EXTRACTIVES FROM THE HYACINTHACEAE

By

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TO MY MOST FAVOURITE
NATURAL PRODUCTS :
FATHER, MOTHER,
BRENDA & CHRISTEL

'I hold you in my heart,
for together we have shared
in the b lessings of the Lord....'

Phillipians 1:7

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.

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.

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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

NOESY – nuclear Overhauser effect spectroscopy

HMBC - heteronuclear multiple bond coherence

HSQC – heteronuclear multiple quantum coherence

INADEQUATE – incredible natural abundance double quantum transfer experiment

TOCSY – total correlation spectroscopy

HRMS – high resolution mass spectroscopy

I.R. - infra-red

Mp - melting point

t.l.c - thin layer chromatography

PTLC - preparative thin layer chromatography

MS - mass spectroscopy

ppm - parts per million

s - singlet

d - doublet

t - triplet

q - quartet

m - multiplet

dd - doublet of doublets

Hz - Hertz

c - concentration

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ABSTRACT

Four species belonging to the Hyacinthaceae family were investigated. The taxonomy of the Hyacinthaceae is currently under review and therefore compounds isolated from these plants could provide valuable chemical evidence which taxonomists could find very useful.

The bulbs of the species investigated have been reported to have widespread uses in traditional African medicine, being used by different local tribes to treat a variety of ailments ranging from use as a soothing medicine for pregnant women to their being used as an ethnoveterinary medicine. The bulbs of *Ledebouria cooperi* are specifically used as an anti-inflammatory agent during circumcision ceremonies.

The bulbs investigated were found to contain homoisoflavonoids, a class of compounds known to be specifically responsible for the anti-inflammatory properties of these plants when used by traditional healers. The bulbs of *L. cooperi* were found to contain two known homoisoflavonoids as well as a triterpenoid. Malic acid was also isolated from the methanol extract. A further homoisoflavonoid of the 3-benzyl-4-chromanone type was isolated from *Scilla plumbea. Drimiopsis maculata* was found to be an abundant source of natural products from which two scillascillin-type homoisoflavonoids as well as two aromatic compounds were isolated. Investigation into the fourth species, *Drimia robusta*, yielded an uncommon bufadienolide. As no spectroscopic information was available for this compound, the complete assignment of the compound was performed using 2-D NMR spectroscopy.

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CHAPTER 1 : INTRODUCTION

1.1 GENERAL

Despite advances in modern medicine, the popularity of traditional medicine remains undiminished among the natives in every continent around the globe. The widespread and long-standing practice of using plants in medicine has been transcribed to us through the pictographs of the Egyptians, the tablet ideographs of the Babylonians and the Vedic Sanskrit. This alone gives us an idea of how ancient the practice of traditional/herbal remedies really is. A postulated reason for the popularity of traditional medicine is that it is the general belief among many natives that "white" doctors take care of certain physical needs such as infections and surgery, while their "medicine men" minister to the major problems of the mind and spirit. They believe such difficulties to be far more important, for during one's life, harmony and order impart strength and inner peace, which in turn assures physical safety and emotional security. In Africa, another reason for the popularity of traditional medicine could be the non-availability of affordable health care.

There are well documented accounts of the uses and successes of traditional medicine in almost every continent.[1] In America, these healers are referred to as medicine men. In Africa they are known as sangomas, herbalists or witch doctors and in Asia they are called shamans. It is a common belief that these healers treat patients and obtain results not unlike the formally trained therapists of the cosmopolitan western world. It is with this trail of thought that recently, many efforts are being made to bridge the gap between traditional healers and mainstream health care.

Traditional medicine is nowhere as extensive or as widely accepted as in China. There, traditional medicinal techniques uniquely involving herbology and acupuncture have been fused with cosmopolitan medicine. Some examples include the use of purgatives in the treatment of appendicitis in conjunction with herbs and acupuncture and the employment of ancient Chinese herbs for treating skin burns.

India, however, presents a different picture. Here, the ancient practices known as Ayurvedic or Hindu or Muslim medicines are largely ignored by the trained physicians. This, however, does not discourage the majority of the Indians from

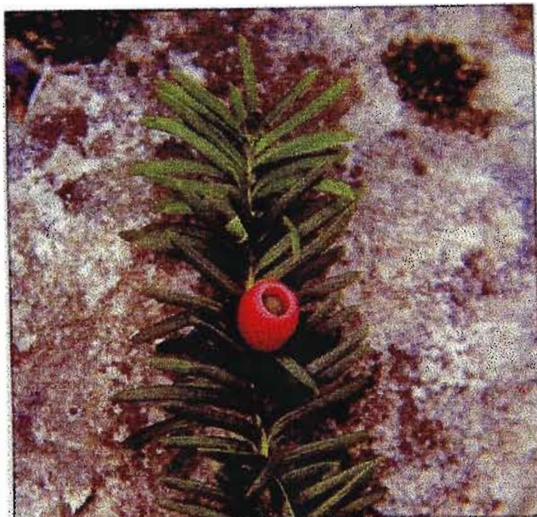
ascribing to these traditional healers and this could again be attributed to the lack of affordable medical insurances. This is unfortunate for it is believed that much knowledge could be shared to mutual advantage. For example, the efficacy of the Ayurvedic techniques of oil and milk herbal messages for the treatment of headaches, rheumatism and eye ailments has since found a scientific basis in new evidence showing lipid permeability of skin.[2]

The North American Indians have also documented the use of the hallucinogenic plant *Datura inoxia* for the successful treatment of schizophrenia.[3] *Datura* species contain several tropane alkaloids of which atropine and scopolamine are the major ones.[4]

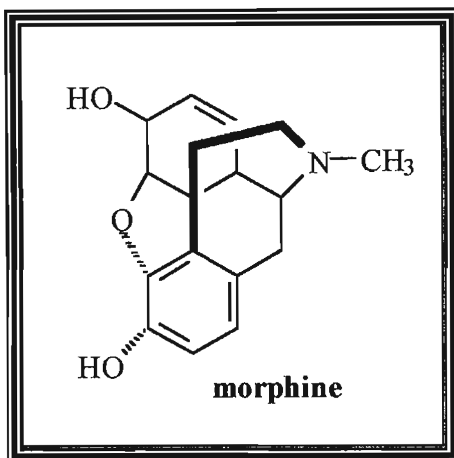
Many drugs on the pharmaceutical market today are of plant or animal origin.[5] However, what examples are there of the correlations between empirical applications and proven efficacy? Firstly, there is no doubt that natural products of plant origin can be very harmful to man. In various environments, urban and rural, there are hundreds of plants that may be injurious if ingested and are capable of causing any number of symptoms including death. Included in this category are many house plants which are very attractive but are often poisonous. Few people know of the effects of the common bedding plant English yew which contains the alkaloid taxine. Taxine is rapidly absorbed in the body and causes sudden death. Similarly, boxwood, which is a common hedging plant, produces buxine, an alkaloid which contributes to respiratory failure in humans and domestic animals. Other plants such as oleanders, caladiums and philodendrons should also be avoided. Ingesting leaves of oleander, or its sweet nectar, may lead to severe vomiting, irregular heartbeat and respiratory paralysis followed by death.[1]

However, there are also numerous plants that have been found to have remedial properties. Of these none has been more welcomed than those that help in the fight against cancer. Thousands of lives have been saved or extended by the antineoplastic agents of microorganisms or by compounds such as taxol from *Taxus brevifolius* or vincristine and vinblastine of the Madagascan periwinkle.[6] Due to a shortage of the pacific taxus yew, now semi-synthetic derivatives are produced from the leaves of *Taxus baccata*. The gigantic leaps in the medical field for the treatment of

Hodgkin's disease has been widely demonstrated in tests showing up to 80% remission while using alkaloids such as vincristine and vinblastine in combination chemotherapy.[1]


Yew tree (*Taxus baccata*)

Cosmopolitan medicine has also learned of the pain-relieving effects of the opium alkaloids and morphine is now an all important analgesic. Today, the world consumption of opium alkaloids for medicinal purposes is nearly 200 metric tons per annum.[6]



Opium poppy (*Papaver somniferum*)

FIG. 1.1 : structure of morphine derived from opium poppy (right)

Without foxglove or other plants producing cardiotonic compounds, congestive heart failure and death would occur inevitably.

High blood pressure was also at one time a quick killer. Before 1950, many people suffering from high blood pressure inevitably died of stroke, heart failure or kidney failure. Today, extracts of *Rauvolfia* are used for the effective control of hypertension in a large percentage of sufferers. Reserpine is the well-known antihypertensive.

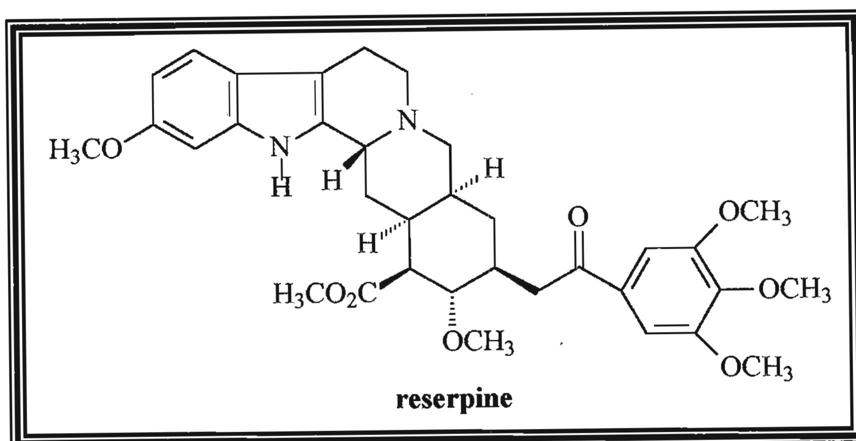
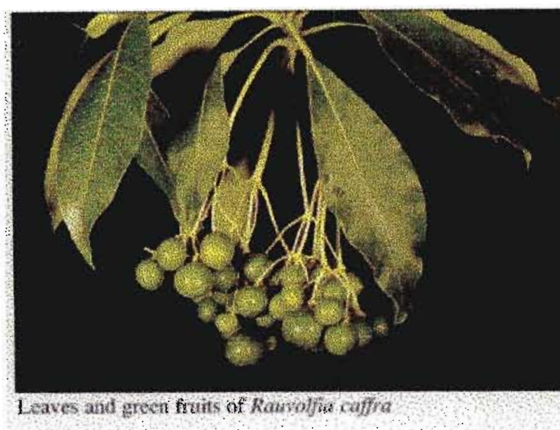


FIG. 1.2 : structure of reserpine from *Rauvolfia caffra* (below)



Leaves and green fruits of *Rauvolfia caffra*

The treatment of glaucoma with alkaloids from the calabar bean of Nigeria can prevent blindness. These compounds relieve the pressure within the eye by acting on neural receptor sites.

With the onset of tooth and gum disorders, one often wonders if preventive dentistry has been adequate, especially when we observe that the teeth of indigenous Asians and Africans are often free of caries. Recent research has, in fact, shown that their traditional chewing stick may contain anticariogenic properties. Furthermore, the oil from an Indian chewing stick, when incorporated into toothpaste, has been found to promote the healing of inflamed gums.

Licorice has a long history in European domestic medicine for the treatment of indigestion and for alleviating inflamed stomachs. Research has also shown that using derivatives of *Glycyrrhiza glabra,*[6] the common licorice from which candy is made, leads to the reduction in size of peptic ulcers. One such derivative is liquiritin.

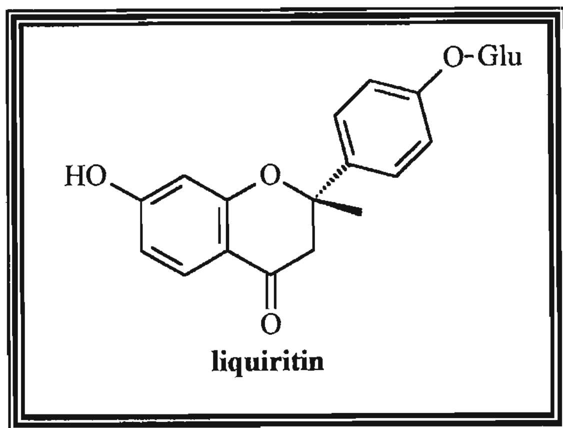


FIG. 1.3 : structure of liquiritin from *Glycyrrhiza glabra* (right)

More commonly, there are also those plant extracts which are used in treatment of symptoms of the common cold such as ipecac syrup from *Cephaelis* or the mucolytic agents from the leaves of the Malabar nut tree.

An area in which the use of plants has had one of the greatest impacts is in that of producing substances from which sex hormones are manufactured. Few realize the great contribution made by yams, for example, in stabilizing world population. These plants provide the basic compounds from which steroidal compounds are now cheaply available as oral contraceptives are synthesized. A good example is diosgenin which is extracted from *Dioscorea dregeana*. This is the starting material in steroid hormone semi-synthesis.

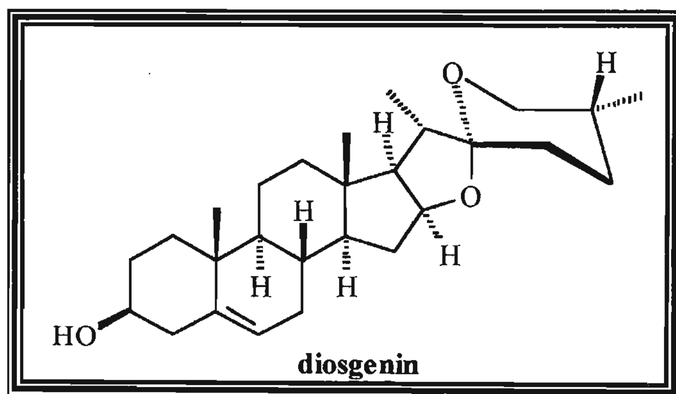


FIG. 1.4 : structure of diosgenin

The skin, which is man's largest tissue, has also benefitted from the array of plant derived soaps, oils, shampoos, lotions and perfumes.

Plants having psychoactive properties have always been popular. Stimulants like cocaine from *Erythroxylum coca* and nicotine from tabacco have for centuries been

used to give a general sense of well-being and exhilaration. But man has also had his depressants which includes alcohol. All such drugs are enormously useful in medicine but all are also subject to abuse.

All these uses that have been documented are the results of centuries of experiments performed by trial and error by our forefathers and thanks to them we now have a broader insight into the preparations that are useful, harmful and enjoyable to man. With technological advances and the scientific expertise available today, there is now greater opportunity for us to utilise this invaluable information. This is therefore a field of research that encourages one to study the practices of indigenous populations before they are lost, either through complacency and indifference or the ability of man to destroy the vegetation around him.

1.2 ETHNOBOTANY

Since the beginning of time, man has relied on products from nature to improve his lifestyle and ward off diseases. Despite advances in modern medicine, a large proportion of the globe's native population still rely heavily on traditional medicine. Ethnobotany has always been to some extent an interdisciplinary science. In particular, there has been considerable co-operation between botanist and chemists, for example, work done on the various hallucinogenic plants of the Amazon.[7,8] The term "ethnobotany", first coined by Harshberger in 1968, refers to the study of plants related to people.[9] As we approach a new millennium, many efforts are being made, in South Africa specifically, to bridge the gap between traditional healers and mainstream health care. In this investigation, all four species studied belong to the Hyacinthaceae family but they each belong to a different genus.

The first plant investigated, *Ledebouria cooperi* has found widespread use in traditional medicine. Well documented accounts have illustrated its typical digitalis-like activity.[10] The Zimbabweans report *L. cooperi* to be highly poisonous, capable of inducing a comatose state in children, as well as a number of symptoms associated with cardiac glycoside activity. [11] However, Steyn[12] reported that he was unable to prove the toxicity of *L. cooperi* in a rabbit and sheep feeding experiment. This, he ascertained, could be associated with the edaphic, climatic and developmental factors influencing the plant. *L. cooperi* is also used as a substitute for *L. ovatifolia* when applied as a soap, as an enema for children, or internally to cattle. The Sotho prepare a medicinal mixture with *Phygelius capensis* to inebriate boys during circumcision ceremonies. This reportedly sedates the boys and makes them appear stupified.[13] The Basotho tribe also employ *L. cooperi* as a soothing medicine for women in their fourth month of pegnancy and also as an ethnoveterinary medicine for cows which supposedly ensures a succession of calves of the same gender.

The bulbs of several *Scilla* species are also widely used medicinally by the indigenous people in southern Africa. Many of the local tribes employ these *Scilla* species to treat ailments in both humans as well as in animals. Traditional healers have used decoctions from these plants to treat rheumatism, venereal diseases, mental illness, infertility in women and a variety of other ailments.[13-18] However, cardiotoxic

glycosides of the bufadienolide group have also been known to occur in some of these species and these have proved to be fatal to animals in various feeding trials.[19]

Drimiopsis maculata is also commonly known as little white soldier or small snake lily. Its Zulu names are *injobo* or *ucibicibane*. Infusions are made from the pounded bulbs of *Drimiopsis maculata* and they are very mucilaginous.[15] These decoctions are first steeped in cold water and then warmed and administered as enemas to young children. This is reported to treat stomach ailments. In the Transkei, the bulb infusions are traditionally used for newly-born babies suffering from a disease known as ipleyti.[15] This is likely to be a marasmic condition. Feeding experiments carried out with fresh flowering and seedling plants seemed to produce no ill effects.[13]

Plants belonging to the genus *Drimia* are generally known to be poisonous. The potency of this plant cannot be underestimated as bulbs cause severe itching even though gloves are worn. In *Drimia ciliaris*, this irritation is thought to be attributed to the presence of raphides of calcium oxalate. The juice of *D. robusta* is also known to blister the skin. *Drimia robusta* is known to the native people as *umqumba* or *isiklenama.* The fruit of this plant is roundish and deeply lobed and is poisonous to stock. In a study involving the ingestion of these bulbs by rabbits, a post-mortem revealed poisoning by general cyanosis[13] and kidney and liver failure. Decoctions of this plant are used in traditional medicine for the relief of symptoms of pain and fever. It was formerly used in the Cape for the treatment of common colds as an expectorant and emetic. Sangomas or spiritual healers also believe that *Drimia robusta* can be worn as a protective charm to ward off evil spirits and danger. Ethnobotanical research is entering into a new era with many dimensions. In America alone, many university courses in ethnobotany are now being offered and rapid progress is being made in this field. From a chemist's perspective, understanding the ethnobotany of the plants under investigation also gives an idea of the type of compounds that might be present in a plant.

1.3 SPECIES IN THIS STUDY

1.3.1 ***Ledebouria cooperi*** (Hook)

Ledebouria cooperi (Hook) is a member of the Hyacinthaceae family and has widespread occurrence in southern Africa. The plant used in this particular investigation was harvested in Richmond, in the Kwa-Zulu Natal midlands during February 1999. The taxonomy of this genus is still currently under revision because of contrasting accounts of its toxicity and pharmacological activity. *Ledebouria* is only a very recently formed genus. Previously, most of the plants belonging to this genus were classified as *Scilla* species.

Ledebouria cooperi has many synonyms, for example, *Scilla cooperi, Scilla rogersii* and *Scilla saturata. Ledebouria cooperi* consists of a very small bulb with a long slender leaf. Plants tend to grow in clusters in shady, damp grasslands. The leaves that are visible above the ground look much like coarse grass while the bulbs, at their biggest, are only about 1-2cm in diameter. The flowers of *Ledebouria cooperi* are bright pink in colour and they protrude from a short stem. For this investigation, only the bulbs were extracted.

FIG. 1.5 : *Ledebouria cooperi* growing in the Natal Midlands

1.3.2 ***Scilla plumbea*** (Lindl.)

This genus also falls under the Hyacinthaceae family. In general, plants belonging to the genus *Scilla* have widespread location from Europe through to Asia and Africa. There are approximately forty *Scilla* species in all but only about six species are found in southern Africa. The South African species are found to occur mostly along the eastern belt of the country from the eastern Cape to Mpumalanga. The only species not found in this eastern belt is the one undertaken in this study, namely, *Scilla plumbea* which is found in the south-western Cape region. It was thus interesting to see if its geographical location has any influence on its chemotaxonomical constitution. *Scilla* is an evergreen genus that blooms in the period December to January. It only grows to between 20-40cm and requires moisture throughout the year. *Scilla plumbea* is rather rare and is seldom grown as a garden plant but these plants have definite potential as pot subjects. Their flowers bloom in shades of blue and hence the common name blouberglelie.

FIG. 1.6 : *Scilla plumbea* from the south-western Cape region

1.3.3 ***Drimiopsis maculata*** (Lindl.)

This species is also known as *injobo* or *ucibicibane* to the native people. *Drimiopsis* means resembling *Drimia* and it is a bulb with no papery outer scales. There are approximately fifteen species in Africa of which five are known to occur in South Africa. *Drimiopsis maculata* grows in shady places with a bulb of approximately 40mm which narrows to a slender tip. The leaves are deeply lobed with waxy margins. *Drimiopsis maculata* has dark green spotted leaves and hence the name *maculata* which means blotched or spotted. The flowers occur in small, compact inflorescences which are grey-green in colour. They bloom during the period September to April. *Drimiopsis maculata* is also used as an attractive garden plant in deep shaded areas. This species is found all along the eastern coast of South Africa. In this investigation only the bulbs were extracted and analyzed.

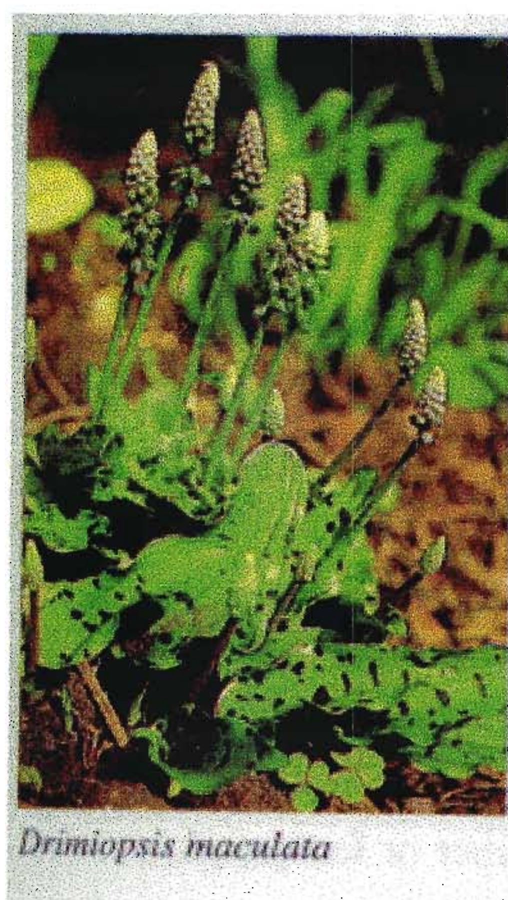


FIG. 1.7 : spotted leaves of *Drimiopsis maculata*

1.3.4 ***Drimia robusta*** (Bak.)

The genus *Drimia* comes from the word *drymis* which means acrid or pungent. This implies that all parts of some species in this genus are poisonous or irritants.[20] The genus, in general, contains bulbs which are usually above the ground and have very loose scales. In South Africa alone there are approximately thirteen species belonging to this genus. *Drimia robusta* is also known as *Drimia elata* (which means tall) or *Drimia alta*. *Drimia robusta* usually grows in colonies and can be found growing to a height of about 1.2m. They are found along the coastal belt of the southern Cape as well as in the interior. The bulbs are approximately 40-100mm in diameter with overlapping red scales. The stalks are erect and they end in a cluster of small flowers. *Drimia robusta* is also faintly scented and is an attractive garden plant. It is closely related to the genus *Urginea* and this seems logical since the compound isolated in this work from *Drimia robusta* has also previously been reported from *Urginea maritima* (Hyacinthaceae).

FIG. 1.8 : bulbs of *Drimia robusta*

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CHAPTER 2 : TYPES OF COMPOUNDS

2.1 HOMOISOFLAVONOIDS

2.1.1 Classification and Occurrence

Homoisoflavonoids are also known as homoisoflavanones. The numbering system that is generally used when referring to these compounds is shown below :

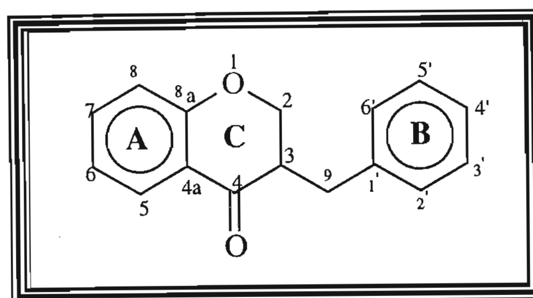


FIG. 2.1 : Numbering system for homoisoflavonoids

The A-ring is numbered using counting numbers while the B-ring is numbered using primed numbers. Homoisoflavonoids differ from isoflavonoids in that they have an extra carbon as compared to the 15-C skeleton of an isoflavonoid shown below :

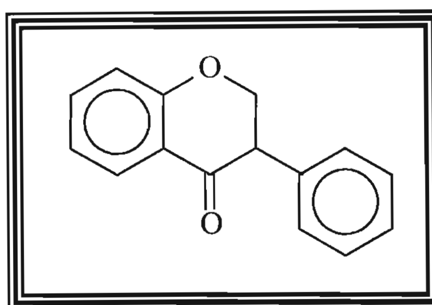


FIG. 2.2 : Structure of an isoflavonoid

The 16-C skeleton of homoisoflavonoids has a chromane, chromone or chromanone moiety to which is attached a benzyl or benzylidene grouping at the 3-position[1]. Besides these common homoisoflavonoids, there are three other types which each contain a fourth ring, namely, the brazillin, scillascillin and dracaenone types.

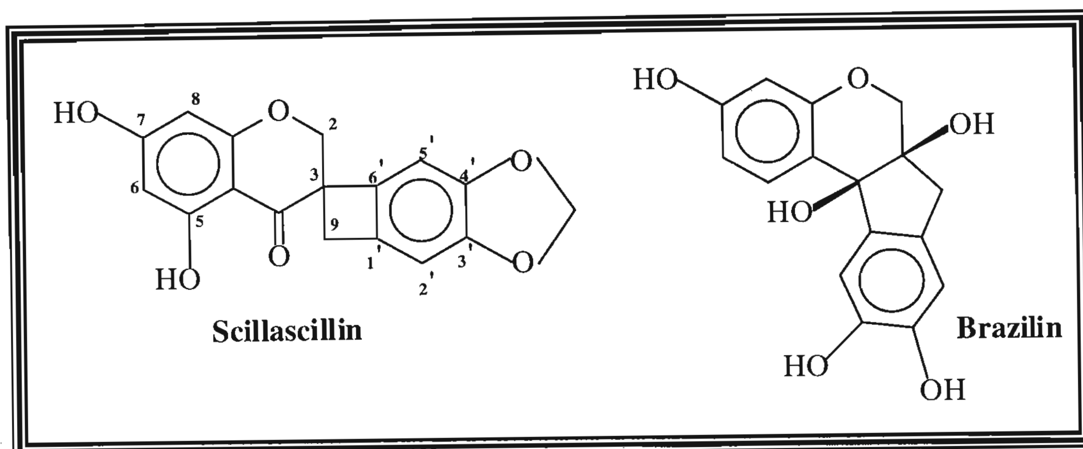


FIG. 2.3 : Structure and numbering system of scillascillin and structure of brazilin

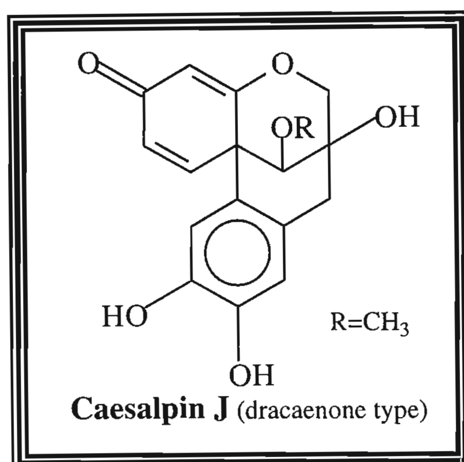


FIG. 2.4 : Structure of Caesalpin J

Homoisoflavonoids vary one from the other by the substitution patterns present in the A and B-rings. Common substituents are hydroxy, methoxy or acetoxy groups but sometimes even methyl or aldehydo groups can occur.

Homoisoflavonoids are a small group of compounds within the natural products family and their occurrence, thus far, has been largely restricted to the Hyacinthaceae family. Among the few reported to occur outside this family are bonducellin and intricatin.[2,3] In 1967, the first homoisoflavonoids were reported to occur in the *Eucomis* genus. Eucomin was one of the first homoisoflavonoids to be isolated and characterised. There are well documented accounts of the occurrence of these compounds from many other genera within the Hyacinthaceae such as *Bellevalia*[4], *Ophiopogon*[5,6] and *Veltheimia*[7] but they appear to be most dominant in genera such as *Scilla, Eucomis* and *Muscari*. The homoisoflavonoids are known to be concentrated

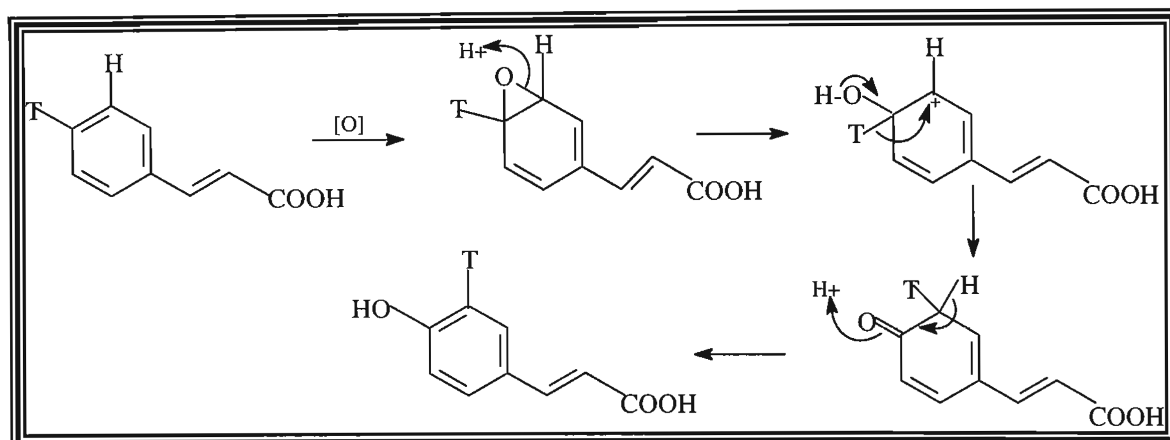
in the waxy, scale-like layers of the bulbs.[8] In this work, the homoisoflavonoids were indeed isolated from the bulb extracts. Homoisoflavonoids are known to possess anti-inflammatory, anti-mutagenic, anti-bacterial and analgesic properties and this is closely related to their ethnobotanical usage by traditional healers.[9] The anti-inflammatory properties of bulbs within the Hyacinthaceae family have been widely investigated. Homoisoflavonoid-rich fractions from the bulbs of *Muscari comosum* have been shown to inhibit mouse ear dermatitus.[10] In fact, the effect of this administration was comparable to the potent anti-inflammatory drug, indomethacin. In other similar experiments, the antimutagenic properties of two well known homoisoflavonoids were investigated. Conclusive evidence showed that intricatin and intricatinol inhibited the mutagenic effects of *Salmonella typhimurium.*[11] Intricatinol was, however, found to be more effective, and it was postulated that this could have been attributed to the presence of extra hydroxy moieties. Phenolics, in general, are known for their antibiotic properties.

2.1.2 Biosynthesis

There are two general routes by which phenolic compounds, such as homoisoflavonoids, can be biosynthesized *viz.* the shikimate pathway which starts from a carbohydrate, or the polyketide pathway which starts from acetyl and malonyl coenzyme A.[12] Often compounds like flavonoids and homoisoflavonoids are of mixed biosynthetic origin. These compounds with more than one phenolic ring have the A-ring which is polyketide derived and the B-ring shikimate derived. Shikimate derived phenols are always oxidised in the *para*-position but further oxidations may occur in the advanced biosynthetic stages to yield oxygenation substitution at the adjacent positions. The polyketide pathway, on the other hand, produces a 1,3,5-substitution pattern.[12] This is very common among many natural polyphenols but it is possible for one or more of these groups to disappear during polyketide biosynthesis.

The first step in the biosynthetic route to homoisoflavonoids involves the formation of a chalcone where chalcones are the biosynthetic precursors to homoisoflavonoids.
The shikimate pathway for the chalcone formation begins with the conversion of L-phenylalanine to cinnamic acid. This reaction is catalyzed by the enzyme L-phenylalanine ammonia lyase.[13] Cinnamic acid is then oxidised in the *para*-position to give 4-coumaric acid. The proton in the 4-position is oxidised to a hydroxy group,

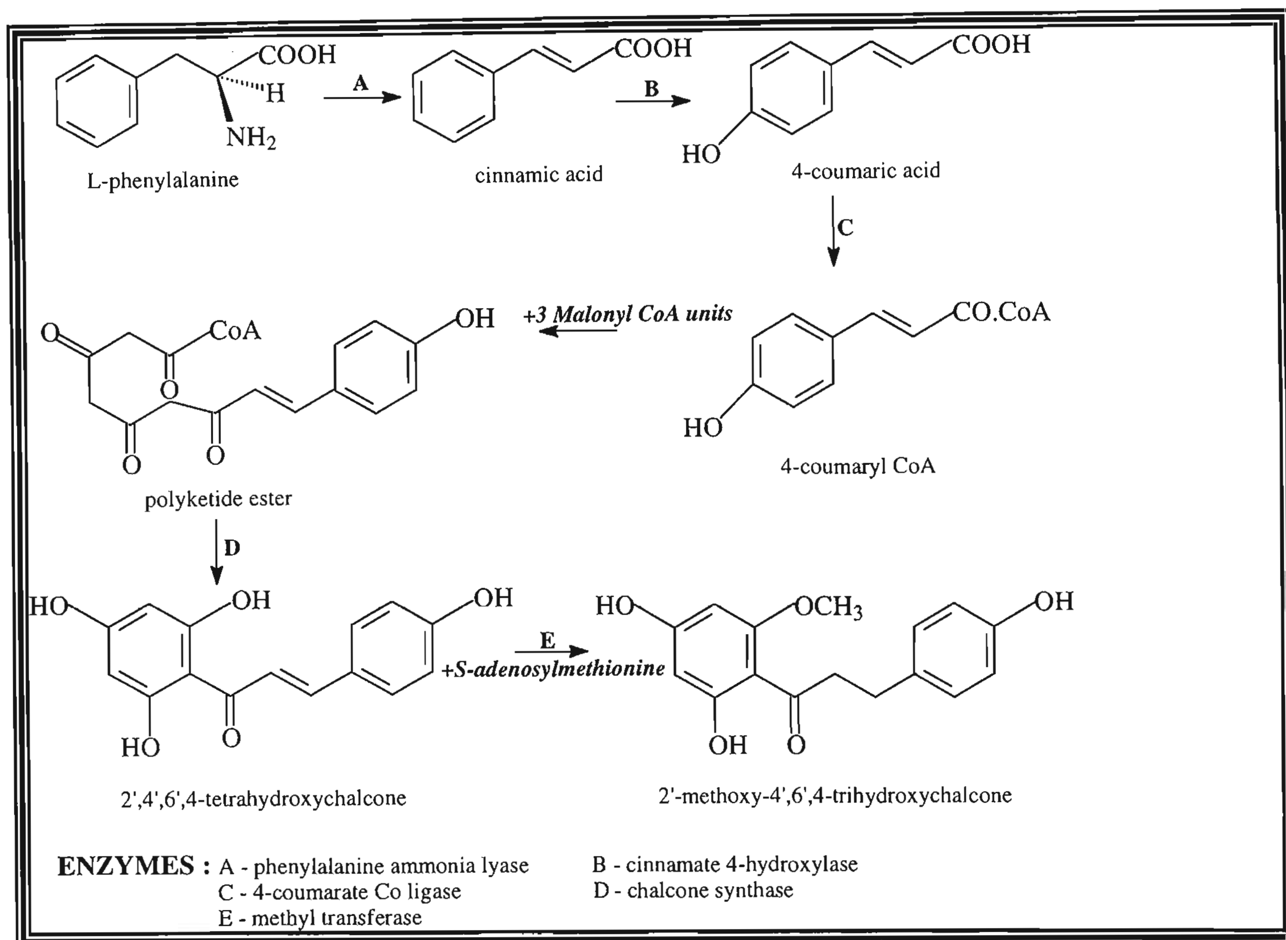
being moved itself to the 3-position. This shift was established by tritium-labelling experiments performed by the National Institute of Health at Bethesda, Washington D.C and is thus called the NIH shift. The mechanism for this conversion is shown in Scheme 2.1.[12]



SCHEME 2.1 : Mechanism showing the NIH shift[12]

From here, 4-coumaric acid is converted to 4-coumaryl CoA by the enzyme 4-coumarate: CoA ligase. The 4-coumaryl CoA molecule then combines with 3 malonyl CoA units to give the polyketide ester. Cyclisation of the polyketide occurs *via* a postulated Claisen type condensation and this affords the tetrahydroxychalcone. Chalcone synthase is the catalytic enzyme in this step.

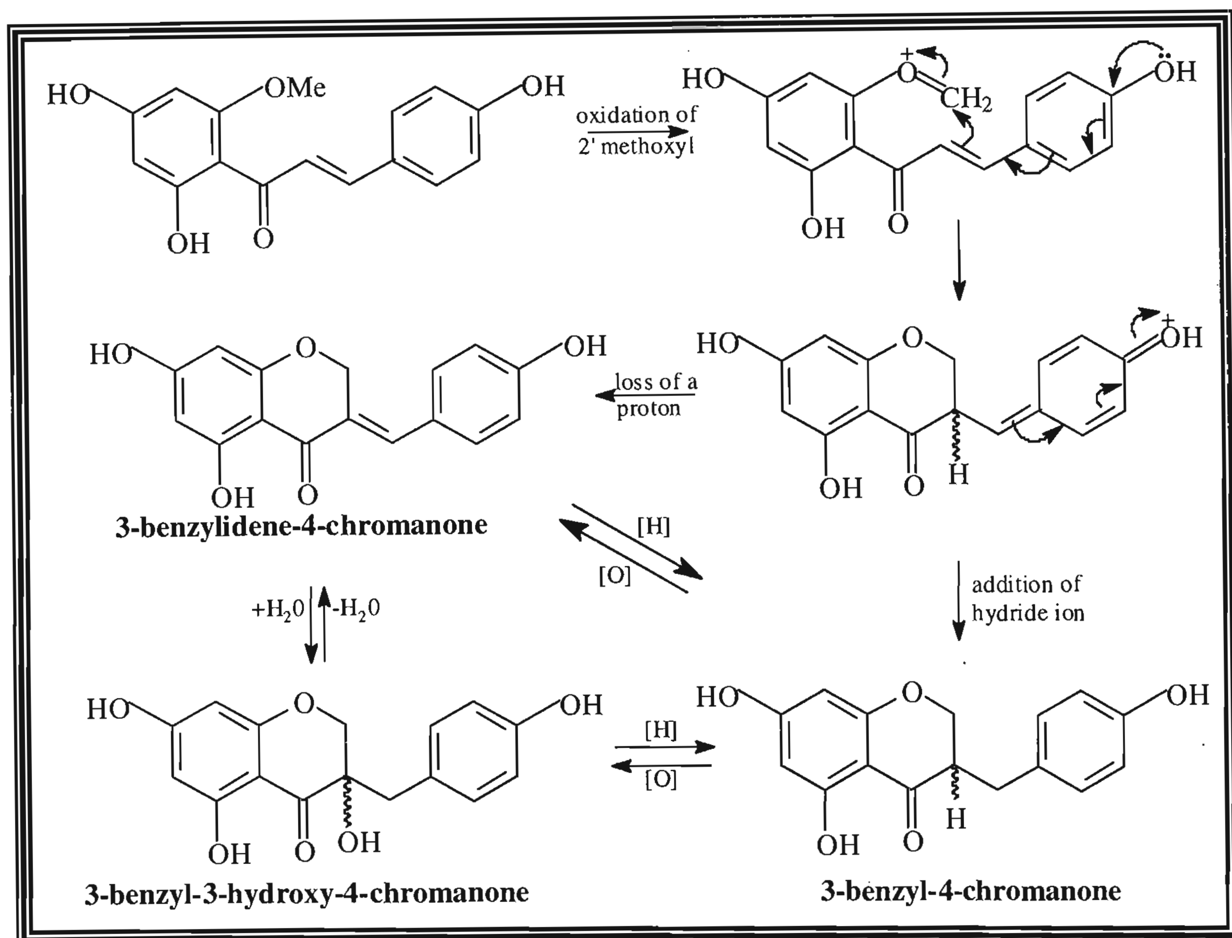
In many general biosynthetic pathways, S-substituted methionine is often the source of an additional carbon atom and, here too, the tetrahydroxychalcone is converted to the 2'-methoxy-4,4',6'-trihydroxychalcone by methylation with the S-adenosylmethionine group (Scheme 2.2).



SCHEME 2.2 : Chalcone phase formation

A scheme for the conversion of chalcones to homoisoflavonoids has been proposed by Dewick[14] whereby the methoxy group is thought to be oxidised and subsequent cyclisation produces the three basic types of homoisoflavonoids. The addition of a hydride ion produces the 3-benzyl-4-chromanone types while the loss of a proton leads to the formation of the 3-benzylidene-4-chromanones.

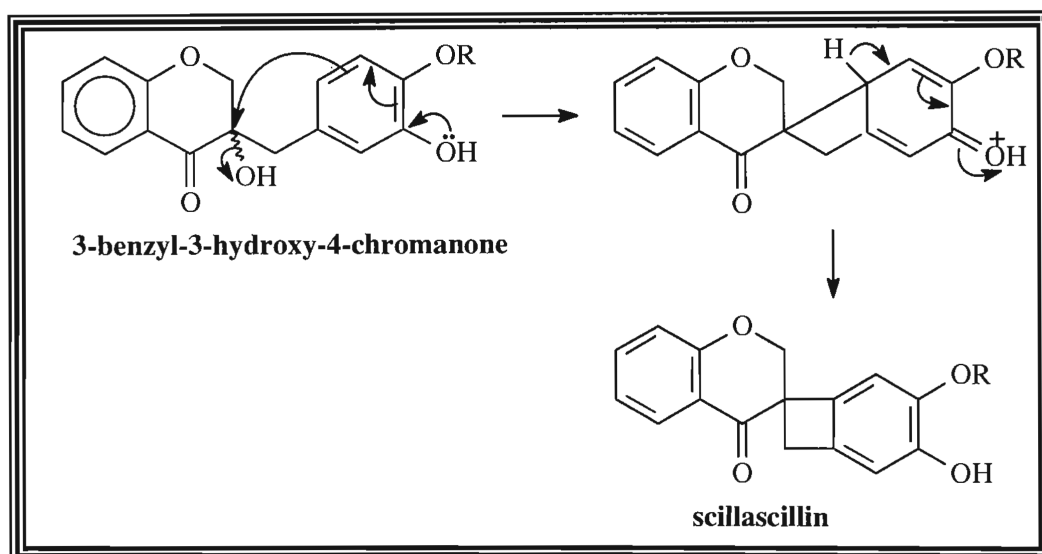
Hydration or oxidation at the C-3 position of a 3-benzyl-4-chromanone leads to a 3-benzyl-3-hydroxy-4-chromanone. These postulations are illustrated in Scheme 2.3.



SCHEME 2.3 : Conversion of chalcones to homoisoflavonoids

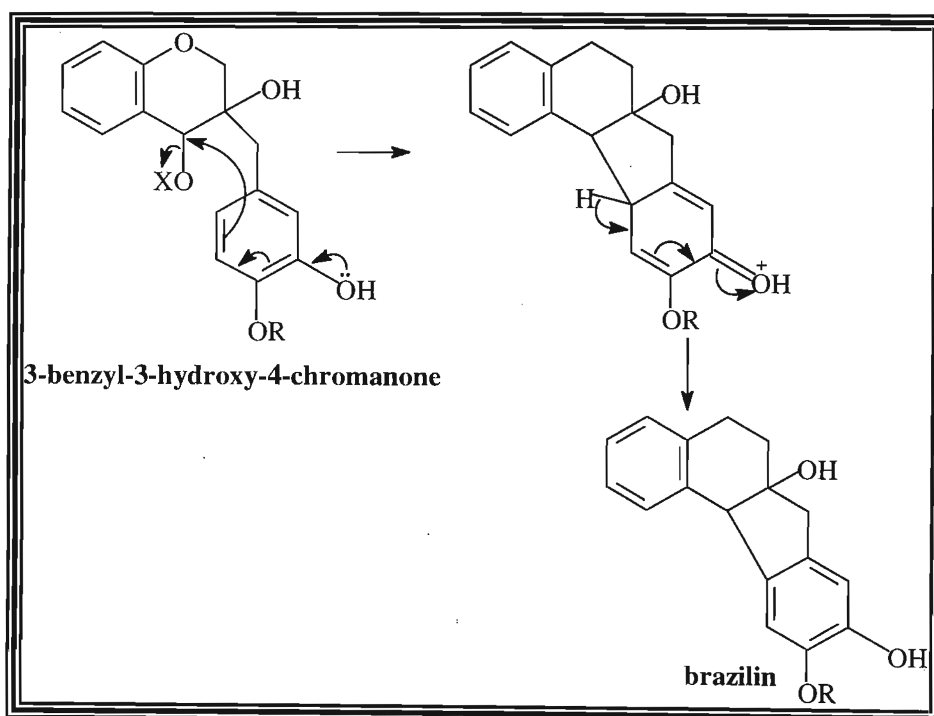
Scillascillin, which carries an extra 3-spirocyclobutenyl ring, brazilin a cyclopentenyl ring and dracaenone-type compounds an extra cyclohexene ring are thought to be derived from more complex mechanisms. Some of these mechanisms have been suggested by Dewick[14] and Bhandari[13] and co-workers.

Scillascillin-type homoisoflavonoids are thought to be formed from the 3-benzyl-3-hydroxy-4-chromanone precursors.

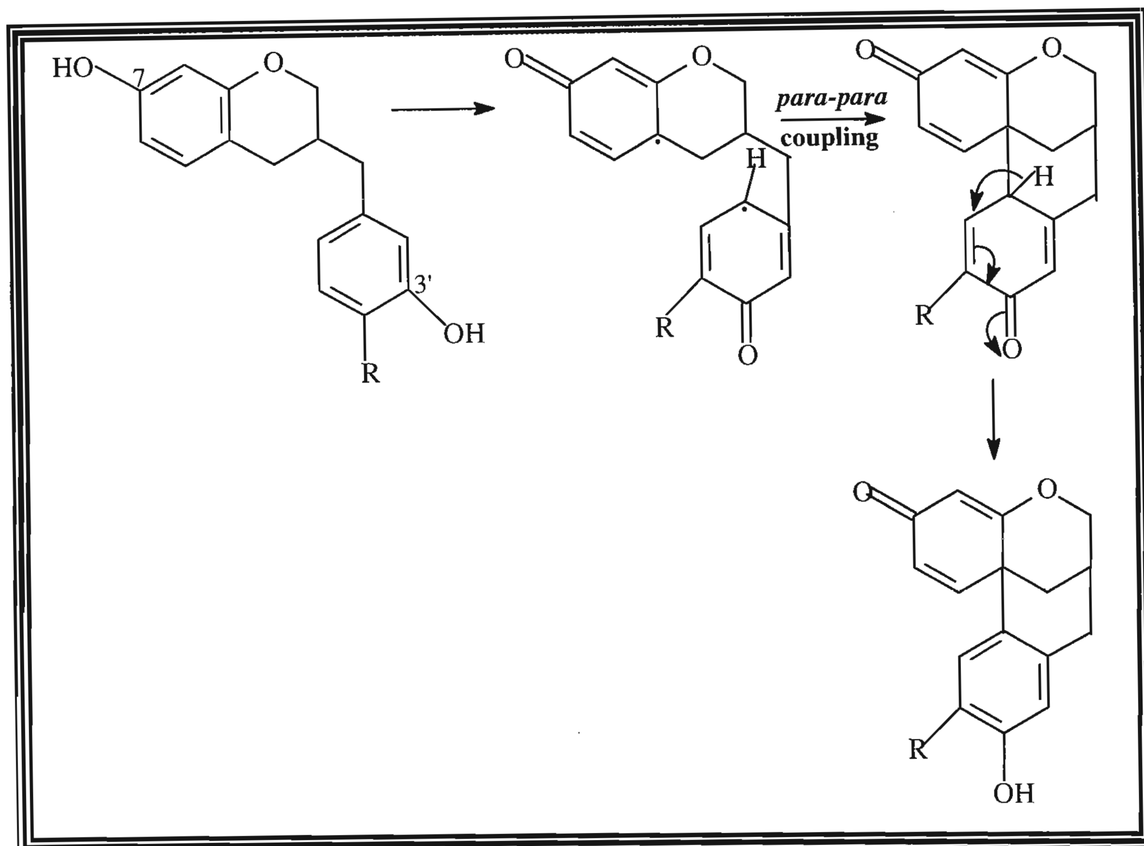


SCHEME 2.4 : Proposed formation of scillascillin

The biosynthetic route to brazillin occurs in much the same way except that the activated precursor now produces a cyclopentene ring.



SCHEME 2.5 : Biosynthetic route to brazilin

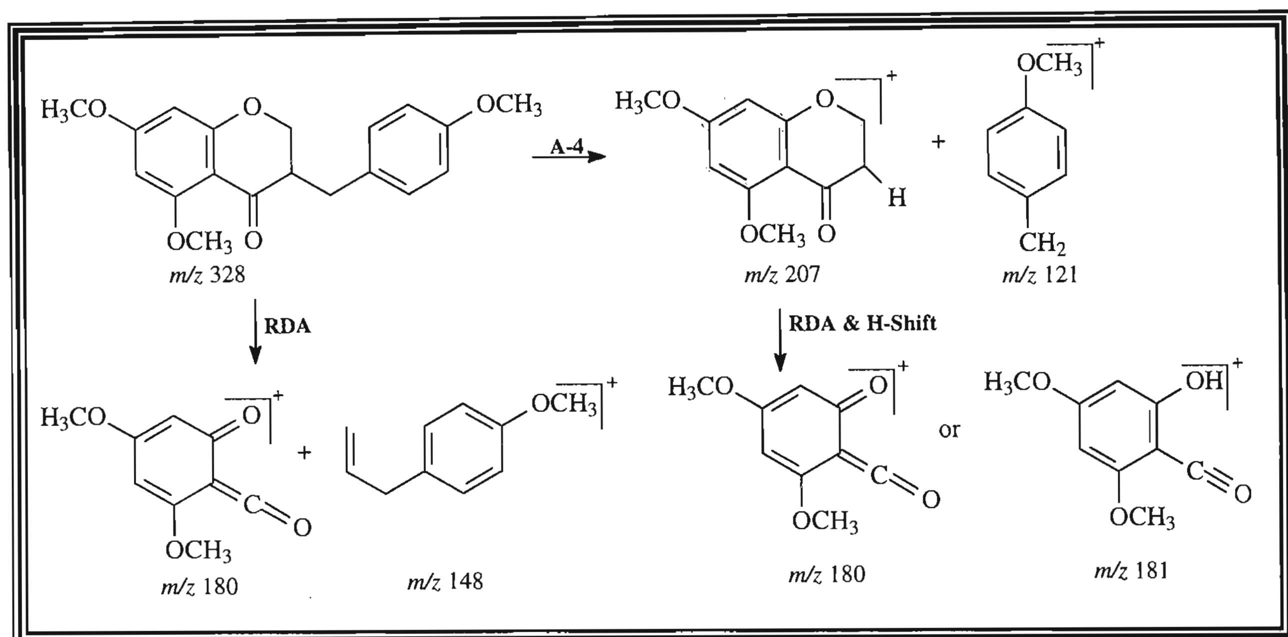


SCHEME 2.6 : Biosynthetic route to dracaenone type compounds

In summary, homoisoflavonoids are biosynthesized by the modifications of the C6.C3.C6. chalcone skeleton. Methylation by the addition of S-adenosylmethionine provides the extra carbon which becomes C-2 of the heterocyclic ring. The shikimate pathway starting with phenylalanine leads to the formation of the C-4, C-3, C-9 skeleton with the aromatic ring-B while the A-ring is acetate/mevalonate derived.

In general, there are three main techniques employed for the structural determination of homoisoflavonoids. These are mass spectrometry, ultra-violet (UV) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy.

A mass spectrometer is the instrument used for determining the masses of atoms, molecules and molecular fragments. For homoisoflavonoids, there are certain peaks which show the characteristic fragmentation patterns. For example, an intense peak at *m/z* 121 is indicative of an A-4 and RDA fragmentation pattern and this corresponds to a methoxybenzyl/tropylium ion. This fragmentation pattern is shown in the scheme below :



SCHEME 2.7 : Fragmentation pattern corresponding to a methoxybenzyl ion

On the other hand, a base peak at *m/z* 107 is indicative of a hydroxybenzyl ion. The fragmentation pattern for all such compounds is shown in Scheme 3.1 in chapter 3. Other peaks which occur in the mass spectrum are shown in these schemes and these are the typical fragments which arise from the breakdown of these compounds.

The UV spectra are valuable in the determination of substitution patterns on the A and B-rings of homoisoflavonoids. The compounds were analysed in the presence of NaOAc and $AlCl_3$ solutions. The original UV spectra gave absorption maxima between 295-310nm. The addition of NaOAc and $AlCl_3$ causes these maxima to be shifted in some instances. When bathochromic shifts are observed with NaOAc these indicate the presence of a hydroxy group at the C-7 position on the A-ring. A bathochromic shift with $AlCl_3$ implies a hydroxy group at the C-5 position.[22]

The NMR spectra are probably the most important means of structural determination. The ^{1}H NMR spectra of 3-benzyl-4-chromanone-type homoisoflavonoids show a coupling pattern due to the 2H-2, H-3, 2H-9 grouping. A pair of double doublets within the chemical shift range 4.10-4.32ppm [AB of ABX - 2H-2], one multiplet in the range 2.72-2.87ppm [H-3] and two double doublets in the ranges 2.65-2.73ppm

and 3.10-3.26ppm [2H-9] are indicative of this arrangement. The signals between 6.50-7.20ppm and their coupling constants generally indicate the substitution pattern on the B-ring.

In the ^{13}C NMR spectrum, particular emphasis is placed on the C-5, C-6 and C-8 resonances which help to determine the A-ring substitution pattern. When there is a 5,7-dihydroxy substitution pattern, the C-5 resonance should occur at δ165.8ppm. However, when there is a methoxy substituent at C-6, C-5 should resonate about 9.0ppm upfield from the norm.[1] Moreover, when C-6 or C-8 are unsubstituted, these resonances should occur at 97.1-97.3ppm and 95.8-96.0ppm respectively when C-7 carries a hydroxy group.[1] When C-7 carries a methoxy group, these resonances are at a higher field.

These are some of the main points to consider when working out the structure of a homoisoflavonoid

2.2 TRITERPENOIDS

2.2.1 Occurrence and Classification

The numbering that is generally used when referring to nortriterpenoids is shown below :

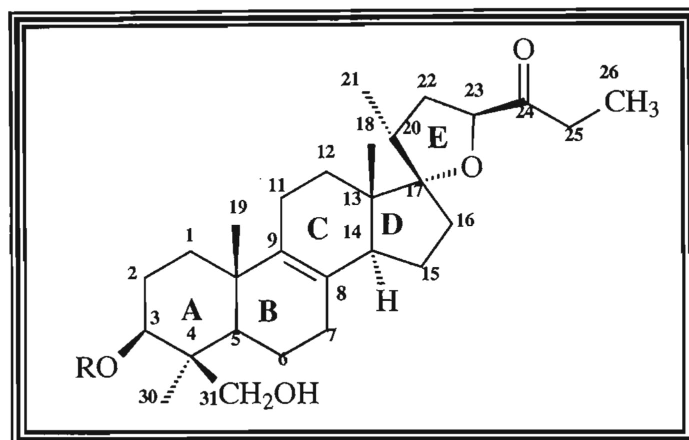


FIG. 2.5 : Numbering system referring to nortriterpenoids

Triterpenoids form a very large group of naturally occurring compounds and they are abundant throughout the plant kingdom. They occur in the plant kingdom as esters, glycosides or in the free state.[15] The term 'triterpene' refers to C_{30} compounds that are derived from squalene.[16] They have been known and investigated for over 100 years. Squalene was first obtained from shark liver oil and a number of the earliest tetracyclic triterpenoids were isolated from wool fat.

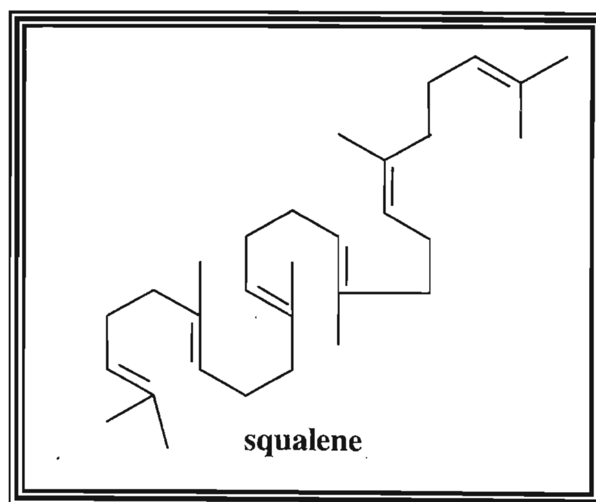


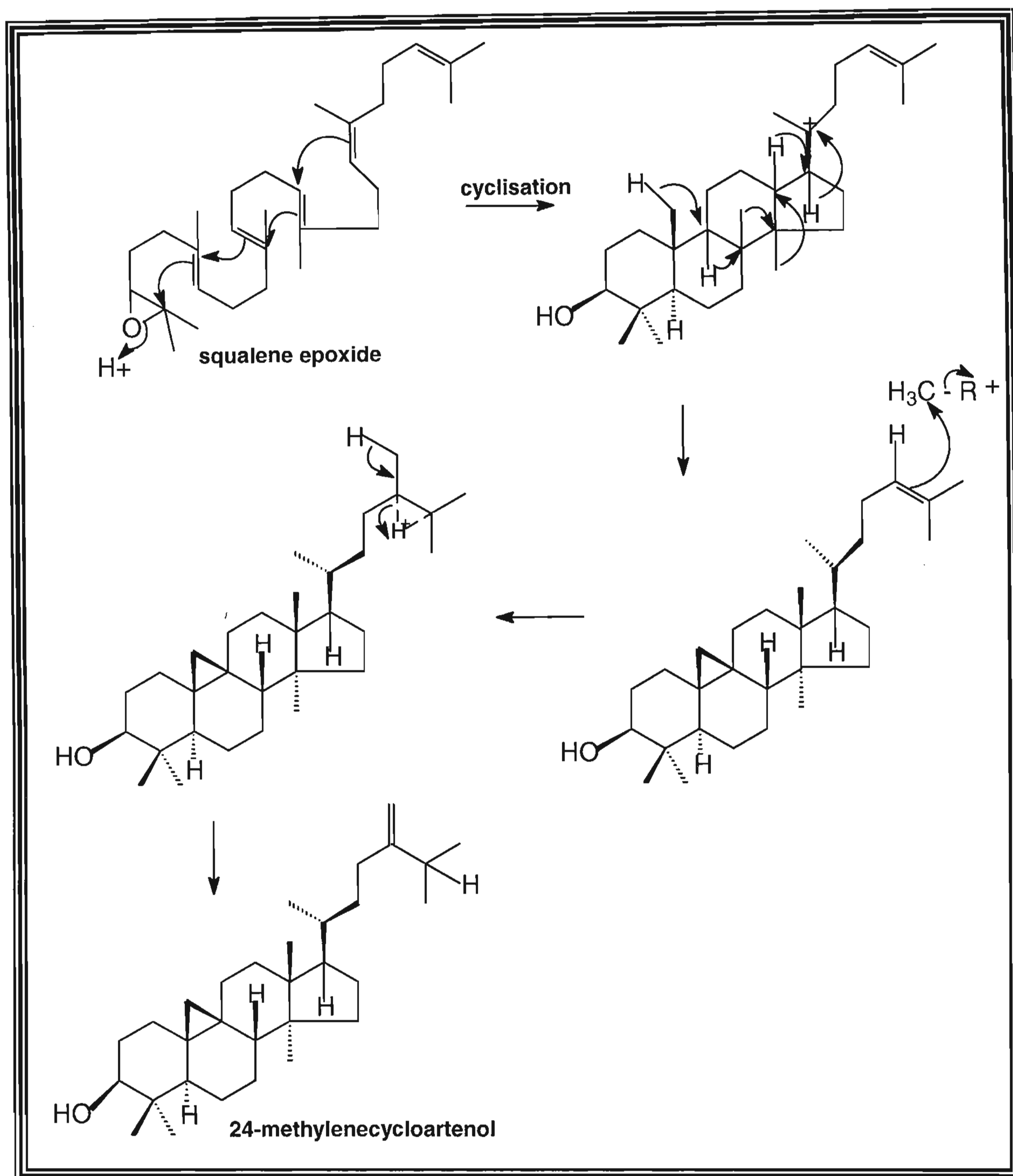
FIG. 2.6 : Structure of squalene

The acyclic precursor, squalene, undergoes a series of cyclisations and is elaborated to give the different classes.[17] Spirocyclic triterpenoids have been well-documented within the Hyacinthaceae family for example, the derivative 22-acetoxy-15-deoxoeucosterol has been reported from *Veltheimia viridifolia*[18]. The spirocyclic structure is made up of a five-ring system and derivatives often have substituents at the C-22 position. Many other eucosterols have also been reported from *Scilla scilloides*.[18]

2.2.2 Biosynthesis

According to the Biogenetic Isoprene Rule, the different types of tetracyclic and pentacyclic triterpenoids are formed according to the conformation that squalene epoxide adopts at an enzyme surface prior to cyclisation. It is generally recognised that all steroids in animals originate from lanosterol and are termed zoosterols while cycloartenol is the precursor of the steroids in plants and these are termed phytosterols. Experimental investigations have proven that all triterpenoids are derived from squalene by a series of cyclisation and rearrangement reactions.

Cyclisation of squalene yields the intermediate squalene-2,3-oxide and this reaction is catalysed by a flavoprotein. The epoxide then undergoes a series of concerted Wagner-Meerwein migrations of methyls and hydrides to ultimately yield cycloartenol.[19] Cycloartenol was the first triterpenoid to be discovered containing a cyclopropane ring. The mechanism for this conversion is shown in Scheme 2.8.



SCHEME 2.8 : Biosynthesis of 24-methylenecycloartenol[20,21]

The cyclopropane ring in cycloartenol is generated by cyclisation of the methyl group at C-10 but for many plant steroids this ring has to be re-opened. A ring-opening mechanism is shown in Scheme 2.9. Manipulation of the cycloartenol skeleton then results in the formation of the eucosterol class of compounds.

2.3 CARDIOACTIVE GLYCOSIDES

2.3.1 Occurrence and Classification

The numbering system employed when referring to the aglycones of cardiac glycosides is shown below :

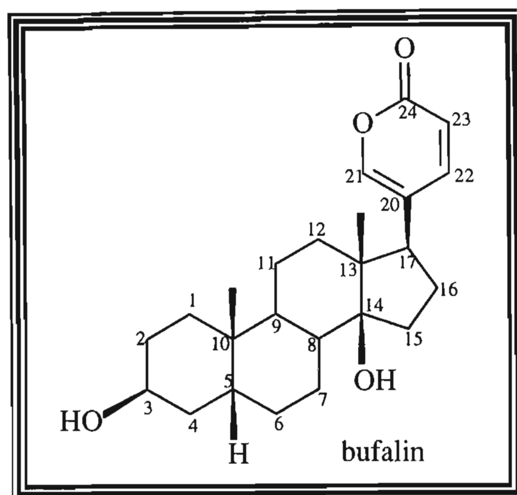


FIG. 2.7 : Numbering system referring to bufadienolides

Generally, there are two types of aglycones that are recognized *viz.* cardenolides, for example digitoxigenin from *Digitalis purpurea* and bufadienolides, for example, hellebrigenin from *Helleborus niger*.

