been investigated previously. Overall, little investigation has been done on *Cyrtanthus*. Only seven alkaloids have been reported previously from the genus i.e. lycorine (**1**), parkamine (**2**), hippeastrine (**3**), haemanthamine (**4**), haemanthidine (**5**), narciclasine (**6**) and galanthamine (**7**) [1]. The marked role of *Cyrtanthus* in traditional medicinal usage prompted further investigation [1]. The ethanol extract of the bulbs, leaves and roots of *Cyrtanthus breviflorus* yielded four alkaloids, lycorine (**I**), haemanthamine (**VIII**), crinamine hydrochloride (**IX**) and tazettine (**X**), after separation by column chromatography over silica gel (**figure 4.6**). Crinamine hydrochloride (**IX**) and tazettine (**X**) have not been reported previously from *Cyrtanthus*.

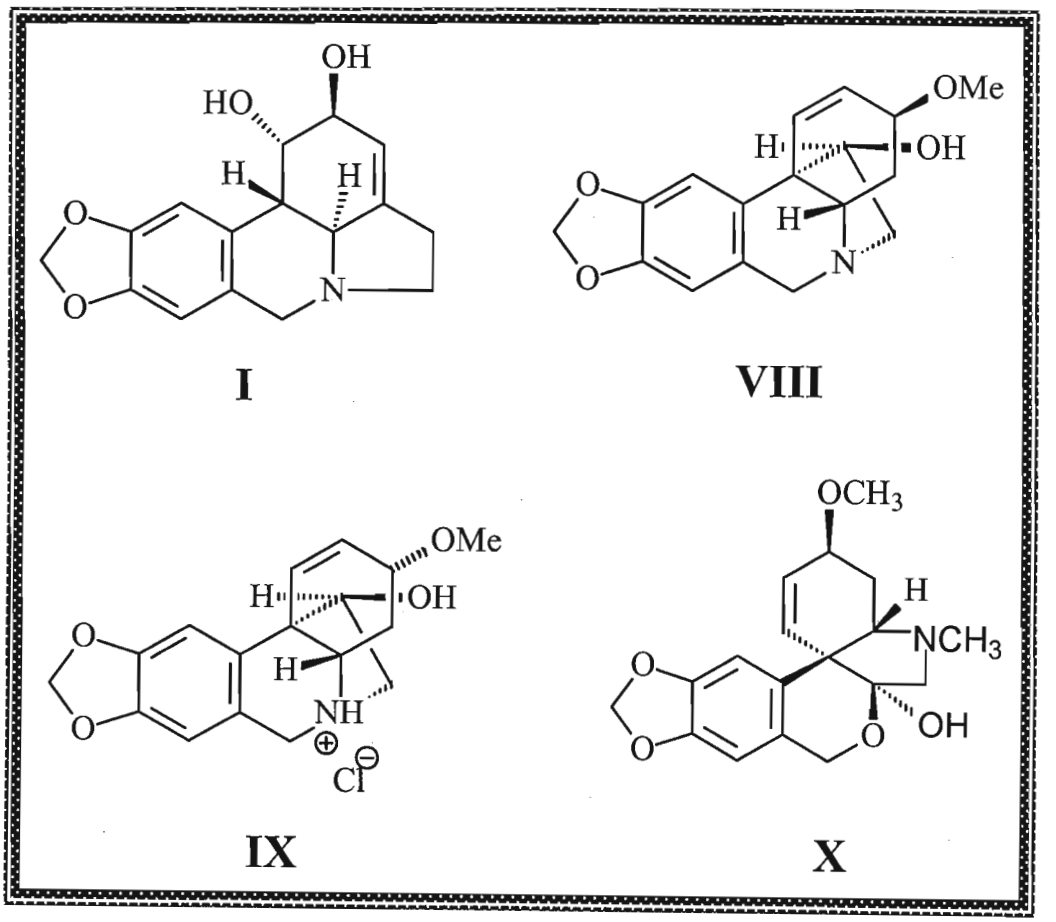


Figure 4.6: Compounds isolated from the ethanol extract of *Cyrtanthus breviflorus*

The hexane extract of *Cyrtanthus breviflorus* yielded five triterpenoids, lupeol (**XI**), lupenone (**XII**), glochidone (**XIII**), 3β,27-dihydroxylup-20(29)-ene (**XIV**) and betulinaldehyde (**XV**), after being subjected to column chromatography. Triterpenoids have not been reported previously from *Cyrtanthus*.

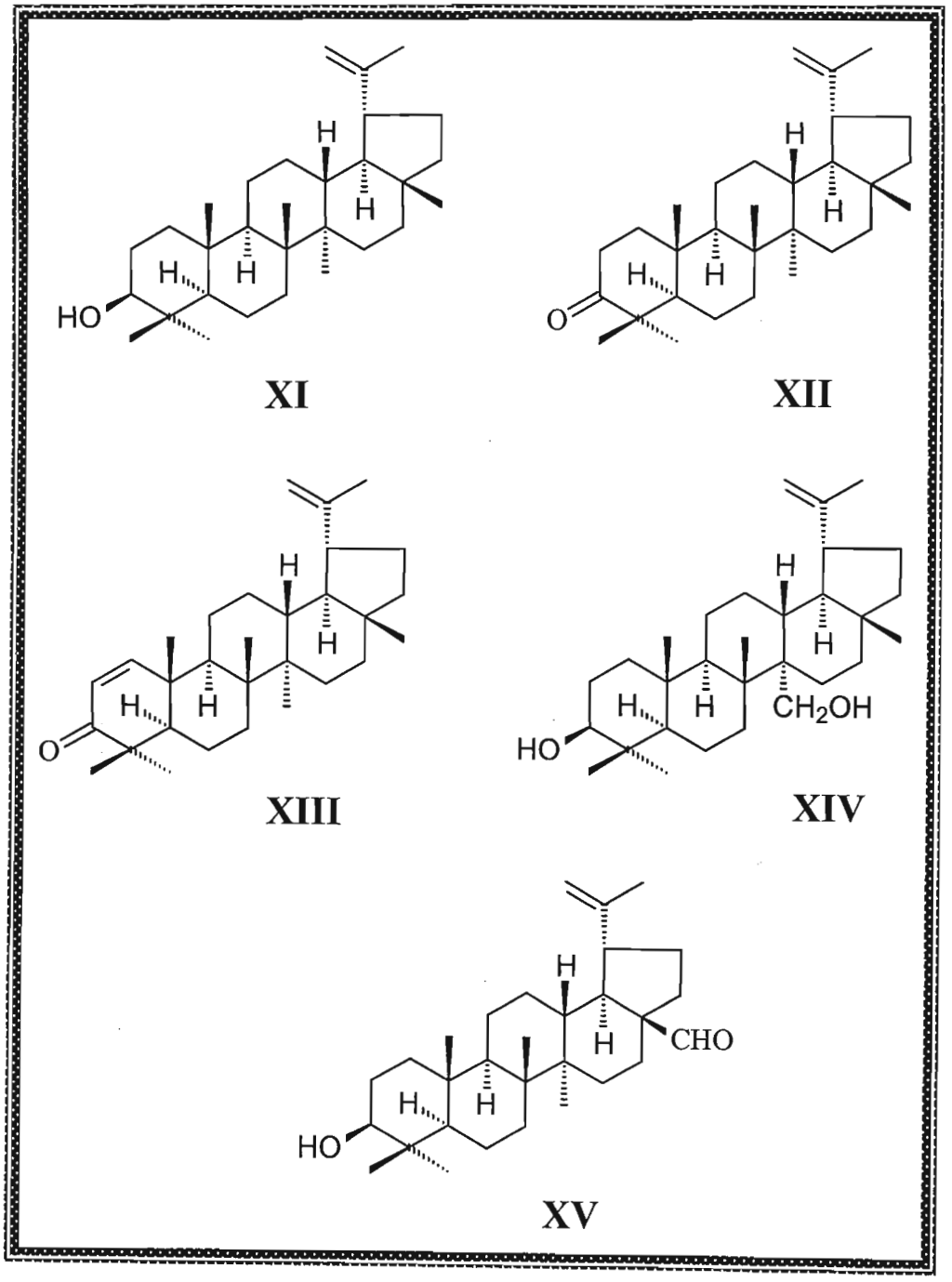


Figure 4.7: Compounds isolated from the hexane extract of *Cyrtanthus breviflorus*

4.2.1 The structural elucidation of compound VIII

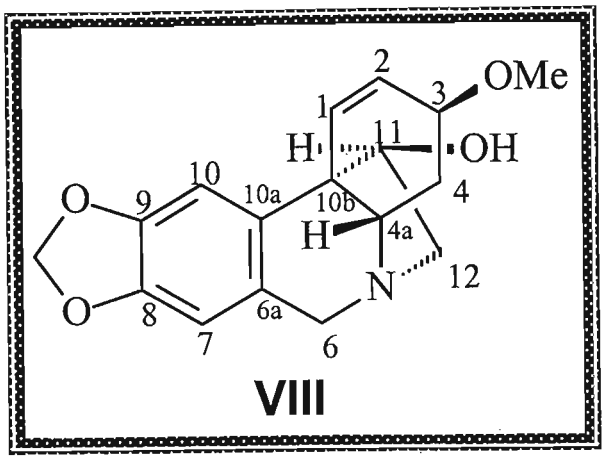


Figure 4.8: COMPOUND VIII: haemanthamine

Compound **VIII** was isolated as white crystalline material (27 mg). Its physical and spectral properties were consistent with those of haemanthamine [24]. Haemanthamine has been isolated previously from *Cyrtanthus elatus* and is frequently isolated from the Amaryllidaceae family [1,10]. Spectra for compound **VIII** are given on pages 209-218 in appendix A.

The mass spectrum of compound **VIII** showed a molecular ion peak at *m/z* 301 and fragments at *m/z* 269, 257, 240, 227, 225, 211 and 181. The molecular ion peak corresponded to the molecular formula of haemanthamine ($C_{17}H_{19}NO_4$). Haemanthamine (**VIII**) is stereoisomeric with crinamine (**V**). The difference between **VIII** and **V** is that in the former compound, the methoxy substituent on C-3 is in the β position whereas in the latter compound it is in the α position. Thus both alkaloids constitute a C-3 epimeric pair. Ghosal *et al.* and Duffield *et al.* have reported on the similarities and differences in the mass spectrum of C-3 epimeric pairs of 5,10b-ethanophenanthridine alkaloids [22,23]. They have shown for C-3 epimeric pairs, such as vittatine/epicrinine or haemanthamine/crinamine, that the first alkaloid in each pair i.e. the alkaloid with the C-3 oxygen substituent in the β position, had its molecular ion appear as a prominent peak in the mass spectrum. In the corresponding epimeric pair i.e. the C-3 oxygen substituent in the α position, the molecular ion was not detectable [22,23].

However, it was reported that the rest of the fragmentation pattern is similar [23]. The fragments observed in the mass spectrum of haemanthamine (**VIII**) arise through an analogous manner as that of crinamine (pages **51-54**) [22,23].

The infra red spectrum of compound **VIII** was consistent with that of haemanthamine [24]. The infra red spectrum showed bands at V_{max} 3448 cm^{-1} (O – H stretching) and 1029 cm^{-1} (C – O stretching), 1483 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1242 cm^{-1} (C – N stretching) indicative of carbon nitrogen bonds and 929 cm^{-1} (C – O – C stretching), suggestive of the presence of a methylenedioxy group.

The ^1H NMR data of compound **VIII** was consistent with that of haemanthamine (**table 4.3**) [24]. The ^1H NMR spectrum, recorded in $CDCl_3$, showed three singlets in the downfield region at δ_H 5.86, 6.52 and 6.89. These resonances were assigned to the protons of the methylenedioxy group and to the *p*-oriented aromatic protons, H-7 and H-10, respectively. The HSQC correlation between the methylenedioxy group protons and a methylene carbon resonance at δ_C 101.10, as well as the NOESY interactions between H-10 and H-1/H-11 and the NOESY interactions between H-7 and H-6α/H-6β were consistent with their assignments.

The olefinic region also showed a doublet at δ_H 6.44 (J = 10.1) which was assigned to H-1 and a double doublet at δ_H 6.23 (J = 10.1, 4.9), assigned to H-2. The splitting pattern of the H-2 resonance is characteristic for compounds having a *trans* orientation for the 5,10b-ethane bridge and the C-3 oxygen substituent (page **49**). The H-2 resonance is split by H-1 and the H-3 proton. The H-3 resonance was observed as a multiplet at δ_H 3.85. The NOESY interaction between H-2 and H-3 confirmed their assignments. NOESY interactions were also observed between the H-3 and the two H-4 protons at δ_H 2.14 and δ_H 1.97. These resonances were assigned to H-4α and H-4β respectively. The H-4a resonance was observed as a multiplet at δ_H 3.30. This resonance showed an HSQC correlation to a methine carbon at δ_C 62.90. The NOESY interaction between H-4a and H-4β confirmed their assignments.

The H-6 protons were observed resonating as doublets (J_{gem}=16.7) at δ_H 4.28 and δ_H 3.76. H-6β was assigned to δ_H 4.28 due to its NOESY interaction with H-4a. H-11_{endo} was assigned to the multiplet at δ_H 3.94. The HSQC spectrum showed a correlation between H-11_{endo} and a methine carbon at δ_C 79.72. The NOESY interaction between H-11_{endo} and H-10 confirmed their assignments. The remaining two resonances at δ_H 3.44 and δ_H 3.14 were assigned to the H-12 protons. H-12_{endo} was assigned to the more downfield resonance due to its NOESY correlation with H-6α.

The ^{13}C NMR spectrum showed seventeen carbon atoms. Their chemical shifts were consistent with those reported for haemanthamine (**table 4.4**) [25].

Table 4.3: ^{1}H NMR data for compound VIII and literature data for haemanthamine [24] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound VIII	**^{1}H NMR lit. data for haemanthamine [24]**	**Observed NOESY interactions**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**	
H-1	6.44 d (10.1)	6.40 d (10.1)	2, 10, 11_{endo}
H-2	6.23 dd (10.1, 4.9)	6.30 dd (10.1, 4.8)	1, 3, OMe
H-3	3.85 m	3.88 m	2, 4α, 4β, OMe
H-4α	2.14 ddd (13.5, 9.2, 4.2)	2.12 ddd (13.5, 13.4, 3.6)	3, 4α, 12_{exo}
H-4β	1.97 ddd (13.5, 9.0, 4.3)	2.06 ddd (13.5, 6.1, 1.9)	3, 4α, 4a
H-4a	3.30 m	3.32 dd (13.4, 6.1)	4β, 6β
H-6α	3.76 d (16.7)	4.30 d (16.8)	6β, 12_{endo}, 7
H-6β	4.28 d (16.7)	3.68 d (16.8)	6α, 4a, 7
H-7	6.52 s	6.48 s	6α, 6β
H-10	6.89 s	6.84 s	1, 11
H-11_{endo}	3.94 m	3.98 dddd (6.6, 3.5, 1.1)	1, 10, 12_{endo}, 12_{exo}
H-12_{exo}	3.14 dd (13.9, 7.0)	3.39 dd (14.0, 6.6)	4α, 11_{endo}, 12_{exo}
H-12_{endo}	3.44 dd (13.9, 3.2)	3.19 dd (14.0, 3.5)	6α, 11_{endo}, 12_{exo}
OCH_3	3.33 s	3.36 s	2, 3
OCH_2O	5.86 s	5.90 s	----------

$^{\otimes}$ ^{1}H NMR spectrum recorded in CD_3OD, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 200 MHz.

Table 4.4: ^{13}C NMR data of compound VIII and literature data for haemanthamine [25]

	^{13}C NMR data of COMPOUND VIII	^{13}C NMR lit. data of haemanthamine [25]	Observed HMBC correlations
Carbon No.	**$^{\otimes}$ shift/δ_C ppm**	**$^{\otimes\otimes}$shift/δ_C ppm**	**C→H**
C-1	127.9 CH	127.3 CH	2, 3
C-2	129.4 CH	131.6 CH	1, 3, 4β
C-3	72.9 CH	72.7 CH	1, 2, 4α, 4β, 4a, OMe
C-4	27.8 CH_2	28.1 CH_2	2, OMe
C-4a	62.9 CH	62.6 CH	1, 3, 4α, 4β, 12_{endo}
C-6	60.4 CH_2	61.2 CH_2	4a, 7, 12_{endo}, 12_{exo}
C-6a	125.6 C	126.5 C	6α, 6β, 10
C-7	106.7 CH	106.7 CH	6α, 6β
C-8	146.9 C	146.0 C	OCH_2O, 7, 10
C-9	146.5 C	146.3 C	11
C-10	103.1 CH	103.2 CH	6β, 7
C-10a	135.5 C	135.2 C	1, 4a, 6α, 6β, 7, 11
C-10b	50.3 C	49.9 C	1, 2, 4α, 4β, 4a, 10, 12_{endo}
C-11	79.7 CH	80.0 CH	4a. 6α, 6β, 12_{exo}
C-12	62.6 CH_2	63.5 CH_2	4a, 6α, 6β
$O\underline{C}H_3$	55.6 CH_3	56.5 CH_3	3, 4β
$O\underline{C}H_2O$	101.1 CH_2	100.7 CH_2	----------

$^{\otimes}$ ^{13}C NMR spectrum recorded in CD_3OD, 100 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 75 MHz.

4.2.2 The structural elucidation of compound IX

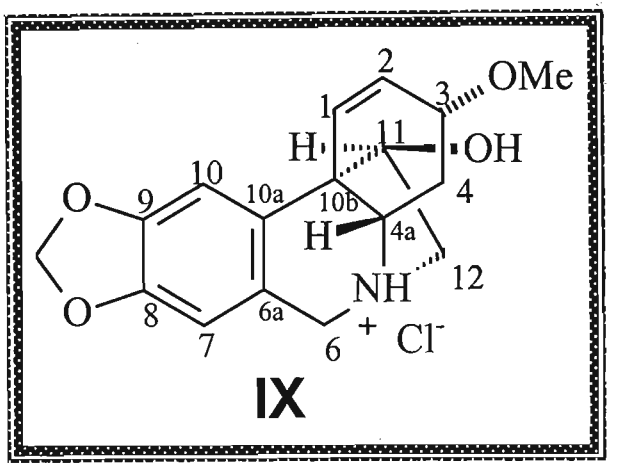


Figure 4.9: COMPOUND IX: crinamine hydrochloride

Compound **IX** was isolated as white crystalline material (13 mg). The ^{1}H NMR spectrum of **IX** was similar to that of crinamine (**V**) with the exception that certain resonances were shifted downfield. Pronounced downfield shifts were evident for the protons surrounding the nitrogen atom, suggesting that compound **IX** was a crinamine salt. The subsequent base treatment of **IX** yielded crinamine (**V**). Crinamine or its salt has not been isolated previously from the genus *Cyrtanthus*. It is unlikely that compound **IX** is an artefact, arising from crinamine, as no acid or base was used in the extraction or isolation procedure. Spectra for compound **IX** are given on pages 219-228 in appendix A.

The mass spectrum of compound **IX** showed a very weak molecular ion peak at *m/z* 301, consistent with a compound of the crinamine type [23]. The spectrum was very similar to that of crinamine (**V**). The mass spectral fragmentation pattern was previously discussed on pages **51-53**.

The infra red spectrum of compound **IX** was similar to crinamine (**V**) [26]. The infra red spectrum showed bands at V_{max} 3322 cm^{-1} (O – H stretching) and 1038 cm^{-1} (C – O stretching), 1492 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1242 cm^{-1} (C – N stretching) indicative of carbon nitrogen bonds and 940 cm^{-1} (C – O – C stretching), suggestive of the presence of a methylenedioxy group.

The ^{1}H NMR spectrum of compound **IX**, recorded in deuterated methanol, exhibited three downfield signals at δ_H 5.98, 6.57 and 6.84. These resonances were assigned to the protons of the methylenedioxy group and to the *p*-oriented aromatic protons, H-7 and H-10, respectively. The HSQC spectrum suggested that these assignments were correct. Furthermore, the assignments of H-7 and H-10 were consistent with literature [22,27]. In addition to this, a NOESY interaction was observed between H-10 and the H-1 proton.

The olefinic protons, H-1 and H-2, were observed as a double doublet and a doublet in the olefinic region of the ^{1}H NMR spectrum. Strong coupling between these protons were observed in the COSY spectrum. H-1 is split by H-2 and H-3 (J = 10.4, 2.7 Hz) whereas H-2 is split only by H-1 (J = 10.4 Hz). The dihedral angle between H-2 and H-3 is approximately 90°, hence no coupling is observed. The resonances at δ_H 6.15 and δ_H 6.26 were accordingly assigned to H-1 and H-2 respectively. As observed previously, the above splitting pattern is important because information about the orientation of the 5,10b-ethane bridge and the C-3 oxygen substituent can be derived from it (discussed on page **49**). From the splitting pattern it can be inferred that the 5,10b-ethane bridge and the C-3 methoxy substituent have the same orientation. This is consistent with the mass spectral suggestion.

NOESY correlations between the H-2 proton and the resonances at δ_H 3.97 and δ_H 3.39 were observed in the NOESY spectrum. The HSQC spectrum showed a correlation between the δ_H 3.97 resonance and a methine carbon. The resonance at δ_H 3.39 correlated to a methyl carbon. This suggested that the methoxyl group was located on C-3 and that H-3 resonated at δ_H 3.97. The COSY spectrum showed that the H-3 resonance coupled to two resonances at δ_H 2.95 and δ_H 2.32. H-4α and H-4β were assigned to these resonances respectively. The HSQC spectrum suggested that these assignments were correct. The NOESY correlation between the methoxyl group protons and the H-4α proton was consistent with their assignments. The H-4 protons were observed, in the COSY spectrum, to couple additionally with a resonance at δ_H 3.70. This resonance was assigned to H-4a.

The HSQC spectrum showed a correlation between the H-4a resonance and a methine carbon at δ_C 67.2 suggesting that its assignment was correct. The H-6 protons were observed resonating as doublets (geminal coupling) at δ_H 4.11 and 4.76. The HSQC spectrum showed a correlation between the H-6 proton resonances and a methylene carbon. The lower field resonance was assigned to H-6β due to its co-planarity with the nitrogen atom [28,29]. The NOESY correlation between H-4a and H-6β confirmed their assignments.

The remaining resonance, at δ_H 4.22, which had an HSQC correlation to a methine carbon was assigned to H-11$_{endo}$. The two remaining H-12 protons were assigned to the remaining resonances at δ_H 3.64 and δ_H 4.00. The HSQC spectrum was consistent with these assignments. NOESY correlations were observed between H-11$_{endo}$ and H-10/H-1 confirming their assignments. COSY correlations were observed between H-11$_{endo}$ and the two H-12 protons, suggesting that their assignments were correct. H-12$_{endo}$ was assigned to the resonance at δ_H 3.64. The NOESY correlation between H-11$_{endo}$ and H-12$_{endo}$ confirmed their assignments

Table 4.5 compares the ^{1}H NMR data of crinamine hydrochloride (**IX**) with that of its free base. Marked downfield shifts are observed for those protons that are spatially proximate to the protonated nitrogen atom (shown in **bold**) i.e. the two H-4 protons, the two H-6 protons, the H-11 proton and H-4a.

Table 4.5: ^{1}H NMR data for compound IX and literature data for crinamine [26] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound IX	**^{1}H NMR lit. data for crinamine [26]**	***Observed NOESY interactions**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**	**H→H**
H-1	6.15 dd (10.4, 2.7)	6.23 br s	2, 10, 11
H-2	6.26 d (10.4)		1, OMe, 3
H-3	3.97 m	3.98 m	2, OMe
H-4α	**2.95** m	**2.08** m	OMe, 4β
H-4β	**2.32** dd (13.0, 10.4)		4α
H-4a	**3.70** dd (13.0,2.9)	**3.22** dd (13.2, 3.2)	6β
H-6α	**4.11** d (16.8)	**3.67** d (16.9)	----------
H-6β	**4.76** d (16.8)	**4.29** d (16.9)	4a
H-7	6.57 s	6.46 s	----------
H-10	6.84 s	6.78 s	1, 11
H-11$_{endo}$	**4.22** m	**3.92** m	1, 10, 12$_{endo}$
H-12$_{exo}$	**4.00** m	**3.40** m	----------
H-12$_{endo}$	**3.64** m		11$_{endo}$
OC<u>H</u>$_3$	3.39 s	3.38 s	2, 3, 4α
OC<u>H</u>$_2$O	5.98 s	5.87 s	----------

$^{\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 300 MHz.

* A weak NOESY spectrum was obtained due to small amount of sample isolated.

Table 4.6: ^{13}C NMR data of compound IX and literature data for crinamine [26]

	^{13}C NMR data of COMPOUND IX	^{13}C NMR lit. data of crinamine [26]
Carbon No.	⊗ **shift/δ_C ppm**	⊗⊗ **shift/δ_C ppm**
C-1	121.2 CH	123.60 CH
C-2	137.2 CH	135.99 CH
C-3	75.0 CH	76.02 CH
C-4	27.8 CH_2	30.14 CH_2
C-4a	67.2 CH	66.10 CH
C-6	58.6 CH_2	63.44 CH_2
C-6a	118.4 C	126.55 C
C-7	107.4 CH	106.87 CH
C-8	148.7 C	146.22 C
C-9	148.2 C	146.50 C
C-10	104.2 CH	103.18 CH
C-10a	132.0 C	135.32 C
C-10b	50.9 C	50.26 C
C-11	77.9 CH	79.99 CH
C-12	61.1 CH_2	61.18 CH_2
O$\underline{C}H_3$	56.2 CH_3	55.80 CH_3
O$\underline{C}H_2$O	102.1 CH_2	100.87 CH_2

⊗ ^{13}C NMR spectrum recorded in $CDCl_3$, 100 MHz.

⊗⊗ ^{13}C NMR spectrum recorded in $CDCl_3$, 50 MHz.

Due to the strength of the sample, only a weak HMBC spectrum was obtained. This spectrum was, nevertheless, sufficient to assign all carbon singlets fully. Protonated carbons were assigned using the HSQC spectrum.

4.2.3 The structural elucidation of compound X

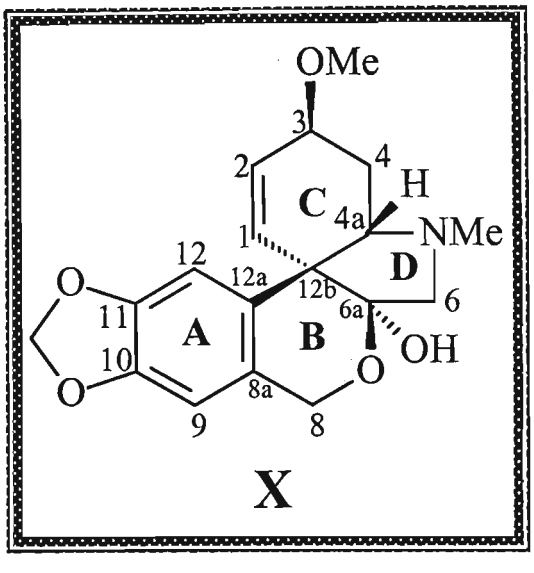
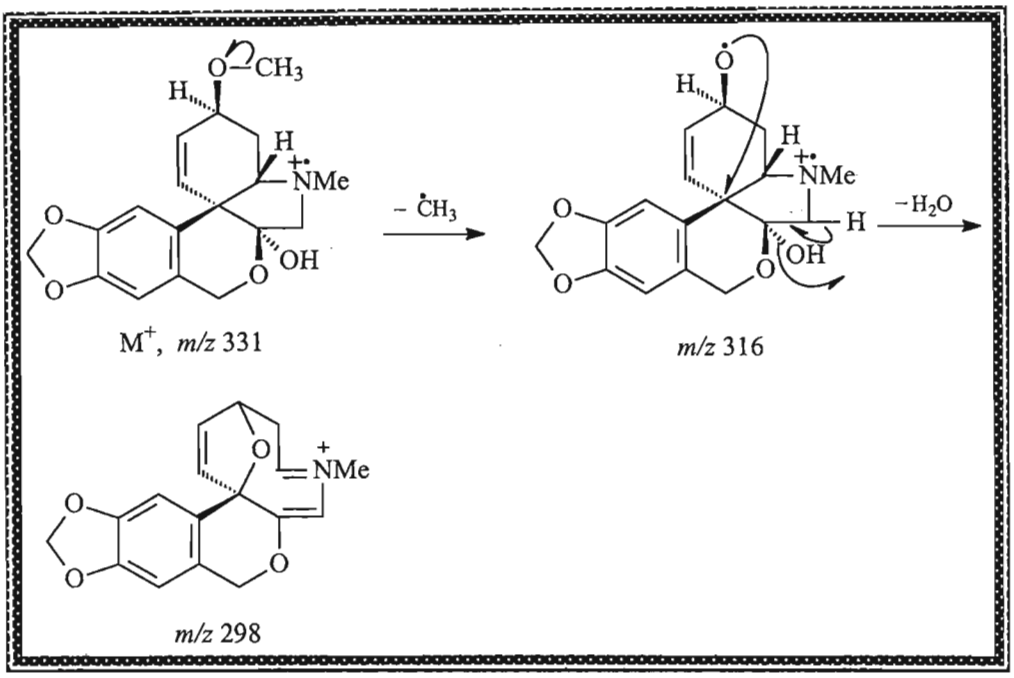


Figure 4.10: COMPOUND X: tazettine

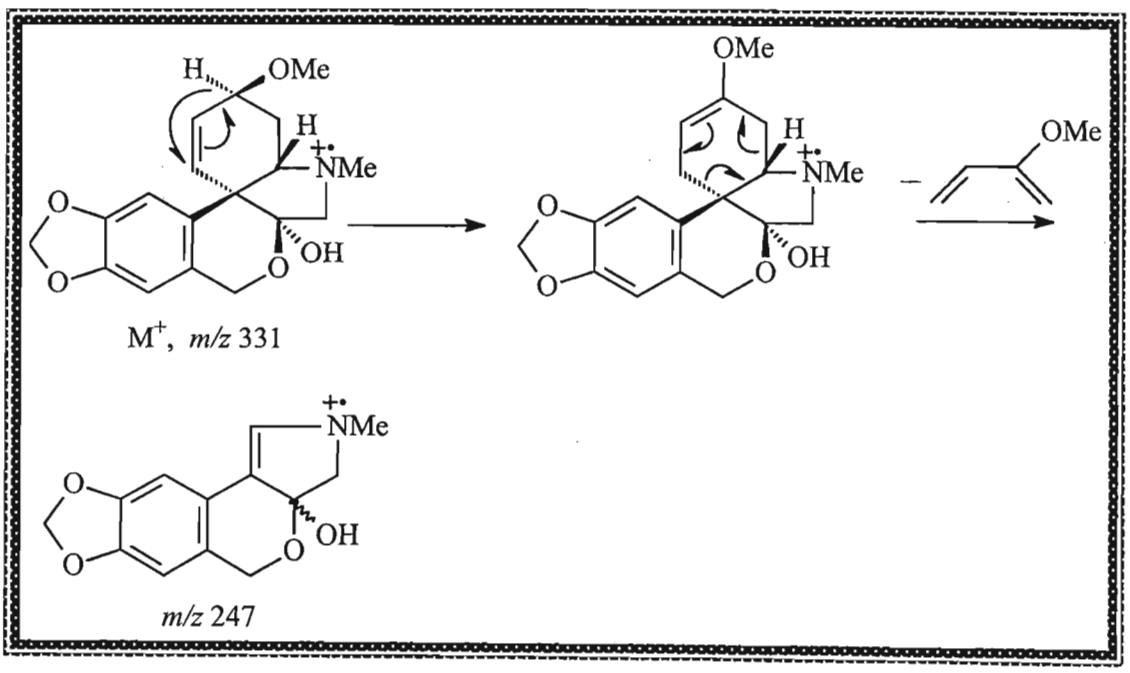
Compound **X** was isolated as shiny brown needles (36 mg). The physical and spectral properties of compound **X** were consistent with those of tazettine [1,30,31]. This is the first report of tazettine in *Cyrtanthus*. Tazettine has been isolated from a large number of species in the Amaryllidaceae family [19]. Spectra for compound **X** are given on pages 229-238 in appendix A.

The low resolution mass spectrum of compound **X** showed a molecular ion peak at *m/z* 331, corresponding to the formula of $C_{18}H_{21}NO_5$. Significant peaks were also observed at *m/z* 316, 298, 247 and 201. The peak at 316 is due to the loss of a methyl radical i.e. $[M^+ - \bullet CH_3]$. The peak at *m/z* 298 is due to the further loss of water i.e. $[M^+ - \bullet CH_3 - H_2O]$ (**scheme 4.1**) [23].



Scheme 4.1: Mass spectral fragmentation pattern of tazettine (X) [23]

The base peak in the mass spectrum of tazettine (**X**) occurs at *m/z* 247. A mechanism consistent with high resolution spectral measurements is shown in **scheme 4.2**. The double bond in ring C in tazettine rearranges. Ring C is then fragmented by a reverse Diels Alder process to yield the fragment at *m/z* 247 [23].



Scheme 4.2: Mechanism for the formation of the base peak in tazettine (X) [23]

The infra red spectrum of compound **IX** was consistent with that of tazettine and pretazettine [31]. The infra red spectrum showed a broad band at V_{max} 3329 cm^{-1} (O – H stretching) and sharper bands at 1038 cm^{-1} (C – O stretching), 1664 and 1493 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1249 cm^{-1} (C – N stretching) indicative of carbon nitrogen bonds and 939 cm^{-1} (C – O – C stretching), suggestive of the presence of a methylenedioxy group.

The ^{1}H, ^{13}C and DEPT NMR spectra showed signals for one methoxyl group, one N–methyl, one methylenedioxy group, four olefinic/aromatic protons, two aliphatic methines, three methylenes, four olefinic/aromatic quaternary carbons and two aliphatic quaternary carbons, suggesting that compound **X** was a tazettine type alkaloid. The methylenedioxy group protons and the aromatic, H-9 and H-12, protons were observed as singlets at δ_H 5.87, 6.52 and 6.76 respectively. The resonance for the methylenedioxy group protons was confirmed by the HSQC spectrum, which showed the resonance to correlate to a methylene carbon resonance at δ_C 101.2. The NOESY correlation between H-12 and H-1 and between H-9 and H-8α/H-8β confirmed their assignments. The H-8 protons were observed as a pair of doublets at δ_H 4.95 and δ_H 4.62. The COSY spectrum showed strong coupling between these protons. Due to the absence of vicinal protons, the NOESY spectrum could not be used to differentiate between H-8α and H-8β. They were assigned by comparison to literature [31].

The olefinic protons, H-1 and H-2, were observed as a pair of double doublets at δ_H 5.65 and δ_H 6.10 respectively. The COSY spectrum showed strong coupling between H-1 and H-2. Weaker coupling between H-1 and H-3 and between H-2 and H-3 were observed. H-3 was assigned to the multiplet at δ_H 4.13. NOESY interactions between H-1 and H-2/H12 as well as those between H-2 and H-3 confirmed their assignments. Additionally, H-2 showed a NOESY interaction with a methoxy proton group resonance at δ_H 3.45. This suggested that the methoxy group was positioned at C-3.

The COSY spectrum showed H-3 coupling to two protons at δ_H 2.30 and δ_H 1.60. The HSQC spectrum showed these resonances to belong to geminal protons. These resonances were assigned to the H-4 protons. H-4α was assigned to the more downfield resonance. The NOESY interaction between H-4α and H-3α was consistent with their assignments. The COSY spectrum showed strong coupling between the H-4 protons and a resonance at δ_H 2.98. This resonance was assigned to H-4a. The NOESY interaction between H-4a and H-4β was consistent with their assignments.

The singlet at δ_H 2.49, which integrated for three protons, was assigned to the *N*–methyl group. The presence of a *N*–methyl group in tazettine type alkaloids, distinguishes them from the haemanthamine type, from which they formed biosynthetically [32]. The NOESY spectrum showed interactions between the *N*–methyl group and the H-4α, H-6β and the H-4a protons. The remaining pair of doublets at δ_H 3.30 and δ_H 2.72 were assigned to H-6α and H-6β respectively. The NOESY spectrum showed an interaction between the two H-6 protons and the *N*–methyl group.

Table 4.7: ^{1}H NMR data for compound X and literature data for tazettine [31] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound X	**^{1}H NMR lit. data for tazettine** [31]	**Observed NOESY interactions**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**	**H→H**
H-1	5.65 dd (10.5, 2.0)	5.60 ddd (10.5, 2.0, 1.5)	2, 12
H-2	6.10 dd (10.5, 2.0)	6.15 ddd (10.5, 2.0, 1.2)	1, 3, OMe
H-3α	4.13 m	4.13 m	2, 4α, OMe
H-4α	2.30 m	2.20 m	3, 4β, OMe
H-4β	1.60 m	1.60 m	4α, 4a, 12
H-4a	2.98 m	2.83 m	4β, N-Me
H-6α	3.30 d (11.7)	3.30 d (10.5)	6β, N-Me
H-6β	2.72 d (10.8)	2.65 d (10.5)	6α, N-Me
H-8α	4.95 d (14.5)	4.95 dd (14.7, 0.5)	9, 8β
H-8β	4.62 d (14.5)	4.65 dd (14.7, 0.5)	9, 8α
H-9	6.52 s	6.50 s	8α, 8β
H-12	6.76 s	6.85 s	1, 4β
OCH_2O	5.87 s	5.90 s	----------
OCH_3	3.45 s	3.45 s	2, 3
N-CH_3	2.49 s	2.40 s	4α, 6β, 4a

$^{\otimes}$ ^{1}H NMR spectrum recorded in CD_3OD, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 90 MHz.

Table 4.8: ^{13}C NMR data of compound X and literature data for tazettine [30]

	^{13}C NMR data of COMPOUND X		^{13}C NMR lit. data of tazettine [30]		Observed HMBC correlations
Carbon No.	**$^{\otimes}$ shift/δ_C ppm**		**$^{\otimes\otimes}$ shift/δ_C ppm**		**C→H**
C-1	129.0	CH	128.6	CH	3
C-2	129.9	CH	130.6	CH	3, 4
C-3	72.5	CH	72.9	CH	1, 4, OMe
C-4	26.0	CH_2	26.7	CH_2	2
C-4a	70.9	CH	70.0	CH	1, 4, 6, N-Me, OMe
C-6	64.7	CH_2	65.6	CH_2	N-Me
C-6a	101.1	C	102.1	C	1, 2, 6, 8, N-Me
C-8	61.6	CH_2	62.1	CH_2	9
C-8a	126.0	C	125.5	C	8, 12
C-9	103.9	CH	104.0	CH	8
C-10	146.6	C	146.4	C	9, 12, OCH_2O
C-11	146.8	C	146.6	C	9, 12, OCH_2O
C-12	108.8	CH	109.3	CH	----------
C-12a	127.6	C	128.0	C	8, 9
C12b	49.8	C	49.9	C	2, 4, 6, 12
$O\underline{C}H_2O$	101.2	CH_2	100.9	CH_2	----------
$O\underline{C}H_3$	55.4	CH_3	56.2	CH_3	----------
N-$\underline{C}H_3$	42.1	CH_3	41.9	CH_3	6

$^{\otimes}$ ^{13}C NMR spectrum recorded in CD_3OD, 100 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 75.5 MHz.

4.2.4 The structural elucidation of compound XI and XII

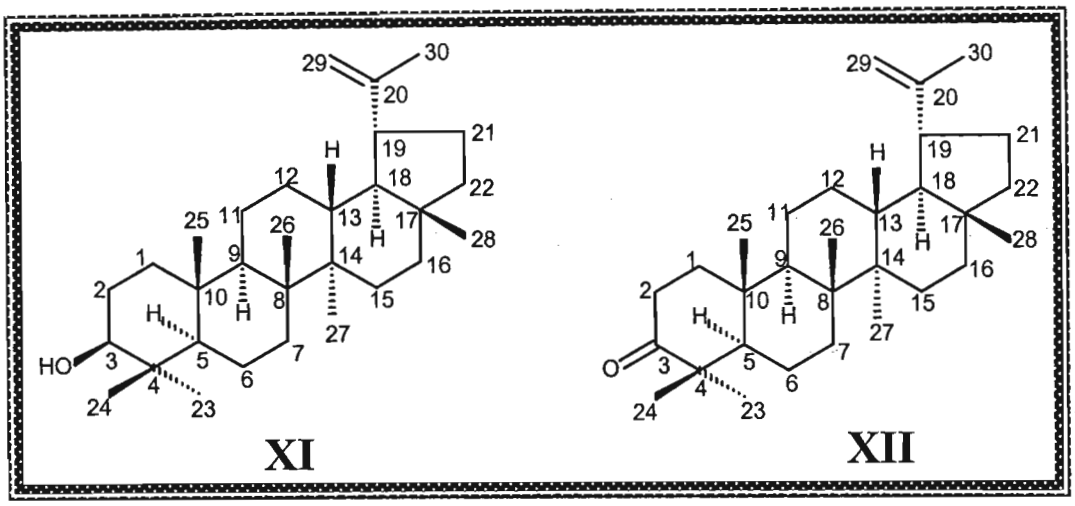


Figure 4.11: COMPOUND XI: lupeol and COMPOUND XII: lupenone

Compounds **XI** and **XII** were isolated as white crystalline materials. The physical and spectral properties of **XI** were consistent with those of 3β-hydroxylup-20(29)-ene and the spectral properties of **XII** with that those of 3-oxolup-20(29)-ene. Compounds **XI** and **XII** are commonly known as lupeol and lupenone respectively. Lupeol and lupenone are common triterpenoids and they are frequently isolated from a wide range of plant species [33]. The difference between the two structures is the substituent at C-3. In the case of lupeol, there is a β-hydroxy group at C-3 whereas in lupenone, there is a keto group. Spectra for compounds XI and XII are give on pages 239-244 and 245-246 in appendix A.

The high resolution mass spectrum of **XI** showed a molecular ion at 426.3867, which was in agreement with the molecular formula $C_{30}H_{50}O$. Significant peaks were also observed at *m/z* 411, 315, 257, 218, 207, 203 and 189. The base peak occurred at *m/z* 218. Compound **XII** decomposed before a mass spectrum could be obtained.

The infra red spectrum of compound **XI** showed peaks at ν_{max} 3379 cm^{-1} (O – H stretching), 2929 cm^{-1} (saturated C – H stretching) and at 1690 cm^{-1} (isolated C=C stretching).

The ^{1}H NMR spectrum suggested that compound **XI** had a lupane type structure [34,35,36]. The ^{1}H NMR spectrum showed a vinylic methyl proton resonance at δ_H 1.66. Resonances for six other methyl group protons were observed as intense singlets at δ_H 0.74, 0.77, 0.81, 0.92, 0.95 and 1.01. By comparison to literature, these resonances were attributed to H-24, H-28, H-25, H-27, H-23 and H-26 respectively [33].

Two broad singlets at δ_H 4.55 and δ_H 4.67 were assigned to the two non-equivalent geminal protons of the terminal methylene group. The HETCOR spectrum showed a correlation between the geminal protons and an olefinic methylene carbon at δ_C 109.3, confirming their assignments. In addition to this, the geminal proton resonances were seen to be coupled to 3H-30, the vinylic methyl group proton resonance, in the COSY spectrum.

The double doublet resonance observed at δ_H 3.16 (J=5.4, 10.9) was assigned to H-3β. A literature survey of similar lupane type compounds indicated that the observed chemical shift and coupling constants were typical for such H-3α protons. In the HETCOR spectrum, the resonance at δ_H 3.16 was seen to be coupled to a methine carbon at δ_C 79.0. By comparison to literature, the multiplets at δ_H 2.35 and δ_H 1.90 were assigned to H-19 and H-21A respectively. The HETCOR spectrum was consistent with these assignments.

The ^{1}H NMR spectrum of **XII** was similar to that of **XI** with the exception that the H-3α resonance was missing. This suggested the presence of a keto group at C-3. Comparison of the ^{13}C NMR data confirmed the presence of a ketone group at the C-3 position. In lupeol (**XI**) the resonance for C-3 occurs at δ_C 79.0 whereas in the spectrum of **XII** it occurs at 218.1.

The ^{1}H NMR spectrum revealed the presence of seven methyl proton group resonances. The downfield shifted methyl resonance at δ_H 1.66 was assigned to the vinyl methyl protons at C-30. The remaining six methyl proton group resonances at δ_H 0.77, 0.91, 0.93, 1.00, 1.05 and 1.23 were assigned, by comparison to literature, to

H-28, H-25, H-24, H-27, H-26 and H-23 respectively. The vinylic protons, H-29A and H-28B were observed as broad singlets at δ_H 4.67 and δ_H 4.55.

The ^{13}C NMR spectrum indicated the presence of thirty carbons for **XI** and **XII.** The ^{13}C NMR data for compounds **XI** and **XII** were assigned by comparison to literature **(table 4.10)**.

Table 4.9: ^1H NMR data for compounds XI and XII and literature data for lupeol and lupenone [33] (*J* given in Hz in parenthesis)

	^1H NMR data for compound XI	**Literature data for lupeol** [33]	**^1H NMR data for compound XII**	**Literature data for lupenone** [33]
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes\otimes}$shift / δ_H ppm**
H-3	3.16 dd (5.4, 10.9)	3.18 dd	----------	----------
H-23	0.95 s	0.98 s	1.23 s	1.53 s
H-24	0.74 s	0.77 s	0.93 s	0.92 s
H-25	0.81 s	0.84 s	0.91 s	0.89 s
H-26	1.01 s	1.04 s	1.05 s	1.04 s
H-27	0.92 s	0.97 s	1.00 s	0.99 s
H-28	0.77 s	0.79 s	0.77 s	0.76 s
H-29A	4.55 brs	4.56 s	4.67 s	4.66 s
H-29B	4.67 brs	4.69 s	4.55 s	4.54 s
H-30	1.66 s	1.69 s	1.66 s	1.65 s

$^{\otimes}$ ^1H NMR spectrum recorded in $CDCl_3$, 300 MHz.

$^{\otimes\otimes}$ ^1H NMR spectrum recorded in $CDCl_3$, 400 MHz.

$^{\otimes\otimes\otimes}$ ^1H NMR spectrum recorded in $CDCl_3$, 60 MHz.

Table 4.10: ^{13}C NMR data for compounds XI and XII and literature data for lupeol and lupenone [33]

	^{13}C NMR data for compound XI	^{13}C literature data for lupeol [33]	^{13}C NMR data for compound XII	^{13}C literature data for lupenone [33]
Carbon No.	$^{\otimes}$ shift/δ_C ppm	$^{\otimes\otimes}$shift/δ_C ppm	$^{\otimes}$ shift/δ_C ppm	$^{\otimes\otimes\otimes}$shift/δ_C ppm
C-1	38.7 CH_2	38.6 CH_2	39.6 CH_2	39.6 CH_2
C-2	27.4 CH_2	27.3 CH_2	34.1 CH_2	34.1 CH_2
C-3	79.0 CH	78.9 CH	218.1 C	217.9 C
C-4	38.8 C	38.8 C	47.3 C	47.3 C
C-5	55.3 CH	55.2 CH	54.9 CH	55.0 CH
C-6	18.3 CH_2	18.2 CH_2	19.6 CH_2	19.6 CH_2
C-7	34.2 CH_2	34.2 CH_2	33.5 CH_2	33.6 CH_2
C-8	40.8 C	40.7 C	40.7 C	40.9 C
C-9	50.4 CH	50.3 CH	49.7 CH	49.8 CH
C-10	37.1 C	37.1 C	36.8 C	36.9 C
C-11	20.9 CH_2	20.9 CH_2	21.4 CH_2	21.5 CH_2
C-12	25.1 CH_2	25.0 CH_2	25.1 CH_2	25.2 CH_2
C-13	38.0 CH	38.0 CH	38.1 CH	38.2 CH
C-14	42.8 C	42.7 C	42.9 C	42.9 C
C-15	27.7 CH_2	27.4 CH_2	27.4 CH_2	27.4 CH_2
C-16	35.5 CH_2	35.5 CH_2	36.5 CH_2	35.6 CH_2
C-17	43.0 C	42.9 C	42.9 C	42.9 C
C-18	48.3 CH	48.2 CH	48.2 CH	48.3 CH
C-19	47.9 CH	47.9 CH	47.9 CH	47.9 CH
C-20	150.9 C	150.8 C	150.8 C	150.7 C
C-21	29.8 CH_2	29.8 CH_2	29.8 CH_2	29.9 CH_2
C-22	40.0 CH_2	39.9 CH_2	39.9 CH_2	40.0 CH_2
C-23	28.0 CH_3	27.9 CH_3	26.6 CH_3	26.6 CH_3
C-24	15.3 CH_3	15.3 CH_3	21.0 CH_3	21.0 CH_3
C-25	16.1 CH_3	16.1 CH_3	16.9 CH_3	15.8 CH_3
C-26	15.9 CH_3	15.9 CH_3	15.7 CH_3	15.9 CH_3
C-27	14.5 CH_3	14.5 CH_3	14.4 CH_3	14.4 CH_3
C-28	18.0 CH_3	17.9 CH_3	18.0 CH_3	18.0 CH_3
C-29	109.3 CH_2	109.3 CH_2	109.4 CH_2	109.2 CH_2
C-30	19.3 CH_3	19.2 CH_3	19.3 CH_3	19.3 CH_3

$^{\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 75 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 100 MHz.

$^{\otimes\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 25.2 MHz.

4.2.5 The structure elucidation of compound XIII

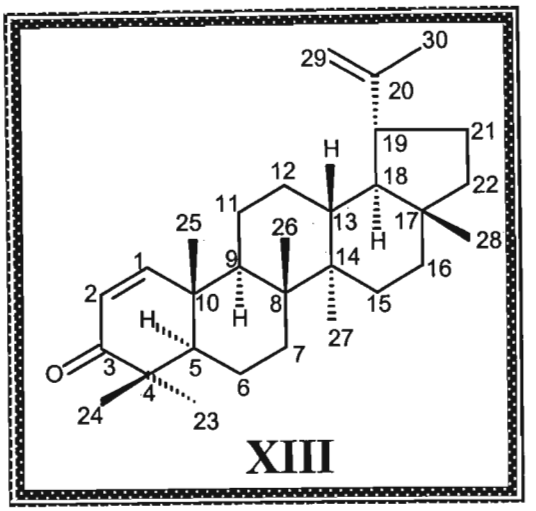


Figure 4.12: COMPOUND XIII: glochidone

Compound **XIII** was isolated as white crystalline material. The physical and spectral properties of compound **XIII** were consistent with those of 1,20(29)-lupadien-3-one. Compound **XIII** is commonly referred to as glochidone. Spectra for compound **XIII** are given on pages 247-256 in appendix A.

The low resolution mass spectrum of compound **XIII** showed a molecular ion at *m/z* 422, which is in agreement with the molecular formula of $C_{30}H_{46}O$. Significant fragments occurred at *m/z* 405, 379, 311, 285, 229, 191, 150, 121 and 95. The base peak occurred at *m/z* 150.

The infra red spectrum obtained for compound **XIII** was consistent with the proposed structure. Peaks were observed at ν_{max} 2927 cm^{-1} (saturated C – H stretching), 1466 and 1387 cm^{-1} (bending of CH_2 groups) and a strong peak was observed at 1677 cm^{-1} (C=O stretching).

The ^1H NMR spectrum suggested the presence of a lupane type structure [34,35,36]. The ^1H NMR spectrum of **XIII** was similar to that of **XII** except for a downfield pair of doublets, at δ_H 7.08 and δ_H 5.76 (J=10.1). Two broad singlets were observed at δ_H 4.57 and δ_H 4.68.

These resonances were assigned to the non-equivalent geminal H-29 protons. These two resonances were seen to be coupled to a methylene carbon at δ_C 109.7 in the HSQC spectrum. The COSY spectrum showed long range coupling between the H-29 protons and a vinylic methyl group at δ_H 1.67. The H-20 protons were assigned to this resonance. The NOESY spectrum confirmed these assignments.

Six other methyl resonances were observed at δ_H 1.11, 1.09, 1.06, 1.05, 0.94 and 0.79. The resonances at δ_H 1.05 and 0.79 were assigned to the H-25 and H-28 protons respectively. HMBC, NOESY and HSQC data were consistent with these assignments.

The pair of doublets at δ_H 7.08 and δ_H 5.76, were assigned to the enonic protons, H-1 and H-2 respectively. NOESY, COSY, HSQC and HMBC data were consistent with these assignments. The double double doublet at δ_H 2.38 was assigned to H-19. A NOESY interaction was observed between one of the H-29 protons and the H-19 proton. The HMBC spectrum also confirmed this assignment. The H-19 resonance was seen to be coupled to a methylene proton at δ_H 1.90. This multiplet was assigned to one of the H-21 protons. The NOESY and COSY spectra confirmed this assignment.

The ^{13}C NMR spectra indicated the presence of 30 carbons. The ^{13}C NMR data for compound **XIII** was assigned by comparison to literature (**table 4.12**).

Table 4.11: ^{1}H NMR data for compound XIII and literature data for glochidone [33] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound XIII	**Literature data for glochidone [33]**
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**
H-1	7.08 d (10.1)	7.02 d (10.0)
H-2	5.76 d (10.1)	5.73 d (10.0)
H-29	4.68 brs	4.60 brs
H-29	4.57 brs	4.50 brs
H-19	2.38 ddd (11.0, 7.2, 1.8)	unassigned
H-21	1.90 m	unassigned
H-25	1.05 s	unassigned
H-28	0.79 s	unassigned
H-30	1.67 s	1.60 s

$^{\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 400 or 90 MHz.

Table 4.12: ^{13}C NMR data for compound XIII and literature data for glochidone [33]

	^{13}C NMR data for compound XIII	^{13}C literature data for glochidone [33]
Carbon No.	**$^{\otimes}$ shift/δ_C ppm**	**$^{\otimes\otimes}$shift/δ_C ppm**
C-1	160.0 CH	159.6 CH
C-2	125.3 CH	125.1 CH
C-3	205.6 CH	205.2 CH
C-4	44.9 C	44.6 C
C-5	53.6 CH	53.4 CH
C-6	19.5 CH_2	19.2 CH_2
C-7	34.1 CH_2	33.7 CH_2
C-8	42.0 C	41.7 C
C-9	44.7 CH	44.4 CH
C-10	39.8 C	39.5 C
C-11	21.5 CH_2	21.2 CH_2
C-12	25.4 CH_2	25.0 CH_2
C-13	38.5 CH	38.2 CH
C-14	43.2 C	42.7 C
C-15	27.6 CH_2	27.3 CH_2
C-16	35.8 CH_2	35.4 CH_2
C-17	43.4 C	43.0 C
C-18	48.4 CH	48.1 CH
C-19	48.1 CH	47.8 CH
C-20	150.9 C	150.5 C
C-21	30.1 CH_2	29.8 CH_2
C-22	40.2 CH_2	39.9 CH_2
C-23	28.1 CH_3	27.8 CH_3
C-24	21.7 CH_3	21.4 CH_3
C-25	19.3 CH_3	19.0 CH_3
C-26	16.7 CH_3	16.4 CH_3
C-27	14.7 CH_3	14.4 CH_3
C-28	18.3 CH_3	18.0 CH_3
C-29	109.7 CH_2	109.5 CH_2
C-30	19.6 CH_3	19.3 CH_3

$^{\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 100 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 50 MHz.

4.2.6 The structural elucidation of compound XIV and XV

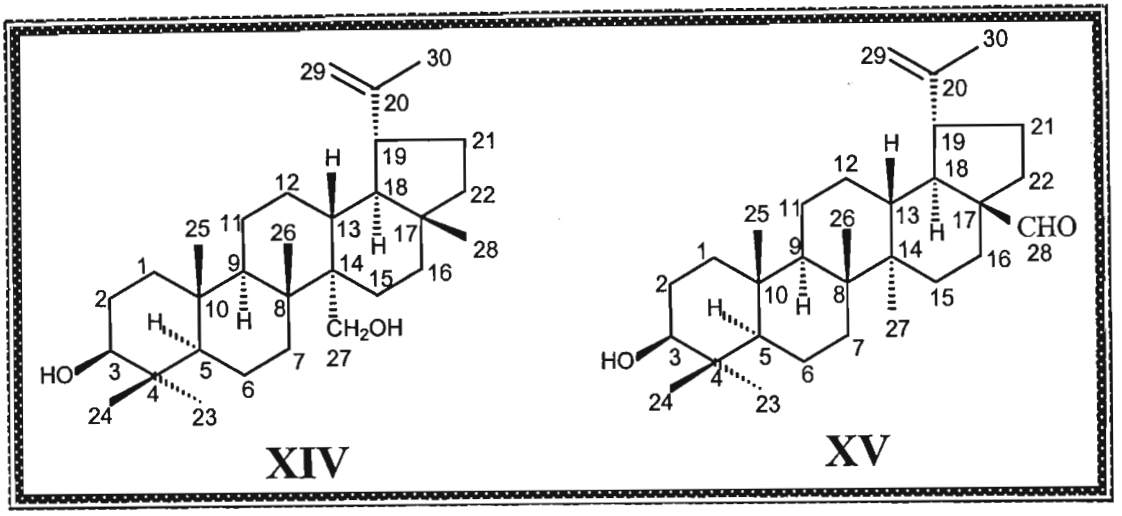


Figure 4.13: COMPOUND XIV: 3β,27-dihydroxylup-20(29)-ene and COMPOUND XV: betulinaldehyde

Compound **XIV** was isolated as white crystalline material. **XIV** had consistent spectral and physical properties with those of 3β,27-dihydroxylup-20(29)-ene [33,37]. 3β,27-Dihydroxylup-20(29)-ene has been previously reported from *Lithocarpus cornea* [36]. Compound **XV** was isolated as yellowish-white amorphous material. Repeated column chromatography was only partially successful in purifying compound **XV**. Compound **XV** was identified to be 3β-hydroxy-20(29)-lupen-28-al and is commonly referred to as betulinaldehyde. Spectra for compounds **XIV** and **XV** are given on pages 257-266 and 267-270, respectively, in appendix A.

The low resolution mass spectrum of compound **XIV** showed a molecular ion at *m/z* 442, in agreement with the molecular formula $C_{30}H_{50}O_2$. Significant fragments were also observed at *m/z* 411, 385, 288, 257, 234, 207, 189, 135, 95 and 69. The base peak of **XIV** was observed at *m/z* 95. Compound **XV** decomposed before an infra red, HETCOR, NOESY and mass spectrum could be obtained.

The infra red spectrum of compound **XIV** showed absorption bands at ν_{max} 3450 cm^{-1} (O – H stretching), 2942 and 2871 cm^{-1} (saturated C – H stretching) and at 1690 cm^{-1} (isolated C=C stretching).

The ^{1}H NMR spectrum of compound **XIV** and **XV** had similarities to that of lupeol (**XI**), suggesting the presence of a lupane type structure [34,35,36]. Comparison of the ^{1}H NMR spectra of **XIV** and **XV** to that of **XI** suggested that one methyl group had been oxidised to a primary alcohol for compound **XIV** and to an aldehyde for compound **XV**.

Both the ^{1}H NMR spectra of **XIV** and **XV** showed a vinylic methyl group proton resonance at approximately δ_H 1.66, and five other methyl resonances. In both cases, the H-29 protons appeared as doublets at approximately δ_H 4.70 and δ_H 4.60 (J_{gem}=2.0 Hz). In the COSY spectra the H-29 protons were seen to be coupled to the terminal vinylic methyl group resonance. In both ^{1}H NMR spectra, H-3 was observed at δ_H 3.16 as a double doublet (J=11.0,6.0). In the ^{1}H NMR spectrum of **XIV**, two proton resonances were observed at δ_H 3.78 and δ_H 3.31. The HETCOR spectrum showed these resonances to be coupled to a methylene carbon at δ_C 60.5, suggesting a –CH_2OH group. A comparison of the NMR data for compound **XIV** to that of lupeol suggested that C-27 or C-28 possessed a hydroxyl group. The exact location of the –CH_2OH group was determined using the NOESY spectrum. A NOESY interaction was observed between the protons of the –CH_2OH and the vinylic methyl group. This suggested that the –CH_2OH group was located at C-27 (from a molecular model). Furthermore the C-28 position was ruled out as the ^{1}H NMR spectrum of compound **XIV** was not identical with that of betulin [33,36,37,38]. Whereas the ^{1}H NMR of betulin shows an H-19β resonance δ_H 2.99, compound **XIV** showed a H-19β resonance at δ_H 2.35. Compound **XIV** was acetylated to yield the diacetate, thus confirming the presence of two hydroxyl groups in the molecule. The ^{13}C NMR data of compound **XIV** compared favourably with that of literature (**table 4.15**) [37].

The ^{1}H NMR spectrum of compound **XV** showed a proton resonance at δ_H 9.66, indicating the presence of an aldehyde group. The ^{13}C NMR spectrum showed a methine carbon at δ_C 206.7, confirming the presence of an aldehyde group. Compound **XV** decomposed before a HETCOR and NOESY spectrum could be run. The carbon and proton NMR data were consistent with literature data for betulinaldehyde (**table 4.14** and **table 4.15**) [38,39].

Table 4.13: ^{1}H NMR data for compound XIV and the literature data for 3β,27-dihydroxylup-20(29)-ene [33] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound XIV	Literature data for 3β,27-dihydroxylup-20(29)-ene [33]
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**
H-29	4.66 d (2.0)	4.68 d
H-29	4.55 d (2.0)	4.57 d
H-28	3.78 d (10.7)	3.77 d (11.0)
H-28	3.31 d (10.7)	3.32 d (11.0)
H-3	3.16 dd (11.0,6.2)	3.20 dd (9.0, 7.0)
H-30	1.66 s	1.68 s
H-27	1.01 s	1.05 s
H-26	0.96 s	0.97 s
H-23	0.88 s	0.85 s
H-25	0.80 s	0.80 s
H-24	0.77 s	0.77 s

$^{\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 400 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 300 MHz.

Table 4.14: ^{1}H NMR data for compound XV and the literature data for betulinaldehyde [38] (*J* given in Hz in parenthesis)

	^{1}H NMR data for compound XV	Literature data for betulinaldehyde [38]
Proton No.	**$^{\otimes}$shift / δ_H ppm**	**$^{\otimes\otimes}$shift / δ_H ppm**
H-28	9.66 s	9.65 s
H-29	4.74 d (2.0)	4.72 brs
H-29	4.61 d (2.0)	4.60 brs
H-3	3.16 dd (11.0,5.3)	3.15 dd (11.0, 6.0)
H-19	2.84 ddd (11.0,11.0, 6.0)	2.80 m
H-30	1.67 s	1.68 s
H-24	0.95 s	0.95 s
H-23	0.94 s	0.95 s
H-26	0.89 s	0.90 s
H-25	0.80 s	0.80 s
H-27	0.73 s	0.73 s

$^{\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 300 MHz.

