EXTRACTIVES FROM *EUCOMIS MONTANA* AND *AGAPANTHUS INAPERTUS*

by

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As the candidates supervisor I have/have not approved this thesis for submission

Signed: _____ **Name:** Prof. D. A. Mulholland   **Date: 10/03/2003**

TO MY DEAR FAMILY:

MUM, DAD, PRE AND NAVEN

To selves belong the loveless ones;
To oth'rs the loving e'en to bones.
Thiruvalluvar

PREFACE

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of Natal, Durban, from February 2002 to February 2003, under the supervision of Professor D.A. Mulholland and co-supervision of Dr. N.A. Koorbanally.

This study represents the original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use was made of the work of others, it has been duly acknowledged in the text.

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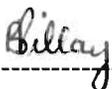
B. Pillay, BSc., BSc.(Hons.) (Natal)

I hereby certify that the above statement is correct.

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Prof. D.A. Mulholland, PhD. (Natal)

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ACKNOWLEDGEMENTS

To my family: This thesis is dedicated to you. To my parents: Babs and Saroj, you have provided me with a life that many others dream of. You have instilled me with values that have made me who I am today. Thanks for allowing me the opportunity to study and for your continuous support and encouragement. Mum, so giving and hardworking, you are a great inspiration. Dad, your strength and compassion make you a remarkable person. Mum and dad you have fulfilled your roles in every possible way and I am eternally grateful. The sacrifices you have made will never be forgotten. To my sister, Pravani, it has been a pleasure growing up with you. Thanks for always lending a helping hand and for your sound words of advice. There were times I felt as if you were the older sister. To my brother, Naven, your cheerful nature has always brought a smile to my face. Your life is just beginning and be assured, I will be there every step of the way. To my aunty, Savy, thanks for everything you have done. There were many times I could count on you.

Finally, I would like to thank God who has blessed me with love and knowledge, the greatest gifts of life!

LIST OF ABRREVIATIONS

^{1}H NMR spectroscopy	proton nuclear magnetic spectroscopy
^{13}C NMR spectroscopy	carbon-13 nuclear magnetic resonance spectroscopy
COSY	correlated nuclear magnetic resonance spectrum
HMBC	heteronuclear multiple bond coherence
NOESY	nuclear Overhauser effect
HSQC	heteronuclear single quantum coherence
ADEPT	a distortionless enhancement by polarization transfer
IR	infrared
UV	ultra violet
MS	mass spectrometry
mp	melting point
s	singlet
d	doublet
dd	doublet of doublets
t	triplet
q	quartet
m	multiplet
Hz	Hertz
HRMS	high resolution mass spectrometry
IPP	isoprene pyrophosphate
FPP	farnesyl pyrophosphate
GPP	geranyl pyrophosphate
DMAPP	dimethylallyl pyrophosphate
PAL	phenylalanine ammonia lyase
C4H	cinnamate-4-hydroxylase
PPE	phosphodiesterase
NIH	National Institute of Health
NADPH	nicotinamide adenine dinucleotide phosphate
SAM	*S*-adenosylmethionine
RDA	*retro*-Diels-Alder
H-shift	hydrogen-shift

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ABSTRACT

Two species belonging to different families were investigated, *Eucomis montana* from the Hyacinthaceae and *Agapanthus inapertus* from the Agapanthaceae.

To date no previous chemical investigation on *Eucomis montana* has been reported. *Eucomis* species are routinely harvested, processed and sold for the treatment of various ailments ranging from toothache, gastro-intestinal ailments, pain-producing ailments and venereal and urinary diseases. Members of the genus have shown to contain steroidal compounds and homoisoflavonoids. In this work one nortriterpenoid, a eucosterol type derivative and eleven homoisoflavonoids belonging to four classes, the 3-benzyl-4-chromanone, the 3-benzyl-3-hydroxy-4-chromanone, the 3-benzylidenyl-4-chromanone and the scillascillin type were found in the bulbs of *Eucomis montana*.

Agapanthus species are also used by a number of African tribes medicinally. The bulbs and rhizomes of this family have been reported to contain steroidal saponins and sapogenins. In this work the roots of *Agapanthus inapertus* have been investigated and a lignan precursor and a lignan have been found.

Structures of the compounds isolated were determined using spectroscopic techniques.

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Chapter 5: Extractives from *Agapanthus inapertus*

CHAPTER 1: INTRODUCTION

1.1. General

The use of plants as a source of medicine dates back to antiquity. Plants were thought to have healing powers, perhaps as early as by Neanderthal man. The earliest documented uses are found in Babylon *circa* 1770 BC in the code of Hammurabi and in ancient Egypt *circa* 1550 BC. In fact, ancient Egyptians believed medicinal plants to be useful in the afterlife of their Pharaohs.[1]

Plants play a fundamental role in many cultures, particularly for their medicinal purposes and a close tie between the study of plants and medicine exists.[1] Around the nineteenth century, emphasis was placed on the extraction of pure substances from plant material. Of these substances, few proved satisfactory as therapeutic agents, others being more toxic. The search for less toxic medicines based on natural sources resulted in the introduction of synthetic substances as drugs. These are often based on pharmacologically active compounds isolated from plants.[2]

Despite advances in modern medicine, traditional medicine still remains popular among indigenous people throughout the world. For instance, in Africa, many plants are used for medicinal purposes. Although there have been a number of documentations on traditional African medicine, this form of therapy is often overshadowed by modern medicine. As there is a gradual decline in the number of traditional healers, valuable information on medicinal plants is being lost. To counteract this problem, certain African countries have introduced measures to preserve the traditional healer network. The list of biologically active principles isolated from African medicinal plants is growing all the time.[3] Already African plants such as the calabar bean (*Physostigma venenosum*) and Devil's claw (*Harpagophylum procumbens*) have made it onto the pharmaceutical market.

Papaver somniferum, the opium poppy, is one of the earliest reported plants to be used medicinally. Its use was first documented in the writings of Theophrastus in the third century BC and it became popular among the Arabian, Chinese, Portuguese and English physicians.[4] Today the main producer of medicinal opium is India. Several

alkaloids have been extracted from opium with morphine being the major component. Morphine is known to be one of the best analgesics for severe pain relief. Codeine, the methoxy derivative of morphine, is a minor opium constituent. It has valuable antitussive effects, helping in the prevention and relief of coughs. Papaverine, another alkaloid isolated from the seed, possesses spasmolytic and vasodilatory activity. Several analgesic drugs using morphine as a model have been synthesized. Of these heroin, originally synthesized as a cough suppressant, is most addictive and this has led to the creation of a drug problem.[5]

morphine $R_1=R_2=H$
codeine $R_1=H; R_2=CH_3$
heroin $R_1=R_2=COCH_3$
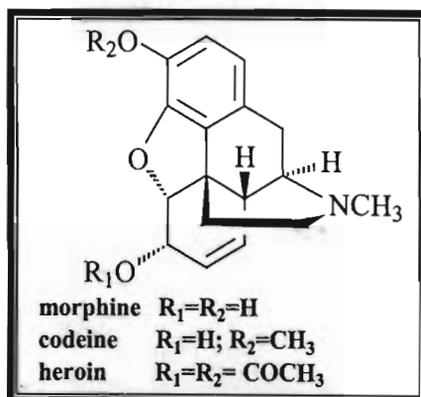
Figure 1.1: Structures of alkaloids from *Papaver somniferum*

Around the 1600's, the bark from the tree *Cinchona officinalis* indigenous to South America drew much interest. It was used as an infusion to treat fevers by the European consumers and was referred to as the fever bark. Jesuit priests became the main importers and distributors of cinchona and the name "Jesuit bark" was later adopted.[4] The bark's active ingredient is the alkaloid quinine, which is used in the treatment of malaria caused by the parasite *Plasmodium falciparum*. Other important alkaloids found in the cinchona bark are quinidine, cinchonidine and cinchonine.[5]
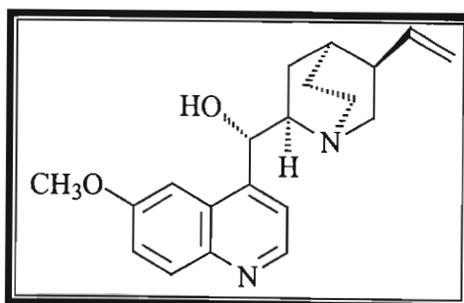
Figure 1.2: Structure of quinine

Another plant, *Artemisia annua*, known as "ginghao" in Chinese traditional medicine, is also used in the treatment of fevers and malaria. The antimalarial drug artemisinin isolated from this plant is effective against drug-resistant strains of *P. falciparum*.[5]

The coca plant has proven to be useful from ancient times. In Andes cultures the coca leaves are mixed with lime to activate the pharmacologically active part of coca, cocaine, and then chewed. Cocaine acts as an antifatigue agent and this allows labourers to ignore hunger, fatigue and cold, enhancing physical activity and endurance. Cocaine was found to paralyse nerve endings, and was used as a local anaesthetic in several surgical and dental procedures. *Erythroxylum coca* and *E. truxillense* are the most prominent sources of cocaine. Procaine, the synthetic drug based on the cocaine structure is employed to provide a safer, less toxic local anaesthetic.[5]

The leaves of the jaborandi tree, *Pilocarpus jaborandi*, native to Brazil, are popular as a folk medicine. In the form of tea, they are used as a diuretic and may be applied to the scalp to prevent baldness. It is also used to treat diabetes, asthma and a number of diseases including pleurisy and rheumatism. The leaves produce a gland rich in alkaloids such as pilocarpine. Pilocarpine is used to treat the blinding disease glaucoma.[6] Currently pilocarpine and its analogues are being investigated for the treatment of Alzheimer's disease.[5]
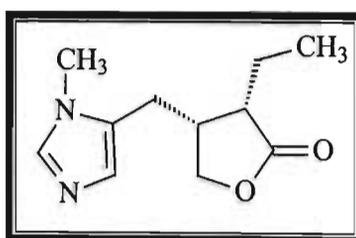
Figure 1.3: Structure of pilocarpine

The use of *Rauwolfia* plant extracts may be traced back to ancient Hindu Ayurvedic writings. They were used to prepare primitive remedies for diseases such as hypertension, insomnia and insanity.[4] *Rauwolfia* species found in India, Africa and the Caribbean produce large amounts of the therapeutically active compounds, reserpine and deserpidine. These alkaloids are used as antihypertensives and mild tranquilizers.[5]

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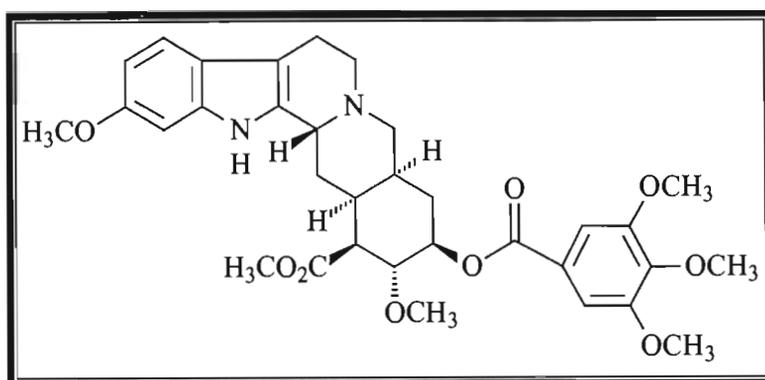
Figure 1.4: Structure of reserpine

The Madagascan periwinkle plant, *Vinca rosea*, has been shown to possess beneficial medicinal properties. As a folk medicine it is used as a tea for diabetes. Investigation into the possible hypoglycaemic activity of the plant indicated its anticancer potential. This has led to the isolation of some one hundred and fifty alkaloids, many of which have demonstrated useful antitumor activity, including vinblastine, vinrosidine, vinleurosine and vincristine. Of these, vincristine and vinblastine are considered to be extremely valuable drugs in cancer chemotherapy. Vinblastine is used mainly in the treatment of Hodgkin's disease while vincristine is more neurotoxic.[5]

Another plant known for its anticancer properties is the Pacific Yew, *Taxus brevifolia*. The bark of this plant has produced the anticancer drug Taxol (paclitaxel). Taxol is used clinically to treat ovarian, breast and lung cancers and cancers of the brain and neck. The problem experienced with Taxol, however, is that the amount of active ingredient produced from the plant is enough to treat only one patient and it takes several years to grow the plant. Currently a semi-synthetic form of Taxol is being produced.[5]
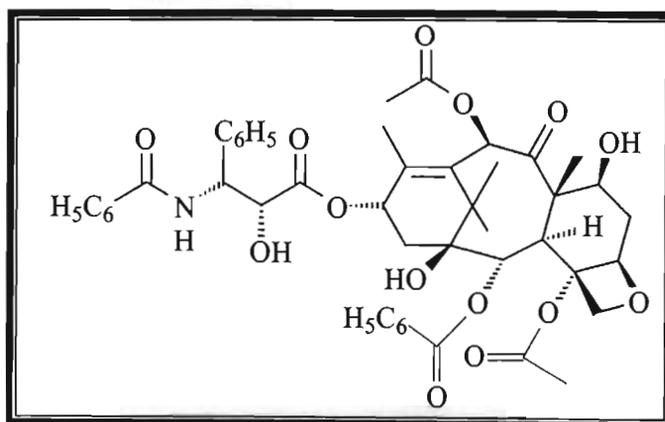
Figure 1.5: Structure of Taxol

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These are a few, yet classic, examples of plant-derived medicinals. The Plant Kingdom has provided us with a virtually untapped wealth of healing gifts and will continue to provide a rich, virtually inexhaustible supply of new potential drugs. Problems with drug resistant micro-organisms, side effects of modern drugs and emerging diseases where no drugs are available encourages the investigation of plants as a vital source of medicine. It is therefore imperative that modern-day researchers fully appreciate the vast medicinal knowledge of indigenous people in their quest for drug discovery.

1.2. The Hyacinthaceae and the Agapanthaceae

1.2.1. Introduction to the Hyacinthaceae

The Hyacinthaceae is the family of hyacinths and related plants. Formerly part of the Liliaceae *sensu lato*, the family has two centres of diversity, one being southern Africa, while the other, stretches from the Mediterranean to South-West Asia.[7] It comprises sixty-seven genera and nine hundred species worldwide, with three hundred and sixty-eight species and twenty-seven genera locally represented.[8,9]

Although there has been great difficulty in the classification of the family, five subfamilies have now been identified. Of these, the Urgineoideae, Ornithogaloideae and Hyacinthoideae are found in southern Africa.[7,8]

Members of the Hyacinthaceae are bulbous perennial herbs. They are widespread and well adapted to the moist, arid climate in southern Africa.[7]

The compounds isolated from the Hyacinthaceae are generally classified into four groups: homoisoflavonoids, steroidal type compounds, bufadienolides and miscellaneous type compounds.[10] The occurrence and biosynthesis of homoisoflavonoids are described in detail in chapter 2. Steroidal type compounds include spirocyclic nortriterpenoids and cholestane glycosides/steroidal saponins and their derivatives. The characteristic feature of cholestane glycosides is a basic cholestane triterpenoid skeleton.[5,10] Classification and biosynthesis of spirocyclic nortriterpenoids is described in chapter 2. Bufadienolides are one of a group of two types of cardiac glycosides. Cardiac glycosides are characterized by a steroidal aglycone attached to a sugar moiety. The cardenolides have a β-substituted, unsaturated, five membered lactone ring at C-17 and the bufadienolides have a β-substituted, doubly unsaturated, six membered lactone ring at the same position.[5,10] Miscellaneous type compounds include compounds such as chromones, stilbenes, chalcones, acids, pyrones, phenolic compounds and two alkaloids.[10]
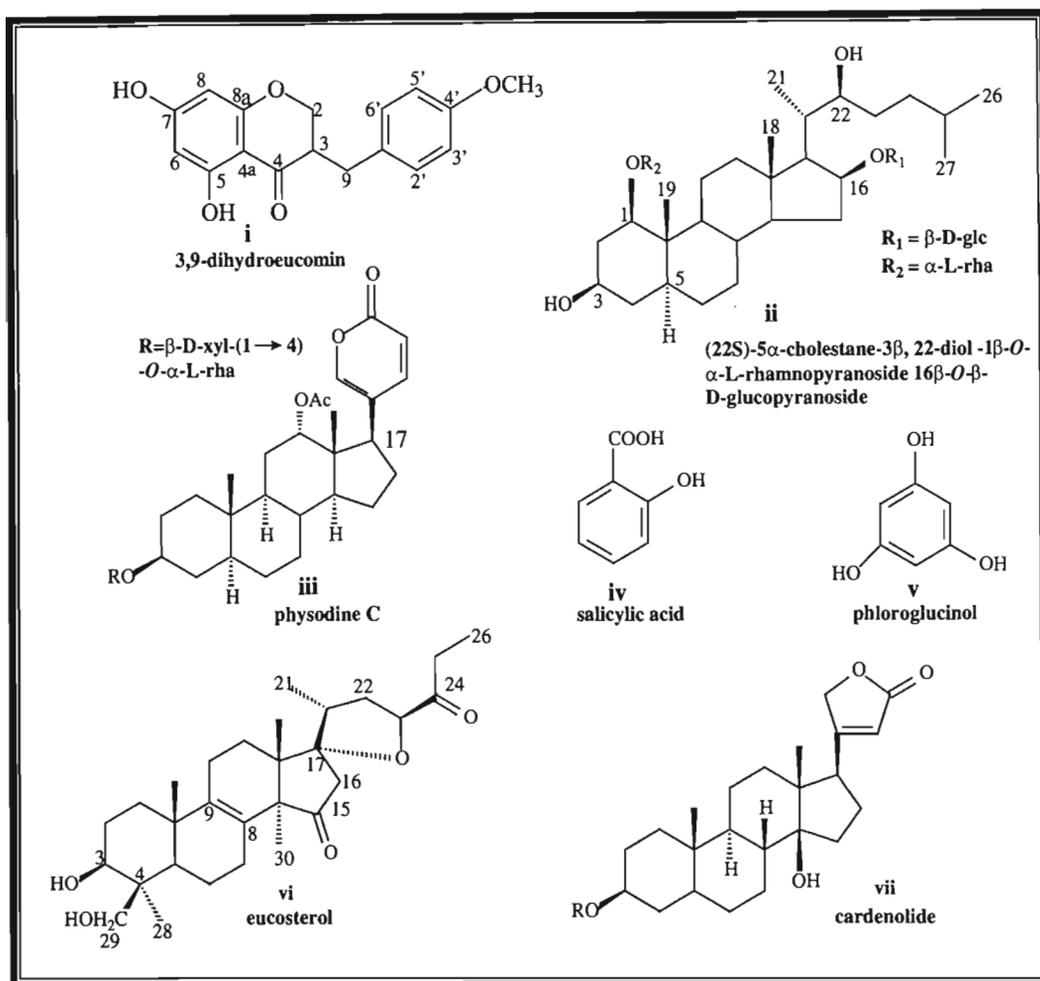
Figure 1.6: Examples of some types of compounds isolated from Hyacinthaceae, (i) homoisoflavonoid, (ii) cholestane glycoside, (iii) bufadienolide, (iv) acid, (v) phenolic compound, (vi) spirocyclic nortriterpenoid and (vii) cardenolide

1.2.2. Introduction to the Agapanthaceae

The genus *Agapanthus* was initially included in the Aliaceae, and then moved to the Amaryllidaceae. It is now placed in its own family, the Agapanthaceae. These previous inclusions were based on whether the umbellate inflorescence is considered to be of greater taxonomic importance than its superior ovary.[11,12,13]

Species of the family Agapanthaceae have been found to contain steroidal saponins.[11,12] Steroidal saponins are C_{27} sterols in which the cholesterol side chain has

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been modified to produce a spiroketal and usually a sugar residue is attached at C-3. Hydrolysis of the sugar unit at C-3 produces compounds called sapogenins.[5]

i. R=sugar residue
ii. R=H
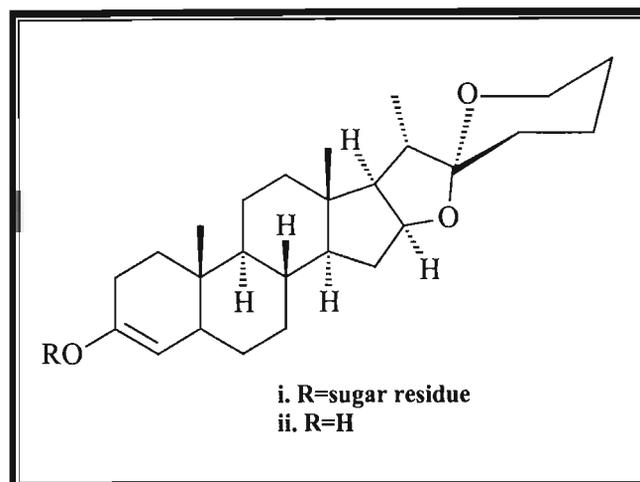
Figure 1.7: Structure of a (i) saponin and (ii) sapogenin

The Agapanthaceae is a monotypic family *i.e.* it consists of only one genus, *Agapanthus*. This family is indigenous to southern Africa. Agapanthaceae comprises ten variable species widespread in all provinces of South Africa, except for the Northern Cape. It is also found in Lesotho and Swaziland.[13,14] They are found in areas where the rainfall is more than 500 mm per annum, from sea level to 2000 m.[13] Its natural distribution extends from the Cape Peninsula in the south-west, along the southern and eastern coast of southern Africa, then inland and northwards into the mountainous regions of the Limpopo River.[13,14]

The name *Agapanthus* is derived from the Greek word *agapé*, meaning love and *anthos*, meaning, flower.[13] These plants require sun or lightly shaded positions for optimum growth and flowering. Both deciduous and evergreen species occur.[14]

1.3. Species investigated

1.3.1. *Eucomis montana* (Hyacinthaceae)

Eucomis montana is one of ten species of the genus *Eucomis* L'Heritier. Members of this genus are mostly endemic to southern Africa and are found in the eastern and north-eastern regions, except *Eucomis regia* which occurs in the Western and South-Western Cape.[14]

All species of *Eucomis* are deciduous geophytes and summer growing. They have large bulbs that produce a rosette of smooth, usually shiny leaves. The inflorescence is a spike of scented white, pale green or yellow flowers, bearing a tuft of leaf-like bracts at its tip that resemble a pineapple. The name pineapple-flower is often employed.[14,15]

Figure 1.8: Photograph of *Eucomis montana* inflorescence by Neil Crouch

Although actual uses of *Eucomis montana* have not been documented, indigenous people of southern Africa use other members of *Eucomis* in the treatment of various ailments. The Southern Sotho use *E. bicolor* as a colic remedy and *E. regia* as a remedy for venereal disease. Decoctions of the bulbs of *E. punctata* are used by the Xhosa as a treatment for rheumatism and given as an enema to children during teething while decoctions of the bulbs of *E. undulata* is a Pedi remedy for abdominal pain. The Zulu take decoctions of a *Eucomis sp.* (probably *E. regia*) for the treatment of respiratory conditions, especially cough, and as an enema for biliousness.[16]

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Previous investigations of the *Eucomis* genus have yielded homoisoflavonoids **(I-III)**, spirocyclic nortriterpenes **(IV)**, lanosterol oligosaccharides **(V)**, acids **(VI)**, dibenzo-α-pyrones **(VII)** and chromanones **(VIII)**. These are listed below.

I) 3-benzyl-4-chromanone type homoisoflavonoids
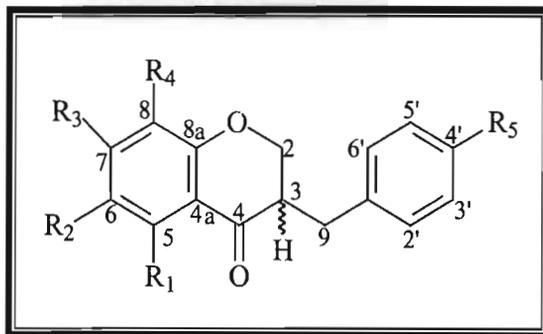
Name	R_1	R_2	R_3	R_4	R_5	Ref
1. 3,9-dihydroeucomin	OH	H	OH	H	OCH_3	17,18
2. 7-*O*-methyl-3,9-dihydro-eucomin	OH	H	OCH_3	H	OCH_3	18
3. 3,9-dihydropunctatin	OH	H	OH	OCH_3	OH	19
4. 4'-*O*-methyl-3,9-dihydro-punctatin	OH	H	OH	OCH_3	OCH_3	19
5. 4'-demethyl-5-*O*-methyl-3,9-dihydroeucomin	OCH_3	H	OH	H	OH	19
6. 3,9-dihydroautumnalin	OH	OCH_3	OH	H	OH	20

II) 3-benzyl-3-hydroxy-4-chromanone type homoisoflavonoids
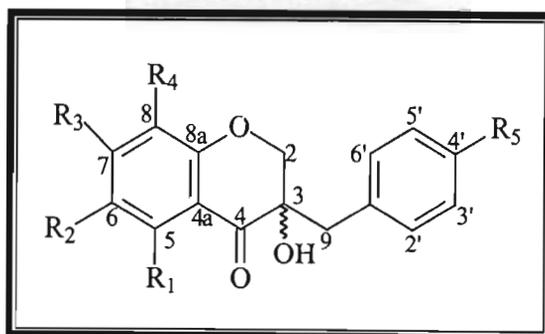
Name	R_1	R_2	R_3	R_4	R_5	Ref
7. eucomol	OH	H	OH	H	OCH_3	21
8. 7-*O*-methyleucomol	OH	H	OCH_3	H	OCH_3	18
9. 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone	OH	H	OH	H	OH	17

III) 3-benzylidenyl-4-chromanone type homoisoflavonoids
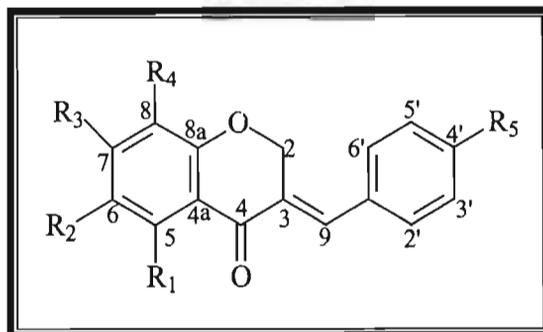
Name	R₁	R₂	R₃	R₄	R₅	Ref
10. eucomin	OH	H	OH	H	OCH₃	21
11. punctatin	OH	H	OH	OCH₃	OH	19
12. 4'-*O*-methylpunctatin	OH	H	OH	OCH₃	OCH₃	19,20
13. 4'-demethyleucomin	OH	H	OH	H	OH	19
14. autumnalin	OH	OCH₃	OH	H	OH	20

IV) spirocyclic nortriterpenes

Name	R_1	R_2	R_3	R_4	R_5	Ref
15. eucosterol	OH	CH₃	=O	H	H	22
16. 16β-hydroxyeucosterol	OH	CH₃	=O	OH	H	22
17. scillascilloside D-1	[O-α-L-rha-(1→2)-O-β-D-glc-(1→2)-O-α-L-arab-(1→6)-β-D-glc]	CH₃	H	H	H	23
18. muscaroside C	[O-β-D-*apio*-D-furan-(1→2)- O-β-D-glc-(1→2)-O-α-L-arab-(1→6)-β-D-glc]	CH₂OH	H	H	H	23
19. 16β-hydroxyeucosterol 3β-*O*-[*O*-α-L-rhamnopyranosyl-(1→2)-*O*- β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside]	[O- α –L-rha-(1→2)-O-β-D-glc-(1→2)-O-α-L-arab-(1→6)-β-D-glc]	CH₃	=O	OH	H	23
20. 15-deoxo-30-hydroxyeucosterol 3β-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)- *O*-[β-D-xylopyranosyl-(1→3)]-*O*-β- D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}	{O- α –L-rha-(1→2)-O-[β-D-xyl -(1→3)] O-β-D-glc-(1→2)-O-α-L-arab-(1→6)-β-D-glc}	CH₂OH	H	H	H	23
21. eucosterol 3β-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)] -*O*- β-D-glucopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside}	{O- α –L-rha-(1→2)-O-[β-D-xyl -(1→3)] O-β-D-glc-(1→2)-O-α-L-arab-(1→6)-β-D-glc}	CH₃	=O	H	H	23

V) lanosterol oligosaccharides
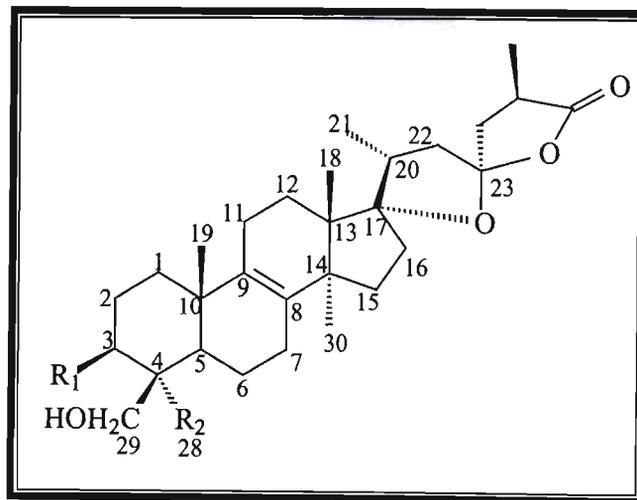
Name	R_1	Ref
22. scillasaponin A	*O*-α-L-rha-(1→2)-*O*-[β-D-xyl-(1→3)]-*O*-β-D-glc-(1→2)-*O*- α-L-arab-(1→6)-β-D-glc	23

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VI) acids
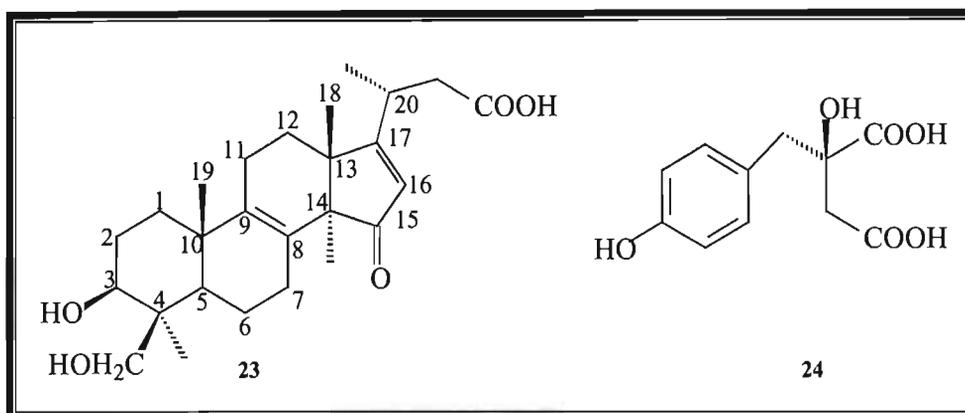
Name	Ref
23. 3β-hydroxy-4β-hydroxymethyl-4α,14 α-dimethyl-15-oxo-24-nor-5α-chola-8,16-dien-23-oic acid	22
24. eucomic acid	22

VII) dibenzo-α-pyrones
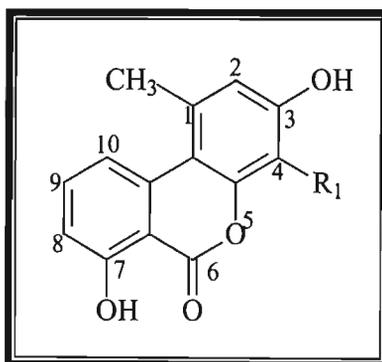
Name	R_1	Ref
25. autumnariol*	H	24
26. autumnariniol*	OCH₃	24

* These published structures are incorrect. They are actually xanthones and structures are being corrected in a PhD thesis currently being prepared by C. Koorbanally of the Natural Products Research Group, UND.

VIII) chromanones
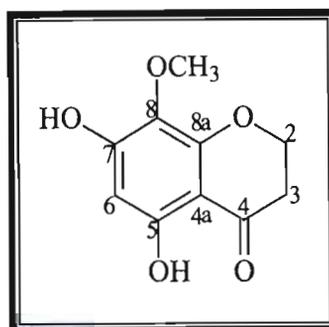
Name	Ref
27. 5,7-dihydroxy-8-methoxy-4-chromanone	25

Table 1.1: List of compounds isolated from various *Eucomis* species

Eucomis species	Compounds isolated
E. bicolor	1,2,7,8,9,10,15,16,17,18,19,20,21,22
E. comosa (syn E. punctata)	3,4,5,11,12,13,15,24,27
E. autumnalis (syn E. undulata)	6, 12,14,15,23,25,26
E. pole-evansii	15

1.3.2. *Agapanthus inapertus* (Agapanthaceae)

Agapanthus inapertus is also known as the Drakensburg *Agapanthus*, drooping *Agapanthus*, bloulelie and hlakahla. *Agapanthus inapertus* is an attractive, tuberous plant, which occurs naturally in open grassland and forest margins through Mpumalanga, Swaziland, northern Kwazulu-Natal, Gauteng and the Northern Province where it is found in the mountainous, rocky areas and commonly along the Drakensberg Escarpment.

A striking feature of these plants is the beautiful dark blue to violet flowers held in dense stalks up to 1.5 m high. Individual flowers are tubular and pendulous hence the name "drooping agapanthus" is often used. There are five subspecies of *Agapanthus inapertus*, which are distinguished from each other on the basis of shape and size of the flowers.[26]

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**Figure 1.9: Photograph of *Agapanthus inapertus* inflorescence
by Dr Neil Crouch**

Members of *Agapanthus* have been reported to have medicinal uses. South African traditional healers use *A. africanus* plants in herbal remedies to treat pregnancy-related ailments and to allow an easy childbirth. The leaves and roots are prepared as antenatal medicines in the form of decoctions or infusions.[16,27] Infusions of the root are used by the Zulu to help with chest pains and cough. The bulb of *A. orientalis* is used as a Zulu aphrodisiac.[16]

Steroidal saponins and sapogenins that have been isolated from *Agapanthus* are given below.
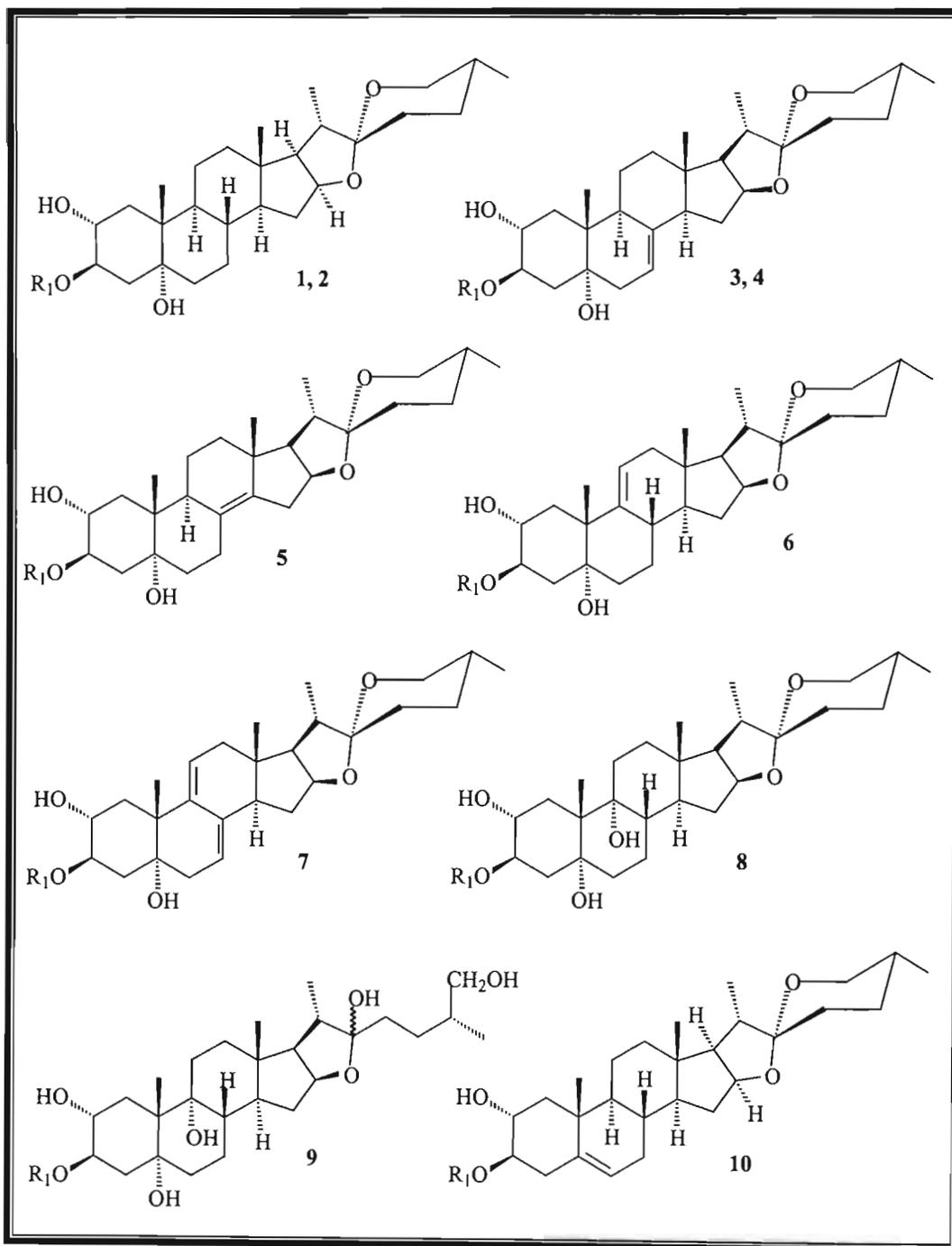
Figure 1.10: Compounds isolated from *Agapanthus* species

Name	R_1	Ref
1. Agapanthagenin	H	28
2. Agapanthussaponin A	3-O-[α-L-Rahmnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside]	29
3. 7-dehydroagapanthagenin	H	28
4. Agapanthussaponin B	3-O-[α-L-Rahmnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside]	29
5. 8(14)-dehydroagapanthagenin	H	28
6. 9(11)-dehydroagapanthagenin	H	30
7. Agapanthussaponin C	3-O-[α-L-Rahmnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside]	29
8. Agapanthussaponin D	3-O-[α-L-Rahmnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside]	29
9. Furostanolsaponin	3-O-[α-L-Rahmnopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside],26-O-β-D-glucopyranoside,22-methylether	29
10. Yuccagenin	H	28

Table 1.2: List of compounds isolated from *Agapanthus* species (see Figure 1.10)

Agapanthus species	Compounds isolated
A. africanus	1,3,5,6,10
A. inapertus	2,4,7,8,9

17

1.4. References

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CHAPTER 2: THE CLASSIFICATION AND BIOSYNTHESIS OF TRITERPENOIDS AND HOMOISOFLAVONOIDS

In this work a eucosterol type nortriterpenoid and eleven homoisoflavonoids were isolated from *Eucomis montana*. The biosynthesis of these types of compounds will be discussed in this chapter.

2.1. Occurrence and Classification of Triterpenoids

Terpenoids are a large, diverse group of natural products widely distributed throughout the Plant Kingdom. A small, yet important group such as lanosterol, is of animal origin. The basic building blocks of terpenoids are C-5 isoprene units. The classification of triterpenoids is based on the number of isoprene units incorporated in the carbon skeleton.[1]
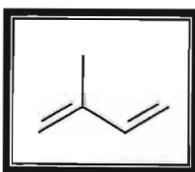
Figure 2.1: Structure of the isoprene unit

Compounds that have thirty carbons are referred to as "triterpenoids" and are derived from squalene, which was first isolated from shark liver oil.[2] Triterpenoids occur as esters, glycosides or in the free state.[3]
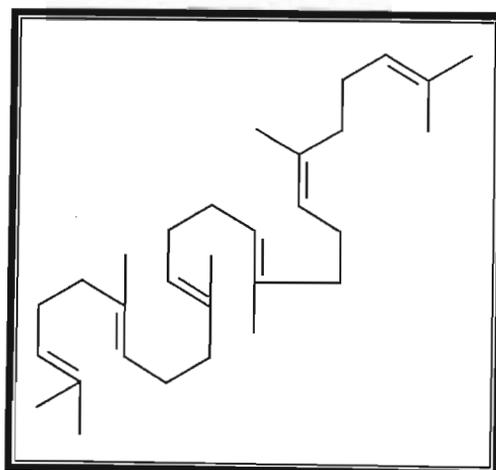
Figure 2.2: Structure of squalene

Spirocyclic nortriterpenoids are a class of compounds with a basic lanosterol triterpenoid skeleton that have been isolated from members of the Hyacinthaceae. The two main types are the eucosterol and 16β-hydroxyeucosterol types.[4] In addition, lanosterol oligosaccharides with aglycone skeletons have been reported to occur in *Velthemia viridifolia* and *Eucomis bicolor* and spirocyclic nortriterpenoid glycosides from *Scilla scilloides*.[5,6,7] These contain skeletons closely related to eucosterol. The variation in these compounds is due to the differing degree of oxygenation of the aglycone and different combinations of sugars.

Two numbering systems are found in the literature for sterols. Older literature makes use of IUPAC-IUB rules for sterol nomenclature of 1967, but the more recent literature makes use of IUPAC-IUB rules for sterol nomenclature of 1989. Both systems are given below. The IUPAC-IUB rules of 1989 are used in this work.
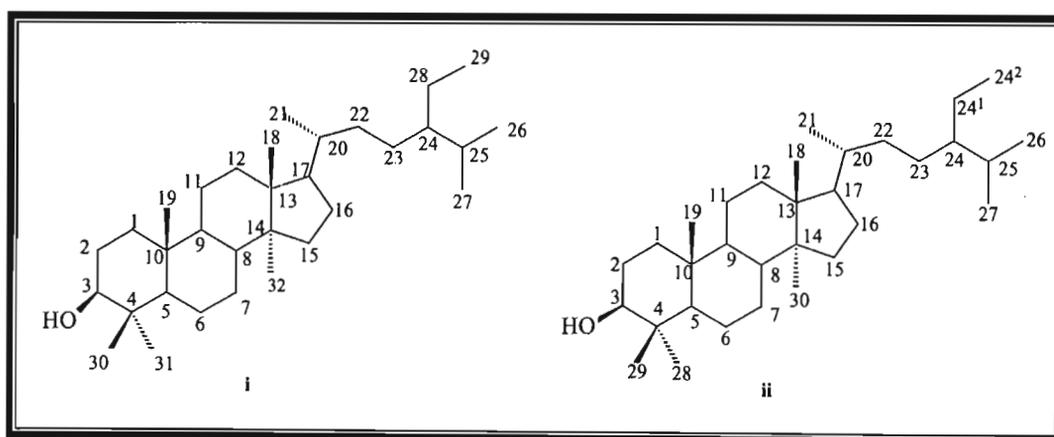
Figure 2.3: Numbering system for sterols according to IUPAC-IUB rules of (i) 1967, (ii) 1989

2.1.1. Eucosterol type Triterpenoids
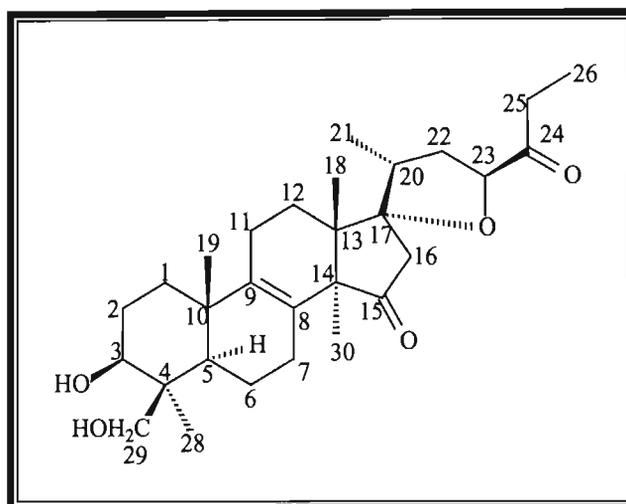
Figure 2.4: Structure of eucosterol

Eucosterol is found to be a secondary metabolite of several *Eucomis* species. The structure and absolute configuration of eucosterol was determined by chemical and spectroscopic data and provides the basis for the structural elucidation of related compounds.[4] The unique structural feature of eucosterol-type compounds is the spirofused α -furanoic ether linkage between C-17 and C-23.

A summary of characteristic resonances found in the ^{1}H NMR and ^{13}C NMR spectra for eucosterol-type compounds is given below.[4]

The ^{1}H NMR spectra show a multiplet between δ_H 3.40 and δ_H 3.60 for the H-3 proton and a pair of doublets at δ_H 3.30-3.50 and δ_H 4.00-4.20 for the methylene protons of the CH$_2$OH group (2H-29). The methine proton at C-23 resonates as a triplet between δ_H 4.64 and δ_H 4.80.

Six methyl groups are usually present in the molecule. The 3H-18 and 3H-19 methyl group protons resonate as singlets between δ_H 0.87 and δ_H 1.05 and the singlet resonances at approximately δ_H 1.05-1.30 and δ_H 1.27-1.41 belong to the methyl protons of 3H-28 and 3H-30 respectively. The 3H-26 methyl group proton resonance occurs as a triplet at approximately δ_H 1.04-1.12.

The ^{13}C NMR spectrum usually shows two carbonyl resonances at about δ_C 215.0 and δ_C 211.0 belonging to C-15 and C-24 respectively. The molecular formula for eucosterol itself shows the presence of five oxygen atoms, however the ^{13}C NMR spectrum shows six oxygen bonded carbons and this indicates the presence of the ether linkage between C-17 and C-23 forming the heterocyclic ring E. This can be proven by the fact that on acetylation of eucosterol, acetylation occurs only at C-3β and C-29 indicating only two free hydroxyl groups.

The ^{13}C NMR spectrum also shows the presence of two fully substituted alkene resonances at about δ_C 133.0-135.2 and δ_C 133.1-137.0. With the aid of HMBC correlations these resonances can be assigned to C-8 and C-9. This accounts for one of the eight double bond equivalents expected in eucosterol with two belonging to the keto groups and the remaining five to the pentacyclic ring structure.

The stereochemistry of the molecule can be determined from the NOESY spectrum. NOESY correlations between H-20 and 3H-18, 3H-21, H-22β and H-25 indicate that the stereochemistry of C-20 is *R*. Correlations between H-23 and H-22α and 3H-21 indicate that H-23 is α. Correlations between H-5α and H-3 and 3H-28 shows that H-3 and 3H-28 are α. No correlation between H-5 and H-19 is seen which confirms that H-19 is β as expected. The 3H-21 and 3H-18 resonances show correlations with each other, as a result of the C-17, C-20 bond being β.

2.2. Biosynthesis of Triterpenoids

It is believed that the acyclic hexa-ene all *trans*-squalene is the biological precursor in the biosynthesis of triterpenoids.[1] This molecule undergoes a series of cyclizations to give different types of compounds. The Biogenetic Isoprene Rule proposes that the type of triterpenoid produced is dependent on the conformation that squalene epoxide adopts presumably at the enzyme surface prior to cyclization.[1,8] It is recognised that all sterols in animals originate from lanosterol and the precursor to sterols in plants is cycloartenol.[1]
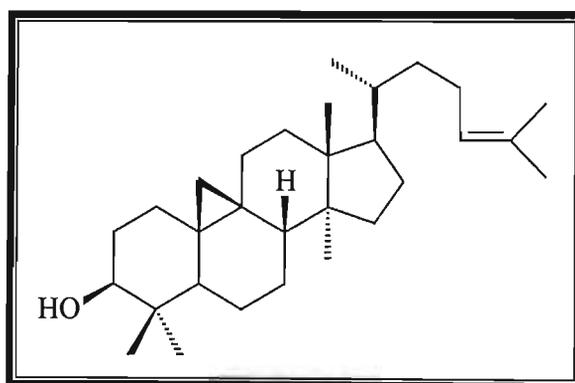
Figure 2.5: The structure of cycloartenol
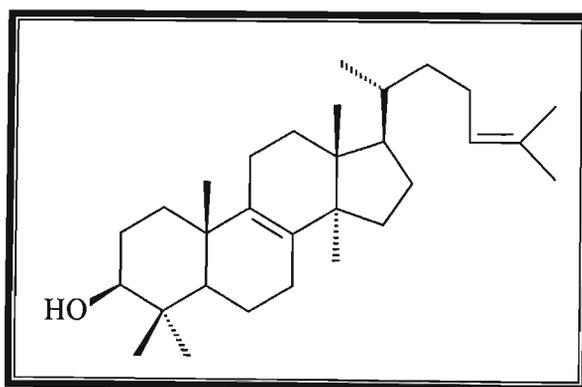
Figure 2.6: The structure of lanosterol

The biochemically active isoprene units involved in squalene formation are the diphosphate (pyrophosphate) esters, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP).[9]

Combination of DMAPP and IPP *via* the enzyme prenyl transferase generates geranyl pyrophosphate (GPP), a ten carbon compound. This reaction involves ionization of

DMAPP to the allylic carbocation. This cation is then added to the double bond of IPP in a head to tail fashion. Loss of a stereospecific proton results in GPP[9] (**Scheme 2.1**).
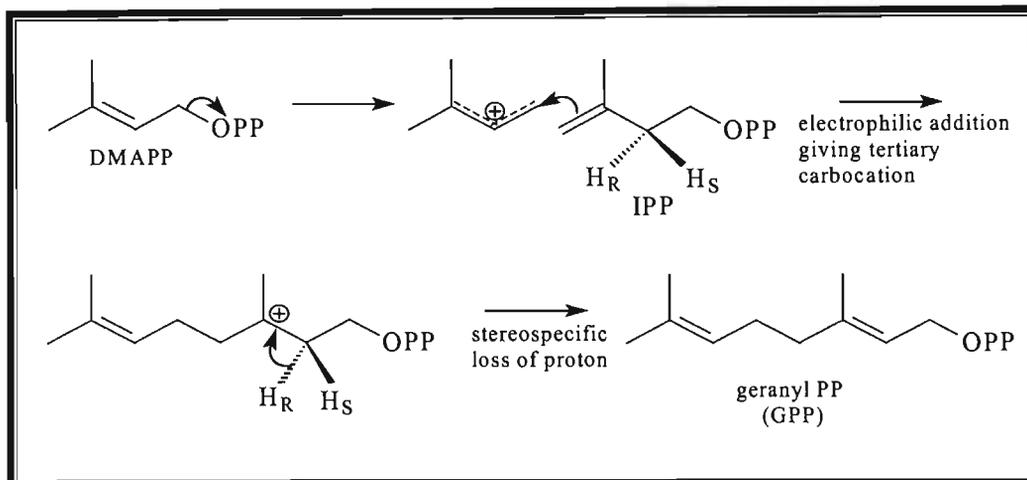
Scheme 2.1: The formation of geranyl prophosphate[9]

Addition of another molecule of IPP to GPP generates the fifteen carbon compound farnesyl pyrophosphate (FPP).[9] This mechanism (**Scheme 2.2**) proceeds in a similar manner to GPP formation.
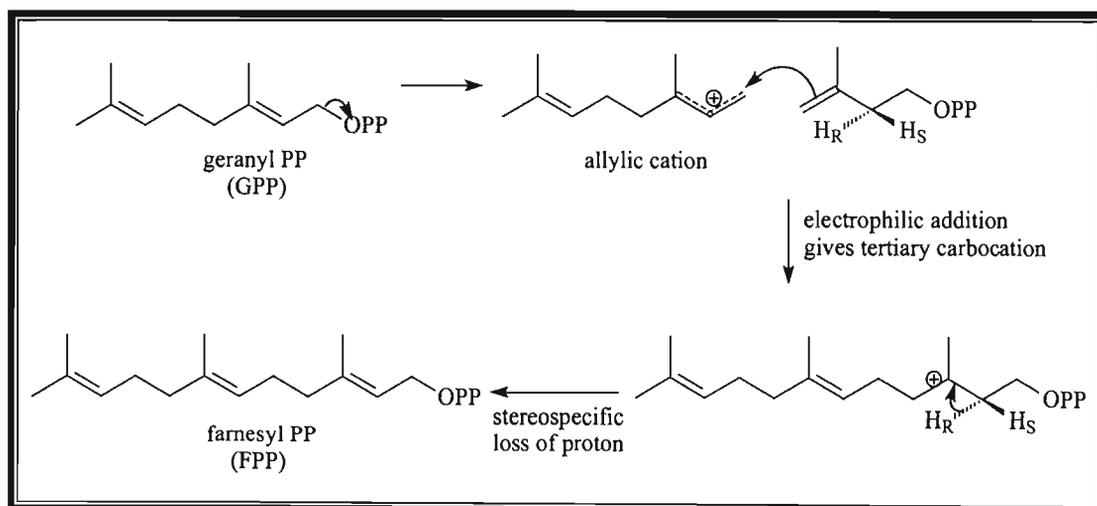
Scheme 2.2: The formation of farnesyl pyrophosphate[9]

Squalene is formed from two molecules of FPP joined by a tail to tail linkage (**Scheme 2.3**). A proton from C-1 of one molecule of FPP is lost and replaced with a proton from NADPH. This involves attack of the 2,3-double bond of FPP onto the farnesyl carbocation in a manner similar to chain extension using IPP. Loss of a

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proton from the tertiary carbocation formed, results in a cyclopropane ring and formation of presqualene PP.

The loss of a diphosphate from presqualene PP results in an unstable primary carbocation, which undergoes a Wagner-Meerwein migration to generate a more stable tertiary carbocation. This is followed by bond cleavage in the cyclopropane ring and generation of a favourable allylic carbocation. Addition of a hydride ion from NADPH to the cation gives squalene.[9]
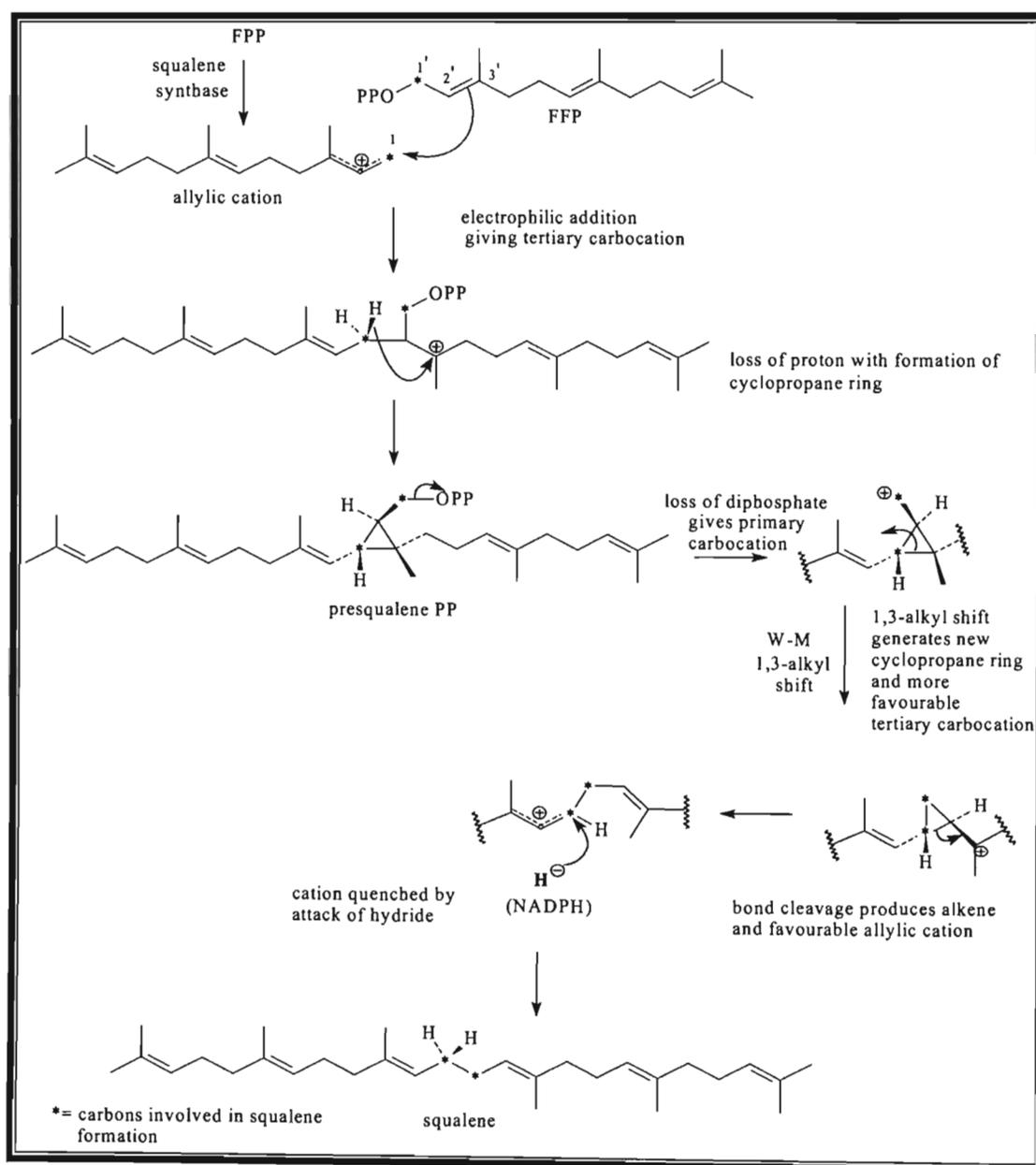
Scheme 2.3: The formation of squalene[9]

Squalene 2,3-epoxide is the intermediate through which cyclization of squalene occurs. This reaction is catalysed by a flavoprotein requiring O_2 and NADPH as cofactors. Protonation of the epoxide group allows opening of the epoxide ring and generation of the preferred tertiary carbocation. Electrophilic addition to the double bond follows and this allows for the formation of a six-membered ring. The process of generating a new carbocation after each ring formation continues until a tertiary protosteryl carbocation is formed. The cation then undergoes a sequence of Wagner-Meerwein rearrangements. Loss of a proton results in formation of a double bond and formation of lanosterol **(Scheme 2.4)**.[9]
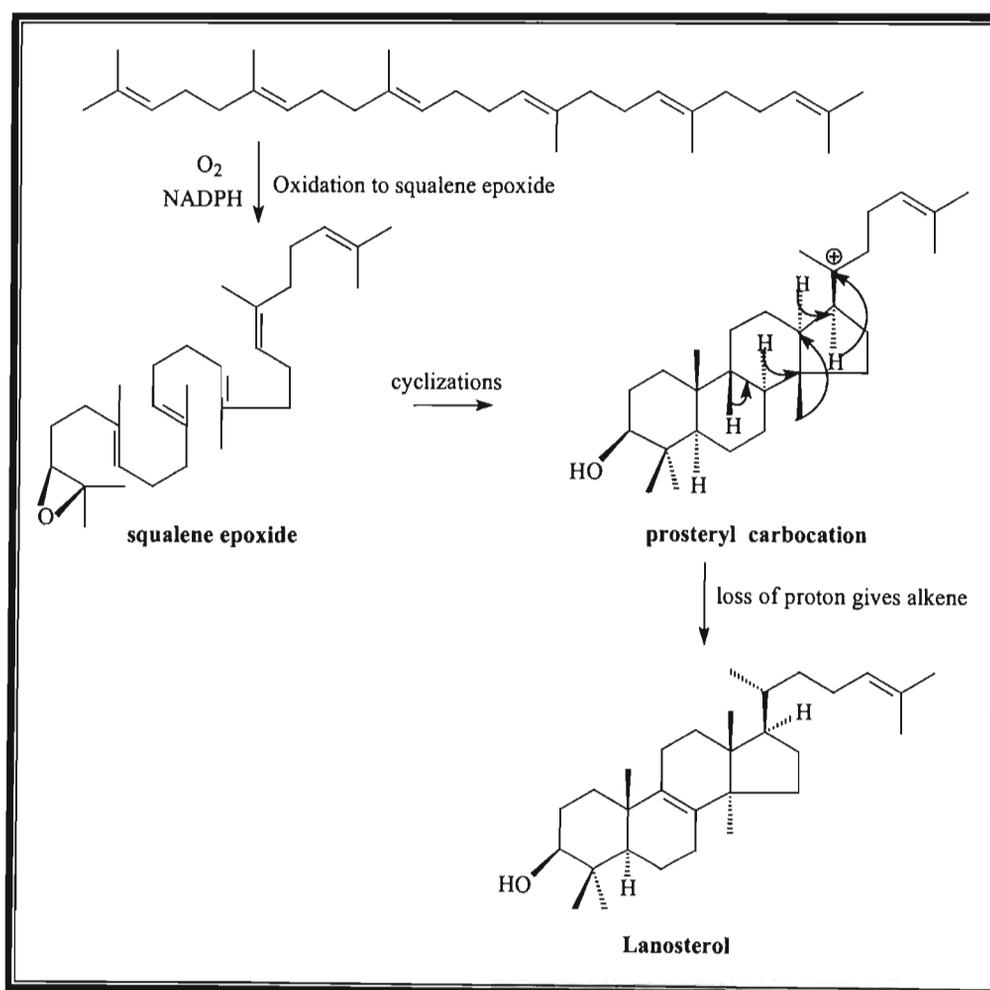
Scheme 2.4: Proposed biosynthesis of lanosterol[9]

Lanosterol is thought to be the precursor of eucosterol type nortriterpenoids. The postulated biosynthetic pathway to these compounds is given below **(Scheme 2.5)**.[10]
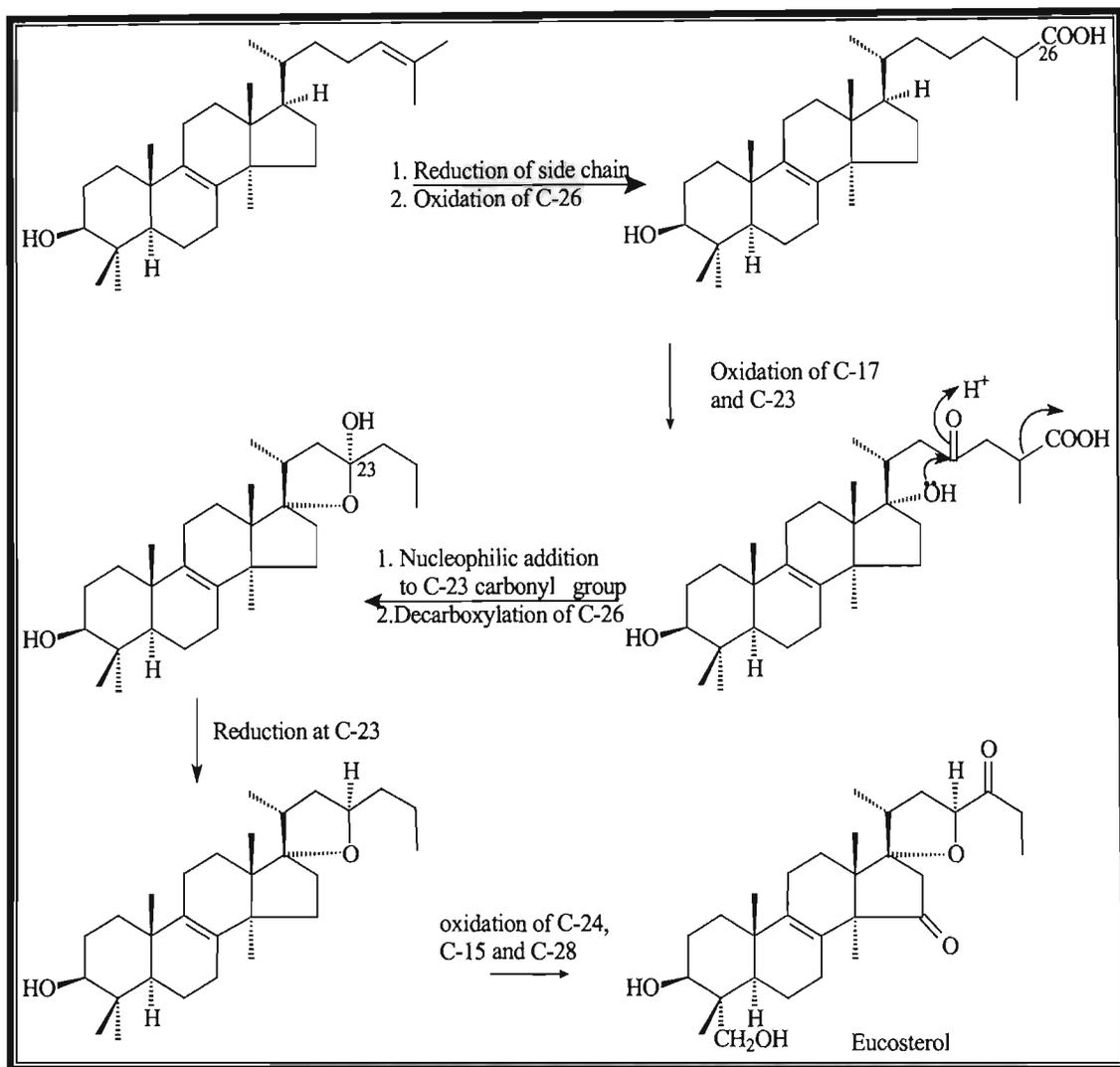
Scheme 2.5: Proposed biosynthesis of eucosterol[10]

2.3. Biological Activity of Triterpenoids

There have been reports on the biological activity of triterpenoids which indicates their potential as drugs.

The anticancer activity of some pentacyclic and tetracyclic triterpenoids was tested against human cancer cell lines and showed interesting results.[11] Among the pentacyclic triterpenoids, *epi*maniladiol was found to be cytotoxic against HEC-1-A, CAMA-1, ME-180, u-87MG, CALAU-1 and SK-OV-3. Maniladiol, the 16β-epimer, was cytotoxic against ME-180 and CAMA-1 and sophoradiol, which lacks the 16-hydroxy group, showed cytotoxicity against ME-180 only. Furthermore, glycyrrhetinic acid and 11-oxo-β-amyrin, both with free 3β-hydroxy groups, were active against SK-OV-3 and CAMA-1 respectively. These results suggested that the presence of a 16α-hydroxy group is important for the cytotoxicity of 12-oleanenes.[11]
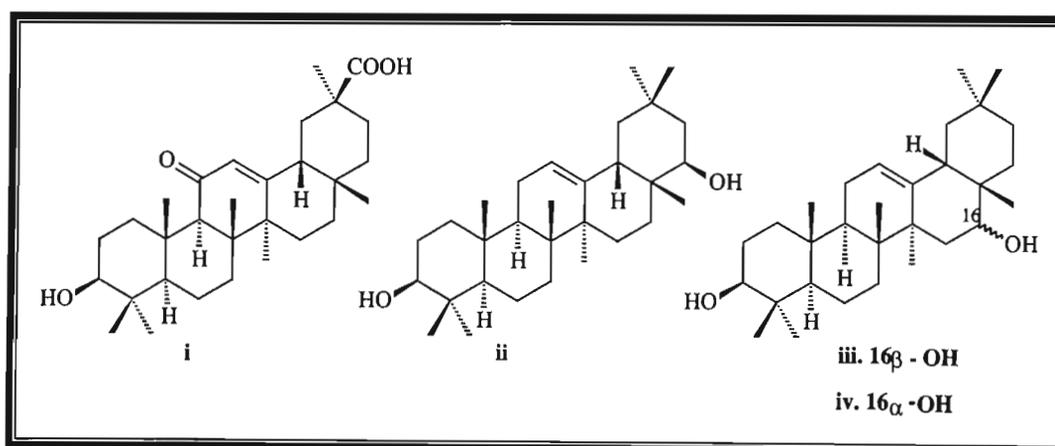
Figure 2.7: Structure of (i) glycyrrhetinic acid, (ii) sophoradiol, (iii) maniladiol and (iv) *epi*maniladiol

The hexacyclic nortriterpene pfaffic acid, isolated from the roots of *Pfaffia paniculata*, exerts high inhibitory effects on the growth of cultured tumor cells, such as melanoma (B-16), Hela (S-3) and Lewis Lung carcinoma cells.[12]
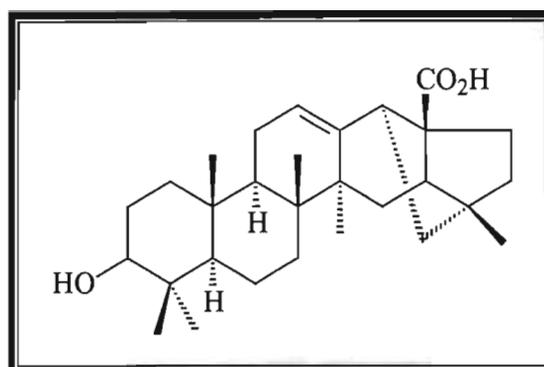
Figure 2.8: Structure of pfaffic acid

A number of lanosterol oligosaccharides from *Eucomis bicolor*, *Scilla peruviana*, *Chionodoxa gigantea* and *Chionodoxa luciliae* have been screened for antitumor-promoter activity *in vitro*. They were found to have an inhibitory effect on the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-stimulated 32P incorporation into phospholipids of HeLa cells. It is worth noting that introduction of a 16β-hydroxy group into the eucosterols markedly reduced their activity.[6]

22β-Hydroxy-15-deoxoeucosterol, isolated from *Ledebouria zebrina*, was recently tested against *Fusarium moliniforme* for antifungal properties and showed fungal growth inhibition. No other work on the biological activity of eucosterol type triterpenoids has been conducted.[10]

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2.4. Occurrence and Classification of Homoisoflavonoids

Homoisoflavonoids are a group of natural products of which the first representatives eucomol and eucomin, from the plant species *Eucomis bicolor* were reported in 1967.[13] Homoisoflavonoids are found predominantly in the Hyacinthaceae family, particularly in the genera *Scilla, Eucomis* and *Muscari* [13-22], but a few have been reported from others. For instance, intricatin and intricatinol are two homoisoflavonoids found in the Fabaceae family.[23]

Homoisoflavonoids, also referred to as homoisoflavanones, are a group of naturally occurring oxygen heterocycles.[24] They have a sixteen-carbon skeleton instead of the fifteen carbon skeleton characteristic of isoflavonoids.[20,25,26] It is believed that homoisoflavonoids are biosynthesized by modification of the $C_6.C_3.C_6$ chalcone-flavonoid skeleton by insertion of an extra carbon atom. The term "homoisoflavonoid", however, is somewhat misleading, since the characteristic 2,3-aryl migration step in the biosynthesis of isoflavonoids does not occur in that of homoisoflavonoids. The systematic name 3-benzyl-4-chromanone is often preferred.[20]

The numbering system for homoisoflavonoids is shown below. The A and C ring is numbered with counting numbers and the B-ring, with primed numbers.
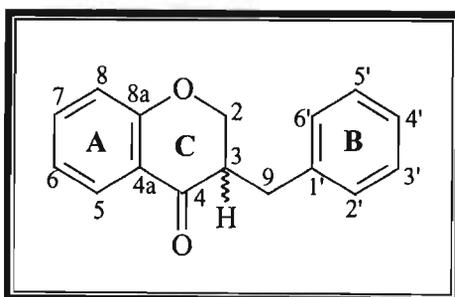
Figure 2.9: Numbering system for homoisoflavonoids

The carbon skeleton of homoisoflavonoids has a chromane, chromone or chromanone moiety to which is attached, in most cases, a benzyl or benzylidenyl group at position 3.[25] Substitution patterns on the A and B rings distinguish homoisoflavonoids from each other. Structural features of homoisoflavonoids have allowed classification into three types: (1) the 3-benzyl-4-chromanone or dihydroeucomin type, (2) the 3-benzyl-

3-hydroxy-4-chromanone or eucomol type and (3) the 3-benzylidenyl-4-chromanone or eucomin type.[24,27]

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

2.4.1. The 3-benzyl-4-chromanone or dihydroeucomin type

3-benzyl-4-chromanone
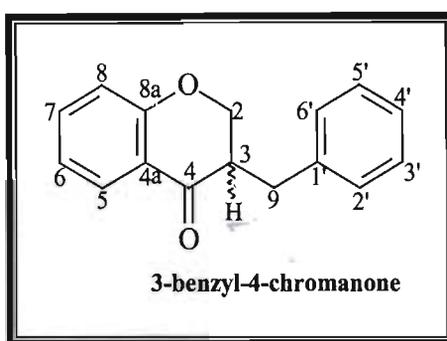
Figure 2.10: The 3-benzyl-4-chromanone type homoisoflavonoids

The ^{1}H NMR spectrum shows a characteristic coupling pattern due to the coupled 2H-2, H-3 and 2H-9 proton system for this type of homoisoflavonoid. A pair of double doublets is observed for the two non-equivalent H-2 protons at δ_H 4.10-4.32 and another pair of double doublets is observed for the non-equivalent H-9 protons in the range δ_H 2.65-2.73 and δ_H 3.10-3.26. The H-3 resonance occurs as a multiplet at about δ_H 2.72-2.87.

2.4.2. The 3-benzyl-3-hydroxy-4-chromanone or eucomol type

3-benzyl-3-hydroxy-4-chromanone
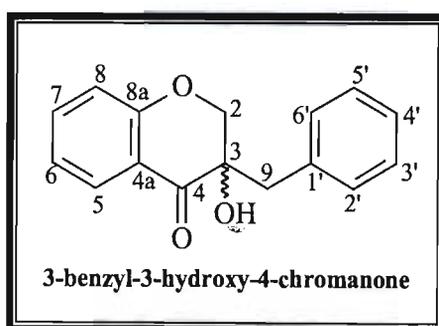
Figure 2.11: The 3-benzyl-3-hydroxy-4-chromanone type homoisoflavonoids

In the ^{1}H NMR spectrum a pair of doublets is observed for the two H-2 protons in the range δ_H 4.20-4.36 and δ_H 4.12-4.17. A second pair of doublets is observed for the 2H-9 protons at δ_H 3.10-3.20 and δ_H 2.65-2.75. The multiplet at δ_H 2.72-2.87 is absent and this indicates the presence of a hydroxy substituent at C-3.[28]

2.4.3. The 3-benzylidenyl-4-chromanone or eucomin type

(Z)-configuration   (E)-configuration
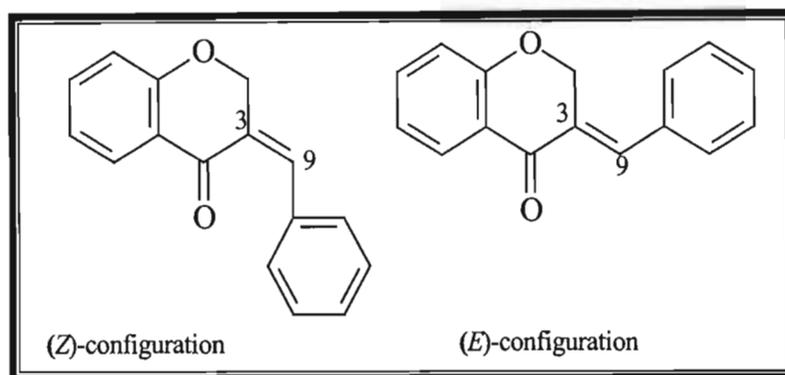
Figure 2.12: The 3-benzylidene-4-chromanone type homoisoflavonoids

The typical feature for this type of compound is the 3,9-double bond which may be in either the (E) or (Z) configuration. In the ^{1}H NMR spectrum a pair of doublets is observed for the two H-2 protons at approximately δ_H 5.20-5.50. The H-9 resonance occurs as a singlet whose chemical shift depends on the configuration of the molecule. In the (E) configuration the H-9 proton is in the anisotropic region of the carbonyl group and causes it to resonate at about δ_H 7.60-7.70. In the (Z) configuration, the proton at C-9 is away from the anisotropic region of the carbonyl group and the resonance for this vinyl group proton occurs at approximately δ_H 5.50.[28,29]

In the ^{13}C NMR spectrum, certain shifts are characteristic for homoisoflavonoids. The chemical shifts of C-2 in 3-benzylidenyl-4-chromanones, 3-benzyl-4-chromanones and 3-benzyl-3-hydroxy-4-chromanones occur at δ_C 67.5, δ_C 69.3 and δ_C 73.0 respectively and can be used to distinguish one type of homoisoflavonoid from the other. The C-4 carbonyl group resonance also differs in its chemical shifts for the three types of homoisoflavonoids. For 3-benzylidenyl-4-chromanones, C-4 occurs at δ_C 182.0 because the carbonyl group is conjugated. The C-4 shifts for 3-benzyl-4-chromanones and 3-benzyl-3-hydroxy-4-chromanones are distinctly different from the

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3-benzylidenyl-4-chromanone type and appear at about δ_C 197.0 and δ_C 195.0 respectively.[24] The C-3 resonance occurs as an aliphatic methine resonance for the 3-benzyl-4-chromanone type homoisoflavonoid and as an oxygenated, aliphatic quaternary resonance for the 3-benzyl-3-hydroxy-4-chromanone type. For the 3-benzylidenyl-4-chromanone type homoisoflavonoids, the C-3 resonance occurs as a quaternary resonance in the double bond region.

Other features of the ^{1}H NMR spectrum may assist in determining the substitution pattern on the homoisoflavonoid. The signals at approximately δ_H 6.50-7.20 usually indicate substitution on the B-ring. This is either an ABX or an AA'BB' system. The substituents present are attached at C-4' (AA'BB' system) or C-3' and C-4' (ABX system). The substitution pattern on the A-ring is indicated by resonances in the region δ_H 5.80-6.30. There are usually only one or two proton resonances in this region, which are normally due to H-6 and/or H-8. The position of the proton is normally assigned through the NOESY spectrum, which shows NOESY correlations with the substituents at C-5 and C-7.

UV spectra are also important in the determination of the substitution pattern on the A ring of homoisoflavonoids. The original UV spectrum of homoisoflavonoids gave an absorption maximum at 285-367 nm.[28] Addition of NaOAc and AlCl$_3$ solutions causes the maximum to shift in some instances.[28,30] These shifts are referred to as bathochromic shifts. A bathochromic shift observed with NaOAc indicates the presence of a hydroxy group at the C-7 position while a bathochromic shift with AlCl$_3$ implies a hydroxy group at the C-5 position.[28,31,32]

The mass spectrum is another diagnostic tool used in the determination of the structure of compounds. There are certain peaks that occur for homoisoflavonoids due to characteristic fragmentation patterns. In eucomol, cleavage of the C-3, C-9 bond in what is known as an A-4 type cleavage leads to fragments at m/z 195 and m/z 121 as shown in **Scheme 2.6**.[28] The intense peak at m/z 121 is due to the methoxytropylium ion, which is the base peak of the spectrum. In eucomol, the chromanone fragment ion at m/z 195 may eliminate water, CO or undergo a *retro*-Diels-Alder (RDA) cleavage to give a fragment ion at m/z 152 or, due to a hydrogen shift, at m/z 153. The major

pathway is usually the RDA cleavage and hydrogen-shift. The minor pathway is usually the subsequent loss of water, CO and methyl from the molecular ion.[28]
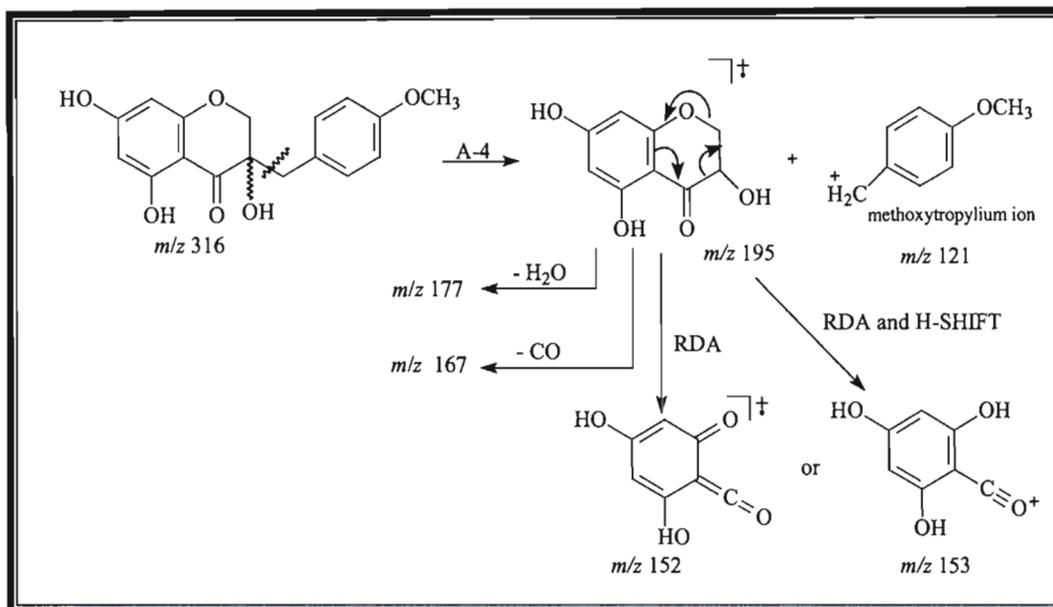
Scheme 2.6: Mass spectral fragmentation pattern for homoisoflavanones[28]

Apart from these three basic structural types, several unusual homoisoflavonoids characterized by an additional fourth ring have been found.[24,26,27] Scillascillin-type compounds possess a 3-spiro-cyclobutenyl ring whereas brazilin homoisoflavonoids and haematoxylin homoisoflavonoids contain a cyclopentenyl ring. In scillascillin the C-4 carbonyl group is retained, however in brazilin and haematoxylin it is lost. [20]
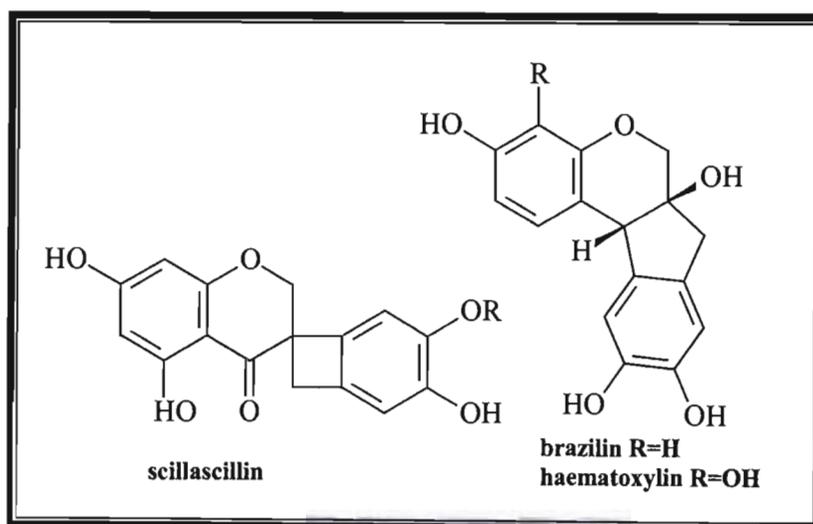
Figure 2.13: Structure of scillascillin, brazilin and haematoxylin type homoisoflavonoids

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2.5. Biosynthesis of Homoisoflavonoids

Phenolic compounds can be biosynthesized by two routes: the shikimate pathway or the polyketide pathway. Often compounds, such as flavonoids and homoisoflavonoids, are of mixed biosynthetic origin. In homoisoflavonoids the A-ring is polyketide derived and the B-ring is shikimate derived (**Figure 2.14**). Polyketide derived phenols produce a 1,3,5-oxygenation substitution pattern but it is common for one or more of these groups to disappear during the biosynthesis. Phenols from the shikimate pathway are always oxidised in the *para*-position but further oxidations on adjacent positions may occur in advanced biosynthetic stages.[33,34-36]
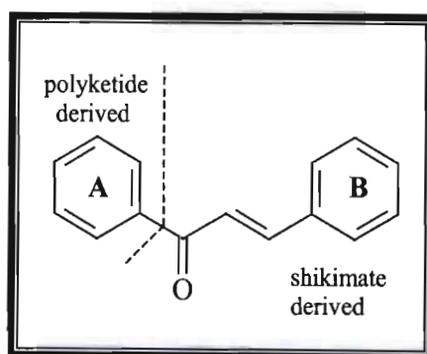
Figure 2.14: The origin of the A and B rings of homoisoflavonoids

The first step in the biosynthetic route leading to homoisoflavonoids involves chalcone formation as chalcones are the precursors to homoisoflavonoids.[20,26,28] Formation of the chalcone precursor involves conversion of phenylalanine to cinnamic acid, which is catalysed by the enzyme phenylalanine ammonia lyase (PAL). PAL catalyses the *anti*-elimination of ammonia and the *pro*-3*S*-proton from L-phenylalanine to yield *trans*-cinnamic acid.[34-36]
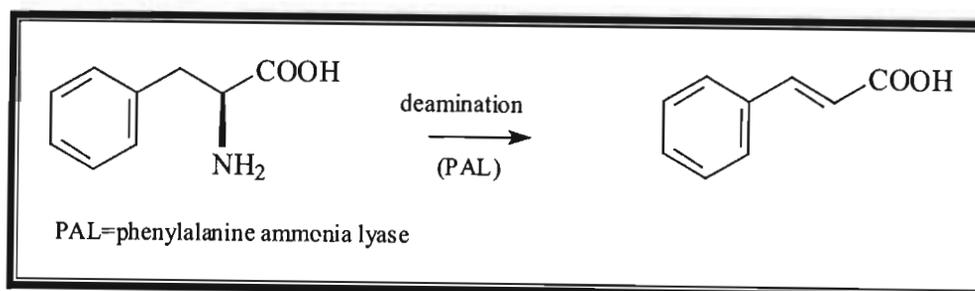
Figure 2.15: Conversion of L-phenylalanine to *trans*-cinnamic acid[34-36]

Cinnamate-4-hydroxylase (C4H) catalyses oxidation of *trans*-cinnamic acid at the *para*-position. This reaction requires NADPH and molecular oxygen and exhibits properties characteristic of plant P450 enzymes.[34-36] In this reaction mechanism the proton at the 4-position is oxidised to a hydroxyl group and in the process moved to the 3-position. This shift was established by tritium-labelling experiments performed at the National Institute of Health at New Bethesda Hospital, Washington D.C. and is thus called the NIH shift.[9,34-36] (**Scheme 2.7**)
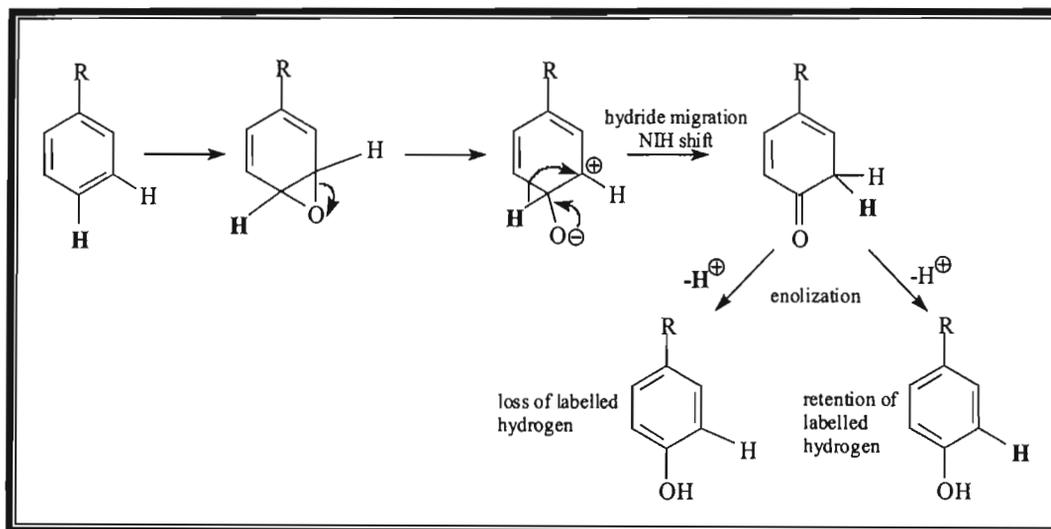
Scheme 2.7: Mechanism of the NIH shift [9]

The *p*-coumaric acid is then converted to *p*-coumaryl-CoA by the enzyme 4-coumarate: CoA ligase. The formation of the CoA ester requires ATP and Mg^{2+} as cofactors.[34-36]
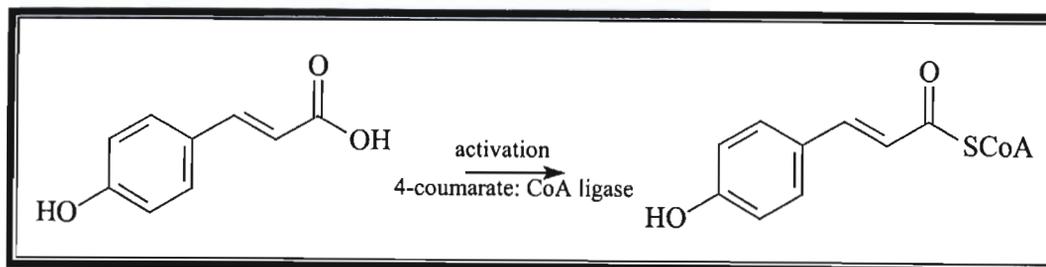
Figure 2.16: Conversion of 4-coumaric acid to 4-coumaryl CoA

In the next step three malonyl-CoA molecules combine with *p*-coumaryl-CoA to give the polyketide ester. The result is the elongation of the aliphatic side chain of 4-coumarate by six carbon atoms. Cyclization of the polyketide ester *via* a postulated

Claisen-type reaction produces the tetrahydroxychalcone. This step is catalysed by the enzyme chalcone synthase.
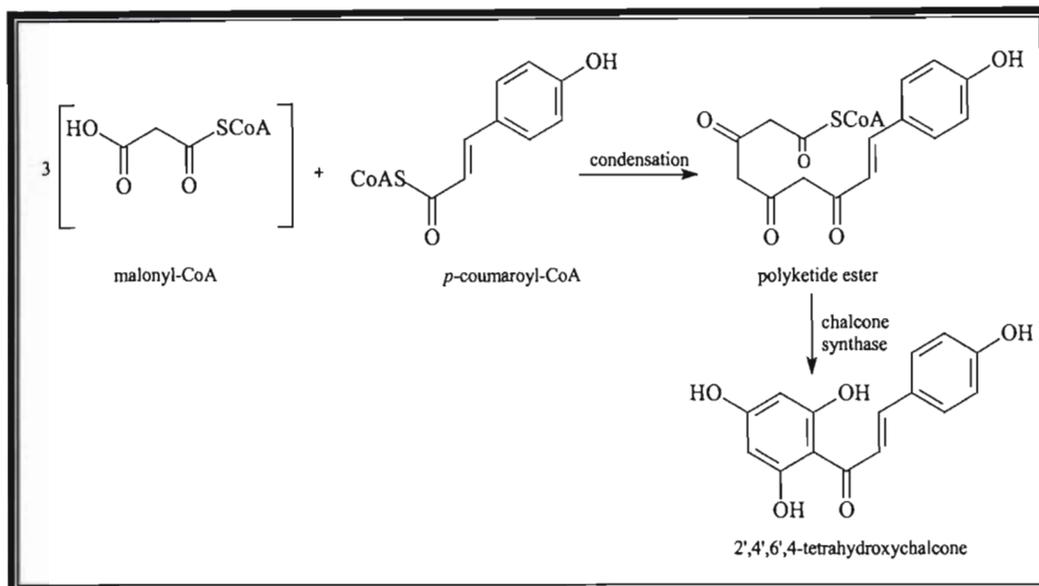
Scheme 2.8: The biosynthesis of 2',4',6',4- tetrahydroxychalcone[34-36]

As already mentioned, chalcones are the intermediates to all flavonoids. Since chalcone formation is catalysed by chalcone synthase, this enzyme may be regarded as the most essential enzyme in flavonoid biosynthesis. Generally, methionine is often the source of an additional carbon in many biosynthetic pathways. In this case the 2',4',6',4-tetrahydroxychalcone is converted to the 2'-methoxy-4',6',4-trihydroxychalcone with S-adenosylmethionine. The reaction is catalysed by the enzyme, methyl transferase.
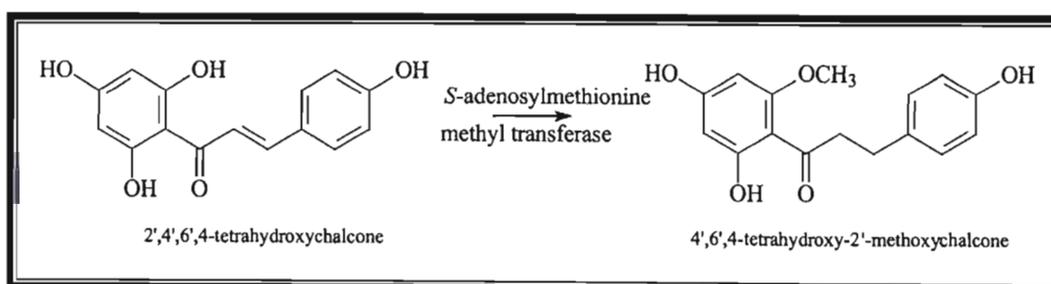
Figure 2.17: Formation of 4',6',4-tetrahydroxy-2'-methoxychalcone[34-36]

A scheme for the conversion of chalcones to homoisoflavanoids has been proposed by Dewick.[20] It is believed that oxidation of the 2'-methoxy group, followed by

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cyclization of the intermediate produces the three basic types of homoisoflavonoids. The addition of a hydride ion leads to formation of 3-benzyl-4-chromanones while loss of a proton produces the 3-benzylidenyl-4-chromanone types. Hydration of 3-benzylidenyl-4-chromanone or hydroxylation of 3-benzyl-4-chromanone produces 3-benzyl-3-hydroxy-4-chromanones.[20] The postulated mechanism is given in **Scheme 2.9**.
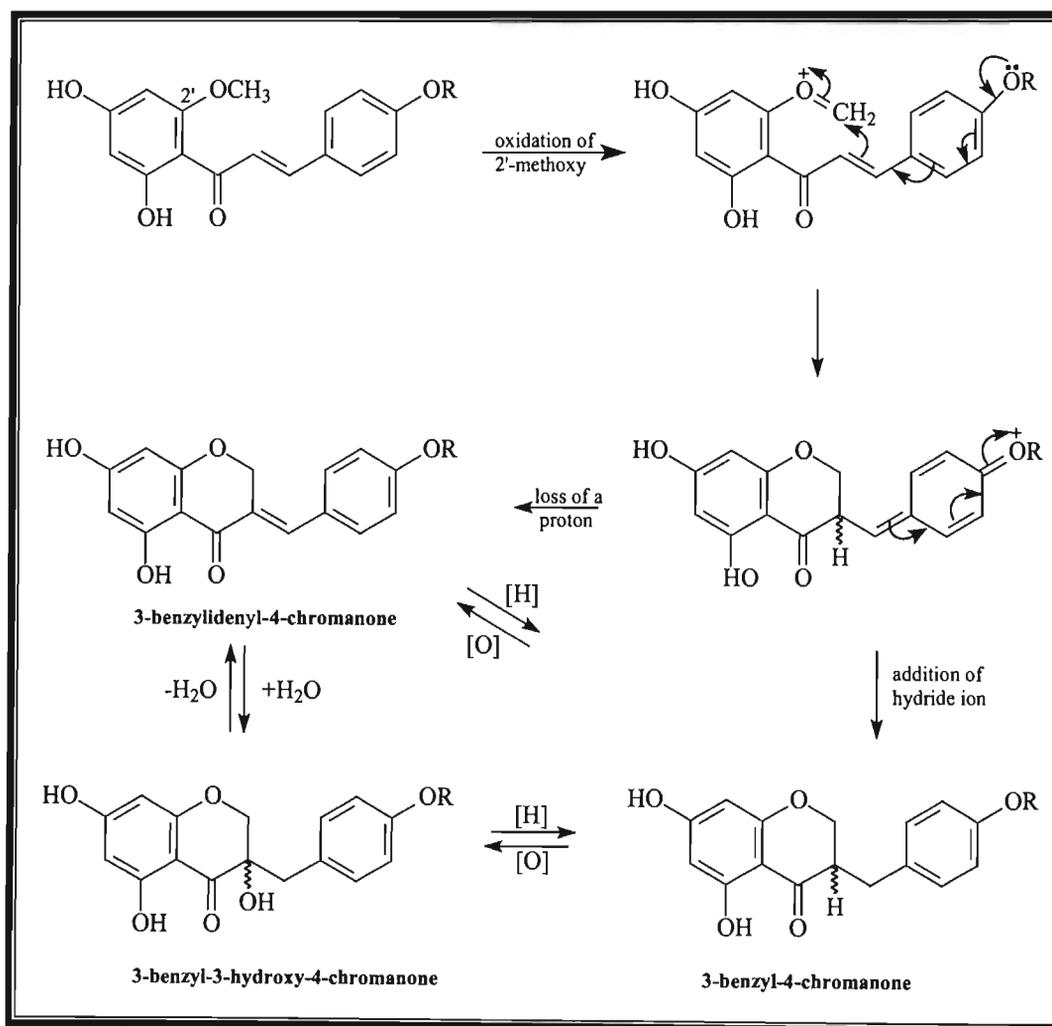
Scheme 2.9: Postulated biosynthetic routes for the conversion of chalcones to homoisoflavonoids[20]

The more unusual homoisoflavonoids, scillascillin, brazilin and haematoxylin are thought to be derived from more complex mechanisms. The precursor to scillascillin is the 3-benzyl-3-hydroxy-4-chromanone-type homoisoflavonoid.[20]
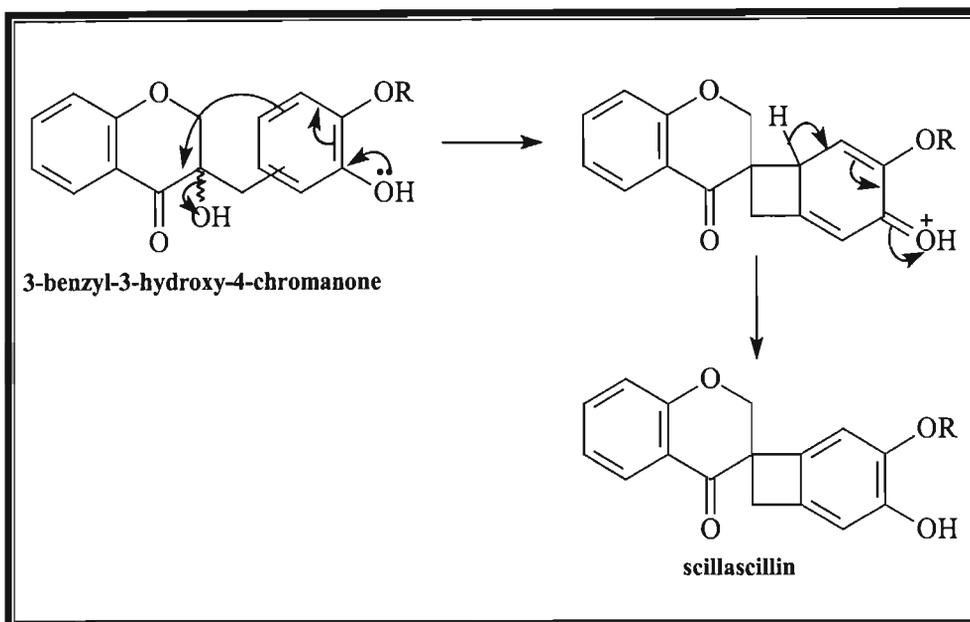
Scheme 2.10: Proposed biosynthetic route to scillascillin type homoisoflavonoids[20]

For brazilin and haematoxylin the mechanism is thought to be similar except that the activated precursor, 3-benzyl-3-hydroxy-4-chromanol produces a cyclopentene ring.[20]
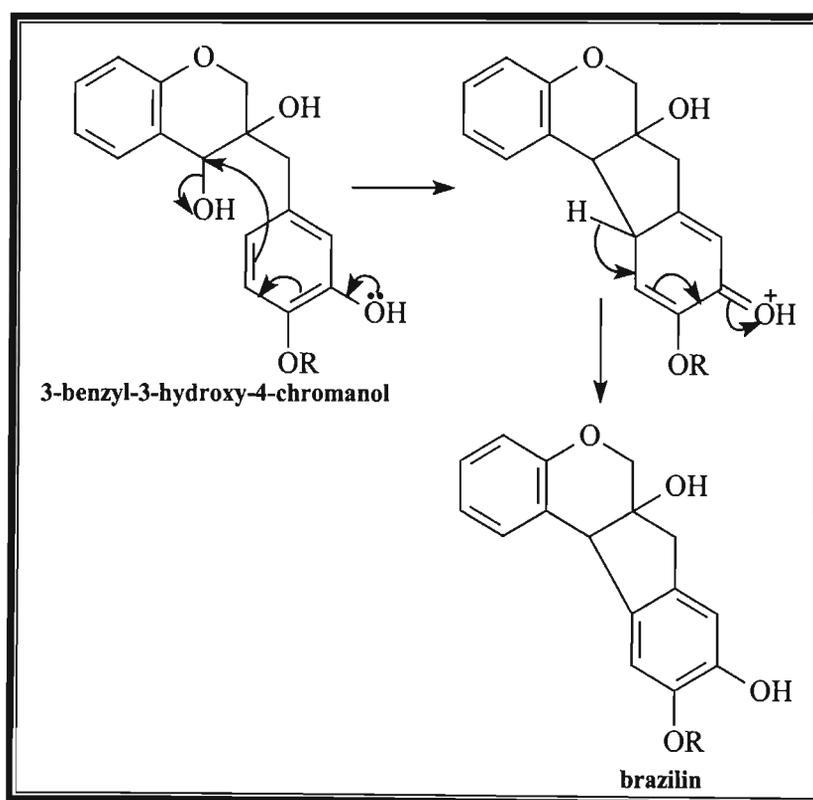
Scheme 2.11: Proposed biosynthesis of brazilin type homoisoflavonoids[20]

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2.6. Biological Activity of Homoisoflavonoids

Homoisoflavonoids are known to be concentrated in the waxy, scale-like layers of bulbs.[28] In this investigation homoisoflavanones were isolated from the bulb extracts. Homoisoflavonoids have been reported to have anti-inflammatory, antimutagenic, antibacterial and antioxidative properties and this may be related to the medicinal use of plants that contain them.[23,28,37,38,39]

There has been evidence to show that two closely related homoisoflavonoids intricatin and intricatinol isolated from the roots of *Hoffmanosseggia intricata* (Fabaceae) possess antimutagenic properties. They inhibit the mutagenicity of the carcinogens 2-aminoanthracene, acetylaminofluorene and ethyl methanesulfonate in *Salmonella typhimurium*. Intricatinol is the more active homoisoflavonoid and it is believed that this may be due to the presence of the extra hydroxy group on the A-ring.[23]
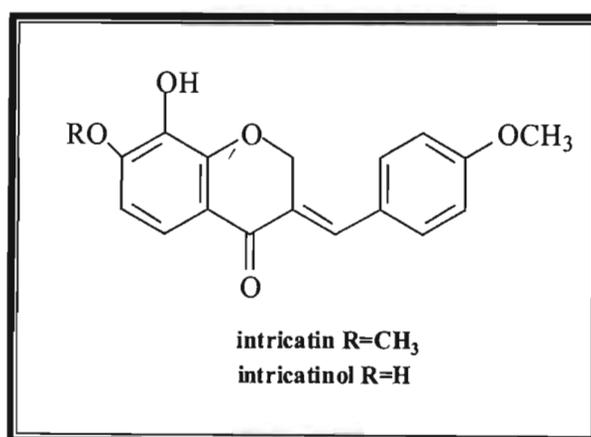
Figure 2.18: Structures of intricatin and intricatinol

The homoisoflavonoid *R*-(-)-3-(4-hydroxybenzyl)-5-hydroxy-6,7,8-trimethoxy-chroman-4-one from the bulbs of *Veltheimia viridifolia* was put through screening tests. This compound was first tested against phosphodiesterase (PDE) isoenzymes and shown to possess a weak selective profile for PDE IV and V. *In vitro* tests concerned with the inhibition of A 23187-stimulated leukotriene synthesis in human polymorphonuclear leucocytes showed weak activity. The homoisoflavanone was also tested for *in vivo* activity on respiration and the influence on the cardiac system of guinea pigs. It was found that after administration, expiratory flow increased slightly and there was an increase in the respiratory rate. Also, bronchoplasmolysis was

detected as well as a decrease in the heart rate. It was concluded that this compound has only weak and especially short biological activity in this *in vivo* test system.[38]

The anti-inflammatory activity of bulbs within the Hyacinthaceae has been studied. The crude extracts of the bulbs of *Muscari comosum*, rich in homoisoflavonoids, have shown to inhibit croton oil-induced dermatitis in mouse ear as effectively as the potent anti-inflammatory drug, indomethacin.[39]

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CHAPTER 3: THE OCCURRENCE AND BIOSYNTHESIS OF LIGNANS

3.1. Introduction

Lignans are naturally occurring dimers of phenylpropanoid (C_6-C_3) units linked by the central carbons of their side chain.[1] Lignan precursors are substituted cinnamic alcohols that are joined by C_8-C_8' linkages, the most important of which are 4-hydroxycinnamyl alcohol, coniferyl alcohol and sinapyl alcohol.[1,2]
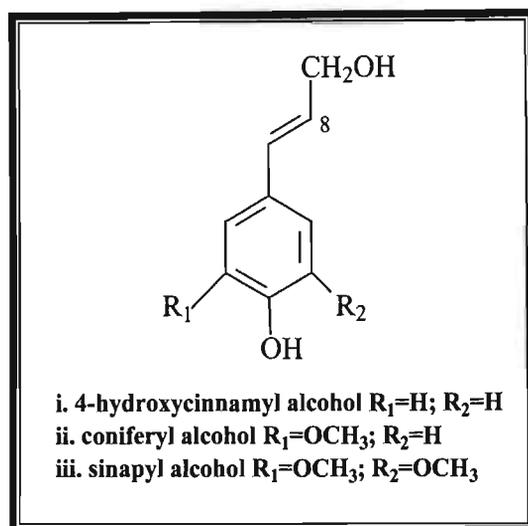
Figure 3.1: Structures of lignan precursors

Lignans have been reported to occur in all parts of the plant. They are important constituents of the wood and have also been found in the roots, leaves, fruits, flowers and seeds.[1]

A broad range of lignans have been identified, which is attributed to the great diversity in the chemical assembly of the two characteristic phenylpropanoid groups, as well as the degree of oxidation and the types of substituents.[1]
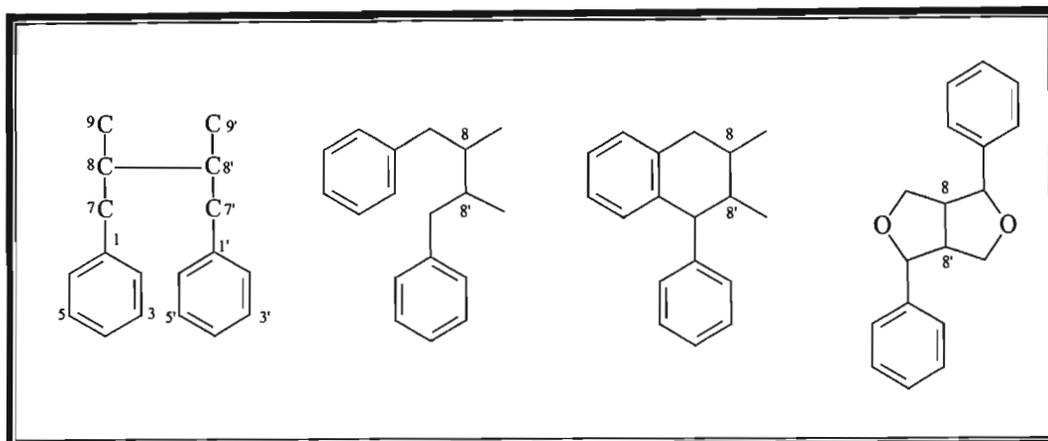
Figure 3.2: Examples of the structural diversity in lignans showing C$_8$-C$_8$' linkages[3]

3.2. Biosynthesis of Lignans

Biosynthesis of lignans begins with elimination of ammonia from the side chain of *L*-phenylalanine to generate the appropriate *trans*-cinnamic acid. This is achieved *via* the enzyme phenylalanine ammonia lyase (PAL). Various derivatives of cinnamic acid are formed by further hydroxylation and methylation reactions, sequentially building up the substitution pattern typical of the shikimate pathway metabolites *i.e.* an *ortho* oxidation pattern **(Scheme 3.1)**. [2]
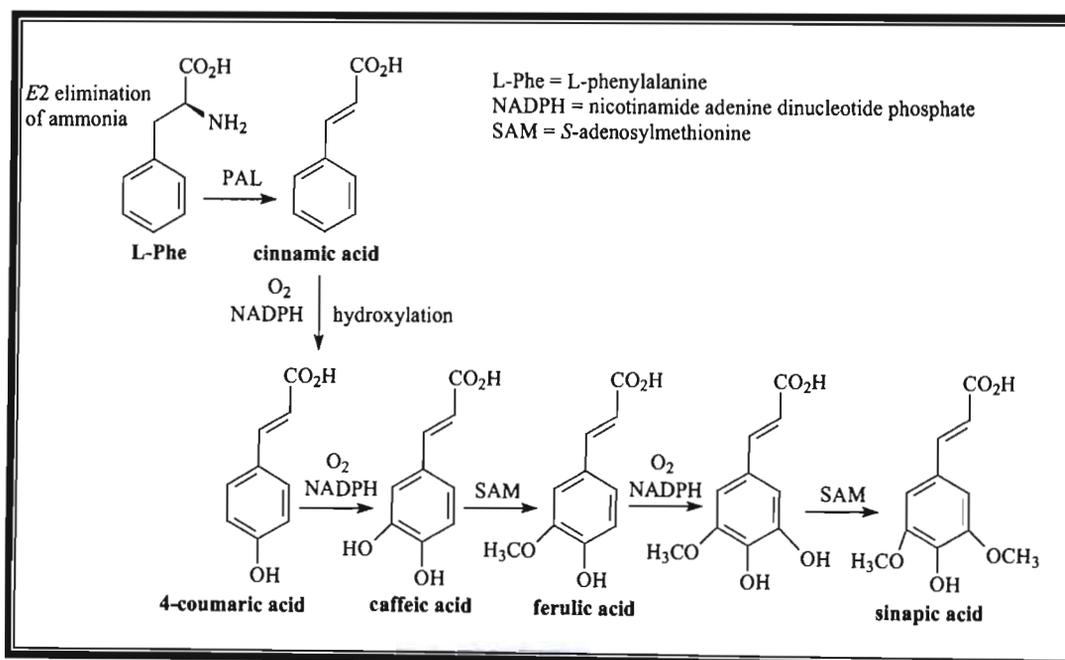
Scheme 3.1: Shikimate pathway showing formation of various cinnamic acid derivatives[2]

The next step in the biosynthesis is the formation of hydroxycinnamyl alcohol monomer units. This involves the reduction of the corresponding cinnamic acids *via* coenzyme A esters and aldehydes. Formation of the coenzyme A ester facilitates reduction to the aldehyde by introducing a better leaving group (CoAS) for the NADPH-dependent reaction. Reduction of the aldehyde to the phenolic monomer utilizes a further molecule of NADPH and is a reversible reaction **(Scheme 3.2)**.[2]
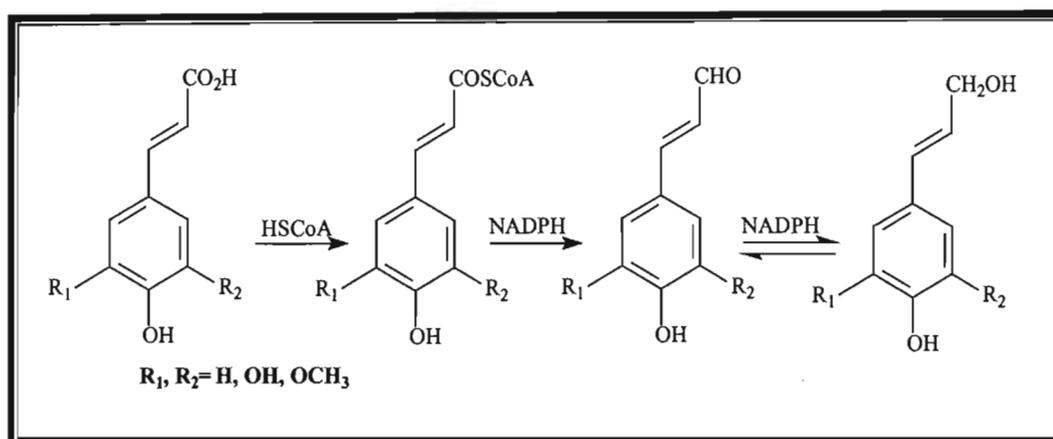
Scheme 3.2: Formation of phenolic monomers from cinnamic acid derivatives[2]

Phenolic oxidative coupling of alcohol monomers catalysed by peroxidase enzymes form lignans. The peroxidase enzyme generates a free radical species. One electron oxidation of a simple phenol allows delocalization of the unpaired electron, giving resonance forms in which the free electron resides at positions *ortho* and *para* to the oxygen function. Conjugation also allows the unpaired electron to be delocalized to the side chain **(Scheme 3.3)**. Radical pairing of the resonance structures, cyclization and other modifications can thus provide a wide variety of lignans.[2]
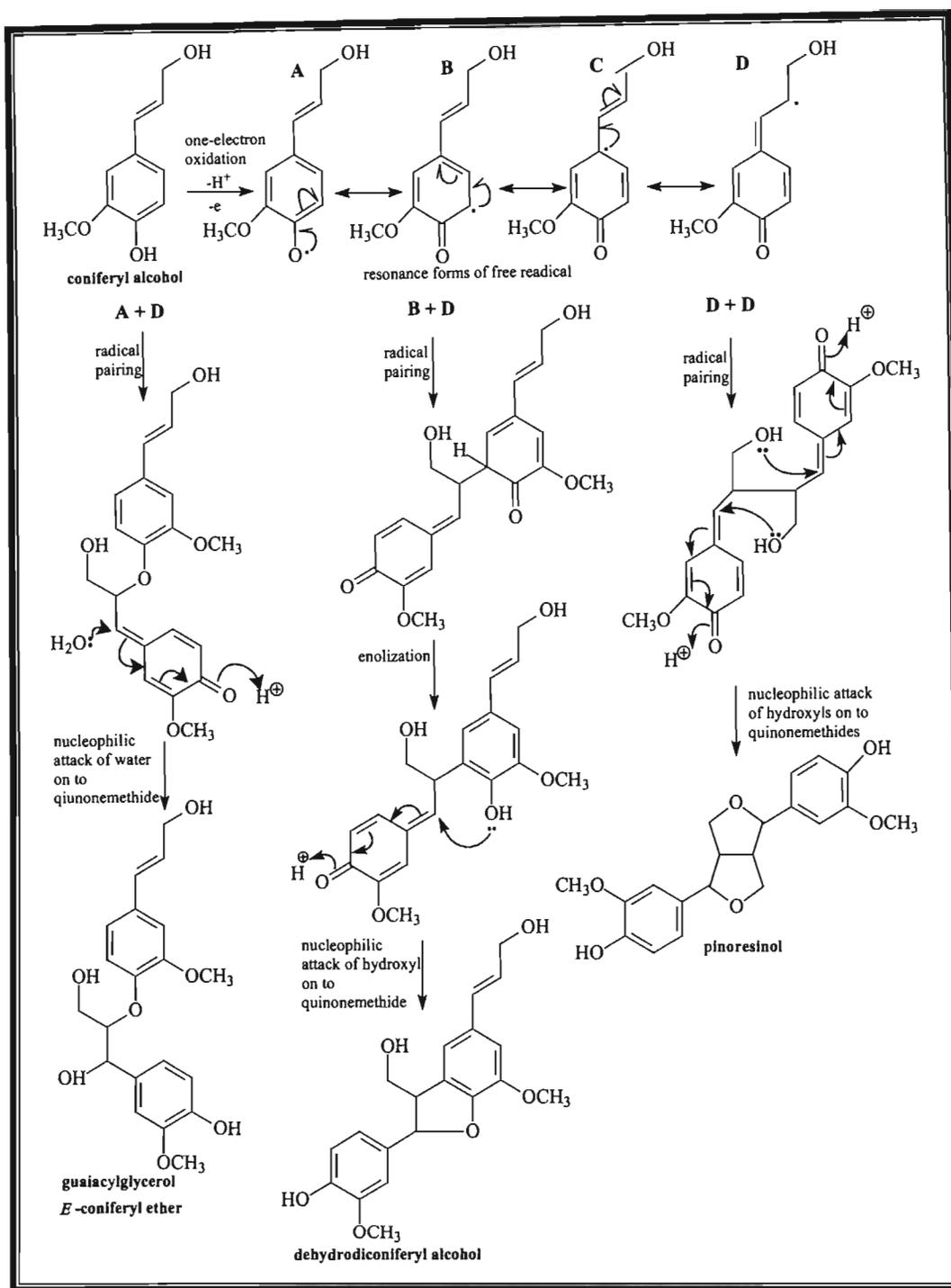
Scheme 3.3: Formation of lignans from phenolic monomers[2]

It was initially believed that coupling of phenolic monomer radicals to form lignans is random, requiring enzymes only to generate the corresponding free radicals. Random coupling, however, could not explain the optically active lignans found in nature. The discovery of dirigent (guiding) proteins gave a new perspective to the mechanism involved in lignan biosynthesis.[4]

This protein shows no catalytic activity but has sites that bind either the monomers or monomer radicals in specific orientations that lead to specific coupling.[4,5,6] The dirigent protein was first isolated from *Forsythia intermedia*, a plant that both produces and further metabolizes the enantiomerically pure lignan, (+)-pinoresinol which is formed from *(E)*-coniferyl alcohol monomers. It was found that an *in vitro* system containing the protein, an oxidant and *(E)*-coniferyl alcohol monomers produced (+)-pinoresinol in almost 100% optical purity while a system with only an oxidant and the monomer gave racemic mixtures of random coupling products.[4]

How the protein binds and orientates the substrate is somewhat unclear. The dirigent protein controls the monomers that will be incorporated and the type of linkage formed through specificity and orientations of the binding sites. Kinetic studies suggest that the protein functions in a unique manner, whereby the peroxidase first generates the free-radical intermediate, which is then presumed to capture the dirigent protein. These are bound and orientated in such a manner that coupling can provide only an optically pure product.[5,6]

Little is known about the mechanism for the stereoselectivity and enantiospecificity in lignan formation. The enantiomeric composition of a particular lignan may vary with the plant species. *Forsythia suspensa* produces (+)-pinoresinol while *Zanthoxylum ailanthoides* produces (-)-pinoresinol. It was found that species of the genera *Forsythia*, *Arctium* and *Wikstroemia* accumulate different enantiomers with various enantiomeric compositions.[3]

3.3. Biological Activity of Lignans

Lignans have been found to have a broad range of uses, particularly in human health where their biological activities have come to be appreciated only fairly recently. Their beneficial cardioprotective, antiviral, antibacterial, antifungal and anticancer properties have been reviewed.[7]

Investigations into the biological activity of lignans have led scientists to believe that lignans interfere with estrogen metabolism and may be significant in combating the onset of hormone dependent cancers such as breast, endometrium and prostate cancers. The estrogenic effects of lignans have also been associated with the prevention of bone resorption and promotion of increased bone density, thus preventing beginning of osteoporosis.[7]

Podophyllotoxin, a constituent of *Podophyllum peltatum*, has been identified for its antitumor activity as a result of screening for cytostatic activity. It is used to treat venereal warts and is a precursor to the drug etoposide, which is used to treat testicular cancer. Secoisolariciresinol, secoisolariciresinol diglucoside, and matairesinol are the chemopreventive agents of many edible plants and their metabolism in the gut helps protect against the onset of breast and prostate cancers.[6]

Lignans have also been tested for cardioprotective benefits and the lignan cinnamophilin has been studied for the ability to reduce platelet aggregation and vasoconstriction.[8] Gomisin A from the plant *Schisandra chinensis* is used in the treatment of liver disorders and kadsurenone from *Piper futokadsura* is a platelet-activating factor. Sesamin isolated from the seed oil of the plant *Sesamum indicum* is an anti-oxidant.[6]
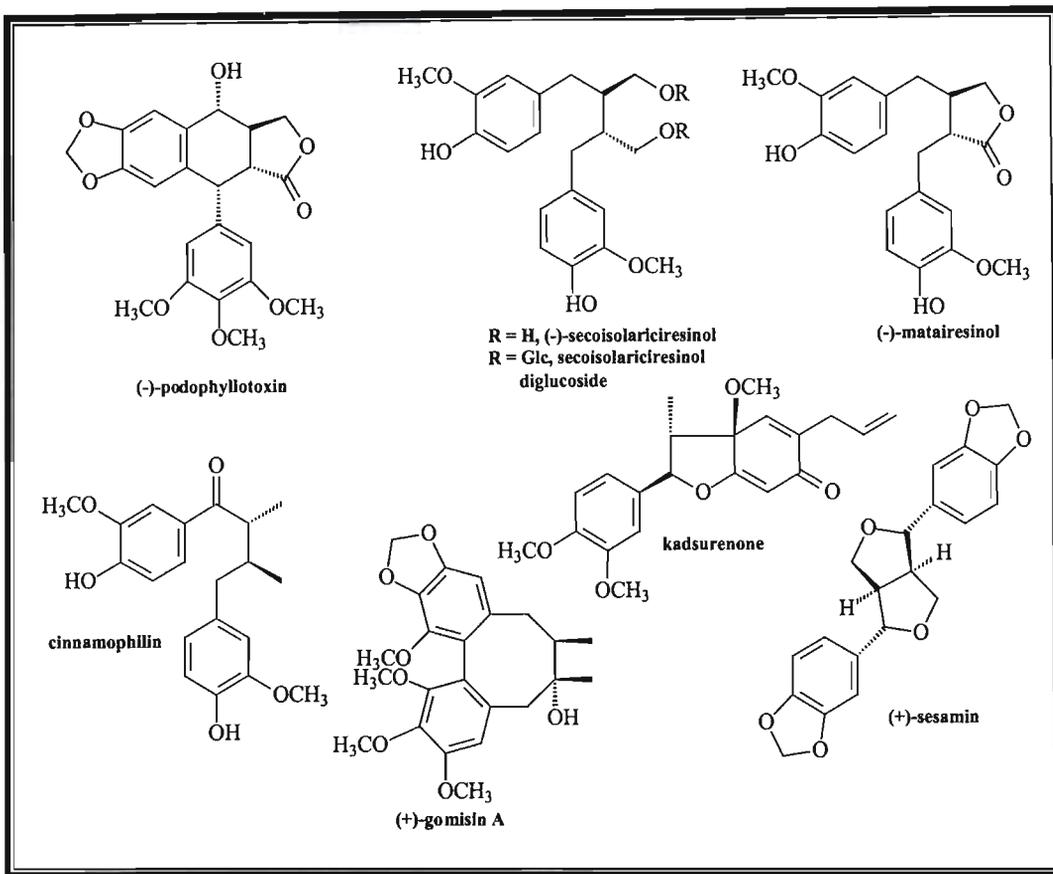
Figure 3.3: Structures of some biologically important lignans[6]

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CHAPTER 4: EXTRACTIVES FROM *EUCOMIS MONTANA*

4.1 Introduction

This is the first reported phytochemical study of *Eucomis montana*. The dichloromethane, ethyl acetate and the methanol extracts were investigated. This has led to the isolation of one nortriterpenoid and four homoisoflavonoids from the dichloromethane extract, five homoisoflavonoids from the ethyl acetate extract and two homoisoflavonoids from the methanol extract. The compounds isolated were **(1)** (23S)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one, **(2)** 4'-demethyl-3,9-dihydroeucomin (5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone), **(3)** 3,9-dihydroeucomin (5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone), **(4)** 4'-demethyl-5-*O*-methyl-3,9-dihydroeucomin (7-hydroxy-3-(4'-hydroxybenzyl)-5-methoxy-4-chromanone, **(5)** 8-*O*-demethyl-7-*O*-methyl-3,9-dihydropunctatin (5,8-dihydroxy-3-(4'-hydroxybenzyl)-7-methoxy-4-chromanone), **(6)** 7-*O*-methyl-3,9-dihydropunctatin (5-hydroxy-3-(4'-hydroxybenzyl)-7,8-dimethoxy-4-chromanone), **(7)** 7-hydroxy-3-(4'-hydroxybenzyl)-5,6-dimethoxy-4-chromanone, **(8)** 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone, **(9)** eucomol (3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone, **(10)** 7-*O*-methyleucomol (3,5-dihydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone), **(11)** (*E*)-eucomin ((*E*)-5,7-dihydroxy-3-(4'-methoxybenzylidenyl)-4-chromanone) and **(12)** 3',5,7-trihydroxy-4'-methoxyspiro-[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one. Of these twelve compounds 7-hydroxy-3-(4'-hydroxybenzyl)- 5,6-dimethoxy-4-chromanone has not been reported previously.
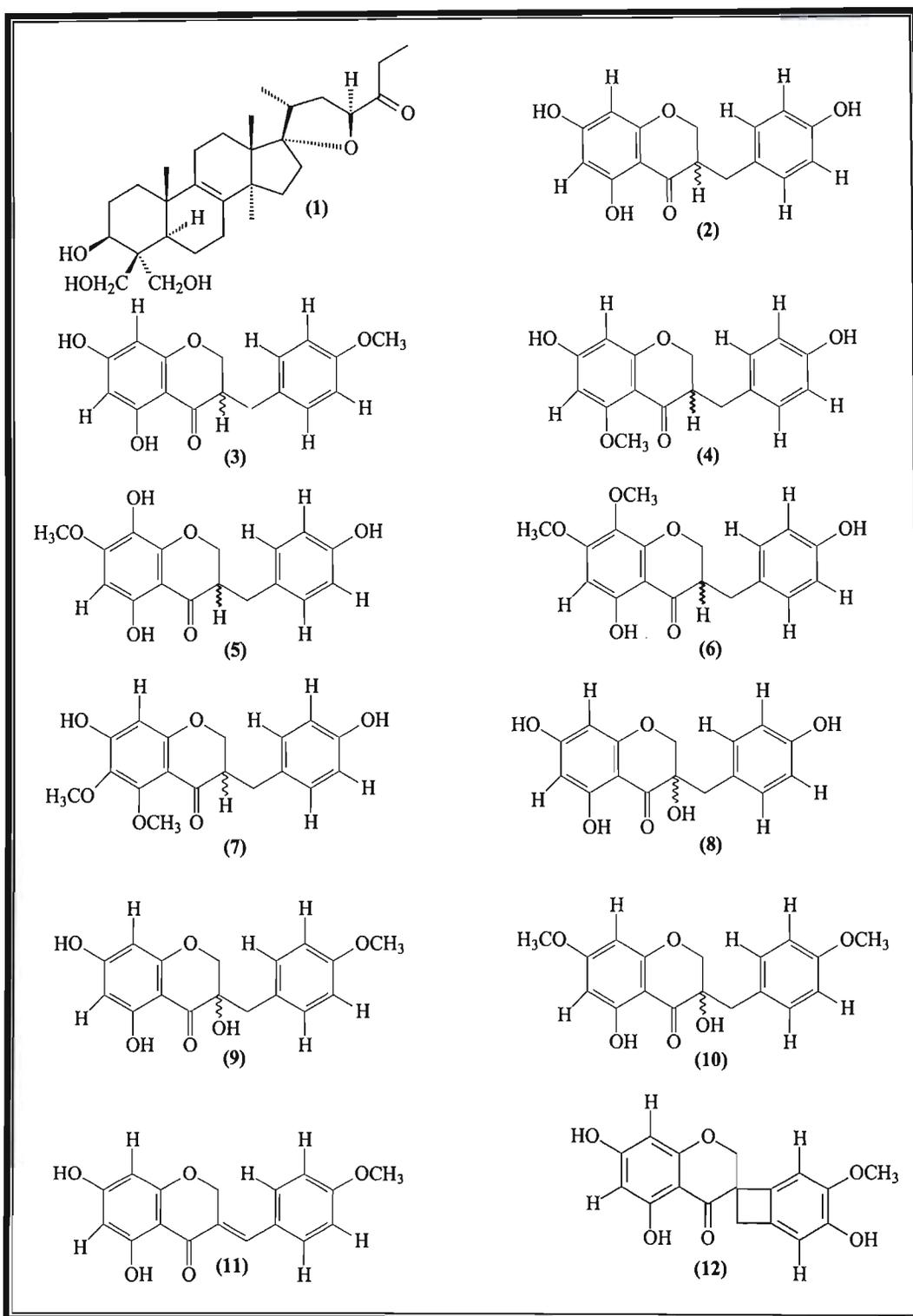
Figure 4.1: Compounds isolated from *Eucomis montana*

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4.2. Results and Discussion

4.2.1. Structural elucidation of compound 1: (23S)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one (spectra 1-1.9, pp 143-152)

This compound was isolated as an orange gum.
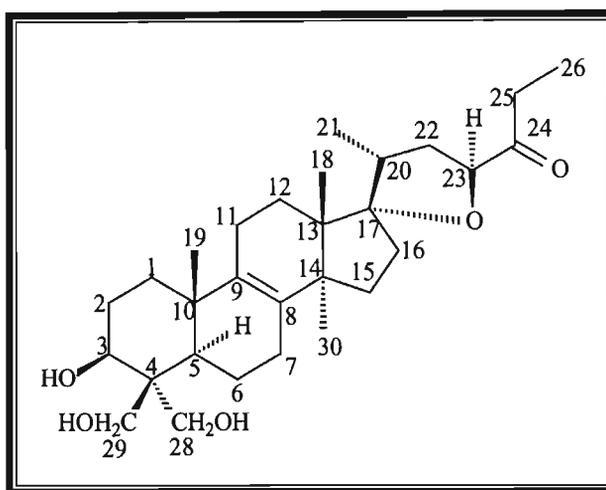
Figure 4.2: Structure of compound 1: (23S)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one

Compound 1 was found to belong to the class of compounds known as eucosterol-type nortriterpenoids. The high resolution mass spectrum **[spectrum 1.8]** showed a molecular ion [M$^+$] peak at m/z 474.33398 which corresponded to a molecular formula of $C_{29}H_{46}O_5$. From this, a double bond equivalence of seven was deduced.