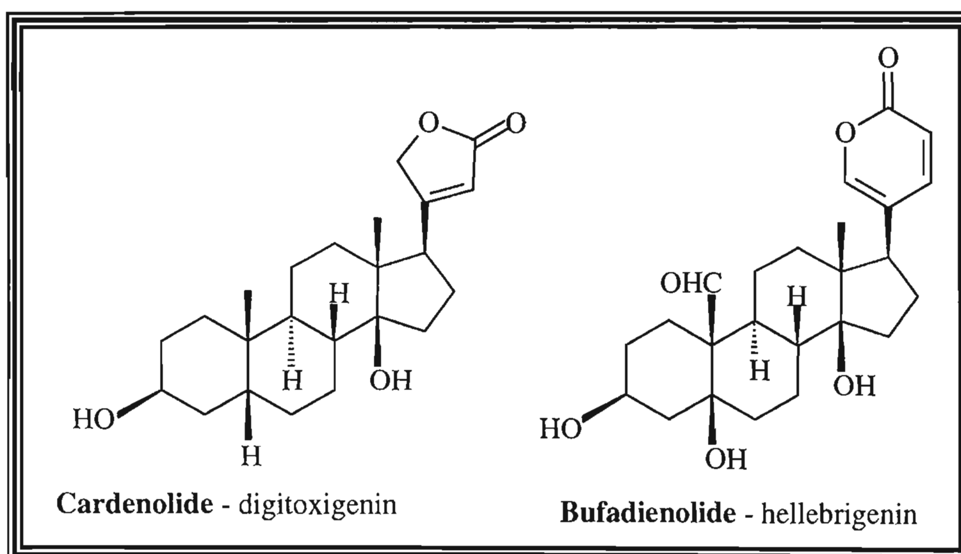


FIG. 2.8 : Structures of digitoxigenin and hellebrigenin

The structure of the aglycone and the type and number of sugar units attached determine the therapeutic action of the cardioactive glycoside. Cardenolides are C_{23}

compounds whereas bufadienolides have an extra carbon, giving it a six-membered lactone ring. Although most of the pharmacological activity resides in the aglycone portion of these compounds, the nature of the sugar attached at C-3 gives considerable modifications to its activity. They increase water solubility and heart muscle binding capacity.[19] Cardenolides are the more common of the two classes of glycosides and are known to occur in a number of plant families such as *Apocynaceae, Hyacinthaceae* and *Scrophulariaceae.* The rarer bufadienolides are reported in families such as *Hyacinthaceae* (eg. *Urginea*) and *Ranunculaceae* (eg. *Helleborus*).[19] Bufadienolides such as bufalin are also known to be a constituent in toads. In plants, cardiac glycosides are confined to the Angiosperms, but are found in both monocotyledons and dicotyledons.

Digitalis-like compounds have also been detected in very small quantities in mammalian tissue. The compound 19-norbufalin is found in the human eye lenses and at higher levels if they are cataract afflicted, the bufadienolides are believed to regulate ATPase activity.[19]

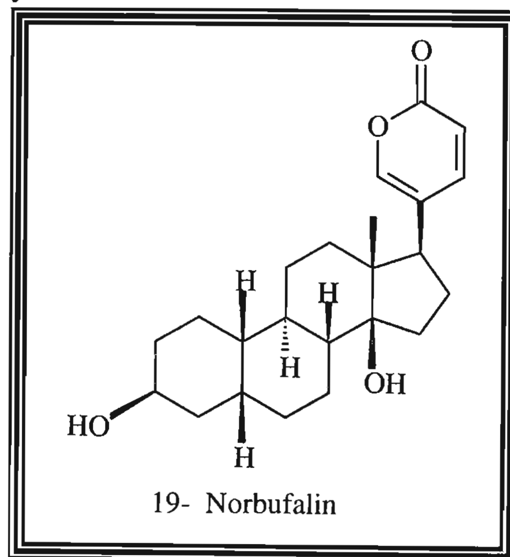


FIG. 2.9 : Structure of 19-norbufalin

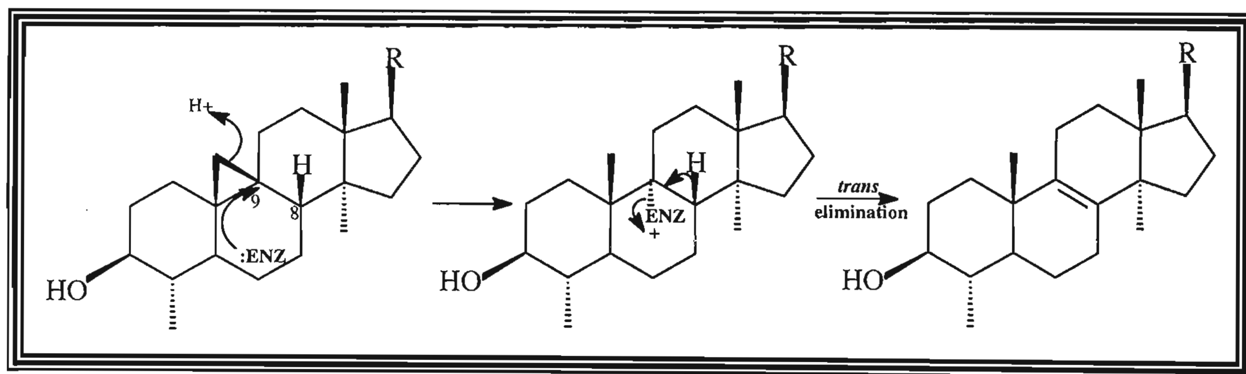
Plants containing cardiotonic glycosides were known for their use as arrow poisons. These days they are used as heart drugs. *Digitalis* is a well known source of these compounds. Although these drugs are used to strengthen a weakened heart, the toxic dose is so close to the therapeutic dose that optimal dosage control must be exercised. Extracts of *Digitalis* are also reported to be used in the successful treatment of dropsy,

which is an accumulation of water in the body tissues. The extract was found to alleviate dropsy by improving the blood supply to the kidneys and thereby removing excess fluid.[19]

In general, plants containing cardioactive glycosides are widely grown for ornamental purposes but these are considered toxic and must be treated with due care and caution.

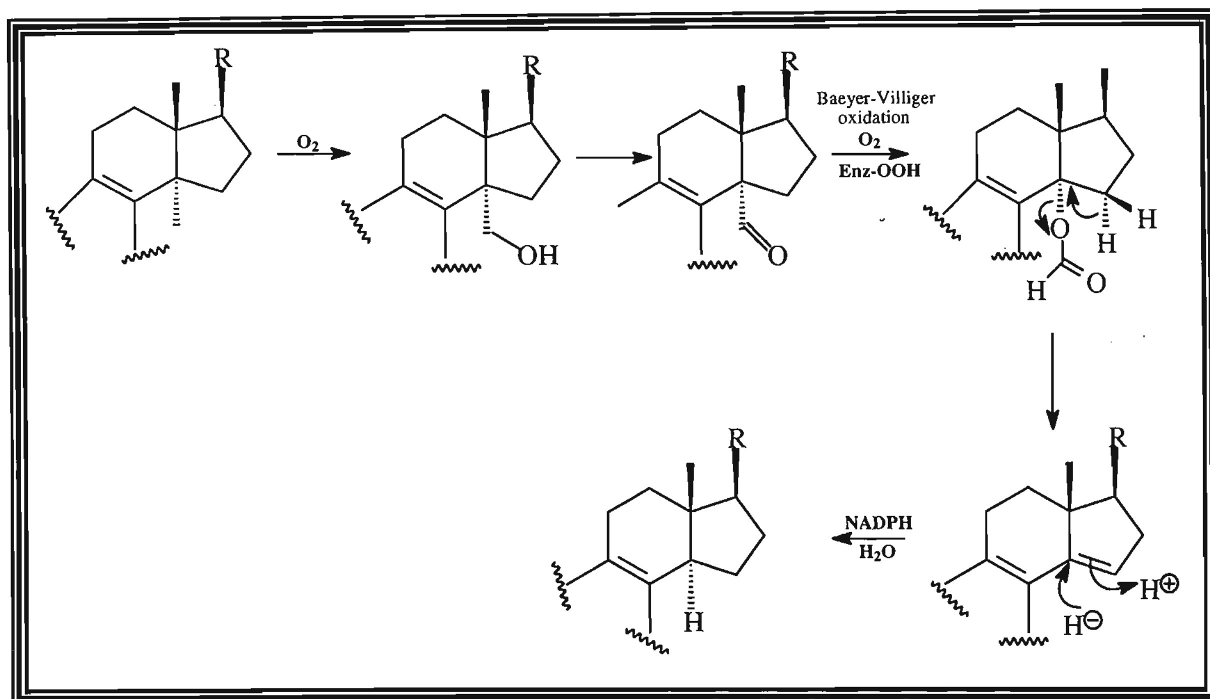
2.3.2 Biosynthesis

The basic structures of cardioactive glycosides arise from the biosynthetic manipulation and metabolism of cholesterol. In photosynthetic organisms, the route to cholesterol begins with the cyclopropane triterpenoid cycloartenol.[19] The first step involves a ring-opening mechanism. Since the stereochemistry at C-8 (H-β) is unfavourable for a concerted mechanism involving the loss of H-8 and hence ring opening, an alternate route involves attack by a nucleophilic group at C-9. This opens the cyclopropane ring and incorporates a proton from water. A *trans* elimination then incorporates the double bond at C-8. The specificity of the cyclopropane ring-opening enzyme means that cycloartenol is not converted into lanosterol and thus lanosterol is absent from virtually all plant tissue.



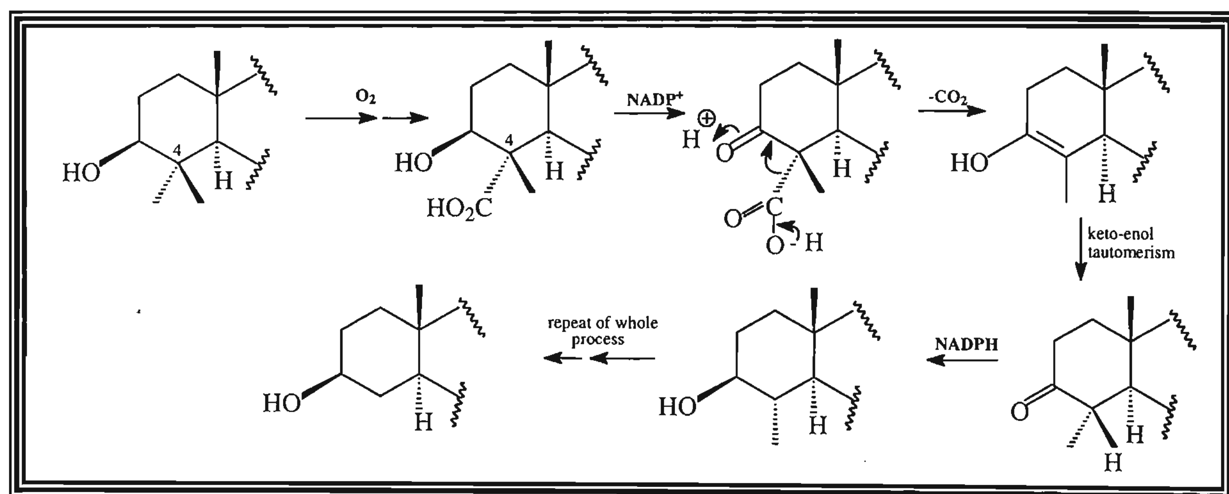
SCHEME 2.9 : Cyclopropane ring-opening mechanism[19]

The next step involves the loss of the C-14 methyl group. This is removed as formic acid and the reaction is catalysed by cytochrome P-450 mono-oxygenase. The loss of the formyl group and subsequent formation of the diene proceeds via a Baeyer-Villiger type oxidation reaction. Finally, the C-14 methyl is lost as formic acid and the last step involves reduction by NADPH which reduces the double bond on the D-ring (Scheme 2.10).



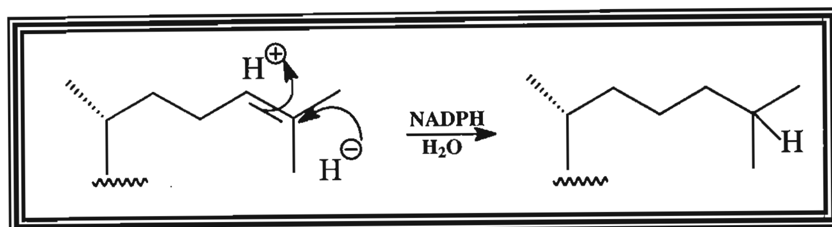
SCHEME 2.10 : Loss of C-14 methyl group[19]

Repetitive decarboxylations result in the loss of the C-4 methyl groups and the C-24 double bond on the side chain is reduced by an NADPH-dependant reductase.

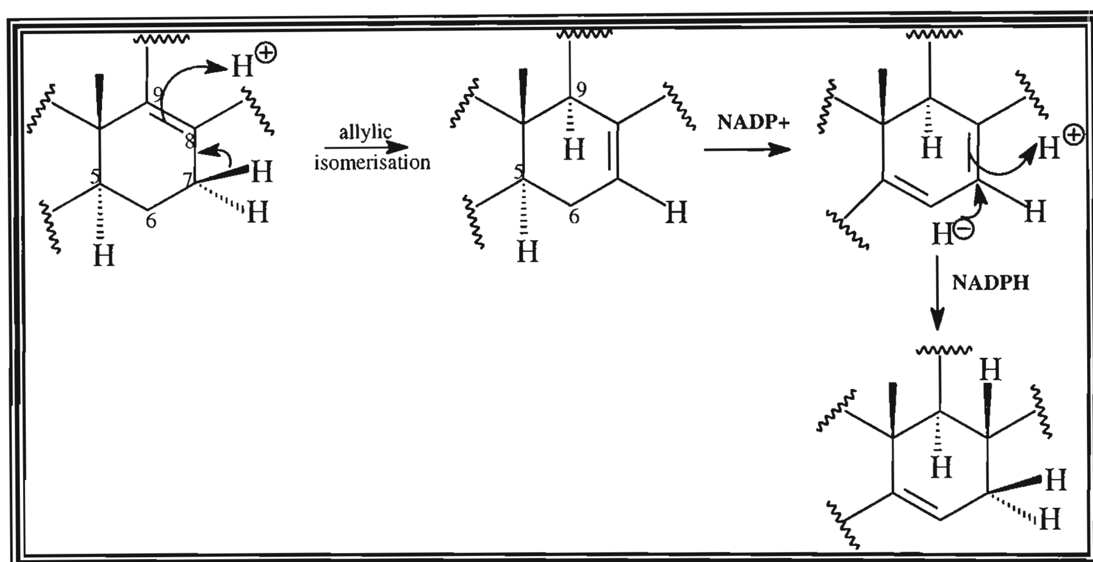


SCHEME 2.11 : Loss of C-4 methyl groups[19]

The double bond between C-8 and C-9 then migrates to C-5 *via* an allylic isomerisation process.[19] Protons at C-8 and C-9 are added on from water while that at C-7 comes from NADPH.

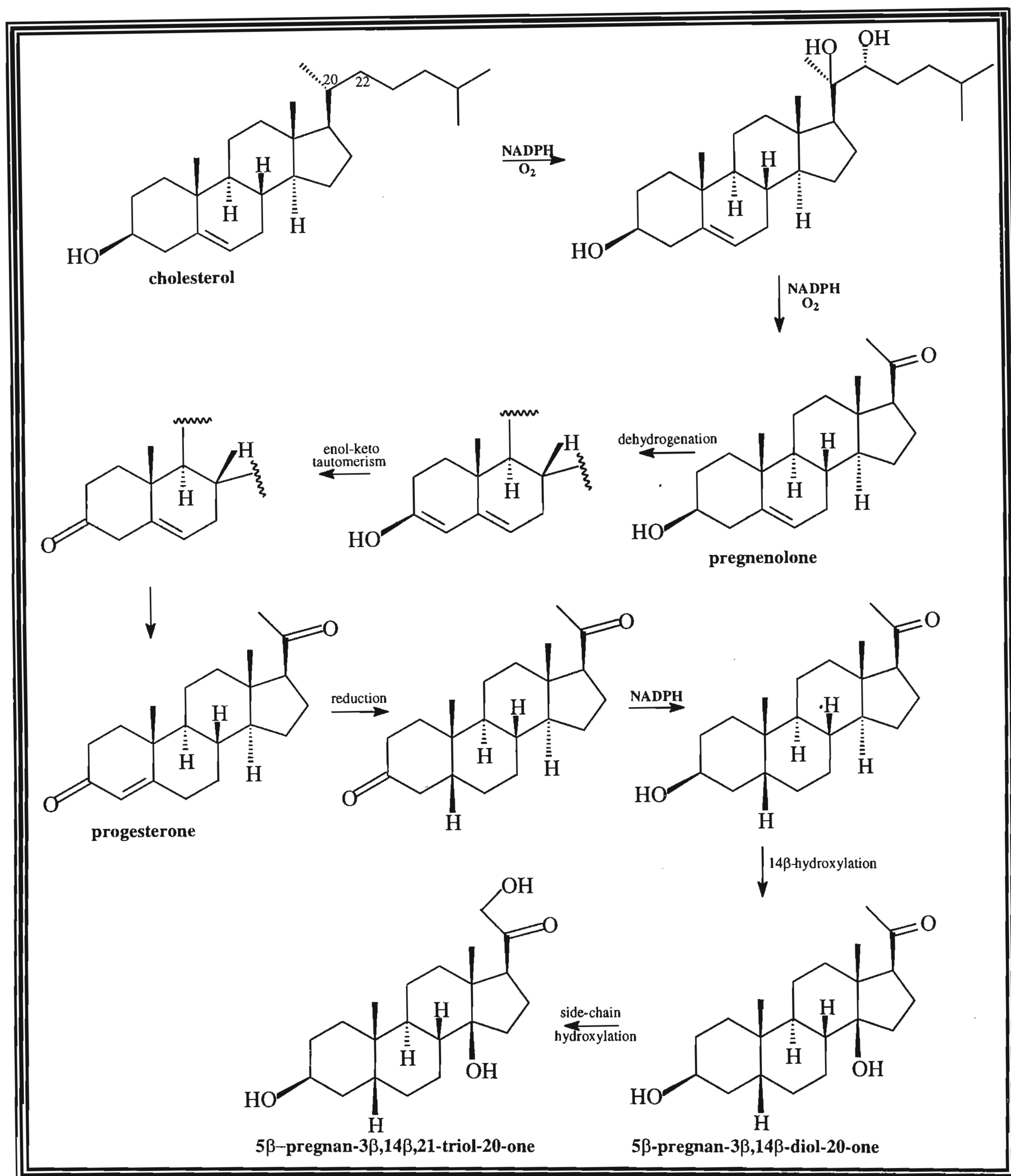


SCHEME 2.12 : Reduction of C-24 double bond



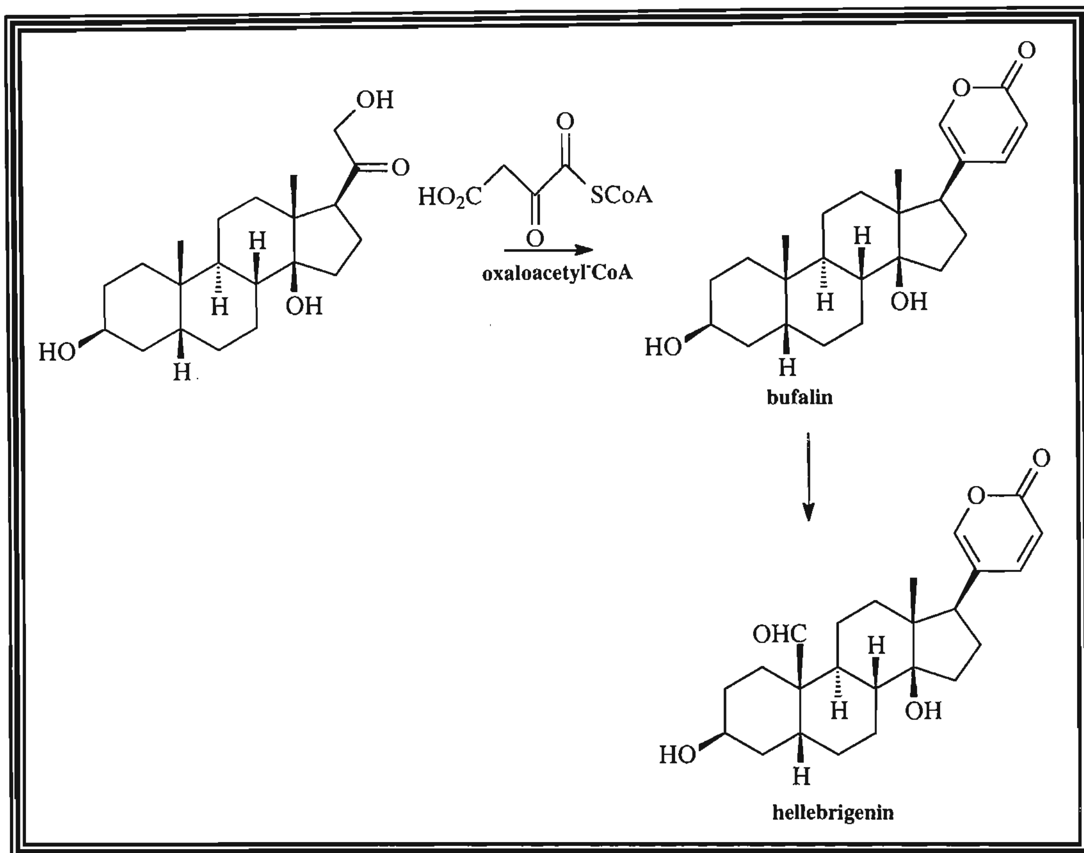
SCHEME 2.13 : Migration of double bond *via* an allylic isomerisation process[19]

The side chain of cholesterol is then cleaved to a two-carbon acetyl group. This shortening is achieved by successive oxidation, hydration and cleavage of the C-20/22 bond to give pregnenolone which is, in turn, oxidised to give progesterone (Scheme 2.14).



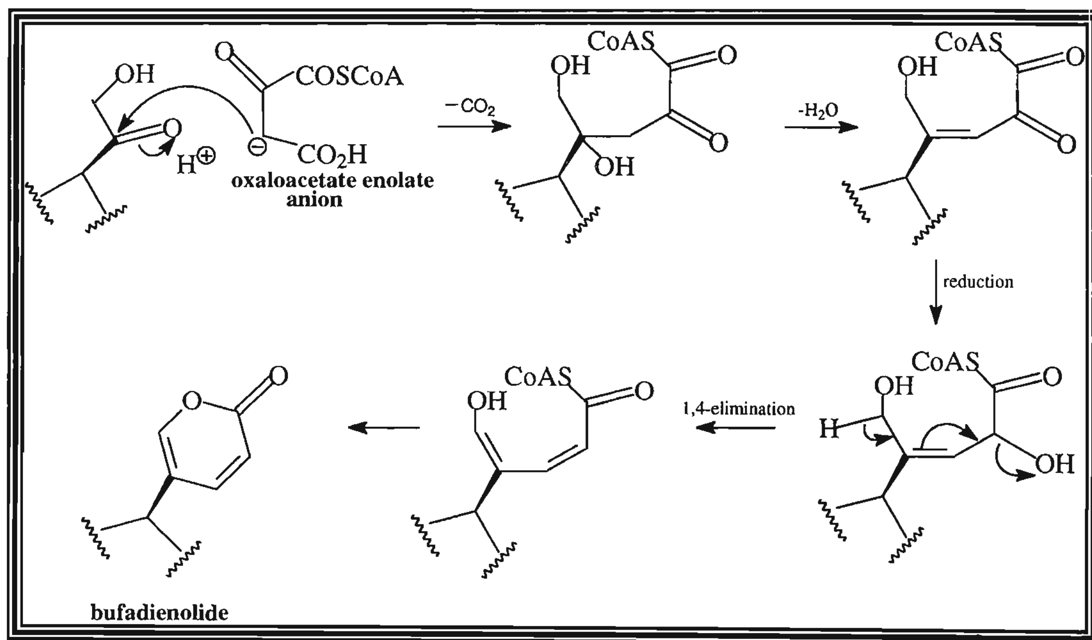
SCHEME 2.14 : Conversion of cholesterol to 5β-pregnan-3β,14β,21-triol-20-one

Creation of the lactone ring is achieved by the addition of either two or three carbons for cardenolides or bufadienolides respectively. An oxaloacetyl moiety is added in the case of bufadienolides.



SCHEME 2.15 : Addition of an oxaloacetyl moiety[19]

A detailed mechanism for the formation of the lactone ring is shown in Scheme 2.16.



SCHEME 2.16 : Mechanism for the lactone ring formation[19]

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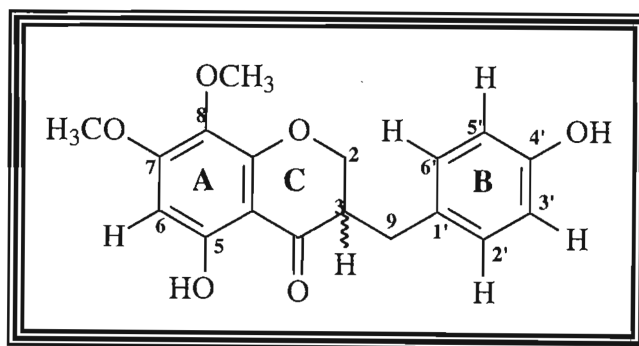
CHAPTER 3 : RESULTS AND DISCUSSION

3.1 EXTRACTIVES FROM *LEDEBOURIA COOPERI* (Hook)

Ledebouria cooperi was harvested as very small bulbs from the Natal Midlands area in KwaZulu-Natal. The bulbs were chopped and extracted first with $MeCl_2$ and then with methanol. The first compound, 7-O-methyl-3,9-dihydropunctatin, was isolated from the methanol extract after column chromatography. Further screening of the methanol extract yielded only sugars. The second homoisoflavonoid as well as the eucosterol-type triterpenoid was isolated from the methylene chloride extract after column chromatography. A free acid also crystallised out of the methanol extract and this has not been previously reported to occur in this genus.

3.1.1 Structural elucidation of COMPOUND 1

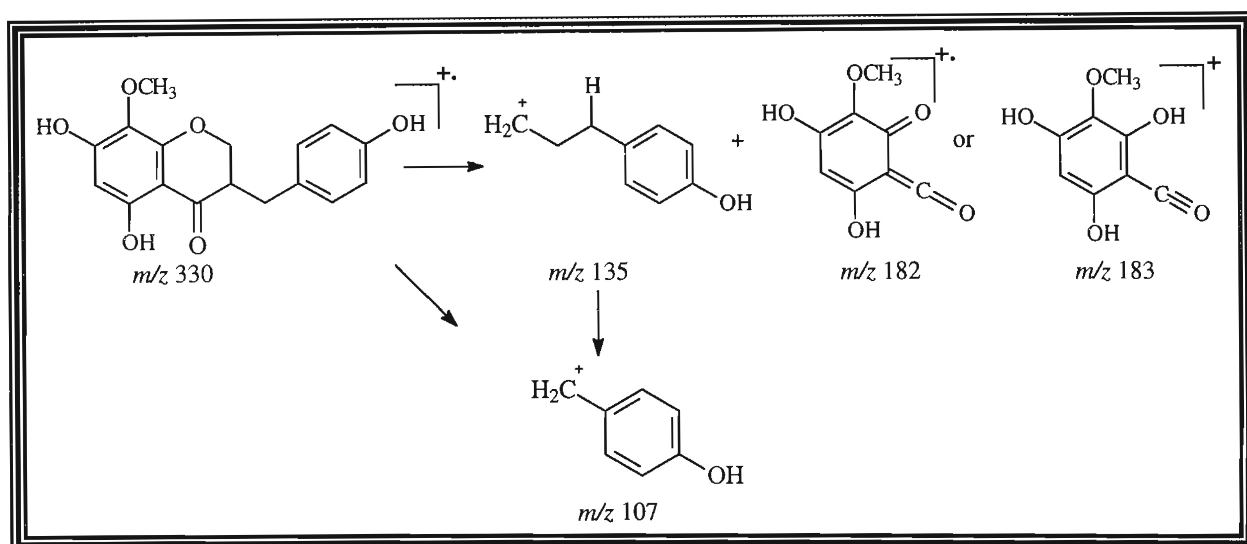


The high resolution mass spectrum of compound 1 gave a molar mass of 330.1104 $g.mol^{-1}$ (calculated 330.1103 $g.mol^{-1}$). This corresponded to a molecular formula of $C_{18}H_{18}O_6$. A double bond equivalence of 10 was deduced.

The proton NMR spectrum of compound 1 showed the typical splitting pattern of the 2H-2, H-3 and 2H-9 protons. This is indicative of 3-benzyl-4-chromanone type homoisoflavonoids. Each of the H-2 protons was split by the H-3 proton and this resulted in the pair of double doublets at δ4.18ppm and δ4.30ppm assignable to the two H-2 protons. The same splitting pattern was displayed by the interaction of the H-3 and the two H-9 protons and the double doublet resonances of the H-9 protons are found at δ2.69ppm and δ3.10ppm. The H-3 proton resonates as a multiplet at δ2.85ppm. The proton spectrum also show the presence of two methoxy group proton resonances at δ3.67ppm and δ3.85ppm. The pair of doublets at δ6.72ppm and

δ7.04ppm (*J*=8.5Hz), each integrating to two protons, are typical of a *para*-disubstituted B-ring system. A single proton resonance was also evident slightly downfield of 6.00ppm. This was assigned to a proton on ring A.

The mass spectrum was used to confirm the substitution pattern on the B-ring. The peak at *m/z* 107 which was thought to be a hydroxybenzyl ion was unexpected given previous reports on the fragmentation of these compounds as presented in Scheme 3.1.[11]



SCHEME 3.1 : Hydroxybenzyl fragmentation pattern[11]

This proves that the B-ring is hydroxylated[10] and further, the hydroxy group can be placed at the C-4 position since this ring is *para*-disubstituted.

The substitution on the A-ring could be inferred from the UV and mass spectra. The UV spectrum of compound 1 showed a bathochromic shift (+23nm) with $AlCl_3$. This indicated the presence of a hydroxy group at the C-5 position.[5] This was further confirmed by the presence of the C-4 carbonyl resonance at δ199.6ppm. The carbonyl resonance is deshielded due to chelating effects with the hydroxy group and this causes it to be shifted downfield to almost δ200.0ppm.[5] In the event of a methoxy group being present at C-5, the carbonyl would resonate much further upfield of δ200.0ppm. No bathochromic shift was observed with NaOAc and this confirms the presence of a methoxy group at C-7. The single proton resonance in the proton spectrum was also found to occur downfield of 6.00ppm. This implies that there is no hydroxy group at the C-7 position. One more methoxy group remained to

be placed at either the C-6 or C-8 position of ring-A. This assignment was made by comparison with literature references.[1-4]

Table 1.1 shows that if a methoxy group were placed at C-8, the methoxy group protons would resonate at δ3.66ppm but if it were placed at C-6, they would resonate at δ3.72ppm. The methoxy group proton resonance in compound 1 occurred at δ3.67ppm which suggested its placement at the C-8 position. The position of the methoxy group also affects the C-5, C-6 and C-8 resonances. According to Table 1.2 (^{13}C NMR data) the resonances in compound 1 were in agreement with the values for the known compound 7-O-methyl-3,9-dihydropunctatin.[5] The substituent on C-8 would also have an effect on the C-8a resonance. The literature values for C-8a in the compounds 3,9-dihydropuctatin and 3,9-dihydroeucomnalin, which have the methoxy group at the C-8 and the C-6 positions respectively, are very different. When the methoxy group is positioned at C-8, the chemical shift of C-8a is δ154.9ppm. When the methoxy group is at C-6, the C-8a resonance occurs at δ160.6ppm. The C-8a resonance of compound 1 occurs at δ154.8ppm and this provides further evidence for the placement of the methoxy group at C-8.

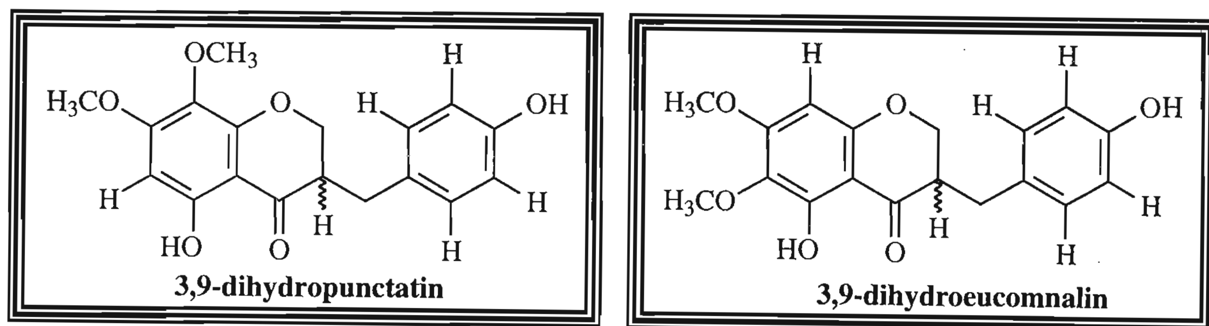


FIG. 3.1 : Structures of 3,9-dihydropunctatin and 3,9-dihydroeucomnalin

Spectrum 1.4 also shows a nOe correlation between the C-7 methoxy group proton resonance at δ3.67ppm and the single proton resonance of H-6. The COSY spectrum of compound 1 shows coupling between the H-3 and 2H-2 protons and between the H-3 and the 2H-9 protons. The COSY spectrum shows further coupling between the H-2'/6' and the H-3'/5' protons. This is typical of a *para*-disubstituted system.

Table 1.3 shows the HMBC correlations. Two, three and four bond correlations can be seen between carbons and protons. The assigning of the C-8 carbon atom

resonance was initially a problem. The HMBC spectrum also confirmed the presence of the C-8 resonance at δ129.6ppm which is superimposed on the C-1' resonance, as a correlation between the peak at δ129.6ppm and the H-3'/5' protons can be seen. From this it could be deduced that this represents the three-bond HMBC correlation between C-1' and H-3'/5'. However, a further correlation could be seen with the resonance at δ129.6ppm and H-6. Since there can be no correlation between H-6 and any of the B-ring protons, this could only represent the three-bond correlation between C-8 and H-6. Hence it could be deduced that the peak at δ129.6ppm represents C-1' as well as C-8. The stereochemistry at C-3 could not be determined.

Compound 1 was shown to be the known compound 7-O-methyl-3,9-dihydropunctatin which was previously reported to be isolated from *Muscari comosum* (Hyacinthaceae).[5]

TABLE 1.1 : Comparison of proton NMR data for compound 1 with 3,9-dihydroeucomnalin[5] and 3,9-dihydropunctatin[5]

^{1}H	3,9-dihydro-eucomnalin[5] (CD_3OD)	3,9-dihydro-punctatin[5] (CD_3OD)	COMPOUND 1 (CD_3OD)
H -2·	4.07dd , 4.25dd (*J*=4.2, 7.2, 11.4Hz)	4.12dd , 4.29dd (*J*=4.4, 7.3, 11.4Hz)	4.18dd , 4.29dd (*J*=4.2, 7.1Hz)
H -3	2.81m	2.83m	2.84m
7 -OCH_3	3.84s	3.84s	3.85s
H -9	2.60dd (*J*=10.2,13.50Hz) 3.07dd (*J*=4.2, 13.5Hz)	2.61dd (*J*=9.6, 13.6Hz) 3.08dd (*J*=4.0, 13.6Hz)	2.69dd (*J*=10.0, 13.9Hz) 3.08dd (*J*=4.5, 13.7Hz)
6 -OCH_3 or H -6	3.72s	6.11	6.14
8 -OCH_3 or H -8	6.10	3.66s	3.67s
H-2'/6'	7.03d (*J*=8.4Hz)	7.03d (*J*=8.5Hz)	7.03d (*J*=8.4Hz)
H -3'/5'	6.72d (*J*=8.4Hz)	6.72d (*J*=8.5Hz)	6.72d (*J*=8.4Hz)

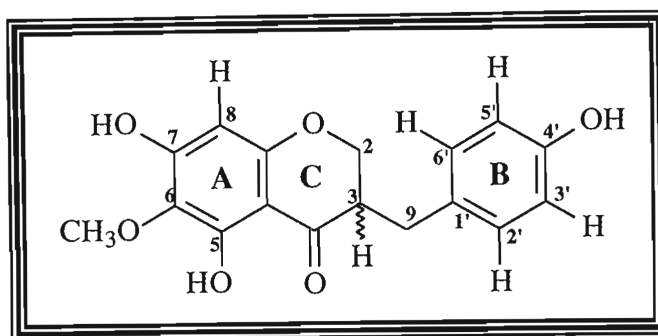
TABLE 1.2 : Comparison of carbon NMR data for compound 1 with 3,9-dihydroeucomnalin[5] and 3,9-dihydropunctatin[5]

^{13}C	3,9-dihydro-eucomnalin[5] (CD_3OD)	3,9-dihydro-punctatin[5] (CD_3OD)	COMPOUND 1 (CD_3OD)
2	70.6	70.4	70.2
3	48.1	48.8	48.4
4	200.5	199.8	199.6
4a	103.7	103.3	102.9
5	156.2	161.3	161.1
6	131.4	93.8	93.4
7	162.3	162.5	162.3
8	92.6	130.3	129.6
8a	160.6	154.9	154.8
9	32.9	32.0	32.6
1'	130.1	129.7	129.6
2'	131.2	131.0	130.8
3'	116.5	116.4	116.0
4'	157.3	157.2	156.9
5'	116.5	116.4	116.0
6'	131.3	131.0	130.8
6 - OCH_3	61.1		
7 - OCH_3	56.7	56.7	56.3
8 - OCH_3		61.5	61.1

TABLE 1.3: HMBC correlations (C→H) for compound 1

δ	^{13}C	^{1}H
199.6	C -4	H -2α , H -2β
162.3	C -7	OCH_3 (δ3.85ppm) , H -6
161.1	C -5	H -6
156.9	C -4'	H -2'/6'
154.8	C -8a	H -2α or H -2β
130.8	C -2'/6'	H -9α ,H -9β , H -2'/6'
154.8	C -1'	H -3'/5' , H -9α
129.6	C -8	H -6
102.9	C -4a	H -6

3.1.2 Structural elucidation of COMPOUND 2



Compound 2 was found to have a molar mass of 316.09469g.mol^{-1} (calculated 316.09468g.mol^{-1}), corresponding to a molecular formula of $C_{17}H_{16}O_6$. A double bond equivalence of 10 was deduced.

The proton NMR spectrum showed the presence of one methoxy group three proton resonance at δ3.75ppm. The splitting pattern for the C-9, C-3 and C-2 proton coupled system appeared as a pair of double doublets at δ2.62ppm and δ3.05ppm, a multiplet at δ2.75ppm and another pair of double doublets at δ4.01ppm and δ4.19ppm respectively. The splitting pattern was typical of a 3-benzyl-4-chromanone type homoisoflavonoid.

The resonances with chemical shifts at δ6.72ppm and δ7.01ppm (*J*=8.60Hz) were indicative of a *para*-disubstituted benzene ring. The substitution pattern on the A and B rings could be inferred from analysis of the UV and mass spectral data. The appearance of peaks at *m/z* 209, 183 and 182 corresponding to A-4 and RDA fragments was an indication of the presence of two hydroxy and one methoxy group on ring A while the base peak at *m/z* 107 was indicative of a hydroxybenzyl moiety. Hence, ring B was hydroxylated. From this, the hydroxy group could be assigned to the C-4' position since the B-ring also showed a *para*-disubstituted benzene ring as mentioned previously. The resonance at δ7.01ppm represents H-2'/6' while the resonance at δ6.72ppm represents H-3'/5'.

Placement of the two hydroxy groups and the methoxy group on the A-ring was carried out using the UV spectra. Bathochromic shifts with $AlCl_3$ (+22nm) and NaOAc (+13nm) were a clear indication of the presence of hydroxy groups at the C-5

and C-7 positions.[1,2] This implied that the methoxy group could be at either the C-6 or the C-8 position. The methoxy group was assigned on the basis of the ^{13}C NMR spectrum. Adinolfi[5] ascertained that when there is no methoxy group present at the C-6 position, the chemical shift of C-5 occurs at approximately δ165.8ppm. The shift assigned to C-5 of compound 2 was δ156.5ppm. This is about 9.0ppm upfield from that reported earlier, which, according to Adinolfi[5], suggests that there is a methoxy group present at the C-6 position. Furthermore, the range for a C-8 resonance in the case of an unsubstituted C-6 is 97.1-97.3ppm. The chemical shift of C-8 in compound 2 occurs at δ95.4ppm. This is out of the range for an unsubstituted C-6 and hence the methoxy group must be at the C-6 position on the A-ring.

The NOESY spectrum showed the expected correlations between the H-3, H-9 and H-2 protons. A further correlation could be seen between the H-2'/6' protons and those of H-9 and H-3.

The HMBC data are tabulated in Table 2.3 and serve to confirm the position of the substituents.

Finally, the structure of compound 2 was confirmed by comparison with literature values.[6] Compound 2 was found to be the known 3,9-dihydroautumnalin [5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one]. It was first isolated from *Eucomis autumnalin* (Hyacinthaceae).[6]

TABLE 2.1: Comparison of proton NMR data for compound 2 with 3,9-dihydroautumnalin[5]

^{1}H	3,9-dihydroautumnalin[5] (CD_3OD)	COMPOUND 2 (CD_3OD)
2H – 2	4.00dd, 4.17dd (*J*=4.3, 7.7, 11.4Hz)	4.01dd, 4.19dd (*J*=4.2, 7.3Hz)
H – 3	2.75m	2.75m
H – 8	5.89s	5.89s
2H – 9	2.59dd (*J*=10.6, 13.0Hz) 3.07dd (*J*=4.0, 13.0Hz)	2.62dd (*J*=10.2, 13.7Hz) 3.05dd (*J*=4.3, 13.7Hz)
H – 2'/6'	7.03d (*J*=8.0Hz)	7.01d (*J*=8.5Hz)
H – 3'/5'	6.72d (*J*=8.0Hz)	6.72d (*J*=8.6Hz)
6-OCH_3	3.76s	3.75s

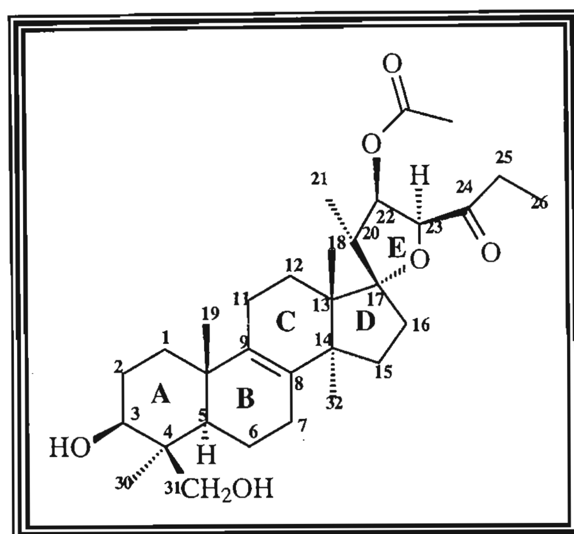
TABLE 2.2: Comparison of carbon NMR data of compound 2 with 3,9-dihydroautumnalin[5]

^{13}C	3,9-dihydroautumnalin[5] (CD_3OD)	COMPOUND 2 (CD_3OD)
C – 2	70.5	69.9
C – 3	49.1	49.3
C - 4	199.9	199.8
C – 4a	102.7	102.6
C – 5	156.7	156.5
C - 6	130.4	130.0
C – 7	161.1	160.3
C – 8	95.9	95.4
C – 8a	160.0	159.7
C – 9	32.9	32.6
C – 1'	130.0	129.7
C – 2'	131.0	130.8
C – 3'	116.3	116.0
C – 4'	157.1	156.8
C – 5'	116.3	116.0
C – 6'	131.0	130.8
6-OCH_3	60.9	60.6

TABLE 2.3: HMBC correlations (C→H) for compound 2

δ (ppm)	^{13}C	C→H
199.8	C – 4	2H-9, 2H-2, H-3, H-8
160.3	C – 7	H-8
159.7	C – 8a	2H-2
156.8	C – 4'	H-3'/5', H-2'/6'
156.5	C – 5	H-8
130.8	C – 2'/6'	2H-9, H-3'/5'
130.0	C – 6	OCH_3(3.75), H-8
116.0	C – 3'/5'	H-3'/5', H-2'/6'
102.6	C – 4a	H-8
69.2	C – 2	2H-9, H-3
49.3	C – 3	2H-9, 2H-2
32.6	C - 9	2H-2, H-2'/6', H-3

3.1.3 Structural elucidation of COMPOUND 3



The third compound isolated from this plant species belongs to a different class of compounds known as the eucosterol-type triterpenoids. The mass spectrum showed a molar mass of 516.34619g.mol^{-1} (calculated 516.34509g.mol^{-1}). This corresponded to a molecular formula of $C_{31}H_{48}O_6$ and a double bond equivalence of 8 was deduced.

The proton NMR spectrum showed the presence of six methyl groups as well as an acetoxy group methyl proton resonance. A pair of doublets at δ5.18ppm and δ4.58ppm was assigned to the protons at C-22 and C-23 respectively. The COSY spectrum did not show coupling between H-20 and H-22, however, this coupling could be seen in the TOCSY spectrum.

The ^{13}C NMR spectrum showed 31 carbon signals which were resolved by an ADEPT experiment into seven methyl, ten methylene, five methine and (by comparison with the ^{13}C NMR spectrum) eight fully substituted carbon atoms. The protonated carbon signals were able to be assigned to their corresponding protons by means of a HETCOR experiment. The ^{13}C NMR spectrum also showed the lowfield shifts at δ211.1ppm, δ171.1ppm, δ136.0ppm and δ135.4ppm which were assigned to a ketone group carbon, C-24, the carbonyl carbon of the acetate group and the two quarternary carbons of the double bond (C-8 and C-9) respectively. The five remaining double bond equivalents were attributed to the pentacyclic ring structure which included a heterocyclic ring E.

From the TOCSY experiment, the six different spin systems could be identified as shown in Fig. 3.2 below and all proton and carbon atoms could be assigned.

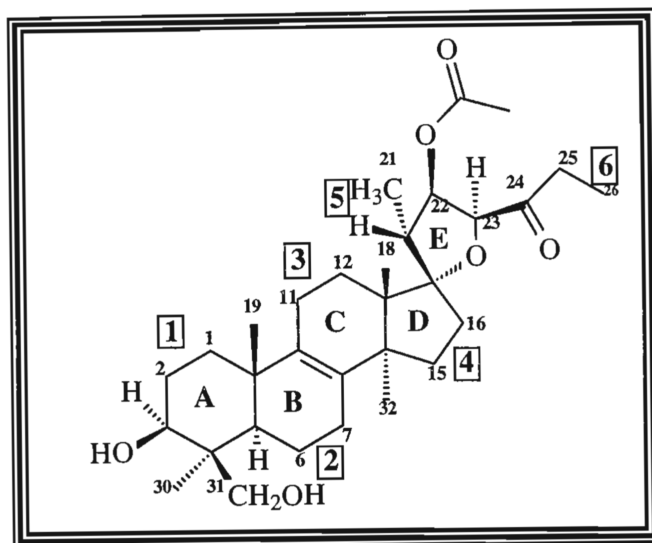


FIG. 3.2 : Different spin systems

The HMBC spectrum showed the two and three-bond corrrelations which were used to confirm the structure of compound 3. Table 3.3 shows the carbon to hydrogen correlations.

The stereochemistry of the side-chain was confirmed by a NOESY experiment.

H-20 gave a positive nOe with 3H-18 (strong), the acetate methyl group protons and 3H-21 (strong). From this it could be concluded that the stereochemistry at H-20 was β. H-22 also gave strong positive nOes with H-23 and 3H-21. This implied that H-22 was α. H-23 showed correlations with H-22 and 3H-21 again placing the proton in the α-position. The methylene group protons of the side-chain (H-25) showed a weak nOe with H-23 and a strong nOe with the methyl group protons of H-26. C-31 was shown to be β by the positive nOes between the H-31 protons at δ3.39ppm and δ4.15ppm and 3H-19

Finally, the structure was confirmed by comparison with literature.[7] The reference compound, however, was run in pyridine while compound 3 was run in deuteriomethanol. To confirm that the NMR data was identical, compound 3 was then also run in deuterated pyridine to get a better comparison with the literature values. From Table 3.1 it is evident that the values obtained for both pyridine runs are slightly different but this was attributed to the fact that the spectrum from literature

was not correctly referenced for pyridine. When the spectrum of compound 3 was run uncalibrated, the values corresponded very well. The values recorded in Table 3.1 are for the spectrum calibrated for pyridine at δ8.57ppm (the calibration of the reference peak in the compound in the literature is at δ8.70ppm). From this, compound 3 was deduced as (22*R*, 23*S*)-22-acetoxy-3β, 31-17, 23-epoxy-dihydroxy-27-nor-5α-lanost-8-en-24-one (22-acetoxy-15-deoxo-eucosterol). It has been previously isolated from *Veltheimia viridifolia* (Hyacinthaceae).[7]

TABLE 3.1 : Comparison of proton NMR data for compound 3 with 22*R*-acetoxy-15-deoxoeucosterol from literature[7]

^{1}H	COMPOUND 3 in CD_3OD	COMPOUND 3 in C_5D_5N	COMPOUND from lit.[7]- C_5D_5N
H - 1a H - 1b	* *	1.22 1.67	1.23 1.73
H - 2a H - 2b	2.07 2.05	1.97 2.05	1.97 2.06
H - 3	3.38	3.69	3.65
H - 4	*	-	-
H - 5	2.65	1.30	1.32
H - 6a H - 6 b	* *	1.94 1.55	1.87 1.55
H - 7a H - 7b	* *	2.08 2.10	2.06 2.10
H - 11a H - 11b	* *	 2.02	2.17 2.03
H - 12a H - 12b	* *	2.40 1.47	2.42 1.48
H - 13	-	-	-
H - 14	-	-	-
H - 15a H - 15b	* *	1.53 1.71	1.53 1.77
H - 16a H - 16b	* *	2.53 2.04	2.64 2.02
H - 17	-	-	-
H - 18	0.93	0.91	0.94
H - 19	1.00	1.05	1.06
H - 20	2.33	2.49	2.43
H - 21	1.12	1.09	1.12
H - 22	5.17	5.42 (J=5.3Hz)	5.43
H - 23	4.58	4.96 (J=5.3Hz)	4.98
H - 24		-	-
H - 25a,b	2.51	2.52	2.55
H - 26	1.09	1.07	1.07
H - 30	1.19	1.40	1.56
H - 31a H - 31b	3.39 (J=10.8Hz) 4.15 (J=10.8Hz)	3.72 (J=10.8Hz) 4.58 (J=10.8Hz)	3.75 4.59
H - 32	1.24	1.42	1.58
CH_3COO	1.89	1.84	1.99

* = signal too weak to be deciphered or it is buried under other peaks

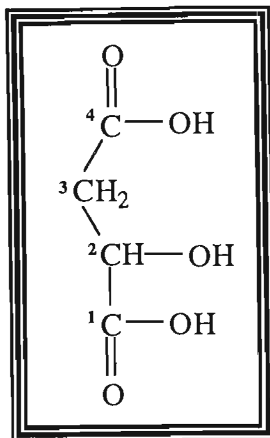
TABLE 3.2 : Comparison of carbon NMR data for compound 3 with 22*R*-acetoxy-15-deoxoeucosterol from literature[7]

^{13}C	COMPOUND 3 (CD_3OD)	COMPOUND. from lit.[7] (C_5D_5N)
C - 1	36.3	35.8 (t)
C - 2	28.6	28.9 (t)
C - 3	80.7	79.9 (d)
C - 4	43.2	43.2 (s)
C - 5	50.9	51.6 (d)
C - 6	19.3	18.9 (t)
C - 7	26.2	26.8 (t)
C - 8	136.0	134.9 (s)
C - 9	135.4	134.7 (s)
C - 10	37.6	37.1 (s)
C - 11	21.4	21.1 (t)
C - 12	25.7	25.2 (t)
C - 13	50.1	50.0 (s)
C - 14	50.5	50.4 (s)
C - 15	32.8	32.5 (t)
C - 16	40.5	40.0 (t)
C - 17	98.2	97.4 (s)
C - 18	19.3	19.3 (q)
C - 19	19.8	20.1 (q)
C - 20	49.3	49.5 (d)
C - 21	15.3	15.4 (q)
C - 22	82.8	82.0 (d)
C - 23	85.4	84.9 (d)
C - 24	211.1	208.6 (s)
C - 25	33.8	33.3 (t)
C - 26	7.1	7.5 (q)
C - 30	22.7	23.3 (q)
C - 31	64.8	64.3 (t)
C - 32	27.2	26.4 (q)
CH_3COO	20.5	20.8 (q)
CH_3COO	171.1	169.8 (s)

TABLE 3.3 : HMBC correlations (C→H) for compound 3

δ (ppm)	^{13}C	^{1}H
36.3	C - 1	H - 19
80.7	C - 3	H - 30
43.2	C - 4	H - 6b
50.9	C - 5	H -3, 10
136.0	C - 8	H - 32
135.4	C - 9	H - 19
37.6	C - 10	H - 19
25.7	C - 12	H - 18
50.1	C - 13	H - 18, 32
50.5	C - 14	H - 18, 32
32.8	C - 15	H - 32
98.2	C - 17	H - 18, 21, 22
49.3	C - 20	H - 21
82.8	C - 22	H - 21
211.1	C - 24	H - 25, 26
33.8	C - 25	H - 26
7.1	C - 26	H - 25
64.8	C - 31	H - 30
20.5	$\underline{C}H_3COO$-	OAc, H - 22

3.1.4 Structural elucidation of COMPOUND 4



The methanol extract of *Ledebouria cooperi* yielded a further simple aliphatic compound.

Compound 4 showed a simple proton NMR spectrum when run in deuterium oxide. The powdery crystals of compound 4 did not dissolve in deuterated methanol or deuterated chloroform. The ^{1}H NMR spectrum showed a double doublet at δ3.83ppm which integrated to one proton. A pair of double doublets was also evident centred at δ2.80ppm and δ2.70ppm. These integrated to a single proton each and were indicative of a methylene group.

The ^{13}C NMR spectrum showed the presence of just four carbon signals. This was resolved by an ADEPT spectrum into one methylene carbon and one methine carbon and (by comparison with the ^{13}C spectrum) two fully substituted carbon atoms. The methylene carbon resonance occurred at δ35.4ppm while the methine carbon resonance occurred at δ52.2ppm. The two fully substituted carbon signals occurred at δ174.2ppm and δ175.4ppm. These signals were attributed to two carbonyl groups present in the compound. These were deduced to be carboxylic acid groups. It was thus deduced that compound 4 was malic acid with a molecular formula $C_4H_6O_5$.

An IR spectrum was also run and this was compared to that of malic acid from literature.[10] The IR spectrum confirmed the presence of hydroxy groups by the presence of broad peaks at 3447cm^{-1} and 3388cm^{-1}. The carbonyl stretch appeared at 1650cm^{-1} while the peaks at approximately 1433cm^{-1} and 1367cm^{-1} were indicative of CH_2 and CH bending and stretching vibrations.

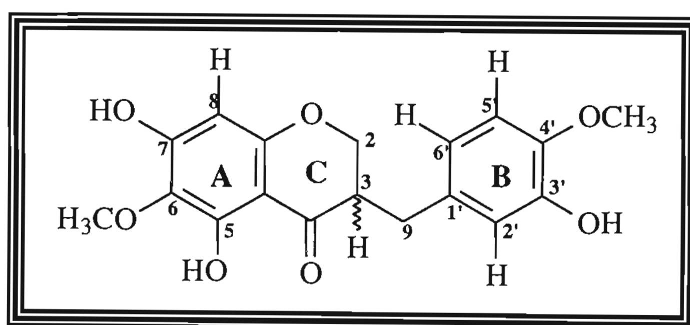
TABLE 4.1 : Proton and carbon NMR shifts for compound 4

^{1}H	δ(ppm)	^{13}C	δ(ppm)
		C-1	174.2
H-2	3.83 dd (J=7.8Hz, 4,3Hz)	C-2	52.2
H-3	2.65 - 2.71 dd (J=17.0Hz, 7.6Hz) 2.76 - 2.81 dd (J=16.8Hz, 4.2Hz)	C-3	35.4
		C-4	175.3

3.2 EXTRACTIVES FROM *SCILLA PLUMBEA* (Lindl.)

Scilla plumbea is the only species in this genus that grows outside KwaZulu-Natal. The bulbs of *Scilla plumbea* were harvested in the Western Cape region. The bulbs were extracted into hexane, ethyl acetate, methylene chloride and methanol. However, the hexane and ethyl acetate extracts were too small in quantity for any sufficient amount of compound to be isolated. The methylene chloride extract contained homoisoflavonoids that co-eluted. Again, the small quantity of the extract made separation difficult. Compound 5 was isolated from the methanol extract using preparative thin layer chromatography.

3.2.1 Structural elucidation of COMPOUND 5



Compound 5 was found to have a molar mass of 346.1059g.mol^{-1}(calculated 346.1052g.mol^{-1}). This corresponded to a molecular formula of $C_{18}H_{18}O_7$ with a double bond equivalence of 10.

The proton NMR spectrum was typical of a 3-benzyl-4-chromanone type homoisoflavonoid. However, instead of the pair of double doublets found in compounds 1 and 2 between 6-7ppm, compound 5 showed a more complex splitting pattern in this region. The resonances at δ6.84ppm (*J*=8.0Hz), δ6.69ppm (*J*=2.38Hz) and δ6.66ppm (*J*= 8.24Hz, 2.38Hz) were indicative of the presence of three non-equivalent protons on the B-ring. This also showed that the B-ring was 1',3'4'-trisubstituted and the protons could thus be assigned at the 5', 2' and 6' positions respectively. The corresponding carbon atoms could also be assigned. The proton spectrum also showed the presence of two methoxy group protons with resonances at δ3.74ppm and δ3.80ppm.

The substitution pattern on the A-ring could be deduced from the UV, ^{1}H NMR and mass spectra. The existence of a peak at *m/z* 209 in the mass spectrum was evidence of a dihydroxy-methoxychromanone fragment ion. This implied that the A-ring contained two hydroxy and one methoxy group. One hydroxy group was assigned to the C-5 position due to a bathochromic shift (+19nm) in the UV spectrum with $AlCl_3$.[1,2] This was also confirmed by the very downfield shift of the carbonyl peak in the ^{13}C NMR spectrum. The carbonyl group becomes deshielded due to chelating effects with the hydroxy group and this causes its very downfield shift. A bathochromic shift (+36nm) was also recorded with NaOAc and this implied that the second hydroxy group was at the C-7 position.[1,2]

The appearance of a singlet integrating to one proton at δ5.83ppm (normally due to H-6 or H-8) in the proton NMR spectrum indicated that there was only one unsubstituted position on ring A. The occurrence of this peak upfield of 6.00ppm provided further evidence of an hydroxyl substituent at C-7.[5] The position of the methoxy group was determined by comparing carbon chemical shift data of compound 5 with that of the known compound, 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one, from literature.[8] The carbon chemical shift data for C-5, C-6 and C-8 were looked at specifically. These are the shifts that would be affected by the presence of a methoxy group at C-6. In compound 5, these chemical shifts occurred at δ156.8, δ129.2 and δ95.8 respectively. These were a very good match to the literature values reported for a methoxy substituent at C-6. From this it was concluded that the methoxy group was at C-6.

The appearance of an ABX system in the proton NMR spectrum revealed that the B-ring was 1',3',4'-trisubstituted. The mass spectrum showed a peak at *m/z* 137 which was a clear indication of a hydroxymethoxybenzyl fragment ion. The substitution pattern was deduced through a NOESY experiment. The NOESY spectrum showed correlations between the methoxy group proton resonance at δ3.80ppm and a doublet at δ6.84ppm (J=8.0Hz). Since the resonance at δ6.84ppm belonged to H-5' (^{1}H NMR Table), it could be concluded that the methoxy group on the B-ring was positioned at C-4'. In an nOe experiment, irradiation of the doublet at δ6.84ppm was carried out. This led to the positive enhancement of the signal at δ3.80ppm ($-OCH_3$) and the

double doublet at δ6.66ppm ($J_{5'6'}$=8.24Hz, $J_{2'6'}$=2.38Hz). This substantiated the presence of the methoxy group at C-4' and it could also be concluded that the resonance at δ6.66ppm was due to H-6' adjacent to H-5'. Irradiation of the signal at δ6.69ppm also led to the enhancement of the resonances corresponding to H-3 and H-9. This showed that the signal at δ6.69ppm could be attributed to H-2' due to its close proximity to H-3 and H-9. The hydroxy group was placed at the C-4 position. The stereochemistry at C-3 could not be determined.

The ^{1}H and ^{13}C NMR data corroborated with those of the known compound from literature . It could thus be concluded that compound 5 was 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one which was previously isolated from *Muscari armeniacum* (Hyacinthaceae).[8]

TABLE 5.1: Comparison of proton NMR data of compound 5 with 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one[8]

^1H	5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4 methoxybenzyl)chroman-4-one[8] (CD_3OD)	COMPOUND 5 (CD_3OD)
2H -2	4.06dd , 4.24dd (*J*=4.1, 7.3, 11.4Hz)	4.04dd , 4.20dd (*J*=4.3, 6.9Hz)
H -3	2.81m	2.79m
H -8	5.91s	5.83s
2H -9	2.62dd (*J*=9.9, 13.6Hz) 3.06dd (*J*=4.8, 13.6Hz)	2.63dd (*J*=13.7Hz) 3.03dd (*J*=4.2Hz)
H -2'	6.71d (*J*=1.8Hz)	6.69d (*J*=2.0Hz)
H -5'	6.85d (*J*=8.1Hz)	6.84d (*J*=7.9Hz)
H -6'	6.66dd (*J*=1.8, 8.1Hz)	6.66dd (*J*=2.1, 8.2Hz)
6 -OCH_3	3.77s	3.74s
4' -OCH_3	3.82s	3.80s

TABLE 5.2 : Comparison of carbon NMR data for compound 5 with 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one[8]

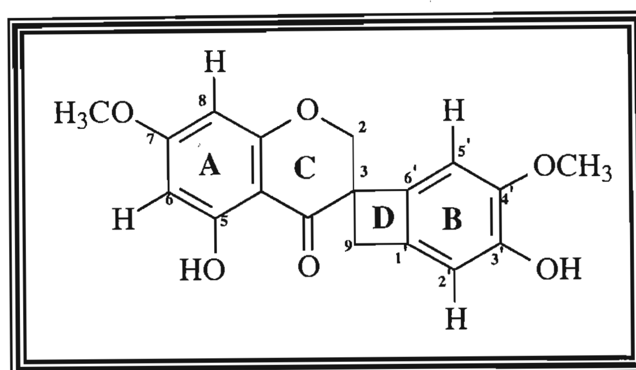
^{13}C	5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one[8] (CD_3OD)	COMPOUND 5 (CD_3OD)
2	70.3	70.3
3	*	*
4	200.0	200.0
4a	103.0	103.0
5	156.8	156.8
6	129.2	129.2
7	160.9	160.9
8	95.8	95.8
8a	160.1	160.1
9	33.1	33.1
1'	132.3	132.3
2'	117.0	117.0
3'	147.8	147.8
4'	147.8	147.8
5'	112.9	112.9
6'	121.4	121.4
6 -OCH_3	61.0	61.0
4' -OCH_3	56.4	56.4

*= hidden under solvent peak

3.3 EXTRACTIVES FROM *DRIMIOPSIS MACULATA* (Lindl.)

The bulbs of *Drimiopsis maculata* were harvested in the Kloof area in KwaZulu-Natal. The bulbs were chopped and extracted with methylene chloride and methanol for periods of 72 hours each. All the compounds reported were isolated from the methylene chloride extract. The methanol extract was not investigated as a proton NMR spectrum of the crude extract only revealed the presence of sugars.

3.3.1 Structural elucidation of COMPOUND 6



The high resolution mass spectrum gave a molar mass of 328.09469g.mol^{-1} corresponding to the molecular formula $C_{18}H_{16}O_6$. Compound 6 had a calculated molar mass of 328.09468g.mol^{-1}. A double bond equivalence of 11 was deduced.

The proton spectrum of compound 6 showed the presence of two methoxy group proton resonances at δ3.81ppm and δ3.75ppm. The presence of a pair of doublets at δ6.67ppm and δ6.63ppm (*J*=<1Hz) each integrating to one hydrogen were indicative of protons *para* to each other and this implied a 1',3',4',6'-tetra-substituted ring B. The proton at C-3 is absent and this is typical of scillascillinoids which have the methylene protons of C-2 and C-9 each appearing as two sets of doublets. The former is centred at δ4.54ppm and the latter at δ3.25ppm. The signals at δ6.06ppm and δ6.04ppm with *J*=2.38Hz are indicative of the *meta*-coupled protons, H-6 and H-8 respectively. The mass spectrum showed a peak at *m/z* 162 which was indicative of one hydroxyl and one methoxyl B-ring substituent. Since the B-ring is tetra-substituted with two protons *para* to each other, the methoxy group had to be at either the C-3' or C-4' position.