$^{\otimes\otimes}$ ^{1}H NMR spectrum recorded in $CDCl_3$, 90 MHz.

Table 4.15: ^{13}C NMR data for compounds XI and XII and literature data for 3β,27-dihydroxylup-20(29)-ene and betulinaldehyde [37,39]

	^{13}C NMR data for compound XIV	^{13}C literature data for 3β,27-dihydroxylup-20(29)-ene [37]	^{13}C NMR data for compound XV	^{13}C literature data for betulinaldehyde [39]
Carbon No.	**$^{\otimes}$ shift/δ_C ppm**	**$^{\otimes\otimes}$shift/δ_C ppm**	**$^{\otimes}$shift/δ_C ppm**	**$^{\otimes\otimes}$shift/δ_C ppm**
C-1	38.7 CH_2	38.7 CH_2	38.7 CH_2	38.7 CH_2
C-2	[a]27.4 CH_2	[a]27.4 CH_2	27.4 CH_2	27.3 CH_2
C-3	79.0 CH	79.0 CH	79.0 CH	78.9 CH
C-4	38.9 C	38.9 C	38.9 C	38.8 C
C-5	55.3 CH	55.3 CH	55.3 CH	55.5 CH
C-6	18.3 CH_2	18.3 CH_2	18.3 CH_2	18.2 CH_2
C-7	[b]34.2 CH_2	[b]34.2 CH_2	34.3 CH_2	34.3 CH_2
C-8	40.9 C	40.9 C	40.8 C	40.8 C
C-9	50.4 CH	50.4 CH	50.5 CH	50.4 CH
C-10	37.1 C	37.2 C	37.2 C	37.1 C
C-11	20.8 CH_2	20.8 CH_2	20.8 CH_2	20.7 CH_2
C-12	25.2 CH_2	25.2 CH_2	25.5 CH_2	25.5 CH_2
C-13	37.3 CH	37.3 CH	38.7 CH	38.7 CH
C-14	42.7 C	42.7 C	42.6 C	42.5 C
C-15	[a]27.0 CH_2	[a]27.0 CH_2	29.3 CH_2	29.2 CH_2
C-16	[c]29.1 CH_2	[c]29.2 CH_2	28.8 CH_2	28.8 CH_2
C-17	47.8 C	47.8 C	59.3 C	59.3 C
C-18	47.8 CH	47.8 CH	48.1 CH	48.0 CH
C-19	48.7 CH	48.8 CH	47.5 CH	47.5 CH
C-20	150.5 C	150.5 C	149.7 C	149.7 C
C-21	[c]29.7 CH_2	[c]29.7 CH_2	29.9 CH_2	29.8 CH_2
C-22	[b]33.9 CH_2	[b]34.0 CH_2	33.2 CH_2	33.2 CH_2
C-23	28.0 CH_3	28.0 CH_3	28.0 CH_3	27.9 CH_3
C-24	15.4 CH_3	15.4 CH_3	15.3 CH_3	15.4 CH_3
C-25	[d]16.1 CH_3	[d]16.1 CH_3	15.9 CH_3	15.9 CH_3
C-26	[d]16.0 CH_3	[d]16.0 CH_3	16.1 CH_3	16.1 CH_3
C-27	60.5 CH_2	60.5 CH_2	14.3 CH_3	14.2 CH_3
C-28	14.7 CH_3	14.7 CH_3	206.7 CH	205.6 CH
C-29	109.7 CH_2	109.7 CH_2	110.2 CH_2	110.1 CH_2
C-30	19.1 CH_3	19.1 CH_3	19.0 CH_3	19.0 CH_3

$^{\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 100 MHz.

$^{\otimes\otimes}$ ^{13}C NMR spectrum recorded in $CDCl_3$, 62.9 MHz.

[a, b, c, d] – values are interchangeable

4.3 Conclusion

Whole plants of *Cyrtanthus breviflorus* were investigated. The ethanol extract of the bulbs yielded four alkaloids, lycorine (**1**), crinamine hydrochloride (**IX**), haemanthamine (**XIII**) and tazettine (**X**), after separation by column chromatography over silica gel. Compounds **IX** and **X** have not been previously found in the genus. Crinamine is isolated for the first time in its salt form (**IX**).

The hexane extract yielded five known pentacyclic triterpenoids: lupeol (**XI**), lupenone (**XII**), glochidone (**XIII**), 3β,27-dihydroxylup-20(29)-ene (**XIV**) and betulinaldehyde (**XV**). Triterpenoids have not been previously isolated from *Cyrtanthus*.

The four alkaloids isolated from *Cyrtanthus breviflorus* all display significant bioactivity. Lycorine (**I**) is the most active. Haemanthamine (**XIII**) has been shown to be cytotoxic against a variety of cultured cells [16,17,18]. Tazettine (**X**) is a known hypotensive agent [19]. Lupeol (**XI**), lupenone (**XII**) and glochidone are antineoplastic agents [19].

4.4 Experimental

Cyrtanthus breviflorus Harv. was obtained from Pinetown, Kwazulu-Natal and identified by Dr. N. Crouch. A voucher specimen (*Crouch 830*) was deposited at the Natal Herbarium. Whole plants (3.1 kg) were dried, and then finely cut and extracted, first with hexane and then with 99% ethanol on a Labcon shaker for 48 hours each. The extract was evaporated under reduced pressure.

Chromatographic separation of the hexane extract using a methylene chloride–methanol step gradient (50:0, 49:1, 48:2, 46:4, 40:10) as the eluant and collecting approximately 30 x 35 ml fractions for each step, gave lupenone (**XII**), present in fractions 7-13, glochidone (**XIII**), present in fractions 15-22, lupeol (**XI**), present in fractions 23-25, betulinaldehyde (**XV**), found in fractions 29-33 and 3β,27-dihydroxylup-20(29)-ene (**XIV**), present in fractions 39-41.

Chromatographic separation of the ethanol extract using a methylene chloride–methanol step gradient (19:1, 18:2, 16:4, 12:8, 10:10, 8:12, 0:20) as the eluant and collecting approximately 30 x 35 ml fractions for each step, gave haemanthamine (**XIII**), present in fractions 11-14, crinamine hydrochloride (**IX**), present in fractions 17-23, lycorine (**I**), present in fractions 44-51, and tazettine (**X**), found in fractions 64-73.

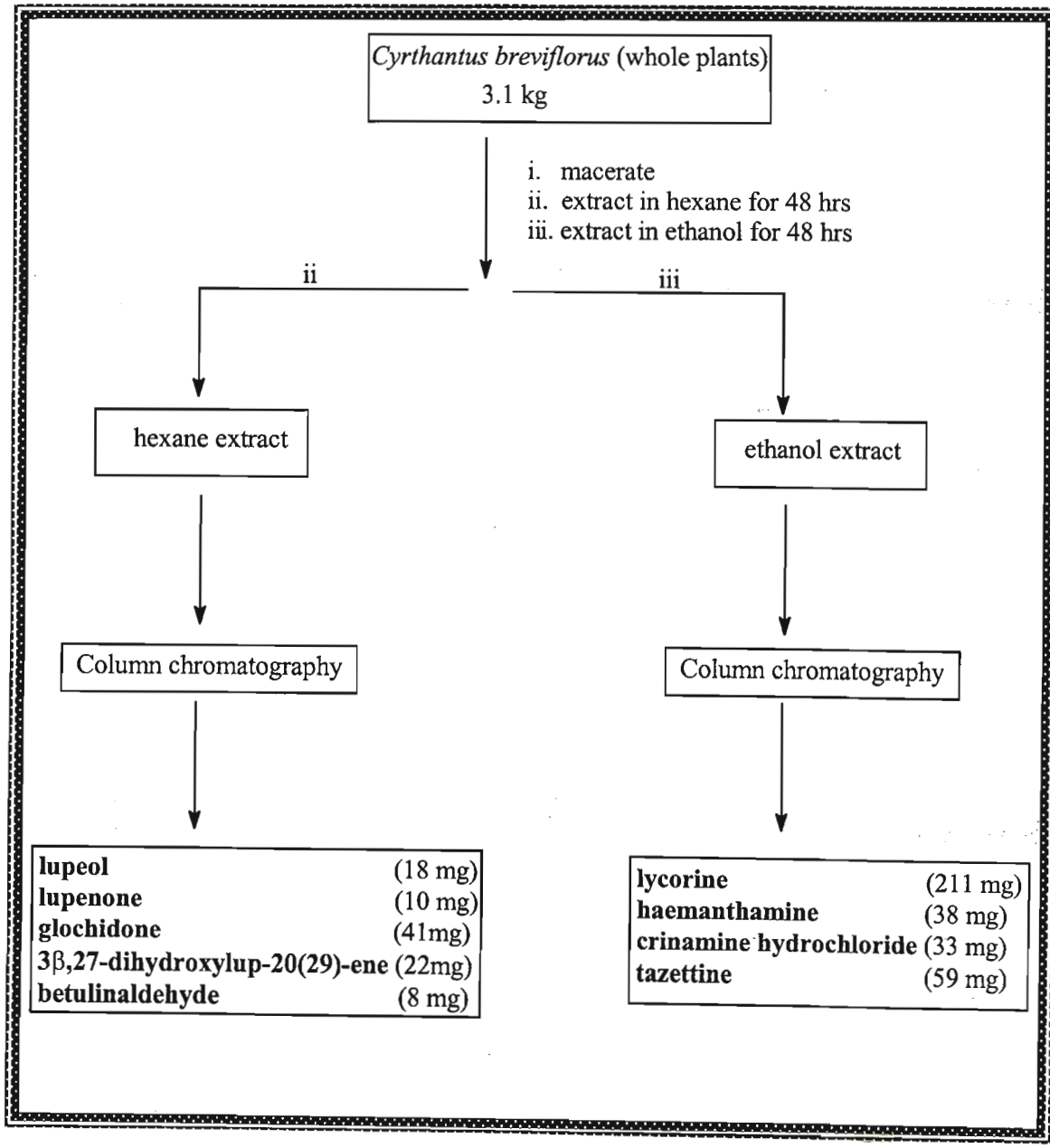
4.4.1 The base treatment of compound IX

4M NaOH (2 cm^3) was added to 10 mg crinamine hydrochloride (**IX**). The mixture was shaken and then allowed to stand at room temperature for 10 minutes. The mixture was then extracted with dichloromethane. The solvent was evaporated and the base treated compound was analysed by NMR. The physical and spectral properties of the base treated product were consistent with those of crinamine (**V**)

4.4.2 Acetylation of compound XIV

3β,27-Dihydroxylup-20(29)-ene (**XIV**), was acetylated. 3β,27-Dihydroxylup-20(29)-ene **XIV** (20 mg) was placed in a round bottom flask with pyridine (3 cm^3) and acetic

anhydride (3 cm^3). The flask was heated on a steam bath for 10 minutes and then left at room temperature for approximately 48 hours. Thereafter, excess acetic anhydride was removed by the addition of methanol (3x10 ml). This was followed by the removal of pyridine by adding toluene (3x10 ml). After each addition, the solvent was evaporated off on the Rotavapor. Traces of toluene were then removed by the addition of excess methanol. The sample was then spotted on a TLC plate to test for the completion of the reaction.



Scheme 4.3: Extraction of *Cyrtanthus breviflorus*

4.4.3 PHYSICAL DATA FOR COMPOUND VIII

NAME: haemanthamine

Yield: 27 mg

Melting point: 201 °C (lit. 203 – 203.5 °C [19])

Optical rotation: $[\alpha]_D$ = +47° (c=1.0, MeOH); lit = +19.7° (c=3.25, MeOH) [19]

Mass: HRMS: $[M^+]$ at *m/z* 301.1310, $C_{17}H_{19}NO_4$ requires 301.1314
EIMS: *m/z* (rel. int.): 301 (<1) $[M^+]$, 269 (57), 257 (57), 258 (17), 227 (93), 225 (70), 211 (35), 181 (59)

Infra red: ν_{max} (cm^{-1}): 3448, (O – H stretching), 1483 (aromatic C=C stretching), 1242 (C – N stretching), 1029 (C – O stretching), 929 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 6.44 (1H, d, *J*=10.1, H-1), 6.23 (1H, dd, *J*=10.1, 4.9, H-2), 3.85 (1H, m, H-3), 2.14 (1H, ddd, *J*=13.5, 9.2, 4.2, H-4α),1.97 (1H, ddd, *J*=13.5, 9.0, 4.3, H-4β),3.30 (1H, m, H-4a), 3.76 (1H, d, *J*=16.7, H-6α), 4.28 (1H, d, *J*=16.7, H-6β),6.52 (1H, s, H-7), 6.89 (1H, s, H-10), 3.94 (1H, m, H-11_{endo}), 3.14 (1H, dd, *J*=13.9, 7.0, H-12_{exo}), 3.44 (1H, dd, *J*=13.9, 3.2, H-12_{endo}), 3.33 (3H, s, $OC\underline{H}_3$), 5.86 (2H, s, $OC\underline{H}_2O$)

^{13}C NMR: (CD_3OD): 127.9 (CH, C-1), 129.4 (CH, C-2), 72.9 (CH, C-3), 27.8 (CH_2, C-4), 62.9 (CH, C-4a), 60.4 (CH_2, C-6), 125.6 (C, C-6a), 106.7 (CH, C-7), 146.9 (C, C-8), 146.5 (C, C-9), 103.1 (CH, C-10), 135.5 (C, C-10a), 50.3 (C, C-10b), 79.7 (CH, C-11), 62.6 (CH_2, C12), 55.6 (CH_3, $O\underline{C}H_3$), 101.1 (CH_2, $O\underline{C}H_2O$)

4.4.4 PHYSICAL DATA FOR COMPOUND IX

NAME: crinamine hydrochloride

Yield: 13 mg

Melting point: 197 °C

Optical rotation: $[\alpha]_D = +133°$ (c=0.5, MeOH)

Mass: HRMS: $[M^+]$ at *m/z* 301.1298, $C_{17}H_{19}NO_4$ requires 301.1313

EIMS: *m/z* (rel. int.): 301 (<1) $[M^+]$, 270 (37.03), 269 (100.00), 268 (28.49), 252 (17.49), 250 (11.08), 240 (28.20), 226 (32.41), 211 (13.80), 181 (26.10), 149 (7.47), 115 (6.05) 60 (12.45), 45 (11.88), 43 (15.69)

Infra red: ν_{max} (cm^{-1}): 3322, (O – H stretching), 1493 (aromatic C=C stretching), 1242 (C – N stretching), 1038 (C – O stretching), 94 (C – O – C stretching)

^{1}H NMR: ($CDCl_3$): 2.95 (1H, m, H-4α), 2.32 (1H, dd, H-4β, *J*=13.0, 10.4), 4.00 (1H, m, H-12_{exo}), 3.70 (1H, dd, H-4a, *J*=13.0, 2.9), 3.39 (3H, s, $OC\underline{H}_3$), 3.64 (1H, m, H-12_{endo}), 4.11 (1H, d, H-6α, *J*=16.8), 4.22 (1H, m, H-11_{endo}), 3.98 (1H, m, H-3), 4.76 (1H, d, H-6β, *J*=16.8), 5.98 (2H, s, $OC\underline{H}_2O$), 6.26 (1H, d, H-2, *J*=10.4), 6.15 (1H, dd, H-1, *J*=10.4, 2.7), 6.57 (1H, s, H-7), 6.84 (1H, s, H-10)

^{13}C NMR: ($CDCl_3$): 121.2 (CH, C-1), 137.2 (CH, C-2), 75.0 (CH, C-3), 27.8 (CH_2, C-4), 67.2 (CH, C-4a), 58.9 (CH_2, C-6), 118.4 (C, C-6a), 107.4 (CH, C-7), 148.7 (C, C-8), 148.2 (C, C-9), 104.2 (CH, C-10), 132.0 (C, C-10a), 5.9 (C, C-10b), 77.9 (CH, C-11), 61.1 (CH_2, C-12), 56.2 (CH_3, $O\underline{C}H_3$), 102.1 (CH_2, $O\underline{C}H_2O$)

Base treatment of crinamine hydrochloride (**IX**) yielded crinamine (**V**). The physical data for crinamine (**V**) is given on page **76**.

4.4.5 PHYSICAL DATA FOR COMPOUND X

NAME: tazettine

Yield: 36 mg

Melting point: 210 °C (lit. 210 – 211 °C [19])

Optical rotation: $[\alpha]_D$ = +97° (c=1.0, MeOH); lit = +150° (c=3.25, $CHCl_3$) [19]

Mass: LRMS: [M^+] at *m/z* 331, $C_{17}H_{19}NO_4$ requires 331.368

EIMS: *m/z* (rel. int.): 331 (19.76), 316 (10.23), 298 (18.01), 272 (3.40), 247 (100.00), 230 (16.88), 201 (20.09), 181 (21.22), 153 (19.02), 135 (6.01), 115 (28.66), 96 (3.89), 70 (32.67), 42 (28.00)

Infra red: ν_{max} (cm^{-1}): 3329, (O – H stretching), 1663 and 1492 (aromatic C=C stretching), 1249 (C – N stretching), 1038 (C – O stretching), 939 (C – O – C stretching)

^{1}H NMR: (CD_3OD): 5.65 (1H, dd, *J*=10.5, 2.0, H-1), 6.10 (1H, dd, *J*=10.5, 2.0, H-2), 4.13 (1H, m, H-3α), 2.30 (1H, m, H-4α), 1.60 (1H, m, H-4β), 2.98 (1H, m, H-4a), 3.30 (1H, d, *J*=11.7, H-6α), 2.72 (1H,d, *J*=10.8, H-6β), 4.95 (1H, d, *J*=14.5, H-8α), 4.62 (1H, d, *J*=14.5, H-8β), 6.52 (1H, s, H-9), 6.76 (1H, s, H-12), 5.87 (2H, s, OCH_2O), 3.45 (3H, s, OCH_3), 2.49 (3H, s, NCH_3)

^{13}C NMR: (CD_3OD): 129.0 (CH, C-1), 129.9 (CH, C-2), 72.5 (CH, C-3), 26.0 (CH_2, C-4), 70.9 (CH, C-4a), 64.7 (CH_2, C-6), 101.1 (C, C-6a), 61.6 (CH_2, C-8), 126.0 (C, C-8a), 103.9 (CH, C-9), 146.6 (C, C-10), 146.8 (C, C-11), 108.8 (CH, C-12), 127.6 (C, C-12a), 49.8 (C, C12b), 101.2 (CH_2, O<u>C</u>H_2O), 55.4 (CH_3, O<u>C</u>H_3), 42.1 (CH_3, N<u>C</u>H_3)

4.4.6 PHYSICAL DATA FOR COMPOUND XI

NAMES: 3β-hydroxylup-20(29)-ene, lupeol

Yield: 18 mg

Melting point: 210 °C (lit. 215 – 216 °C [19])

Mass: HRMS: $[M^+]$ at *m/z* 426.3867, $C_{30}H_{50}O$ requires 426.3862
EIMS: *m/z* (rel. int.): 411 (11.00), 218 (100.00), 203 (24.12)

Infra red: ν_{max} (cm^{-1}): 3378, (O – H stretching), 2929 (saturated C – H stretching), 1690 (isolated C=C stretching)

^{1}H NMR: ($CDCl_3$): 3.16 (1H, dd, H-3, *J* = 5.4, 10.9), 0.95 (3H, s, H-23), 0.74 (3H, s, H-24), 0.81 (3H, s, H-25), 1.01 (3H, s, H-26), 0.92 (3H, s, H-27), 0.77 (3H, s, H-28), 4.55 (1H, brs, H-29A), 4.67 (1H, brs, H-29B), 1.66 (3H, s, H-30)

^{13}C NMR: ($CDCl_3$): 38.7 (CH_2, C-1), 27.4 (CH_2, C-2), 79.0 (CH, C-3), 38.8 (C, C-4), 55.3 (CH, C-5), 18.3 (CH_2, C-6), 34.2 (CH_2, C-7), 40.8 (C, C-8), 50.4 (CH, C-9), 37.1 (C, C-10), 20.9 (CH_2, C-11), 25.1 (CH_2, C-12), 38.0 (CH, C-13), 42.8 (C, C-14), 27.7 (CH_2, C-15), 35.5 (CH_2, C-16), 43.0 (C, C-17), 48.3 (CH, C-18), 47.9 (CH, C-19), 150.9 (C, C-20), 29.8 (CH_2, C-21), 40.0 (CH_2, C-22), 28.0 (CH_3 C-23), 15.3 (CH_3, C-24), 16.1 (CH_3, C-25), 15.9 (CH_3 C-26), 14.5 (CH_3 C-27), 18.0 (CH_3, C-28), 109.3 (CH_2, C-29), 19.3 (CH_3, C-30)

4.4.7 PHYSICAL DATA FOR COMPOUND XII

NAMES: 3-oxolup-20(29)-ene, lupenone

Yield: 10 mg

^{1}H NMR: ($CDCl_3$): 1.23 (3H, s, H-23), 0.93 (3H, s, H-24), 0.91 (3H, s, H-25), 1.05 (3H, s, H-26), 1.00 (3H, s, H-27), 0.77 (3H, s, H-28), 4.67 (1H, s, H-29A), 4.55 (1H, s, H-29B), 1.66 (3H, s, H-30)

^{13}C NMR: ($CDCl_3$): 39.6 (CH_2, C-1), 34.1 (CH_2, C-2), 218.1 (C, C-3), 47.3 (C, C-4), 54.9 (CH, C-5), 19.6 (CH_2, C-6), 33.5 (CH_2, C-7), 40.7 (C, C-8), 49.7 (CH, C-9), 36.8 (C, C-10), 21.4 (CH_2, C-11), 25.1 (CH_2, C-12), 38.1(CH, C-13), 42.9 (C, C-14), 27.4 (CH_2, C-15), 36.5 (CH_2, C-16), 42.9 (C, C-17), 48.2 (CH, C-18), 47.9 (CH, C-19), 150.8 (C, C-20), 29.8 (CH_2, C-21), 39.9 (CH_2, C-22), 26.6 (CH_3, C-23), 21.0 (CH_3, C-24), 16.9 (CH_3, C-25), 15.7 (CH_3, C-26), 14.4 (CH_3, C-27), 18.0 (CH_3, C-28), 109.4 (CH_2, C-29), 19.3 (CH_3, C-30)

4.4.8 PHYSICAL DATA FOR COMPOUND XIII

NAMES: 1,20(29)-lupadien-3-one, glochidone

Yield: 41 mg

Melting point: 165 °C (lit. 164 – 165 °C [19])

Mass: LRMS: $[M^+]$ at *m/z* 422, $C_{30}H_{50}O$ requires 422.693

EIMS: *m/z* (rel. int.): 405 (7.06), 379 (6.16), 311 (6.76), 285 (10.09), 229 (87.25), 191 (43.46), 150 (100.00), 121 (80.47) , 95 (81.08)

Infra red: ν_{max} (cm^{-1}): 2927, (saturated C – H stretching), 1466 and 1387 (CH_2 bending), 1677 (C=O stretching)

^{1}H NMR: ($CDCl_3$): 7.08 (1H, d, H-1, J = 10.1), 5.76 (1H, d, H-2, J = 10.1), 4.68 (1H, brs, H-29), 4.57 (1H, brs, H-29), 2.38 (1H, ddd, H-19, J = 11.0, 7.2, 1.8), 1.90 (1H, m, H-21), 1.05 (3H, s, H-25), 0.79 (3H, s, H-28), 1.67 (3H, s, H-30)

^{13}C NMR: ($CDCl_3$): 160.0 (CH, C-1), 125.3 (CH, C-2), 205.6 (CH, C-3), 44.9 (C, C-4), 53.6 (CH, C-5), 19.5 (CH_2, C-6), 34.1 (CH_2, C-7), 42.0 (C, C-8), 44.7 (CH, C-9), 39.8 (C, C-10), 21.5 (CH_2, C-11), 25.4 (CH_2, C-12), 38.5 (CH, C-13), 43.2 (C, C-14), 27.6 (CH_2, C-15), 35.8 (CH_2, C-16), 43.4 (C, C-17), 48.4 (CH, C-18), 48.1 (CH, C-19), 150.9 (C, C-20), 30.1 (CH_2, C-21), 40.2 (CH_2, C-22), 28.1 (CH_3, C-23), 21.7 (CH_3, C-24), 19.3 (CH_3, C-25), 16.7 (CH_3, C-26), 14.7 (CH_3, C-27), 18.3 (CH_3, C-28), 109.7 (CH_2, C-29), 19.6 (CH_3, C-30)

4.4.9 PHYSICAL DATA FOR COMPOUND XIV

NAMES: 3β,27-dihydroxylup-20(29)-ene

Yield: 22 mg

Melting point: 210-211 °C (lit. 214 – 215 °C [19,33])

Mass: LRMS: $[M^+]$ at *m/z* 426, $C_{30}H_{50}O$ requires 426.3862

EIMS: *m/z* (rel. int.): 411 (11.00), 218 (100.00), 203 (24.12)

Infra red: ν_{max} (cm^{-1}): 3378, (O – H stretching), 2929 (saturated C – H stretching), 1690 (isolated C=C stretching)

^{1}H NMR: ($CDCl_3$): 4.66 (1H,d, H-29, *J* = 2.0), 4.55 (1H, d, H-29, *J* = 2.0), 3.78 (1H, d, H-28, *J* = 10.7), 3.31 (1H, d, H-28, *J* = 10.7), 3.16 (1H, dd, H-3,*J* = 11.0,6.2), 1.66 (3H, s, H-30), 1.01 (3H, s, H-27), 0.96 (3H, s, H-26), 0.88 (3H, s, H-23), 0.80 (3H, s, H-25), 0.77 (3H, s, H-24)

^{13}C NMR: ($CDCl_3$): 38.7 (CH_2, C-1), 27.4 (CH_2, C-2), 79.0 (CH, C-3), 38.9 (C, C-4), 55.3 (CH, C-5), 18.3 (CH_2, C-6), 34.2 (CH_2, C-7), 40.9 (C, C-8), 50.4 (CH, C-9), 37.1 (C, C-10), 20.8 (CH_2, C-11), 25.2 (CH_2, C-12), 37.3 (CH, C-13), 42.7 (C, C-14), 27.0 (CH_2, C-15), 29.1 (CH_2, C-16), 47.8 (C, C-17), 47.8 (CH, C-18), 48.7 (CH, C-19), 150.5 (C, C-20), 29.7 (CH_2, C-21), 33.9 (CH_2, C-22), 28.0 (CH_3, C-23), 15.4 (CH_3, C-24), 16.1 (CH_3, C-25), 16.0 (CH_3, C-26), 60.5 (CH_2, C-27), 14.7 (CH_3, C-28), 109.7 (CH_2, C-29), 19.1 (CH_3, C-30)

4.4.10 PHYSICAL DATA FOR COMPOUND XV

NAMES: 3β-hydroxylup-20(29)-en-28-al, betulinaldehyde

Melting point: sample decomposed (lit. 192-193 °C [19])

Yield: 8 mg

^{1}H NMR: ($CDCl_3$): 9.66 (1H, s, H-28), 4.74 (1H, d, H-29, J = 2.0) , 4.61 (1H, d, H-29, J = 2.0), 3.16 (1H, dd, H-3, J = 11.0,5.3), 2.84 (1H, ddd, H-19, J = 11.0,11.0, 6.0), 1.67 (3H, s, H-30), 0.95 (3H, s, H-24), 0.94 (3H, s, H-23), 0.89 (3H, s, H-26), 0.80 (3H, s, H-25), 0.73 (3H, s, H-27)

^{13}C NMR: ($CDCl_3$): 38.7 (CH_2, C-1), 27.4 (CH_2, C-2), 79.0 (CH, C-3), 38.9 (C, C-4), 55.3 (CH, C-5), 18.3 (CH_2, C-6), 34.3 (CH_2, C-7), 40.8 (C, C-8), 50.5 (CH, C-9), 37.2 (C, C-10), 20.8 (CH_2, C-11), 25.5 (CH_2, C-12), 38.7 (CH, C-13), 42.6 (C, C-14), 29.3 (CH_2, C-15), 28.8 (CH_2, C-16), 59.3 (C, C-17), 48.1 (CH, C-18), 47.5 (CH, C-19), 149.7 (C, C-20), 29.9 (CH_2, C-21), 33.2 (CH_2, C-22), 28.0 (CH_3, C-23), 15.3 (CH_3, C-24), 15.9 (CH_3, C-25), 16.1 (CH_3, C-26), 14.3 (CH_3, C-27), 206.7 (CH, C-28), 110.2 (CH_2, C-29), 19.0 (CH_3, C-30)

4.5 References

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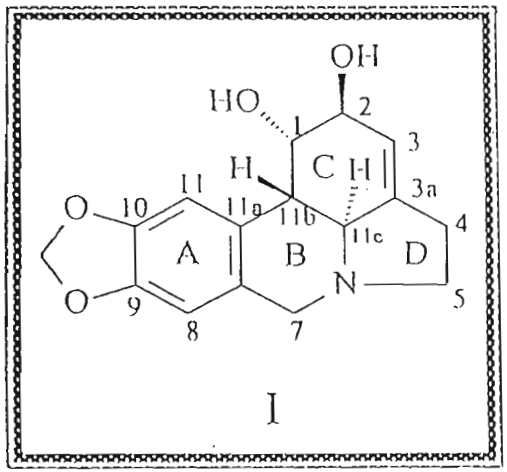
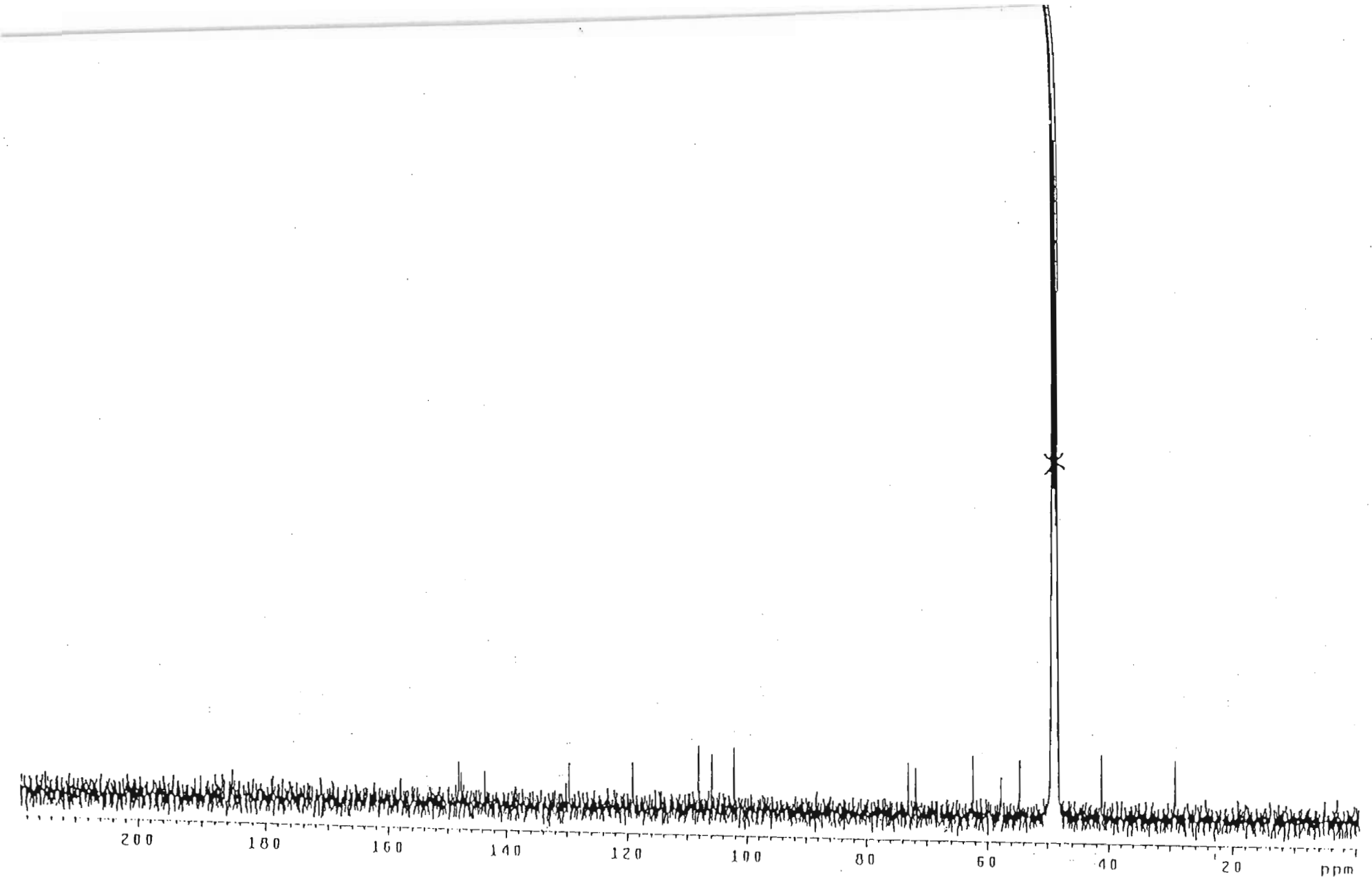
Appendix A

List of Spectra

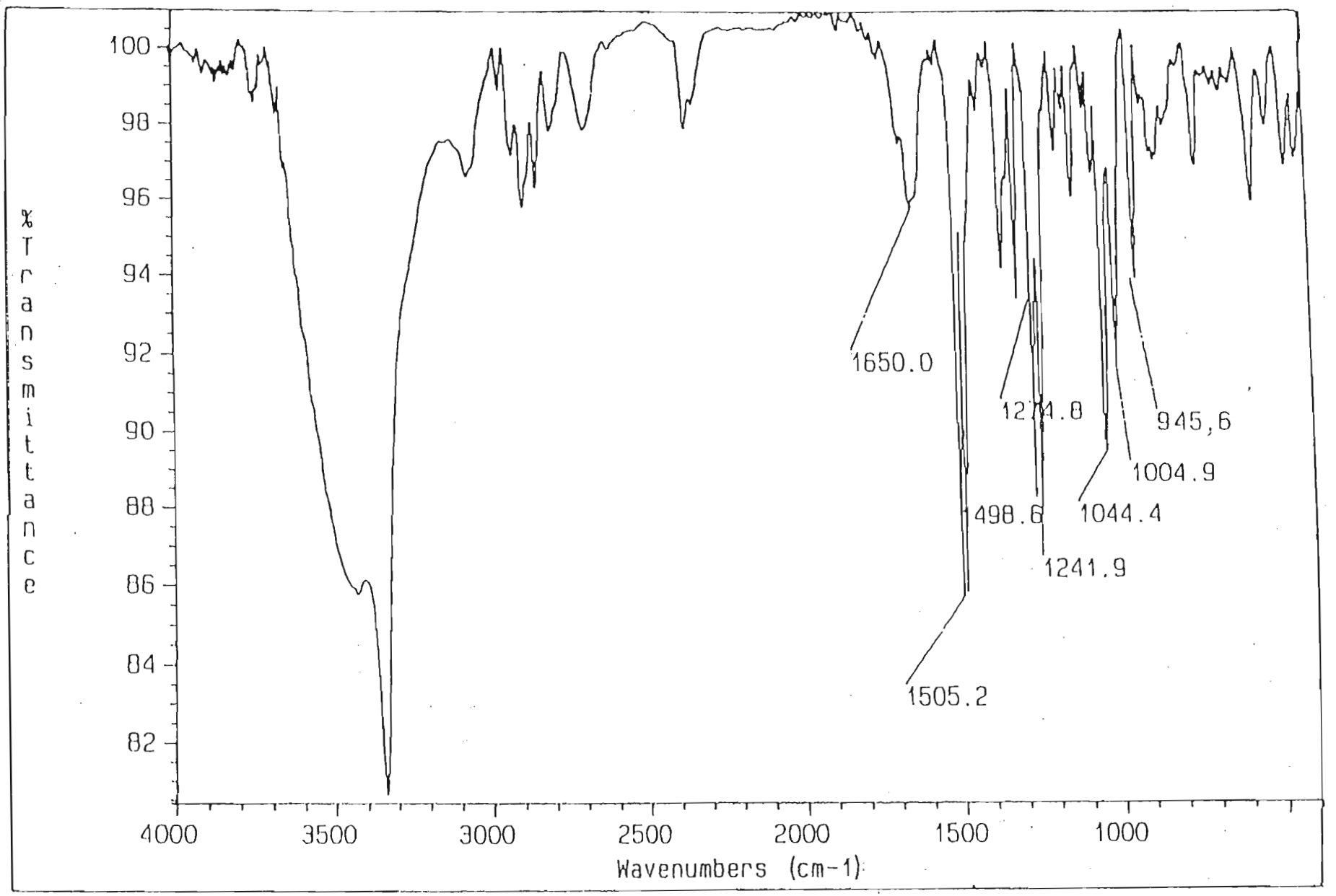


I

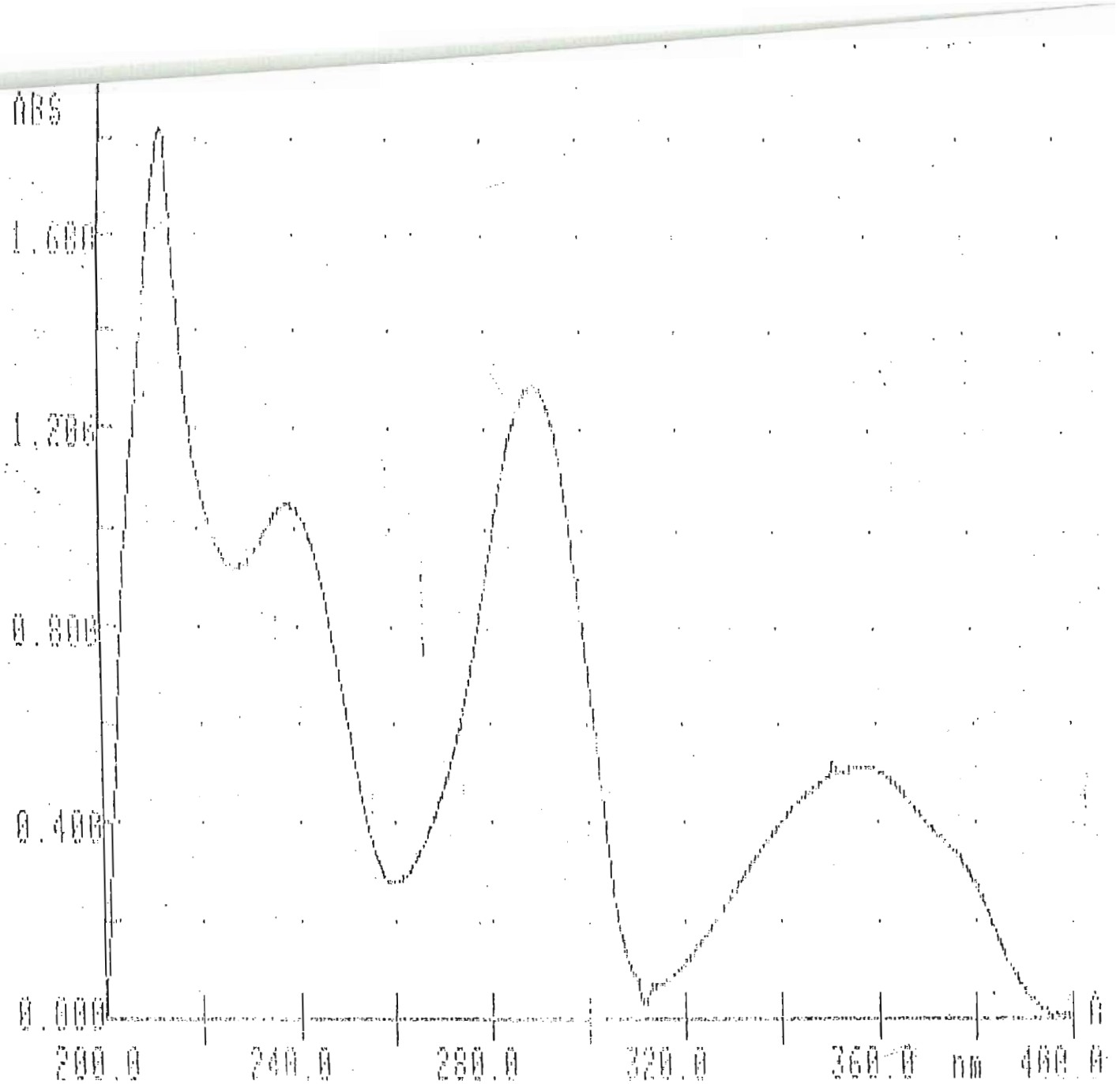
Spectrum [1-A]: ^{1}H NMR spectrum of lycorine(**I**) in CD_3OD



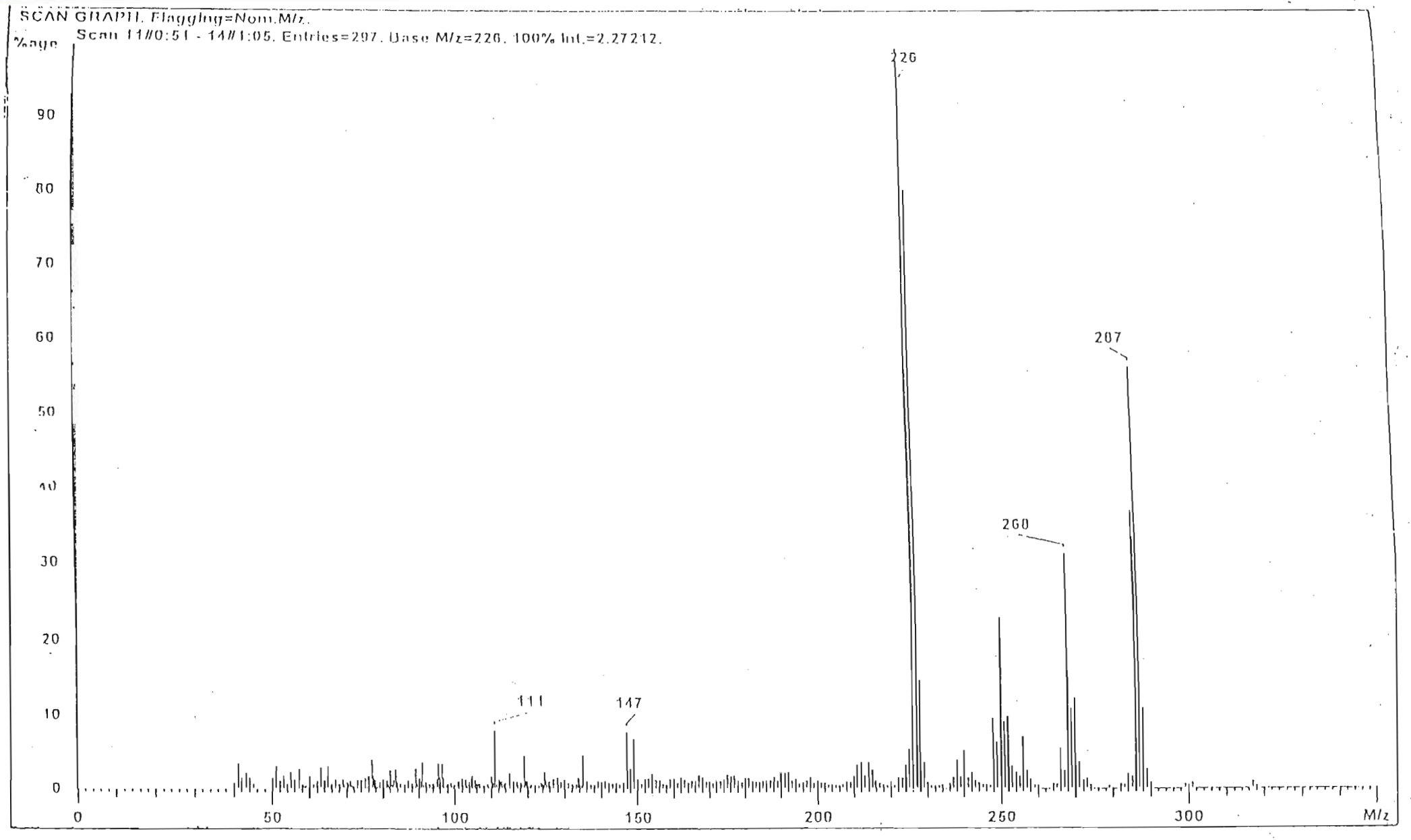
Spectrum [1-B]: ^{13}C NMR spectrum of lycorine(I) in CD_3OD



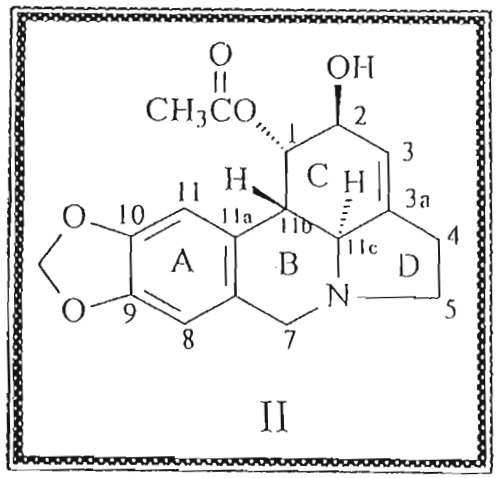
Spectrum [I-C]: Infra red spectrum of lycorine(I)



Spectrum [1-D]: UV spectrum of lycorine(**I**) in CH_3OH

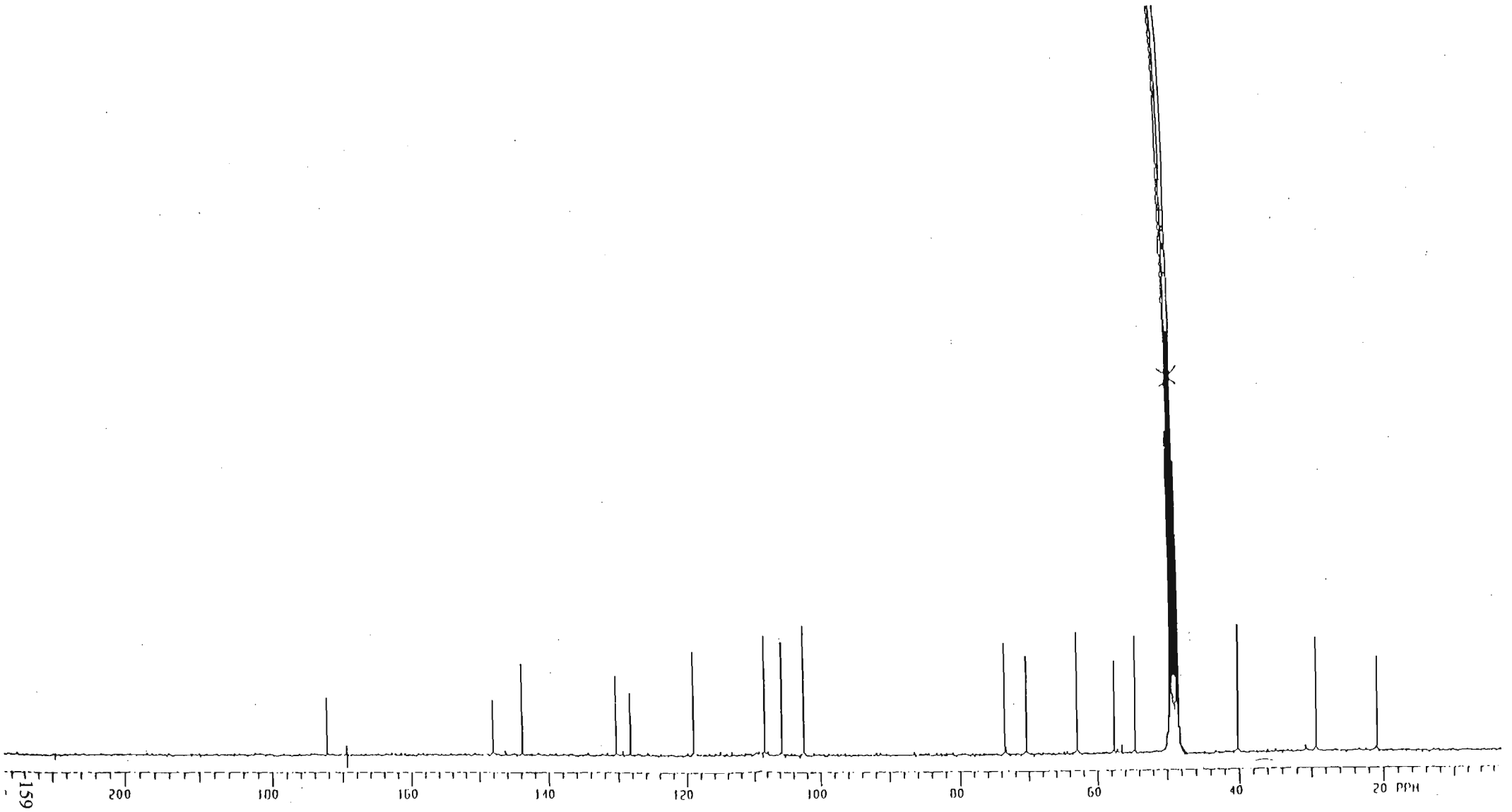


Spectrum [1-E]: Mass spectrum of lycorine(**I**)

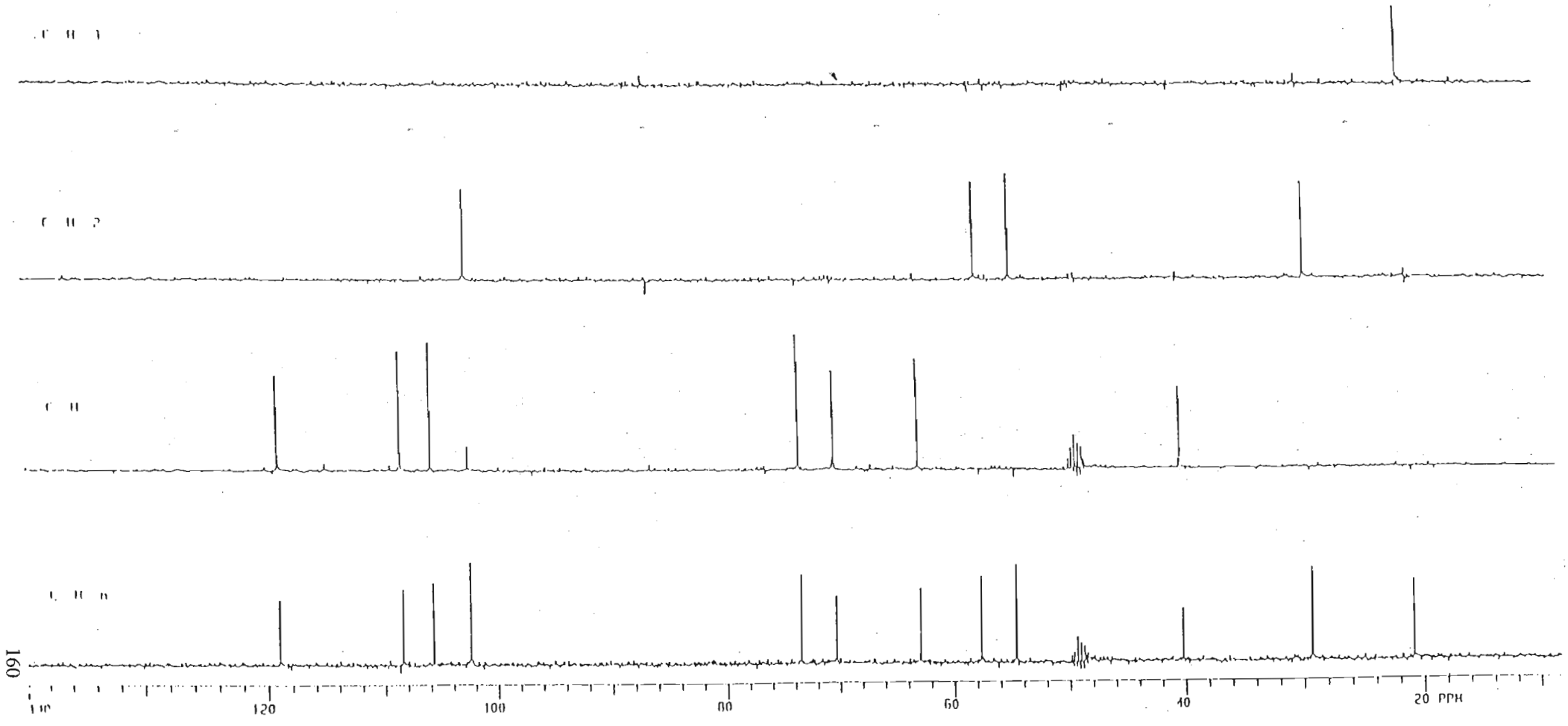


II

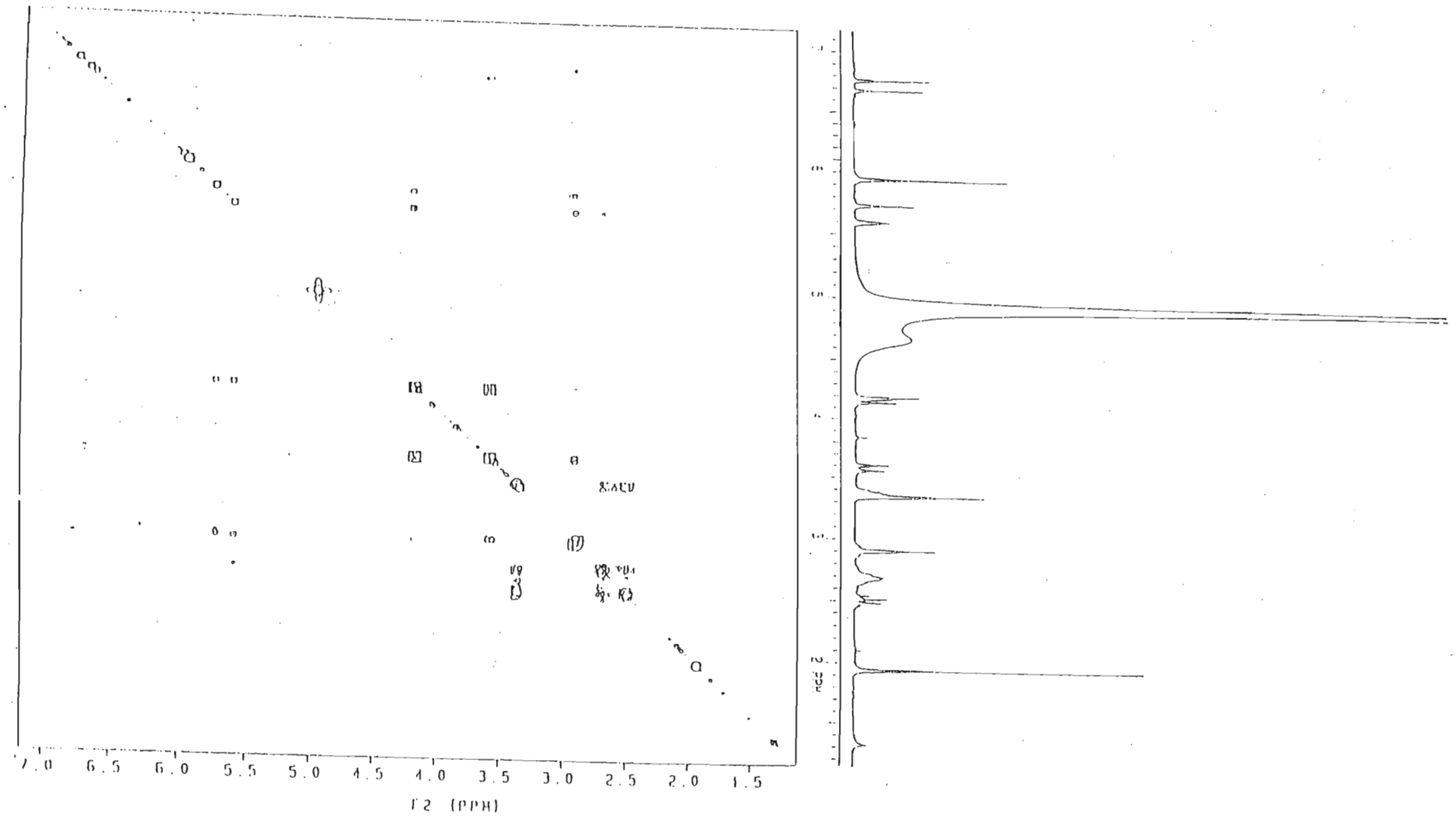
Spectrum [2-A]: ^{1}H NMR spectrum of 1-*O*-acetyllycorine (**II**) in CD_3OD



Spectrum [2-B]: ^{13}C NMR spectrum of 1-*O*-acetyllycorine (**II**) in CD_3OD

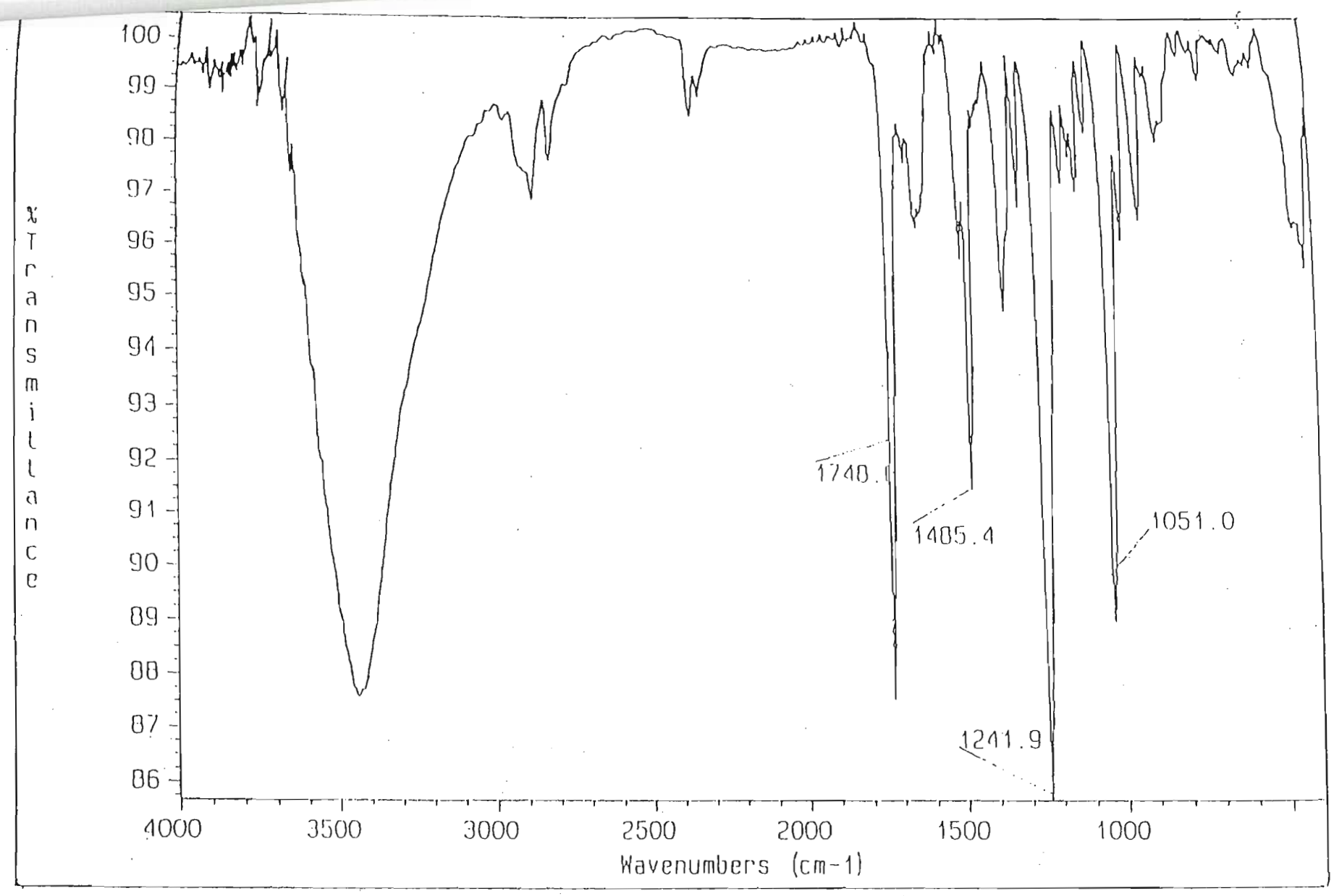


Spectrum [2-C]: ADEPT spectrum of 1-*O*-acetyllycorine (**II**) in CD_3OD

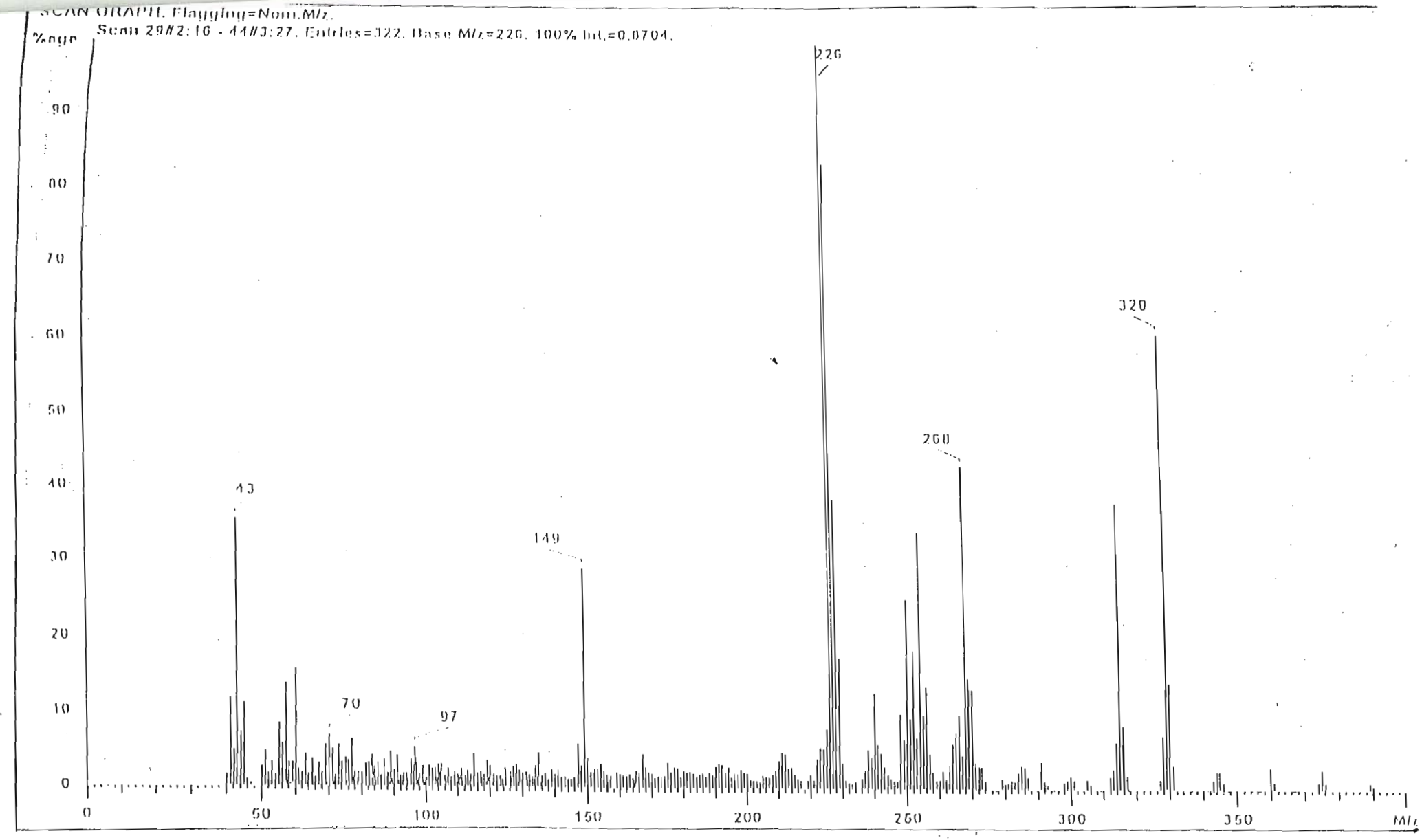


Spectrum [2-D]: COSY spectrum of 1-*O*-acetyllycorine (**II**) in CD_3OD

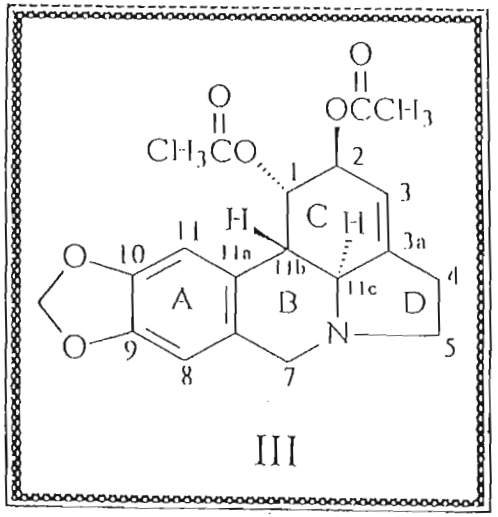
Spectrum [2-E]: HETCOR spectrum of 1-*O*-acetyllycorine (**II**) in CD_3OD



