Extractives from the Amaryllidaceae

and

the Fabaceae

by

Neil Anthony Koorbanally
BSc (HONS) (Natal)

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for the degree of
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in the
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To my late mother

Yvonne Evelyn Bernadette Koorbanally,

for giving me the greatest gift of all,

the gift of life.

To my late grandfather,

Anthony Koorbanally,

for teaching me about life,

and

To my grandmother

Marie Louise Koorbanally,

for being beside me throughout my life.

This is for the three of you.

Knowledge and wisdom is acquired,

but the thirst for knowledge and wisdom is within.

Preface

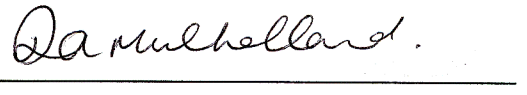
The experimental work described in this thesis was carried out in the Department of Chemistry, University of Natal, Durban, under the supervision of Professor D.A. Mulholland and Dr N. Crouch.

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 : 

N. A. Koorbanally
BSc (HONS) (Natal)

I hereby certify that the above statement is correct.

Signed : 

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

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A special thank you to my fiance, Chantal, whose patience and understanding has been tremendous throughout this period. It has all come to an end. Thank you also for your help with the final touches to this thesis.

Finally, I acknowledge the support of the University of Natal, Durban and the Foundation for Research and Development for awarding me the scholarship to continue with my studies. This award has been most valuable.

List of Abbreviations

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

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

COSY - correlated nuclear magnetic resonance spectrum

HETCOR - heteronuclear shift correlation nuclear magnetic resonance

NOE - nuclear overhauser effect

i.r. - infra red

m.p. - melting point

t.l.c. - thin layer chromatography

MS - mass spectroscopy

CD - circular dichroism

ppm - parts per million

s - singlet

d - doublet

t - triplet

q - quartet

m - multiplet

dd - doublet of doublets

td - triplet of doublets

brs - broad singlet

brd - broad doublet

Hz - Hertz

c - concentration

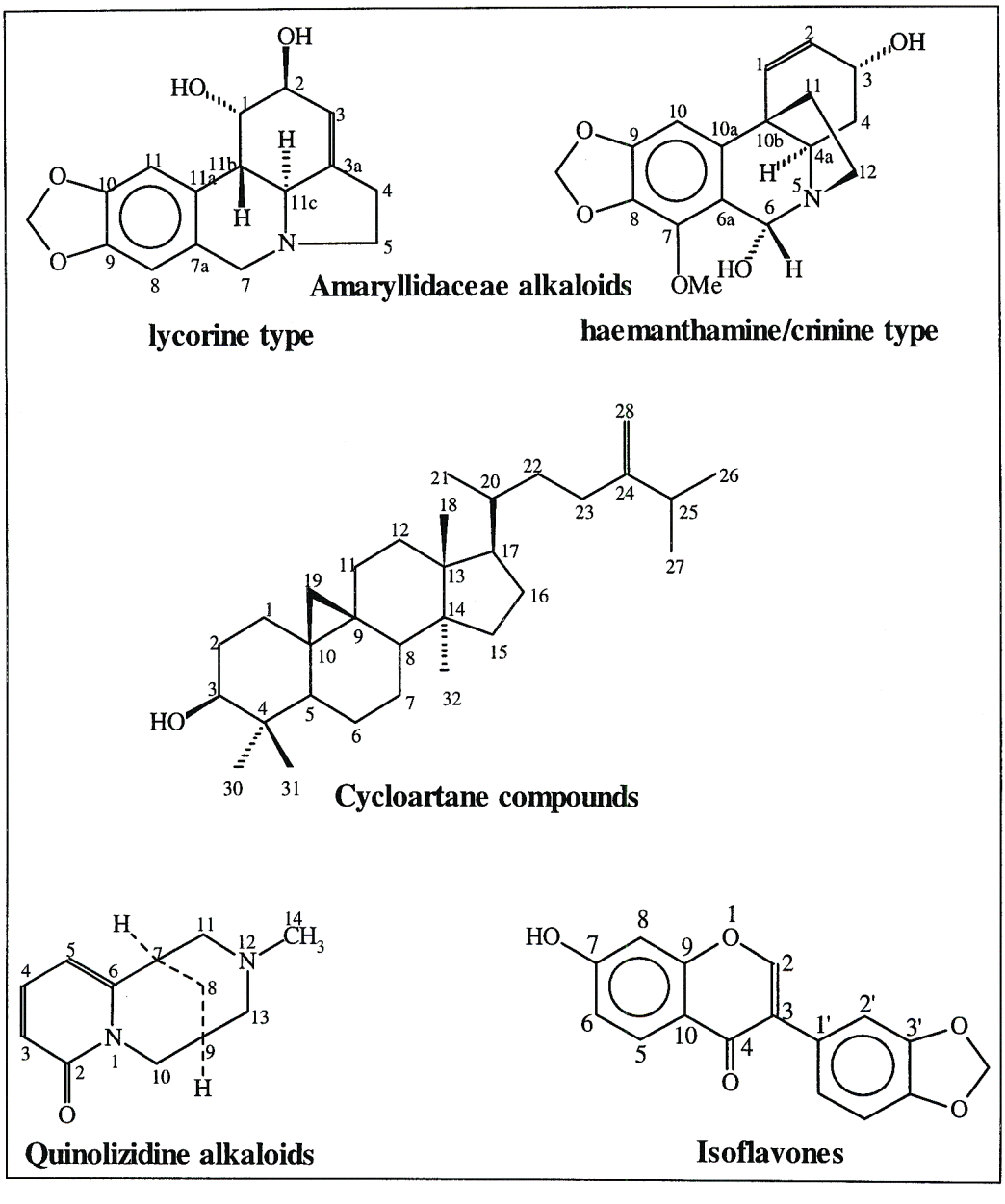
ax - axial

eq - equatorial

AA - ascorbic acid

Nomenclature

The numbering of the four classes of compounds described in the text are depicted below :



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Abstract

This work is an account of investigations into the chemistry of one of the members of the Amaryllidacae family, *Ammocharis coranica*, and one of the members of the Fabaceae family, *Sophora velutina.*

Chapter one is an account of the extractives from the bulbs of *Ammocharis coranica.* In all, twelve compounds, eight alkaloids and four cycloartane compounds have been isolated of which one alkaloid and one cycloartane compound have not been described previously. Plants belonging to the Amaryllidacae family have been used by traditional healers, especially in Africa, to treat a range of illnesses and diseases. The alkaloids isolated from these plants have been shown to exhibit responses to muscle stimulant, antiviral, antifungal, antiyeast, antimalarial, cytotoxic and antitumoural activities. *Ammocharis coranica* is used by the Zulu tribe in South Africa to treat any illness believed to be caused by witchcraft. Alkaloids from the three most common types among the isoquinoline group were found in this species. These are lycorine, 1-O-acetyllycorine, hippadine, acetylcaranine, and the novel 1-O-acetyl-9-norpluviine from the lycorine type, 6-α-hydroxypowelline from the crinine type and hamayne and crinamine from the haemanthamine type. Cycloartane compounds have not been reported previously from the Amaryllidaceae family. All four cycloartane compounds had a common side chain, containing an olefinic methylene group at position 24, but differed in their substituents at positions 3 and 4. These compounds were found to be 24-methylenecycloartan-3β-ol, cycloeucalenol, cycloeucalenone and the novel compound 24-methylenepollinastanone.

Chapter two is an account of the extractives from the seeds of *Sophora velutina.* The seeds of other *Sophora* species have been used in traditional ceremonies by the Indians of the Southwest United States and adjacent Mexico because of their hallucinogenic activity. The seeds of *Sophora velutina* subsp. *zimbabweensis* found in Zimbabwe are suspected to have historically been used by the natives for their hallucinogenic properties. These plants have been known to contain several quinolizidine alkaloids, flavonoids and isoflavonoids. One alkaloid, N-methylcytisine and two isoflavones, pseudobaptigenin and calycosin, as well as the common phytosterol, β-sitosterol were isolated from the seeds of this species.

N-methylcytisine is a common quinolizidine alkaloid, isolated previously from several *Sophora* species and pseudobaptigenin and calycosin are well known isoflavones, isolated previously from several species in the Fabaceae.

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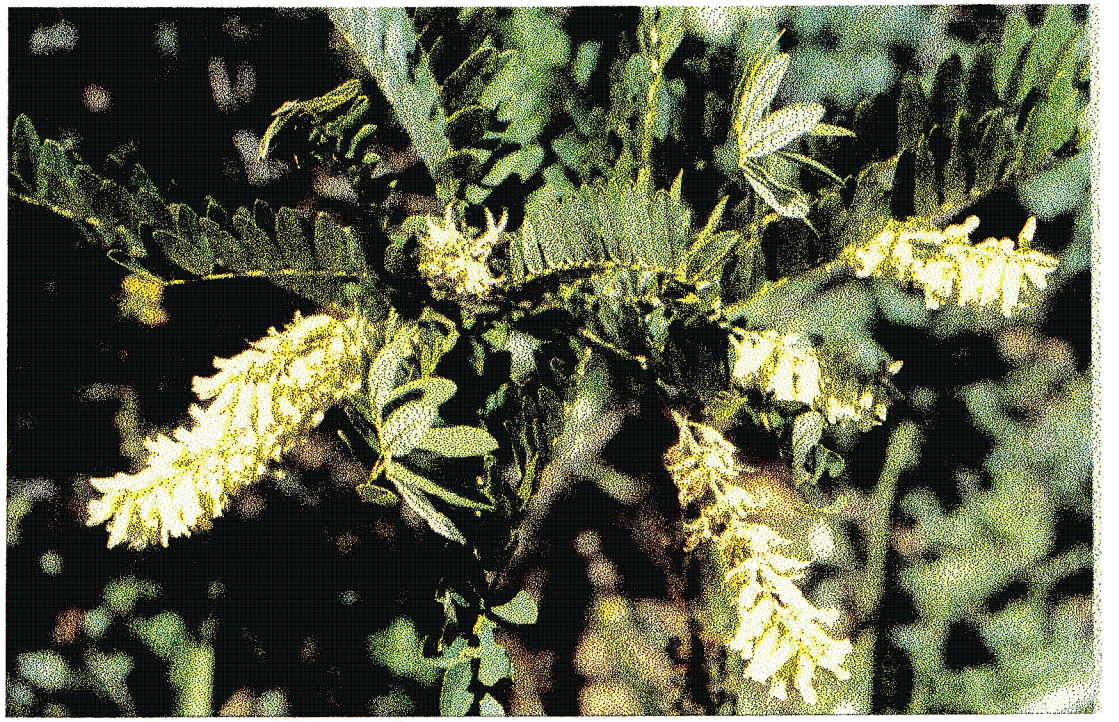
Ammocharis coranica

Ammocharis coranica

Sophora velutina subsp. *zimbabweensis*



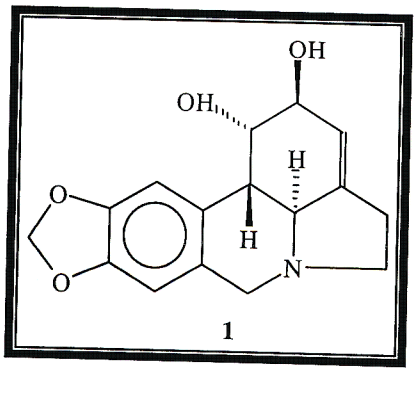
Sophora velutina subsp. *zimbabweensis*

Chapter 1. Extractives from the Amaryllidaceae. A chemical investigation of the bulbs of *Ammocharis coranica*.

1.1 An Introduction to Amaryllidaceae Alkaloids

Alkaloids are defined as basic, nitrogen-containing compounds of plant origin with complex molecular structures and significant pharmacological activity [1]. The term alkaloid, or "alkali-like" was first proposed by the pharmacist, W. Meissner, in 1819. Alkaloid chemistry dates back nearly two hundred years to when F.W. Serturner announced the isolation of morphine in 1805. Other pioneers in the field were Gomes who isolated an alkaloid known as "cinchonino" from an extract of cinchona bark in 1810 and P. J. Pelletier and J. B. Caventou who, in 1820, showed that "cinchonino" was a mixture which they separated into two new alkaloids named quinine and cinchonine. Because of the great contribution by P. J. Pelletier in the early nineteenth century to this field, the discoverer of emetine (1817), colchicine (1819), quinine (1820), cinchonine (1820), strychnine (1820), brucine (1820), caffeine (1820), piperine (1821) and thebaine (1835), the pelletierine group of alkaloids has been named after him [1].

In this work, alkaloids from the Amaryllidaceae family have been investigated. Previously, one-hundred and ten different alkaloids from sixty-two of the approximately two-hundred and fifty known species of the family Amaryllidaceae have been isolated and identified. The Amaryllidaceae alkaloids are a group of isoquinoline alkaloids found abundantly in this family. Lycorine (**1**) is probably the most featured alkaloid in this family, being isolated from twenty-eight of the sixty-two species which have been examined phytochemically [2].



1

A number of plants in this family have been used in medicinal preparations by the indigenous people of South Africa with physiological effects ranging from the facilitation of successful childbirth to use as a stimulant to induce trances and hallucinations [2]. Due to their exploitation in traditional medicine, these plants have become the focus of many pharmacological investigations and accordingly have been found to contain many biologically active alkaloids including some highly toxic ones[2-4]. Apart from having use in the medicinal field, a number of these plants are used as ornamentals, while others are grown for other economic uses [5,6].

Amaryllidaceae representatives are distributed through the three major areas of South Africa, from the savannah terrain in the north, to the tropical environment of the east and the winter rainfall area of the south-west. The Amaryllideae and Haemantheae are the two prominent tribes that feature in these areas [2].

1.1.1 Classification of Amaryllidaceae Alkaloids

The nomenclature of alkaloids has not been systematised. This is due mainly to the complexity of the compounds involved and for historical reasons. The two commonly used systems classify alkaloids either according to the plant genera in which they occur or on the basis of similarity of molecular structure. The latter system derives alkaloid names based on the skeletal feature which members of a group possess in common. For example, indole alkaloids contain an indole or modified indole nucleus and the isoquinoline alkaloids contain an isoquinoline or modified isoquinoline skeleton [1].

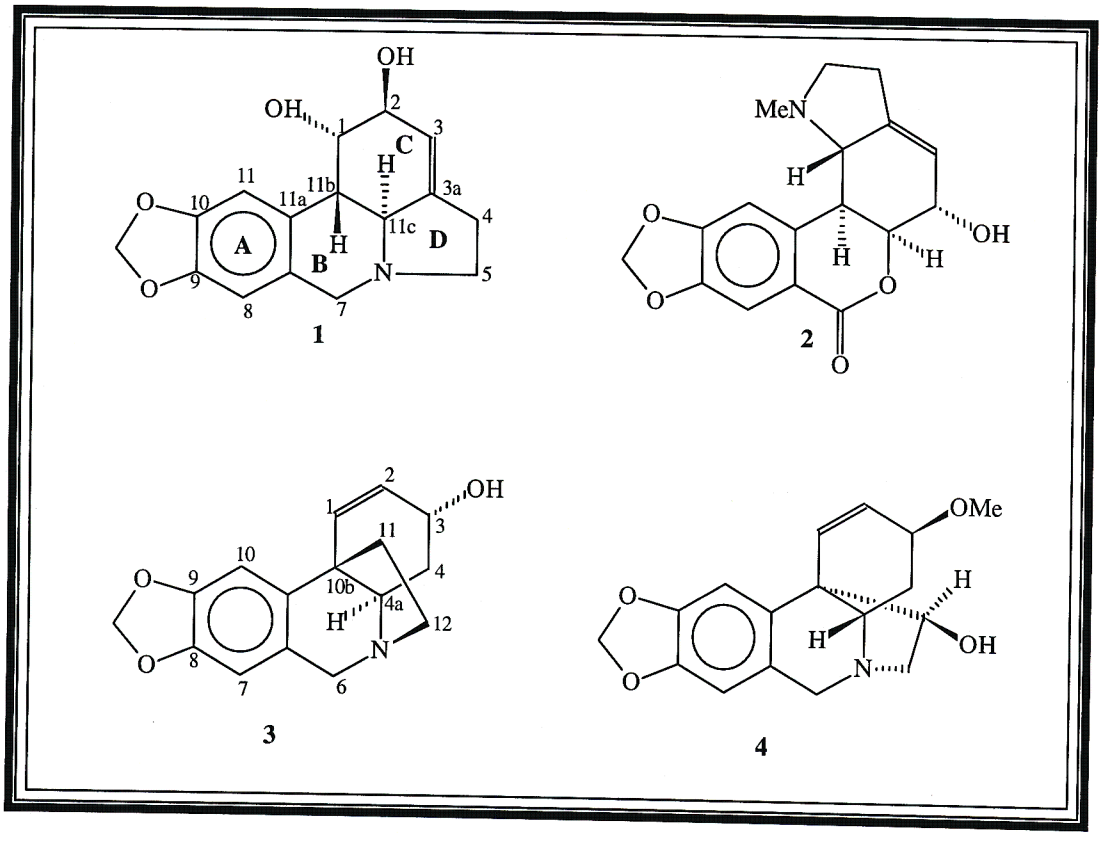
The alkaloids from the Amaryllidaceae make use of both systems. The alkaloids found in this family are a group of isoquinoline alkaloids found almost exclusively* in this family and hence can either be referred to as Amaryllidaceae alkaloids or isoquinoline alkaloids. The Amaryllidaceae alkaloids are classified according to the position of rings C and D and the heteroatom present in ring B. Using this as a basis, these alkaloids can be classified into ten groups. These ten groups are listed in Table 1. The characteristics of the tazettine, montanine, bufalvine and miscellaneous types somewhat differ from the norm and will be mentioned separately.

In the other six groups, rings A, B and C are six-membered rings while ring D is five-membered. The heteroatom in ring B is nitrogen but this is replaced by oxygen in the homolycorine and tazettine type alkaloids. Referring to the numbering system of lycorine (**1**), ring C is usually attached to ring B at the 11b and 11c positions. The position of the five membered ring D, absent in the narciclasine group, usually determines which of the six groups the alkaloid should be assigned to.

* There are three exceptions, lycorine and acetylcaranine which were isolated from *Urginea altissima*[24] (Hyacinthaceae) and crinamine which was reported in *Dioscorea dregeana* [25] (Dioscoreaceae).

Table 1. Categorization of Amaryllidaceae alkaloids (all with intact A ring)

Group	example	ring B	ring C	ring D
Lycorine	lycorine (**1**)	intact	intact	intact
Homolycorine	hippeastrine (**2**)	O replaces N	intact	moves to 1,2 position
Crinine	crinine (**3**)	intact	intact	β-5-10b-ethano bridge
Haemanthamine	haemanthamine (**4**)	intact	intact	α-5-10b-ethano bridge
Tazettine	tazzetine (**5**)	O replaces N	At 1 position on ring B	between rings B and C
Narciclasine	narciclasine (**6**)	intact	intact	absent
Montanine	montanine (**7**)	7-membered	intact	bridge within ring B
Galanthamine	galanthamine (**8**)	open with N-Me group	intact	α-5-10b-ethano bridge
Buflavine	buflavine (**9**)	8-membered with N-Me	intact	absent
Miscellaneous	belladine (**10**)	variable	variable	variable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C
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11b
11a
3a
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B
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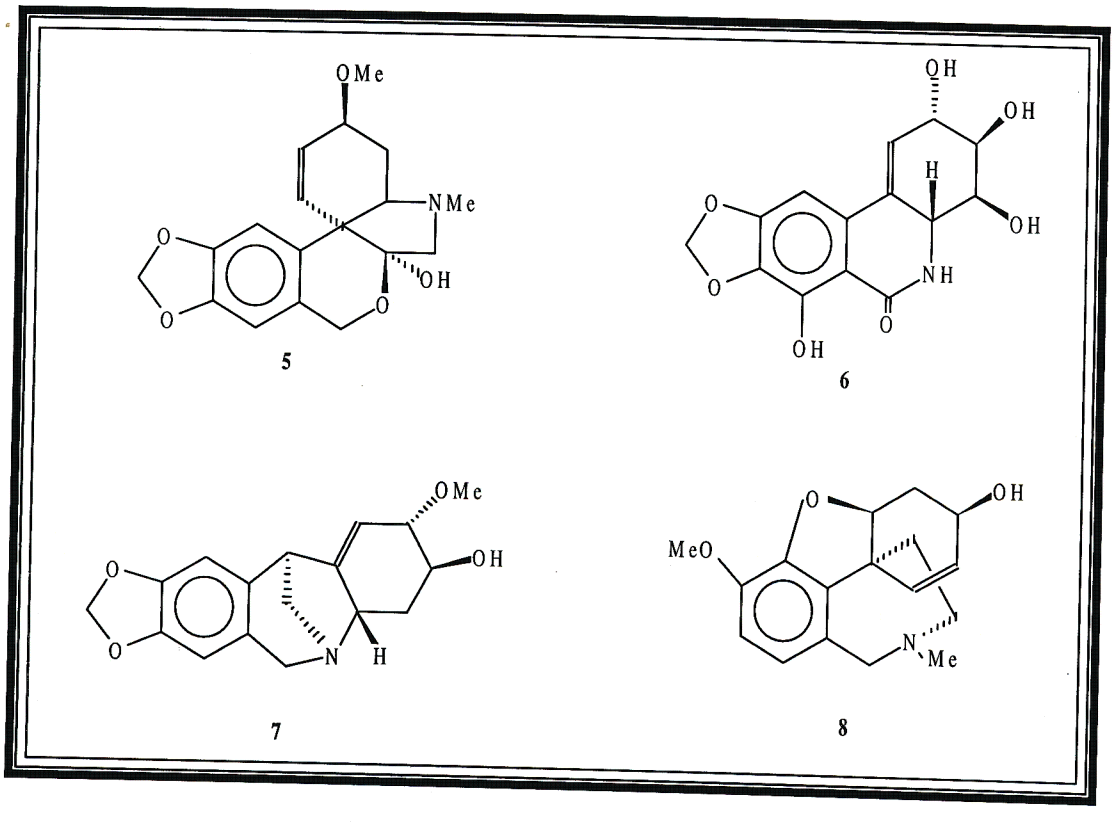
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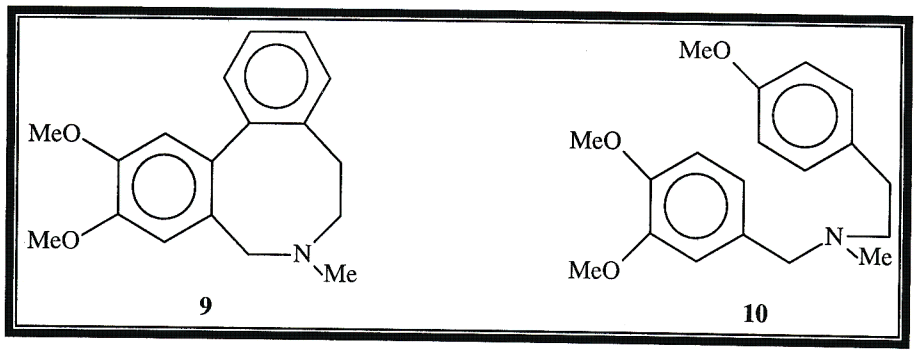
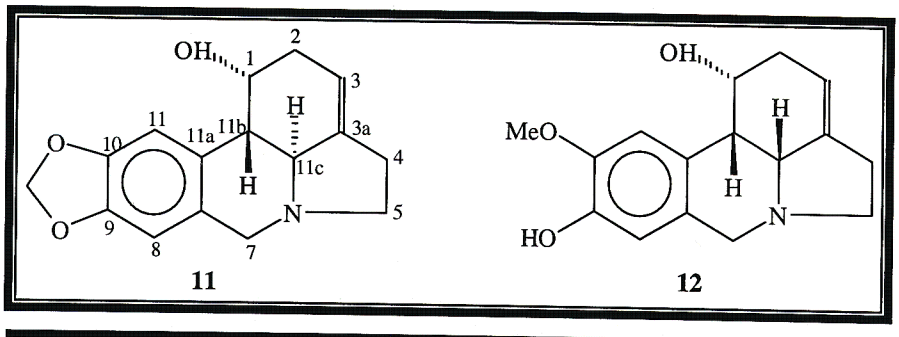
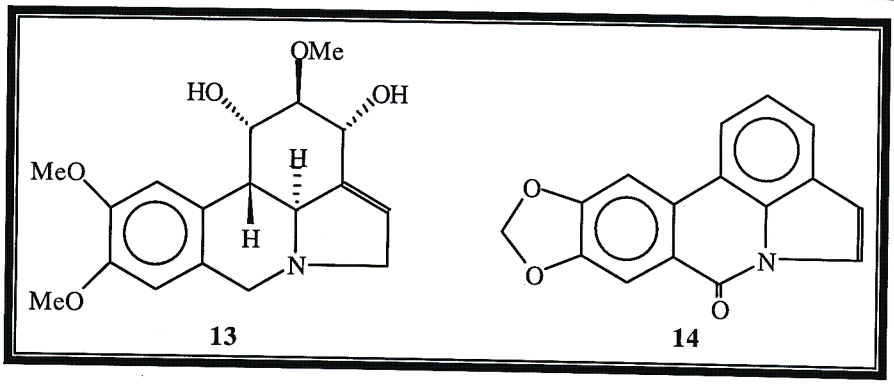


9 **10**

Since only alkaloids of the lycorine, crinine and haemanthamine type have been isolated in this work, these types will be discussed in detail. The other seven groups will be discussed briefly. The numbering systems for lycorine and crinine/haemanthamine differ because of the difference in position of ring D.

The lycorine type alkaloids (e.g. lycorine (**1**)) consist of an A, B, C and D ring. The A and B ring form the isoquinoline skeleton. Ring C is attached to the isoquinoline skeleton at C-11b and C-11c and ring D is attached at the nitrogen and C-3a. At position 1, either a hydroxy or acetoxy group is attached at the α position and a hydroxy, acetoxy or methoxy group can replace the β hydrogen at position 2. In some instances, such as in caranine (**11**), no substituent is present. A double bond is characteristic at the 3-3a position and a methylenedioxy group is present at the 9 and 10 positions but a hydroxy and/or a methoxy group can also occur here instead. At C-11, a methoxy group can replace the hydrogen. The hydrogen atoms at C-11b and C-11c are in the β and α positions respectively with the exception of kirkine (**12**) which has both hydrogen atoms orientated in the β position. The 3-3a double bond can also shift to the 3a-4 position as in narcissidine (**13**) with the addition of an α hydroxyl group situated at the 3 position. There are also lycorine type alkaloids with two aromatic rings, the A and C rings, such as in hippadine (**14**) which incidentally also has a double bond at the Δ^4 position and a carbonyl group at C-7.

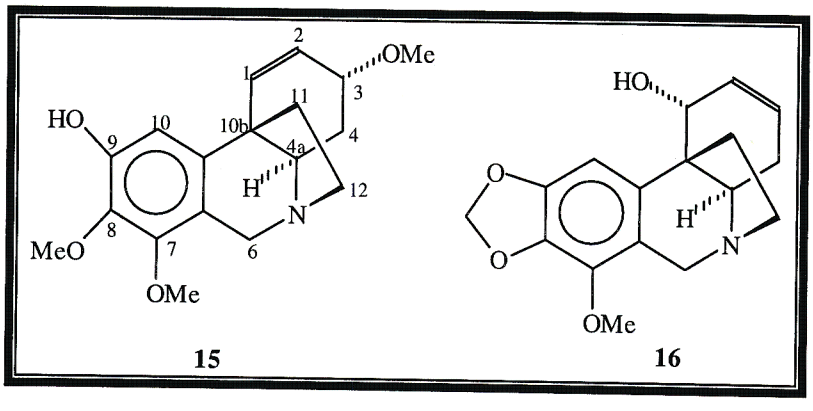




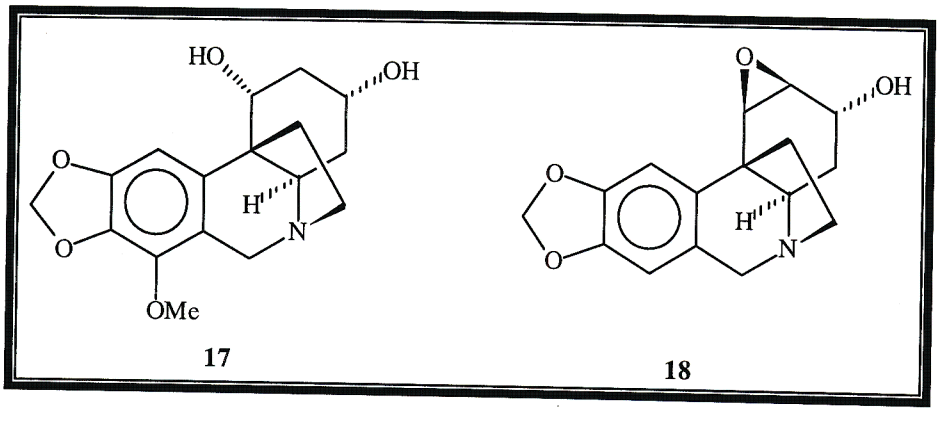
The homolycorine type alkaloids differ from the lycorine type alkaloids in that the heteroatom in ring B is an oxygen instead of a nitrogen. In this group, the nitrogen containing five membered ring is attached at the 1 and 2 positions.

The 5-10b-ethanophenanthridine alkaloids consist of the crinine and haemanthamine type alkaloids. In the crinine type alkaloids, the 5-10b-ethano bridge (ring D) is situated in a β position. This is the distinction between crinine alkaloids and haemanthamine alkaloids where the 5-10b ethano bridge is situated in an α position. A double bond is present at the Δ^1 position with a hydroxy or methoxy substituent at the 3 position which can be either α or β. At C-6, a hydroxy group can replace the hydrogen at the α or β position. In some of these alkaloids, a methoxy substituent replaces the hydrogen at position 7 on the aromatic ring. With the exception of amaryllisine (**15**), all the crinine alkaloids have a methylenedioxy group attached at the 8 and 9 positions of the aromatic ring. Amaryllisine (**15**) has a methoxy and hydroxy group at these positions. At C-11, a hydroxy or acetoxy group may replace one of the hydrogen atoms. The double bond at the Δ^1 position may also shift to the Δ^2 position with the addition of a hydroxy group at the α position as in buphanamine

(**16**). This Δ^1 double bond may also be reduced and hydroxy or acetoxy groups can appear at these positions e.g. nerbowdine (**17**). Furthermore, the Δ^1 double bond can be oxidised into an epoxide as in flexinine (**18**).

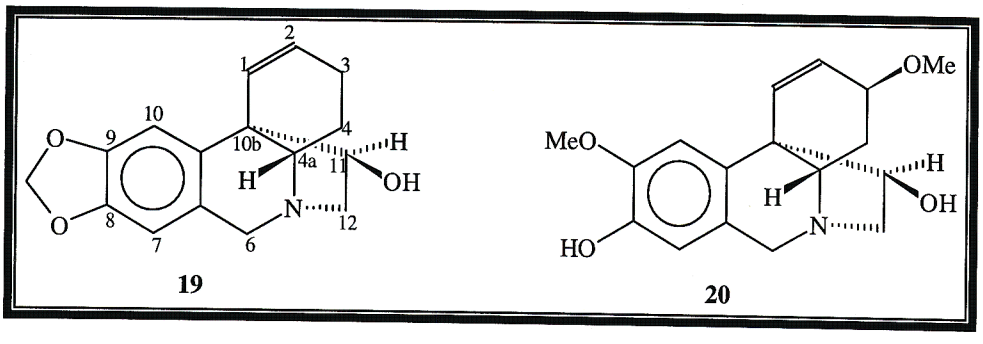


15 **16**

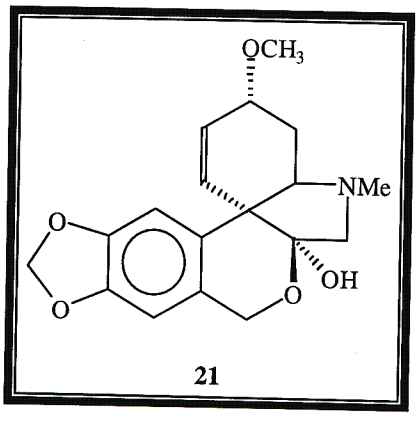


17 **18**

The haemanthamine alkaloids, also known as the α-5-10b-ethanophenanthridine alkaloids, have a double bond at the Δ^1 position and a hydroxy, methoxy or acetoxy substituent at C-3 which can either be α or β. Only haemultine (**19**) has no substituent at this position. An α or β hydroxy group can occupy one of the positions at C-6. With the exception of narcidine (**20**) which has a hydroxy and methoxy substituent at C-8 and C-9, all the other alkaloids in this class have a methylenedioxy group attached at these positions. At C-11, a hydroxy substituent may replace either one of the hydrogens.



The tazzetine type alkaloids have four rings, an aromatic A ring and a six membered B ring with an oxygen atom, a six membered ring (ring C) and a nitrogen containing five membered ring (ring D) attached to ring B. A methylenedioxy group is attached to ring A, a methoxy group is present on ring C and a N-methyl group on ring D. There is evidence that in some cases, tazettine (**5**) as well as criwelline (**21**) are artifacts arising from isolation techniques [7].



Narciclasine type alkaloids have a lycorine type skeleton except for the absence of ring D, the presence of a double bond at the 1 and 11b positions instead of the Δ^1 positions and a carbonyl group at C-7. A hydroxy group is attached to position 8 of the aromatic A ring.

The montanine type alkaloids consist of an aromatic A ring with a methylenedioxy group, a seven membered B ring with a methylene bridge extending from the nitrogen atom to the other side of the ring such that a six membered and a five membered ring

are formed, and another six membered ring attached to the seven membered ring containing a double bond, hydroxy and methoxy groups.

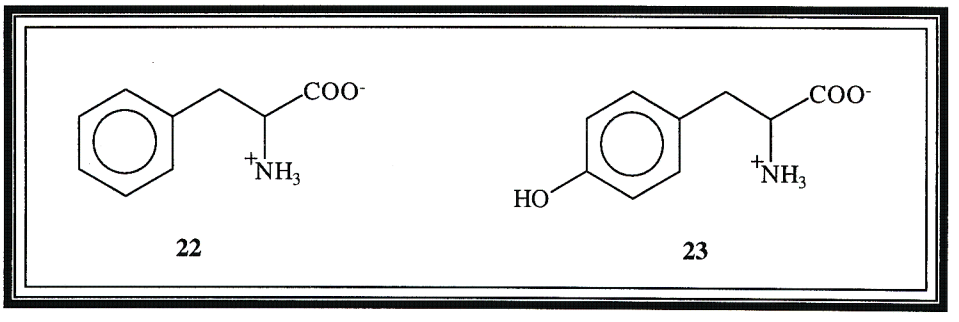
The galanthamine type alkaloids have a haemanthamine type skeleton with an open B ring, a methyl group attached to the nitrogen atom of ring B and an oxygen bridge attached at the 10 and 1 positions on ring A and ring C respectively.

The buflavine type alkaloids consist of three rings, an aromatic A ring with a methoxy substituent, an eight membered B ring containing an N-Me group and another aromatic C ring attached to ring B.

The miscellaneous type alkaloids consist of two or three rings, each containing one or more aromatic rings. These compounds do not find a place in the other nine groups and are therefore termed miscellaneous alkaloids.

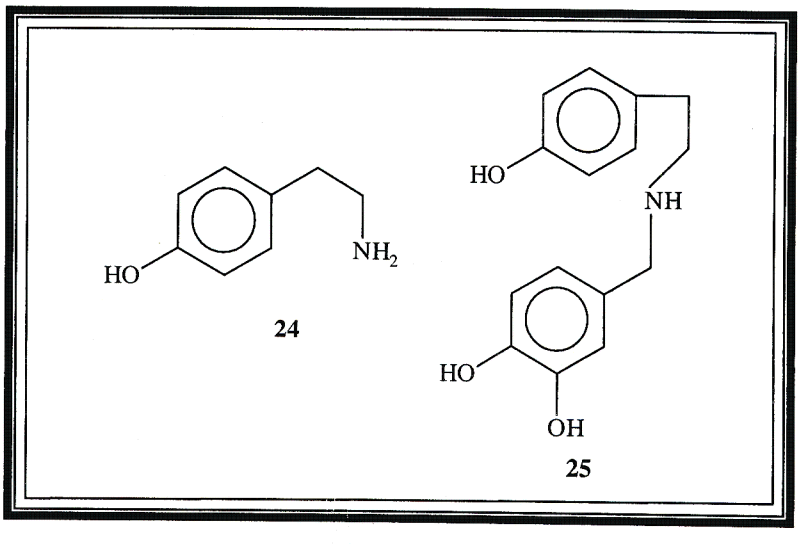
1.1.2 Biosynthesis of Amaryllidaceae alkaloids

A phenylethylamine fragment occurs in most of the Amaryllidaceae alkaloids and all of the alkaloids isolated from *Ammocharis coranica*. This fragment is present in two ubiquitous amino acids, phenylalanine (**22**) and tyrosine (**23**).



22 **23**

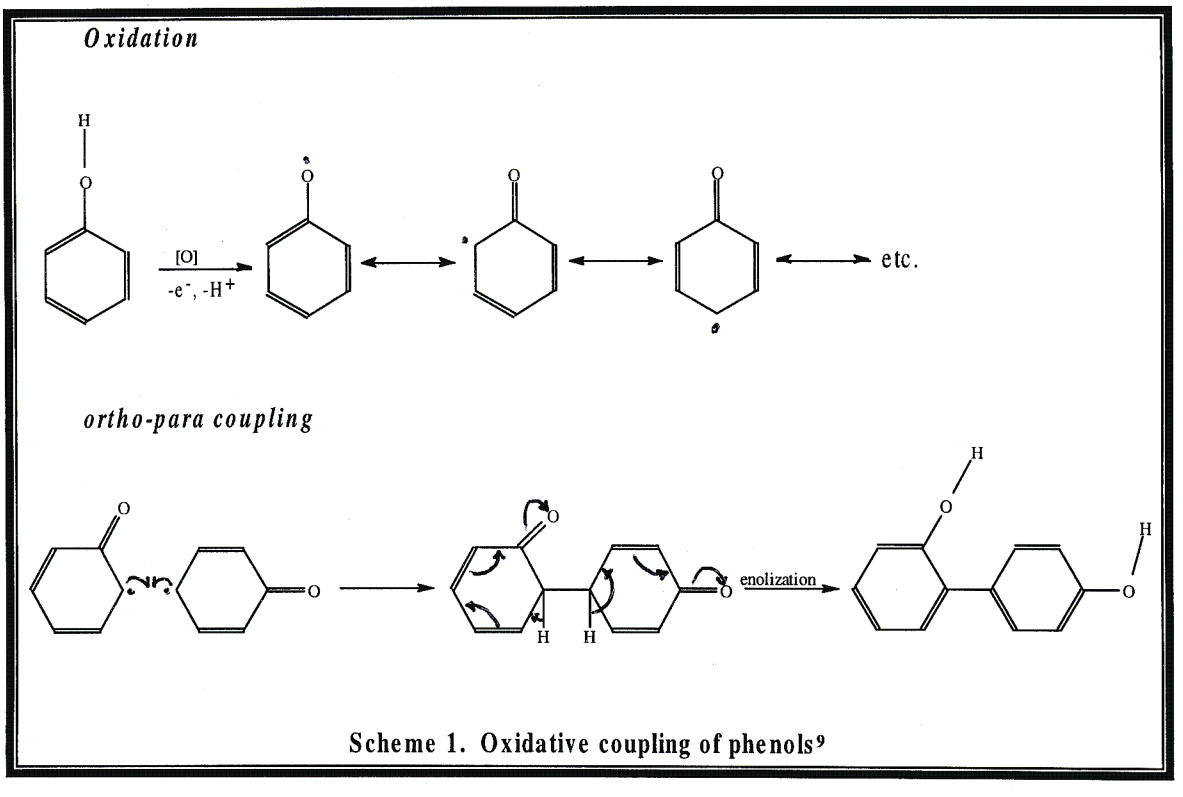
Union of such a phenylethylamine molecule with a second aromatic fragment gives rise to the polycyclic bases isolated from the Amaryllidaceae family. The skeletal structures found amongst the Amaryllidaceae alkaloids are all related to an intermediate known as norbelladine (**25**). Norbelladine (**25**) is derived by interaction of a tyramine (**24**) moiety with a C_6-C_1 unit from phenylalanine [8].



24 **25**

The oxidative coupling of phenols is one of the reactions essential in alkaloid biosynthesis. This is a free radical process that is catalysed by enzymes in plants. Loss of an electron and a proton from phenol leads to a resonance stabilized free radical. Two free radicals can then undergo coupling in a variety of ways. In most

cases, oxidative coupling occurs intramolecularly. An example is illustrated below using *ortho-para* coupling (**Scheme 1**)[9].



Scheme 1. Oxidative coupling of phenols[9]

Norbelladine (**25**) consists of a C_6-C_2-N-C_1-C_6 system. Tracer evidence indicates that norbelladine (**25**) originates by reduction of the condensation product of tyramine (**24**) with protocatechuich aldehyde (**29**), intermediates which originate from tyrosine and phenylalanine respectively (**Scheme 2**) [8].

Hydroxylation of the C_6-C_1 precursor takes place at the level of cinnamic acid (**26**) to yield *p*-coumaric acid (**27**) which is further hydroxylated to yield caffeic acid (**28**). Side chain cleavage of caffeic acid (**28**) then produces protocatechuic aldehyde (**Scheme 2**) [8].

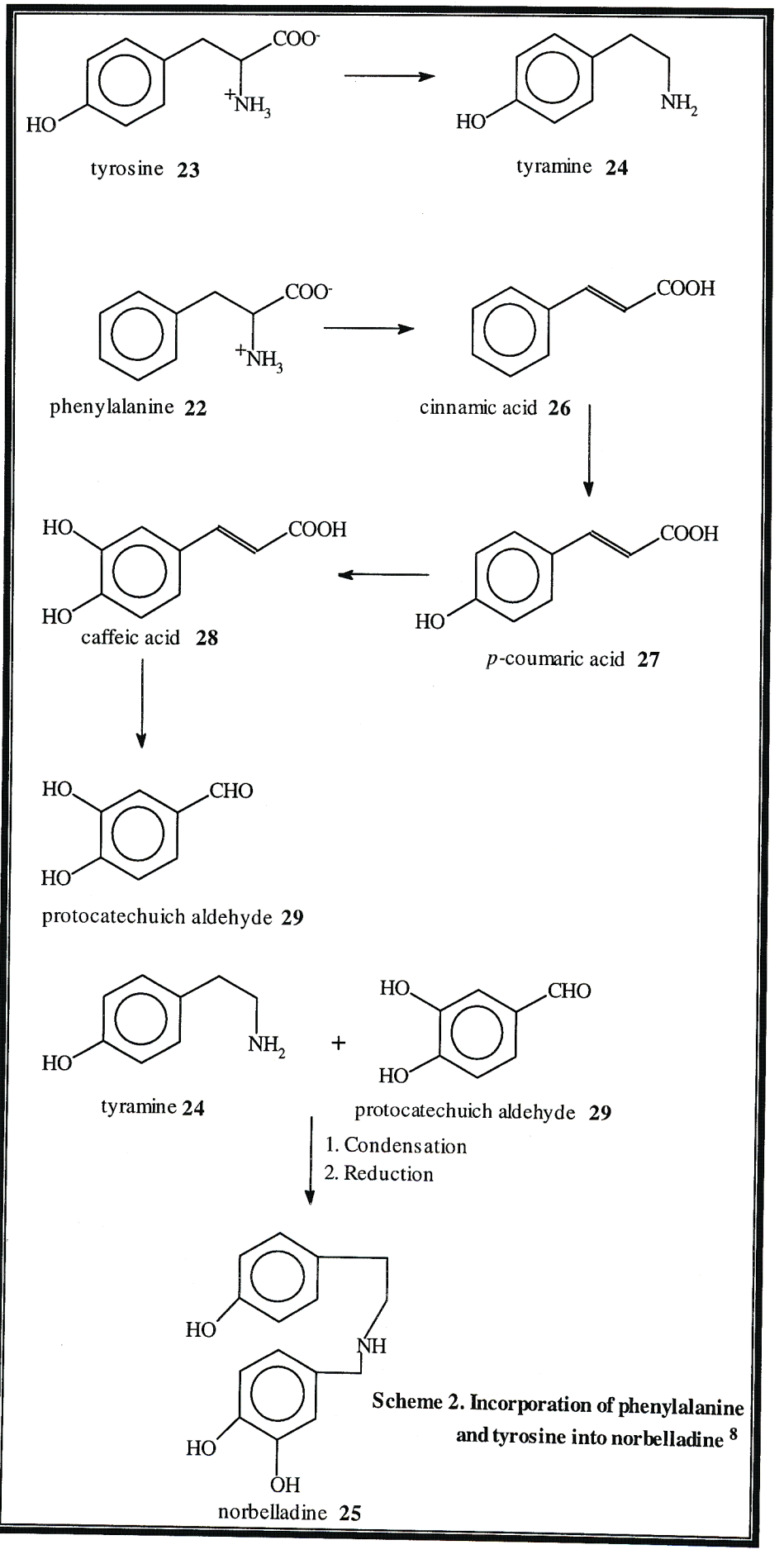
The three major structural groups among the isoquinoline alkaloids, the lycorine, galanthamine, and crinine/haemanthamine types are all derived from the parent compound, norbelladine, by intramolecular oxidative coupling of its two aromatic rings (**Scheme 3**). This hypothesis is sustained by tracer evidence [8]. Radioactivity

from singly and multiply labelled samples of norbelladine and *p'-O-methylnorbelladine is incorporated into alkaloids belonging to each of the three series[8].

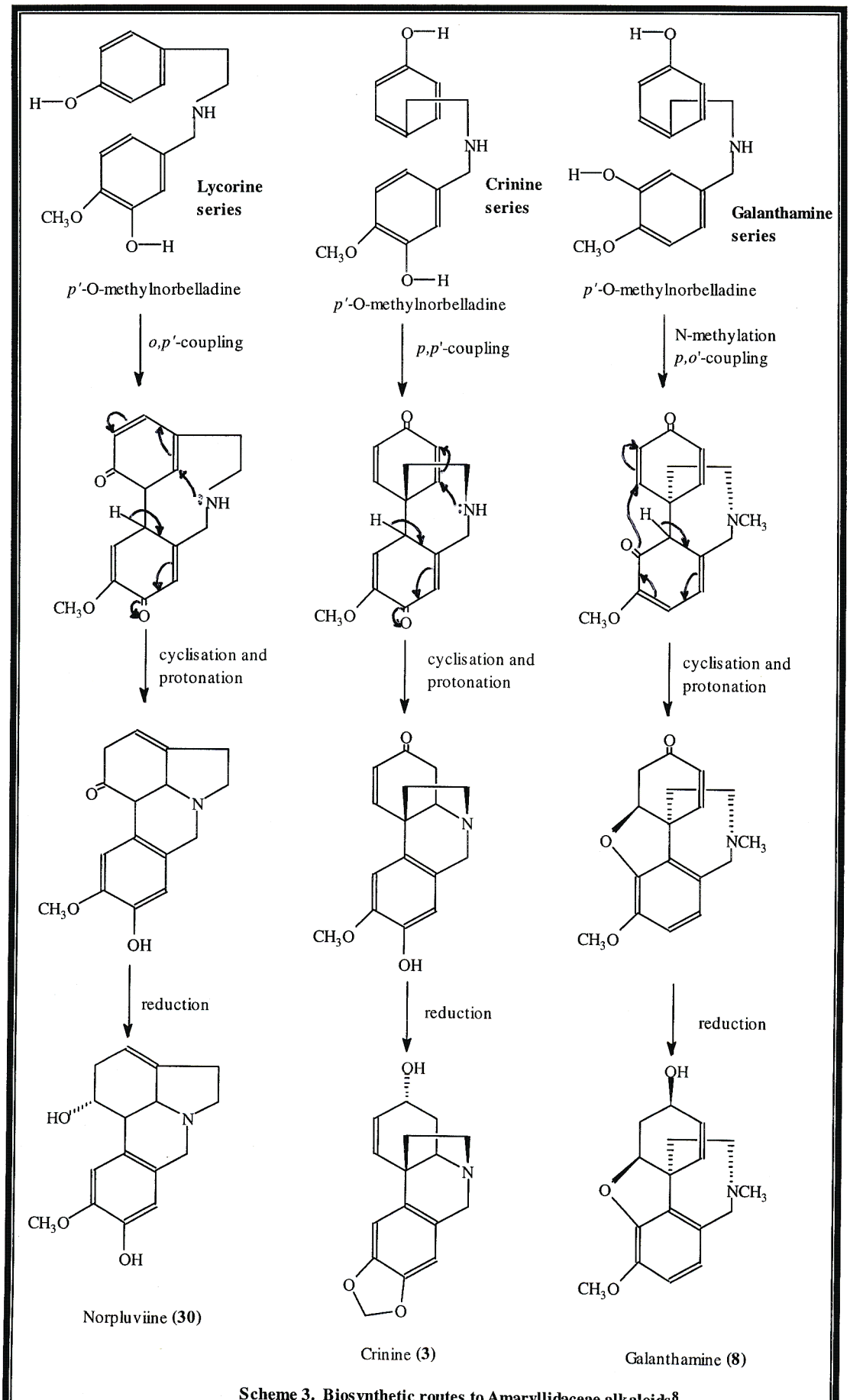
The labelled methoxy group of *p'*-O-methylnorbelladine enters into the methylenedioxy group of haemanthamine which provides experimental proof of the biosynthetic origin of this one-carbon group [8].

The nitrogen atom of galanthamine type alkaloids is methylated, but not the lycorine and crinine types. Therefore N-methylation of norbelladine plays an important part in determining which of the three series are formed. The N-methyl group blocks entry of this precursor into the lycorine and crinine/haemanthamine type alkaloids but does not inhibit incorporation into the galanthamine series.

* *p'* refers to the position *para* to the hydroxy group of the C_6-C_1 unit whereas *p* refers to the position *para* to the hydroxy group of the C_6-C_2 unit.



Scheme 2. Incorporation of phenylalanine and tyrosine into norbelladine [8]



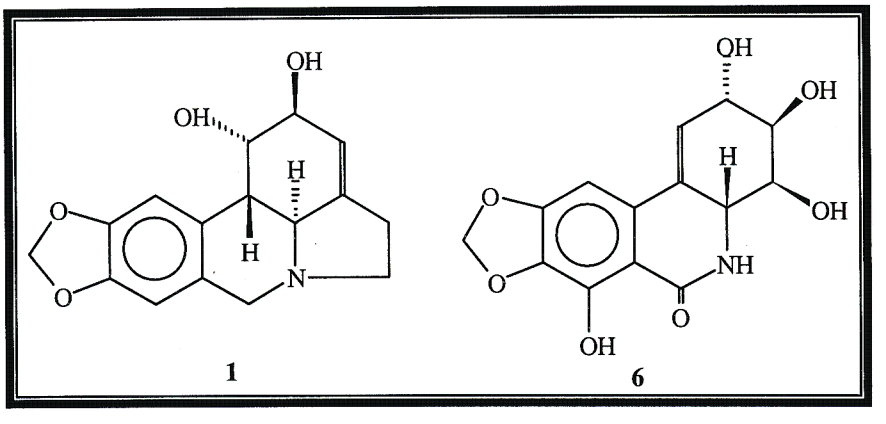
Scheme 3. Biosynthetic routes to Amaryllidaceae alkaloids[8]

1.1.3 Ethnobotanical use and biological activity of Amaryllidaceae alkaloids

Traditional medicine has always been popular amongst the different ethnic groups in South Africa and in some instances, although modern medicine is available, people still prefer to go to the traditional healers, who have had their knowledge passed down to them from generation to generation. Bulbs of many species of the Amaryllidaceae have been used by these traditional healers for medicinal purposes [2]. Of the different ethnic groups in South Africa, the Zulu have probably the best documented system of traditional medicine [10]. Their medicinal plants are sold and used all over South Africa. The bulb is the part of the plant most frequently used and most remedies, prepared from single plants, are administrated from hot or cold water infusions [2].

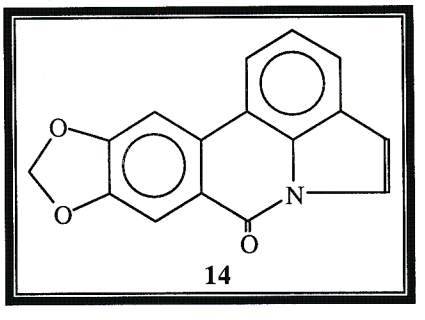
The ubiquitous Amaryllidaceae alkaloid, lycorine (**1**) can be considered the most important of the phenanthridine alkaloids because of its biological activities such as inhibition of growth and cell division in higher plants and algae, its inhibition of cyanide resistant respiration and peroxidase enhancement. The effects of lycorine (**1**) on these physiological processes have been ascribed to its ability to inhibit ascorbic acid (AA) biosynthesis *in vivo* [3,11]. Previous tests done on twenty-three lycorine derivatives and related compounds as inhibitors of AA biosynthesis in potato tubers have shown the following relationships between structure modification and activity: (a) cleavage of the acetalic bonds on the dioxole ring had no effect on activity; (b) derivatives with a methoxy group at C-8 (A ring) were inactive; (c) oxidation of N-CH_2-7 to an amide group (B ring) caused loss of activity; (d) modification of the C/D ring junction had no effect on activity when the B ring assumed a β configuration whereas a great decrease in activity was observed when the ring assumed an α configuration; (e) selective or complete acetylation of the hydroxy groups of the C ring and epimerization or oxidation of the hydroxy group on C-2 led to a loss of activity; (f) a compound with a double bond located at the Δ^1 position showed activity almost identical to lycorine; (g) stereoselective hydrogenation of the double bond of the C ring induced a considerable increase of the activity; (h) protonation of the nitrogen atom had no effect on activity [3]. In addition to the twenty-three compounds used in their preliminary screening tests, Evidente *et al.* [11] have tested another seventeen compounds all related to lycorine for their inhibitory effect on ascorbic acid

biosynthesis. They found that narciclasine (**6**), containing a double bond at the 11b-1 position, an α hydroxy group at the 2- position and β hydroxy groups at the 3- and 4- positions displayed the highest potency, followed by compounds having an aromatic C ring. Derivatives modified at C-1 and/or C-2 were inactive while the compound with a double bond between these positions was found to be a weak inhibitor. These results confirm that the presence of an appropriately substituted C-ring is a necessary requirement for optimal 'response triggering' contact between the lycorine derivatives and the specific receptor. Functional groups jutting out from the α- side of the molecule do not allow a good fit with the binding sites [11].



A number of *Crinum* species, *Crinum latifolium* [12], *C. pratense* and *C. bulbispermum* [5] as well as *Brunsvigia josephinae* [13], *Hippeastrum vittatum* and *Lycoris sanguinea maximum* [14] contain an alkaloid called hippadine (**14**) which has been shown to reversibly inhibit fertility in male rats [4]. The physiological actions of hippadine on albino rats (3 mg in saline/ rat / day) for three days had the following effects : (a) the DNA content of the testis was depleted; (b) a loss of tissue weight of the testis was observed; (c) steroidogenic cells were active and the weight of the ventral prostrate was increased which suggested growing hormonal activity in the animals; (d) the protein concentration of the testis was marginally, but consistently, increased, suggesting a continued cellular activity. Although groups of rats treated with hippadine (**14**) showed loss of fertility, 8 out of 10 rats regained fertility between 8 to 10 weeks and two remained sterile up to 12 weeks. There is also a delayed onset of infertility effects of hippadine with loss of DNA and an increase in concentration of protein. This, together with the reversibility of the damage to the germinal activity, suggests that the alkaloid possibly acted on the germ cells in their earlier stages of

spermatocytogenesis. These observations suggest that hippadine exerts its effects at the genetic level and may prove to be a useful agent in fertility control. The ontogenic variation of hippadine and its precursor lycorine, showed an increase in the concentration of the former and a decrease in the concentration of the latter during the pre- and post- flowering stages when the plants were harvested for analysis. This suggests a close association of hippadine with the reproductive activity of plants in which it is found [5].



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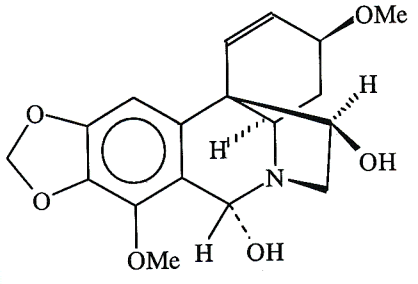
Extracts of different parts of *Crinum latifolium* have been reported to be used in popular medicine for a variety of purposes, e.g. a rubefacient in rheumatism and piles, and for abscess treatment to promote suppuration [12]. Nineteen alkaloids have been isolated and characterised from this species, including hippadine (**14**), crinafoline (**31**), crinafolidine (**32**) and 1,2-β-epoxyambelline (**33**). Crinafoline and crinafolidine, in several concentrations, produced significant reduction in the viability and *in vivo* growth of S-180 ascites tumour cells [15]. 1,2-β-Epoxyambelline is an immuno-stimulant alkaloid [16]. *Crinum kirkii*, a common grassland plant of East Africa, is used in Kenya for the treatment of sores. In Tanzania, the fruit and inner parts of the bulb are used as a purgative and the outer scales are used as rat poison [17]. Extracts of the bulbs of *Crinum pratense* is used in popular medicine as a bitter tonic, a laxative and in chest ailments [5]. From the bulbs of *Crinum amabile*, the cytotoxic and antimalarial compounds, lycorine (**1**), augustine (**34**) and crinamine (**35**) were isolated and characterised. Among the 5-10b-ethanophenanthridines, augustine (**34**) appeared to be the most active alkaloid, demonstrating a significant cytotoxic response in all cell lines tested except drug resistant KB (KB-V1). This compound also exhibited antimalarial activity in both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum.* These activities were probably due to the presence of the epoxide group, which can result in the formation of adducts with nucleophiles in

biological systems, leading to non-selective toxicity. Structures lacking the oxirane ring between C-1 and C-2, amabiline (**36**) and buphanisine (**37**), were not active, however crinamine (**35**) demonstrated strong cytotoxic and moderate antimalarial activities. It can therefore be concluded that the epoxide group is not essential for activity and other elements of structure may be important. This is also suggested by lycorine (**1**) which was found to be very cytotoxic in all cancer cell lines tested. Crinamine (**35**) also showed moderate antimalarial activity. However, the three compounds were not very selective having low selectivity indices which could lead to non-selective toxicity [18]. The water extract of the bulbs of *Crinum macowanii* is drunk as a remedy in the treatment of sexually transmitted diseases, as an emetic, to treat backache and to increase lactation in women [2]. *Crinum macowanii* also showed response against Punta toro virus, yellow fever virus and Japanese encephalitis virus [2]. A decoction of *Crinum bulbispermum* is used for gall sickness and the roasted bulbs are applied externally for aching joints, rheumatism, varicose veins and backache, as poultices for septic sores and ulcers [2] and also for haemorrhoids and abscesses [19]. The infusions of chopped leaves are also administered for rheumatic fever [10]. This species is also a remedy for colds and scrofula and the juice of the leaves are used to treat earache[19]. Extracts of the plant are slightly effective against malaria [19]. To treat urinary tract problems and swelling, the Zulu use *Crinum delagoense* and *Crinum moorei* [10]. *Crinum giganteum* is used as a remedy for leprosy [19].

The bulb extracts of *Haemanthus albiflos* have been attributed strong antiviral activity[20]. This species is active against Coxsackie B2 virus, Echo virus 11, Polio virus 1, Rotavirus SA 11, Herpes simplex 1 virus and vesicular estomatitus virus. It also has cytotoxic activity against Hela cells, Ca-mammary- ma- 104 and buffalo green monkey kidney cells (BGM) with DNA, RNA and protein synthesis inhibition[2]. Extracts of the roots, bulbs and flowers of *Haemanthus kalbreyeri* are used in popular medicine in the treatment of common cold, cough, asthma and in healing wounds [6]. Two compounds, kalbretorine (**38**) and kalbreclasine (**39**) isolated from this species show significant biological properties. Kalbretorine (**38**) markedly inhibited the growth of S-180 tumour cells (transplantable ascites tumour in mice) and their viability while kalbreclasine (**39**), in doses of 20 μg and above, produced



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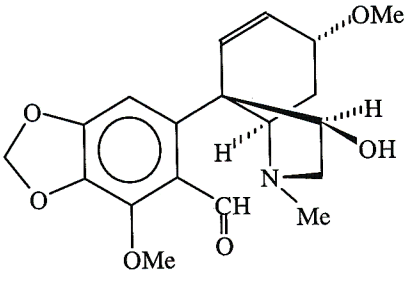

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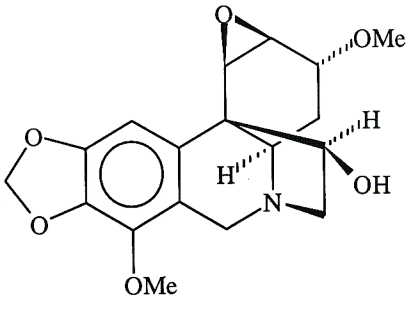

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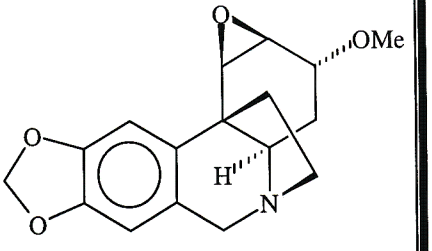

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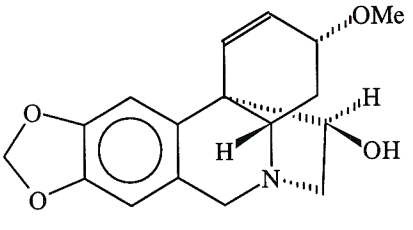

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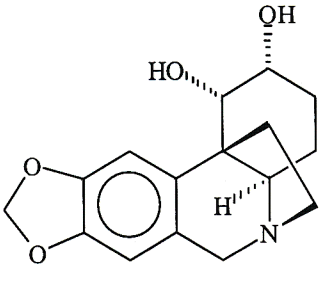

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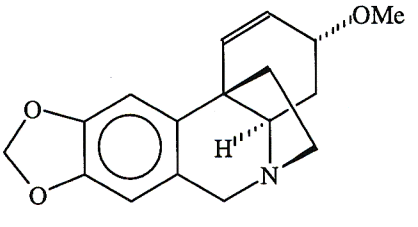

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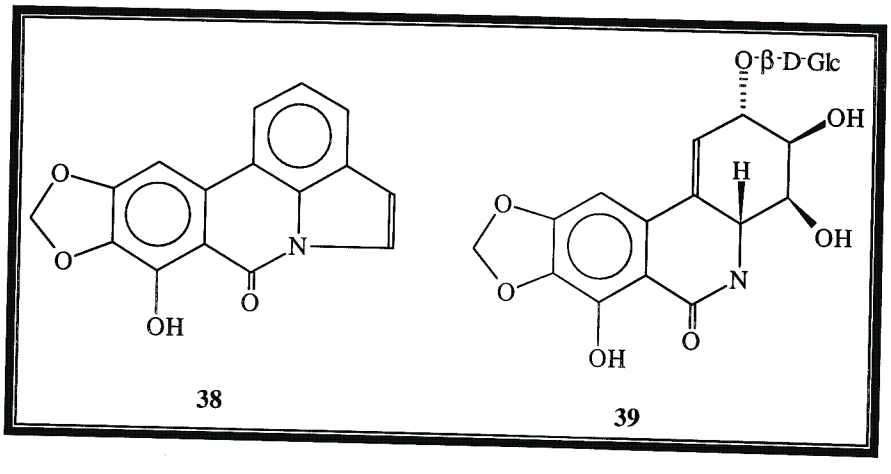
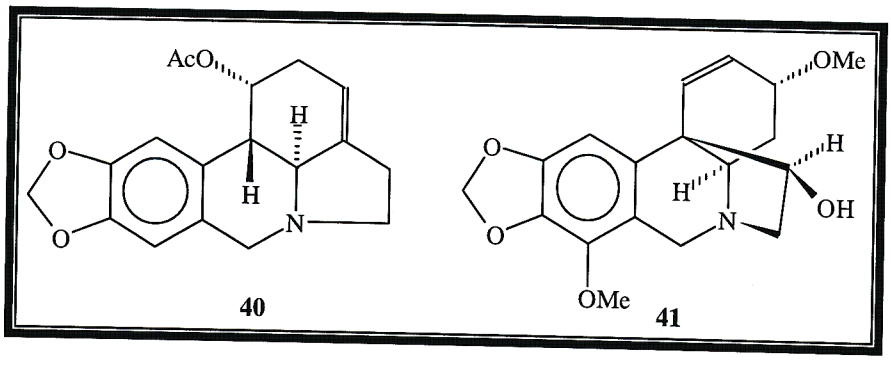
extensive proliferation of the splenic lymphocytes in healthy adult male mice. The mitogenic activation produced by kalbreclasine (**39**) was comparable to that of the known mitogen, concavalin A [6]. *Haemanthus amarylloides* administered to sheep resulted in their death. *Post mortem* examination revealed haemorrhagic gastro-

enteritis and degenerative changes and haemorrhages in various organs [19]. *Haemanthus coccineus* is used as a diuretic and as an asthma remedy while the fresh leaves of this species have been reported to have antiseptic properties and was applied to foul ulcers, sores and anthrax pustules [19]. The juice of *Scadoxus multiflorus* (syn. *Haemanthus multiflorus*) is highly poisonous and has reportedly caused dangerous swellings of the lips and tongue [19]. However, a smear of bulb preparation of the same plant is applied over scarifications of the breasts as a galactagogue [2]. Ingestion of the bulb of *Scadoxus puniceus* (syn. *Haemanthus natalensis*) has resulted in death and reports of emesis and malaise in dogs have been recorded [19]. The alkaloids isolated from this plant, which were not named, were found to be toxic to sheep [19].



38 **39**

Crude material from *Amaryllis belladonna* was found to be toxic to animals, resulting in death by respiratory paralysis [21]. This same species, also referred to as the "belladonna lily" or "naked lady" has found use in Java for the treatment of cancer. Their flowers have an antispasmodic action [2]. Two other members of this genus, *Amaryllis formossisima* and *Amaryllis zeylanica* have also been employed in folk medicine as a cancer treatment [22]. Australian-grown material of *A. belladonna* bulbs was shown to yield fractions with a confirmed level of activity against the *in vivo* murine P-388 lymphocytic leukemia (3 PS system). One of the antineoplastic components was found to be lycorine (**1**). Acetylcaranine (**40**) and ambelline (**41**) were shown to have *in vitro* activity against the P-388 system, however, 3 PS *in vivo* activity was not realized at the dose levels tested [22]. The bulbs of this species are reported to produce a cardiotonic poison [2].



Aqueous extracts of several species of the genus *Brunsvigia* have been used in the traditional medicine of the southern Sotho and Zulu tribes of South Africa for the treatment of coughs and colds, in renal and hepatic conditions, for the relief of backpains and as a remedy for barrenness. A decoction of leaves and roots of *Brunsvigia* species is drunk in order to treat infertility in women[2]. However, an excess of this hot water extract has hepatotoxic activity, causing a necrodegenerative hepatitus [2]. The Xhosa use the outer skin of the bulb of *B. grandiflora* to dress circumcision wounds [10]. Extracts of *B. radulosa* showed inhibitory activity against P388 lymphocytic leukaemia in mice [13]. Taken in small amounts, this species can also induce visual hallucinations [2].

Apodolirion buchananii bulb infusions are used to treat stomach complaints amongst the Zulu people [10].

Extracts of *Boophane* species have been used in the treatment of leprosy, ulcers, febrile colds, asthma, coughs and wounds [10,19]. *Boophane disticha* bulb decoctions are used by the Zulu traditional healers to treat headaches, chest pains, bladder pains, cramp-like pains in the calf muscles and are sometimes administered to hysterical adolescent females [19]. This species has been shown to cause the smooth muscle to relax and lose its spontaneous activity having both antihistaminic and adrenergic activities. Extracts also exhibit high activity against different rhinovirus strains [2]. Other ethnic groups use the bulbs as outer dressings for circumcision and other wounds, burns, boils and abscesses and the fresh leaves to stop bleeding and in the treatment of skin disease [10]. The Xhosa use this plant to combat 'red water' in cattle[23]. The extract of these bulbs has been used as an arrow poison by the Bushmen and Hottentots and as an agent in suicide [2]. This plant is also capable of producing

profound hallucinations and is used in initiation ceremonies of Basuto boys who are directed to consume a decoction of the bulb. This decoction produces intoxication, which is a sign that the spirit of manhood has entered their bodies [2].

The Zulu in South Africa use the root of *Clivia miniata* as a snake-bite remedy and in treating febrile conditions. The herb is used to facilitate delivery at childbirth or to initiate parturition when its onset is retarded [19]. However, in large doses, this plant is potentially fatal, causing salivation, vomiting, diarrhoea and depression of the central nervous system [2]. The decoction of dried leaves is also drunk to augment or induce labour while the bulb decoctions are used to treat urinary infections and infertility [2]. *Clivia miniata* has been shown to have smooth muscle stimulant activity on the ileum and uterus of guinea pigs, inducing spontaneous contractions of the uterus. *Clivia miniata* also has strong antiviral activity against Coxsackie B2 virus, Herpes virus type 1, Measles virus, Polio virus 1, and Semlicki Forest virus [2]. *Clivia nobilis* is known to have low toxicity and a strong decoction in large doses is feebly emetic [19].

The roots of *Cyrtanthus obliquus* find applications in South Africa for stomach aches and for the treatment of leprosy [2]. The dried dark portion of the root is used as a snuff for the relief of headache resulting from old skull wounds and the same powder is rubbed into incisions at the site of fracture with a view to aiding union. The bulb is also one of the ingredients in a medicine for scrofula and in another for troublesome coughs [19]. *Cyrtanthus breviflorus* bulbs are used to treat roundworm and tapeworm while *Cyrtanthus sanguineus* is used during pregnancy to assist in easy labour [10]. The bulb of *Cyrtanthus elatus* (syn. *Vallota speciosa*) has been proven experimentally toxic to cattle, sheep, dogs and rabbits. The symptoms are those of an acute irritant poisoning and death from consequent exhaustion [19].

The fruit of *Gethyllis afra, Gethyllis ciliaris, Gethyllis linearis,* and *Gethyllis spiralis* are used as a remedy for the relief of colic, flatulence and indigestion. More recent applications of *Gethyllis spiralis* include using a diluted infusion of the flower for teething problems, using the skin of the fruit as a local application to bring a boil to a head and using the plant to heal bruises and insect bites [19].

The bulb and flower of *Narcissus jonquilla* are emetic and have been used for diarrhoea, cramp and epilepsy and externally on sores, however the experimental administration of 50 grams of the bulb to a rabbit resulted in an asphyxial death within twenty-four hours [19].

The bulb of *Nerine angustifolia* has shown positive tests for haemolysis. Experimental administration of 200 grams of *Nerine laticoma* (syn. *Nerine lucida*) killed a sheep within twenty-four hours. *Post mortem* examination revealed that the heart was in diastole with the muscle flabby, the spleen was markedly swollen and hyperaemic, the lungs slightly hyperaemic and the rumen distended with gas [19].

The bulb of *Periphanes zeyheri* has given positive tests for haemolysis [19].

The leaf of *Zeyphyranthes candida* is used as a remedy for diabetes mellitus [19].

The San in Dobe, Botswana use *Pancratium tenuifolium* to induce visual hallucinations [2].

In conclusion, the medicinal effects among the Amaryllidaceae family range from treating infertility in woman to cancer treatments. It is because of this that this family has become the focus of many pharmacological and phytochemical investigations. Further work in this family could lead to important medical applications.

1.1.4. References

1. Pelletier, S.W. (1970) *in* **Chemistry of the Alkaloids**, Ed. Pelletier, S.W.,van Nostrand Reinhold Company, New York, pp 1-9.

2. Viladomat, F., Bastida, J., Codina, C., Campbell, W.E. and Nair, J.J. (1997) **Alkaloids of the South African Amaryllidaceae**, *in Recent Research Developments in Phytochemistry*, **1,** Research Signpost Publisher, Trivandrum, pp 131-171.

3. Evidente, A., Cicala, M.R., Randazzo, G., Riccio, R., Calabrese, G., Liso, R. and Arrigoni, O. (1983) **Lycorine structure-activity relationships**, *Phytochemistry,* **22**, 2193-2196.

4. Chattopadhyay, S., Chattopadhyay, U., Mathur, P. P., Saini, K.S. and Ghosal, S. (1983) **Effects of hippadine, an Amaryllidaceae alkaloid on testicular function in rats**, *Planta Medica,* **49,** 252-254.

5. Ghosal, S., Rao, P.H., Jaiswal, D.K., Kumar, Y. and Frahm, A.W. (1981) **Alkaloids of *Crinum pratense,*** *Phytochemistry*, **20**, 2003-2007.

6. Ghosal, S., Lochan, R., Ashutosh, Kumar, Y. and Srivastava, R.S. (1985) **Alkaloids of *Haemanthus kalbreyeri***, *Phytochemistry,* **24**, 1825-1828.

7. Wildman, W.C. (1970) **Amaryllidaceae alkaloids**, *in* **Chemistry of the Alkaloids**, Ed. Pelletier, S.W., van Nostrand Reinhold Company, New York, pp 51-170.

8. Spenser, I.D. (1970) **Biosynthesis of alkaloids**, *in* **Chemistry of the Alkaloids**, Ed. Pelletier, S.W., van Nostrand Reinhold Company, New York, pp 688-695.

9. Solomons, T.W.G. (1992) *in* **Solomons Organic Chemistry**, 5th Ed., John Wiley and Sons Inc., New York, pp 931-934.

10. Hutchings, A., Scott, A.H., Lewis, G. and Cunningham, A.B. (1996) *in* **Zulu medicinal plants. An inventory.** University of Natal Press, Pietermaritzburg.

11. Evidente, E., Arrigoni, O., Liso, R., Calabrese, G. and Randazzo, G. (1986) **Further experiments on structure activity relationships among the lycorine alkaloids**, *Phytochemistry,* **25**, 2739-2743.

12. Ghosal, S., Saini, K.S. and Frahm, A.W. (1983) **Alkaloids of *Crinum latifolium,*** *Phytochemistry*, **22**, 2305-2309.

13. Viladomat, F., Bastida, J., Codina, C., Campbell, W.E. and Mathee, S. (1994) **Alkaloids from *Brunsvigia josephinae,*** *Phytochemistry,* **35**, 809-812.

14. Ali, A.A., Mesbah, M.K. and Frahm, A.W. (1981) **Phytochemical investigation of *Hippeastrum vittatum* growing in Egypt**, *Planta Medica*, **43**, 407-409.

15. Ghosal, S. and Singh, S.K. (1986) **Chemical constituents of Amaryllidaceae. Part 24. Crinafoline and crinafolidine, two anti-tumour alkaloids from *Crinum latifolium***, *Journal of Chemical Research (S)*, 312-313.

16. Ghosal, S., Saini, K.S. and Arora, V.K. (1984) **1,2-β-Epoxyambelline, an immuno-stimulant alkaloid from *Crinum latifolium***, *Journal of Chemical Research (S)*, 232-233.

17. Bastida, J., Codina, C., Peeters, P., Rubiratta, M., Orozco, M., Luque, F.J. and Chhabra, S.C. (1995) **Alkaloids from *Crinum kirkii***, *Phytochemistry*, **40**, 1291-1293.

18. Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M. and Cordell, G.A. (1983) **Cytotoxic and antimalarial alkaloids from the bulbs of *Crinum amabile***, *Journal of Natural Products*, **56**, 1331-1338.

19. Watt, J.M. and Breyer-Brandwijk, M.G. (1962) *in* **The medicinal and poisonous plants of southern and eastern Africa**., E. and S. Livingstone Ltd., Edinburgh, pp 19-42.

20. Baudoin, G., Tillequin, F. and Koch, M. (1994) **Albiflomanthine – A crinane alkaloid from *Haemanthus albiflos* (Jacq)**, *Heterocycles*, **38**, 965-970.

21. Mason, L.H., Puschett, E.R. and Wildman, W.C. (1954) **Alkaloids of the Amaryllidaceae. IV. Crystalline alkaloids of *Ammocharis coranica* (Ker-Gawl.) Herb., *Brunsvigia rosea* (Lam.) Hannibal and two *Crinum* species,** *Journal of the American Chemical Society*, **77**, 1253-1256.

22. Pettit, G.R., Gaddamidi, V., Goswami, A. and Cragg, G.M. (1984) **Antineoplastic agents, 99. *Amaryllis belladonna***, *Journal of Natural Products*, **47**, 796-801.

23. Bates, A.N., Cooke, J.K., Dry, L.J., Goosen, A., Krusi, H. and Warren, F.L. (1957) **The alkaloids of the Amaryllidaceae. Part II. Haemanthamine and a new alkaloid distichine from *Boophane disticha* Herb.,** *Journal of the Chemical Society*, 2537-2540.

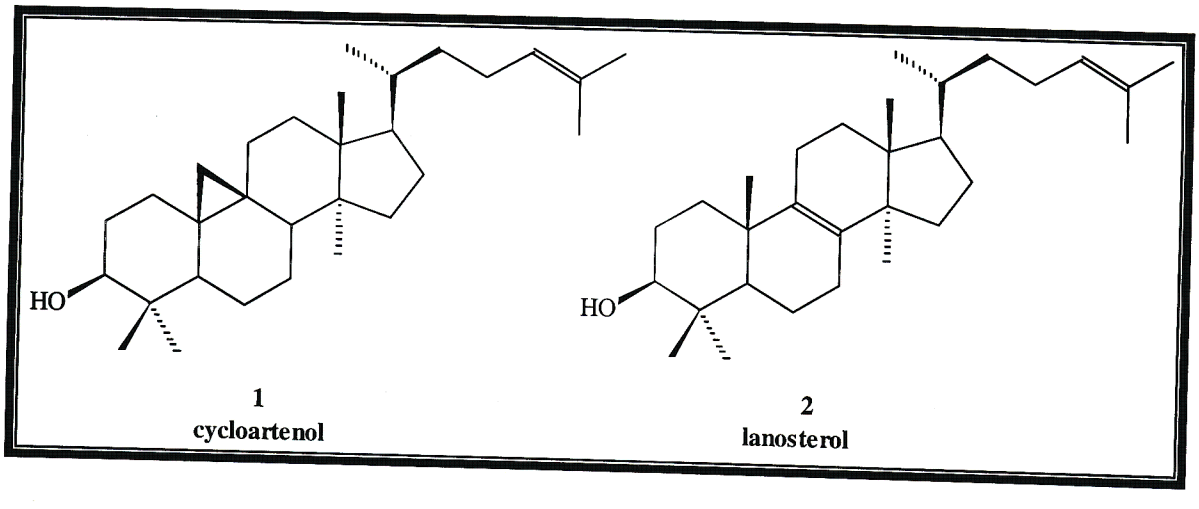
24. Miyakado, M., Kato, T., Ohmo, N. and Koshimizu, K. (1975) **Alkaloids of *Urginea altissima* and their antimicrobial activity against *Phytophthora capsici***, *Phytochemistry*, **14**, 2717.

25. Page, B. A. (1998) *in* **Extractives of three Southern African medicinal plants**, MSc dissertation, University of Natal, Durban, South Africa, pp 36-40.

1.2 An Introduction to Cycloartane Compounds

Cycloartane compounds are compounds derived from the parent compound, cycloartenol (**1**) which is derived from the precursor of all triterpenoids, squalene. Cycloartenol (**1**) was the first triterpenoid to be discovered containing a cyclopropane ring [1]. In green plants, cycloartenol is the intermediate in phytosterol biosynthesis [2,3]. It is characterised by a 9,10,19-cyclopropane ring instead of the methyl group at C-19 and the double bond between C-8 and C-9 which occurs in lanosterol (**2**), the intermediate in cholesterol biosynthesis in animals and fungi [3].

Cycloartenol is nearly as effective as cholesterol, *in vivo* and *in vitro* in stabilizing membranes composed of phospholipid bilayers in eukaryotic organisms [3].



1
cycloartenol

2
lanosterol

Nomenclature

Although other numbering systems have been recommended[4], the most commonly accepted numbering system for all sterols remains that of the IUPAC-IUB Rules for Sterol Nomenclature of 1967 (**Fig. 1.**)[4].

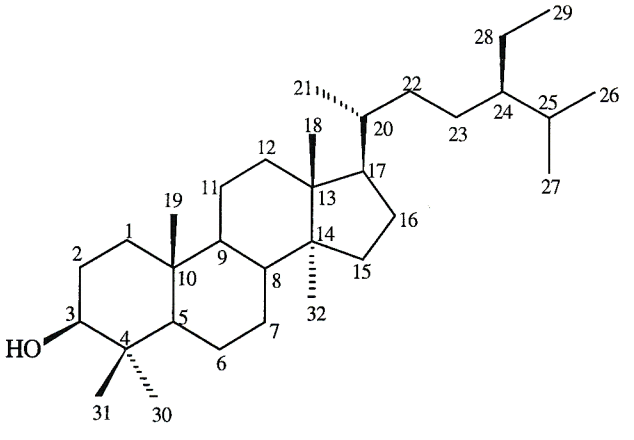
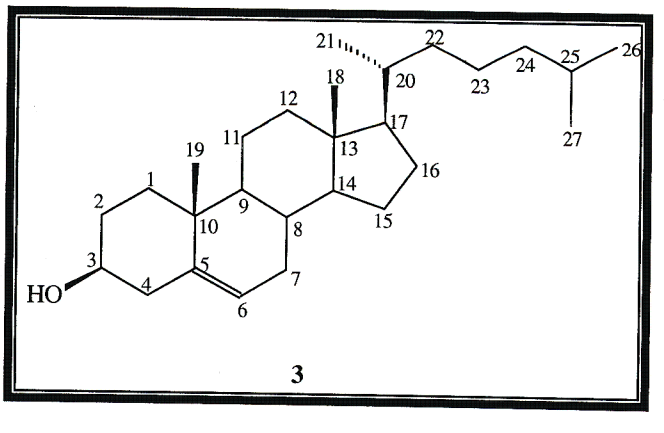


Fig. 1. Basic structure of a sterol with carbon numbering[4]

1.2.1 Classification of cycloartane compounds

Sterol was the name originally proposed to describe a 3β-monohydroxy compound based upon the perhydro-1,2-cyclopentanophenanthrene ring system and with methyl substitution at C-10 and C-13 as typified by the structure of cholesterol (**3**)[4].



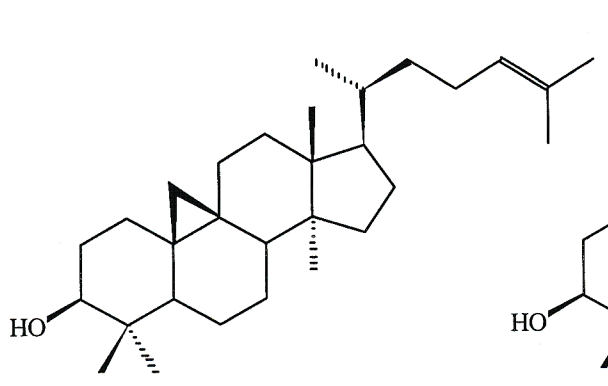
3

The term triterpene was assigned to C_{30} compounds derived from squalene[4]. Tetracyclic triterpenes, which superficially resemble the sterols, are also produced by

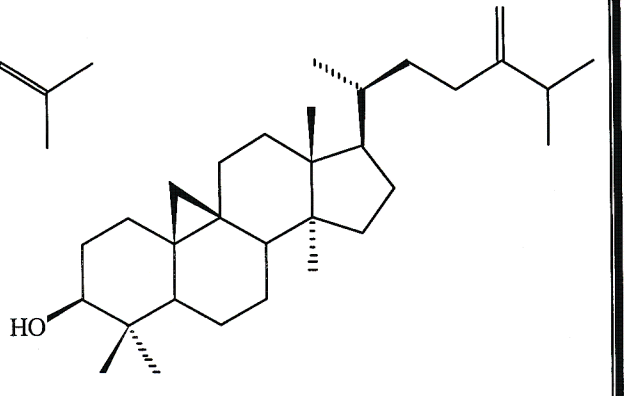
some plants and are sometimes referred to as triterpenols[4]. With the realisation that lanosterol (**2**) and cycloartenol (**1**) are the biosynthetic precursors of sterols in animals and plants respectively, it has become usual to call them 4,4-dimethylsterols rather than tetracyclic triterpenes or triterpenols[4]. Those biosynthetic intermediates with one methyl group at C-4, usually in the α configuration are termed 4-monomethyl- or 4α-methylsterols and those compounds with no methyl substitution at C-4 are termed 4-desmethylsterols or simply sterols[4].

The IUPAC recommendations also permit the use of root names in naming sterols[4]. The root name cholestane is used for the naming of all sterols, ergostane to name compounds with a 24*S* (or 24β) methyl side chain, stigmastane for 24*R* (or 24α) ethylsterols, lanostane for compounds related to the lanostane skeleton, cycloartane for 9β-19-cyclolanostanes, campestane for 24*R* (or 24α) methylsterols, poriferastane for 24*S* (or 24β) ethylsterols and gorgostane for compounds with a 22*R*,23*R*-cyclopropane-23-methyl-24*R*-methyl side chain[4].

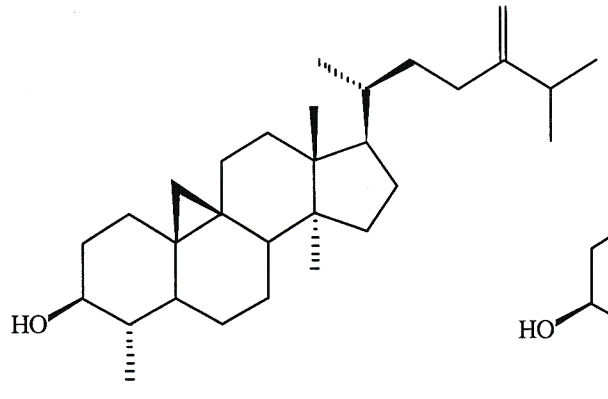
Cycoartenol (**1**), a pentacyclic alcohol was the first triterpenoid to be shown to contain a three membered ring[5]. It is this 9,10,19-cyclopropane ring that is characteristic of all cycloartane compounds. Oxidation of the 3-hydroxy group to a ketone, demethylations at C-4 and changes to the side chain, result in a number of compounds belonging to the cycloartane group. Among the 4,4-dimethylsterols, cycloartenol and 24-methylenecycloartanol are common in most seed bearing plants, while in the 4-methylsterols, cycloeucalenol is one of the most dominant components of flowering plants[6]. Examples of some cycloartane type compounds are given in **Fig. 2**.



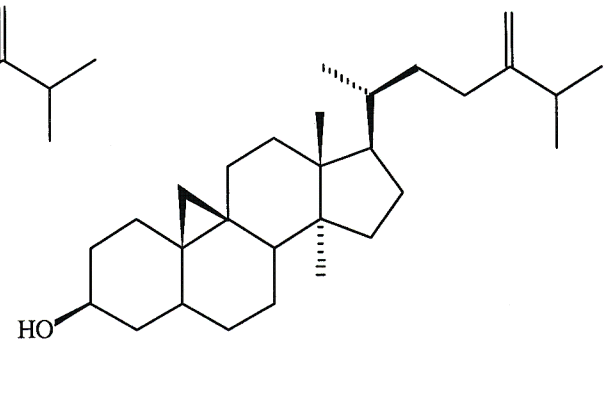
cycloartenol



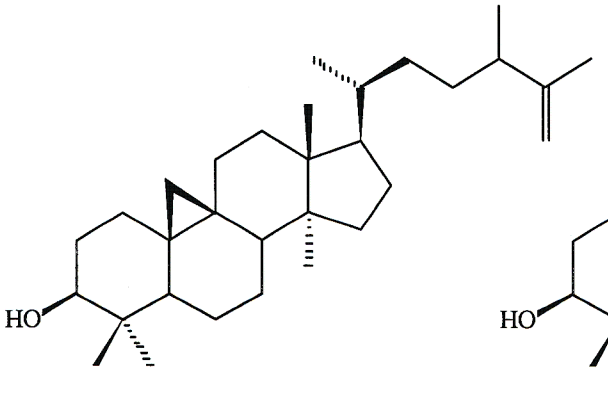
24-methylenecycloartanol



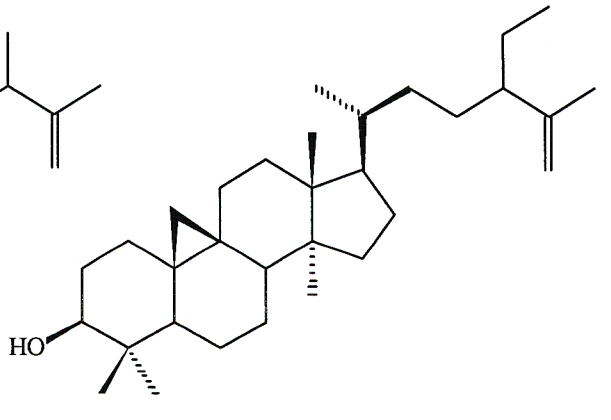
cycloeucalenol



24-methylenepollinastanol



cyclolaudenol



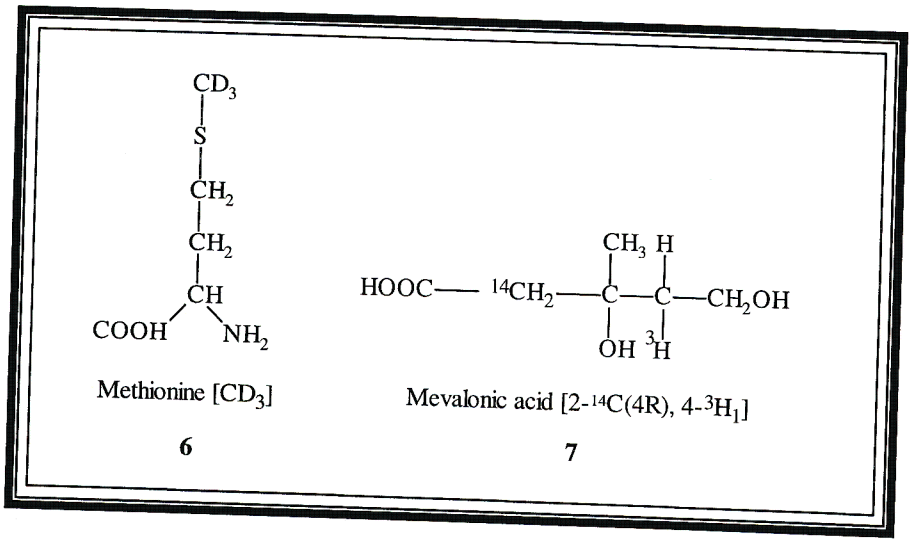
cyclomargenol

Fig. 2. Examples of some cycloartane type compounds

1.2.2 Biosynthesis of cycloartane compounds

It has been established beyond doubt that squalene is an obligatory precursor in the biosynthesis of lanosterol and cholesterol and the logical inference that all triterpenoids are derived from squalene by a series of cyclisation and rearrangement reactions has received substantial experimental verification[5]. The genesis of the lanostane skeleton from squalene epoxide (**4**) requires, in addition to an oxidative cyclization process, the migration of a number of groups. Thus, a straightforward cyclization of squalene epoxide (**4**) would give the ionic species (**5**) which must undergo five 1,2-shifts and a proton elimination to form cycloartenol[5] which is the biosynthetic precursor of all phytosterols[4]. Since only cycloartane compounds with a 24-methylene side chain were isolated in this work, only their biosynthesis will be discussed.

Information on the various biosynthetic pathways of 24-methylenecycloartane compounds has been obtained by feeding experiments using either methionine [CD_3] (**6**) or mevalonic acid [2-^{14}C(4R), 4-$^{3}H_1$] (**7**)[7]. Mevalonic acid [2-^{14}C(4R), 4-$^{3}H_1$] – labelled cycloartenol at C-24 with tritium and methionine [CD_3] gives an indication of where in the side chain methylation had taken place. With these two substrates, the 24-methylene phytosterol is obtained with labelling patterns in the side chain in accordance with the alkylation mechanism employed[7] (**Scheme 4**).



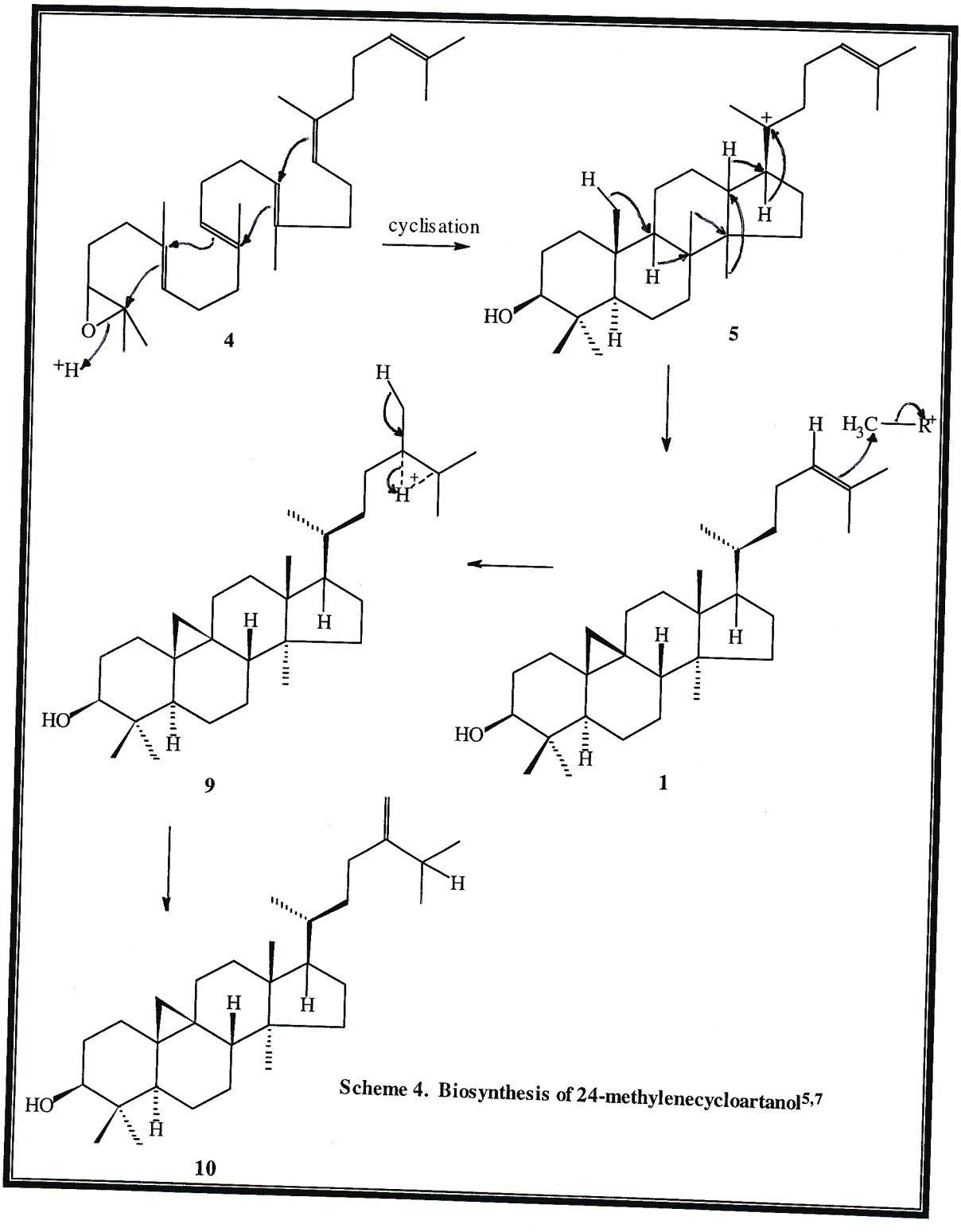
Methionine [CD_3]

6

Mevalonic acid [2-^{14}C(4R), 4-$^{3}H_1$]

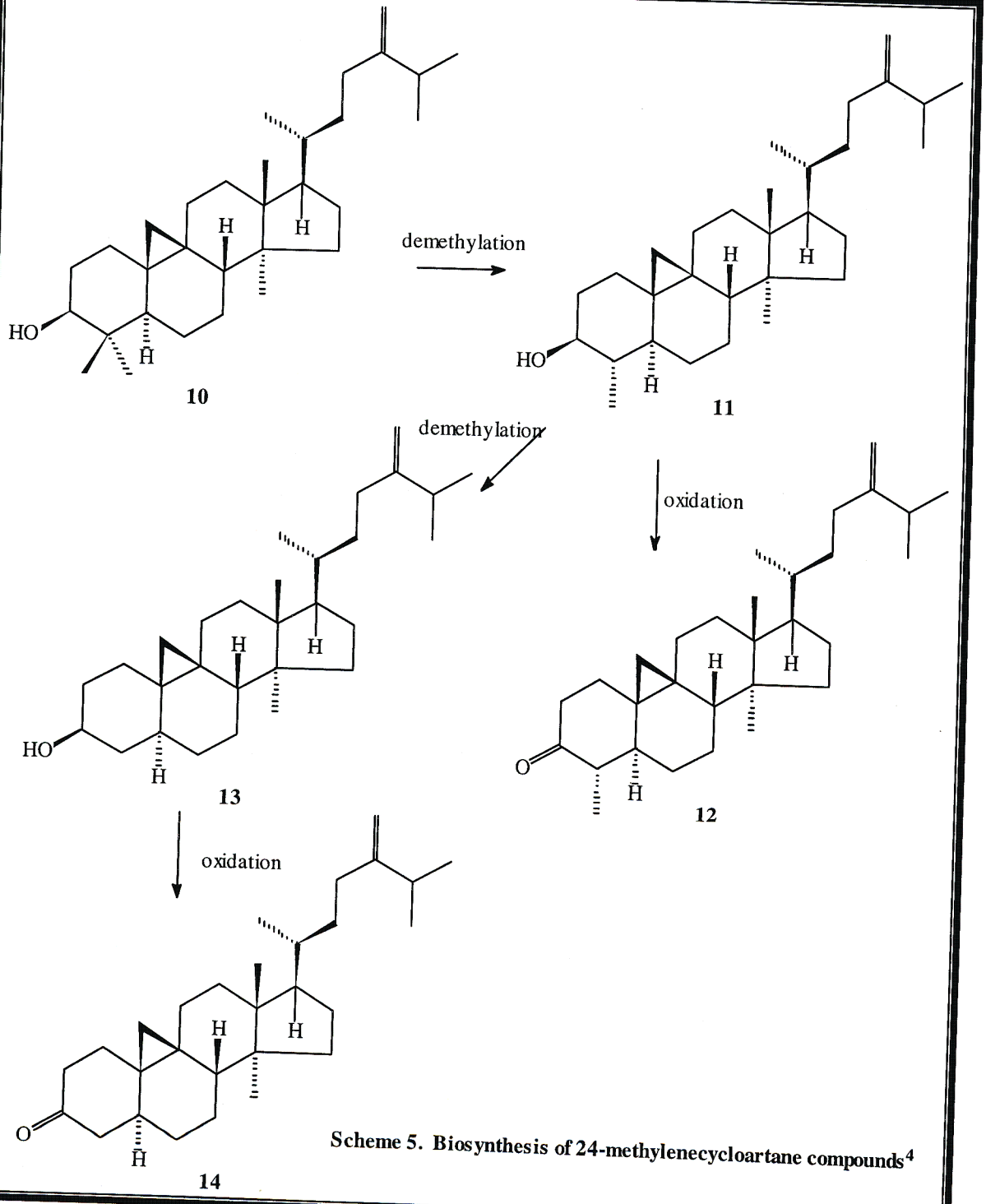
7

The C-24 methylene group arises by the *trans*-methylation from the methyl group of methionine (**6**). The hydrogen at C-24 of (**1**) migrates to C-25 in the stabilization of cation (**9**) [7] (**Scheme 4**). This results in the formation of 24-methylenecycloartanol (**10**).