A NOESY correlation could be seen between the H-2 protons and the signal at δ6.63ppm. This confirmed the signal at δ6.63ppm to be H-5'. This implied that the two proton signals at δ6.63ppm and δ6.67ppm belong to the *para*-protons of H-5' and H-2' respectively. The methoxy group proton resonance at δ3.75ppm showed a NOESY correlation with H-5' at δ6.63ppm. This confirmed the presence of this methoxy group at C-4' and hence C-3' was occupied by the hydroxy group. Furthermore, a COSY correlation could also be seen between the H-9 protons and the signal at δ6.67ppm. This is typical of benzylic coupling. This confirmed that the signal at δ6.67ppm belonged to H-2'. Irradiation of the second methoxy group at δ3.81ppm gave a positive correlation with the H-8 and H-6 protons. This was evidence of the presence of the methoxy group at the C-7 position. This is further supported by the proton shifts of H-6 and H-8 downfield of 6.00ppm. When these shifts occur upfield of 6.00ppm, the presence of a hydroxy group at C-7 is implied.[5]

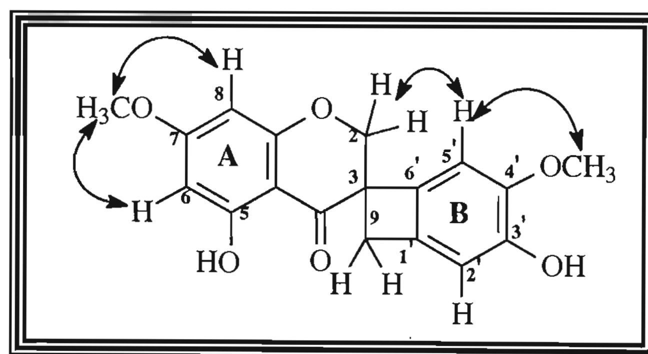


FIG. 3.3 : Diagram showing NOESY signals

The assignments of the ^{13}C NMR chemical shifts was based on comparisons with literature data.[8] Due to the absence of a proton at C-3, the C-3 carbon chemical shift appeared as a fully-substituted carbon atom resonance at δ54.4ppm. The presence of the carbonyl peak of C-4 at δ198.0ppm also confirmed the presence of a hydroxy group at C-5. A hydroxy group at C-5 imparts a deshielding effect on the carbonyl group and this results in the downfield shift of C-4 to close to 200.0ppm.[5] On the other hand, a methoxy group at C-5 would result in absence of the chelation between the carbonyl and the hydroxyl and this would impart an upfield shift to approximately δ193ppm.

The COSY spectrum of compound 6 confirmed the substitution pattern by showing correlations between the methoxy group proton resonance at δ3.81ppm (assigned to methoxy group at C-7) and the H-6 and H-8 protons. The H-9 protons gave a benzylic correlation with H-2'.

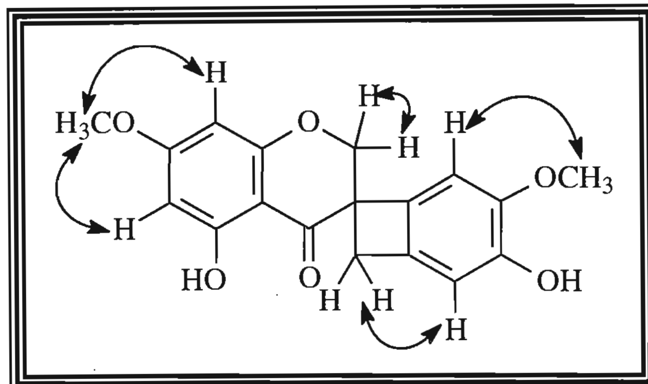


FIG. 3.4 : Diagram showing COSY correlations

The UV spectrum revealed a bathochromic shift (+17) with $AlCl_3$ and this was further evidence of the existence of a hydroxy group at C-5. No bathochromic shift was observed with NaOAc confirming that a methoxy group occurred at C-7. The stereochemistry at C-3 could not be ascertained.

Finally, compound 6 was compared to a compound reported in literature. [8] The ^{13}C and ^{1}H NMR data are tabulated in Tables 6.1 and 6.2 and this provided conclusive evidence that compound 6 was the known compound 3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one.

TABLE 6.1: Comparison of proton NMR data of compound 6 with compound A* from literature[8]

^{1}H	COMPOUND A*[8] (CD_3OD)	COMPOUND 6 (CD_3OD)
2H -2	4.54 , 4.56AB (*J*=9.0Hz)	4.53 , 4.54AB (*J*=12.2Hz)
H -6	6.08	6.06
H -8	6.05	6.05
2H -9	2.99d , 3.51d (*J*=13.2Hz)	2.99d , 3.51d (*J*=13.3Hz)
H -2'	6.68	6.67
H - 5'	6.65	6.63
OH (C-5)	12.09	OH
OH (C-3')	8.88	OH
OCH_3 (C-7)	3.83	3.81
OCH_3 (C-4')	3.76	3.75

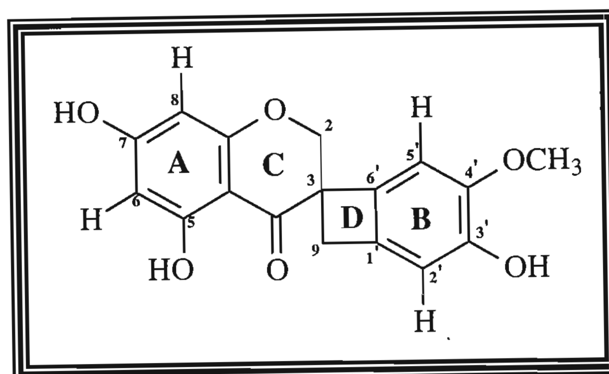
* = 3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

TABLE 6.2: Comparison of carbon NMR data of compound 6 with compound A* from literature[8]

^{13}C	COMPOUND A*[8] (CD_3OD)	COMPOUND 6 (CD_3OD)
2	74.4	74.6
3	54.8	55.0
4	197.7	198.0
4a	102.8	102.9
5	164.9	165.3
6	95.6	95.5
7	168.4	169.2
8	94.6	94.4
8a	164.0	164.5
9	35.9	35.5
1'	134.7	135.2
2'	111.7	111.7
3'	148.8	149.3
4'	148.8	149.2
5'	106.5	106.8
6'	134.7	135.3
7 -OCH_3	56.6	56.5
4' -OCH_3	56.1	55.9

* = 3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

3.3.2 Structural elucidation of COMPOUND 7



Compound 7 was found, from the mass spectrum, to have a molar mass of 314.07854g.mol^{-1}(calculated 314.07903g.mol^{-1}). This corresponded to a molecular formula of $C_{17}H_{14}O_6$. A double bond equivalence of 11 was deduced. The molecular formula implied the presence of a fourth ring in addition to rings A, B and C. This is typical of a scillascillinoid-type homoisoflavonoid.

The proton NMR spectrum of compound 7 was very similar to that of compound 6 except for the presence of just one methoxy group proton resonance. The pair of doublets at δ6.63ppm and δ6.66ppm (J=<1Hz) were indicative of *para* B-ring protons. The two singlets at δ5.90ppm and δ5.91ppm with J=2.20Hz were again indicative of a *meta*-substitution pattern in ring A. The methylene protons at C-2 and C-9 appeared as two AB quartets which was a further indication of a scillascillin type compound.

The substitution pattern on ring A was deduced using UV and NMR spectra. The UV absorption at 295nm was shifted upon addition of NaOAc (+32nm) and $AlCl_3$ (+17nm), indicating the presence of hydroxy groups at the C-5 and C-7 positions.[1,2] The presence of a hydroxy group at C-5 was further supported by the presence of the C-4 carbonyl peak at δ196.6ppm. The chelating effect between the hydroxy group and the carbonyl group causes the C-4 resonance to be deshielded. The presence of a hydroxy group at C-7 can be confirmed by the chemical shifts of the H-6 and H-8 protons which occur upfield of 6.00ppm.

The proton NMR spectrum also showed a 1',3',4',6'-tetra-substituted scillascillin B-ring pattern. To fulfil this, the protons must be at the C-2' and C-5' positions. The

presence of a fragment ion peak at *m/z* 162 is evidence of a hydroxy and methoxy substituent on the B-ring. It then remains for the hydroxy and the methoxy groups to be assigned at either the C-3' or C-4' position. The substitution pattern was deduced from a NOESY experiment. The NOESY experiment gave an enhancement of the H-2 protons upon irradiation of the signal at δ6.63ppm. The only proton close to the 2H-2 protons is that of C-5' and thus the signal at δ6.63ppm was deduced to be the resonance for H-5'. Upon irradiation of the methoxy group proton signal at δ3.75ppm, an enhancement was also observed for the singlet at δ6.63ppm. This provided evidence of the presence of the methoxy group at C-4'. Further, upon irradiation of the H-2' proton at δ6.66ppm, an enhancement of the H-9 signal was achieved. This provided conclusive evidence of the presence of the hydroxyl group at C-3' and the methoxy group at C-4' on the B-ring.

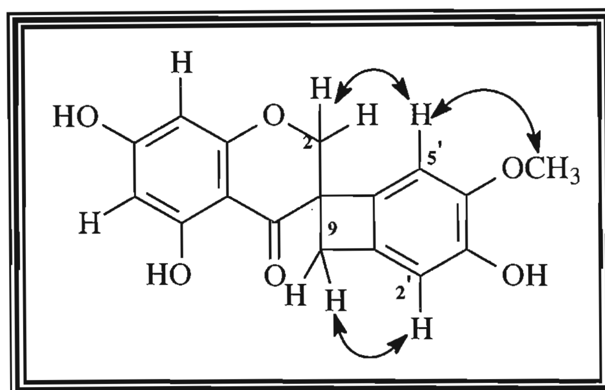
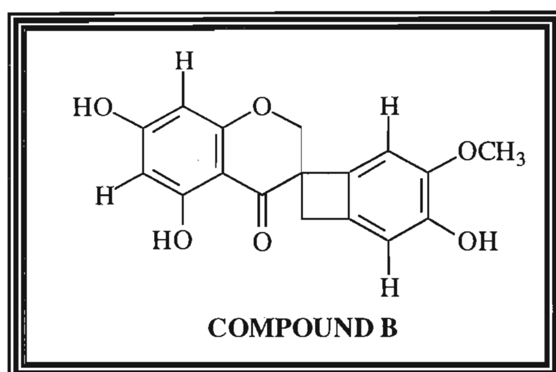


FIG. 3.5 : Diagram showing NOESY correlations

The HMBC spectrum was used to confirm the proposed structure. The HMBC carbon to hydrogen correlations are shown in Table 7.3.

The ^{1}H and ^{13}C NMR data for compound 7 were compared to literature values.[8] While one of the compounds in the literature, compound B, showed the methoxy group at the C-4' position, the other compound, muscomosin, had the methoxy at the C-3' position. Compound 7 was found to be in closest agreement with compound B that had the methoxy group at the C-4' position. Compound 7 was thus deduced to be the known 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one which was previously isolated from both *Muscari armeniacum* and *Muscari botryoides*.[8]



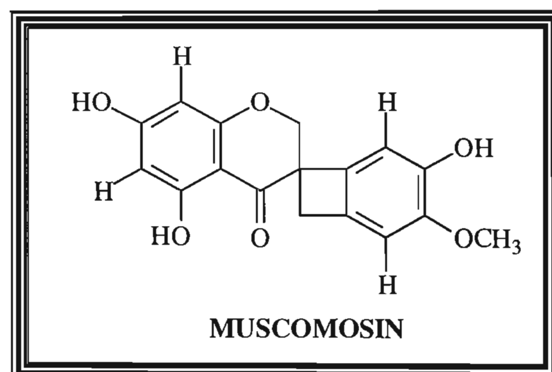


FIG. 3.6 : Structures of compound B and Muscomosin

TABLE 7.1 : Comparison of proton NMR data of compound 7 with compound B and Muscomosin from literature[8]

^{1}H	COMPOUND B*[8] (CD_3OD)	MUSCOMOSIN[8] (CD_3OD)	COMPOUND 7 (CD_3OD)
2H -2	4.51 , 4.53AB (J=9.0Hz)	4.50	4.51 , 4.53AB (J=11.3Hz)
H -6	5.93	5.91	5.90
H -8	5.90	5.89	5.90
2H -9	2.97 , 3.49AB (J=13.2Hz)	2.98 , 3.51AB (J=13.2Hz)	2.97 , 3.46AB (J=13.3Hz)
H -2'	6.68	6.82	6.66
H -5'	6.65	6.51	6.63
OH(C-5)	12.12	12.11	OH
OH(C-7)	10.82	8.50-9.30	OH
OH(C-3')	8.86	3.83	OH
OCH_3(C-4')	3.77	8.50-9.30	3.75

*= 3',5,7-trihydroxy-4'-methoxy-spiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

TABLE 7.2: Comparison of carbon NMR data of compound 7 with compound B and Muscomosin from literature[8]

^{13}C	COMPOUND B[8] (CD_3OD)	MUSCOMOSIN[8] (CD_3OD)	COMPOUND 7 (CD_3OD)
2	74.9	74.7	73.7
3	55.3	55.2	54.1
4	198.4	197.9	196.6
4a	102.6	102.4	101.3
5	165.9	165.8	164.6
6	97.3	97.3	96.0
7	168.4	168.5	167.1
8	96.0	96.0	94.8
8a	165.0	165.0	163.7
9	36.0	35.9	34.8
1'	135.7	134.1	134.2
2'	112.1	109.0	110.8
3'	149.6	150.8	148.2
4'	149.6	148.1	148.2
5'	107.3	110.1	105.8
6'	135.7	137.2	134.5
3'-OCH_3		56.7	
4'-OCH_3	56.9		55.6

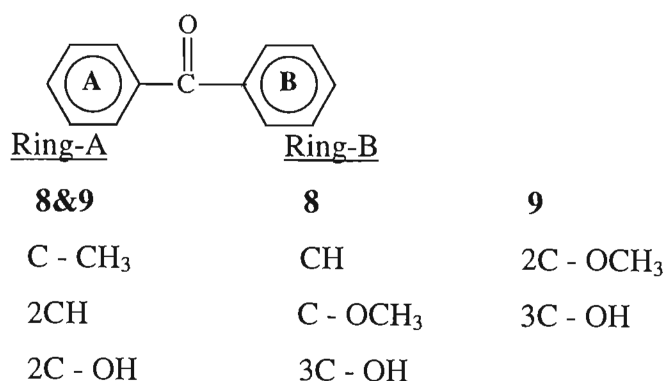
TABLE 7.3 : HMBC correlations (C→H) for compound 7

^{13}C	δ(ppm)	C→H
C - 4	196.6	H - 9α, H - 2
C - 3'	148.2	2H - 9
C - 4'	148.2	OCH_3
C - 6'	134.5	2H - 9, H - 2, H - 2'
C - 4a	101.3	H - 6, H - 8
C - 6	96.0	H - 8
C - 8	94.8	H - 6
C - 2	73.7	H -9α(weak),H- 9β(strong)
C - 3	54.1	2H - 9, 2H - 2, H - 2'
C - 9	34.8	H - 2, H - 2', H - 5'

3.3.3 Structural elucidation of COMPOUNDS 8 and 9

Compounds 8 and 9 were found to be two very closely related polyhydroxy/ methoxy methylbenzophenones. The one ring, ring A, was found to be the same in both compounds but they differed in that compound 8 had an unsubstituted position in ring-B, whereas ring-B in compound 9 was fully substituted with three hydroxy groups and two methoxy groups.

The ^{13}C NMR spectra of compound 8 showed 15 carbon atom resonances, thirteen for the benzophenone structure, one for an aromatic methyl group carbon and one for a methoxy group carbon. Using HMBC data, it was possible to separate the carbon resonances into those associated with ring-A and those associated with ring-B. The ^{13}C NMR data for ring-A was the same for compounds 8 and 9 indicating the same ring-A. HMBC data indicated that two of the carbons in ring-A were methine carbons (δ116ppm, δ100ppm) one carbon was attached to the methyl carbon (δ22.5ppm) placing the methyl group on ring-A. The carbonyl had to be attached to the ring at the carbon atom resonating at δ100.2ppm, leaving two further carbons (δ163ppm, δ159ppm) at which hydroxy groups were placed. Use of COSY and HMBC spectra of compound 8 to assign the structure of ring-A was made difficult by the fact that the two proton resonances were superimposed, in compound 8, so spectra from both compounds 8 and 9 had to be used together to propose a tentative structure.
In compound 8, ring-B had a methine carbon, the carbon atom to which the keto group was attached and a methoxy group attached to it, leaving three positions to be occupied by hydroxy groups. Ring B of compound 9 had no methine carbon, two methoxy, three hydroxy and the carbonyl carbon atom attached to the ring. This may be summarized in the diagram below :



8&9	**8**	**9**
C - CH_3	CH	2C - OCH_3
2CH	C - OCH_3	3C - OH
2C - OH	3C - OH	

The presence of so few hydrogen atoms on the aromatic rings made the assignment of a structure to these two compounds impossible using the ^{1}H, ^{13}C, COSY, NOESY and HMBC NMR spectra which were available. A structure for ring-A can be proposed, but more plant material has to be obtained when available to isolate more of these compounds in order to prepare derivatives and obtain enough material to run INADEQUATE NMR spectra. This technique uses $^{13}C/^{13}C$ coupling, and as ^{13}C is present in very low natural abundance, larger samples or very long experiment times are required to obtain a spectrum.

The structure of ring-A could be determined in the following manner. The coupling constant of J=2.2Hz indicates *meta*-coupling between protons on the A-ring. There is a COSY and NOESY correlation between the methyl group proton resonance at δ2.76ppm and the proton resonating at δ6.59ppm, but not between the methyl group resonance and the proton resonating at δ6.66ppm. Thus the following partial structure may be proposed :

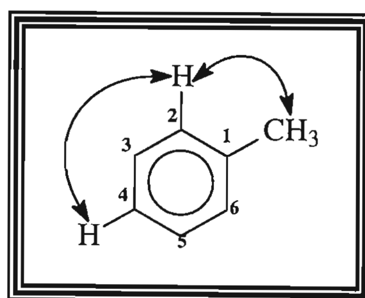


FIG. 3.7 : Partial structure for ring-A

If the carbonyl carbon was placed at positions 3 or 5, one would expect a ^{3}J correlation in the HMBC spectra between the resonance at δ182.5ppm and either H-2 or H-4. This is not observed. Thus the carbonyl carbon atom is attached to C-6, and hydroxy groups are placed at C-3 and C-5 to give the following partial structure :

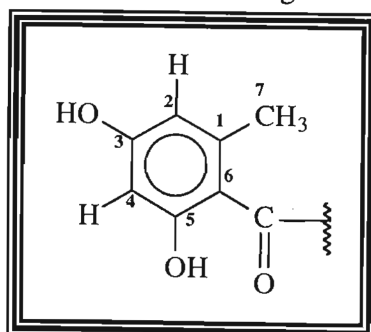


FIG. 3.8 : Structure of ring-A

Using the HMBC spectra, and by comparison with standard literature values for substituents on aromatic rings, the resonances for ring-A could be assigned as in Tables 8.1 and 9.1.

The structure of ring-B in compounds 8 and 9 differed so they will be discussed separately. Ring-B of compound 8 has one aromatic proton. An HMBC correlation between the carbonyl carbon atom resonance at δ182.5ppm and this proton (δ6.29ppm) suggested placement of the proton in the *ortho*-position to give a ^{3}J correlation. (Note no ^{4}J correlation was seen between the carbonyl carbon resonance and protons in ring A).

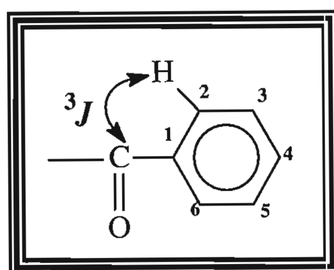


FIG. 3.9 : Structure showing ^{3}J correlation in ring-B

HMBC correlations were seen between four carbons in the aromatic ring and this proton. These resonances occurred at δ157.4ppm, δ152.6ppm, δ130.4ppm and δ103.0ppm. By comparison with literature data,[12] the first two resonances were assigned to carbons to which hydroxy groups are attached and the resonances at δ103.0ppm is assigned C-1 on the above structure. The resonance at δ130.4ppm is assigned to a carbon atom to which a methoxy group is attached as it gives a HMBC correlation to the methoxy group proton resonance at δ3.75ppm. There is no nOe correlation between the aromatic proton and the methoxy group protons, thus the methoxy group is not *ortho* to the aromatic proton. Thus a hydroxy group was placed at C-3, *ortho* to the aromatic proton. As there was no HMBC correlation between the resonance at δ154.5ppm and the aromatic proton, this resonance was assigned to C-5 and a hydroxy group was placed at this position. This gives the part structure for ring-B :

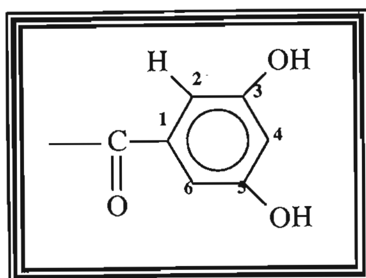


FIG. 3.10 : Partial structure of ring-B

Thus the methoxy group would have to be placed at either C-4 or C-6. A model shows that if the methoxy group was placed at C-6, nOe correlations might be expected between the methyl group from ring-A and the methoxy group from ring-B. This is not the case. Thus the methoxy group is tentatively placed at C-4 and the tentatively suggested structure for compound 8, showing HMBC correlations in ring-B, is given below :

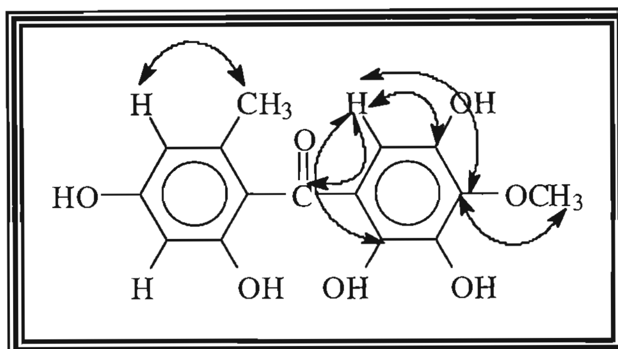


FIG. 3.11 : HMBC correlations for compound 8

The structure of ring-B in compound 9 is far more difficult to determine as there is no aromatic proton present and the only HMBC correlations seen are between the two methoxy group protons and the aromatic ring carbon atoms to which they are attached. There is no nOe correlation between the two methoxy groups, suggesting they are not *ortho* to each other. Due to the small quantity of sample isolated, an acetylation reaction could not be carried out on compound 9.

The possibility of these compounds being xanthones was investigated. If water was lost between the hydroxy groups on the two rings, compound 8 would be the following xanthone :

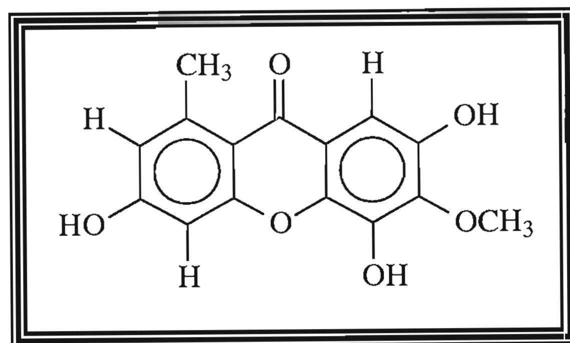


FIG. 3.12 : Proposed xanthone structure of compound 8

The IR carbonyl stretch of a benzophenone occurs at approximately 1700cm^{-1} and this is the same for that of a xanthone.[10] Therefore, it is also difficult to confirm either structure from IR data.

In the first proposed structure, acetylation would be expected to give the penta-acetate, but if the compound was the xanthone, the tri-acetate was expected. Compound 8 was acetylated and, although the product was not obtained pure, and was obtained in too small a yield for purification, the proton NMR spectrum appeared to have more than three acetate group methyl proton resonances (Spectrum 8.1B). This favours a benzophenone-type structure. The HRMS of compound 8 gave a molecular formula of 328g.mol^{-1}. The HRMS of compound 9 gave a molar mass of 318 g.mol^{-1}. This is the molar mass required for the xanthone structure, however, the loss of water in obtaining a mass spectrum cannot be ruled out.

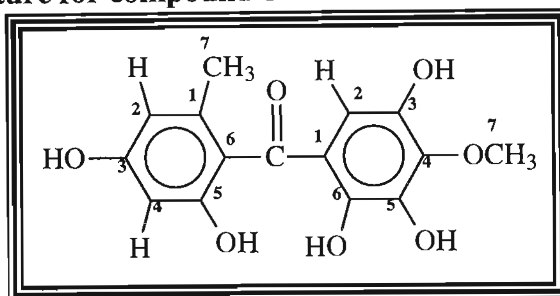
Further work on these two compounds will be undertaken as more plant material becomes available as these compounds seem to be novel and compounds of this type have not been reported previously from the Hyacinthaceae family.

TABLE 8.1 : ^{1}H, ^{13}C, COSY, NOESY and HMBC data for compound 8

Carbon	^{13}C (ppm)	^{1}H (ppm)	COSY	HMBC C→H	NOESY
1	162.9	-	-	6.57	-
2	159.6	-	-	6.57	-
3	143.4	-	-	2.75	-
4	115.8	6.57	-	6.57 + 2.75	-
5	111.3	-	-	6.57 + 2.75	-
6	100.2	6.57	-	-	-
7	22.5	2.75	6.57	6.57	6.57
Ketone 8	182.5	-	-	6.29	-
9	157.4	-	-	6.29	-
10	154.5	-	-	-	-
11	152.6	-	-	6.29	-
12	130.4	-	-	6.29 + 3.75	-
13	103.0	-	-	6.29	-
14	93.0	6.29	-	-	-
15	59.7	3.75	-	-	-
16	-		-	-	-

*Note : chemical shifts are listed from largest to smallest for each of the rings and do not correspond to a structure

TABLE 8.2 : Table of ^{1}H and ^{13}C NMR data assigned according to a numbered structure for compound 8



Ring-A Carbon Nos.	^{1}H (ppm)	^{13}C (ppm)
C-1		143.4
C-2	6.57 s	115.8 (CH)
C-3		162.9
C-4	6.57 s	100.2 (CH)
C-5		159.6
C-6		111.3
C-7	2.75 s	22.5 (CH_3)
Ketone C-8		182.5 (C=O)
Ring-B Carbon Nos.		
C-1		103.0
C-2	6.29 s	93.0 (CH)
C-3		152.6
C-4		130.4
C-5		154.5
C-6		157.4
C-7	3.75 s	59.7 (OCH_3)

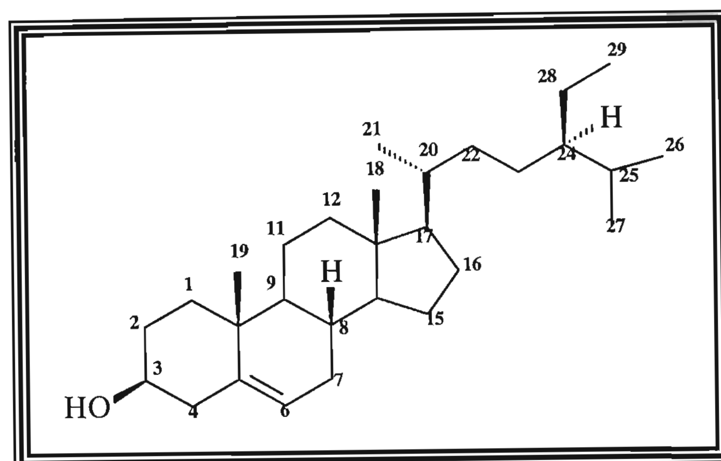
TABLE 9.1 : ^{1}H, ^{13}C, COSY, NOESY and HMBC data for compound 9

Carbon	^{13}C (ppm)	^{1}H (ppm)	COSY	HMBC C→H	NOESY
1	163.0	-	-	6.66	-
2	159.4	-	-	6.66	-
3	143.5	-	-	2.76	-
4	116.0	6.59 (*J*=1.5Hz)	6.66 + 2.76	6.66 + 2.76	-
5	111.3	-	-	6.59, 6.66, 2.76	-
6	100.3	6.66 (*J*=2.2Hz)	6.59	6.59	-
7	22.5	2.76	6.59	6.59	6.59
Ketone 8	182.5	-	-	-	-
9	151.1	-	-	-	-
10	150.4	-	-	-	-
11	145.2	-	-	-	-
12	130.5	-	-	-	-
13	127.1	-	-	-	-
14	102.0	-	-	-	-
15	60.7	3.85	-	-	-
16	59.9	3.89	-	-	-

*Note 1: chemical shifts are listed from largest to smallest for each of the rings and do not correspond to a structure.

Note 2: Ring-A is the same in both compounds 8 and 9, however the B-ring for compound 9 was difficult to assign due to the absence of aromatic protons.

3.3.5 Structural elucidation of COMPOUND 10



This compound is a common triterpenoid and although it was isolated from *Drimiopsis maculata*, it is found in most other plant specimens as well; it occurs widely in the plant kingdom.

High resolution mass spectrometry showed a molecular ion peak at *m/z* 414.3848 (calculated 414.3861g.mol^{-1}) which was consistent with a molecular formula of $C_{29}H_{50}O$. A peak at *m/z* 396 was due to the loss of one H_2O molecule and this indicated the possible presence of one hydroxy group.
An infrared spectrum showed absorption peaks at 3446cm^{-1} (O-H stretching), 2940 and 2849cm^{-1} (saturated C-H stretching), 1474cm^{-1} (C-H deformation), 1375cm^{-1} (CH_3 symmetrical deformation), 1242 and 1046cm^{-1} (C-O stretching), 758cm^{-1} (C-H out-of-plane deformations).
Comparison of the ^{1}H NMR spectrum of compound 10 with that of library spectra suggested that this compound was β-sitosterol.
The ^{13}C NMR and ADEPT spectra showed a methine carbon resonance occurring at δ71.8ppm and this was typical for a carbon atom attached to an oxygen. This was therefore assigned to C-3. The fully-substituted carbon resonance at δ141.0ppm and the methine carbon resonance at δ121.9ppm indicated the presence of one trisubstituted double bond and these resonances were assigned to C-5 and C-6 respectively.
A multiplet at δ5.32ppm in the ^{1}H NMR spectrum was assigned to the H-6 proton. Another multiplet at δ3.50ppm could be ascribed to H-3α, with a hydroxyl group

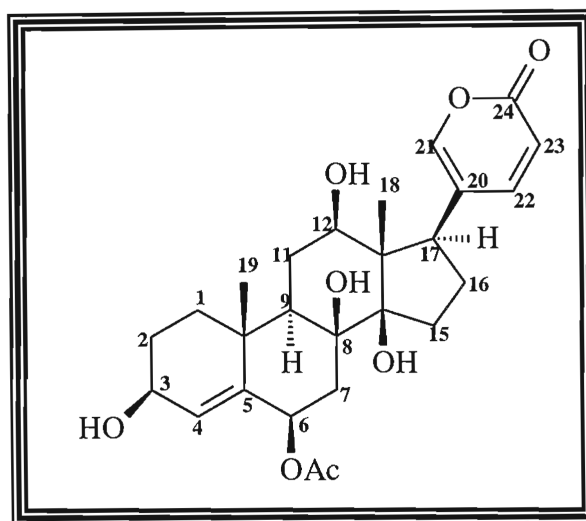
attached at C-3β. The $W_{1/2}$ of 16Hz for the H-3 resonance confirmed it to be α. Chemical shifts for the methyl group protons correlated with the literature values and were assigned as follows : δ0.66ppm (3H, s, H-18), δ0.78ppm (3H, d, *J*=7.1Hz, H-27), δ0.80ppm (3H, d, *J*=7.9Hz, H-26), δ0.82ppm (3H, t, *J*=7.2Hz, H-29), δ0.91ppm (3H, d, *J*=6.4Hz, H-21), δ0.99 (3H, s, H-19).

The physical properties of compound 10 were identical to those of an authentic sample of β-sitosterol.

3.4 EXTRACTIVES FROM *DRIMIA ROBUSTA* (Bak.)

The large, purple bulbs of *Drimia robusta* were purchased at the Warwick Triangle market in Durban, KZN, in June 1999. The bulbs were extracted with methylene chloride and methanol, however, only the methylene chloride extract was investigated. The bufadienolide isolated could be identified as a navy blue spot on the t.l.c plate. A proton NMR spectrum of the crude methanol extract showed an abundance of sugar in the sample.

3.4.1 Structural elucidation of COMPOUND 11



This compound belongs to a class of cardioactive compounds known as the bufadienolides. These compounds often have a sugar moiety attached to them (at C-3) but compound 11 was isolated without the sugar.

Use was made of the ^{1}H, ^{13}C, HSQC, HMBC, COSY, TOCSY and NOESY NMR spectra to determine the structure of the compound and to assign all ^{1}H and ^{13}C NMR resonances (Table 11.1). The presence of a bufadienolide side chain at C-17 was indicated by the coupled resonances at δ7.94ppm, δ7.41ppm and δ6.27ppm in the ^{1}H NMR spectrum ascribable to H-22, H-21 and H-23 respectively and δ123.8ppm, δ149.6ppm, δ148.1ppm, δ114.2ppm and δ163.5ppm ascribable to C-20, 21, 22, 23 and 24 respectively in the ^{13}C NMR spectrum.

Two secondary hydroxy groups and a secondary acetoxy group were indicated by resonances at δ4.10ppm, δ3.43ppm and δ5.43ppm and a three proton singlet

resonance at δ1.99ppm in the ^{1}H NMR spectrum and the corresponding methine resonances at δ67.1ppm, δ76.1ppm and δ76.1ppm in the ^{13}C NMR spectrum. The presence of two tertiary hydroxy groups was suggested by carbon resonances at δ75.7ppm and δ85.6ppm and a trisubstituted double bond was indicated by resonances at δ133.3ppm(CH) and δ140.7ppm(C). The alkene proton was present at δ5.73 in the ^{1}H NMR spectrum and this proton was coupled to the methine resonance at δ4.10ppm.

The TOCSY and HETCOR spectra indicated the presence of two further methylene groups in the same spin system as the alkene and methine proton resonances and these were assigned to 2H-1, 2H-2 while the hydroxy group was placed at C-3 and the vinylic proton at C-4. The acetoxy group was placed at C-6. The H-6 resonance at δ5.43ppm was seen to be coupled to 2H-7. The HETCOR spectrum showed ^{3}J correlation between C-6 and H-4. The tertiary hydroxy groups were placed at C-8 and C-14 as the TOCSY spectrum showed that H-17 was only coupled to 2H-16 and 2H-15 and that no coupling was present between 2H-7 and H-8. The COSY spectrum showed coupling between H-9(δ1.41ppm), 2H-11(δ1.34ppm and δ1.36ppm) and H-12(δ3.43ppm). Thus the remaining hydroxy group was placed at C-12.
The stereochemistry at C-8 and C-14 could not be determined from NMR spectra but a literature search showed that bufadienolides in which hydroxy groups are present at these positions have them in the β-orientation.[3]

The stereochemistry at C-3, C-6 and C-12 was determined from the NOESY spectrum. The resonance ascribed to H-9α gave a positive NOE correlation with H-17α and H-12α indicating that the hydroxy group was present at C-12β. The methyl group protons of the acetate group at C-6 showed a positive NOE with 3H-19 at C-10β, indicating that the acetate group was at C-6β. H-6α showed a positive NOE interaction with H-4 and H-4 showed a positive correlation with H-3α (J=1.6Hz). Thus the hydroxy group was placed at C-3β.
Compound 11 was deduced to be 6β-acetoxy-3β, 8β, 12β, 14β-tetrahydroxybufa-4, 20, 22-trienolide. This compound required a molecular formula of $C_{26}H_{34}O_8$ (calculated molar mass 474.22536g.mol^{-1}). However, the mass spectrum of

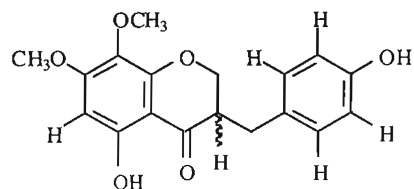
compound 11 gave a molar mass of 414.20568g.mol^{-1} corresponding to the molecular formula $C_{24}H_{30}O_6$. This implied the loss of an acetic acid molecule. The loss of acetic acid is common when acetate groups are present. Compound 11 was reported previously from *Urginea maritema* (Hyacinthaceae)[9], however, no spectral information was provided.

Confirmation of the presence of hydroxy groups at C-3 and C-12 was carried out by performing an acetylation reaction on compound 11. Spectrum 11.8 shows the proton NMR spectrum of the acetylated product. The presence of two extra acetate methyl proton peaks could be seen at δ2.10ppm and δ2.15ppm. The two acetate groups were deduced to be the products of the acetylation of the hydroxy groups at C-3 and C-12. This was further supported by the shifting of the proton resonances at δ4.10ppm and δ3.45ppm corresponding to H-3 and H-12 respectively downfield to δ5.30ppm and δ4.75ppm.

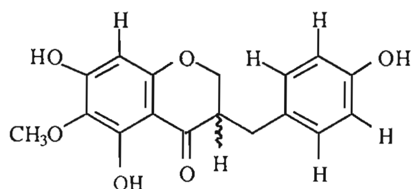
TABLE 11.1 : ^{1}H, ^{13}C, HMBC and NOESY NMR data for compound 11 (CD_3OD)

	^{1}H	^{13}C	HMBC C→H	NOESY
1	1.82, 1.32	37.9	19	
2	1.88, 1.60	27.9	4	3
3	4.10	67.1		2, 4
4	5.73 *s*	133.3		3, 6
5	-	140.7	7, 19	-
6	5.43 *br t*	76.1	4	4, 7
7	2.32, 1.63	37.6		6
8	-	75.7	6	-
9	1.41 *dd*	47.9	11, 19, 1, 7	12, 17
10	-	36.6	4, 19	-
11	1.92, 1.60	26.7	12	
12	3.43 *dd*	76.1	9, 11, 17, 18	9, 17
13	-	55.4	11, 12, 17, 18	-
14	-	85.6	15, 18	-
15	1.66	33.9		
16	2.10	29.1	17	17
17	3.04 *dd*	47.1	18	12, 16, 21
18	0.80 *s*	12.0	12	21, 22
19	1.34 *s*	21.7		
20	-	123.8	17, 21, 23	-
21	7.41(J=1.7Hz)	149.6	17, 22	17, 18
22	7.94(J=2.4Hz)	148.1	17, 21	16, 18, 23
23	6.27(J= 9.7Hz)	114.2		22
24	-	163.5	21, 22, 23	-
Acetate C=O	-	170.1	$OCOCH_3$	
Acetate CH_3	1.99 *s*	20.4		

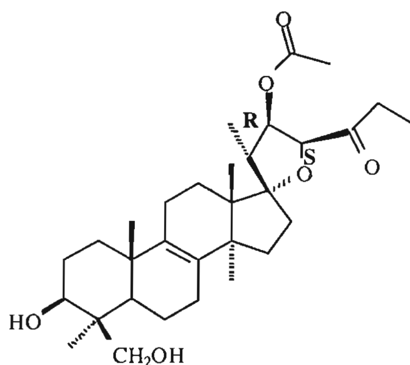
3.5 STRUCTURAL SUMMARY OF ISOLATED COMPOUNDS



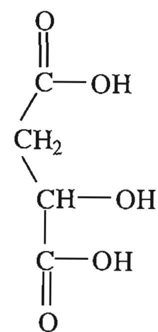
COMPOUND 1



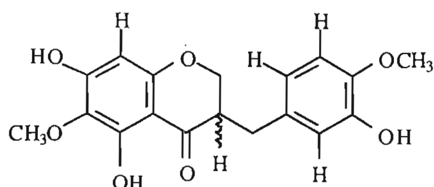
COMPOUND 2



COMPOUND 3



COMPOUND 4



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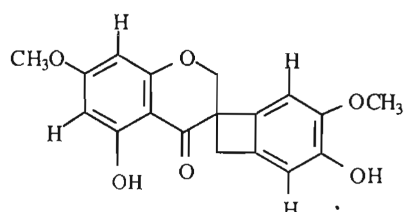
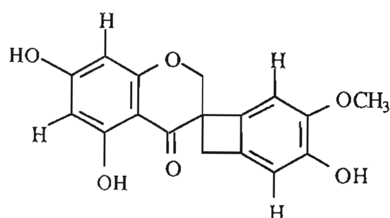
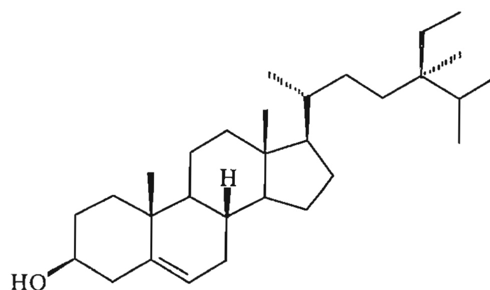


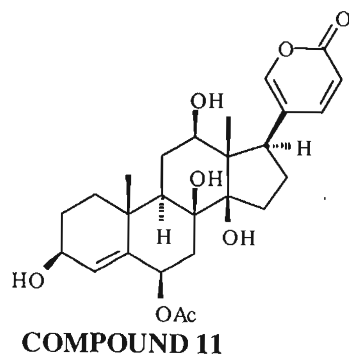
COMPOUND 6



COMPOUND 7



COMPOUND 10



COMPOUND 11

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CHAPTER 4 : EXPERIMENTAL

4.1 FOREWORD TO EXPERIMENTAL

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR SPECTROSCOPY)

Nuclear magnetic resonance spectroscopy was carried out on a 400MHz Varian UNITY-INOVA spectrophotometer. All spectra were recorded at room temperature in deuteriomethanol (CD_3OD) with the exception of compounds 4 and 10 which were run in deuterium oxide (D_2O) and deuteriochloroform ($CDCl_3$) respectively. The chemical shifts were all recorded in ppm relative to TMS.

For deuteriomethanol, the spectra were referenced according to the central line at δ_C=49.0ppm and δ_H=4.80 or δ_H=3.30ppm.

INFRARED SPECTROSCOPY (I.R SPECTROSCOPY)

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

ULTRAVIOLET ABSORPTION SPECTROMETRY/SPECTROSCOPY (U.V)

The ultra-violet absorption spectra were obtained on a Varian DMS 300 UV-visible spectrometer. The solvent in which the spectra were recorded was methylene chloride.

MELTING POINTS (Mp)

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 either methanol or chloroform using an Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (4x200mm) unjacketed flow tube.

MASS SPECTROMETRY (M.S)

High resolution mass spectra were recorded on a Kratos 9/50 HRMS instrument. The mass spectrometry was performed by Dr P. Boshoff at the Cape Technikon.

GENERAL CHROMATOGRAPHY

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

PREPARATIVE THIN LAYER CHROMATOGRAPHY (PTLC)

Compounds which were visible under UV light were isolated using this technique. The glass-backed t.l.c plates were lined with the extract sample 1cm from the bottom of the plate. The plates were lined by dipping a capillary tube in the extract sample and allowing it to run onto the silica-gel by touching it to the plate. The plates were then developed in a chromatography tank and the compound of interest was marked under UV light. The marked portion was then scraped off the plate, boiled in methanol and the solvent decanted.

ACETYLATION

Pyridine (1ml) and acetic anhydride (1ml) were added to the sample (15mg) in a round bottom flask. The sample was left to stand for 48 hours. MeOH (5ml) was then added to the sample to remove the excess acetic anhydride and toluene (4×10ml) was added successively to remove the pyridine. After each addition, the solvent was

evaporated off on the Rotavapor. Thereafter, MeOH (5×10ml) was added to remove the toluene. The sample was then spotted on a t.l.c plate see whether the reaction had gone to completion or needed to be separated from the starting material.

4.2 GENERAL PLANT EXTRACTION PROCEDURES

All the species in this study were collected by Dr Neil Crouch of the National Botanical Institute. In all instances, the part of the plant investigated was the bulbs. The bulbs were chopped into small pieces, air dried for approximately 48 hours and then extracted successively with dichloromethane and methanol by agitation on a Labcon Mechanical shaker at 140rpm. Extraction with each solvent was carried out for approximately 96 hours. The extracts obtained were then filtered and the solvent was evaporated using a BUCHI Rotavapor.

4.3 PHYSICAL DATA

4.3.1 Extractives from ***Ledebouria cooperi*** (Hook)

Crude plant material (1156.90g) was collected under voucher specimen number N.Crouch and T.Edwards 775. The masses of the methylene chloride and methanol extracts after evaporation were 2971.4mg and 5332.9mg respectively.

4.3.1.1 **Physical Data for compound 1**

Name : 7-O-methyl-3,9-dihydropunctatin

Yield : 4.5mg

Physical Description : yellow amorphous substance

$[\alpha]_D$ = too little material available, no lit. value available[1]

Mass spectrum : HRMS : $[M^+]$ at *m/z* 330.1104, $C_{18}H_{18}O_6$ requires 330.1103g.mol^{-1}
EIMS : *m/z* (rel. int.) : 315(3.49) 271(3.56), 224(31.72), 209(37.06), 197(7.92), 153(5.53), 133(5.00), 107(100.00), 57(21.17)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3423 (O-H stretching, H-bonded), 2937, 2846 (C-H aromatic and aliphatic stretching), 1641 (C=O stretching), 1460, 1381, 1314 (CH_2 and CH_3 bending), 1211, 1127 (C-O stretching)

UV Data: λ_{max} nm (log ε) : 290(6.58)

^1H and ^{13}C NMR data are presented in Tables 1.1 and 1.2 in Ch. 3

4.3.1.2 **Physical Data for compound 2**

Name : 5,7-dihydroxy-6-methoxy-3(4-hydroxybenzyl)chroman-4-one, (3,9-dihydroautumnalin)

Yield : 10.2mg

Physical Description : fine white crystals

Mp : 205-206°C, lit. value : 207-209°C[2,3]

$[\alpha]_D^{25°}$ = -8° (MeOH, *c*=0.062g/100ml) , lit. value : -10° (dioxane)[3]

Mass spectrum : HRMS : $[M^+]$ at *m/z* 316.09475, $C_{17}H_{16}O_6$ requires 316.09468g.mol^{-1}

EIMS : *m/z* (rel. int.) : 301(4.69), 287, 283, 210(46.51), 195(33.86), 183(8.61), 166(9.04), 133(6.22), 107(100.00), 77(9.70)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3352 (O-H stretching, H-bonded), 2939 (C-H aromatic and aliphatic stretching), 1639 (C=O stretching), 1452, 1303, 1237 (CH_2 and CH_3 bending), 1166, 1100 (C-O stretching)

UV Data : λ_{max} nm (log ε) : 315(8.76)

^1H and ^{13}C NMR data are presented in Tables 2.1 and 2.2 in Ch. 3

4.3.1.3 **Physical Data for compound 3**

Name : (22*R*,23*S*)-22-acetoxy-3β, 31-17,23-epoxy-dihydroxy-27-nor-5α-lanost-8-en-24-one, (15-deoxo-22*R*-acetoxyeucosterol)

Yield : 52.4mg

Physical Description : white crystalline solid

Mp : 178°C, lit. value : 176°C[4]

$[\alpha]_D^{25°}$ = -16.6° (MeOH, *c*=0.090g/100ml), lit. value : -17.0° (MeOH)[4]

Mass spectrum : HRMS : [M^+] at *m/z* 516.34619, $C_{31}H_{48}O_6$ requires 516.34509g.mol^{-1}

EIMS : *m/z* (rel. int.) : 501(67.87), 483(22.31), 456(25.67), 423(23.53), 405(18.77), 399(16.28), 329(47,42), 315(100.00), 289(62.88), 271(51.29), 185(33.57), 159(62.15), 119(70.72), 93(40.55)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3464 (O-H stretching, H-bonded), 2937 (C-H aromatic and aliphatic stretching), 1650 (C=O stretching), 1465, 1267 (CH_2 and CH_3 bending), 1124, 1034 (C-O stretching)

^{1}H and ^{13}C NMR data are presented in Tables 3.1 and 3.2 in Ch 3

4.3.1.4 **Physical Data for compound 4**

Name : Malic Acid
Yield : 26.6mg
Physical Description : white, well-formed, needle-like crystals
Mp : 102°C, lit. value : 100-101°C[7]

IR Data : ν_{max} [NaCl](cm^{-1}) : 3387 (O-H stretching, H-bonded), 2953 (C-H aliphatic stretching), 1650 (C=O stretching), 1367, 1314, 1235 (CH_2 bending), 1150 (C-O stretching)

^{1}H and ^{13}C NMR data are presented in Table 4.1 in Ch. 3

4.3.2 Extractives from ***Scilla plumbea*** (Lindl.)

Crude plant material (175.88g) was collected with voucher specimen number Crouch 771. The mass of the crude methanol extract was 8589.0mg.

4.3.2.1 **Physical Data for compound 5**

Name : 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one
Yield : 22.8mg

Physical Description : yellow vitreous solid

$[\alpha]_D^{25°}$ = -10° (MeOH, *c*=0.050g/100ml) , no lit. value available[5]

Mass spectrum : HRMS : $[M^+]$ at *m/z* 346.10595, $C_{18}H_{18}O_7$ requires 346.10520g.mol^{-1}

EIMS : *m/z* (rel. int.) : 331(2.86), 330(2.80), 210(1.85)], 209(2.58), 183(3.38), 150(3.84), 137(100.00), 122(4.08), 107(5.65)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3367 (O-H stretching, H-bonded), 2933, 2863 (C-H aromatic and aliphatic stretching), 1647 (C=O stretching), 1457, 1384 (CH_2 and CH_3 bending), 1168, 1278 (C-O stretching)

UV Data : λ_{max} nm (log ε) : 295(3.92)

^{1}H and ^{13}C NMR data are presented in Tables 5.1 and 5.2 in Ch. 3

4.3.3 Extractives from ***Drimiopsis maculata*** (Lindl.)

The crude plant material (737.56g) was harvested in the Kloof area, KZN, with voucher specimen number Crouch 790. The mass of the methylene chloride extract was 1037.4mg.

4.3.3.1 **Physical Data for compound 6**

Name : 3',5-dihydroxy-4',7-dimethoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

Yield : 69.2mg

Physical Description : off-white, well formed crystals

Mp : 185-187°C , lit. value : 187-188°C[5]

$[\alpha]_D^{25°}$ = +8.7° (MeOH, *c*=0.086g/100ml), lit. value : +9° (MeOH)[5]

Mass spectrum : HRMS : $[M^+]$ at *m/z* 328.09442, $C_{18}H_{16}O_6$ requires 328.09468g.mol^{-1}

EIMS : *m/z* (rel. int.) : 328(100.00)[M+], 313(4.20), 299(4.39), 285(2.63), 167(9.60), 150(5.45)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3330 (O-H stretching, H-bonded), 2921, 2965 (C-H aromatic and aliphatic stretching), 1625 (C=O stretching),1445, 1483,1293 (CH_2 and CH_3 bending), 1161, 1210 (C-O stretching)

UV Data : λ_{max} nm (log ε) : 295(4.40)

^1H and ^{13}C NMR data are found in Tables 6.1 and 6.2 in Ch. 3

4.3.3.2 **Physical Data for compound 7**

Name : 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H), 7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

Yield : 197.7mg

Physical Description : creamy-coloured, powder-like solid

Mp : 210-212°C, lit. value : 209-211°C[5]

$[\alpha]_D^{25°}$ = +12.1° (MeOH, *c*=0.082g/100ml), lit. value : +11° (MeOH)[5]

Mass spectrum : HRMS : [M^+] at *m/z* 314.07854, $C_{17}H_{14}O_6$ requires 314.07903g.mol^{-1}
EIMS : *m/z* (rel. int.) : 314(100.00)[M+], 285(4.21), 195(6.58), 162(53.77), 153(83.05), 105(15.82), 91(15.67), 71(23.10)

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3356 (O-H stretching, H-bonded), 2927 (C-H aromatic and aliphatic stretches), 1636 (C=O stretching), 1331, 1269, 1207 (CH_2 and CH_3 bending), 1166, 1088 (C-O stretching)

UV Data : λ_{max} nm (log ε) : 295(4.93)

^1H and ^{13}C NMR data are presented in Tables 7.1 and 7.2 in Ch 3

4.3.3.3 **Physical Data for compound 8**

Name : benzophenone or xanthone-type compound

Yield : 21.5mg

Physical Description : fine, white crystals

[a]$_D$: unable to obtain

Mass spectrum : HRMS : [M^+] at *m/z* 328, $C_{15}H_{12}O_6$ requires 288g.mol^{-1}

EIMS : *m/z* (rel. int.) : 306.9 (15.08), 288.9 (31.08), 273 (10.79), 154 (100.00), 136 (85.56), 107 (32.44)

I.R Data : ν_{max} [NaCl](cm^{-1}) : 3387 (O-H stretching, H-bonded), 2918, 2848 (C-H aromatic and aliphatic stretching), 1622 (C=O stretching), 1467, 1273 (CH_2 and CH_3 bending), 1158 (C-O stretching)

UV Data : unable to obtain

^1H and ^{13}C NMR data are presented in Table 8.1 in Ch. 3

4.3.3.4 **Physical Data for compound 9**

Name : benzophenone or xanthone-type compound

Yield : 3.7mg

Physical Description : fine, yellow crystals

[a]$_D$ = unable to obtain

Mass spectrum : HRMS : [M^+] at *m/z* 318, $C_{16}H_{14}O_6$ requires 302g.mol^{-1}

EIMS : *m/z* (rel. int.) : 318 (100.0), 287 (4.0), 248 (5.1), 210.9 (5.2), 130.1 (4.0)

I.R Data : ν_{max} [NaCl](cm^{-1}) : 3404 (O-H stretching, H-bonded), 2931, 2858 (C-H aromatic and aliphatic stretching), 1633 (C=O stretching), 1463, 1324 (CH_2 and CH_3 bending), 1185 and 1052 (C-O stretching)

UV Data : unable to obtain

^{1}H and ^{13}C NMR data are presented in Table 9.1in Ch 3

4.3.3.5 **Physical Data for compound 10**

Name : β-sitosterol

Yield : 37.3mg

Physical Description : off-white, powdery crystals

Mp : 137-140°C

$[a]_D^{25°}$ = -37° ($CHCl_3$)

I.R Data : ν_{max} [NaCl](cm^{-1}) : 3446 (O-H stretching), 2940,2849 (saturated C-H stretching), 1474,1375 (CH_2 and CH_3 bending), 1242, 1046 (C-O stretching)

4.3.4 Extractives from ***Drimia robusta*** (Bak.)

The crude plant material was obtained from the Warwick Triangle area, KZN. 3477.97g of crude material was purchased under voucher specimen number Crouch 793. The mass of the methylene chloride extract was 1675.4mg.

4.3.4.1 Physical Data for compound 11

Name : 6β-acetoxy-3β, 8β, 12β,14β-tetrahydroxybufa-4, 20, 22-trienolide

Yield : 80.1mg

Physical Description : cream-coloured, powdery solid

Mp : 169-171°C , no lit. value available[6]

$[\alpha]_D^{25°}$ = +8° (MeOH, *c*=0.092g/100ml) , no lit. value available[6]

Mass spectrum : HRMS : [M^+] at *m/z* 414.20568, $C_{24}H_{30}O_6$ requires 414.20424g.mol^{-1}

EIMS : *m/z* (rel. int.) : 414(100.00)[M^+], 396(42.49), 381(10.71), 378(11.45), 363(7.64), 279(9.13), 261(12.76), 223(17.35), 193(29.70), 177(34.45), 136(59.47), 121(37.15), 79

I.R Data : ν_{max}[NaCl](cm^{-1}) : 3450 (O-H stretching, H-bonded), 2948, 2876 (C-H

aromatic and aliphatic stretches), 1711 (C=O stretch), 1379, 1252 (CH_2 and CH_3 bending), 1136, 1037 (C-O stretching)

UV Data : λ_{max} nm (log ε) : 300(3.00)

^{1}H and ^{13}C NMR data are presented in Table 11.1 in Ch. 3

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CONCLUSION

The findings contained in this work have proved to be particularly interesting since the taxonomy of this family is currently under review. This investigation of the Hyacinthaeae family has provided compounds which are predominantly homoisoflavonoids. This was not surprising given the well-documented occurrence of these compounds in this family.[1-12] Of particular interest though was the isolation of two uncommon type homoisoflavonoids *viz.* the scillascillin types and the bufadienolide isolated from *Drimia robusta.*

Ledebouria cooperi yielded two homoisoflavonoids of the 3-benzyl-4-chromanone type and one eucosterol-type triterpenoid. The isolation of this type of triterpenoid is not a very common occurrence within the Hyacinthaceae family and this compound has only once been previously isolated from *Veltheimia viridifolia.*[13] These spirocyclic nortriterpenes, as they are known, are most often isolated with a pentaglycoside attached at the C-3 position. A number of homoisoflavonoids have also been reported from *Veltheimia ,viridifolia*[14] and this serves to clarify its chemotaxonomical classification within the Hyacinthaceae family. Of the two homoisoflavonoids isolated from *Ledebouria cooperi*, one was of the autumnalin-type and the other was a punctatin-type homoisoflavonoid. Each differed only by the position of one methoxy substituent. Malic acid was also isolated from *Ledebouria cooperi.*

Scilla plumbea yielded just one homoisoflavonoid, having two substituents on the B-ring. The investigation of this plant was undertaken as a comparative study against all the other *Scilla* species investigated. It was thought that this species of *Scilla* would be particularly interesting since it was the only species growing outside Kwa-Zulu Natal. The bulbs of *Scilla plumbea* were harvested in the Western Cape. The isolation of just one homoisoflavonoid proved to be insufficient evidence to draw any conclusions about the influence of different geographical locations on the growth and activity of similar plant species. The homoisoflavonoid isolated, 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one, was also reported previously from *Scilla natalensis.*[15] The 5,7-dioxy substitution pattern is also a notable feature of all homoisoflavonoids isolated within this genus.

Drimiopsis maculata proved to be a rich source of homoisoflavonoids as well as some other compounds. The two scillascillin-type homoisoflavonoids were isolated from this plant genus. These compounds are different to the 3-benzyl-4-chromanone types in that they lack the single proton resonance at C-3 due to the presence of a cyclobutane ring. The occurrence of these scillascillin-type compounds is widespread in the genus *Muscari* and the two isolated in this work were previously reported from *Muscari armeniacum* and *Muscari botryoides*.[4] Two further aromatic compounds were isolated from *Drimiopsis maculata*. These appeared to have simple proton NMR spectra but the lack of proton signals made the assignment of a structure difficult. The compounds consisted of two aromatic rings joined by an open or closed six-membered ring. From the lack of proton signals it was inferred that the aromatic rings were almost fully hydroxylated. Further investigation of these compounds will be undertaken when more plant material becomes available.

The methylene chloride extract of *Drimia robusta* yielded one bufadienolide. This compound belongs to the class of cardioactive glycosides. The isolation of this compound has confirmed the chemotaxonomical similarities between the genus *Drimia* and *Urginea*. Although the isolation of this bufadienolide has been previously reported from *Urginea maritima*,[16,17] no spectral data has been reported previously. Structural elucidation of this compound and complete assignment of the NMR spectra was carried out by 2-D NMR techniques such as TOCSY, HSQC, HMBC and NOESY.