Spectrum [2-F]: Infra red spectrum of 1-*O*-acetyllycorine **(II)**

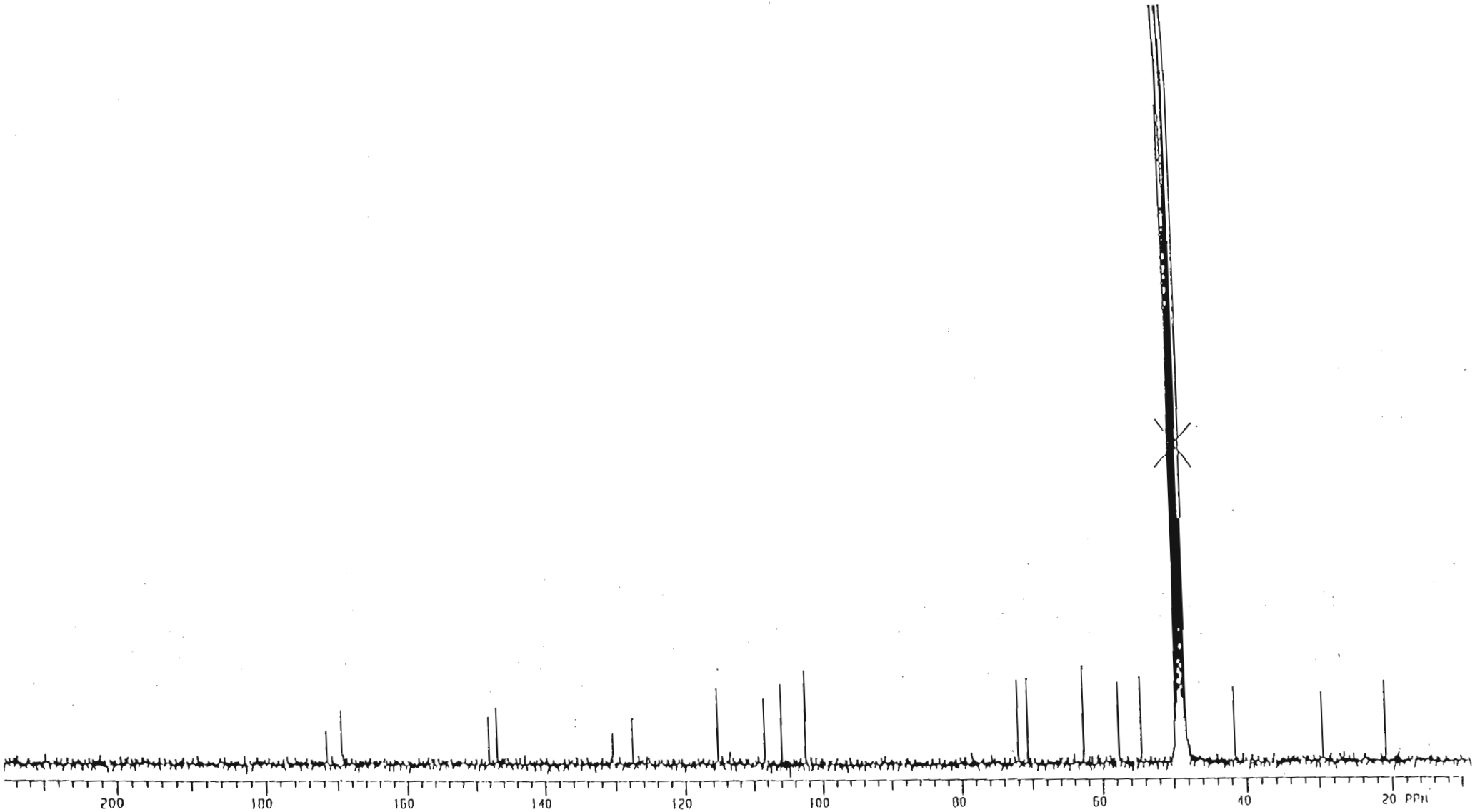


Spectrum [2-G]: Mass spectrum of 1-*O*-acetyllycorine **(II)**

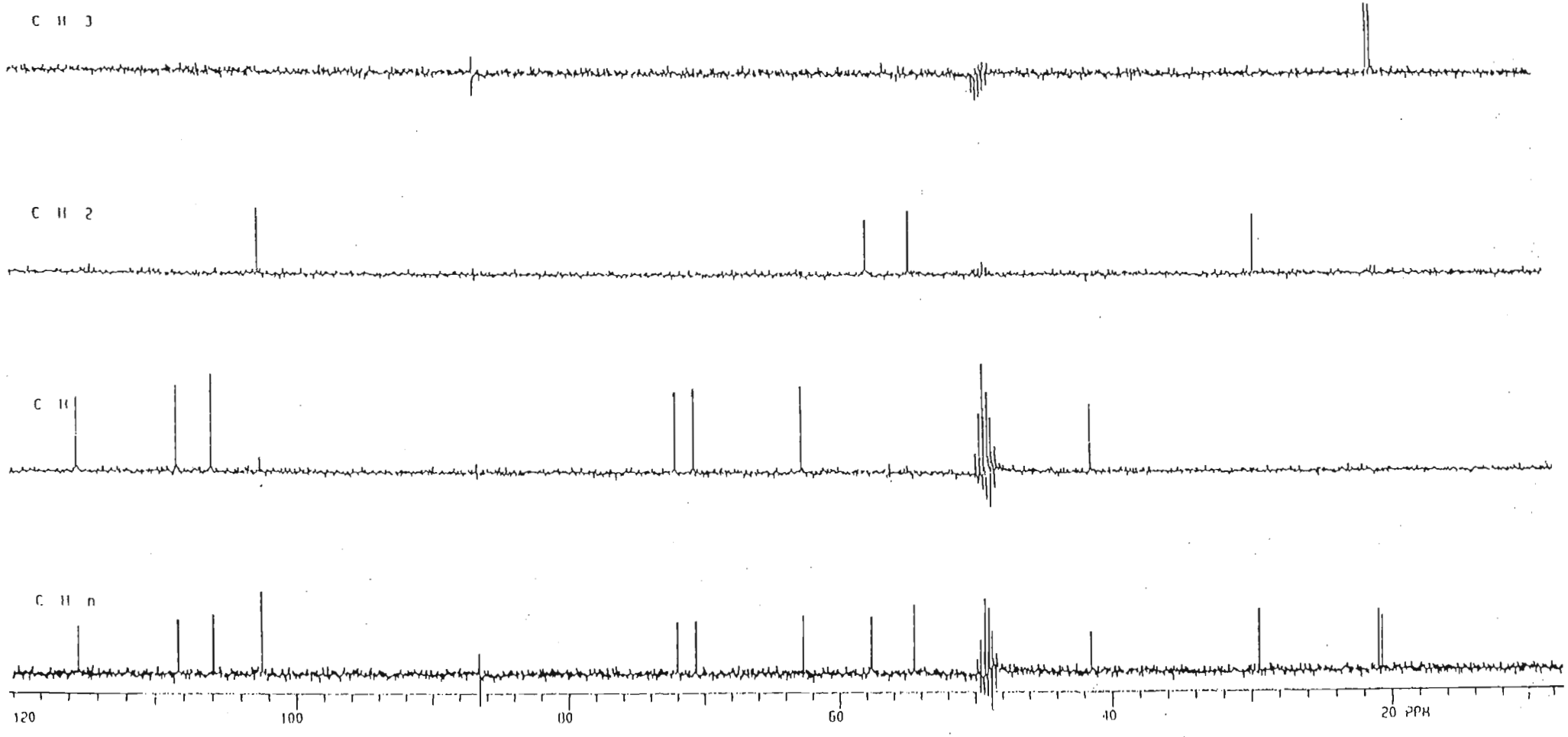


III

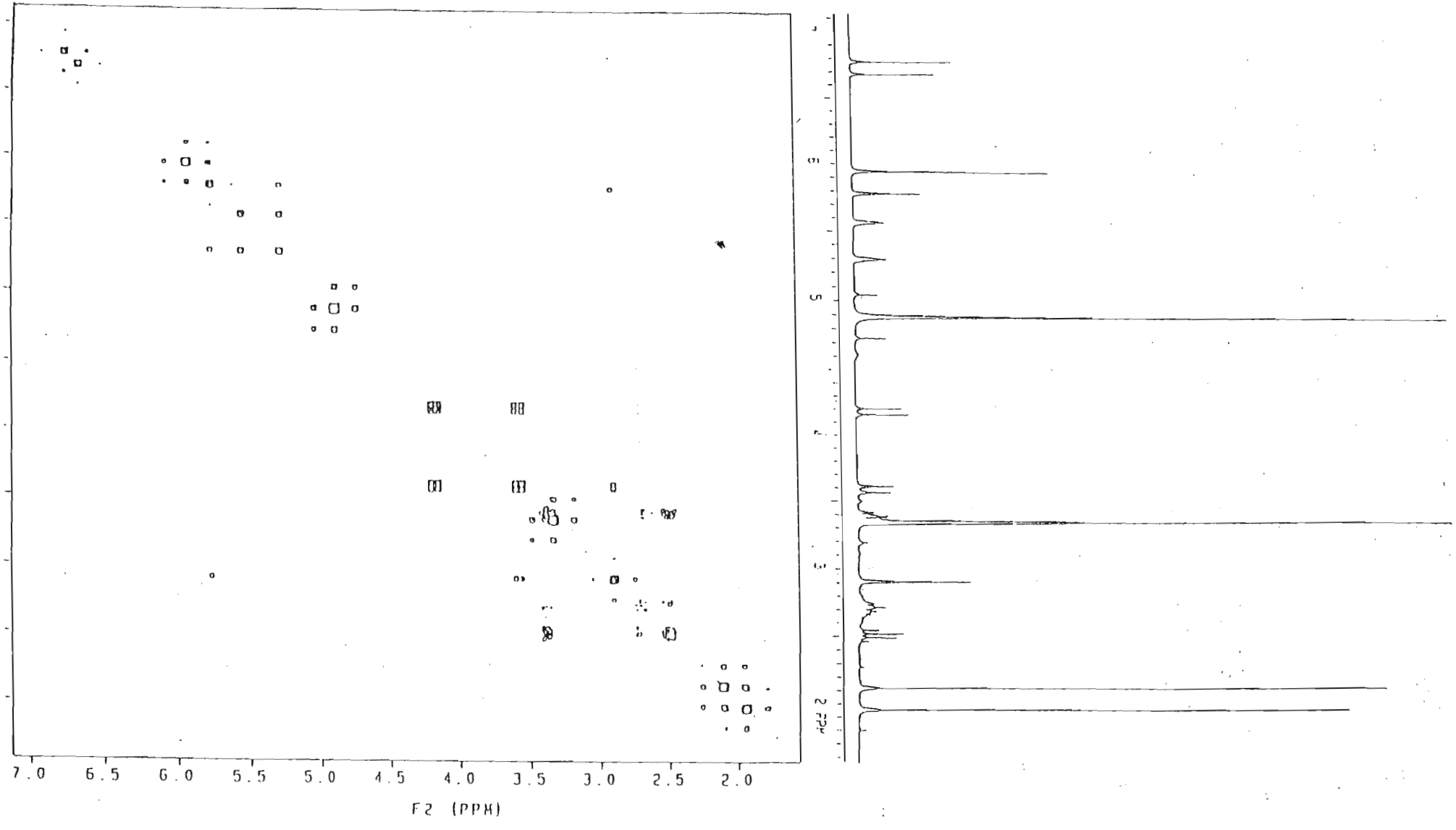
Spectrum [3-A]: ^{1}H NMR spectrum of 1,2-di-*O*-acetyllycorine (**III**) in CD_3OD



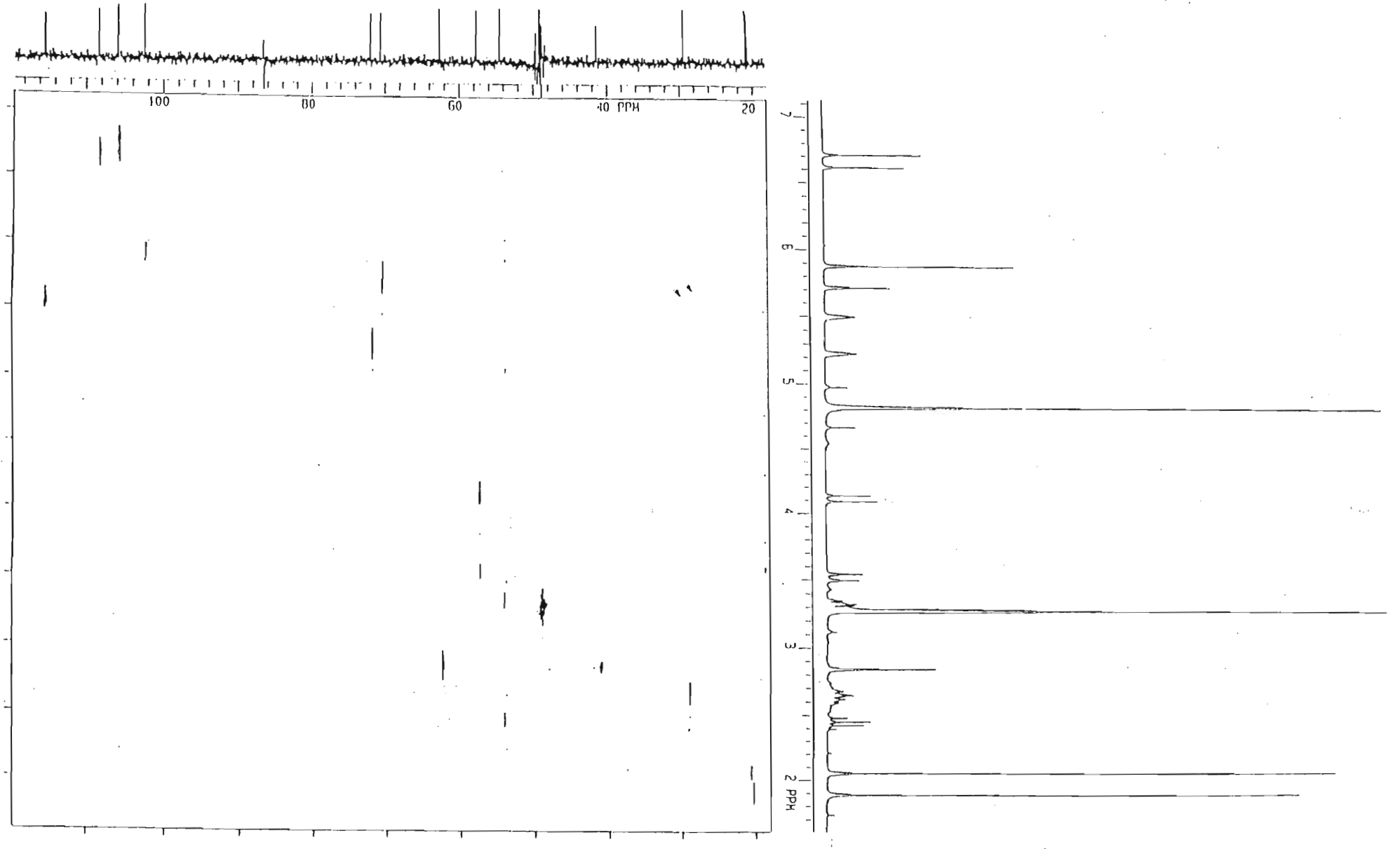
Spectrum [3-B]: ^{13}C NMR spectrum of 1,2-di-*O*-acetyllycorine (**III**) in CD_3OD



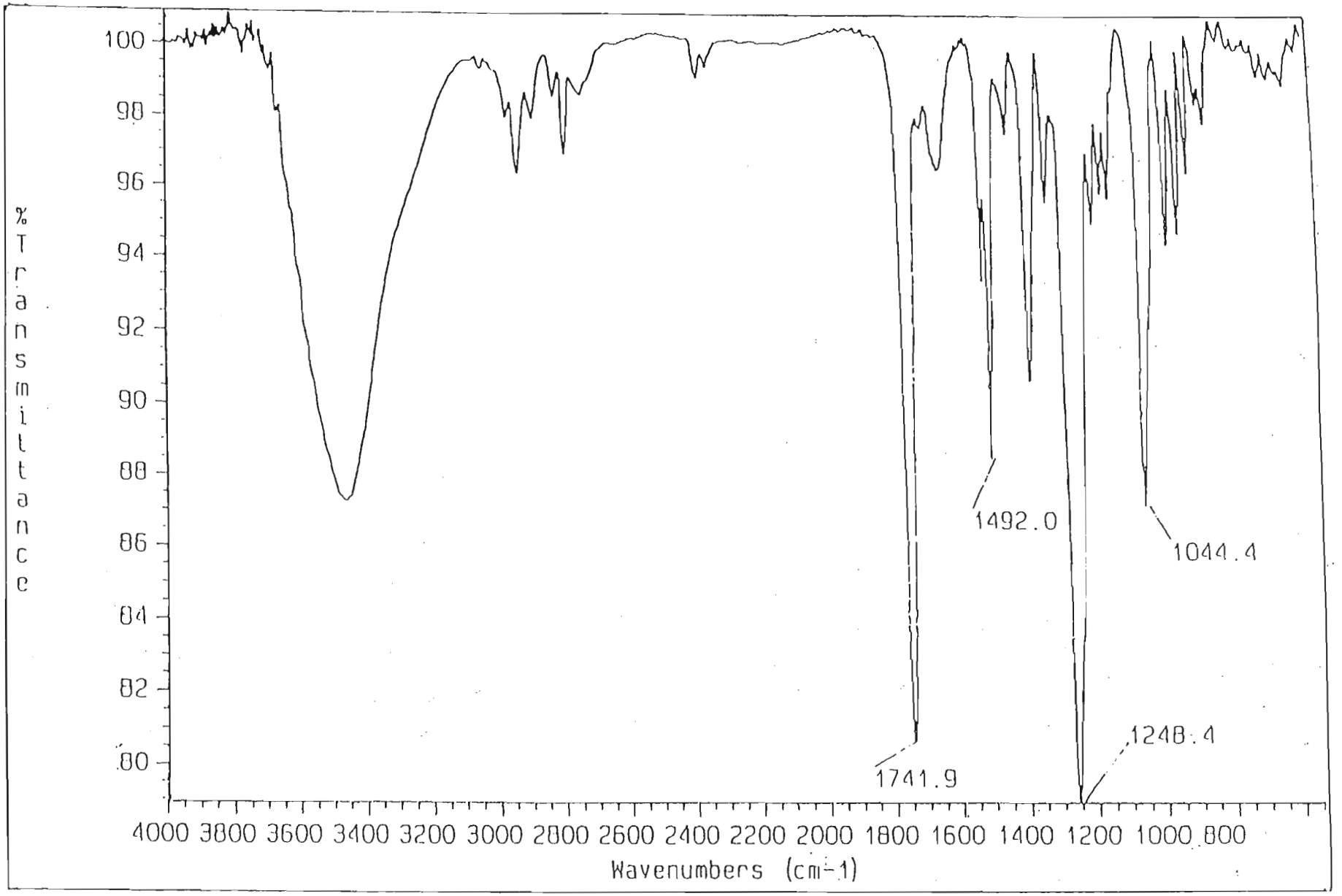
Spectrum [3-C]: ADEPT spectrum of 1,2-di-*O*-acetyllycorine (**III**) in CD_3OD



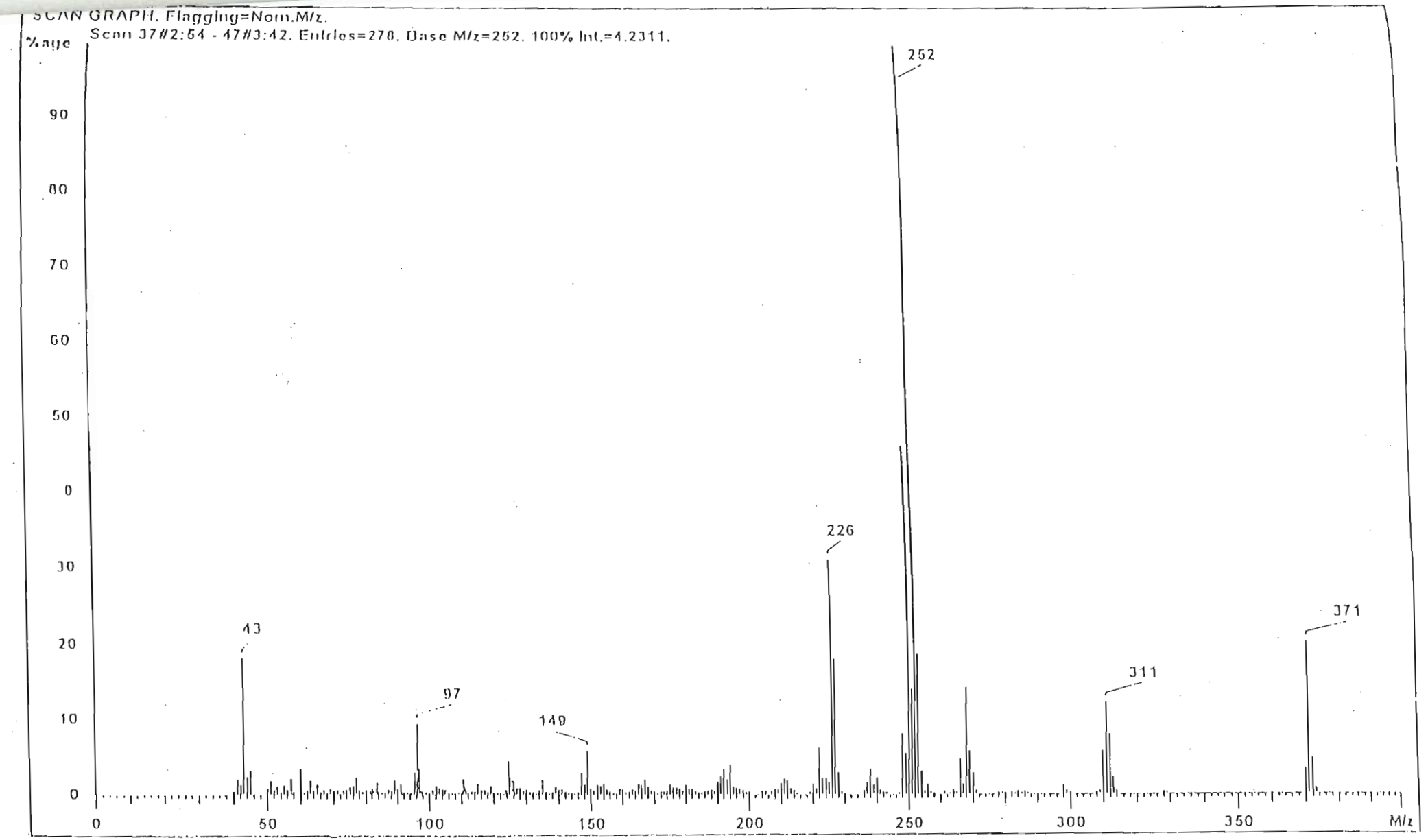
Spectrum [3-D]: COSY spectrum of 1,2-di-*O*-acetyllycorine (**III**) in CD_3OD



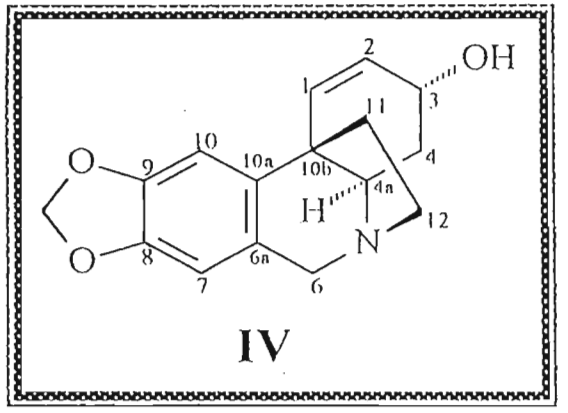
Spectrum [3-E]: HETCOR spectrum of 1,2-di-*O*-acetyllycorine (**III**) in CD_3OD



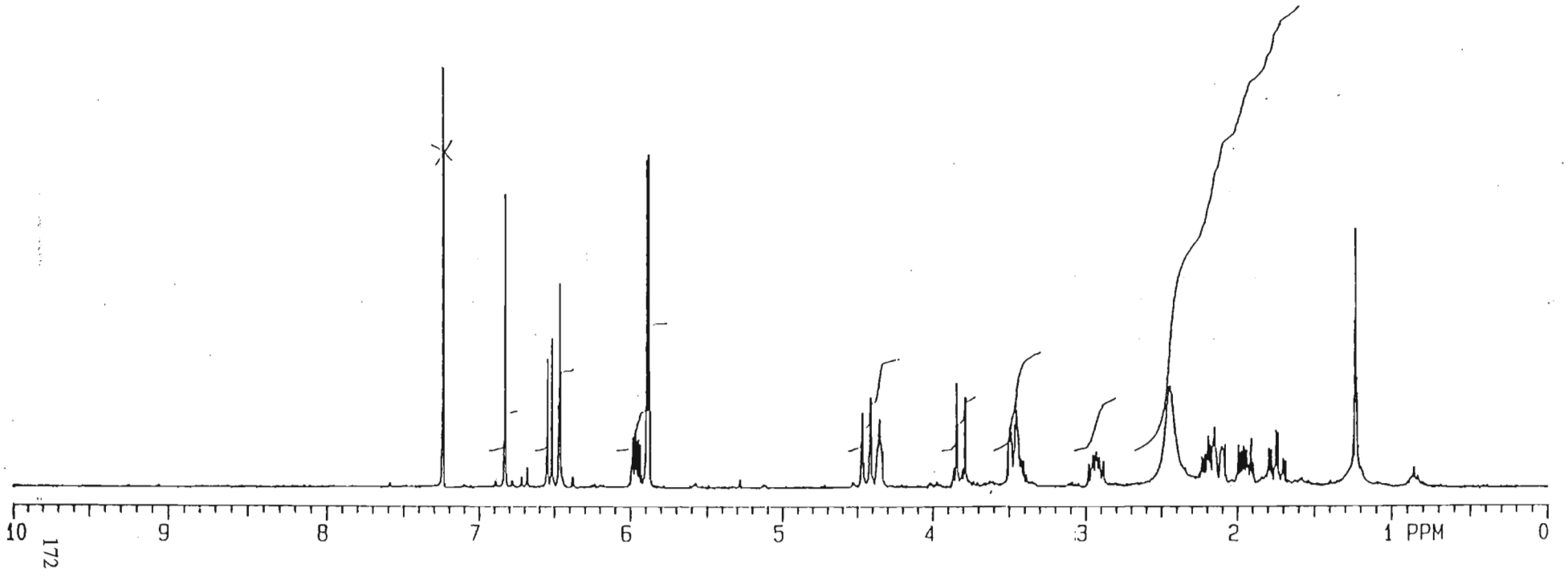
Spectrum [3-F]: Infra red spectrum of 1,2-di-*O*-acetyllycorine (**III**)



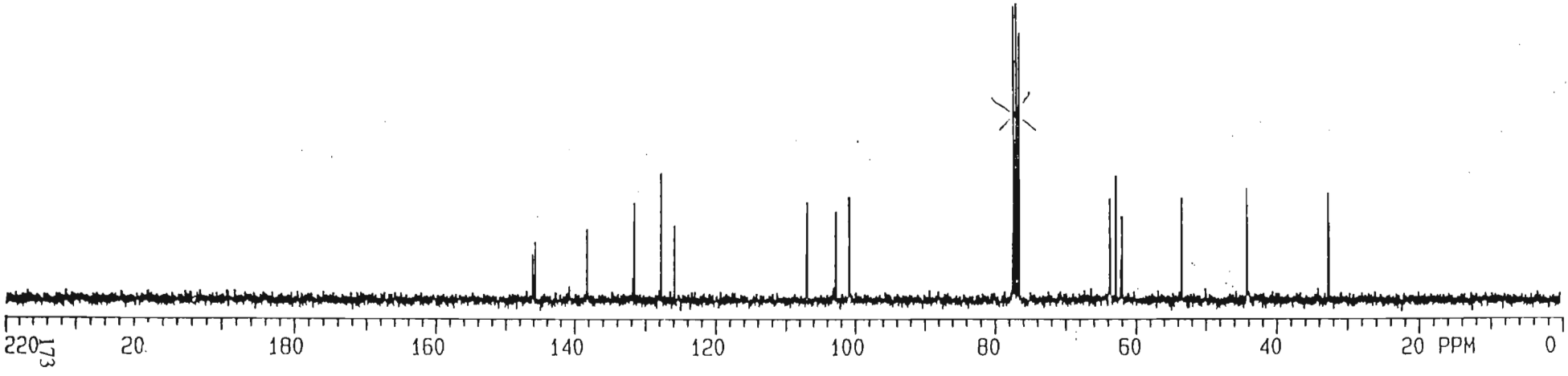
Spectrum [3-G]: Mass spectrum of 1,2-di-*O*-acetyllycorine (**III**)



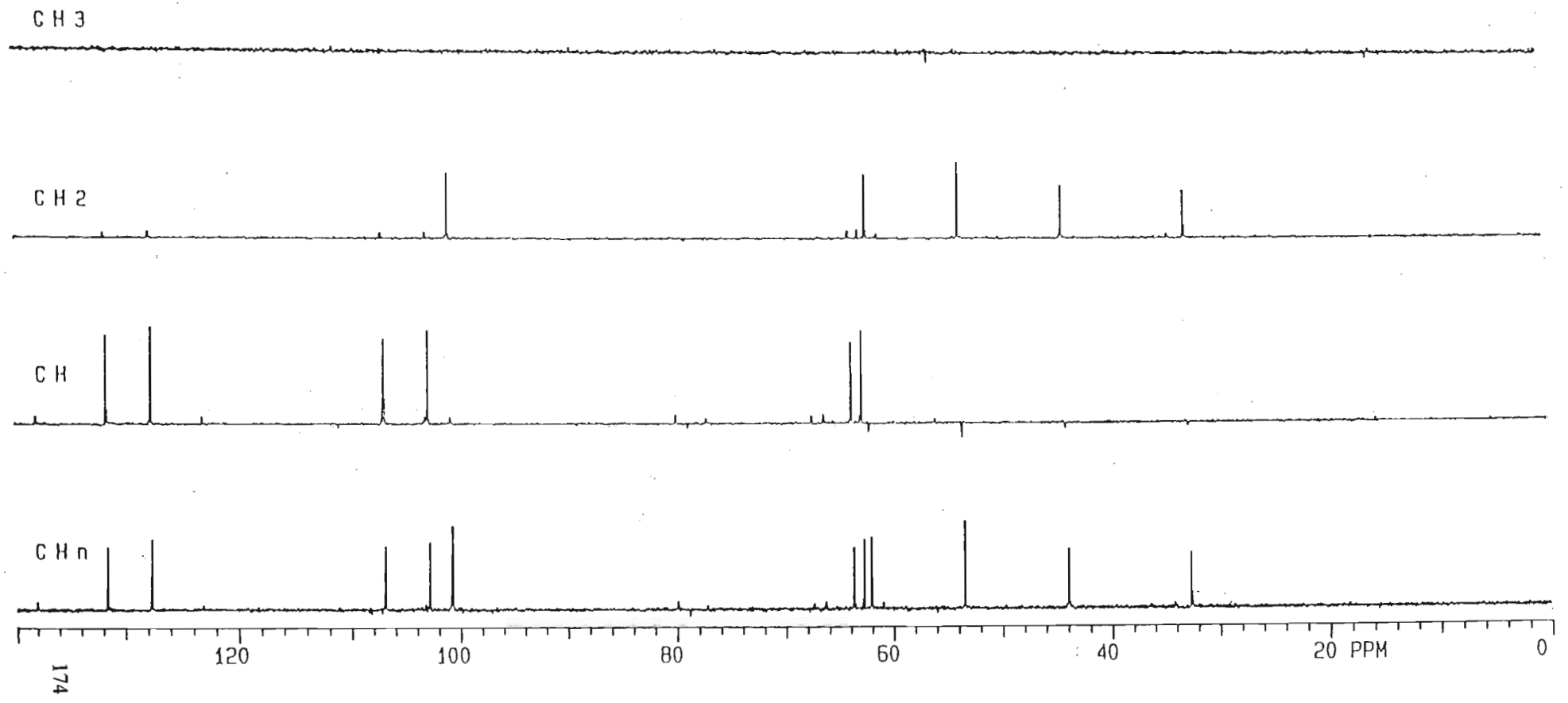
IV



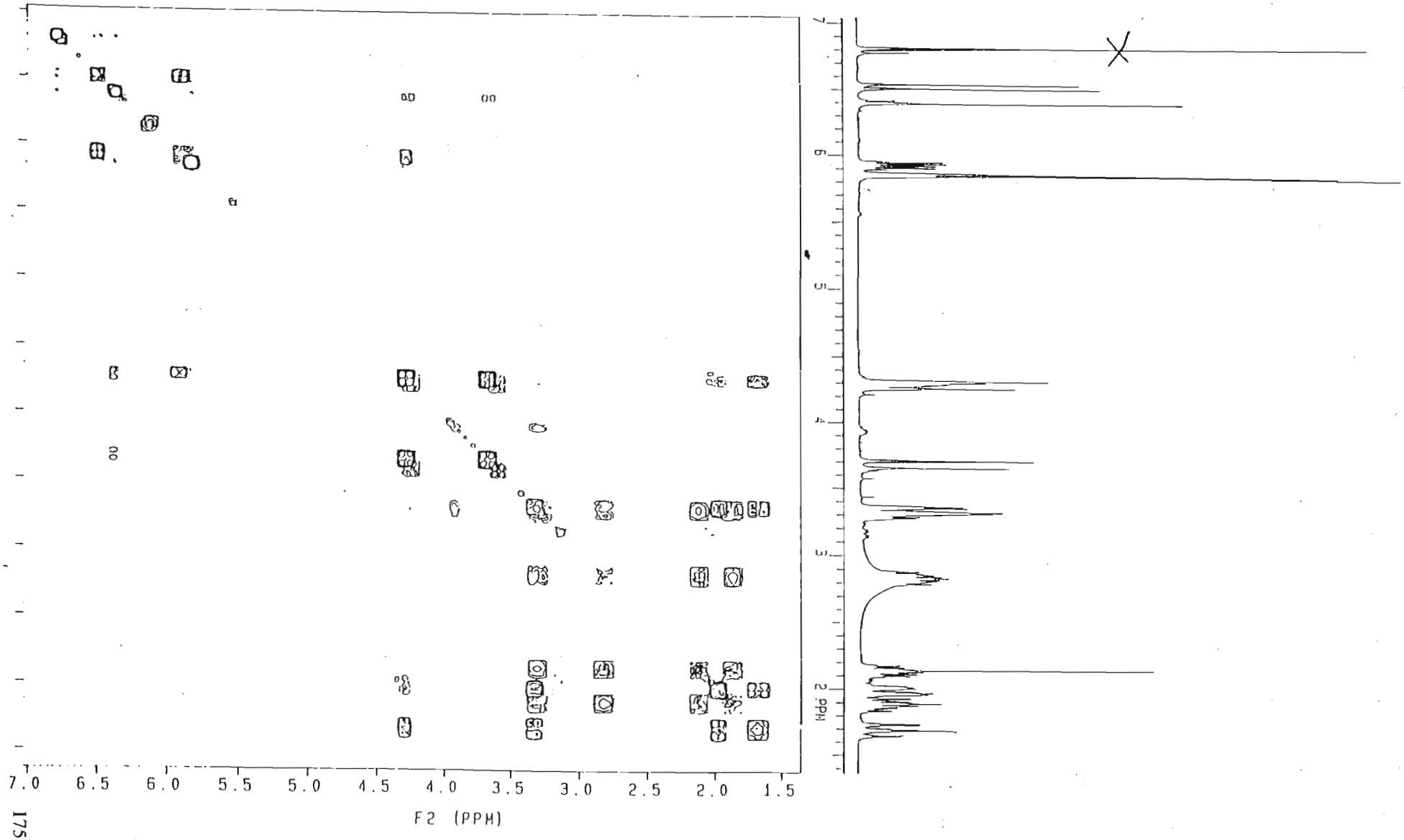
Spectrum [4-A]: ^{1}H NMR spectrum of crinine(**IV**) in $CDCl_3$



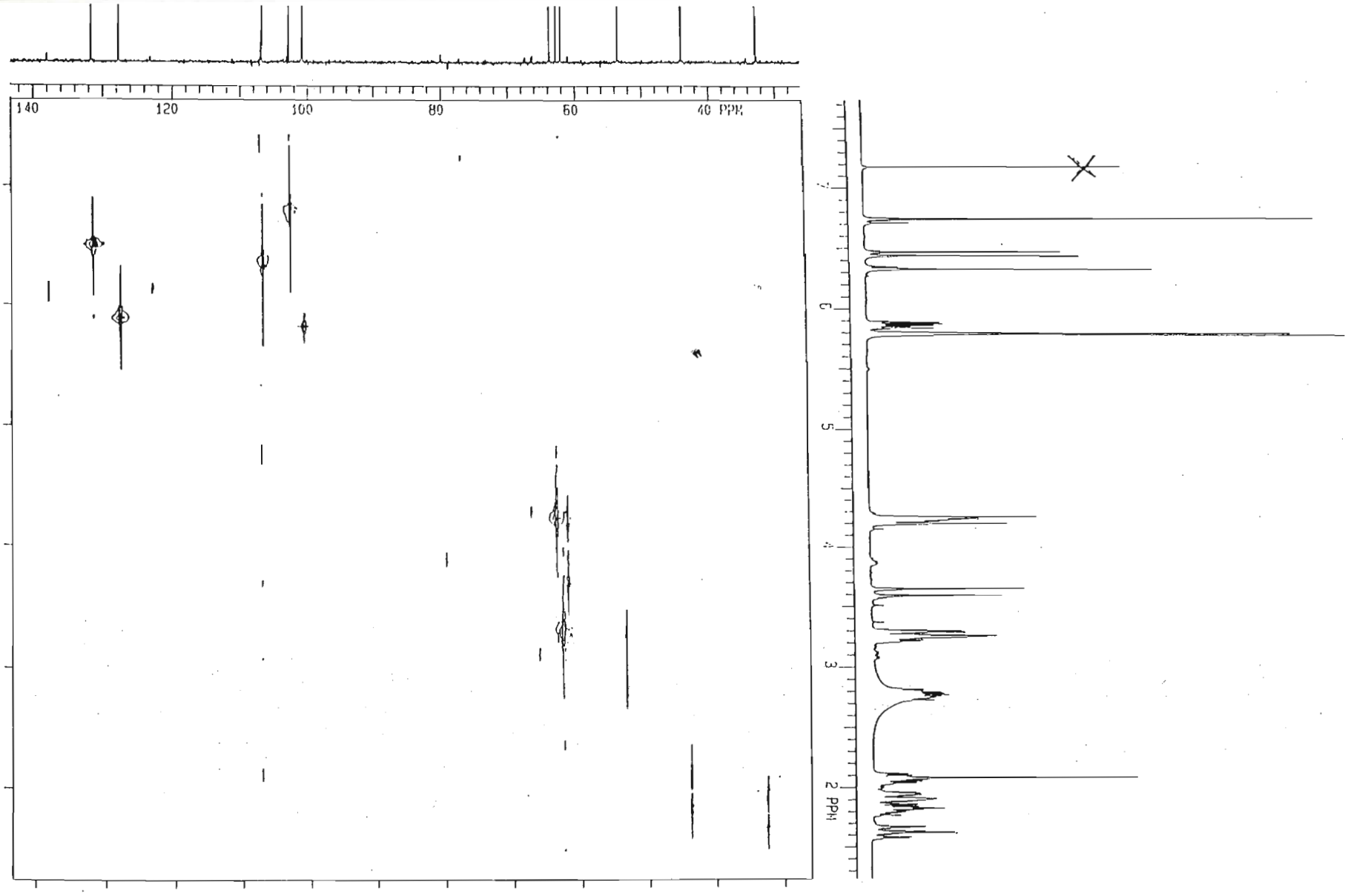
Spectrum [4-B]: ^{13}C NMR spectrum of crinine(IV) in $CDCl_3$



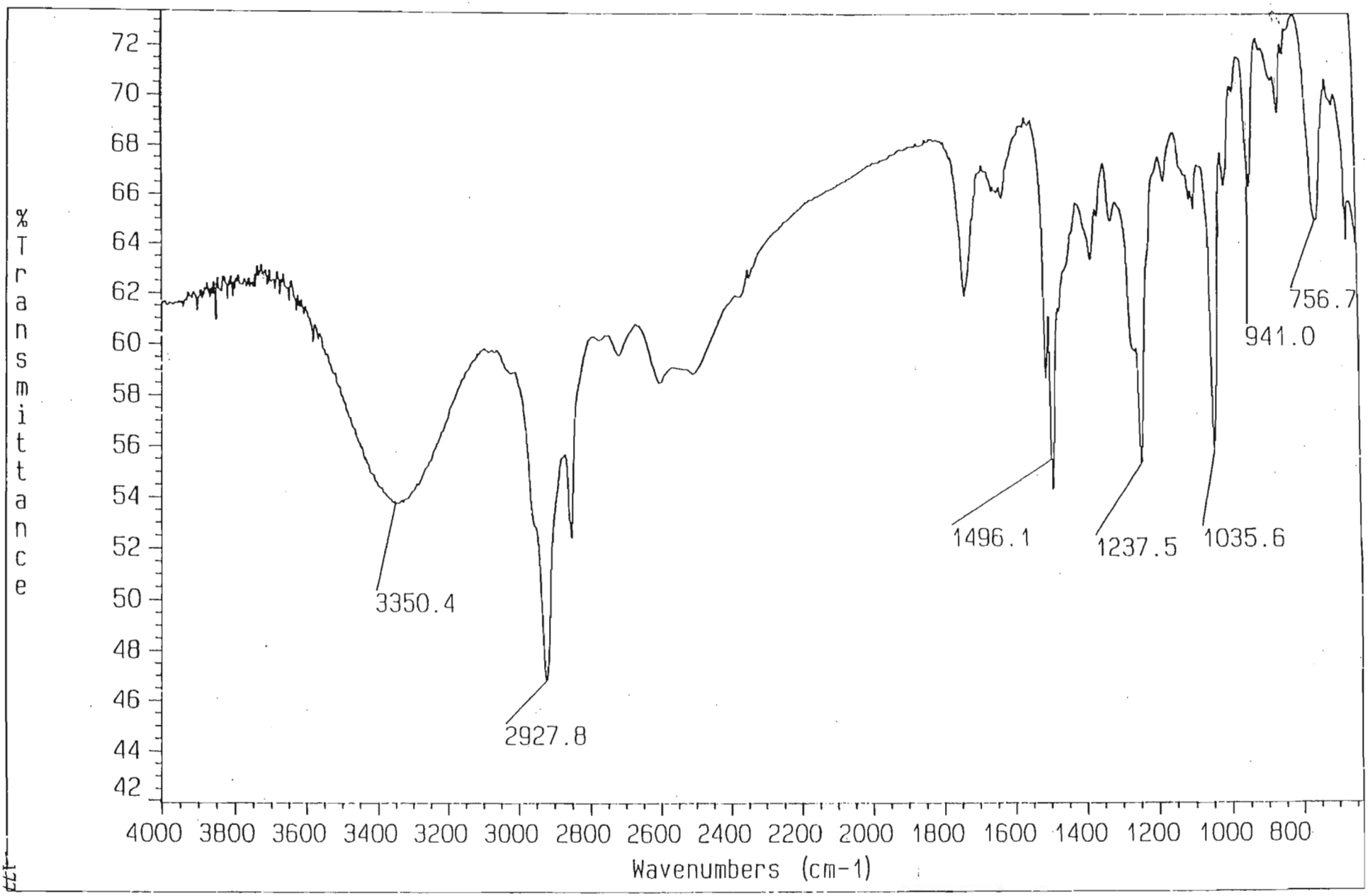
Spectrum [4-C]: ADEPT spectrum of crinine(**IV**) in $CDCl_3$



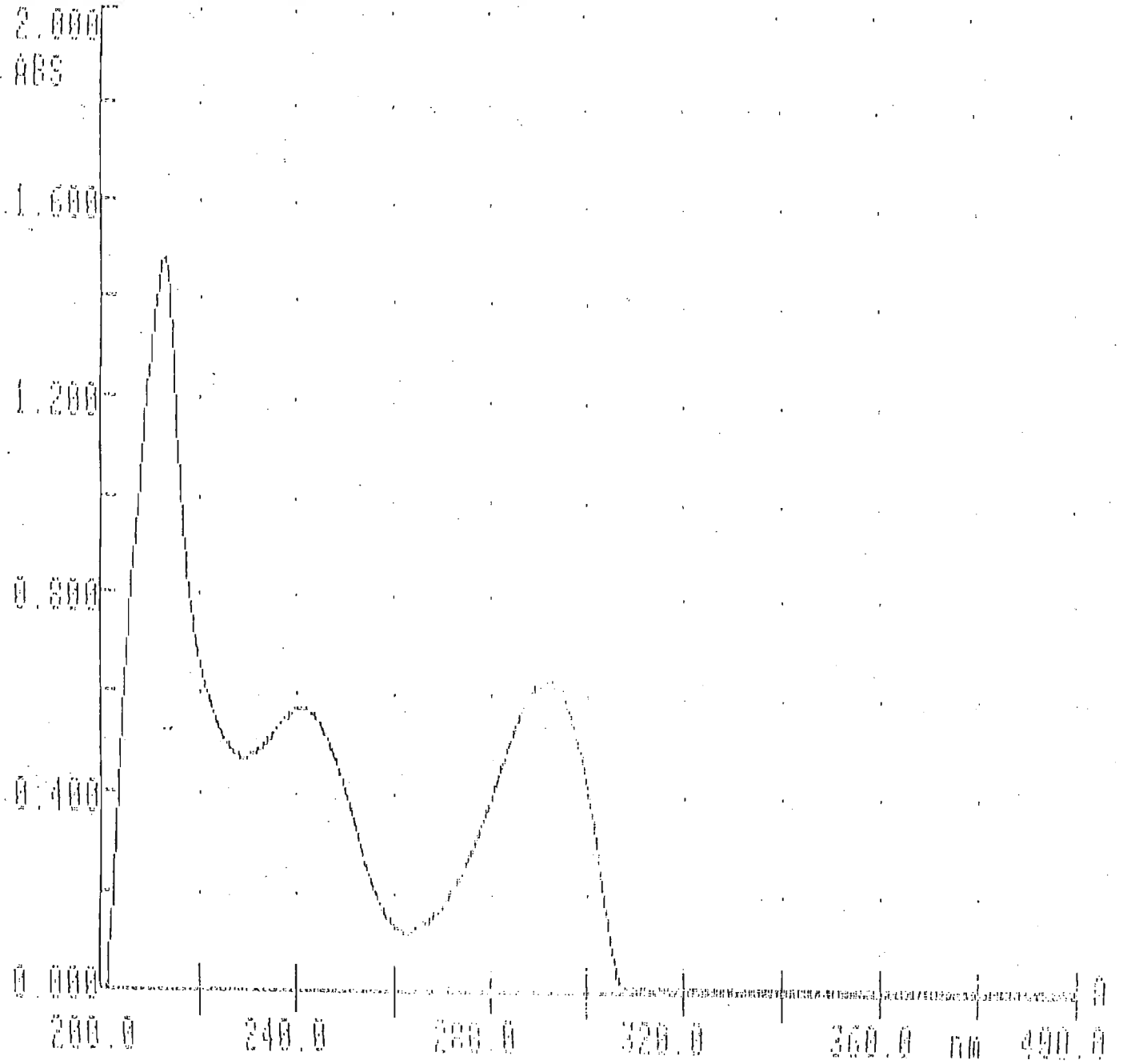
Spectrum [4-D]: COSY spectrum of crinine(**IV**) in $CDCl_3$



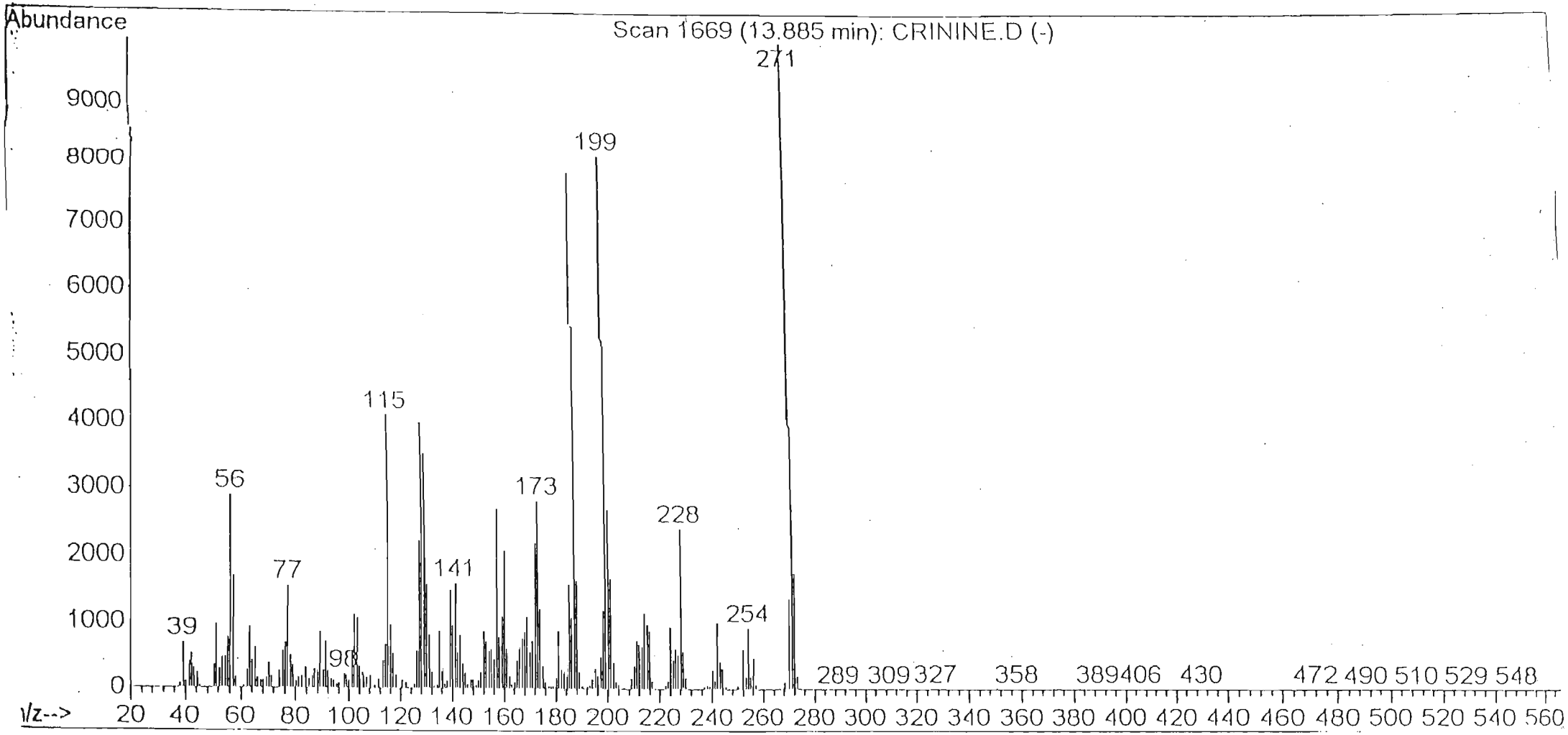
Spectrum [4-E]: HETCOR spectrum of crinine(IV) in $CDCl_3$



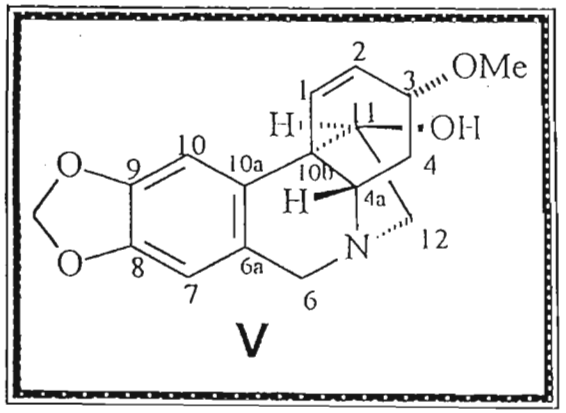
Spectrum [4-F]: Infra red spectrum of crinine(IV)



Spectrum [4-G]: UV spectrum of crinine(IV) in CH_3OH

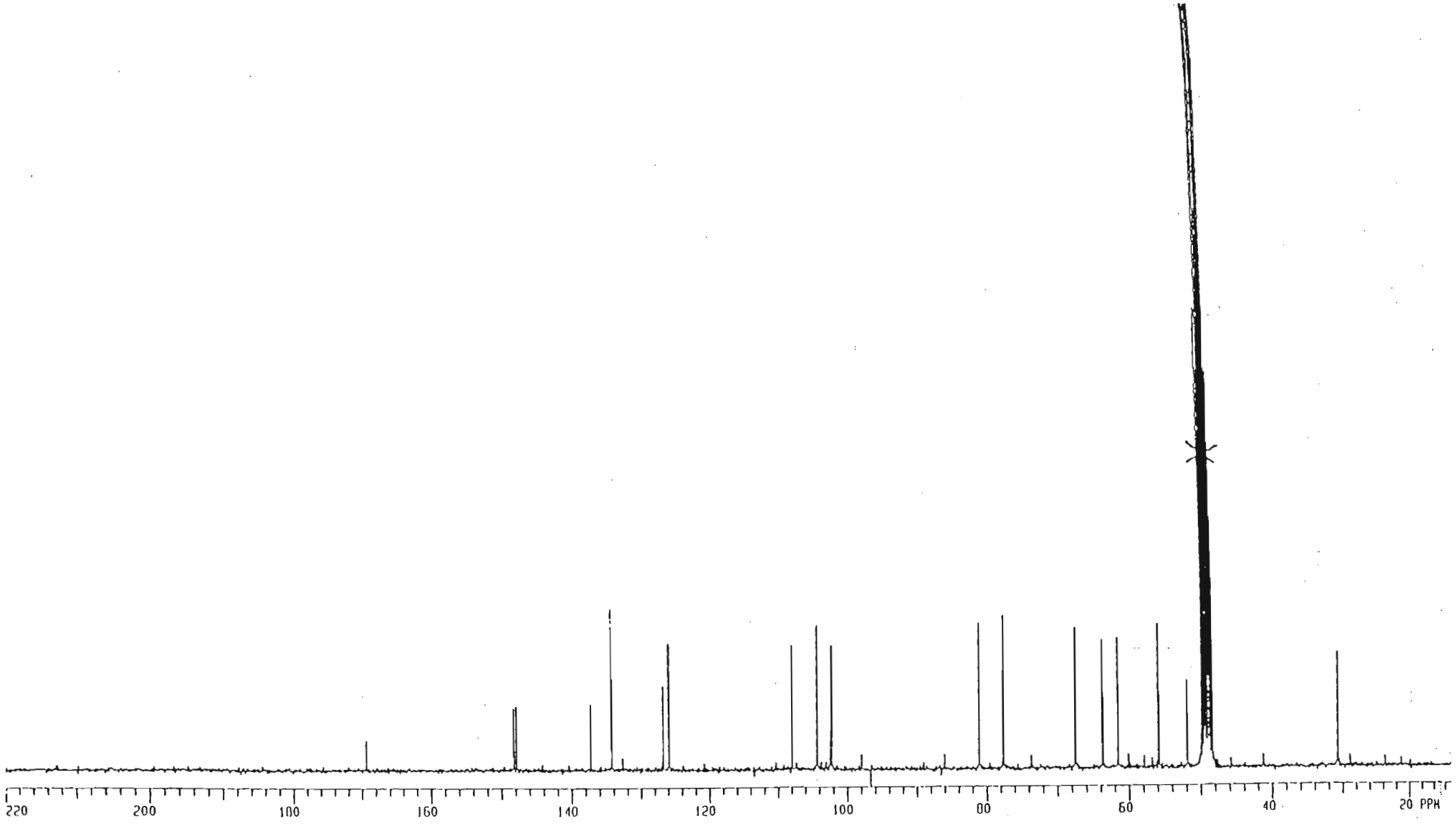


Spectrum [4-H]: Mass spectrum of crinine(IV)

