EXTRACTIVES FROM THE PTAEROXYLACEAE AND THE MESEMBRYANTHEMACEAE

EXTRACTIVES FROM

THE

PTAEROXYLACEAE

AND THE

MESEMBRYANTHEMACEAE

by

Neil Anthony Koorbanally

BSc, BSc (HONS), MSc (Natal)

Submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the

School of Pure and Applied Chemistry,

University of Natal,

Durban, South Africa

2001

In loving memory of

my father

Colin Anthony Paul Koorbanally

Your memory will live on
in the hearts
of those who loved you.

PREFACE

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of Natal, Durban, South Africa, under the supervision of Professor D. A. Mulholland.

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

Signed:

@Koorbanally

N.A. Koorbanally

BSc, BSc (HONS), MSc (Natal)

I hereby certify that the above statement is correct.

Signed: D A Mulholland.

Professor D.A. Mulholland

Ph.D. (Natal)

Professor of Organic Chemistry, University of Natal, Durban

Acknowledgements

I thank my superviser, Professor D.A. Mulholland for taking a keen interest in me and getting me started in Natural Products Chemistry in 1996. Her enthusiasm, hard work and dedication are evident in her work and those are qualities that I've tried to attain over the years. She has always been kind, patient and generous, not only to myself, but also to the rest of the students in her group. Professor Mulholland has always been there to offer me advice whenever I needed it and her endeavors in helping me succeed is something that I will always remember. Thank you Prof. for making my studies a pleasant and enjoyable experience as well as a very fruitful one. I will pass on to other students what I have learnt from you.

I thank my colleagues in the Natural Products Research Group, Philip Coombes for never being too busy to discuss any problem I may have encountered over the past three years, Tracy Pohl for working alongside me for the past five years and offering suggestions and thoughts whenever I need it, Peter Cheplogoi, Chantal Koorbanally, Kathryn McFarland, Angela Langlois, Brenda Moodley, Dashnie Naidoo, Niven Moodley, Leonard Gitu and Martin Dovey for creating a pleasant and enjoyable atmosphere in which to work and for always being willing to help whenever I needed it.

Thanks must also go out to Dr J.P Gerber and Dr H.A. Mahomed, whose advice on many an occasion has had a positive influence in my study career. I thank Dr Neil Crouch for all his assistance throughout my studies and for the collection of *Khadia alticola*. I must also thank Prof. K. H. Pegel, who was always willing to help me in whatever way he could.

I thank Mr Bret Parel for his excellent running and management of the laboratory and his willingness to assist me in whatever way he could. I also thank him for the running of the Mass Spectrometer. Mr Dilip Jagjivan has also played a vital role during the duration of my studies in running all my NMR samples in a professional and efficient manner. Dilip, thank you for all your hard work and tireless effort. I thank Mr Earnest Mchunu for keeping the laboratory neat and tidy and for always being eager to help me whenever I asked him to. I thank the rest of the technical staff,

Mr R. Suchipersadh, Mr K. Singh, Mr J. Couling, Mr G. Moodley and Mrs Anita Moodley for their eagerness to help and assist me whenever I approached them. Thanks must also go to Mrs J. Govender who helped with all the financial aspects and administrative tasks throughout my studies. I must also thank Dr P. Boshoff for the running of the high resolution mass spectra and Prof. Albert J.J. van den Berg from the Department of Medicinal Chemistry, Faculty of Pharmacy, University of Utrecht in the Netherlands for screening the prenylated chalcones, flavanones and coumarins in this work.

I would also like to thank all the staff, both academic and technical, in the School of Pure and Applied Chemistry who has assisted me in some way during the past three years.

A great deal of thanks must go out to my family, my father Colin, my two brothers, Vincent and Paul, my granny, Marie Louis Koorbanally and my uncle and aunt Jerry and Lynette, for all their support and encouragement. I have worked hard to make you proud of me. A thank you must also go out to the Moodley family, uncle Dennis, aunty Saras, Brenda and Chrissie who have made me part of their family and always kept me in their thoughts and prayers. A very special thank you to my wife Chantal for all her patience and understanding. You are my colleague, my partner and my best friend and I could not have done all this without you. Thank you for always being there for me and beside me.

To my father, Colin Anthony Paul Koorbanally, who never lived to see me complete my degree. I have inherited your looks, your intellect and your intelligence. Though you are with us no more, your memory will continue to live in our hearts and minds. Thank you for being my father in the best way you knew how. They say you can choose you friends, but you cannot choose your parents. If I had a choice, I still would have picked you.

Finally, I acknowledge the support of the University of Natal, Durban and the National Research Foundation for awarding me the scholarship to continue with my PhD. This award has been a tremendous help to me and allowed me the freedom to carry out research in a field I enjoyed.

LIST OF ABBREVIATIONS

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

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

COSY – correlated nuclear magnetic resonance spectroscopy.

HSQC – heteronuclear multiple quantum coherence.

HMBC – heteronuclear multiple bond coherence.

NOESY – nuclear Overhauser effect spectroscopy.

UV – ultra violet.

IR – infrared.

HRMS – high resolution mass spectrometry.

MS – mass spectrometry.

mp – melting point.

t.l.c. – thin layer chromatography.

s – singlet.

bs – broad singlet.

d – doublet.

dd – double doublet.

ddd – triplet of doublets.

t – triplet.

q – quartet.

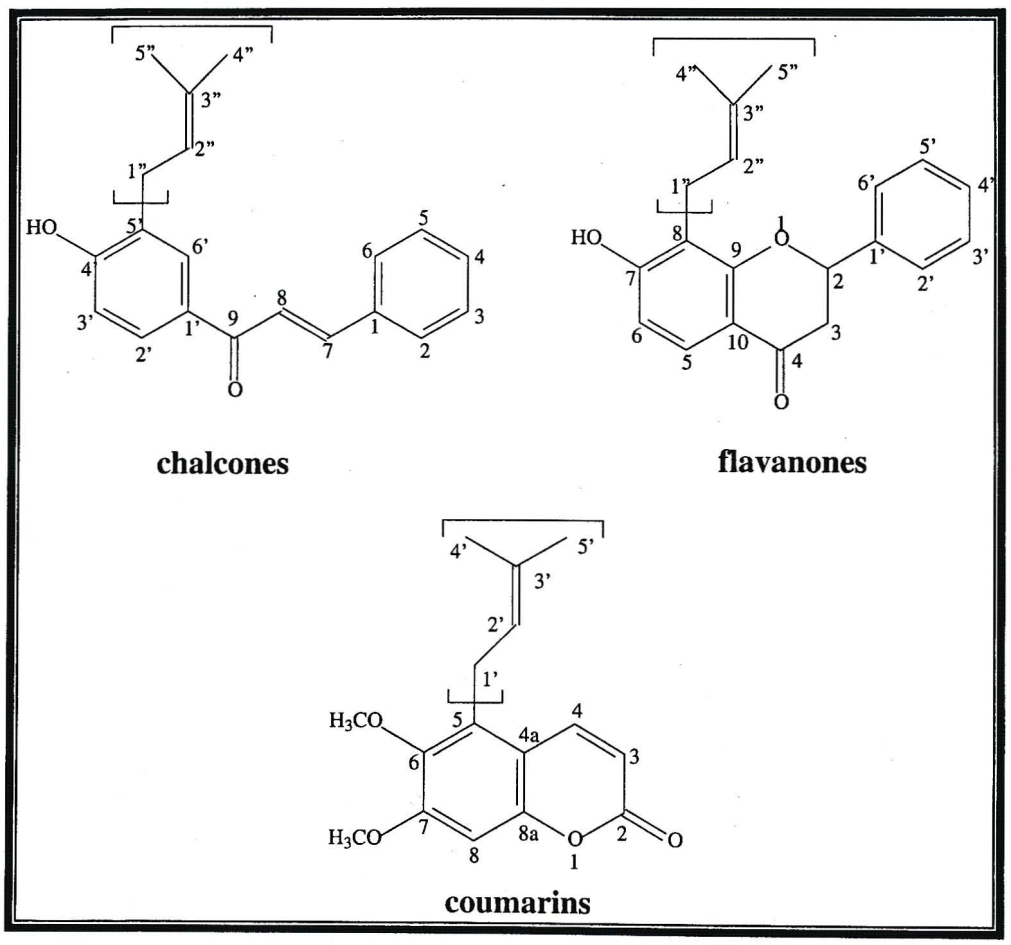
m – multiplet.

Hz – hertz.

c – concentration.

NOMENCLATURE

The numbering system used for the classes of compounds described in the text are depicted below:



LIST OF SCHEMES

LIST OF FIGURES

Chapter 4

Chapter 5

Chapter 6

LIST OF TABLES

Chapter 5

Chapter 6

ABSTRACT

This work is an account of the investigations into the chemistry of two *Cedrelopsis* species from the Ptaeroxylaceae, *Cedrelopsis grevei* and *Cedrelopsis microfoliata* and a species from the Mesembryanthemaceae, *Khadia alticola*, as well as investigations into the synthesis of hydroxylated and prenylated chalcones.

Cedrelopsis grevei, commonly called Katrafay, is amongst the many medicinal plants of Madagascar, being used to relieve muscle fatigue when the bark is soaked in hot water. Previous investigations of the wood and stem bark of this plant, have yielded chromones and coumarins and a recent investigation of the stem bark of a specimen collected in the north of Madagascar has yielded two novel limonoids of unusual structure, cedmilinol and cedmiline. The fruit and seed of *Cedrelopsis grevei* have not been studied previously and a phytochemical investigation of these plant parts was undertaken in this work. The dichloromethane extract of the fruit and seeds yielded, after column chromatography, a dihydrochalcone, uvangoletin, a flavanone, 5,7-dimethoxypinocembrin, two simple chalcones, cardamonin and flavokawin B and three prenylated chalcones, 2'-methoxyhelikrausichalcone, cedreprenone and cedrediprenone. Three of these compounds, 2'-methoxyhelikrausichalcone, cedreprenone and cedrediprenone have not been isolated previously.

Cedrelopsis microfoliata is another medicinal plant used in Madagascar. The leaves of this plant are used to prepare a decoction for woman to drink after childbirth. This is the first phytochemical investigation of *Cedrelopsis microfoliata.* The hexane extract of the dried stem bark yielded three compounds after column chromatography, a chalcone, microfolian and two flavanones, microfolione and (+)-agrandol. The dicholoromethane extract of this compound yielded four compounds after column chromatography, three coumarins, cedrecoumarin A, obliquin and microfolicoumarin and a sesquiterpenoid, sesquichamaenol. Four of the compounds isolated from *Cedrelopsis microfoliata*, microfolidione, microfolione, (+)-agrandol and microfolicoumarin have not been isolated previously.

Khadia alticola is one of the species added to "Khadi", a Tswana/South Sotho name for beer brewed traditionally using the fleshy roots of a variety of taxa. *Khadia* is also

reported to be used medicinally by the Manyika people of the Umtali district of Zimbabwe. The phytochemical investigation of the roots of *Khadia alticola*, which have not been studied previously, was undertaken to determine whether mesembrine type alkaloids were present in this species and thus contributing to the "potency" of the beer brewed traditionally. No mesembrine alkaloids were isolated in this work, however, a common sterol, sitosterol was isolated from the acidic chloroform fraction of the roots of this species and a flavonoid, 3,4',5,7-tetrahydroxyflavan was isolated from the basic chloroform fraction.

Two chalcones, 3',5'-dihydroxychalcone and 2'-hydroxychalcone were synthesised using the Claisen condensation. An isoprenylated acetophenone intermediate and an isoprenylated chalcone were also synthesised.

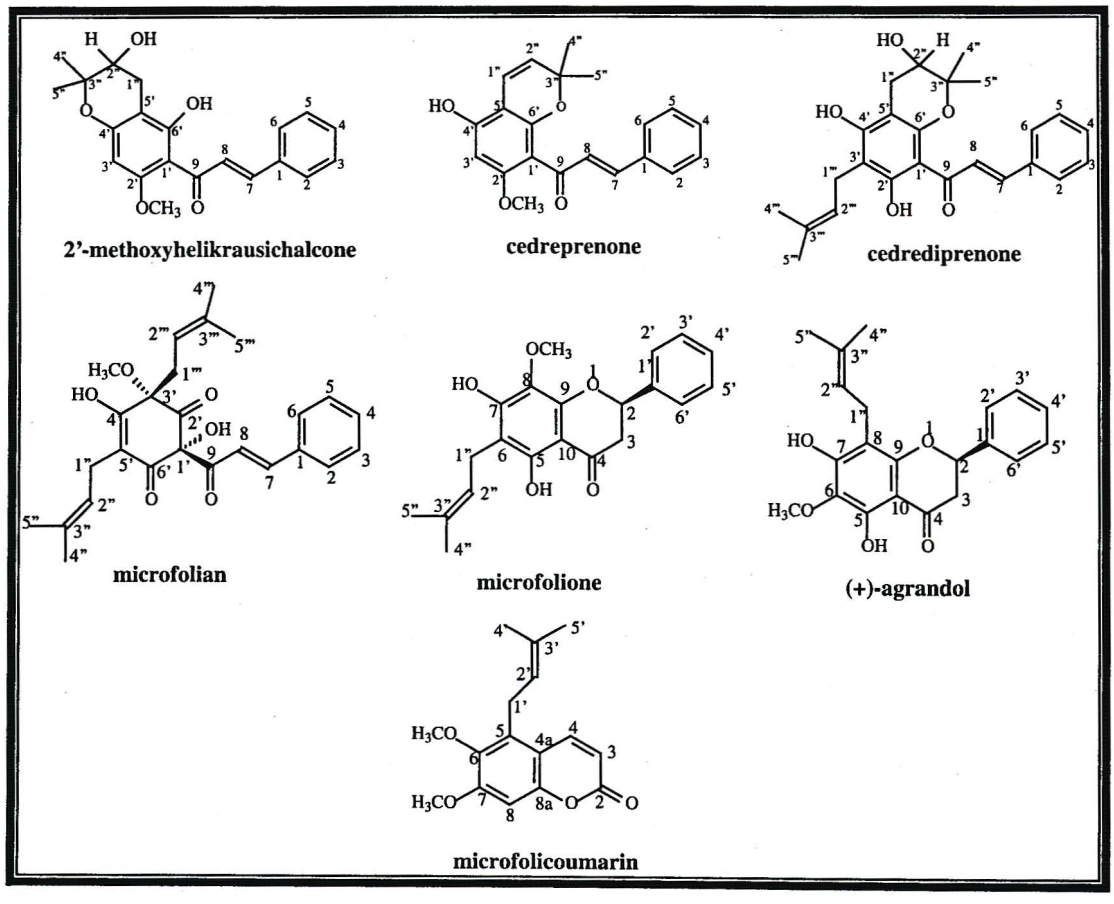


Figure 1. Novel compounds isolated in this work

FOREWORD

This thesis consists of six chapters. Chapters one and two are introductory chapters to the three classes of compounds most commonly featured in this thesis, i.e. chalcones, flavanones and coumarins.

Chapter three is an account of the extractives from *Cedrelopsis grevei*. This chapter contains mainly a discussion on the structural elucidation of both hydroxylated and three novel prenylated chalcones as well as a discussion of a flavanone.

Chapter four is an account of the extractives from *Cedrelopsis microfoliata*. This chapter contains a discussion of a novel unusual prenylated chalcone, a discussion of two flavanones, one of them being novel, a discussion of three coumarins, one of them being novel as well and a discussion of a sesquiterpenoid.

Chapter five is an account of the extractives from *Khadia alticola*. This chapter contains a discussion on the Mesembryanthemaceae and the class of compounds most commonly found in this family, the mesembrine alkaloids as well as a discussion of a flavan isolated from this plant.

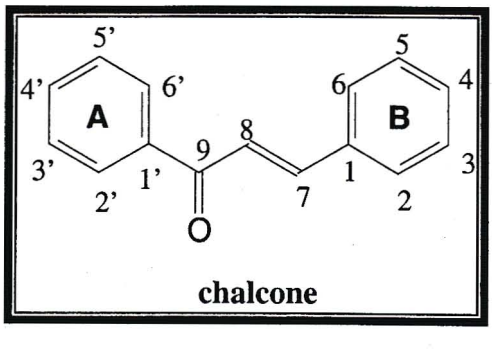
Chapter six is an account of the investigations into the synthesis of hydroxylated and isoprenylated chalcones. This chapter contains a discussion on the hydroxylated and isoprenylated chalcones synthesised in this work.

TABLE OF CONTENTS

Chapter 1. Chalcones and Flavanones

1.1 Introduction to chalcones

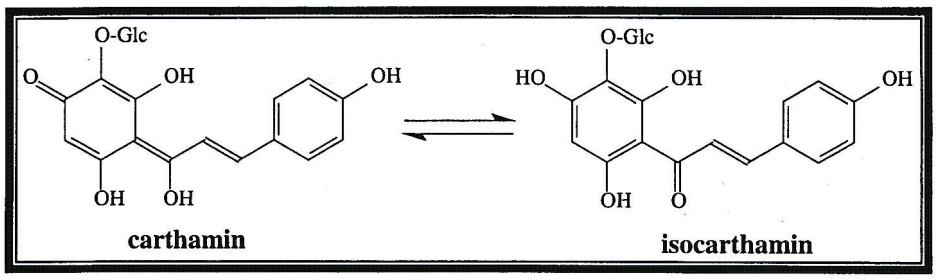
The term "chalcone" was first used by Kostanecki who did pioneering work in the synthesis of natural colouring compounds[1]. Chalcones are benzylideneacetophenones that constitute a class of naturally occurring pigments. The parent molecule of this class of compounds, chalcone (benzylideneacetophenone) consists of a C-15 skeleton, which comprises two aromatic rings joined by a three carbon α,β-unsaturated carbonyl system. The alternative names given to chalcone are phenyl styryl ketone, benzalacetophenone, β-phenylacrylophenone, γ-oxo-α,γ-diphenyl-α-propylene and α-phenyl-β-benzoylethylene[1]. The A ring in chalcones is situated adjacent to the carbonyl group and is numbered 1'-6', whereas the B ring is found adjacent to the double bond and is numbered 1-6. The numbering system in the chalcone nucleus is reversed from that of most flavonoids.



chalcone

Chalcone, the parent compound is not known as a natural product. Naturally occurring chalcones are all hydroxylated to a greater or lesser extent[1-4]. The A ring substitution pattern is usually based upon the phloroglucinol system (2',4',6'-trihydroxy), since this part of the molecule is acetate derived. The B ring originates from a phenylpropanoid precursor and thus most commonly exhibits a 4-mono-, 3,4-di-, or 3,4,5-trihydroxylation pattern[3]. Chalcones are important biosynthetically as they were the first isolatable C-15 precursors in flavonoid biosynthesis[3]. The chalcones play an ecological role in nature in relation to plant colour[3]. These brightly coloured compounds are found in many plant organs, but most conspicuously in flowers[3]. Carthamin, the red pigment of the flowers of safflower, a composite plant native to Southern Asia and cultivated widely throughout the world, was the first

example of a natural chalcone[2]. It was shown that when carthamin was treated with dilute hydrochloric acid, it changes into an isomeric yellow compound, isocarthamin[2].



At present over a hundred chalcones have been isolated from natural sources[1-4]. Some naturally occurring chalcones are given in figure 1.

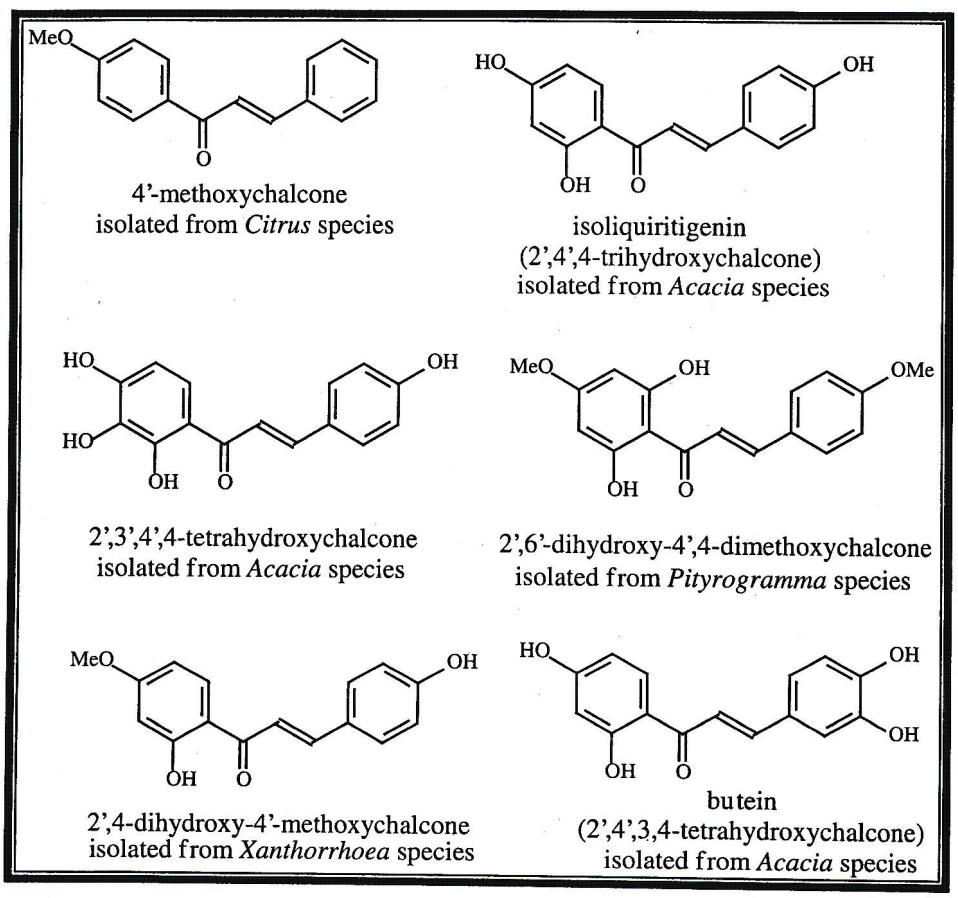


Fig. 1. Some naturally occurring chalcones[3]

Dihydrochalcones are chalcones in which the 7,8-position is saturated and thus lacks the conjugation of a double bond between the carbonyl group and the B ring. The numbering system is the same as for the chalcones. Dihydrochalcones have substitution patterns similar to those of chalcones. Structures of naturally occurring

dihydrochalcones have been reported by Bohm[4]. A few common dihydrochalcones are given in figure 2.

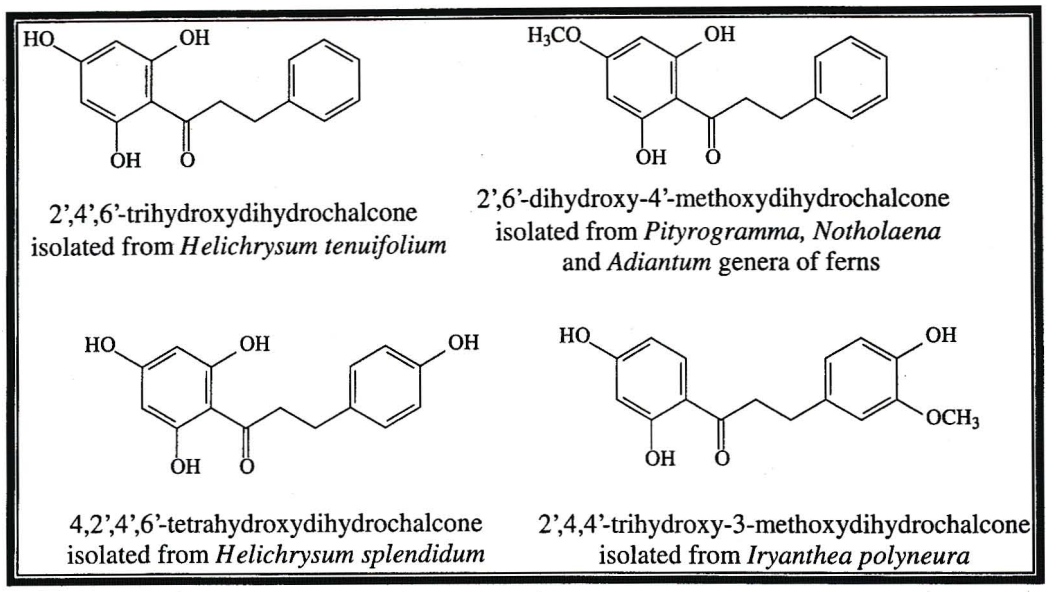
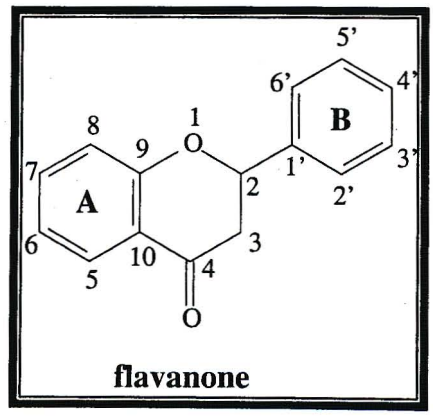


Fig. 2. Some naturally occurring dihydrochalcones[4]

1.2 Introduction to flavanones

Flavanones, like all other flavonoid compounds, possess a C-15 skeleton which can be regarded as being made up of two distinct units. There is a C_6-C_3 group, containing the B ring and a C_6 group, the A ring. Both these groups are of different biosynthetic origin and while each can be found represented in nature in many organisms, from bacteria to higher plants, their combination into the C-15 skeleton of flavonoid compounds is confined almost entirely to flowering plants and ferns[2]. Flavanones are based upon 2-phenylbenzopyran-4-one, which is flavanone itself. The numbering system of the flavonoid nucleus is numbered 1-10 for the benzopyranone system and 1'-6' for the B ring.



Flavanone is a colourless substance and has not yet been found in nature. Hydroxylated flavanones, however, occur in the free form or as glycosides in flowers, fruits, leaves, bark and roots and appear to be of fairly general distribution, especially in the higher plants[2]. The simplest naturally occurring plant flavanone has a hydroxyl group at position 7[5]. Flavanones have a center of asymmetry at C-2 and naturally occurring flavanones are often optically active[5]. Some naturally occurring flavanones are given in figure 3.

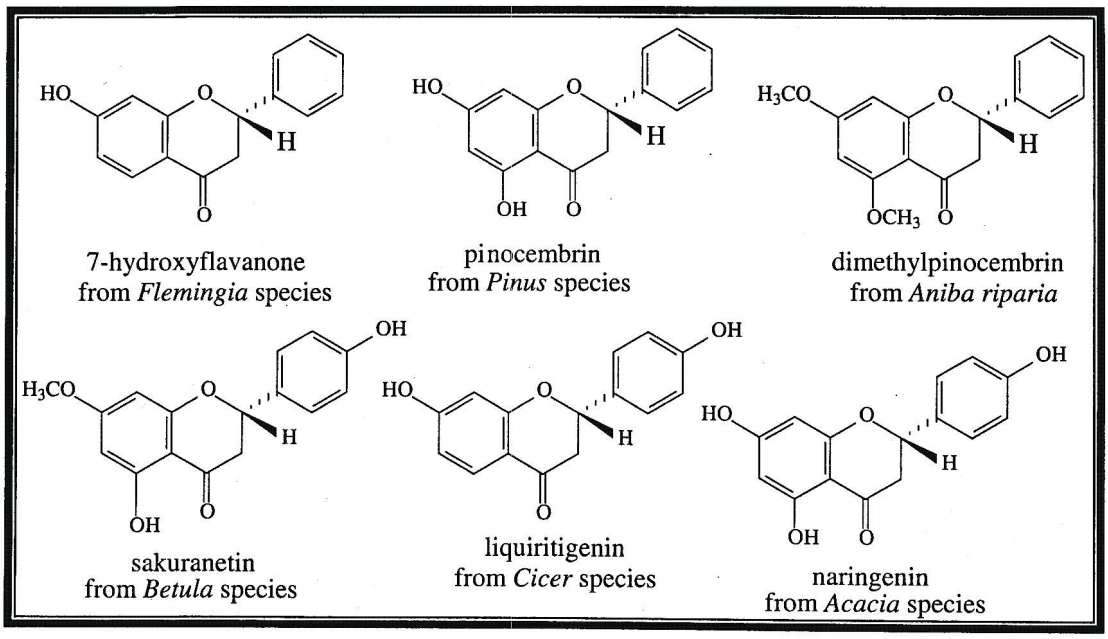


Fig. 3 Some naturally occurring flavanones[4]

1.3 Interconversion of flavanones and chalcones

The dihydropyrone ring of the flavanones is far more unstable than the pyrone ring of flavones or flavonols and is known to open between positions 1- and 2-, giving rise to a chalcone compound[2]. Chalcones and flavanones are interconvertible by acid or alkali-catalysed ring-chain tautomerism[2]. The ring opening of a flavanone to a chalcone occurs when the flavanone is treated with acetic anhydride[2]. Flavanones in an alkaline medium are readily converted to chalcones by ring fission[2]. An equilibrium may exist in the interchange and this equilibrium is shifted to the chalcone in alkaline medium and to the flavanone in acid medium[2].

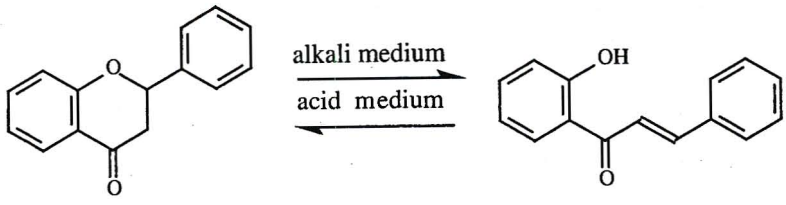


Fig. 4 Interconversion of flavanones and chalcones

It was found that naringenin (4',5,7-trihydroxyflavanone), isosakuranetin (5,7-dihydroxy-4'-methoxyflavanone) and naringenin 4',7-dimethyl ether, dissolve readily in cold (10%) sodium hydroxide and are precipitated unchanged by acidification, whereas 5-methoxy- and 5,7-dimethoxyflavanone dissolve in this reagent only on warming and give the corresponding 2'-hydroxychalcones on acidification[2]. Thus, when the 5-hydroxyl group is present in the flavanone, the chalcone-flavanone equilibrium is strongly on the side of the flavanone because of the resulting hydrogen bond stabilisation of the ring[2].

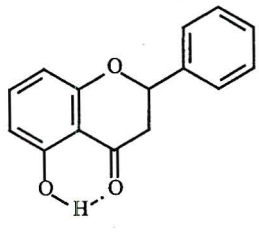


Fig. 5 Hydrogen bonding stabilisation of the ring in flavanones

Thus, interconversion of flavanones and chalcones is important when choosing a medium to synthesise either the chalcones or the flavanones.

1.4 Biosynthesis of flavanones and chalcones

Flavonoids originate from acetate units and from a phenylpropanoid intermediate derived from the shikimic acid pathway[6]. This was deduced from feeding experiments with radioactively labelled compounds[6]. Ring A is formed by head to tail condensation of three acetate units while ring B as well as carbon atoms 2,3 and 4 arise from a phenylpropanoid precursor. CoA esters of malonic and cinnamic acids are the substrates of an enzyme-mediated condensation reaction[6].

Phenylalanine and/or tyrosine are converted to activated cinnamic acids. The enzymes responsible for this conversion are phenylalanine ammonia-lyase (PAL),

cinnamic acid 4-hydroxylase (CAH), *p*-coumarate:CoA ligase, and possibly phenolases and methyltransferases[6]. Phenylalanine ammonia-lyase catalyses the formation of *trans*-cinnamic acid from L-phenylalanine or its hydroxy derivatives. Cinnamic acid 4-hydroxylase is the enzyme that converts cinnamic acid to 4-hydroxycinnamic acid[6]. P-coumarate:CoA ligase converts cinnamic acids to their CoA thiol esters thereby activating them[6]. Phenolases are the enzymes responsible for hydroxylation of the aromatic ring[6]. Formation of the phenylpropanoid precursor is shown in figure 6.

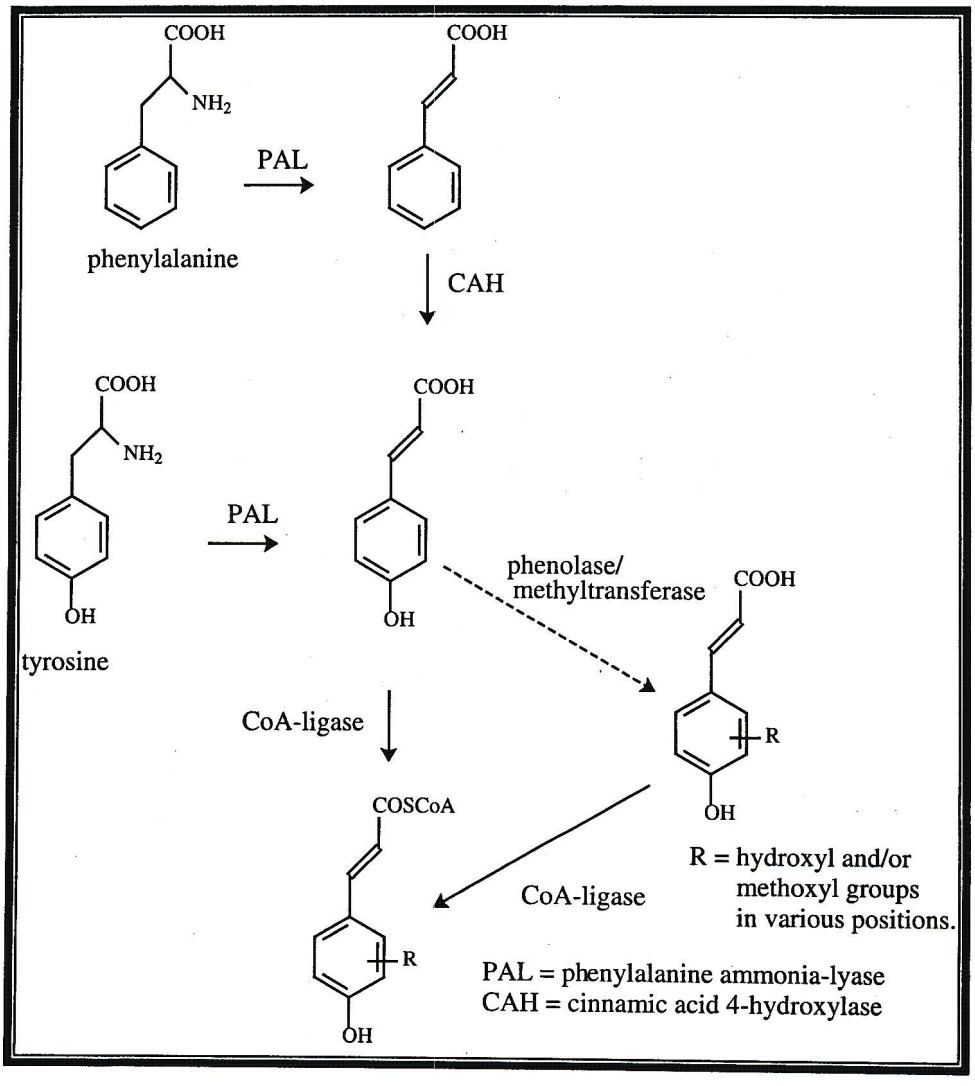


Fig. 6 Formation of the phenylpropanoid precursor in flavanone\chalcone biosynthesis[6]

There is no evidence as to whether cinnamic acids or flavonoids (or both) are the natural substrates of the phenolase enzyme. It is also not certain whether O-methyl groups are introduced into flavonoids at the phenylpropanoid stage or at a later stage

in flavonoid biosynthesis or both. Hydroxycinnamic acid 3-*O*-methyltransferases are responsible for specific methylation in the 3-*O*-position of various hydroxycinnamic acids[6]. Methyltransferase catalyses the transfer of the methyl group from S-adenosyl-L-methionine to hydroxyflavonoids[6].

Flavanones and chalcones are formed by the enzyme-mediated condensation of an activated cinnamic acid with three molecules of malonyl CoA. The enzyme involved in this condensation reaction is chalcone/flavanone synthetase[6]. Chalcone/flavanone isomerase catalyses the formation of the 6-membered heterocyclic ring of flavanones from their corresponding chalcones[6]. The formation of 2',4',6',4-tetrahydroxychalcone and 5,7,4'-trihydroxyflavanone from malonyl CoA and activated cinnamic acid is shown in figure 7.

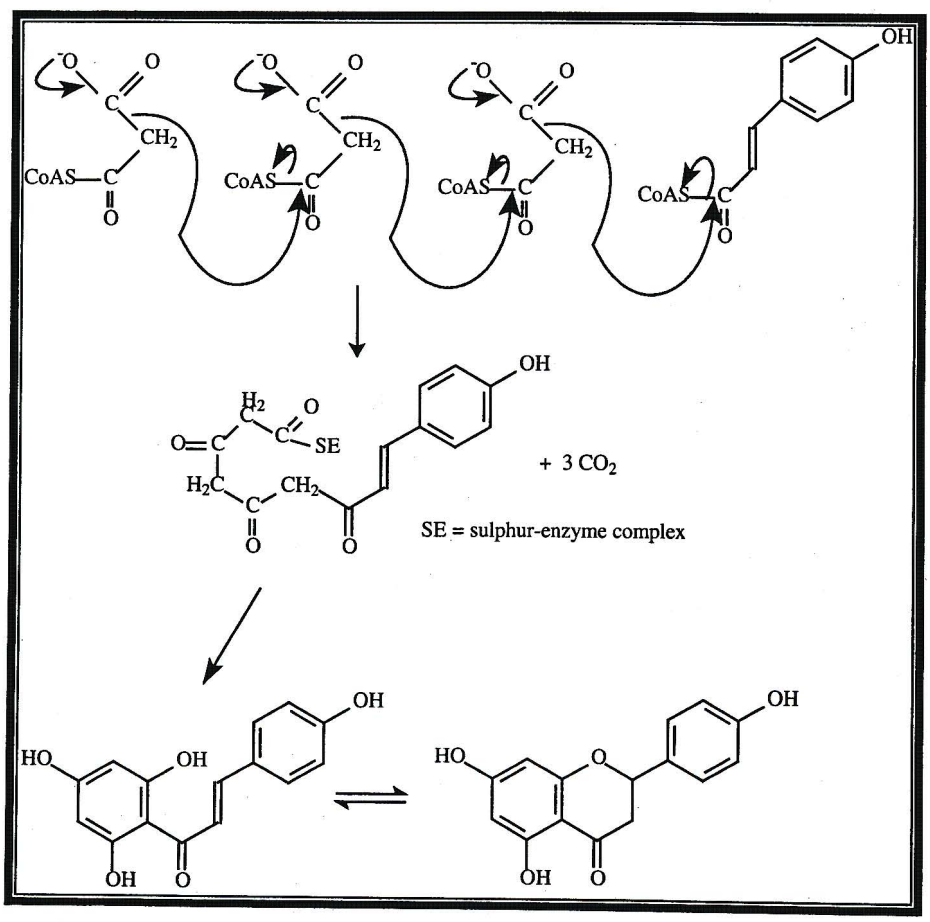


Fig 7. Condensation of an activated cinnamic acid with three molecules of malonyl CoA[6].

Chalcones and flavanones are the precursors of all other types of flavonoids.

1.5 Biological activity of chalcones

The chalcones have a broad range of biological activities from bactericidal, antimicrobial and antiobiotic activity to antispasmodic, anti-inflammatory and analgesic activity[1]. The biological activities of the chalcones have been summarised in the review by Dhar[1].

The enone function in the chalcone molecule is responsible for antibiotic activity[7]. The parent molecule, chalcone, shows significant bacteriostatic action against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus mycoides*, *Bacillus subtilis* and *Sarcina lutea*[7]. Chalcone was tested against these strains of bacteria as it contained a –CH=HC-C=O group, common to both clavacin and penicillic acid, known antibiotic compounds[7]. Various unsaturated ketones, including chalcones have also shown bacteriostatic activity against *Brucella abortus*[8]. In *S. aureus*, the bacteriostatic effect could not be reversed by cystein[1]. Compounds containing an α,β–unsaturated carbonyl group like that of chalcone also exhibited antibacterial action against *S. aureus*, *E. coli*, *Trichophyton mentagrophytes*, *Candida albicans*, *Willia anomala*, *Torula utilis*, *Aspergillus usami*, *Penicillium chrysogenum* Q 176 and *Saccharomyces sake*[9]. The prenylated chalcone, 4-hydroxyderricin exhibited marked *in vitro* inhibitory activity against Gram positive microorganisms but was inactive against Gram negative mycobacteria and fungi[10]. From a study of the antibacterial activity of ninety-seven nitrohydroxychalcone derivatives on *Staphylococcus albus* and *S. aureus*, it was shown that optimal bacteriostatic effect was observed with a structure containing the hydroxyl group in the 4' position[11]. Derivatives with a hydroxyl group at the 2' or 3' position were shown to be less active. The activity was enhanced by the introduction of halogen substituents at positions 2 and 4[11]. Fluoro substituents were shown to have better antibacterial activity when compared to bromo- or chlorochalcones. The nitro group had a lesser though favourable effect on activity[11]. Nitrohydroxychalcones have shown antibacterial activity *in vitro* against *S. albus*[12]. Of these compounds, 3'-nitro-4'-hydroxy-2,3-dimethoxychalcone was found to have the highest antibacterial activity followed by 3'-nitro-4'-hydroxy-2,3-dimethoxychalcone and 3'-nitro-4'-hydroxy-2,5-dimethoxychalcone[12]. The 4- and 4'-aminochalcones showed bacteriostatic activity against *S. aureus* and *Streptococcus hemolyticus*[13]. Chlorohydroxychalcone[14] and bromohydroxychalcones[1] are

considered antibacterial compounds while iodohydroxychalcones[15] are considered as possible antibacterial agents. Eighteen alkylthiochalcones showed an intense antibacterial activity against *B. subtilis* NRRL. The 4-choro-5'-methyl-2'-thioalkylchalcone also showed antibacterial activity against *E. coli* O-55, while 4'-thioalkylchalcone showed antibacterial action against *S. aureus* only[16]. Sulfonic acid and carboxylic acid derivatives of chalcones were also shown to have bactericidal activity[1]. Chalcone sulfanilamides are also potential antibacterial agents[17]. Hydroxychalcone penicillanates prepared by the esterification of the appropriate penicillin derivative with hydroxychalcones were useful as bactericides[18]. Furan analogues of chalcone (chalcones in which one of the benzene rings have been replaced by a furan ring) have shown significant bacteriostatic action against *E. coli*, *S aureus*, *B. mycoides*, *B. subtilis* and *S. lutea*[7]. The hydroxycarboxychalcones, 4'-hydroxy-5'-carboxychalcone and 4-chloro-4'-hydroxy-5'-carboxychalcone and the dihydrochalcone, 4-methoxy-4'-hydroxy-5'-carboxydihydrochalcone showed bacteriostatic activity against *B. subtilis*, *S. hemolyticus* and other bacteria *in vitro*[20].

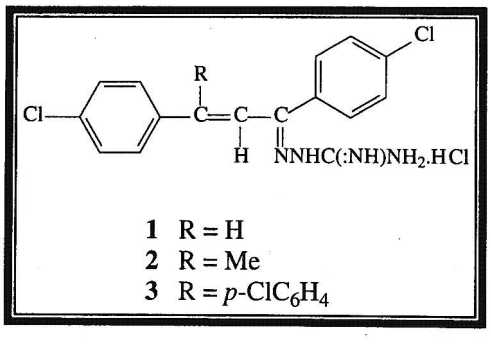
Antimicrobial activity was shown by α-substituted chalcones, hydroxycarboxychalcones and their dihydro derivatives, chalcone derivatives and α-bromochalcones[19,20,21,22]. Chalcones generally showed antimicrobial activity against *Trichophyton* and the activity was increased by α- bromination of these compounds[19]. The methylene dithiodiacetic acid derivative of chalcone produced by the reaction of chalcone with monothiol acetic acid showed marked growth inhibition for *Trichomonas vaginalis*[21]. No antibacterial action was observed by the introduction of nitro or chloro groups, but the presence of phenolic hydroxyl groups were necessary for growth inhibition of *Shigella dysenteriae*[21].

Antibiotic activity was shown by the parent compound, chalcone[1]. This antibiotic activity was associated with the –CH=HC-C=O group of the chalcone molecule which was enhanced when nitro and bromo substitutions were made at the α postion and when bromo and hydroxy substitutions were made at the β position[1]. The antibiotic activity was also hampered when cystein or serum was added to the chalcones owing to their reduction by the SH group[1]. The salicylic chalcone 3'-carboxy-4'-

hydroxychalcone was reported to have antibiotic properties[23] and a furan analogue of chalcone exhibited an antibiotic action against *E. coli* [24].

Tuberculostatic and antitubercular activity was shown by a furan analogue of chalcone in rats[25]. Chalcone-2-hydroxy-4-acetamidobenzene sulfonyl hydrazone was shown to be a potential antitubercular compound[26]. Semicarbazone and thiosemicarbazones of chalcone or its analogue were highly effective antitubercular compounds when tested against tubercle bacilli[27]. By treating chalcones with hydrazines, chalcone hydrazones were prepared which had tuberculostatic activity[28].

Thiophene analogues of chalcone were shown to be possible antiparasitic compounds[1]. Chalcone derivatives **1**, **2** and **3** were shown to have antimalarial activity[29].

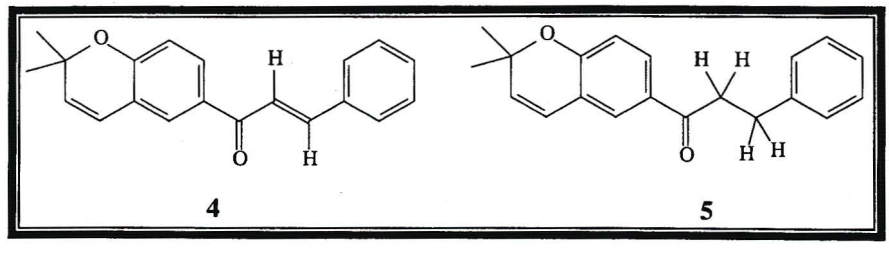


1 R = H
2 R = Me
3 R = *p*-ClC$_6$H$_4$

Many chalcones and some furan analogues of chalcones were shown to exhibit acaricidal activity[30]. Thienylpyrazolines derived from appropriate chalcones are described as having potential schistosomicidal activity[31]. Antihelmintic activity was shown by 2',4'-dihydroxychalcone against *Ascaris*[32] and 2-hydroxychalcone and 2,2'-dihydroxychalcone has shown this antihelmintic activity against amoeba[33]. Chalcone, dihydrochalcone and the chalcone derivative, 3,5-diphenylisoxazoline were shown to be active against pinworms[33].

It has also been claimed that some chalcone derivatives have insect repellant properties[34]. The *N*-substituted *ortho*-carbamoyloxime of chalcone has been reported to have weak herbicidal and insecticidal activity[35]. When chalcone α,β-dichloride combined with DDT was tested on DDT-resistant female flies, the mortality rate was shown to be 92%[36]. A furan analogue of chalcone was shown to be markedly toxic to rats[25] and hydroxy and methoxy chalcones demostrated toxicity against freshwater fish[1]. Pyrazolium salts of chalcone were shown to be very effective herbicides[37].

The substituted chalcone, 2'-hydroxychalcone sulfonic acid was shown to have weak antifungal activity when tested on mold fungi[38]. Carboxylic and sulfonic acid derivatives of chalcone have shown fungicidal activity[1]. The prenylated chalcone (**4**) and dihydrochalcone (**5**) has shown antifungal activity against *Helminthosporium oryzae*[1] and 2',4'-dihydroxydihydrochalcone showed antifungal activity against *Alternaria solani* and *Curvularia lunate in vitro*[1]. Chalcone[7,9] and heterocyclic analogues of chalcone, furan and 8-hydroxyquinoline type compounds[1,7] were also shown to exhibit antifungal activity. Fungistatic activity was also shown by α-chalcone and the α-2-furan analogue of chalcone when tested against *Fusarium graminearum*, *Penicillium digitatum* and *Botrytis allii*[39]. Antifungal activity was exhibited by 2-hydroxy-2'-carboxylic chalcone when tested against cucumber powdery mildew[40].

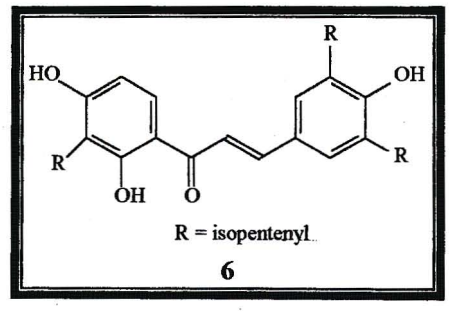


4 **5**

Chalcones and dihydrochalcones are also known to act on enzymes. In rats, 3,3',4,4'-tetrahydroxychalcone is an effective inhibitor of liver xanthine oxidase[41] and is also useful in the treatment of gouty arthritis[42]. The carboxychalcones, 3,4'-dihydroxy-3'-carboxychalcone and 3,4,4'-trihydroxy-3'-carboxychalcone were shown to inhibit 5-hydroxytryptophan decarboxylase[43]. When tested on pig kidney, naringenin chalcone, hesperidin chalcone, coreopsin, phlorizin and asebotin were found to be inhibitors of sodium-potassium-dependant ATPase, the extent of inhibition depending upon the number and position of hydroxy groups in their skeletons[44]. The free hydroxy group at the 4' position of phlorizin was found to play an important role in the inhibitory power[44]. Some salicyclic chalcones inhibited the aromatic L-amino acid carboxylases in guinea pig kidney[45]. Chalcone and the furan analogue of chalcone were shown to inhibit cholinesterase in horse serum[46]. The furan analogue of chalcone was found to have a marked ability to inhibit the activity of the enzyme dihydroxyphenylalanine decarboxylase[47]. In wheat root, phlorizin and some related dihydrochalcone glycosides, as well as the structurally similar compounds, naringenin and 2',4,4'-

trihydroxychalcone are potent stimulators of indole acetic acid oxidase[48]. The aglycones, phloretin, naringenin and 2',4,4'-trihydroxychalcone have been shown to stimulate wheat root growth, inhibit the absorption of sugars and of 2,4-dichlorophenoxyacetic acid and inhibit oxidative phosphorylation[48]. Chalcones related to resorcinol, have inhibitory activity on L-dopa decarboxylase[1].

The isopentenyl chalcone (**6**) and similar isopentenyl chalcones have been shown to be effective in the treatment of peptic ulcers in rats[49,50]. Prenylated chalcones with peptic ulcer inhibiting properties have also been reported[51,52,53]. Chalcone ethers were also reported to be antiulcer agents[54]. Chalcone derivatives have proved useful as inflammation inhibitors, in the treatment of allergies, in the treatment of gastrointestinal and peptic ulcers in rats and in the treatment of stomach and duodenal ulcers[55-62]. Prenyloxychalcones were found to be effective in the treatment of rat stomach ulcers[63] and prenylated chalcones exhibit antipeptic ulcer activity in mice[64].



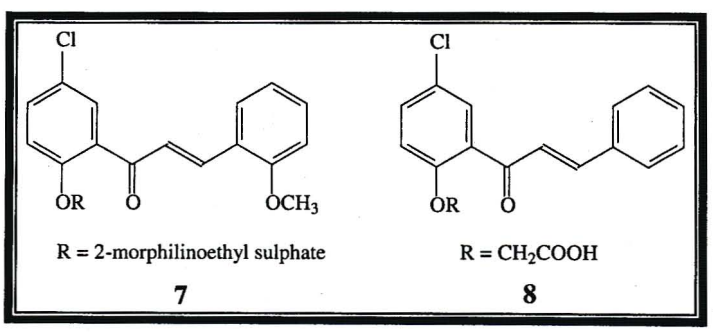
6

Hypotensive or antihypertensive activity as well as hypocholeretic and anticoagulating activity is associated with 2',4',6'-trihydroxychalcone[65]. Other chalcones with antihypertensive properties are ω-aminoalkoxychalcones and acid addition salts[66,67], N,N-disubstituted-2-(ω-aminoalkoxy)-3',4',5'-trimethoxychalcone[68], and reduced benzofuran chalcone derivatives[69,70]. Indole analogues of chalcone are reported to be weakly hypotensive[71] and 2-[3-(4-methyl-1-piperazinyl)propoxy]chalcone hydrochlorides and related compounds[72,73] and 4-aminohalogenochalcone (with a 2'-hydroxy substituent)[74] are reported to have hypotensive activity.

In a study on the antineoplastic effect of chalcones on Ehrlich's ascitic sarcoma, it was found that the most active compound was 2',4,4',6'-tetrahydroxychalcone, followed by

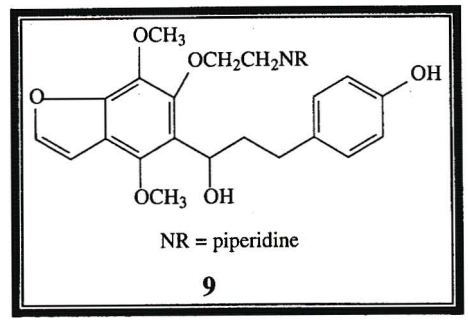
2,4,4'- and 2,2',4-trihydroxychalcone[75]. Nitrochalcones, having nitro groups in the 2- (and 2'-) and 4- (and 4') positions have shown cytotoxic activity when tested against normal and Rous virus-transformed hamster fibroblasts[76]. In HeLa cells, 4-nitro-3'-isothiocyanatochalcone is the most active compound in respect of cytotoxicity and cancerostatic effects[77]. Other active compounds were found to be 4-chloro-4'-isothiocyanatochalcone and 4-bromo-3'-isothiocyanatochalcone[77]. Uvaretin, a dihydrochalcone from *Uvaria acuminata* showed inhibitory activity against the P-388 lymphocytic leukemia test system[78]. The flavonol derived from 2'-hydroxy-2,4',5',6,6'-pentamethoxychalcone was shown to be a potential antitumour compound[79].

The chalcones, 4,4'-dihydroxy-2'-methoxychalcone and 2',4,4'-trimethoxychalcone (vesidryl) was shown to exhibit choleretic activity in rats[80]. The nitrochalcones, 3'-nitro-4'-hydroxy-2-methoxychalcone, 3'-nitro-4'-hydroxy-2,3-dimethoxychalcone and 3'-nitro-4'-hydroxy-2,5-dimethoxychalcone were shown to possess choleretic effects *in vivo* when tested in rats[12]. The pyridine analogue of chalcone also possessed choleretic properties[81]. Hypocholeretic action was demonstrated by 2',4',6'-trihydroxychalcone and the chlorochalcones, compound **7** and compound **8** [65,82].



7 **8**

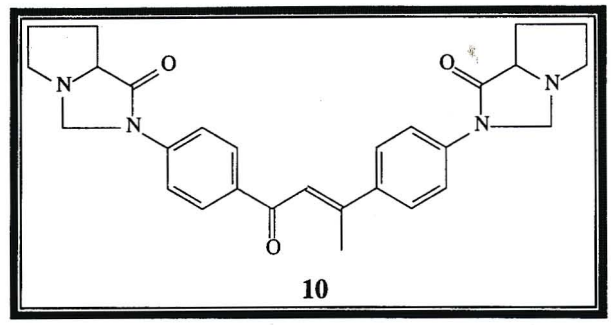
Chalcone, 2 (and 2'-)-hydroxychalcones, 2,4-dihydroxychalcone, 2,2'-dihydroxychalcone, 2,4,4'-trihydroxychalcone and 2,2',4-trihydroxychalcone all exhibited spasmolytic action when tested in the isolated bowels and stomach of rats[83]. Salts of *N*-substituted 4'-aminoalkoxy-2',4-dihydroxychalcones had relatively high muscular spasmolytic and low neurospasmolytic properties[84]. Reduced benzofuran-chalcone derivatives also showed spasmolytic activity with compound **9** having the highest activity[69]. The bis(phenylalkyl)amines, the catalytic reduction products of chalcone oxime were shown to be potential spasmolytic compounds[85]. The chalcone, licurzid, had a spasmolytic action on isolated sections of rat and guinea pig intestines[86].



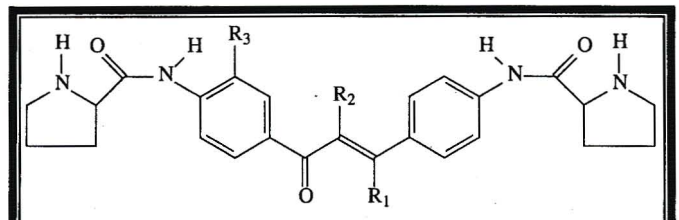
NR = piperidine

9

Compound **10**[87] and compounds **11, 12,** and **13**[88,89] were shown to have antispasmodic and tranquilizing action.

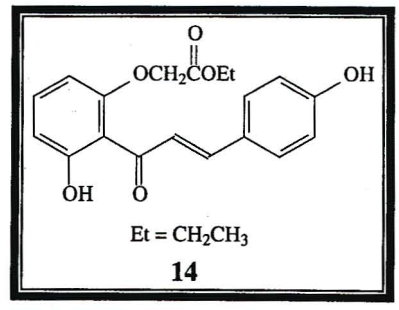


10



11 $R_1 = R_2 = H, R_3 = CH_3$
12 $R_1 = H, R_2 = CH_3, R_3 = H$
13 $R_1 = CH_3, R_2 = R_3 = H$

Substituted chalcones were shown to have anti-inflammatory and analgesic action[20,74]. The 5-(4-chlorocinnamoyl)salicyclic acid had an analgesic effect similar to that of aspirin[20]. Substituted chalcones have shown antithrombic activity[74] while the flavone derived from the chalcone ester (**14**) was shown to be a potential antithrombic active compound[90].

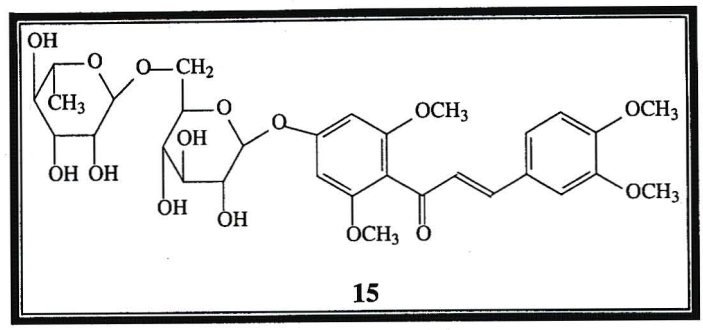


Et = CH_2CH_3

14

Various chalcones have been shown to restore capillary resistance in guinea pigs[91]. Compound **15** has been shown to decrease capillary fragility and in addition, affect

venous circulation[92]. In mice, chalcone and hesperidinmethylchalcone have been found to affect the fragility of capillaries present in the inner surface of the abdominal skin[1].



15

Coronary vasodilatory properties have been associated with ω-aminoalkoxychalcones and acid addition salts[67,69,70,93]. Mecinarone, a benzofuranic chalcone showed vasodilatory activity on the peripheral and cerebral circulation in experimental animals[94]. Coronary vasodilative properties have also been shown by pyridine analogues of chalcone[81].

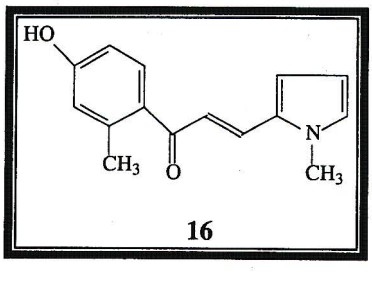
In mice, 2'-chloro-4,4'-difluorochalcone and 2'-hydroxy (and 2'-chloro-)-3,4-methylenedioxy-4'-fluorochalcone, have produced uterotonic effects, decreased the weight and size of the testes and seminal vesicles and inhibited implantation[95]. In rats, 4,2',4'-trimethoxychalcone epoxide and 4-methoxy-2'-4'-dibenzoyloxychalcone epoxide have shown estrogenic activity[1].

The pyrazole derived from 4-dimethylamino chalcone has shown some local anaesthetic activity[96]. Anticonvulsant as well as narcosis potentiation activity has been shown by 3,4-methylenedioxychalcone[97]. Cyanomethylchalcones are claimed as valuable medicinal agents for cardiovascular diseases and endocrine dysfunctions[98]. Hesperidinmethylenecarboxychalcone exerts therapeutic action in the treatment of chronic diseases of the eye and kidney, including rheumatoid diseases such as bursitis and osteoarthritis[99]. Hesperetin methyl chalcone, when incorporated in the diet (0.2%), shows an inhibitory effect on the incidence of dental caries in cotton rats[100]. Reduced benzofuranochalcone derivatives have been shown to possess antiangiotensin, antiarrhythmic and diuretic activity[69].

In isolated strips of intestine, chalcone, 2-hydroxychalcone, 2',3-dimethoxychalcone and hesperidinchalcone are able to protect epinephrine from destruction *in vitro*[101].

Dialkylaminoalkoxyderivatives of chalcone have been shown to be potential adrenergic blocking agents[1] and aminoazachalcones have been shown to act as adrenal cortex inhibitors in rats[102]. In rats, azachalcones have been shown to be suprarenal gland inhibitors of the amphenone type[103]. The most potent compound in pharmacological activity was shown to be 2-(2-dimethylaminoethoxy)chalcone, but this compound did not compare with the available therapeutic agents in specificity, potency and duration of action[1]. When tested in rats and mice, the chalcone, licurzid, was found to reduce stomach motility, inhibit evacuation of water from stomach to the duodenum and inhibit development of exudative processes in the inflammation and prevent development of neurogenic and butadione stomach ulcers[86].

In dogs and rats, 2-[2-dimethylaminoethoxy-3',4',5'-trimethoxy]chalcone hydrochloride was found to be an effective and long acting depressor agent[1]. There was also an electrolytic alteration in blood vascular smooth muscles following treatment with the chalcone[1]. The sulphur-containing derivative of chalcone ($PhCOCH_2S$-C_6H_4Cl), obtained by the interaction of the chalcone epoxide with thiol was shown to have biological activity[104]. In grasshoppers, the rejoining of broken ends of chromosomes and chromatids was found to be accelerated by the addition of 2',4'-dihydroxychalcone[105]. A decrease in the incidence of blood spots in chicken eggs was brought about by 3-pyrrole-2-aldehydechalcone and compound **16**[1]. The chalcone 3,2',4'-trihydroxy-4-methoxydihydrochalcone 4'-glycoside was shown to have an inhibitory effect on the growth of the plant *Triticale*[106].



16

The search for more novel chalcones from plant sources as well as the synthesis of novel chalcones and chalcone derivatives, followed by biological assays could be important in medicinal applications as known chalcones have many biological activities.

1.6 References

1. Dhar, D.N. (1981) *The Chemistry of Chalcones and Related Compounds*, John Wiley and Sons, New York, pp 3-5, 201-208, 213-237.
2. Shimokoriyama, M. (1962) Flavanones, chalcones and aurones, in *The Chemistry of Flavonoid Compounds*, Ed. Geissman, T.A., Pergamon Press, New York, pp 286-289,298-303.
3. Bohm, B.A. (1975) Chalcones, aurones and dihydrochalcones, in *The Flavonoids*, Ed. Harborne, J.B. and Mabry, T.J and Mabry, H., Chapman and Hall, London, pp 443-466.
4. Bohm, B.A. (1982) The minor flavonoids, in *The Flavonoids, Advances in Research*, Ed. Harborne, J.B. and Mabry, T.J, Chapman and Hall, London, pp 313-372.
5. Bohm, B.A. (1975) Flavanones and dihydroflavanols, in *The Flavonoids*, Ed. Harborne, J.B. and Mabry, T.J and Mabry, H., Chapman and Hall, London, pp 561-582.
6. Hahlbrock, K. and Grisebach, H. (1975) Biosynthesis of flavonoids, in *The Flavonoids*, Ed. Harborne, J.B. and Mabry, T.J and Mabry, H., Chapman and Hall, London, pp 866-900.
7. Geiger, W.B. and Conn, J.E. (1945) The mechanism of the antibiotic action of clavacin and penicillic acid, *Journal of the American Chemical Society*, **67**, 112-116.
8. Jeney, E. and Zsolnai, T. (1955) Experimental information on the chemotherapy of brucellosis, *Acta Microbiol. Acad. Sci. Hung.*, **2**, 249-256; *Chem. Abstr.* (1955) **49**, 13501g.
9. Ishida, S., Matsuda, A., Kawamura, Y. and Yamanaka, K. (1960) Antifungal agent. I. Antibacterial and antifungal activities *in vitro* of several organic compounds, *Chemotherapy* (Tokyo), **8**, 146-151; *Chem. Abstr.* (1960) **54**, 22844c.
10. Goncalves de Lima, O., Francisco de Mello, J., Coelho, J.S. de Barros, Lyra, F.D. de Andrade, Fernandes de Albuquerque, M. Machado, Marini-Bettolo, G.B, Delle Monache, G., Delle Monache, F. (1975) New prenylated chalcones from *Lonchocarpus neuroscapha* (Cordoa piaca), *Farmaco, Ed. Sci.*, **30**, 326-342 (Eng); *Chem. Abstr.* (1975) **83**, 91495t.

11. Gabor, M., Sallai, J., Szell, T. and Sipos, G. (1967) Relation of the antibacterial activity and chemical structure of chalcone derivatives, *Acta. Microbiol. Acad. Sci. Hung.*, **14**, 45-64; *Chem. Abstr.* (1967) **67**, 51338g.
12. Gabor, M., Sallai, J. and Szell, T. (1968) Pharmacological studies of new synthetic chalcones, *Kiserl. Orvostud.*, **20**, 540-544; *Chem Abstr.* (1969) **70**, 56156t.
13. Marrian, D.H., Russell, P.B. and Todd, A.R. (1947) The preparation and bacteriostatic action of some aminochalcones and related compounds, *Journal of the Chemical Society*, 1419.
14. Gudi, N., Hiremath, S., Badiger, V. and Rajagopal, S. (1962) Antibacterial compounds. I. Synthesis of several hydroxy chloro chalcones, *Arch Pharm*, **295**, 16-19; *Chem Abstr.* (1962) **57**, 7154e.
15. Covello, M., Dini, A. and Piscopo, E. (1970) Synthetic iodoorganic compounds. Iodinated α,β-unsaturated ketones having presumable antibacterial and antifungal activity, *Rend. Accad. Sci. Fis. Mat., Naples*, **37**, 56-62.; *Chem Abstr.* (1972) **76**, 33900r.
16. Hirose, K., Ukai, S., Hattori, T. (1971) Synthesis and biological activity of suphur containing derivatives of chalcone. I. Synthesis and antibacterial action of alkylthiochalcones, *Yakugaku Zasshi*, **91**, 804-810; *Chem Abstr.* (1971) **75**, 129467k.
17. Calcinari, R. (1972) Research on chalcones. III. Aminochalcones and sulfanilamidochalcones, *Farmaco, Ed. Sci*, **27**, 397-407; *Chem Abstr.* (1972) **77**, 48007z.
18. Aries, R. (1974) Antibacterial hydroxychalcone penicillanates, German patent, 2,341,514; *Chem Abstr.* (1974) **80**, 146152z.
19. Gasha, M., Tsuji, A., Sakurai, Y., Kurumi, M., Endo, T., Sato, S. and Yamaguchi, K. (1972) Antimicrobial activity of chalcones. I., *Yakugaku Zasshi*, **92**, 719-723; *Chem Abstr.* (1972) **77**, 97221y.
20. Lespagnol, A., Lespagnol, C., Lesieur, D., Cazin, J.C., Cazin, M., Beerens, H. and Romond, C. (1972) Analgesic, antiinflammatory and antimicrobial activities of chalcones and dihydrochalcones derived from salicylic acid, *Chim. Ther.*, **7**, 365-369; *Chem Abstr.* (1973) **78**, 52753c.

21. Kishimoto, Y., Akabori, Y. and Horiguchi, T. (1958) (Methylene)dithiodiacetic acid derivatives. I. Antimicrobial and antiprotozoal activity. 1., *Yakugaku Zasshi*, **78**, 447-450; *Chem Abstr.* (1958) **52**, 13862i

22. Yamaguchi, K., Sakurai, Y. and Kurumi, M. (1972) α-Bromochalcones, Japanese Patent, 72 47,016; *Chem Abstr.* (1973) **78**, 97330d.

23. Lespagnol, A., Lespagnol, Ch., Lesieur, D., Bonte, J.P., Blain, Y. and Labiau, O. (1971) Preparation of chalcones having salicylic structure, *Chim. Ther.*, **6**, 192-195; *Chem Abstr.* (1971) **75**, 98285t.

24. Kamoda, M. and Ito, N. (1954) The antibiotic action of unsaturated ketones. II. Unsaturated halogenated ketones., *J. Agr. Chem. Soc. Japan*, 28, 791-794; *Chem Abstr.* (1955) **49**, 5571f.

25. Jeney, E., Zsolnai, T. and Lazar, J. (1955) The bacteriostatic action of the furan compounds, *Zentr. Bakteriol. Parasitenk.*, Abt I, Orig. **163**, 294-301; *Chem Abstr.* (1955) **49**, 15074g.

26. Lora-Tamayo, M., Martin Municio, A. and Ruiz, J.L. (1960) Potential antitubercular compounds. V. 2-hydroxy-4-acetamidobenzenesulfonic acid derivatives., *Anales Real Soc. Espan. Fiz. Y Quim.*, **56B**, 403-406; *Chem Abstr.* (1961) **55**, 7410i.

27. Bayer, F. (1954) Chemotherapeutic thiosemicarbazones, British patent 708,013; *Chem Abstr.* (1956) **50**, 1086b.

28. Tomcufcik, A.S., Wikinson, R.G. and Child, R.G. (1975) Substituted chalcones, German patent 2,502,490; *Chem Abstr.* (1975) **83**, 179067r.

29. Henry, D.W. (1973) Antimalarial 1,3-bis(4-chlorophenyl)-2-propen-1-one guanylhydrazone hydrochloride and analogues, U.S. Patent 3,726,920; *Chem Abstr.*(1973) **79**, 18450n.

30. Velarde, E. (1970) Synthesis and biological activity of chalcones and of furan analogs, *Bol. Soc. Quim. Peru*, **36**, 127-157; *Chem Abstr.* (1971) **75**, 19808q.

31. El-Kerdawy, M.M., Samour, A.A. and El-Agamey, A.A. (1975) Thienyl acrylamides and thienyl pyrazolines as potential schistosomicidal agents, *Pharmazie*, **30**, 76-77; *Chem Abstr.* (1975) **83**, 9888s.

32. Takayanagi, Y. (1954) Studies on anthelmintic action of chalcone and flavanone, *Ann. Rept. Tohoku Coll. Pharm.*, **1**, 10-15; *Chem Abstr.* (1956) **50**, 4389f.

33. Laliberte, R., Campbell, D. and Bruderlein, F. (1967) Anthelmintic activities of chalcones and related compounds, *Can. J. Pharm. Sci.*, **2**, 37-43; *Chem Abstr.* (1967) **67**, 98058f.

34. Nakanishi, M. and Tsuda, A. (1964) Insect repellant, Japanese patent 74 46,056; *Chem Abstr.* (1975) **83**, 38818z.

35. Ruchkin, V.E., Shvetsova-Shilovskaya, K.D. and Mel'nikov, N.N. (1967) Synthesis of some N-substituted carbamoyl oximes, *Probl. Poluch. Poluprod. Prom. Org. Sin., Akad. Nauk SSSR, Otd. Obshch. Tekh. Khim.*, 66-70; *Chem Abstr.* (1968) 68, 95417g.

36. Clark, S.F.(1957) Chalcone dichloride, its derivative and its synergistic action with DDT, U.S. Patent 2,805,184; *Chem Abstr.* (1958) **52**, P 2325i.

37. Cross, B. (1975) Pyrazolinium compounds as herbicides, U.S. Patent 3,925,408; *Chem Abstr.* (1976) **84**, 135646v.

38. Wurm, G. and Lachmann, Ch. (1974) 2'-Hydroxychalcone and 3-hydroxyflavonesulfonic acid derivatives, *Arch. Pharm. (Weinheim, Ger.)*, **307**, 695-700; *Chem Abstr.* (1974) **81**, 169399v.

39. McGowan, J.C., Brian, P.W. and Hemming, H.G. (1948) Fungistatic activity of ethylenic and acetylenic compounds. I. Effect of the affinity of the substituents for electrons upon the biological activity of ethylenic compounds., *Ann. Applied Biol.*, **35**, 25-36; *Chem Abstr.* (1949) **43**, 9334b.

40. Ko, K, Iseki, K., Adachi, Y., Konno, K., Yokoyama, K. and Misato, T. (1978) Effects of chalcones and chromenes on powdery mildew and anthracnose of cucumber plants, *Nippon Noyaku Gakkaishi*, **3**, 155-160; *Chem Abstr.* (1978) **89**, 192310h.

41. Beiler, J.M., Graf, M. and Martin, G.J. (1952) Inhibition of liver xanthine oxidase activity in rats, *Am. J. Digestive Diseases*, **19**, 333; *Chem Abstr.* (1953) **47**, 1198e.

42. Martin, G.J., Beiler, J.M. and Avakian, S. (1956) Chalcones, U.S. Patent 2,769,817; *Chem Abstr.* (1957) **51**, 14815d.

43. Yuwiler, A., Geller, E. and Eiduson, S. (1960) Studies on 5-hydroxytryptophan decarboxylase. II. Additional inhibition studies and suggestions on the nature of the enzymic site., *Arch. Biochem. Biophys.*, **89**, 143-147; *Chem Abstr.* (1960) **54**, 21222c.

44. Hase, J., Kobashi, K. and Kobayashi, R. (1973) Inhibitors of the (sodium-potassium ion)–dependant adenosinetriphosphatase. I. Effect of phlorizin and

related natural products., *Chem. Pharm. Bull.*, **21**, 1076-1079; *Chem Abstr.* (1974) **80**, 10261b.

45. Lesieur-Demarquilly, I., Mizon, J. and Osteux, R. (1973) Inhibitory effect of some salicylic chalcones on the aromatic L-amino acid decarboxylases, *Ann. Pharm. Fr.*, **31**, 705-708; *Chem Abstr.* (1974) **81**, 59921b.
46. Kamoda, M.,Chiba, T., Mori, K. and Ito, N. (1953) Inhibition of enzymes by antibiotics. II. Inhibition of cholinesterase by unsaturated ketones., *Botyu-Kagaku*, **18**, 117-122; *Chem Abstr.* (1954) **48**, 778c.
47. Deutsch, D.H. and Garcia, E.N. (1956) Heterocyclic chalcones, U.S. Patent 2,754,299; *Chem Abstr.* (1957) **51**, 4437i.
48. Stenlid, G. (1968) The physiological effects of phlorizin, phloretin and some related substances upon higher plants, *Physiol. Plant.*, **21**, 882-894; *Chem Abstr.* (1968) **69**, 84194b.
49. Komatsu, M., Kyogoku, K., Hatayama, K., Suzuki, K. and Yokomori, S. (1972) Isopentenyl chalcones, Japanese Patent 74 05,950; *Chem Abstr.* (1974) **80**, 120551d.
50. Kyogoku, K., Hatayama, K., Yokomori, S., Seki, T. (1973) Isopentenyl chalcones, Japanese Patent 74,126,654; *Chem Abstr.* (1975) **83**, 78864t.
51. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1973) Isoprenylchalcones, Japanese Patent 75 24,258; *Chem Abstr.* (1975) **83**, 178566r.
52. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1973) Isoprenylchalcones, Japanese Patent 75 24,257; *Chem Abstr.* (1975) **83**, 178567s.
53. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1975) Isoprenylchalcones, Japanese Patent 75,140,430; *Chem Abstr.* (1976) **84**, 121467a.
54. Kyogoku, K., Hatayama, K., Yokomori, S., Sawada, J. and Tanaka, I. (1977) Chalcone ethers, Japanese Patent 77 97,950; *Chem Abstr.* (1978) **88**, 74200d.
55. Vanstone, A.E. and Maile, G.K. (1977) Chalcone derivatives, German Patent 2,810,253; *Chem Abstr.* (1979) **90**, 22628w.
56. Kyogoku, K., Hatakeyama, M., Yokomori, S., Sawada, J. and Tanaka, I. (1978) Chalcone derivatives, Japan Kokai Tokkyo Koho 78,116,355; *Chem Abstr.* (1979) **90**, 87056c.