Scheme 4. Biosynthesis of 24-methylenecycloartanol[5,7]

A single demethylation at C-4 of (**10**) results in cycloeucalenol (**11**) and a further oxidation of the 3β-hydroxy group results in cycloeucalenone (**12**). Two demethylations at C-4 of (**10**) results in 24-methylenepollinastanol (**13**). An oxidation of the 3β-OH group of (**13**) results in 24-methylenepollinastanone (**14**), which has been isolated for the first time in this work (**Scheme 5**).



Scheme 5. Biosynthesis of 24-methylenecycloartane compounds[4]

1.2.3 References

1. de Mayo, P. (1959) **The Triterpenoids: I**, *in* **The Higher Terpenoids**, Ed. Bentley, K. W., Interscience Publishers Inc., New York, pp 64, 92.

2. Conolly, J.D. and Overton, K.H. (1972) **The triterpenoids**, *in* **Chemistry of terpenes and terpenoids**, Ed. Newman, A.A., Academic Press, London, pp 207-211.

3. Milon, A., Nakatani, Y., Kintzinger, J. and Ourisson, G. (1989) **The conformation of cycloartenol investigated by NMR and molecular mechanics**, *Helvetica Chimica Acta,* **72,** 1-13.

4. Goad, L. J. (1991) **Phytosterols,** *in* **Methods in plant biochemistry,** Volume 7, series Eds. Dey, P.M. and Harborne, J. B., Eds. Charlwood, B. V. and Banthorpe, D.V., Academic Press, London, pp 369-383.

5. Templeton, W. (1969) **The triterpenoids**, *in* **An introduction to the chemistry of the terpenoids and steroids**, Butterworths and Co. Ltd., London, pp 124, 129-135.

6. Akihisa, T., Kokke, W. C. M. C. and Tamura, T. (1991) **Naturally occurring sterols and related compounds from plants,** *in* **Physiology and biochemistry of sterols,** Ed. Patterson, G. W. and Nes, W. D., American Oil Chemists' Society, Champaigne, Illinois, pp 205-206.

7. Lenton, J.R., Goad, L.J. and Goodwin, T.W. (1975) **Sitosterol biosynthesis in *Hordeum vulgare***, *Phytochemistry*, **14,** 1523-1528.

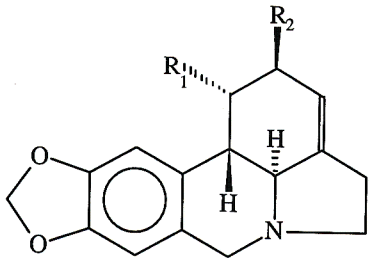
1.3. Extracts from *Ammocharis coranica*

1.3.1. Introduction

Ammocharis coranica (Ker-Gawl) Herb., belongs to the Amaryllidaceae family and is a large bulb with a scale-like covering and large green leaves which is often found growing along old riverbeds and old lands and bears a head of beautiful red flowers[1]. It belongs more specifically to the subtribe Crininae which is one of two subtribes of the tribe Amaryllideae. The Amaryllideae tribe consists of 11 genera and approximately 155 species. *Ammocharis, Boophane, Crinum,* and *Cybistetes* all belong to the Crininae subtribe[2]. The distribution pattern of the subtribe Crininae indicates that it is widespread in the tropical and temperate regions of sub-saharan Africa. *Ammocharis* is a widely distributed sub-Saharan plant[2].

The bulbs are used by the Zulu people to treat any illness believed to be caused by witchcraft, which could be any illness from nausea and vomiting to epilepsy[2]. Another use, totally isolated from any medicinal effect, is the use of the charred and crushed bulb scale of *Ammocharis coranica* as an adhesive[2]. The Swazi people partially burn the outside layers of the bulb, and chew the charred portions until a pitch-like substance is formed. This is then used to make the headring of the chiefs and head-men[1].

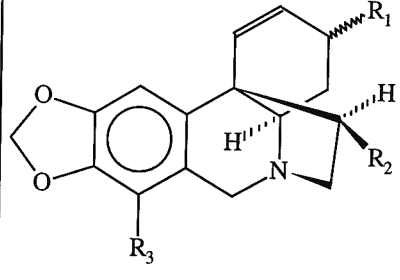
Ammocharis coranica is one of sixty-two species of the approximately two hundred and fifty species of the Amaryllidaceae family which have been investigated phytochemically. Eleven alkaloids have been previously isolated from *Ammocharis coranica*. These are lycorine (**1**), caranine (**2**) and acetylcaranine (**3**) from the lycorine group[3,4], buphanisine (**4**), *epi*buphanisine (**5**), buphanidrine (**6**) and ambelline (**7**) from the crinine group[4] and crinamine (**8**)[3,4], 6-hydroxycrinamine (**9**) and epivittatine (**10**)[4] from the haemanthamine group and an uncharacterised alkaloid, coranicine[4].



(**1**) $R_1 = R_2 = OH$

(**2**) $R_1 = OH, R_2 = H$

(**3**) $R_1 = OAc, R_2 = H$

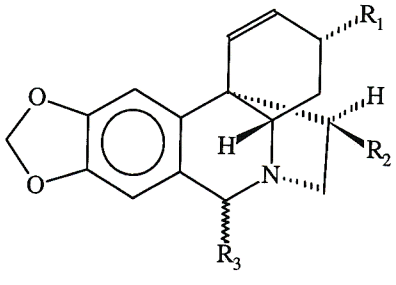


(**4**) $R_1 = OMe\alpha, R_2 = R_3 = H$

(**5**) $R_1 = OMe\beta, R_2 = R_3 = H$

(**6**) $R_1 = OMe\alpha, R_2 = H, R_3 = OMe$

(**7**) $R_1 = OMe\alpha, R_2 = OH, R_3 = OMe$



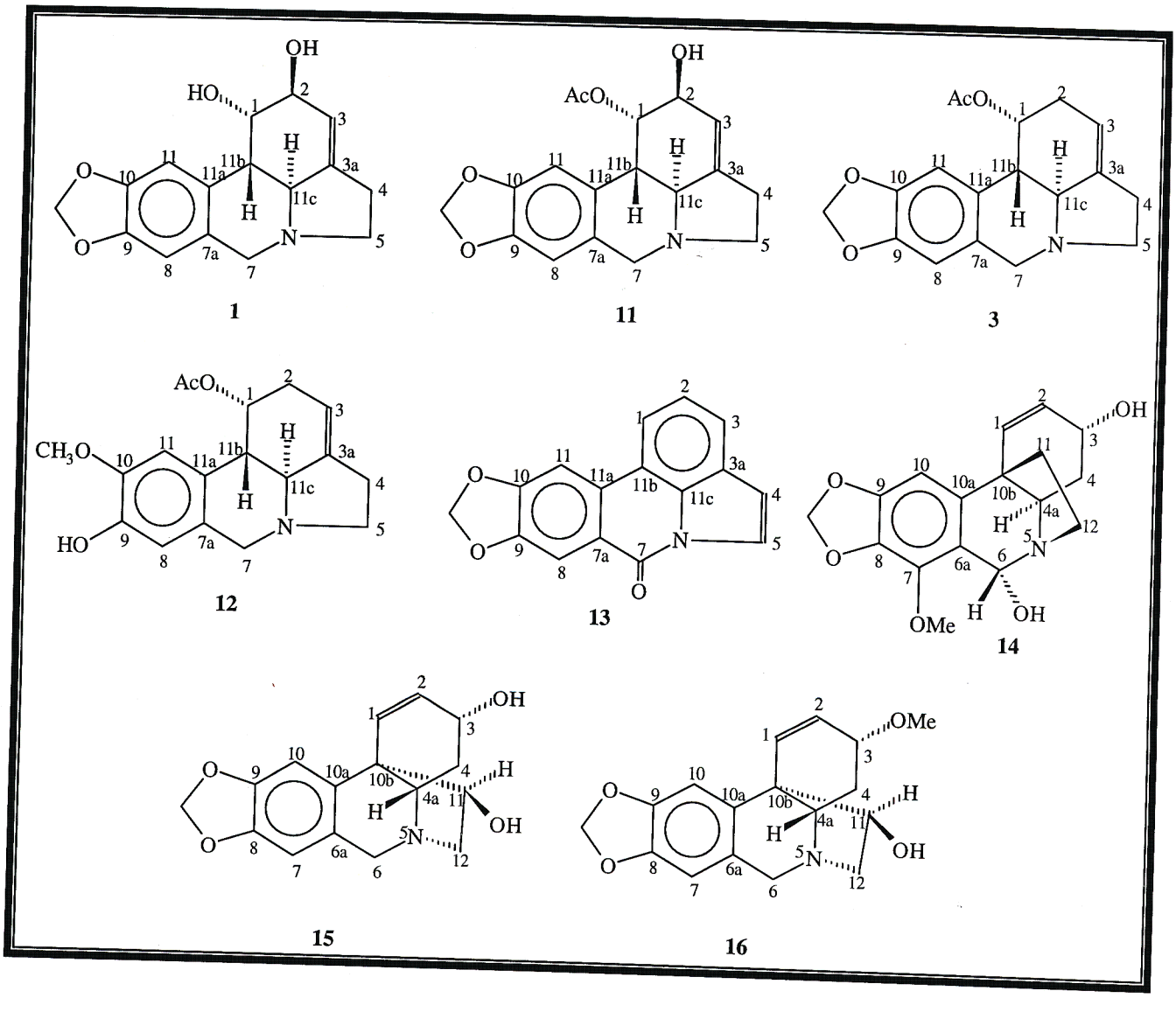
(**8**) $R_1 = OMe, R_2 = OH, R_3 = H$

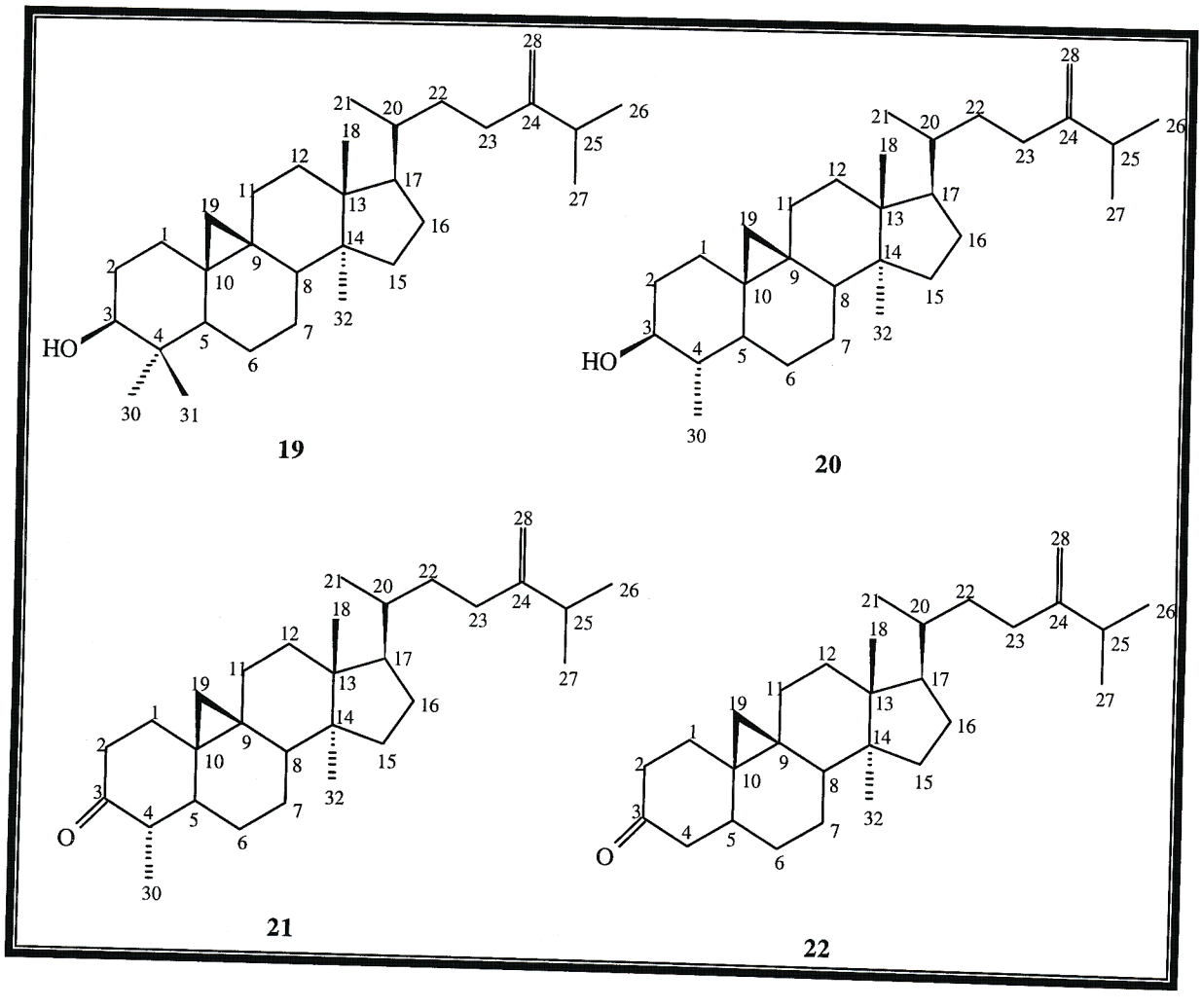
(**9**) $R_1 = OMe, R_2 = OH, R_3 = OH$

(**10**) $R_1 = OH, R_2 = H, R_3 = H$

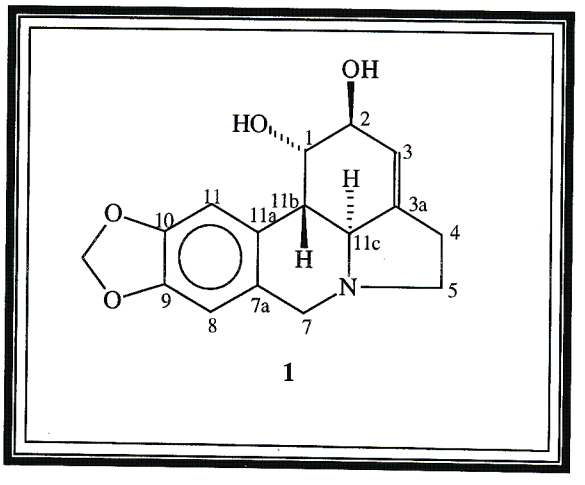
1.3.2 Results and Discussion

The basic chloroform extract of the cut bulbs of this species afforded the Amaryllidaceae alkaloids, lycorine (**1**), 1-O-acetyllycorine (**11**), acetylcaranine (**3**), 1-O-acetyl-9-norpluviine (**12**), hippadine (**13**), 6α-hydroxypowelline (**14**), hamayne (**15**) and crinamine (**16**). Also isolated from the basic chloroform extract were four cycloartane compounds, 24-methylenecycloartan-3-ol (**19**), cycloeucalenol (**20**), cycloeucalenone (**21**) and 24-methylenepollinastanone (**22**).




HO
30
31
32
27
28
O
19
20
21
22

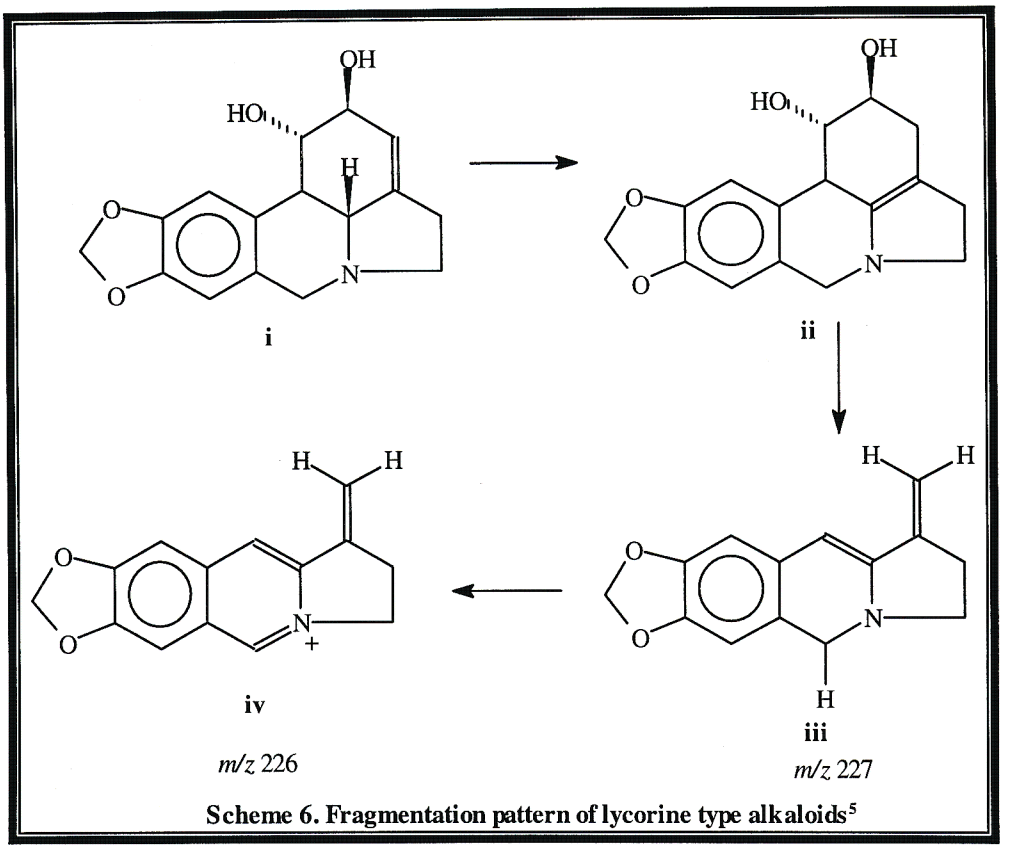
1.3.2.1 Alkaloids from *Ammocharis coranica*. The Amaryllidaceae alkaloids



1

Compound (**1**), lycorine [$C_{16}H_{17}NO_4$, M^+ at *m/z* 287.1156] was isolated as a white crystalline material. Lycorine was previously isolated from *Ammocharis coranica* by Mason *et al.*[3] and Hauth and Stauffacher[4]. This compound, which is ubiquitous amongst the Amaryllidaceae family, is known to inhibit ascorbic acid biosynthesis *in vivo*. Its biological activity is discussed in section 1.1.3. Spectra for lycorine are given on pages 150-153 in appendix A.

The mass spectrum of lycorine showed an intense molecular ion peak at *m/z* 287.1156 corresponding to $C_{16}H_{17}NO_4$. Twin base peaks occurred at *m/z* 227 (M^+ - 60) and *m/z* 226 (M^+ - 61). The formation of these intense fragment ions was the result of the loss of C-1 and C-2 and their substituents (CHOHCHOH = 60; CHOHCHOH + H = 61) by the fragmentation pattern shown in **scheme 6** to form **iii** and **iv** respectively. These daughter ions are known to occur in all lycorine type alkaloids containing a 3-3a double bond[5]. The other fragment ion of reasonable intensity at *m/z* 268 (M^+ - 19) was attributed to the loss of water followed by the loss of an additional hydrogen atom (H_2O + H = 19). The ease of water loss from the molecular ion has been found to be greatly dependant on the stereochemistry of the C-2 hydroxy group[5]. In 2-*epi*lycorine, the peak at *m/z* 269 is five times as intense as the molecular ion while in lycorine, this peak is much smaller than the molecular ion peak[5]. Since the peak at *m/z* 268 was half the intensity of the molecular ion peak, the structure of compound **1** was confirmed to be lycorine.

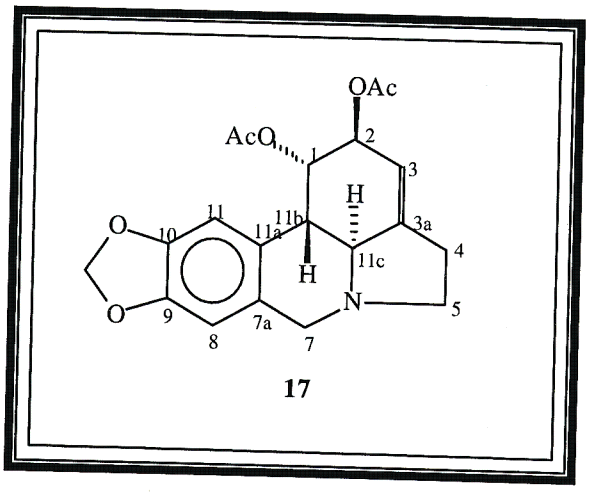


Scheme 6. Fragmentation pattern of lycorine type alkaloids[5]

The i.r. spectrum showed two broad bands at ν_{max} 3330 and 3410 cm^{-1} (O – H stretching) and sharp bands at 1044 cm^{-1} and 1004 cm^{-1} (C – O stretching) indicative of the hydroxy groups, 1505 cm^{-1} and 1498 cm^{-1} (aromatic C = C stretching) suggestive of an aromatic ring, 1274 cm^{-1} and 1241 cm^{-1} (C – N stretching) indicating C – N bonds and 945 cm^{-1} (C – O – C stretching) 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, attributed to H-11 and H-8 of ring A. A singlet integrating to two protons at δ_H 5.94 was assigned to the methylenedioxy group protons attached to ring A. In the olefinic region of the spectrum at δ_H 5.58, a broad singlet integrating to one proton was assigned to H-3. Resonances ascribed to H-1 and H-2 appeared as broad singlets at δ_H 4.50 and 4.20 of one proton each. The pair of doublets (J=14.28 Hz) at δ_H 3.60 and δ_H 4.16, each integrating to one proton, was assigned to H-7α and H-7β respectively. The multiplet at δ_H 3.38 and the double doublet at δ_H 2.48 (J = 17.58 Hz, 3.38 Hz) were

attributed to the two protons at position 5. The two protons at position 4 appeared at δ_H 2.65 as a multiplet. The H-11b resonance appeared in the multiplet at δ_H 2.70. The resonance ascribed to H-11c appeared as a doublet at δ_H 2.93 (J = 11.36 Hz).



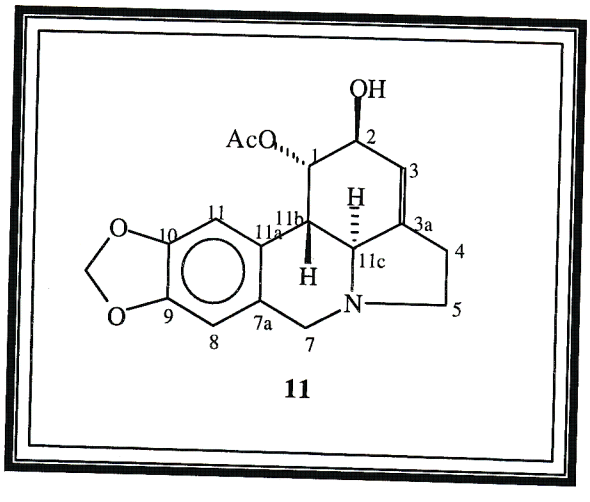
17

Acetylation of lycorine (**1**) afforded the di-acetate (**17**). Spectra for 1,2-di-O-acetyllycorine are given on pages 154-160 in appendix A. The ^{1}H NMR spectrum of the di-acetate clearly showed a downfield shift of H-1 from δ_H 4.50 to δ_H 5.78. The H-2 resonance also shifted downfield from δ_H 4.20 to δ_H 5.29. This was evidence that acetylation had occurred at both positions 1 and 2 of ring C. The appearance of two singlets, each integrating to three protons at δ_H 1.95 and δ_H 2.12 attributed to the methyl group protons of the acetyl substituents further indicated that the diacetyl derivative was made.

The i.r. spectrum showed intense bands at ν_{max} 1742 cm^{-1} (C = O stretching), 1044 cm^{-1} and 1004 cm^{-1} (C – O stretching) indicative of the acetate groups. Also present were bands at ν_{max} 1492 cm^{-1} (aromatic C = C stretching) indicating an aromatic ring, 1248 cm^{-1} (C – N stretching) suggestive of C – N bonds and 945 cm^{-1} (C – O – C stretching) indicative of the methylenedioxy group.

The mass spectrum of 1,2-di-O-acetyllycorine showed a molecular ion peak at *m/z* 371.1360 corresponding to $C_{20}H_{21}NO_6$. The peak at *m/z* 311 (M^+- 60) was due to the loss

of acetic acid (CH_3COOH = 60) from either position 1 or 2. The peak at *m/z* 250 was due to the loss of two acetic acid molecules and a hydrogen atom. The base peak at *m/z* 252 (M^+- 119) was due to the loss of acetic acid followed by the loss of an acetyl fragment ($CH_3COOH + CH_3COO$ = 119). The peaks at *m/z* 227 (M^+- 144) and *m/z* 226 (M^+- 145) were due to the loss of C-1 and C-2 along with their acetyl substituents to form **iii** and **iv** (**scheme 6**).



11

1-O-Acetyllycorine (**11**), [$C_{18}H_{19}NO_5$, M^+ at *m/z* 329.1272] was isolated as a white crystalline material. This compound had been reported to occur in the bulbs of *Crinum latifolium* and *Crinum bulbispermum*[6]. This is the first report of 1-O-acetyllycorine in *Ammocharis coranica*. Spectra for 1-O-acetyllycorine are given on pages 161-167 in appendix A.

The mass spectrum of 1-O-acetyllycorine showed a molecular ion peak at *m/z* 329.1272 corresponding to $C_{18}H_{19}NO_5$. The peak at *m/z* 268 (M^+- 61) indicated the loss of acetic acid and the further loss of a hydrogen atom ($CH_3COOH + H$ = 61). The intense twin peaks at *m/z* 227 (M^+-102) and *m/z* 226 (M^+- 103) were due to the loss of C-1 and C-2 with their substituents, to form **iii** and **iv** respectively (**scheme 6**).

The i.r. spectrum showed bands at ν_{max} 3440 cm^{-1} (O – H stretching) indicating the presence of a hydroxy group, 1748 cm^{-1} (C = O stretching) indicative of the acetate group, 1051 cm^{-1} and 1004 cm^{-1} (C – O stretching), confirming the hydroxy and acetate

groups, 1485 cm^{-1} (aromatic C = C stretching) suggestive of an aromatic ring, 1241 cm^{-1} (C – N stretching) indicating C – N bonds and 945 cm^{-1} (C – O – C stretching) indicative of the methylenedioxy group.

The ^1H NMR spectrum had two noticeable differences to that of lycorine (**1**). The broad singlet that initially appeared at δ_H 4.50 attributed to H-1 in the lycorine spectrum now appeared at δ_H 5.73. The singlet at δ_H 6.91 attributed to H-11 in the lycorine spectrum occurred at δ_H 6.76 in the spectrum of 1-O-acetyllycorine. These two changes in the spectrum were both due to the influence of the acetyl group at position 1 of ring C. The methyl group protons of the acetyl group occurred as a singlet integrating to three protons at δ_H 1.93. A one proton singlet ascribed to H-8 occurred at δ_H 6.67, while the methylenedioxy group protons appeared as a singlet at δ_H 5.94 (2H). The broad singlet at δ_H 5.58 (1H) was attributed to H-3. At δ_H 4.19, a broad singlet integrating to one proton appeared and was attributed to H-2. Strong coupling between the pair of doublets at δ_H 4.16 (14.28 Hz) and δ_H 3.60 (14.28 Hz) could be seen in the COSY spectrum. These were attributed to H-7β and H-7α respectively. This was confirmed in the HETCOR spectrum where both these resonances could be seen coupling to C-7 at δ_C 57.6. The COSY spectrum also showed coupling between the resonances at δ_H 3.33 and δ_H 2.50, a multiplet and double doublet respectively, each integrating to one proton. These resonances were attributed to both the protons at position 5. This was confirmed in the HETCOR spectrum as both these proton resonances coupled to C-5 at δ_C 54.6. These resonances also showed coupling in the COSY spectrum to the multiplet at δ_H 2.68 (2H) attributed to both protons at position 4. At δ_H 2.90 a broad singlet integrating to two protons occurred and was attributed to H-11b and H-11c.

The chemical shifts of the proton resonances may differ in spectra run with different solvents, however this effect is not so pronounced in ^{13}C NMR spectra. Previous authors have reported NMR data of lycorine (**1**) in DMSO-d_6[7] and CD_3OD : CD_3COOD (3:1)[8] because of its insolubility in the normal organic solvents. NMR spectra of 1-O-acetyllycorine[6,9] and 1,2-di-O-acetyllycorine[6] were reported in $CDCl_3$. ^1H and ^{13}C NMR

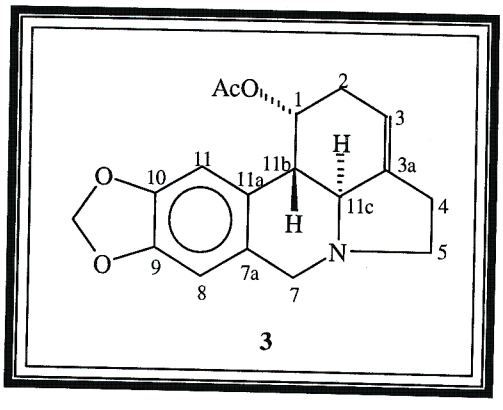
resonances of lycorine (**1**), 1-O-acetyllycorine (**11**) and 1,2-di-O-acetyllycorine (**17**) with CD_3OD as solvent are reported here for the first time (**Table 2 and 3**).

Table 2. ^1H NMR data of lycorine (1), 1-O-acetyllycorine (11) and 1,2-di-O-acetyllycorine (17) (300 MHz, CD_3OD)

	lycorine (1)	1-O-acetyllycorine (11)	1,2-di-O-acetyllycorine (17)
H-1	4.50 (s)	5.73 (s)	5.78 (s)
H-2	4.20 (brs)	4.19 (brs)	5.29 (brs)
H-3	5.58 (brs)	5.58 (brs)	5.56 (brs)
H-4	2.65 (m)	2.68 (m)	2.71 (m)
H-5	2.48 (dd) *J* = 17.58 Hz, 8.72 Hz 3.38 (m)	2.50 (dd) *J* = 17.64 Hz, 8.79 Hz 3.33 (m)	2.52 (dd) *J* = 17.71 Hz, 8.86 Hz 3.40 (m)
H-7α H-7β	3.60 (d) *J* = 14.28 Hz 4.16 (d) *J* = 14.28 Hz	3.60 (d) *J* = 14.28 Hz 4.16 (d) *J* =14.28 Hz	3.59 (d) *J* = 14.29 Hz 4.18 (d) *J* = 14.29 Hz
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) *J* = 11.36 Hz	2.90 (brs)	2.91 (s)
OCH_2O	5.94 (s)	5.94 (s)	5.94 (s)
$OCOCH_3$-1	-	1.93 (s)	1.95 (s)
$OCOCH_3$-2	-	-	2.12 (s)

Table 3. ^{13}C NMR data of lycorine (1), 1-O-acetyllycorine (11) and 1,2-di-O-acetyllycorine (17) (75 MHz, CD_3OD)

	lycorine (1)	1-O-acetyllycorine (11)	1,2-di-O-acetyllycorine (17)
C-1	73.1 (d)	73.4 (d)	70.6 (d)
C-2	71.9 (d)	70.3 (d)	72.0 (d)
C-3	119.2 (d)	119.0 (d)	115.3 (d)
C-3a	143.6 (s)	143.8 (s)	146.9 (s)
C-4	29.3 (t)	29.3 (t)	29.3 (t)
C-5	54.7 (t)	54.6 (t)	54.5 (t)
C-7	57.7 (t)	57.6 (t)	57.6 (t)
C-7a	130.4 (s)	130.4 (s)	130.4 (s)
C-8	108.2 (d)	108.4 (d)	108.3 (d)
C-9	148.2 (s)	148.1 (s)	148.1 (s)
C-10	147.7 (s)	147.9 (s)	146.9 (s)
C-11	106.0 (d)	105.7 (d)	105.9 (d)
C-11a	129.7 (s)	128.3 (s)	127.5 (s)
C-11b	41.3 (d)	40.1 (d)	41.4 (d)
C-11c	62.5 (d)	62.9 (d)	62.6 (d)
$OCO\underline{C}H_3$-1	-	20.8 (q)	20.6 (q)
$O\underline{C}OCH_3$-1	-	172.2 (s)	171.6 (s)
$OCO\underline{C}H_3$-2	-	-	20.9 (q)
$O\underline{C}OCH_3$-2	-	-	171.4 (s)
$O\underline{C}H_2O$	102.3 (t)	102.4 (t)	102.4 (t)



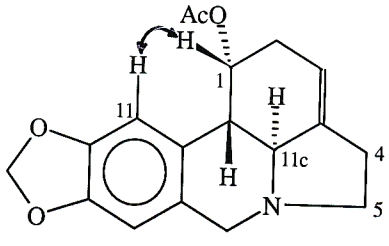
3

Acetylcaranine (**3**), [$C_{18}H_{19}NO_4$, M^+ at *m/z* 313.1316] was isolated as white crystalline material. This compound was previously isolated from *Ammocharis coranica*[3,4]. Acetylcaranine was also reported previously from the bulbs of *Amaryllis belladonna* and showed *in vitro* activity against the murine P388 lymphocytic leukemia system[10]. Spectra for acetylcaranine are given on pages 168-175 in appendix A.

The mass spectrum of acetylcaranine showed a molecular ion peak at *m/z* 313.1316 that corresponded to the molecular formula $C_{18}H_{19}NO_4$. A peak at *m/z* 270 (M^+- 43), indicated the loss of a CH_3CO fragment. The loss of acetic acid with the further loss of a hydrogen atom was indicated by the intense peak *m/z* 252 (M^+- 61) (CH_3COOH + H = 61). The intense peaks at *m/z* 227 (M^+- 86) and *m/z* 226 (M^+- 87) are the structures **iii** and **iv** in **scheme 6**, resulting from the loss of C-1 and C-2 with their substituents in the manner shown in **scheme 6**. This is common among lycorine type alkaloids with a 3-3a double bond.

The i.r. spectrum showed bands at ν_{max} 1729 cm^{-1} (C = O stretching) and 1037 cm^{-1} (C – O stretching) indicative of the acetate group, 1496 cm^{-1} and 1380 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1241 cm^{-1} (C – N stretching) indicating C – N bonds and 939 cm^{-1} (C – O – C stretching), indicative of the methylenedioxy group.

The ^{1}H NMR spectrum showed two singlets of one proton each at δ_H 6.56 and δ_H 6.68 attributed to the two aromatic protons, H-11 and H-8 respectively. A broad singlet at δ_H 5.82 was attributed to H-1 while the broad singlet at δ_H 5.39 was attributed to H-3 (1H). The assignments of H-11 and H-1 were confirmed in an NOE experiment where the singlet at δ_H 6.56 was irradiated and this showed the enhancement of the H-1 signal at δ_H 5.82.

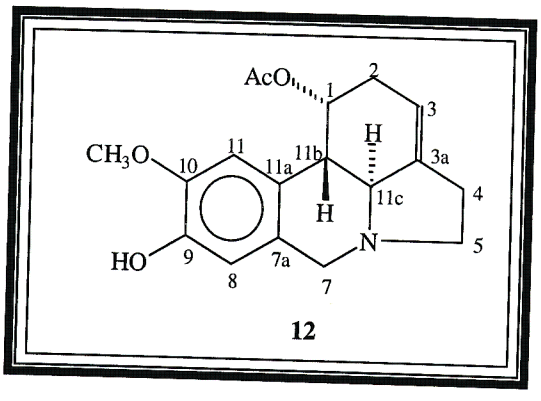


3

The methylenedioxy group proton resonance was evident at δ_H 5.89 as a singlet integrating to two protons. A pair of doublets at δ_H 3.60 and δ_H 4.11, integrating to one proton each was attributed to H-7α and H-7β respectively. These two resonances showed strong coupling to each other in the COSY spectrum. Further confirmation is given in the HETCOR spectrum, where both these proton resonances were coupled to the C-7 resonance at δ_C 55.9.

The resonances at δ_H 3.29, a multiplet integrating to one proton, and δ_H 2.38, a broad singlet also integrating to one proton, were attributed to the protons at position 5. Both these resonances were coupled to the C-5 resonance at δ_C 53.7. The resonance at δ_H 2.38 also showed strong coupling (COSY) to the broad singlets at δ_H 2.64 and δ_H 2.68 attributed to the protons at position 4. The protons at position 2 appeared at δ_H 2.32 (1H, brs) and δ_H 2.60, another broad singlet integrating to one proton. Coupling could also be seen between the two H-2 resonances in the COSY spectrum. Furthermore, these two H-2 resonances were coupled to the C-2 resonance at δ_C 33.1. The resonances ascribed to H-11b and H-11c both appeared as broad singlets each integrating to one proton at δ_H 2.48 and δ_H 2.87. The protons of the acetyl group appeared at δ_H 1.91, a singlet which

integrated to three protons. The ^{1}H and ^{13}C NMR data compare well with that from literature (**Tables 4 and 5**)[10].



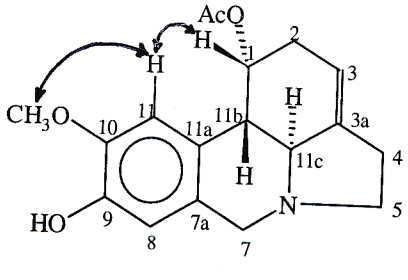
12

Compound (**12**), 1-O-acetyl-9-norpluviine, [$C_{18}H_{21}NO_4$, M^+ at *m/z* 315.1466] was isolated as white crystalline material. This is the first report of this compound, however 1-O-acetyl-10-norpluviine is a known Amaryllidaceae alkaloid. Spectra for 1-O-acetyl-9-norpluviine are given on pages 176-183 in appendix A.

The mass spectrum of 1-O-acetyl-9-norpluviine showed a molecular ion peak at *m/z* 315.1466, corresponding to the molecular formula $C_{18}H_{21}NO_4$. This compound had a similar fragmentation pattern to acetylcaranine, the difference being that all the major peaks were two units higher, attributed to two extra protons as a result of the presence of a methoxy and a hydroxy group at C-10 and C-9 respectively instead of a methylenedioxy group between these two carbons. The loss of acetic acid, together with a hydrogen atom (CH_3COOH + H = 61), resulted in the peak at *m/z* 254 (M^+- 61). The intense peaks at *m/z* 229 (M^+- 86) and *m/z* 228 (M^+- 87) were the result of the loss of C-1 and C-2 along with their substituents. This occurred in the manner indicated in **scheme 6**.

The i.r. spectrum showed bands at ν_{max} 3440 cm^{-1} (O – H stretching) and 1235 cm^{-1} (C – O stretching) indicative of the hydroxy group, 1735 cm^{-1} (C = O stretching) and 1037 cm^{-1} (C – O stretching) indicating the presence of an acetate group, 1100 cm^{-1} (C – O stretching) indicative of the methoxy group, 1518 cm^{-1} (aromatic C = C stretching) suggestive of an aromatic ring and 1255 cm^{-1} (C – N stretching) indicating C – N bonds.

The ^1H NMR spectrum of 1-O-acetyl-9-norpluviine (**12**) was similar to that of acetylcaranine (**3**) except for one noticeable difference, the disappearance of the two proton singlet at δ_H 5.89 and the appearance of a three proton singlet at δ_H 3.83. This was indicative of the presence of a hydroxy group and a methoxy group in place of the methylenedioxy group. Two singlets at δ_H 6.86 and δ_H 6.61 were attributed to the aromatic protons of ring A, H-11 and H-8 respectively. The position of the methoxy and hydroxy groups as well as the assignment of the H-11 and H-8 protons were ascertained in an NOE experiment where the singlet at δ_H 6.86 assigned to H-11 was irradiated and this showed the enhancement of the three proton singlet at δ_H 3.83 assigned to the methoxy group and the doublet at δ_H 6.06 (1H, J = 3.42 Hz) attributed to H-1, which confirmed that the methoxy group was at C-10 and that the resonance at δ_H 6.86 was due to the H-11 proton.



12

The H-1 resonance at δ_H 6.06 showed coupling (COSY) to the resonance at δ_H 2.79, a one proton doublet (J = 10.44 Hz) attributed to H-11b. At δ_H 5.48, another one proton doublet (J = 2.39 Hz) occurred and was attributed to H-3. A pair of doublets at δ_H 3.56 (J = 14.00 Hz) and δ_H 4.14 (J = 14.00 Hz), each integrating to one proton, was attributed to the two protons at position 7. These two proton resonances showed strong coupling to each other in the COSY spectrum and were coupled to the C-7 resonance in the

HETCOR spectrum at δ_C 57.1 which confirmed their assignment. The protons at position 5 appeared as a one proton multiplet at δ_H 3.33, and at δ_H 2.48, a one proton double doublet (J = 17.70 Hz, 8.91 Hz). These assignments were confirmed by strong coupling in the COSY spectrum between these proton resonances which were also coupled to the C-5 resonance at δ_C 54.7 in the HETCOR spectrum. The resonance at δ_H 2.89, a broad doublet integrating to one proton (J = 10.44 Hz) was assigned to H-11c. This resonance was coupled to the doublet (J = 10.44 Hz, 1H) at δ_H 2.79 in the COSY spectrum assigned to H-11b. The protons at position 2 appeared at δ_H 2.40 and δ_H 2.68, both of which were multiplets each integrating to one proton. These resonances showed very weak coupling in the COSY spectrum, but both these proton resonances were coupled to the C-2 resonance at δ_C 34.2 in the HETCOR spectrum. At δ_H 2.64, a two proton multiplet appeared which was attributed to the protons at position 4. The methyl proton resonance of the acetyl group appeared as a singlet at δ_H 1.90.

Table 4. ^{1}H NMR data of acetylcaranine (3) and 1-O-acetyl-9-norpluviine (12) (300 MHz)

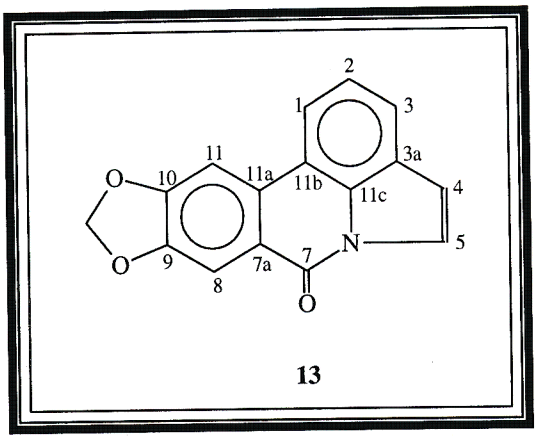
	Acetylcaranine (3) in $CDCl_3$	**Acetylcaranine (3) in $CDCl_3$ [10]**	**1-O-acetyl-9-norpluviine (12) in CD_3OD**
H-1	5.82 (brs) J = 3.17 Hz	5.40 (brs) *	6.06 (d) J = 3.42 Hz
H-2a	2.32 (brs)	2.48 (m)	2.40 (m)
H-2b	2.60 (brs)	2.48 (m)	2.68 (m)
H-3	5.39 (brs)	5.90 (brs) *	5.48 (d) J = 2.39 Hz
H-4a	2.64 (brs)	2.48 (m)	2.64 (m)
H-4b	2.68 (brs)	2.48 (m)	2.64 (m)
H-5a	2.38 (brs)	2.48 (m)	2.48 (dd) J = 17.70 Hz, 8.79 Hz
H-5b	3.29 (m)	3.32 (m)	3.33 (m)
H-7α	3.60 (d) J = 14.16 Hz	3.54 (d) J = 15 Hz	3.56 (d) J = 14.00 Hz
H-7β	4.11 (d) J = 13.86 Hz	4.16 (d) J = 15 Hz	4.14 (d) J = 14.00 Hz

H-8	6.68 (s)	6.58 (s) *	6.61 (s)
H-11	6.56 (s)	6.74 (s) *	6.86 (s)
H-11b	2.48 (brs)	2.48 (m)	2.79 (d) J = 10.44 Hz
H-11c	2.87 (brs)	2.48 (m)	2.89 (bd) J = 10.44 Hz
OCH_3	-	-	3.83 (s)
OCH_2O	5.89 (s)	5.92 (s)	-
$OCOCH_3$	1.91 (s)	1.92 (s)	1.90 (s)

* NOE experiments show these literature assignments to be incorrect.

Table 5. ^{13}C NMR data of acetylcaranine (3) and 1-O-acetyl-9-norpluviine (12) (75 MHz)

	Acetylcaranine (3) in $CDCl_3$	**Acetylcaranine (3) in $CDCl_3$[10]**	**1-O-acetyl-9-norpluviine (12) in CD_3OD**
C-1	66.1 (d)	66.52 (d)	67.6 (d)
C-2	33.1 (t)	33.34 (t)	34.2 (t)
C-3	115.3 (d)	114.19 (d)	115.9 (d)
C-3a	138.0 (s)	139.53 (s)	139.8 (s)
C-4	28.9 (t)	28.62 (t)	29.2 (t)
C-5	53.7 (t)	53.71 (t)	54.7 (t)
C-7	55.9 (t)	56.99 (t)	57.1 (t)
C-7a	127.7 (s)	129.55 (s)	129.3 (s)
C-8	104.9 (d)	105.02 (d)	114.9 (d)
C-9	146.3 (s)	146.42 (s)	147.8 (s)
C-10	147.0 (s)	146.13 (s)	146.2 (s)
C-11	107.5 (d)	107.29 (d)	109.5 (d)
C-11a	127.7 (s)	127.73 (s)	126.3 (s)
C-11b	42.5 (d)	43.57 (d)	44.1 (d)
C-11c	60.8 (d)	61.25 (d)	62.8 (d)
OCH_2O	101.0 (t)	100.89 (t)	-
OCH_3	-	-	56.4 (q)
$OCOCH_3$	21.2 (q)	21.22 (q)	21.0 (q)
$OCOCH_3$	170.8 (s)	170.79 (s)	172.4 (s)



13

Hippadine (**13**), [$C_{16}H_{19}NO_3$, M^+ at *m/z* 263.0584] was isolated as a white crystalline material. This is the first report of hippadine in *Ammocharis coranica.* This compound, which is present in a number of *Crinum* species including *Crinum pratense*[11], *Crinum latifolium*[12] and also *Haemanthus kalbreyeri*[13] and *Hippeastrum vittatum*[14] has been shown to be a very active compound. This is discussed in section 1.1.3. Spectra for hippadine are given on pages 184-191 in appendix A.

The mass spectrum of hippadine showed a molecular ion peak at *m/z* 263.0584 corresponding to the molecular formula $C_{16}H_9NO_3$. The molecular ion was the base peak, which showed the high stability of the pyrrolophenanthridone skeleton of hippadine.

The i.r. spectrum showed bands at ν_{max} 1676 cm^{-1} (C = O stretching) indicative of the carbonyl group, 1465 cm^{-1} (aromatic C = C stretching) suggestive of an aromatic ring, 1314 cm^{-1} (C – N stretching) indicating C – N bonds, 1037 cm^{-1} (C – O stretching) and 939 cm^{-1} (C – O – C stretching) indicative of the methylenedioxy group.

The ^1H NMR spectrum of compound (**13**) displayed a pair of doublets at δ_H 7.90 (1H, J = 7.69 Hz) and δ_H 7.73 (1H, J = 7.70 Hz) each integrating to one proton attributed to H-1 and H-3 respectively. The H-1 and H-3 resonances both showed coupling to the resonance at δ_H 7.47, a double doublet (J = 7.70 Hz, 7.69 Hz) ascribed to H-2 (COSY). Another pair of doublets at δ_H 8.03 (1H, J = 3.66 Hz) and δ_H 6.88 (1H, J = 3.72 Hz) also

showed strong coupling in the COSY spectrum. These resonances were assigned to H-5 and H-4 respectively. The resonances ascribed to H-8 and H-11 appeared as one proton singlets at δ_H 7.97 and δ_H 7.65 respectively. The methylenedioxy group appeared as a singlet integrating to two protons at δ_H 6.15.

Literature values for ^{1}H NMR data for H-3 and H-5 for hippadine (**13**) differ from each other[11, 14] as shown in **Table 6**. NMR data obtained in this work for H-3 agreed with that of Ali *et al.*[14] and for H-5 agreed with that of Ghosal *et al.*[11]. There is no other possible structure to fit the NMR data obtained. ^{1}H NMR assignments of hippadine (**13**) in DMSO-d_6 were also reported in literature[15] but these resonances were slightly shifted when compared to those using $CDCl_3$ as solvent. The ^{13}C NMR resonances of hippadine compare well with those from literature ($CDCl_3$)[11, 14].

Table 6. ^{1}H NMR data of hippadine (13) (300 MHz, $CDCl_3$)

	Hippadine (13)	**Hippadine (13)[14]**	**Hippadine (13) [11]**
H-1	7.90 (d) *J* = 7.69 Hz	7.87 (dd) *J* = 7.55 Hz, 1.00 Hz	7.87 (dd) *J* = 7.60 Hz, 1.00 Hz
H-2	7.47 (dd) *J* = 7.70 Hz, 7.69 Hz	7.44 (dd) *J* = 7.60 Hz, 7.55 Hz	7.44 (dd) *J* = 7.60 Hz, 7.55 Hz
H-3	7.73 (d) *J* = 7.70 Hz	7.73 (dd) *J* = 7.6 Hz, 1.0 Hz	7.33 (dd) *J* = 7.60 Hz, 1.00 Hz
H-4	6.88 (d) *J* = 3.72 Hz	6.88 (d) *J* = 3.66 Hz	6.88 (d) *J* = 3.66 Hz
H-5	8.03 (d) *J* = 3.66 Hz	8.23 (d) *J* = 3.66 Hz	8.03 (d) *J* = 3.66 Hz
H-8	7.97 (s)	7.95 (s)	7.95 (s)
H-11	7.65 (s)	7.61 (s)	7.61 (s)
OCH_2O	6.15 (s)	6.15 (s)	6.15 (s)

Table 7. ^{13}C NMR data of hippadine (13) (75 MHz, $CDCl_3$)

	Hippadine (13)	Hippadine (13)[14]	Hippadine (13)[11]
C-1	118.4 (d)	118.20 (d)	118.20 (d)
C-2	124.0 (d)	123.82 (d)	123.82 (d)
C-3	122.6 (d)	122.42 (d)	122.42 (d)
C-3a	*	128.28 (s)	128.28 (s)
C-4	110.8 (d)	110.59 (d)	110.59 (d)
C-5	123.6 (d)	123.34 (d)	123.34 (d)
C-7	*	157.95 (s)	157.95 (s)
C-7a	*	131.51 (s)	131.51 (s)
C-8	108.0 (d)	107.24 (d)	107.84 (d)
C-9	*	148.37 (s)	148.37 (s)
C-10	*	152.45 (s)	152.45 (s)
C-11	101.7 (d)	101.55 (d)	101.55 (d)
C-11a	*	107.84 (s)	119.58 (s)
C-11b	*	116.56 (s)	116.56 (s)
C-11c	*	130.85 (s)	130.85 (s)
O$\underline{C}H_2O$	102.3 (t)	102.11 (t)	102.11 (t)

* The singlets could not be seen in the ^{13}C NMR spectra as this compound was not isolated in sufficient amounts to get a strong ^{13}C NMR spectrum.

The next three compounds discussed all have a double bond at the Δ^1 position, a methoxy substituent at the 3 position, and a 5-10b-ethano bridge. The methoxy substituent at C-3 and the 5-10b-ethano bridge can either be on the same side (both α or both β) or on opposite sides (one α and the other β). This information can be derived from the splitting pattern of the H-2 resonance.

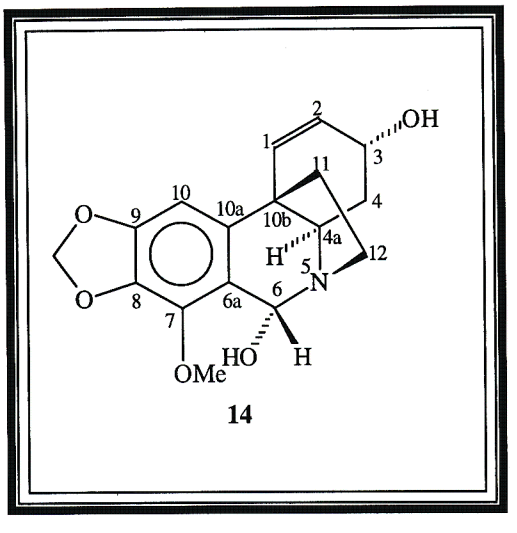
The hydrogen atoms, H-1 and H-2 have a dihedral angle of approximately 0^0 (from molecular models). They split each other into doublets of coupling constant 10 Hz. If the methoxy substituent at C-3 and the 5-10b-ethano bridge are on the same side, then the dihedral angle between H-2 and H-3 is approximately 90^0 and the H-2 resonance remains as a doublet of 10 Hz. However, if the methoxy substituent and the ethano bridge are on opposite sides, then the dihedral angle between H-2 and H-3 is approximately 30^0 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 split into a doublet of 10 Hz indicates a *cis* relationship between the methoxy substituent at C-3 and the 5-10b-ethano bridge while a H-2 resonance split into a double doublet of 10 Hz and 5 Hz is indicative of a *trans* relationship[16-18]. This is consistent with the ^{1}H NMR data published for compounds of these types, i.e. the crinine and haemanthamine type alkaloids with a Δ^1 double bond and a 5-10b-ethano bridge.

Examples with a *trans* relationship are haemanthamine, 11-*epi*haemanthamine, haemanthidine, vittatine, 11-hydroxyvittatine[19], albiflomanthine[20], 6-hydroxycrinine, 6-hydroxybuphanisine[16], krepowiine (also known as O-acetylcrinine)[21], powelline[6], 6α-hydroxypowelline, 6α-hydroxybuphanidrine[22], amaryllisine[23], crinine, buphanisine, buphanidrine, ambelline and 11-O-acetylambelline[18].

Examples with a *cis* relationship are brunsbelline[18], *epi*buphanisine, *epi*vittatine[24], 6-hydroxycrinamine[25], and 3-O-acetylhamayne[6]. There are two exceptions, crinamine[7] and hamayne[26] where the methoxy and the ethano bridge have a *cis* relationship, yet H-1 and H-2 resonances are superimposed (in $CDCl_3$) to give one singlet integrating to two protons. This unusual appearance of a singlet can be interpreted as a consequence of the

near co-incidence of the inner two lines of the pair of doublets and the virtual disappearance of the outer two lines[17].

The stereochemistry of the 5-10b-ethano bridge can only be established by circular dichroism. Those compounds displaying a negative peak at 244 nm similar in sign and magnitude to that observed for haemanthamine, have an α bridge[20], while those displaying a positive peak at 244 nm, similar in sign and magnitude to crinine have a β bridge[18].



14

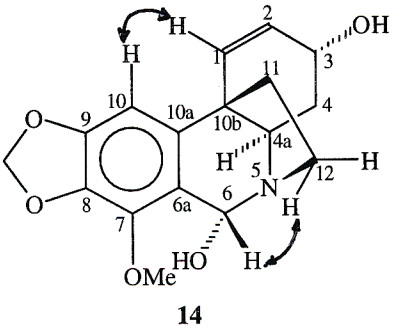
Compound **14**, 6α-hydroxypowelline [$C_{17}H_{19}NO_5$, M^+ at *m/z* 317.1254] was isolated as a white crystalline material. This is the first report of this compound in *Ammocharis coranica*. It was previously isolated from the bulbs of *Nerine bowdenii*[22]. Spectra for 6α-hydroxypowelline are given on pages 192-201 in appendix A.

The mass spectrum of 6α-hydroxypowelline showed a strong molecular ion peak at *m/z* 317.1254 corresponding to the molecular formula $C_{17}H_{19}NO_5$. The peak at *m/z* 299 (M^+ - 18) was attributed to the loss of water. The peak at *m/z* 273 (M^+ - 44) was a result of a hydrogen migration from C-11 to C-10b along with the loss of the 5-10b ethano bridge and a hydroxyl radical at position 3.

The i.r. spectrum showed bands at ν_{max} 3350 cm^{-1} (O – H stretching), 1097 cm^{-1} and 1044 cm^{-1} (C – O stretching) indicative of the hydroxy groups, 1630 cm^{-1} and 1498 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring and 939 cm^{-1} (C – O – C stretching) indicative of the methylenedioxy group.

The CD spectrum of 6α-hydroxypowelline (**14**) displayed positive peaks at 243 nm and 251 nm and a negative peak at 284 nm indicative of a β 5-10b ethano bridge[44].

The ^1H NMR spectrum showed a doublet and double doublet with resonances at δ_H 6.62 (1H) and δ_H 6.00 (1H) assignable to H-1 and H-2 respectively with coupling constants, $J_{1,2}$ = 10.01 Hz and $J_{2,3}$ = 5.01 Hz suggestive of a *trans* relationship between the 5-10b ethano bridge and the substituent at C-3[18]. Strong coupling between these two resonances was also evident in the COSY spectrum. The aromatic proton H-10 appeared as a one proton singlet at δ_H 6.75 and the methylenedioxy group protons appeared as a singlet at δ_H 5.96 integrating to two protons. The assignments of H-1 and H-10 was confirmed in an NOE experiment where the singlet at δ_H 6.75 attributed to H-10 was irradiated and this resulted in the enhancement of the doublet at δ_H 6.62 attributed to H-1.



14

At δ_H 5.40, a one proton singlet occurred which was attributed to H-6. A broad triplet of one proton at δ_H 4.33 was attributed to H-3. This resonance was strongly coupled to the one proton double doublet assigned to H-2 at δ_H 6.00 and the one proton doublet doublet at δ_H 1.88, which was attributed to one of the protons at position 4. The other proton at position 4 appeared in the multiplet at δ_H 2.00, which integrated to three protons, and which also contained the resonances of the two protons at C-11. This resonance also

showed coupling (COSY) to the resonance at δ_H 4.00, a double doublet attributed to H-4a and the resonances at δ_H 2.95 (1H) and δ_H 3.42 (1H), attributed to H-12_{endo} and H-12_{exo} respectively. These H-12 protons were assigned endo and exo positions based on an NOE experiment where the singlet at δ_H 5.40 attributed to H-6 was irradiated and this showed a positive effect on the multiplet at δ_H 2.95 which confirmed that this resonance was due to the H-12_{endo} proton. The two resonances assigned to the protons at C-4 are also coupled to the C-4 resonance at δ_C 32.5 (HETCOR) and the two resonances assigned to the protons at C-12 showed coupling to the C-12 resonance at δ_H 48.2 in the HETCOR spectrum, which confirmed these assignments. Coupling between the H-4 resonance that appeared at δ_H 1.88 and the H-4a resonance at δ_H 4.00 was also evident in the COSY spectrum with long range coupling between the H-4a and H-3 resonances evident as well. A three proton singlet appeared at δ_H 4.06 and was attributed to the methoxy substituent at C-7. Irradiation of the H-6 proton resonance at δ_H 5.40 (s) showed a positive effect on the singlet at δ_H 4.06 which confirmed that the methoxy group was located on C-7.

^{1}H NMR data of 6α-hydroxypowelline (**14**) in literature is given using $CDCl_3$ as solvent[22], however these assignments are incomplete and lack multiplicities and coupling constants. ^{13}C NMR data of 6α-hydroxypowelline was unavailable and therefore a comparison was made with the ^{13}C NMR data of powelline (**17**) in $CDCl_3$ [27], which posesses two protons at C-6.

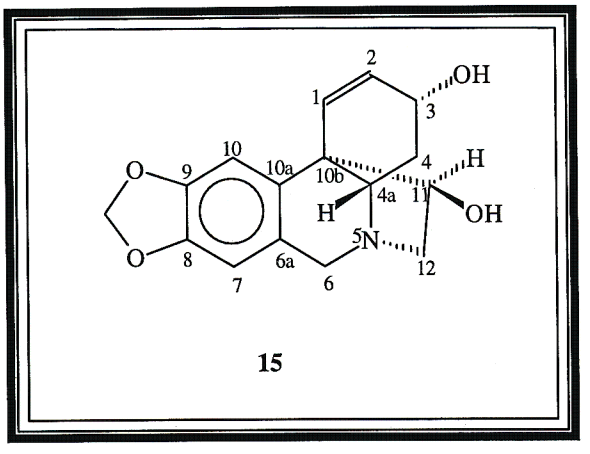
Table 8. ^{1}H NMR data of 6α-hydroxypowelline (14) (300 MHz)

	6α-hydroxypowelline (14) in CD_3OD	6α-hydroxypowelline (14) in $CDCl_3$[22]
H-1	6.62 (d) J = 10.01 Hz	6.38 multiplicity not given
H-2	6.00 (dd) J = 5.01 Hz, 9.95 Hz	5.85 multiplicity not given
H-3	4.33 (brt)	not assigned
H-4	1.88 (1H, dd) J =4.28 Hz, 13.43 Hz 2.00 (1H, m)	not assigned
H-4a	4.00 (dd) J = 4.39 Hz, 13.19 Hz	not assigned
H-6	5.40 (s)	5.38 (s)
H-10	6.75 (s)	6.55 (s)
H-11$_{endo}$ **H-11$_{exo}$**	2.00 (m) 2.00 (m)	not assigned
H-12$_{endo}$ **H-12$_{exo}$**	2.95 (m) 3.42 (m)	not assigned
OCH_3	4.06 (s)	4.02 (s)
OCH_2O	5.96 (s)	5.88 (s)

Table 9. ^{13}C NMR data of 6α-hydroxypowelline (14) (75 MHz)

	6α-hydroxypowelline (14) in CD_3OD	powelline in $CDCl_3$ (17) [27]
C-1	131.0 (d)	129.5 (d)*
C-2	129.3 (d)	127.2 (d)*
C-3	64.0 (d)	63.5 (d)
C-4	32.5 (t)	33.5 (t)
C-4a	57.7 (d)	62.6 (d)
C-6	86.1 (d)	58.6 (t)
C-6a	119.0 (s)	116.0 (s)
C-7	151.6 (s)	140.0 (s)
C-8	140.1 (s)	132.5 (s)
C-9	144.1 (s)	147.0 (s)
C-10	98.1 (d)	96.0 (d)
C-10a	135.9 (s)	138.0 (s)
C-10b	45.7 (s)	44.7 (s)
C-11	41.0 (t)	44.7 (t)
C-12	48.2 (t)	54.8 (t)
$O\underline{C}H_3$	60.2 (q)	59.3 (q)
$O\underline{C}H_2O$	102.6 (t)	99.3 (t)

*assignments may be interchanged



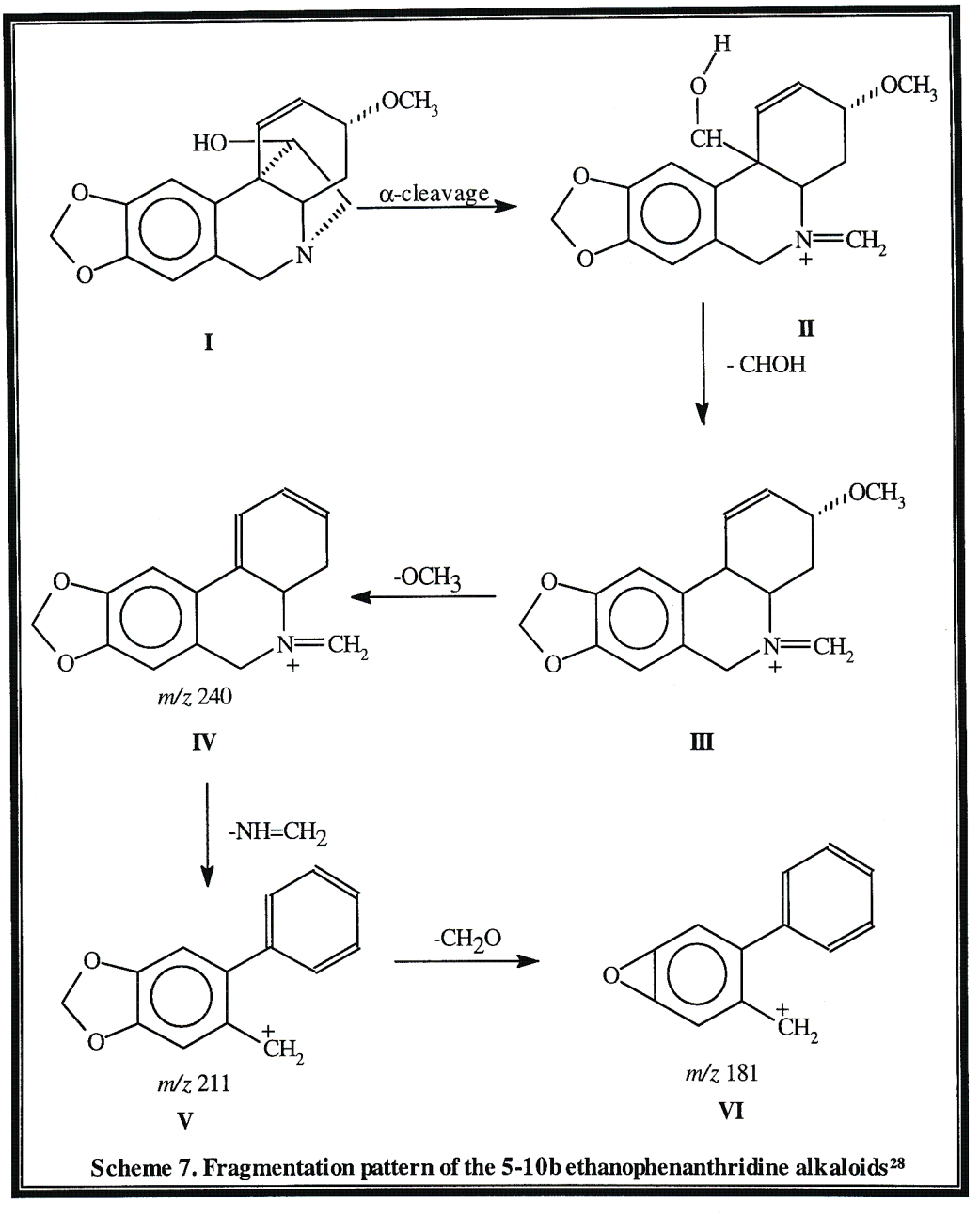
15

Compound **15** was found to be hamayne, $C_{16}H_{17}NO_4$, which was isolated as white crystalline material. This is the first report of this compound in *Ammocharis coranica*. It was isolated previously from *Crinum latifolium*[6], and *Brunsvigia josephinae*[26]. Spectra for hamayne are given on pages 202-211 in appendix A.

The mass spectrum of hamayne ($C_{16}H_{17}NO_4$) showed a weak molecular ion peak at *m/z* 287. The base peak at *m/z* 269 (M^+-18) was due to the loss of H_2O. The peaks at *m/z* 240, *m/z* 211 and *m/z* 181, fragments **IV**, **V** and **VI** are formed as in **scheme 7**[28].

The i.r. spectrum showed bands at ν_{max} 3400 cm^{-1} (O – H stretching), 1044 cm^{-1} and 1037 cm^{-1} (C – O stretching) indicative of the hydroxy groups, 1478 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1235 cm^{-1} (C – N stretching) suggestive of C – N bonds and 945 cm^{-1} (C – O – C stretching), indicative of the methylenedioxy group.

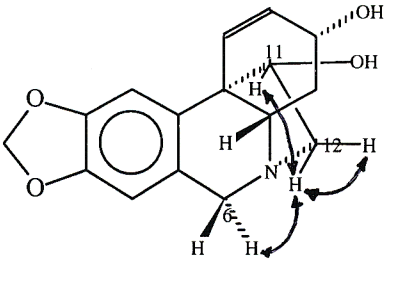
The CD spectrum of hamayne (**15**) showed negative peaks at 234 nm, 242 nm and 249 nm and positive peaks at 279 nm, 294 nm and 301 nm indicative of an α 5-10b ethano bridge[44].



Scheme 7. Fragmentation pattern of the 5-10b ethanophenanthridine alkaloids[28]

The ^{1}H NMR spectrum showed two singlets at δ_H 6.92 and δ_H 6.60, each integrating to one proton, attributed to H-10 and H-7 respectively. These assignments were made based on the consistency of the lowfield resonance being assigned to H-10[17]. The methylenedioxy group appeared as a two proton singlet at δ_H 5.93. A pair of doublets appeared at δ_H 6.25 and δ_H 6.09, each integrating to one proton and was attributed to H-1

and H-2 respectively. The coupling constant between these resonances was 10.31 Hz. H-1 was further finely split into a double doublet with $J_{1,3}$ = 2.26 Hz due to the coupling between H-1 and H-3. The H-3 resonance was assigned to the one proton multiplet at δ_H 4.37. The coupling constant $J_{2,3}$ = 0 since the dihedral angle between these two protons is 90^0. Strong coupling in the COSY spectrum between the H-1 and H-2 resonances as well as weaker coupling between the H-1 and H-3 resonances was evident, confirming these assignments. The H-3 resonance also exhibited strong coupling to the resonances at δ_H 2.11, a one proton multiplet and δ_H 2.20, a one proton double doublet attributed to the two protons at C-4. These two H-4 resonances were also coupled to the C-4 resonance at δ_C 33.5 in the HETCOR spectrum, confirming their assignments. Furthermore, they were coupled strongly (COSY) to the resonance at δ_H 3.48, a double doublet of one proton assigned to H-4a. The H-6α and H-6β resonances appeared as a pair of doublets of one proton each with a coupling constant of 16.48 Hz at δ_H 3.95 and δ_H 4.47 respectively. These resonances also showed strong coupling in the COSY spectrum. They were also coupled to the C-6 resonance at δ_C 60.7 in the HETCOR spectrum. The H-11 proton resonance appeared as a one proton multiplet at δ_H 4.05. This resonance was also coupled to the resonances at δ_H 3.38, a double doublet of one proton assigned to H-12_{exo} and δ_H 3.66, a double doublet of one proton assigned to H-12_{endo}. These H-12 resonances were also coupled to the C-12 resonance at δ_C 63.2 in the HETCOR spectrum. The 11-OH group was assigned an exo position based on an NOE experiment where the H-12_{endo} resonance, a double doublet at δ_H 3.66 was irradiated and this showed an enhancement of the resonances at δ_H 3.38 (dd), δ_H 3.95 (d) and δ_H 4.05 (m) assigned to H-12_{exo}, H-6α and H-11_{endo} respectively.



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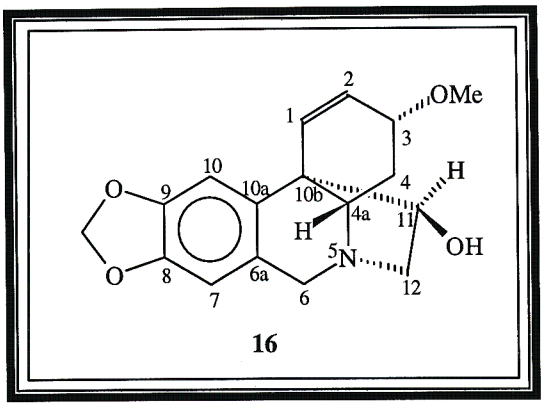


16

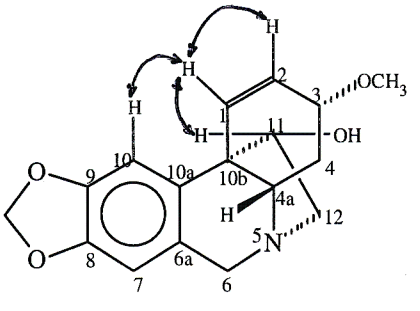
Compound **16** was found to be crinamine, [$C_{17}H_{19}NO_4$, M^+ at *m/z* 301.1298] which was isolated as a white crystalline material. Crinamine is a well known Amaryllidaceae alkaloid found previously in *Ammocharis coranica*[3,4], *Crinum latifolium*[6], *Crinum amabile*[7], *Brunsvigia orientalis*[25] and *Brunsvigia josephinae*[26]. It has also been reported to occur in *Dioscorea dregeana*, a member of the Dioscoreaceae[45]. Spectra for crinamine are given on pages 212-222 in appendix A.

The mass spectrum of crinamine had a weak molecular ion peak at *m/z* 301.1298 which corresponded to $C_{17}H_{19}NO_4$. The base peak at *m/z* 269 (M^+-32) was attributed to the loss of methanol. The driving force for the elimination of methanol was presumably the release of steric strain resulting from the proximity of the methoxy group and the two-carbon bridge. As a result, compounds having a *cis* relationship between the two-carbon bridge and the methoxy substituent will have a weak molecular ion peak. The peak at *m/z* 226 (M^+-75) was due to the loss of the methoxy group together with the loss of the ethylene bridge and its hydroxy substituent. The peaks at *m/z* 211 and *m/z* 181 are due to fragments **V** and **VI**, formed as in **scheme 7**[28].

The i.r. spectrum showed bands at ν_{max} 3400 cm^{-1} (O – H stretching) and 1044 cm^{-1} (C – O stretching) indicative of the hydroxy group, 1630 cm^{-1} and 1492 cm^{-1} (aromatic C = C stretching) indicating the presence of an aromatic ring, 1241 cm^{-1} (C – N stretching) suggestive of C – N bonds and 945 cm^{-1} (C – O – C stretching), indicative of the methylenedioxy group.

The CD spectrum displayed a negative peak at 249 nm and positive peaks at 276 nm and 305 nm indicating that the 5-10b ethano bridge has an α orientation[44].

The ^{1}H NMR spectrum showed two one proton singlets at δ_H 6.89 and δ_H 6.56 attributable to H-10 and H-7 respectively. From literature, the H-10 signal was located at a consistently lower field than the H-7 signal[17]. This was confirmed in an NOE experiment where the H-10 signal at δ_H 6.89 was irradiated, and this resulted in the enhancement of the H-1 resonance at δ_H 6.31, a double doublet integrating to one proton with $J_{1,2}$ = 10.44 Hz and $J_{1,3}$ = 2.13 Hz.



16

The methylenedioxy protons appeared as a singlet at δ_H 5.91 (2H). The other doublet at δ_H 6.11 integrating to one proton had a coupling constant of 10.44 Hz and was attributed to H-2. The H-3 resonance appeared as a one proton multiplet at δ_H 4.10. Strong coupling in the COSY spectrum between the H-1 and H-2 resonances as well as long range coupling between the H-1 and H-3 resonances were evident. The resonances at δ_H 2.08, a one proton multiplet, and at δ_H 2.15, a one proton double doublet, were assigned to the two protons at C-4. These H-4 resonances, in turn, showed coupling to the double doublet at δ_H 3.28 assigned to H-4a and the multiplet at δ_H 4.10 assigned to H-3 (COSY). Both H-4 resonances showed coupling to the C-4 resonance at δ_C 30.6 in the HETCOR spectrum. The H-6α and H-6β resonances appeared as one proton doublets at δ_H 3.78 and δ_H 4.33 respectively with a large coupling constant of 16.61 Hz. These two resonances were also coupled to the C-6 resonance at δ_C 61.5 in the HETCOR spectrum. H-11$_{endo}$ appeared as a one proton double doublet at δ_H 4.00 and showed strong coupling

(COSY) to the resonances at δ_H 3.23 and δ_H 3.50, each double doublets of one proton assigned to H-12_{exo} and H-12_{endo}. These two H-12 resonances showed coupling to the C-12 resonance at δ_C 63.6 in the HETCOR spectrum. The stereochemistry of the H-11 proton was assigned the endo position based on an NOE experiment where the double doublet at δ_H 6.31 assigned to H-1 was irradiated and this showed a positive effect on the H-11 resonance at δ_H 4.00 (dd). A positive effect on the H-10 and H-2 resonances at δ_H 6.89 (s) and δ_H 6.11 (d) could also be seen, as was expected. The H-12 protons were assigned endo and exo positions by comparison with data obtained for hamayne (**15**). The resonance ascribed to the protons of the methoxy substituent at C-3 appeared as a three proton singlet at δ_H 3.42.

Table 10. ^{1}H NMR data of hamayne (15) and crinamine (16) (300 MHz)

	hamayne (15) (CD_3OD)	**hamayne (15) ($CDCl_3$)[26]**	**crinamine (16) (CD_3OD)**	**crinamine (16) ($CDCl_3$)[7]**
H-1	6.25 (dd) J = 10.38 Hz, 2.26 Hz	6.19 (s)	6.31 (dd) J = 10.44 Hz, 2.13 Hz	6.23 (s)
H-2	6.09 (d) J = 10.31 Hz		6.11 (d) J = 10.32 Hz	
H-3	4.37 (m)	4.35 (m)	4.10 (m)	unassigned
H-4α **H-4β**	2.11 (m) 2.20 (dd) J = 13.43 Hz, 10.44 Hz	2.10 (m)	2.08 (m) 2.15 (dd) J = 13.06 Hz, 10.44 Hz	unassigned
H-4a	3.48 (dd) J = 13.62 Hz, 4.40 Hz	3.25 (dd) J = 13.5 Hz, 4.5 Hz	3.28 (dd) J = 13.25 Hz 3.30 Hz,	unassigned
H-6α **H-6β**	3.95 (d) J = 16.48 Hz 4.47 (d) J = 16.48 Hz	3.65 (d) J = 16 Hz 4.30 (d) J = 16 Hz	3.78 (d) J = 16.61 Hz 4.33 (d) J = 16.91 Hz	4.29 (d) J = 16.9 Hz 3.67 (d) J = 16.9 Hz
H-7	6.60 (s)	6.47 (s)	6.56 (s)	6.46 (s)

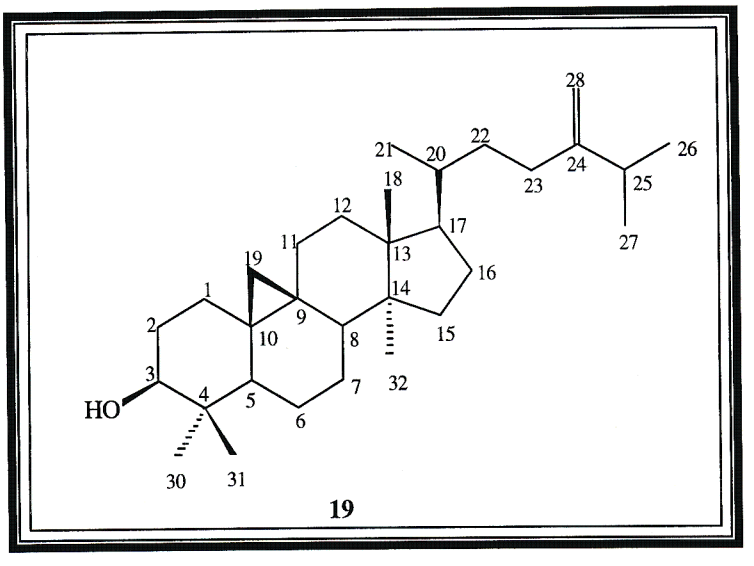
H-10	6.92 (s)	6.81 (s)	6.89 (s)	6.78 (s)
H-11$_{endo}$	4.05 (m)	4.00 (m)	4.00 (dd) J = 6.17 Hz, 2.75 Hz	unassigned
H-12$_{exo}$	3.38 (dd) J = 13.73 Hz, 3.42 Hz	3.35 (m)	3.23 (dd) J = 13.73 Hz, 3.45 Hz	unassigned
H-12$_{endo}$	3.66 (dd) J = 13.73 Hz, 6.96 Hz		3.50 (dd) J = 13.73 Hz, 6.16 Hz	
OCH_3	-	-	3.42 (s)	3.38 (s)
OCH_2O	5.93 (s)	5.90 (s)	5.91 (s)	5.87 (s)

Table 11. ^{13}C NMR data of hamayne (15) and crinamine (16) (75 MHz)

	hamayne (15) CD_3OD	**hamayne (15) in $CDCl_3$[26]**	**crinamine (16) CD_3OD**	**crinamine (16) in $CDCl_3$[7]**
C-1	124.0 (d)	122.9 (d)	125.8 (d)	123.60 (d)
C-2	137.4 (d)	137.4 (d)	134.2 (d)	135.99 (d)
C-3	67.7 (d)	67.0 (d)	77.6 (d)	76.02 (d)
C-4	33.5 (t)	33.2 (t)	30.6 (t)	30.14 (t)
C-4a	67.9 (d)	65.6 (d)	67.4 (d)	66.10 (d)
C-6	60.7 (t)	63.0 (d)	61.5 (t)	63.44 (t)
C-6a	124.7 (s)	125.2 (s)	126.6 (s)	126.55 (s)
C-7	107.9 (d)	106.8 (d)	107.8 (d)	106.87 (d)
C-8	148.1 (s)	146.3 (s)	147.8 (s)	146.22 (s)
C-9	148.6 (s)	146.8 (s)	148.2 (s)	146.50 (s)
C-10	104.4 (d)	103.3 (d)	104.3 (d)	103.18 (d)
C-10a	136.4 (s)	135.4 (s)	137.2 (s)	135.32 (s)
C-10b	51.6 (s)	49.8 (s)	51.7 (s)	50.26 (s)
C-11	80.2 (d)	79.5 (d)	81.0 (d)	79.99 (d)
C-12	63.2 (t)	60.5 (t)	63.6 (t)	61.18 (t)
O$\underline{C}H_3$	-	-	55.8 (q)	55.80 (q)
O$\underline{C}H_2O$	102.5 (t)	101.0 (t)	102.2 (t)	100.87 (t)

1.3.2.2. Cycloartane compounds from *Ammocharis coranica*

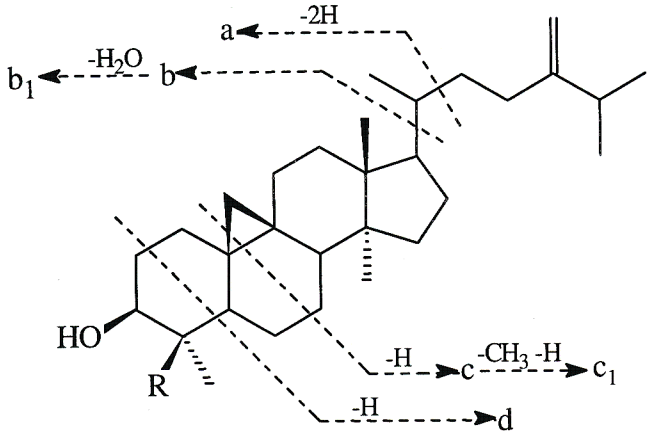
The next four compounds isolated were cycloartane compounds, immediately recognisable by the pair of upfield doublets at approximately δ_H 0.30 and δ_H 0.60 attributed to the H-19 protons of the cyclopropane ring system. These four compounds differ in their ^1H NMR spectra in that the resonance at δ_H 3.26 attributed to the proton at C-3 was present in the spectra of compounds **19** and **20** but not compounds **21** and **22**, which indicated that the former two compounds had a hydroxy group attached to C-3 and the latter two had a carbonyl group at C-3 (**Table 13 and 16**). Compounds **21** and **22** had a carbonyl carbon resonance at δ_C 212 in their ^{13}C NMR spectra which was absent in the spectra of compounds **19** and **20** (**Table 14 and 17**). The carbon resonances of C-2 - C-6, C-29 and C-30 were noticably shifted (**Table 14 and 17**) as a result of the changes that took place at C-3 and C-4. The side chain however remained the same in all four compounds as indicated by the same chemical shifts in the ^{13}C NMR spectra of all four compounds (**Table 14 and 17**). The carbon atoms of ring D all have the same carbon resonances in the ^{13}C NMR spectra as well, which indicated that no changes had occurred in this ring, nor was it influenced by any changes that occurred in ring A. The resonances of C-7 and C-8 remained fairly consistent in the ^{13}C NMR spectra of all four compounds while the resonances of C-9 and C-10 to which the cyclopropane ring is attached, differed slightly (**Table 14 and 17**).



19

Compound (**19**), 24-methylenecycloartan-3β-ol, [$C_{31}H_{52}O$, M^+ at *m/z* 440.4029] was isolated as white crystalline material. This compound, a constituent of rice bran oil, has also been isolated from the woods of several plant species including, *Tristania conferta, Angophora subvelutina,* and *Cephalosphaera usambarensis*[29]. It is also found in *Garcinia kola*[30], *Polypodium formosanum*[31] and *Costus tonkinensis*[32]. It is a major compound in the latex of *Euphorbia broteri*, a plant which has an irritant effect on the skin and mucous membranes[33]. This is the first report of compound **19** in *Ammocharis coranica* or any other Amaryllidaceae species. Spectra for 24-methylenecycloartan-3β-ol are given on pages 223-227 in appendix A.

The mass spectrum of compound **19** showed a molecular ion peak at *m/z* 440.4029 corresponding to the molecular formula $C_{31}H_{52}O$. The fragmentation pattern of 24-methylenecycloartanol (**19**) is characterised by cleavages in the side chain as well as in ring A and ring B (**Scheme 8, Table 12**). Also evident in this spectrum was the loss of H_2O at *m/z* 422 and the loss of a methyl fragment at *m/z* 425. The loss of both the methyl fragment and H_2O could be seen at *m/z* 407.



24-methylenecycloartanol (**19**), R = CH_3

cycloeucalenol (**20**), R = H

Scheme 8. Fragmentation pattern of 24-methylenecycloartane alcohols[32]

Table 12. Key ions in the EI-mass spectra of the 24-methylenecycloartane alcohols

Compound	M^+	a	b	b_1	c	c_1	d
19	440	341	315	297	300	285	353
20	426	327	301	283	300	285	353

The i.r. spectrum showed O – H stretching bands at ν_{max} 3400 cm^{-1} and C – O stretching bands at 1056 cm^{-1} indicative of the hydroxy group, C – H symmetric stretching bands at ν_{max} 2870 cm^{-1} and 2850 cm^{-1}, C – H antisymmetric stretching bands at ν_{max} 2960 cm^{-1} and 2925 cm^{-1} and C – H antisymmetric and symmetric bending bands at ν_{max} 1475 cm^{-1} and 1373 cm^{-1} indicative of the methyl and methylene groups and also C = C stretching bands and = CH_2 out of plane bending bands at ν_{max} 1652 cm^{-1} and 891 cm^{-1} respectively indicative of the olefinic methylene group.

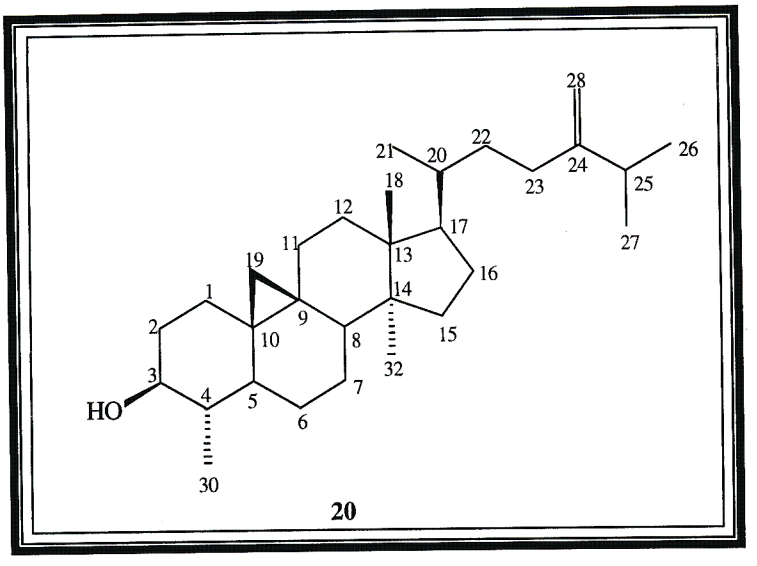
The proton chemical shifts of compound **19** were similar to those from literature (**Table 13**)[33]. The ^{1}H NMR spectrum showed two cyclopropane protons at δ_H 0.31 (d, J = 4.27 Hz) and δ_H 0.53 (d, J = 4.27 Hz) attributed to the two protons at C-19 which appeared as a methylene carbon resonance at δ_C 29.9 in the ^{13}C NMR spectrum. This is characteristic of a cycloartane skeleton with a cyclopropane ring at positions 9 and 10. Three methyl proton singlets could be seen at δ_H 0.79 and δ_H 0.88, each integrating to three protons and at δ_H 0.95 which integrated to six protons indicating that two methyl signals were superimposed on each other. These singlet methyl group proton resonances were attributed to H-31, H-32, H-18 and H-30 respectively. These were assigned by comparison with values from literature (**Table 13**)[33]. Methyl carbon signals due to C-31, C-32, C-18 and C-30 appeared in the ^{13}C NMR spectrum at δ_C 25.4, δ_C 19.3, δ_C 18.0 and δ_C 14.0 respectively (**Table 14**)[33]. Three doublet methyl proton signals could be seen at δ_H 0.88 (J = 4.03 Hz), δ_H 1.00 (J = 6.78 Hz) and δ_H 1.01 (J = 6.78 Hz) each integrating to three protons. These resonances, by comparison with literature values, were attributed to H-21, H-26 and H-27 respectively[33]. The C-21, C-26 and C-27 resonances could be seen at δ_C 18.3 (q), δ_C 21.9 (q) and δ_C 22.0 (q) respectively. A broad doublet of doublets resonating at δ_H 3.26 (J = 11.11 Hz and 4.52 Hz) ($W_{1/2}$ = 16 Hz) integrating to one proton was attributed to H-3α and the hydroxy group was given the β orientation. The stereochemistry at this position was given based on the $W_{1/2}$ value for this resonance[34]. At δ_C 78.9 in the ^{13}C NMR spectrum, the resonance ascribed to C-3 occurred. Two singlets, integrating to one proton each, at δ_H 4.64 and δ_H 4.70 were attributed to the two methylene protons at C-28. These signals appeared as singlets because the coupling constants between these two protons is extremely small. The resonance of C-28 appeared in the ^{13}C NMR spectrum at δ_C 105.9. A fully substituted carbon resonance in the ^{13}C NMR spectrum at δ_C 156.9 was attributed to C-24.

Table 13. ^{1}H NMR assignments of 24-methylenecycloartan-3β-ol (19) (300 MHz, $CDCl_3$)

	Compound 19	Compound 19[33]		Compound 19	Compound 19[33]
H-3	3.26 (dd, *J* = 11.1, 4.5 Hz)	3.28 (m)	**H-27**	1.01 (d) *J* = 6.8 Hz	1.03 (d) *J* = 6.8 Hz
H-18	0.95 (s)	0.97 (s)	**H-28a** **H-28b**	4.64 (s) 4.70 (s)	Not assigned
H-19a **H-19b**	0.31 (d) *J* = 4.27 Hz 0.53 (d) *J* = 4.27 Hz	0.33 (d) *J* = 4.2 Hz 0.56 (d) *J* = 4.2 Hz	**H-30**	0.95 (s)	0.97 (s)
H-21	0.88 (d) *J* = 4.0 Hz	0.90 (d) *J* = 6.2 Hz	**H-31**	0.79 (s)	0.81 (s)
H-26	1.00 (d) *J* = 6.8 Hz	1.03 (d) *J* = 6.8 Hz	**H-32**	0.88 (s)	0.90 (s)

Table 14. ^{13}C NMR assignments of 24-methylenecycloartan-3β-ol (19) (75 MHz, $CDCl_3$)

	Compound 19	Compound 19 [33]		Compound 19	Compound 19 [33]
C-1	32.0 (t)	32.1 (t)	C-17	52.3 (d)	52.4 (d)
C-2	30.4 (t)	30.5 (t)	C-18	18.0 (q)	18.1 (q)
C-3	78.9 (d)	78.8 (d)	C-19	29.9 (t)	29.9 (t)
C-4	40.5 (s)	40.5 (s)	C-20	36.1 (d)	36.2 (d)
C-5	47.1 (d)	47.2 (d)	C-21	18.3 (q)	18.4 (q)
C-6	21.1 (t)	21.2 (t)	C-22	35.0 (t)	35.2 (t)
C-7	28.1 (t)	28.2 (t)	C-23	31.3 (t)	31.4 (t)
C-8	48.0 (d)	48.0 (d)	C-24	156.9 (s)	156.8 (s)
C-9	20.0 (s)	20.1 (s)	C-25	33.8 (d)	33.9 (d)
C-10	26.1 (s)	26.2 (s)	C-26	21.9 (q)	21.9 (q)
C-11	26.1 (t)	26.1 (t)	C-27	22.0 (q)	22.1 (q)
C-12	35.6 (t)	35.7 (t)	C-28	105.9 (t)	106.1 (t)
C-13	45.3 (s)	45.4 (s)	C-30	14.0 (q)	14.1 (q)
C-14	48.8 (s)	48.9 (s)	C-31	25.4 (q)	25.5 (q)
C-15	32.9 (t)	33.0 (t)	C-32	19.3 (q)	19.4 (q)
C-16	26.5 (t)	26.6 (t)			



20

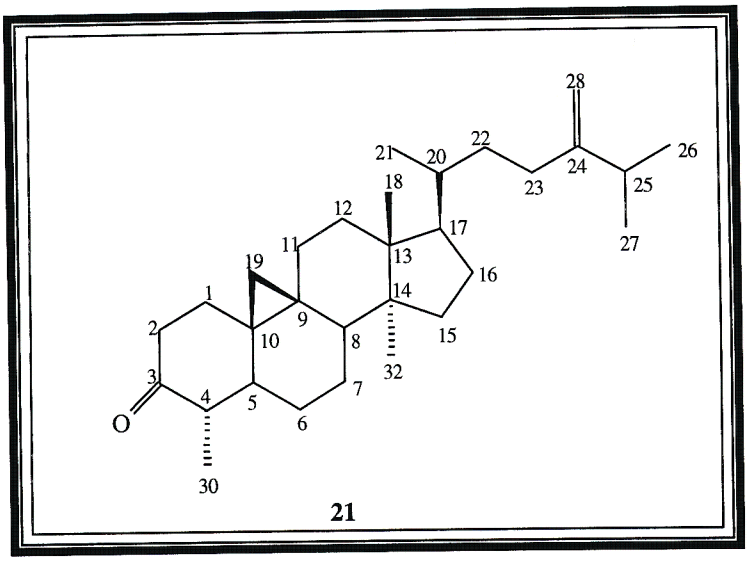
Compound **20**, cycloeucalenol, [$C_{30}H_{50}O$, M^+ at *m/z* 426.3852] was isolated as white crystalline material. This compound which is also a constituent of rice bran oil[35] was found previously in many plant species including *Eucalyptus microcorys*[38], *Erythrophleum guineense*[38], *Costus tonkinensis*[32], *Psuedotsuga menziessi* (Douglas fir) [36] and *Euphorbia boetica*[37] and *Cephalosphaera usambarensis*[29]. This is the first report of cycloeucalenol in an Amaryllidaceae species. Spectra for cycloeucalenol are given on pages 228-233 in appendix A.

The mass spectrum of cycloeucalenol (**20**) showed a molecualr ion peak at *m/z* 426.3852 corresponding to the molecular formula $C_{30}H_{50}O$. The fragmentation pattern of cycloeucalenol (**20**) was similar to that of 24-methylenecycloartanol with cleavages in the side chain, ring A and ring B (**Scheme 8, Table 12**). The fragments resulting from the loss of a methyl fragment and the loss of H_2O were evident at *m/z* 411 and *m/z* 408 respectively, while the peak at *m/z* 393 resulted from the loss of both the methyl fragment and H_2O.

The i.r. spectrum showed O – H stretching bands at ν_{max} 3400 cm^{-1} and C – O stretching bands at 1056 cm^{-1} indicative of the hydroxy group. Also evident were C – H symmetric stretching bands at ν_{max} 2870 cm^{-1} and 2850 cm^{-1}, C – H antisymmetric stretching bands

at ν_{max} 2960 cm^{-1} and 2925 cm^{-1} and C – H antisymmetric and symmetric bending bands at ν_{max} 1468 cm^{-1} and 1380 cm^{-1} indicative of the methyl and methylene groups and also C = C stretching bands and = CH_2 out of plane bending bands at ν_{max} 1640 cm^{-1} and 885 cm^{-1} respectively indicative of the olefinic methylene group.

The ^1H NMR spectrum showed a pair of doublets at δ_H 0.12 (J = 4.15 Hz) and δ_H 0.36 (J = 3.85 Hz) attributed to the protons at C-19 which appeared as a methylene carbon resonance at δ_C 27.3 in the ^{13}C NMR spectrum. This suggested a cycloartane skeleton with a 9, 10, 19-cyclopropane ring. H-32 and H-18 appeared as three proton singlets at δ_H 0.87 and δ_H 0.95 respectively while the C-32 and C-18 signals appeared at δ_C 19.1 and δ_C 17.8 respectively in the ^{13}C NMR spectrum and were assigned by comparison with values from literature[37]. Four doublet methyl group proton resonances could also be seen in the ^1H NMR spectrum and were assigned in accordance with literature[37]. These appeared at δ_H 0.87 (J = 6.23 Hz), δ_H 0.96 (J = 6.10 Hz), δ_H 1.00 (J = 6.90 Hz) and δ_H 1.01 (J = 6.90 Hz) and were attributed to H-21, H-30, H-26 and H-27 respectively. The C-21, C-30, C-26 and C-27 resonances could be seen in the ^{13}C NMR spectrum at δ_C 18.3, δ_C 14.4, δ_C 21.9 and δ_C 22.0 respectively. A multiplet at δ_H 3.19 was attributed to H-3α ($W_{1/2}$ = 16 Hz) and the hydroxy group at C-3 was thus given the β orientation. Again, the stereochemistry at this position was given based on the $W_{1/2}$ value for this resonance [34]. At δ_H 4.65 and δ_H 4.70, two singlets occurred, which were attributed to the two olefinic protons at C-28. The olefinic carbon signals, a methylene carbon resonance at δ_C 105.9 and a fully substitued carbon resonance at δ_C 156.9 in the ^{13}C NMR spectrum, were attributed to C-28 and C-24 respectively which compared well with values from literature (**Table 17**)[37]. The physical characteristics, m.p. and i.r., confirmed that this compound was cycloeucalenol and not its epimer, 4-*epi*cycloeucalenol.