Finally, the field of natural products research is proving to be a dynamic one. There is a constant quest to find new or better drugs for the diseases that plague man. Southern Africa has well over 30 000 species of higher plants and the Cape Floral Kingdom has nearly 9 000 species making it the most diverse temperate flora on earth. With South Africa's remarkable biodiversity and cultural diversity, it is not surprising to find that approximately 3 000 species of plants are used as medicines, and of these, approximately 350 species are the most commonly used and traded medicinal plants. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world today.[18]

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APPENDIX

LIST OF SPECTRA

SPECTRUM 1.1 : ^{1}H NMR spectrum of compound 1 (CD_3OD)
SPECTRUM 1.2 : ^{13}C NMR spectrum of compound 1 (CD_3OD)
SPECTRUM 1.3 : COSY spectrum of compound 1 (CD_3OD)
SPECTRUM 1.4 : NOESY spectrum of compound 1 (CD_3OD)
SPECTRUM 1.5A : HMBC spectrum of compound 1 (CD_3OD)
SPECTRUM 1.5B: expanded HMBC spectrum of compound 1 (CD_3OD)
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SPECTRUM 1.7 : Mass spectrum of compound 1
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SPECTRUM 4.4 : Mass spectrum of compound 4
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SPECTRUM 4.6A : ^{1}H NMR spectrum from literature
SPECTRUM 4.6B : Infrared spectrum from literature

SPECTRUM 5.1 : ^{1}H NMR spectrum of compound 5 (CD_3OD)
SPECTRUM 5.2 : ^{13}C NMR spectrum of compound 5 (CD_3OD)
SPECTRUM 5.3 : NOESY spectrum of compound 5 (CD_3OD)
SPECTRUM 5.4A : NOE spectrum of compound 5 (CD_3OD)
SPECTRUM 5.4B : NOE spectrum of compound 5 (CD_3OD)
SPECTRUM 5.5 : Mass spectrum of compound 5
SPECTRUM 5.6A : UV spectrum of compound 5 (original and with $AlCl_3$)
SPECTRUM 5.6B : UV spectrum of compound 5 (original and with NaOAc)
SPECTRUM 5.7 : Infrared spectrum of compound 5

SPECTRUM 6.1 : ^{1}H NMR spectrum of compound 6 (CD_3OD)
SPECTRUM 6.2 : ^{13}C NMR spectrum of compound 6 (CD_3OD)
SPECTRUM 6.3 : ADEPT spectrum of compound 6 (CD_3OD)
SPECTRUM 6.4 : COSY spectrum of compound 6 (CD_3OD)
SPECTRUM 6.5 : NOESY spectrum of compound 6 (CD_3OD)
SPECTRUM 6.6 : Mass spectrum of compound 6
SPECTRUM 6.7A : UV spectrum of compound 6 (original and with $AlCl_3$)
SPECTRUM 6.7B : UV spectrum of compound 6 (original and with NaOAc)
SPECTRUM 6.8 : Infrared spectrum of compound 6

SPECTRUM 7.1 : ^{1}H NMR spectrum of compound 7 (CD_3OD)
SPECTRUM 7.2 : ^{13}C NMR spectrum of compound 7 (CD_3OD)
SPECTRUM 7.3 : ADEPT spectrum of compound 7 (CD_3OD)
SPECTRUM 7.4 : COSY spectrum of compound 7 (CD_3OD)

SPECTRUM 7.5 : NOESY spectrum of compound 7 (CD_3OD)
SPECTRUM 7.6 : HMBC spectrum of compound 7 (CD_3OD)
SPECTRUM 7.7 : Mass spectrum of compound 7
SPECTRUM 7.8A : UV spectrum of compound 7 (original and with $AlCl_3$)
SPECTRUM 7.8B : UV spectrum of compound 7 (original and with NaOAc)
SPECTRUM 7.9 : Infrared spectrum of compound 7

SPECTRUM 8.1A : ^{1}H NMR spectrum of compound 8 (CD_3OD)
SPECTRUM 8.1B : ^{1}H NMR spectrum of acetylated compound 8 (CD_3OD)
SPECTRUM 8.2 : ^{13}C NMR spectrum of compound 8 (CD_3OD)
SPECTRUM 8.3 : COSY spectrum of compound 8 (CD_3OD)
SPECTRUM 8.4 : NOESY spectrum of compound 8 (CD_3OD)
SPECTRUM 8.5 : HMBC spectrum of compound 8 (CD_3OD)
SPECTRUM 8.6 : Mass spectrum of compound 8
SPECTRUM 8.7 : Infrared spectrum of compound 8

SPECTRUM 9.1 : ^{1}H NMR spectrum of compound 9 (CD_3OD)
SPECTRUM 9.2 : ^{13}C NMR spectrum of compound 9 (CD_3OD)
SPECTRUM 9.3 : COSY spectrum of compound 9 (CD_3OD)
SPECTRUM 9.4 : HETCOR spectrum of compound 9 (CD_3OD)
SPECTRUM 9.5 : NOESY spectrum of compound 9 (CD_3OD)
SPECTRUM 9.6 : HMBC spectrum of compound 9 (CD_3OD)
SPECTRUM 9.7 : Mass spectrum of compound 9
SPECTRUM 9.8A : UV spectrum of compound 9 (original and with $AlCl_3$)
SPECTRUM 9.8B : UV spectrum of compound 9 (original and with NaOAc)
SPECTRUM 9.9 : Infrared spectrum of compound 9

SPECTRUM 10.1 : ^{1}H NMR spectrum of compound 10
SPECTRUM 10.2 : ^{13}C NMR spectrum of compound 10
SPECTRUM 10.3 : Infrared spectrum of compound 10

SPECTRUM 11.1A : ^{1}H NMR spectrum of compound 11 (CD_3OD)
SPECTRUM 11.1B : ^{1}H NMR spectrum of acetylated compound 11 (CD_3OD)

SPECTRUM 11.2 : ^{13}C NMR spectrum of compound 11 (CD_3OD)

SPECTRUM 11.3 : ADEPT spectrum of compound 11 (CD_3OD)

SPECTRUM 11.4 : HETCOR spectrum of compound 11 (CD_3OD)

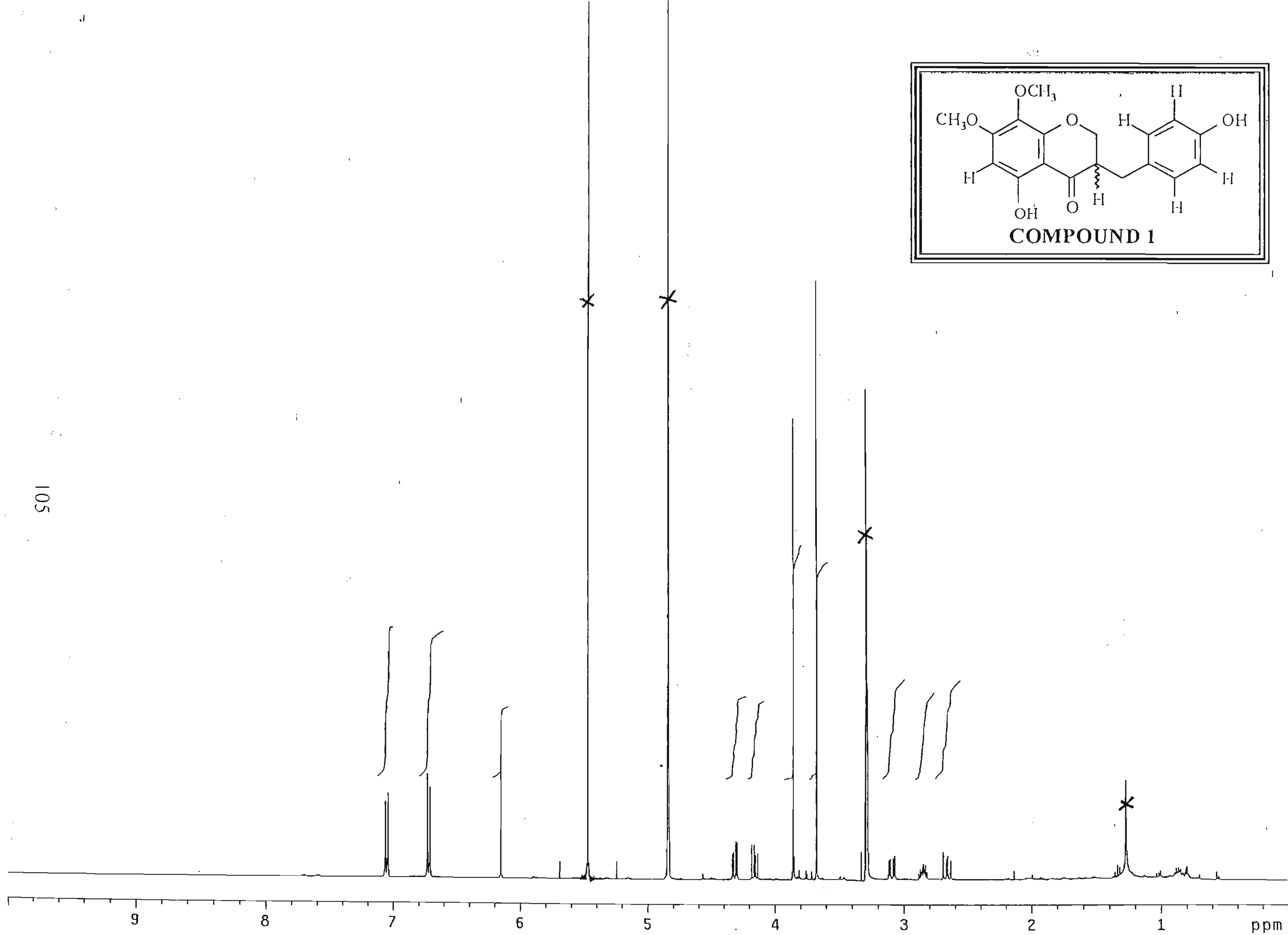
SPECTRUM 11.5 : NOESY spectrum of compound 11 (CD_3OD)

SPECTRUM 11.6 : HMBC spectrum of compound 11 (CD_3OD)

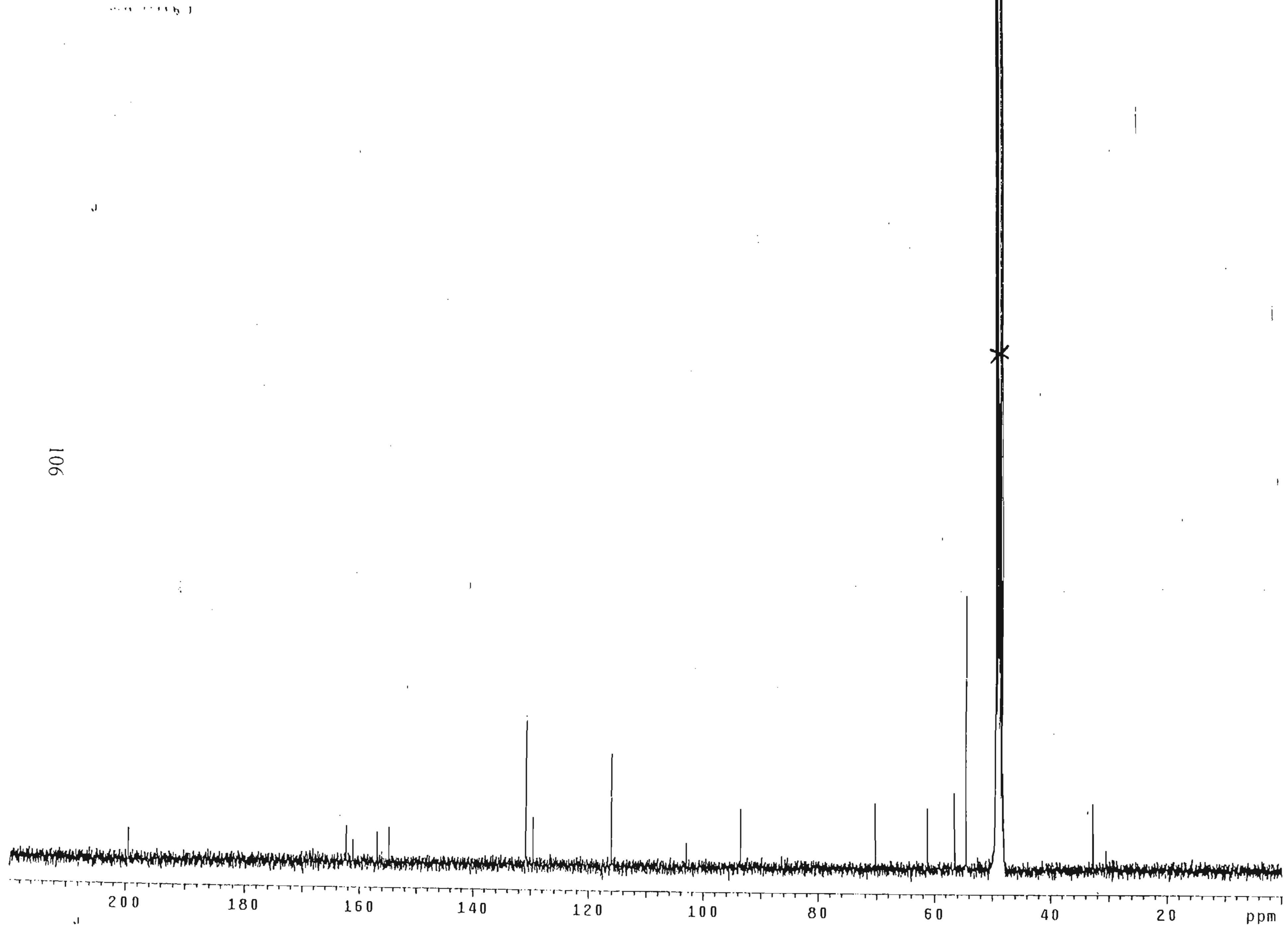
SPECTRUM 11.7 : Mass spectrum of compound 11

SPECTRUM 11.8 : UV spectrum of compound 11

SPECTRUM 11.9 : Infrared spectrum of compound 11



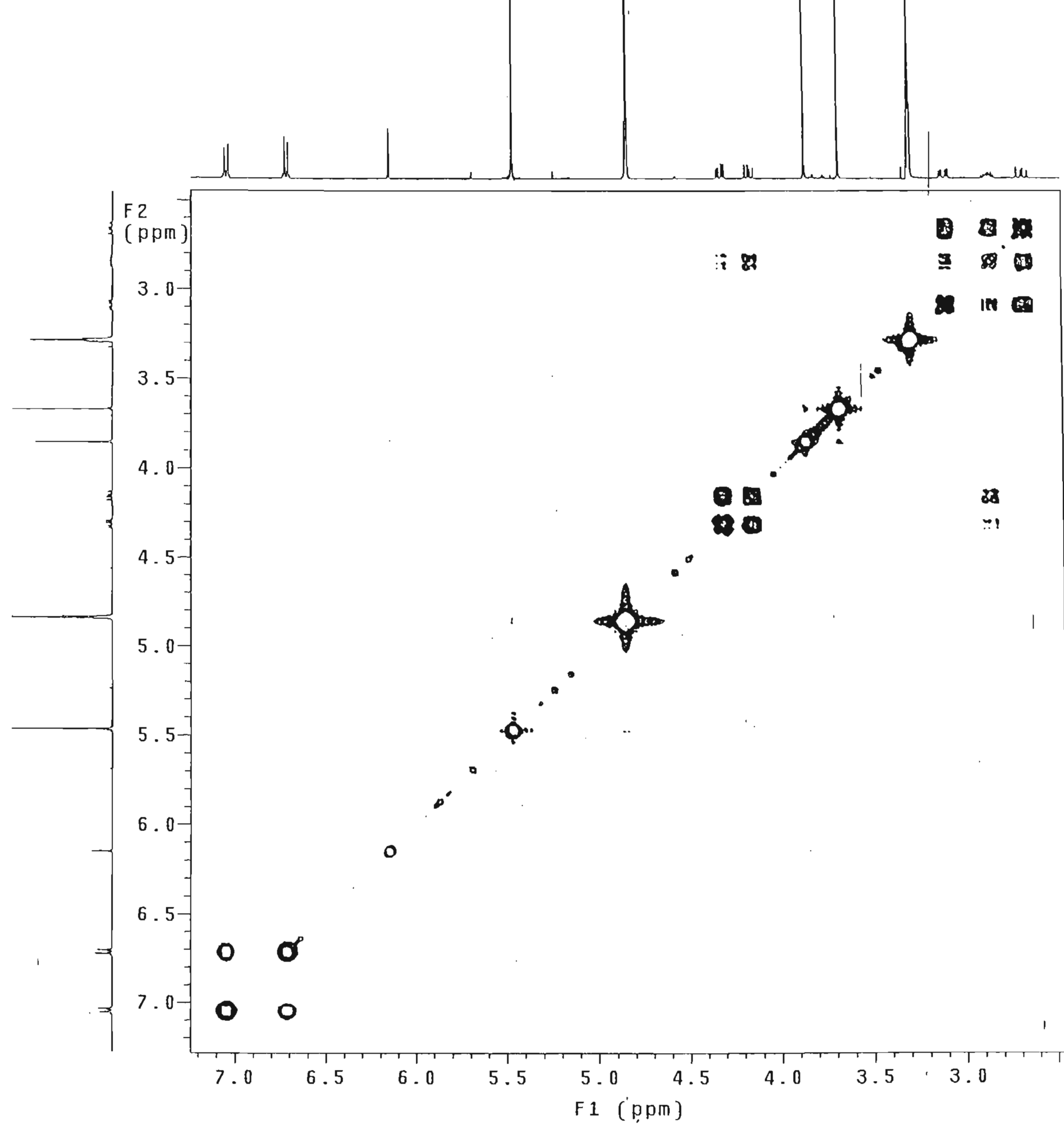
SPECTRUM 1.1 : ^{1}H NMR spectrum of compound 1 (CD_3OD)



SPECTRUM 1.2 : ^{13}C NMR spectrum of compound 1 (CD_3OD)

ambient temperature
INOVA-400 "undnmr400"

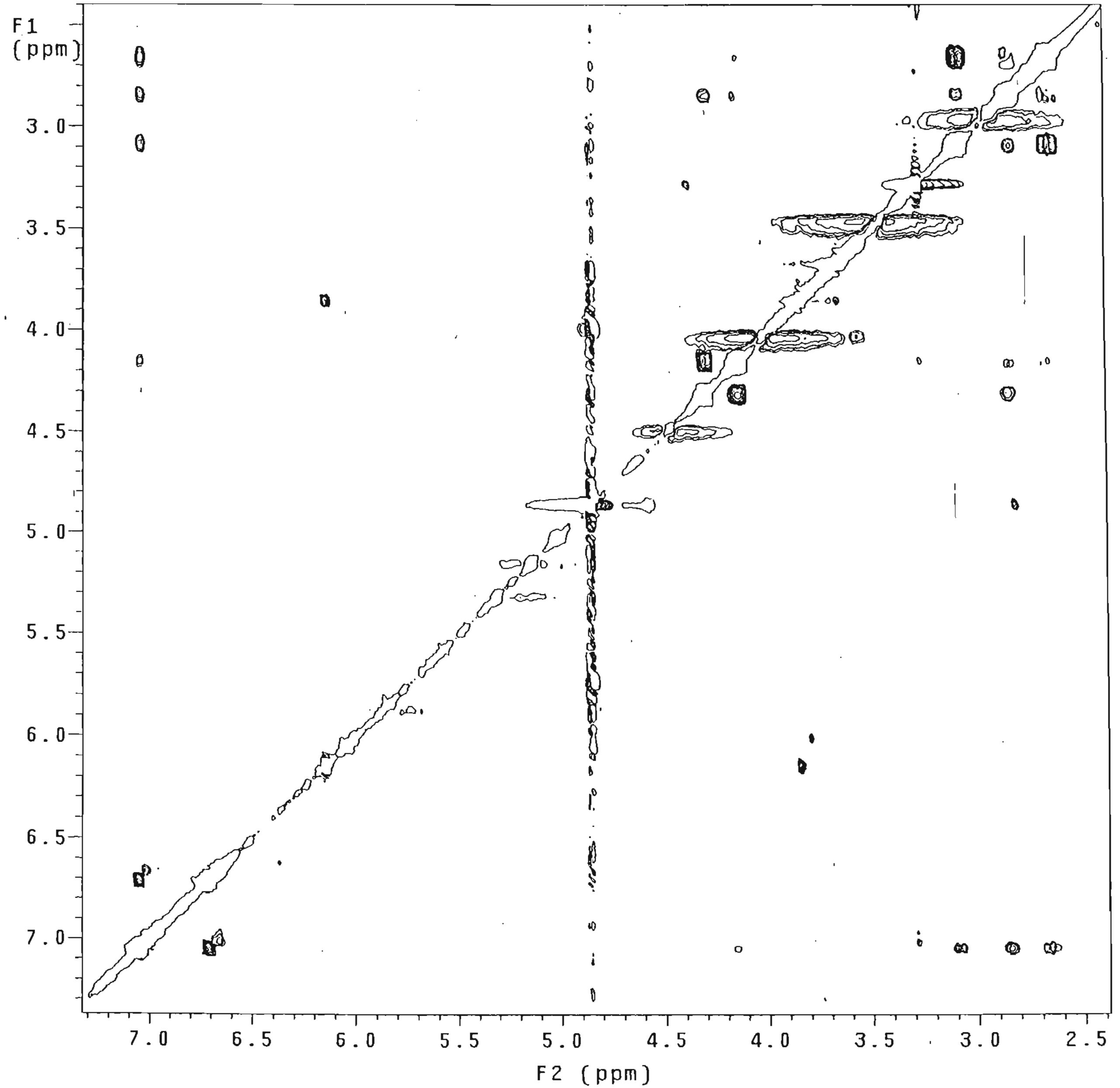
PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.169 sec
Width 3031.1 Hz
2D Width 3031.1 Hz
32 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.084 sec
F1 DATA PROCESSING
Sine bell 0.042 sec
FT size 1024 x 1024
Total time 2.8 hours



SPECTRUM 1.3 : COSY spectrum of compound 1 (CD3OD)

Pulse Sequence: noesy_da
Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

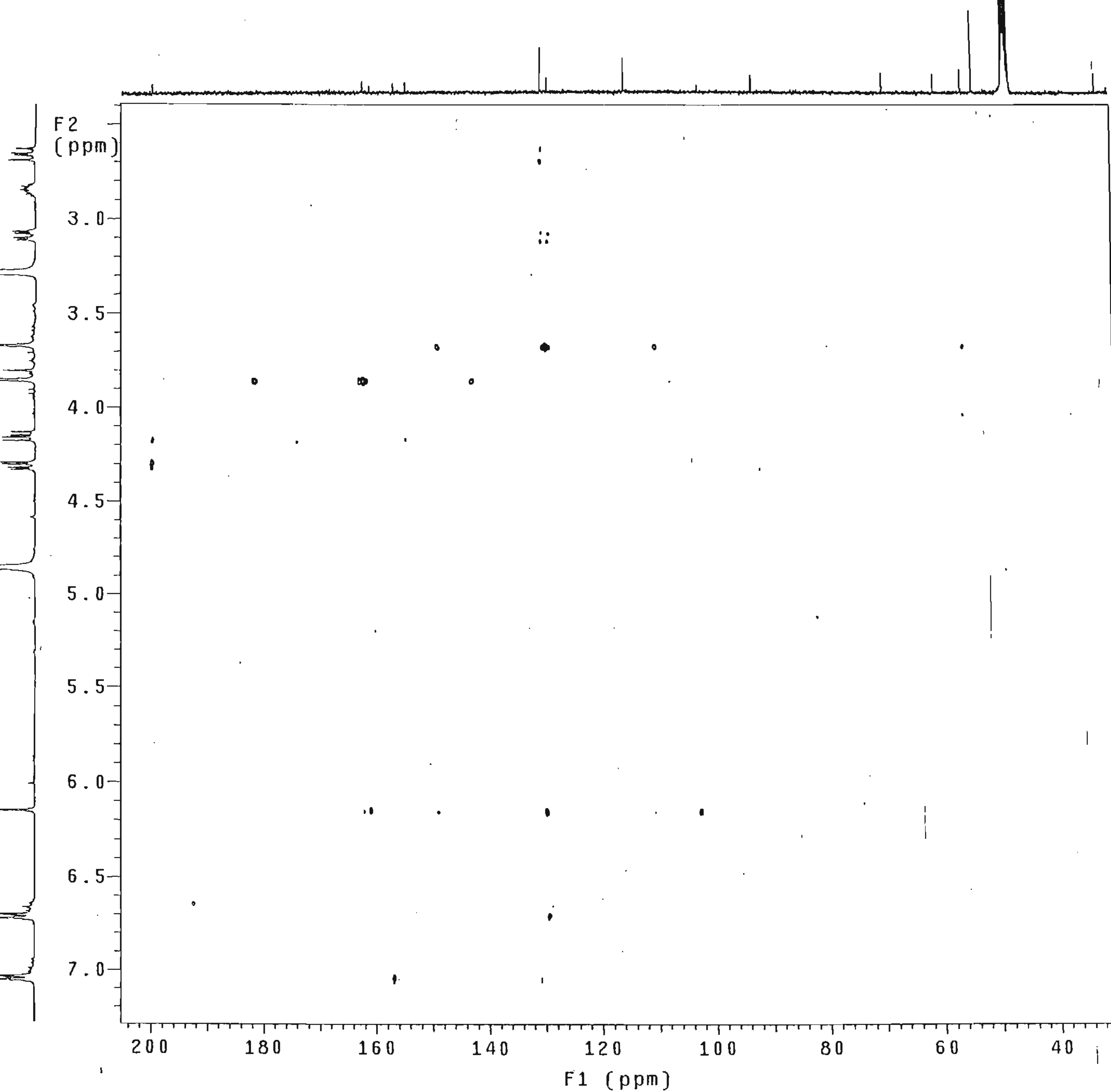
PULSE SEQUENCE: noesy_da
Relax. delay 2.500 sec
Mixing 1.000 sec
Acq. time 0.150 sec
Width 3410.1 Hz
2D Width 3410.1 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sq. sine bell 0.150 sec
Shifted by -0.150 sec
F1 DATA PROCESSING
Sq. sine bell 0.075 sec
Shifted by -0.075 sec
FT size 1024 x 1024
Total time 4 hr, 12 min, 55 sec



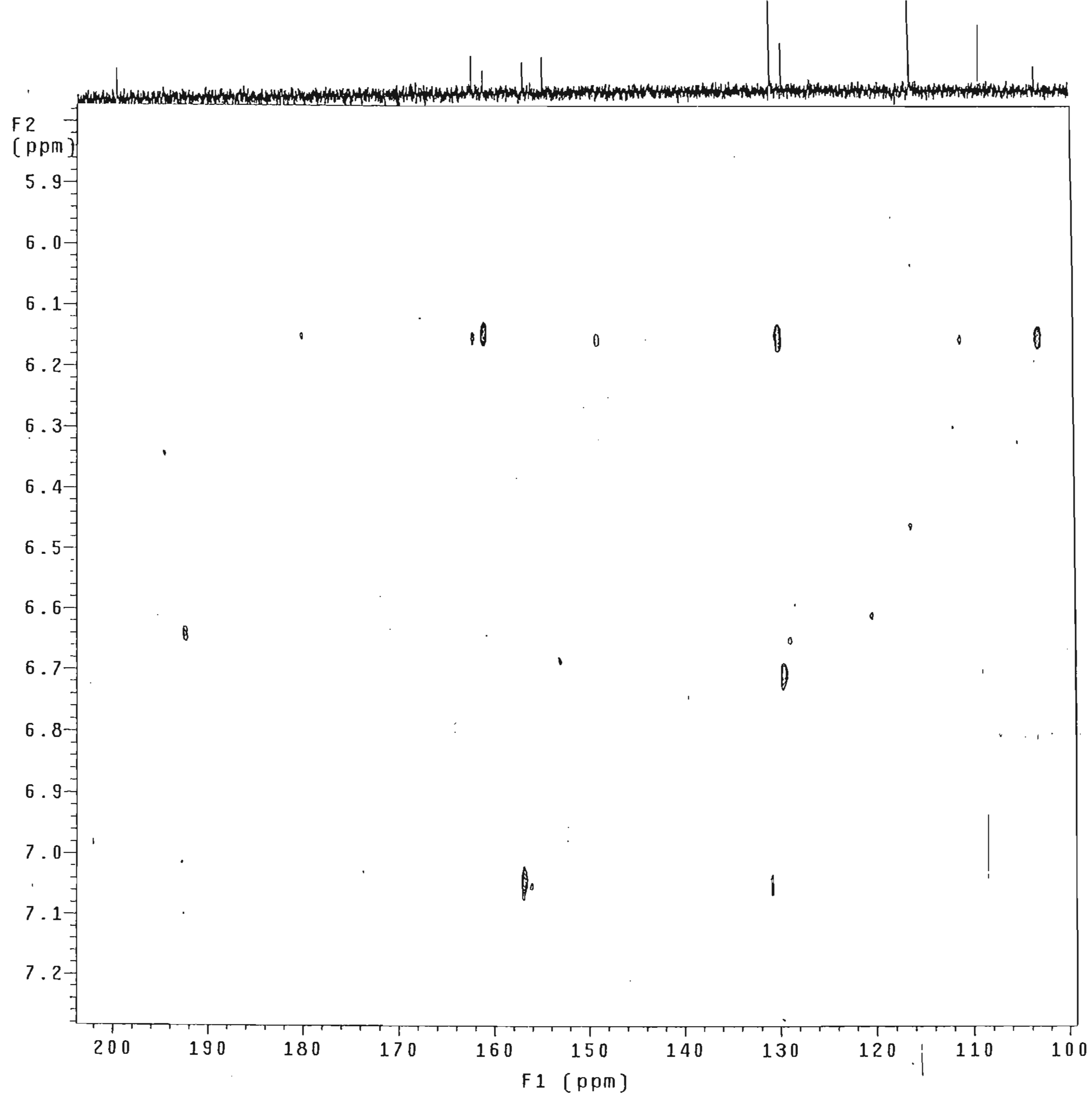
SPECTRUM 1.4 : NOESY spectrum of compound 1 (CD3OD)

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

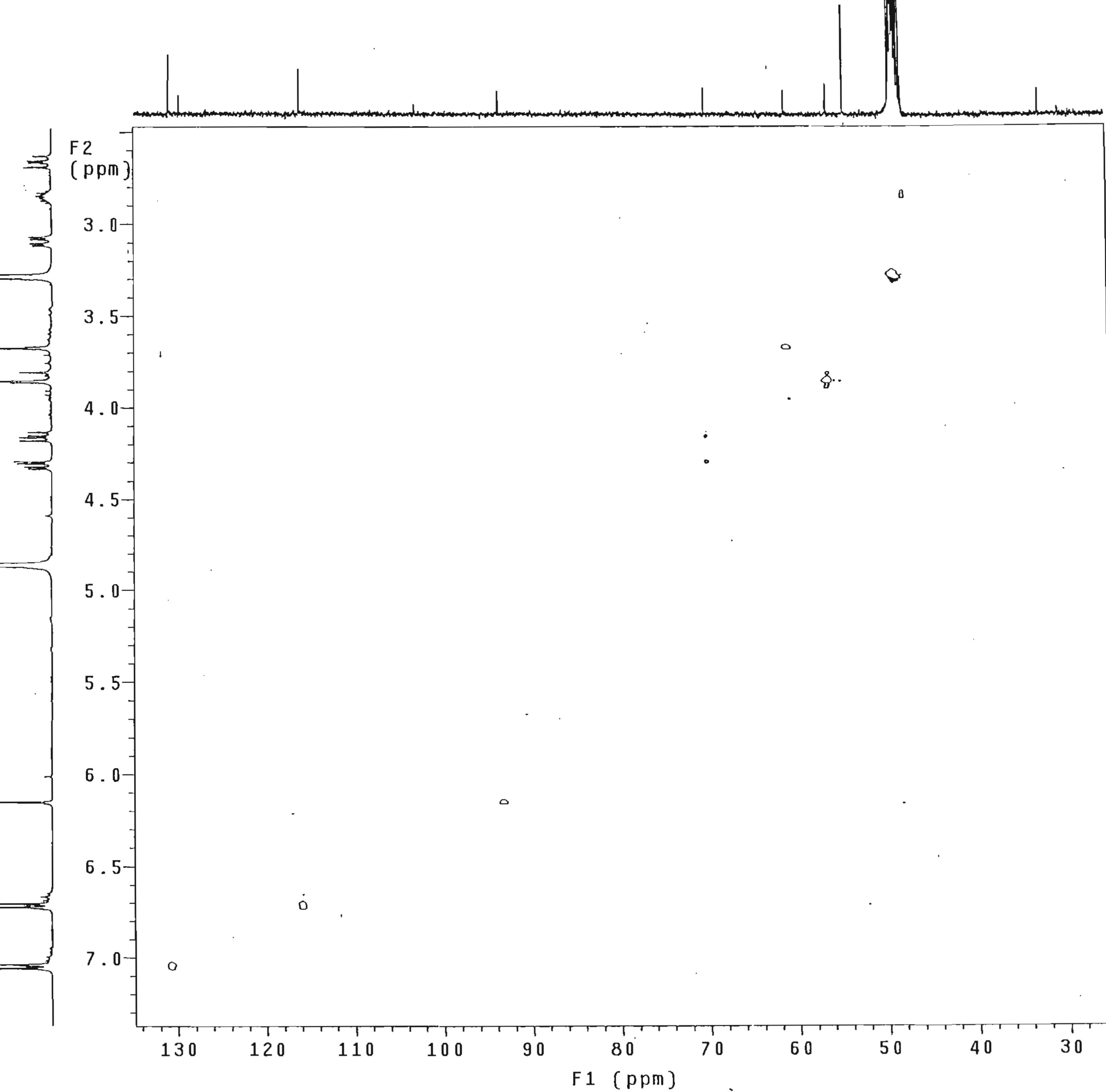
PULSE SEQUENCE: ghmqc_da
Relax. delay 1.500 sec
Acq. time 0.148 sec
Width 3450.1 Hz
2D Width 20639.8 Hz
32 repetitions
512 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.074 sec
F1 DATA PROCESSING
Sine bell 0.012 sec
FT size 2048 x 2048
Total time 7 hr, 50 min, 1 sec



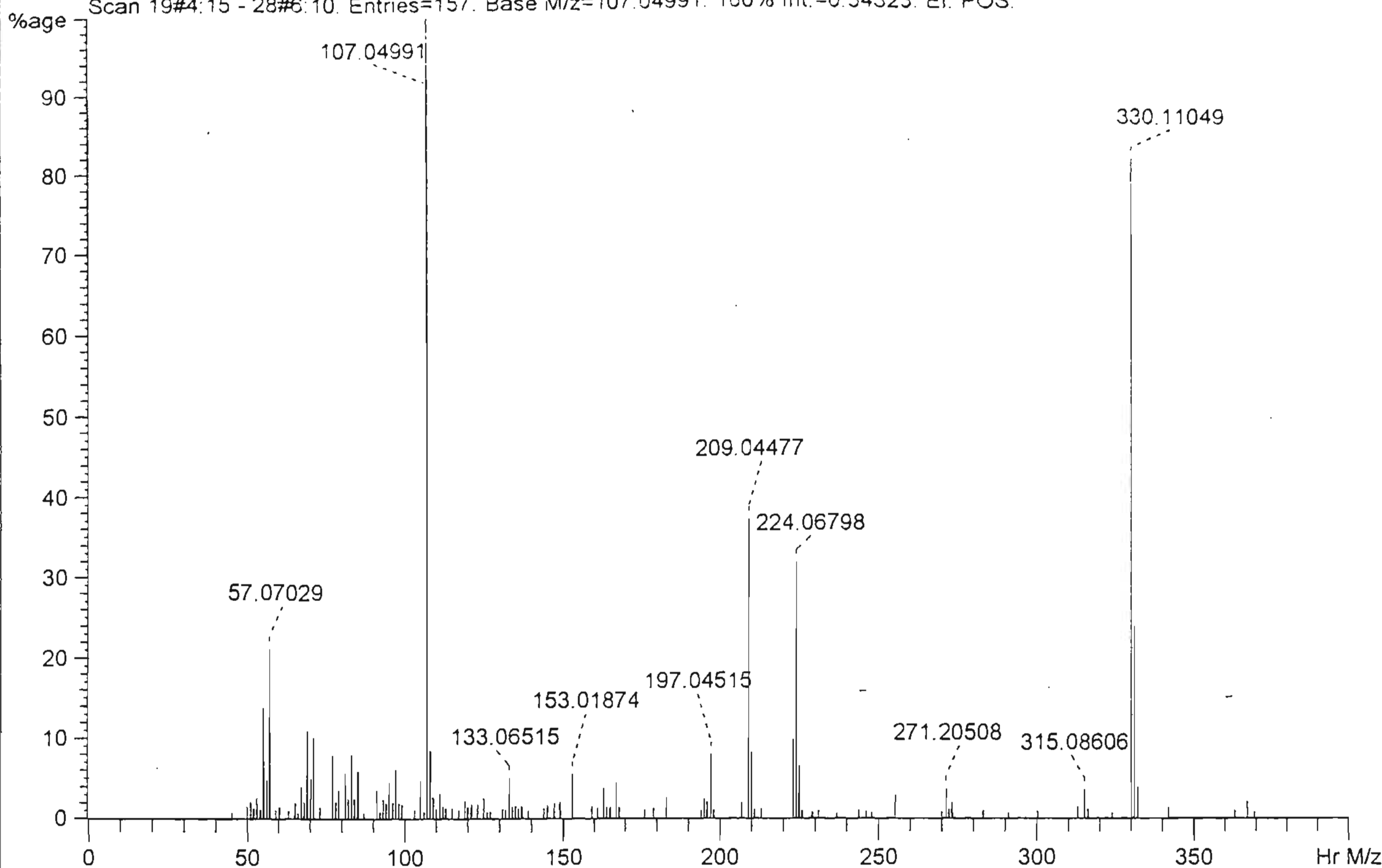
SPECTRUM 1.5A : HMBC spectrum of compound 1 (CD3OD)



SPECTRUM 1.5B: expanded HMBC spectrum of compound 1 (CD_3OD)



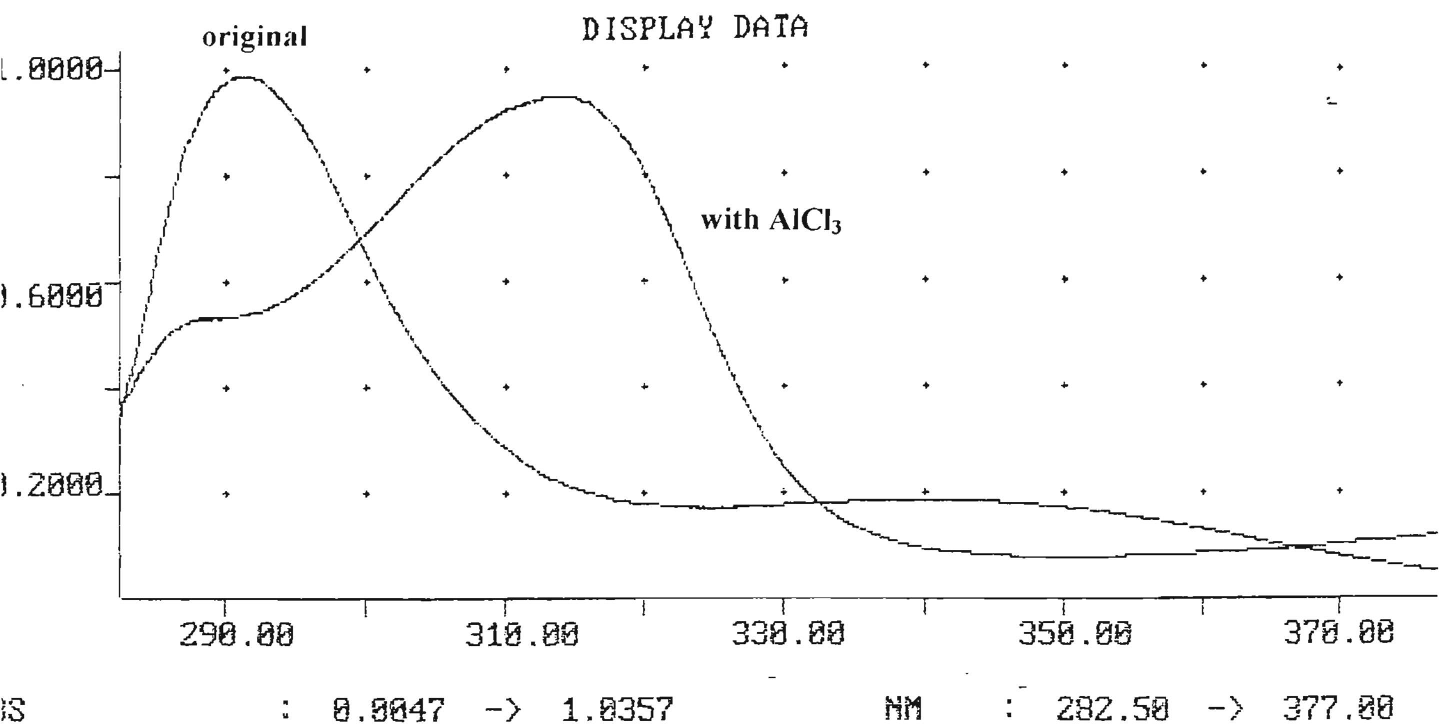
SPECTRUM 1.6 : HSQC spectrum of compound 1 (CD_3OD)



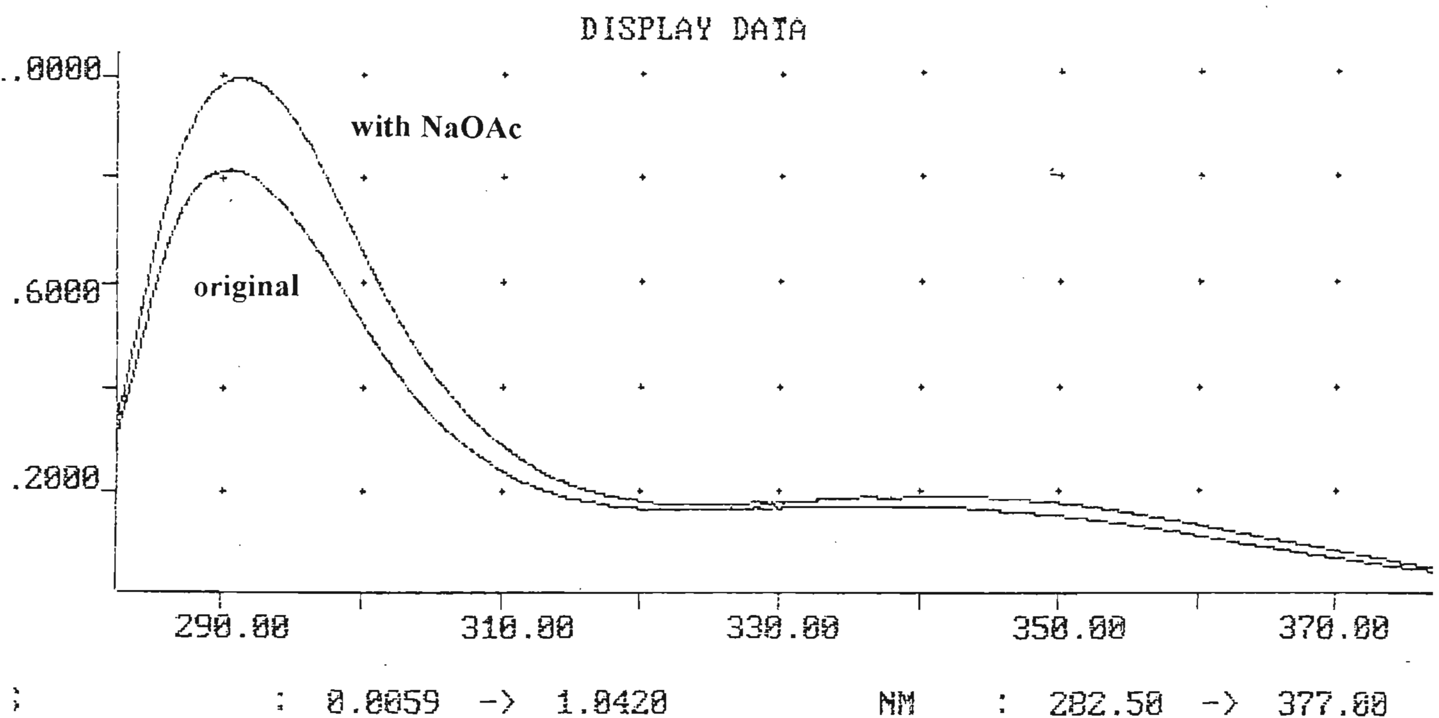
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:2%. Range:0-340. Excl: Ref/Ex.].
Scan 19#4:15 - 28#6:10. Entries=49. Base M/z=107.04991. 100% Int.=0.54323. EI. POS.

Hr M/z	%age	Width
51.02317	2.08	33
53.03890	2.55	35
55.01851	9.42	40
55.05473	13.83	40
56.06197	4.74	39
57.07029	21.17	40
67.05441	4.01	36
69.07002	10.86	39
70.07754	5.00	38
71.08561	10.09	39
77.03897	7.88	39
78.04573	2.07	35
79.05436	3.47	37
81.07016	5.64	37
82.07739	2.45	33
83.08567	7.95	37
84.09263	2.44	34
85.10126	5.91	37
91.05426	3.52	35
93.07003	2.33	33
95.08572	4.48	36
96.09317	2.02	31
97.10122	6.05	37
105.07028	4.74	36
107.04991	100.00	40
107.08582	3.20	33
108.05348	8.45	38
109.10174	2.62	32
111.11704	3.11	32
119.08566	2.21	32
125.02354	2.55	36
133.06515	5.00	36
153.01874	5.53	37
163.03956	3.77	36
167.03401	4.43	37
183.00553	2.55	44
195.06488	2.34	42
197.04515	7.92	36
209.04477	37.06	37
210.04991	8.19	34
223.06084	9.77	34
224.06798	31.72	36
225.07233	6.47	34
255.21083	2.78	31
271.20508	3.56	32
315.08606	3.49	32
330.11049	81.33	36
331.11418	23.71	35
332.11722	3.83	31

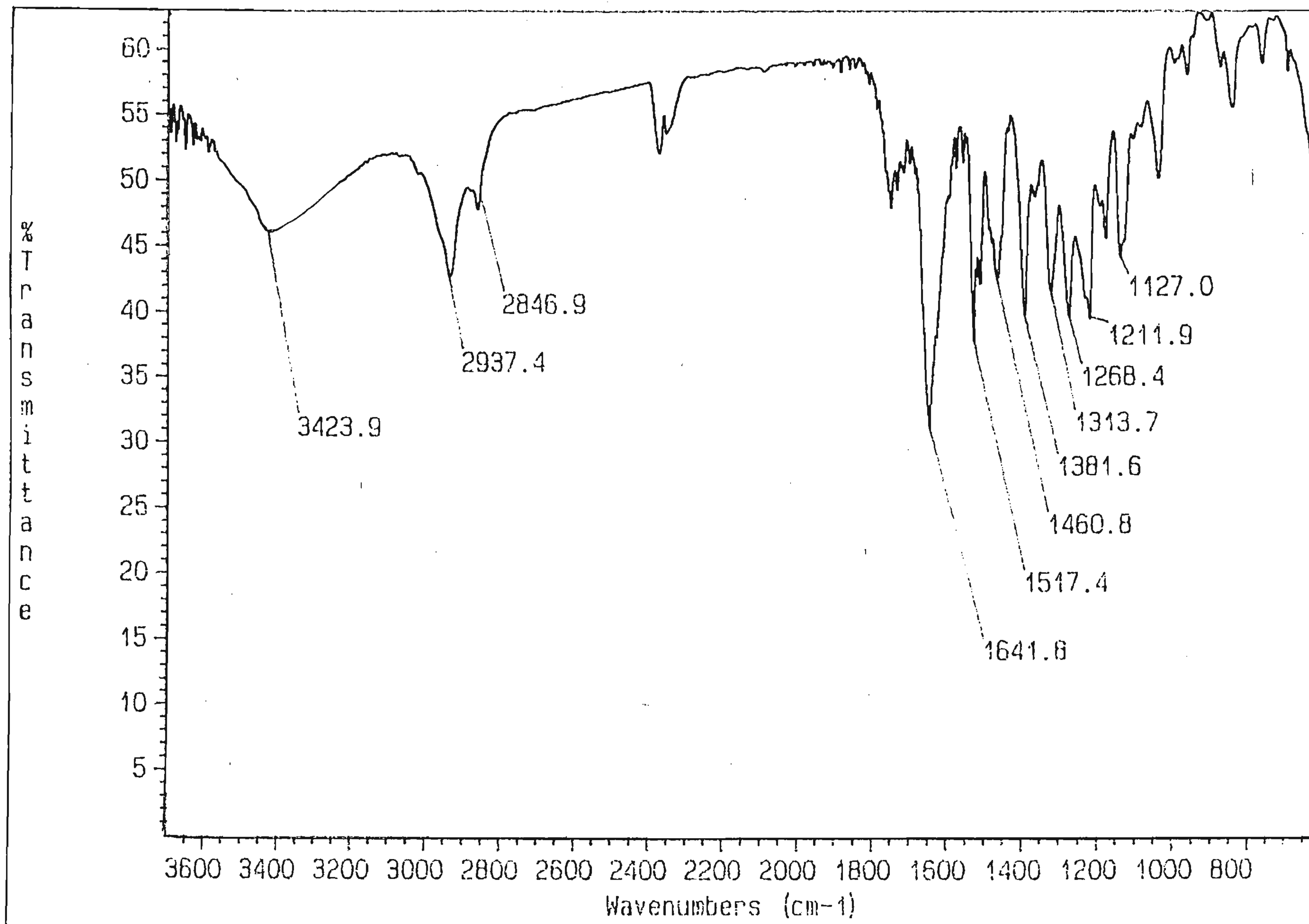
SPECTRUM 1.7 : Mass spectrum of compound 1



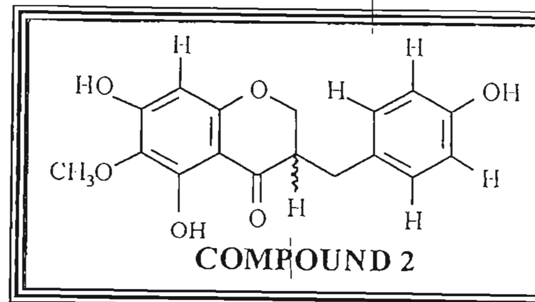
SPECTRUM 1.8A : UV spectrum of compound 1 (original and with $AlCl_3$)



SPECTRUM 1.8B : UV spectrum of compound 1 (original and with NaOAc)

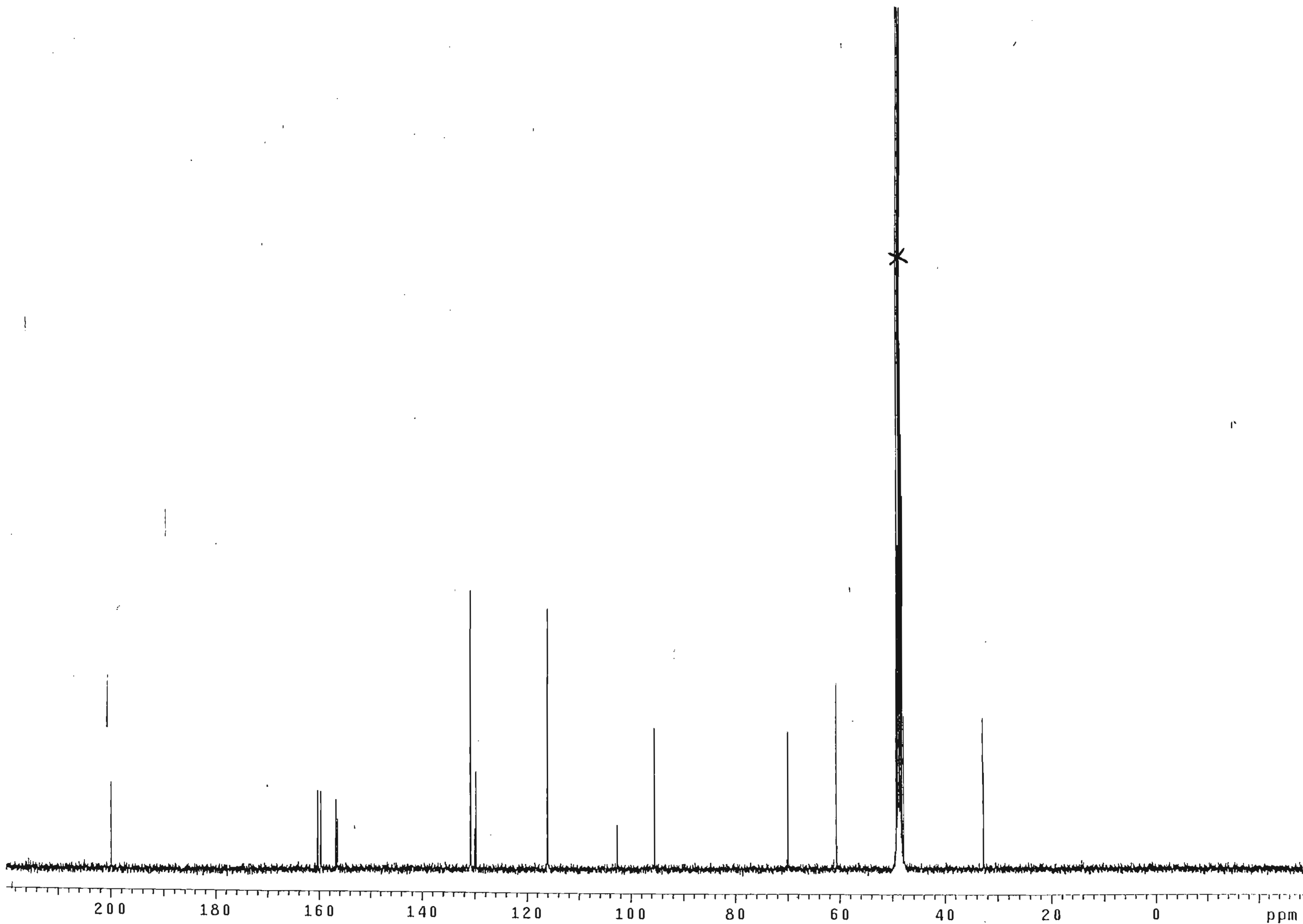


SPECTRUM 1.9 : Infrared spectrum of compound 1



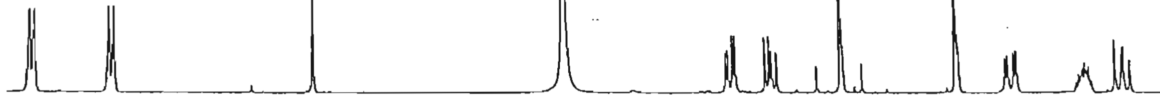
9 8 7 6 5 4 3 2 1 ppm

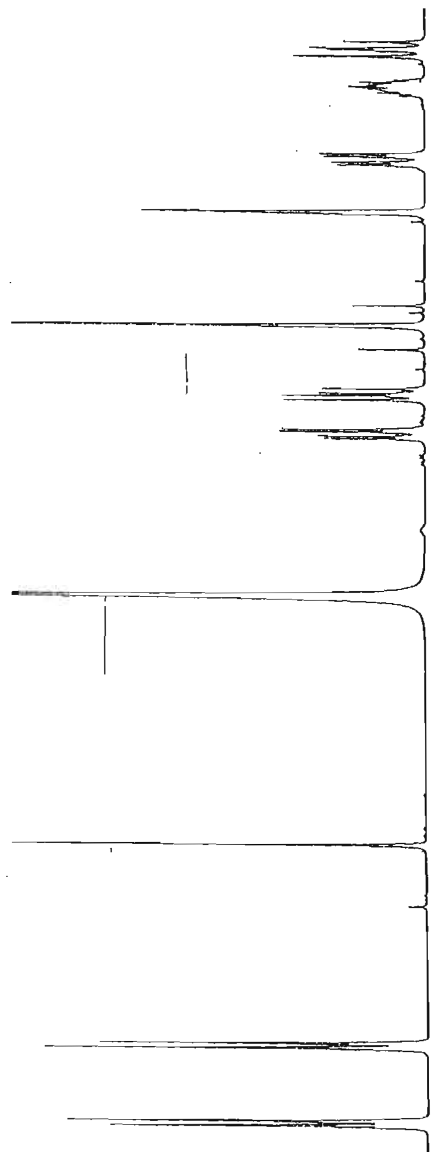
SPECTRUM 2.1 : ^{1}H NMR spectrum of compound 2 (CD_3OD)



SPECTRUM 2.2 : ^{13}**C NMR spectrum of compound 2 (CD_3OD)**

Pulse Sequence: noesy_da





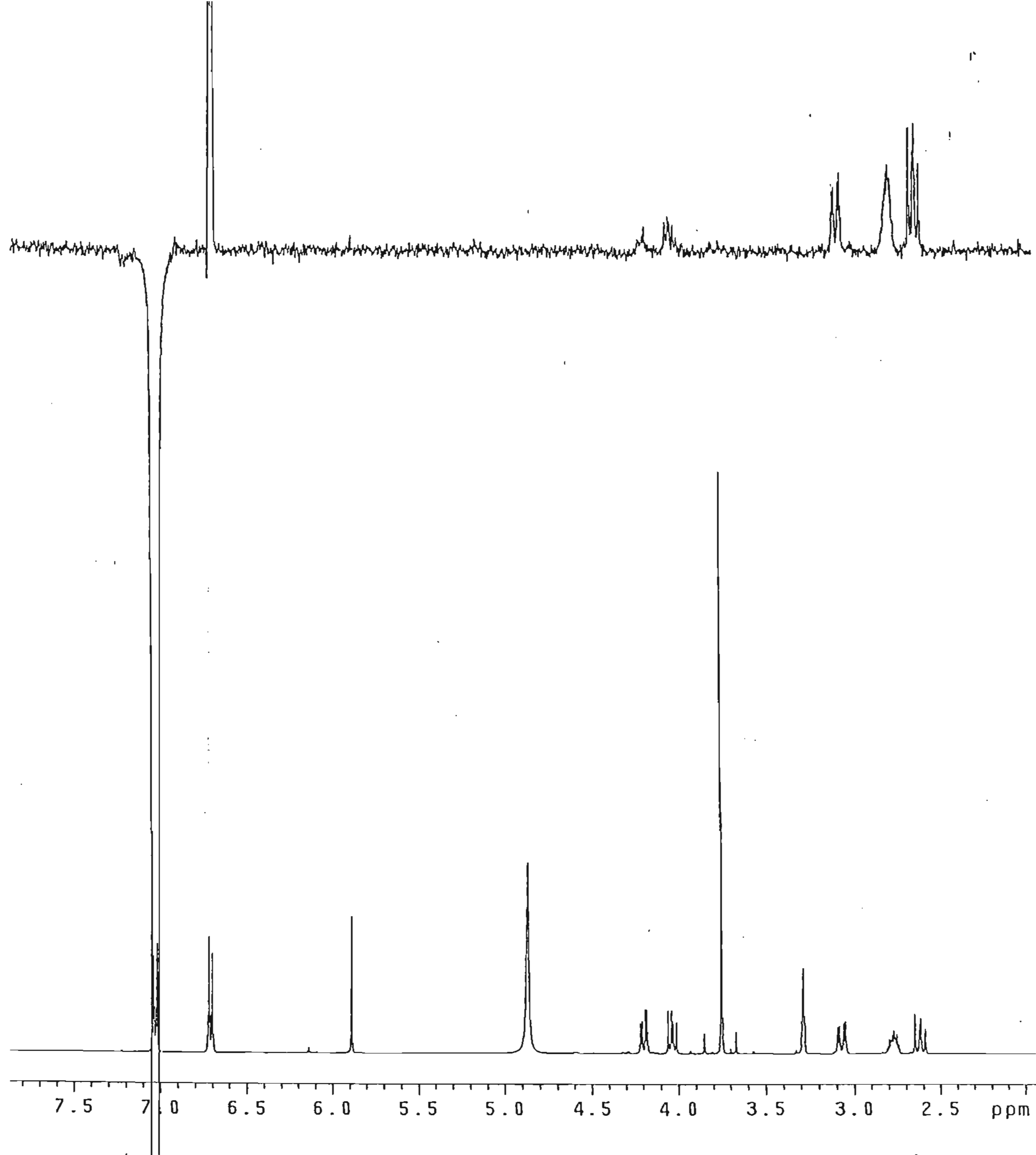
F1 (ppm)

3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0

7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5

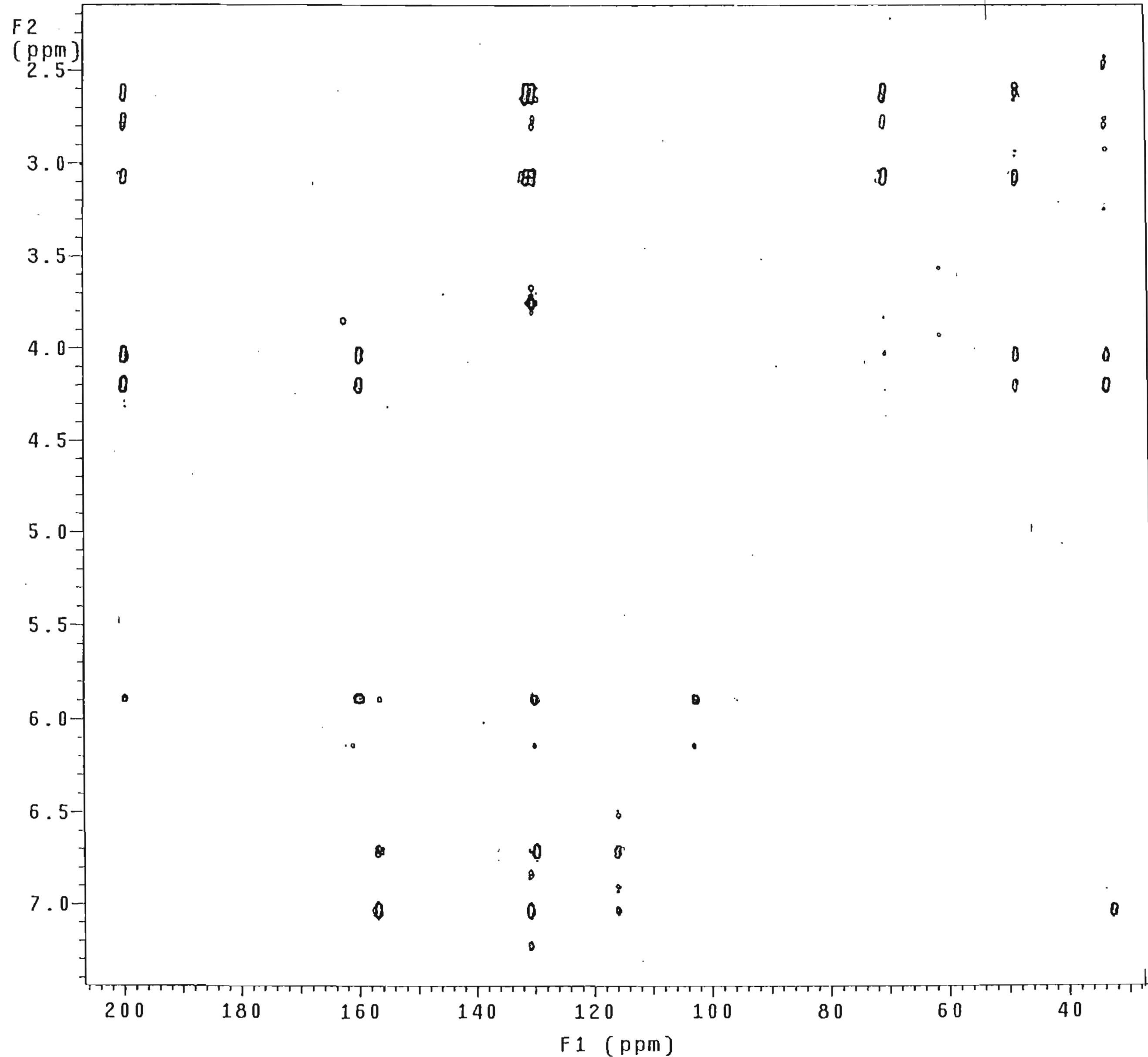
F2 (ppm)

SPECTRUM 2.3 : NOESY spectrum of compound 2 (CD_3OD)

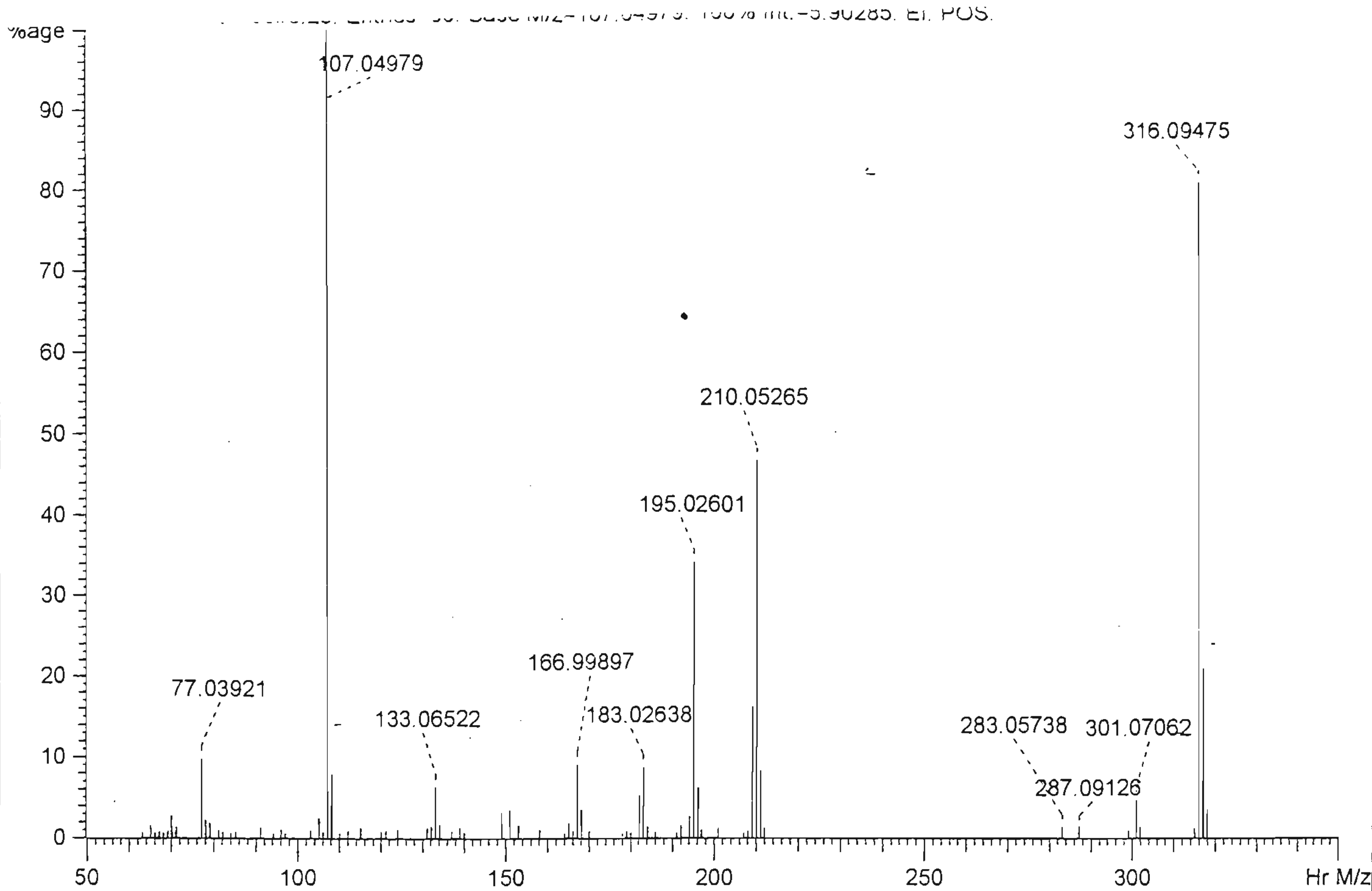


SPECTRUM 2.4 : NOE spectrum of compound 2 (CD_3OD)

Pulse Sequence: ghmqc_da



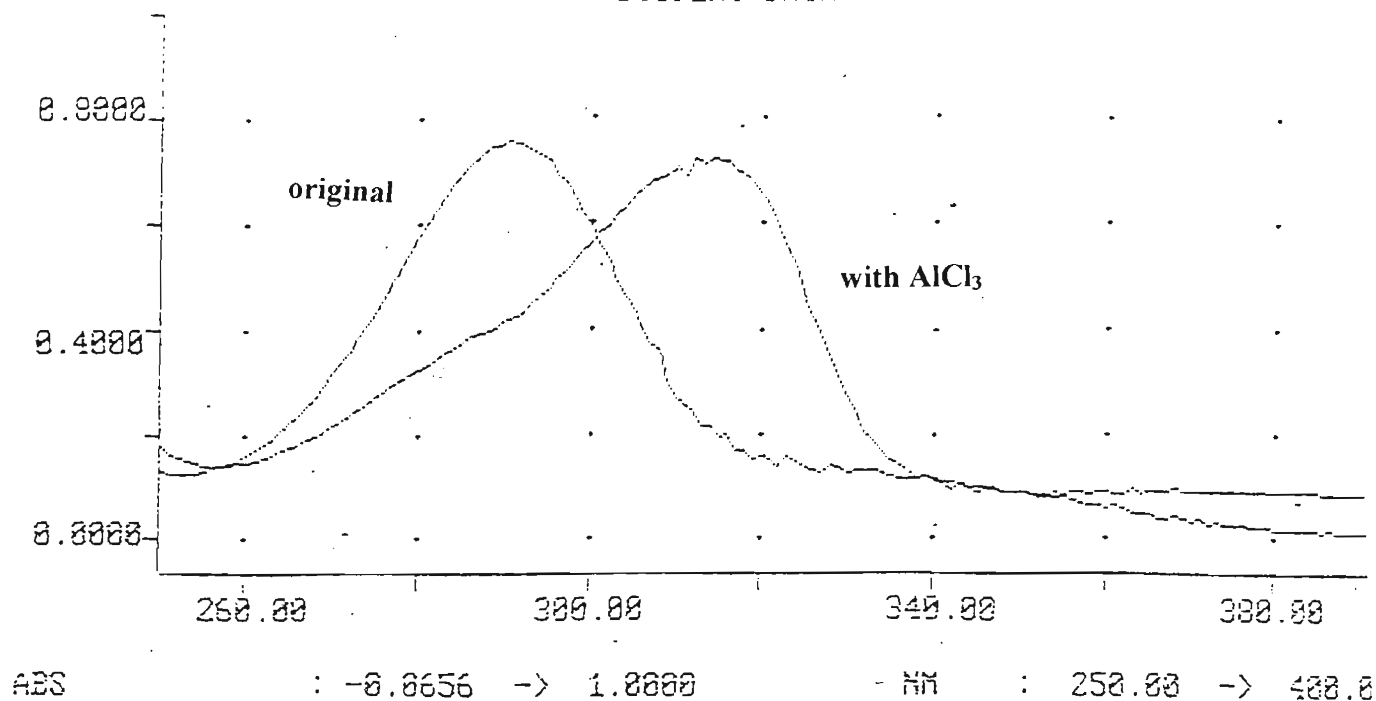
SPECTRUM 2.5 : HMBC spectrum of compound 2 (CD_3OD)



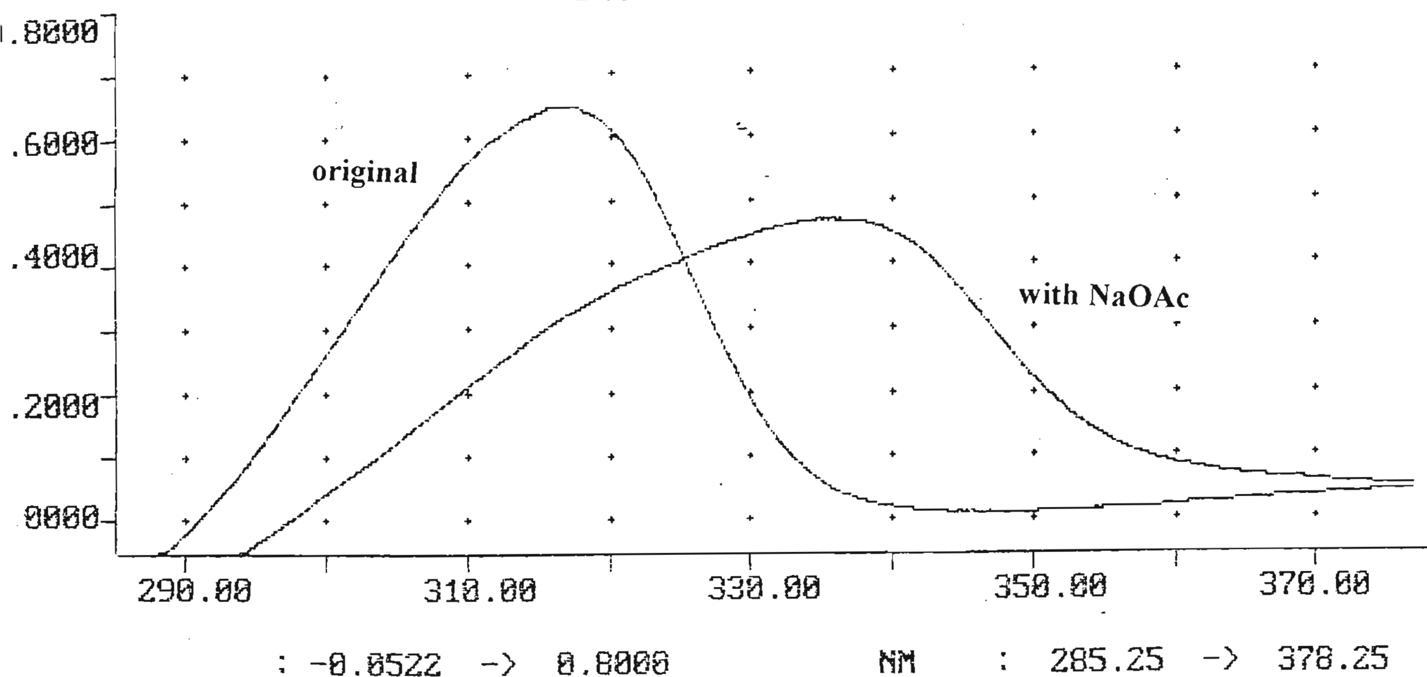
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:2%. Range:0-320. Excl: Ref/Ex.].
Scan 26#5:36 - 30#6:25. Entries=23. Base M/z=107.04979. 100% Int.=5.90285. EI. POS.