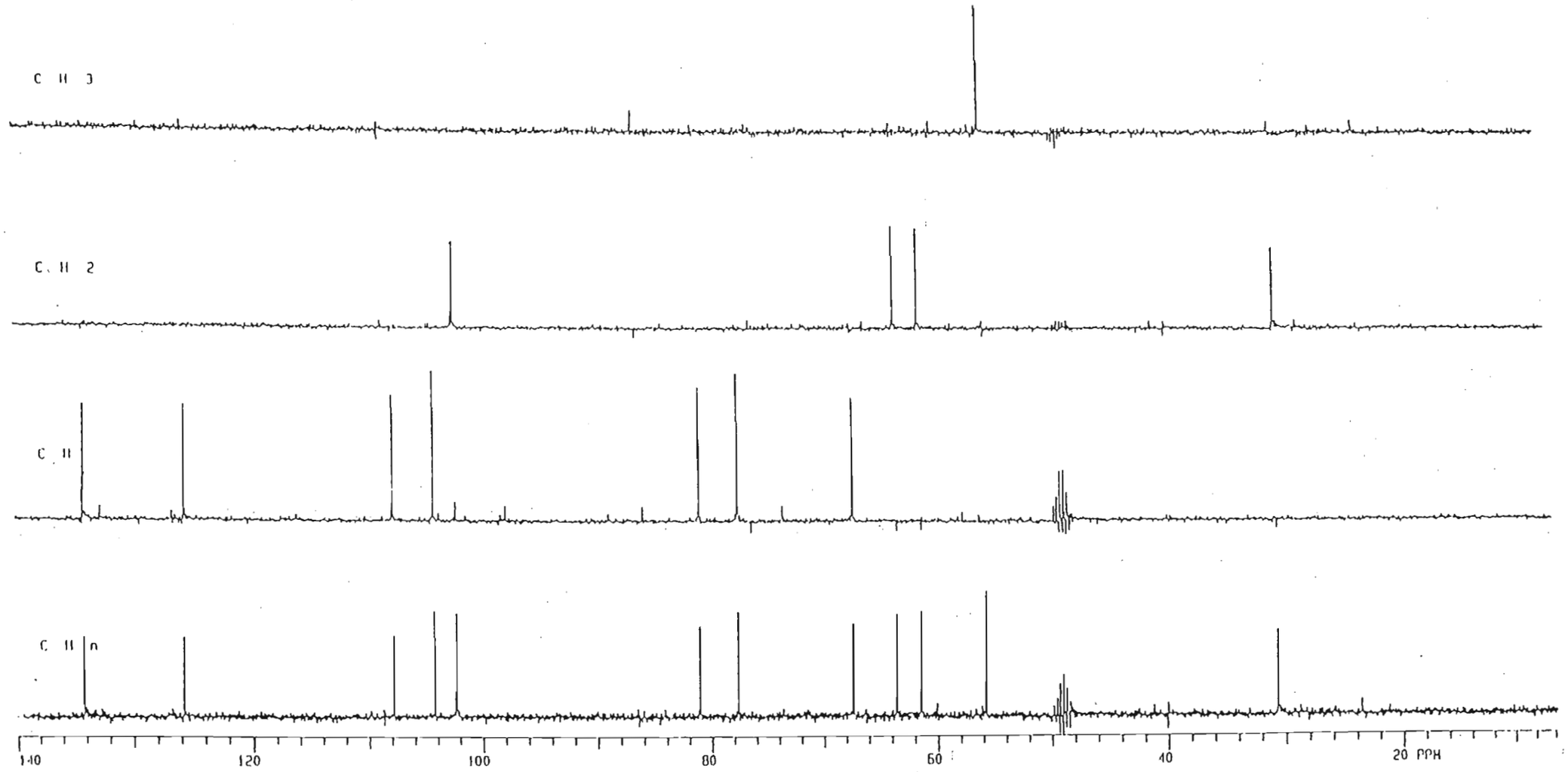


V

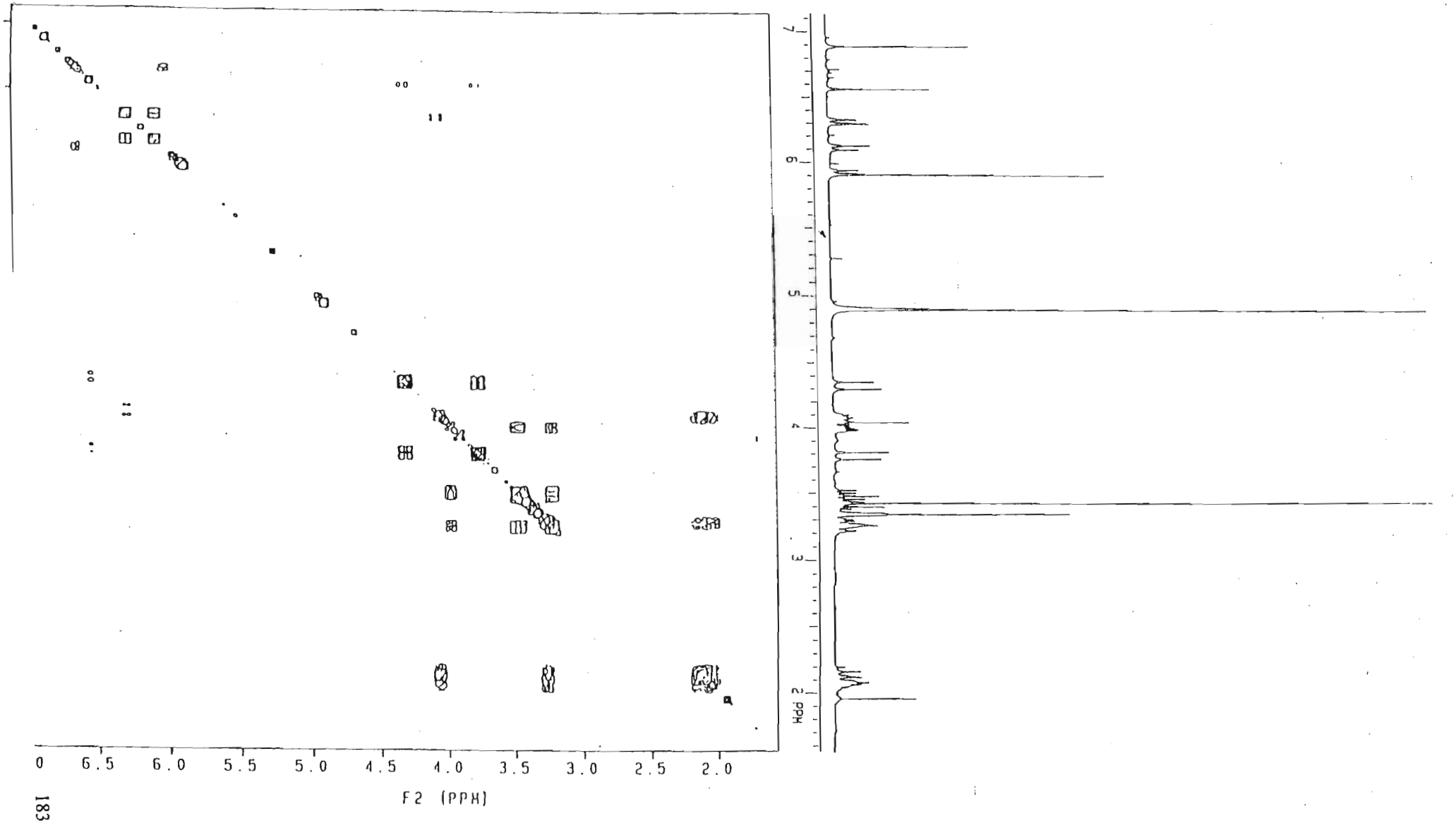
Spectrum [5-A]: ^{1}H NMR spectrum of crinamine(V) in CD_3OD



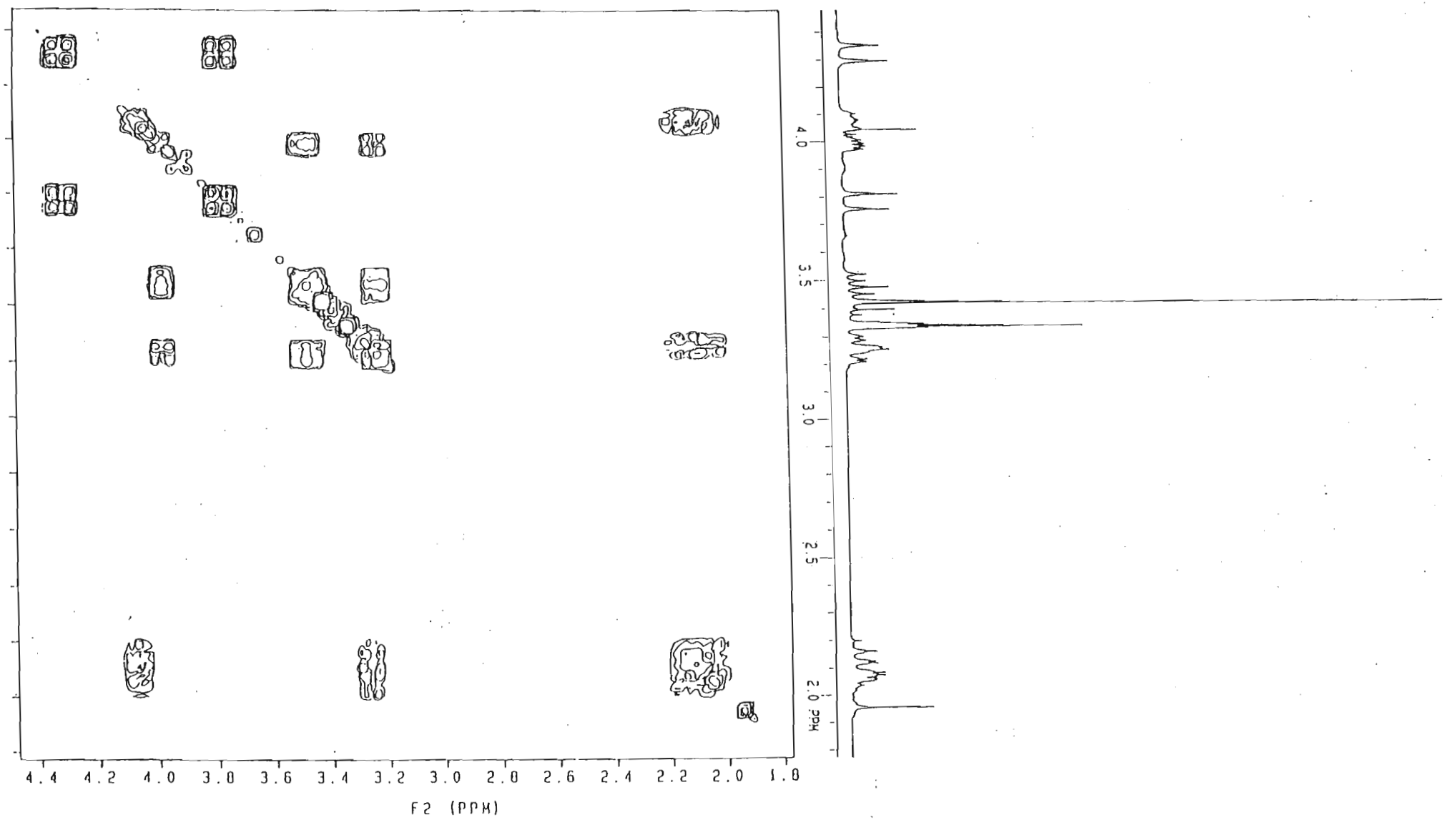
Spectrum [5-B]: ^{13}C NMR spectrum of crinamine(V) in CD_3OD



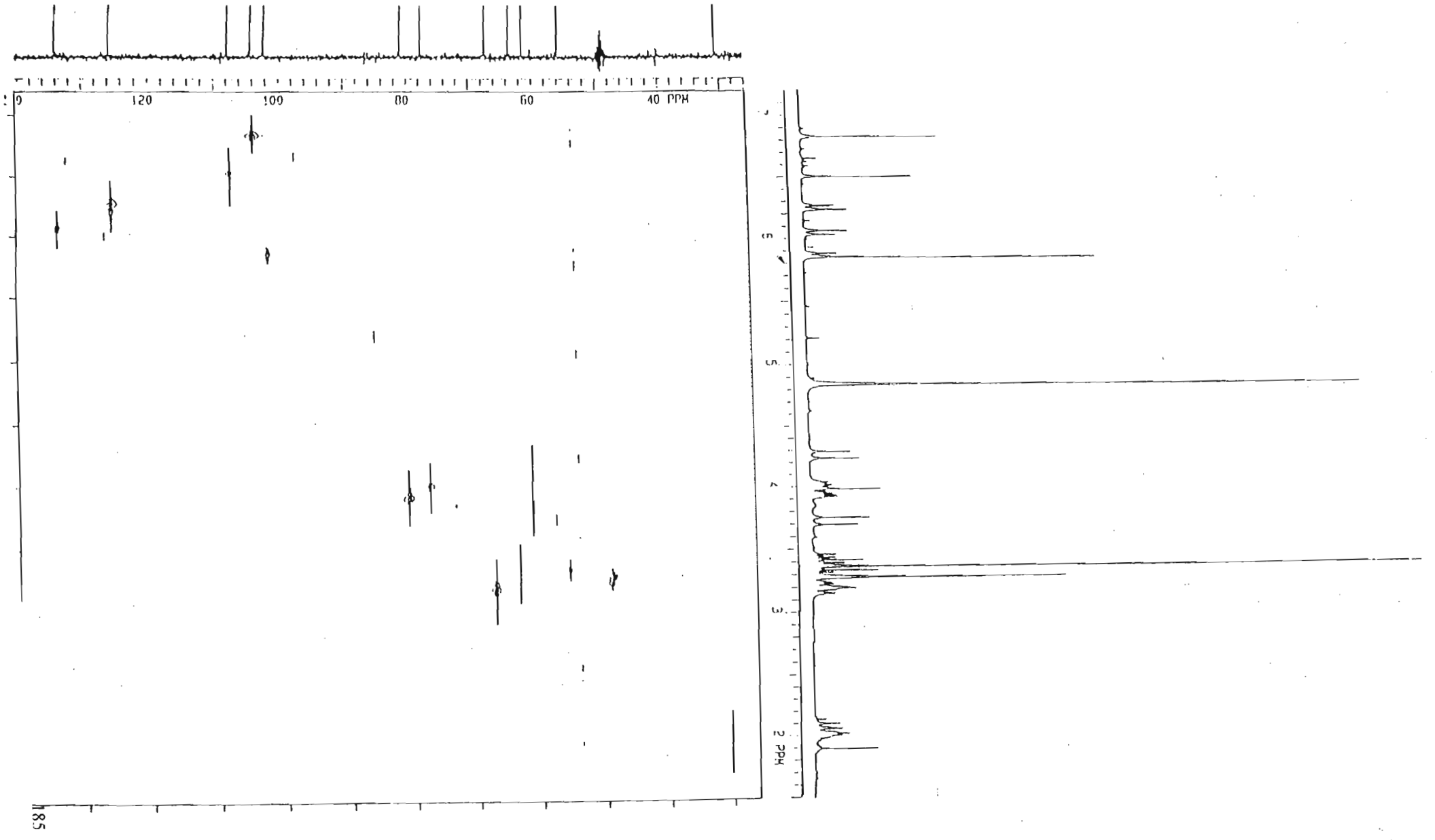
Spectrum [5-C]: ADEPT spectrum of crinamine(V) in CD_3OD



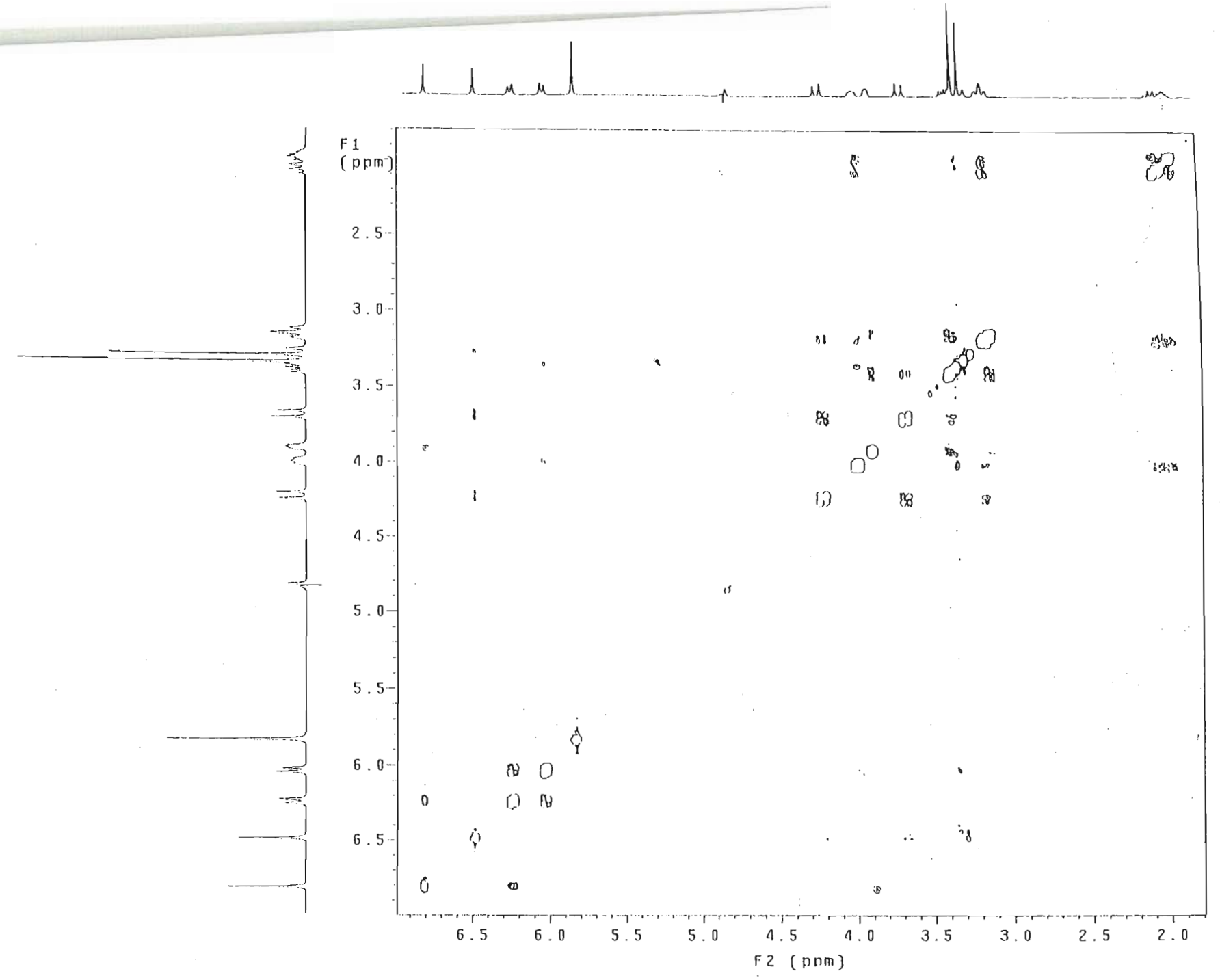
Spectrum [5-D]: COSY spectrum of crinamine(V) in CD_3OD



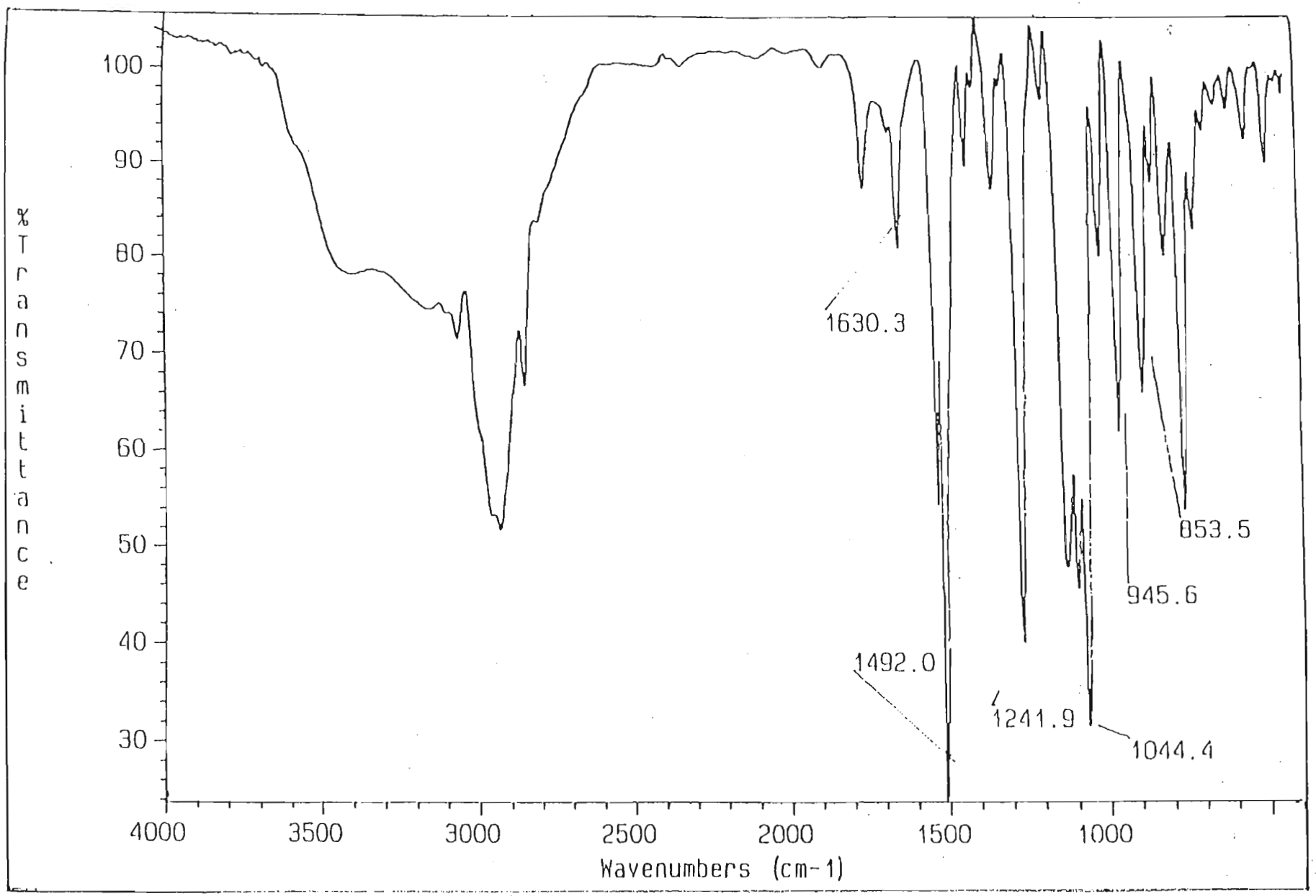
Spectrum [5-E]: Expanded COSY spectrum of crinamine(V) in CD_3OD



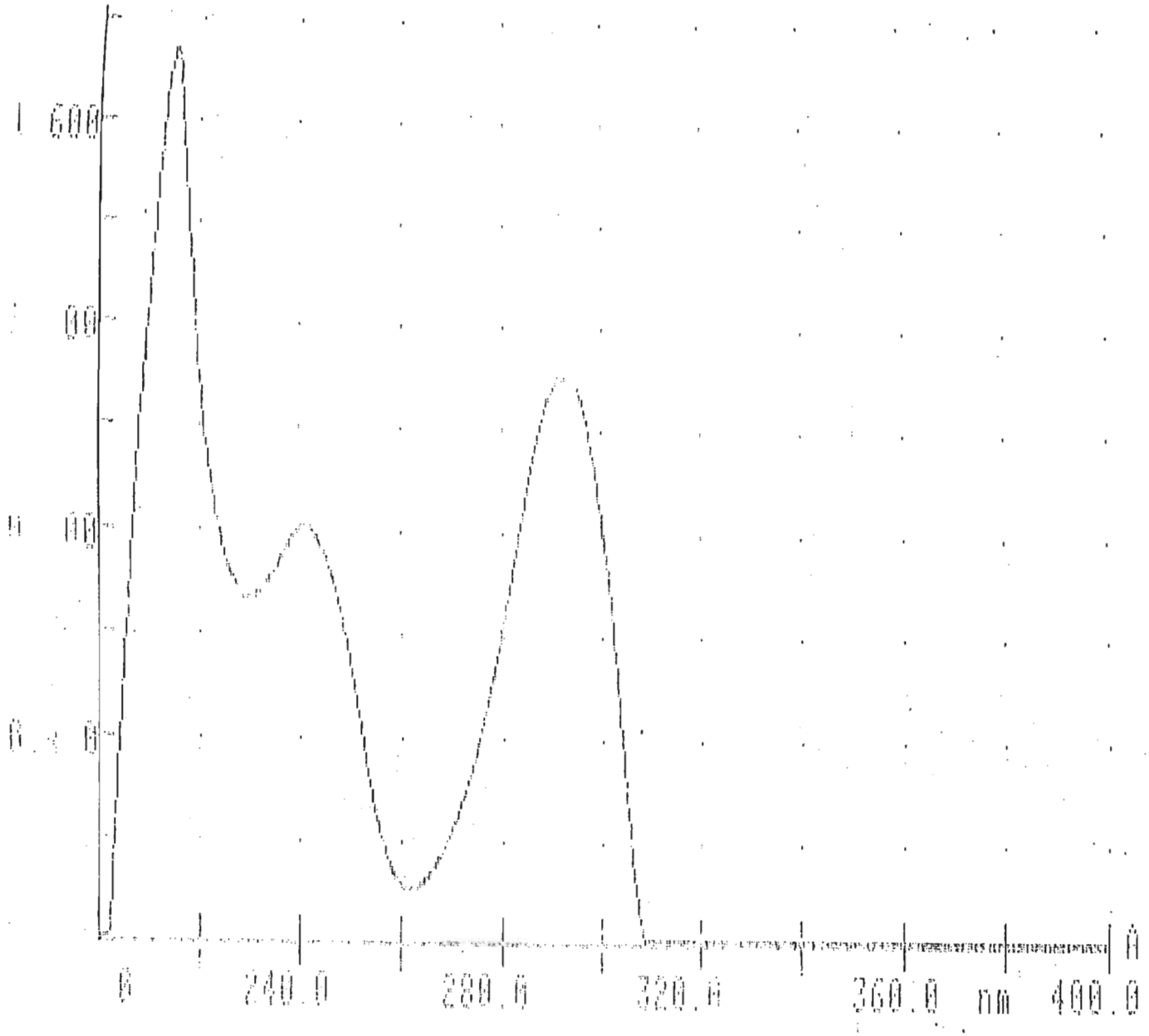
Spectrum [5-F]: HETCOR spectrum of crinamine(V) in CD_3OD



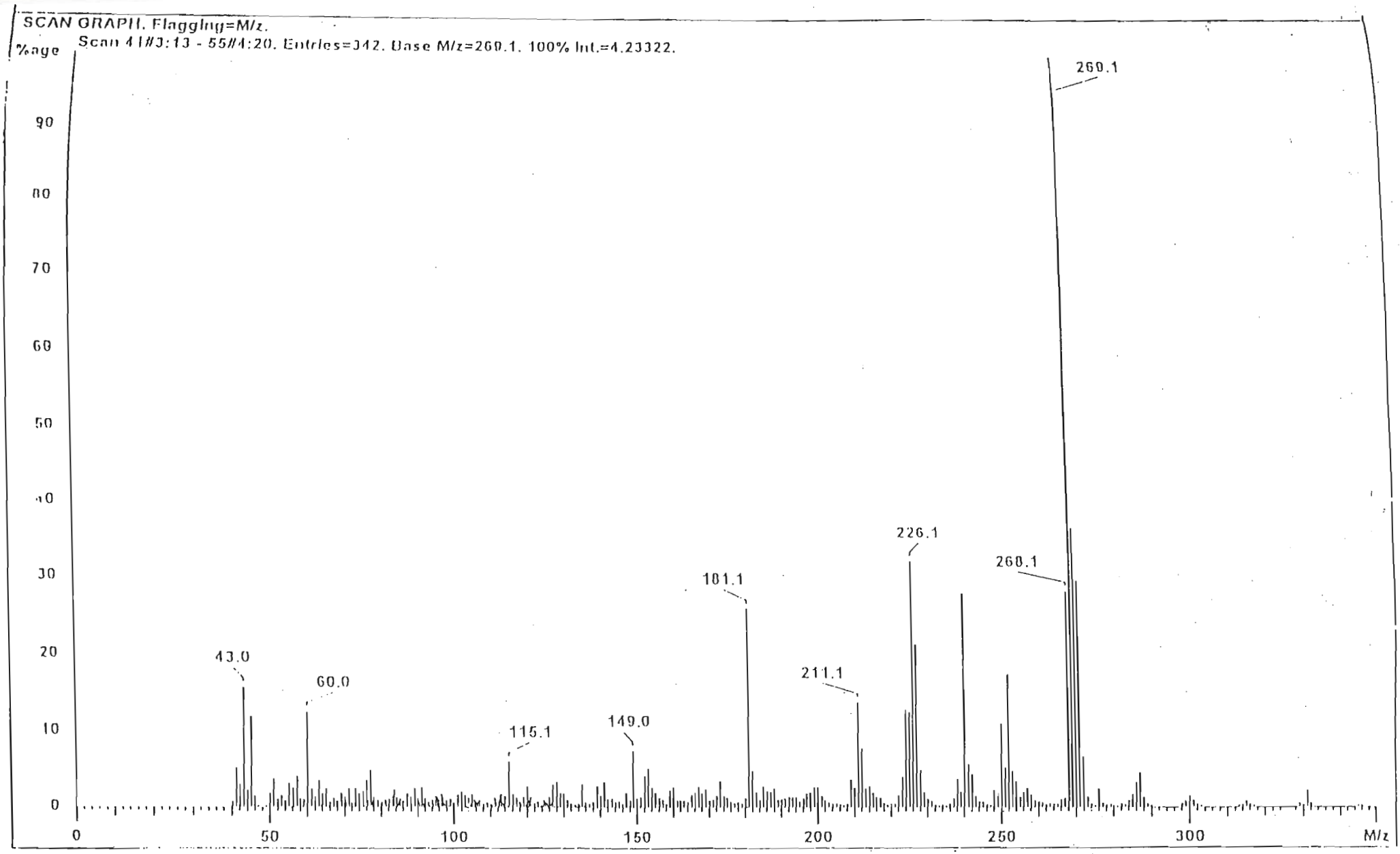
Spectrum [5-G]: NOESY spectrum of crinamine(V) in CD_3OD



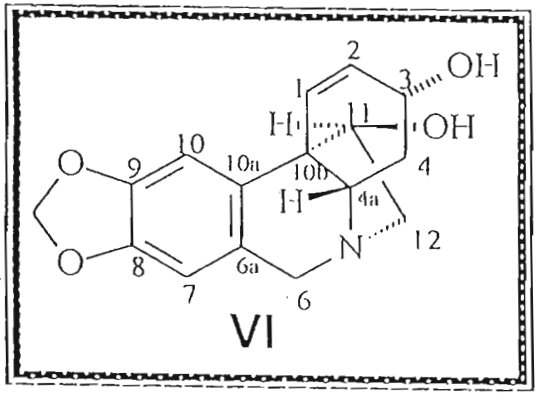
Spectrum [5-H]: Infra red spectrum of crinamine(**V**)



Spectrum [5-I]: UV spectrum of crinamine(V) in CD_3OD

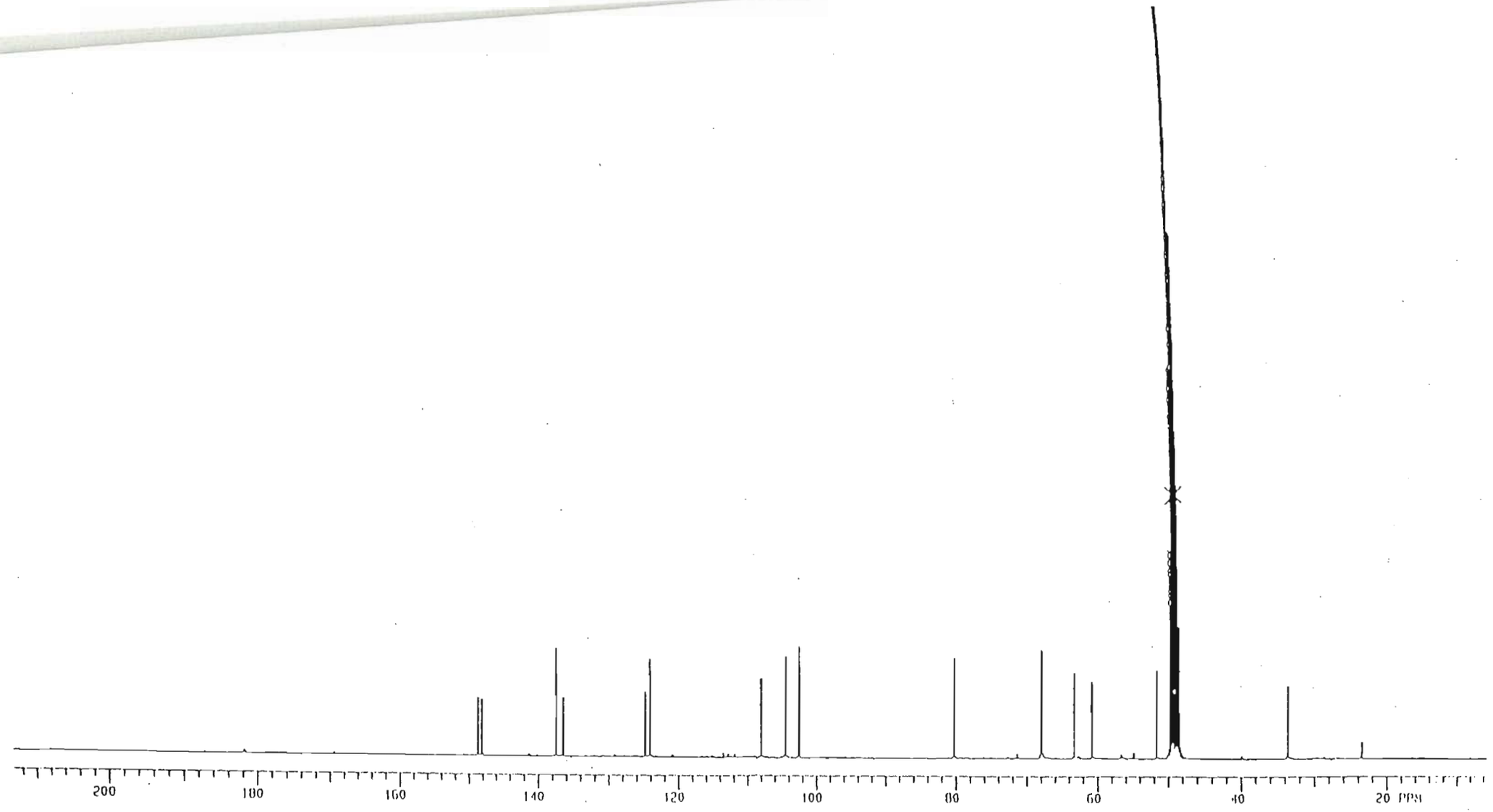


Spectrum [5-J]: Mass spectrum of crinamine(V)

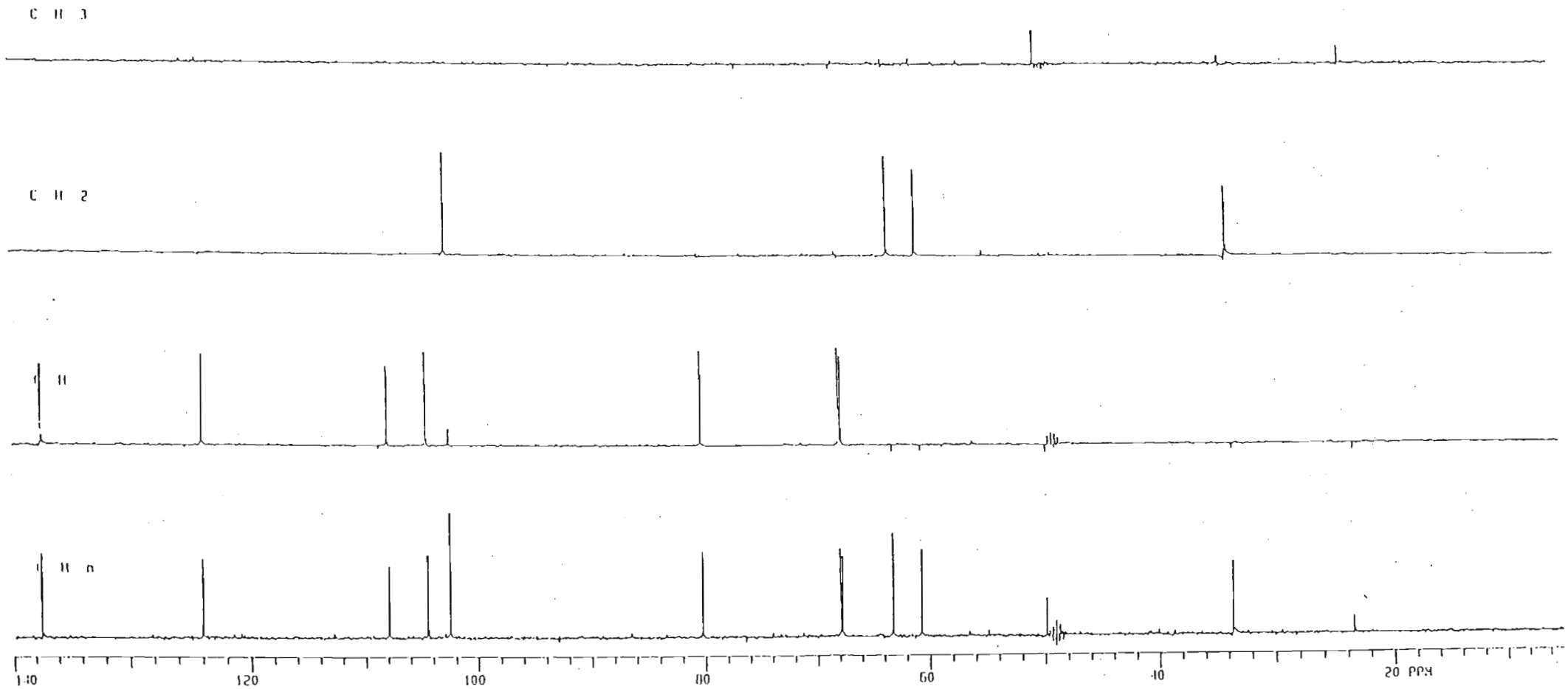


VI

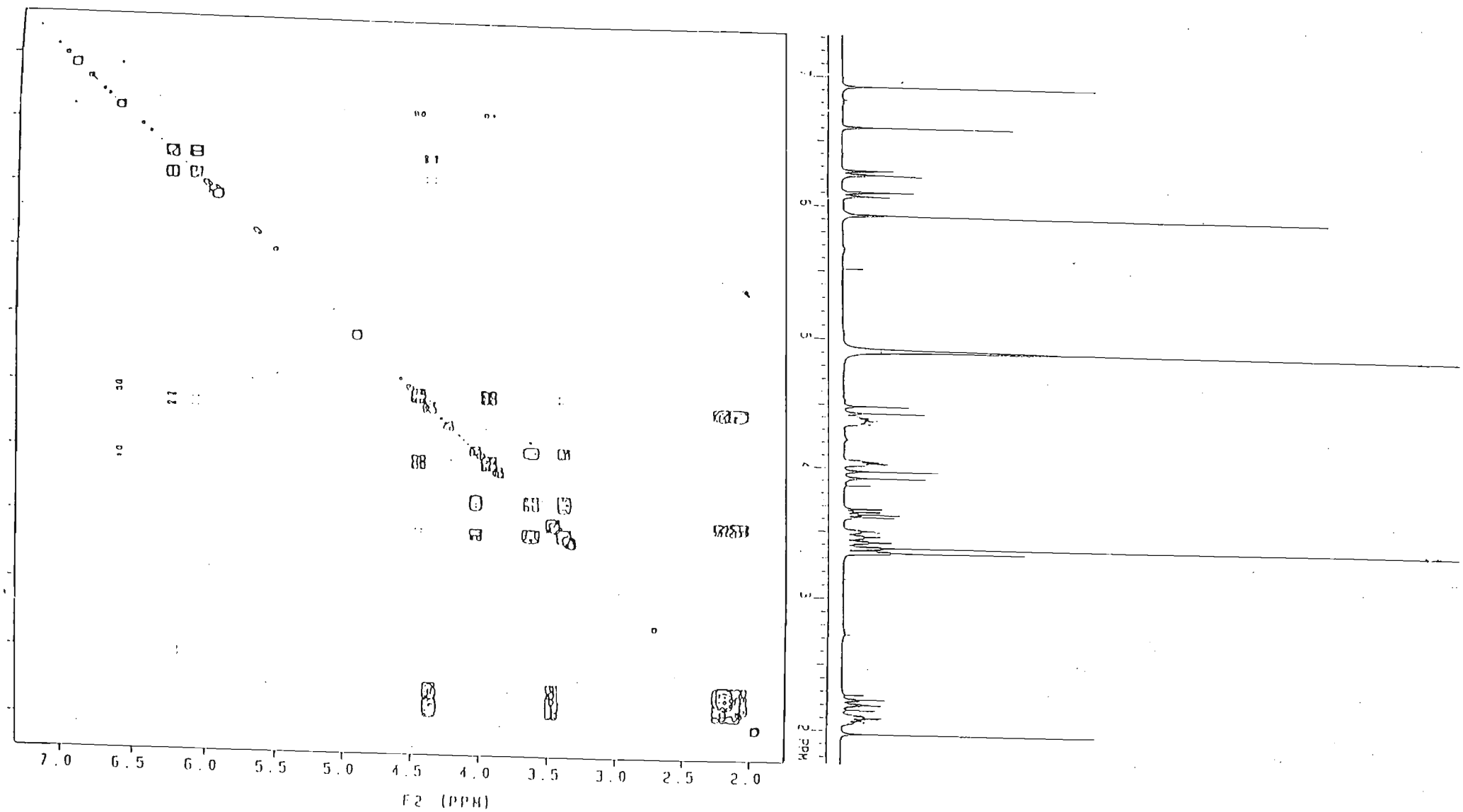
Spectrum [6-A]: ^{1}H NMR spectrum of hamayne(**VI**) in CD_3OD



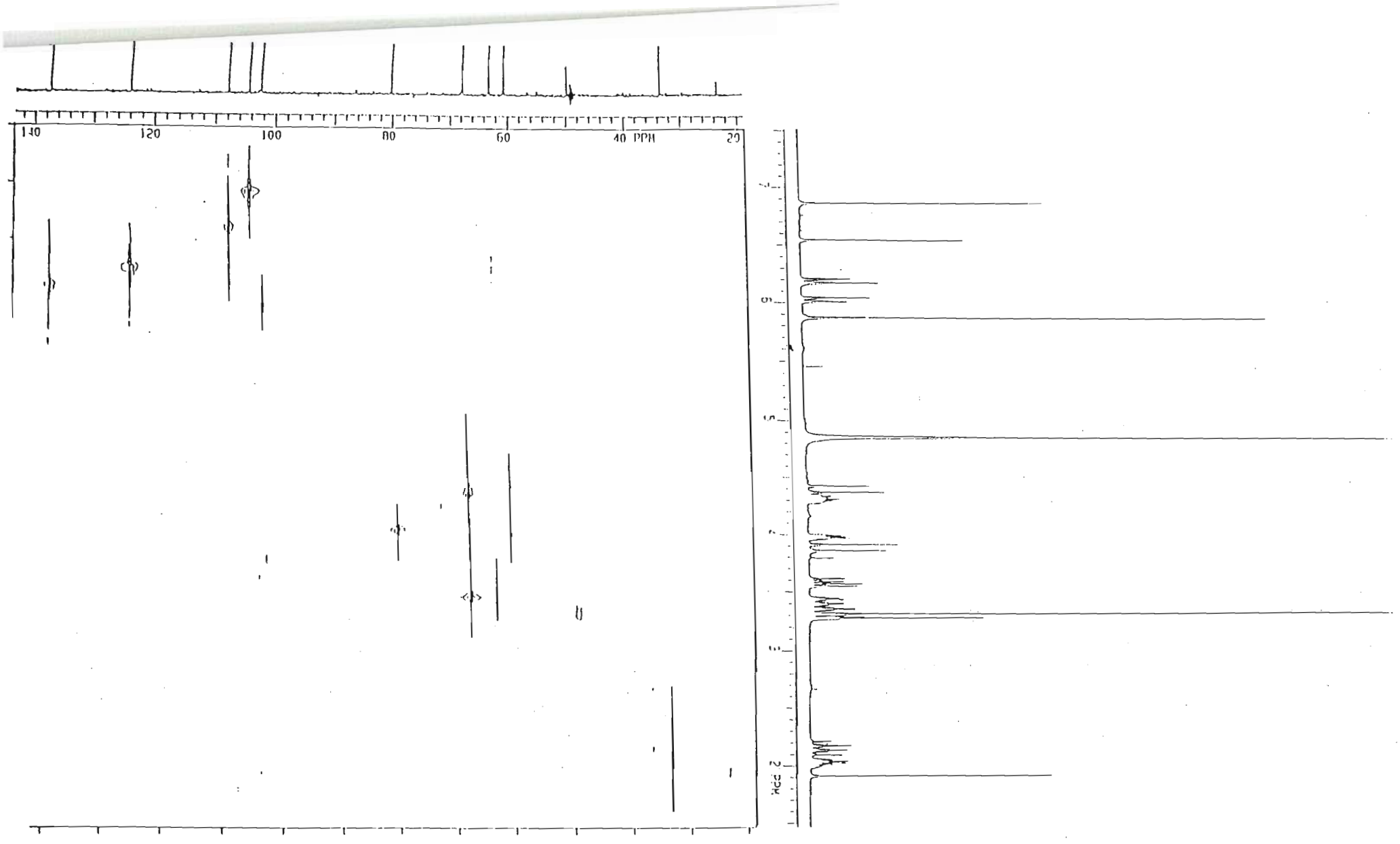
Spectrum [6-B]: ^{13}C NMR spectrum of hamayne(**VI**) in CD_3OD



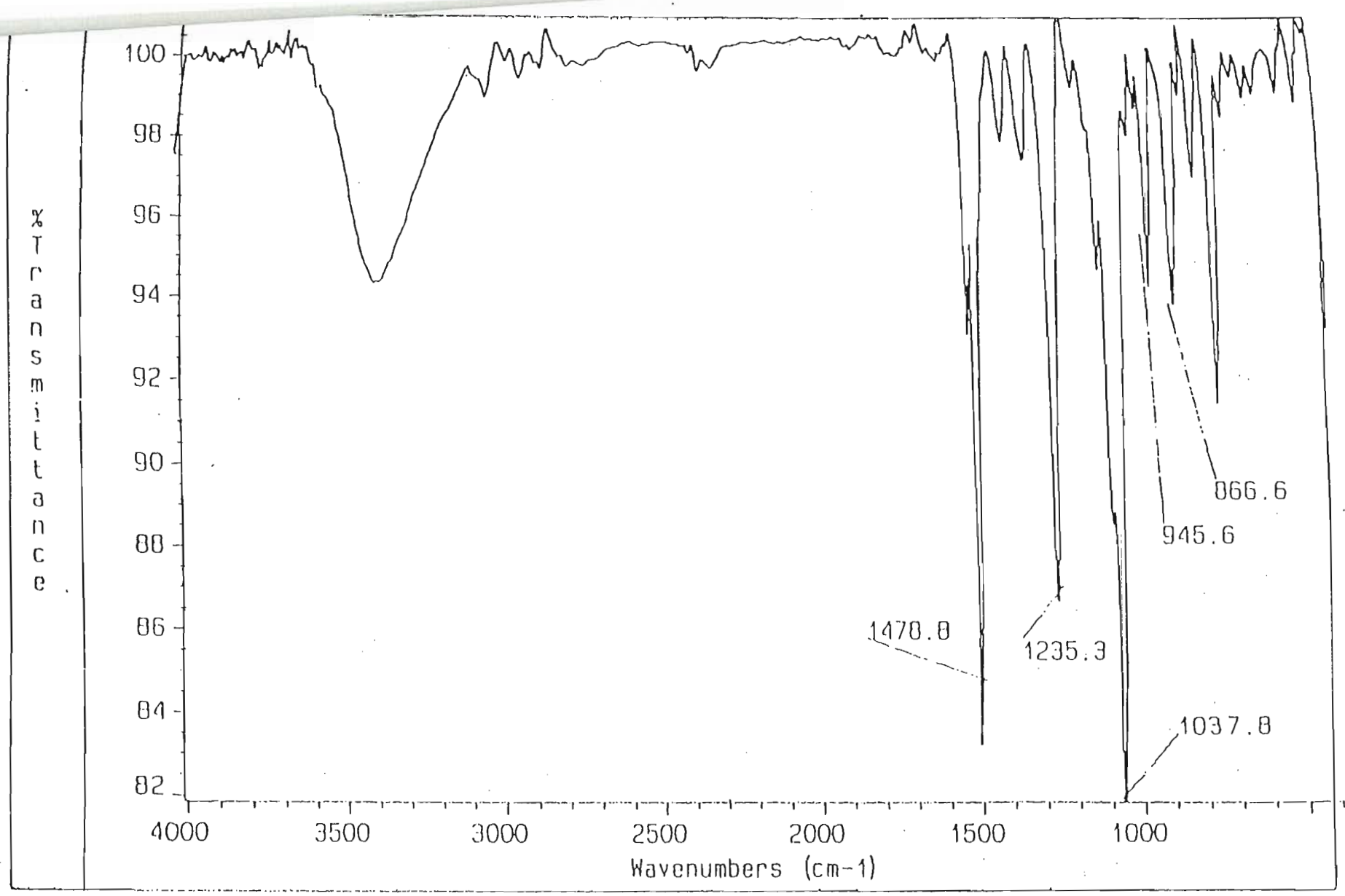
Spectrum [6-C]: ADEPT spectrum of hamayne(**VI**) in CD_3OD



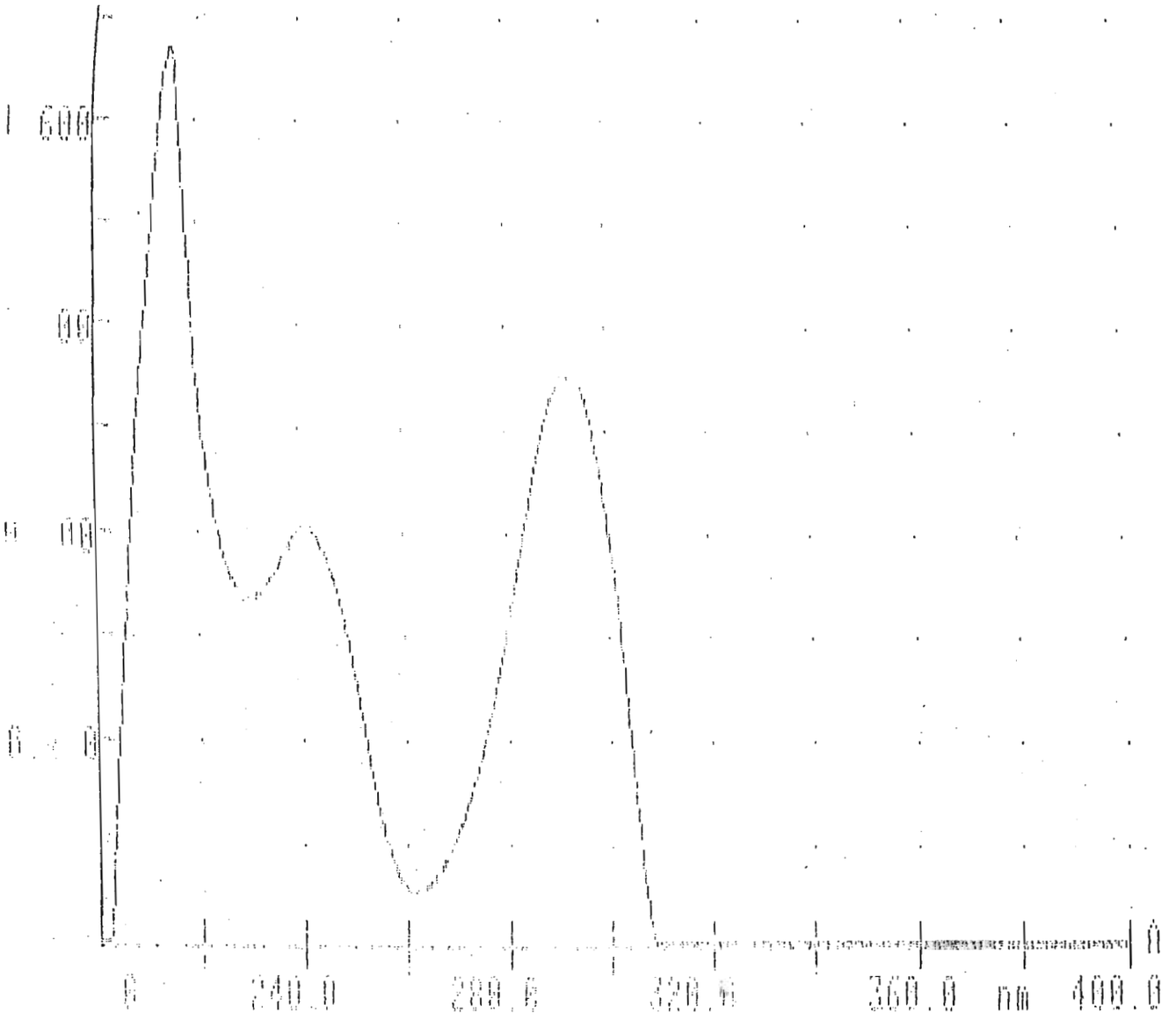
Spectrum [6-D]: COSY spectrum of hamayne(**VI**) in CD_3OD



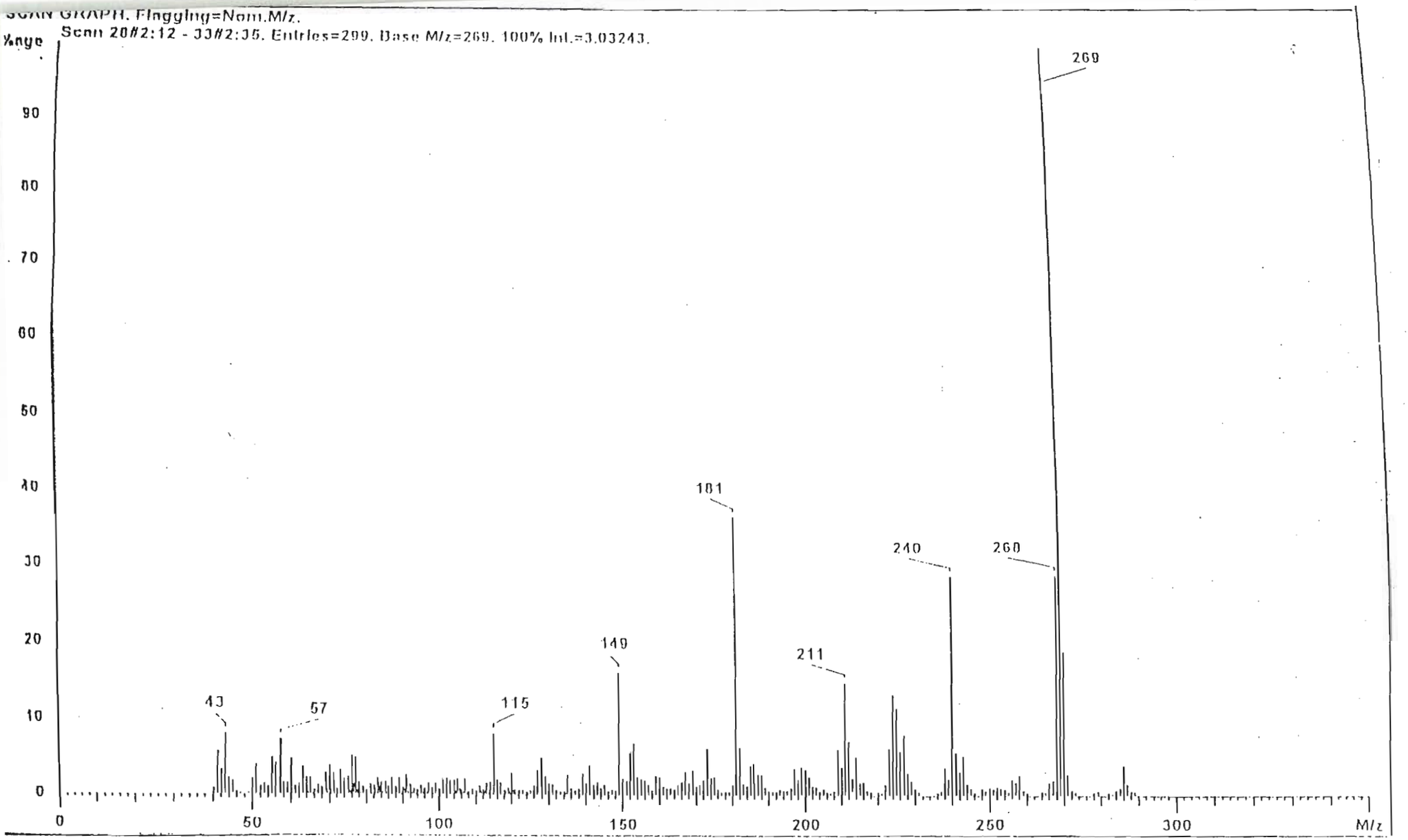
Spectrum [6-E]: HETCOR spectrum of hamayne(**VI**) in CD_3OD



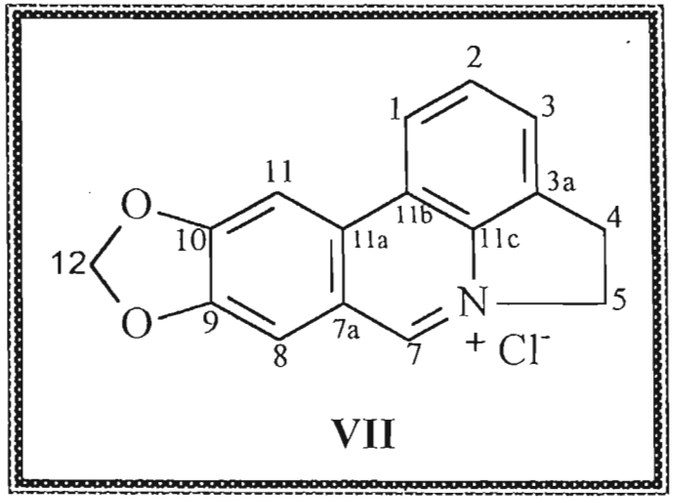
Spectrum [6-F]: Infra red spectrum of hamayne(VI)



Spectrum [6-G]: UV spectrum of hamayne(**VI**) in CD_3OD

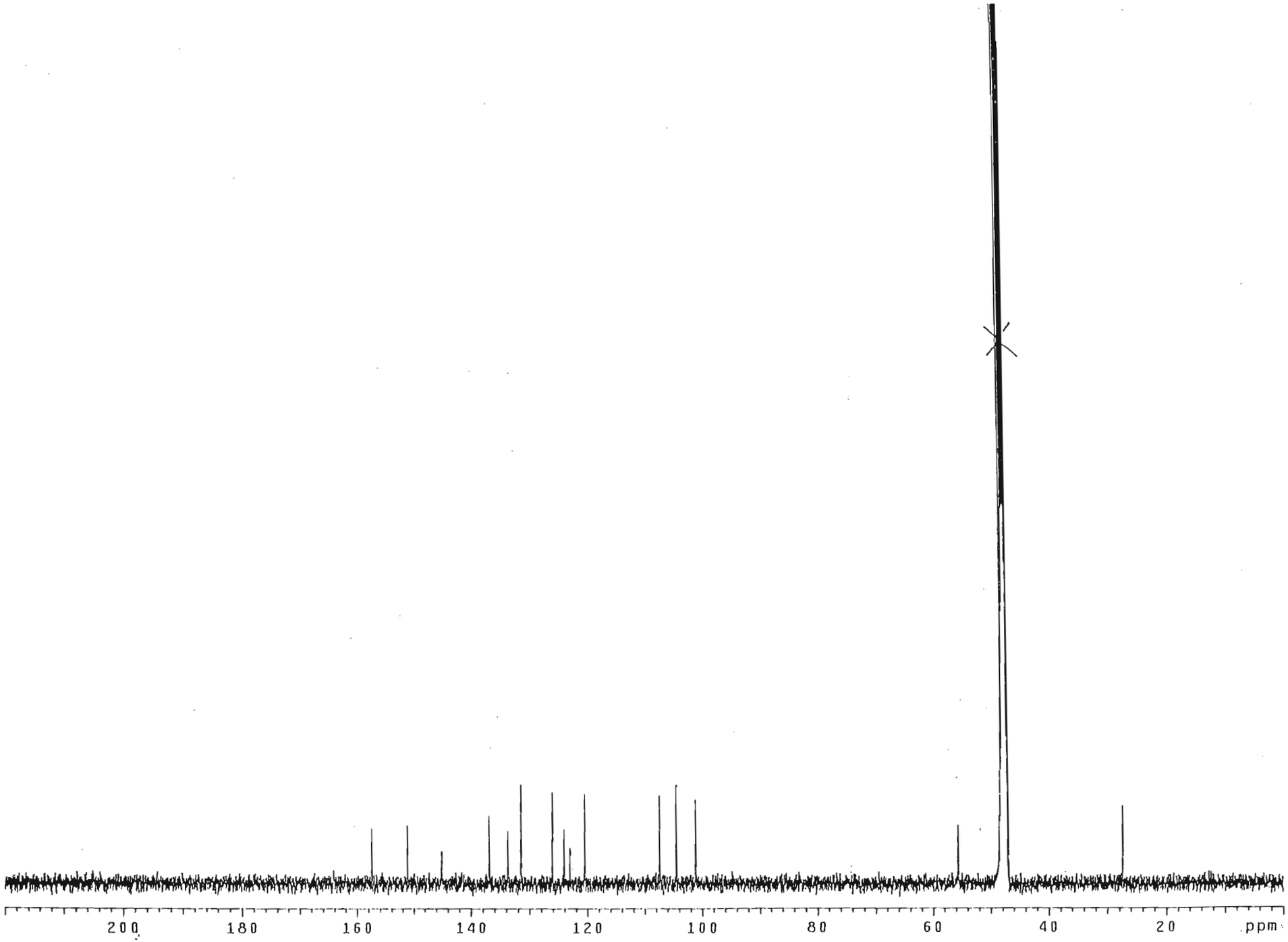


Spectrum [6-H]: Mass spectrum of hamayne(**VI**)