The ^{1}H NMR spectrum **[spectrum 1.1]** showed a multiplet at δ_H 3.74 attributed to H-3 and its corresponding carbon resonance occurred at δ_C 77.6 in the ^{13}C NMR spectrum **[spectrum 1.2]**. The ^{1}H NMR spectrum also showed a pair of doublet resonances at δ_H 3.76 and δ_H 4.34 (d, J = 11.6 Hz) belonging to the methylene protons of 2H-29. The other pair of doublet resonances at δ_H 3.76 and δ_H 4.14 (d, J = 11.2 Hz) was assigned to 2H-28 because the HMBC spectrum **[spectrum 1.5]** showed a correlation between C-3 and C-5 and these resonances. This indicated that the methyl group at C-28 was oxidised to an alcohol. The H-23 resonance occurred as a double doublet downfield at δ_H 4.50 (dd, J = 10.4, 7.3 Hz).

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The ^{1}H NMR spectrum showed the presence of five methyl groups. Of these, three were tertiary methyl groups: 3H-18 (δ_H 0.86), 3H-19 (δ_H 0.92) and 3H-30 (δ_H 1.19). These were assigned using the HMBC spectrum. The methyl group protons at C-18 were assigned because the HMBC spectrum showed a correlation from C-17 to the methyl group proton singlet at δ_H 0.86. A correlation from C-9 to the methyl group proton singlet at δ_H 0.92 allowed assignment of the methyl group protons at C-19. The HMBC spectrum showed a correlation from C-8 to the methyl group proton singlet at δ_H 1.19 and this methyl group proton resonance was assigned to 3H-30. The remaining two methyl group protons were difficult to distinguish because they were both superimposed at δ_H 1.04 in the HSQC spectrum [spectrum 1.4]. A correlation of C-22 with the 3H-21 resonance and C-25 with the 3H-26 resonance in the HMBC spectrum allowed assignment of the methyl groups at C-21 and C-26. Their corresponding carbon resonances occurred at δ_C 17.1 and δ_C 7.4 respectively in the ^{13}C NMR spectrum.

The ^{13}C NMR spectrum showed the presence of twenty-nine carbon signals, which were resolved by the ADEPT spectrum [spectrum 1.3] into five methyl, twelve methylene and four methine groups. This indicated that there were eight fully substituted carbon atoms. The low field shifts at δ_C 133.9 and δ_C 135.2 were assigned to the two quaternary carbons of the double bond, C-8 and C-9 respectively. These were assigned accordingly because the resonance at δ_C 133.9 showed an HMBC correlation with 3H-30 and the resonance at δ_C 135.2 showed HMBC correlations to H-5 and 3H-19.

The mass spectrum showed a peak at m/z 417.29715 that resulted from elimination of an ethyl ketone radical (M$^+$ -57). This allowed placement of the ketone group resonating at δ_C 213.7 to the C-24 position. HMBC correlations between C-24 and the 2H-25 and 3H-26 proton resonances confirmed placement of the ketone group at the C-24 position. The fifth oxygen atom indicated by the molecular formula was placed between C-17 and C-23. This was determined by the presence of the two further signals at δ_C 97.0 and δ_C 81.4 attributed to C-17 and C-23 in addition to the three carbinolic carbon resonances in the ^{13}C NMR spectrum.[1] The pentacyclic ring structure accounted for the five remaining double bond equivalents.

The NOESY spectrum [spectrum 1.7] was used to determine the stereochemistry of the compound. NOESY correlations between H-23, H-22α and 3H-21 indicated that H-23 was α. No correlations between H-5 and H-19 could be seen which indicated that H-19 was β as expected. The 3H-21 and 3H-18 resonances showed correlations with each other due to the C-17, C-20 bond being β.

The infrared spectrum [spectrum 1.9] showed a broad signal at 3421 cm^{-1} due to hydroxy group stretching and the strong peak at 1737 cm^{-1} indicated the presence of a ketone carbonyl group. The peak observed at 2934 cm^{-1} was due to aliphatic C-H stretching.

Finally, a literature search for the proposed structure was undertaken and it was found that ^{13}C NMR data of compound **1** compared favourably with that of **(23S)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one. Table 4.2** shows this comparison. This compound has been previously isolated from *Muscari comosum* and *Ledebouria zebrina*.[1,2]

Table 4.1: ^{1}H , ^{13}C , HMBC, COSY and NOESY data for compound 1 (CDCl$_3$)

	^{1}H /ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
1	1.71 m	35.1	3H-19	2H-2	
2	1.76 m	27.3	2H-1	H-3	3H-19
3	3.74 m	77.6	H-28a,b, H-29a,b	2H-2	H-28b, H-5
4		45.8	H-5		
5	1.23 m	47.0	3H-19, H-3, H-28a,b, H-29a,b	2H-6	2H-7, H-28a
6	1.46 m	18.6			
7	1.98 m	26.1			H-5, 3H-30
8		133.9	3H-30		
9		135.2	H-5, 3H-19		
10		36.6	H-5, 3H-19		
11	α-2.15 m β-1.95 m	20.7		H-12α,β, H-11β H-12α,β, H-11α	3H-19,H-12α,β
12	α-1.38 m β-2.18 m	24.8	3H-18	H-11α,β, H-12β H-11α,β, H-12α	3H-21 3H-18
13		48.5	3H-18, 3H-30		
14		50.4	3H-18, 3H-30		
15	α-1.61 m β-1.33 m	31.6	3H-30	H-16α,β, H-15β H-16α,β, H-15α	3H-30 H-16β
16	α-2.15 m β-1.61 m	39.6		H-15α,β, H-16β H-15α,β, H-16α	3H-30 3H-21, H-20, 3H-18, H-15β
17		97.0	H-15β, 3H-18, 3H-21, 2H-22		
18	0.86 s	19.4	3H-21		3H-21, H-15β, H-20, H-12β, H-16β
19	0.92 s	19.1	2H-2		2H-1, 2H-7, H-29b
20	2.16 s	43.5	3H-21	3H-21	3H-18, 3H-21, H-16β
21	1.03 d, 7.1	17.1	2H-22	H-20	3H-18, H-23, H-12β, 2H-22
22	1.76 m	36.7	3H-21	H-23	3H-21, H-23
23	4.50 dd, 10.4, 7.3	81.4		2H-22	2H-25, 3H-21, 2H-22
24		213.7	3H-26, 2H-25		
25	2.52 q, 7.33	32.3	3H-26	3H-26	H-23, 3H-26
26	1.04 t*	7.4	2H-25	2H-25	2H-25, H-23
28	a-3.76 d, 11.2 b-4.14 d, 11.2	70.8	H-5, H-29b	H-29b H-29a	H-29a,b, H-5 H-3
29	a-3.76 d, 11.6 b-4.34 d, 11.6	63.7	H-3, H-5	H-29b H-28a	H-28b 3H-19, H-29a
30	1.19 s	25.8	3H-18		H-15α, H-16α

* Peaks obscured, J values could not be determined

Table 4.2: Comparison of ^{13}C NMR data for compound **1** and (23*S*)-17α,23-epoxy-3β, 28,29-trihydroxy-27-norlanost-8-en-24-one[1]

	^{13}C NMR data of compound 1 (CDCl$_3$)/ ppm	^{13}C NMR data from literature (CDCl$_3$)[1]/ ppm
1	35.1	35.3
2	27.3	27.5
3	77.6	77.9
4	45.8	46.0
5	47.0	47.3
6	18.6	18.7
7	26.1	26.2
8	133.9	134.1
9	135.2	135.5
10	36.6	36.5
11	20.7	20.8
12	24.8	24.9
13	48.5	48.7
14	50.4	50.6
15	31.6	31.7
16	39.6	39.7
17	97.0	97.2
18	19.4	19.5
19	19.1	19.2
20	43.5	43.6
21	17.1	17.2
22	36.7	36.8
23	81.4	81.5
24	213.7	213.6
25	32.3	32.3
26	7.4	7.4
28	70.8	71.3
29	63.7	63.8
30	25.8	25.9

4.2.2. Structural elucidation of compound 2: 4'-demethyl-3,9-dihydroeucomin (spectra 2.1-2.12, pp 153-163)

This compound was isolated as a yellow, crystalline solid with a melting point of 96-99 °C.
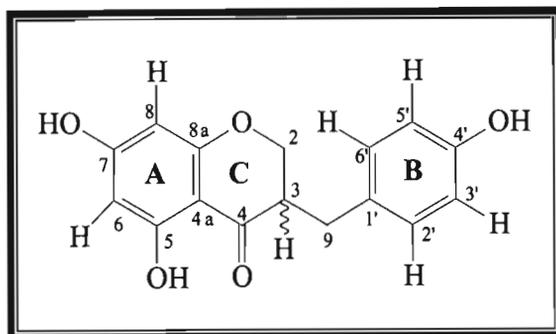
Figure 4.3: Structure of compound 2: 4'-demethyl-3,9-dihydroeucomin

The low resolution mass spectrum [spectrum 2.11] gave a molecular ion $[M^+]$ peak at m/z 286. This corresponded to a molecular formula of $C_{16}H_{14}O_5$, from which a double bond equivalence of ten was deduced. The compound gave UV absorption maxima at 324 nm (log ε 4.00), 267 nm (log ε 4.46) and 238 nm (log ε 4.76) [spectrum 2.8].

The ^1H NMR spectrum [spectrum 2.1] of compound 2, showed a typical splitting pattern for the 2H-2, H-3 and 2H-9 protons. The pair of double doublet resonances at δ_H 4.07 (dd, J = 11.4 and 7.1 Hz) and δ_H 4.23 (dd, J = 11.4 and 4.4 Hz) ascribed to H-2a and H-2b respectively, the multiplet resonance at δ_H 2.77 ascribed to H-3, and a pair of double doublet resonances at δ_H 3.08 (dd, J = 13.7 and 4.6 Hz) and δ_H 2.63 (dd, J = 13.7 and 10.3 Hz) assigned to H-9a and H-9b are characteristic of 3-benzyl-4-chromanone type homoisoflavonoids.

The H-2a proton resonance was split into a double doublet because of coupling with the H-3 and H-2b protons. The resonances for H-2b, H-9a and H-9b were split into double doublets in the same manner. The H-3 proton resonance was split into a multiplet because this proton is coupled to the H-2a, H-2b, H-9a and H-9b protons. The resonances for the H-2 protons were more deshielded and shifted downfield because the methylene group was attached to an oxygen atom. The COSY spectrum [spectrum 2.6] showed coupling between 2H-2 and H-3, and then H-3 with 2H-9, which confirmed assignment of these protons. The ^{13}C NMR resonances for C-2, C-3

and C-9 occurred at δ_C 70.1, δ_C 48.1 and δ_C 33.0 respectively [spectrum 2.2]. The absolute stereochemistry at C-3 can only be determined with circular dichroism techniques. Future work requires collaboration with other institutes to determine this. The carbon resonance at δ_C 199.5 was ascribed to the carbonyl group at C-4 as this resonance showed correlations to the 2H-9, H-3 and 2H-2 resonances in the HMBC spectrum [spectrum 2.5].

An A-4 type cleavage of the molecular ion (m/z 286) resulted in the chromanone fragment at m/z 179 represented in **Scheme 4.1**. The occurrence of peaks at m/z 152 and 153 in the mass spectrum was due to a *retro*-Diels-Alder (RDA) cleavage and hydrogen-shift of the chromanone fragment. This indicated the presence of two hydroxy groups on ring A. The A-4 type fragmentation pattern is common for 3-benzyl-4-chromanone type homoisoflavonoids. The hydroxytropylium peak at m/z 107 indicated the presence of one hydroxy group on the B ring.
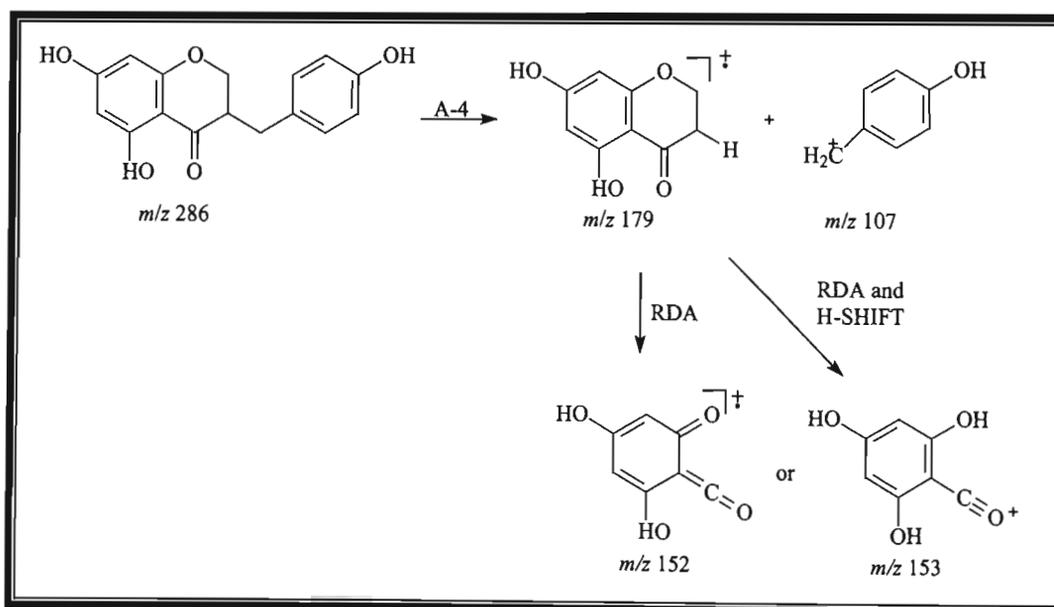
Scheme 4.1: Mass spectral fragmentation pattern for compound 2

The substitution pattern on the A-ring was determined using the ^{1}H NMR, UV and mass spectra. The pair of doublets at δ_H 5.87 ($J = 2.2$ Hz) and δ_H 5.84 ($J = 2.2$ Hz) indicated *meta*-coupled protons: the H-6 and H-8 protons respectively (see **Section 2.4**). Their corresponding carbon resonances occurred at δ_C 97.1 and δ_C 95.8 in the ^{13}C NMR spectrum. The HMBC spectrum showed a correlation between the resonance at δ_H 5.84 and the C-8a resonance, which distinguished H-8. The remaining doublet was therefore that of H-6. The carbon resonance at δ_C 164.7 was assigned to

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C-8a because it showed HMBC correlations to the 2H-2 protons. Bathochromic shifts with AlCl$_3$ (+20 nm) and NaOAc (+36 nm) in the UV spectrum **[spectra 2.9** and **2.10]** indicated hydroxy groups at the C-5 and C-7 positions.[3] The deshielded carbon resonances of C-5 and C-7 at δ_C 165.8 and δ_C 168.2 were assigned to their respective positions because of correlations of C-5 with H-6 and C-7 with H-6 and H-8 in the HMBC spectrum.

The pair of doublet resonances at δ_H 6.73 ($J = 8.6$ Hz) and δ_H 7.05 ($J = 8.6$ Hz), each integrating to two protons, were typical resonances of a *para*-disubstituted aromatic ring system. These resonances were assigned to the H-3'/5' and H-2'/6' protons respectively of the B ring. The HMBC spectrum showed a correlation between the methine resonance at δ_H 7.05 and C-9 and was therefore assigned H-2'/6'. The remaining doublet resonance had to be that of H-3'/5'. The resonance at δ_C 157.2 was assigned to C-4' because of correlations with the H-2'/6' and H-3'/5' protons in the HMBC spectrum. A hydroxy group indicated by the hydroxytropylium ion in the mass spectrum was placed at the C-4' position because the B ring was *para*-substituted. The resonance at δ_C 130.2 was assigned to C-1' because it showed a HMBC correlation to H-9a,b and H-3'/5'.

The IR spectrum **[spectrum 2.12]** further supported the proposed homoisoflavonoid structure. Peaks were observed at 3374 cm^{-1} (O-H stretching), 2920 cm^{-1} (aliphatic C-H stretching), 1641 cm^{-1} (C=O stretching), 1520 cm^{-1} (aromatic C=C stretching).

Finally, a literature search for compound **2** was undertaken. By comparison of the ^{1}H and ^{13}C NMR data (**Tables 4.4** and **4.5**) it was found that compound **2** was the known compound **4'-demethyl-3,9-dihydroeucomin** (or **5,7-dihydroxy-3-(4'-hydroxy-benzyl)-4-chromanone**) which has been previously isolated from *Muscari comosum* and *Ledebouria ovatifolia*.[3,4,5]

Table 4.3: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 2 (CD$_3$OD)

	^{1}H / ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
2	a-4.07 dd, 11.4, 7.1 b-4.23 dd, 11.4, 4.4	70.1	H-9a,b	H-3	H-3, H-9a, H-2b H-3, H-2a, H-2'/6'
3	2.77 m	48.1	H-9a,b, H-2a	H-9a,b, H-2a,b	H-9b, H-2a,b, H-2'/6'
4		199.5	H-2a,b, H-3, H-9a,b		
4a		102.8	H-6, H-8		
5		165.8	H-6		
6	5.87 d, 2.2	97.1	H-8	H-8	
7		168.2	H-8, H-6		
8	5.84 d, 2.2	95.8	H-6	H-6	
8a		164.7	H-2a,b, H-8		
9	a-3.08 dd, 13.7, 4.6 b-2.63 dd, 13.7, 10.3	33.0	H-2a,b, H-3, H-2'/6'	H-3, H-9b H-3, H-9a	H-9b, H-2'/6' H-9a, H-3, H-2'/6'
1'		130.2	H-3'/5', H-9a,b		
2'/6'	7.05 d, 8.6	131.1	H-9a,b	H-3'/5'	H-2a, H-3, H-9a,b, H-3'/5'
3'/5'	6.73 d, 8.6	116.4	H-2'/6'	H-2'/6'	H-2'/6'
4'		157.2	H-2'/6', H-3'/5'		

Table 4.4: Comparison of the ^{1}H NMR data for compound 2 with 4'-demethyl-3,9-dihydroeucomin [4]

	^{1}H NMR data for compound 2 (CD$_3$OD)/ ppm	^{1}H NMR data from literature (CD$_3$OD)[4]/ ppm
2	a-4.07 dd, 11.4, 7.1 b-4.23 dd, 11.4, 4.4	a-4.08 dd, 11.4, 7.3 b-4.25 dd, 11.4, 4.4
3	2.77 m	2.79 m
6	5.87 d, 2.2	5.87 d, 2.1
8	5.84 d, 2,2	5.85 d, 2.1
9	a-3.08 dd, 13.7, 4.6 b-2.63 dd, 13.7, 10.3	a-3.04 dd, 13.4, 3.8 b-2.65 dd, 13.4, 10.4
2'/6'	7.05 d, 8.6	7.06 d, 8.2
3'/5'	6.73 d, 8.6	6.74 d, 8.2

Table 4.5: Comparison of ^{13}C NMR data for compound 2 with 4'-demethyl-3,9-dihydroeucomin [4]

	^{13}C data of compound 2 (CD$_3$OD)/ ppm	^{13}C data from literature (CD$_3$OD)[4]/ ppm
2	70.1	70.1
3	48.1	48.2
4	199.5	199.4
4a	102.8	102.8
5	165.8	165.8
6	97.1	97.1
7	168.2	168.2
8	95.8	95.9
8a	164.7	164.6
9	33.0	32.9
1'	130.2	130.2
2'/6'	131.1	131.2
3'/5'	116.4	116.4
4'	157.2	157.2

4.2.3. Structural elucidation of compound 3: 3,9-dihydroeucomin (spectra 3.1-3.12, pp 164-174)

This compound was isolated as a white crystalline solid, with a melting point of 152-154°C.
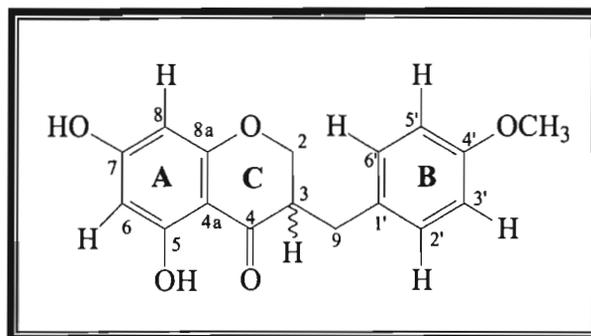
Figure 4.4: Structure of compound 3: 3,9-dihydroeucomin

Compound **3** gave a UV absorption maximum at 293 nm (log ε 4.24) **[spectrum 3.8]**. The mass spectrum **[spectrum 3.11]** gave a molecular ion [M⁺] peak at m/z 300 and this corresponded to a molecular formula of $C_{17}H_{16}O_5$. From this a double bond equivalence of ten was deduced.

The ^1H NMR spectrum **[spectrum 3.1]** of compound **3** showed a characteristic splitting pattern for 3-benzyl-4-chromanone type homoisoflavonoids. These were the H-2a and H-2b double doublet resonances at δ_H 4.06 (dd, $J = 11.4, 6.5$ Hz) and δ_H 4.22 (dd, $J = 11.4, 4.0$ Hz), the multiplet resonance of H-3 at δ_H 2.74 and the pair of double doublet resonances of H-9a and H-9b at δ_H 2.67 (dd, $J = 13.4, 10.6$ Hz) and δ_H 3.13 (dd, $J = 13.4, 3.9$ Hz).

The *meta*-coupled protons, H-6 and H-8 on the A-ring were indicated by the doublet resonances at δ_H 5.94 ($J = 2.2$ Hz) and δ_H 5.88 ($J = 2.2$ Hz) in the ^1H NMR spectrum. The peaks at m/z 153 and 179 in the mass spectrum indicated the presence of two hydroxy groups on ring A **(Scheme 4.2)**.
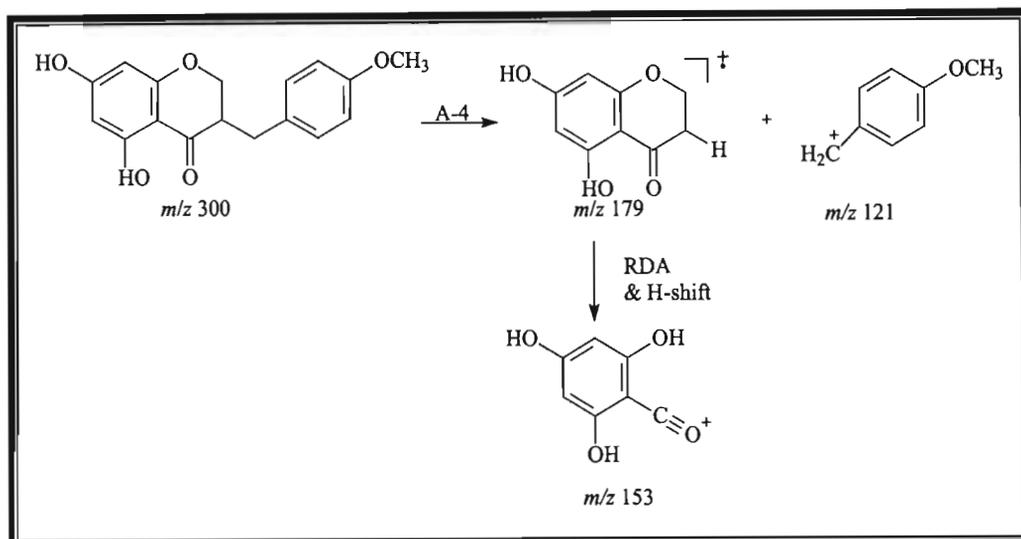
Scheme 4.2: Mass spectral fragmentation pattern for compound 3

Bathochromic shifts of +32 nm with AlCl$_3$ and +32 nm with NaOAc [spectra 3.9 and 3.10] were observed and this indicated hydroxy groups at the C-5 and C-7 positions.[6] The deshielded carbon resonances of C-5 and C-7 at δ_C 164.4 and δ_C 166.0 were assigned to their respective positions because of HMBC correlations of C-5 with H-6 and C-7 with H-6 and H-8 [spectrum 3.5]. The broad singlet at δ_H 12.14 indicated the strongly hydrogen bonded proton of the hydroxy group at C-5.[7] This was confirmed by the downfied shift of the C-4 resonance that occurred at δ_C 197.5 in the ^{13}C NMR spectrum [spectrum 3.2] due to the chelating effects of the C-5 hydroxy group with that of the carbonyl group. Placement of the hydroxy group at the C-7 position was confirmed by the presence of the C-6 and C-8 resonances at δ_C 96.5 and δ_C 95.2. If a methoxy group was attached to C-7 an upfield shift of 1.3 ppm and 3.3 ppm would have been observed for the C-6 and C-8 resonances in the ^{13}C NMR spectrum.[4]

The two proton doublet resonances at δ_H 6.83 (J = 8.6 Hz) and δ_H 7.11 (J = 8.6 Hz) in the ^{1}H NMR spectrum indicated a *para*-disubstituted B-ring system. These resonances were assigned to the H-3'/5' and H-2'/6' protons respectively. The methoxy group, indicated by a proton resonance at δ_H 3.76 integrating to three protons, was placed at the C-4' position because of a NOESY interaction with the H-3'/5' protons in the NOESY spectrum [spectrum 3.7]. This was further supported by the presence of an intense peak at *m/z* 121 in the mass spectrum that corresponded to a methoxytropylium ion.

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The IR spectrum [spectrum 3.12] showed peaks typical of homoisoflavonoids. These were observed at 3364 cm⁻¹ (O-H stretching), 2929 cm⁻¹ (aliphatic C-H stretching), 1644 cm⁻¹ (C-O stretching) and 1515 cm⁻¹ (aromatic C=C stretching).

Finally, a literature search was undertaken for compound **3** and it was found that the ¹H NMR data **(Table 4.7)** matched that of the known compound **3,9-dihydro-eucomin** (or **5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone**), previously isolated from *Eucomis bicolor*.[8]

Table 4.6: ¹H, ¹³C, HMBC, COSY and NOESY data for compound 3 (CDCl₃)

	¹H /ppm	¹³C/ ppm	HMBC C→H	COSY	NOESY
2	a-4.06 dd, 11.4, 6.5 b-4.22 dd, 11.4, 4.0	68.8	H-3, H-9a,b	H-2b, H-3 H-2a, H-3	H-9a, H-2b H-9a, H-2a
3	2.74 m	46.8	H-9a,b, H-2b	H-9a,b	H-9b, H-2a,b
4		197.5	H-9a,b, H-3, H-2a,b		
4a		102.0	H-8, H-6		
5-OH	12.14 bs	164.4	H-6		
6	5.94 d, 2.2	96.5	H-8		
7		166.0	H-6, H-8		
8	5.88 d, 2.2	95.2	H-6		
8a		163.1	H-2a,b, H-8		
9	a-2.67 dd, 13.4, 10.6 b-3.13 dd, 13.4, 3.9	32.0	H-3, H-2a,b, H-2'/6'	H-9b, H-3 H-9a, H-3	H-2a, b H-3
1'		129.8	H-9a,b, H-3, H-3'/5',H-2'/6'		
2'/6'	7.11 d, 8.6	130.0	H-3'/5',H-9a,b	H-3'/5'	
3'/5'	6.83 d, 8.6	114.1	H-2'/6'	H-2'/6'	4'-OCH₃
4'		158.3	4'-OCH₃, H-3'/5', H-2'/6'		
4'-OCH₃	3.76 s	55.4			H-3'/5'

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Table 4.7: Comparison of ^{1}H NMR data for compound 3 and 3,9-dihydro-eucomin[8]

	^{1}H NMR data for compound 3 (CDCl$_3$)/ ppm	^{1}H NMR data from literature (CDCl$_3$)[8]/ ppm
2	a-4.06 dd, 11.4, 6.5 b-4.22 dd, 11.4, 4.0	a-4.12 dd, 11.5, 6.5 b-4.28 dd, 11.5, 4.2
3	2.74 m	2.80 m
5-OH	12.14 bs	12.13 s
6	5.94 d, 2.2	5.98 d, 2.3
8	5.88 d, 2.2	5.91 d, 2.3
9	a-2.67 dd, 13.4, 10.6 b-3.13 dd, 13.4, 3.9	2.80 m 3.20 m
2'/6'	7.11 d, 8.6	7.14 d, 8.8
3'/5'	6.83 d, 8.6	6.86 d, 8.8
4'-OCH$_3$	3.76 s	3.80 s

4.2.4. Structural elucidation of compound 4: 4'-demethyl-5-O-methyl-3,9-dihydroeucomin (spectra 4.1-4.13, pp 175-186)

Compound **4** was isolated as a colourless crystalline solid with a melting point of 201-203°C.
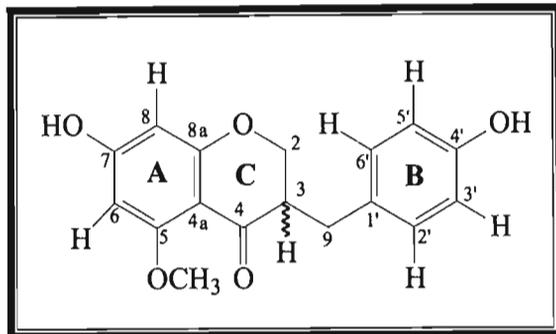
Figure 4.5: Structure of compound 4: 4'-demethyl-5-O-methyl-3,9-dihydroeucomin

This compound gave a molecular ion $[M^+]$ peak at m/z 300 in the mass spectrum **[spectrum 4.12]** that corresponded to a molecular formula of $C_{17}H_{16}O_5$. From this a double bond equivalence of ten was deduced. UV absorption maxima were obtained at 283 nm (log ε 2.83) and 232 nm (log ε 3.77) **[spectrum 4.9]**.

The ^{1}H NMR spectrum **[spectrum 4.1]** showed a splitting pattern for the C-2, C-3 and C-4 proton coupled system that was typical for the 3-benzyl-4-chromanone type homoisoflavonoids. These resonances were similar to those found in compound **2** and **3**.

The ^{1}H NMR spectrum indicated *meta*-coupled protons at δ_H 6.09 (d, $J = 2.2$ Hz) and δ_H 5.98 (d, $J = 2.2$ Hz) attributed to H-6 and H-8 of the A ring. The UV spectrum **[spectrum 4.11]** showed a bathochromic shift (+20 nm) with NaOAc, which indicated the presence of a hydroxy group at the C-7 position. No shift was observed with AlCl₃ **[spectrum 4.10]** and this indicated that a hydroxy group was not present at C-5.[9] The methoxy group indicated by the singlet resonance at δ_H 3.84 was placed at this position as this resonance showed a NOESY correlation to the H-6 proton in the NOESY spectrum **[spectrum 4.8]**. This was confirmed by the appearance of the C-4 carbonyl resonance at δ_C 194.0. It would be further downfield if a hydroxy group was present in the C-5 position due to its chelating effects.[3]

Substitution on the B-ring was the same as compound **2**. The pair of doublets at δ_H 7.07 (J = 8.6 Hz) and δ_H 6.75 (J = 8.6 Hz) were those of the respective H-2'/6' and H-3'/5' protons which indicated a *para*-disubstituted aromatic ring system.

The mass spectrum confirmed the substitution patterns on the A and B rings. The peak at *m/z* 193 was due to the A-4 type fragmentation and confirmed the presence of the methoxy and the hydroxy groups on the A ring while the peak at *m/z* 107, typical of the hydroxytropylium ion, confirmed the substitution pattern on the B ring. The peaks at *m/z* 166 and 167 are formed from the RDA cleavage and hydrogen-shift of the chromanone fragment.
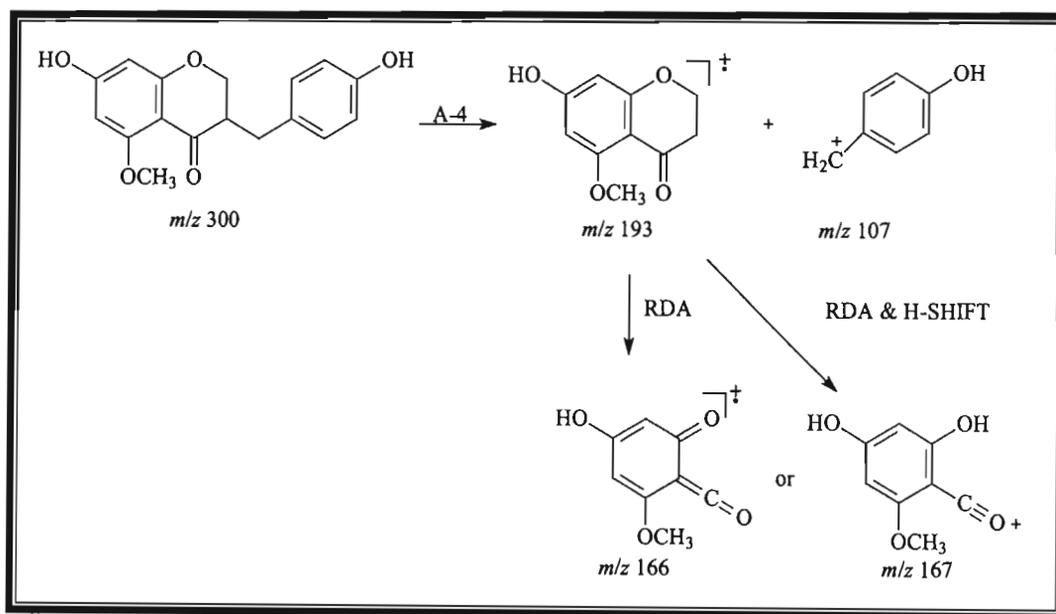
Scheme 4.3: Mass spectral fragmentation pattern for compound 4

The IR data for compound **4 [spectrum 4.13]** was consistent with that of the proposed structure. Peaks were observed at 3419 cm^{-1} (O-H stretching), 2925 cm^{-1} (aliphatic C-H stretching), 1736 cm^{-1} (C=O stretching) and 1590 cm^{-1} (aromatic C=C stretching).

A literature search for compound **4** showed that the ^{1}H NMR data **(Table 4.9)** agreed with that of **4'-demethyl-5-*O*-methyl-3,9-dihydroeucomin** (or **7-hydroxy-3-(4'-hydroxybenzyl)- 5-methoxy-4-chromanone**).[6] This compound has been previously isolated from *Eucomis bicolor* and *Eucomis punctata*.[6,9]

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Table 4.8: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 4 (CD$_3$OD)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
2	a-4.07 dd, 11.6, 6.5 b-4.23 dd, 11.6, 3.8	69.8	H-9a,b, H-3	H-3, H-9a, H-2b H-9a, H-3, H-2a	H-2b, H-2'/6', H-3, H-9a H-2a, H-3, H-9a
3	2.65 m*	49.9	H-9a,b, H-2a,b	H-9a,b, H-2a,b, H-3	H-9b, H-2a,b, H-2'/6'
4		194.0	H-9a,b, H-3, H-2a,b		
4a		105.2	H-6, H-8		
5		164.5	5-OCH$_3$, H-6		
6	6.09 d, 2.2	94.2	H-8	5-OCH$_3$	5-OCH$_3$
7		166.5	H-6, H-8		
8	5.98 d, 2.2	96.7	H-6		
8a		166.7	H-2a,b, H-8		
9	a-2.65 m* b-3.04 dd, 11.0, 4.8	33.4	H-3, H-2a,b, H-2'/6'	H-3 H-3, H-9a	H-9b H-9a, H-3, H-2'/6'
1'		130.6	H-9a,b, H-3, H-2'/6', H-3'/5'		
2'/6'	7.07 d, 8.6	131.1	H-9a,b, H-3'/5'	H-3'/5'	H-3'/5', H-9a,b, H-3, H-2a
3'/5'	6.75 d, 8.6	116.3	H-2'/6'	H-2'/6'	H-2'/6'
4'		157.1	H-2'/6', H-3'/5'		
5-OCH$_3$	3.84 s	56.2		H-6	H-6

* Peaks overlapping, not possible to determine coupling constants

Table 4.9: Comparison of ^{1}H NMR data for compound 4 with 4'-demethyl-5-O-methyl-3,9-dihydroeucomin [6]

	^{1}H NMR data for compound 4 (CD$_3$OD)/ ppm	^{1}H NMR data for compound 4 ((CD$_3$)$_2$CO)/ ppm	^{1}H NMR data from literature ((CD$_3$)$_2$CO)[6]/ ppm
2	a-4.07 dd, 11.6, 6.5 b-4.23 dd, 11.6, 3.8	a-4.07 dd, 11.2, 8.2 b-4.27 dd, 11.2, 4.2	a-4.06 dd, 11.2 b-4.26 dd, 11.2
3	2.65 m*	2.68 m	2.2-3.5 m
6	6.09 d, 2.2	6.15 d, 2.2	6.13 d, 2.3
8	5.98 d, 2.2	6.02 d, 2.2	6.00 d, 2.3
9	a-2.65 m* b-3.04 dd, 11.0, 4.8	a-2.55 m b-3.03 m	2.2-3.5 m
2'/6'	7.07 d, 8.6	7.11 d, 8.4	7.09 d, 8.8
3'/5'	6.75 d, 8.6	6.81 d, 8.4	6.79 d, 8.8
5-OCH$_3$	3.84 s	3.79 s	3.79 s

* Peaks overlapping, not possible to determine coupling constants

4.2.5. Structural elucidation of compound 5: 8-*O*-demethyl-7-*O*-methyl-3,9-dihydropunctatin (spectra 5.1-5.14, pp 187-199)

This compound was isolated as a yellow, crystalline solid with a melting point of 178-180°C.
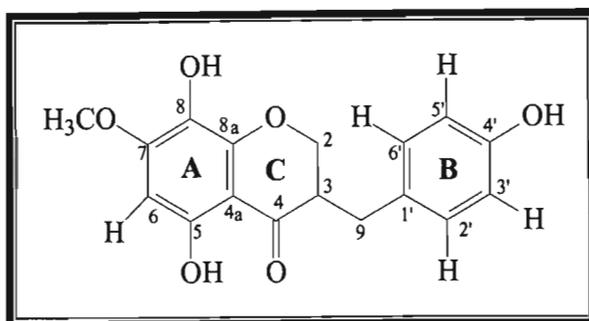
Figure 4.6: Structure of compound 5: 8-*O*-demethyl-7-*O*-methyl-3,9-dihydropunctatin

Compound **5** showed a molecular ion [M$^+$] peak at *m/z* 316 in the mass spectrum **[spectrum 5.13]**. This corresponded to a molecular formula of $C_{17}H_{16}O_6$ from which a double bond equivalence of ten was deduced. UV absorption maxima were obtained at 368 nm (log ε 3.13), 295 nm (log ε 3.53) and 235 (log ε 4.60) **[spectrum 5.10]**.

The ^{1}H NMR spectrum **[spectrum 5.1]** showed the characteristic 2H-2, H-3 and 2H-9 resonances for a 3-benzyl-4-chromanone type homoisoflavonoid. This was similar to those of compounds **2-4**.

The singlet resonance at δ_H 6.14 indicated a pentasubstituted A-ring system. The existence of a peak at *m/z* 209 in the mass spectrum was evidence for a dihydroxy-methoxychromanone fragment ion. This indicated that the A-ring contained two hydroxy groups and one methoxy group. One hydroxy group was assigned to the C-5 position due to a bathochromic shift (+14 nm) with AlCl$_3$ in the UV spectrum **[spectrum 5.11]**. This was confirmed by the downfield shift of the C-4 resonance at δ_C 200.1 in the ^{13}C spectrum **[spectrum 5.3]** due to the chelating effects of the C-5 hydroxy group with that of the carbonyl group. No shift was observed with NaOAc **[spectrum 5.12]** and this indicated the absence of a hydroxy group at the C-7 position.[3] The methoxy group indicated by the singlet resonance in the ^{1}H NMR spectrum at δ_H 3.87 was therefore placed in this position.
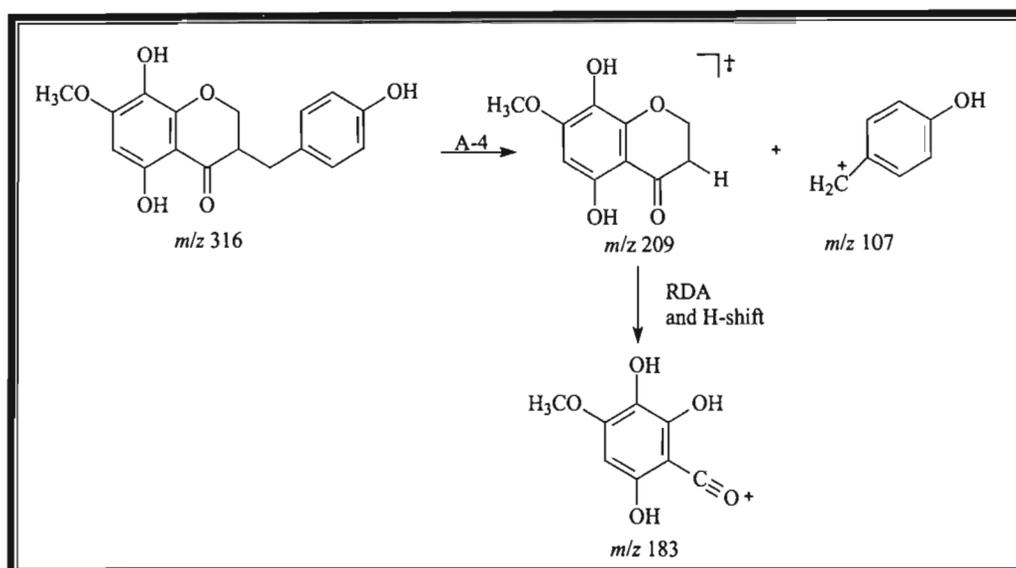
Scheme 4.4: Mass spectral fragmentation pattern for compound 5

A NOESY correlation between the 5-OH proton resonance at δ_H 11.74 with the singlet resonance at δ_H 6.12 in the NOESY spectrum [**spectrum 5.9**] run in deuterated chloroform, indicated that the singlet resonance at δ_H 6.12 belonged to H-6. No HMBC correlation was seen between the C-8a resonance and this singlet resonance, which confirmed that it did not occur at C-8. The 5-OH proton and the methoxy group at the C-7 position showed NOESY correlations with the H-6 proton, confirming its assignment. Furthermore, the corresponding C-6 resonance occurred at an appreciably higher field at δ_C 93.4 in the ^{13}C NMR spectrum. This was attributed to the stronger electron-releasing effect of the 7-methoxy group.[4] The second hydroxy group was then placed at the remaining C-8 position.

Substitution on the B-ring was the same as compounds **2**, **4** and **5** *i.e. para*-disubstituted benzene ring with a hydroxy group attached at the C-4' position. The peak at *m/z* 107 in the mass spectrum indicated a hydroxytropylium ion that confirmed this assignment.

The IR spectrum [**spectrum 5.14**] supported the proposed structure of compound **5**. Peaks were observed at 3414 cm^{-1} (O-H stretching), 2920 cm^{-1} (aliphatic C-H stretching), 1652 cm^{-1} (C=O stretching) and 1514 cm^{-1} (aromatic C=C stretching).

Finally, a literature search for the compound was undertaken and it was found that the ^{1}H and ^{13}C NMR data of compound **5** matched that of **8-*O*-demethyl-7-*O*-methyl-**

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3,9-dihydropunctatin (or **5,8-dihydroxy-3-(4'-hydroxybenzyl)-7-methoxy-4-chromanone**, previously isolated from *Muscari comosum*.[4,7]

Table 4.10: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 5 (CD$_3$OD)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
2	a-4.30 dd, 11.4, 4.2 b-4.14 dd, 11.4, 7.1	70.5	H-9a,b	H-3	H-2b, H-3 H-2a, H-3
3	2.83 m	48.5	H-9a,b, H-2b	H-9a,b H-2a,b	H-9a,b, H-2a,b, H-2'/6'
4		200.1	H-9a,b, H-3, H-2a,b		
4a		103.2	H-6		
5-OH	11.74 s*	158.2			H-6*
6	6.14 s	93.4		7-OCH$_3$	7-OCH$_3$
7		158.0	H-6, 7-OCH$_3$		
8		127.6	H-6		
8a		149.2	H-2		
9	a-3.10 dd, 13.9, 4.8 b-2.66 dd, 13.9,10.4	32.9	H-2a,b	H-9a, H-3 H-9b, H-3	H-9b, H-3, H-2'/6' H-9a, H-2b, H-2'/6'
1'		130.2	H-9a,b, H-3'/5'		
2'/6'	7.06 d, 8.6	131.2	H-9a,b, H-3'/5'	H-3'/5'	H-9a,b, H-3, H-3'/5'
3'/5'	6.73 d, 8.6	116.4	H-2'/6'	H-2'/6'	
4'		157.2	H-3'/5', H-2'/6'		
7-OCH$_3$	3.87 s	56.7			H-6

* Observed with CDCl$_3$

Table 4.11: Comparison of ^{1}H NMR data for compound 5 with 8-O-demethyl-7-O-methyl-3,9-dihydropunctatin[4]

	^{1}H NMR data of compound 5 (CDCl$_3$)/ ppm	^{1}H NMR data of compound 5 (CD$_3$OD)/ ppm	^{1}H NMR data from literature[4] (CD$_3$OD)/ ppm
2	a-4.34 dd, 11.3, 3.2 b-4.19 dd, 11.3, 6.6	a-4.30 dd, 11.4, 4.2 b-4.14 dd, 11.4, 7.1	a-4.29 dd, 11.4, 4.4 b-4.12 dd, 11.4, 7.3
3	2.80 m	2.83 m	2.82 m
5-OH	11.74 s		
6	6.12 s	6.14 s	6.12 s
9	a-3.13 dd, 13.5, 4.2 b-2.72 dd, 13.5, 10.5	a-3.10 dd, 13.9, 4.8 b-2.66 dd, 13.9, 10.4	a-3.10 dd, 13.6, 4.0 b-2.64 dd, 13.6, 10.3
2'/6'	7.08 d, 8.3	7.06 d, 8.6	7.06 d, 8.1
3'/5'	6.78 d, 8.3	6.73 d, 8.6	6.72 d, 8.1
7-OCH$_3$	3.90 s	3.87 s	3.86 s

Table 4.12: Comparison of ^{13}C NMR data for compound 5 with 8-O-demethyl-7-O-methyl-3,9-dihydropunctatin[4]

	^{13}C NMR data of compound 5 (CD$_3$OD)/ ppm	^{13}C NMR data from literature (CD$_3$OD)[4]/ ppm
2	70.5	70.5
3	48.5	48.8
4	200.1	200.1
4a	103.2	103.2
5	158.2	158.2
6	93.4	93.5
7	158.0	158.1
8	127.6	127.6
8a	149.2	149.2
9	32.9	32.8
1'	130.2	130.2
2'/6'	131.2	131.1
3'/5'	116.4	116.4
4'	157.2	157.2
7-OCH$_3$	56.7	56.7

4.2.6. Structural elucidation of compound 6: 7-*O*-methyl-3,9-dihydropunctatin

(spectra 6.1-6.14, pp 200-212)

Compound **6** was isolated as a yellow amorphous solid.
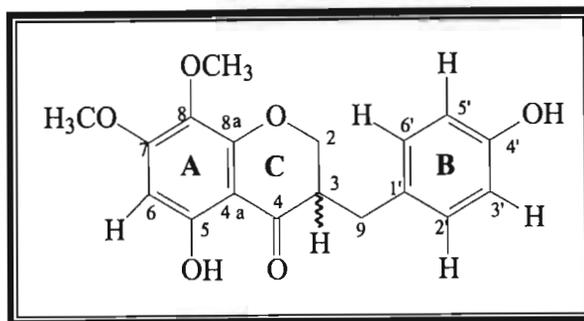
Figure 4.7: Structure of compound 6: 7-*O*-methyl-3,9-dihydropunctatin

This compound gave a molecular ion [M$^+$] peak at *m/z* 330 in the mass spectrum [**spectrum 6.13**] that corresponded to a molecular formula of $C_{18}H_{18}O_6$. A double bond equivalence of ten was deduced. UV absorption maxima were obtained at 287 (log ε 3.18) and 229 nm (log ε 3.21) [**spectrum 6.10**].

The splitting pattern for the 2H-2, H-3 and 2H-9 protons in the ^{1}H NMR spectrum [**spectrum 6.1**] was characteristic for the 3-benzyl-4-chromanone-type homoisoflavonoids. These were similar to compounds **2-5**.

The ^{1}H NMR spectrum showed the presence of two methoxy group proton resonances at δ_H 3.86 and δ_H 3.68 integrating to three protons each. The single proton resonance at δ_H 6.16 was assigned to the A ring. The aromatic doublet resonances at δ_H 7.06 (*J* = 8.5 Hz) and δ_H 6.73 (*J* = 8.5 Hz) indicated *ortho* coupling and a *para*-disubstituted B ring. These resonances were attributed to the H-2'/6' and H-3'/5' protons respectively.

The intense signals observed at *m/z* 223 and *m/z* 107 in the mass spectrum were due to the A-4 type fragmentation. The peak at *m/z* 223 indicated that the A-ring had one hydroxy and two methoxy substituents. The signal at *m/z* 197 was typically formed *via* the RDA and hydrogen-shift of the chromanone fragment. The hydroxytropylium ion at *m/z* 107 indicated a single hydroxy group on the B-ring and this was assigned to the C-4' position since the B ring was *para*-substituted.

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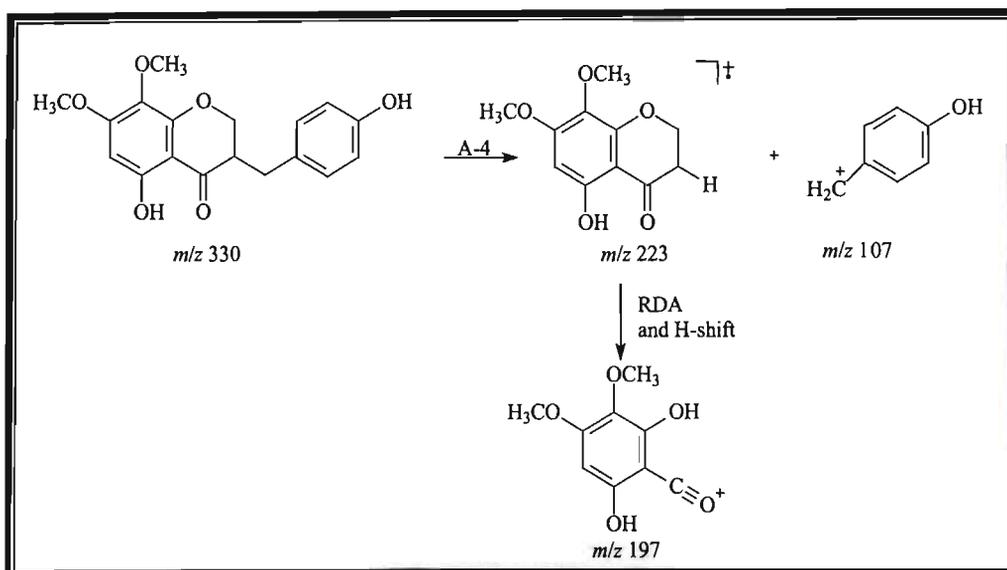
Scheme 4.5: Mass spectral fragmentation pattern for compound 6

UV spectra were used to assign the substituents on the A ring. A bathochromic shift (+25 nm) was observed with AlCl$_3$ **[spectrum 6.11]** and this indicated the presence of a hydroxy group at the C-5 position.[6] This strongly hydrogen-bonded 5-hydroxy group proton resonance was observed at δ_H 12.03 in the ^{1}H NMR spectrum **[spectrum 6.2]** run in deuterated chloroform. This was confirmed by the downfield resonance of C-4 at δ_C 200.0 in the ^{13}C NMR spectrum **[spectrum 6.3]**. No bathochromic shift was observed with NaOAc **[spectrum 6.12]** and this indicated a methoxy group at the C-7 position.[6] The singlet proton resonance in the ^{1}H NMR spectrum occurred downfield of δ_H 6.00 and this confirmed the absence of a hydroxy group at C-7.[4]

A NOESY correlation between the 5-OH proton and the singlet resonance at δ_H 6.09 was observed in the NOESY spectrum **[spectrum 6.9]** run in deuterated chloroform. This resonance was therefore assigned to H-6. The methoxy group proton resonance at δ_H 3.86 was placed at the C-7 position because it showed a NOESY correlation **[spectrum 6.8]** to the H-6 proton. The second methoxy group at δ_H 3.68 was then placed at the remaining C-8 position.

Placement of the methoxy group at the C-8 position was confirmed by comparison of the ^{13}C NMR data with the literature values of 7-*O*-methyl-3,9-dihydropunctatin and 7-*O*-methyl-3,9-dihydroeucomnalin **(Table 4.15)**.[4] It was found that placement of the methoxy group at the C-6 or C-8 position has an effect on the chemical shift of the C-

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5, C-6, C-8 and the C-8a resonances. As shown in **Table 4.15,** the resonances of compound **6** agreed with those of 7-*O*-methyl-3, 9-dihydropunctatin.
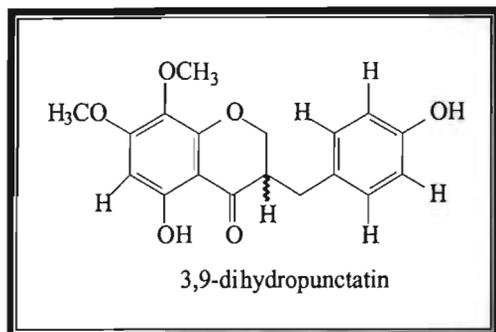
Figure 4.8: Structure of 7-*O*-methyl-3,9-dihydropunctatin
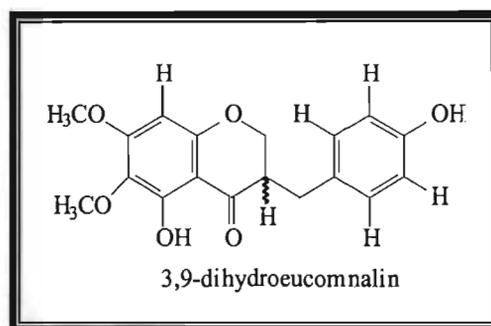
Figure 4.9: Structure of 7-*O*-methyl-3,9-dihydroeucomnalin

The IR spectrum **[spectrum 6.14]** further supported the proposed structure. Peaks were observed at 3414 cm^{-1} (O-H stretching), 2932 cm^{-1} (aliphatic C-H stretching), 1641 cm^{-1} (C=O stretching) and 1520 cm^{-1} (aromatic C=C stretching).

Compound **6** was found to be the known compound **7-*O*-methyl-3,9-dihydropunctatin** (or **5-hydroxy-3-(4'-hydroxybenzyl)-7,8-dimethoxy-4-chromanone**). This compound has been previously isolated from *Muscari comosum* and *Ledebouria cooperi*.[4,10]

Table 4.13: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 6 (CD$_3$OD)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	NOESY	COSY
2	a-4.16 dd, 11.4, 7.1 b-4.32 dd, 11.4, 4.4	70.6	H-9a,b	H-2b, H-3 H-2a, H-3	H-3
3	2.85 m	48.2	H-9a,b, H-2a	H-2b, H-9a,b, H-2'/6'	H-9a,b, H-2a
4		200.0	H-2a,b, H-9a,b		
4a		103.2	H-6		
5-OH	12.03 s*	161.5	H-6	H-6*	
6	6.16 s	93.8		7-OCH$_3$	
7		162.7	7-OCH$_3$, H-6		
8		130.4	8-OCH$_3$, H-6		
8a		155.1	H-2a,b		
9	a-2.67 dd, 13.9, 10.1 b-3.10 dd, 13.9, 4.2	33.0	H-2a,b, H-2'/6'	H-9b, H-2'/6' H-9a, H-3, H-2'/6'	H-3, H-9b H-9a
1'		129.9	H-9a,b, H-3'/5'		
2'/6'	7.06 d, 8.5	131.2		H-2a,b, H-3, H-3'/5'	H-3'/5'
3'/5'	6.73 d, 8.5	116.4	H-9a,b	H-2'/6'	H-2'/6'
4'		157.3	H-2'/6', H-3'/5'		
7-OCH$_3$	3.86	56.7	8-OCH$_3$	H-6	
8-OCH$_3$	3.68 s	61.5	7-OCH$_3$		

*Observed with CDCl$_3$

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Table 4.14: Comparison of ^{1}H NMR data for compound 6 with 7-*O*-methyl-3,9-dihydropunctatin[4]

	^{1}H NMR data of compound 6 (CDCl$_3$)/ ppm	^{1}H NMR data of compound 6 (CD$_3$OD)/ ppm	^{1}H NMR data from literature (CD$_3$OD)[4]/ ppm
2	a-4.18 dd, 11.4, 6.9 b-4.33 dd, 11.4, 4.2	a-4.16 dd, 11.4, 7.1 b-4.32 dd, 11.4, 4.4	a-4.12 dd, 11.4, 7.3 b-4.29 dd, 11.4, 4.4
3	2.80 m	2.85 m	2.83 m
5-OH	12.03 s		
6	6.09 s	6.16 s	6.11 s
9	a-2.70 dd, 13.7, 10.4 b-3.14 dd, 13.7, 4.4	a-2.67 dd, 13.9, 10.1 b-3.10 dd, 13.9, 4.2	a-2.61 dd 13.6, 9.6 b-3.08 dd, 13.6, 4.0
2'/6'	7.08 d, 8.5	7.06 d, 8.5	7.03 d, 8.5
3'/5'	6.77 d, 8.5	6.73 d, 8.5	6.72 d, 8.5
7-OCH$_3$	3.87 s	3.86 s	3.84 s
8-OCH$_3$	3.76 s	3.68 s	3.66 s

Table 4.15: Comparison of ^{13}C NMR data for compound 6 with 7-*O*-methyl-3,9-dihydroeucomnalin and 7-*O*-methyl-3,9-dihydropunctatin[4]

	^{13}C data of compound 6 (CD$_3$OD)/ ppm	^{13}C data of 3,9-dihydroeucomnalin (CD$_3$OD)[4]/ ppm	^{13}C data of 3,9-dihydropunctatin (CD$_3$OD)[4]/ ppm
2	70.6	70.6	70.4
3	48.2	48.1	48.8
4	200.0	200.5	199.8
4a	103.2	103.7	103.3
5	161.5	156.2	161.3
6	93.8	131.4	93.8
7	162.7	162.3	162.5
8	130.4	92.6	130.3
8a	155.1	160.6	154.9
9	33.0	32.9	32.0
1'	129.9	130.1	129.7
2'/6'	131.2	131.2	131.0
3'/5'	116.4	116.5	116.4
4'	157.3	157.3	157.2
6-OCH$_3$		61.1	
7-OCH$_3$	56.7	56.7	56.7
8-OCH$_3$	61.5		61.5

4.2.7. Structural elucidation of compound 7: 7-hydroxy-3-(4'-hydroxybenzyl)-5,6-dimethoxy-4-chromanone (spectra 7.1-7.13, pp 213-224)

This compound was isolated as a yellow amorphous substance.
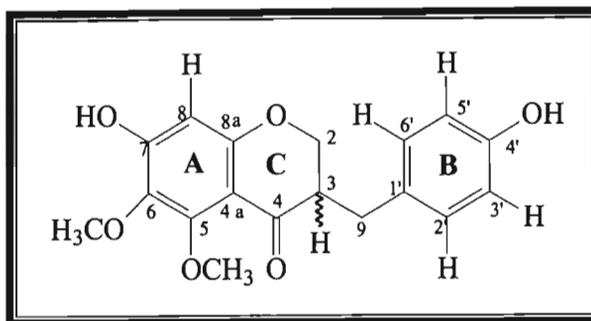
Figure 4.10: Structure of compound 7: 5,6-dimethoxy-7-hydroxy-3-(4'-hydroxybenzyl)-4-chromanone

Compound **7** gave a molecular ion $[M^+]$ peak at m/z 330 in the mass spectrum **[spectrum 7.12]**. This corresponded to a molecular formula of $C_{18}H_{18}O_6$ from which a double bond equivalence of ten was deduced. UV absorption maxima were obtained at 321 (log ε 3.53), 277 nm (log ε 3.87) and 232 nm (log ε 4.56) **[spectrum 7.9]**.

The distinctive 2H-2, H-3 and 2H-9 resonances for 3-benzyl-4-chromanone type homoisoflavonoids were evident in the aliphatic region of the ^1H NMR spectrum **[spectrum 7.1]** for this compound.

The fragmentation pattern for this compound was the same as compound **6**. A hydroxydimethoxy fragment at m/z 223 in the mass spectrum indicated the substituents on the A ring whereas the hydroxytropylium fragment at m/z 107 indicated a hydroxylated B ring. The aromatic resonances at δ_H 7.06 (d, J = 8.4 Hz) and δ_H 6.76 (d, J = 8.4 Hz) assigned to H-2'/6' and H-3'/5' indicated a *para*-disubstituted B ring. A hydroxy group was assigned to the C-4' position.

The ^1H NMR and UV spectra confirmed substituents on the A ring that were indicated by the mass spectrum. The singlet at δ_H 6.29 was assigned to H-8 because of the HMBC correlation with C-8a. The presence of a singlet proton resonance indicated

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only one unsubstituted position on the A ring. Two overlapped singlets integrated to six protons at δ_H 3.89 indicated that two methoxy groups were present. One methoxy group was placed at the C-5 position because no bathochromic shift was observed with AlCl$_3$ in the UV spectrum [spectrum 7.10].[6] The appearance of a carbonyl group at δ_C 191.9 in the ^{13}C NMR spectrum [spectrum 7.2] further supported the placement of the methoxy group at the C-5 position. If a hydroxy group was present at C-5, the C-4 resonance would have resonated further downfield as in compound 6. A hydroxy group was placed at the C-7 position because a bathochromic shift (+43 nm) was observed with NaOAc [spectrum 7.11].[6] The other methoxy group was then placed at the remaining C-6 position. One methoxy group showed an HMBC correlation to the C-5 resonance at δ_C 153.6 and the other to the C-6 resonance at δ_C 135.2 and this confirmed placement of the methoxy groups at the C-5 and C-6 positions.
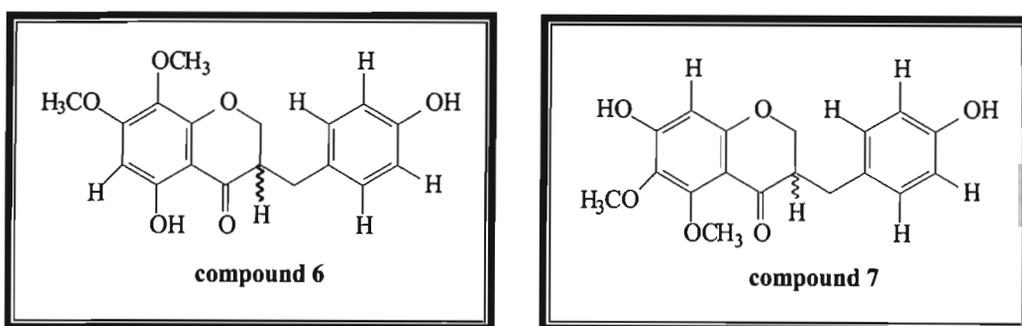
Figure 4.11: Comparison of structures of compound 6 and compound 7

The IR spectrum [spectrum 7.13] further supported the postulated structure of the homoisoflavonoid. Peaks were observed at 3368 cm^{-1} (O-H stretching), 2921 cm^{-1} (aliphatic C-H stretching), 1618 cm^{-1} (C=O stretching) and 1525 cm^{-1} (aromatic C=C stretching).

The ^{13}C NMR data of compound 7 was compared to the literature values of 7-*O*-methyl-3,9-dihydropunctatin and 7-*O*-methyl-3,9-dihydroeucomnalin (Table 4.17) and it was found that the values did not match either of the compounds.[4] Compound 7 was found to be the novel compound **7-hydroxy-3-(4'-hydroxybenzyl)-5,6-dimethoxy-4-chromanone**.

Table 4.16: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 7 (CDCl$_3$)

	^{1}H/ppm	^{13}C/ppm	HMBC C→H	NOESY	COSY
2	a-4.06 dd, 11.4, 6.8 b-4.23 dd, 11.4, 3.8	68.8	H-9b, H-3	H-9b, H-3 H-9b	H-2b H-2a
3	2.68 m	48.5	H-9a,b	H-9a, 2a	H-9a, H-2a,b
4		191.9	H-2a,b, H-8		
4a		108.6	H-8		
5		153.6	5-OCH$_3$		
6		135.2	6-OCH$_3$, H-8		
7		155.7	H-8		
8	6.29 s	98.9			
8a		159.8	H-2a,b, H-8		
9	a-2.63 dd, 12.8, 10.4 b-3.12 dd, 12.8, 3.7	32.0	H-2a,b, H-2'/6'	H-9b, H-3, H-2'/6' H-9a, H-2b, H-2'/6'	H-9b, H-3 H-9a,
1'		130.1	H-3, H-9a,b, H-2'/6', H-3'/5'		
2'/6'	7.06 d, 8.4	130.2	H-9a,b, H-3'/5'	H-9a,b, H-3, H-2b, H-3'/5'	H-3'/5'
3'/5'	6.76 d, 8.4	115.5	H-2'/6'	H-2'/6'	H-2'/6'
4'		154.5	H-2'/6', H-3'/5'		
5-OCH$_3$	3.89 s	61.4			
6-OCH$_3$	3.89 s	61.5			

Table 4.17: Comparison of ^{13}C NMR data for compound 7 with 7-*O*-methyl-3,9-dihydroeucomnalin and 7-*O*-methyl-3,9-dihydropunctatin[4]

	^{13}C data of compound 7 (CDCl$_3$)/ ppm	^{13}C data of compound 7 (CD$_3$OD)/ ppm	^{13}C data of 3,9-dihydroeucomnalin (CD$_3$OD)[4]/ ppm	^{13}C data of 3,9-dihydropunctatin (CD$_3$OD)[4]/ ppm
2	68.8	70.0	70.6	70.4
3	48.5	50.1	48.1	48.8
4	191.9	194.0	200.5	199.8
4a	108.6	108.9	103.7	103.3
5	153.6	155.9	156.2	161.3
6	135.2	137.7	131.4	93.8
7	155.7	159.6	162.3	162.5
8	98.9	100.5	92.6	130.3
8a	159.8	161.4	160.6	154.9
9	32.0	33.2	32.9	32.0
1'	130.1	130.4	130.1	129.7
2'/6'	130.2	131.2	131.2	131.0
3'/5'	115.5	116.4	116.5	116.4
4'	154.5	157.1	157.3	157.2
5-OCH$_3$	61.4	61.9		
6-OCH$_3$	61.5	61.6	61.1	
7-OCH$_3$			56.7	56.7
8-OCH$_3$				61.5

4.2.8. Structural elucidation of compound 8: 3,5,7-trihydroxy-3-(4'-hydroxy-benzyl)-4-chromanone (spectra 8.1-8.12, pp 225-235)

Compound **8** was isolated as a yellow crystalline solid with a melting point of 99-101°C.
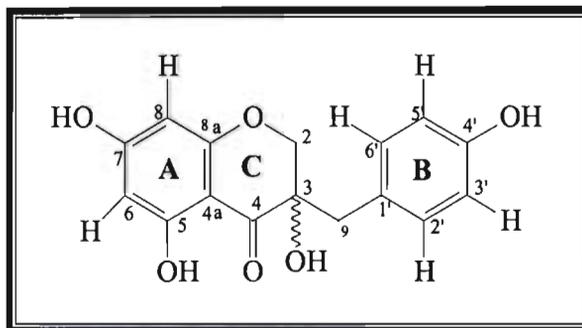
Figure 4.12: Structure of compound 8: 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone

The mass spectrum [**spectrum 8.11**] gave a molecular ion [M$^+$] peak at *m/z* 302. This corresponded to a molecular formula of $C_{16}H_{14}O_6$ from which a double bond equivalence of ten was deduced. UV absorption maxima were obtained at 291 nm (log ε 3.69) [**spectrum 8.8**].

Compound **8** was similar to compound **2** but differed in that it had a hydroxy group at C-3 rather than a hydrogen atom as in compound **2**. This was indicated by the lack of the H-3 resonance in the ^{1}H NMR spectrum [**spectrum 8.1**]. The ^{13}C NMR spectrum [**spectrum 8.3**] indicated an extra C-O signal at δ$_C$ 73.5 and this indicated that a hydroxy group was present at C-3.
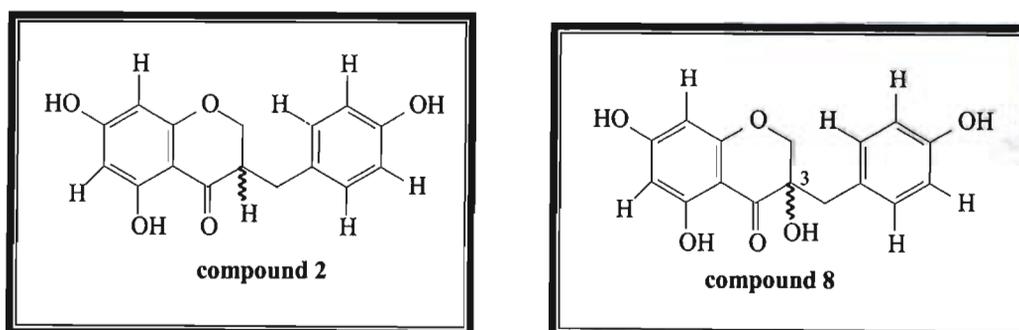
Figure 4.13: Comparison of structures of compound 2 and compound 8

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The ^{1}H NMR spectrum showed aliphatic resonances that were definitive for 3-benzyl-3-hydroxy-4-chromanone type homoisoflavonoids. The doublet resonances at δ_H 3.94 (J = 11.3 Hz) and δ_H 4.04 (J = 11.3 Hz) were attributed to the two non-equivalent H-2 protons. The resonance at δ_H 2.88 was attributed to the two non-equivalent 2H-9 protons. This resonance was typical of a non-first-order resonance. In general, first-order resonances only arise if the chemical shift difference between signals is much larger than the coupling constant. However, if signals from the coupling protons are closer together in the spectrum, and the chemical shift difference is small, distortion of the signals arise. As a result, the signals move together, the inner peaks become even larger at the expense of the outer peaks, and the position of the lines also change: the original chemical shift positions are no longer found at the midpoints of the doublets, but lie approximately at the 'center of gravity' of the doublets.[11]

The substitution pattern on the A and B rings were the same as in compound **2** and this was indicated by the ^{1}H NMR, mass and UV spectra. The ^{1}H NMR spectrum showed the *meta*-coupled protons: H-6 at δ_H 5.91 (d, J = 2.1 Hz) and H-8 at δ_H 5.89 (d, J = 2.1 Hz) of the A-ring. Hydroxy groups were placed at the C-5 and C-7 positions because bathochromic shifts were observed with $AlCl_3$ (+16 nm) and NaOAc (+37 nm) in the UV spectrum [**spectra 8.9** and **8.10**].[6] Placement of the hydroxy group at the C-5 position was confirmed by the downfield C-4 resonance at δ_C 200.0 while placement of the hydroxy at the C-7 position was confirmed by the appearance of the C-6 resonance at δ_C 97.4 and the C-8 resonance at δ_C 96.1.[4]

The mass spectrum showed peaks at m/z 195 and m/z 107 that resulted from an A-4 type fragmentation pattern. The peak at m/z 195 indicated that the chromanone fragment contained three hydroxy groups. A RDA and hydrogen-shift of the chromanone fragment resulted in the peak at m/z 153. This ion was primary evidence for a C-3 hydroxy substituent. If a third hydroxy substituent of the chromone fragment was positioned on the A ring, the RDA cleavage would have produced an ion at m/z 169.
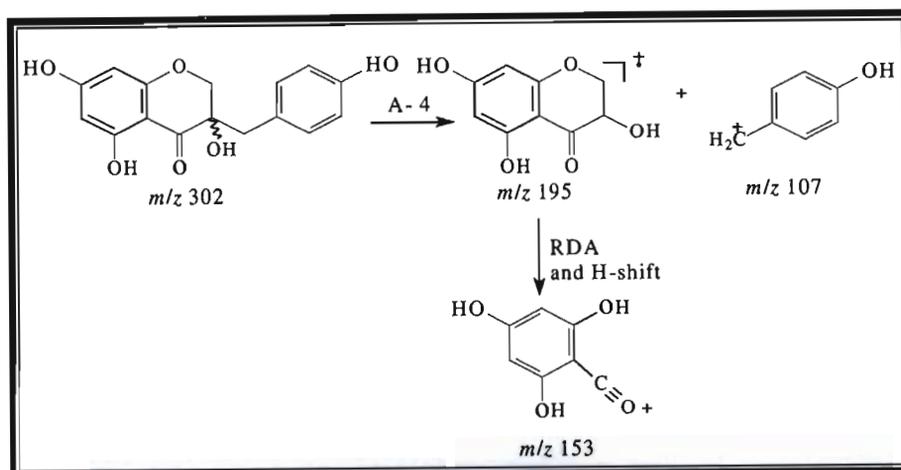
Scheme 4.6: Mass spectral fragmentation pattern for compound 8

A *para*-disubstituted B-ring was indicated from the H-2'/6' resonance at δ_H 7.05 (d, J = 8.5 Hz) and the H-3'/5' resonance at δ_H 6.70 (d, J = 8.5 Hz). The resonance at δ_C 157.5 showed HMBC correlations to the H-2'/6' and H-3'/5' protons in the HMBC spectrum **[spectrum 8.5]** and was assigned to C-4'. The mass spectrum indicated a hydroxytropylium peak at *m/z* 107 therefore a hydroxy group was placed at the C-4' position since the B ring was *para*-disubstituted.

The IR data **[spectrum 8.12]** obtained for this compound was consistent with the proposed structure. Peaks were observed at 3337 cm^{-1} (O-H stretching), 2915 cm^{-1} (aliphatic C-H stretching), 1646 cm^{-1} (C=O stretching) and 1521 cm^{-1} (aromatic C=C stretching).

Finally, a literature search for compound **8** revealed that it was **3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone.**[6] Compound **8** gave an optical rotation of +71° and differs from that isolated from *Eucomis bicolor* found in literature which gave an optical rotation of −90° and for which the hydroxy group at C-3 was shown as α.[6] A thorough literature search was then undertaken to determine how these researchers had proven the absolute stereochemistry. The literature reference referred to a PhD thesis by W. Schaad (1976) so an attempt was made to search the Chemical Abstracts to locate related publications. The publication found based on his PhD work, did not explain how the absolute stereochemistry had been determined.[8] On the basis of this lack of evidence relating to the assignment of the absolute stereochemistry at C-3 of 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone in the paper referenced[8], the absolute stereochemistry would is in doubt. The absolute configuration of a

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compound can only be proven by using the technique of circular dichroism and no mention is made of this in the publication. Thus from the optical rotations it would seem as though the compounds isolated from the two sources, *Eucomis montana* and *Eucomis bicolor*, have opposite stereochemistry at C-3. However, *Eucomis montana* and *Eucomis bicolor* belong to the same genus and one would have expected them to have the same absolute configuration at C-3.

Table 4.18: ^{1}H, ^{13}C, HMBC, NOESY and COSY data for compound 8 (CD$_3$OD)

	^{1}H/ ppm	^{13}C / ppm	HMBC C→H	NOESY	COSY
2	a-3.94 d, 11.3 b-4.04 d, 11.3	72.3	H-9	H-2b H-2'/6', H-2a	
3		73.5	H-9, H-2a,b		
4		200.0	H-9, H-2a,b		
4a		101.4	H-6, H-8		
5		165.8	H-6		
6	5.91 d, 2.1	97.4	H-8		H-8
7		168.6	H-8		
8	5.89 d, 2.1	96.1	H-6		H-6
8a		164.4	H-2a,b, H-8		
9	2.88*	40.7	H-2a,b, H-2'/6'	H-2'/6'	
1'		126.9	H-9, H-3'/5'		
2'/6'	7.05 d, 8.5	132.8	H-9	H-9, H-3'/5'	H-3'/5'
3'/5'	6.70 d, 8.5	115.9		H-2'/6'	H-2'/6'
4'		157.5	H-2'/6', H-3'/5'		

* The two H-9 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

Table 4.19: ^{1}H NMR data for compound 8 and 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone[6]

	^{1}H NMR data for compound 8 (CD$_3$OD)/ ppm	^{1}H NMR data for compound 8 ((CD$_3$)$_2$CO)/ ppm	^{1}H NMR data for 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone ((CD$_3$)$_2$CO)[6]/ ppm
2	a-3.94 d, 11.3 b-4.04 d, 11.3	a-4.05 d, 11.3 b-4.13 d, 11.3	a-4.04 d, 11.3 b-4.11 d, 11.3
6	5.91 d, 2.1	6.01 d, 2.2	5.99 d, 2.3
8	5.89 d, 2.1	6.00 d, 2.2	5.97 d, 2.3
9	2.88*	2.94*	2.93 s
2'/6'	7.05 d, 8.5	7.14 d, 8.5	7.12 d, 8.8
3'/5'	6.70 d, 8.5	6.79 d, 8.5	6.77 d, 8.8
5-OH		11.79 s	11.75 s

* The two H-9 protons resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

4.2.9. Structural elucidation of compound 9: eucomol (spectra 9.1-9.12, pp 236-246)

This compound was isolated as a yellow crystalline solid with a melting point of 128-130°C.
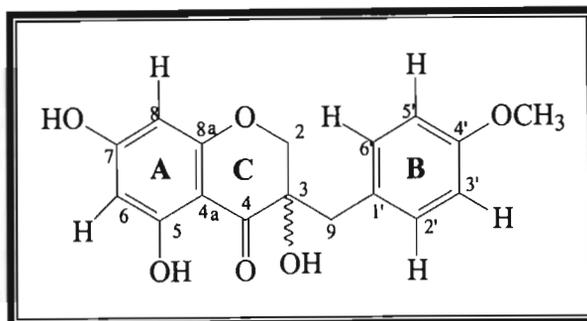
Figure 4.14: Structure of compound 9: eucomol

The mass spectrum [spectrum 9.11] gave a low intensity molecular ion $[M^+]$ peak at m/z 316 that corresponded to a molecular formula of $C_{17}H_{16}O_6$. From this a double bond equivalence of ten was deduced. UV absorption maxima [spectrum 9.8] were obtained at 293 nm (log ε 4.15) and 235 nm (log ε 4.59).

The splitting pattern shown in the ^1H NMR spectrum [spectrum 9.1] for the 2H-2 and 2H-9 protons were typical of 3-benzyl-3-hydroxy-4-chromanone type homoisoflavonoids. The doublet resonances at δ_H 4.17 ($J = 11.1$ Hz) and δ_H 4.03 ($J = 11.1$ Hz) assigned to 2H-2 and the resonance at δ_H 2.93 assigned to 2H-9 were similar to those of compound **8**. Furthermore an oxygenated C-3 resonance was indicated from the appearance of a peak at δ_C 72.2 in the ^{13}C NMR spectrum [spectrum 9.2] that showed HMBC correlations to the 2H-2 and 2H-9 proton resonances in the HMBC spectrum [spectrum 9.5].

The ^1H NMR, UV and mass spectra showed that the substitution pattern of the A and B rings were the same as in compound **3**. The pair of doublet resonances at δ_H 6.02 ($J = 2.2$ Hz) and δ_H 5.97 ($J = 2.2$ Hz) in the ^1H NMR spectrum were assigned to H-6 and H-8 respectively and this indicated substituents at C-5 and C-7 on the A ring. The UV spectra [spectra 9.9 and 9.10] showed bathochromic shifts with $AlCl_3$ (+15 nm) and NaOAc (+34 nm) and this allowed placement of hydroxy groups at the C-5 and C-7

positions.[6] The C-4 carbonyl resonance observed at δ_C 198.1 in the ^{13}C NMR spectrum confirmed the hydroxy group at the C-5 position whereas the C-6 resonance at δ_C 97.1 and the C-8 resonance at δ_C 95.8 confirmed the hydroxy group at the C-7 position.[4]
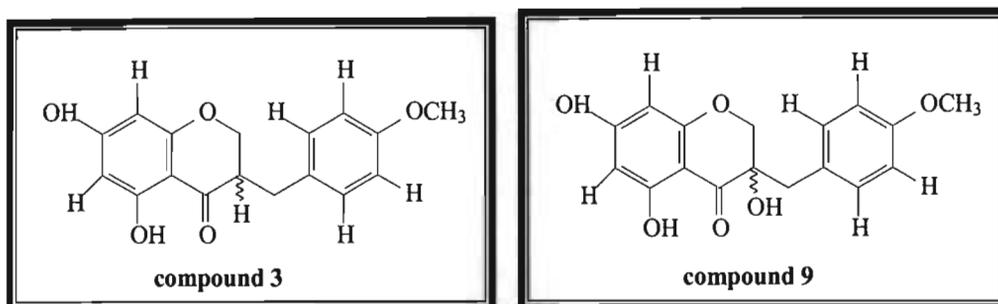
Figure 4.15: Comparison of structures of compound 3 and compound 9

The A-4 fragmentation pattern produced peaks at m/z 195, 153 and 121 as shown in the mass spectrum. The peak at m/z 153 was due to a RDA and hydrogen-shift of the chromanone fragment and indicated that the A ring contained two hydroxy groups. The A-4 fragmentation ion at m/z 195 indicated that the third hydroxy group belonged to C-3 of the chromanone fragment. The methoxytropylium ion at m/z 121 indicated that the B ring contained a methoxy group.
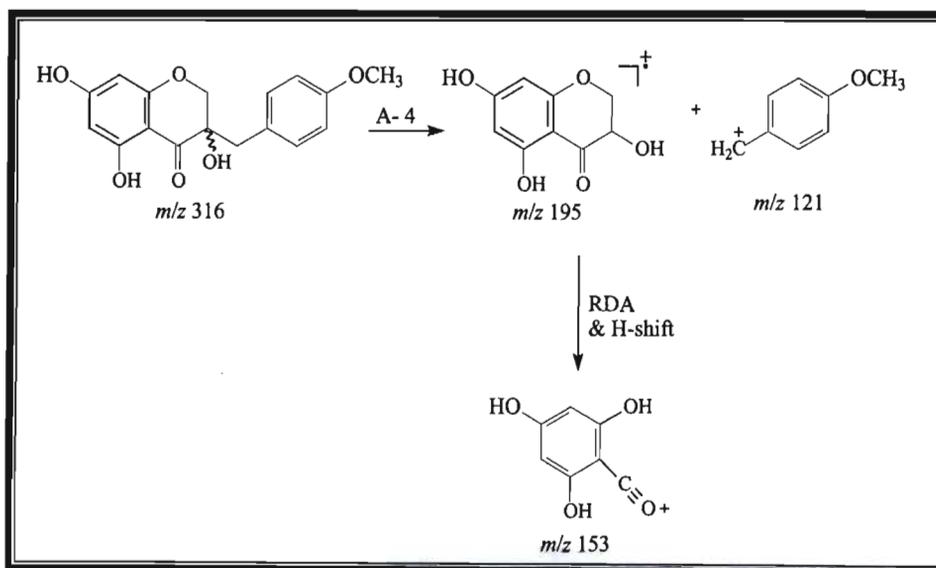
Scheme 4.7: Mass spectral fragmentation pattern for compound 9

A *para*-disubstituted B ring was indicated by the pair of doublets integrating to two protons each at δ_H 7.10 (J = 8.8 Hz) and δ_H 6.83 (J = 8.8 Hz) assigned to H-2'/6' and H-3'/5' respectively. The methoxy group resonance at δ_H 3.77 showed a positive NOESY correlation [spectrum 9.7] to the H-3'/5' doublet resonance and was thus assigned to the C-4' position.

The IR spectrum [spectrum 9.12] showed peaks that agreed with the proposed structure of compound 9. The peak at 3408 cm^{-1} indicated O-H stretching and the peak at 1638 cm^{-1} indicated C=O stretching. Peaks were also observed for aliphatic C-H stretching at 2922 cm^{-1} and for the aromatic C=C stretching at 1513 cm^{-1}.

Finally, a literature search for compound 9 was undertaken and it was found that the ^{1}H NMR data (Table 4.21) matched that of the known compound eucomol (or 3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone), previously isolated from *Eucomis bicolor* and *Scilla dracomontana*.[8,12]

Table 4.20: ^{1}H, ^{13}C, HMBC, NOESY and COSY data for compound 9 (CDCl$_3$)

	^{1}H /ppm	^{13}C /ppm	HMBC C→H	COSY	NOESY
2	a-4.17 d, 11.1 b-4.03 d, 11.1	71.7	2H-9	H-2b 2H-9	H-2b, 2H-9, H-2'/6' H-2a
3		72.2	H-2a,b, 2H-9		
4		198.1	2H-9, H-2a,b		
4a		100.4	H-8, H-6		
5	11.28 s	164.1	H-6		
6	6.02 d, 2.2	97.1	H-8		
7		165.8	H-8, H-6		
8	5.97 d, 2.2	95.8	H-6		
8a		163.0	H-2a,b, H-8		
9	2.93*	40.6	H-2a,b, H-2'/6'	H-2b	H-2'/6'
1'		126.0	H-3'/5', 2H-9		
2'/6'	7.10 d, 8.8	131.6	H-3'/5', 2H-9	H-3'/5'	2H-9, H-2a, H-3'/5'
3'/5'	6.83 d, 8.8	113.8	H-2'/6'	H-2'/6'	H-2'/6', 4'-OCH$_3$
4'		158.7	H-3'/5', H-2'/6'		
4'-OCH$_3$	3.77 s	55.3			H-3'/5'

* The two H-9 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

Table 4.21: Comparison of ^{1}H NMR data for compound 9 and eucomol[6,8]

	^{1}H NMR data of compound 9 (CDCl$_3$)/ ppm	^{1}H NMR data from literature (CDCl$_3$)[6,8]/ ppm
2	a-4.17 d, 11.1 b-4.03 d, 11.1	a-4.21 d, 11.2 b-4.06 d, 11.2
5-OH	11.28 bs	11.26 s
6	6.02 d, 2.2	6.03 d, 2.3
8	5.97 d, 2.2	5.99 d, 2.3
9	2.93*	2.95 s
2'/6'	7.10 d, 8.8	7.11 d, 8.8
3'/5'	6.83 d, 8.8	6.87 d, 8.8
4'-OCH$_3$	3.77 s	3.80 s

* The two H-9 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

4.2.10. Structural elucidation of compound 10: 7-*O*-methyleucomol (spectra 10-10.12, pp 247-258)

This compound was isolated as a cream crystalline solid with a melting point of 78-81°C.
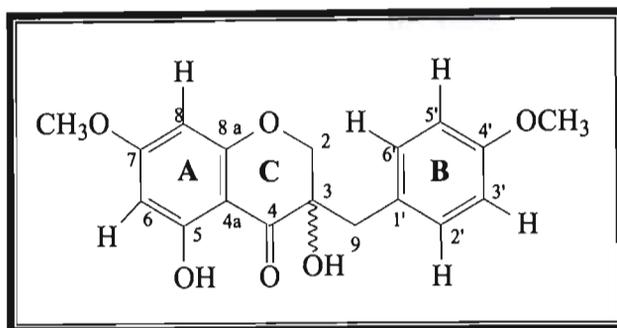
Figure 4.16: Structure of compound 10: 7-*O*-methyleucomol

The mass spectrum [**spectrum 10.11**] gave a low intensity molecular ion [M$^+$] peak at *m/z* 330. This corresponded to a molecular formula of $C_{18}H_{18}O_6$ from which a double bond equivalence of ten was deduced. UV absorption maxima were obtained at 293 nm (log ε 3.42) and 232 nm (log ε 3.50) [**spectrum 10.8**].

The ^{1}H NMR spectrum [**spectrum 10.1**] showed resonances in the aliphatic region that were typical of 3-benzyl-3-hydroxy-4-chromanone type homoisoflavonoids. These were the doublet resonances at δ_H 4.04 (*J* = 11.2 Hz) and δ_H 4.19 (*J* = 11.2 Hz) attributed to 2H-2 and the resonance at δ_H 2.93, integrating to two protons, attributed to 2H-9.

The *meta*-coupled protons H-6 and H-8 were indicated from the pair of doublets at δ_H 6.10 (*J* = 2.2 Hz) and δ_H 6.03 (*J* = 2.2 Hz) integrating to one proton each in the ^{1}H NMR spectrum. The HMBC correlation [**spectrum 10.5**] observed between the C-8a resonance and the doublet at δ_H 6.03 allowed assignment of H-8. The remaining doublet was then assigned to H-6. The two substituents that were assigned to the C-5 and C-7 position were determined with the aid of the UV and mass spectrum. The UV spectrum [**spectrum 10.9**] showed a bathochromic shift of +18 nm with AlCl$_3$. This indicated that a hydroxy group was present at the C-5 position.[6] The C-4 carbonyl resonance was observed at δ_C 198.3 in the ^{13}C NMR spectrum [**spectrum 10.2**] and

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this confirmed placement of the C-5 hydroxy group. No shift was observed with NaOAc. The methoxy group proton resonance at δ_H 3.82 was placed in the C-7 position as this resonance showed NOESY correlations [spectrum 10.7] to the H-6 and H-8 resonances. Both the A ring doublet resonances were deshielded (>6.00 ppm) which further supported this assignment of the methoxy group at the C-7 position.[4]

A *para*-dibsubstituted B ring was indicated from the pair of doublet resonances at δ_H 7.10 (J = 8.6 Hz) and δ_H 6.83 (J = 8.6 Hz) assigned to H-2'/6' and H-3'/5' respectively. The methoxy group proton resonance at δ_H 3.78 was placed at the C-4' position because it showed a positive NOESY correlation to the H-3'/5' protons.

The mass spectrum confirmed the substitution pattern on the A and B rings. The A-4 fragmentation pattern resulted in fragment ions at m/z 209 and m/z 121. The peak at m/z 209 indicated the presence of two hydroxy groups and one methoxy group on the chromanone fragment. The chromanone fragment upon a RDA and hydrogen-shift resulted in the peak at m/z 167 which confirmed the placement of the hydroxy and methoxy substituents on the A ring and the C-3 hydroxy substituent. The methoxytropylium ion, indicated by the fragment ion at m/z 121, confirmed the substitution pattern on the B ring (Scheme 4.8).
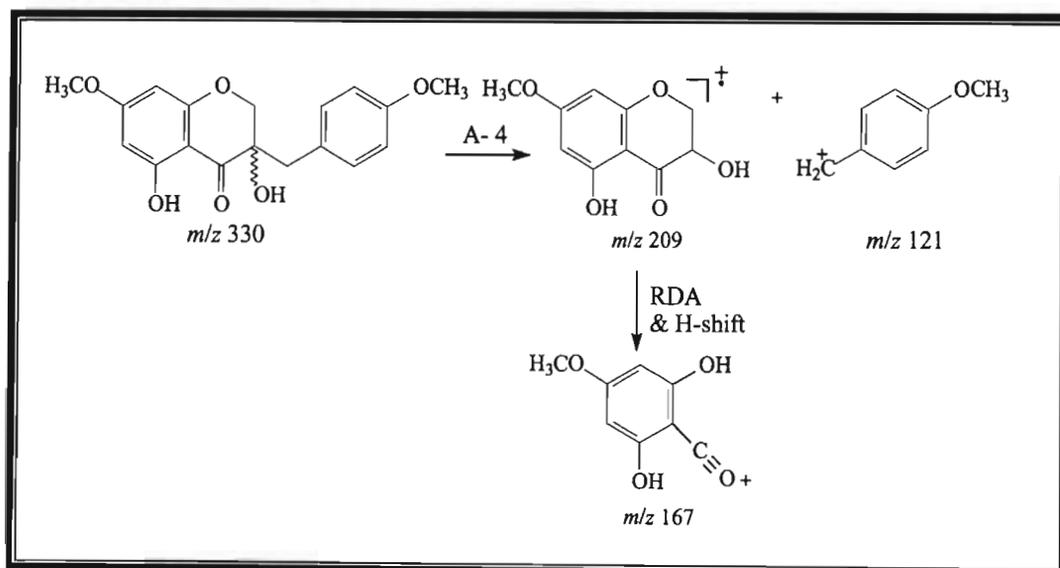
Scheme 4.8: Mass spectral fragmentation pattern for compound 10

The IR spectrum [spectrum 10.12] further supported the proposed structure. Peaks were observed at 3381 cm^{-1} (O-H stretching), 2921 cm^{-1} (aliphatic C-H stretching), 1651 cm^{-1} (C=O stretching) and 1512 cm^{-1} (aromatic C=C stretching).

Finally, a literature search for compound **10** was undertaken and it was found that the ^{1}H NMR data matched that of the known compound, **7-*O*-methyleucomol** (or **3,5-dihydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone**) that has been isolated previously from *Eucomis bicolor* and *Ornithogalum longibracteatum*.[8,13]

Table 4.22: ^{1}H, ^{13}C, HMBC, NOESY and COSY data for compound 10 (CDCl$_3$)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	NOESY	COSY
2	a-4.04 d, 11.2 b-4.19 d, 11.2	71.8	2H-9	H-2b H-2a, 2H-9,H-2'/6'	H-2b H-2a
3		72.2	H-2a,b		
4		198.3	H-2a,b, 2H-9		
4a		100.5	H-6, H-8		
5-OH	11.23 s	164.0	H-6		
6	6.10 d, 2.2	95.4	H-8	7-OCH$_3$	
7		168.6	H-6, H-8, 7-OCH$_3$		
8	6.03 d, 2.2	94.5	H-6	7-OCH$_3$	
8a		162.7	H-2b, H-8		
9	2.93*	40.7	H-2'/6', H-2a,b	H-2'/6', H-2b	
1'		126.1	H-3'/5', 2H-9		
2'/6'	7.10 d, 8.6	131.5	H-3'/5', 2H-9	2H-9, H-2b, H-3'/5'	H-3'/5'
3'/5'	6.83 d, 8.6	113.7	H-2'/6'	4'-OCH$_3$, H-2'/6'	H-2'/6'
4'		158.8	4'-OCH$_3$, H-2'/6', H-3'/5'		
4'-OCH$_3$	3.78 s	55.2		H-3'/5'	H-3'/5'
7-OCH$_3$	3.82 s	55.8		H-6, H-8	

* The 2H-9 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

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Table 4.23: Comparison of ^{1}H NMR data for compound 10 with 7-*O*-methyl-eucomol [8]

	^{1}H NMR of compound 10 (CDCl$_3$)/ ppm	^{1}H NMR data from literature (CDCl$_3$)[8]/ ppm
2	4.04 d, 11.2	4.06 d, 11.2
	4.19 d, 11.2	4.21 d, 11.2
5-OH	11.23 s	11.24 s
6	6.10 d, 2.2	6.12 d, 2.3
8	6.03 d, 2.2	6.05 d, 2.3
9	2.93 *	2.95 s
2'/6'	7.10 d, 8.6	7.12 d, 8.8
3'/5'	6.83 d, 8.6	6.85 d, 8.8
4'-OCH$_3$	3.78 s	3.80 s
7-OCH$_3$	3.82 s	3.85 s

* The 2H-9 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

4.2.11. Structural elucidation of compound 11: (*E*)-eucomin (spectra 11.1- 11.13, pp 259-270)

This compound was isolated as a yellow crystalline solid with a melting point of 206-208°C.
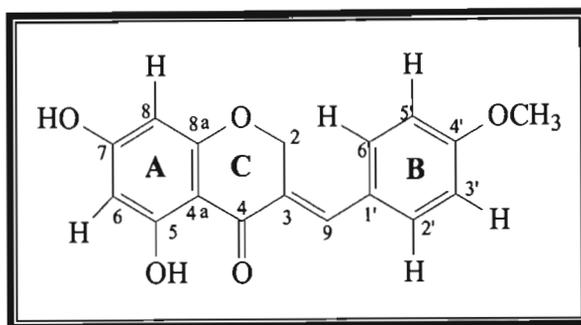
Figure 4.17: Structure of compound 11: (*E*)-eucomin

The mass spectrum [**spectrum 11.12**] gave a molecular ion [M$^+$] peak at *m/z* 298. This corresponded to a molecular formula of $C_{17}H_{14}O_5$ from which a double bond equivalence of eleven was deduced. UV absorption maxima were obtained at 357 nm (log ε 3.44) and 232 nm (log ε 4.27) [**spectrum 11.9**].