57. Kyogoku, K., Hatakayema, M., Yokomori, S., Miyata, Y., Sawada, J. and Tanaka, I. (1979) Chalcone derivatives, Japan Kokai Tokkyo Koho 79 19,948; *Chem Abstr.* (1979) **91**, 20115y.

58. Biorex Laboratories Ltd. (1978) Chalcone derivatives, Fr. Demande 2,383,157;*Chem Abstr.* (1979) **91**, 39136c.

59. Kyogoku, K., Hatakayema, M., Yokomori, S., Miyata, Y. Sawada, J. and Tanaka, I. (1979) Chalcone derivatives, Japan Kokai Tokkyo Koho 79 19,947; *Chem Abstr.* (1979) **91**, 20114x.

60. Kyogoku, K., Hatakayema, M., Yokomori, S., Miyata, Y., Sawada, J. and Tanaka, I. (1979) Chalcone derivatives, Japan Kokai Tokkyo Koho 79 19,949; *Chem Abstr.* (1979) **90**, 203699r.

61. Kyogoku, K., Hatakayema, M., Yokomori, S., Miyata, Y., Sawada, J. and Tanaka, I. (1979) Chalcones, Japan Kokai Tokkyo Koho 79 22,349; *Chem Abstr.* (1979) **90**, 203690f.

62. Kyogoku, K., Hatayama, K.,Yokomori, S., Sawada, J. and Tanaka, I. (1976) 2'-(Carboxymethoxy)chalcones and their use in treating stomach and duodenal ulcers, Fr. Demande 2,340,924; *Chem Abstr.* (1978) **88**, 104910e.

63. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1974) Chalcone ethers with activity against stomach ulcers, Belgium Patent 816,463; *Chem Abstr.* (1975) **83**, 43051y.

64. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1974) Isoprenylchalcones, Japanese Patent 75,140,429; *Chem Abstr.* (1976) **85**, 5913q.

65. Lafon, L. (1970) Hypocholeretic, hypotensive, and anticoagulating 2',4',6'-trihydroxychalcones, German Patent 2,010,180; *Chem Abstr.* (1970) 73, 120342s.

66. Mizuta, Y., Igasa, H., Uno, J. and Tsukamoto, M. (1974) ω-Aminoalkoxychalcones and acid addition salts, Japanese Patent 74 21,125; *Chem Abstr.* (1975) **82**, 86208b.

67. Igasa, H., Mizuta, Y., Tsukamoto, M.,Uno, J. (1970) ω-Aminoalkoxychalcones and acid addition salts, Japanese Patent 74 21,126; *Chem Abstr.* (1975) **82**, 97841d.

68. Igasa, H., Tsukamoto, M. and Uno, J. (1973) N,N-Disubstituted 2-(ω-aminoalkoxy)-3',4',5'-trimethoxychalcones, Japanese Patent 73 37,027; *Chem Abstr.* (1974) **80**, 120553f.

69. Raynaud, G., Pourrias, B., Thomas, J., Thomas, M., Roblin, B., Hecaen, V. and Perroud, F. (1974) Benzofurans. IV. Reduced chalcone derivatives with vasodilatory and antihypertensive action. Pharmacodynamic screening, *Eur. J. Med. Chem. – Chim. Ther.*, **9**, 85-93; *Chem Abstr.* (1974) **81**, 99240k.

70. Raynaud, G., Pourrias, B., Thomas, J. and Thomas, M. (1973) Benzofurans. II. Chalcones with vasodilating effect. Pharmacological study., *Chim. Ther.*, **8**, 479-486; *Chem Abstr.* (1974) **81**, 45196g.

71. Venturella, P., Bellino, A. and Piozzi, F. (1971) Synthesis of indolylchalcones and indolylchromonols, *Farmaco. Ed. Sci.*, **26**, 591-596; *Chem Abstr.* (1971) **75**, 88432z.

72. Kinugasa, H., Tsukamoto, M., Mizuta, Y. and Uno, J. (1970) Chalcone derivatives and salts, Japanese Patent 74 06,904; *Chem Abstr.* (1974) **80**, 145797b.

73. Mizuta, Y., Kinugasa, H., Uno, J. and Tsukamoto, M. (1974) ω-aminoalkoxychalcones and salts, Japanese Patent 74 06,903; *Chem Abstr.* (1974) **80**, 146198u.

74. Hsu, K.K. and Chen, F.C. (1973) Structure-activity relations of substituted flavonoids. I., *Tai-Wan K'o Hsueh*, **27**, 23-26; *Chem Abstr.* (1974) **80**, 66597h.

75. Kabiev, O.K. and Vermenichev, S.M. (1971) Kinetics on the antineoplastic effect of chalcones on Ehrlich's ascitic sarcoma, *Izv. Akad. Nauk Kaz. SSR. Ser. Biol.*, **9**, 72-75; *Chem Abstr.* (1971) **75**, 47091u.

76. Dore, J.C. and Viel, C. (1974) Antitumour chemotherapy. IX. Cytotoxic activity of substituted chalcones and related compounds on tumor cells in culture, *J. Pharm. Belg.*, **29**, 341-351; *Chem Abstr.* (1975) **83**, 90650c.

77. Horakova, K., Drobnica, L. and Nemec, P. (1971) Cytotoxic and cancerostatic effect of isothiocyanates and related compounds. VII. Effect of isothiocyanatechalcones on HeLa cells., *Neoplasma*, **18**, 355-359; *Chem Abstr.* (1971) **75**, 107958u.

78. Arora, S.K. and Bates, R.B. (1976) Uvaretin, a new antitumour agent from *Uvaria acuminata* (Annonaceae), *Journal of Organic Chemistry*, **41**, 1852-1855.

79. Uong, T., Yan, C.F. and Chen, F.C. (1973) Synthesis of tumour-inhibitory flavonoids, *Hua Hsueh* (4), 133-134; *Chem Abstr.* (1974) **81**, 120380t.

80. Chabannes, B., Grandgeorge, M., Duperray, B. and Pacheco, H. (1973) Metabolism of a choleretic drug, 2',4,4'-trimethoxychalcone (Vesidryl), *Chim. Ther.*, **8**, 621-625; *Chem Abstr.* (1974) **81**, 20752x.

81. Laboratoires Gerda (1968) Pyridine chalcone analogues having choleretic and coronary vasodilative properties, Fr. M. 6427; *Chem Abstr.* (1971) **75**, 40406s.

82. Flammang, M., Wermuth, C.G., Delassue, H. (1970) Choleretic chalcones: synthesis, spectral study and pharmacological properties, *Chim. Ther.*, **5**, 431-440; *Chem Abstr.* (1971) **74**, 125043a.

83. Formanek, K., Holler, H.J. and Kowac, W. (1958) Relations between structure and activity of some flavonoids, *Pharm. Acta. Helv.*, **33**, 437-444; *Chem Abstr.* (1959) **53**, 15380g.

84. Woelm, F.M. (1964) Salts of N-substituted 4'-aminoalkoxy-2',4-dihydroxychalcones, German Patent 1,174,311; *Chem Abstr.* (1964) **61**, 11933g.

85. Dornow, A. and Frese, A. (1952) Preparation of papaverinelike compounds. II., *Arch, Pharm.*, **285**, 463-471; *Chem Abstr.* (1954) **48**, 11373g.

86. Obolentseva, G.V. and Khadzhai, Y.I. (1966) Pharmacological data on certain aspects of the action of Glycyrrhiza flavonoids, *Vop. Izuch. Ispol'z. Solodki Nauka.*, 163-166; *Chem Abstr.* (1967), **67**, 107314r.

87. Susumu, O., Nagura, T., Sugihara, Y., Okamoto, K., Ishida, R. and Shintomi, K. (1973) Chalcone derivatives, Japanese Patent 73 19,594; *Chem Abstr.* (1973) **78**, 147963k.

88. Susumu, O., Nagura, T., Sugihara, Y., Okamoto, K., Ishida, R. and Shintomi, K. (1973) Chalcone derivatives, Japanese Patent 73 19,569; *Chem Abstr.* (1973) **78**, 147788g.

89. Susumu, O., Nagura, T., Sugihara, Y., Okamoto, K., Ishida, S. and Shintomi, K. (1973) Chalcone derivatives, Japanese Patent 73 19,570; *Chem Abstr.* (1973) **78**, 147790b.

90. Yan, C.F.,Uong, T. and Chen, F.C. (1973) Synthesis of antithrombic flavonoids, *Hua Hsueh* (4), 135-136; *Chem Abstr.* (1974) **81**, 120382v.

91. Hughes, E.G. and Parkes, M.W. (1946) Studies on dietry factors affecting capillary resistance, *Jubilee Vol. Emil Barell*, 216-228; *Chem Abstr.* (1947) **41**, 2141a.

92. Fabre, P. (1972) Water-soluble derivatives of flavanone glucosides, Fr. Demande 2,183,612; *Chem Abstr.* (1974) **80**, 121294j.

93. Uno, J., Kinugasa, H., Tsukamoto, M. and Mizuta, Y. (1970) (ω-Aminoalkoxy)chalcones, Japanese Patent 72 49,581; *Chem Abstr.* (1973) **78**, 147569m.

94. Pourrias, B., Sergant, M., Thomas, J., Gouret, C. and Raynaud, G. (1975) Pharmacological study of a new substance, mecinarone., *Arzneim.-Forsch.*, **25**, 782-786; *Chem Abstr.* (1975) **83**, 91023n.

95. Jacob, D. and Kaul, D.K. (1973) Estrogenic and antifertility effects of chalcone derivatives, *Acta Endocrinol.*, **74**, 371-378; *Chem Abstr.* (1974) **80**, 22992d.

96. Hosni, G. and Saad, S.F. (1975) Synthesis of some new pyrazolines from 4-dimethylaminochalcone and 2-*p*-dimethylaminocinnamoyl-1-naphthol, *Acta Chim. Acad. Sci. Hung.*, **86**, 263-267; *Chem Abstr.* (1976) **84**, 30959w.

97. UNICLER S.A. (1972) Chalcones and chalcols derived from piperonals, French Patent 2,253,503; *Chem Abstr.* (1976) **84**, 89825c.

98. Rorig, K.J. (1956) *Ar'*-cyanomethylchalcones, U.S. Patent 2,755,299; *Chem Abstr.* (1957) **51**, 2868c.

99. Kubler, D.G. (1959) 2-Alkoxy- and 2-alkoxyalkoxy-5,6-dihydro-1,2-pyrans, U.S. Patent 2,905,699; *Chem Abstr.* (1960) **54**, 12161g.

100. Thompson, D.T., Vogel, J.J. and Philips, P.H. (1965) Certain organic substances and their effects on the incidence of dental caries in the cotton rat, *J. Dental Res.*, **44**, 596-599; *Chem Abstr.* (1965) **63**, 7526d.

101. Wilson, R.H., DeEds, F. (1949) The *in vitro* protection of adrenaline by flavonoids, *J. Pharmacol. Exptl. Therap.*, **95**, 399-406; *Chem Abstr.* (1949) **43**, 5542a.

102. Durinda, J., Szucs, L., Kolena, J., Nagy, A., Misikova, E. and Heger, J. (1977) Studies of amphenone-type adrenal cortex inhibitors. III. Aminoazachalcones., *Cesk. Farm.*, **26**, 140-149; *Chem Abstr.* (1978) **88**, 6671h.

103. Durinda, J., Szucs, L., Struharova, L., Kolena, J. and Heger, J. (1972) Suprarenal gland inhibitors of the amphenone type. II. Azachalcone derivatives and analogs., *Cesk. Farm.*, **21**, 276-282; *Chem Abstr.* (1973) **78**, 29576p.

104. Ukai, S., Hirose, K., Hattori, T., Kayano, M. and Yamamoto, C. (1972) Synthesis and biological activity of sulphur containing derivatives of chalcone. II. Reaction of chalcone oxides with thiols., *Yakugaku Zasshi*, **92**, 278-283; *Chem Abstr.* (1972) **77**, 34207c.

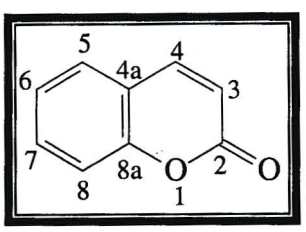
105. Saha, A.K. and Khudabaksh, A.R. (1974) Action of 2',4'-dihydroxychalcone on grasshopper spermatocyte chromosomes, *Cytologia*, **39**, 357-366; *Chem Abstr.* (1974) **81**, 146348p.

106. Tetenyi, P. (1977) Inhibitory effect of chalcones and dihydrochalcones on the growth of plants, *Agrokem. Talajtan*, **26**, 391-398; *Chem Abstr.* (1978) **88**, 164842u.

Chapter 2. Coumarins

2.1 An Introduction to Coumarins

Coumarins are a class of compounds named after the parent compound, coumarin which contains a C_6-C_3 nucleus. Coumarins contain two rings, a benzene ring and a pyrone ring fused in linear fashion and are considered to be δ-lactones or more specifically α-benzopyrones. Coumarin itself has a pleasant odour likened to that of new-mown hay and was first isolated from tonka beans (*Coumaraouna odorata*) and then from various melilot species e.g. *Melilotus officinalis* and from *Asperula odorata*[1].



Coumarin

Most coumarins are oxygenated at the 7- position and umbelliferone (7-hydroxycoumarin) is the true parent of nearly all naturally occurring coumarins. Further hydroxyl or methoxyl groups are often present and may occupy any of the vacant sites on the umbelliferone system. Hydroxylation at the 3- and 4- positions is uncommon[1].

Many of the properties of coumarins are modified properties of simple α-pyrones and there is usually a simple relation between the patterns of behaviour in the two systems[1]. Coumarins absorb infrared radiation in the same region as α-pyrones, at approximately 1700 cm^{-1}, the exact value depending on the substituents on the benzene ring[1]. There are also three strong absorption bands in the double bond region[1] between 1600 cm^{-1} and 1660 cm^{-1}. As α-pyrones, coumarins absorb ultraviolet light at approximately 300 nm, the exact wavelength depending on the substituents present[1]. There are usually additional strong bands between 250 nm and 340 nm[1].

Dilute alkali slowly hydrolyses coumarin into the salt of the coumarinic acid, *o*-hydroxy-*cis*-cinnamic acid[1]. The 7-methoxycoumarin is much harder to hydrolyse than coumarin and the 7-hydroxycoumarin harder still[1]. This effect can be accounted for in figure 1. and does not work for either the 6- or the 8-hydroxycoumarins which are not hard to hydrolyse[1]. It is useful that acidification regenerates the original coumarin because this can be used to separate coumarins form neutral, acidic and phenolic contaminants when dealing with complex plant extracts[1].

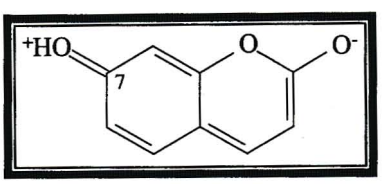


Fig. 1 Effect accounting for the difficulty of hydrolysing 7-hydroxycoumarin

Coumarins can be placed into one of five groups *viz.* coumarins substituted on the benzene ring only (group A), coumarins substituted on the pyrone ring (group B), furanocoumarins (group C), 3,4-benzocoumarins (group D) and pyranocoumarins (group E)[1]. A brief description and examples and structures of each group taken from Dean[1]are given in this chapter.

2.1.1 The group A coumarins

The group A coumarins, consist of coumarin itself and the widespread umbelliferone (7-hydroxycoumarin). Other coumarins in group A occur as methyl ethers or with isoprenoid substituents on the benzene ring, especially at the 8- position. Examples of some of the coumarins belonging to the group A coumarins are: aurapten (**1**), a constituent of grapefruit peel and of the root barks of *Feronia elephantum* and *Aegle marmelos*[1], marmin (**2**) isolated from *Aegle marmelos*[1], umbelliprenin (**3**), one of the many constituents of the seeds of *Angelica archangelica*[1], osthol (**4**), one of several coumarins in the rhizome of *Imperatoria ostruthium* and in the roots of *Angelica archangelica*[1], the epoxide meranzin (**5**) which occurs in the oils of orange peel and lemon-grass[1] and suberosin (**6**), isomeric with osthol (**4**) and isolated from the bark of *Xanthoxylum suberosum* and the heartwood of *X. flavum*[1]. Structures of compounds **1-6** are given in figure 2.

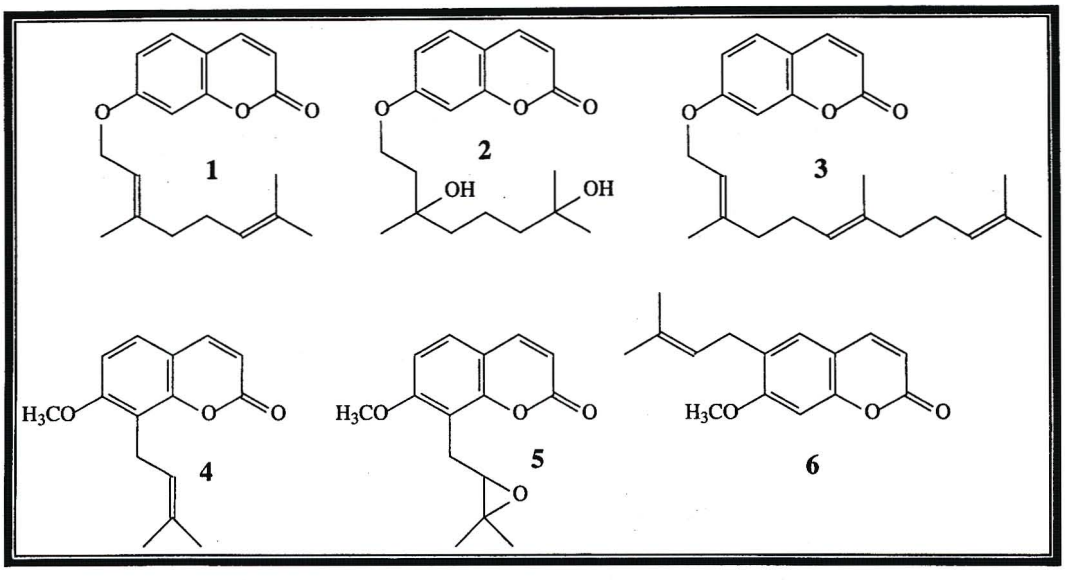


Figure 2. Examples of coumarins belonging to group A.

2.1.2 The group B coumarins

In group B (coumarins substituted on the pyrone ring), dalbergin (**7**) was the first phenylcoumarin to be isolated from a natural source, the heartwood of *Dalbergia sissoo*, where it occurred with its methyl ether[1]. This phenyl group was attached to the coumarin at the 4- position. Calophyllolide (**8**), a pyranocoumarin, also contained a phenyl group at the 4- position[1]. Another example of a coumarin with a phenyl group attached at the 4- position is 5,7-dihydroxy-8-isopentenyl-4-phenyl-6-isovalerylcoumarin (**9**) which occurs in the pulp of the mamey fruit (*Mamea americana*)[1]. Another coumarin, mammein (**10**), isolated from the seeds of *Mamea americana*[1] has a C_3H_7 group attached at the 4- position. Pachyrrhizin (**11**) isolated from the seeds of the yam bean, *Pachyrrhizus erosus*[1], is a 3-phenylcoumarin, a rare arrangement like that in isoflavones. Ammoresinol (**12**), isolated from *Dorema ammoniacum* is an example of a rare 4-hydroxycoumarin[1]. Dicoumarol (**13**) was found to be the active principle in spoiled sweet clover (*Melilotus alba*). Isoshehkagenin (**14**) was isolated from *Iris watti*[1] and novobiocin (**15**) was isolated as a metabolite of *Streptomyces niveus*[1]. The structures of compounds **7-15** are given in figure 3.

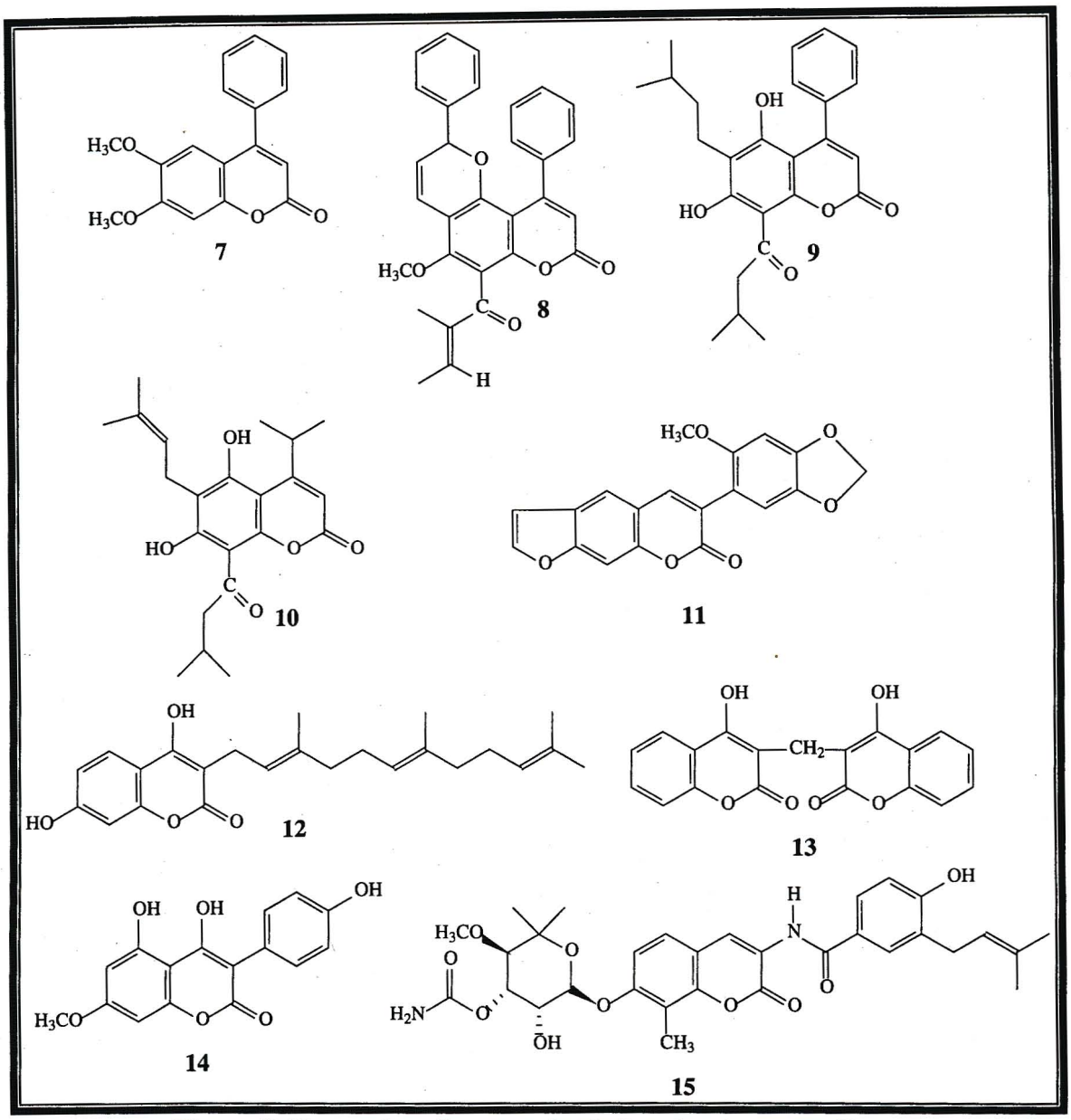


Figure 3. Examples of coumarins belonging to group B.

2.1.3 The group C coumarins

In furanocoumarins (group C), rings fused in a linear fashion are more common than those fused in an angular fashion[1]. Furanocoumarins often have isoprenoid groups attached either to oxygen or to carbon. The isopropyldihydrofuran ring can be regarded as arising from cyclisation of isoprenoid groups onto neighbouring hydroxyl groups in appropriately substituted coumarins[1]. Angelicin (**16**), isolated from *Angelica archangelica* and *Psoralea corylifolia* is an example of a furocoumarin where the rings are fused in an angular fashion[1]. Psoralene (**17**), first isolated in the seeds of *Psoralea corylifolia*, is an example of a furocoumarin where the rings are fused in a linear fashion[1]. Psoralene (**17**) was also isolated from several other sources

e.g. *Ficus carica, Xanthoxylum flavum* and also some species of *Coronilla*[1]. Bergapten (**18**) was isolated as early as 1839 and occurs in various species of *Heracleum, Fagara, Ruta, Skimmia, Ficus, Ligusticum, Angelica, Seseli, Levisticum, Citrus, Ammi, Pimpinella, Petroselinum, Casimiroa* and *Pastinaca*[1]. Bergaptol (**19**) occurs in Calabrian bergamot oil and in the oil of *Citrus aurantifolia*, but is relatively rare[1]. Isobergapten (**20**) was isolated from *Pimpinella saxifraga, P. magna, Heracleum sphondylium, H. lasatum* and *H. nipponicum*[1]. Isoimperatorin (**21**) is a furocoumarin with an isoprenoid group attached to the oxygen on the coumarin nucleus and was isolated from *Imperatoria ostruthium*[1]. A phenolic isomer of isoimperatorin (**21**), psoralidin (**22**) was reported from the pericarp of *Psoralea corylifolia*[1]. Ostruthol (**23**) was found in the roots of *Imperatoria ostruthium*[1]. Xanthotoxin (**24**) has been isolated from a number of plants e.g. *Fagara xanthoxyloides, Angelica archangelica, Ammi majus, Luvanga scandens* and various species of *Ruta*[1]. Xanthotoxol (**25**) is a minor component in *Angelica archangelica*[1]. Imperatorin (**26**) is the γγ-dimethylallyl ether of xanthotoxol (**25**) and has been isolated from *Aegle marmelos, Imperatoria ostruthium, Angelica archangelica, A. glabra, Ammi majus* and *Pastinaca sativa*[1]. Isopimpinellin (**27**) with methoxy groups at both the 5- and 8- positions was isolated from the roots of *Pimpinella saxifraga, Luvanga scandens, Heracleum sphondylium, H. panaces, Casimiroa edulis, Fagara ailanthoides* and *Flindersia Bennetiana*[1]. Pimpinellin (**28**) is found with isopimpinellin (**27**)[1]. Phellopterin (**29**) was extracted from the fruit of *Phellopterus littoralis* and from the roots of *Angelica glabra*[1]. Sphondin (**30**) is a minor component in *Heracleum sphondylium, H. lanatum* and *Pimpinella saxifraga*[1]. Halfordin (**31**) and isohalfordin (**32** or **32a**)[1] were isolated from the bark of *Halfordia scleroxyla*[1]. These two compounds have a methoxy group in the pyrone ring. Nodakenetin (**33**) was found in the roots of *Peucedanum decursivum*[1]. Peucedanin (**34**) was isolated from *Peucedanum officinale* in 1833[1]. Athamantin (**35**) was isolated from *Athamantia oreoselinum* nearly a century before a structural formula was proposed[1]. The bark of *Ekebergia pterophylla* yielded pterophyllin 1 (**36**) and pterophyllin 2 (**37**) and the wood of the same plant yielded pterophyllins 4 (**38**) and 5 (**39**)[13]. Compounds **36-39** are unique in that the furan ring is now at the 3,4- position. The structures of compounds **16-35** are given in figure 4 and **36-39** in figure 5.

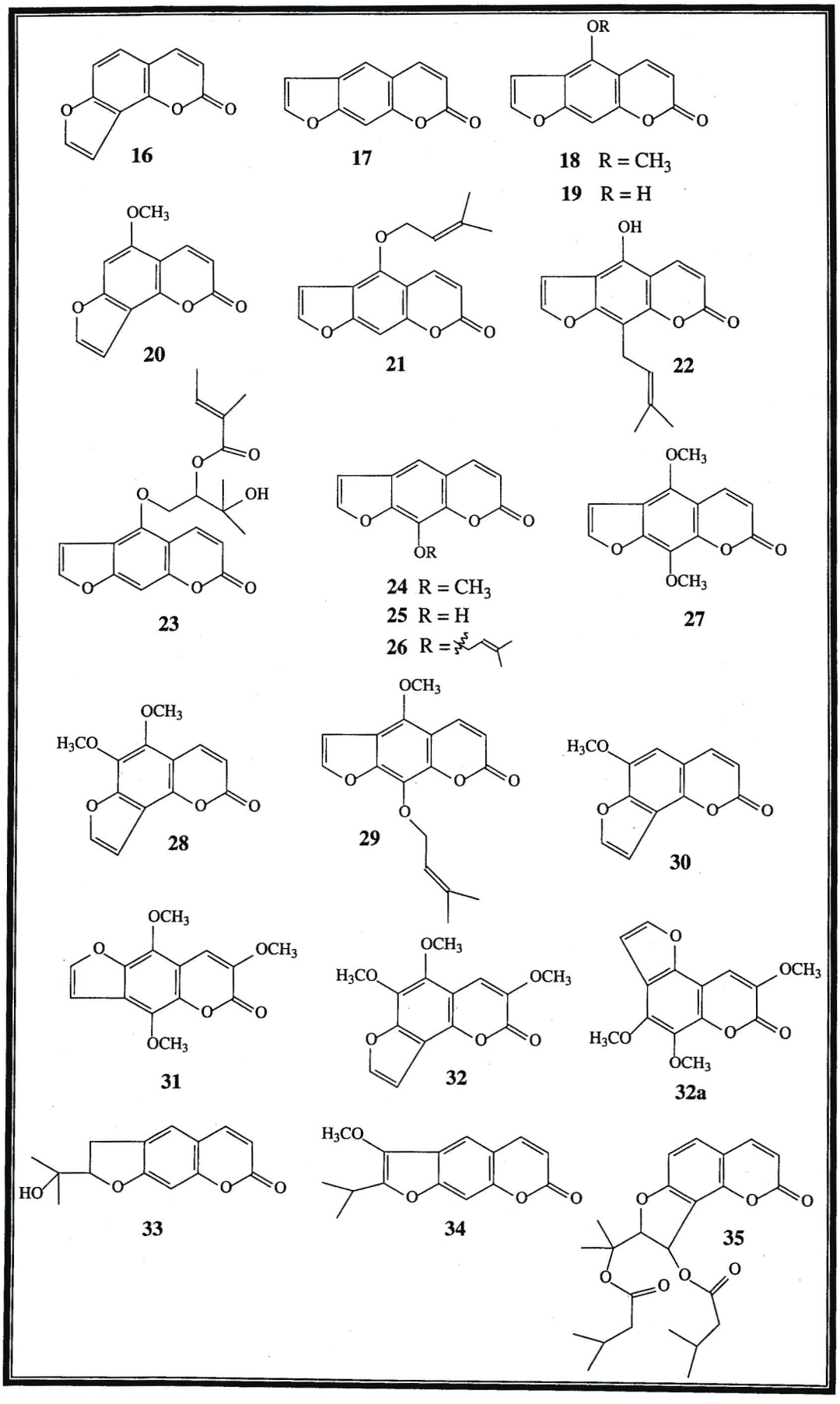


Figure 4. Examples of coumarins belonging to group C.

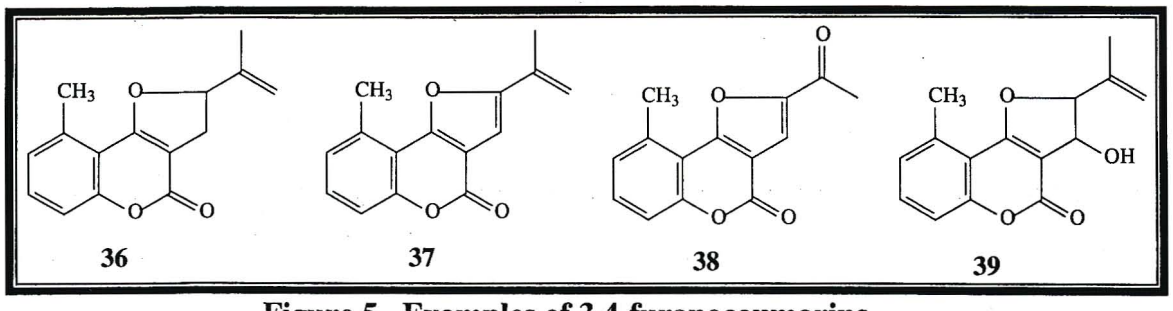


Figure 5. Examples of 3,4-furanocoumarins

2.1.4 The group D coumarins

Group D consists of coumarins in which a benzene ring is fused at the 3,4-position. The fungus *Alternaria tenuis*[1] produces alternariol (**40**). Ellagic acid (**41**) is a common yellow pigment found in about seventy-five families of dicotyledons. Ellagic acid (**41**) is found consistently in species of *Fagales*, *Myrtiflorae*, *Rosales*, *Sapindales* and *Geraniales*[1]. The dimethyl ether (**42**) of ellagic acid was isolated from the roots of *Euphorbia formosana* and the trimethyl ether (**43**) was isolated from the bark of *Eugenia maire*[1]. Compounds **40-43** are given in figure 6.

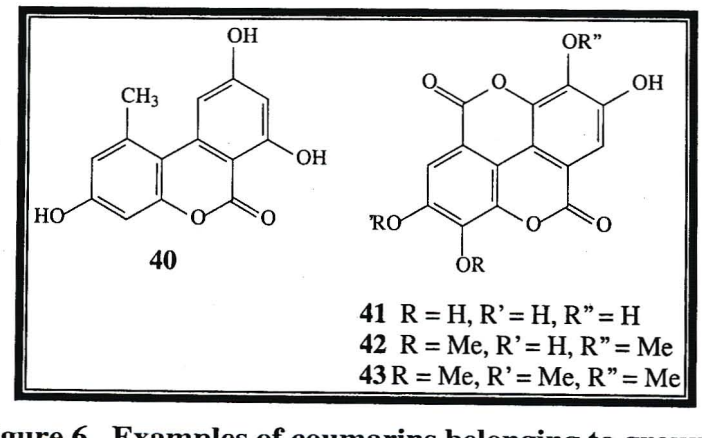
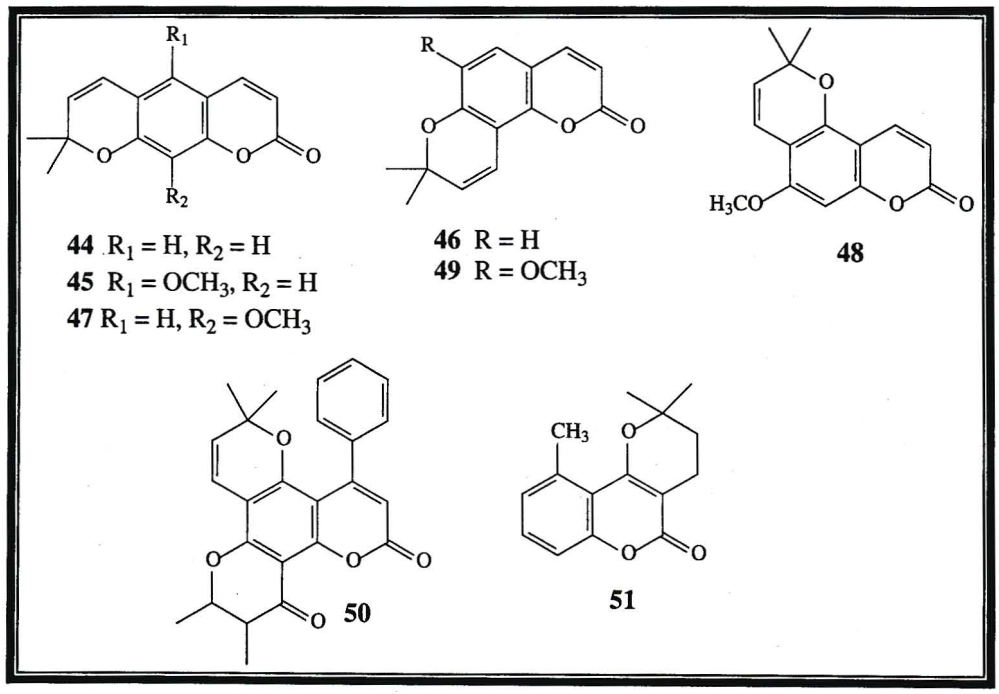


Figure 6. Examples of coumarins belonging to group D

2.1.5 The group E coumarins

When an isoprenoid unit is attached to a carbon and oxygen atom on the coumarin nucleus simultaneously, a pyran ring system results, giving rise to a group of coumarins known as pyranocoumarins (Group E). These pyranocoumarins can be linear or angular. The linear form is best illustrated by xanthyletin (**44**), a coumarin isolated from the bark of *Xanthoxylum americanum*, the fruits of *Luvanga scandens*, the bark of *Citrus acida* and the heartwood of *Chloroxylon swietenia*[2]. Another linear pyranocoumarin is xanthoxyletin (**45**), occurring with xanthyletin (**44**) in the bark of *Xanthoxylum americanum* and the heartwood of *Chloroxylon swietenia*[2]. Seselin (**46**)

is an example of an angular pyranocoumarin, isolated from the leaves of *Skimmia repens* and *S. japonica* and from the fruit of *Seseli indicum*[2]. Luvangetin (**47**) is one of several coumarins in *Luvanga scandens*[2]. Alloxanthoxyletin (**48**) is another pyranocoumarin from *Xanthoxylum americanum*[2]. Braylin (**49**) was isolated from the bark of the Australian plant, *Flindersia brayleyana*[2]. Calophyllolide (**8**) was isolated from the nuts of the Tahitian tree, *Calophyllum inophyllum*[2]. Inophyllolide (**50**) was obtained from the same source as calophyllolide (**8**) but from a different batch of nuts[2]. A 3,4-pyranocoumarin, pterophyllin 3 (**51**) was isolated from the wood of *Ekebergia pterophylla*[13].



44 $R_1 = H, R_2 = H$
45 $R_1 = OCH_3, R_2 = H$
47 $R_1 = H, R_2 = OCH_3$

46 $R = H$
49 $R = OCH_3$

48

50

51

Figure 7. Examples of coumarins belonging to group E.

2.2 Biosynthesis of Coumarins

The biosynthesis of simple coumarins involves cinnamic acid derivatives, beginning with *ortho-* hydroxylation, a reaction that is catalysed by the enzyme, phenolase and involving the NIH shift. The ortho- hydroxyl group can undergo glucosylation, which allows both the *trans-* and the *cis-* isomers to be formed. This is followed by hydrolysis of the sugar *via* enzymatic cleavage of the glucoside and spontaneous cyclisation (lactone formation), resulting in the final coumarin product[3,4,5]. The biosynthesis of simple coumarins is illustrated in Figure 8, which shows the biosynthesis of coumarin and 7-hydroxycoumarin (umbelliferone) which is regarded as the parent compound of all other 7-oxygenated coumarins[1,7]. The biosynthetic

pathway of the 7-oxygenated coumarins and those which are not hydroxylated at the 7- position is similar, but in the 7-oxygenated coumarins, *p*-hydroxylation precedes *o*-hydroxylation, which is followed by lactone formation[8]. Once *o*-hydroxylation occurs, it is followed directly by spontaneous lactone formation.

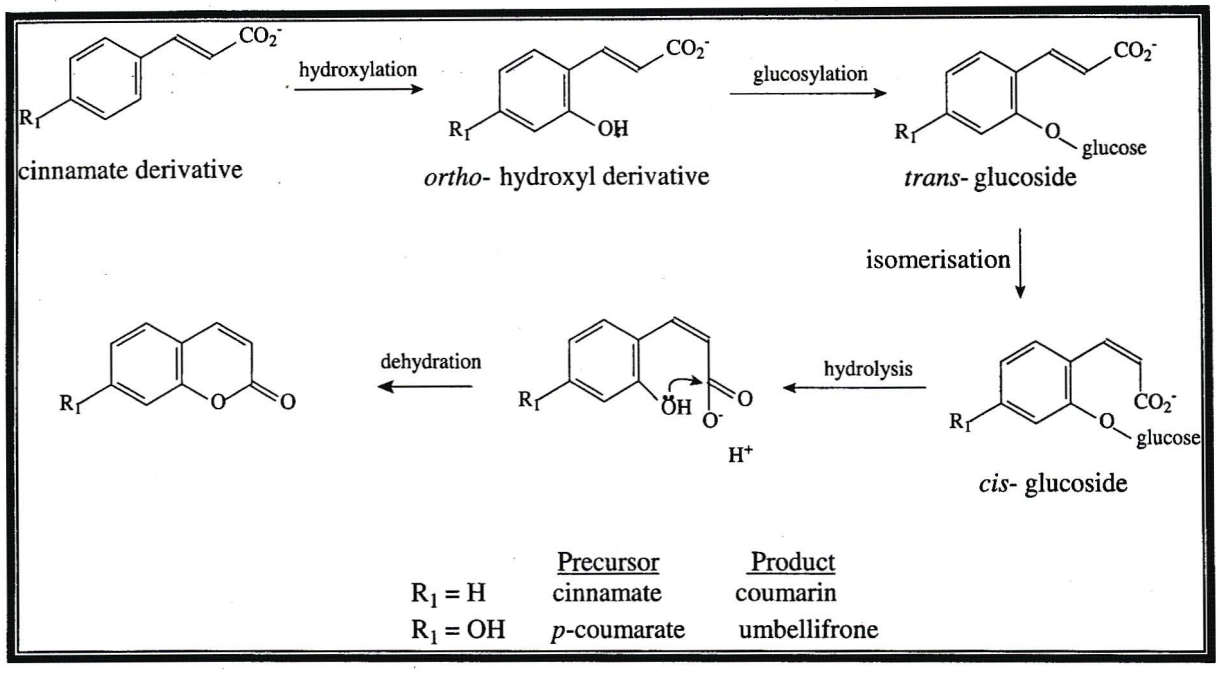
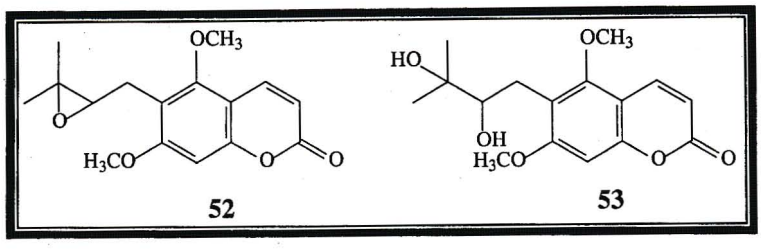


Figure 8. Biosynthetic route to coumarin production[3,4,5,6]

Many coumarins and other phenolic compounds e.g. flavanones and chalcones, have isoprenoid chains of 1,2 or 3 isoprene units attached to oxygen or carbon atoms on the phenolic nucleus. These isoprenoid residues arise from isopentyl pyrophosphate (IPP), which is also involved in the biosynthesis of steroids and terpenoids[9]. In coumarins, both C- and O- prenylations are known to occur, leading to numerous polyisoprenoid derivatives, which can, in turn, be further modified.

There are generally two types of isoprenoid derivatives, αα-dimethylallyl and γγ-dimethyl allýl derivatives. The γγ-dimethylallyl derivatives are more common due to steric reasons. The biosynthesis of prenylated coumarins involves the γγ-dimethylallyl pyrophosphate units reacting with the appropriate sites on the phenolic nucleus[9]. The mechanism for such C- and O- prenylations for the γγ-dimethylallyl coumarins is illustrated in figure 9. The double bond of the isoprenoid group can be further modified by oxygenation to the isopentenyl epoxide as in asculeatin (**52**), which can, in turn, hydrolyse to an isopentane diol[9] as in toddalolactone (**53**).



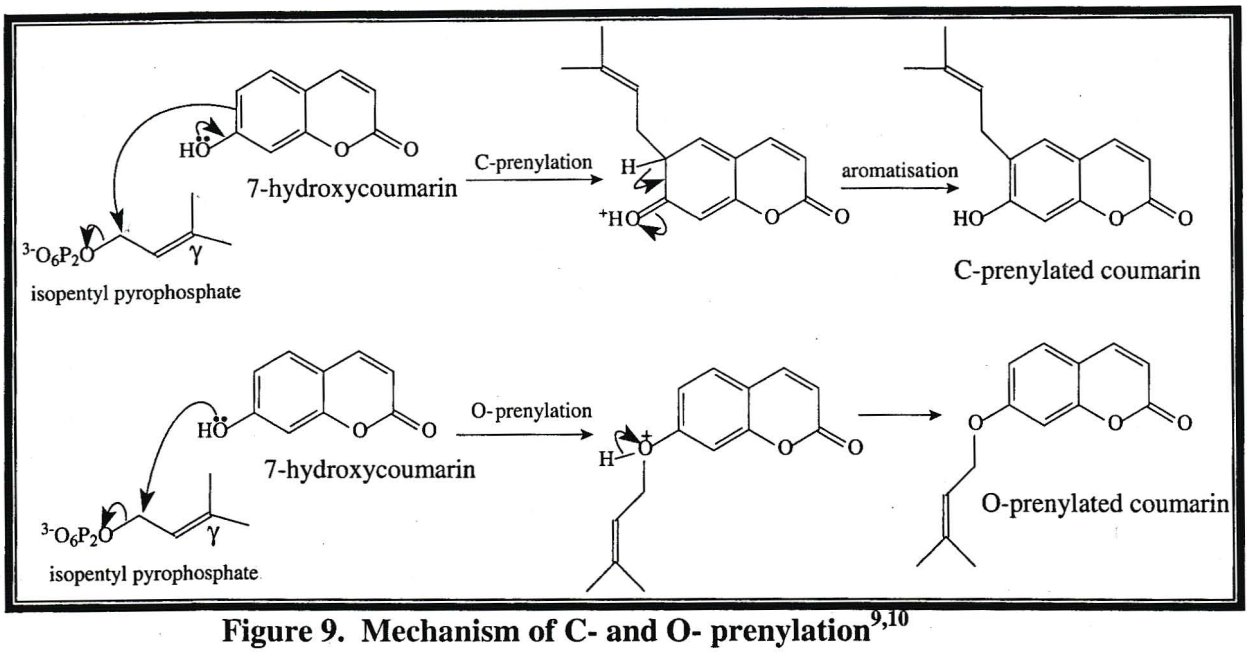


Figure 9. Mechanism of C- and O- prenylation[9,10]

Cyclisation of the prenylated side chains with neighbouring hydroxyl groups is very common, resulting in either fused furanyl or chromane rings and thus forming furano- and pyranocoumarins respectively[1]. The biosynthesis of the pyranocoumarins will be discussed further as compounds containing a pyran ring were isolated from the *Cedrelopsis* species in this work. Pyranocoumarins are 2',2'-dimethylchromene derivatives which can be derived from both the C- and O- prenylated derivatives. In the C- prenylated derivatives, oxidation of the phenolate anion is followed by a proton loss from the prenyl side chain, resulting in a quinone intermediate which then cyclises to form the 2',2'-dimethylchromene derivative[7,11]. Direct cyclisation can also occur in the presence of NADPH, which then results in a 2',2'-dimethylchroman derivative[9]. The mechanisms for these cyclisations are given in figure 10.

Pyranocoumarins can also be formed by acid catalysed ring closure of γγ-dimethylallyl epoxides. This is shown in figure 11, for the coumarins, xanthyletin and seselin[7,9,10].

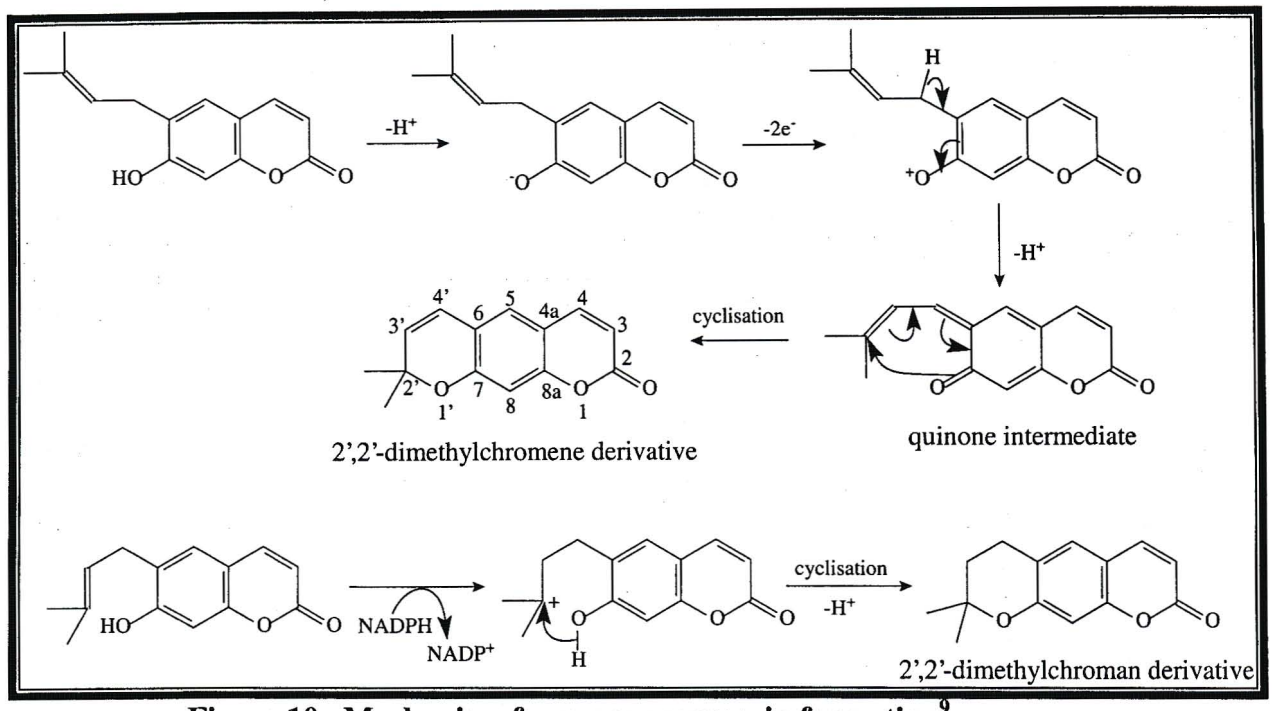


Figure 10. Mechanism for pyranocoumarin formation[9]

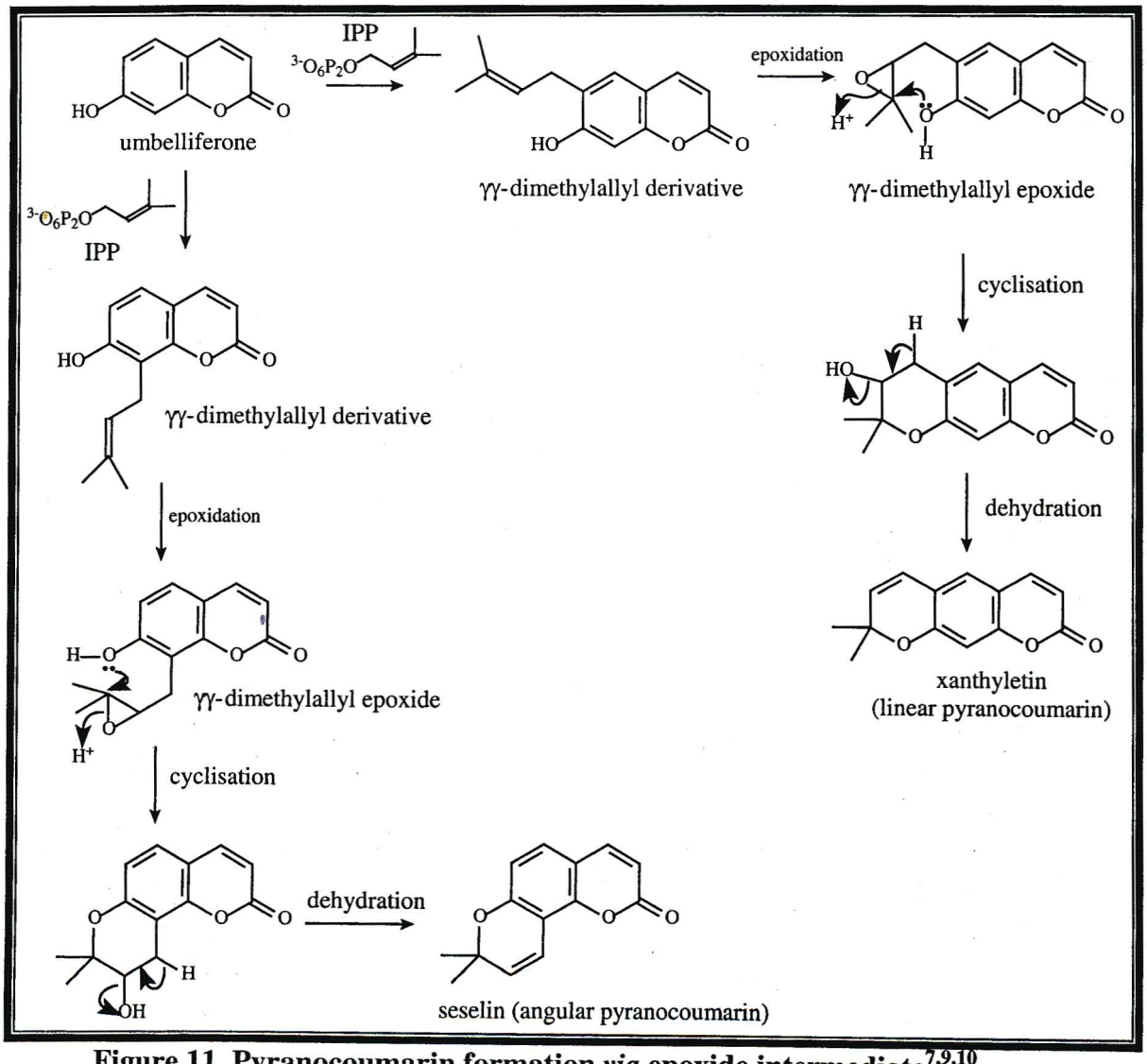


Figure 11. Pyranocoumarin formation *via* epoxide intermediate[7,9,10]

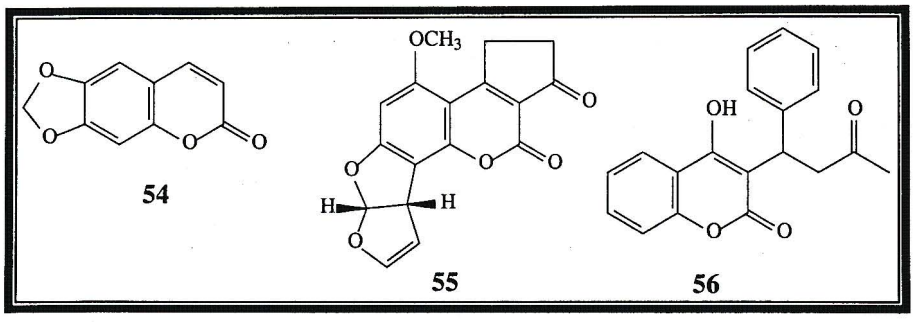
2.3 Biological activity of coumarins

Coumarins have shown biological activity, with coumarin itself being toxic to mammals[12]. Derivatives of umbelliferone (7-hydroxycoumarin) have attracted attention as sun-burn preventatives, as they absorb a wide range of ultraviolet frequencies, dissipate the energy as fluoresence and, in addition, modify the erythermal response to ultra violet light[1]. Umbelliferone itself is active against undulant fever (infection by *Brucella malitensis* and *B. abortus*)[1]. Mammein (**10**) has insecticidal properties, which made the compound of special interest as insecticidal properties had not been found in coumarins before[1]. Dicoumarol (**13**) was found to be the active compound in spoiled sweet clover (*Melilotus alba*), which, when eaten by cattle, interferes with the mechanism of blood clotting, so that even slightly injured animals may bleed to death[1]. Derivatives of dicoumarol are now used clinically to prevent the incidence of thrombosis and to aid the dissolution of clots already formed[1]. Not much is known of the mechanism by which the compound operates, but the methylene ether ayapin (**54**) has similar activities, suggesting that the methylene group in the molecule may have a role in the mechanism[1]. Novobiocin (**15**) is a useful antibiotic compound[1,5].

Furanocoumarins have an outstanding toxic action on fish, but relatively little on other animals[1]. This has important applications as plant extracts containing these coumarins can be added to streams to paralyse the fish, which can then be caught readily, without affecting their edibility[1]. Many plants, e.g. *Ruta graveolens* owe their poisonous character to their furocoumarin content[1]. Furanocoumarins, in general appear to produce photosensitivity in skin[1]. Psoralene (**17**) and its derivatives have skin photosensitising abilities, causing dermatitis with the exposure of skin to light[5,12]. Psoralene (**17**) has also been recommended for the treatment of alopecia[1]. The seeds of *Psoralea corylifolia*, an important plant in the Hindu Ayurvedic system of medicine, contain many coumarins and have found uses as laxatives, diuretics and anthelmintics[1].

Aflotoxins, metabolites of *Aspergillus flavus* are intense liver poisons and amongst the most potent known carcinogens[5]. Aflotoxin B1 (**55**) is an example of an aflotoxin. The 3-phenylcoumarins have estrogenic activity[5] and a synthetic

derivative of 4-hydroxycoumarin (**56**) is an effective rat poison that has been sold for many years under the commercial name, Warfarin[5].



2.4 References

1. Dean, F.M. (1963) Coumarins, *in Naturally Occurring Oxygen Ring Compounds*, Butterworths and Co. Ltd, London, pp 176-219.
2. Dean, F.M. (1963) Chromenes and chromans, *in Naturally Occurring Oxygen Ring Compounds*, Butterworths and Co. Ltd, London, pp 220-250.
3. Herbert, R.B. (1989) *The Biosynthesis of Secondary Metabolites*, 2nd Edition, Chapman and Hall Ltd., London, Chapter 5, pp 96-119.
4. Vickery, M.L. and Vickery, B. (1981) *Secondary Plant Metabolism*, MacMillan Press Ltd., London, Chapter 6., pp 157-182.
5. Brown, S.A. (1980) The biochemistry of coumarins, *in Secondary Plant Products*, Editors: Bell, E.A. and Charlwood, B.V., Springer-Verlag Berlin-Heidelberg, Germany, pp 59-89.
6. Dewick, P.M. (1986) The biosynthesis of shikimate metabolites, *Natural Product Reports*, **3** (6), 565-585.
7. Murray, R.D.H., Mendez, J. and Brown, S.A. (1982) *The Natural Coumarins – Occurrence, Chemistry and Biochemistry*, John Wiley and Sons Ltd., Chichester, Chapters 1,2,8, pp 1-20, 163-207.
8. Stafford, H.A. (1974) The metabolism of aromatic compounds, *Ann. Rev. Plant Physiol.*, **25**, 459-486.

9. Ollis, W.D. and Sutherland, I.O. (1961) Isoprenoid units in natural phenolic compounds, *in Recent Developments in the Chemistry of Natural Phenolic Compounds*, Editor: Ollis, W.D., Pergamon Press Ltd., London, pp 74-118.
10. Torsell, K.B.G. (1983) *Natural Product Chemsitry – A Mechanistic and Biosynthetic Approach to Secondary Metabolism*, John Wiley and Sons Ltd., Chichester, Chapter 2, 58-106.
11. Floss, H.G. (1980) The shikimate pathway, *in Secondary Plant Products*, Editors: Bell, E.A. and Charlwood, B.V., Springer-Verlag Berlin-Heidelberg, Germany, pp 59-89.
12. Brown, S.A. (1979) Biosynthetic studies on coumarins, *Planta Medica*, **36** (4), 299-310.
13. Mulholland, D.A., Iourine, S.E., Taylor, D.A.H and Dean, F.M. (1998) Coumarins from *Ekebergia pterophylla*, *Phytochemistry*, **47**, 1641-1644.

Chapter 3. Extractives from *Cedrelopsis grevei* (Ptaeroxylaceae)

3.1 Introduction

The genus *Cedrelopsis* has been placed in the Sapindaceae, Rutaceae and the Meliaceae families, but is now placed in the family Ptaeroxylaceae with the monospecific genus *Ptaeroxylon* after showing similarities in morphology and structure to the secondary xylem of *Ptaeroxylon obliquum*[1]. Furthermore, the pollen of *Cedrelopsis* and *Ptaeroxylon* has also been found to be very similar[1]. *Cedrelopsis grevei* and *Ptaeroxylon obliquum* both contain ptaeroxylin (**13**) and ptaeroglycol (**15**)[2], providing chemical evidence that a relationship between the two genera does exist.

Cedrelopsis grevei Baill. (fig. 1), commonly called Katrafay, is amongst the many medicinal plants of Madagascar, being used to relieve muscle fatigue when the bark is soaked in hot water[3]. Previous investigations have found this plant to contain chromones and coumarins[4,5,6,7] (fig. 2 and fig. 3). Recently, two novel limonoids of unusual structure, cedmilinol (**21**) and cedmiline (**22**) have been isolated from *C. grevei*[3]. These compounds were isolated from a stem bark specimen collected in the north of Madagascar. The present study was undertaken to ascertain whether seed of the same species contained compounds similar to **21** and **22**. Other compounds isolated previously from the wood and bark of *Cedrelopsis grevei* include the coumarins, cedrelopsin (**1**)[4], *O*-methylcedrelopsin (**2**), scoparone (**3**), cedrecoumarin A (**4**), norbraylin (**5**) and cedrecoumarin B (**6**)[5] (fig.2), the chromones peucinin (**7**), heteropeucinin (**8**), greveiglycol (**9**), alloptaeroxylin (**10**), alloptaeroxylin methyl ether (**11**), greveichromenol (**12**)[6], ptaeroxylin (**13**)[6,7], ptaeroxylinol (**14**)[5] and ptaeroglycol (**15**)[6] (fig. 3), a chromene, alloevodionol (**16**)[8], two acetophenones, 2,4-dihydroxy-6-methoxy-3-formylacetophenone (**17**) and 2,6-dihydroxy-4-methoxyacetophenone (**18**)[8], an acetylcoumaran, 2-(2*-hydroxyisopropyl)-6-hydroxy-4-methoxy-7-acetylcoumaran (**19**)[8] and a common terpenoid, β-amyrin (**20**)[3] (fig. 4). The seed has not been examined previously.

Figure 1. *Cedrelopsis grevei* in a Madagascan forest

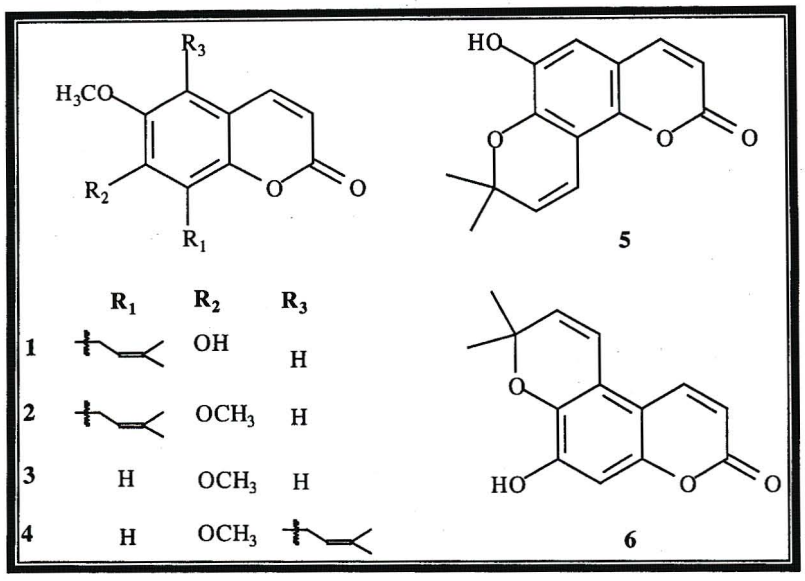


Fig. 2. Coumarins isolated from *Cedrelopsis grevei*.[4,5]

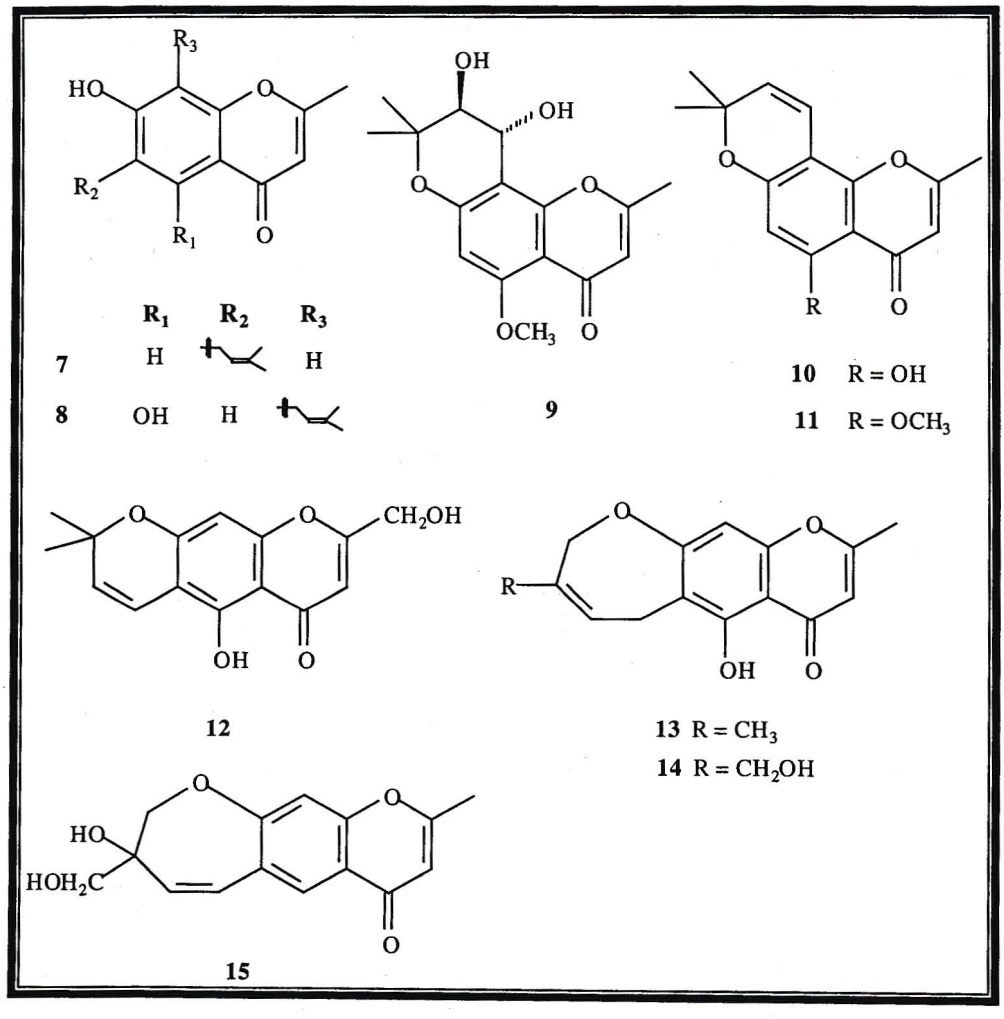


Fig. 3. Chromones isolated from *Cedrelopsis grevei*.[5-7]

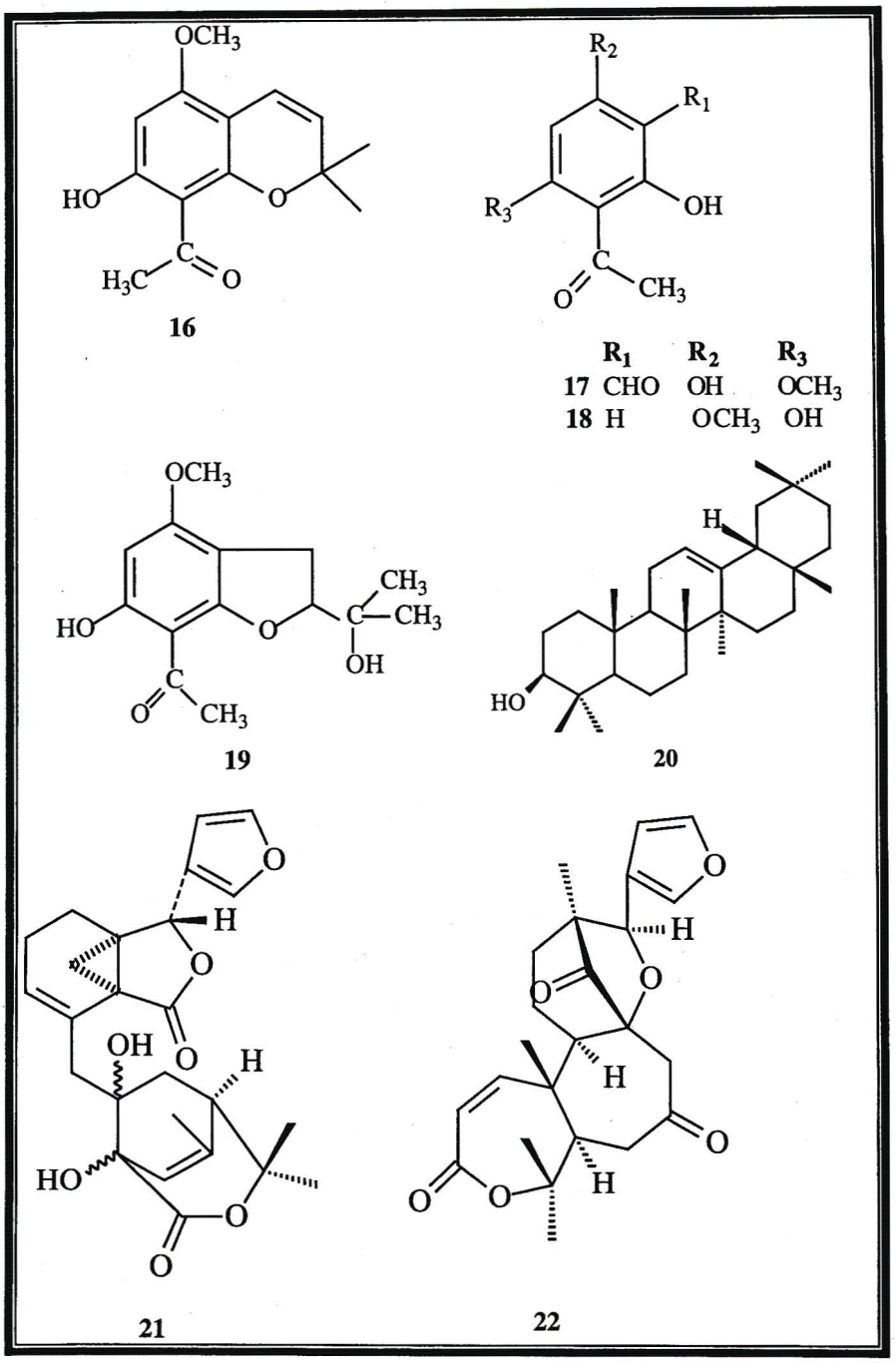


Fig. 4. Miscellaneous compounds isolated from *Cedrelopsis grevei*.[3,8]

3.2 Results and Discussion

The dried fruit and seed of *Cedrelopsis grevei* Baill. were extracted successively with dichloromethane and methanol. Proton NMR spectroscopy of the crude dichloromethane and methanol extracts showed similar components. The dichloromethane extract was separated by column chromatography using silica gel as the stationary phase and an ethyl acetate : hexane step gradient as the mobile phase. This yielded a dihydrochalcone, uvangoletin (**I**), a flavanone, 5,7-dimethoxypinocembrin (**II**), cardamonin (**III**), flavokawin B (**IV**), 2'-methoxyhelikrausichalcone (**V**), cedreprenone (**VI**) and cedrediprenone (**VII**) (fig. 5). Three of these compounds, 2'-methoxyhelikrausichalcone (**V**), cedreprenone (**VI**) and cedrediprenone (**VII**) have not been isolated previously.

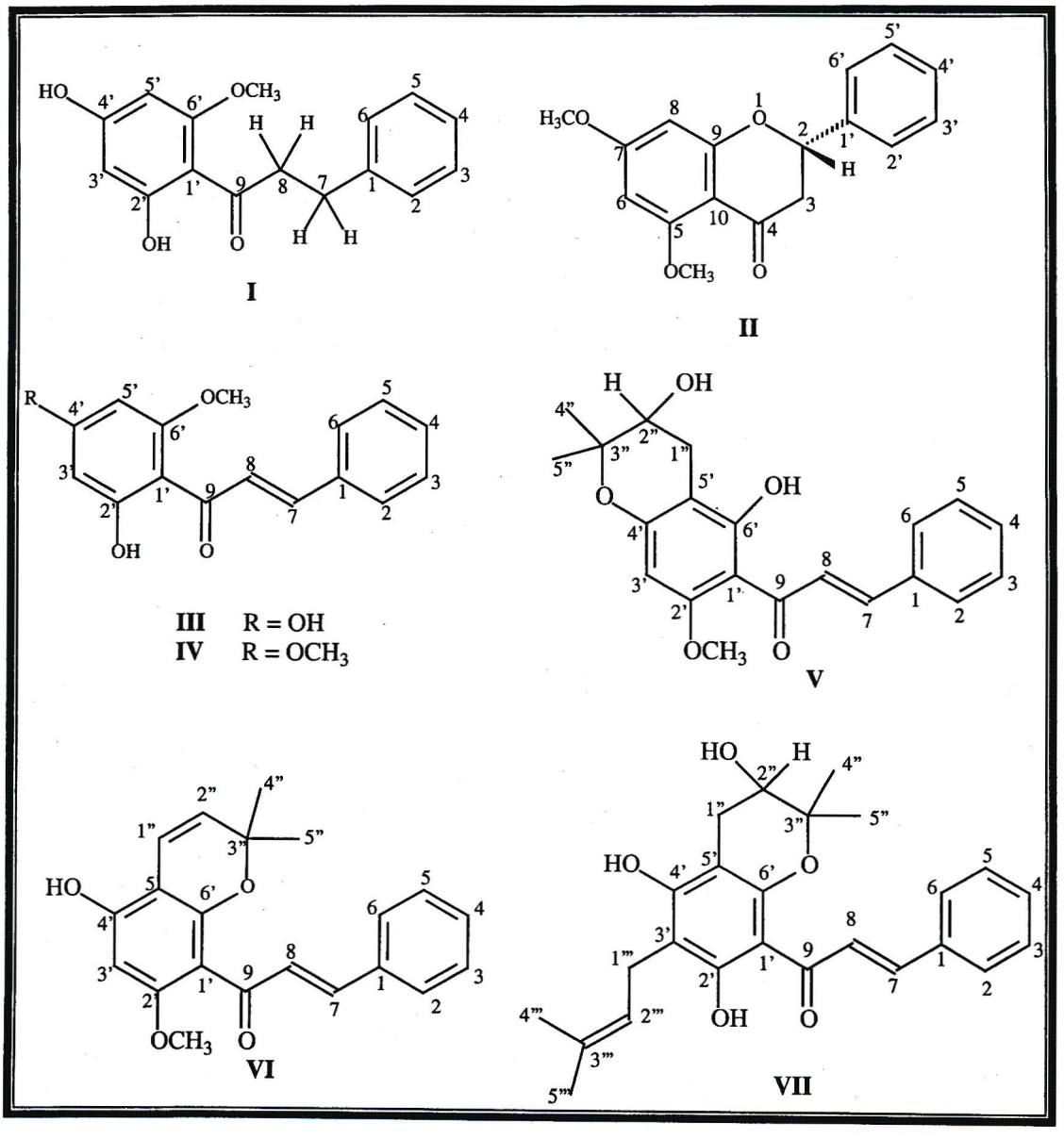
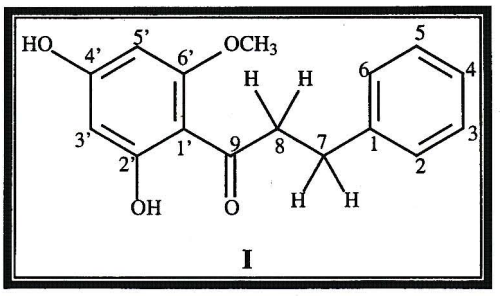


Fig. 5. Compounds isolated from the fruit of *Cedrelopsis grevei*.