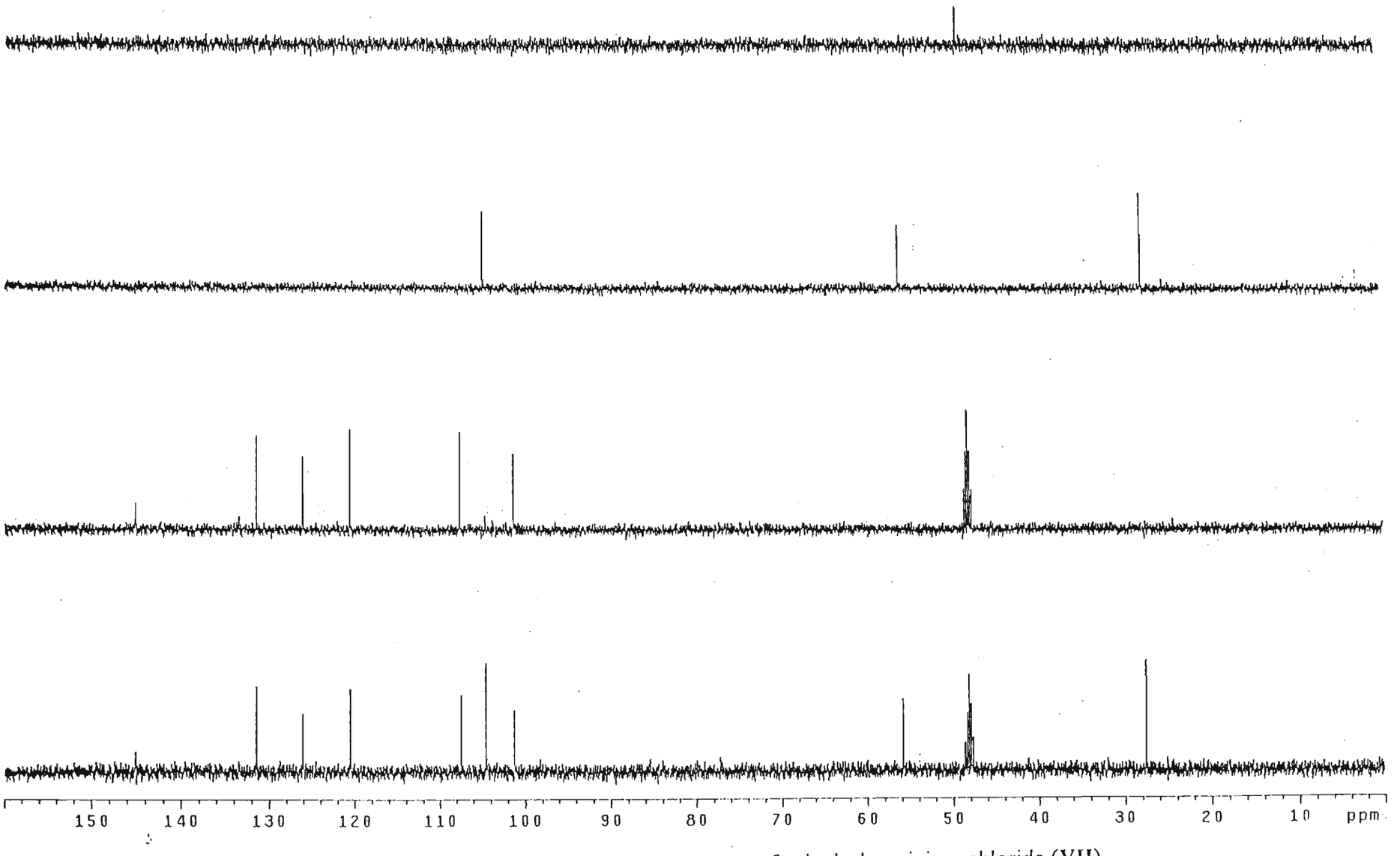


VII

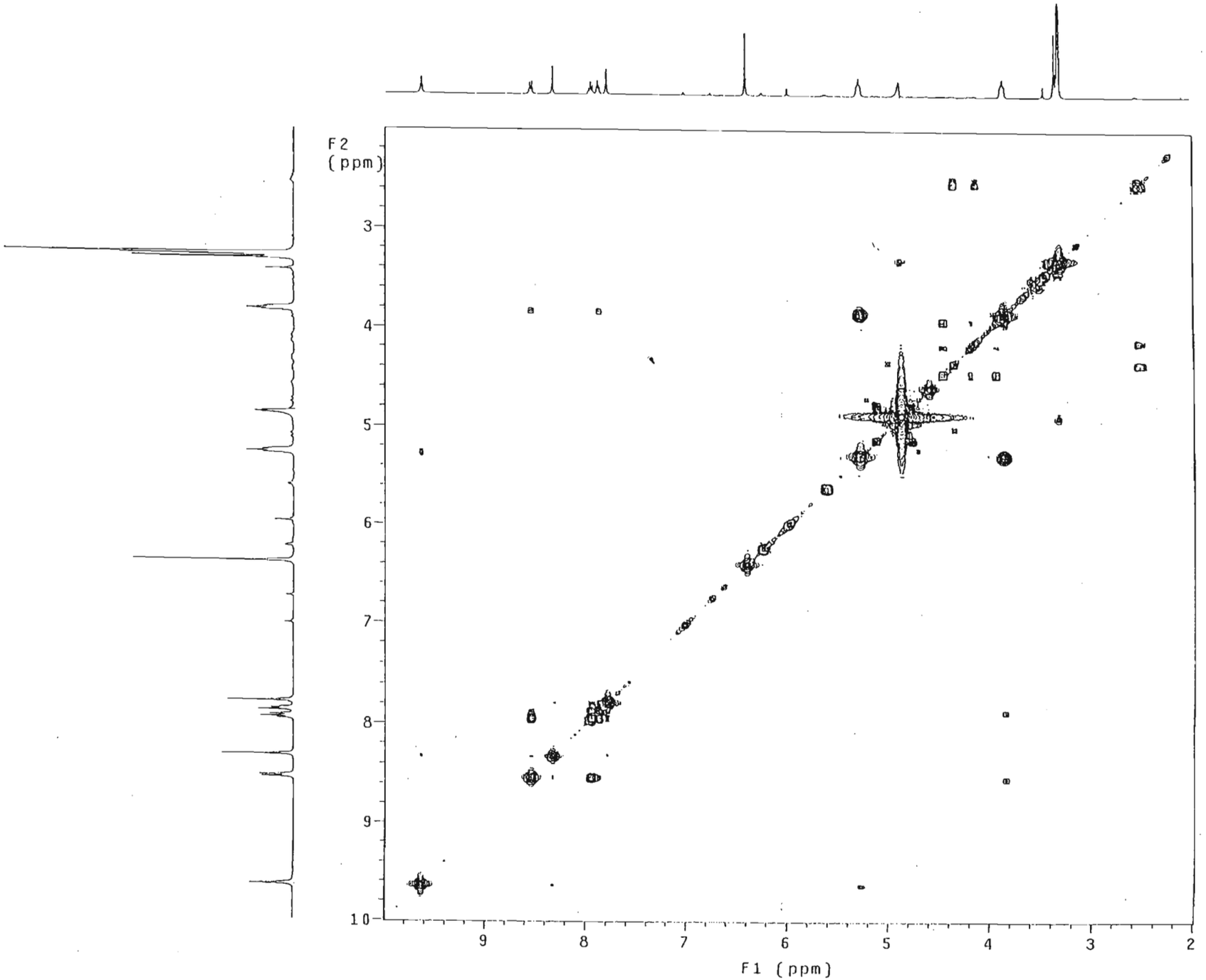
Spectrum [7-A]: ^{1}H NMR spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



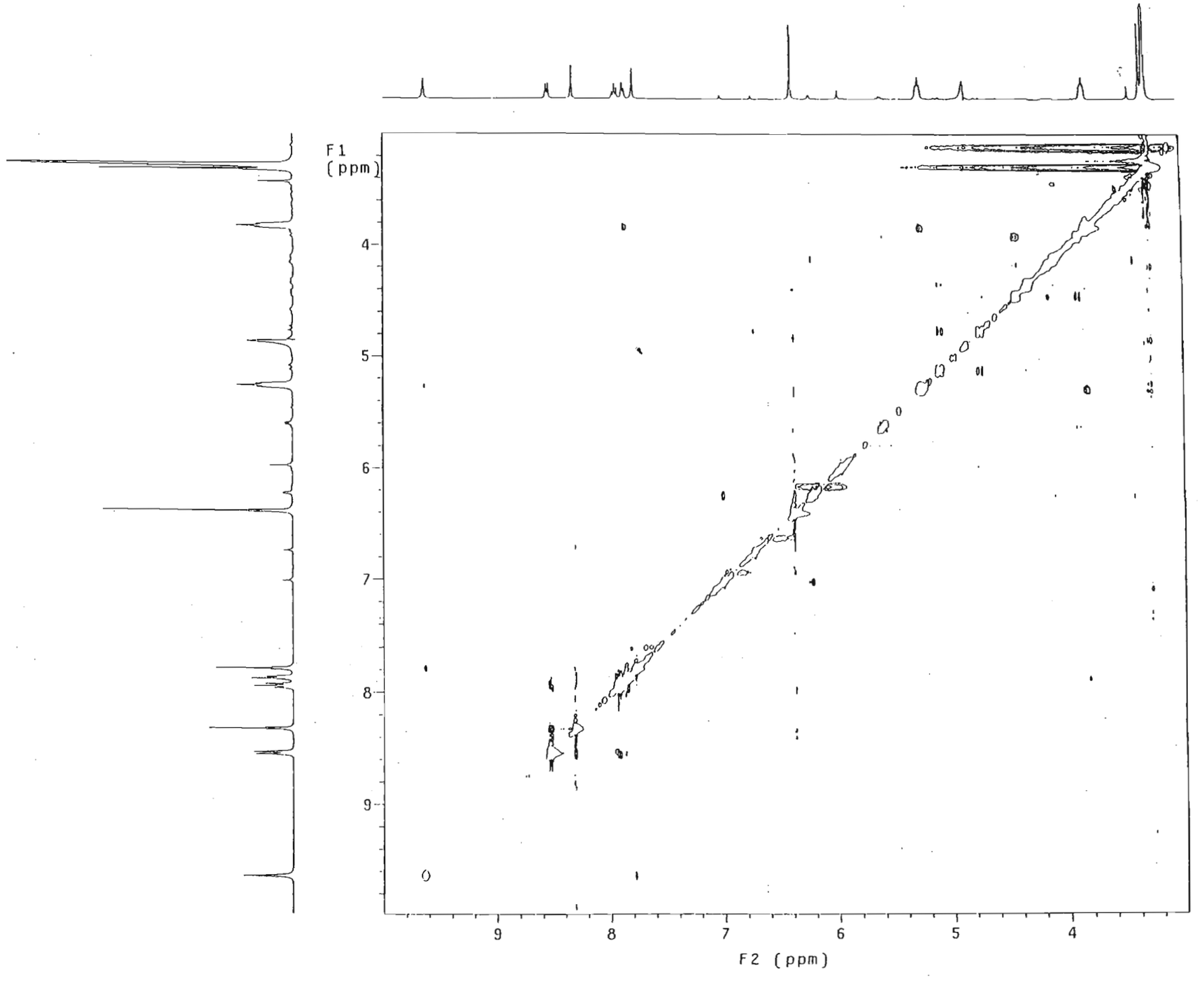
Spectrum [7-B]: ^{13}C NMR spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



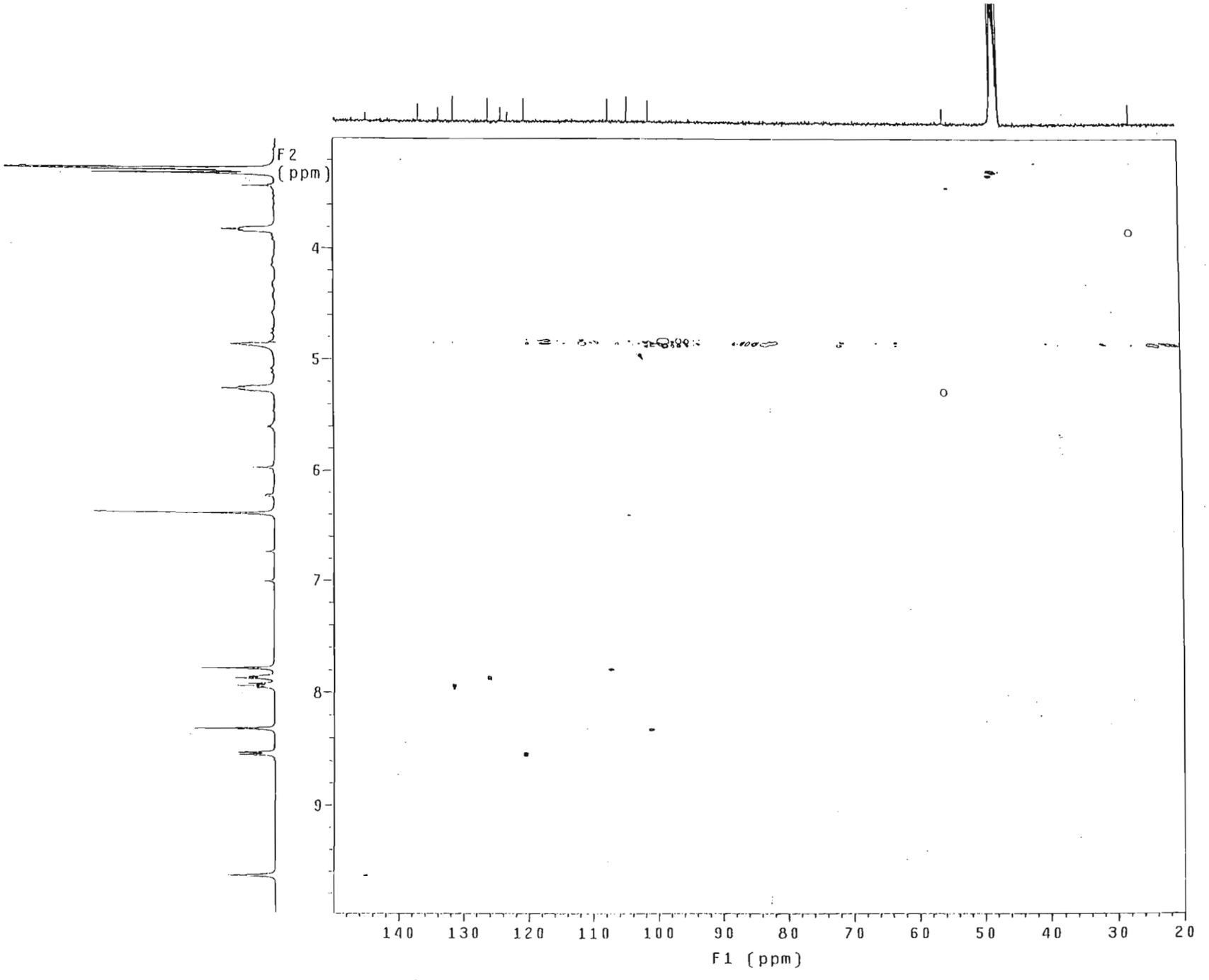
Spectrum [7-C]: ADEPT spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



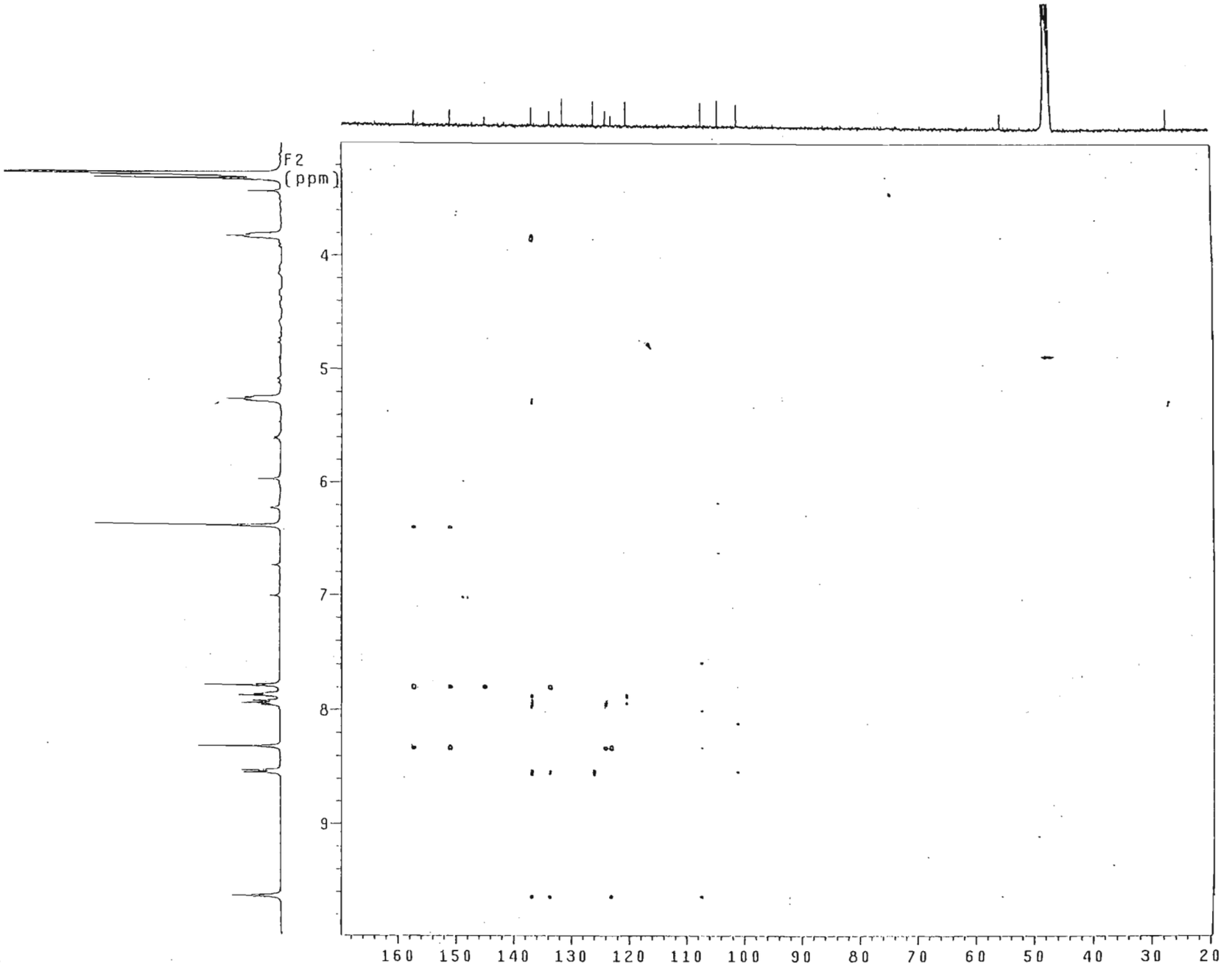
Spectrum [7-D]: COSY spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



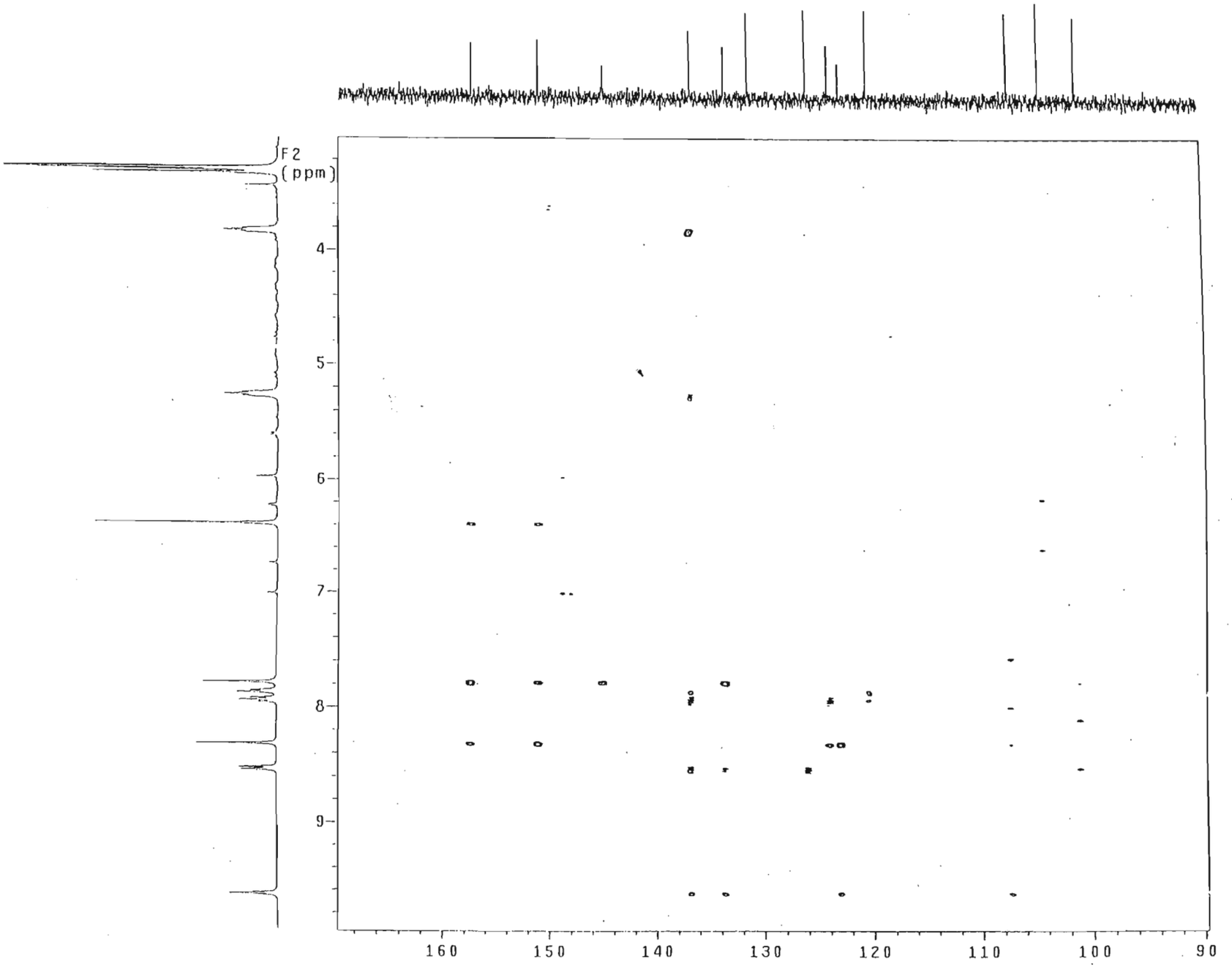
Spectrum [7-E]: NOESY spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



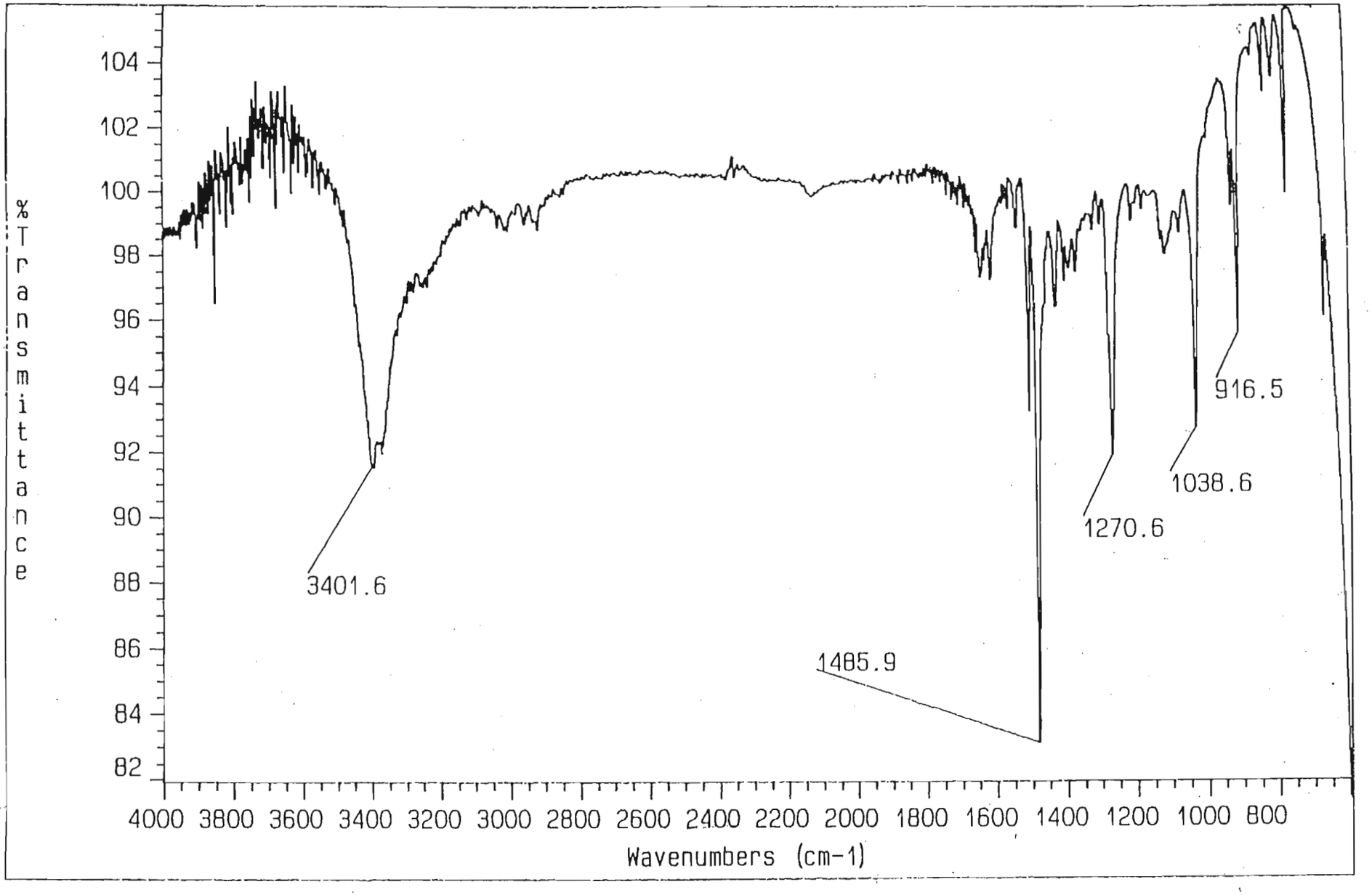
Spectrum [7-F]: HSQC spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



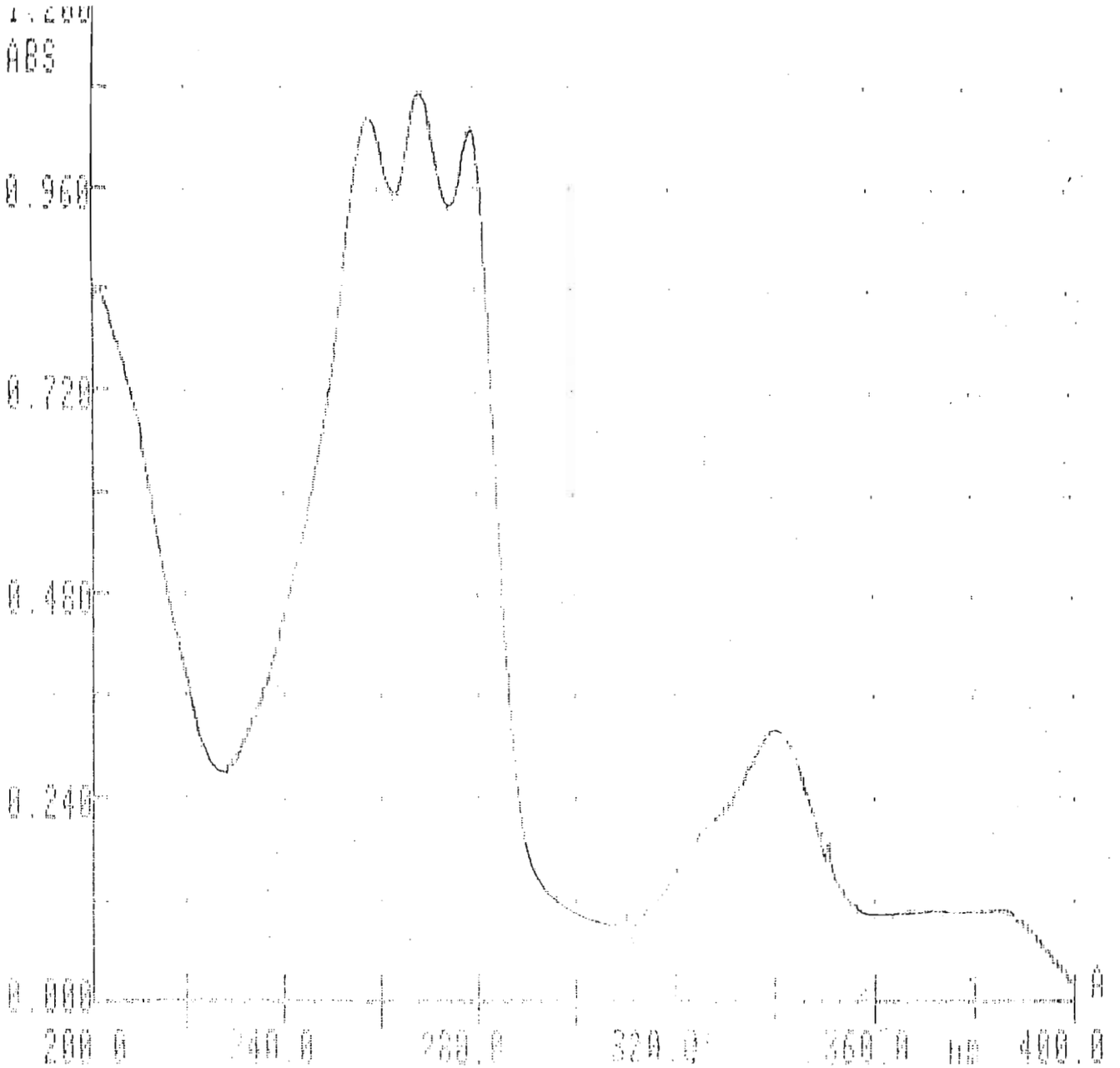
Spectrum [7-G]: HMBC spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



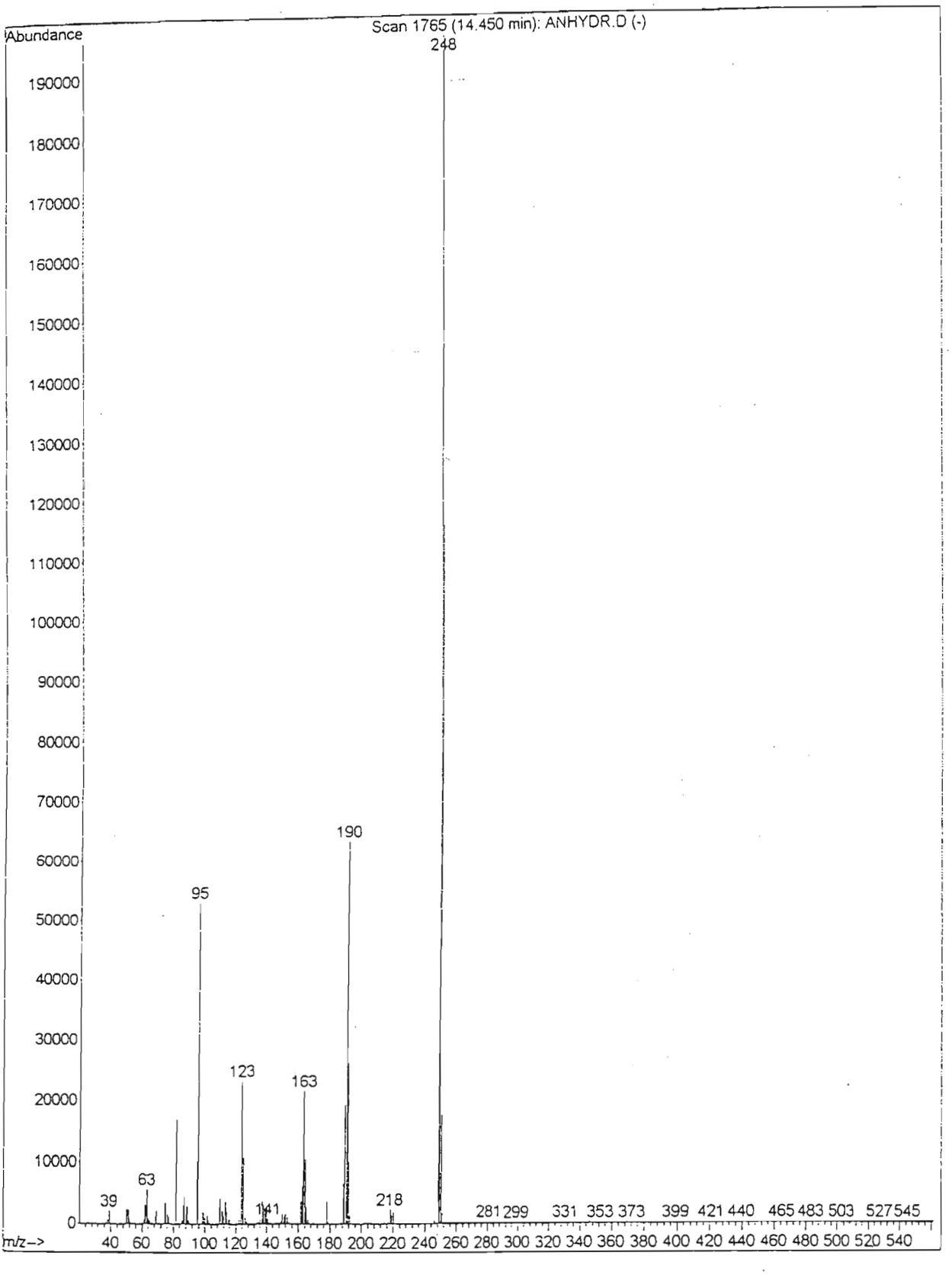
Spectrum [7-H]: Expanded HMBC spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD



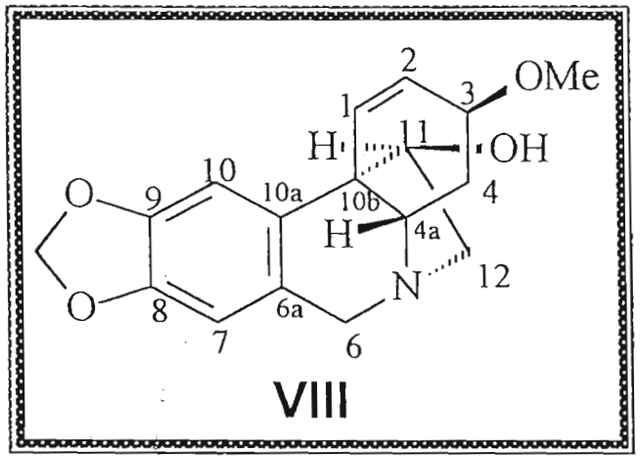
Spectrum [7-I]: Infra red spectrum of anhydolycorinium chloride (**VII**) in CD_3OD



Spectrum [7-J]: UV spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD

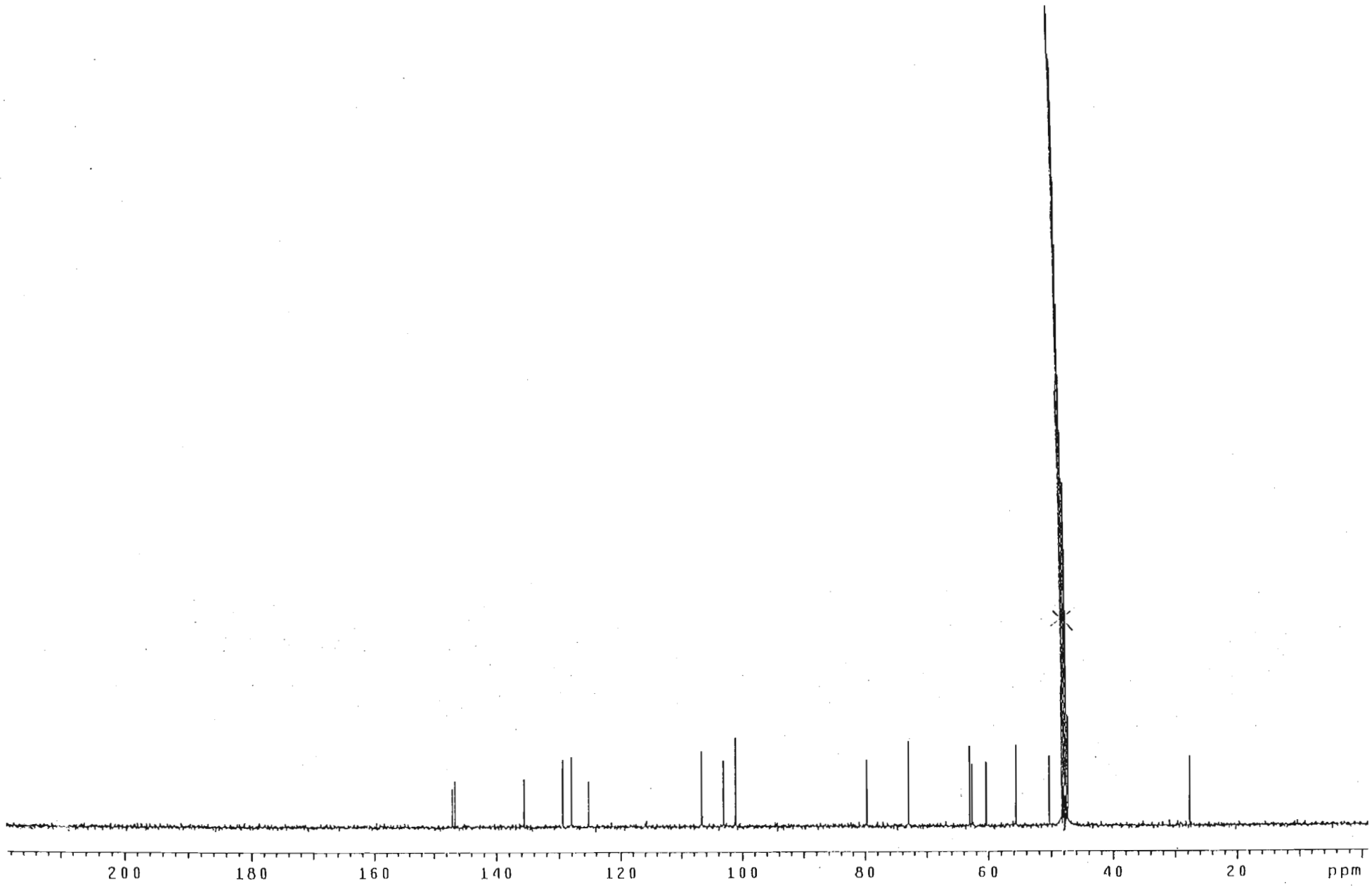


Spectrum [7-K]: Mass spectrum of anhydrolycorinium chloride (**VII**) in CD_3OD

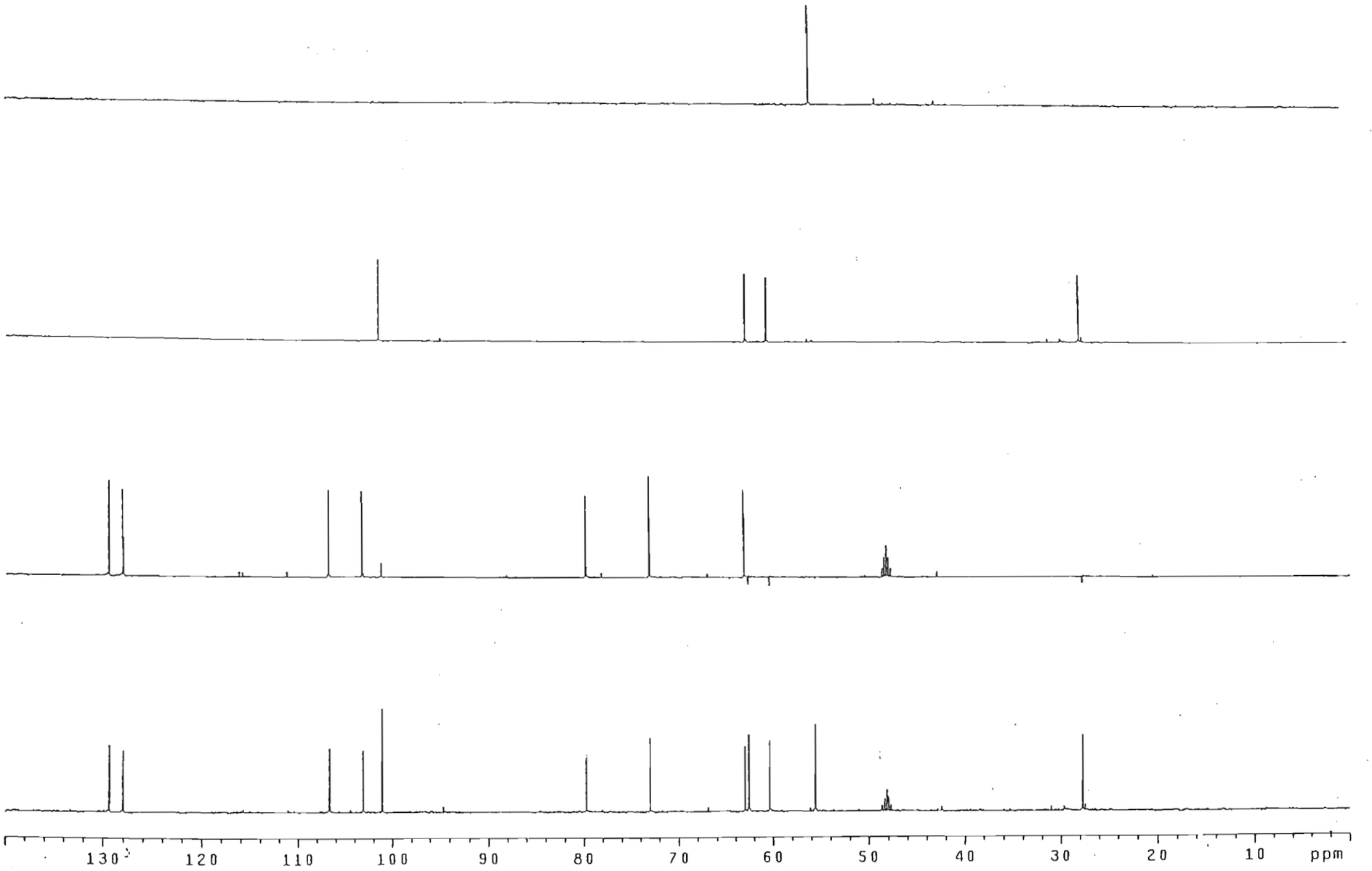


VIII

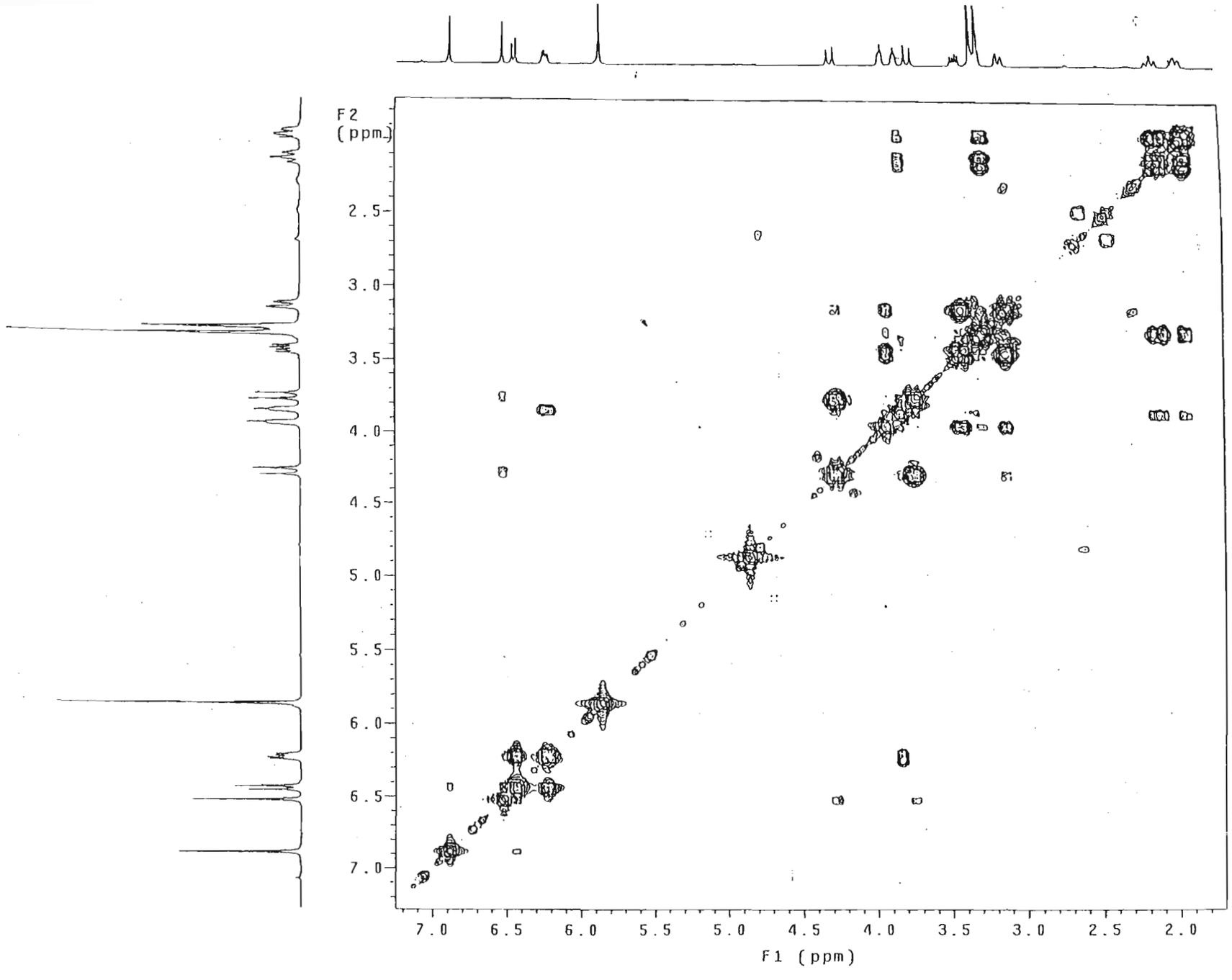
Spectrum [8-A]: ^{1}H NMR spectrum of haemanthamine (**VIII**) in CD_3OD



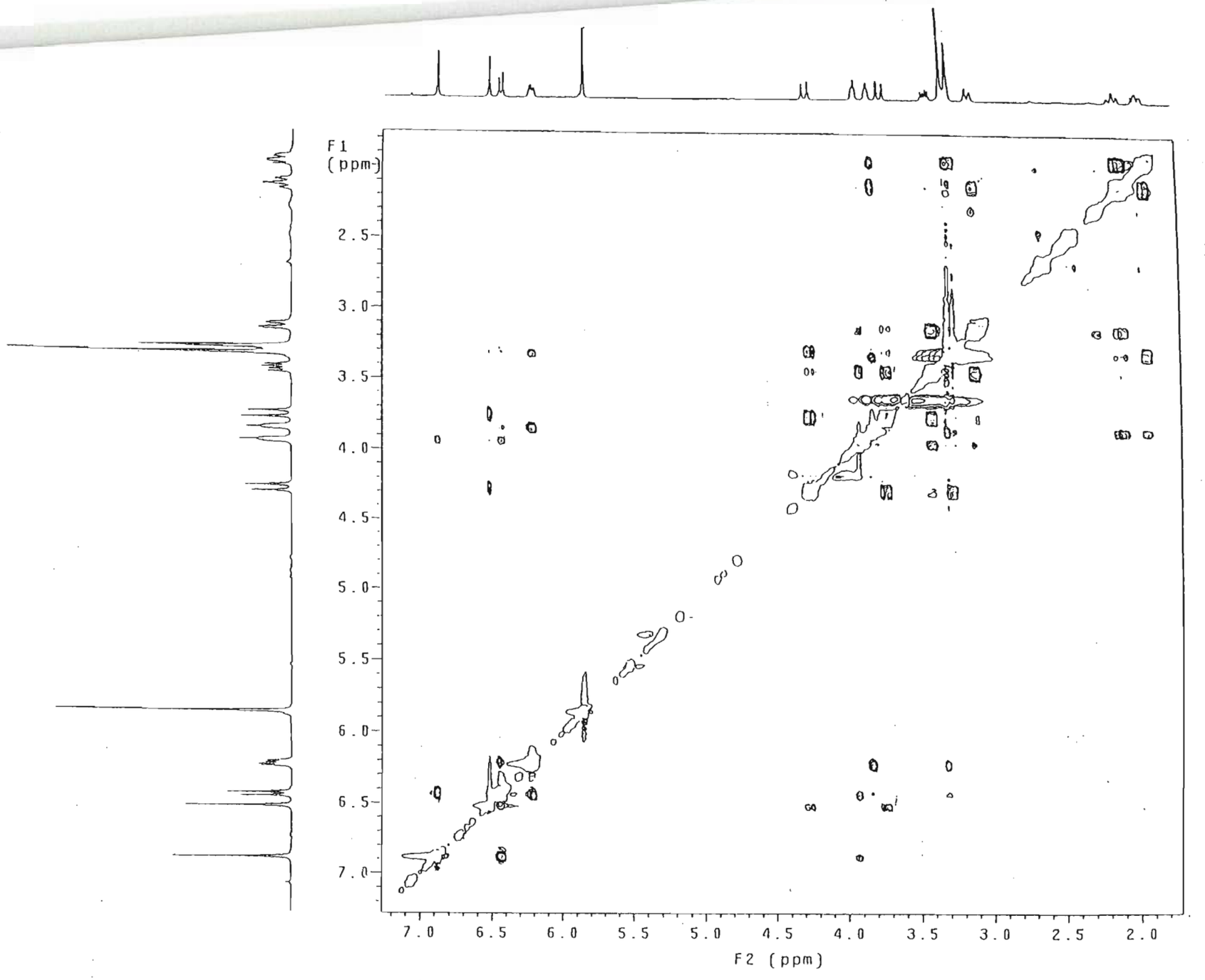
Spectrum [8-B]: ^{13}C NMR spectrum of haemanthamine (**VIII**) in CD_3OD



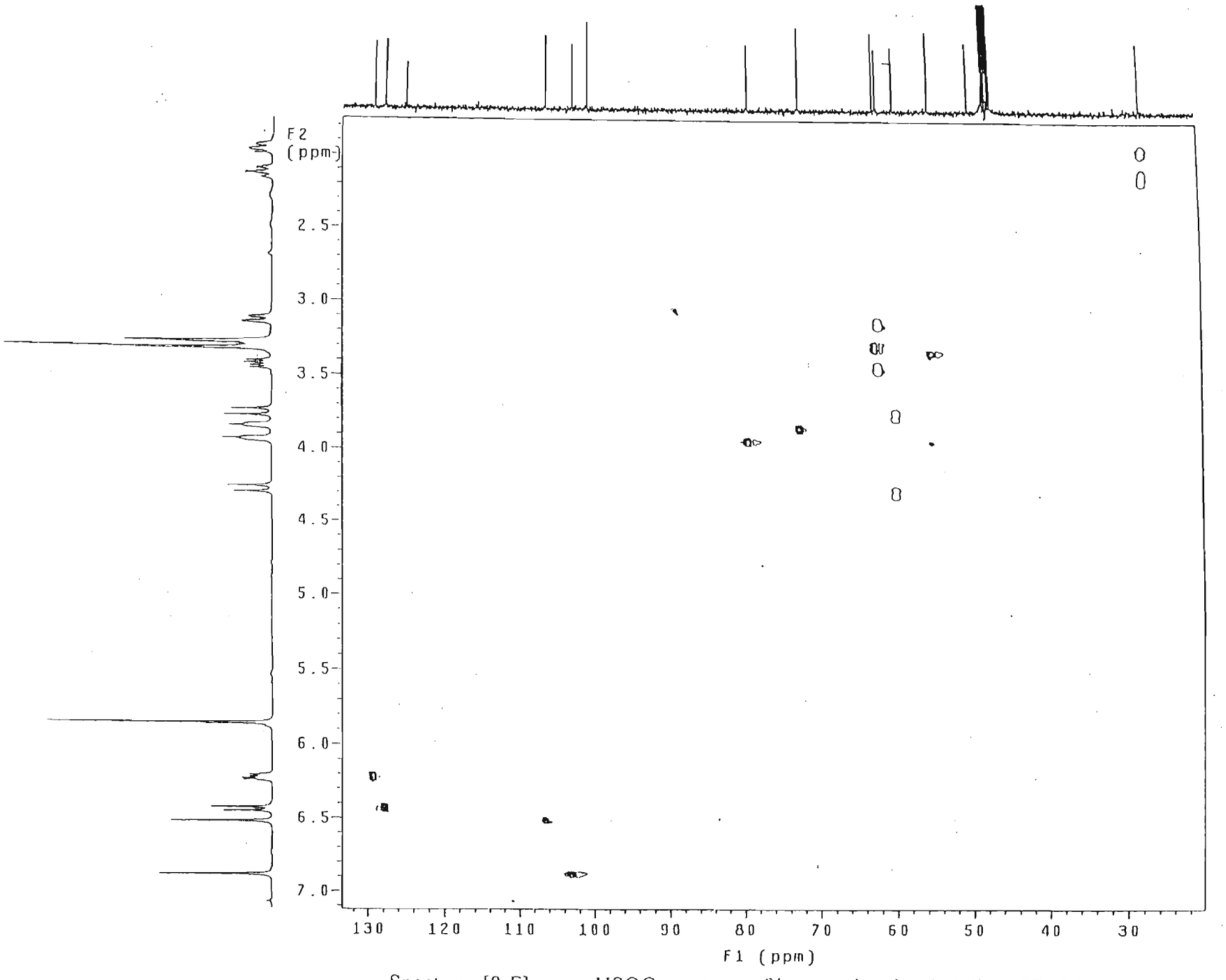
Spectrum [8-C]: ADEPT spectrum of haemanthamine (**VIII**) in CD_3OD



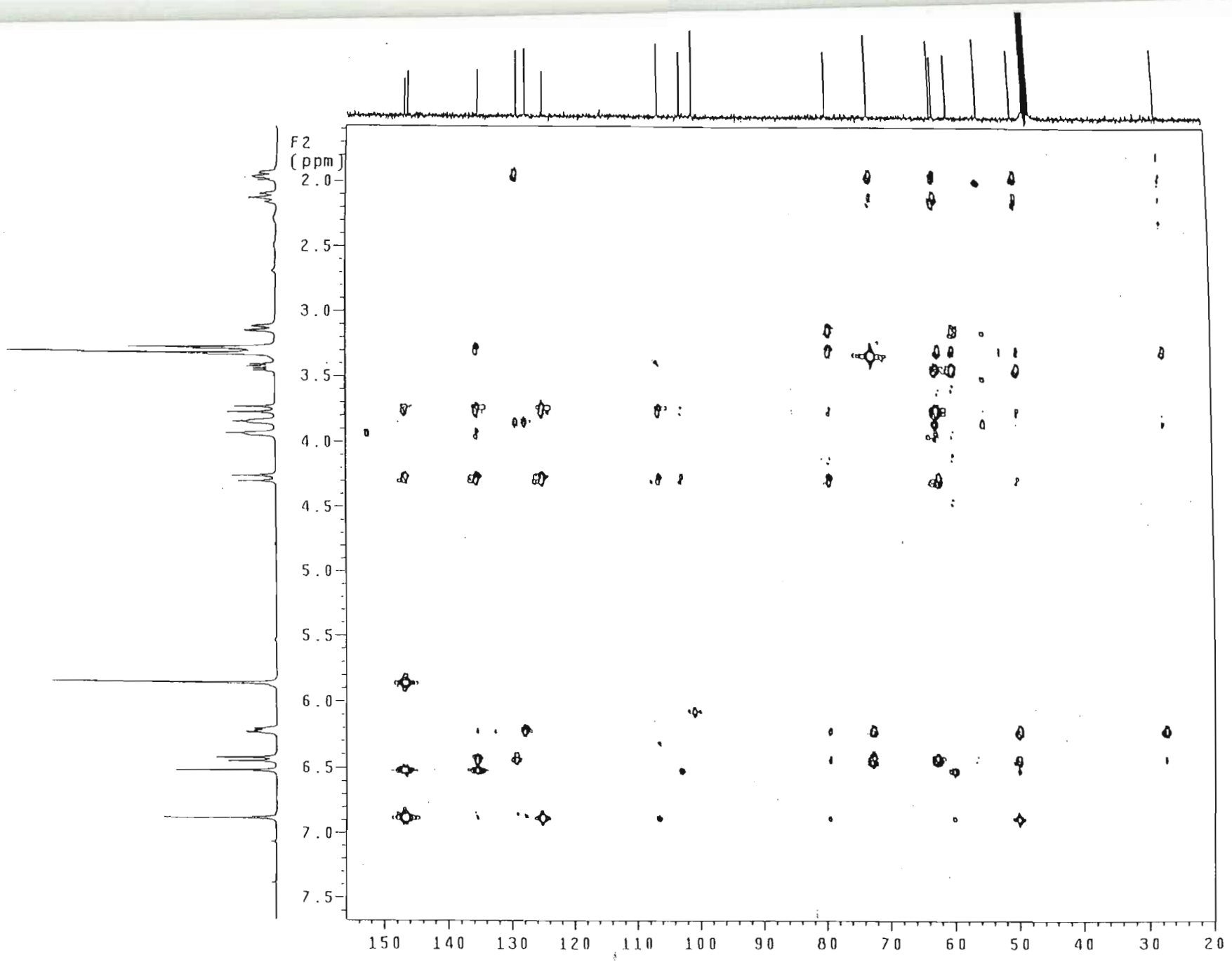
Spectrum [8-D]: COSY spectrum of haemanthamine (**VIII**) in CD_3OD



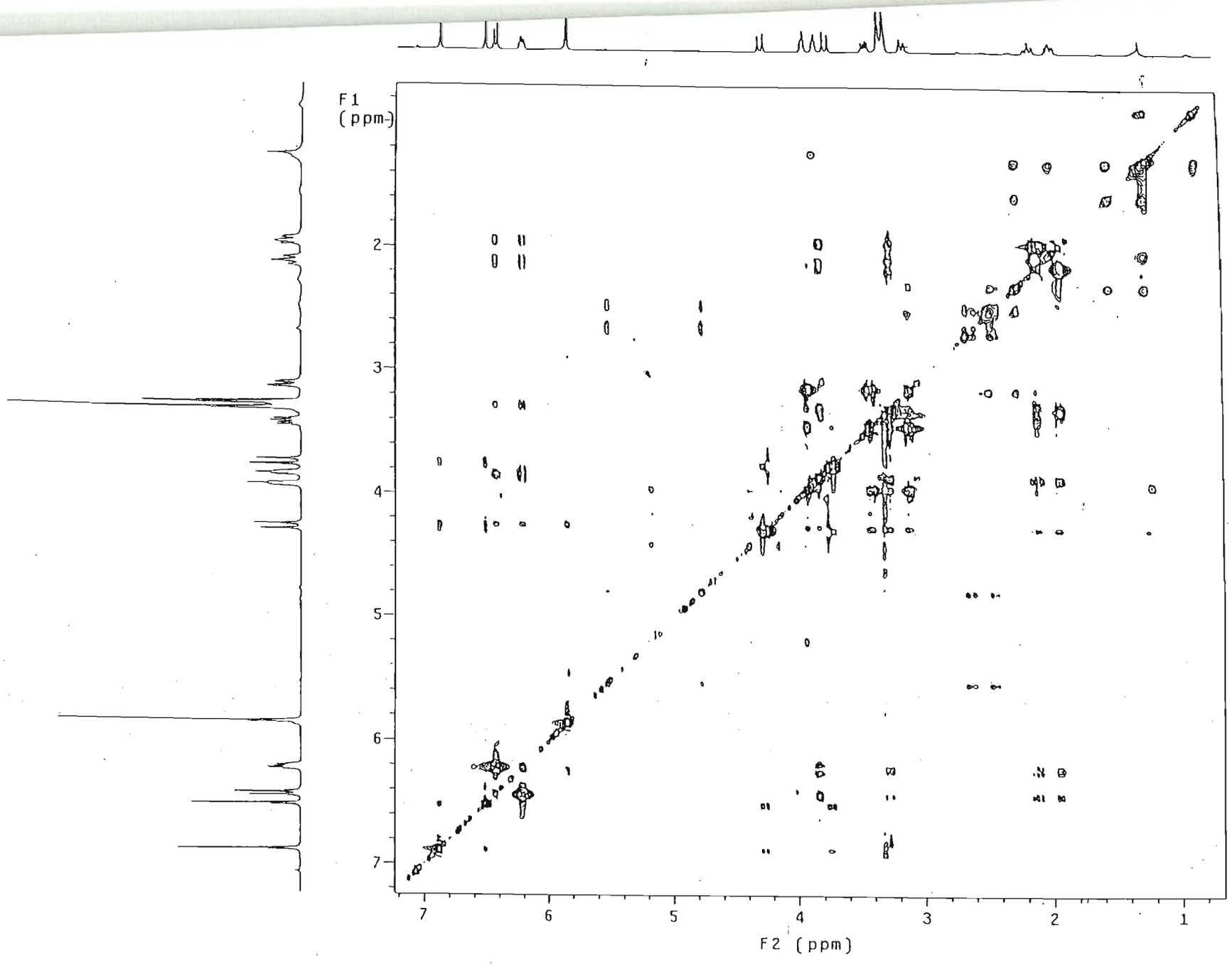
Spectrum [8-E]: NOESY spectrum of haemanthamine (**VIII**) in CD_3OD



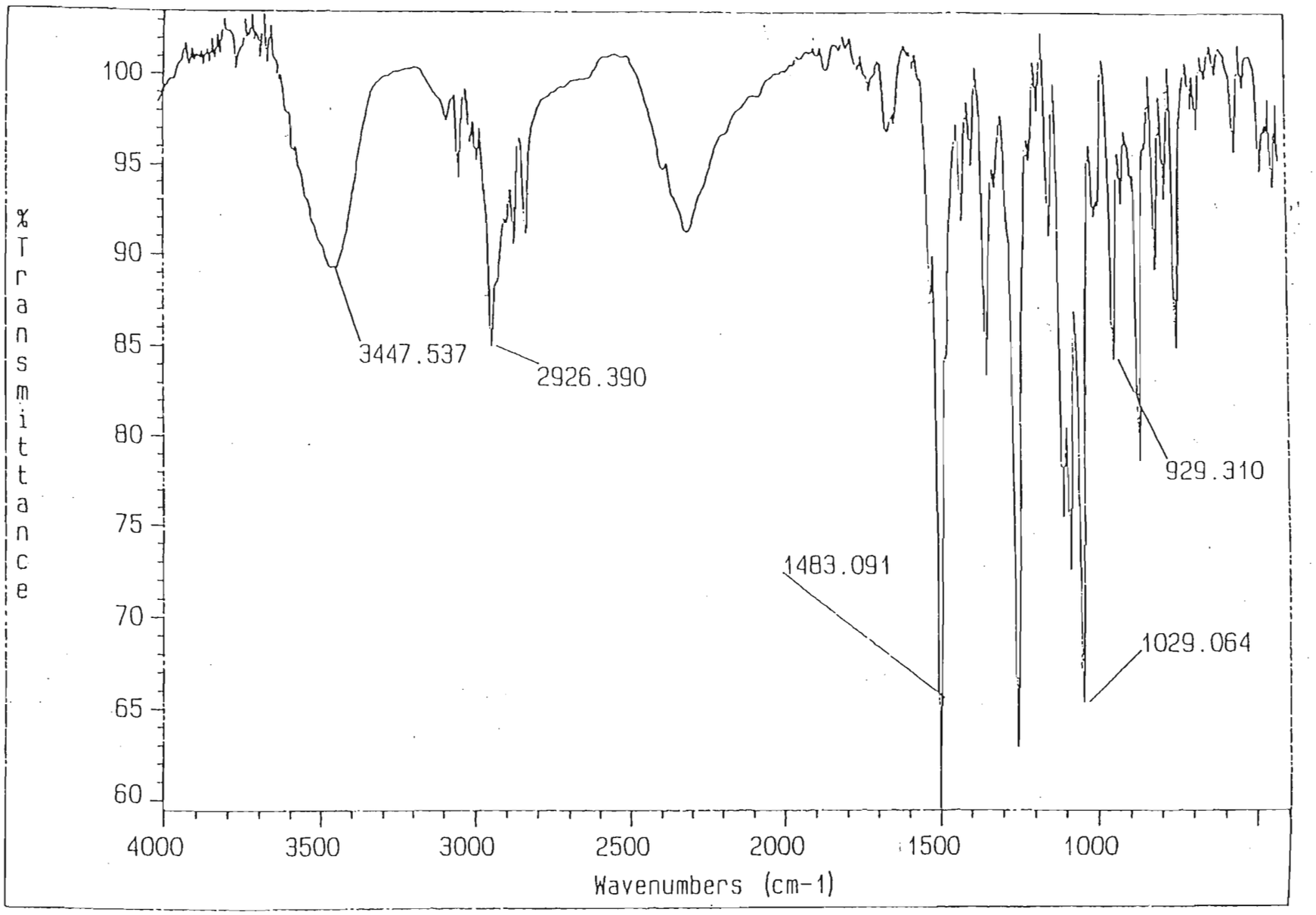
Spectrum [8-F]: HSQC spectrum of haemanthamine (**VIII**) in CD_3OD



Spectrum [8-G]: HMBC spectrum of haemanthamine (VIII) in CD_3OD



Spectrum [8-H]: TOCSY spectrum of haemanthamine (**VIII**) in CD_3OD

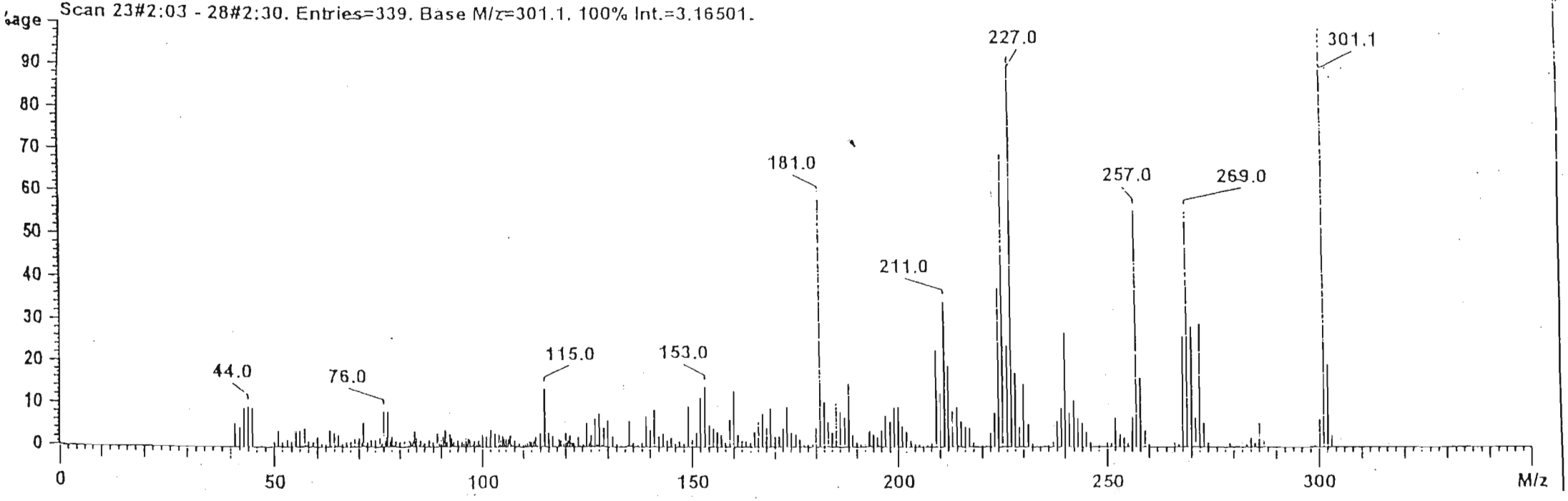


Spectrum [8-1]: Infra red spectrum of haemanthamine (**VIII**)

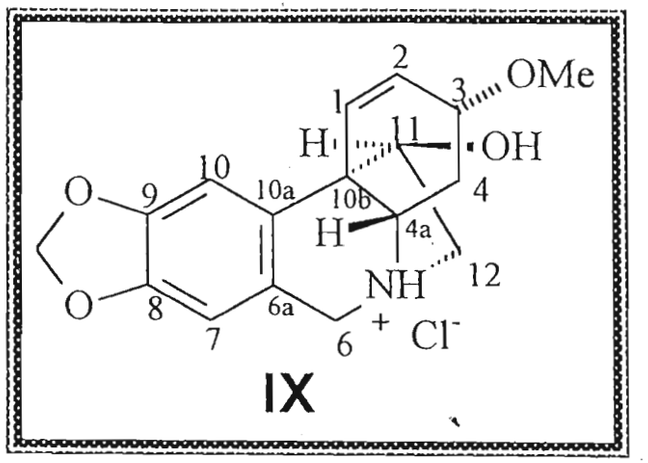
File Title : II-CC1A2-12/15
Operator : Dr Philip Boshoff/NMSC
Instrument : VG70-250SEQ-MS2

SCAN GRAPH. Flagging=M/z.