The ^{1}H NMR spectrum [**spectrum 11.1**] for compound **11** showed resonances that were typical of a 3-benzylidenyl-4-chromanone type homoisoflavonoid. This was indicated from the doublet resonance at δ_H 5.34 (*J* = 1.7 Hz) attributed to 2H-2 and the broad singlet at δ_H 7.77 attributed to the H-9 resonance. Furthermore a characteristic quaternary carbon resonance in the double bond region for C-3 at δ_C 128.8 as well as a resonance corresponding to a α,β-unsaturated carbonyl group carbon at δ_H 186.3 was evident in the ^{13}C NMR spectrum [**spectrum 11.3**]. The upfield C-4 carbonyl group resonance is due to the conjugative effect of the 3,9-double bond.[6]

Substitution on the A and B rings was the same as compound **3**. The ^{1}H NMR spectrum showed a pair of doublets at δ_H 5.96 (*J* = 2.2 Hz) and δ_H 5.90 (*J* = 2.2 Hz) attributed to the H-6 and H-8 protons. The UV spectrum [**spectra 11.10** and **11.11**] indicated hydroxy groups at the C-5 and C-7 positions because bathochromic shifts were observed with AlCl₃ (+39 nm) and NaOAc (+20 nm).[6] The mass spectrum

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showed a peak at *m/z* 153 due to a RDA cleavage and H-shift and this confirmed the two hydroxy groups on the A ring.
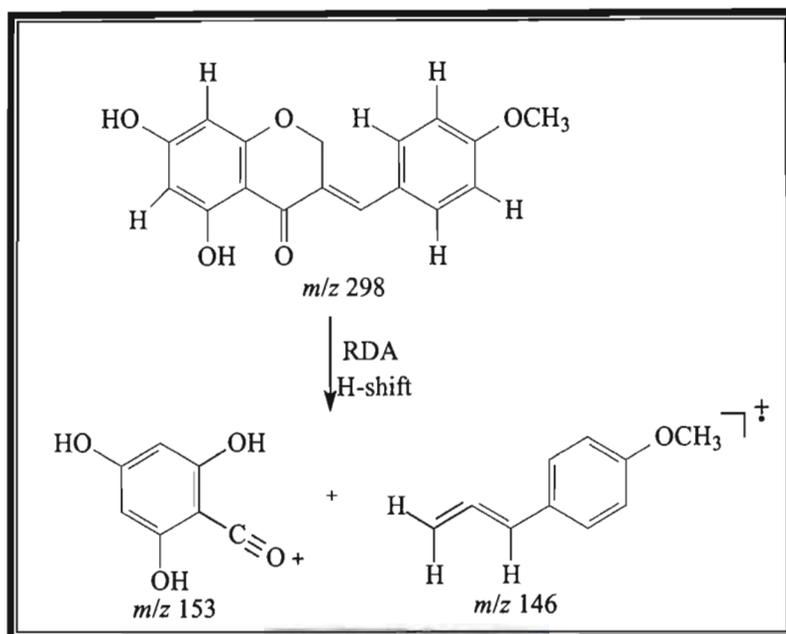
Scheme 4.9: Mass spectral fragmentation pattern for compound 11[6]

A *para*-disubstituted B ring was indicated from the doublet resonances at δ_H 7.36 (*J* = 8.8 Hz) and δ_H 7.06 (*J* = 8.8 Hz) attributed to the H-2'/6' and H-3'/5' protons respectively. The fragment ion at *m/z* 146 indicated that the methoxy group was on the B ring, where it was placed on the C-4' position because it showed a NOESY correlation **[spectrum 11.8]** to the H-3'/5' protons.

To determine the configuration of the 3,9-double bond, molecular models were constructed for both geometric isomers (*E* and *Z*). The model for the (*E*)-isomer showed that only the H-2'/6' protons were close to H-9 and therefore positive NOESY correlations should be observed for these protons in the NOESY spectrum. The model for the (*Z*)-isomer showed that the H-9 proton was close to the 2H-2 and H-2'/6' protons and therefore positive NOESY correlations should be obtained for these protons.

A positive NOESY correlation was seen for the H-9 proton with the H-2'/6' protons only and this suggested the 3,9-double bond being in the (*E*)-configuration. Also, the 2H-2 protons showed a correlation to the H-2'/6' protons only and the H-2'/6' protons

showed positive NOESY correlations to the 2H-2, H-9 and the H-3'/5' protons. This provided evidence that the geometry was indeed (*E*) as these results would not be expected for the (*Z*)-configuration. NOESY correlations of H-2'/6' to the mentioned protons can be attributed to free rotation across the C-9, C-1' bond. Further support of the (*E*)-configuration was the H-9 proton at δ_H 7.77 due to the presence of this resonance in the anisotropic region of the carbonyl group.[6]
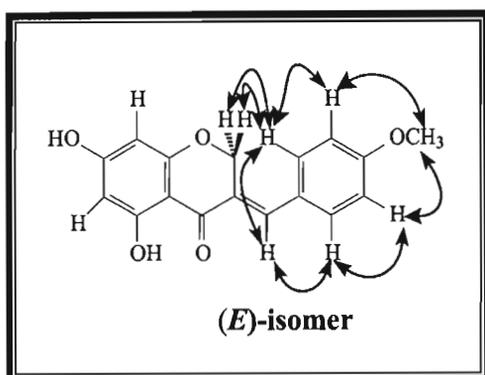
Figure 4.18: Diagram showing NOESY correlations for the (*E*)-isomer
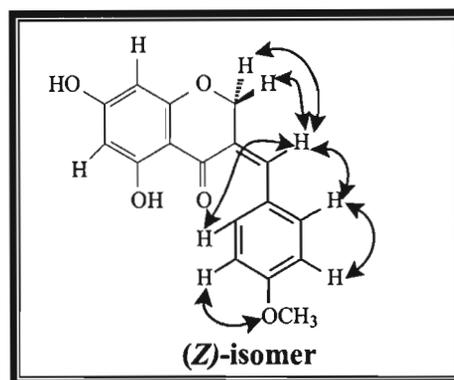
Figure 4.19: Diagram showing NOESY correlations for the (*Z*)-isomer

The IR data **[spectrum 11.13]** obtained for compound **11** supported the proposed structure. Peaks were observed at 3374 cm^{-1} (O-H stretching), 2926 cm^{-1} (aliphatic C-H stretching), 1646 cm^{-1} (C=O stretching) and 1515 cm^{-1} (aromatic C=C stretching).

Finally, a literature search for compound **11** showed that the ^{1}H and ^{13}C NMR data **(Tables 4.25** and **4.26)** matched those of the known compound **(*E*)-eucomin** (or **(*E*)-5,7-dihydroxy-3-(4'-methoxybenzylidenyl)-4-chromanone**), isolated previously from *Eucomis bicolor*.[6,8]

Table 4.24: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 11 (CD$_3$OD)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
2	5.34 d, 1.7	68.4	H-9		H-2'/6'
3		128.8	2H-2		
4		186.3	2H-2, H-9		
4a		103.8	H-6, H-8		
5		165.9			
6	5.96 d, 2.2	97.4	H-8		
7		168.2	H-8		
8	5.90 d, 2.2	96.1	H-6		
8a		163.7	2H-2, H-8		
9	7.77 bs	137.8	2H-2, H-2'/6'	2H-2	H-2'/6'
1'		127.8	H-3'/5'		
2'/6'	7.36 d, 8.8	133.2	H-9	H-3'/5'	2H-2, H-3'/5', H-9
3'/5'	7.06 d, 8.8	115.3		H-2'/6'	H-2'/6', 4'-OCH$_3$
4'		162.3	4'-OCH$_3$, H-3'/5', H-2'/6'		
4'-OCH$_3$	3.89 s	56.0			H-3'/5'

Table 4.25: Comparison of ^{1}H NMR data for compound 11 with the literature[6,8]

	^{1}H NMR data of compound 11 (CD$_3$OD)/ ppm	^{1}H NMR data of compound 11(CDCl$_3$)/ ppm	^{1}H NMR data of (Z)-eucomin (CDCl$_3$)[6,8]/ ppm	^{1}H NMR data of (E)-eucomin (CDCl$_3$)[6,8]/ ppm
2	5.34 d, 1.7	5.27 d, 1.8	4.91 s	5.31 d, 1.7
6	5.96 d, 2.2	5.97 d, 2.2	5.93 d, 2.3	5.90 d, 2.3
8	5.90 d, 2.2	5.87 d, 2.2	5.99 d, 2.3	6.00 d, 2.3
9	7.77 bs	7.77 bs	6.87 s	7.80 t, 1.7
2'/6'	7.36 d, 8.7	7.24 d, 8.8	7.81 d, 8.8	7.27 d, 8.8
3'/5'	7.06 d, 8.7	6.94 d, 8.8	6.90 d, 8.8	6.96 d, 8.8
4'-OCH$_3$	3.89 s	3.84 s	3.85 s	3.87 s

Table 4.26: Comparison of ^{13}C NMR data for compound 11 with the literature[6]

	^{13}C data of compound 11 (CD$_3$OD)/ ppm	^{13}C data of compound 11 ((CD$_3$)$_2$SO)/ ppm	^{13}C data of (Z)-eucomin ((CD$_3$)$_2$SO)[6]/ ppm	^{13}C data of (E)-eucomin ((CD$_3$)$_2$SO)[6]/ ppm
2	68.4	67.3	74.1	67.1
3	128.8	127.4	125.5	127.1
4	186.3	184.0	186.6	184.1
4a	103.8	102.0	103.2	101.6
5	165.9	164.7	164.8	164.5
6	97.4	96.4	96.2	96.2
7	168.2	167.1	166.7	166.9
8	96.1	95.0	94.7	94.9
8a	163.7	162.1	162.5	161.9
9	137.8	136.4	140.5	136.0
1'	127.8	126.4	126.6	126.2
2'/6'	133.2	132.7	133.1	132.4
3'/5'	115.3	114.6	113.4	114.3
4'	162.3	160.8	160.6	160.6
4-OCH$_3$	56.0	55.6	55.2	55.2

4.2.12. Structural elucidation of compound 12: 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one (spectra 12.1-12.12, pp 271-281)

This compound was isolated as a cream coloured crystalline solid with a melting point of 215-217 °C.
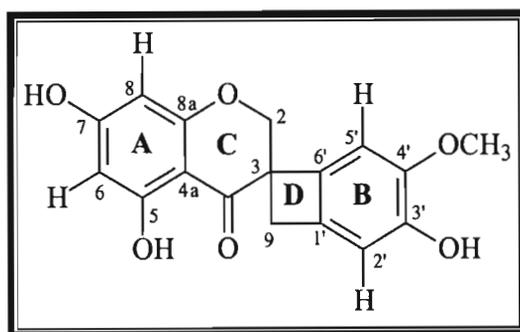
Figure 4.20: Structure of compound 12: 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one

The mass spectrum [spectrum 12.11] gave a molecular ion [M$^+$] peak at *m/z* 314. This corresponded to a molecular formula of $C_{17}H_{14}O_6$. A double bond equivalence of eleven was deduced which implied presence of either another double bond or an additional ring compared to the usual A, B, C rings and carbonyl group found in the previous homoisoflavonoids. UV absorption maxima were obtained at 289 nm (log ε 4.16) and 231 nm (log ε 4.15) [spectrum 12.8].

The ^{1}H NMR spectrum [spectrum 12.1] showed a splitting pattern for the methylene protons of 2H-2 and 2H-9 that are typical of scillascillin type compounds. This was indicated from the appearance of non-first order resonances at $δ_H$ 4.51 and $δ_H$ 4.53 attributed to 2H-2, the pair of doublets at $δ_H$ 2.97 (*J* = 13.4 Hz) and $δ_H$ 3.49 (*J* = 13.4 Hz) attributed to 2H-9 and the absence of the H-3 proton resonance. The fully substituted C-3 carbon resonance occurred at $δ_C$ 55.3 in the ^{13}C NMR spectrum [spectrum 12.2].

The ^{1}H NMR spectrum showed the presence of a methoxy group at $δ_H$ 3.77. The resonances at $δ_H$ 5.92 (d, *J* = 2.2 Hz) and $δ_H$ 5.90 (d, *J* = 2.2 Hz) indicated a *meta*-coupled proton system on the A ring and were assigned to the H-8 and H-6 protons

respectively. The UV spectra showed bathochromic shifts with $AlCl_3$ (+17 nm) and NaOAc (+36 nm) [spectra 12.9 and 12.10] and this indicated hydroxy groups at the C-5 and C-7 positions.[14] The presence of a hydroxy group at C-5 was confirmed by the downfield resonance of the C-4 carbonyl group at δ_C 198.0 in the ^{13}C NMR spectrum. Placement of the hydroxy group at the C-7 position was confirmed by the chemical shifts of the H-6 and H-8 protons which occur upfield of 6.00 ppm in the ^{1}H NMR spectrum.[4]
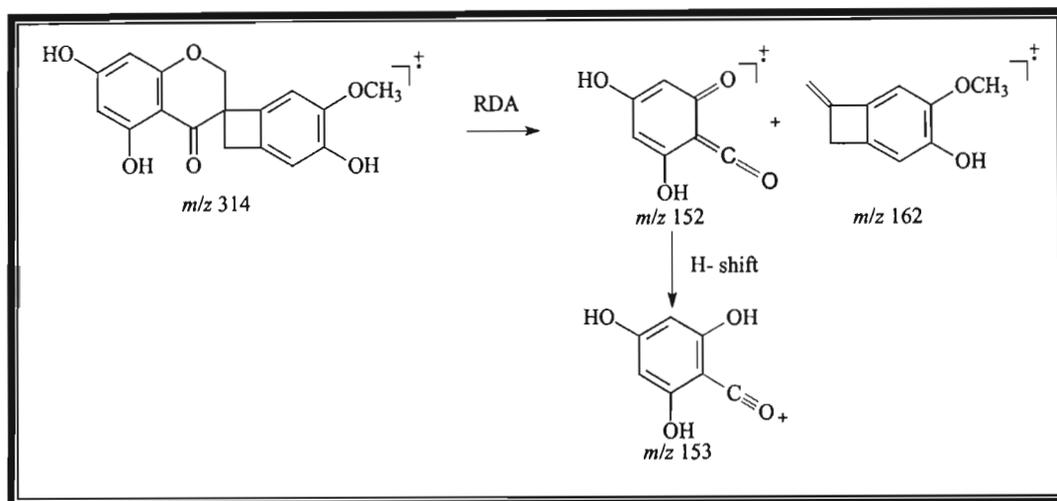
Scheme 4.10: Mass spectral fragmentation pattern for compound 12[14]

The pair of doublet resonances at δ_H 6.68 and δ_H 6.65 (J = < 1 Hz) indicated protons *para* to each other and this implied a 1',3',4',6'- tetrasubstituted B ring. The mass spectrum showed a peak at *m/z* 162 which indicated one methoxy and one hydroxy group on the B ring. Since the B ring was tetrasubstituted with two protons *para* to each other, the methoxy group had to be placed at either at the C-3' or C-4' position. This was determined by the NOESY spectrum [spectrum 12.7]. A NOESY correlation was seen between the H-2 proton and the signal at δ_H 6.65. This confirmed the signal at δ_H 6.65 to be H-5' and implied that the proton *para* to it was the H-2' proton at δ_H 6.68. The methoxy group proton resonance at δ_H 3.77 showed a NOESY correlation with the H-5' proton. This indicated the presence of a methoxy group at the C-4' position and the C-3' position was occupied by the hydroxy group. Benzylic coupling between the 2H-9 protons and the signal at δ_H 6.68 was seen in the COSY spectrum [spectrum 12.6] and this confirmed that the resonance belonged to H-2'.

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The IR spectrum [**spectrum 12.12**] showed peaks at 3381 cm^{-1} (O-H stretching), 2930 cm^{-1} (aliphatic C-H stretching), 1644 cm^{-1} (C-O stretching) and 1476 cm^{-1} (aromatic C=C stretching). These were consistent with that of the proposed structure.

Assignment of ^{13}C NMR chemical shifts was done using the HMBC spectrum [**spectrum 12.5**] and comparison with the literature (**Table 4.29**).[15] The NMR data of compound **12** was compared to that of 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa[1,3,5]-trien]-4-one (COMPOUND A) which had a methoxy group at the C-4' position and muscomosin (COMPOUND B) which had the methoxy group at the C-3' position. From this comparison it was found that the ^{1}H and ^{13}C NMR data of compound **12 (Table 4.28** and **4.29)** agreed more closely with that of **3',5,7- trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa[1,3,5]-triene-4-one**, confirming what was expected from NOESY correlations. This compound was previously isolated from *Muscari armeniacum*, *Muscari botryoides* and *Drimiopsis maculata*.[15,16]
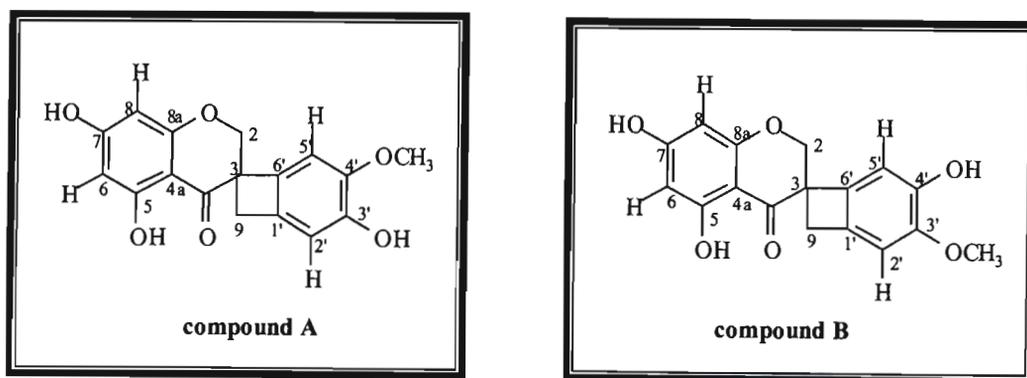
Figure 4.21: Structures of compounds A and B

Table 4.27: ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound 12 (CD$_3$OD)

	^{1}H/ ppm	^{13}C/ ppm	HMBC C→H	COSY	NOESY
2	a-4.51 d* b-4.53 d*	74.9	H-9a		H-9a H-5'
3		55.3	H-9a,b, H-2a,b, H-5'		
4		198.0	H-9b, H-2a,b		
4a		102.5	H-8		
5		165.9	H-6		
6	5.90 d, 2.2	97.2	H-8		
7		168.4			
8	5.92 d, 2.2	96.0	H-6		
8a		165.0	H-2a,b, H-8		
9	a-2.97 d, 13.4 b-3.49 d, 13.4	35.9	H-2a,b, H-2'	H-9b, H-2' H-9a, H-2'	H-9b, H-2', H-2 H-9a, H-2'
1'		135.5	H-9a,b, H-2a,b, H-5', H-2'		
2'	6.68	112.0		H-9a,b	H-9a, b
3'		149.6	H-5', H-2'		
4'		149.6	4'-OCH$_3$, H-5', H-2'		
5'	6.65	107.1			4'-OCH$_3$, H-2b
6'		135.8	H-9a,b, H-2a,b, H-2'		
4'-OCH$_3$	3.77 s	56.8			H-5'

* The 2H2 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

Table 4.28: Comparison of ^{1}H NMR data for compound 12 with the literature [15]

	^{1}H NMR data of compound 12 (CD$_3$OD)/ ppm	^{1}H NMR data of compound A from literature (CD$_3$OD)[15]/ ppm	^{1}H NMR data of compound B from literature (CD$_3$OD)[15]/ ppm
2	a-4.51* b-4.53*	4.51 d, 9.0 4.53 d, 9.0	4.50 s
6	5.90 d, 2.2	5.93 d, 2.2	5.91 d, 1.8
8	5.92 d, 2.2	5.90 d, 2.2	5.89 d, 1.8
9	a-2.97 d, 13.4 b-3.49 d, 13.4	2.97 d, 13.2 3.49 d, 13.2	2.98 d, 13.2 3.51 d, 13.2
2'	6.68 s	6.68 s	6.82 s
4'-OCH$_3$	3.77 s	3.77 s	
3'-OCH$_3$			3.83 s
5'	6.65 s	6.65 s	6.51s

* The 2H-2 proton resonances are superimposed in the ^{1}H NMR spectrum (non-first order resonances)

Table 4.29: Comparison of ^{13}C NMR data for compound 12 with literature[15]

	^{13}C data for compound 12 (CD$_3$OD)/ ppm	^{13}C NMR data of compound A from literature (CD$_3$OD)[15]/ ppm	^{13}C NMR data of compound B from literature (CD$_3$OD)[15]/ ppm
2	74.9	74.9	74.7
3	55.3	55.3	55.2
4	198.0	198.4	197.9
4a	102.5	102.6	102.4
5	165.9	165.9	165.8
6	97.2	97.3	97.3
7	168.4	168.4	168.5
8	96.0	96.0	96.0
8a	165.0	165.0	165.0
9	35.9	36.0	35.9
1'	135.5	135.7	134.1
2'	112.0	112.1	109.0
3'	149.6	149.6	150.8
4'	149.6	149.6	148.1
5'	107.1	107.3	110.1
6'	135.8	135.7	137.2
4'-OCH$_3$	56.8	56.9	
3'-OCH$_3$			56.7

4.2.13. Summary of ^{13}C NMR data for homoisoflavonoids isolated

2. $R_1=R_3=R_5=OH$, $R_2=R_4=H$
3. $R_1=R_3=OH$, $R_2=R_4=H$, $R_5=OCH_3$
4. $R_1=OCH_3$, $R_2=R_4=H$, $R_3=R_5=OH$
5. $R_1=R_4=R_5=OH$, $R_2=H$, $R_3=OCH_3$
6. $R_1=R_5=OH$, $R_2=H$, $R_3=R_4=OCH_3$
7. $R_1=R_2=OCH_3$, $R_3=R_5=OH$, $R_4=H$

8. $R_1=R_3=R_5=OH$, $R_2=R_4=H$
9. $R_1=R_3=OH$, $R_2=R_4=H$, $R_5=OCH_3$
10. $R_1=OH$, $R_2=R_4=H$, $R_3=R_5=OCH_3$
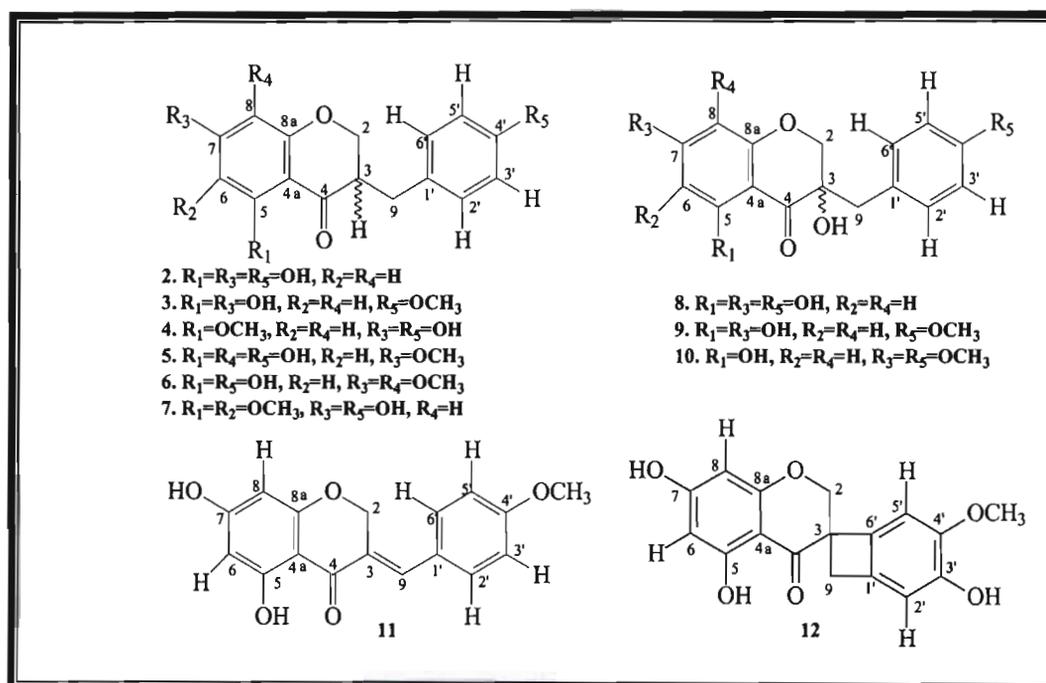
Figure 4.22: Structures of compounds 2-12

Table 4.30: ^{13}C NMR data for compounds 2-12 *CD$_3$OD, **CDCl$_3$

	2*	3**	4*	5*	6*	7**	8*	9**	10**	11*	12*
2	70.1	68.8	69.8	70.5	70.6	68.8	72.3	71.7	71.8	68.4	74.9
3	48.1	46.8	49.9	48.5	48.2	48.5	73.5	72.2	72.2	128.8	55.3
4	199.5	197.5	194.0	200.1	200.0	191.9	200.0	198.1	198.3	186.3	198.0
4a	102.8	102.0	105.2	103.2	103.2	108.6	101.4	100.4	100.5	103.8	102.5
5	165.8	164.4	164.5	158.2	161.5	153.6	165.8	164.1	164.0	165.9	165.9
6	97.1	96.5	94.2	93.4	93.8	135.2	97.4	97.1	95.4	97.4	97.2
7	168.2	166.0	166.5	158.0	162.7	155.7	168.6	165.8	168.6	168.2	168.4
8	95.8	95.2	96.7	127.6	130.4	98.9	96.1	95.8	94.5	96.1	96.0
8a	164.7	163.1	166.7	149.2	155.1	159.8	164.4	163.0	162.7	163.7	165.0
9	33.0	32.0	33.4	32.9	33.0	32.0	40.7	40.6	40.7	137.8	35.9
1'	130.2	129.8	130.6	130.2	129.9	130.1	126.9	126.0	126.1	127.8	135.5
2'	131.1	130.0	131.1	131.2	131.2	130.2	132.8	131.6	131.5	133.2	112.0
3'	116.4	114.1	116.3	116.4	116.4	115.5	115.9	113.8	113.7	115.3	149.6
4'	157.2	158.3	157.1	157.2	157.3	154.5	157.5	158.7	158.8	162.3	149.6
5'	116.4	114.1	116.3	116.4	116.4	115.5	115.9	113.8	113.7	115.3	107.1
6'	131.1	130.0	131.1	131.2	131.2	130.2	132.8	131.6	131.5	133.2	135.8
4'-OCH$_3$		55.4						55.3	55.2	56.0	56.8
5-OCH$_3$			56.2			61.4					
6-OCH$_3$						61.5					
7-OCH$_3$				56.7	56.7					55.8	
8-OCH$_3$					61.5						

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4.3. Foreward to Experimental

4.3.1. Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

Nuclear magnetic resonance spectroscopy was carried out on either a 400 MHz Varian UNITY-INOVA spectrometer or a 300 MHz Gemini spectrometer. All spectra were aquired at ambient temperature in either deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD), deuterated acetone ($(CD_3)_2CO$) or deuterated dimethylsulfoxide ($(CD_3)_2SO$). The chemical shifts (δ) were recorded in ppm relative to the internal standard, tetramethylsilane (TMS), and coupling constants (J) are given in Hertz (Hz).

4.3.2. Infrared Spectroscopy (IR spectroscopy)

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

4.3.3. Ultraviolet Absorption Spectroscopy (UV spectroscopy)

Ultraviolet absorption spectra were obtained on a Varian DMS 300 UV-visible spectrometer. The solvent used to record spectra was dichloromethane or methanol when bathochromic shift measurements were being obtained. The NaOAc and $AlCl_3$ solutions used for bathochromic shifts were prepared by dissolving 0.5 g of each anhydrous salt in 100 mL of methanol.

4.3.4. Melting Points

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

4.3.5. Optical Rotations

Optical rotations were measured at room temperature in either methanol, chloroform or acetone using an Optical activity AA-5 Polarimeter together with a series A2 stainless steel (4 X 200 mm) unjacketed flow tube.

The optical rotation value was calculated as follows.

$[\alpha]_D = 100 \, \alpha/lc$

where α = reading in °, l = tube length in dm and c = concentration (g/100ml)

4.3.6. Mass Spectrometry

The high resolution mass spectrum for compound **1** [**spectrum 1.8**] was recorded on a Kratos 9/50 HRMS instrument. All other mass spectra were recorded on an Agilent MS 5973 instrument connected to a GC 6890, except the mass spectrum for compound **13** [**spectrum 13.9**] which was recorded on a Perkin Elmer Turbo Mass mass spectrometer connected to a Perkin Elmer Autosystem GC.

4.1.7. General Chromatography

The isolation process employed column and thin layer chromatographic techniques. In column chromatography, different sized columns were used ranging from 2-8 cm in diameter depending on the amount of sample available and purification stage. Separation of the crude extracts was generally carried out on a column using Merck Art. 9385 silica gel. All separations were carried out under gravity. Both column and thin layer chromatographic techniques made use of varying ratios of hexane, dichloromethane, ethyl acetate and methanol. Thin layer chromatography was carried out on 0.2 mm SiO_2, 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 (254 and 366 nm) and then by heating.

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4.4. Experimental

Plant material was collected by Dr Neil Crouch of the National Botanical Institute from Long Tom Pass. A voucher specimen is retained at the Natal Herbarium (N. Crouch 857 NH).

The bulbs (dry mass 980 g) were chopped into small pieces, air dried for approximately 48 hours and then extracted successively with dichloromethane (mass of extract-3.45 g), ethyl acetate (mass of extract-3.81 g) and methanol (mass of extract- 2.53 g) by agitation on a Labcon Mechanical Shaker at 140 rpm. Extraction with each solvent was carried out for approximately 96 hours. The extracts obtained were then filtered and the solvent was removed under reduced pressure at room temperature using a BUCHI rotavapor. General chromatographic techniques lead to the isolation of twelve compounds: (**1**) (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one, (**2**) 4'-demethyl-3,9-dihydroeucomin, (**3**) 3,9-dihydroeucomin, (**4**) 4'-demethyl-5-*O*-methyl-3,9-dihydroeucomin, (**5**) 8-*O*-demethyl-7-*O*-methyl-3,9-dihydropunctatin, (**6**) 7-*O*-methyl-3,9-dihydropunctatin, (**7**) 7-hydroxy-3-(4'-hydroxybenzyl)-5,6-dimethoxy-4-chromanone, (**8**) 3,5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone, (**9**) eucomol, (**10**) 7-*O*-methyleucomol, (**11**) (*E*)-eucomin and (**12**) 3',5,7-trihydroxy-4'-methoxyspiro[2H-1-benzopyran-3(4H),7'-bicyclo[4.2.0]octa [1,3,5]-trien]-4-one.

4.4.1. Isolation of compounds 1-12

The dichloromethane extract was eluted with a dichloromethane: ethyl acetate: methanol step gradient [100% dichloromethane (fractions 1-160), 10% ethyl acetate in dichloromethane (fractions 161-199), 15% ethyl acetate in dichloromethane (fractions 200-322), 20% ethyl acetate in dichloromethane (fractions 323-343), 40% ethyl acetate in dichloromethane (fractions 344-387), 60% ethyl acetate in dichloromethane (fractions 388-404), 80% ethyl acetate in dichloromethane (fractions 405-439), 100% ethyl acetate (fractions 440-470), 10% methanol in ethyl acetate (fractions 471-491) and 20% methanol in ethyl acetate (fractions 492-512). Fractions 30-42 yielded compound **10** that was further purified by chromatography with 100% dichloromethane as eluent. Compound **3** was isolated in fractions 93-100 that was

further purified by chromatography with 20% ethyl acetate in hexane as eluent. Fractions 177-180 yielded compound **9** and fractions 211-223 yielded compound **7**. Compound **1** was isolated in fractions 405-427 and was further purified by chromatography using 60% ethyl acetate in hexane as eluent. All purification steps were carried out using Pasteur pipette columns.

The ethyl acetate extract was eluted with a dichloromethane: ethyl acetate: methanol step gradient [100% dichloromethane (fractions 1-30), 10% ethyl acetate in dichloromethane (fractions 31-60), 20% ethyl acetate in dichloromethane (fractions 61-90), 40% ethyl acetate in dichloromethane (fractions 91-120), 60% ethyl acetate in dichloromethane (fractions 121-147), 80% ethyl acetate in dichloromethane (fractions 148-161), 100% ethyl acetate (fractions 162-184), 5% methanol in ethyl acetate (fractions 185-215). Fractions 1-30 yielded crystalline material that on purification by chromatography with 10% methanol in ethyl acetate as eluent yielded compounds **4** and **11**. Compound **11** was purified further with 100% dichloromethane. Compound **5** was isolated from fractions 47-51 and was further purified by chromatography with 100% dichloromethane as eluent. Fractions 64-67 yielded compound **12**, which was further purified by chromatography with 100% dichloromethane as eluent. Fractions 76-80 yielded compound **6** that was further purified by chromatography with 10% ethyl acetate in dichloromethane and then finally 100% ethyl acetate.

The methanol extract was eluted with a dichloromethane: ethyl acetate step gradient [100% dichloromethane (fractions 1-10), 20% ethyl acetate in dichloromethane (fractions 11-15) and 40% ethyl acetate in dichloromethane (fractions 16-25). Fraction 11 yielded compound **8**, which was further purified by chromatography with 100% dichloromethane as the eluent. Fractions 18-19 yielded compound **2**.

4.5. PHYSICAL DATA

4.5.1. Physical data for compound 1

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

Yield: 7.0 mg

Physical description: Orange gum

Optical rotation: not determined because sample decomposed

Mass spectrum*: [M$^+$] at m/z 474.33398, $C_{29}H_{46}O_5$ requires 474.33453 g.mol^{-1}

HRMS: m/z (rel. int) 474.33398 (51), 459.30952 (27), 456.32257 (8), 417.29715 (25), 345.24182 (39), 305.21341 (30), 271.20423 (35), 167.10605 (50), 121.10149 (58), 83.08548 (62), 57.03385 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3421, 2934, 1737, 1461

^{1}H NMR data**: CDCl$_3$ (Table 4.1)

^{13}C NMR data**: CDCl$_3$ (Tables 4.1 and 4.2)

4.5.2. Physical data for compound 2

Name: 4'-demethyl-3,9-dihydroeucomin

Yield: 6.3 mg

Physical description: Yellow crystals

Melting point: 96-99˚C (lit: 103-104˚C)[3]

Optical rotation: [α]$_D$ -30˚ MeOH, c=0.05 (lit: [α]$_D$ -34˚ MeOH)[3]

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

EIMS: m/z (rel. int) 286 (30), 179 (65), 153 (42), 152 (11), 107 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3374, 2920, 2852, 1641, 1520, 1457

UV data: $\lambda_{max}^{CH_2Cl_2}$ (log ε): 324 (4.00), 267 (4.46), 238 (4.76)
Bathochromic shift: AlCl$_3$ (20 nm), NaOAc (36 nm)

^{1}H NMR data: CD$_3$OD (Tables 4.3 and 4.4)

^{13}C NMR data: CD$_3$OD (Tables 4.3 and 4.5)

* Sample had decomposed prior to running of mass spectrum therefore spectrum from N. Moodley's thesis[2] was used.

** Own NMR spectra was not well resolved (see original ^{1}H NMR spectrum [spectrum 1]) therefore spectra from N. Moodley's earlier work on *Ledebouria zebrina*[2] which had been shown on computer were used.

4.5.3. Physical data for compound 3

Name: 3,9-dihydroeucomin

Yield: 5.8 mg

Physical description: White crystals

Melting point: 152-154°C (lit 161-163 °C)[8]

Optical rotation: sample size too small to carry out optical rotation (lit: $[\alpha]_D$ +38°, CHCl$_3$)[8]

Mass spectrum: [M$^+$] at *m/z* 300, C$_{17}$H$_{16}$O$_5$ requires 300 g.mol^{-1}

EIMS: *m/z* (rel. int) 300 (11), 179 (1.9), 153 (2.5), 121(100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3364, 2929, 1644, 1515, 1478

UV data: λ_{max}^{CH2Cl2} nm (log ε): 293 (4.24)

Bathochromic shift: AlCl$_3$ (32 nm), NaOAc, (32 nm)

^{1}H NMR data: CDCl$_3$ (Tables 4.6 and 4.7)

^{13}C NMR data: CDCl$_3$ (Table 4.6)

4.5.4. Physical data for compound 4

Name: 4'-demethyl-5-*O*-methyl-3,9-dihydroeucomin

Yield: 6 mg

Physical description: Colourless crystals

Melting point: 201-203°C (lit 196-197 °C)[9]

Optical rotation: sample size too small to carry out optical rotation (lit: $[\alpha]_D$ -38°, dioxane)[9]

Mass spectrum: [M$^+$] at *m/z* 300, C$_{17}$H$_{16}$O$_5$ requires 300 g.mol^{-1}

EIMS: *m/z* (rel. int) 300 (78), 193 (48), 167 (100), 166 (60), 107 (54)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3419, 2925, 2853, 1736, 1590

UV data: λ_{max}^{CH2Cl2} nm (log ε): 283 (2.83), 232 (3.77)

Bathochromic shifts: AlCl$_3$ (0 nm), NaOAc (20 nm)

^{1}H NMR data: CD$_3$OD (Tables 4.8 and 4.9), (CD$_3$)$_2$CO (Table 4.9)

^{13}C NMR data: CD$_3$OD (Table 4.8)

4.5.5. Physical data for compound 5

Name: 8-O-demethyl-7-O-methyl-3,9-dihydropunctatin

Yield: 8.1 mg

Physical description: Yellow crystals

Melting point: 178-180°C (lit 172-174°C)[7]

Optical rotation: $[\alpha]_D$ −17° MeOH, c=0.06 (no literature value available)

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

        EIMS: m/z (rel. int) 316 (53), 209 (58), 183 (11), 107 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3414, 2920, 2857, 1652, 1514, 1383

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 368 (3.13), 295 (3.53), 235 (4.60)

        Bathochromic shifts: AlCl$_3$ (14 nm), NaOAc (0 nm)

^{1}H NMR data: CDCl$_3$ (Table 4.11), CD$_3$OD, (Tables 4.10 and 4.11)

^{13}C NMR data: CD$_3$OD (Tables 4.10 and 4.12)

4.5.6. Physical data for compound 6

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

Yield: 5.5 mg

Physical description: Yellow amorphous solid

Optical rotation: sample size too small to carry out optical rotation (no literature value available)

Mass spectrum: [M$^+$] at m/z 330, $C_{18}H_{18}O_6$ requires 330 g.mol^{-1}

        EIMS: m/z (rel. int) 330 (50), 223 (12), 197 (5), 107 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3414, 2932, 2852, 1641, 1520, 1463

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 287 (3.18), 229 (3.21)

        Bathochromic shifts: AlCl$_3$ (25 nm), NaOAc (0 nm)

^{1}H NMR data: CD$_3$OD (Tables 4.13 and 4.14), CDCl$_3$ (Table 4.14)

^{13}C NMR data: CD$_3$OD (Tables 4.13 and 4.15)

4.5.7. Physical data for compound 7

Name: 7-hydroxy-3-(4'-hydroxybenzyl)- 5,6-dimethoxy-4-chromanone

Yield: 8.0 mg

Physical description: Yellow amorphous solid

Optical rotation: $[\alpha]_D$ –30° MeOH, c=0.05

Mass spectrum: [M$^+$] at m/z 330, $C_{18}H_{18}O_6$ requires 330 g.mol^{-1}

EIMS: m/z (rel. int) 330 (71), 223 (28), 197 (32), 196 (38), 107 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3368, 2921, 2855, 1618, 1525, 1486

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 321 (3.53), 277 (3.87), 232, (4.56)

Bathochromic shifts: AlCl$_3$ (0 nm), NaOAc (43 nm)

^{1}H NMR data: CDCl$_3$ (Table 4.16)

^{13}C NMR data: CDCl$_3$ (Tables 4.16 and 4.17), CD$_3$OD (Table 4.17)

4.5.8. Physical data for compound 8

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

Yield: 7.7 mg

Physical description: Yellow crystals

Melting point: 99-101°C (lit 105-107 °C)[6]

Optical rotation: $[\alpha]_D$ +71° acetone, c=0.07 (lit: $[\alpha]_D$ –90° acetone)[6]

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

EIMS: m/z (rel. int) 302 (11), 195 (50), 153 (62), 107 (100)

Infrared data: ν_{max}^{NaCl} cm^{-1}: 3337, 2915, 2845, 1646, 1521

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 291 (3.69)

Bathochromic shifts: AlCl$_3$ (16 nm), NaOAc (37 nm)

^{1}H NMR data: CD$_3$OD (Table 4.18 and 4.19), (CD$_3$)$_2$CO (Table 4.19)

^{13}C NMR data: CD$_3$OD (Table 4.18)

4.5.9. Physical data for compound 9

Name: eucomol

Yield: 15.6 mg

Physical description: Yellow crystals

Melting point: 128-130°C (lit 133-134 °C)[6]

Optical rotation: $[\alpha]_D$ -31° CHCl$_3$, c=0.08 (lit: $[\alpha]_D$ –32°/-26° CHCl$_3$)[6]

Mass spectrum: [M$^+$] at m/z 316, C$_{17}$H$_{16}$O$_6$ requires 316 g.mol^{-1}

EIMS: m/z (rel. int) 316 (1.5), 195 (2.4), 153 (4.1), 121 (100)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3408, 2922, 2848, 1638, 1513

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 293 (4.15), 235 (4.59)

Bathochromic shifts: AlCl$_3$ (15 nm), NaOAc (34 nm)

^{1}H NMR data: CDCl$_3$ (Tables 4.20 and 4.21)

^{13}C NMR data: CDCl$_3$ (Table 4.20)

4.5.10. Physical data for compound 10

Name: 7-*O*-methyleucomol

Yield: 2 mg

Physical description: Cream crystals

Melting point: 78-81°C (lit 83-84 °C)[6]

Optical rotation: sample size too small to carry out optical rotation (lit: $[\alpha]_D$ -27° CHCl$_3$)[6]

Mass spectrum: [M$^+$] at m/z 330, C$_{18}$H$_{18}$O$_6$ requires 330 g.mol^{-1}

EIMS: m/z (rel. int) 330 (2), 209 (15), 167 (7), 121 (100)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3381, 2921, 2848, 1651, 1512

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 293 (3.42), 232 (3.50)

Bathochromic shifts: AlCl$_3$ (18 nm), NaOAc (0 nm)

^{1}H NMR data*: CDCl$_3$ (Tables 4.22 and 4.23)

^{13}C NMR data*: CDCl$_3$ (Table 4.22)

* Sample was weak (see original ^{1}H NMR spectrum [spectrum 10]) and NMR spectra weren't well resolved therefore spectra from T. Pohl's earlier work on *Ornithogalum longibracteatum*[13] which had been shown on computer were used.

4.5.11. Physical data for compound 11

Name: (*E*)-eucomin

Yield: 9.8 mg

Physical description: Yellow crystals

Melting point: 206-208 (lit 199-201 °C)[6]

Optical rotation: sample was contaminated (lit: $[\alpha]_D$ 0°)[6]

Mass spectrum: [M$^+$] at *m/z* 298 requires 298 g.mol^{-1}

    EIMS: *m/z* (rel. int) 298 (100), 153 (75), 146 (48)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3374, 2961, 2926, 2857, 1646, 1515

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 357 (3.44), 232 (4.27)

    Bathochromic shifts: AlCl$_3$ (39 nm), NaOAc (20 nm)

^{1}H NMR data: CDCl$_3$ (Table 4.25), CD$_3$OD (Tables 4.24, 4.25)

^{13}C NMR data: (CD$_3$)$_2$SO (Table 4.26), CD$_3$OD (Tables 4.24, 4.26)

4.5.12. Physical data for compound 12

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: 5.9 mg

Physical description: cream crystals

Melting point: 215-217°C (lit 209-211 °C)[15]

Optical rotation: sample size too small to carry out optical rotation (lit: $[\alpha]_D + 11°$
    MeOH)[15]

Mass spectrum: [M$^+$] at *m/z* 314 requires 314 g.mol^{-1}

    EIMS: *m/z* (rel.int) 314 (44), 162 (16), 153 (100), 152 (42)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3381, 2930, 2849, 1644, 1476

UV data: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 231 (4.15), 289 (4.16)

    Bathochromic shifts: AlCl$_3$ (17 nm), NaOAc (36 nm)

^{1}H NMR data: CD$_3$OD (Tables 4.27 and 4.28)

^{13}C NMR data: CD$_3$OD (Tables 4.27 and 4.29)

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CHAPTER 5: EXTRACTIVES FROM *AGAPANTHUS INAPERTUS*

5.1. Introduction

Investigation of the acidic chloroform extract *of Agapanthus inapertus* has led to the isolation of a lignan precursor **(13)** dihydroconiferyl alcohol (3-(4'-hydroxy-3'-methoxyphenyl)-1-propanol) and a lignan **(14)** isolariciresinol. The ^{1}H NMR spectrum of the crude basic chloroform extract did not show any characteristic peaks for saponins and sapogenins of interest and was therefore not examined.
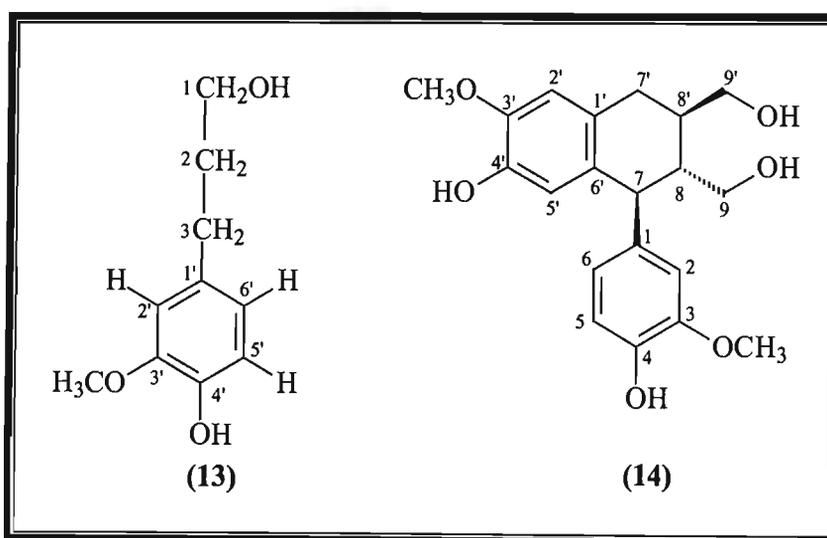
Figure 5.1: Compounds isolated from *Agapanthus inapertus*

5.2. Results and Discussion

5.2.1. Structural elucidation of compound 13: dihydroconiferyl alcohol (spectra 13.1-13.10, pp 282-291)

This compound was isolated as a yellow amorphous solid.
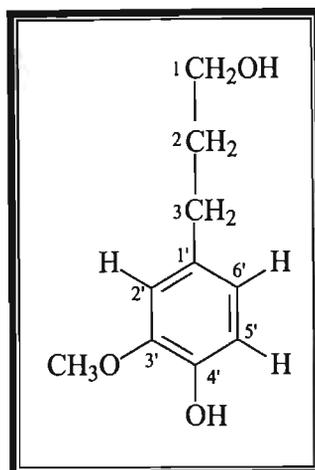
Figure 5.2: Structure of compound 13: dihydroconiferyl alcohol

The mass spectrum [**spectrum 13.9**] of compound **13** gave a molecular ion [M$^+$] peak of m/z 182, which corresponded to a molecular formula of $C_{10}H_{14}O_3$. Fragmentation between C-2 and C-3 resulted in the loss of 45 mass units and gave a peak at m/z 137. This corresponded to a hydroxymethoxytropylium ion.

The infrared spectrum [**spectrum 13.10**] showed a peak at 3379 cm^{-1} that indicated the presence of hydroxy substituents. The strong peaks at 2932 and 2852 cm^{-1} corresponded to the C-H stretching of methine and methylene groups. The peaks at 1601 and 1515 cm^{-1} corresponded to the C=C stretching of the aromatic ring. The peaks at 1279 and 1033 cm^{-1} are characteristic of C-O stretching.

The resonances at δ_H 3.66 (2H, t, J = 6.4 Hz), δ_H 1.85 (2H, m) and δ_H 2.62 (2H, t, J = 7.5 Hz) in the ^{1}H NMR spectrum [**spectrum 13.1**] indicated the presence of three methylene groups. The resonance at δ_H 3.66 was deshielded because it was attached to an oxygen atom. Its corresponding carbon resonance occurred at δ_C 62.3 in the ^{13}C NMR spectrum [**spectrum 13.2**] and was assigned to the C-1 position because this resonance showed HMBC correlations [**spectrum 13.5**] to 2H-2 (δ_H 1.85) and 2H-3

(δ_H 2.62). The 2H-2 and 2H-3 resonances were assigned through the HMBC spectrum. Their corresponding carbon resonances occurred at δ_C 34.5 and δ_C 31.7 respectively.

The strong singlet peak at δ_H 3.85 integrating to three protons in the ^{1}H NMR spectrum was characteristic of protons of a methoxy group. The doublet resonance at δ_H 6.66 (J = 2.0 Hz) indicated a *meta*-coupled proton which was assigned to H-2' because its corresponding carbon resonance at δ_C 120.9 showed HMBC correlations to 2H-3 and the double doublet resonance at δ_H 6.67 (J = 7.8, 2.0 Hz). The double doublet resonance at δ_H 6.67 (J = 7.8, 2.0 Hz) indicated *ortho*-coupling as well as *meta*-coupling and was therefore assigned to the protons at the C-6' position. The doublet resonance at δ_H 6.81 (J = 7.8 Hz) was attributed to the H-5' proton.

The C-1' position had to be substituted because there was *meta*-coupling between the H-2' and H-6' protons. The other positions that were substituted were the C-3' and C-4' positions. The methoxy group proton resonance showed a NOESY correlation [**spectrum 13.7**] to the H-2' proton resonance and was therefore placed at the C-3' position. NOESY correlations between 2H-3 and H-2' and H-6' allowed placement of the propanol group at the C-1' position. The remaining hydroxy group was then placed at the C-4' position. NOESY correlations confirmed the structure.
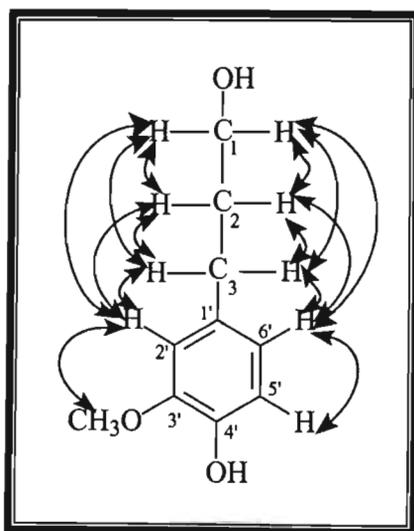
Figure 5.3: Diagram showing NOESY correlations for compound 13

Coupling of protons in the COSY spectrum **[spectrum 13.6]** further supported the structure.
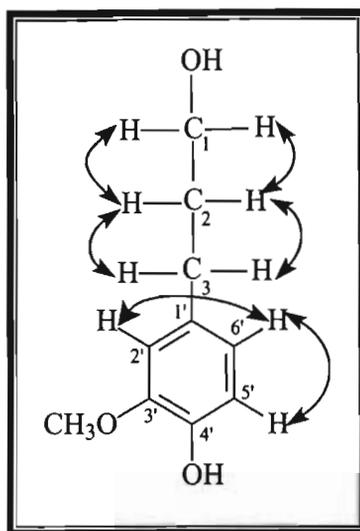
Figure 5.4: Diagram showing COSY coupling for compound 13

Compound **13** was found to be the known compound **dihydroconiferyl alcohol** (or **3-(4'-hydroxy-3'-methoxyphenyl)-1-propanol**), which is a typical lignan precursor.[1]

Table 5.1: ^{1}H, ^{13}C, HMBC, NOESY and COSY data for compound 13 (CDCl$_3$)

	^{1}H/ ppm	^{13}C/ ppm	HMBC	NOESY	COSY
1'		133.7	2H-2, 2H-3, H-5'		
2'	6.66 d, 2.0	120.9	2H-3, H-6'	3'-OCH$_3$, 2H-2, 2H-3, 2H-1	H-6'
3'		146.4	3'-OCH$_3$, H-2', H-5'		
4'		143.7	H-5', H-2', H-6'		
5'	6.81 d, 7.8	114.2		H-6'	H-6'
6'	6.67 dd, 2.0, 7.8	111.0	2H-3, H-2'	2H-2, 2H-3, H-5', 2H-1	H-2', H-5'
1	3.66 t, 6.4	62.3	2H-2, 2H-3	2H-2, 2H-3, H-2', H-6'	2H-2
2	1.85 m	34.5	2H-3, 2H-1	2H-3, 2H-1 H-2', H-6'	2H-3, 2H-1
3	2.62 t, 7.5	31.7	2H-2, 2H-1, H-2', H-6'	2H-2, 2H-1, H-2', H-6'	2H-2
3'-OCH$_3$	3.85 s	55.8		H-2'	

5.2.2. Structural elucidation of compound 14: isolariciresinol (spectra 14.1-14.10, pp 292-301)

Compound **14** was isolated as a white crystalline solid with a melting point of 148-150°C.
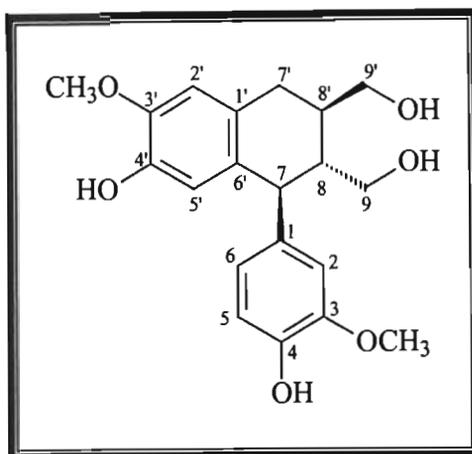
Figure 5.5: Structure of compound 14: isolariciresinol

The mass spectrum [**spectrum 14.9**] of compound **14** showed a molecular ion [M^+] peak at m/z 360. This corresponded to a molecular formula of $C_{20}H_{24}O_6$. The peaks at m/z 342 (M^+ -18) and m/z 311 (M^+ -18-31) were due to the loss of water and loss of water and a CH_2OH fragment respectively, which is typical of a primary alcohol.

The IR spectrum [**spectrum 14.10**] showed peaks at 3374 cm^{-1} due to O-H stretching, 2927 cm^{-1} due to C-H stretching and 1603 cm^{-1} due to aromatic C=C stretching. The peak at 1031 cm^{-1} was due to C-O stretching.

The ^{13}C NMR spectrum [**spectrum 14.2**] indicated the presence of twenty carbon signals: two methoxy group resonances, twelve aromatic (five methine and seven quaternary) resonances and six aliphatic (three methylene and three methine) resonances.

The ^1H NMR spectrum [**spectrum 14.1**] indicated the presence of two aromatic ring systems. A third ring had to be present to account for the nine double bond equivalents indicated from the molecular formula. One aromatic ring was found to be 1,3,4-trisubstituted. The doublet resonances at δ_H 6.57 (J = 1.8 Hz) and δ_H 6.82 (J =

127

8.0 Hz) were attributed to H-2 and H-5 respectively and the double doublet resonance at δ_H 6.62 ($J = 8.0$, 1.8 Hz) was attributed to H-6. The methoxy group indicated by the singlet resonance integrating to three protons at δ_H 3.80 was placed at the C-3 position because a NOESY correlation was seen between this resonance and H-2 in the NOESY spectrum [**spectrum 14.7**]. The resonance at δ_C 146.7 was assigned to C-3 because the HMBC spectrum [**spectrum 14.5**] showed correlations between this resonance and with the methoxy group at δ_H 3.80 and H-5.

The second aromatic ring was found to be 1',3',4',6'-tetrasubstituted. The methoxy group resonance at δ_H 3.83 was placed at C-3' because this resonance showed a NOESY correlation to H-2'. The singlet at δ_H 6.55 was assigned to H-2' because it showed a correlation with 2H-7' in the NOESY spectrum. HMBC correlations were also seen between C-3' and C-4' and the H-2' resonance. The other singlet resonance at δ_H 6.27 was assigned to H-5' because NOESY correlations were seen between this resonance and those of H-7 and H-5. HMBC correlations were also seen between C-3', C-1', C-4', C-7 and the H-5' resonance.

HMBC correlations was observed between C-1, C-2, C-6, C-9 and C-6' and the doublet resonance at δ_H 3.70 attributed to H-7. Coupling was seen between the multiplet resonance at δ_H 1.84, attributed to H-8, with H-8', H-7, and H-9a, and between the multiplet resonance at δ_H 1.99, attributed to H-8', with H-8, H-7' and 2H-9' in the COSY spectrum [**spectrum 14.6**]. This confirmed arrangement of the two phenylpropanoid groups (see **Section 3.1**).

The relative stereochemistry of the molecule was determined using the NOESY spectrum. A NOESY correlation was seen between H-7 and H-8' that confirmed the *cis*-stereochemistry of these protons. The β-stereochemistry of H-8 was based on the NOESY interaction between H-8 and H-2.

A literature search of compound **14** showed that the ^{13}C NMR data matched that of the known compound **(+)-isolariciresinol** (**Table 5.3**), previously isolated from *Araucaria angustifolia*.[2] However, the optical rotation for compound **14** could not be obtained because the sample had decomposed.

Table 5.2: ^{1}H, ^{13}C, HMBC, NOESY and COSY data for compound 14 (CDCl$_3$)

	^{1}H/ ppm	^{13}C/ ppm	HMBC	NOESY	COSY
1		132.8	H-7, H-2, H-6		
2	6.57 d, 1.8	111.4	H-7	H-8, H-7, 3-OCH$_3$	
3		146.7	3-OCH$_3$, H-5		
4		143.5			
5	6.82 d, 8.0	114.2		H-5', H-6	H-6
6	6.62 dd, 8.0, 1.8	122.4	H-2, H-7	H-7, H-8, H-5	H-5
7	3.70 d*	47.9	H-9b, H-5', H-2	H-2, H-6, H-5', H-8, H-8', H-9b'	H-8, H-9a
8	1.84 m	48.0	H-9b	H-2, H-6, H-7	H-9a, H-7, H-8'
9	a-3.50 dd, 11.3, 5.5	63.0	H-7	H-9b	H-8, H-9b, H-7
	b-3.76 dd, 11.1, 2.9			H-9a	H-9a
1'		127.5	H-7', H-5'		
2'	6.55 s	110.1		3'-OCH$_3$, H-7'	
3'		144.8	3'-OCH$_3$, H-5', H-2'		
4'		144.2	H-5', H-2'		
5'	6.27 s	115.5		H-5, H-7	
6'		136.9	H-7		
7'	2.75 dd, 11.4, 5.1	33.3	H-2'	H-2', H-8'	H-8'
8'	1.99 m	40.1	H-9a,b,	H-9b', H-7', H-7, H-9a'	H-7', H-8, H-9a'
9'	a-3.70 dd*	66.4		H-9b', H-8'	H-8', H-9b'
	b-3.86 dd, 11.0, 3.6			H-9a', H-7, H-8'	H-9a'
3'-OCH$_3$	3.83 s	55.9		H-2'	
3-OCH$_3$	3.80 s	56.0		H-2	

* Peaks superimposed therefore coupling constants could not be determined

Table 5.3: Comparison of ^{13}C NMR data for compound 14 with (+)-isolariciresinol[2]

	^{13}C (CDCl$_3$)/ ppm	^{13}C lit (CDCl$_3$)[2]/ ppm
1	132.8	132.6
2	111.4	112.0
3	146.7	145.2*
4	143.5	143.5
5	114.2	114.5
6	122.4	121.9
7	47.9	47.4
8	48.0	47.5
9	63.0	62.1
1'	127.5	127.2
2'	110.1	110.6
3'	144.8	147.1*
4'	144.2	144.1
5'	115.5	115.8
6'	136.9	136.8
7'	33.3	32.8
8'	40.1	39.5
9'	66.4	65.7
3'-OCH$_3$	55.9	55.6
3-OCH$_3$	56.0	55.6

* Values appear incorrect in the literature[1], corrected with HMBC experiment

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5.3. Experimental

The roots of *Agapanthus inapertus* were collected from God's Window, Mpumalanga and a voucher specimen is retained at the National Botanical Institute, Durban (N. Crouch 946 NH). The dried roots (dry mass- 850g) were extracted on a Labcon shaker at room temperature for forty-eight hours with 95% ethanol and the solvent was removed under reduced pressure to yield 3.51 g of extract. Th extract was dissolved in 100 mL of water, acidified to pH 5 and extracted five times with 200 mL portions of chloroform. The acidic chloroform extracts were combined and the solvent removed under reduced pressure to yield 1.03 g of acidic chloroform extract. The aqueous portion was then basified to pH 10 and extracted five times with 200 mL portions of chloroform as above. The solvent was removed under reduced pressure to yield 0.67 g of basic chloroform extract. Only the acidic chloroform extract was worked on. The ^{1}H NMR spectrum of the crude basic chloroform extract did not show interesting results and was not worked on.

5.3.1. Isolation of compounds 13 and 14

The acidic chloroform extract was chromatographed over silica gel using a column and eluted with a dichloromethane: ethyl acetate step gradient starting with 100% dichloromethane (fractions 1-16), then 25% (fractions 17-22), 50% (fractions 23-32), 75% (fractions 33-36) ethyl acetate in dichloromethane and finally 100% (fractions 37-42) ethyl acetate. 100 mL fractions were collected. Fraction 5 yielded compound **13**, which was further purified by repeated column chromatography using 5% ethyl acetate in dichloromethane. Fractions 18-21 yielded compound **14**. This was further purified by repeated column chromatography using 20% ethyl acetate in dichloromethane.

5.4. Physical data

5.4.1 Physical data for compound 13

Name: dihydroconiferyl alcohol

Yield: 3.7 mg

Physical description: Yellow amorphous solid

Optical rotation: sample size too small to carry out optical rotation

Mass spectrum: [M$^+$] at m/z 182, $C_{10}H_{14}O_3$ requires 182 g.mol^{-1}

EIMS: m/z (rel. int) 182 (77), 138 (75), 137 (100), 123 (25), 122 (35)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3379, 2932, 2852, 1601, 1515, 1279,1033

UV data: $\lambda_{max}^{CH_2Cl_2}$ (log ε): 281 (3.58), 233 (3.76)

^{1}H NMR data: CDCl$_3$ (Table 5.1)

^{13}C NMR data: CDCl$_3$ (Table 5.1)

5.4.2. Physical data for compound 14

Name: isolariciresinol

Yield: 8.0 mg

Physical description: White crystals

Melting point: 148-150˚C (lit 155-157 ˚C)[1]

Optical rotation: sample decomposed (lit: [α]$_D$ + 68˚ acetone)[1]

Mass spectrum: [M$^+$] at m/z 360, $C_{20}H_{24}O_6$ requires 360 g.mol^{-1}

EIMS: m/z (rel. int) 360 (88), 342 (24), 311(100)

Infrared data: v_{max}^{NaCl} cm^{-1}: 3374, 2927, 2853, 1603, 1512, 1467, 1286, 1031

UV data: $\lambda_{max}^{CH_2Cl_2}$ (log ε): 283 (3.58), 231 (3.88)

^{1}H NMR data: CDCl$_3$ (Table 5.2)

^{13}C NMR data: CDCl$_3$ (Table 5.2 and 5.3)

5.5. References

1. Dewick, P.M., 1997. Medicinal Natural Products. A Biosynthetic Approach, John Wiley and Sons Ltd.: West Sussex. pp 119-124.

2. Fonseca, S. F., Campello, J., Barata, L. E. S. and Ruveda, E. A., 1978. ^{13}C NMR spectral analysis of lignans from *Araucaria angustifolia*. Phytochemistry. 17, 499-502.

CHAPTER 6

6.1. Conclusion

Recently the taxonomy of the Hyacinthaceae has been under review. The findings of this work are interesting in that the types of compounds isolated from *Eucomis montana* are similar to those previously isolated from the Hyacinthoideae subfamily of the Hyacinthaceae. Given the well documented occurrence of homoisoflavonoids in this family, it is not surprising that this is the major class of compound isolated in this work. Six 3-benzyl-4-chromanone, three 3-benzyl-3-hydroxy-4-chromanone, one 3-benzylidenyl-4-chromanone and one scillascillin type homoisoflavonoids were isolated. The 5,7-dioxy substitution pattern is a notable feature of all of the homoisoflavonoids isolated from species of the genus *Eucomis*. Of the eleven homoisoflavonoids belonging to the four different classes that have been found here all were *para*-disubstituted, except the scillascillin type. A novel homoisoflavonoid, 7-hydroxy-3-(4'-hydroxybenzyl)-5,6-dimethoxy-4-chromanone was isolated. A eucosterol type nortriterpenoid, which is a less common type of compound isolated from the family, was also isolated.

In this chemical investigation of *Agapanthus inapertus* a lignan precursor and a lignan were found. Dihydroconiferyl alcohol is a common lignan precursor. It is the first time that a lignan (isolariciresinol) has been reported from the *Agapanthus* family.

The structures of the compounds isolated are shown in **Figure 6.1**.

Future work will involve collaboration with other universities to screen compounds isolated for anti-inflammatory, antiviral, antibacterial and antifungal properties. The biologically active compounds may provide an important link in the drug industry.
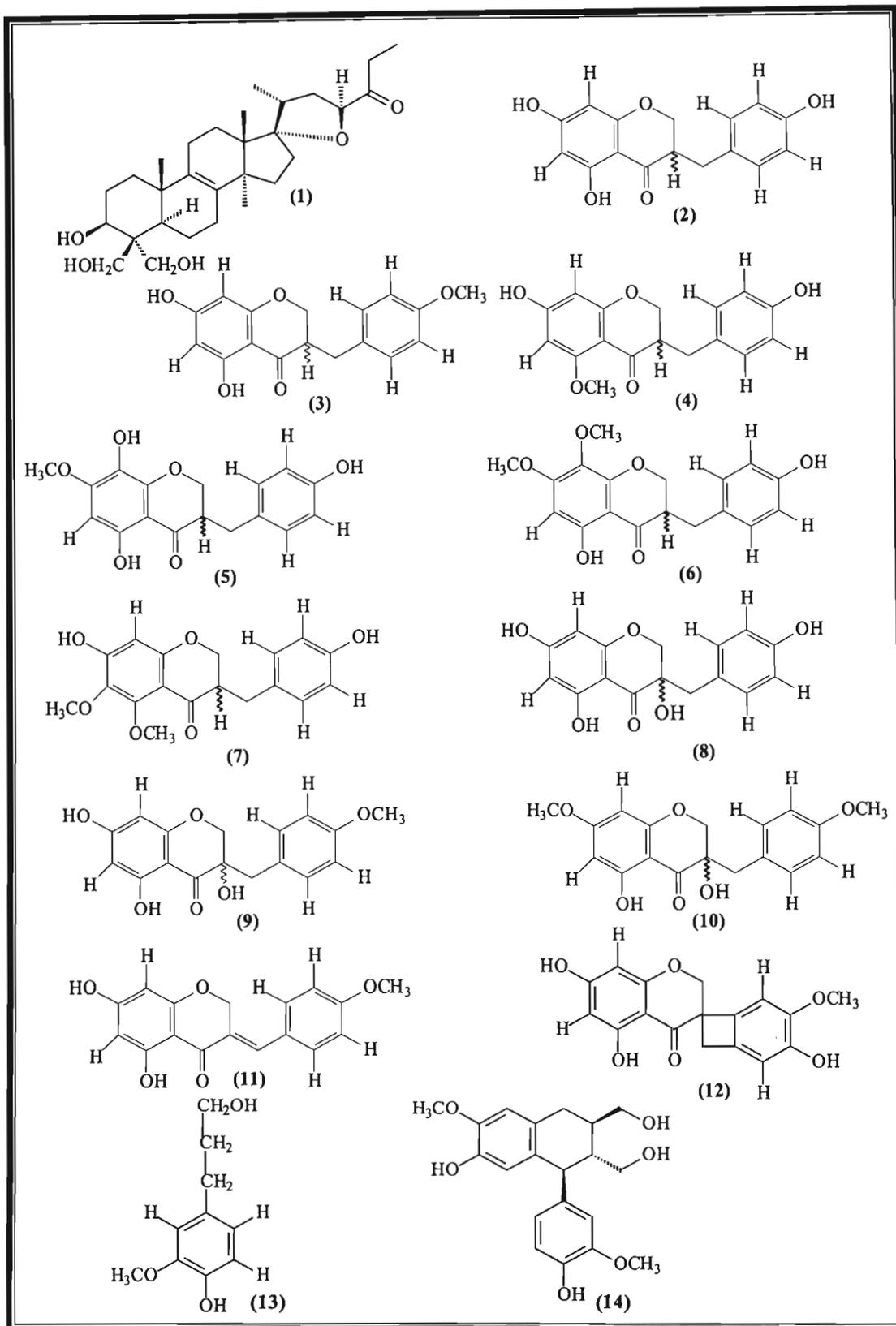
Figure 6.1: Compounds isolated from *Eucomis montana* (1-12) and *Agapanthus inapertus* (13-14)

APPENDIX A

LIST OF SPECTRA

Spectrum 1: ^{1}H NMR spectrum of compound 1 (CDCl$_3$) original

143

Spectrum 1.1: ^{1}H NMR spectrum of compound 1 (CDCl$_3$)

Spectrum 1.2: ^{13}C NMR spectrum of compound 1 (CDCl$_3$)
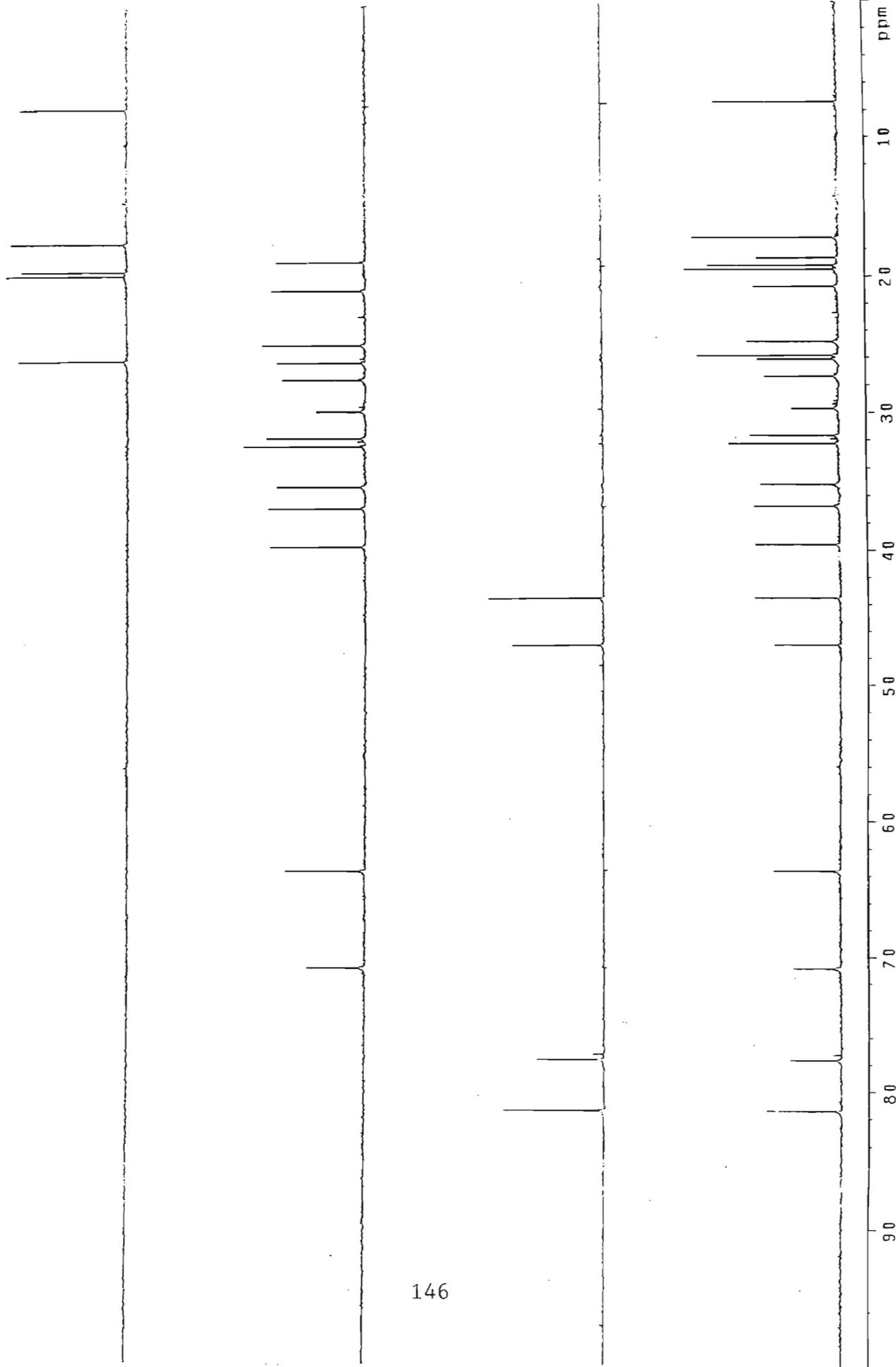
Spectrum 1.3: ADEPT spectrum of compound 1 (CDCl₃)
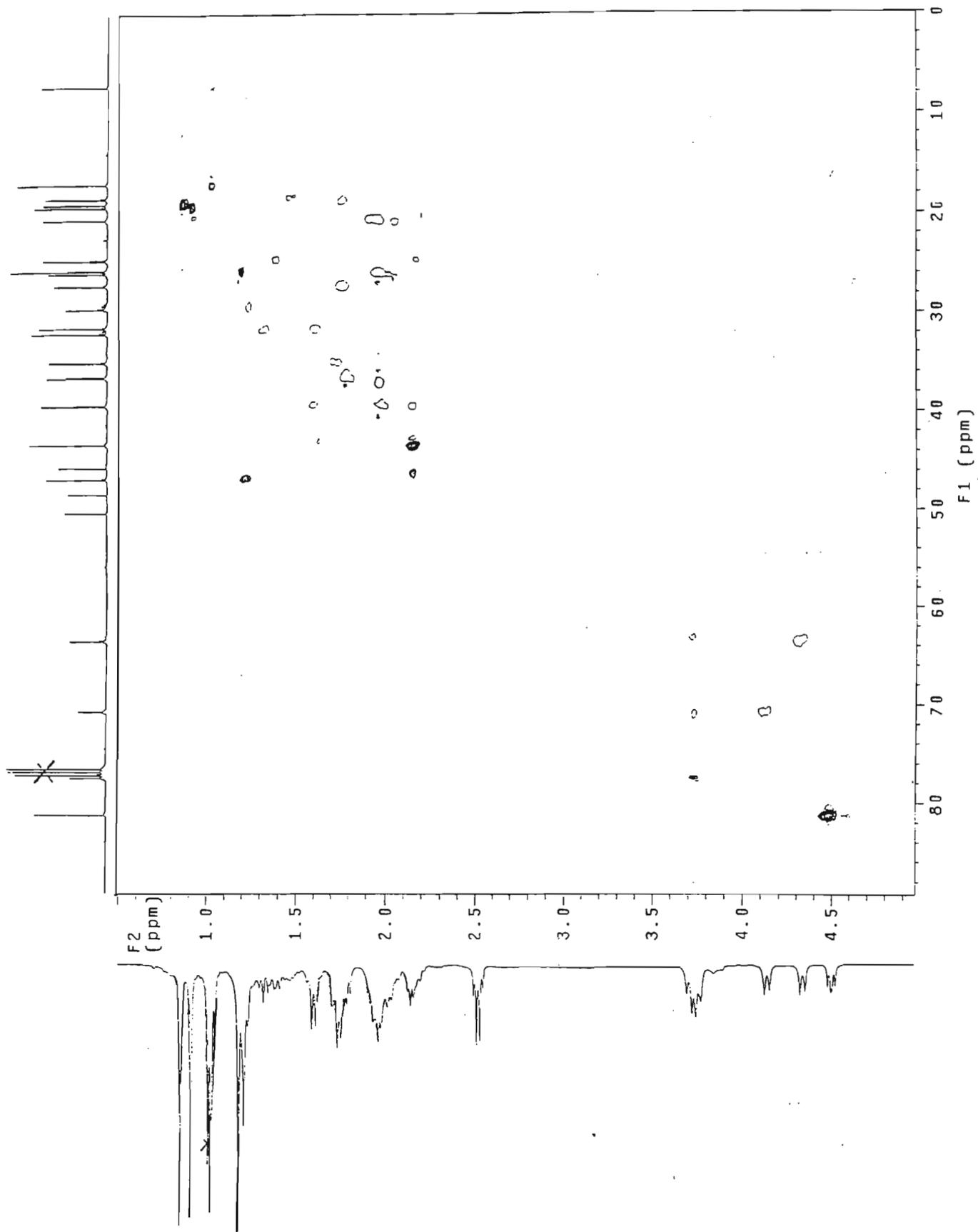
Spectrum 1.4: HSQC spectrum of compound 1 (CDCl₃)

F1 (ppm)

F2 (ppm)

147
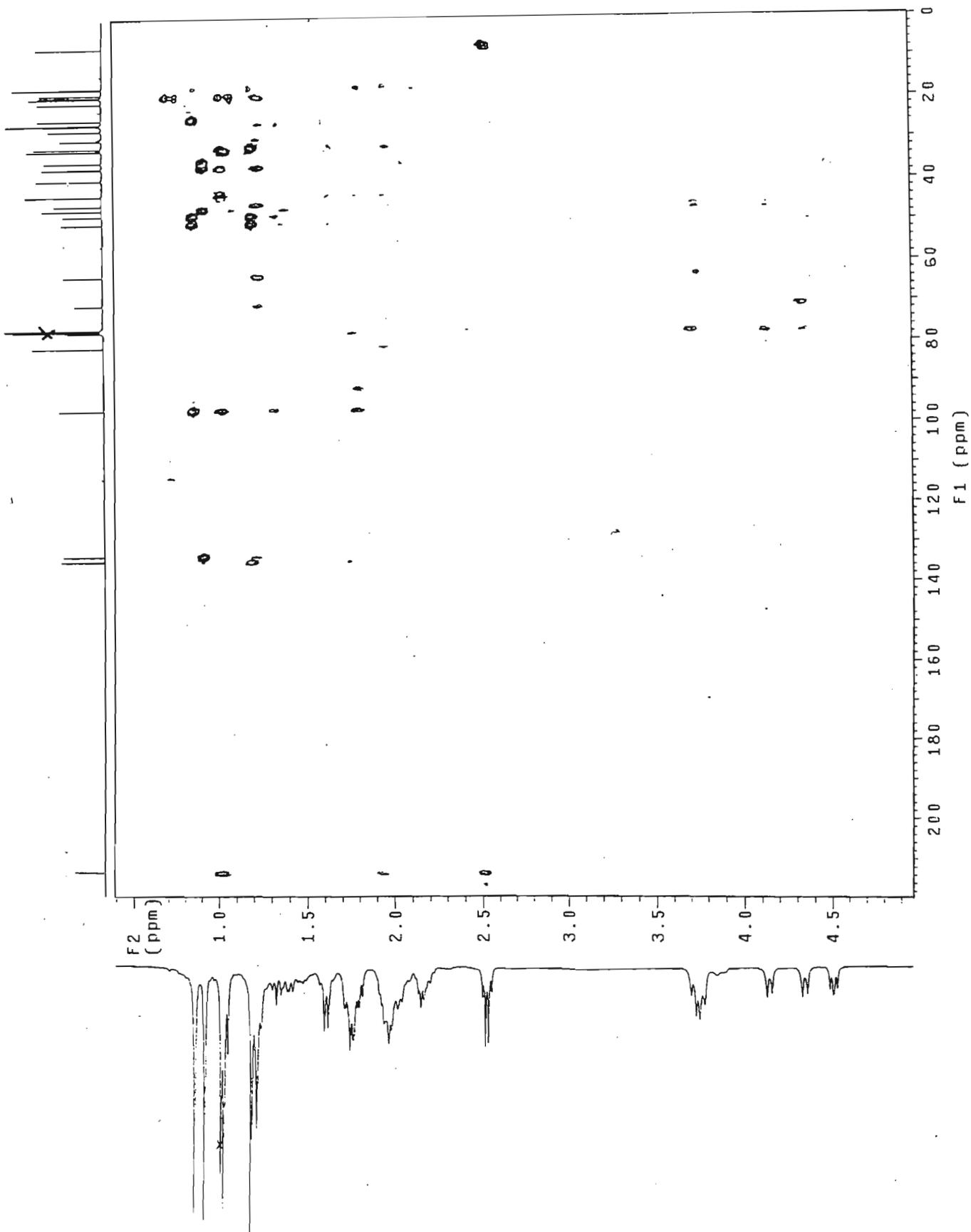
Spectrum 1.5: HMBC spectrum of compound 1 (CDCl₃)

148
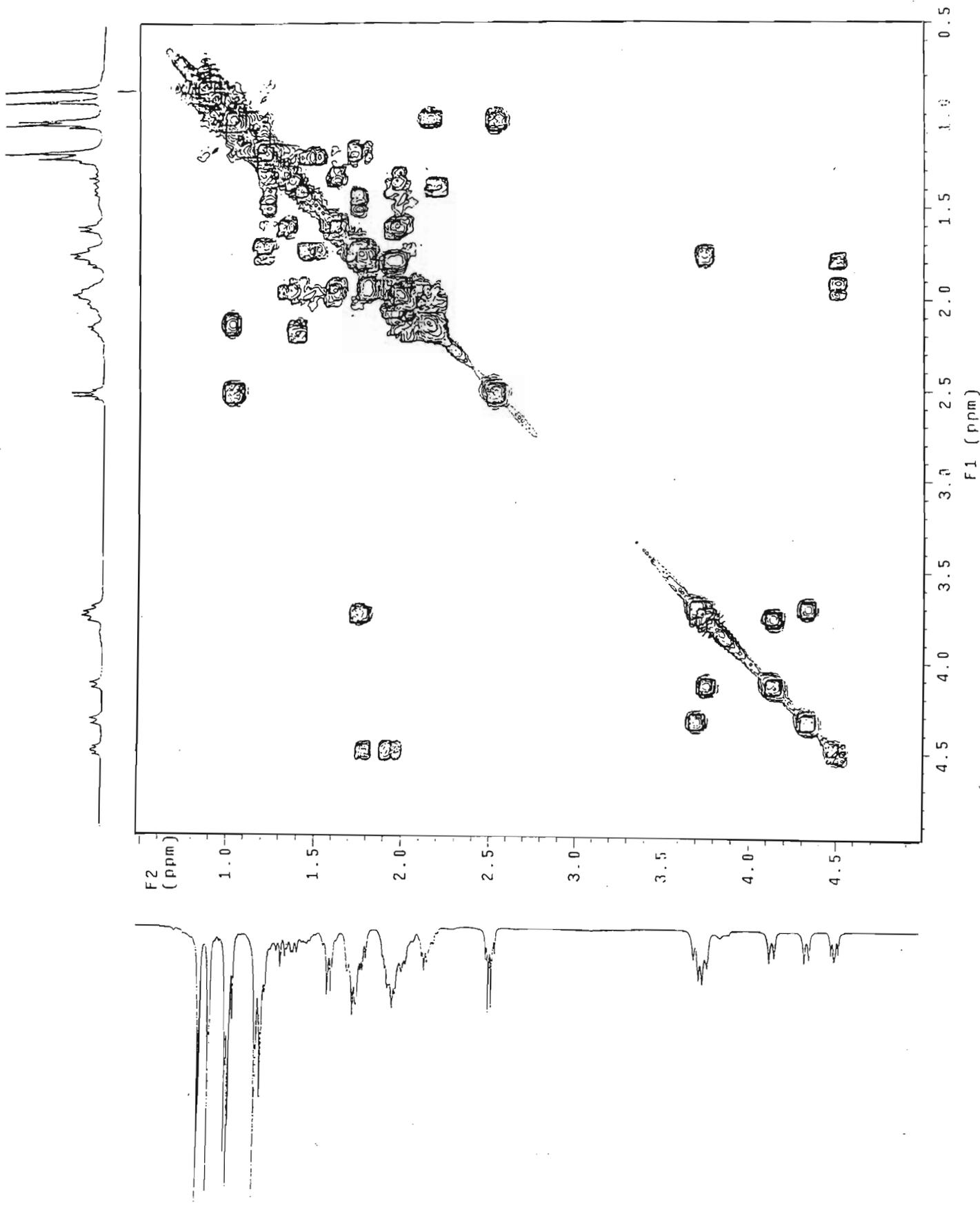
Spectrum 1.6: COSY spectrum of compound 1 (CDCl₃)

149
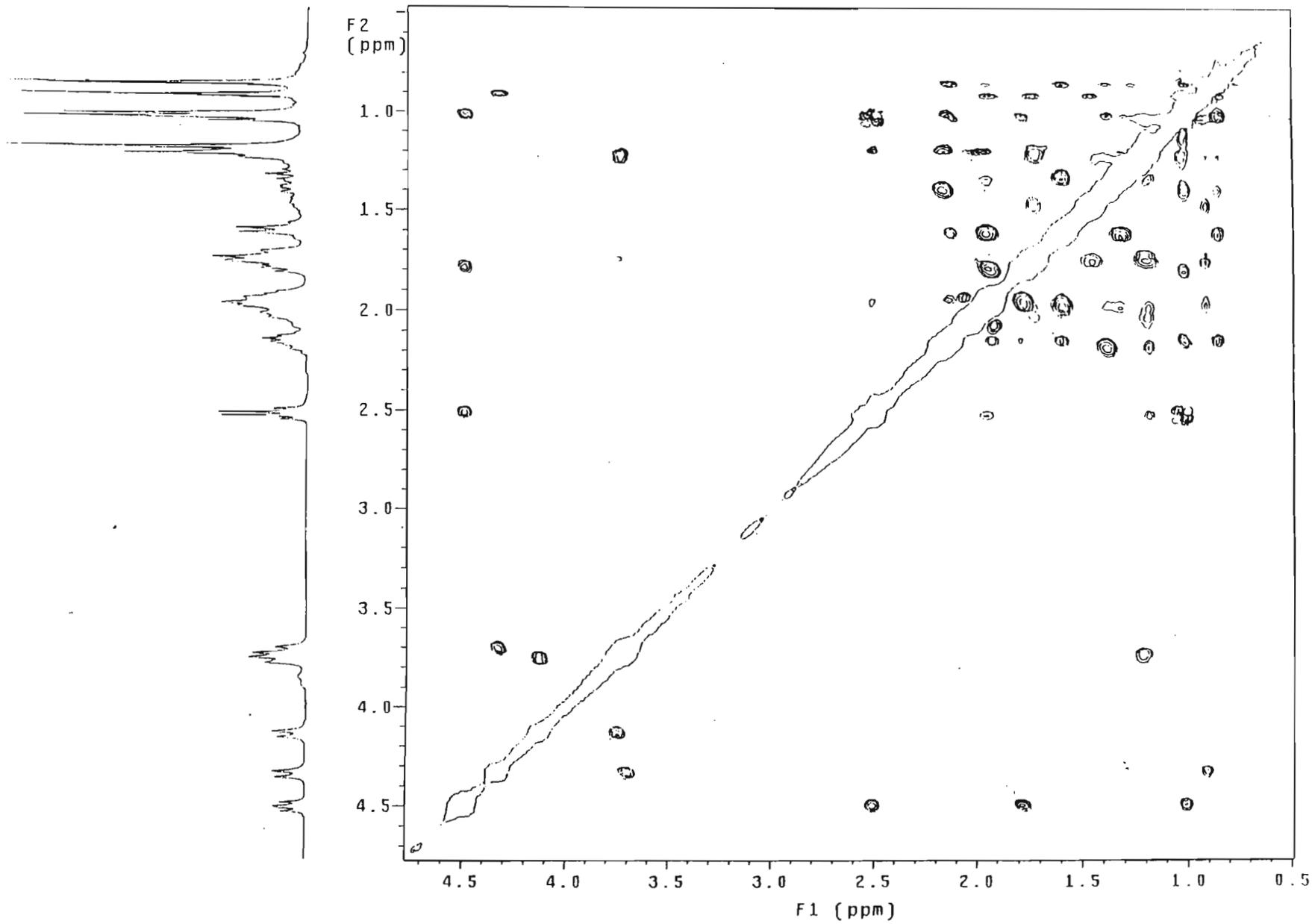
Spectrum 1.7: NOESY spectrum of compound 1 (CDCl₃)
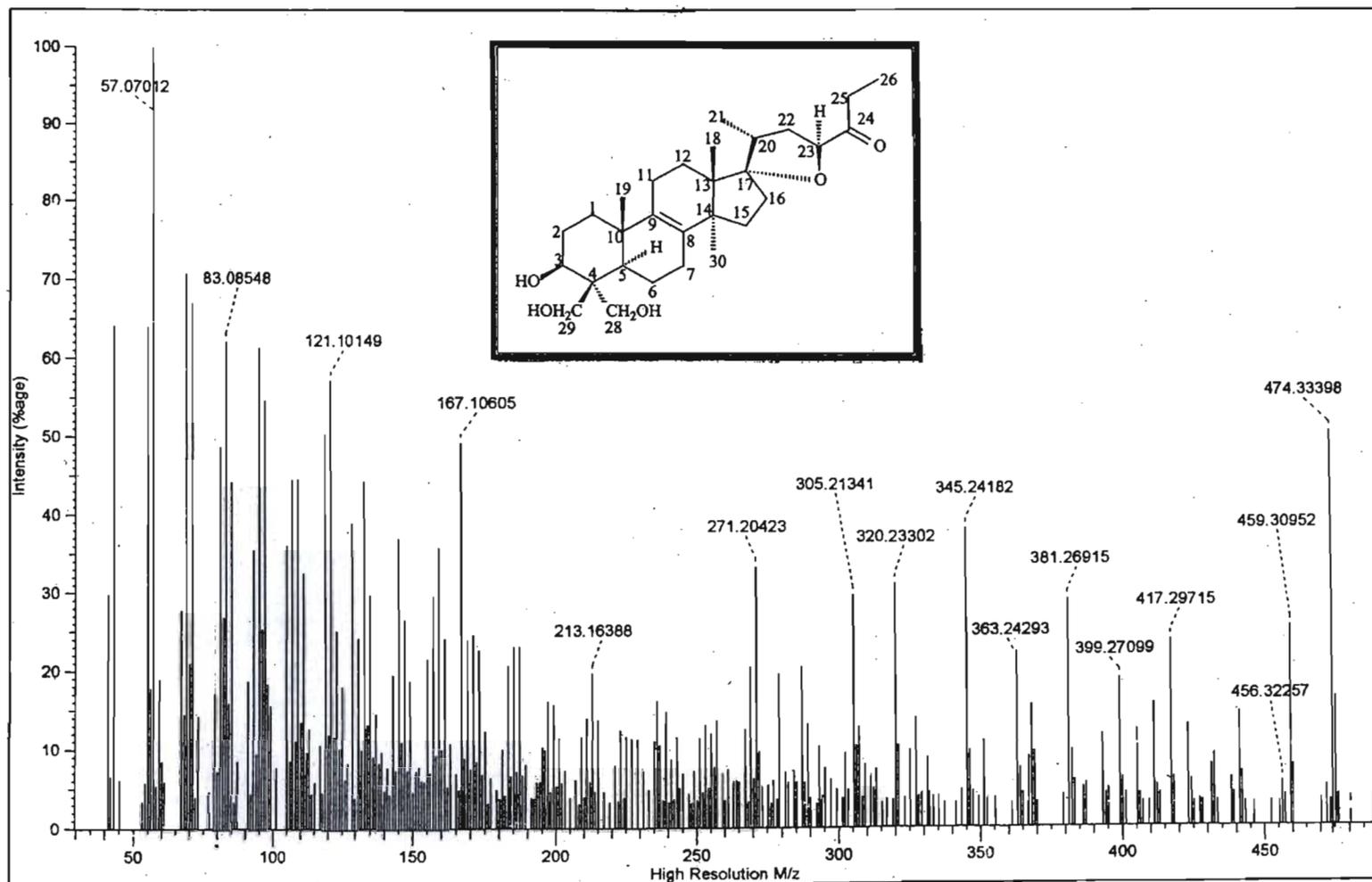
Spectrum 1.8: Mass spectrum of compound 1

100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
70
68

% Transmittance

3421.3

2933.9

1736.5

1461.0

1376.2 1037.1

3500 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000

Wavenumbers (cm-1)

152
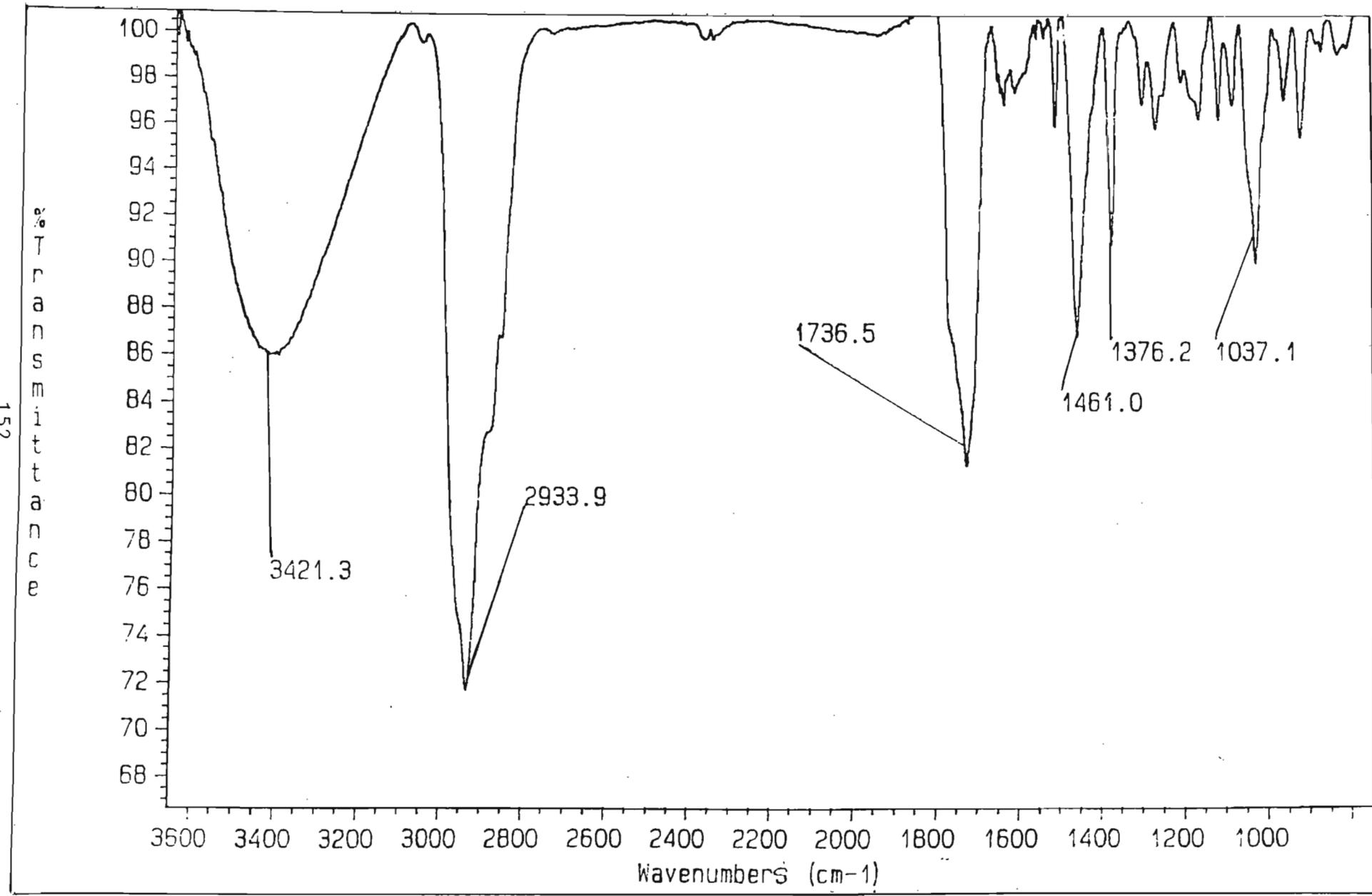
Spectrum 1.9: IR spectrum of compound 1
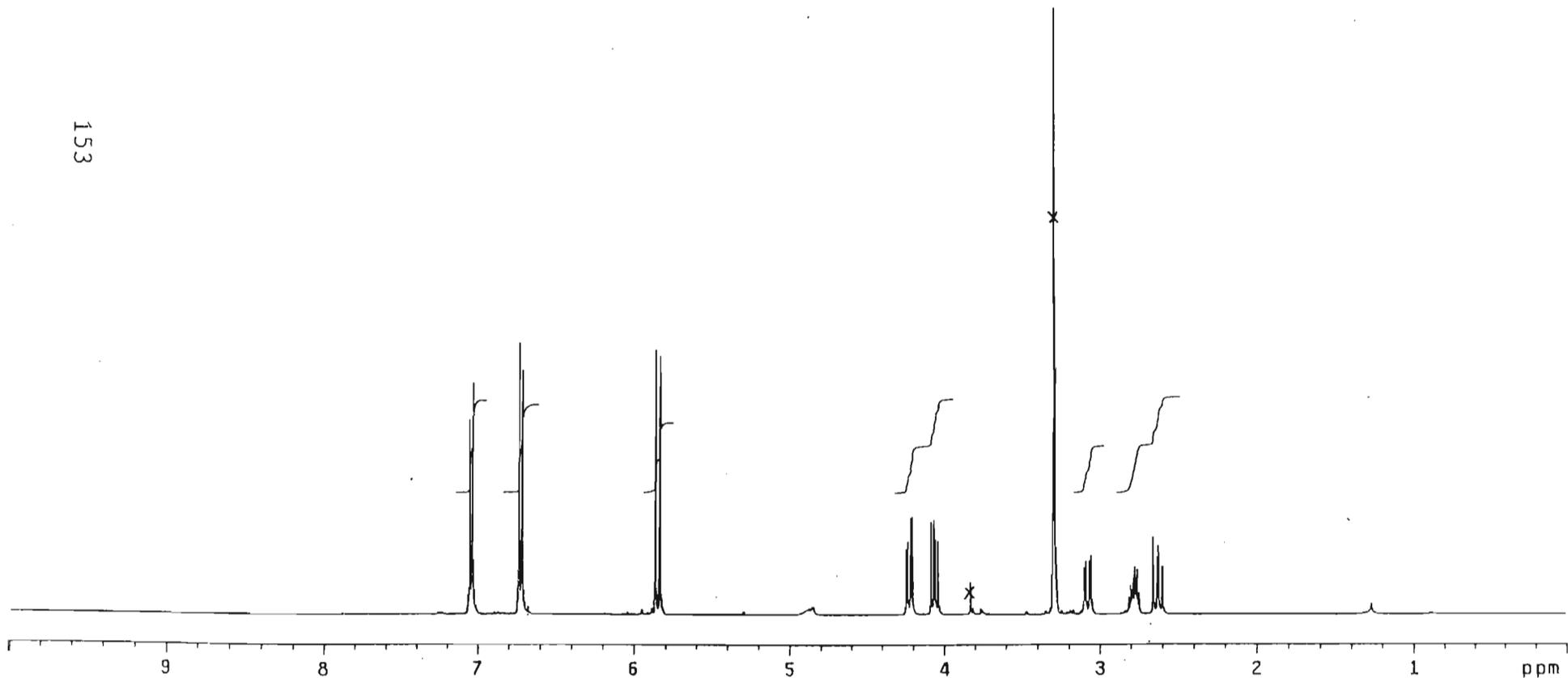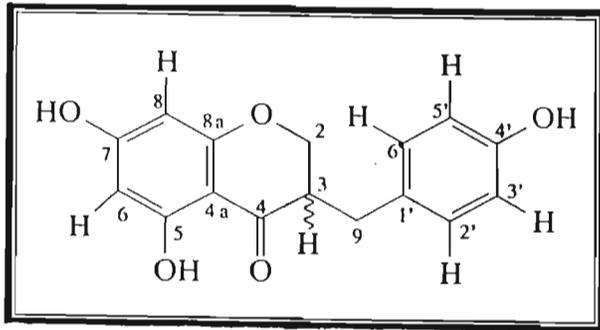
Spectrum 2.1: ^{1}H NMR spectrum of compound 2 (CD$_3$OD)

153
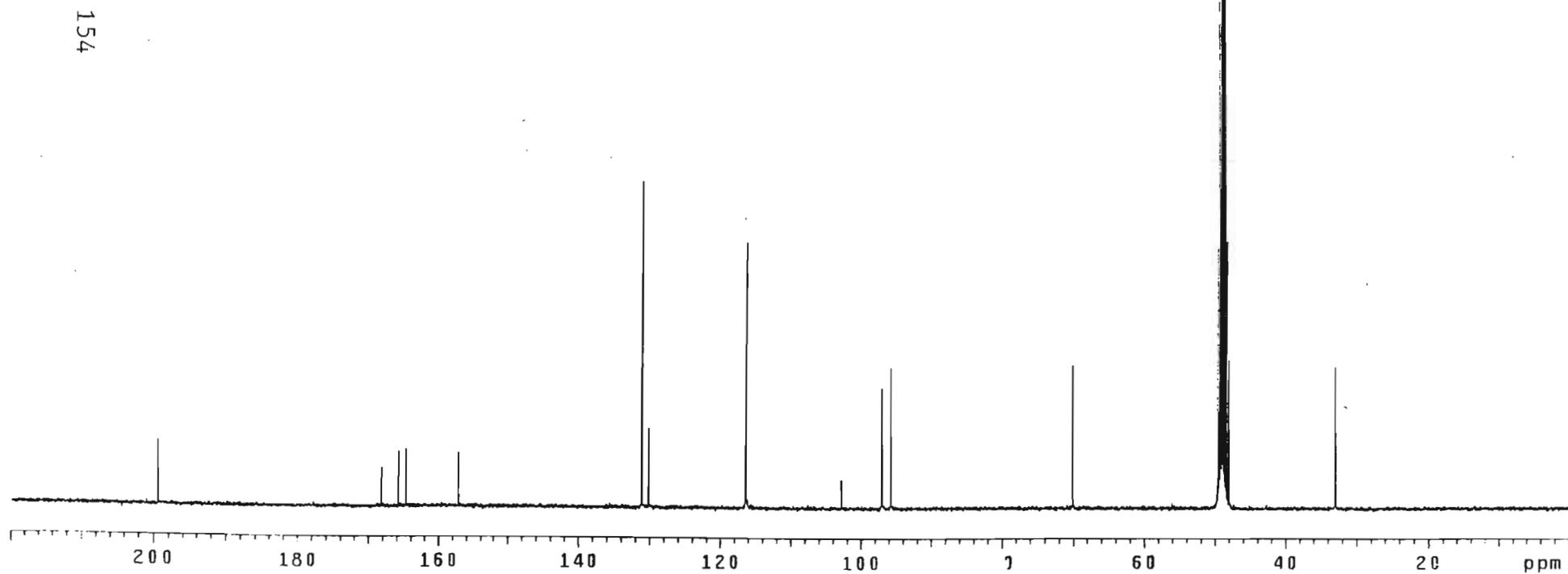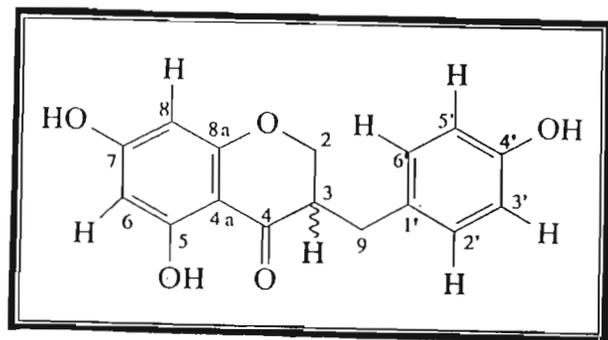
Spectrum 2.2: ^{13}C NMR spectrum of compound 2 (CD$_3$OD)
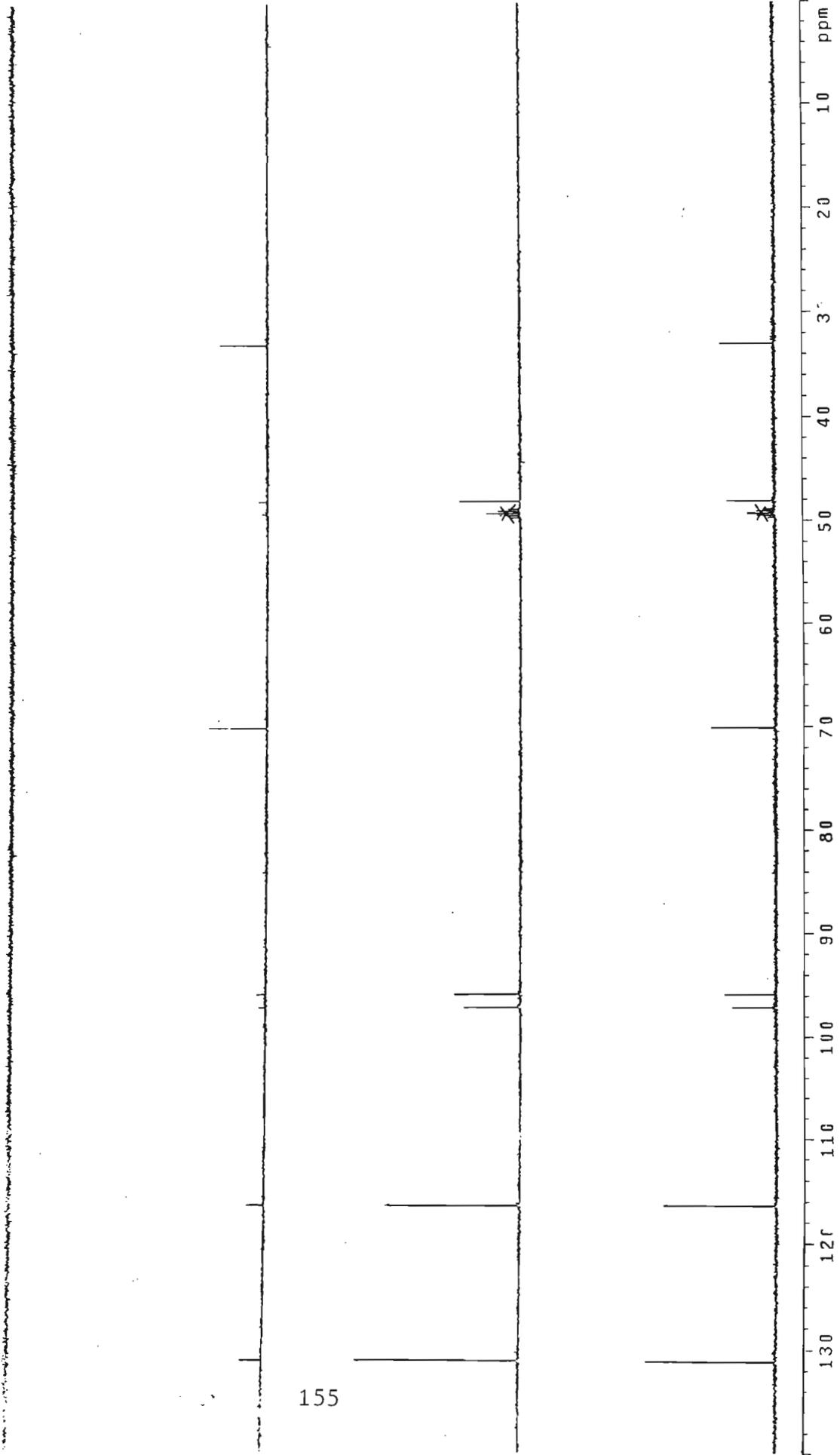
Spectrum 2.3: ADEPT spectrum of compound 2 (CD₃OD)
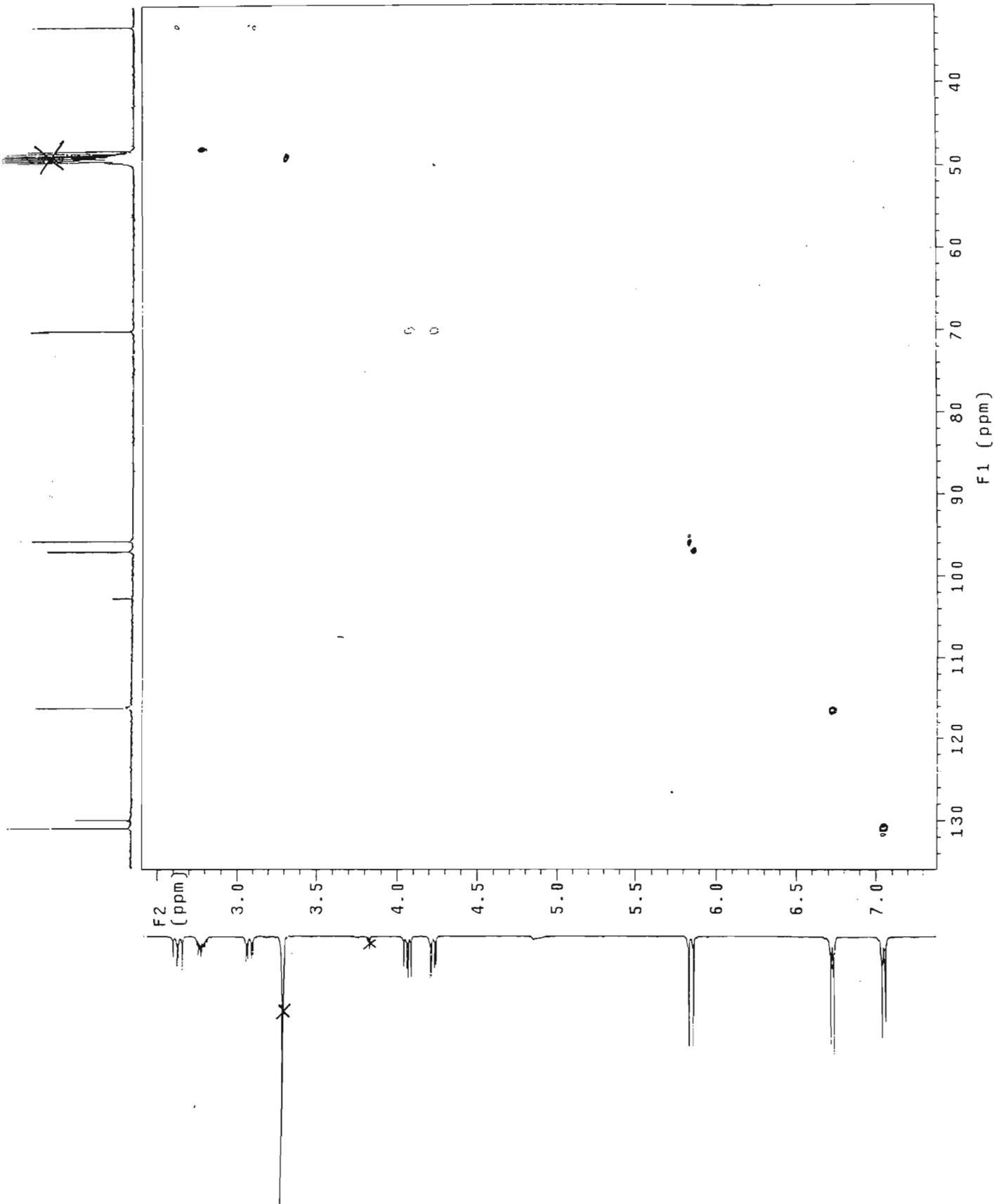
Spectrum 2.4: HSQC spectrum of compound 2 (CD3OD)
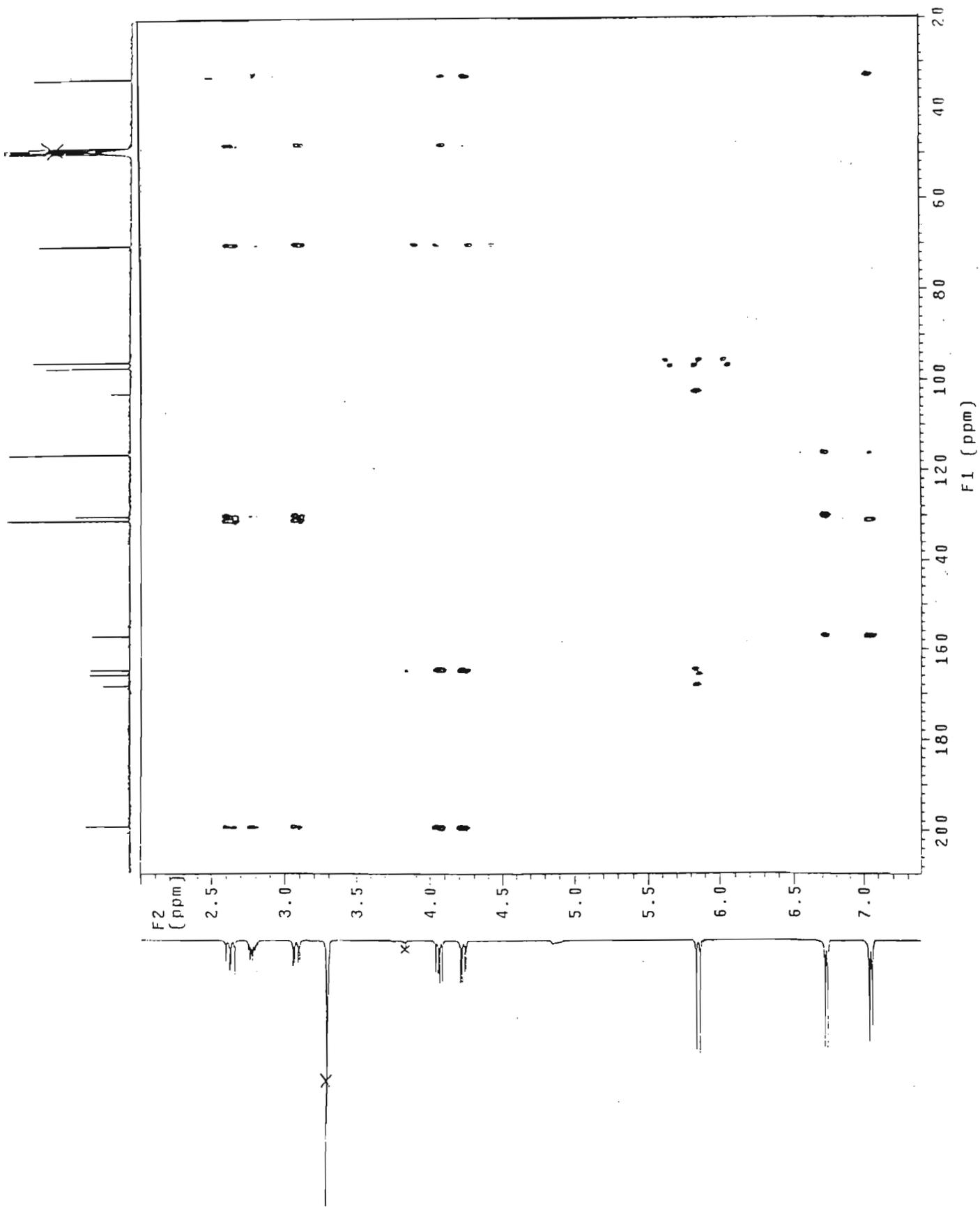
Spectrum 2.5: HMBC spectrum of compound 2 (CD₃OD)