Hr M/z	%age	Width
70.00081	2.64	62
77.03921	9.70	58
78.04552	2.14	60
105.07041	2.41	94
107.04979	100.00	57
108.05311	7.88	60
133.06522	6.22	59
149.02177	3.13	59
150.99647	3.46	60
166.99897	9.04	83
168.00461	3.46	74
182.01878	5.22	74
183.02638	8.61	63
194.01567	2.65	65
195.02601	33.86	60
196.03113	6.08	60
209.04514	16.13	61
210.05265	46.51	60
211.05692	8.26	61
301.07062	4.69	69
316.09475	80.15	61
317.09852	20.67	62
318.10142	3.43	61

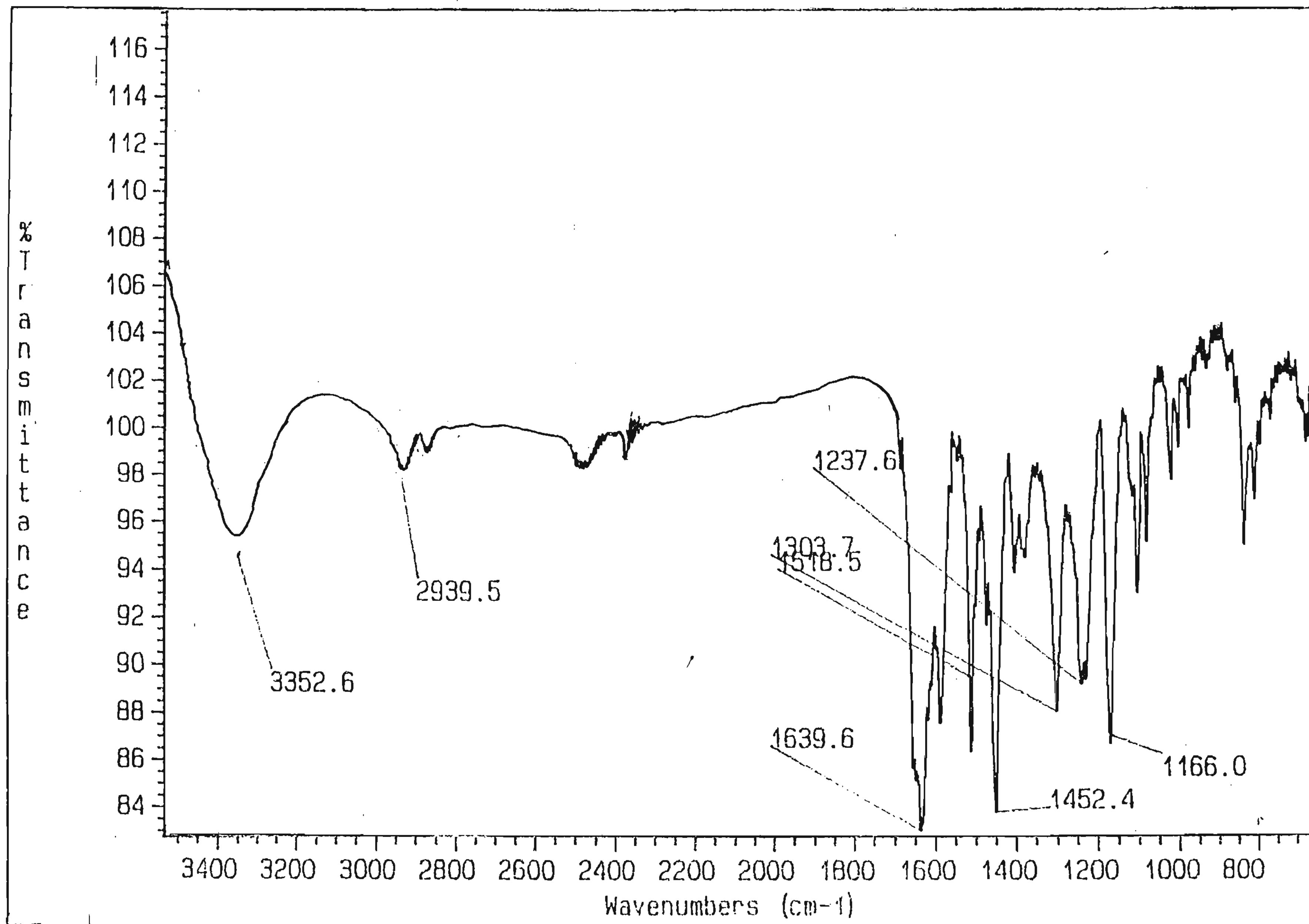
SPECTRUM 2.6 : Mass spectrum of compound 2



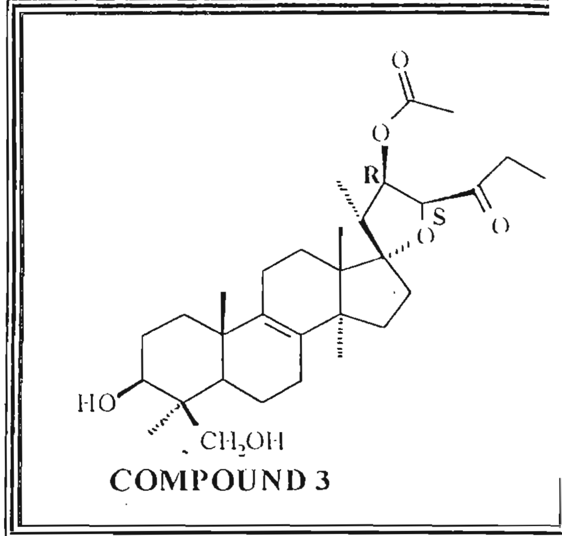
SPECTRUM 2.7A : UV spectrum of compound 2 (original and with $AlCl_3$)



SPECTRUM 2.7B : UV spectrum of compound 2 (original and with NaOAc)

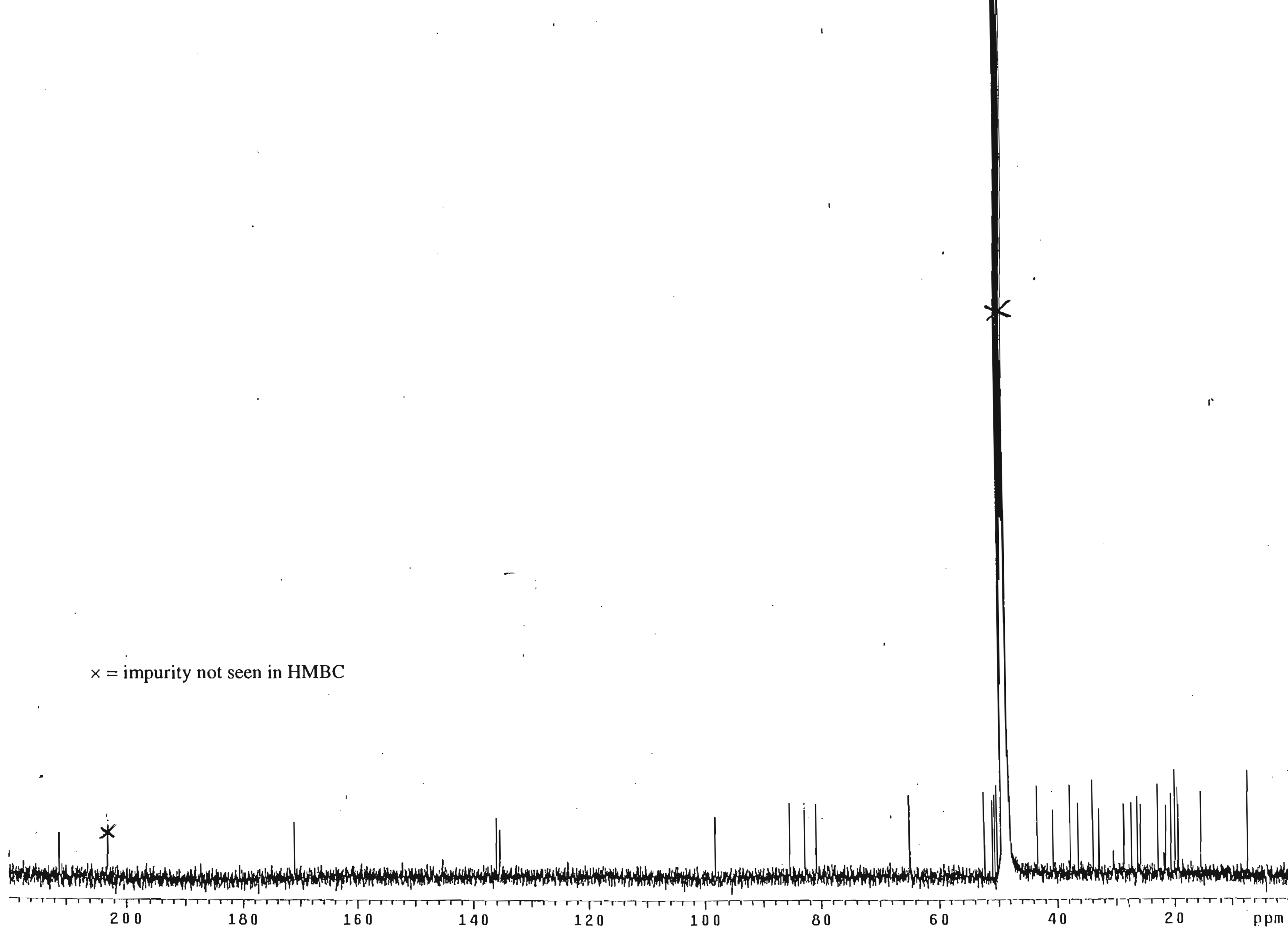


SPECTRUM 2.8 : Infrared spectrum of compound 2

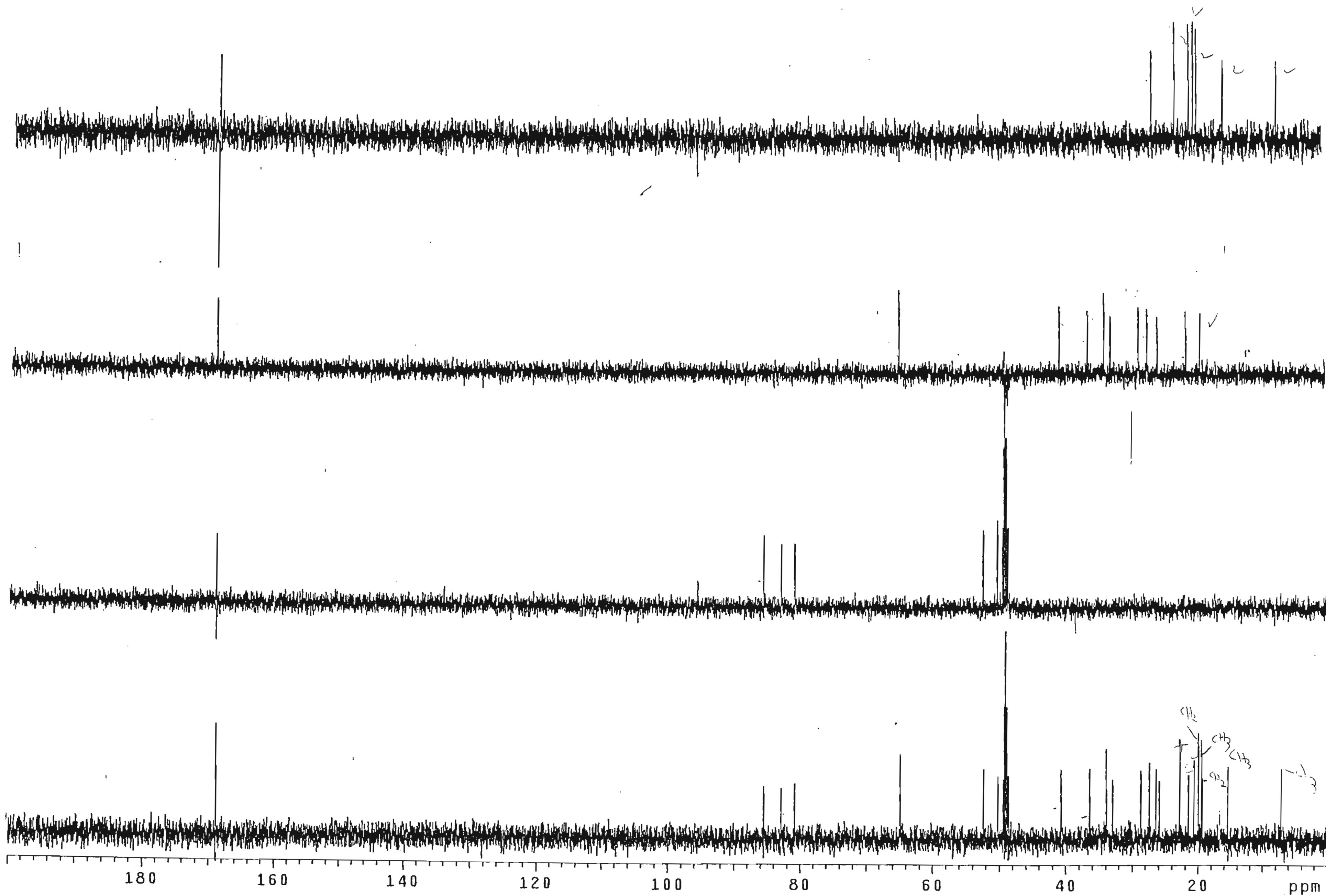


5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

SPECTRUM 3.1 : ^{1}H NMR spectrum of compound 3 (CD_3OD)

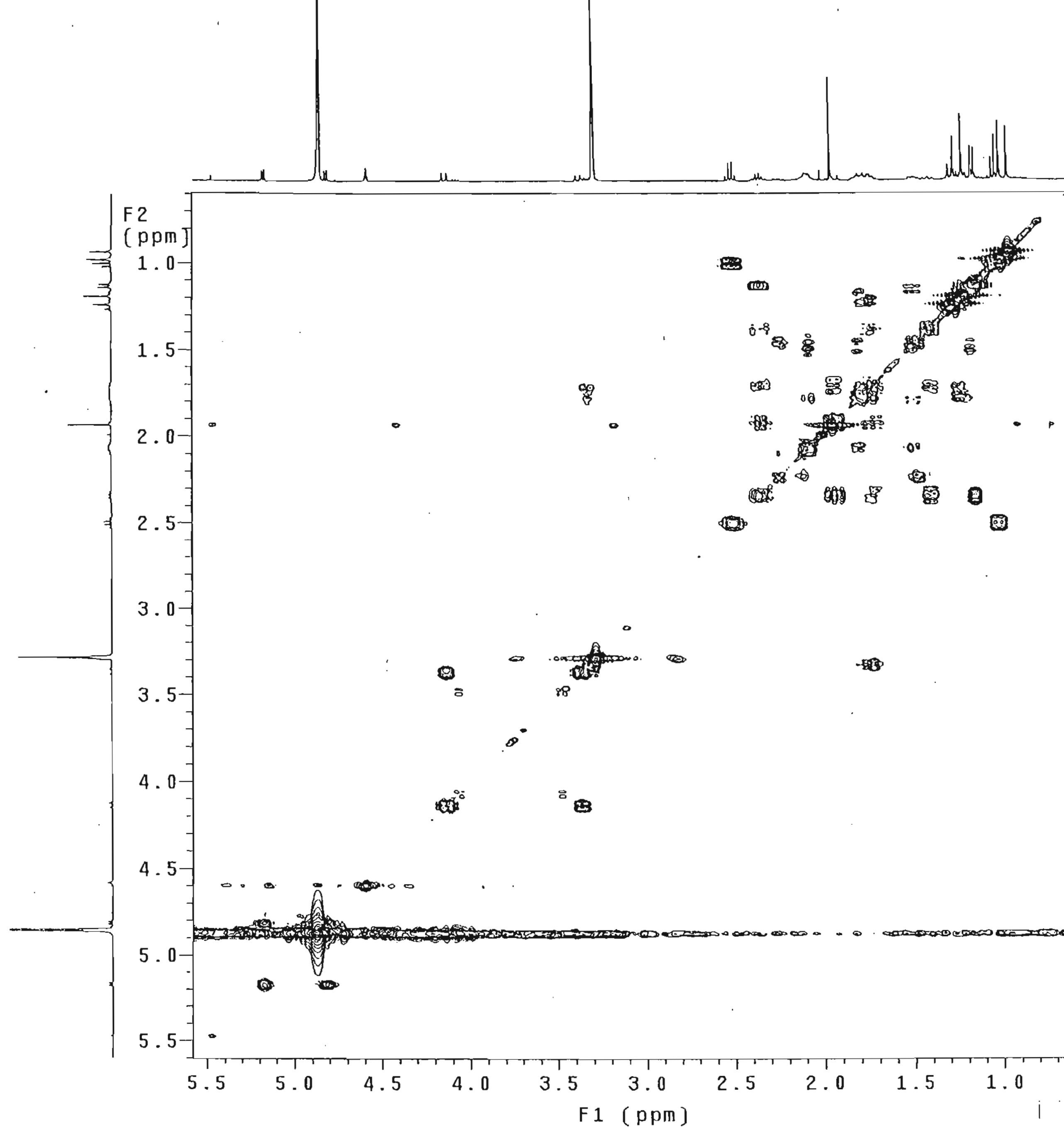


SPECTRUM 3.2 : ^{13}C NMR spectrum of compound 3 (CD_3OD)



SPECTRUM 3.3 : ADEPT spectrum of compound 3 (CD_3OD)

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.213 sec
Width 2399.6 Hz
2D Width 2399.6 Hz
128 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.107 sec
F1 DATA PROCESSING
Sine bell 0.053 sec
FT size 1024 x 1024
Total time 11.5 hours

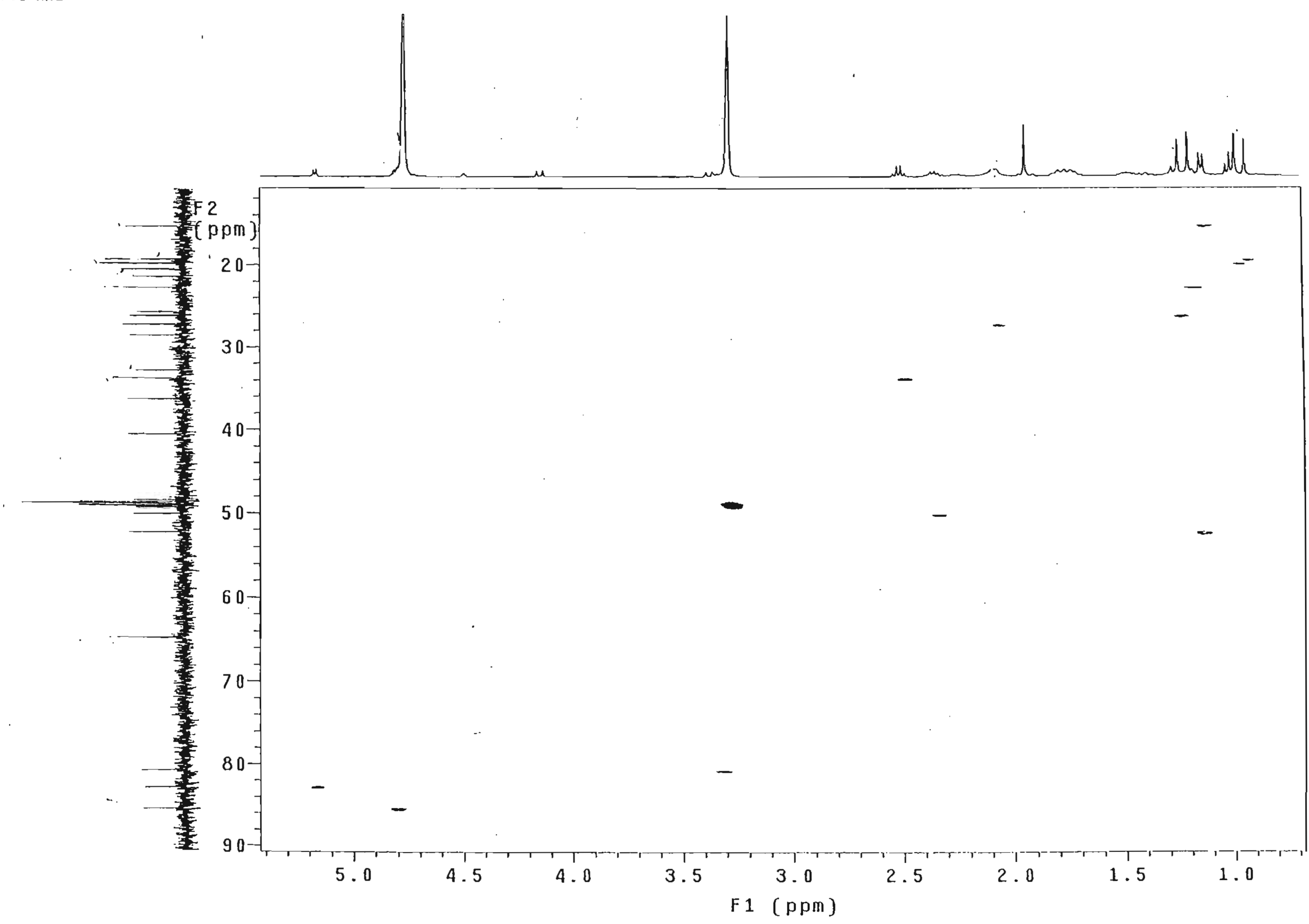


SPECTRUM 3.4 : COSY spectrum of compound 3 (CD_3OD)

1H/13C hetcor

Solvent: cd3od
Temp. 30.0 C / 303.1 K
INOVA-400 "undnmr400"

PULSE SEQUENCE: hetcor
Relax. delay 1.000 sec
Acq. time 0.056 sec
Width 18186.0 Hz
2D Width 2238.6 Hz
1600 repetitions
128 increments
OBSERVE C13, 100.5674605 MHz
DECOUPLE H1, 399.9514701 MHz
Power 38 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Sine bell 0.028 sec
F1 DATA PROCESSING
Sine bell 0.028 sec
FT size 2048 x 512
Total time 61.7 hours



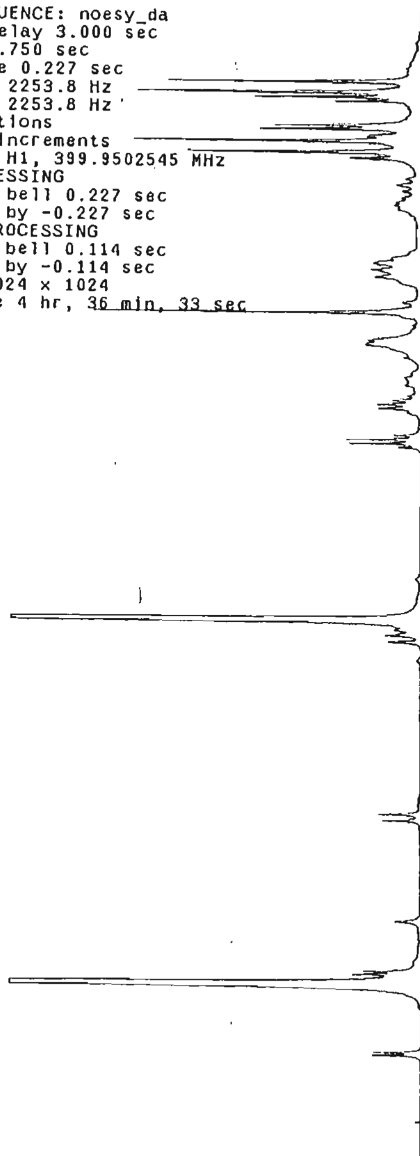
SPECTRUM 3.5 : HETCOR spectrum of compound 3 (CD_3OD)

NOESY expt.
mix=0.75
probe=5mmASW

Pulse Sequence: noesy_da

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: noesy_da
Relax. delay 3.000 sec
Mixing 0.750 sec
Acq. time 0.227 sec
Width 2253.8 Hz
2D Width 2253.8 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sq. sine bell 0.227 sec
Shifted by -0.227 sec
F1 DATA PROCESSING
Sq. sine bell 0.114 sec
Shifted by -0.114 sec
FT size 1024 x 1024
Total time 4 hr, 36 min, 33 sec



F1 (ppm)

1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5

5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0

F2 (ppm)

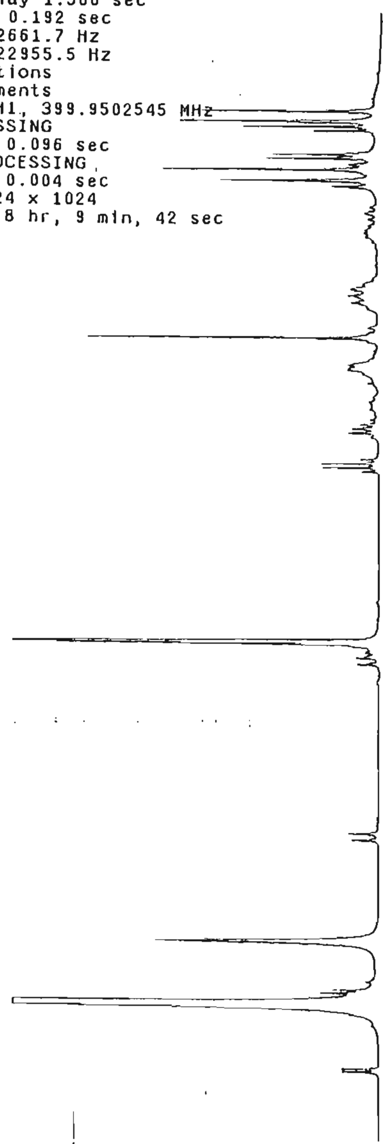
SPECTRUM 3.6 : NOESY spectrum of compound 3 (CD_3OD)

probe=5mm ASW

Pulse Sequence: ghmqc_da

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

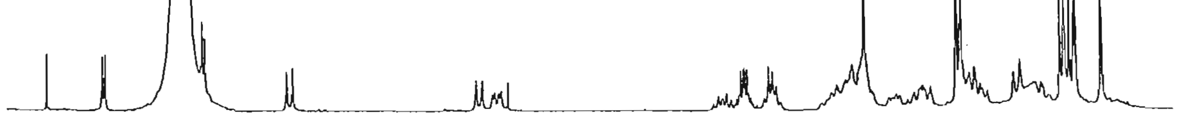
PULSE SEQUENCE: ghmqc_da
Relax. delay 1.500 sec
Acq. time 0.192 sec
Width 2661.7 Hz
2D Width 22955.5 Hz
32 repetitions
512 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.096 sec
F1 DATA PROCESSING
Sine bell 0.004 sec
FT size 1024 x 1024
Total time 8 hr, 9 min, 42 sec

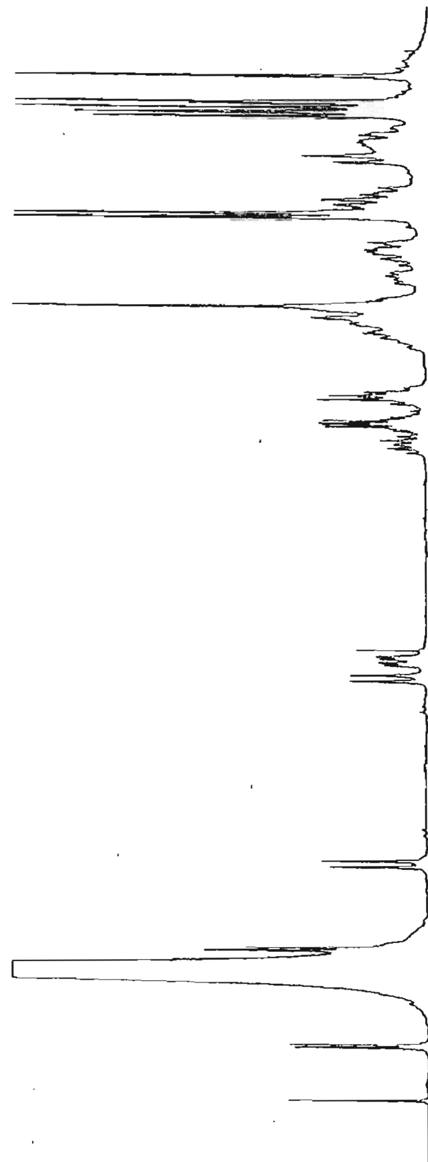


SPECTRUM 3.7 : HMBC spectrum of compound 3 (CD_3OD)

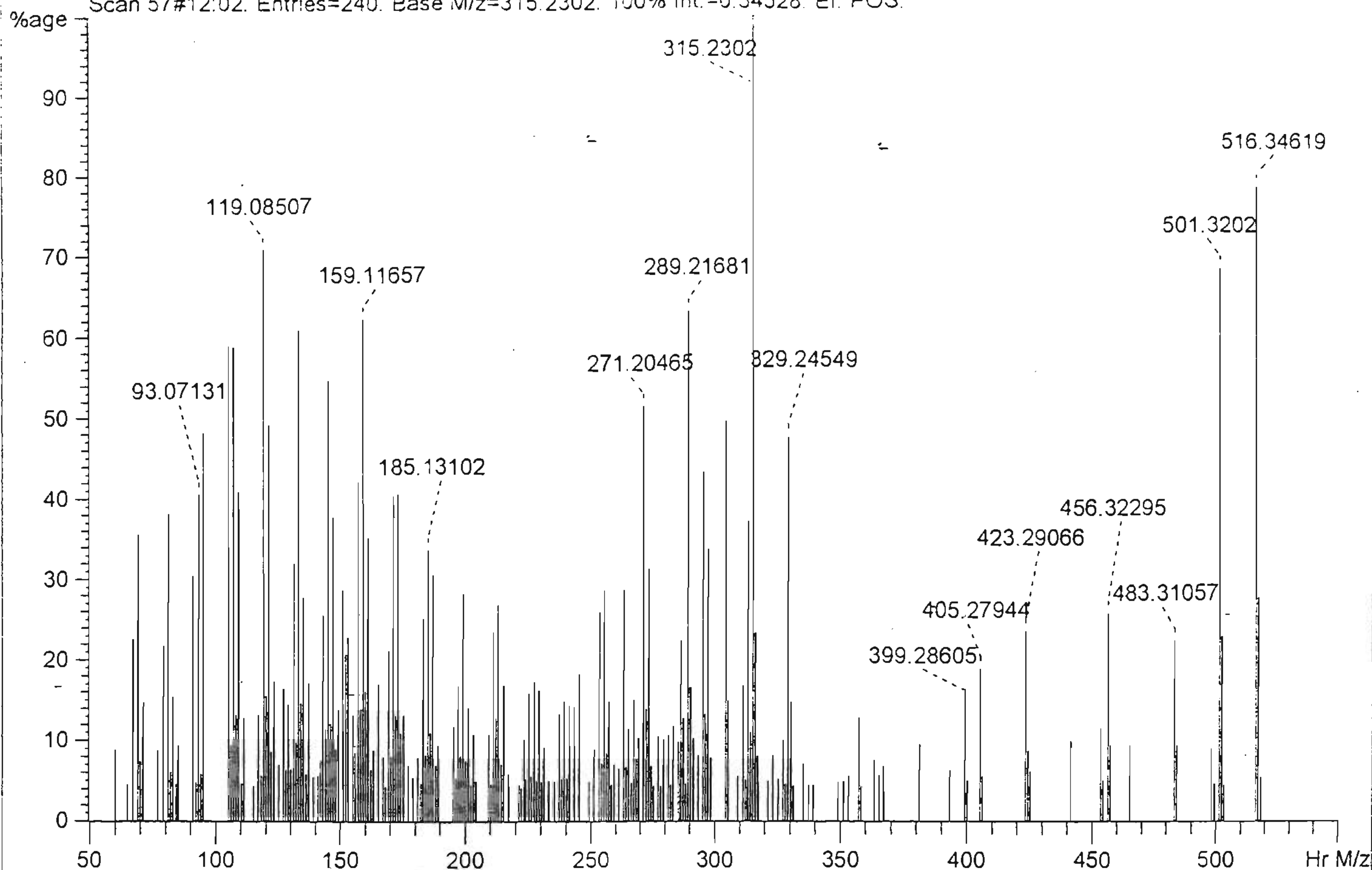
Gradient TOCSY expt.
probe=5mmASW

Pulse Sequence: tocsy_da





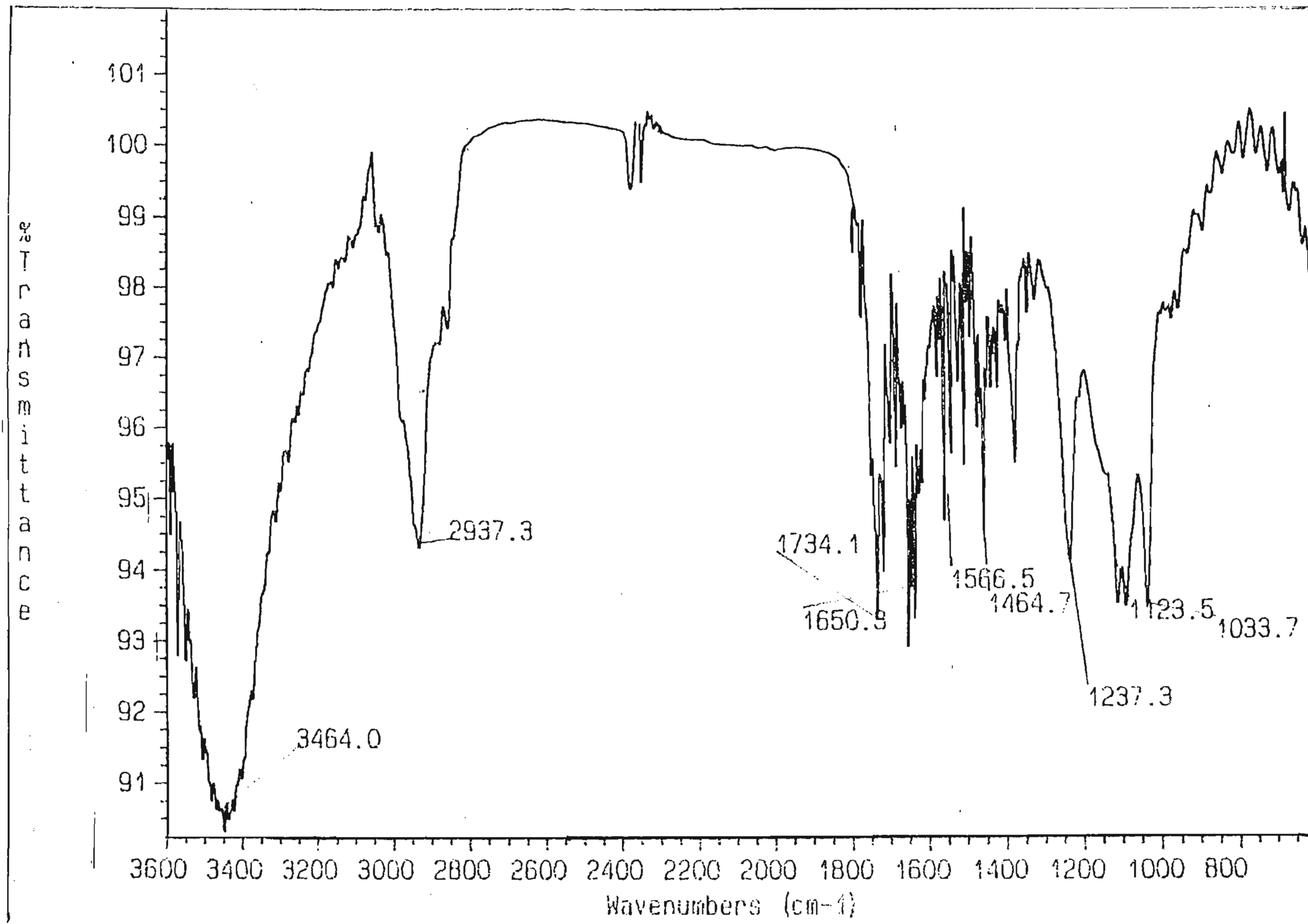
SPECTRUM 3.8 : TOCSY spectrum of compound 3 (C_5D_5N)



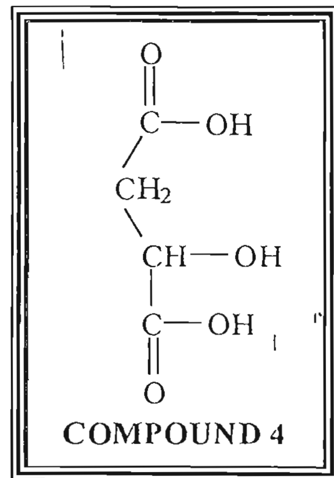
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:12%. Range:0-520. Excl: Ref/Ex.].
Scan 57#12:02. Entries=100. Base M/z=315.2302. 100% Int.=0.54528. EI. POS.

Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width
67.05214	22.50	58	146.10742	12.09	62	237.16438	13.18	64	423.29066	23.53	63
69.03344	35.53	56	147.11749	37.71	68	239.17780	14.74	69	456.32295	25.67	64
69.06912	29.52	57	149.12719	13.70	89	241.18648	14.25	75	483.31057	22.31	65
71.08474	14.64	56	150.99944	28.61	59	243.18309	14.09	78	501.32020	67.87	66
79.05347	21.68	58	152.08400	20.69	64	245.19191	18.16	68	502.32230	22.74	66
81.06902	38.06	57	153.09107	22.78	66	253.19079	25.91	71	516.34619	78.17	66
83.08575	15.32	57	155.08495	13.06	68	255.20503	28.52	73	517.34955	27.49	65
91.05426	30.37	58	157.10166	42.08	61	257.18788	14.71	69			
93.07131	40.55	58	158.10585	13.86	61	263.19705	28.52	65			
95.05144	43.98	55	159.11657	62.15	62	267.19001	14.97	76			
95.08718	48.12	55	160.12286	16.00	64	271.20465	51.29	66			
105.07170	58.86	58	161.13046	35.15	74	272.20438	13.78	66			
107.08688	58.71	60	165.09124	16.92	82	273.21874	31.16	67			
108.09280	13.15	72	169.10433	21.10	65	286.22550	22.30	69			
109.10236	40.79	100	171.11782	40.40	62	287.21068	12.64	71			
111.08368	12.65	102	172.12083	13.02	64	289.21681	62.88	64			
117.06970	13.16	60	173.13352	40.61	67	290.21874	16.43	63			
119.08507	70.72	60	175.14024	13.05	82	295.20875	43.10	65			
120.09262	15.45	59	183.11566	25.12	63	296.21455	13.11	63			
121.10121	49.00	69	185.13102	33.57	64	297.22237	33.60	63			
123.11081	17.26	98	187.14710	30.46	73	304.24229	49.38	63			
127.07592	16.31	64	197.13273	22.19	64	305.24220	14.77	66			
129.07019	14.41	71	199.14826	28.17	65	311.23447	16.73	69			
131.08548	31.90	59	201.15181	13.97	81	313.22037	37.12	64			
133.00657	13.34	58	211.15193	23.41	64	315.23020	100.00	63			
133.10061	60.83	61	211.98915	12.66	61	316.23683	23.20	63			
134.10666	14.55	63	213.16618	26.73	71	329.24549	47.42	64			
135.11611	27.67	87	215.17452	16.78	78	330.25024	14.69	63			
137.09654	17.01	94	225.16296	15.76	69	357.27707	12.76	64			
143.08395	25.41	59	227.17131	17.18	76	399.28605	16.28	63			
145.10126	54.67	60	229.16200	16.11	75	405.27944	18.77	63			

SPECTRUM 3.9 : Mass spectrum of compound 3



SPECTRUM 3.10 : Infrared spectrum of compound 3



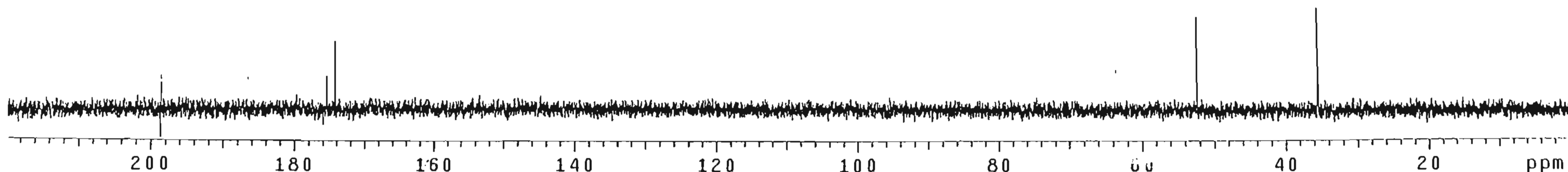
COMPOUND 4

9 8 7 6 5 4 3 2 1 ppm

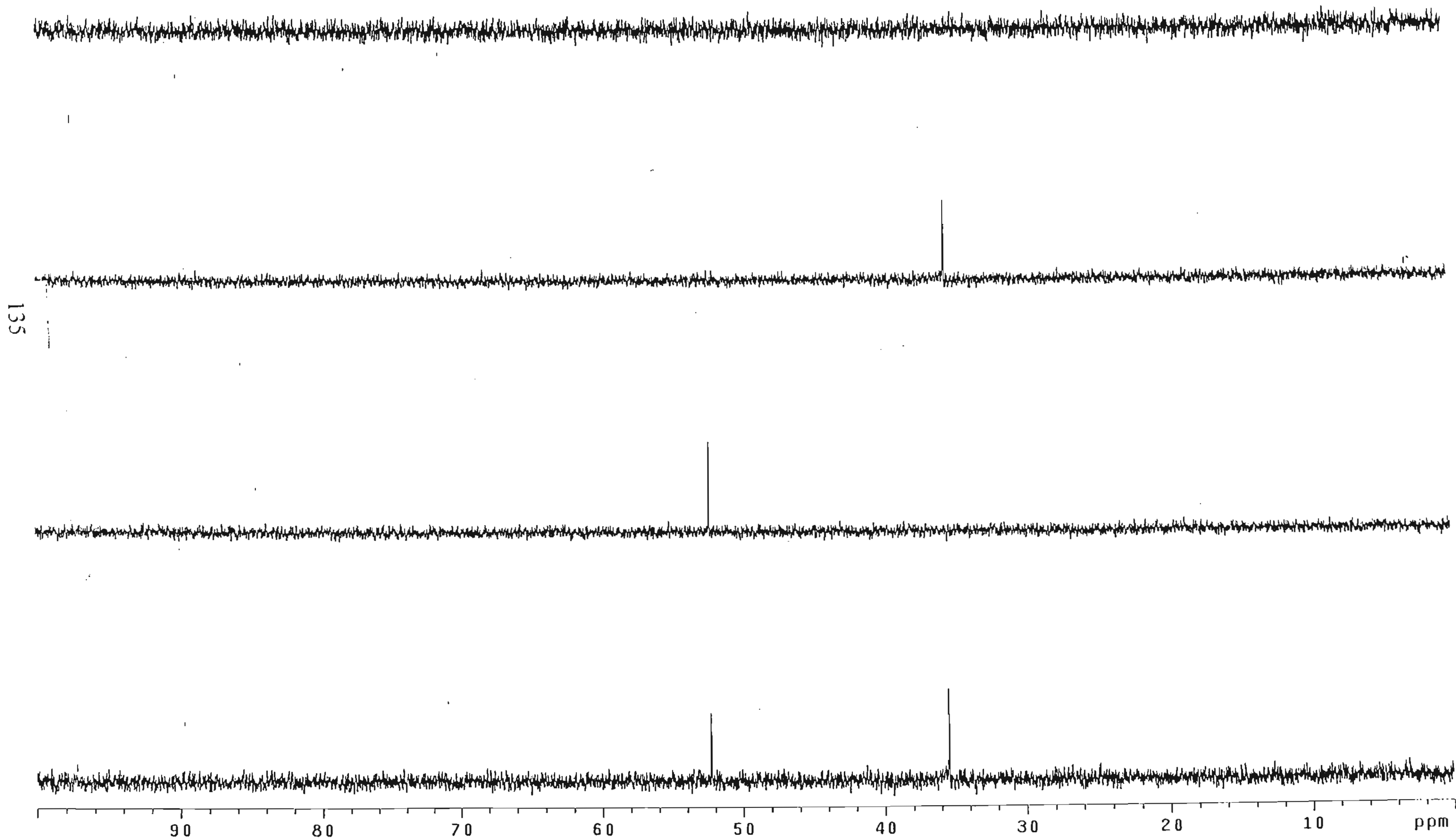
SPECTRUM 4.1 : ^{1}H NMR spectrum of compound 4 (D_2O)

```
clbxtl1.lb-meoh xstal1 in d2o

exp6  std13c

      SAMPLE                  DEC. & VT
date    Jan 27 1999   dfrq        399.952
solvent         D2O   dn               H1
file            exp   dpwr             38
   ACQUISITION        dof               0
sfrq        100.577   dm              yyy
tn              C13   dmm               w
at            1.199   dmf           12500
np            59968   dseq
sw          25000.0   dres            1.0
fb            14000   homo              n
bs               64       PROCESSING
tpwr             54   lb             1.00
pw              3.0   wtfile
d1            1.000   proc             ft
tof               0   fn         not used
nt            32000   math              f
ct             2304
alock             s   werr
gain       not used   wexp
      FLAGS           wbs
il                n   wnt
in                n
dp                y
hs               nn
     DISPLAY
sp            -24.0
wp          22127.4
vs               17
sc                0
wc              250
hzmm          88.51
is           500.00
rfl         15234.4
rfp         12337.8
th                4
ins           1.000
nm       ph
```

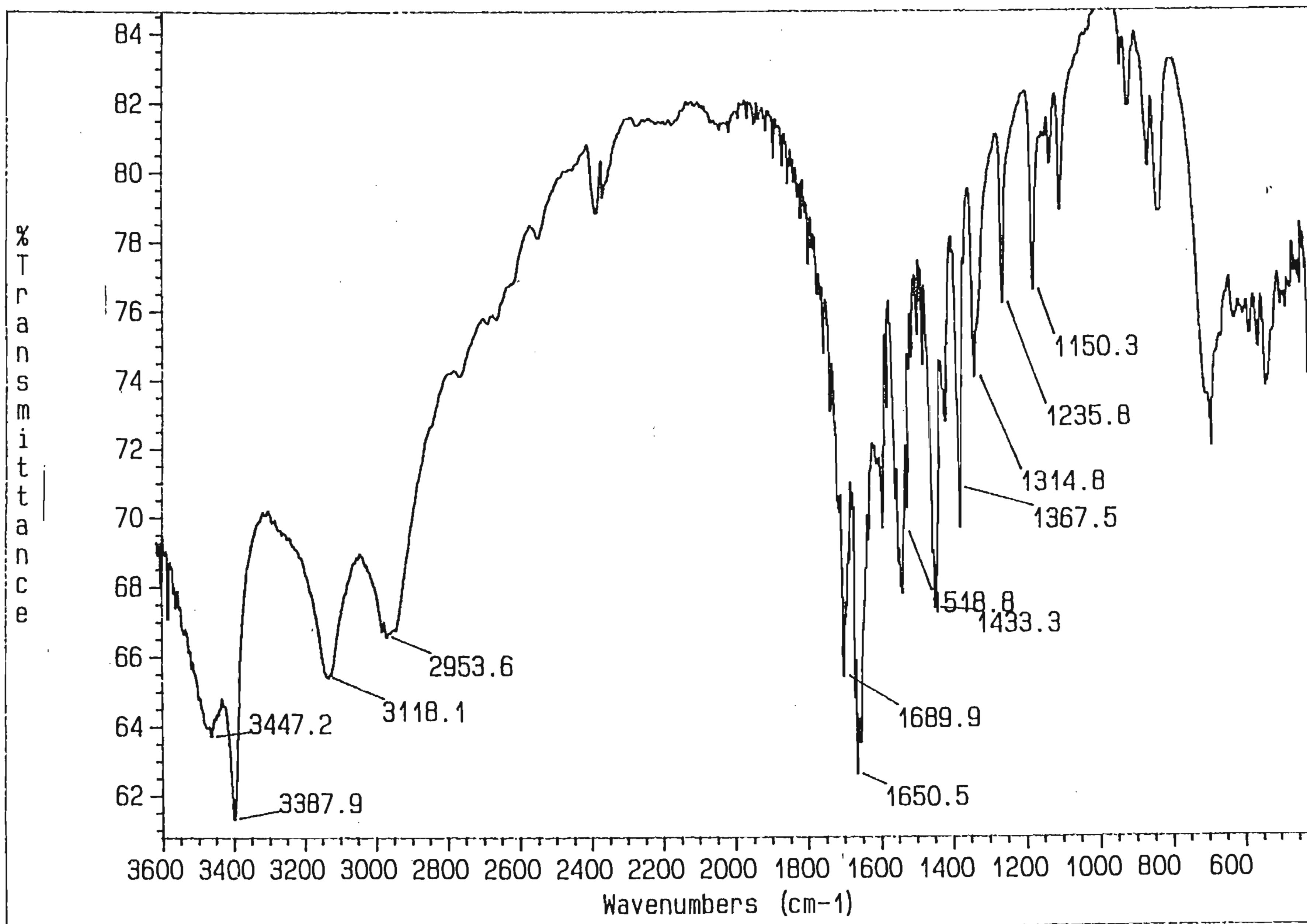
INDEX	FREQUENCY	PPM	HEIGHT
1	17639.325	175.377	5.9
2	17524.881	174.239	11.6
3	5254.914	52.246	15.8
4	3563.426	35.429	17.1



SPECTRUM 4.2 : ^{13}C NMR spectrum of compound 4 (D_2O)



SPECTRUM 4.3 : ADEPT spectrum of compound 4 (D_2O)

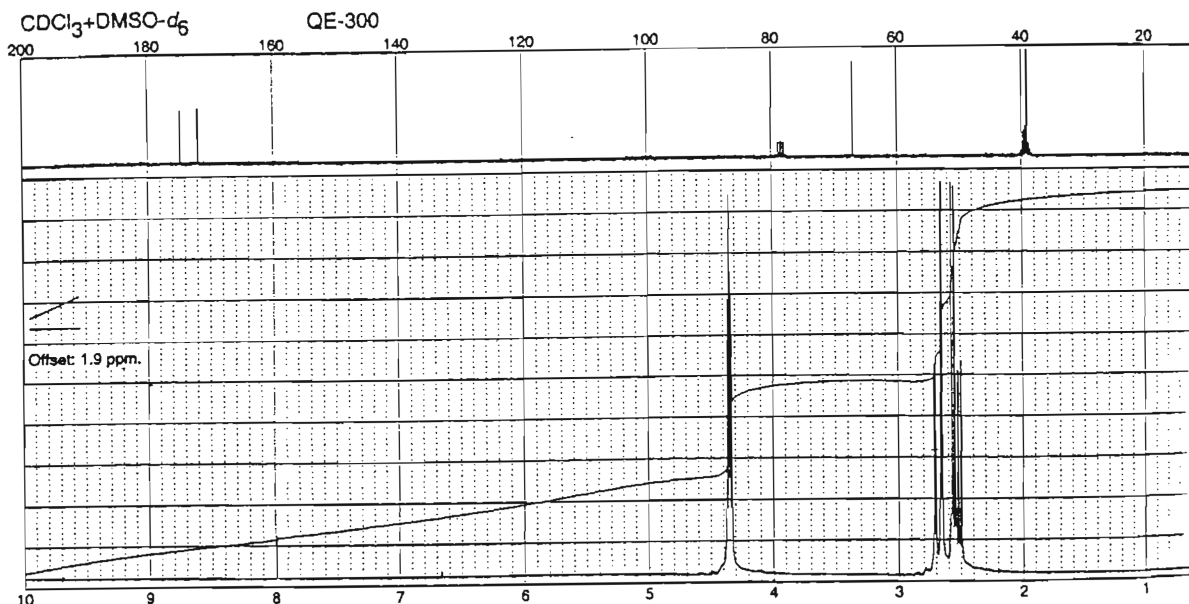


SPECTRUM 4.5 : Infrared spectrum of compound 4

D-Malic acid, 99%

FW 134.09
mp 100°C

FT-IR: *1*, 522B

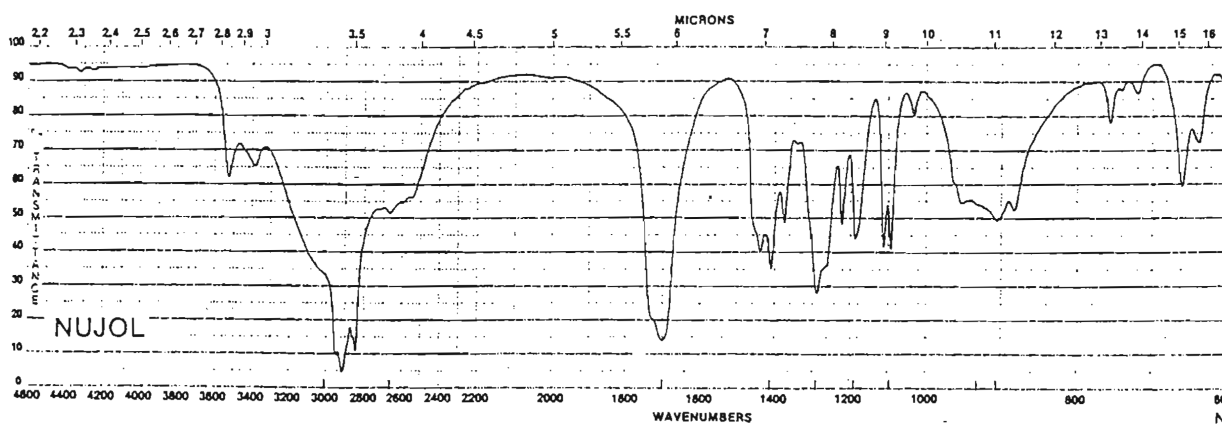


SPECTRUM 4.6A : ^{1}H NMR spectrum from literature

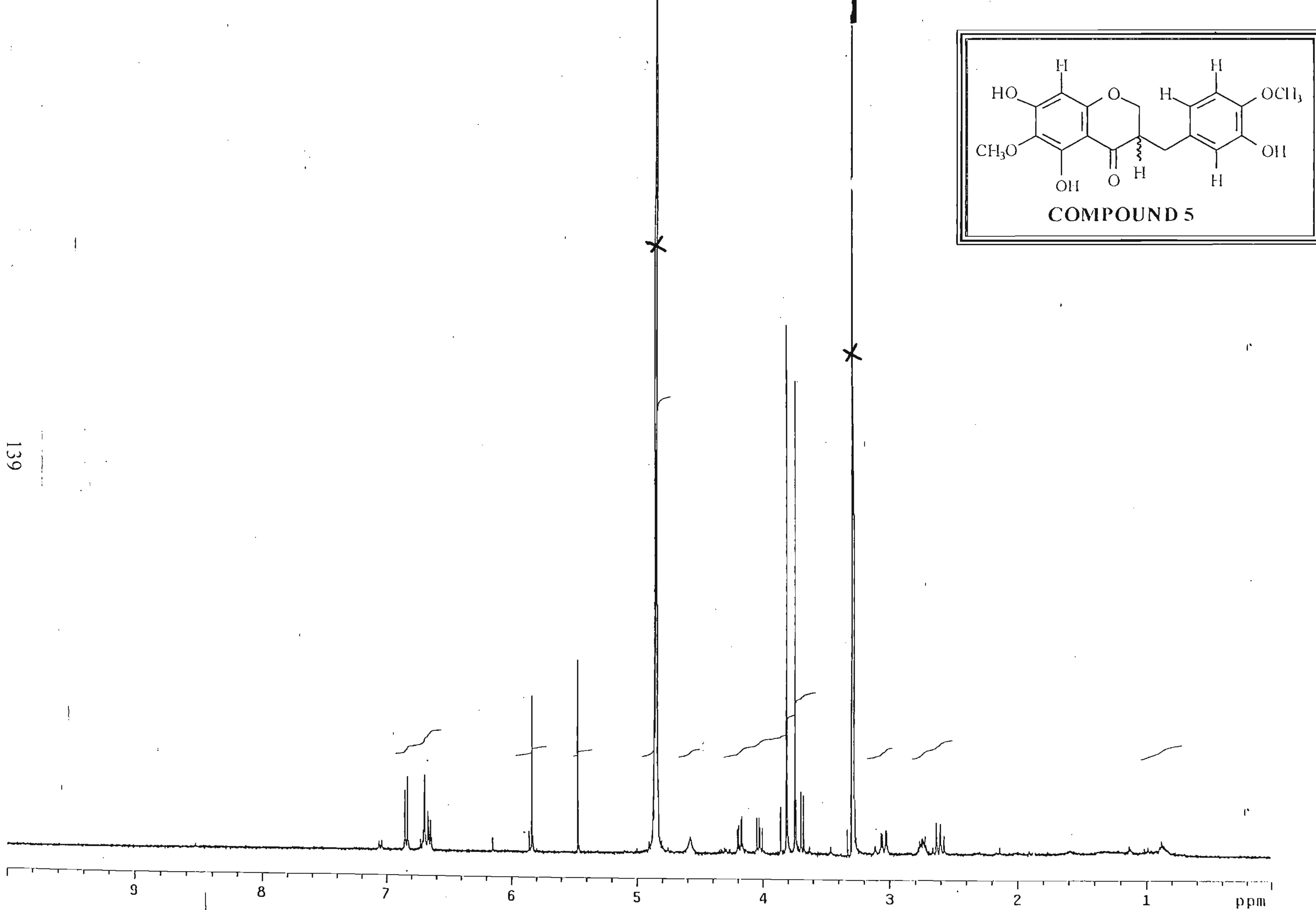
M0750 *CAS* [636-61-3]
D(+)MALIC ACID 98-100%

FW 134.1
mp 98-102°C
$[\alpha]_D^{23}$ +27.0° (c = 5.0, PYRIDINE)