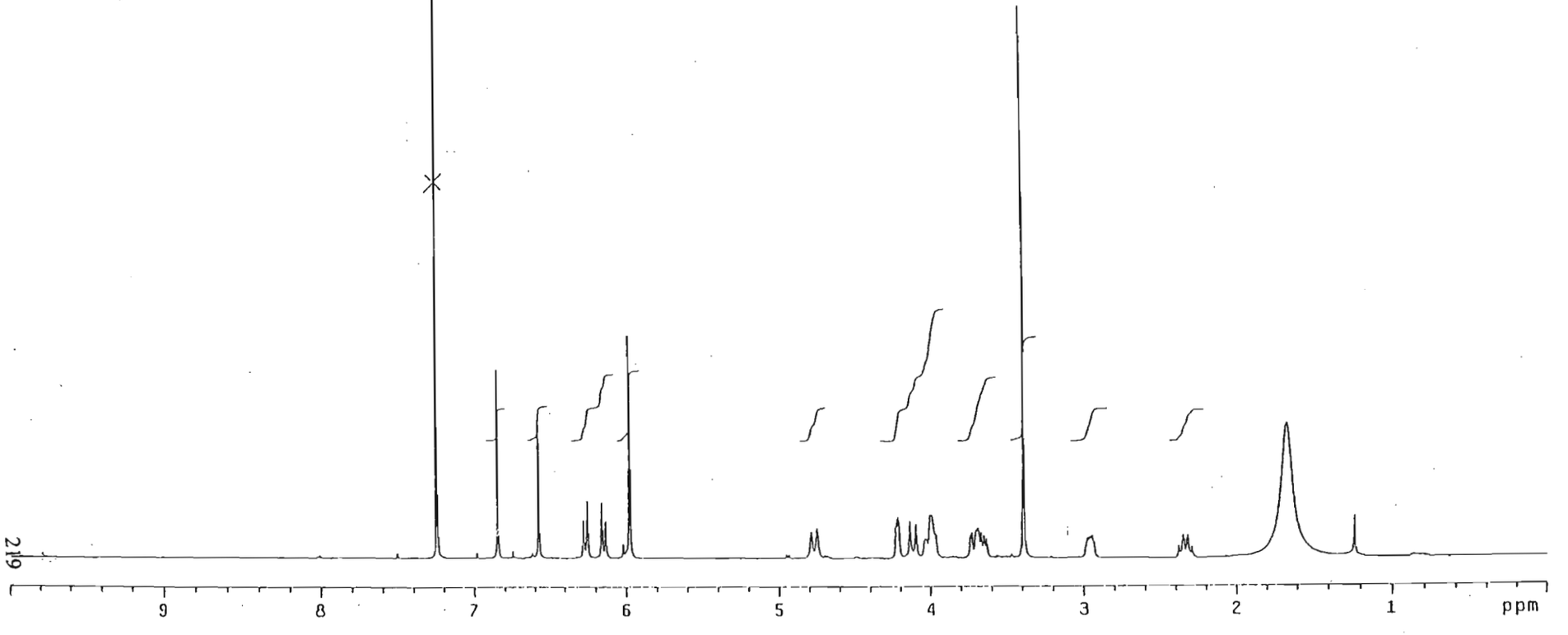
Scan 23#2:03 - 28#2:30. Entries=339. Base M/z=301.1. 100% Int.=3.16501.



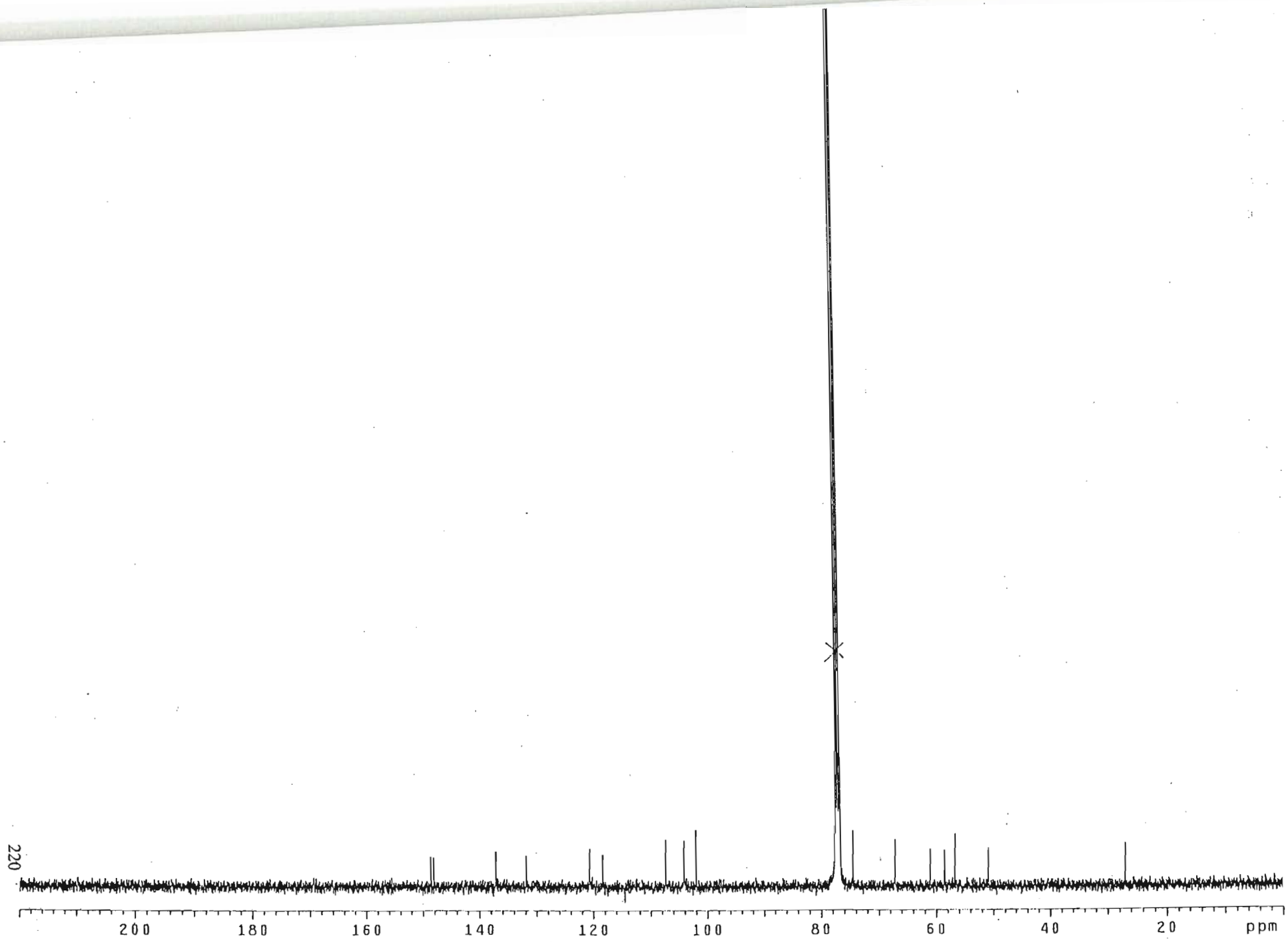
Spectrum [8-J]: Mass spectrum of haemanthamine (**VIII**)



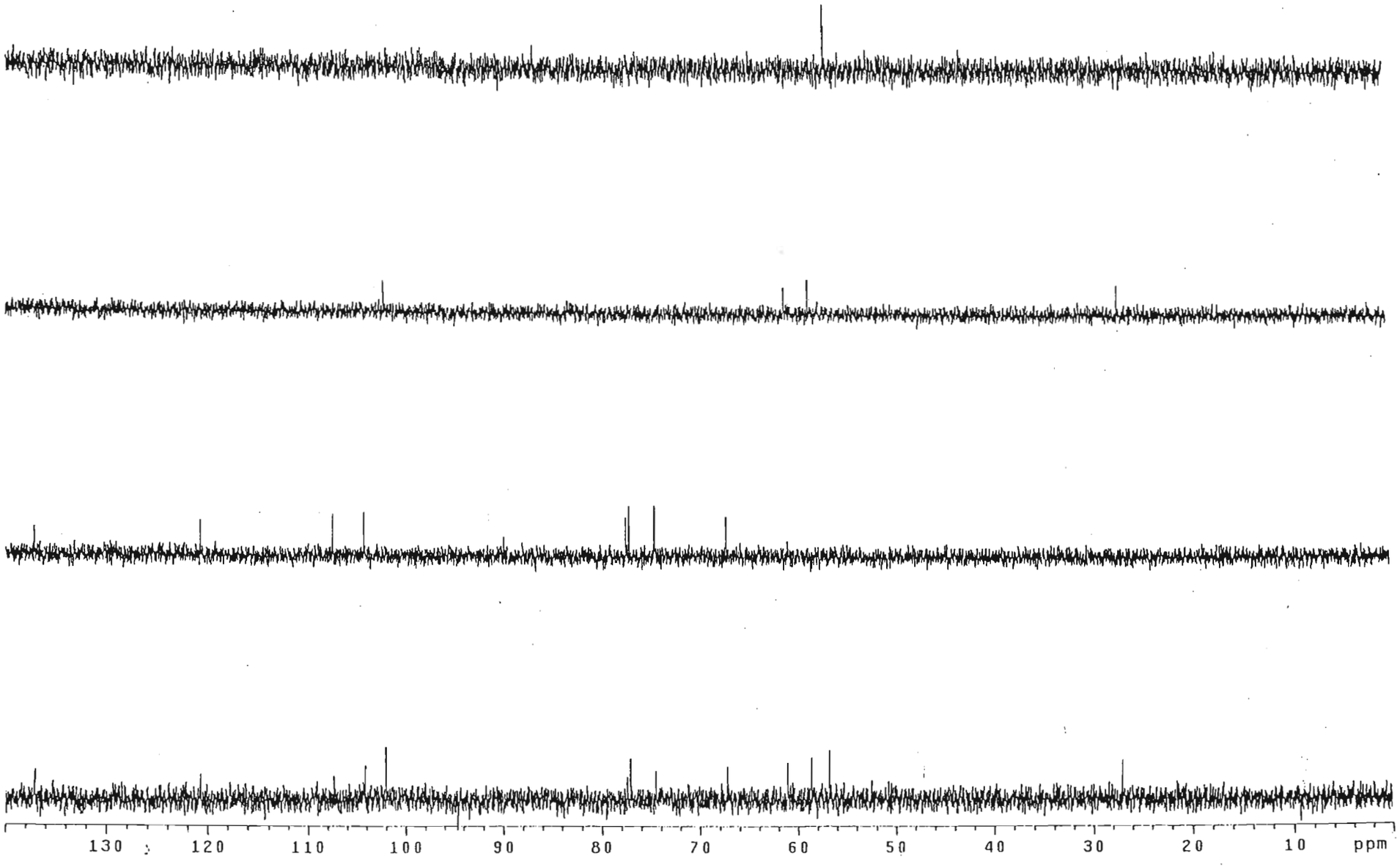
IX



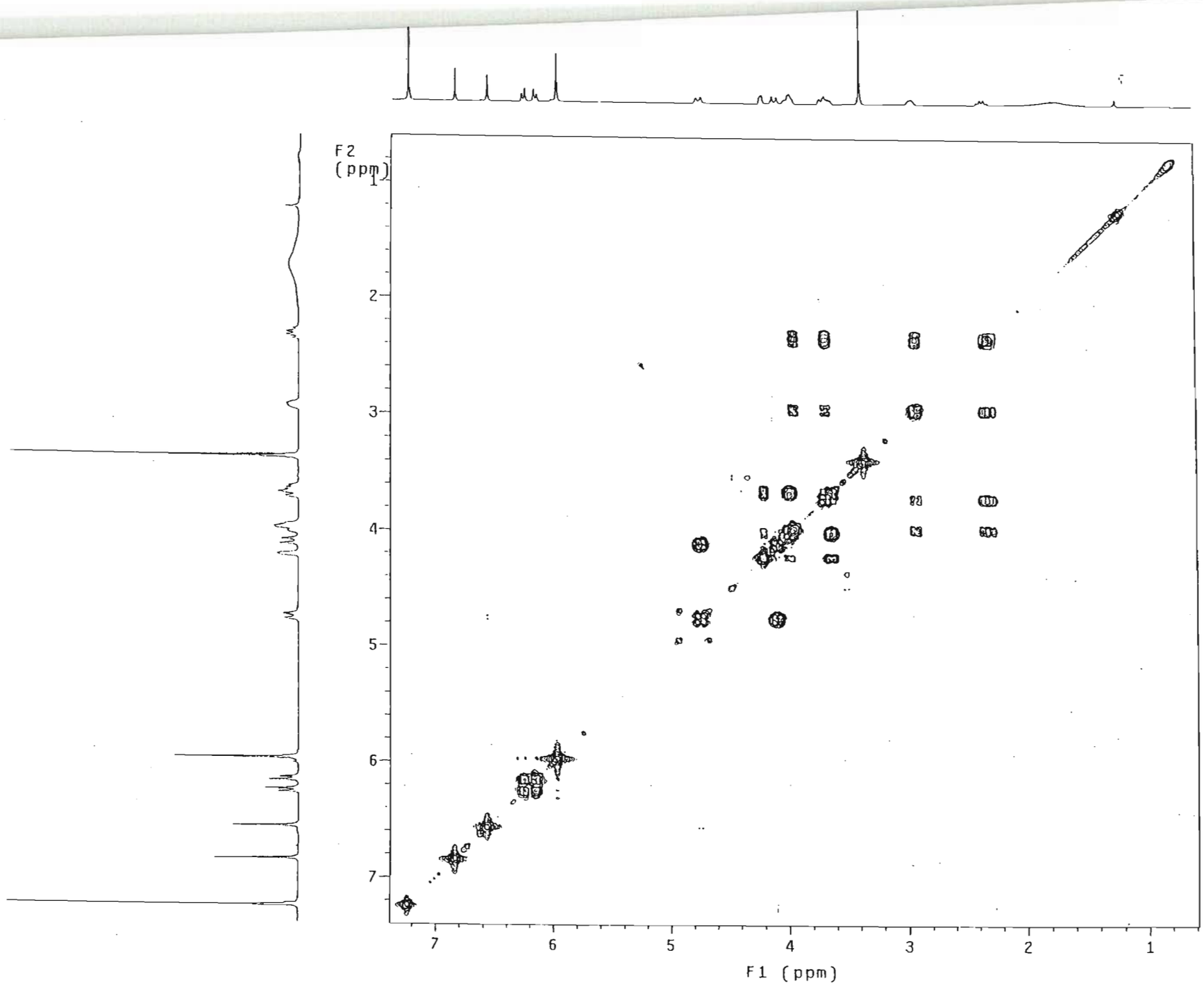
Spectrum [9-A]: ^{1}H NMR spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



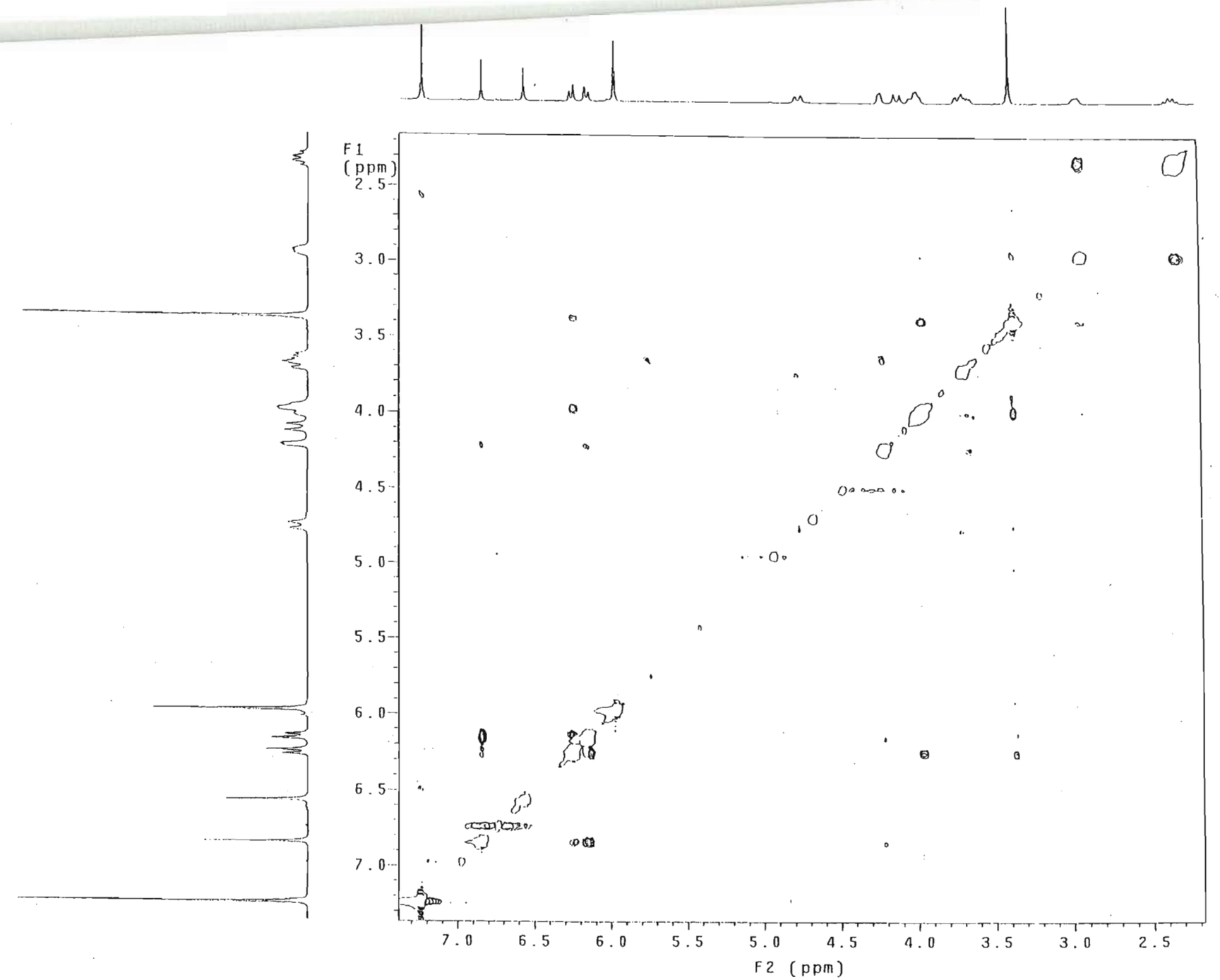
Spectrum [9-B]: ^{13}C NMR spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



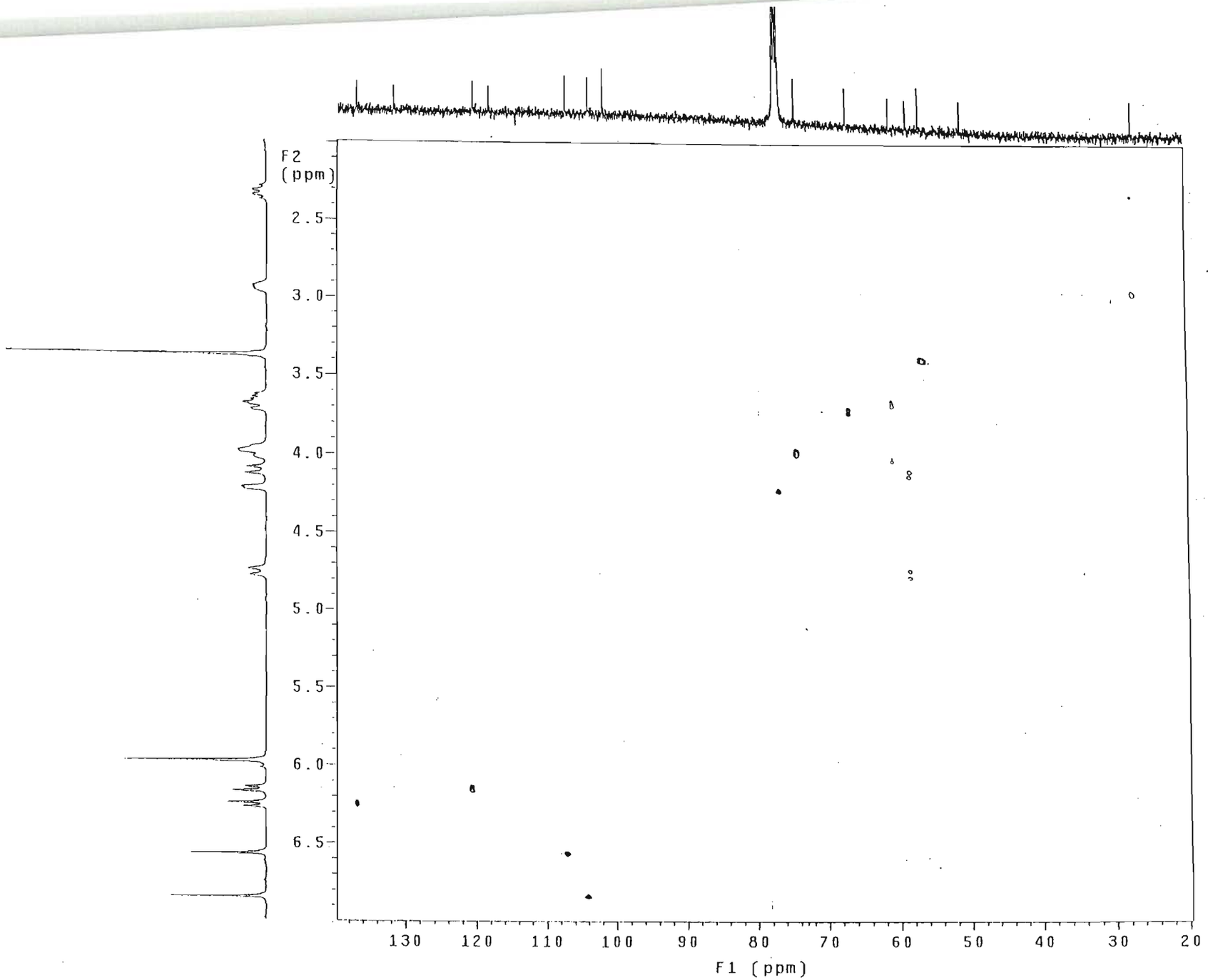
Spectrum [9-C]: ADEPT spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



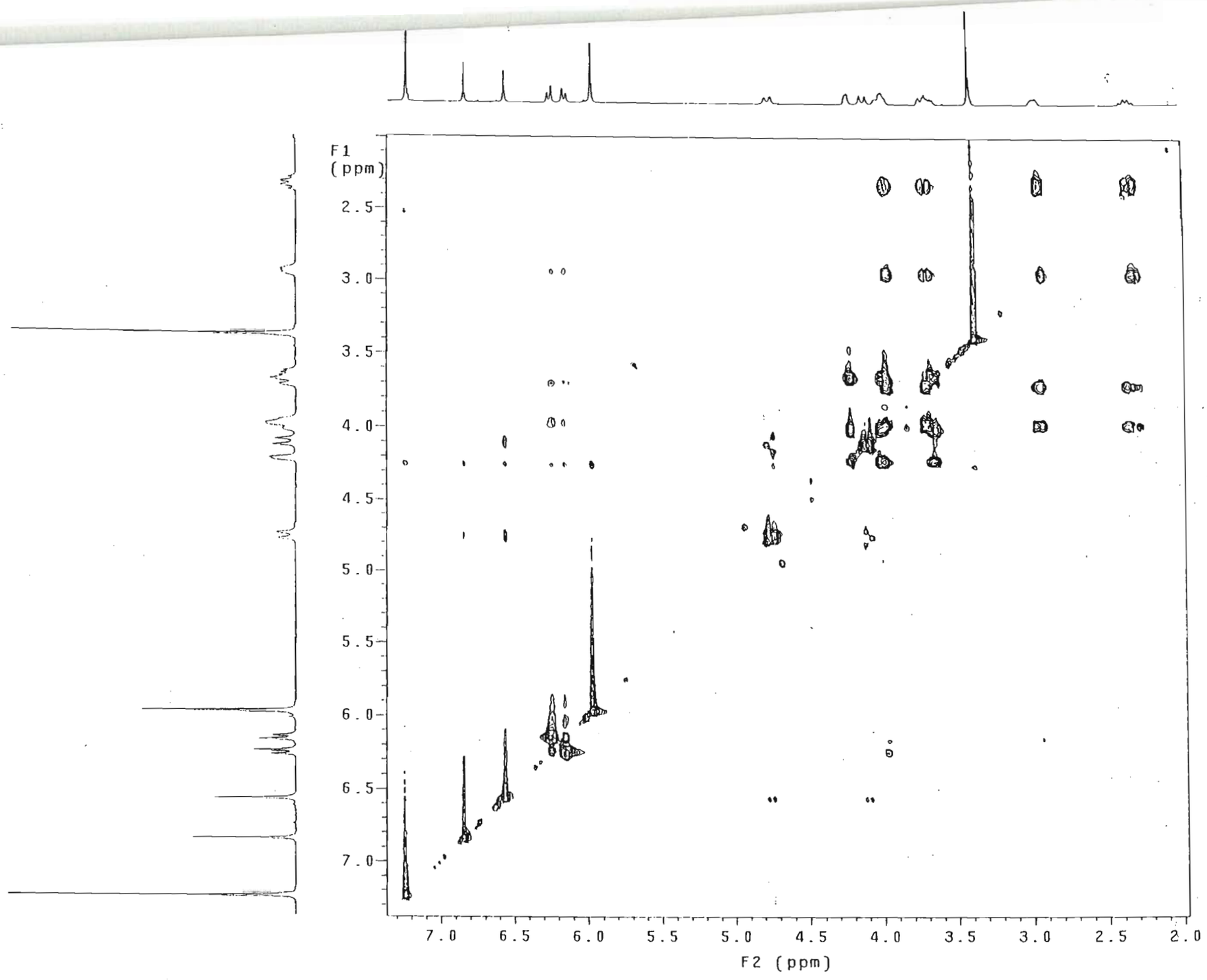
Spectrum [9-D]: COSY spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



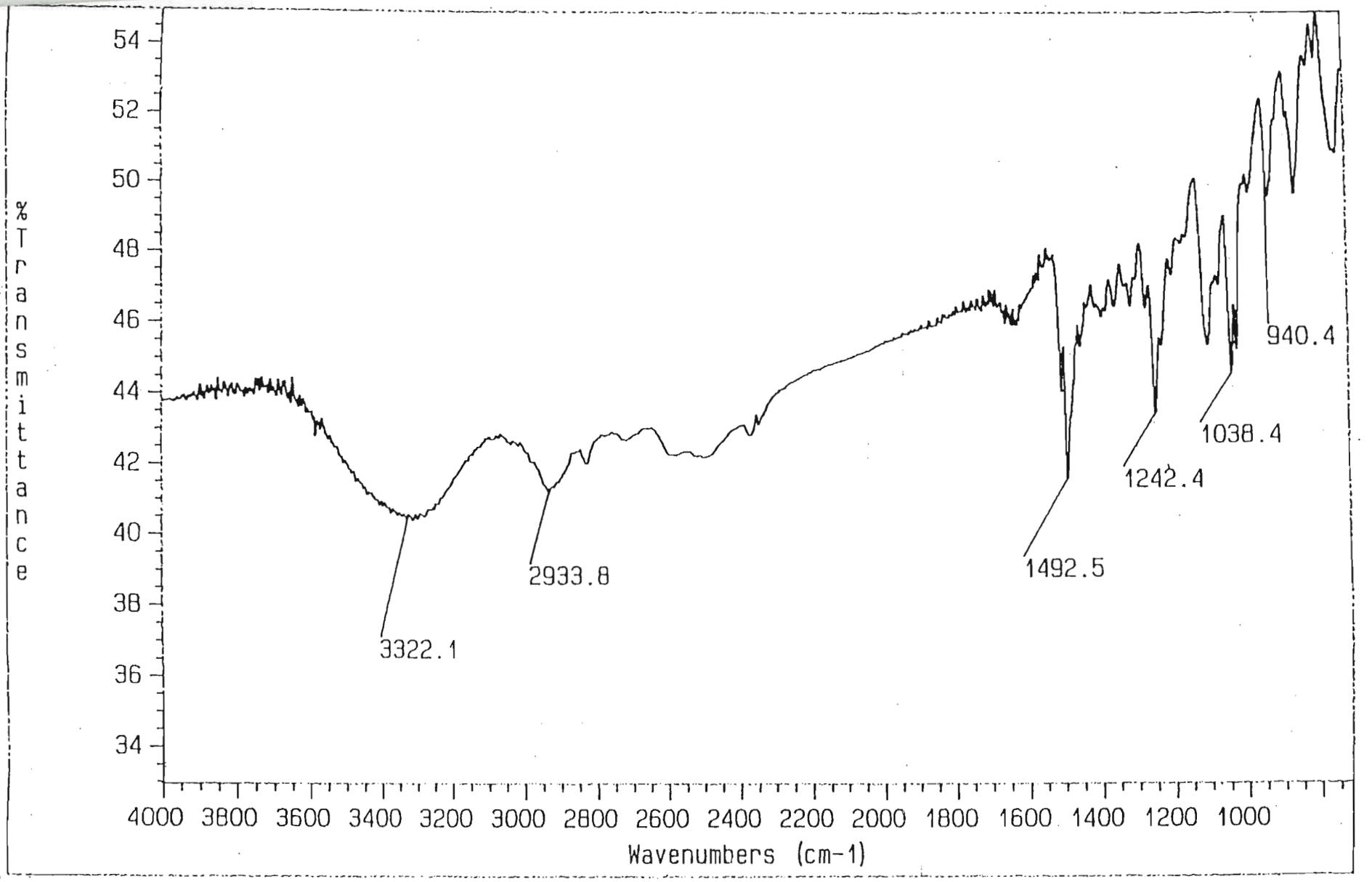
Spectrum [9-E]: NOESY spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



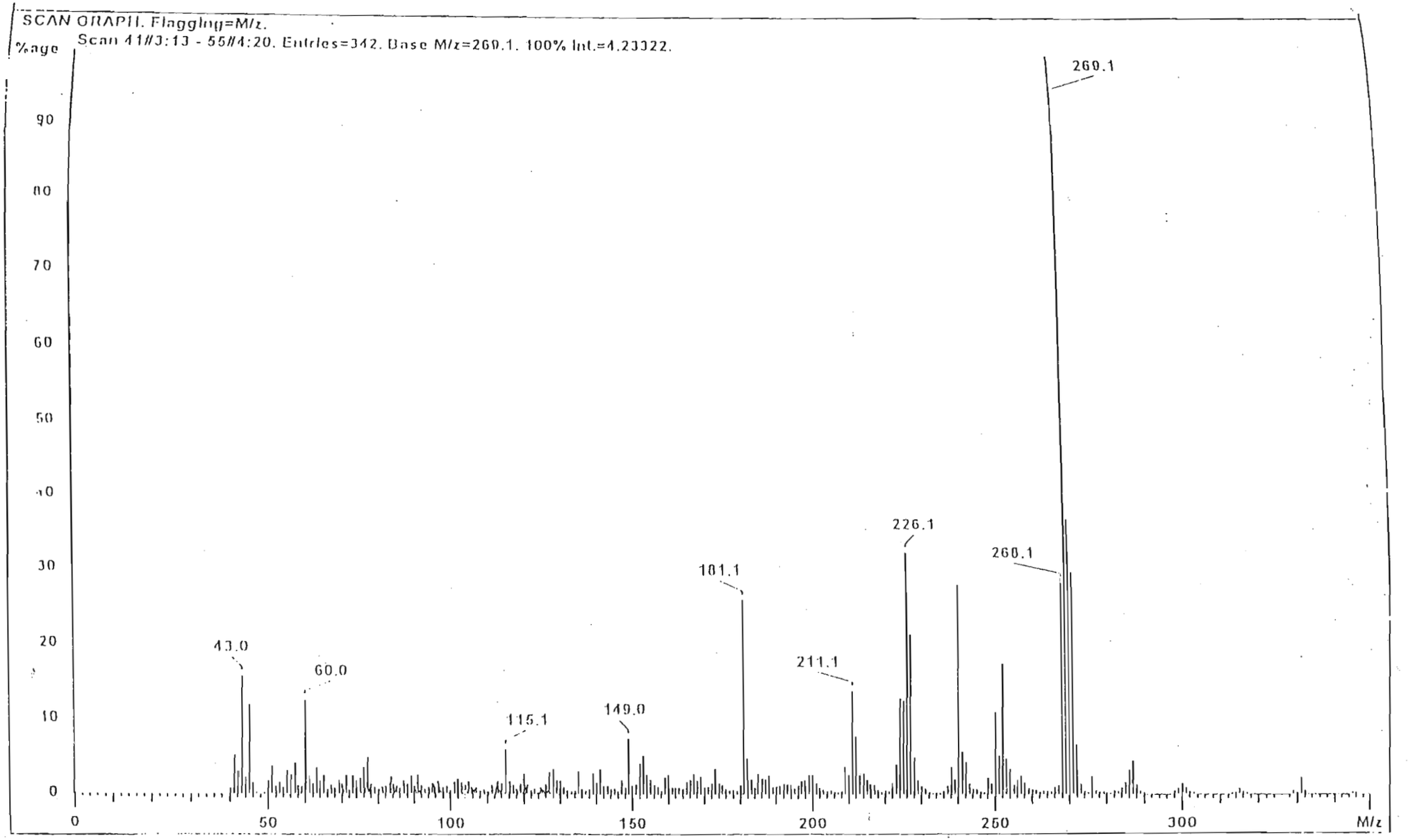
Spectrum [9-F]: HSQC spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



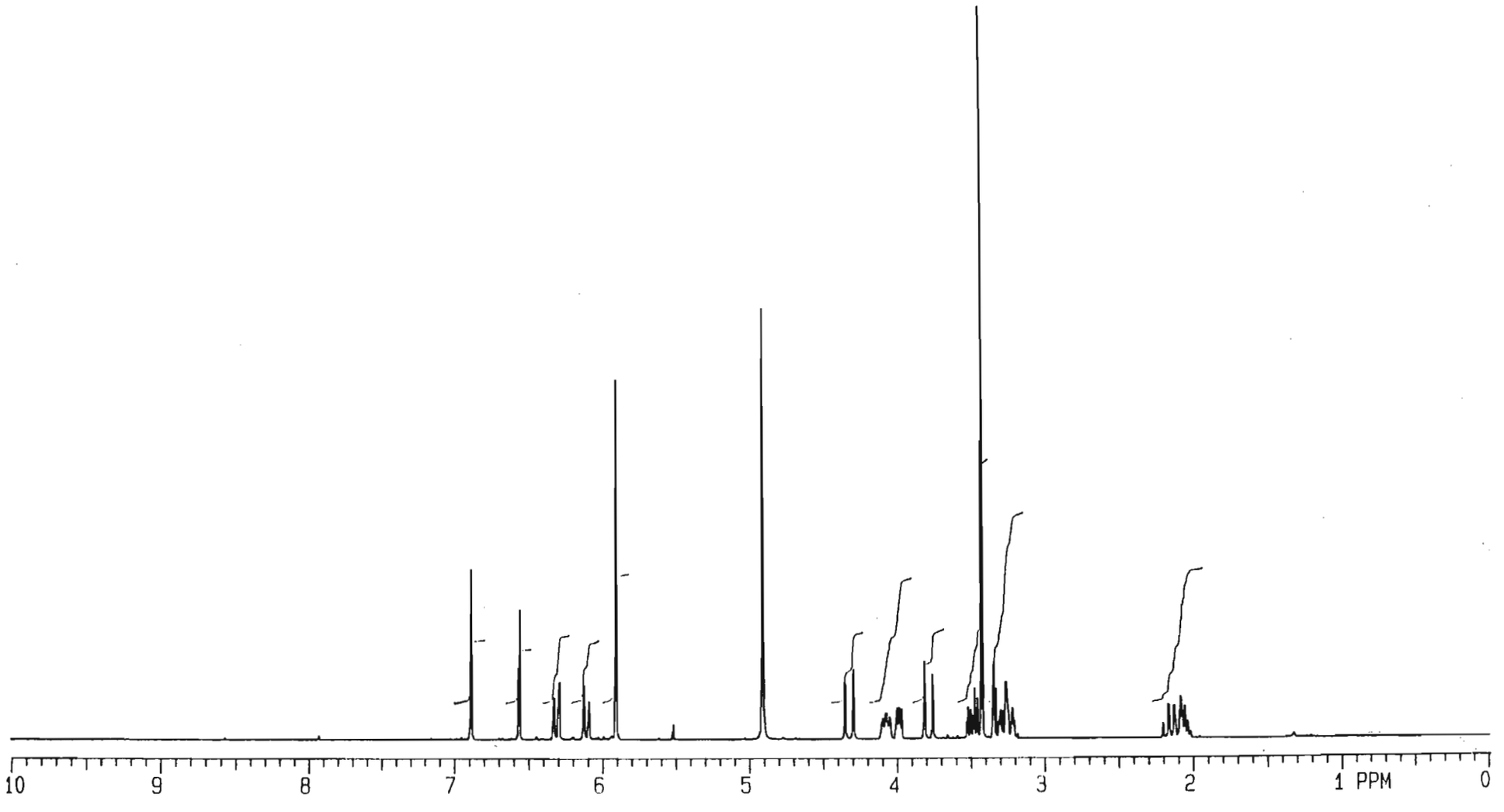
Spectrum [9-G]: TOCSY spectrum of crinamine hydrochloride (**IX**) in $CDCl_3$



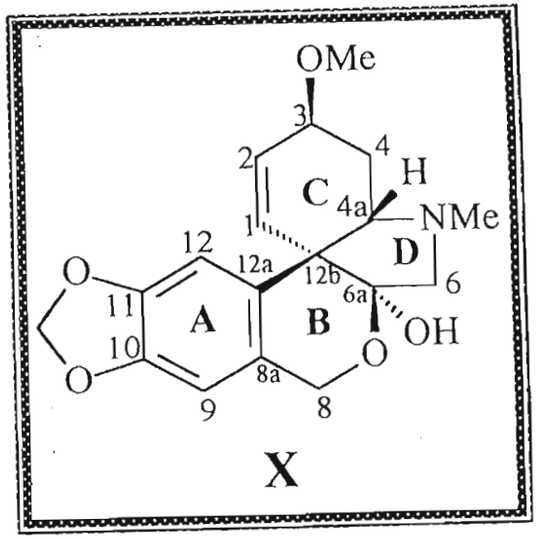
Spectrum [9-H]: Infra red spectrum of crinamine hydrochloride (**IX**)



Spectrum [9-1]: Mass spectrum of crinamine hydrochloride (**IX**)

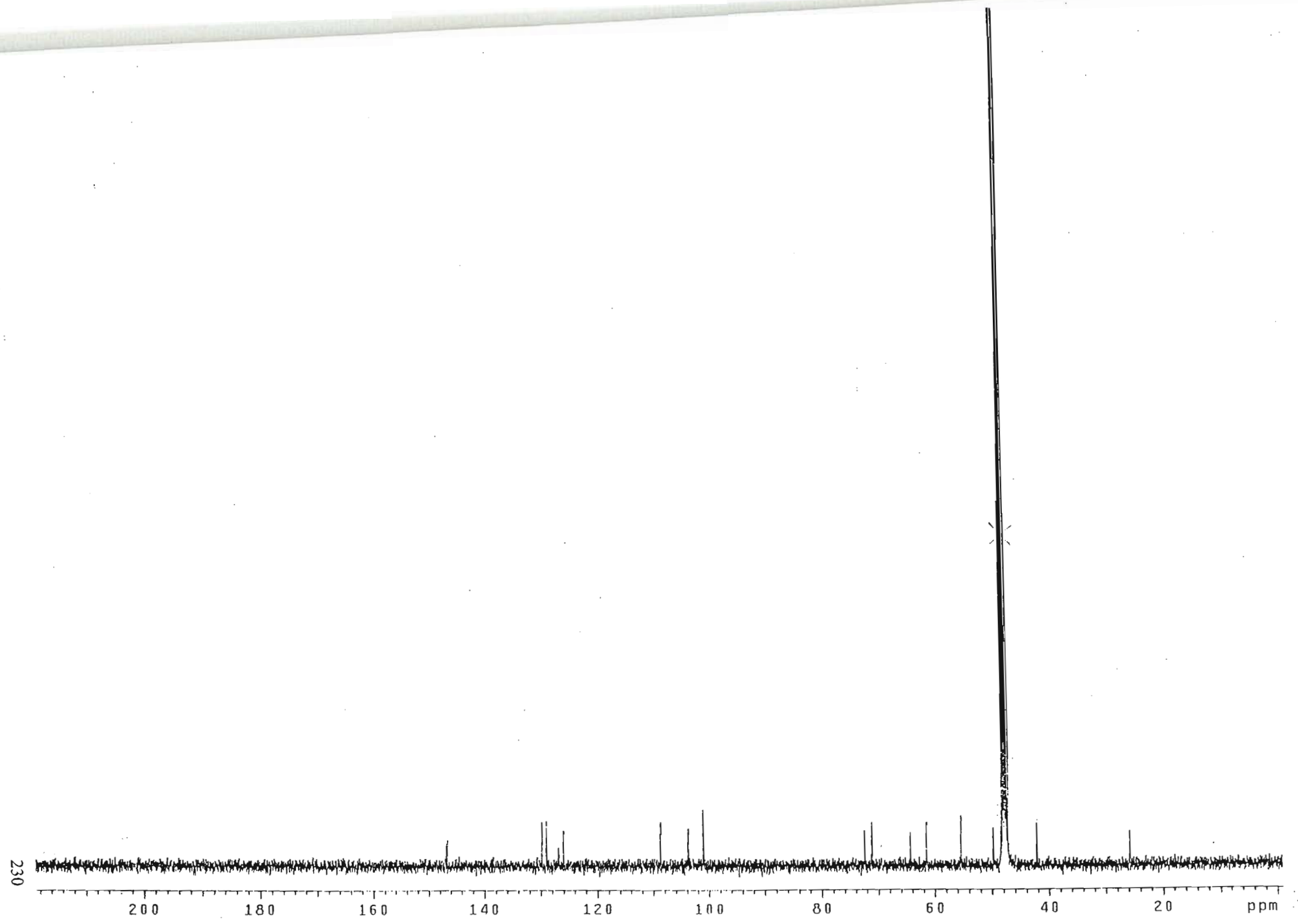


Spectrum [10-I]: ^{1}H NMR spectrum of base treated compound (**IX**) in CD_3OD

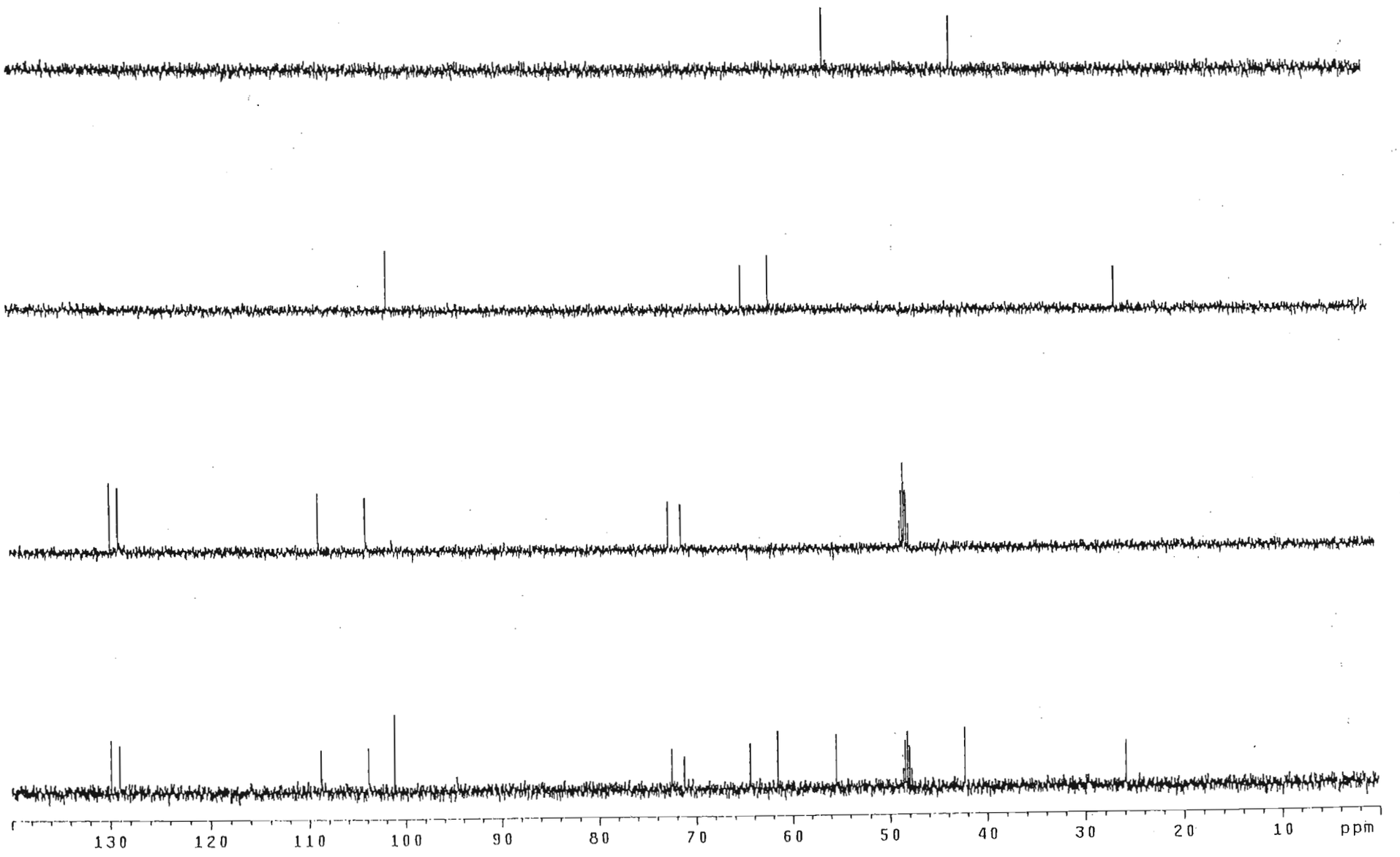


X

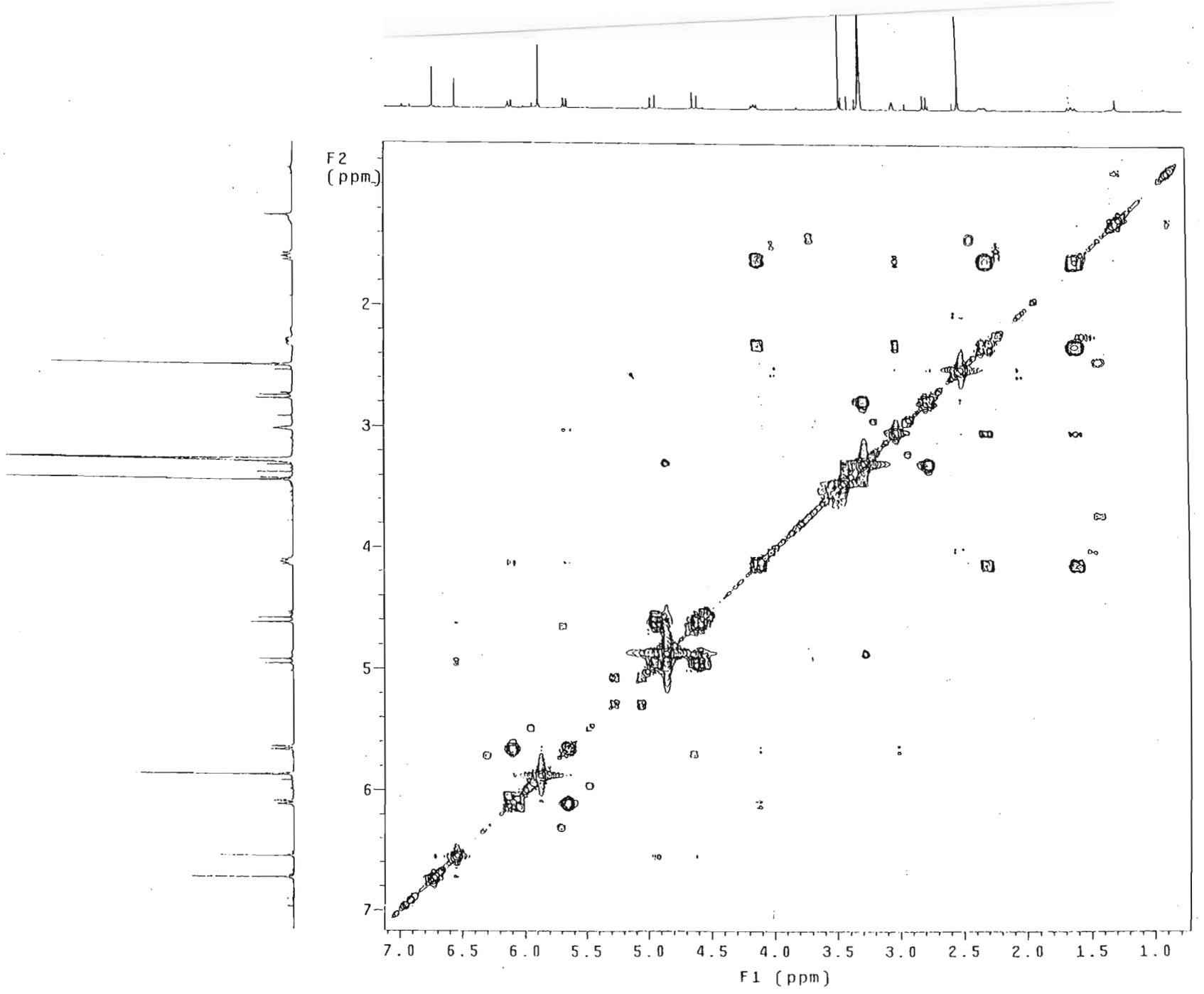
Spectrum [10-A]: ^{1}H NMR spectrum of tazettine (**X**) in $CDCl_3$



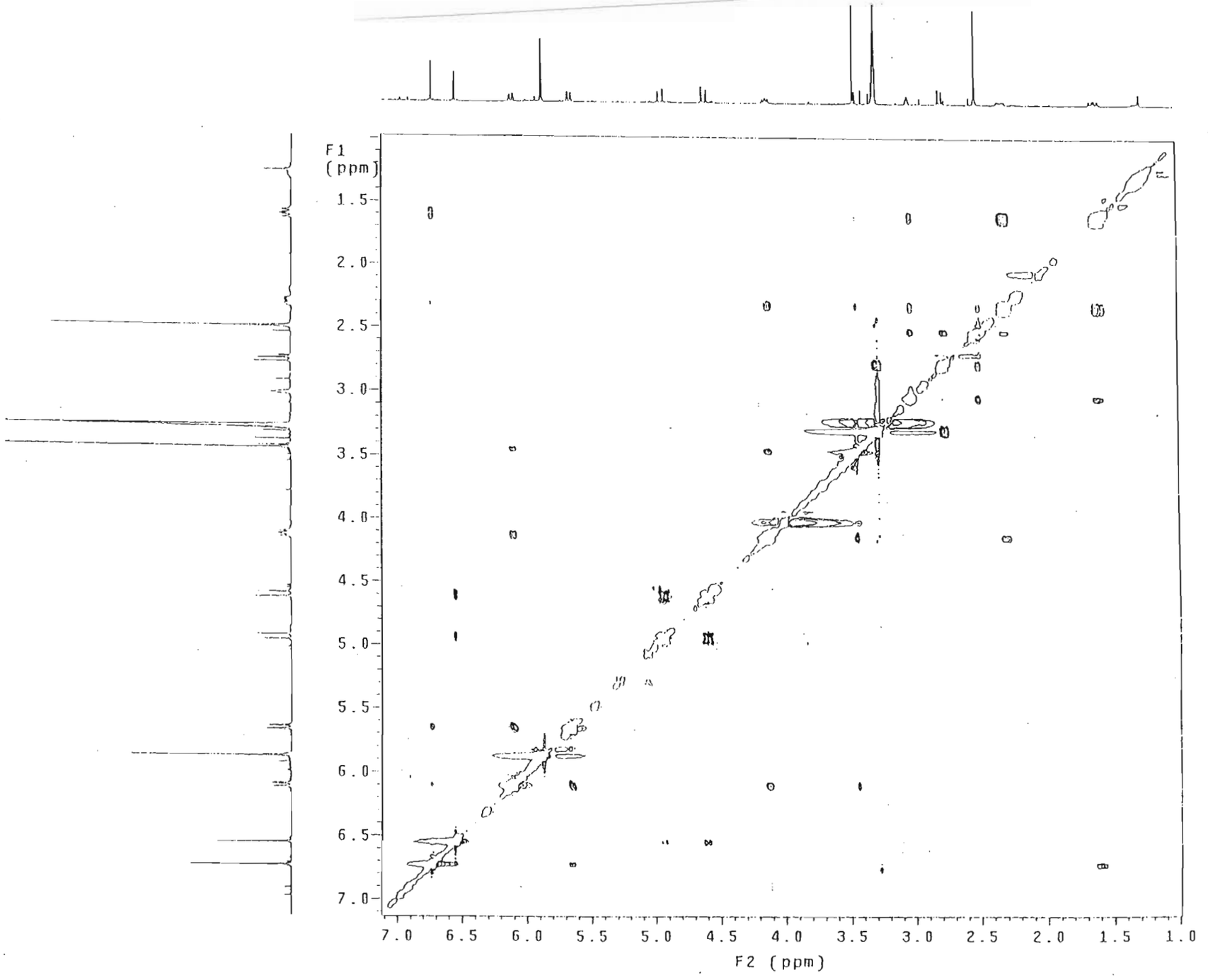
Spectrum [10-B]: ^{13}C NMR spectrum of tazettine (**X**) in $CDCl_3$



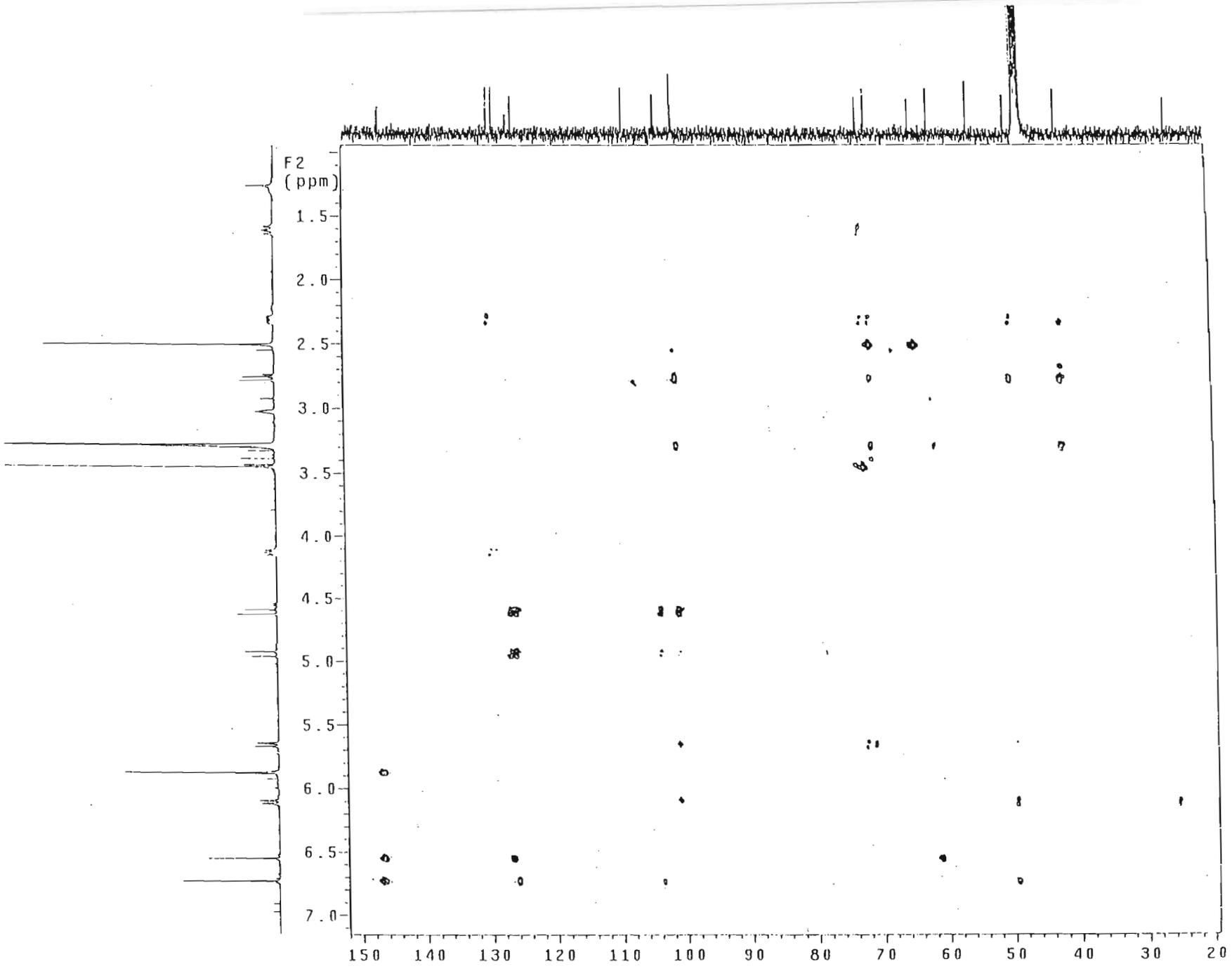
Spectrum [10-C]: ADEPT spectrum of tazettine (X) in $CDCl_3$