157
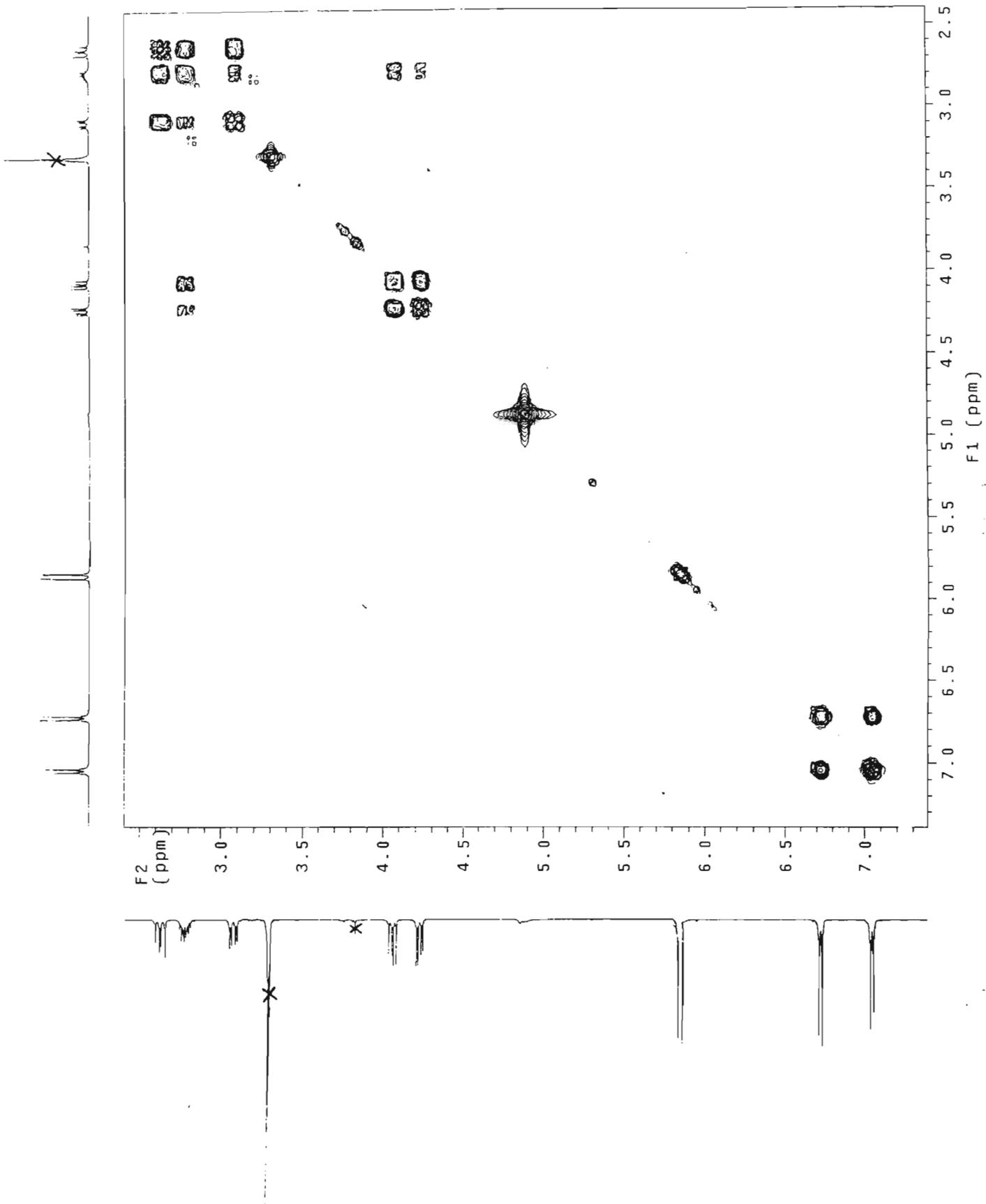
Spectrum 2.6: COSY spectrum of compound 2 (CD₃OD)
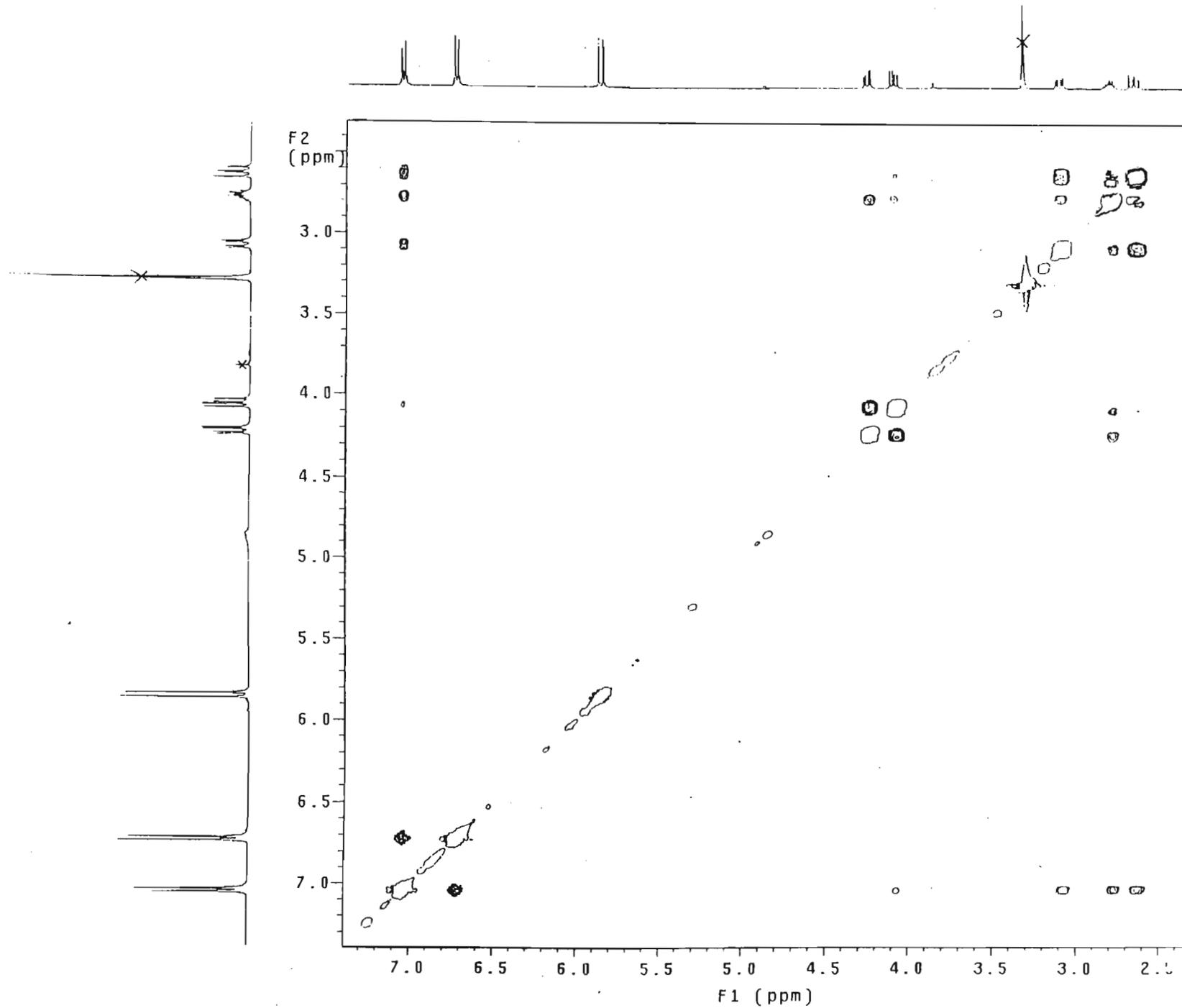
Spectrum 2.7: NOESY spectrum of compound 2 (CD₃OD)
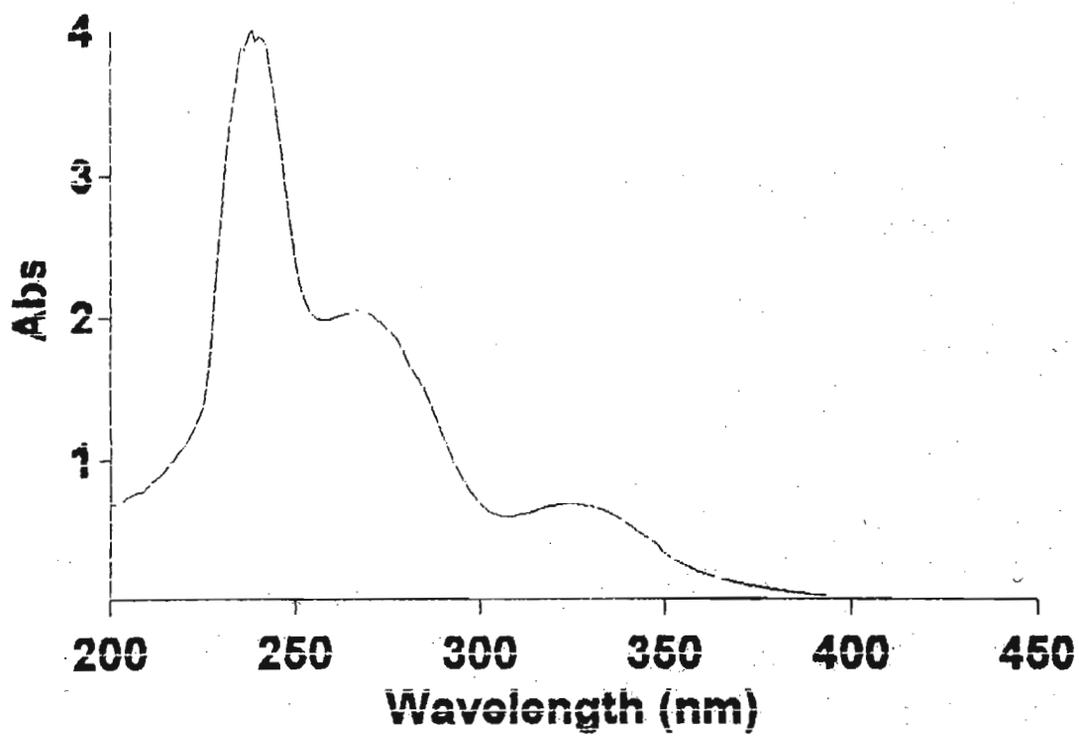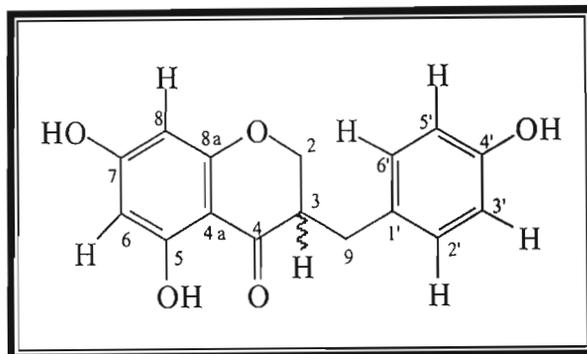
Spectrum 2.8: UV spectrum of compound 2

160
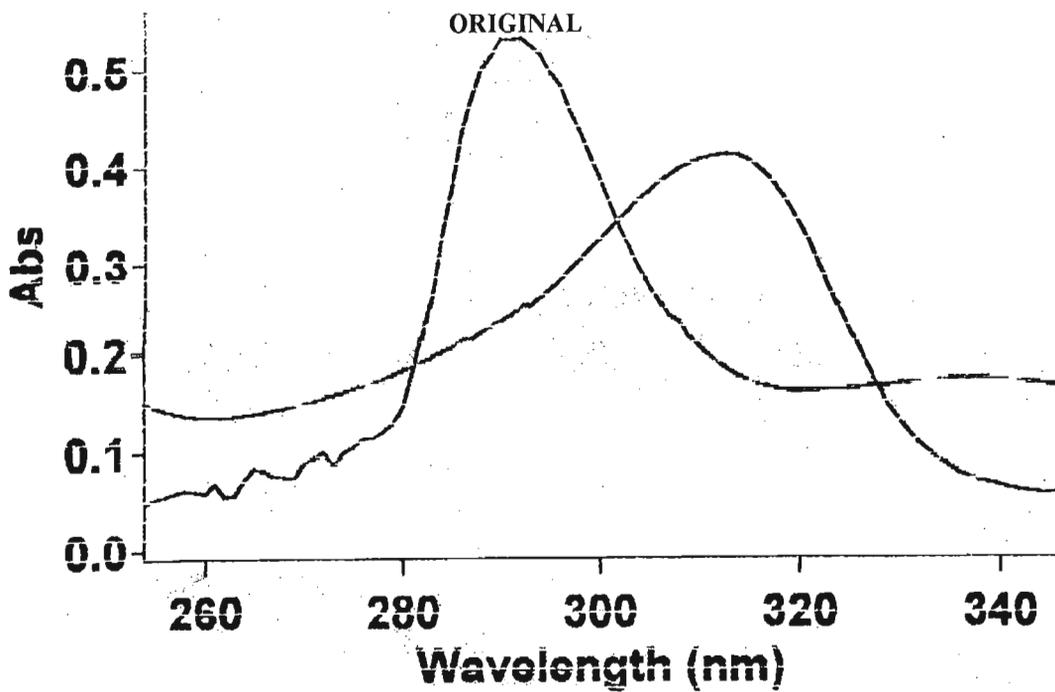
Spectrum 2.9: UV spectrum of compound 2 with AlCl$_3$
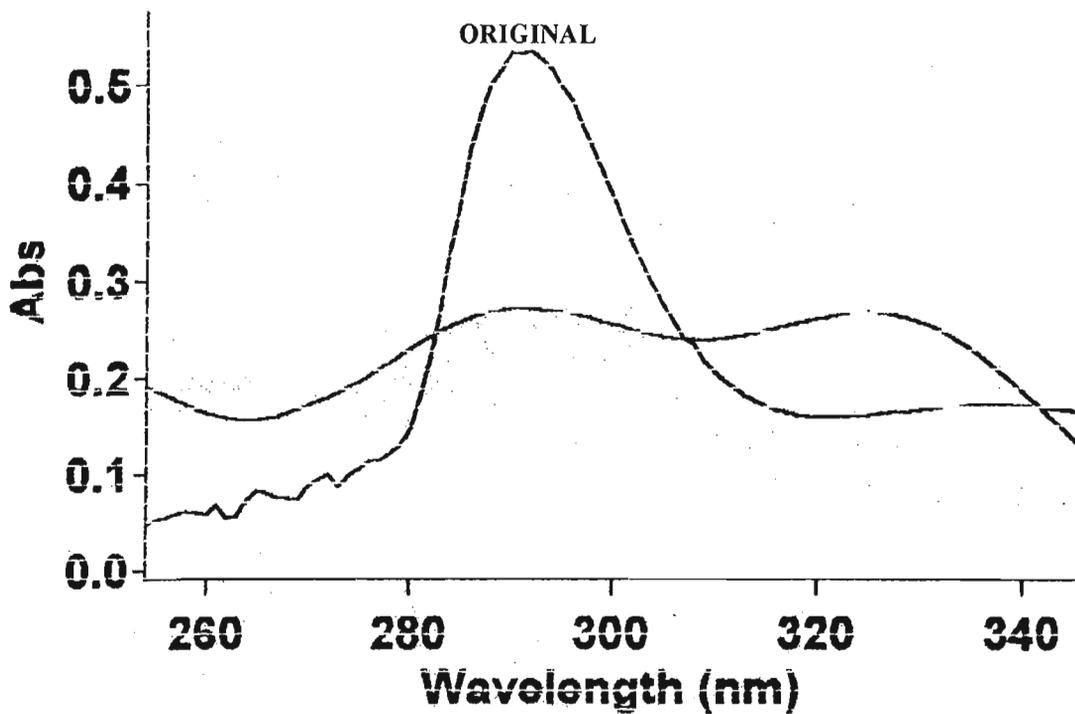
Spectrum 2.10: UV spectrum of compound 2 with NaOAc
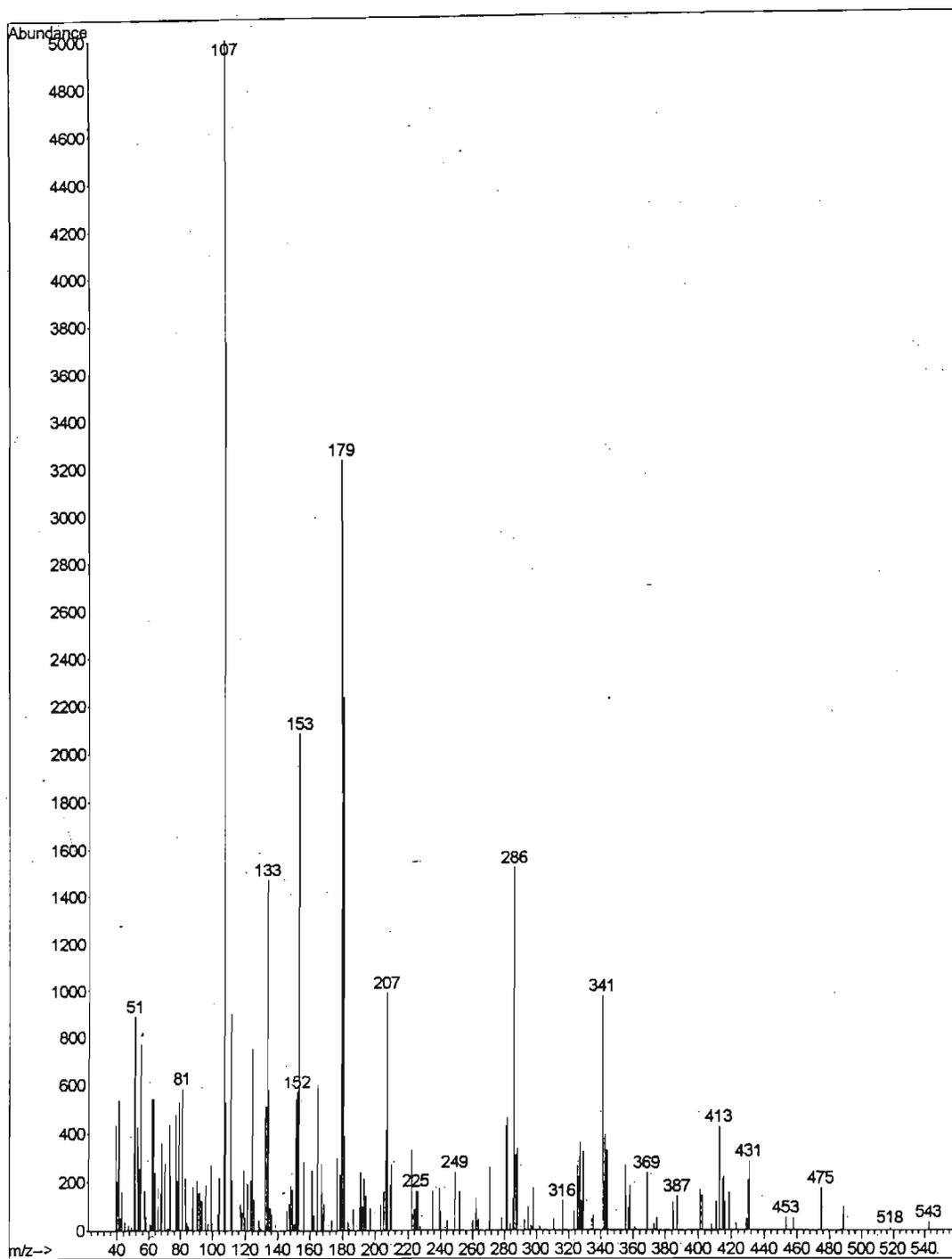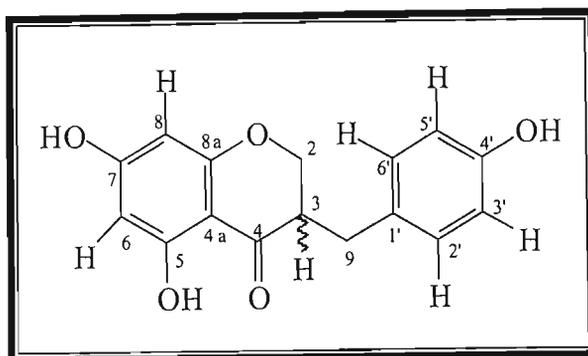
Spectrum 2.11: Mass spectrum of compound 2

162

100

99

98

97 — 2851.5

96 — 2920.4

95

94 — 3373.7 1640.7

93 — 1520.2

92

91

90

89

88

3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000

Wavenumbers (cm-1)

1267.7

1457.1

1170.2

%Transmittance

163
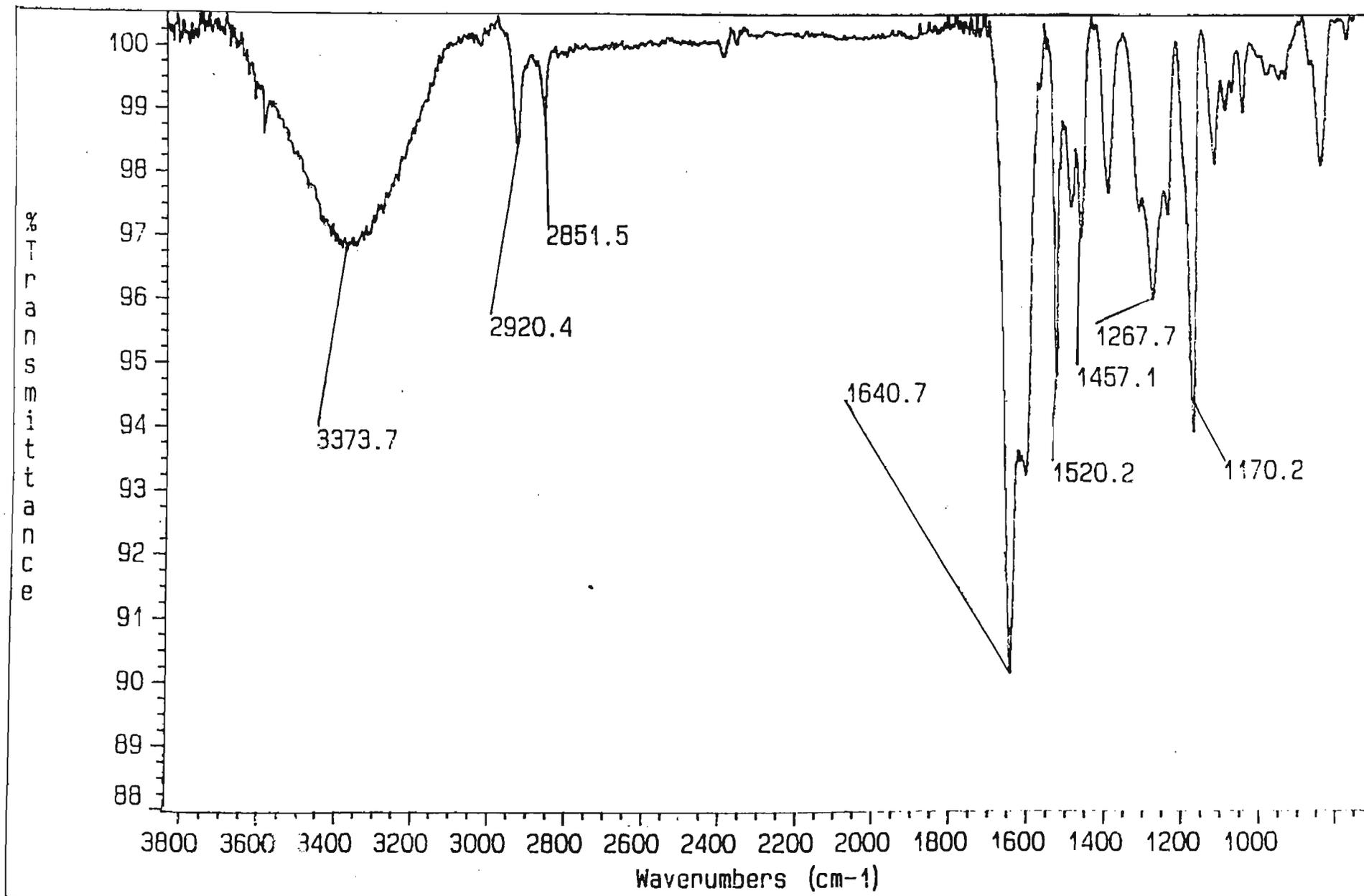
Spectrum 2.12: IR spectrum of compound 2
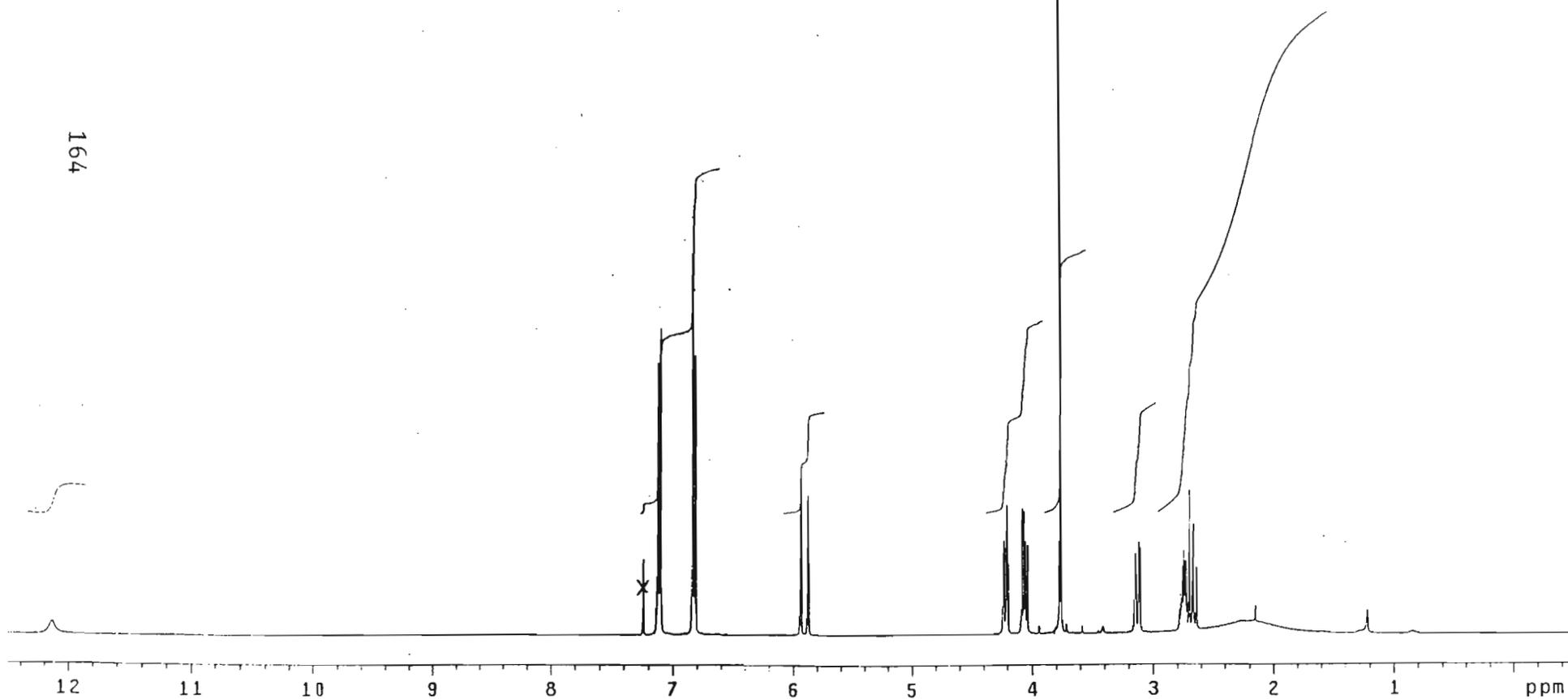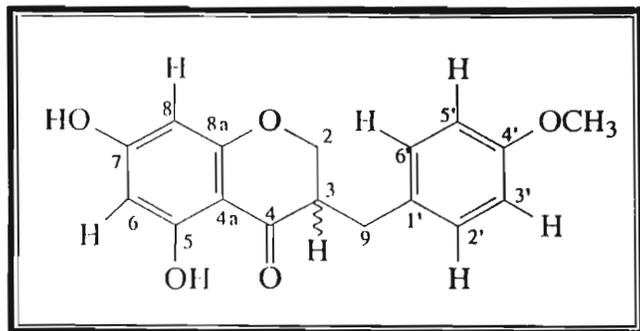
Spectrum 3.1: ^{1}H NMR spectrum of compound 3 (CDCl$_3$)
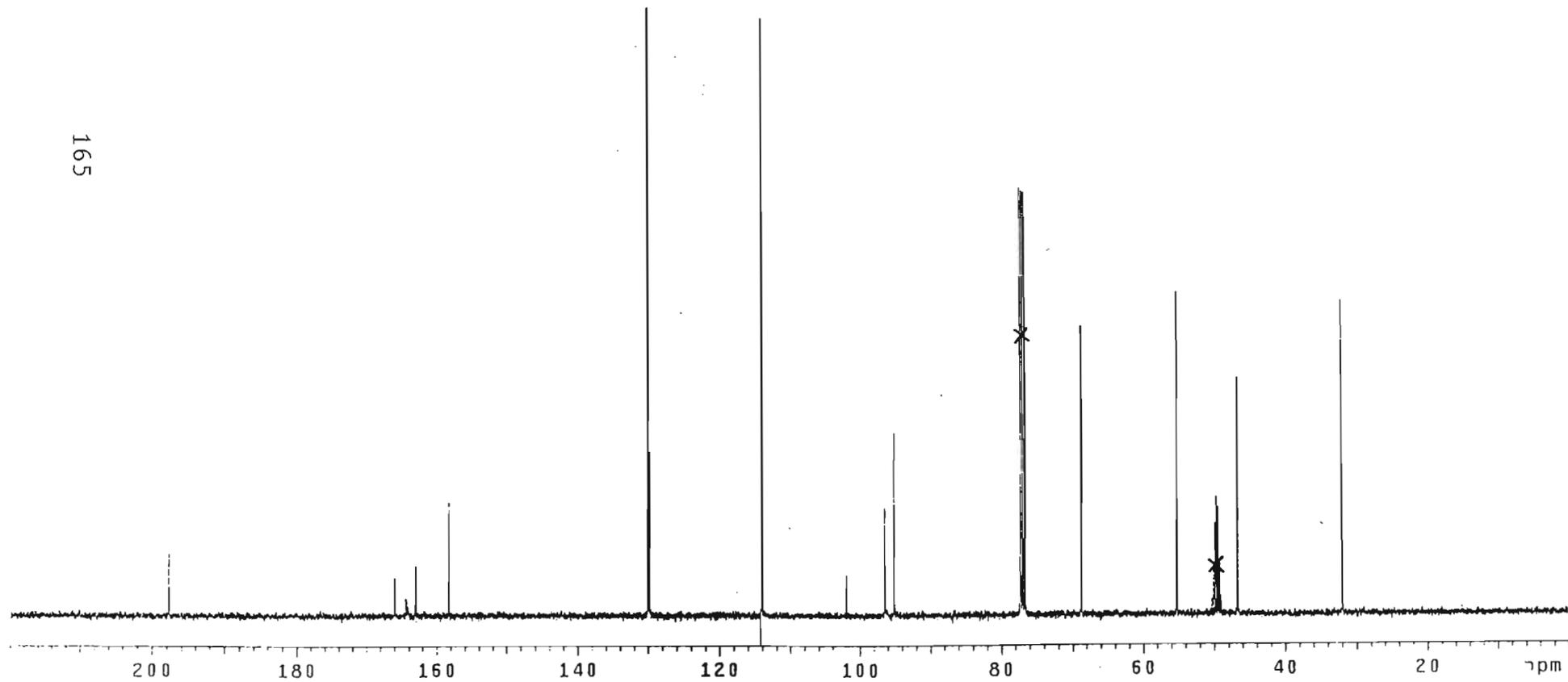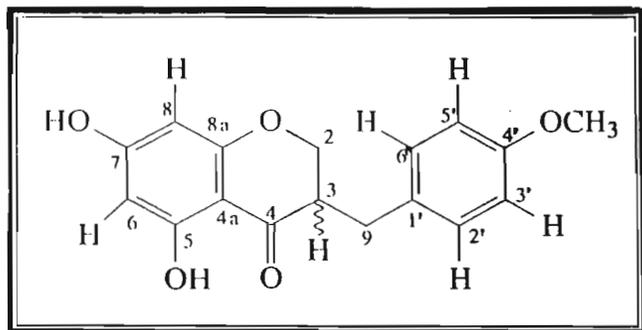
Spectrum 3.2: ^{13}C NMR spectrum of compound 3 (CDCl$_3$)
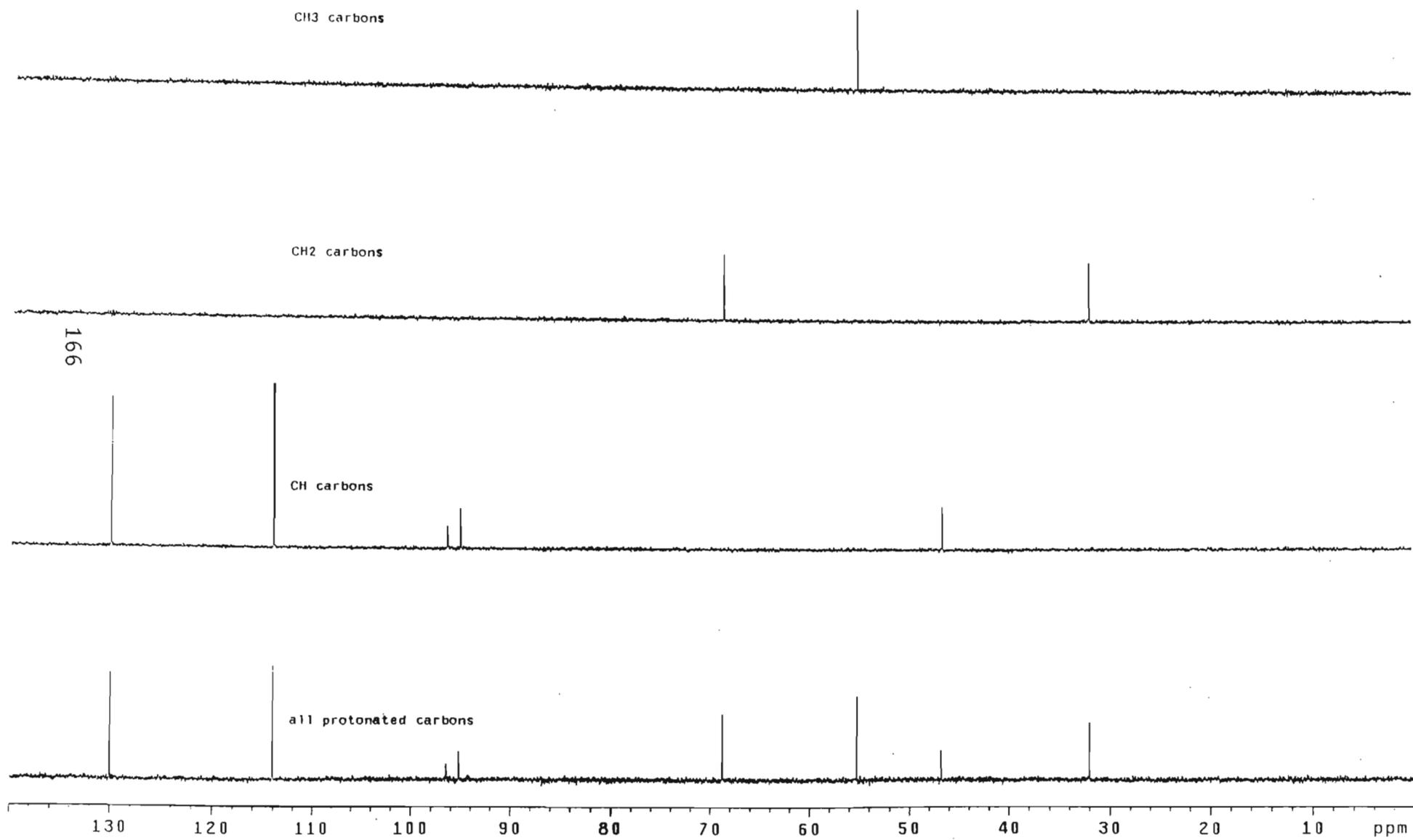
Spectrum 3.3: ADEPT spectrum of compound 3 (CDCl₃)
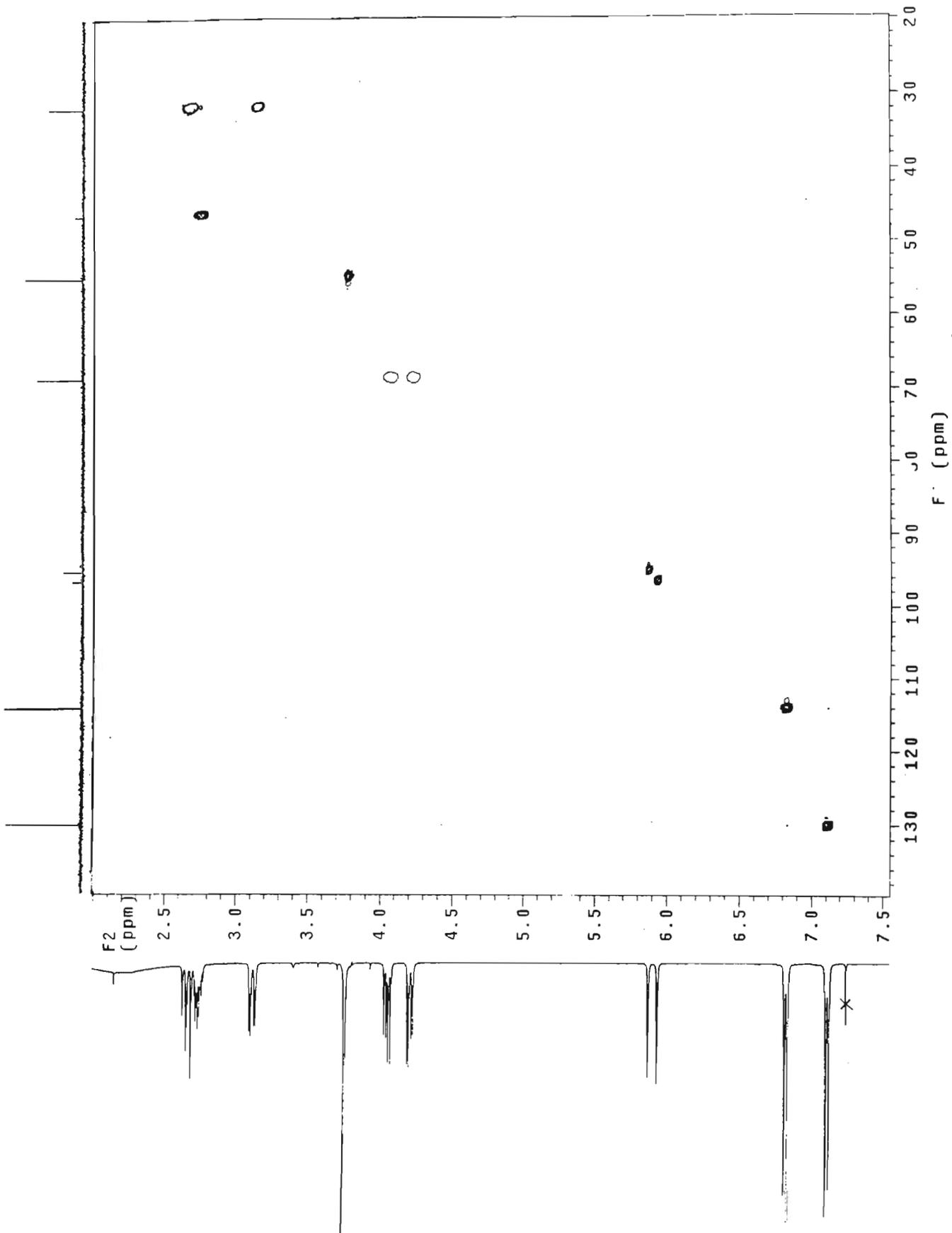
Spectrum 3.4: HSQC spectrum of compound 3 (CDCl₃)

167
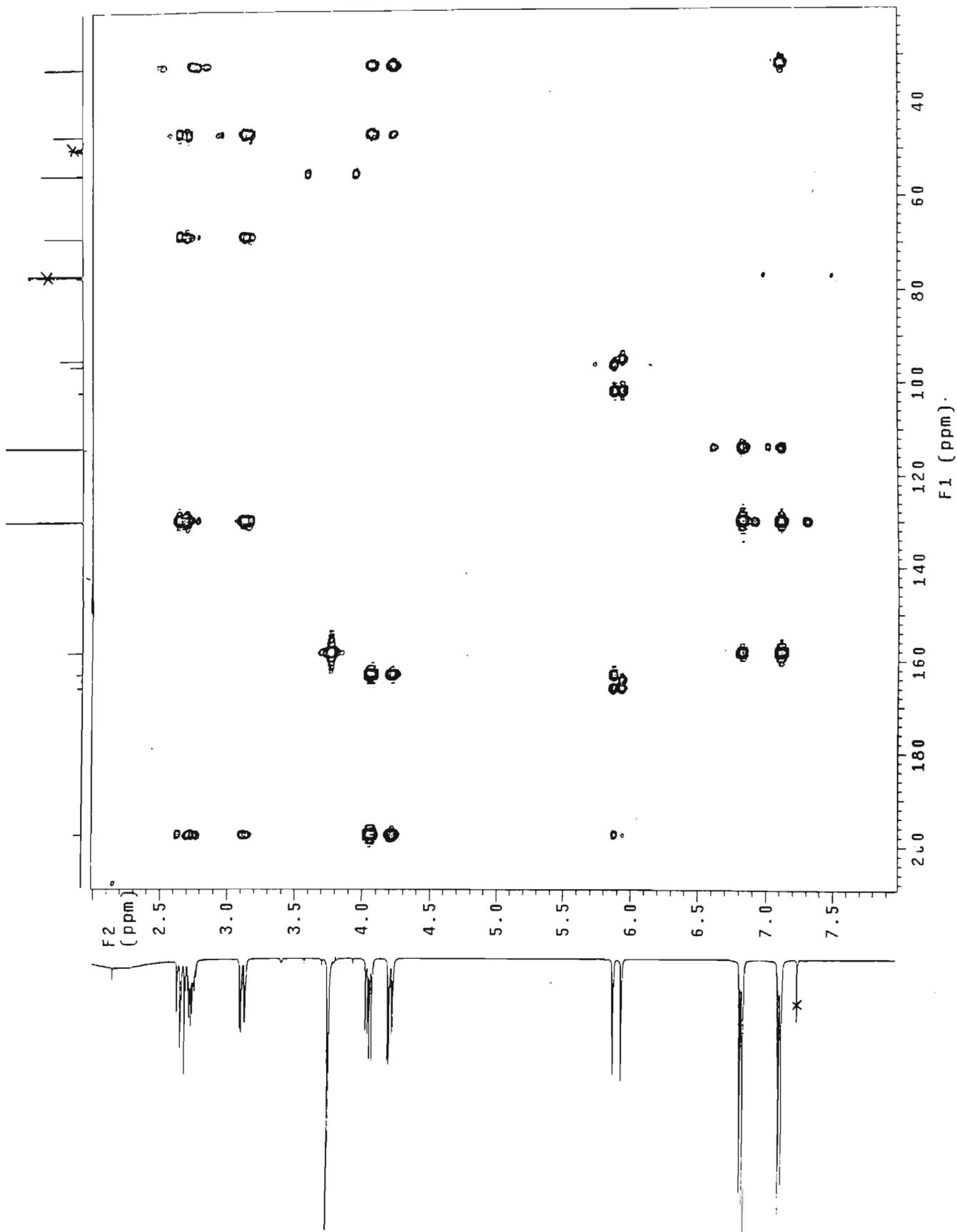
Spectrum 3.5: HMBC spectrum of compound 3 (CDCl₃)
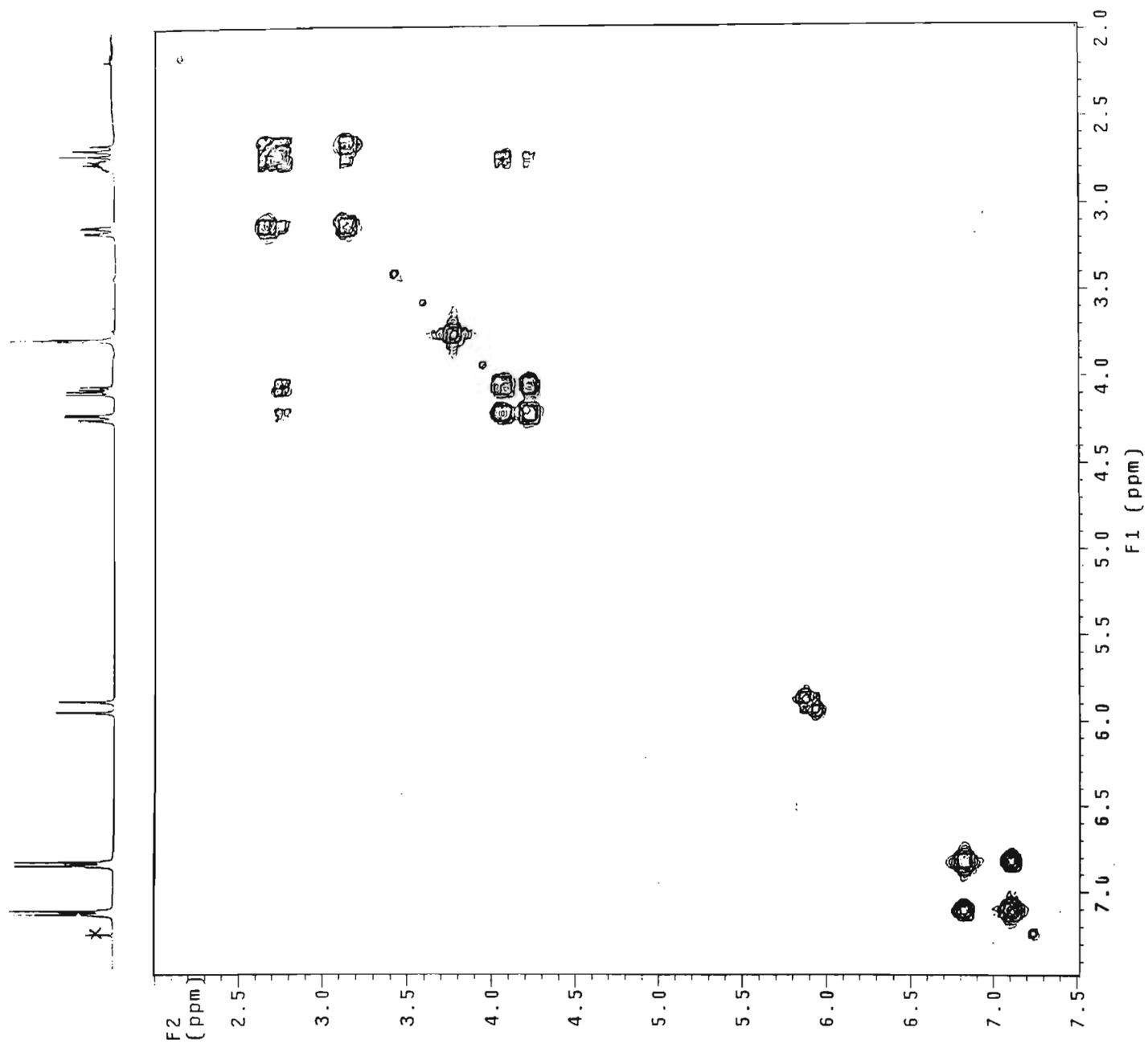
Spectrum 3.6: COSY spectrum of compound 3 (CDCl₃)

F1 (ppm)

F2 (ppm)
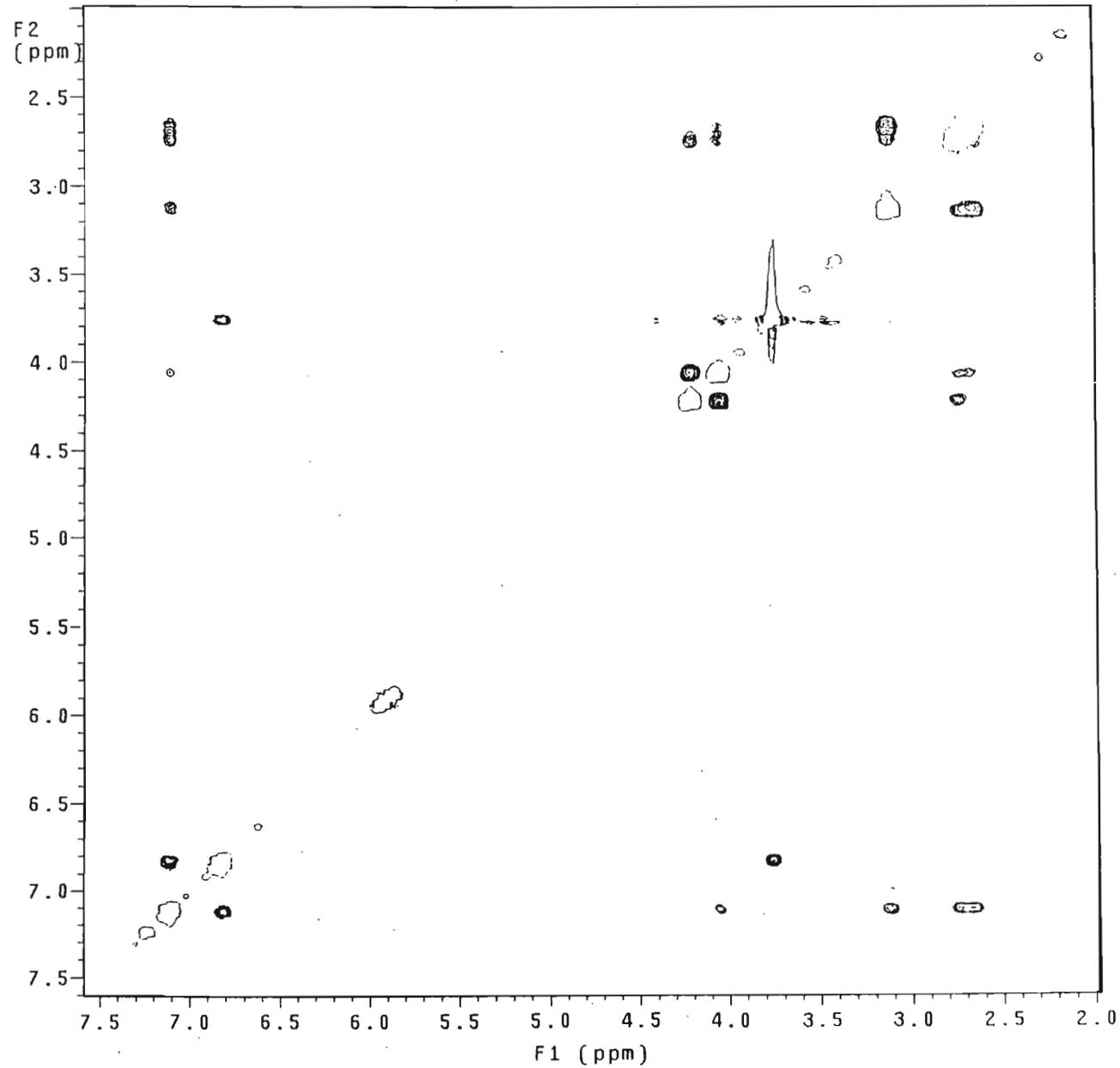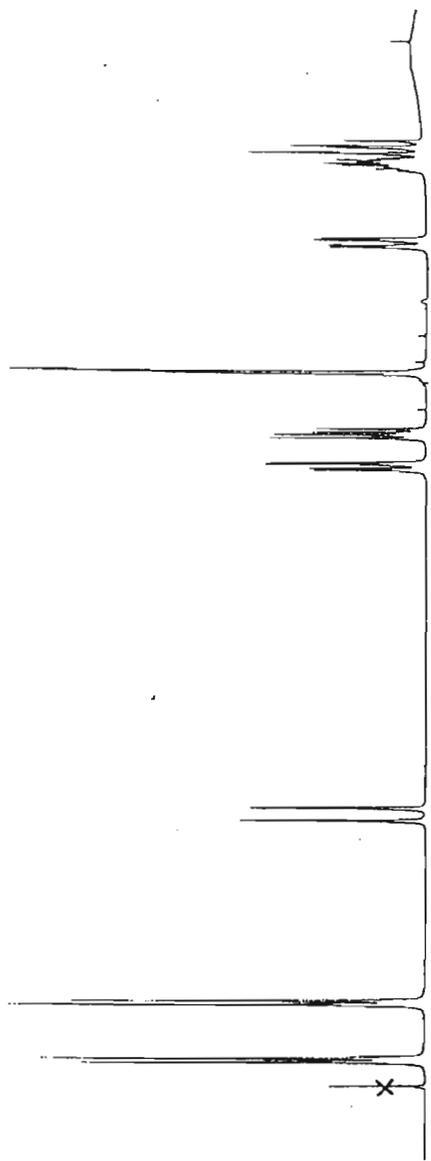
Spectrum 3.7: NOESY spectrum of compound 3 (CDCl₃)
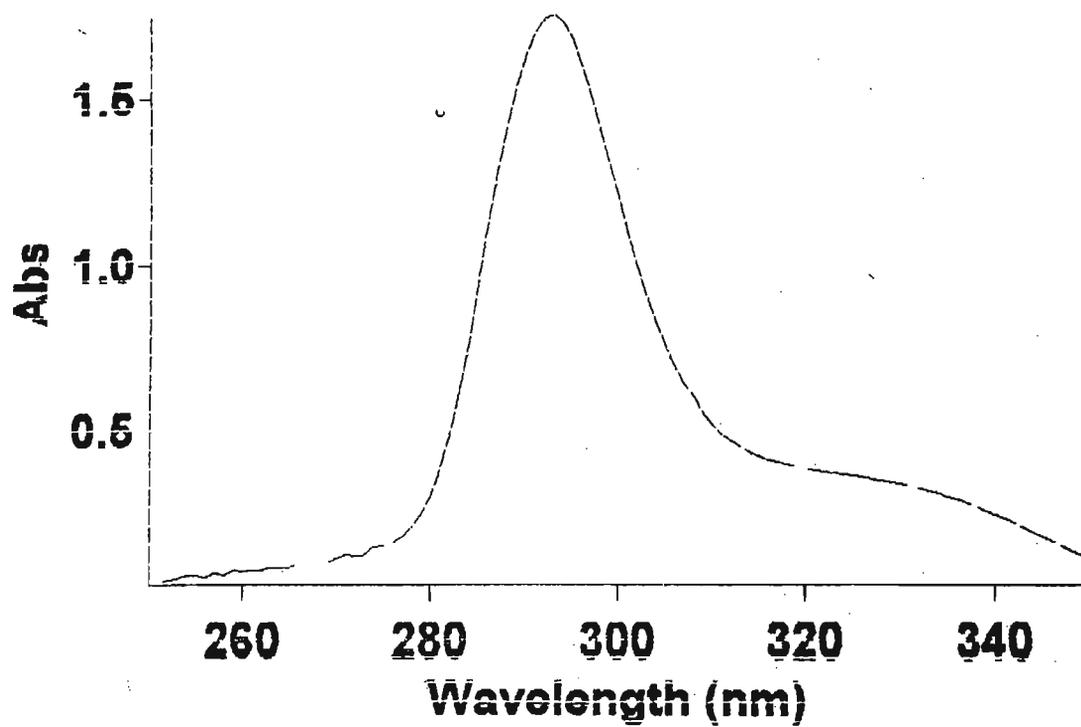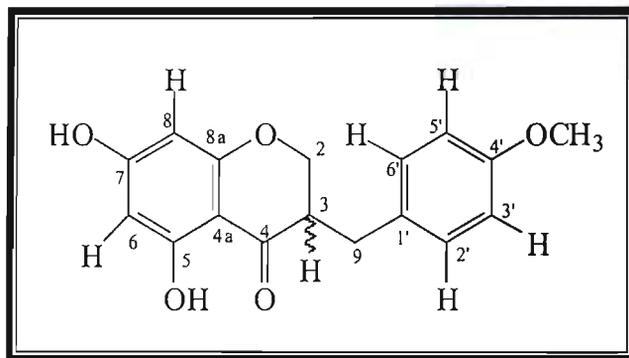
Spectrum 3.8: UV spectrum of compound 3

ORIGINAL
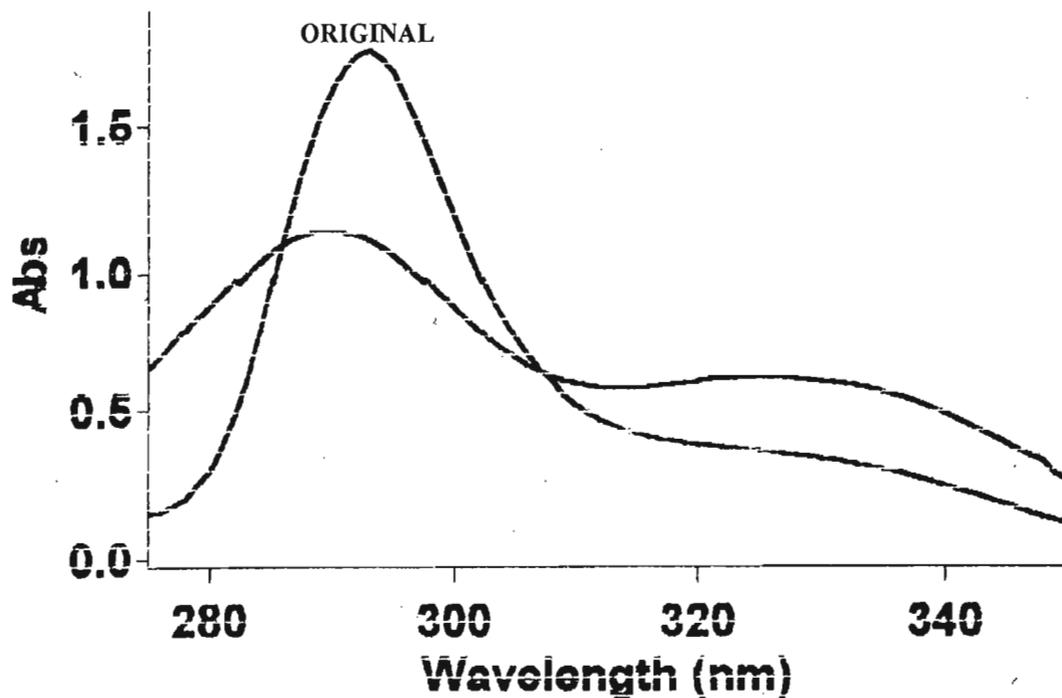
Spectrum 3.9: UV spectrum of compound 3 with AlCl₃

ORIGINAL
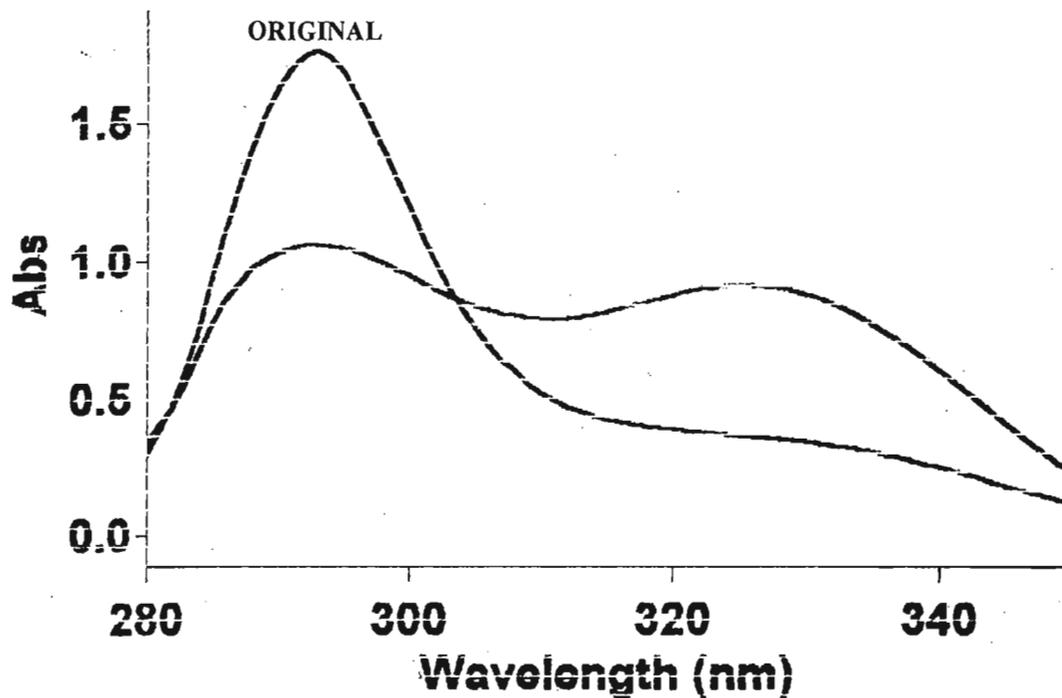
Spectrum 3.10: UV spectrum of compound 3 with NaOAC

172
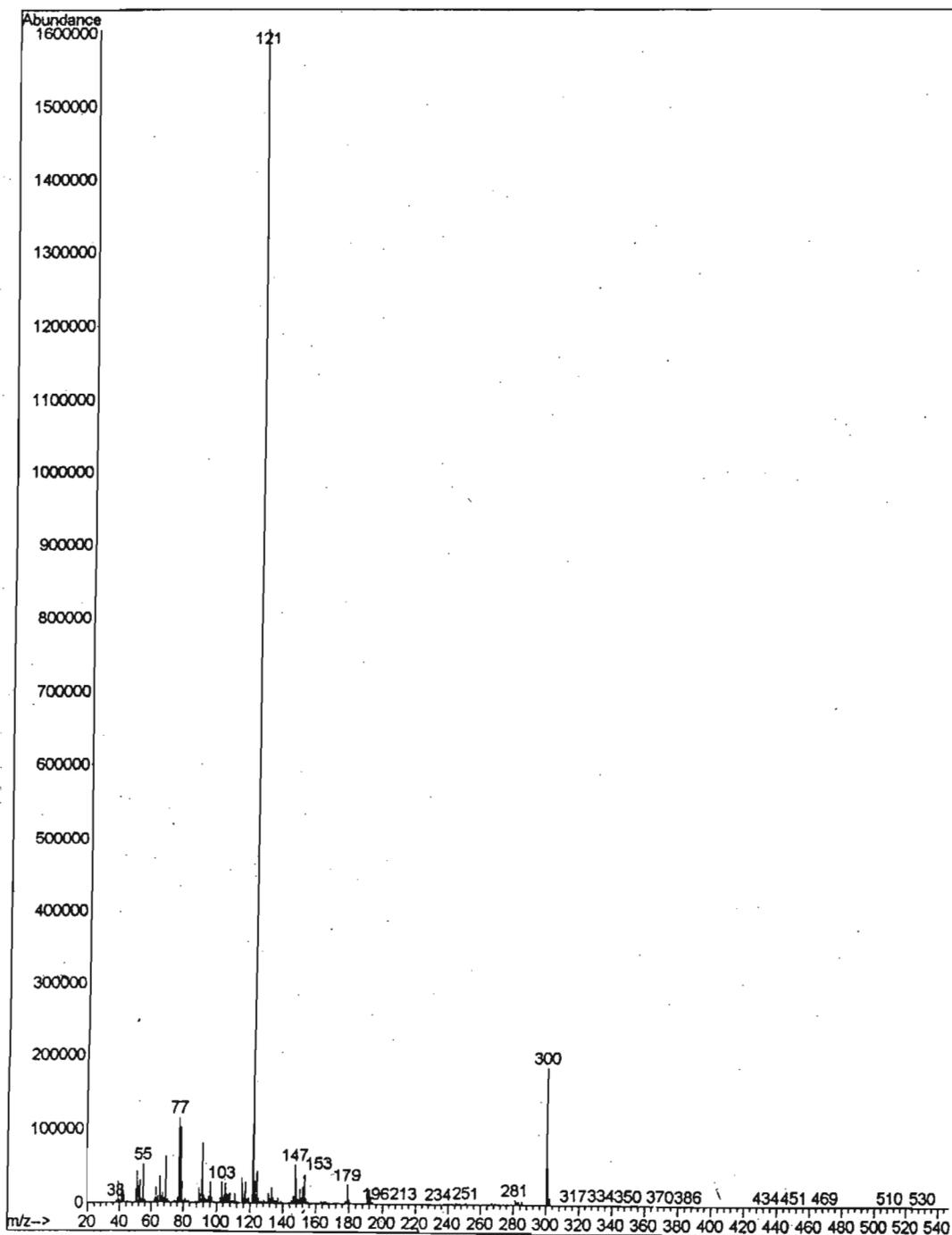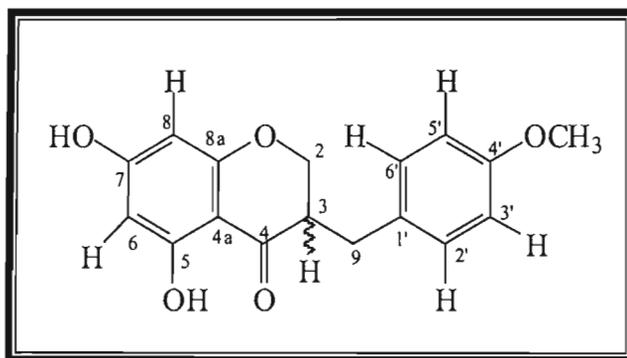
Spectrum 3.11: Mass spectrum of compound 3

173
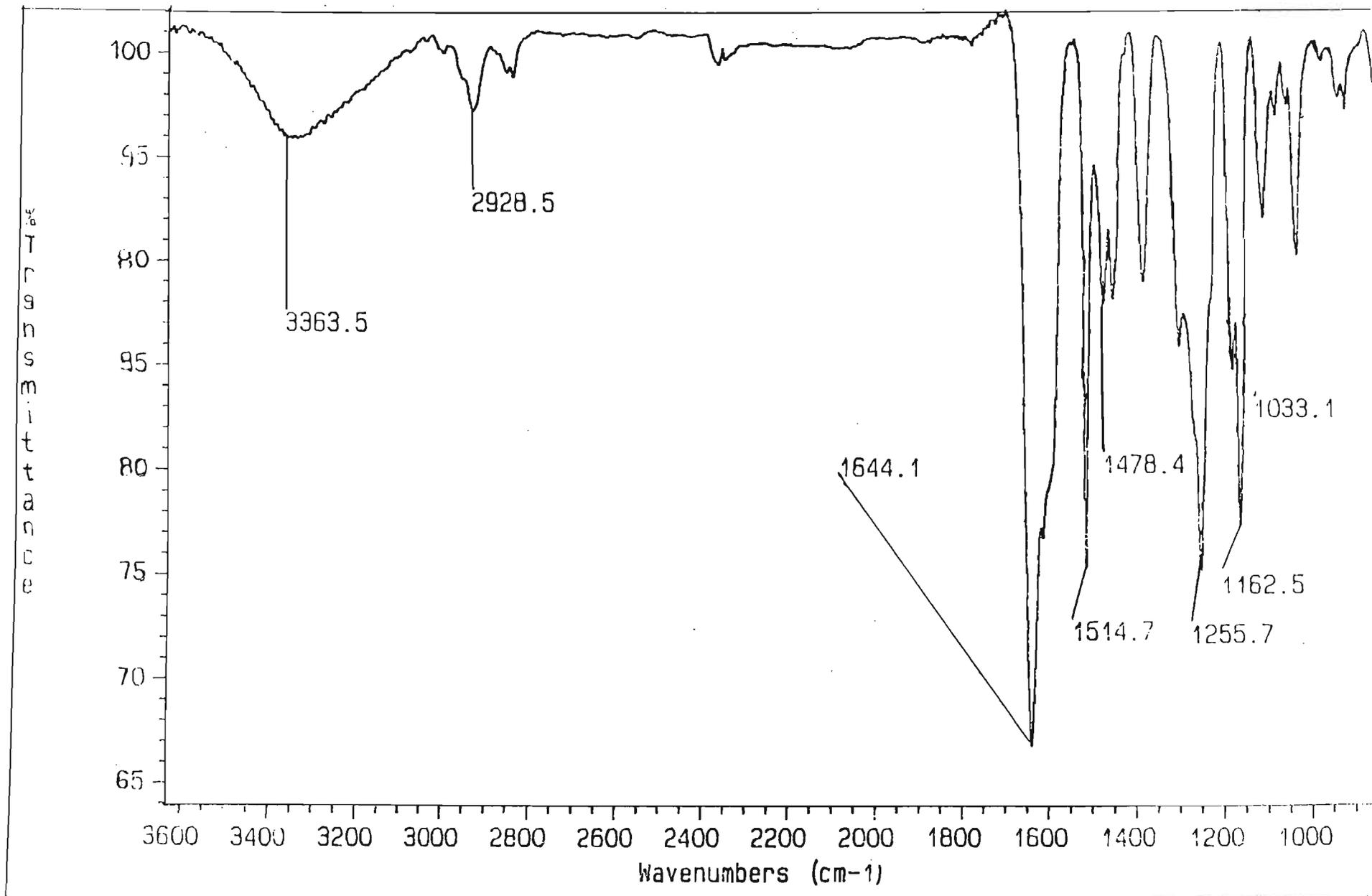
Spectrum 3.12: IR spectrum of compound 3
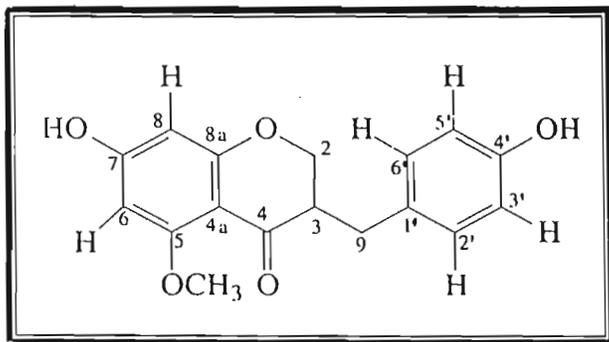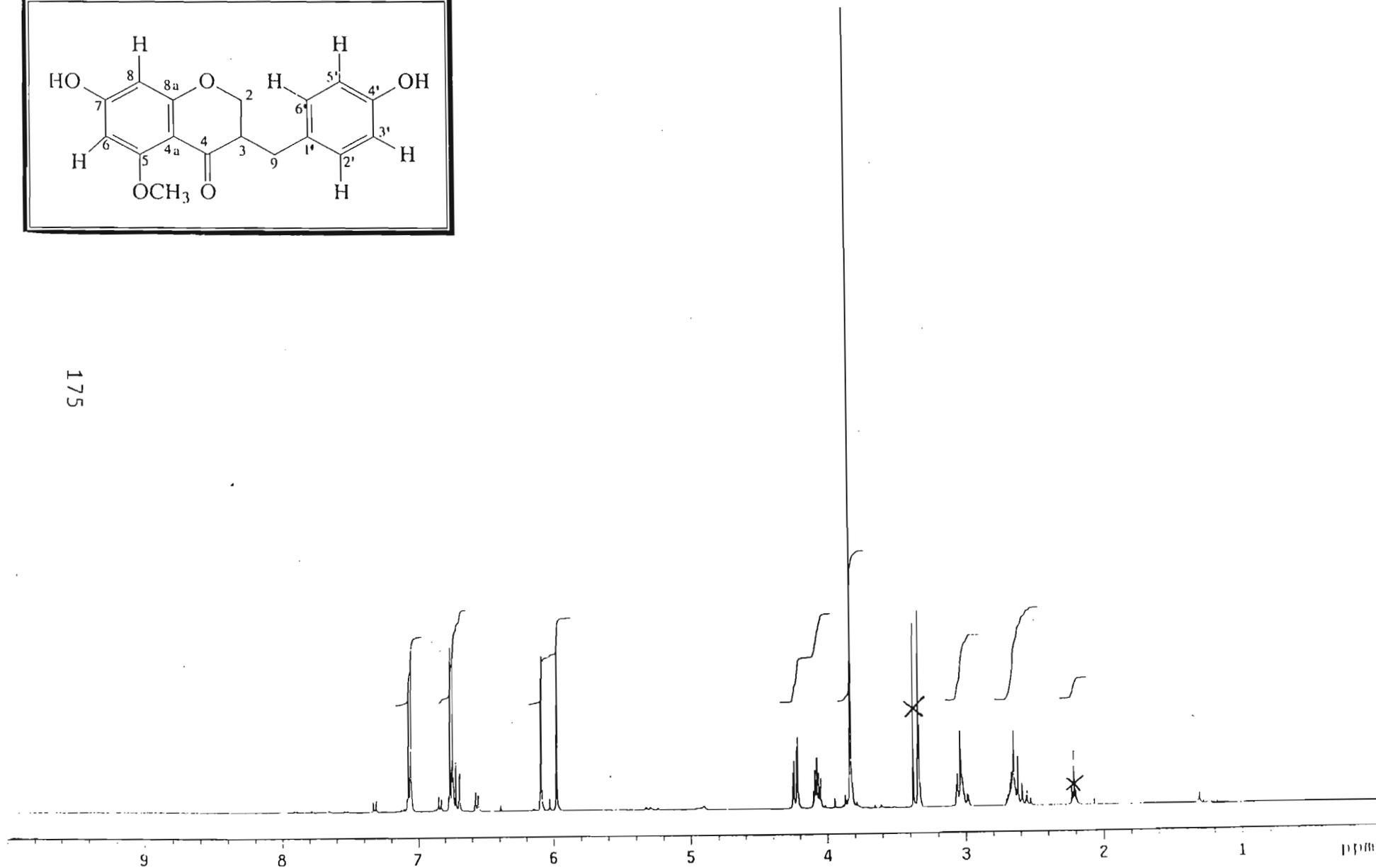
Spectrum 4.1: ¹H NMR spectrum of compound 4 (CD₃OD)

175
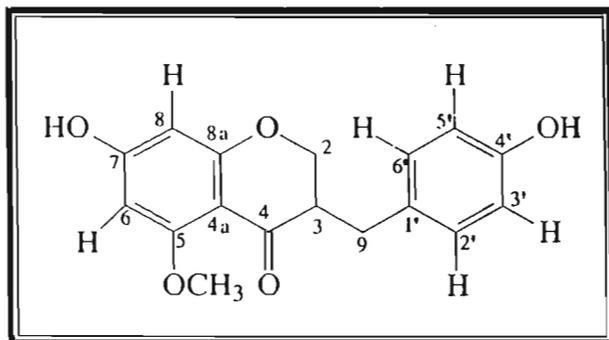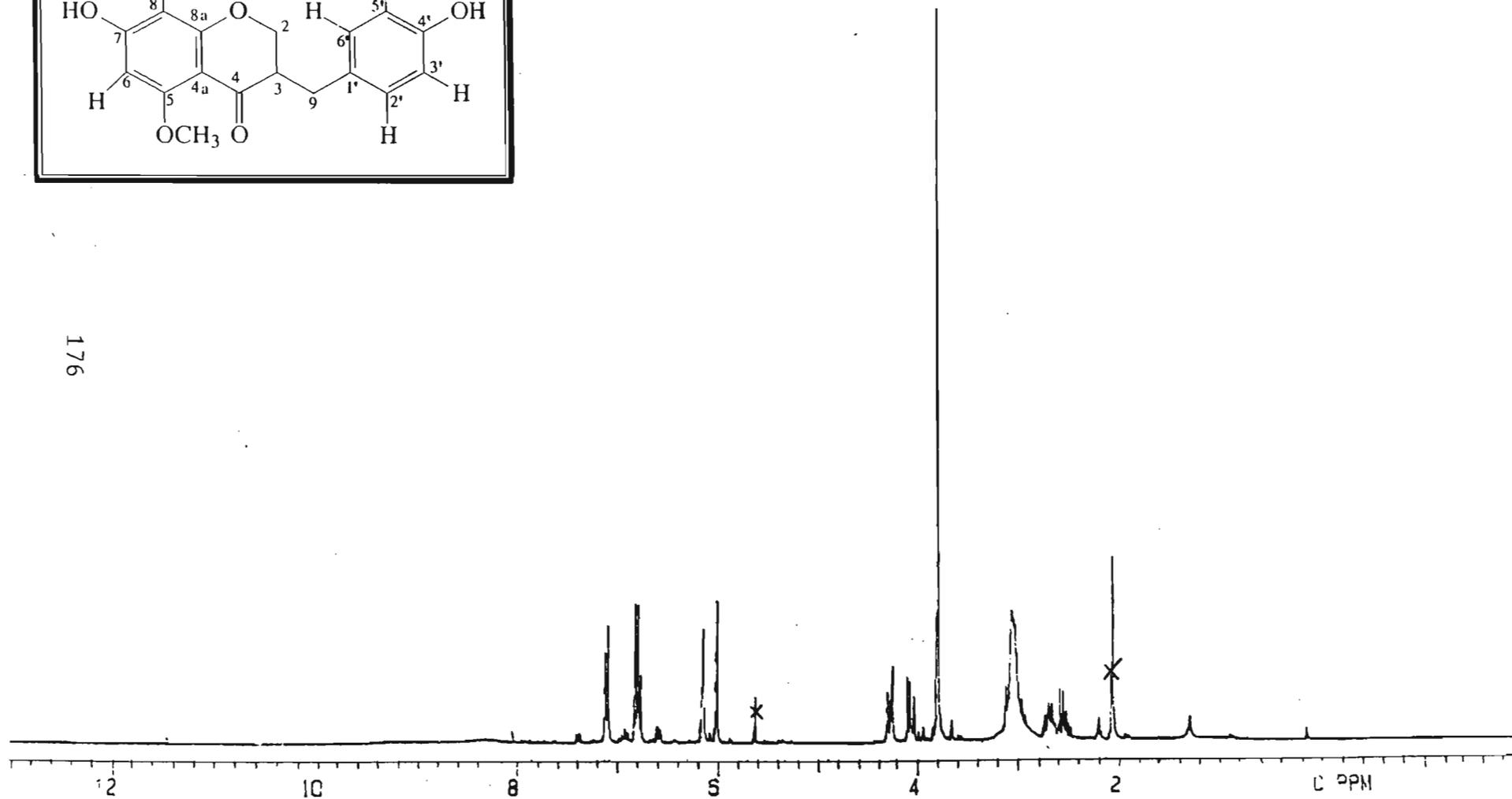
Spectrum 4.2: ^{1}H NMR spectrum of compound 4 ((CD$_3$)2CO)

176
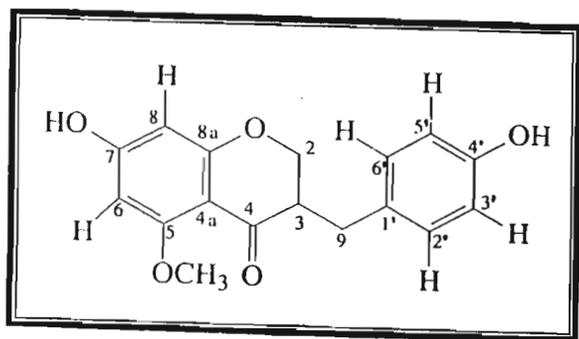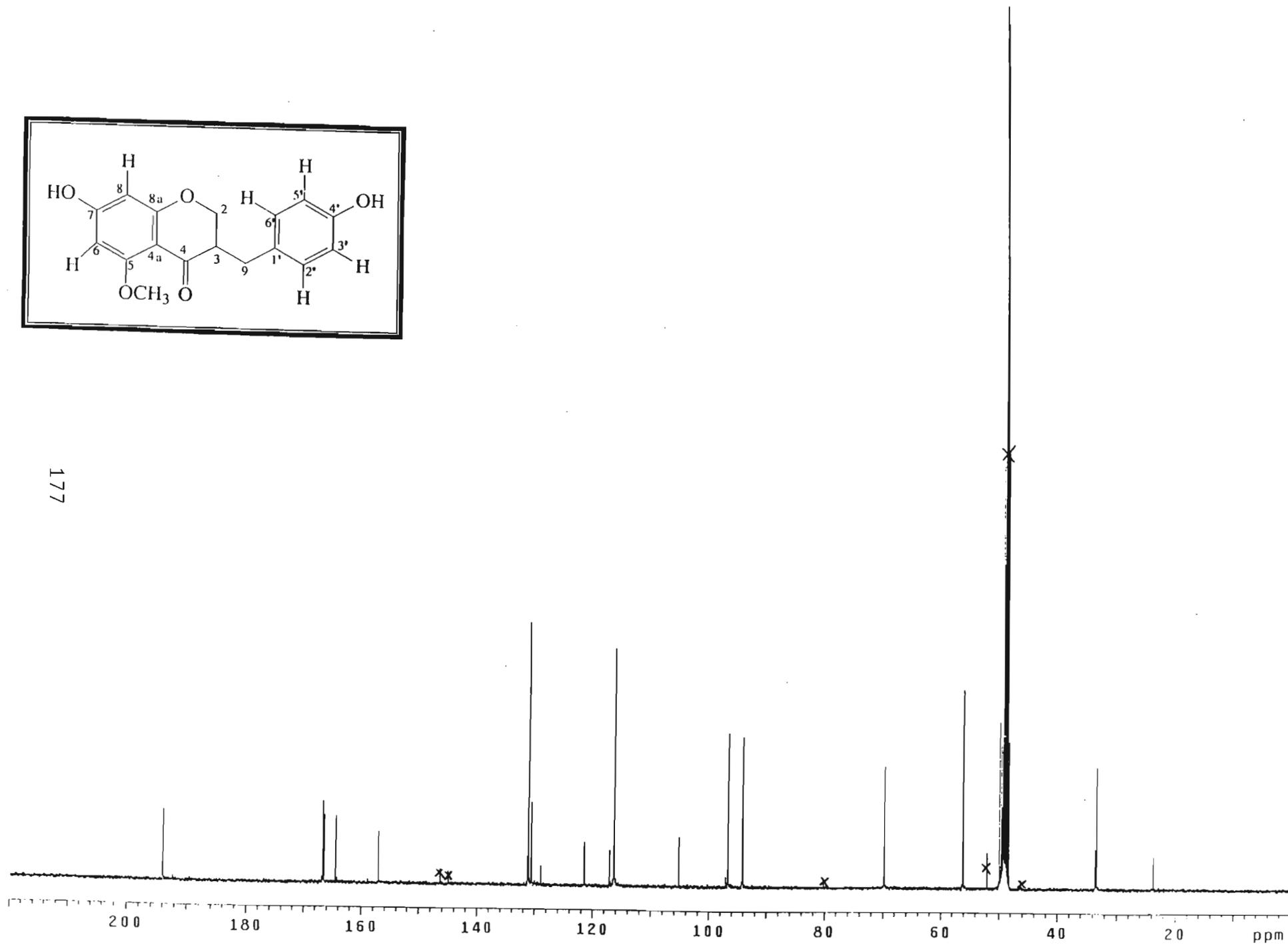
Spectrum 4.3: ^{13}C NMR spectrum of compound 4 (CD$_3$OD)

177
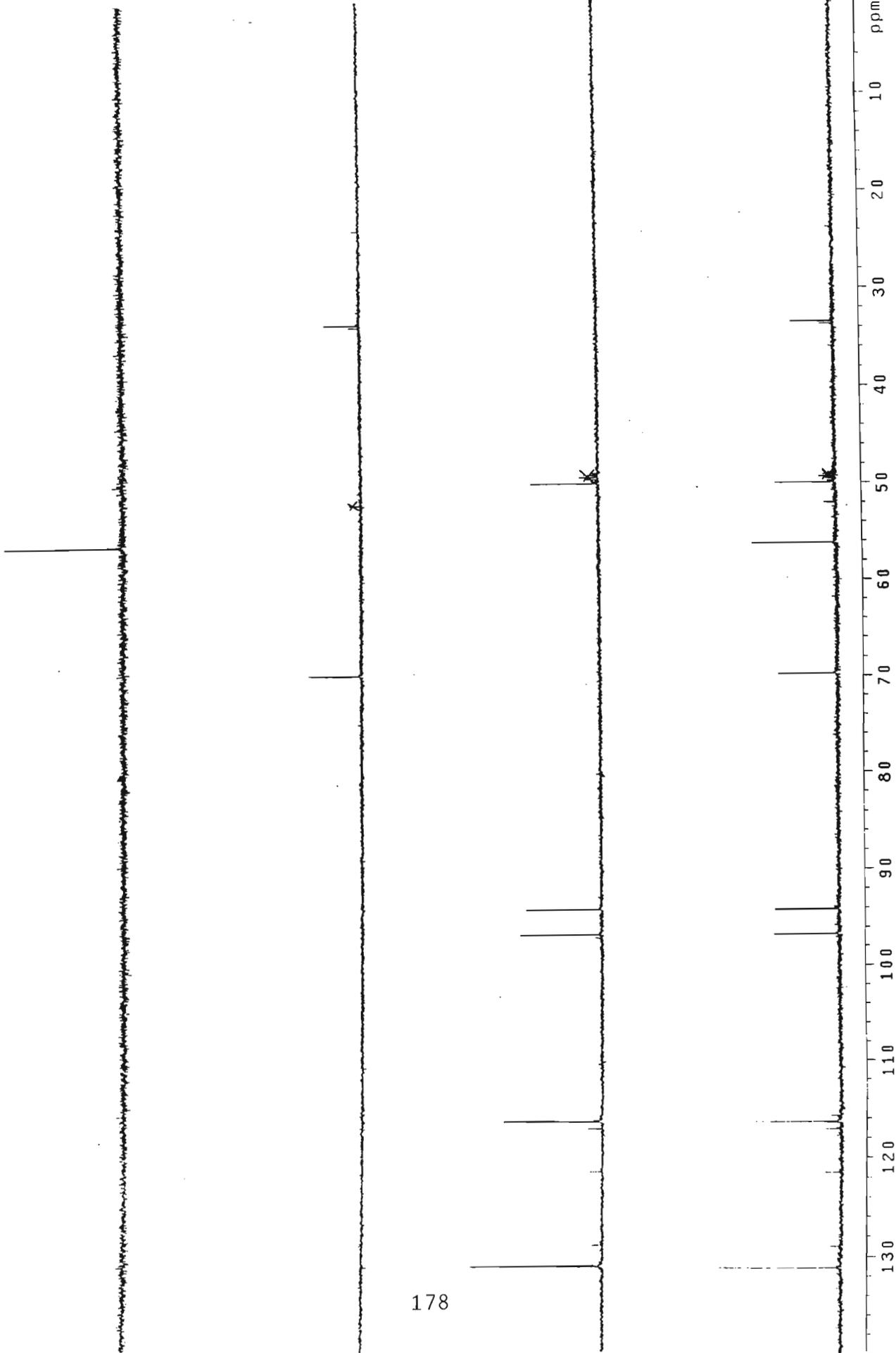
Spectrum 4.4: ADEPT spectrum of compound 4 (CD₃OD)
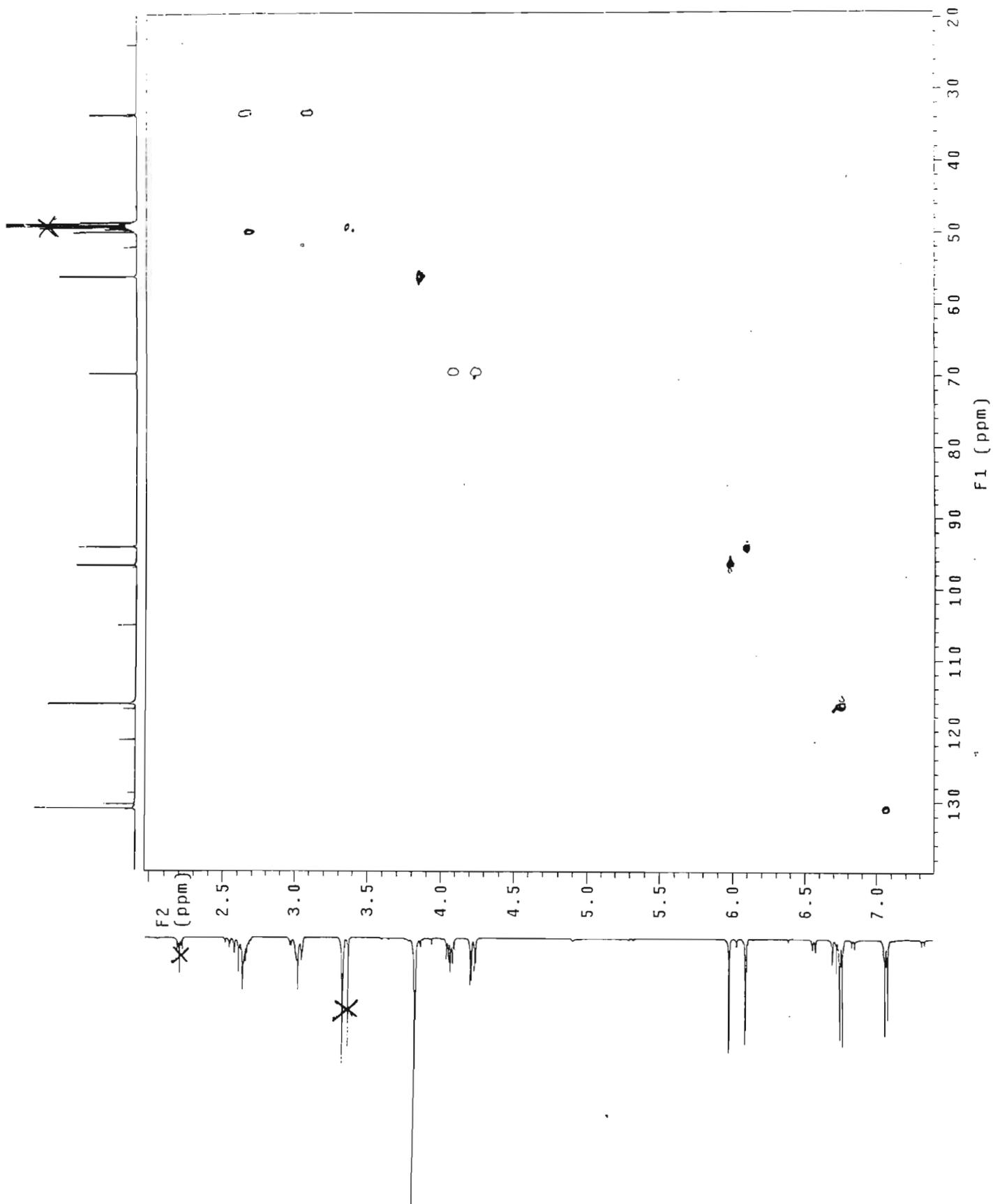
Spectrum 4.5: HSQC spectrum of compound 4 (CD$_3$OD)
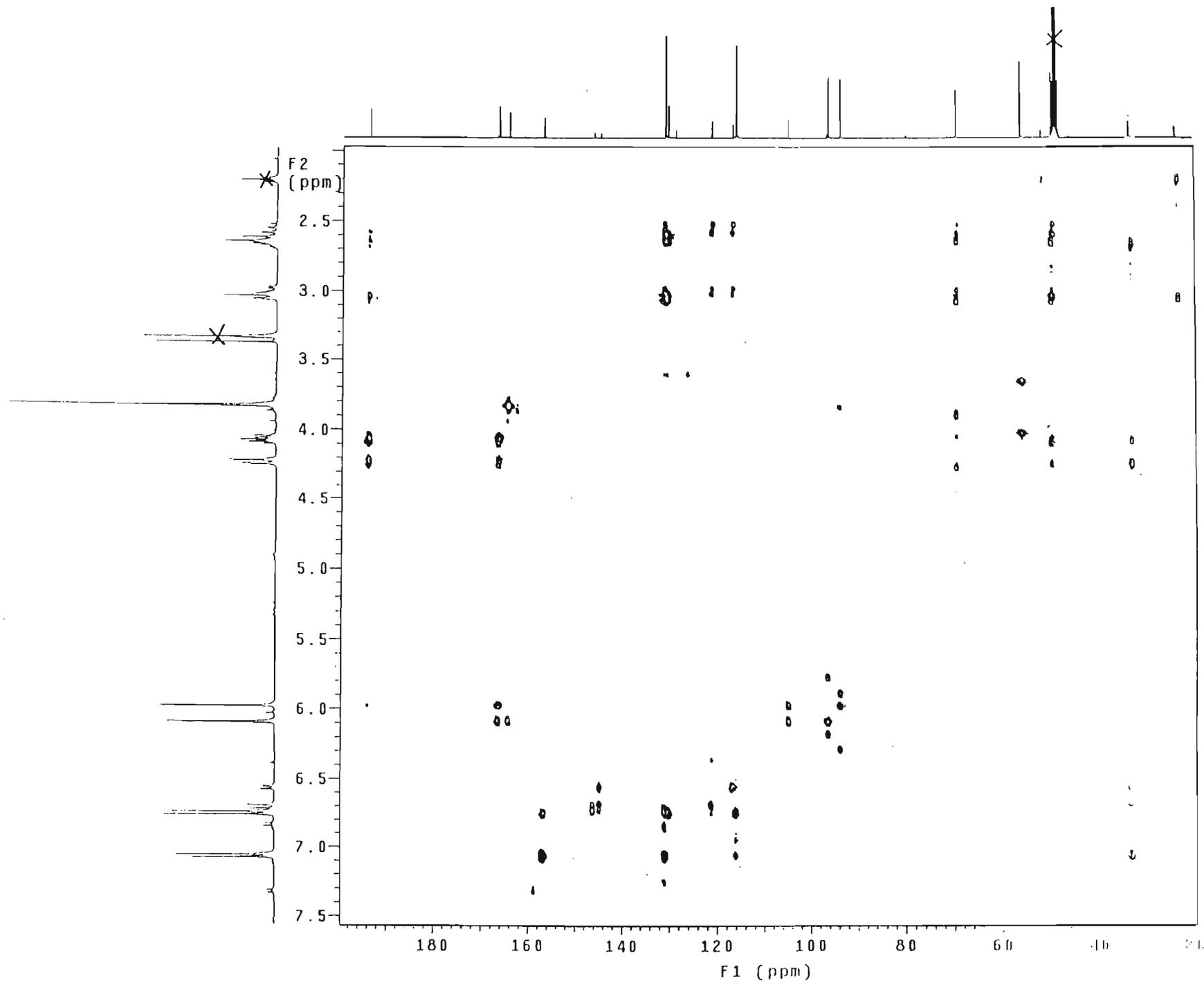
Spectrum 4.6: HMBC spectrum of compound 4 (CD₃OD)
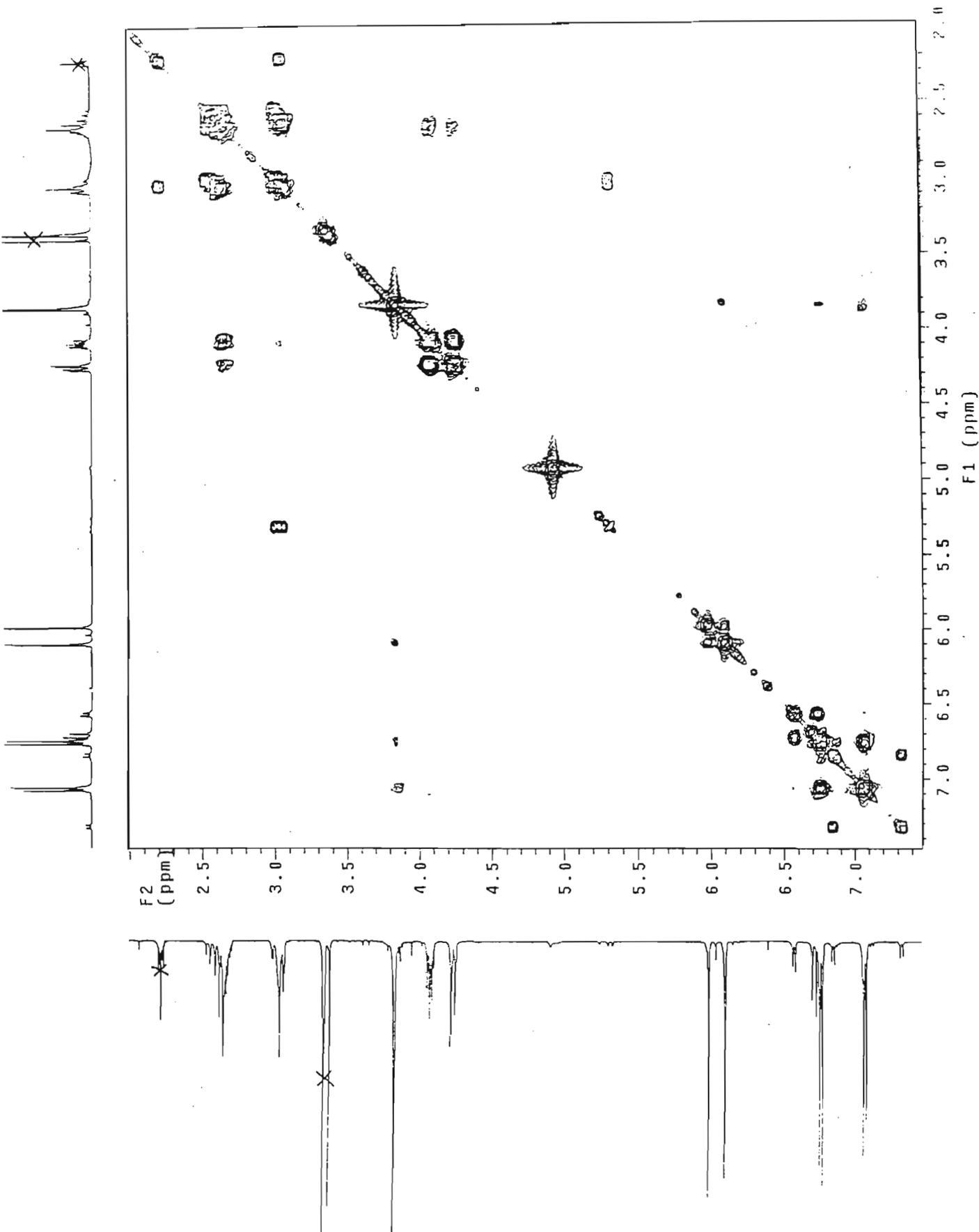
Spectrum 4.7: COSY spectrum of compound 4 (CD₃OD)

181
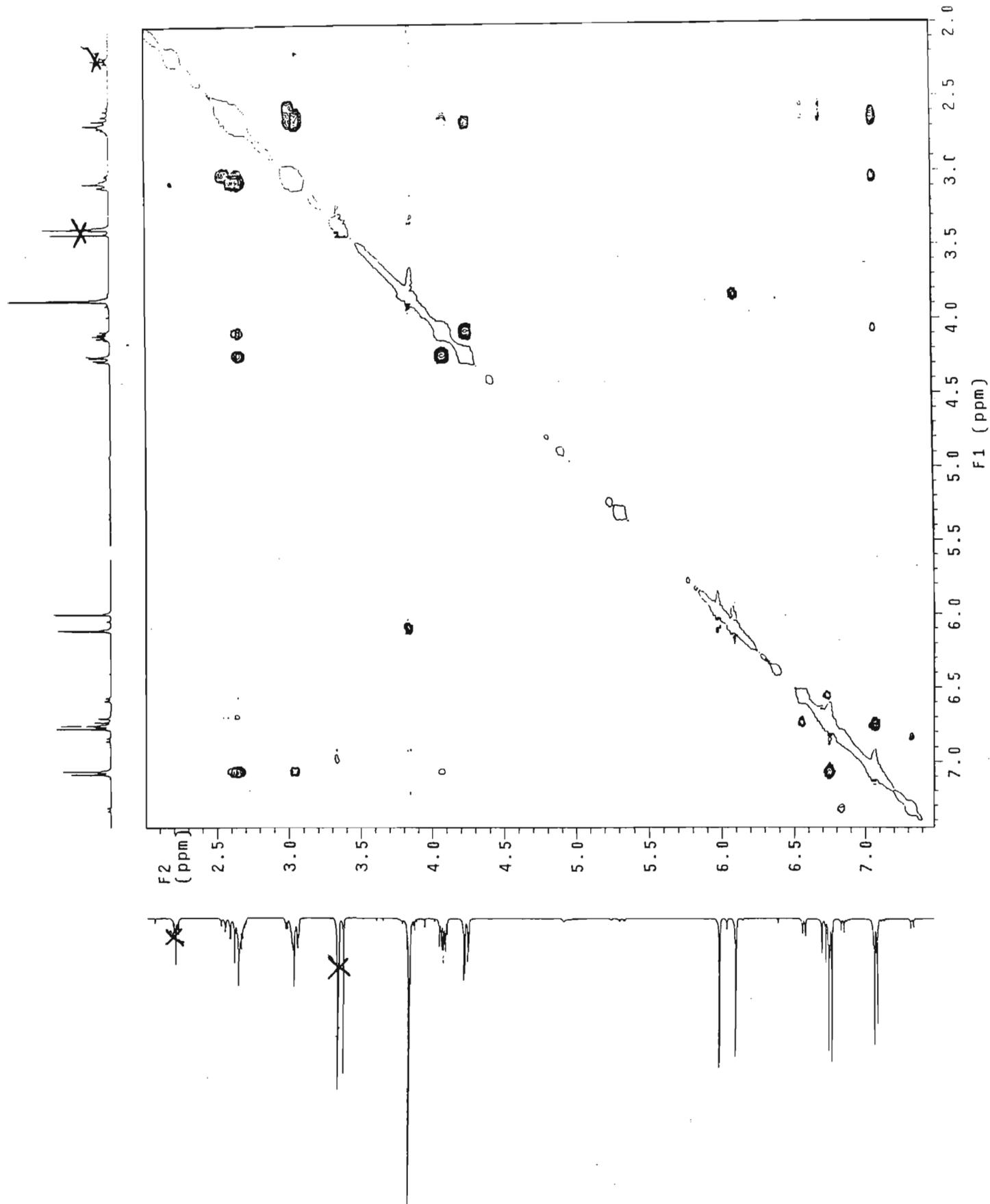
Spectrum 4.8: NOESY spectrum of compound 4 (CD₃OD)

F1 (ppm)

F2 (ppm)
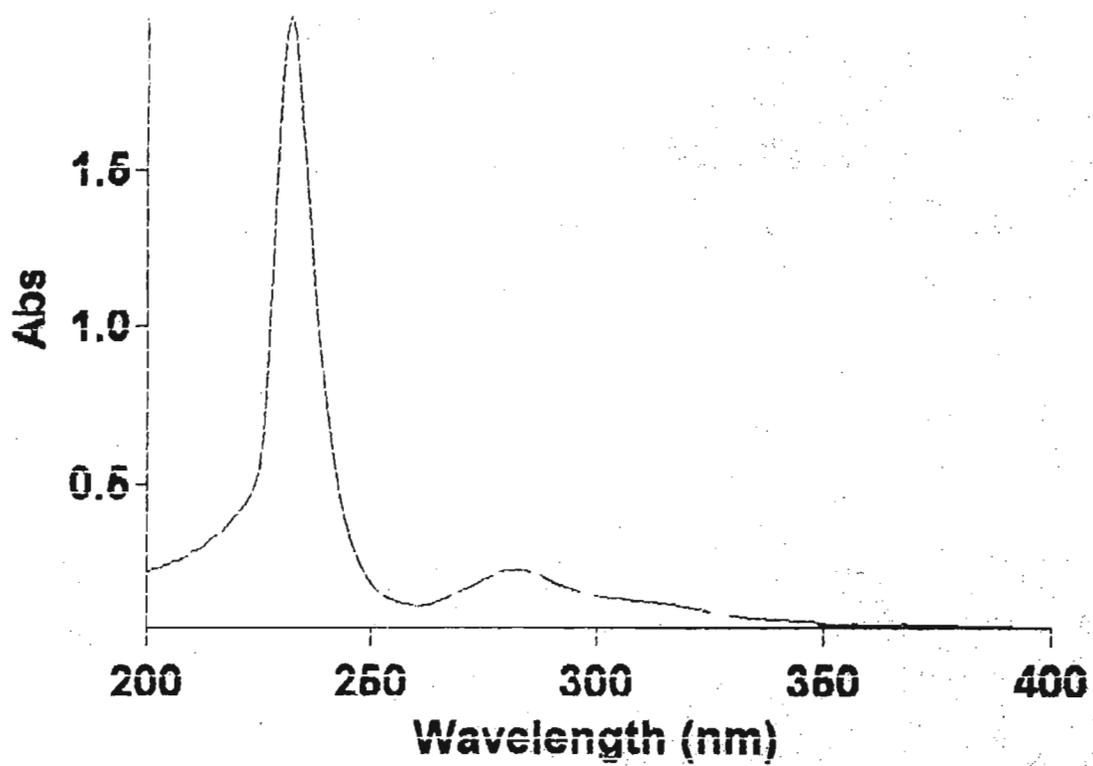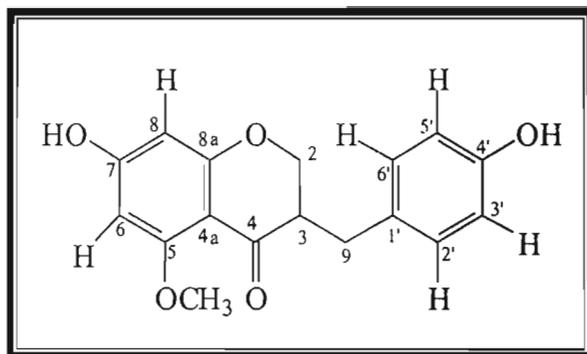
Spectrum 4.9: UV spectrum of compound 4
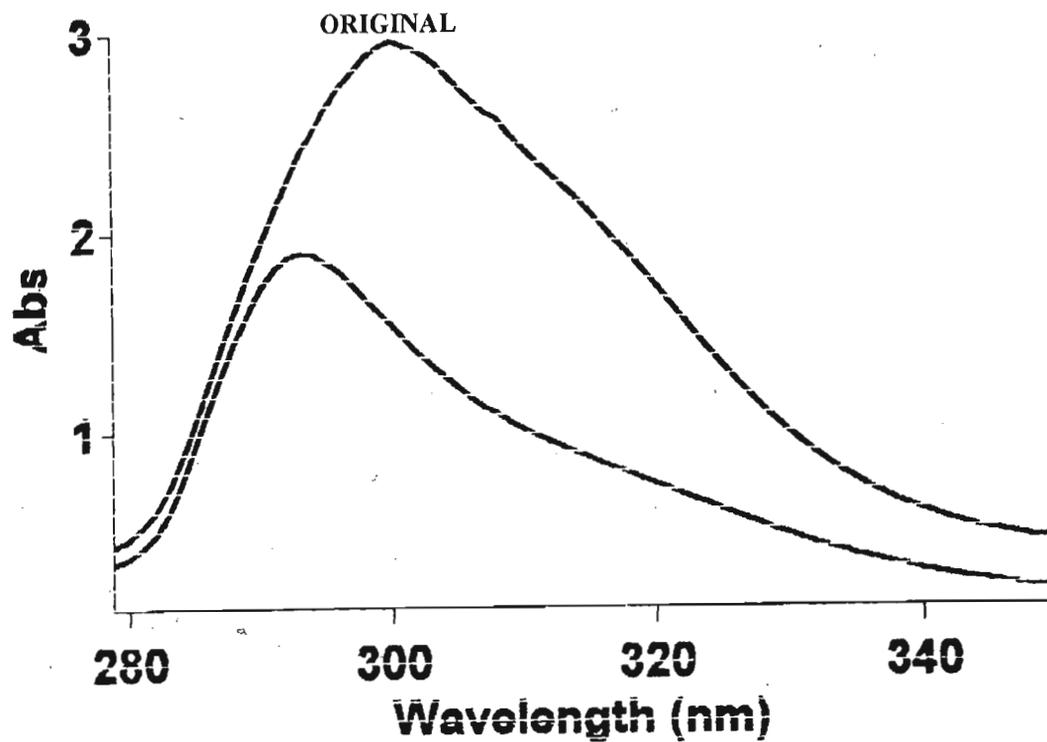
Spectrum 4.10: UV spectrum of compound 4 with AlCl₃
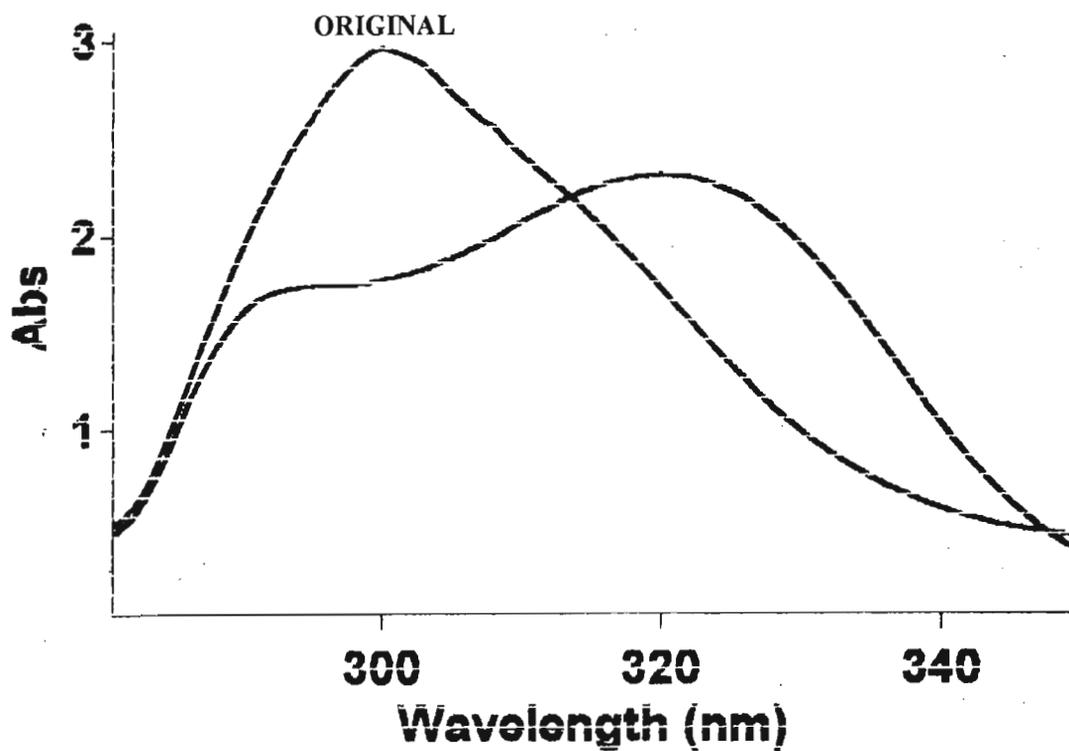
Spectrum 4.11: UV spectrum of compound 4 with NaOAc