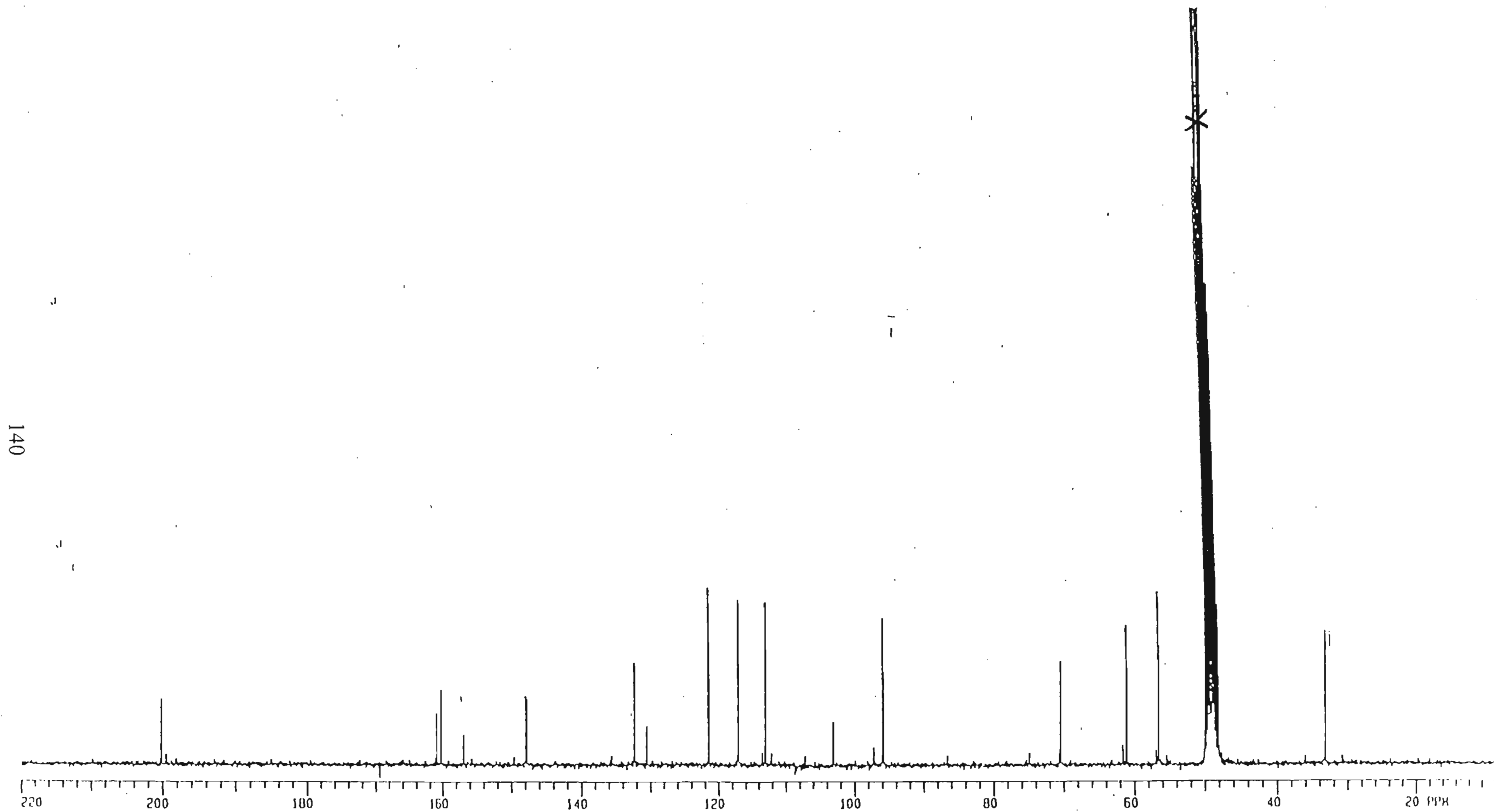
3016.2
2671.4
1704.1



SPECTRUM 4.6B : Infrared spectrum from literature

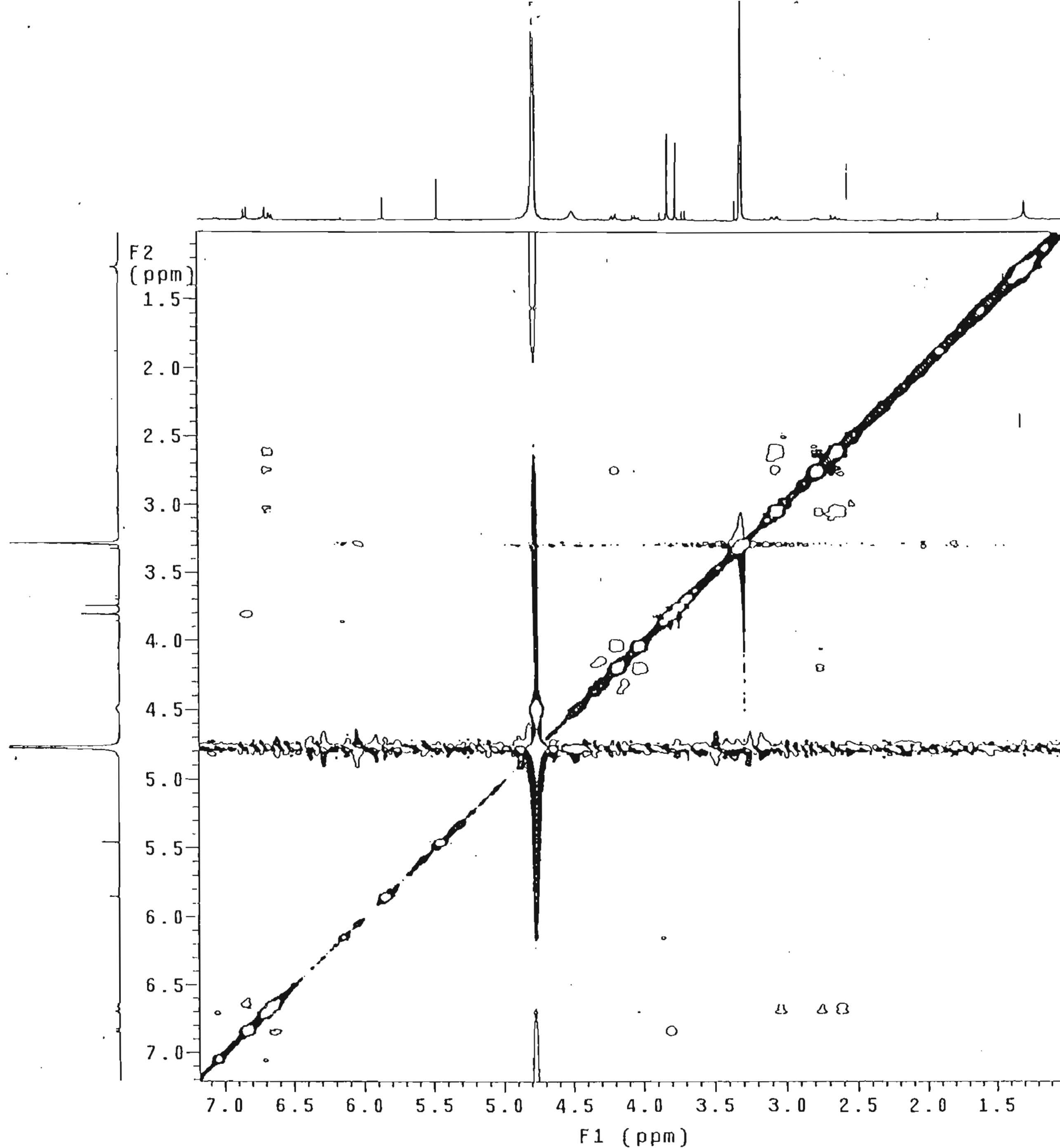


SPECTRUM 5.1 : ^{1}H NMR spectrum of compound 5 (CD_3OD)



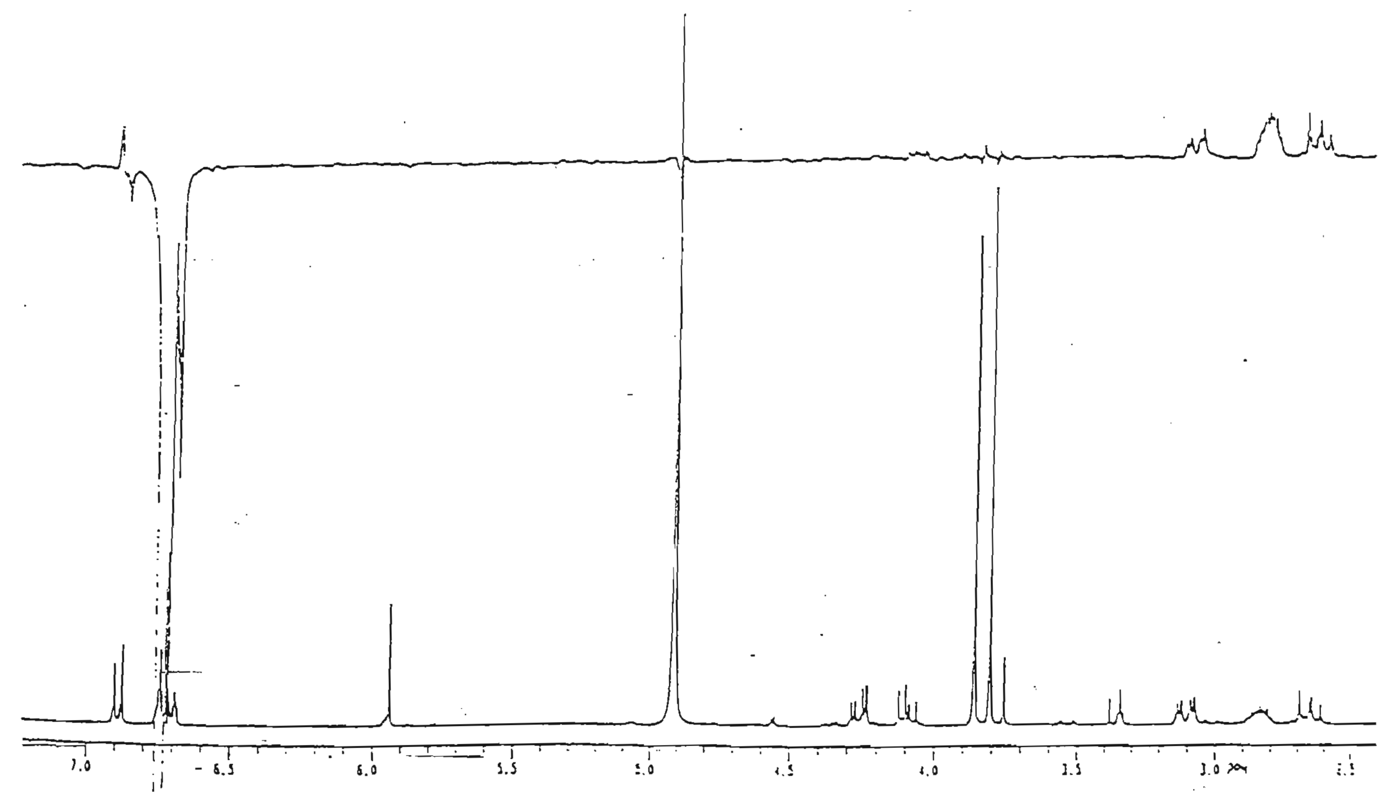
SPECTRUM 5.2 : ^{13}C NMR spectrum of compound 5 (CD_3OD)

Solvent: cd3od
Temp. 30.0 C / 303.1 K
INOVA-400 "undnmr400"
PULSE SEQUENCE: noesy
Relax. delay 3.000 sec
Mixing 0.750 sec
Acq. time 0.181 sec
Width 2832.7 Hz
2D Width 2832.7 Hz
32 repetitions
2 x 256 increments
OBSERVE H1, 399.9502544 MHz
DECOUPLE H1, 399.9522542 MHz
Power 30 dB
off during acquisition
on during delay
single frequency
DATA PROCESSING
Gauss apodization 0.084 sec
F1 DATA PROCESSING
Gauss apodization 0.042 sec
FT size 1024 x 1024
Total time 18.1 hours

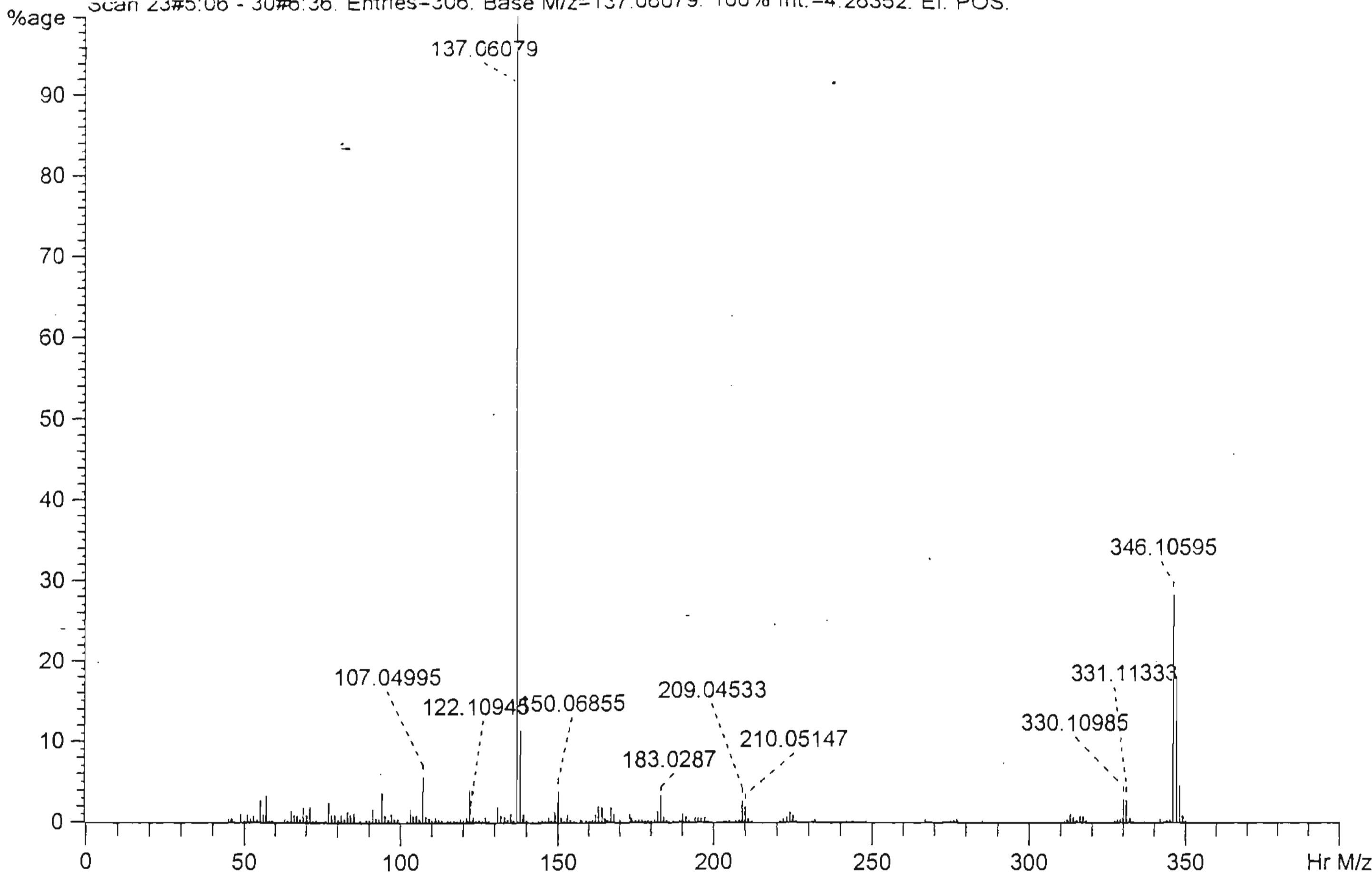


SPECTRUM 5.3 : NOESY spectrum of compound 5 (CD_3OD)

SPECTRUM 5.4A : NOE spectrum of compound 5 (CD_3OD)



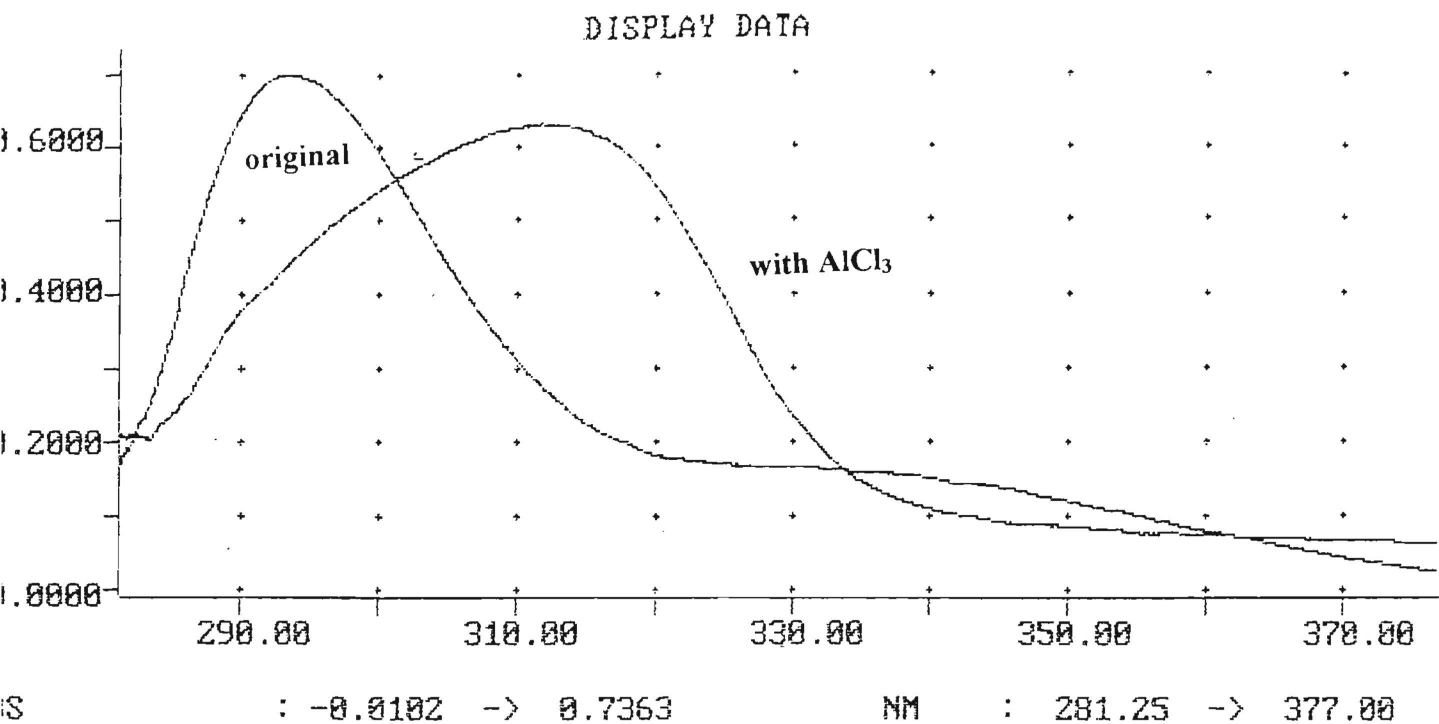
SPECTRUM 5.4B : NOE spectrum of compound 5 (CD_3OD)



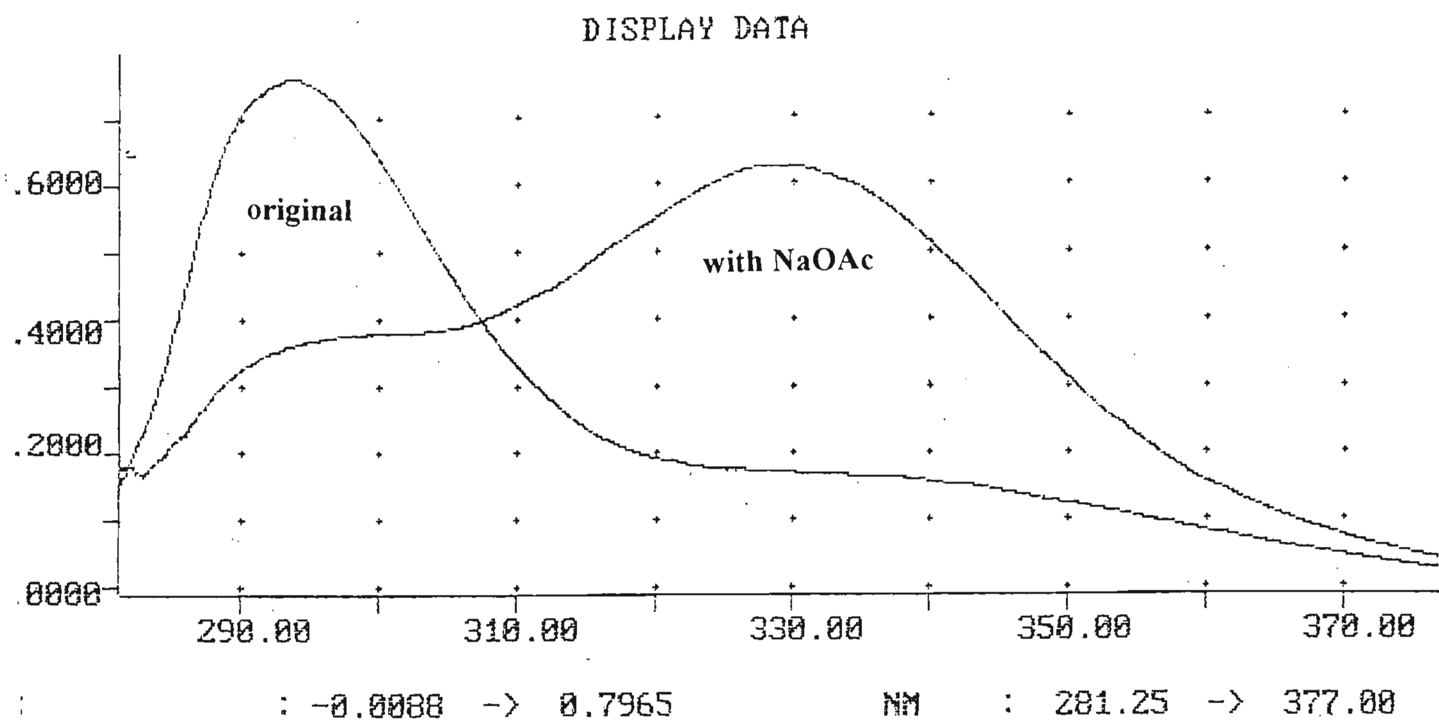
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:1%. Excl: Ref/Ex.].
Scan 23#5:06 - 30#6:36. Entries=39. Base M/z=137.06079. 100% Int.=4.26352. EI. POS.

Hr M/z	%age	Width
55.01864	2.75	39
55.05515	2.53	38
57.07085	3.31	39
65.03904	1.48	38
69.07044	1.85	37
71.08605	1.90	37
77.03862	2.44	38
83.08579	1.28	36
85.10153	1.11	35
91.05465	1.59	37
94.04193	3.65	38
97.10226	1.04	35
103.05499	1.58	37
107.04995	5.65	38
122.03691	4.08	38
131.05009	1.88	36
135.04478	1.10	35
137.06079	100.00	36
138.06485	11.35	37
139.06708	1.02	51
149.02488	1.32	35
150.06855	3.84	36
162.06857	1.02	34
163.07638	1.99	37
164.08326	1.82	35
166.99796	1.85	36
168.00473	1.01	48
173.05320	1.00	33
182.02093	1.30	60
183.02870	3.38	42
209.04533	2.58	34
210.05147	1.85	33
224.06810	1.25	34
313.07153	1.04	33
330.10985	2.80	37
331.11333	2.68	42
346.10595	28.01	34
347.11137	17.77	34
348.11539	4.54	34

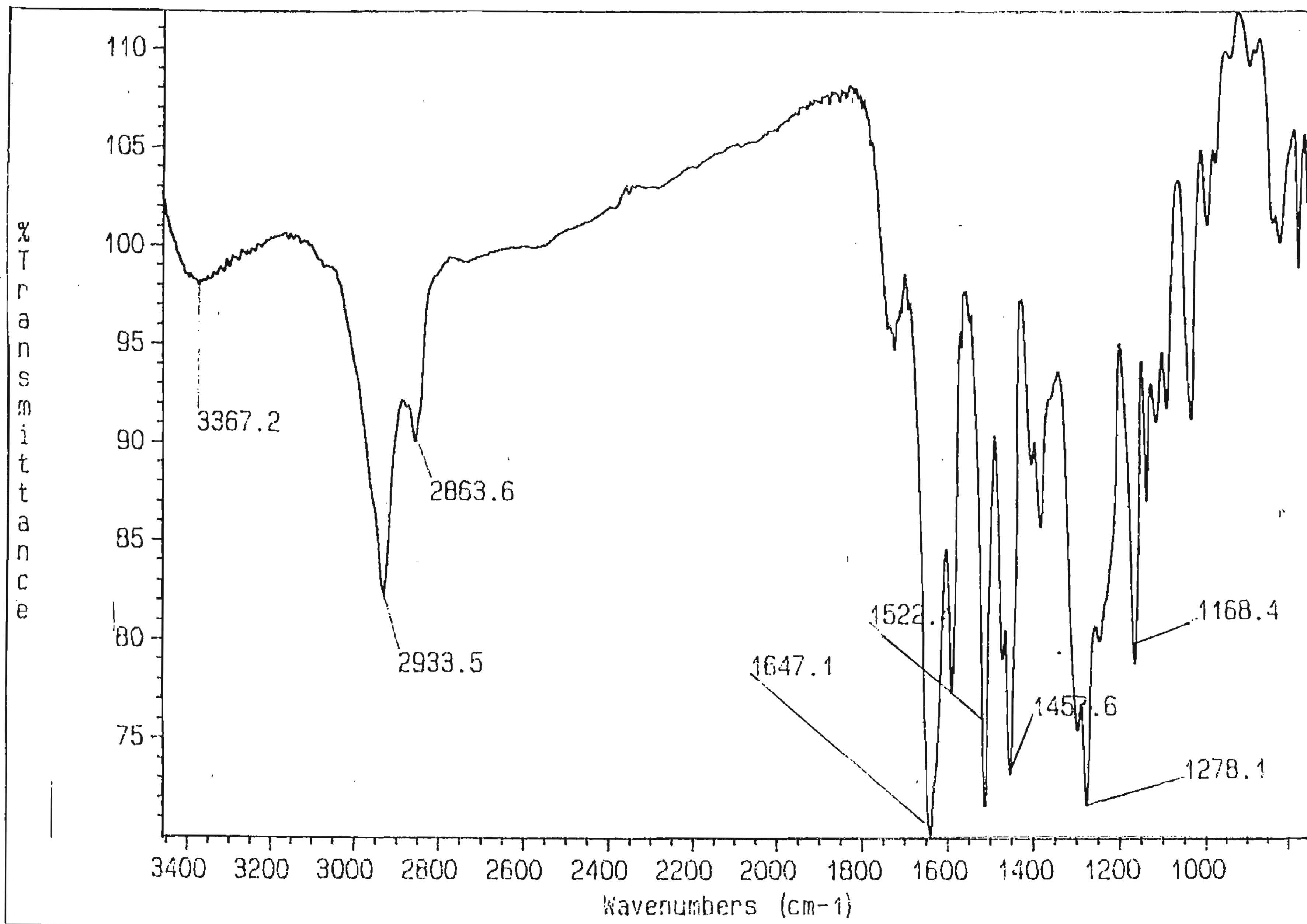
SPECTRUM 5.5 : Mass spectrum of compound 5



SPECTRUM 5.6A : UV spectrum of compound 5 (original and with $AlCl_3$)

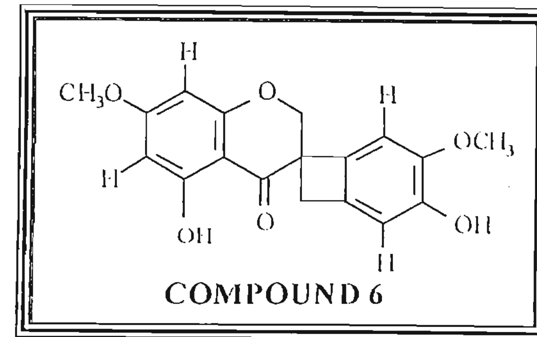


SPECTRUM 5.6B : UV spectrum of compound 5 (original and with NaOAc)



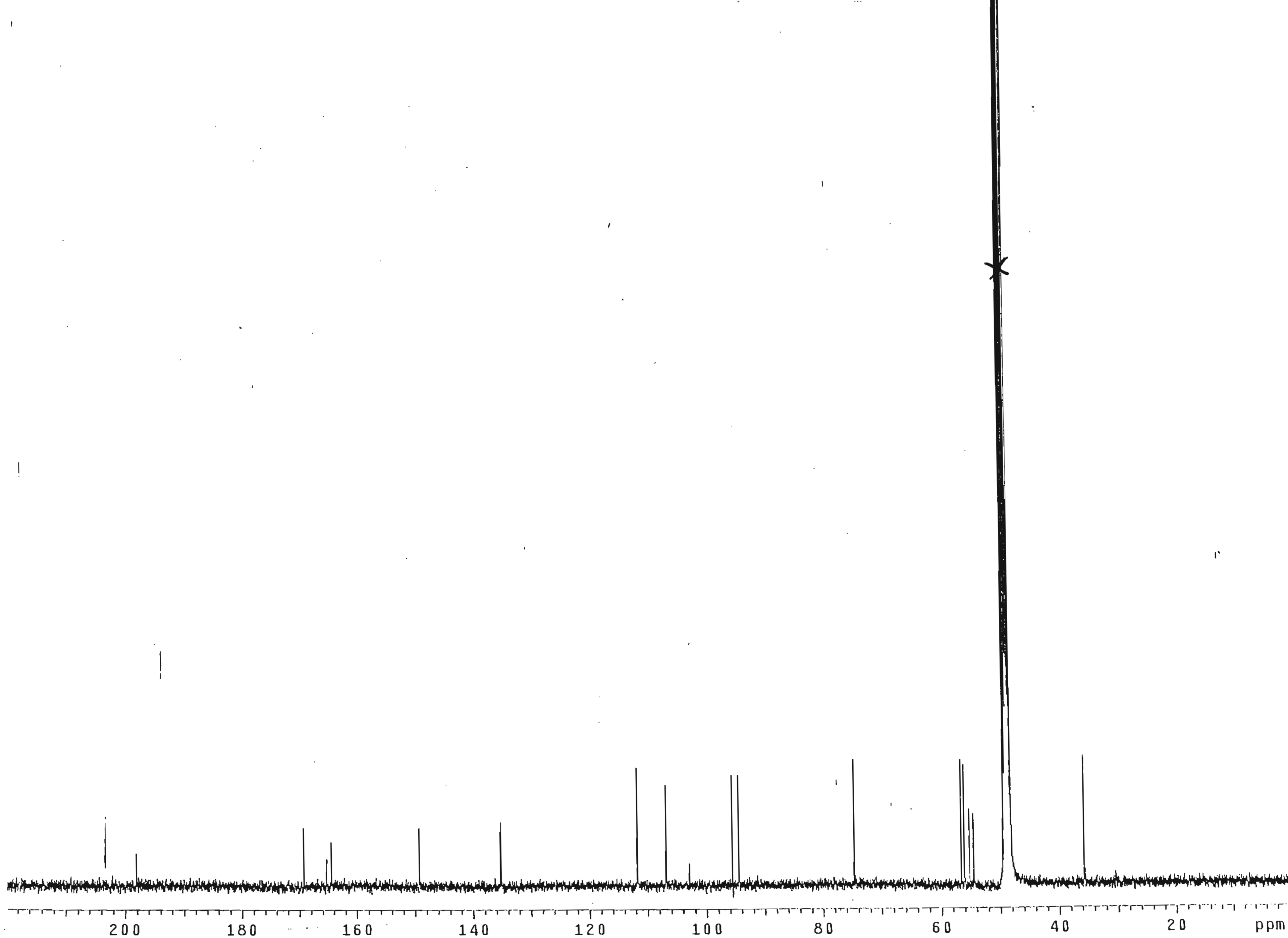
SPECTRUM 5.7 : Infrared spectrum of compound 5

Pulse Sequence: s2pul



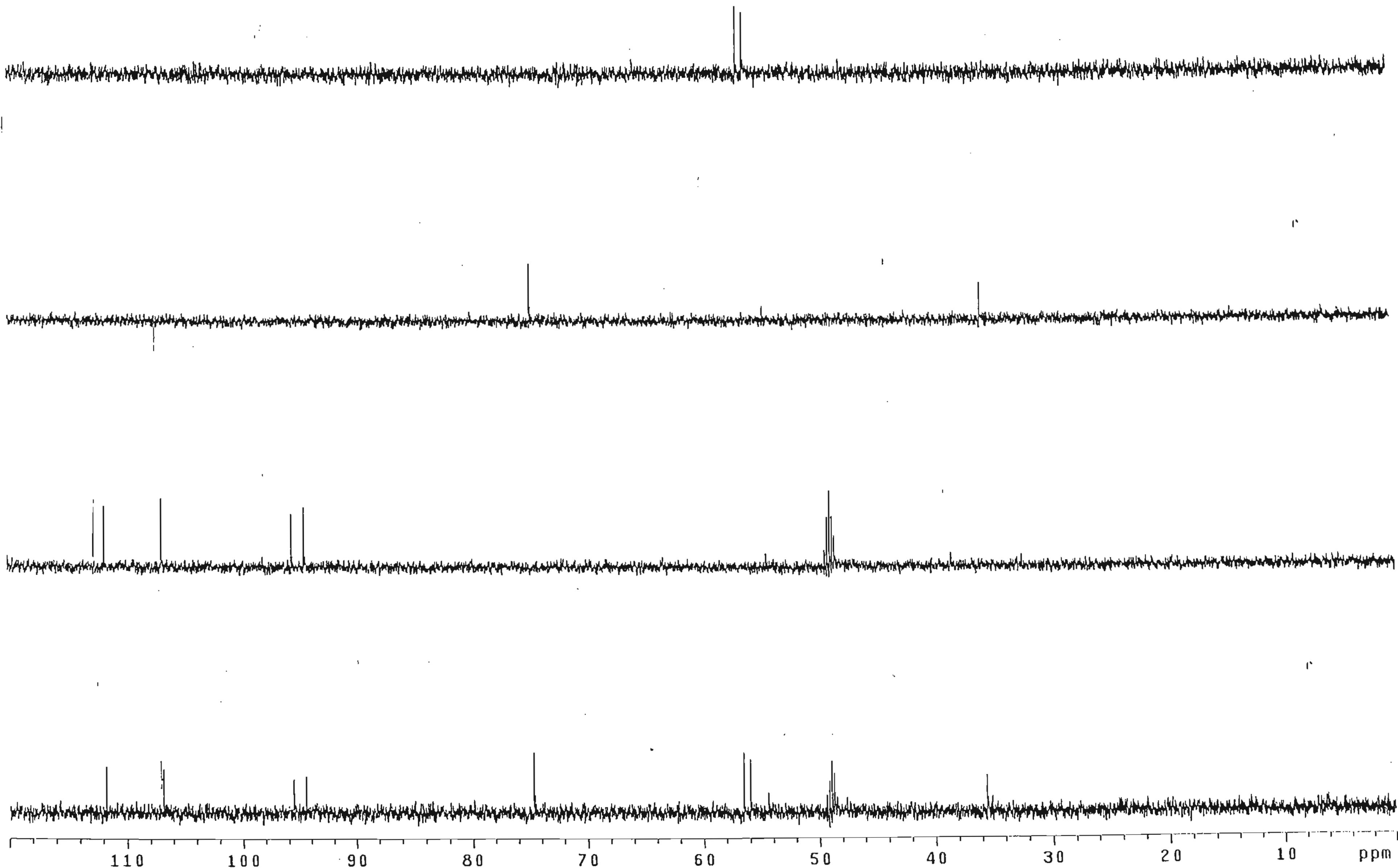
9 8 7 6 5 4 3 2 1 ppm

SPECTRUM 6.1 : ^{1}H NMR spectrum of compound 6 (CD_3OD)



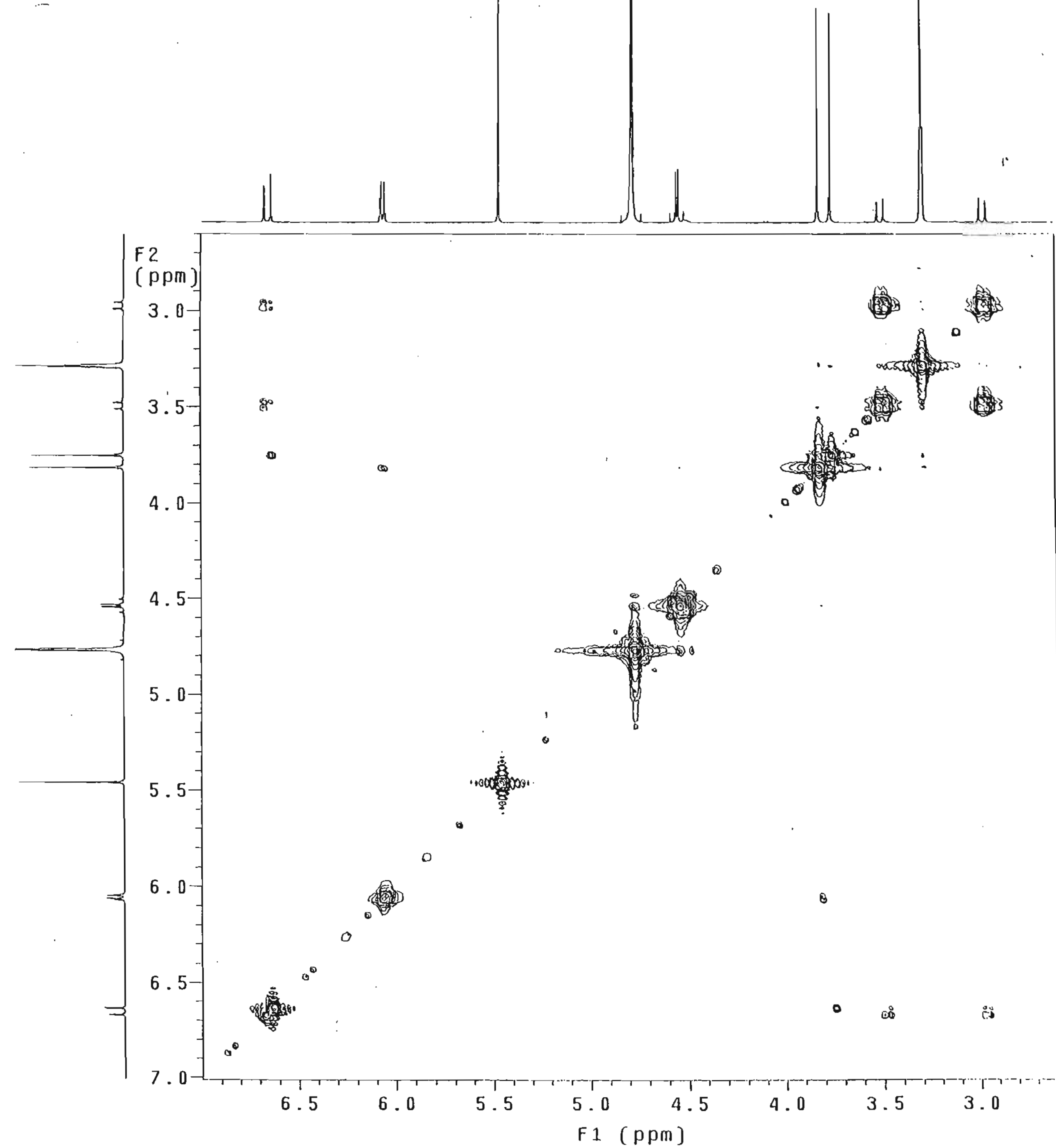
SPECTRUM 6.2 : ^{13}C NMR spectrum of compound 6 (CD_3OD)

Pulse Sequence: dept



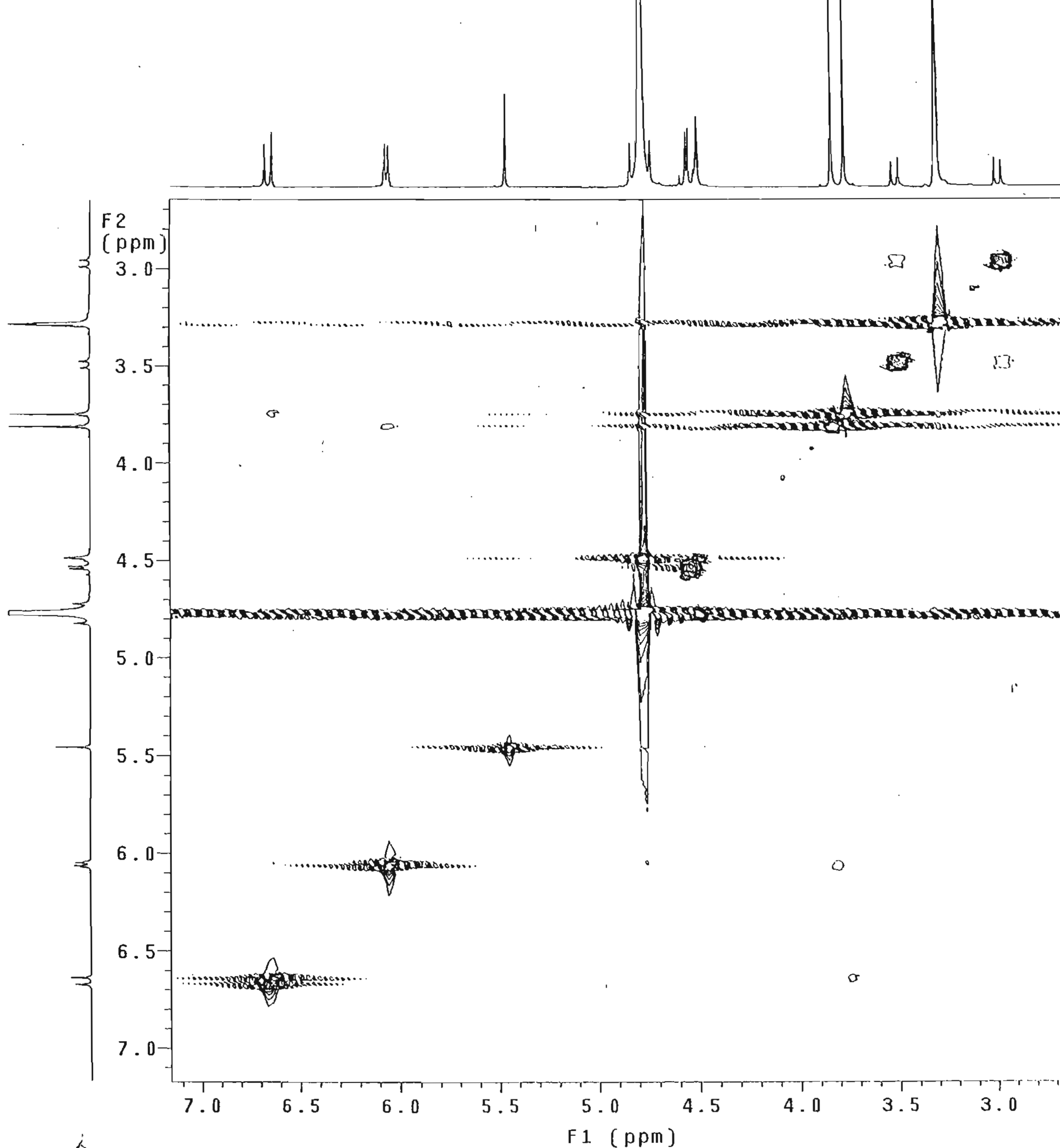
SPECTRUM 6.3 : ADEPT spectrum of compound 6 (CD_3OD)

Solvent: cd3od
Temp. 30.0 C / 303.1 K
INOVA-400 "undnmr400"
PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.187 sec
Width 2740.4 Hz
2D Width 2740.4 Hz
16 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.093 sec
F1 DATA PROCESSING
Sine bell 0.047 sec
FT size 1024 x 1024
Total time 84 minutes

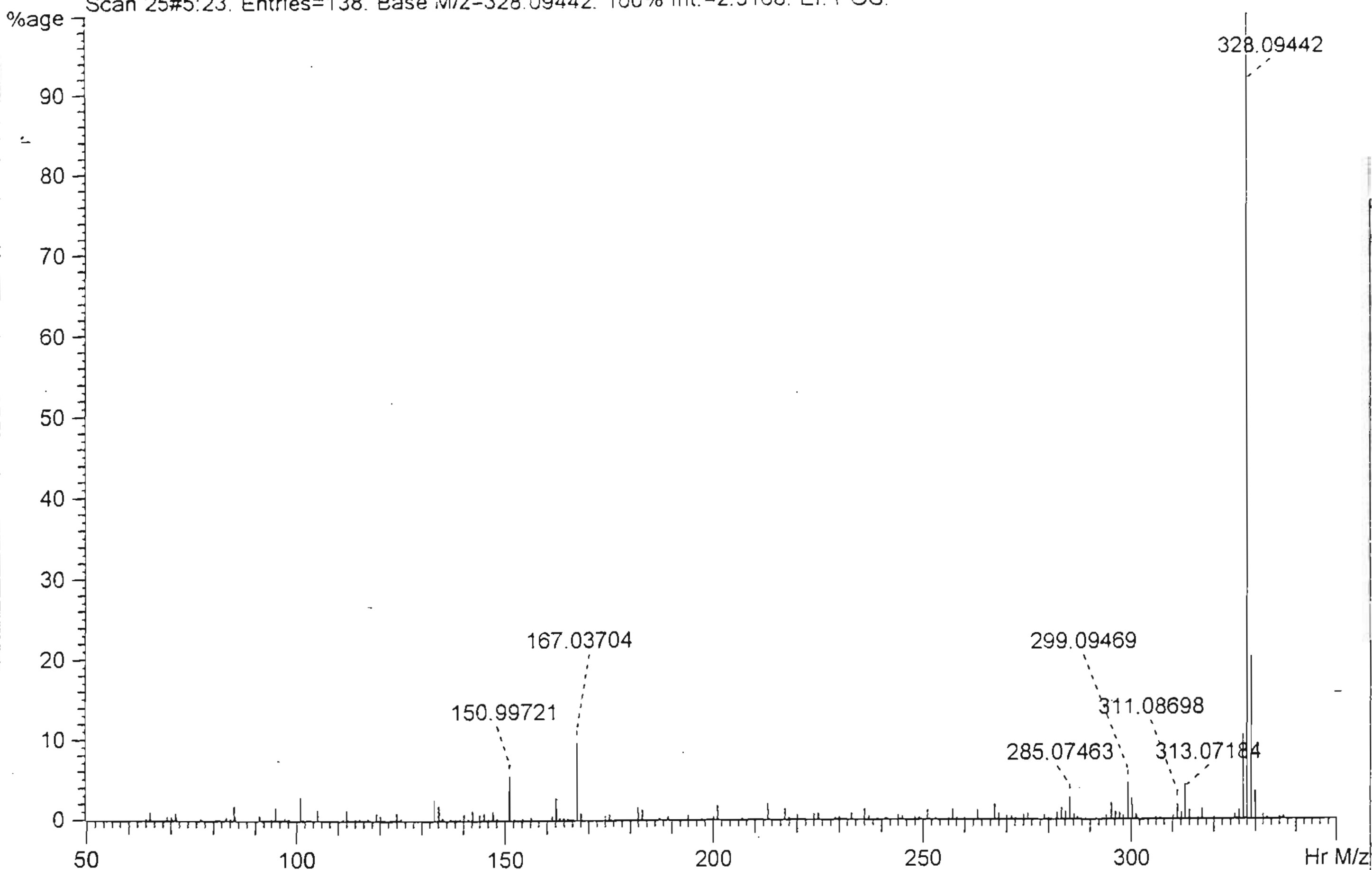


SPECTRUM 6.4 : COSY spectrum of compound 6 (CD_3OD)

```
temp=30

Pulse Sequence: noesy
 Solvent: cd3od
 Temp. 30.0 C / 303.1 K
INOVA-400  "undnmr400"
PULSE SEQUENCE: noesy
 Relax. delay 3.000 sec
 Mixing 0.750 sec
 Acq. time 0.173 sec
 Width    2953.1 Hz
 2D Width 2953.1 Hz
 16 repetitions
 2 x 123 increments
OBSERVE   H1, 399.9502545 MHz
DECOUPLE  H1, 399.9522542 MHz
 Power 38 dB
 off during acquisition
 on during delay
 single frequency
DATA PROCESSING
 Line broadening 1.0 Hz
F1 DATA PROCESSING
 Line broadening 0.3 Hz
FT size 1024 x 512
Total time 4 hr, 20 min, 2 sec
```



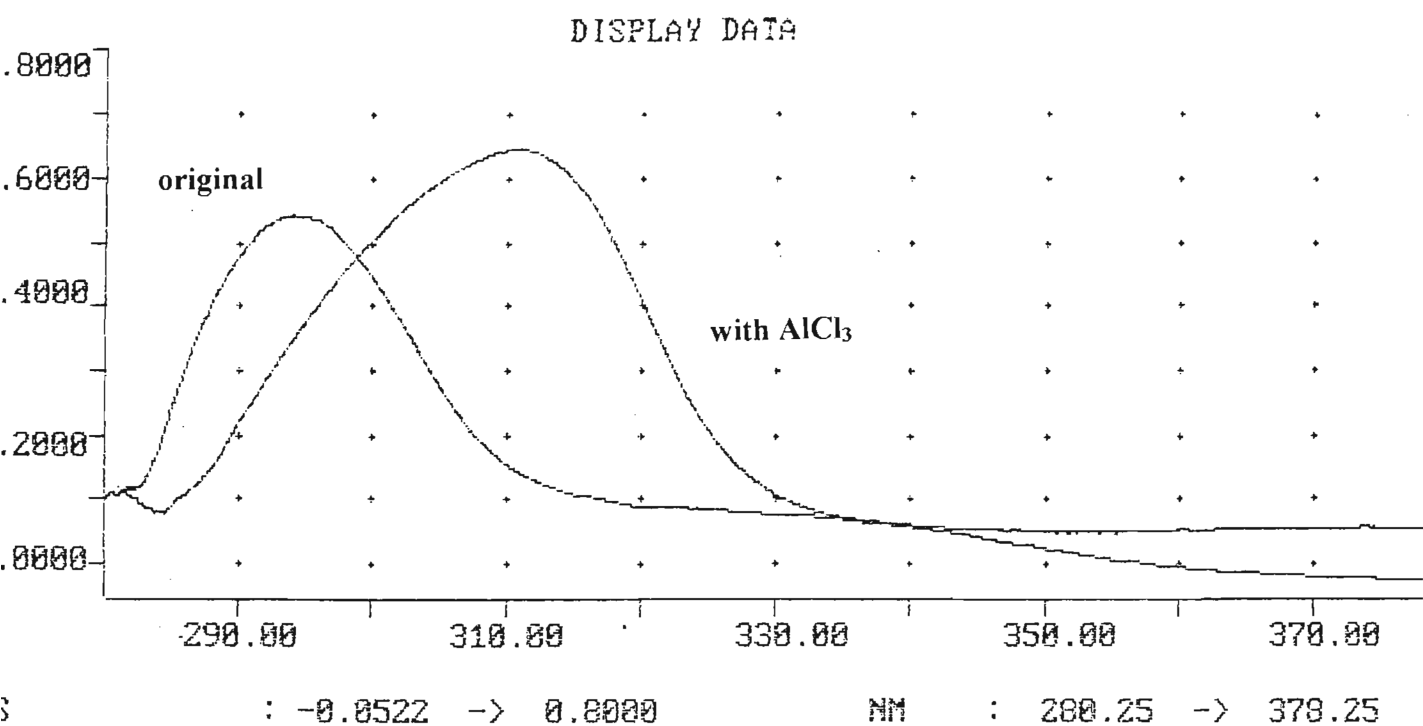
SPECTRUM 6.5 : NOESY spectrum of compound 6 (CD_3OD)



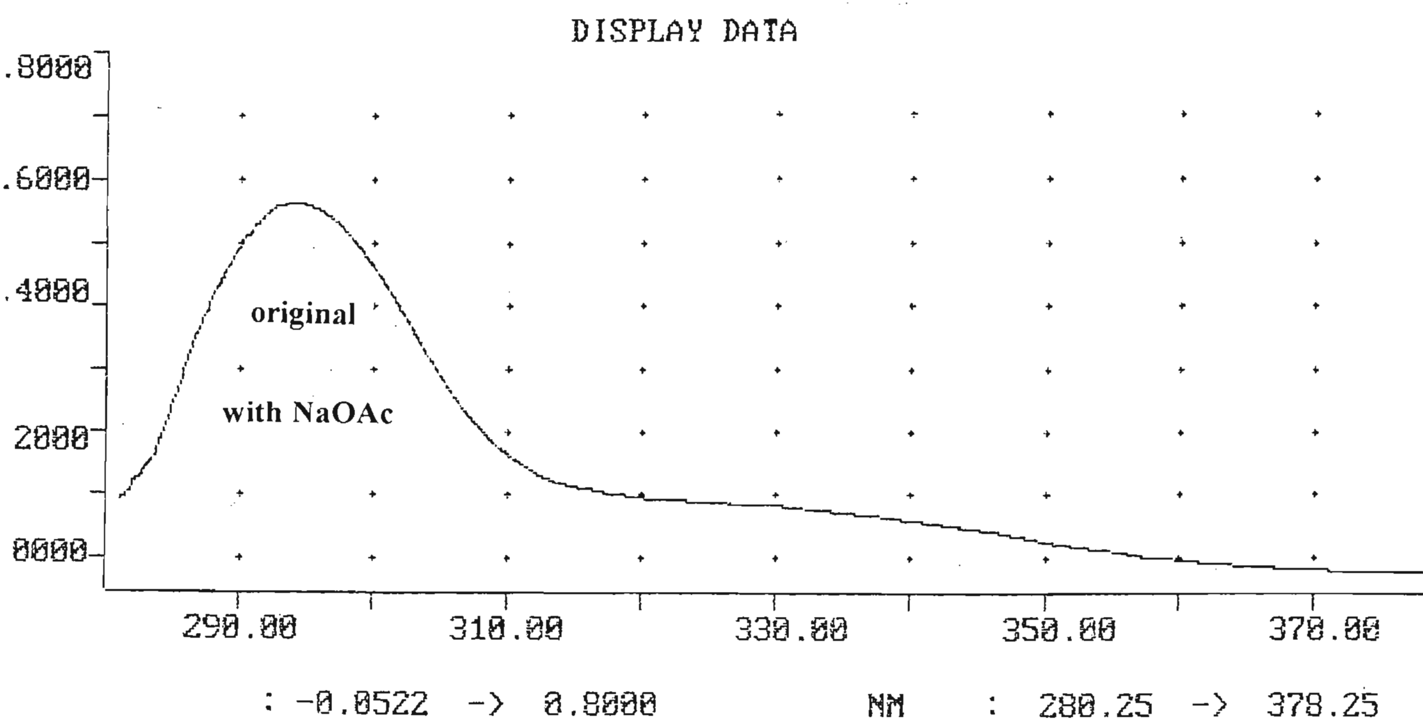
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:2%. Range:0-340. Excl: Ref/Ex.].
Scan 25#5:23. Entries=13. Base M/z=328.09442. 100% Int.=2.3168. EI. POS.

Hr M/z	%age	Width
100.99964	2.86	56
133.00901	2.51	56
150.99721	5.45	57
162.06758	2.78	56
167.03704	9.60	60
285.07463	2.63	58
299.09469	4.39	63
300.10292	2.60	60
313.07184	4.20	80
327.08552	10.38	66
328.09442	100.00	61
329.09705	20.10	62
330.10087	3.36	60

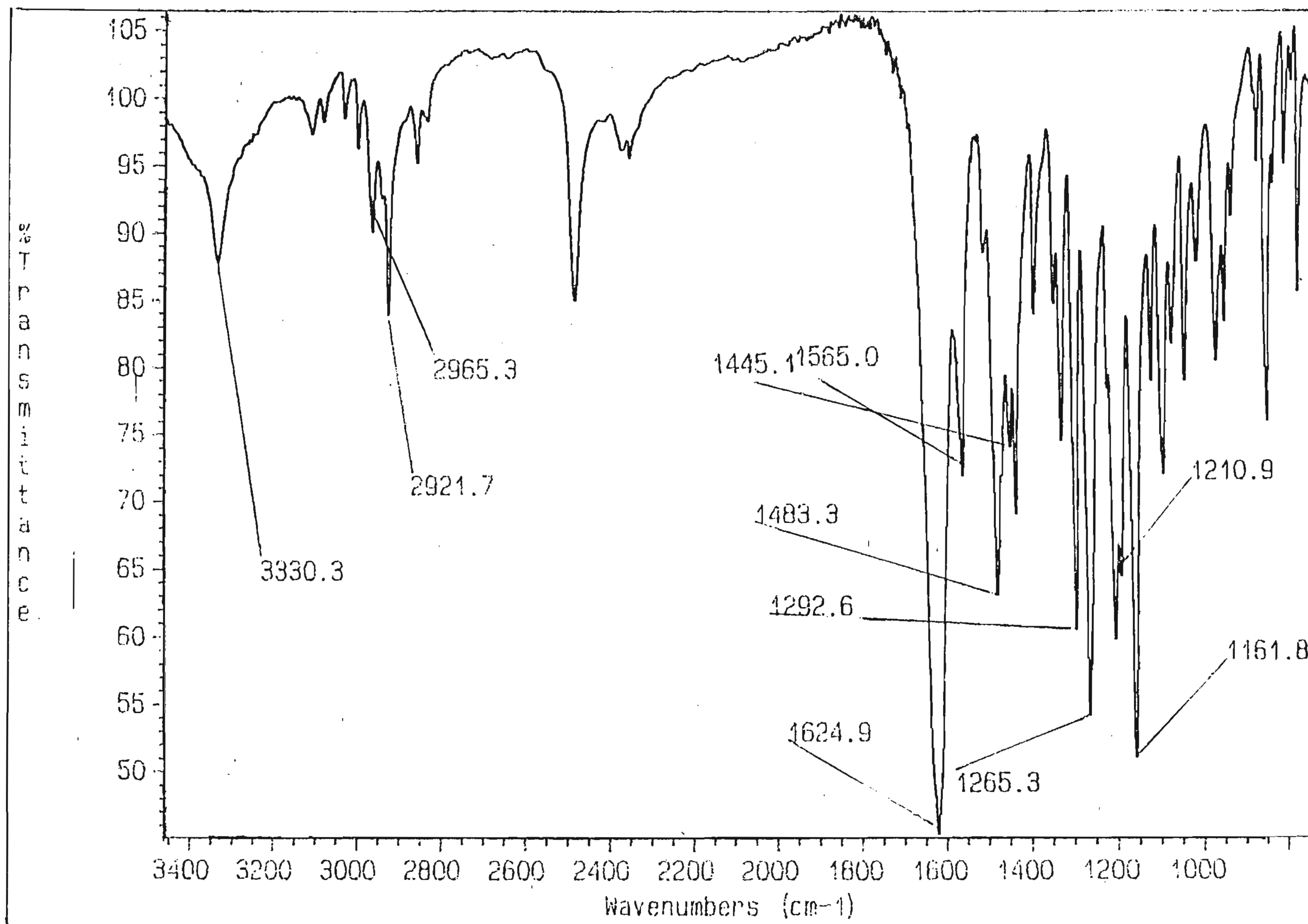
SPECTRUM 6.6 : Mass spectrum of compound 6



SPECTRUM 6.7A : UV spectrum of compound 6 (original and with $AlCl_3$)

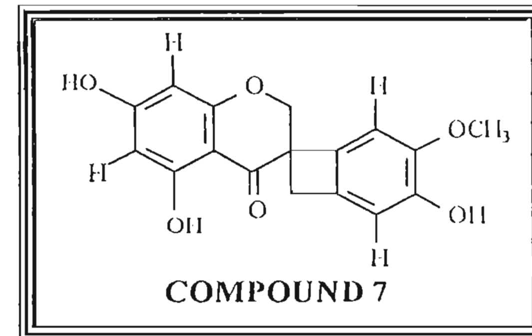


SPECTRUM 6.7B : UV spectrum of compound 6 (original and with NaOAc)



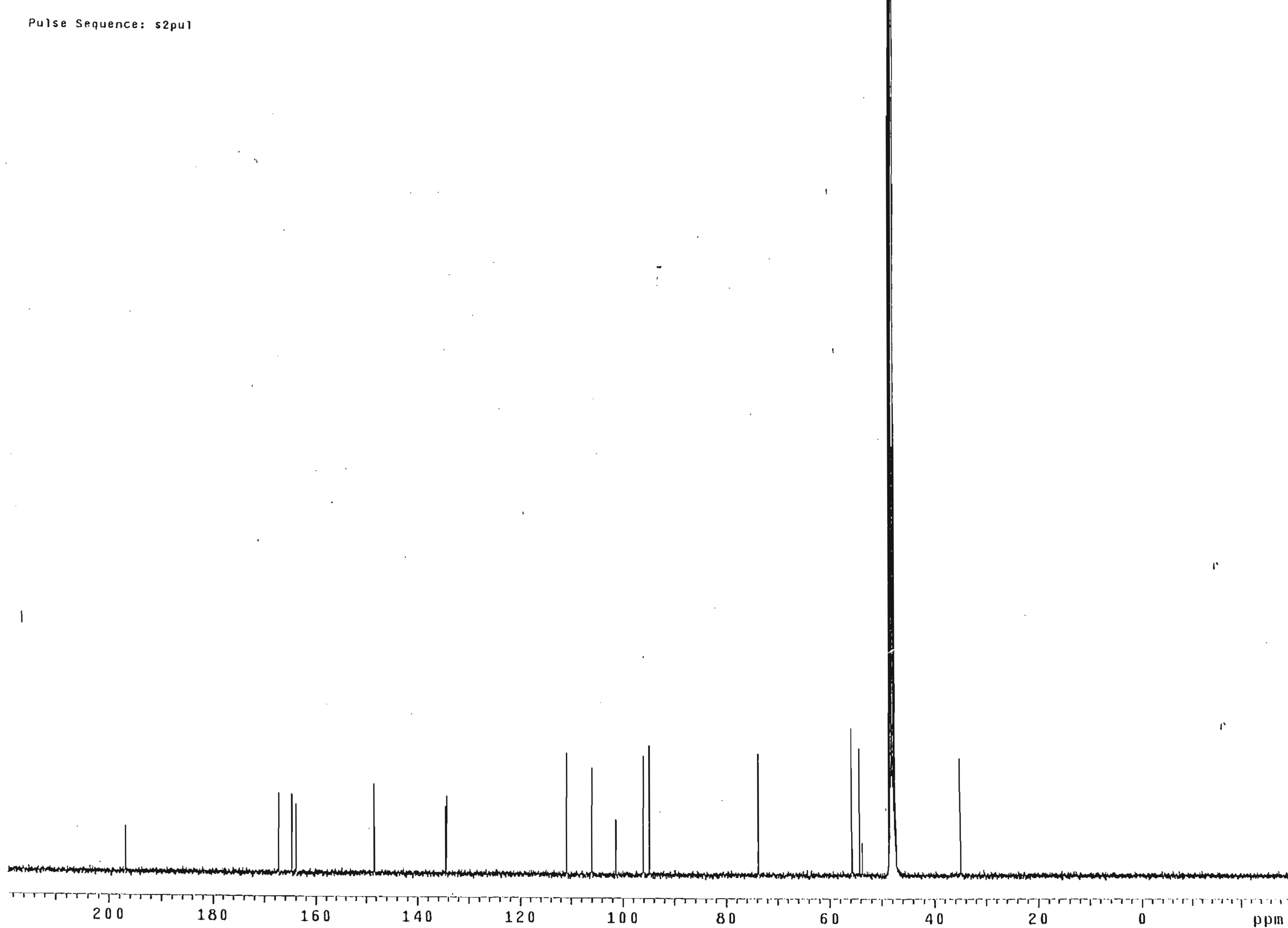
SPECTRUM 6.8 : Infrared spectrum of compound 6

Pulse Sequence: s2pul

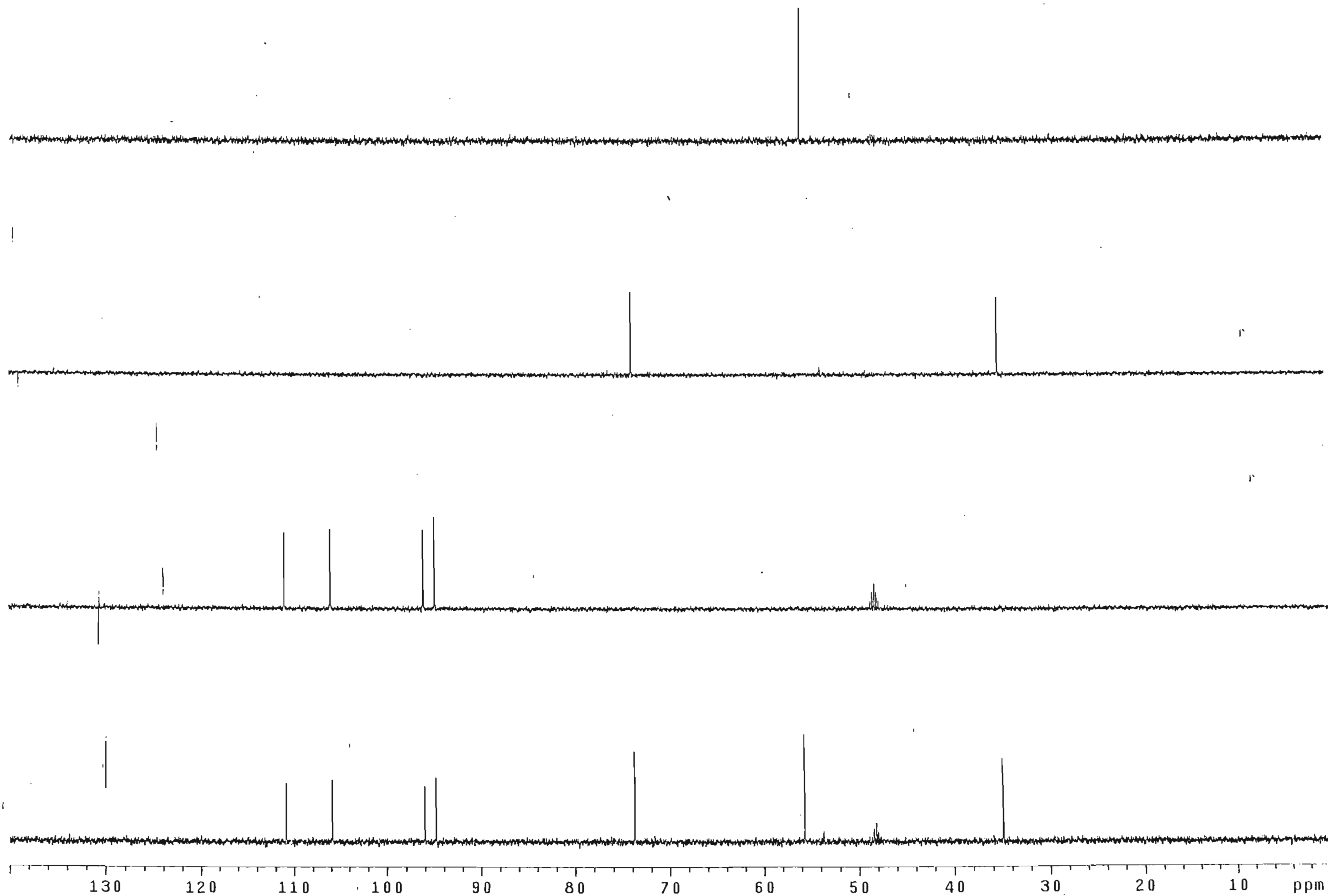


9 8 7 6 5 4 3 2 1 ppm

SPECTRUM 7.1 : ^{1}H NMR spectrum of compound 7 (CD_3OD)