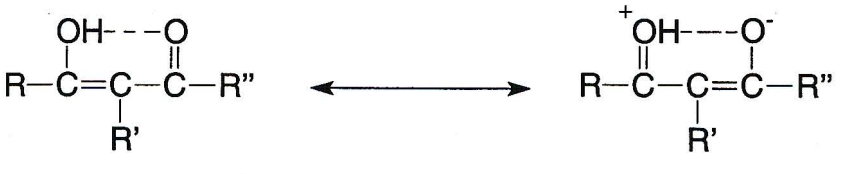
3.2.1 Structural elucidation of 2',4'-dihydroxy-6'-methoxydihydrochalcone, uvangoletin (I) (CED58A)

Compound **I** was identified as a dihydrochalcone by its ^{1}H NMR, UV, IR and mass spectra. It was isolated as a yellow crystalline material with a melting point of 190 °C and had a molecular formula of $C_{16}H_{16}O_4$ based on high resolution MS (Found *m/z* 272.10427, required 272.10485).



I

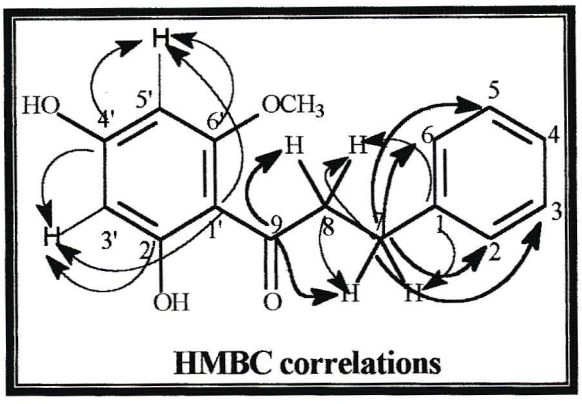
Compound **I** gave UV absorption maxima at λ_{max}^{MeOH} nm 290 (log ε = 4.11), 218 (3.84). These UV absorption maxima are characteristic of a dihydrochalcone[9]. The IR spectrum of compound **I** showed absorptions at 3376 cm^{-1} and 1635 cm^{-1}. The broad peak at 3376 cm^{-1} was due to a hydroxy group stretch and the intense peak at 1635 cm^{-1} was due to the carbonyl stretch. β-hydroxy ketones have a chelation effect which gives rise to very considerable shifts of the carbonyl frequency[10]. This absorption arises from a carbonyl group which has had its double bond character reduced by resonance between the following forms:



This resonance effect is described as conjugate chelation[10] and explains the low frequency carbonyl stretch of compound **I**.

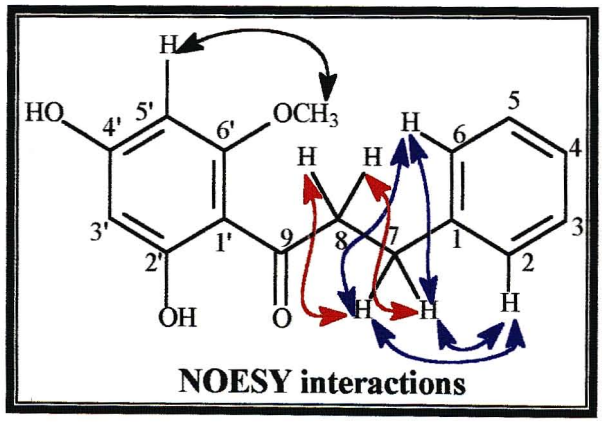
The ^{1}H NMR spectrum of compound **I** showed the presence of a monsubstituted aromatic ring with the appearance of a four-proton multiplet at δ_H 7.22, attributed to H-2, H-3, H-5 and H-6 and a one-proton multiplet attributed to H-4. The two-proton methylene resonances appearing as triplets at δ_H 2.91 and δ_H 3.24 (J = 8.06 Hz) were

attributed to 2H-7 and 2H-8 respectively. COSY correlations could also be seen between these two resonances. The resonance ascribed to C-7 showed HMBC correlations with the H-2/3/5/6 proton resonance indicating that C-7 was adjacent to the monosubstituted aromatic ring. The carbon resonance at δ_C 204.2 was attributed to the carbonyl group at C-9. This carbonyl group showed HMBC correlations with 2H-7 and 2H-8.



HMBC correlations

Meta coupling between the two aromatic proton resonances at δ_H 5.87 (H-3') and δ_H 5.93 (H-5') was evident (J = 2.2 Hz). These proton resonances were assigned to H-3' and H-5' since the 2',4' and 6' positions are usually oxygenated. The other possibilities for *meta* coupling to occur are either the 2' and 4' positions or the 4' and 6' positions. These substitution patterns are very uncommon. The methoxy group proton signal at δ_H 3.81 must be placed at the 6'- position as if it were placed at the 4'-position then the benzene ring would be symmetrical. If the benzene ring were symmetrical, H-3' and H-5' would be equivalent and would appear as one resonance in the ^{1}H NMR spectrum, which is not the case. NOESY interactions could be seen for H-5' and the 6'-methoxy group proton resonance.



NOESY interactions

A molecular ion peak at *m/z* 272 in the mass spectrum, as well as the base peak at *m/z* 167, due to the cleavage between the C-8/C-9 bond provided further evidence for the structure of compound **I**.

^{1}H and ^{13}C NMR assignments were made with the aid of HSQC and HMBC spectra and are reported in table 1. The isolation of compound **I** has been reported previously from the roots of *Uvaria angolensis* (Annonaceae)[11]. A derivative of this compound, 2',4',6'-trihydroxydihydrochalcone has been shown to have antimicrobial activity[12].

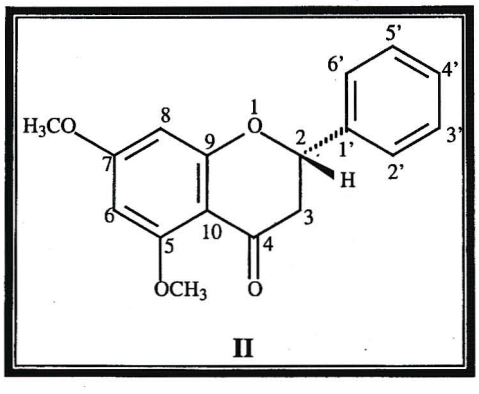
Table 1. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound I, uvangoletin.

Pos.	δ_H, 400 MHz, CD_3OD	δ_H (lit.[11]), 60 MHz, Me_2CO-d_6)	δ_C, 100 MHz, CD_3OD	δ_C (lit[11])	HMBC correlations	COSY correlations	NOESY interactions
1			141.8	142.7	H-2/3/5/6,2H-7,2H-8		
2/6	7.22, m	7.20, m	128.2	129.1	2H-7	H-4	2H-7
3/5	7.22, m	7.20, m	128.2	129.2	2H-7	H-4	
4	7.15, m	7.20, m	125.7	126.6	H-2/3/5/6	H-2/3/5/6	
7	2.91, t (8.06)	2.77-3.47, m	31.0	31.4	H-2/3/5/6,2H-8	2H-8	H-2/6, 2H-8
8	3.24, t (8.06)	2.77-3.47, m	45.9	46.1	2H-7	2H-7	2H-7
9			204.2	205.0	2H-7,2H-8		
1'			104.7	105.7	H-3',5'		
2'			167.0 #	164.5 #	H-3'		
3'	5.87, d (2.2)	5.91, d (2)	95.8	96.9		H-5'	
4'			165.2	165.6	H-3',5'		
5'	5.93, d (2.2)	5.97, d (2)	90.9	91.9		H-3'	6'-OCH_3
6'			163.6 #	168.3 #	H-5'		
6'-OCH_3	3.81, s	3.87, s	55.0	56.1			H-5'
OH		13.80, s					

#, assignments were revised based on HMBC data.

3.2.2 Structural elucidation of 5,7-dimethoxypinocembrin (II) (CED90E)

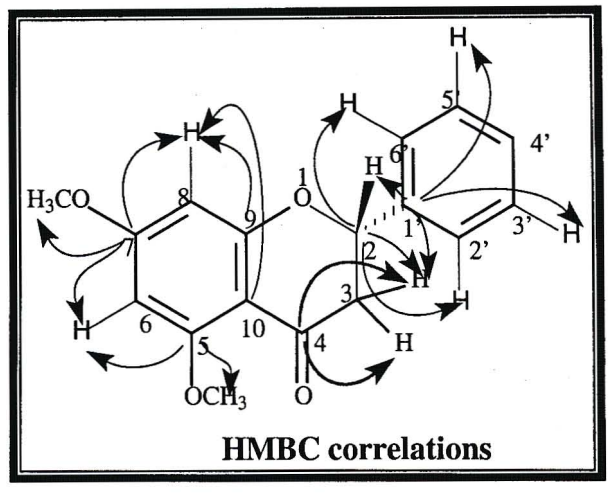
Compound **II** was identified as a flavanone from its characteristic ^{1}H NMR spectrum, UV, IR and mass spectra. It was isolated as white needles with a melting point of 159 °C and had a molecular formula of $C_{17}H_{16}O_4$ based on high resolution MS (Found *m/z* 284.10392, required 284.10485).



II

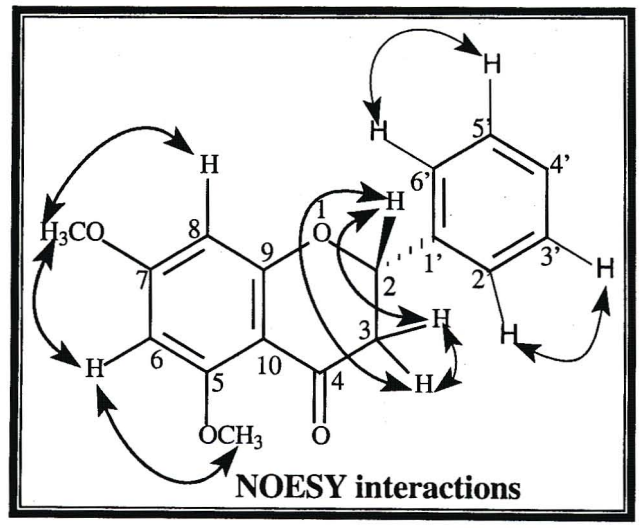
Compound **II** gave UV absorption maxima at λ_{max}^{MeOH} nm and 283 (3.65) and 215 (3.50). These UV absorption maxima are characteristic for a flavanone[9]. The IR spectrum of compound **II** showed an absorption at 1613 cm^{-1} which was due to the carbonyl stretch. The low carbonyl frequency is not unusual, as considerable carbonyl frequency shifts have also been known to occur in β-keto enol ethers, where neither hydrogen bonding nor chelation occurs[10].

In the ^{1}H NMR spectrum, the monosubstituted aromatic ring appeared as a two-proton multiplet at δ_H 7.47 attributed to H-2' and H-6' and a three-proton multiplet at δ_H 7.36 attributed to H-3', H-4' and H-5'. An AMX coupled system could be seen with resonances at δ_H 5.44, δ_H 3.00 and δ_H 2.72. These proton resonances integrated to one proton each and were attributed to H-2, H-3a and H-3b respectively. The C-2 carbon resonance at δ_C 79.2 showed HMBC correlations to the H-2'/6' proton resonance. COSY coupling could also be seen between the H-2, H-3a and H-3b proton resonances. The carbonyl group at C-4 appeared at δ_C 190.5 in the ^{13}C NMR spectrum and this resonance showed HMBC correlations to H-3a and H-3b as well as a weak HMBC correlation to H-2.



HMBC correlations

Meta coupling between the two aromatic proton resonances at δ_H 6.20 (H-8) and δ_H 6.18 (H-6) was evident (J = 2.3 Hz). These proton resonances were assigned to H-8 and H-6 based on HMBC correlations. The methoxy group proton resonance integrated to six protons, which indicated that two methoxy groups were present. These methoxy groups had to be placed at the 5- and 7- positions. NOESY interactions could be seen between this six proton methoxy group proton resonance and H-6 and H-8.



NOESY interactions

The molecular ion peak at *m/z* 284 in the mass spectrum as well as the base peak at *m/z* 180, due to the cleavage between the oxygen at the 1- position and C-2 and cleavage between the C-3/C-4 bond provided further evidence for the structure of compound **II**.

^{1}H and ^{13}C NMR assignments were made with the aid of HSQC and HMBC spectra and are reported in table 2. Compound **II** had an optical rotation of -46.10^0 which

was consistent with the (*S*)-form of 5,7-dimethoxypinocembrin which has been isolated previously from the leaves of *Eucalyptus sieberi* (Myrtaceae)[20] and from the trunk wood of *Aniba riparia* (Lauraceae)[21].

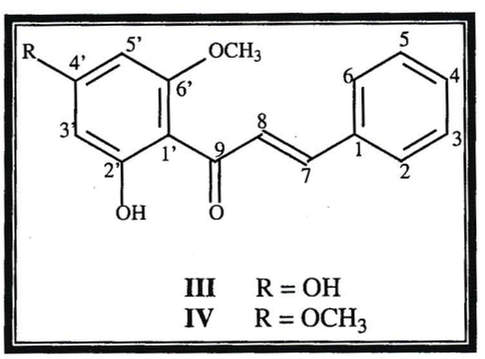
Table 2. ^{1}H, ^{13}C, COSY, HMBC and NOESY data for compound II; 5,7-dimethoxypinocembrin (400 MHz, CD_3OD)

Pos.	δ_H	δ_H, * lit. 20	δ_C	HMBC correlations	COSY correlations	NOESY interactions
2	5.44, dd (12.82, 2.96)	5.40, q	79.2	H-2',6',3a	H-3a, 3b	H-3a, 3b
3a	3.00, dd (16.67, 12.82)	3.02, q	45.2		H-2, 3b	H-2, 3b
3b	2.72, dd (16.67, 3.10)	2.77, q			H-2, 3a	H-2, 3a
4			190.5	H-2,H-3a,3b		
5			162.5	H-6, 5-OCH_3		
6	6.18, d (2.3)	6.10, d	93.8		5-OCH_3, 7-OCH_3	5-OCH_3, 7-OCH_3
7			167.0	H-6,8,7-OCH_3		
8	6.20, d (2.3)	6.18, d	92.7		7-OCH_3	7-OCH_3
9			165.4	H-8		
10			105.4	H-8		
1'			139.2	H-3'/5',2,3a		
2'/6'	7.47, m	7.42, m	126.0	H-3'/4'/5',2	H-3'/5'	H-3'/5'
3'/5'	7.36, m	7.42, m	128.4	H-2'/6'	H-2'/6'	H-2'/6'
4'	7.36, m	7.42, m	128.3	H-2'/6'		
5-OCH_3	3.82 , s	3.89, s	55.2		H-6	H-6
7-OCH_3	3.82, s	3.74, s	55.1		H-6, 8	H-6, 8

* 100 MHz, $CDCl_3$

3.2.3 Structural elucidation of 2',4'-dihydroxy-6'-methoxychalcone (cardamonin) III (CED67C2) and 2'-hydroxy-4',6'-dimethoxychalcone (flavokawin B) IV (CED27B)

Compound **III** was isolated as a yellow crystalline material with a melting point of 195 ^{0}C, having the molecular formula $C_{16}H_{14}O_4$ based on high resolution MS (Found *m/z* 270.09123, required 270.08920). Compound **IV** was isolated as a yellow crystalline material with a lower melting point of 92 ^{0}C and was found to have the molecular formula $C_{17}H_{16}O_4$ based on high resolution MS (Found *m/z* 284.10572, required 284.10485).



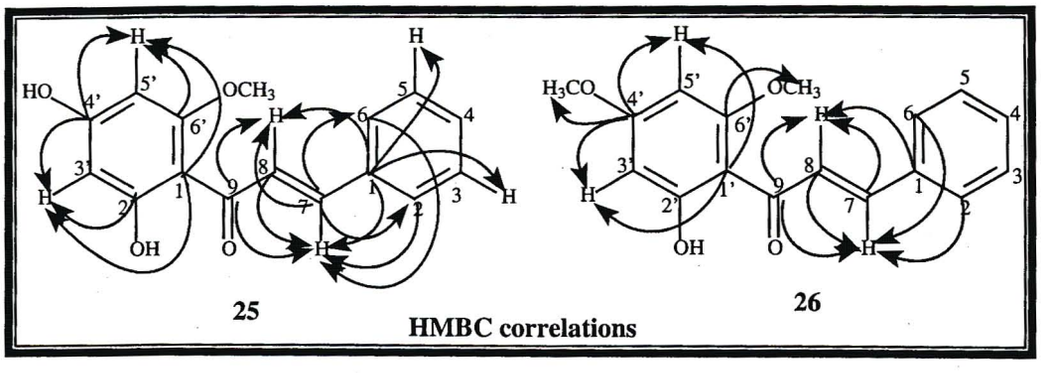
III R = OH
IV R = OCH_3

These compounds gave UV absorption maxima characteristic of chalcones[9] at λ_{max}^{MeOH} nm 341 (log ε = 3.81), 268 (3.97) and 218 (3.41) for compound **III** and λ_{max}^{MeOH} nm 338 (log ε = 4.22) and 231(4.54) for compound **IV**. The IR spectra of compounds **III** and **IV** showed absorptions at ν_{max}^{NaCl} 1629 cm^{-1} and ν_{max}^{NaCl} 1635 cm^{-1} respectively, typical of carbonyl stretches with conjugated chelation[10]. In the IR spectrum of compound **III,** a broad hydroxy group stretch at 3412 cm^{-1} could be seen but in the IR spectrum of compound **IV**, a hydroxy group peak was not clearly visible.

The ^{1}H NMR spectra of compounds **III** and **IV** were similar to those of compounds **I** and **II** in that they both showed the presence of a monosubstituted aromatic ring and two *meta* coupled aromatic protons in ring A, although in compound **IV**, these two *meta* coupled proton resonances were superimposed. As in compound **II**, the monosubstituted aromatic ring was indicated by the presence of a three-proton multiplet at δ_H 7.40 attributed to H-3, H-4 and H-5 and a two-proton multiplet at δ_H 7.62 attributed to H-2 and H-6 for both compounds **III** and **IV**. The *meta* coupled

aromatic protons in ring B in compound **III** appeared at δ_H 5.91 and δ_H 5.99 and were attributed to H-3' and H-5' respectively. In the ^{1}H NMR spectrum of compound **IV**, both these resonances occured at δ_H 6.09. Compound **III** had one methoxy group present at δ_H 3.90 and compound **IV** had two methoxy groups present at δ_H 3.82 and δ_H 3.93. In compound **III**, the methoxy group had to be placed at the 6'- position and in compound **IV** these methoxy groups had to be placed at the 4'- and 6'- positions respectively. Placing the methoxy group at the 4'- position in compound **III** and the 2' and 6' positions in compound **IV** would make the molecule symmetrical. This is not the case as the H-3' and H-5' proton resonances as well as the C-3' and C-5' carbon resonances are not equivalent.

Like compounds **I** and **II**, compounds **III** and **IV** also had carbonyl carbon resonances in their ^{13}C NMR spectra at δ_C 192.5 and δ_C 192.9 respectively. The ^{1}H NMR spectra of these two compounds differed from compounds **I** and **II** in that no methylene proton resonances could be seen, but a pair of downfield doublets with large coupling constants (15.8 Hz for compound **III** and 15.5 Hz for compound **IV**) appeared. These were characteristic of *trans* olefinic protons and are typical for chalcones with a *trans*-7,8-double bond. These resonances appeared at δ_H 7.70 (H-7) and δ_H 7.90 (H-8). The resonance at δ_H 7.70 was assigned to H-7 as the C-7 carbon resonance occurring at δ_C 141.6 in compound **III** showed HMBC correlations with the H-2/6 proton resonance. COSY coupling between H-7 and H-8 was also seen. The carbonyl group carbon at C-9 showed HMBC correlations with both H-7 and H-8.



25 **26**

HMBC correlations

In the mass spectrum of compound **III**, the molecular ion peak at *m/z* 270 was the base peak and in the mass spectrum of compound **IV**, a molecular ion peak at *m/z* 284

could be seen with the base peak at *m/z* 207, which was due to cleavage of the monosubstituted B ring.

^{1}H and ^{13}C NMR assignments were made with the aid of HSQC and HMBC spectra and are reported in tables 3 and 4. The isolation of compound **III** has been previously reported from *Alpinia speciosa*[13] and *Alpinia katsumadai* (Zingiberaceae)[13]. Compound **IV** has been previously reported together with compound **III,** in the rhizomes of *Alpinia speciosa* and *Alpinia japonica* (Zingiberaceae)[13] and from the roots of *Piper methysticum* (Piperaceae)[14].

Table 3. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound III, cardamonin.

Pos.	δ_H, 400 MHz, CD_3OD	δ_H (lit[13]*)	δ_C, 100 MHz, CD_3OD	δ_C (lit[13]*)	HMBC correlations	COSY correlations	NOESY interactions
1			135.6	136.5	H-3,5,7,8		
2/6	7.62, m	7.36-7.76, m	128.0	129.0	H-3,4,5,7	H-3,5,7	H-3,5
3/5	7.40, m	7.36-7.76, m	128.7	129.7	H-2/6	H-2/6	H-2/6
4	7.40, m	7.36-7.76, m	129.9	130.7	H-2,6		
7	7.70, d (15.8)	7.80, d (16)	141.6 #	128.6 #	H-2,6,8	H-8	H-2,6
8	7.90, d (15.8)	7.94, d (16)	127.6 #	142.4 #	H-7	H-7	H-2,6
9			192.5	193.0	H-7,8		
1'			105.4	106.4	H-3',5'		
2'			167.4	168.3	H-3'		
3'	5.91, d (2.2)	6.02, d (3)	95.9 ##	92.3 ##			
4'			165.5	165.8	H-3',5'		
5'	5.99, d (2.2)	6.08, d (3)	91.3 ##	97.0 ##		6'-OCH_3	6'-OCH_3
6'			163.5	164.3	H-5'		
6'-OCH_3	3.90, s	3.98, s	55.2	56.3		H-5'	H-5',8

#, assignments were revised based on HMBC data.
##, assignments were revised based on HMBC, COSY and NOESY data.
* Me_2CO-d_6 – solvent.

Table 4. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound IV, flavokawin B.

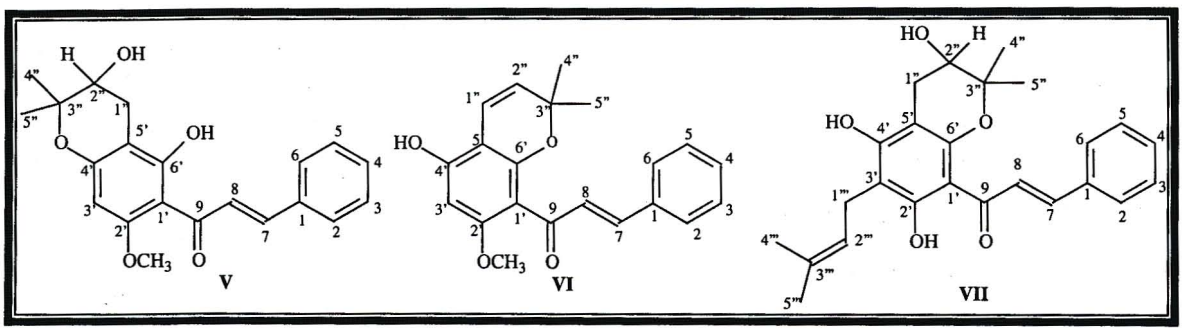
Pos.	δ_H, 400 MHz, CD_3OD	δ_H (lit[13] *), 60 MHz, Me_2CO-d_6)	δ_C, 100 MHz, CD_3OD	δ_C (lit[13] *)	HMBC correlations	COSY correlations	NOESY interactions
1			135.6	135.5	H-8		
2/6	7.62, m	7.32-7.92, m	128.1	128.3	H-7	H-3/5	H-3/5
3/5	7.40, m	7.32-7.92, m	128.8	128.7		H-2/6	H-2/6
4	7.40, m	7.32-7.92, m	130.0	130.0	H-2,6		
7	7.70, d (15.5)	unassigned	142.0 #	127.5 #	H-8	H-8	
8	7.90, d (15.5)	unassigned	127.5 #	142.2 #	H-7	H-7	
9			192.9	192.5	H-7,8		
1'			106.5	106.3	H-3', 5'		
2'			167.5	166.1			
3'	6.10, d (2.3)	6.10, d (3)	90.9	91.2		4'-OCH_3	4'-OCH_3
4'			166.7	168.3	H-3',5',4'-OCH_3		
5'	6.10, d (2.3)	5.96, d (3)	93.6	93.8		4',6'-OCH_3	4',6'-OCH_3
6'			162.9	162.4	6'-OCH_3		
4'-OCH_3	3.82, s	3.89, s	55.0	55.5		H-3',5'	H-3',5'
6'-OCH_3	3.93, s	3.88, s	55.4	55.5		H-5'	H-5'

#, assignments were revised based on HMBC data.
* Me_2CO-d_6 – solvent.

3.2.4 Structural elucidation of 2'-methoxyhelikrausichalcone (V) (CED 58B), cedreprenone (VI) (CED18) and cedrediprenone (VII) (CED 90C).

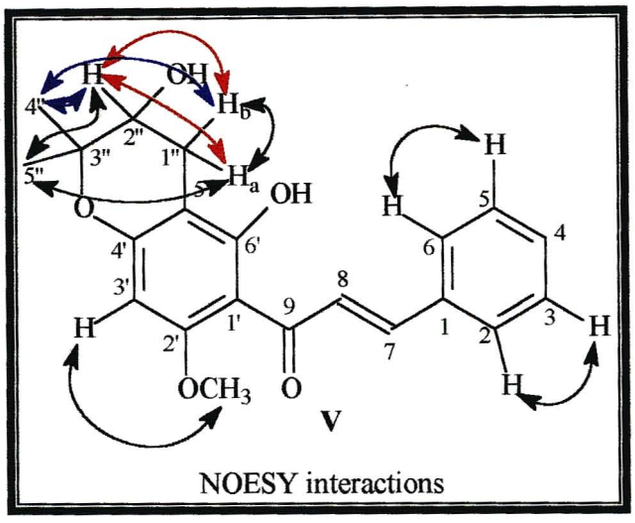
Compounds **V, VI** and **VII** were all prenylated at either the 5' position or the 3' and 5' positions. All three compounds were isolated as yellow crystalline compounds. Compound **V** had a melting point of 127-128 ^{0}C and had the molecular formula $C_{21}H_{22}O_5$, compound **VI** had a melting point of 134 °C and a molecular formula of $C_{21}H_{20}O_4$ and compound **VII** had a melting point of 153 °C with a molecular formula of $C_{25}H_{28}O_5$. The molecular formulae of compounds **V, VI** and **VII** were based on high resolution mass spectrometry (Found *m/z* 354.14690, required 354.14672

(compound **V**); found *m/z* 336.13675, required 336.13616 (compound **VI**); found *m/z* 408.19489, required 408.19367 (compound **VII**)).



These compounds gave UV absorption bands characteristic of chalcones[9]. Compound **V** had bands at λ_{max}^{MeOH} nm 338 (log ε = 4.36) and 205 (4.19), compound **VI** showed bands at λ_{max}^{MeOH} nm 336 (5.40), 291 (5.40) and 218 (5.05) and compound **VII** exhibited bands at λ_{max}^{MeOH} nm 346 (4.74), 268 (4.84), 261(4.83) and 216 (4.11). Compounds **V** and **VII** had absorptions in the IR spectrum exhibited by broad peaks at ν_{max}^{NaCl} 3425 cm^{-1} and 3377 cm^{-1} respectively. These were due to the hydroxy group stretch. In the IR spectrum of compound **VI**, the hydroxy band could not clearly be seen. All three compounds however showed IR absorptions of the carbonyl stretch at ν_{max}^{NaCl} 1639 cm^{-1}, 1649 cm^{-1} and 1629 cm^{-1} for compounds **V**,**VI** and **VII** respectively[10].

The ^{1}H NMR spectra of all three compounds showed the characteristic peaks for the monosubstituted benzene ring and the *trans* olefinic protons and the ^{13}C NMR spectrum showed the presence of the carbonyl group carbon. This indicated that they were chalcones similar to compounds **III** and **IV**. However, compounds **V** and **VI** possessed only one aromatic proton resonance on the B ring. In both cases this proton resonance was assigned to the 3' position because of NOESY interactions with the methoxy group proton resonance in both compounds **V** and **VI**. Compound **VII** had no aromatic proton resonances other than those for the monosubstituted benzene ring.



The ^{1}H NMR spectrum of compound **V** showed, in addition to the resonances mentioned above, an AMX coupled system with resonances at δ_H 3.77 (dd, 6.78, 5.49 Hz), δ_H 2.80 (dd, 17.03, 5.49) and δ_H 2.47 (dd, 17.03, 6.78). These were attributed to H-2", H-1a" and H-1b" respectively. Coupling in the COSY spectrum could also be seen between these three resonances. These resonances indicated that these protons were in a fixed system and must form part of a ring. The deshielded proton resonance at δ_H 3.77 suggested that a hydroxy group was present at the 2"- position. Two methyl group proton resonances appearing at δ_H 1.36 and δ_H 1.41 as two singlets in the ^{1}H NMR spectrum of compound **V**, together with the deshielded C-3" carbon resonance at δ_C 78.6 completes the resonances necessary for the isoprenyl group. The deshielded C-3" carbon resonance suggests that an oxygen substituent is attached to this carbon and since H-2", H-1a" and H-1b" are in a fixed system, C-3" must either be bonded to the oxygen at the 4'- or the 6'- positions. Since this carbon had no protons attached, the two methyl groups had to be at this position. The 3H-5" proton resonance showed NOESY interactions with the H-2" proton resonance only and the 3H-4" proton resonance showed NOESY interactions with both the H-2" and the H-1a" proton resonances. The C-3" carbon was therefore joined to the 4'- oxygen because if it were joined to the 6'- oxygen, molecular models showed that NOESY interactions would be seen between the methyl groups and the H-8 and H-2/6 protons.

The mass spectrum of compound **V** showed the molecular ion peak at *m/z* 354 and loss of the phenyl group resulted in the ion peak at *m/z* 277.

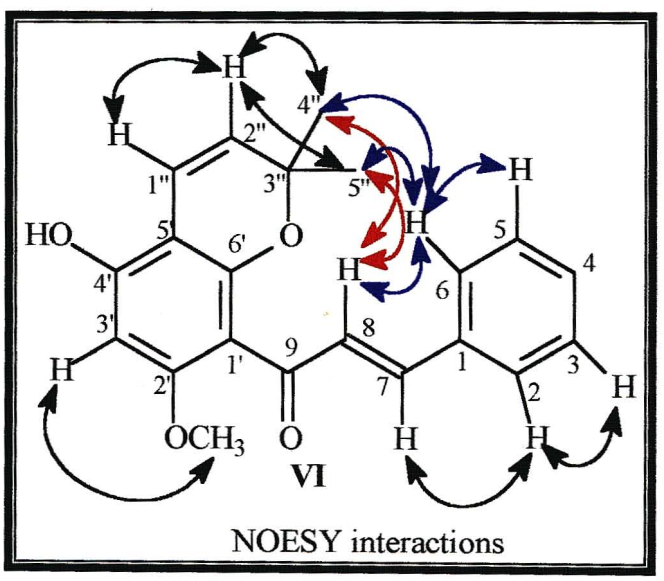
This compound was identified as 2'-methoxyhelikrausichalcone. The isolation of compound **V** has not been reported previously*. The 2'-hydroxy analogue, helikrausichalcone has been isolated from the aerial parts of *Helichrysum krausii*[16]. ^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are reported in table 5.

Table 5. ^{1}H, ^{13}C, COSY, NOESY and HMBC data for 2'-methoxyhelikrausichalcone, compound V.

Pos.	δ_H, 400 MHz, CD_3OD	δ_C, 100 MHz, CD_3OD	HMBC correlations	COSY correlations	NOESY interactions
1		135.7	H-3,5,7,8		
2/6	7.61, m	128.0	H-7	H-3/5	H-3/5
3/5	7.40, m	128.8		H-2/6	H-2/6
4	7.40, m	129.9			
7	7.65,d (15.75)	141.2	H-2,6	H-8	
8	8.04, d (15.75)	128.0		H-7	
9		193.0	H-7,8		
1'		106.1	H-3'		
2'		165.9	H-3',2'-OCH_3		
3'	6.10, s	91.8		2'-OCH_3	2'-OCH_3
4'		164.2	H-3',1a"		
5'		100.3	H-3',1a",2"		
6'		155.0	H-1a"		
2'-OCH_3	3.84, s	55.2		H-3'	H-3'
1a"	2.80, dd (17.03, 5.49)	25.6		H-1b",2"	1b", 2", 5"
1b"	2.47, dd (17.03, 6.78)			H-1a",2"	1a", 2", 4"
2"	3.77, dd (6.78, 5.49)	68.1	H-1a",3H-4",3H-5"	H-1a",1b"	H-1a", 1b", 3H-4", 3H-5"
3"		78.6	H-1a",3H-4",3H-5"		
4"	1.36, s	20.5	3H-5"		H-2"
5"	1.41, s	24.7	3H-4"		H-2", 1a"

* Although the Dictionary of Natural Products (1999) lists this compound as being isolated from *Helichrysum aphelexioides*, the three references listed[15,16,17], do not contain compound **V**. Only one reference[15], lists compounds isolated from *H. aphelexioides* and a similar compound to **V** is listed as one of the compounds isolated.

The ^{1}H NMR spectrum of compound **VI** showed that it was related to compound **V.** However the AMX coupled system was now replaced by a pair of doublets in the olefinic region. These proton resonances occurred at δ_H 6.56 and δ_H 5.52 with a coupling constant of 9.89 Hz. This is characteristic of *cis* coupling of olefinic protons and prompted the positioning of a double bond at the Δ1" position in compound **VI.** The deshielded carbon resonance of C-3" at δ_C 78.6 suggested that this carbon atom was attached to an oxygen atom. The methyl group proton resonances appeared as one singlet integrating to six protons at δ_H 1.52. The methyl carbon resonances were also present as one resonance at δ_C 27.0. Molecular models and a computer simulation showed that both the methyl groups on the isoprenyl ring were equivalent due to the planarity of the molecule, explaining why these methyl groups appeared as one resonance. This methyl resonance now showed NOESY interactions with the H-8 and the H-2/6 proton resonances.



The mass spectrum of compound **VI** showed a molecular ion peak at *m/z* 336 and the loss of a methyl group resulted in the ion peak at *m/z* 321, providing further evidence for the structure of compound **VI**.

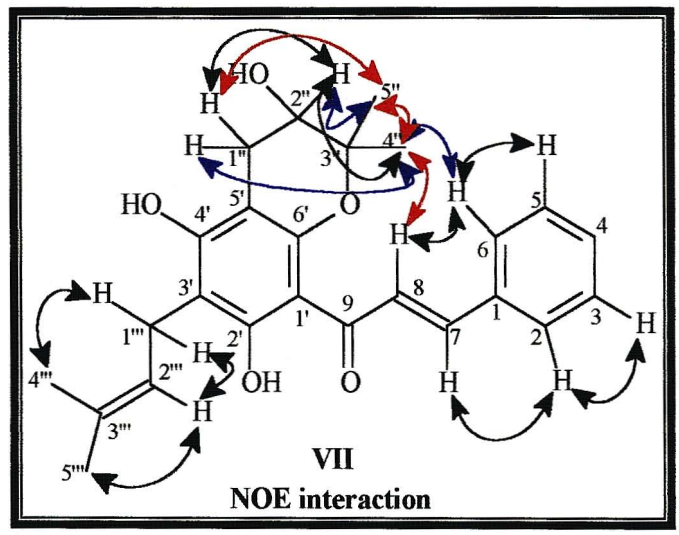
Compound **VI** has not been isolated previously and was given the name cedreprenone. ^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are given in table 6.

Table 6. ^{1}H, ^{13}C, COSY, HMBC and NOESY data for cedreprenone, compound VI.

Pos.	δ_H, 400 MHz, CD_3OD	δ_C, 100 MHz, CD_3OD	HMBC correlations	COSY correlations	NOESY interactions
1		135.6	H-8		
2/6	7.62, m	128.0	H-7	H-3/5	H-7,8,3/5
3/5	7.41, m	128.8		H-2/6	H-2/6
4	7.41, m	130.1			
7	7.71, d (15.75)	142.0		H-8	H-2/6
8	8.09, d (15.75)	127.4		H-7	H-2/6
9		192.9	H-7,8		
1'		106.0	H-3'		
2'		161.4	H-3',2'-OCH_3		
3'	6.10, s	92.3		2'-OCH_3	2'-OCH_3
4'		166.8	H-3'		
5'		103.3	H-3',2"		
6'		155.5	H-1"		
1"	6.56, d (9.89)	116.3		H-2"	H-2"
2"	5.52, d (9.89)	124.8	3H-4",3H-5"	H-1"	H-1", 3H-4", 3H-5"
3"		78.1	3H-4",3H-5"		
4"	1.52, s	27.0	3H-5"		H-8, 2/6, 2"
5"	1.52, s	27.0	3H-4"		H-8, 2/6, 2"
2'-OCH_3	3.86, s	55.3		H-3'	H-3'

The ^{1}H NMR spectrum of compound **VII** showed the presence of four methyl groups, indicating that two isoprenyl groups must be present in the molecule. This was confirmed by the absence of aromatic proton resonances in the B ring. The two isoprenyl groups must therefore be positioned at the 3' and the 5' positions since the 2', 4' and 6' positions are usually oxygenated and this was confirmed by the HMBC spectrum. The resonance at δ_H 5.16, a broad triplet was assigned to an olefinic proton next to a freely rotating methylene group and was attributed to H-2"' on the uncyclised isoprenyl unit. This olefinic proton, H-2"' split the methylene group proton resonance attributed to 2H-1"' into a doublet, which appeared at δ_H 3.25. COSY correlations could be seen between these two proton resonances, which confirms their assignment. Since the resonance at δ_H 3.04 was a double doublet (J = 8.59, 5.32 Hz), this meant that the protons at the 1"- position were not equivalent, no free rotation could occur

and the second prenyl group had to be part of a ring. This proton resonance was coupled in the COSY spectrum to the other deshielded triplet proton resonance at δ_H 4.78, which had to be bonded to an oxygen atom. This resonance was therefore assigned to H-2". This ring formed by the isoprenyl group could be formed with either the 6'- oxygen atom or the 4'- oxygen atom, but since the 3H-4" methyl resonance showed NOESY interactions with the H-8 and H-2/6 proton resonances, it was formed with the 6'-oxygen atom. The methyl resonances at δ_H 1.38 and δ_H 1.28 were attributed to 3H-4" and 3H-5" respectively because of HMBC correlations with C-2" and C-3". Likewise, the methyl resonances at δ_H 1.74 and 1.63 were attributed to 3H-4"' and 3H-5"' because of HMBC correlations with C-2"' and C-3"'.



VII

NOE interaction

A molecular model showed that all NOESY interactions observed are possible. Further evidence for the proposed structure was shown in the mass spectrum, which showed the molecular ion peak at *m/z* 408 as the base peak and the ion peak at *m/z* 393, which was due to the loss of a methyl group.

Compound **VII** is a novel compound and has been named cedrediprenone. Compound **VII** was found to inhibit the (luminol-enhanced) chemiluminescence of reactive oxygen metabolites generated by activated human granulocytes with an IC_{50} value of 8.1 ug/ml. It was also found to scavenge superoxide anions in a cell-free system with an IC_{50} value of 0.2 ug/ml. The inhibition of the chemiluminescence of reactive oxygen metabolites and the superoxide scavenging activity, suggest anti-inflammatory activity of cedrediprenone **VII**.

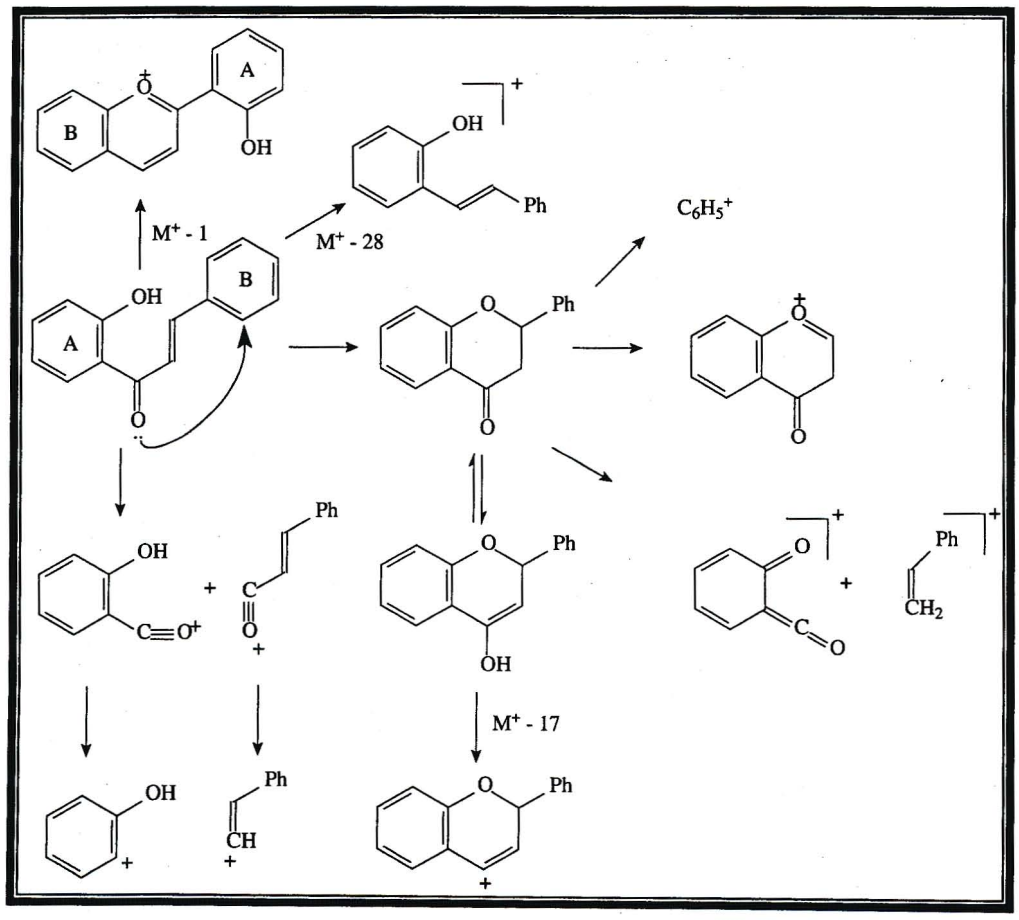
^{1}H and ^{13}C NMR assignments were made with the aid of HSQC and HMBC spectra and are reported in table 7.

Table 7. ^{1}H, ^{13}C, COSY, HMBC and NOESY data for cedrediprenone, compound VII.

Pos.	δ_H, 400 MHz, CD_3OD	δ_C, 100 MHz, CD_3OD	HMBC correlations	COSY correlations	NOESY interactions
1		135.6	H-3,5,7,8		
2/6	7.65, m	128.1	H-3/5,4,7	H-3/5	H-8,7,3/5
3/5	7.37, m	128.7	H-2/6	H-2/6	H-2/6
4	7.37, m	130.0	H-2,6		
7	7.74, d (15.57)	142.2		H-8	H-2,6
8	8.20, d (15.57)	126.5		H-7	H-2,6,4"
9		191.1	H-7,8		
1'		101.6			
2'		162.9	2H-1"'		
3'		108.2	2H-1"'		
4'		158.7	2H-1",2H-1"'		
5'		103.9	2H-1"		
6'		160.8	2H-1",H-2"		
1"	3.04, 2H, dd (8.59, 5.32)	27.0		H-2"	H-2",3H-4",3H-5"
2"	4.78, t (8.59)	91.0	2H-1",3H-4",3H-5"	2H-1"	2H-1",3H-4",3H-5"
3"		71.0	2H-1",3H-4",3H-5"		
4"	1.38, s	24.7	H-2",3H-5"		H-8,2/6,2H-1",H-2",3H-5"
5"	1.28, s	24.7	H-2",3H-4"		2H-1",H-2",3H-4"
1"'	3.25, 2H, d (7.57)	21.2		H-2"',3H-4"', 3H-5"'	H-2"',3H-4"'
2"'	5.16, bt (6.75)	123.1	2H-1"',3H-4"',3H-5"'	2H-1"',3H-5"',3H-4"'	2H-1"',3H-5"'
3"'		130.1	2H-1"',3H-4"',3H-5"'		
4"'	1.74, s	16.8	3H-5"'	2H-1"', H-2"'	2H-1"'
5"'	1.63, s	24.8	3H-4"'	2H-1"', H-2"'	H-2"'

3.2.5 Mass spectral fragmentation patterns for compounds I-VII

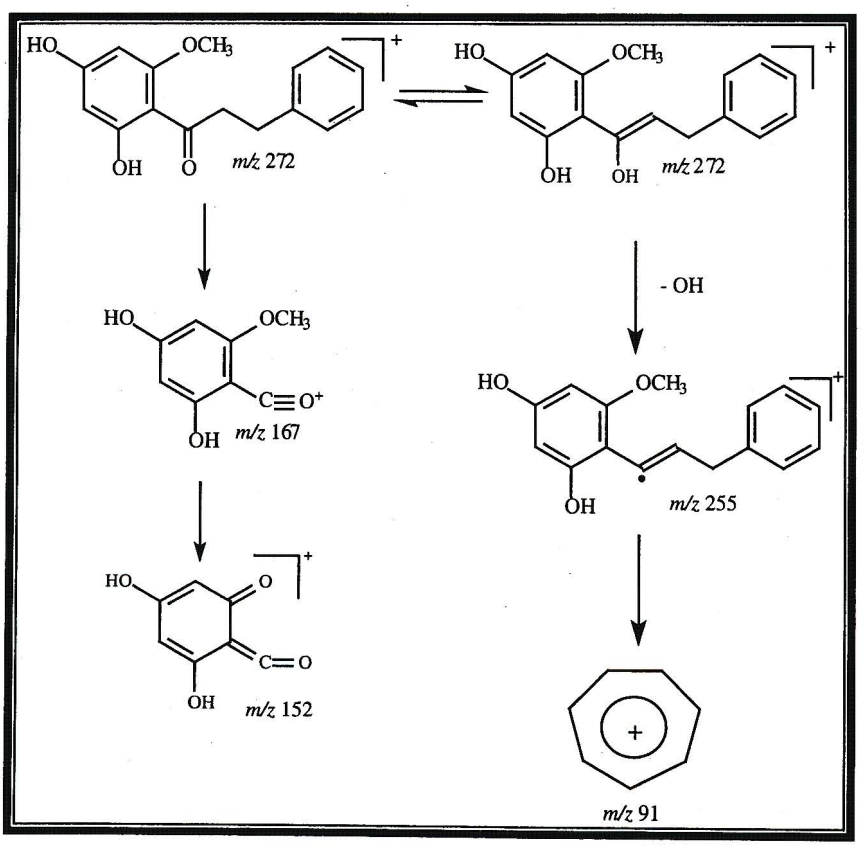
Chalcones give strong molecular ion peaks and $[M-H]^+$ and $[M-CH_3]^+$ ions[18]. Fragments are also derived by fission on either side of the carbonyl group and these are very useful in the structural elucidation of chalcones[18]. The relative intensities of these ions depend upon the substitution pattern of the parent compounds[18]. Chalcones with 2'-hydroxyl groups give ions in the mass spectrum, which are derived by fragmentation of both the chalcone and its corresponding flavanone[18]. An intramolecular equilibrium exists between the chalcone and the flavanone type molecular ions[18] (scheme 1), although complete isomerisation to one or the other molecular ion does not occur[18]. This isomerisation occurs when the hydrogen from the 2'-hydroxyl group in a 2'-hydroxychalcone shifts to C-3, forming the flavanone ion. In some cases, cleavage of the chalcone adjacent to the carbonyl group is much faster than isomerisation to the flavanone and the chalcone fragmentation path predominates[18]. The main fragmentation path of 2'-hydroxychalcone is illustrated in scheme 1[18,19].



Scheme 1. Mass spectral fragmentation pattern for 2'-hydroxychalcone [18,19]

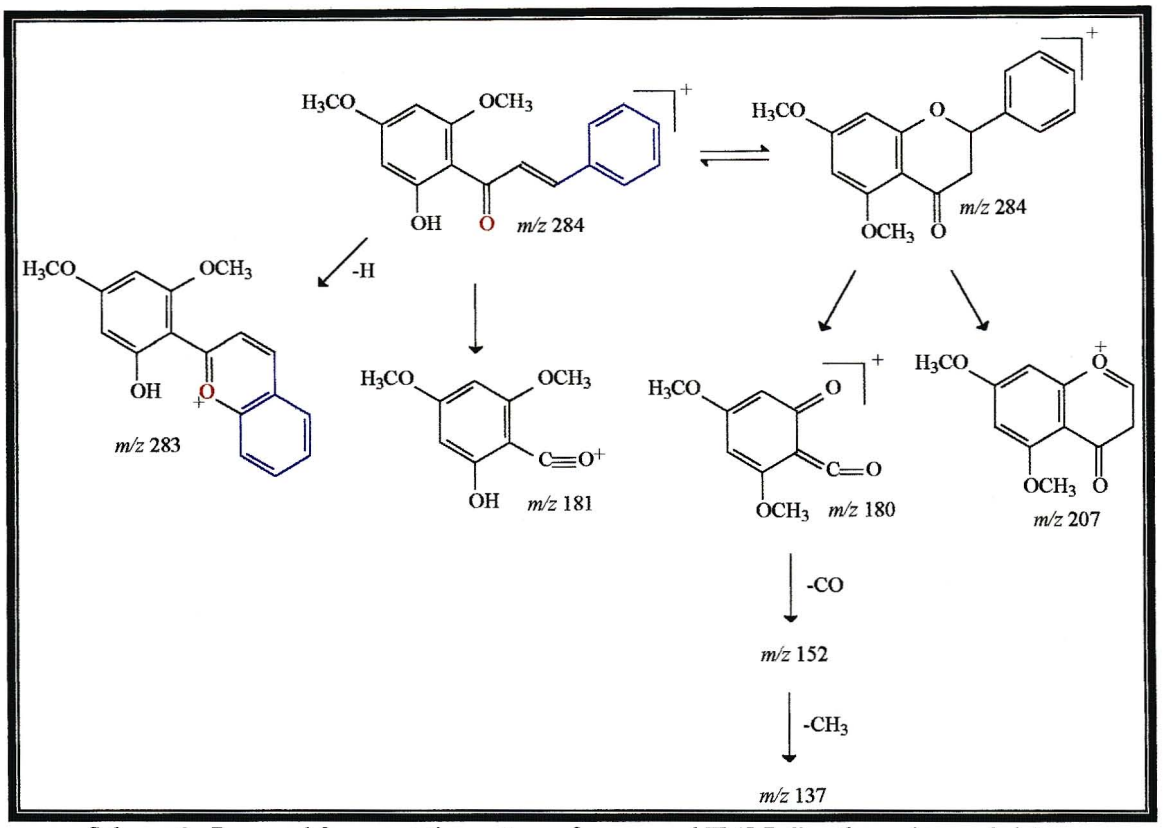
The mass spectra of the six chalcones isolated (compounds **I, III, IV, V, VI** and **VII**) and the flavanone (**II**) showed intense molecular ion peaks. Beside the dihydrochalcone (**I**), the flavanone (**II**) and the diprenylated chalcone (**VII**) all the other chalcones showed an M^+ -1 ion which results from the cyclisation between the carbonyl group and the C-2 carbon on the phenyl ring (ring B) in the manner shown in scheme 1. Compound **VII** may have too much steric hindrance for this to occur. Compounds **I, II** and **III** showed the loss of a hydroxy group, probably in the manner depicted in scheme 1.

Although fragmentation on both sides of the carbonyl group is known to occur, the dihydrochalcone and the flavanone only showed fragmentation on one side of the carbonyl group, that which was adjacent to the *trans* double bond (schemes 2 and 3).



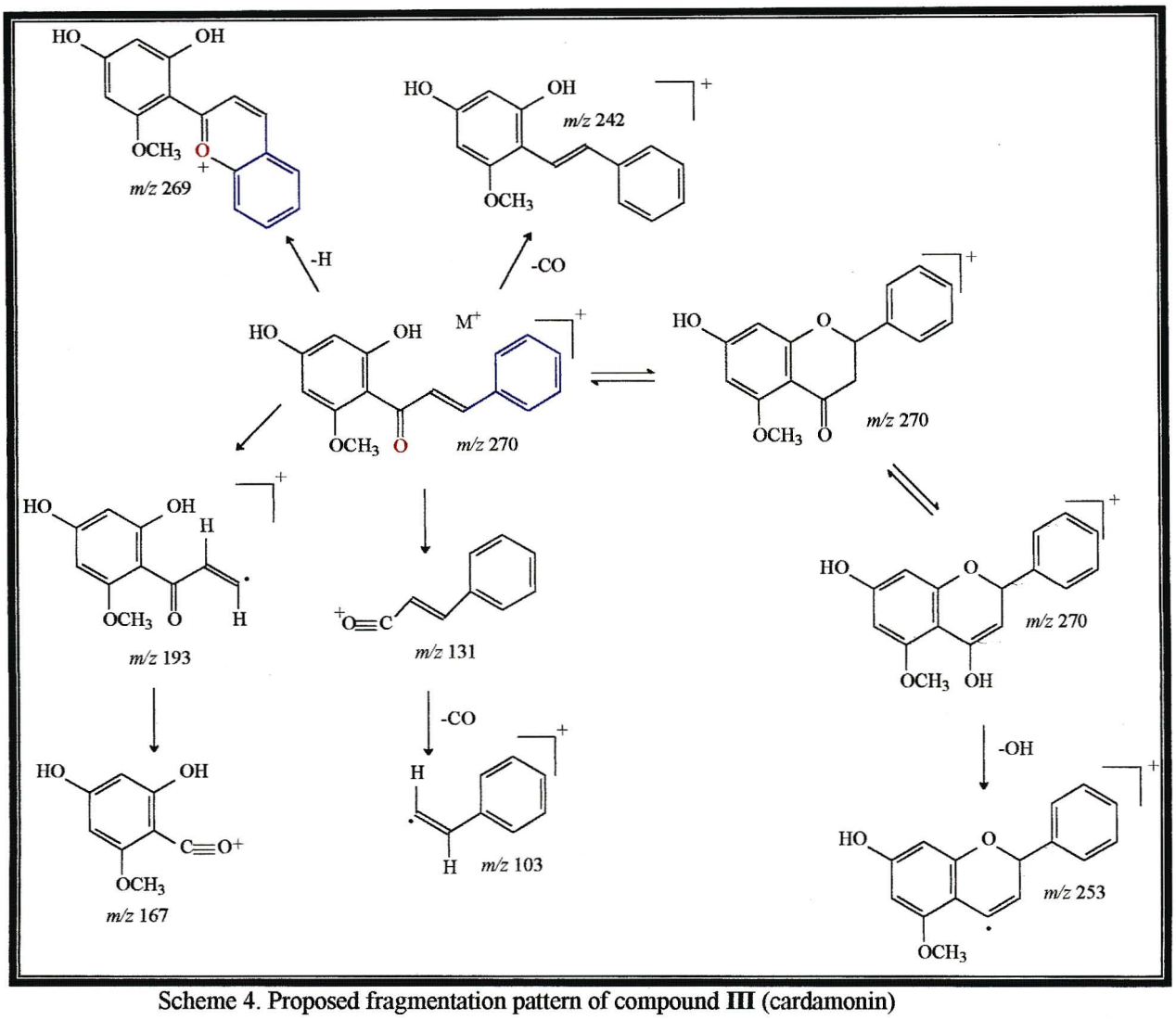
Scheme 2. Proposed fragmentation pattern of compound **I** (uvangoletin)

A loss of the carbonyl group from the parent compound (M^+ - 28) was only shown by compounds **II** and **III** (schemes 3 and 4).



Scheme 3. Proposed fragmentation pattern of compound **II** (5,7-dimethoxypinocembrin)

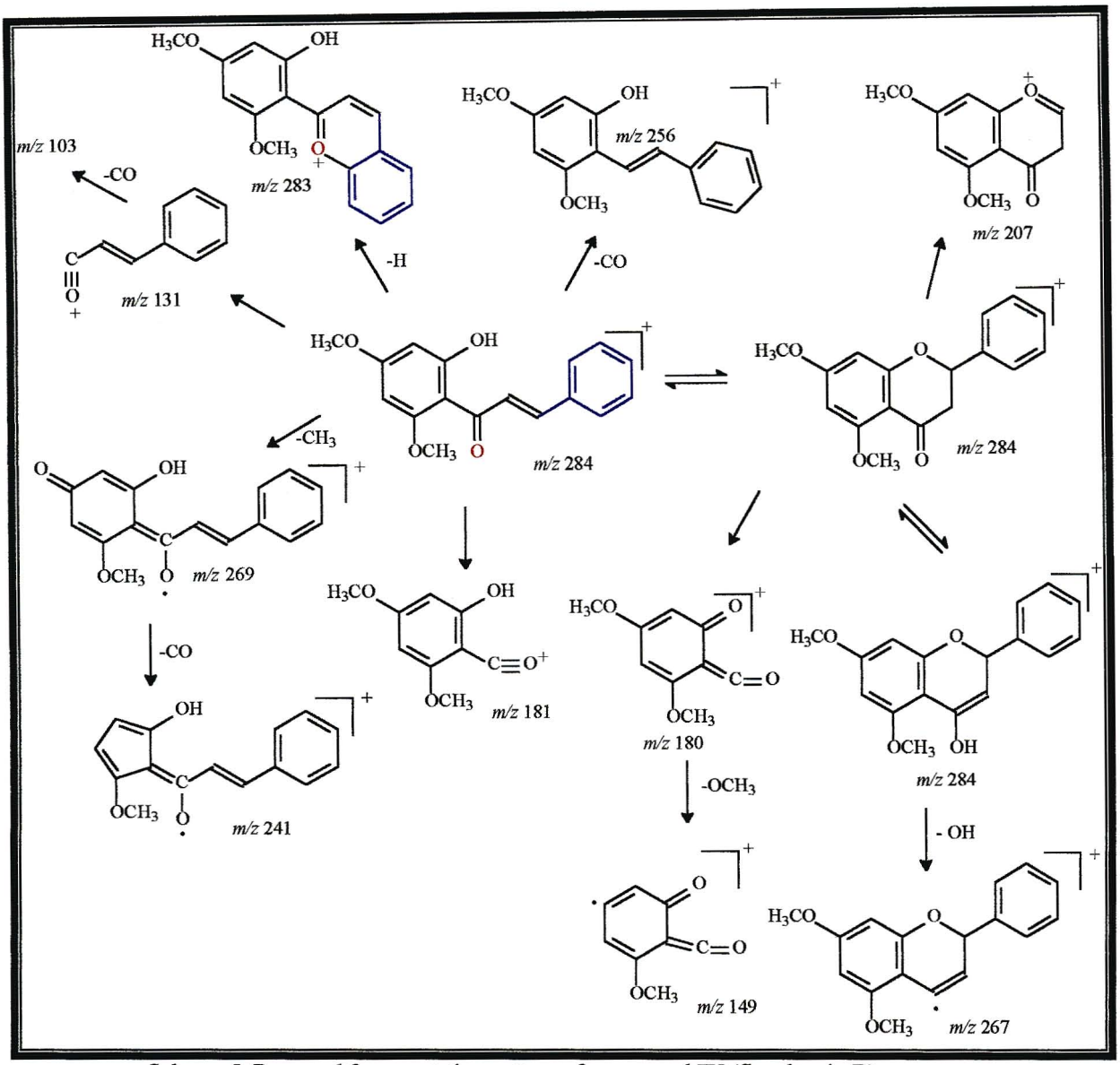
All the chalcones showed fragmentation on both sides of the carbonyl group. Since all the compounds have a monosubstituted B ring, fragmentation on the side of ring A in all compounds would result in an ion at *m/z* 131. In compound **VI**, although this ion was present, it was not as prominent as in the other four chalcones (**III, IV, V** and **VII**). This ion at *m/z* 131, then loses the carbonyl group to yield the phenyl ring with a CH=CH group attached. This ion appears at *m/z* 103 and can be seen in compounds **III-VII**.



Scheme 4. Proposed fragmentation pattern of compound **III** (cardamonin)

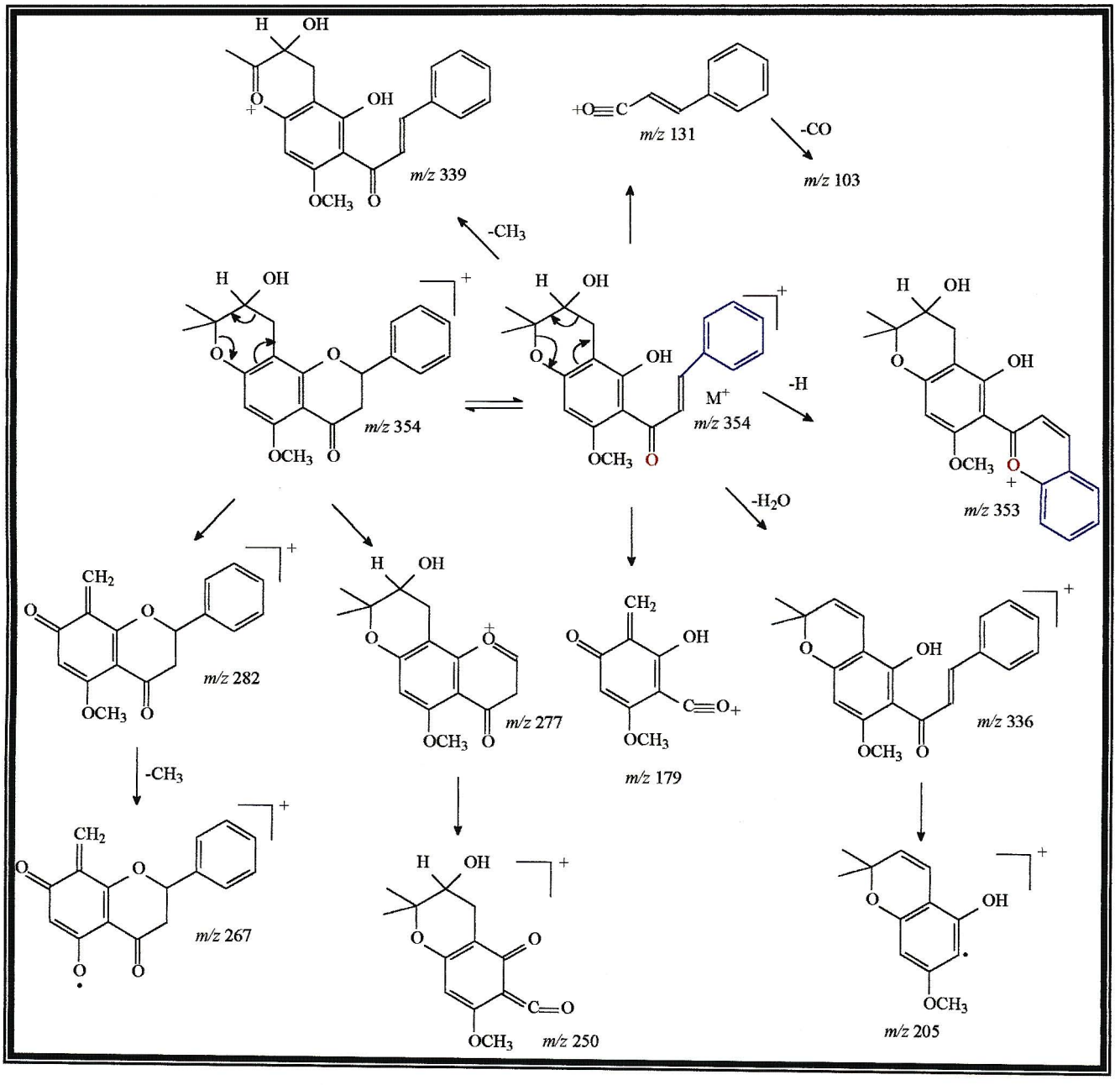
The phenyl ring was also lost from the parent compound in compounds **III-V**. This was characterised by an M^+ -77 ion in the mass spectrum. In compounds **III** and **IV**, the ion resulting from the loss of this phenyl group was an intense peak at *m/z* 193 and the base peak at *m/z* 207 respectively.

Another common feature of the mass spectra of these compounds was the loss of methyl groups. These methyl groups were lost from the methoxy groups on the A ring, which occurred after fragmentation adjacent to the carbonyl group. This occurred in compounds **I, II** and **IV**. In compound **IV**, a methyl group was lost from the parent compound as well. This methyl group was again lost from the methoxy substituent on the A ring to give the ion at *m/z* 269 which then loses a carbonyl group which results in the ion at *m/z* 241.

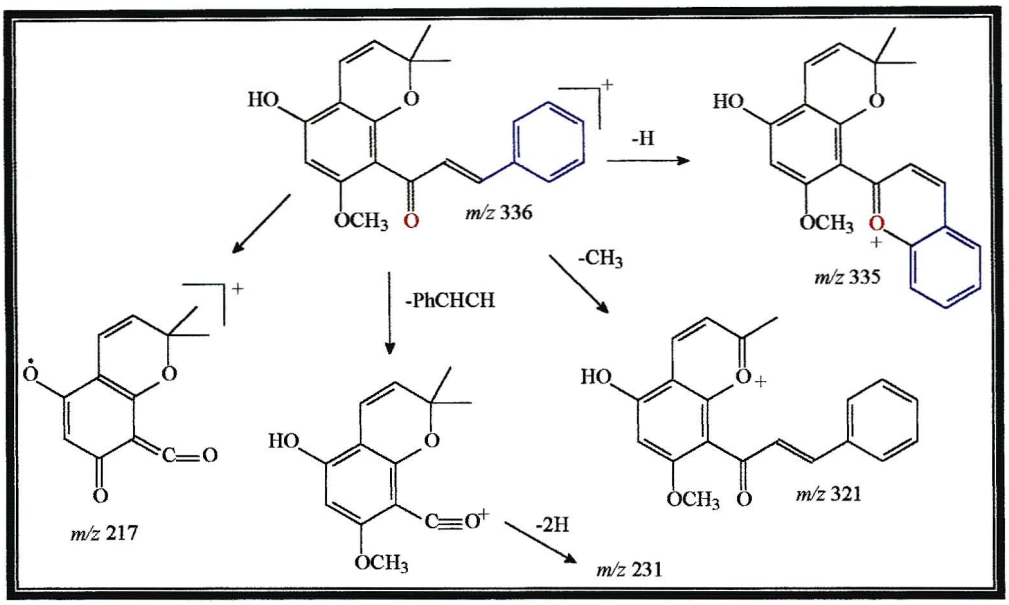


Scheme 5. Proposed fragmentation pattern of compound **IV** (flavokawin B)

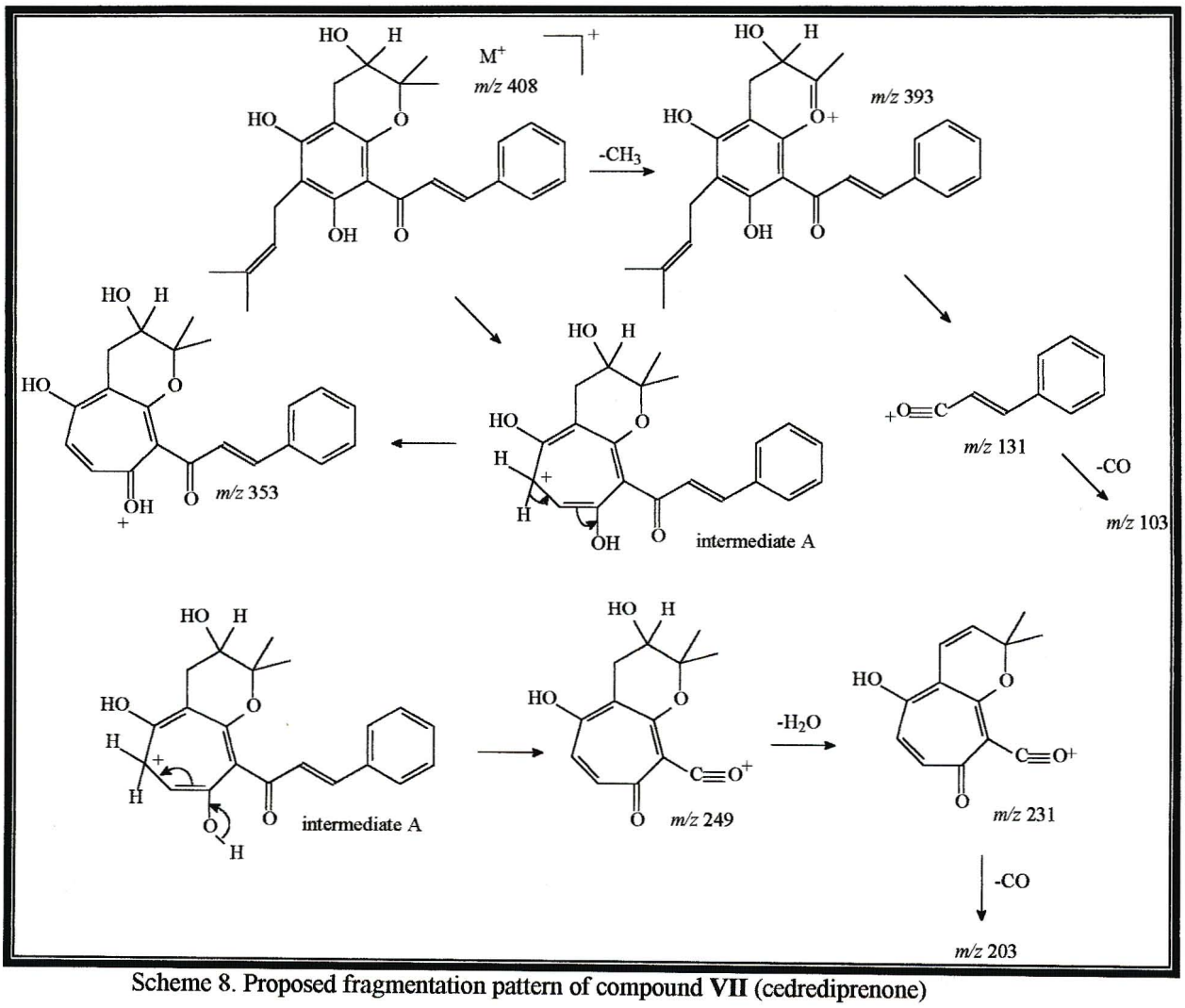
In the prenylated chalcones, a loss of one of the geminal methyl groups was evident. This appeared as an M^+-15 ion in compounds **V**, **VI** and **VII** (schemes 6-8).



Scheme 6. Proposed fragmentation pattern of compound **V** (2'-methoxyhelikrausichalcone)



Scheme 7. Proposed fragmentation pattern of compound **VI** (cedreprenone)



Scheme 8. Proposed fragmentation pattern of compound **VII** (cedrediprenone)

3.3 Foreword to Experimental

Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

NMR spectra were recorded in $CDCl_3$ and CD_3OD on a Varian Unity Inova 400 MHz NMR spectrometer. For the NMR data, chemical shifts are expressed in δ (ppm) from tetramethylsilane as an internal standard and coupling constants (J) are given in Hz. The proton spectra were recorded at 400 MHz and the carbon spectra at 100 MHz. The spectra were referenced against the central line of the deuteriochloroform signal at δ_C 77.0, the $C\underline{H}Cl_3$ singlet at δ_H 7.24, the deuteriomethanol signal at δ_C 49.0 or the $C\underline{H}D_2OD$ signal at δ_H 3.34.

Infra red spectroscopy (IR)

IR spectra were recorded on a Nicolet Impact 400D spectrometer which was calibrated against an air background. Spectra were recorded using NaCl windows with $CHCl_3$ as solvent.

Melting points (mp)

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

Optical Rotations (α_D)

Optical rotations were measured at room temperature in methanol or chloroform using an Optical Activity AA-5 Polarimeter together with a series A2 stainless steel (4 x 200 mm) unjacketed flow tube. Concentrations are quoted as g/100 ml.

Mass Spectrometry

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

Ultra violet spectroscopy (UV)

UV absorption spectra were obtained on a Varian DMS 300 UV-visible spectrophotometer using methanol as solvent.

General Chromatography

Silica gel 60 (Merck 1.09385, 230-400 mesh ASTM - particle size 0.040-0.063 mm) was used for column chromatography and silica gel plates 60 F_{254} (Merck 1.05553) was used for thin layer chromatography (TLC). Detection was carried out by spraying with anisaldehyde:H_2SO_4:MeOH (1:2:97) and heating.

3.4 Experimental

Extraction of *Cedrelopsis grevei*.

The fruit of *Cedrelopsis grevei* Baill. (628 grams) was collected at Beza Mahafaly, in the south of Madagascar in March,1999, and a voucher specimen retained at the Labarotoire de Pharmacodynamie of the Faculty of Science at the University of Antananarivo (MJ/MDUL01-99). The dried fruit (432 g) was extracted on a labcon shaker with dichloromethane (2L) and the solvent removed under reduced pressure to yield 2.76 g of extract. The contents were then extracted with methanol (2L) and the solvent removed under reduced pressure to yield 4.83 g of methanol extract. TLC of the crude dichloromethane and methanol extracts indicated similar components, thus the methanol extract was not examined further.

Isolation of compounds I-VII.

The dichloromethane extract (2.76 g) was chromatographed over silica gel (150 g) using a column (3cm in diameter) and eluted with an ethyl acetate:hexane step gradient, 5%, 10%, 20%, 40%, 75% and 100% ethyl acetate in hexane, collecting 30 x 30ml fractions for the first step (5%), 20 x 30ml fractions for the next two steps (10%, 20%), 40 x 40ml fractions for steps four and five (40%, 75%) and 25 x 30 ml fractions for the last step (100%). Elution with 5% EtOAc in hexane afforded a yellow oil and a yellow crystalline material which were purified by repeated column chromatography (CC) using 5% EtOAc in hexane to afford **VI** (12 mg) and **IV** (17 mg). Elution with 20% EtOAc in hexane afforded three yellow crystalline products which were purified by repeated CC using 20% EtOAc in hexane to yield **I** (24 mg) and **V** (13 mg) and with 100% dichloromethane to yield **III** (27 mg). Compounds **II** (36 mg) and **VII** (18 mg) were eluted with 40% EtOAc in hexane. Compound **VII** was purified using 10% EtOAc in dichloromethane and **II** was purified using 40% EtOAc in hexane.

Physical Data for compound I

Uvangoletin (CED58A)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 12 mg

Melting point: 190 ^{0}C (lit[11] 189-191 ^{0}C)

Mass: HRMS [M^+] at *m/z* 272.10427, $C_{16}H_{16}O_4$ requires 272.10485

EIMS: *m/z* (rel. int.): 272 (31.70), 255 (2.50), 179 (4.03), 168 (12.40), 167 (100), 152 (4.52), 149 (4.52), 140 (19.54), 124 (3.38), 104 (4.12), 91 (10.28), 77 (3.55), 71 (6.48), 57 (10.04), 43 (7.17)

Infra red: ν_{max}^{NaCl} cm^{-1}: 3376, 2924, 2851, 1635, 1470, 1207, 1170, 1115

Ultra violet: λ_{max}^{MeOH} nm (log ε): 290 (4.11), 218 (3.84)

^{1}H and ^{13}C NMR data are given in table 1.

Physical Data for compound II

5,7-dimethoxypinocembrin (CED90E)

Description: White needles (CH_2Cl_2)

Yield: 22 mg

Melting point: 159 ^{0}C (lit.[20] 159-160^{0}C)

Mass: HRMS [M^+] at *m/z* 284.10392, $C_{17}H_{16}O_4$ requires 284.10485.