184
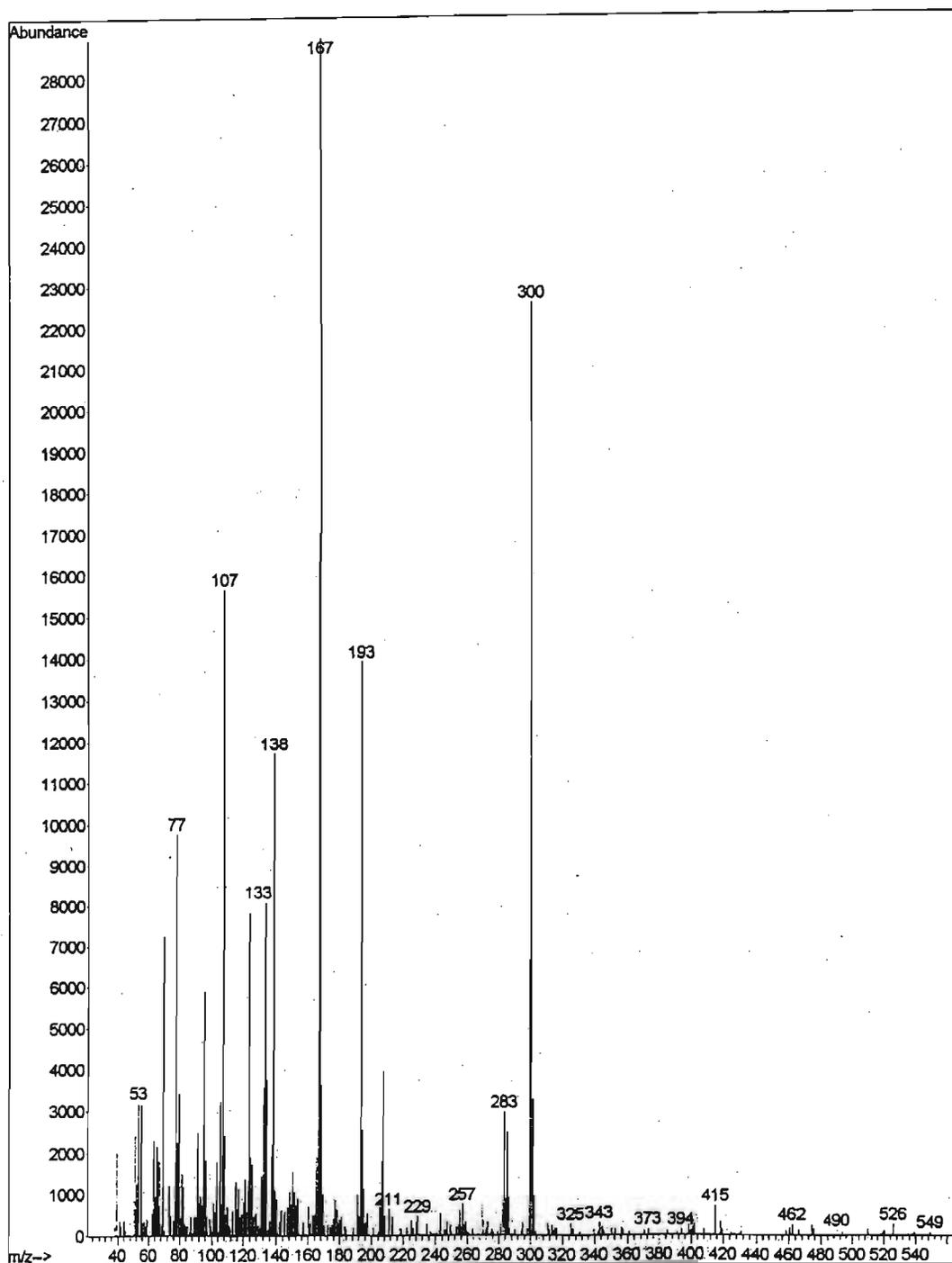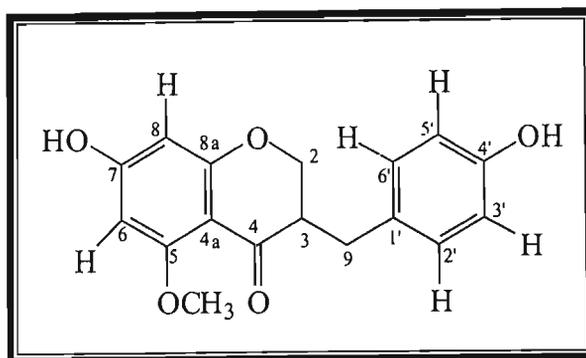
Spectrum 4.12: Mass spectrum of compound 4

185
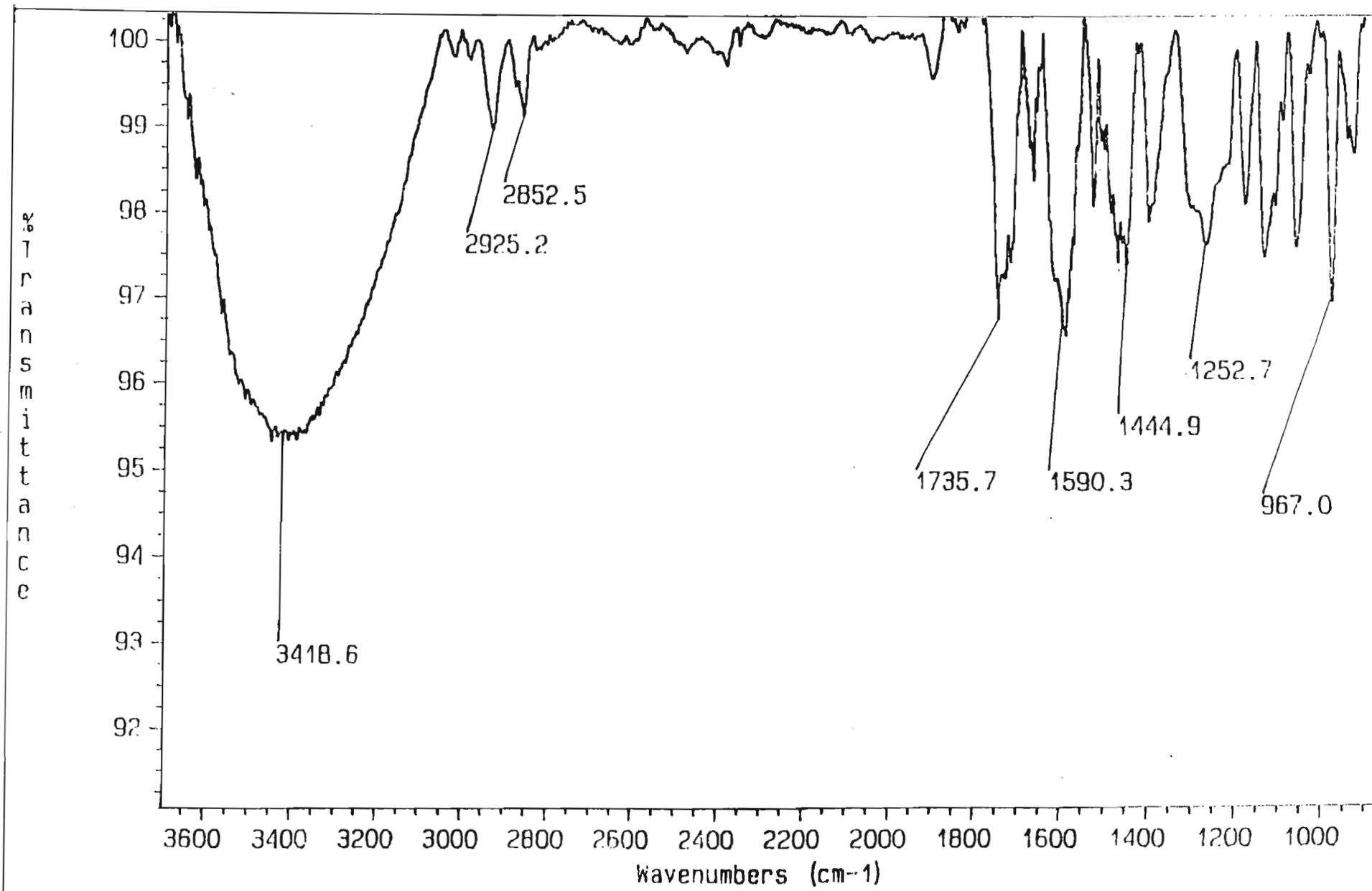
Spectrum 4.13: IR spectrum of compound 4
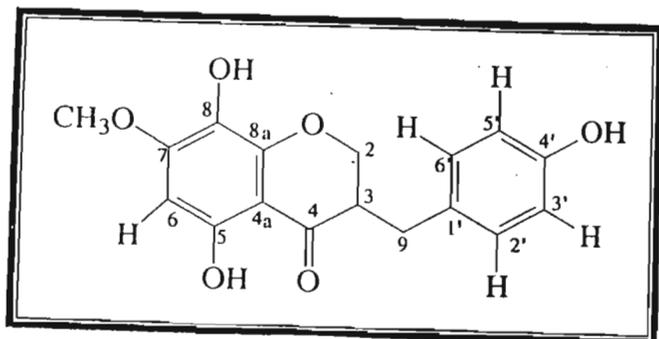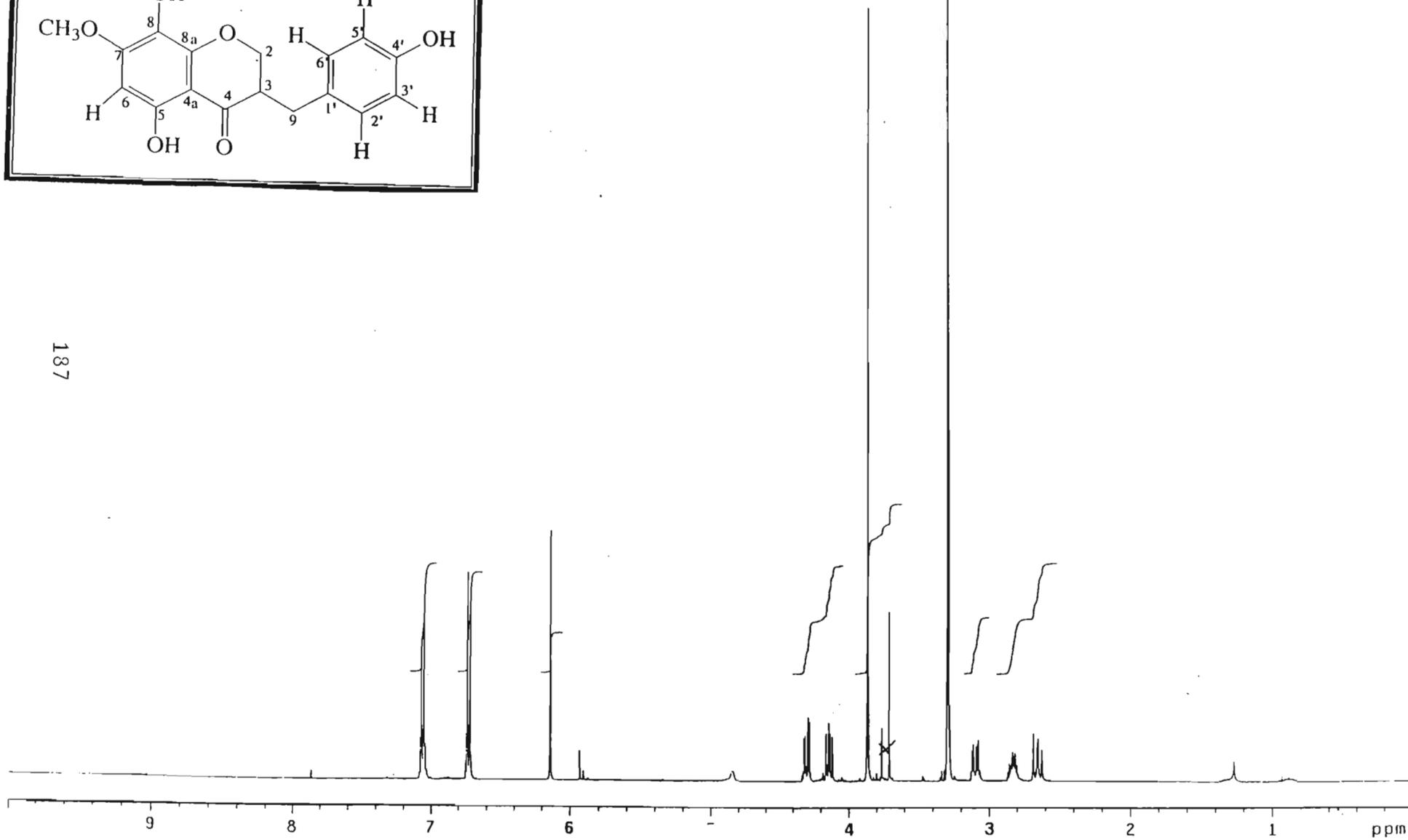
Spectrum 5.1: ^{1}H NMR spectrum of compound 5 (CD$_3$OD)

187
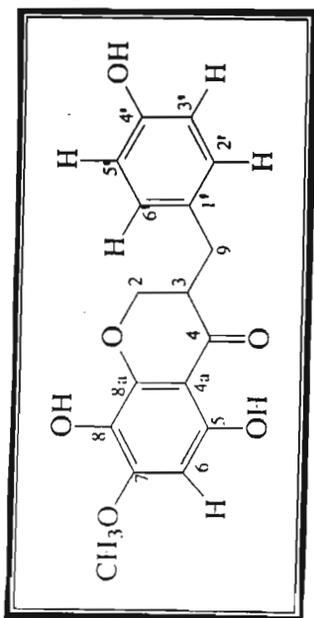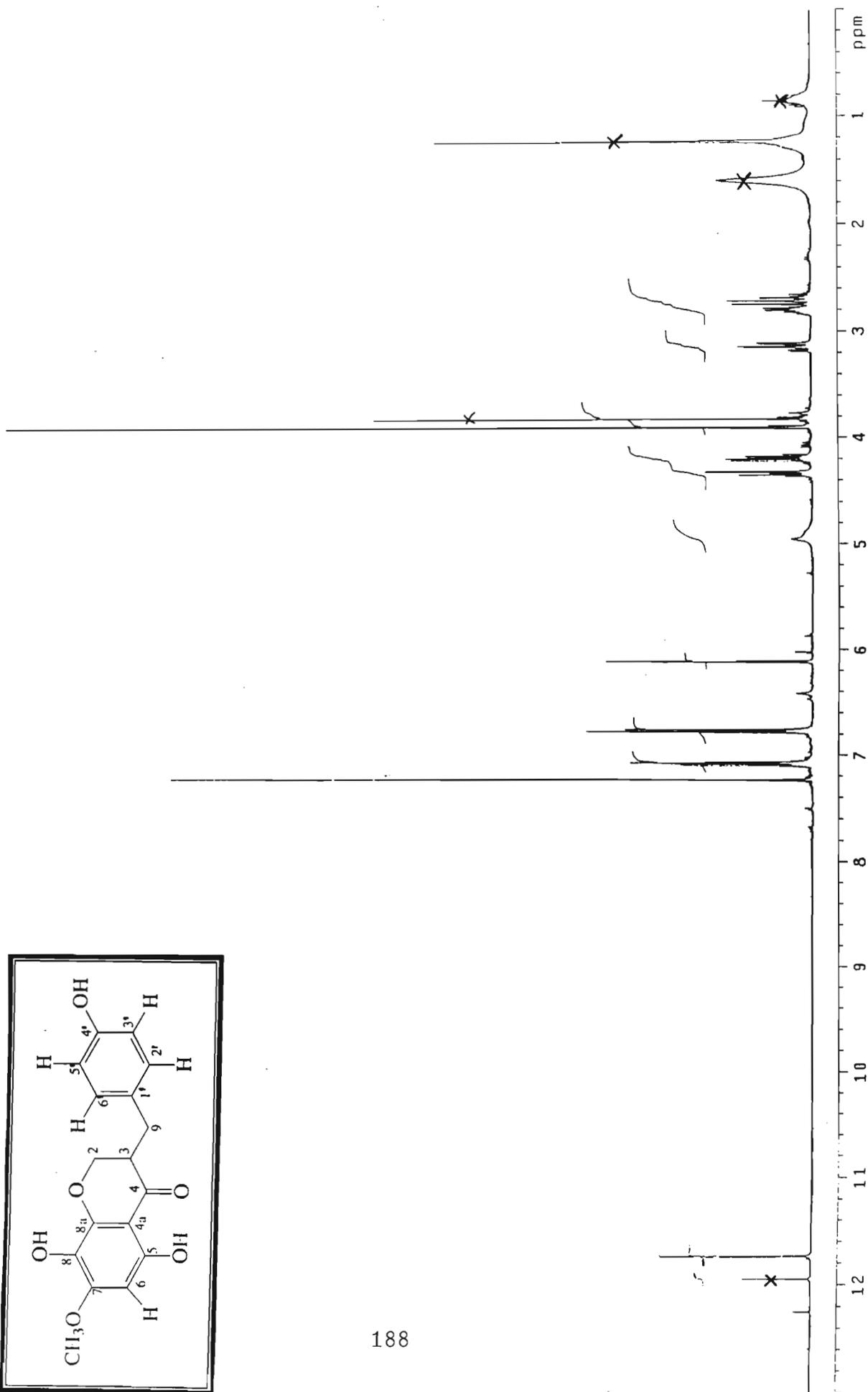
Spectrum 5.2: ^{1}H NMR spectrum of compound 5 (CDCl$_3$)

188
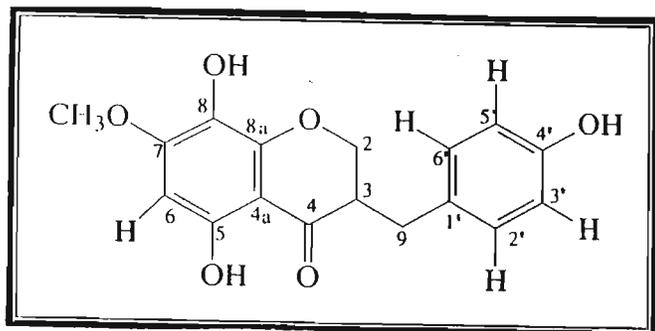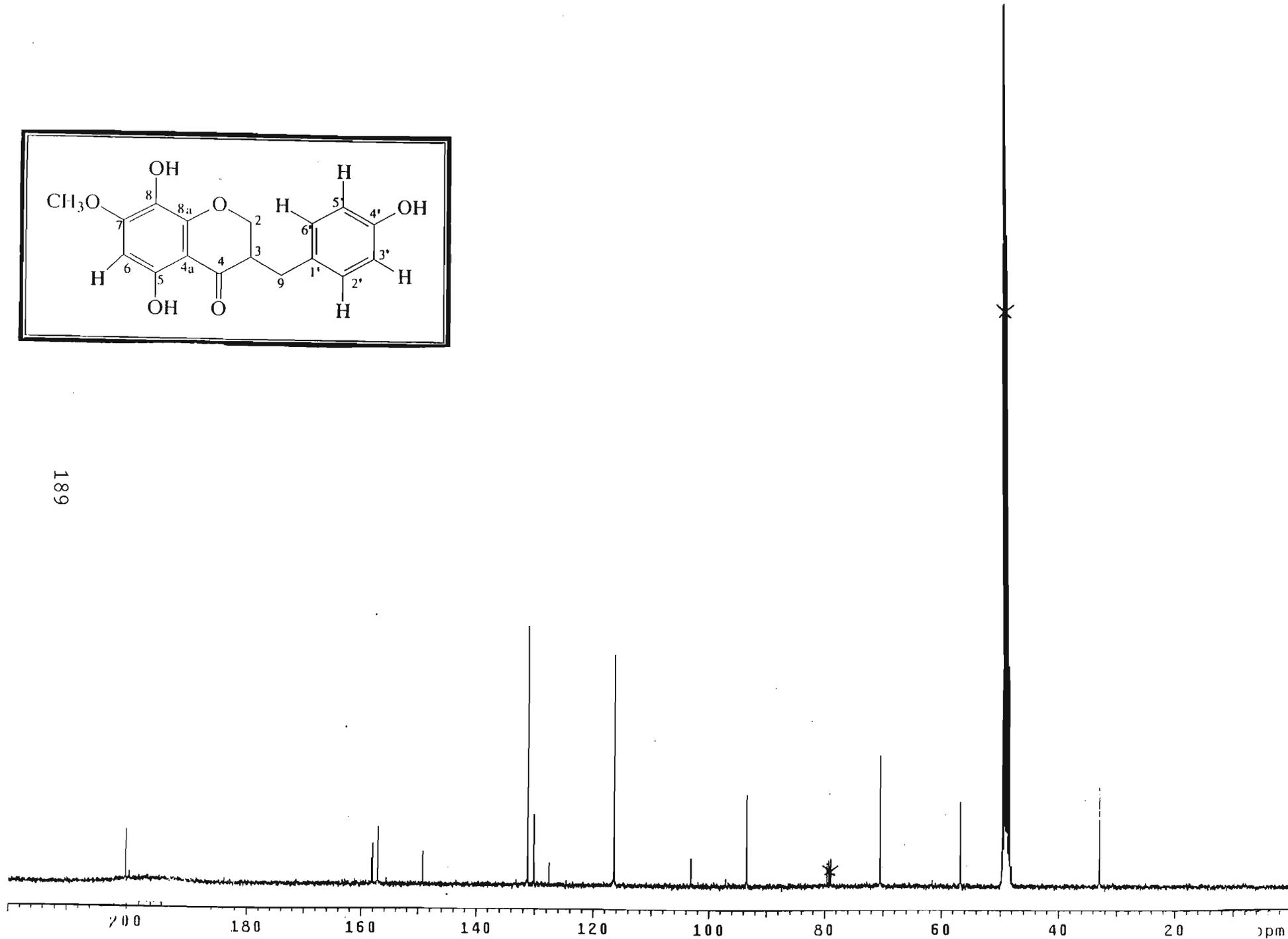
Spectrum 5.3: ^{13}C NMR spectrum of compound 5 (CD$_3$OD)

189
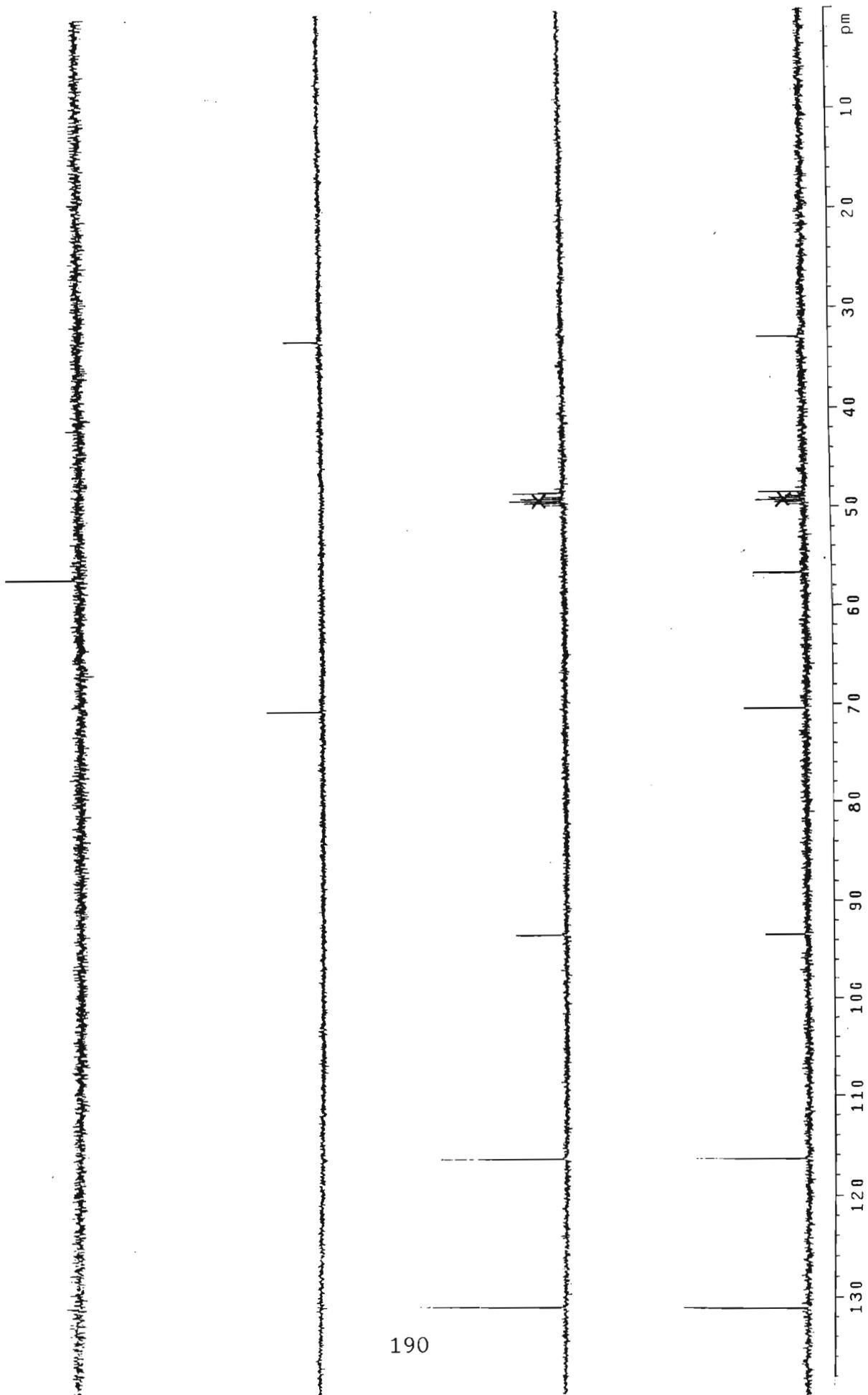
Spectrum 5.4: ADEPT spectrum of compound 5 (CD₃OD)

190
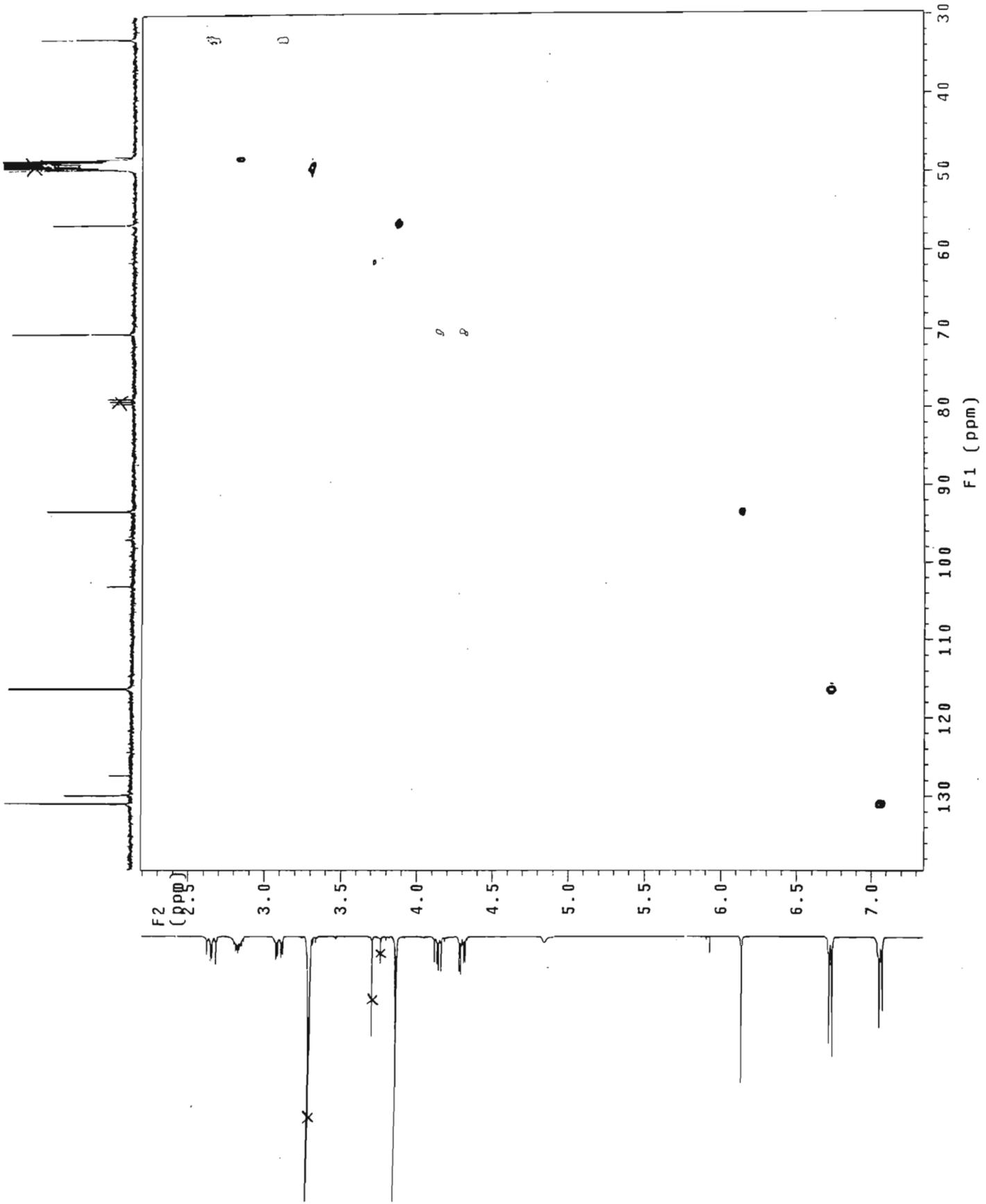
Spectrum 5.5: HSQC spectrum of compound 5 (CD₃OD)

191
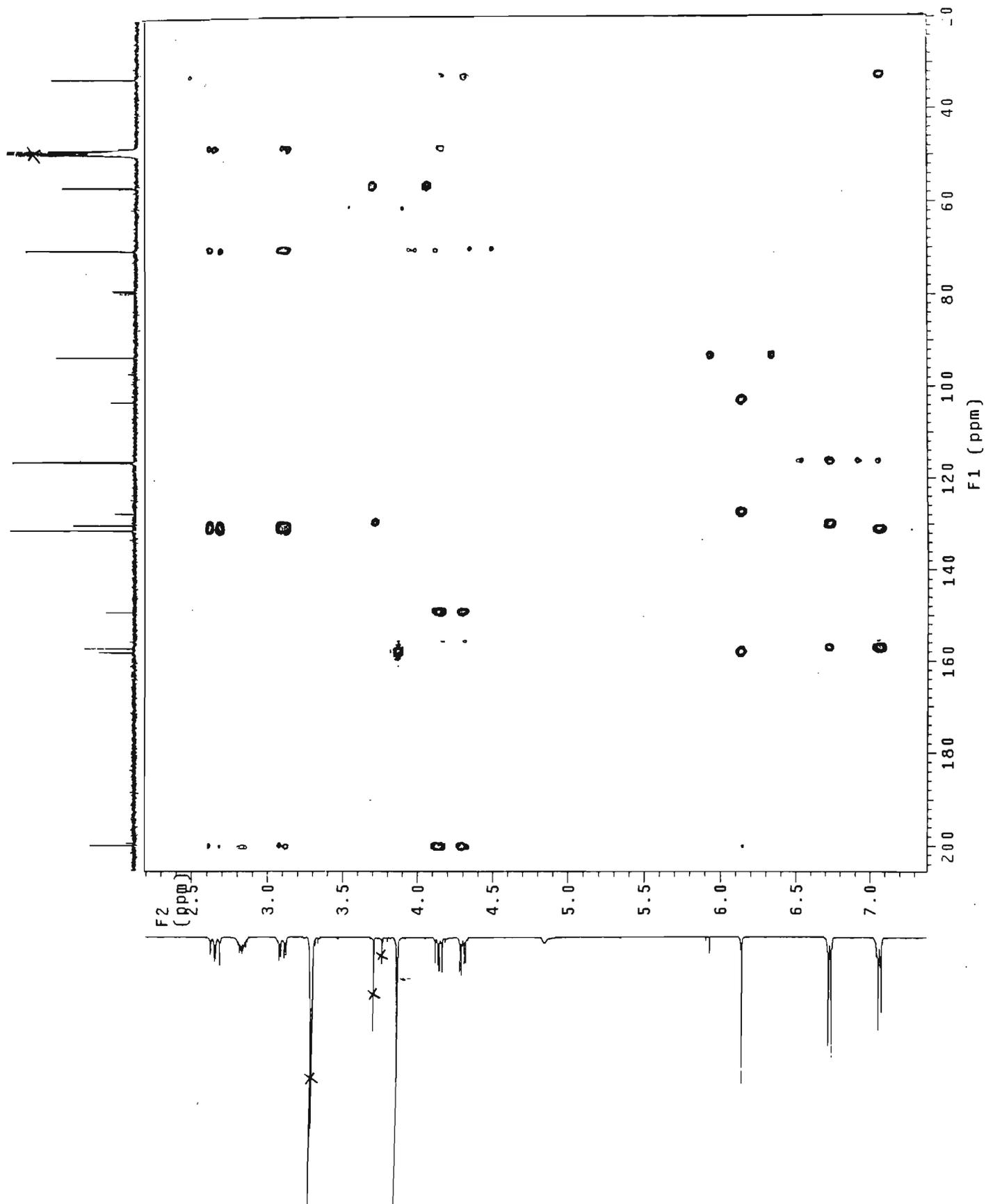
Spectrum 5.6: HMBC spectrum of compound 5 (CD₃OD)

192
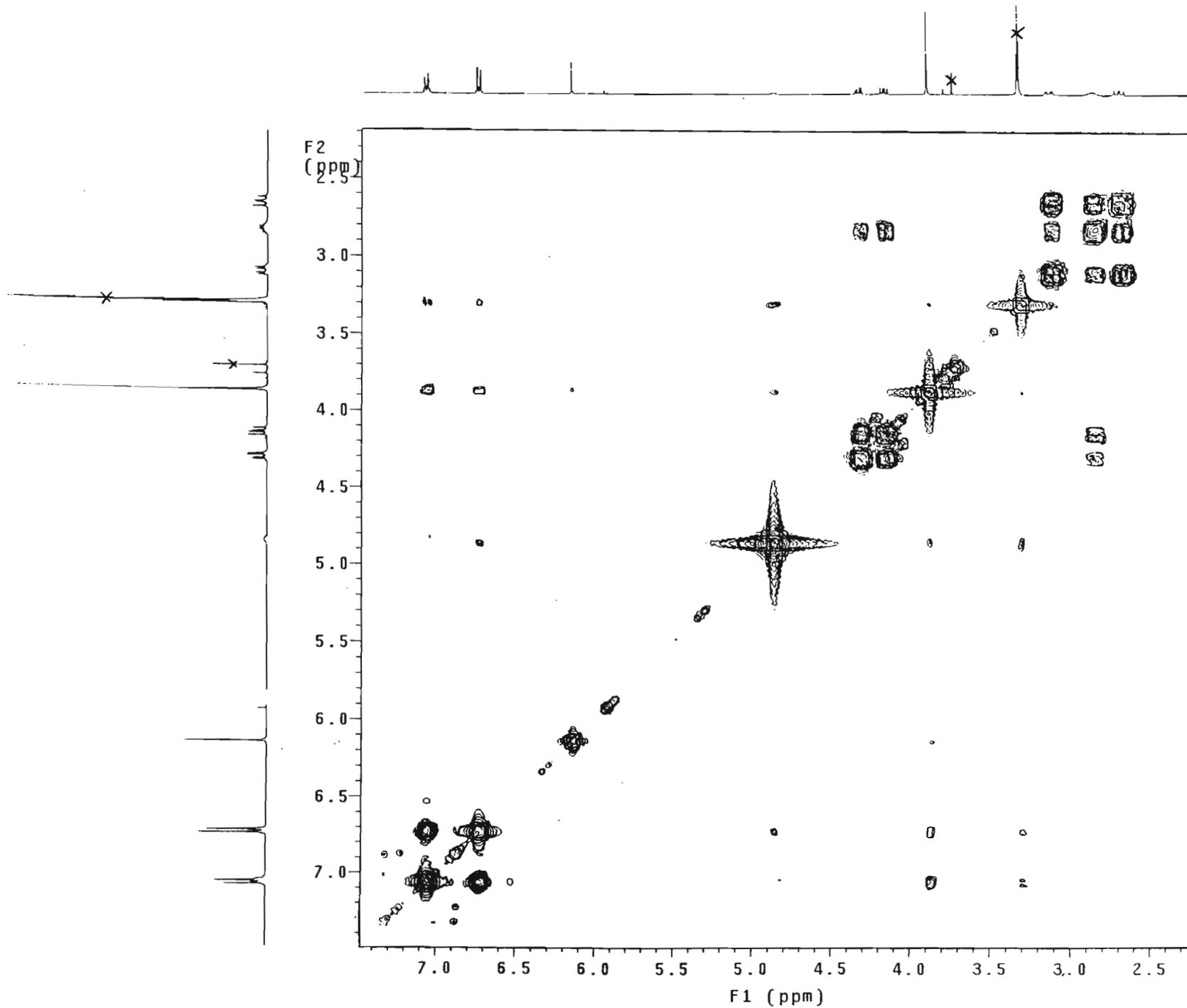
Spectrum 5.7: COSY spectrum of compound 5 (CD₃OD)
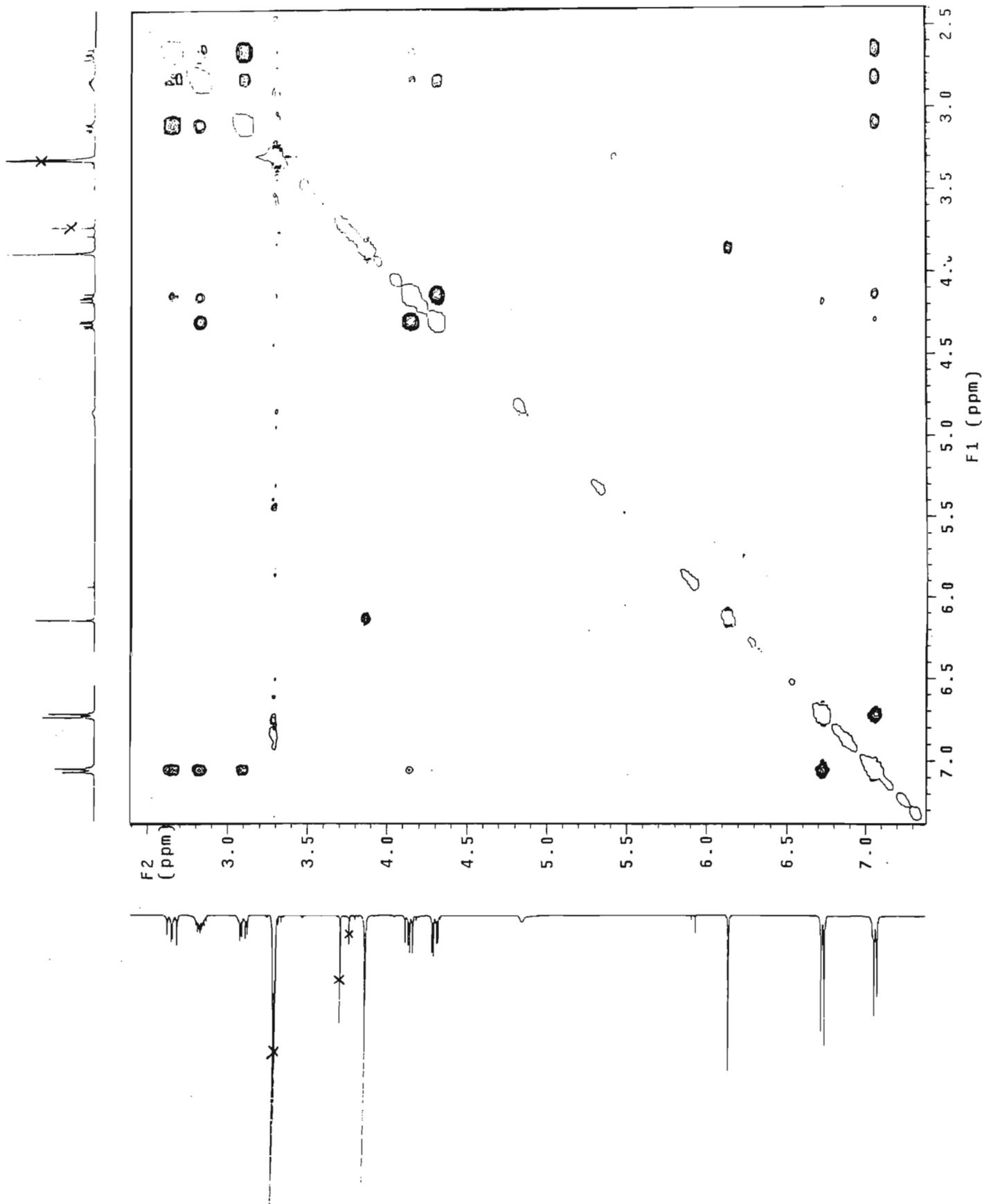
Spectrum 5.8: NOESY spectrum of compound 5 (CD₃OD)
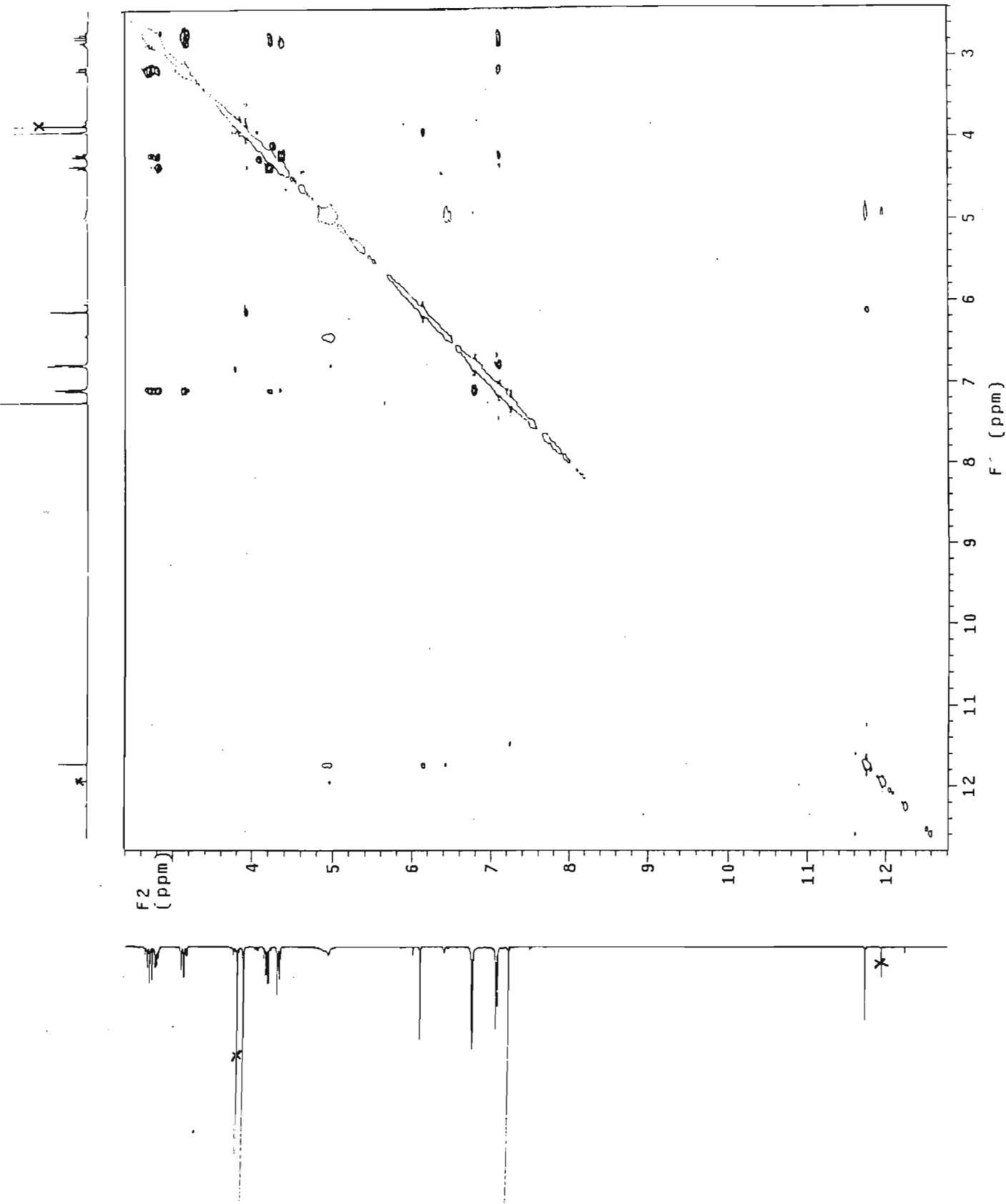
Spectrum 5.9: NOESY spectrum of compound 5 (CDCl₃)
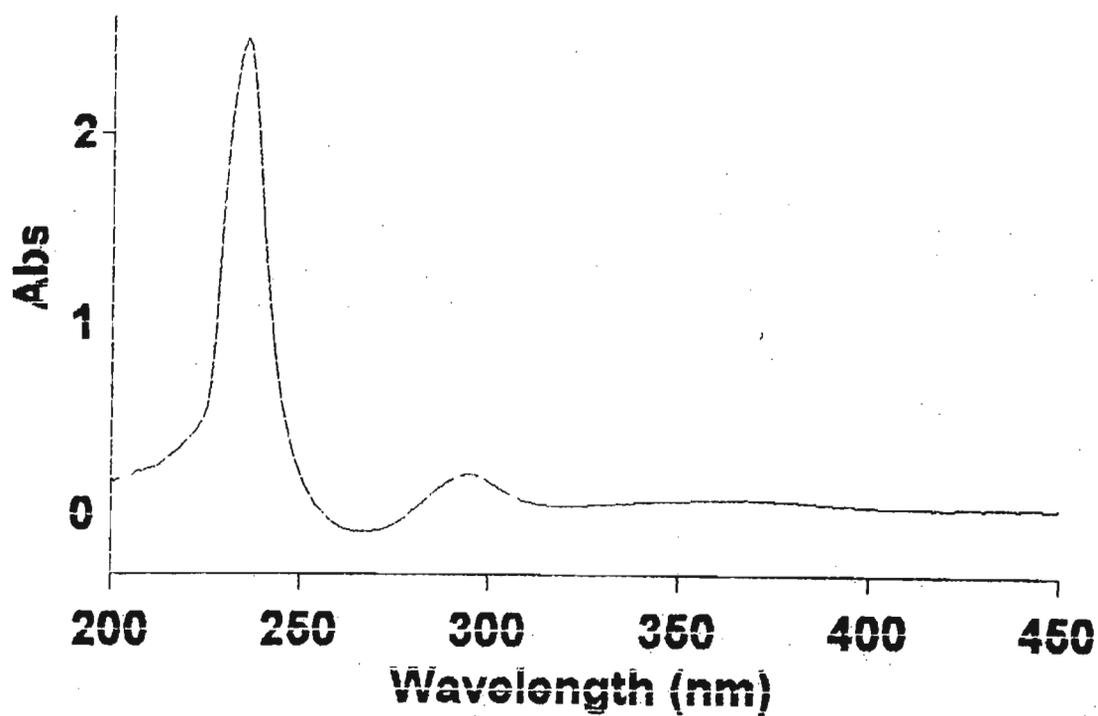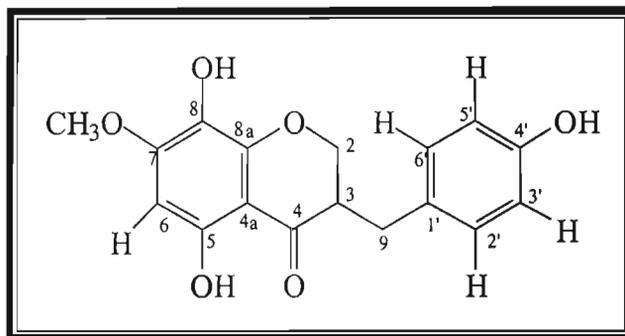
Spectrum 5.10: UV spectrum of compound 5
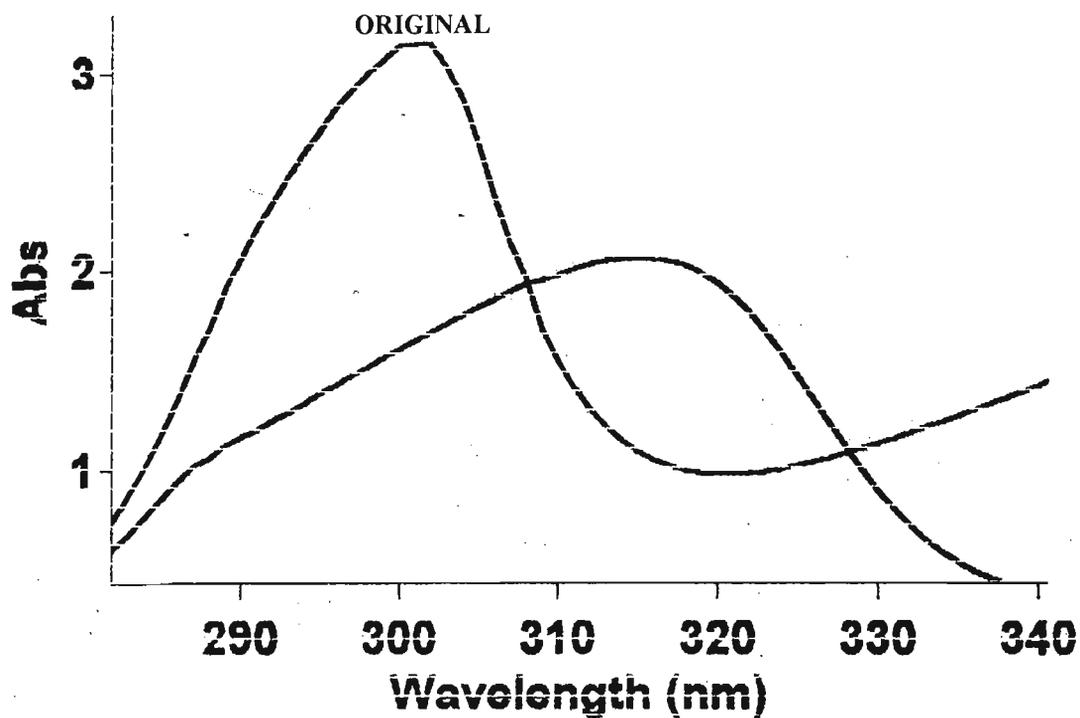
Spectrum 5.11: UV spectrum of compound 5 with AlCl₃
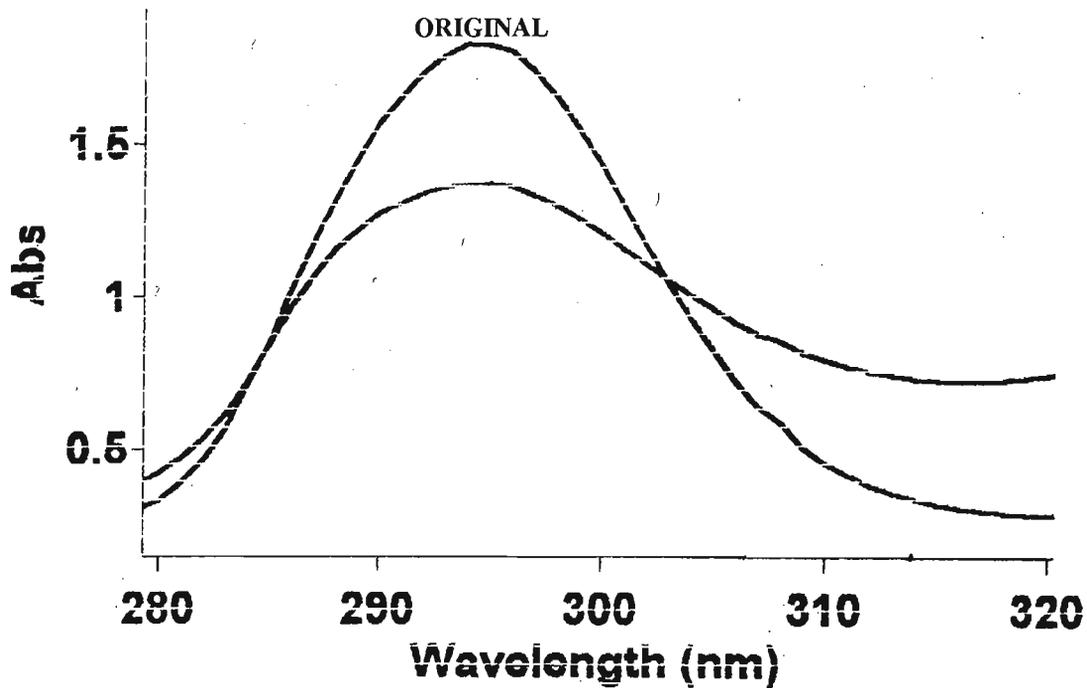
Spectrum 5.12: UV spectrum of compound 5 with NaOAc

197
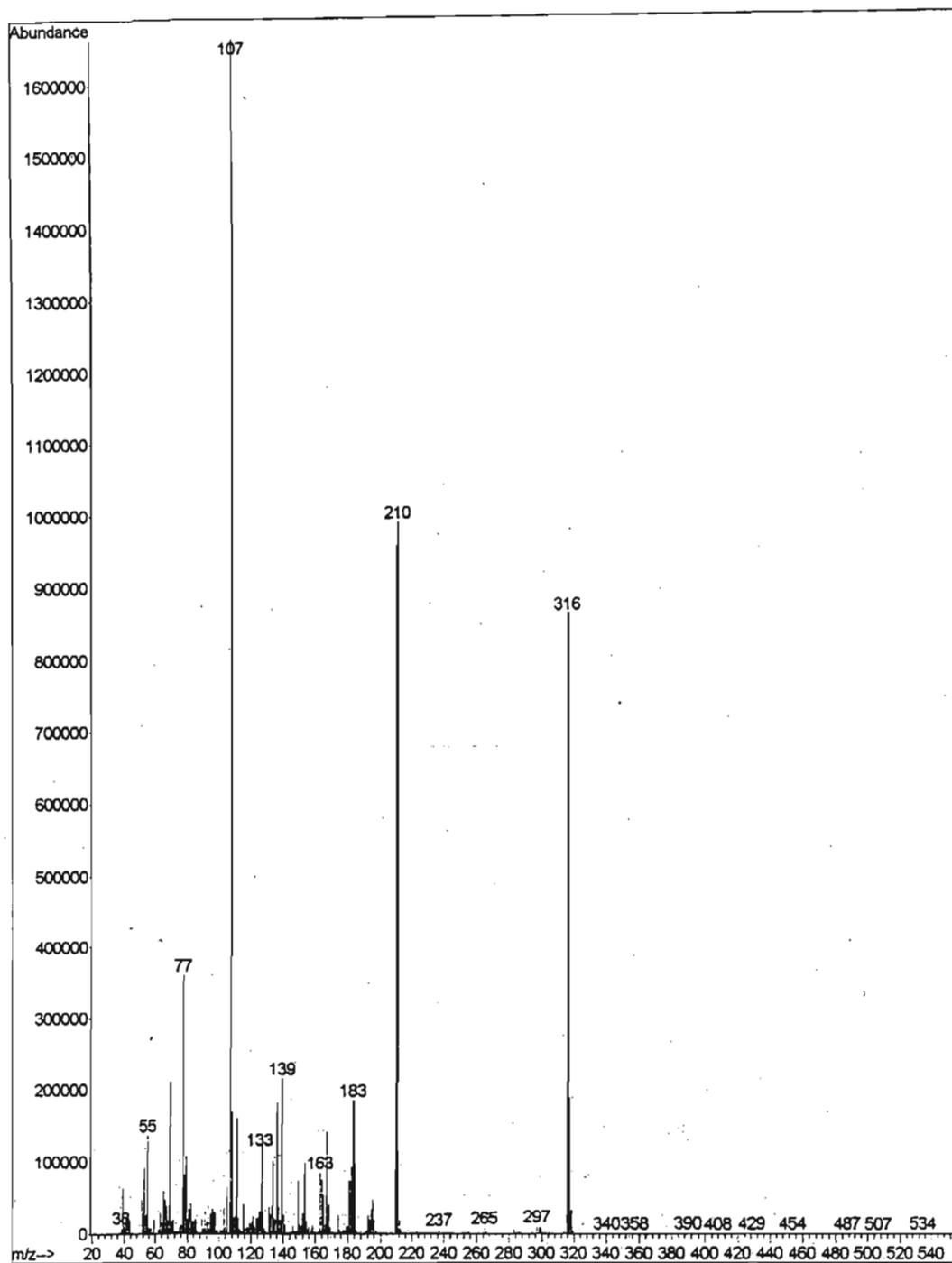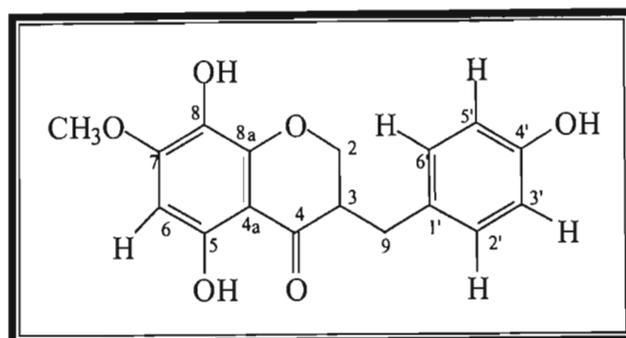
Spectrum 5.13: Mass spectrum of compound 5
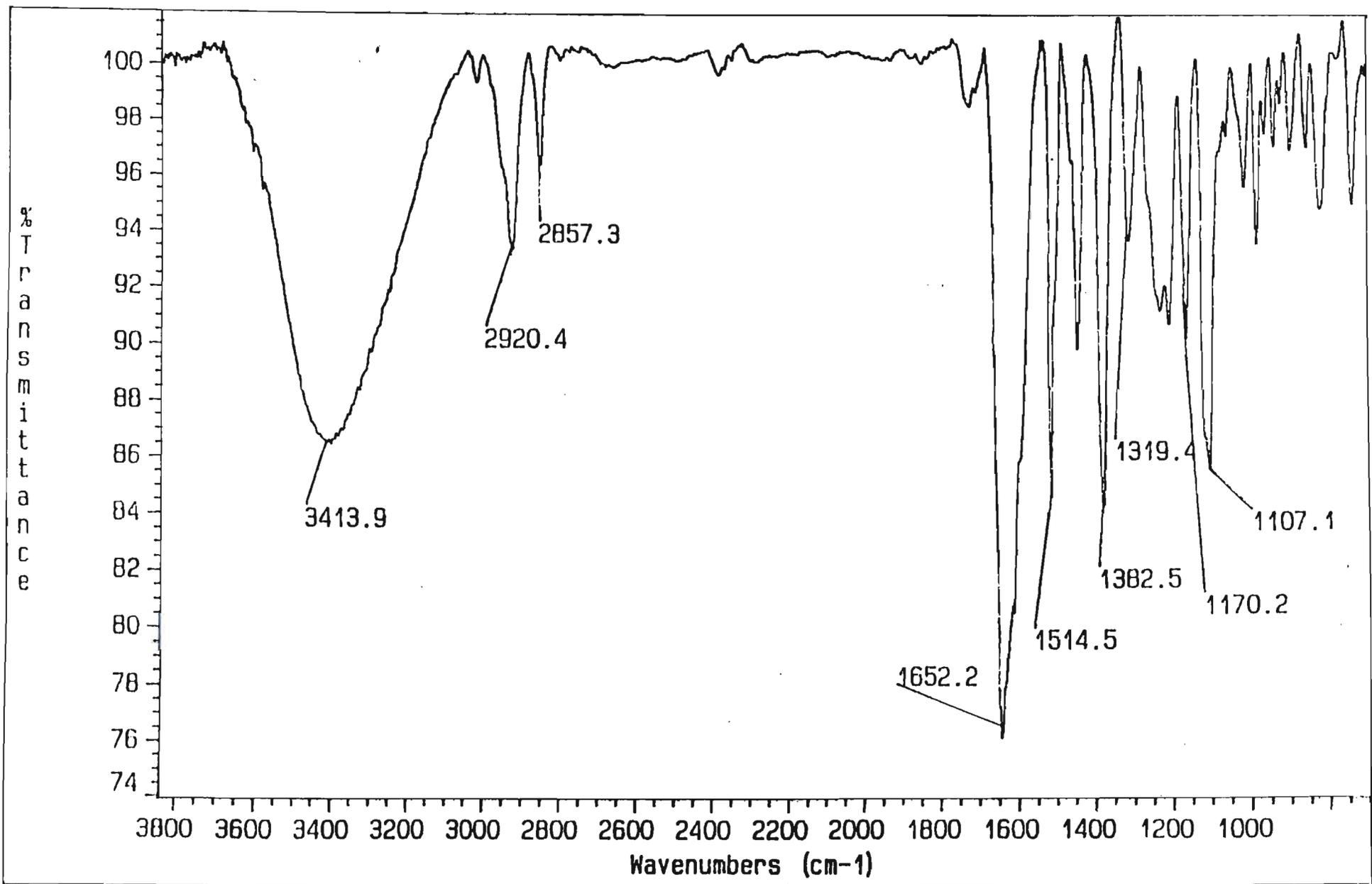
Spectrum 5.14: IR spectrum of compound 5
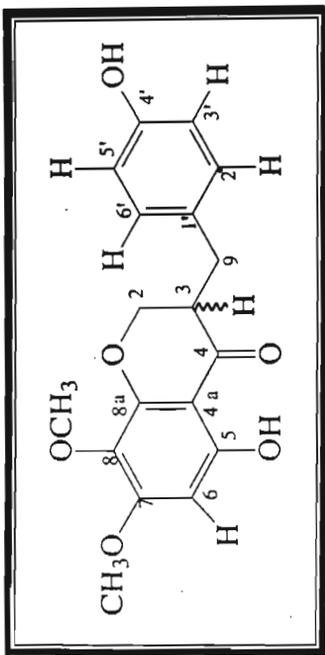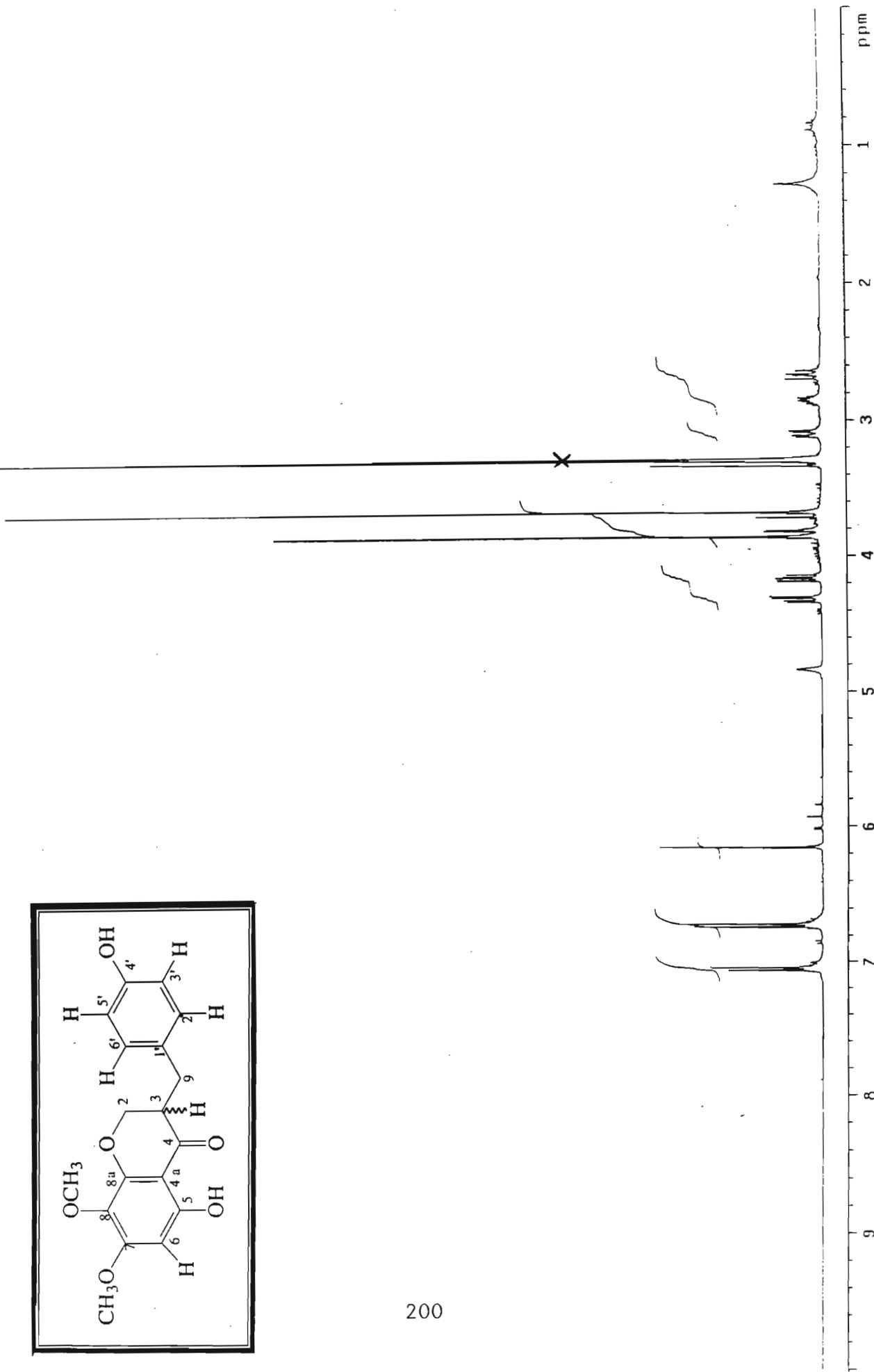
Spectrum 6.1: ^{1}H NMR spectrum of compound 6 (CD$_3$OD)

200
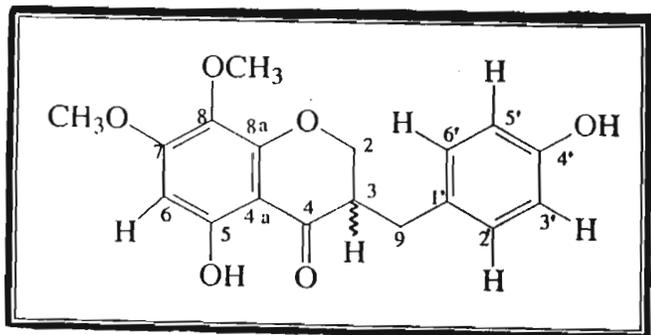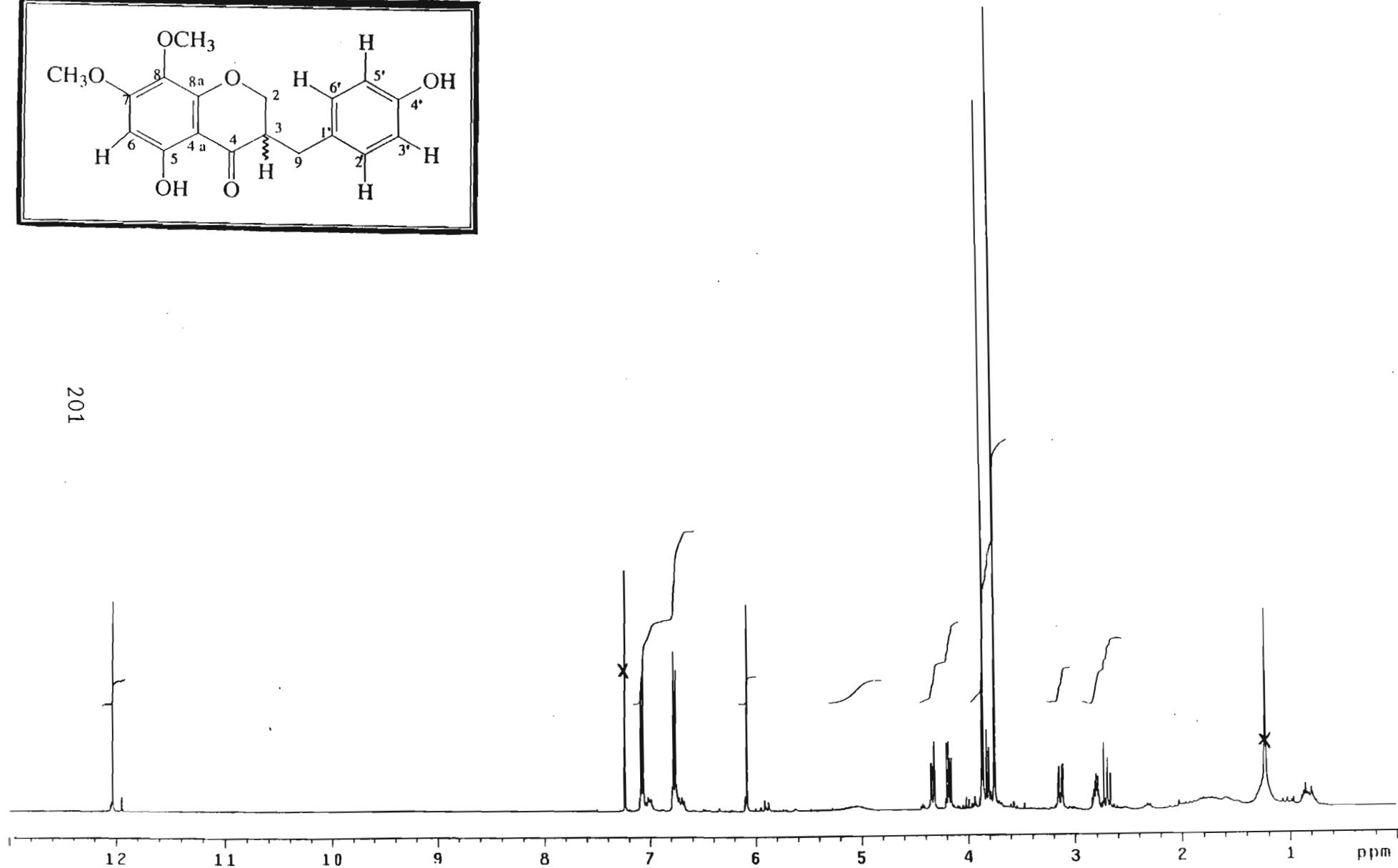
Spectrum 6.2: ^{1}H NMR spectrum of compound 6 (CDCl$_3$)
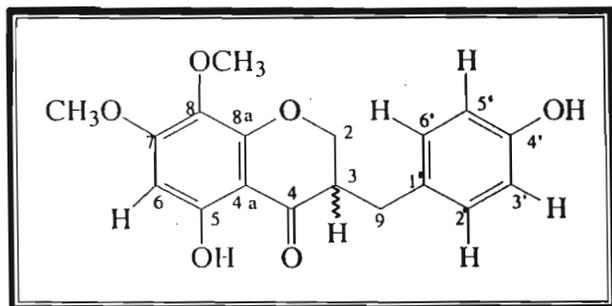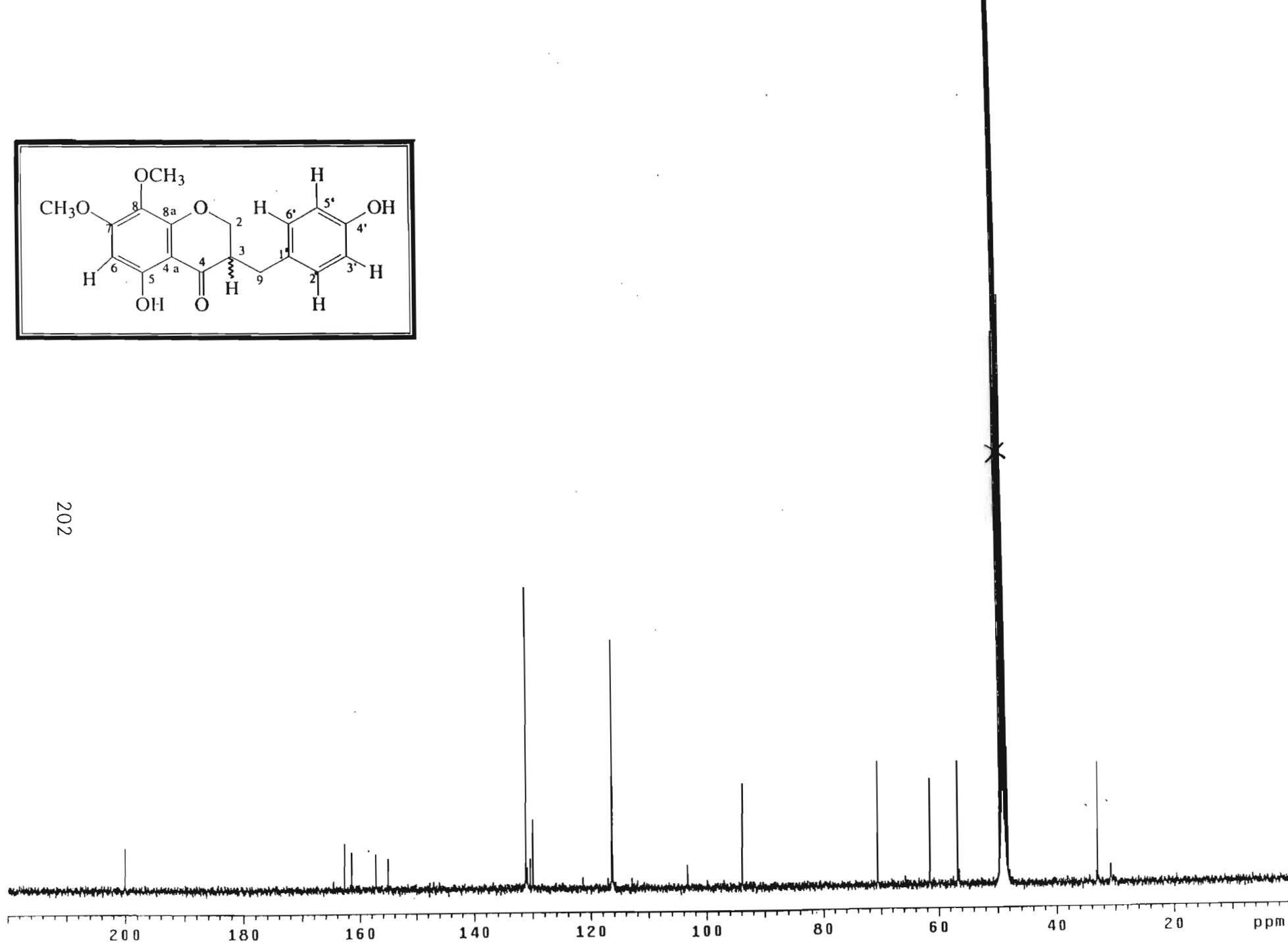
Spectrum 6.3: ^{13}C NMR spectrum of compound 6 (CD₃OD)

202
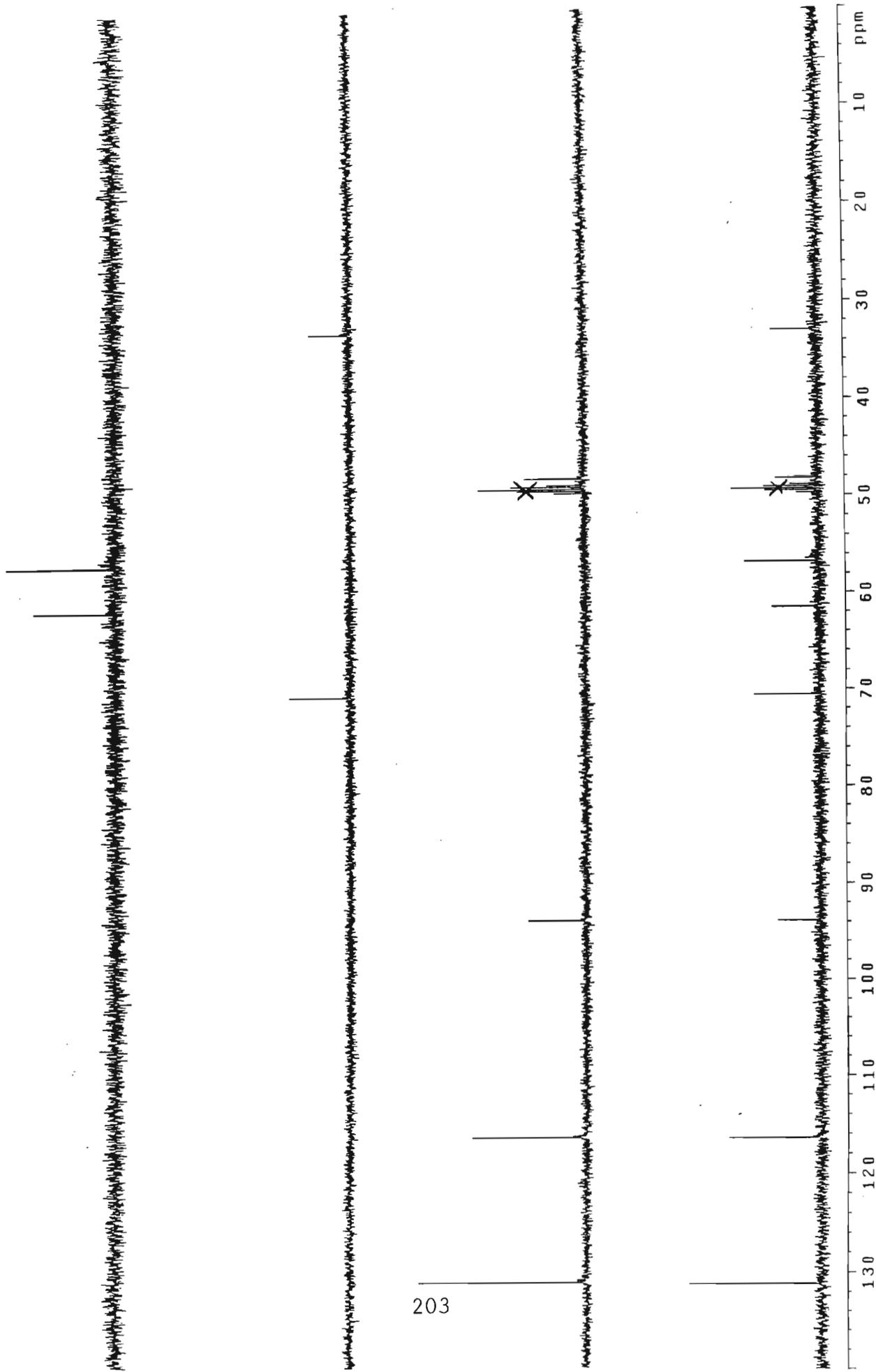
Spectrum 6.4: ADEPT spectrum of compound 6 (CD₃OD)

203
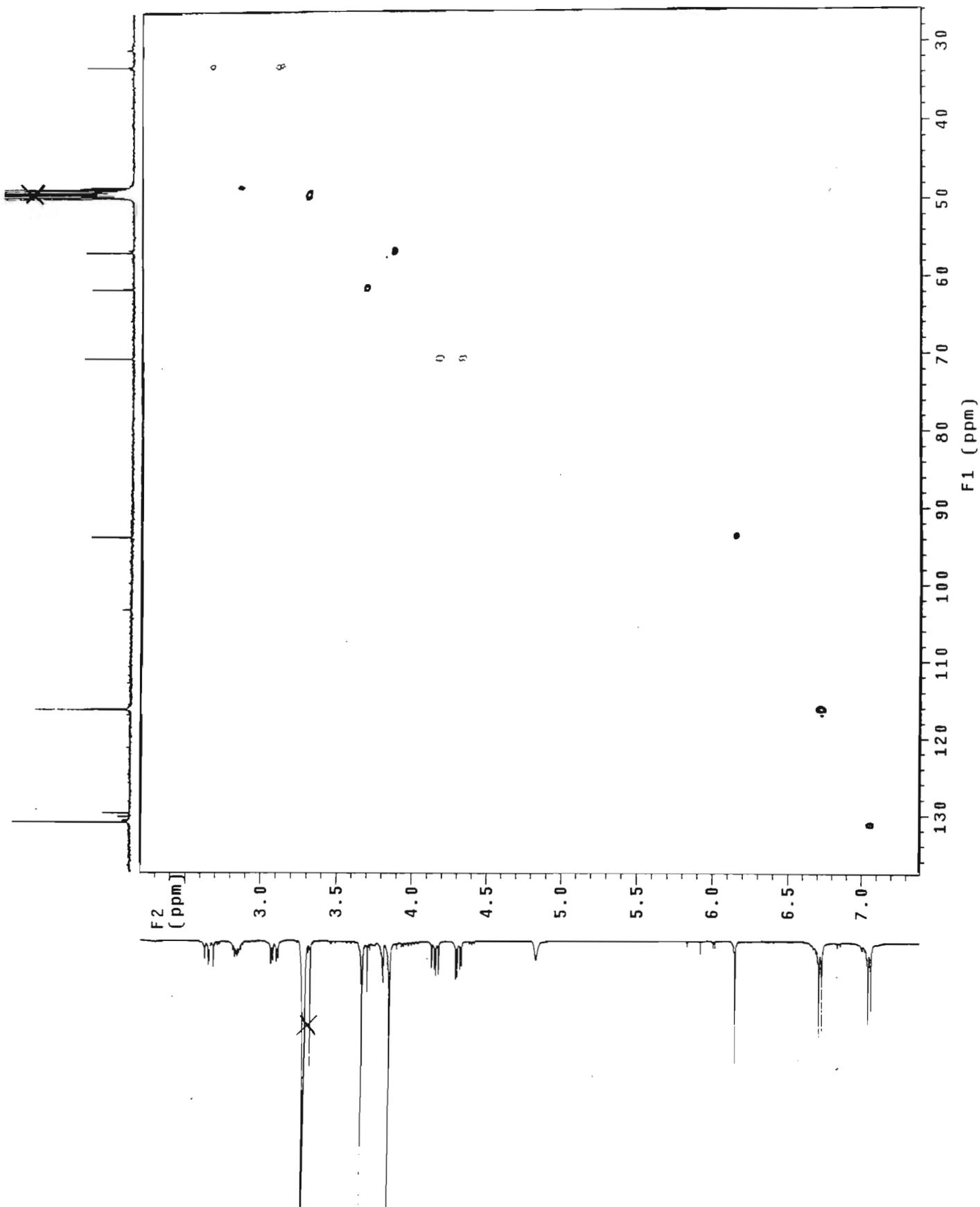
Spectrum 6.5: HSQC spectrum of compound 6 (CD₃OD)

204
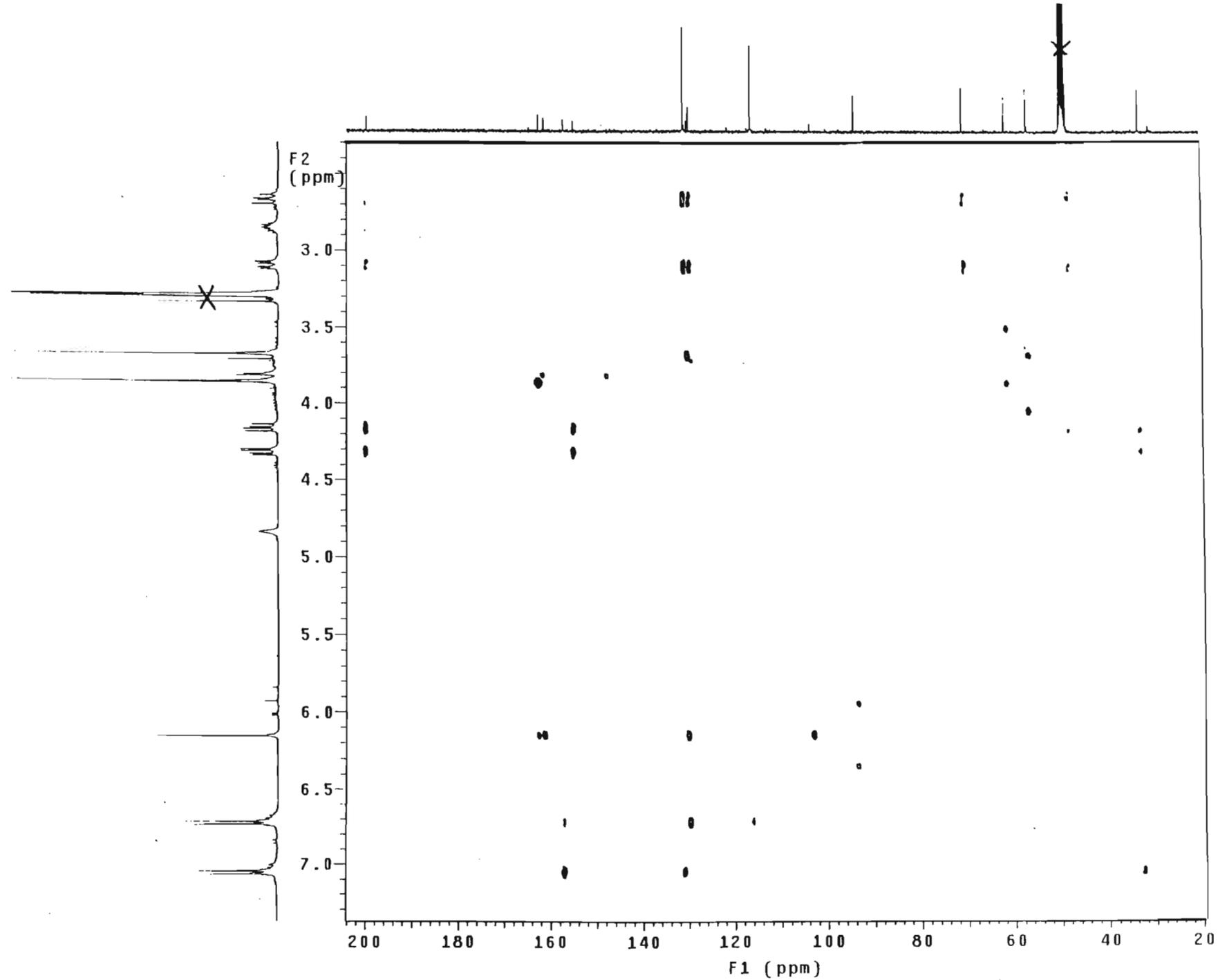
Spectrum 6.6: HMBC spectrum of compound 6 (CD₃OD)
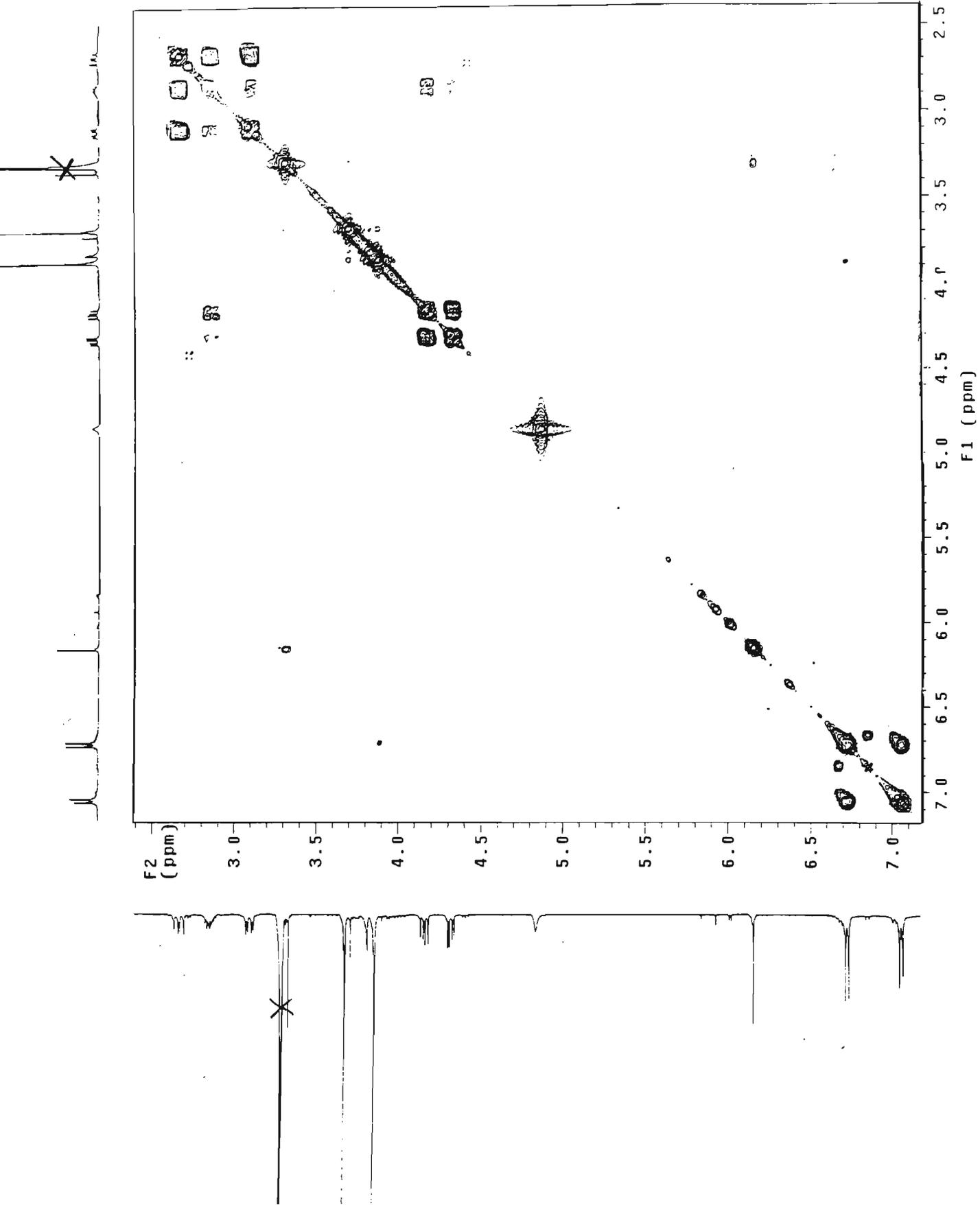
Spectrum 6.7: COSY spectrum of compound 6 (CD₃OD)
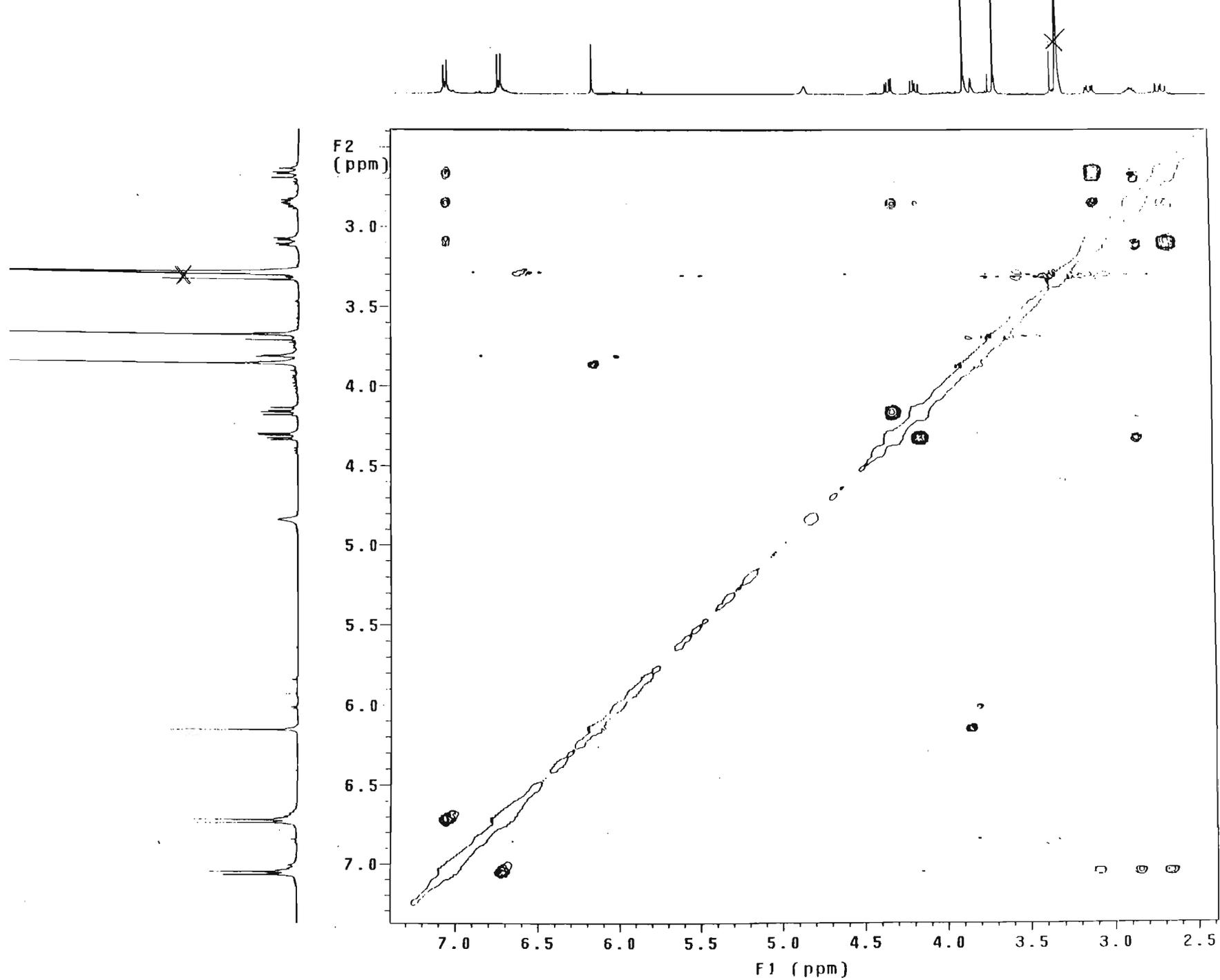
Spectrum 6.8: NOESY spectrum of compound 6 (CD₃OD)
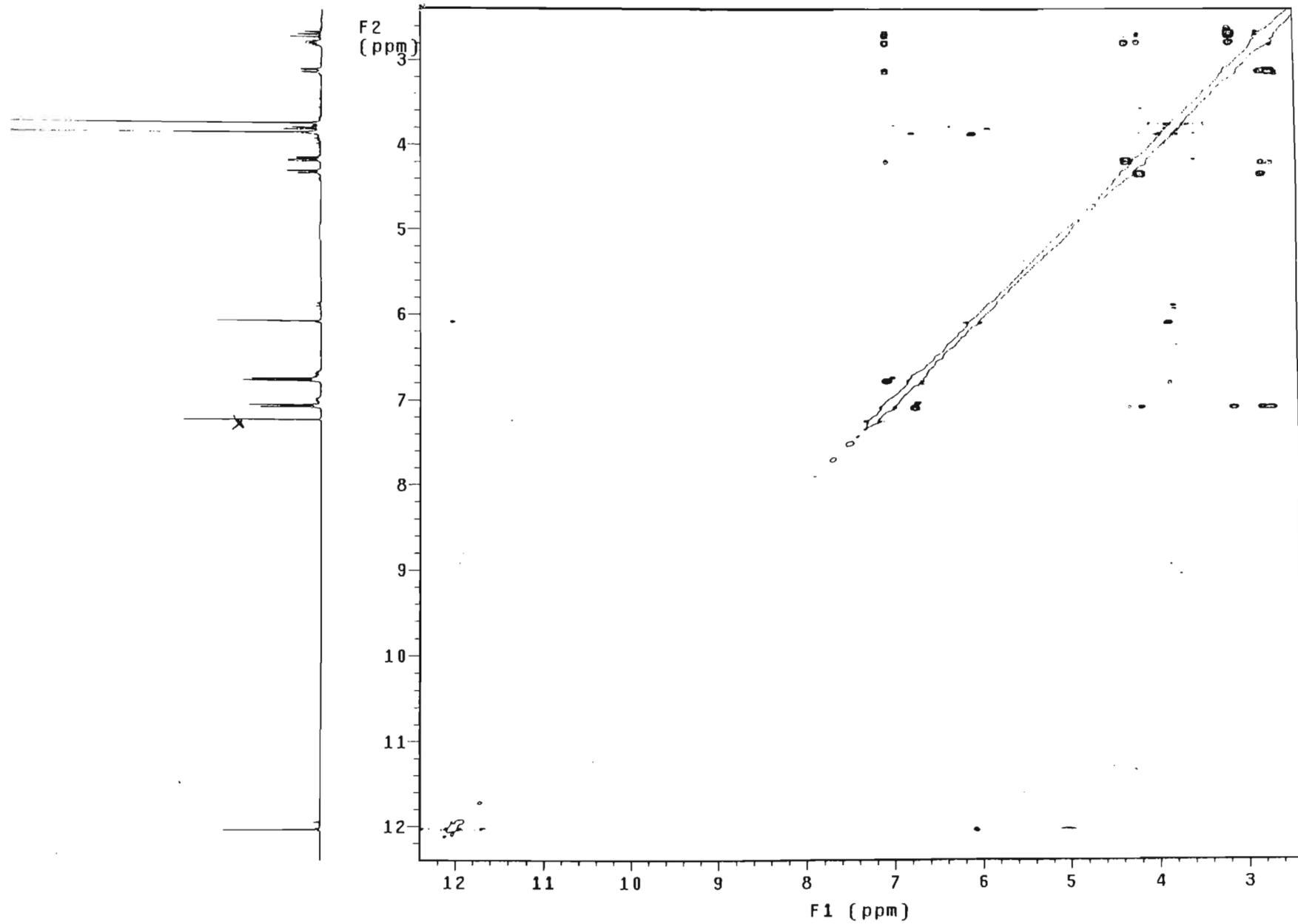
Spectrum 6.9: NOESY spectrum of compound 6 (CDCl₃)
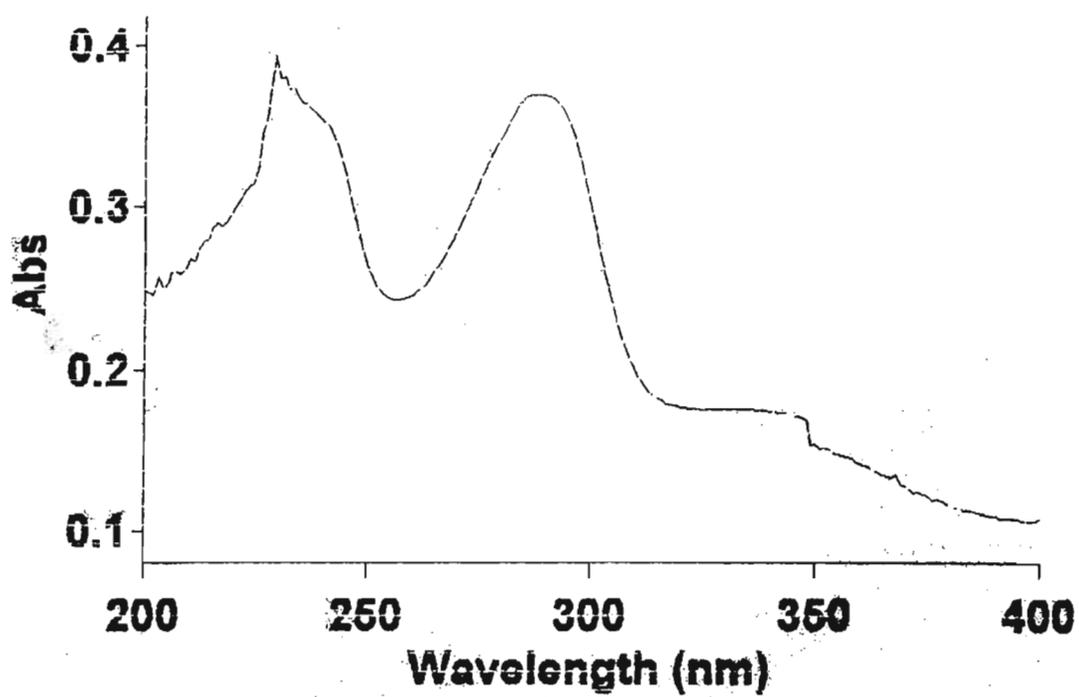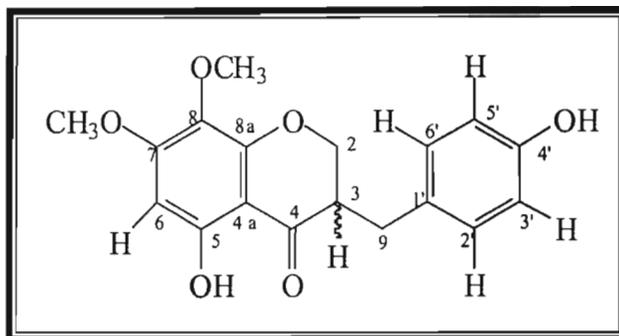
Spectrum 6.10: UV spectrum of compound 6
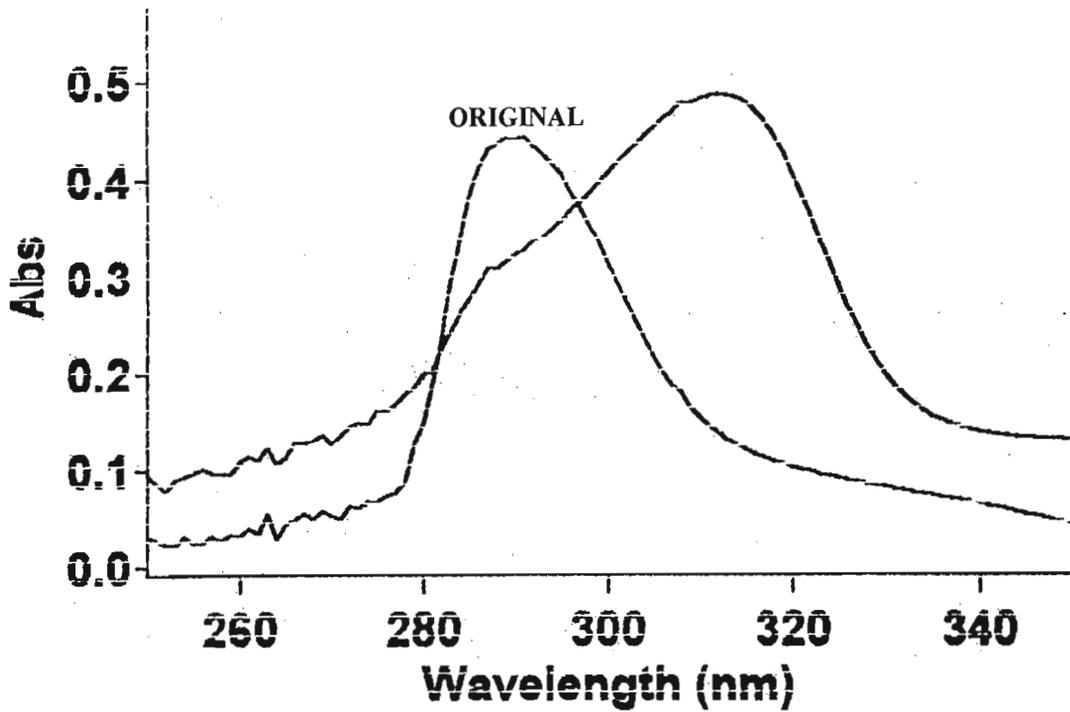
Spectrum 6.11: UV spectrum of compound 6 with AlCl₃
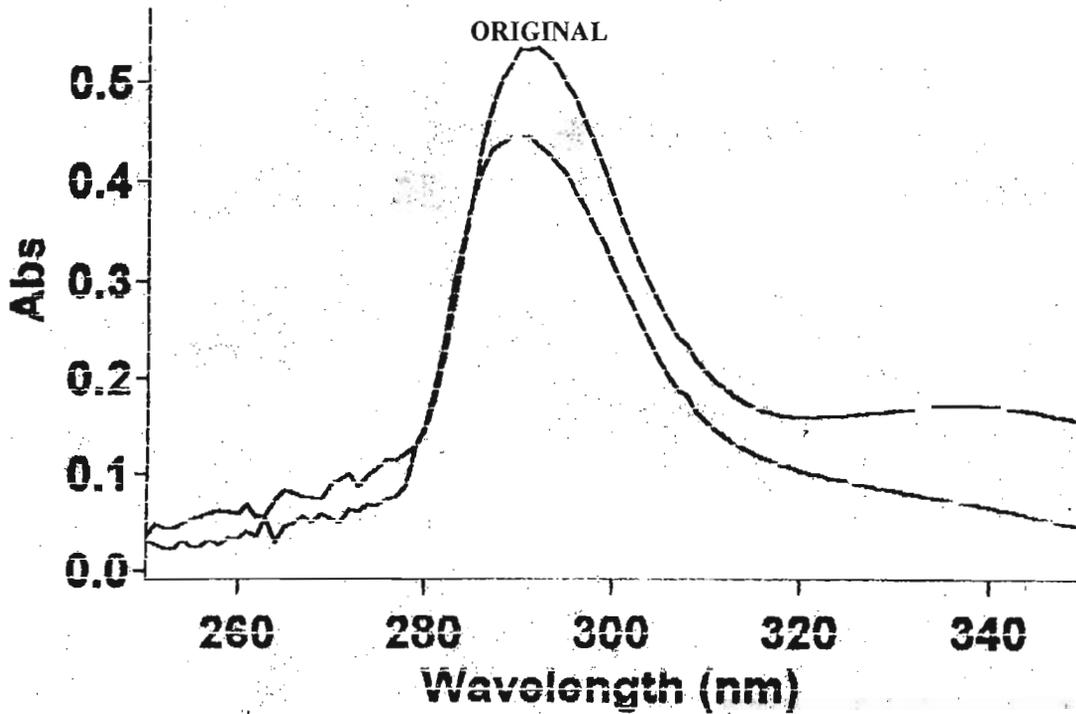
Spectrum 6.12: UV spectrum of compound 6 with NaOAc
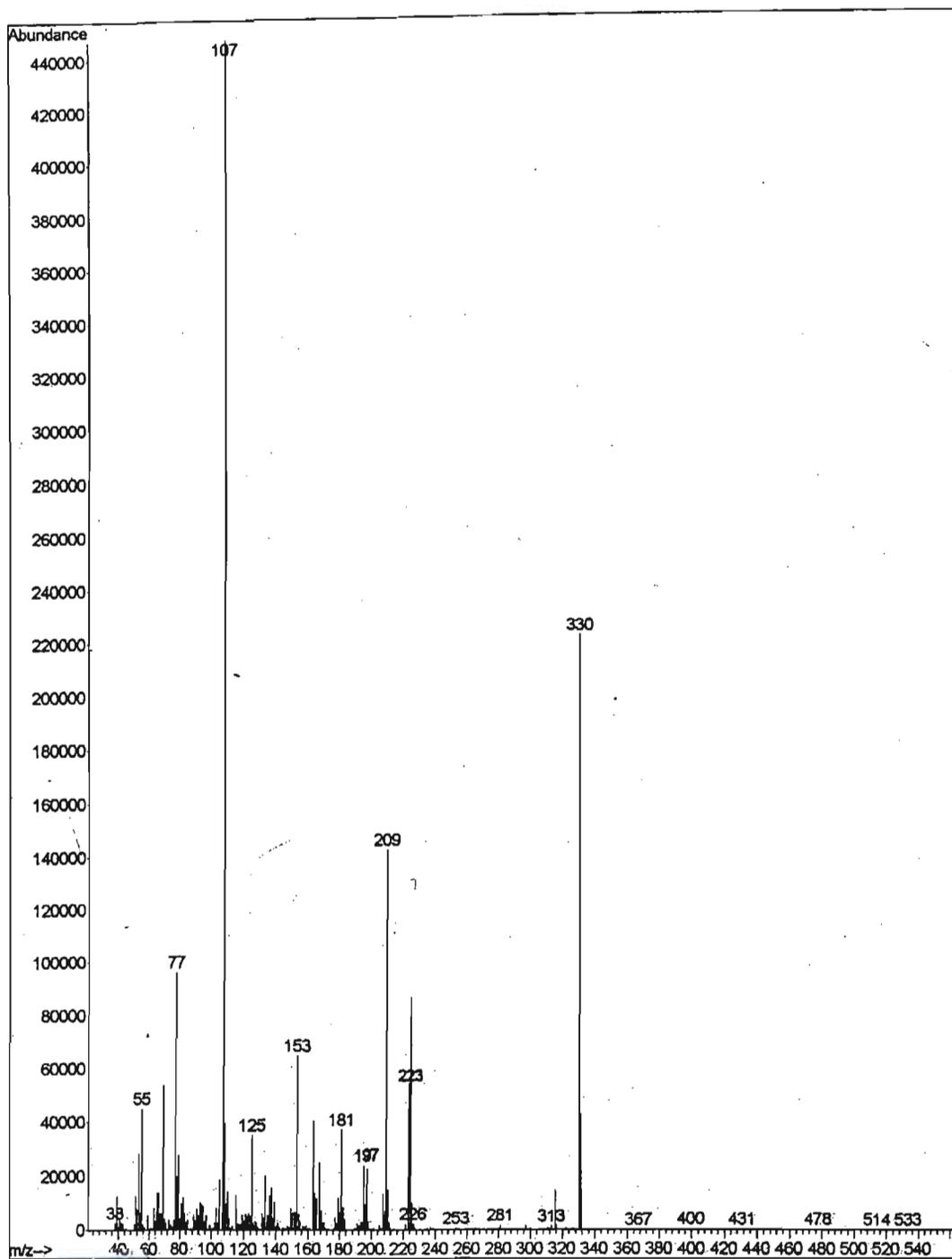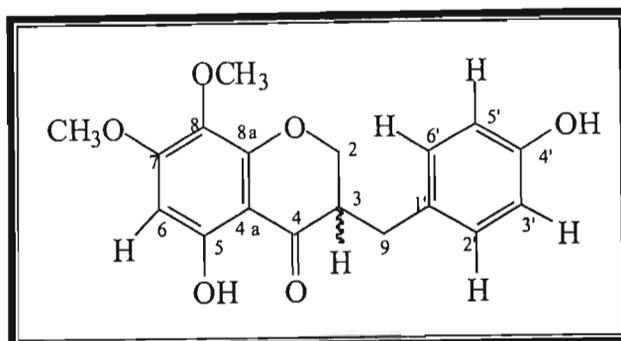
Spectrum 6.13: Mass spectrum of compound 6
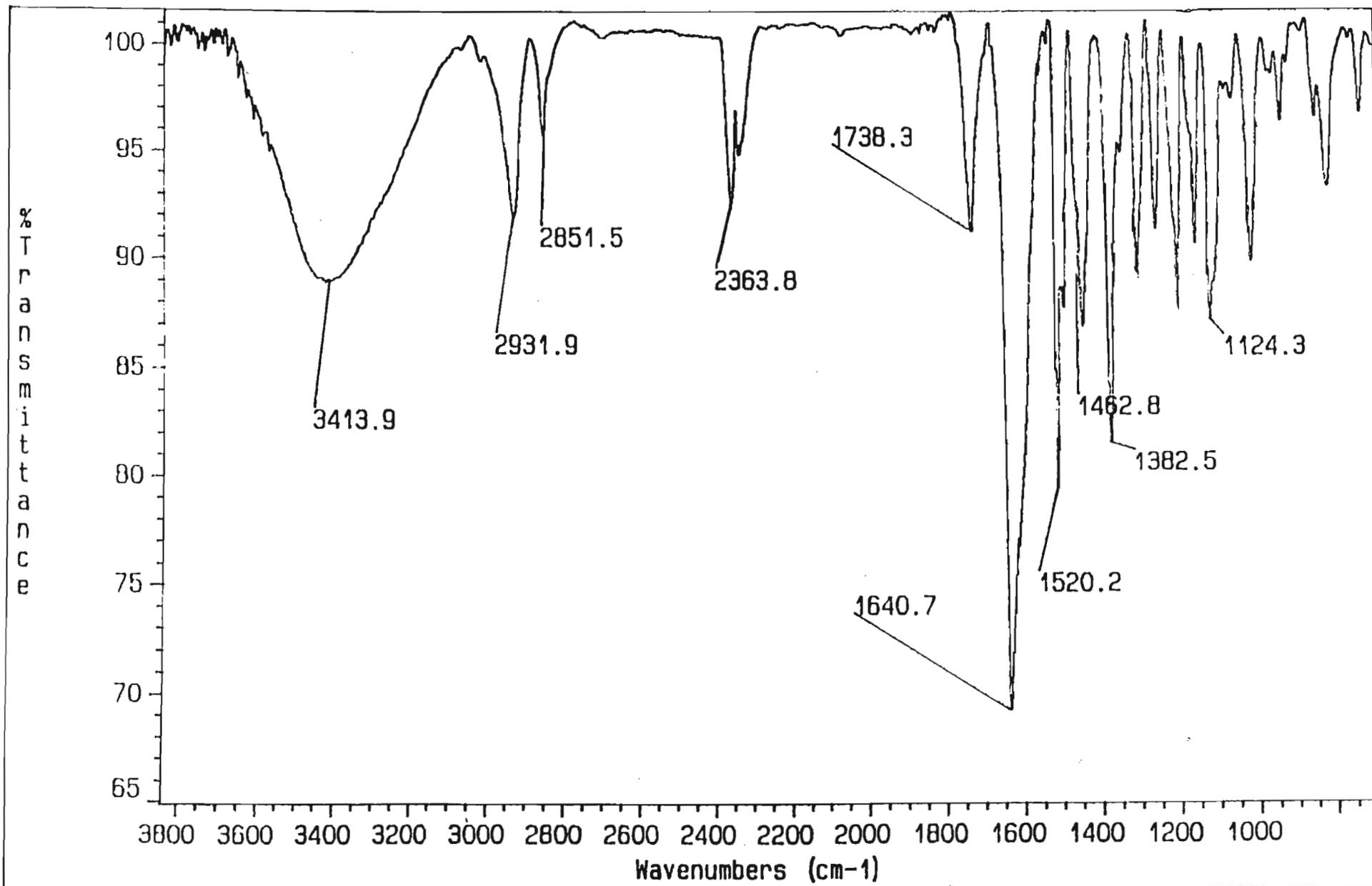
Spectrum 6.14: IR spectrum of compound 6
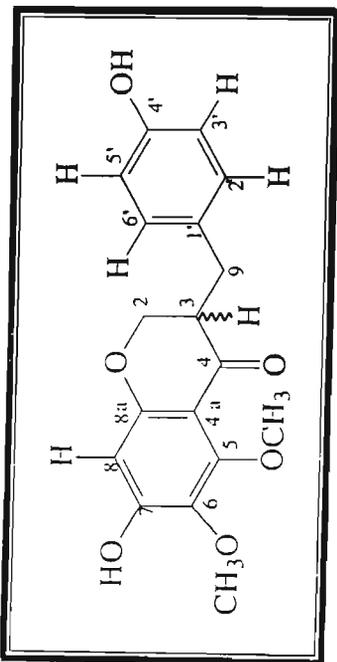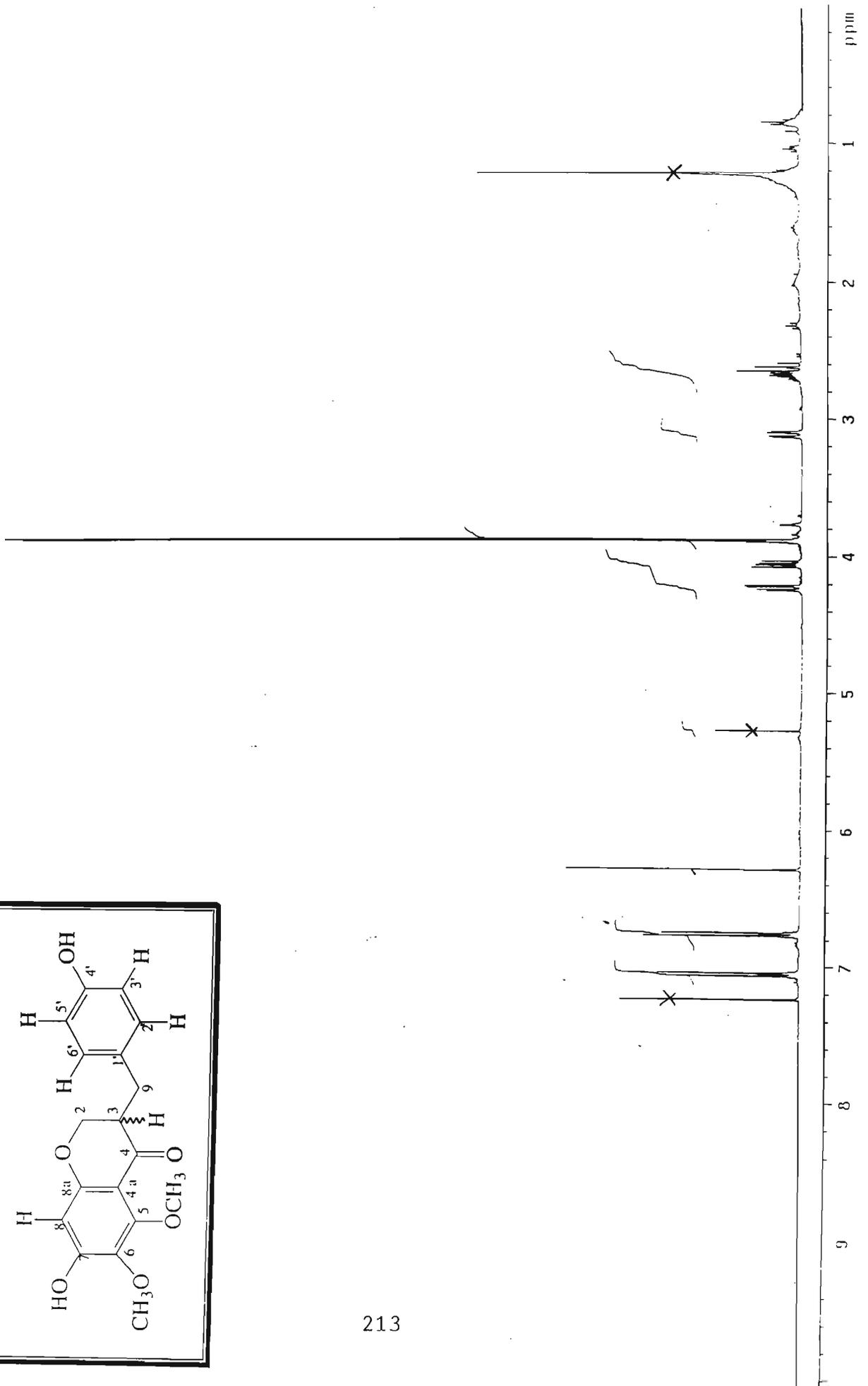
Spectrum 7.1: ^1H NMR spectrum of compound 7 (CDCl$_3$)