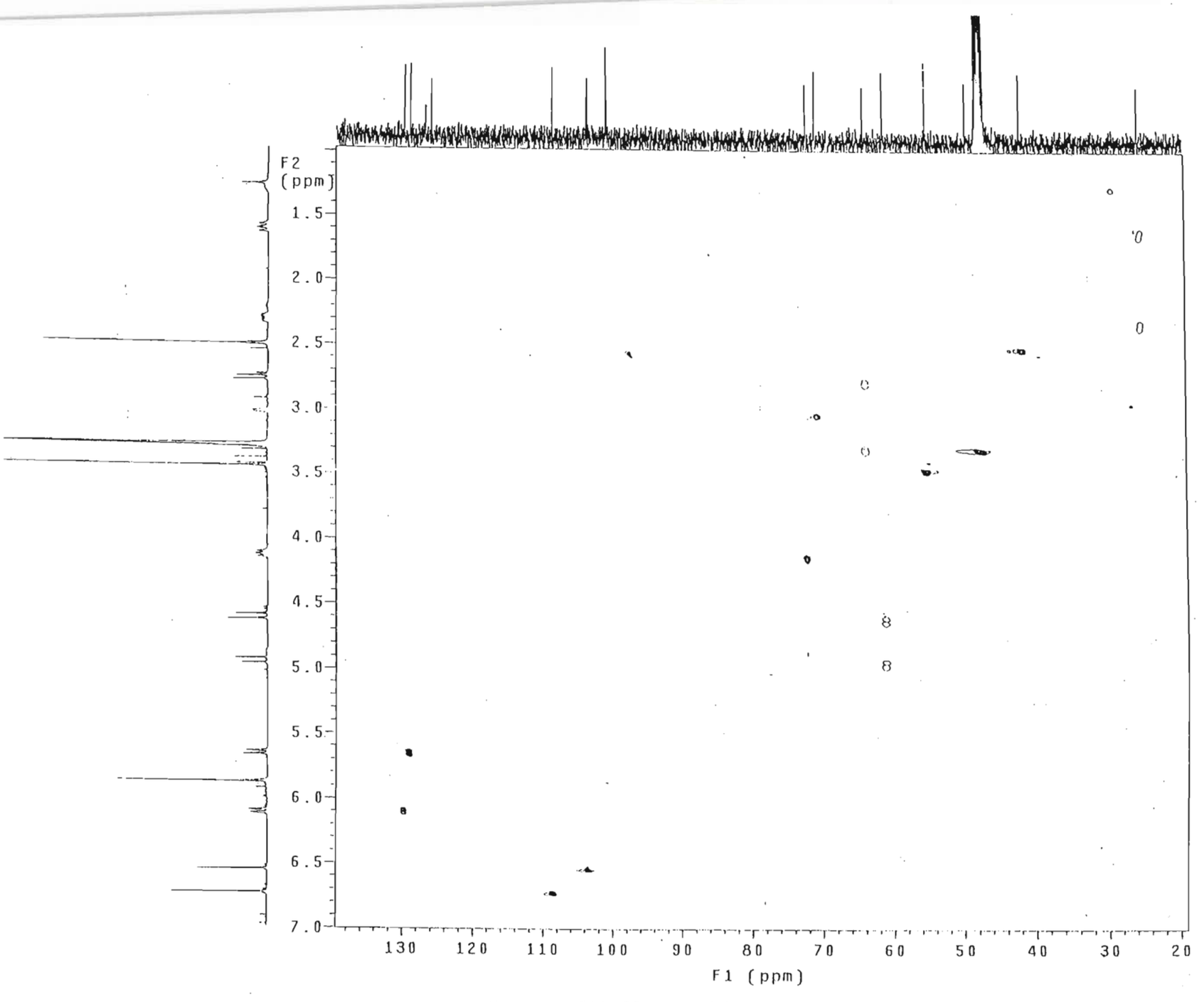
Spectrum [10-D]: COSY spectrum of tazettine (**X**) in $CDCl_3$



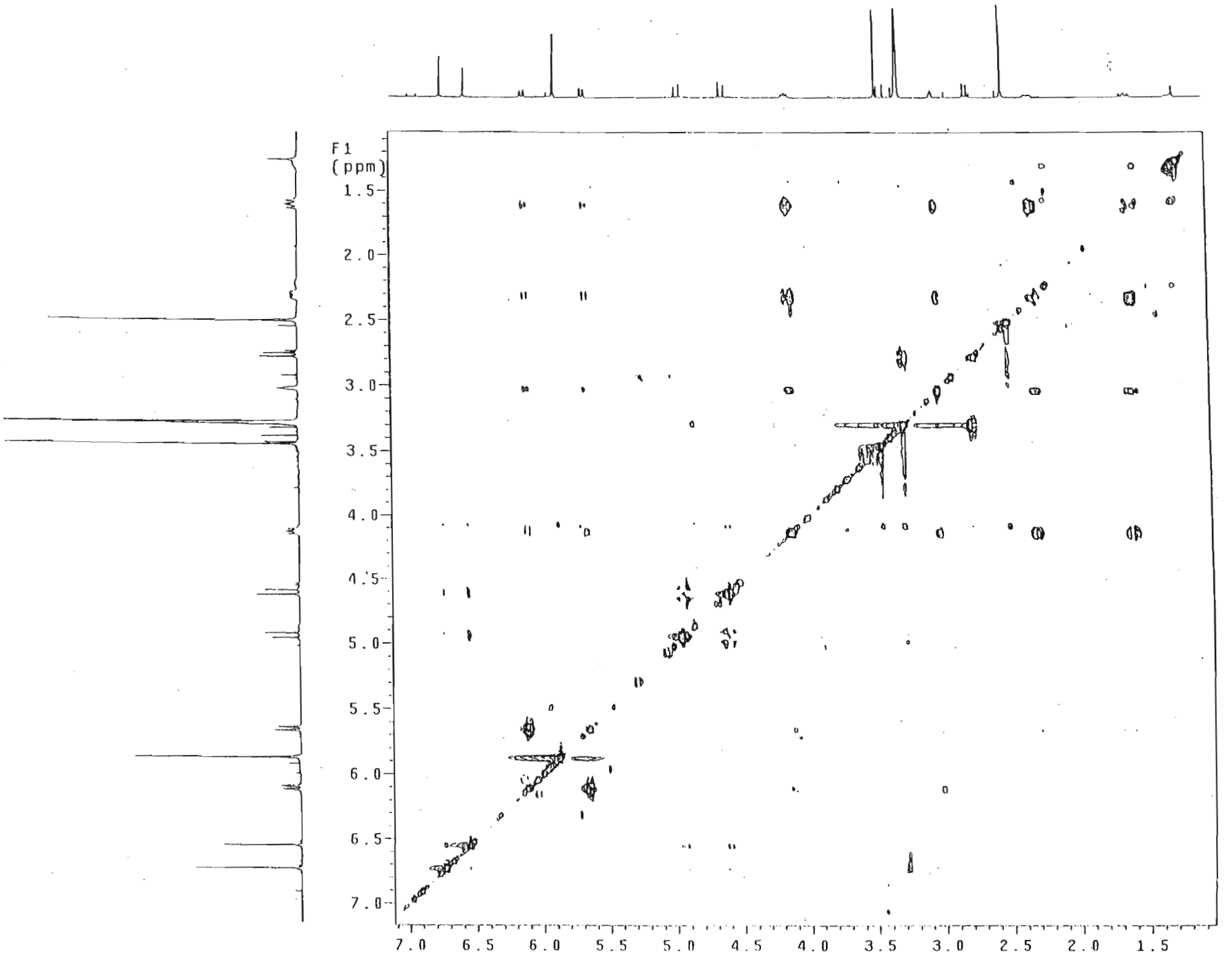
Spectrum [10-E]: NOESY spectrum of tazettine (**X**) in $CDCl_3$



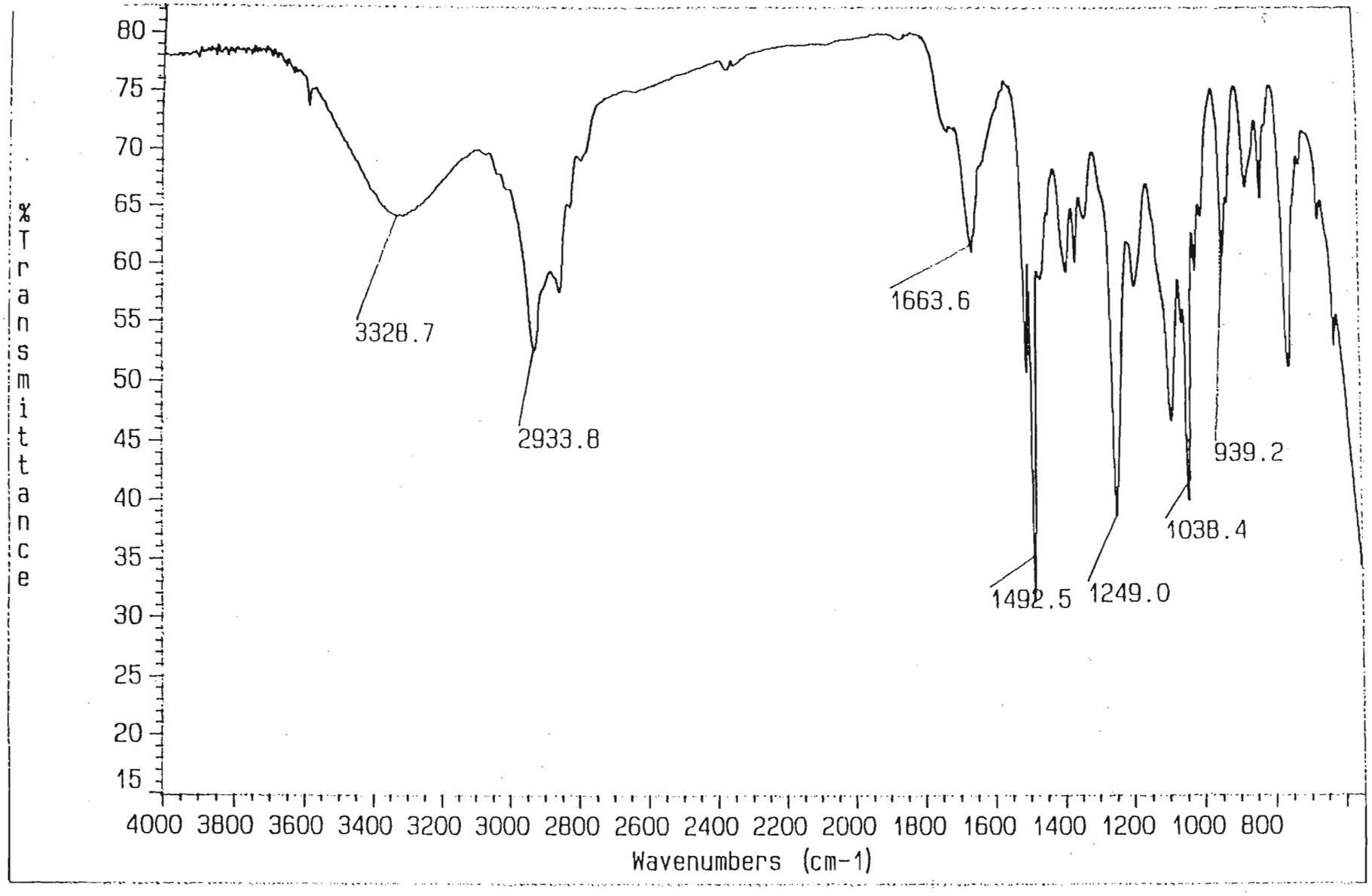
Spectrum [10-F]: HMBC spectrum of tazettine (**X**) in $CDCl_3$



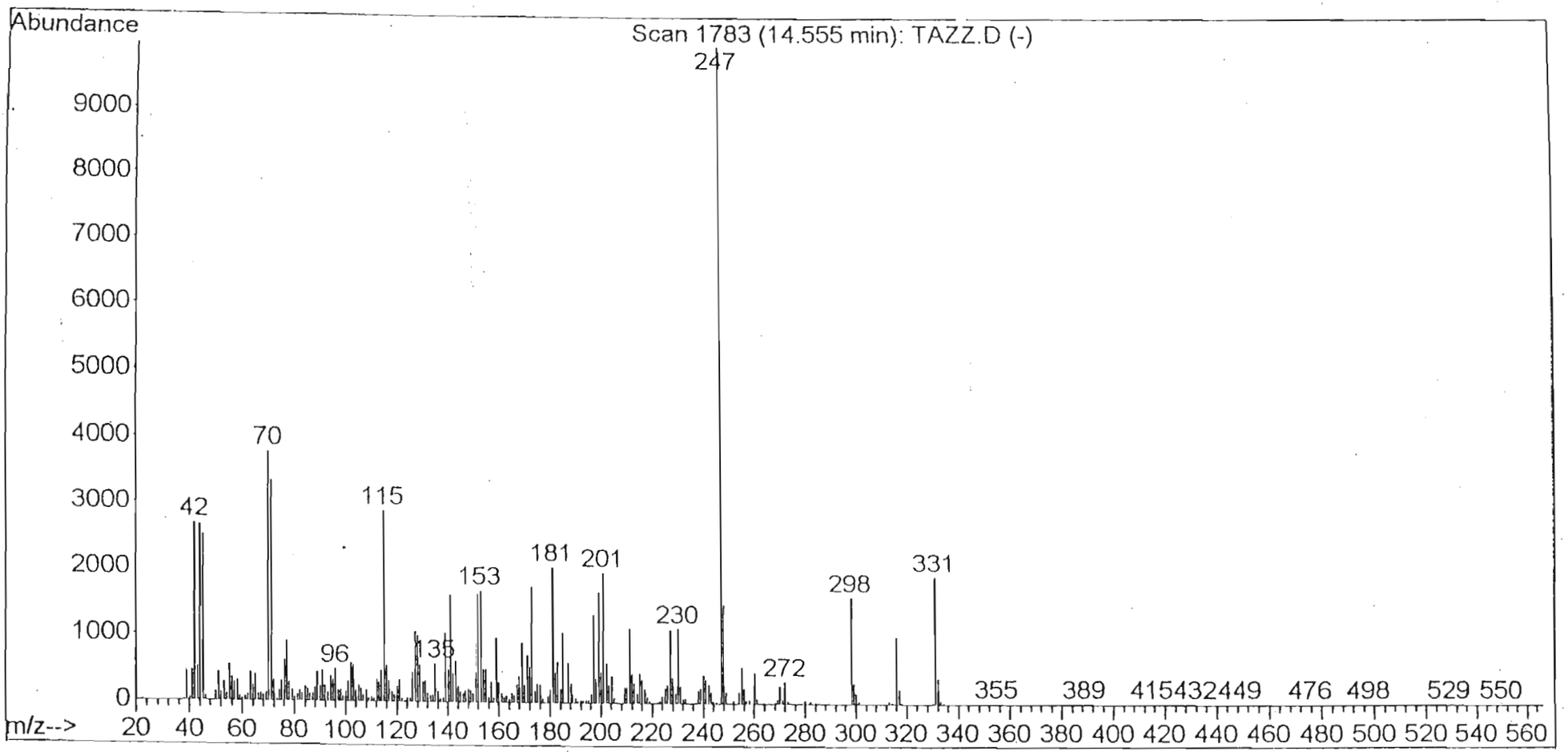
Spectrum [10-G]: HSQC spectrum of tazettine (**X**) in $CDCl_3$



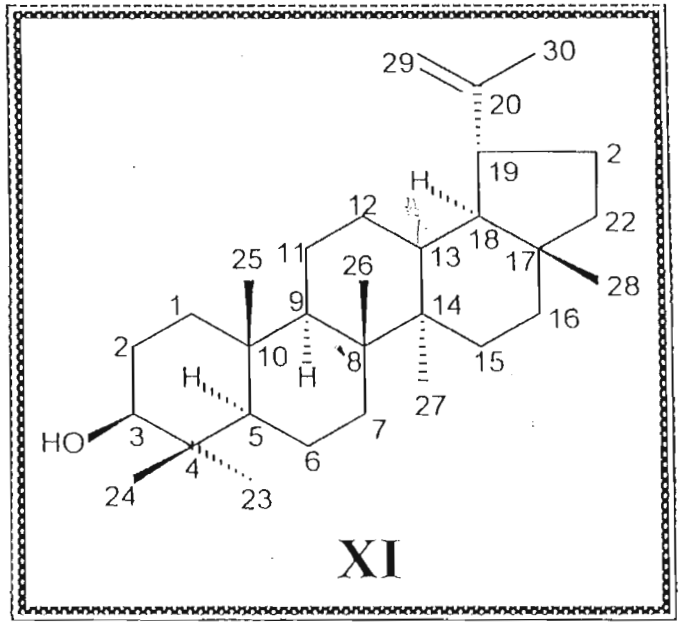
Spectrum [10-H]: TOCSY spectrum of tazettine (X) in $CDCl_3$



Spectrum [10-I]: Infra red spectrum of tazettine (X)

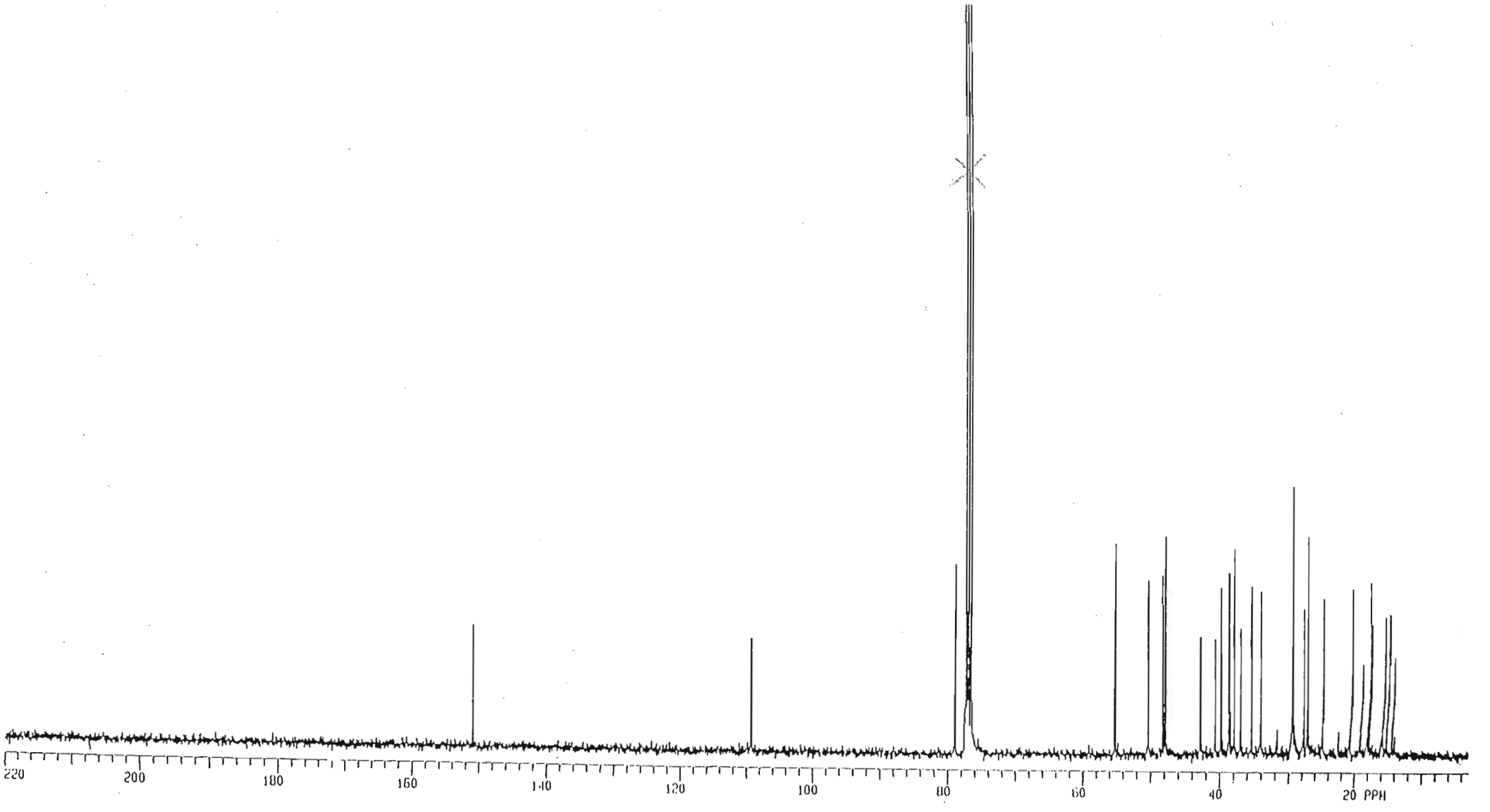


Spectrum [10-J]: Mass spectrum of tazettine (**X**)

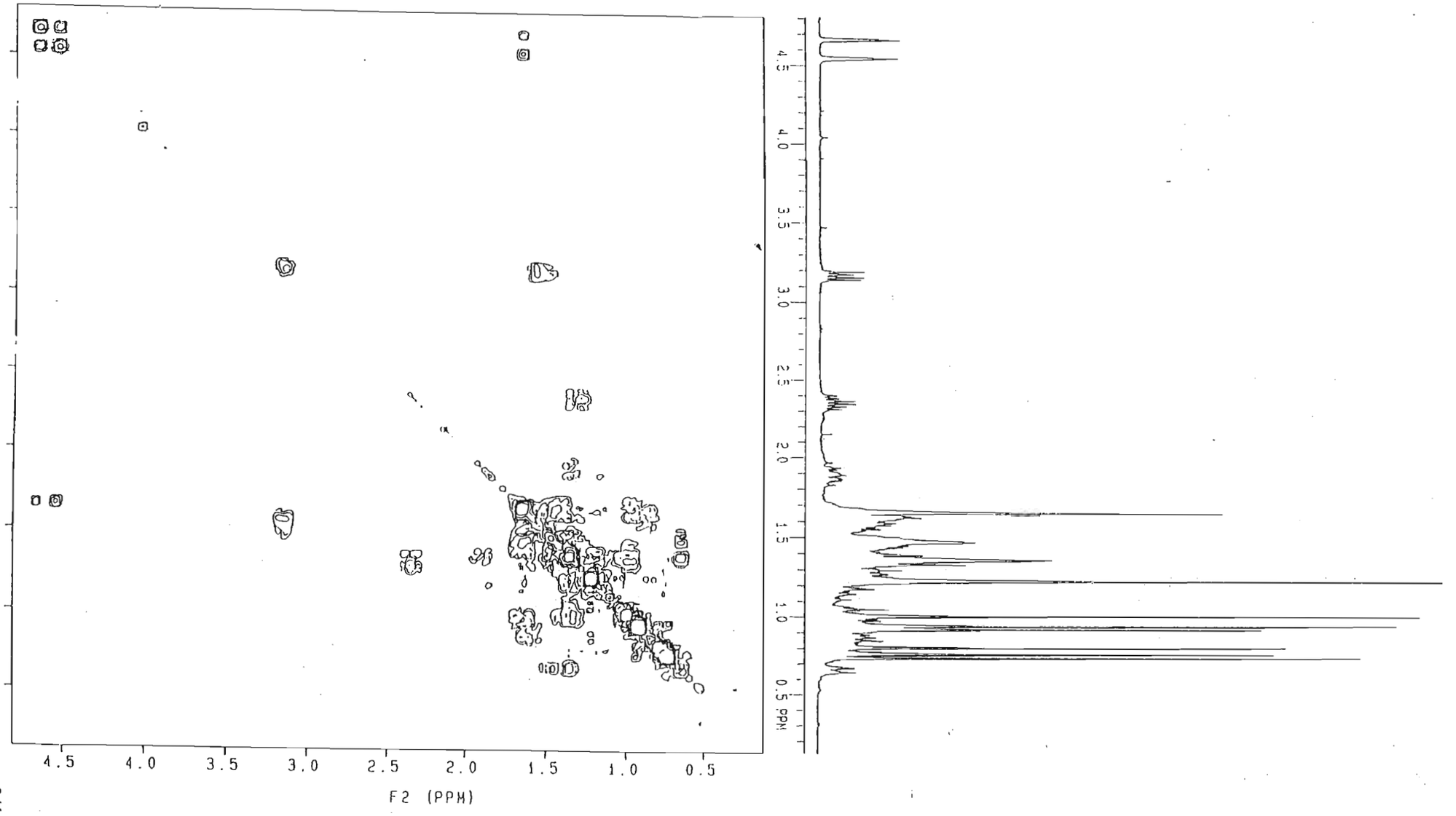


XI

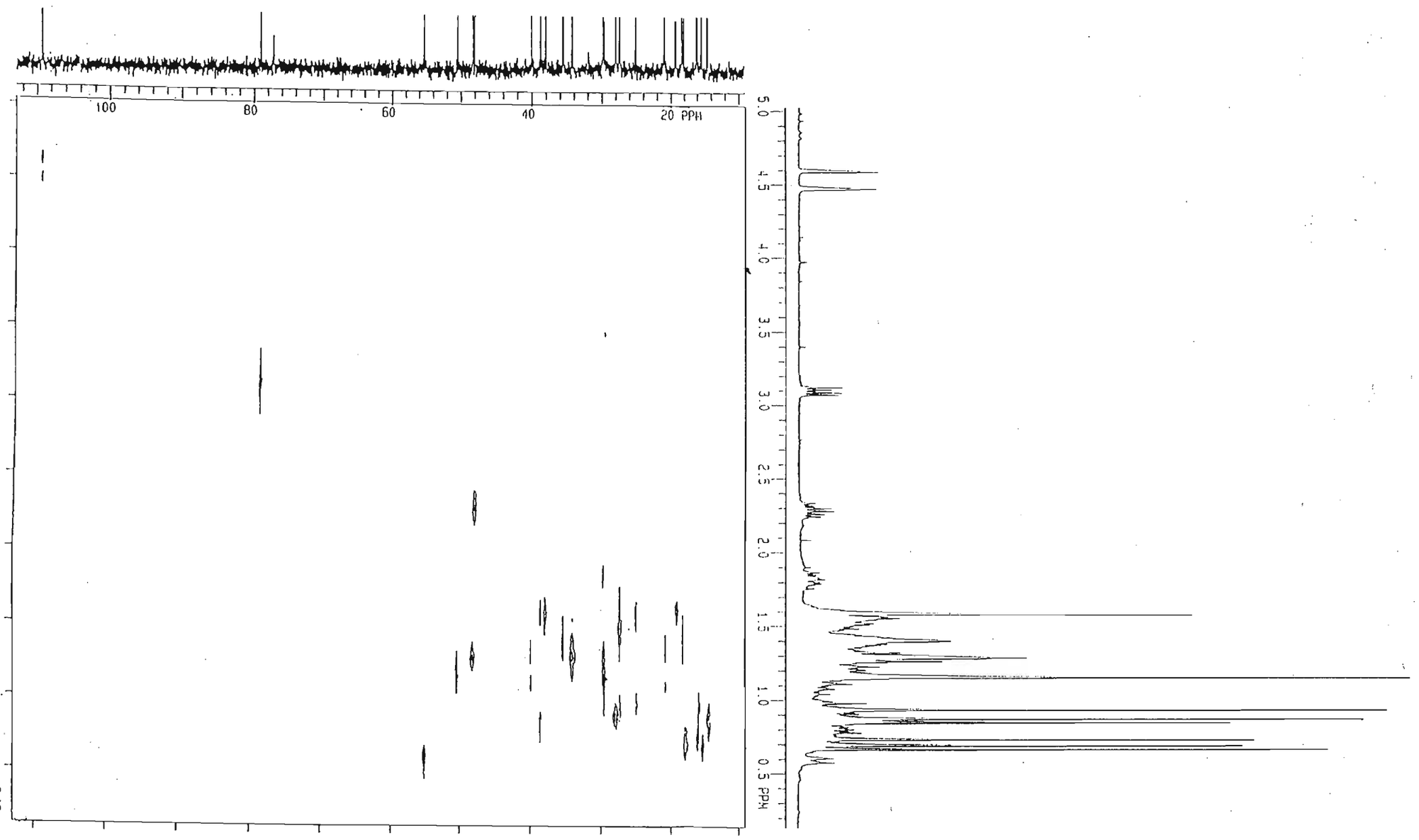
Spectrum [11-A]: ^{1}H NMR spectrum of lupeol (**XI**) in $CDCl_3$



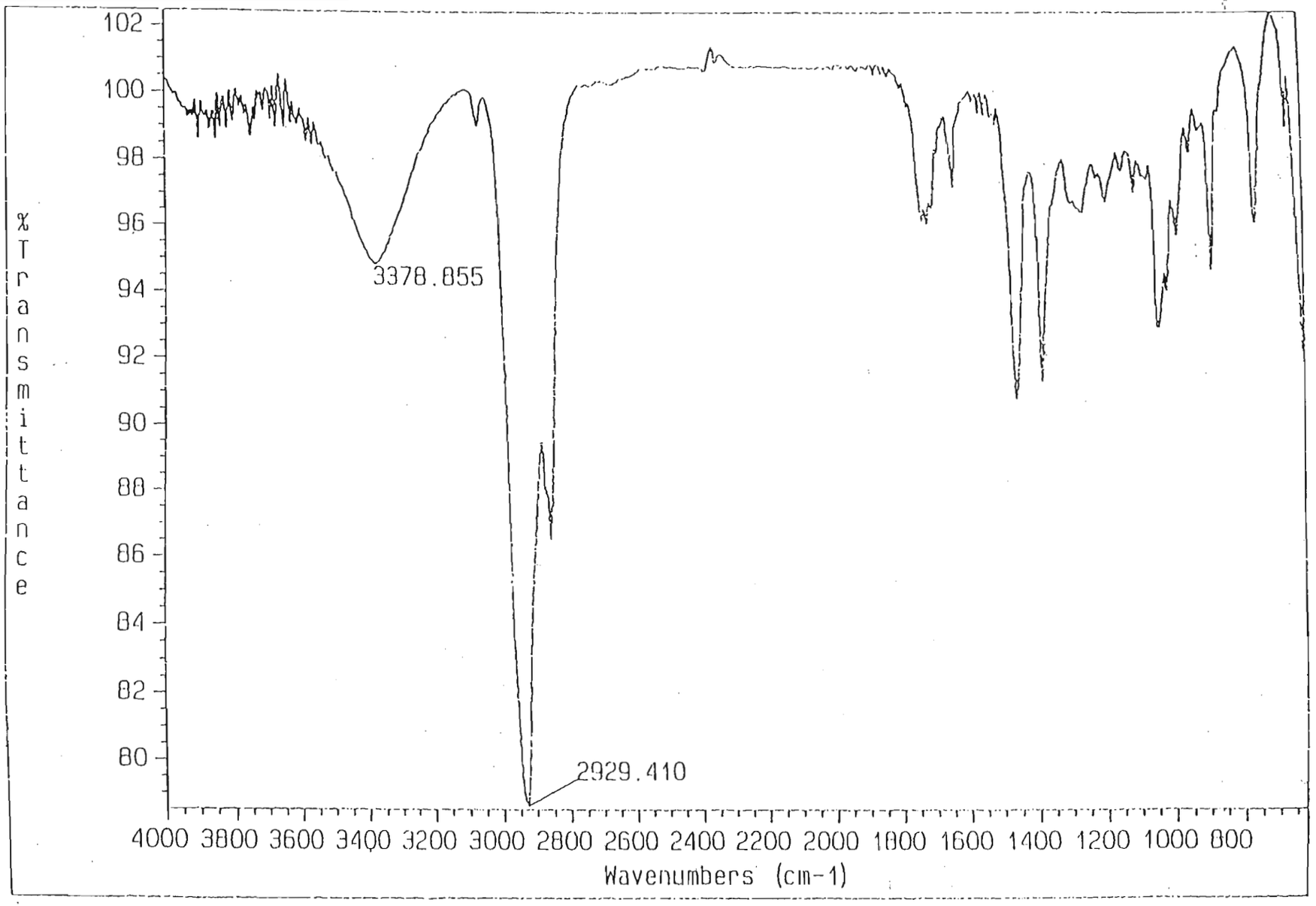
Spectrum [11-B]: ^{13}C NMR spectrum of lupeol (XI) in $CDCl_3$



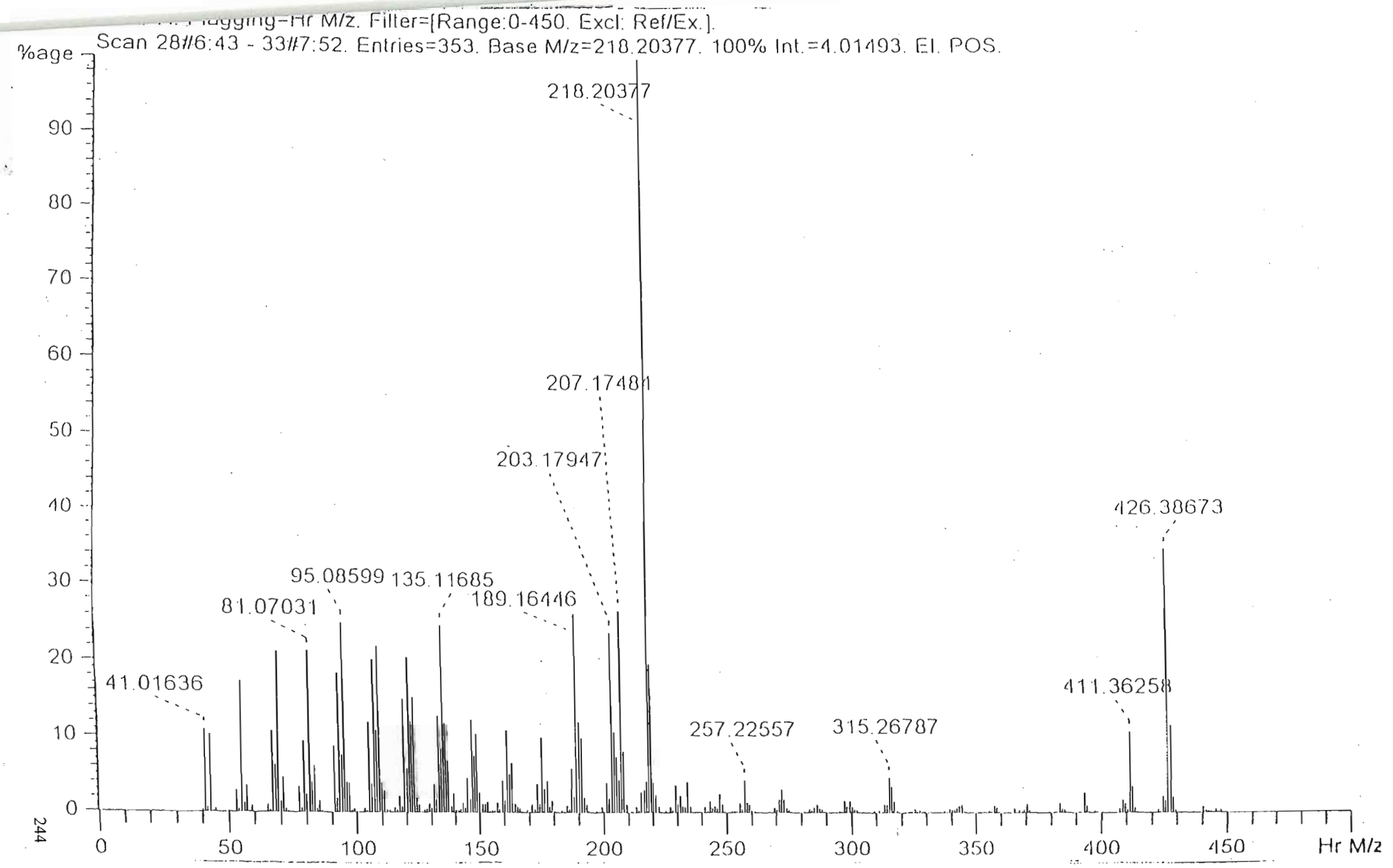
Spectrum [11-C]: COSY spectrum of lupeol (**XI**) in $CDCl_3$



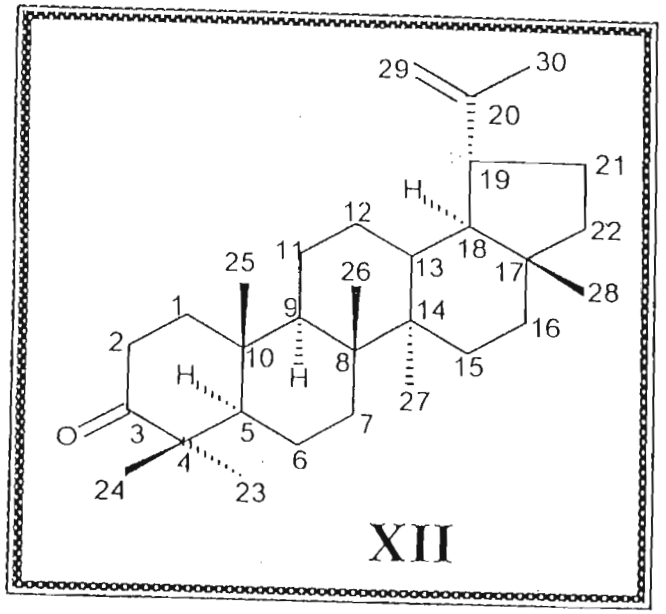
Spectrum [11-D]: HETCOR spectrum of lupeol (**XI**) in $CDCl_3$



Spectrum [11-E]: Infra red spectrum of lupeol (**XI**)

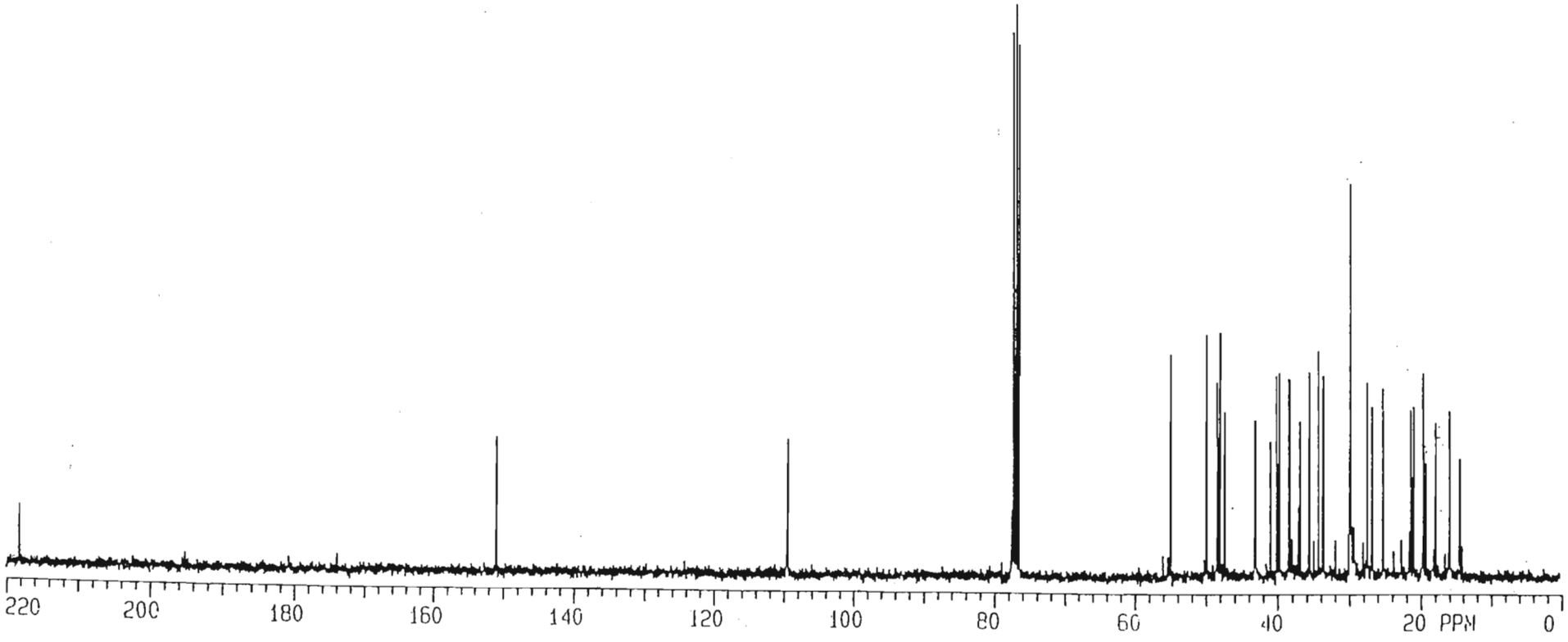


Spectrum [11-F]: Mass spectrum of lupeol (**XI**)

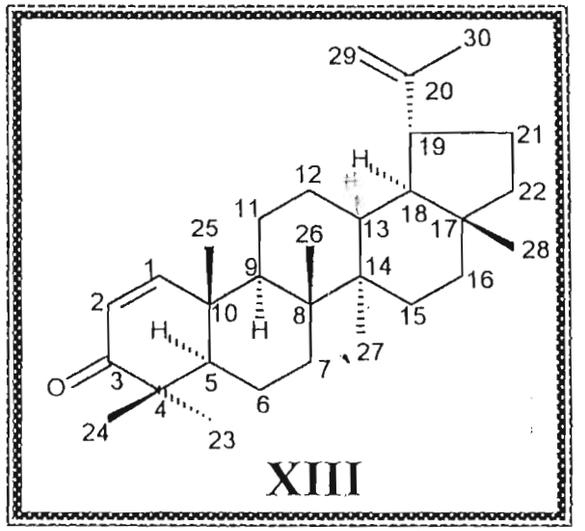


XII

Spectrum [12-A] ^{1}H NMR spectrum of lupenone (**XII**) in $CDCl_3$

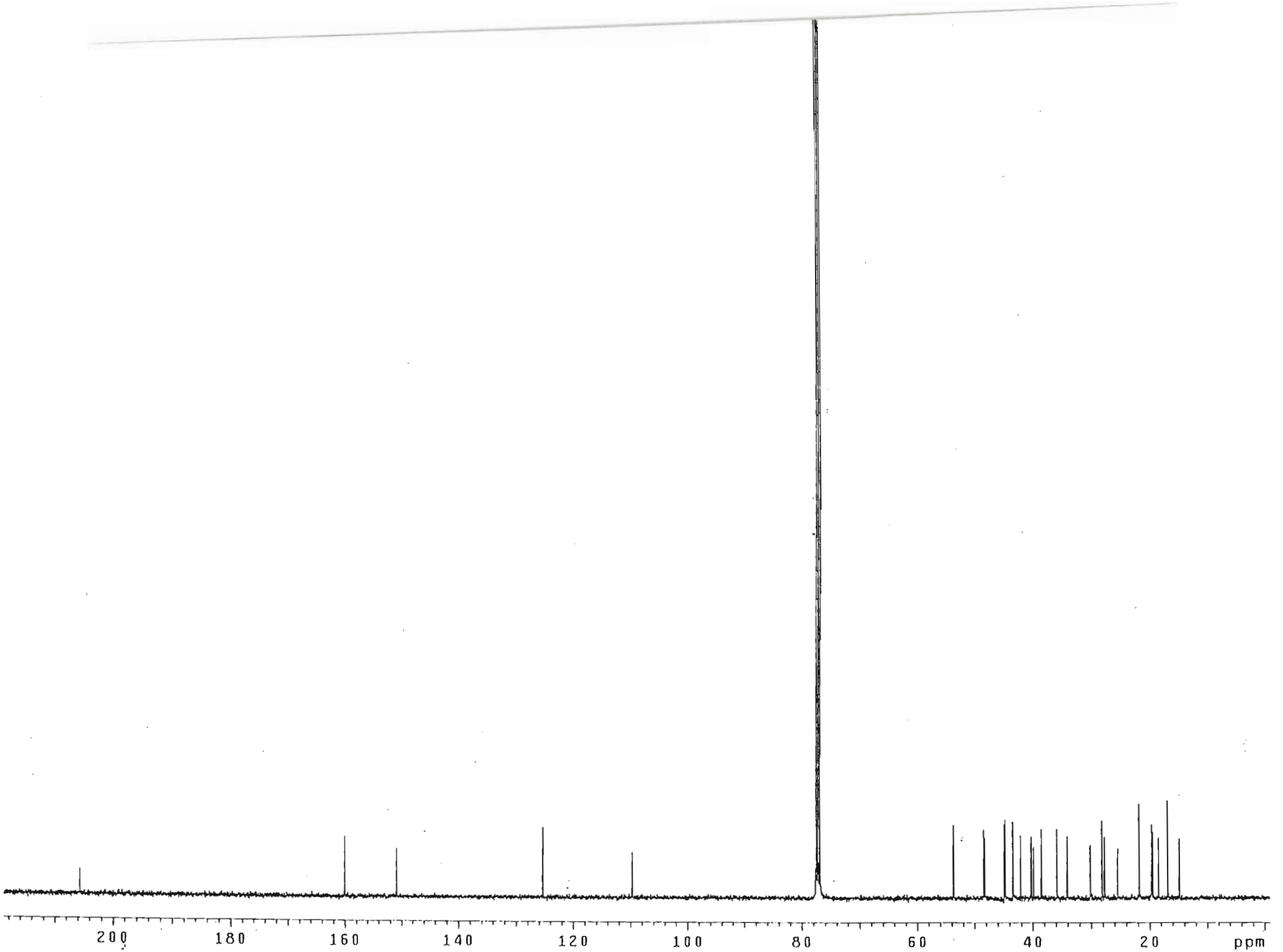


Spectrum [12-B]: ^{13}C NMR spectrum of lupenone (**XII**) in $CDCl_3$

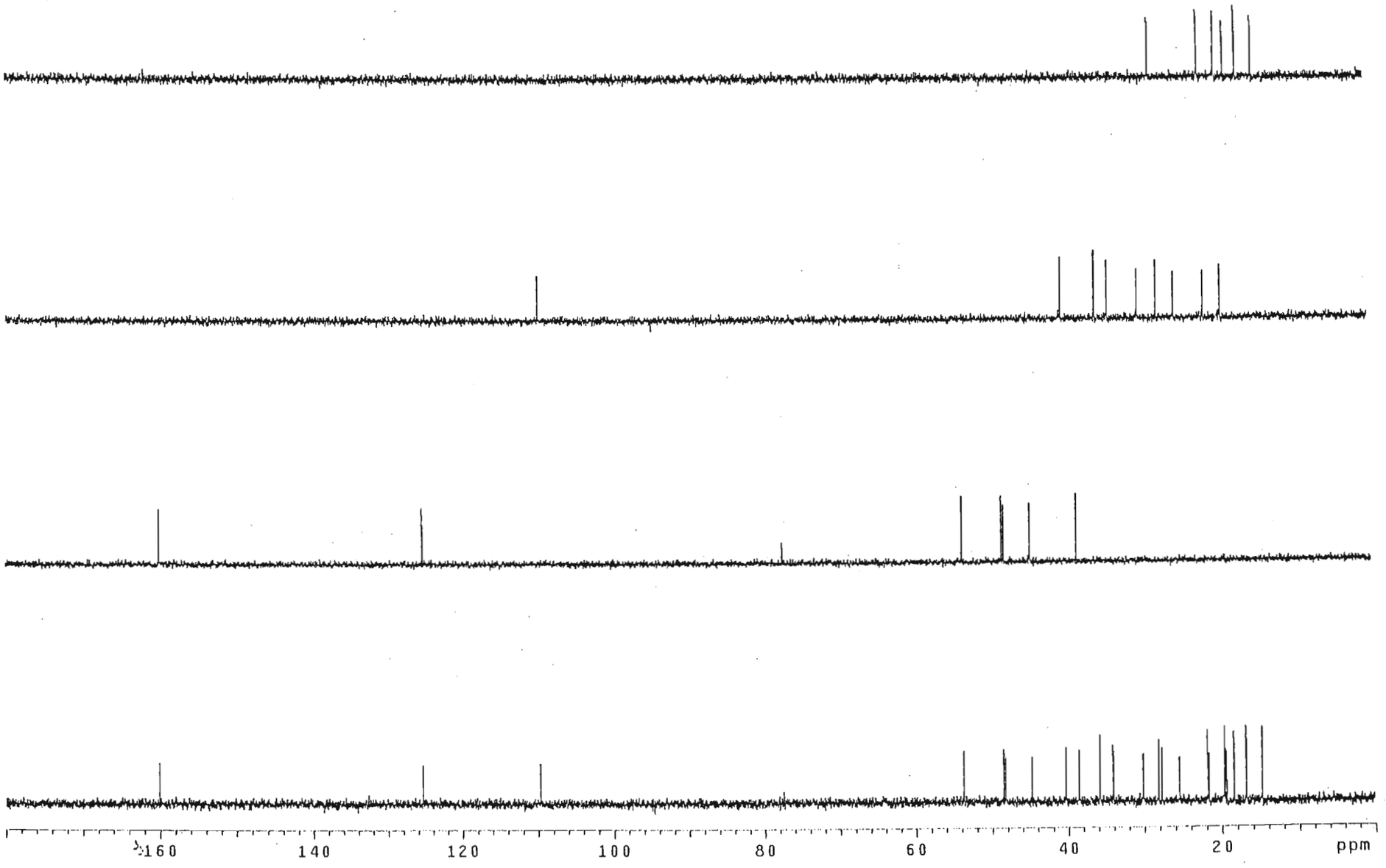


XIII

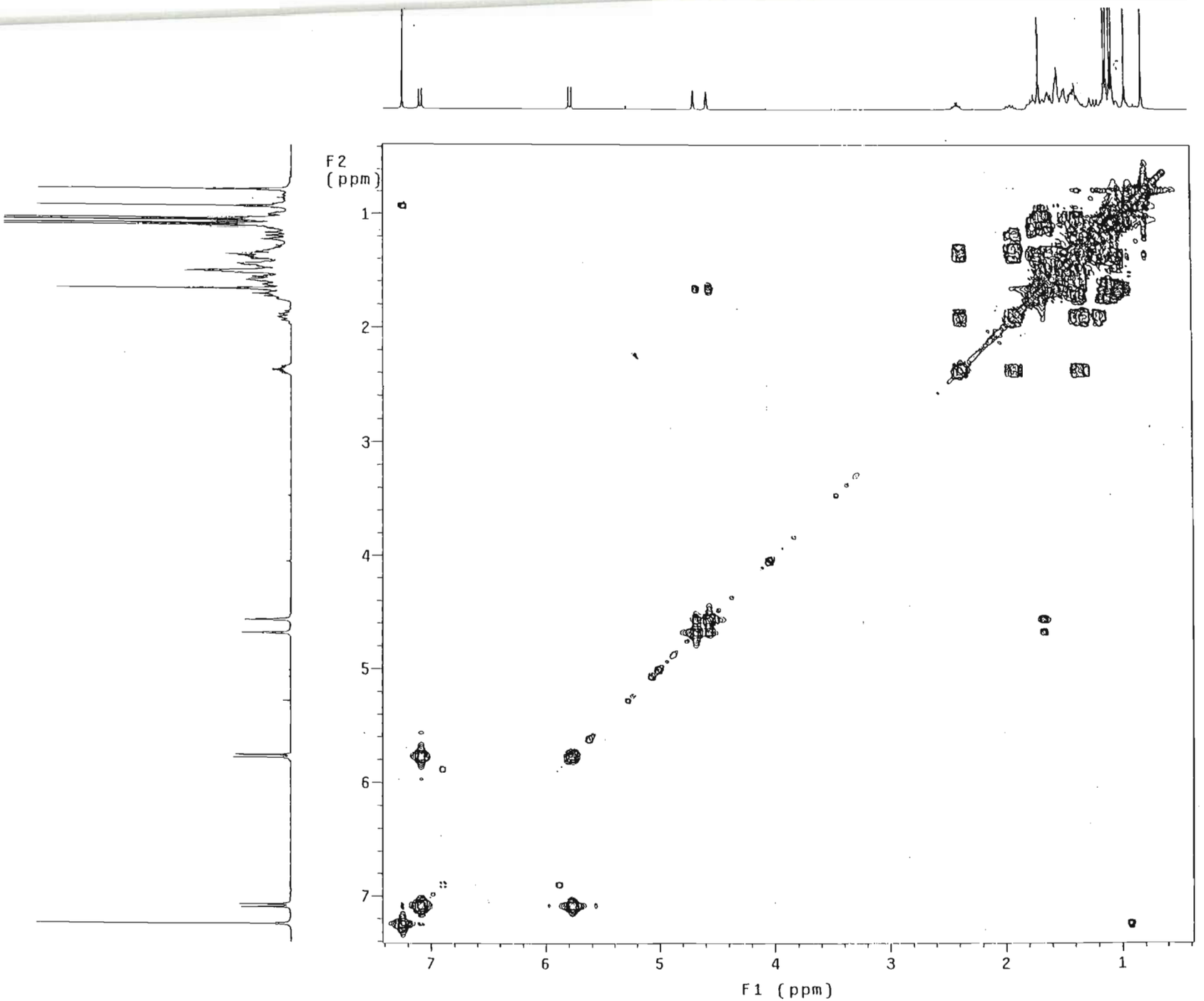
Spectrum [13-A]: ^{1}H NMR spectrum of glochidone (**XIII**) in $CDCl_3$



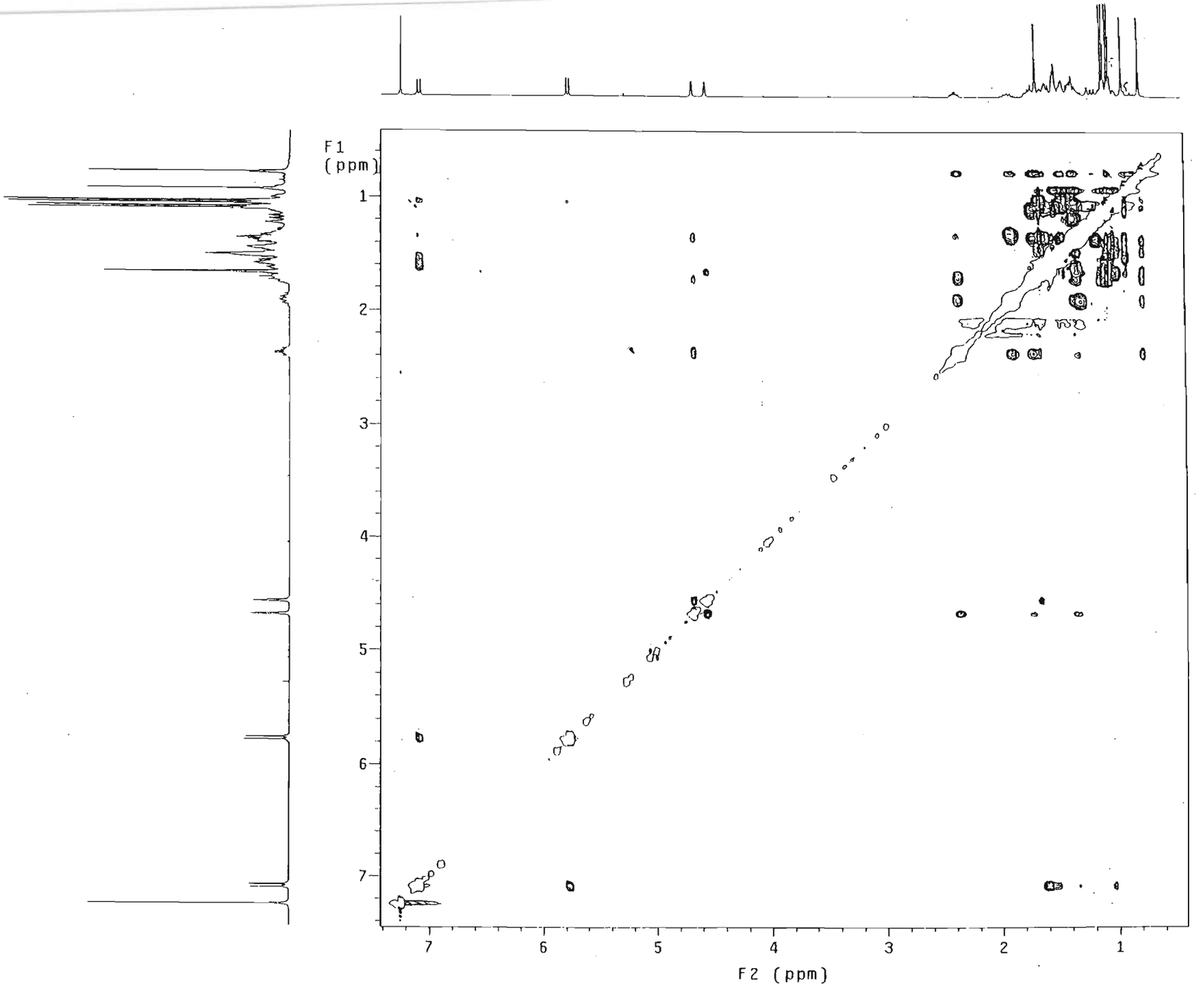
Spectrum [13-B]: ^{13}C NMR spectrum of glochidone (**XIII**) in $CDCl_3$



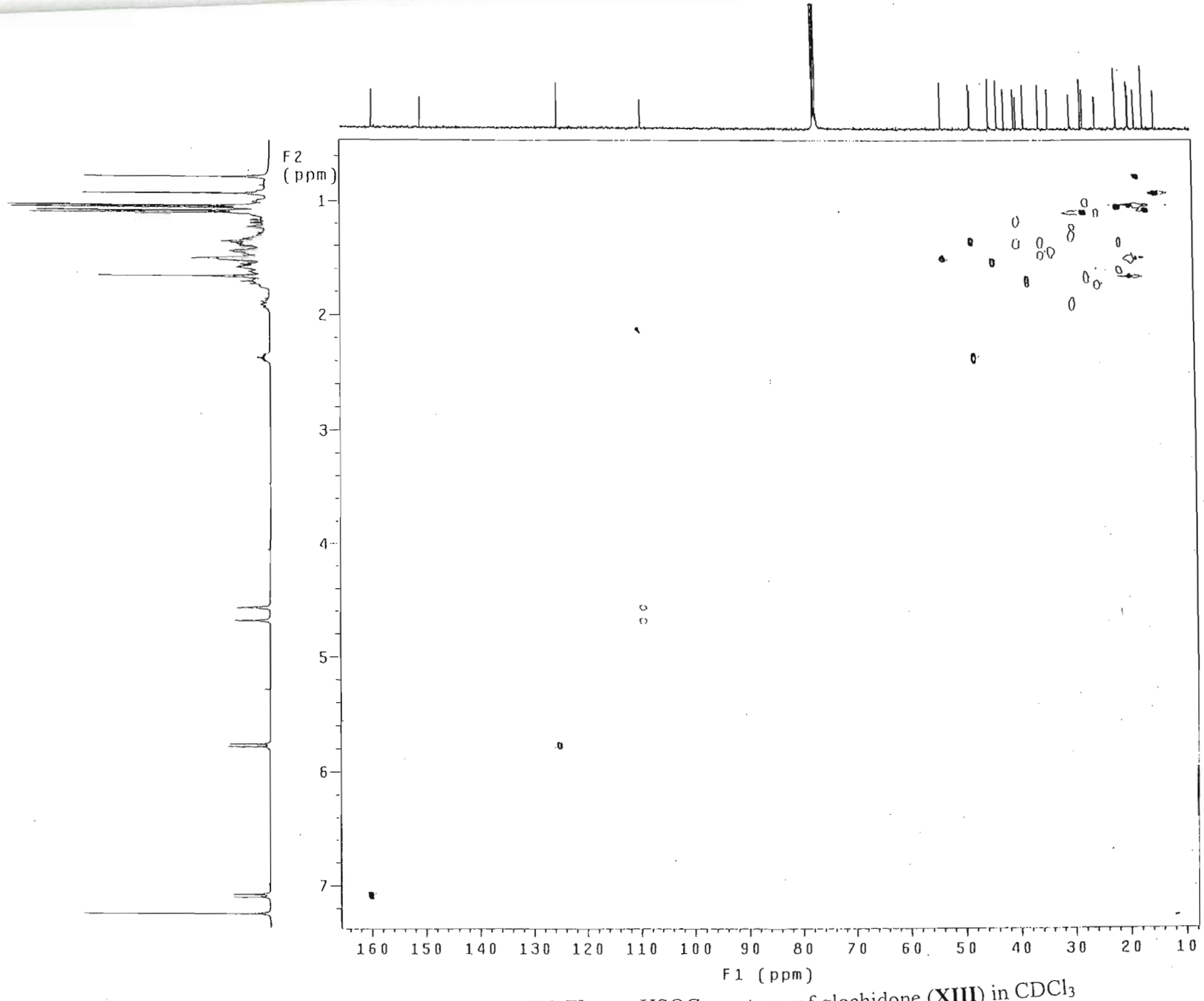
Spectrum [13-C]: ADEPT spectrum of glochidone (**XIII**) in $CDCl_3$



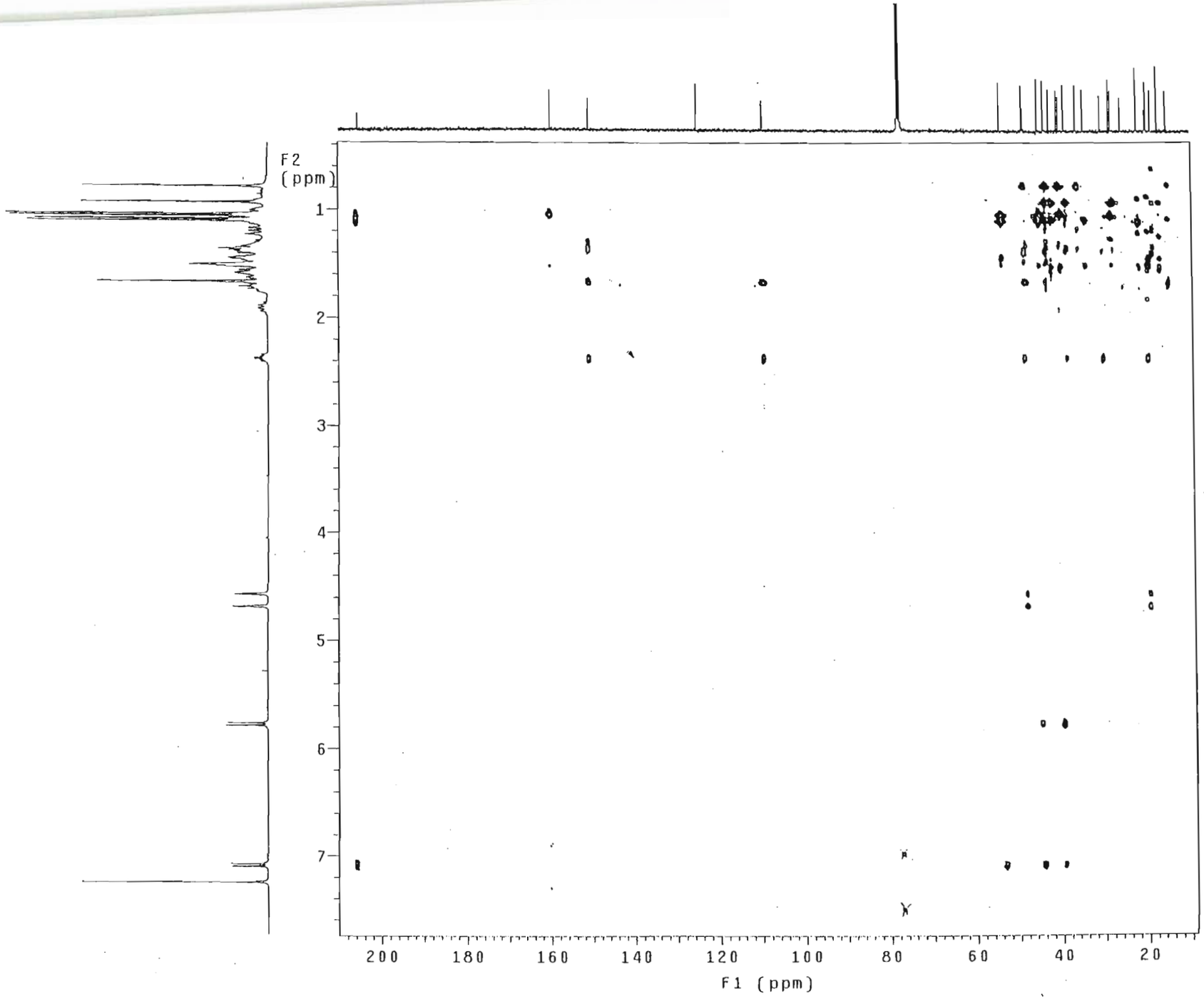
Spectrum [13-D]: COSY spectrum of glochidone (**XIII**) in $CDCl_3$