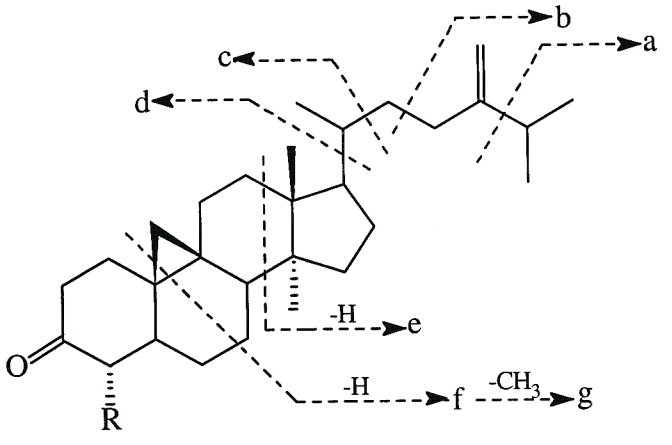


21

Compound (**21**), cycloeucalenone, [$C_{30}H_{48}O$, M^+ at *m/z* 424.3717] was isolated as white crystalline material. This compound was found previously in the roots and the aerial parts of *Costus tonkinensis*[32]. This is the first report of cycloeucalenone in an Amaryllidaceae species. Spectra for cycloeucalenone are given on pages 234-242 in appendix A.

The mass spectrum of cycloeucalenone (**21**) showed a molecular ion peak at *m/z* 424.3717, corresponding to the molecular formula $C_{30}H_{48}O$. The fragmentation pattern of cycloeucalenone is characterised by cleavages in the side chain as well as in ring B and ring C (**Scheme 9, Table 15**). The loss of a methyl fragment resulting in an ion at *m/z* 409, could also be seen in the spectrum.

The i.r. spectrum showed C = O stretching bands at ν_{max} 1717 cm^{-1} indicative of the carbonyl group, C – H symmetric stretching bands at ν_{max} 2870 cm^{-1} and 2850 cm^{-1}, C – H antisymmetric stretching bands at ν_{max} 2960 cm^{-1} and 2925 cm^{-1} and C – H antisymmetric and symmetric bending bands at ν_{max} 1461 cm^{-1} and 1374 cm^{-1} indicative of the methyl and methylene groups and also = CH_2 out of plane bending bands at ν_{max} 896 cm^{-1} indicative of the olefinic methylene group.



cycloeucalenone (21), R = CH_3

24-methylenepollinastanone (22), R = H

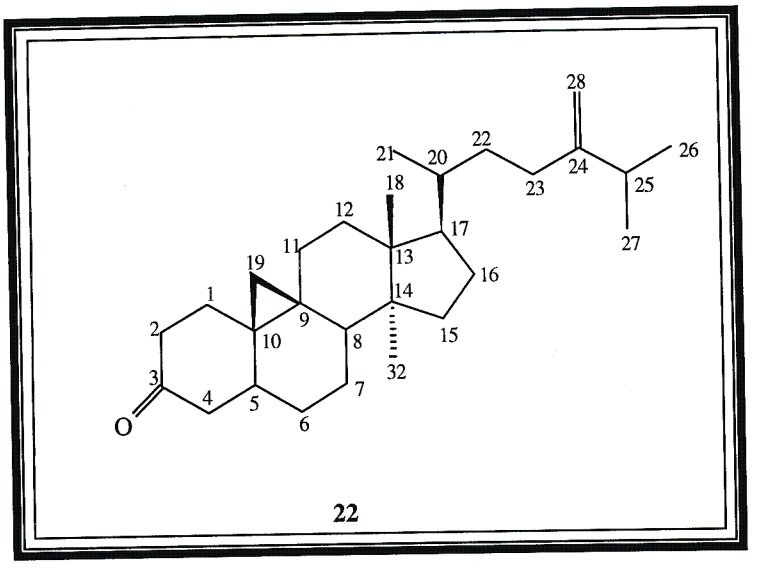
Scheme 9. Fragmentation pattern of 24-methylenecycloartane ketones[32]

Table 15. Key ions in the EI-mass spectra of the 24-methylenecycloartane ketones

Compound	M^+	a	b	c	d	e	f	g
21	424	381	341	327	299	219	300	285
22	410	367	327	313	285	219	300	285

A pair of doublets at δ_H 0.37 (J = 4.09 Hz) and δ_H 0.60 (J = 3.84 Hz) appeared in the ^1H NMR spectrum and was attributed to the protons at C-19. C-19 appeared as a methylene carbon resonance in the ^{13}C NMR spectrum at δ_C 27.0. This was again indicative of a cycloartane skeleton with a cylopropane ring at positions 9 and 10 on ring C. Two singlets each integrating to three protons which appeared at δ_H 0.89 and δ_H 0.96 were attributed to H-32 and H-18 respectively. The methyl carbon resonances of C-32 and C-18 appeared at δ_C 19.2 and δ_C 17.9 respectively. These values compared well with those

from literature[39]. The four doublet methyl signals in the ^{1}H NMR spectrum appeared at δ_H 0.88 (J = 4.40 Hz), δ_H 0.98 (J = 1.65 Hz), δ_H 1.00 (J = 6.84 Hz) and δ_H 1.01 (J = 6.84 Hz) and were attributed to H-21, H-30, H-26 and H-27 respectively. The ^{13}C NMR resonances of C-21, C-30, C-26 and C-27 appeared at δ_C 18.3, δ_C 10.8, δ_C 21.9 and δ_C 22.0 respectively. These resonances were assigned with the aid of values from literature (**Table 16 and Table 17**)[37, 39]. Two singlets at δ_H 4.64 and δ_H 4.70 were attributed to the methylene protons at C-28. The olefinic carbon resonances of C-24 and C-28 were seen at δ_C 156.8 and δ_C 105.9 in the ^{13}C NMR spectrum (**Table 16 and Table 17**) [37,39]. The C-3 resonance could be seen at δ_C 213.4 in the ^{13}C NMR spectrum. The other possibilty, 4-*epi*cycloeucalenone was ruled out as the NMR data was quite different from that of literature[40].



22

Compound (**22**), 24-methylenepollinastanone, [$C_{29}H_{46}O$, M^+ at *m/z* 410.3559] was isolated as white crystalline material. This is the first report of this novel compound, however 24-methylenepollinastanol has been isolated from *Musa sapientum*[41]. Spectra for 24-methylenepollinastanone are given pages 243-249 in appendix A.

The mass spectrum of 24-methylenepollinastanone (**22**) showed a molecular ion peak at *m/z* 410.3559, corresponding to $C_{29}H_{46}O$. The fragmentation pattern of 24-methylenepollinastanone was similar to that of cycloeucalenone with cleavages in the side chain and in rings B and C (**Scheme 9, Table 15**). At *m/z* 395, an ion resulting from the loss of a methyl fragment was observed.

The i.r. spectrum showed C = O stretching bands at ν_{max} 1715 cm^{-1} indicative of the carbonyl group. C – H symmetric stretching bands at ν_{max} 2870 cm^{-1} and 2850 cm^{-1}, C – H antisymmetric stretching bands at ν_{max} 2960 cm^{-1} and 2925 cm^{-1} and C – H antisymmetric and symmetric bending bands at ν_{max} 1468 cm^{-1} and 1377 cm^{-1} could also be seen and were indicative of the methyl and methylene groups. Also evident was a $=CH_2$ out of plane bending band at ν_{max} 904 cm^{-1} indicative of the olefinic methylene group.

The ^{1}H NMR spectrum of compound (**22**) showed a pair of doublets at δ_H 0.33 (J = 4.21 Hz) and δ_H 0.60 (J = 3.85 Hz) attributed to the protons at C-19. The resonance ascribed to C-19 appeared as a methylene carbon resonance in the ^{13}C NMR spectrum at δ_C 28.4. This was suggestive of a cycloartane skeleton with a cylopropane ring at positions 9 and 10. Two singlets, each integrating to three protons appeared in the ^{1}H NMR spectrum at δ_H 0.90 and δ_H 0.98 and were assigned to H-32 and H-18 respectively[41]. The resonances of C-32 and C-18 appeared in the ^{13}C NMR spectrum at δ_C 19.1 and δ_C 17.8 respectively (**Table 17**). The H-21, H-26 and H-27 resonances appeared as doublets in the ^{1}H NMR spectrum at δ_H 0.89 (J = 6.60 Hz), δ_H 1.00 (J = 6.90 Hz) and δ_H 1.01 (J = 6.84 Hz). This compared well with values from literature[41]. Resonances ascribed to C-21, C-26 and C-27 appeared as fully substituted carbons in the ^{13}C NMR spectrum at δ_C 18.3, δ_C 21.9 and δ_C 22.0. The methylene carbon resonance ascribed to C-4 appeared in the ^{13}C NMR spectrum at δ_C 48.5. Two singlets δ_H 4.65 and δ_H 4.70 appeared in the ^{1}H NMR spectrum and were attributed to the two protons at C-28. Again, these resonances appeared as singlets because of the small coupling constant between them. The C-28 signal appeared in the ^{13}C NMR spectrum at δ_C 105.9. The C-24 resonance, to which C-28 is bonded by means of a double bond, appeared as a fully substituted carbon at δ_C 156.9 in the ^{13}C NMR spectrum. The carbonyl group carbon resonance, C-3, appeared as a singlet at δ_C 212.0.

Table 16. ^{1}H NMR assignments of cycloeucalenol (20), cycloeucalenone (21) and 24-methylenepollinastanone (22) (300 MHz, $CDCl_3$)

	Compound 20	Compound 20[37]	Compound 21	Compound 22	* Compound 23[41]
H-3	3.19 (m)	3.22 (m)	-	-	-
H-18	0.95 (s)	0.97 (s)	0.96 (s)	0.98 (s)	0.96 (s)
H-19a	0.36 (d) J = 3.85 Hz	0.38 (d) J = 4.2 Hz	0.37 (d) J = 4.09 Hz	0.33 (d) J = 4.21 Hz	not assigned
H-19b	0.12 (d) J = 4.15 Hz	0.14 (d) J = 4.2 Hz	0.60 (d) J = 3.84 Hz	0.60 (d) J = 3.85 Hz	
H-21	0.87 (d) J = 6.2 Hz	0.89 (d) J = 6.3 Hz	0.88 (d) J = 4.40 Hz	0.89 (d) J = 6.60 Hz	0.90 (d) J = 6.1 Hz
H-26	1.00 (d) J = 6.9 Hz	1.02 (d) J = 6.9 Hz	1.00 (d) J = 6.8 Hz	1.00 (d) J = 6.9 Hz	1.02 (d) J = 7.0 Hz
H-27	1.01 (d) J = 6.9 Hz	1.02 (d) J = 6.9 Hz	1.01 (d) J = 6.8 Hz	1.01 (d) J = 6.8 Hz	1.03 (d) J = 6.7 Hz
H-28a	4.65 (s)	4.66 (d) J = 1.2 Hz	4.64 (s)	4.65 (s)	4.66 (s)
H-28b	4.70 (s)	4.71 (s)	4.70 (s)	4.70 (s)	4.71 (s)
H-30	0.96 (d) J = 6.1 Hz	0.98 (d) J = 6.0 Hz	0.98 (d) J = 1.7 Hz	-	-
H-32	0.87 (s)	0.89 (s)	0.89 (s)	0.90 (s)	0.90 (s)

* 24-methylenepollinastanol (acetate)

Table 17. ^{13}C NMR assignments of cycloeucalenol (20), cycloeucalenone (21) and 24-methylenepollinastanone (22) (75 MHz, $CDCl_3$)

	Compound 20	Compound 20[37]	Compound 21	Compound 21[39]	Compound 22
C-1	30.8 (t)	30.81 (t)	32.8 (t)	32.8 (t)	32.1 (t)
C-2	34.8 (t)	34.84 (t)	41.0 (t)	40.8 (t)	41.2 (t)
C-3	76.5 (d)	76.59 (d)	213.4 (s)	212.2 (s)	212.0 (s)
C-4	44.6 (d)	44.62 (d)	50.0 (d)	49.8 (d)	48.5 (t)
C-5	43.3 (d)	43.36 (d)	46.0 (d)	45.9 (d)	39.8 (d)
C-6	24.7 (t)	24.69 (t)	25.2 (t)	25.1 (t)	25.8 (t)
C-7	28.1 (t)	28.13 (t)	28.1 (t)	28.0 (t)	28.1 (t)
C-8	46.9 (d)	46.89 (d)	47.1 (d)	46.9 (d)	46.9 (d)
C-9	23.5 (s)	23.58 (s)	25.0 (s)	24.9 (s)	29.2 (s)
C-10	29.5 (s)	29.70 (s)	29.3 (s)	29.3 (s)	24.5 (s)
C-11	25.2 (t)	25.18 (t)	25.9 (t)	25.8 (t)	24.9 (t)
C-12	35.3 (t)	35.37 (t)	35.4 (t)	35.3 (t)	35.3 (t)
C-13	45.4 (s)	45.38 (s)	45.3 (s)	45.2 (s)	45.4 (s)
C-14	48.9 (s)	48.93 (s)	48.8 (s)	48.7 (s)	48.9 (s)
C-15	32.9 (t)	32.84 (t)	32.9 (t)	32.8 (t)	32.8 (t)
C-16	27.0 (t)	27.00 (t)	27.0 (t)	26.9 (t)	27.2 (t)
C-17	52.2 (d)	52.23 (d)	52.2 (d)	52.1 (d)	52.2 (d)
C-18	17.8 (q)	17.80 (q)	17.9 (q)	17.9 (q)	17.8 (q)
C-19	27.3 (t)	27.25 (t)	27.2 (t)	27.1 (t)	28.4 (t)
C-20	36.1 (d)	36.15 (d)	36.1 (d)	36.0 (d)	36.1 (d)
C-21	18.3 (q)	18.36 (q)	18.3 (q)	18.3 (q)	18.3 (q)
C-22	35.0 (t)	35.04 (t)	35.0 (t)	35.0 (t)	35.0 (t)
C-23	31.3 (t)	31.34 (t)	31.3 (t)	31.3 (t)	31.3 (t)
C-24	156.9 (s)	156.93 (s)	156.8 (s)	156.1 (s)	156.9 (s)
C-25	33.8 (d)	33.83 (d)	33.8 (d)	33.7 (d)	33.8 (d)
C-26	21.9 (q)	21.89 (q)	21.9 (q)	21.8 (q)	21.9 (q)
C-27	22.0 (q)	22.01 (q)	22.0 (q)	21.8 (q)	22.0 (q)
C-28	105.9 (t)	105.95 (t)	105.9 (t)	105.6 (t)	105.9 (t)
C-30	14.4 (q)	14.41 (q)	10.8 (q)	10.7 (q)	-
C-32	19.1 (q)	19.15 (q)	19.2 (q)	19.1 (q)	19.1 (q)

Conclusion

The alkaloids isolated in this work were all Amaryllidaceae alkaloids of the lycorine, crinine and haemanthamine groups. The compounds, 1-O-acetyllycorine, hippadine, 1-O-acetyl-9-norpluviine, 6α-hydroxypowelline and hamayne have not been isolated previously from *Ammocharis coranica.* This is the first report of 1-O-acetyl-9-norpluviine.

Cycloartane compounds have not been reported previously in *Ammocharis coranica.* This is also the first report of these compounds from the Amaryllidaceae family. The four cycloartane compounds isolated all had a common side chain with a methylene group attached to position 24 by means of a double bond. This is the first report of 24-methylenepollinastanone.

The alkaloids isolated from *Ammocharis coranica* in this work have been known to have important pharmacological activities. Lycorine, the most common of the Amaryllidaceae alkaloids caused scorbutic symptoms in experimental animals, is a respiratory stimulant and plant growth inhibitor by inhibition of protein synthesis[8, 9]. This alkaloid also shows moderate antitumour activity, is an antiviral agent and a weak protozoicide[42]. It was also found to be cytotoxic in all cancer cell lines tested[7]. Hippadine acts on the germ cells in their earlier stages of spermatocytogenesis and was found to reversibly inhibit fertility in male rats[43]. Caranine is a weak analgesic, convulsant and hypotensive agent as well as an acetylcholinesterase inhibitor, while acetylcaranine is cytotoxic to the murine P388 lymphocytic leukemia *in vitro*[10]. Crinamine is a powerful transient hypotensive agent in dogs, a respiratory depressant and antineoplastic agent. Accordingly, it is not surprising that the traditional healers in South Africa use these plants to treat a wide variety of illnesses and diseases.

The isolation of these bioactive compounds from *Ammocharis coranica* is a good indication that natural sources are a reservoir of useful bioactive compounds.

1.3.3 Foreword to experimental

Nuclear magnetic Resonance Spectroscopy (NMR)

^{1}H NMR and ^{13}C NMR spectra were recorded with a 300 MHz Gemini spectrometer using $CDCl_3$ and CD_3OD as solvents. The proton spectra were recorded at 300 MHz and the carbon spectra at 75 MHz. The spectra were 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 (i.r.)

Infra red spectra were recorded using a Nicolet Impact 400 D spectrometer which was calibrated against an air background. Compounds **1**, **3**, **11** and **17** were recorded using KBr disks and compounds **12-16** and **19-22** were recorded using KBr windows with $CHCl_3$ as solvent.

Melting points (m.p.)

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 mm) unjacketed flow tube. Concentrations are quoted as g/100 ml.

General Chromatography

Silica gel (0.2 mm) containing fluorescent indicator (F_{254}) on aluminium backed plates (Merck: Art 5554) was used for t.l.c. analysis and silica gel (Merck Art: 9385) was used for gravity column chromatography. The t.l.c. plates were developed using anisaldehyde: conc. H_2SO_4: methanol [1:2:97] as spray reagent, followed by heating.

Mass Spectrometry

GC/MS spectra were recorded using a Finnigan 1020 GC/MS spectrometer using both injection and solid probe methods. High resolution mass spectra were recorded on a Kratos 9/50 HRMS instrument. Mass spectrometry was performed by Dr. P. Boshoff at the Cape Technikon.

1.3.4 Experimental

1.3.4.1 Extractives from the bulbs of *Ammocharis coranica*

Ammocharis coranica (Ker.-Gawl.) Herb. was obtained from Ashburton, Kwazulu-Natal and identified by Dr. N. Crouch. A voucher specimen (*Crouch 766*) was deposited at the Natal Herbarium. The leaves were removed and the dried and cut bulbs (2.3 kg) were extracted with 95% ethanol on a Labcon shaker for 96 hours yielding, after evaporation of solvent, 7.2 g of extract. This was dissolved in water (100 ml) and acidified to pH 4. The acidic extract was then extracted with chloroform (3 x 200 ml) to yield the acidic chloroform extract (2.8 g). The aqueous solution was then made basic to pH 10 and extracted further with chloroform (3 x 200 ml) to yield the basic chloroform extract (3.5 g).

^{1}H NMR spectroscopy of the crude basic chloroform extract indicated the presence of isoquinoline alkaloids and this extract was purified further. Chromatographic separation of the basic chloroform extract using a methylene chloride : methanol step gradient (19:1, 18:2, 16:4, 12:8, 8:12, 4:16, 0:20) as the eluant and collecting 30 x 30 ml fractions for each step, gave lycorine (**1**), which crystallized out of fractions 69-76, 1-O-acetyllycorine (**11**), present in fractions 31-40, acetylcaranine (**3**), which precipitated out of fractions 22-30, 1-O-acetyl-9-norpluviine (**12**), present in fractions 41-50, hippadine (**13**), present in fractions 10-18, 6α-hydroxypowelline (**14**), contained in fractions 61-65, hamayne (**15**), contained in fractions 61-65, and crinamine (**16**), present in fractions 31-40. Also isolated from fractions 10-18 were four cycloartane compounds, 24-methylenecycloartanol (**19**), cycloeucalenol (**20**), cycloeucalenone (**21**) and 24-methylenepollinastanone (**22**).

Physical Data for compound (1)

lycorine [AMM75PP] *

Yield : 110 mg

Melting point : 251-252 ^{0}C (lit.[7] 250^{0}C)

Mass : HRMS: [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)

Optical rotation : $[\alpha]_D^{22}$ –68^0 (c = 2.5; EtOH)

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

^{1}H NMR : δ_H (ppm) (CD_3OD, 300 MHz)

2.48 (1H, dd, H-5, *J* = 17.58 Hz, 8.72Hz), 2.65 (2H, m, H-4), 2.70 (1H, d, H-11b, *J* = 10.44 Hz), 2.93 (1H, d, H-11c, *J* = 11.36Hz), 3.38 (1H , m, H-5), 3.60 (1H, d, H-7α, *J* = 14.28Hz), 4.16 (1H, d, H-7β, *J* = 14.28Hz), 4.20 (1H, brs, H-2), 4.50 (1H, s, H-1), 5.58 (1H, brs, H-3), 5.94 (2H, s, OCH_2O), 6.67 (1H, s, H-8), 6.91 (1H, s, H-11)

^{13}C NMR : δ_H (ppm) (CD_3OD, 75 MHz)

29.3 (t, C-4), 41.3 (d, C-11b), 54.7 (t, C-5), 57.7 (t, C-7), 62.5 (d, C-11c), 73.1 (d, C-1), 71.9 (d, C-2), 102.3 (t, OCH_2O), 106.0 (d, C-11), 108.2 (d, C-8), 119.2 (d, C-3), 129.7 (s, C-11a), 130.4 (s, C-7a), 143.6 (s, C-3a), 147.7 (s, C-10), 148.2 (s, C-9)

* Numbers in square brackets e.g. [AMM75PP], refer to the original sample numbers, included for reference purposes.

Acetylation of compound (1)

Compound (**1**) (25 mg) was dissolved in pyridine (2 ml). The resulting solution was treated with acetic anhydride (2 ml), heated briefly on a steam bath and allowed to stand for 24 hours. Treatment of the mixture with methanol (2-3 ml) and removal of the solvents *in vacuo*, followed by column chromatography yielded the di-acetate (**17**).

Compound (17)

1,2-di-O-acetyllycorine [AMM75AC]

Melting point: 211-212 ^{0}C (lit.[6] 208 – 210^{0}C)

Mass : HRMS: [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 (100.00) [M^+ - CH_3COOH - CH_3COO], 251 (14.02) [M^+ - 2 X CH_3COOH], 250 (46.41) [M^+ - 2 X CH_3COOH - H], 227 (18.24) [M^+ - 2 X $CHOC(O)CH_3$], 226 (31.17) [M^+ - 2 X $CHOC(O)CH_3$ - H], 149 (5.99), 97 (9.56), 43 (18.24)

Optical rotation: $[\alpha]_D^{22}$ –76^0 (c = 1.0; MeOH)

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

^{1}H NMR : δ_H (ppm) (CD_3OD, 300 MHz)

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* = 17.71 Hz, 8.86Hz), 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* = 14.29Hz), 4.18 (1H, d, H-7β, *J* = 14.29Hz), 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 : δ_H (ppm) (CD_3OD, 75 MHz)

20.6 (q, $OCO\underline{C}H_3$-1), 20.9 (q, $OCO\underline{C}H_3$-2), 29.3 (t, C-4), 41.4 (d, C-11b), 54.5 (t, C-5), 57.6 (t, C-7), 62.6 (d, C-11c), 70.6 (d, C-1), 72.0 (d, C-2), 102.4 (t, OCH_2O), 105.9 (d, C-11), 108.3 (d, C-8), 115.3 (d, C-3), 127.5 (s, C-11a), 130.4 (s, C-7a), 146.9 (s, C-3a), 146.9 (s, C-10), 148.1 (s, C-9), 171.4 (s, $O\underline{C}OCH_3$-2), 171.6 (s, $O\underline{C}OCH_3$-1)

Physical Data for compound (11)

1-O-acetyllycorine [AMM31M]

Yield: 20 mg

Melting point: 217-218 ^{0}C (lit.[6] 217 – 219^{0}C)

Mass: HRMS: [M^+] at *m/z* 329.1272, $C_{18}H_{19}NO_5$ requires 329.1263

EIMS: *m/z* (rel. int.): 329 (61.41) [M^+], 315 (38.24), 270 (13.39) [M^+ - CH_3COO], 269 (14.88) [M^+ - CH_3COOH], 268 (43.31) [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 (100.00) [M^+ - $CHOC(O)CH_3$ - CHOH – H], 149 (29.09), 43 (35.61)

Optical rotation: $[\alpha]_D^{22}$ –98^0 (c = 0.4; MeOH)

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

^{1}H NMR: δ_H (ppm) (CD_3OD, 300 MHz)

1.93 (3H, s, $OCOC\underline{H}_3$), 2.50 (1H, dd, H-5, *J* = 17.64 Hz, 8.79 Hz), 2.68 (2H, m, H-4), 2.90 (2H, brs, H-11b, H-11c), 3.33 (1H, m, H-5), 3.60 (1H, d, H-7α, *J* = 14.28Hz), 4.16 (1H, d, H-7β, *J* = 14.28 Hz), 4.19 (1H, brs, H-2), 5.58 (1H, brs, H-3), 5.73 (1H, s, H-1), 5.94 (2H, s, OCH_2O), 6.67 (1H, s, H-8), 6.76 (1H, s, H-11)

^{13}C NMR: δ_C (ppm) (CD_3OD, 75 MHz)

20.8 (q, $OCO\underline{C}H_3$), 29.3 (t, C-4), 40.1 (d, C-11b), 54.6 (t, C-5), 57.6 (t, C-7), 62.9 (d, C-11c), 70.3 (d, C-2), 73.4 (d, C-1), 102.4 (t, OCH_2O), 105.7 (d, C-11), 108.4 (d, C-8), 119.0 (d, C-3), 128.3 (s, C-11a), 130.4 (s, C-7a), 143.8 (s, C-3a), 147.9 (s, C-10), 148.1 (s, C-9), 172.2 (s, $O\underline{C}OCH_3$)

Physical Data for compound (3)

acetylcaranine [AMM22-30PP]

Yield: 50 mg

Melting point: 193-194 ^{0}C (lit.[10] 195^{0}C)

Mass: HRMS: [M^+] at *m/z* 313.1316, $C_{18}H_{19}NO_4$ requires 313.1314

EIMS: *m/z* (rel. int.): 313 (100.00) [M^+], 312 (12.50) [M^+ - H], 270 (11.29) [M^+ - CH_3CO], 254 (31.02) [M^+ - CH_3COO], 253 (24.22) [M^+ - CH_3COOH], 252 (99.67) [M^+ - CH_3COOH - H], 250 (18.04), 240 (11.49), 228 (20.59), 227 (45.67) [M^+ - $CHOC(O)CH_3$ - CH_2], 226 (86.89) [M^+ - $CHOC(O)CH_3$ - CH_2 - H], 149 (16.87), 57 (14.09), 43 (16.12)

Optical rotation: $[\alpha]_D^{22}$ –165^0 (c = 1.2; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 1729 (C = O stretching); 1496, 1380 (aromatic C = C stretching); 1241 (C – N stretching); 1037 (C – O stretching), 939 (C – O – C stretching)

^{1}H NMR: δ_H (ppm) ($CDCl_3$, 300 MHz)

1.91 (3H, s, $OCOC\underline{H}_3$), 2.32 (1H, brs, H-2), 2.38 (1H, brs, H-5a), 2.48 (1H, brs, H-11b), 2.60 (1H, brs, H-2), 2.64 (1H, brs, H-4a), 2.68 (1H, brs, H-4b), 2.87 (1H, brs, H-11c), 3.29 (1H, m, H-5b), 3.60 (1H, d, H-7α, *J* = 14.16 Hz), 4.11 (1H, d, H-7β, *J* = 13.86 Hz), 5.39 (1H, brs, H-3), 5.82 (1H, brd, H-1, *J* = 3.17 Hz), 5.89 (2H, s, OCH_2O), 6.56 (1H, s, H-11), 6.68 (1H, s, H-8)

^{13}C NMR: δ_C (ppm) ($CDCl_3$, 75 MHz)

21.2 (q, $OCO\underline{C}H_3$), 28.9 (t, C-4), 33.1 (t, C-2), 42.5 (d, C-11b), 53.7 (t, C-5), 55.9 (t, C-7), 60.8 (d, C-11c), 66.1 (d, C-1), 101.0 (t, OCH_2O), 104.9 (d, C-8), 107.5 (d, C-11), 115.3 (d, C-3), 127.7 (s, C-7a), 127.7 (s, C-11a), 138.0 (s, C-3a), 146.3 (s, C-9), 147.0 (s, C-10), 170.8 (s, $O\underline{C}OCH_3$)

Physical Data for compound (12)

1-O-acetyl-9-norpluviine [AMM31K]

Yield: 20 mg

Melting point: 173 ^{0}C

Mass: HRMS: [M^+] at *m/z* 315.1466, $C_{18}H_{21}NO_4$ requires 315.1469

EIMS: *m/z* (rel. int.): 315 (100.00) [M^+], 314 (13.70) [M^+ - H], 256 (16.39) [M^+ - CH_3COO], 255 (21.97) [M^+ - CH_3COOH], 254 (78.08) [M^+ - CH_3COOH - H], 229 (38.58) [M^+ - $CHOC(O)CH_3$ - CH_2], 228 (63.29) [M^+ - $CHOC(O)CH_3$ - CH_2 - H], 43 (9.61)

Optical rotation: $[\alpha]_D^{22}$ -106^0 (c = 0.4; MeOH)

Infra red: ν_{max} (cm^{-1}): 3440 (O – H stretching); 1735 (C = O stretching); 1518 (aromatic C = C stretching); 1255, 1235 (C – N stretching); 1235, 1100, 1037 (C – O stretching)

^{1}H NMR : δ_H (ppm) (CD_3OD, 300 MHz)

1.90 (3H, s, $OCOC\underline{H}_3$), 2.40 (1H, m, H-2a), 2.48 (1H, dd, H-5a, *J* = 17.70 Hz, 8.79 Hz), 2.64 (2H, m, H-4), 2.68 (1H, m, H-2b), 2.79 (1H, d, H-11b, *J* = 10.44 Hz), 2.89 (1H, bd, H-11c, *J* = 10.44 Hz), 3.33 (1H, m, H-5b), 3.56 (1H, d, H-7α, *J* = 14.00 Hz), 3.83 (3H, s, OCH_3), 4.14 (1H, d, H-7β, *J* = 14.00 Hz), 5.48 (1H, d, H-3, *J* = 2.39 Hz), 6.06 (1H, d, H-1, *J* = 3.42 Hz), 6.61 (1H, s, H-8), 6.86 (1H, s, H-11)

^{13}C NMR : δ_C (ppm) (CD_3OD, 75 MHz)

21.0 (q, $OCO\underline{C}H_3$), 29.2 (t, C-4), 34.2 (t, C-2), 44.1 (d, C-11b), 54.7 (t, C-5), 56.4 (q, OCH_3), 57.1 (t, C-7), 62.8 (d, C-11c), 67.6 (d, C-1), 109.5 (d, C-11), 114.9 (d, C-8), 115.9 (d, C-3), 126.3 (s, C-11a), 129.3 (s, C-7a), 139.8 (s, C-3a), 146.2 (s, C-10), 147.8 (s, C-9), 172.4 (s, $O\underline{C}OCH_3$)

Physical Data for compound (13)

hippadine [AMM10-18A3C]

Yield: 10 mg

Melting point: 212-213 ^{0}C (lit.[18] 212-214^{0}C)

Mass: HRMS: [M^+] at *m/z* 263.0584, $C_{16}H_9NO_3$ requires 263.0583

EIMS: *m/z* (rel. int.): 263 (100.00) [M^+], 177 (14.36), 97 (16.44), 71 (23.20), 69 (18.72), 57 (27.82), 43 (22.64)

Optical rotation: $[\alpha]_D^{22}$ +32^0 (c = 0.2; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 1676 (C = O stretching); 1465 (aromatic C = C stretching); 1314 (C – N stretching); 1037 (C – O stretching), 939 (C – O – C stretching)

^{1}H NMR : δ_H (ppm) ($CDCl_3$, 300 MHz)

6.15 (2H, s, OCH_2O), 6.88 (1H, d, H-4, *J* = 3.72 Hz), 7.47 (1H, dd, H-2, *J* = 7.70 Hz, 7.69 Hz), 7.65 (1H, s, H-11), 7.73 (1H, d, H-3, *J* = 7.70 Hz), 7.90 (1H, d, H-1, *J* = 7.69 Hz), 7.97 (1H, s, H-8), 8.03 (1H, d, H-5, *J* = 3.66 Hz)

^{13}C NMR : δ_C (ppm) ($CDCl_3$, 75 MHz)

101.7 (d, C-11), 102.3 (t, OCH_2O), 108.0 (d, C-8), 110.8 (d, C-4), 118.4 (d, C-1), 122.6 (d, C-3), 123.6 (d, C-5), 124.0 (d, C-2)

Physical Data for compound (14)

6α-hydroxypowelline [AMM61B1A]

Yield: 30 mg

Melting point: 234-235 ^{0}C (lit.[22] 233-235 ^{0}C)

Mass: HRMS: [M^+] at m/z 317.1254, $C_{17}H_{19}NO_5$ requires 317.1264

EIMS: *m/z* (rel. int.): 317 (100.00) [M^+], 300 (14.95) [M^+ - OH], 299 (21.92) [M^+ - H_2O], 273 (35.12) [M^+ - CH_2CH - OH], 262 (30.14), 244 (47.87), 60 (39.97), 56 (28.41), 45 (39.32), 43 (45.34)

Optical rotation: $[\alpha]_D^{22}$ -42^0 (c = 0.6; MeOH)

Circular dichroism: molar ellipticity [θ] (deg. cm^2. $decimole^{-1}$): $[\theta]_{243}$ +1578, $[\theta]_{251}$ +3286, $[\theta]_{284}$ -1756

Infra red: ν_{max} (cm^{-1}): 3350 (O – H stretching); 1630, 1498 (aromatic C = C stretching); 1097, 1044 (C – O stretching), 939 (C – O – C stretching)

^{1}H NMR : δ_H (ppm) (CD_3OD, 300 MHz)

1.88 (1H, dd, H-4, *J* = 13.43 Hz, 4.28 Hz), 2.00 (3H, m, H-4, H-11a, H-11b), 2.95 (1H, m, H-12a), 3.42 (1H, m, H-12b), 4.00 (1H, dd, H-4a, *J* = 13.19 Hz, 4.39 Hz), 4.06 (3H, s, OCH_3), 4.33 (1H, brs, H-3), 5.40 (1H, s, H-6), 5.96 (2H, s, OCH_2O), 6.00 (1H, dd, H-2, *J* = 5.01 Hz, 9.95 Hz), 6.62 (1H, d, H-1, *J* = 10.01 Hz), 6.75 (1H, s, H-10)

^{13}C NMR : δ_C (ppm) (CD_3OD, 75 MHz)

32.5 (t, C-4), 41.0 (t, C-11), 45.7 (s, C-10b), 48.2 (t, C-12), 57.7 (d, C-4a), 60.2 (q, OCH_3), 64.0 (d, C-3), 86.1 (d, C-6), 98.1 (d, C-10), 102.6 (t, OCH_2O), 119.0 (s, C-6a), 129.3 (d, C-2), 131.0 (d, C-1), 135.9 (s, C-10a), 140.1 (s, C-8), 144.1 (s, C-9), 151.6 (s, C-7)

Physical Data for compound (15)

hamayne [AMM61B2]

Yield: 40 mg

Melting point: 83-85 ^{0}C (lit.[26] 82-84^{0}C)

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) $[C_{13}H_9O]^+$, 149 (16.19), 115 (8.24), 57 (7.40), 43 (8.13)

Optical rotation: $[\alpha]_D^{22}$ +88^0 (c = 0.8; MeOH)

Circular dichroism: molar ellipticity [θ] (deg. cm^2. decimole^{-1}): $[\theta]_{235}$ –1827, $[\theta]_{242}$ –3582, $[\theta]_{249}$ –3078, $[\theta]_{279}$ +2332, $[\theta]_{294}$ +2728, $[\theta]_{301}$ +3277 (lit.[26] $[\theta]_{245}$ –5750, $[\theta]_{290}$ +4420)

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

^{1}H NMR : δ_H (ppm) (CD_3OD, 300 MHz)

2.11 (1H, m, H-4α), 2.20 (1H, dd, H-4β, *J* = 13.43, 10.44 Hz), 3.41 (1H, d, H-12a, *J* = 3.45 Hz), 3.48 (1H, dd, H-4a, *J* = 13.62, 4.40 Hz), 3.66 (1H, dd, H-12b, *J* = 13.73, 6.96 Hz), 3.95 (1H, d, H-6α, *J* = 16.48 Hz), 4.05 (1H, m, H-11), 4.37 (1H, m, H-3), 4.47 (1H, d, H-6β, *J* = 16.48 Hz), 5.93 (2H, s, OCH_2O), 6.09 (1H, d, H-2, *J* = 10.31 Hz), 6.25 (1H, dd, H-1, *J* = 10.38, 2.26 Hz), 6.60 (1H,s, H-7), 6.92 (1H, s, H-10)

^{13}C NMR: δ_C (ppm) (CD_3OD, 75 MHz)

33.5 (t, C-4), 51.6 (s, C-10b), 60.7 (t, C-6), 63.2 (t, C-12), 67.7 (d, C-3), 67.9 (d, C-4a), 80.2 (d, C-11), 102.5 (t, OCH_2O), 104.4 (d, C-10), 107.9 (d, C-7), 124.0 (d, C-1), 124.7 (s, C-6a), 136.4 (s, C-10a), 137.4 (d, C-2), 148.1 (s. C-8), 148.6 (s, C-9)

Physical Data for compound (16)

crinamine [AMM31V]

Yield: 30 mg

Melting point: 189-190 ^{0}C (lit.[7] 188^{0}C)

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)

Optical rotation: $[\alpha]_D^{22}$ +103^0 (c = 0.6; MeOH)

Cicular dichroism: molar ellipticity [θ] (deg. cm^2. decimole^{-1}): $[\theta]_{249}$ –5508, $[\theta]_{276}$ +2765, $[\theta]_{305}$ +2682, (lit.[26] $[\theta]_{245}$ –7285, $[\theta]_{285}$ +5395)

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: δ_H (ppm) (CD_3OD, 300 MHz)

2.08 (1H, m, H-4α), 2.15 (1H, dd, H-4β, *J* = 13.06, 10.44 Hz), 3.23 (1H, d, H-12a, *J* = 3.45 Hz), 3.28 (1H, dd, H-4a, *J* = 13.25, 3.30 Hz), 3.42 (3H, s, OMe), 3.50 (1H, dd, H-12b, *J* = 13.73, 6.16 Hz), 3.78 (1H, d, H-6α, *J* = 16.61 Hz), 4.00 (1H, dd, H-11, *J* = 6.17, 2.75 Hz), 4.10 (1H, m, H-3), 4.33 (1H, d, H-6β, *J* = 16.91 Hz), 5.91 (2H, s, OCH_2O), 6.11 (1H, d, H-2, *J* = 10.32 Hz), 6.31 (1H, dd, H-1, *J* = 10.44, 2.13 Hz), 6.56 (1H, s, H-7), 6.89 (1H, s, H-10)

^{13}C NMR: δ_C (ppm) (CD_3OD, 75 MHz)

30.6 (t, C-4), 51.7 (s, C-10b), 55.8 (q, OCH_3), 61.5 (t, C-6), 63.6 (t, C-12), 67.4 (d, C-4a), 77.6 (d, C-3), 81.0 (d, C-11), 102.2 (t, OCH_2O), 104.3 (d, C-10), 107.8 (d, C-7), 125.8 (d, C-1), 126.6 (s, C-6a), 134.2 (d, C-2), 137.2 (s, C-10a), 147.8 (s, C-8), 148.2 (s, C-9)

Physical Data for compound (19)

24-methylenecycloartan-3β-ol [AMM10-18A4]

Yield: 20 mg

Melting point: 120-121 ^{0}C (lit.[33] 120-121^{0}C)

Mass: HRMS: [M^+] at *m/z* 440.4029, $C_{31}H_{52}O$ requires 440.4018

EIMS: *m/z* (rel. int.): 440 (25.97) (M^+), 425 (33.83) [M^+ - CH_3], 422 (49.80) [M^+ - H_2O], 408 (20.51) [M^+ - CH_3 - OH], 407 (48.03) [M^+ - CH_3 - H2O], 379 (17.77) [M^+ - CH_3CHCH_3 - H_2O], 353 (11.57) [M^+ - $CH_2CHOHCCH_3CH_3$ - H], 341 (6.25) [M^+ - C_7H_{13} - 2H], 315 (16.27) [M^+ - C_9H_{17}], 300 (42.38) [M^+ - $C_9H_{15}O$ – H], 297 (15.83) [M^+ - C_9H_{17} - H_2O], 285 (13.24) [M^+ - $C_9H_{15}O$ - H - CH_3], 239 (26.93), 229 (11.48), 219 (12.06), 217 (18.77), 216 (20.87), 215 (12.52), 203 (40.08), 201 (26.04), 189 (22.50), 187 (26.38), 177 (21.13), 175 (58.94) $[C_{13}H_{19}]^+$, 173 (35.10), 163 (29.64), 161 (40.97), 159 (29.52), 149 (36.92), 148 (26.62), 147 (50.54), 145 (25.86), 137 (21.25), 135 (60.92), 134 (33.09), 133 (48.46), 123 (45.42), 121 (63.50), 119 (45.29), 109 (67.18), 107 (69.75), 105 (37.35), 97 (29.56) $[C_7H_{13}]^+$, 95 (98.25) $[C_7H_{11}]^+$, 93 (50.66), 83 (46.26), 81 (63.26), 69 (100.00) $[C_5H_9]^+$, 55 (78.94) $[C_4H_7]^+$, 43 (44.28) $[C_3H_7]^+$

Optical rotation: $[\alpha]_D^{22}$ +38^0 (c = 0.4; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 3400 (O – H stretching), 2960, 2925 (C – H antisymmetric stretching), 2870, 2850 (C – H symmetric stretching), 1652 (C = C stretching), 1475 (C – H antisymmetric bending), 1373 (C – H symmetric bending), 1056 (C – O stretching), 891 (= CH_2 out of plane bending)

^{1}H NMR : δ_H (ppm) ($CDCl_3$, 300 MHz)

0.31 (1H, d, H-19a, *J* = 4.09 Hz), 0.53 (1H, d, H-19b, *J* = 4.27 Hz), 0.79 (3H, s, H-31), 0.88 (3H, d, H-21, *J* = 4.03 Hz), 0.88 (3H, s, H-32), 0.95 (6H, s, H-18, H-30), 1.00 (3H, d, H-26, *J* = 6.78 Hz), 1.01 (3H, d, H-27, *J* = 6.78 Hz), 3.26 (1H, dd, H-3, *J* = 11.11 Hz, 4.52 Hz), 4.64 (1H, s, H-28a), 4.70 (1H, s, H-28b)

^{13}C NMR : δ_H (ppm) ($CDCl_3$, 75 MHz)

14.0 (q, C-30), 18.0 (q, C-18), 18.3 (q, C-21), 19.3 (q, C-32), 20.0 (s, C-9), 21.1 (t, C-6), 21.9 (q, C-26), 22.0 (q, C-27), 25.4 (q, C-31), 26.1 (t, C-11), 26.1 (s, C-10), 26.5 (t, C-

16), 28.1 (t, C-7), 29.9 (t, C-19), 30.4 (t, C-2), 31.3 (t, C-23), 32.0 (t, C-1), 32.9 (t, C-15), 33.8 (d, C-25), 35.0 (t, C-22), 35.6 (t, C-12), 36.1 (d, C-20), 40.5 (s, C-4), 45.3 (s, C-13), 47.1 (d, C-5), 48.0 (d, C-8), 48.8 (s, C-14), 52.3 (d, C-17), 78.9 (d. C-3), 105.9 (t, C-28), 156.9 (s, C-24)

Physical Data for compound (20)

cycloeucalenol [AMM10-18A5]

Yield: 20 mg

Melting point: 138-139 ^{0}C (lit.[37] 138-140^{0}C)

Mass: HRMS: [M^+] at *m/z* 426.3852, $C_{30}H_{50}O$ requires 426.3861

EIMS: *m/z* (rel. int.): 426 (66.81) [M^+], 411 (100.00) [M^+ -CH_3], 409 (25.26) [M^+ - OH], 408 (65.61) [M^+ - H_2O], 394 (17.51) [M^+ - CH_3 – OH], 393 (45.82) [M^+ - CH_3 - H_2O], 383 (18.27) [M^+ - CH_3CHCH_3], 353 (6.58) [M^+ - $CH_2CHOHCHCH_3$ - H], 327 (13.80) [M^+ - C_7H_{13} - 2H], 301 (18.33) [M^+ - C_9H_{17}], 300 (34.22) [M^+ - $C_8H_{13}O$ - H], 299 (35.21) [M^+ - $C_8H_{15}O$], 285 (12.69) [M^+ - $C_8H_{13}O$ - CH_3 - H], 283 (10.47) [M^+ - C_9H_{17} - H_2O], 245 (24.66), 233 (15.21), 201 (18.80), 189 (20.87), 175 (28.70) $[C_{13}H_{19}]^+$, 173 (25.77), 163 (24.06), 161 (27.25), 159 (22.47), 149 (23.52), 147 (31.37), 145 (17.70), 135 (30.66), 134 (15.30), 133 (27.10), 123 (30.66), 121 (37.09), 119 (26.00), 109 (41.15), 107 (38.11), 105 (23.41), 97 (29.64) $[C_7H_{13}]^+$, 95 (58.42) $[C_7H_{11}]^+$, 93 (24.97), 83 (32.17), 81 (39.96), 71 (16.09), 69 (50.43) $[C_5H_9]^+$, 57 (25.60), 55 (43.49) $[C_4H_7]^+$, 43 (19.15) $[C_3H_7]^+$

Optical rotation: $[\alpha]_D^{22}$ +48^0 (c = 0.4; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 3400 (O – H stretching), 2960, 2925 (C – H antisymmetric stretching), 2870, 2850 (C – H symmetric stretching), 1640 (C = C stretching bands), 1468 (C – H antisymmetric bending), 1380 (C – H symmetric bending), 1056 (C – O stretching), 885 (= CH_2 out of plane bending)

^{1}H NMR : δ_H (ppm) ($CDCl_3$, 300 MHz)

0.12 (1H, d, H-19a, J = 4.15 Hz), 0.36 (1H, d, H-19b, J = 3.85 Hz), 0.87 (3H, d, H-21, J = 6.23 Hz), 0.87 (3H, s, H-32), 0.95 (3H, s, H-18), 0.96 (3H, d, H-30, J = 6.1 Hz), 1.00 (3H, d, H-26, J = 6.9 Hz), 1.01 (3H, d, H-27, J = 6.9 Hz), 3.19 (1H, m, H-3), 4.65 (1H, s, H-28a), 4.70 (1H, s, H-28b)

^{13}C NMR : δ_C (ppm) ($CDCl_3$, 75 MHz)

14.4 (q, C-30), 17.8 (q, C-18), 18.3 (q, C-21), 19.1 (q, C-32), 21.9 (q, C-26), 22.0 (q, C-27), 23.5 (s, C-10), 24.7 (t, C-6), 25.2 (t, C-11), 27.0 (t, C-16), 27.3 (t, C-19), 28.1 (t, C-7), 29.5 (s, C-9), 30.8 (t, C-2), 31.3 (t, C-23), 32.9 (t, C-15), 33.8 (d, C-25), 34.8 (t, C-1), 35.0 (t, C-22), 35.3 (t, C-12), 36.1 (d, C-20), 43.3 (d, C-4), 44.6 (d, C-8), 45.4 (s, C-13), 46.9 (d, C-5), 48.9 (s, C-14), 52.2 (d, C-17), 76.5 (d, C-3), 105.9 (t, C-28), 156.9 (s, C-24)

Physical Data for compound (21)

cycloeucalenone [AMM10-18A2]

Yield: 20 mg

Melting point: 85-87 ^{0}C (lit.[41] 85-86 ^{0}C)

Mass: HRMS: [M^+] at *m/z* 424.3717, $C_{30}H_{48}O$ requires 424.3705

EIMS: *m/z* (rel. int.): 424 (72.79) [M^+], 409 (31.27) [M^+ - CH_3], 381 (28.19) [M^+ - CH_3CHCH_3], 341 (29.76) [M^+ - C_6H_{11}], 340 (29.20) [M^+ - C_6H_{11} - H], 328 (20.60), 327 (24.39) [M^+ - C_7H_{13}]$^+$, 326 (18.77) [M^+ - C_7H_{13} - H], 300 (27.70) [M^+ - $C_8H_{11}O$ - H], 299 (59.67) [M^+ - C_9H_{17}], 297 (17.46), 285 (10.63) [M^+ - $C_8H_{11}O$ - CH_3 - H], 257 (16.13), 219 (25.97) [$C_{16}H_{28}$ - H]$^+$, 217 (17.18), 216 (16.44), 203 (20.48), 189 (23.91), 187 (15.71), 177 (22.99), 175 (37.43) [$C_{13}H_{19}$]$^+$, 173 (20.90), 163 (35.72), 161 (34.48), 159 (19.63), 150 (16.75), 149 (45.90), 148 (22.89), 147 (43.89), 145 (21.09), 136 (51.24), 135 (46.96), 133 (47.99), 123 (70.95), 121 (64.03), 119 (38.66), 109 (61.76), 107 (68.78), 105 (33.53), 97 (26.85) [C_7H_{13}]$^+$, 95 (100.00) [C_7H_{11}]$^+$, 93 (49.28), 83 (42.53), 81 (65.62), 69 (86.68) [C_5H_9]$^+$, 55 (75.48) [C_4H_7]$^+$, 43 (29.24) [C_3H_7]$^+$

Optical rotation: $[\alpha]_D^{22}$ +82^0 (c = 0.4; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 1717 (C = O stretching), 2960, 2925 (C – H antisymmetric stretching), 2870, 2850 (C – H symmetric stretching), 1461 (C – H antisymmetric bending), 1374 (C – H symmetric bending), 896 (= CH_2 out of plane bending)

^{1}H NMR: δ_H (ppm) ($CDCl_3$, 300 MHz)

0.37 (1H, d, H-19a, *J* = 4.09 Hz), 0.60 (1H, d, H-19b, *J* = 3.84 Hz), 0.88 (3H, d, H-21, *J* = 4.40 Hz), 0.89 (3H, s, H-32), 0.96 (3H, s, H-18), 0.98 (3H, d, H-30, *J* = 1.65 Hz), 1.00 (3H, d, H-26, *J* = 6.84 Hz), 1.01 (3H, d, H-27, *J* = 6.84 Hz), 4.64 (1H, s, H-28a), 4.70 (1H, s, H-28b)

^{13}C NMR : δ_C (ppm) ($CDCl_3$, 75 MHz)

10.8 (q, C-30), 17.9 (q, C-18), 18.3 (q, C-21), 19.2 (q, C-32), 21.9 (q, C-26), 22.0 (q, C-27), 25.0 (s, C-10), 25.2 (t, C-11), 25.9 (t, C-6), 27.0 (t, C-19), 27.2 (t, C-16), 28.1 (t, C-7), 29.3 (s, C-9), 31.3 (t, C-23), 32.8 (t, C-1), 32.9 (t, C-15), 33.8 (d, C-25), 35.0 (t, C-22), 35.4 (t, C-12), 36.1 (d, C-20), 41.0 (t, C-2), 45.3 (s, C-13), 46.0 (d, C-4), 47.1 (d, C-5), 48.8 (s, C-14), 50.0 (d, C-8), 52.2 (d, C-17), 105.9 (t, C-28), 156.8 (s, C-24), 213.4 (s, C-3)

Physical Data for compound (22)

24-methylenepollinastanone [AMM10-18A3]

Yield: 20 mg

Melting point: 76-77 ^{0}C

Mass: HRMS: $[M^+]$ at *m/z* 410.3559, $C_{29}H_{46}O$ requires 410.3548

EIMS: *m/z* (rel. int.): 410 (40.82) $[M^+]$, 395 (22.89) $[M^+ - CH_3]$, 367 (20.33) $[M^+ - CH_3CHCH_3]$, 327 (21.73) $[M^+ - C_6H_{11}]$, 326 (19.66) $[M^+ - C_6H_{11} - H]$, 286 (20.59), 285 (81.47) $[M^+ - C_9H_{17}]$, 283 (18.79), 243 (16.81), 229 (17.36), 219 (19.29) $[C_{16}H_{28} - H]$, 217 (20.75), 203 (19.55), 189 (22.95), 175 (41.48) $[C_{13}H_{19}]^+$, 173 (17.86), 163 (44.45), 161 (32.01), 159 (20.20), 149 (45.67), 148 (21.41), 147 (41.22), 145 (19.39), 137 (23.32), 135 (46.28), 133 (37.81), 123 (44.62), 121 (60.84), 119 (42.61), 109 (64.60), 107 (71.68), 105 (35.80), 97 (30.85) $[C_7H_{13}]^+$, 95 (100.00) $[C_7H_{11}]^+$, 93 (52.14), 91 (27.10), 83 (41.26), 81

(72.38), 69 (78.50) $[C_5H_9]^+$, 67 (34.25), 57 (25.34), 55 (84.05) $[C_4H_7]^+$, 43 (46.90) $[C_3H_7]^+$

Optical rotation: $[\alpha]_D^{22}$ $+37^0$ (c = 0.4; $CHCl_3$)

Infra red: ν_{max} (cm^{-1}): 1715 (C = O stretching), 2960, 2925 (C – H antisymmetric stretching), 2870, 2850 (C – H symmetric stretching), 1468 (C – H antisymmetric bending), 1377 (C – H symmetric bending), 904 (= CH_2 out of plane bending)

^{1}H NMR : δ_H (ppm) ($CDCl_3$, 300MHz)

0.33 (1H, d, H-19a, *J* = 4.21 Hz), 0.60 (1H, d, H-19b, *J* = 3.85 Hz), 0.89 (3H, d, H-21, *J* = 6.60 Hz), 0.90 (3H, s, H-32), 0.98 (3H, s, H-18), 1.00 (3H, d, H-26, *J* = 6.90 Hz), 1.01 (3H, d, H-27, *J* = 6.84 Hz), 4.65 (1H, s, H-28a), 4.70 (1H, s, H-28b)

^{13}C NMR : δ_H (ppm) ($CDCl_3$, 75 MHz)

17.8 (q, C-18), 18.3 (q, C-21), 19.1 (q, C-32), 21.9 (q, C-26), 22.0 (q, C-27), 24.5 (s, C-10), 24.9 (t, C-11), 25.8 (t, C-6), 27.2 (t, C-16), 28.1 (t, C-7), 28.4 (t, C-19), 29.2 (s, C-9), 31.3 (t, C-23), 32.1 (t, C-1), 32.8 (t, C-15), 33.8 (d, C-25), 35.0 (t, C-22), 35.3 (t, C-12), 36.1 (d, C-20), 39.8 (d, C-8), 41.2 (t, C-2), 45.4 (s, C-13), 46.9 (d, C-5), 48.5 (t, C-4), 48.9 (s, C-14), 52.2 (d, C-17), 105.9 (t, C-28), 156.9 (s, C-24), 212.0 (s, C-3)

1.3.5 References

1. Pole Evans, I. B. (1938) ***Ammocharis coranica,*** *Flowering Plants of South Africa,* **18**: t.712.

2. Viladomat, F., Bastida, J., Codina, C., Nair, J.J. and Campbell, W.E. (1997) **Alkaloids of the South African Amaryllidaceae,** *in Recent Research Developments in Phytochemistry,* (**1**), Ed. S.G. Pandalai, Research Signpost Publisher, Trivandrum, pp 131-171.

3. Mason, L.H., Puschett, E.R. and Wildman, W.C. (1955) **Alkaloids of the Amaryllidaceae. IV. Crystalline alkaloids of *Ammocharis coranica* (Ker-Gawl.) Herb., *Brunsvigia rosea* (Lam.) Hannibal and two *Crinum* species,** *Journal of the American Chemical Society,* **77,** 1253-1256.

4. Hauth, H. and Stauffacher, D. (1962) **Die Alkaloide von *Ammocharis coranica* (Ker-Gawl.) Herb. *emend.* M-Redh.**, *Helvetica Chimica Acta*, **44**, 491-502.

5. Kinstle, T. H., Wildman, W.C. and Brown, C.L. (1966) **Mass spectra of Amaryllidaceae alkaloids. The structure of narcissidine,** *Tetrahedron Letters,* **7**, 4659-4666.

6. Kobayashi, S., Tokumoto, T., Kihara, M., Imakura, Y., Shingu, T. and Taira, Z. (1984) **Alkaloidal constituents of *Crinum latifolium* and *Crinum bulbispermum* (Amaryllidaceae),** *Chemical and Pharmaceutical Bulletin,* **32**, 3015-3022.

7. Likhitwitayawuid, K., Angerhofer, C.K., Chai, H., Pezzuto, J.M. and Cordell, G.A. (1993) **Cytotoxic and antimalarial alkaloids from the bulbs of *Crinum amabile,*** *Journal of Natural Products,* **56**, 1331-1338.

8. Evidente, A., Cicala, M.R., Giudicianni, I., Randazzo, G. and Riccio, R. (1983) **^{1}H and ^{13}C NMR analysis of lycorine and α-dihydrolycorine,** *Phytochemistry,* **22**, 581-584.

9. Evidente, A. (1986) **Isolation and structural characterisation of lutessine, a new alkaloid from bulbs of *Sternbergia lutea*,** *Journal of Natural Products,* **49**, 90-94.

10. Pettit, G.R., Gaddamidi, V., Goswami, A. and Cragg, G.M. (1984) **Antineoplastic agents, 99. *Amaryllis belladonna*,** *Journal of Natural Products,* **47**, 796-801.

11. Ghosal, S., Rao, P.H., Jaiswal, D.K., Kumar, Y. and Frahm, A.W. (1981) **Alkaloids of *Crinum pratense*,** *Phytochemistry,* **20**, 2003-2007.

12. Ghosal, S., Saini, K.S., and Frahm, A.W. (1983) **Alkaloids of *Crinum latifolium*,** *Phytochemistry,* **22,** 2305-2309.

13. Ghosal, S., Lochan, R., Ashutosh, Kumar, Y. and Srivastava, R.S. (1985) **Alkaloids of *Haemanthus kalbreyeri*,** *Phytochemistry,* **24**, 1825-1828.

14. Ali, A.A., Mesbah, M.K. and Frahm, A.W. (1981) **Phytochemical investigation of *Hippeastrum vittatum* growing in Egypt. Part III: Structural elucidation of hippadine,** *Planta Medica,* **43,** 407-409.

15. Lauk, U., Durst, D. and Fischer, W. (1991) **Total synthesis of the Amaryllidaceae alkaloid ungeremine**, *Tetrahedron Letters*, **32**, 65-68.

16. Ali, A.A., Kating, H. and Frahm, A.W. (1981) **Four 6-hydroxylated alkaloids in the crinine series from *Crinum augustum,*** *Phytochemistry,* **20**, 1731-1733.

17. Haugwitz, R.D., Jeffs, P.W. and Wenkert, E. (1965) **Proton magnetic resonance studies of some Amaryllidaceae alkaloids of the 5-10b-ethanophenanthridine series and of criwelline and tazettine,** *Journal of the Chemical Society,* 2001-2009.

18. Viladomat, F., Codina, C., Bastida, J., Mathee, S. and Campbell, W.E. (1995) **Further alkaloids from *Brunsvigia josephinae,*** *Phytochemistry,* **40**, 961-965.

19. Pabuccuoglu, V., Richomme, P., Gozler, T., Kivcak, B., Freyer, A.J. and Shamma, M. (1989) **Four new crinine type alkaloids from *Sternbergia* species,** *Journal of Natural Products,* **52**, 785-791.

20. Baudoin, G., Tillequin, F., Koch, M. (1994) **Albiflomanthine – A crinane alkaloid from *Haemanthus albiflos* (Jacq.),** *Heterocycles,* **38**, 965-970.

21. Ali, A.A., El Sayed, H.M., Abdallah, O.M. and Steglich, W. (1986) **Oxocrinine and other alkaloids from *Crinum americanum,*** *Phytochemistry,* **25**, 2399-2401.

22. Slabaugh, M.R. and Wildman, W.C. (1971) **6-Hydroxybuphanidrine and 6-hydroxypowelline,** *Journal of Organic Chemistry,* **36**, 3202-3207.

23. Burlingame, A.L., Fales, H.M. and Highet, R.J. (1964) **The structure of Amaryllisine,** *Journal of the American Chemical Society,* **86,** 4976-4979.

24. Viladomat, F., Bastida, J., Codina, C., Campbell, W.E. and Mathee, S. (1995) **Alkaloids from *Boophane flava,*** *Phytochemistry,* **40**, 307-311.

25. Viladomat, F., Almanza, G.R., Codina, C., Bastida, J., Campbell, W.E and Mathee, S. (1996) **Alkaloids from *Brunsvigia orientalis*,** *Phytochemistry*, **43**, 1379-1384.

26. Viladomat, F., Bastida, J., Codina, C., Campbell, W.E. and Mathee, S. (1994) **Alkaloids from *Brunsvigia josephinae*,** *Phytochemistry*, **35**, 809-812.

27. Zetta, L., Gatti, G. and Fuganti, C. (1973) **^{13}C Nuclear Magnetic Resonance spectra of Amaryllidaceae alkaloids**, *Journal of the Chemical Society. Perkin 2,* 1180-1184.

28. Duffield, A.M., Aplin, R.T., Budzikiewicz, H., Djerassi, C., Murphy, C.F., and Wildman, W.C. (1965) **Mass spectrometry in structural and stereochemical problems. LXXXII. A study of the fragmentation of some Amaryllidaceae alkaloids,** *Journal of the American Chemical Society,* **87,** 4902-4912.

29. Cocker, W., McMurray, T.B.H. and Ntamila, M.S. (1965) **Extractives from woods. Part VII. Extractives from *Cephalosphaera usambarensis* Warb.**, *Journal of the Chemical Society*, 1692-1699.

30. Aplin, R.T., and Hornby, G.M. (1966) **Application of mass spectrometry to the structural investigation of 9,19-cyclosterols and triterpenes**, *Journal of the Chemical Society (B)*, 1078-1079.

31. Ageta, H. and Arai, Y. (1984) **Fern constituents: Cycloartane triterpenoids and allied compounds from *Polypodium formosanum* and *P. niponicum***, *Phytochemistry*, **23**, 2875-2884.

32. Bohme, F., Schmidt, J., Van Sung, T. and Adam, G. (1997) **24-methylpollinastanone, and related triterpenoids and sterols from *Costus tonkinensis***, *Phytochemistry*, **45**, 1041-1044.

33. de Pascual Teresa, J., Urones, J. G., Marcos, I.S., Basabe, P., Cuadrado, M.J.S and Moro, R.F. (1987) **Tritepenes from *Euphorbia broteri***, *Phytochemistry*, **26**, 1767-1776.

34. Ahmed, V. U. (1994), **Handbook of Natural Products Data – Volume 2 - Pentacyclic Triterpenoids,** *Elsevier Science B. V.,* London, pp 21-22.

35. Itoh, T., Uchikawa, K., Tamura, T. and Matsumoto, T. (1977) **Two new 4α-methylsterols in the seeds of *Brassica napus***, *Phytochemistry*, **16**, 1448-1450.

36. Conner, A.H. and Foster, D.O. (1981) **Triterpenes from Douglas fir sapwood**, *Phytochemistry*, **20**, 2543-2546.

37. Tavares, O.S., Duarte, N.D., Cunha, S.O, Ascenso, J.R. and Ferreira, M.J.U. (1995) **Triterpenoids from *Euphorbia boetica***, *Fitoterapia*, **66**, 551-552.

38. Cox, J.S.G., King, F.E. and King, T.J. (1959) **The structure of cycloeucalenol**, *Journal of the Chemical Society,* 514-518.

39. Khuong-Hu, F., Sangare, M., Chari, V.M., Bekaert, A., Devys, M., Barbier, M. and Lukass, G. (1975) **Carbon-13 nuclear resonance spectral analysis of cycloartenol and related compounds**, *Tetrahedron Letters*, **16**, 1787-1790.

40. Akihisa, T., Kimura, Y., Kokke, W. C. M. C., Takase, S., Yasukawa, K., Jin – Nai, A. and Tamura, T. (1997) **4-*Epi*cycloeucalenone and 4-*Epi*cyclomusalenone: Two 3-oxo-28-norcycloartanes from the fruit peel of *Musa sapientum.* L.,** *Chemical and Pharmaceutical Bulletin,* **45**, 744-746.

41. Akihisa, T., Shimizu, N., Tamura, T. and Matsumoto, T. (1986) **(24S)-14α,24-dimethyl-9β,19-cyclo-5α-cholest-25-en-3β-ol: A new sterol and other sterols in *Musa sapientum*, *Lipids*, 21**, 494-497.

42. Suffness, M. and Cordell, G. A. (1985) **Amaryllidaceae alkaloids. Antitumour activity and biochemical actions,** *in* ***The Alkaloids. Chemistry and Pharmacology*, 25,** Ed. Arnold, B., Academic Press, Orlando Florida, pp. 198-211.

43. Chattopadhyay, S., Chattopadhyay, U., Mathur, P.P., Saini, K.S. and Ghosal, S. (1983) **Effects of hippadine, an Amaryllidaceae alkaloid, on testicular function in rats,** *Planta Medica*, **49**, 252-254.

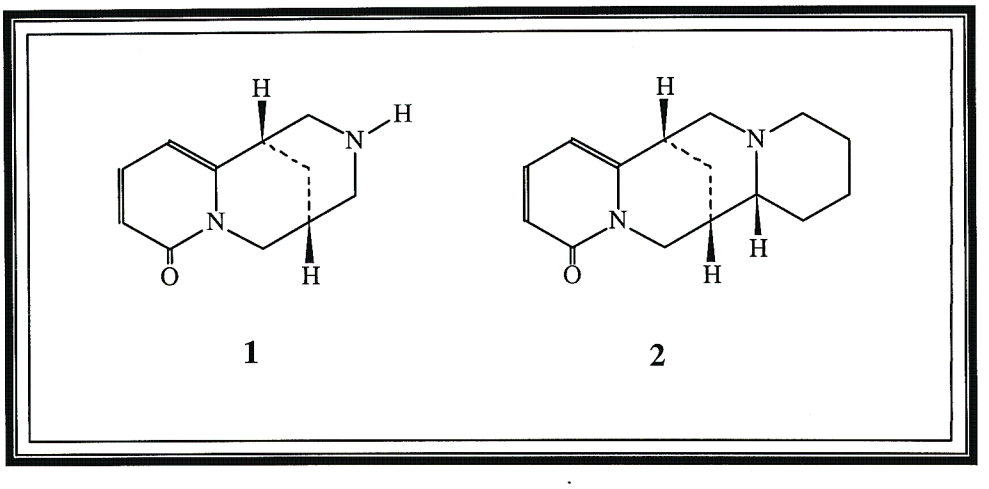
44. Wagner, J., Pham, H.L. and Dopke, W. (1996) **Alkaloids from *Hippeastrum equestre* Herb. –5. Circular dichroism studies**, *Tetrahedron*, **52**, 6591-6600.

45. Page, B. A. (1998) *in* **Extractives of three Southern African medicinal plants**, MSc dissertation, University of Natal, Durban, South Africa, pp. 36-40.

Chapter 2. Extractives from the Fabaceae. An investigation of the seeds of *Sophora velutina*.

2.1 An introduction to Quinolizidine Alkaloids

Plants of the Fabaceae are known to contain a group of alkaloids, sometimes referred to as the quinolizidine or lupine alkaloids. These alkaloids may be tricyclic or tetracyclic compounds. The tricyclic quinolizidine alkaloids all have the same basic skeleton as cytisine (**1**) and the tetracyclic quinolizidine alkaloids have the same basic skeleton as that of anagyrine (**2**).

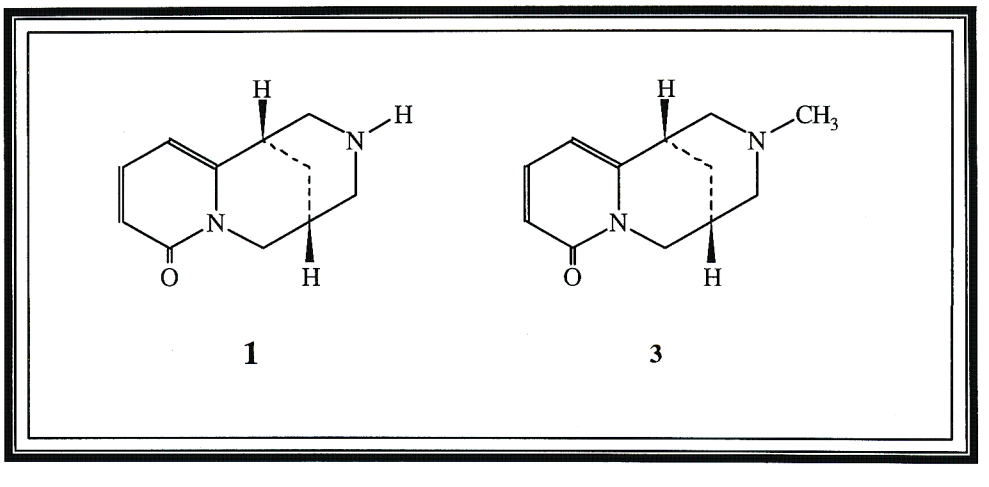


1 **2**

2.1.1 Cytisine and N-methylcytisine

The most common and well known tricyclic quinolizidine alkaloid isolated from the Fabaceae is cytisine (**1**). In the genus *Sophora*, this compound has been isolated from *Sophora secundiflora* [1-3], *Sophora tomentosa* [4] and *Sophora exigua* [5]. Cytisine is a very toxic compound (LD_{50} 101 mg/kg), similar to nicotine in its pharmacological effects, but twice as toxic when administered orally to mice. Cytisine appears to contribute most to the toxicity of *Sophora secundiflora* [1].

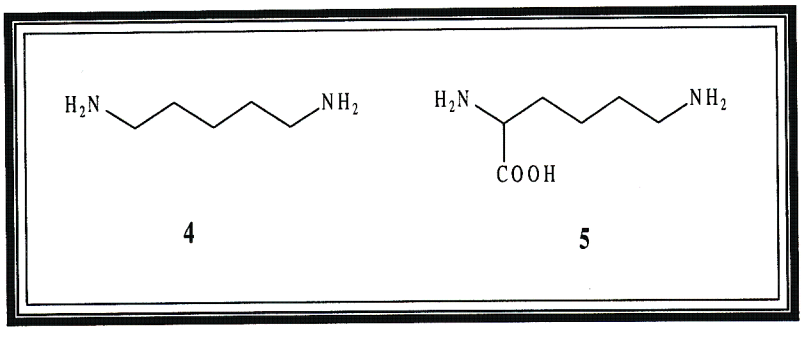
N-methylcytisine (**3**) is another toxic quinolizidine alkaloid found in *Sophora secundiflora* [1-3,6], *Sophora flavescens* [7], *Sophora tomentosa* [4], *Sophora exigua* [5] and *Sophora macrocarpa* [8]. N-methylcytisine was also found to contribute to the toxicity of *Sophora secundiflora* but is present in amounts far less than that of cytisine. Cytisine and N-methylcytisine were found to cause cardiac depression, stoppage in diastole, hypotension and produced peristalsis in mice [9].



1 **3**

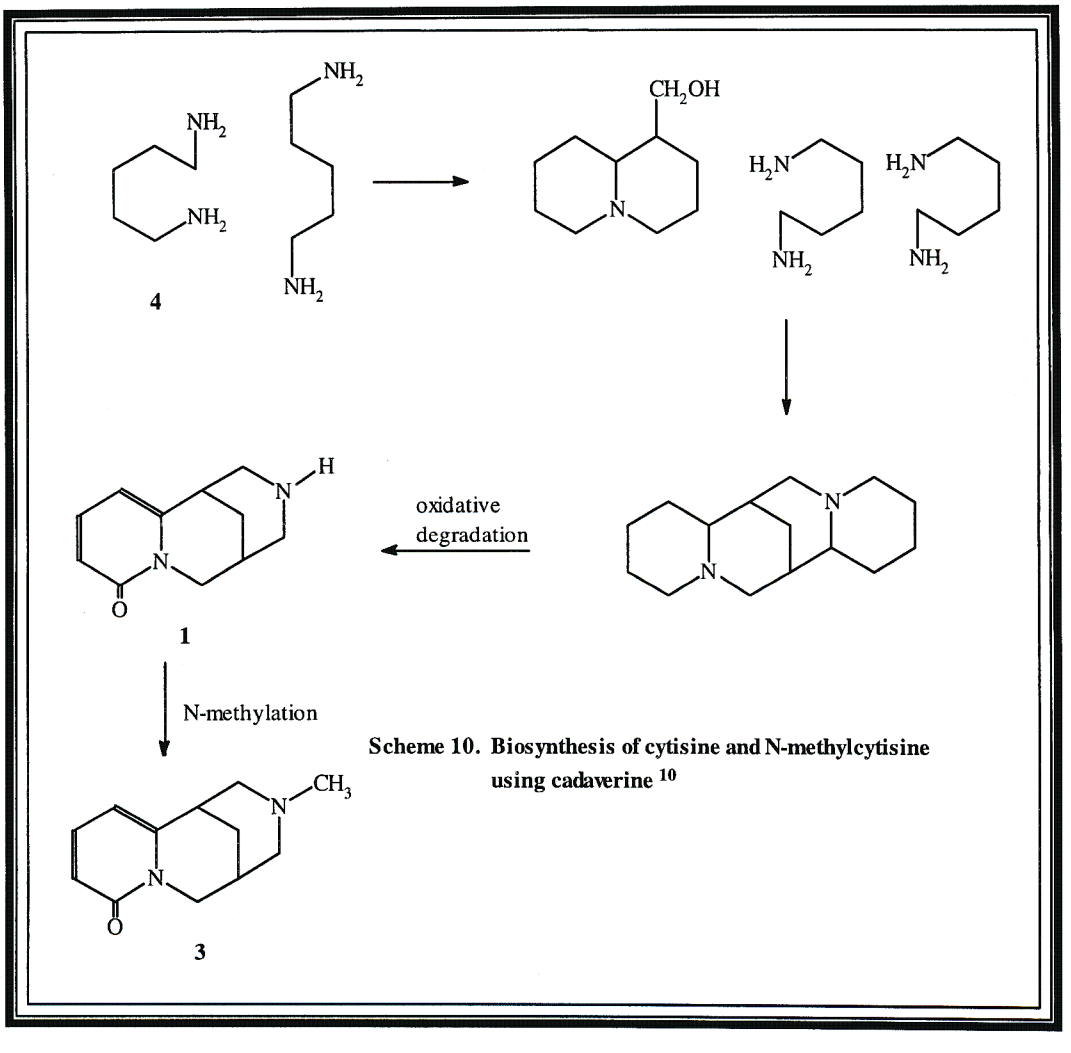
2.1.2 Biosynthesis of cytisine and N-methylcytisine

The biosynthetic precursors of cytisine and N-methylcytisine were shown to be cadaverine (**4**) and lysine (**5**). Cadaverine -1,5- ^{14}C and lysine -2- ^{14}C have been shown to be incorporated into cytisine and N-methylcytisine [10].



4 **5**

A schematic diagram outlining the biosynthesis of cytisine and N-methylcytisine using cadaverine (**4**) is given in **scheme 10**.



Scheme 10. Biosynthesis of cytisine and N-methylcytisine using cadaverine [10]

2.1.3 *Sophora* species containing quinolizidine alkaloids. Their ethnobotanical use and biological activity.

The seeds of the Texas Mountain Laurel, *Sophora secundiflora* are used by many different North American tribes as an oracular or divinatory medium, to induce visions during the initiation rites and as a ceremonial stimulant. *Sophora secundiflora*, which is commonly called "mescal beans", "red beans", "big drunk beans" and "dry whiskey" is considered by these Indian tribes to be alive and consequently attach a great deal of symbolic and religious importance to the seeds [6]. Three of the seeds most concentrated alkaloids, cytisine [2, 6, 11], N-methylcytisine [2, 6, 11], and sparteine [2, 6] showed responses similar to those produced by known hallucinogenic drugs [6]. The reported toxicity of these seeds has been due mainly to cytisine, with N-methylcytisine and sparteine being far less toxic than cytisine [1]. The seeds of *Sophora secundiflora* were also reported to have emetic and purgative properties [1]. Also contained in these seeds are the quinolizidine alkaloids anagyrine[11,12], thermopsine [11] and Δ^5-dehydrolupanine [1, 12]. The leaves of *Sophora secundiflora* were shown to contain the additional alkaloids, 11-oxocytisine, baptifoline, N-formylcytisine and N-acetylcytisine along with cytisine, N-methylcytisine and anagyrine, which is contained in the seeds as well [3]. Rhombifoline, lupanine, 11-allylcytisine and β-isosparteine were also found in the unripe fruits of *Sophora secundiflora* [12].

Sophora exigua, which is used as a folk medicinal plant in Thailand for antipyretic and respiratory diseases, contains nine quinolizidine alkaloids in its roots, 12-cytisineacetamide, cytisine, 12-hydroxycytisine, N-methylcytisine, N-formylcytisine, lupanine, 5,6-dehydrolupanine, anagyrine and baptifoline [5].

The quinolizidine alkaloids, cytisine, N-methylcytisine, N-acetylcytisine, baptifoline and anagyrine has been isolated from the aerial parts of *Sophora tomentosa* [4].

The roots of *Sophora flavescens* are used as an indigenous crude drug in folk stomachics, diuretics, antipyretics, analgesics and as an insecticide. The flowers of *Sophora flavescens* are known to contain N-methylcytisine and anagyrine [7].

Sophora griffithii is used in indigenous medicine in Pakistan as a stomachic, diuretic, antipyretic and analgesic as well as being employed as an insecticide. Two quinolizidine alkaloids, sophorasine A and sophorasine B were isolated from the leaves of *Sophora griffithii* [13]. The upper parts of *Sophora griffithii* also contained cytisine and N-methylcytisine as well as argentine [14]. The leafy shoots of this plant contained a new type of tetracyclic quinolizidine alkaloid, sophazrine [15].

Three cage quinolizide alkaloids, tsukushinamine-A, tsukushinamine-B and tsukushinamine-C have been isolated from the fresh epigeal parts of *Sophora franchetiana*, a very rare shrub in Japan, along with cytisine, N-formylcytisine, rhombifoline, anagyrine and baptifoline [16].

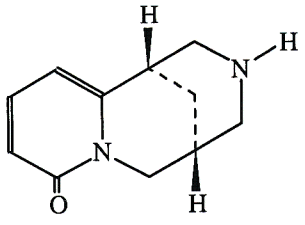
A list of the quinolizidine alkaloids isolated from *Sophora* species is given in **Table 18**.

Table 18. Quinolizidine alkaloids and the *Sophora* species in which they occur.

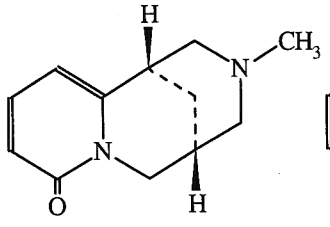
Quinolizidine alkaloid	*Sophora* species
Tricyclic quinolizidine alkaloids	
cytisine	*S. secundiflora* [1, 2, 3, 6, 11] *S. tomentosa* [4] *S. exigua* [5] *S. franchetiana* [16] *S. griffithii* [14]
N-methylcytisine	*S. secundiflora* [1, 2, 3, 6] *S. flavescens* [7] *S. tomentosa* [4] *S. exigua* [5] *S. macrocarpa* [8] *S. griffithii* [14]
rhombifoline	*S. secundiflora* [12] *S. franchetiana* [16]
12-cytisineacetamide	*S. exigua* [5]
sophorasine A	*S. griffithii* [13]
sophorasine B	*S. griffithii* [13]
N-formylcytisine	*S. secundiflora* [3] *S. exigua* [5] *S. franchetiana* [16]
N-acetylcytisine	*S. secundiflora* [3] *S. tomentosa* [4]
12-hydroxycytisine	*S. exigua* [5]
11-oxocytisine	*S. secundiflora* [3]
11-allylcytisine	*S. secundiflora* [12]

Table 18. continued.

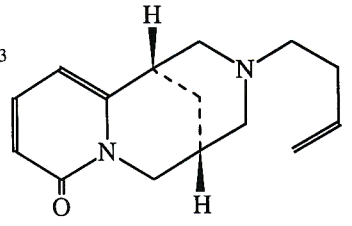
Tetracyclic quinolizidine alkaloids	
anagyrine	*S.secundiflora* [11, 12] *S. exigua* [6] *S. tomentosa* [4] *S. flavescens* [7] *S. franchetiana* [16]
thermopsine	*S. secundiflora* [11]
baptifoline	*S. secundiflora* [3] *S. exigua* [5] *S. tomentosa* [4] *S. franchetiana* [16]
Δ^5-dehydrolupanine	*S. secundiflora* [1, 12] *S. exigua* [5]
lupanine	*S. secundiflora* [12] *S. exigua* [5]
sparteine	*S. secundiflora* [2, 6]
β–isosparteine	*S. secundiflora* [12]
Miscellaneous quinolizidine alkaloids	
sophazrine	*S. griffithii* [15]
argentine	*S. griffithii* [14]
tsukushinamine-A	*S. franchetiana* [16]
tsukushinamine-B	*S. franchetiana* [16]
tsukushinamine-C	*S. franchetiana* [16]



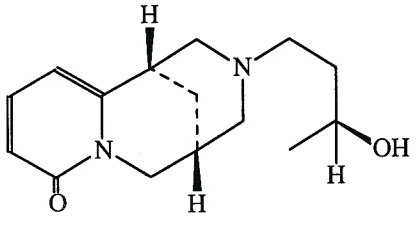
cytisine



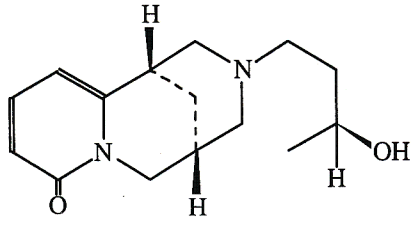
N-methylcytisine



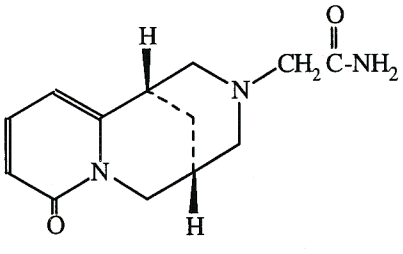
rhombifoline



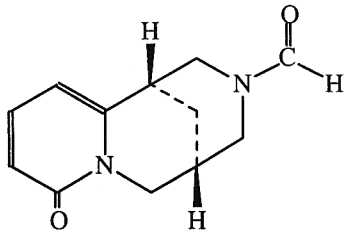
sophorasine A



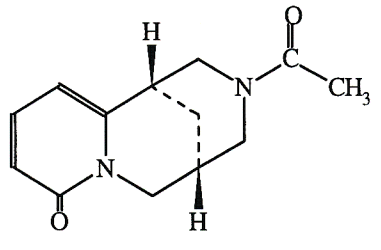
sophorasine B



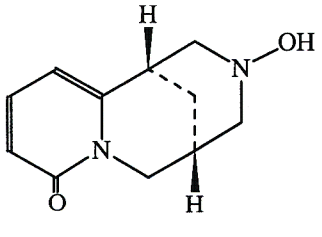
12-cytisineacetamide



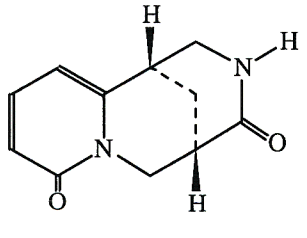
N-formylcytisine



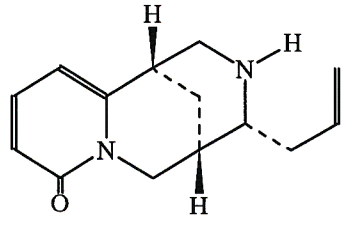
N-acetylcytisine



12-hydroxycytisine

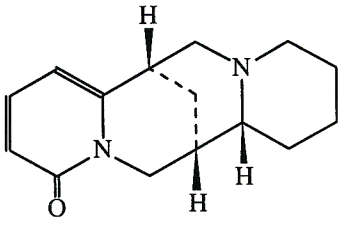


11-oxocytisine

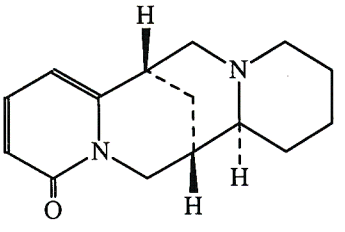


11-allylcytisine

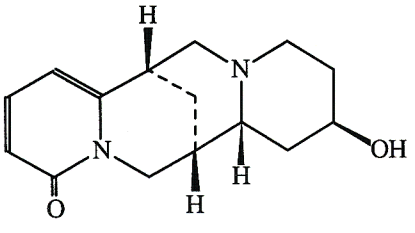
Fig. 3. Tricyclic quinolizidine alkaloids



anagyrine



thermopsine



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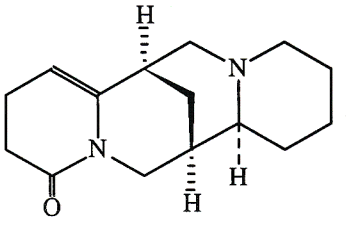
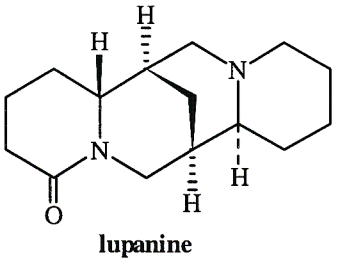
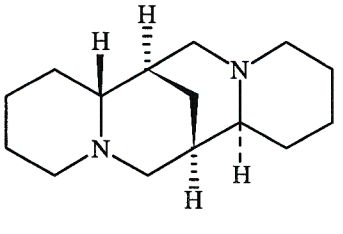


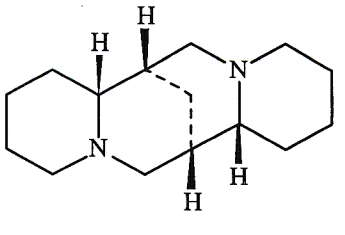
Δ^5-dehydrolupanine



lupanine

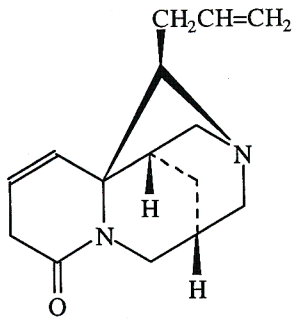


sparteine



β-isosparteine

Fig. 4. Tetracyclic quinolizidine alkaloids



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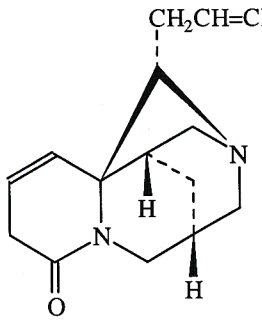

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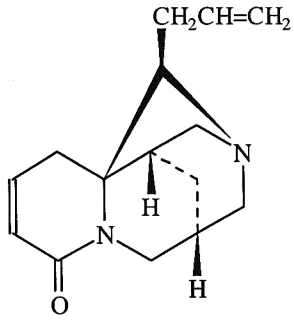

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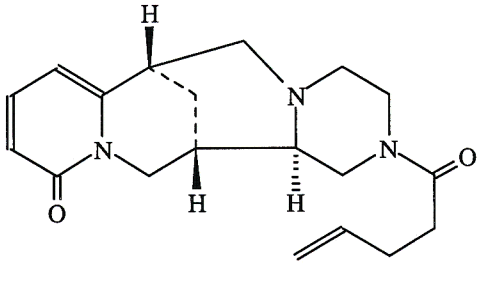
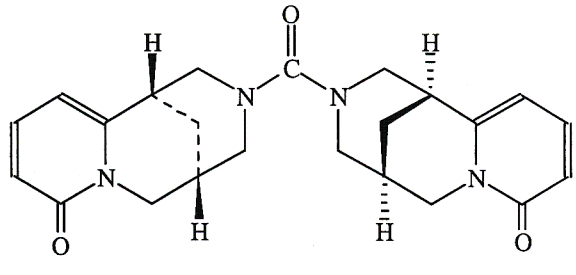


sophazrine



argentine

Fig. 5. Miscellaneous quinolizidine alkaloids

2.1.4 References

1. Hatfield, G. M., Valdes, L. J. J., Keller, W. J., Merrill, W. L. and Jones, V. H. (1977) **An investigation of *Sophora secundiflora* seeds (Mescalbeans)**, *Lloydia*, **40**, 374 - 383.

2. Keller, W. J. (1975) **Alkaloids from *Sophora secundiflora***, *Phytochemistry*, **14**, 2305 - 2306.

3. Murakoshi, I., Kubo, H., Ikram, M., Israr, M., Shafi, N., Ohmiya, S. and Otomasu, H. (1986) **(+) – 11 – oxocytisine, a lupine alkaloid from leaves of *Sophora secundiflora***, *Phytochemistry*, **25**, 2000 - 2002.

4. Ohmiya, S., Otomasu, H., Murakoshi, I., and Haginiwa, J. (1974) **N-acetylcytisine from *Sophora tomentosa***, *Phytochemistry*, **13**, 1016.

5. Takamatsu, S., Saito, K., Ohmiya, S., Ruangrungsi, N. and Murakoshi, I. (1991) **Lupin alkaloids from *Sophora exigua***, *Phytochemistry*, **30**, 3793 - 3795.

6. Bourn, W. M., Keller, W. J. and Bonfiglio, J. F. (1979) **Comparisons of mescal bean alkaloids with mescaline, Δ^9 - THC and other psychotogens**, *Life sciences*, **25**, 1043 - 1054.

7. Murakoshi, I., Kidoguchi, E., Haginiwa, J., Ohmiya, S., Higashiyama, K. and Otomasu, H. (1981) **(+) – Kuraramine, a possible metabolite of (-) - N - methylcytisine in flowers of *Sophora flavescens***, *Phytochemistry*, **20**, 1407-1409.

8. Silva, M., Medina, M. V. and Sammes, P. G. (1968) **Alkaloids of *Sophora macrocarpa***, *Phytochemistry*, **7**, 661 - 663.

9. Oung-Boran, Lebreton, P. and Netien, G. (1969) **Biochemical and pharmacological study of *Baptista australis***, *Planta Medica*, **17**, 301 - 318.

10. Schutte, V. H. R. and Lehfeldt, J. (1964) **Zur biosynthese der Papilionaceen alkaloide. XII. Biosynthese des cytisins**, *Journal für Praktische Chemie*, **24**, 143 - 147.

11. Izaddoost, M., Harris, B. G. and Gracy, R. W. (1976) **Structure and toxicity of alkaloids and amino acids of *Sophora secundiflora***, *Journal of Pharmaceutical Sciences*, **65**, 352 – 354.

12. Keller, W. J. and Hatfield, M. (1979) **11-Allylcytisine and other minor alkaloids from unripe *Sophora secundiflora* fruits**, *Phytochemistry*, **18**, 2068-2069.

13. Atta-ur-Rahman, Pervin, A., Perveen, S., Nasir, H. and Hasan, N. (1991) **Two lupin alkaloids from *Sophora griffithii***, *Phytochemistry*, **30**, 1001-1003.

14. Primukhamedov, I., Aslanov, Kh. A. and Sadykov, A. S. (1968) **Alkaloids from *Sophora griffithii,*** *Chemical Abstracts*, **72**, 79280j.

15. Atta-ur-Rahman, Pervin, A., Choudhary, M. I., Hasan, N. and Sener, B. (1991) **Sophazrine - A novel quinolizidine alkaloid from *Sophora griffithii***, *Journal of Natural Products*, **54**, 929 – 935.

16. Ohmiya, S., Higashiyama, K., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1981) **Two cage-type lupin alkaloids from *Sophora franchetiana***, *Phytochemistry*, **20**, 1997 – 2001.

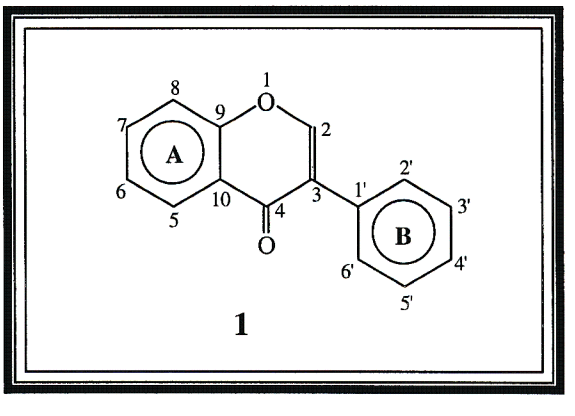
2.2 An introduction to isoflavones

The isoflavonoids are biogenetically related to the flavonoids but contain a rearranged C_{15} skeleton and are therefore considered a separate class from the flavonoids [1].

2.2.1 Classification of isoflavones

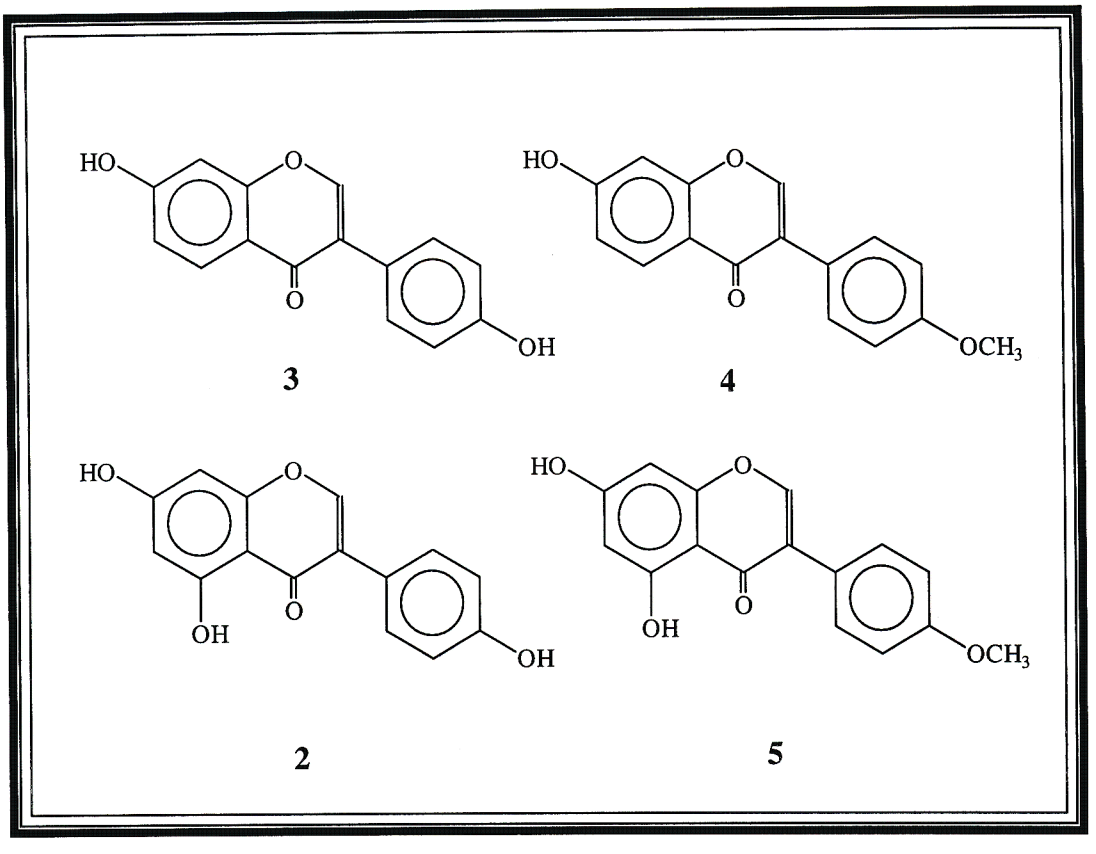
With regard to structure, the isoflavonoids may be subdivided into several classes according to oxidation levels in the skeleton. The structural variety within the isoflavonoids is surprisingly large. Isoflavones are the most abundant of the natural isoflavonoid derivatives, with over three hundred and sixty isoflavone aglycones isolated [2].

Isoflavones have the basic skeleton of isoflavone (**1**). Substitution patterns in ring A and ring B lead to the many isoflavone derivatives which occur in nature. The general isoflavone structures include the basic 5,7,4'- or 7,4'- oxygenation patterns associated with genistein (**2**) and daidzein (**3**) precursors [2]. Isoflavones with 5,6,7- and 5,7,8- as well as 6,7- oxygenation patterns in ring A are known. The oxygenation patterns in ring B are usually 3',4'-, but the more unusual 2',3'- and 3',5'- are also known [2].



1

Isoflavones which are extremely common are daidzein (**3**), formononetin (**4**), genistein (**2**) and biochanin A (**5**) [1].