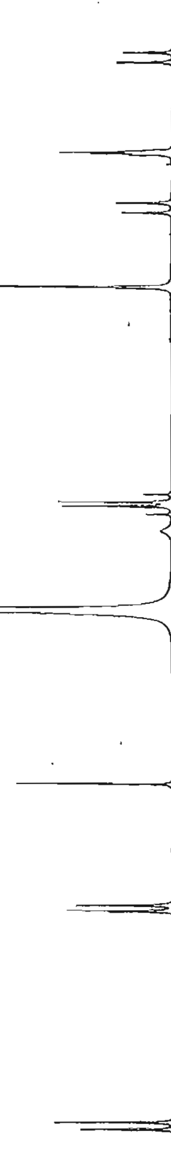


SPECTRUM 7.2 : ^{13}C NMR spectrum of compound 7 (CD_3OD)



SPECTRUM 7.3 : ADEPT spectrum of compound 7 (CD_3OD)

```
in cosy-90
probe=5mmASW

Pulse Sequence: relayh
 Solvent: CD3OD
 Ambient temperature
INOVA-400  "undnmr400"
PULSE SEQUENCE: relayh
 Relax. delay 1.000 sec
 COSY 90-90
 Acq. time 0.218 sec
 Width     2343.6 Hz
 2D Width 2343.6 Hz
 8 repetitions
 256 increments
OBSERVE   H1, 399.9502545 MHz
DATA PROCESSING
 Line broadening 0.5 Hz
 Sine bell 0.109 sec
F1 DATA PROCESSING
 Sine bell 0.054 sec
FT size 1024 x 1024
Total time 43 min, 50 sec
```



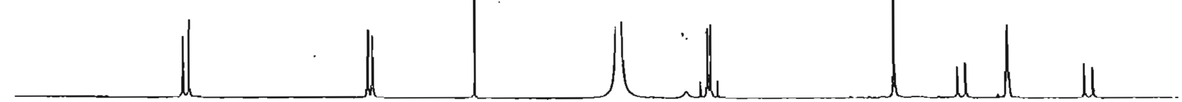
F2 (ppm)

3.5 4.0 4.5 5.0 5.5 6.0 6.5

6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0

F1 (ppm)

SPECTRUM 7.4 : COSY spectrum of compound 7 (CD$_3$OD)



Probe-5mmASW

Pulse Sequence: noesy_da
Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: noesy_da
Relax. delay 2.500 sec
Mixing 1.000 sec
Acq. time 0.171 sec
Width 3001.2 Hz
2D Width 3001.2 Hz
16 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sq. sine bell 0.171 sec
Shifted by -0.171 sec
F1 DATA PROCESSING
Sq. sine bell 0.085 sec
Shifted by -0.085 sec
FT size 1024 x 1024
Total time 8 hr, 29 min, 19 sec

F1 (ppm)

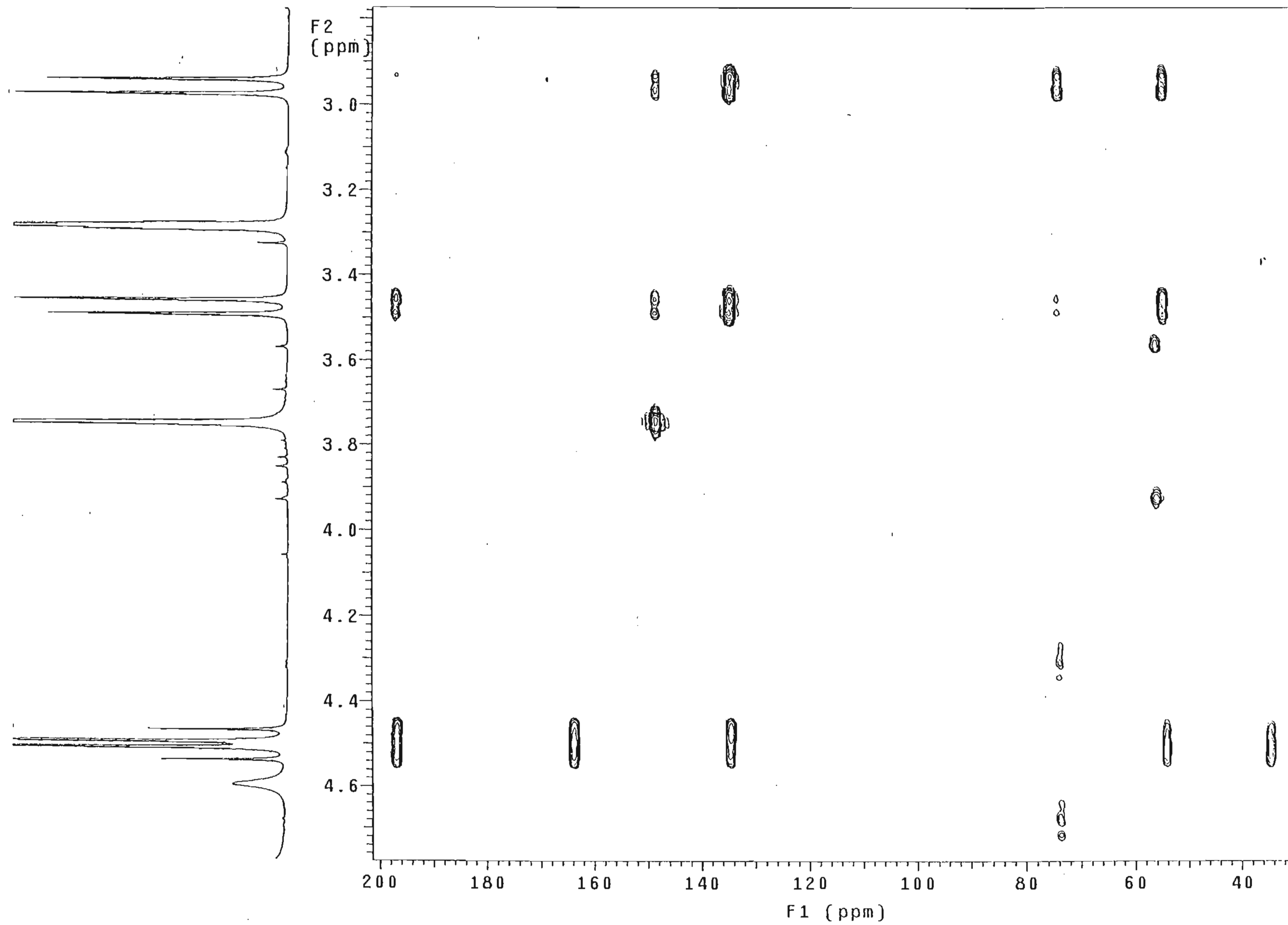
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0

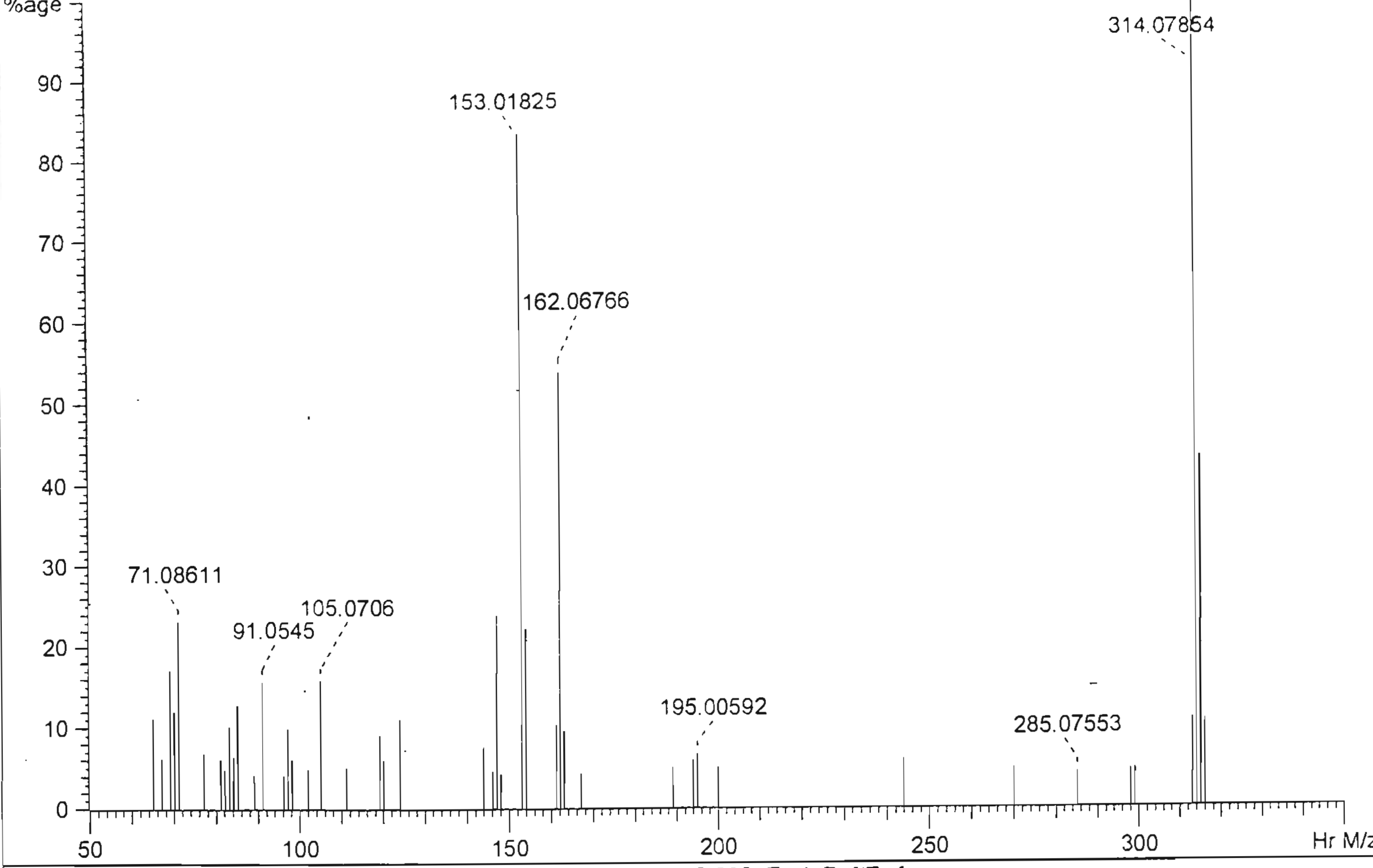
F2 (ppm)

SPECTRUM 7.5 : NOESY spectrum of compound 7 (CD_3OD)

Pulse Sequence: ghmqc_da



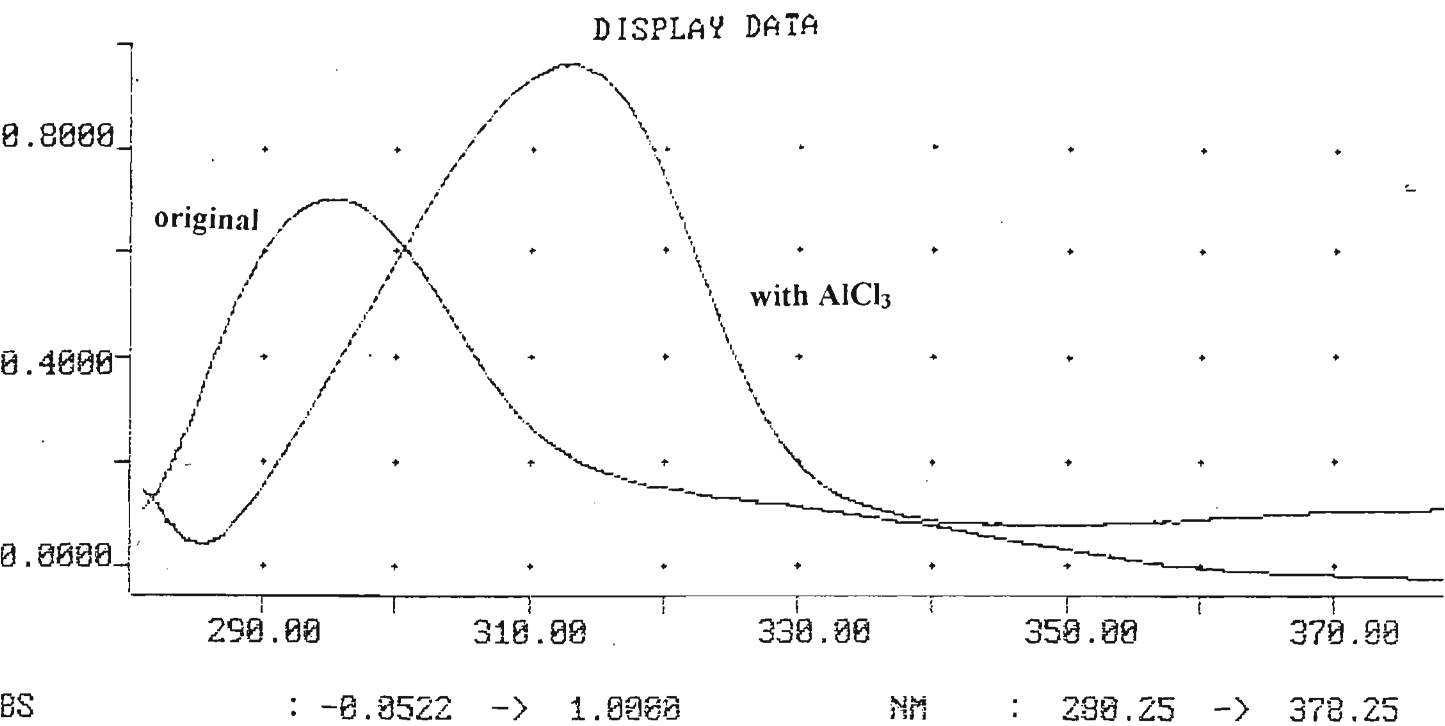
SPECTRUM 7.6 : HMBC spectrum of compound 7 (CD_3OD)



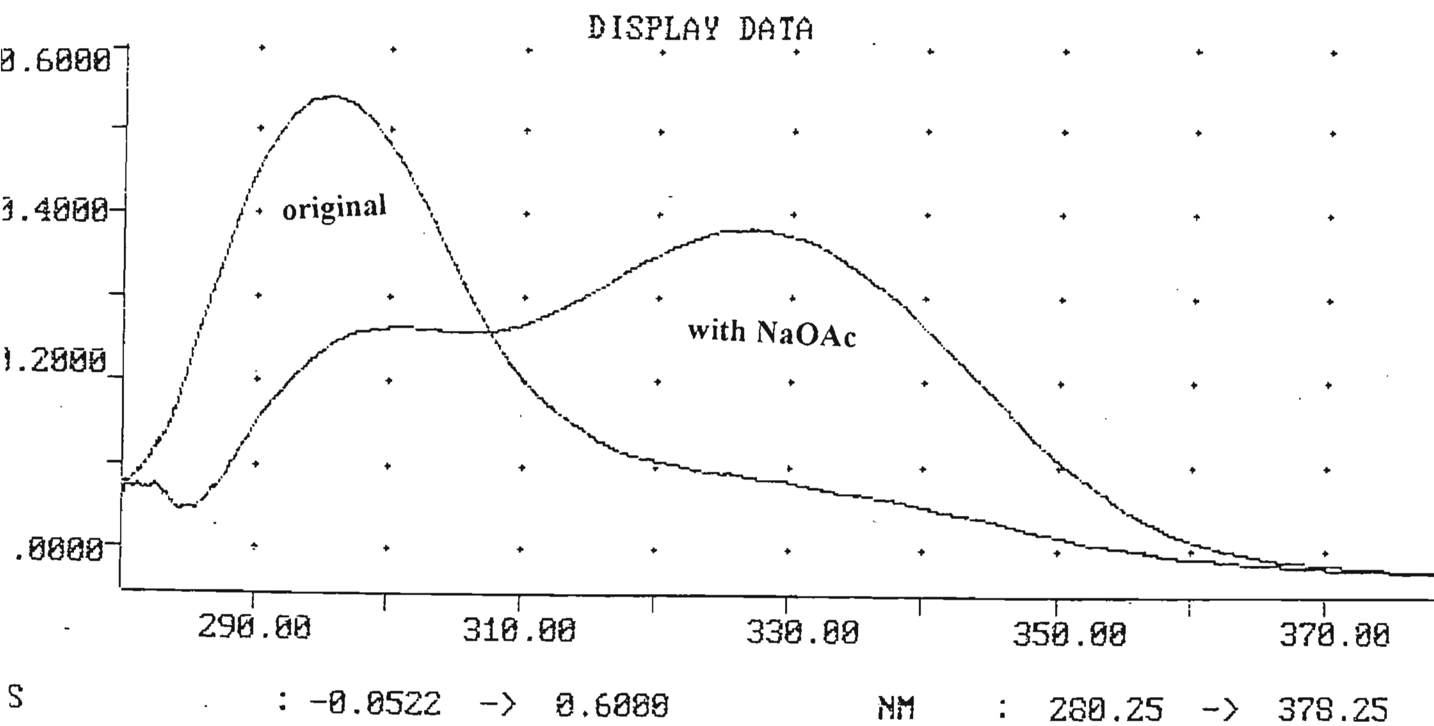
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:2%. Range:0-320. Excl: Ref/Ex.].
Scan 66#13:55 - 70#14:45. Entries=98. Base M/z=314.07854. 100% Int.=0.32038. EI. POS.

Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width
60.01035	2.04	55	103.05452	2.83	48	163.07193	9.48	57	313.02397	10.66	95
63.01657	3.31	51	105.07060	15.82	56	164.93011	2.44	44	314.07854	100.00	60
64.00787	2.33	68	107.05044	2.04	50	167.01311	4.21	88	315.08363	42.69	60
65.01955	11.11	94	111.00817	2.70	46	189.05545	4.93	59	316.08848	10.52	55
65.03295	8.35	90	111.11735	5.06	52	193.99521	5.79	53	319.98368	2.87	48
67.01670	2.25	43	112.12444	3.39	50	195.00592	6.58	56			
67.05289	6.13	51	113.13225	3.26	47	196.96317	2.28	50			
68.06158	3.18	47	114.00598	2.30	46	198.99717	2.73	64			
69.07040	17.07	55	115.05481	3.59	79	199.98876	4.98	55			
70.07834	11.97	54	118.04150	3.86	50	205.99397	2.78	47			
71.08611	23.10	55	119.05004	9.00	49	207.00447	2.37	47			
73.02927	2.43	45	119.08518	4.65	40	217.99499	2.67	48			
77.03877	6.86	72	120.09323	5.96	78	228.98991	3.09	68			
79.05428	3.14	49	123.99505	10.98	70	240.99052	2.40	50			
81.07046	6.00	51	125.00355	2.42	57	243.99018	5.89	54			
82.07758	4.70	49	125.13272	2.17	43	247.98692	3.66	50			
83.08564	10.13	53	127.02171	2.15	74	248.99325	2.79	50			
84.09321	6.35	52	131.04951	2.83	48	249.98776	2.09	48			
85.10168	12.84	54	134.96273	3.16	47	257.00192	2.50	51			
89.03901	4.13	51	137.00310	2.67	83	259.98685	2.37	42			
90.04603	2.78	46	140.15625	2.15	44	267.99209	2.38	60			
91.05450	15.67	55	143.99859	7.48	53	269.98799	4.77	82			
92.05979	2.04	46	146.03547	4.56	55	271.05905	3.14	55			
95.08580	3.96	48	147.04498	23.78	57	285.07553	4.21	53			
96.01987	2.17	47	148.04883	4.23	51	286.08147	2.72	44			
96.09367	4.10	48	152.00554	3.38	52	294.99848	3.01	50			
97.10180	9.84	53	153.01825	83.05	57	297.06546	2.38	75			
98.07410	2.03	44	154.02348	22.15	56	297.98651	4.45	80			
98.10893	6.05	57	157.02260	2.84	77	299.03725	4.66	78			
99.11699	3.23	46	161.05947	10.21	56	305.98796	2.84	48			
102.04695	4.88	52	162.06766	53.77	58	311.98468	3.48	82			

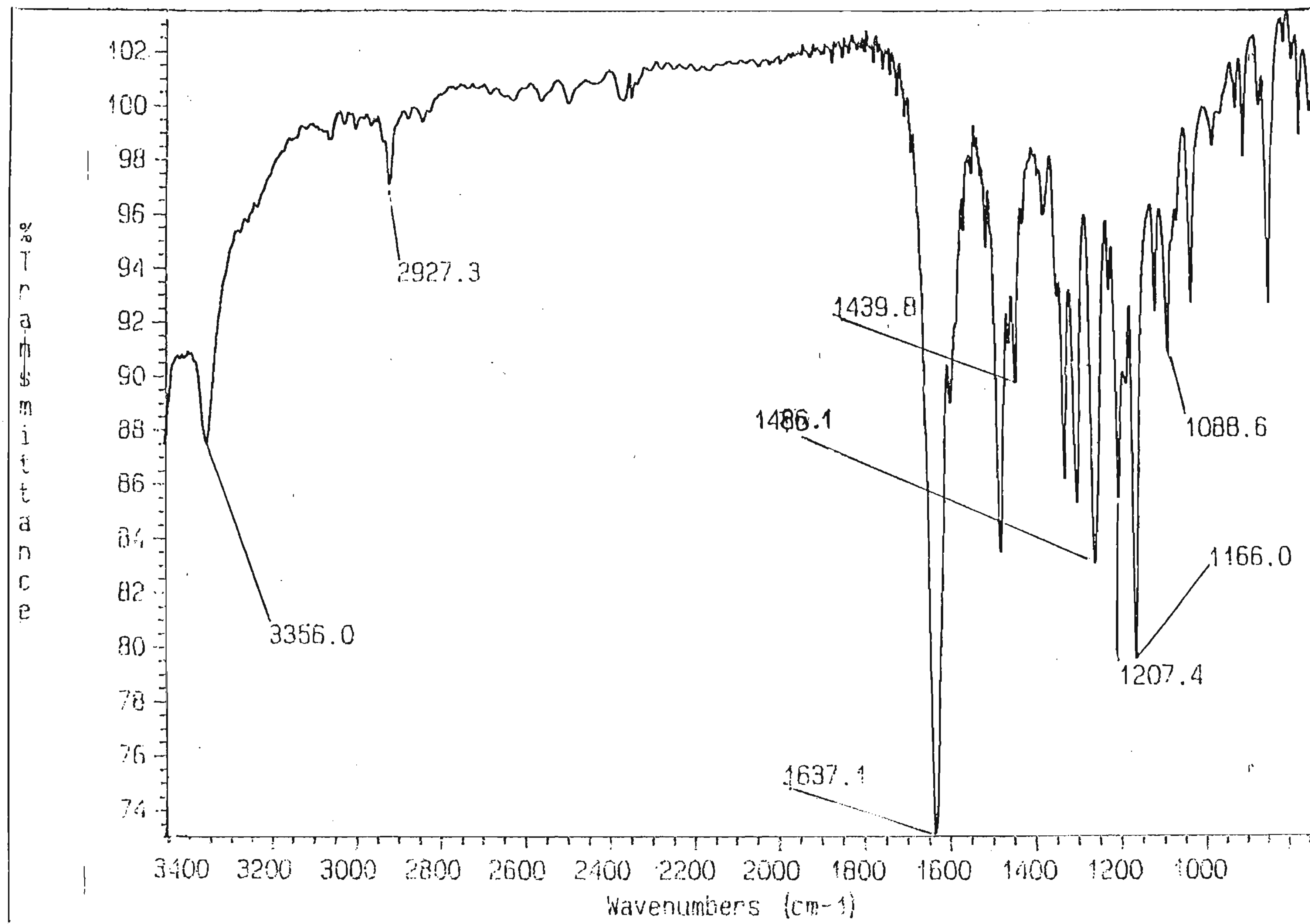
SPECTRUM 7.7 : Mass spectrum of compound 7



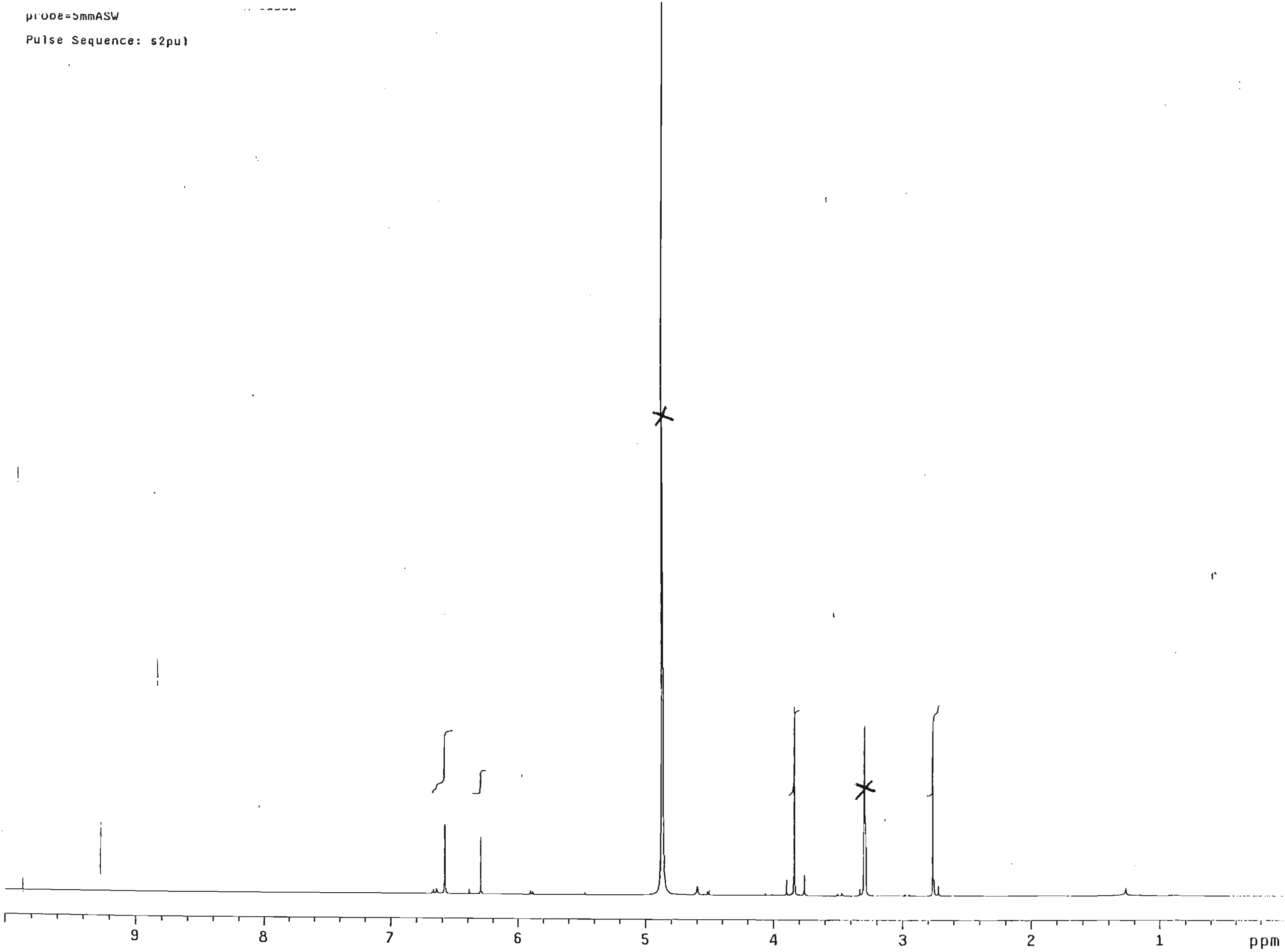
SPECTRUM 7.8A : UV spectrum of compound 7 (original and with $AlCl_3$)



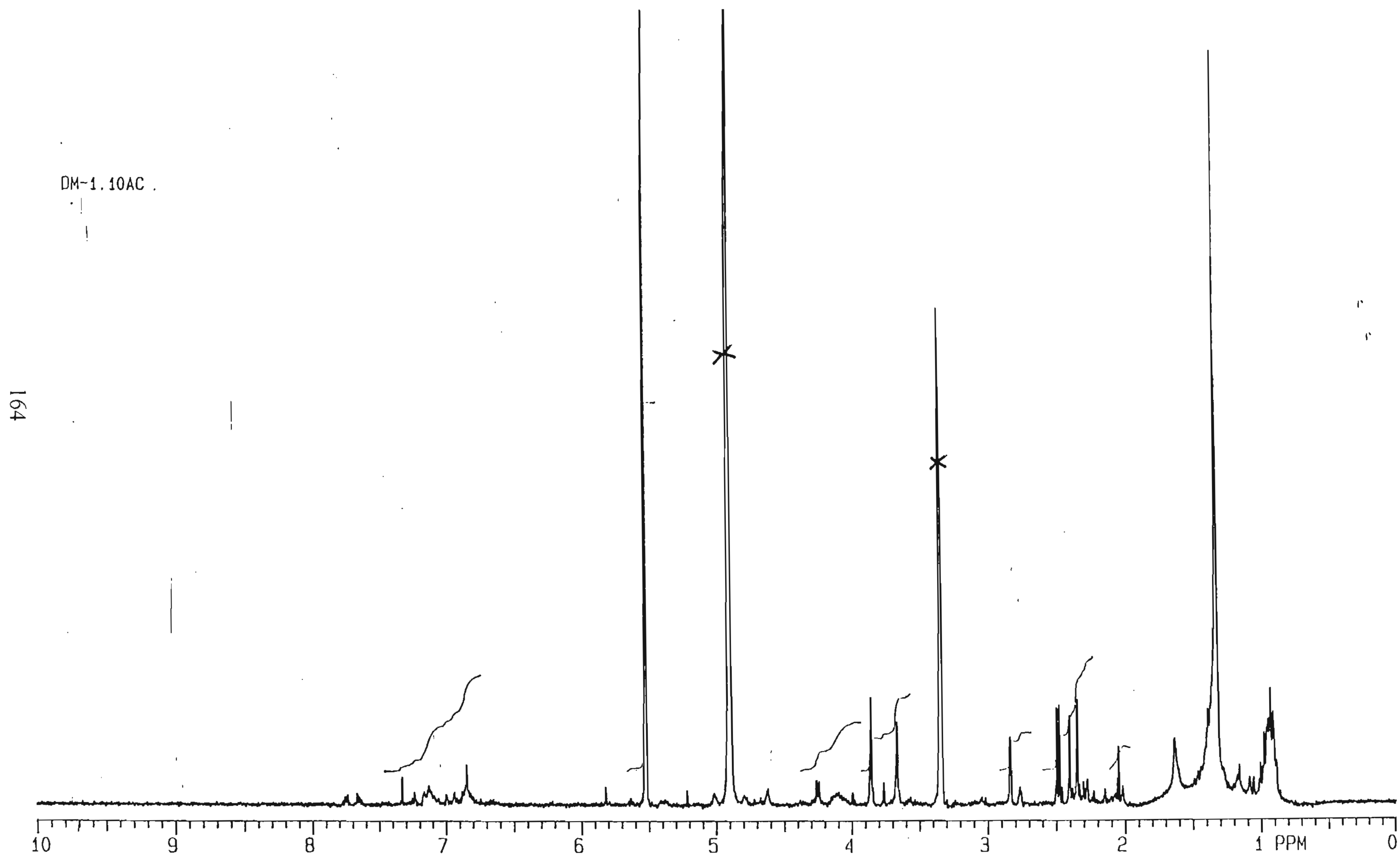
SPECTRUM 7.8B : UV spectrum of compound 7 (original and with NaOAc)



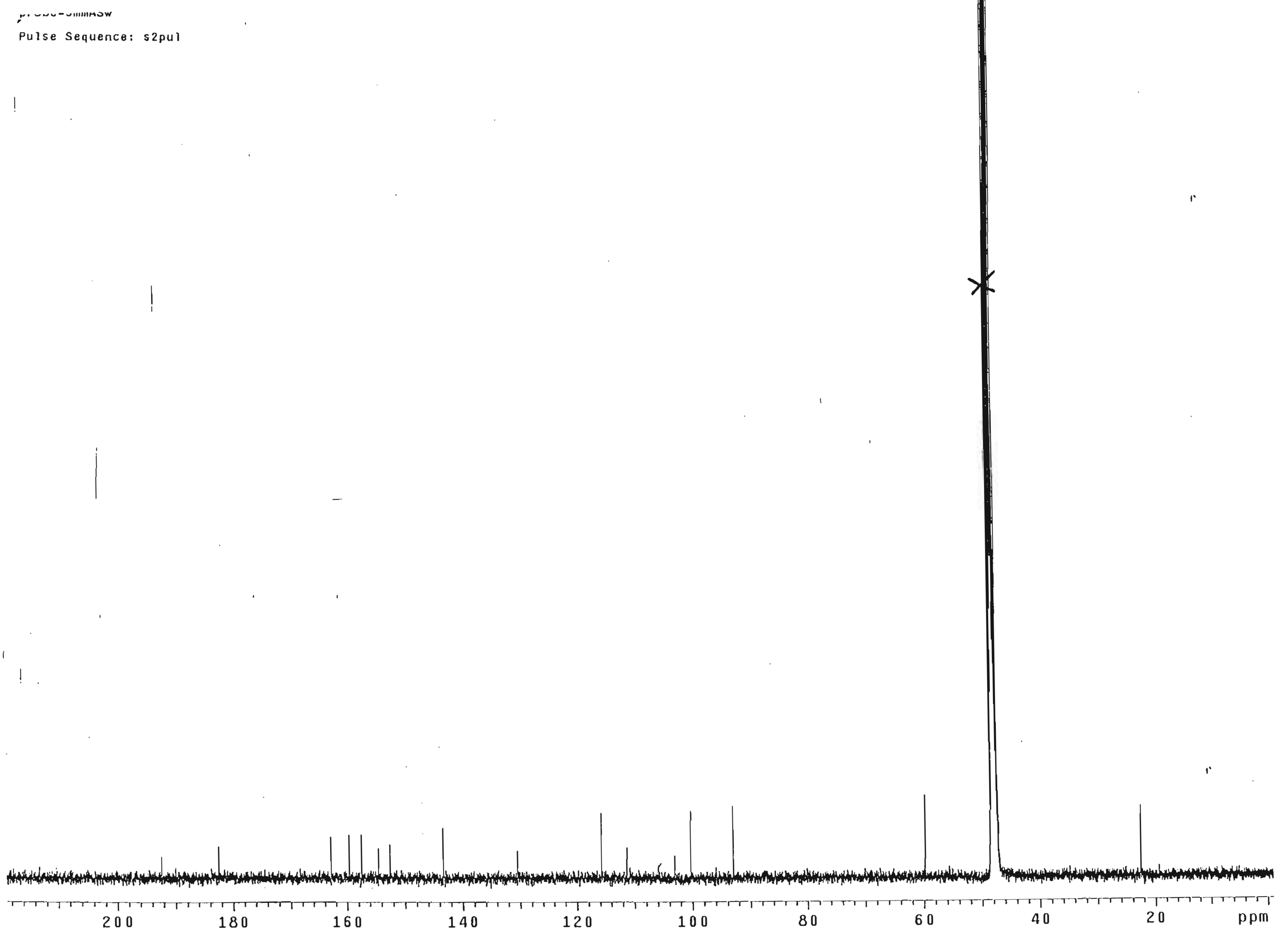
SPECTRUM 7.9 : Infrared spectrum of compound 7



SPECTRUM 8.1A : ^{1}H NMR spectrum of



SPECTRUM 8.1B : ^{1}H NMR spectrum of acetylated compound 8 (CD_3OD)



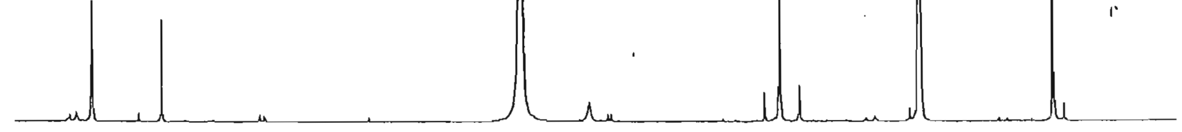
SPECTRUM 8.2 : ^{13}C NMR spectrum of compound 8 (CD_3OD)

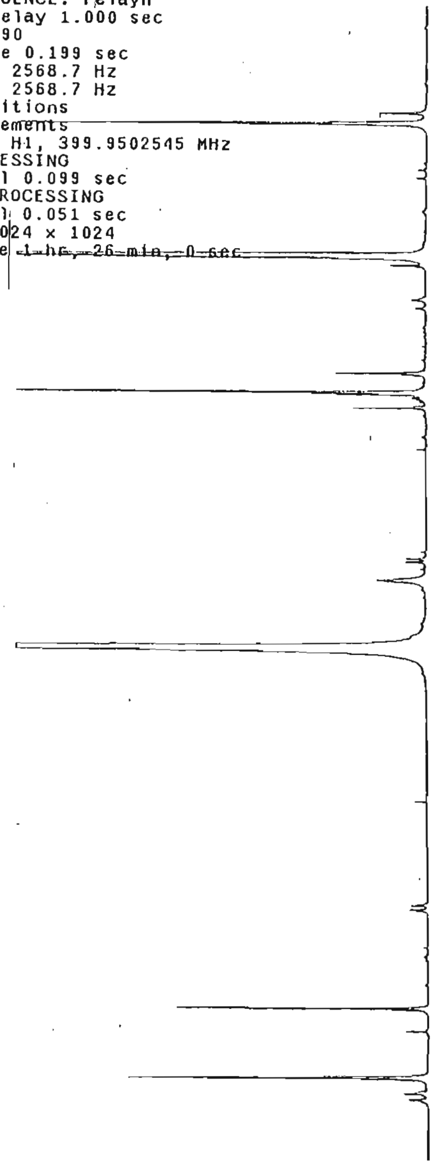
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.199 sec
Width 2568.7 Hz
2D Width 2568.7 Hz
16 repetitions
256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.099 sec
F1 DATA PROCESSING
Sine bell 0.051 sec
FT size 1024 x 1024
Total time 1 hr, 26 min, 0 sec





F2 (ppm)

3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5

6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5

F1 (ppm)

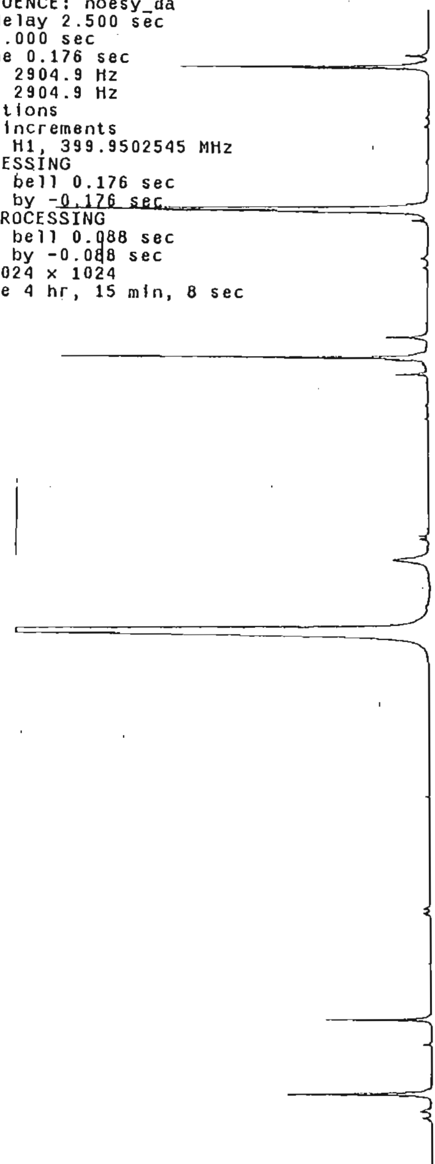
SPECTRUM 8.3 : COSY spectrum of [illegible]

Gradient NOESY expt.
probe=5mmASW

Pulse Sequence: noesy_da

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

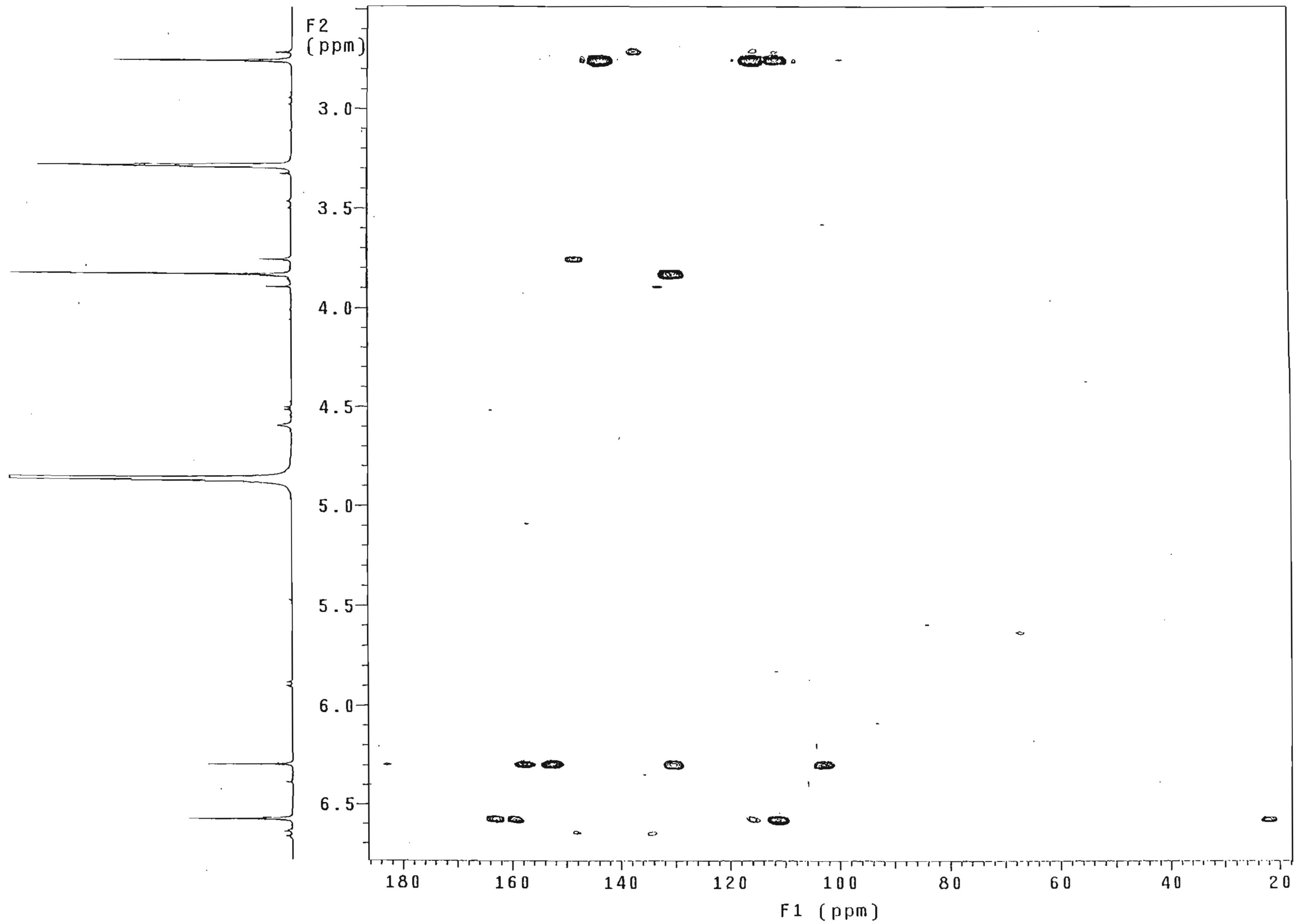
PULSE SEQUENCE: noesy_da
Relax. delay 2.500 sec
Mixing 1.000 sec
Acq. time 0.176 sec
Width 2904.9 Hz
2D Width 2904.9 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sq. sine bell 0.176 sec
Shifted by -0.176 sec
F1 DATA PROCESSING
Sq. sine bell 0.088 sec
Shifted by -0.088 sec
FT size 1024 x 1024
Total time 4 hr, 15 min, 8 sec



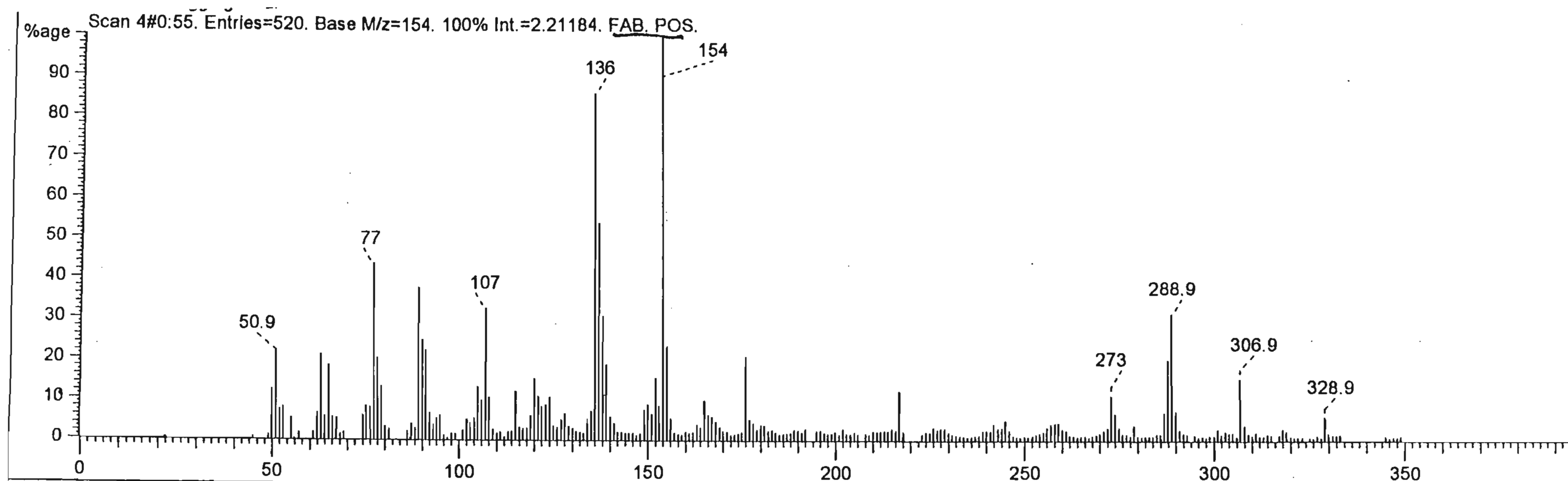
SPECTRUM 8.4 : NOESY spectrum of compound 8 (CD_3OD)

Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da



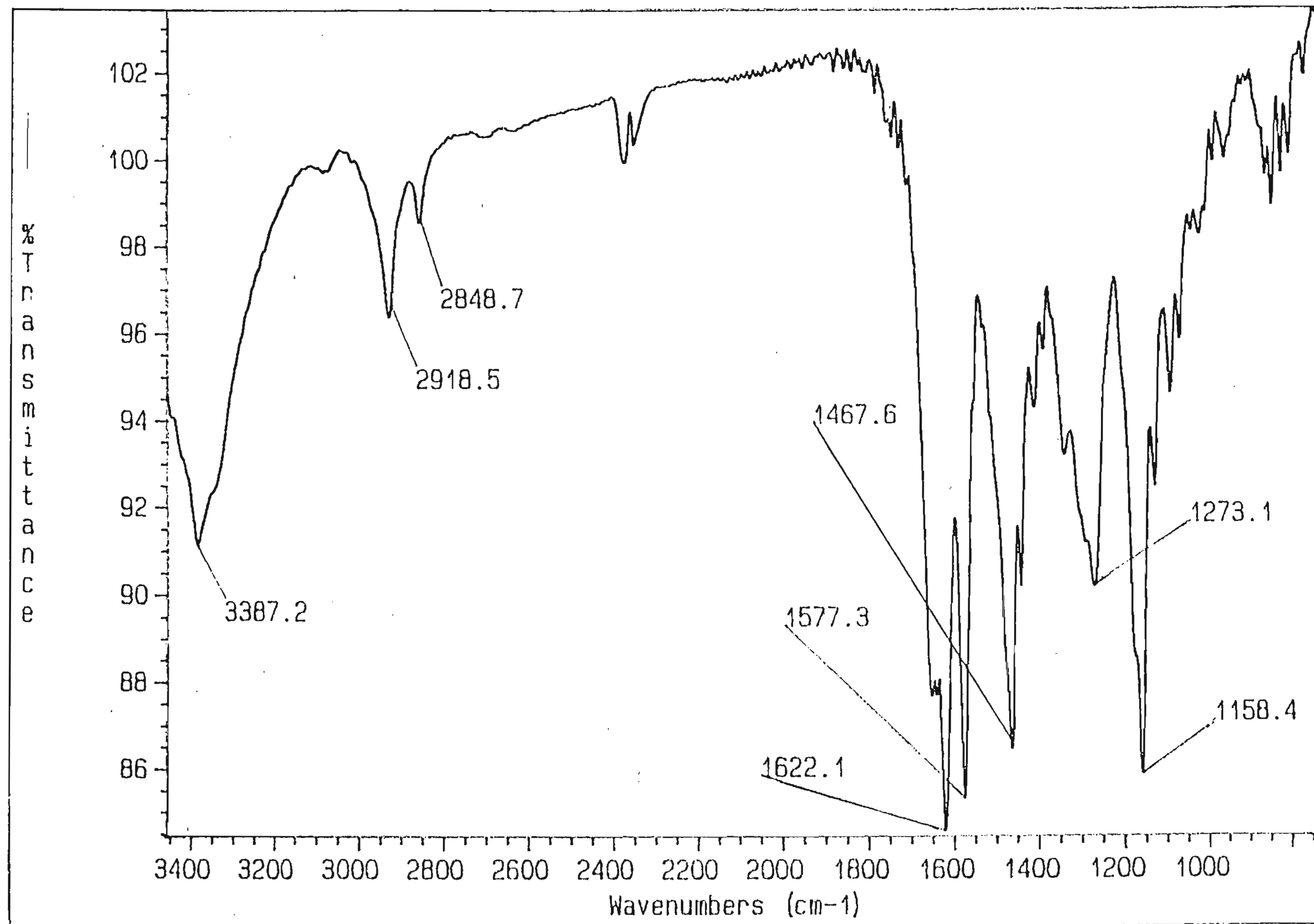
SPECTRUM 8.5 : HMBC spectrum of compound 8 (CD_3OD)



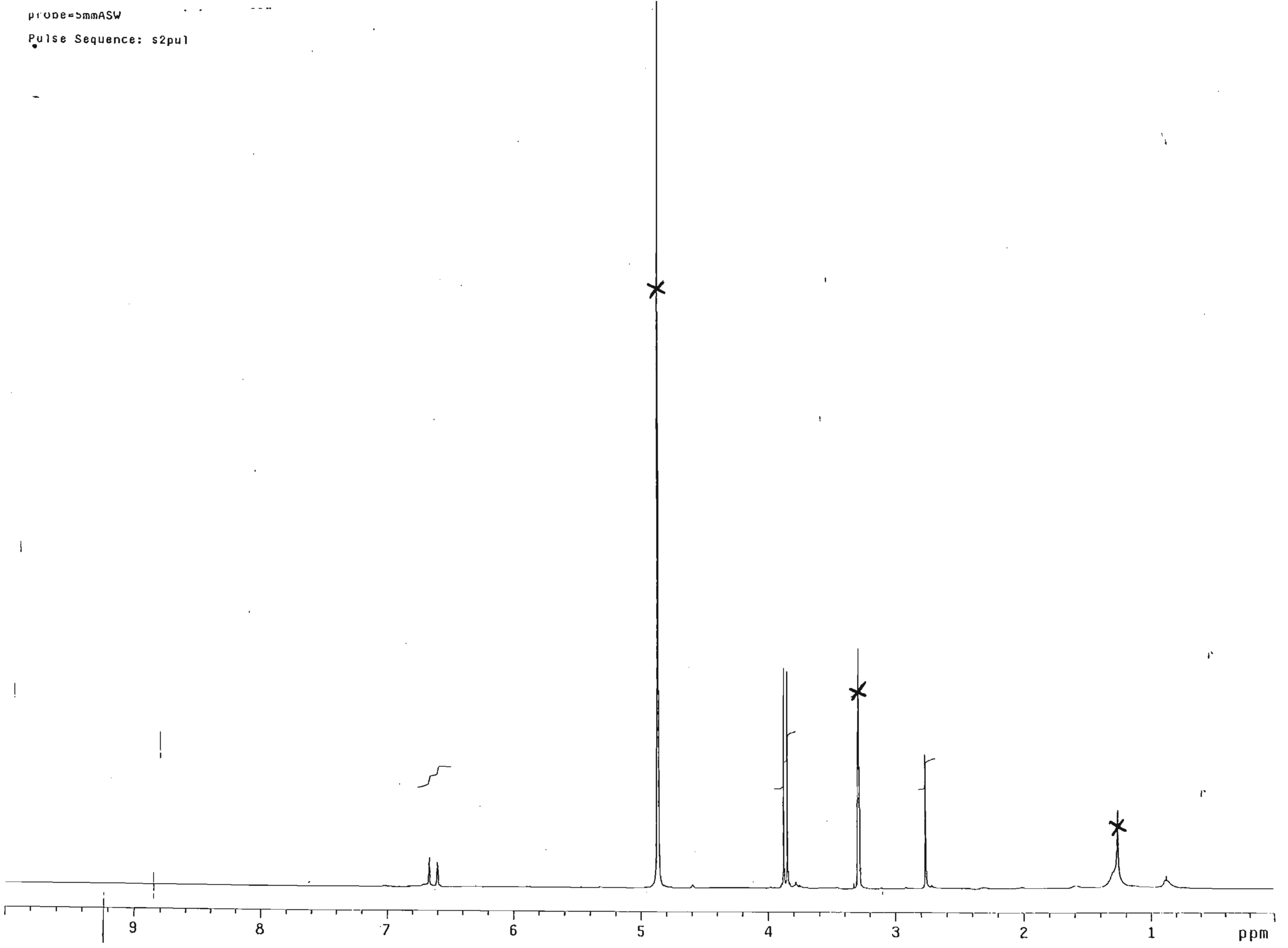
SCAN TEXT. Sorted on M/z (ascending). Filter=[Int:1.5%.].
Scan 4#0:55. Entries=211. Base M/z=154. 100% Int.=2.21184. FAB. POS.

M/z	%age	M/z	%age	M/z	%age	M/z	%age	M/z	%age	M/z	%age	M/z	%age	M/z	%age	M/z	%a
91.0	22.16	115.0	11.94	133.0	1.74	153.0	8.50	176.0	20.57	196.0	2.37	217.0	12.06	254.0	1.64	287.9	19.
92.0	6.64	116.0	3.16	134.0	4.99	154.0	100.00	177.0	4.98	197.0	1.79	218.0	2.07	255.0	2.03	288.9	31.
93.0	3.77	117.0	2.75	135.0	6.92	155.0	23.09	178.0	4.11	197.0	1.71	218.1	1.75	255.0	1.75	290.0	7.
93.0	3.70	118.0	2.91	136.0	85.56	156.0	5.34	179.0	2.56	198.0	1.55	224.0	1.93	256.0	3.08	291.0	2.
94.0	5.13	119.0	5.92	137.0	53.59	157.0	1.82	180.0	3.58	199.0	1.66	225.0	1.95	257.0	3.82	292.0	1.
95.0	6.03	120.0	14.99	138.0	30.58	160.0	2.02	181.0	3.53	200.0	1.99	226.0	3.14	258.0	4.04	301.0	2.
101.0	2.25	121.0	10.65	139.0	18.48	161.0	1.65	182.0	2.34	202.0	2.61	227.0	2.54	259.0	4.31	302.9	2.
102.0	4.94	122.0	8.03	140.0	5.65	162.0	1.56	183.0	2.53	203.0	1.58	228.0	2.91	260.0	2.80	303.0	1.
103.0	4.25	123.0	8.56	141.0	4.09	162.0	1.87	183.0	2.11	204.0	1.60	229.0	2.75	261.0	2.44	304.0	1.
104.0	5.25	124.0	10.40	142.0	1.91	163.0	3.71	184.0	1.94	205.0	1.93	230.0	1.91	270.0	1.80	304.9	1.
105.0	12.91	125.0	3.21	143.0	1.91	164.0	3.17	186.0	1.57	208.0	1.53	239.0	2.33	271.0	2.42	306.9	15
106.0	9.60	125.0	3.49	144.0	1.65	165.0	9.61	187.0	1.67	210.0	2.12	240.0	2.39	272.0	3.12	308.0	3
107.0	32.44	126.0	3.08	144.1	1.51	166.0	6.16	188.0	1.83	210.0	1.67	240.0	2.13	273.0	10.79	308.9	1
108.0	10.45	127.0	4.90	145.0	1.67	167.0	5.67	189.0	2.48	211.0	2.07	241.0	2.26	274.0	6.33	309.0	1.
109.0	2.70	128.0	6.42	146.0	1.64	168.0	4.47	189.0	2.37	212.0	2.20	242.0	4.00	275.0	3.18	310.9	1.
110.0	1.73	129.0	3.35	148.0	1.58	169.0	3.07	190.0	2.36	213.0	2.28	243.0	2.92	276.0	1.58	317.9	2.
111.0	1.94	130.0	2.84	149.0	7.35	170.0	2.26	191.0	1.88	213.0	2.22	243.1	1.84	279.0	3.57	318.9	2.
113.0	1.97	131.0	2.17	150.0	8.70	171.0	1.99	191.9	2.79	214.0	2.27	244.0	3.00	285.0	1.55	328.9	5.
113.0	1.77	132.0	1.80	151.0	6.36	174.0	1.55	192.0	2.59	215.0	2.73	245.0	4.74	285.9	1.59	329.9	1.
114.0	2.21	132.0	1.95	152.0	15.10	175.0	1.88	195.0	2.21	216.0	2.41	246.0	2.36	287.0	6.71	461.9	2.