EIMS: *m/z* (rel. int.): 284 [M^+] (56.91), 283 (10.86), 207 (18.71), 181 (15.19), 180 (100), 152 (18.51), 137 (17.14), 121 (3.20), 109 (3.70), 104 (5.93), 103 (5.44), 77 (4.73)

Optical rotation: $[\alpha]_D^{22}$ -46.10^0 (CH_2Cl_2, c=0.041); (lit.[20] $[\alpha]_D^{15}$ -45.8^0 (50% MeOH/$CHCl_3$, c=2.0))

Infra red: ν_{max}^{NaCl} cm^{-1}: 3441, 2926, 2852, 1681, 1613, 1570, 1466, 1263, 1233, 1159, 1110

Ultra violet: λ_{max}^{MeOH} nm (log ε): 283 (3.65), 215 (3.50)

^{1}H and ^{13}C NMR data are given in table 2.

Physical Data for compound III

cardamonin (CED67C2)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 15 mg

Melting point: 195 ^{0}C (lit[13] 195-196 ^{0}C)

Mass: HRMS [M^+] at *m/z* 270.09123, $C_{16}H_{14}O_4$ requires 270.08920

EIMS: *m/z* (rel. int.): 270 (100), 269 (57), 253 (8.33), 242 (8.30), 212 (10.55), 211 (11.53), 194 (13.37), 193 (92.43), 167 (62.54), 166 (23.84), 163 (17.32), 138 (8.08), 131 (8.43), 103 (17.33), 95 (11.52), 93 (10.72), 91 (15.61), 83 (11.35), 81 (16.97), 77 (17.35), 71 (11.28), 69 (41.37), 67 (9.61), 57 (20.64), 55 (21.10), 43 (29.66)

Infra red: ν_{max}^{NaCl} cm^{-1}: 3412, 2936, 2855, 1629, 1467, 1348, 1216, 1123

Ultra violet: λ_{max}^{MeOH} nm (log ε): 341 (3.81), 268 (3.97), 218 (3.41)

^{1}H and ^{13}C NMR data are given in table 3.

Physical Data for compound IV

flavokawin B (CED27B)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 8 mg

Melting point: 92 ^{0}C (lit[13] 91.5-92 ^{0}C)

Mass: HRMS [M^+] at *m/z* 284.10572, $C_{17}H_{16}O_4$ requires 284.10485

EIMS: *m/z* (rel. int.): 284 (90.56), 283 (61.33), 270 (10.61), 269 (7.47), 267 (7.62), 256 (11.42), 241 (10.03), 216 (7.12), 214 (23.36), 208 (17.41), 207 (100), 193 (9.92), 181 (37.07), 180 (34.42), 167 (12.29), 166 (10.92), 149 (26.37), 131 (9.11), 111 (18.07), 109 (14.42), 103 (19.61), 97 (26.97), 95 (19.76), 85 (28.75), 83 (28.59), 81 (19.81), 77 (15.49), 71 (43.94), 70 (14.06), 69 (37.10), 57 (64.94), 56 (11.57), 55 (31.62), 43 (41.33)

Infra red: ν_{max}^{NaCl} cm^{-1}: 2930, 2845, 1635, 1561, 1341, 1219, 1170

Ultra violet: λ_{max}^{MeOH} nm (log ε): 338 (4.22), 231 (4.54)

^{1}H and ^{13}C NMR data are given in table 4.

Physical Data for compound V

6'-methoxyhelikrausichalcone (CED58B)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 18 mg

Melting point: 127-128 ^{0}C*

Mass: HRMS [M^+] at *m/z* 354.14690, $C_{21}H_{22}O_5$ requires 354.14672

EIMS: *m/z* (rel. int.): 354 (94.29), 353 (25.07), 339 (2.29), 336 (2.31), 296 (6.38), 295 (7.73), 284 (7.85), 283 (30.25), 282 (18.23), 281 (19.24), 278 (10.43), 277 (40.95), 267 (11.31), 253 (7.08), 250 (9.30), 206 (8.99), 205 (39.67), 180 (19.03), 179 (100), 167 (16.08), 164 (8.58), 152 (6.96), 149 (9.86), 131 (11.29), 103 (20.50), 91 (12.09), 83 (11.76), 77 (15.83), 71 (14.35), 69 (16.20), 57 (24.59), 55 (16.69), 43 (25.57)

Optical rotation: $[\alpha]_D^{22}$ -263^0 (MeOH, c=0.19)*

Infra red: ν_{max}^{NaCl} cm^{-1}: 3425, 2937, 2850, 1639, 1450, 1348, 1231, 1157, 1114, 1052

Ultra violet: λ_{max}^{MeOH} nm (log ε): 338 (4.36), 205 (4.19)

^{1}H and ^{13}C NMR data are given in table 5.

* Although the Dictionary of Natural Products, 1999 lists compound **V** as being isolated previously from *Helichrysum aphelexioides*, the reference cited[15] does not contain compound **V**, but a similar compound to that of **V**. Furthermore no physical characteristics of this related compound are reported in this paper[15].

Physical Data for compound VI

cedreprenone (CED18)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 18 mg

Melting point: 134 ^{0}C

Mass: HRMS [M^+] at *m/z* 336.13675, $C_{21}H_{20}O_4$ requires 336.13616

EIMS: *m/z* (rel. int.): [M^+] 336 (37.94), 335 (21.77), 323 (8.33), 322 (12.29), 321 (52.84), 283 (8.70), 279 (17.57), 251 (15.34), 231 (29.13), 217 (61.67), 179 (9.94), 167 (39.00), 160 (10.70), 150 (11.79), 149 (100), 129 (11.40), 113 (19.72), 112 (16.14), 111 (11.20), 103 (13.50), 97 (18.68), 95 (11.76), 85 (28.10), 83 (27.31), 77 (13.84), 71 (59.32), 70 (29.38), 69 (30.64), 57 (87.25), 56 (17.31), 55 (38.78), 43 (58.91)

Infra red: ν_{max}^{NaCl} cm^{-1}: 3750, 2925, 2859, 2377, 1649, 1571, 1406, 1348, 1168, 1131, 1041

Ultra violet: λ_{max}^{MeOH} nm (log ε): 336 (5.40), 291 (5.40), 218 (5.05)

^{1}H and ^{13}C NMR data is given in table 6.

Physical Data for compound VII

cedrediprenone (CED90C)

Description: Yellow crystalline (CH_2Cl_2)

Yield: 17 mg

Melting point: 153 ^{0}C

Mass: HRMS [M^+] at *m/z* 408.19489, $C_{25}H_{28}O_5$ requires 408.19367

EIMS: *m/z* (rel. int.): 408 (100), 393 (23.22), 375 (9.64), 365 (12.80), 353 (20.00), 347 (7.50), 335 (6.07), 305 (3.57), 293 (6.92), 289 (9.17), 276 (14.59), 249 (9.63), 231 (13.12), 217 (8.65), 215 (8.21), 203 (6.04), 191 (7.21), 190 (7.53), 189 (18.00), 177 (13.98), 149 (3.87), 131 (14.57), 103 (12.96), 69 (16.75), 59 (12.25), 57 (13.05), 55 (10.13), 43 (14.39)

Optical rotation: $[\alpha]_D^{22}$ -3.62^0 (CH_2Cl_2, c=0.069)

Infra red: ν_{max}^{NaCl} cm^{-1}: 3377, 2934, 2862, 1629, 1569, 1432, 1366, 1258, 1180, 1085

Ultra violet: λ_{max}^{MeOH} nm (log ε): 346 (4.74), 268 (4.84), 261 (4.83), 216 (4.11)

^{1}H and ^{13}C NMR data is given in table 7.

3.5 References

1. Styles, B.T. and Pennington, T.D. (1975) A generic monograph of the Meliaceae, *Blumea*, **22**, 476.
2. Dean, F.M., Parton, B., Somvichien, N. and Taylor, D.A.H. (1967) Chromones, containing an oxepin ring, from *Ptaeroxylon obliquum*, *Tetrahedron Letters*, **36**, 3459.
3. Mulholland, D., Mahomed, H., Kotsos, M., Randrianarivelojosia, M., Lavaud, C., Massiot, G. and Nuzillard, J. (1999) Limonoid derivatives from *Cedrelopsis grevei*, *Tetrahedron*, **55**, 11547-11552.
4. Eshiett, I.T. and Taylor, D.A.H. (1968) The isolation and structure elucidation of some derivatives of dimethylallyl-coumarin, chromone, quinoline and phenol from *Fagara* species and from *Cedrelopsis grevei*, *Journal of the Chemical Society (C)*, 481.
5. Kotsos, M., Mahomed, H. A., Mulholland, D.A., Koorbanally, N. A. and Milijaona Randrianarivelojosia, M., Coumarins from *Cedrelopsis grevei*, *Phytochemistry*, *in press*.
6. Dean, F.M. and Robinson, M.L. (1971) The heartwood chromones of *Cedrelopsis grevei*, *Phytochemistry*, **10**, 3221.
7. McCabe, P.M., McCrindle, R. and Murray, R.D.H. (1967) Constituents of sneezewood, *Ptaeroxylon obliquum* (Thunb.) Radlk. Part 1. Chromones, *Journal of the Chemical Society (C)*, 145.
8. Schulte, K.E., Rucker, G. and Klewe, U. (1973) Einige inhaltsstoffe der rinde von *Cedrelopsis grevei* Baillon (German), *Arch. Pharm. (Weinheim. Ger.)*, **306**, 857.
9. Markham, K.R. and Mabry, T.J. (1975) Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids, in. *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 51.
10. Bellamy, L.J. (1954) *The Infra-red Spectra of Complex Molecules*, Methuen and Co. Ltd., London, p. 133-145.
11. Hufford, C.D. and Oguntimein, B.O. (1980) Dihydrochalcones from *Uvaria angolensis*, *Phytochemistry*, **19**, 2036-2038.
12. Hufford, C.D. and Lasswell, W.L. Jr. (1978) *Lloydia*, **41**, 156.
13. Itokawa, H., Morita, M. and Mihashi, S. (1981) Phenolic compounds from the rhizomes of *Alpinia speciosa*, *Phytochemistry*, **20**, 2503-2506.

14. Sauer, H. and Haensel, R. (1967) Components of *Piper* species. XVI . Kava lactones and flavonoids from an endemic *Piper* species of New Guinea, *Planta Medica*, **15**, 443.

15. Randriaminahy, M., Proksch, P., Witte, L. and Wray, V. (1992) Lipophilic phenolic constituents from *Helichrysum* species endemic to Madagascar, *Zeitschrift fur Naturforschung. C*, **47**, 10.

16. Jakupovic, J., Zdero, C., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M. and Bohlmann, F. (1989) Twenty-one acylphloroglucinol derivatives and further constituents from South African *Helichrysum* species, *Phytochemistry*, **28**, 1119-1131.

17. Iinuma, M., Okawa, Y. and Tanaka, T. (1994) Three new cinnamoylphenols in heartwood of *Erythrina crista-galli*, *Phytochemistry*, **37**, 1153-1155.

18. Mabry, T.J. and Markham, K.R. (1975) Mass spectrometry of flavonoids, in. *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 105-107.

19. Dhar, D.N. (1981) *The Chemistry of Chalcones and Related Compounds*, John Wiley and Sons, New York, p 188-189.

20. Bick, I.R.C, Brown, R.B. and Hillis, W.E. (1972) Three flavanones from leaves of *Eucalyptus sieberi*, *Australian Journal of Chemistry*, **25**, 449-451.

21. Fernandes, J.B., Gottlieb, O.R. and Xavier, L.M. (1978) Chemosystematic implications of flavonoids in *Aniba riparia*, *Biochemical Systematics and Ecology*, **6**, 55-58.

Chapter 4. Extractives from *Cedrelopsis microfoliata* (Ptaeroxylaceae)

4.1 Introduction

Cedrelopsis microfoliata J.-F. Leroy. is a Madagascan species belonging to the family Ptaeroxylaceae. It is a tree of 5 to 10 meters high with leaves approximately 7-16 cm long and 3-5 cm wide. The leaves are characterised by 8-14 pairs of leaflets, ove-oblong or oblong-elliptic. The leaflet is very small, 1-2.5 cm long and 0.3-0.7 cm wide (Fig 1.)[8]. The specimen studied in this work was collected in Ankarafantsika in the north west part of Madagascar.

Cedrelopsis microfoliata, also called by the vernacular names, tamotamo hazo, mantaora, maninjo and fandroihosy, is used for its wood for building traditional houses[8]. The wood of this species is bright yellow in colour and very characteristic of the species. The leaves of this plant also have ethnopharmacological importance as they are used to prepare a decoction for woman to drink after childbirth[8]. This is the first phytochemical investigation of *Cedrelopsis microfoliata*.

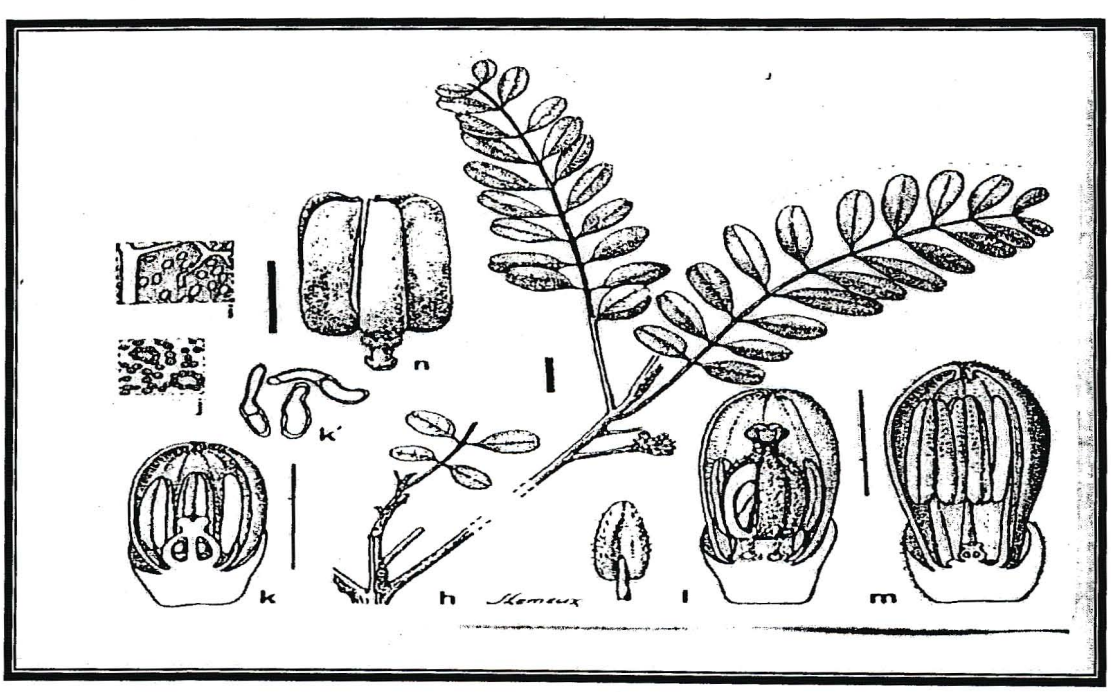


Fig. 1. *Cedrelopsis microfoliata* profile[8].
h – rameau inflorescentiel en boutons (branch inflorescent in bud), i – ponctuations du limbe vues par transparence (transparent view through sections of the leaf), j – papilles et stomates du limbe grossis – face inferieure (papilla and stomata of the leaf, magnified – lower surface, k – bouton (male and female bud), k' – trichome du calice grossi (trichome of the calyx magnified), l – bouton et antherode (female bud and anther), m – bouton (male bud), n – fruit (fruit).

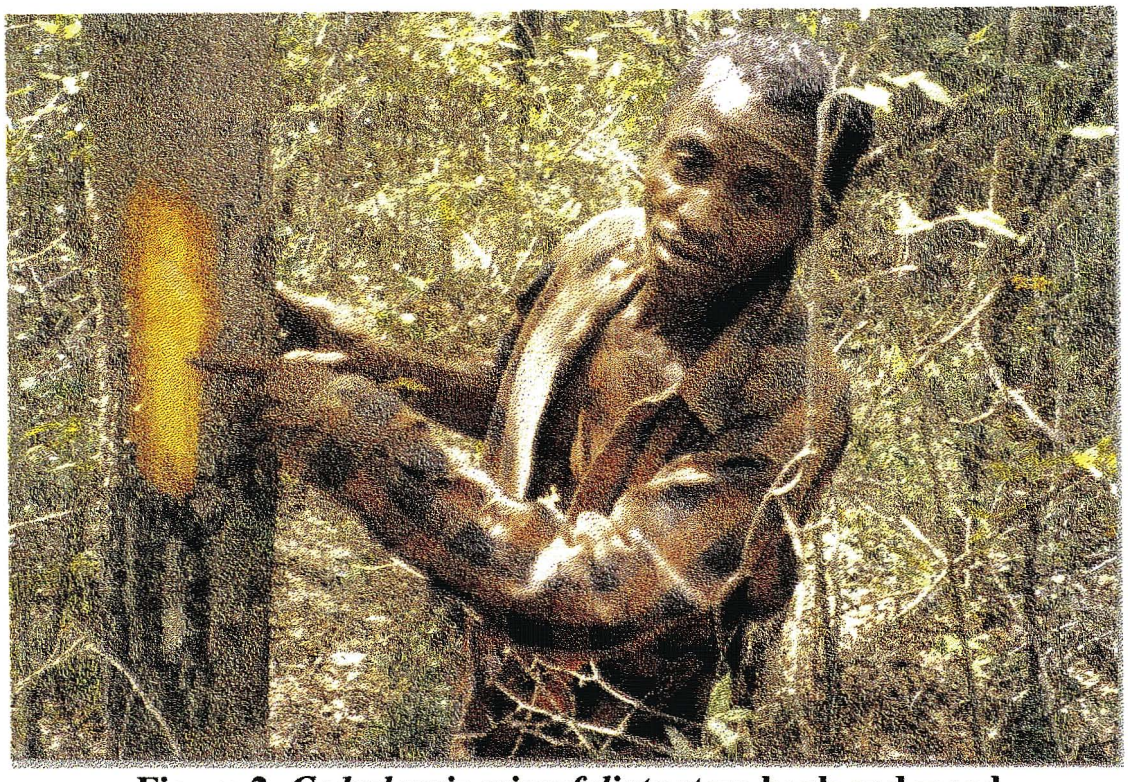


Figure 2. *Cedrelopsis microfoliata* stem bark and wood (the bright yellow wood is characteristic of the plant)

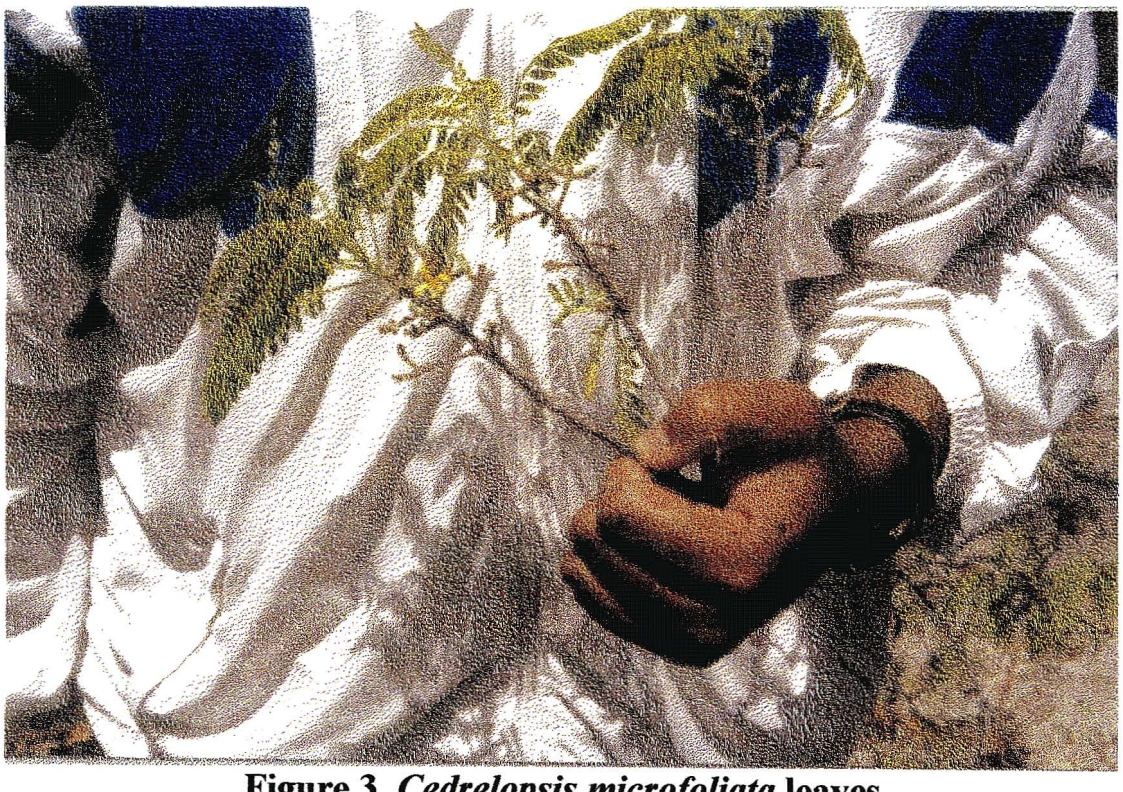


Figure 3. *Cedrelopsis microfoliata* leaves

4.2 Results and Discussion

The dried stem bark of *Cedrelopsis microfoliata* was extracted successively with hexane, dichloromethane and methanol. Proton NMR spectroscopy of the crude hexane and dichloromethane extracts showed the presence of aromatic compounds. Proton NMR spectroscopy of the crude methanol extract only indicated the presence of sugars. The hexane extract was separated by column chromatography using silica gel as the stationary phase and a hexane : dichloromethane : methanol step gradient as the mobile phase. This yielded three compounds, a chalcone, microfolian (**VIII**) and two flavanones (microfolione (**IX**) and agrandol (**X**)). The dichloromethane extract was separated using the same stationary phase and a dichloromethane : ethyl acetate mobile phase. This yielded four compounds, three coumarins (cedrecoumarin A (**XI**), obliquin (**XII**), microfolicoumarin (**XIII**), and a sesquiterpenoid (sesquichamaenol (**XIV**)).

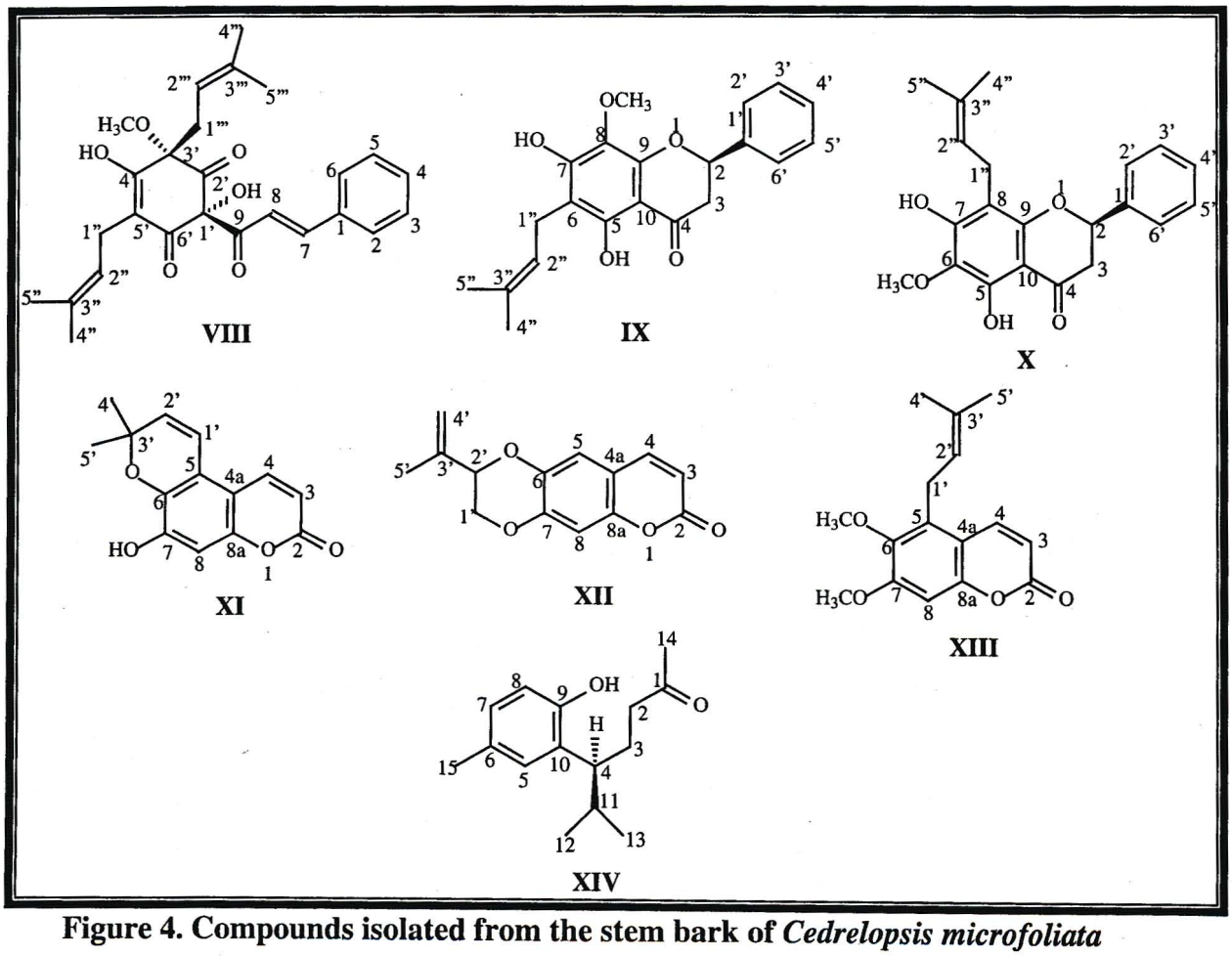
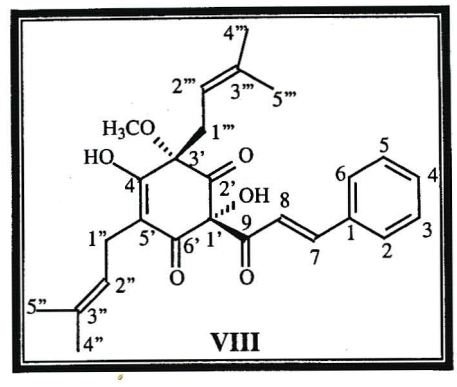


Figure 4. Compounds isolated from the stem bark of *Cedrelopsis microfoliata*

4.2.1 Structural elucidation of compound VIII, microfolian (MIC40)



VIII

Compound **VIII** was identified as a prenylated chalcone derivative from its ^{1}H NMR spectrum. It was isolated as a yellow oil and had a molecular formula of $C_{26}H_{30}O_6$ based on high resolution MS (Found *m/z* 438.20428, required 438.20424). This compound gave UV absorption maxima at $\lambda_{max}^{CH_2Cl_2}$ nm 380 (log ε= 4.23), 238 (4.08) and 223 (3.99). The IR spectrum of compound **VIII** showed absorptions at υ_{max}^{NaCl} 3462 cm^{-1}, a broad absorption band characteristic of a hydroxy group stretch and at 1620 cm^{-1}, 1677 cm^{-1} and 1714 cm^{-1}, which were identified as the carbonyl stretching bands[13].

The ^{1}H NMR spectrum of compound **VIII** showed the presence of a monosubstituted aromatic ring by the presence of a three-proton multiplet at δ_H 7.40 attributed to H-3, H-4 and H-5 and a two-proton multiplet at δ_H 7.62 attributed to H-2 and H-6. A pair of olefinic protons was indicated by a pair of downfield doublets at δ_H 7.88 and δ_H 8.20 with a large coupling constant ($J_{7,8}$ = 15.75 Hz) indicating that these protons were *trans* to each other. These proton resonances were attributed to H-7 and H-8 respectively. Both these proton resonances showed NOESY interactions with the H-2/6 proton resonance. The C-7 carbon resonance at δ_C 144.29 showed HMBC correlations to the H-2/6 proton resonance and the C-8 carbon resonance at δ_C 122.53 showed an HMBC correlation to the H-7 proton resonance. This indicated that the Δ^7 double bond was attached to the monosubstituted aromatic ring.

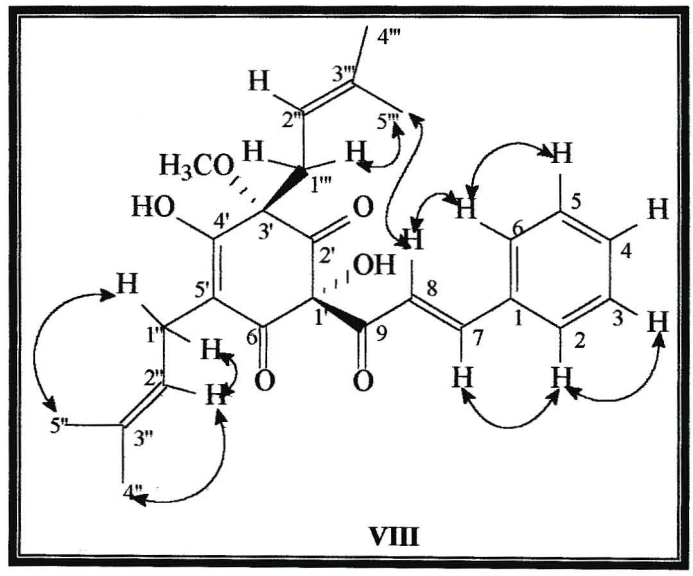
Two isoprenyl groups were evident from the ^{1}H NMR spectrum acquired in deuterated chloroform. Two methylene resonances at δ_H 3.15 and δ_H 2.59, two methine proton resonances at δ_H 5.15 and δ_H 4.96 and two pairs of methyl proton

resonances at δ_H 1.70, δ_H 1.75, δ_H 1.63 and δ_H 1.50 could clearly be seen. Also present in the ^1H NMR spectrum was a methoxy proton resonance at δ_H 3.21. The sample was unstable in deuterated chloroform and the other spectra had to be acquired in deuterated methanol. In the ^1H NMR spectrum acquired in deuterated methanol, one of the methine resonances overlapped with the solvent peak and one of the methylene resonances overlapped with the methoxy proton resonance. In the COSY spectrum, the methylene proton resonance at δ_H 3.10, the methine proton resonance at δ_H 5.15 and the two methyl proton resonances at δ_H 1.66 and δ_H 1.74 were all seen to be coupled with each other. These were attributed to H-1", H-2" and H-4" and H-5" respectively. Similarly, the methylene proton resonance at δ_H 2.57, the methine proton resonance at δ_H 4.86 and the two methyl resonances at δ_H 1.54 and δ_H 1.47 were all seen to be coupled in the COSY spectrum. These were attributed to H-1"', H-2"' and H-4"' and H-5"'.

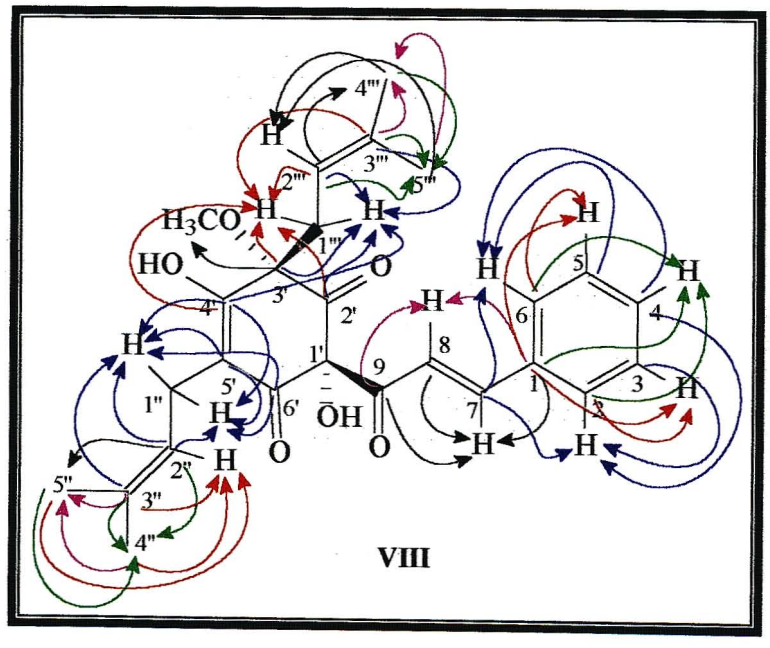
Unlike the ^{13}C NMR spectra of the other chalcones isolated from *Cedrelopsis grevei* which showed a single carbonyl carbon resonance, that of the C-9 carbonyl group, the ^{13}C NMR spectrum of compound **VIII** showed the presence of three carbonyl group carbon resonances at δ_C 184.59, δ_C 191.56 and δ_C 194.82. The carbon resonance at δ_C 184.59 showed HMBC correlations to the H-7 and H-8 proton resonances and was therefore attributed to C-9. The other two carbonyl carbon resonances were assigned to positions on the A ring. The carbon resonances of the A ring were assigned using HMBC correlations. The carbonyl carbon resonance ascribed to C-2' at δ_C 194.82, showed an HMBC correlation with the 2H-1"' proton resonance. The C-3' carbon resonance at δ_C 84.43 also showed HMBC correlations to the 2H-1"' proton resonance at δ_H 2.57 and the 3'-OCH_3 proton resonance at δ_H 3.10. The C-4' carbon resonance at δ_C 168.26 showed HMBC correlations to both the 2H-1" and 2H-1"' proton resonances. This indicated that the C-4' carbon atom was situated between the two isoprenyl groups. Both the C-5' and the C-6' carbon resonances at δ_C 114.59 and δ_C 191.56 showed an HMBC correlation to the 2H-1" proton resonance. The C-1" carbon resonance was more downfield than expected at δ_C 50.89, but this observed resonance could be due to the presence of the adjacent carbonyl group. The C-1' carbon resonance at δ_C 108.17 was also more downfield than is expected for a C-

O carbon resonance but this carbon atom is also attached to three carbonyl carbon atoms, which could explain the observed resonance.

A NOESY interaction exists between the 3H-5''' proton resonance of the prenyl group at the 3' position and the H-8 proton resonance, which indicates that the prenyl group and the Ph-CH=CH-C(O)- group are *cis* to each other. The stereochemistry given for compound **VIII** is the relative stereochemistry.

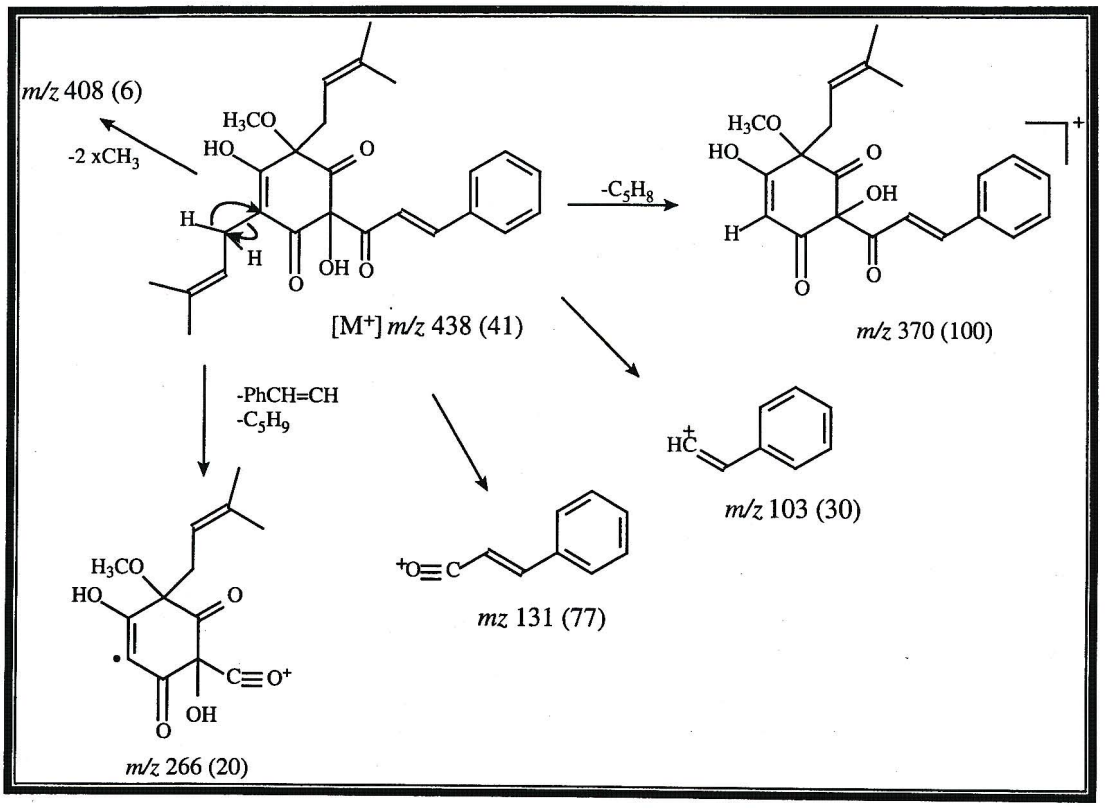


NOESY interactions for compound VIII



HMBC correlations for compound VIII

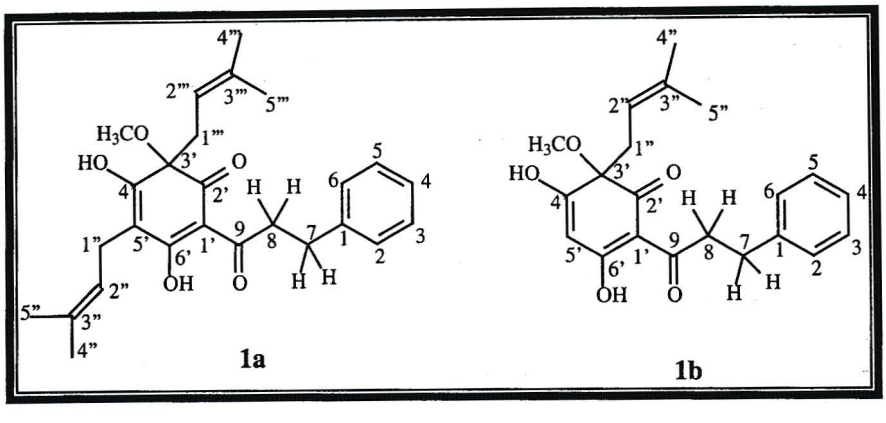
The mass spectrum of compound **VIII** showed the molecular ion peak at *m/z* 438. The loss of two methyl groups, either from the methoxy group and the prenyl group or from the two prenyl groups resulted in the ion peak at *m/z* 408. The ion peak at *m/z* 370, the base peak, was due to the loss of a C_5H_8 fragment[9]. Cleavage of one of the prenyl groups as well as between the C-8/C-9 bond results in the ion peak at *m/z* 266. The ion peaks at *m/z* 131 and *m/z* 103, the $[O{=}C\text{-}HC{=}CH\text{-}Ph]^+$ and the $[HC{=}CH\text{-}Ph]^+$ ion fragments respectively are due to cleavages on both sides of the C-9 carbonyl group[16]. The proposed mass spectral fragmentation pattern is given in scheme 1.



Scheme 1. Proposed mass spectral fragmentation pattern of microfolian (**VIII**)[9,16]

Chalcones with carbonyl groups on the A ring have been found previously. Examples are helihumulon (**1a**), which was isolated from *Helichrysum cymosum* (Compositae)[9] and another similar dihydrochalcone (**1b**) without the additional isoprenyl group, which was isolated from *Helichrysum aphelexoides* (Compositae)[11]. The ^1H and ^{13}C NMR data of helihumulon (**1a**) is tabulated alongside that of compound **VIII**. The ^{13}C NMR data for C-2' and C-6' in helihumulon and compound **VIII** are very similar and the downfield carbon resonance at δ_C 190.4 of C-6' is unusual for an aromatic C-O carbon resonance. It could possibly be explained by keto-enol tautomerism

occurring. The structure proposed for helihumulon was in agreement with its high resolution mass spectral data[9]. High resolution mass spectral data of compound **VIII** indicate that a hydroxy group is present at C-1' and hence keto-enol tautomerism is not possible.



^{1}H and ^{13}C NMR assignments were made with the aid of HMBC and NOESY spectra and are reported in table 1. Compound **VIII** has not been isolated previously and was named microfolian.

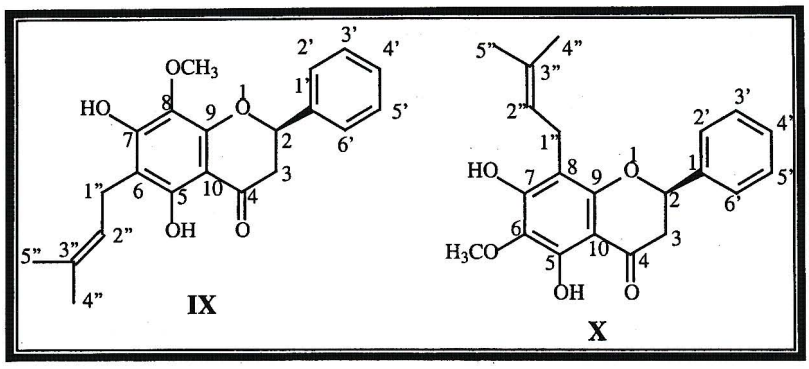
Table 1. ^{1}H, ^{13}C, HMBC and NOESY data for compound VIII, microfolian and helihumulon (1a)

Pos	^{1}H # VIII	^{1}H ## VIII	^{1}H * 1a	^{13}C# VIII	^{13}C * 1a	HMBC correlations VIII	NOESY interactions VIII
1				135.33	141.1	H-3/4/5, H-7,8	
2/6	7.62, m	7.63, m	7.25, m	128.40	128.5	H-3/4/5, H-7	H-7,8, H-3/5
3/5	7.40, m	7.38, m	###	128.90	128.4	H-2/6	H-2/6
4			###	130.56	126.1	H-2/6	
7	7.88, d (15.75)	7.90, d (15.75)	3.00, dd (15, 8) 2.93, dd (15, 8)	144.29	39.7	H-2/6	H-2/6
8	8.20, d (15.75)	8.22, d (15.75)	3.31, t (8)	122.53	42.1	H-7	H-2/6, 3H-5'''
9				184.59	201.3	H-7,8	
1'				108.17	108.3		
2'				194.82	192.5	H-1'''	
3'				84.43	84.7	H-1''', 3'-OCH_3	

4'				168.26	166.4	H-1", 1'''	
5'				114.59	113.3	H-1"	
6'				191.56	190.4	H-1"	
1"	3.10**	3.15, t (7.10)	3.11, dd (13,7) 3.10, dd (13, 7)	50.89	21.3		3H-5", H-2"
2"	5.13, t (7.14)	5.15, t (7.10)	5.13, tqq (7,1,1)	121.76	121.3	2H-1", 3H-4",3H-5"	2H-1", 3H-4"
3"				131.31	132.4	2H-1",3H-4",3H-5"	
4"	1.66, s	1.70, s	1.69, s	24.99	25.7	H-2", 3H-5"	H-2"
5"	1.74, s	1.75, s	1.74, s	16.89	17.8	H-2", 3H-4"	2H-1"
1'''	2.57, t (7.51)	2.59, t (7.55)	2.55, dd (14,8) 2.48, dd (14,8)	39.23	31.3		3H-5'''
2'''	4.86 ***	4.96, t (7.55)	4.96, tqq (8,1,1)	115.54	115.4	2H-1''', 3H-4''', 3H-5'''	3H-4'''
3'''				137.04	138.0	2H-1''', 3H-4''', 3H-5'''	
4'''	1.54, s	1.63, s	1.63, s	24.94	25.8	H-2''', 3H-5'''	H-2'''
5'''	1.47, s	1.50, s	1.50, s	16.80	17.8	H-2''', 3H-4'''	2H-1''', H-8
3'-OCH_3	3.10, s	3.21, s	3.20, s	52.86	54.0		

400 MHz, CD_3OD, ## 300 MHz, $CDCl_3$, ### data not provided
* lit.[9] (^{1}H NMR of helihumulon, 270 MHz, $CDCl_3$), ** signal overlaps with methoxy signal, *** signal underneath solvent peak.

4.2.2 Structural elucidation of compound IX, (+)-microfolione (MIC19A) and compound X, (+)-agrandol (MIC30)



Compounds **IX** and **X** were identified as flavanones by their characteristic ^{1}H NMR patterns, UV, IR and mass spectra. Compound **IX** was isolated as a white crystalline compound with a melting point of 172-173^{0}C and had a molecular formula of $C_{21}H_{22}O_5$, based on high resolution MS (Found *m/z* 354.14543, required 354.14672). Compound **X** was also isolated as a white crystalline compound, with the same molecular formula as compound **IX**, with a M^+ ion peak at *m/z* 354 in the mass spectrum. However, compound **X** had a lower melting point than compound **IX** of 142-143^{0}C.

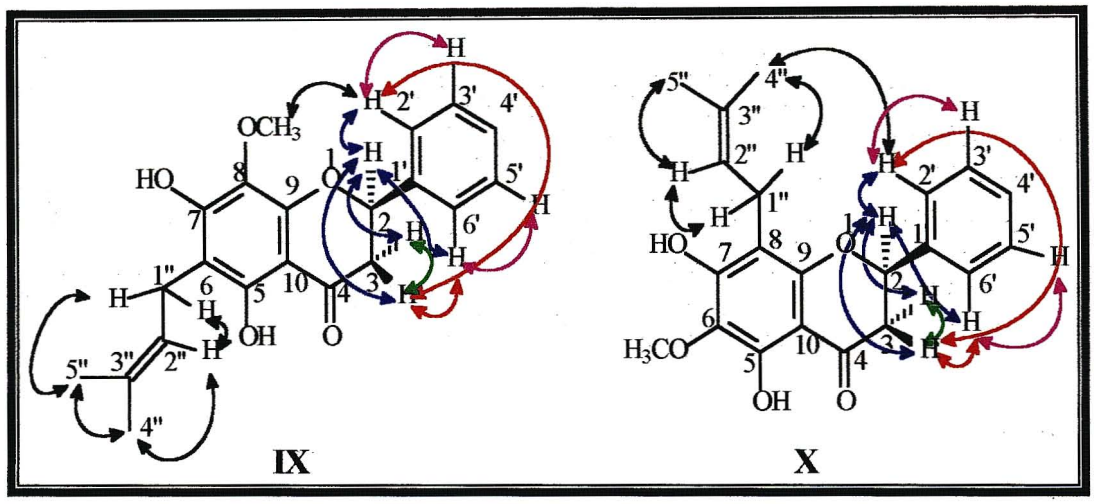
Compound **IX** gave UV absorption maxima at λ_{max}^{MeOH} nm 345 (4.53), 297 (5.13) and 208 (5.44), while compound **X** gave UV absorption maxima at λ_{max}^{MeOH} nm 344 (3.68), 298 (4.19) and 204 (4.54). These UV absorption maxima were characteristic for flavanones[12]. The IR spectrum of compound **IX** showed a broad band at 3382 cm^{-1}, due to the hydroxy group stretch and a stretch of the carbonyl group at 1637 cm^{-1}. Compound **X** showed the hydroxy group stretch at 3192 cm^{-1} and the carbonyl stretch at 1637 cm^{-1}.

The ^{1}H NMR spectra of compounds **IX** and **X** also suggested that they were flavanones. Their spectra were very similar except for the two methyl group three-proton resonances in the high-field region. The spectra showed three characteristic patterns. The characteristic AMX coupling system of H-2, H-3β and H-3α appeared at δ_H 5.48 (dd, J = 12.82, 3.11 Hz, H-2), δ_H 3.09 (dd, J = 17.21, 12.82 Hz, H-3β) and

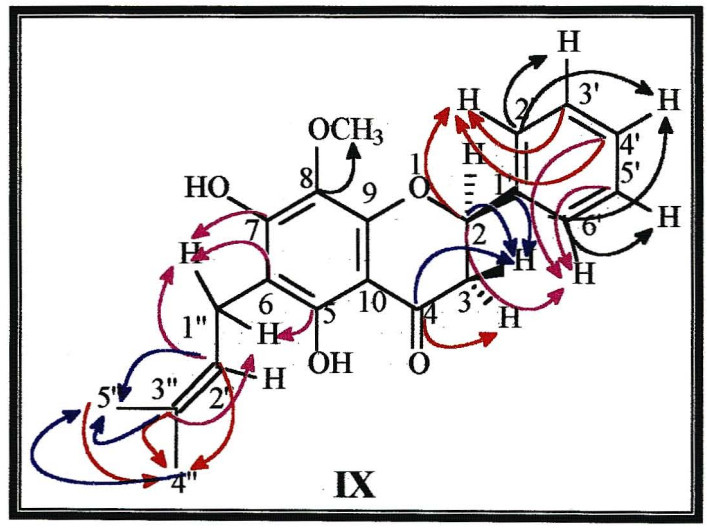
δ_H 2.78 (dd, J = 17.03, 3.11 Hz, H-3α) for compound **IX** and δ_H 5.40 (dd, J = 12.64, 3.11 Hz, H-2), δ_H 3.05 (dd, J = 17.21, 12.64 Hz, H-3β) and δ_H 2.78 (dd, J = 17.21, 3.11 Hz, H-3α) for compound **X**. The large vicinal coupling constant of 12.64 Hz indicated a *trans* relationship between the H-2 and H-3β protons[1,19]. These three signals also showed COSY correlations with each other for each compound.

Another characteristic pattern was that of the monosubstituted aromatic B ring. The proton resonances on this B ring occurred as a two- proton doublet at δ_H 7.51 (2H, d, J = 7.13 Hz, H-2'/6'), another doublet at δ_H 7.40 (2H, d, J = 7.52 Hz, H-3'/5') and a multiplet at δ_H 7.36 (1H, m, H-4') for compound **IX** and δ_H 7.48 (2H, d, J = 7.14 Hz, H-2'/6'), δ_H 7.35 (2H, d, J = 7.10 Hz, H-3'/5') and a triplet at δ_H 7.37 (1H, t, J = 7.51 Hz, H-4') for compound **X**. In both compounds **IX** and **X**, the proton resonances for H-3'/5' and H-4' overlapped. COSY coupling between H-2'/6' and H-3'/5' and between H-2'/6' and H-4' were evident.

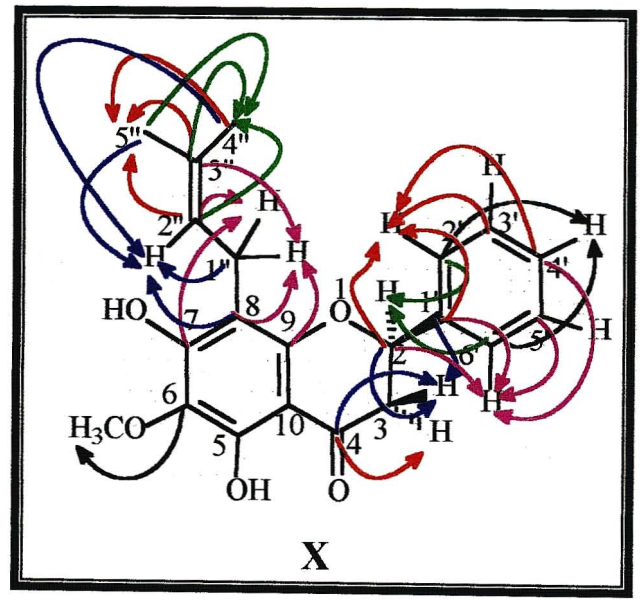
The third characteristic ^{1}H NMR pattern was that of the isoprenyl group on the A ring. The proton resonances of the isoprenyl group could be seen at δ_H 3.22 (2H, d, J = 7.14 Hz, H-1"), δ_H 5.18 (1H, t, J = 7.14 Hz, H-2"), δ_H 1.63 (3H,s, H-4") and δ_H 1.74 (3H, s, H-5") for compound **IX** and δ_H 3.21 (2H, d, J = 7.14 Hz, H-1"), δ_H 5.14 (1H, t, J = 7.14 Hz, H-2"), δ_H 1.56 (3H,s, H-4") and δ_H 1.60 (3H, s, H-5") for compound **X**. Coupling in the COSY spectrum could be seen between 2H-1" and H-2" and NOESY interactions could be seen between 2H-1" and 3H-5" and between H-2" and 3H-4" for both compounds **IX** and **X**. The isoprenyl group in compound **IX** was placed at the 6-position as both C-5, C-6 and C-7 all showed HMBC correlations to 2H-1". The methoxy group proton resonance at δ_H 3.73 (3H, s) showed NOESY interactions in the COSY spectrum to H-2'/6' and was therefore placed at the 8-position. Molecular models show that this NOESY interaction is possible. The hydroxy groups were thus placed in the remaining 5- and 7- positions, which are usually the most common positions on the A ring to be hydroxylated and this was confirmed by the HMBC spectra.



NOESY interactions for compounds **IX** and **X**



HMBC correlations for compound **IX**



HMBC correlations for compound **X**

In compound **X**, the isoprenyl group was placed at the 8- position as the 3H-4" proton resonance showed NOESY interactions with the H-2'/6' proton resonance. Furthermore, C-7, C-8 and C-9 showed HMBC correlations to the 2H-1" proton resonance. The methoxy group could not be placed at the 7- position as NOESY interactions were not observed between the methoxy group proton resonance and the 2H-1" proton resonance. The methoxy group was placed at the 6- position and not at the 5- position, since the presence of a hydroxy group at the 5- position was implied by the carbonyl carbon resonance of 197.14[1]. Hydrogen bonding between the C-5 hydroxy group and the C-4 carbonyl group, results in a carbonyl carbon resonance between 195.6 and 197.3 ppm[1] whereas in flavonoids where the hydroxy group is absent, the C-4 carbonyl resonance is observed between 189.7 and 191.7 ppm[1].

Compound **X** had an optical rotation of $+46.05^0$ and was therefore identified as (+)-agrandol which has not been isolated previously. (-)-Agrandol isolated previously from the root bark of *Dioclea grandiflora* (Leguminosae)[1] had an optical rotation of -41.2^0 and had the phenyl group at C-2 in the α position (2*S* configuration) as is the case in most naturally occurring flavanones[18]. Naturally occurring laevorotatory flavanones are given the 2*S* configuration[18]. (+)-Agrandol was therefore given the 2*R* configuration with the phenyl group at C-2 in the β position, as this compound was dextrorotatory. Naturally occurring dextrorotary flavanones are rare[18]. Compound **IX,** had an optical rotation of $+37.5^0$, similar in sign and magnitude to compound **X** and was therefore also given the 2*R* configuration with the phenyl group at C-2 in the β position as in (+)-agrandol. The absolute stereochemistry, which is omitted in many flavanones in the literature, can be unequivocally determined by optical rotatory dispersion data (ORD curves) and by comparison of the resultant curve to that of a flavanone whose absolute configuration is known[18].

Most flavonoid compounds yield intense peaks for the molecular ion, M^+, which is often the base peak[16]. In addition, flavonoids usually exhibit major peaks for $[M-H]^+$ and when methoxylated, $[M-CH_3]^+$ [16]. The most useful fragmentations in terms of flavonoid identification are those which involve cleavage of intact A and B ring fragments[16]. Pathway 1 and pathway 2 (figure 5) are two most common fragmentation pathways for flavonoids[16]. Pathway 1 corresponds to a retro-Diels-

Alder cleavage. Flavanones typically fragment by the retro-Diels-Alder reaction (pathway 1 in figure 5) to yield ions which correspond to the same A_1^+ and $[A_1 + H]^+$ ions observed for flavones, however, the B ring ion (B_3^+ in figure 6) from pathway 1 now contains an ethylene group. The mass spectral fragmentation pattern for 4'-methoxyflavanone is given in figure 6 and shows the fragmentation pathway observed for most flavanones[16].

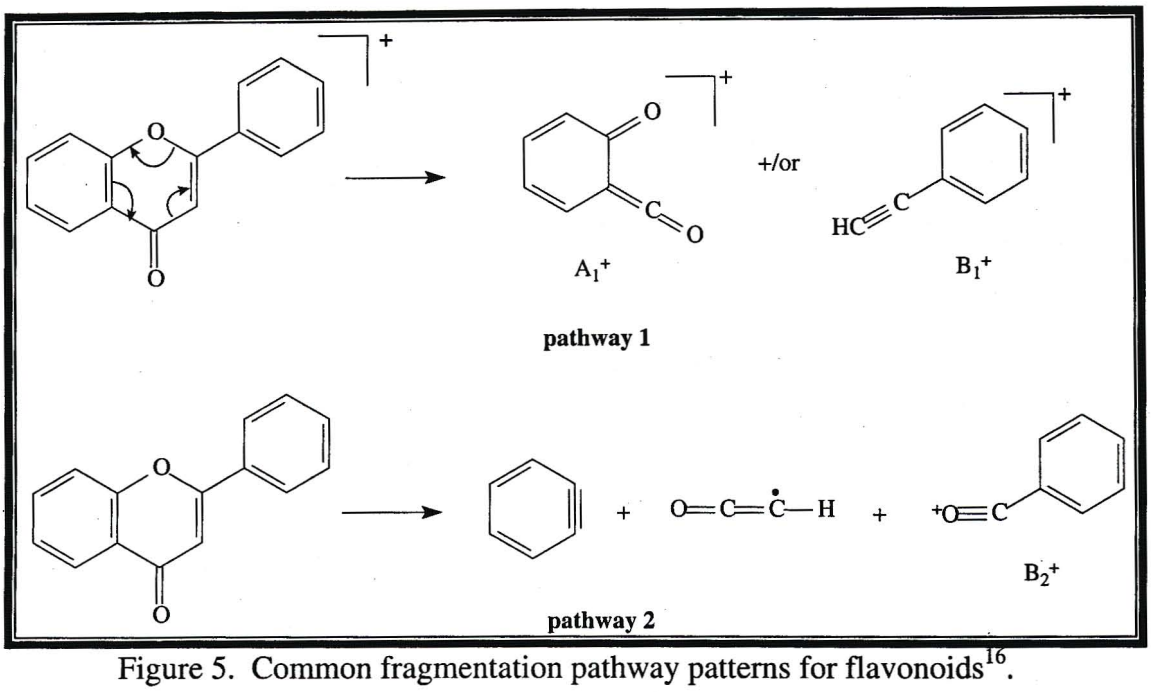


Figure 5. Common fragmentation pathway patterns for flavonoids[16].

The mass spectra of compounds **IX** and **X** were very similar. This was expected since the only difference between the two compounds was that the methoxy group and the prenyl group changed positions on the A ring. Both compounds showed the molecular ion peaks at *m/z* 354 as the base peaks, which is the case in most flavonoid compounds[16]. The loss of a methyl group in both compounds showed a fragment ion peak at *m/z* 339. The loss of a methyl group usually occurs in methoxylated flavonoid compounds[16]. This is followed by the loss of a C=O group[1], resulting in the ion fragment at *m/z* 311. The fragment peak at *m/z* 250 (A_1^+), results from the retro-Diels-Alder cleavage of the molecular ion[1,16]. The A_1^+ Diels-Alder fragment then loses a methyl group, followed by the loss of a C=O group, resulting in the fragment ion peaks at *m/z* 235 and *m/z* 207 respectively. The loss of 55 amu corresponds to a loss of a $C_4H_7^+$ fragment, a common fragmentation process whenever a prenyl sidechain undergoes allylic cleavage[1]. This fragmentation process is evident in the

fragment ion peaks at *m/z* 299 (M^+ - C_4H_7) and *m/z* 195 (A_1^+ - C_4H_7)[1]. The proposed mass spectral fragmentation pattern of compounds **IX** and **X** are given in schemes 2 and 3.

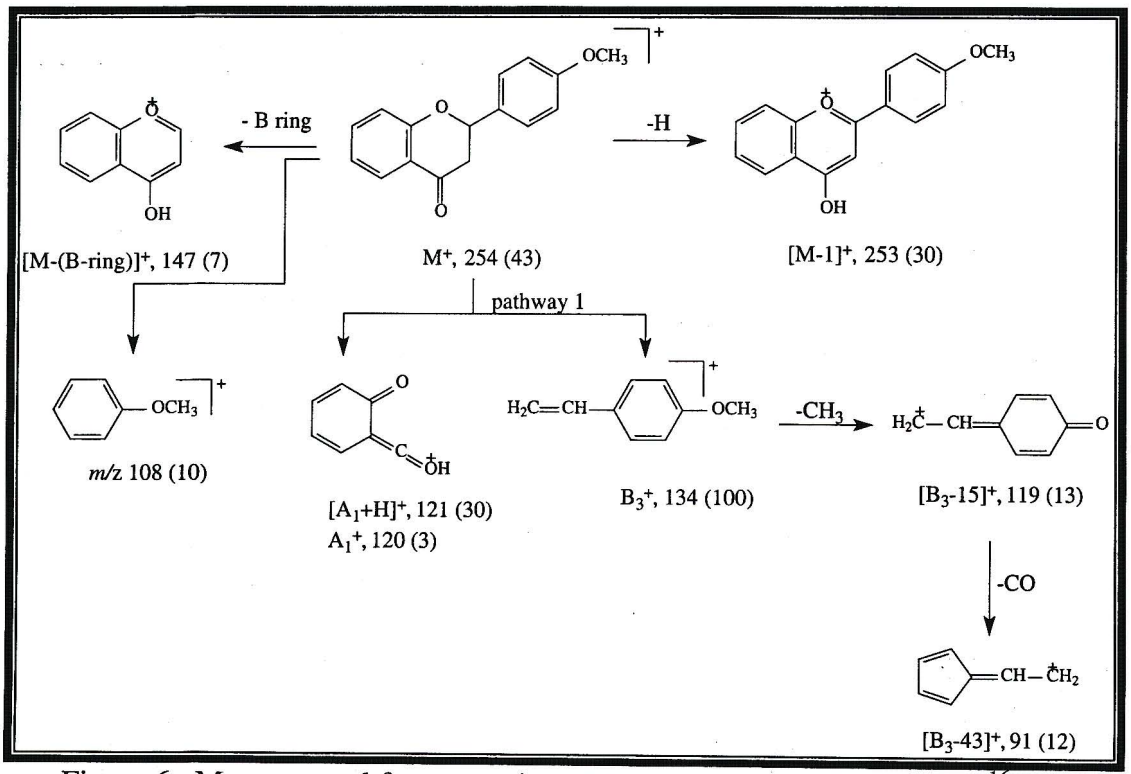
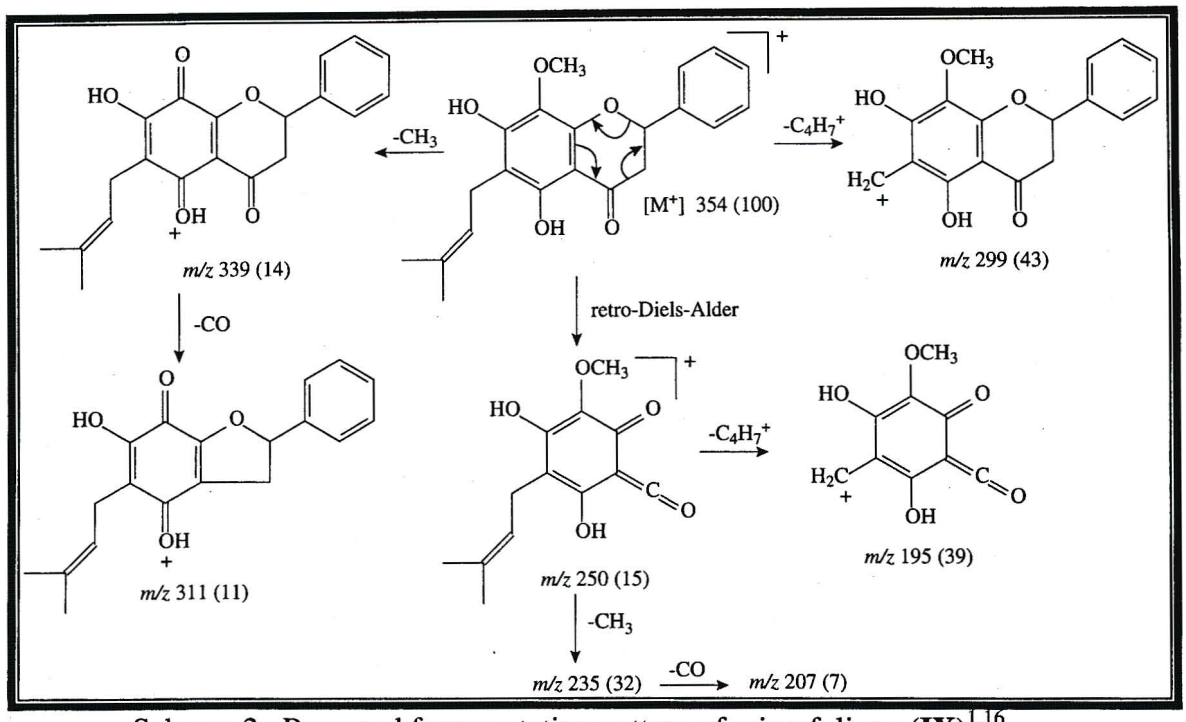
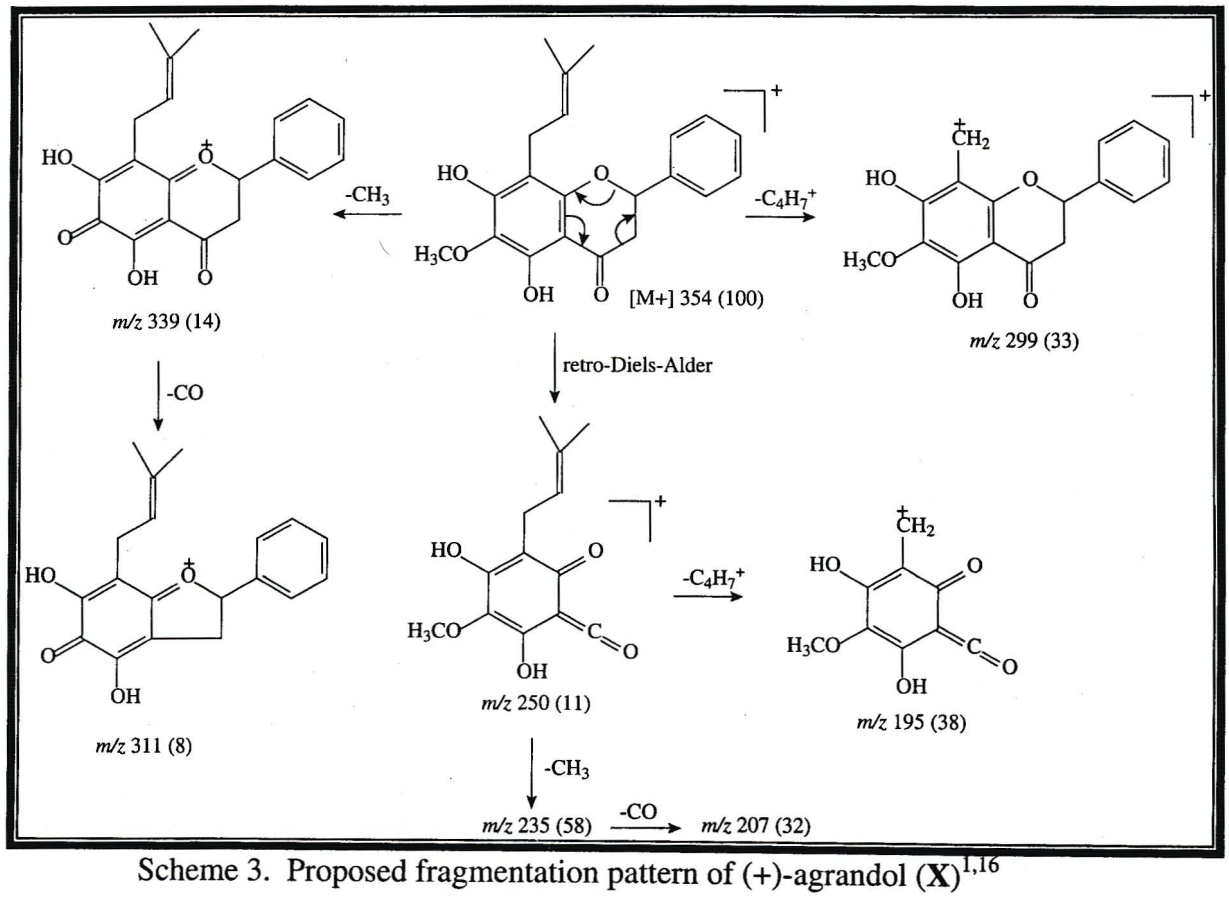


Figure 6. Mass spectral fragmentation pattern for 4'-methoxyflavanone[16] (the numbers in brackets indicate the relative percentages of the ion peaks).

The melting point of (+)-agrandol was 142-143 ^{0}C, which was fourteen degrees lower than that of (-)-agrandol which had a melting point of 156-157 ^{0}C[1]. The ^{1}H and ^{13}C NMR data compared well with those from literature[1]. ^{1}H and ^{13}C NMR assignments for compounds **IX** and **X** were made with the aid of HMBC, COSY and NOESY data and are tabulated in tables 2 and 3. Compound **IX,** (+)-microfolione and compound **X,** (+)-agrandol have not been isolated previously.



Scheme 2. Proposed fragmentation pattern of microfolione (**IX**)[1,16]



Scheme 3. Proposed fragmentation pattern of (+)-agrandol (**X**)[1,16]

Table 2. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound IX, (+)-microfolione (CD_3OD, 400 MHz)

Pos.	^{1}H	^{13}C	HMBC interactions	COSY correlations	NOESY interactions
2	5.48, dd (12.82, 3.11)	79.58	H-3β, H-2′/6′	H-3β,3α	H-2′/6′, H-3β,3α
3β 3α	3.09, dd (17.21, 12.82) 2.78, dd (17.03, 3.11)	43.24		H-2, H-3α H-2, H-3β	H-2′/6′, H-2,3α H-2,H-3β
4		196.21	H-3β,3α		
5		156.88*	2H-1"		
6		108.44	2H-1"		
7		157.69*	2H-1"		
8		128.28	8-OCH_3		
9		151.79			
10		101.77			
1′		139.24	H-3β		
2′/6′	7.51, 2H, d (7.13)	126.03	H-3′/4′/5′	H-3′/4′/5′	H-2,3β,3′/5′,8-OCH_3
3′/5′	7.40, 2H, d (7.52)	128.49	H-2′/6′	H-2′/6′	H-2′/6′
4′	7.36, m	128.40	H-2′/6′	H-2′/6′	
1"	3.22, 2H, d (7.14)	21.07		H-2", 3H-4", 3H-5"	H-2", 3H-5"
2"	5.18, t (7.14)	122.35	2H-1", 3H-4", 3H-5"	2H-1", 3H-4", 3H-5"	2H-1",3H-4"
3"		130.66	2H-1", 3H-4", 3H-5"		
4"	1.63, 3H, s	24.85	3H-5"	H-2", 2H-1", 3H-5"	H-2", 3H-5"
5"	1.74, 3H, s	16.76	3H-4"	H-2", 2H-1", 3H-4"	2H-1", 3H-4"
OCH_3	3.73, 3H, s	60.46			H-2′/6′

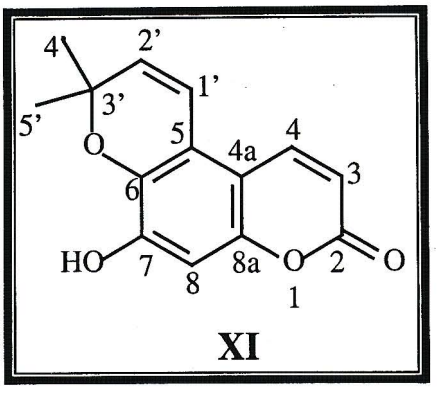
* Assignments may be interchanged.

Table 3. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for compound X, (+)-agrandol and (-) – agrandol[1] (CD_3OD, 400 MHz)

Pos.	^{1}H (+)-agrandol	^{1}H (Lit.[1])# (-)-agrandol	^{13}C (+)-agrandol	^{13}C (lit [1])## (-)-agrandol	HMBC interactions	COSY correlations	NOESY interactions
2	5.40, dd (12.64, 3.11)	5.40, dd (12.8, 3.3)	79.18	79.1	H-3β, H-2′/6′	H-3β,3α	H-2′/6′,3β,3α
3β 3α	3.05, dd (17.21, 12.64) 2.78, dd (17.21, 3.11)	3.03, dd (17.2, 12.6) 2.85, dd (17.3, 3.5)	43.18	43.8		H-2, 3α H-2, 3β	H-2′/6′,2, 3α H-2, 3β
4			197.14	197.2	H-3β,3α		
5			152.91	152.4			
6			128.88	128.3	OCH_3		
7			157.35 *	155.9	2H-1"		
8			107.78	107.4	2H-1", H-2"		
9			155.73 *	152.4	2H-1"		
10			102.03	103.0			
1′			139.39	139.0	H-3β, 2′/6′		
2′/6′	7.48, 2H, d (7.14)	7.40, m	126.03	126.1	H-2, 4′	H-3′/4′/5′	H-2, 3β, 3α, 3′/5′, 3H-4"
3′/5′	7.35, 2H, d (7.10)	7.40, m	128.39	129.0	H-2′/6′	H-2′/6′	H-2′/6′
4′	7.37, t (7.51)	7.40, m	128.27	128.8	H-2′/6′	H-2′/6′	
1"	3.21, 2H, d (7.14)	3.29, d (7.2)	21.66	22.1	H-2"	H-2", 3H-5"	H-2", 3H-4"
2"	5.14, t (7.14)	5.21, t	122.44	122.1	2H-1", 3H-4", 3H-5"	2H-1", 3H-4"	2H-1", 3H-5"
3"			130.60	132.2	2H-1", 3H-4", 3H-5"		
4"	1.56, 3H, s	1.68, s	24.85	26.0	H-2", 3H-5"	H-2", 2H-1"	2H-1", H-2′/6′
5"	1.60, 3H, s	1.64, s	16.80	18.0	H-2", 3H-4"	H-2", 2H-1"	H-2"
OCH_3	3.78, 3H, s	3.96, s	59.90	61.2			
5-OH		12.20, s					
7-OH		10.30, s					

* Assignments may be interchanged, # Data recorded in $CDCl_3$ (250 MHz), ## Data recorded in $CDCl_3$ and CD_3OD (250 MHz)

4.2.3 Structural elucidation of compound XI, cedrecoumarin A (MICM23A)



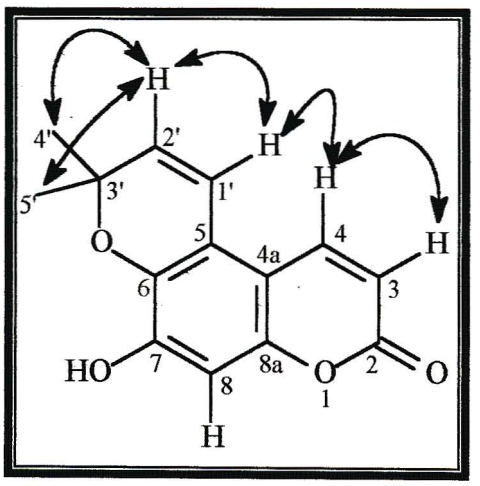
XI

Compound **XI** was isolated as a yellow crystalline material with a melting point of 148-149^{0}C. This compound was found to have a molecular formula of $C_{14}H_{12}O_4$. This compound gave UV absorption maxima at λ_{max}^{MeOH} nm 325 (log ε = 3.92), 277 (3.72), 228 (4.17) and 205 (4.18), typical of coumarins[15]. The IR spectrum showed peaks at 3394 cm^{-1}, a broad peak due to hydroxy stretching, 2976 cm^{-1} and 2933 cm^{-1} due to C-H stretching and at 1710 cm^{-1} which was typical of the α-pyrone carbonyl stretch[14].