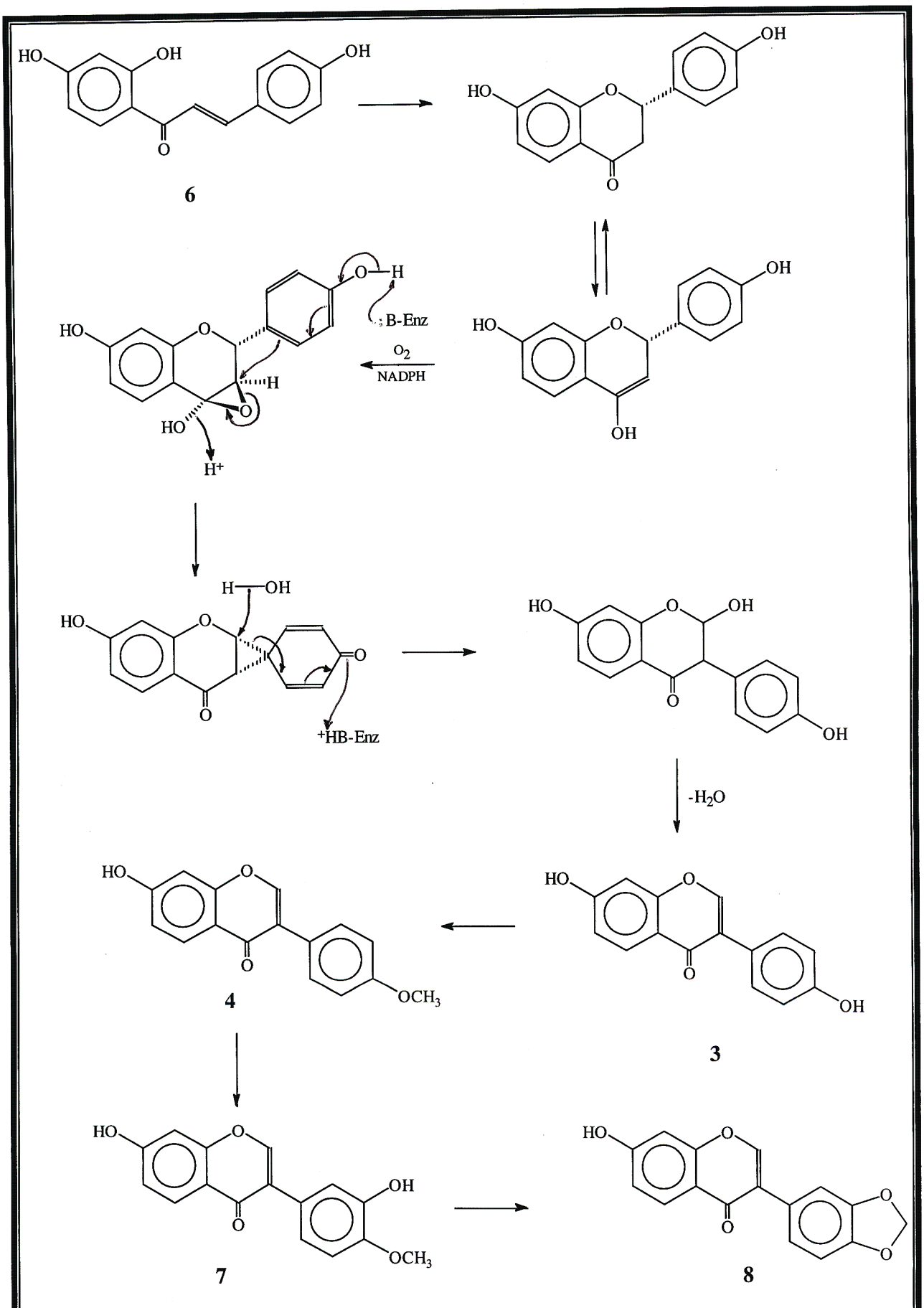
^{1}H NMR spectra of isoflavones differ from those of flavones in that the H-2 signal in isoflavones resonates at a lower field than the H-3 signal in flavones. However, in the ^{13}C NMR spectra of isoflavones and flavones, C-2 in isoflavones resonates at a higher field than in flavones and C-3 at a lower field [3].

2.2.2 Biosynthesis of isoflavones

The isoflavonoids share a common biosynthetic pathway with the flavonoids in being formed from a chalcone intermediate. A 1,2- aryl migration then occurs to give the characteristic rearranged isoflavonoid skeleton [1].

Chalcone intermediates are synthesised by the condensation of three molecules of malonyl-CoA with a suitable hydroxycinnamic acid CoA ester, ordinarily 4-coumaryl-CoA [4]. This reaction is catalysed by chalcone synthase. Both these flavonoid precursors are derived from carbohydrates. Malonyl-CoA is synthesised from acetyl-CoA and carbon dioxide and the synthesis of 4-coumaryl-CoA involves the shikimate/arogenate pathway, the main route to the aromatic amino acids, phenylalanine and tyrosine in higher plants [4]. These biosynthetic routes are catalysed by specific enzymes.

The biosynthesis of calycosin (**7**) and pseudobaptigenin (**8**) isolated in this work proceeds via 2',4',4-trihydroxychalcone (**6**), daidzein (**3**) and formononetin (**4**). The biosynthetic pathway is given in **scheme 11**.



Scheme 11. Biosynthetic pathway of calycosin (7) and pseudobaptigenin (8) [4,5]

2.2.3 Biological activity of isoflavones

Isoflavonoids have a broad range of biological activities [1, 5]. Isoflavones have been reported to exhibit oestrogenic activity, suppression of the growth of wheat coleoptiles, inhibition of soybean lipase activity and they act as phytoalexins[1].

The oestrogenic activity of simple isoflavones such as daidzein, formononetin, genistein and biochanin A has been detected in many leguminous plants and oestrogenic isoflavonoids feature among the important toxic constituents of leguminous plants [5].

Phytoalexins are antimicrobial (especially anti-fungal) compounds produced by plants as a response to fungal or bacterial attack and may be considered as part of a plant's natural defence against micro-organisms. Antimicrobial activity in roots of *Sophora angustifolia* was traced to the isoflavonoid maackiain[5]. Strongly antifungal isoflavones, luteone and wighteone have been isolated from healthy *Lupinus* tissues [1].

Several simple isoflavonoids, pseudobaptigenin, calycosin, maackiain and irilone, present in red clover (*Trifolium pratense*) root, suppress the growth of wheat coleoptiles *in vitro*. This is also evident in the glycosides of genistein and pseudobaptigenin from *Lupinus luteus*. These compounds could act as endogenous growth regulators [1].

Isoflavone activity also includes the inhibition of soybean lipase activity, especially genistein derivatives [5].

Another biological activity associated with isoflavonoids is insect-feeding deterrent activity. This property was first discovered in a number of isoflavonoid phytoalexin structures and a proposal was made that such compounds may serve two different roles, as phtoalexins and as insect feeding deterrents [5].

2.2.4 References

1. Dewick, P. M. (1982) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research**, Eds. Harborne, J. B. and Mabry, T. J., Chapman and Hall Ltd., New York, pp 535-609, 620-623.

2. Dewick, P. M. (1994) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research since 1986**, Ed. Harborne, J. B., Chapman and Hall Ltd., New York, pp 120-121.

3. Wenkert, E. and Gottlieb, H. E. (1977) **Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds**, *Phytochemistry*, **16**, 1811-1816.

4. Heller, W. and Forkman, G. (1968) **Biosynthesis,** *in* **The Flavonoids. Advances in Research since 1980**, Ed. Harborne, J. B., Chapman and Hall Ltd., New York, pp 399-407.

5. Dewick, P. M. (1988) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research since 1980**, Ed. Harborne, J. B., Chapman and Hall Ltd., New York, pp 137-147, 192-194, 202-204.

2.3 Extracts from *Sophora velutina*

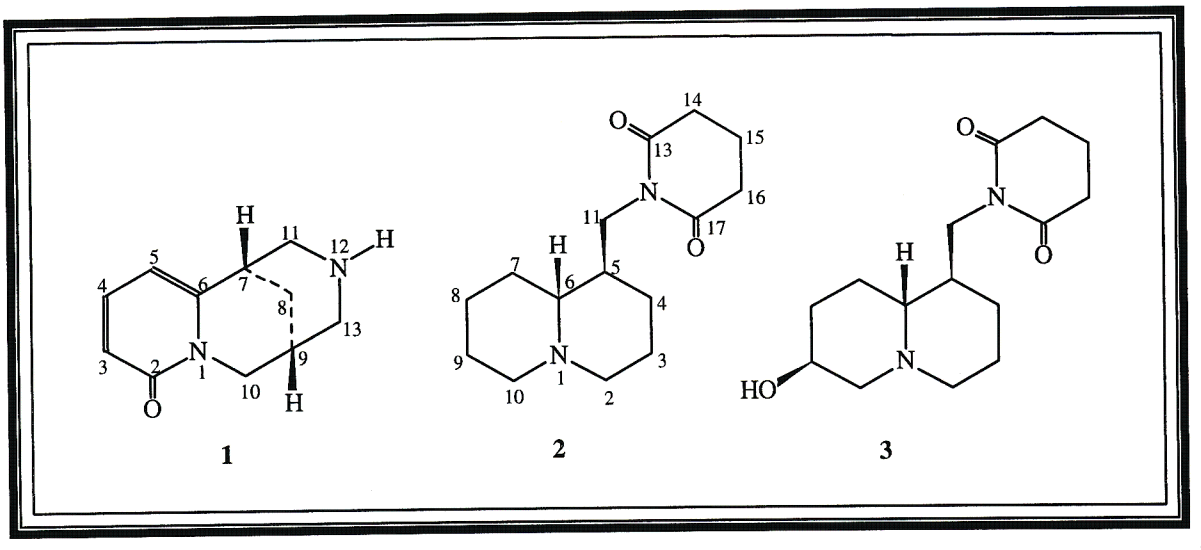
2.3.1 Introduction

Sophora velutina, subsp. *zimbabweensis* Gillet and Brummit, belongs to the tribe Sophoreae of the Fabaceae. *Sophora velutina* subsp. *zimbabweensis* is native to the immediate vicinity of Zimbabwe Ruins in Zimbabwe where it grows to a height of about two and a half metres, each plant having up to twelve stems from soil level. When exposed to the sun, these plants become very floriferous and produce an abundance of seed pods [1]. This species is distinct from other *Sophora* species represented on the African continent, having affinities with typical *Sophora velutina* Lindl., which is distributed from west and east-central India to south-west China and Indonesia [1]. Since this plant was found only 7-8 km from the ruins at Zimbabwe, which has yielded Chinese porcelain, Ming ware, Caledon and Persian glazed ware as well as Arab glass, it is possible that this plant could have been imported from Asia by people visiting Zimbabwe several centuries ago[1]. Since then it could have evolved its own distinguishing characters (calyx shape, petal shape and short bracts) in isolation in Africa[1].

Several species of *Sophora* have been shown to contain quinolizidine alkaloids which are found abundantly in the Fabaceae. *Sophora velutina* is one of over twenty species of *Sophora* which have been investigated for their alkaloidal contents[2]. Three alkaloids have been isolated previously from the leaves of *Sophora velutina* [2], one of which is the well known quinolizidine alkaloid cytisine (**1**). The other two alkaloids, (+)-lamprolobine (**2**) and (+)-9β-hydroxylamprolobine (**3**) are of the lipinane-type alkaloids which were found for the first time in the genus *Sophora* [2].

Another two classes of compounds quite commonly found in the Fabaceae are the flavonoids and isoflavonoids [13-15]. Although there have been reports of flavonoids and isoflavonoids within the Sophoreae tribe [13-15], there has been no previous reports of these compounds in *Sophora velutina*.

This is the first investigation of the seeds of *Sophora velutina*.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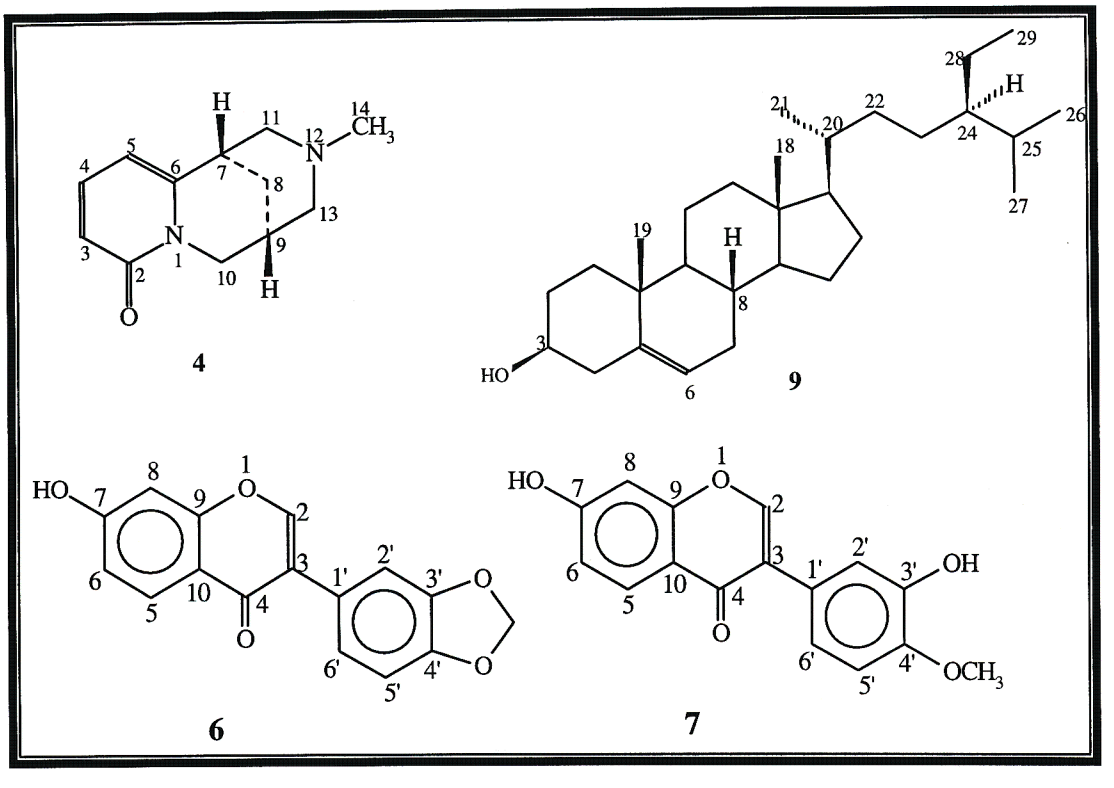
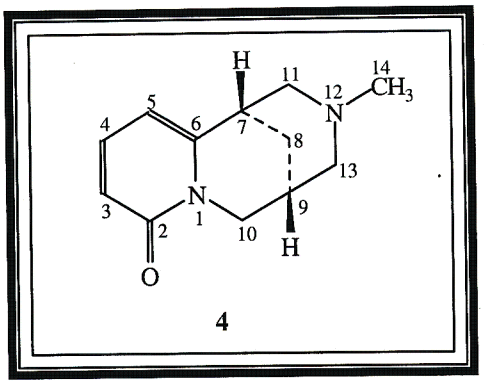
3

2.3.2 Results and Discussion

The basic chloroform extract of the seeds of this species afforded the quinolizidine alkaloid N-methylcytisine (**4**) and the acidic chloroform extract afforded the isoflavones pseudobaptigenin (**6**) and calycosin (**7**) as well as sitosterol (**9**), a common phytosterol. The physical properties and ^{1}H NMR spectrum (page 271) of sitosterol were identical to those of an authentic sample.





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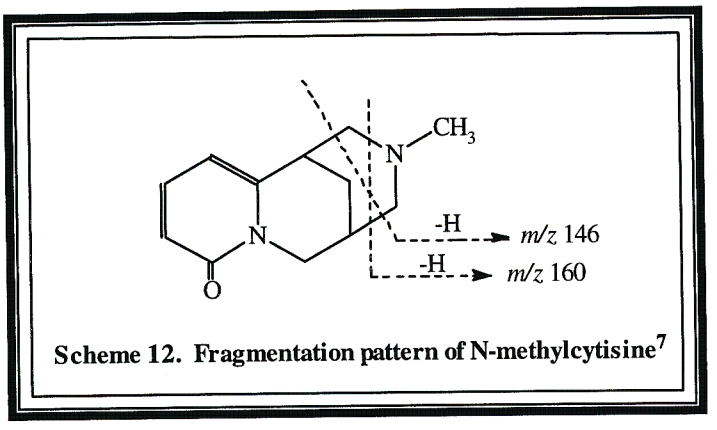
Compound (**4**), N-methylcytisine [$C_{12}H_{16}N_2O$, M^+ at *m/z* 204.1265] was isolated as white crystalline material. N-methylcytisine is a well known alkaloid from the Fabaceae, having being found in *Sophora exigua* [3], *Sophora tomentosa* [4], *Thermopsis chinensis* [5], *Petteria ramentacea* [6] and many other species in this family. This is the first report of this compound from the seeds of *Sophora velutina.* Spectra for N-methylcytisine are given on pages 250-256 in appendix A.

The i.r. spectrum of N-methylcytisine (**4**) showed bands at 1663 cm^{-1} and 1550 cm^{-1} indicative of an α-pyridone system[25-29] and at 2947 cm^{-1} and 2786 cm^{-1}, attributed to the *trans* quinolizidine system[26-29,30].

The mass spectrum of N-methylcytisine (**4**) showed a molecular ion peak at *m/z* 204.1265, corresponding to $C_{12}H_{16}N_2O$. The peak at *m/z* 160 (M^+ - 44) occurred as a result of the loss of a CH_3NCH_2 fragment and a hydrogen atom. At *m/z* 146 (M^+ - 58), the loss of $CH_2N(CH_3)CH_2$ and a hydrogen atom were evident. The base peak at *m/z* 58 was due to the ion, $[C_3H_8N]^+$ and the peak at *m/z* 42 was due to the presence of a $[C_2H_4N]^+$ ion. The fragmentation pattern of N-methylcytisine is given in **scheme 12**[7].



Scheme 12. Fragmentation pattern of N-methylcytisine[7]

The ^{1}H NMR spectrum of compound (**4**) showed a double doublet and two doublets at δ_H 7.50, δ_H 6.44 and δ_H 6.31 respectively, each integrating to one proton and these were attributed to H-4, H-3 and H-5 respectively. The H-4 resonance was coupled to the H-3 and H-5 resonances (COSY), but the resonances of H-3 and H-5 were not seen to be coupled to each other in the COSY spectrum. A doublet and double doublet at δ_H 4.03 (J = 15.45 Hz) and δ_H 3.91 (J = 15.45 Hz, 6.59 Hz) respectively were attributed to H-10$_{eq}$ and H-10$_{ax}$ respectively. These two protons, each split the resonance of the other. The resonance at δ_H 3.91 was further split by H-9, which appeared as a broadened doublet at δ_H 2.50. Coupling between the H-10$_{ax}$ resonance and the H-9 resonance could be seen in the COSY spectrum. The broad double doublets at δ_H 1.86 (J = 12.75 Hz, 2.44 Hz) and δ_H 1.93 (J = 12.75 Hz, 1.71 Hz), each integrating to one proton were attributed to H-8$_{ax}$ and H-8$_{eq}$ respectively. These resonances also showed coupling, in the COSY spectrum, to the H-9 resonance at δ_H 2.50 and the resonance at δ_H 3.12, a broadened doublet integrating to one proton which was attributed to H-7. This H-7 resonance was coupled to the double doublet resonances at δ_H 2.90 (J = 12.46 Hz, 3.18 Hz) and δ_H 2.33 (J = 12.46 Hz, 2.19 Hz), integrating to one proton each, assigned to H-11$_{eq}$ and H-11$_{ax}$ respectively and the double doublet resonances at δ_H 1.86 (J = 12.75 Hz, 2.44 Hz) and δ_H 1.93 (J = 12.75 Hz, 1.71 Hz), assigned to H-8$_{ax}$ and H-8$_{eq}$ respectively. The COSY spectrum also showed strong coupling between the resonances at δ_H 2.90 and δ_H 2.33, which is coupling between the H-11$_{eq}$ and H-11$_{ax}$ protons. Coupling could also be seen between the doublet resonances (COSY) at δ_H 2.96 and δ_H 2.30, J = 12.64 Hz, assigned to H-13$_{eq}$ and H-13$_{ax}$ respectively. At δ_H 2.17, a singlet integrating to three protons appeared and was attributed to the N-methyl protons at C-14. The

assignment of the protons at C-8, C-10, C-11 and C-13 were based on values from literature [3].

The ^{13}C NMR spectrum showed the presence of twelve carbon resonances. Three methine signals at δ_C 116.7, δ_C 141.3 and δ_C 107.8 were attributed to C-3, C-4 and C-5 respectively, while two fully substituted carbon resonances at δ_C 165.7 and δ_C 153.6 were assigned to C-2 and C-6 respectively. Two methylene resonances at δ_C 63.2 and δ_C 63.6 and one methyl resonance at δ_C 46.5 were assigned to C-11, C-13 and C-14 respectively. These resonances were deshielded by the presence of the nitrogen atom at position 12. Two methylene signals, which appeared at δ_C 26.0 and δ_C 51.5 were attributed to C-8 and C-10 respectively, the latter being deshielded by the amide group at positions 1 and 2. The remaining two methylene resonances at δ_C 36.6 and δ_C 29.3 were assigned to C-7 and C-9 respectively.

The ^1H and ^{13}C NMR resonances were assigned with the aid of NMR data of 12-cytisineacetamide (**5**) and cytisine (**1**) (CD_3OD as solvent) from literature (**Table 19 and 20**) [3] as NMR data for N-methylcytisine is not available in the literature.

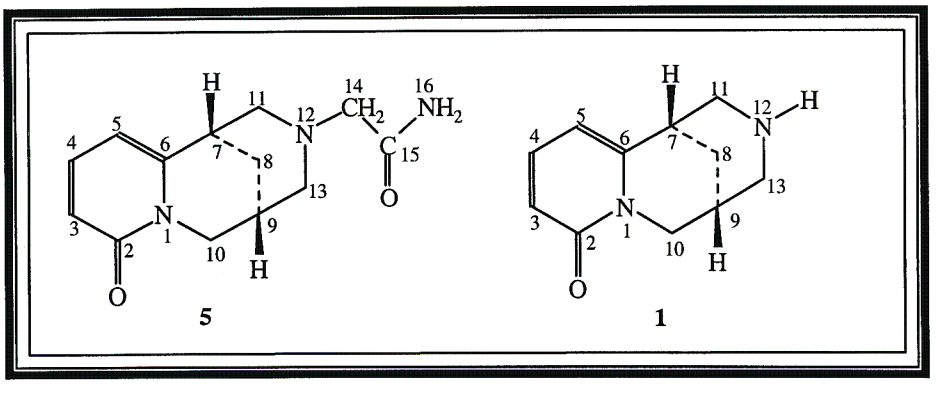


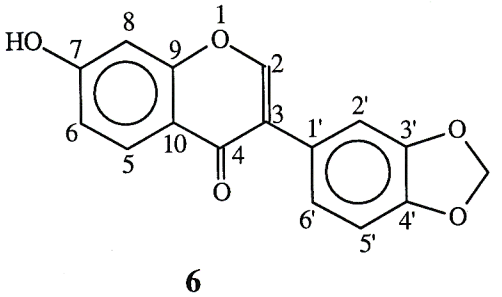
Table 19. ^{13}C **NMR data of cytisine (1), N-methylcytisine (4) and 12-cytisineacetamide (5) (75 MHz, CD_3OD)**

	N-methylcytisine (4)	12-cytisineacetamide (5) [3]	cytisine (1) [3]
C-2	165.7 (s)	165.5 (s)	166.6 (s)
C-3	116.7 (d)	116.8 (d)	117.8 (d)
C-4	141.3 (d)	141.4 (d)	142.1 (d)
C-5	107.8 (d)	107.9 (d)	108.9 (d)
C-6	153.6 (s)	153.2 (s)	153.4 (s)
C-7	36.6 (d)	36.7 (d)	36.9 (d)
C-8	26.0 (t)	26.0 (t)	27.3 (t)
C-9	29.3 (d)	29.5 (d)	29.5 (d)
C-10	51.5 (t)	51.5 (t)	51.8 (t)
C-11	63.2 (t)	61.2 (t)	53.6 (t)
C-13	63.6 (t)	61.8 (t)	54.6 (t)
C-14	46.5 (q)	61.7 (t)	-
C-15	-	175.5 (s)	-

Table 20. ^{1}H **NMR data of N-methylcytisine (4) and 12-cytisineacetamide (5) (300 MHz, CD_3OD)**

	N-methylcytisine (4)	12-cytisineacetamide (5) [3]
H-3	**6.44** (1H, d, J = 8.79 Hz)	**6.43** (1H, dd) J = 8.8 Hz, 1.4 Hz
H-4	**7.50** (1H, dd) J = 8.79 Hz, 6.83 Hz	**7.48** (1H, dd) J = 8.8 Hz, 6.9 Hz
H-5	**6.31** (1H, d, J = 6.83 Hz)	**6.32** (1H, dd) J = 6.9 Hz, 1.1 Hz
H-7	**3.12** (1H, bd, J = 2.50 Hz)	**3.14** (1H, d, J = 2.2 Hz)
$H\text{-}8_{ax}$	**1.86** (1H, dd) J = 12.75 Hz, 2.44 Hz	**1.87** (1H, d, J = 12.9 Hz)
$H\text{-}8_{eq}$	**1.93** (1H, dd) J = 12.75 Hz, 1.71 Hz	**1.96** (1H, dt) J = 12.9 Hz, 1.5 Hz
H-9	**2.50** (1H, bd, J = 2.44 Hz)	**2.50** (1H, d, J = 2.5 Hz)
$H\text{-}10_{ax}$	**3.91** (1H, dd) J = 15.45 Hz, 6.59 Hz	**3.91** (1H, dd) J = 15.5 Hz, 6.0 Hz
$H\text{-}10_{eq}$	**4.03** (1H, d, J = 15.45 Hz)	**4.12** (1H, d, J = 15.5 Hz)

H-11$_{ax}$	**2.33** (1H, dd) J = 12.46 Hz, 2.19 Hz	**2.60** (1H, d, J = 12.0 Hz)
H-11$_{eq}$	**2.90** (1H, dd) J = 12.46 Hz, 3.18 Hz	**3.00** (1H, d, J = 12.0 Hz)
H-13$_{ax}$	**2.30** (1H, d, J = 12.64 Hz)	**2.57** (1H, dd) J = 11.1 Hz, 2.1 Hz
H-13$_{eq}$	**2.96** (1H, d, J = 12.64 Hz)	**2.90** (1H, dm, J = 11.1 Hz)
H-14	**2.17** (3H, s)	**2.87** (1H, d, J = 16.2 Hz) **2.97** (1H, d, J = 16.2 Hz)



6

Compound (**6**), 7-hydroxy-3',4'-methylenedioxyisoflavone (pseudobaptigenin), $C_{16}H_{10}O_5$, was isolated as white crystalline material. Pseudobaptigenin has been previously reported from a variety of species in the Fabaceae [13-20], but this is the first report of pseudobaptigenin from *Sophora*. Spectra for pseudobaptigenin are given on pages 257-263 in appendix A.

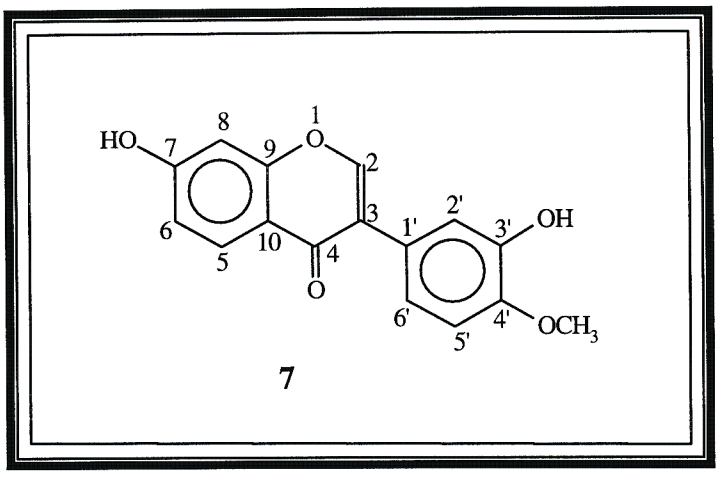
The i.r. spectrum of pseudobaptigenin (**6**) showed the presence of bands at 3312 cm^{-1} (O – H stretching) and 1038 cm^{-1} (C – O stretching), indicative of the hydroxy group, 1747 cm^{-1} (C = O stretching), indicating the presence of a carbonyl group and 940 cm^{-1} (C – O – C stretching) indicative of a methylenedioxy group.

The ^1H NMR spectrum of pseudobaptigenin (**6**) displayed a singlet, integrating to one proton, at δ_H 8.02 which was assigned to H-2. This H-2 resonance is characteristic of compounds having an isoflavone skeleton. This resonance was also coupled to the

methine C-2 resonance at δ_C 152.8 (HETCOR). The doublet at δ_H 7.33 integrating to one proton with $J_{6,8}$ = 1.65 Hz was attributed to H-8 and this proton was coupled to the methine C-8 resonance at δ_C 110.2. The H-5 resonance appears at δ_H 8.29 (1H) as a doublet with $J_{5,6}$ = 8.73 Hz, and shows strong coupling (COSY) to the H-6 resonance which appears in the multiplet at δ_H 7.08. Coupling can also be seen between the H-6 and H-8 resonances in the COSY spectrum. The H-5 and H-6 resonances are coupled in the HETCOR spectrum to the methine resonances at δ_C 128.0 and δ_C 115.8 attributed to C-5 and C-6 respectively. The H-2' resonance appears at δ_H 6.97 as a one proton doublet ($J_{2',6'}$ = 2.26 Hz). This resonance shows coupling to the C-2' methine resonance at δ_C 102.9. The H-5' resonance at δ_H 6.82, a one proton doublet with $J_{5',6'}$ = 8.05 Hz, shows strong coupling in the COSY spectrum to the H-6' resonance which appears in the multiplet at δ_H 7.08. Coupling can also be seen between the H-2' and H-6' resonances in the COSY spectrum. In the HETCOR spectrum, the H-5' proton resonance is coupled to the C-5' methine resonance at δ_c 108.4, and the H-6' proton resonance to the C-6' methine resonance at δ_C 122.7. The methylenedioxy protons appear as a two proton singlet at δ_H 5.82 and showed coupling (HETCOR) to the methylene carbon resonance at δ_C 101.5, attributed to the methylenedioxy carbon atom. The ketonic C-4 resonance appeared as a singlet at δ_C 175.3 in the ^{13}C NMR spectrum.

^1H NMR data of pseudobaptigenin (**6**) are reported in CF_3COOD [8] and CD_3COCD_3 [9] in the literature and due to solvent effects were not comparable to the ^1H NMR of pseudobaptigenin obtained in this work which was run in C_5D_5N.

The melting point of 295-297^{0}C confirmed that compound (**7**) was pseudobaptigenin (m.p. 296^{0}C, lit. [8]).



7

Compound (**7**), 3',7-dihydroxy-4'-methoxyisoflavone (calycosin), $C_{16}H_{12}O_5$, was isolated as a white crystalline material. Calycosin has been previously reported from a number of species in the Leguminosae [13-16,21-24] including *Sophora secundiflora* [23] and *Sophora moocroftiana* [24]. This is the first report of calycosin in *Sophora velutina.* Spectra for calycosin are given on pages 264-270 in appendix A.

The i.r. spectrum of calycosin (**7**) showed bands at 3375 cm^{-1} (O – H stretching) and 1024 cm^{-1} (C – O stretching), indicative of the hydroxy groups and 1620 cm^{-1} (C = O stretching) indicating a carbonyl group.

The ^1H NMR spectrum of calycosin (**7**) showed a singlet at δ_H 8.20 integrating to one proton which was attributed to H-2. This lowfield shift of H-2 is characteristic of isoflavones. This resonance showed coupling in the HETCOR spectrum to the methine C-2 resonance at δ_C 154.9. A doublet at δ_H 7.19 integrating to one proton, with $J_{6,8}$ = 1.89 Hz, was attributed to H-8 which also showed coupling to the methine C-8 resonance at δ_C 114.1 in the HETCOR spectrum. The H-5 resonance at δ_H 8.10, a doublet (J = 8.86 Hz) which integrated to one proton showed coupling to the H-6 resonance (COSY) which appeared in the multiplet at δ_H 7.00. Coupling could also be seen between the H-6 and H-8 resonances in the COSY spectrum. The H-5 and H-6 resonances also showed coupling (HETCOR) to the C-5 and C-6 methine resonances at δ_C 128.5 and δ_C 116.5 respectively. The H-2' and H-5' resonances both appeared in the multiplet at δ_H 6.88 and the H-6' resonance appeared in the multiplet at δ_H 7.00. The H-6' resonance showed strong coupling (COSY) to the H-5' and H-2'

resonances contained in the multiplet at δ_H 6.88, *ortho* and *meta* coupling respectively. The H-2', H-5' and H-6' proton resonances all showed coupling (HETCOR) to the C-2', C-5' and C-6' methine resonances at δ_C 116.2, δ_C 103.2 and δ_C 122.9 respectively. The methoxy group proton resonance was evident at δ_H 3.92, a singlet integrating to three protons. This resonance was coupled to the methyl carbon resonance at δ_C 56.5 in the HETCOR spectrum. The ketonic C-4 resonance was evident at δ_C 178.1 in the ^{13}C NMR spectrum.

^1H NMR data of calycosin (**7**) in the literature is reported in DMSO-d_6 [10] and CF_3COOD [11] and due to solvent effects could not be compared directly with the ^1H NMR data of calycosin in this work, which was run in CD_3OD.

The melting point of 242-243^{0}C confirmed that compound (**7**) was calycosin (m.p. 240-242^{0}C, lit. [11]).

^{13}C NMR data of pseudobaptigenin (**6**) and calycosin (**7**) were not available and that of 7,3',4'-trimethoxyisoflavone (cabreuvin) (**8**)[12] was used for comparison.

Table 21. ^1H NMR data of pseudobaptigenin (6) and calycosin (7) (300 MHz)

	pseudobaptigenin (6) in C_5D_5N	**calycosin (7) in CD_3OD**
H-2	8.02 (1H, s)	8.20 (1H, s)
H-5	8.29 (1H, d) J = 8.73 Hz)	8.10 (1H, d) J = 8.86 Hz
H-6	7.08 (1H, m)	7.00 (1H, m)
H-8	7.33 (1H, d) J = 1.65 Hz	7.19 (1H, d) J = 1.89 Hz
H-2'	6.97 (1H, d) J = 2.26 Hz	6.88 (1H, m)
H-5'	6.82 (1H, d) J = 8.05 Hz	6.88 (1H, m)
H-6'	7.08 (1H, m)	7.00 (1H, m)
O$C\underline{H_2}$O	5.82 (2H, s)	-
O$C\underline{H_3}$	-	3.92 (3H, s)

Table 22. ^{13}C NMR data of pseudobaptigenin (6), calycosin (7) and cabreuvin (8) (75 MHz)

	pseudobaptigenin (6) in C_5D_5N	calycosin (7) in CD_3OD	cabreuvin (8) in $CDCl_3$[12]
C-2	152.8 (d)	154.9 (d)	151.8
C-3	126.7 (s)	126.0 (s)	124.3
C-4	175.3 (s)	178.1 (s)	175.3
C-5	128.0 (d)	128.5 (d)	127.2
C-6	115.8 (d)	116.5 (d)	114.2
C-7	164.0 (s)	164.6 (s)	163.5
C-8	110.2 (d)	114.1 (d)	99.8
C-9	158.3 (s)	159.8 (s)	157.4
C-10	117.6 (s)	118.2 (s)	118.0
C-1'	124.4 (s)	124.8 (s)	124.4
C-2'	102.9 (d)	116.2 (d)	112.3
C-3'	147.8 (s)	147.8 (s)	148.4
C-4'	147.7 (s)	148.7 (s)	148.7
C-5	108.4 (d)	103.2 (d)	110.9
C-6'	122.7 (d)	122.9 (d)	120.6
$O\underline{C}H_2O$	101.5 (t)	-	-
4'-$O\underline{C}H_3$	-	56.5 (q)	55.7
3'-$O\underline{C}H_3$	-	-	55.7
7-$O\underline{C}H_3$	-	-	55.5

2.3.3 Experimental

2.3.3.1 Extractives from the seeds of *Sophora velutina*

Sophora velutina subsp. *zimbabweensis* Gillet and Brummit, was obtained from a cultivated specimen in Pretoria, and identified by Dr. N. Crouch. A voucher specimen (*Crouch 780*) was deposited at the Natal Herbarium. The seeds (400.82 g) were first separated from the pods and then extracted with methanol on a Labcon shaker for 96 hours yielding, after evaporation of solvent, 35.67 g of extract. This was dissolved in water (100 ml) and acidified to pH 4. The acidic extract was then extracted with chloroform (3 X 200 ml) to yield the acidic chloroform extract (5.06 g). The aqueous solution was then made basic to pH 10 and extracted further with chloroform (3 x 200 ml) to yield the basic chloroform extract (2.78 g).

^{1}H NMR spectroscopy of the crude basic chloroform extract indicated the presence of a lupine alkaloid and this extract was purified further. Chromatographic separation of the basic chloroform extract using a methylene chloride : methanol step gradient (19:1, 18:2, 16:4, 12:8) as the eluant and collecting 30 X 40 ml fractions for each step, afforded N-methylcytisine (**4**) which was purified on silica gel using methylene chloride : methanol (19:1) as the eluant.

^{1}H NMR spectroscopy of the acidic chloroform extract indicated the presence of a sterol as well as isoflavanoids and this extract was purified further. Chromatographic separation of the acidic chloroform extract using a methylene chloride : methanol step gradient (19:1, 18:2, 16:4, 12:8) as the eluant and collecting 30 X 40 ml fractions for each step, gave sitosterol (**9**) which was present in fraction 11, 7-hydroxy-3',4'-methylenedioxyisoflavone (pseudobaptigenin) (**6**) which was contained in fractions 24-26, and 3',7-dihydroxy-4'-methoxyisoflavone (calycosin) (**7**) which was present in fractions 27-33. Pseudobaptigenin (**6**) and calycosin (**7**) were purified on silica gel using methylene chloride : ethyl acetate (15:5) as the eluant. The physical properties and ^{1}H NMR data of sitosterol (**9**) were matched with those of an authentic sample.

Physical Data for compound 4

N-methylcytisine [SOPHA13]

Yield: 30 mg

Melting point: 136-137^{0}C (lit.[3] 137^{0}C)

Mass: HRMS: [M^+] at *m/z* 204.1265, $C_{12}H_{16}N_2O$ requires 204.1263

EIMS: m/z (rel. int.): 204 (25.51) [M^+], 160 (5.18) [M^+ - C_2H_5N - H], 146 (6.81) [M^+ - C_3H_7N - H], 58 (100.00) $[C_3H_8N]^+$, 42 (6.64) $[C_2H_4N]^+$

Optical Rotation: $[\alpha]_D^{22}$ –227^0 (c = 0.6, MeOH)

Infra red: ν_{max} **(cm^{-1}):** 2947 (-CH_2 antisymmetric stretching), 2786 (-CH_2 symmetric stretching), 1660 (C = O stretching), 1550 (N – C = O stretching)

^{1}H NMR: δ_H (ppm) (CD_3OD)

1.86 (1H, dd, H-8_{ax}, *J* = 12.75 Hz, 2.44 Hz), 1.93 (1H, dd, H-8_{eq}, *J* = 12.75 Hz, 1.71 Hz), 2.17 (3H, s, H-14), 2.30 (1H, d, H-13_{ax}, *J* = 12.64 Hz), 2.33 (1H, dd, H-11_{ax}, *J* = 12.46 Hz, 2.19 Hz), 2.50 (1H, bd, H-9, *J* = 2.44 Hz), 2.90 (1H, dd, H-11_{eq}, *J* = 12.46 Hz, 3.18 Hz), 2.96 (1H, d, H-13_{eq}, *J* = 12.46 Hz), 3.12 (1H, bd, H-7), 3.91 (1H, dd, H-10_{ax}, *J* = 15.45 Hz, 6.59 Hz), 4.03 (1H, d, H-10_{eq}, *J* = 15.45 Hz), 6.31 (1H, d, H-5, *J* = 6.83 Hz), 6.44 (1H, d, H-3, *J* = 8.79 Hz), 7.50 (1H, dd, H-4, *J* = 8.79 Hz, 6.83 Hz)

^{13}C NMR: δ_C (ppm) (CD_3OD)

26.0 (t, C-8), 29.3 (d, C-9), 36.6 (d, C-7), 46.5 (q, C-14), 51.5 (t, C-10), 63.2 (t, C-11), 63.6 (t, C-13), 107.8 (d, C-5), 116.7 (d, C-3), 141.3 (d, C-4), 153.6 (s, C-6), 165.7 (s, C-2)

Physical Data for compound 6

7-hydroxy-3',4'-methylenedioxyisoflavone (Pseudobaptigenin) [SOPHC24-26]

Yield: 20 mg

Melting point: 295-297^{0}C (lit. [8], m.p. 296^{0}C)

Mass: HRMS: [M^+] at *m/z* 282.0528 corresponding to $C_{16}H_{10}O_5$

Infra red: ν_{max} **(cm^{-1}):** 3312 (O – H stretching), 1747 (C = O stretching), 1600 (aromatic C = C stretching), 1445 (aromatic C = C stretching), 1038 (C – O stretching), 940 (C – O – C stretching)

^{1}H NMR: δ_H (ppm) (C_5D_5N)

5.82 (2H, s, OC<u>H</u>$_2$O), 6.82 (1H, d, H-5', J = 8.05 Hz), 6.97 (1H, d, H-2', J = 2.26 Hz), 7.08 (2H, m, H-6, H-6'), 7.33 (1H, d, H-8, J = 1.65 Hz), 8.02 (1H, s, H-2), 8.29 (1H, d, H-5, J = 8.73 Hz)

^{13}C NMR: δ_C (ppm) (C_5D_5N)

101.5 (t, O<u>C</u>H$_2$O), 102.9 (d, C-2'), 108.4 (d, C-5'), 110.2 (d, C-8), 115.8 (d, C-6), 117.6 (s, C-10), 122.7 (d, C-6'), 124.4 (s, C-1'), 126.7 (s, C-3), 128.0 (d, C-5), 147.7 (s, C-4'), 147.8 (s, C-3'), 152.8 (d, C-2), 158.3 (s, C-9), 164.0 (s, C-7), 175.3 (s, C-4)

Physical Data for compound 7

7,3'-dihydroxy-4'-methoxyisoflavone (Calycosin) [SOPHC27-33]

Yield: 10 mg

Melting point: 242-243 ^{0}C (lit.[11], 240-242 ^{0}C)

Mass: HRMS: [M^{+}] at *m/z* 284.0684 corresponding to $C_{16}H_{12}O_5$

Infra red: ν_{max} **(cm^{-1}):** 3375 (O – H stretching), 1620 (C = O stretching), 1580, 1459 (aromatic C = C stretching), 1024 (C – O stretching)

^{1}H NMR: δ_H (ppm) (CD_3OD)

3.92 (3H, s, $OC\underline{H}_3$), 6.88 (2H, m, H-2', H-5'), 7.00 (2H, m, H-6, H-6'), 7.19 (1H, d, H-8, *J* = 1.89 Hz), 8.10 (1H, d, H-5, *J* = 8.86 Hz), 8.20 (1H, s, H-2)

^{13}C NMR: δ_C (ppm) (CD_3OD)

56.5 (q, $O\underline{C}H_3$), 103.2 (d, C-5'), 114.1 (d, C-8), 116.2 (d, C-2'), 116.5 (d, C-6), 118.2 (s, C-10), 122.9 (d, C-6'), 124.8 (s, C-1'), 126.0 (s, C-3), 128.5 (d, C-5), 147.8 (s, C-3'), 148.7 (s, C-4'), 154.9 (d, C-2), 159.8 (s, C-9), 164.6 (s, C-7), 178.1 (s, C-4)

2.3.4 References

1. Brummit, R. K. and Gillet, J. B. (1966) **Notes on the genus *Sophora* in Africa, including an Asian species found near Zimbabwe**, *Kirkia*, **5**, 259-264.

2. Asres, K., Gibbons, W. A., Philipson, J. D. and Mascagni, P. (1986) **The alkaloids of *Sophora velutina***, *Journal of Natural Products*, **49**, 117-121.

3. Takamatsu, S., Siato, K., Ohmiya, S., Ruangrungsi, N. and Murakoshi, I. (1991) **Lupin alkaloids from *Sophora exigua***, *Phytochemistry*, **30**, 3793-3795.

4. Ohmiya, S., Otomasu, H., Murakoshi, I., and Haginiwa, J. (1974) **N-acetylcytisine from *Sophora tomentosa***, *Phytochemistry*, **13**, 1016.

5. Ohmiya, S., Otomasu, H., Murakoshi, I. and Haginiwa, J. (1974) **N-formylcytisine: A new alkaloid from *Thermopsis chinensis***, *Phytochemistry*, **13**, 643-644.

6. Veen, G., Gunther, S., Greinwald, R., Bachmann, P., Witte, L., Kustrak, D. and Czygan, F. C. (1992) **Quinolizidine alkaloids in *Petteria ramentacea***, *Phytochemistry*, **31**, 3487-3490.

7. Fales, H. M., Lloyd, H. A. and Milne, G. W. A. (1970) **Chemical ionization mass spectrometry of complex molecules. II. Alkaloids**, *Journal of the American Chemical Society*, **92**, 1590-1597.

8. Bevan, C. W. L., Ekong, D. E. U., Obasi, M. E. and Powell, J. W. (1966) **West African timbers. Part XIII. Extracts from the heartwood of *Amphimas pterocarpoides* and *Pterocarpus erinaceous***, *Journal of the Chemical Society (C)*, 509-510.

9. Schuda, P. F. and Price, W. A. (1987) **Total synthesis of isoflavones: Jamaicin, calopogonium isoflavone-B, pseudobaptigenin and maxima substance-B. Friedel Crafts acylation reactions with acid-sensitive substrates**, *Journal of Organic Chemistry*, **52**, 1972-1979.

10. Takai, M., Yamaguchi, H., Saitoh, T. and Shibata, S. (1972) **Chemical studies on the oriental plant drugs. XXXV. The chemical constituents of the heartwood of *Maackia amurensis* var. *buergeri***, *Chemical and Pharmaceutical Bulletin*, **20**, 2488-2490.

11. Kurosawa, K., Ollis, W. D., Sutherland, I. O., Gottlieb, O. R. and de Oliviera, A. B. (1978) **Mucronulatol, mucroquinone and mucronucarpan, isoflavonoids from *Machaerium mucronulatum* and *M. villosum***, *Phytochemistry*, **17**, 1405-1411.

12. Wenkert, E. and Gottlieb, H. E. (1977) **Carbon-13 nuclear magnetic resonance spectroscopy of flavonoid and isoflavonoid compounds**, *Phytochemistry*, **16**, 1811-1816.

13. Dewick, P. M. (1982) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research,** Eds. Harborne, J. B. and Mabry, T. J., Chapman and Hall Ltd., New York, pp 542-543.

14. Dewick, P. M. (1988) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research since 1980,** Ed. Harborne, J. B., Chapman and Hall Ltd., New York, pp 128-129.

15. Dewick, P. M. (1994) **Isoflavonoids,** *in* **The Flavonoids. Advances in Research since 1986,** Ed. Harborne, J. B., Chapman and Hall Ltd., New York, pp 123.

16. Biggs, D. R. and Lane, G. A (1978) **Identification of isoflavones calycosin and pseudobaptigenin in *Trifolium pratense*,** *Phytochemistry*, **17**, 1683-1684.

17. Cook, J. T., Ollis, W. D., Sutherland, I. O. and Gottlieb, O. R. (1978) **Pterocarpans from *Dalbergia spruceana*,** *Phytochemistry*, **17**, 1419-1422.

18. Ohashi, H., Goto, M. and Imamura, H. (1976) **Flavonoids from the wood of *Cladrastis platycarpa*,** *Phytochemistry*, **15**, 354-355.

19. Parthasarathy, M. R., Seshadri, T. R. and Varma, R. S. (1976) **Triterpenoids and flavonoids of *Dalbergia sericea* bark,** *Phytochemistry*, **15**, 226-227.

20. Carlson, R. E. and Dolphin, D. H. (1981) **Chromatographic analysis of isoflavonoid accumulation in stressed *Pisum sativum*,** *Phytochemistry*, **20**, 2281-2284.

21. Kaneko, M., Nakata, H., Takada, F., Matsumura, M., Kitagawa, C., Sakashita, S., Nuno, M. and Saitoh, T. (1988) **Isoflavones from the gall and wood of *Wisteria brachybotrys*,** *Phytochemistry*, **27**, 267-269.

22. Malan, E. and Swinny, E. (1990) **Flavonoids and isoflavonoids from the heartwood of *Virgilia oroboides*,** *Phytochemistry*, **29**, 3307-3309.

23. Minhaj, N., Khan, H. and Zaman, A. (1976) **Unisoflavan, a new isoflavan from *Sophora secondiflora* DC.,** *Tetrahedron Letters*, **17**, 2391-2394.

24. Shiratakai, Y., Yokoe, I., Noguchi, M., Tomimori, T. and Komatsu, M. (1988) **Studies on the constituents of *Sophora* species. XXII. Constituents of the root of *Sophora moorcroftiana* Benth. *ex* Baker.,** *Chemical and Pharmaceutical Bulletin*, **36**, 2220-2225.

25. Murakoshi, I., Kidoguchi, E., Haginiwa, J., Ohmiya, S., Higashiyama, K. and Otomasu, H. (1981) **(+) – Kuraramine, a possible metabolite of (-) – N – methylcytisine in flowers of *Sophora flavescens*,** *Phytochemistry*, **20**, 1407-1409.

26. Keller, W. J. and Hatfield, M. (1979) **11-Allylcytisine and other minor alkaloids from unripe *Sophora secundiflora* fruits,** *Phytochemistry*, **18**, 2068-2069.

27. Murakoshi, I., Fukuchi, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) **N-(3-oxobutyl)cytisine: A new lupin alkaloid from *Echinosophora koreensis,*** *Phytochemistry*, **16**, 1460-1461.

28. Greinwald, R., Bachmann, P., White, L. and Czygan, F. C. (1990) **Cytisine-12-carboxy-ethylester, a quinolizidine alkaloid from *Laburnum watereri* and its occurrence in the Leguminosae**, *Phytochemistry*, **29**, 3553-3554.

29. Atta-ur-Rahman, Pervin , A. Choudhary, M. I. (1991) **Sophazrine – A novel quinolizidine alkaloid from *Sophora griffithii***, *Journal of Natural Products*, **54**, 929-935.

30. Murakoshi, I., Sugimoto, K. and Haginiwa, J. (1975) **(-)-Trans-4'-hydroxycinnamoyl lupinine, a new alkaloid in *Lupinus* seedlings**, *Phytochemistry*, **14**, 2714-2715.

Appendix A

List of Spectra

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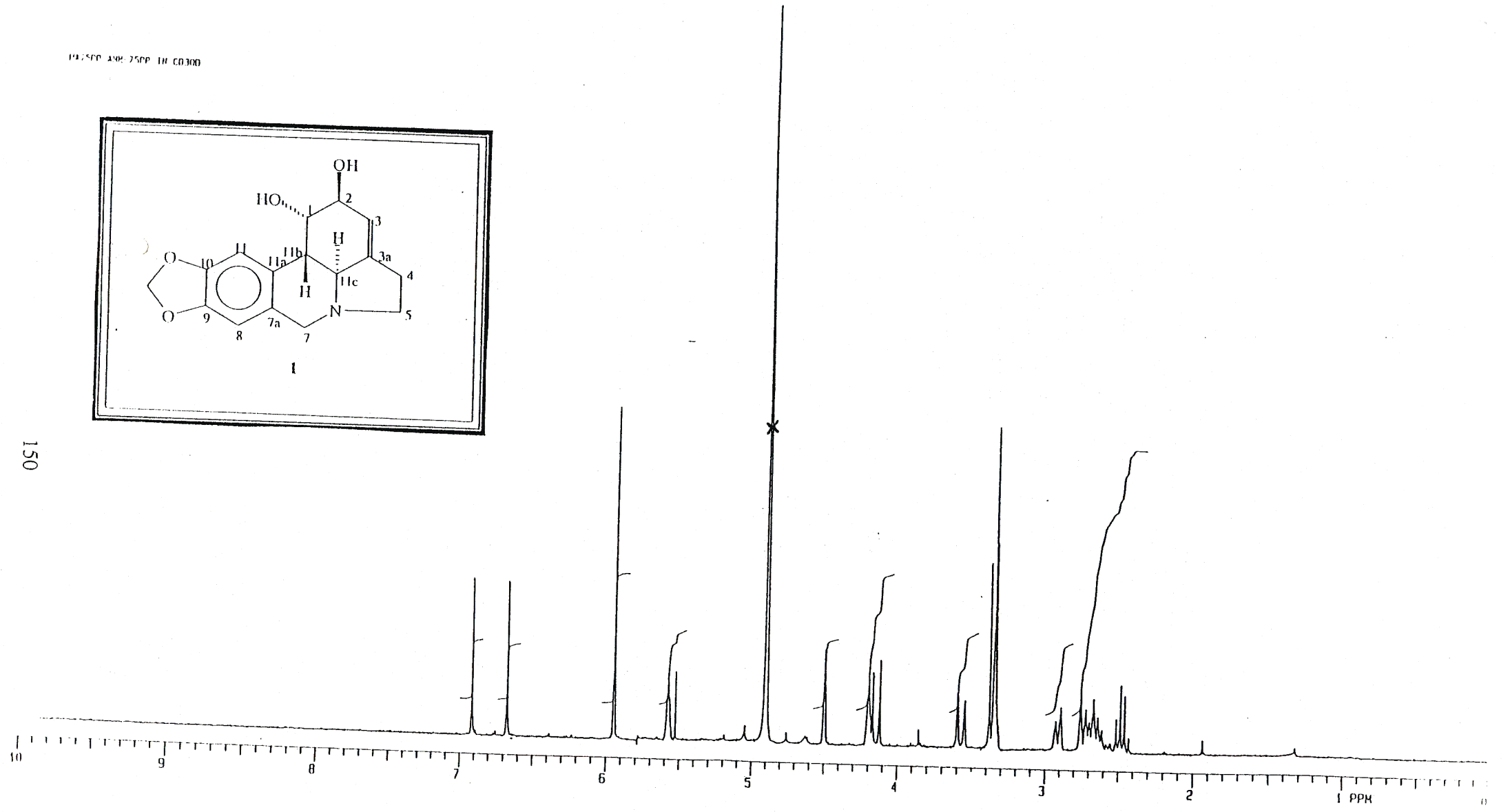
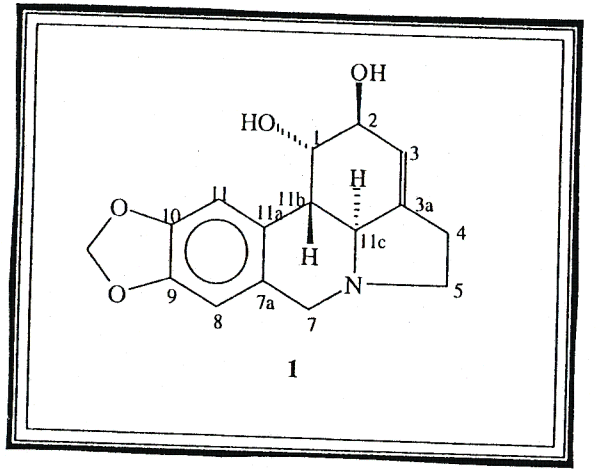


Spectrum [1-A] : ^{1}H NMR spectrum of lycorine (1) in CD_3OD



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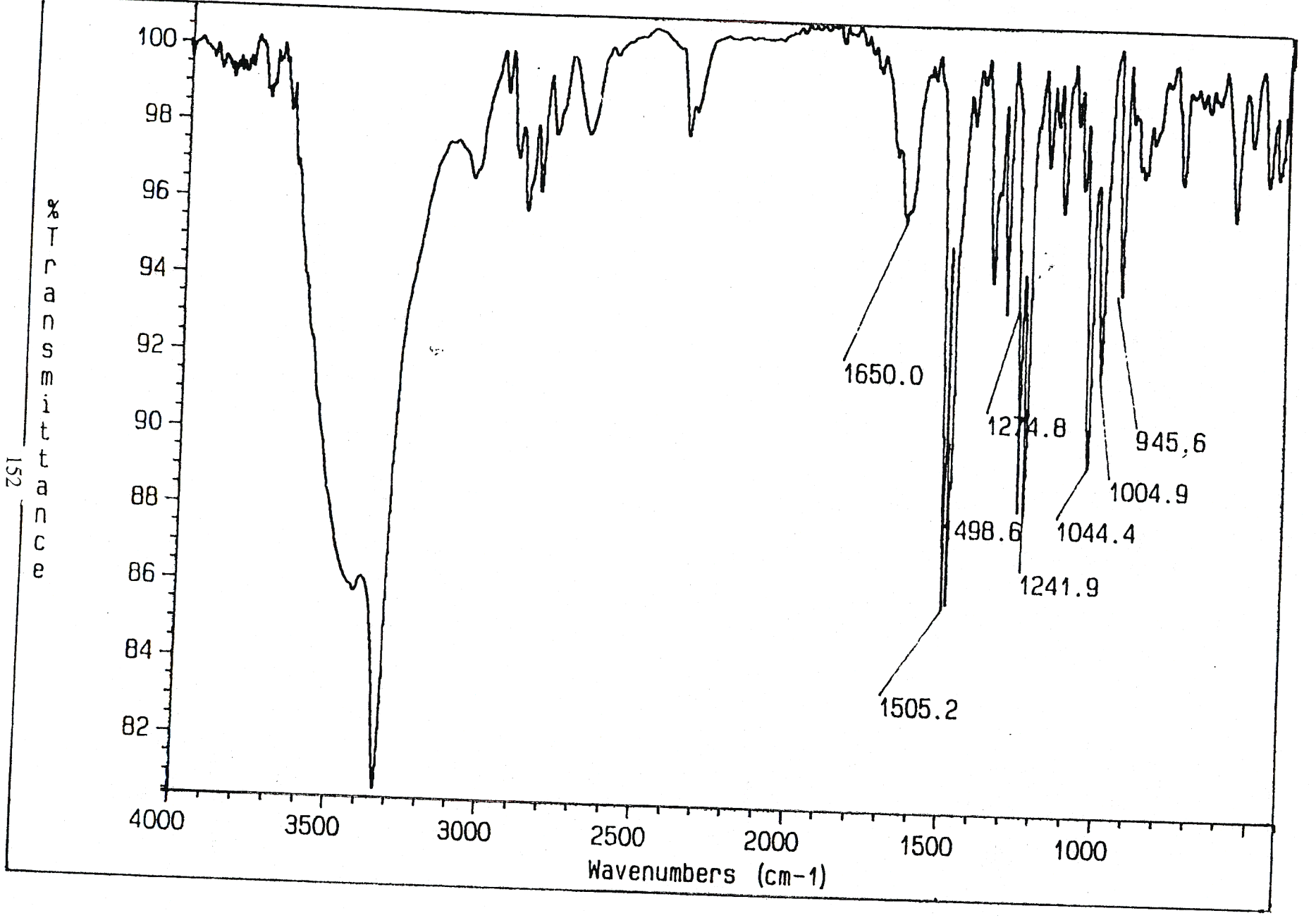
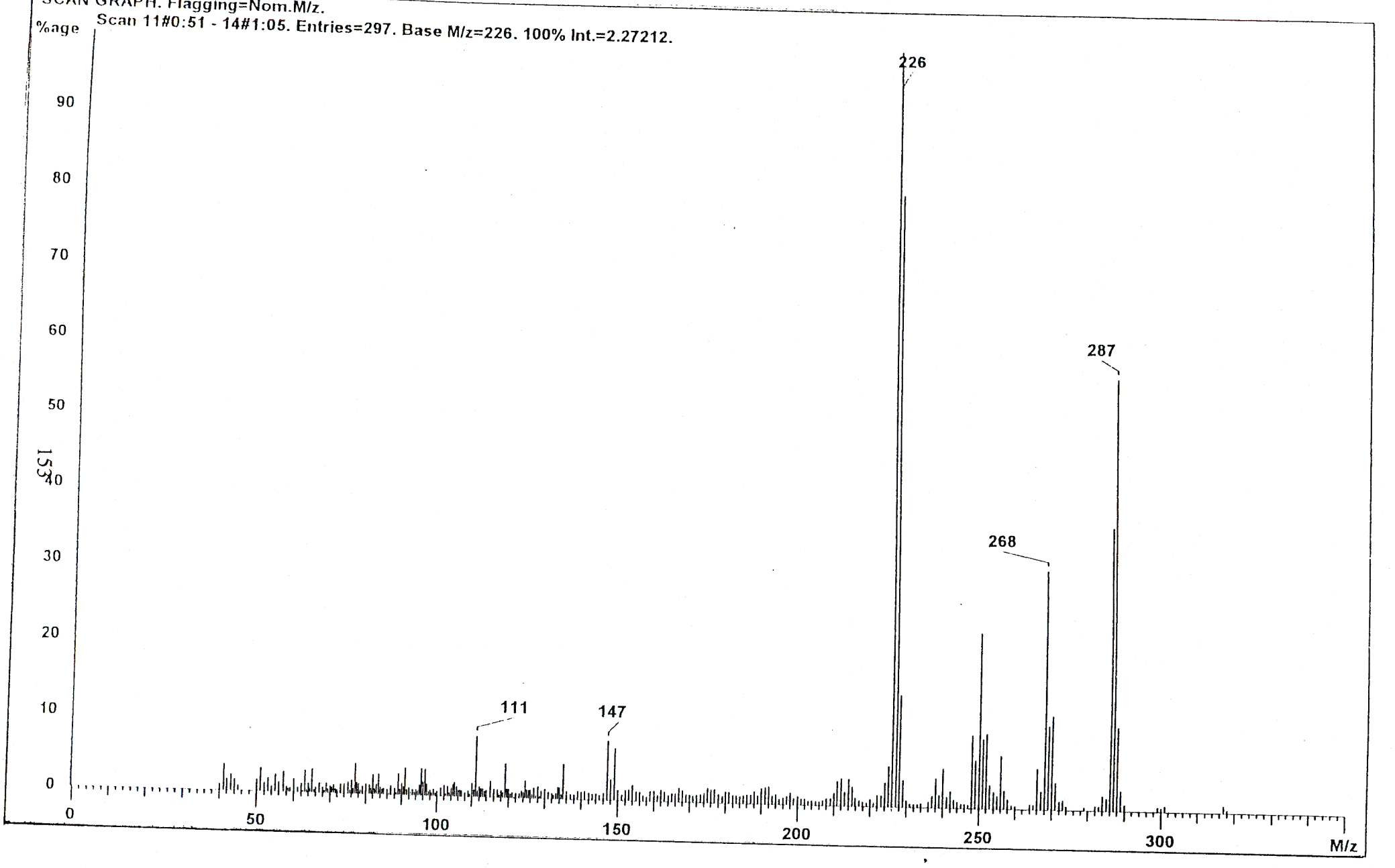
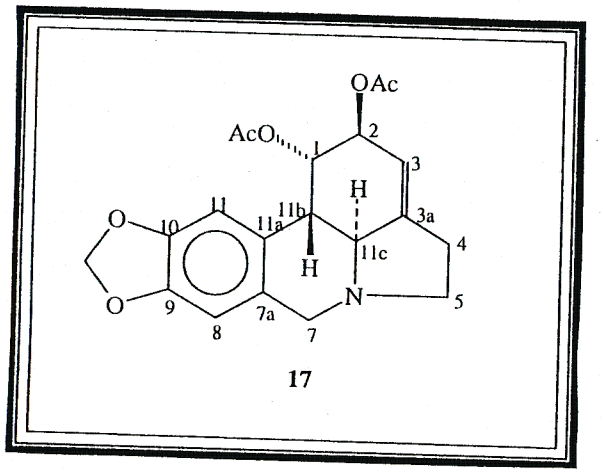
Spectrum [1-B] : ^{13}C NMR spectrum of lycorine (1) in CD_3OD



Spectrum [1-C] : Infra red spectrum of lycorine (1)



Spectrum [1-D] : Mass spectrum of lycorine (1)



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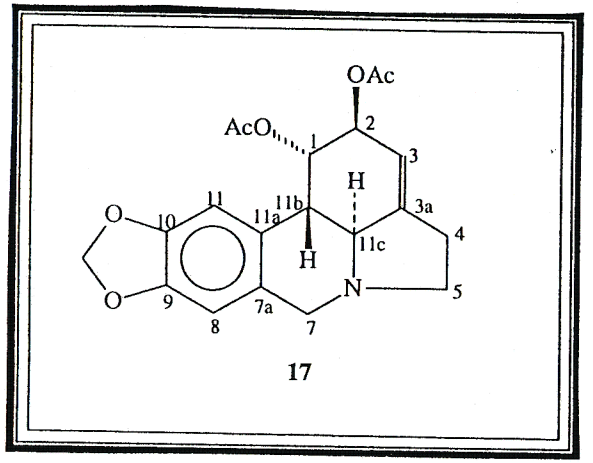
Spectrum [2-A] : ^{1}H NMR spectrum of 1,2-di-O-acetyllycorine (17) in CD_3OD



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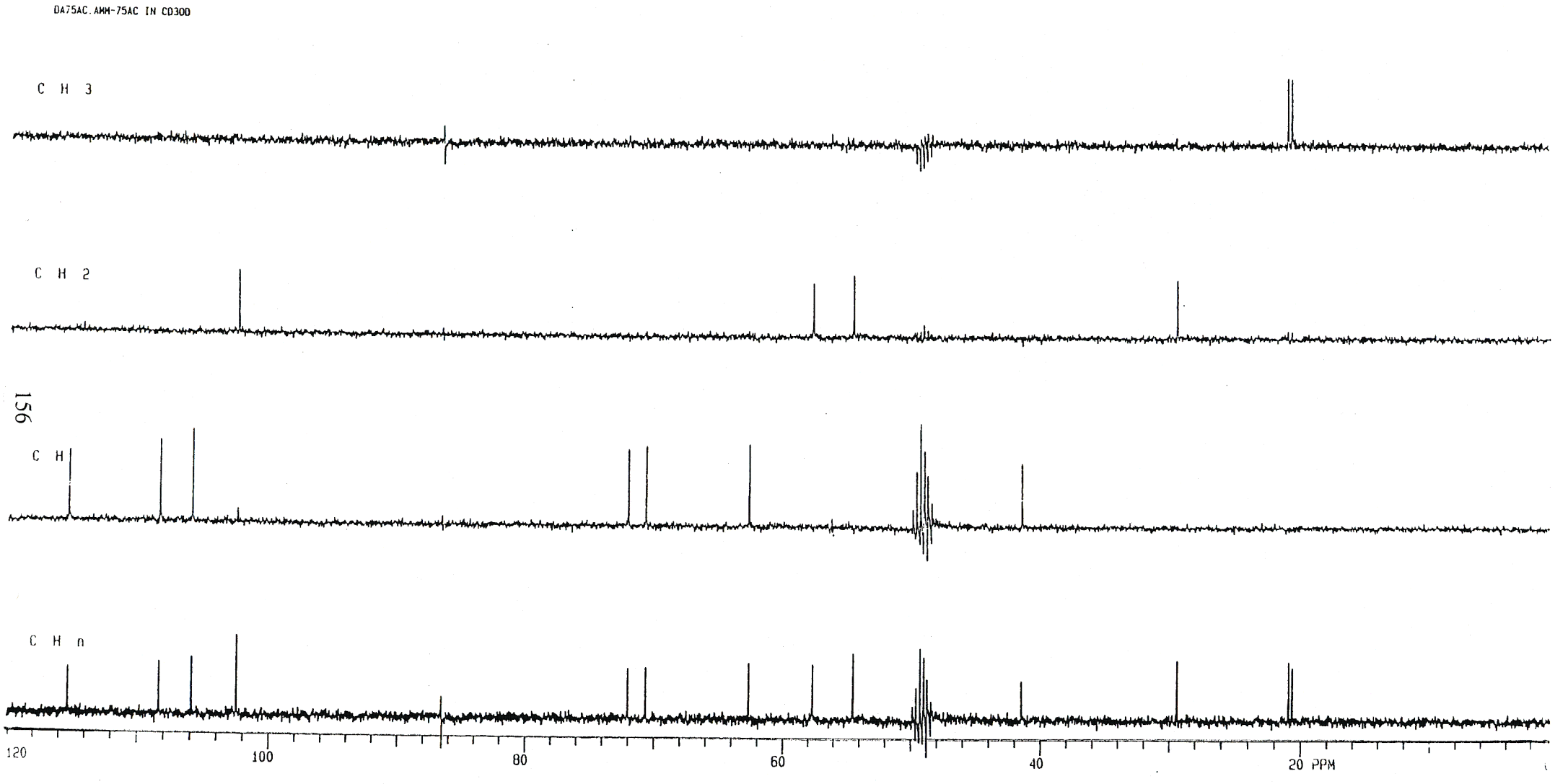
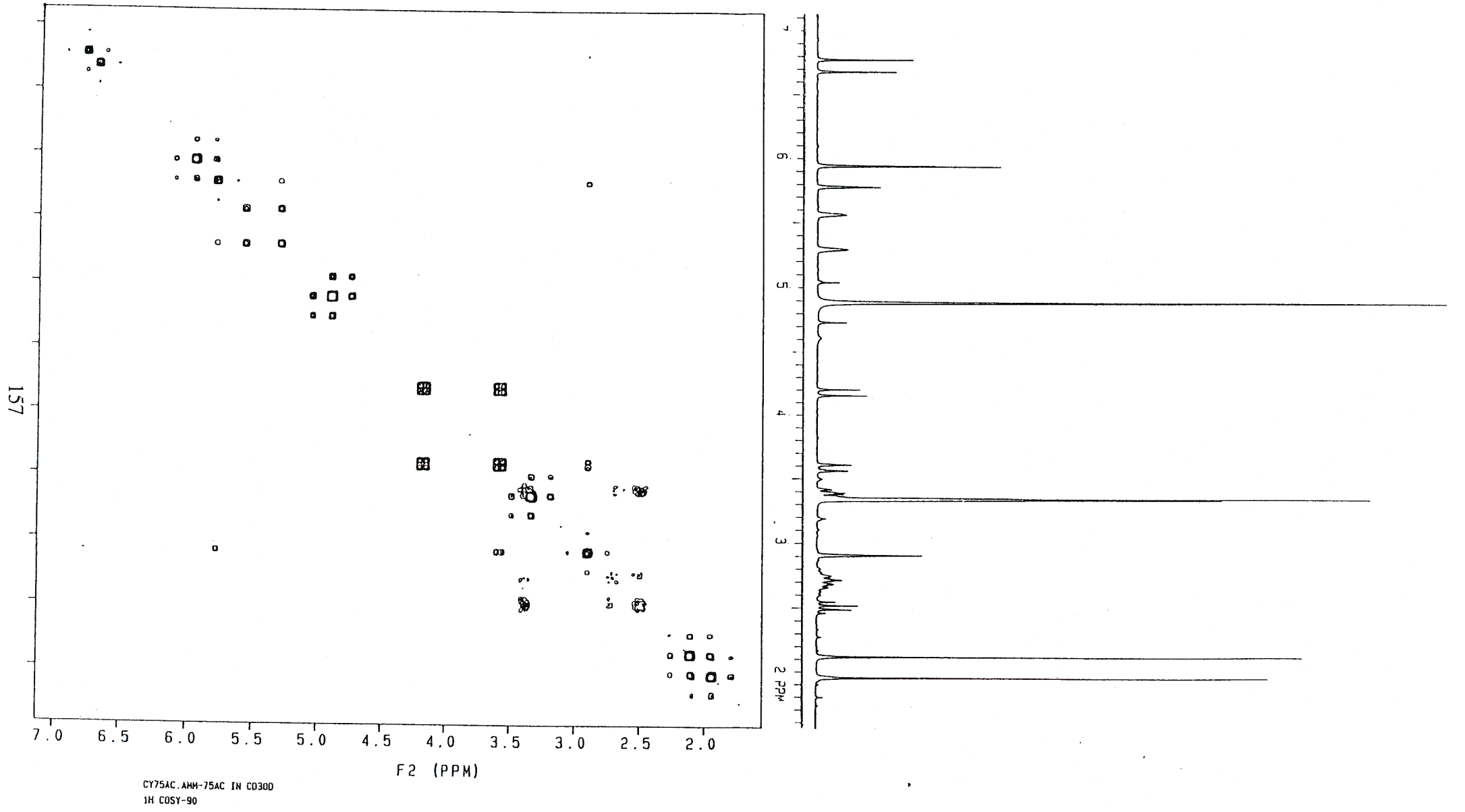
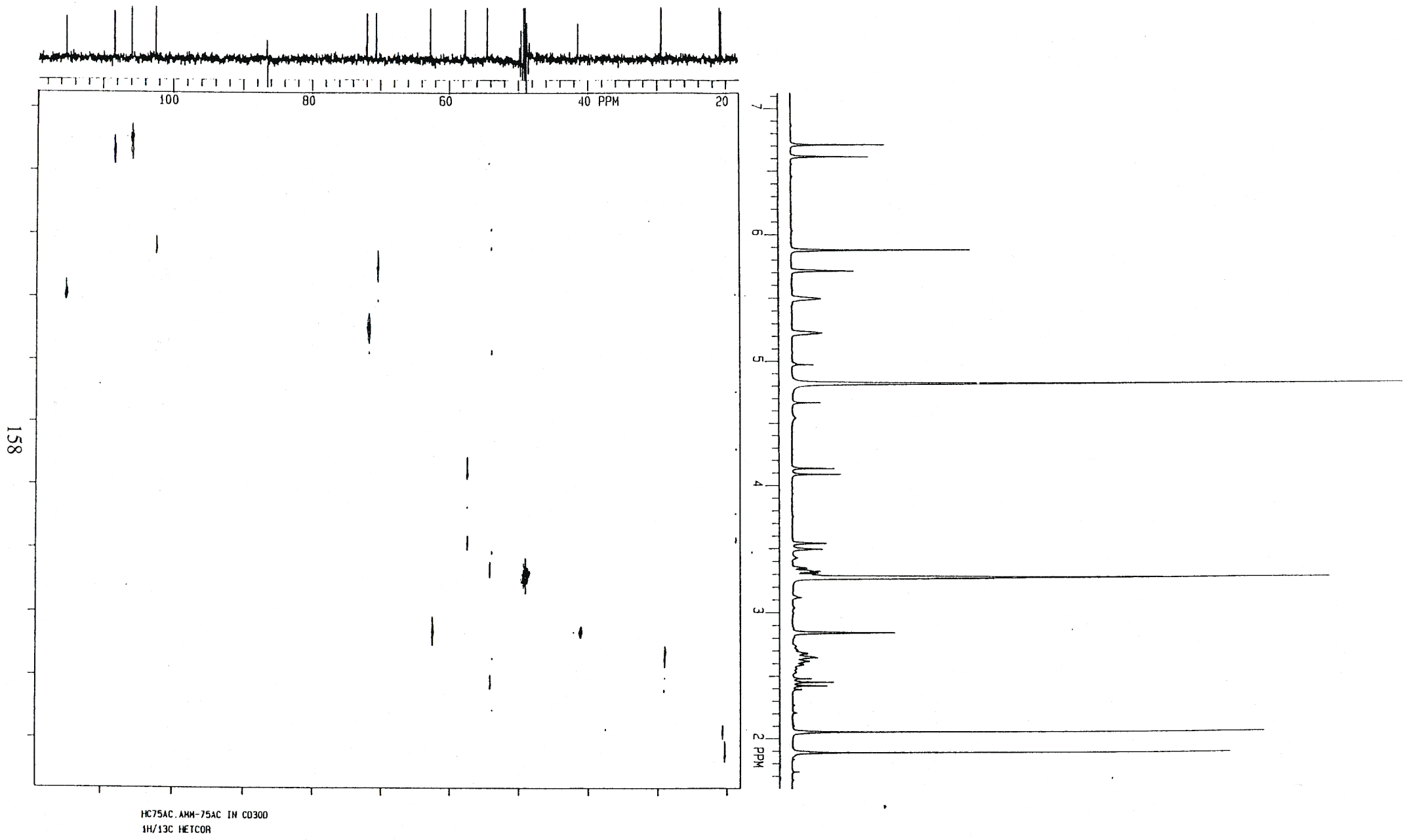
Spectrum [2-B] : ^{13}C NMR spectrum of 1,2-di-O-acetyllycorine (17) in CD_3OD



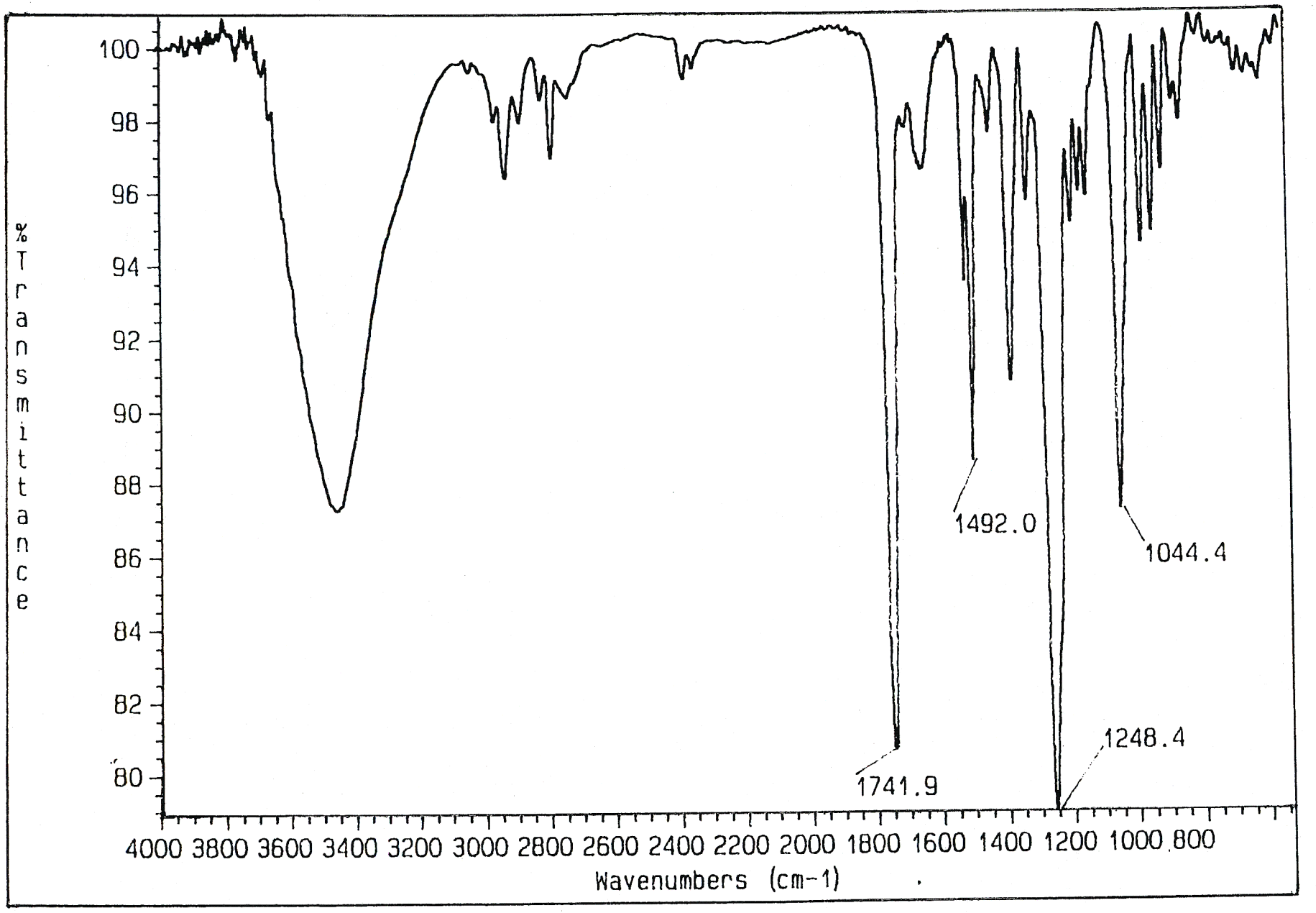
Spectrum [2-C] : ADEPT spectrum of 1,2-di-O-acetyllycorine (17) in CD_3OD



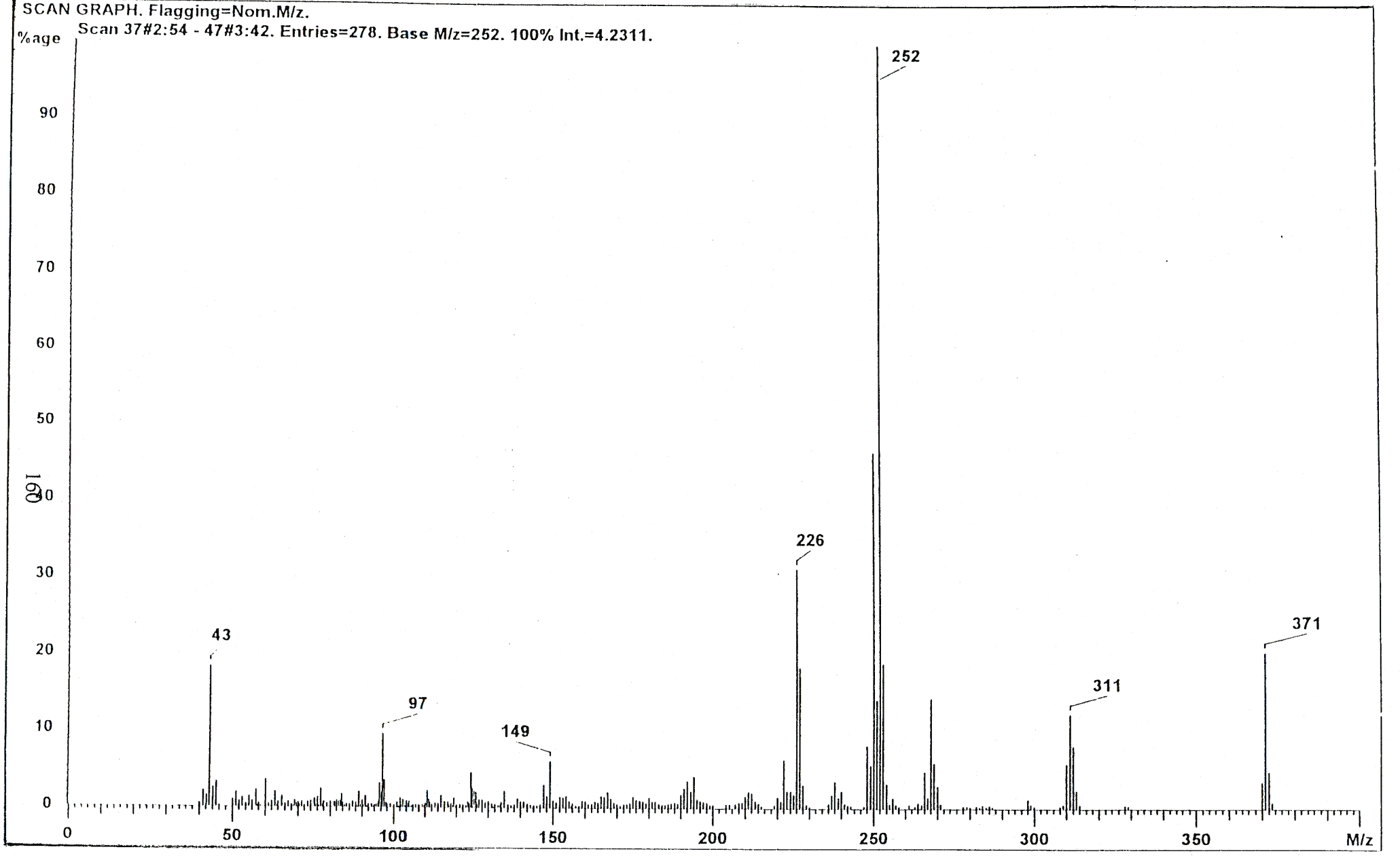
Spectrum [2-D] : COSY spectrum of 1,2-di-O-acetyllycorine (17) in CD_3OD



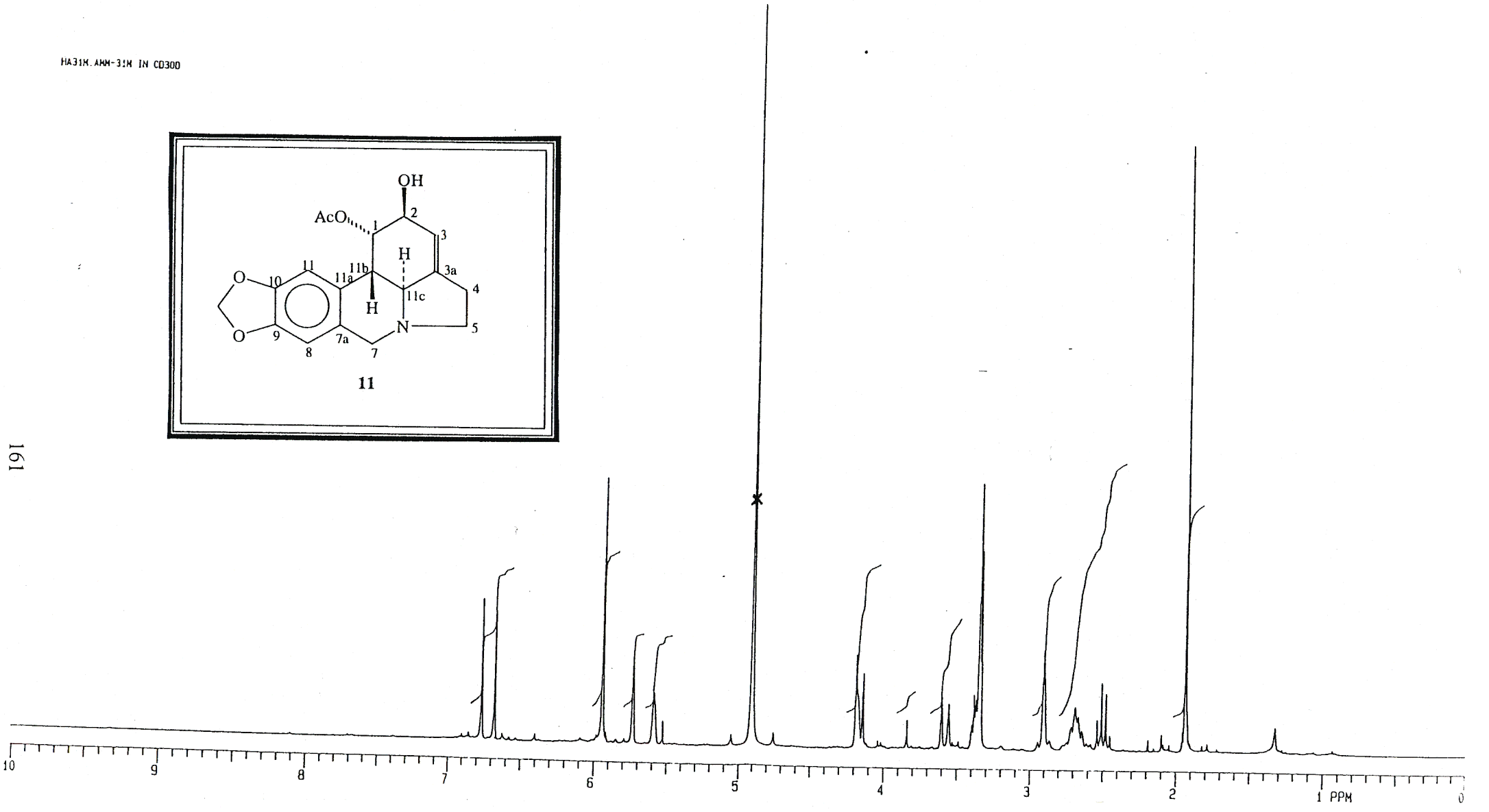
Spectrum [2-E] : HETCOR spectrum of 1,2-di-O-acetyllycorine (17) in CD_3OD



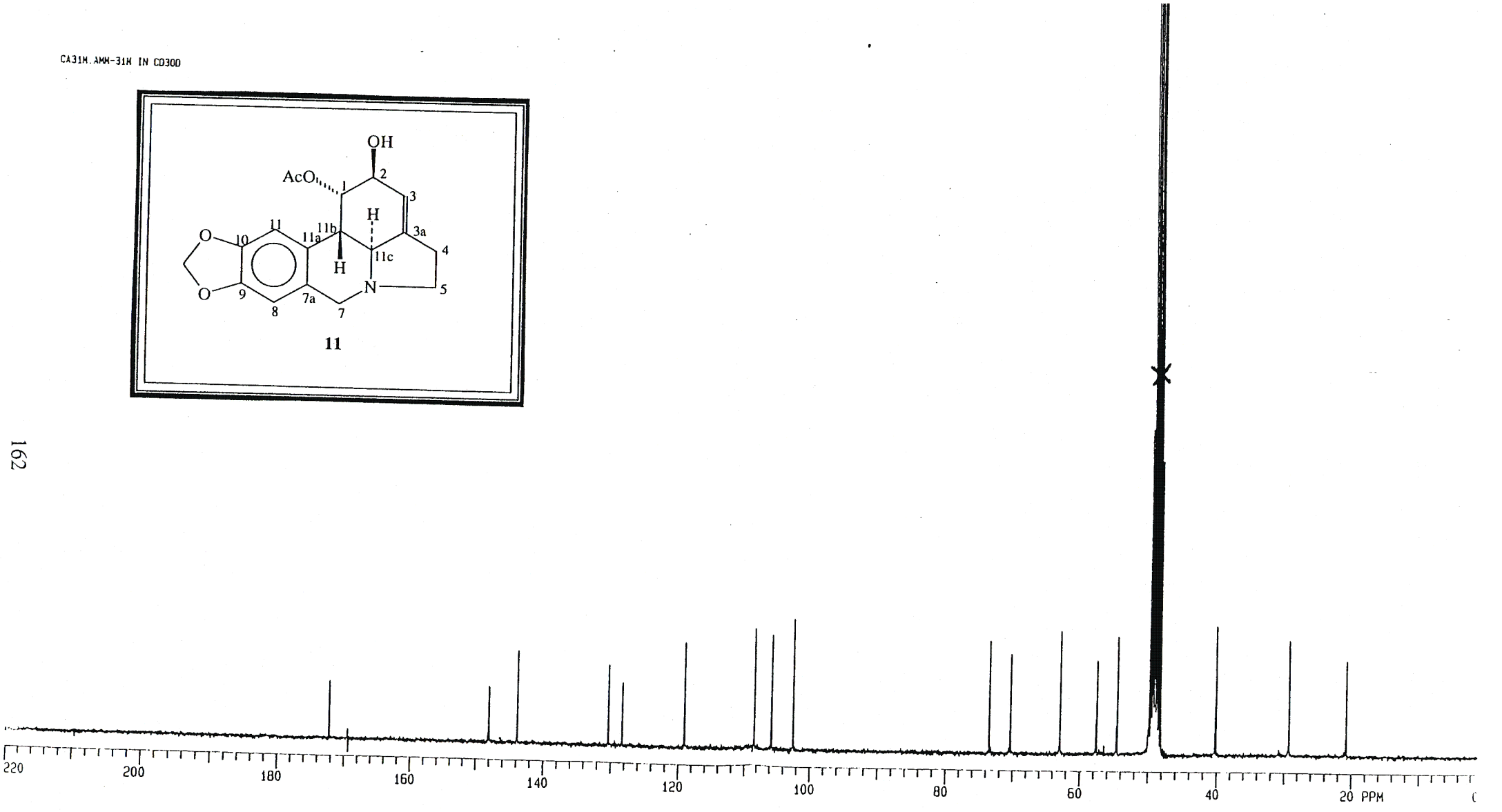
Spectrum [2-F] : Infra red spectrum of 1,2-di-O-acetyllycorine (17)



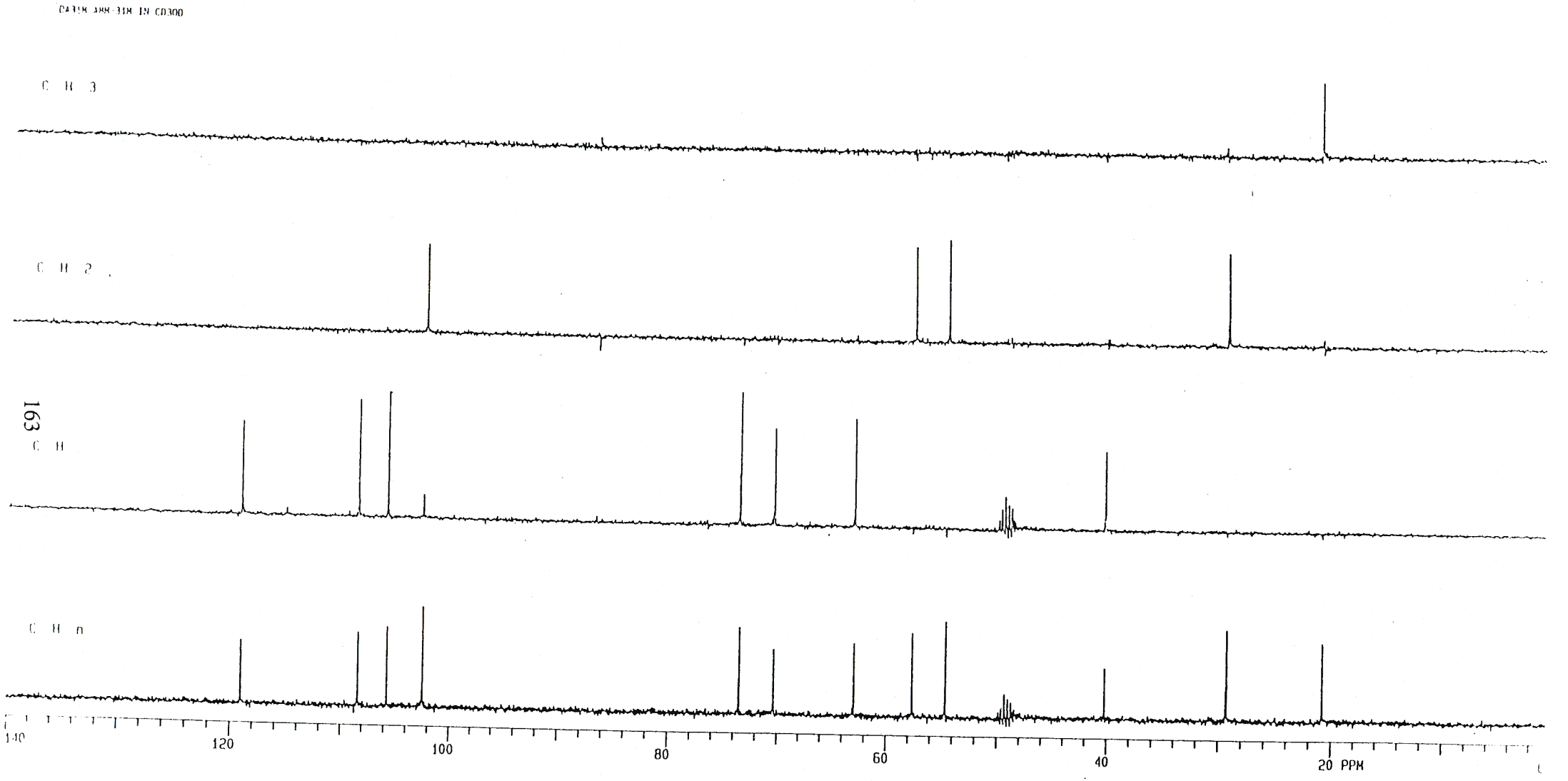
Spectrum [2-G] : Mass spectrum of 1,2-di-O-acetyllycorine (17)



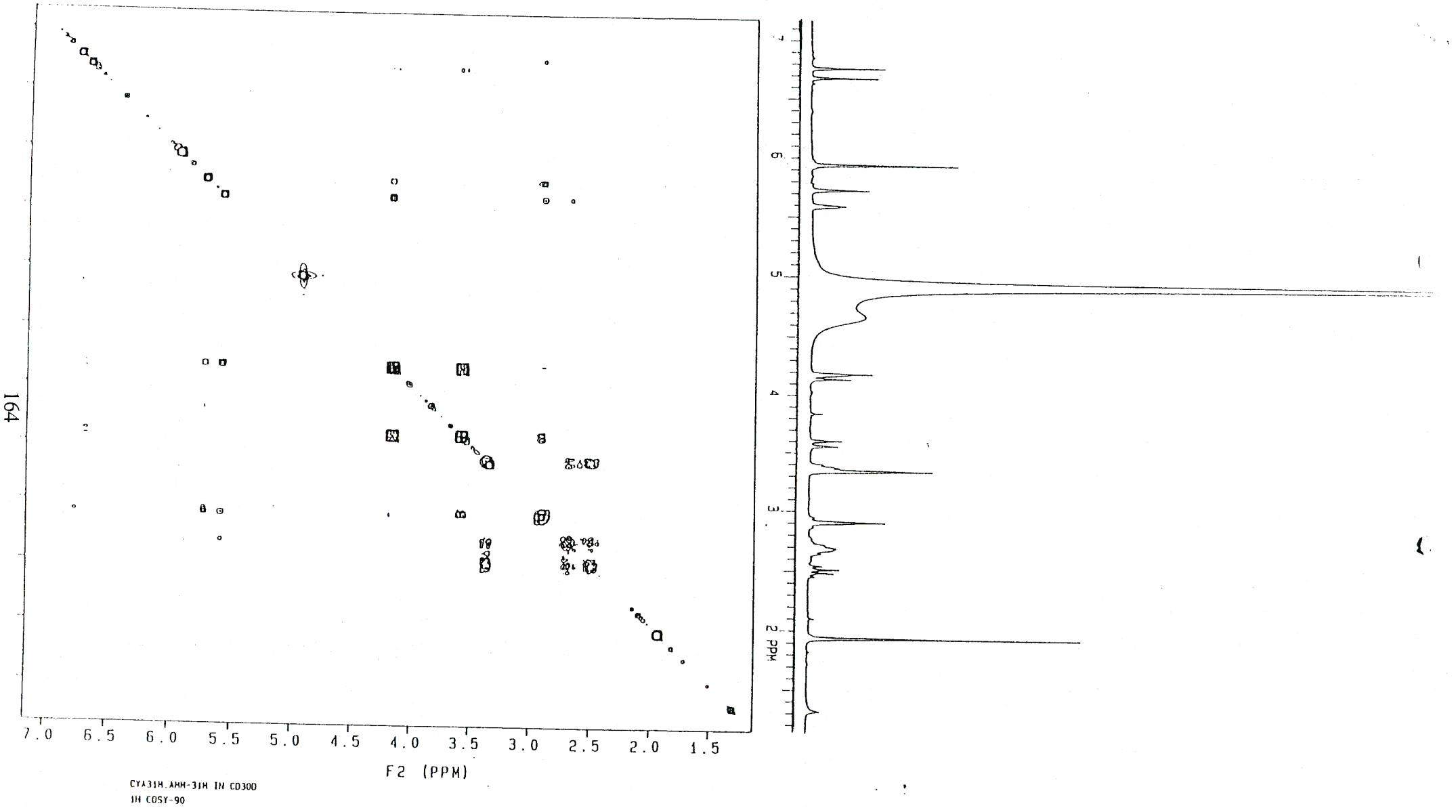
Spectrum [3-A] : ^{1}H NMR spectrum of 1-O-acetyllycorine (11) in CD_3OD