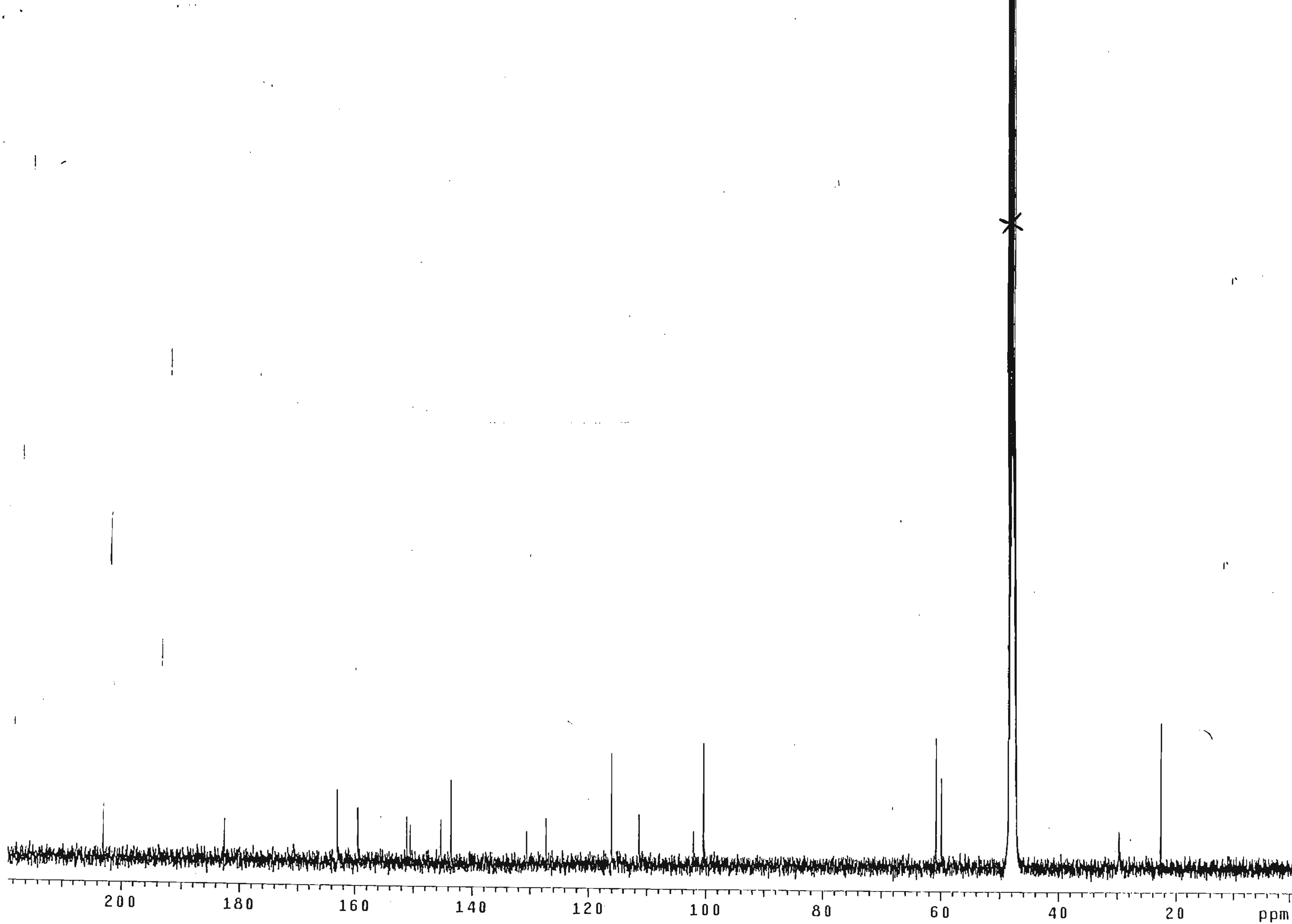
SPECTRUM 8.6 : Mass spectrum of compound 8



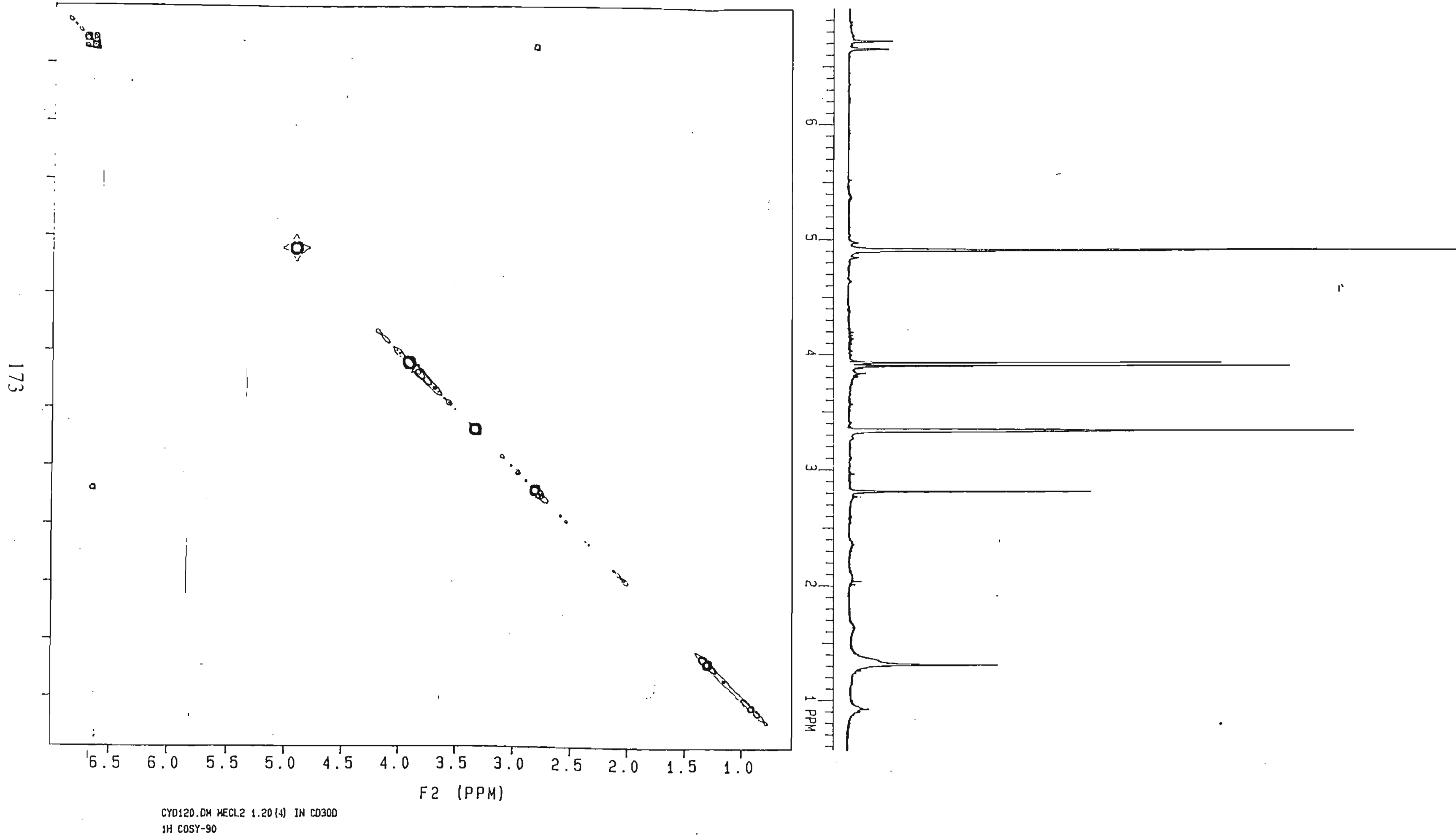
SPECTRUM 8.7 : Infrared spectrum of compound 8



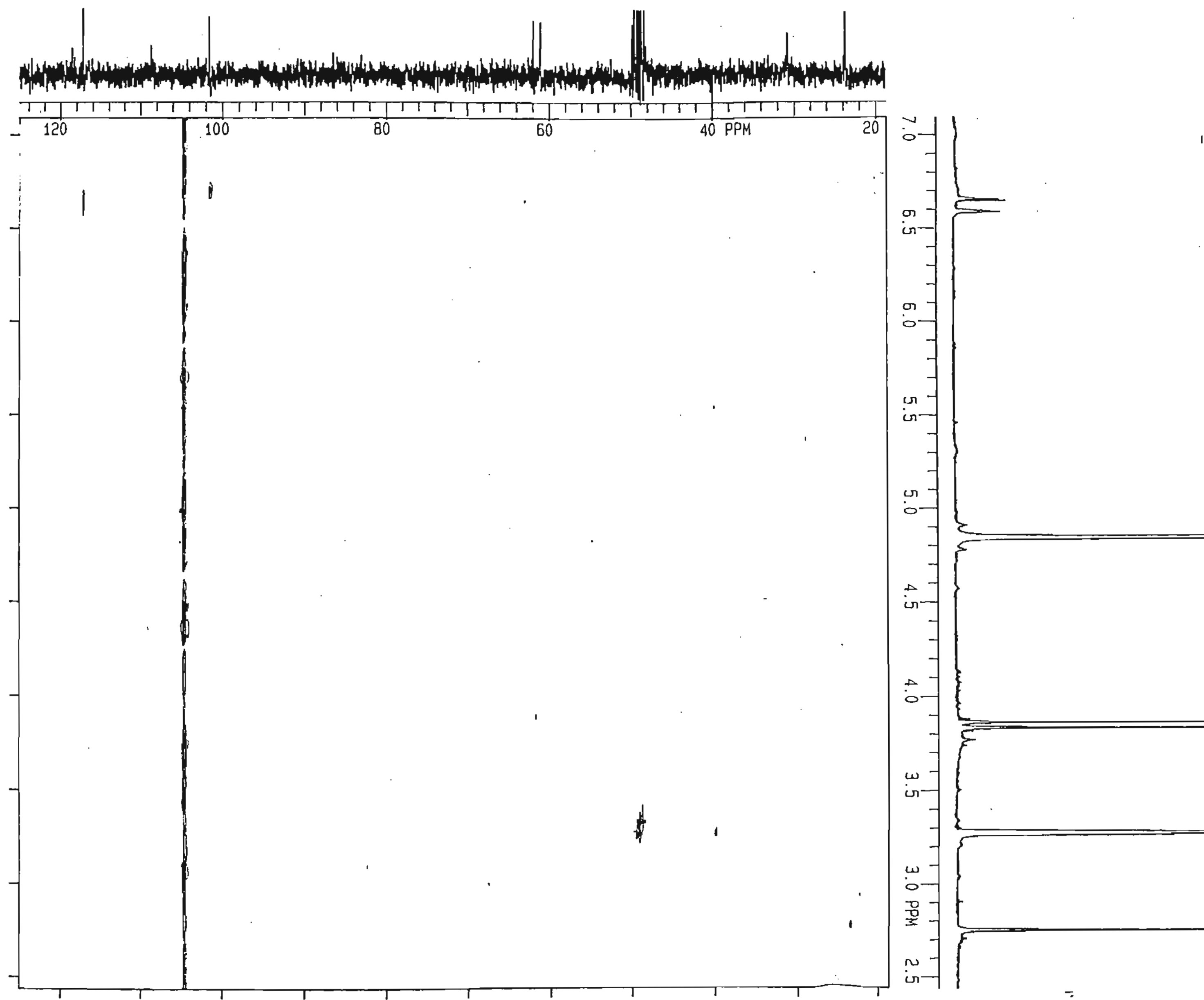
SPECTRUM 9.1 : ^{1}H NMR spectrum of compound 9 (CD_3OD)



SPECTRUM 9.2 : ^{13}C NMR spectrum of compound 9 (CD_3OD)



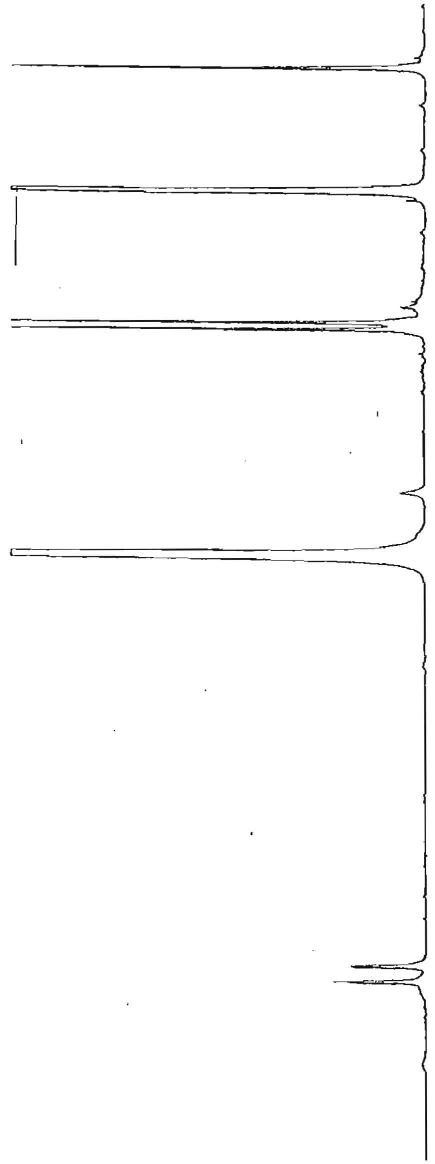
SPECTRUM 9.3 : COSY spectrum of compound 9 (CD_3OD)



SPECTRUM 9.4 : HETCOR spectrum of compound 9 (CD_3OD)

Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



F1 (ppm)

3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5

F2 (ppm)

SPECTRUM 9.5: NOESY

Pulse Sequence: ghmqc_da
Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"
PULSE SEQUENCE: ghmqc_da
Relax. delay 1.500 sec
Acq. time 0.163 sec
Width 3137.3 Hz
2D Width 19323.7 Hz
32 repetitions
512 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.081 sec
F1 DATA PROCESSING
Sine bell 0.013 sec
FT size 1024 x 1024
Total time 8 hr, 2 min, 18 sec

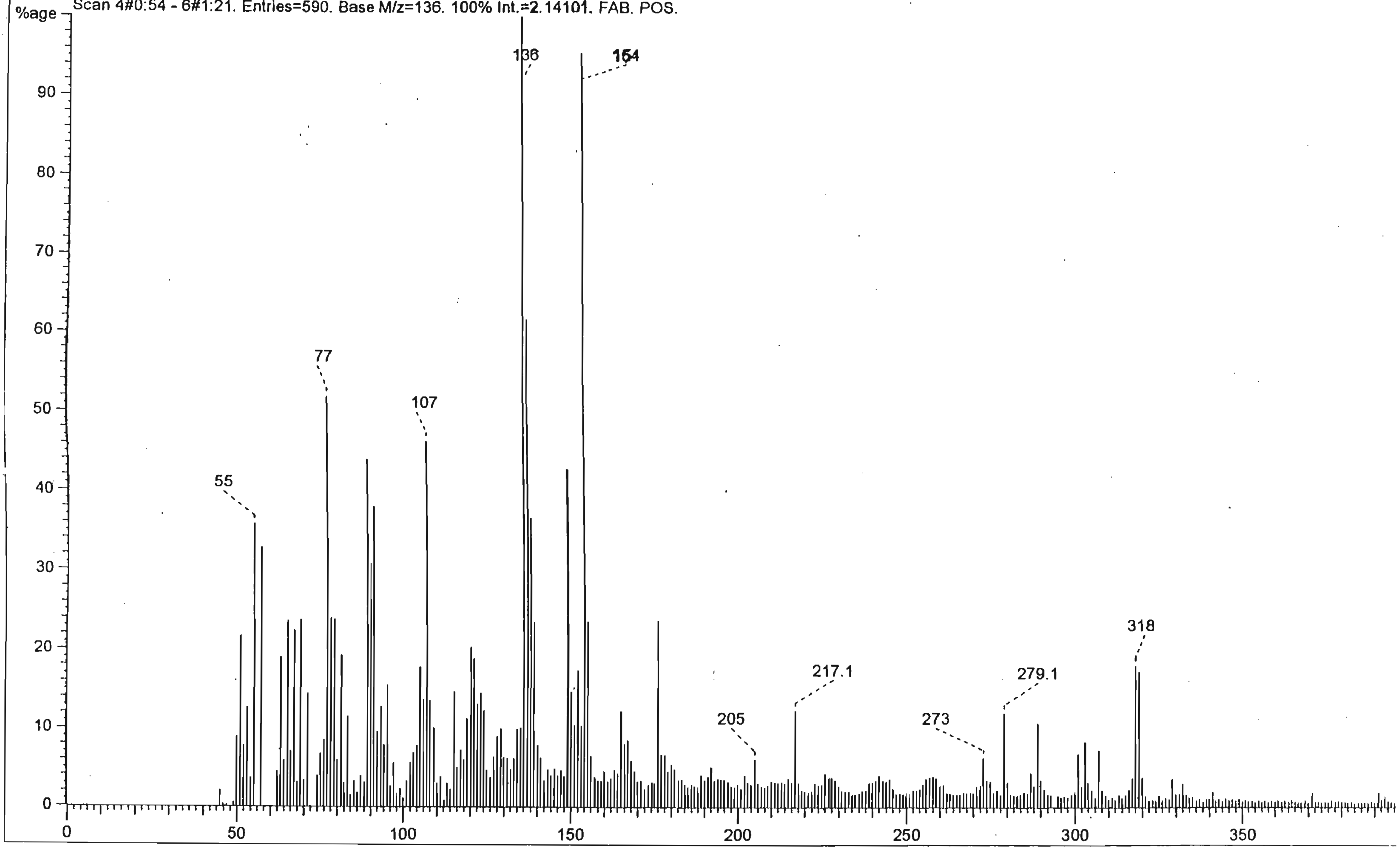
F2 (ppm)

3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

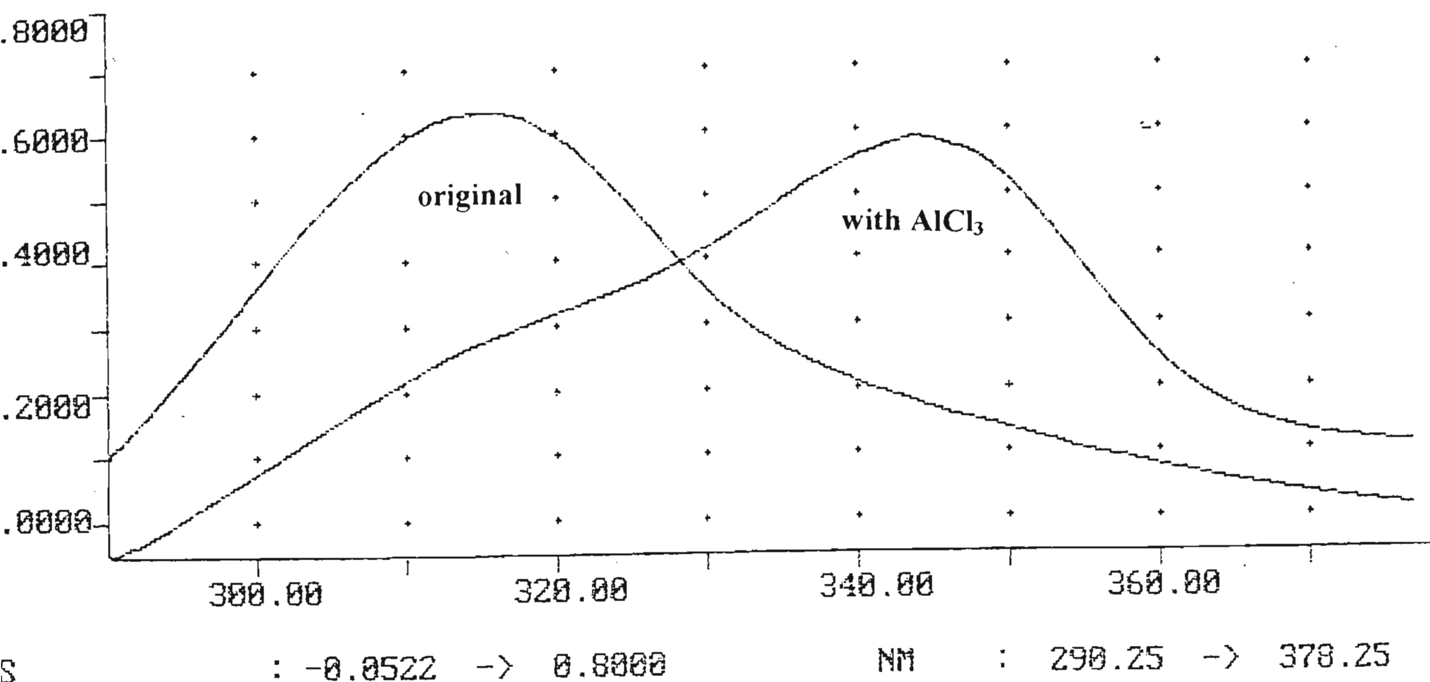
180 170 160 150 140 130 120 110 100 90

F1 (ppm)

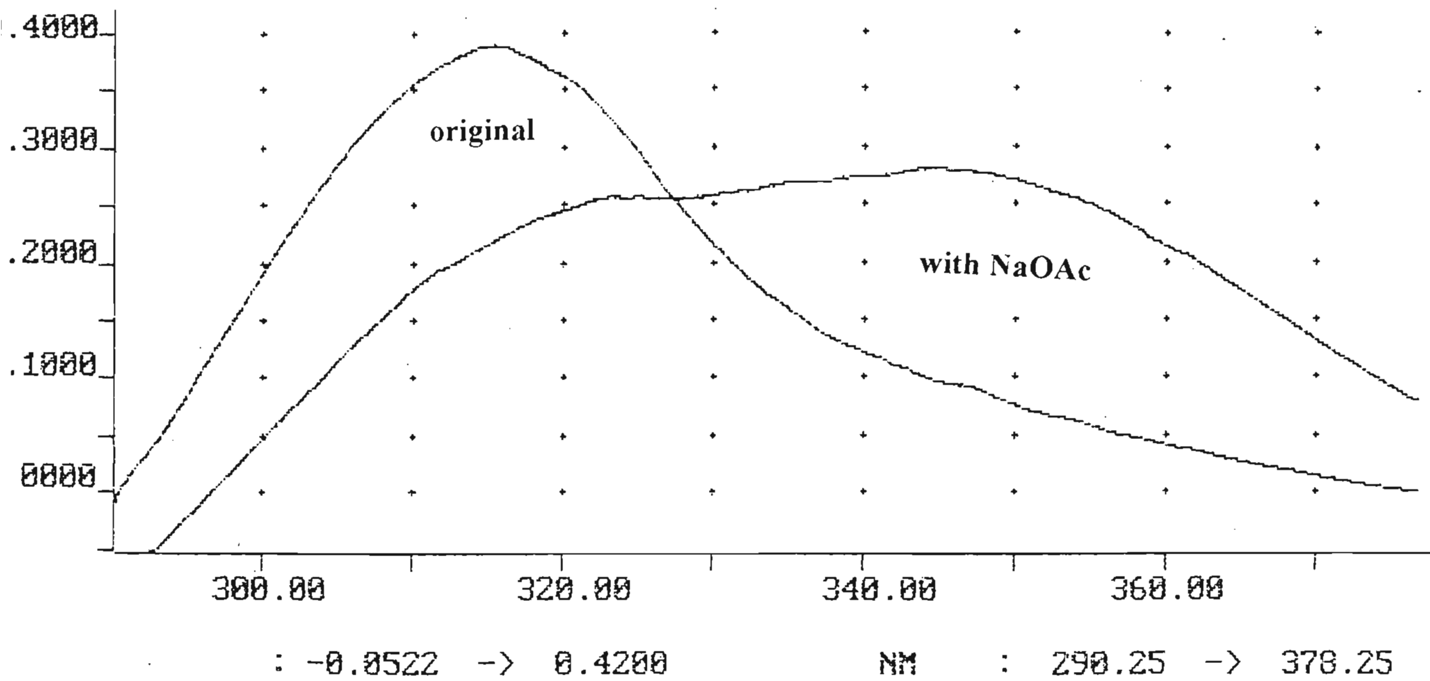
SPECTRUM 9.6 : HMBC spectrum of compound 9 (CD_3OD)



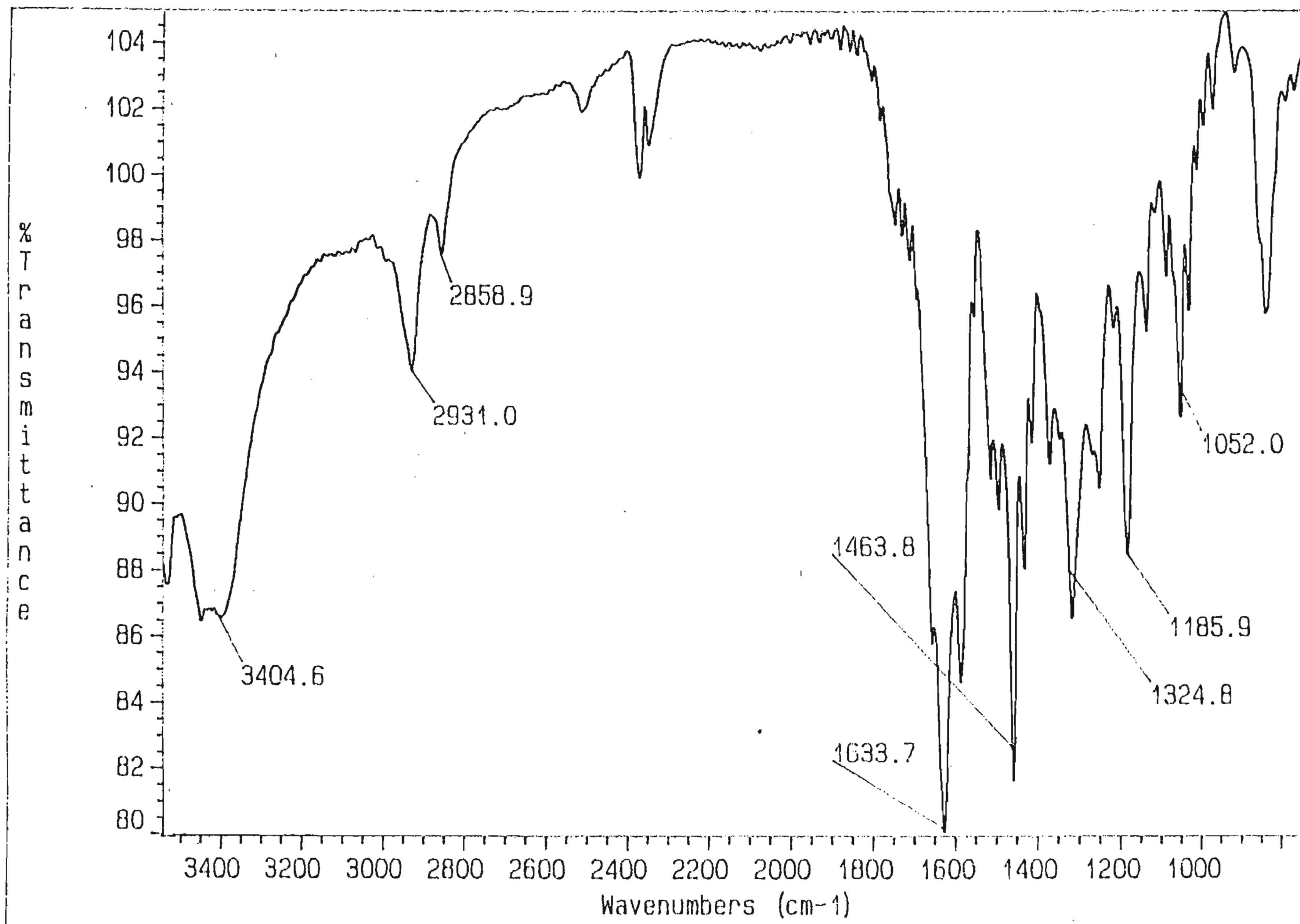
SPECTRUM 9.7 : Mass spectrum of compound 9



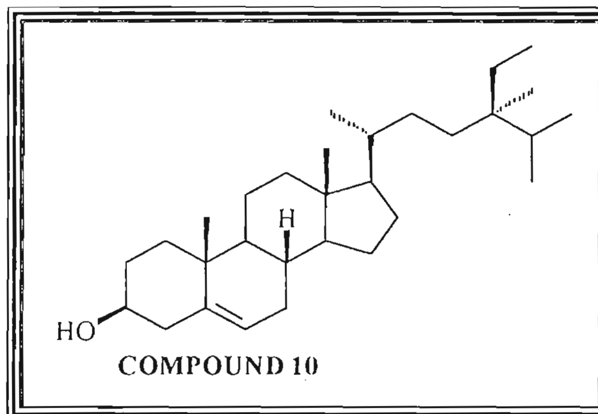
SPECTRUM 9.8A : UV spectrum of compound 9 (original and with $AlCl_3$)



SPECTRUM 9.8B : UV spectrum of compound 9 (original and with NaOAc)

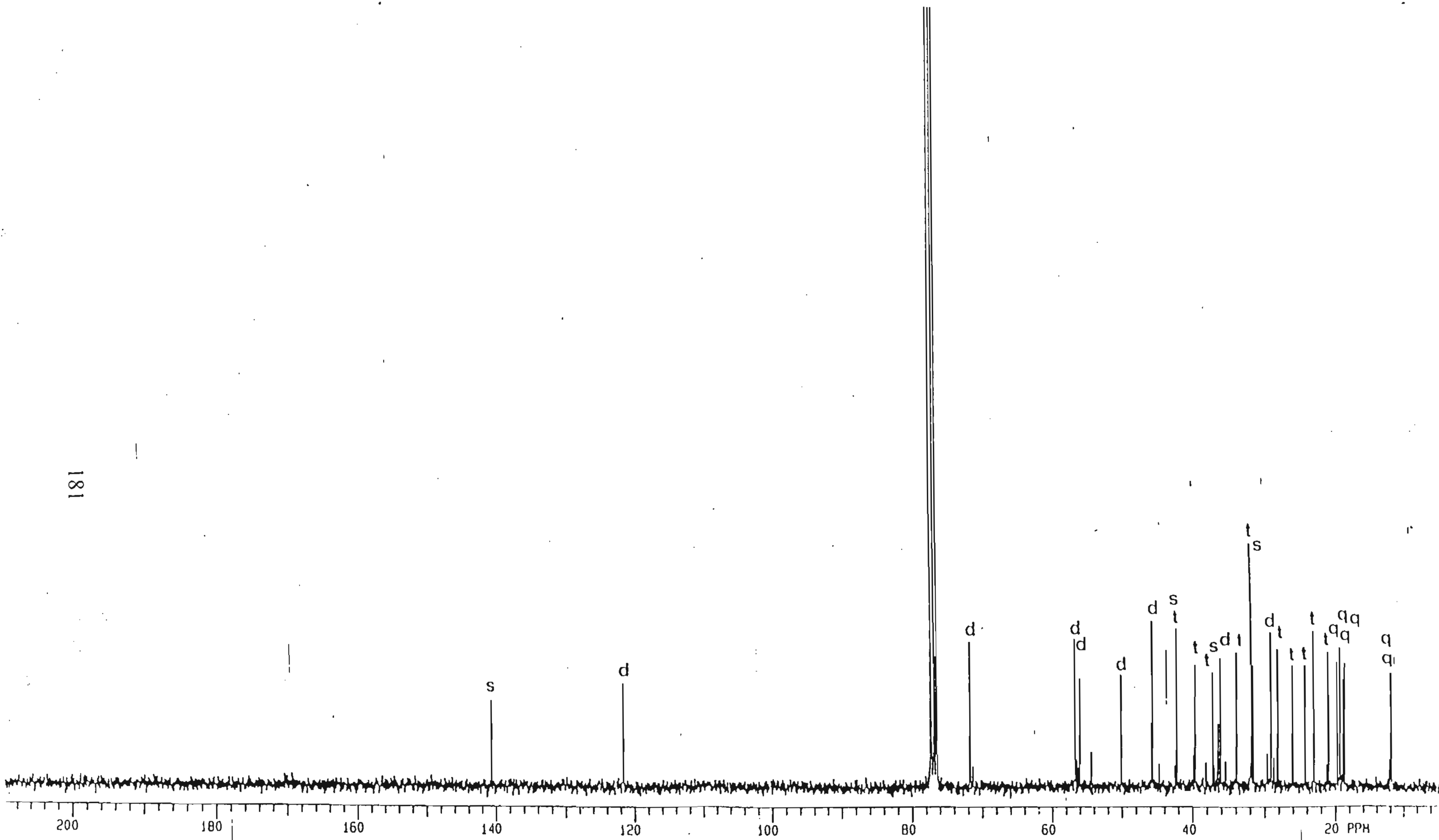


SPECTRUM 9.9 : Infrared spectrum of compound 9

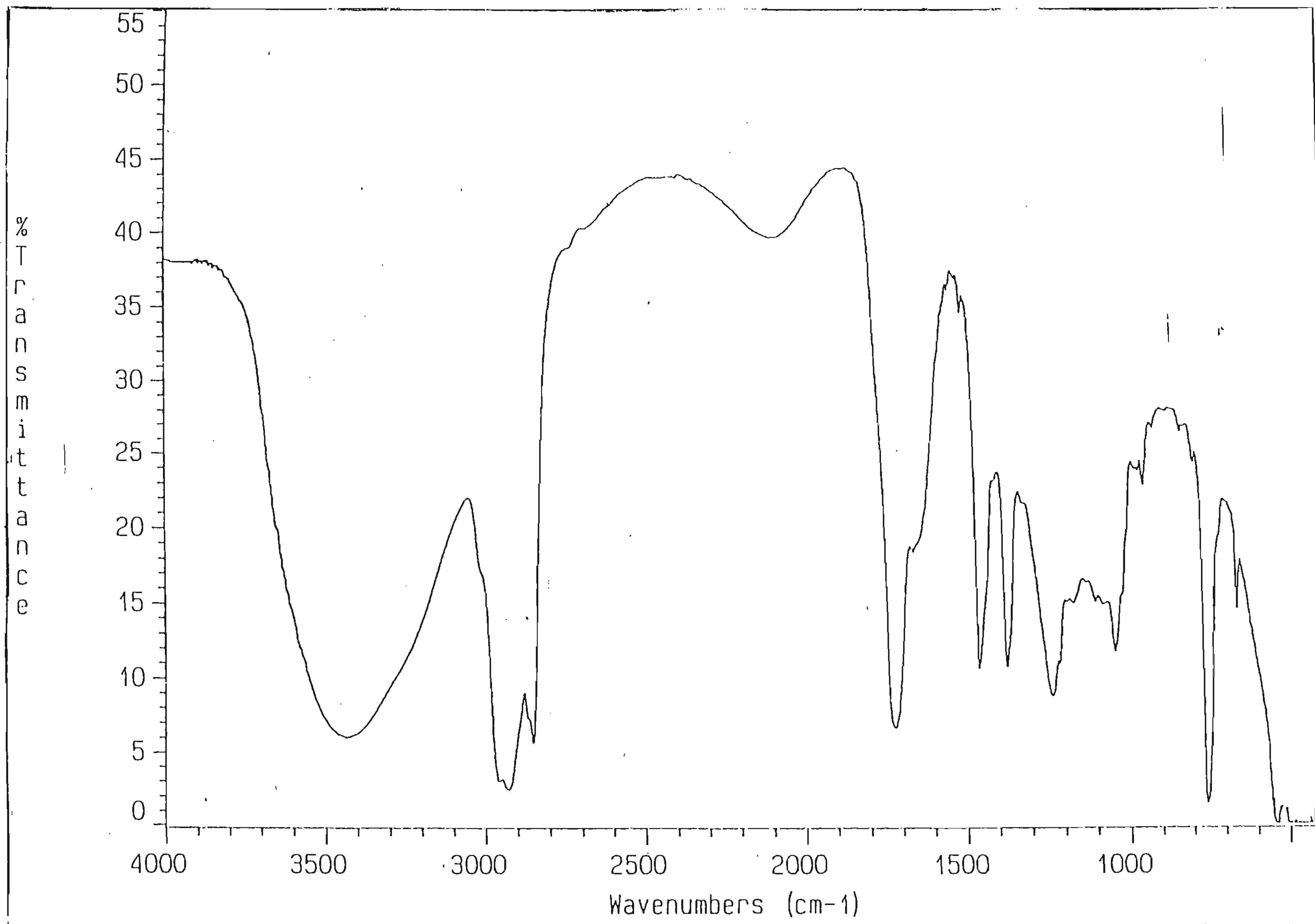


9 8 7 6 5 4 3 2 1 PPM

SPECTRUM 10.1 : ^{1}H NMR spectrum of compound 10

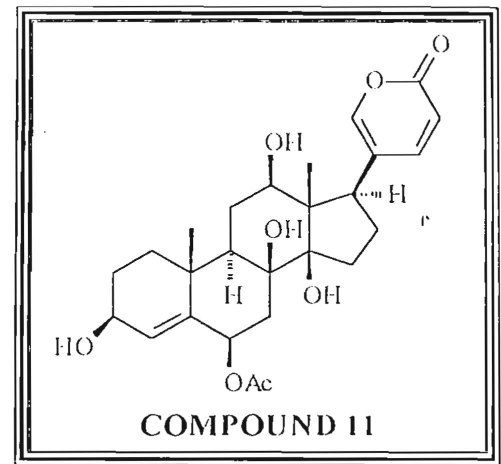


SPECTRUM 10.2 : ^{13}C NMR spectrum of compound 10



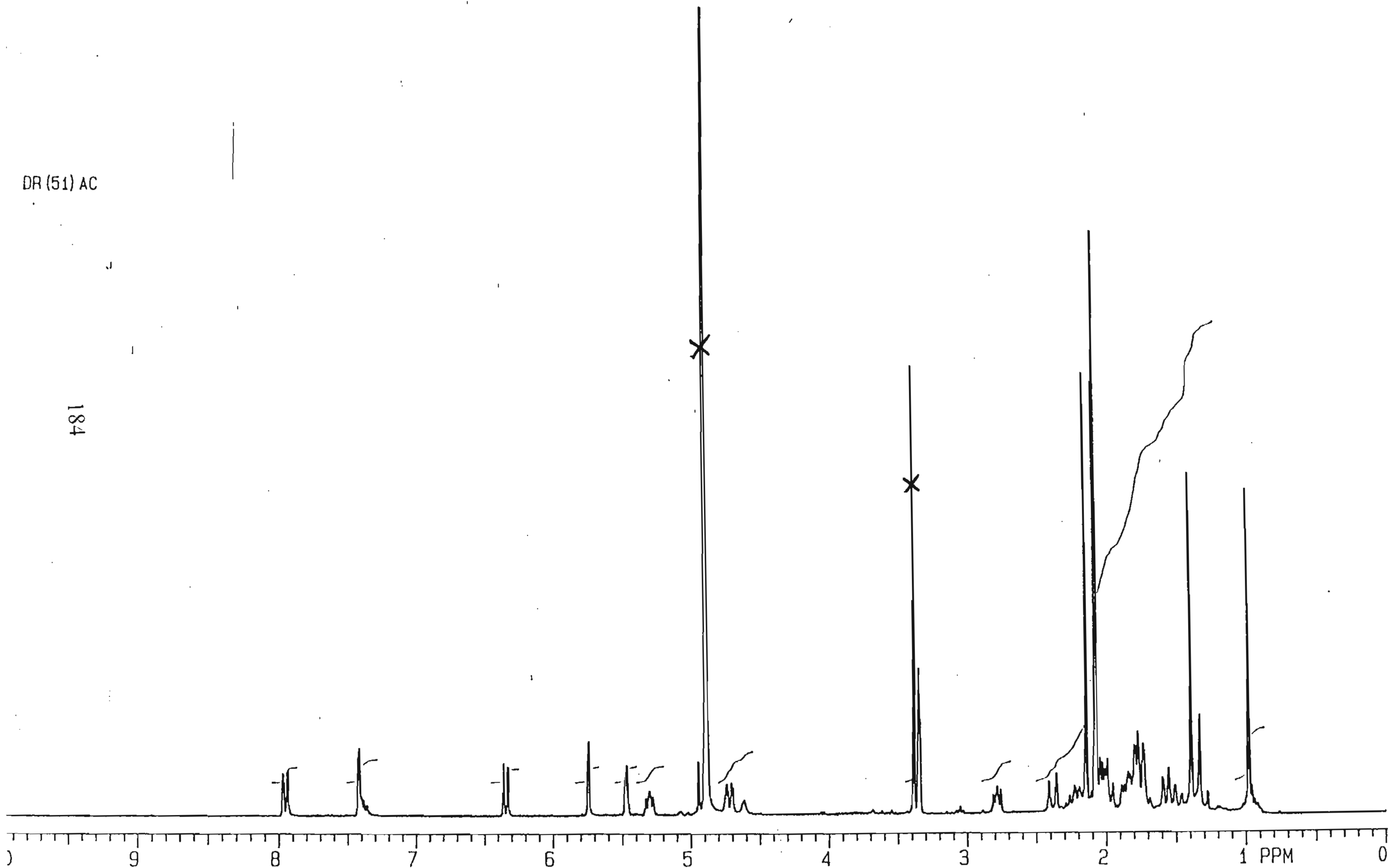
SPECTRUM 10.3 : Infrared spectrum of compound 10

Pulse Sequence: s2pul

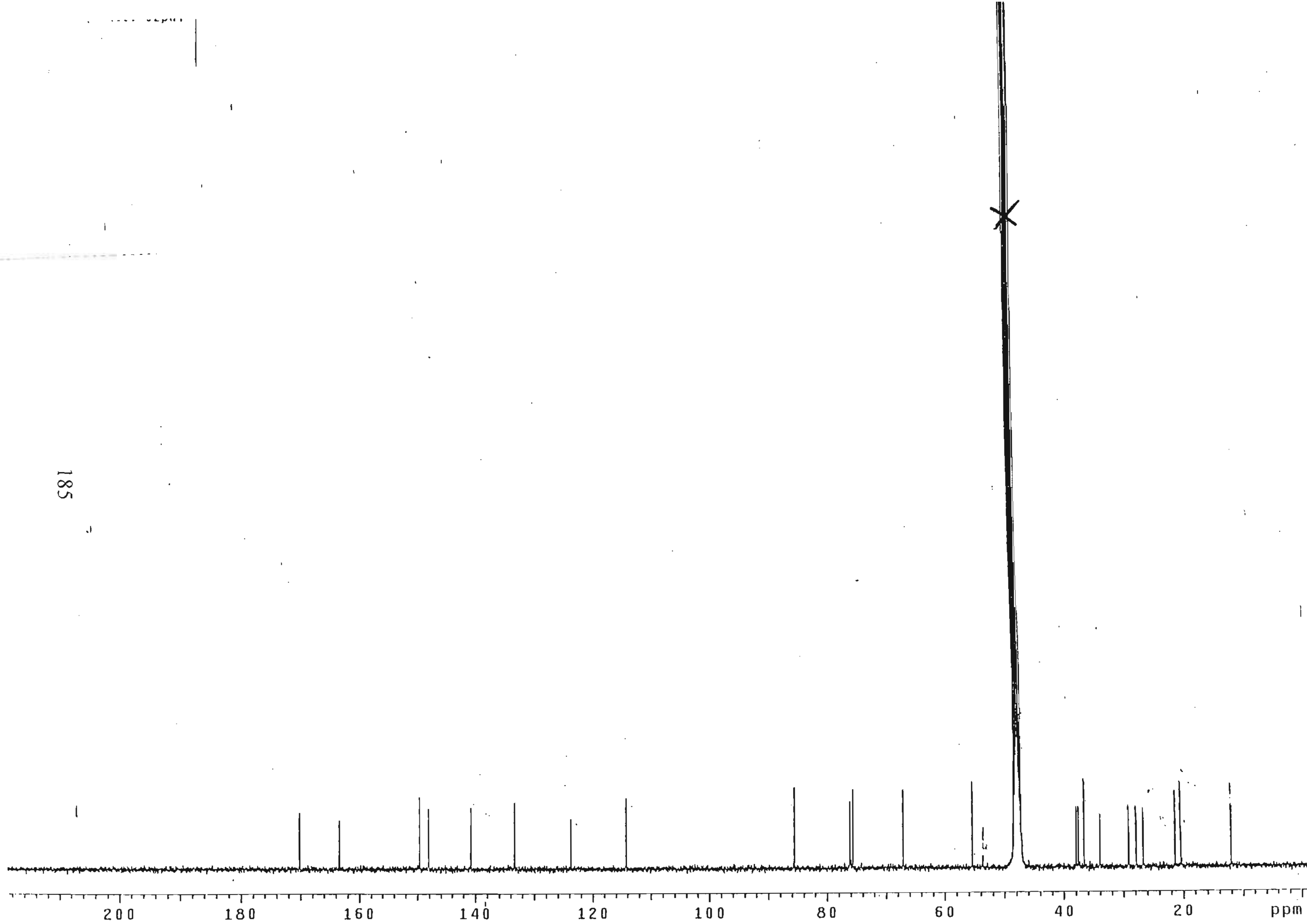


9 8 7 6 5 4 3 2 1 ppm

SPECTRUM 11.1A : ^{1}H NMR spectrum of compound 11 (CD_3OD)



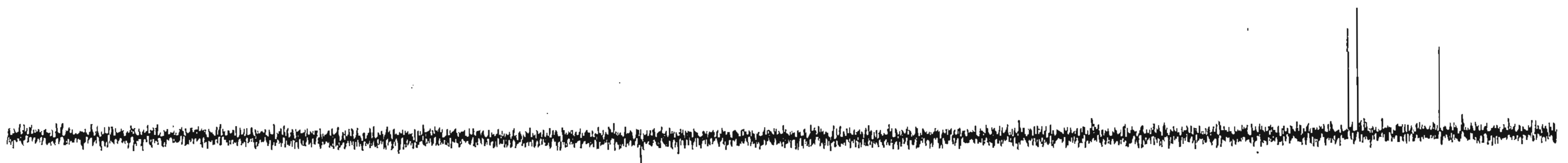
SPECTRUM 11.1B : ^{1}H NMR spectrum of acetylated compound 11 (CD_3OD)

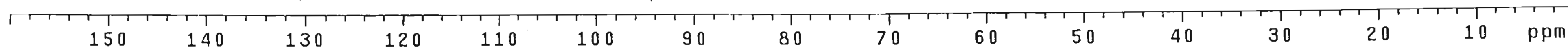


SPECTRUM 11.2 : ^{13}C NMR spectrum of compound 11 (CD_3OD)

probe=5mmASW

Pulse Sequence: dept





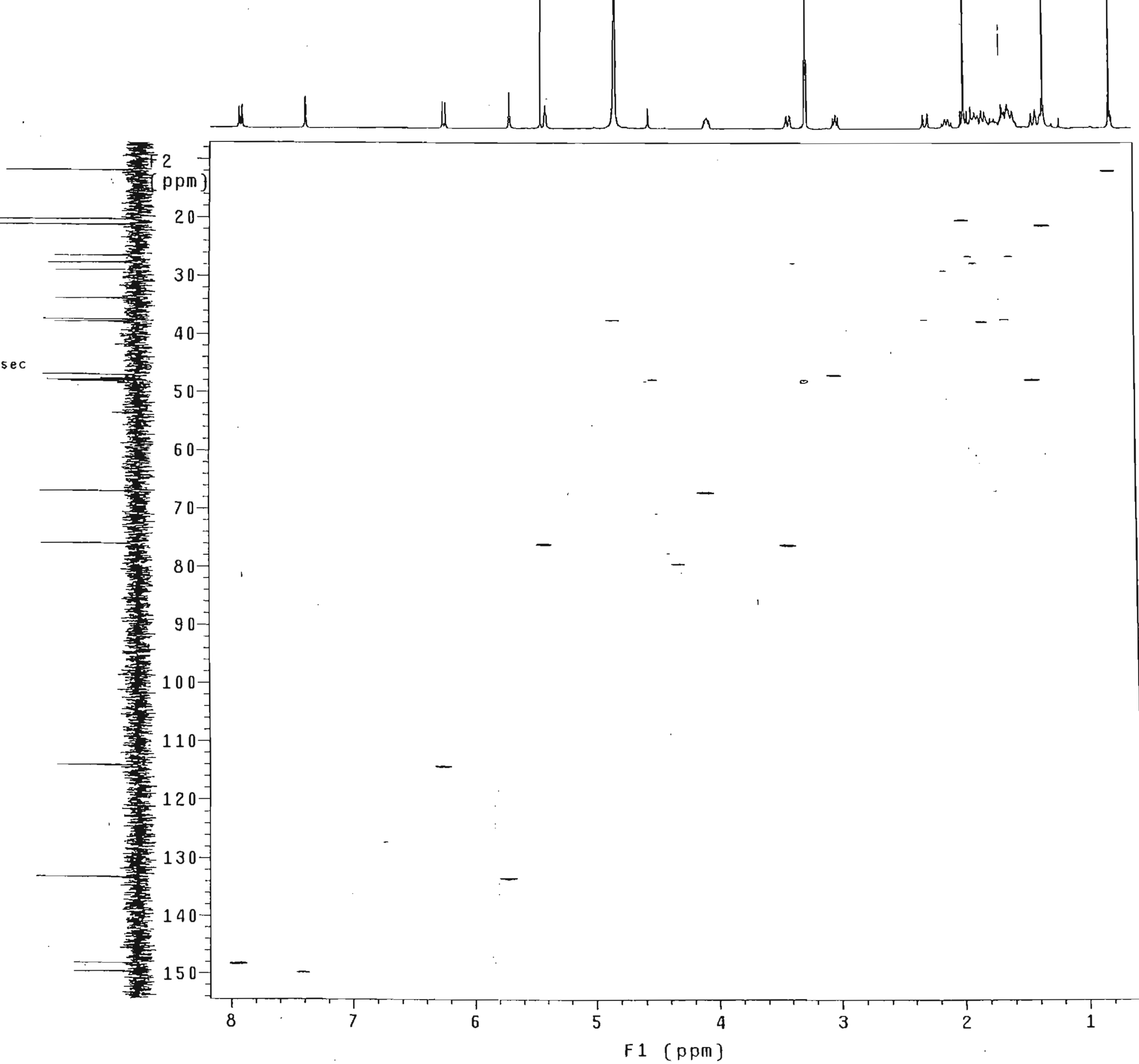
SPECTRUM 11.3 : ADEPT spectrum of compound 11 (CD_3OD)

1H/13C hetcor
probe=5mmASW

Pulse Sequence: hetcor

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

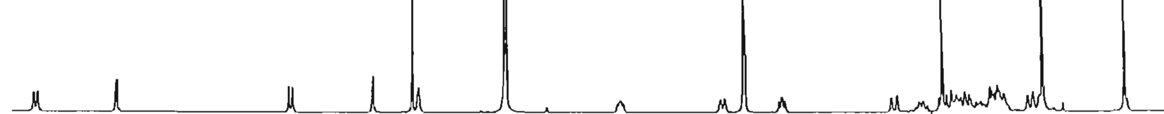
PULSE SEQUENCE: hetcor
Relax. delay 1.000 sec
Acq. time 0.064 sec
Width 16025.6 Hz
2D Width 3249.9 Hz
1920 repetitions
128 increments
OBSERVE C13, 100.5675475 MHz
DECOUPLE H1, 399.9519917 MHz
Power 38 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Sine bell 0.032 sec
F1 DATA PROCESSING
Sine bell 0.020 sec
FT size 2048 x 1024
Total time 75 hr, 37 min, 37 sec

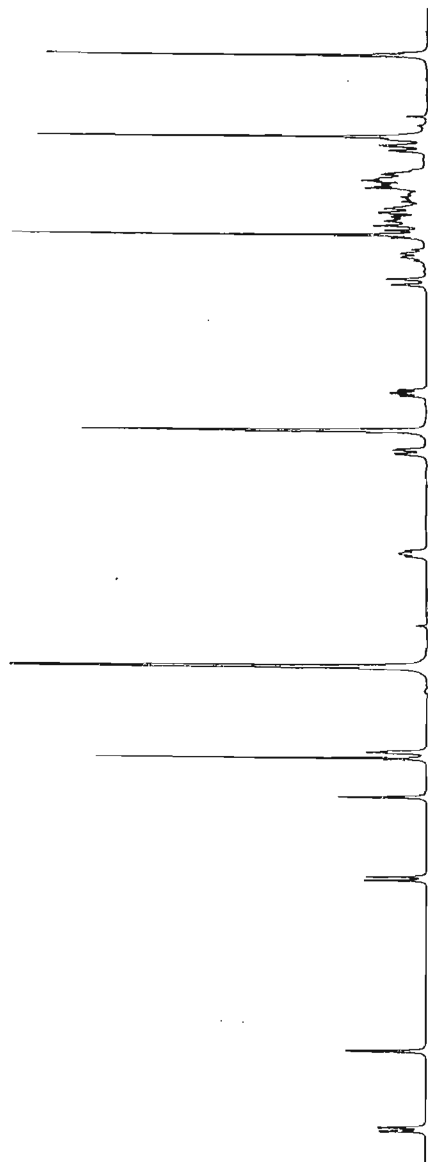


SPECTRUM 11.4 : HETCOR spectrum of compound 11 (CD_3OD)

NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da





F1 (ppm)

1 2 3 4 5 6 7 8

8 7 6 5 4 3 2 1

F2 (ppm)

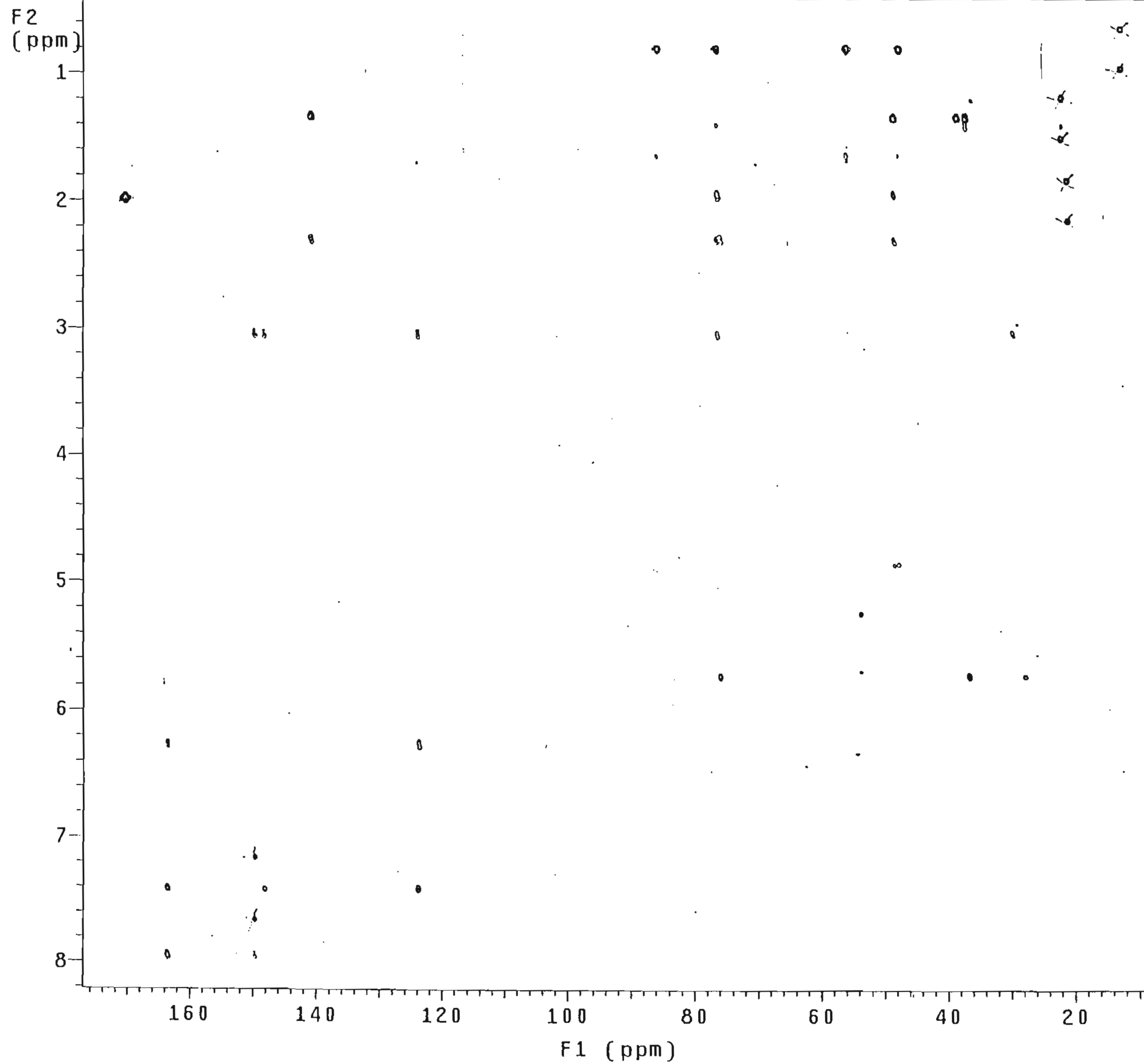
SPECTRUM 11.5 : NOESY spectrum of compound 11 (CD_3OD)

gradient HMBC expt. using GLIDE
probe=5mmASW

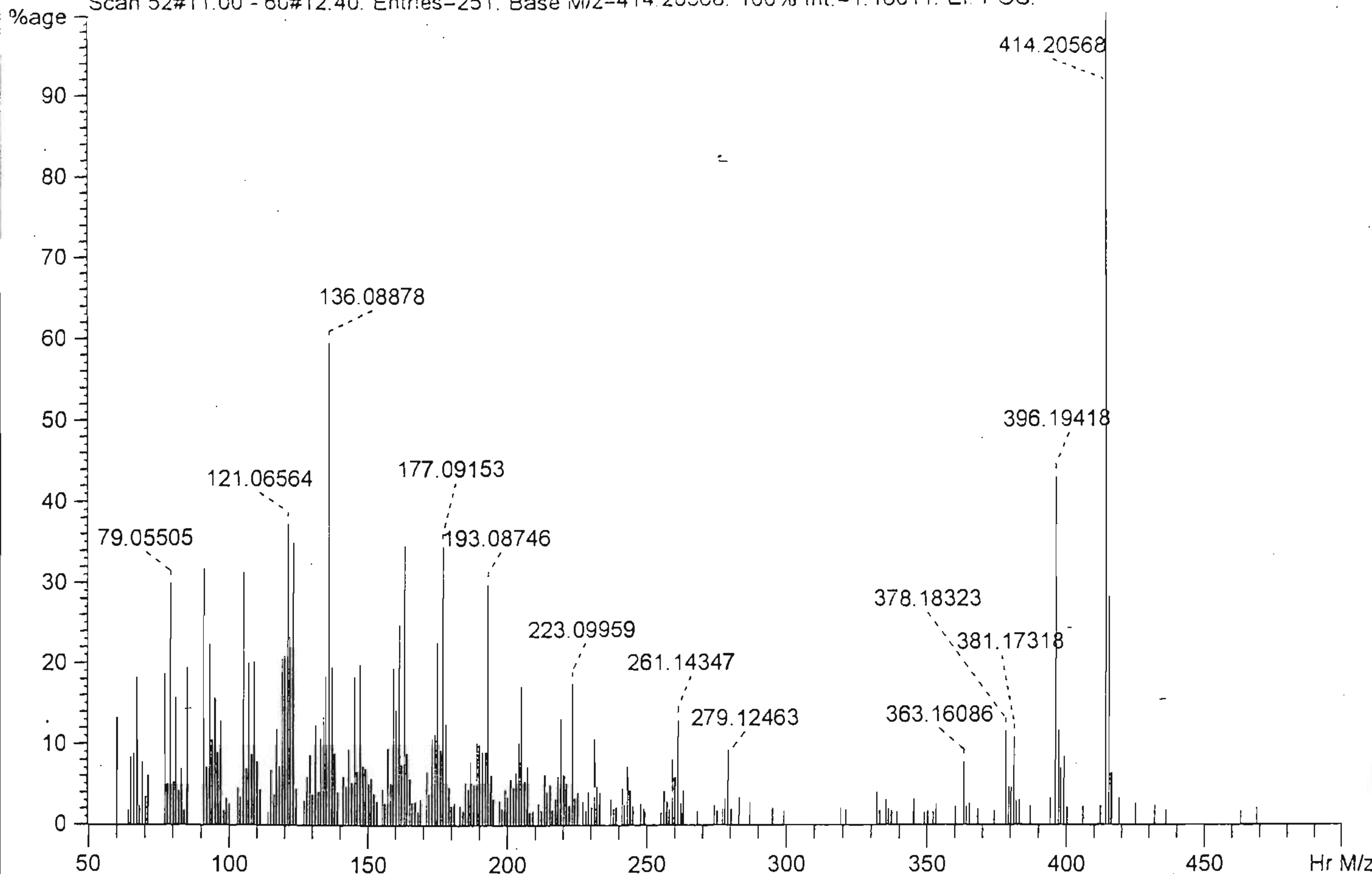
Pulse Sequence: gHMBC

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: gHMBC
Relax. delay 1.000 sec
Acq. time 0.128 sec
Width 4001.6 Hz
2D Width 20115.7 Hz
8 repetitions
512 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.064 sec
F1 DATA PROCESSING
Sine bell 0.013 sec
FT size 1024 x 2048
Total time 1 hr, 24 min, 5 sec



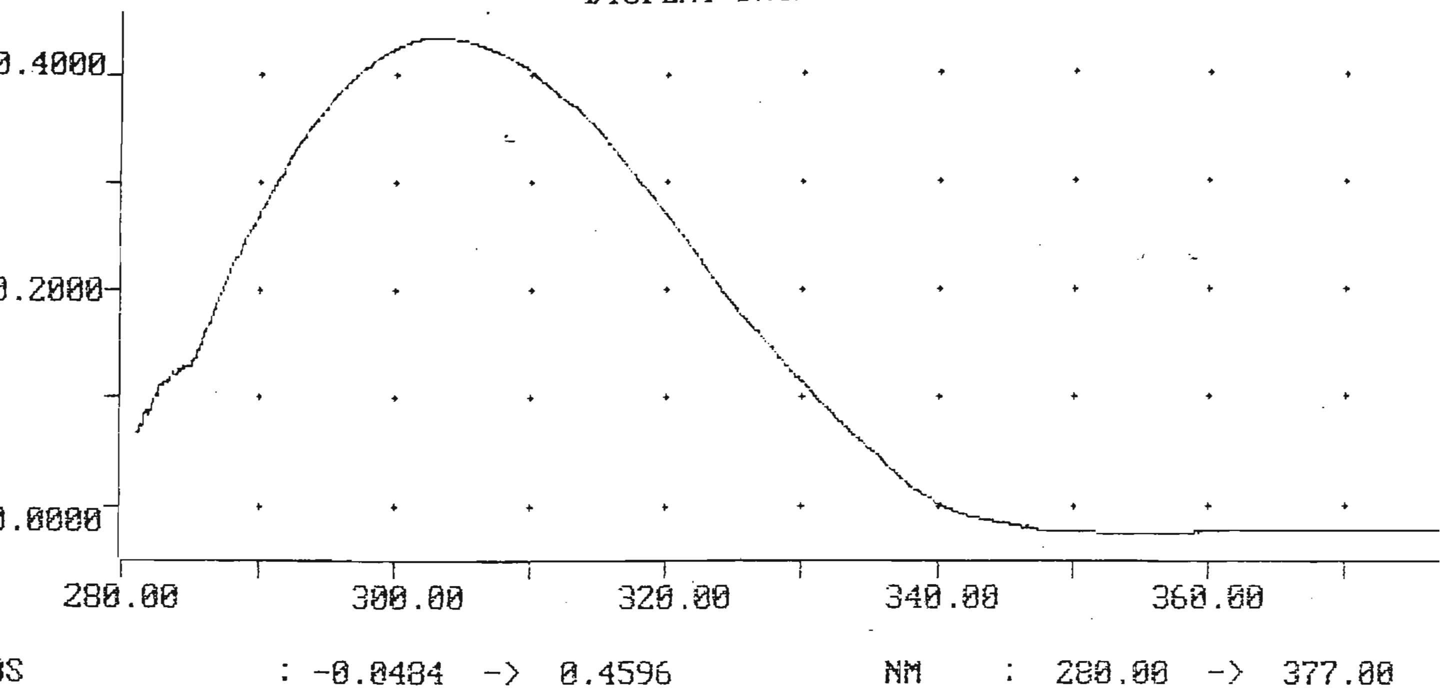
SPECTRUM 11.6 : HMBC spectrum of compound 11 (CD_3OD)



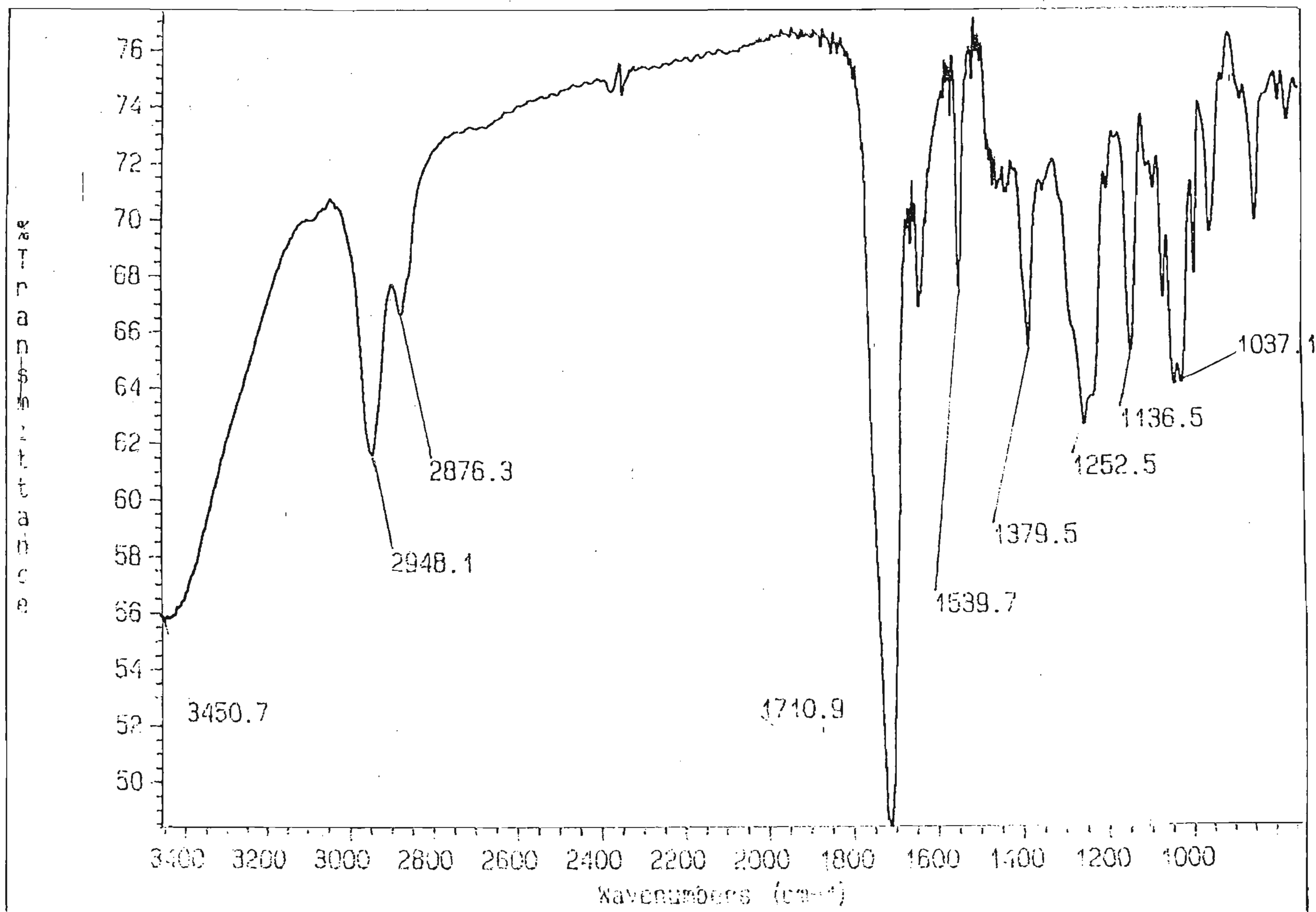
SCAN TEXT. Sorted on Hr M/z (ascending). Filter=[Int:4%. Range:80-470. Excl: Ref/Ex.].
Scan 52#11:00 - 60#12:40. Entries=122. Base M/z=414.20568. 100% Int.=1.16011. EI. POS.

Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width	Hr M/z	%age	Width
80.06119	5.29	59	123.04540	34.91	98	163.07651	34.51	72	218.09615	5.98	68
81.03432	6.32	57	124.05129	4.24	50	164.08180	8.76	83	219.10244	13.02	65
81.07053	15.71	58	124.08704	4.33	48	165.07239	5.68	102	220.10891	6.12	64
82.04176	4.09	52	128.06252	5.85	60	171.08376	6.57	83	221.10236	5.10	81
83.04980	6.83	54	129.06997	8.57	60	173.09318	10.62	83	223.09959	17.35	66
85.02922	19.36	58	131.08568	12.20	87	174.10253	11.15	74	229.12036	4.07	74
91.05495	31.71	58	132.08203	4.05	95	175.08009	22.42	84	231.13162	10.50	75
92.06111	7.05	60	133.06886	10.64	95	176.08662	9.27	86	232.13607	4.69	76
93.07043	22.22	59	134.07321	13.26	83	177.09153	34.45	67	241.12126	4.44	72
94.04215	10.47	59	135.07325	18.32	94	178.09612	12.34	71	243.13200	7.20	72
94.07688	6.00	52	136.08878	59.47	72	179.09476	4.56	77	243.98930	4.23	60
95.04999	15.58	48	137.07670	19.39	96	185.09751	5.67	88	256.14165	4.16	68
95.08596	9.87	53	138.07390	8.75	96	186.10277	4.30	75	259.12928	7.92	70
96.05720	8.86	62	141.06993	5.92	61	187.09193	7.77	91	260.11718	5.77	74
97.06529	12.73	90	142.07705	4.66	60	188.08819	4.87	82	261.14347	12.76	70
103.05483	4.47	58	143.08577	9.33	62	189.09197	10.04	75	263.16032	4.15	69
105.07057	31.15	60	143.99863	5.20	62	190.09825	9.81	67	279.12463	9.13	63
106.07658	6.90	74	145.06713	18.24	93	191.09122	8.94	83	363.16086	7.64	64
107.05037	19.90	61	146.07335	6.54	88	192.09459	9.04	83	378.18323	11.45	63
107.08590	10.15	47	147.08111	19.80	80	193.08746	29.70	65	379.18434	4.56	71
108.05722	8.62	101	148.08688	7.19	82	194.09485	6.10	80	380.19645	4.47	67
109.06571	20.10	133	149.08437	6.88	98	199.10494	4.26	86	381.17318	10.71	67
110.06956	7.71	103	150.07210	4.94	92	201.09638	5.60	83	396.19418	42.49	63
111.07264	4.19	123	151.07535	5.66	78	202.09784	4.60	75	397.19685	11.44	63
115.05494	6.70	82	155.08519	4.34	63	203.09500	6.40	92	398.20626	6.85	63
117.07047	11.72	58	157.07580	9.47	113	204.11155	10.08	73	399.18578	8.34	66
118.07744	7.14	89	158.07817	5.07	88	205.08907	17.06	75	414.20568	100.00	64
119.08602	20.43	90	159.08182	19.38	79	206.09566	5.34	73	415.20896	27.97	63
120.09345	20.85	88	160.08820	14.13	67	207.10317	7.17	67	416.21441	6.30	62
121.06564	37.15	89	161.09487	24.62	78	213.11584	6.15	83			
122.07253	23.09	94	162.09366	7.38	87	215.12456	4.87	91			

SPECTRUM 11.7 : Mass spectrum of compound 11



SPECTRUM 11.8 : UV spectrum of compound 11



SPECTRUM 11.9 : Infrared spectrum of compound 11