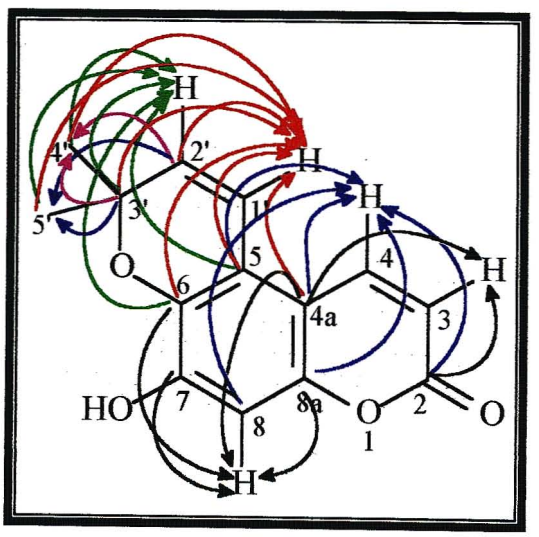
The ^{1}H NMR spectrum of compound **XI** showed the presence of two sets of doublets. The first set of doublets appeared at δ_H 6.19 and δ_H 8.08 and had a coupling constant of 9.70 Hz, suggestive of *cis* olefinic protons and were attributed to H-3 and H-4 respectively of the coumarin nucleus. These two resonances also showed coupling in the COSY spectrum. They were assigned to the 3- and 4- positions as the carbonyl carbon resonance at position 2- at δ_C 162.50 and the C-4a carbon resonance at δ_H 107.31 showed HMBC correlations to both the H-3 and H-4 proton resonances. The carbon resonances at positions 5-, 8- and 8a- all showed HMBC correlations to the doublet at δ_H 8.08 and not to the doublet at δ_H 6.19, therefore the δ_H 8.08 resonance was attributed to H-4.

The other set of doublets at δ_H 6.79 and δ_H 5.90 which were seen to be coupled in the COSY spectrum had a coupling constant of 10.0 Hz also indicative of *cis* olefinic protons. These proton resonances were attributed to H-1' and H-2' respectively on the ring attached at the 5- and 6- positions. The C-2' carbon resonance showed HMBC correlations with H-1', 3H-4' and 3H-5'. The H-1' proton resonance showed a NOESY

interaction with the H-4 proton resonance, which led to the ring being placed at the 5- and 6- positions on the coumarin nucleus. The H-2' proton resonance showed NOESY interactions with the 3H-4', 3H-5' and H-1' proton resonances. The C-3' carbon resonance occurred downfield at δ_C 76.49 in the ^{13}C NMR spectrum, suggesting that it was attached to an oxygen atom. This carbon resonance showed HMBC correlations to H-1', 2', 3H-4' and 3H-5'. The 3H-4' and 3H-5' proton signals occurred as a six-proton singlet at δ_H 1.43 and the C-4' and C-5' carbon resonance occurred as a single resonance at δ_C 26.25 which indicated that these methyl groups must be equivalent, which was predicted and confirmed using a computer simulation and molecular models which showed the methyl groups to be equivalent because of the planarity of the molecule, the A, B and C rings being flat in a plane and the methyl groups sticking out on either side.



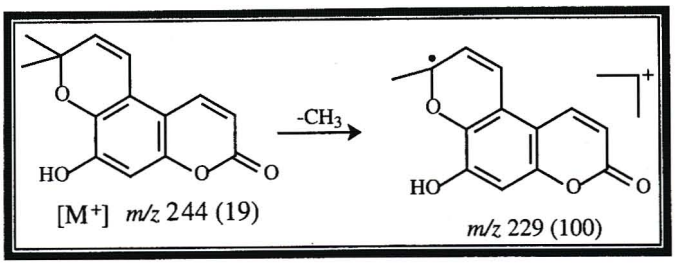
NOESY interactions for compound **XI**



HMBC correlations for compound **XI**

The one-proton singlet at δ_H 6.63 was attributed to H-8. This proton was placed at the 8- position and not in the 7- position as an HMBC correlation was seen between its carbon resonance at δ_C 102.63 and the H-4 proton resonance.

The mass spectrum of compound **XI** showed a molecular ion peak at *m/z* 244 and the base peak at *m/z* 229, was the result of the loss of one of the geminal methyl groups. The proposed fragmentation pattern for compound **XI** can be seen in scheme 4.



Scheme 4. Proposed fragmentation pattern of cedrecoumarin A (**XI**)

^1H and ^{13}C NMR data were assigned with the aid of HSQC, HMBC and NOESY spectra and are given in Table 4. Compound **XI** was previously isolated from the stem bark of *Cedrelopsis grevei* as cedrecoumarin A[2].

Table 4. ^1H, ^{13}C, HMBC, COSY and NOESY data for compound XI, cedrecoumarin A (CD_3OD, 400 MHz)

Pos.	^1H	^{13}C	HMBC interactions	COSY correlations	NOESY interactions
2		162.50	H-3,4		
3	6.19, d (9.70)	111.40		H-4	H-4
4	8.08, d (9.70)	140.28		H-3	H-3,1'
4a		107.31	H-3,4,8,1'		
5		117.64	H-4,1',2'		
6		137.84	H-8,1',2'		
7		149.99	H-8		
8	6.63, s	102.63	H-4		
8a		150.43	H-8,4		
1'	6.79, d (10.0)	116.72		H-2'	H-2',4
2'	5.90, d (10.0)	133.49	H-1',4',5'	H-1'	H-1', 3H-4'/3H-5'
3'		76.49	H-1',2',4',5'		
4'/5'	1.43, 6H, s	26.25	H-1',2'		H-1',2'

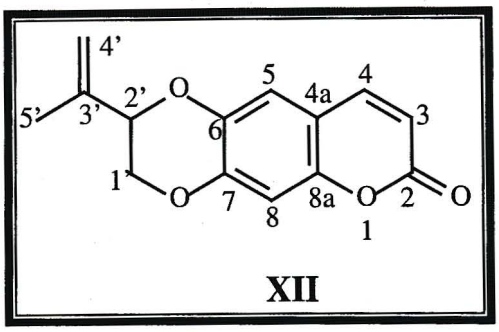
4.2.4 Biological activity of microfolian (VIII), microfolione (IX), (+)-agrandol (X) and cedrecoumarin A (XI).

Compounds **VIII-XI** were tested in an assay for agonistic activity on the alpha and beta estrogen receptors, in an assay for the inhibition of chemiluminescence of reactive oxygen metabolites generated by activated human granulocytes and in an assay with superoxide anions in a cell-free system. All the compounds tested were active in either one or all of the assays.

Microfolione (**IX**), (+)-agrandol (**X**) and cedrecoumarin A (**XI**) were active in the assays for agonistic activity on both alpha and beta estrogen receptors (ER) in ranges from 10-100 ug/ml. The activity in the beta ER assay seemed somewhat more pronounced. However, activity was low when compared with the flavonoid, genistein, a well known phytoestrogen, which showed activity in the same assay in the range of 3-30 ng/ml and has a far more pronounced effect on ER beta. High activity on ER beta and low activity on ER alpha is regarded beneficial in the treatment of menopausal disorders. The different compounds with estrogenic activity, as present in the *Cedrelopsis microfoliata* extract, may together have quite an effect.

Microfolian (**VIII**), (+)-agrandol (**X**) and cedrecoumarin A (**XI**) were found to inhibit the (luminol-enhanced) chemiluminescence of reactive oxygen metabolites generated by activated human granulocytes with IC_{50} values of 13.0, 4.0 and 3.2 ug/ml respectively. The same compounds were also found to scavenge superoxide anions in a cell-free system (IC_{50} values of 0.2, 3.0 and 3.0 ug/ml respectively). In particular, microfolian was found to be a very potent superoxide anion scavenger. The inhibition of the chemiluminescence of reactive oxygen metabolites and superoxide anion scavenging activity, both suggest anti-inflammatory activity for microfolian (**VIII**), (+)-agrandol (**X**) and cedrecoumarin A (**XI**).

4.2.5 Structural elucidation of compound XII, (+)-obliquin (MICM12)

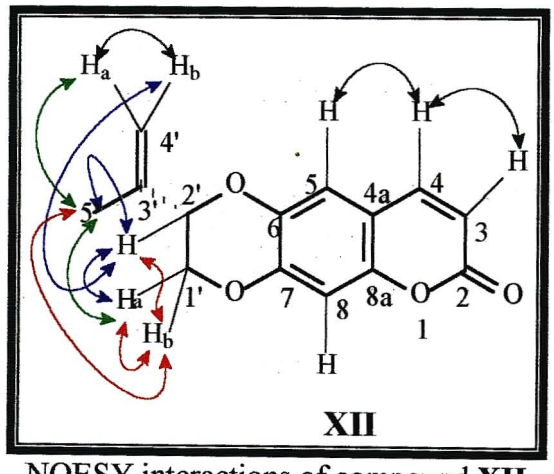


XII

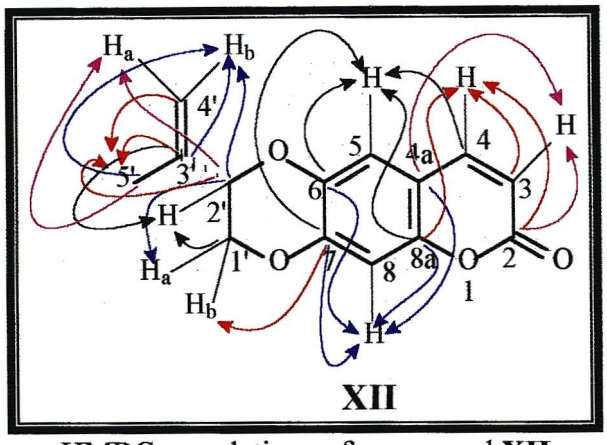
Compound **XII** was isolated as a white crystalline material with a melting point of 160-161^{0}C, having a molecular formula of $C_{14}H_{12}O_4$. This compound gave UV absorption maxima at λ_{max}^{MeOH} nm 341 (log ε = 5.11), 296 (5.06), 260 (4.90), 229 (5.32) and 207 (5.59), characterisitic of coumarins[15]. The IR spectrum showed absorption peaks at υ_{max}^{NaCl} 2971 cm^{-1}, 2916 cm^{-1} and 2849 cm^{-1} characteristic of C-H and C-H_2 stretching and at 1711 cm^{-1} typical of the α-pyrone carbonyl stretch[14]. Compound **XII** had an optical rotation of +80.60^0.

The ^{1}H NMR spectrum of compound **XII** showed the presence of a pair of doublets at δ_H 6.26 and δ_H 7.55 with a coupling constant of 9.52 Hz, characteristic of *cis* olefinic protons. These were attributed to H-3 and H-4 respectively of the coumarin nucleus. These proton resonances were placed at the 3- and 4- positions respectively as the C-2 carbonyl resonance at δ_C 161.38 shows HMBC correlations to both the H-3 and H-4 proton resonances. The resonance at δ_H 6.26 was assigned to H-3 because of an HMBC correlation to C-4a. The resonance at δ_H 7.55 was assigned to H-4 because of an HMBC correlation to C-8a and a NOESY interaction with the singlet at δ_H 6.98. A proton was placed at the 5- position because of this interaction. The remaining singlet at δ_H 6.85 must therefore be placed at the 8- position since, if it were at the 6- or 7-position then either *ortho* or *meta* coupling would result. Very weak *para* coupling could be seen in the COSY spectrum between the H-5 and H-8 proton resonances.

Also evident in the ^{1}H NMR spectrum was an AMX coupled system consisting of three double doublet resonances at δ_H 4.52 (J = 8.05 Hz, 2.38 Hz), δ_H 4.35 (J = 11.35 Hz, 2.38 Hz) and δ_H 4.01 (J = 11.35 Hz, 8.01 Hz). These three resonances were all seen to be coupled in the COSY spectrum. In the HSQC spectrum, the resonances at δ_H 4.35 and δ_H 4.01 both correlated to the same carbon atom at δ_C 67.82. These proton and carbon resonances were attributed to the protons and carbon at the 1'-position. The remaining proton resonance at δ_H 4.52 was attributed to H-2'. The C-1' and C-2' carbons must be part of a ring as the two protons on C-1' are non equivalent. Since the C-1' and C-2' carbon resonances are both deshielded at δ_C 67.82 and δ_C 75.94 respectively, they must both be attached to oxygen atoms on the A ring. These oxygen atoms must be at the 6- and 7- positions as hydrogen atoms were placed at the 5- and 8- postions. This results in a 6-membered ring consisting of C-6, C-7, C-1', C-2' and two oxygen atoms. The C-7 carbon resonance showed HMBC correlations to the 2H-1', H-5 and H-8 proton resonances and the C-6 carbon resonance showed HMBC correlations to the H-5 and H-8 proton resonances only. This resulted in C-1' being placed at the oxygen atom at the 7- position.



NOESY interactions of compound **XII**



HMBC correlations of compound **XII**

A pair of deshielded doublets with a very small coupling constant of 0.73 Hz occurred at δ_H 5.17 and δ_H 5.11. Both these proton resonances correlated to the same carbon resonance at δ_C 115.04 in the HSQC spectrum. These proton and carbon resonances were attributed to the protons and carbon of the methylene group at the 4'- position. The last proton resonance, a three-proton singlet at δ_H 1.84 was attributed to 3H-5'. This 3H-5' proton resonance showed NOESY interactions with one of the H-4' proton resonances and the 2H-1' and H-2' proton resonances. The same H-4' resonance showed NOESY interactions with the H-2' and the 3H-5' proton resonances. COSY coupling could also be seen between the H-4' resonance at δ_H 5.17 and the 3H-5' resonance at δ_H 1.84. The C-3' carbon was bonded to the C-2' carbon as the C-3' carbon resonance showed HMBC correlations to the H-2', H-4'b and the 3H-5' proton resonances.

The mass spectrum of compound **XII** shows the molecular ion peak as the base peak at *m/z* 244 which provides further evidence for the structure of compound **XII**.

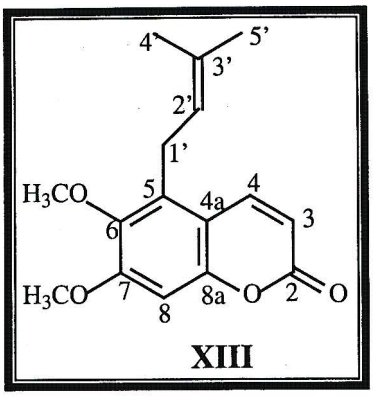
Obliquin (**XII**) was first isolated from the stem bark of *Ptaeroxylon obliquum* (Ptaeroxylaceae)[3]. It was also isolated from three Compositae species, *Helichrysum serpyllifolium*[4], and roots of *Helichrysum dismifolium* and *Helichrysum stirlingii*[5]. ^{1}H and ^{13}C NMR assignments were made with the aid of HSQC, HMBC, COSY and NOESY data and are given in table 5. The ^{13}C NMR data of obliquin (**XII**) is tabulated against that of the 8-methoxy derivative as no ^{13}C NMR data for obliquin (**XII**) is available in the literature. The absolute stereochemistry of (+)-obliquin (**XII**) is unknown.

Table 5. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for obliquin, compound XII ($CDCl_3$, 400 MHz)

Pos.	^{1}H	^{1}H (lit [4])	^{13}C	^{13}C* (lit [6])	HMBC interactions	COSY correlations	NOESY interactions
2			161.38	160.50	H-3,4		
3	6.26, d (9.52)	6.27, d (9.5)	114.38	114.46	H-4	H-4	H-4
4	7.55, d (9.52)	7.57, d (9.5)	143.19	143.39	H-5	H-3	H-3,5
4a			113.21	112.69	H-3,8		
5	6.98, s	7.01, s	114.72	108.92			H-4
6			140.85	141.12	H-5,8		
7			146.80	140.28	H-1'b,5,8		
8	6.85, s	6.87, s	105.08	142.48			
8a			149.45	139.16	H-4,5,8		
1'a 1'b	4.01, dd (11.35, 8.05) 4.35, dd (11.35, 2.38)	4.04, dd (12, 8) 4.39, dd (12, 2)	67.82	67.60	H-2'	H-2'	H-2',3H-5', 1'b H-2',3H-5', 1'a
2'	4.52, dd (8.05, 2.38)	4.54, dd (8, 2)	75.94	75.63	H-1'a,4'a,4'b, 3H-5'	H-1',4', 3H-5'	H-4'b, 3H-5',1'a,b
3'			139.35	136.02	H-2',4'b, 3H-5'		
4'a 4'b	5.11, d (0.73) 5.17, d (0.73)	5.14, br s 5.19, br s	115.04	114.88	3H-5'	H-2',3H-5'	H-2', 3H-5',4'b H-2', 3H-5',4'a
5'	1.84, s	1.86, br s	19.16	18.77	H-4'a,b	H-2',4'	H-4'a,1'a,b,2'
8-OCH_3	-	-		61.69			

8-methoxy derivative[6]

4.2.6 Structural elucidation of compound XIII, microfolicoumarin (MICM95)



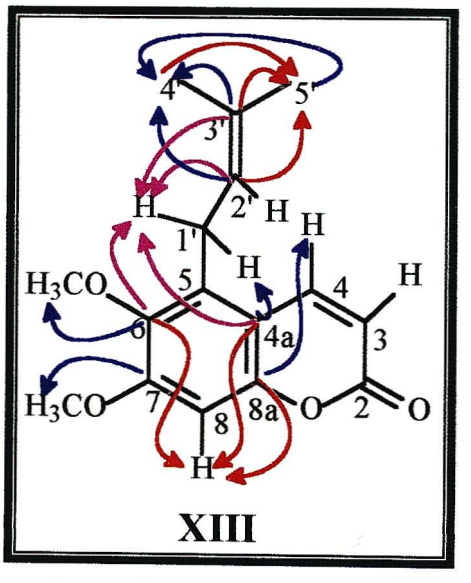
XIII

Compound **XIII** was isolated as a yellow oil and had a molecular formula of $C_{16}H_{18}O_4$ based on high resolution mass spectrometry (Found *m/z* 274.12097, required 274.12050). This compound gave UV absorption maxima at λ_{max}^{MeOH} nm 299 (log ε = 4.54) and 205 (5.08), typical of coumarins[15]. The IR spectrum showed an absorption band at υ_{max}^{NaCl} 1710 cm^{-1}, characteristic of the α-pyrone carbonyl stretch[14].

The ^1H NMR spectrum of compound **XIII**, showed characteristic resonances for a prenylated coumarin. This compound was isolated in small amounts with a minor flavonoid impurity. The amount isolated was too little to purify further but peaks due to the coumarin could easily be separated from those of the minor flavonoid component. A pair of doublets at δ_H 6.25 and δ_H 7.75 with a coupling constant of 9.80 Hz indicative of *cis* proton coupling was attributed to H-3 and H-4 respectively. These two resonances were also seen to be coupled in the COSY spectrum. The carbon resonance at δ_C 152.64 showed an HMBC correlation to the proton resonance at δ_H 7.75, which prompted this carbon resonance to be assigned to the carbon at the 8a- position. The C-2 carbonyl group carbon resonance was evident at δ_C 161.37.

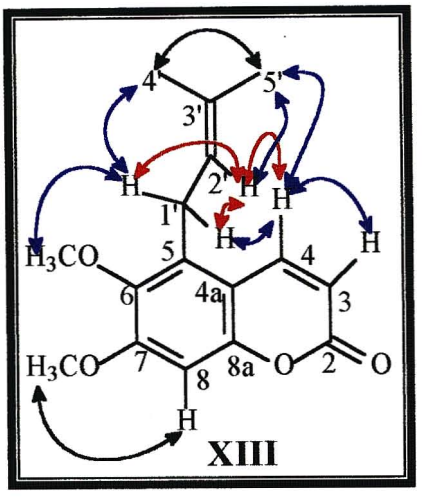
Also present in the ^1H NMR spectrum was an aromatic singlet at δ_H 6.72, two aromatic methoxy proton resonances at δ_H 3.77 and δ_H 3.90 and four characteristic resonances for an isoprenyl group. The isoprenyl group proton resonances consisted of a triplet at δ_H 5.02 (J = 6.59 Hz) attributed to H-2' which was seen to be coupled in the COSY spectrum to the two-proton doublet at δ_H 3.55 (J = 6.59 Hz) attributed to 2H-1' and the two singlets at δ_H 1.80 and δ_H 1.67. The proton resonance at δ_H 1.67

was attributed to the 3H-5' protons as this resonance showed NOESY interactions with the H-2' proton resonance, indicating that this methyl group was *cis* to H-2'. The other methyl resonance was therefore attributed to the 3H-4' protons. The fully substituted carbon resonance at δ_C 133.06 showed HMBC correlations to 2H-1', 3H-4' and 3H-5' and was therefore attributed to the carbon atom at the 3'- position. The H-4 proton resonance showed NOESY interactions with the 2H-1' and H-2' proton resonances, which resulted in the isoprenyl group being placed at the 5-position on the aromatic ring. The 2H-1' proton resonance showed NOESY interactions with the methoxy resonance at δ_H 3.77, which resulted in this resonance being attributed to the methoxy group at the 6- position. The aromatic singlet at δ_H 6.72 was assigned to the H-8 proton as C-4a at δ_C 111.14 and C-8a at δ_C 152.64 both showed HMBC correlations with this proton resonance. The H-8 proton resonance showed a NOESY interaction with the methoxy group proton resonance at δ_H 3.90. This methoxy group was therefore placed at the 7- position on the aromatic ring.



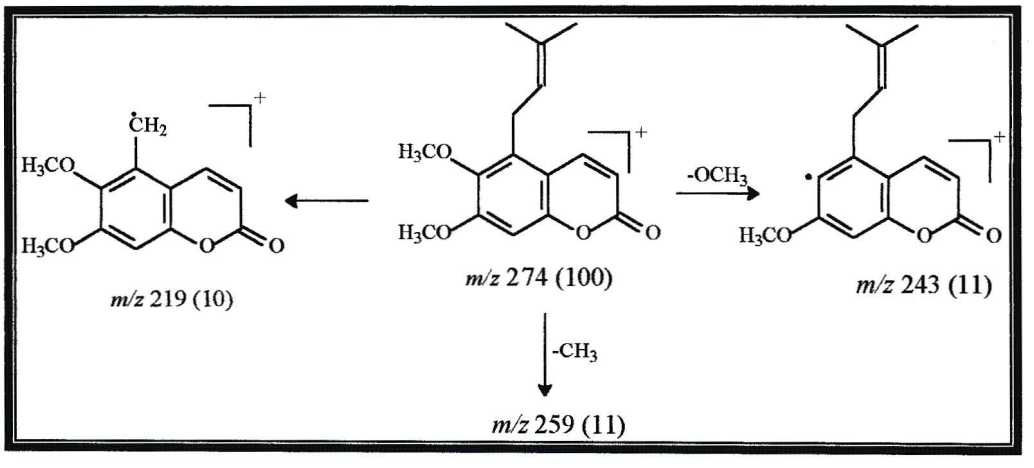
XIII

HMBC correlations of compound XIII



NOESY interactions of compound XIII

The mass spectrum of compound **XIII** showed the molecular ion peak as the base peak at *m/z* 274. Loss of a methyl group and a methoxy group results in fragment peaks with *m/z* 259 and *m/z* 243 respectively. The loss of 55 mass units corresponds to a loss of a $C_4H_7^+$ fragment, which is a common fragmentation process when the prenyl sidechain undergoes allylic cleavage[1] and results in the fragment peak at *m/z* 219. The proposed fragmentation pattern of compound **XIII** is given in scheme 6.



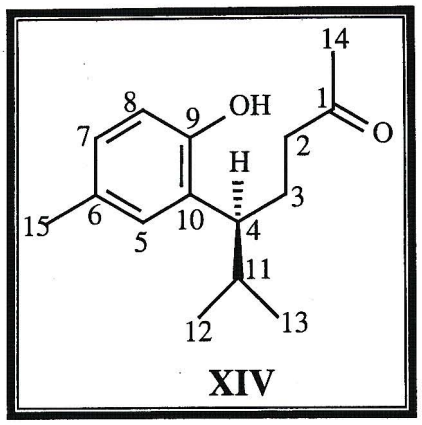
Scheme 5. Proposed fragmentation pattern of microfolicoumarin (**XIII**)

^1H and ^{13}C NMR data were assigned with the aid of COSY, HMBC and NOESY data and are given in table 6. Compound **XIII** has not been isolated previously and has been given the name microfolicoumarin (**XIII**).

Table 6. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for microfolicoumarin XIII (400 MHz, $CDCl_3$)

Pos	^{1}H NMR	^{13}C NMR	COSY correlations	HMBC correlations	NOESY interactions
2		161.37			
3	6.25, d (9.80)	113.05	H-4		H-4
4	7.75, d (9.80)	141.16	H-3		H-3, 2H-1', H-2'
4a		111.14		2H-1', H-8	
5		126.06			
6		143.86		6-OCH_3, 2H-1', H-8	
7		156.36		7-OCH_3	
8	6.72, s	98.74			7-OCH_3
8a		152.64		H-4, H-8	
1'	3.55, d (6.59)	24.97	H-2', 3H-4', 3H-5'		H-2', 3H-4', 6-OCH_3, H-4
2'	5.02, t (6.59)	122.34	2H-1', 3H-4', 3H-5'	2H-1', 3H-4', 3H-5'	2H-1', 3H-5'
3'		133.06		2H-1', 3H-4', 3H-5'	
4'	1.80, s	18.44	2H-1', H-2', 3H-5'	3H-5'	2H-1', 3H-5'
5'	1.67, s	25.94	2H-1', H-2', 3H-4'	3H-4'	H-2', 3H-4'
6-OCH_3	3.77, s	61.43			
7-OCH_3	3.90, s	56.31	H-8		H-8

4.2.7 Structural elucidation of compound XIV, sesquichamaenol (MICM50)



XIV

Compound **XIV** was isolated as a white crystalline material with a melting point of 108-109^{0}C. It was found to have a molecular formula of $C_{15}H_{22}O_2$. The UV spectrum showed absorption maxima at λ_{max}^{MeOH} nm 284 (log ε = 4.22) and 204 (5.00). The IR spectrum showed absorption peaks at 3439 cm^{-1}, a broad band characteristic of O-H stretching, and at 1740 cm^{-1}, characteristic of the carbonyl stretch.

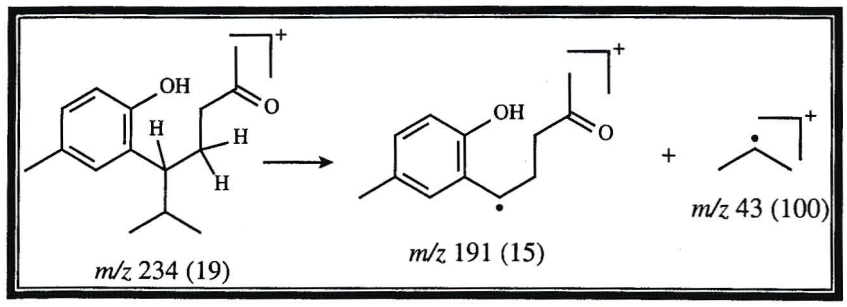
The ^{1}H NMR spectrum of compound **XIV** showed the presence of three aromatic proton signals. These signals occurred as a doublet at δ_H 6.65 (J = 8.06 Hz), a double doublet at δ_H 6.84 (J = 8.06, 1.70 Hz) and a doublet at δ_H 6.82 (J = 1.70 Hz). COSY coupling is evident between the resonances at δ_H 6.65 and δ_H 6.84. These resonances were attributed to H-8 and H-7 respectively. The doublet at δ_H 6.82 with a coupling constant of 1.70 Hz indicates that it is *meta* coupled to H-7, the double doublet at δ_H 6.84 (J = 8.06, 1.70 Hz), and was attributed to H-5. The resonance at δ_H 4.95 disappeared upon addition of D_2O which indicated that it was a hydroxy group proton resonance. This was placed at position 9- on the aromatic ring as this resonance had a NOESY interaction with the H-8 resonance at δ_H 6.65. The C-8 and C-10 carbon resonances showed HMBC correlations to the 9-OH proton resonance. A singlet at δ_H 2.23, integrating to three protons in the ^{1}H NMR spectrum indicated the presence of a methyl group resonance which was placed at position 6- on the aromatic ring as this resonance showed NOESY interactions with H-5 and H-7. Furthermore, the

corresponding C-15 carbon resonance at δ_C 21.08 showed HMBC correlations with the H-5 and H-7 proton resonances.

Another singlet methyl proton resonance integrating to three protons at δ_H 2.03 indicated that a methyl ketone was present. The presence of a carbonyl group was confirmed by the appearance of a carbonyl carbon peak at δ_C 208.63. This carbonyl resonance showed HMBC correlations to the methyl group proton peak at δ_H 2.03. The methyl proton resonance was then assigned to the 14- position and the carbonyl group to the 1- position. This carbonyl group carbon resonance also had HMBC correlations to two other proton resonances. These are the resonances at δ_H 2.20, a two-proton multiplet resonance that overlaps with the aromatic methyl 3H-15 resonance and the resonance at δ_H 1.73, a one-proton multiplet. This multiplet at δ_H 1.73, together with another one-proton multiplet resonance at δ_H 2.08 both correlate to the same carbon resonance at δ_C 27.05 in the HSQC spectrum, indicating that they are non-equivalent protons of a methylene group. The multiplet at δ_H 2.20 was assigned to the protons at position 2- as the C-2 carbon resonance showed HMBC correlations to the 3H-14 proton resonance. The other two resonances at δ_H 1.73 and δ_H 2.08 as well as the carbon resonance at δ_C 27.05 were then assigned to the protons and carbon at the 3- position. The two H-3 proton resonances and the 2H-2 proton resonance were seen to be coupled in the COSY spectrum.

The H-3b proton resonance was also seen coupled to the resonance at δ_H 2.56, a one-proton multiplet. This resonance was assigned to H-4. The H-4 resonance was, in turn, seen to be coupled in the COSY spectrum to the multiplet at δ_H 1.83, a one-proton resonance, which was then assigned to H-11. This H-11 resonance was in turn coupled to the two methyl doublets at δ_H 0.71 and δ_H 0.99 which integrated to three protons each and had a coupling constant of 6.59 Hz. These methyl groups were assigned to the 12- and 13- positions respectively. The C-11 and C-4 carbon resonances also had HMBC correlations to the 3H-12 and 3H-13 proton resonances. This assignment was confirmed when the 3H-12 proton resonance showed NOESY interactions with the H-11, H-4 and H-5 proton resonances and the 3H-13 proton resonance showed NOESY interactions with the H-11, H-4 and H-3a and H-3b proton resonances.

The mass spectrum of compound **XIV** showed a molecular ion peak at *m/z* 234. A peak at *m/z* 191 indicated the loss of an isopropyl group[17] and this isopropyl group fragment ion peak was the base peak at *m/z* 43. The proposed fragmentation pattern of compound **XIV** can be seen in scheme 7.



Scheme 6. Proposed fragmentation pattern of sesquichamaenol (**XIV**)

Compound **XIV** had an optical rotation of -5.95^0, which was consistent with that of (-)-sesquichamaenol, first isolated from the Benihi tree (*Chamaecyparis formosensis*, Cupressaceae)[7] and also from *Juniperus formosana*[10]. The absolute configuration at C-4 of (-)-sesquichamaenol (**XIV**) has been reported as *S*[10]. ^1H and ^{13}C NMR assignments were made with the aid of HSQC, HMBC, COSY and NOESY data and are given in table 7.

Table 7. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for sesquichamaenol, (XIV) ($CDCl_3$, 400 MHz)

Pos.	^{1}H	^{1}H [lit. 10], $CDCl_3$, 300 MHz	^{1}H [lit.. 7], $CDCl_3$, 60 MHz	^{13}C	HMBC interactions	COSY correlations	NOESY interactions
1				208.68	3H-14, H-3b, 2H-2		
2	2.20, m	2.04-2.17,m	#	42.04	3H-14	H-3a,b	H-3b
3	a) 2.08, m b) 1.73, m	a) 2.04-2.17, m b) 1.67-1.89, m	#	27.05	2H-2	H-3b, 2H-2 H-4, H-3a, 2H-2	H-3b,4, 3H-13 H-3a,5/7, 2H-2
4	2.56, m	2.58, td (8.5, 5.2)	2.60, m	44.0*	3H-12, 3H-13	H-11	H-11,3a, 3H-12, 3H-13, H-5,7,9-OH
5	6.82, d (1.7)	6.81, bs	6.5-7.0	128.67	3H-15	3H-15	3H-15, H-11, 3b, 3H-12
6				129.95	3H-15, H-8		
7	6.84, dd (8.06, 1.7)	6.85, bd (8.0)	6.5-7.0	127.46	H-5, 3H-15	H-8	H-8, 3H-15
8	6.65, d (8.06)	6.65, d (8.0)	6.64, d (9.00)	115.88	H-7, 9-OH	H-7	H-7
9				151.95	H-5,7		
10				130.16	9-OH		
11	1.83, m	1.67-1.89, m	#	33.35	3H-12, 3H-13	H-4, 3H-12, 3H-13	H-5/7, 3H-12, 3H-13
12	0.71, d (6.59)	0.71, d (6.6)	0.73, d (6.5)	21.61	3H-13	H-11	H-11,4,5, 3H-13
13	0.99, d (6.59)	0.99, d (6.6)	1.00, d (6.5)	21.23	3H-12	H-11	H-11,4,3a,b, 3H-12
14	2.03, s	2.02, s	2.03, s	30.42	2H-2	2H-2	
15	2.23, s	2.23, s	3.24, s	21.08	H-5/7	H-5/7	H-5/7
9-OH	4.95, s	5.23, bs	5.30, bs				H-4,8

* signal not observed in the ^{13}C NMR spectrum however in the HSQC and HMBC spectra, correlations are observed for a signal approximately 44.0 ppm and which corresponds to C-4.
# data not provided.

4.3 Experimental

Extraction of *Cedrelopsis microfoliata*

The stem bark of *C. microfoliata* J.-F. Leroy. (581.72 g) was collected at Ankarafantsika in the north west part of Madagascar in November 1999 and a voucher specimen (07-99/nJ-nDul (stem bark)) retained at the Département de Botanique of the University of Antananarivo, Madagascar. The dried and milled stem bark (108.82 g) was extracted on a soxhlet apparatus with hexane, dichloromethane and methanol. The solvent was removed under reduced pressure to yield 3.26 g of hexane extract, 2.48 g of dichloromethane extract and 4.83 g of methanol extract. NMR analysis of the crude methanol extract indicated that only sugars were present and was therefore not examined further.

Isolation of compounds VIII-XIV

The hexane and dichloromethane extracts (3.26 g and 2.48 g respectively) were chromatographed over silica gel (150 g) as the stationary phase, using a column (3 cm in diameter). Silica gel (Merck 9385) was used as the stationary phase for all column chromatography.

The mobile phase used for the hexane extract was a hexane : dichloromethane : methanol step gradient (25% dichloromethane in hexane (fractions 1-15), 50% dichloromethane in hexane (fractions (16-40); 100 % dichloromethane (fractions 41-55), 5% methanol in dichloromethane (fractions 56-84), 40% methanol in dichloromethane (fractions 85-110) and 100% methanol (fractions 121-130)). Fractions of 40 ml each were collected in each step. Elution with 50% dichloromethane in hexane afforded two white crystalline compounds, **IX** and **X**, which were purified using the same mobile phase on columns (1 cm in diameter) with silica gel as the stationary phase. This afforded **IX** (35 mg) and **X** (43 mg). Elution with 100% dichloromethane resulted in a yellow oil, which was purified with 100% dichloromethane on Pasteur pipette columns using silica gel as the stationary phase. This afforded **VIII** (32 mg).

The mobile phase used for the dichloromethane extract was a dichloromethane : ethyl acetate step gradient (100% dichloromethane (fractions 1-40), 10% ethyl acetate in

dichloromethane (fractions 41-60), 50% ethyl acetate in dichloromethane (fractions 61-77), 66% ethyl acetate in dichloromethane (fractions 78-90) and 100% ethyl acetate (fractions 90-104). Elution with 100% dichloromethane afforded two crystalline compounds, **XI** and **XII**. Compounds **XI** (12 mg) and **XII** (9 mg) were purified on Pasteur pipette columns packed with silica gel. The mobile phase used to purify compound **XI** was 100% dichloromethane and 5% ethyl acetate in dichloromethane was used to purify compound **XII**. Elution with 10% ethyl acetate in dichloromethane resulted in another crystalline compound, **XIII** (7 mg). This compound was also purified on a Pasteur pipette column packed with silica gel using 10% ethyl acetate in dichloromethane. Compound **XIV** (5 mg) was eluted with 100% ethyl acetate. This compound was again purified on a Pasteur pipette column with 100% dichloromethane.

Physical data for compound VIII

Microfolian (MIC40)

Description: yellow oil

Yield: 32 mg

Mass: HRMS [M^+] at *m/z* 438.20428, $C_{26}H_{30}O_6$ requires 438.20424

EIMS: *m/z* (rel. int.): [M^+] 438 (41.64), 408 (6.60), 371 (20.91), 370 (100.00), 312 (7.77), 300 (9.31), 299 (43.55), 298 (11.20), 266 (20.62), 240 (9.57), 239 (62.90), 233 (9.13), 221 (33.46), 197 (8.26), 196 (10.61), 195 (78.69), 194 (32.95), 181 (7.59), 180 (7.77), 179 (6.72), 132 (8.05), 131 (77.64), 115 (6.86), 103 (30.62), 97 (6.96), 91 (6.48), 77 (11.89)

Optical rotation: $[\alpha]_D^{22}$ $+11.62^0$ (CH_2Cl_2, c=0.086)

Infrared: ν_{max}^{NaCl} cm^{-1}: 3462, 2924, 2850, 1620, 1583, 1522, 1441, 1386, 743.

Ultra violet: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 380 (4.23), 238 (4.08), 223 (3.99).

^{1}H and ^{13}C NMR data are given in table 1.

Physical data for compound IX

Microfolione (MIC19A)

Description: white crystalline (CH_2Cl_2)

Yield: 35 mg

Melting point: 172-173^{0}C

Mass: HRMS [M^+] at *m/z* 354.14543, $C_{21}H_{22}O_5$ requires 354.14672

EIMS: *m/z* (rel. int.): [M^+] 354 (100), 339 (14.29), 311 (11.03), 299 (43.18), 298 (16.17), 250 (15.41), 235 (32.03), 207 (7.65), 195 (39.78), 194 (57.68), 179 (7.56), 166 (9.19), 165 (6.29), 151 (9.84), 131 (7.96), 103 (12.87), 77 (12.80)

Optical rotation: $[\alpha]_D^{22}$ +37.5^0 (CH_2Cl_2, c=1.0)

Infrared: ν_{max}^{NaCl} cm^{-1}: 3382, 2971, 2923, 2849, 1637, 1466, 1380, 1337, 1221, 1184, 1129, 1086, 1031, 921.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 345 (4.53), 297 (5.13), 208 (5.44)

^{1}H and ^{13}C NMR data are given in table 2.

Physical data for compound X

(+)-Agrandol (MIC 30)

Description: white crystalline (CH_2Cl_2)

Yield: 43 mg

Melting point: 142-143 ^{0}C (lit.[1] (-)-agrandol: 156.5-157.5 ^{0}C)

Mass: EIMS: *m/z* (rel. int.): [M^+] 354 (100), 339 (14), 311 (8.62), 299 (33), 250 (11), 249 (9), 235 (58), 207 (32), 195 (38), 194 (34), 192 (21), 180 (8), 166 (8), 131 (5), 103 (10), 77 (9)

Optical rotation: $[\alpha]_D^{22}$ +46.05^0 (CH_2Cl_2, c=2.15), (lit[1] (-)-agrandol: $[\alpha]_D^{22.6}$ -41.2^0 (5.1 x 10^{-4} M))

Infrared: ν_{max}^{NaCl} cm^{-1}: 3192, 2959, 2935, 2843, 1637, 1607, 1454, 1386, 1356, 1307, 1252, 1221, 1172, 1129, 1086, 1037.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 344 (3.68), 298 (4.19), 204 (4.54)

^{1}H and ^{13}C NMR data are given in table 3.

Physical data for compound XI

Cedrecoumarin A (MICM23A)

Description: yellow crystalline (CH_2Cl_2)

Yield: 12 mg

Melting point: 148-149 ^{0}C

Mass: EIMS: *m/z* (rel. int.): [M^+] 244 (19), 230 (14), 229 (100), 201 (11), 115 (9), 100 (7), 91 (5), 77 (4)

Infrared: ν_{max}^{NaCl} cm^{-1}: 3394, 2976, 2933, 2870, 1710, 1635, 1591, 1572, 1498, 1454, 1385, 1323, 1285, 1260, 1142, 992.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 325 (3.92), 277 (3.72), 228 (4.17), 205 (4.18).

^{1}H and ^{13}C NMR data are given in table 4.

Physical data for compound XII

Obliquin (MICM12)

Description: white crystalline (CH_2Cl_2)

Yield: 9 mg

Melting point: 160-161 ^{0}C (lit.[3] 162 ^{0}C)

Mass: EIMS: *m/z* (rel. int.): [M^+] 244 (100), 229 (5), 215 (16), 189 (38), 177 (15), 148 (28), 121 (7), 120 (36), 92 (18), 79 (18), 69 (19), 68 (54), 67 (36)

Optical rotation: $[\alpha]_D^{22}$ +80.60^0 (CH_2Cl_2, c=0.062), (lit.[3] $[\alpha]_D^{25}$ + 76^0 ($CHCl_3$))

Infrared: ν_{max}^{NaCl} cm^{-1}: 2971, 2916, 2849, 1711, 1631, 1570, 1515, 1441, 1313, 1276, 1160, 1025, 884.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 341 (5.11), 296 (5.06), 260 (4.90), 229 (5.32), 207 (5.59)

^{1}H and ^{13}C NMR data are given in table 5.

Physical data for compound XIII

Microfolicoumarin (MICM95)

Description: yellow oil

Yield: 5 mg

Mass: HRMS [M^+] at *m/z* 274.12097, $C_{16}H_{18}O_4$ requires 274.12050

EIMS: *m/z* (rel. int.): [M^+] 274 (100), 259 (11.32), 243 (10.60), 219 (9.65), 218 (6.57), 217 (44.87), 204 (5.99), 202 (6.77), 189 (4.56), 167 (5.82), 161 (4.55), 149 (12.65), 128 (7.38), 115 (5.88), 91 (7.34), 77 (8.75), 69 (11.02).

Infrared: ν_{max}^{NaCl} cm^{-1}: 3403, 2965, 2929, 2873, 1710, 1519, 1457, 1426, 1365, 1266, 1217, 1174, 835.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 299 (4.54), 205 (5.08)

^{1}H and ^{13}C NMR data are given in table 6.

Physical data for compound XIV

(-)-Sesquichamaenol (MICM50)

Description: white crystalline (CH_2Cl_2)

Yield: 7 mg

Melting point: 108-109 ^{0}C (lit.[10] 109-111 ^{0}C)

Mass: EIMS: *m/z* (rel. int.): [M^+] 234 (19), 191 (15), 176 (18), 173 (10), 164 (30), 163 (25), 161 (15), 149 (7), 147 (11), 134 (12), 133 (75), 121 (54), 115 (8), 105 (11), 91 (16), 77 (11), 43 (100)

Optical rotation: $[\alpha]_D^{22}$ -5.95^0 (CH_2Cl_2, c=0.042), (lit.[10] α_D^{25} -4.3^0 (c = 0.6, $CHCl_3$))

Infrared: ν_{max}^{NaCl} cm^{-1}: 3439, 2984, 2935, 2867, 1740, 1648, 1611, 1457, 1390, 1346, 1303, 1254, 1137, 1057.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 284 (4.22), 204 (5.00)

^{1}H and ^{13}C NMR data are given in table 7.

4.4 References

1. Jenkins, T., Bhattacharyya, J., Majetich, G., Teng, Q., de Fatima, A.M. and Almeida, R. (1999) Flavonoids from the root bark of *Dioclea grandiflora*, *Phytochemistry*, **52**, 723-730.
2. Kotsos, M., Mahomed, H.A, Mulholland, D.A, Koorbanally, N.A. and Randrianarivelojosia, M. (2001) Coumarins from *Cedrelopsis grevei*, *Phytochemistry*, in press.
3. Dean, F.M. and Taylor, D.A.H. (1966) Extractives from East African timbers. Part II. *Ptaeroxylon obliquum*. *Journal of the Chemical Society (C)*, 114-116.
4. Bohlmann, F. and Zdero, C. (1980) Nene obliquin –derivative ans *Helichrysum serpyllifolium* (German), *Phytochemistry*, **19**, 331-332.
5. Jakupovic, J., Pathak, V.P., Bohlmann, F., King, R.M. and Robinson, H. (1987) Obliquin derivatives and other constituents from Australian *Helichrysum* species, *Phytochemistry*, **26**, 803-807.
6. Herz, W. and Bruno, M. (1986) Heliangolides, kauranes and other constituents of *Helianthus heterophyllus*, *Phytochemistry*, **25**, 1913-1916.
7. Ando, M., Ibe, S., Kagabu, S., Nakagawa, T., Asao, T. and Takase, K. (1970) Structure and synthesis of a phenolic sesquiterpene, sesquichamaenol, *Chemical Communications*, 1538.
8. Leroy, J.F. and Lescot, M. (1981) Flore de Madagascar et des Comores, Ed. Morat, P.H., Museum National D'Histoire Naturelle, Laboratoire de Phanerogamie, Paris, Famille 107 bis. – Ptaeroxylacees.
9. Bohlmann, F., Ziesche, J. and Mahanta, P.K. (1979) Neue chalkon-derivate und humulon-ahnliche verbindungen aus helichrysum-arten (German), *Phytochemistry*, **18**, 1033-1036.
10. Kuo, Y.H. and Yu, M.T. (1996) Two new sesquiterpenes, (-)-15-hydroxycalamene and (-)-1-hydroxy-1,3,5-bisabolatrien-10-one, from the heartwood of *Juniperus formosana* HAY. var. *concolor* HAY., *Chemical and Pharmaceutical Bulletin*, **44**, 2150-2152.
11. Randriaminahy, M., Proksch, P., Witte, L. and Wray, V. (1992) Lipophilic phenolic constituents from *Helichrysum* species endemic to Madagascar, *Zeitschrift fur Naturforschung. C*, **47**, 10.

12. Markham, K.R. and Mabry, T.J. (1975) Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids, in *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 51.
13. Bellamy, L.J. (1954) *The Infra-red Spectra of Complex Molecules*, Methuen and Co. Ltd., London, p. 133-145.
14. Murray, R.D.H., Mendez, J. and Brown, S.A. (1982) *The Natural Coumarins – Occurrence, Chemistry and Biochemistry*, John Wiley and Sons Ltd, Chichester, pp. 34, 39.
15. Dean, F.M. (1963) Naturally occurring oxygen ring compounds, Butterworths and Co. Ltd., London, pp 177-178.
16. Mabry, T.J. and Markham, K.R. (1975) Mass spectrometry of flavonoids, in *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 79-126.
17. Mulholland, D.A., Iourine, S. and Taylor, D.A.H. (1998) Sesquiterpenoids from *Dysoxylum schiffneri*, *Phytochemistry*, **47**, 1421-1422.
18. Bohm, B.A. (1975) Flavanones and dihydroflavonols, in *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 561-631.
19. Clark-Lewis, J.W. (1968) Flavan derivatives. XXI. Nuclear magnetic resonance spectra, configuration and conformation of flavan derivatives, *Australian Journal of Chemistry*, **21**, 2059-2075.

Chapter 5. Extractives from *Khadia alticola* (Mesembryanthemaceae)

5.1 Introduction

The genus name *Khadia* was named after "Khadi", a Tswana/South Sotho name for beer brewed traditionally using the fleshy roots of a variety of taxa[1]. *Khadia alticola* is one of the species added to this native beer. However, it is unlikely that the plant is used for its active components but rather the fermenting power of the root, which is attributed to the alleged presence of one or two species of fungi[2]. Several species from the Mesembryanthemaceae family are used in fermentation in South Africa. *Khadia* is also reported to be used medicinally by the Manyika people of the Umtali district of Zimbabwe as a local application to sore eyes[3].

Figure 1. *Khadia alticola* in flower in habitat (Photograph by F. Krige)

The Mesembryanthemaceae is one of South Africa's largest succulent plant families, comprising nearly 63% of the Southern African succulent flora[4]. This group comprises one thousand eight hundred species in one hundred and seventeen genera and is almost entirely endemic to Southern Africa[4]. *Khadia* belongs to the subfamily Ruschoideae of the family Mesembryanthemaceae and comprises six species, one of which is *Khadia alticola*[5]. The plants of the genus *Khadia* form mats with needle shaped leaves and have flowers with pink petals[5]. *Khadia alticola* has a thick underground, upward-branching root system and a mat of succulent leaves above ground. It is common at altitudes above two thousand meters in Mpumulanga and

Kwazulu-Natal in shallow, humus rich soil pockets and crevices between rock plates[6]. The large, thick woody rootstock of *Khadia acutipetala* have not been found to contain alkaloids but to contain a large amount of oxalates[2].

Figure 2. *Khadia alticola* with semi-open fruits in dry condition in March in habitat (*Hartmann and Hartzer 32729*, PRE, HBG)

A study done by Smith *et al.*[7] on twenty species from nine genera of the Mesembryanthemaceae (*Aptenia, Bergeranthus, Delosperma, Drosanthemum, Glottiphylum, Lampranthus, Oscularia, Ruschia* and *Sceletium*) revealed that the highest alkaloid levels were found in material of *Sceletium*. *Sceletium tortuosum* contained mesembrine (**1**), mesembrenone (**2**) and 4'-O-demethylmesembrenol (**3**)[7]. *Sceletium strictum* yielded the mesembrine type alkaloids, mesembrine (**1**), mesembrenone (**2**), 4'-O-demethylmesembrenol (**3**), 4'-O-demethylmesembranol (**4**), mesembranol (**5**), mesembrenol (**6**), O-acetylmesembrenol (**7**)[8] and 4'-O-demethylmesembrenone (**8**)[9]. *Sceletium namaquense* yielded mesembrine (**1**), mesembrenone (**2**), mesembranol (**5**), 4'-O-demethylmesembrenone (**8**), N-formyltortuosamine (**9**), sceletium A_4 (**10**), Δ^7-mesembrenone (**11**), and sceletenone (**12**)[9]. *Aptenia cordifolia* contained 4'-O-demethylmesembrenol (**3**) and mesembrine (**1**) but lacked mesembrenone (**2**)[7]. *Delosperma minimum* contained 4'-O-demethylmesembrenol (**3**)[7]. *Lampranthus aureus* and *L. spectabilis* yielded mesembrenol (**6**), while *L. roseus, L. deltoides, L. coccineus* and *L. blanches* yielded mesembrenone (**2**), but all at very low levels[7]. *Drosanthemum hispidum* and *D. bicolor* contained 4'-O-demethylmesembrenol (**3**) and mesembrenone (**2**)[7].

Bergeranthus scapiger was found to have low levels of both 4'-O-demethylmesembrenol (**3**) and mesembrenone (**2**)[7]. *Glottiphylum longum* and *Ruschia lineolata* showed a complete absence of the mesembrine alkaloids[7].

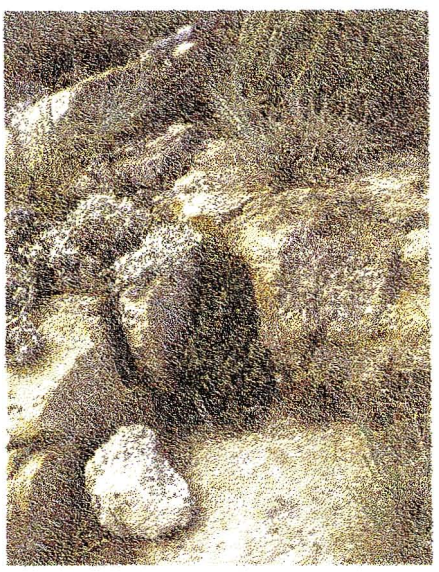


Figure 3. *Khadia alticola* in habitat (*Hartmann and Hartzer 32729*, PRE, HBG)

Amaryllidaceous plants contain primarily Amaryllidaceae alkaloids but Bastida *et al.*[10] have found mesembrenone (**2**) in *Narcissus pallidulus* and the isolation of mesembrenol (**6**) from *Crinum oliganthum* has also been reported[10]. Three broad structural categories of the *Sceletium* alkaloids exist[9]. The first is typified by mesembrine (**1**), mesembrenone (**2**), mesembranol (**5**) and sceletenone (**12**). The second type is typified by the dehydrojoubertiamine molecule (**13**), which was first isolated from *Sceletium joubertii* by Arndt and Kruger[11], who also reported hordenine (**14**) in this species. *Sceletium tortuosum* was found to contain a third structural variant, tortuosamine (**15**)[12].

Tinctures of "kougoed" (crushed plants left to ferment), prepared from plants of *Sceletium* were used by the early white settlers as a sedative. Chewing of the leaves was also a treatment for toothache and stomach pains[13]. A geologist and mining engineer in Namaqualand made the personal observation that "the Nama people were addicted to the use of "kougoed" which produced visions and led to a serious degree of moral degeneration, particularly with regard to veracity and sex"[3]. "Kougoed" is also used by the Nama for the relief of pain of any kind and to relieve hunger[3]. A Nama mother chews the root of *Sceletium tortuosum* and ejects her saliva into the

mouth of her child from an early age[3]. *Sceletium anatomicum* was reported to have anaesthetic effects to the lower jaw, sufficient enough to allow a tooth to be extracted painlessly[14]. *Sceletium tortuosum* was reported to relieve pain and alleviate hunger[15]. Recent studies by Weniger *et al.*[16] have shown mesembrenone (**2**) is moderately effective against cancer cells. It is also less toxic to mouse fibroblasts than twenty-one amaryllidaceous alkaloids tested *in vitro*[16]. The side effects of chewing "kougoed" have been reported to be discomfort, analgesia and slight headches[15].

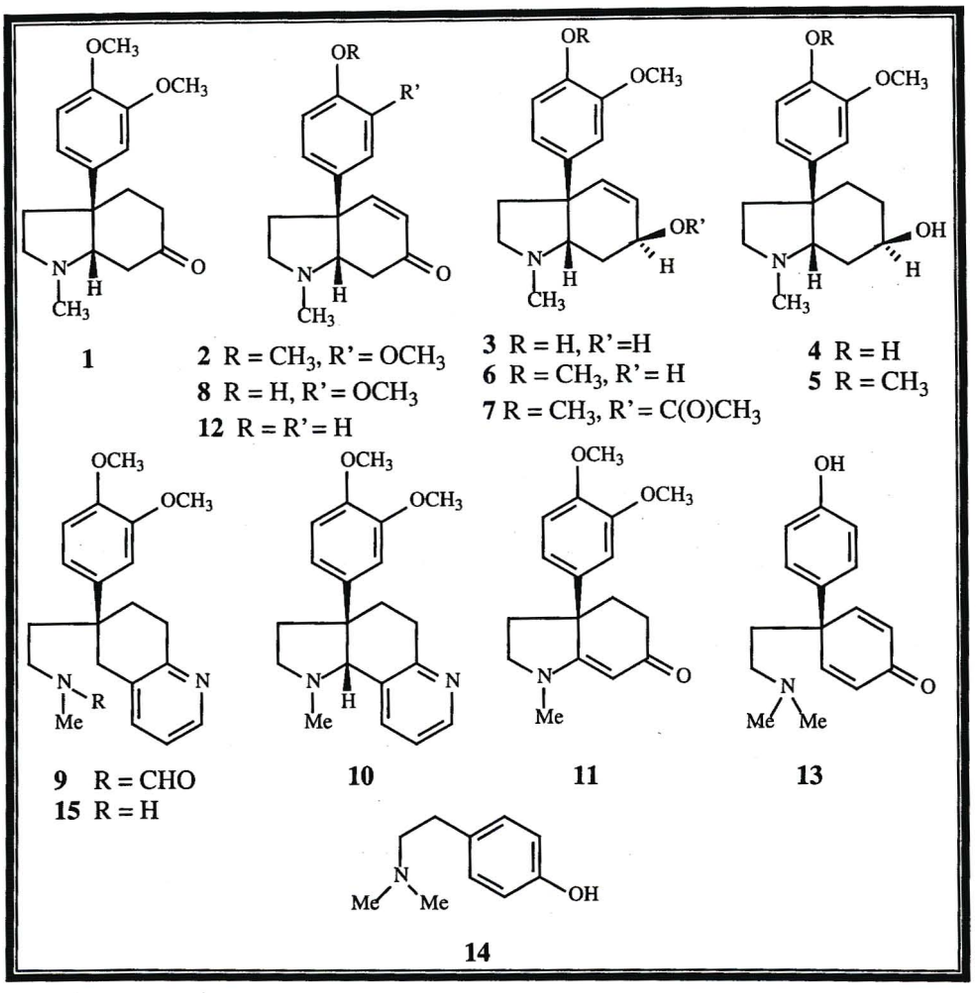


Figure 4. *Sceletium* alkaloids found in the Mesembryanthemaceae

The phytochemical investigation of the roots of *Khadia alticola* was undertaken to determine whether mesembrine type alkaloids were present in this species and thus contributing to the 'potency' of the beer brewed traditionally by the indigenous people of South Africa. The roots of *Khadia alticola* have not been studied previously.

5.2 Results and Discussion

The dried roots and leaves of *Khadia alticola* were extracted with ethanol. The ethanol extract was then acidified to pH 4 and extracted with chloroform. This resulted in an acidic chloroform extract. The aqueous solution was then made basic to pH 10 and extracted with chloroform, resulting in a basic chloroform extract. The crude basic chloroform extract of *Khadia alticola* was separated by column chromatography using silica gel as the stationary phase and a dichloromethane : methanol step gradient as the mobile phase. This yielded one flavonoid, compound **XV** (fig. 5). The crude acidic chloroform extract was separated by column chromatography using silica gel as the stationary phase and again using a dichloromethane : methanol step gradient as the mobile phase. This yielded a common sterol, sitosterol (**XVI**) (fig. 5). The physical properties and ^{1}H NMR spectrum of compound **XVI** were identical to those of an authentic sample of sitosterol.

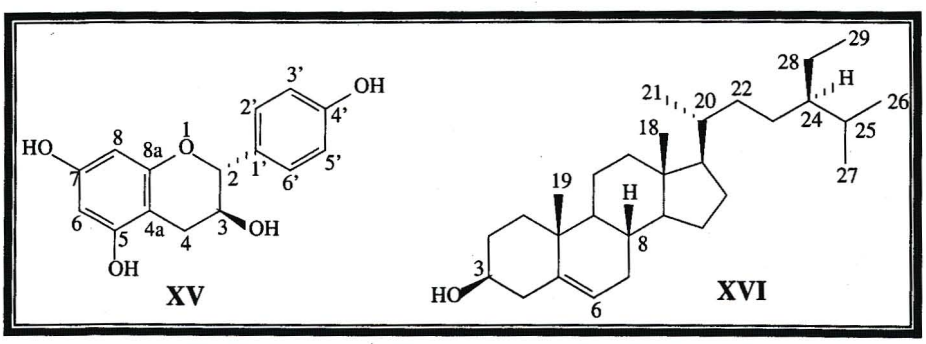
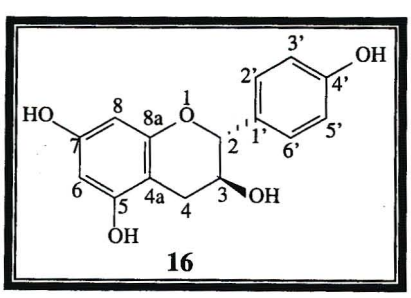


Figure 5. Compounds isolated from *Khadia alticola*

5.2.1 Structural elucidation of (+)-afzelechin (XV)



Compound **XV** was identified as a catechin type flavan by its characteristic ^{1}H NMR, UV, IR and mass spectra. It was isolated as a white crystalline compound with a melting point of 221-222 ^{0}C and had a molecular formula of $C_{15}H_{14}O_5$.

Compound **XV** gave a UV absorption band at 285 nm with log ε = 2.69, which was characteristic of flavan-3-ol compounds[17]. The IR spectrum of compound **XV** showed a strong absorption band at 3343 cm^{-1}, characteristic of a hydroxy group stretch as well as aromatic ring absorption bands at 1621 cm^{-1} and 1516 cm^{-1} [17,18].

The ^1H NMR spectrum of compound **XV** displayed two pairs of doublets in the downfield region of the spectrum. The pair of doublets at δ_H 6.76 (2H, J = 8.24 Hz) and δ_H 7.20 (2H, J = 8.24 Hz) were indicative of a *para* disubstituted aromatic system and these proton resonances were attributed to H-2'/6' and H-3'/5' respectively. Coupling between the H-2'/6' and H-3'/5' proton resonances was also evident in the COSY spectrum. The second pair of doublets at δ_H 5.90 and δ_H 5.82 had a coupling constant of 2.38 Hz, indicative of *meta* coupling and these proton resonances were attributed to H-6 and H-8 respectively. Coupling in the COSY spectrum between these two proton resonances could also be seen.

The doublet at δ_H 4.56 with a coupling constant of 7.87 Hz, was attributed to H-2. This proton resonance showed coupling in the COSY spectrum, with the H-3 proton resonance at δ_H 3.95, a multiplet, which in turn showed coupling in the COSY spectrum to the two one-proton resonances at δ_H 2.85 and δ_H 2.48, attributed to the non-equivalent protons, H-4α and H-4β respectively. The H-4α proton resonance appeared as a double doublet with coupling constants of 16.11 and 5.49 Hz, while H-4β, also appearing as a double doublet, had coupling constants of 16.11 and 8.42 Hz.

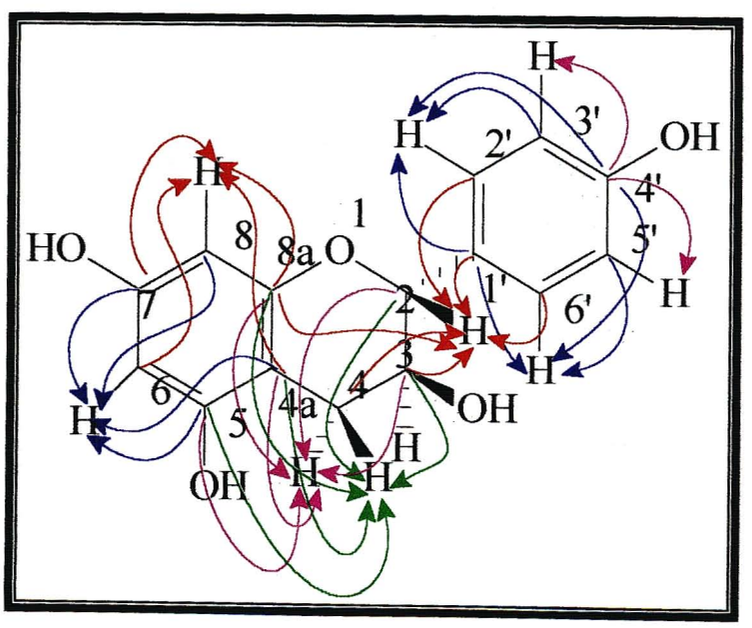
The C-2'/6' carbon resonance at δ_C 129.61 showed HMBC correlations with the H-2 proton resonance and the C-3'/5' carbon resonance at δ_C 116.03 showed HMBC correlations with the H-2'/6' proton resonance. The C-4' carbon resonance at δ_C 158.36 showed HMBC correlations with both the H-2'/6' and the H-3'/5' proton resonances. The C-1' carbon resonance appeared at δ_C 131.47 and this carbon resonance showed HMBC correlations with the H-2 proton resonance and also with the H-2'/6' proton resonance.

The carbon resonance ascribed to C-2 at δ_C 82.84 showed an HMBC correlation with the two H-4 proton resonances and the resonance ascribed to C-4 at δ_C 28.88 showed

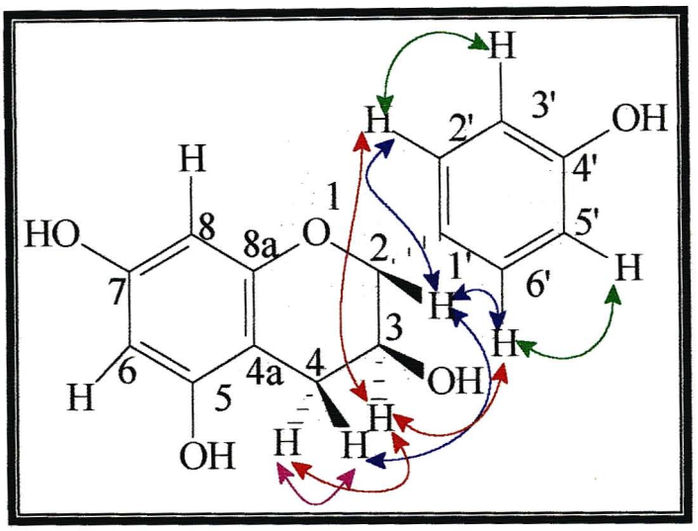
an HMBC correlation with the H-2 proton resonance. The resonance at δ_C 68.82 in the ^{13}C NMR spectrum was assigned to C-3 and this carbon resonance showed HMBC correlations to the H-2 proton resonance as well as the two H-4 proton resonances.

The resonance at δ_C 156.97 was assigned to C-8a as this carbon resonance showed HMBC correlations with H-2, H-8 and the two H-4 proton resonances, while the resonance at δ_C 100.90 was assigned to C-4a because of HMBC correlations with the H-6, H-8, H-4α and H-4β proton resonances. The resonance at δ_C 157.55 showed HMBC correlations with H-6, H-4α and H-4β and was therefore assigned to C-5. The resonance at δ_C 157.82 showed HMBC correlations to H-6 and H-8 and this carbon resonance was assigned to C-7. The C-6 carbon resonance at δ_C 96.30 showed an HMBC correlation with the H-8 proton resonance and the C-8 carbon resonance at δ_C 95.48 showed an HMBC correlation with the H-6 proton resonance.

The H-2'/6' and H-3'/5' proton resonances showed NOESY interactions with each other and the H-2'/6' proton resonance, in addition showed NOESY interactions with the H-2 and H-3 proton resonances. The H-2 proton was given the β- orientation, relative to the H-3 proton as the H-2 proton resonance showed a NOESY interaction with the H-4β proton resonance and the α- positioned H-3 proton, which resonated at δ_H 3.95 in the ^1H NMR spectrum, showed a NOESY interaction with the H-4α proton resonance.



HMBC correlations for compound **XV**

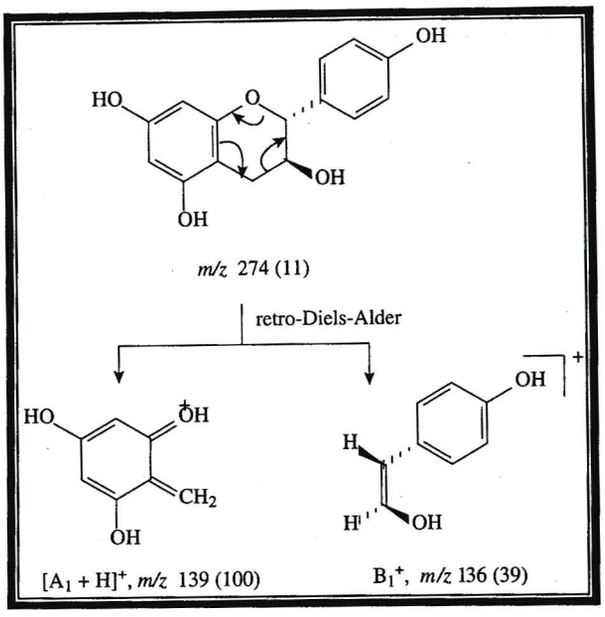


NOESY interactions for compound **XV**

The large coupling constant of the H-2 resonance (J = 7.87 Hz) indicated that the *p*-hydroxyphenyl group at C-2 was in a *trans* orientation to the hydroxy group at C-3[20]. This was consistent with the NMR data of (+)-afzelechin and (+)-afzelechin glycosides[17,20,24,25]. When the phenyl group at C-2 and the hydroxy group at C-3 are in the *cis* configuration, the H-2 resonance has a small coupling constant (J = 1.8 Hz) as in (-)-epiafzelechin 3-*O*-β-D-allopyranoside[18] or occurs as a broad singlet as in (-)-epiafzelechin[21,22] and (-)-epiafzelechin 7-*O*-β-D-glucopyranoside[23]. Furthermore, compound **XV** had an optical rotation of $+23.2^0$ which was in agreement with that of (+)–afzelechin[19].

The mass spectrum of compound **XV** showed a molecular ion peak at *m/z* 274. The retro-Diels-Alder fragmentation[26], a typical fragmentation process for flavonoids[26], resulted in the ion fragment peaks at *m/z* 139 $[A_1 + H]^+$, the base peak, and *m/z* 136 $[B_1]^+$. The fragmentation pattern of compound **XV** is given in scheme 1.

^1H and ^{13}C NMR assignments were made with the aid of HSQC and HMBC spectra and are reported in table 1. (+)-Afzelechin was isolated previously from *Eucalyptus calophylla*[19].



Scheme 1. Fragmentation pattern of compound **XV**, (+)-afzelechin

Table 1. ^{1}H, ^{13}C, HMBC, COSY and NOESY NMR data for (+)-afzelechin, XV (400 MHz, CD_3OD)

Pos.	^{1}H	^{1}H (lit. [17]) *	^{13}C	^{13}C **	HMBC correlations	COSY correlations	NOESY interactions
2	4.56, d (7.87)	4.58, d (7.6)	82.84	81.7	2H-4	H-3	H-2'/6', H-4β
3	3.95, m	4.00, ddd (8.3, 7.6, 5.4)	68.82	67.7	2H-4, H-2	H-2, 2H-4	H-2'/6', H-4α
4	α) 2.85, dd, (16.11, 5.49) β) 2.48, dd (16.11, 8.42)	α) 2.84, dd, (15.9, 5.4) β) 2.49, dd (15.9, 8.3)	28.88	28.1	H-2	H-3, H-4β H-3, H-4α	H-3, H-4β H-2, H-4α
4a			100.90	100.3	H-6,8, 2H-4		
5			157.55	156.5	2H-4, H-6		
6	5.90, d (2.38)	5.99, s	96.30	95.9	H-8	H-8	
7			157.82	156.7	H-6, H-8		
8	5.82, d (2.38)	5.84, s	95.48	95.0	H-6	H-6	
8a			156.97	156.1	2H-4, H-8, H-2		
1'			131.47	130.5	H-2'/6', H-2		

2'/6'	6.76, 2H, d (8.24)	6.78, 2H, d (8.1)	129.61	129.2	H-2	H-3'/5'	H-2,3, H-3'/5'
3'/5'	7.20, 2H, d (8.24)	7.17, 2H, d (8.1)	116.03	115.6	H-2'/6'	H-2'/6'	H-2'/6'
4'			158.36	157.1	H-2'/6', H-3'/5'		

* lit [17] 300 MHz, acetone-d_6, ** lit [17] 75 MHz, acetone-d_6

No alkaloids were found in *Khadia alticola* in this study and only sitosterol and a flavan were found. Either the specimen of *Khadia alticola* was collected at a time when the mesembrine alkaloids were not present or they may have been present in very small amounts that could not be isolated in this work.

5.3. Experimental

Extraction of the roots of *Khadia alticola*

The roots of *Khadia alticola* (1.26 kilograms) were collected at De Berg in December 1998 and a voucher specimen retained at the National Botanical Institute, Durban, South Africa (Crouch 755). The dried roots was extracted on a labcon shaker for forty-eight hours with 95% ethanol (2L) and the solvent removed under reduced pressure to yield 3.65 grams of ethanol extract. The ethanol extract was then dissolved in 100 ml of water and acidified to pH 4. The resulting acidic solution was then extracted five times with 200 ml portions of chloroform. The acidic chloroform extracts were combined and the solvent removed under reduced pressure to yield 0.96 grams of acidic chloroform extract. The aqueous portion was then made basic to pH 10 and extracted five times with 200 ml portions of chloroform as above. The solvent was removed under reduced pressure to yield 0.54 grams of basic chloroform extract. The acidic and basic chloroform extracts were then worked with separately.

Isolation of compounds XV and XVI.

The basic chloroform extract (0.54 grams) was chromatographed over silica gel (150 g) using a column (3 cm in diameter) and eluted with a dichloromethane : methanol step gradient (5%, 10%, 20% and 40% methanol in dichloromethane), collecting 30 X

30 ml fractions for each step. Elution with 5% methanol in dichloromethane yielded a white crystalline compound in fractions 21-23. This was purified further by repeated column chromatography using 40% ethyl acetate in dichloromethane to yield 22 mg of compound **XV**. The acidic chloroform extract (0.96 grams) was chromatographed over silica gel as above using a dichloromethane : methanol step gradient as the eluant, starting with 100% dichloromethane and then 5%, 10%, 20% and 40% methanol in dichloromethane, collecting 50 X 30 ml fractions for the first step and 30 X 30 ml fractions for each subsequent step. Elution with 100% dichloromethane yielded a white crystalline material in fractions 38-40. This was purified by repeated column chromatography to yield compound **XVI** (58 mg).

Physical Data for compound XV

(+)-Afzelechin (KHAD21-22)

Description: white crystalline (MeOH)

Yield: 22 mg

Melting point: 221-222^{0}C (lit[19] 221-222 ^{0}C)

Mass: EIMS: *m/z* (rel. int.): [M^+] at *m/z* 274 (10.59), 224 (4.54), 167 (4.31), 140 (8.10), 139 (100), 137 (11.43), 136 (39.11), 108 (16.00), 107 (41.65), 77 (9.29), 69 (9.38)

Optical rotation: $[\alpha]_D^{22}$ +23.2^0 (MeOH, c=0.05), (lit[19] $[\alpha]_D^{20}$ +20.6^0 (acetone:water (1:1), c=5%))

Infrared: ν_{max}^{NaCl} cm^{-1}: 3343, 2922, 2850, 1621, 1516, 1463, 1365, 1233, 1148, 1043, 826.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 285 (2.69)

^{1}H and ^{13}C NMR data are given in table 1.

5.4. References

1. Brown, N.E. (1930) Mesembryanthemum and some genera separated from it, *Gardener's Chronicle*, ser. **3**, 88: 278-279.
2. Smith, C.A. (1966) Common names of South African plants, *Botanical Survey Memoir No. 35*, Botanical Research Institute, Pretoria, pp 288.
3. Watt and Breyer-Brandwijk (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd Ed., London, Livingstone, pp 8, 11-12.
4. Chesselet, P., Mossmer, M. and Smith, G.F. (1995) Research priorities in the succulent plant family Mesembryanthemaceae Fenzl, *South African Journal of Science*, **91**, 197-209.
5. Chesselet, P and Hartmann, H. E. K. (1995) *Khadia alticola* Chess and H.E.K. Hartm., spec. nov. (Mesembryanthemaceae, Aizoaceae), *Aloe*, **32**, 46-49.
6. Chesselet, P., Hartmann, H.E.K., Hahn, N., Burgoyne, P. and Smith, G.F. (1998) Taxonomic notes on the genus *Khadia* (Mesembryanthemaceae/Aizoaceae), *Bothalia*, **28**, 1: 25-33.
7. Smith, M.T., Field, C.R., Crouch, N.R. and Hirst, M. (1998) The distribution of mesembrine alkaloids in selected taxa of the Mesembryanthamaceae and their modification in the sceletium derived "kougoed", *Pharmaceutical Biology*, **36**(3), 1-7.
8. Jeffs, P.W., Allmann, G., Campbell, H.F., Farrier, D.S., Ganguli, G. and Hawks, R.L. (1970) Alkaloids of *Sceletium* species. III. The structures of four new alkaloids from *Sceletium strictum, Journal of Organic Chemistry*, **35**, 3512-3518.
9. Jeffs, P.W., Capps, T., Johnson, D.B., Karle, J.M., Martin, N.H. and Rauckman, B. (1974) *Sceletium* alkaloids. VI. Minor alkaloids of *Sceletium namaquense* and *Sceletium strictum*, *Journal of Organic Chemistry*, **39**, 2703-2709.
10. Bastida, J., Viladomat, F., Llabres, J.M., Ramires, G., Codina, C. and Rubiralta, M. (1989) Narcissus alkaloids, VIII mesembrenone: an unexpected alkaloid from *Narcissus pallidulus*, *Journal of Natural Products*, **52**, 478-480.
11. Arndt, R. R. and Kruger, P.E.J. (1970) Alkaloids from *Sceletium joubertii* L. Bolus: the structure of joubertiamine, dihydrojoubertiamine and dehydrojoubertiamine, *Tetrahedron Letters*, **37**, 3237-3240.