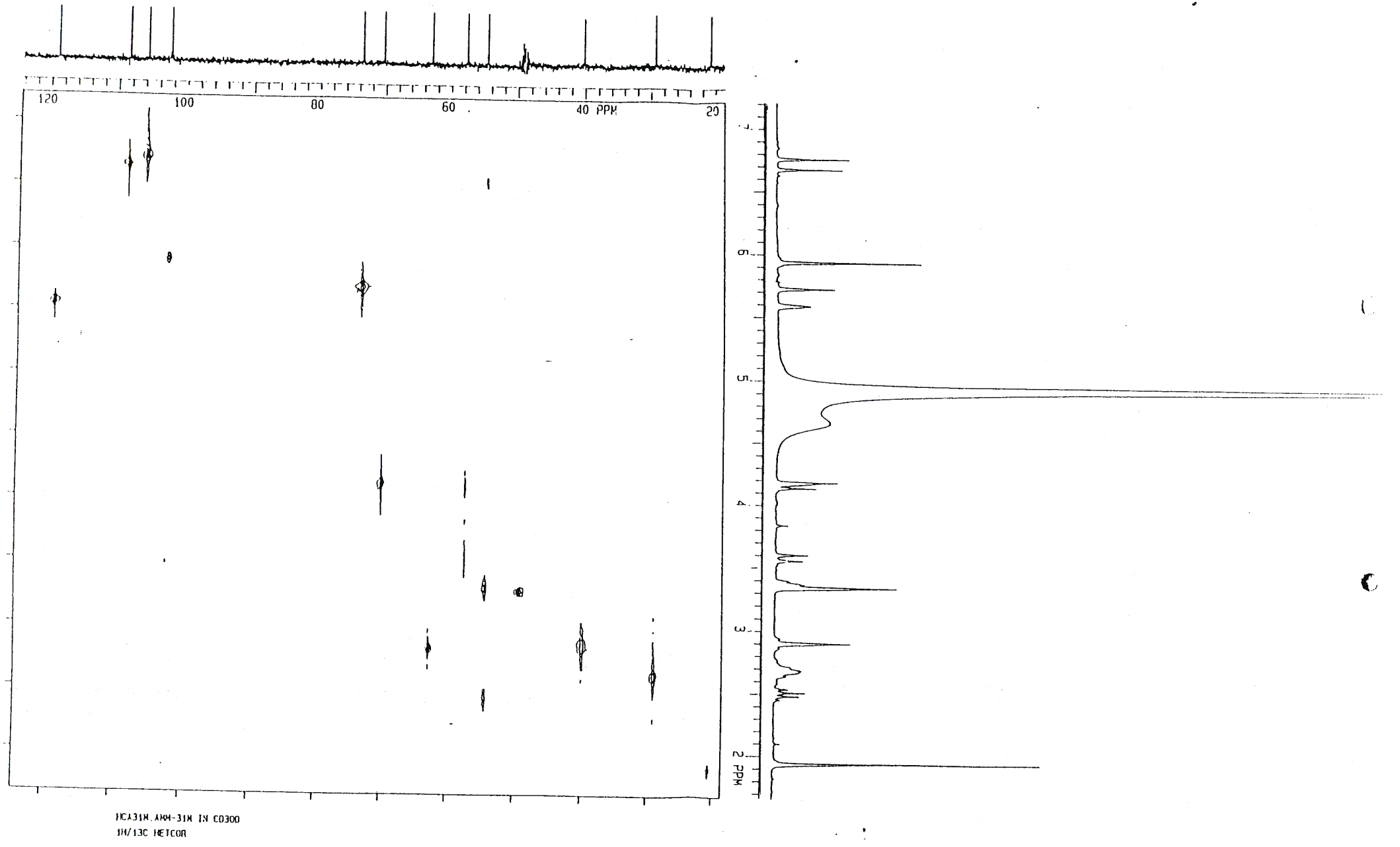
Spectrum [3-B] : ^{13}C NMR spectrum of 1-O-acetyllycorine (11) in CD_3OD



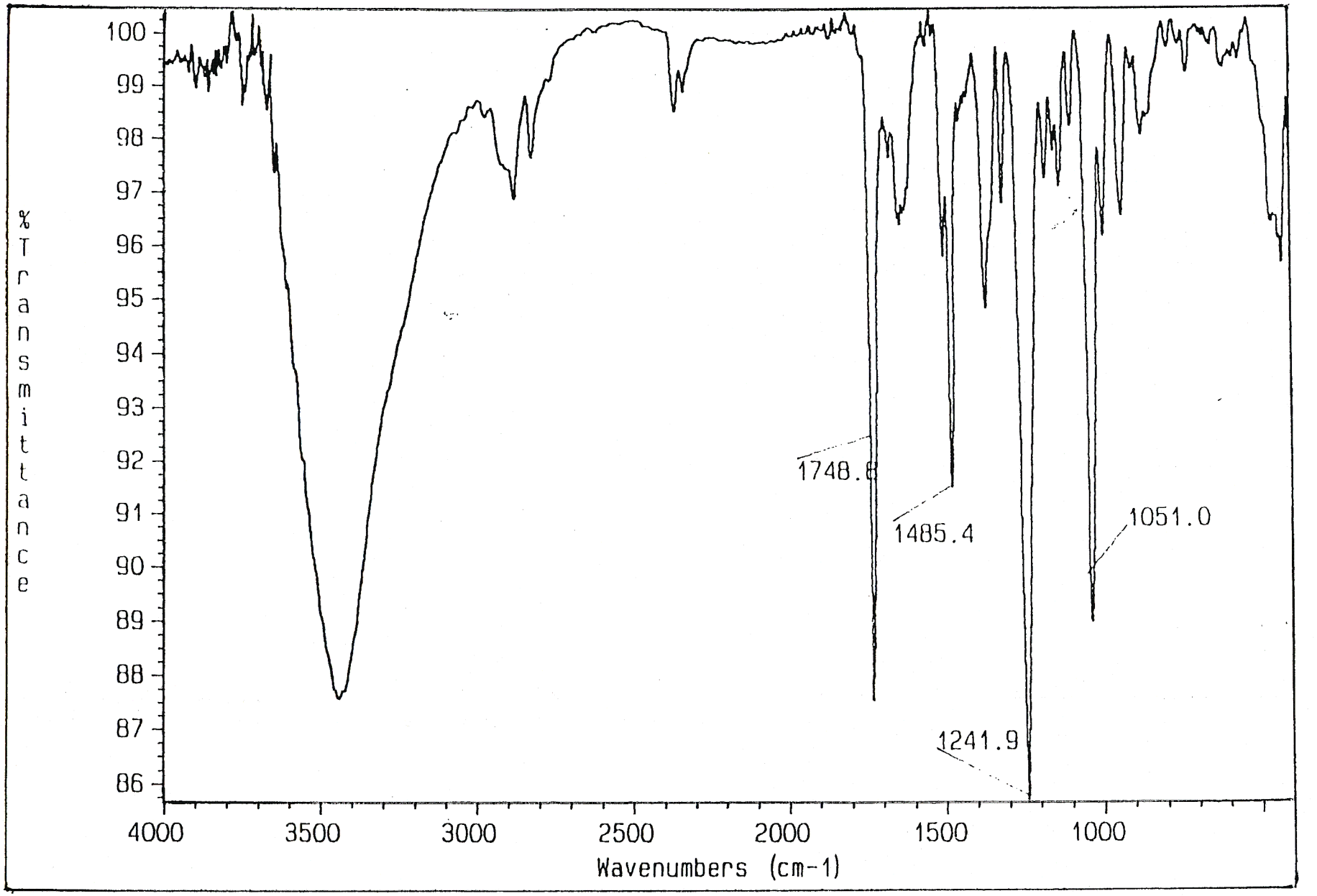
Spectrum [3-C] : ADEPT spectrum of 1-O-acetyllycorine (11) in CD_3OD



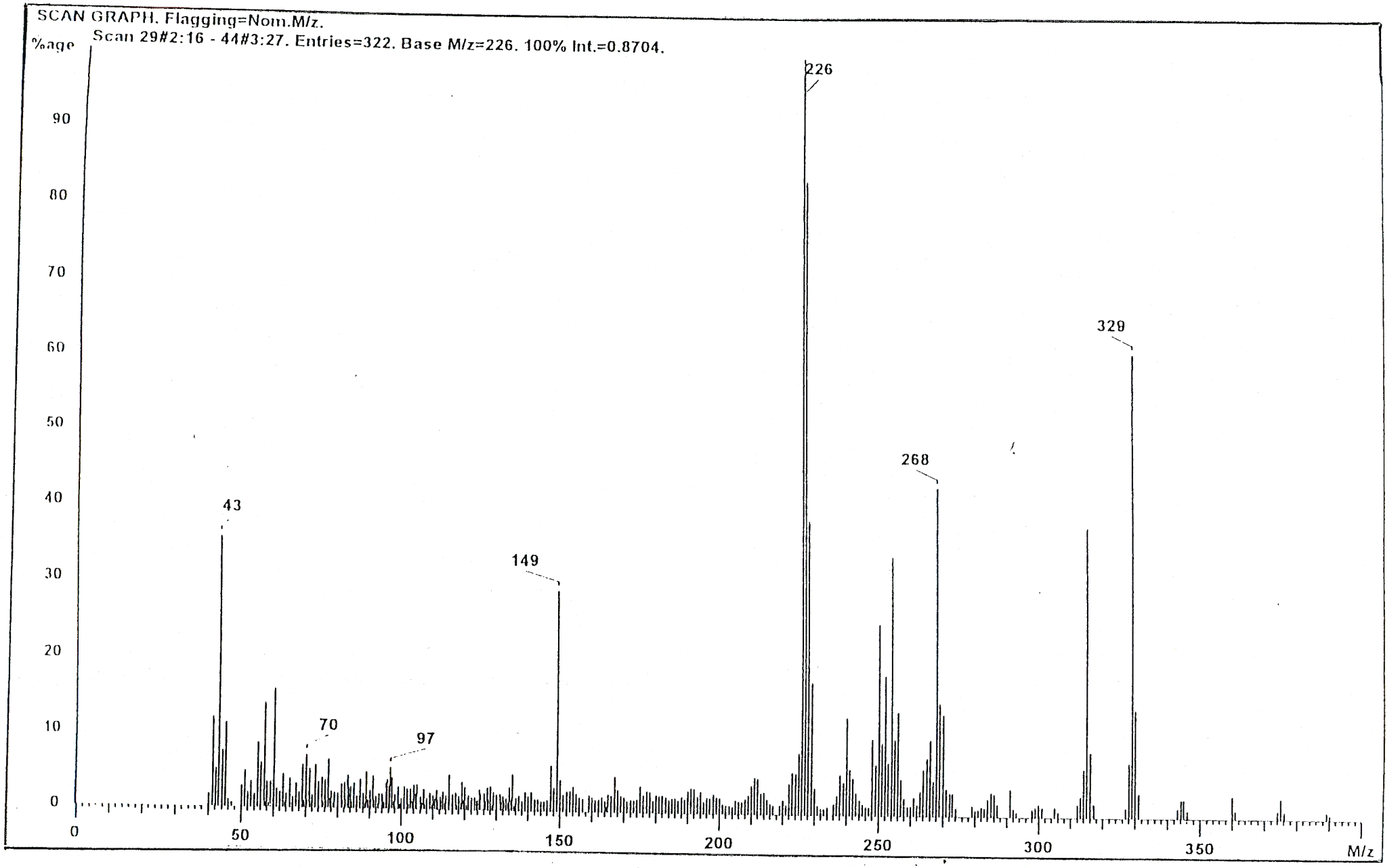
Spectrum [3-D] : COSY spectrum of 1-O-acetyllycorine (11) in CD_3OD



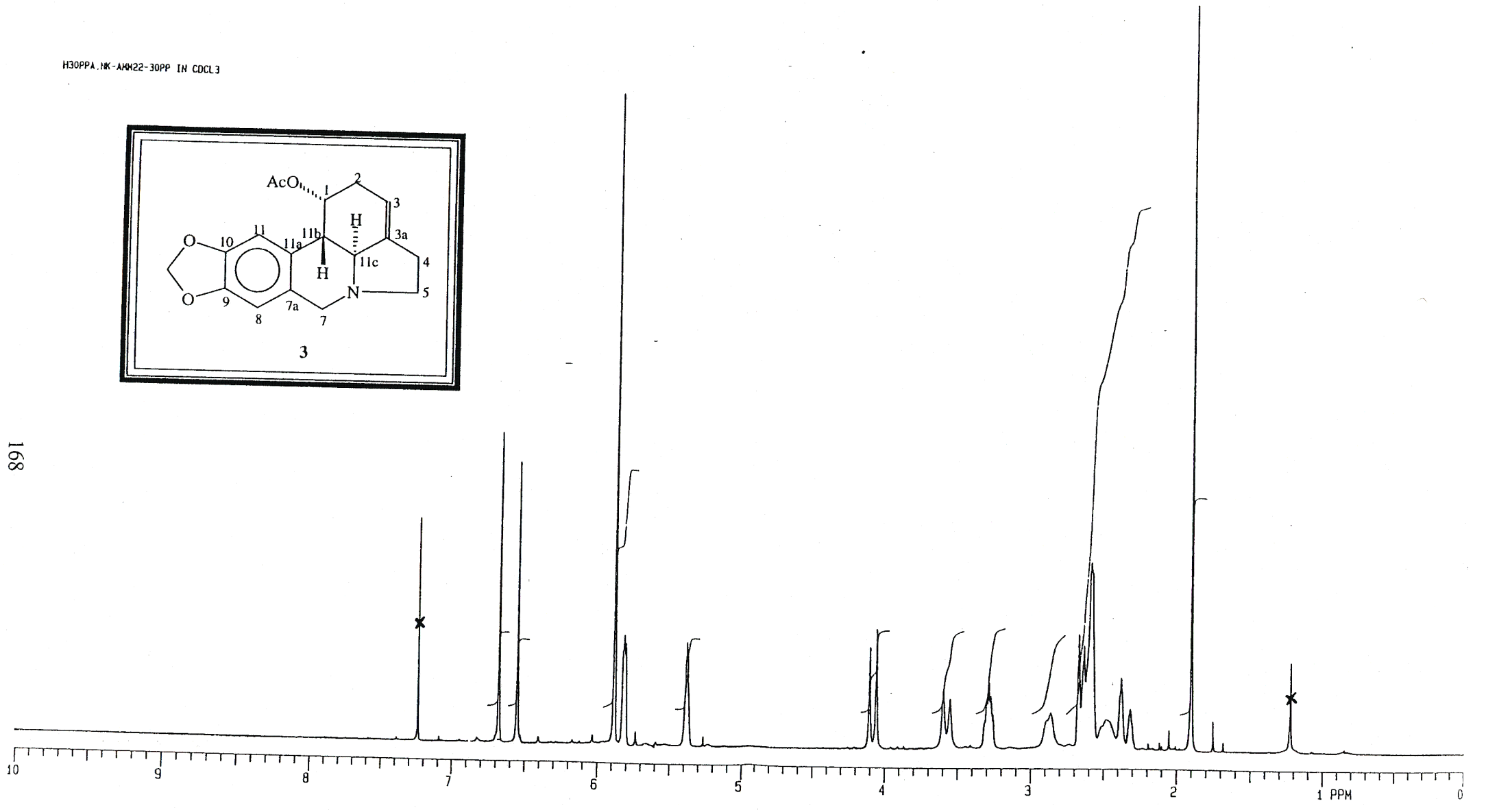
Spectrum [3-E] : HETCOR spectrum of 1-O-acetyllycorine (11) in CD_3OD



Spectrum [3-F] : Infra red spectrum of 1-O-acetyllycorine (11)

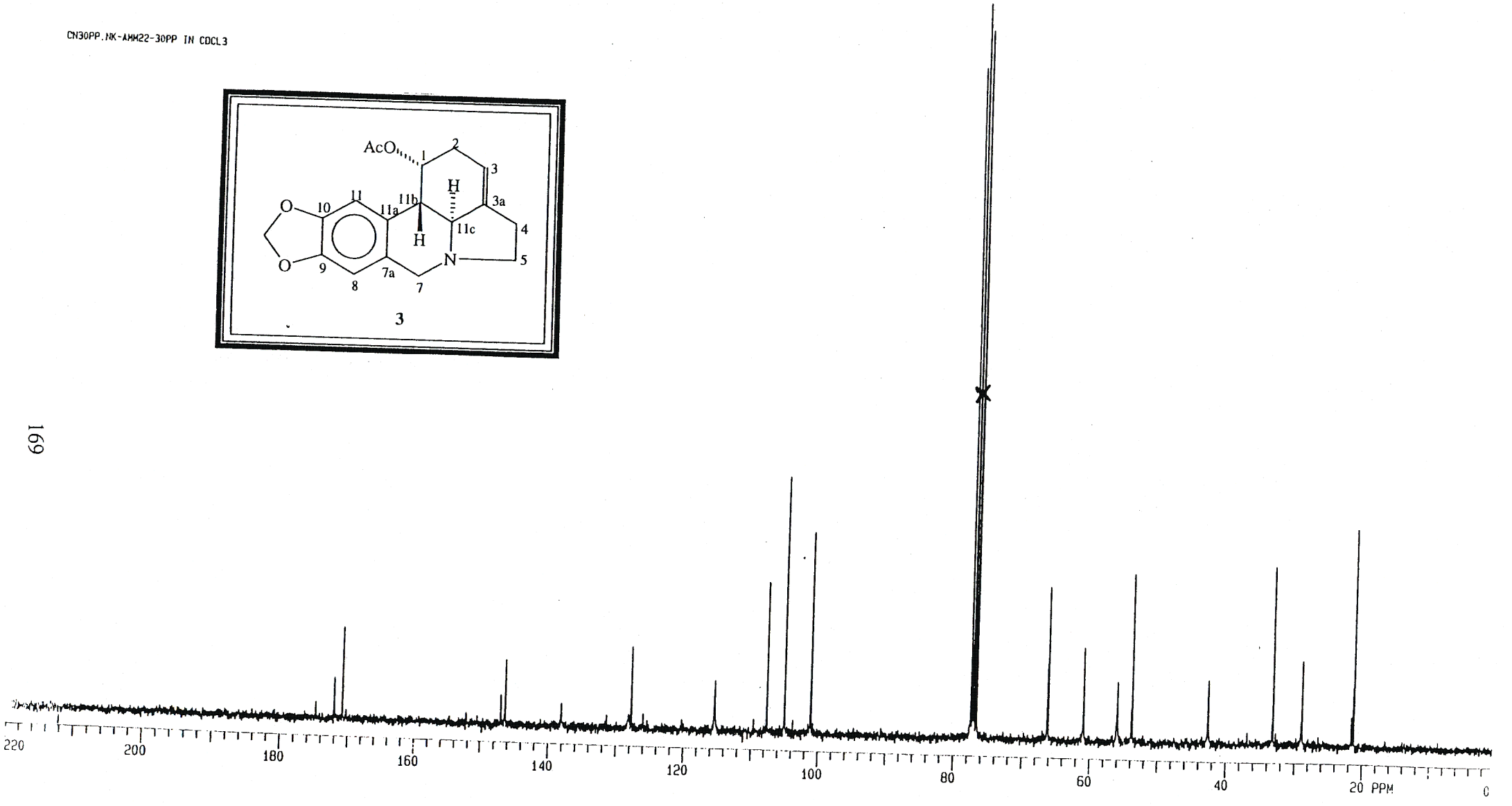


Spectrum [3-G] : Mass spectrum of 1-O-acetyllycorine (11)

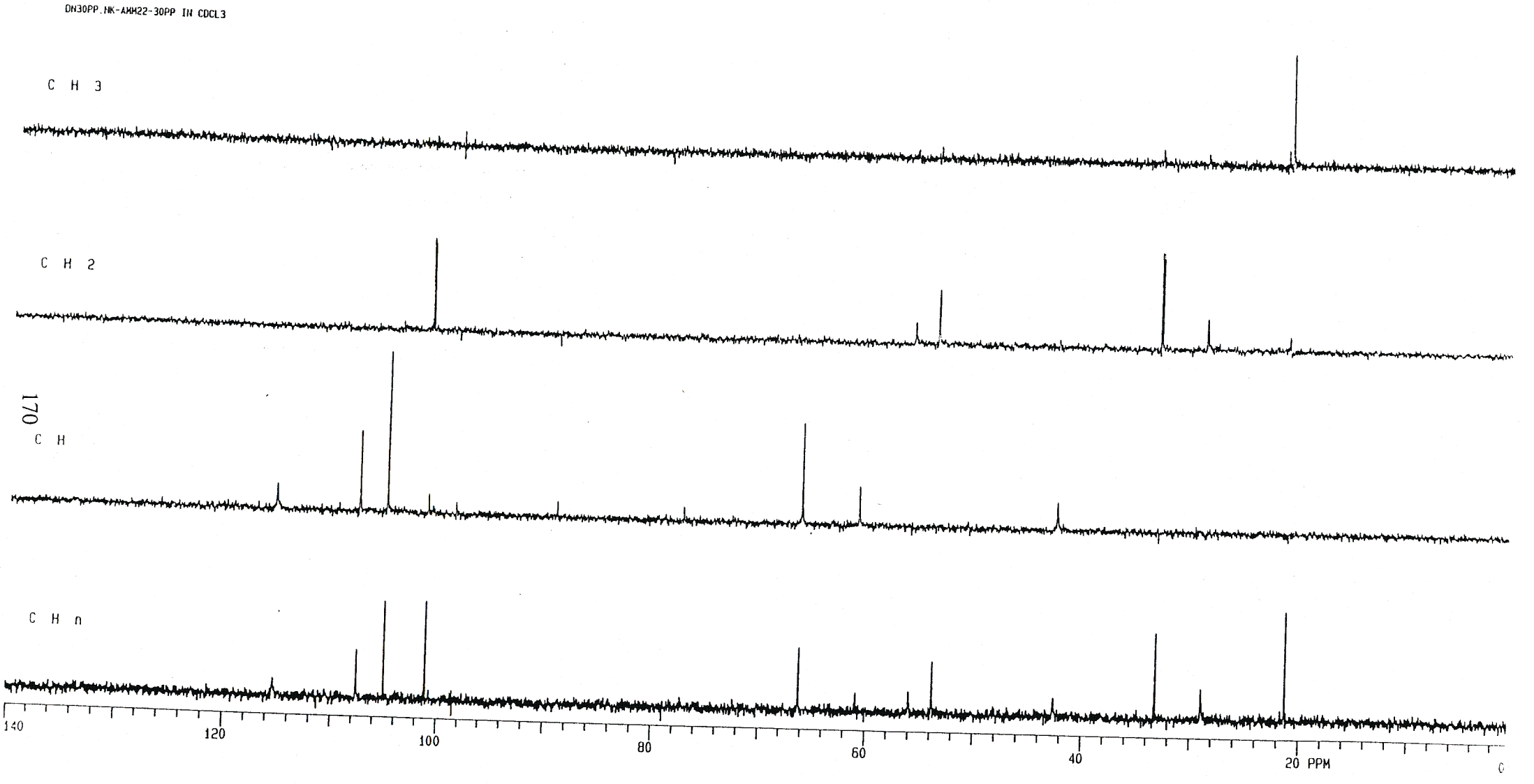


3

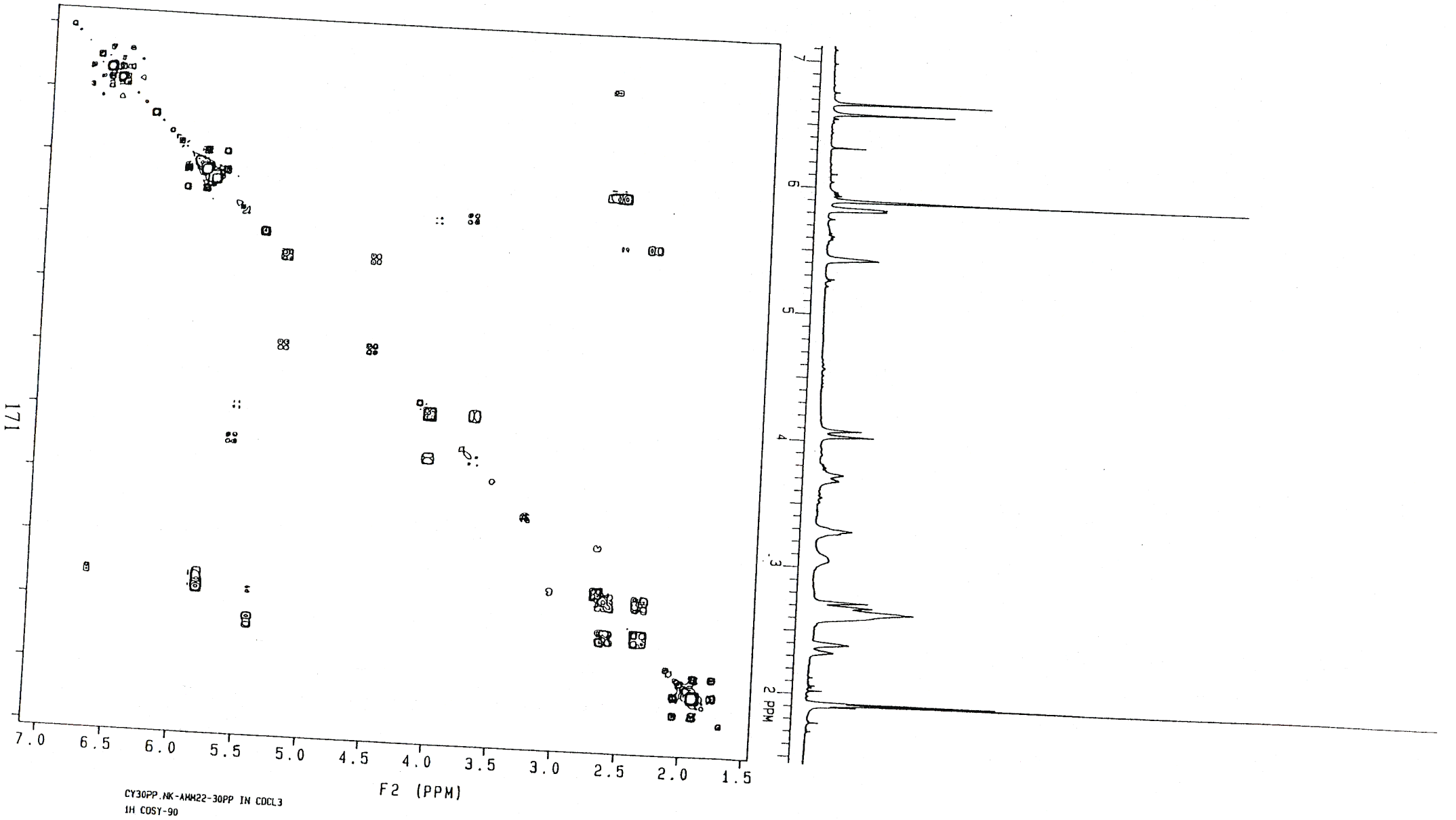
Spectrum [4-A] : ^{1}H NMR spectrum of acetylcaranine (3) in $CDCl_3$



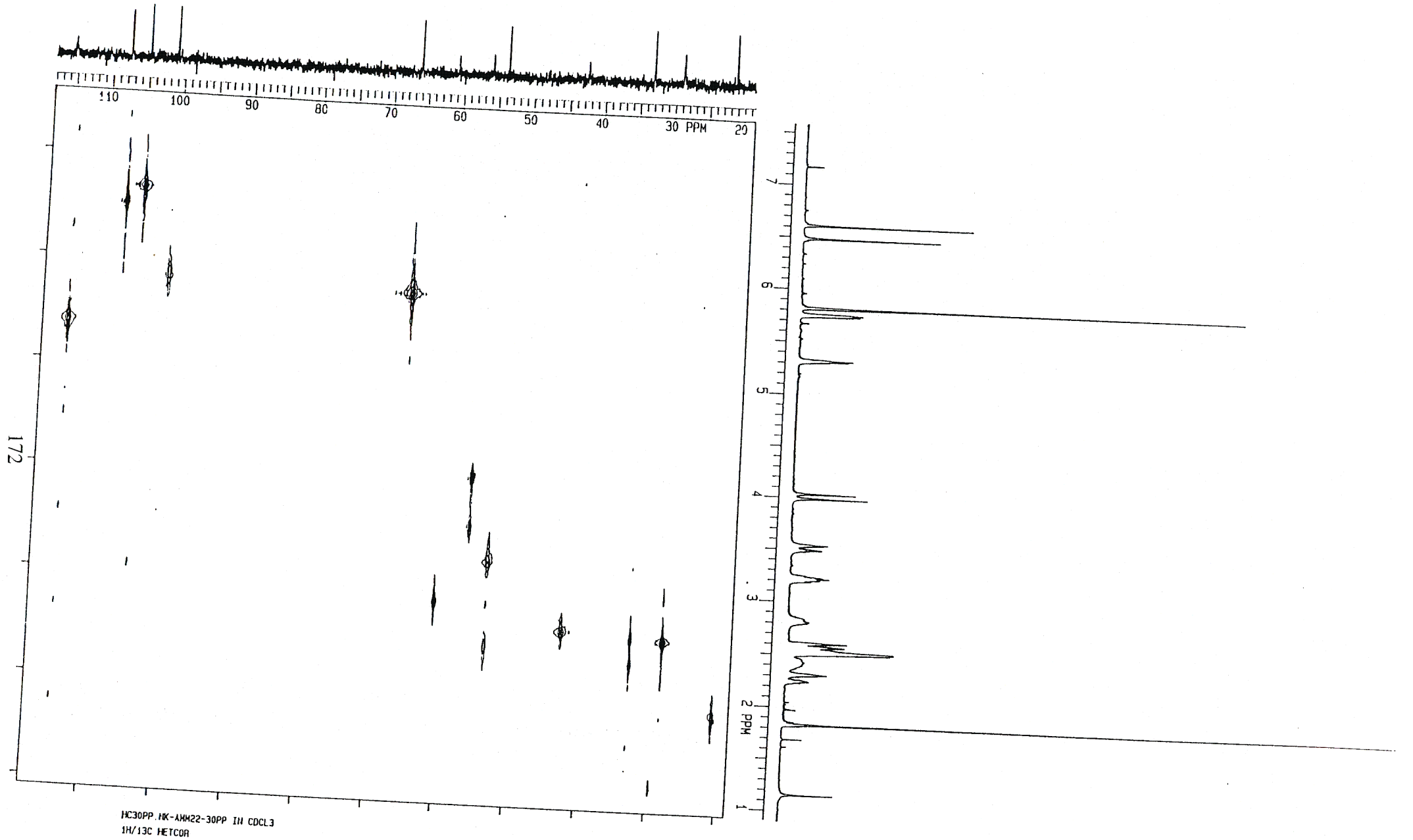
Spectrum [4-B] : ^{13}C NMR spectrum of acetylcaranine (3) in $CDCl_3$



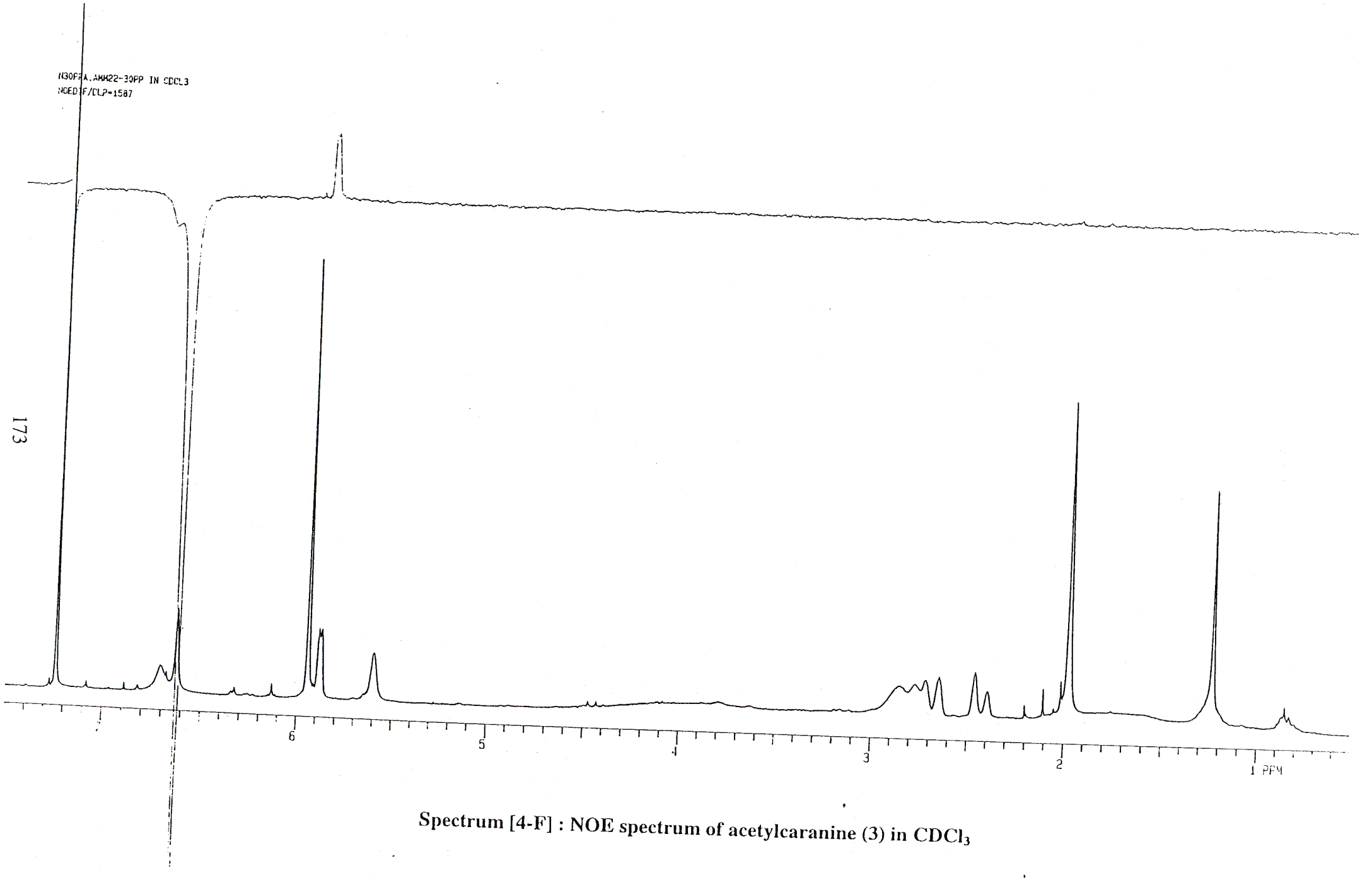
Spectrum [4-C] : ADEPT spectrum of acetylcaranine (3) in $CDCl_3$



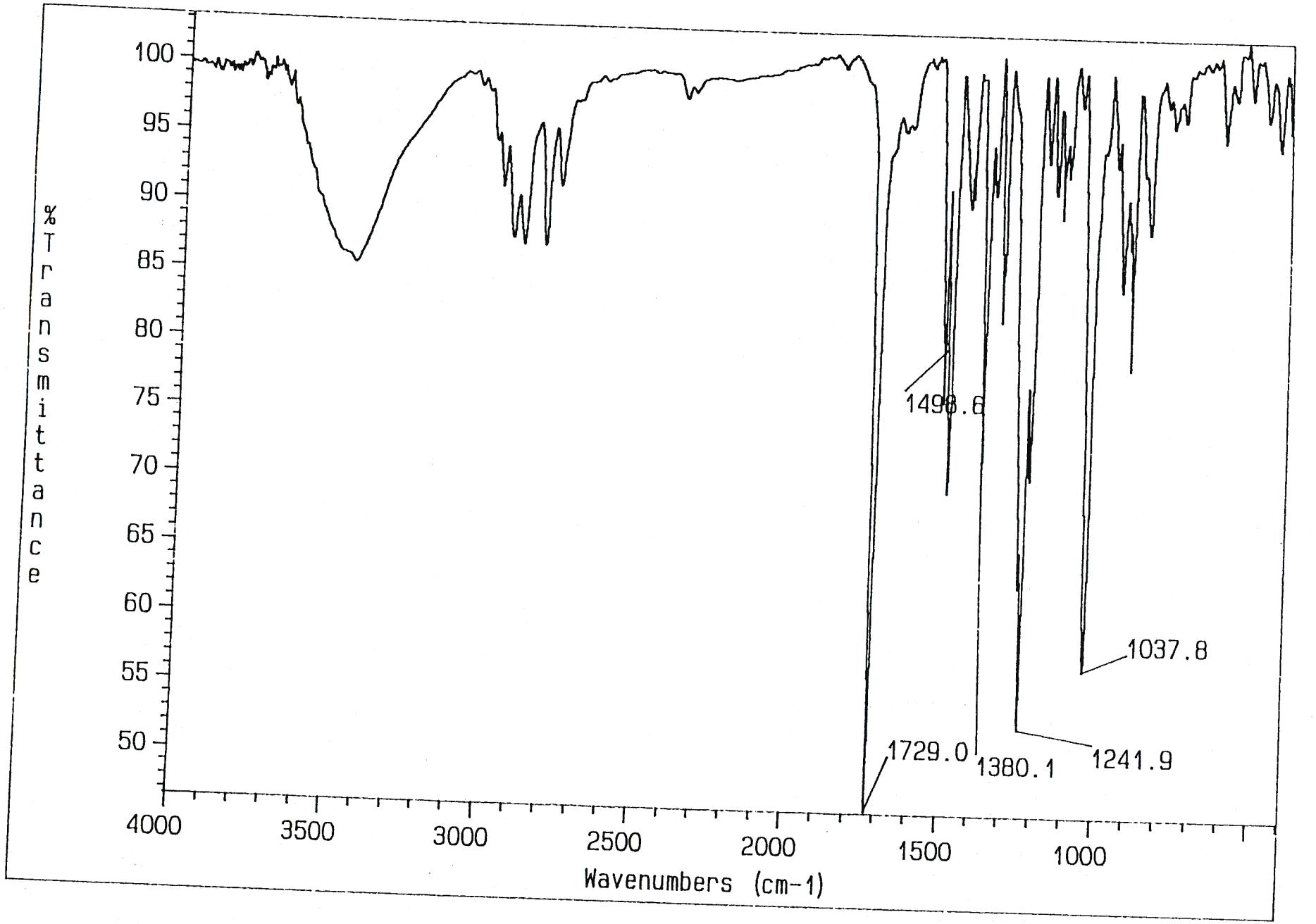
Spectrum [4-D] : COSY spectrum of acetylcaranine (3) in $CDCl_3$



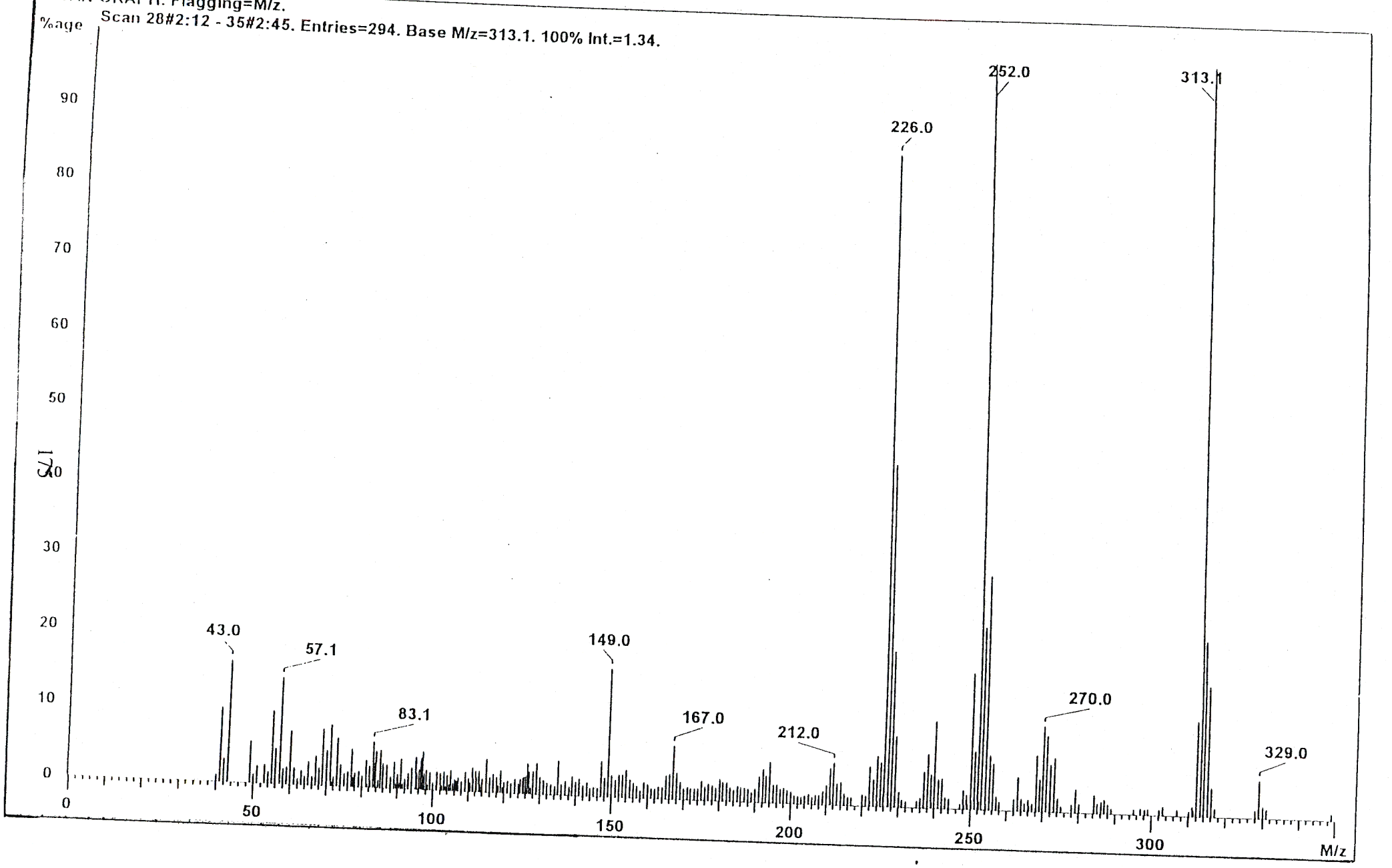
Spectrum [4-E] : HETCOR spectrum of acetylcaranine (3) in $CDCl_3$



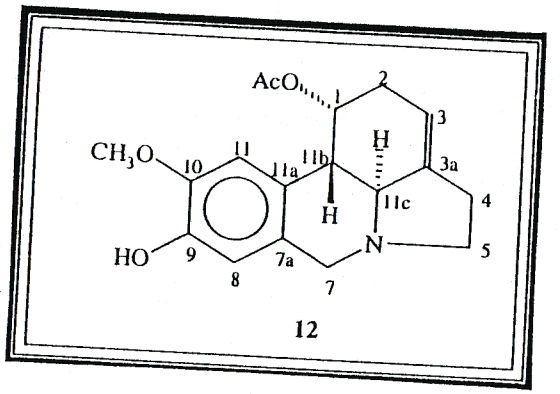
Spectrum [4-F] : NOE spectrum of acetylcaranine (3) in $CDCl_3$



Spectrum [4-G] : Infra red spectrum of acetylcaranine (3)

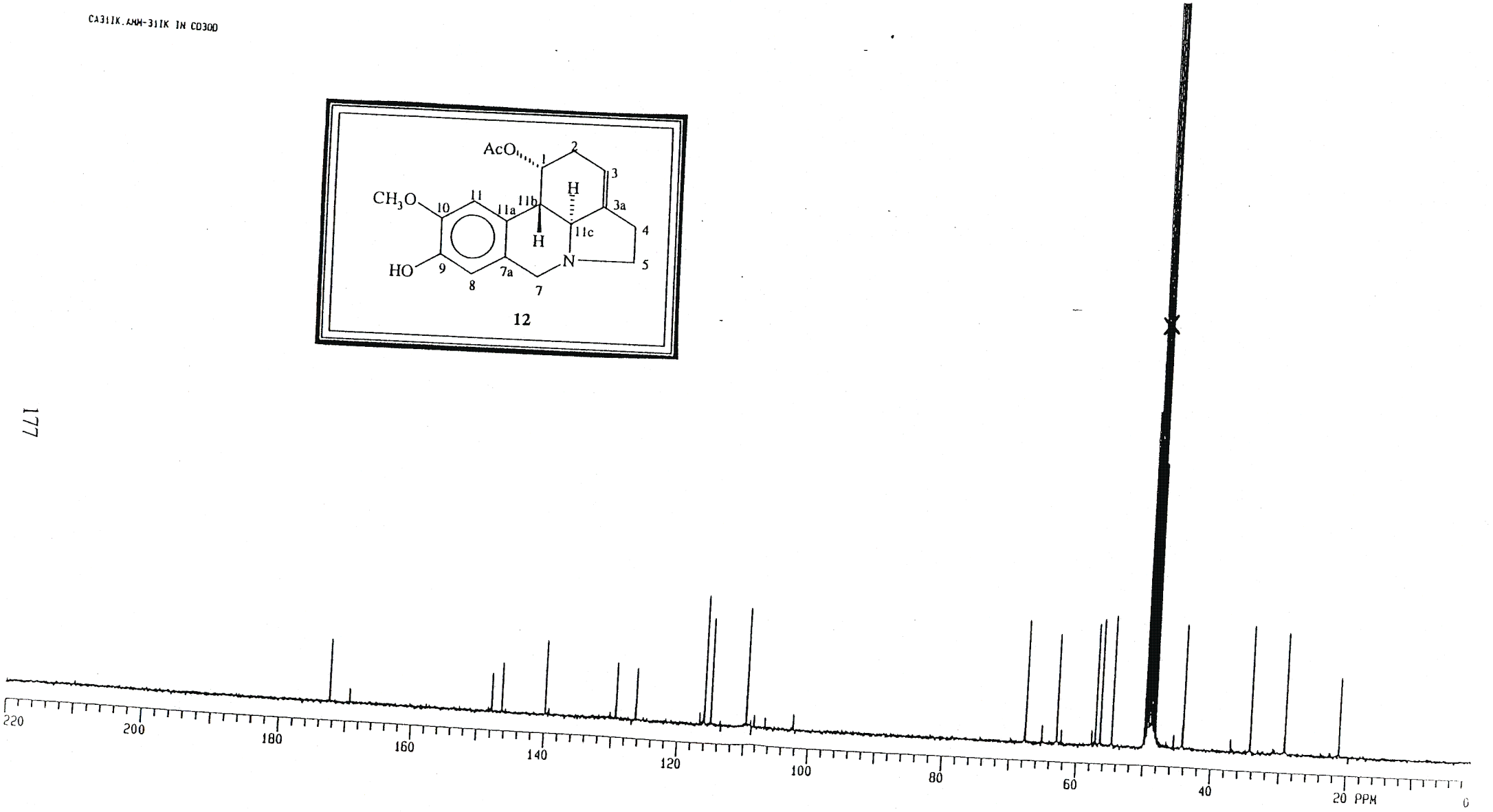


Spectrum [4-H] : Mass spectrum of acetylcaranine (3)



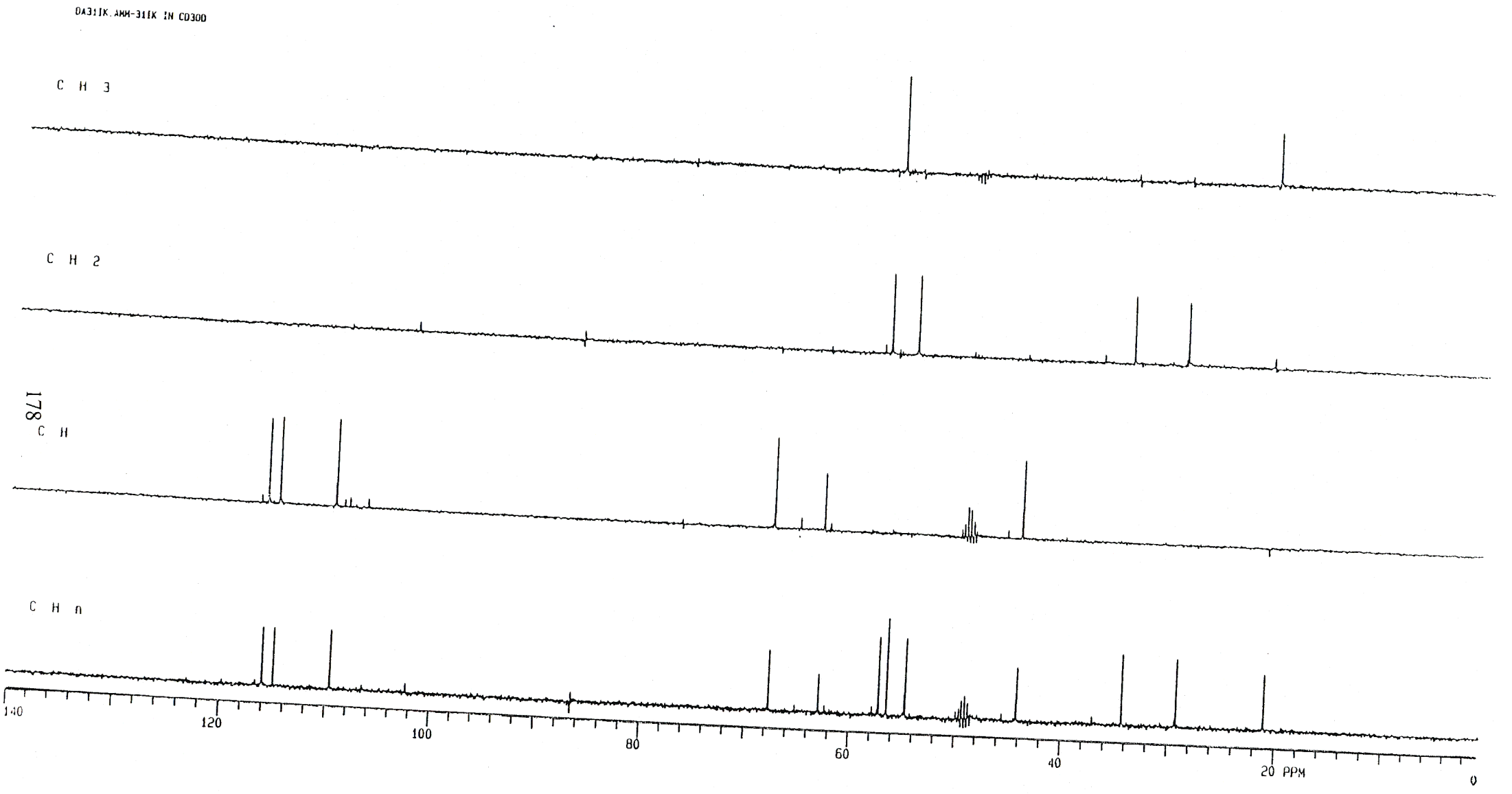
12

Spectrum [5-A] : ^{1}H NMR spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD

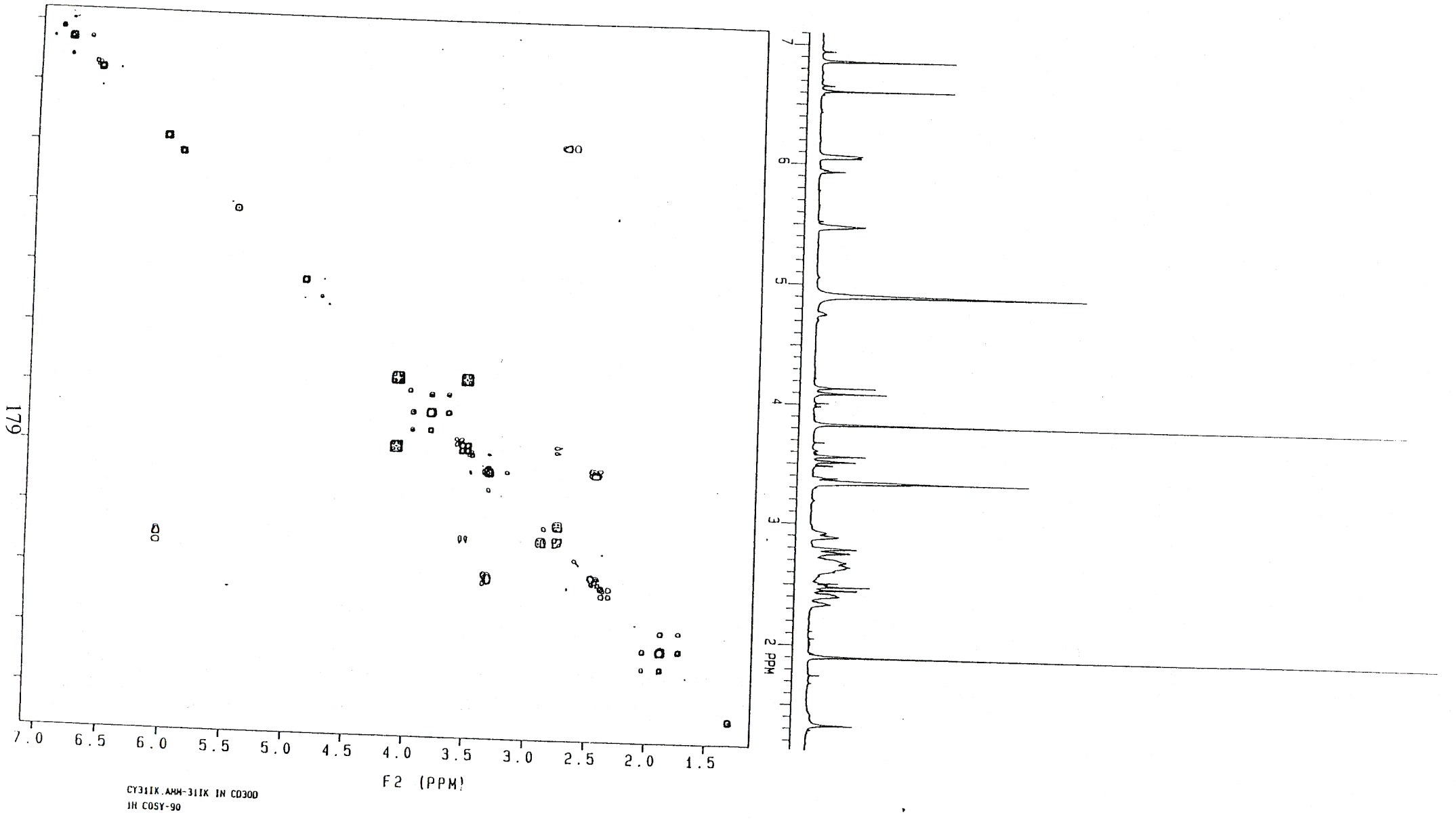


12

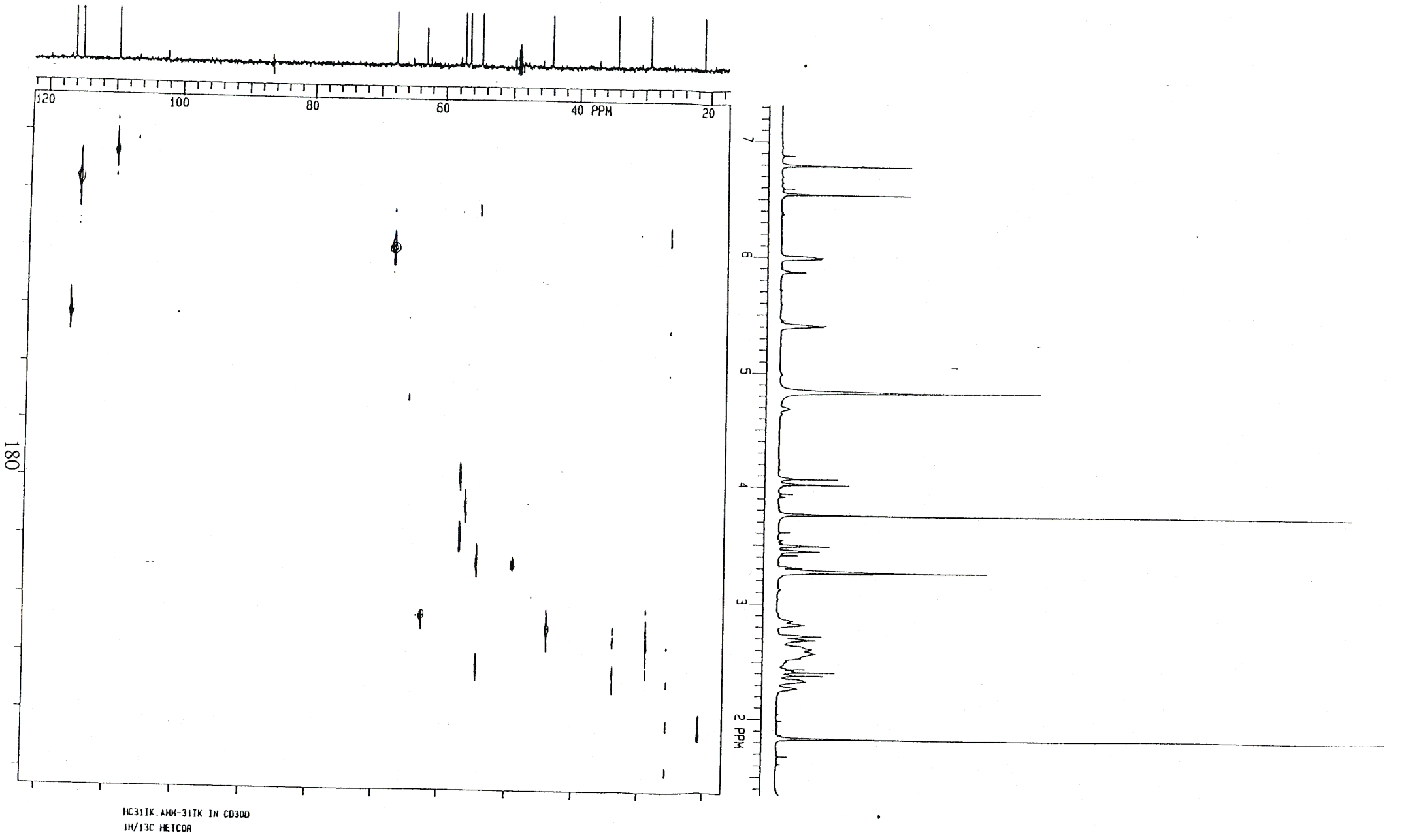
Spectrum [5-B] : ^{13}C NMR spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD



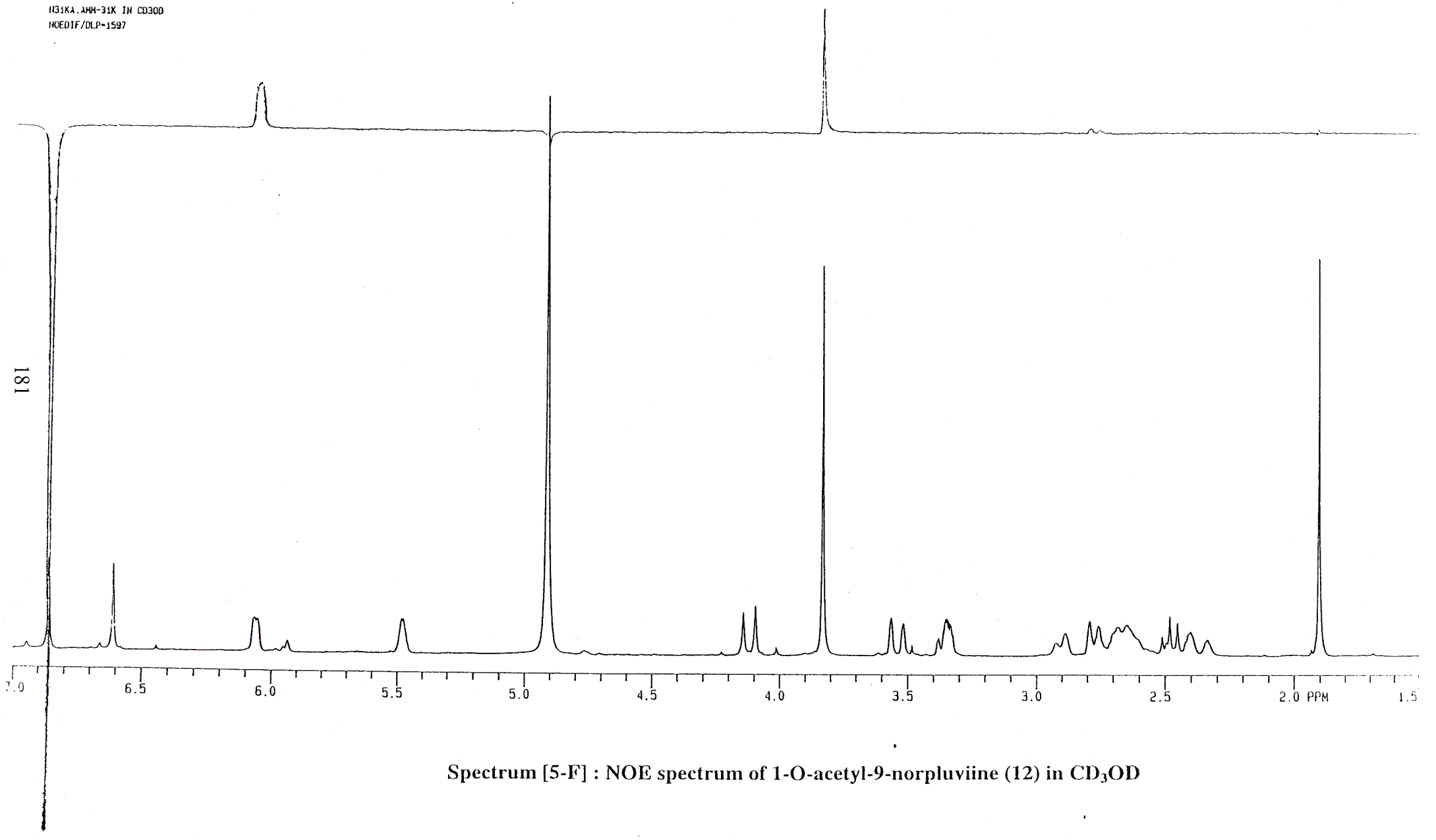
Spectrum [5-C] : ADEPT spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD



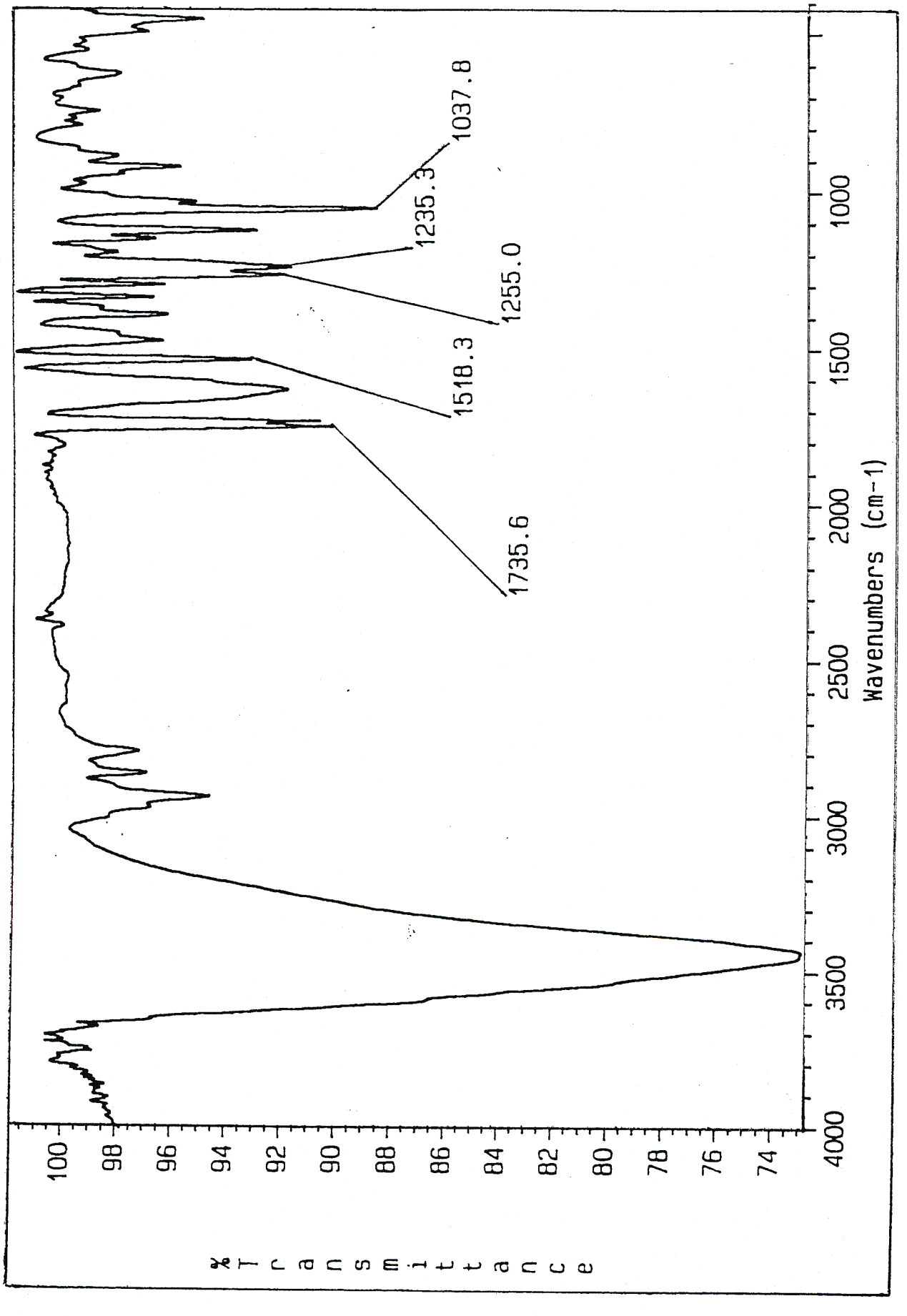
Spectrum [5-D] : COSY spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD .



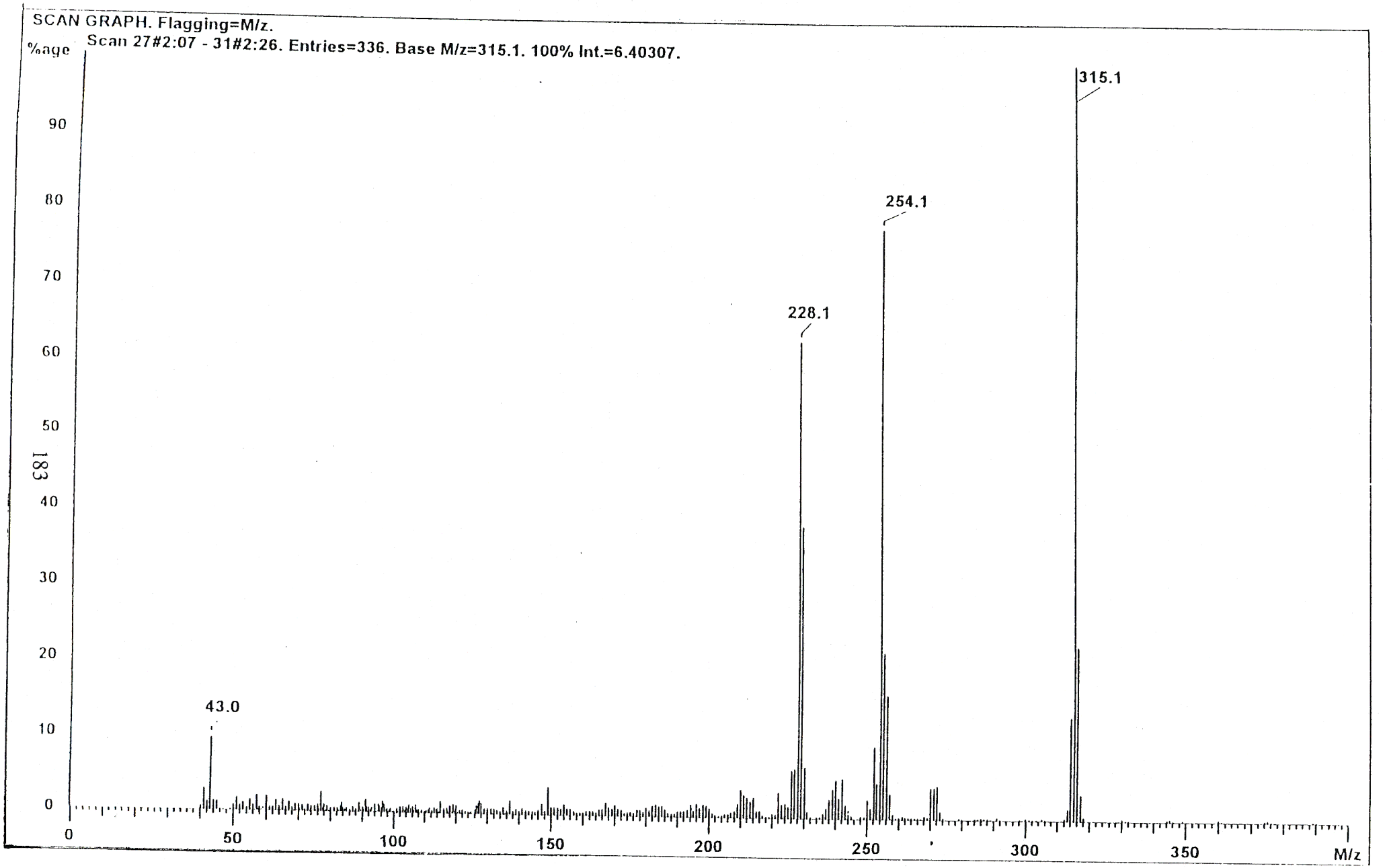
Spectrum [5-E] : HETCOR spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD



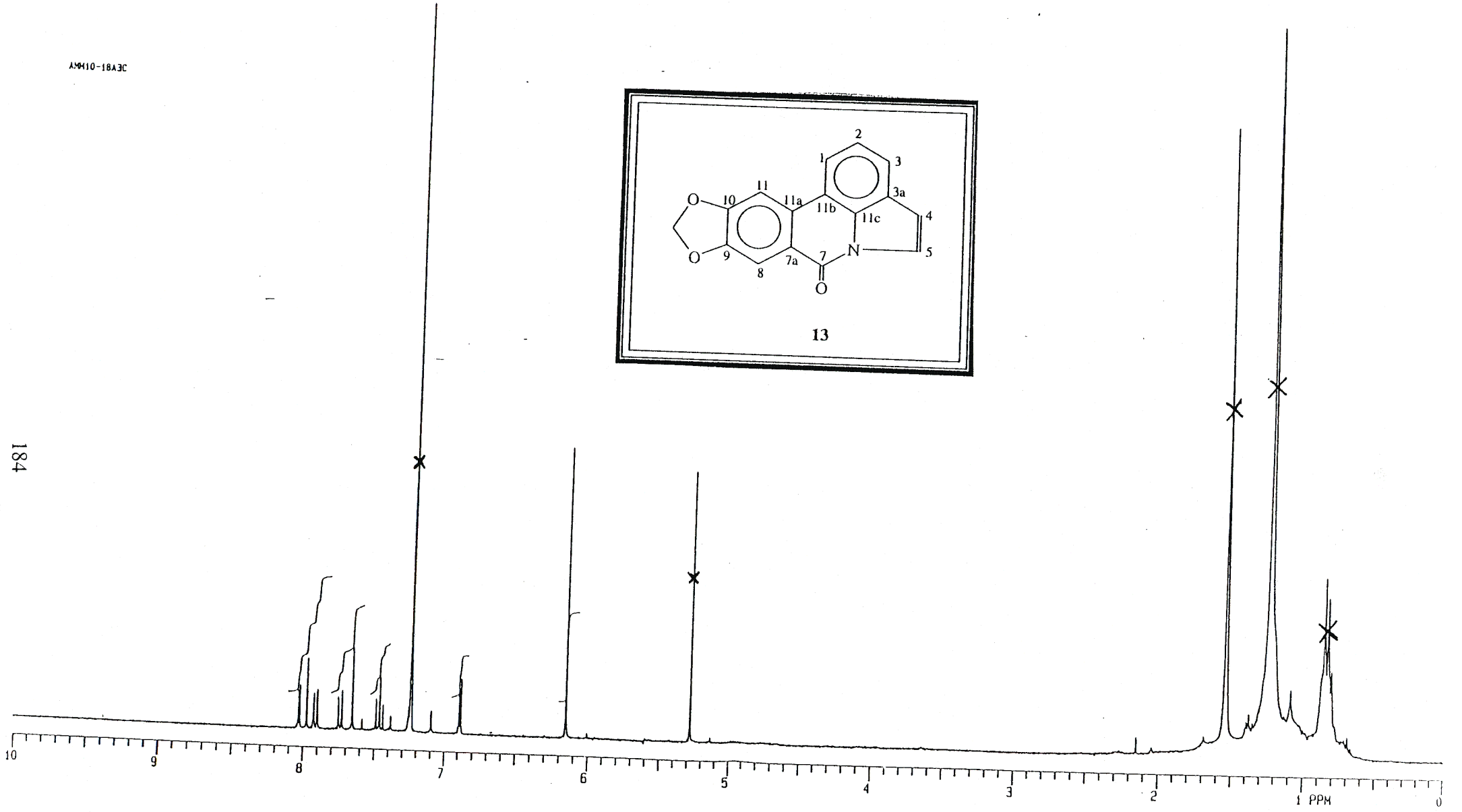
Spectrum [5-F] : NOE spectrum of 1-O-acetyl-9-norpluviine (12) in CD_3OD



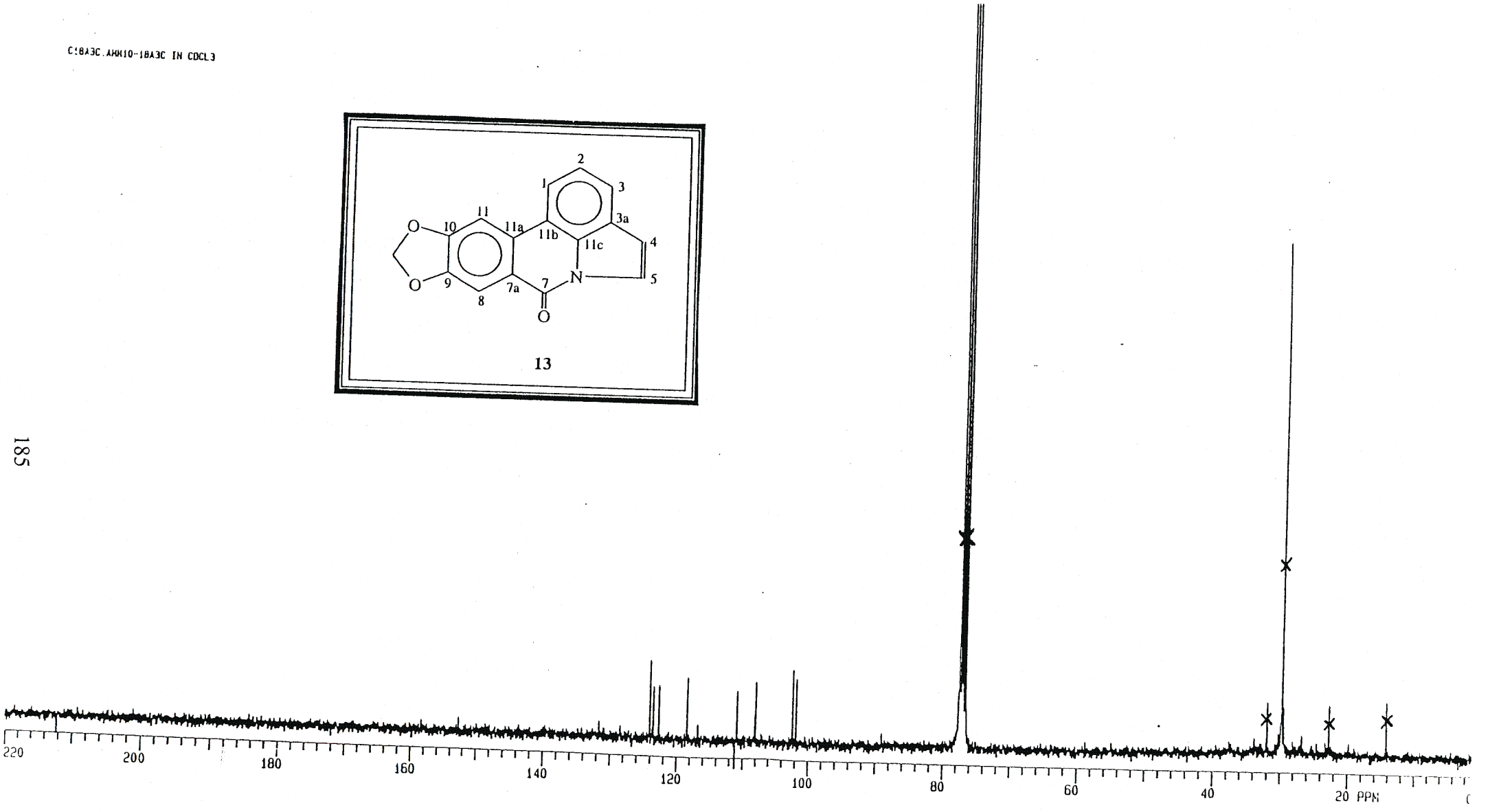
Spectrum [5-G] : Infra red spectrum of 1-O-acetyl-9-norpluviine (12)



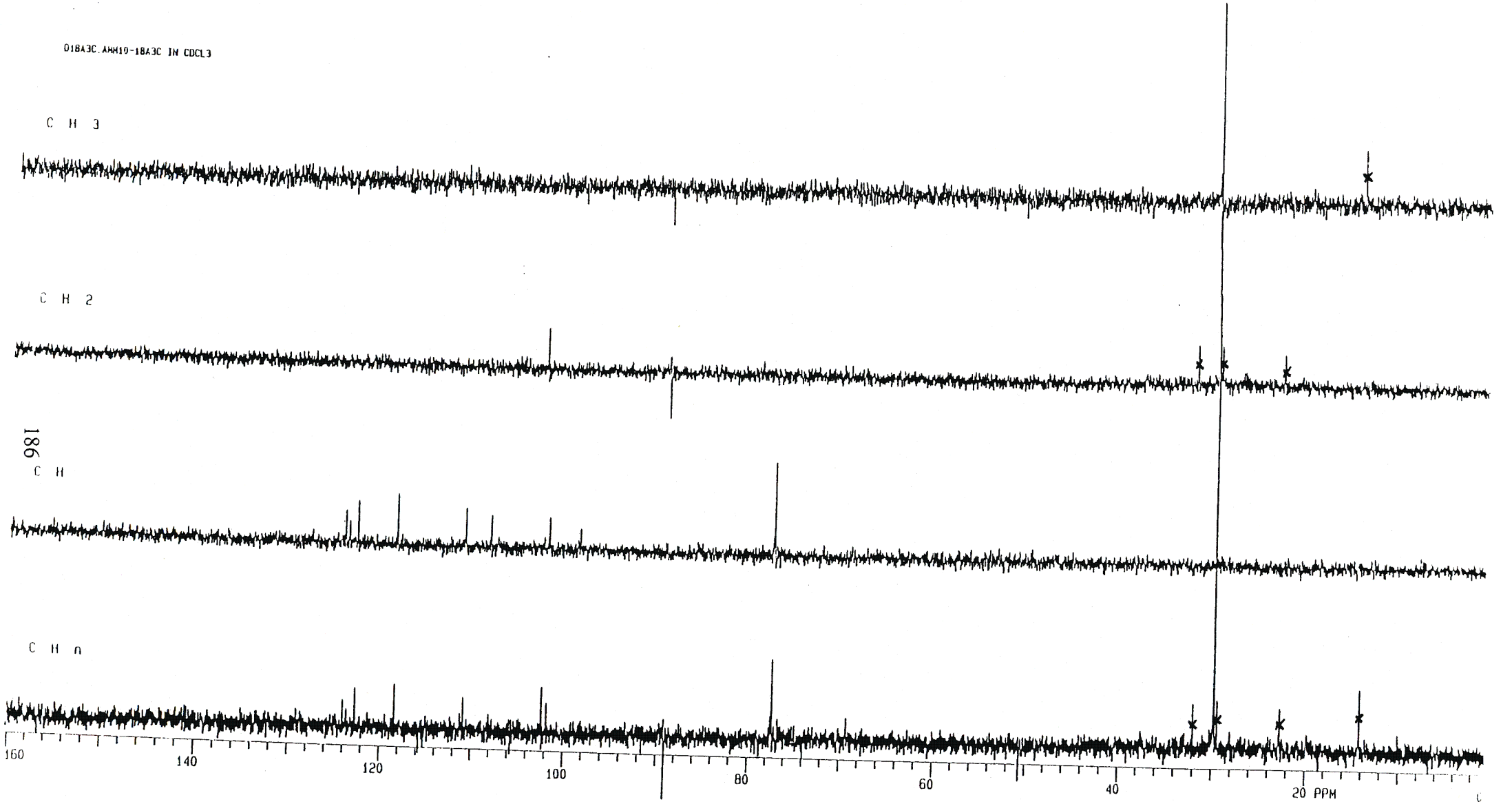
Spectrum [5-H] : Mass spectrum of 1-O-acetyl-9-norpluviine (12)



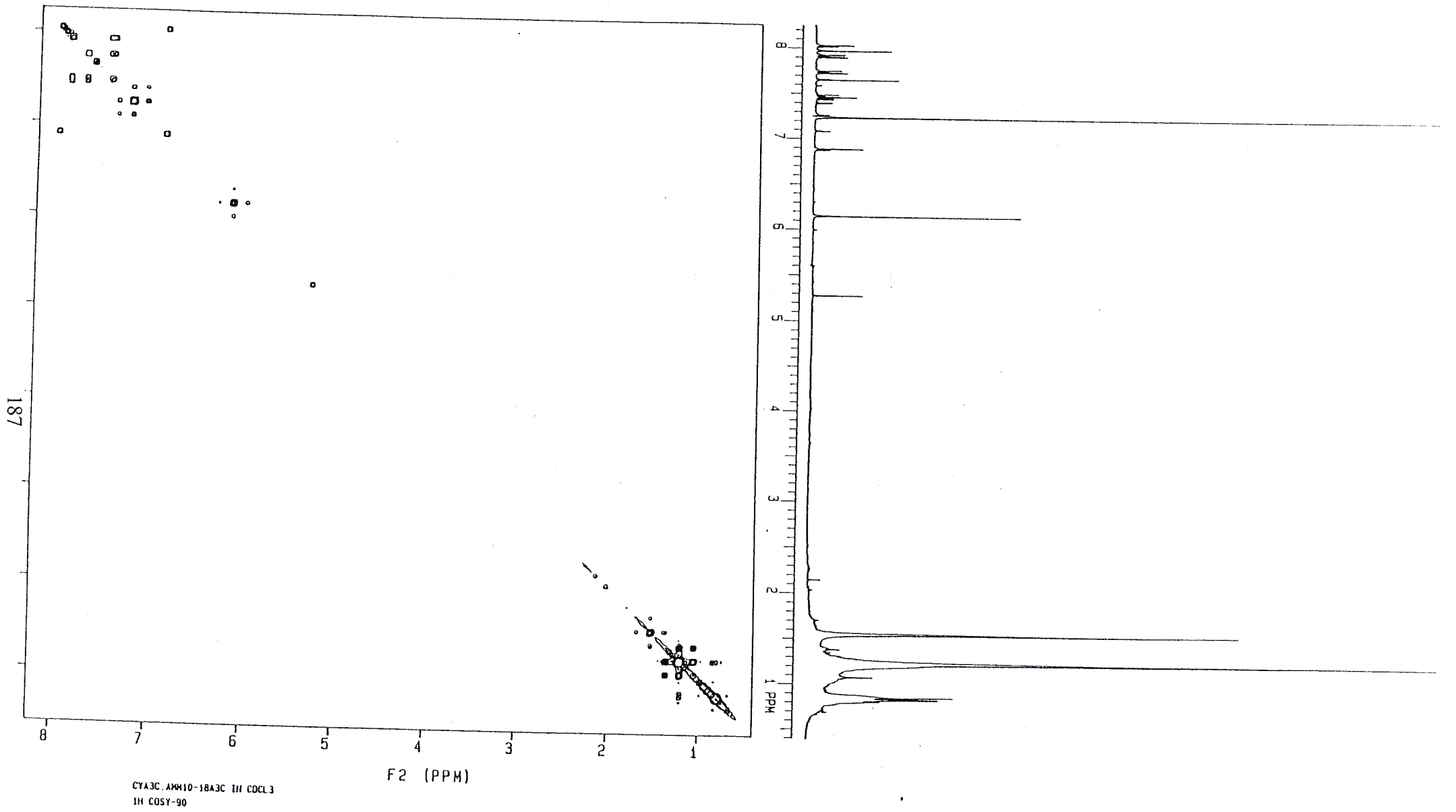
Spectrum [6-A] : ^{1}H NMR spectrum of Hippadine (13) in $CDCl_3$



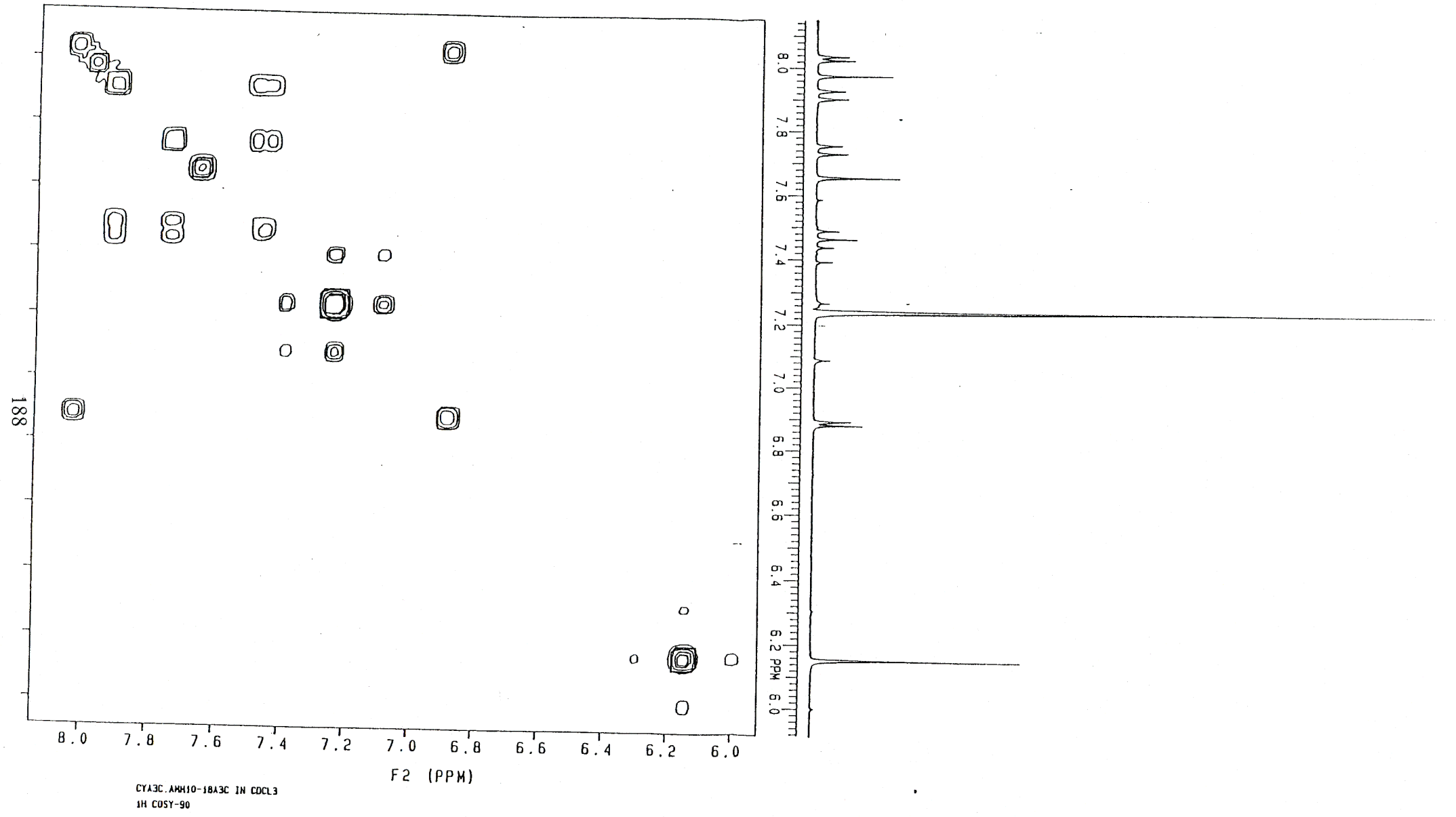
Spectrum [6-B] : ^{13}C NMR spectrum of Hippadine (13) in $CDCl_3$



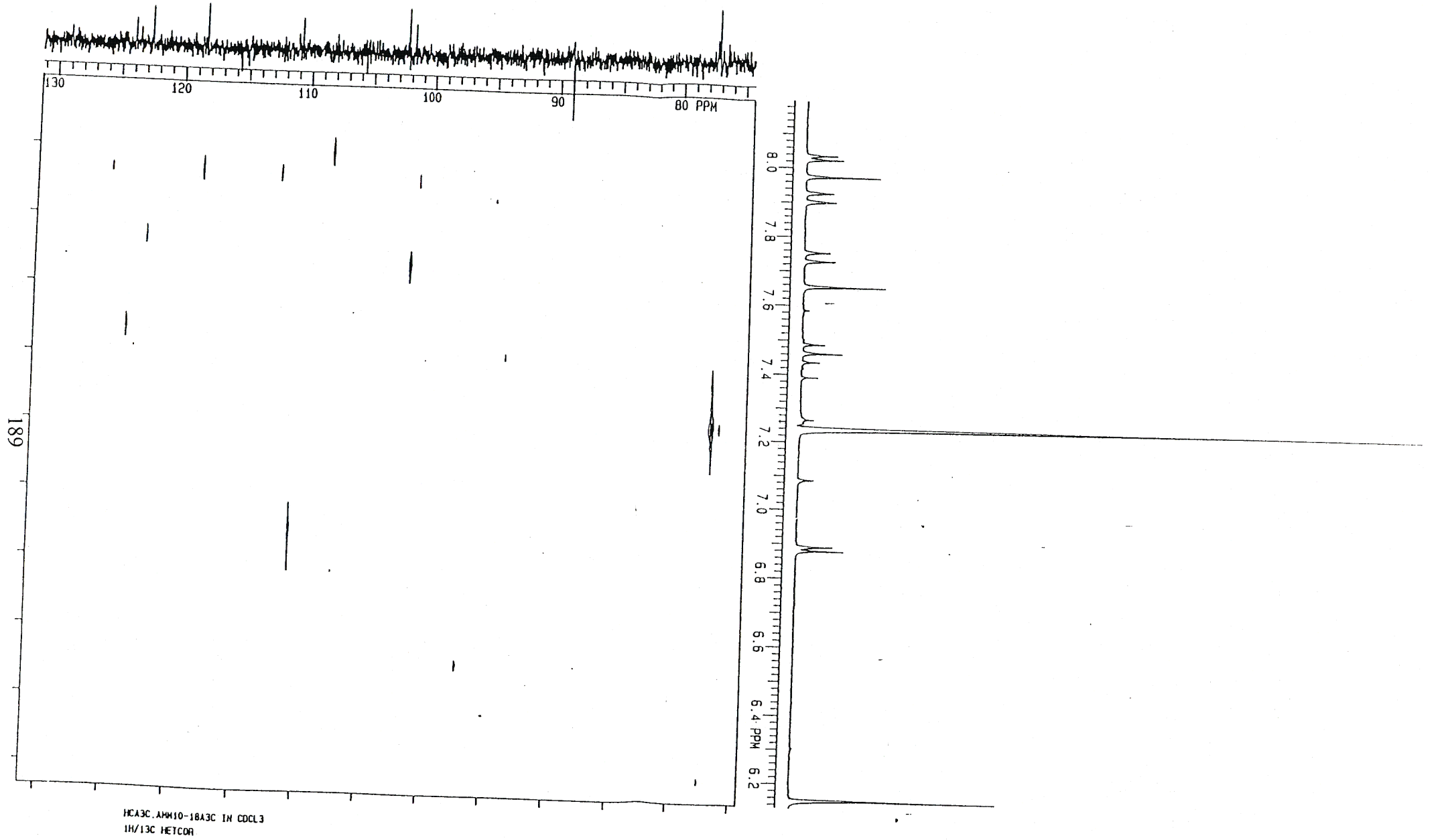
Spectrum [6-C] : ADEPT spectrum of Hippadine (13) in $CDCl_3$



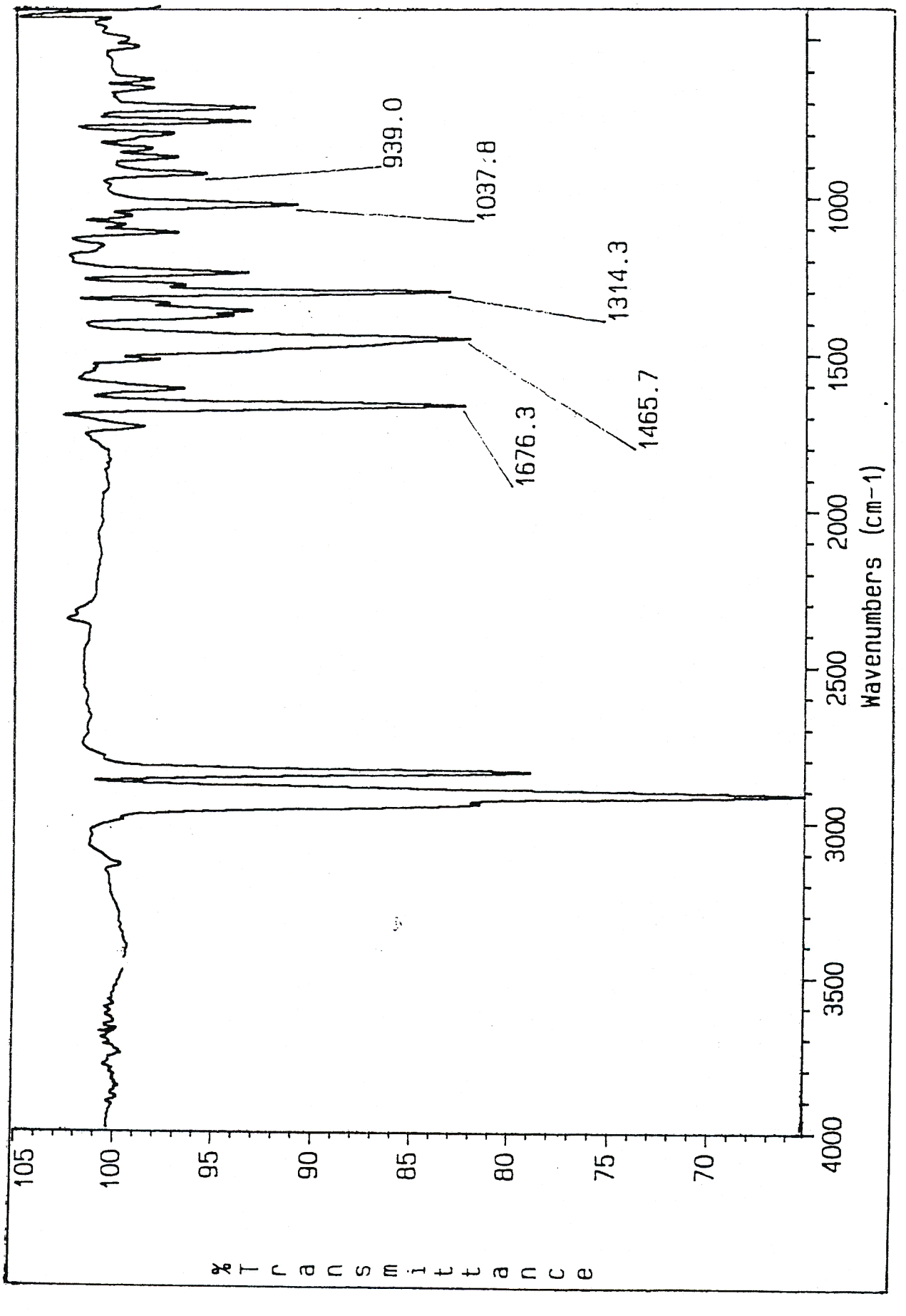
Spectrum [6-D] : COSY spectrum of Hippadine (13) in $CDCl_3$



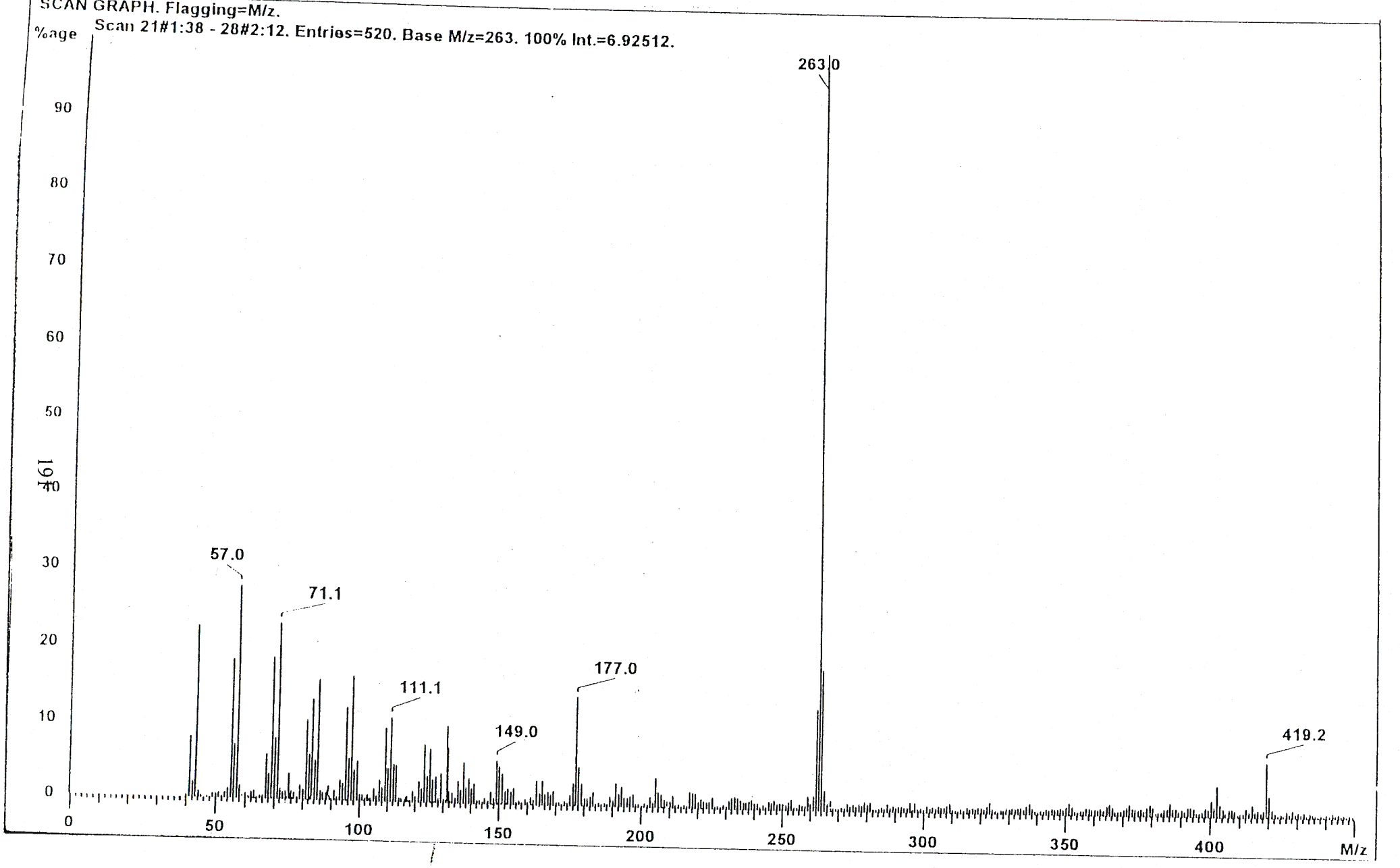
Spectrum [6-E] : Expanded COSY spectrum of Hippadine (13) in $CDCl_3$