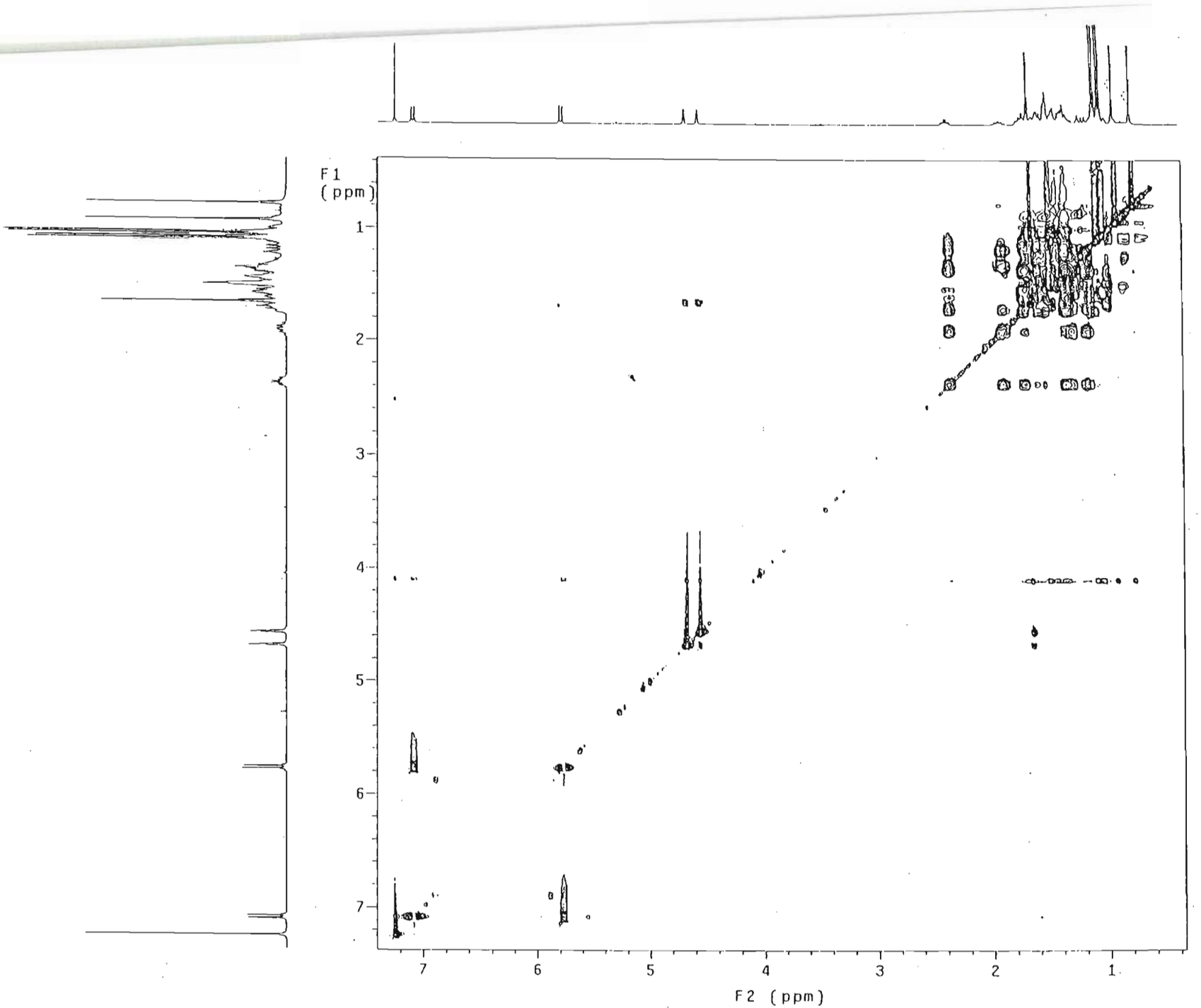
Spectrum [13-E]: NOESY spectrum of glochidone (**XIII**) in $CDCl_3$



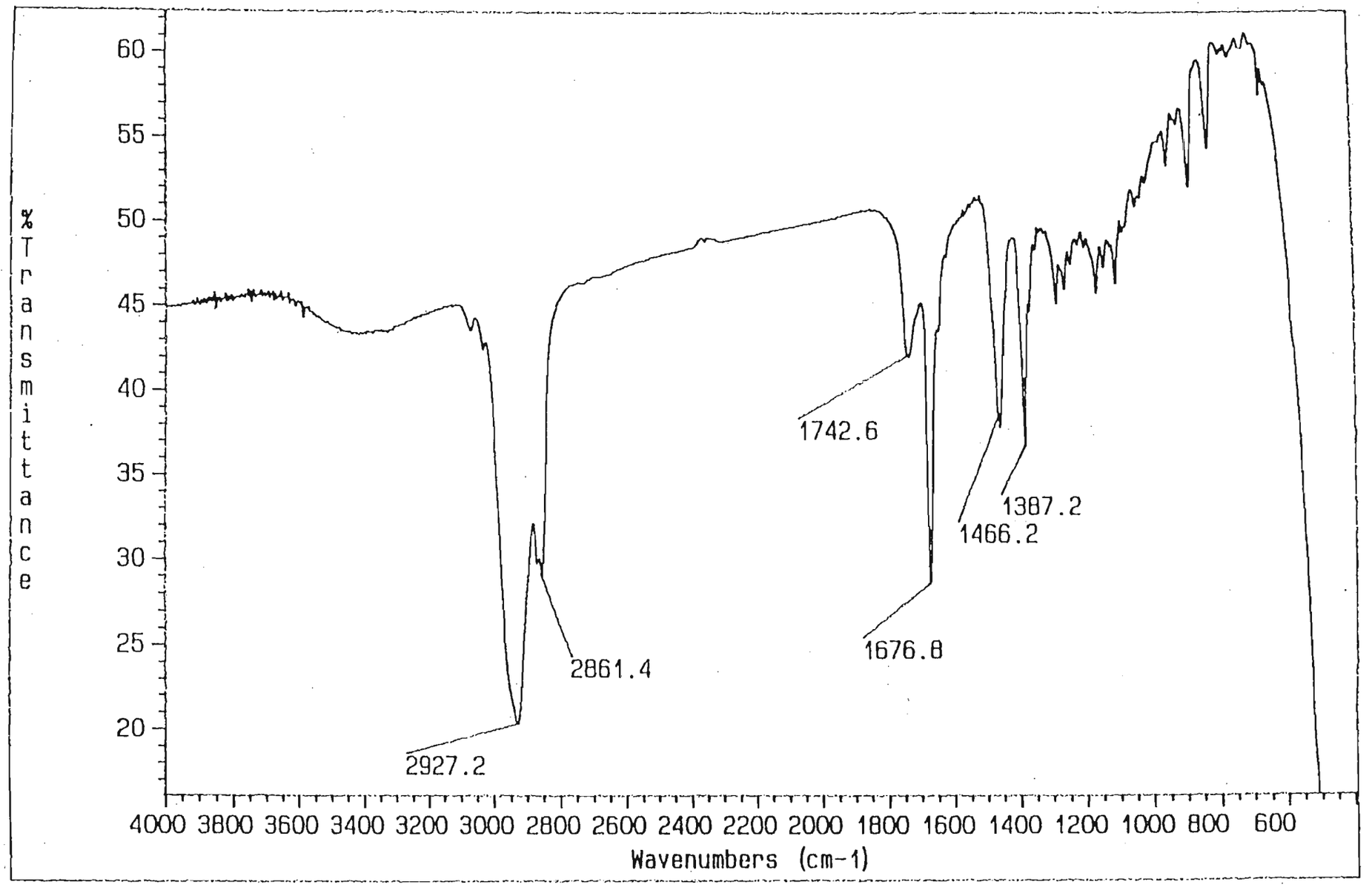
Spectrum [13-F]: HSQC spectrum of glochidone (**XIII**) in $CDCl_3$



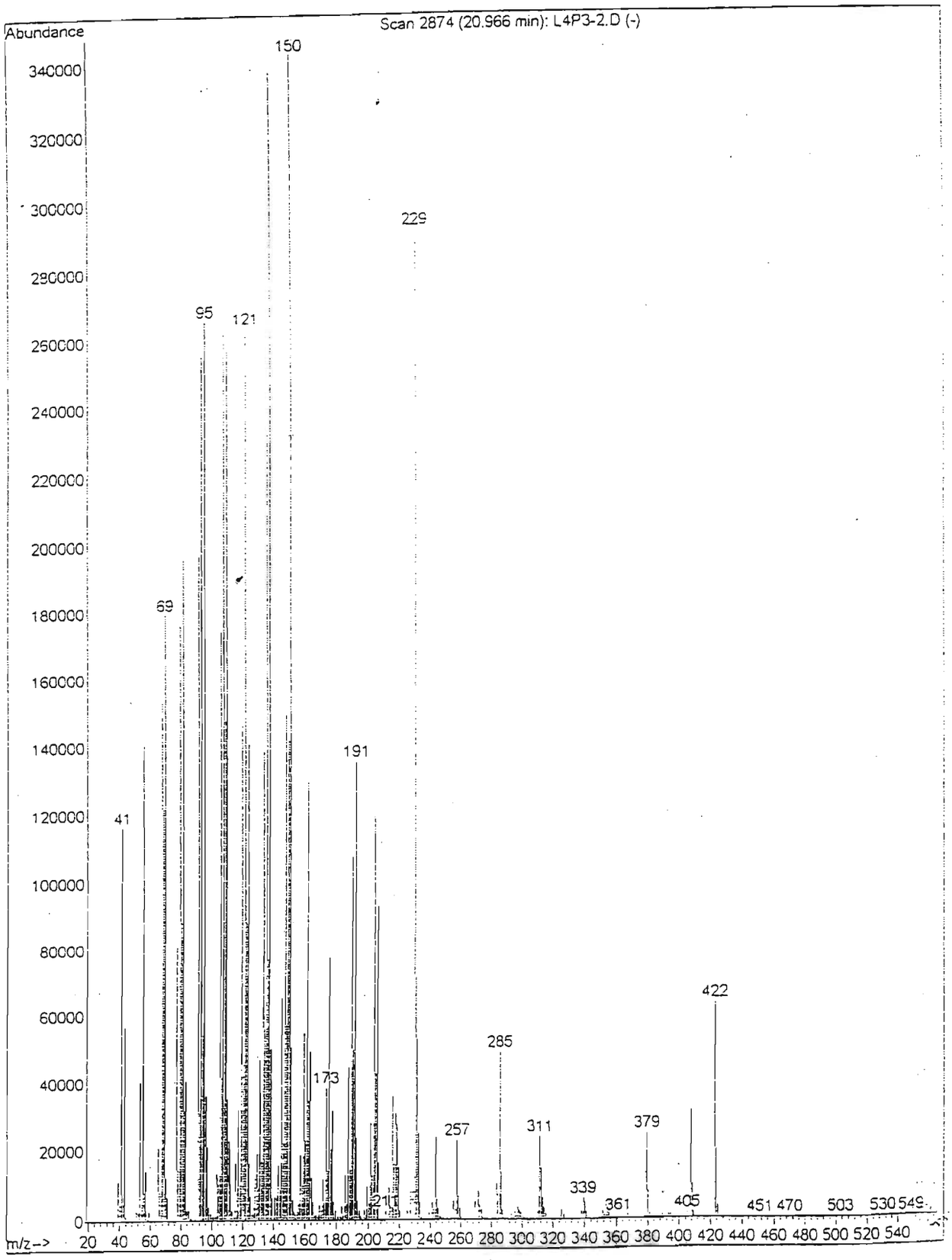
Spectrum [13-G]: HMBC spectrum of glochidone (**XIII**) in $CDCl_3$



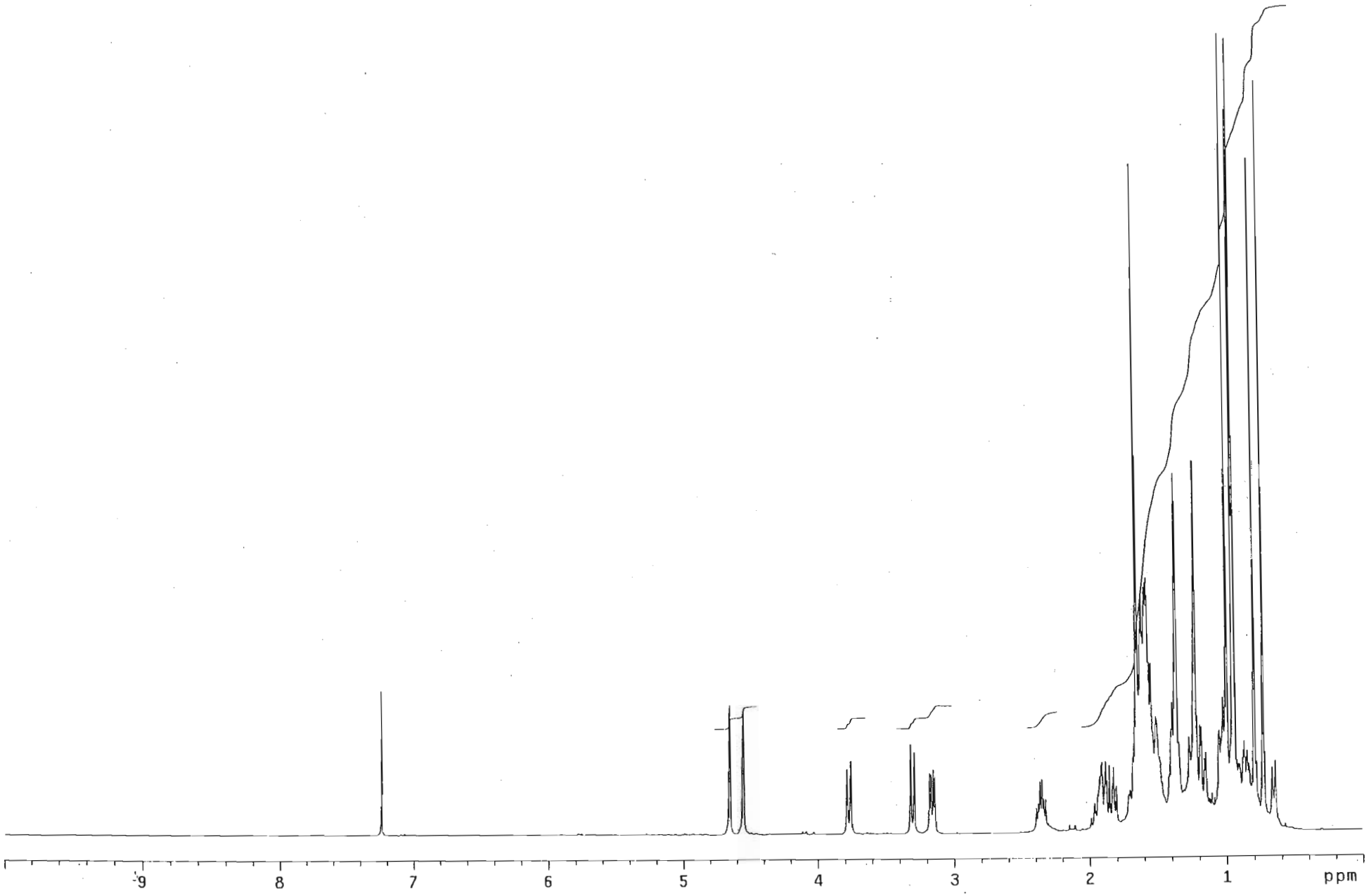
Spectrum [13-H]: TOCSY spectrum of glochidone (**XIII**) in $CDCl_3$



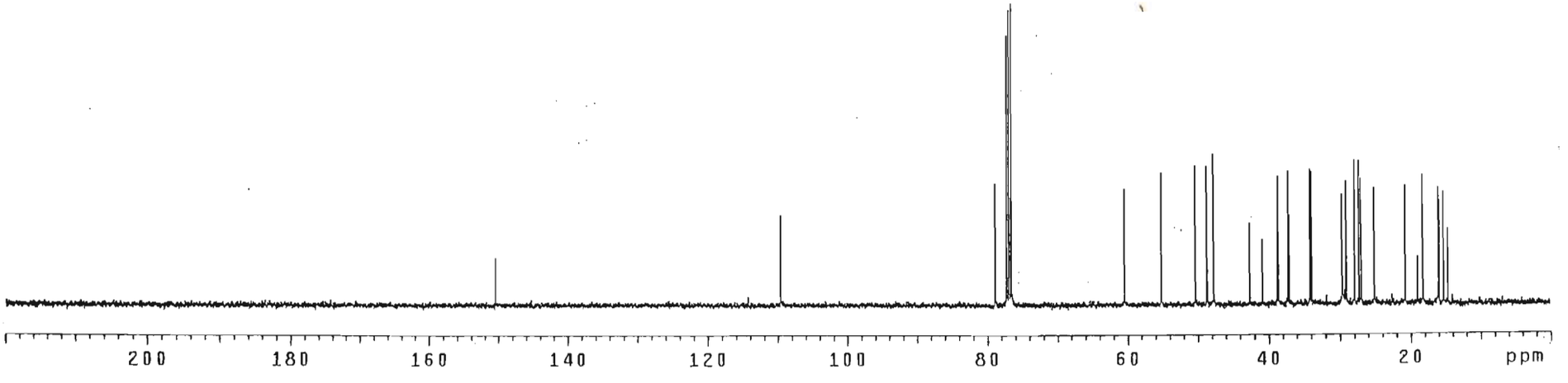
Spectrum [13-I]: Infra red spectrum of glochidone (XIII)



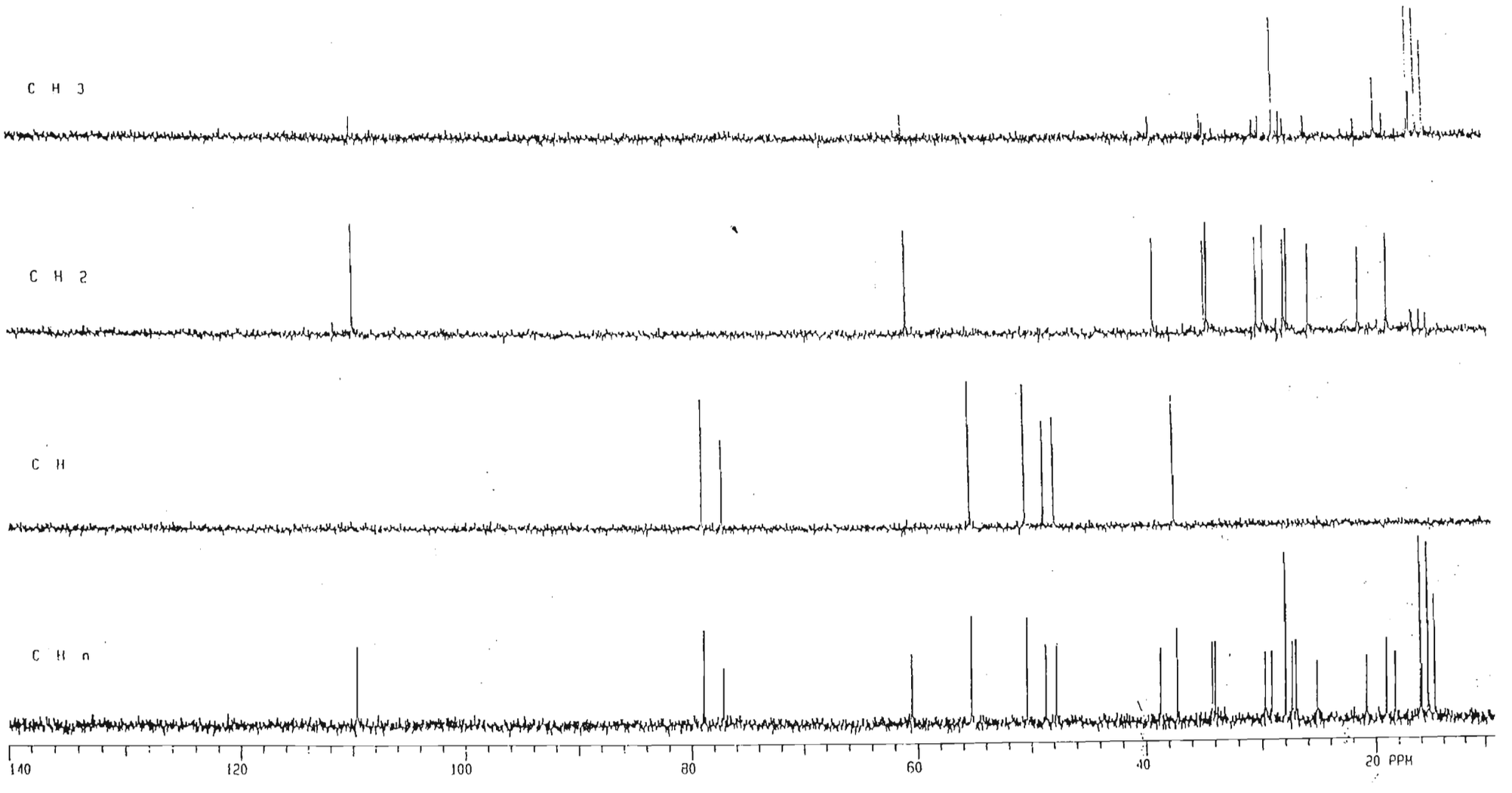
Spectrum [13-J]: Mass spectrum of glochidone (XIII)



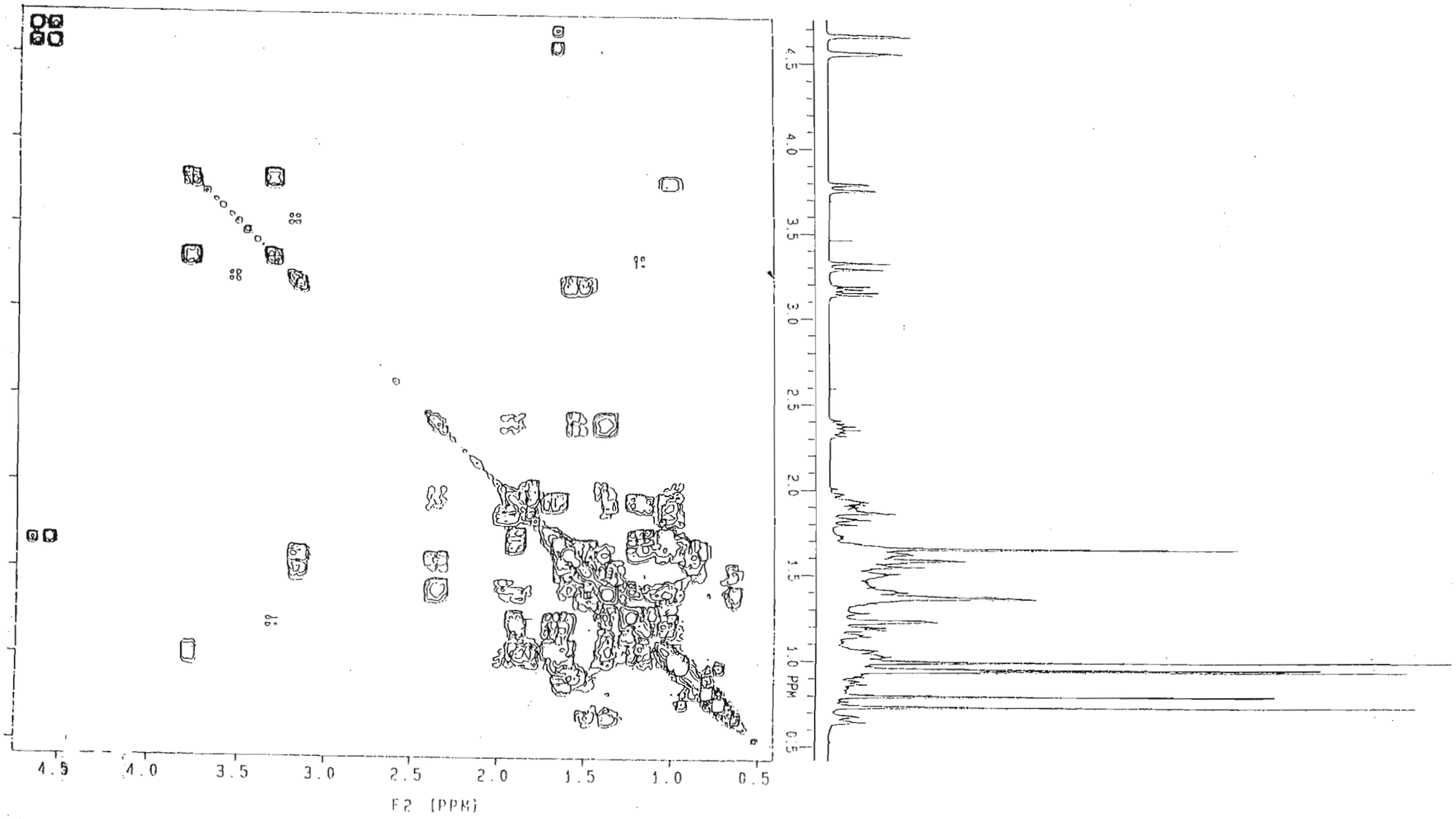
Spectrum [14-A]: ^{1}H NMR spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



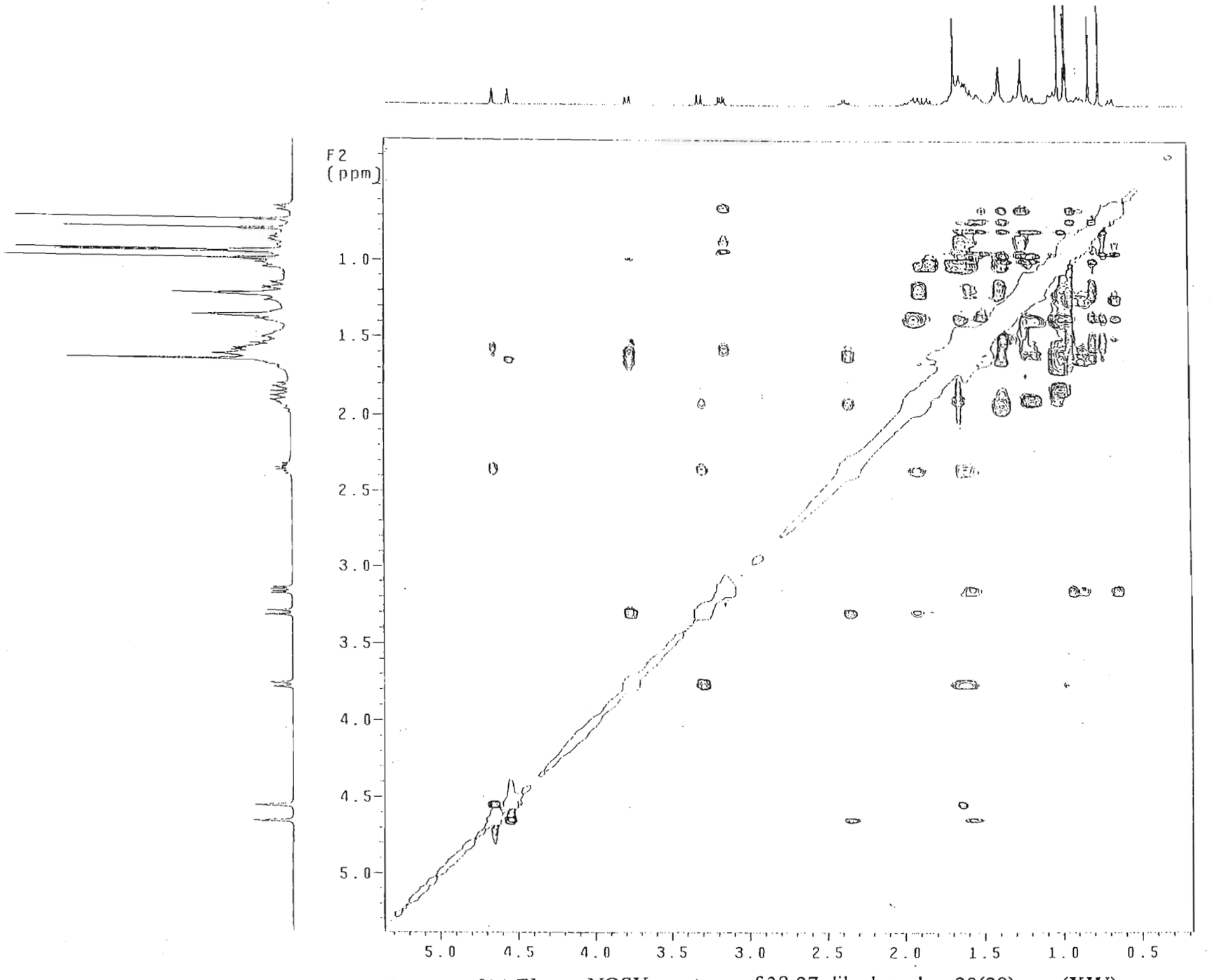
Spectrum [14-B]: ^{13}C NMR spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



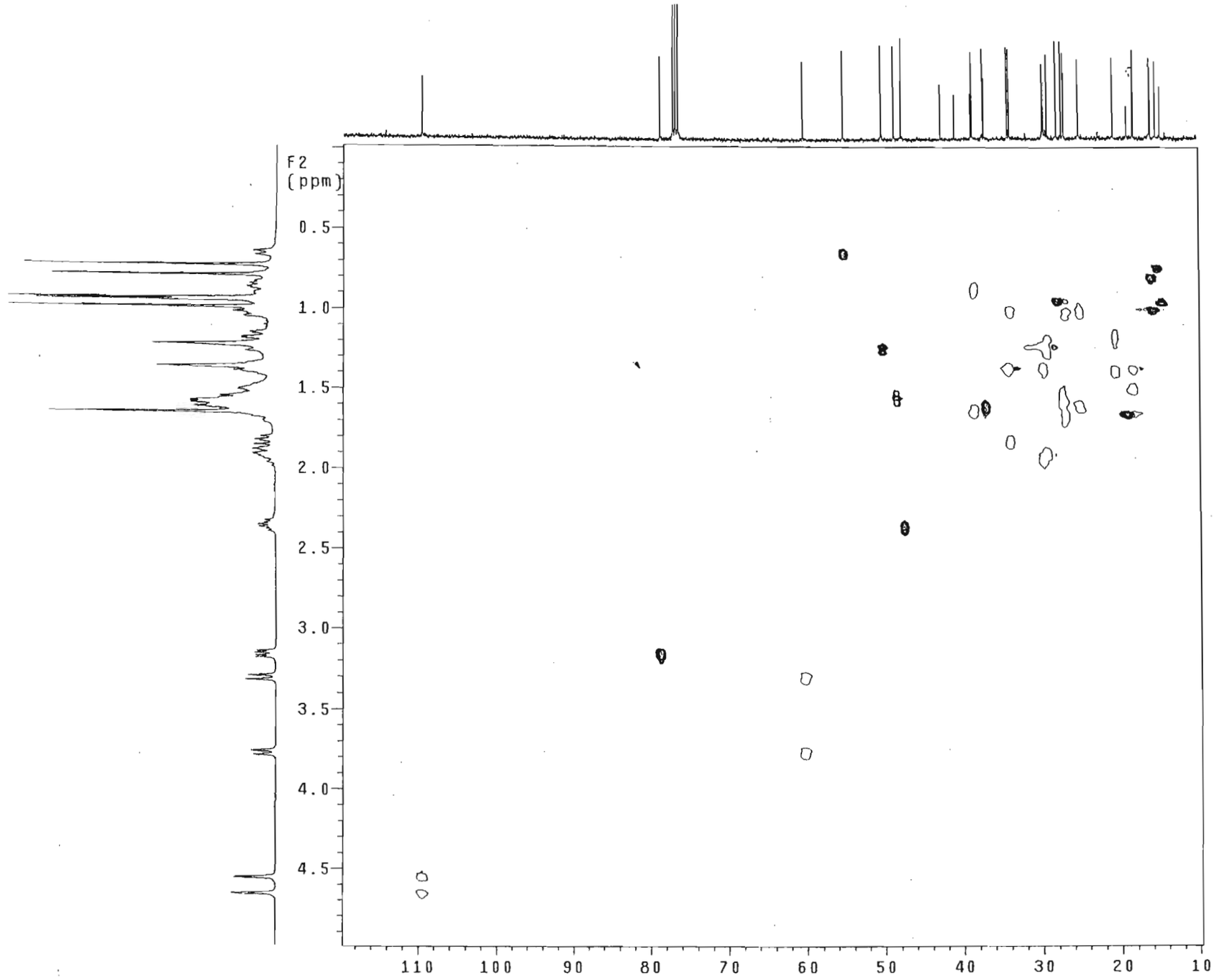
Spectrum [14-C]: ADEPT spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



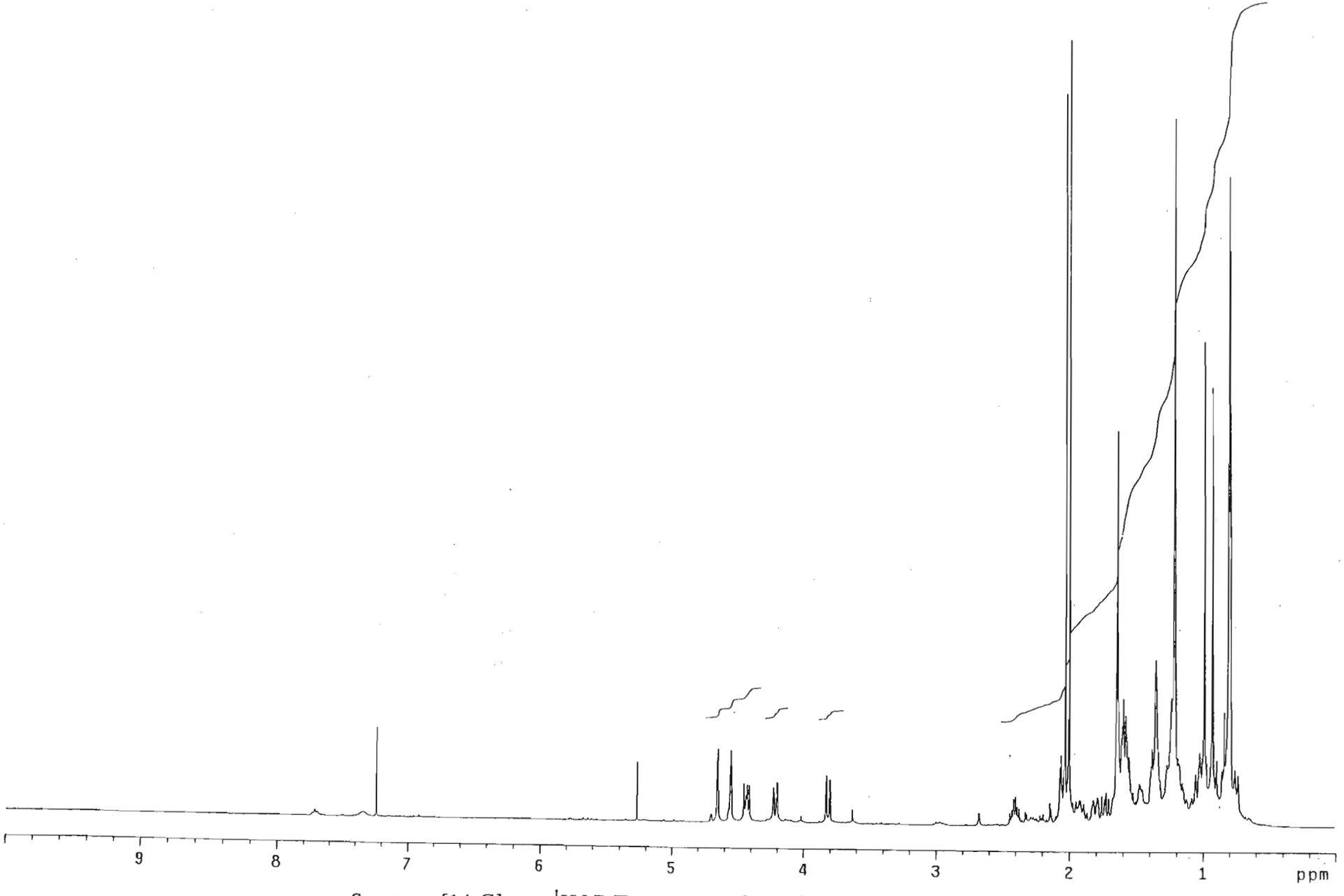
Spectrum [14-D]: COSY spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



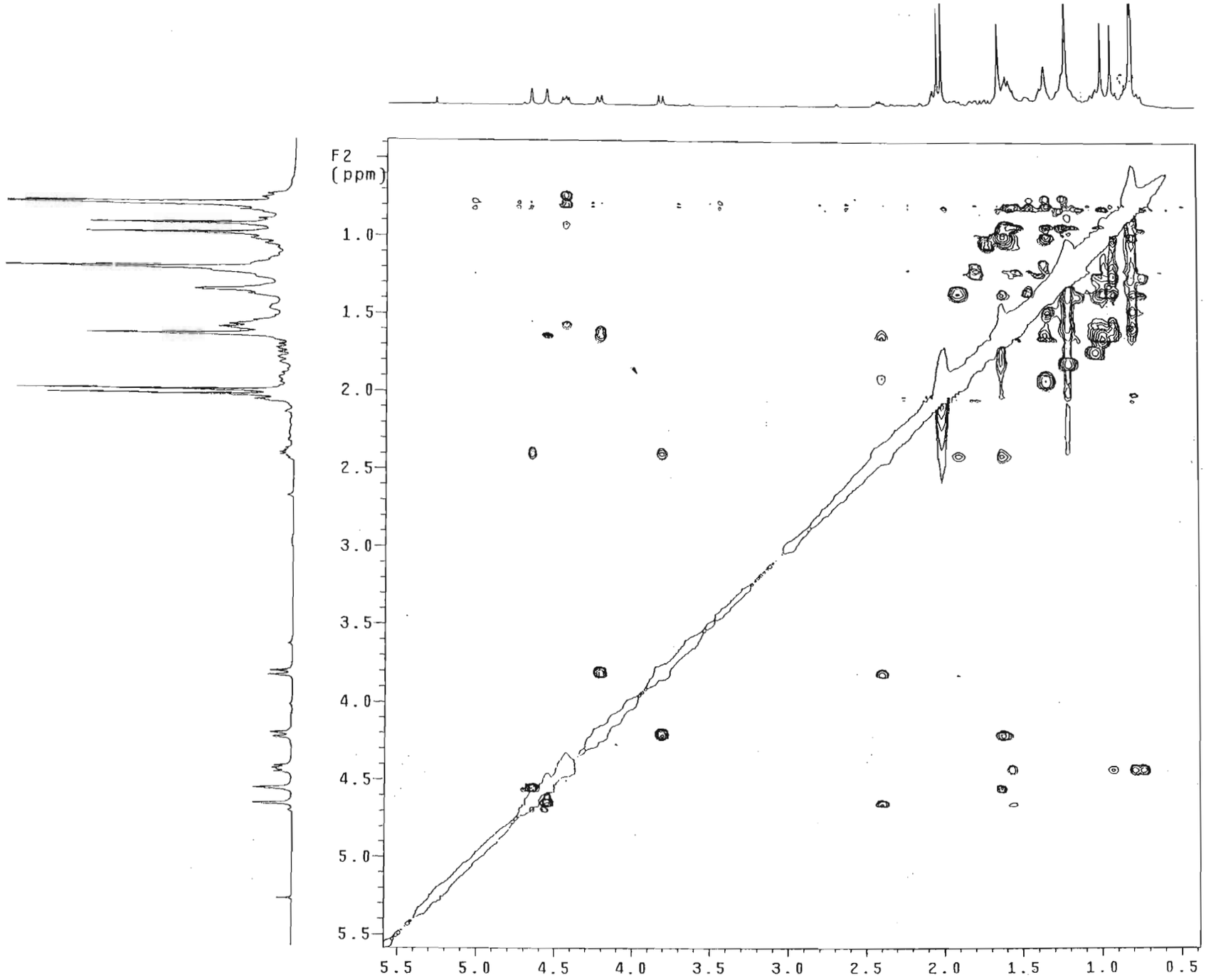
Spectrum [14-E]: NOSY spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



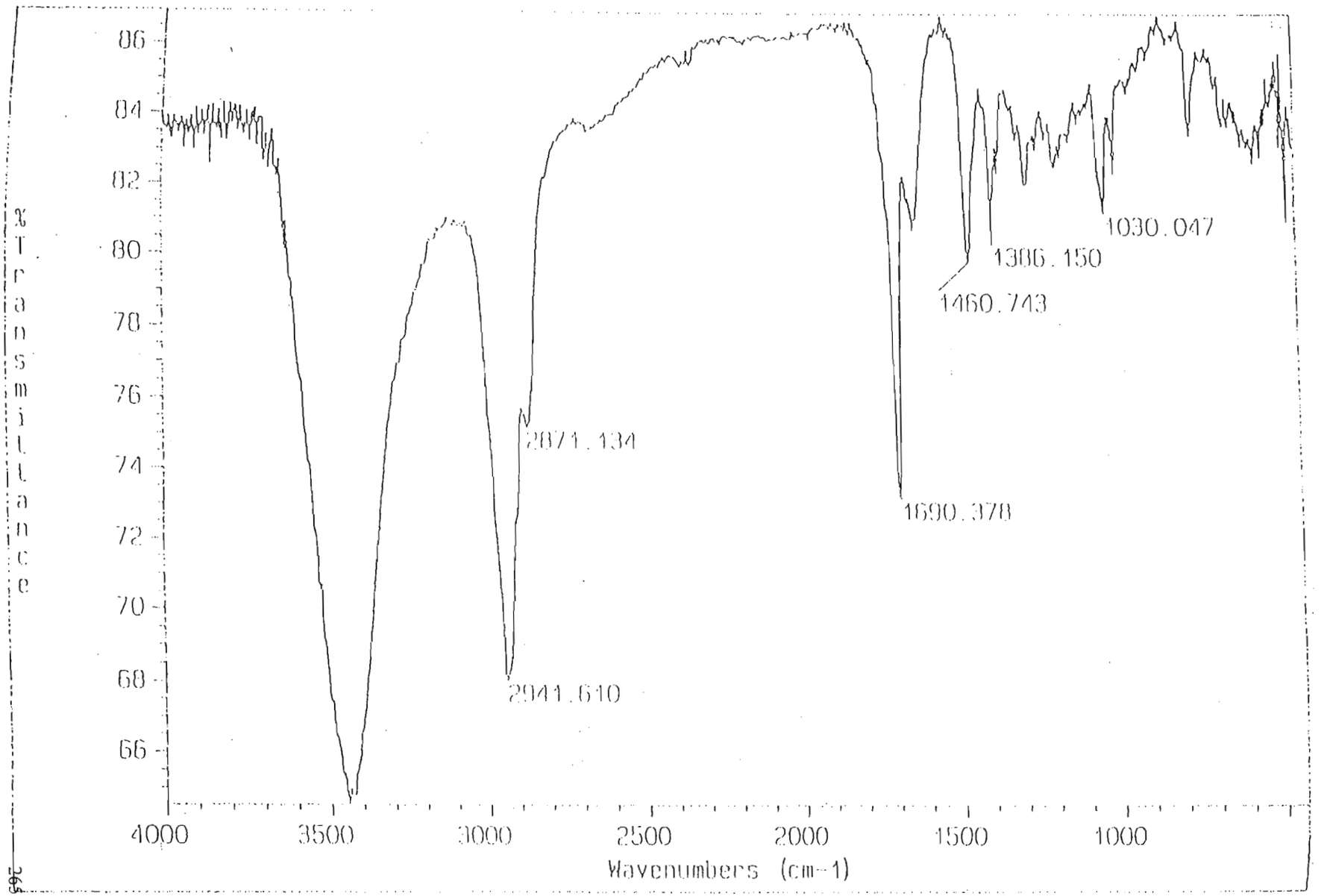
Spectrum [14-F]: HSQC spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



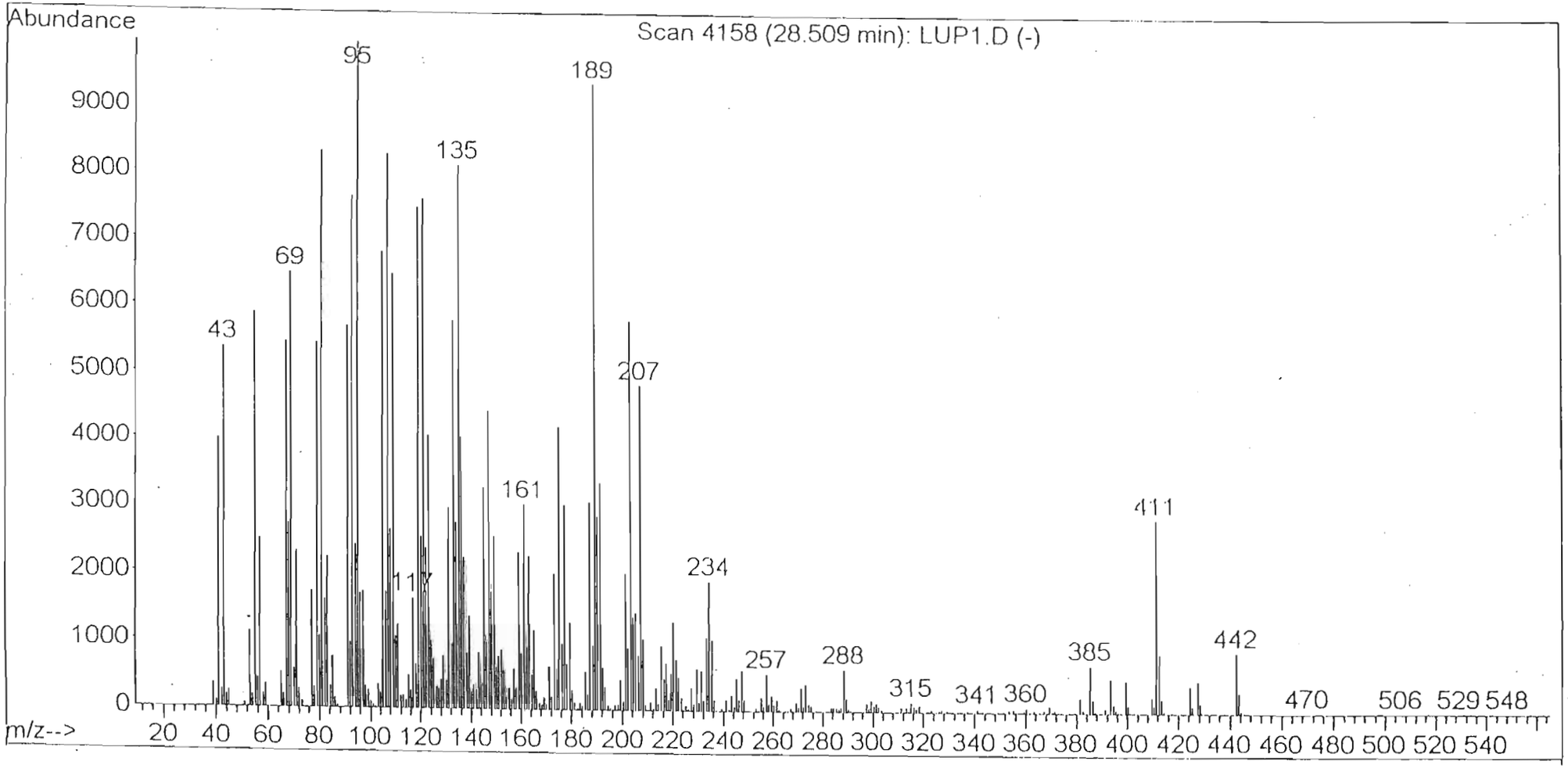
Spectrum [14-G]: ^{1}H NMR spectrum of acetylated 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



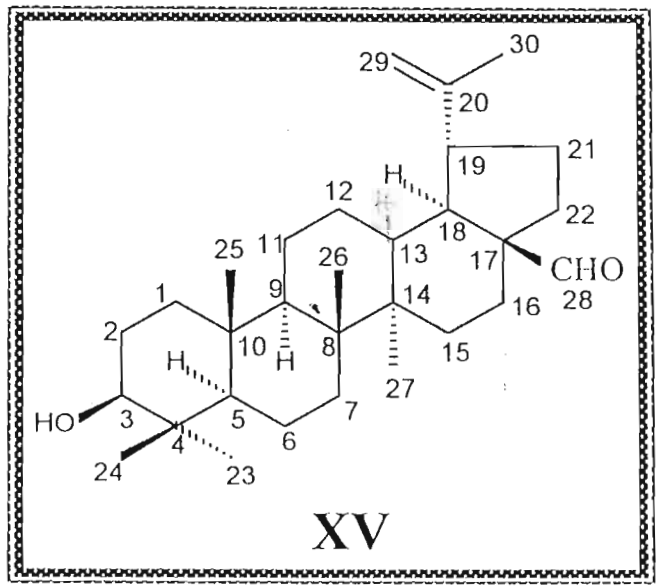
Spectrum [14-H]: NOSEY spectrum of acetylated 3β,27-dihydroxylup-20(29)-ene (**XIV**) in $CDCl_3$



Spectrum [14-I]: Infra red spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**)

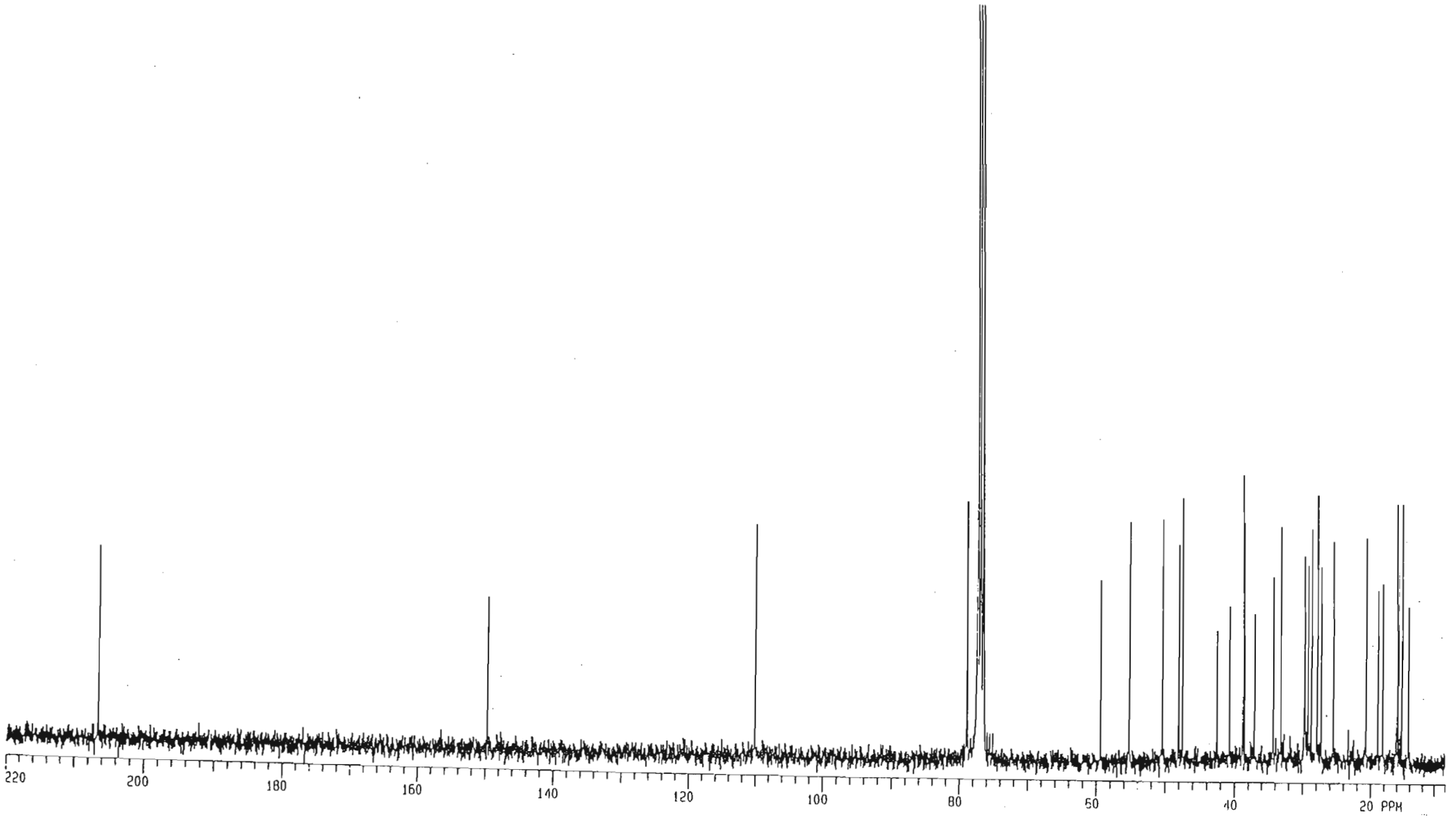


Spectrum [14-J]: Mass spectrum of 3β,27-dihydroxylup-20(29)-ene (**XIV**)

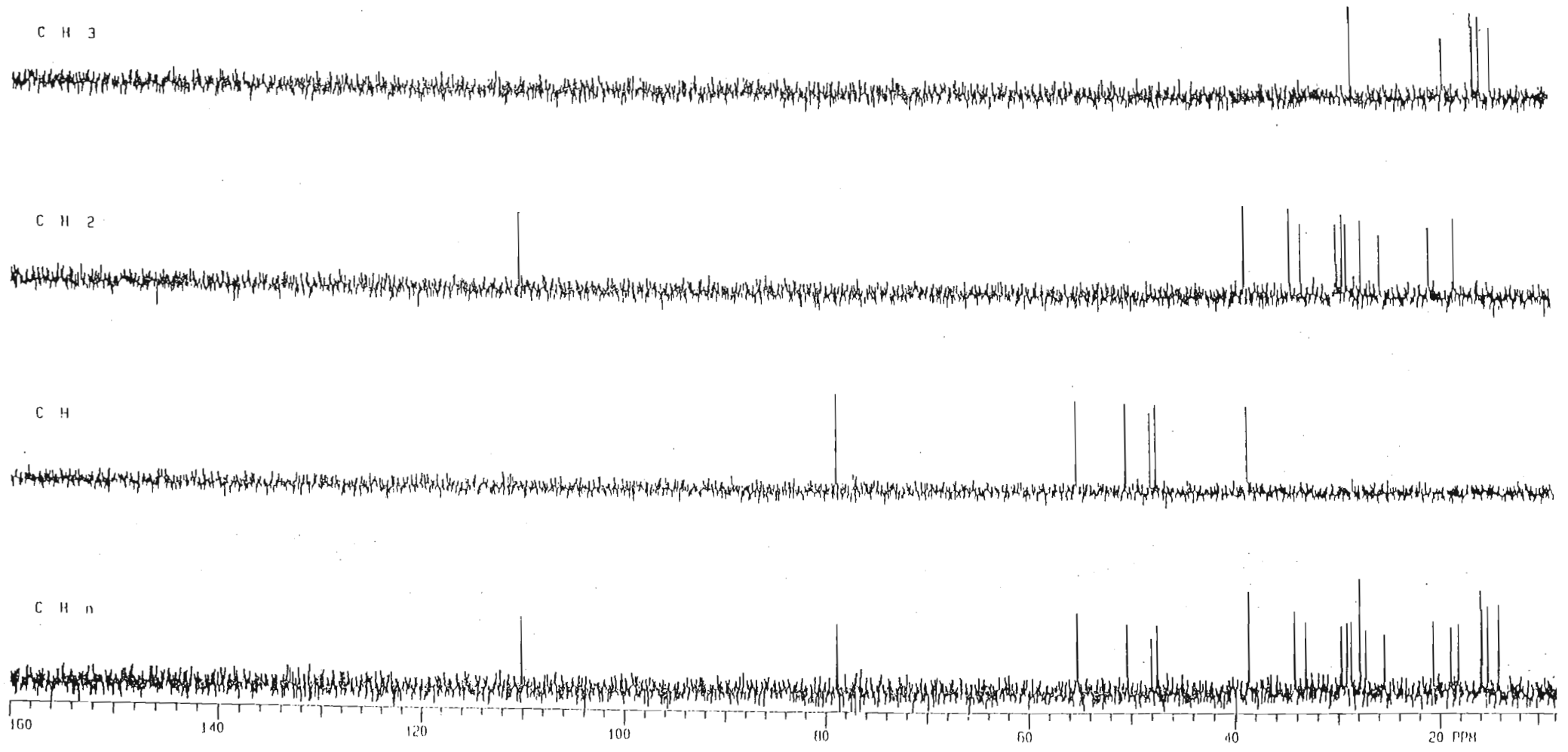


XV

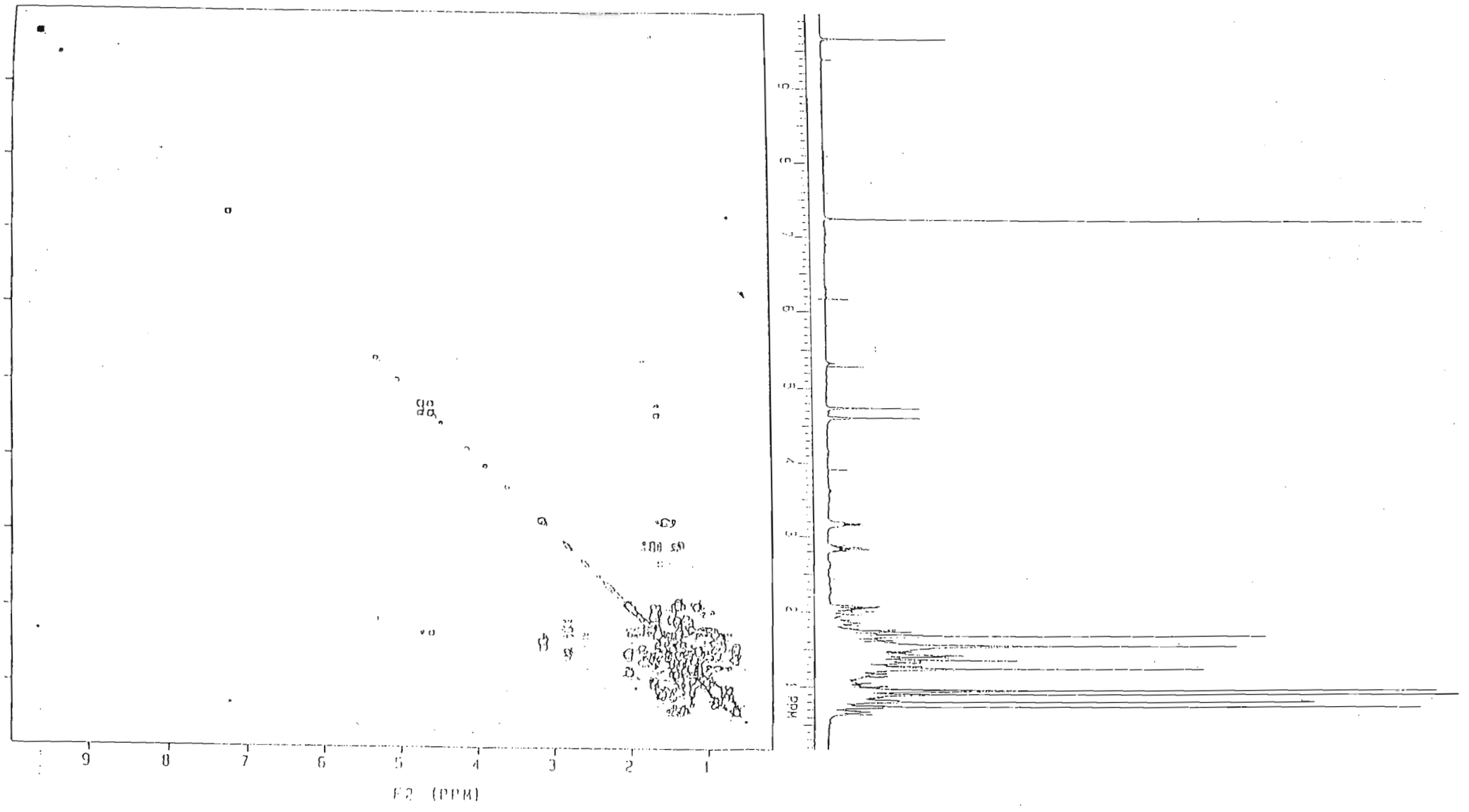
Spectrum [15-A]: ^{1}H NMR spectrum of betulinaldehyde (**XV**) in $CDCl_3$



Spectrum [15-B]: ^{13}C NMR spectrum of betulinaldehyde (**XV**) in $CDCl_3$



Spectrum [15-C]: ADEPT spectrum of betulinaldehyde (**XV**) in $CDCl_3$



Spectrum [15-D]: COSY spectrum of betulinaldehyde (**XV**) in $CDCl_3$