213
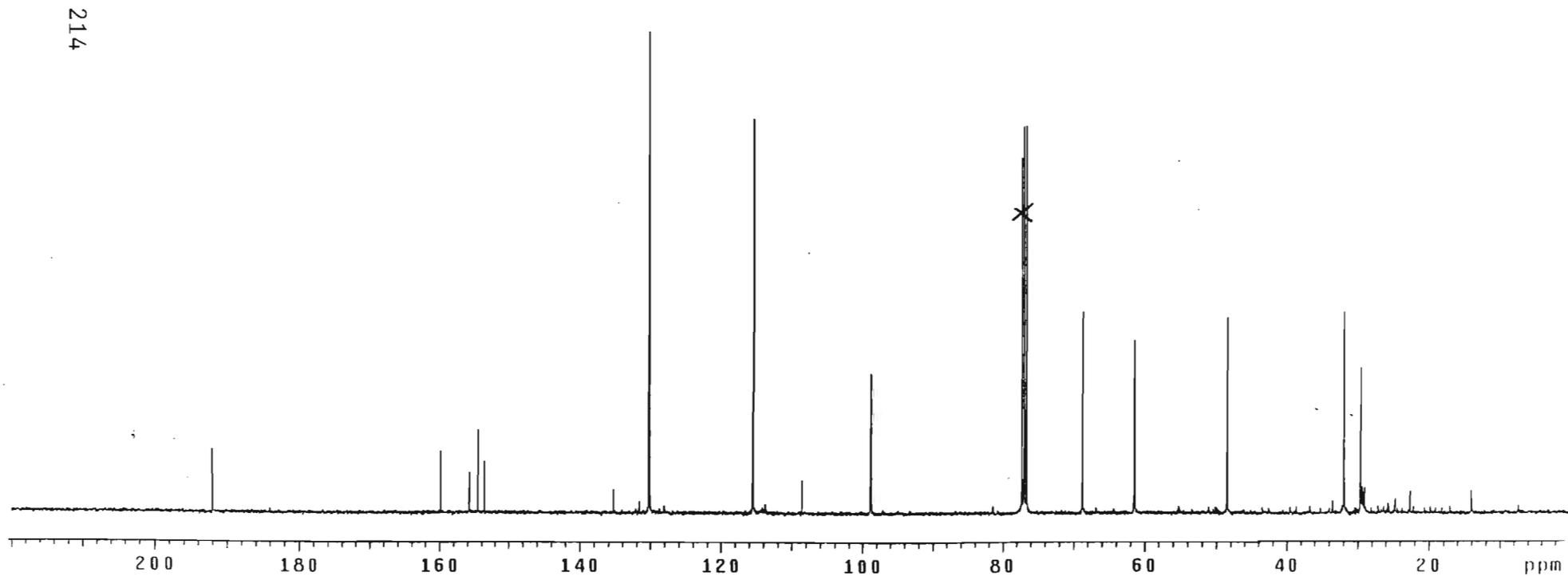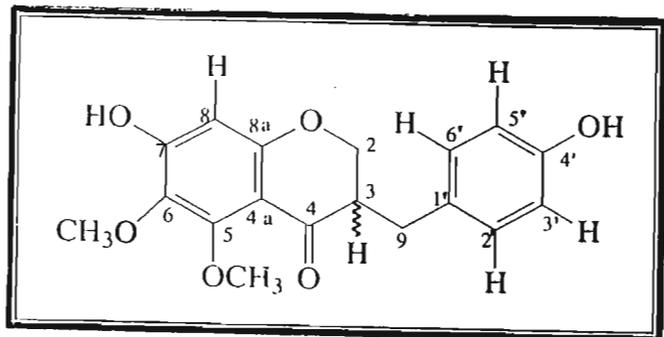
Spectrum 7.2: ^{13}C NMR spectrum of compound 7 (CDCl$_3$)
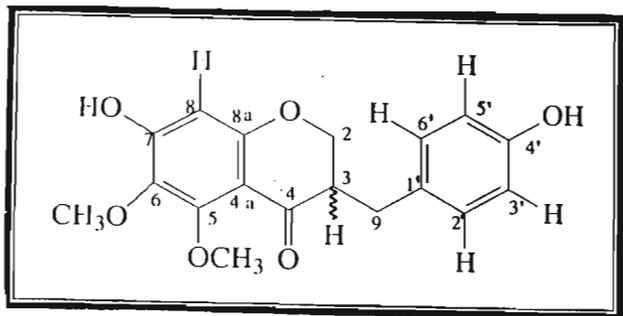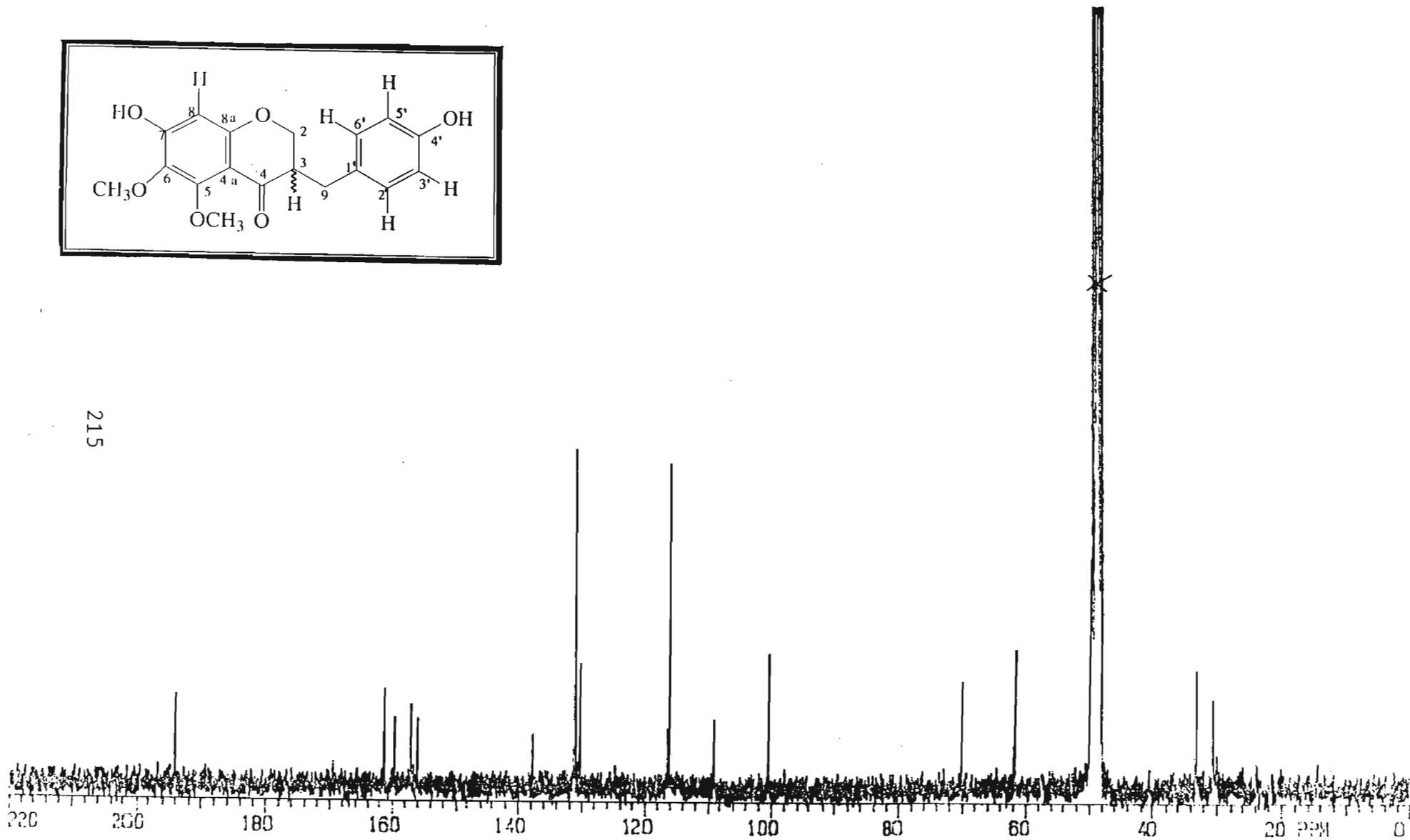
Spectrum 7.3: ^{13}C NMR data of compound 7 (CD$_3$OD)

215

CH3 carbons

CH2 carbons

216

CH carbons

all protonated carbons

130 120 110 100 90 80 70 60 50 40 30 20 10 ppm
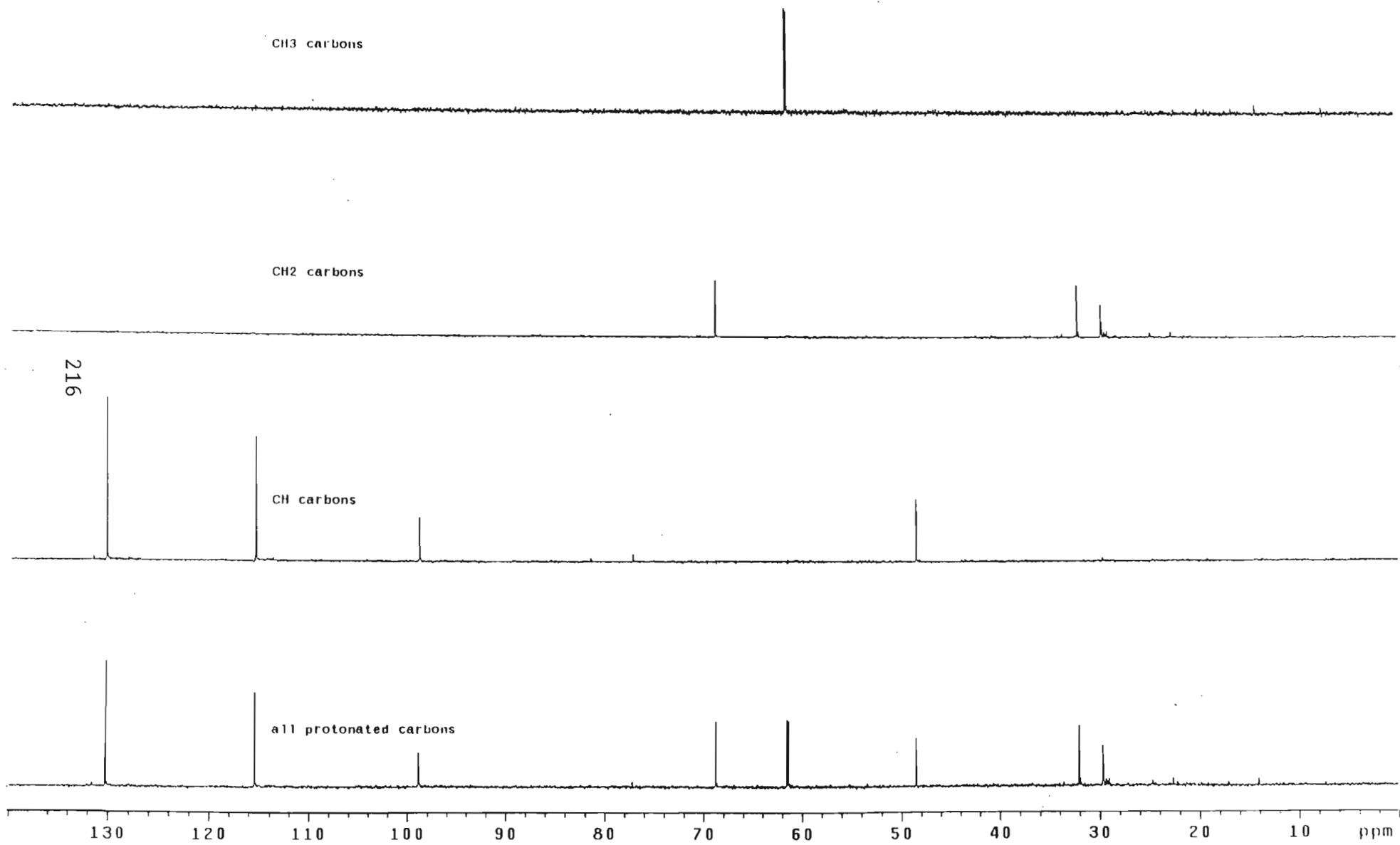
Spectrum 7.4: ADEPT spectrum of compound 7 (CDCl₃)
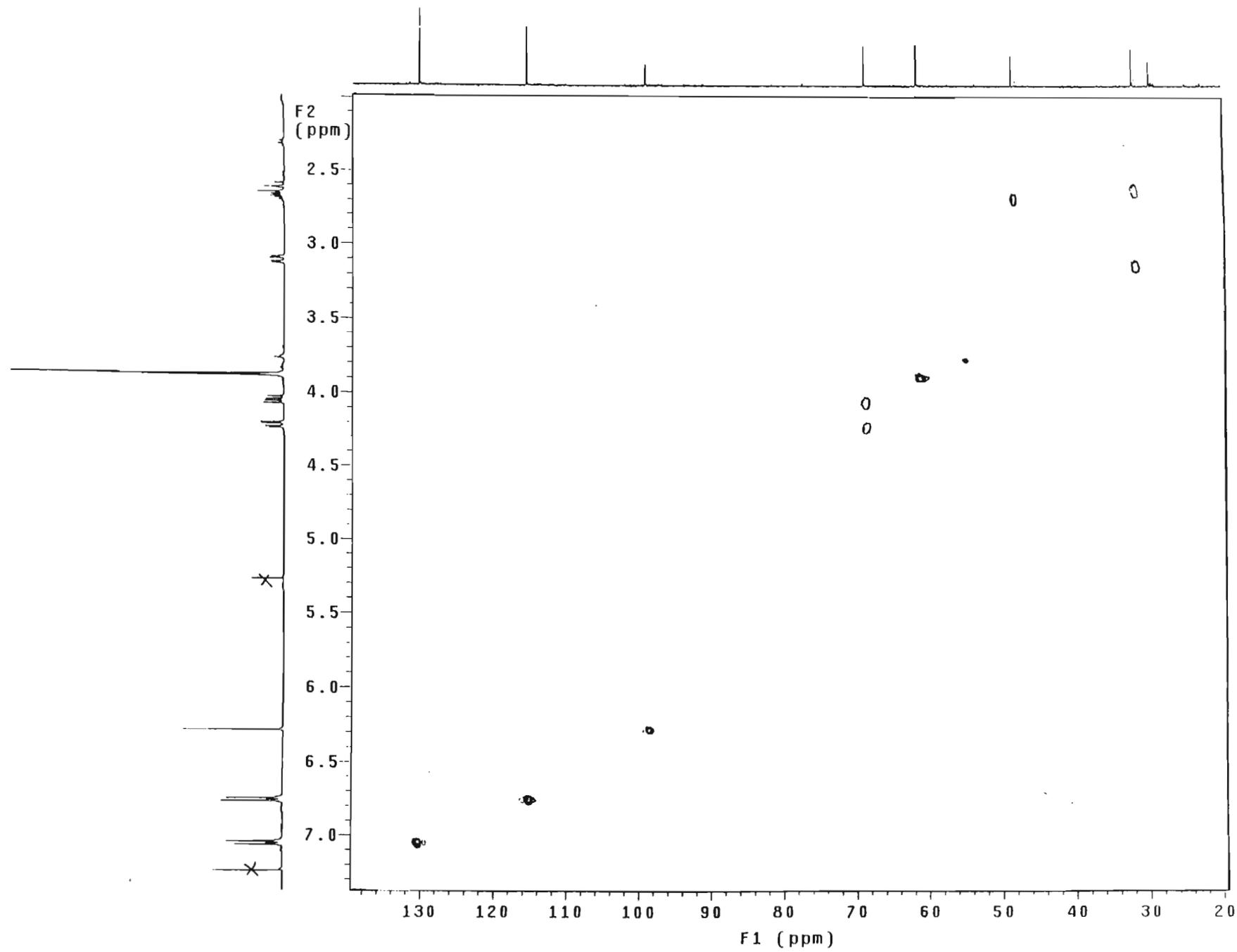
Spectrum 7.5: HSQC spectrum of compound 7 (CDCl₃)
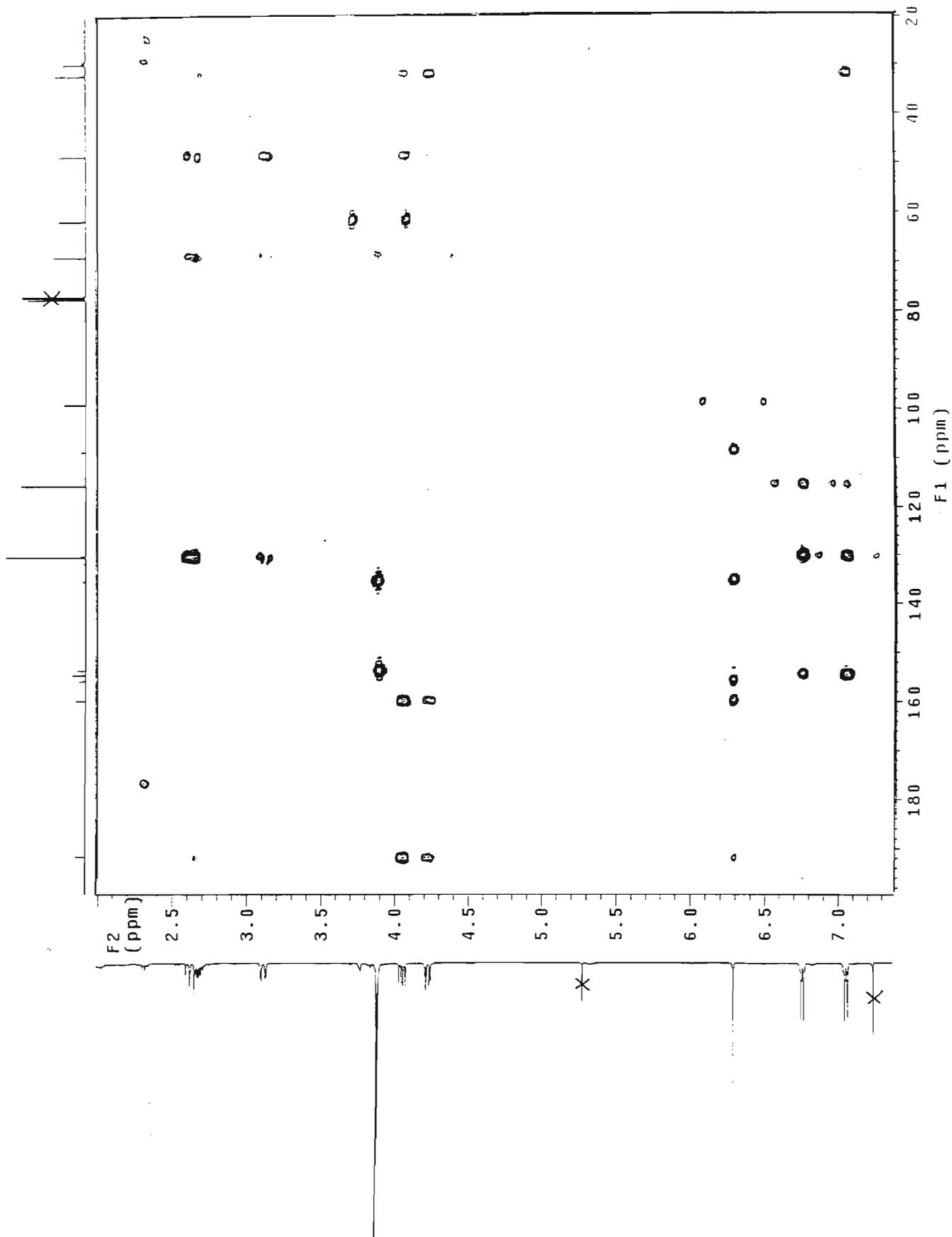
Spectrum 7.6: HMBC spectrum of compound 7 (CDCl₃)

218
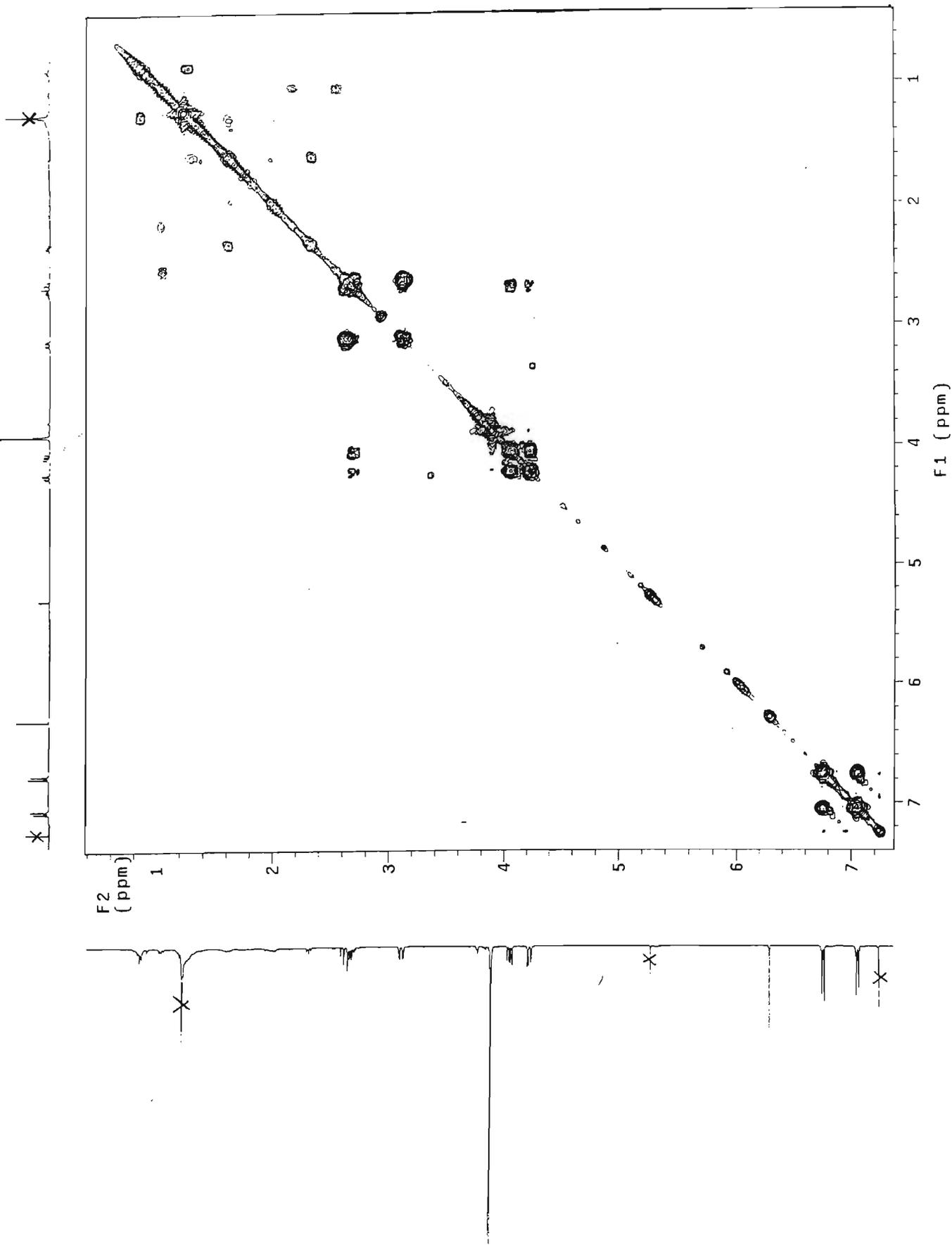
Spectrum 7.7: COSY spectrum of compound 7 (CDCl$_3$)

F1 (ppm)

F2 (ppm)
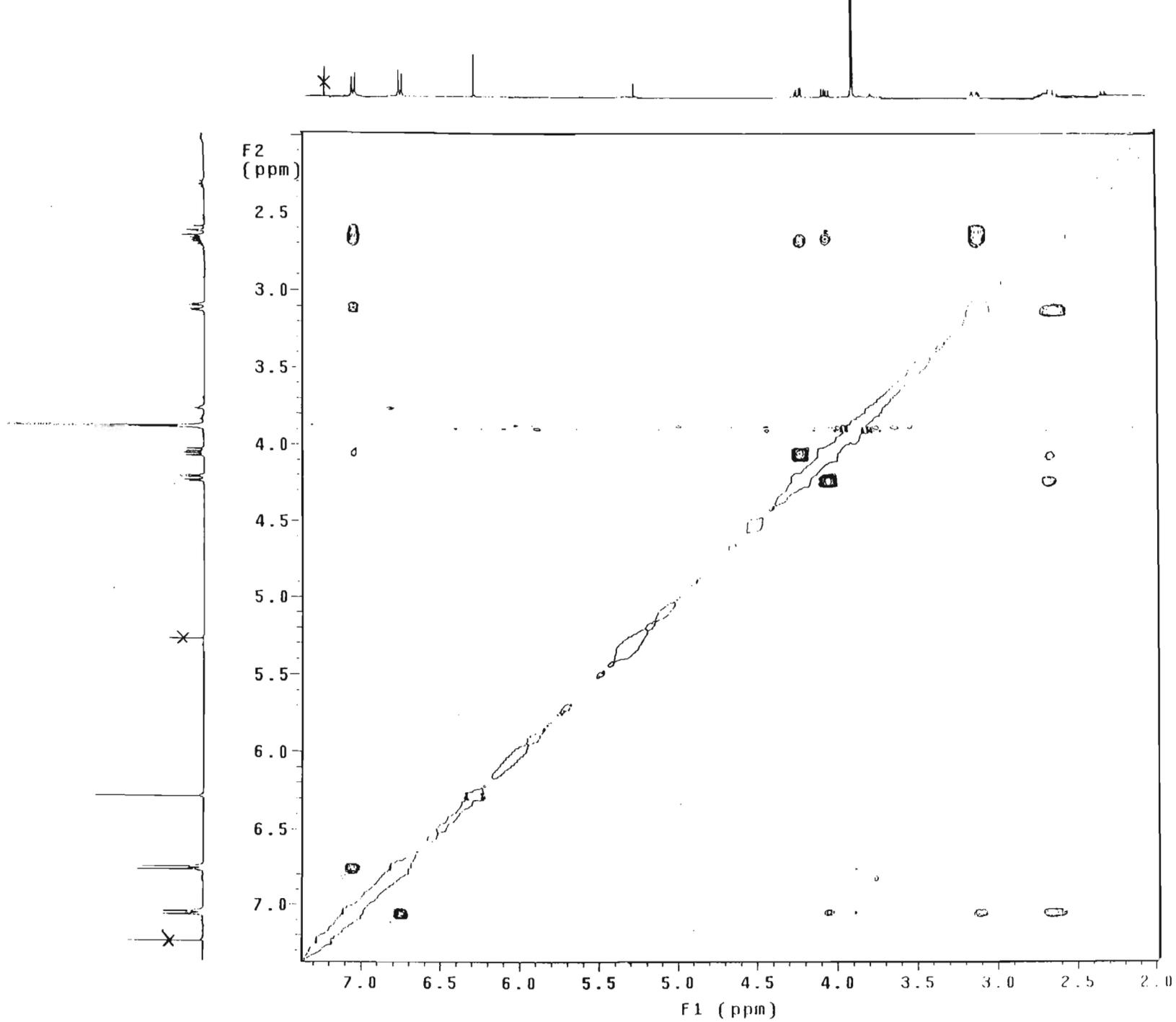
Spectrum 7.8: NOESY spectrum of compound 7 (CDCl₃)
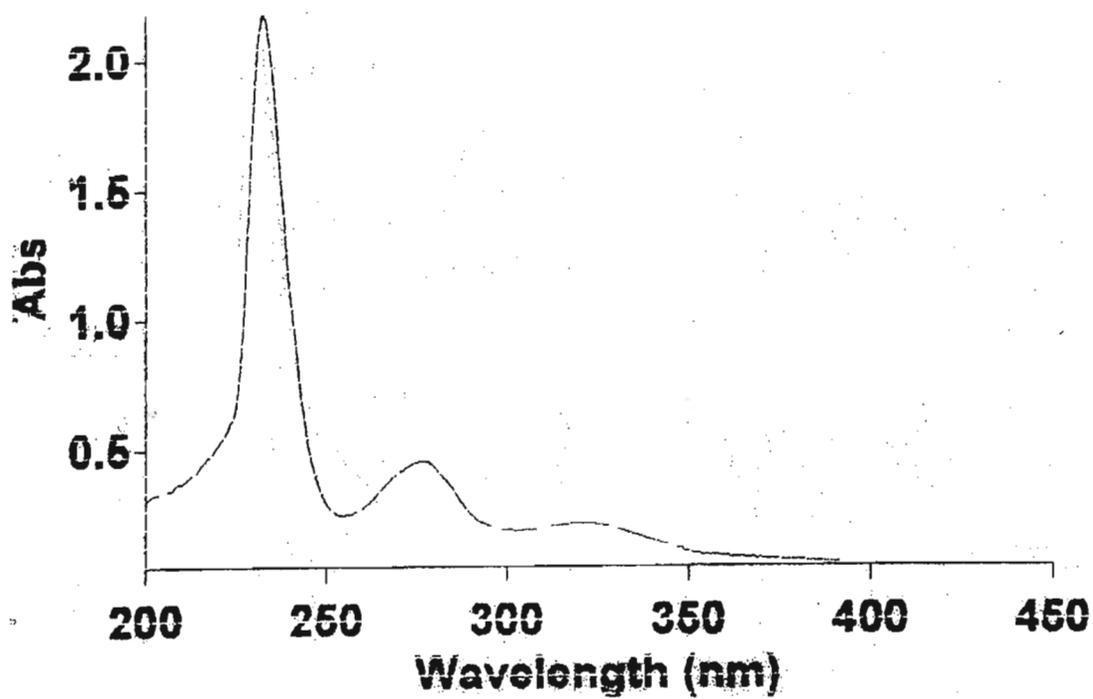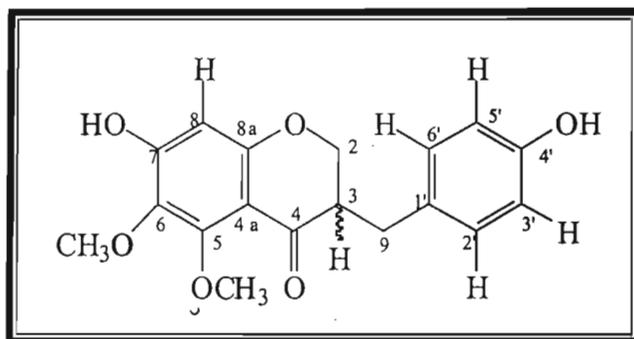
Spectrum 7.9: UV spectrum of compound 7
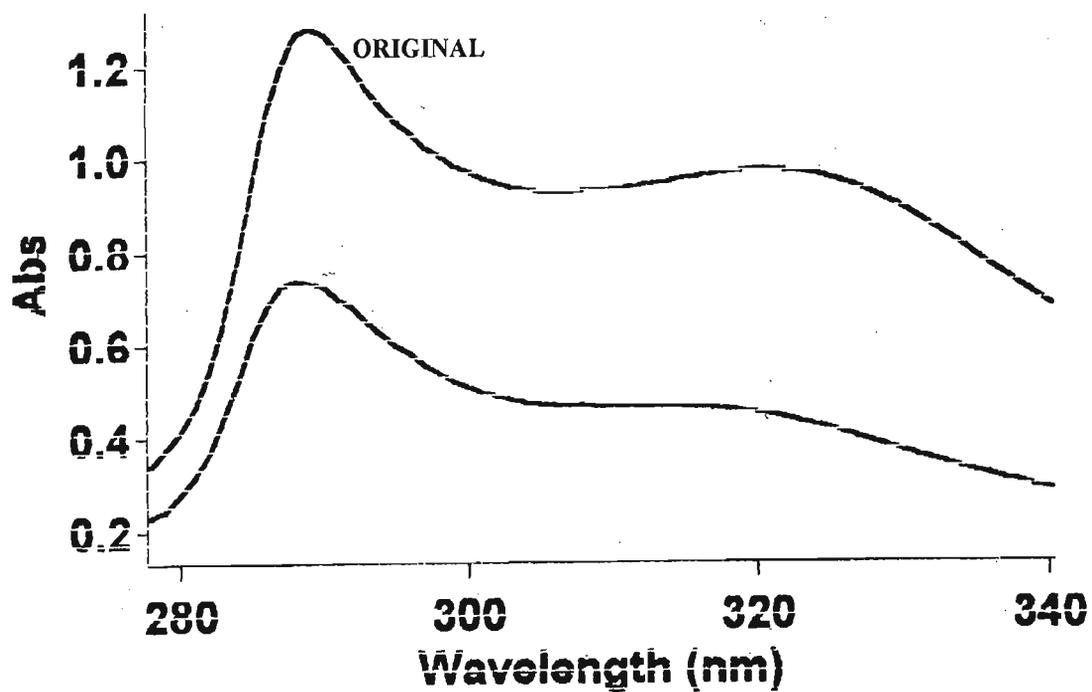
Spectrum 7.10: UV spectrum of compound 7 with AlCl₃
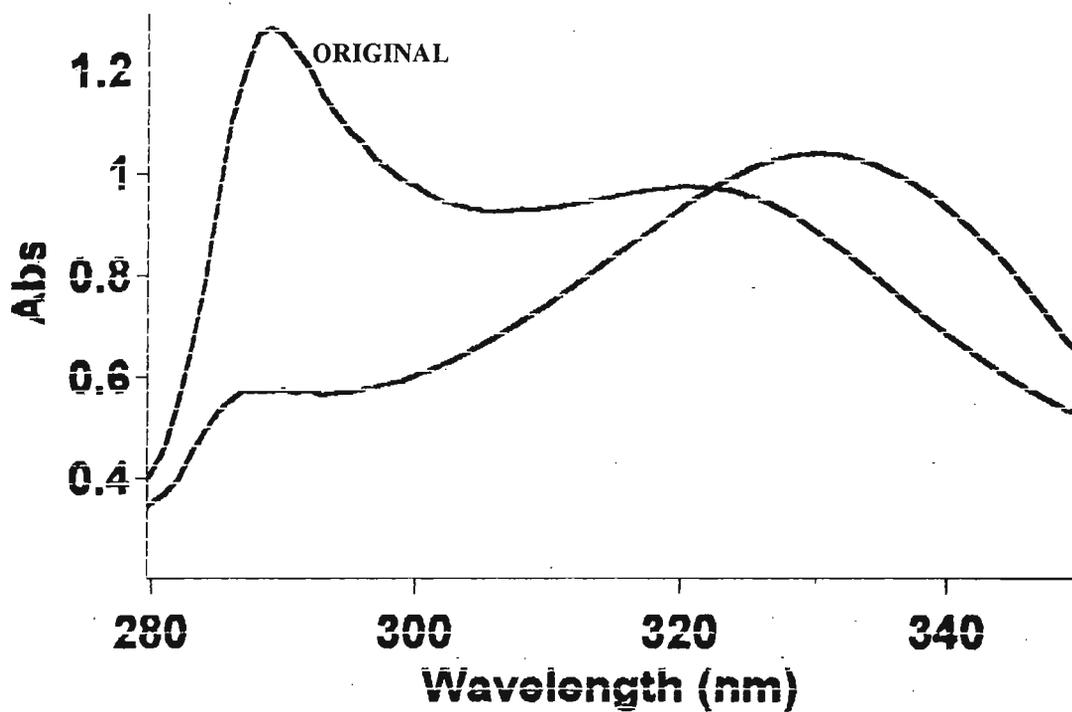
Spectrum 7.11: UV spectrum of compound 7 with NaOAc

222
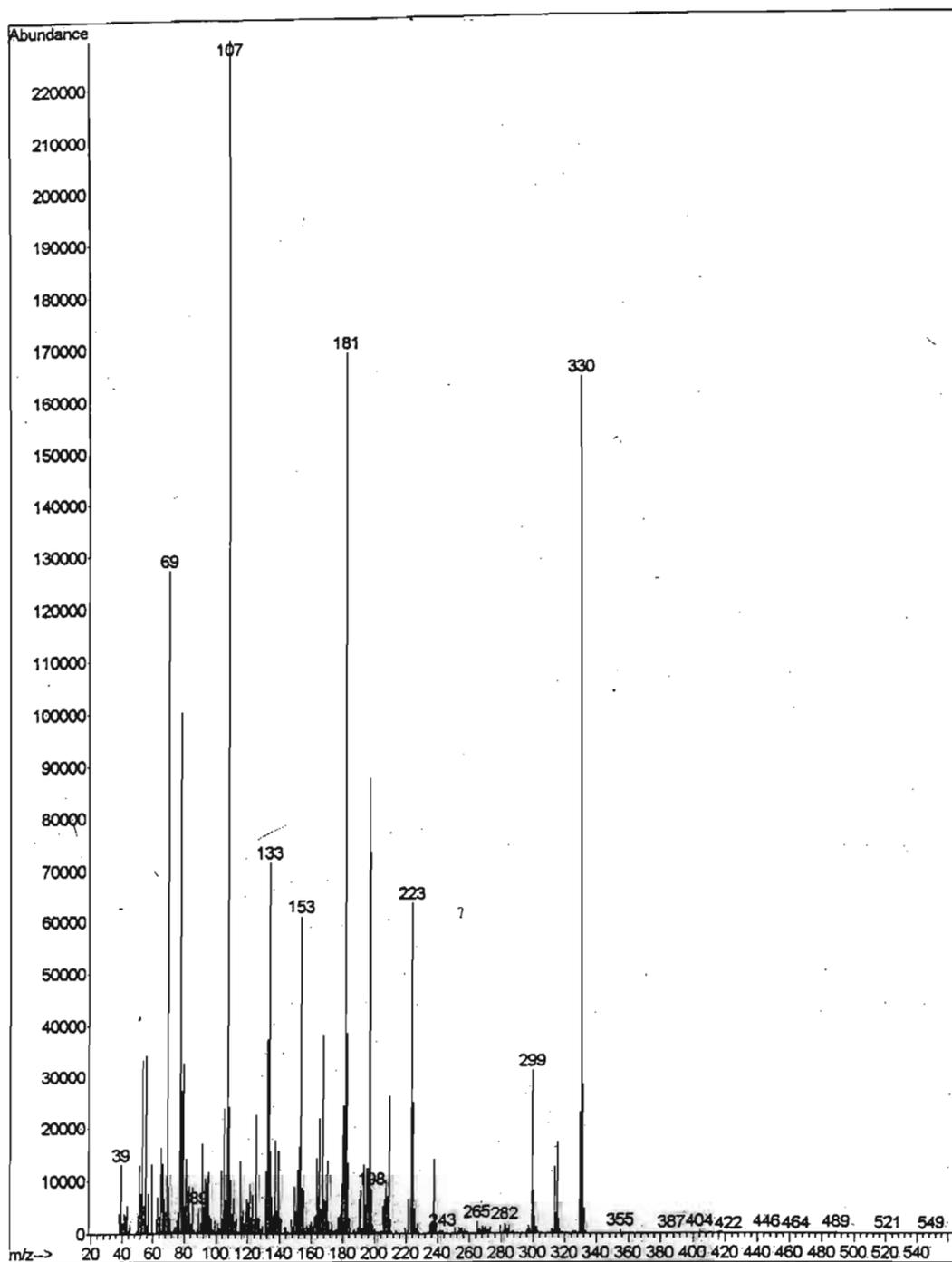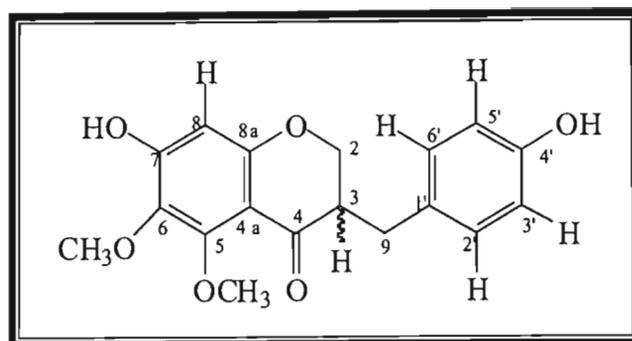
Spectrum 7.12: Mass spectrum of compound 7
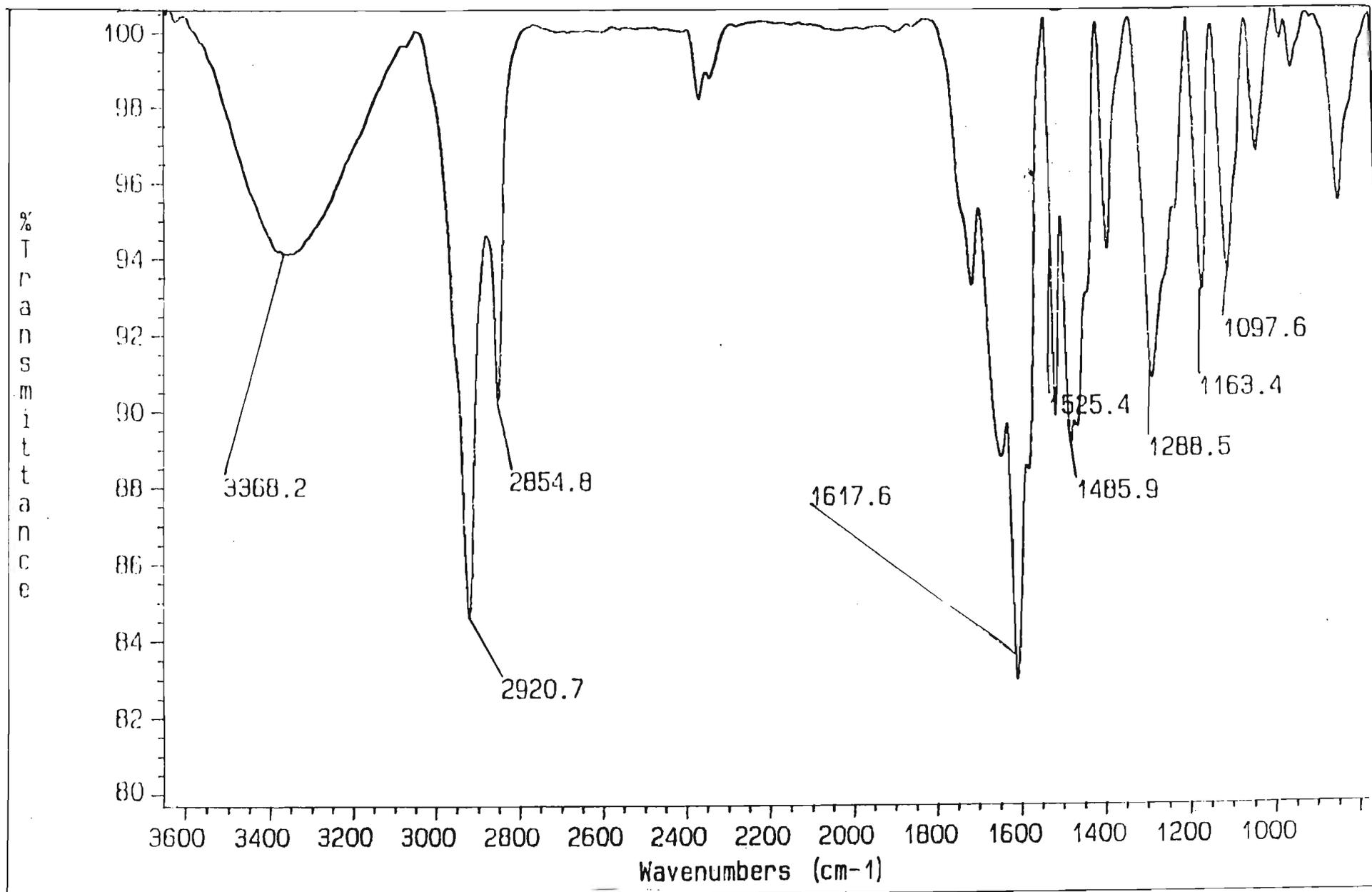
Spectrum 7.13: IR spectrum of compound 7
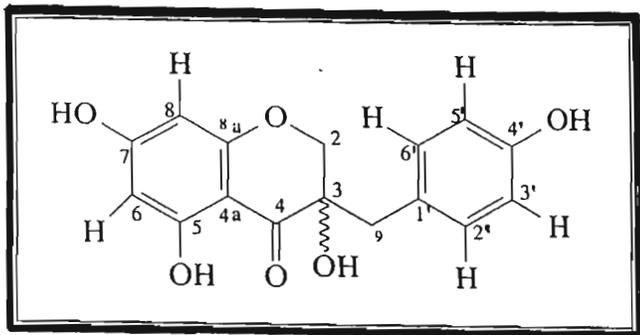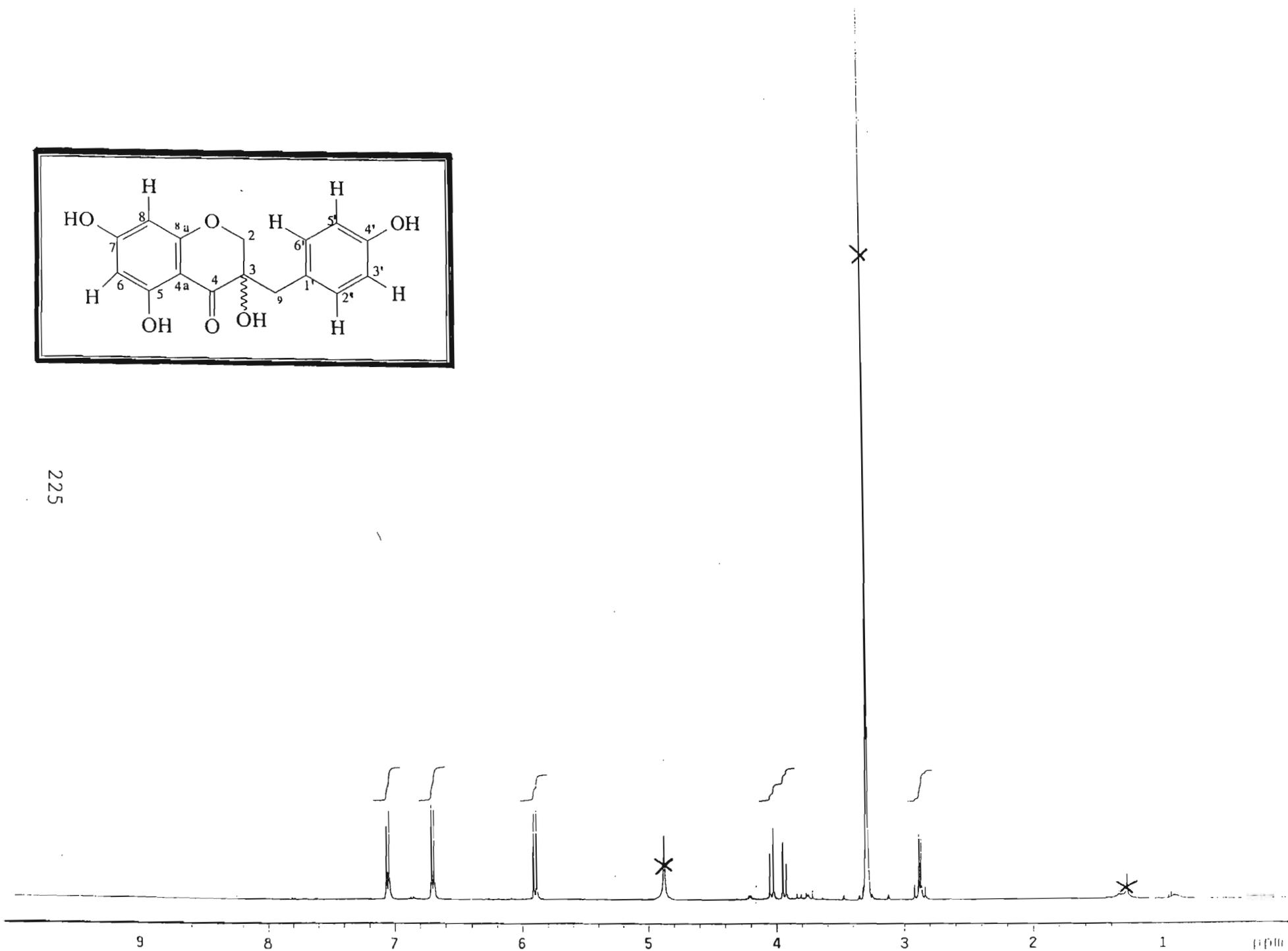
Spectrum 8.1: ^{1}H NMR spectrum of compound 8 (CD$_3$OD)
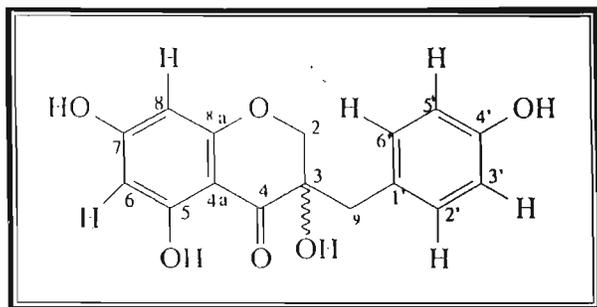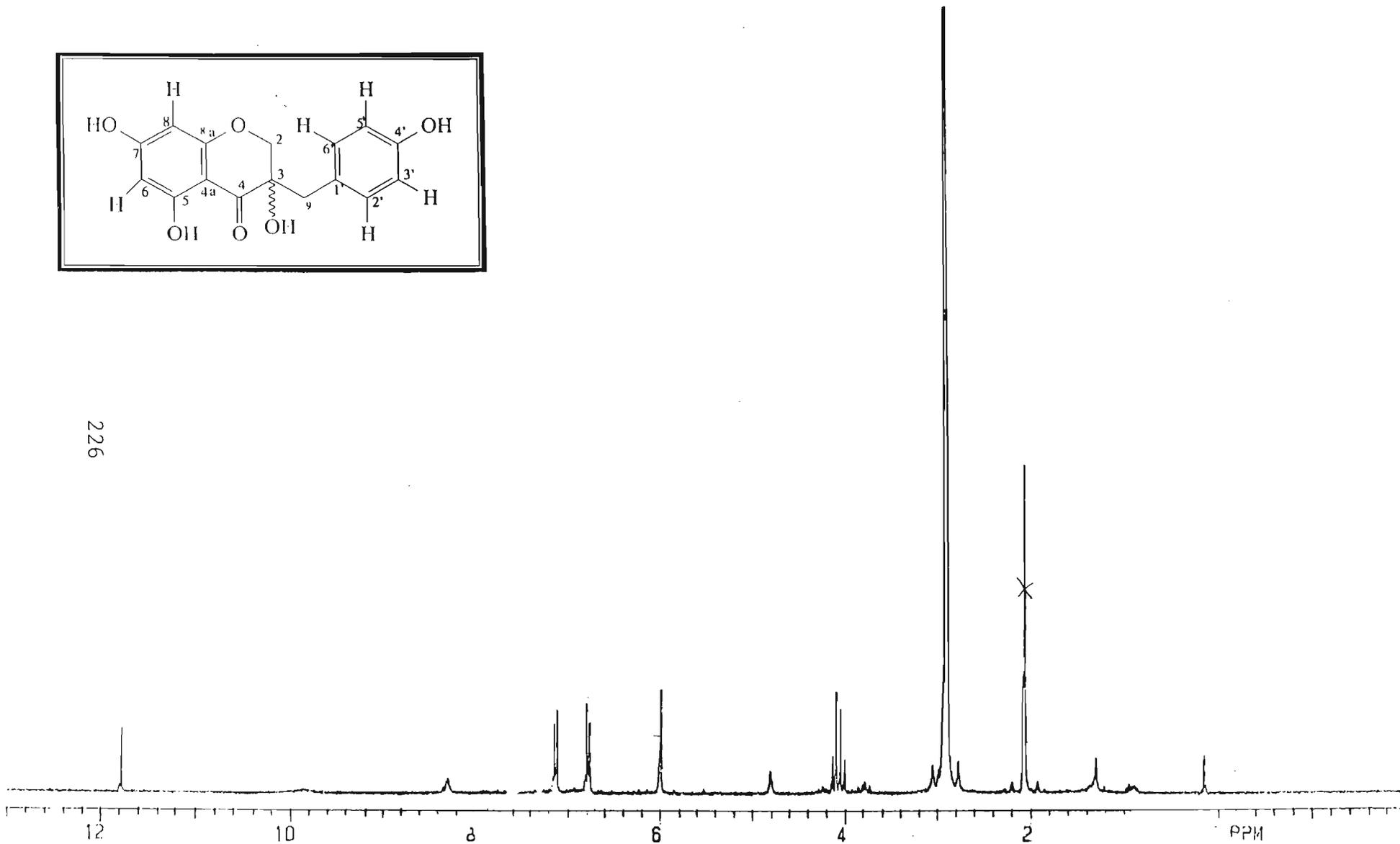
Spectrum 8.2: ¹H NMR spectrum of compound 8 (CD₃)₂CO
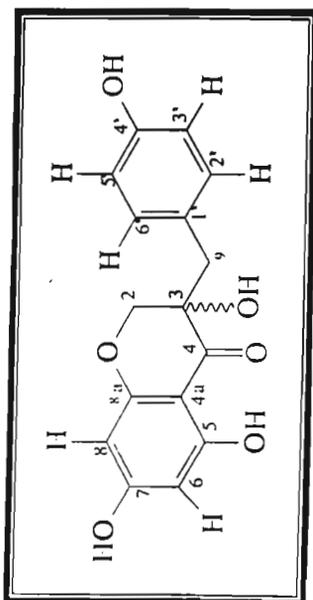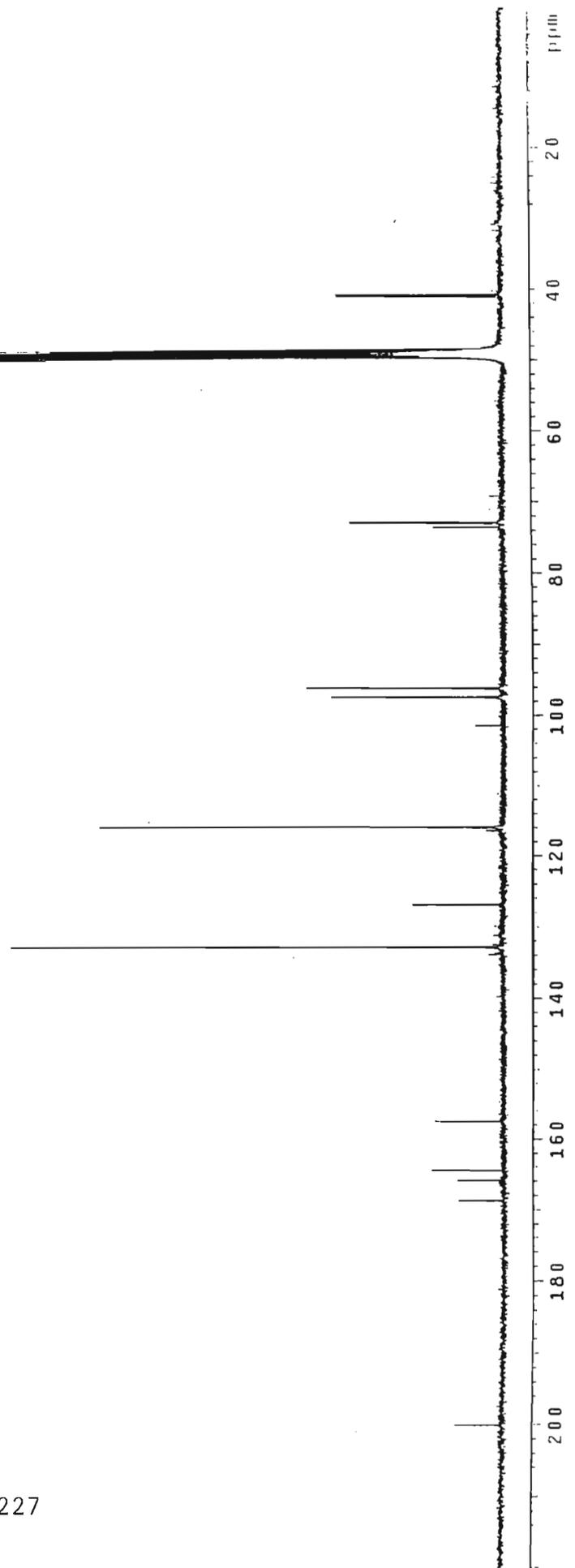
Spectrum 8.3: ^{13}C spectrum of compound 8 (CD$_3$OD)

227
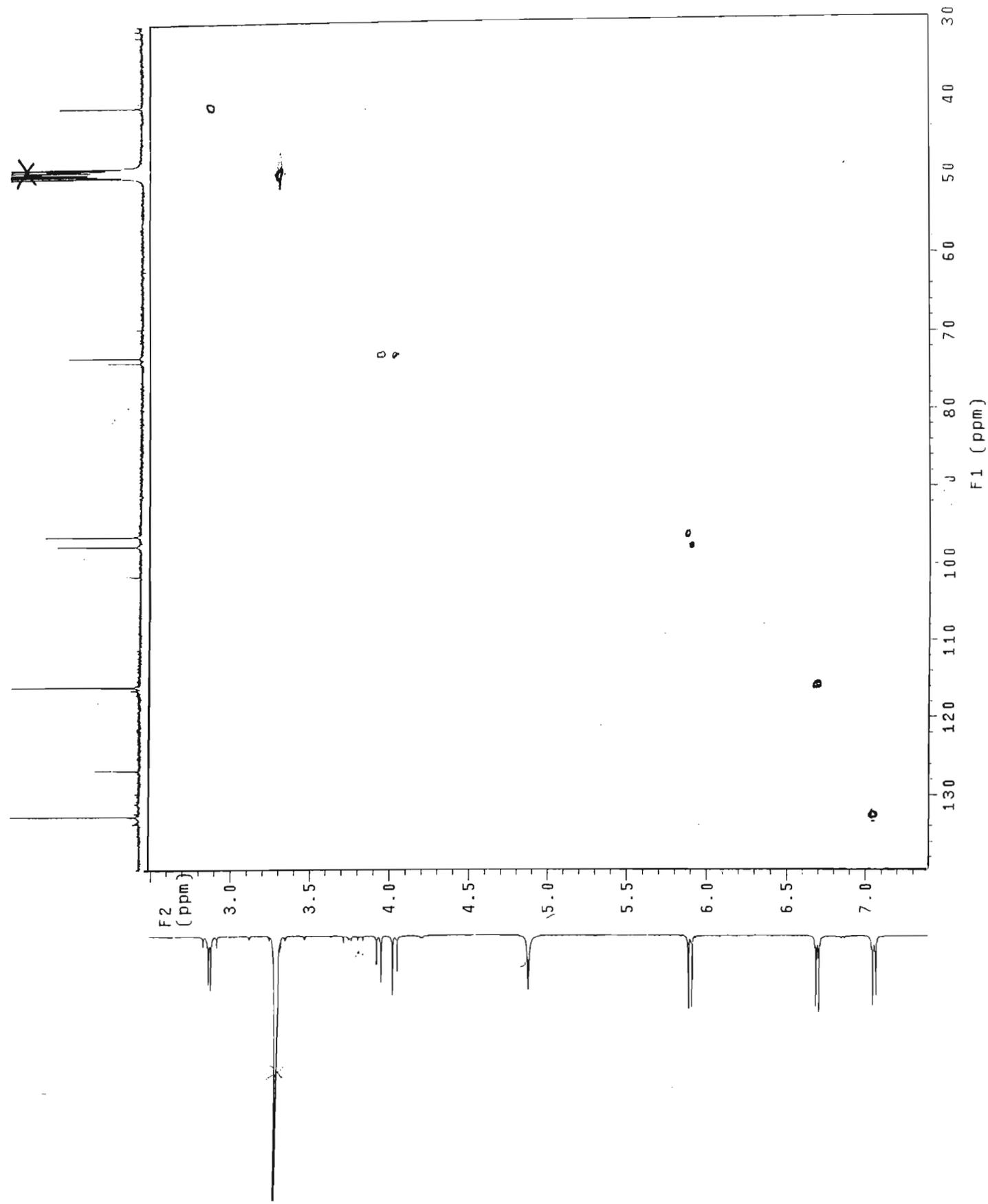
Spectrum 8.4: HSQC spectrum of compound 8 (CD₃OD)

F1 (ppm)

F2 (ppm)
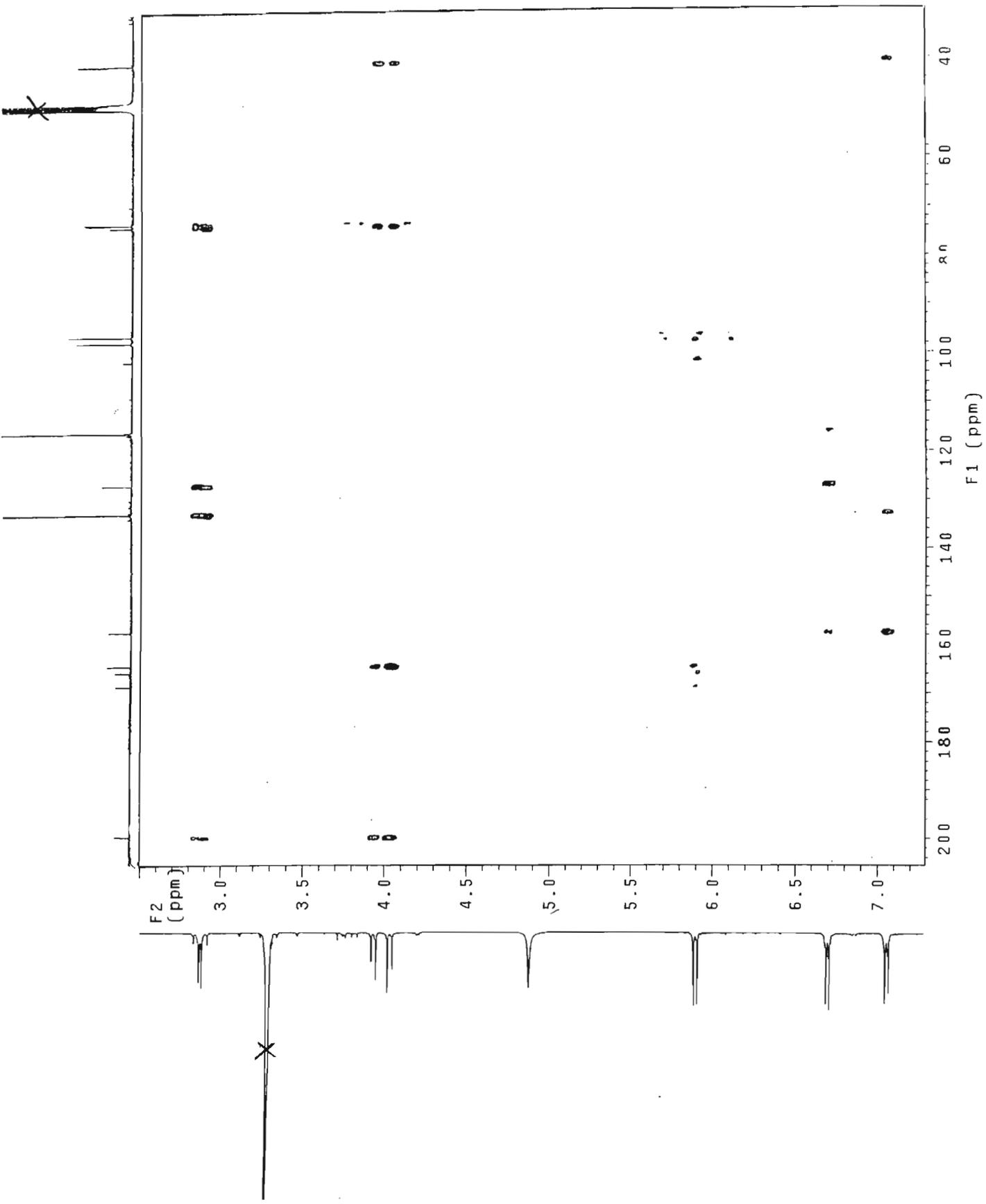
Spectrum 8.5: HMBC spectrum of compound 8 (CD₃OD)

229
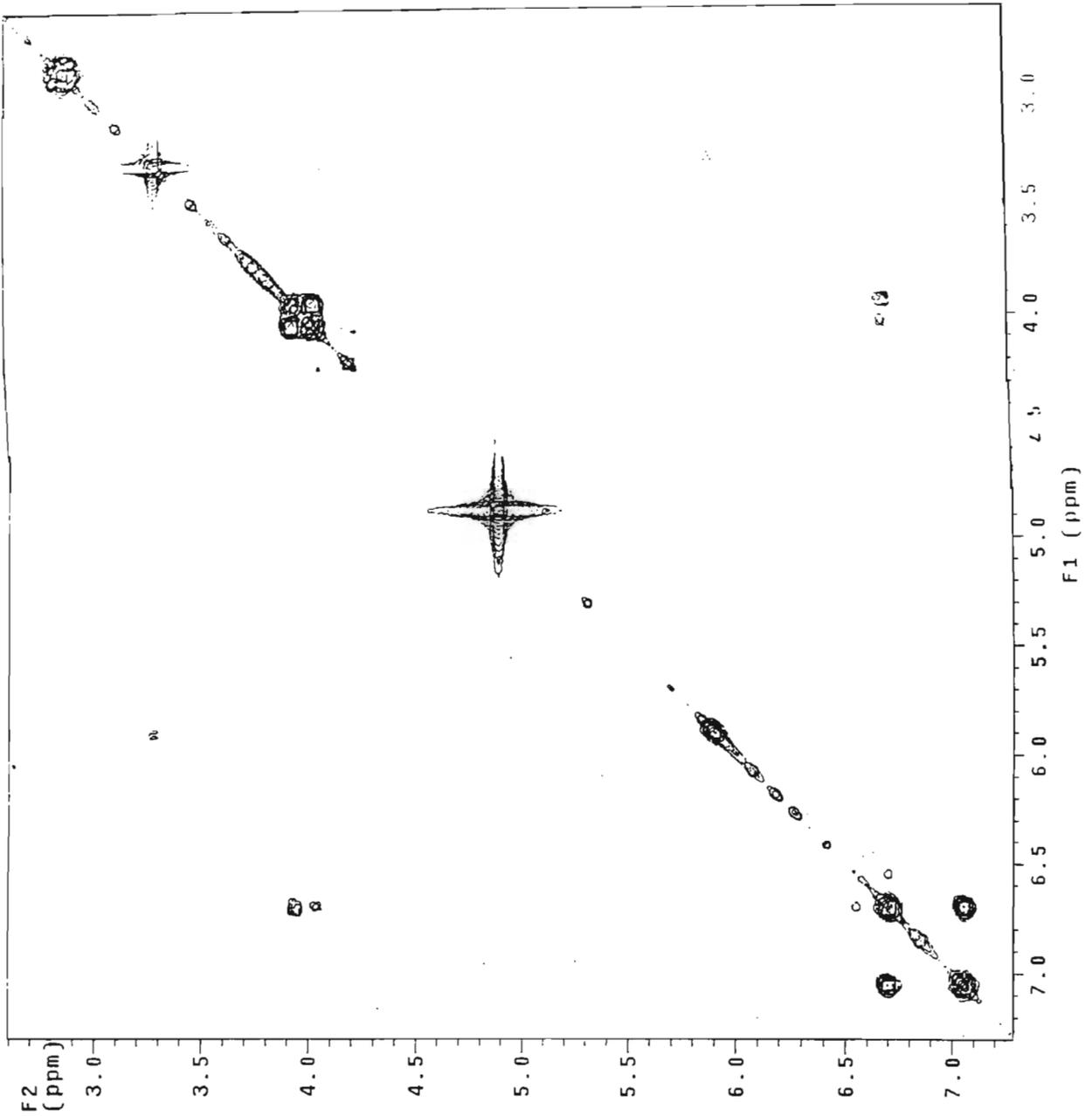
Spectrum 8.6: COSY spectrum of compound 8 (CD₃OD)

230
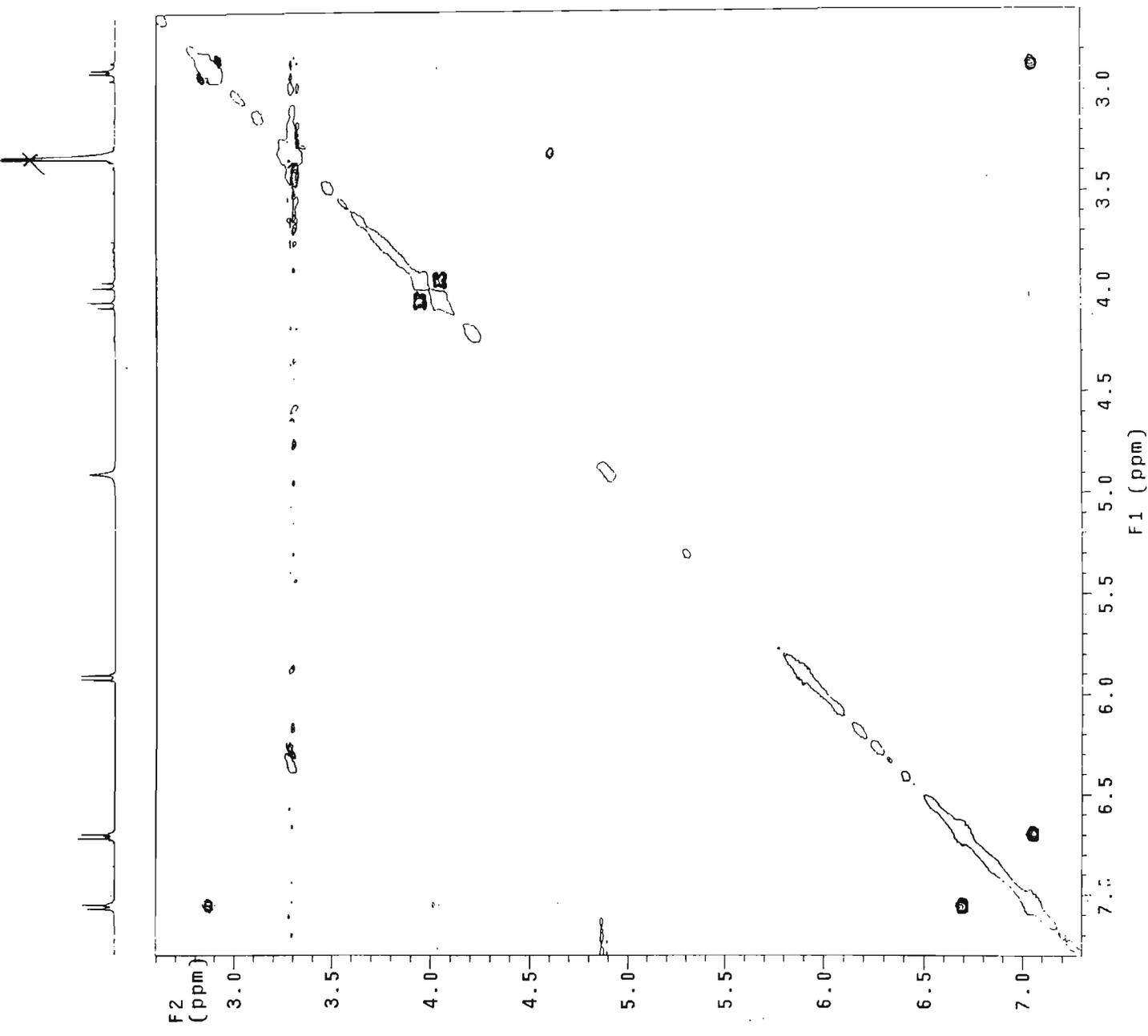
Spectrum 8.7: NOESY spectrum of compound 8 (CD₃OD)

231
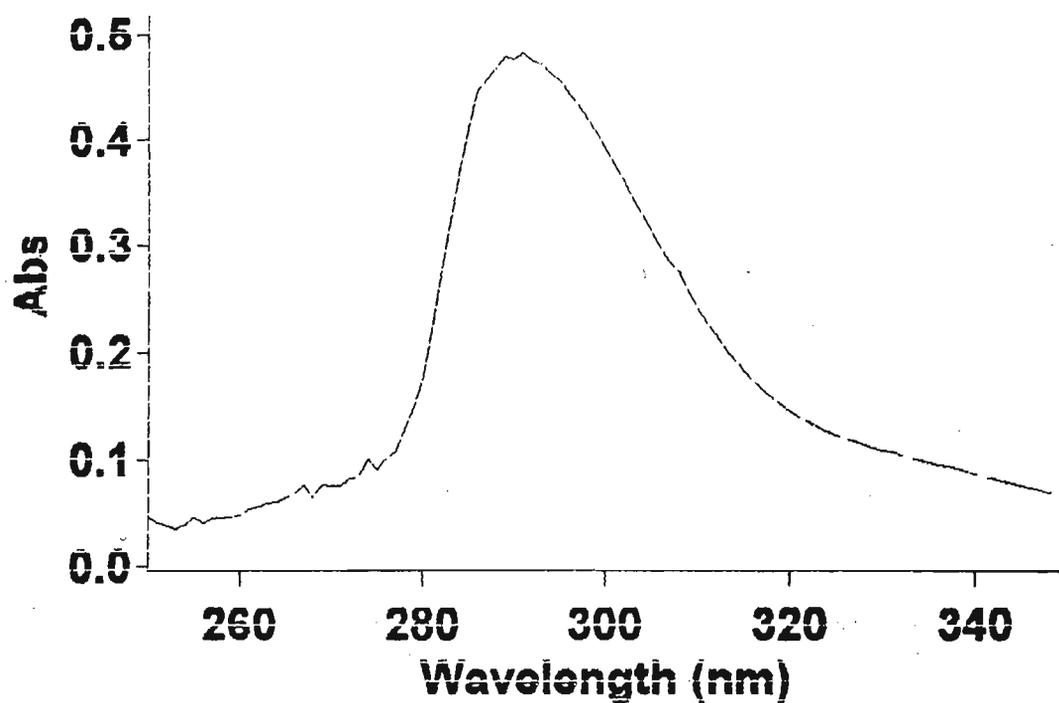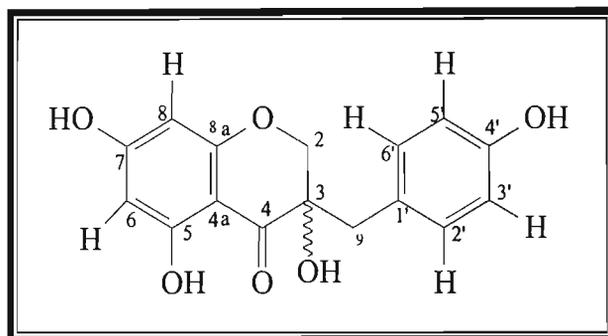
Spectrum 8.8: UV spectrum of compound 8
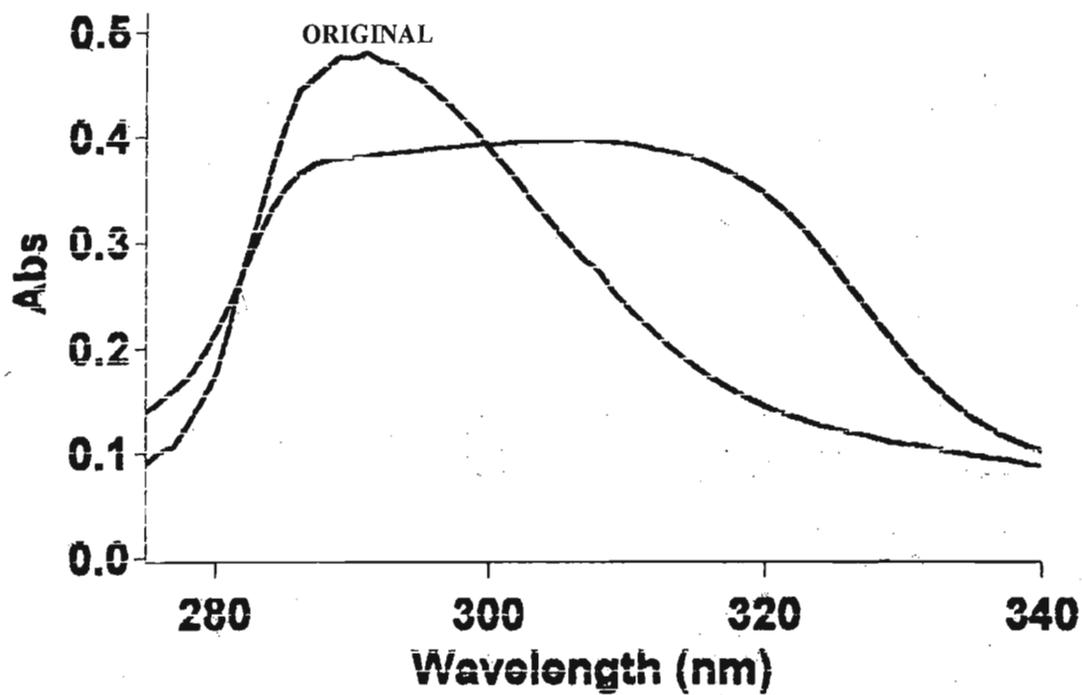
Spectrum 8.9: UV spectrum of compound 8 with AlCl₃
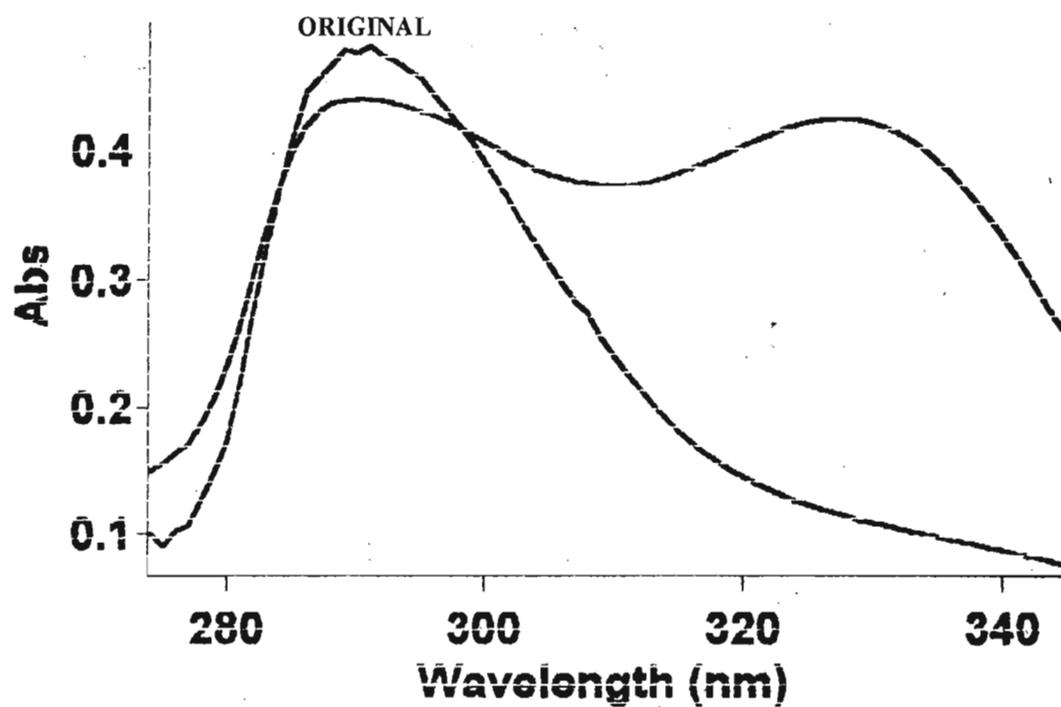
Spectrum 8.10: UV spectrum of compound 8 with NaOAc
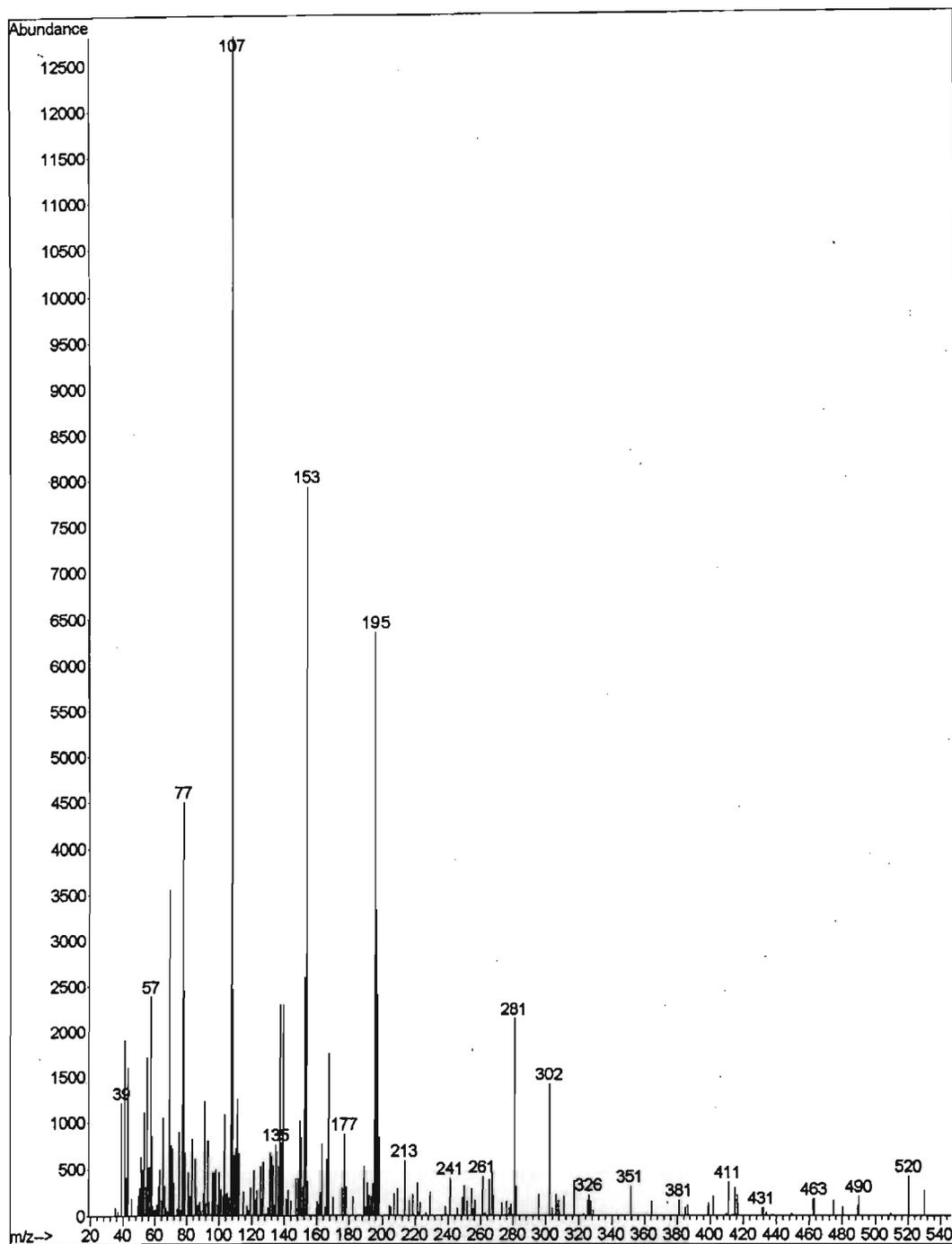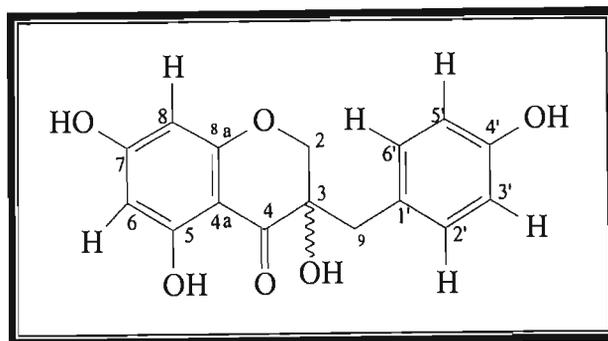
Spectrum 8.11 Mass spectrum of compound 8
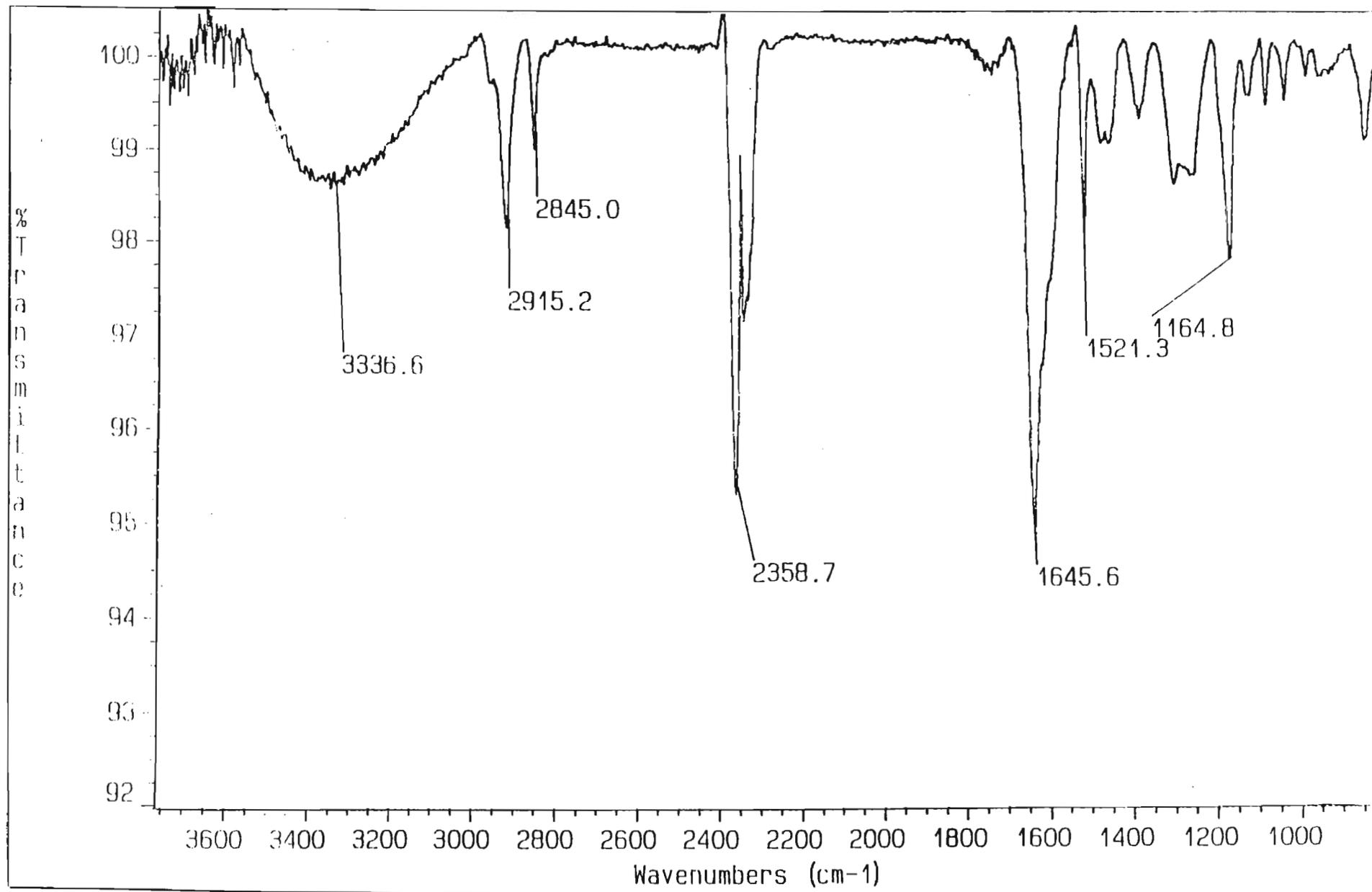
Spectrum 8.12: IR spectrum of compound 8
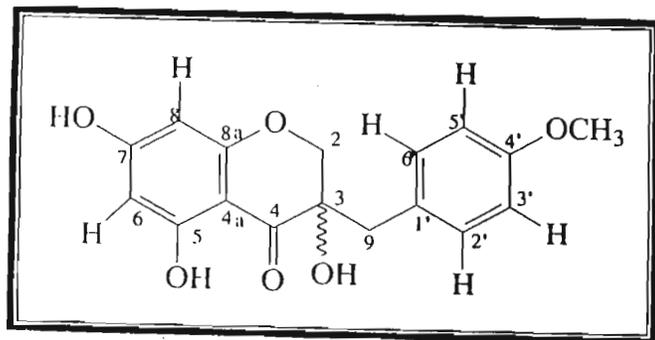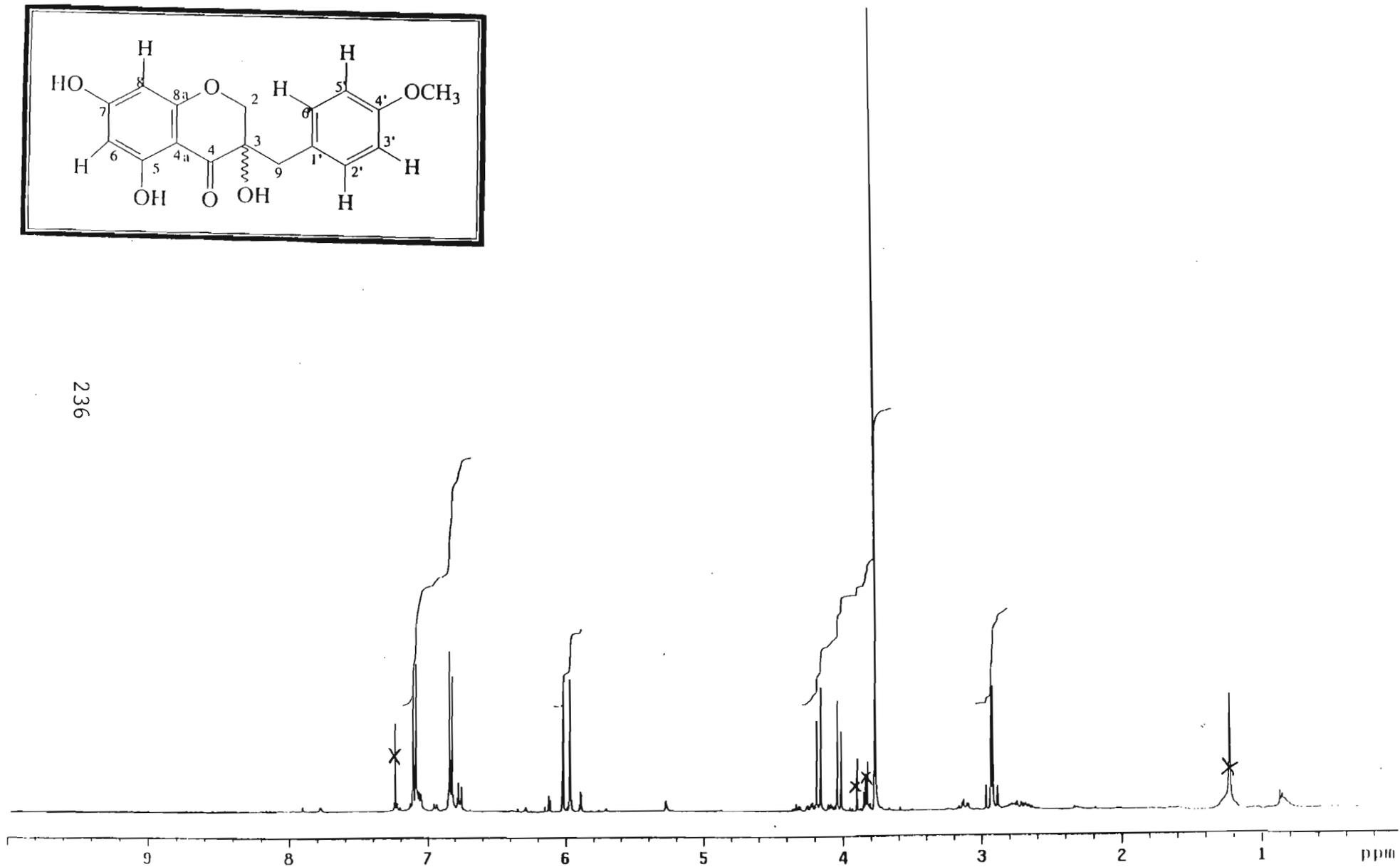
Spectrum 9.1: ^{1}H NMR spectrum of compound 9 (CDCl$_3$)
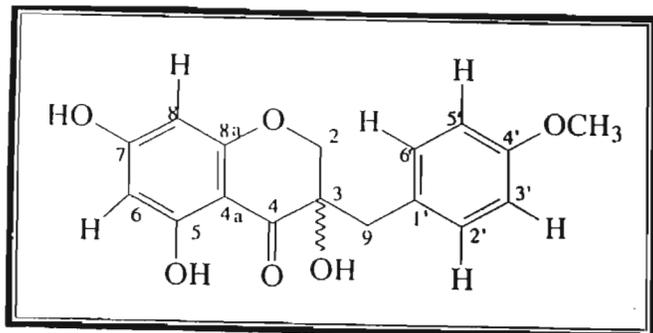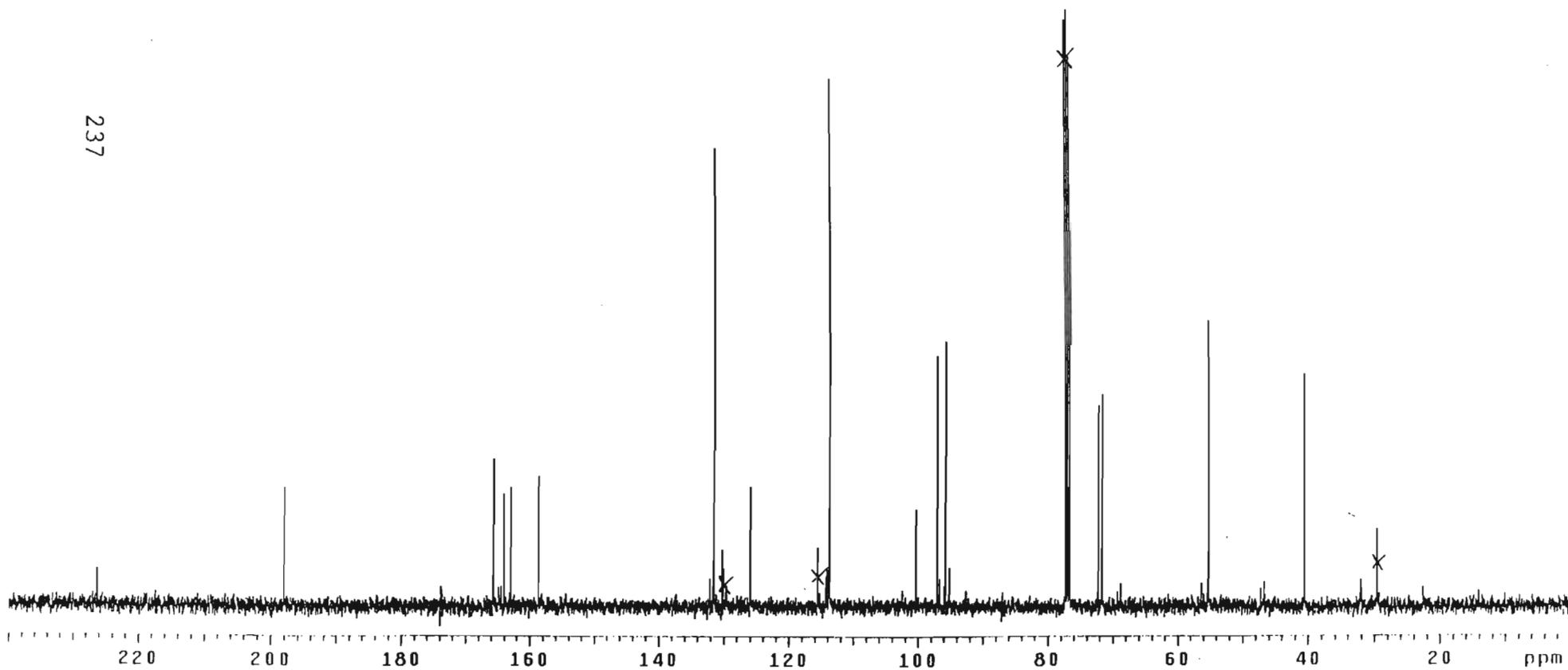
Spectrum 9.2: ^{13}C NMR spectrum of compound 9 (CDCl$_3$)
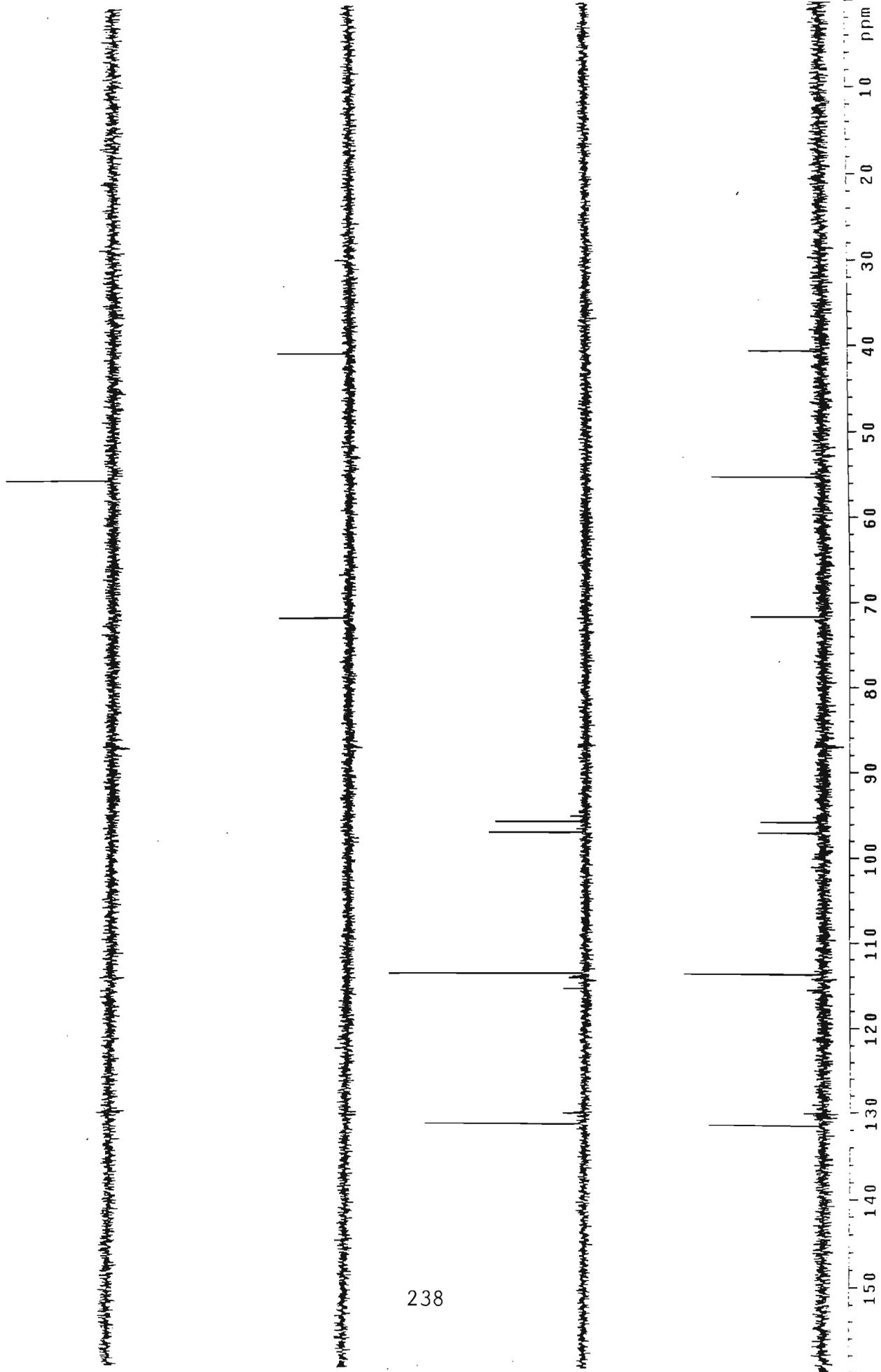
Spectrum 9.3:ADEPT spectrum of compound 9 (CDCl₃)
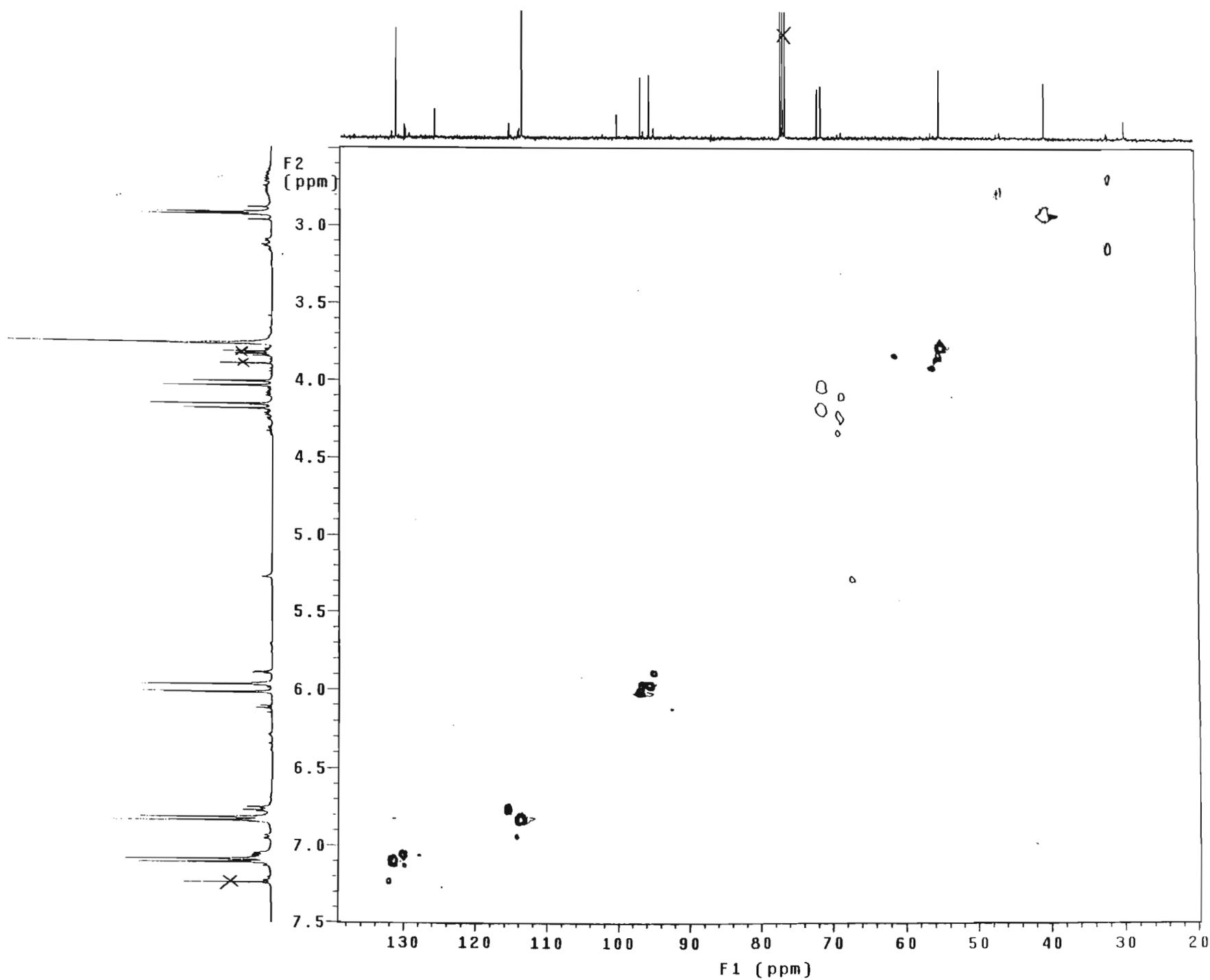
Spectrum 9.4: HSQC spectrum of compound 9 (CDCl₃)

239
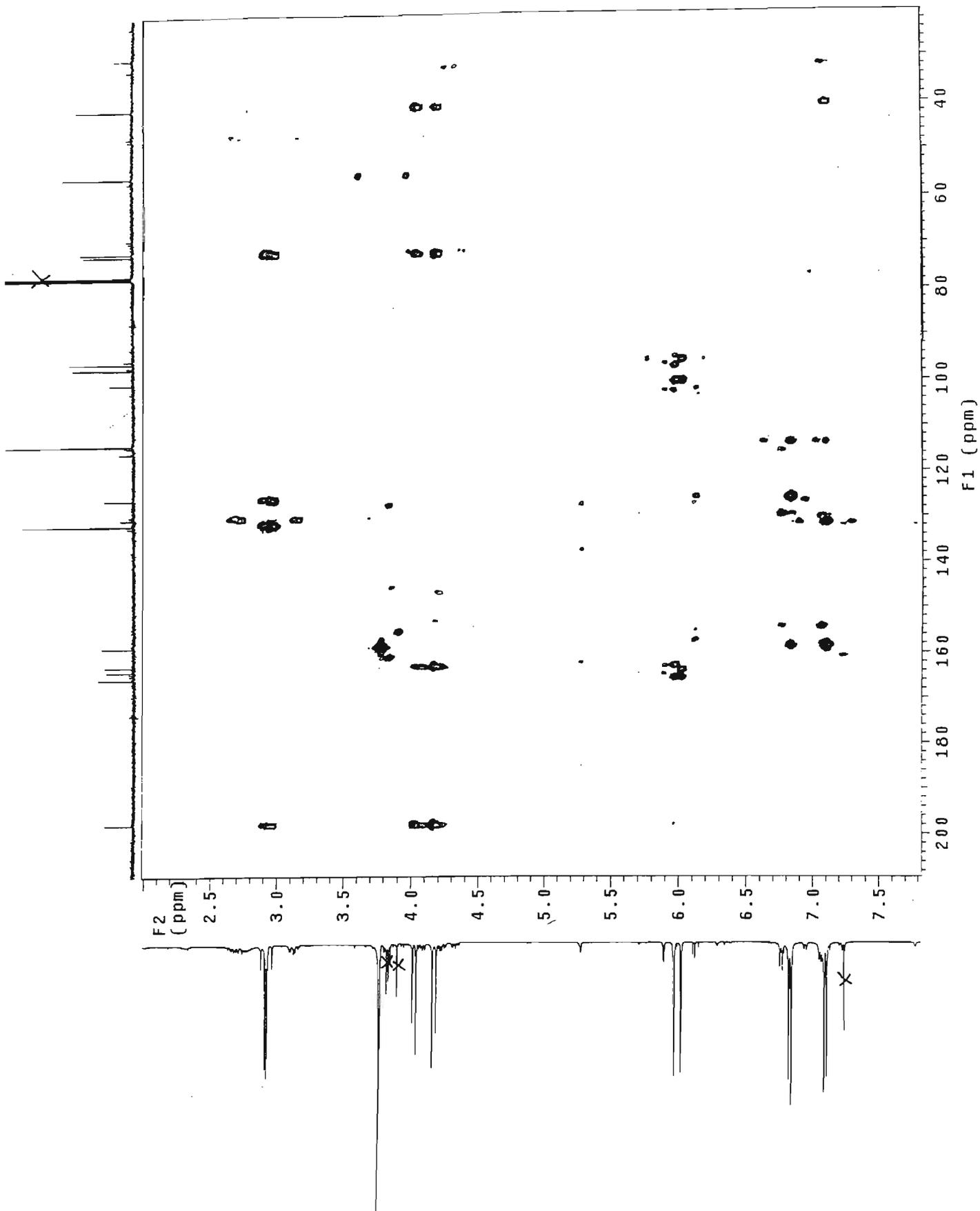
Spectrum 9.5: HMBC spectrum of compound 9 (CDCl₃)