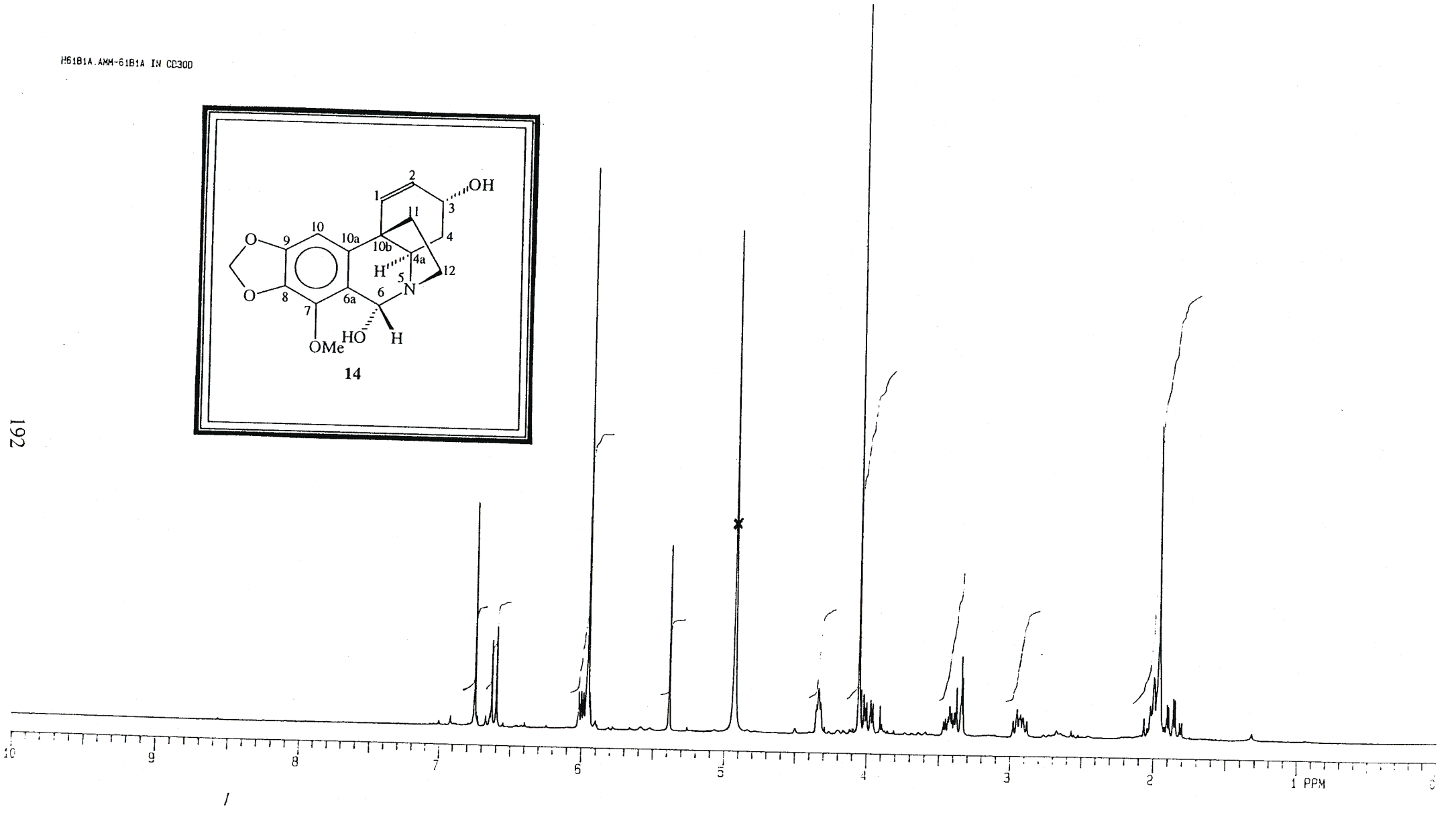
Spectrum [6-F] : HETCOR spectrum of Hippadine (13) in $CDCl_3$



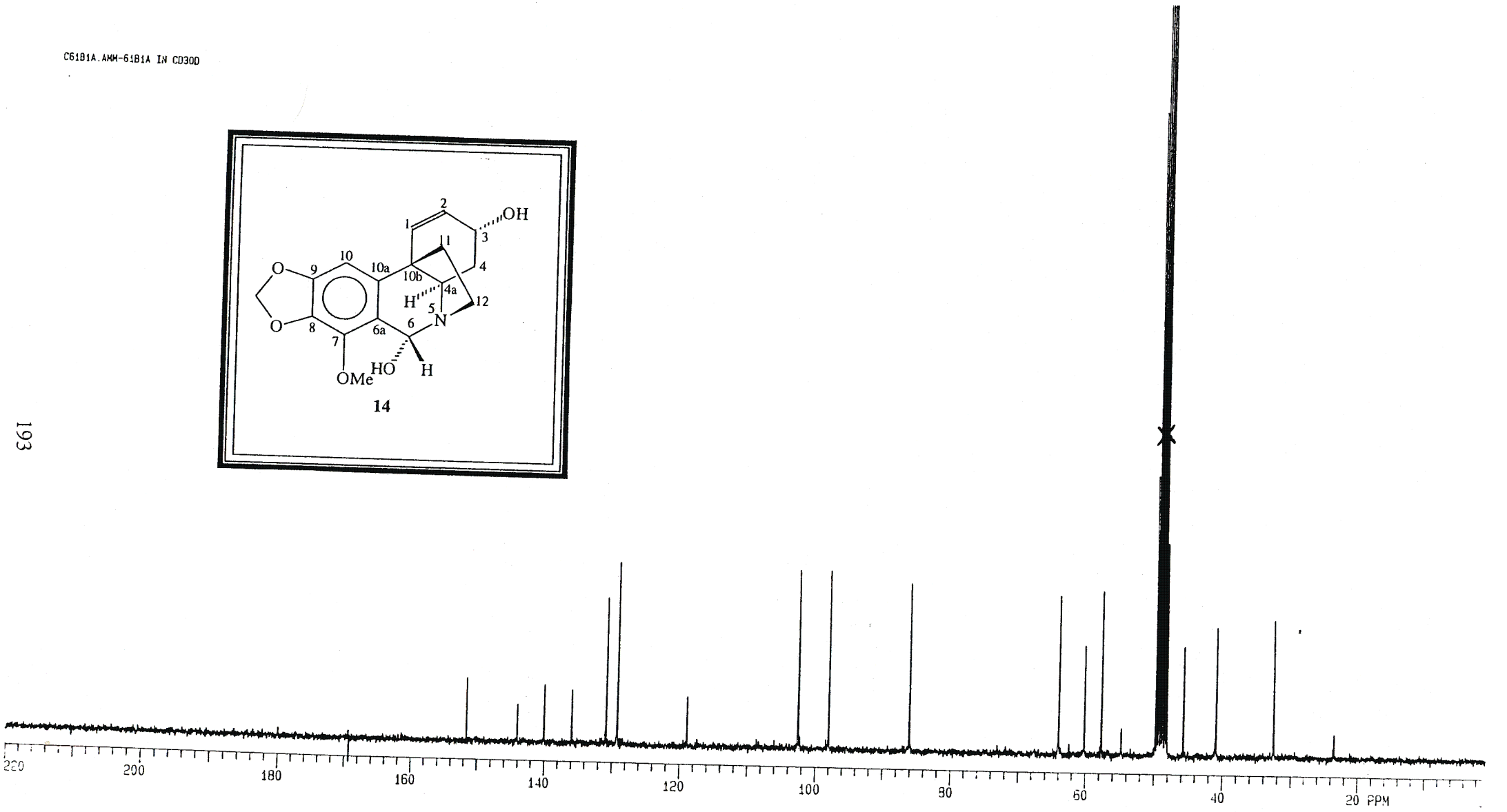
Spectrum [6-G] : Infra red spectrum of Hippadine (13)



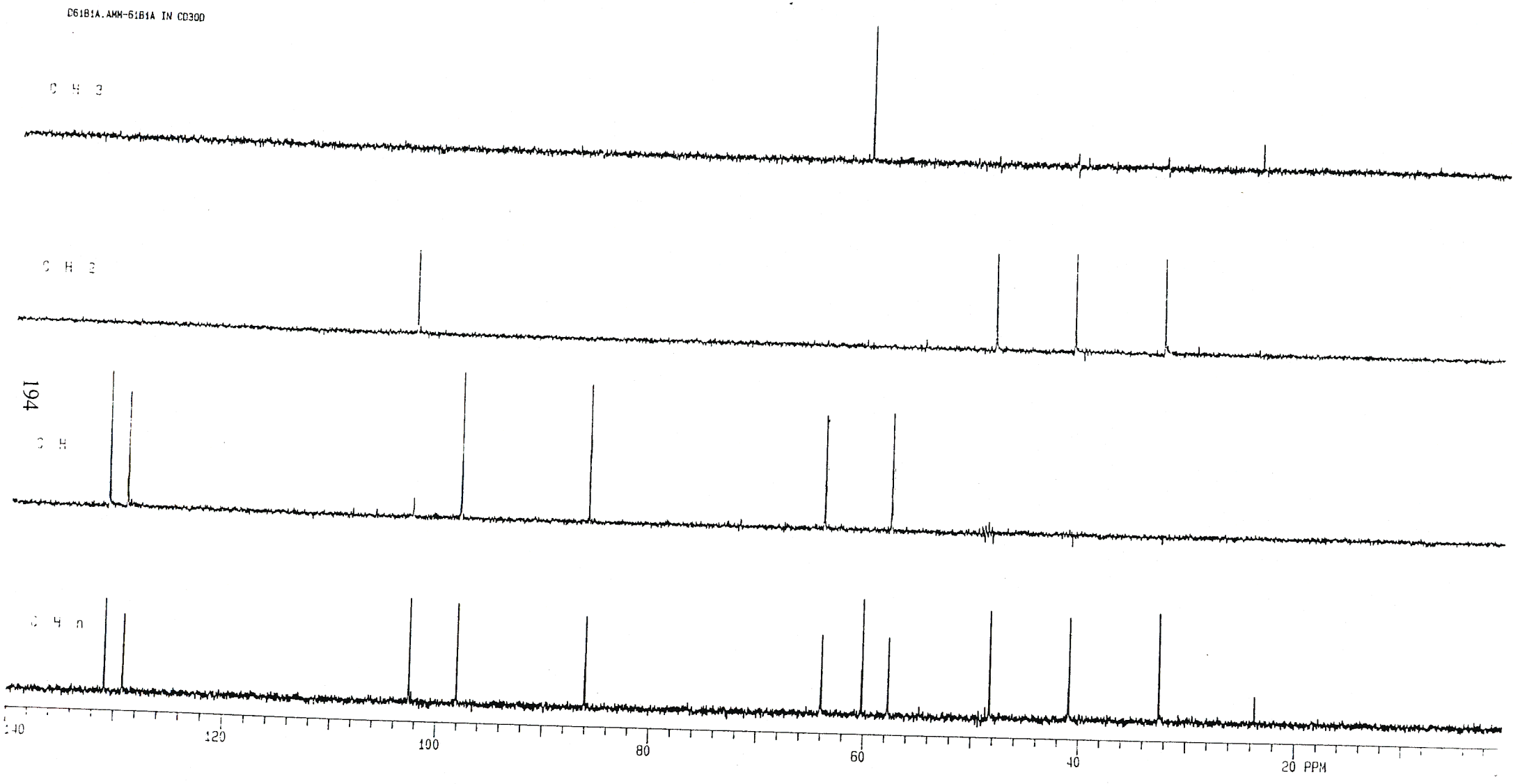
Spectrum [6-H] : Mass spectrum of Hippadine (13)



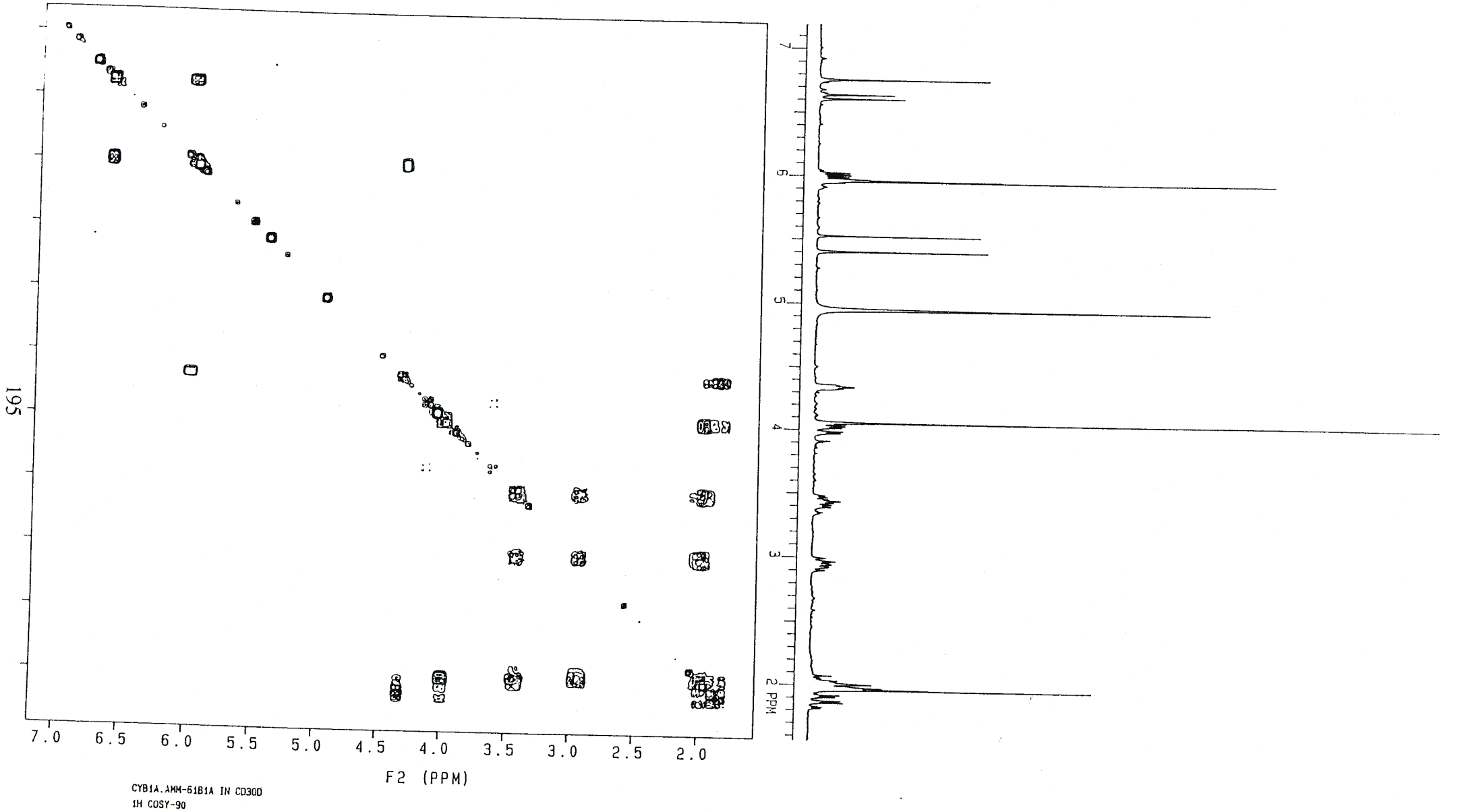
Spectrum [7-A] : ^{1}H NMR spectrum of 6-α-hydroxypowelline (14) in CD_3OD



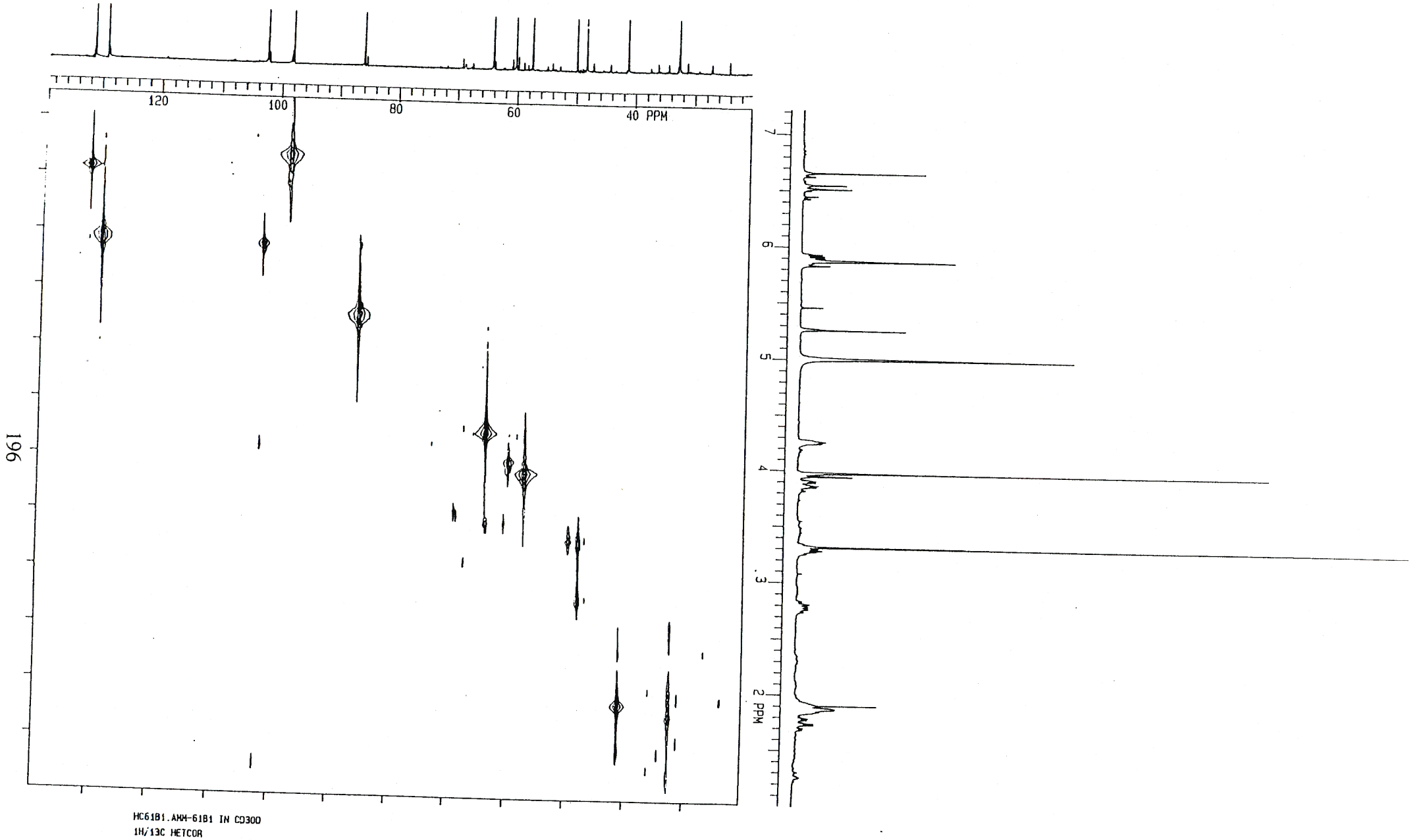
Spectrum [7-B] : ^{13}C NMR spectrum of 6-α-hydroxypowelline (14) in CD_3OD



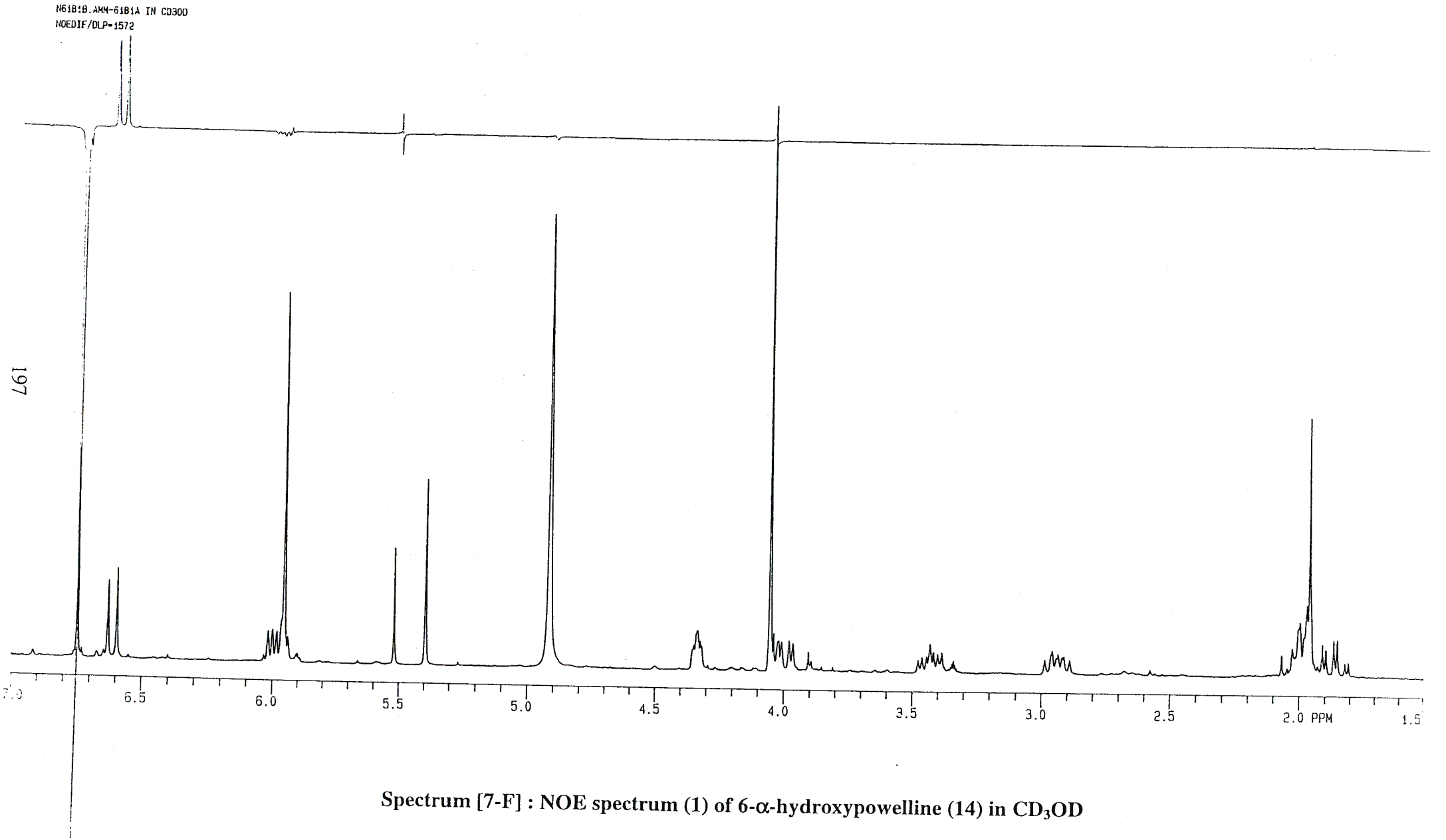
Spectrum [7-C] : ADEPT spectrum of 6-α-hydroxypowelline (14) in CD_3OD



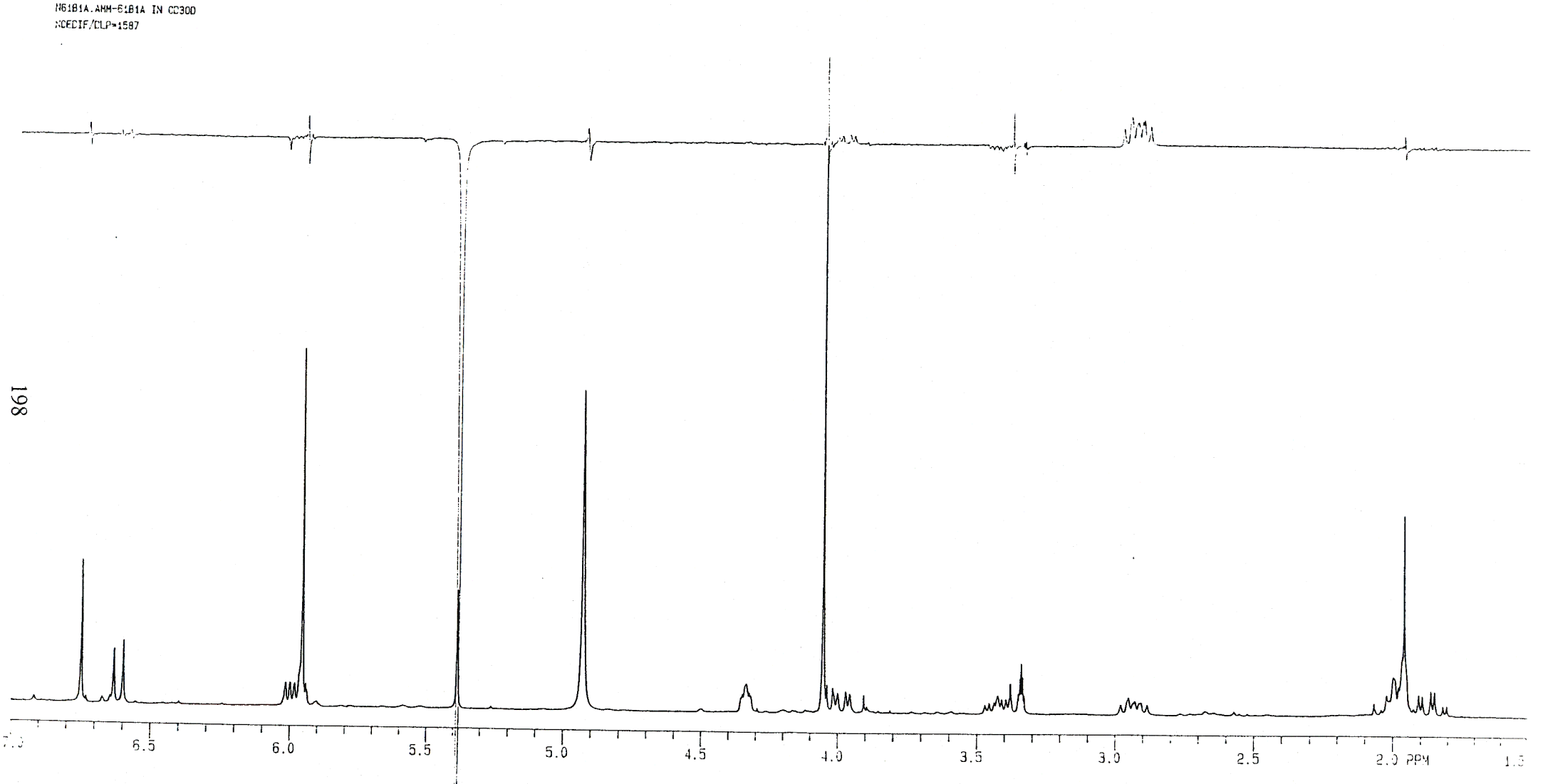
Spectrum [7-D] : COSY spectrum of 6-α-hydroxypowelline (14) in CD_3OD



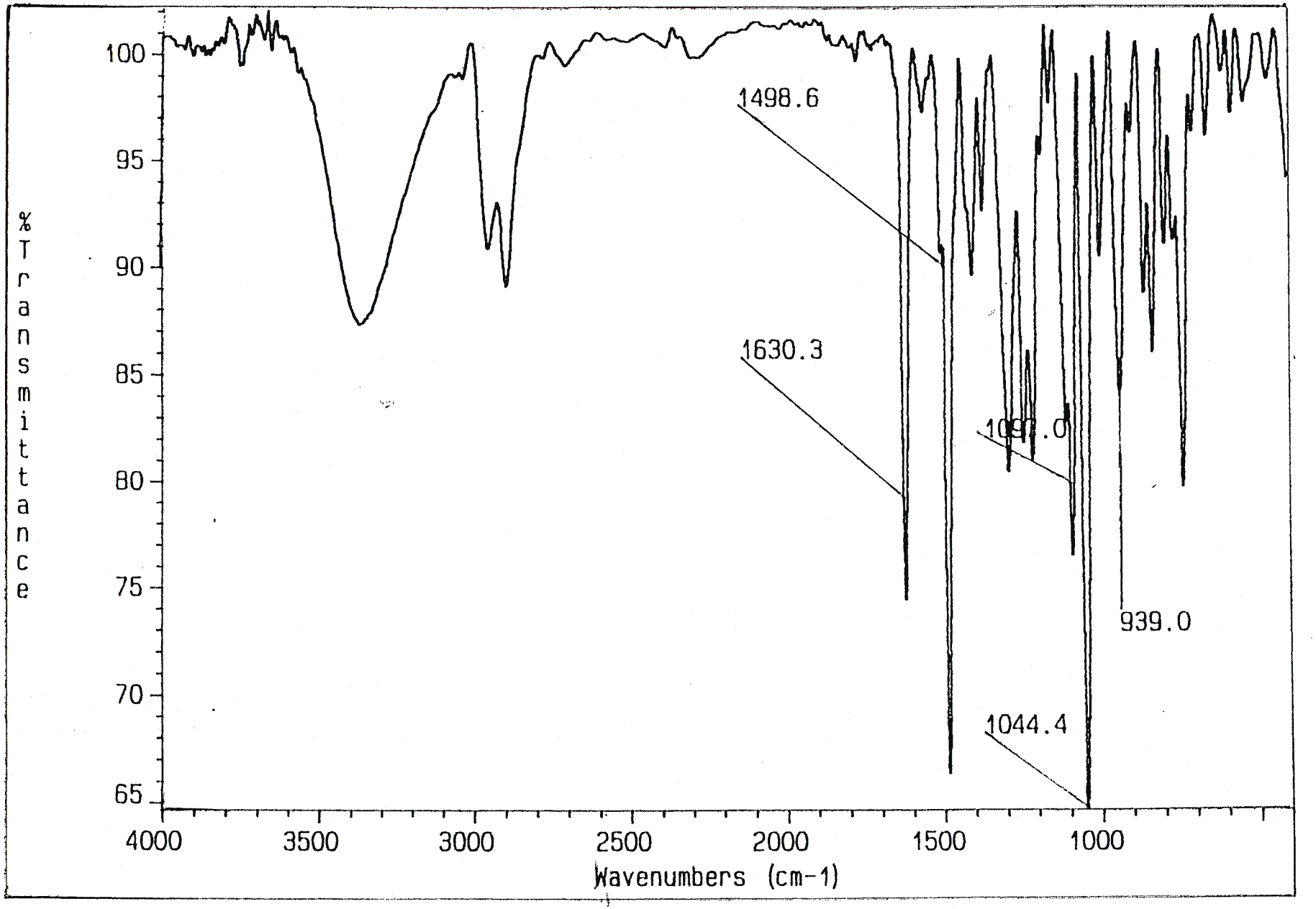
Spectrum [7-E] : HETCOR spectrum of 6-α-hydroxypowelline (14) in CD_3OD



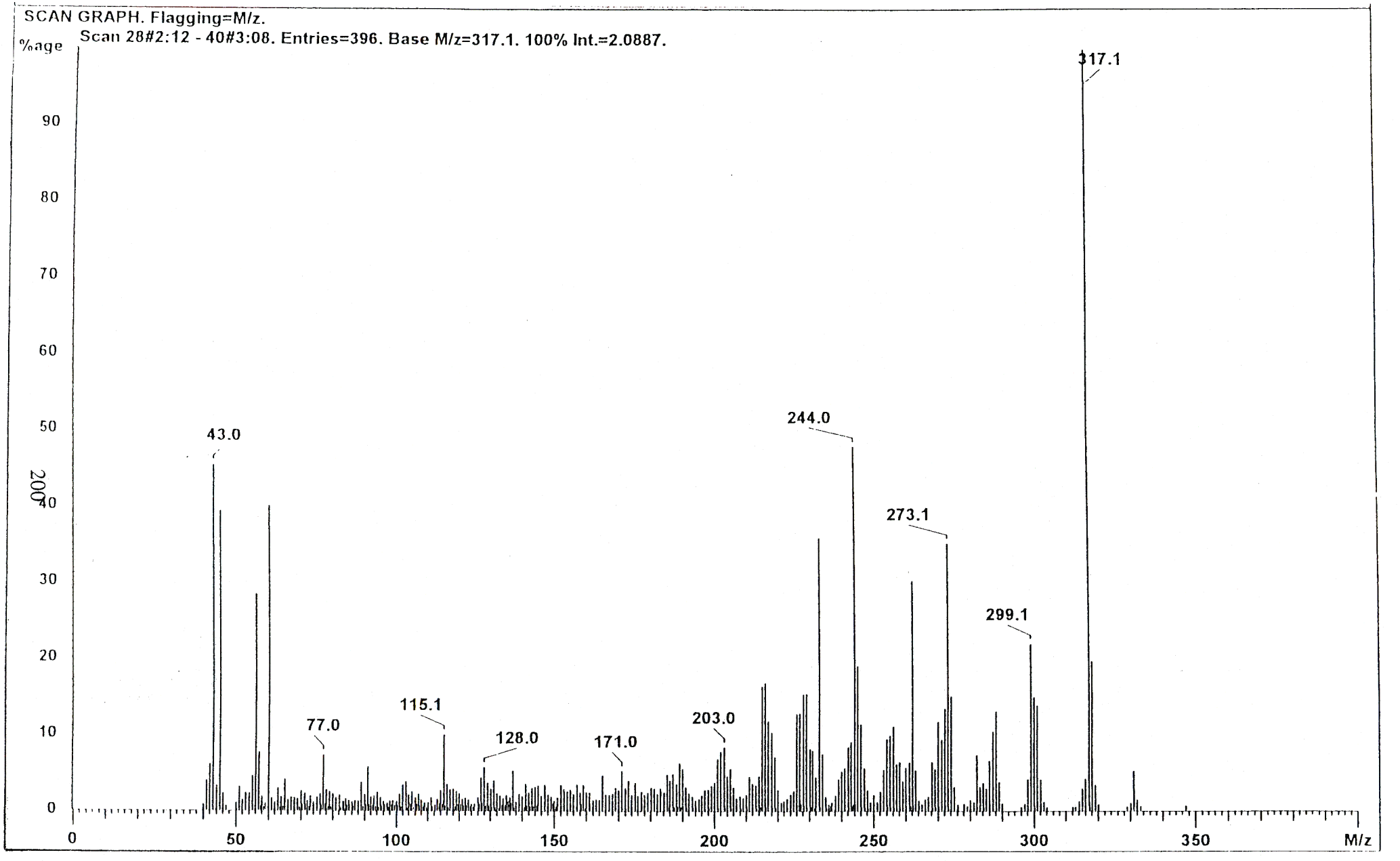
Spectrum [7-F] : NOE spectrum (1) of 6-α-hydroxypowelline (14) in CD_3OD



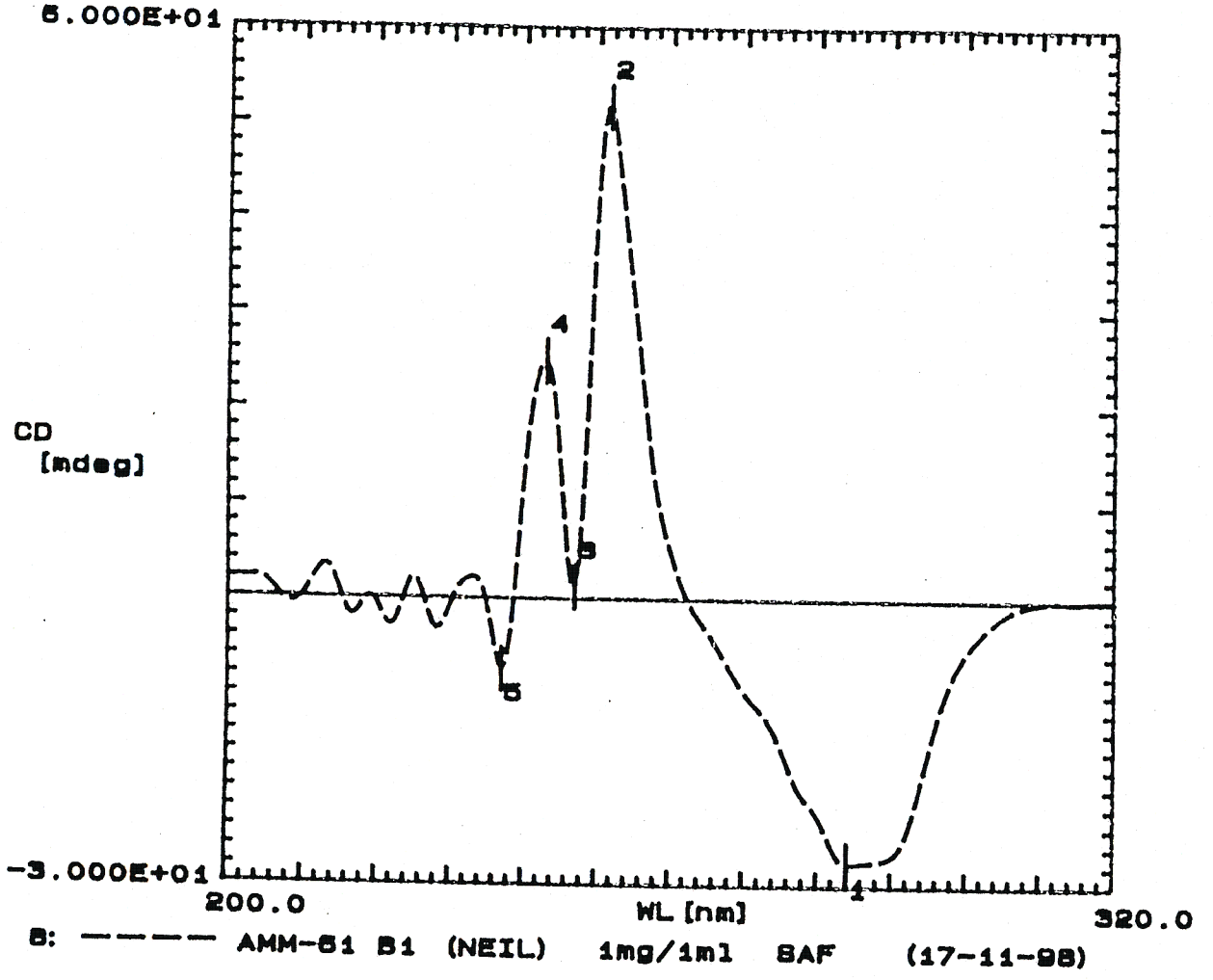
Spectrum [7-G] : NOE spectrum (2) of 6-α-hydroxypowelline (14) in CD_3OD



Spectrum [7-H] : Infra red spectrum of 6-α-hydroxypowelline (14)

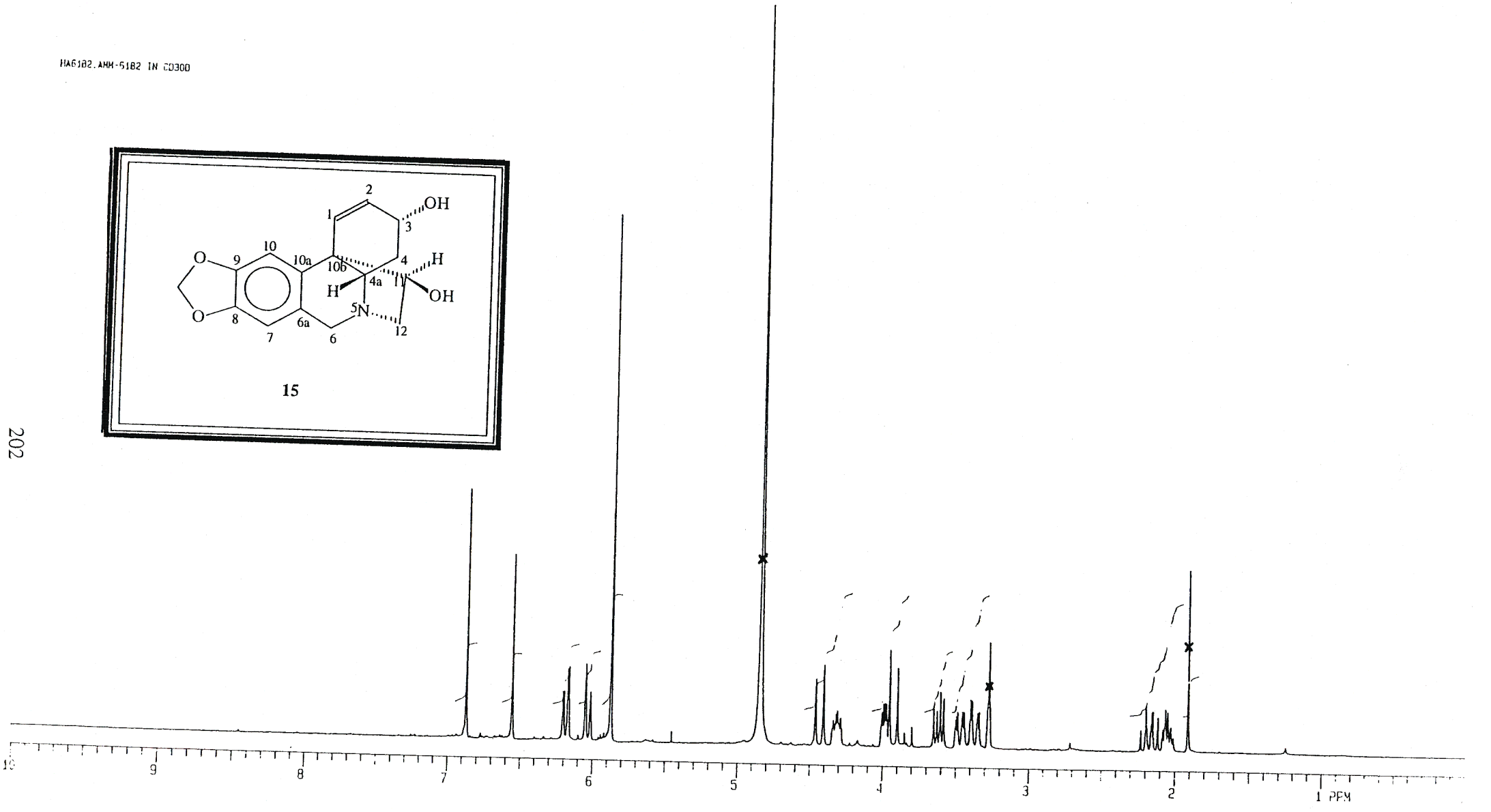


Spectrum [7-I] : Mass spectrum of 6-α-hydroxypowelline (14)

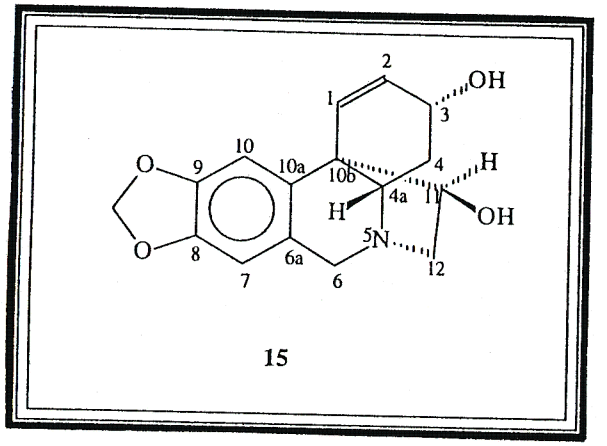


No.	Wavelength	Value
1	284.00 nm	-2.770E+01
2	251.50 nm	5.183E+01
3	247.00 nm	1.223E+00
4	243.00 nm	2.489E+01
5	237.00 nm	-7.498E+00

Spectrum [7-J] : Circular dichroism spectrum of 6-α-hydroxypowelline (14)

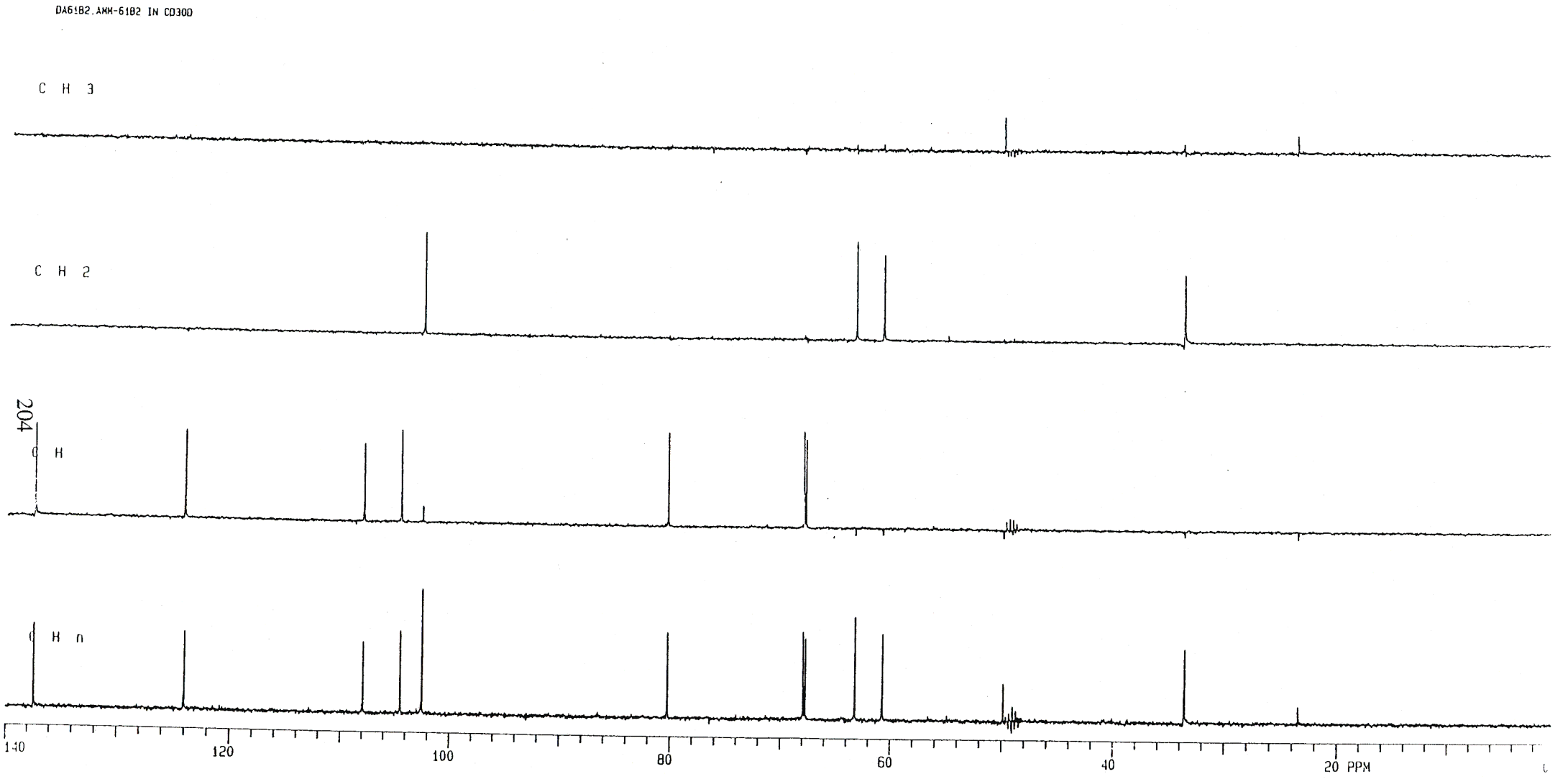


Spectrum [8-A] : ^{1}H NMR spectrum of hamayne (15) in CD_3OD

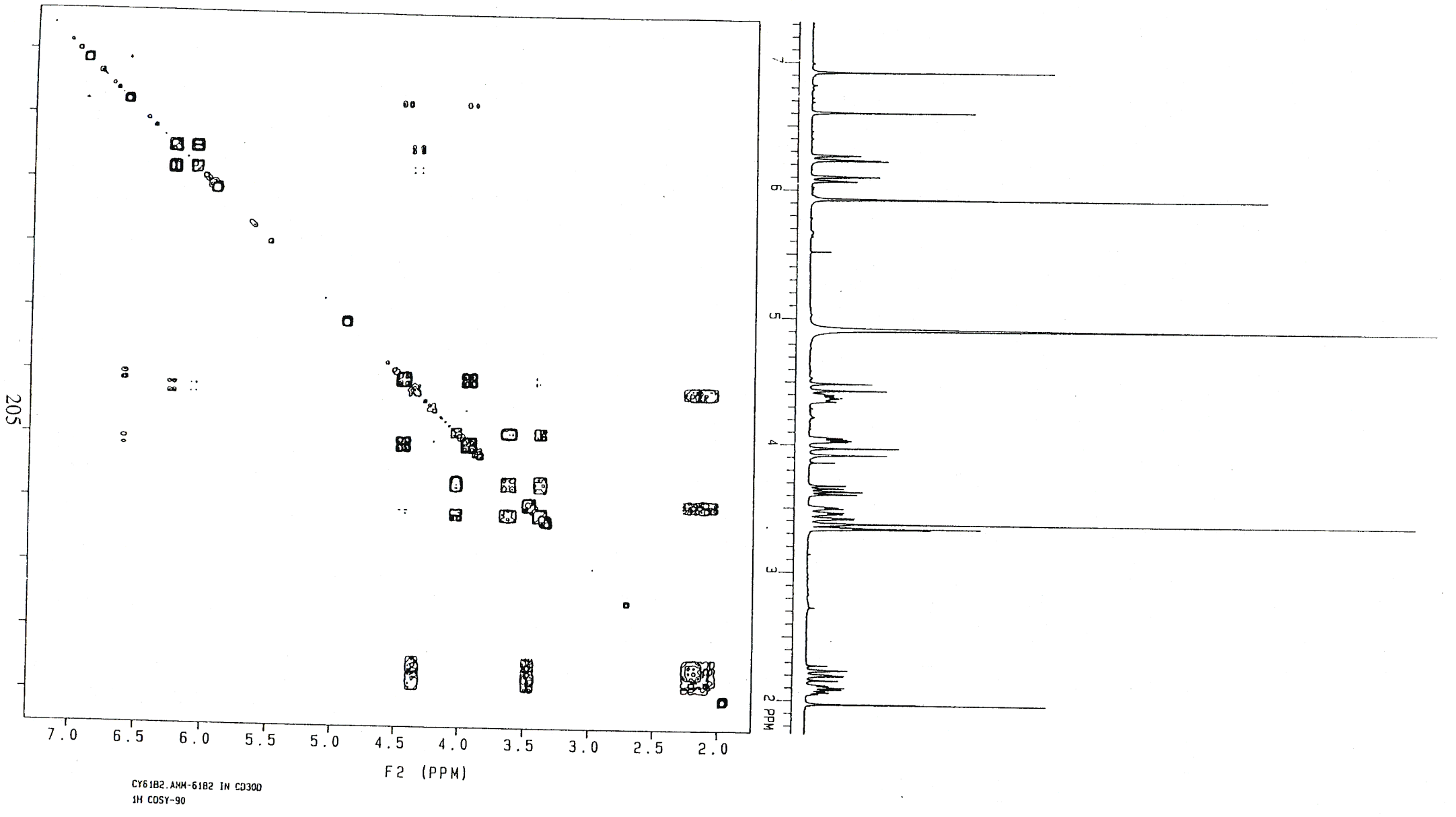


15

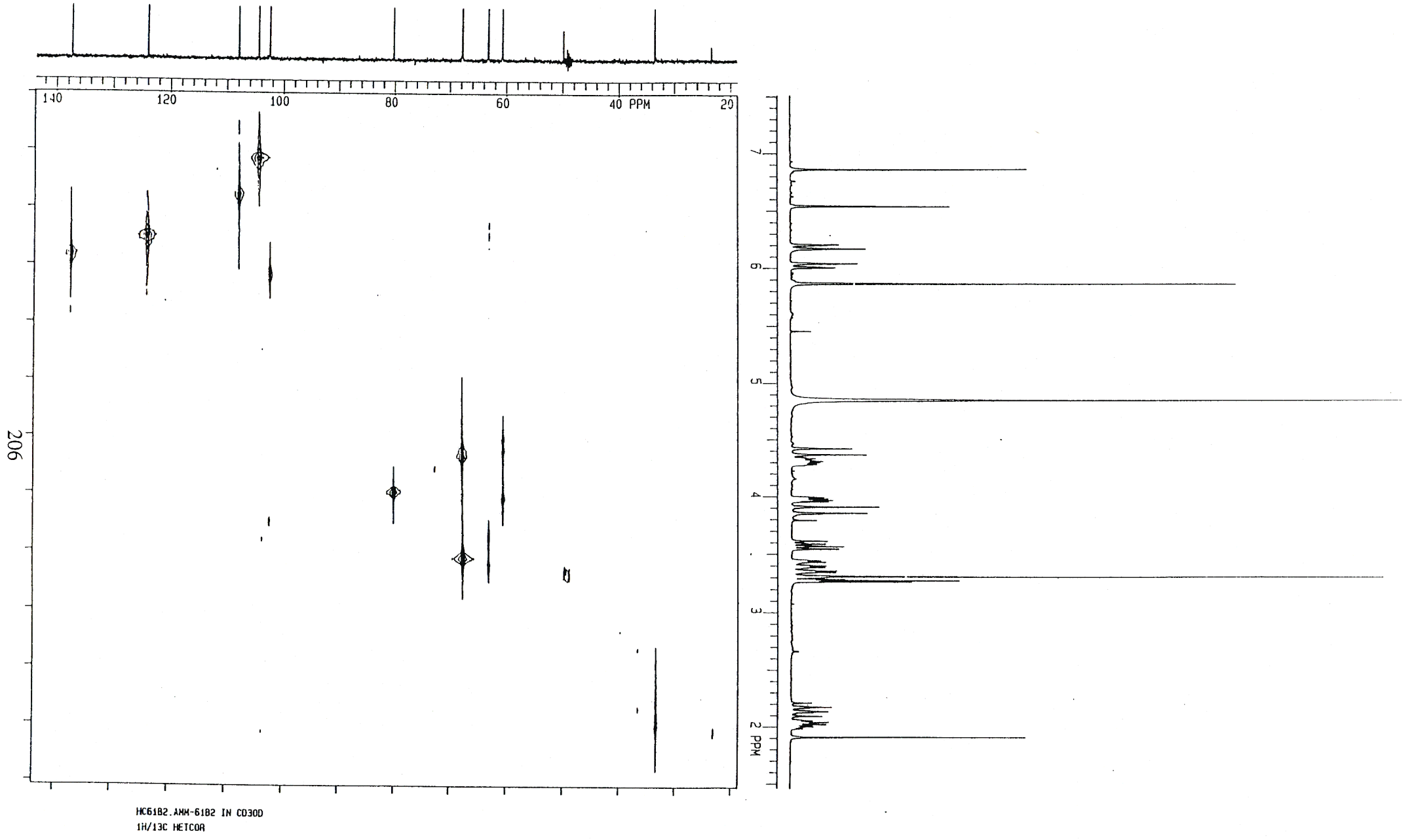
Spectrum [8-B] : ^{13}C NMR spectrum of hamayne (15) in CD_3OD



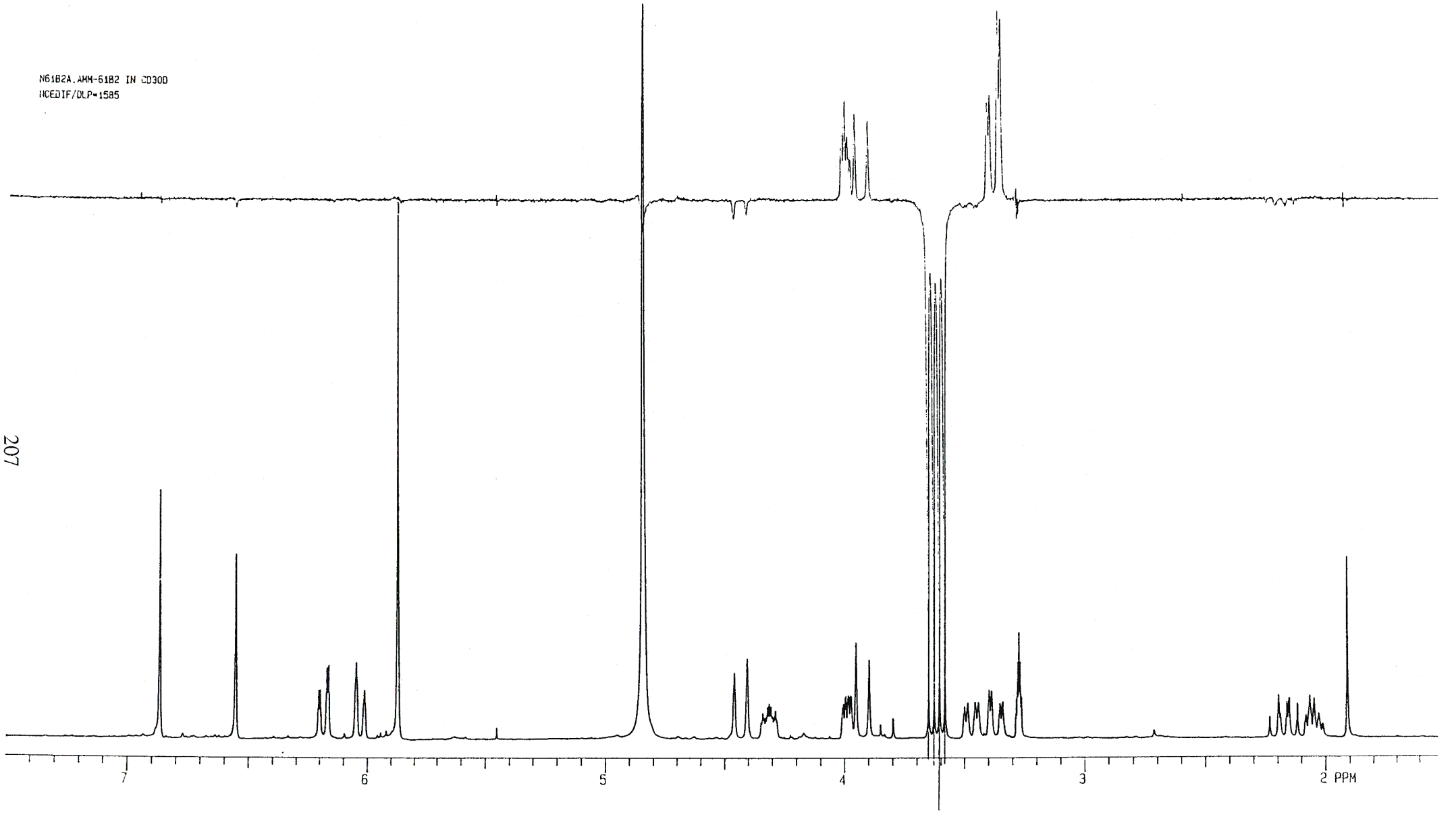
Spectrum [8-C] : ADEPT spectrum of hamayne (15) in CD_3OD



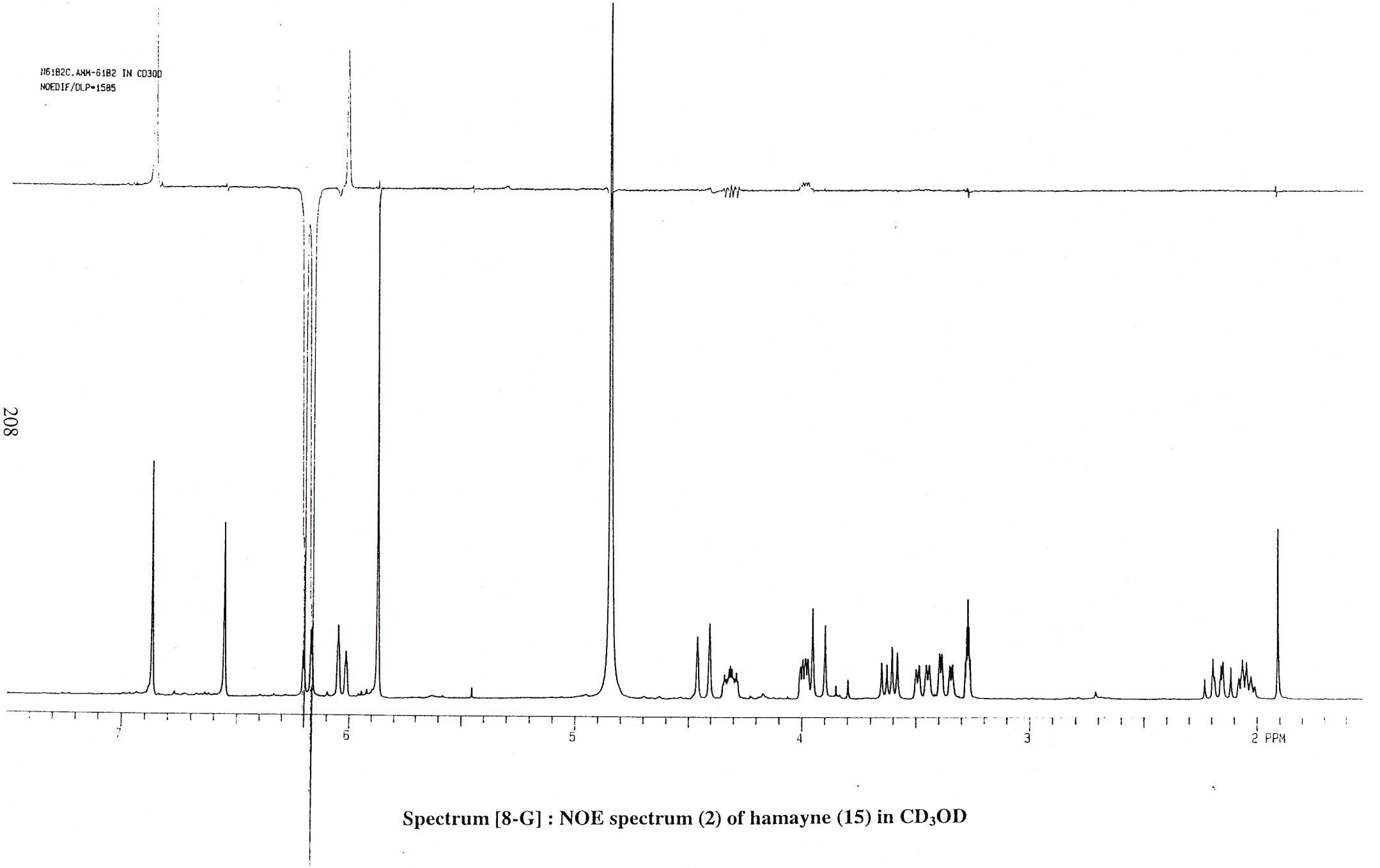
Spectrum [8-D] : COSY spectrum of hamayne (15) in CD_3OD



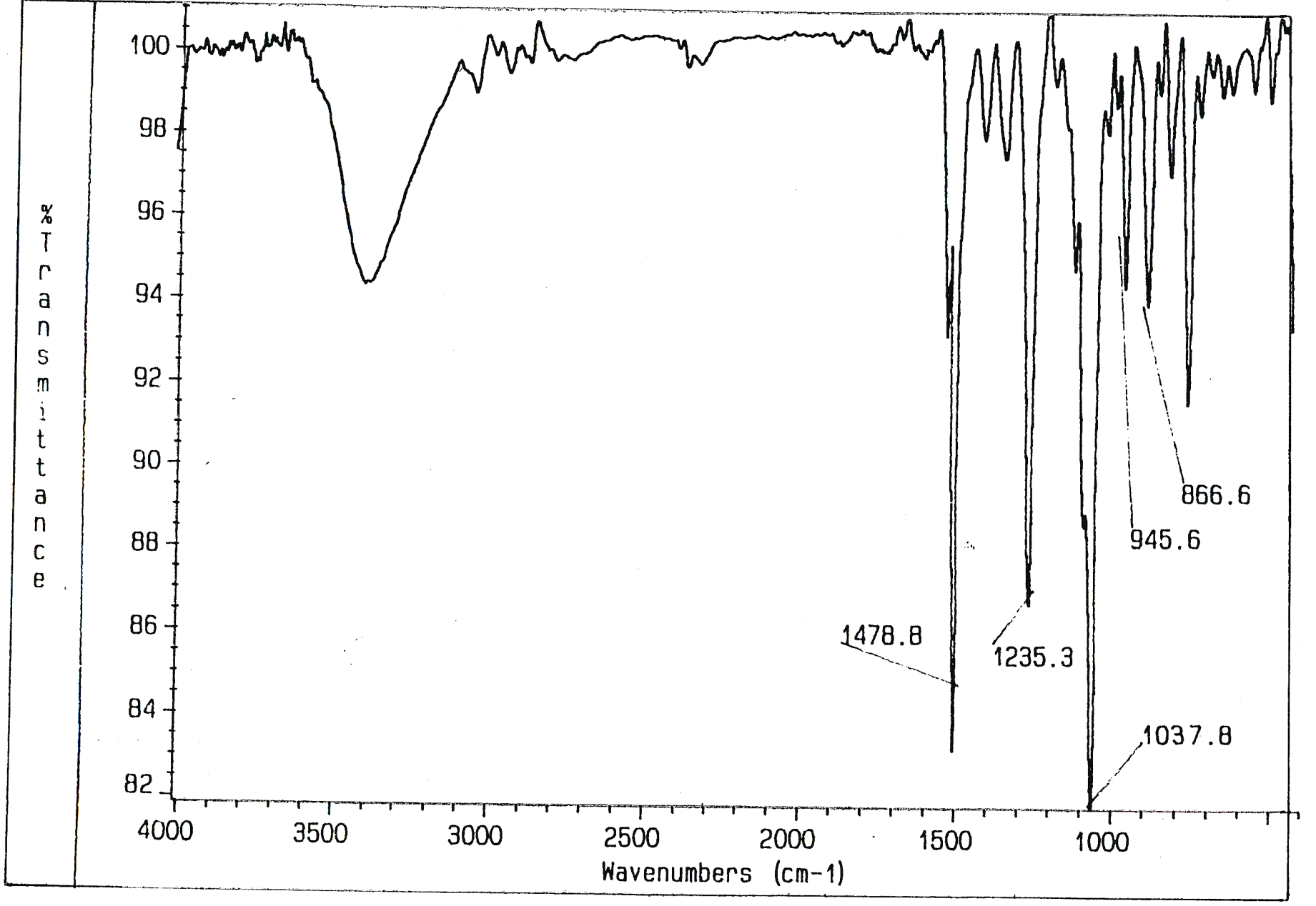
Spectrum [8-E] : HETCOR spectrum of hamayne (15) in CD_3OD



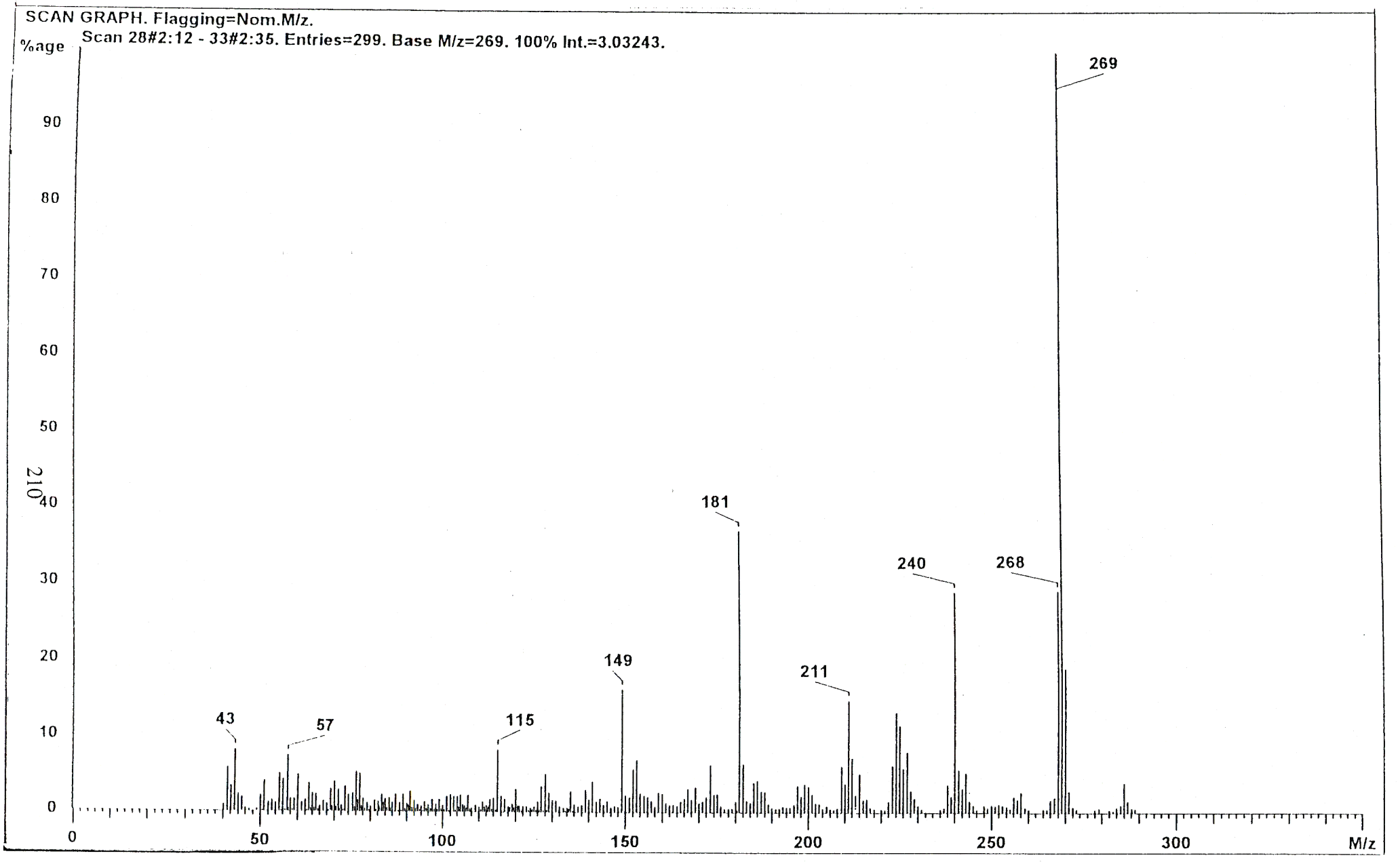
Spectrum [8-F] : NOE spectrum (1) of hamayne (15) in CD_3OD



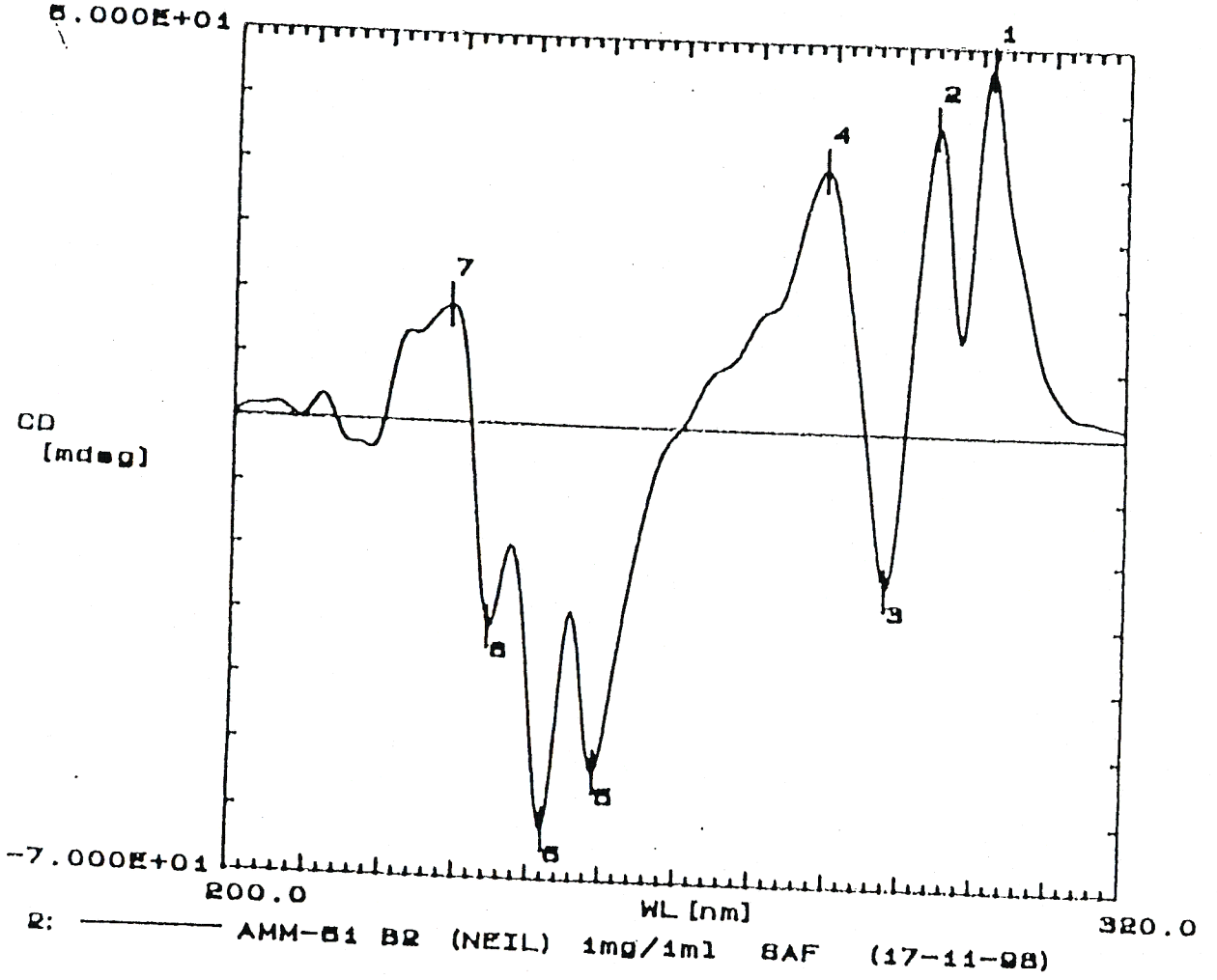
Spectrum [8-G] : NOE spectrum (2) of hamayne (15) in CD_3OD



Spectrum [8-H] : Infra red spectrum of hamayne (15)

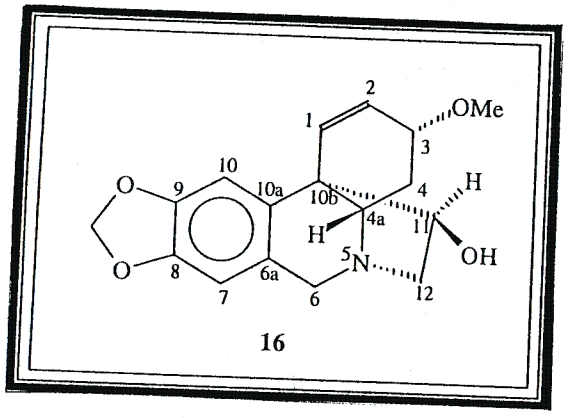


Spectrum [8-I] : Mass spectrum of hamayne (15)



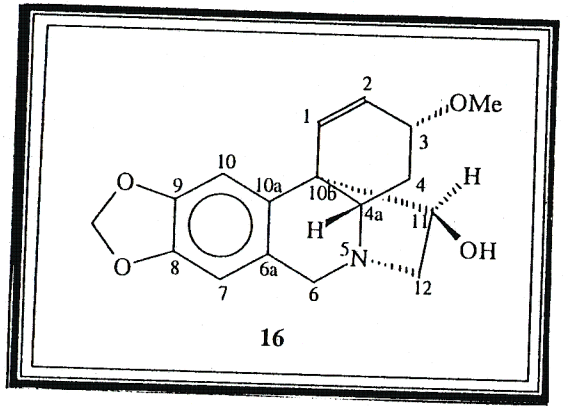
No.	Wavelength	Value
1	301.50 nm	5.709E+01
2	294.00 nm	4.753E+01
3	287.50 nm	-2.422E+01
4	279.00 nm	4.063E+01
5	249.00 nm	-5.364E+01
6	242.00 nm	-5.241E+01
7	226.50 nm	1.514E+01
8	234.00 nm	-3.184E+01

Spectrum [8-J] : Circular dichroism spectrum of hamayne (15)



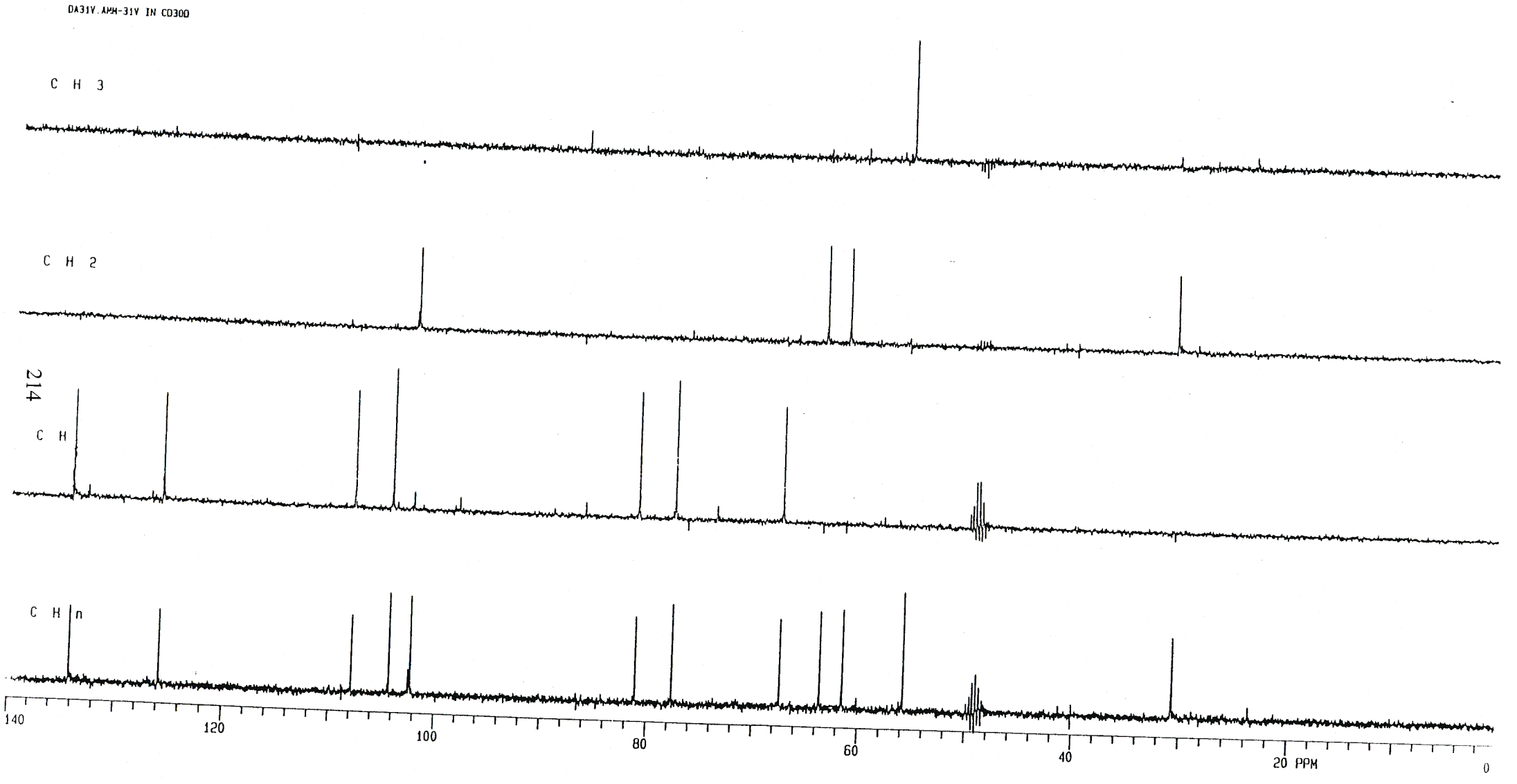
16

Spectrum [9-A] : ^{1}H NMR spectrum of crinamine (16) in CD_3OD

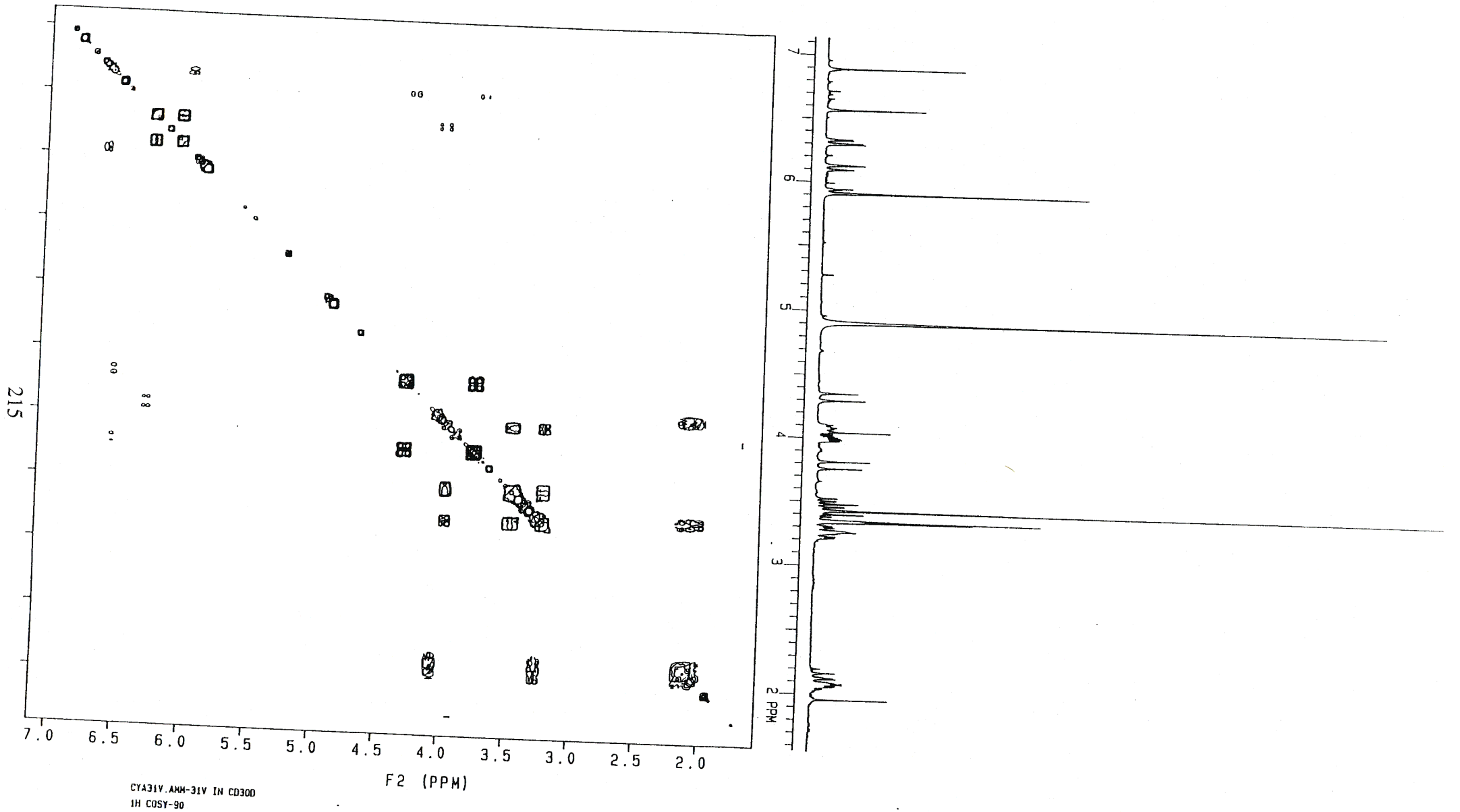


16

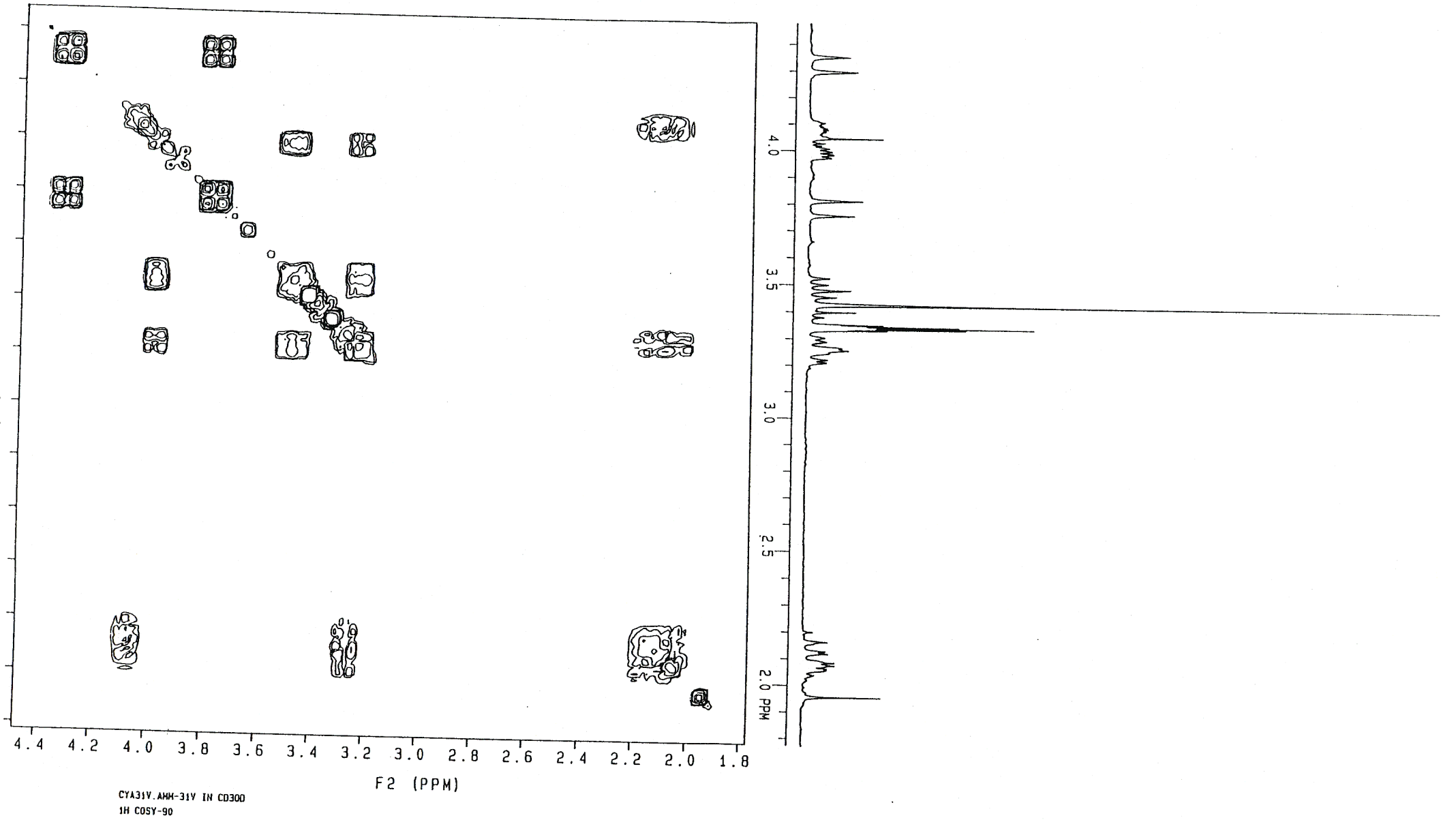
Spectrum [9-B] : ^{13}C NMR spectrum of crinamine (16) in CD_3OD



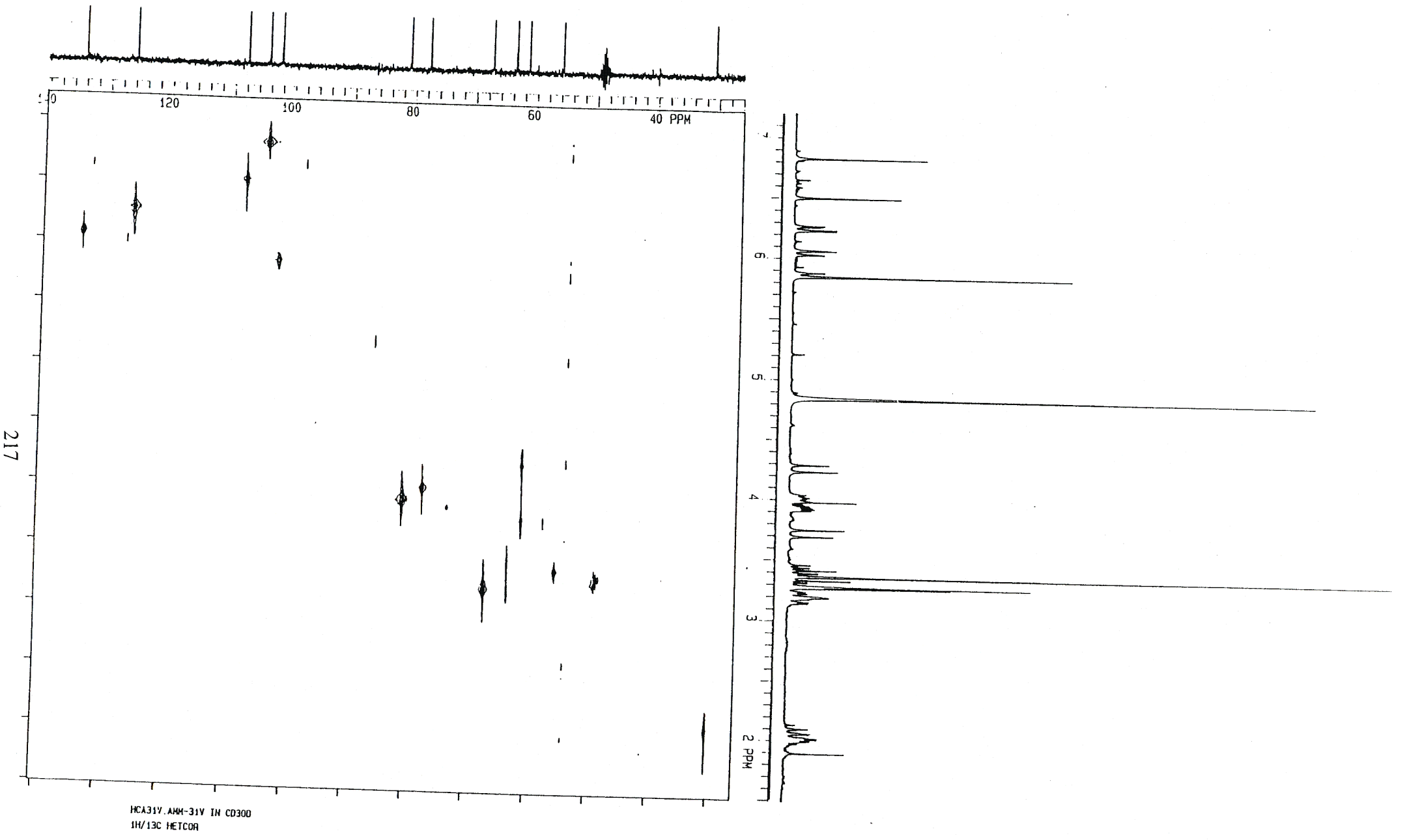
Spectrum [9-C] : ADEPT spectrum of crinamine (16) in CD_3OD



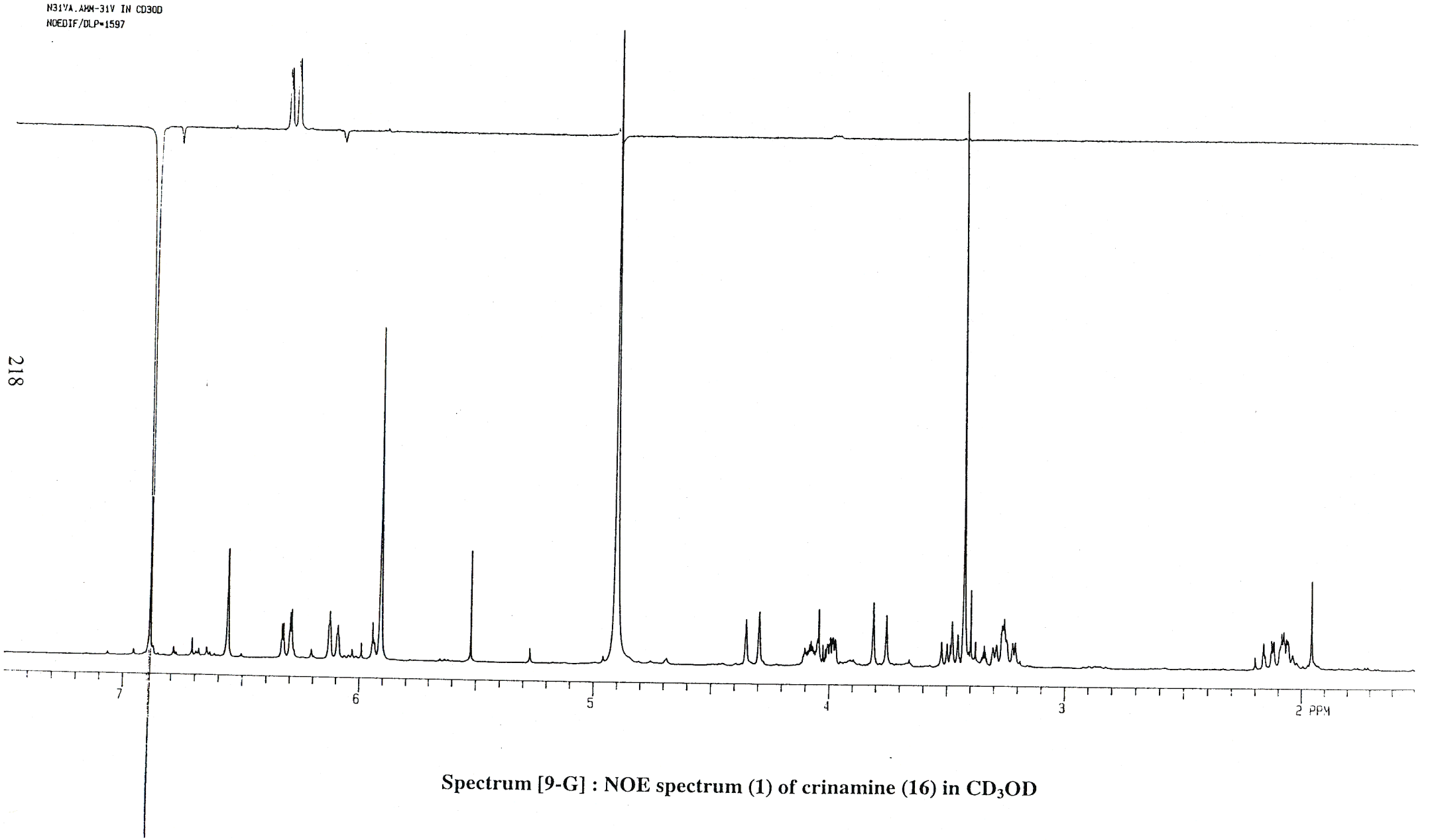
Spectrum [9-D] : COSY spectrum of crinamine (16) in CD_3OD



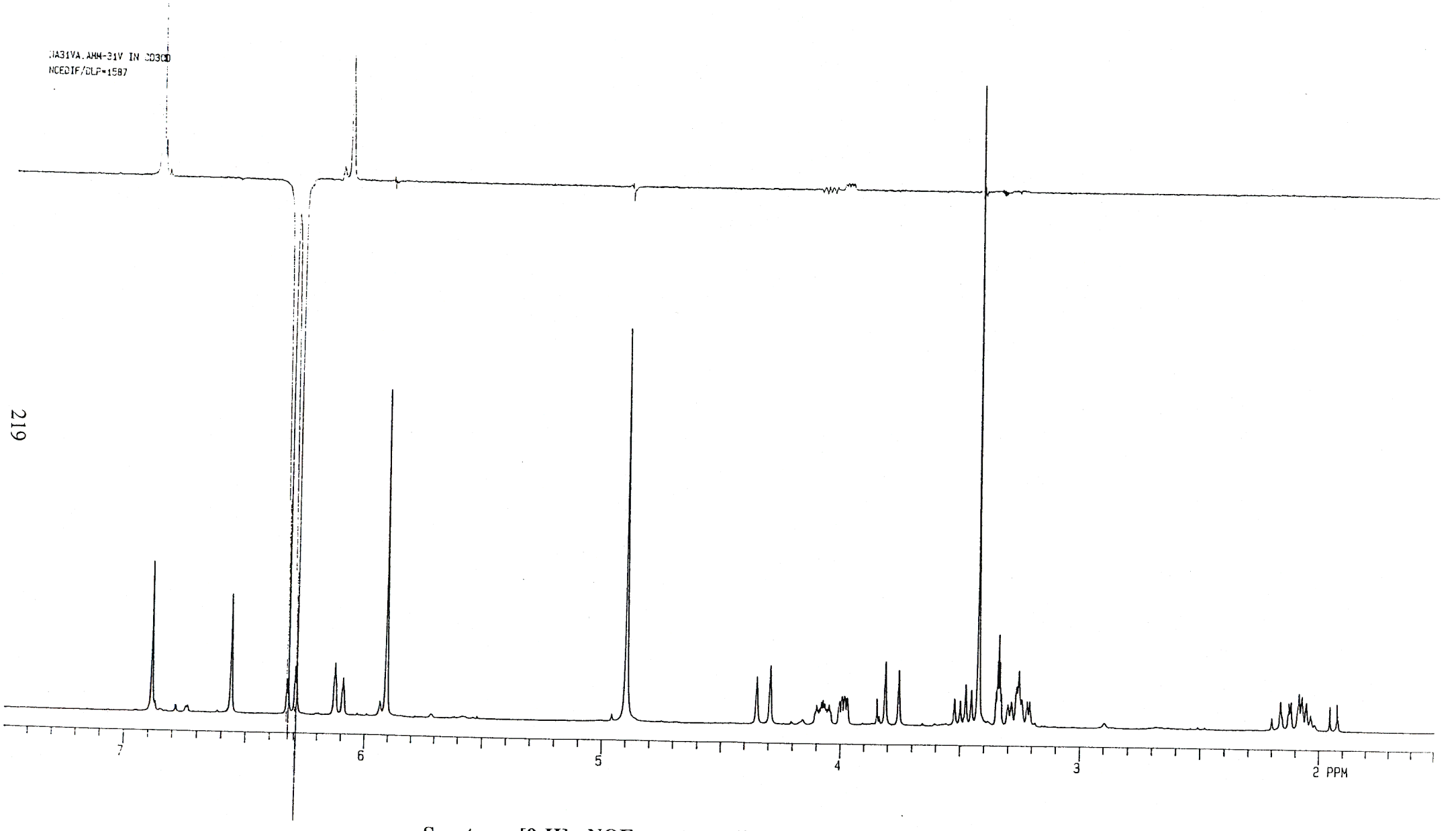
Spectrum [9-E] : Expanded COSY spectrum of crinamine (16) in CD_3OD