12. Snyckers, F.O., Strelow, F. and Weichers, A. (1971) The structures of partial racemic sceletium alkaloids A4 and tortuosamine, pyridine alkaloids from *Sceletium tortuosum* N.E. Br., *Chemical Communications*, 1467-1469.
13. Laidler, P.W. (1928) The magic medicine of the Hottentots, *South African Journal of Science*, **25**, 433-447.
14. Rood, B. (1994) *Uit die Veld-apteek*, Tafelberg-Uitgewers Beperk, Cape Town, pp 72.
15. Smith, M.T., Crouch, N.R., Gericke, N. and Hirst, M. (1996) Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: a review, *Journal of Ethnopharmacology*, **50**, 119-130.
16. Weniger, B., Italiano, L., Beck, J-P., Bastida, J., Bergonon, S., Codina, C., Lobstein, A. and Anton, R. (1995) Cytotoxic activity of Amaryllidaceae alkaloids, *Planta Medica*, **61**, 77-79.
17. Baek, N.I., Kennelly, E.J., Kardono, L.B.S., Tsauri, S., Padmawinata, K., Soejarto, D.D. and Kinghorn, A.D. (1994) Flavonoids and a proanthocyanidin from rhizomes of *Selligua feei*, *Phytochemistry*, **36**, 513-518.
18. Songqing, L., Zhuoyin, X. and Rui, F. (1994) A flavanol glycoside from *Drynaria propinqua*, *Phytochemistry*, **35**, 1595-1596.
19. Hillis, W.E. and Carle, A. (1960) The chemistry of *Eucalypt* kinos. III. (+)-Afzelechin, pyrogallol and (+)-catechin from *Eucalyptus calophylla* kino, *Australian Journal of Chemistry*, **13**, 390-395.
20. Wen-Sheng, Y., Hong, L., Xin-Min, C. and Lei, Y. (1992) Two afzelechin glycosides from *Arthromeris mairei*, *Phytochemistry*, **31**, 4385-4386.
21. Min, K.R., Hwang, B.Y., Lim. H.S., Kang, B.S., Oh, G.J., Lee, J., Kang, S.H., Lee, K.S., Ro, J.S. and Kim, Y. (1999) (-)-Epiafzelechin: cyclooxygenase-1-inhibitor an anti-inflammatory agent from aerial parts of *Celastrus orbiculatus*, *Planta Medica*, **65**, 460-462.
22. Sethi, V.K., Taneja, S.C., Dhar, K.L. and Atal, C.K. (1984) (-)-Epiafzelechin 5-*O*-β-D-glucoside from *Crataeva religiosa*, *Phytochemistry*, **23**, 2402-2403.
23. Yamanaka, M., Shimomura, K., Sasaki, K., Yoshihira, K. and Ishimaru, K. (1995) Glucosylation of phenolics by hairy root cultures of *Lobelia sessilifolia*, *Phytochemistry*, **40**, 1149-1150.

24. Drewes, S.E., Taylor, C.W. and Cunningham, A.B. (1992) (+)-Afzelechin 3-rhamnoside from *Cassipourea gerrardi*, *Phytochemistry*, **31**, 1073-1075.

25. Kim, J. and Kinghorn, A.D. (1987) Use of the selective INEPT NMR technique in the structure elucidation of (+)-afzelechin-7-*O*-β-D-apioside, a bitter principle of *Polypodium glyccyrrhiza*, *Tetrahedron Letters*, **28**, 3655-3658.

26. Mabry, T.J. and Markham, K.R. (1975) Mass spectrometry of flavonoids, in *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 79-126.

Chapter 6. Investigations into the synthesis of chalcones and prenylated chalcones.

6.1 Introduction

The synthesis of chalcone has been accomplished using many different approaches[1], but the simplest approach is the one involving the Claisen-Schmidt condensation. This condensation involves reacting equimolar quantities of acetophenone with benzaldehyde in the presence of aqueous alcoholic alkali (e.g. potassium hydroxide) or sodium ethoxide, resulting in the formation of an α,β-unsaturated ketone (fig 1). The substituted chalcones have been synthesised in the same manner using appropriately substituted acetophenones with substituted benzaldehydes in the presence of aqueous alkali[1].

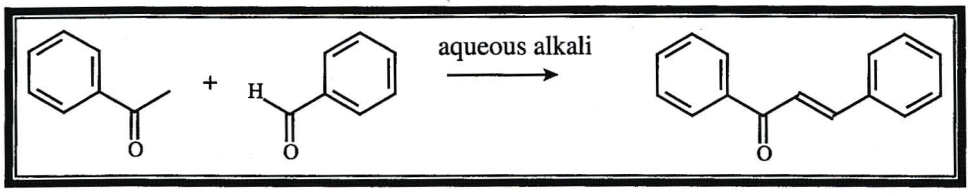


Figure 1. Chalcone formation by the Claisen-Schmidt condensation

From kinetic studies on the base catalysed formation of chalcone[2-4] and its derivatives[3,4], two alternative mechanisms have been proposed for the reaction of benzaldehyde with acetophenone in the presence of a basic catalyst. In the first mechanism (fig. 2), the base abstracts a proton from the methyl group of acetophenone, creating a nucleophile which then attacks the electrophilic carbon atom of benzaldehyde to form the intermediate (i), which then accepts a proton from water to form the intermediate (ii). The intermediate (ii) then undergoes a dehydration step to form the chalcone. This is the currently accepted mechanism[40].

In the second mechanism, the ethoxide anion acts as a nucleophile and attacks the electrophilic carbon atom of benzaldehyde forming intermediate (i) in figure 3. The nucleophilic acetophenone then attacks the electrophilic centre in intermediate (i), with the negative oxygen ion simultaneously accepting a proton from solution to form intermediate (ii), which then undergoes a dehydration step to form the chalcone.

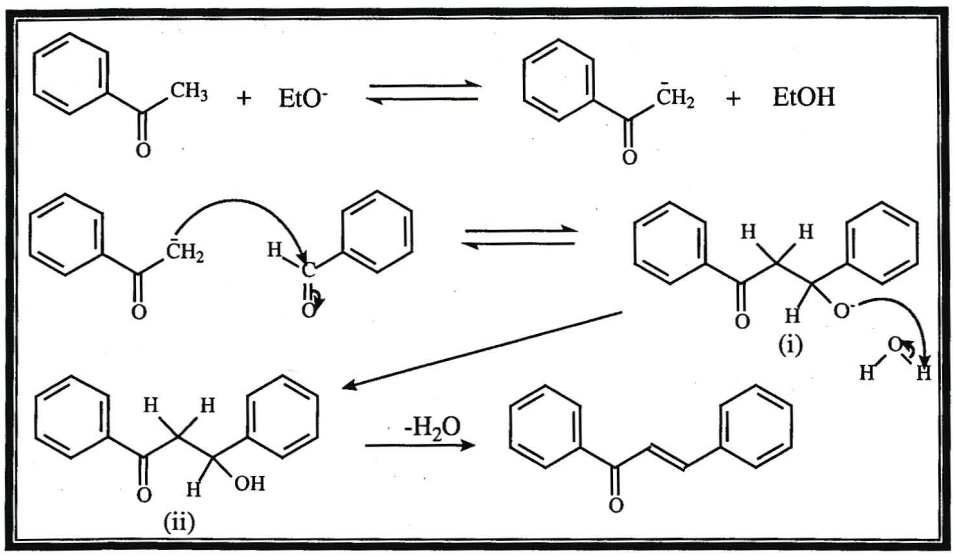


Figure 2. Mechanism of chalcone formation. Alternative 1[2].

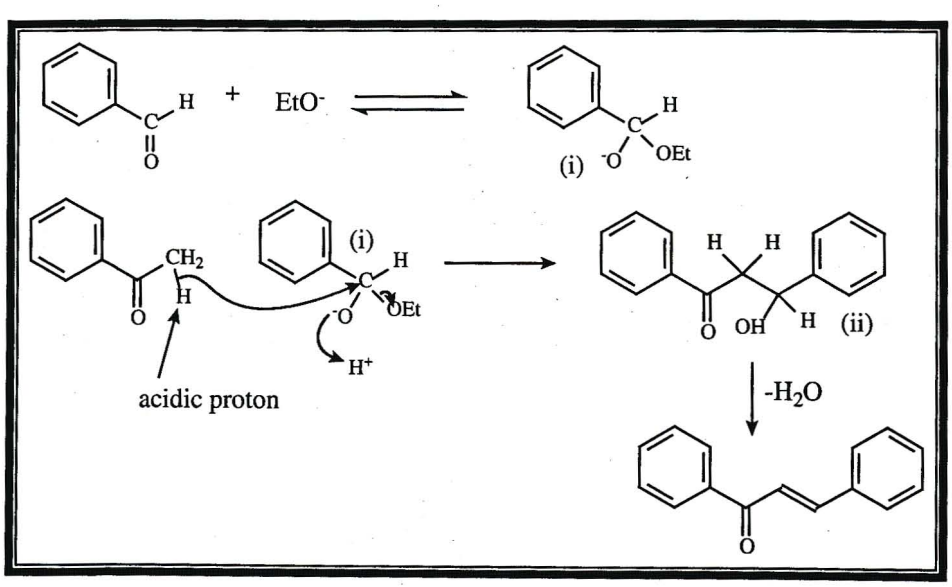


Figure 3. Mechanism of chalcone formation. Alternative 2[2].

In the Claisen-Schmidt reaction, the concentration of alkali usually ranges between 10 and 60%[5-23]. The reaction is carried out at 50 ^{0}C for twelve to fifteen hours or at room temperature for five to seven days[8]. Under these conditions, the Cannizzaro reaction also takes place, decreasing the yield of the desired chalcone. The Cannizzaro reaction is a reaction that occurs when an aldehyde with no α-hydrogen atoms reacts with concentrated aqueous alkali and half the aldehyde is converted to a carboxylic acid and the other half to an alcohol. In the synthesis of polyhydroxychalcones by the Claisen-Schmidt condensation, a higher concentration of alkali is used. Under these conditions, chalcones with a 2'-hydroxy group may cyclise, forming the

corresponding flavanone. This can be overcome by protecting the 2'-hydroxy group of the substituted acetophenone before reacting it with the aldehyde. This procedure has proved quite useful in the synthesis of 2'-hydroxychalcones[36].

Beside aqueous alkali, other condensing agents have also been used. These include alkali metal alkoxides[17,24], magnesium *tert*-butoxide[1], a potassium carbon compound (KC_8)[25], hydrogen chloride[26,27], aluminium chloride[28], boron trifluoride[28], phosphorous oxychloride, boric anhydride, amino acids, borax and an organocadmium compound, $Cd(CH_2CH_3)_2$ in butyl ether[1].

For the synthesis of cyanomethylchalcones[26] as well as hydroxynitrochalcones[27], the use of an acid catalyst, hydrochloric acid, in preference to the alkali has been recommended. Phosphorous oxychloride, claimed to be superior to alkali as a condensing agent has been used in the synthesis of 2'-hydroxy-5-acetamidochalcones[1]. Condensations have also been achieved in good yields using boric anhydride[1]. The water formed in this reaction is azeotropically distilled off with xylene. α-(Phenylthio)- and α-(phenylsulphonyl)chalcones have been prepared by the condensation of aromatic aldehydes with phenacylphenyl sulphide and phenacylsulphone respectively[1]. Glacial acetic acid combined with an organic base such as piperidine or benzylamine was used as the condensing agents in these reactions. Under the conditions of the Perkin reaction, chalcone in 50% yield has been prepared by the reaction of acetophenone and benzaldehyde[1].

Chalcones have also been synthesised using many other methods. These methods are outlined in a review by Dhar[1].

The synthesis of several naturally occurring prenylated chalcones has been accomplished using the Claisen-Schmidt reaction[29-35]. Examples of prenylated chalcones synthesised by the Claisen-Schmidt condensation are cordoin[32], isocordoin[32], 4-hydroxycordoin[32], derricin[32,34], 4-hydroxyderricin[32], sophoradin[29,30,33] and derricidin[34]. In the synthesis of sophoradin[29], prenylation of *para*-hydroxybenzaldehyde with 1-bromo-3-methyl-2-butene in 10% potassium hydroxide gave I (figure 4). The 2,4-dihydroxyacetophenone was prenylated in the same manner to give II. Both I and II were methoxymethylated to give III and IV respectively.

Condensation of III and IV in 50% potassium hydroxide gave chalcone V. Hydrolysis of V with MeOH-HCl gave sophoradin VI.

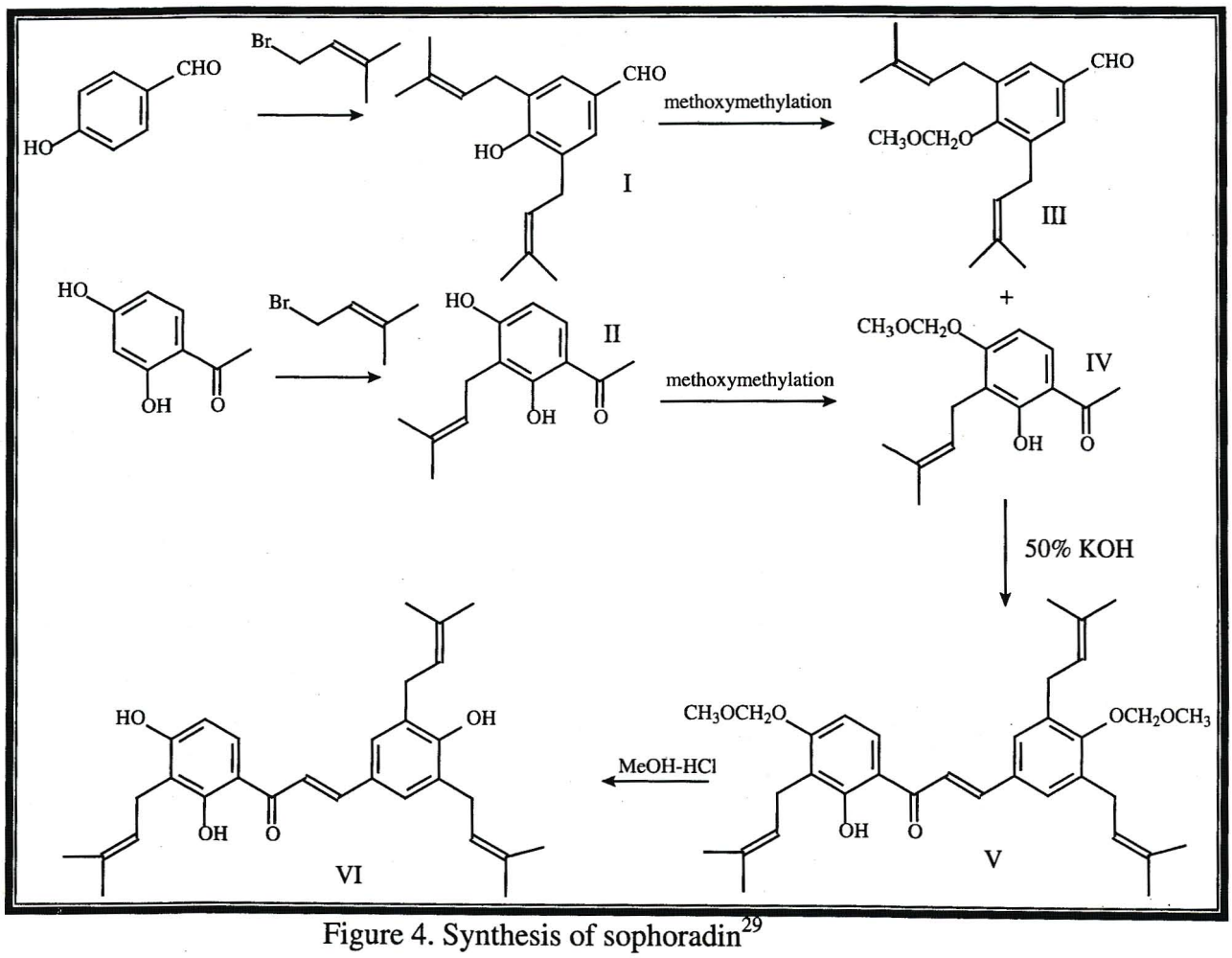


Figure 4. Synthesis of sophoradin[29]

An alternative synthesis of sophoradin[30] is outlined in figure 5. This synthesis involves the use of 2-chloro-2-methyl-3-butyne, hydrogenation and the Claisen rearrangement to achieve the synthesis of sophoradin.

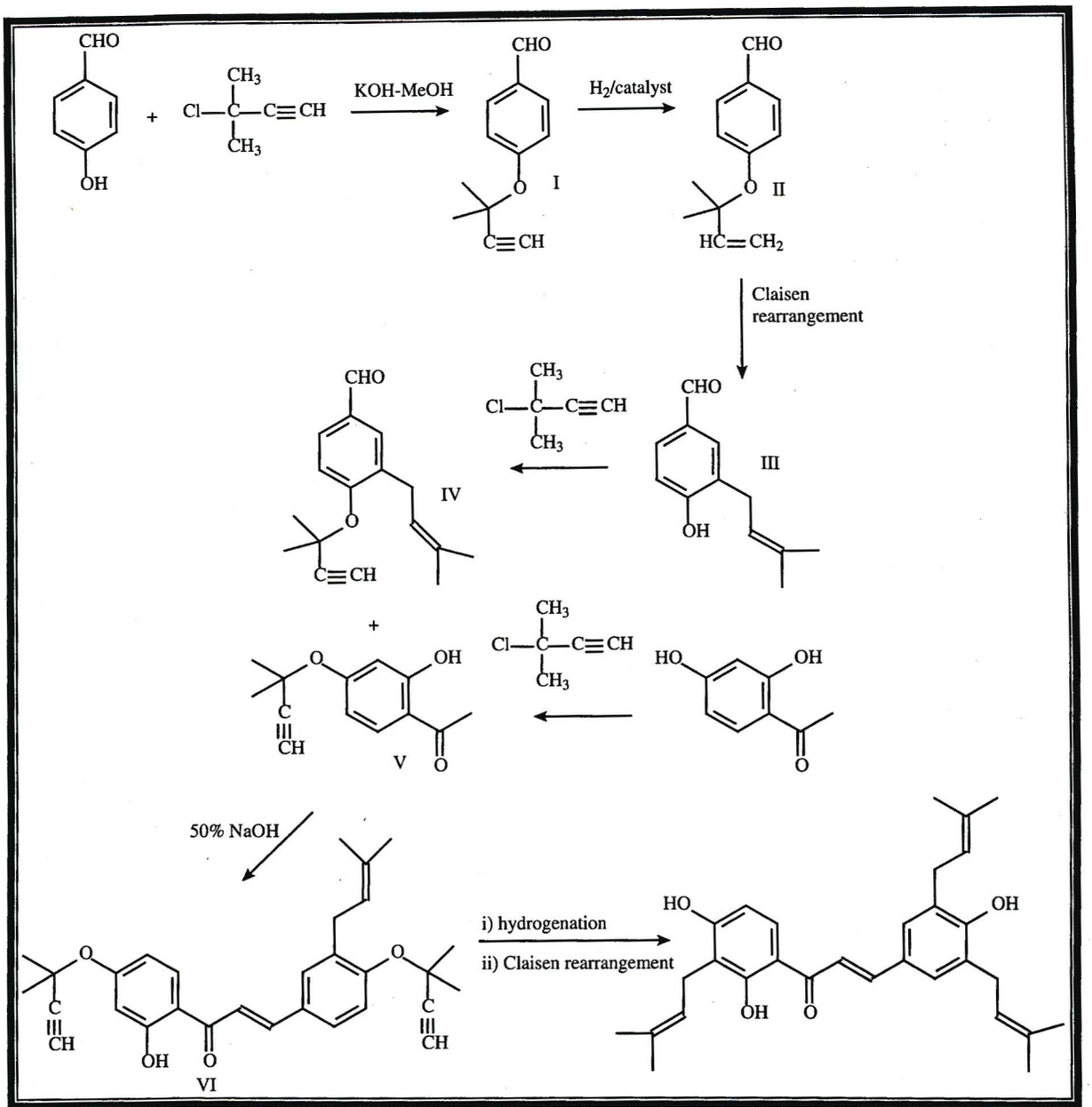


Figure 5. Alternate synthesis of sophoradin[30]

The synthesis of chromenochalcones, including Flemi-Chapparin A has been achieved in 53-63% yield by the reaction of 6-acetyl-5,8-dihydroxy-2,2-dimethylchromene and substituted benzaldehydes[35]. The 6-acetyl-5,8-dihydroxy-2,2-dimethylchromene compound was prepared by reacting β-methylcrotonaldehyde with 2,4,5-trihydroxyacetophenone in pyridine at 150 ^{0}C for ten hours (fig. 6).

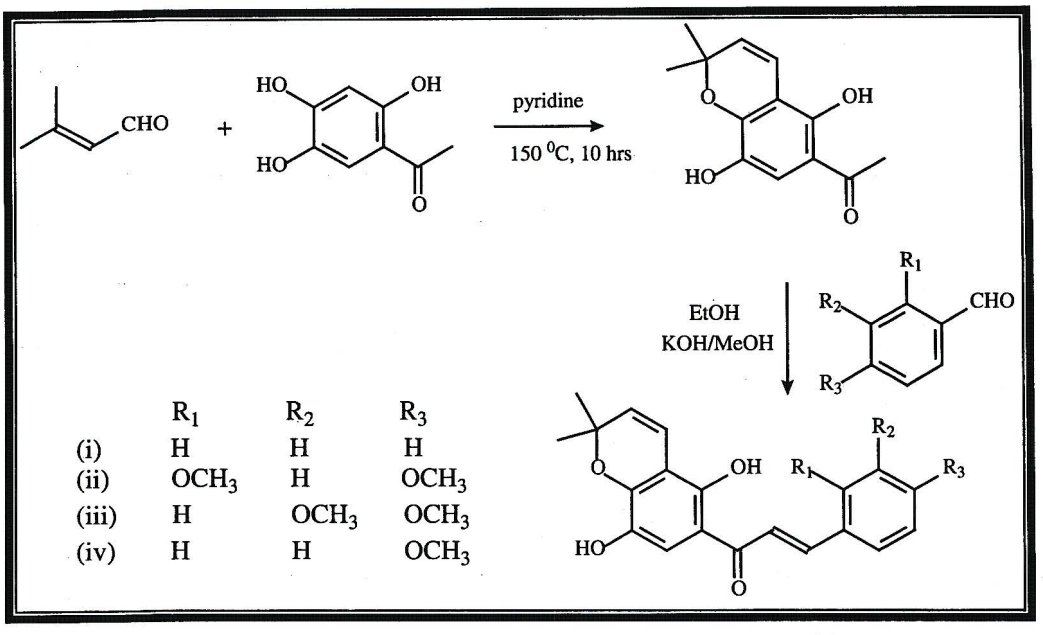


Figure 6. The synthesis of chromenochalcones[35]

Isoprenyl groups have also been added directly onto the chalcones. Reaction of 2',4'-dihydroxychalcone with 2-methyl-3-buten-2-ol yielded 2',4'-dihydroxy-3',5'-di-*C*-prenylchalcone[34].

6.2 Chalcone syntheses

The synthesis of several hydroxylated chalcones was attempted by the Claisen-Schmidt condensation. Difficulty in the synthesis of polyhydroxychalcones using the Claisen-Schmidt condensation is known to occur when compounds with free hydroxyl groups are used in the condensation[6]. Even though these syntheses are difficult, two of the five attempted hydroxychalcone syntheses were successful.

Investigations into the synthesis of prenylated chalcones were also carried out. An isoprenyl intermediate was successfully synthesised. Two isoprenyl groups were successfully attached to the 2',4',6'-trihydroxyacetophenone molecule forming the intermediate in the isoprenylated chalcone synthesis.

6.2.1 Synthesis of hydroxylated chalcones

Synthesis of hydroxychalcones was attempted by the Claisen-Schmidt condensation using 2'-hydroxyacetophenone, 3',5'-dihydroxyacetophenone, 2',5'-dihydroxyacetophenone, 2',3',4'-trihydroxyacetophenone and 2',4',6'-trihydroxyacetophenone and benzaldehyde. Of these, two chalcone syntheses, that of 3',5'-dihydroxychalcone (**XVII**) and 2'-hydroxychalcone (**XVIII**) were successful.

6.2.1.1 Synthesis of 3',5'-dihydroxychalcone (XVII)

Benzaldehyde and 3',5'-dihydroxyacetophenone were reacted in methanol for twenty-four hours at room temperature under basic conditions to yield 3',5'-dihydroxychalcone (**XVII**) (figure 7), a brown crystalline compound with a melting point of 112 ^{0}C.

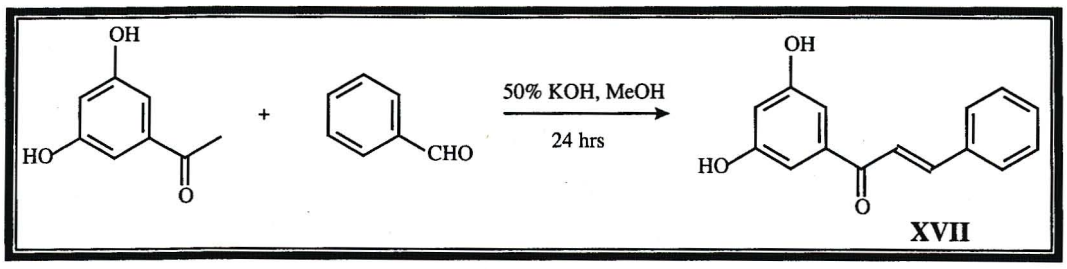
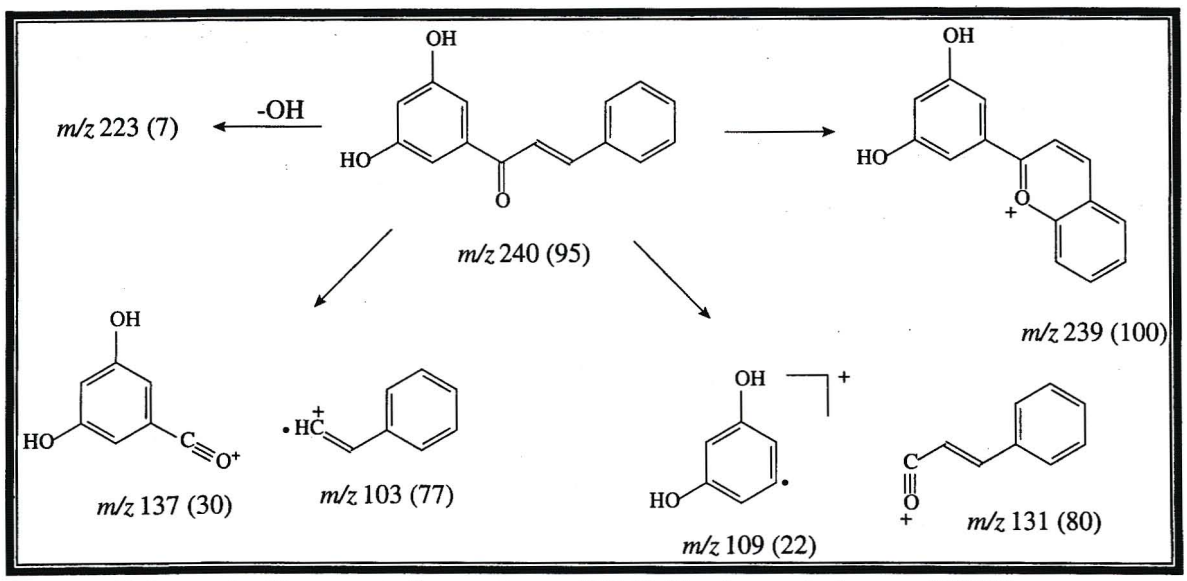


Figure 7. Synthesis of 3',5'-dihydroxychalcone (**XVII**)

Compound **XVII** was found to have a molecular formula of $C_{15}H_{12}O_3$. This compound gave UV absorption maxima at λ_{max}^{MeOH} 310 nm (log $\varepsilon = 4.31$) and 205 nm (4.42) which are characteristic of chalcones[39]. The IR spectrum showed a hydroxy group stretch at 3349 cm^{-1}, a carbonyl stretch at 1666 cm^{-1} and an aromatic C=C stretching band at 1588 cm^{-1}.

The mass spectrum of compound **XVII** showed a molecular ion peak at *m/z* 240. The base peak at *m/z* 239 was the fragment ion resulting from the cyclisation of the B ring with the oxygen of the carbonyl group with the simultaneous loss of a hydrogen atom[38]. Other characteristic peaks in the mass spectrum were at *m/z* 223, the loss of a hydroxy group and *m/z* 137, *m/z* 109, *m/z* 131 and *m/z* 103, resulting from

fragmentation on both sides of the carbonyl group, a common fragmentation process in chalcones[1,39]. The fragmentation pattern of 3',5'-dihydroxychalcone (**XVII**) is given in scheme 1.



Scheme 1. Fragmentation pattern of 3',5'-dihydroxychalcone (**XVII**)

The ^{1}H NMR spectrum of compound **XVII** showed a pair of doublets at δ_H 7.77 and δ_H 7.60 with a large coupling constant of 15.57 Hz which is characteristic for *trans* olefinic protons. These two resonances also showed coupling in the COSY spectrum and were attributed to H-7 and H-8 respectively. A two proton multiplet resonance at δ_H 7.73 and a three proton multiplet resonance at δ_H 7.46 were attributed to H-2,6 and H-3,4,5 respectively on the monosubstituted aromatic ring. These two resonances were seen to be coupled in the COSY spectrum. A two proton doublet at δ_H 6.98 with a coupling constant of 2.19 Hz was attributed to H-2' and H-6' and this resonance was seen to be coupled in the COSY spectrum to a double doublet resonance at δ_H 6.55 which was attributed to H-4'. The H-2'/6' proton resonance showed NOESY interactions with both the H-7 and H-8 proton resonances. The carbon resonance at δ_C 136.23 was attributed to C-1 as this carbon resonance showed HMBC correlations with the H-2/6 and the H-8 proton resonances. The doublet proton resonance at δ_H 7.77 was assigned to H-7 as the C-2/6 carbon resonance at δ_C 129.63 showed HMBC correlations to this proton resonance as well as to the H-3/5 proton resonance. The carbon resonance at δ_C 192.51 was attributed to C-9 and this resonance showed HMBC correlations to the H-7 and H-8 proton resonances as well as the H-2'/6' proton resonances.

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are tabulated in table 1.

Table 1. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 3',5'-dihydroxychalcone (XVII)

Pos.	δ_H	δ_C	HMBC correlations	COSY correlations	NOESY interactions
1		136.23	H-2/6, H-8		
2/6	7.73, m	129.62	H-3/5, H-7	H-3/5	H-3/5
3/5	7.46, m	130.09	H-2/6	H-2/6	H-2/6
4	7.46, m	131.74	H-2/6		
7	7.77, d (15.57)	146.04	H-2/6, H-8	H-8	H-2'/6'
8	7.60, d (15.57)	123.31	H-7	H-7	H-2'/6'
9		192.51	H-7,8, H-2'/6'		
1'		141.35			
2'/6'	6.98, d (2.19)	107.91	H-4'	H-4'	H-7,8
3'/5'		160.11	H-2'/6', H-4'		
4'	6.55, dd (2.19, 2.19)	108.34	H-2'/6'	H-2'/6'	

6.2.1.2 Synthesis of 2'-hydroxychalcone (XVIII)

A mixture of benzaldehyde and 2'-hydroxyacetophenone were reacted at room temperature in methanol under basic conditions to yield 2'-hydroxychalcone (XVIII) (figure 8), yellow needle-like crystals, with a melting point of 88 ^{0}C.

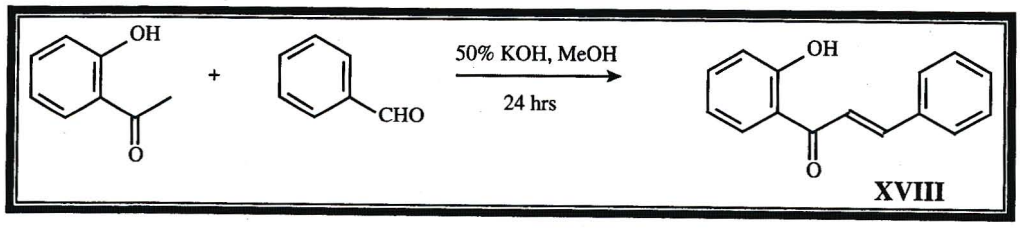
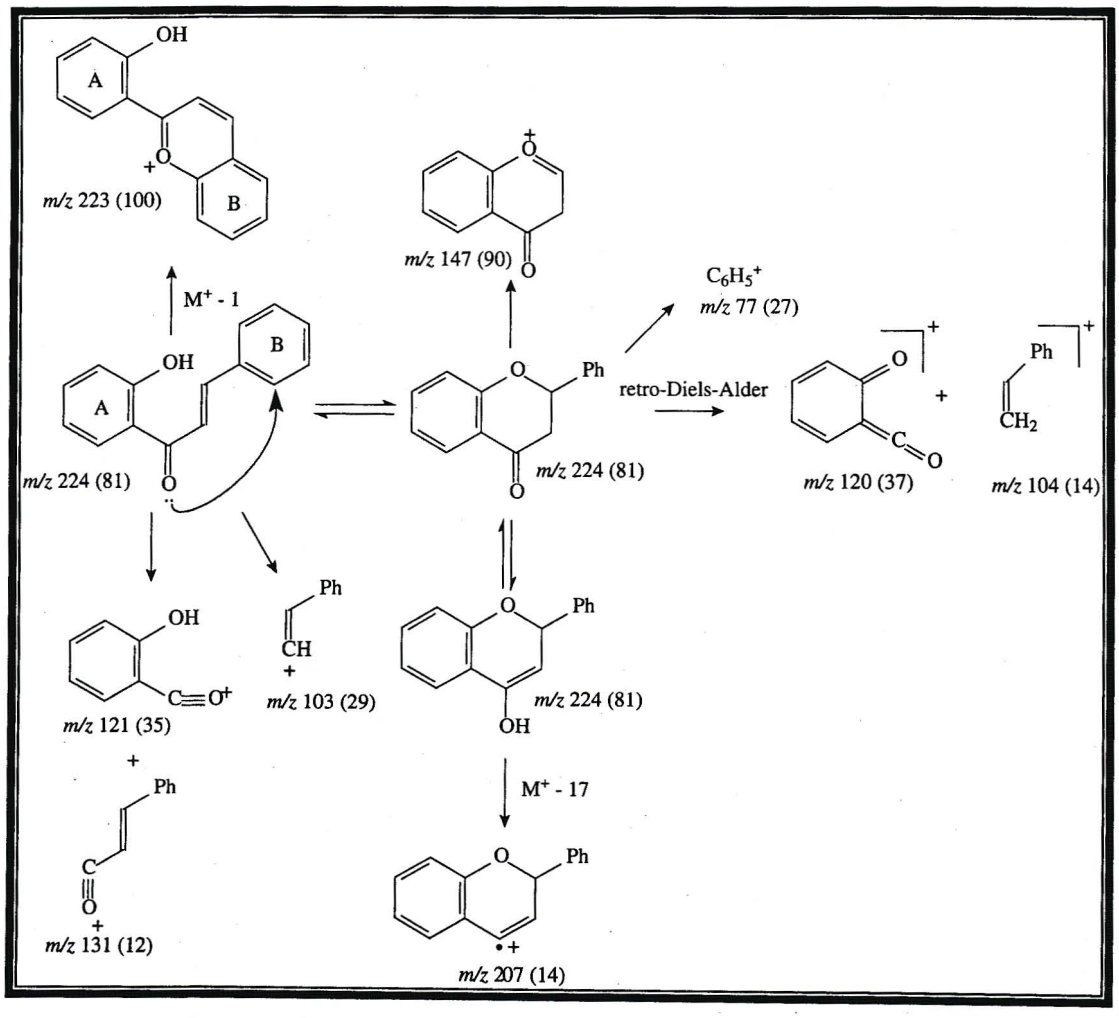


Figure 8. Synthesis of 2'-hydroxychalcone (**XVIII**)

Compound **XVIII** was found to have a molecular formula of $C_{15}H_{12}O_2$. This compound gave UV absorption maxima characteristic of chalcones[39] at $\lambda_{max}^{CH_2Cl_2}$ 317 nm (log $\varepsilon = 4.16$) and 229 nm (3.87). The IR spectrum showed a hydroxy group stretch at 3067 cm^{-1}, a carbonyl stretch at 1644 cm^{-1} and aromatic C=C stretching band at 1577 cm^{-1}.

The mass spectrum of compound **XVIII** showed a molecular ion peak at *m/z* 224. The base peak at *m/z* 223 was due to the cyclisation of the B ring with the oxygen of the carbonyl group and the simultaneous loss of a hydrogen atom[38]. Isomerisation to the flavanone also occurs in 2'-hydroxychalcones[38]. The peak at *m/z* 147 was due to the loss of the phenyl group from the flavanone (scheme 2) and at *m/z* 77, the Ph^+ ion peak was present. Loss of a hydroxy group also occurred from the enol form of the flavanone, in the manner shown in scheme 2[38], resulting in the ion fragment peak at *m/z* 207. Fragmentation on either side of the carbonyl group, a characteristic fragmentation pattern in chalcones[38] resulted in the ion fragment peaks at *m/z* 121, *m/z* 131 and *m/z* 103. The retro-Diels-Alder fragmentation[38] resulted in the fragment ion peaks at *m/z* 120 and *m/z* 104. The fragmentation pattern of 2'-hydroxychalcone, outlined by Mabry and Markham[38] is given in scheme 2.



Scheme 2. Fragmentation pattern of 2'-hydroxychalcone[38].

In the ^{1}H NMR spectrum of compound **XVIII**, a pair of doublets at δ_H 7.89 and δ_H 7.63 with a coupling constant of 15.57 Hz, characteristic of *trans* olefinic coupling could be seen and was attributed to H-7 and H-8 respectively. These two proton resonances were seen to be coupled in the COSY spectrum. The proton resonance at δ_H 7.63 overlapped with a two-proton multiplet, which was attributed to H-2/6. This resonance showed coupling in the COSY spectrum to a three proton multiplet at δ_H 7.42, which was attributed to H-3,4,5. The H-7 resonance at δ_H 7.89 overlapped with a double doublet resonance with coupling constants of 8.06 and 1.47 Hz, suggestive of both *para* and *meta* coupled protons. This proton resonance showed coupling in the COSY spectrum to the two proton resonances at δ_H 6.94 and δ_H 7.47 which both appeared as triplets of doublets (J = 8.06, 7.14 and 1.47 Hz for δ_H 6.94 and J = 8.24, 7.14 and 1.28 Hz for δ_H 7.47) and were attributed to H-5' and H-4' respectively. The resonance at δ_H 7.47 was attributed to H-4' as it showed NOESY correlations to H-5' and the H-3' proton resonance which appeared as a double doublet at δ_H 7.02 (J = 8.24 and 1.28 Hz). The proton resonance at δ_H 6.94 was attributed to H-5' because it showed NOESY interactions with the H-6' and H-4' proton resonances. The carbon resonance at δ_C 134.49 was attributed to C-1 as it showed HMBC correlations with the H-2/6, H-3/5 and the H-7 and H-8 proton resonances. The carbon resonance at δ_C 119.92 was attributed to C-1' as it showed HMBC correlations to H-3',5' and H-6'. The carbon resonance at δ_C 145.39 was attributed to C-7 because of HMBC correlations with the H-2/6 proton resoances. Furthermore, the H-7 proton resonance showed NOESY interactions with the H-2/6 proton resonance. The carbon resonance at δ_C 193.64 showed HMBC correlations with both the H-7 and H-8 proton resonances and was attributed to C-9.

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, COSY and NOESY spectra and are given in table 2.

Table 2. ^{1}H, ^{13}C, HMBC, COSY and NOESY data for 2'-hydroxychalcone (XVIII)

Pos.	δ_H	δ_C	HMBC correlations	COSY correlations	NOESY interactions
1		134.49	H-2/6,H3/5, H-7,H-8		
2/6	7.63, m	128.60	H-3/5,H-7,H-8	H-3/5	H-7, H-3/5
3/5	7.42, m	128.96	H-4, H-2/6, H-7	H-2/6	H-2/6
4	7.42, m	130.86	H-2/6, H-3/5		
7	7.89, d (15.57)	145.39	H-2/6, H-8	H-8	H-2/6
8	7.63, d (15.57)	119.99	H-7	H-7	
9		193.64	H-7, H-8		
1'		119.92	H-3',5',6'		
2'		163.52	H-3',4',6'		
3'	7.02, dd (8.24, 1.28)	118.54	H-5',6'	H-4', H-5'	H-4'
4'	7.47, ddd (8.24, 7.14, 1.28)	136.34	H-6'	H-3', H-5', H-6'	H-3', H-5'
5'	6.94, ddd (8.06, 7.14, 1.47)	118.79	H-3'	H-3', H-4', H-6'	H-4', H-6'
6'	7.89, dd (8.06, 1.47)	129.59	H-4', H-5'	H-4', H-5'	H-5'

6.2.2 Synthesis of prenylated chalcones

The synthesis of prenylated acetophenone intermediates is the first step in the synthesis of prenylated chalcones. A chromene intermediate was successfully synthesised by reacting 3-methyl-2-butenal (β-methylcrotonaldehyde) with 2',4',6'-trihydroxyacetophenone in pyridine (figure 9). The chromene intermediate was then condensed with benzaldehyde in the presence of potassium hydroxide and methanol to yield the dichromenochalcone (figure 9).

6.2.2.1 Synthesis of the chromene intermediate (XIX)

β-methylcrotonaldehyde (3-methyl-2-butenal) was refluxed for twenty-four hours in pyridine with 2',4',6'-trihydroxyacetophenone to yield the chromene intermediate

(**XIX**), a yellow crystalline compound, which had a melting point of 88 ^{o}C and a molecular formula of $C_{18}H_{20}O_4$.

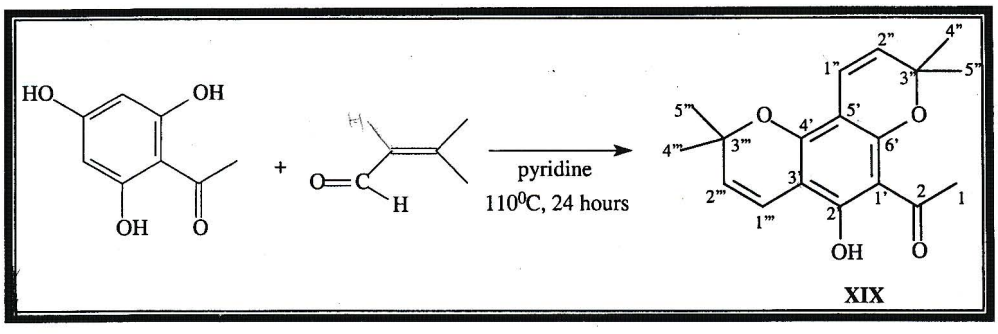


Figure 9. Synthesis of the chromene intermediate (**XIX**)

Compound **XIX** gave UV absorption maxima at 378 nm (log ε = 3.43), 271 nm (4.57) and 218 nm (4.13). The IR spectrum showed a hydroxy group stretch at 3066 cm^{-1}, a carbonyl stretching band at 1649 cm^{-1} and an aromatic C=C stretching band at 1605 cm^{-1}. The mass spectrum of compound **XIX** showed a molecular ion peak at *m/z* 300. The loss of a methyl group resulted in an ion with *m/z* 285 and the loss of an acyl group, O=C-CH_3, resulted in the ion peak at *m/z* 257.

The ^{1}H NMR spectrum of compound **XIX** showed two pairs of doublets in the olefinic region of the spectrum. The doublet proton resonances at δ_H 6.55 and δ_H 5.41 were attributed to H-1" and H-2" of the isoprenyl group which was attached to C-5', which appeared at δ_C 102.13 as this carbon resonance showed HMBC correlations to both the H-1" and H-2" proton resonances. Likewise, the resonance ascribed to C-3', also at δ_C 102.13 showed HMBC correlations to the pair of doublets at δ_H 6.62 and δ_H 5.43 ascribed to H-1'" and H-2'" of the isoprenyl group attached to C-3'. The ^{13}C NMR spectrum showed three separate aromatic C-O resonances at δ_C 160.51, δ_C 154.96 and δ_C 156.64. This indicated that the molecule was not symmetrical and the isoprenyl groups must be cyclised at the 4' and 6' positions as if they were cyclised at the 2' and 6' positions, the molecule would be symmetrical and C-2' and C-6' would be equivalent and have the same carbon resonance. These resonances at δ_C 160.51, δ_C 154.96 and δ_C 156.64 were ascribed to C-2', C-4' and C-6' respectively. Molecular models and a computer simulation (figure 10) showed that the methyl groups on the pyran rings were equivalent because of the planarity of the molecule.

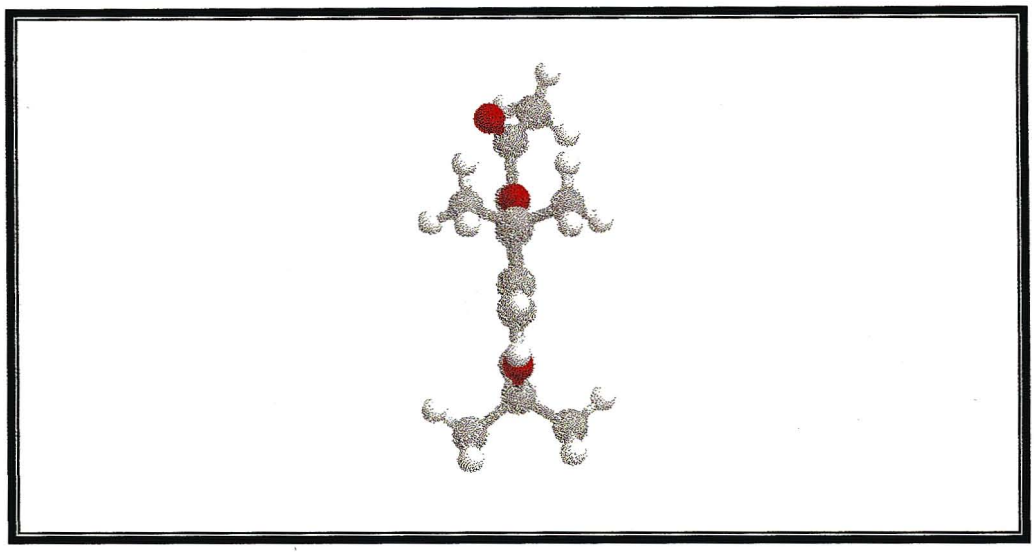


Figure 10. Computer simulation showing the methyl groups of the pyran ring to be equivalent because of the planarity of the molecule.

This agreed with the ^{1}H and ^{13}C NMR data where the 3H-4" and 3H-5" proton resonance appeared as a singlet six-proton resonance at δ_H 1.46 and the corresponding C-4" and C-5" carbon resonance appeared as one resonance at δ_C 27.93. Likewise, the 3H-4"' and 3H-5"' proton resonance appeared at δ_H 1.41 and the C-4"'/C-5"' carbon resonance appeared at δ_C 28.30. This was consistent with the NMR data published for 6-acetyl-5,8-dihydroxy-2,2-dimethylchromene (figure 6) where both methyl groups were equivalent and appeared as one singlet six-proton resonance at δ_H 1.50[35].

The C-4' carbon resonance showed weak HMBC correlations to the 3H-4"'/3H-5"' proton resonances and the C-6' carbon resonance showed weak HMBC correlations to the 3H-4" and 3H-5" proton resonances, providing further evidence that these isoprenyl groups were cyclised at the 4' and 6' positions. The C-3" and C-3"' carbon resonances appeared at δ_C 78.21 and δ_C 78.11 in the ^{13}C NMR spectrum and these carbon resonances showed HMBC correlations to the H-1", H-2", 3H-4"/3H-5" and the H-1"', H-2"', 3H-4"'/3H-5"' proton resonances respectivley.

The methyl group proton resonance at the 1- position appeared as a three-proton singlet at δ_H 2.63 which showed NOESY interactions with the 3H-4"/3H-5" proton resonance. A molecular model and computer simulation (figure 8) showed that these interactions are possible. The C-2 carbonyl resonance appeared at δ_C 203.27 and the

C-1' carbon resonance appeared at δ_C 105.47. Both these carbon resonances showed HMBC correlations with 3H-1 proton resonance.

^1H and ^{13}C NMR data were assigned with the aid of HMBC, COSY and NOESY spectra and are given in table 3.

Table 3. ^1H, ^{13}C, HMBC, COSY and NOESY data for compound XIX

Pos.	δ_H	δ_C	HMBC correlations	COSY correlations	NOESY interactions
1	2.63, 3H, s	33.17			3H-4"/3H-5"
2		203.27	3H-1		
1'		105.47	3H-1		
2'		160.51	H-1"'		
3'		102.13	H-1"', H-2"'		
4'		154.96	H-1", H-1"', 3H-4"'/3H-5"'		
5'		102.13	H-1", H-2"		
6'		156.64	H-1", 3H-4"/3H-5"		
1"	6.55, d (10.07)	116.38	3H-4"/3H-5"	H-2"	H-2"
2"	5.41, d (10.07)	124.67	3H-4"/3H-5"	H-1"	H-1", 3H-4"/3H-5"
3"		78.21*	H-1", H-2", 3H-4"/3H-5"		
4"/5"	1.46, 6H, s	27.93	H-2"		3H-1, H-2"
1"'	6.62, d (10.07)	116.10	3H-4"'/3H-5"'	H-2"'	H-2"'
2"'	5.43, d (10.07)	125.32	3H-4"'/3H-5"'	H-1"'	H-1"', 3H-4"'/3H-5"'
3"'		78.11*	H-1"', H-2"', 3H-4"'/3H-5"'		
4"'/5"'	1.41, 6H, s	28.30	H-2"'		H-2"'

* Assignments may be interchanged.

6.2.2.2 Synthesis of dichromenochalcone (XX)

The chromene intermediate (**XIX**) synthesised in 6.2.2.1 was then condensed with benzaldehyde in the presence of 50% potassium hydroxide and methanol and left to

stir for five days at room temperature to yield the dichromenochalcone (**XX**) (fig. 11), a brown residue with a molecular formula of $C_{25}H_{24}O_4$.

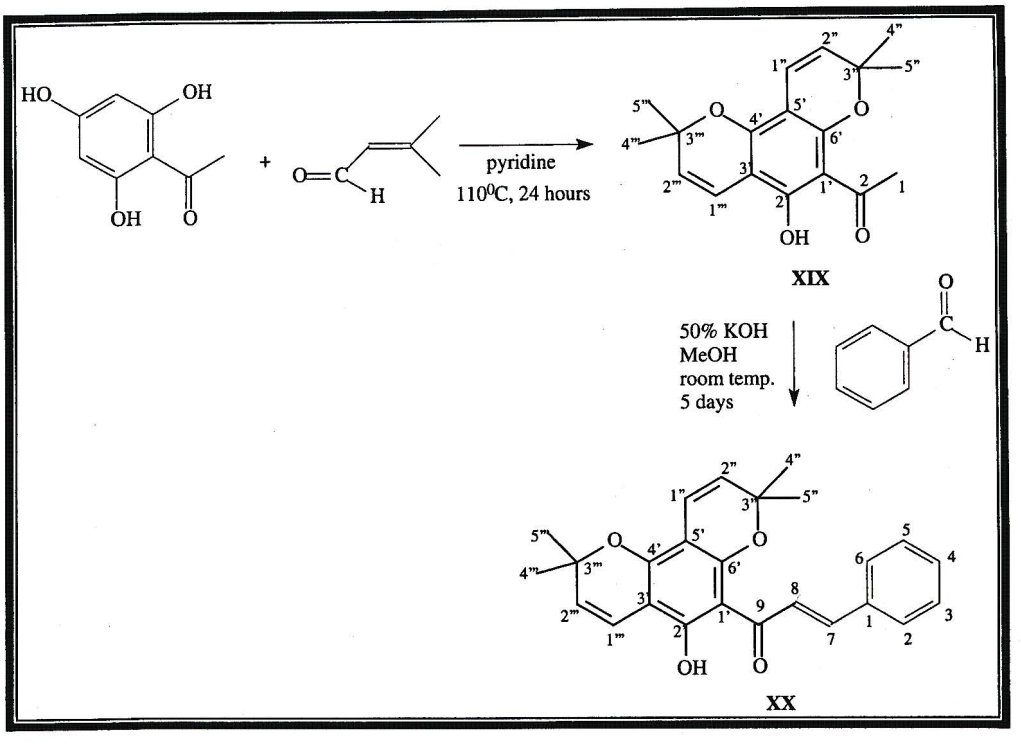
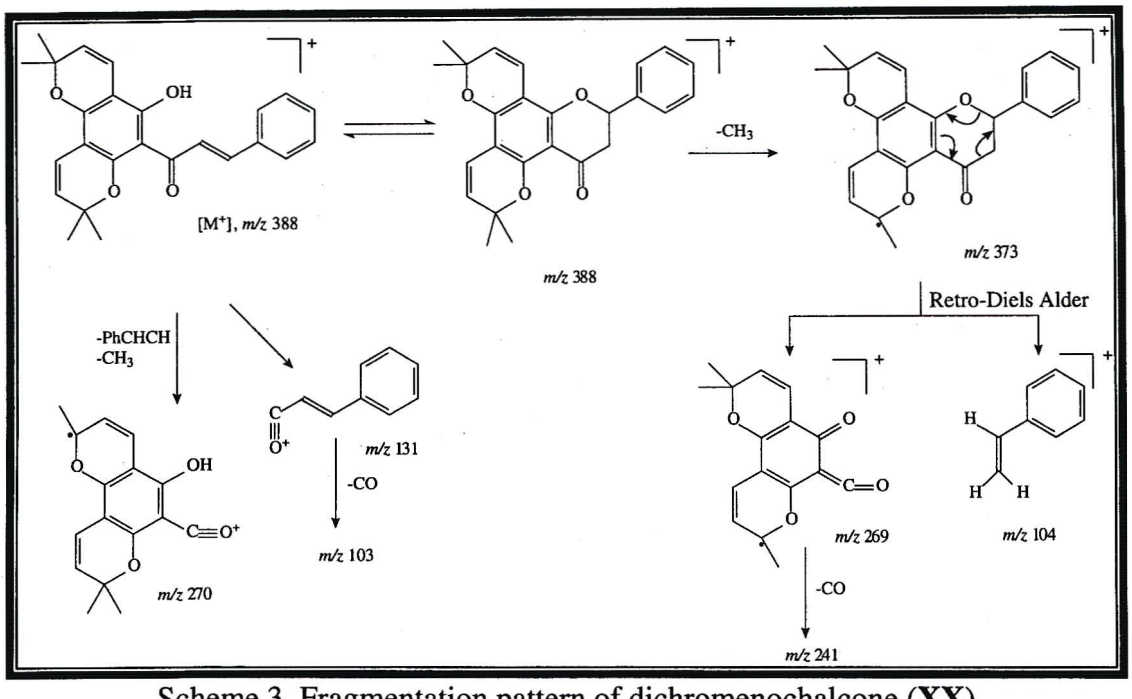


Figure 11. Synthesis of dichromenochalcone (**XX**)

Compound **XX** showed UV absorption maxima at 361 nm (log ε = 4.32), 280 (4.51), 241 (4.29), 224 (4.26), and 212 (4.24), characteristic of chalcones[39]. The IR spectrum showed a hydroxy group stretch at 3064 cm^{-1}, a carbonyl stretching band at 1648 cm^{-1} and an aromatic C=C stretching band at 1594 cm^{-1}. The mass spectrum showed a molecular ion peak at *m/z* 388. The loss of a methyl group resulted in the ion peak at *m/z* 373. Fragmentation on both sides of the carbonyl group resulted in the ion fragment peaks at *m/z* 131 and *m/z* 103, a common fragmentation process in chalcones[38]. Fragmentation adjacent to the carbonyl group on the side of the double bond, followed by loss of a methyl group resulted in the ion peak at *m/z* 270. Isomerisation to the flavanone, followed by the loss of a methyl group and a retro-Diels-Alder cleavage[38] resulted in the ion fragment peaks at *m/z* 269 (the base peak) and *m/z* 104. The loss of a carbonyl group from the base peak fragment resulted in the ion peak at *m/z* 241. The mass spectral fragmentation pattern of dichromenochalcone (**XX**) is given in scheme 3.



Scheme 3. Fragmentation pattern of dichromenochalcone (**XX**)

The ^{1}H NMR spectrum of compound **XX** showed the characteristic pattern for a monosubstituted aromatic ring, at δ_H 7.58 (2H, dd, J = 17.87, 1.83 Hz, H-2/6) and δ_H 7.39 (3H, m, H-3/4/5) with corresponding carbon resonances at δ_C 128.24 (C-2/6) and δ_C 128.94 (C-3/5) and 130.04 (C-4). Also present were a pair of doublets at δ_H 7.74 (d, J = 15.56 Hz) and δ_H 8.08 (d, J = 15.56 Hz) attributed to H-7 and H-8 respectively, with corresponding carbon resonances at δ_C 142.07 (C-7) and δ_C 127.57 (C-8). The large coupling constant (15.56 Hz) is characteristic of *trans* olefinic protons. The proton resonance at δ_H 7.74 was attributed to H-7 as this proton resonance showed HMBC correlations to the C-2/6 carbon resonance at δ_C 128.24. The resonance at δ_H 8.08, attributed to H-8 showed an HMBC correlation to the carbon resonance at δ_C 135.63, which was then assigned to C-1. The carbon resonance at δ_C 192.92 was assigned to C-9 because of HMBC correlations to the H-7 and H-8 proton resonances.

A pair of doublets at δ_H 6.67 and δ_H 6.59 with coupling constants of 10.07 Hz indicative of *cis* olefinic coupling were each coupled to a third doublet at δ_H 5.46 (2H, J = 10.07 Hz) in the COSY spectrum. HSQC correlations of the carbon resonances at δ_C 124.76 and δ_C 125.40 with the doublet at δ_H 5.46 indicated that this resonance was two overlapping methine resonances. These resonances together with the two six-proton methyl group proton resonances at δ_H 1.53 and δ_H 1.44 indicated the presence of two isoprenyl groups. HMBC, COSY and NOESY data allowed the resonances at

δ_H 6.59, δ_H 5.46 and δ_H 1.53 to be attributed to H-1", H-2" and 3H-4"/3H-5" respectively with corresponding carbon resonances at δ_C 116.58, δ_C 124.76 and δ_C 28.08. The second isoprenyl group was shown to have resonances at δ_H 6.67, δ_H 5.46 and δ_H 1.44 for H-1"', H-2"' and 3H-4"'/3H-5"' respectively with corresponding carbon resonances at δ_C 116.22, δ_C 125.40 and δ_C 28.39. The carbon resonances at δ_C 78.31 and δ_C 78.27 showed HMBC correlations to H-1", H-2", H-1"' and H-2"' and were assigned to C-3" and C-3"'.

Three aromatic C-O carbon resonances could be seen in the ^{13}C NMR spectrum at δ_C 161.45, δ_C 156.20 and δ_C 155.31. From the molecular formula, $C_{25}H_{24}O_4$, the oxygen atoms were all accounted for, three for the aromatic ring and one for the carbonyl group at C-9. There were no extra oxygen atoms, which would be needed for hydroxy groups to be positioned at C-3" and C-3"' if the two isoprenyl groups were not cyclised. The cyclisation of the two isoprenyl groups rendered that part of the molecule flat in a plane with each pair of methyl groups in a similar chemical environment on either side of the plane (fig. 12). This explained each pair of methyl groups having one proton and carbon resonance.

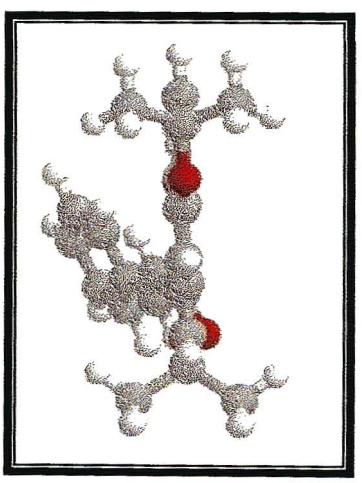


Figure 12. Computer simulation showing the methyl groups of the two pyran rings to be equivalent because of the planarity of the molecule.

The 3H-4"/3H-5" proton resonance showed NOESY interactions with the H-8 and H-2/6 proton resonances and this isoprenyl group was therefore positioned at the 5' position. The carbon resonances at δ_C 102.61 and δ_C 156.20 showed HMBC

correlations to the H-1" proton resonance and was therefore attributed to C-5' and C-6'. The resonances at δ_C 161.45 and δ_C 102.53 showed HMBC correlations to the H-1'" proton resonance and were attributed to C-2' and C-3'. The resonance at δ_C 155.31 showed HMBC correlations to both the H-1" and H-1'" proton resonances and was therefore assigned to C-4'.

^1H and ^{13}C NMR assignments were made with the aid of HMBC, COSY and NOESY data and are tabulated in table 4.

Table 4. ^1H, ^{13}C, HMBC, COSY and NOESY data for dichromenochalcone, XX

Pos.	δ_H	δ_C	HMBC correlations	COSY correlations	NOESY interactions
1		135.63	H-8		
2/6	7.58, dd (7.87, 1.83)	128.24	H-7	H-3/4/5	H-7,8, H-3/5
3/5	7.39, m	128.94	H-4	H-2/6	H-2/6
4	7.39, m	130.04	H-2/6	H-2/6	
7	7.74, d (15.56)	142.07	H-2/6	H-8	H-2/6
8	8.08, d (15.56)	127.57		H-7	H-2/6
9		192.92	H-7,8		
1'		105.94			
2'		161.45	H-1'"		
3'		102.53*	H-1'"		
4'		155.31	H-1", H-1'"		
5'		102.61*	H-1"		
6'		156.20	H-1"		
1"	6.59, d (10.07)	116.58	3H-4"/3H-5"	H-2"	H-2"
2"	5.46, d (10.07)	124.76	3H-4"/3H-5"	H-1"	H-1", 3H-4"/3H5"
3"		78.31**	H-1",2"		
4"/5"	1.53, s (6H)	28.08			H-2", H-8, H-2/6
1'"	6.67, d (10.07)	116.22	3H-4'"/3H-5'"	H-2'"	H-2'"
2'"	5.46, d (10.07)	125.40	3H-4'"/3H-5'"	H-1'"	H-1'", 3H-4'"/3H-5'"
3'"		78.27**	H-1'",2'"		
4'"/5'"	1.44, s (6H)	28.39			H-2'"

*, ** Assignments may be interchanged.

6.3 Experimental

Synthesis of 3',5'-dihydroxychalcone

A potassium hydroxide solution (2 grams of KOH pellets dissolved in 2 ml of water) was added to methanol (10 ml) in a round bottom flask. Benzaldehyde (1 ml) was added to the round bottom flask followed by 3',5'-dihydroxyacetophenone (0.5 grams; 0.00325 moles) and stoppered. The contents were left to stand for twenty-four hours after which it was diluted with water (20 ml) and acidified with dilute HCl (20 ml). The reaction mixture was then extracted with dichloromethane (3 x 20 ml portions). The dichloromethane extract was subject to column chromatography using silica gel as the stationary phase and 5% methanol in dichloromethane as the mobile phase. This yielded 3',5'-dihydroxychalcone, $C_{15}H_{12}O_3$ as a brown crystalline material, with a melting point of 112 ^{0}C and a yield of 72%.

Ultra violet: λ_{max}^{MeOH} nm (log ε): 310 (4.31), 205 (4.42)

Infra-red: ν_{max}^{NaCl} cm^{-1}: 3349 (O-H stretching), 1666 (C=O stretching), 1588 (aromatic C=C stretching), 1449, 1352, 1166, 1015, 864, 768.

Mass: EIMS: *m/z* (rel. int.): [M$^+$] 240 (94.87), 239 (100), 223 (6.85), 211 (11.63), 165 (12.31), 162 (10.98), 137 (30.61), 131 (79.66), 119 (10.16), 110 (10.32), 109 (22.13), 103 (77.09), 102 (14.32), 81 (13.94), 77 (49.94), 69 (23.40), 51 (19.45).

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are tabulated in table 1.

Synthesis of 2'-hydroxychalcone

Methanol, potassium hydroxide and benzaldehyde were added as above. This was followed by adding 2'-hydroxyacetophenone (1 ml; 0.00830 moles). The flask was stoppered and left for twenty-four hours, after which it was diluted, acidified and extracted as above, yielding 2'-hydroxychalcone, $C_{15}H_{12}O_2$ as yellow needles with a melting point of 88 ^{0}C (lit[37] 88-89 ^{0}C) and a yield of 78%.

Ultra violet: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 317 (4.16), 229 (3.87)

Infra-red: ν_{max}^{NaCl} cm^{-1}: 3067 (O-H stretching), 1644 (C=O stretching), 1577 (aromatic C=C stretching), 1489, 1451, 1357, 1307, 1274, 1202, 1158, 1031, 976, 745.

Mass: EIMS: *m/z* (rel. int.): [M^+] 224 (81.02), 223 (100), 207 (14.34), 195 (5.61), 147 (89.57), 131 (12.10), 121 (34.94), 120 (36.80), 104 (13.70), 103 (28.96), 77 (27.35), 65 (18.66).

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are tabulated in table 2.

Synthesis of the chromene intermediate

To 2',4',6'-trihydroxyacetophenone monohydrate (0.93 g; 0.005 moles) in a 10 ml round bottom flask, 3-methyl-2-butenal (β-methylcrotonaldehyde) (0.84 g; 0.010 moles) was added. Pyridine (0.40 g) was added to this mixture and the contents refluxed for twenty hours at 110 ^{0}C. To the reaction mixture, toluene (3 x 10 ml portions) was added and removed under reduced pressure to remove the excess pyridine. Methanol was then added in 3 x 10 ml portions and removed under reduced pressure to remove the excess toluene. The resultant mixture was then chromatographed on a column (2 cm in diameter) packed with silica gel and eluted with hexane:dichloromethane (1:1), collecting 30 ml fractions. Fraction 3 afforded the pure compound **XIX** in 38% yield. Compound **XIX** was a yellow crystalline compound with a molecular formula of $C_{18}H_{20}O_4$ and a melting point of 88 ^{0}C.

Ultra violet: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 378 (3.43), 271 (4.57), 218 (4.13)

Infra-red: ν_{max}^{NaCl} cm^{-1}: 3066 (O-H stretching), 2972, 2928, 2727, 1649 (C=O stretching), 1605 (aromatic C=C stretching), 1467, 1436, 1367, 1279, 1204, 1135, 1003, 890, 727.

Mass: EIMS: *m/z* (rel. int.): [M^+] 300 (24.60), 286 (18.42), 285 (100), 267 (8.81), 257 (8.40), 135 (5.87), 126 (8.36), 91 (2.60), 79 (2.64), 77 (3.60).

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are tabulated in table 3.

Synthesis of the dichromenochalcone

The chromene intermediate (**XIX**) (0.480 g; 0.0016 moles) was dissolved in dichloromethane (2 ml) and added to benzaldehyde (0.27 g; 0.0025 moles) and methanol (15 ml) in a 50 ml round bottom flask. Potassium hydroxide pellets (5.0 g) was dissolved in water (10 ml) and added slowly to the mixture in an ice bath. After leaving the mixture stirring at room temperature for five days, it was poured into

dilute hydrochloric acid (30 ml). The contents were then extracted with dichloromethane (6 x 40 ml portions) and chromatographed on silica gel, using a 1:1 (hexane:dichloromethane) mobile phase. Fractions 3-7 afforded compound **XX** (0.2624 g; 0.000676 moles) in 42% yield. Compound **XX** was isolated as a brown residue with a molecular formula of $C_{25}H_{24}O_4$.

Ultra violet: $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 361 (4.32), 280 (4.51), 241 (4.29), 224 (4.26), 212 (4.24).

Infra-red: ν_{max}^{NaCl} cm^{-1}: 3064 (O-H stretching), 2973, 2936, 2663, 1648 (C=O stretching), 1594 (aromatic C=C stretching), 1551, 1460, 1430, 1369, 1351, 1290, 1187, 1162, 1138, 998, 980, 883.

Mass: EIMS: *m/z* (rel. int.): [M^+] 388 (55.34), 374 (21.36), 373 (82.62), 345 (4.07), 270 (17.32), 269 (100), 256 (7.19), 251 (6.23), 241 (20.74), 215 (7.15), 194 (5.20), 165 (5.88), 131 (18.44), 128 (12.13), 127 (25.68), 115 (14.31), 107 (9.49), 104 (9.41), 103 (33.22), 91 (14.03), 79 (11.70), 77 (28.79), 43 (16.64).

^{1}H and ^{13}C NMR assignments were made with the aid of HMBC, HSQC, COSY and NOESY spectra and are tabulated in table 4.

6.4. References

1. Dhar, D.N. (1981) *The Chemistry of Chalcones and Related Compounds*, John Wiley and Sons, New York, pp 1-32, 188-189.
2. Coombs, E. and Evans, D.P. (1940) Condensations of carbonyl compounds. A kinetic study of the reaction of acetophenone with benzaldehyde, *Journal of the Chemical Society*, 1295-1300.
3. Nayak, P.L. and Rout, M.K. (1975) Kinetics of the formation of chalcone. Linear free energy relationships, *Journal of the Indian Chemical Society*, **52**, 809-811; *Chem. Abstr.* (1976) **84**, 73456x.
4. Sipos, G., Furka, A. and Szell, T. (1960) Kinetic investigation of the base-catalysed formation of chalcones, *Monatsh. Chem.*, **91**, 643-652; *Chem. Abstr.* (1960) **55**, 10384i.
5. Smith, E.H. and Paulson, M.C. (1954) The preparation of chalcones from hydroxy and methoxy aldehydes and ketones, *Journal of the American Chemical Society*, **76**, 4486-4487.
6. Shinoda, J. and Sato, S. (1928) Synthesis of polyhydroxychalcones and flavanones. I. Synthesis of 2',4'-dihydroxychalcone; 2',4'-dihydroxy-4-methoxydihydrochalcone and isosakuranetin, *J. Pharm. Soc.* (Japan), **48**, 791-801; *Chem. Abstr.* (1929) **23**, 836.
7. Kurth, E.F. (1939) The preparation of the polyhydroxychalcones, *Journal of the American Chemical Society*, **61**, 861-862.
8. Geissman, T.A. and Clinton, R.O. (1946) Flavanones and related compounds. I. The preparation of polyhydroxychalcones and flavanones, *Journal of the American Chemical Society*, **68**, 697-700.
9. Martin, G.J., Beiler, J.M. and Avakian, S. (1956) Chalcones, U.S. Patent 2,769,817; *Chem. Abstr.* (1957) **51**, 14815d.
10. Jurd, L. and Horowitz, R.M. (1961) Spectral studies on flavonoid compounds. III. Polyhydroxychalcones, *Journal of Organic Chemistry*, **26**, 2561-2563.
11. Starkov, S.P., Panasenko, A.I. and Volkotrub, M.N. (1969) Synthesis and properties of 6-n-alkyl-7-hydroxy-4-methoxy and 6-n-alkyl-7-hydroxyflavanones, *Izv. Vyssh. Ucheb. Zaved., Khim. Khim. Tekhnol.*, **12**, 1072-1076; *Chem. Abstr.* (1970) **72**, 66738q.

12. Matsuura, S. and Matsuura, A. (1957) Synthesis of 2'-hydroxy-3'(and 5')-acetyl-4,4'-dimethoxychalcone, *Yakugaku Zasshi*, **77**, 330-331; *Chem. Abstr.* (1957) **51**, 11300i.

13. Hsu, K.K., Lo, M.S. and Chen, F.C. (1969) Synthesis of sulfamoyl chalcones, *J. Chin. Chem. Soc. (Taipei)*, **16**, 91-96; *Chem. Abstr.* (1970) **72**, 31393w.

14. Falcao da Fonseca, L. (1968) Synthesis of nonnatural chalcones, *Bol. Fac. Farm., Univ. Coimbra, Ed. Cient*, **28**, 49-52; *Chem. Abstr.* (1970) **72**, 121124p.

15. Ch'en, F-C., Weng, T. and Chang, P-W. (1970) Synthesis of 2,6-dimethyl-4-hydroxy and –methoxy chalcone derivatives, *Taiwan K'o Hsueh*, **24**, 105-106; *Chem. Abstr.* (1971) **75**, 19859g.

16. Takahara, H. and Hamada, M. (1956) Chemical constitution and insecticidal activity. X. Synthesis of 1,1-bis(p-chlorophenyl)-2,2-dichloropropane, *Botyu-Kayaku*, **21**, 20-22; *Chem. Abstr.* (1957) **51**, 3519d.

17. Szell, T. and Bajusz, S. (1955) The effect of substituents on chalcone formation, *Magyar Kem. Fokyoirat*, **61**, 235-236; *Chem. Abstr.* (1958) **52**, 9048g.

18. Loth, H. and Worm, G. (1968) Preparation and properties of some new flavonoid carboxylic acids, *Arch. Pharm. (Weinheim)*, **301**, 897-903; *Chem. Abstr.* (1969) **70**, 68064z.

19. Biletch, H.A. and Rajunas, J.V., Jr. (1968) Preparation of benzalacetophenone, U.S. Patent 3,361 827; *Chem. Abstr.* (1968) **68**, 104756w.

20. Lespagnol, A., Lespagnol, Ch., Lesieur, D., Bonte, J.P., Blain, Y. and Labiau, O. (1971) Preparation of chalcones having salicylic structure, *Chim. Ther.*, **6**, 192-195; *Chem. Abstr.* (1971) **75**, 98285t.

21. Lavrushin, V.F. and Verkhovod, N.N. (1965) Synthesis of some derivatives in the series of chalcone and its vinylogs, *Zh. Organ. Khim.*, **1**, 1220-1222; *Chem. Abstr.* (1965) **63**, 13118b.

22. Hayakawa, G. and Takuya, I. (1971) Chalconesulfonic acids, Japanese Patent 71 07,386; *Chem. Abstr.* (1971) **74**, 143332y.

23. Pomerantseva, L.L., Smirnova, G.A. and Oleinik, A.V. (1972) Synthesis of new organic azides, *Tr. Khim. Khim. Tekhnol*, (1), 14-16; *Chem. Abstr.* (1973) **78**, 159099w.

24. Gilman, H. and Cason, L.F. (1950) Some addition reactions of chalcones. I. The preparation of some γ-ketosulfones, *Journal of the American Chemical Society*, **72**, 3469-3472.

25. Rochus, W. and Kickuth, R. (1957) Potassium-carbon compounds as catalysts for base-catalysed organic reactions, German Patent 1,095,832; *Chem. Abstr.* (1961) **56**, 10976d.

26. Rorig, K.J. (1956) *Ar'*-cyanomethylchalcones, U.S. Patent 2,755,299; *Chem. Abstr.* (1957) **51**, 2868c.

27. Sipos, G., Dobo, I. and Czukor, B. (1962) Chalcone formation in the presence of hydrogen chloride (German), *Acta Univ. Szeged., Acta Phys. Chem.*, **8**, 160-163; *Chem. Abstr.* (1963) **59**, 5059g.