240
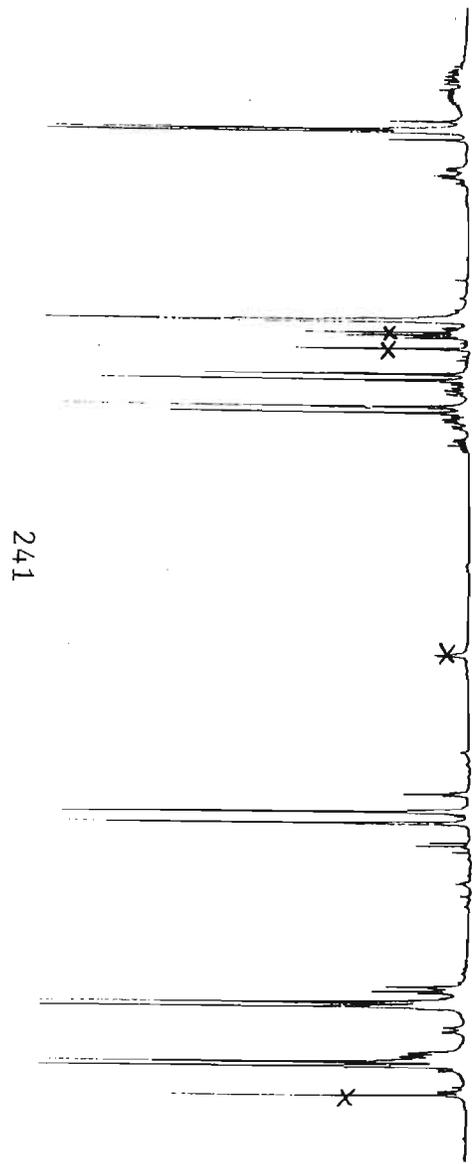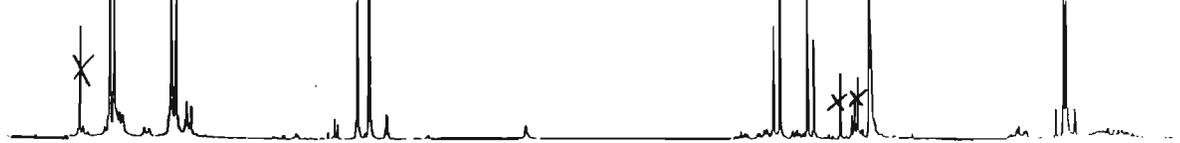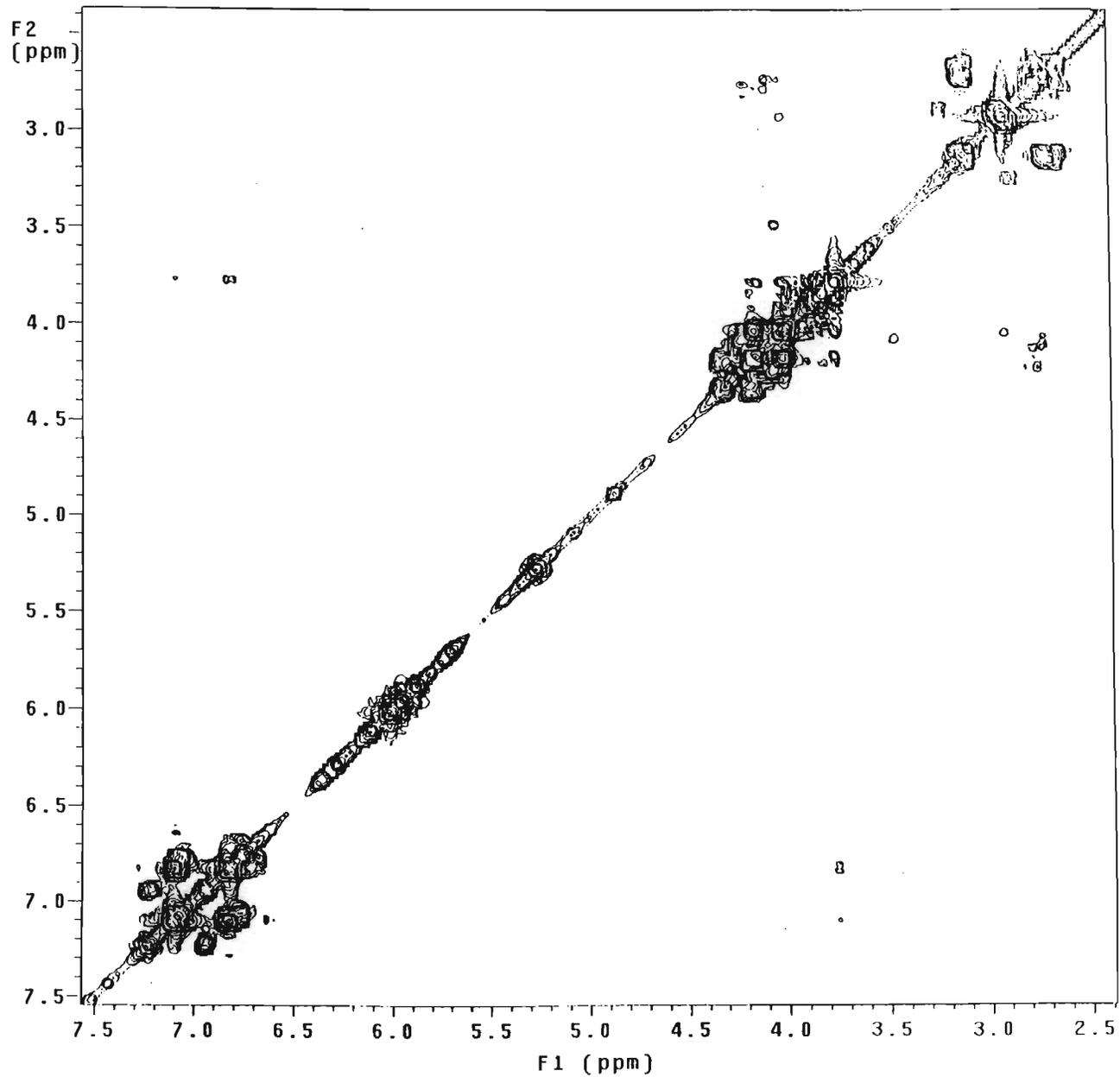
Spectrum 9.6: COSY spectrum of compound 9 (CDCl₃)
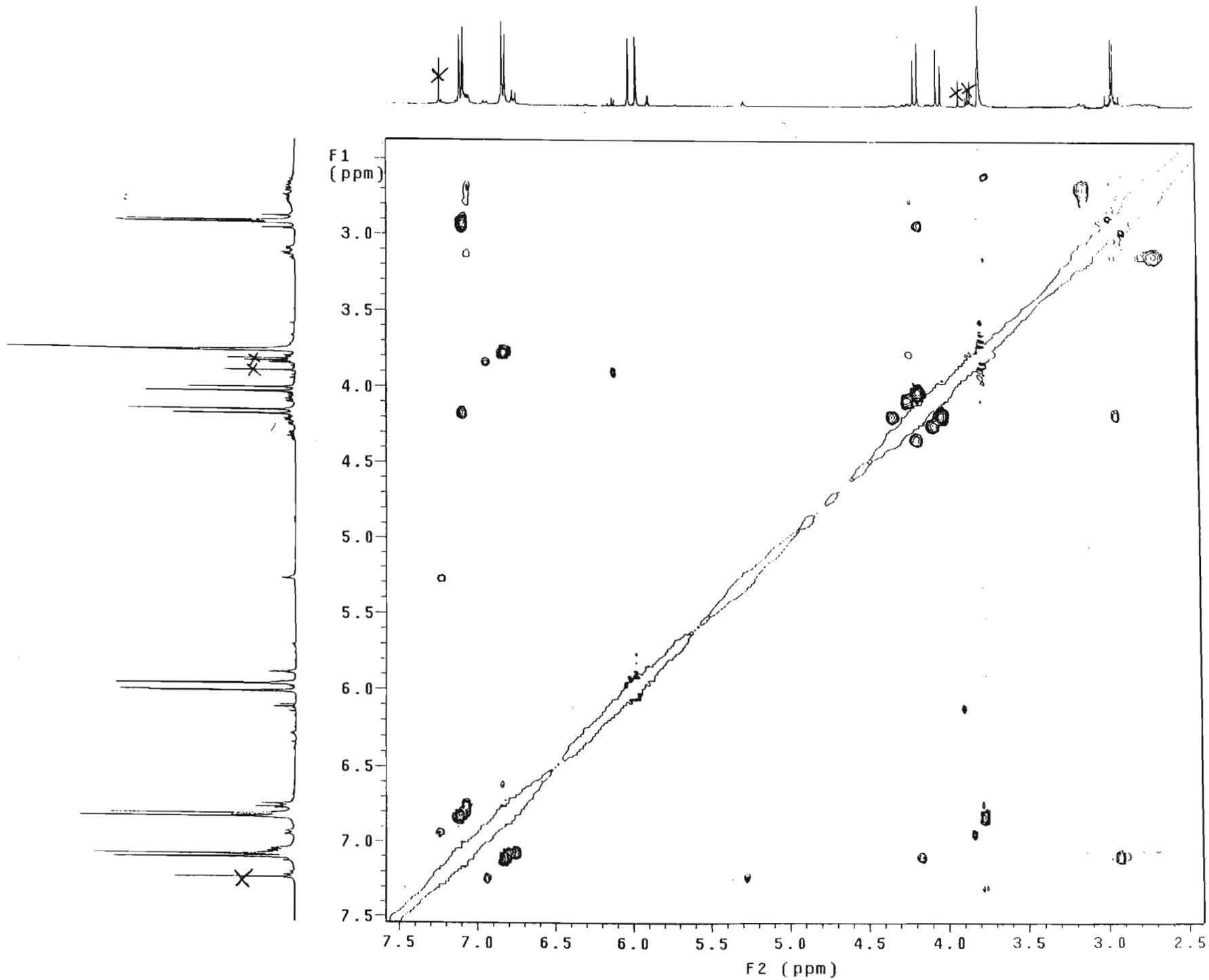
Spectrum 9.7: NOESY spectrum of compound 9 (CDCl₃)
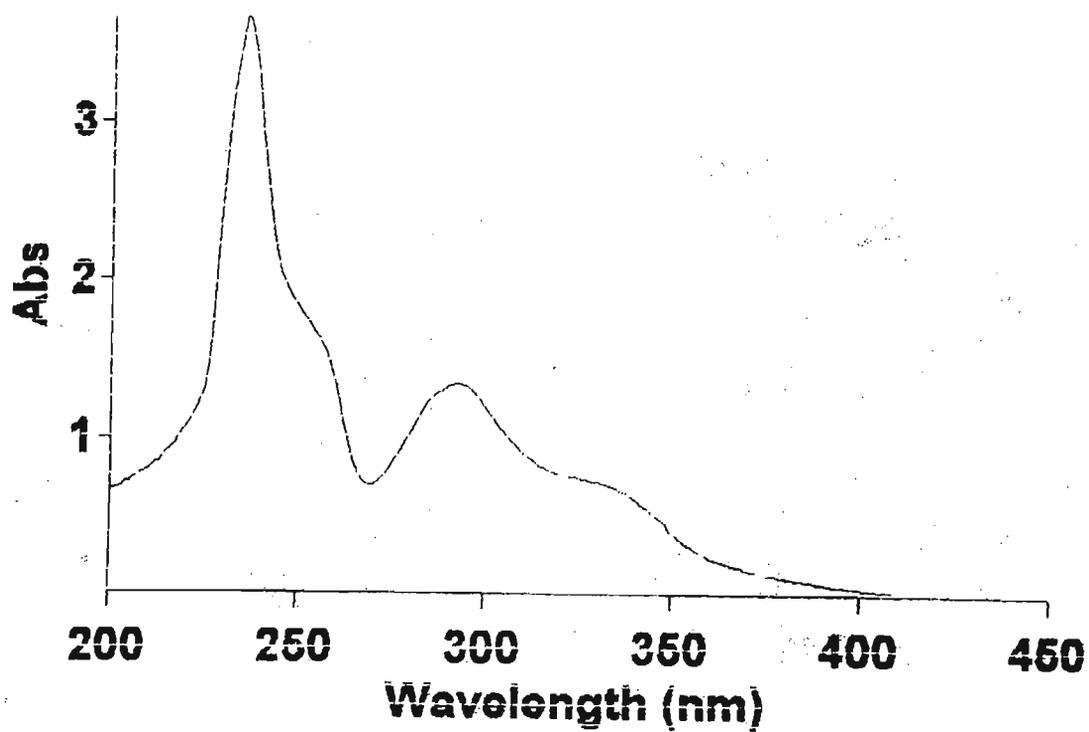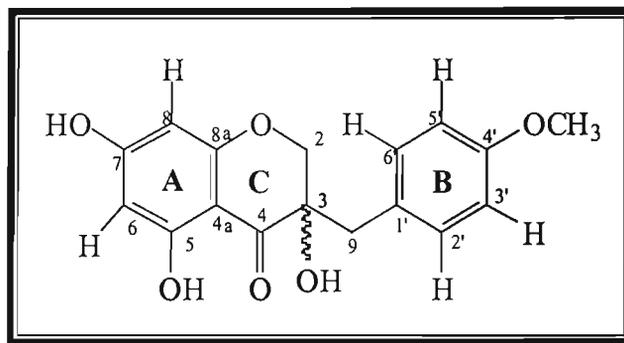
Spectrum 9.8: UV spectrum of compound 9

243
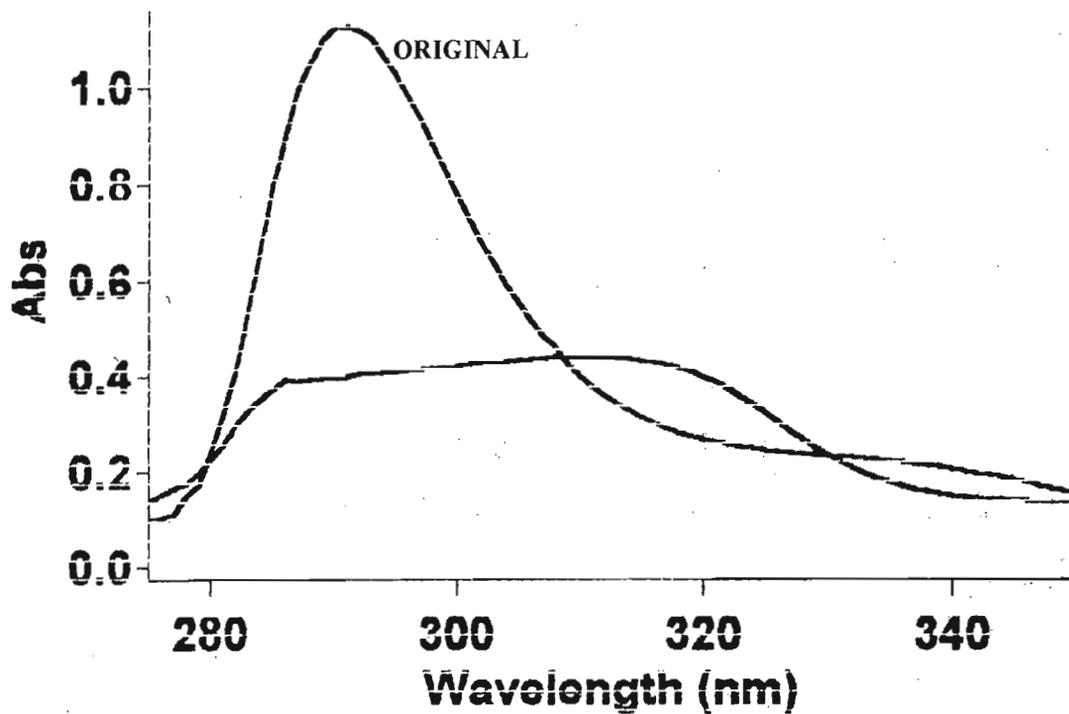
Spectrum 9.9: UV spectrum of compound 9 with AlCl₃
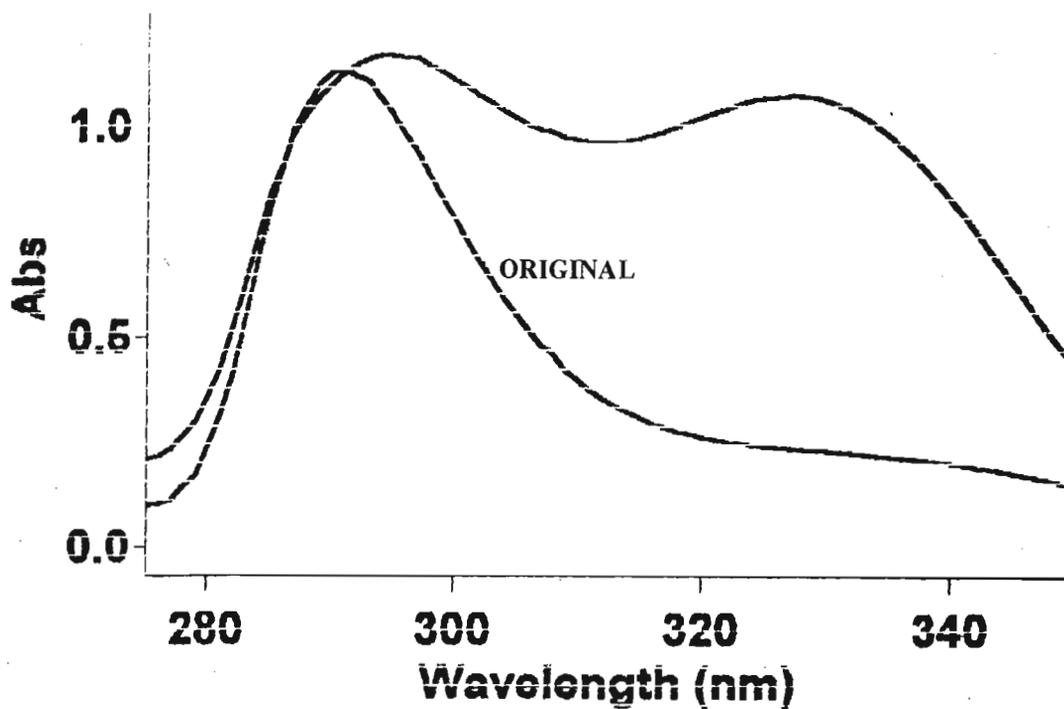
Spectrum 9.10: UV spectrum of compound 9 with NaOAc
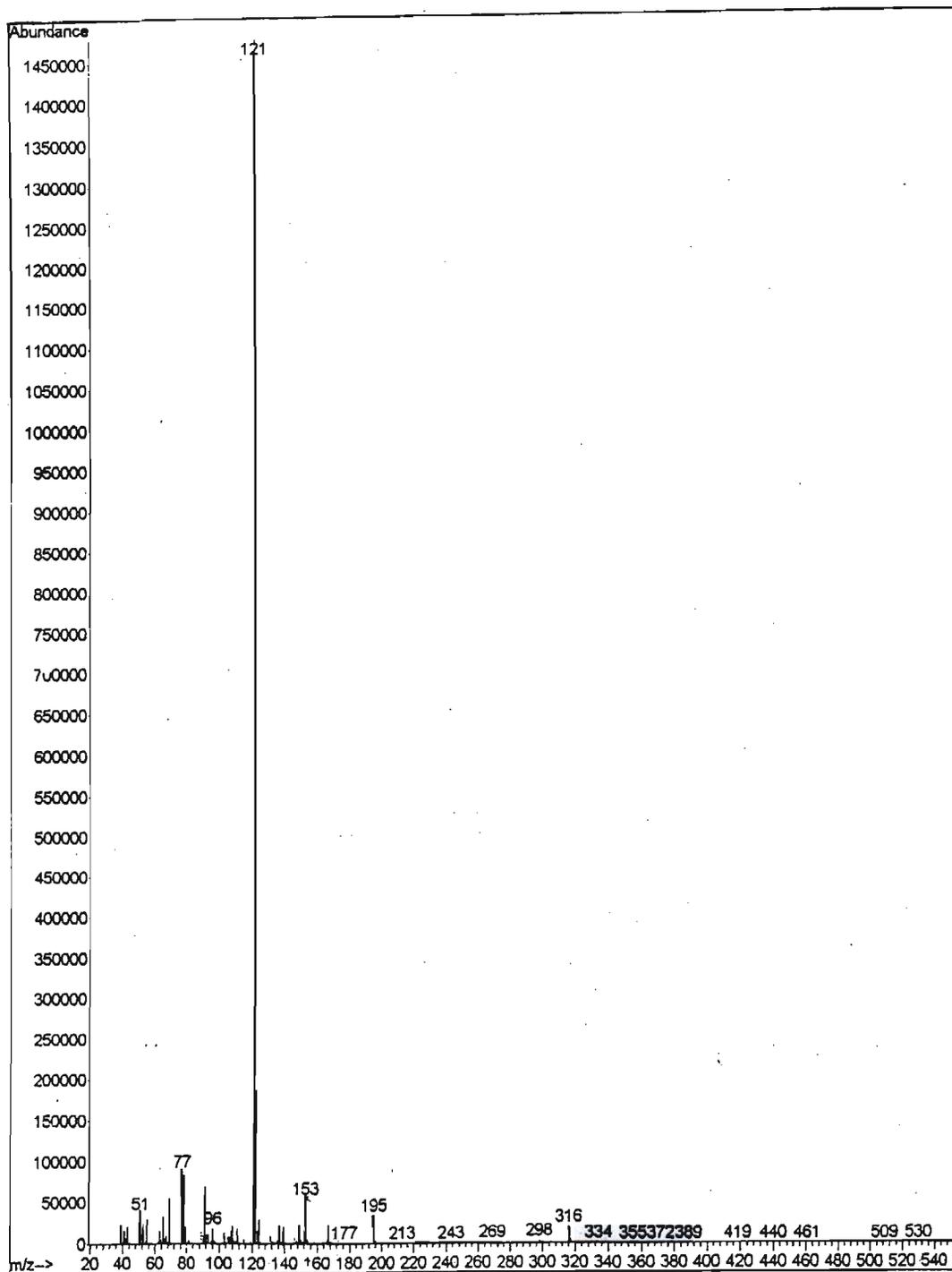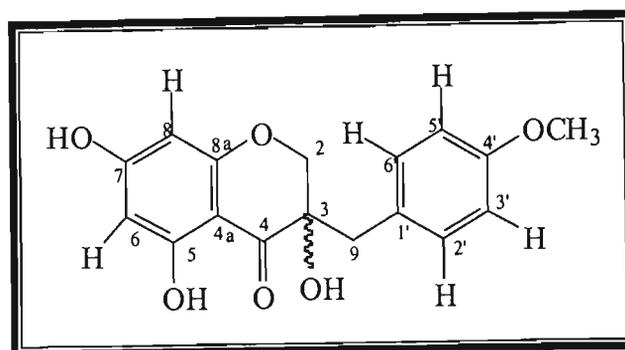
Spectrum 9.11: Mass spectrum of compound 9
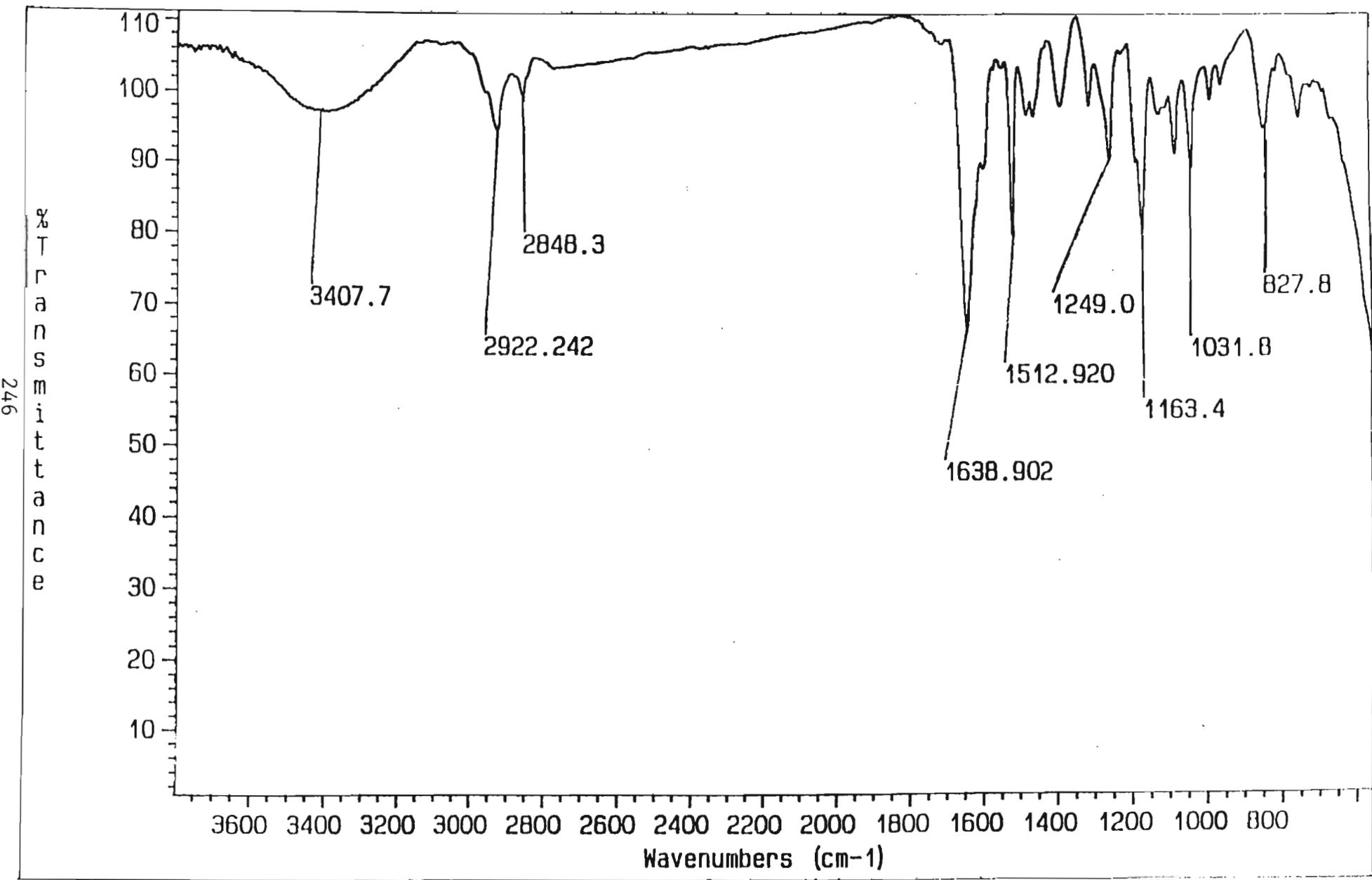
Spectrum 9.12: IR spectrum of compound 9
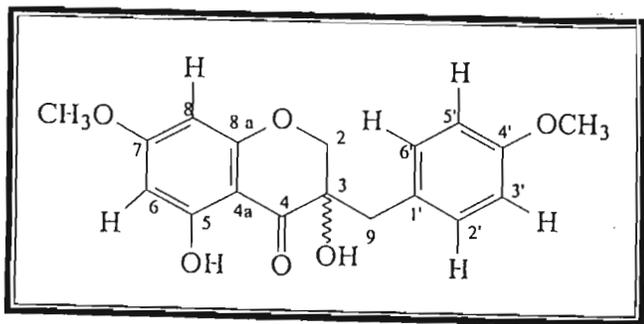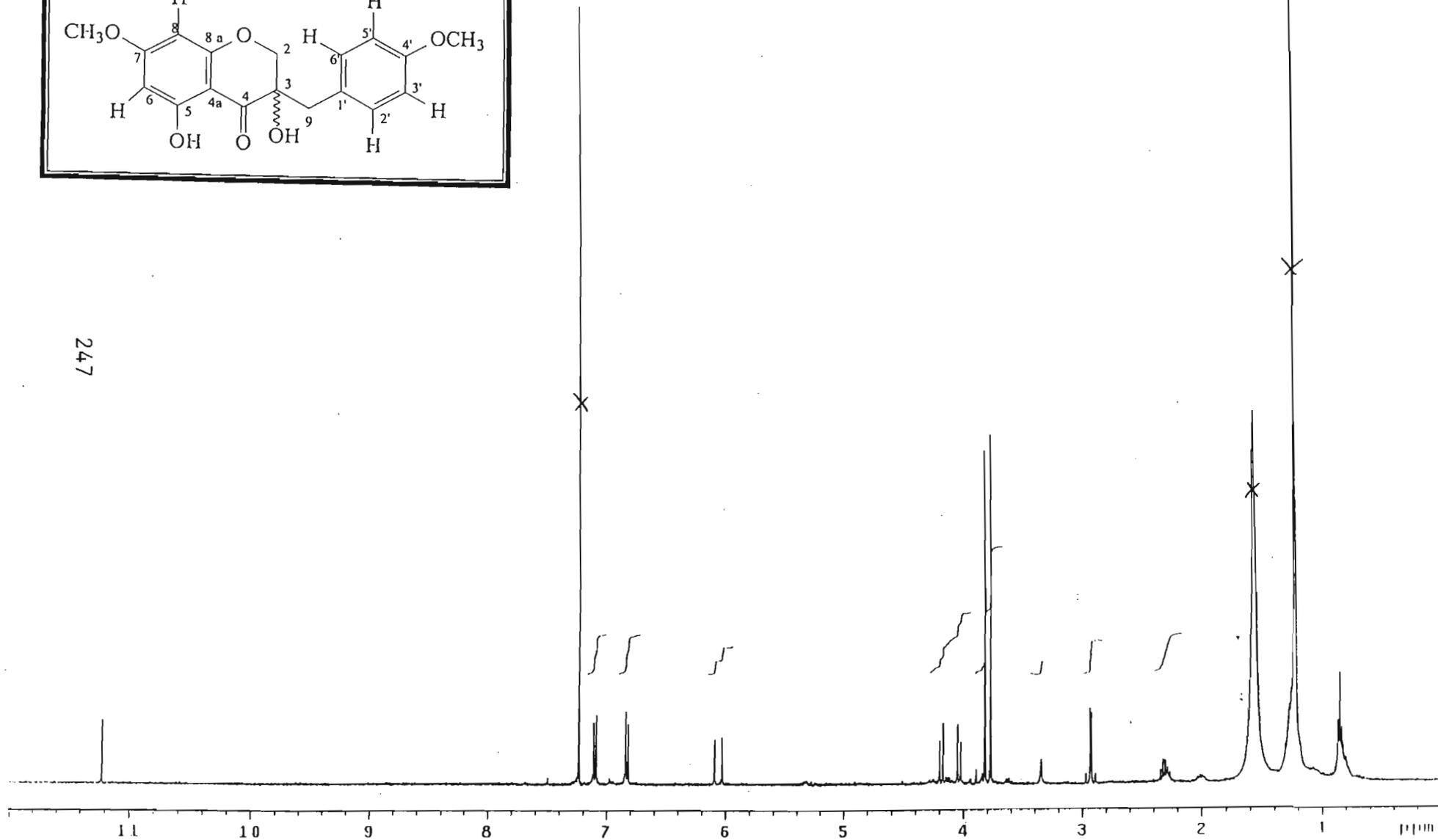
Spectrum 10: ^{1}H NMR spectrum of compound 10 (CDCl$_3$) original
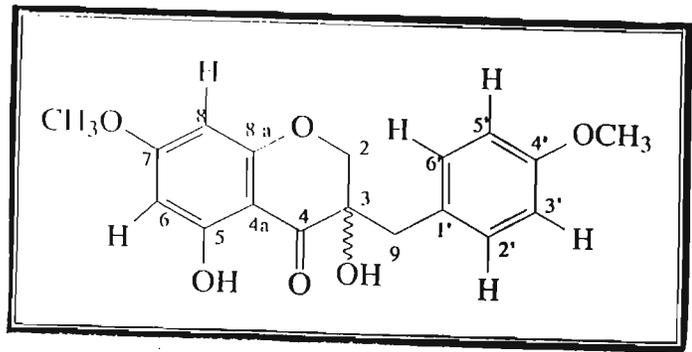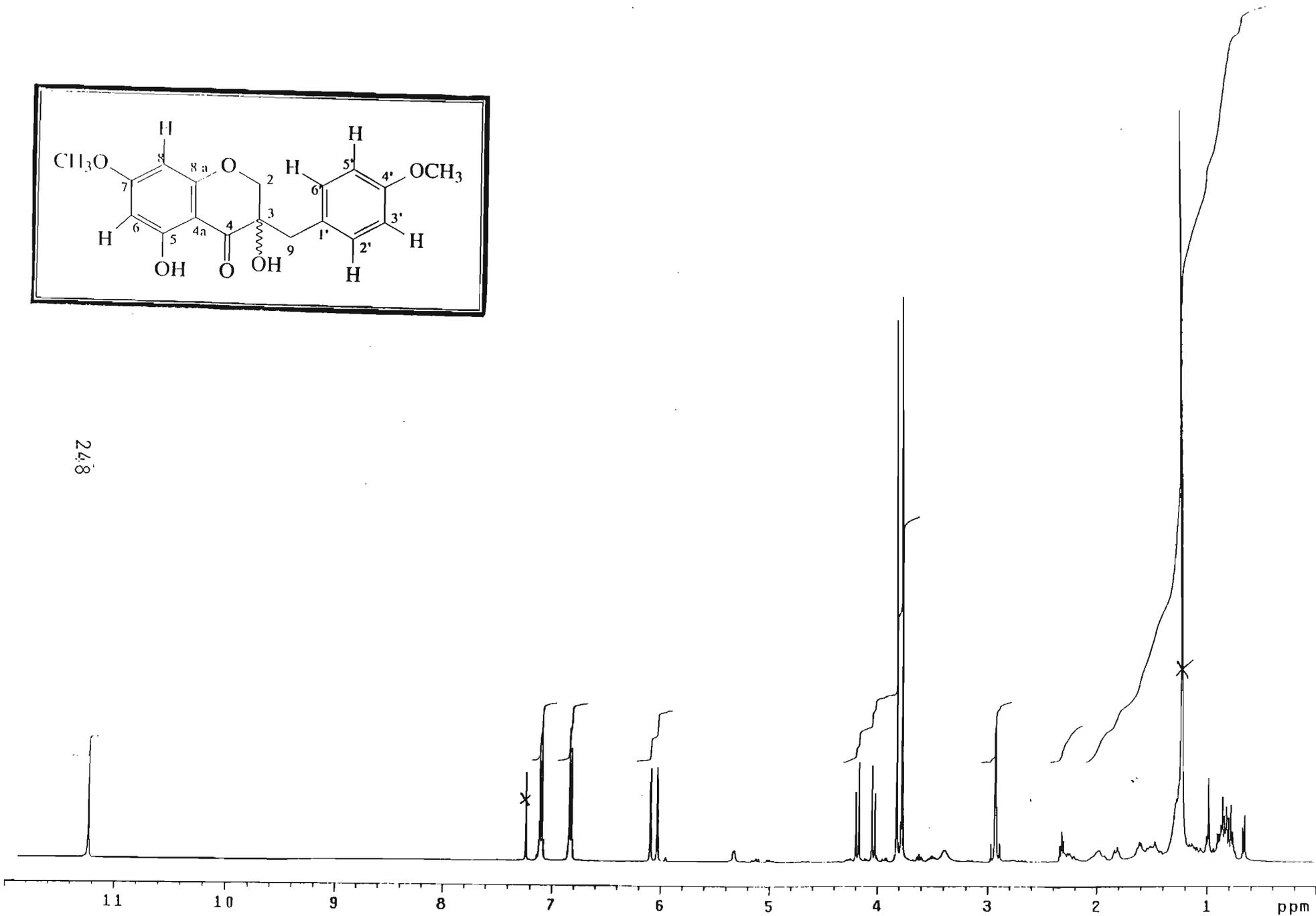
Spectrum 10.1: ^{1}H NMR spectrum of compound 10 (CDCl$_3$)
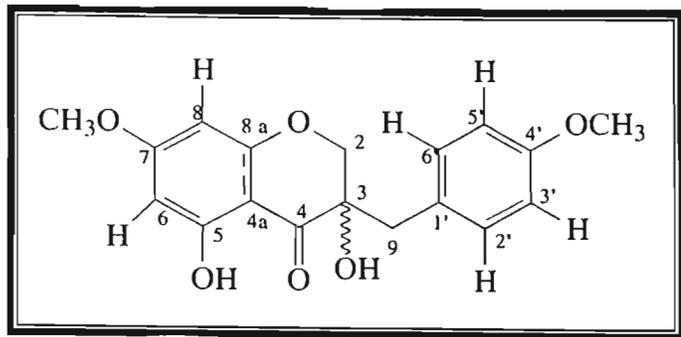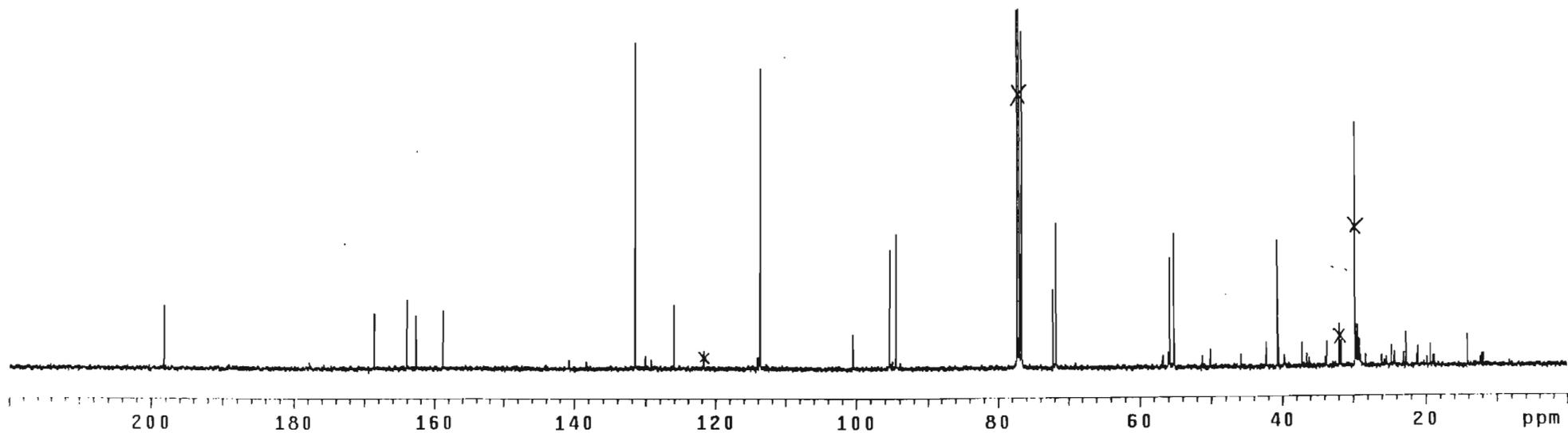
Spectrum 10.2: ^{13} C NMR spectrum of compound **10** (CDCl$_3$)
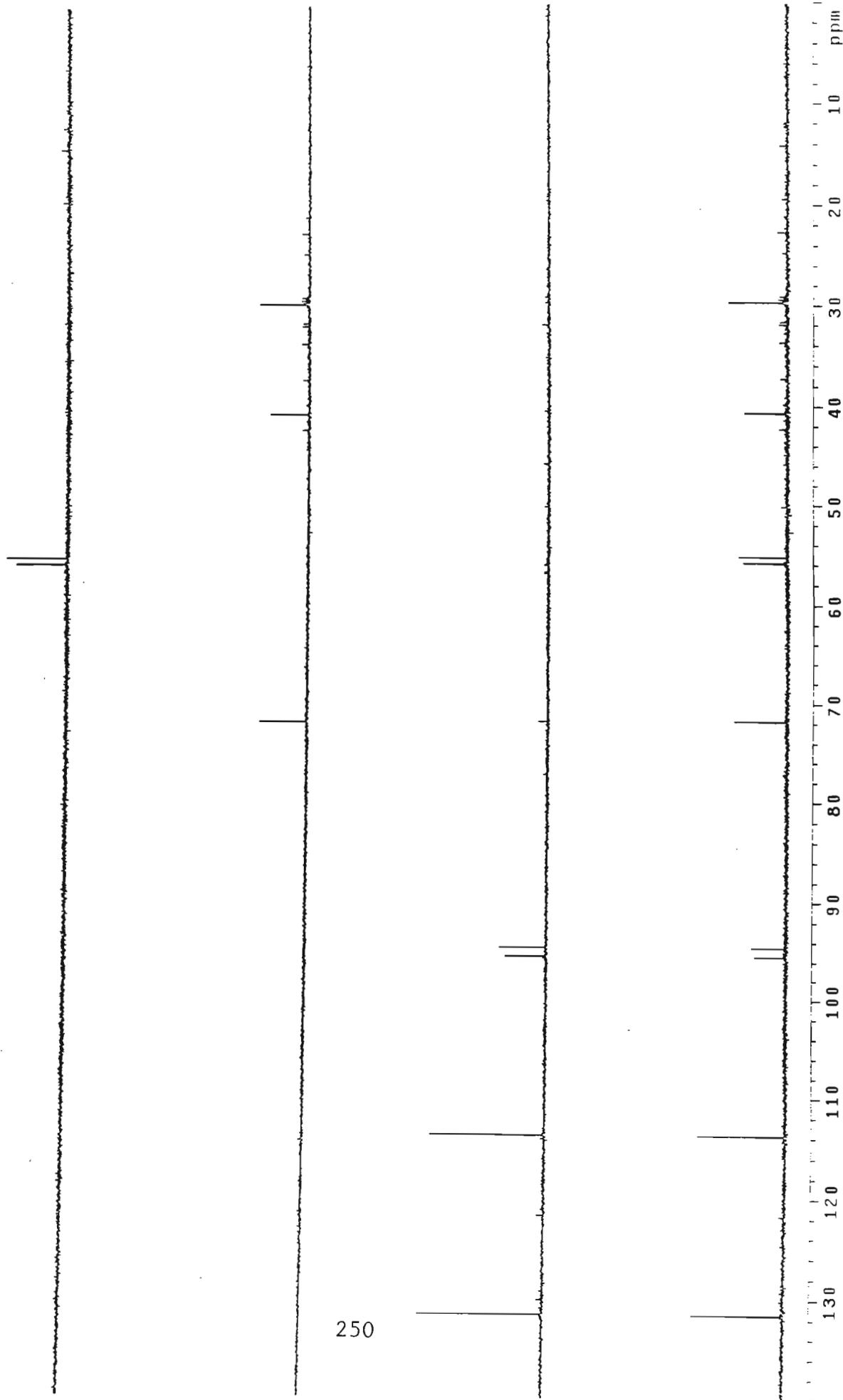
Spectrum 10.3: ADEPT spectrum of compound 10 (CDCl₃)

250
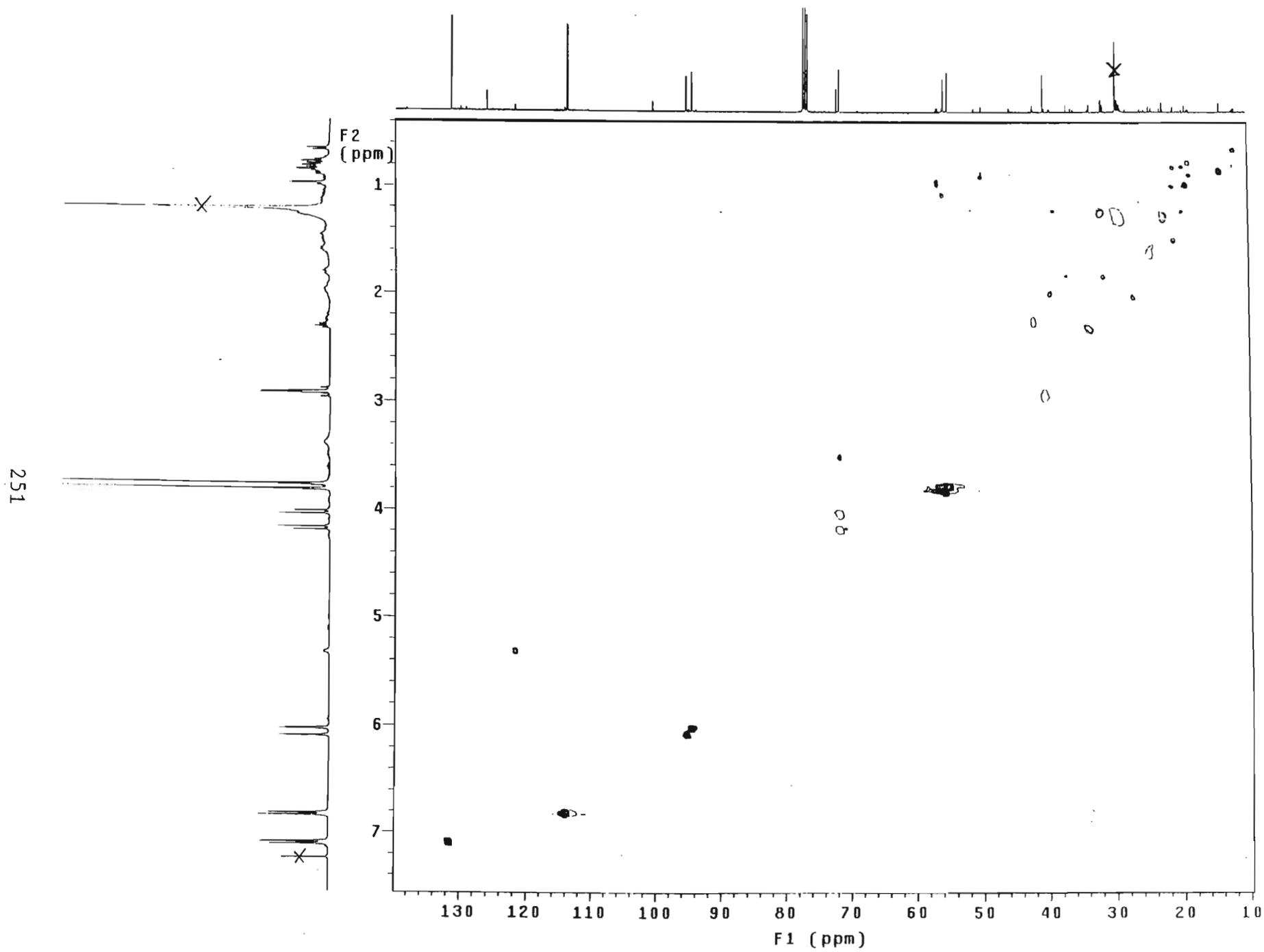
Spectrum 10.4: HSQC spectrum of compound 10 (CDCl₃)
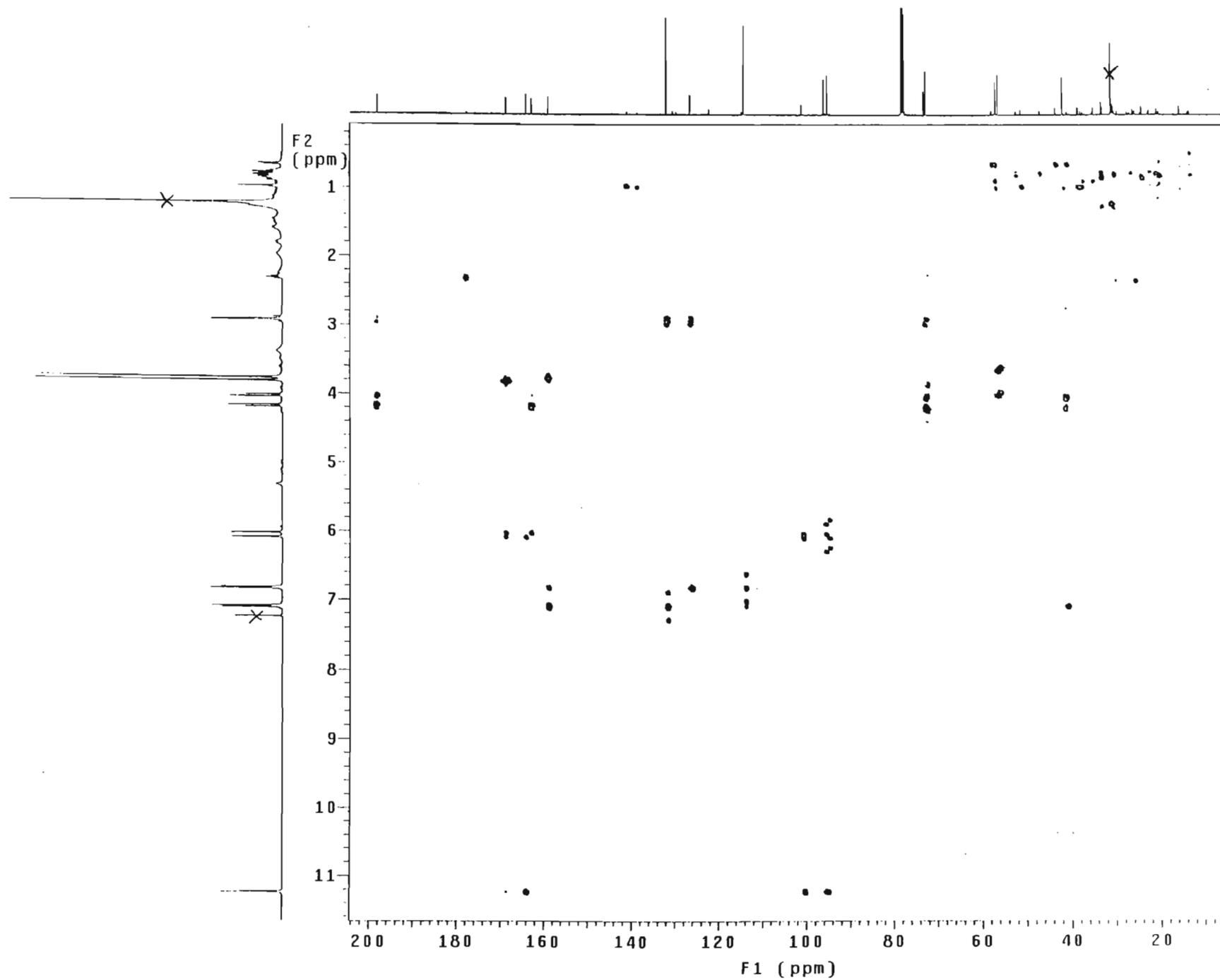
Spectrum 10.5: HMBC spectrum of compound 10 (CDCl₃)
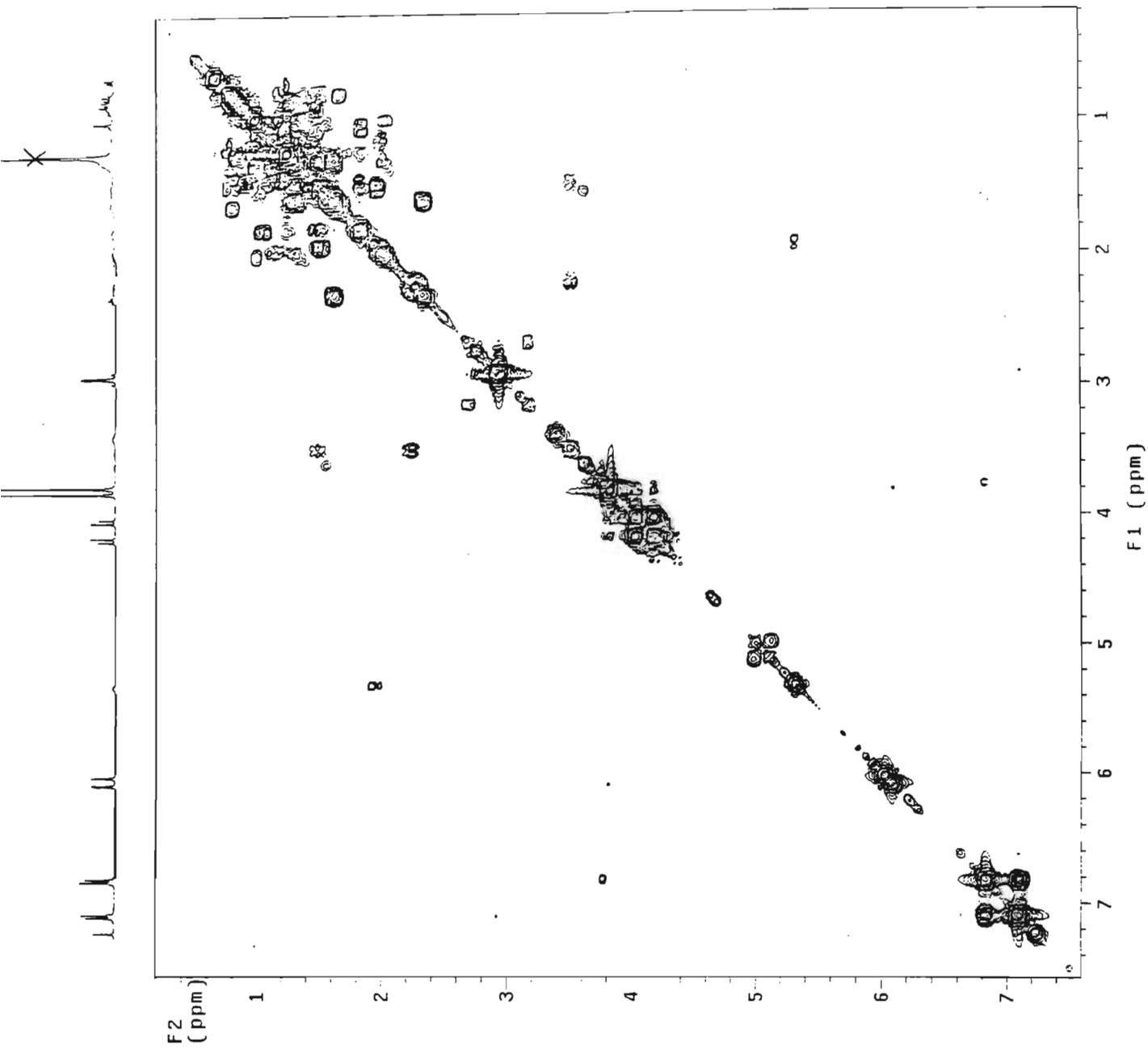
Spectrum 10.6: COSY spectrum of compound 10 (CDCl₃)
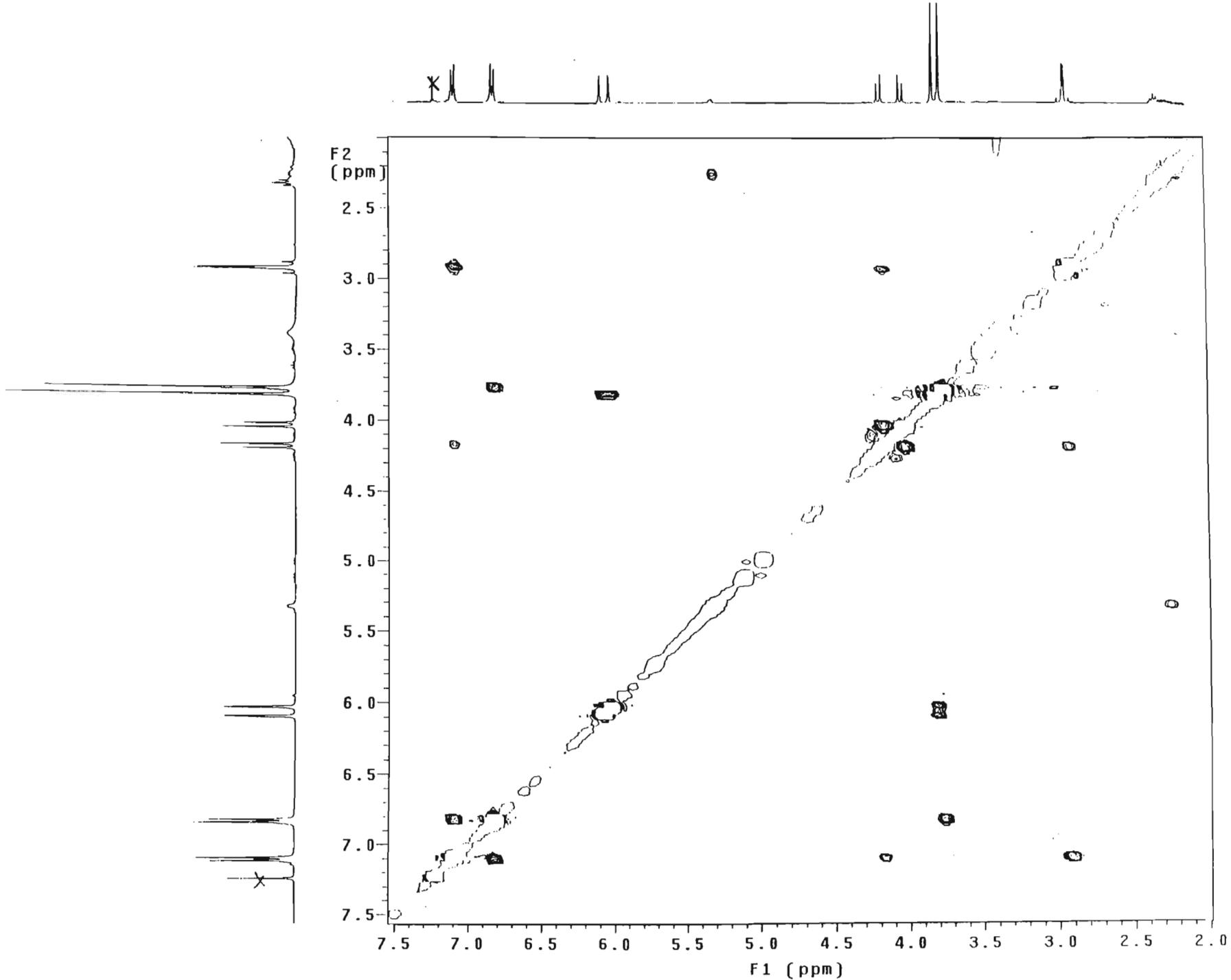
Spectrum 10.7: NOESY spectrum of compound 10 (CDCl₃)
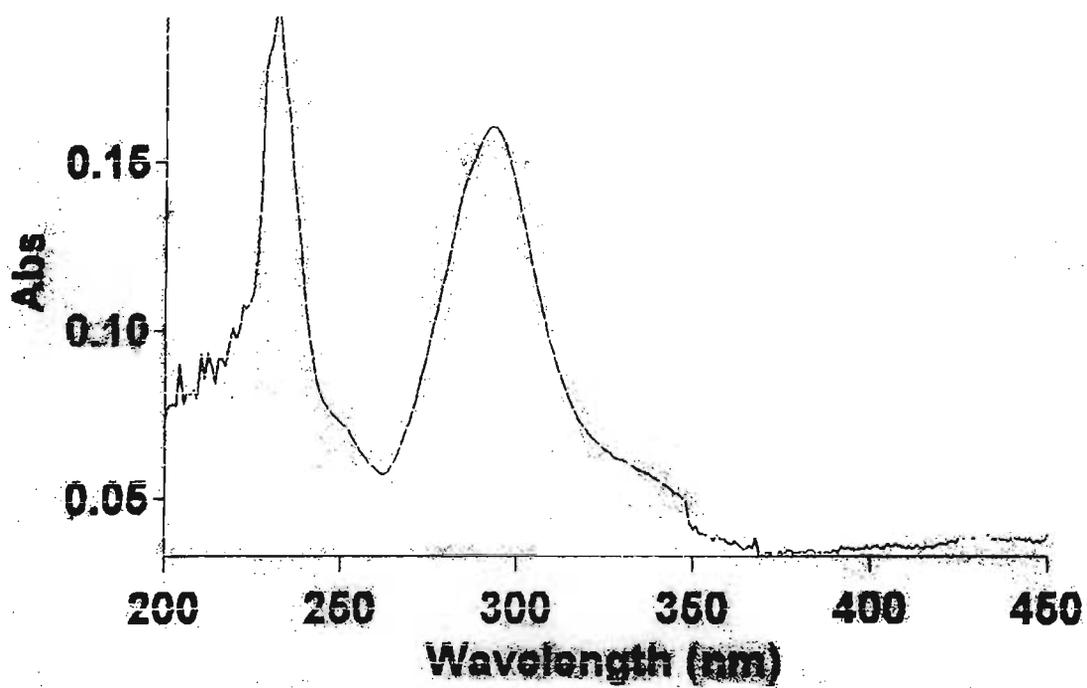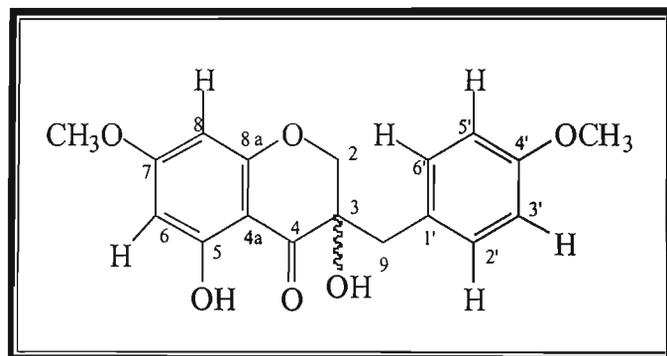
Spectrum 10.8: UV spectrum of compound 10

255
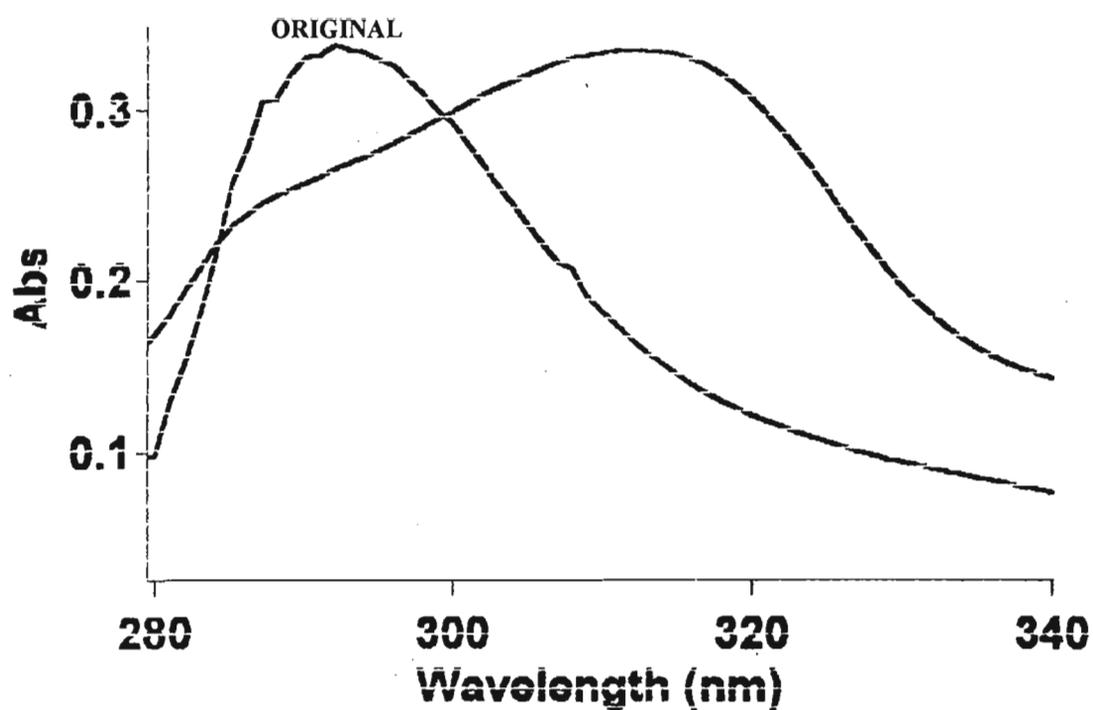
Spectrum 10.9: UV spectrum of compound 10 with AlCl₃
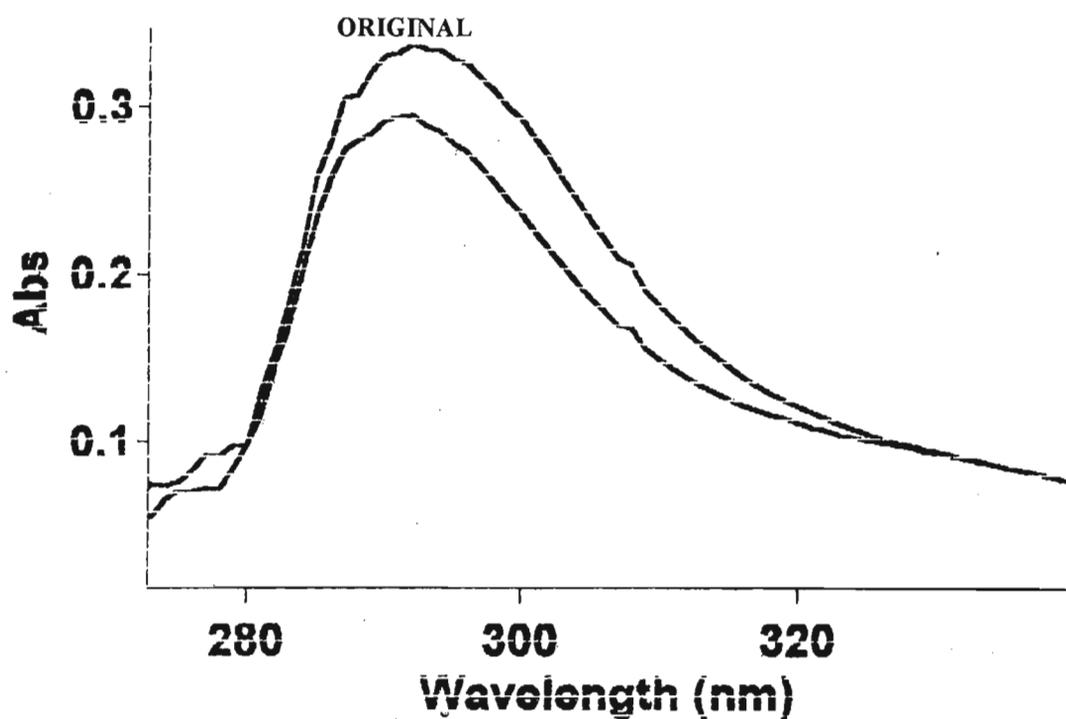
Spectrum 10.10: UV spectrum of compound 10 with NaOAc

256
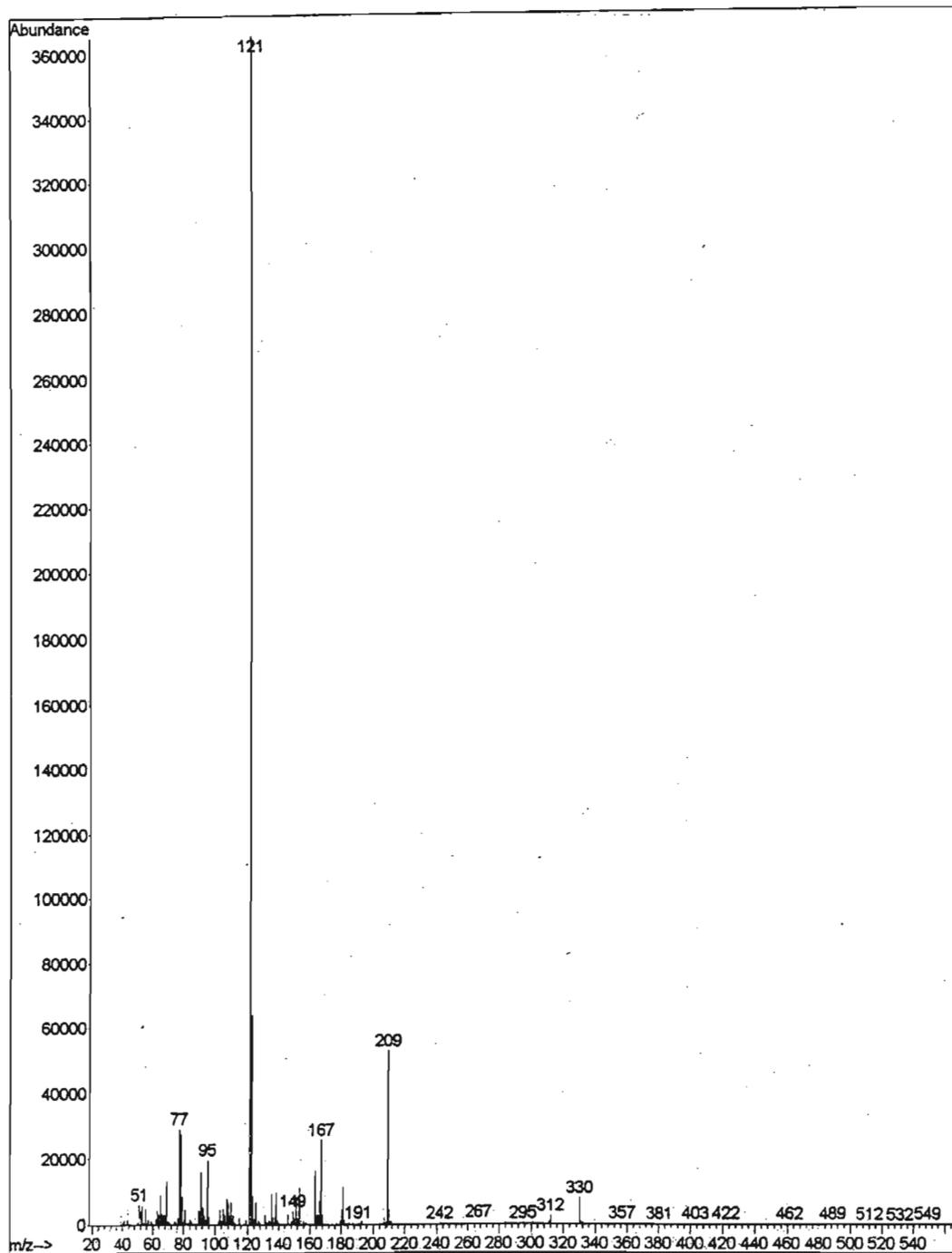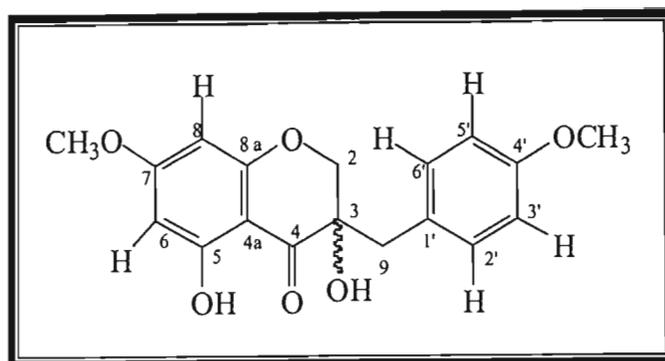
Spectrum 10.11: Mass spectrum of compound 10

257
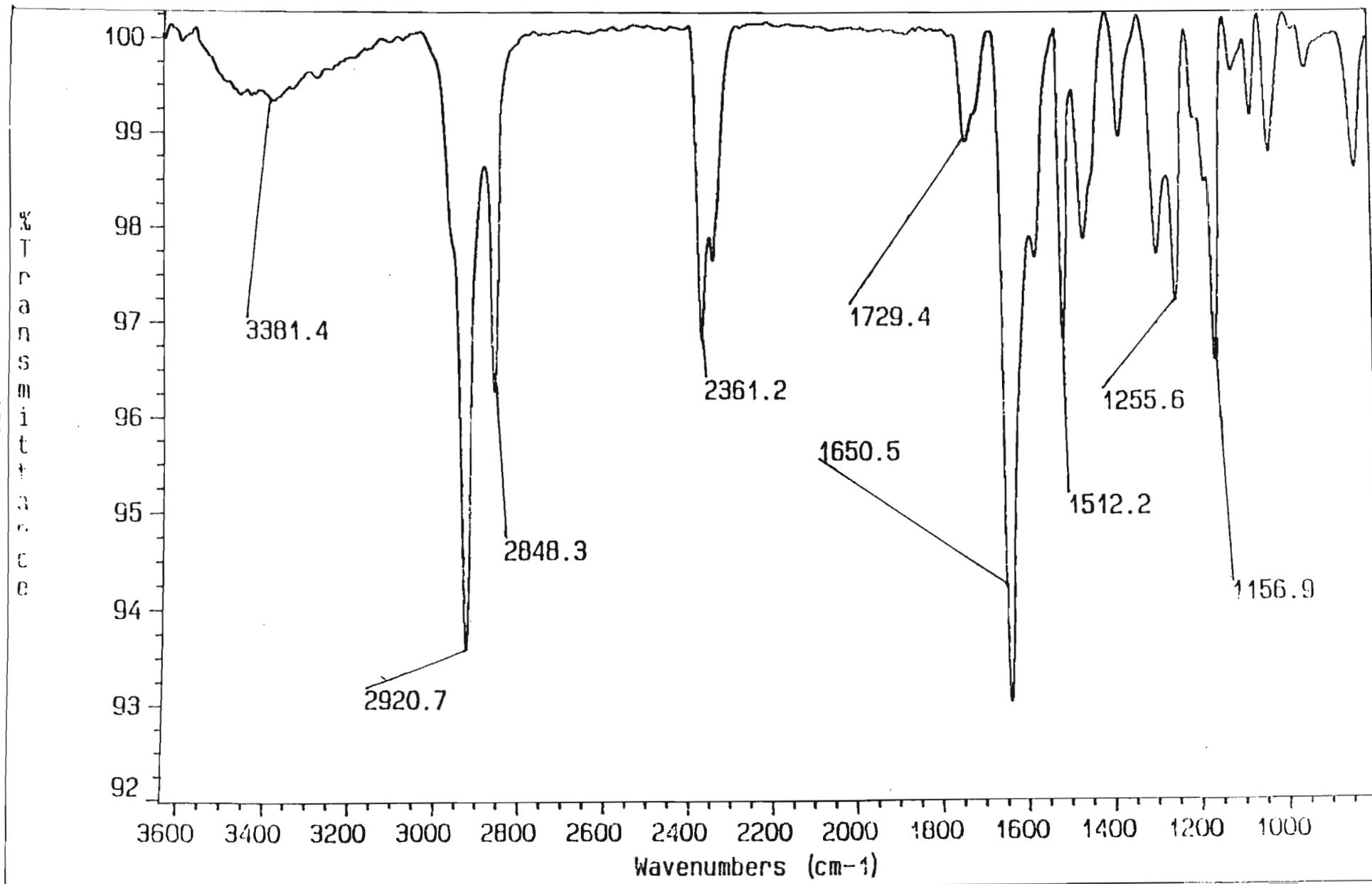
Spectrum 10.12: IR spectrum of compound 10
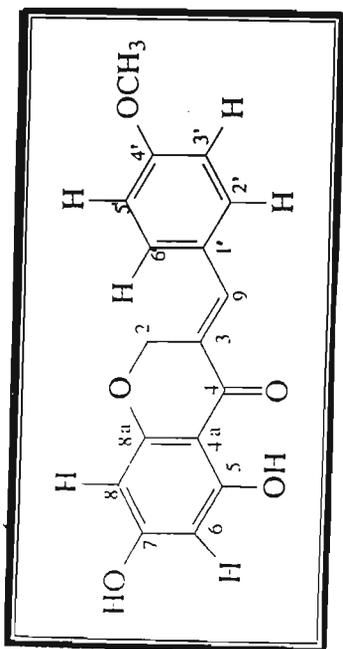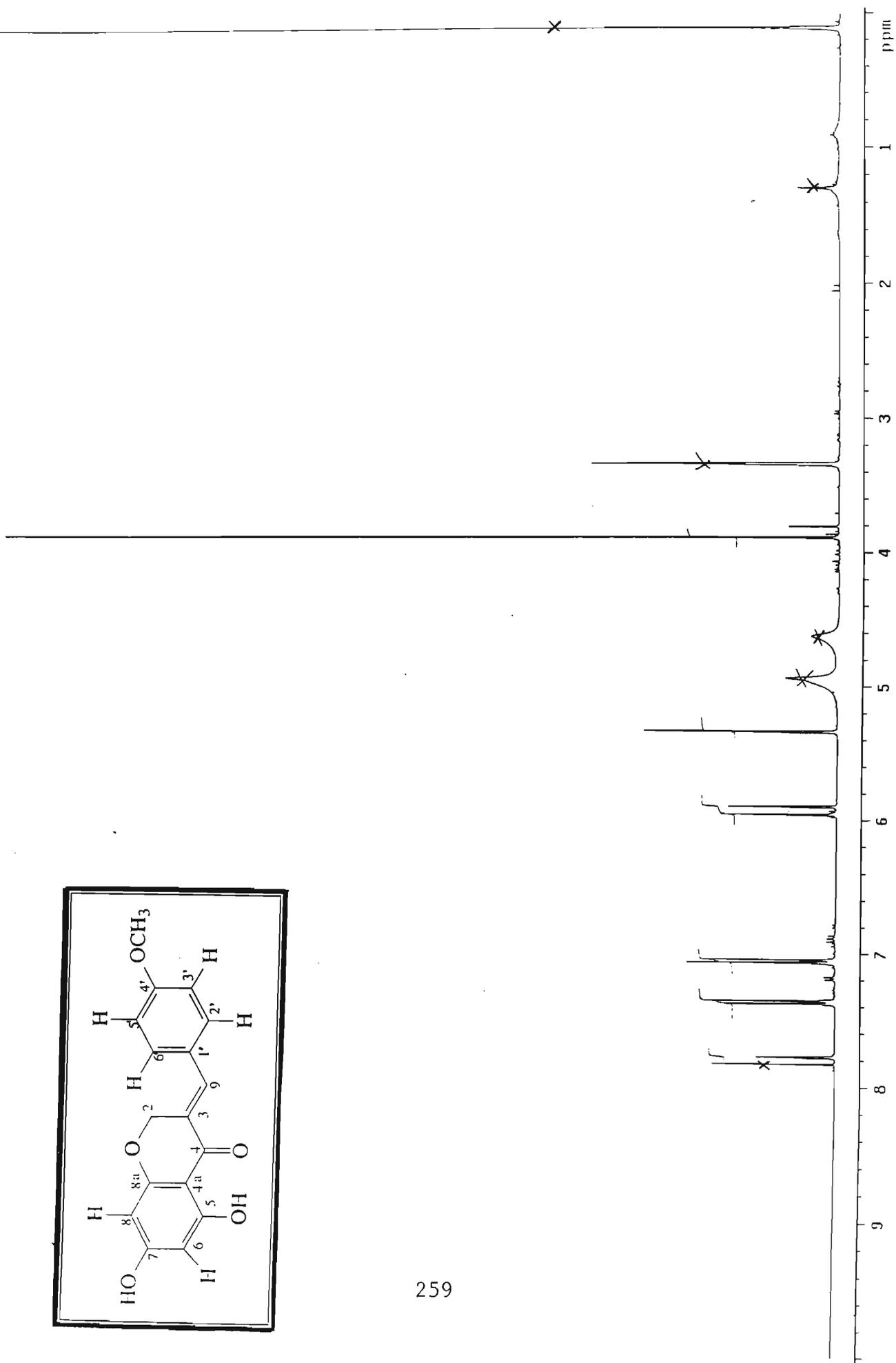
Spectrum 11.1: ^{1}H NMR spectrum of compound 11 (CD$_3$OD)

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Spectrum 11.2: ^{1}H NMR spectrum of compound 11 (CDCl$_3$)

260
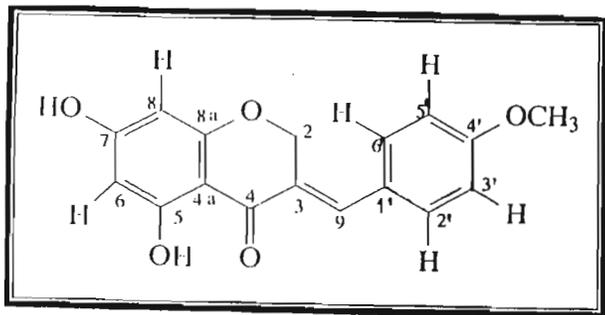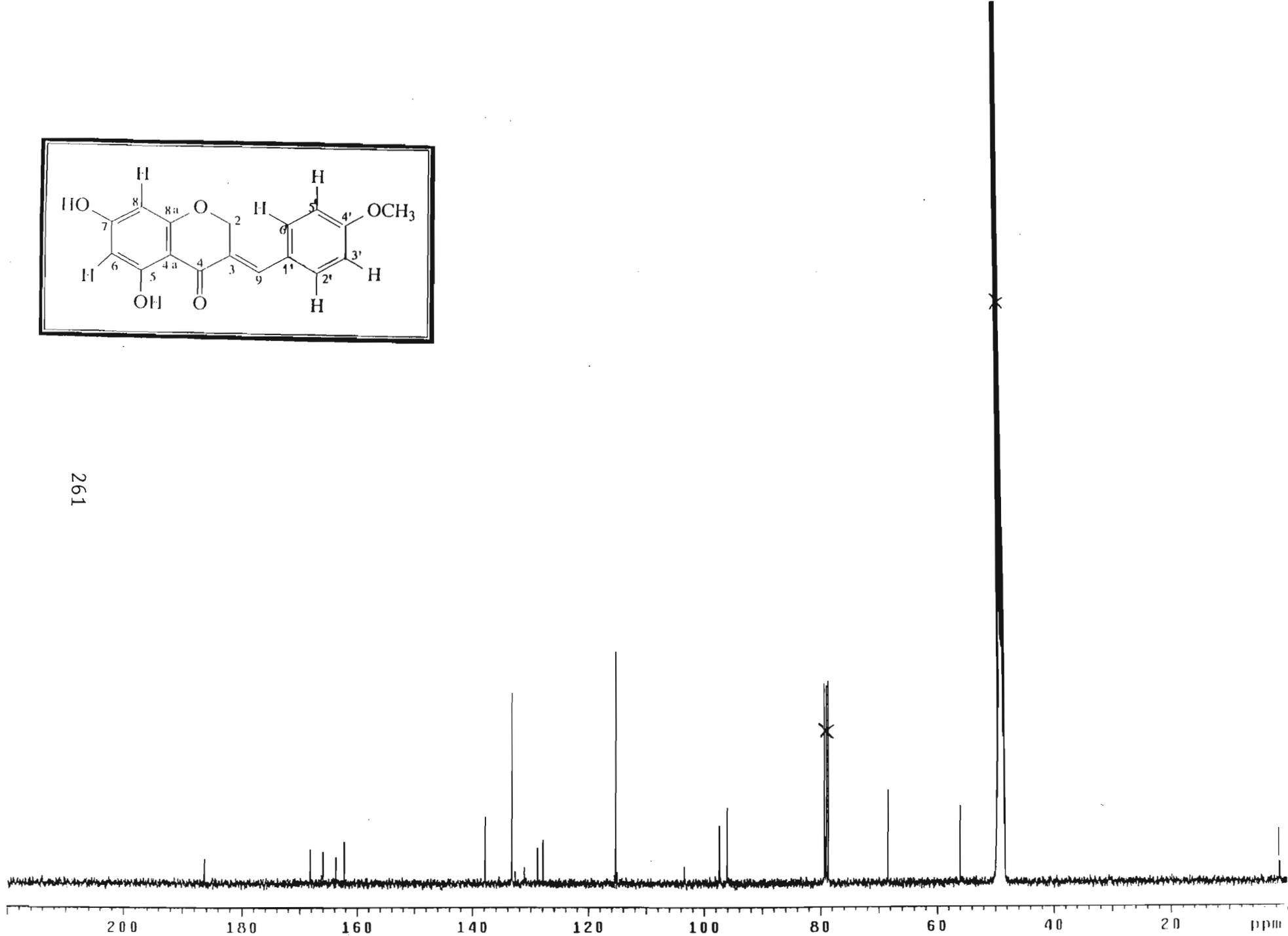
Spectrum 11.3: ^{13}C NMR spectrum of compound 11 (CD$_3$OD)
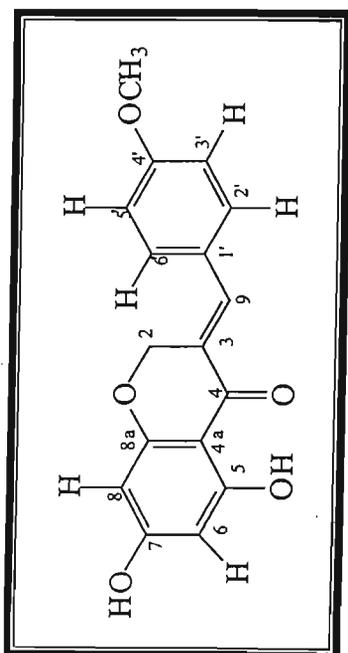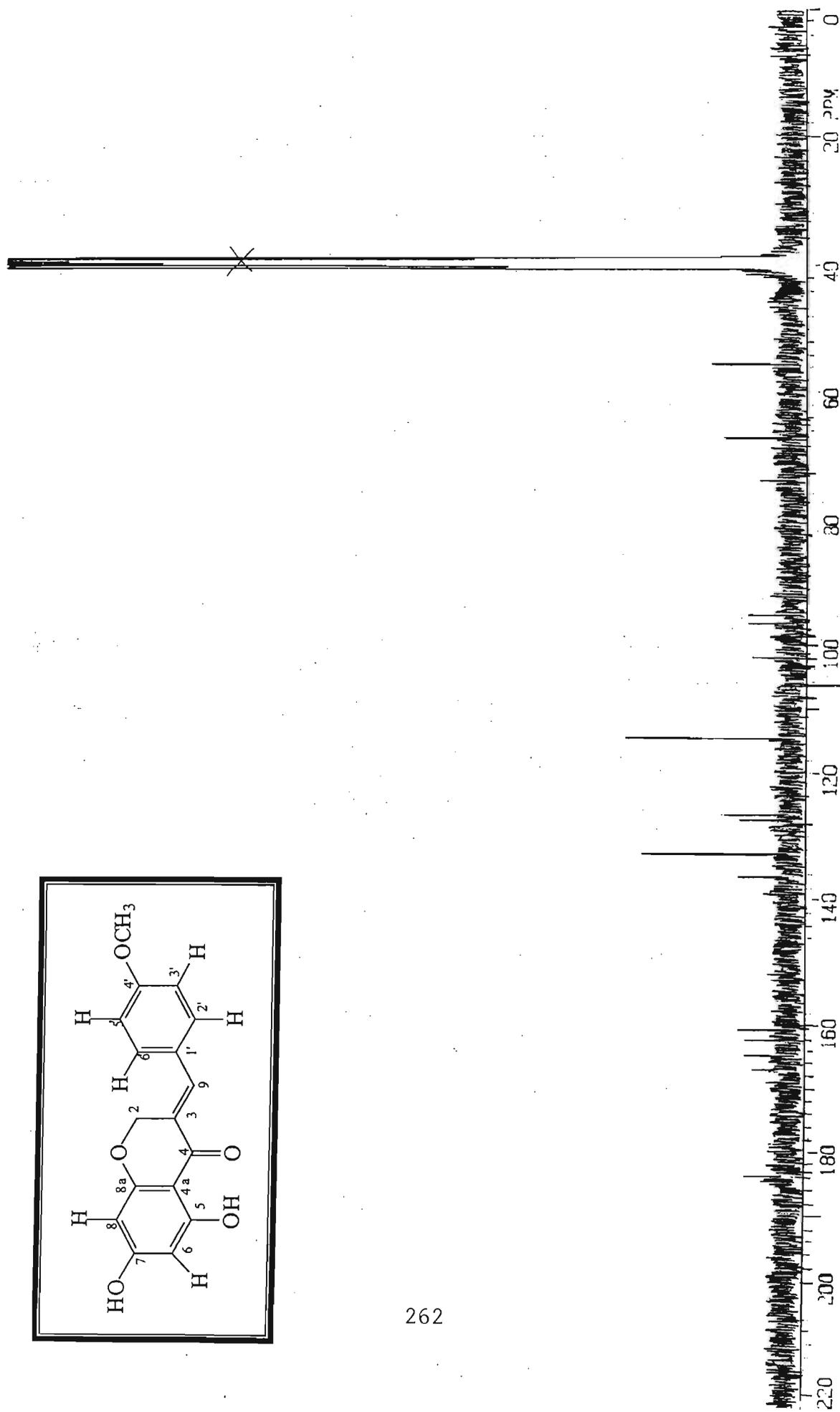
Spectrum 11.4: ^{13}C NMR spectrum of compound 11 $(CD_3)_2SO$

262
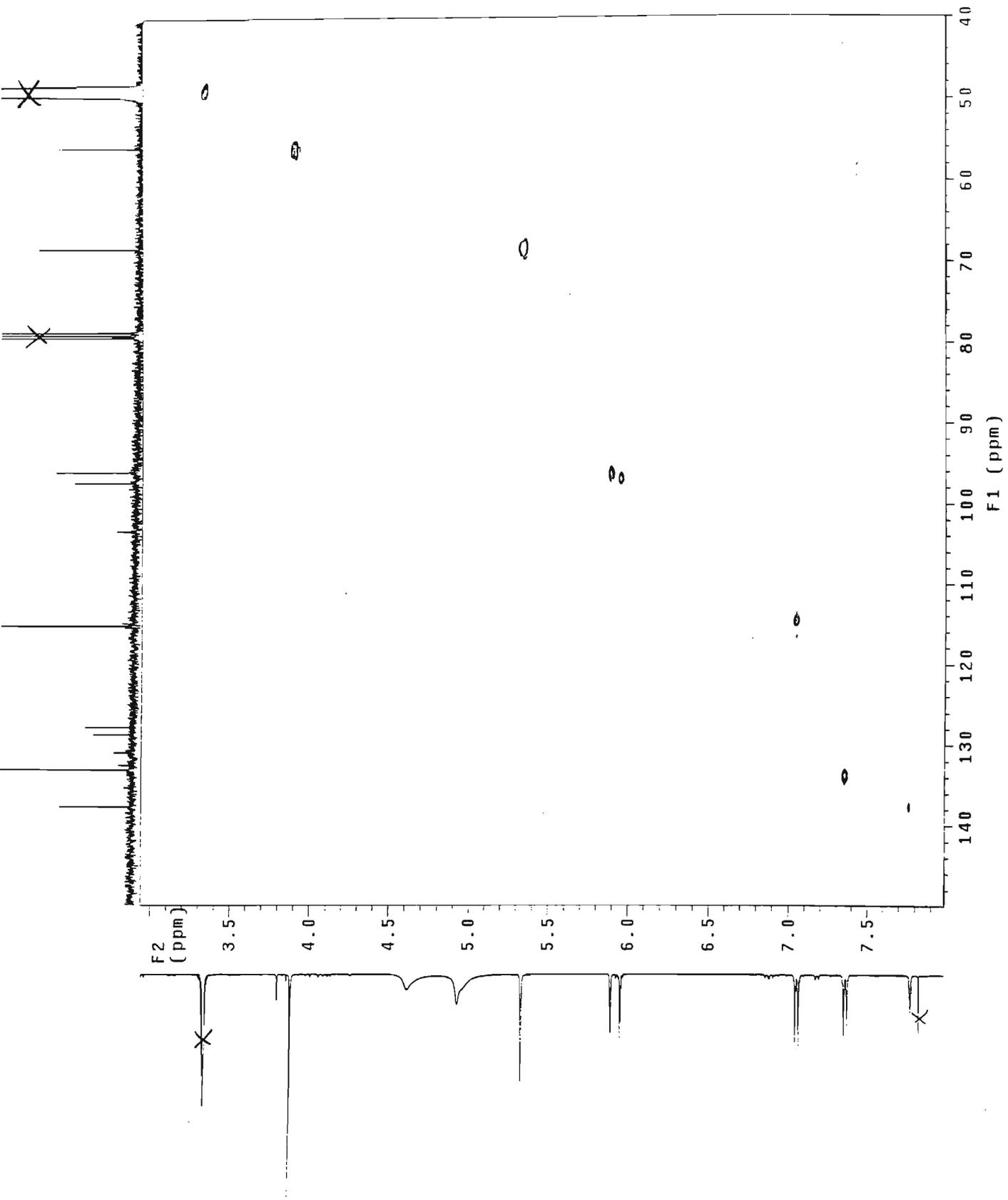
Spectrum 11.5: HSQC spectrum of compound 11 (CD$_3$OD)
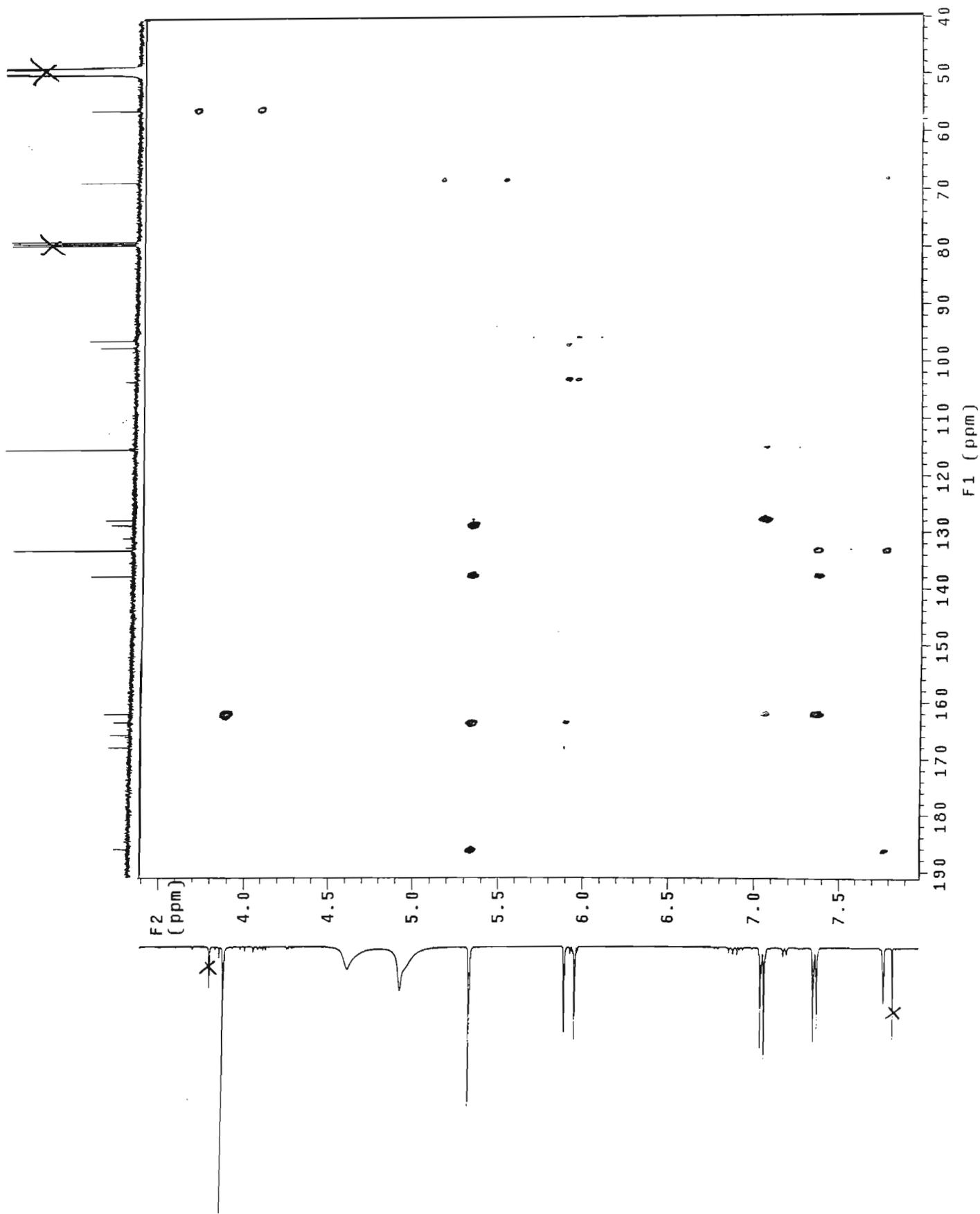
Spectrum 11.6: HMBC spectrum of compound 11 (CD₃OD)
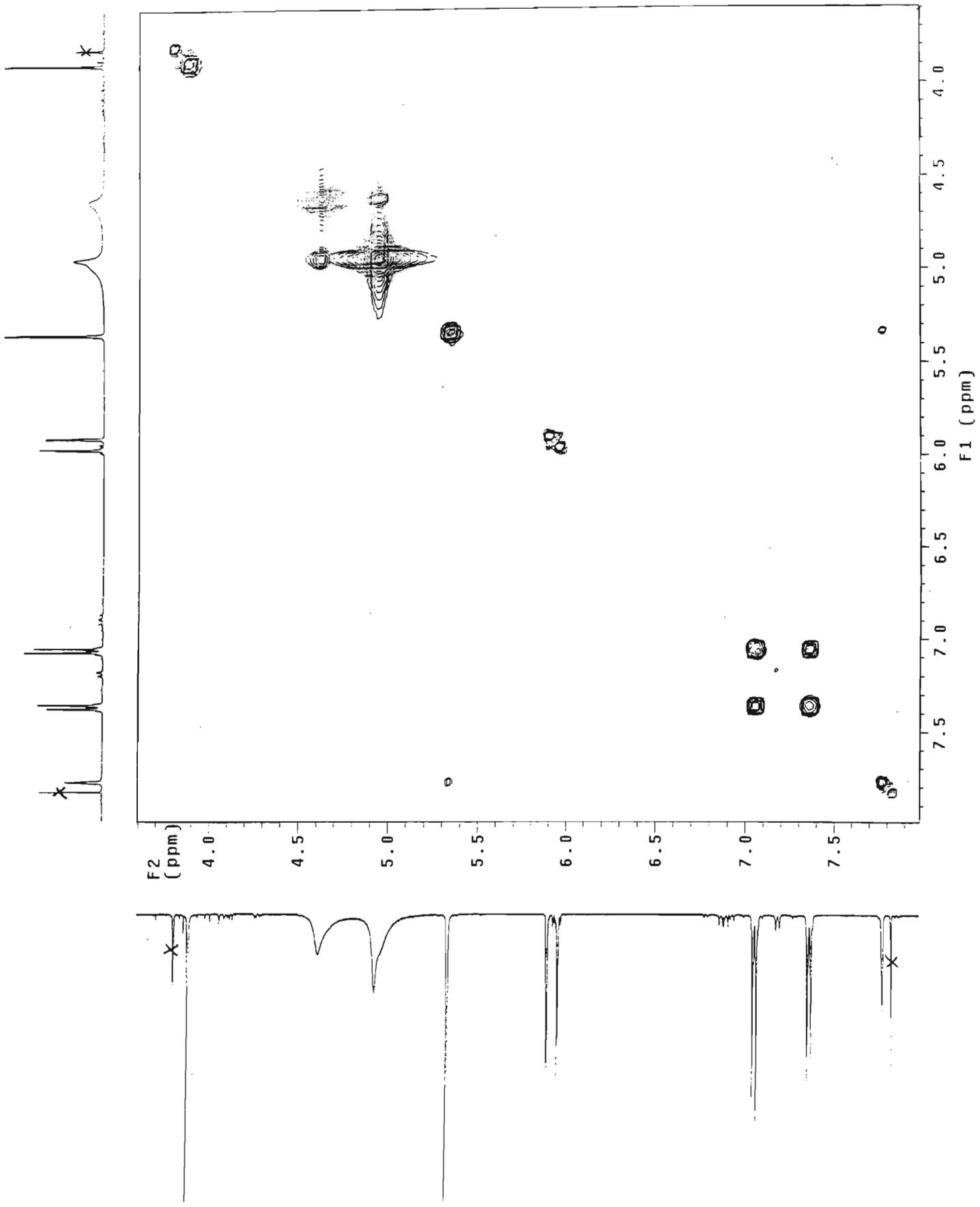
Spectrum 11.7: COSY spectrum of compound 11 (CD₃OD)

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Spectrum 11.8: NOESY spectrum of compound 11 (CD₃OD)
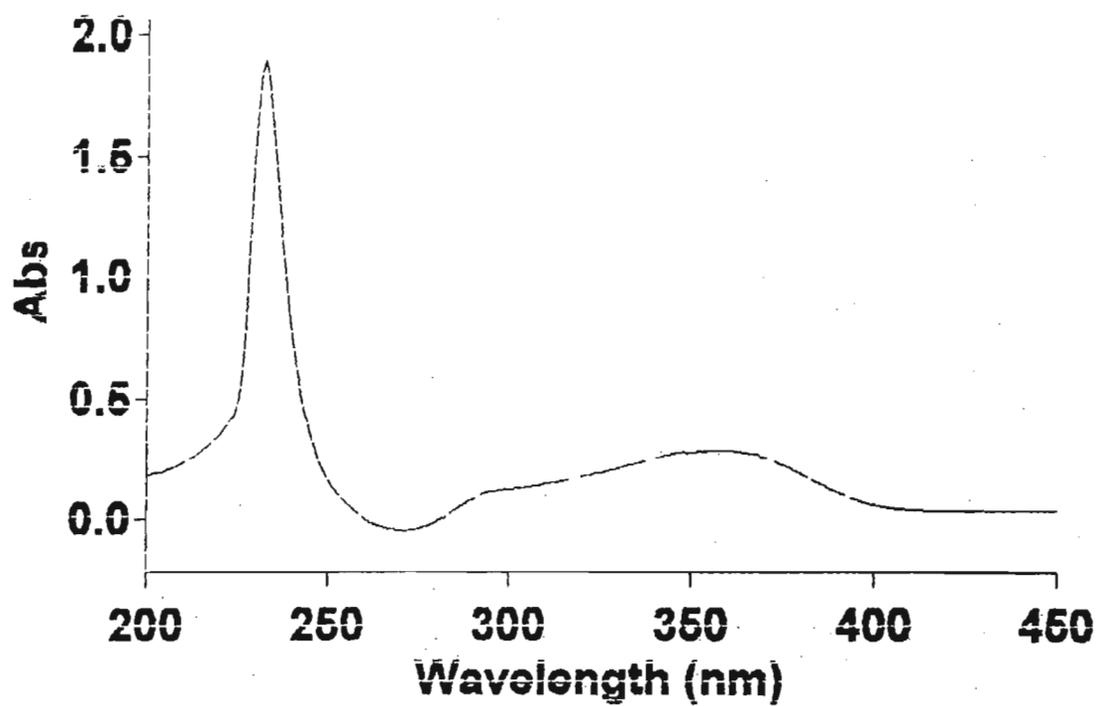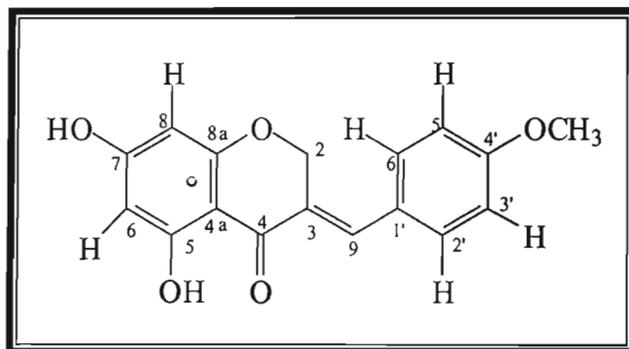
Spectrum 11.9: UV spectrum of compound 11
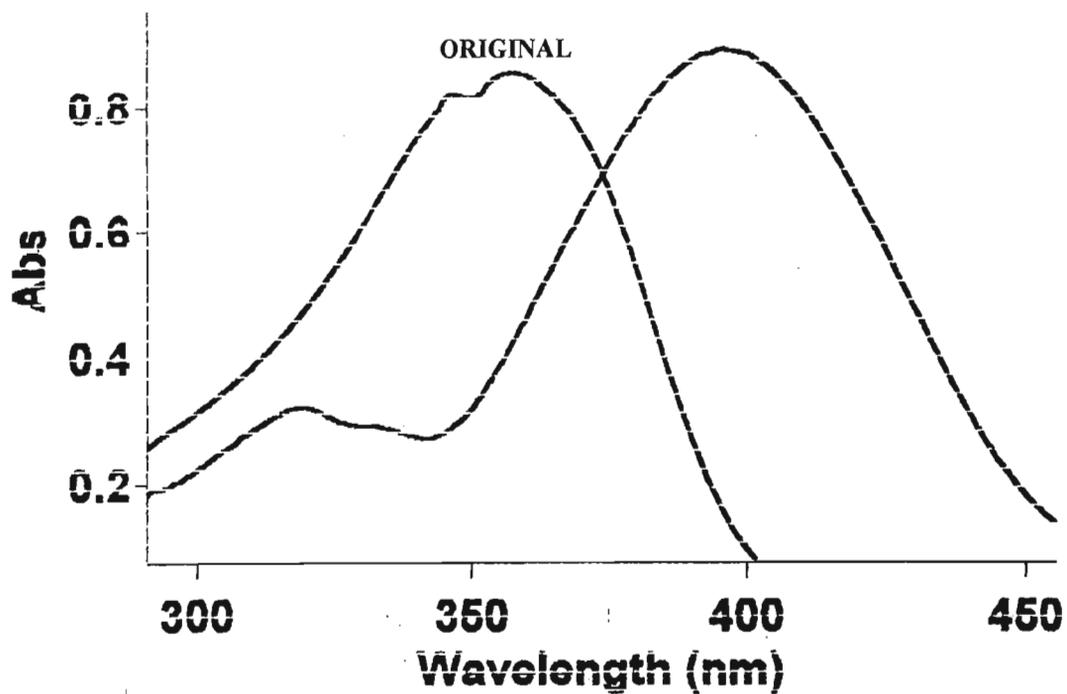
Spectrum 11.10: UV spectrum of compound 11 with AlCl₃
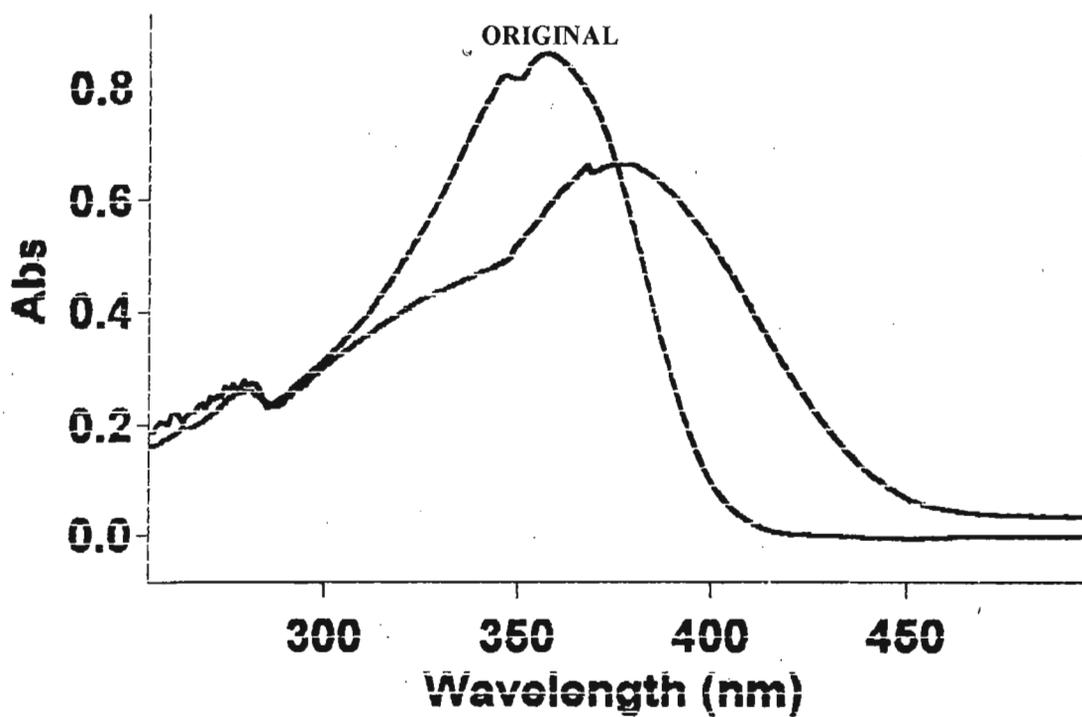
Spectrum 11.11: UV spectrum of compound 11 with NaOAc
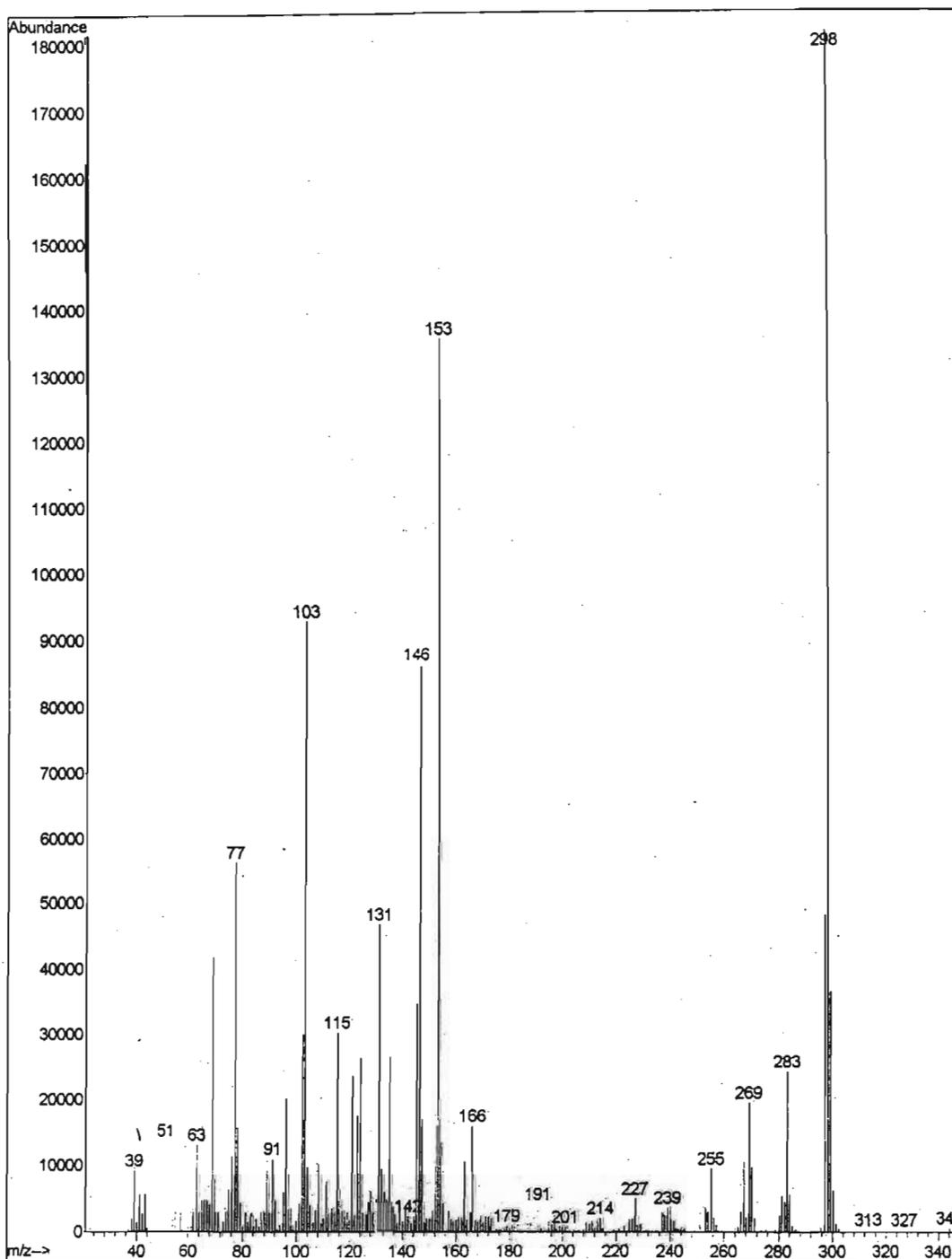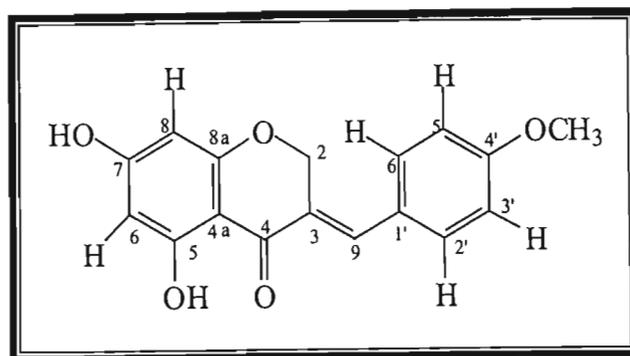
Spectrum 11.12: Mass spectrum of compound 11
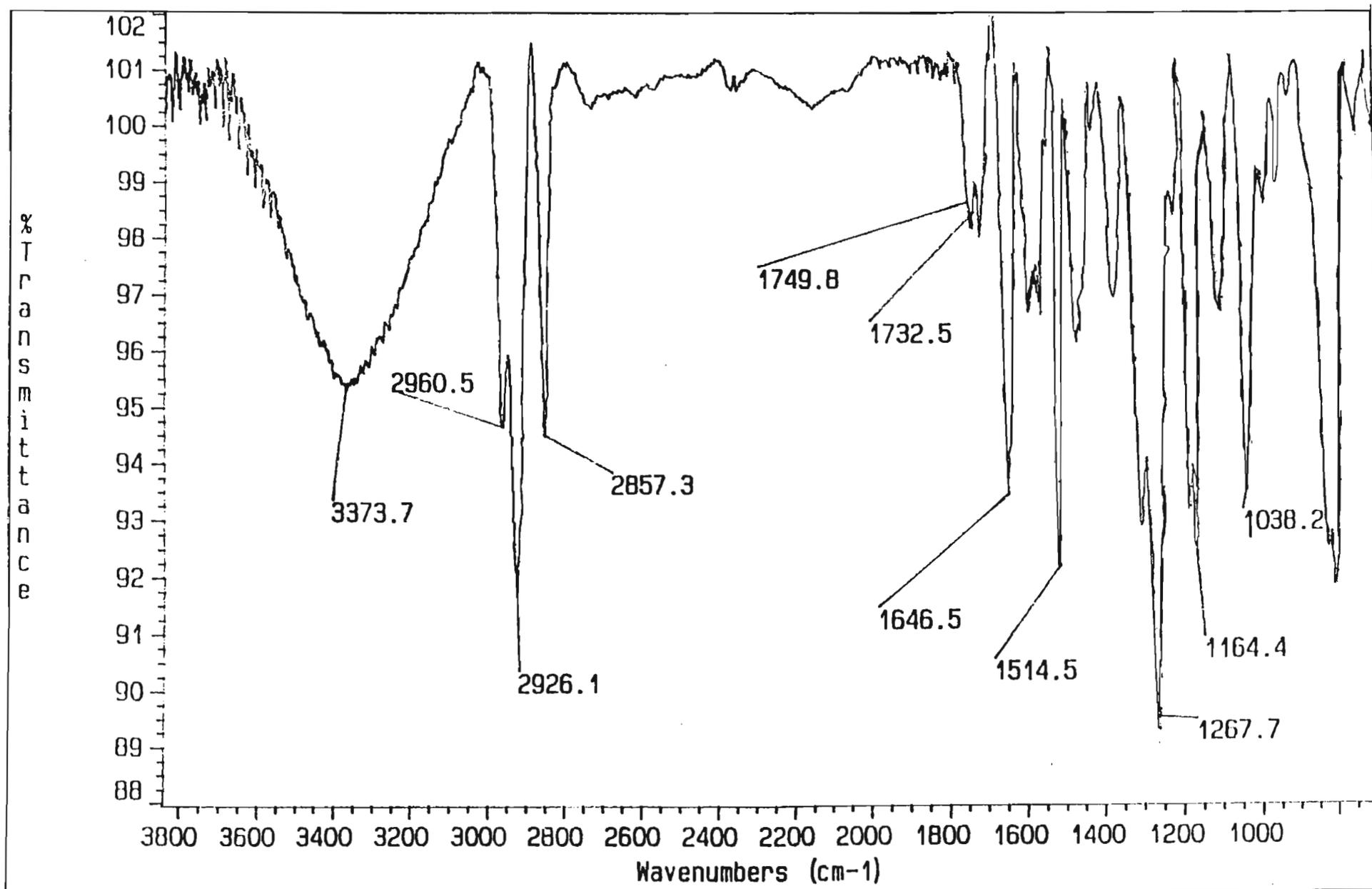
Spectrum 11.13: IR spectrum of compound 11
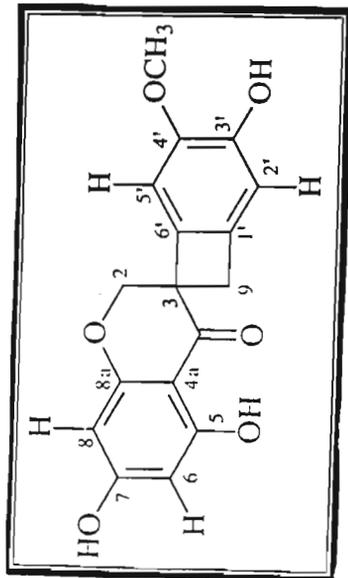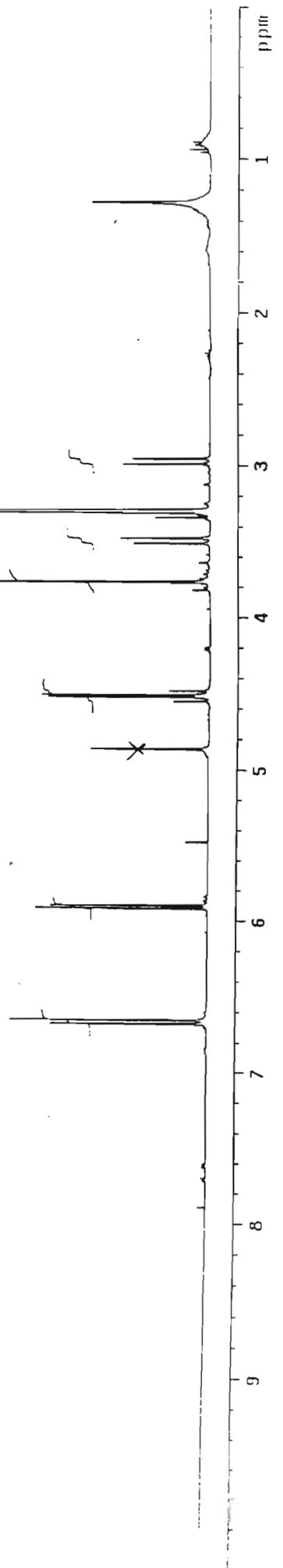
Spectrum 12.1: ^{1}H NMR spectrum of compound 12 (CD$_3$OD)