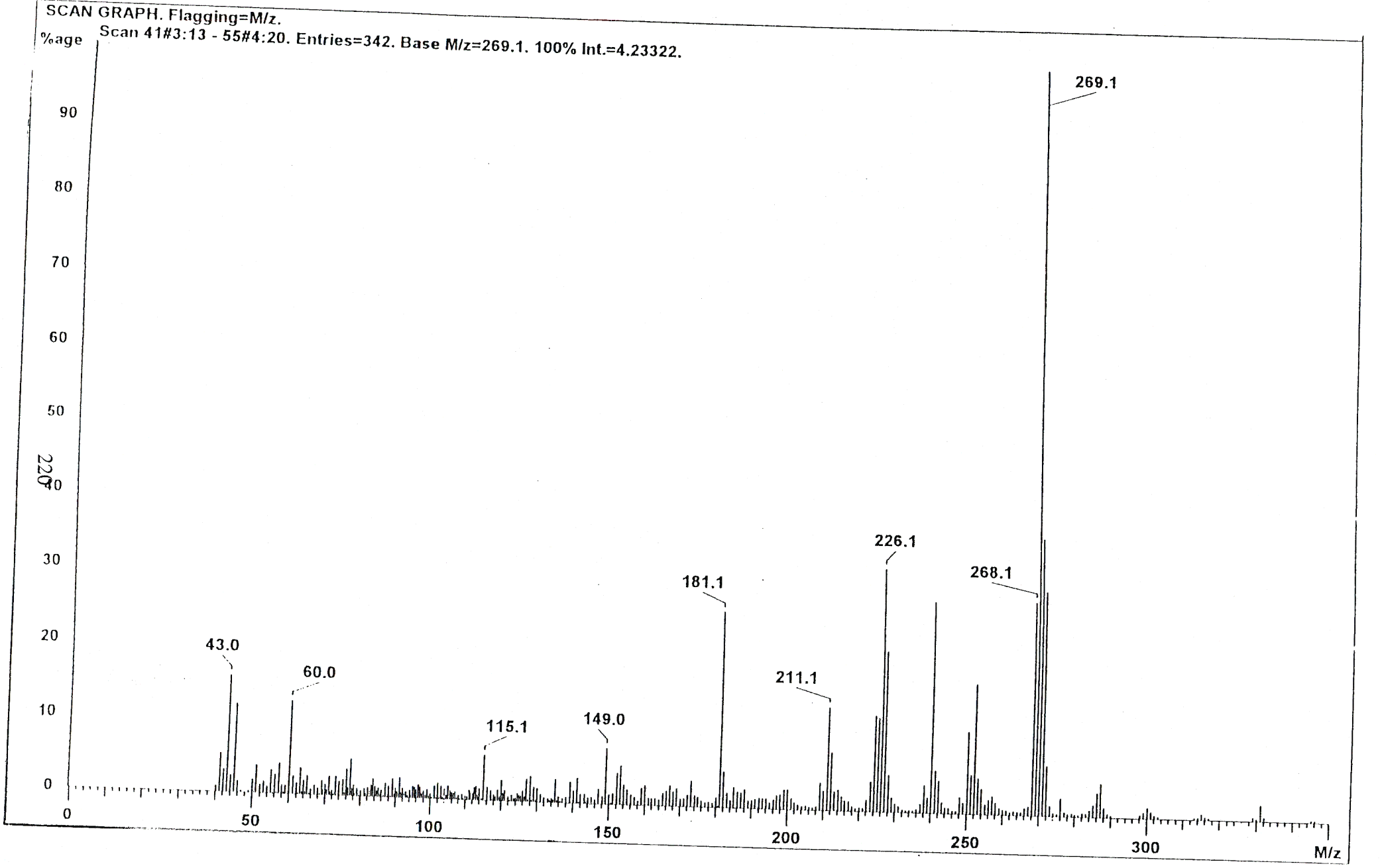
Spectrum [9-F] : HETCOR spectrum of crinamine (16) in CD_3OD



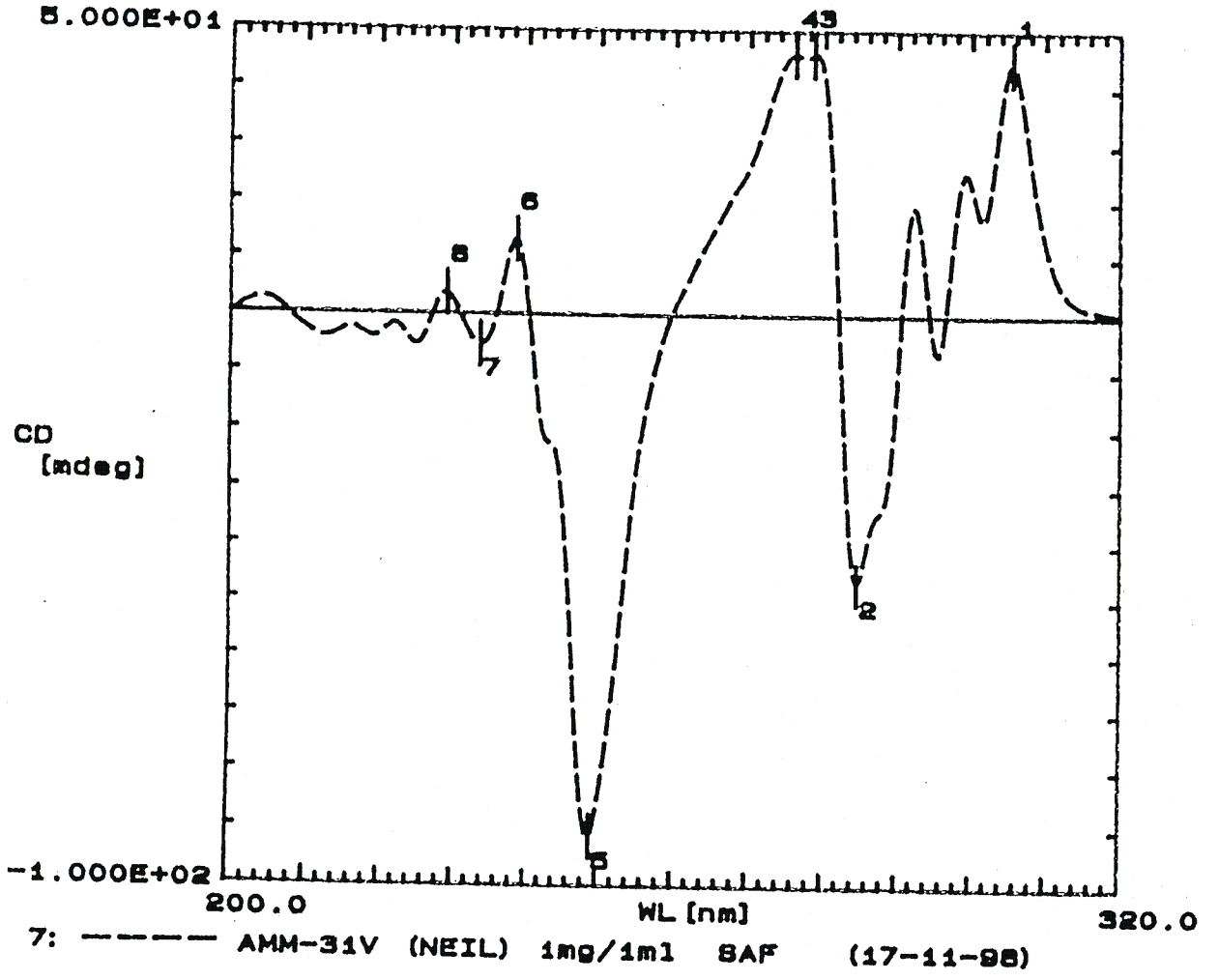
Spectrum [9-G] : NOE spectrum (1) of crinamine (16) in CD_3OD



Spectrum [9-H] : NOE spectrum (2) of crinamine (16) in CD_3OD

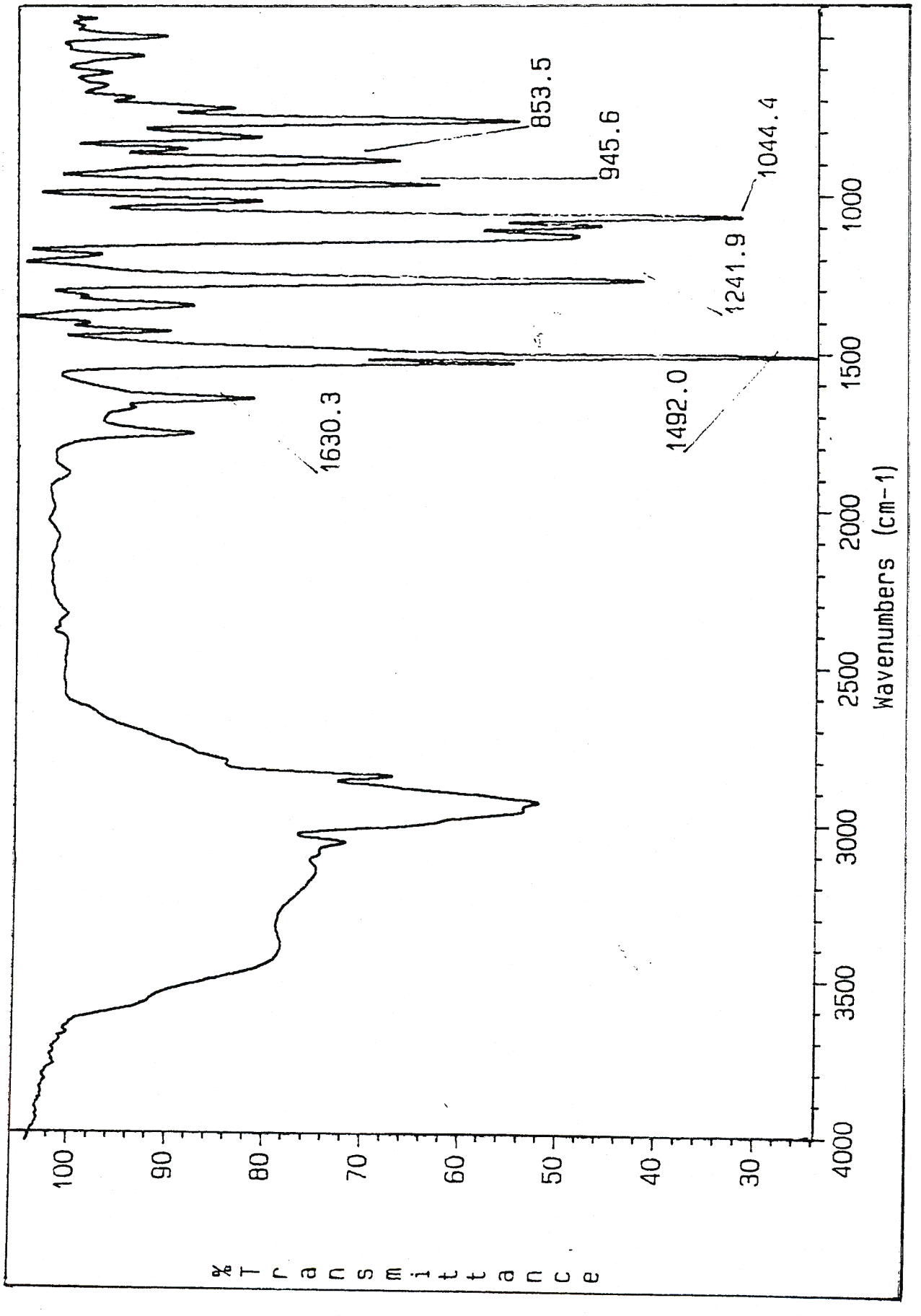


Spectrum [9-I] : Mass spectrum of crinamine (16)

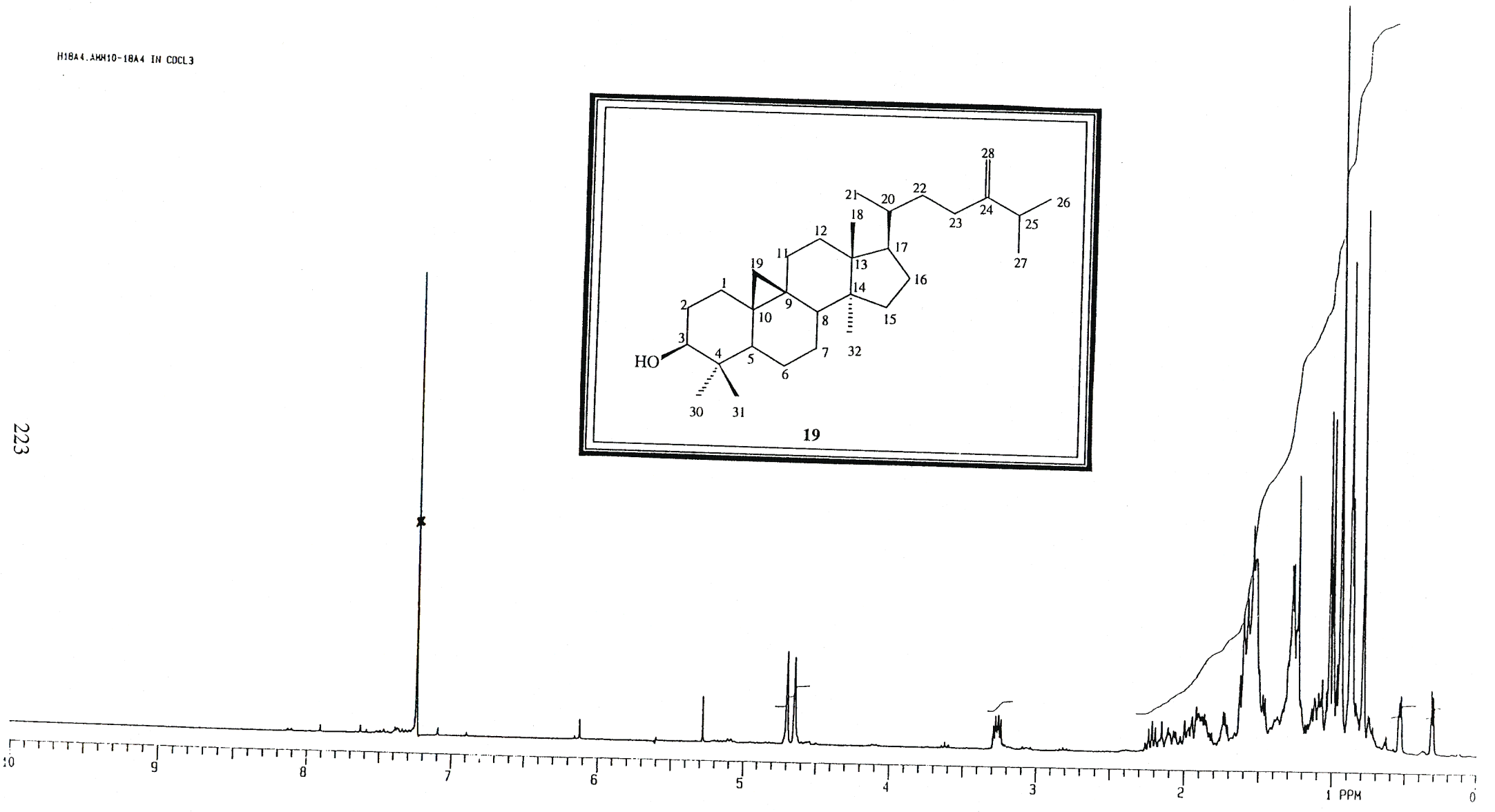


No.	Wavelength	Value
1	305.50 nm	4.456E+01
2	284.50 nm	-4.687E+01
3	276.50 nm	4.594E+01
4	276.00 nm	4.593E+01
5	249.00 nm	-9.151E+01
6	238.50 nm	1.321E+01
7	233.50 nm	-5.332E+00
8	229.00 nm	3.705E+00

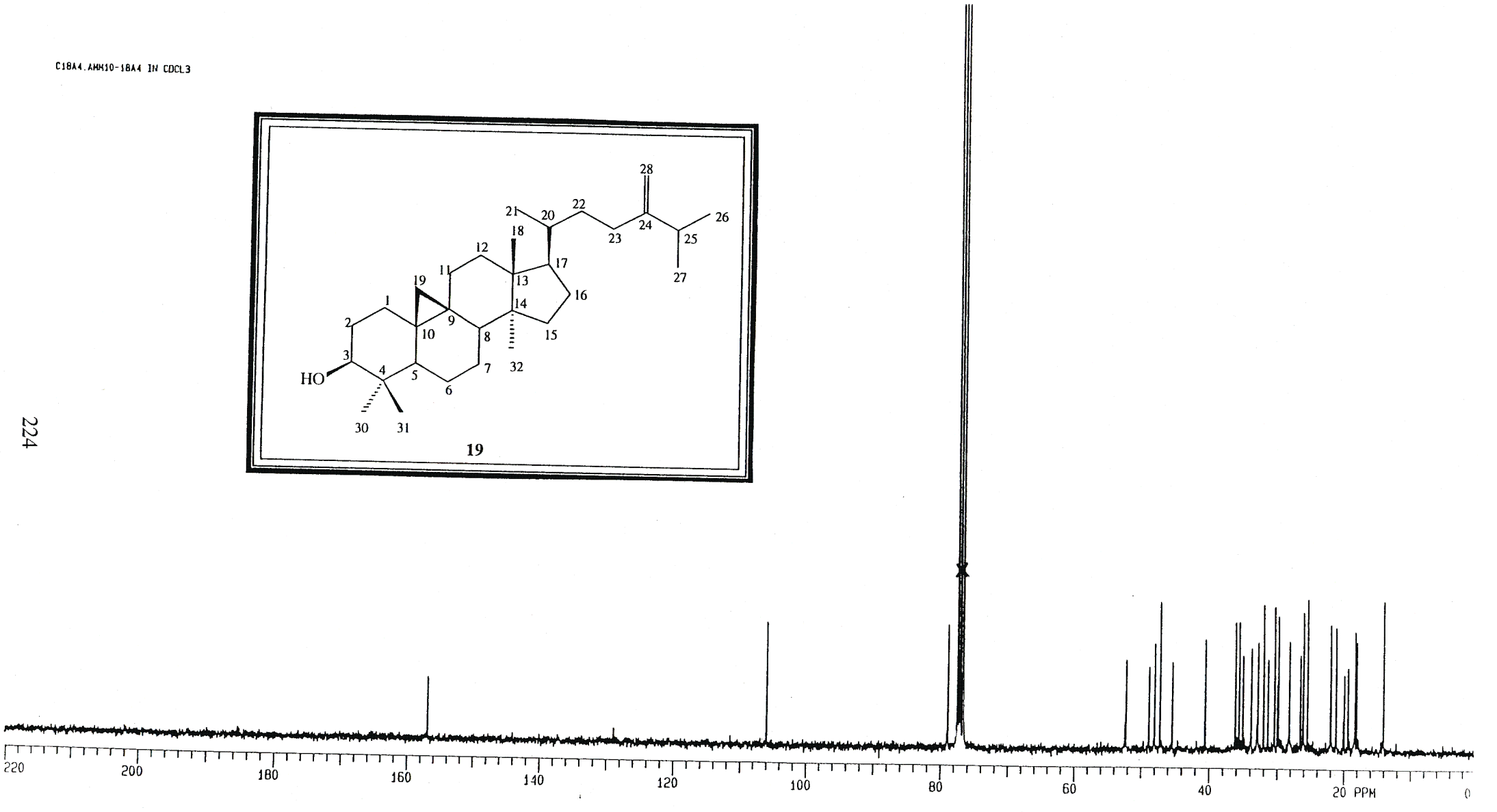
Spectrum [9-J] : Circular Dichroism spectrum of crinamine (16)



Spectrum [9-K] : Infra red spectrum of crinamine (16) in CD_3OD

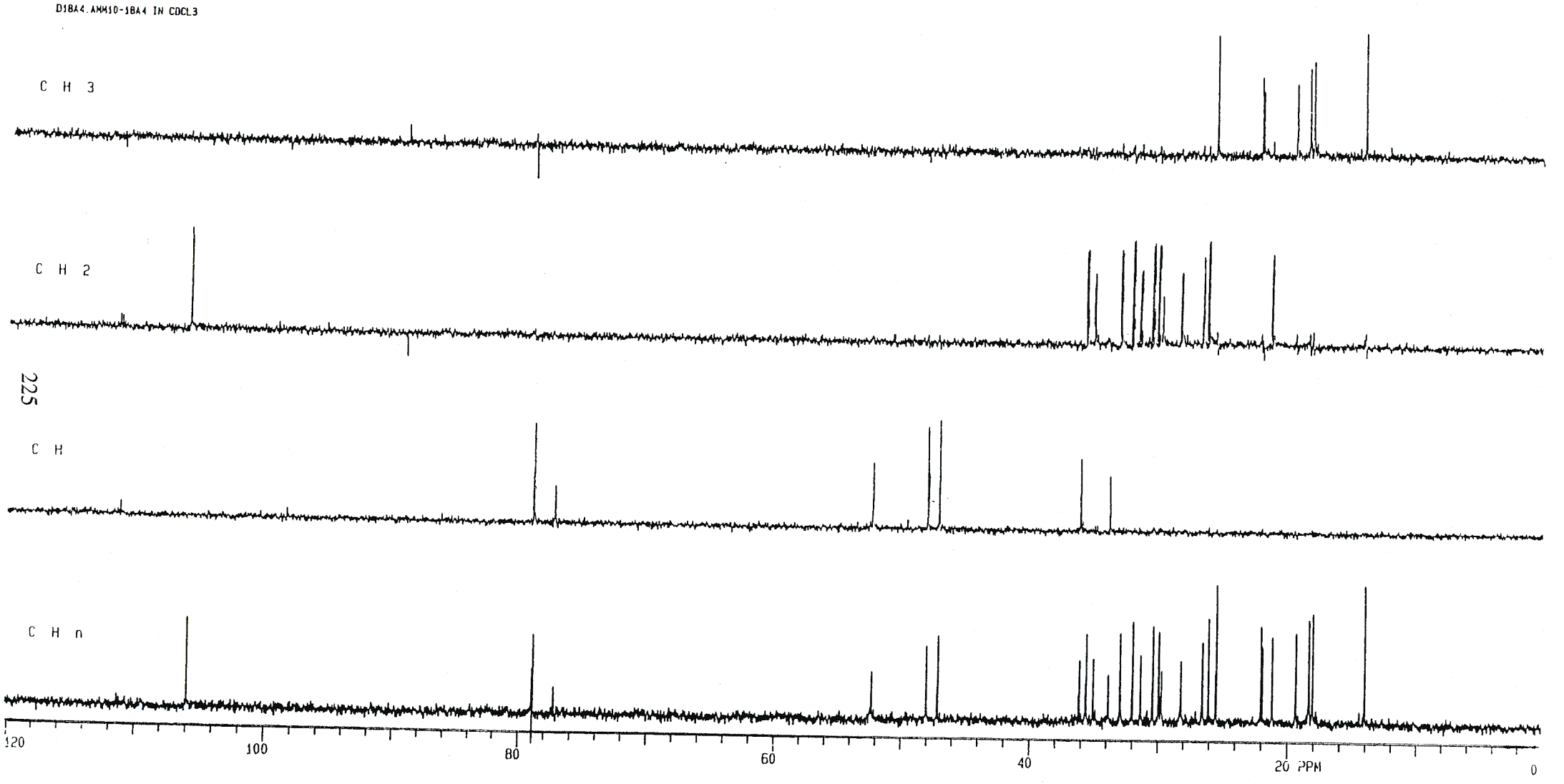


Spectrum [10-A] : ^{1}H NMR spectrum of 24-methylenecycloartanol (19) in $CDCl_3$

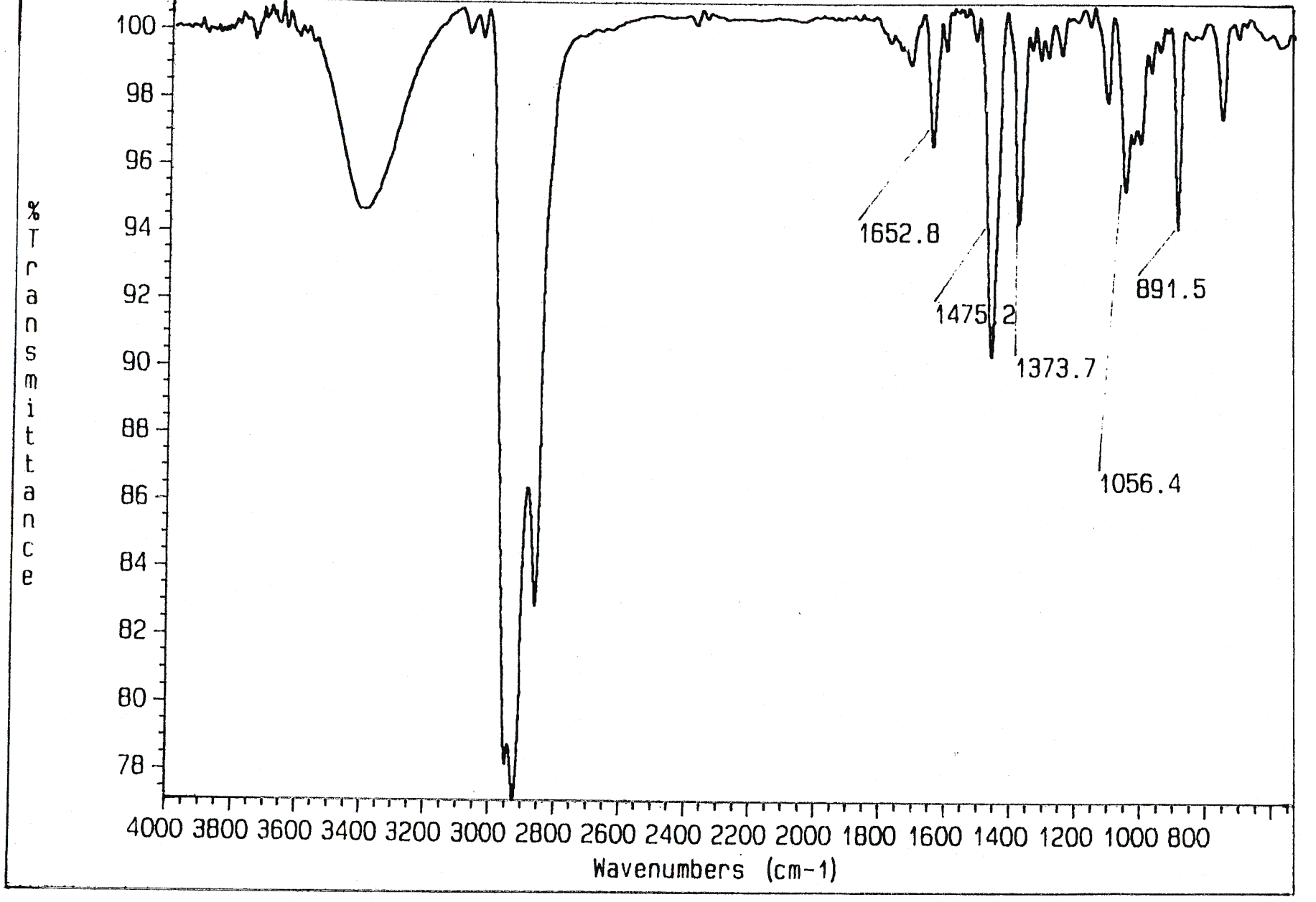


19

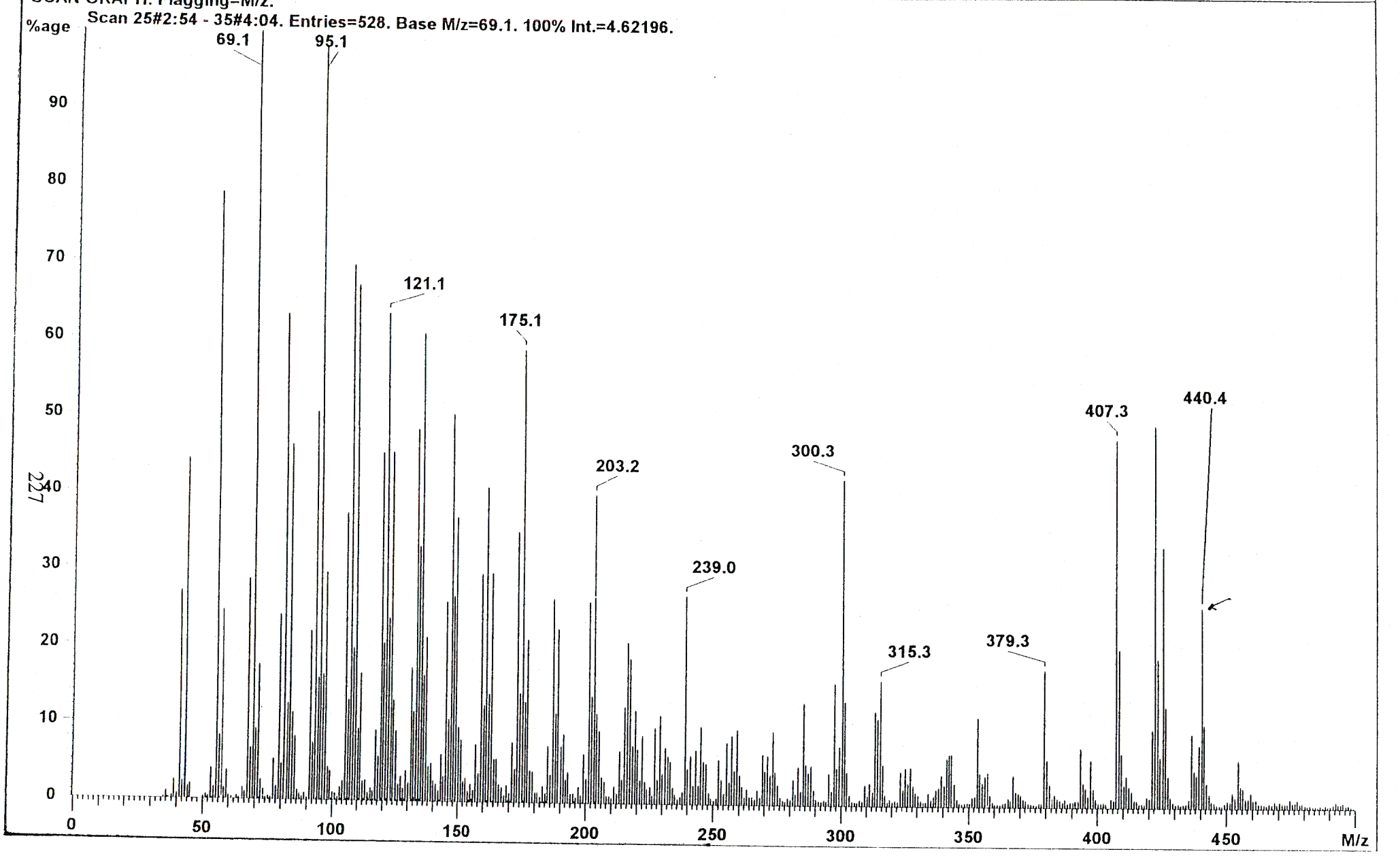
Spectrum [10-B] : ^{13}C NMR spectrum of 24-methylenecycloartanol (19) in $CDCl_3$



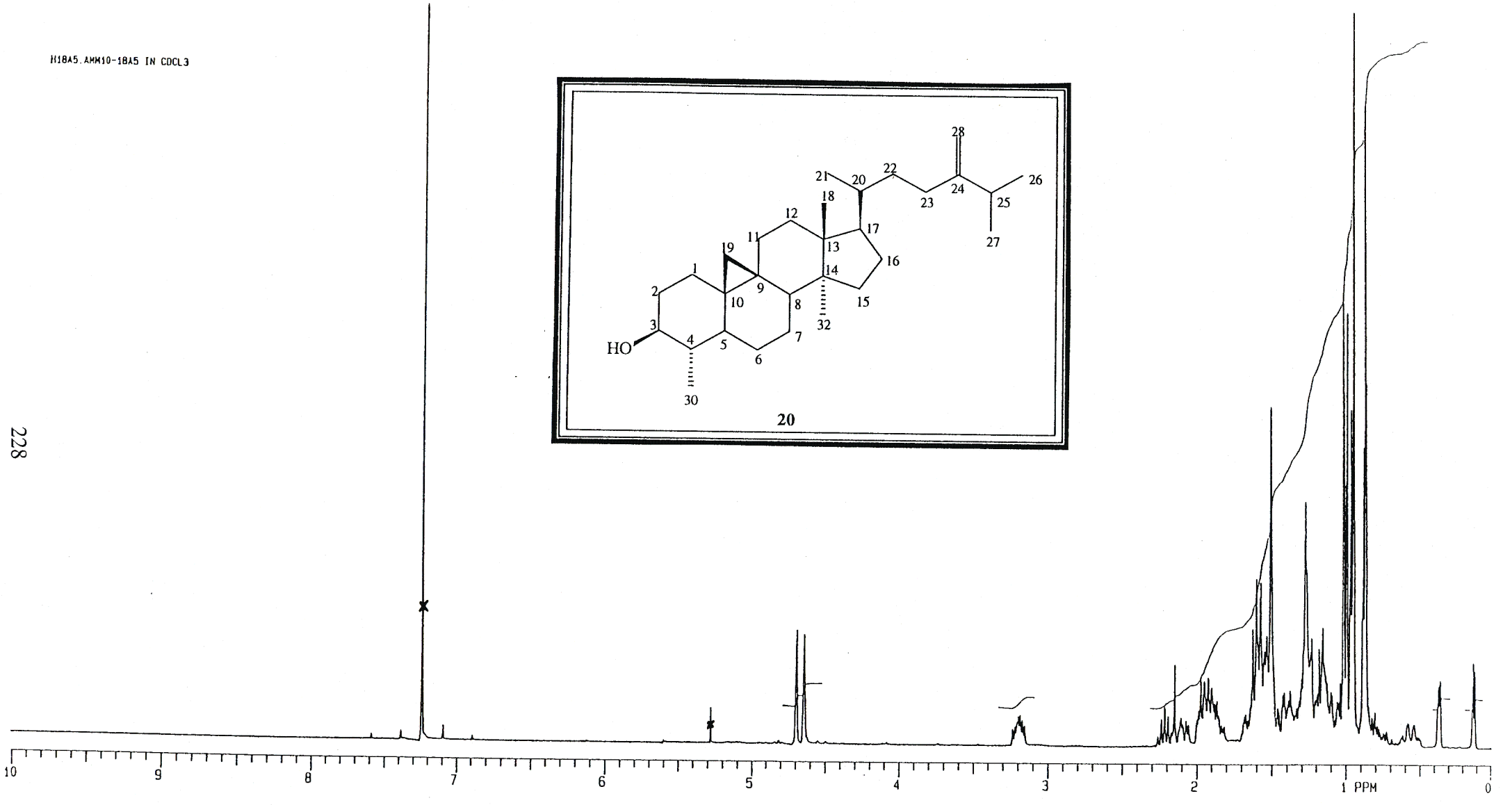
Spectrum [10-C] : ADEPT spectrum of 24-methylenecycloartanol (19) in $CDCl_3$



Spectrum [10-D] : Infra red spectrum of 24-methylenecycloartanol (19)

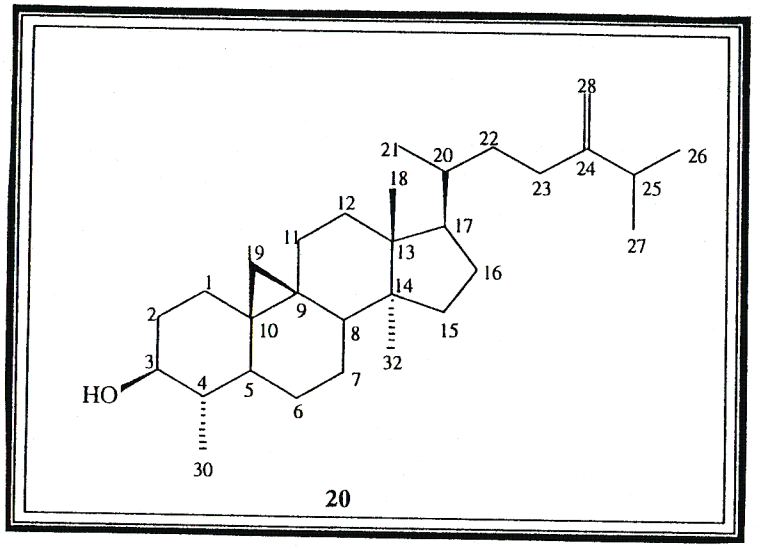


Spectrum [10-E] : Mass spectrum of 24-methylenecycloartanol (19)



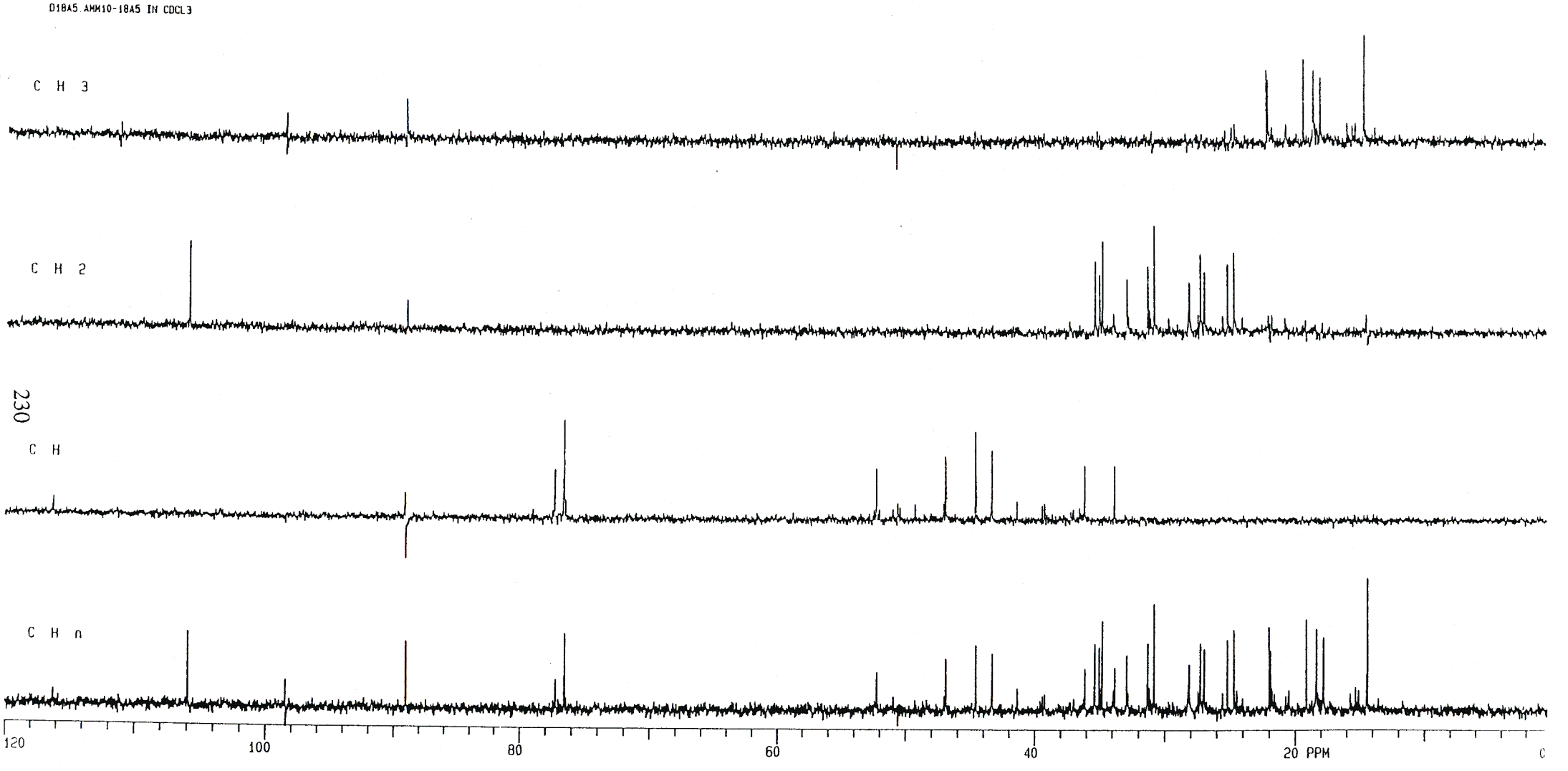
20

Spectrum [11-A] : ^{1}H NMR spectrum of cycloeucalenol (20) in $CDCl_3$

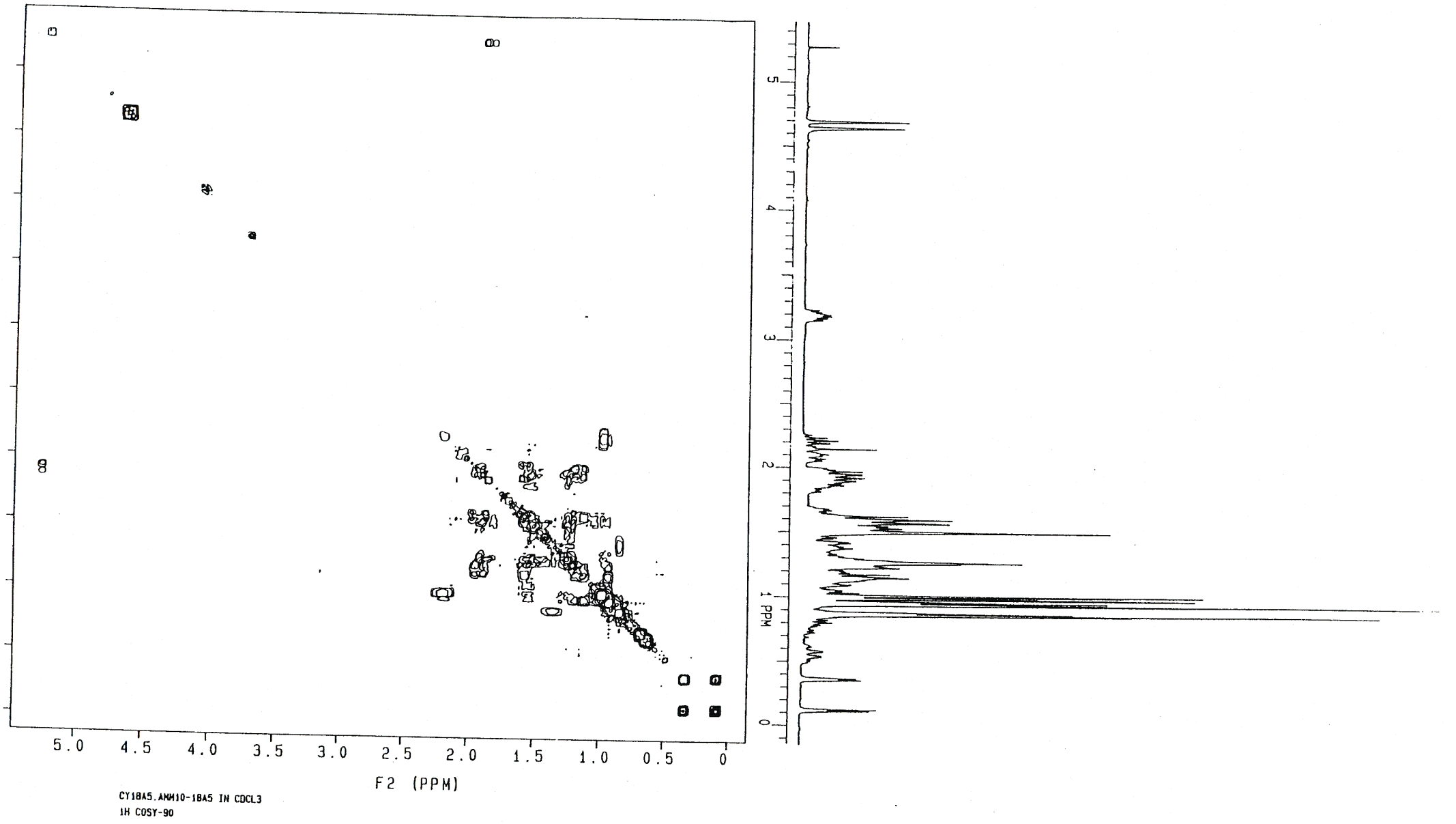


20

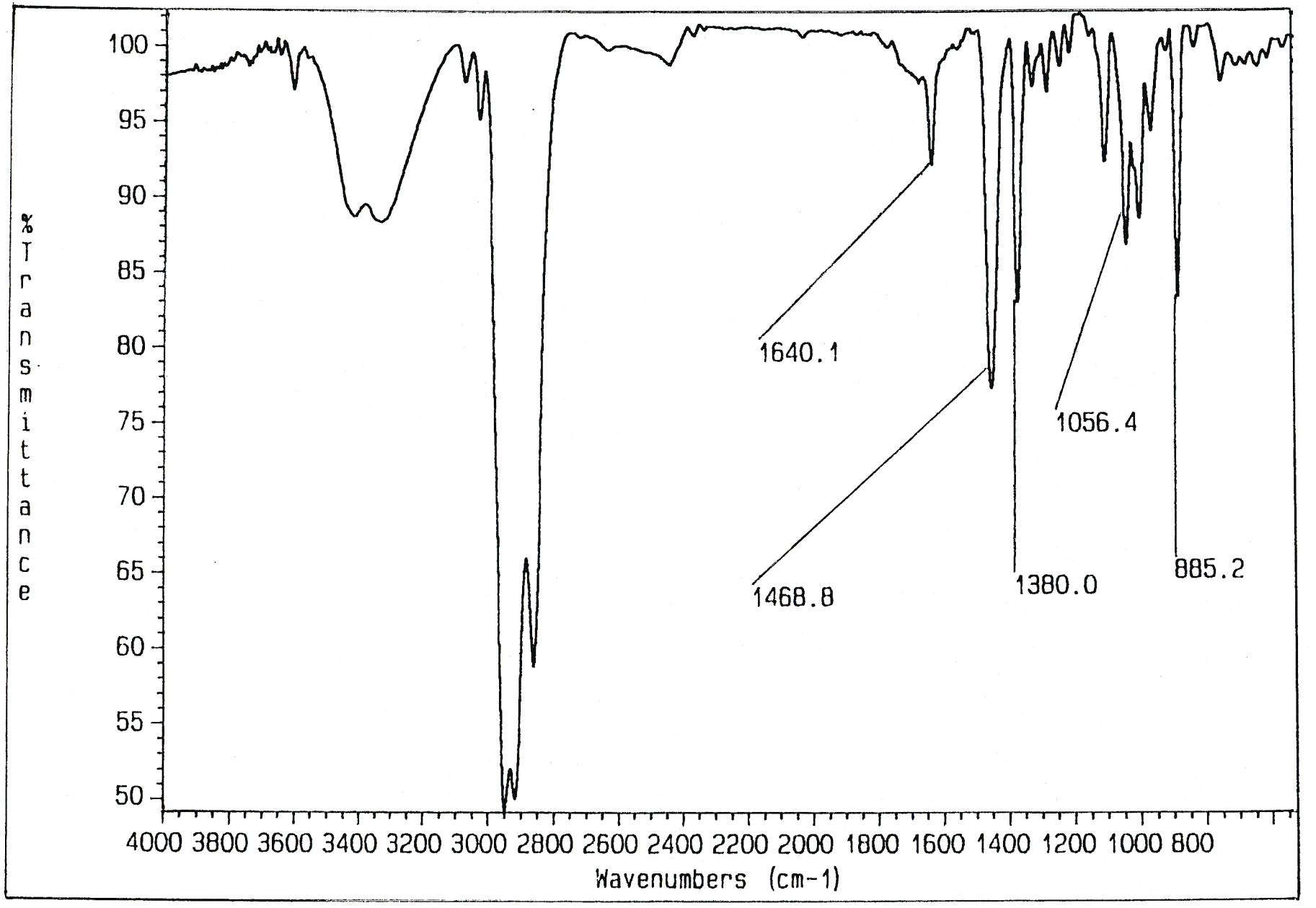
Spectrum [11-B] : ^{13}C NMR spectrum of cycloeucalenol (20) in $CDCl_3$



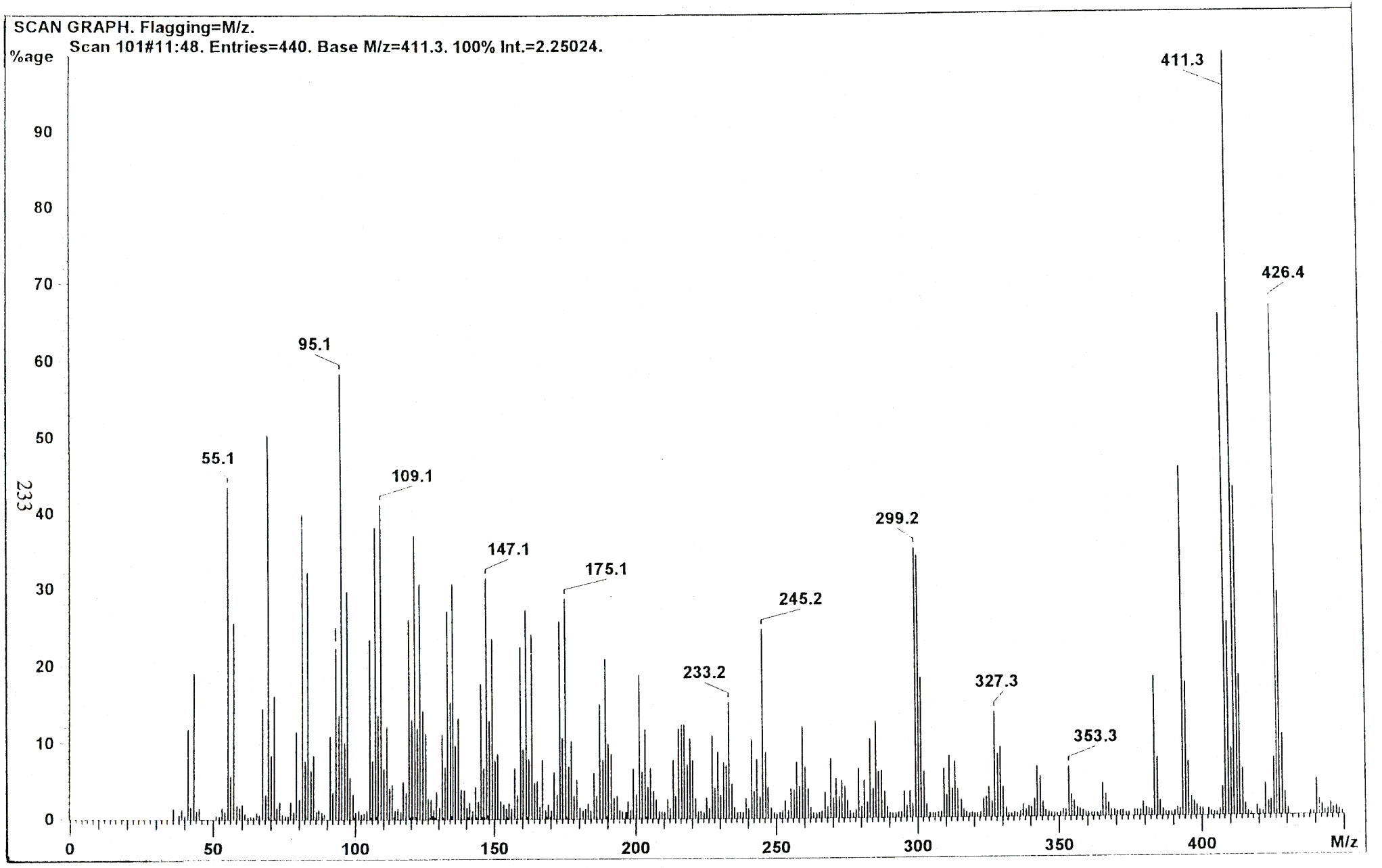
Spectrum [11-C] : ADEPT spectrum of cycloeucalenol (20) in $CDCl_3$



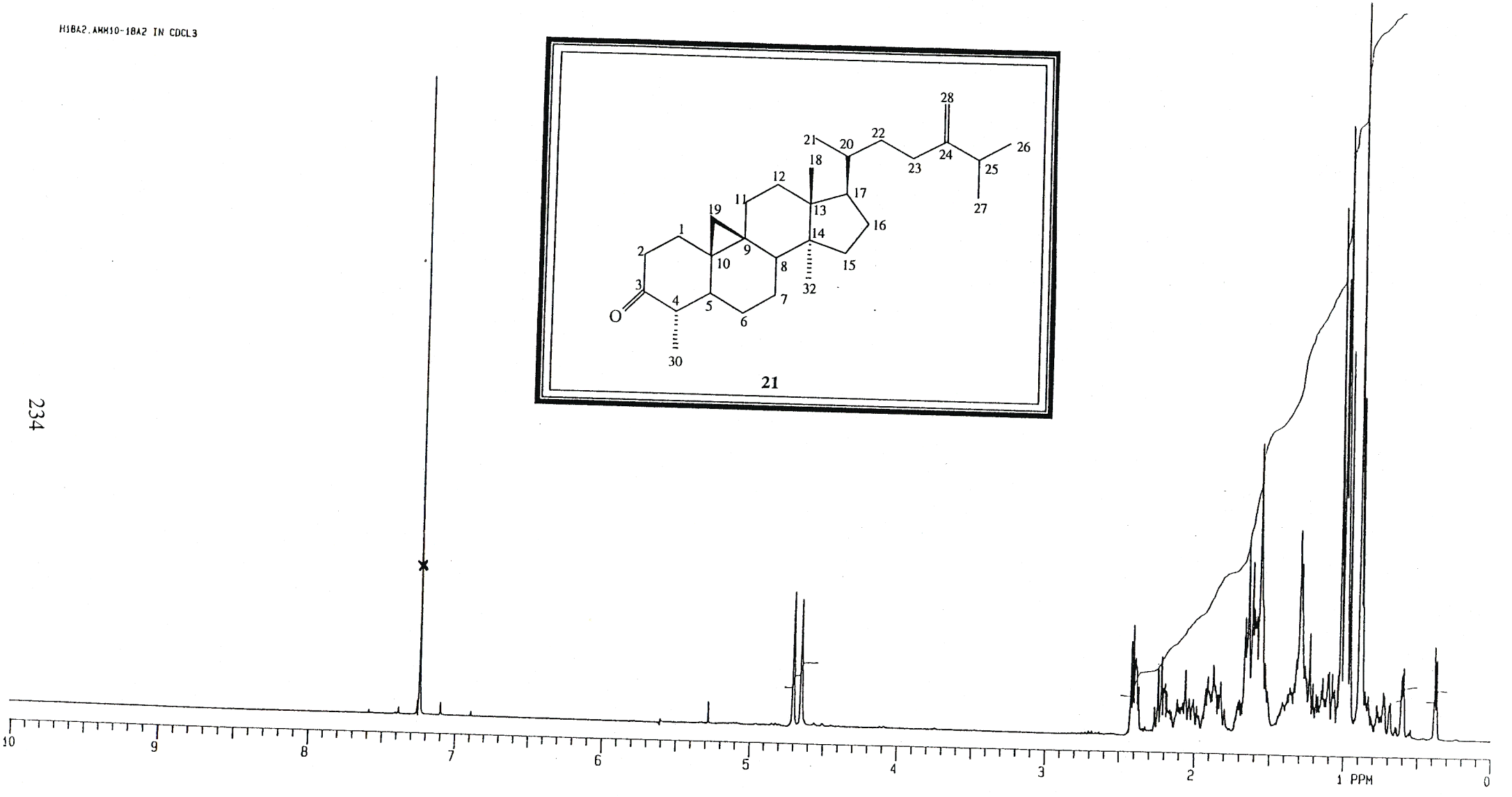
Spectrum [11-D] : COSY spectrum of cycloeucalenol (20) in $CDCl_3$



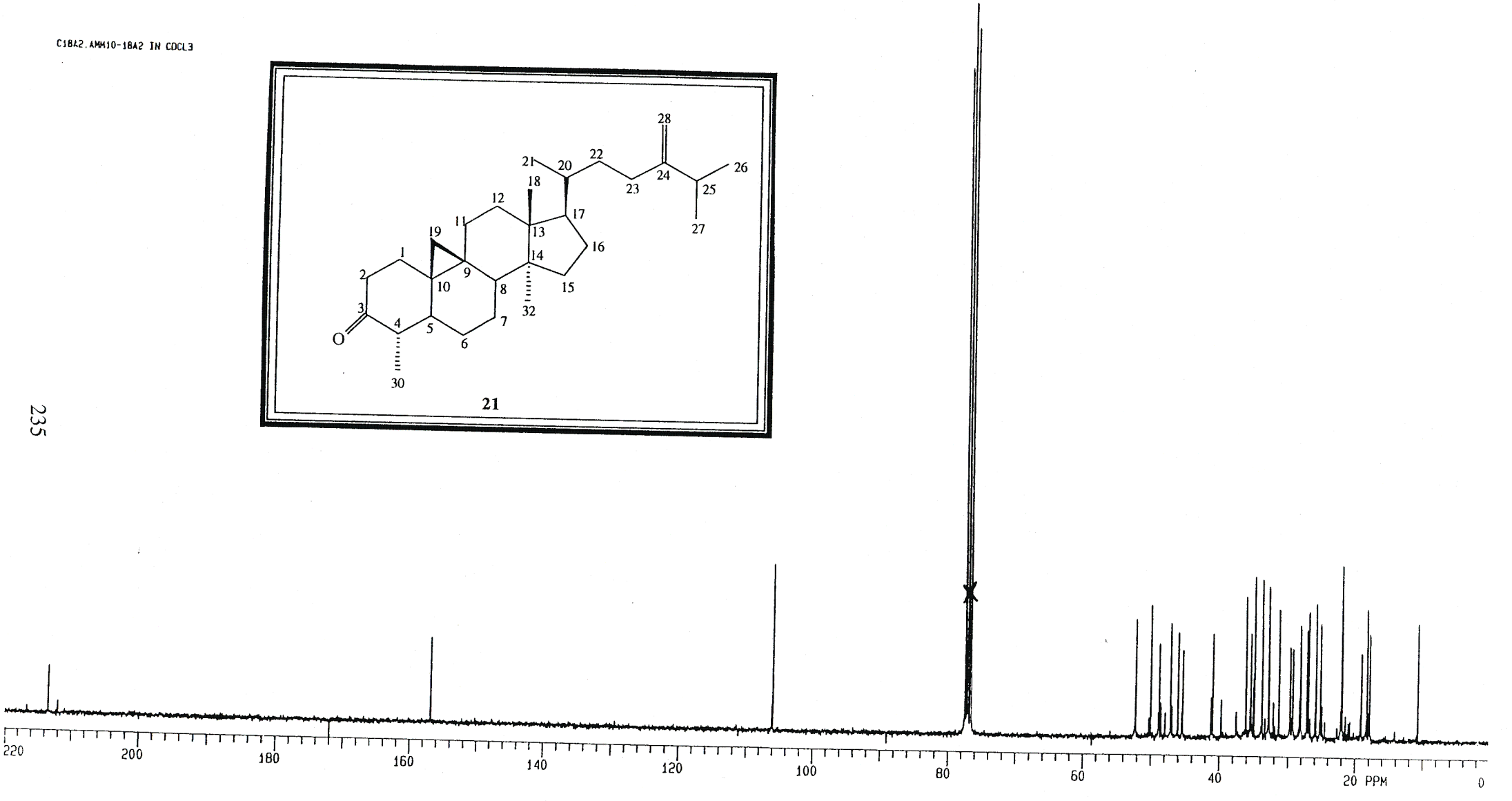
Spectrum [11-E] : Infra red spectrum of cycloeucalenol (20)



Spectrum [11-F] : Mass spectrum of cycloeucalenol (20)

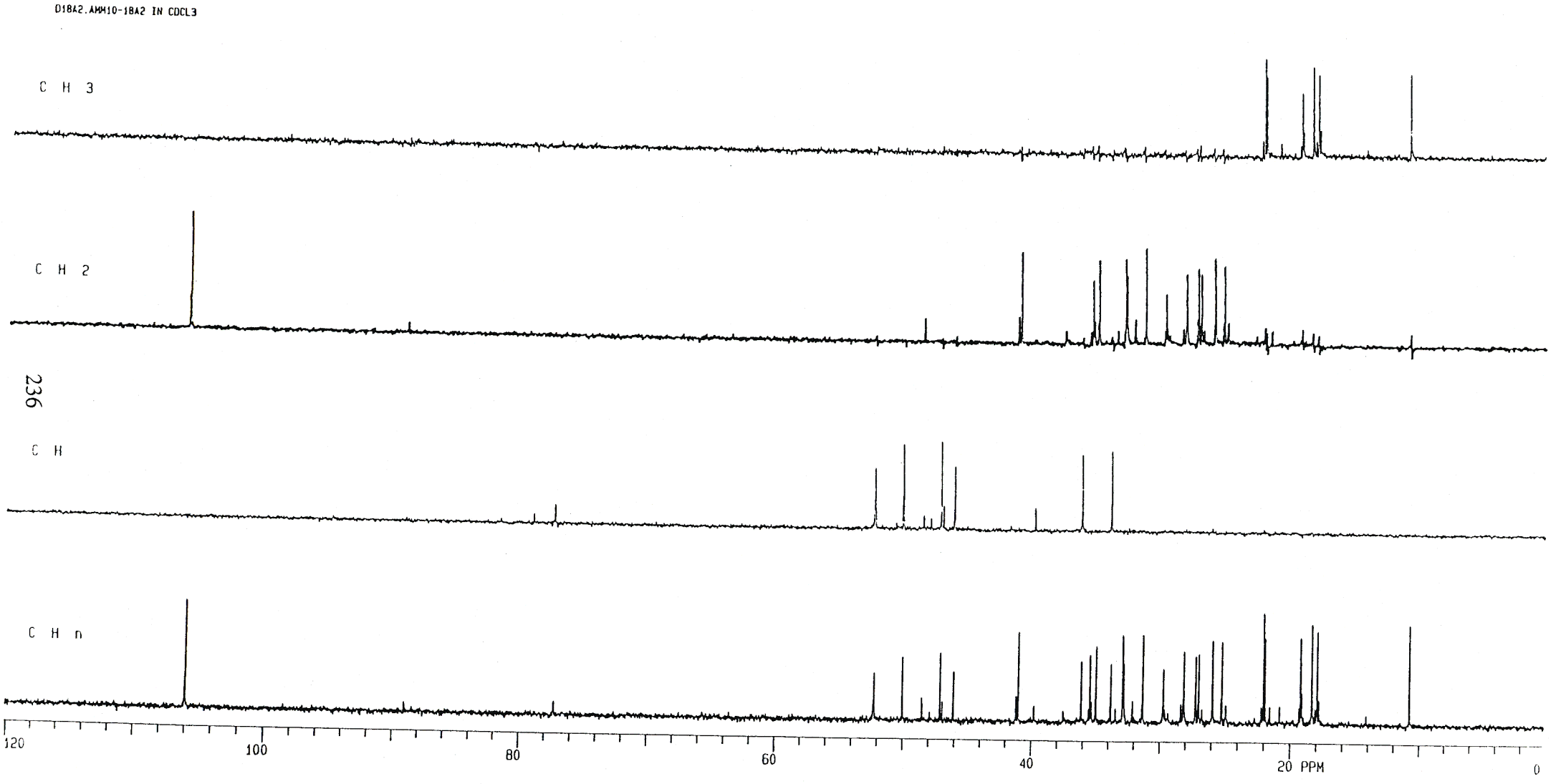


Spectrum [12-A] : ^{1}H NMR spectrum of cycloeucalenone (21) in $CDCl_3$

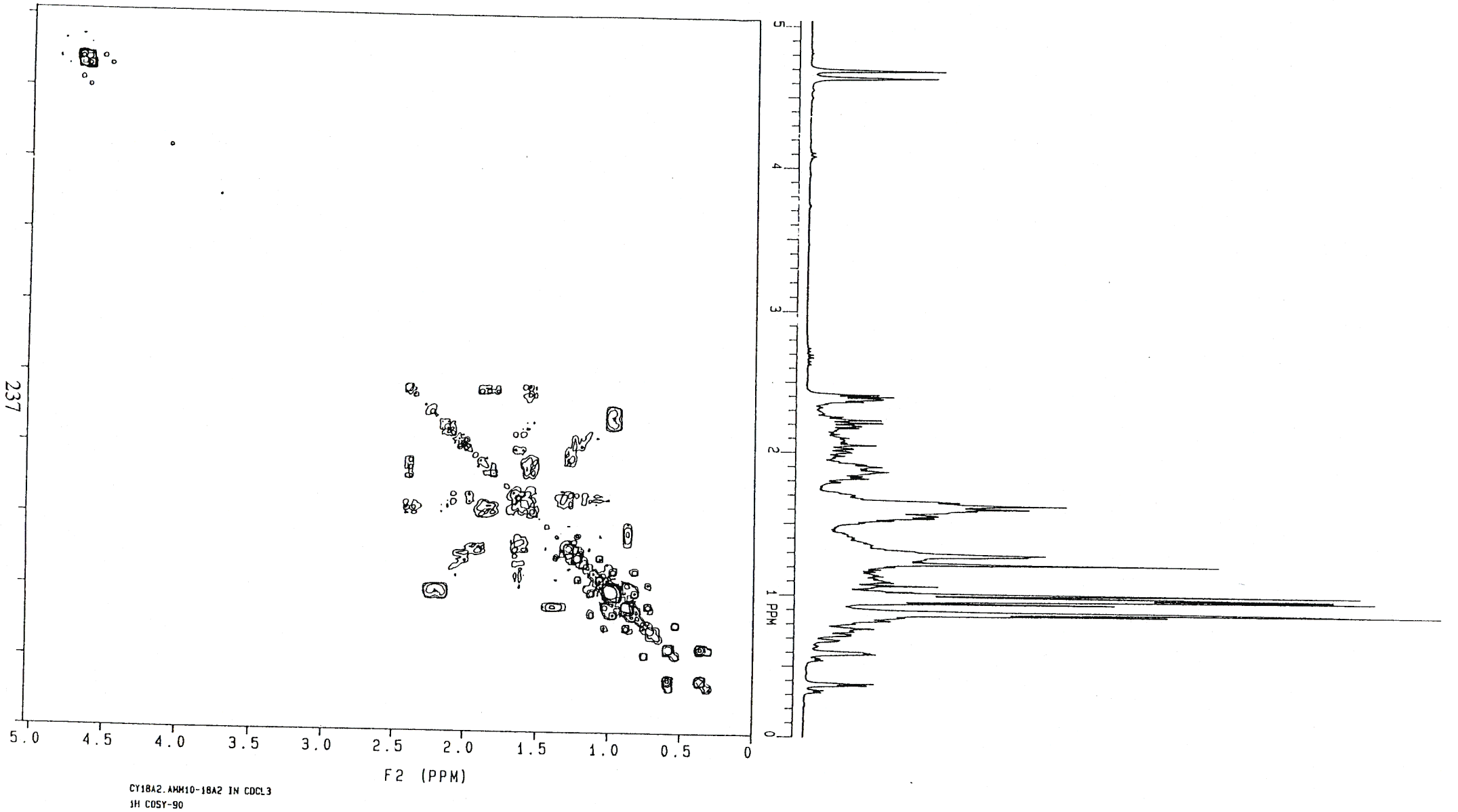


21

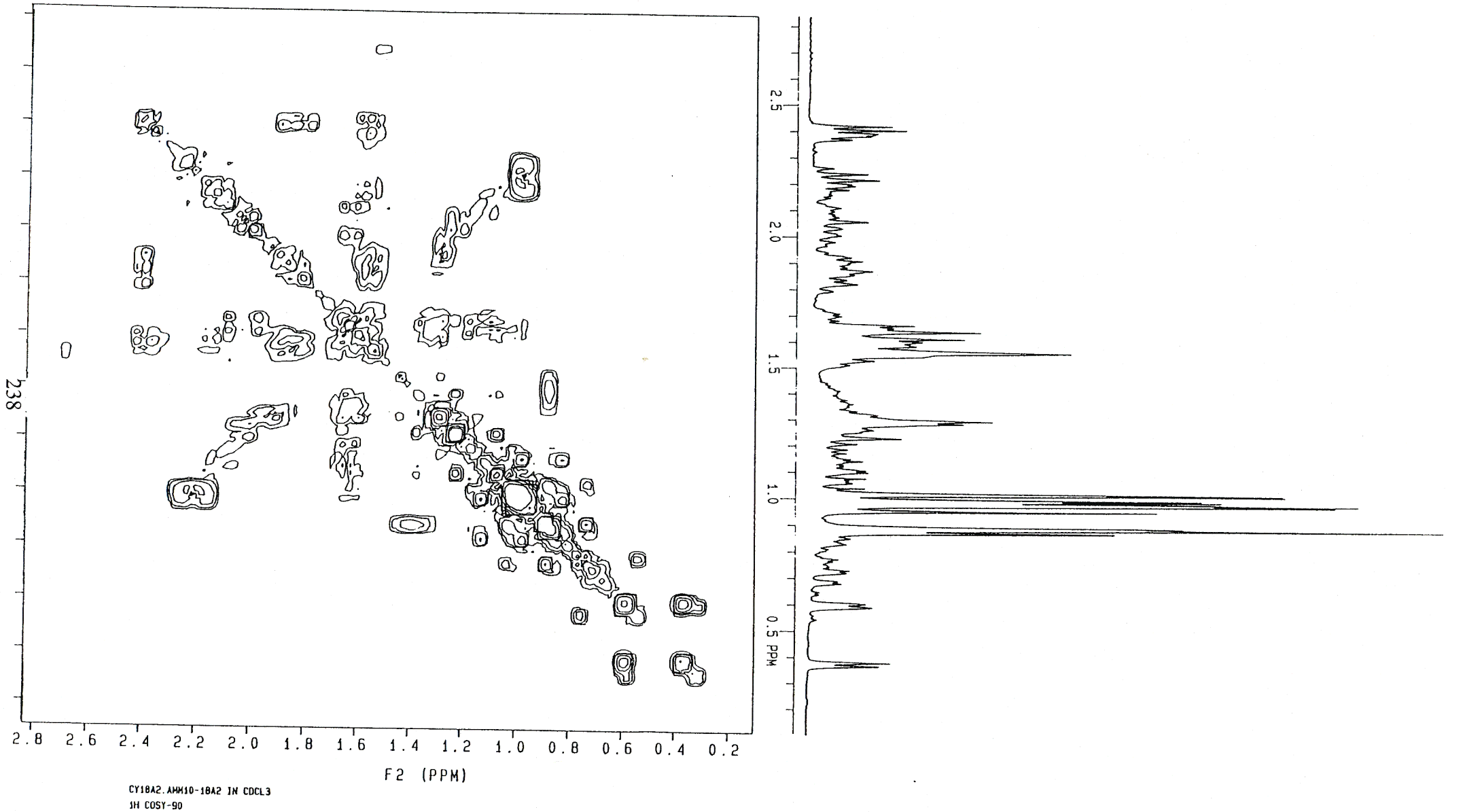
Spectrum [12-B] : ^{13}C NMR spectrum of cycloeucalenone (21) in $CDCl_3$



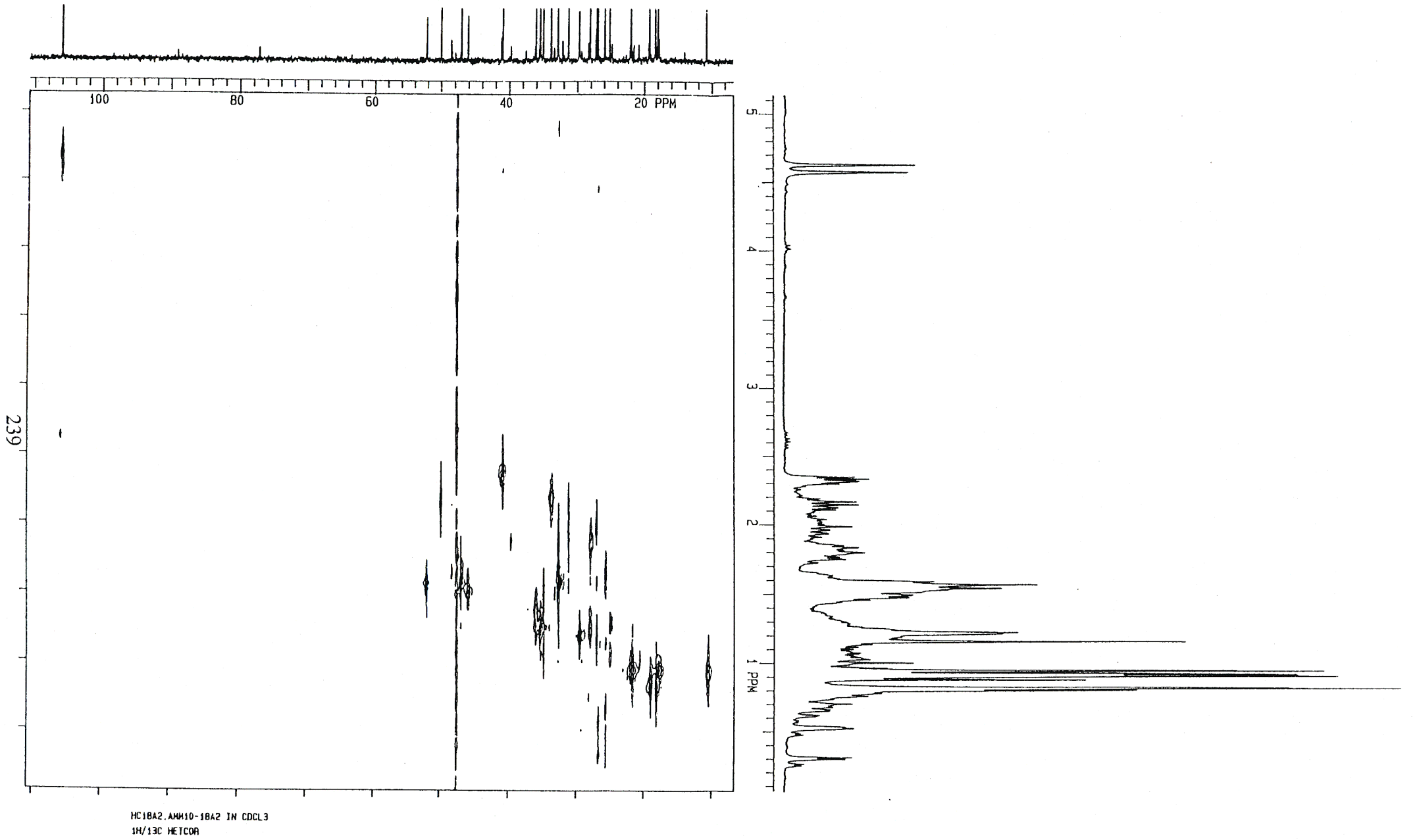
Spectrum [12-C] : ADEPT spectrum of cycloeucalenone (21) in $CDCl_3$



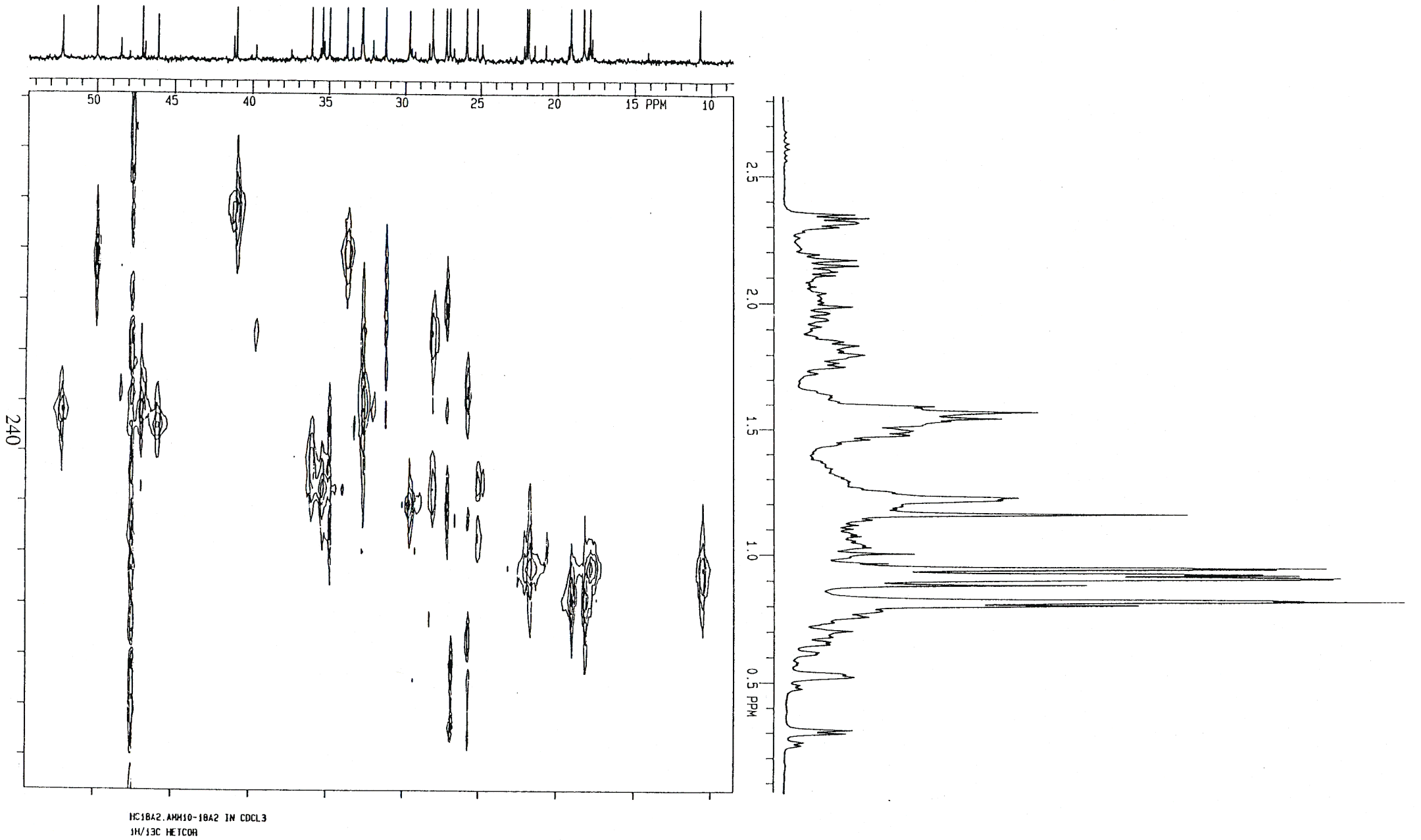
Spectrum [12-D] : COSY spectrum of cycloeucalenone (21) in $CDCl_3$



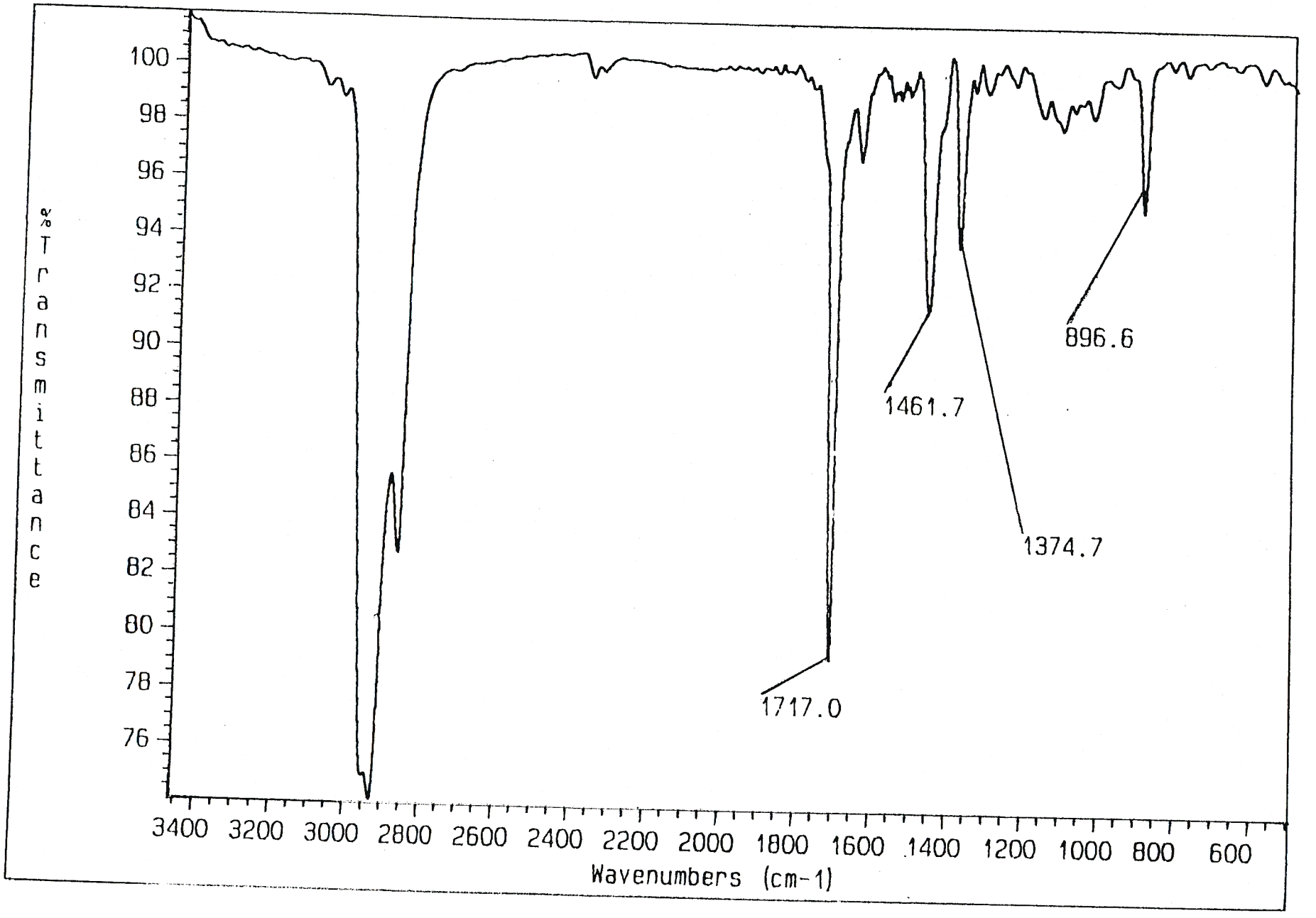
Spectrum [12-E] : Expanded COSY spectrum of cycloeucalenone (21) in $CDCl_3$



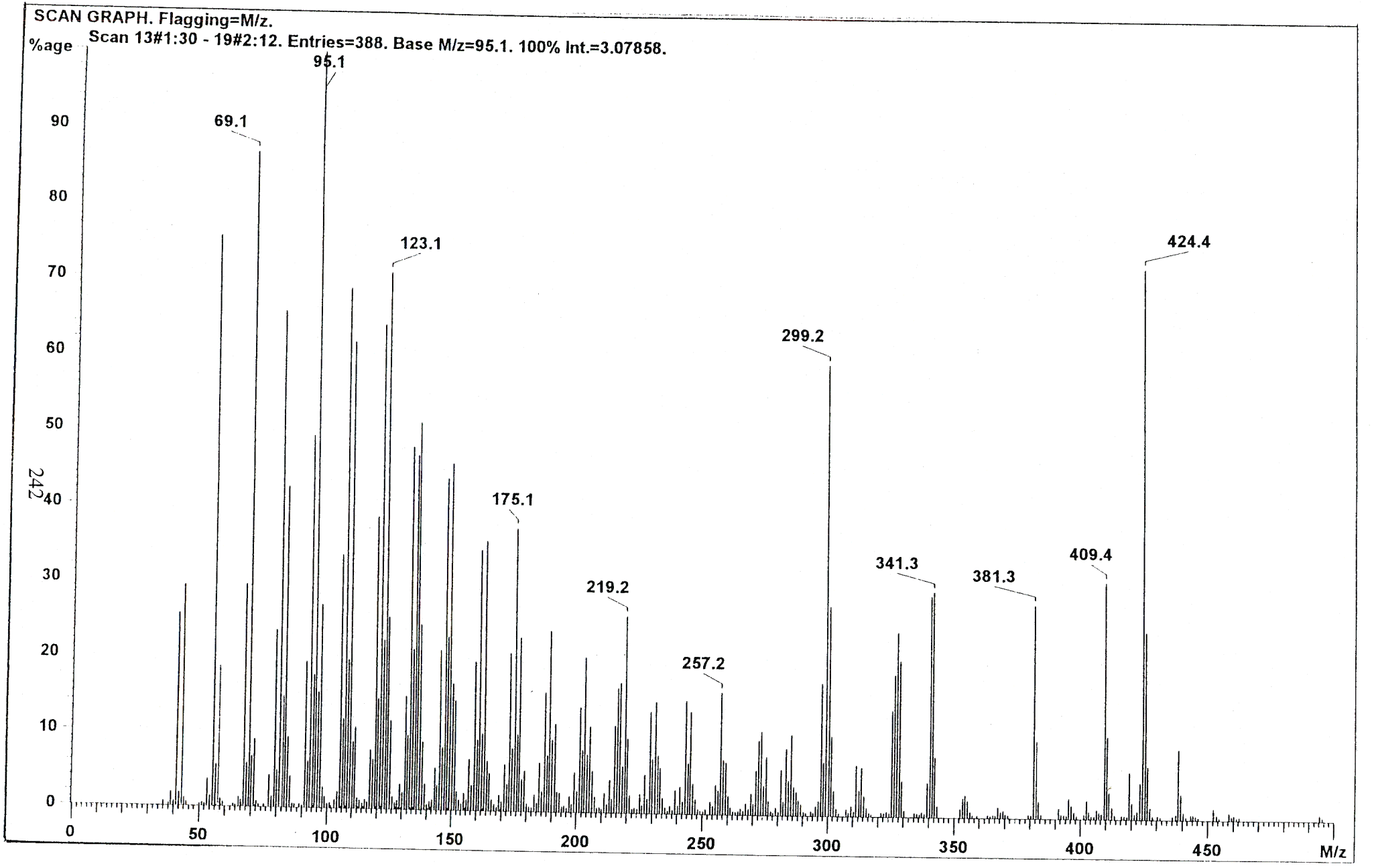
Spectrum [12-F] : HETCOR spectrum of cycloeucalenone (21) in $CDCl_3$



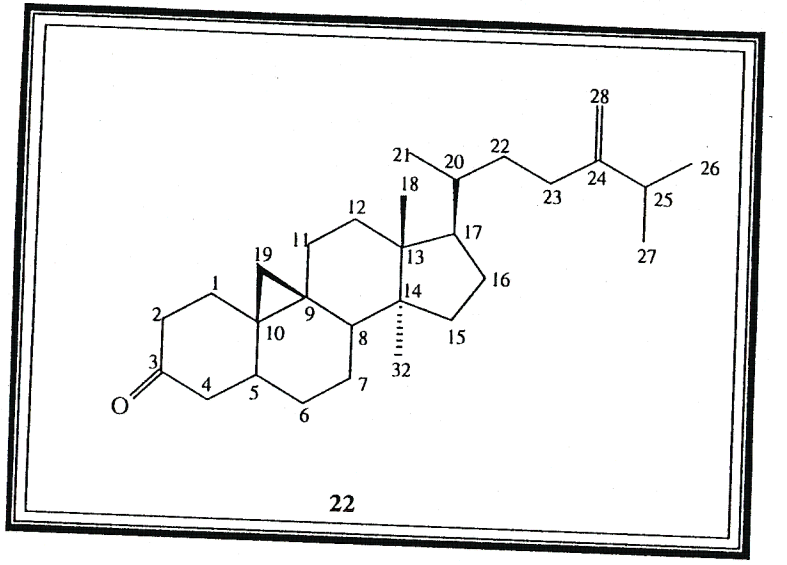
Spectrum [12-G] : Expanded HETCOR spectrum of cycloeucalenone (21) in $CDCl_3$



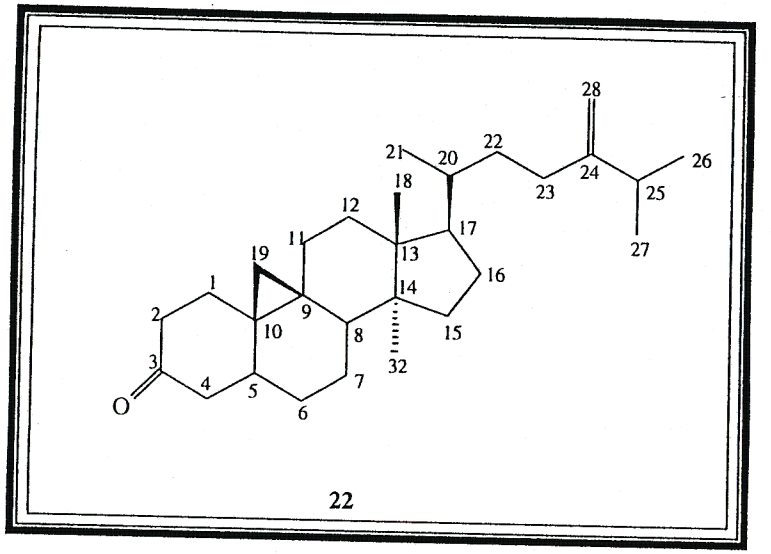
Spectrum [12-H] : Infra red spectrum of cycloeucalenone (21)



Spectrum [12-I] : Mass spectrum of cycloeucalenone (21)

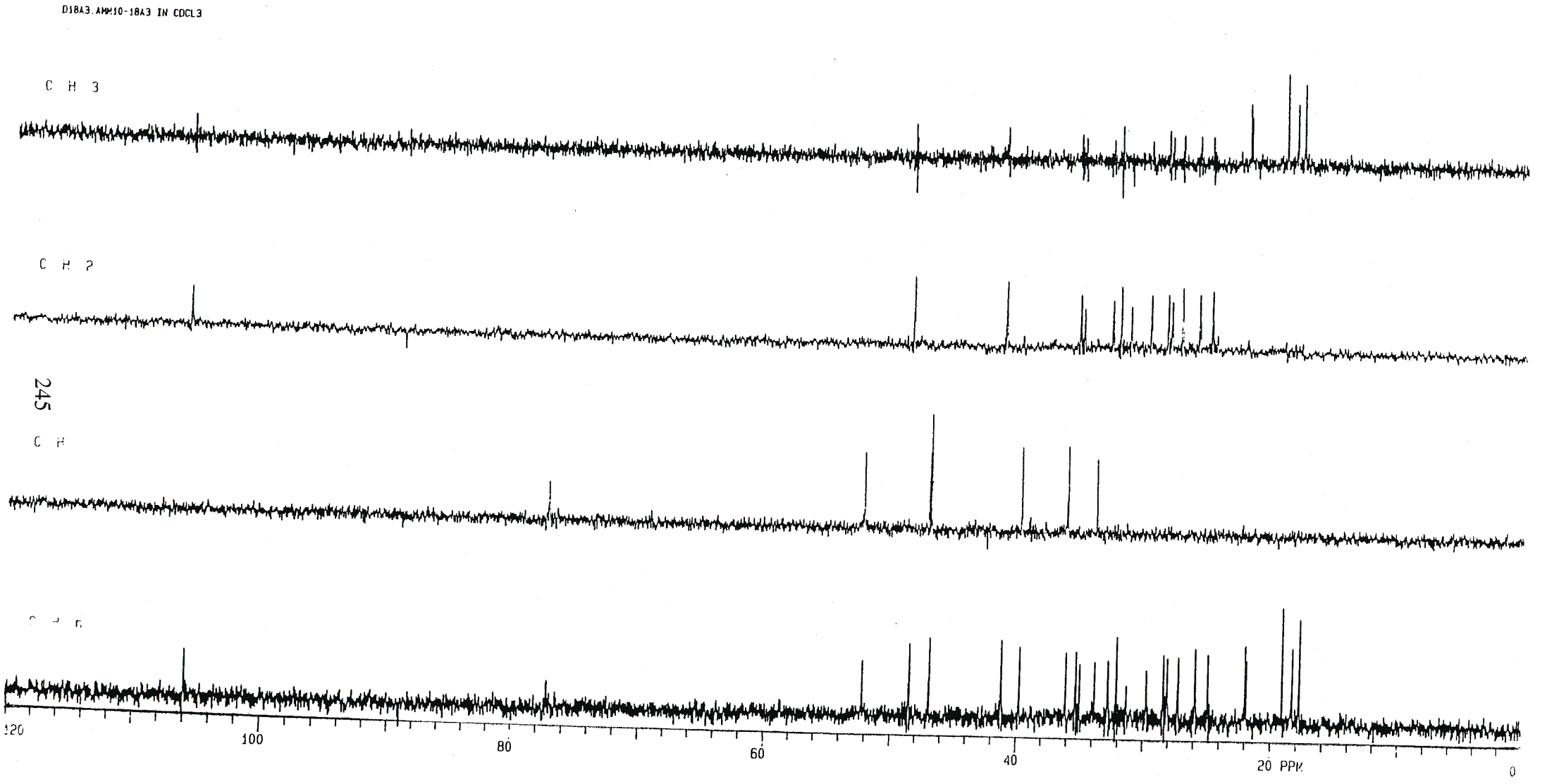


Spectrum [13-A] : ^{1}H NMR spectrum of 24-methylenepollinastanone (22) in $CDCl_3$

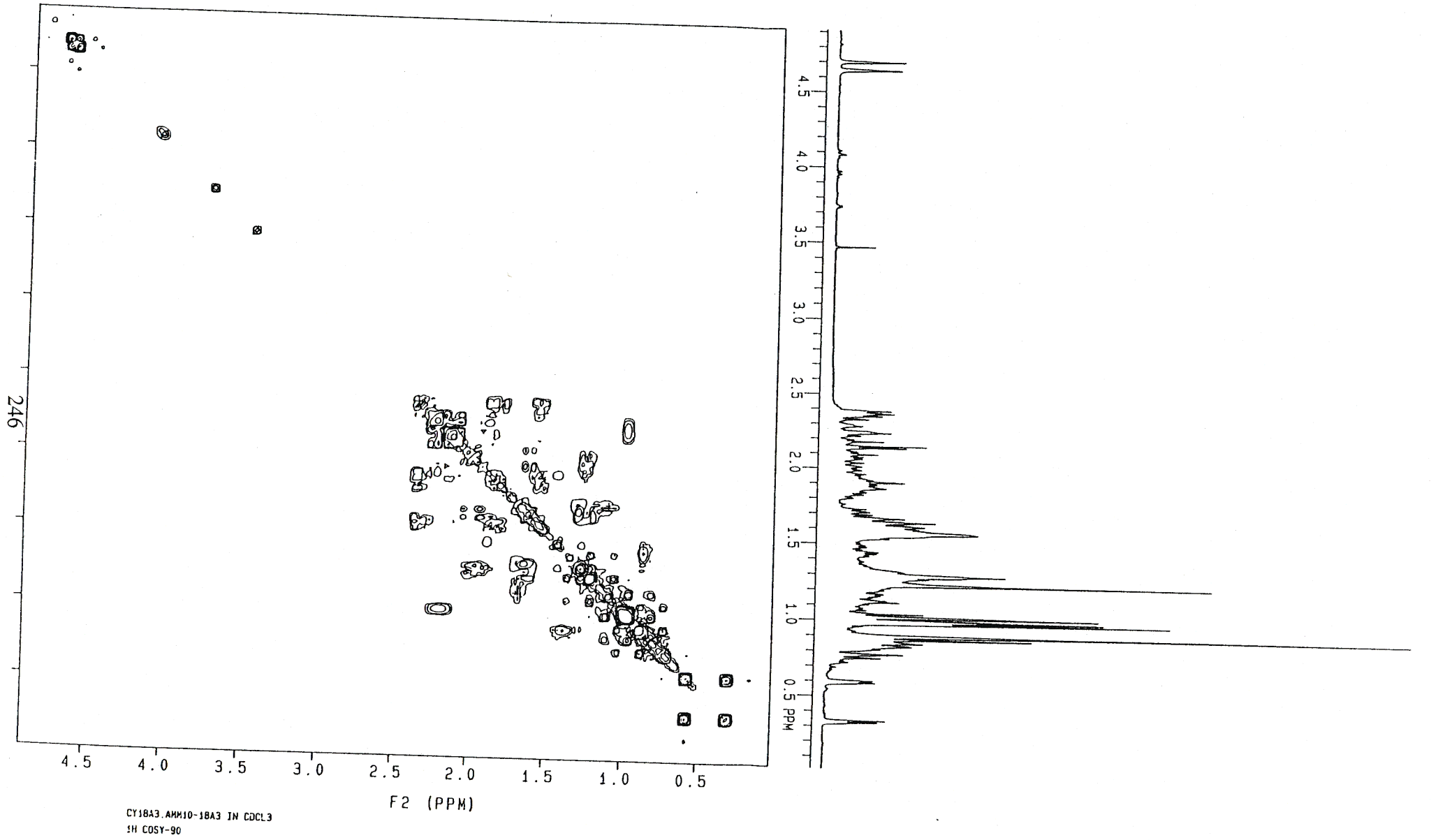


22

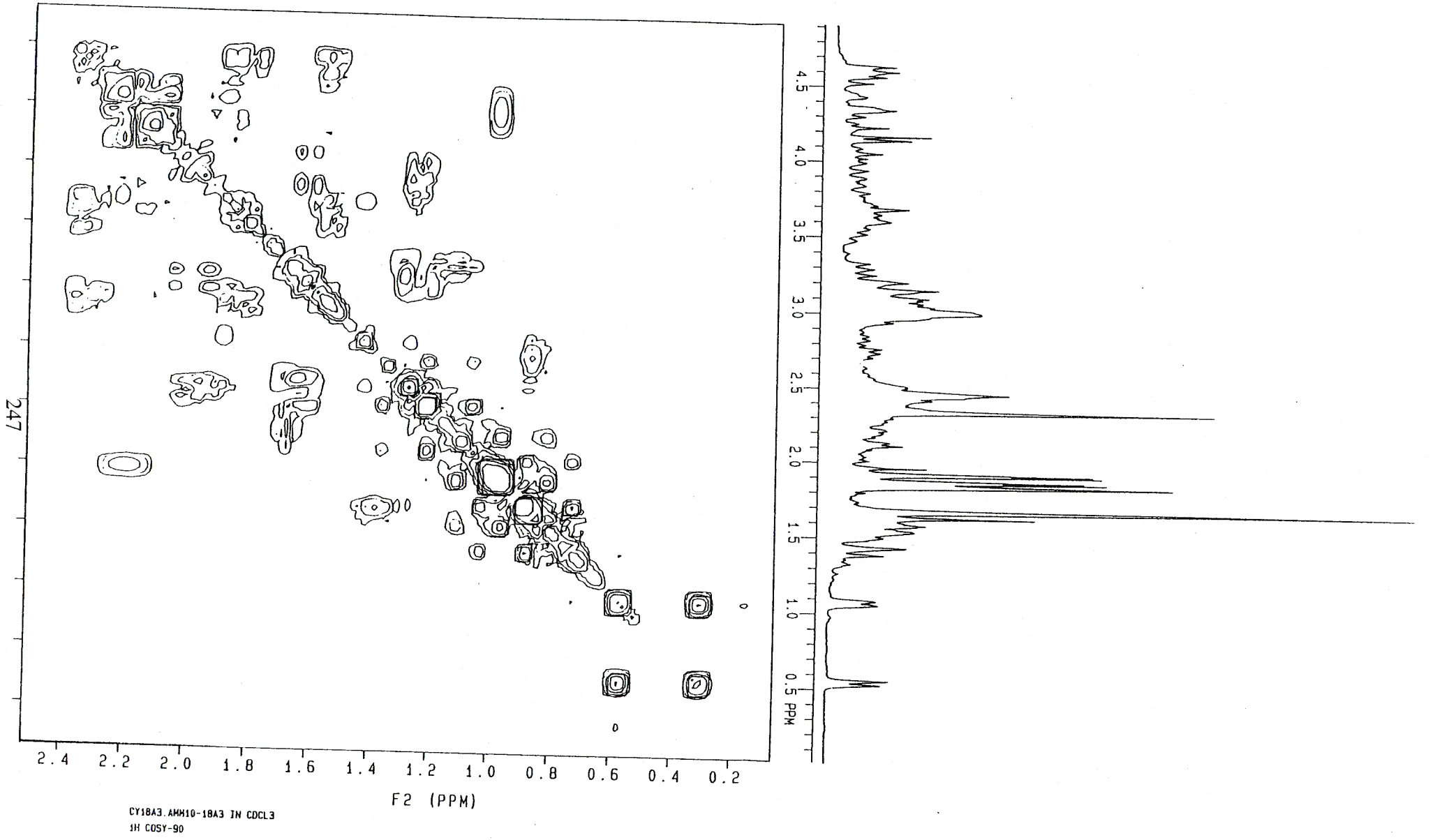
Spectrum [13-B] : ^{13}C NMR spectrum of 24-methylenepollinastanone (22) in $CDCl_3$