28. Breslow, D.S. and Hauser, C.R. (1940) Condensations. XI. Condensations of certain active hydrogen compounds effected by boron trifluoride and aluminium chloride, *Journal of the American Chemical Society*, **62**, 23852388.

29. Kyogoku, K., Hatayama, K., Yokomori, S., Seki, T. and Tanaka, I. (1975) Synthesis of the isoprenyl chalcone sophoradin isolated from *Sophora subprostrata, Agric. Biol. Chem.*, **39**, 133-138; *Chem. Abstr.* (1975) **82**, 156524c.

30. Kyogoku, K., Hatayama, K., Yokomori, S., Seki, T. and Tanaka, I. (1974) Synthesis of the isoprenyl chalcone sophoradin, *Agric. Biol. Chem.*, **38**, 2291-2292; *Chem. Abstr.* (1975) **82**, 97789t.

31. Kyogoku, K., Hatayama, K., Yokomori, S. and Seki, T. (1974) Isopentenyl chalcones, Japanese Patent 74,126,654; *Chem. Abstr.* (1975) **83**, 78864t.

32. Lupi, A., Delle Monache, G., Delle Monache, F., Marini-Bettolo, G.B., Goncalves de Lima, O. and De Mello, J.F. (1975) Synthesis of the prenylated chalcones from Cordoa Piaca (*Lonchocarpus neuroscapha*), *Farmaco, Ed. Sci.*, **30**, 449-455; *Chem. Abstr.* (1975) **83**, 178450y.

33. Kyogoku, K., Hatayama, K., Yokomori, S., Seki, T. and Tanaka, I. (1975) Synthesis of the isoprenyl chalcone sophoradin through Claisen rearrangement, *Agric. Biol. Chem.*, **39**, 667-672; *Chem. Abstr.* (1975) **83**, 28382p.

34. Khanna, R.N., Khanna, P.L., Manchanda, V.P. and Seshadri, T.R. (1973) Synthesis of derricin, derricidin and related compounds, *Indian J. Chem.*, **11**, 1225-1227; *Chem. Abstr.* (1974) **80**, 132959f.

35. Yamada, S., Ono, F., Katagiri, T. and Tanaka, J. (1975) The synthesis of flemichapparin-A and its derivatives, *Bulletin of the Chemical Society of Japan*, **48**, 2391-2392.

36. Rall, G.J.H., Oberholzer, M.E., Ferreira, D. and Roux, D.G. (1976) Crown ethers. Application in the synthesis of chalcones, *Tetrahedron Letters*, 1033-1036.

37. Dictionary of Organic Compounds (1982) Buckingham, J. and Donaghy, S.M. (Editors), Chapman and Hall, London, 5th Edition, H-03208, p. 3220.

38. Mabry, T.J. and Markham, K.R. (1975) Mass spectrometry of flavonoids, in. *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 79-126.

39. Markham, K.R. and Mabry, T.J. (1975) Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids, in. *The Flavonoids*, Ed. Harborne, J.B., Chapman and Hall, London, p. 51.

40. Bruice, P.Y. (2001) Chapter 19. Carbonyl Compounds III: Reactions at the α-carbon, in. *Organic Chemistry*, 3rd Edition, Ed. Challice, J., Murdzek, J. and Schiaparelli, K., Prentice Hall, London, p. 848.

Appendix I

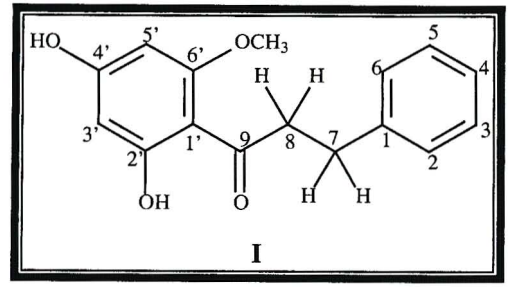
NMR, Ultra violet, Infrared and Mass spectra

for

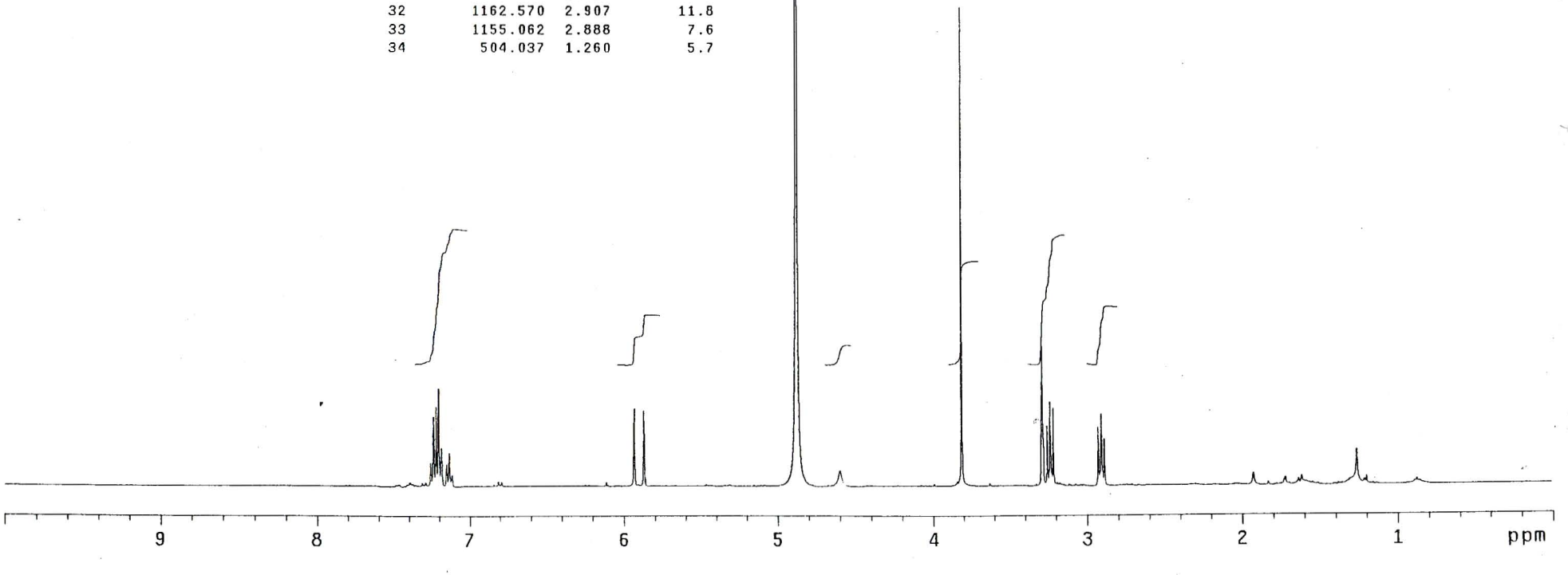
Chapter 3. Extractives from *Cedrelopsis grevei*

Pulse Sequence: s2pul

INDEX	FREQUENC'	PPM	HEIGHT
1	2902.3	7.257	3.8
2	2894.7	7.238	11.4
3	2889.847	7.226	3.7
4	2887.832	7.220	13.0
5	2881.972	7.206	12.1
6	2880.507	7.202	16.0
7	2873.731	7.185	6.2
8	2861.279	7.154	2.5
9	2859.813	7.150	3.6
10	2858.165	7.146	2.0
11	2852.855	7.133	5.4
12	2375.253	5.939	10.4
13	2373.056	5.933	12.8
14	2349.798	5.875	12.4
15	2347.418	5.869	10.6
16	1950.942	4.878	200.0
17	1839.417	4.599	2.5
18	1525.167	3.813	78.7
19	1318.230	3.296	8.2
20	1316.582	3.292	16.1
21	1314.934	3.288	22.8
22	1313.286	3.284	16.6
23	1311.638	3.280	8.7
24	1301.749	3.255	9.8
25	1297.354	3.244	2.7
26	1294.424	3.236	13.8
27	1292.775	3.232	8.1
28	1290.212	3.226	2.8
29	1286.366	3.216	12.8
30	1170.628	2.927	9.6
31	1166.599	2.917	2.2
32	1162.570	2.907	11.8
33	1155.062	2.888	7.6
34	504.037	1.260	5.7



I

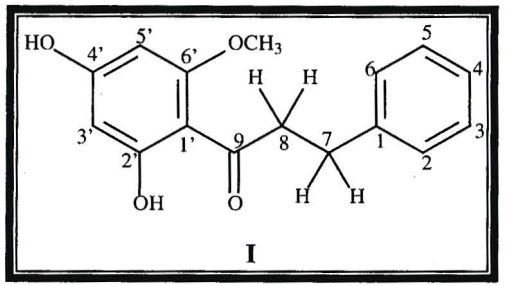


^{1}H NMR spectrum of compound **I,** uvangoletin

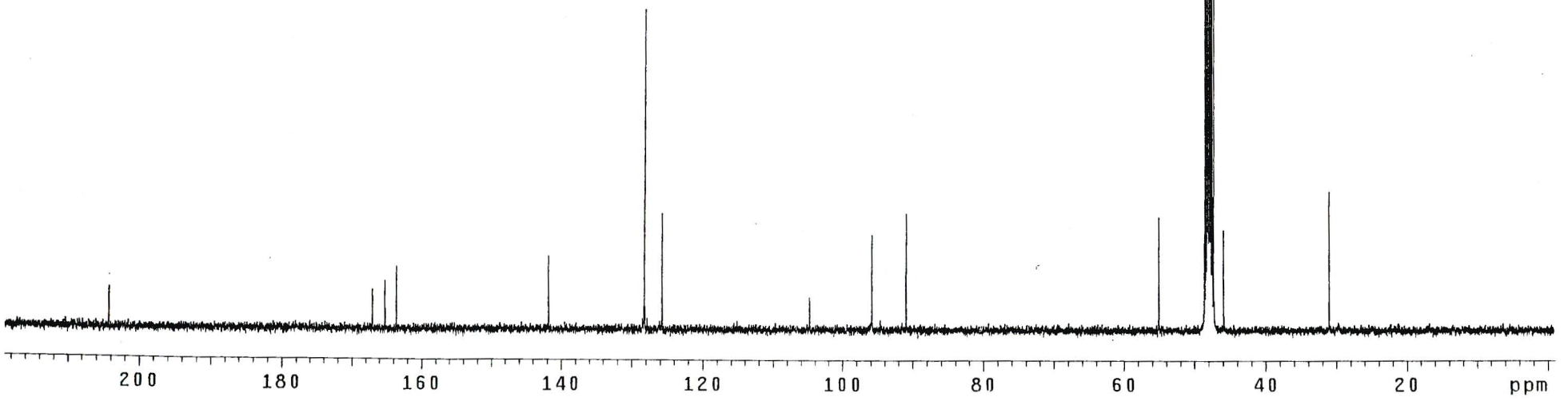
cd58a.ced58a in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	20542.3	204.265	6.7
2	16794.7[illegible]	166.999	6.6
3	16614.657	165.209	7.9
4	16453.672	163.608	10.3
5	14259.391	141.789	12.1
6	12889.873	128.171	52.7
7	12888.347	128.156	45.0
8	12639.621	125.683	19.2
9	10530.029	104.706	5.4
10	9632.022	95.777	15.8
11	9141.437	90.898	19.2
12	5530.335	54.991	18.8
13	4878.764	48.512	121.0
14	4857.401	48.300	356.5
15	4836.038	48.087	698.1
16	4814.676	47.875	800.0
17	4793.313	47.663	673.9
18	4771.950	47.450	334.0
19	4749.824	47.230	110.4
20	4613.253	45.872	16.7
21	3116.320	30.987	23.0



I



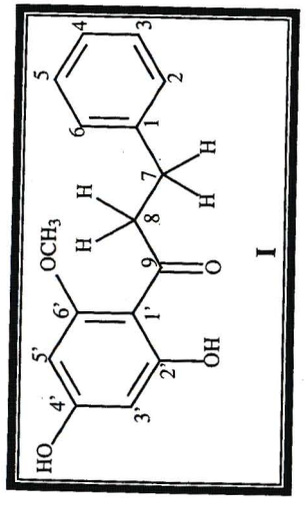
^{13}C NMR spectrum of compound **I**, uvangoletin

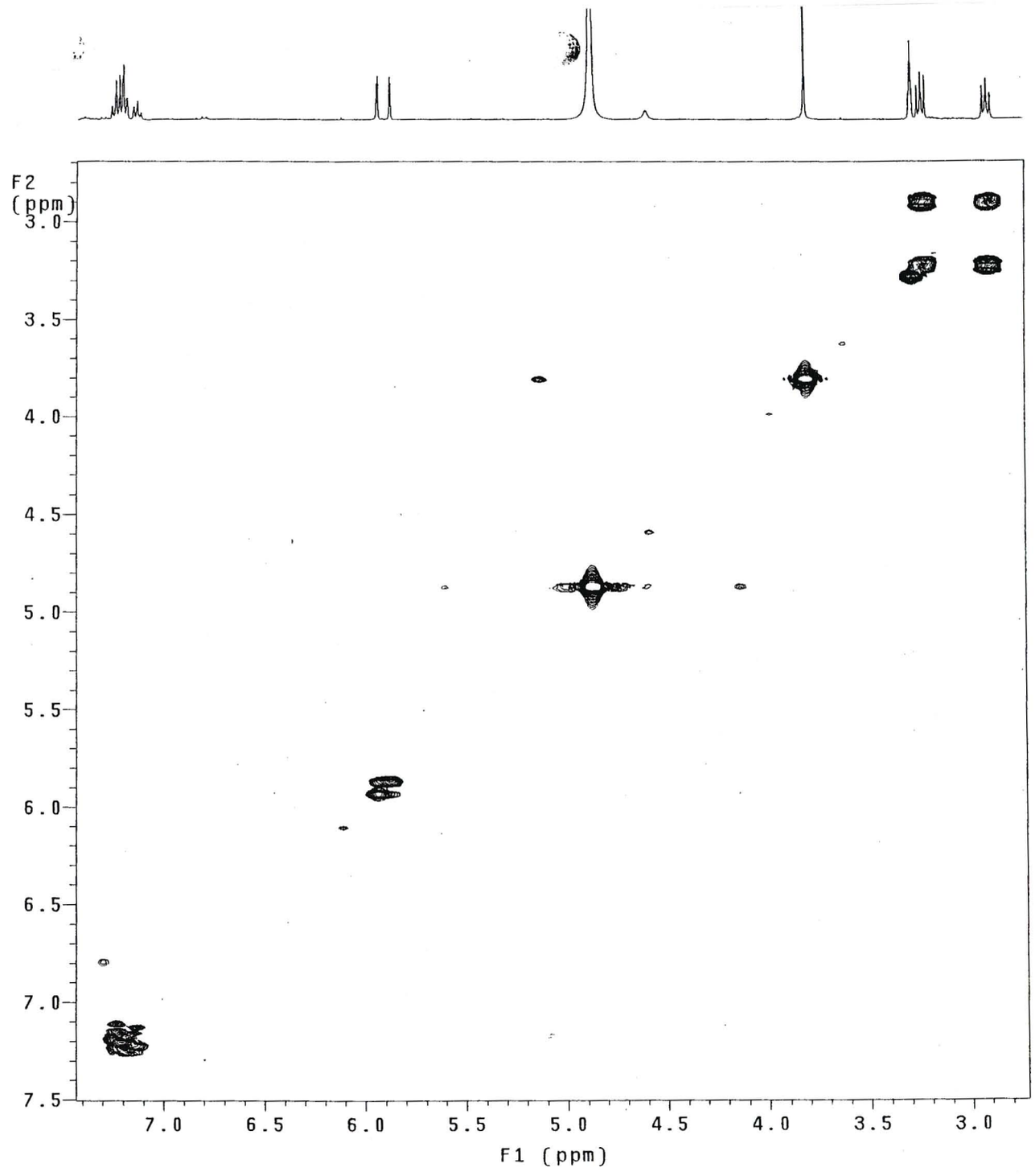
cyd58a.ced58a in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.167 sec
Width 3071.7 Hz
2D Width 3071.7 Hz
16 repetitions
256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.083 sec
F1 DATA PROCESSING
Sq. sine bell 0.042 sec
FT size 1024 x 1024
Total time 1 hr, 23 min, 13 sec

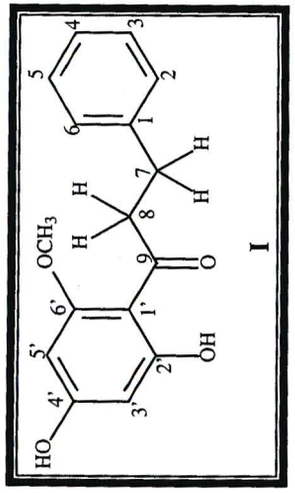


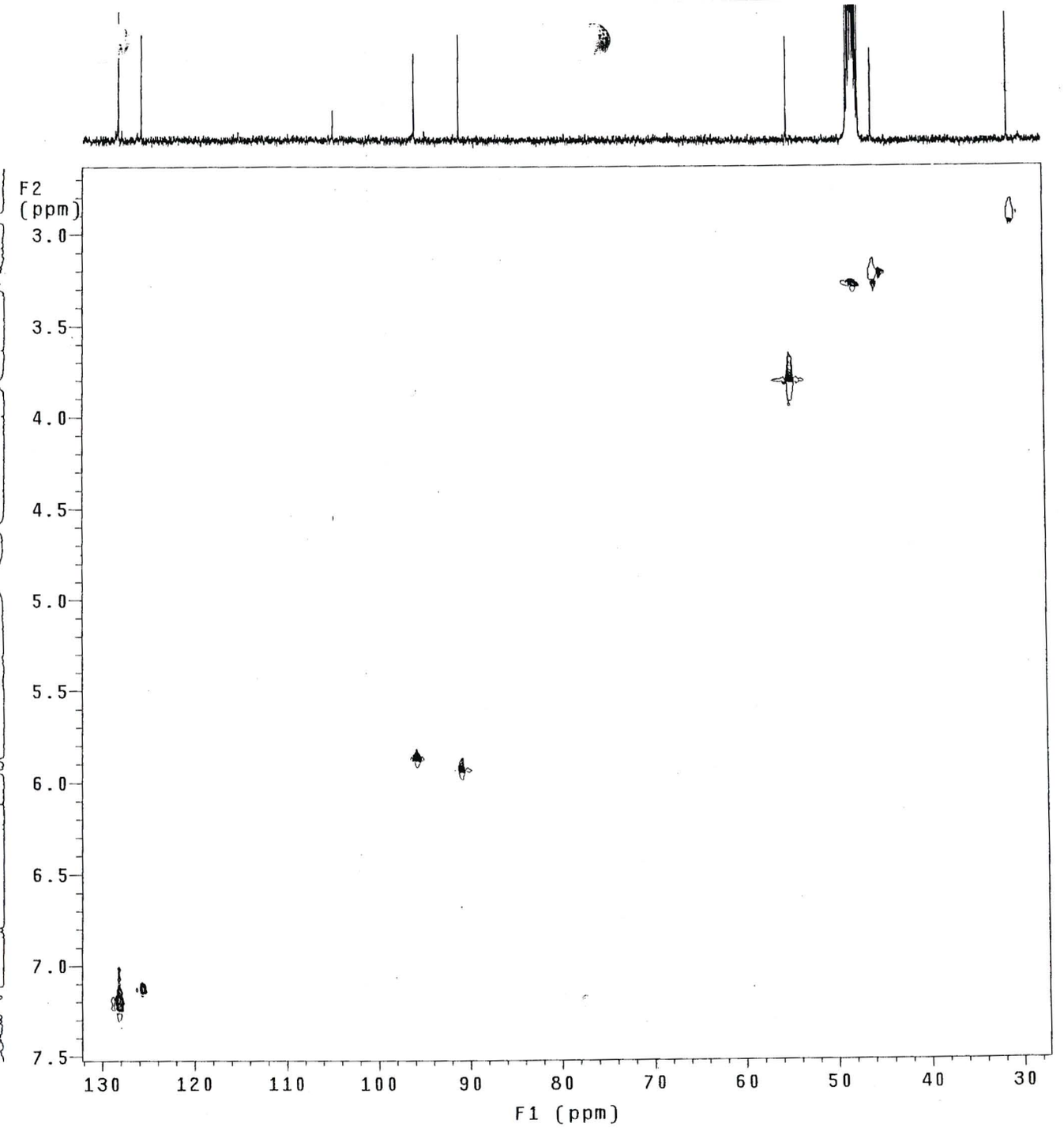


COSY spectrum of compound **I**, uvangoletin

nvd58a.ced58a in cd3od
Gradient HSQC expt.
with multiplicity editing
probe=5mmASW

Pulse Sequence: ghsqc_da
Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"
PULSE SEQUENCE: ghsqc_da
Relax. delay 1.500 sec
Acq. time 0.198 sec
Width 3063.7 Hz
2D Width 14383.3 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DECOUPLE C13, 100.5762034 MHz
Power 39 dB
on during acquisition
off during delay
GARP-1 modulated
DATA PROCESSING
Sq. sine bell 0.199 sec
Shifted by -0.198 sec
F1 DATA PROCESSING
Sq. sine bell 0.014 sec
Shifted by -0.009 sec
FT size 1024 x 1024
Total time 1 hr, 59 min, 11 sec





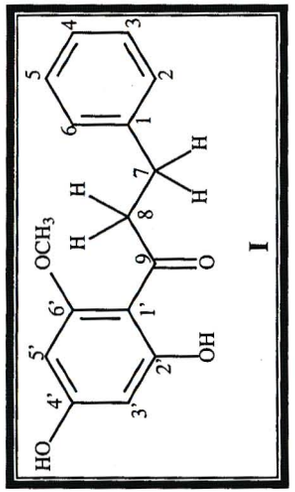
HSQC spectrum of compound **I,** uvangoletin

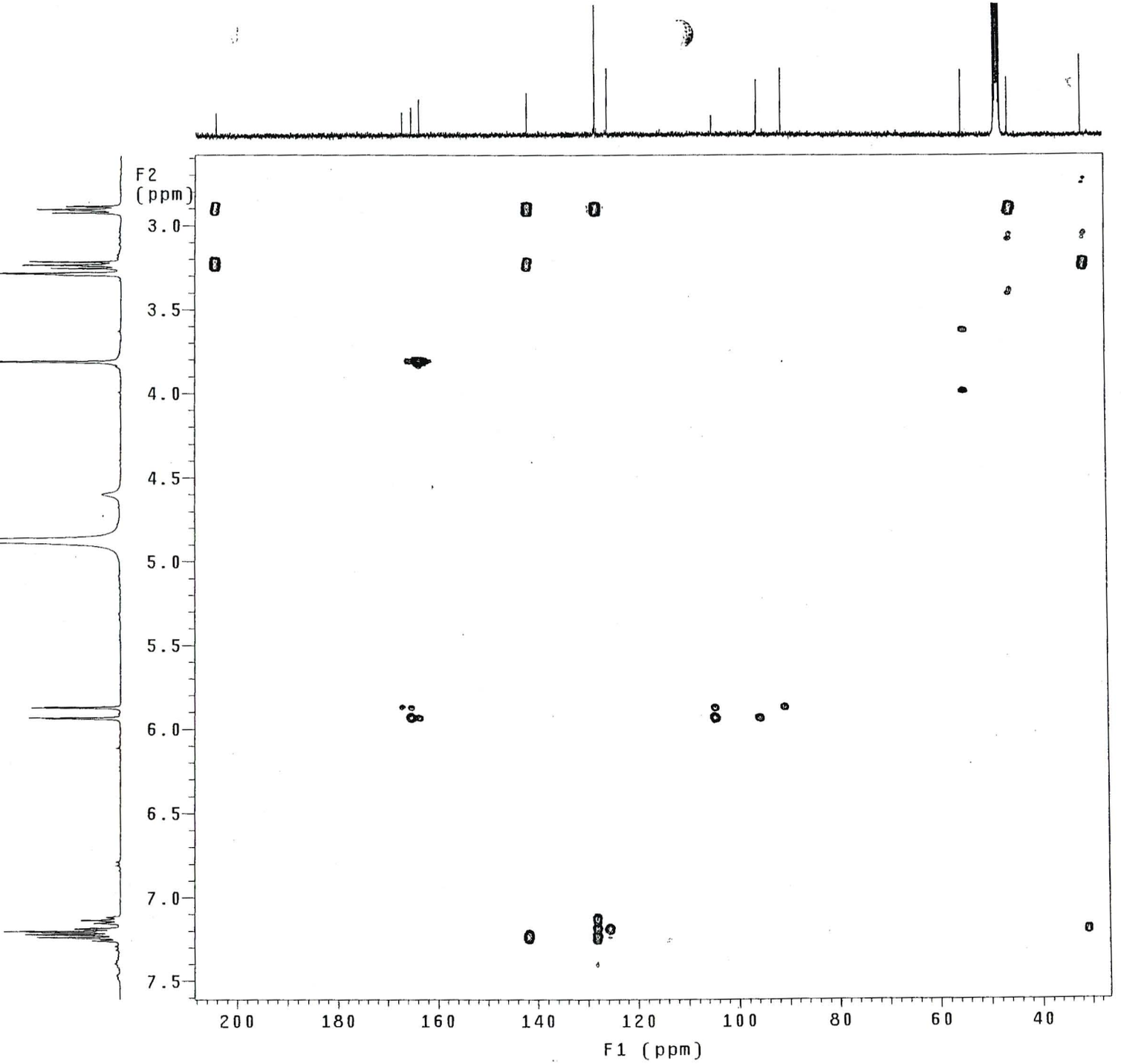
HBd58a.ced58a in cd3od
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da

Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"

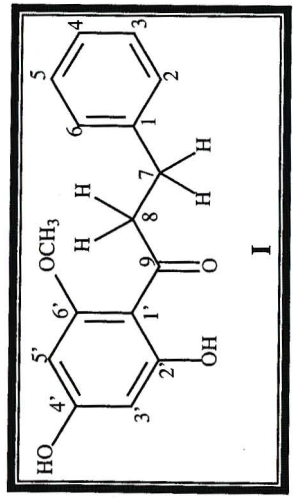
PULSE SEQUENCE: ghmqc_da
Relax. delay 1.500 sec
Acq. time 0.168 sec
Width 3047.9 Hz
2D Width 20639.8 Hz
32 repetitions
256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Sine bell 0.084 sec
F1 DATA PROCESSING
Sine bell 0.006 sec
FT size 1024 x 1024
Total time 4 hr, 1 min, 4 sec

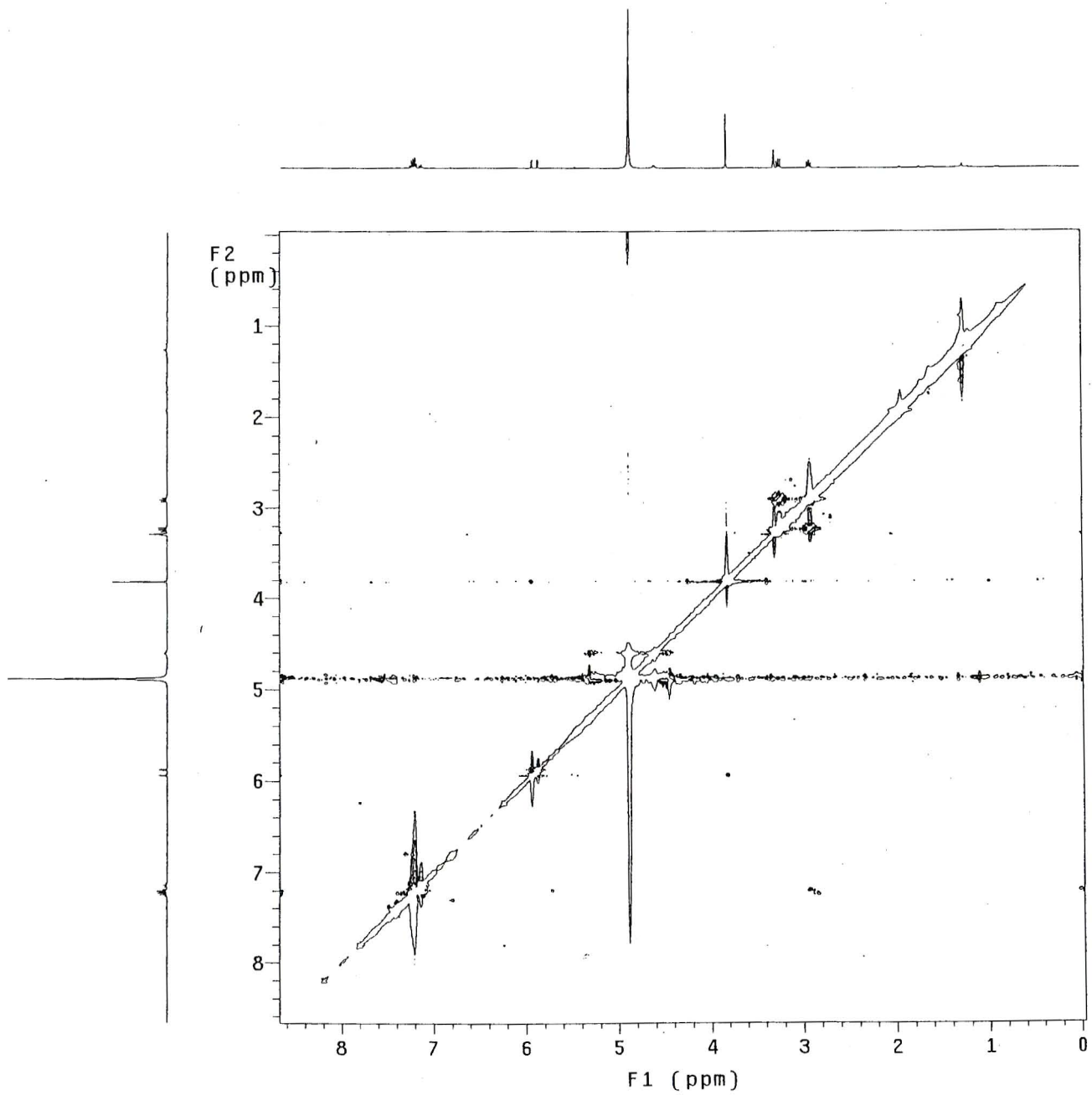




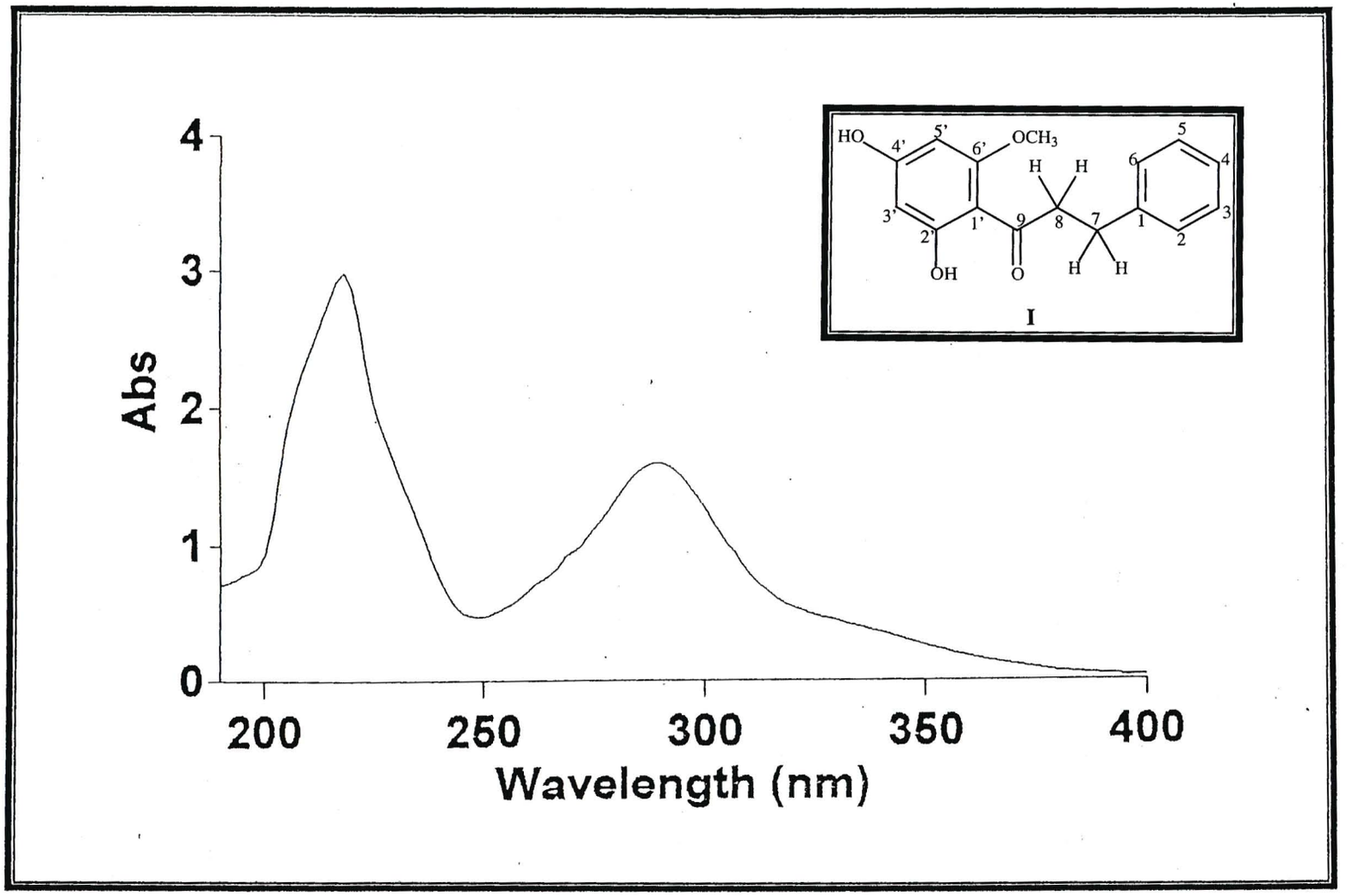
HMBC spectrum of compound **I,** uvangoletin

Pulse Sequence: NOESY
Solvent: CD3OD
Ambient temperature
INOVA-400 "undnmr400"
PULSE SEQUENCE: NOESY
Relax. delay 2.000 sec
Mixing 0.100 sec
Acq. time 0.147 sec
Width 3480.7 Hz
2D Width 3480.7 Hz
8 repetitions
2 x 256 increments
OBSERVE H1, 399.9502545 MHz
DATA PROCESSING
Gauss apodization 0.068 sec
F1 DATA PROCESSING
Gauss apodization 0.068 sec
FT size 2048 x 2048
Total time 2 hr, 38 min, 2 sec

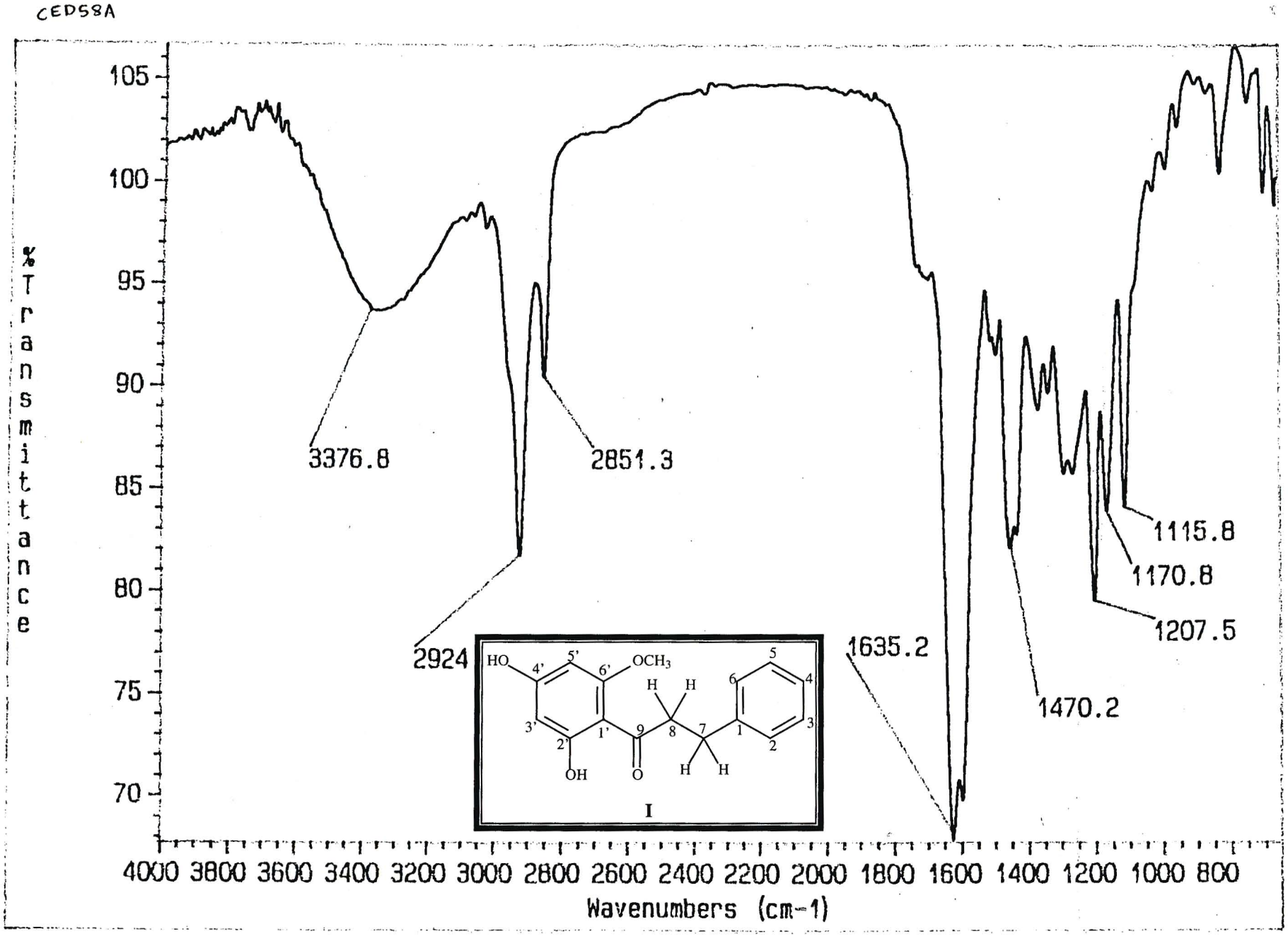




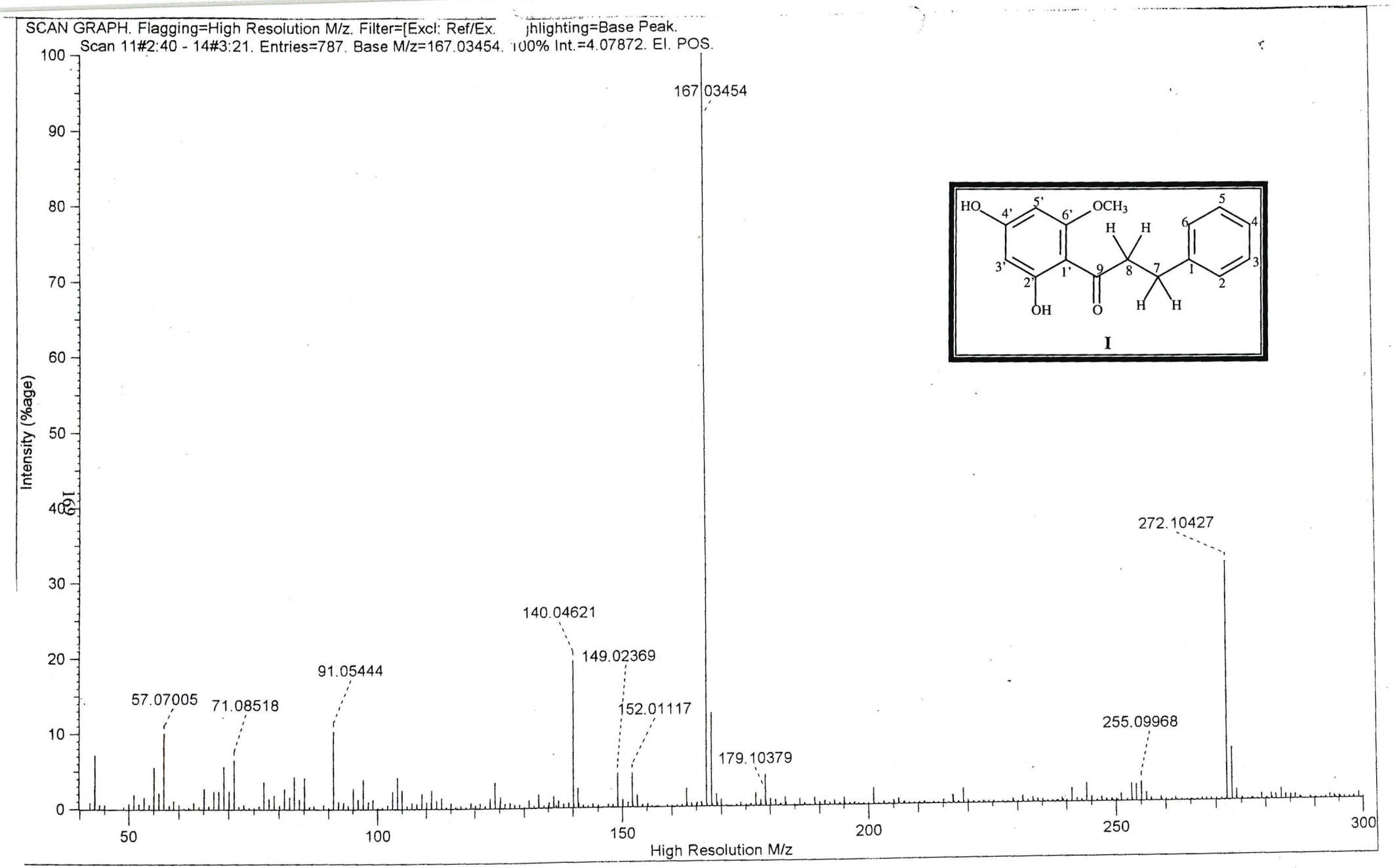
NOESY spectrum of compound **I**, uvangoletin



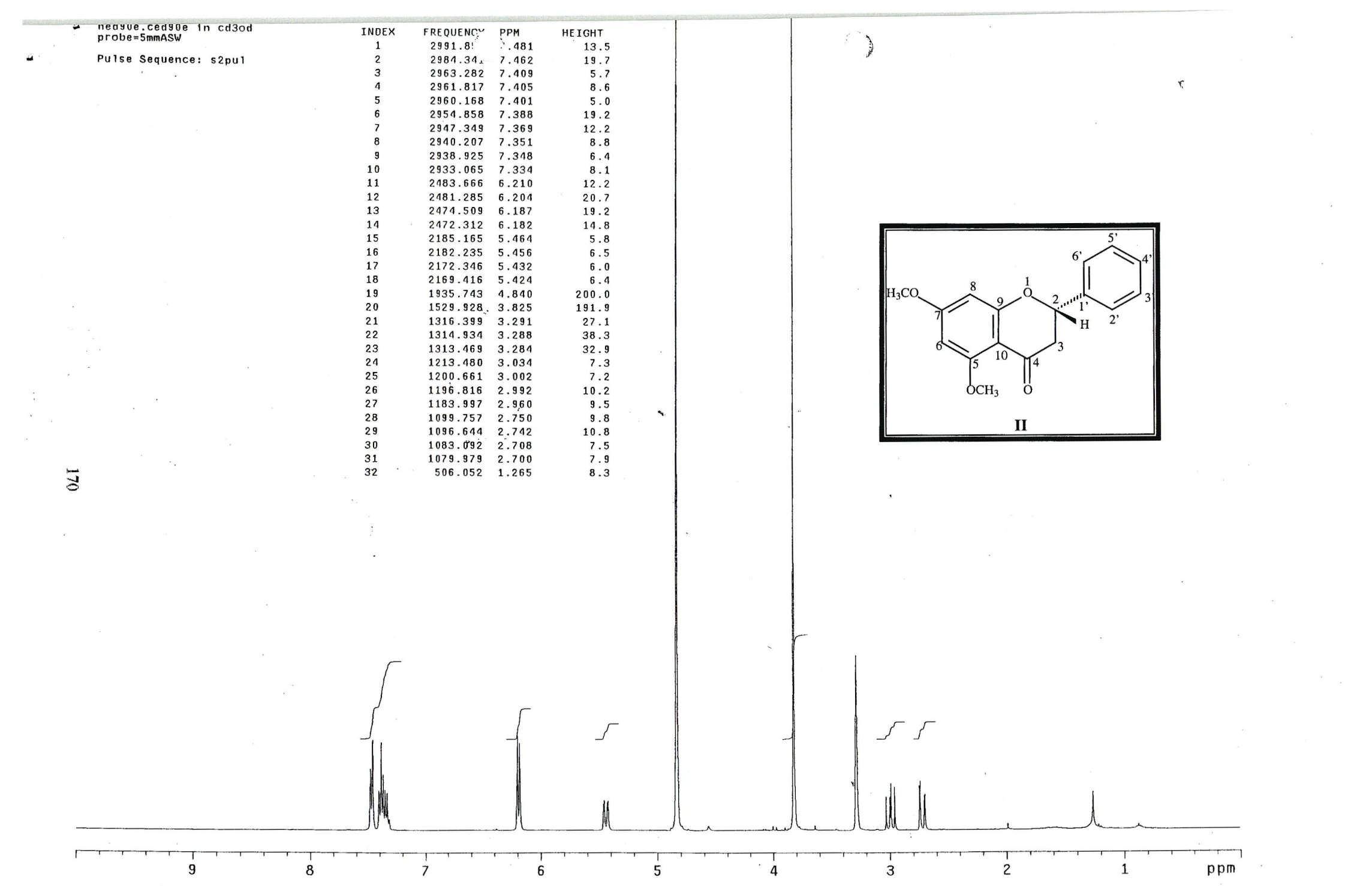
Ultra violet spectrum of compound **I**, uvangoletin



Infrared spectrum of compound **I**, uvangoletin



Mass spectrum of compound **I**, uvangoletin

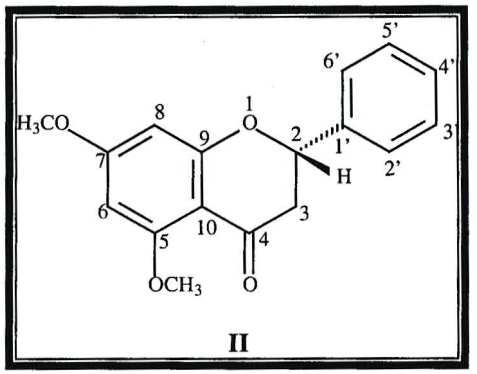


^{1}H NMR spectrum of compound **II,** 5,7-dimethoxypinocembrin

ced90e.ced90e in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	19159.1	190.510	8.7
2	16793.191	166.984	7.9
3	16630.679	165.368	6.6
4	16339.228	162.470	6.5
5	13999.221	139.202	7.3
6	12915.814	128.429	25.8
7	12903.606	128.308	15.2
8	12674.718	126.032	31.3
9	10599.459	105.396	4.2
10	9432.889	93.797	14.4
11	9321.496	92.689	15.4
12	7962.660	79.177	13.9
13	5550.172	55.188	14.3
14	5545.594	55.143	17.4
15	4877.238	48.497	109.1
16	4855.876	48.285	341.6
17	4834.513	48.072	688.2
18	4813.150	47.860	800.0
19	4791.787	47.647	683.4
20	4770.424	47.435	346.3
21	4749.061	47.223	118.8
22	4553.742	45.280	14.7

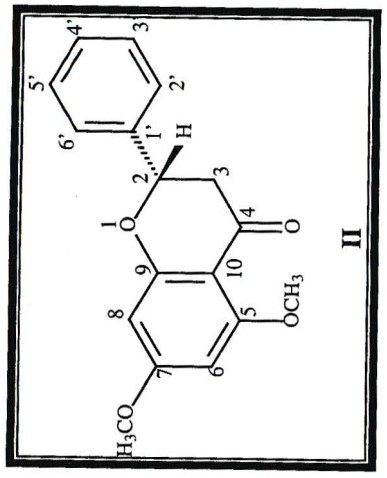


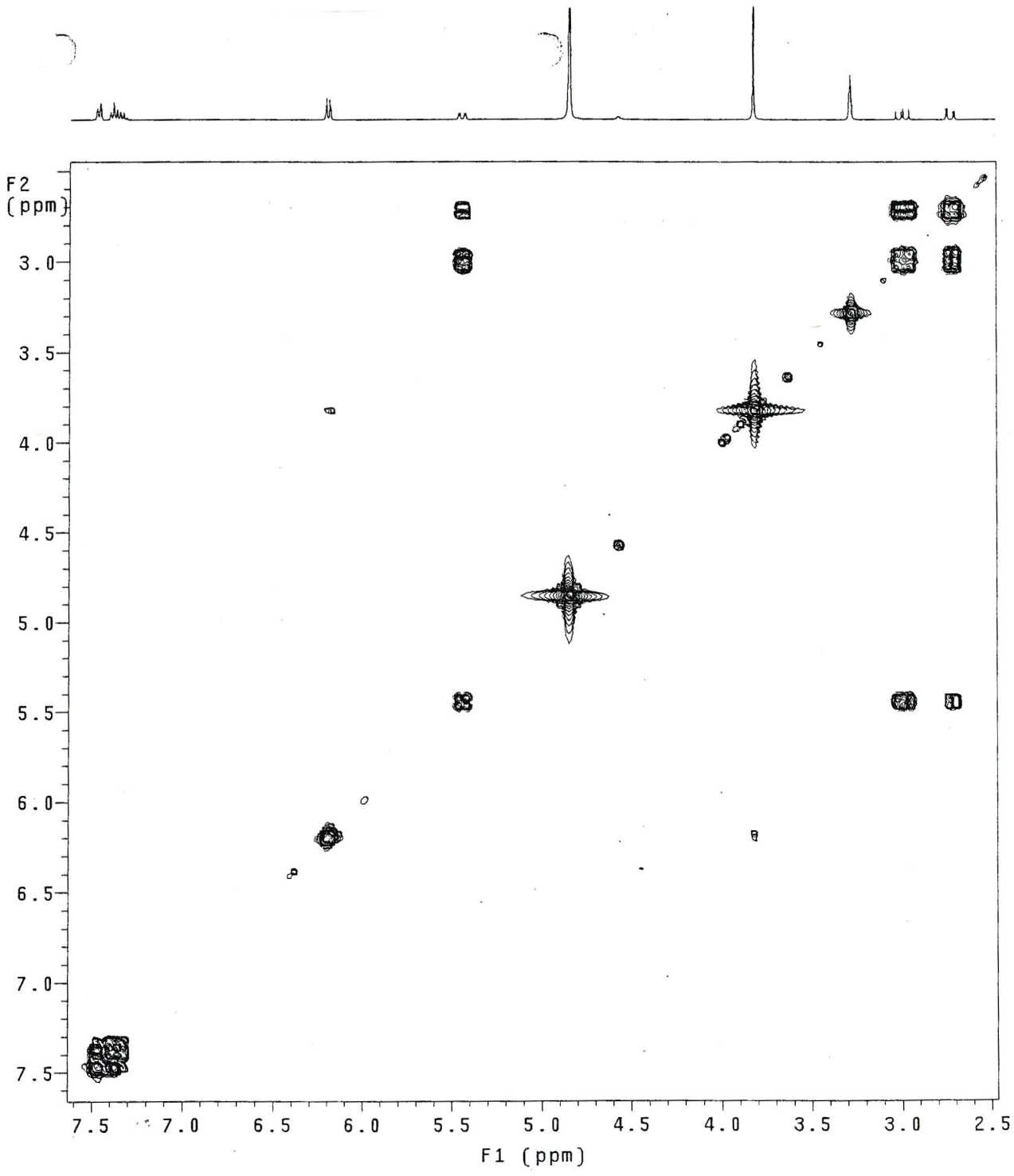
II

^{13}C NMR spectrum of compound **II,** 5,7-dimethoxypinocembrin

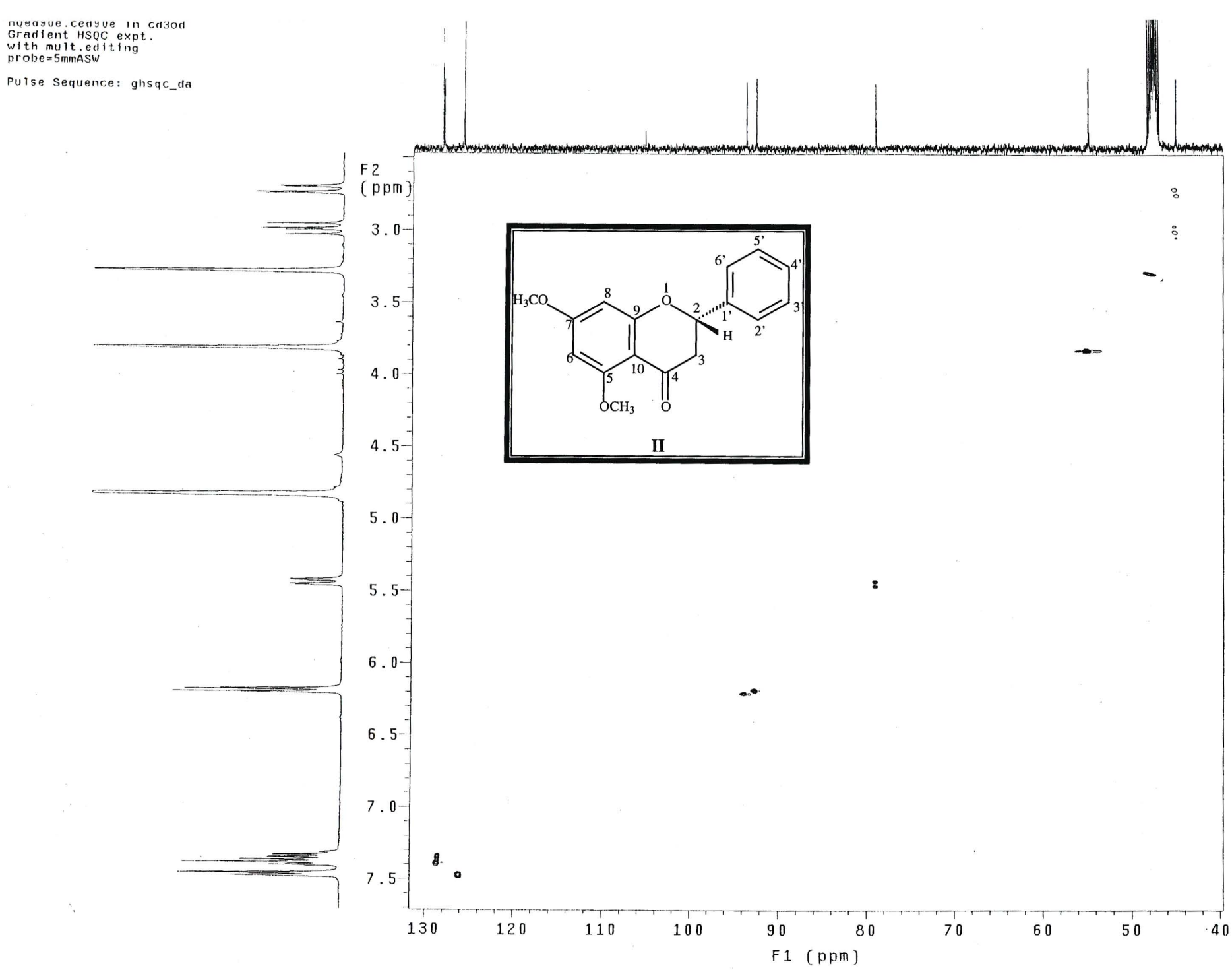
cyed90e.ced90e in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh





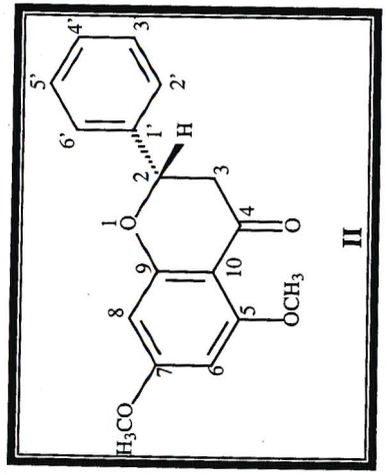
COSY spectrum of compound **II**, 5,7-dimethoxypinocembrin

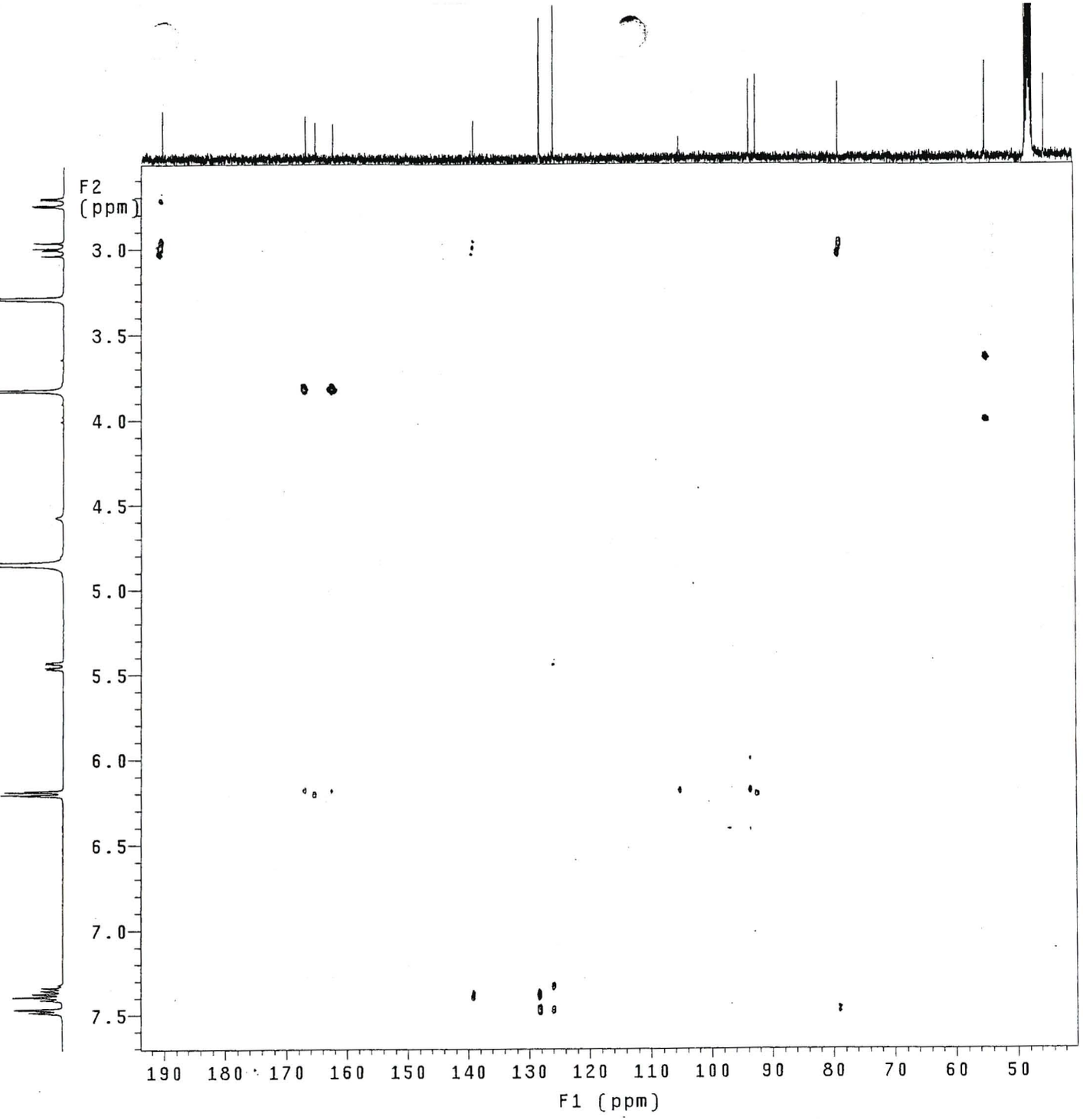


HSQC spectrum of compound **II**, 5,7-dimethoxypinocembrin

HBed90e.ced90e in cd3od
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da

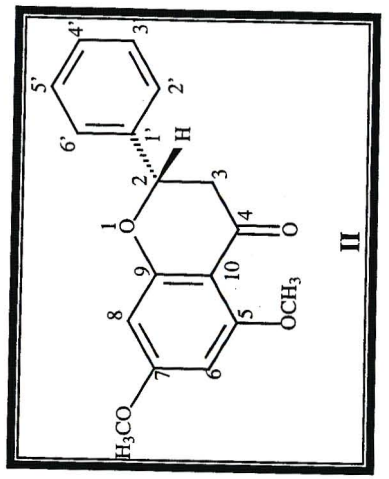




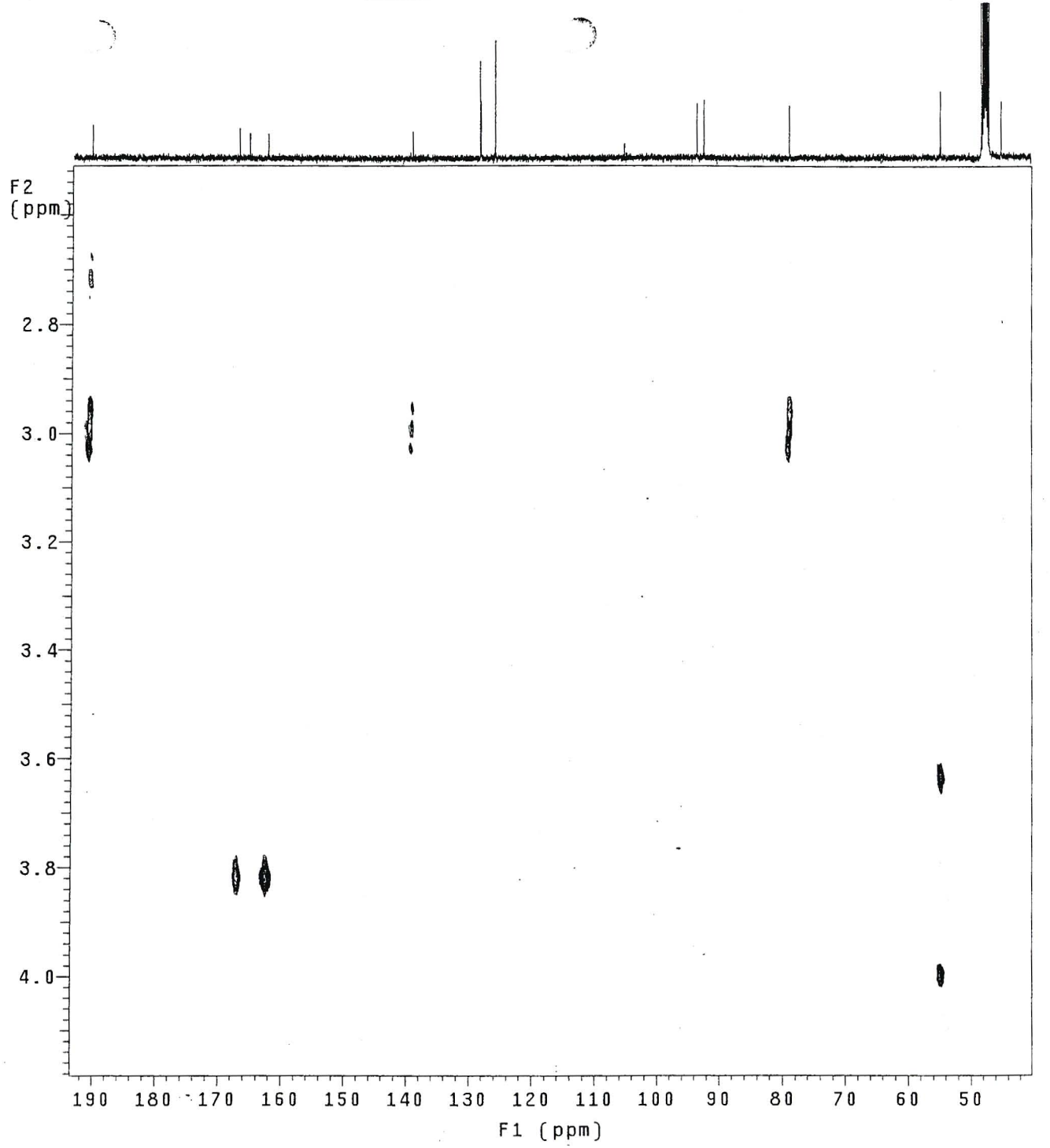
HMBC spectrum of compound **II,** 5,7-dimethoxypinocembrin

HBed90e.ced90e in cd3od
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da



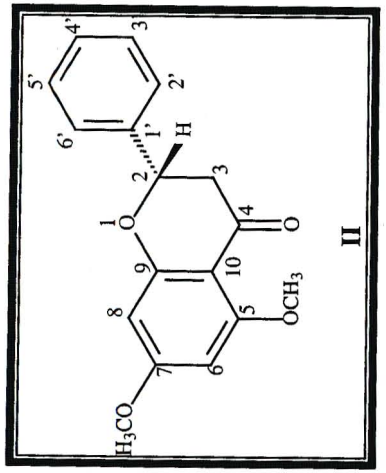
175

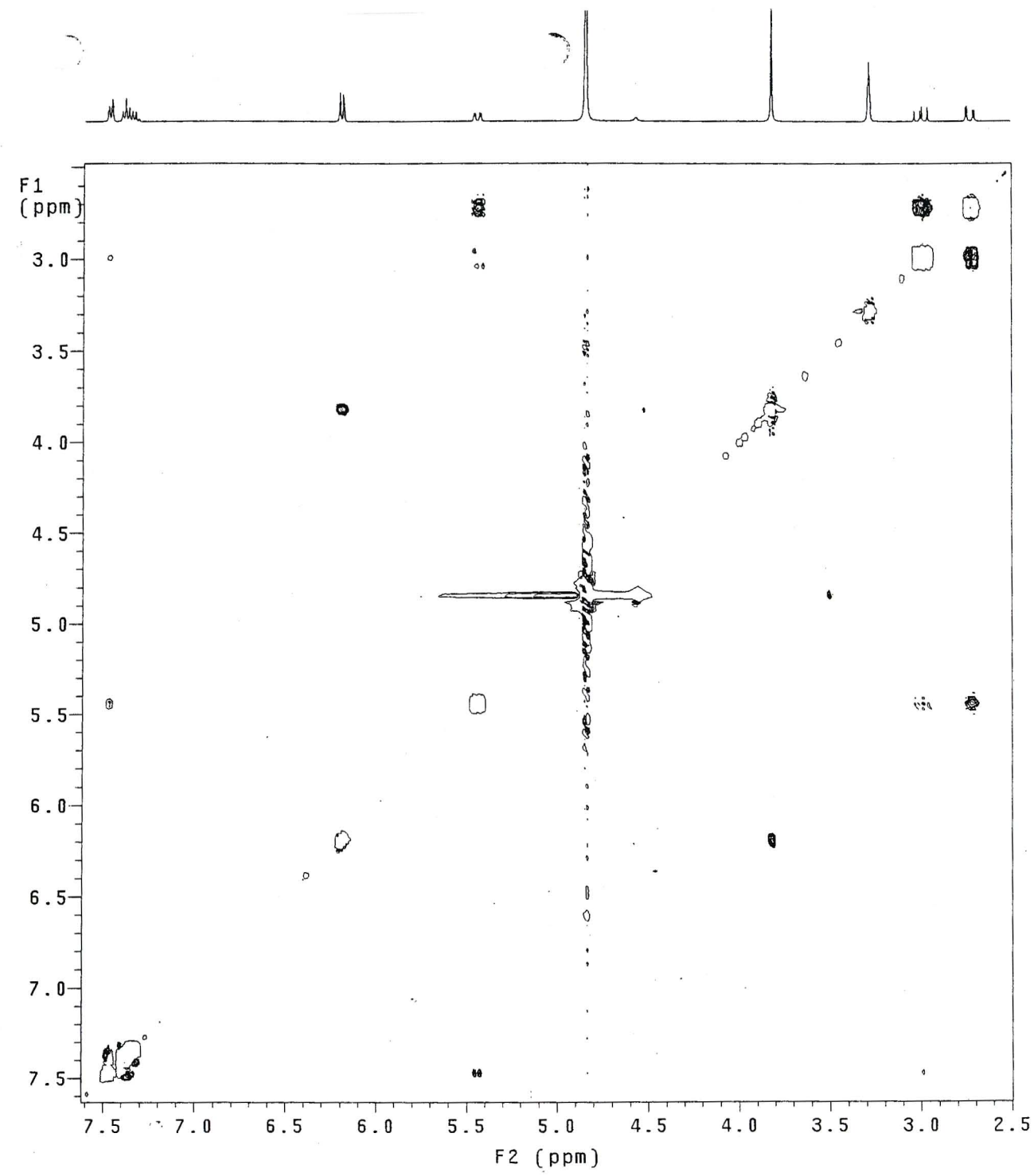


Expanded HMBC spectrum of compound **II,** 5,7-dimethoxypinocembrin

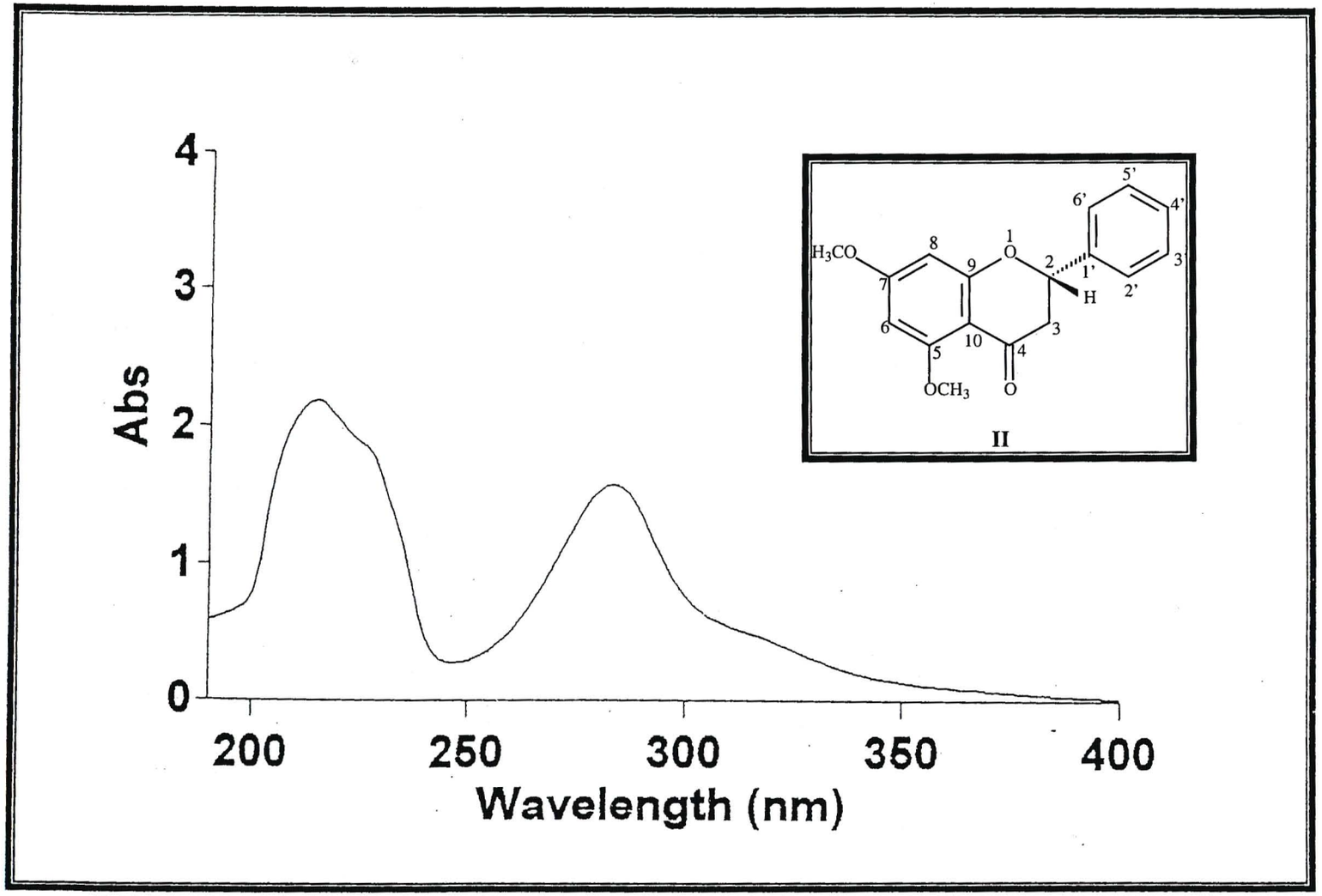
NOed90e.ced90e in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