271
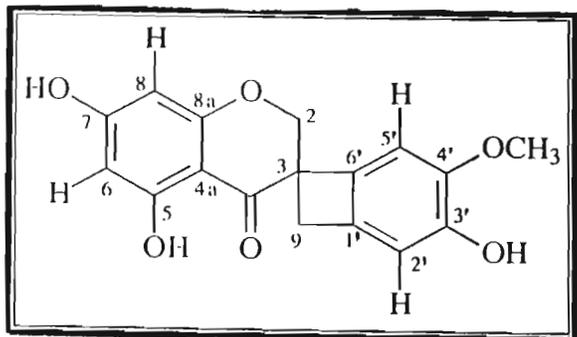
272
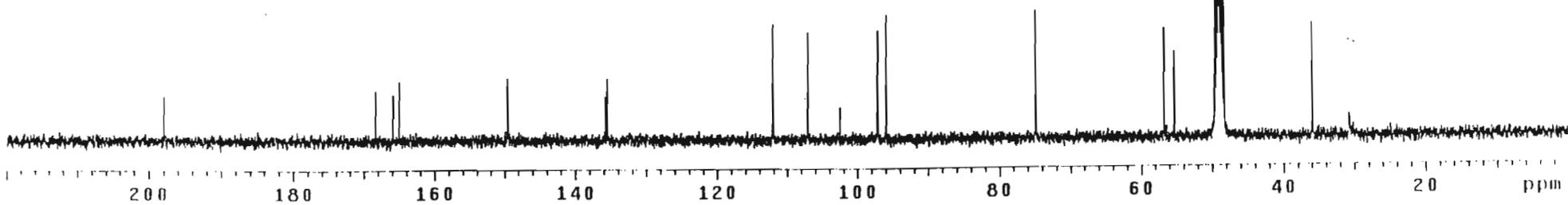
Spectrum 12.2: ^{13}C NMR spectrum of compound 12 (CD$_3$OD)
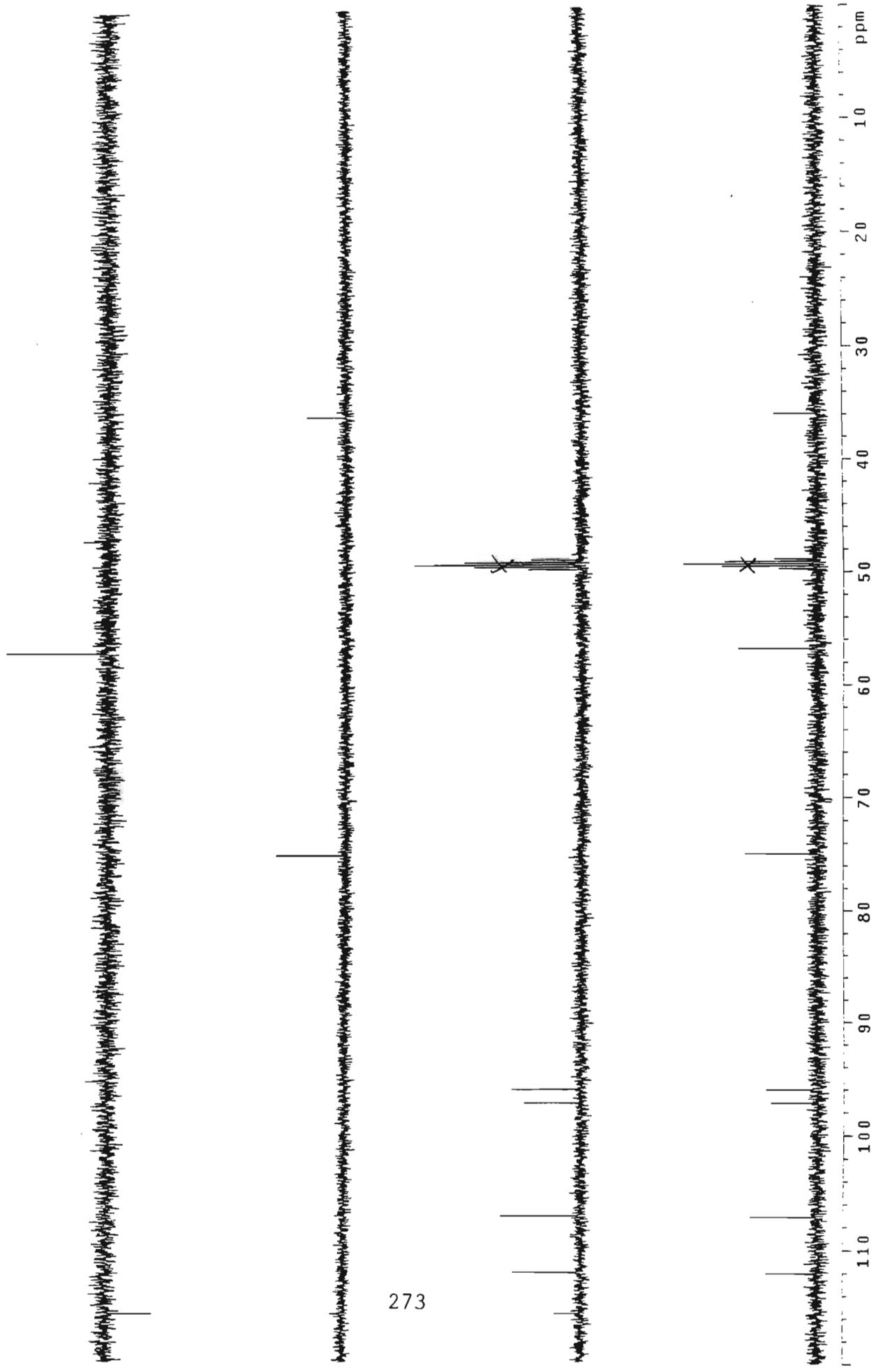
Spectrum 12.3: ADEPT spectrum of compound 12 (CD₃OD)
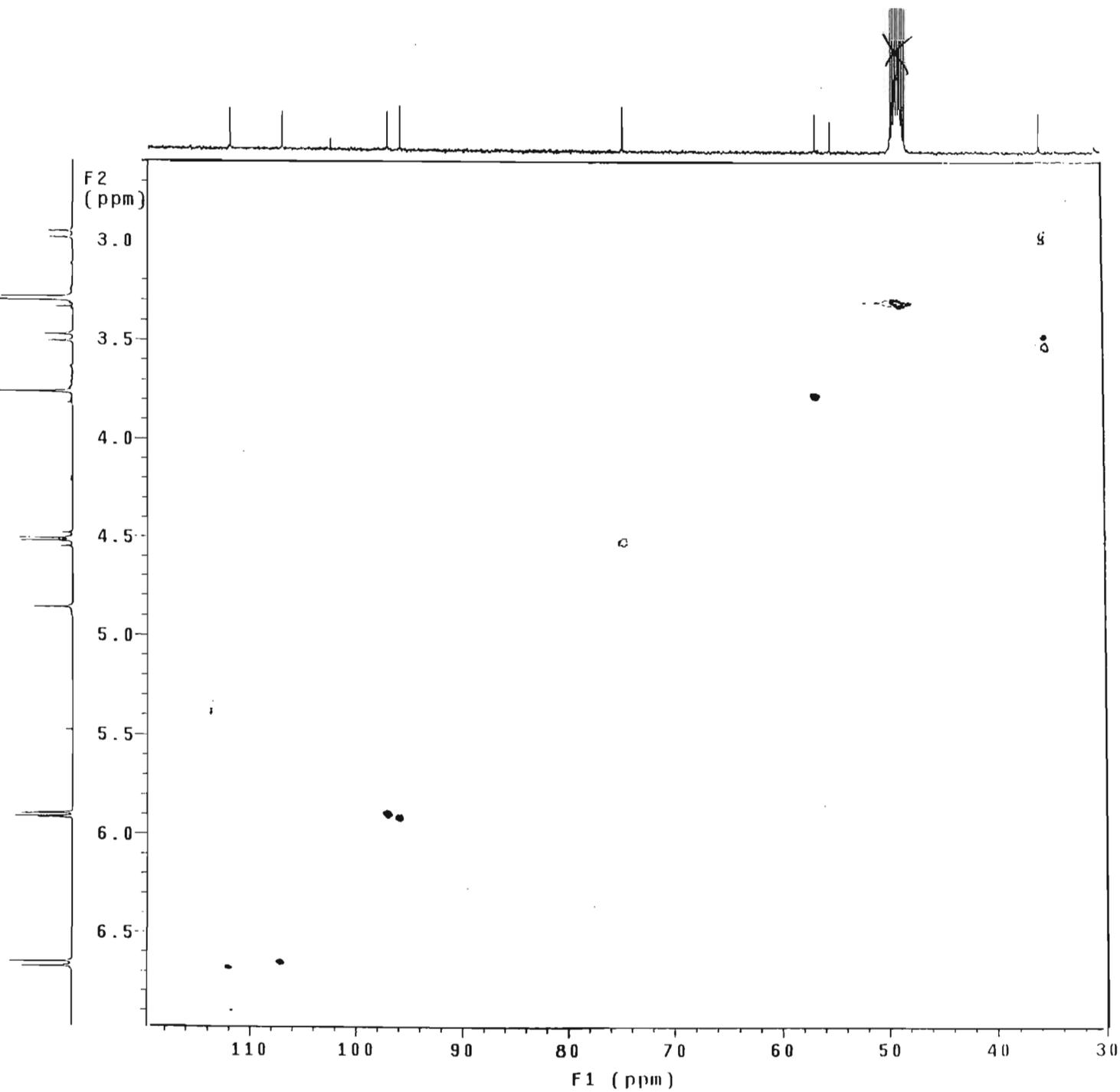
Spectrum 12.4: HSQC spectrum of compound 12 (CD₃OD)
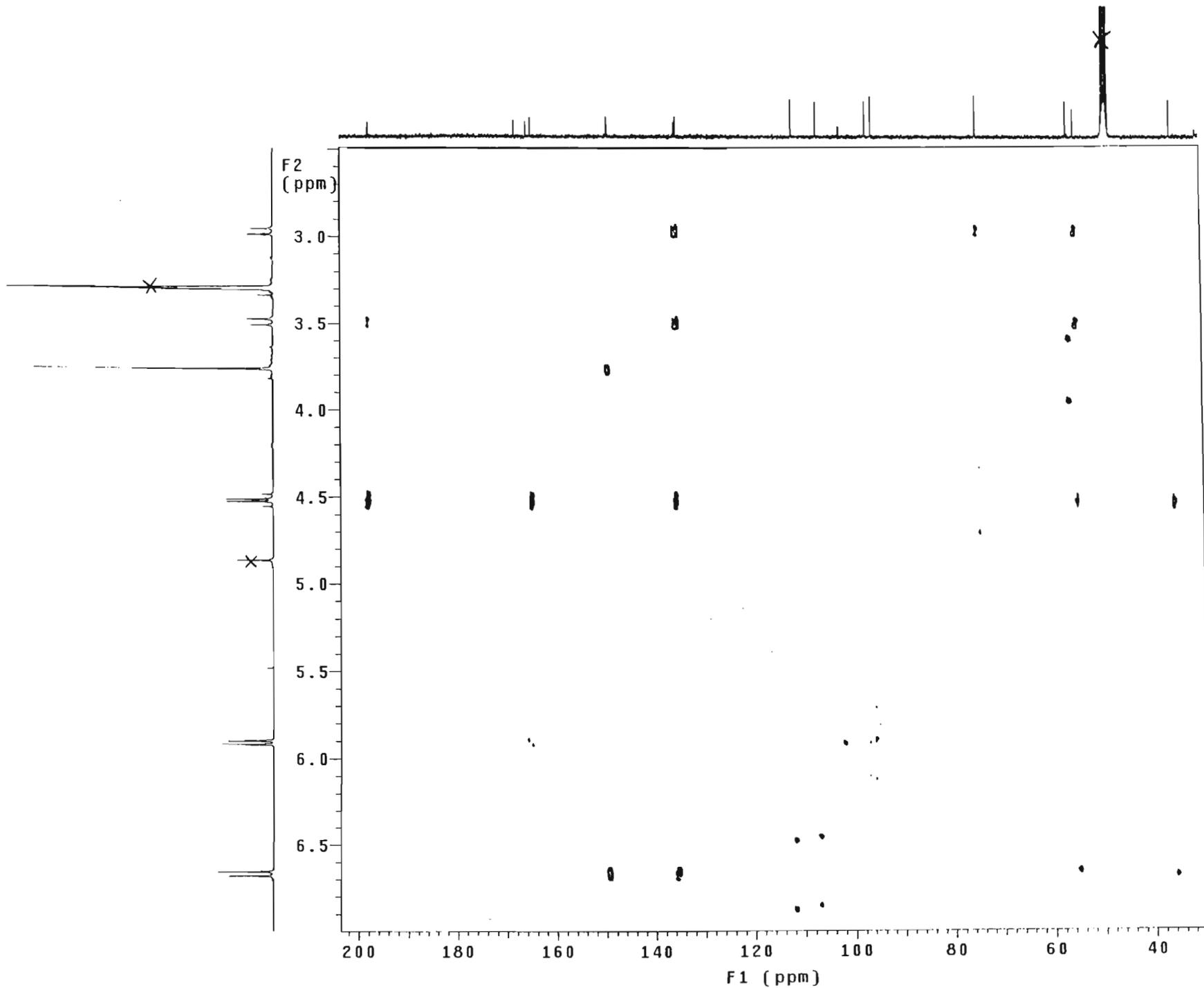
Spectrum 12.5: HMBC spectrum of compound 12 (CD₃OD)
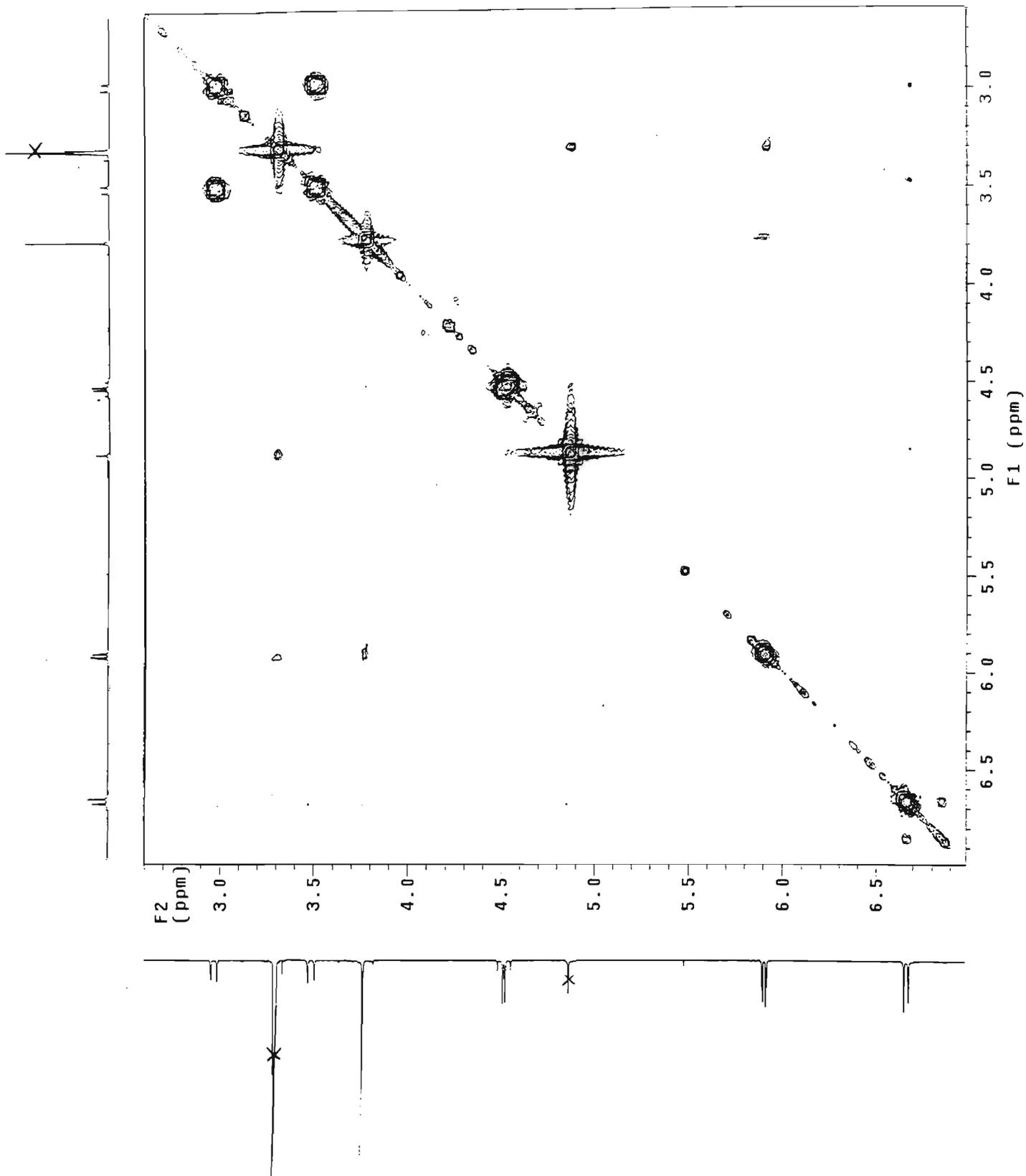
Spectrum 12.6: COSY spectrum of compound 12 (CD₃OD)
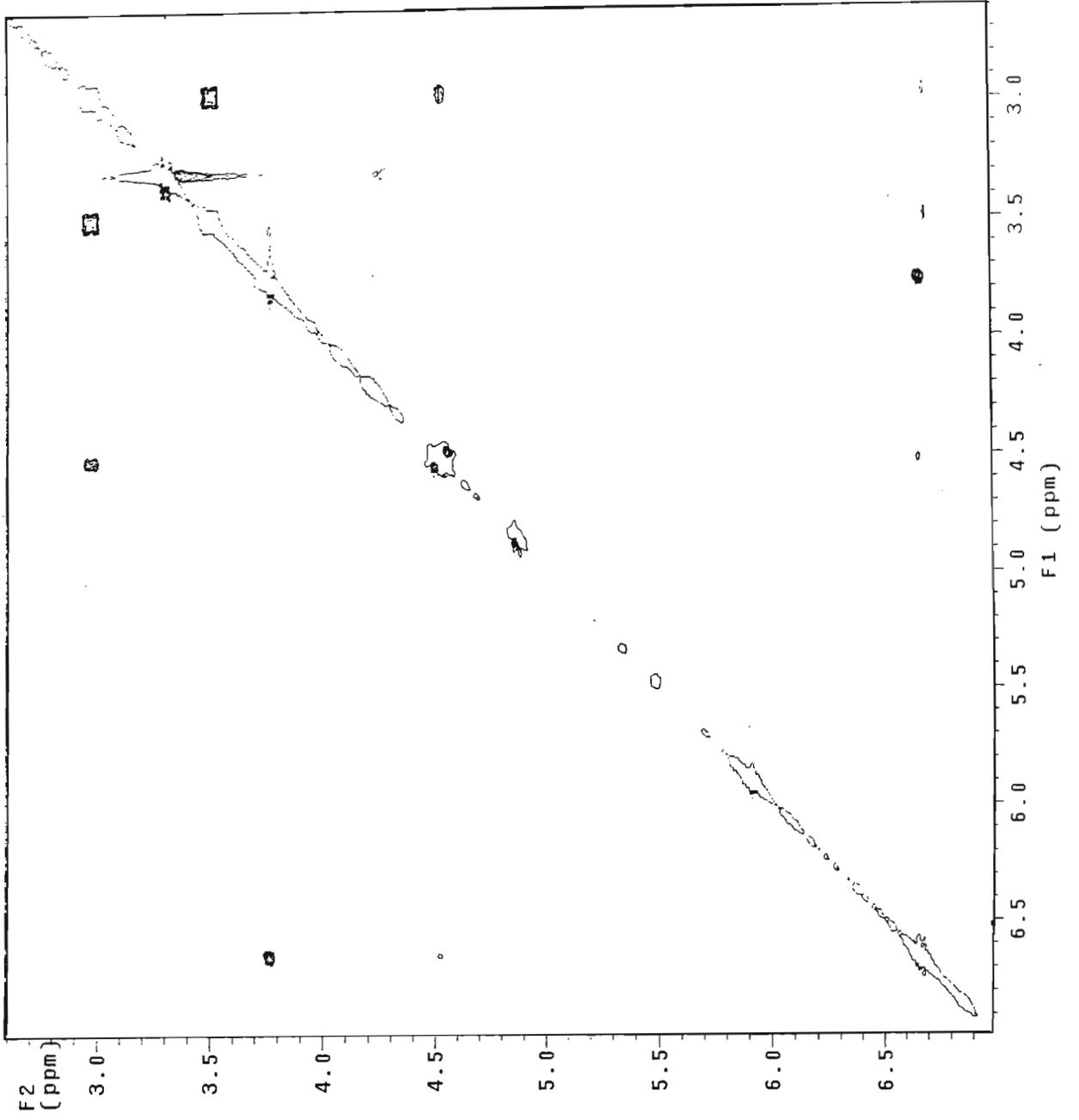
Spectrum 12.7: NOESY spectrum of compound 12 (CD₃OD)
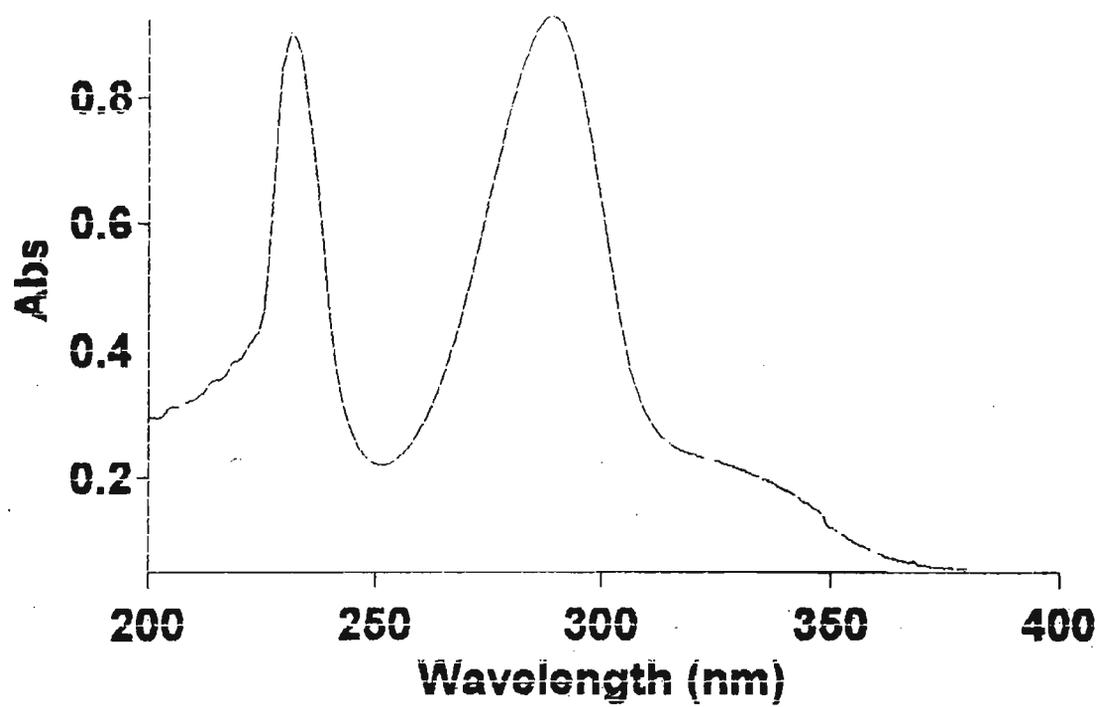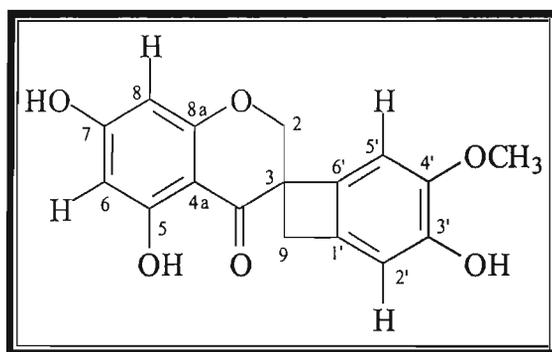
Spectrum 12.8: UV spectrum of compound 12
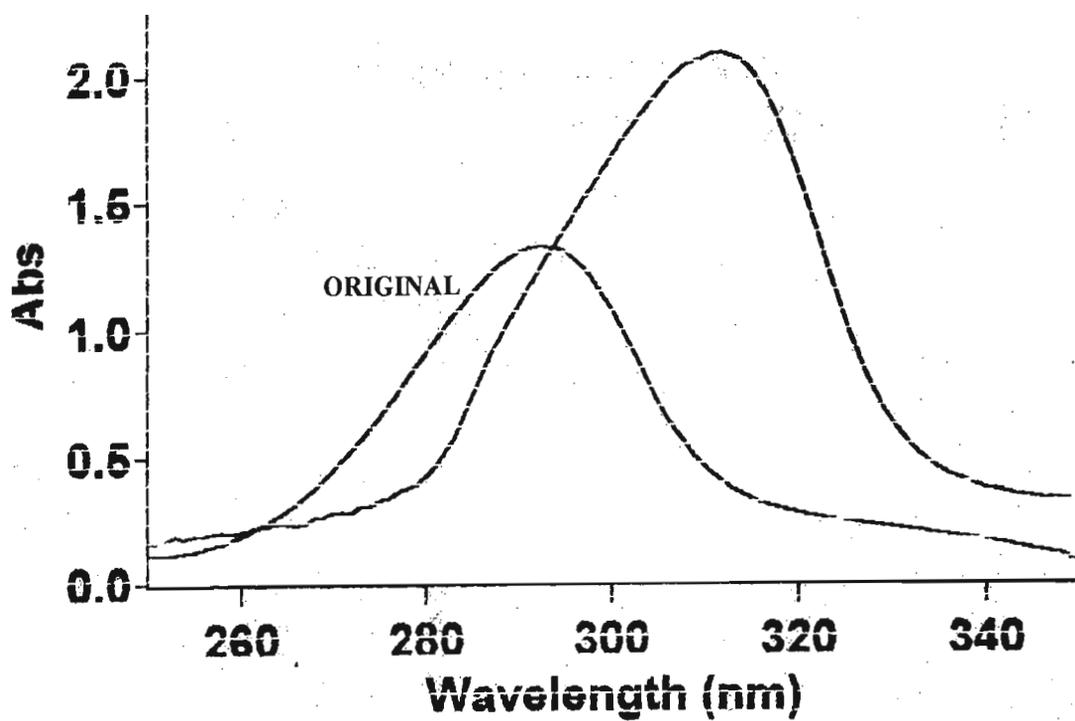
Spectrum 12.9: UV spectrum of compound 12 with AlCl₃
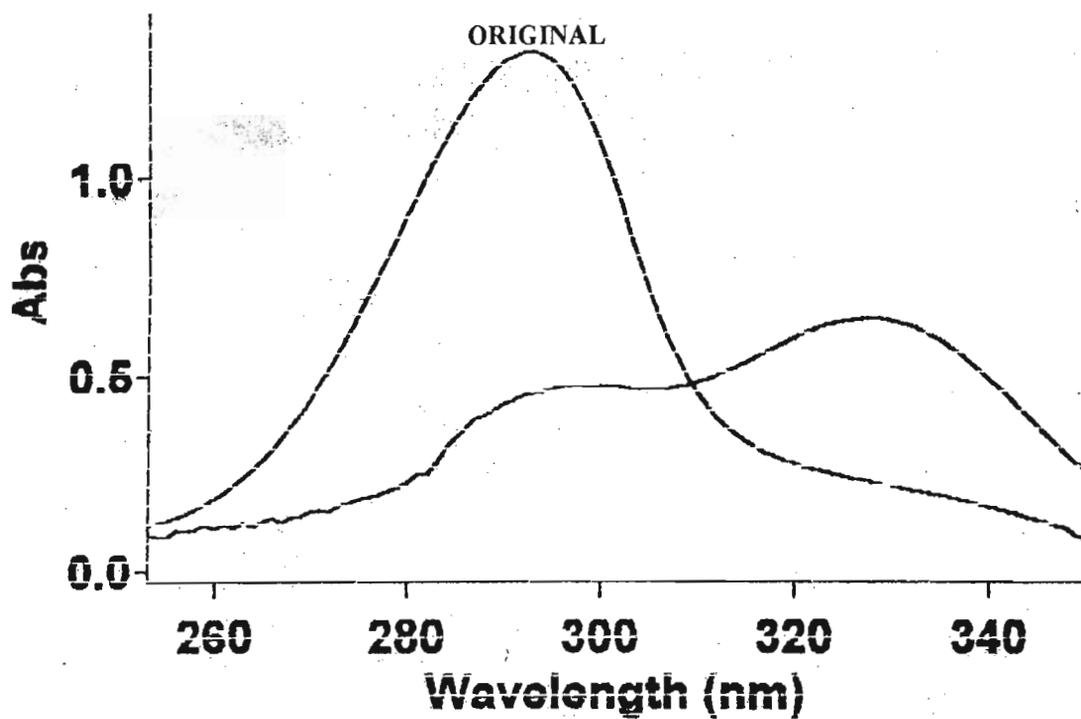
Spectrum 12.10: UV spectrum of compound 12 with NaOAc
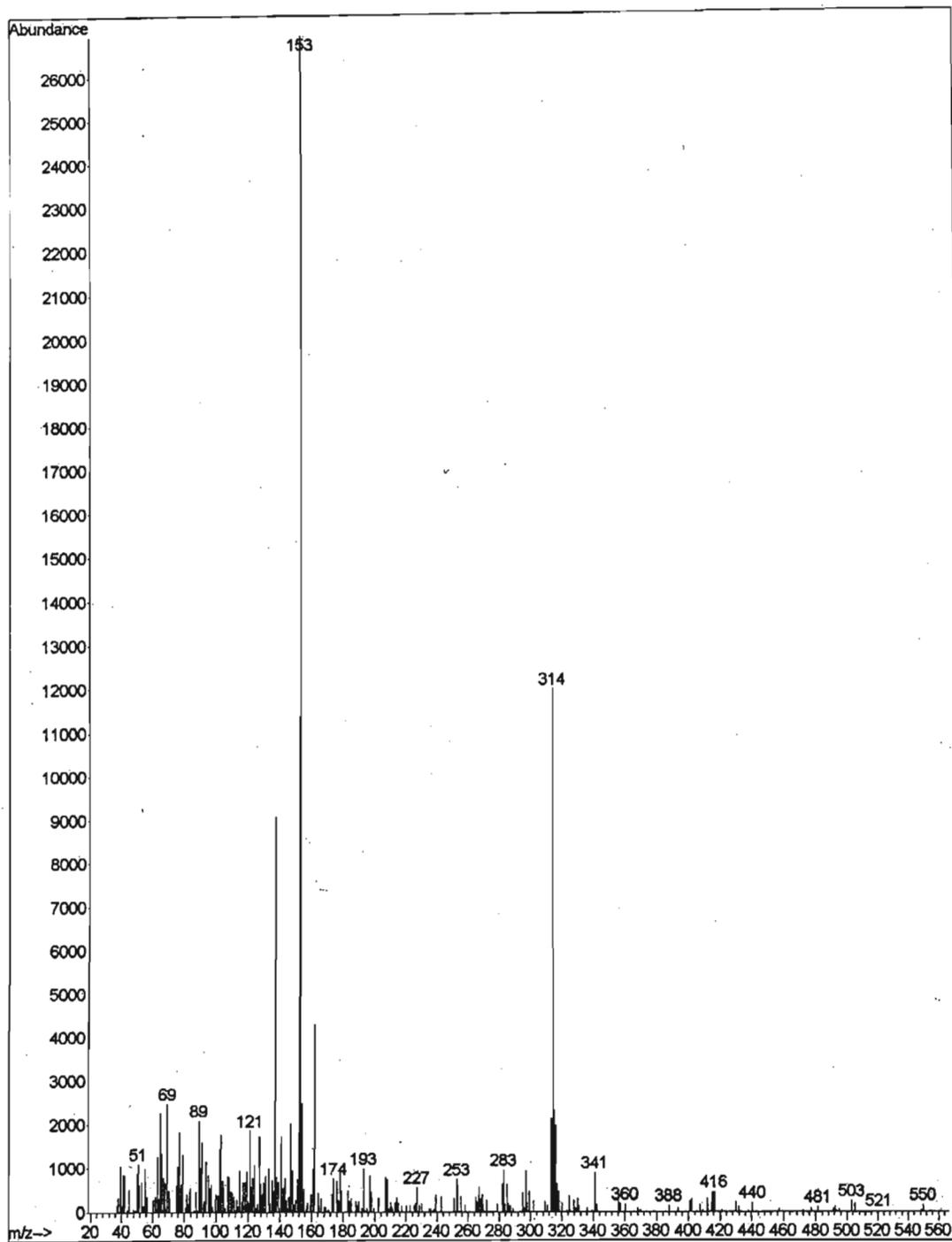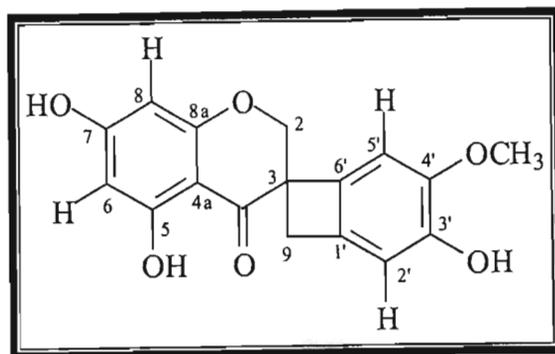
Spectrum 12.11: Mass spectrum of compound 12
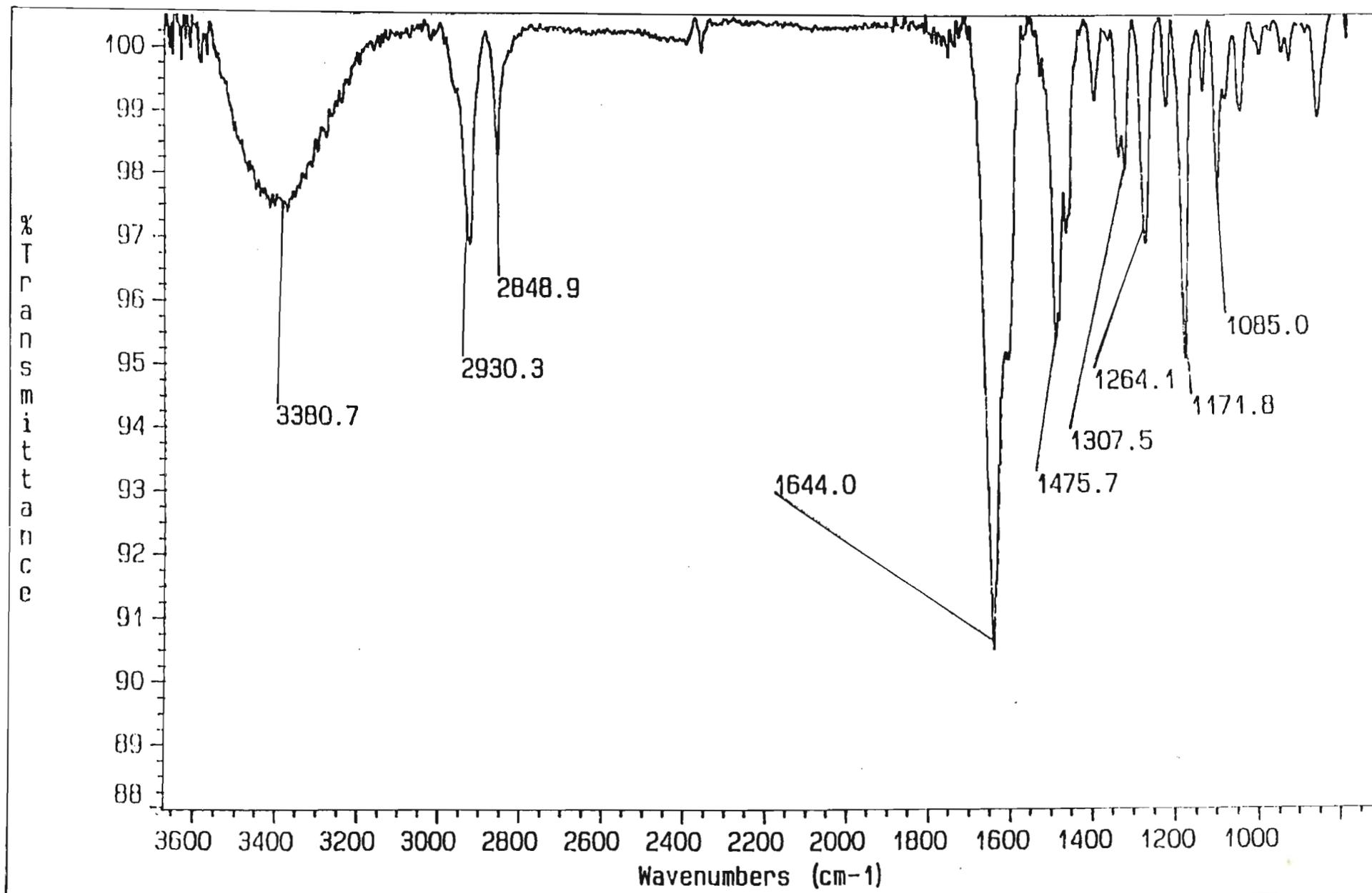
Spectrum 12.12: IR spectrum of compound 12
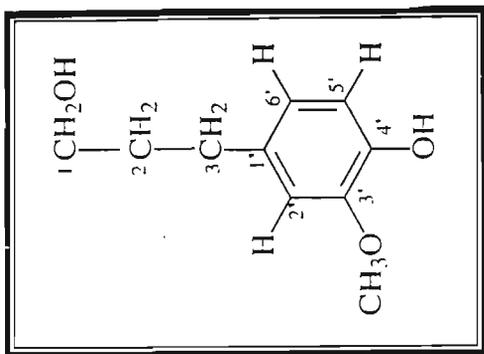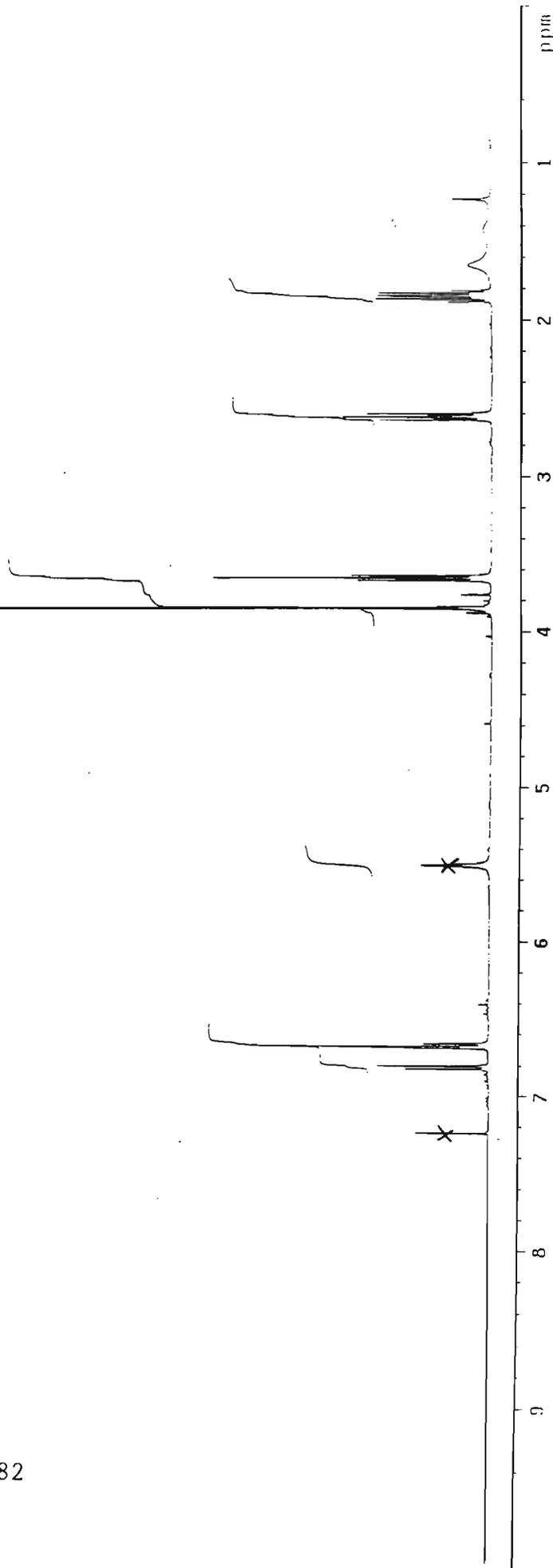
Spectrum 13.1: ^{1}H NMR spectrum of compound 13 (CDCl$_3$)

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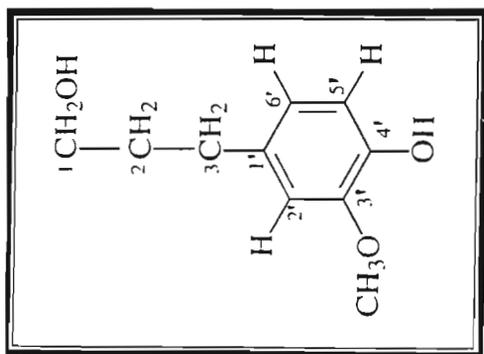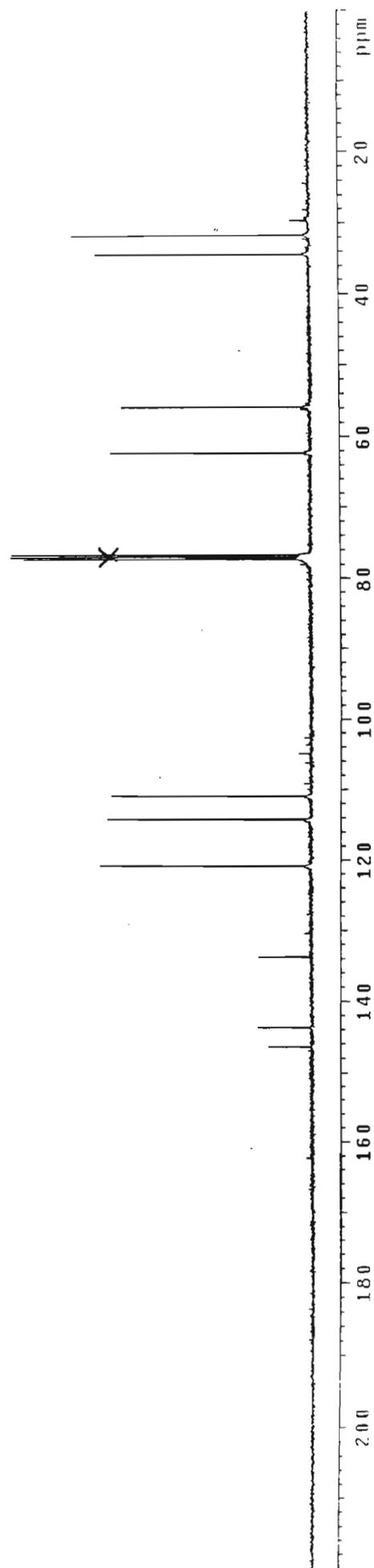
Spectrum 13.2: ^{13}C NMR spectrum of compound 13 (CDCl$_3$)

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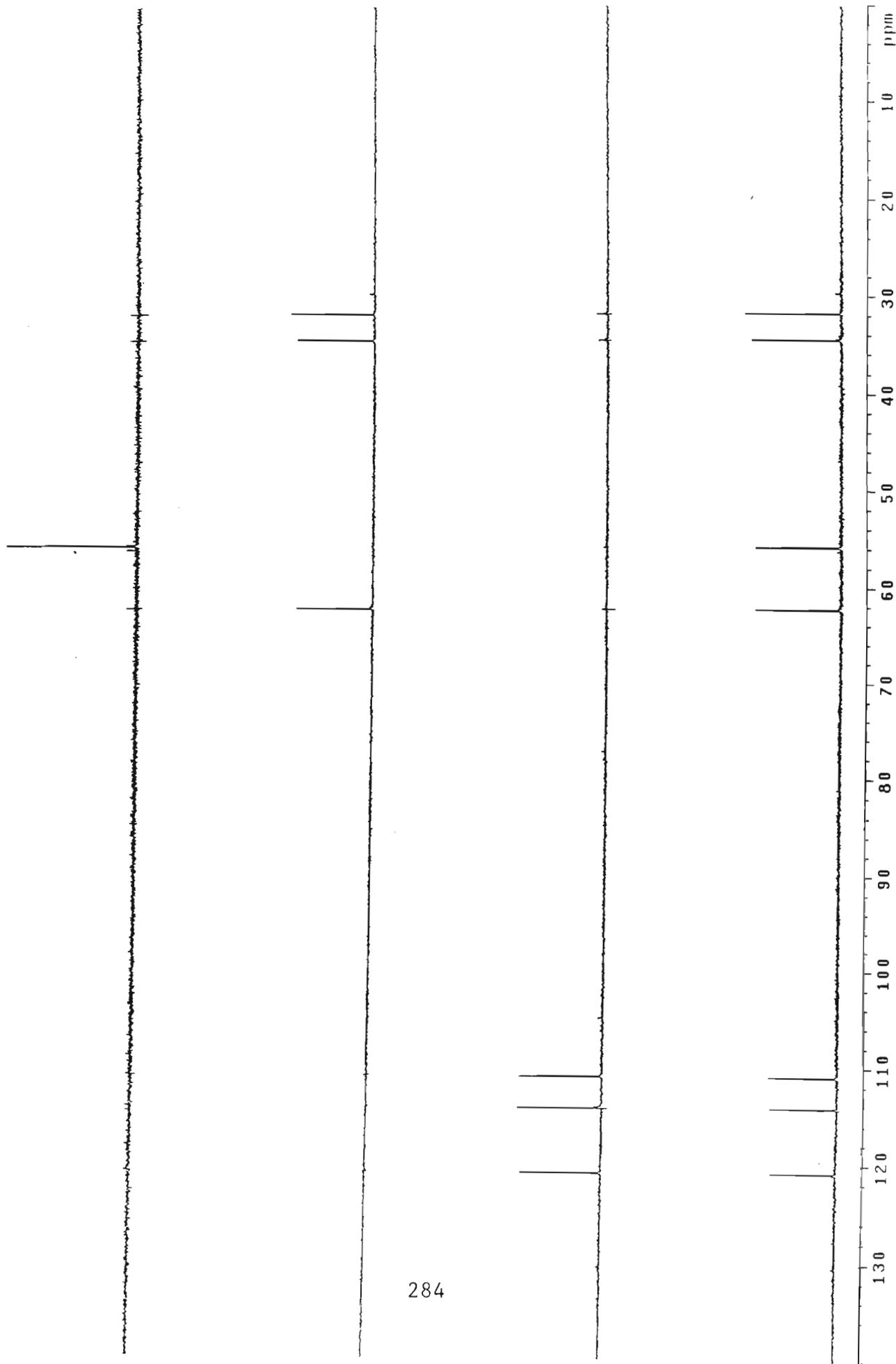
Spectrum 13.3: ADEPT spectrum of compound 13 (CDCl₃)
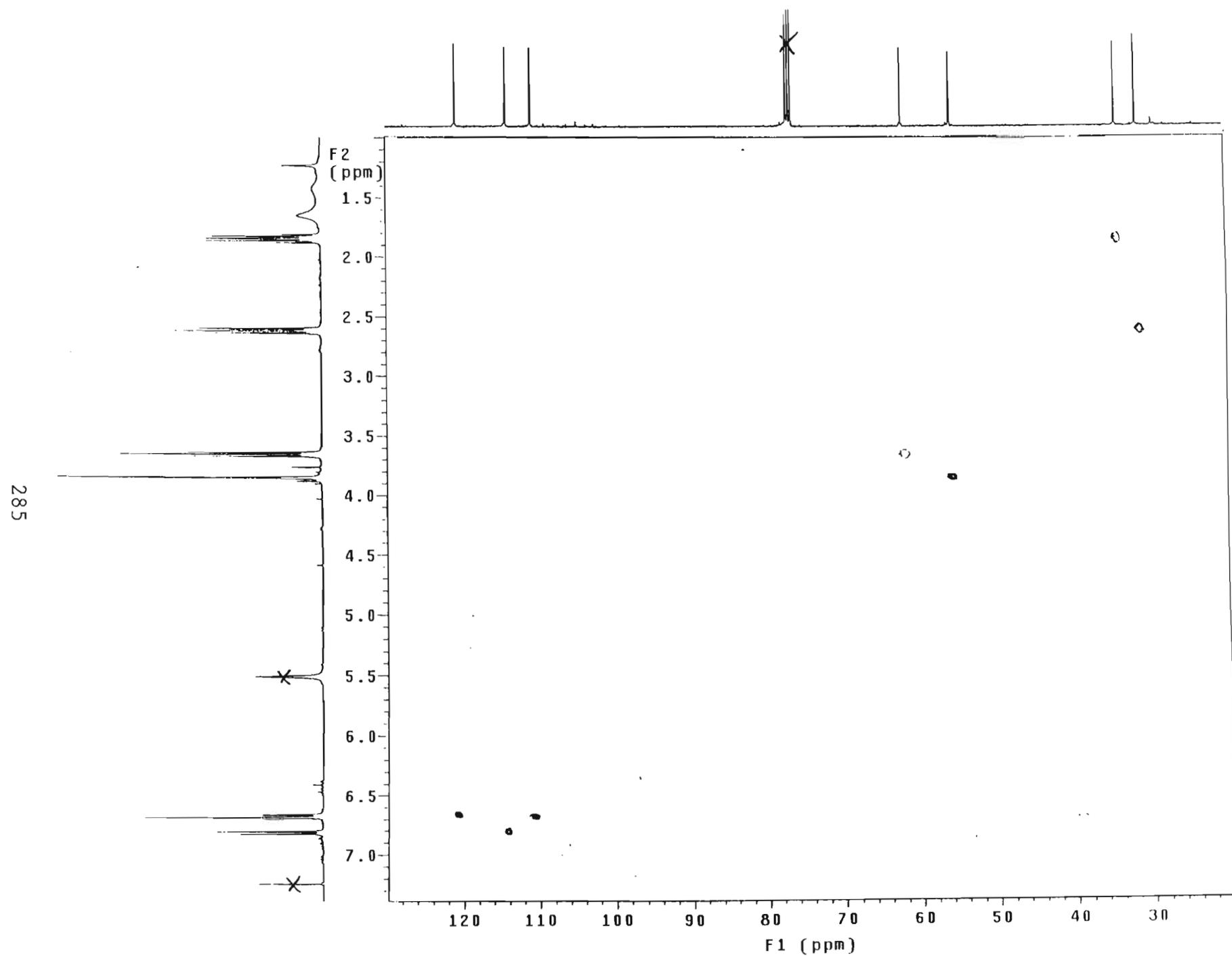
Spectrum 13.4: HSQC spectrum of compound 13 (CDCl₃)
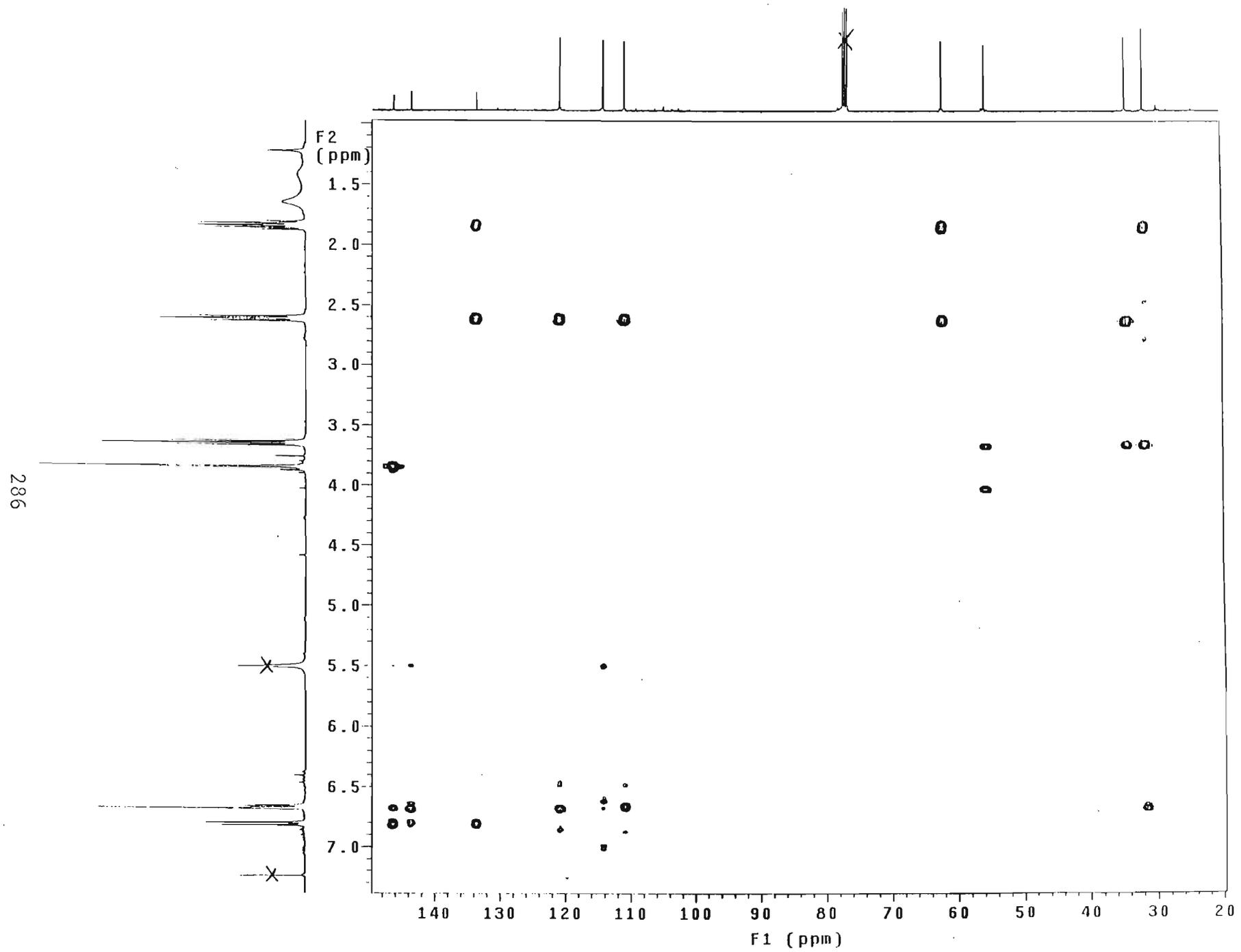
Spectrum 13.5: HMBC spectrum of compound 13 (CDCl₃)
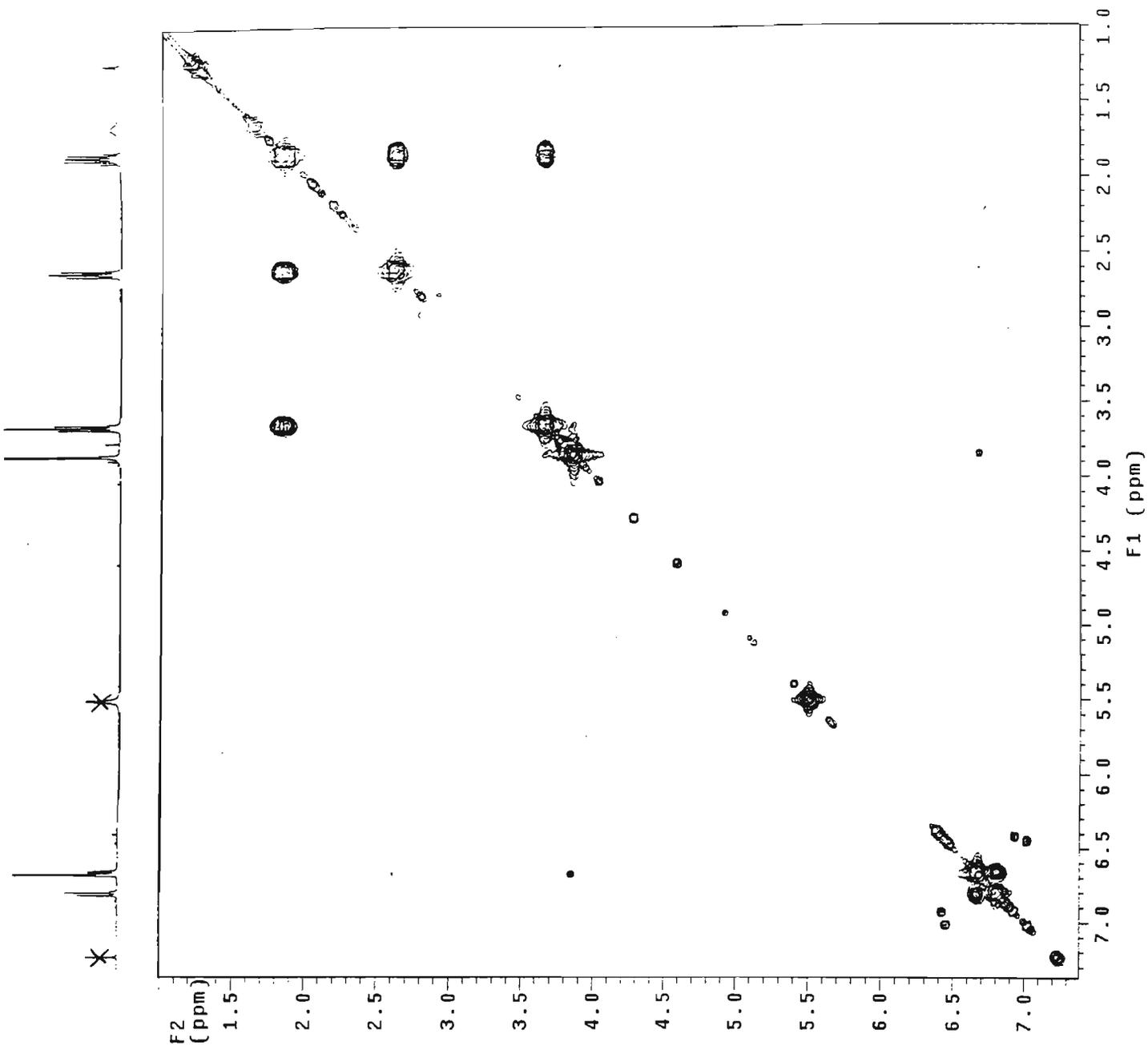
Spectrum 13.6: COSY spectrum of compound 13 (CDCl₃)

F1 (ppm)

F2 (ppm)

Spectrum 13.7: NOESY spectrum of compound 13 (CDCl₃)
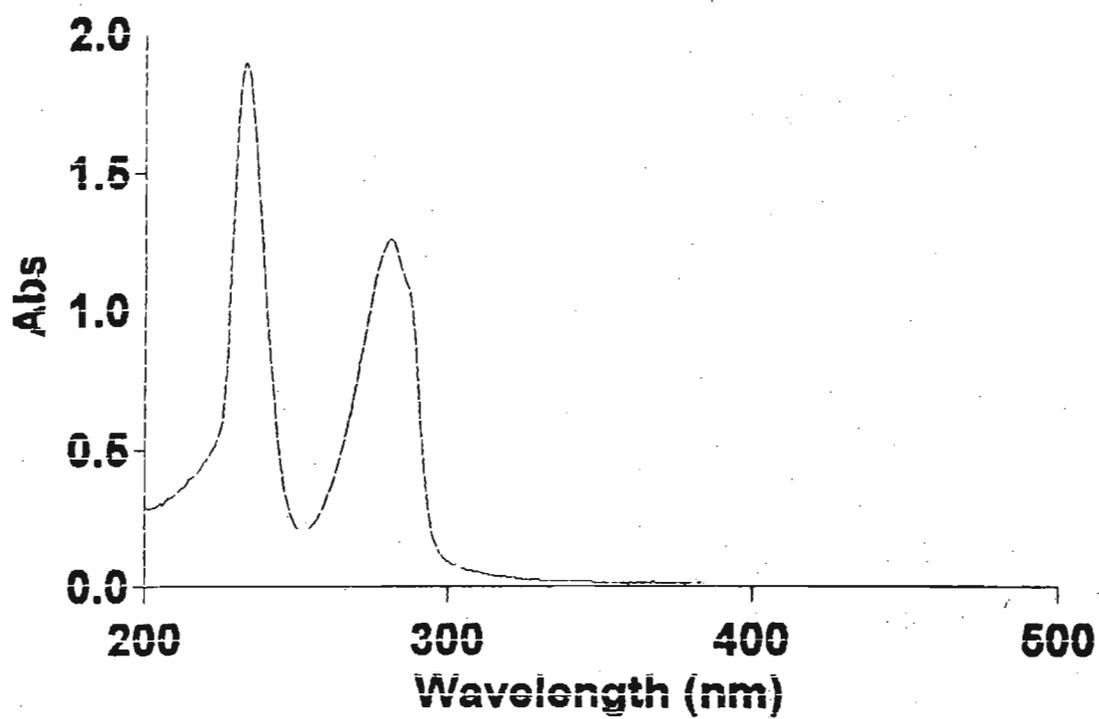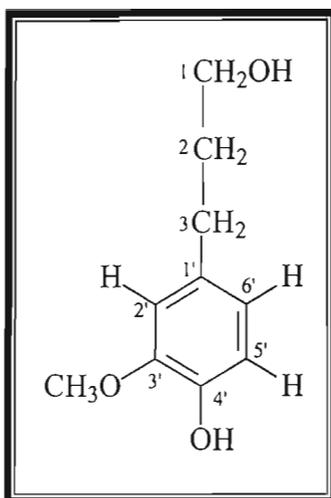
Spectrum 13.8: UV spectrum of compound 13
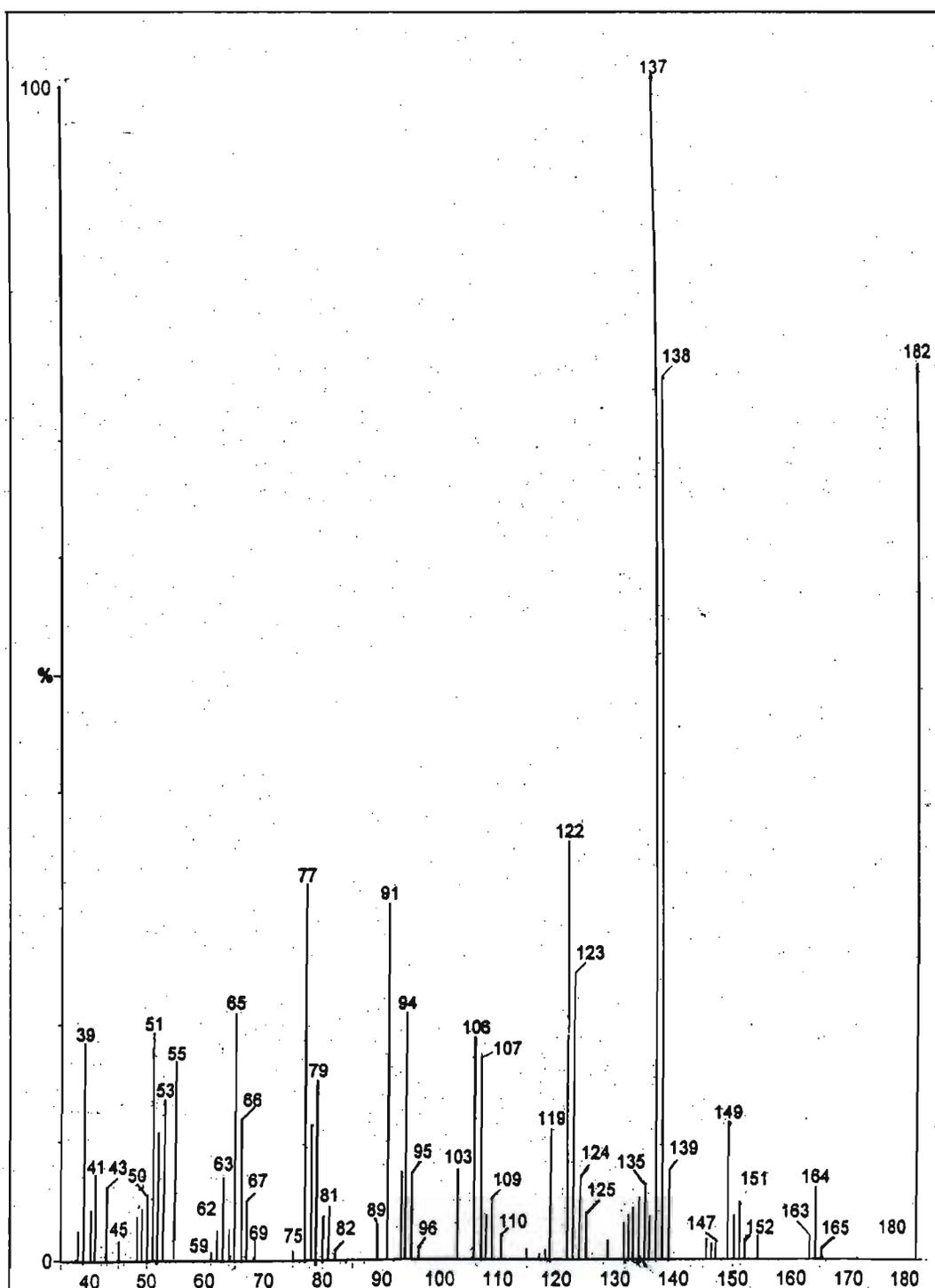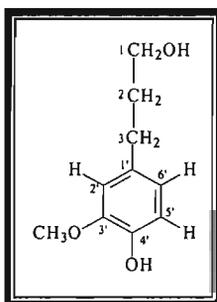
Spectrum 13.9: Mass spectrum of compound 13

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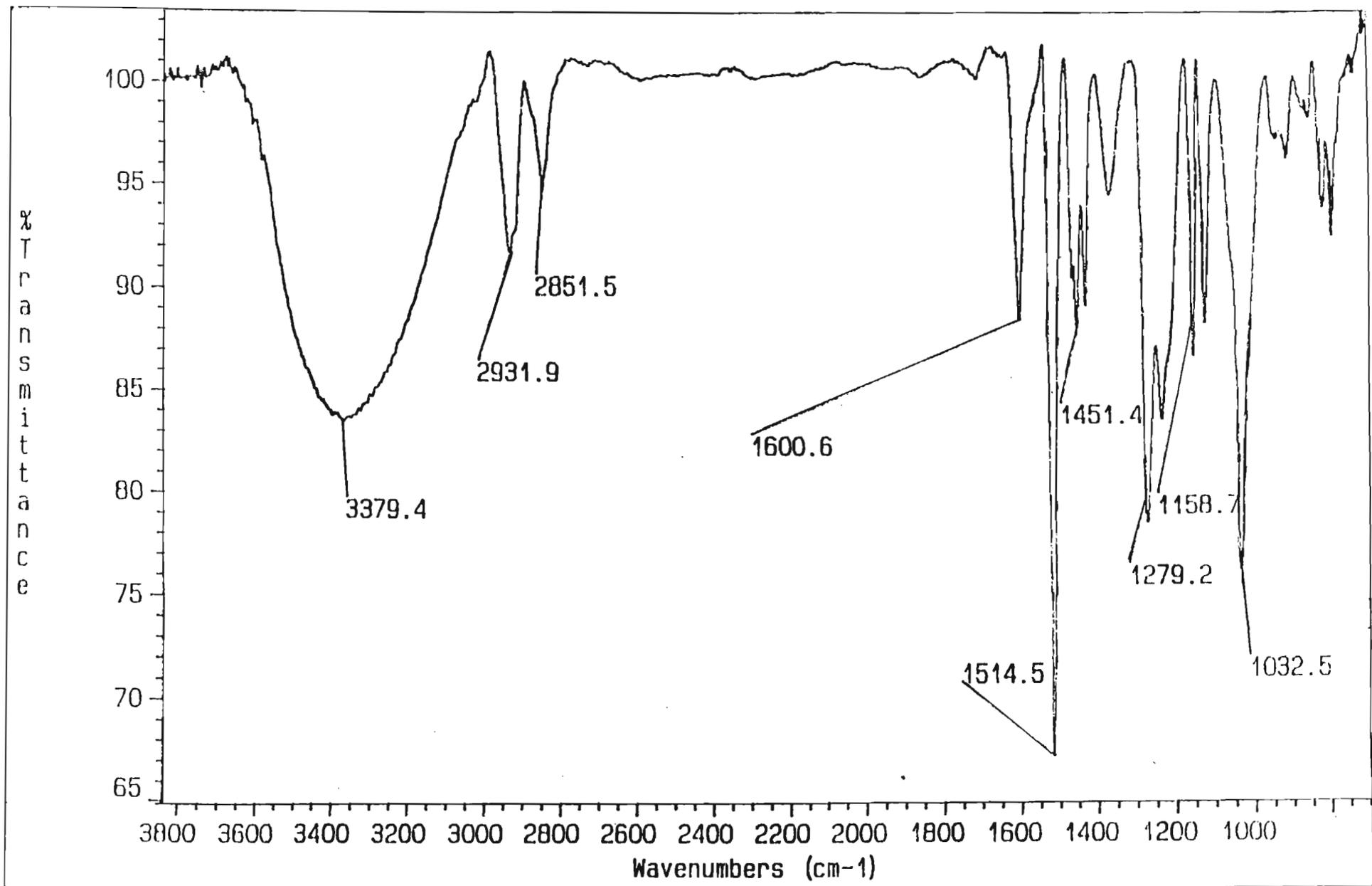
Spectrum 13.10: IR spectrum of compound 13
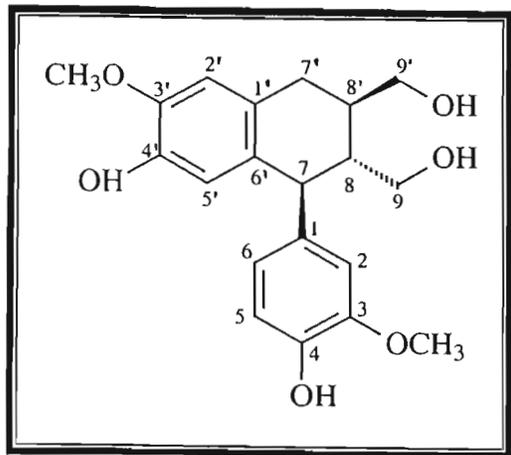
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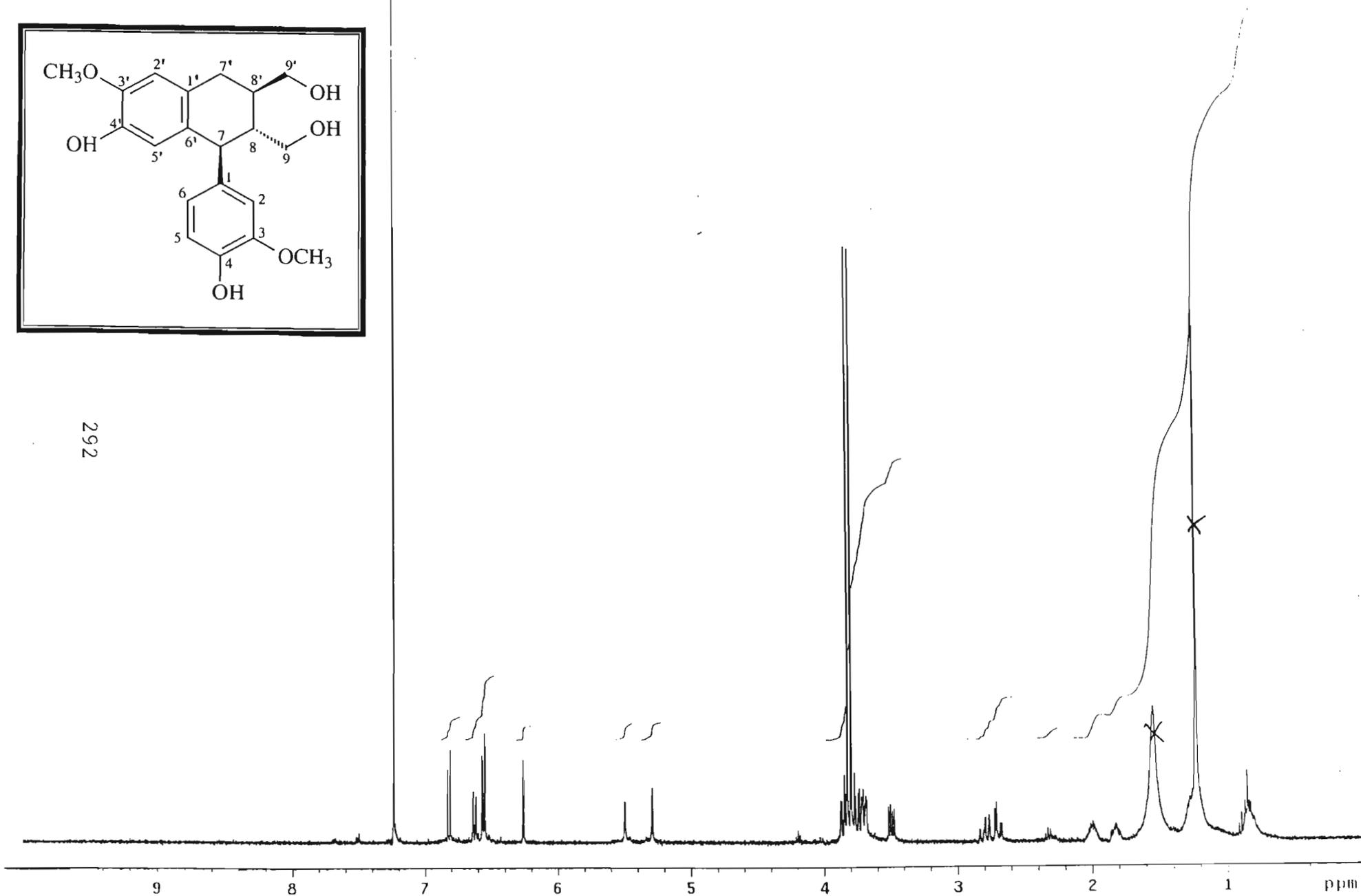
Spectrum 14.1: ^{1}H NMR spectrum of compound 14 (CDCl$_3$)
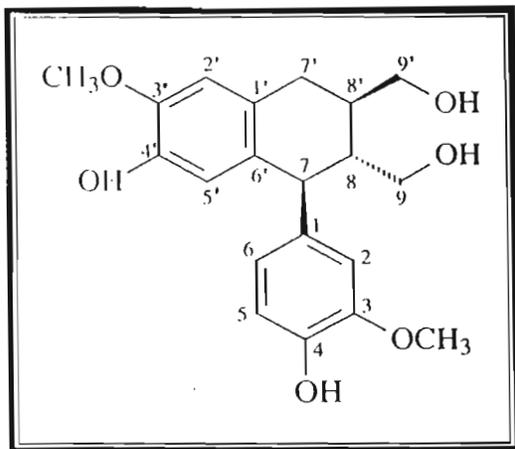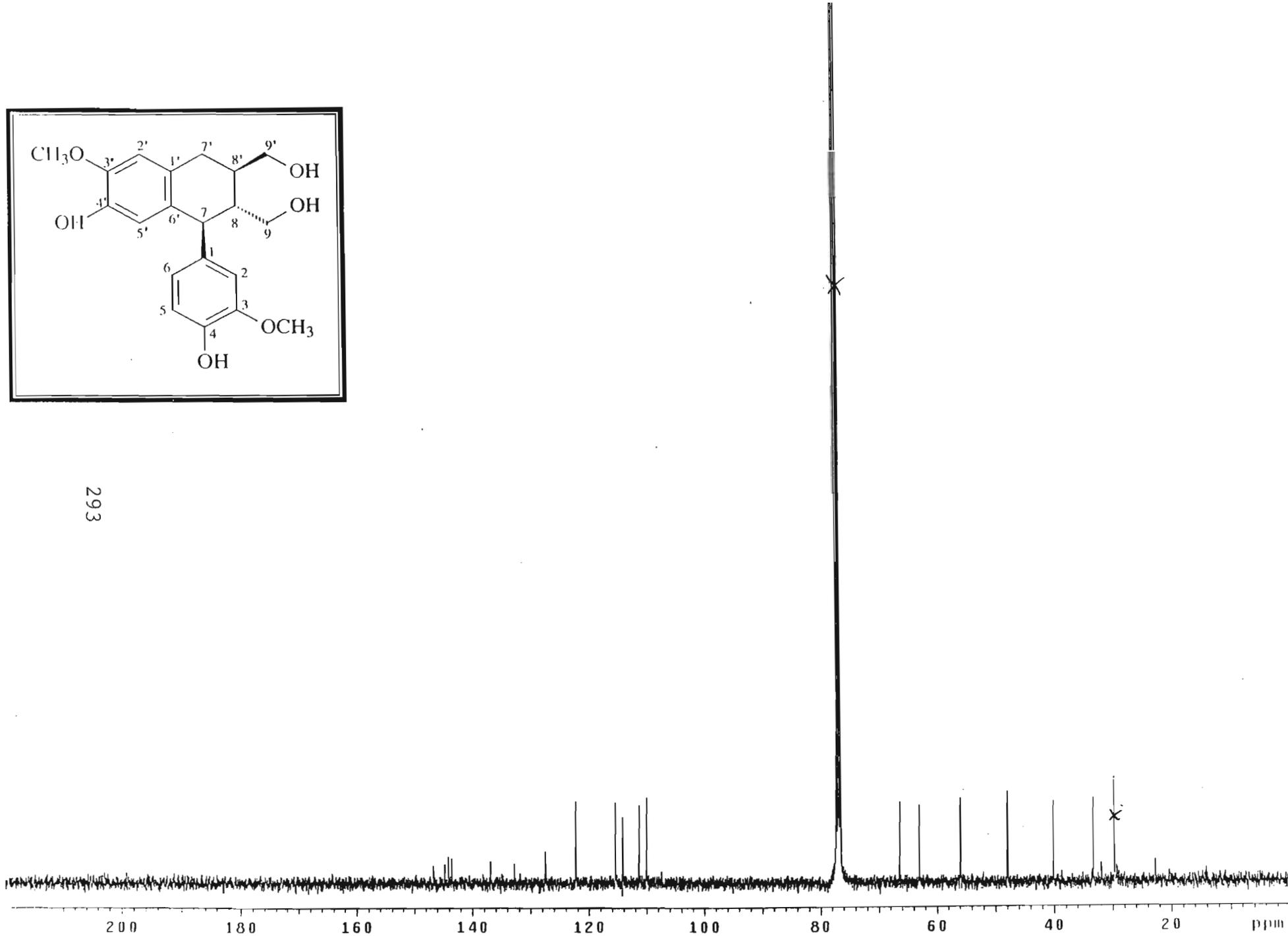
Spectrum 14.2: ^{13}C NMR spectrum of compound 14 (CDCl$_3$)

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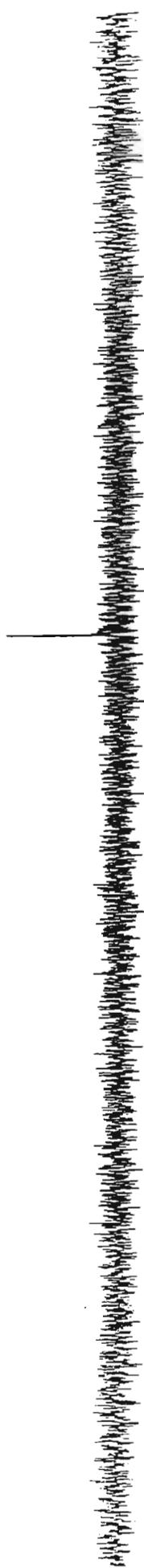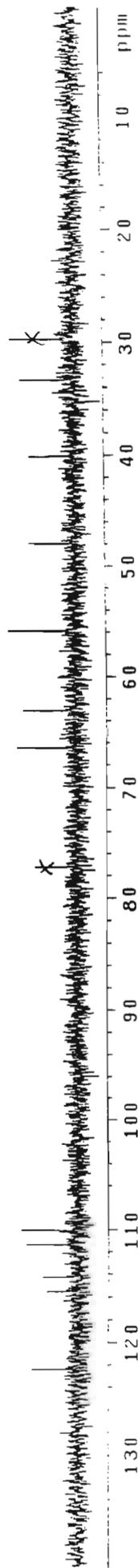
Spectrum 14.3: ADEPT spectrum of compound 14 (CDCl₃)
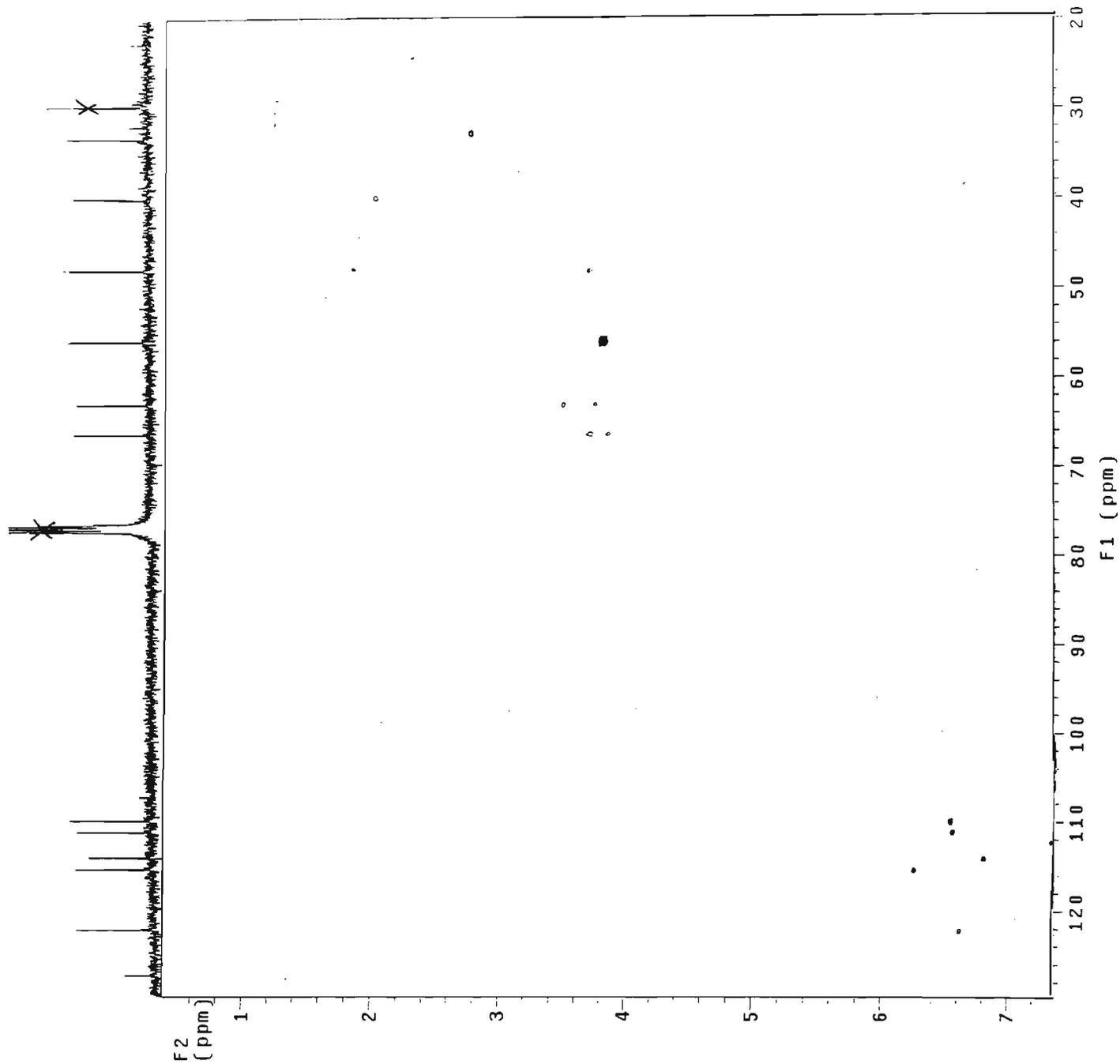
Spectrum 14.4: HSQC spectrum of compound 14 (CDCl₃)

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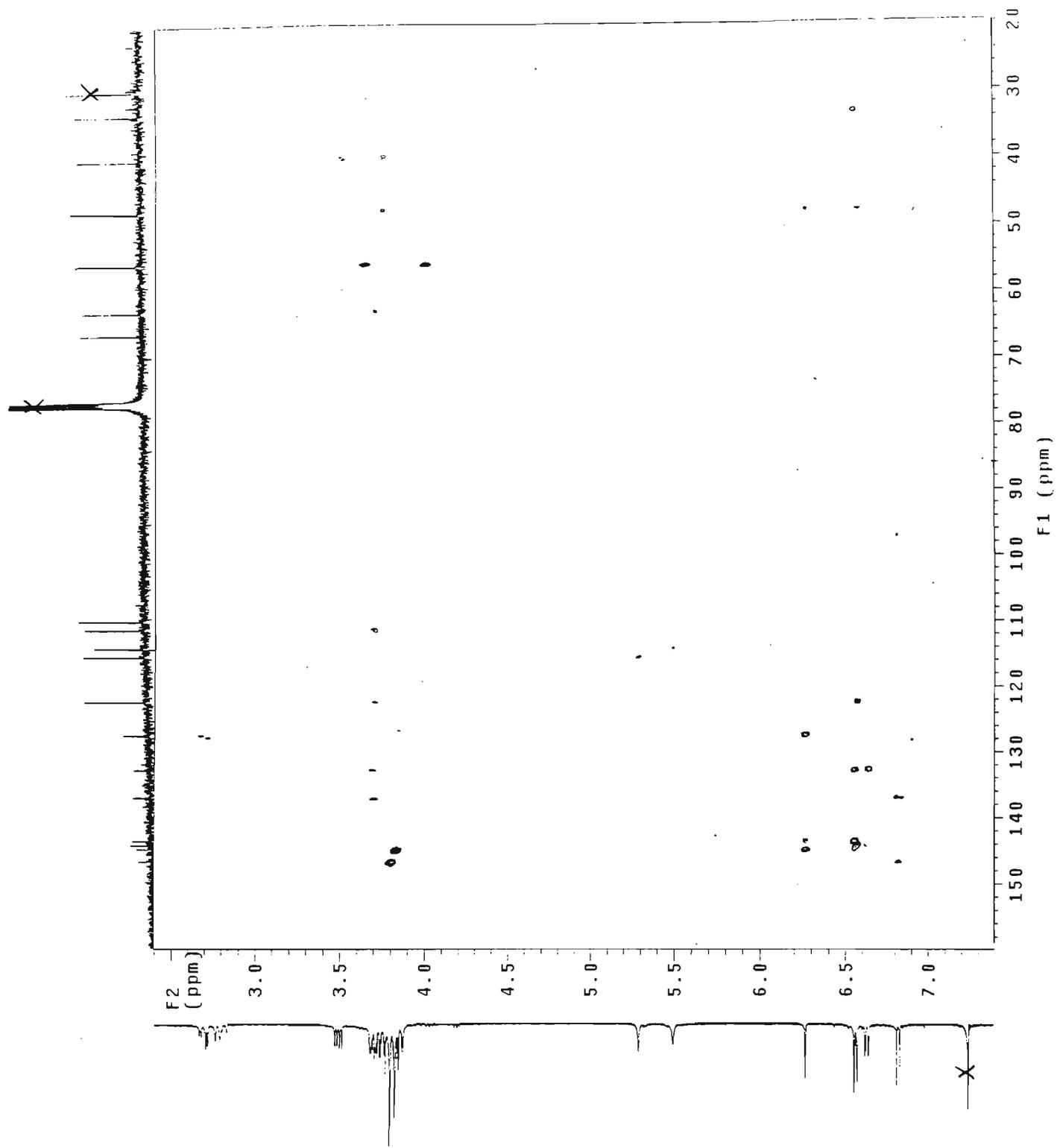
Spectrum 14.5: HMBC spectrum of compound 14 (CDCl₃)

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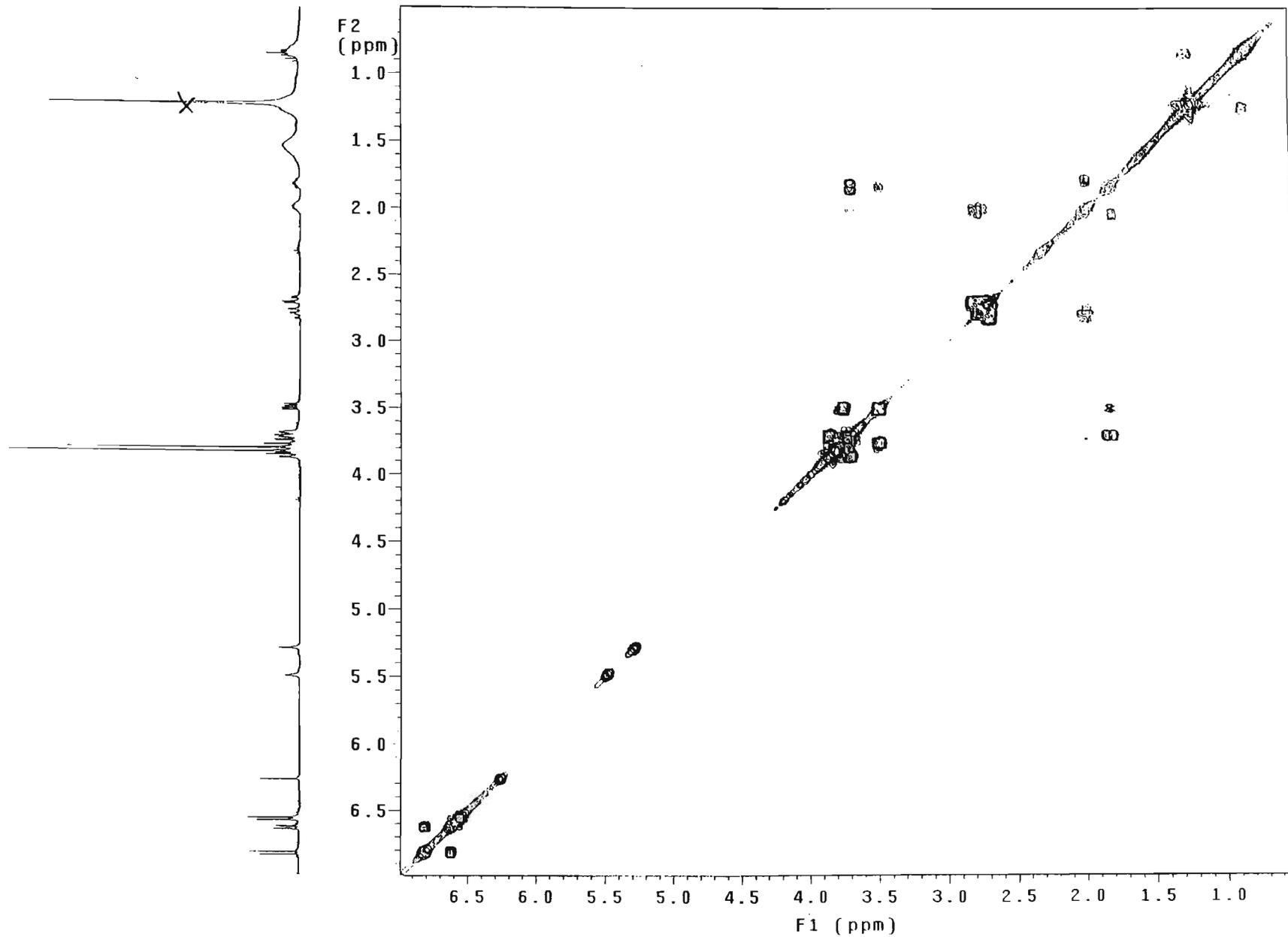
Spectrum 14.6: COSY spectrum of compound 14 (CDCl$_3$)
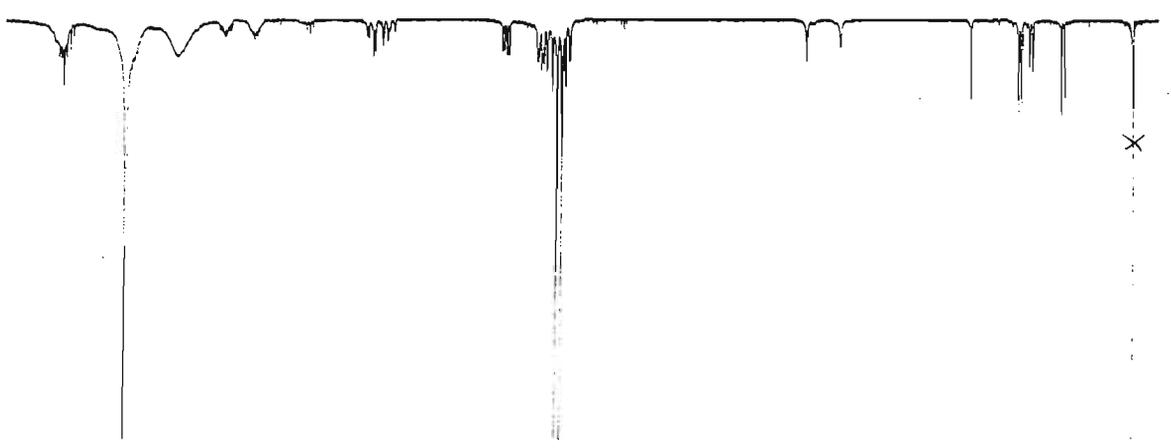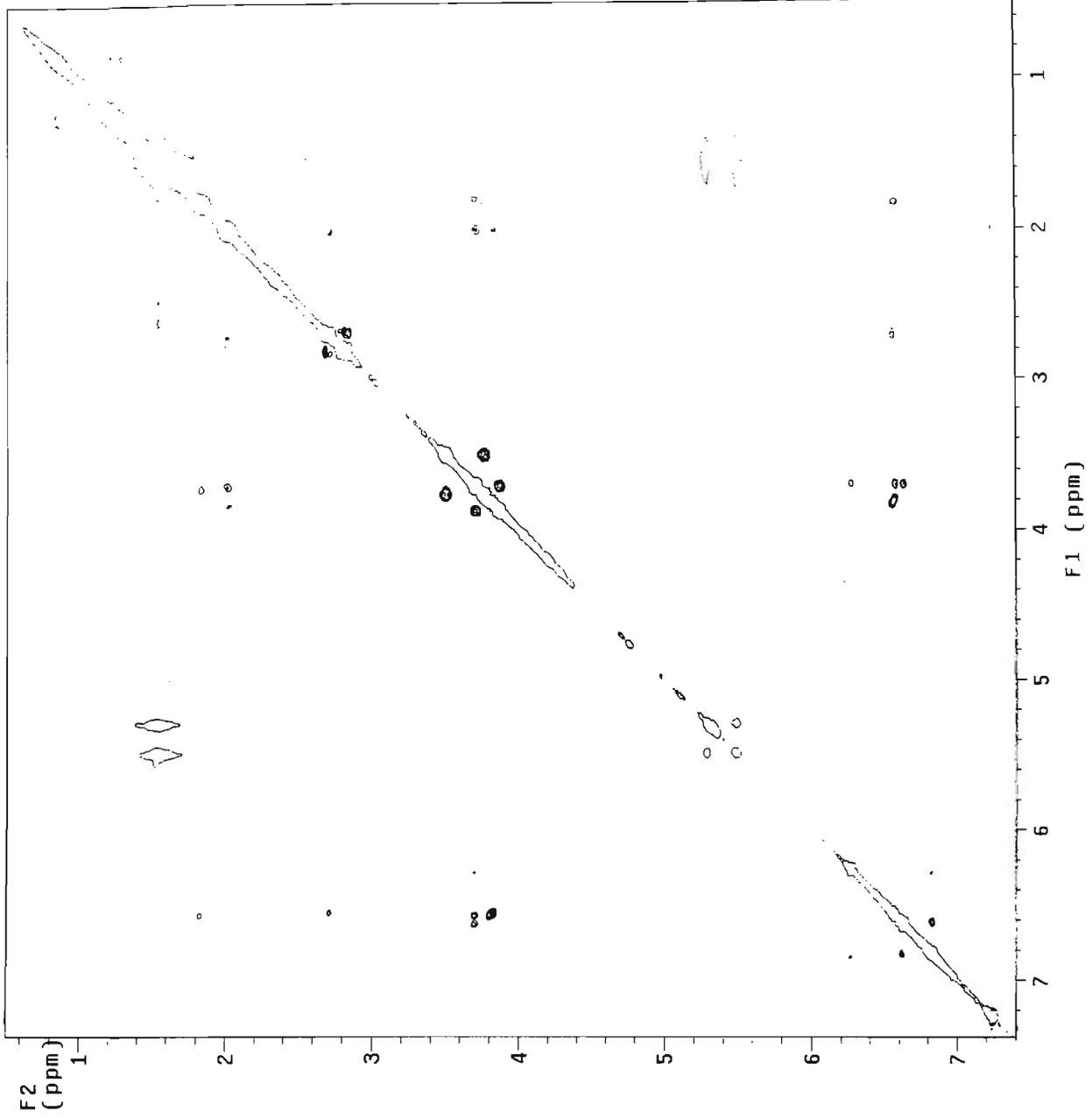
Spectrum 14.7: NOESY spectrum of compound 14 (CDCl₃)
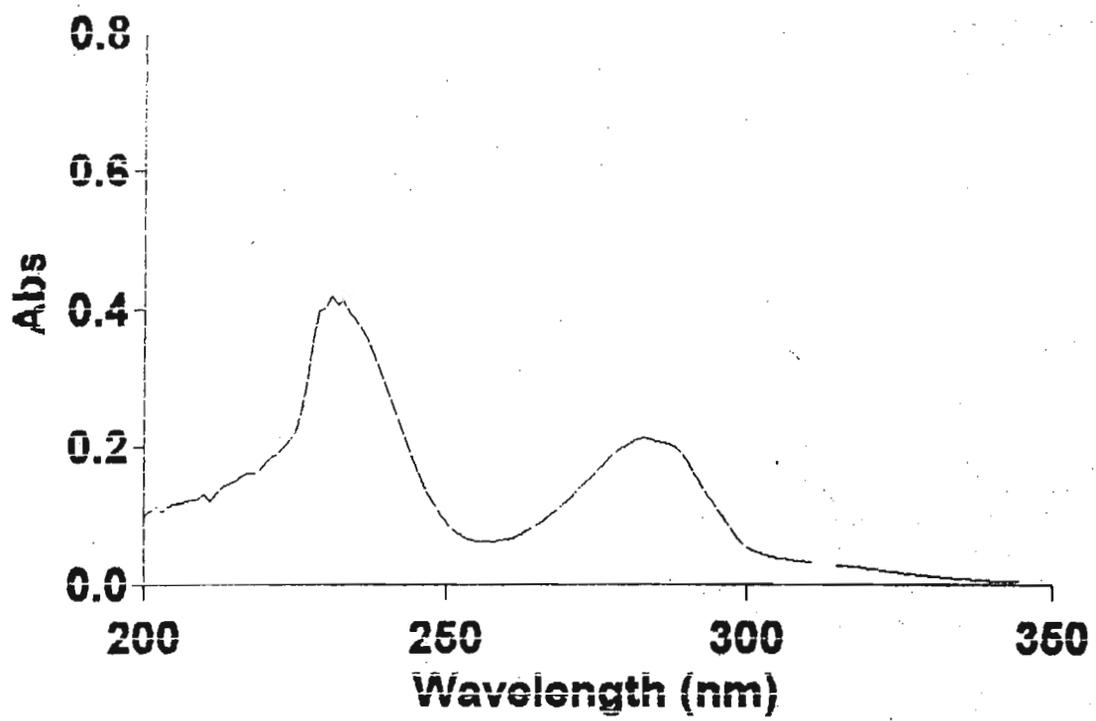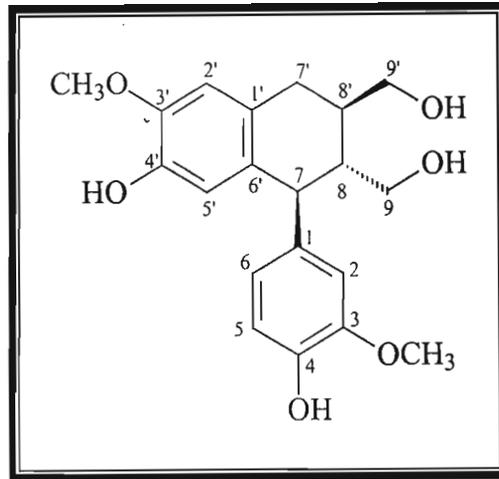
Spectrum 14.8: UV spectrum of compound 14
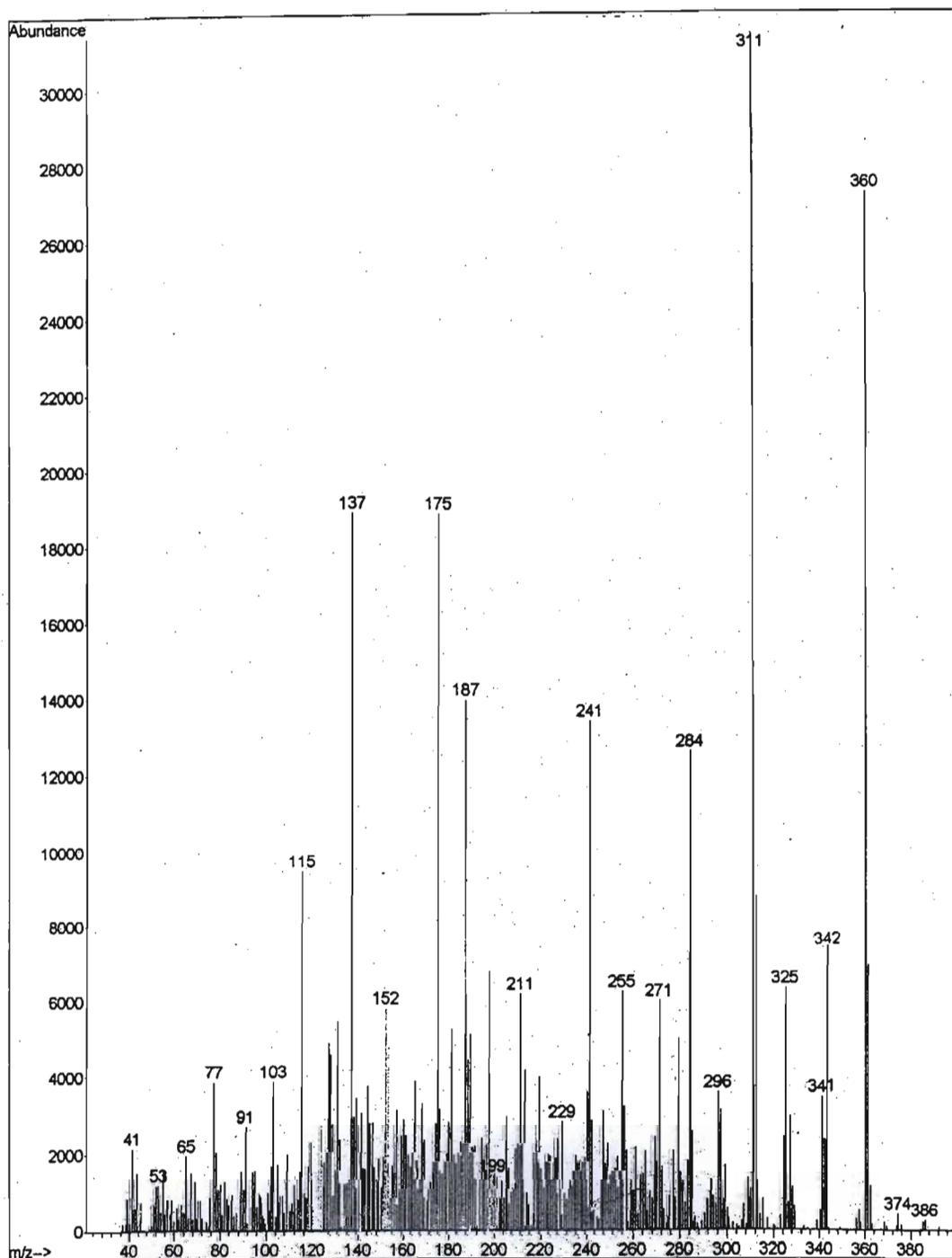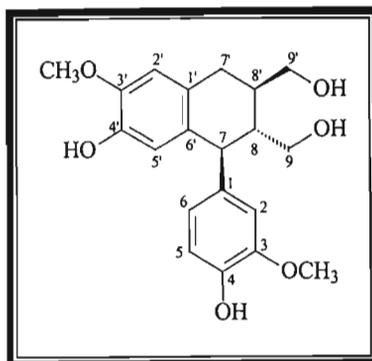
Spectrum 14.9: Mass spectrum of compound 14
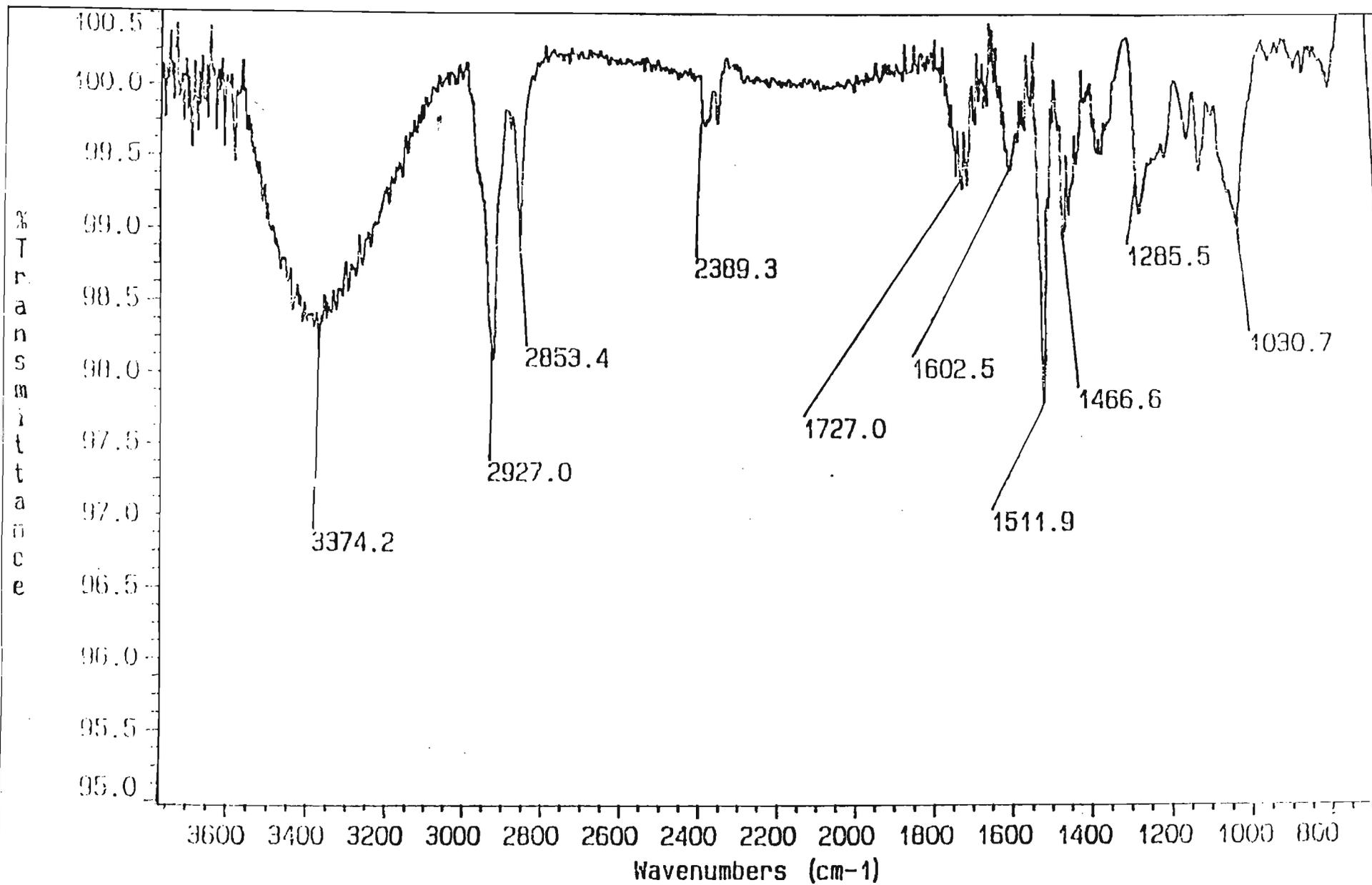
Spectrum 14.10: IR spectrum of compound 14