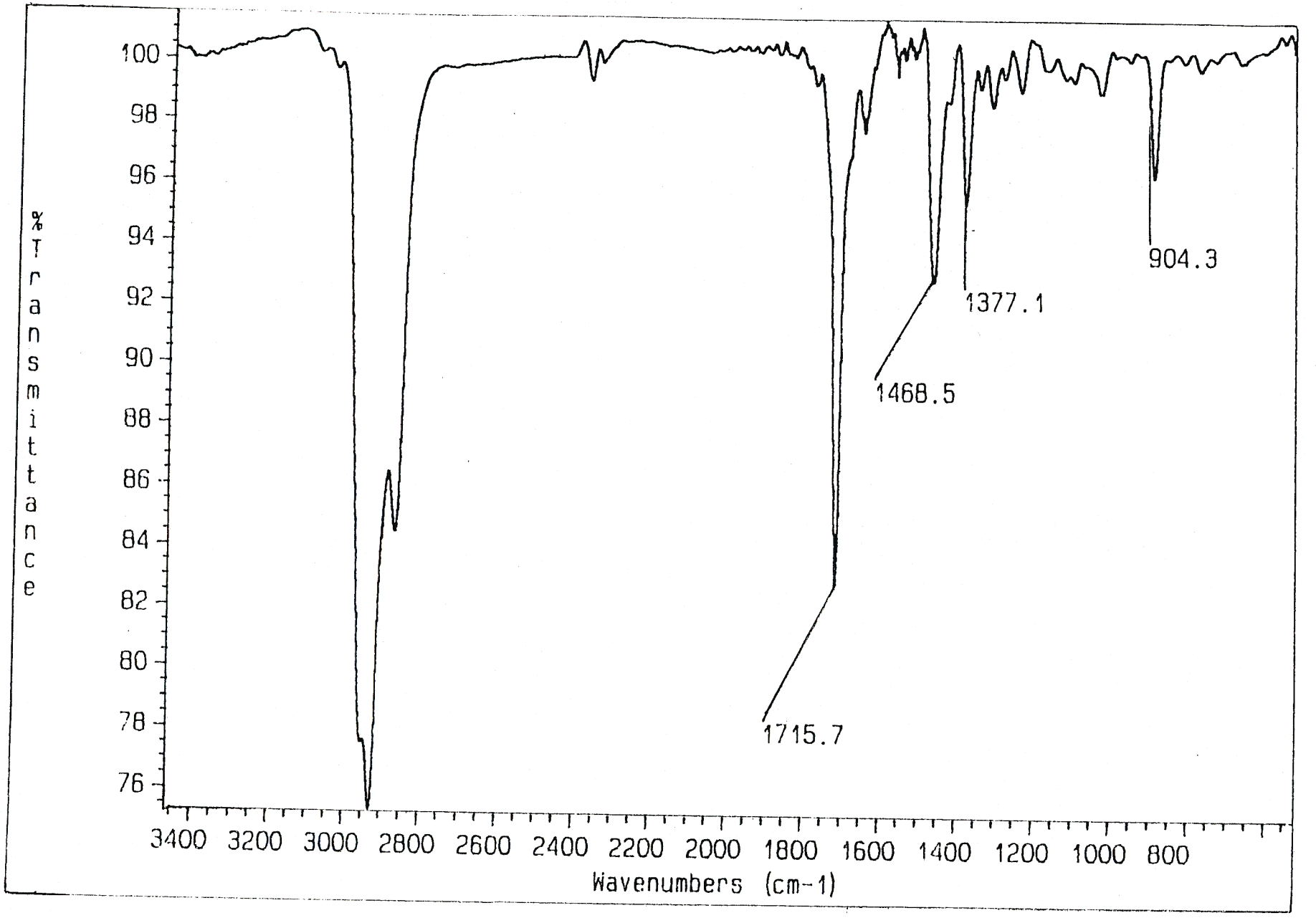
Spectrum [13-C] : ADEPT spectrum of 24-methylenepollinastanone (22) in $CDCl_3$



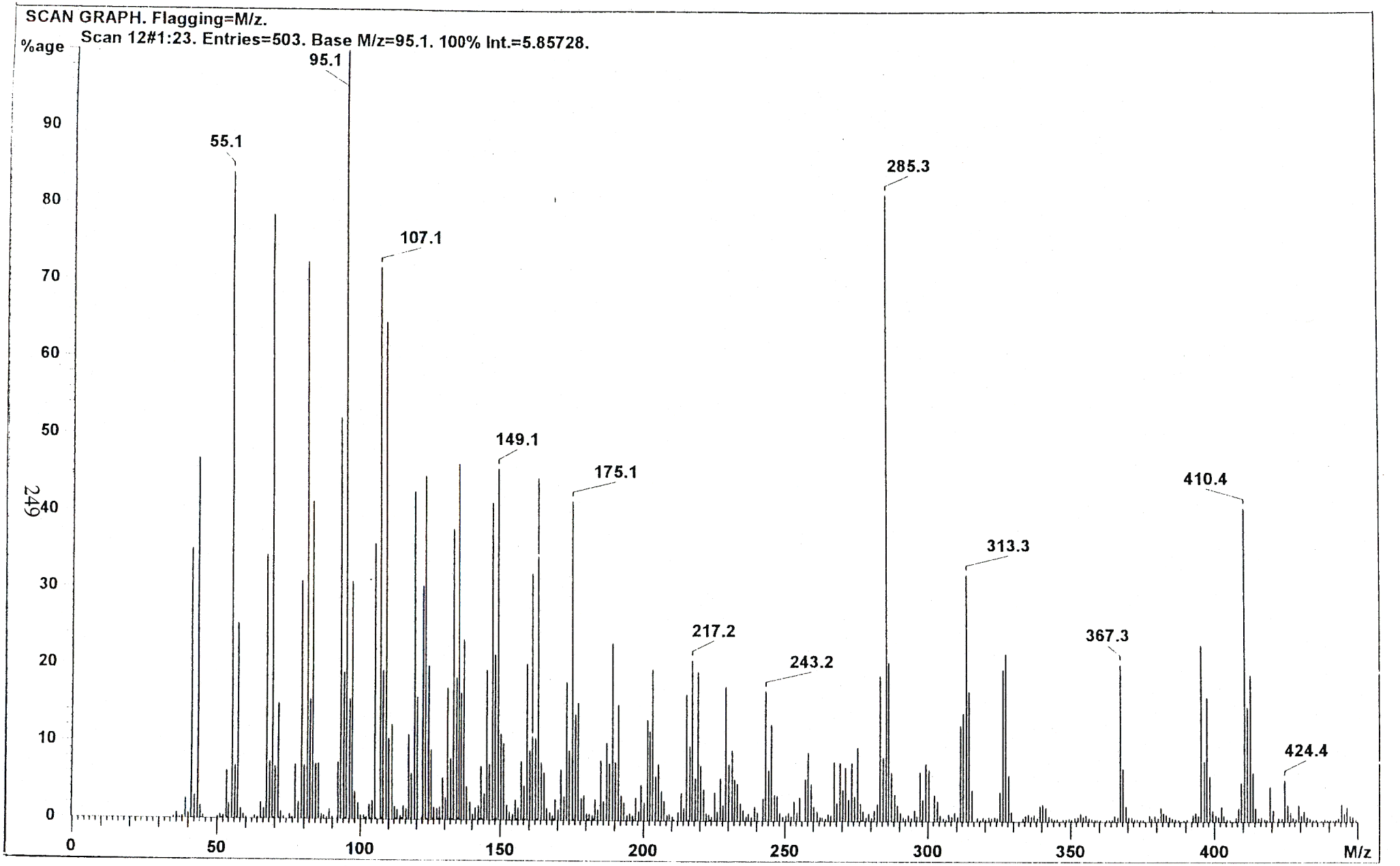
Spectrum [13-D] : COSY spectrum of 24-methylenepollinastanone (22) in $CDCl_3$



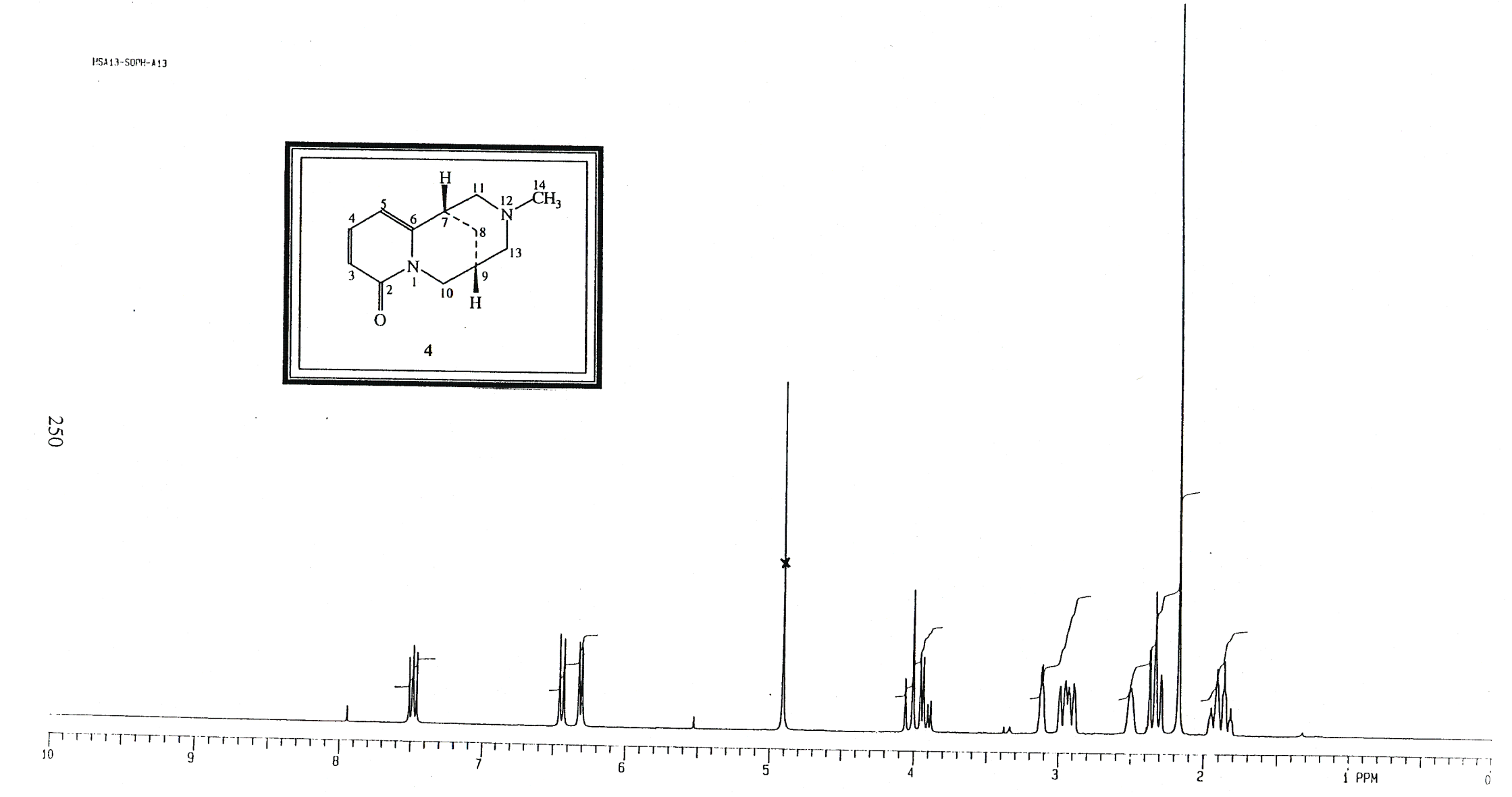
Spectrum [13-E] : Expanded COSY spectrum of 24-methylenepollinastanone (22) in $CDCl_3$



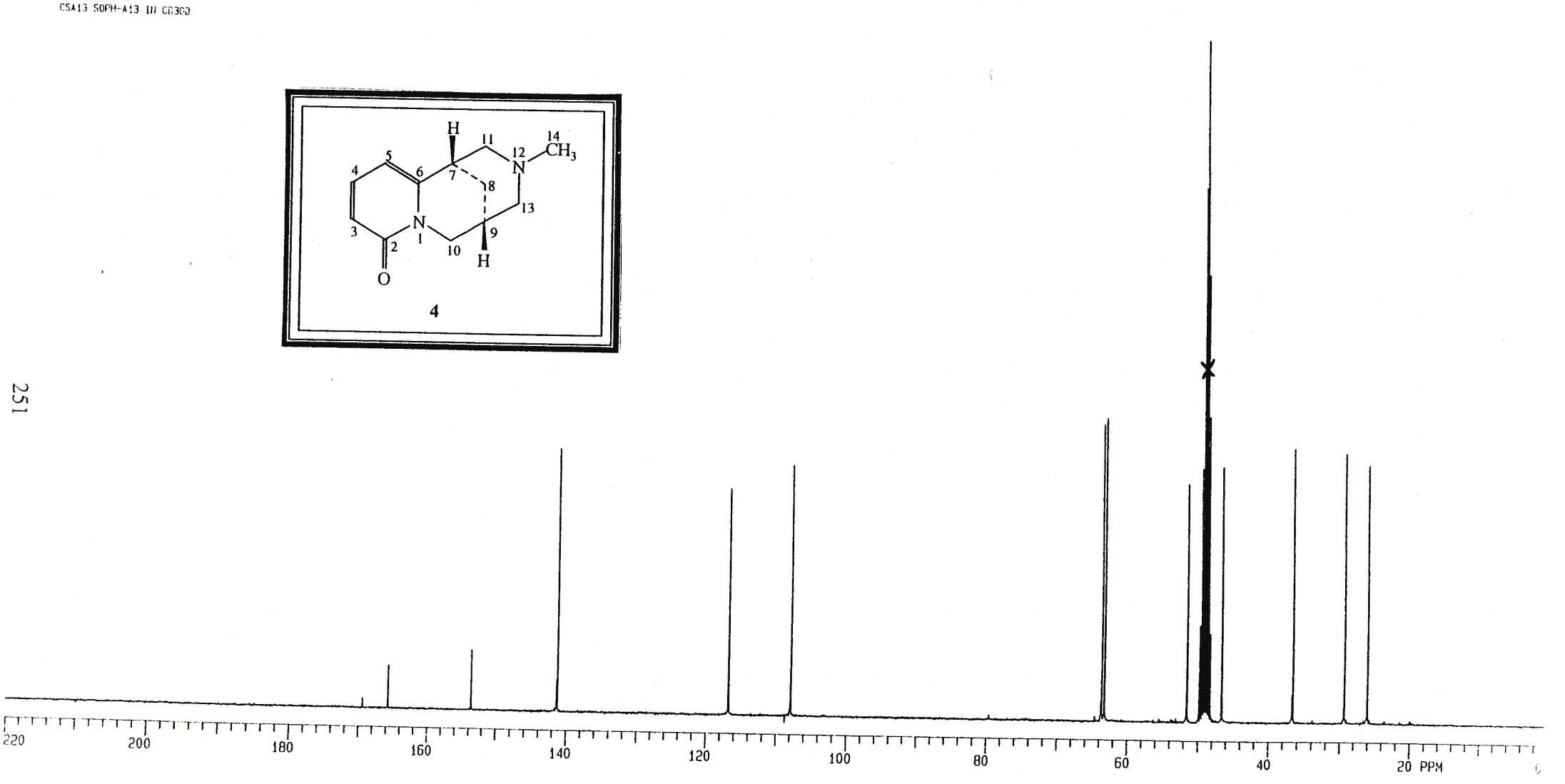
Spectrum [13-F] : Infra red spectrum of 24-methylenepollinastanone (22)



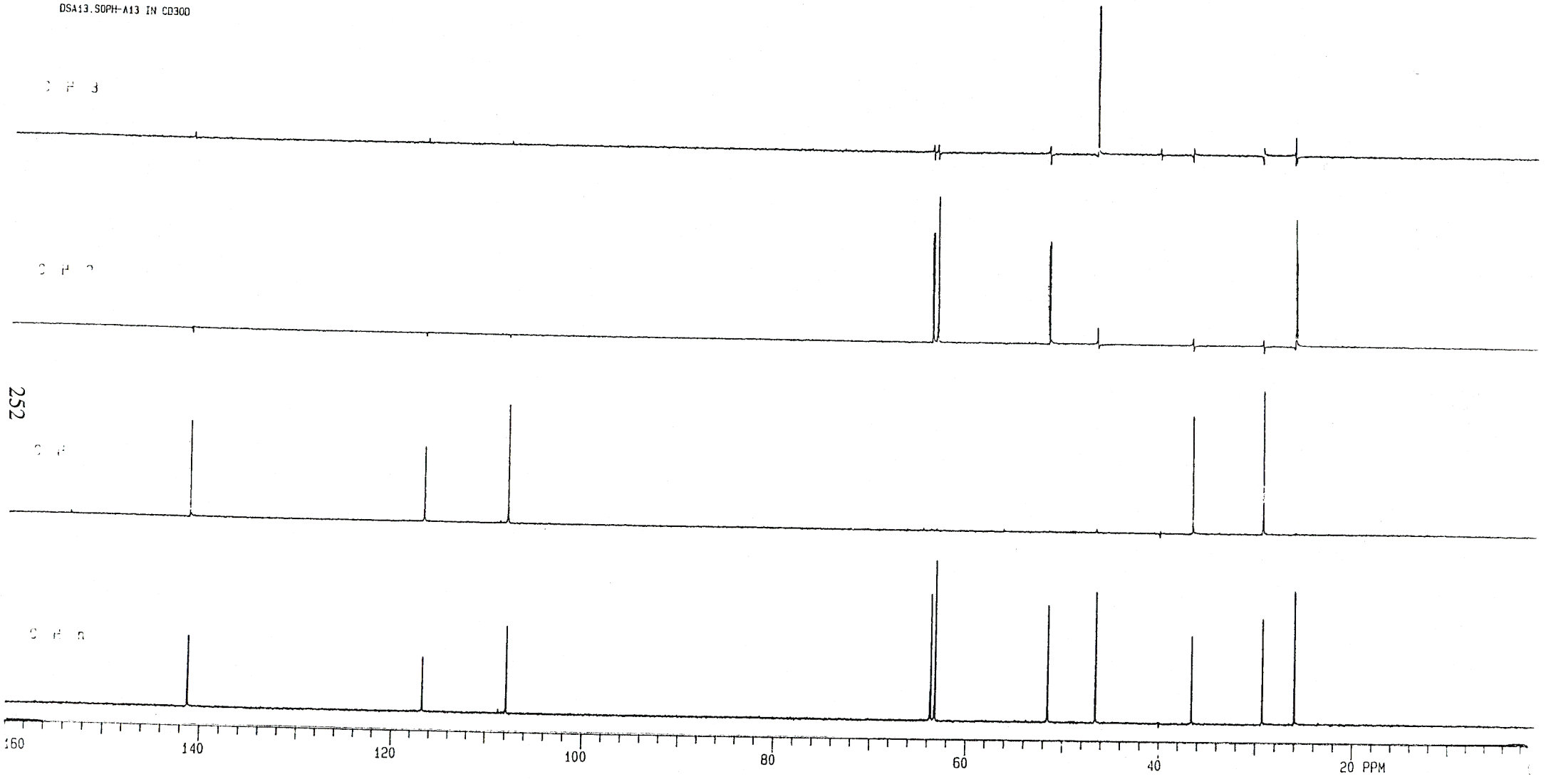
Spectrum [13-G] : Mass spectrum of 24-methylenepollinastanone (22)



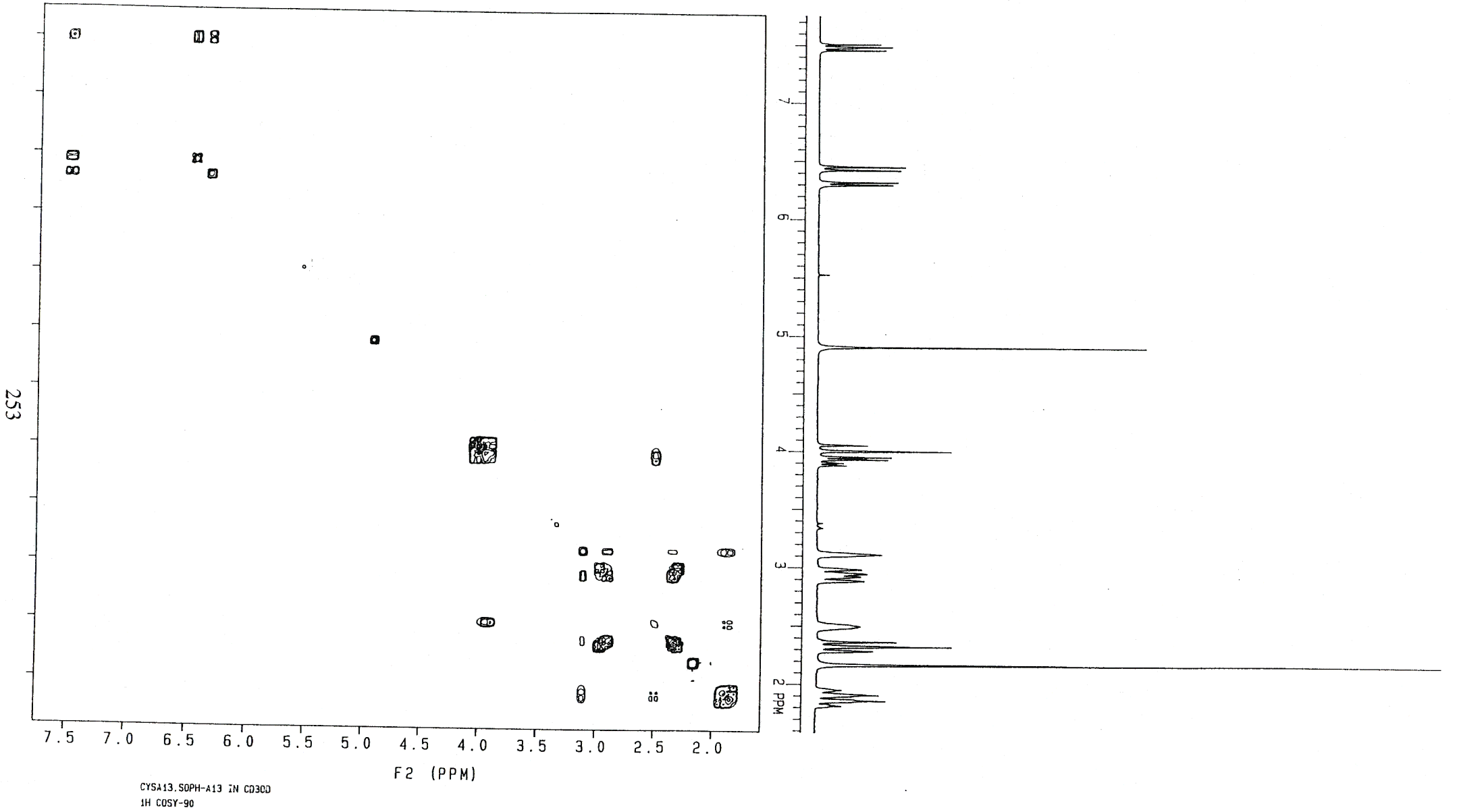
Spectrum [14-A] : ^{1}H NMR spectrum of N-methylcytisine (4) in CD_3OD



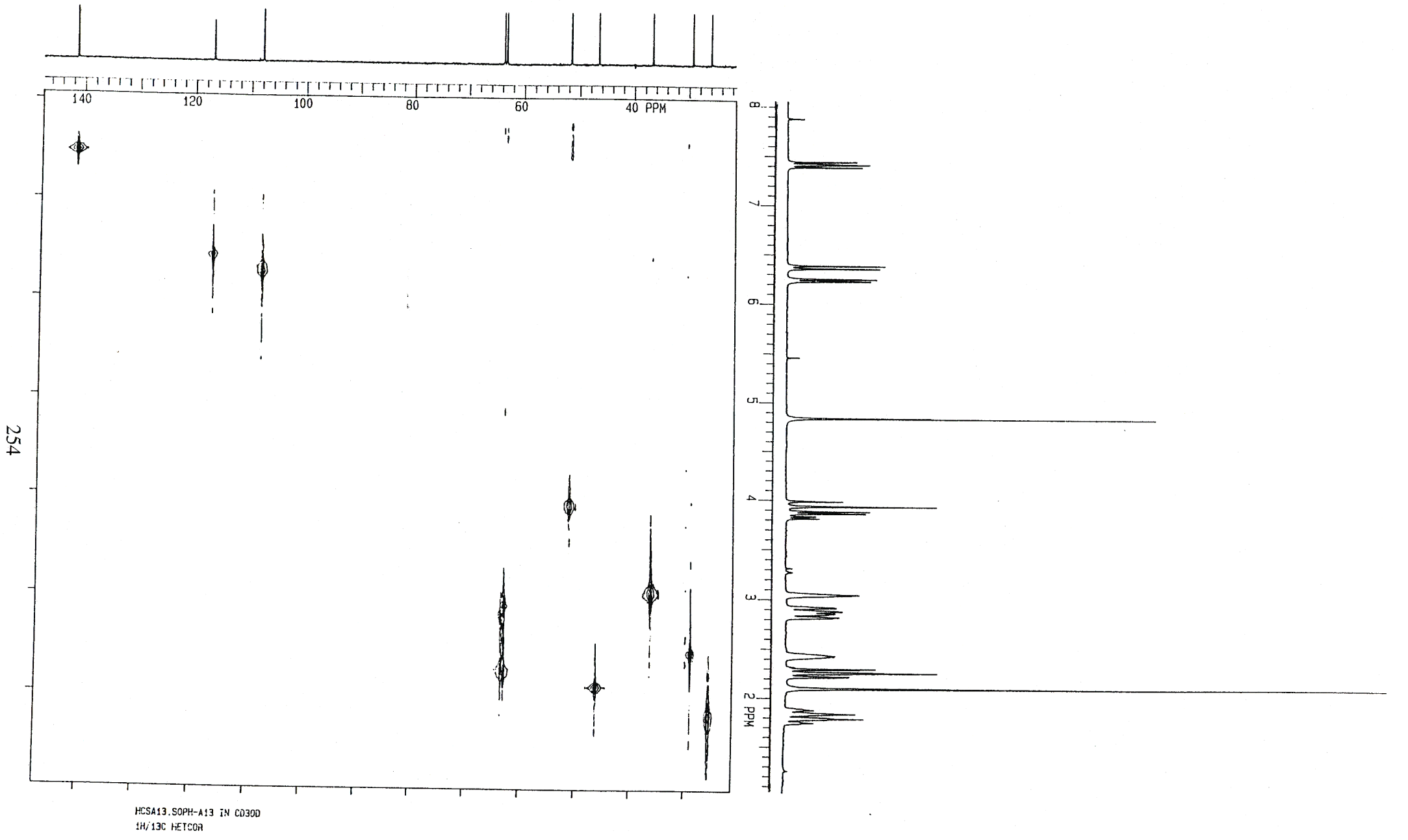
Spectrum [14-B] : ^{13}C NMR spectrum of N-methylcytisine (4) in CD_3OD



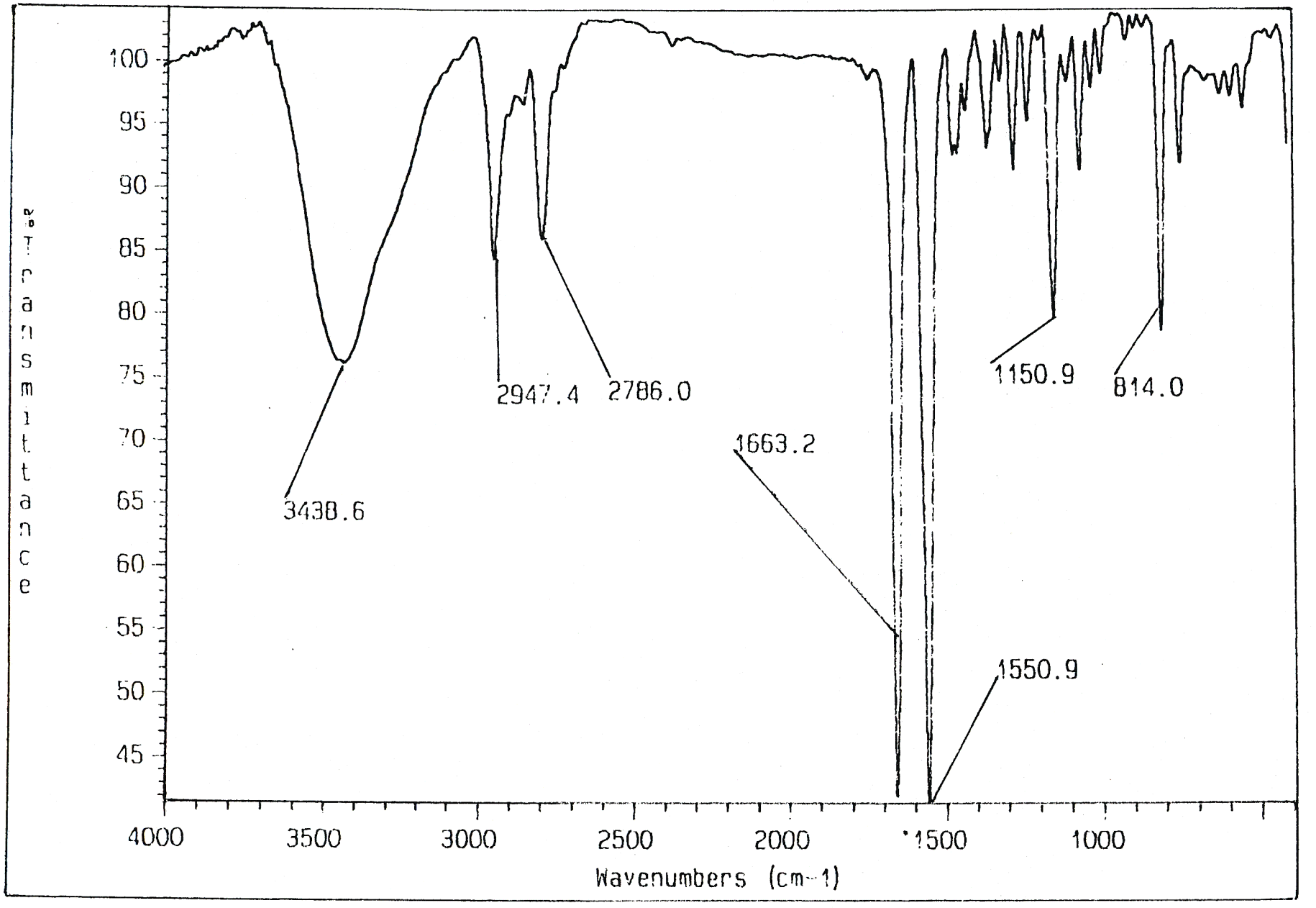
Spectrum [14-C] : ADEPT spectrum of N-methylcytisine (4) in CD_3OD



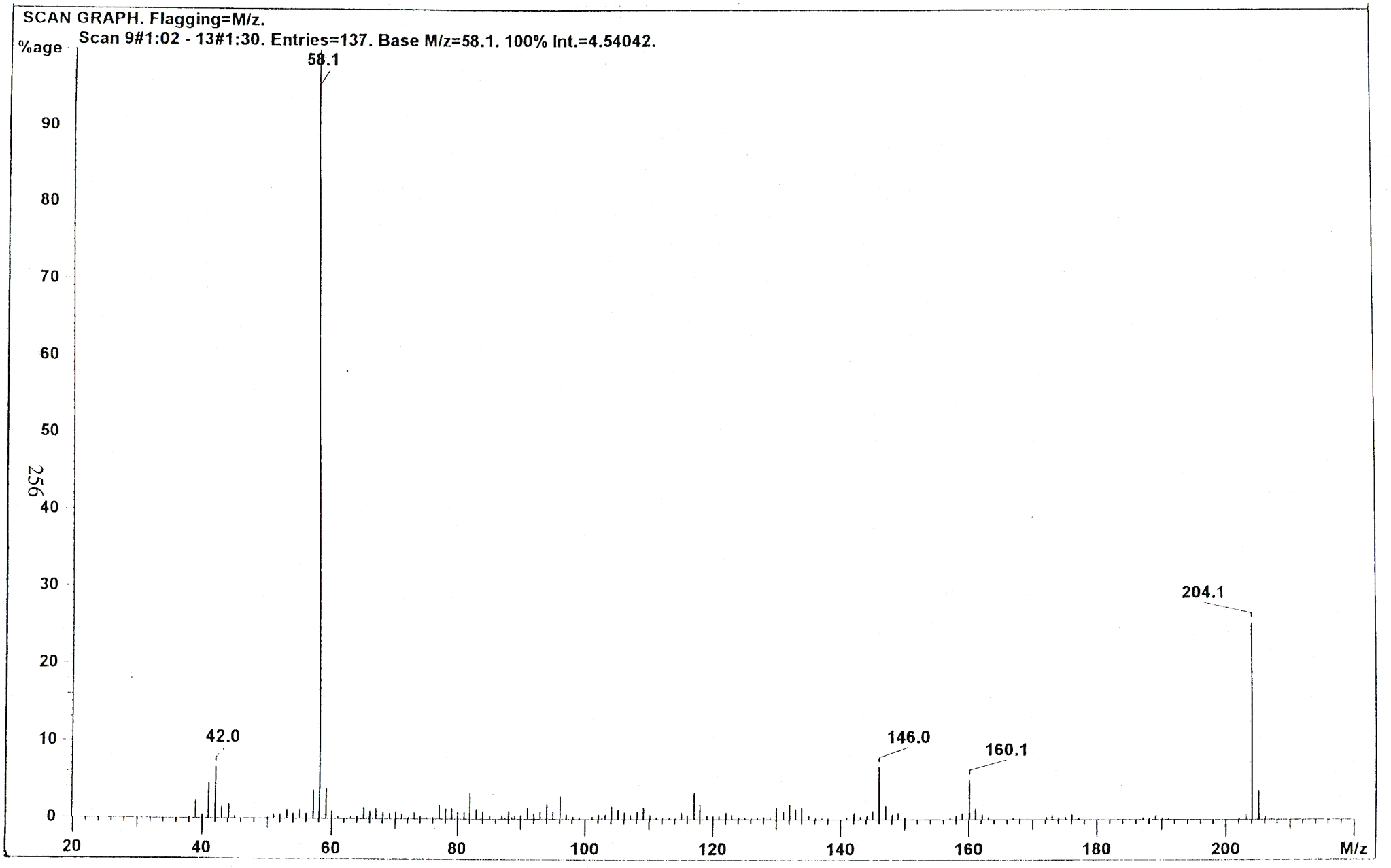
Spectrum [14-D] : COSY spectrum of N-methylcytisine (4) in CD_3OD



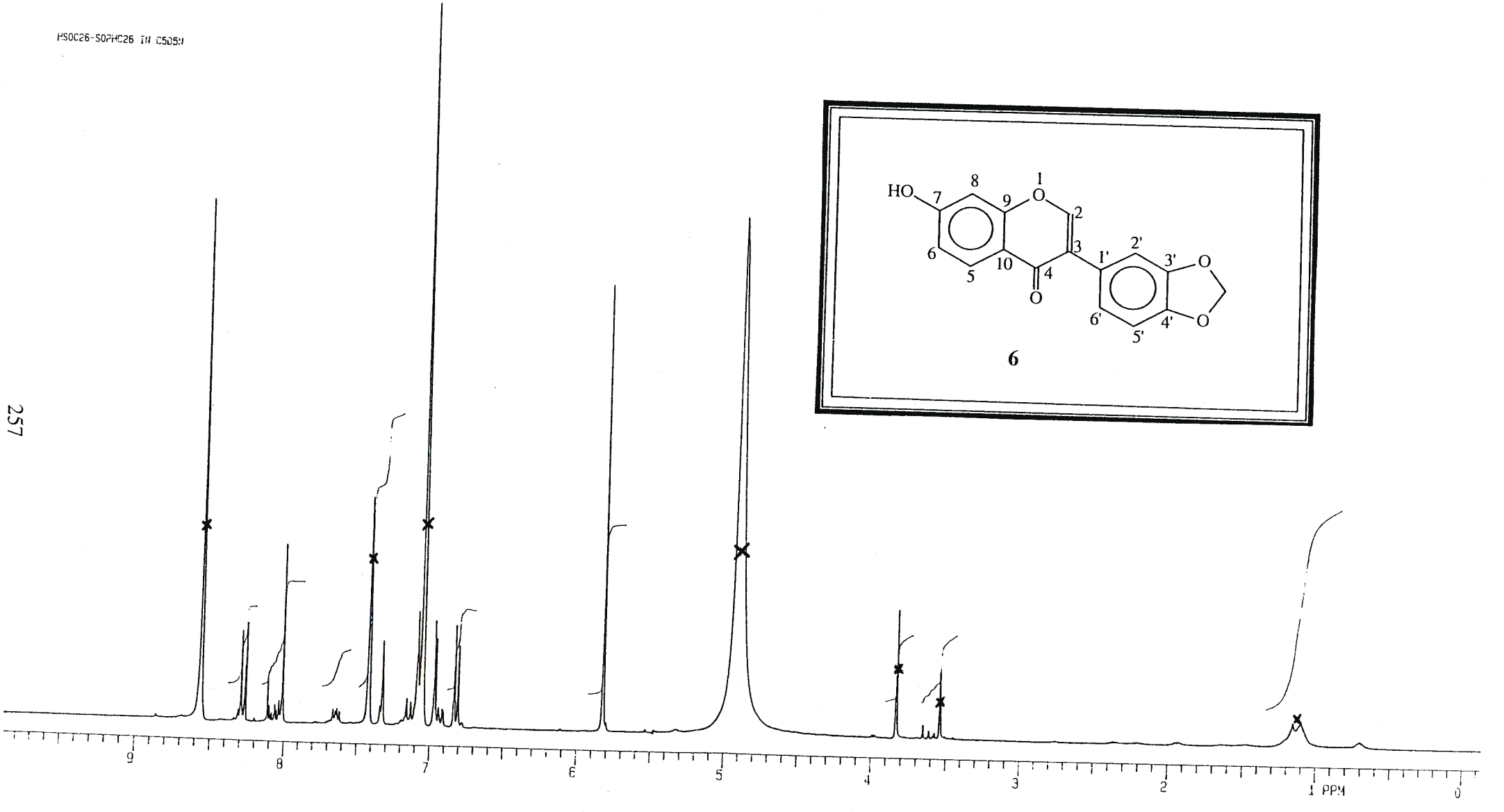
Spectrum [14-E] : HETCOR spectrum of N-methylcytisine (4) in CD_3OD



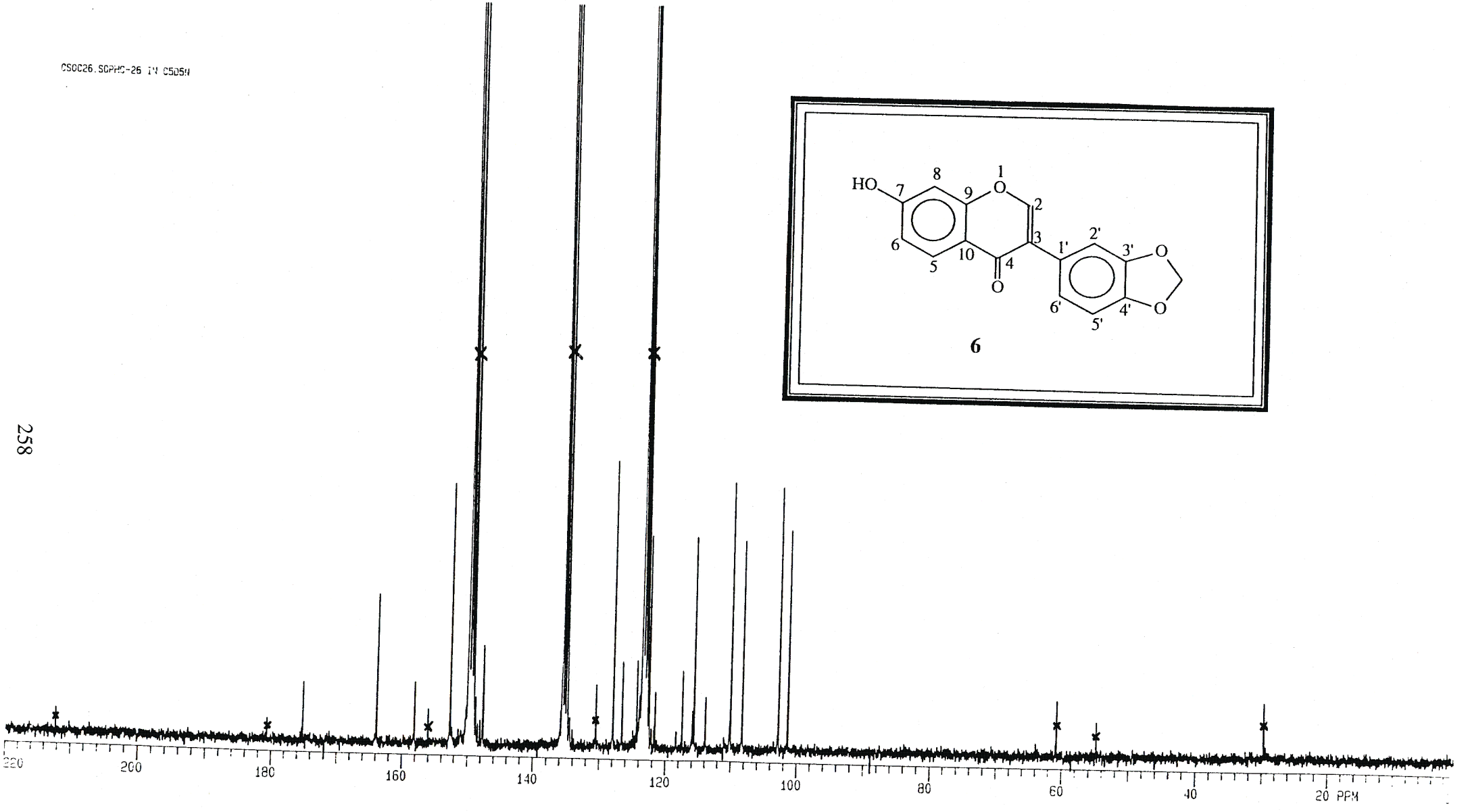
Spectrum [14-F] : Infra red spectrum of N-methylcytisine (4)



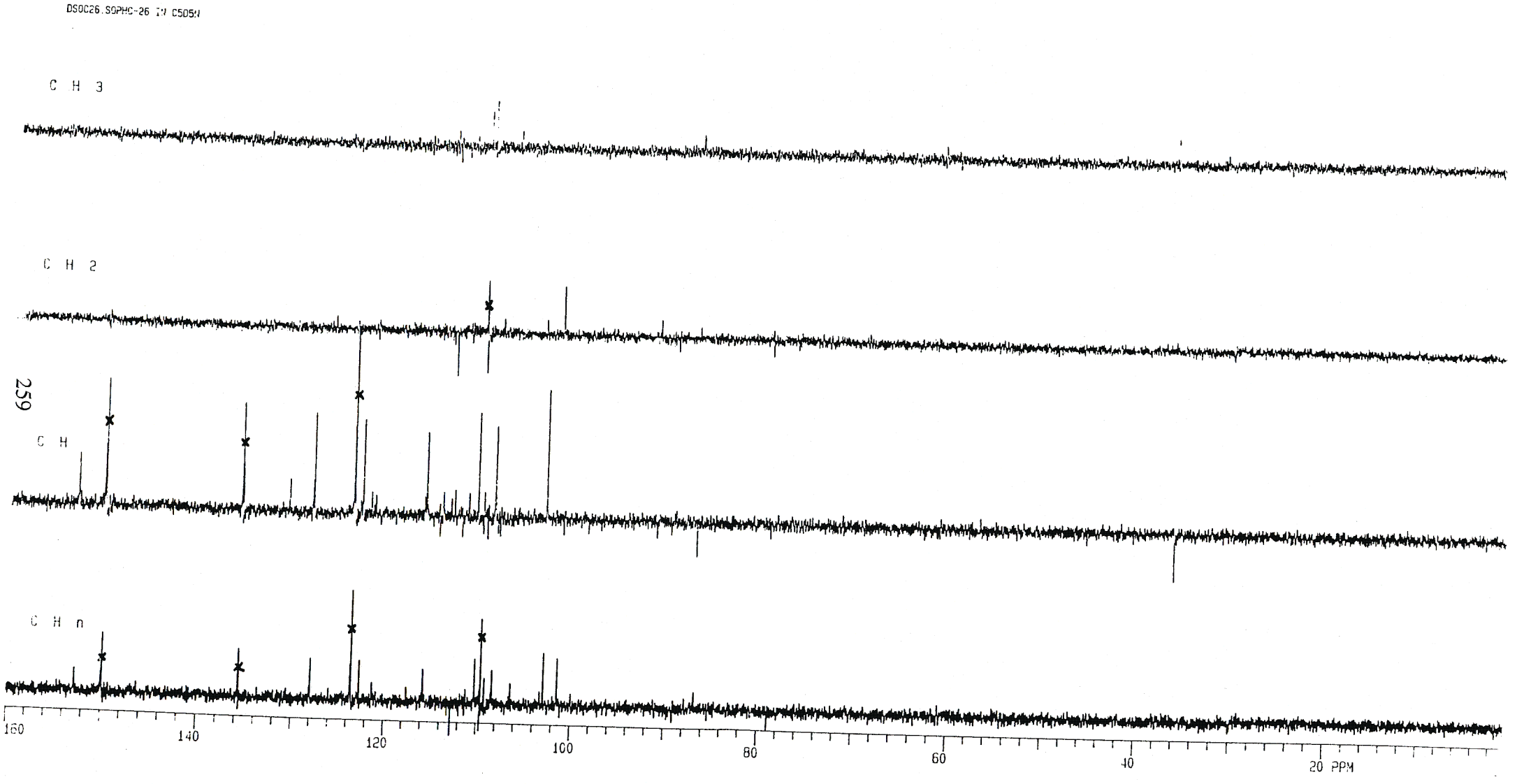
Spectrum [14-G] : Mass spectrum of N-methylcytisine (4)



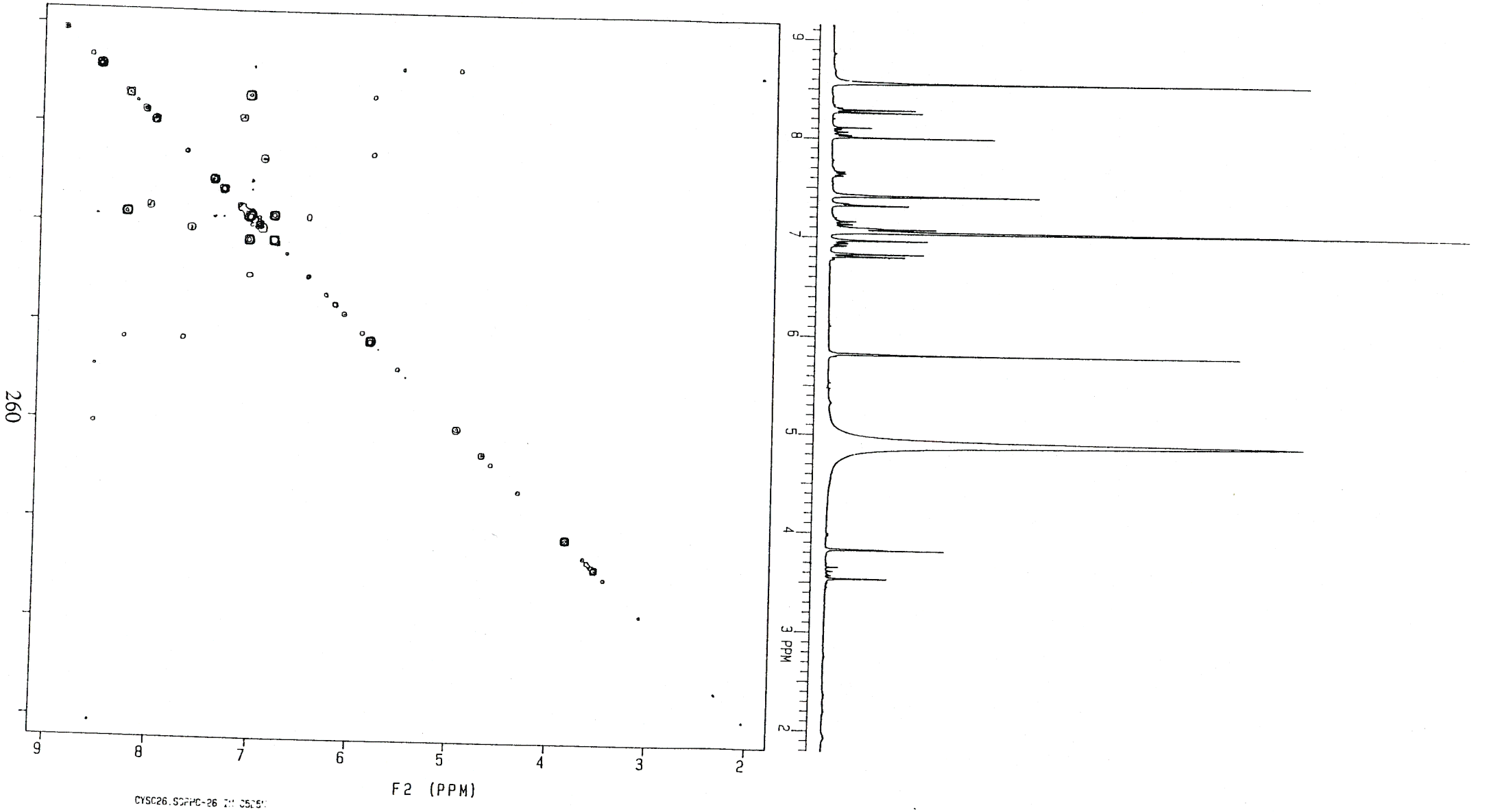
Spectrum [15-A] : ^{1}H NMR spectrum of pseudobaptigenin (6) in C_5D_5N



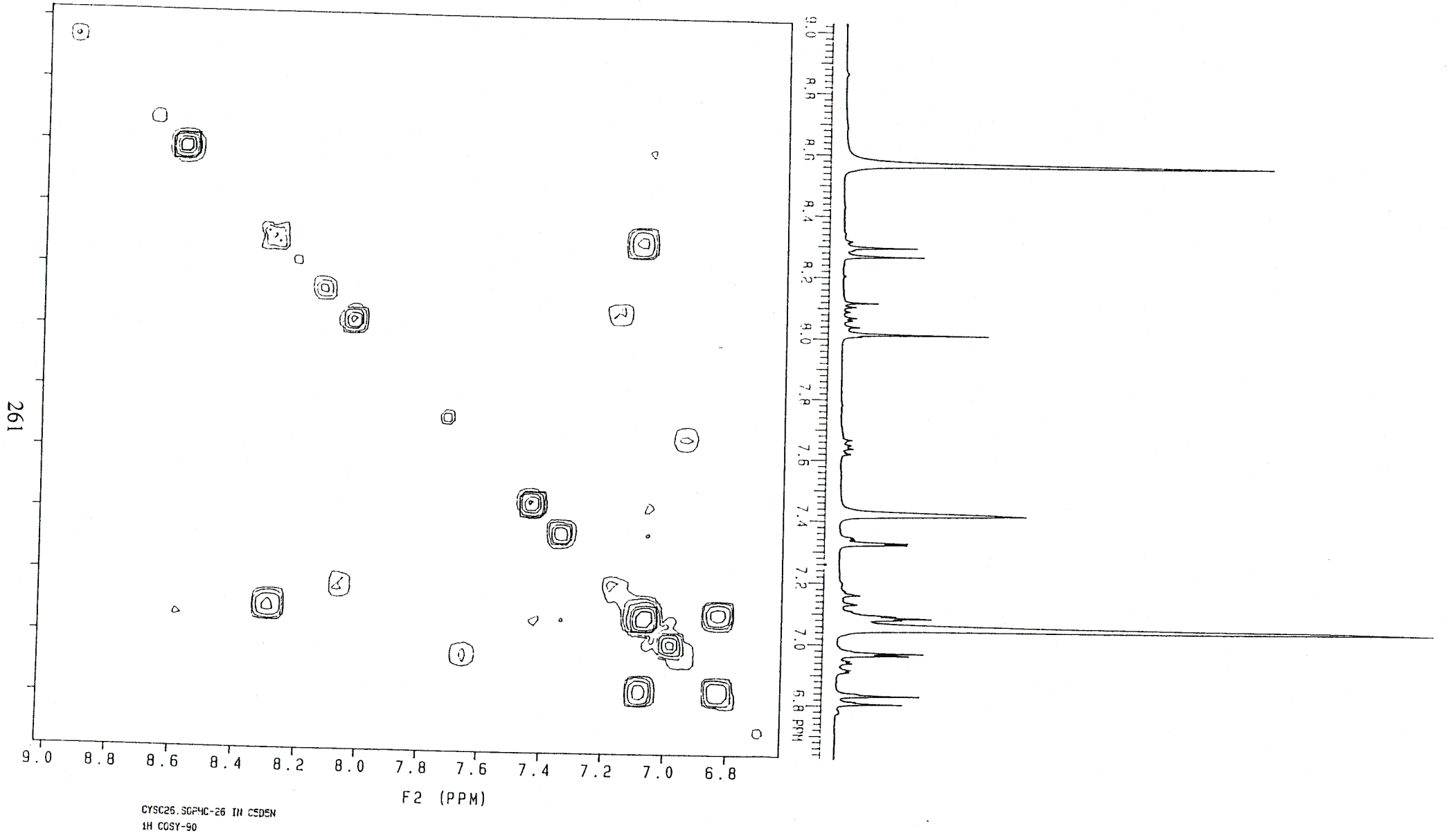
Spectrum [15-B] : ^{13}C NMR spectrum of pseudobaptigenin (6) in C_5D_5N



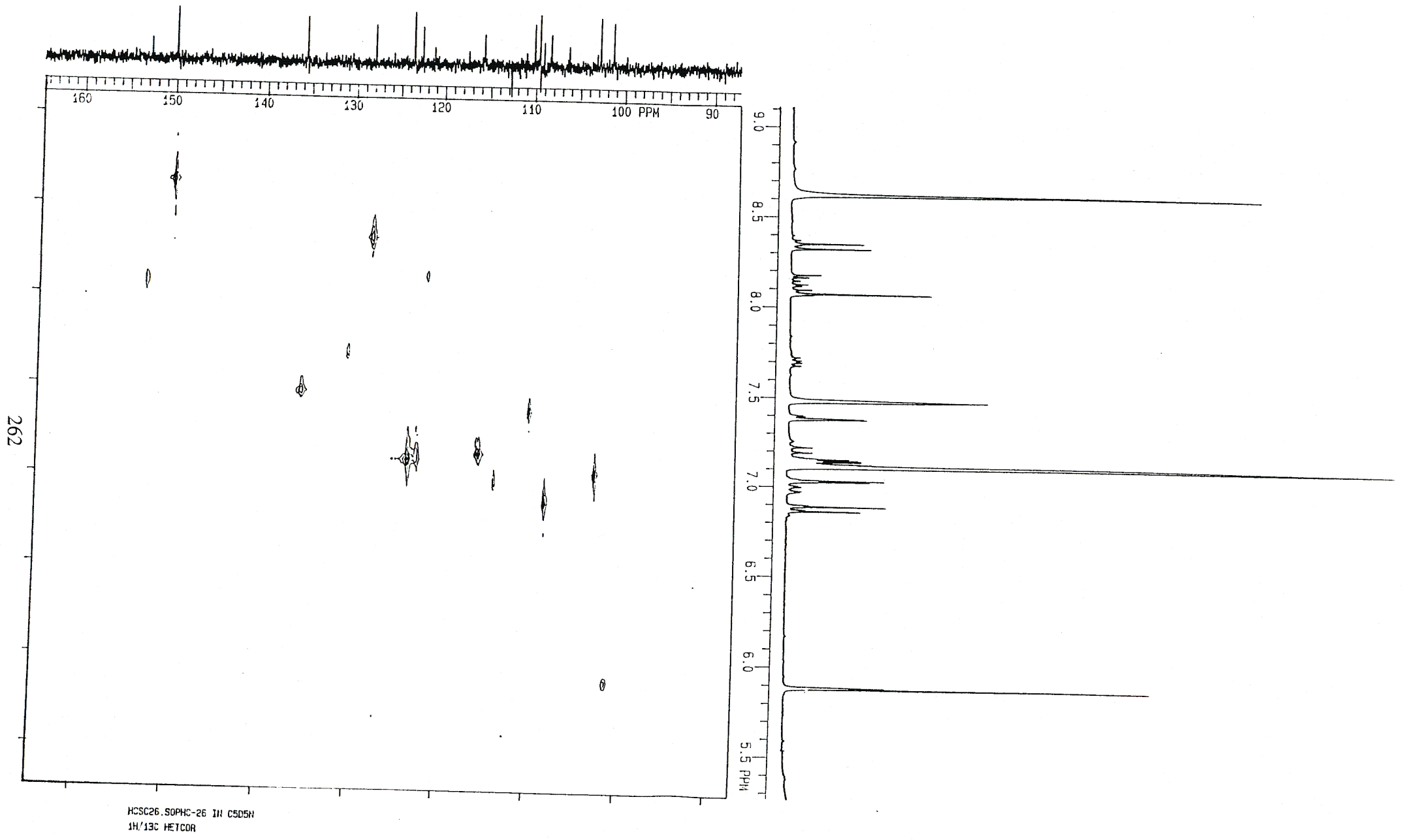
Spectrum [15-C] : ADEPT spectrum of pseudobaptigenin (6) in C_5D_5N



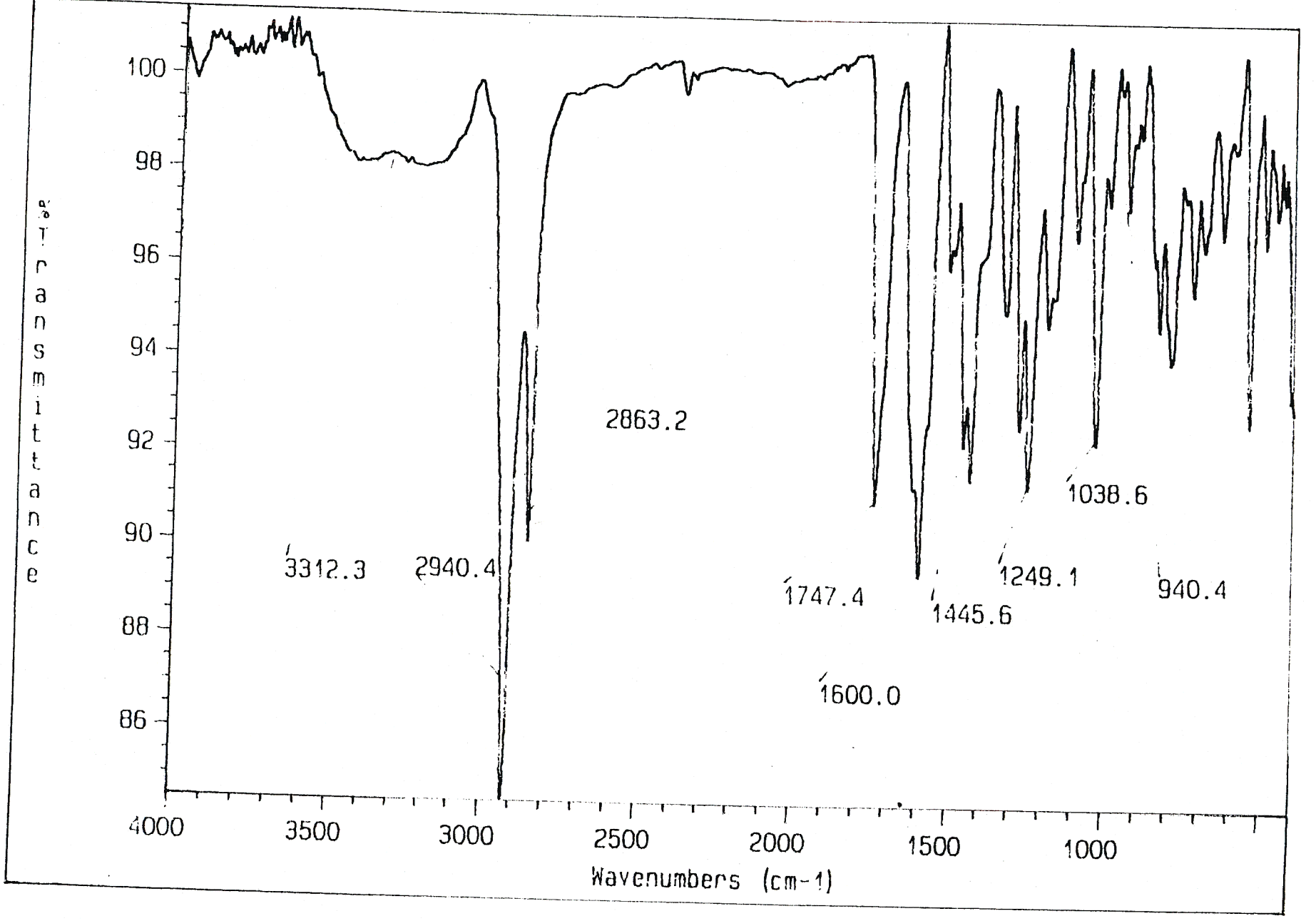
Spectrum [15-D] : COSY spectrum of pseudobaptigenin (6) in C_5D_5N



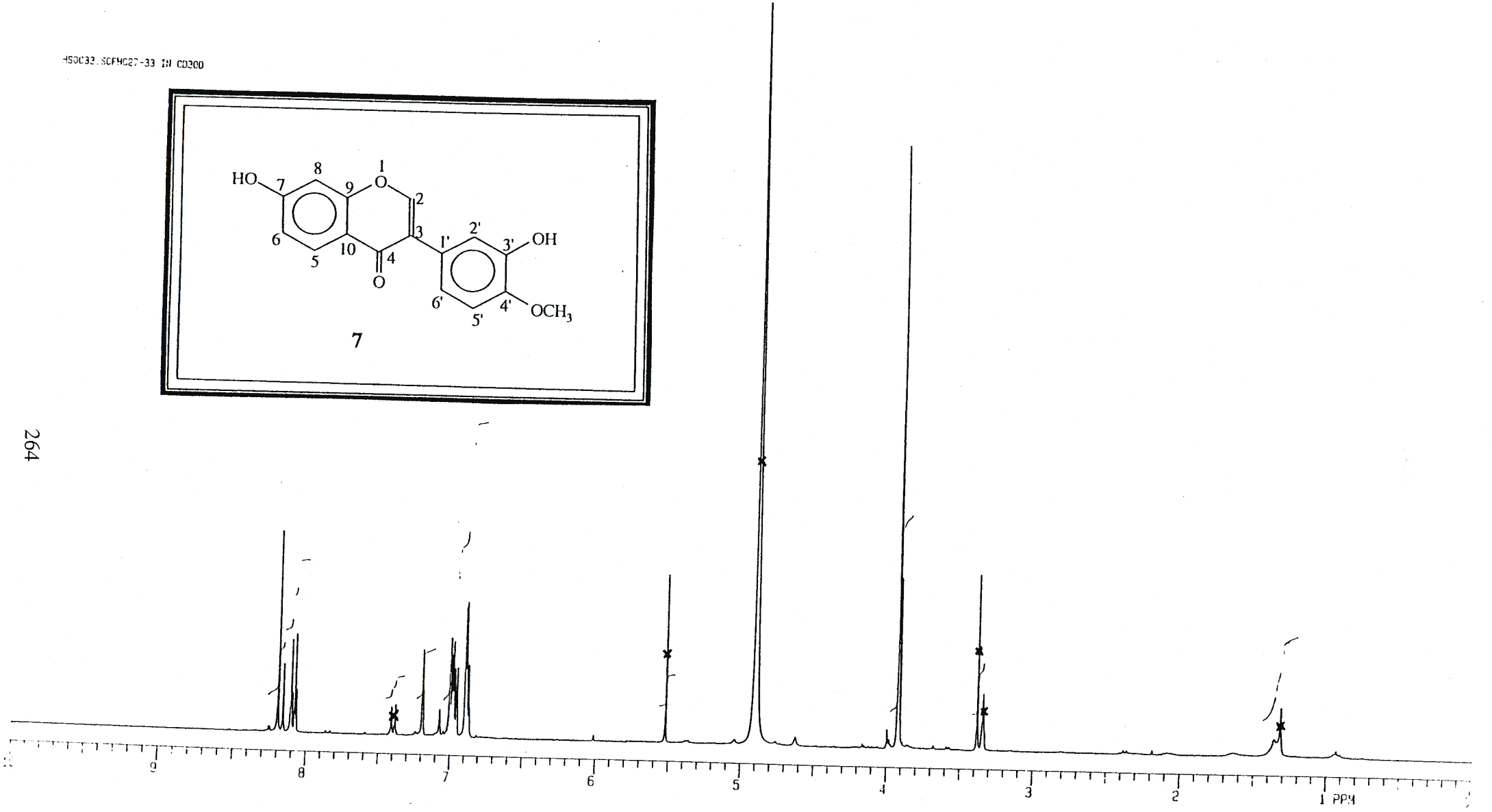
Spectrum [15-E] : Expanded COSY spectrum of pseudobaptigenin (6) in C_5D_5N



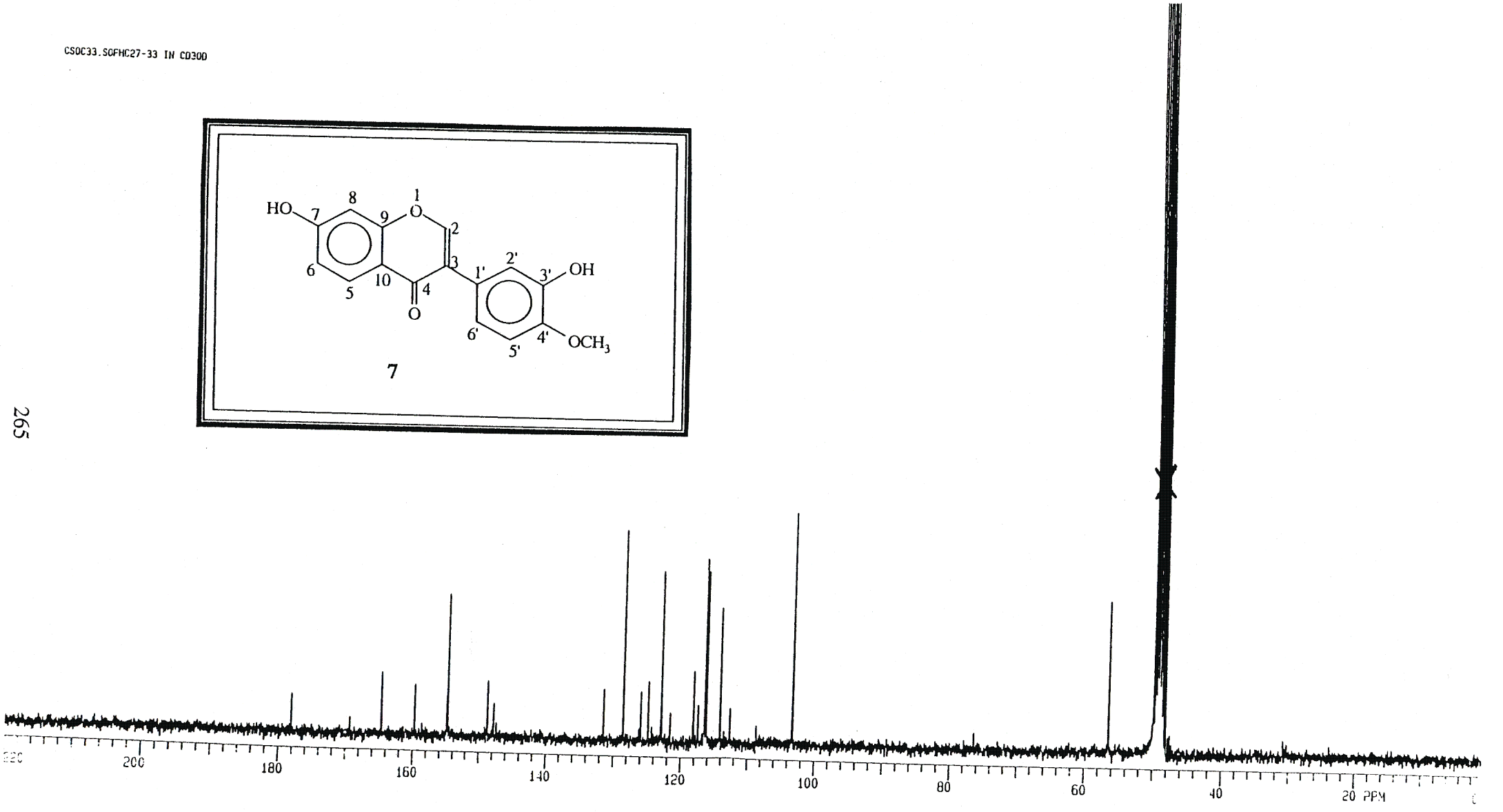
Spectrum [15-F] : HETCOR spectrum of pseudobaptigenin (6) in C_5D_5N



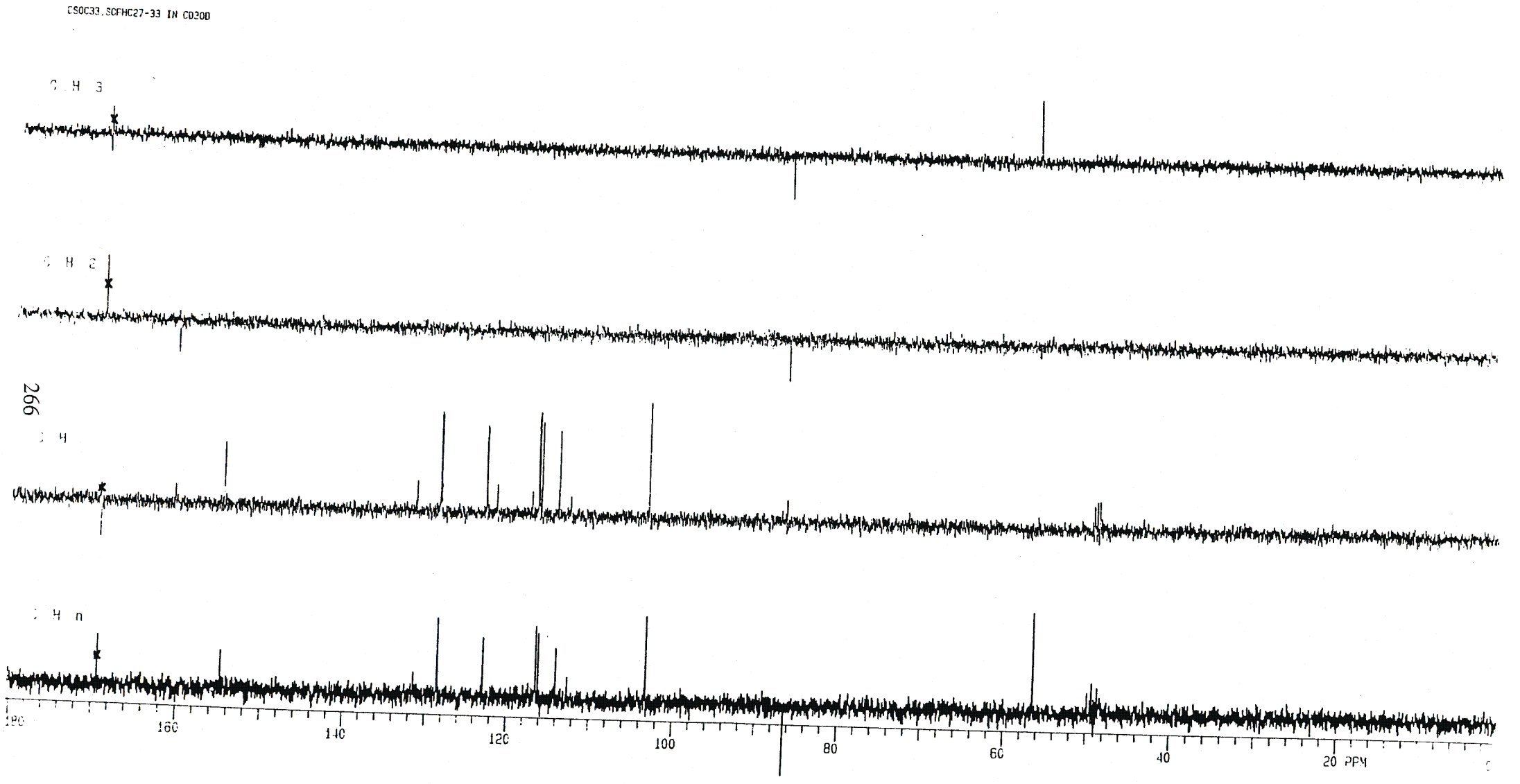
Spectrum [15-G] : Infra red spectrum of pseudobaptigenin (6)



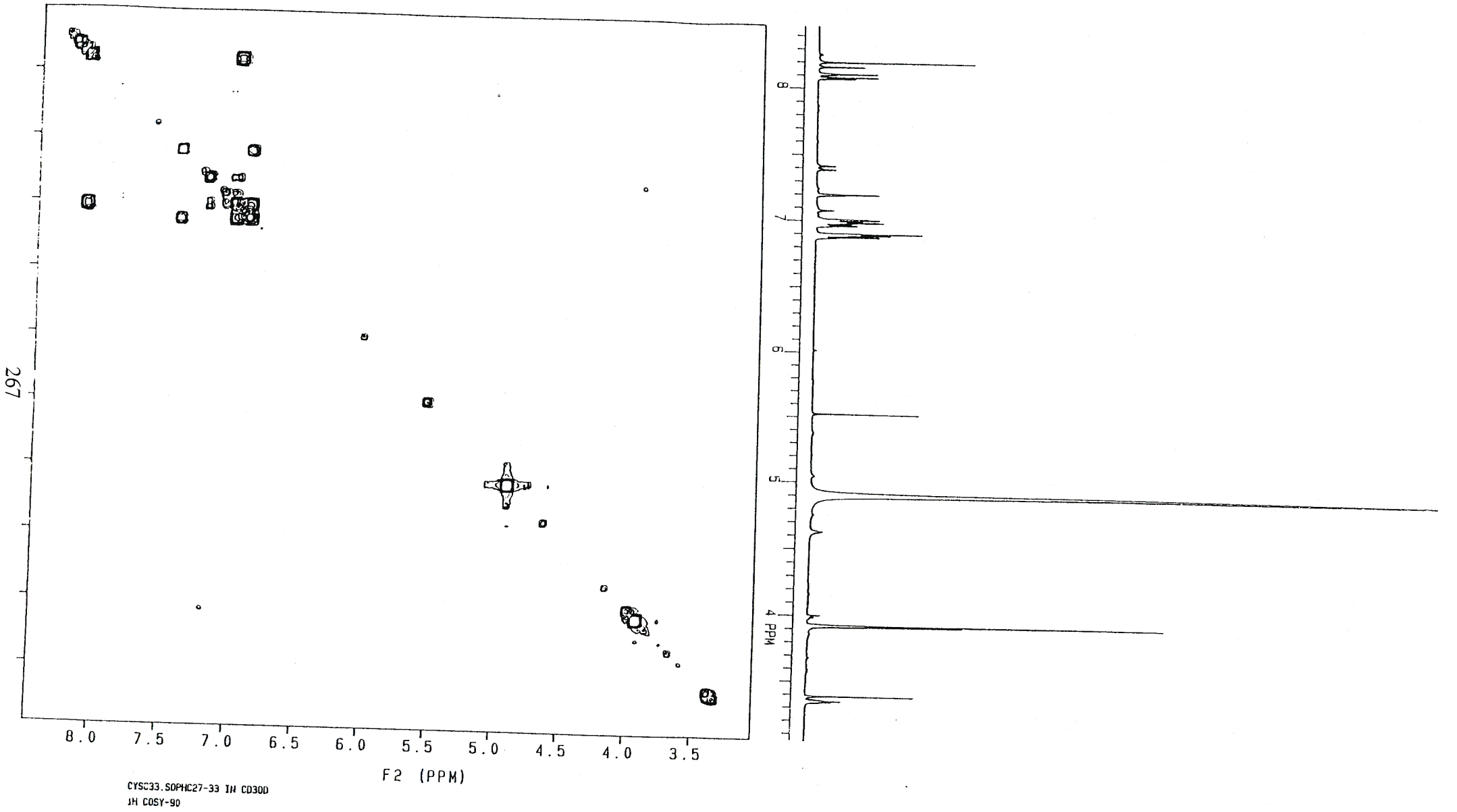
Spectrum [16-A] : ^{1}H NMR spectrum of calycosin (7) in CD_3OD



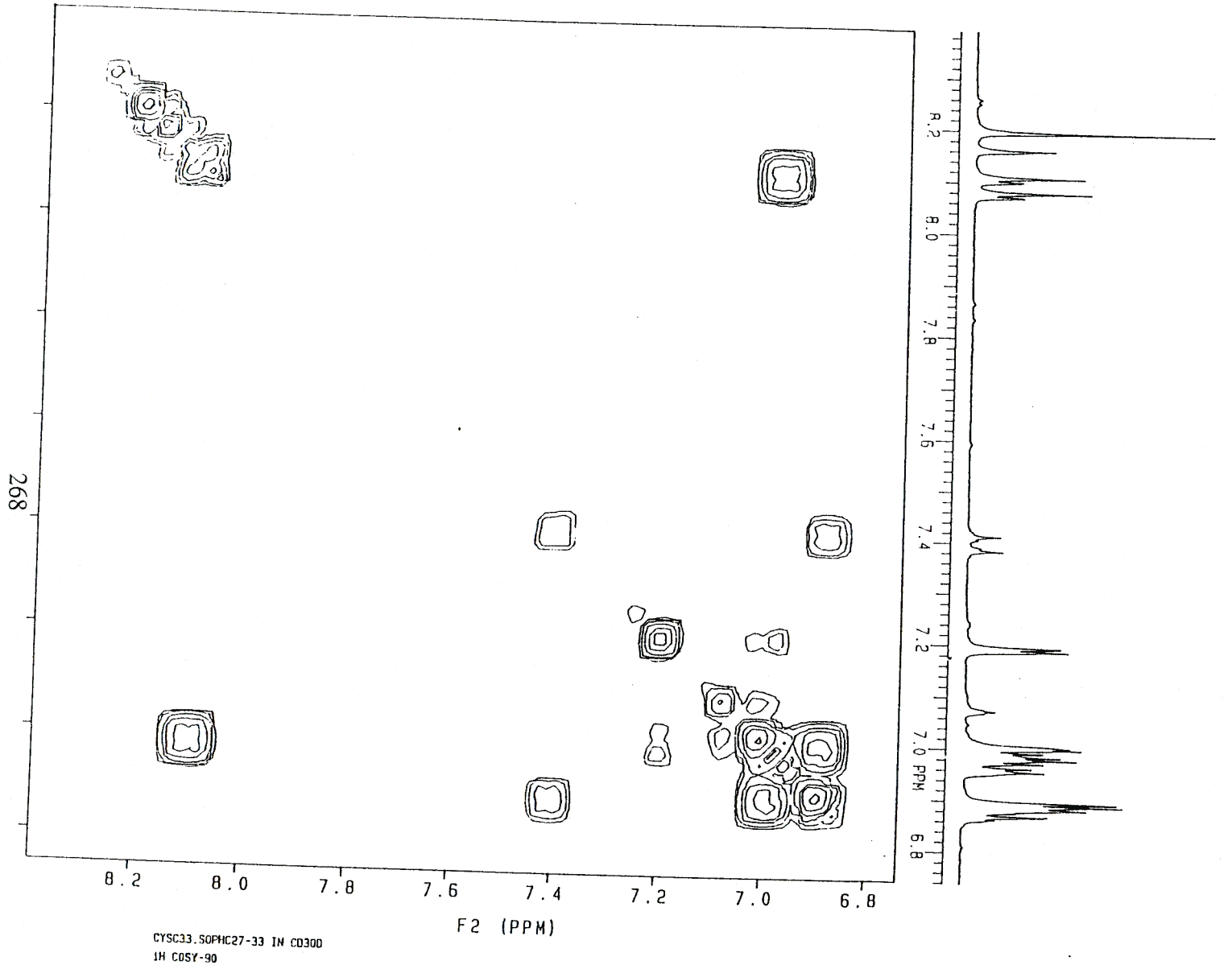
Spectrum [16-B] : ^{13}C NMR spectrum of calycosin (7) in CD_3OD



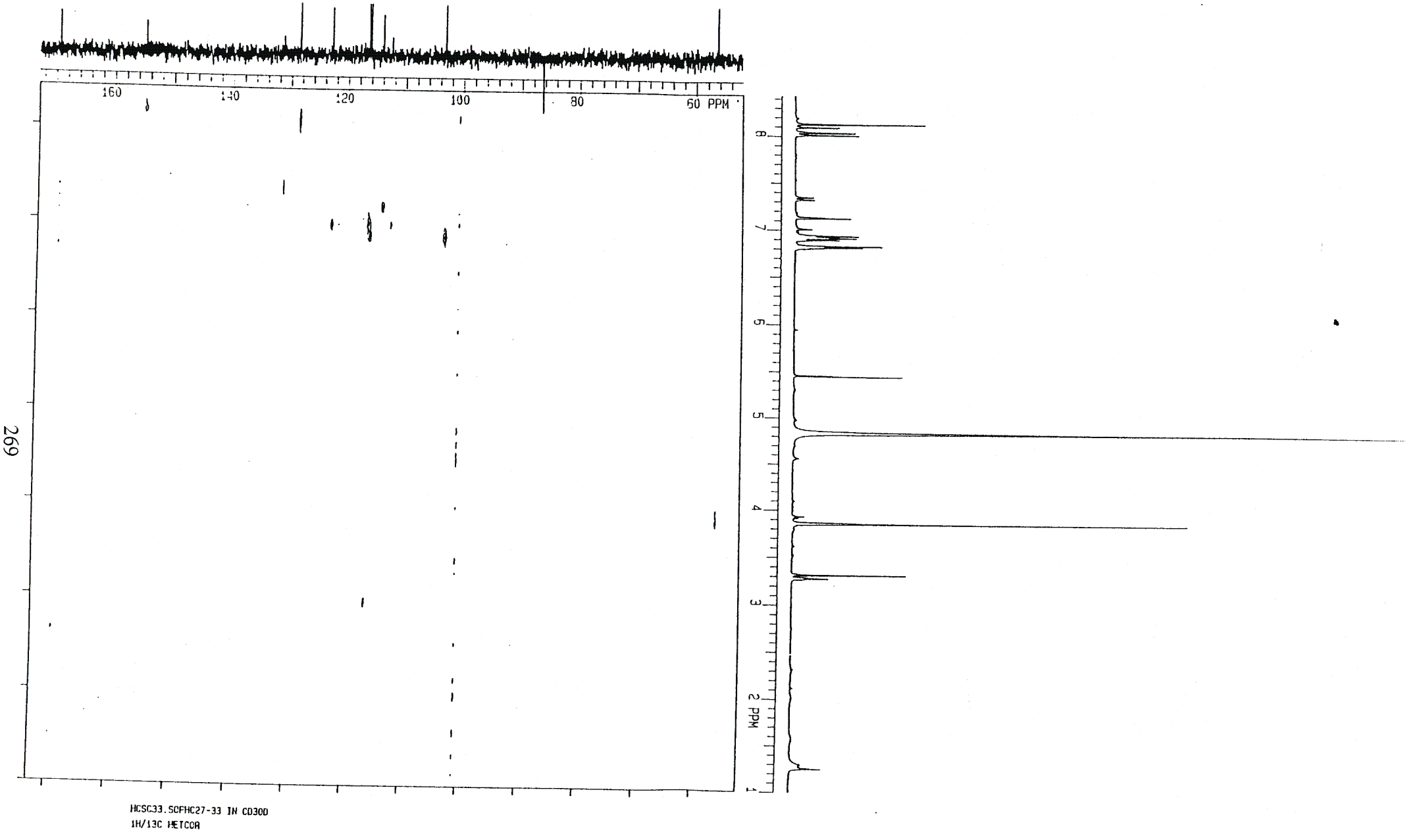
Spectrum [16-C] : ADEPT spectrum of calycosin (7) in CD_3OD



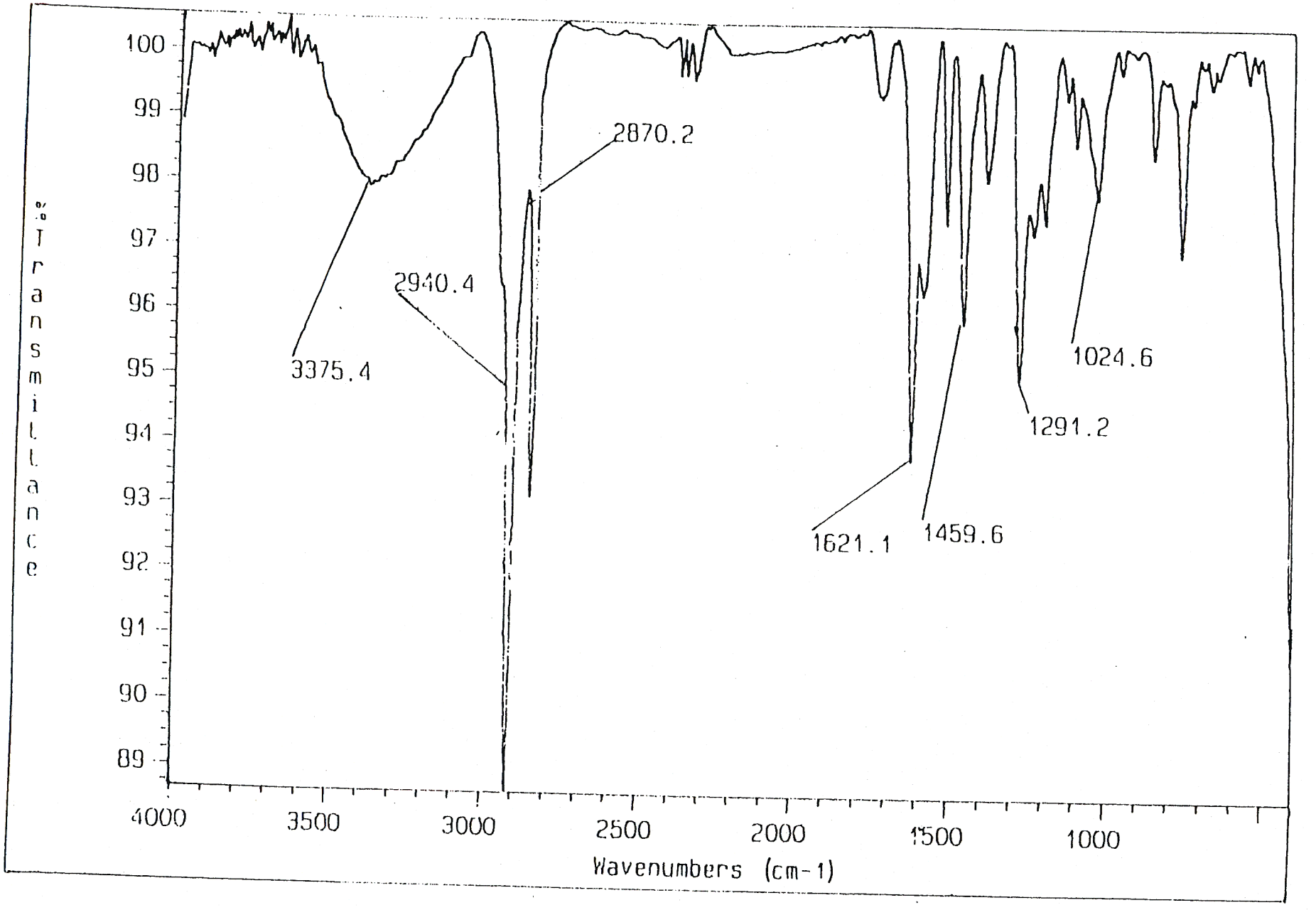
Spectrum [16-D] : COSY spectrum of calycosin (7) in CD_3OD



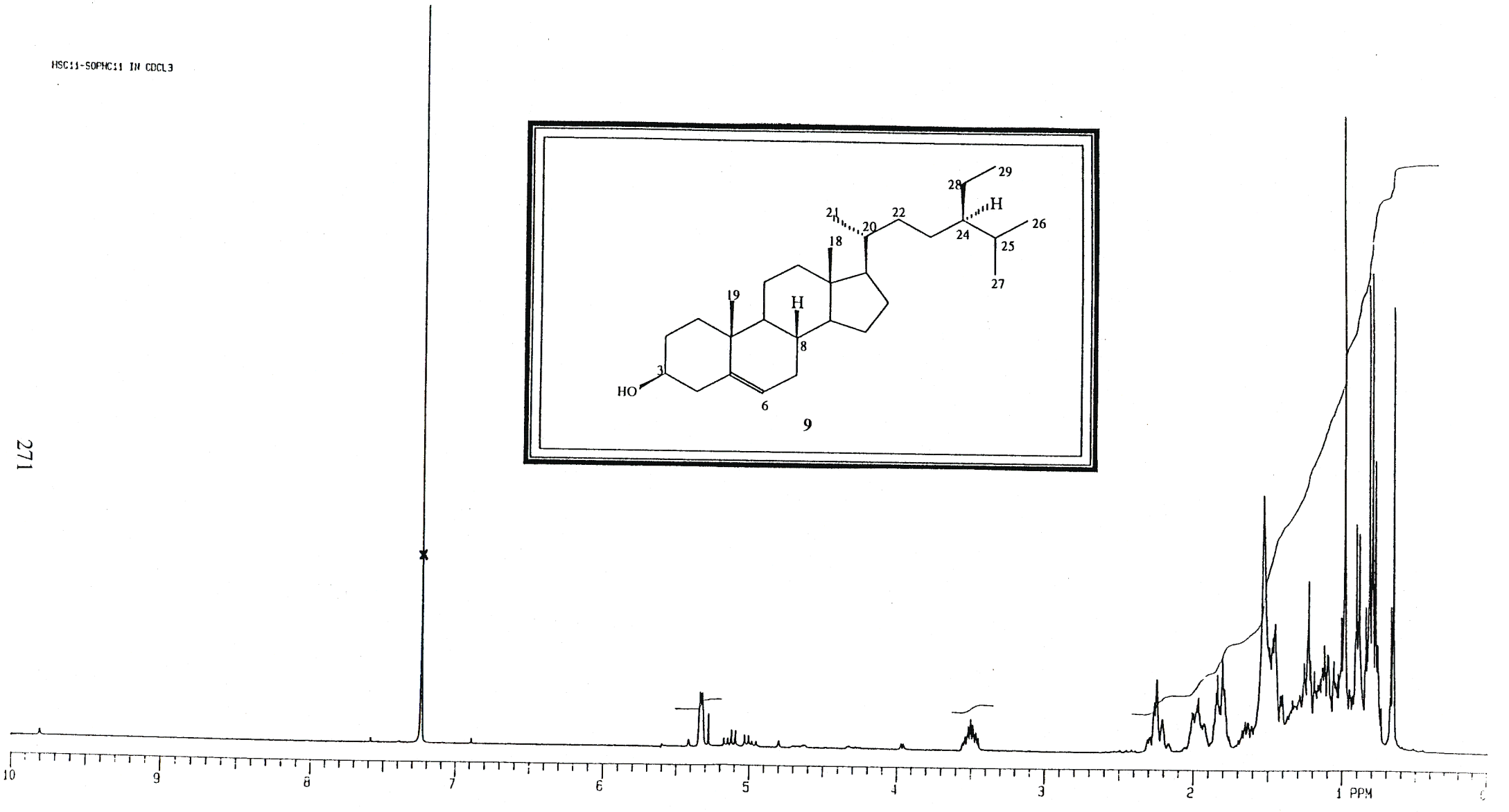
Spectrum [16-E] : Expanded COSY spectrum of calycosin (7) in CD_3OD



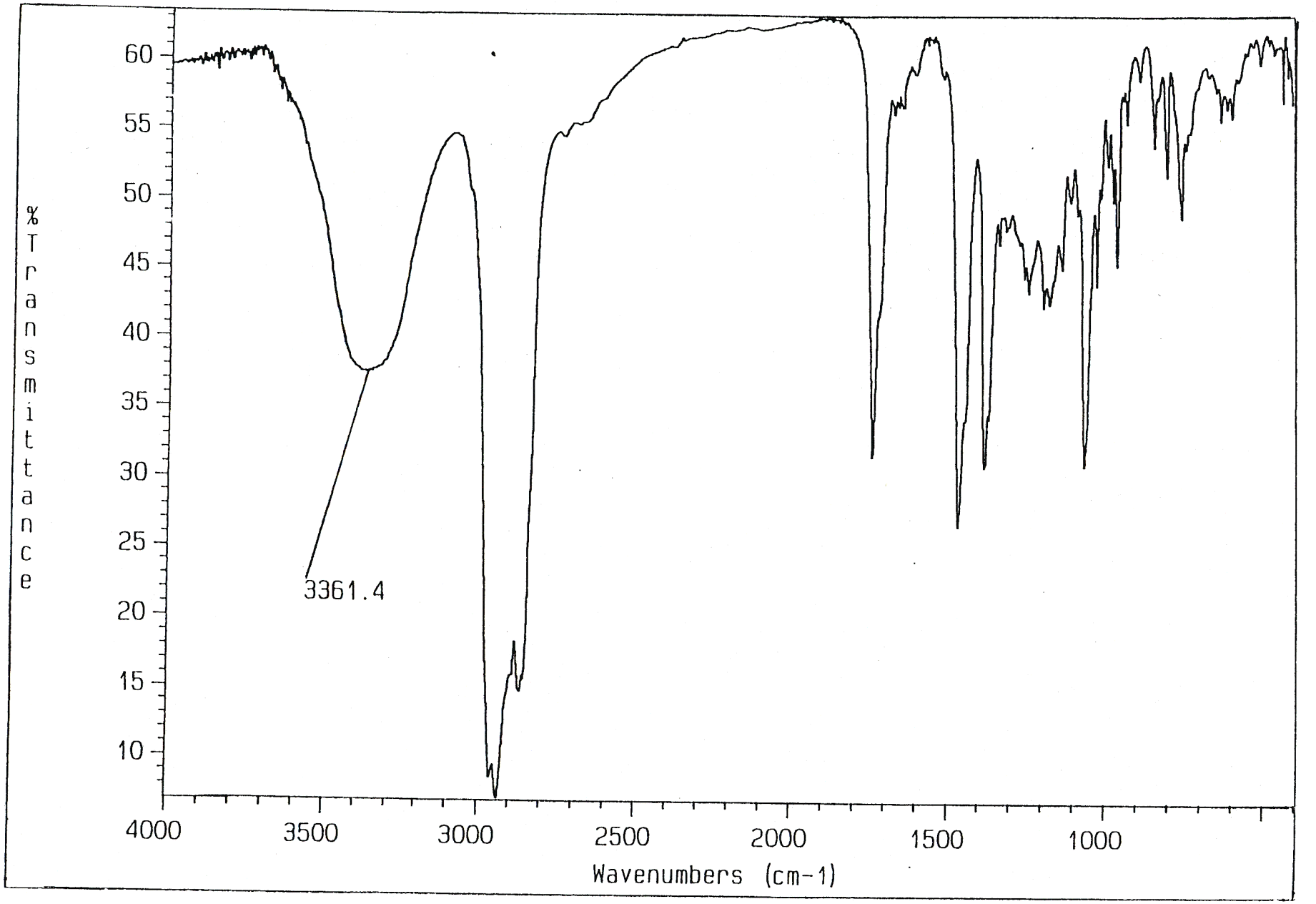
Spectrum [16-F] : HETCOR spectrum of calycosin (7) in CD_3OD



Spectrum [16-G] : Infra red spectrum of calycosin (7)



Spectrum [17-A] : ^{1}H NMR spectrum of β-sitosterol (9) in $CDCl_3$



Spectrum [17-B] : Infra red spectrum of β-sitosterol (9)