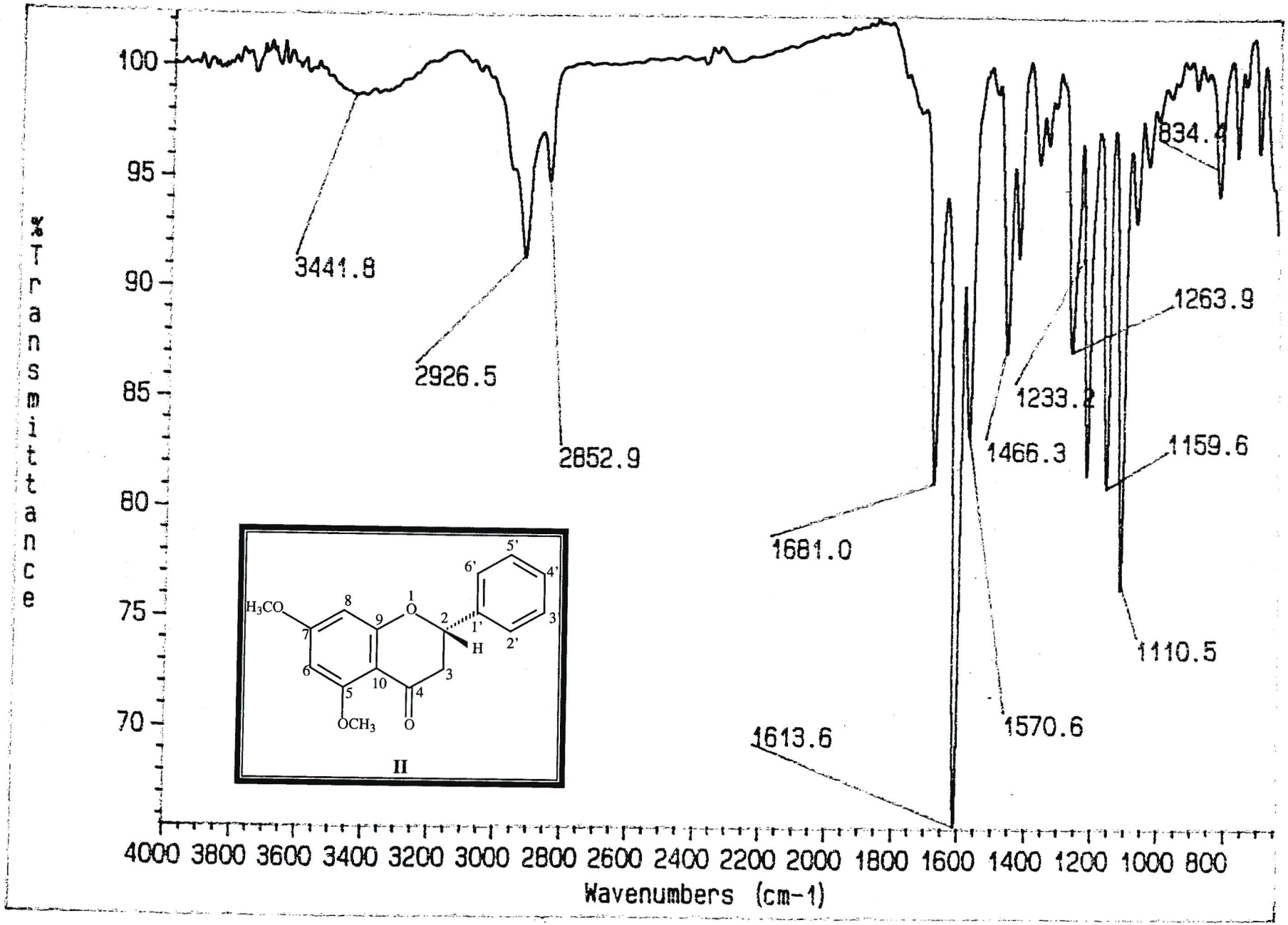


NOESY spectrum of compound **II,** 5,7-dimethoxypinocembrin

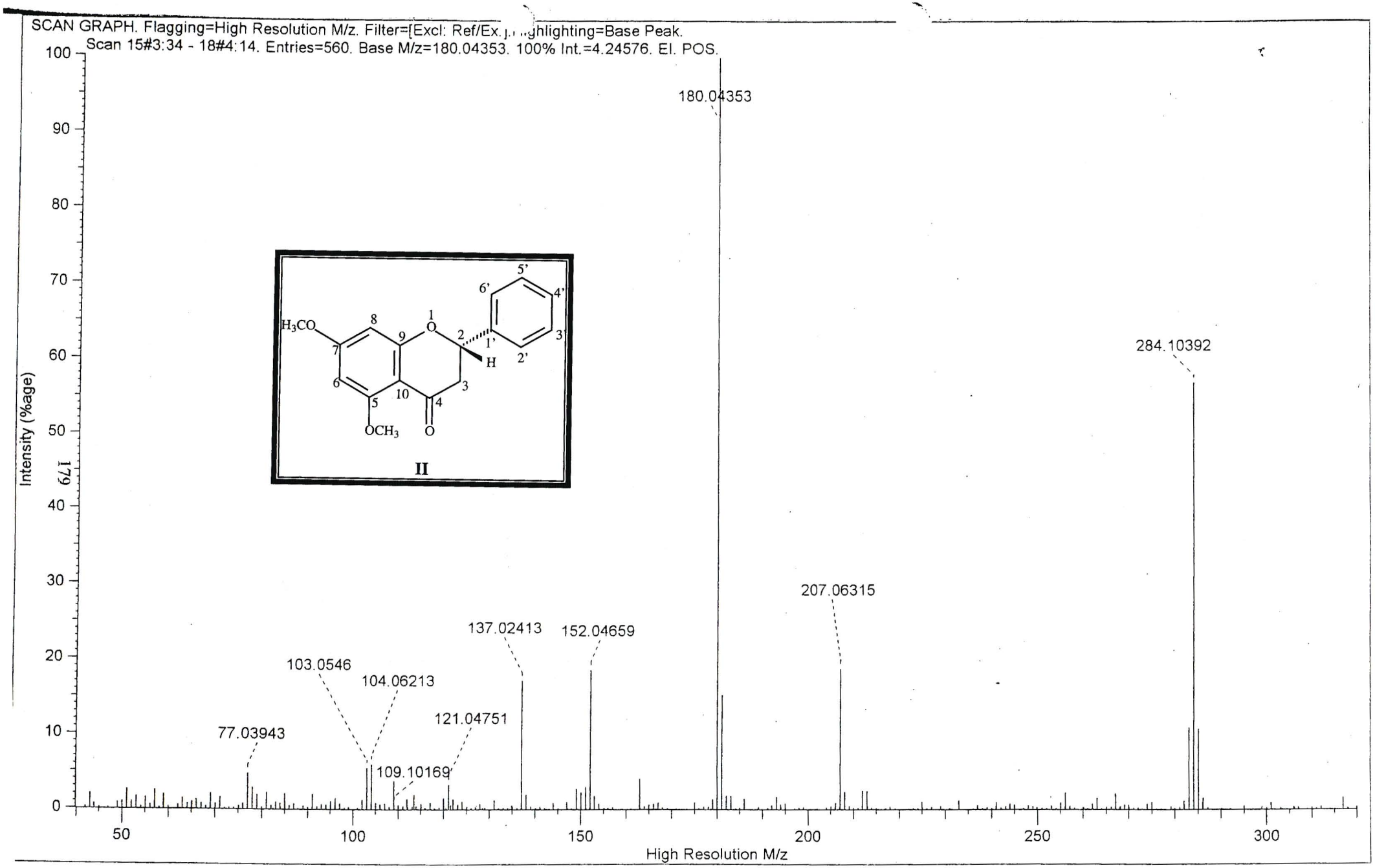


Ultra violet spectrum of compound **II,** 5,7-dimethoxypinocembrin

CED90E



Infrared spectrum of compound **II,** 5,7-dimethoxypinocembrin

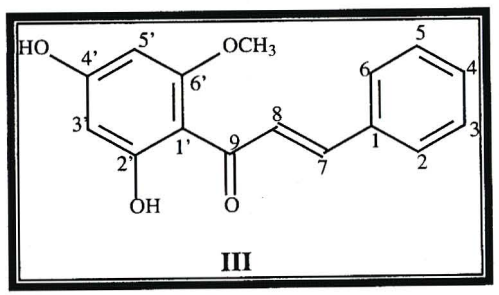


Mass spectrum of compound **II**, 5,7-dimethoxypinocembrin

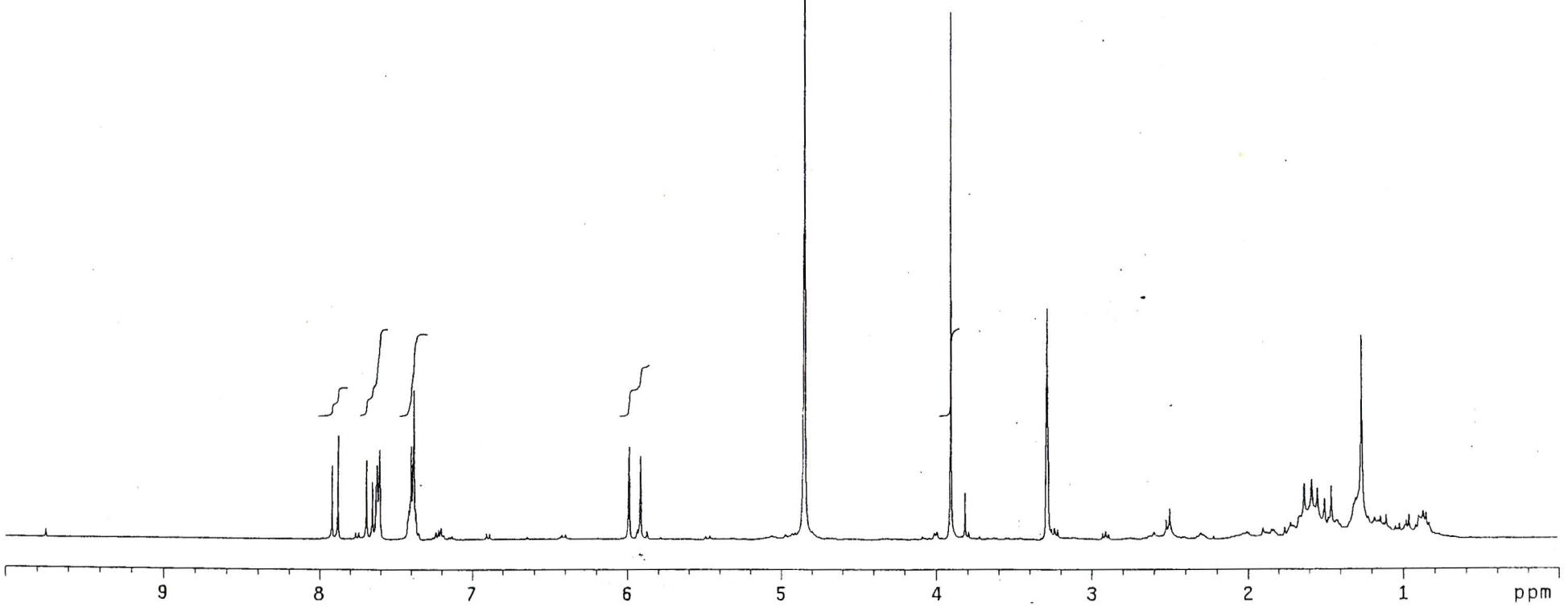
hed67c2.ced67c2 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQU	PPM	HEIGHT
1	3167	7.919	11.9
2	3151.355	7.879	16.8
3	3076.822	7.693	12.9
4	3061.073	7.654	9.4
5	3050.634	7.628	12.0
6	3048.803	7.623	12.0
7	3042.760	7.608	14.5
8	3041.478	7.605	12.7
9	2960.168	7.401	15.1
10	2952.660	7.383	24.1
11	2397.412	5.994	13.4
12	2395.214	5.989	15.0
13	2367.379	5.919	13.6
14	2365.181	5.914	11.7
15	1939.955	4.850	200.0
16	1563.074	3.908	85.6
17	1318.047	3.296	15.2
18	1316.399	3.291	27.6
19	1314.934	3.288	37.4
20	1313.286	3.284	28.2
21	1311.821	3.280	16.1
22	652.555	1.632	9.0
23	632.960	1.583	9.5
24	582.783	1.457	8.6
25	504.220	1.261	33.0



III

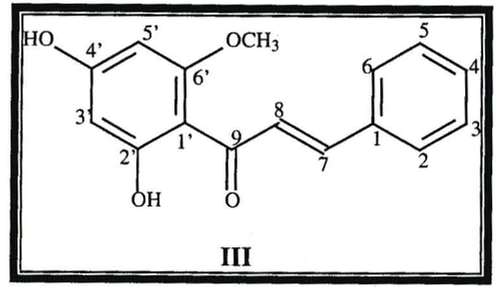


^{1}H NMR spectrum of compound **III**, cardamonin

ced67c2.ced67c2 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUE'	PPM	HEIGHT
1	19363.	192.543	6.8
2	16840.494	167.455	7.4
3	16642.124	165.482	8.0
4	16439.176	163.464	9.1
5	14242.606	141.622	17.9
6	13644.443	135.674	8.3
7	13063.829	129.901	18.1
8	12950.910	128.778	36.0
9	12877.666	128.050	36.8
10	12838.755	127.663	17.9
11	10598.696	105.389	4.5
12	9641.941	95.875	15.0
13	9179.585	91.278	16.5
14	5557.801	55.264	18.9
15	4878.001	48.505	177.4
16	4856.638	48.292	535.8
17	4835.276	48.080	1049.2
18	4813.913	47.867	1200.0
19	4792.550	47.655	1018.7
20	4771.187	47.443	517.0
21	4749.824	47.230	177.3
22	2983.565	29.667	4.3



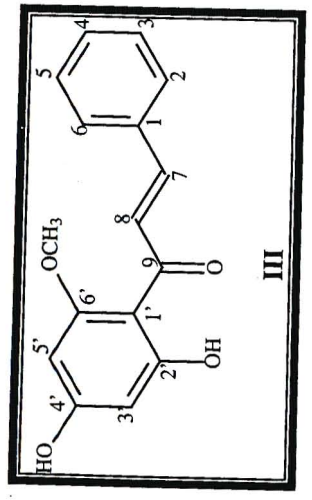
III

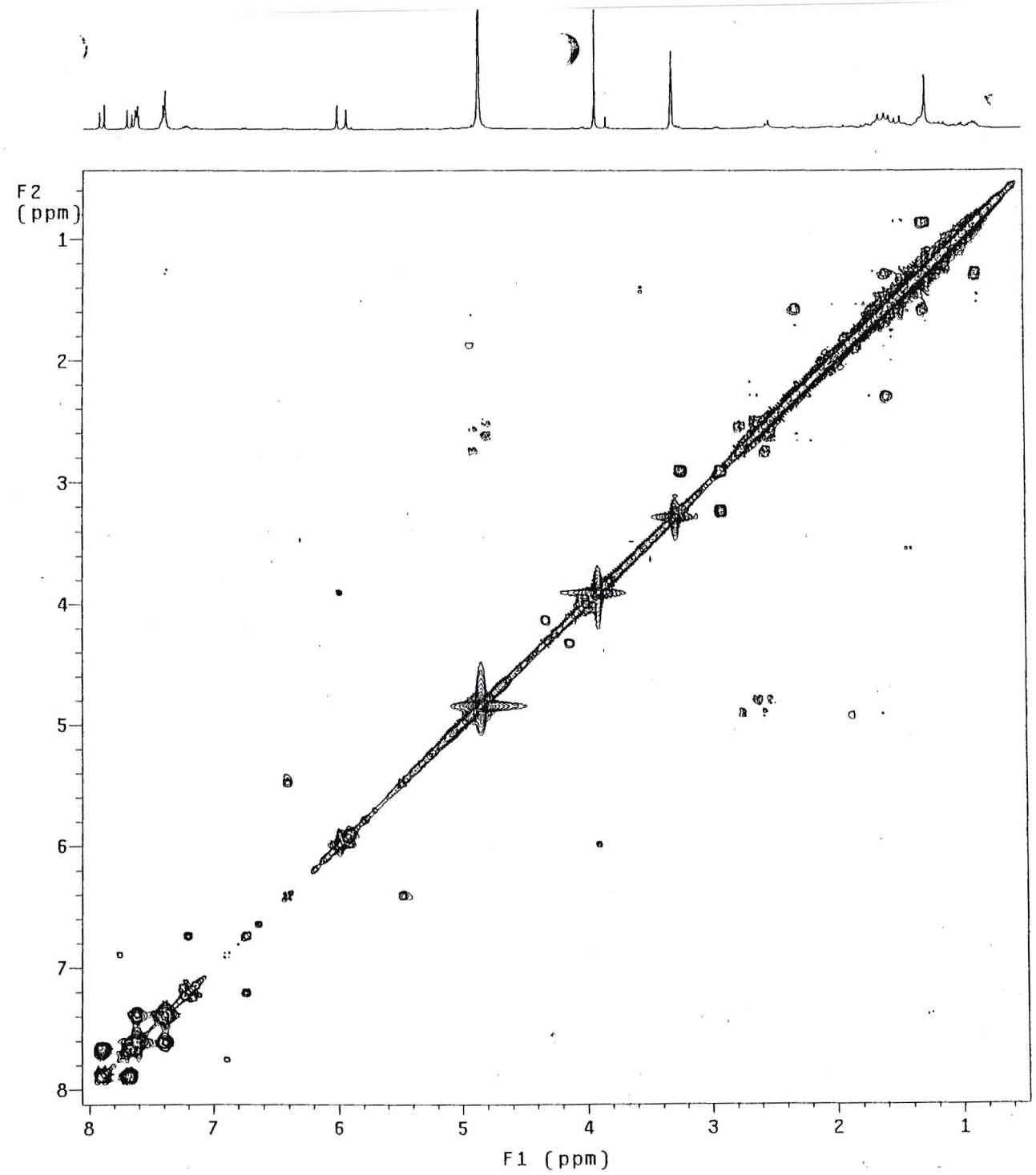
181

^{13}C NMR spectrum of compound **III**, cardamonin

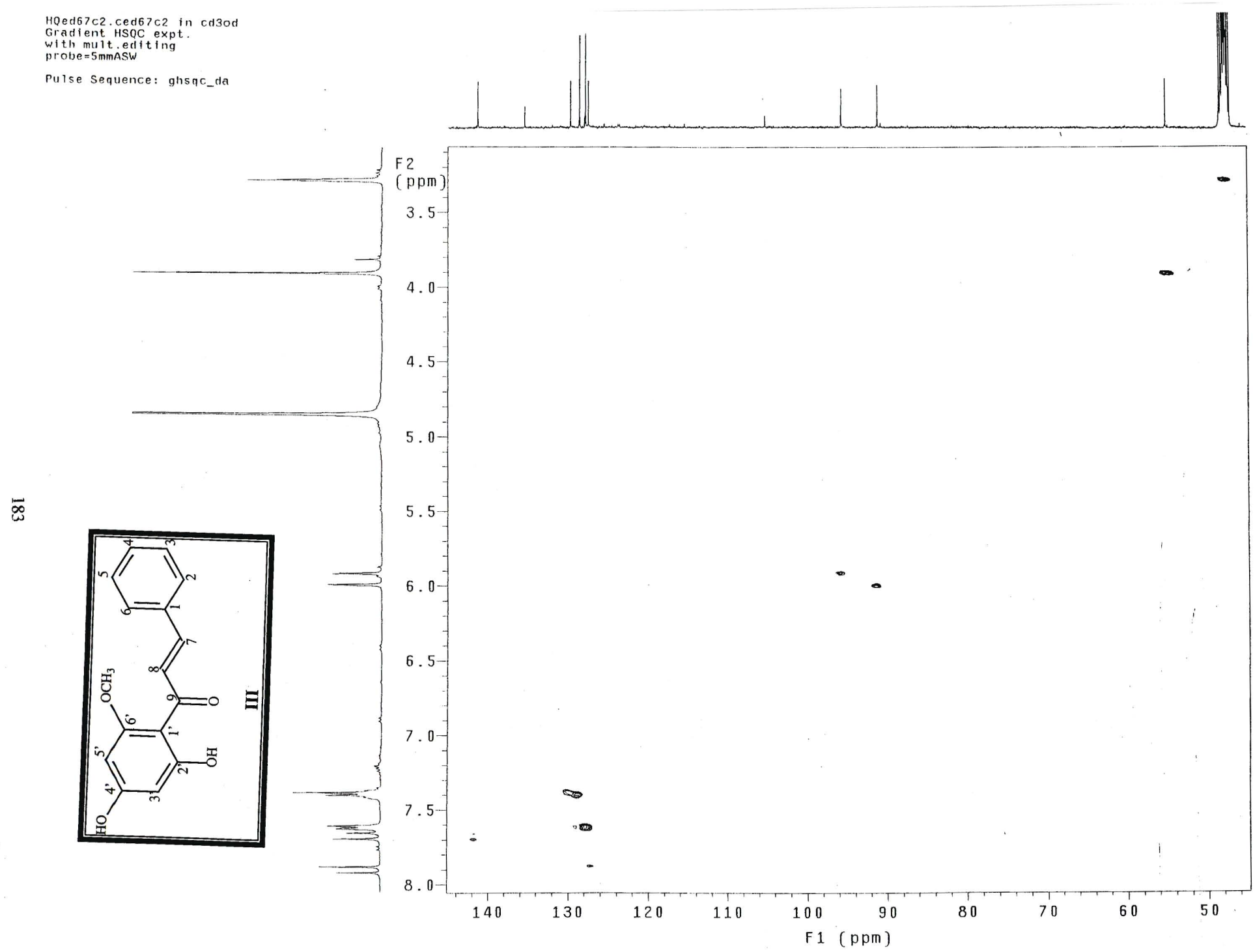
cyed67c2.ced67c2 in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

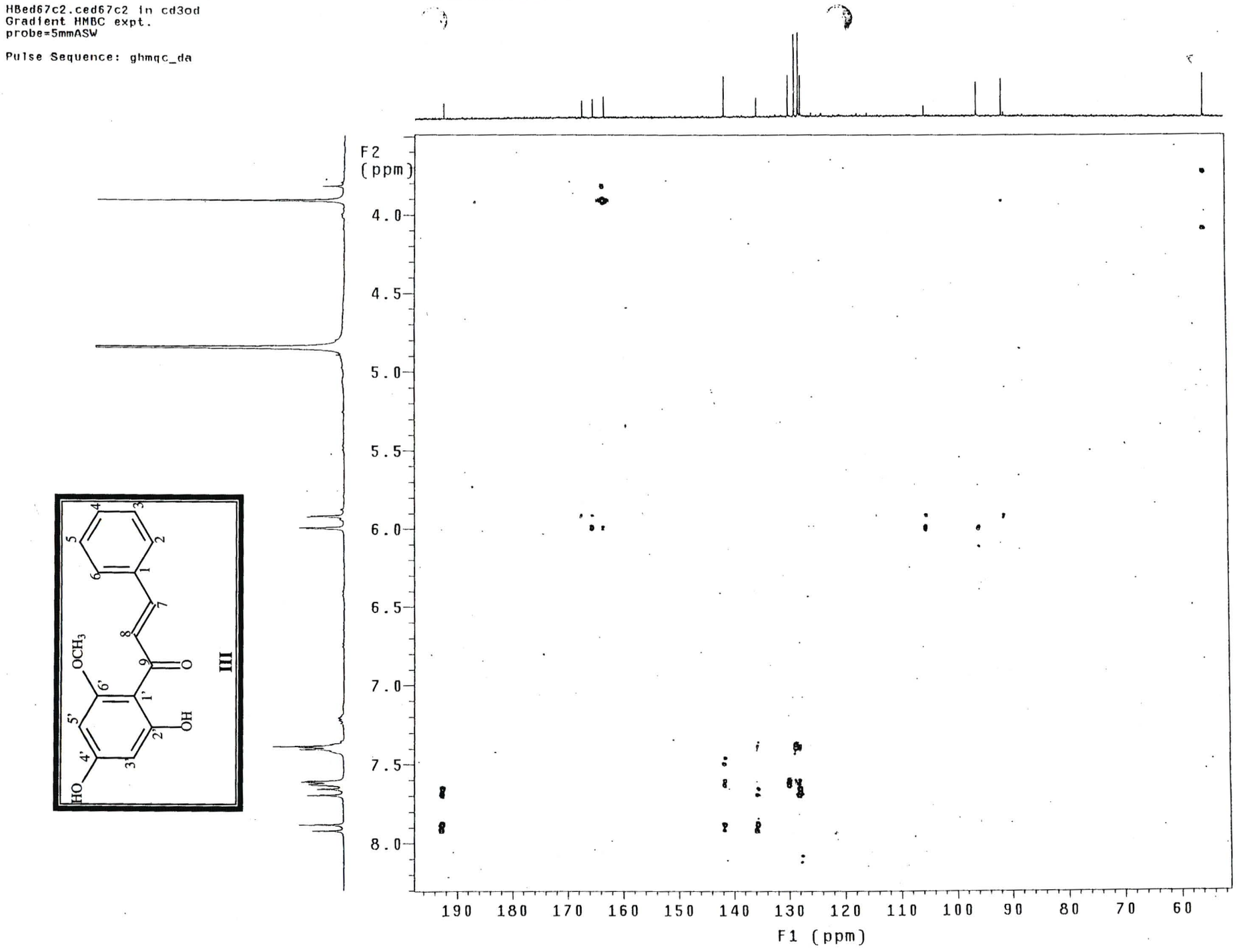




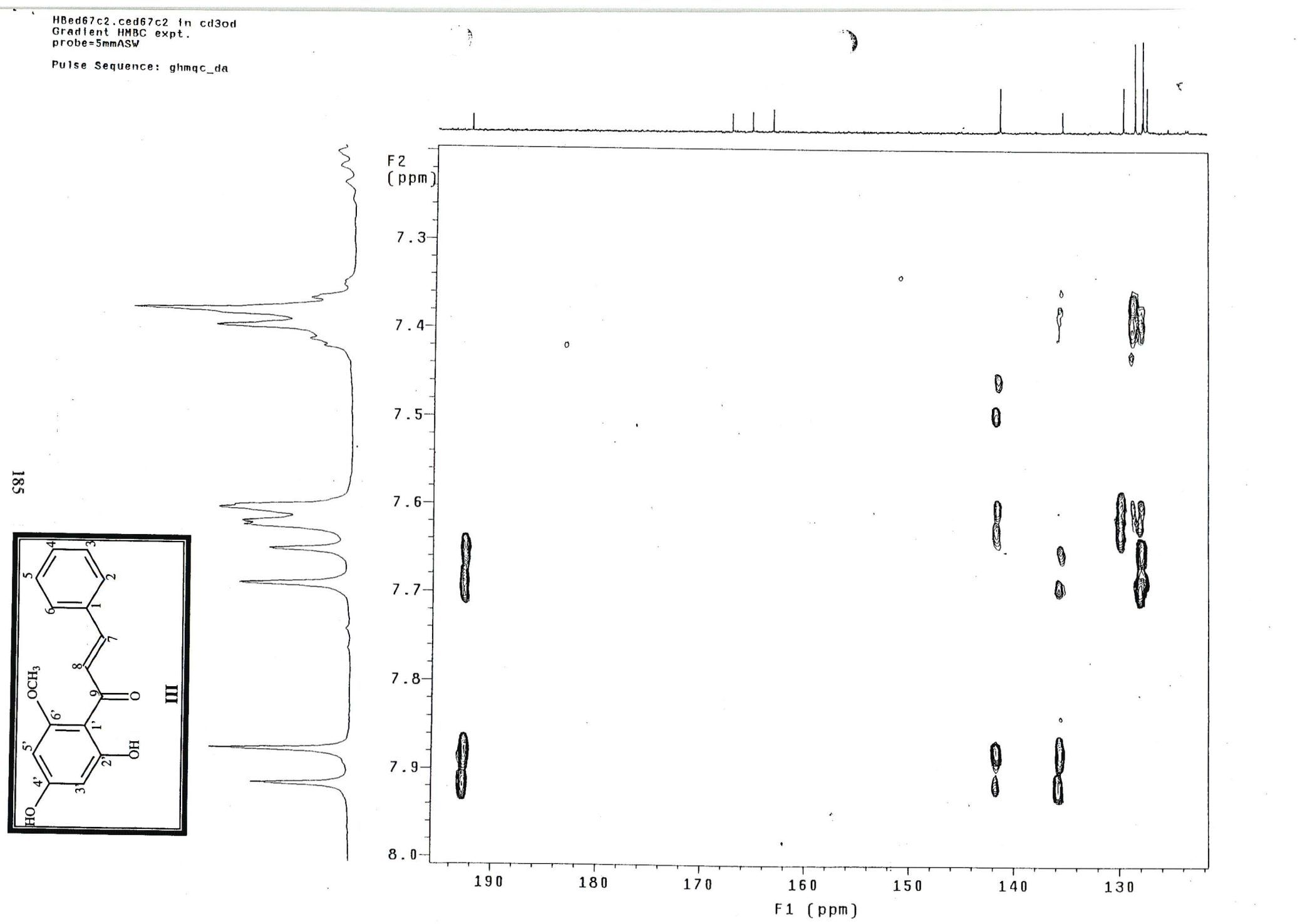
COSY spectrum of compound **III**, cardamonin



HSQC spectrum of compound **III**, cardamonin



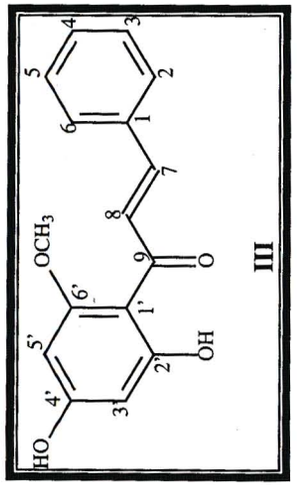
HMBC spectrum of compound **III**, cardamonin

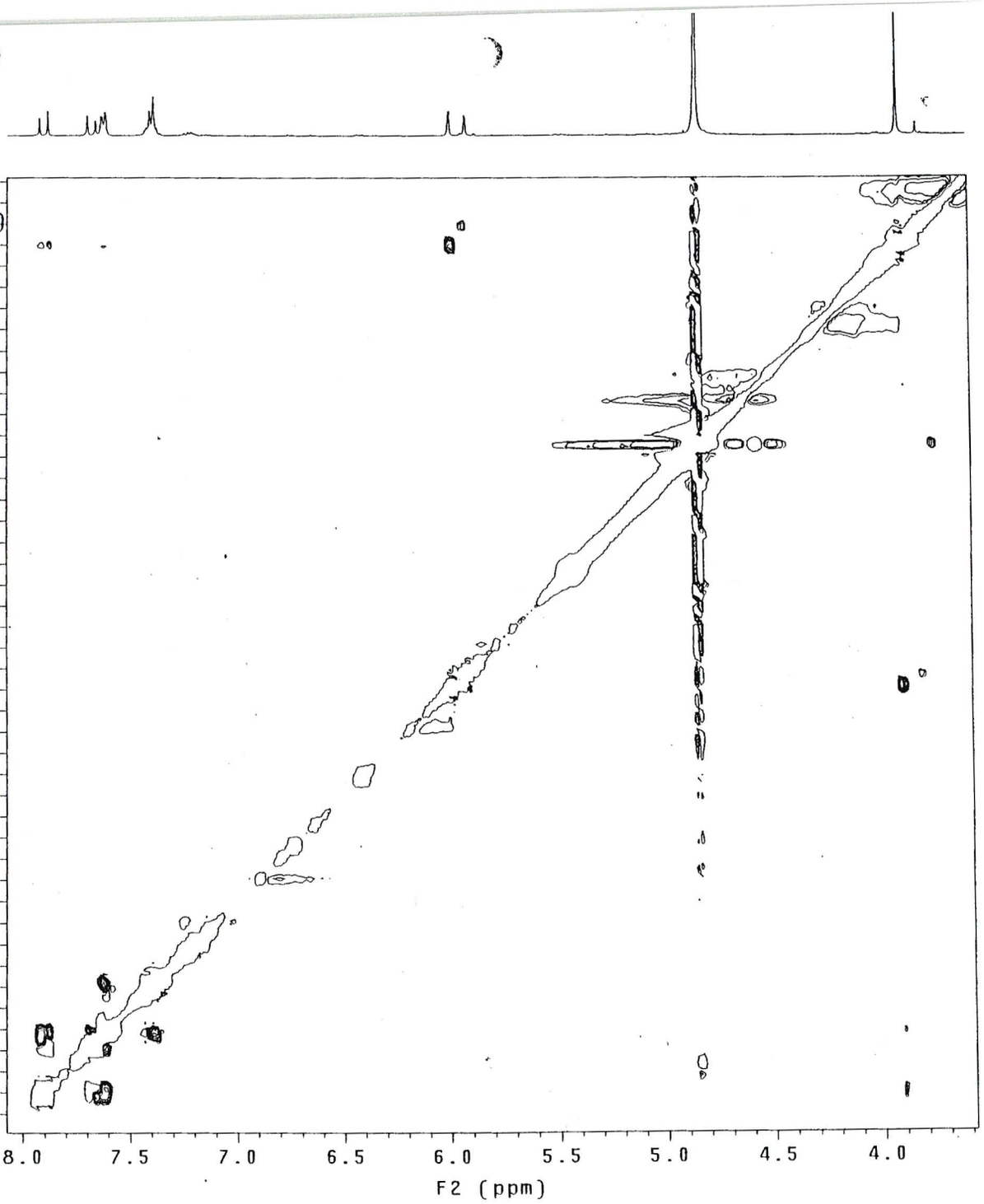


Expanded HMBC spectrum of compound **III**, cardamonin

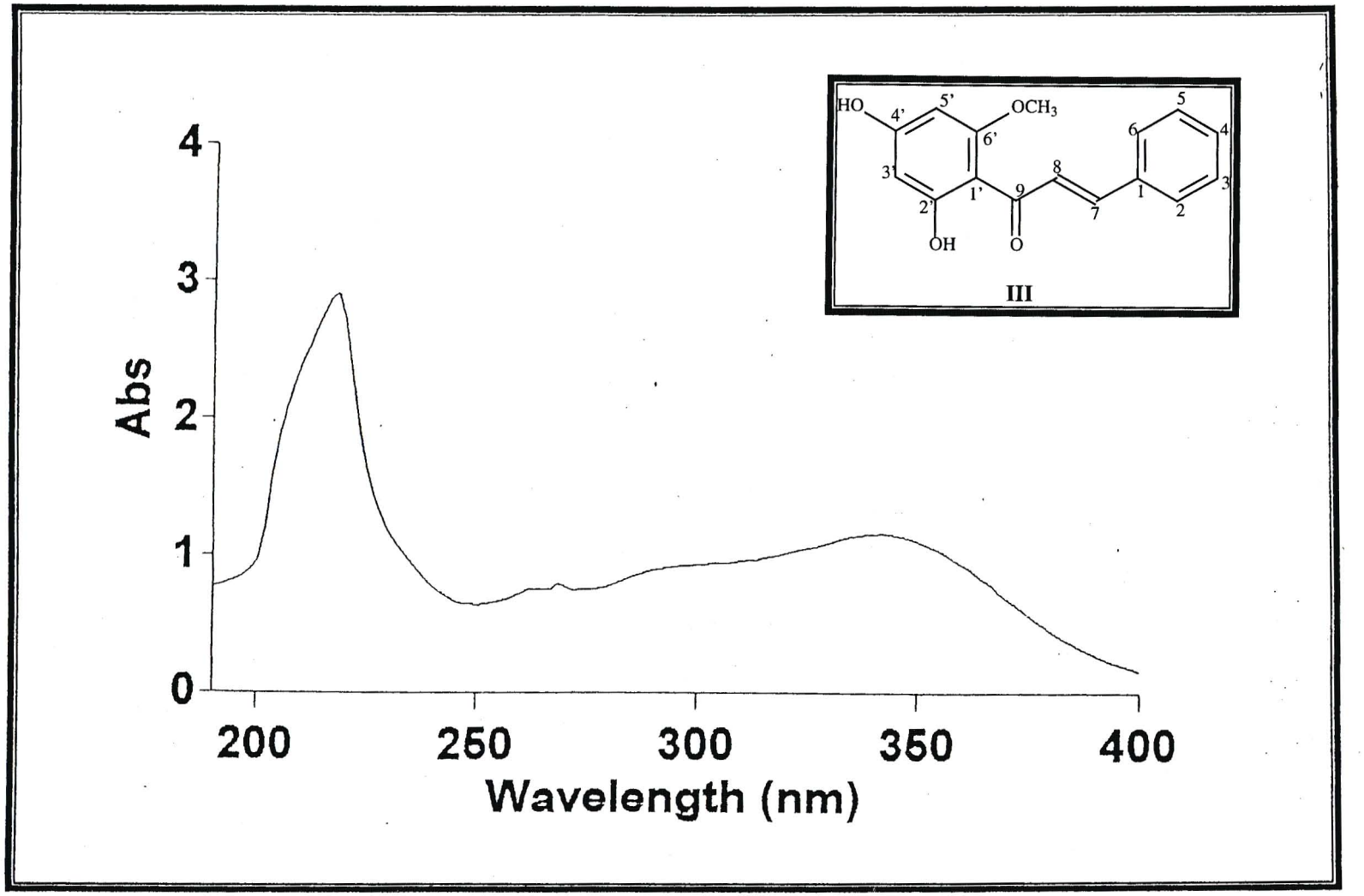
NOed67c2.ced67c2 in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

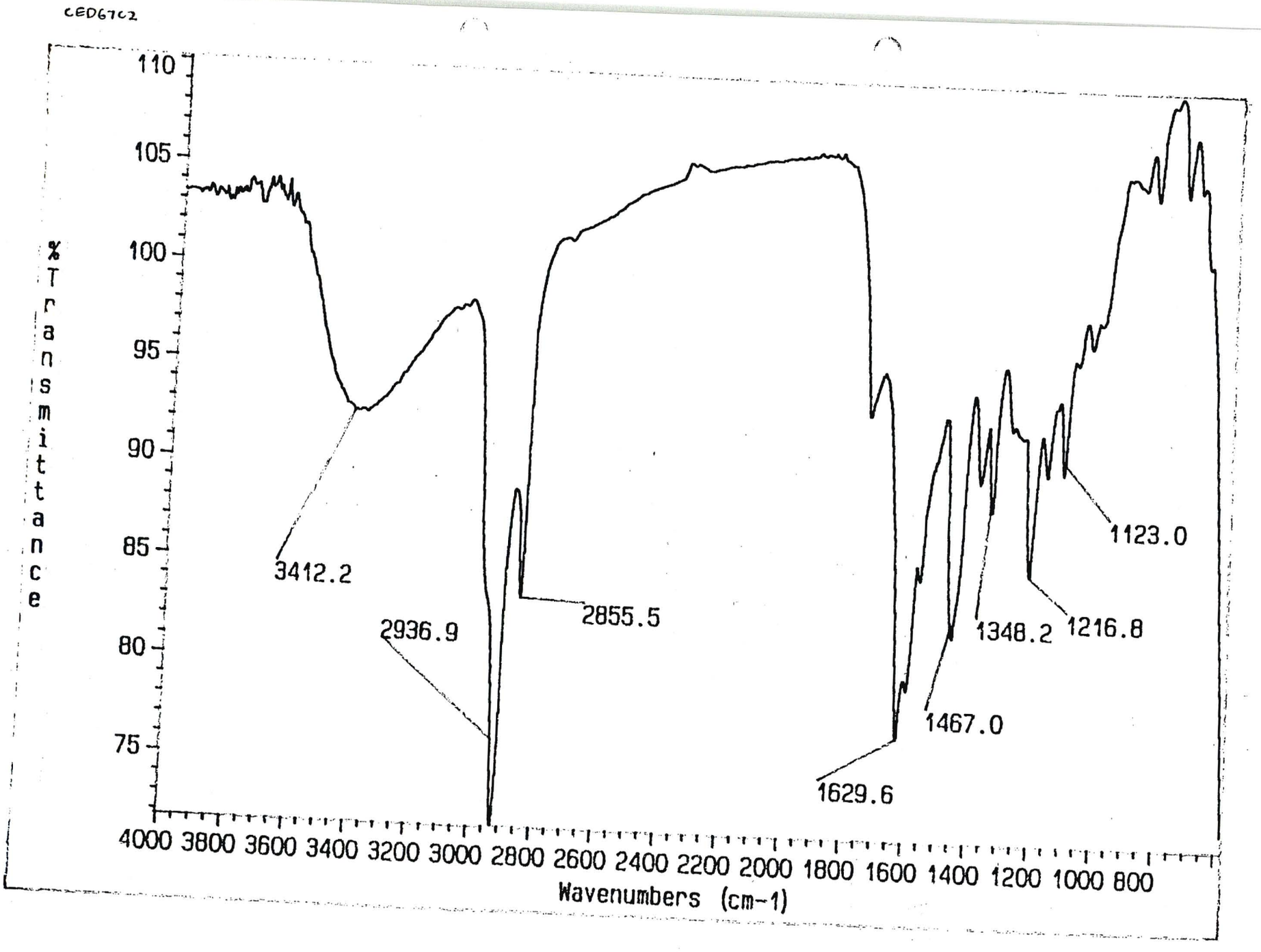




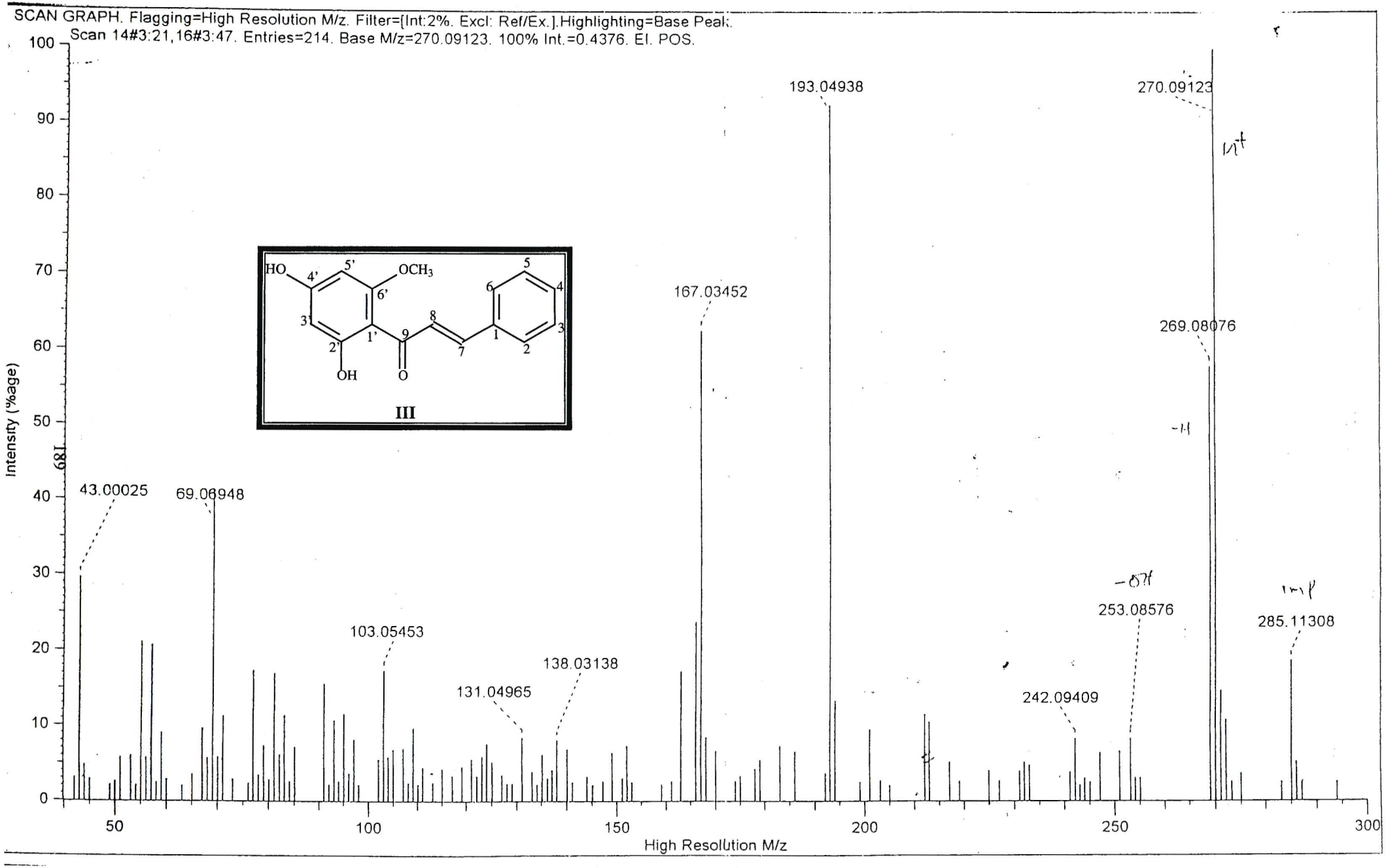
NOESY spectrum of compound **III**, cardamonin



Ultra violet spectrum of compound **III**, cardamonin



Infrared spectrum of compound **III**, cardamonin

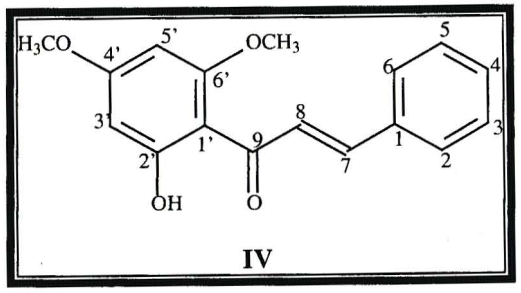


Mass spectrum of compound **III**, cardamonin

5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3166.5[illegible]	.917	7.0
2	3150.96[illegible]	7.878	10.9
3	3088.359	7.722	8.0
4	3072.793	7.683	5.7
5	3055.396	7.639	8.3
6	3053.564	7.635	8.9
7	3047.521	7.620	10.2
8	3046.239	7.617	9.9
9	2966.944	7.418	6.6
10	2963.098	7.409	11.7
11	2956.323	7.392	16.7
12	2951.378	7.379	5.5
13	2440.447	6.102	5.6
14	2438.250	6.096	13.3
15	2435.686	6.090	14.6
16	2433.305	6.084	8.1
17	1933.545	4.834	312.1
18	1607.758	4.020	4.8
19	1594.390	3.986	4.9
20	1572.780	3.932	55.2
21	1565.821	3.915	11.4
22	1531.027	3.828	56.9
23	1519.307	3.799	6.9
24	1516.010	3.790	10.3
25	1316.399	3.291	84.6
26	1314.934	3.288	113.6
27	1313.469	3.284	95.1
28	506.601	1.267	4.8



IV

190

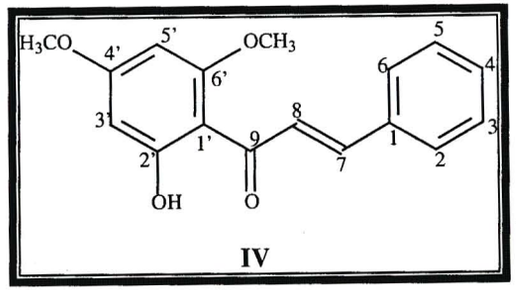
9 8 7 6 5 4 3 2 1 ppm

^{1}H NMR spectrum of compound **IV**, flavokawin B

probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	19396.41	192.870	2.8
2	16840.41	167.455	2.7
3	16767.250	166.726	5.1
4	16378.139	162.857	4.2
5	14284.569	142.040	12.7
6	13634.525	135.576	4.5
7	13121.814	130.478	2.1
8	13075.273	130.015	13.5
9	12964.643	128.915	4.2
10	12952.436	128.793	26.3
11	12918.866	128.460	2.7
12	12904.369	128.315	4.7
13	12897.503	128.247	2.3
14	12883.769	128.111	24.6
15	12819.681	127.473	9.7
16	9417.630	93.645	8.0
17	9142.200	90.906	10.5
18	6179.616	61.447	2.3
19	5569.246	55.378	12.2
20	5531.861	55.006	13.7
21	4877.238	48.497	478.7
22	4868.846	48.414	21.7
23	4862.742	48.353	36.1
24	4855.876	48.285	1466.3
25	4847.483	48.201	40.3
26	4841.379	48.141	60.7
27	4834.513	48.072	2973.1
28	4826.120	47.989	53.8
29	4813.150	47.860	3500.0
30	4791.787	47.647	2986.4
31	4770.424	47.435	1488.8
32	4749.061	47.223	496.0

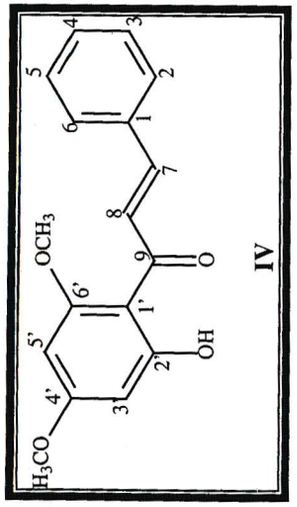


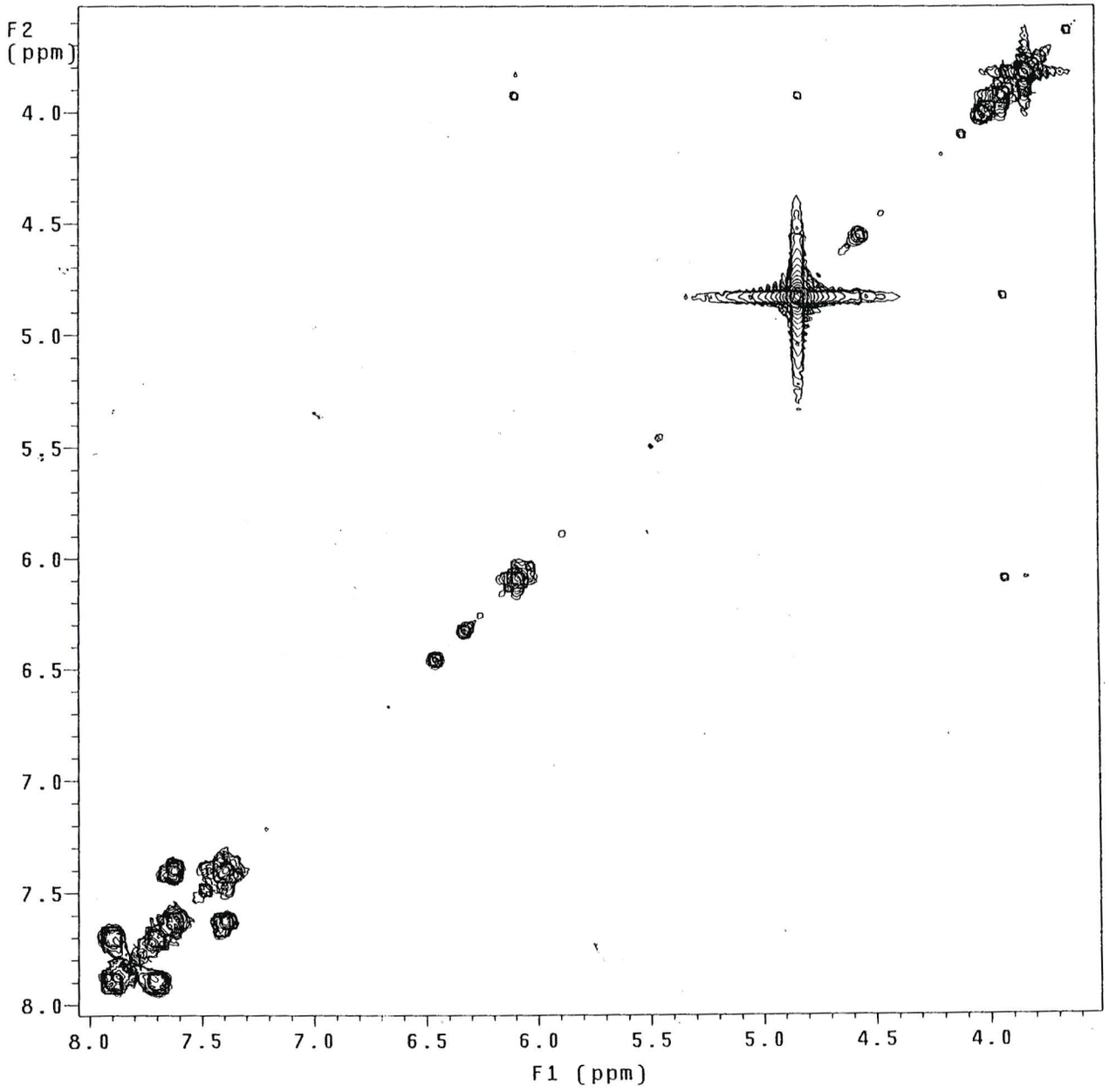
IV

^{13}C NMR spectrum of compound **IV**, flavokawin B

cyed27b.ced27b in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

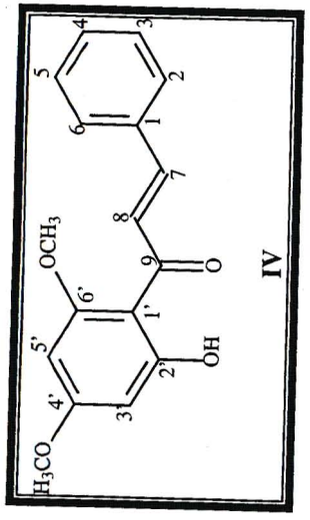


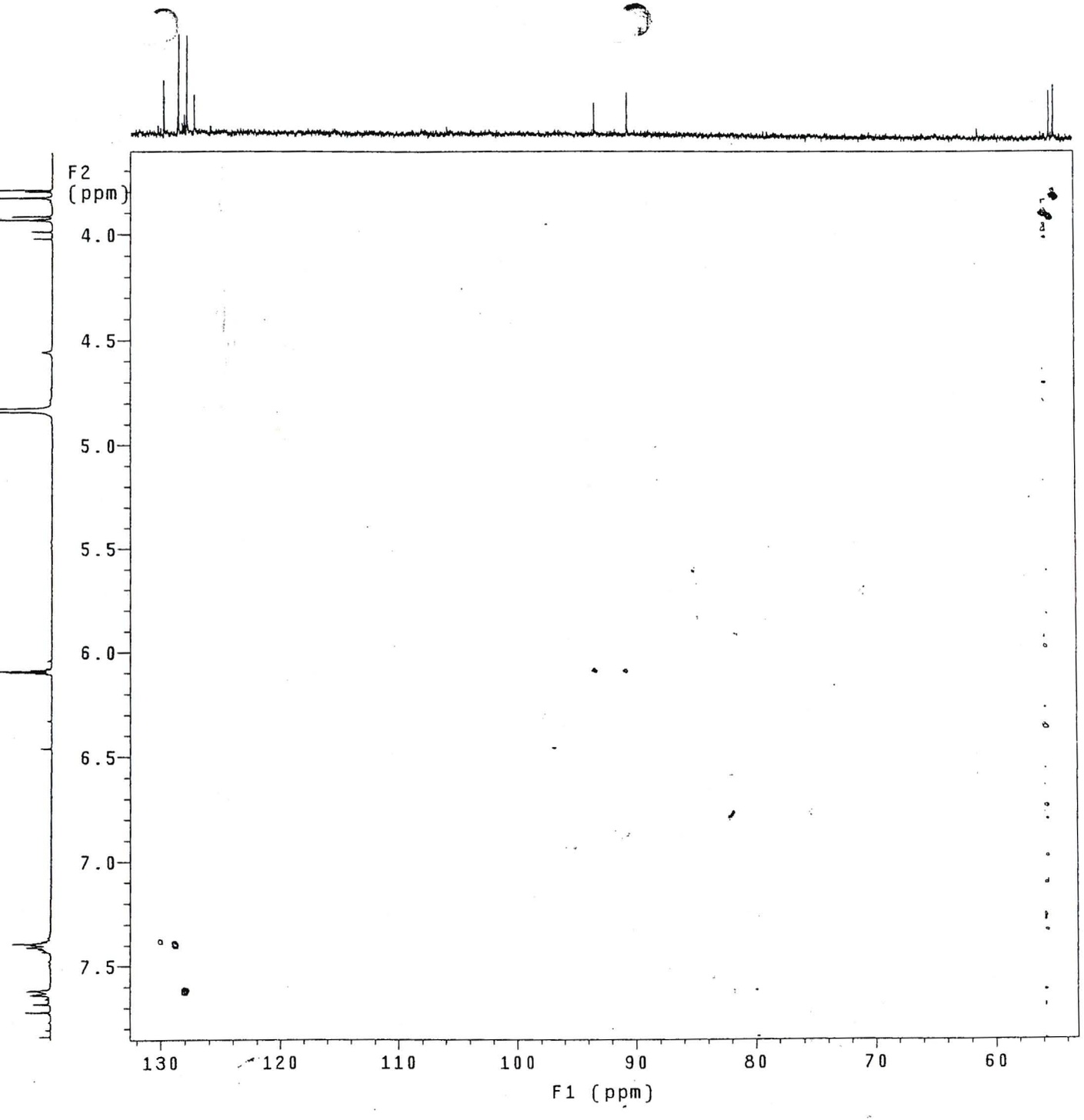


COSY spectrum of compound **IV**, flavokawin B

HQed27b.ced27b in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

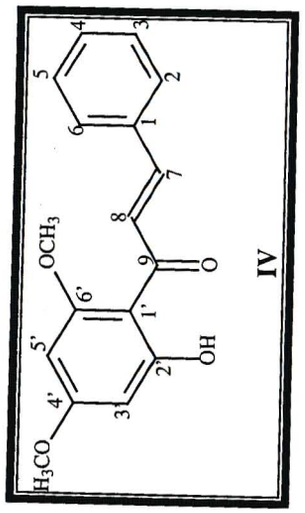


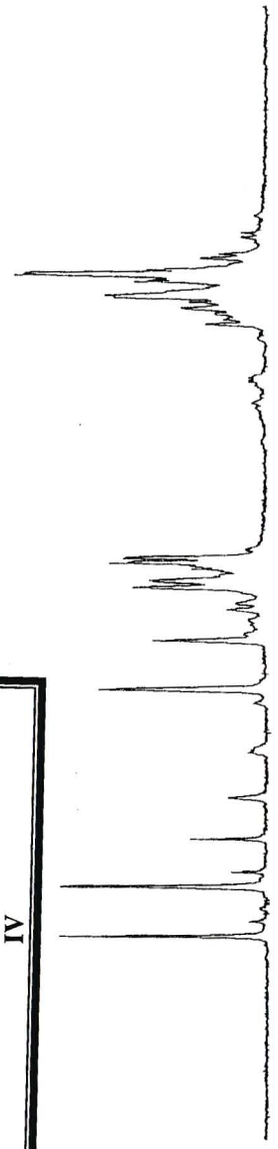


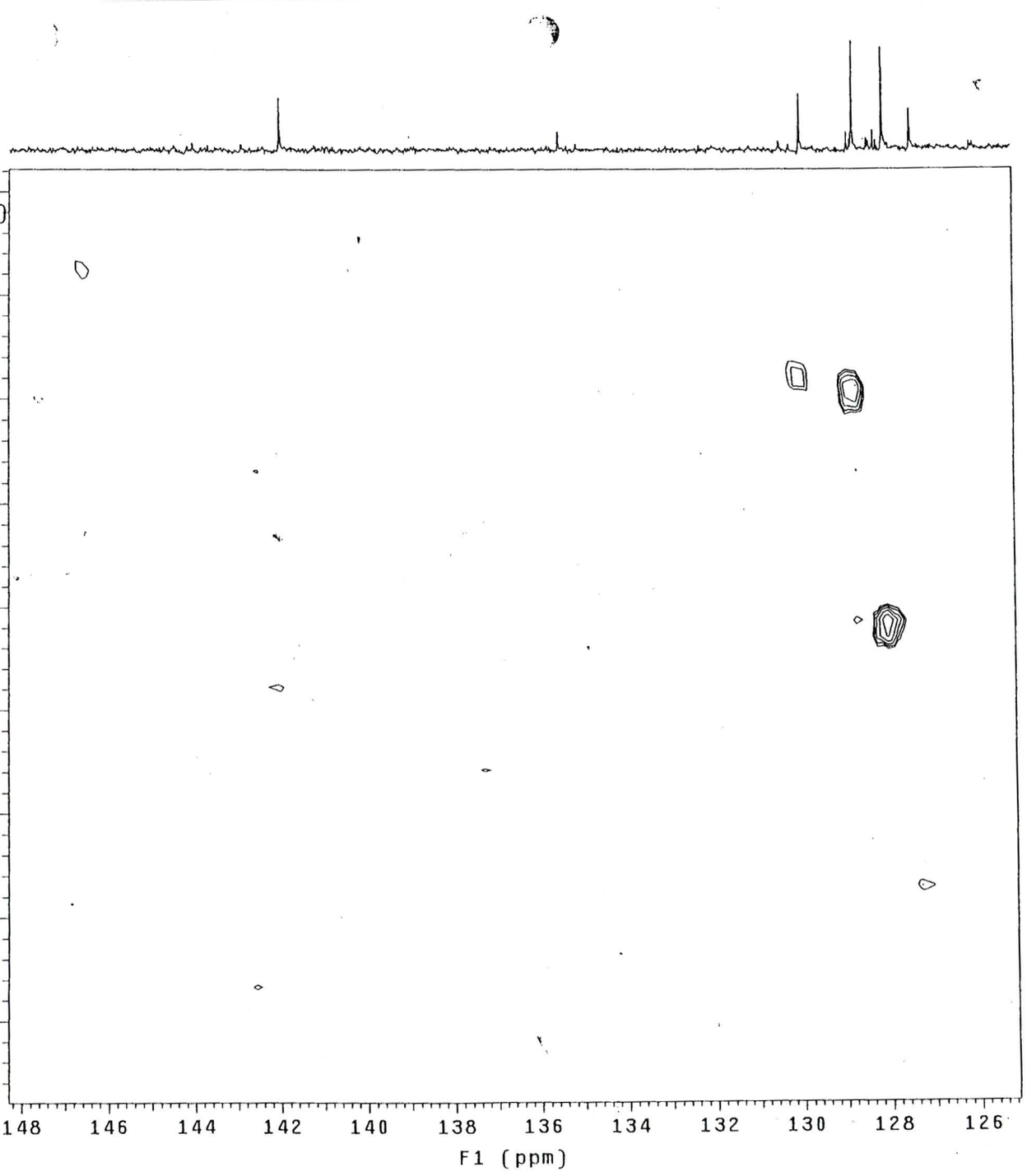
HSQC spectrum of compound **IV**, flavokawin B

HQed27b.ced27b in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



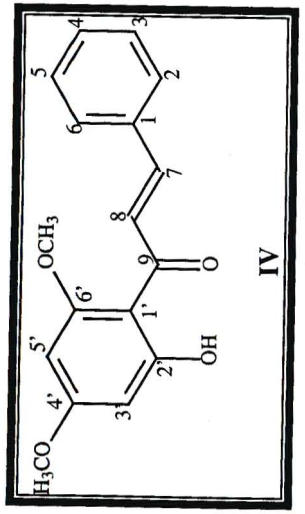




Expanded HSQC spectrum of compound **IV**, flavokawin B

HBed27b.ced27b in cd3od
Gradient HMBC expt.
probe=5mmASW

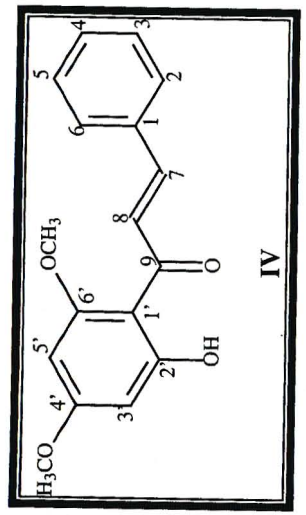
Pulse Sequence: ghmqc_da

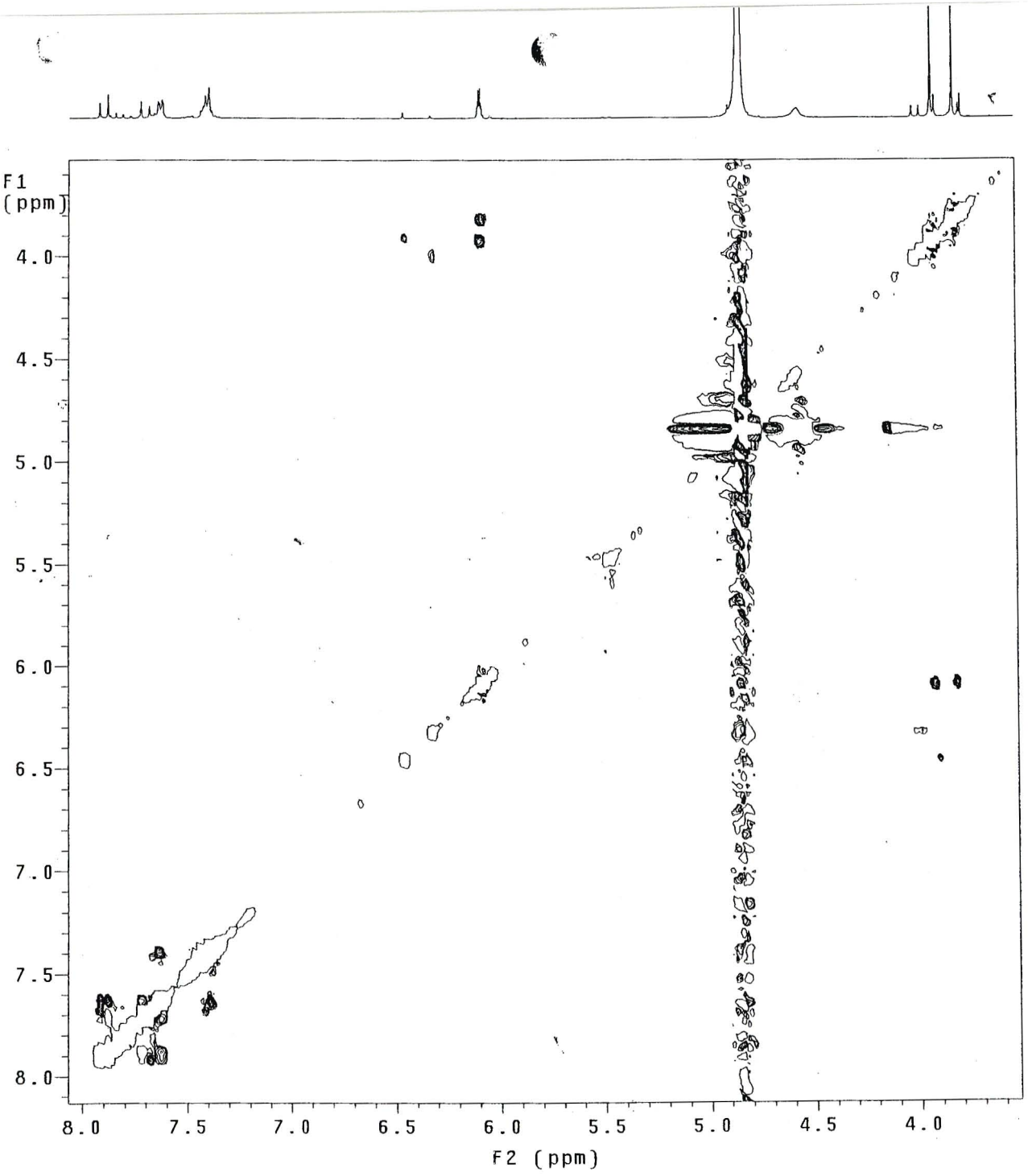


HMBC spectrum of compound **IV**, flavokawin B

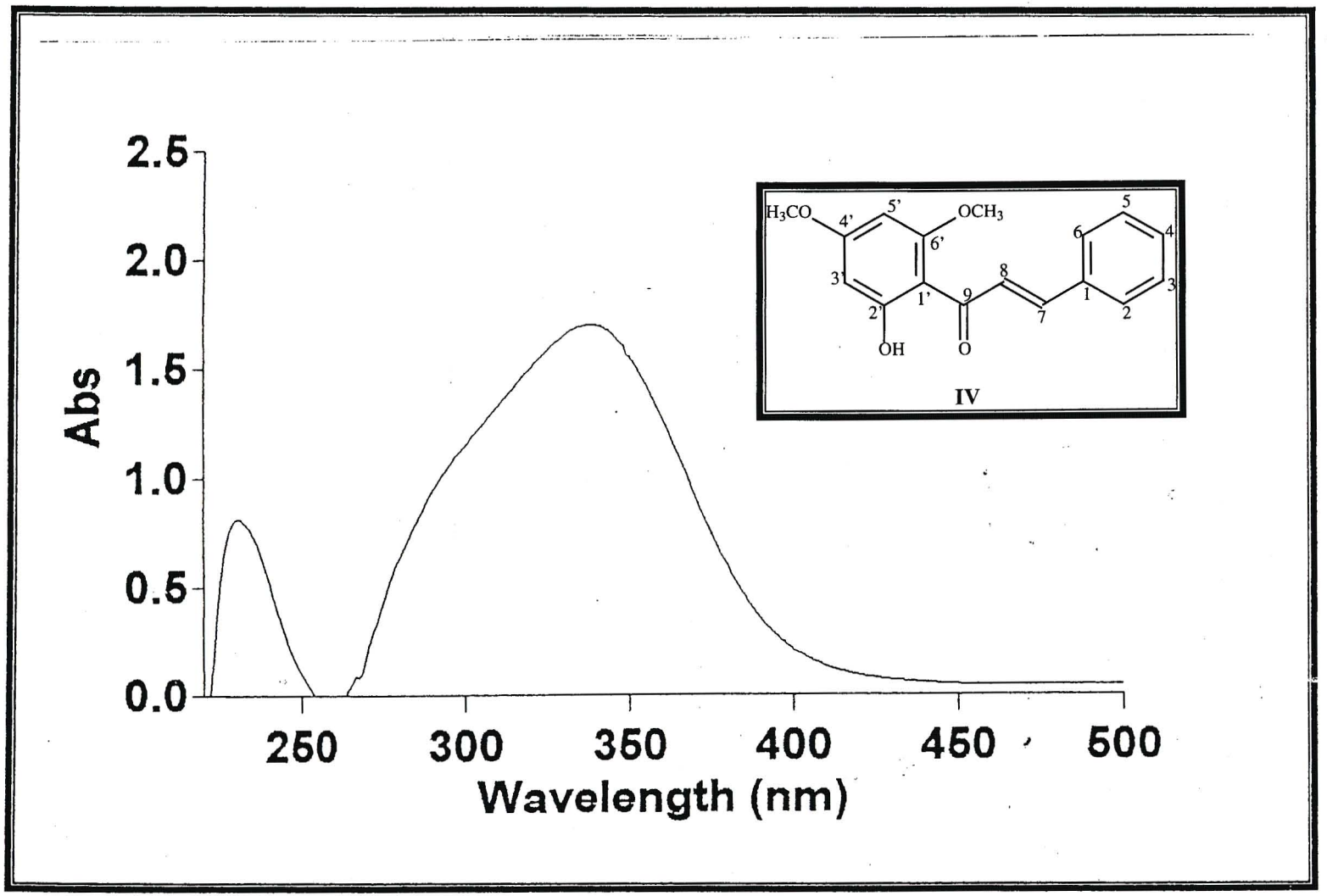
NOed27b.ced27b in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

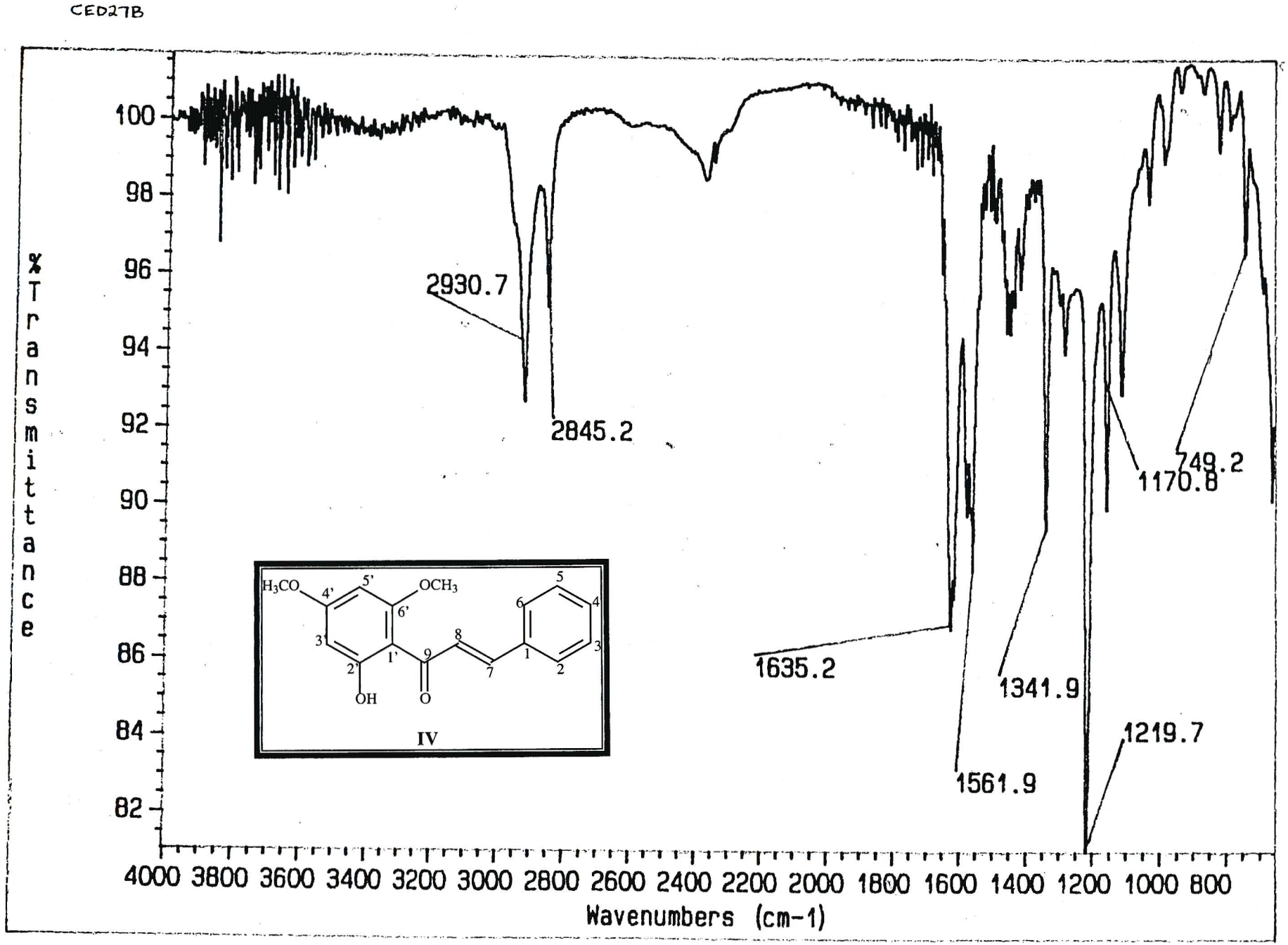




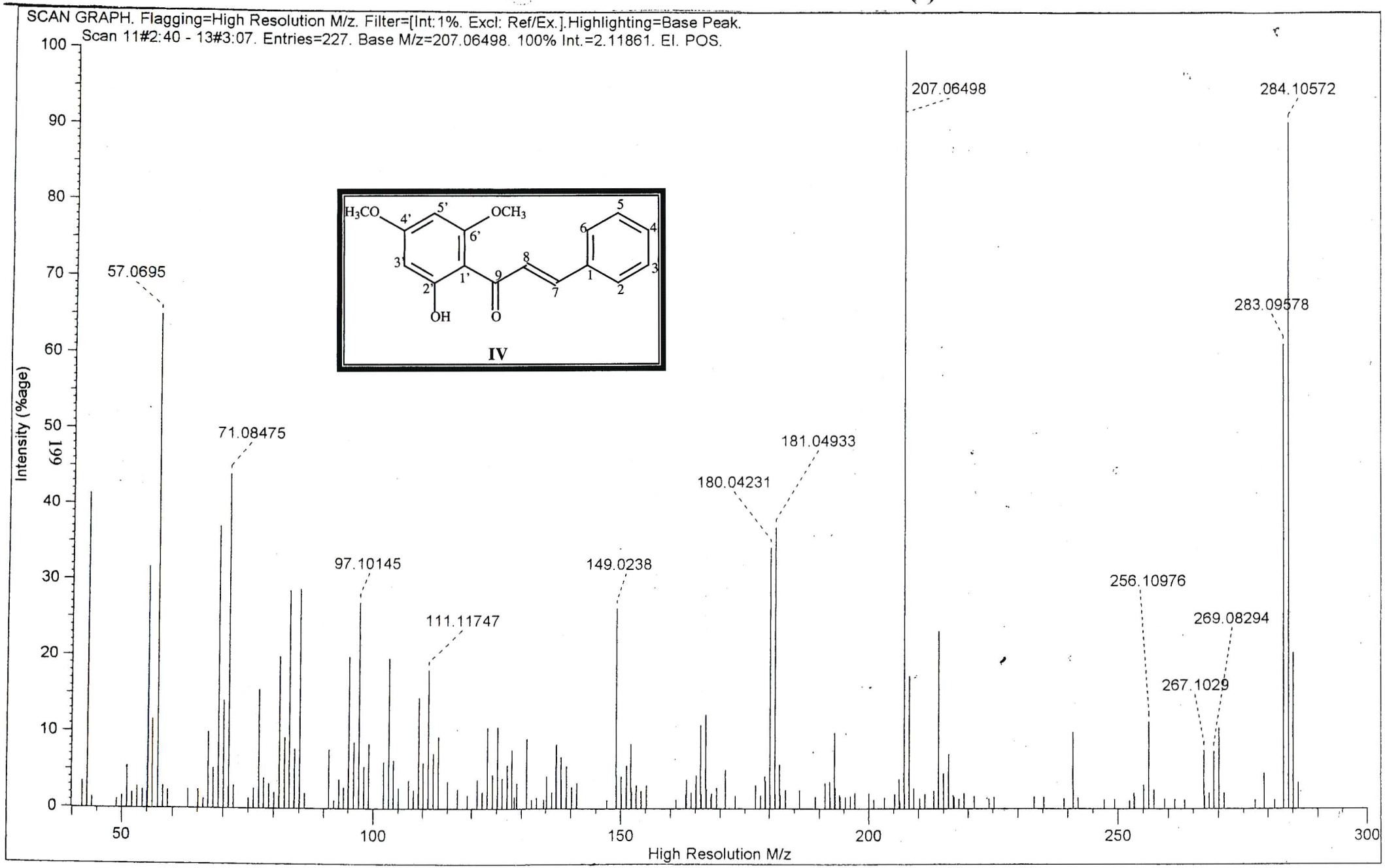
NOESY spectrum of compound **IV**, flavokawin B



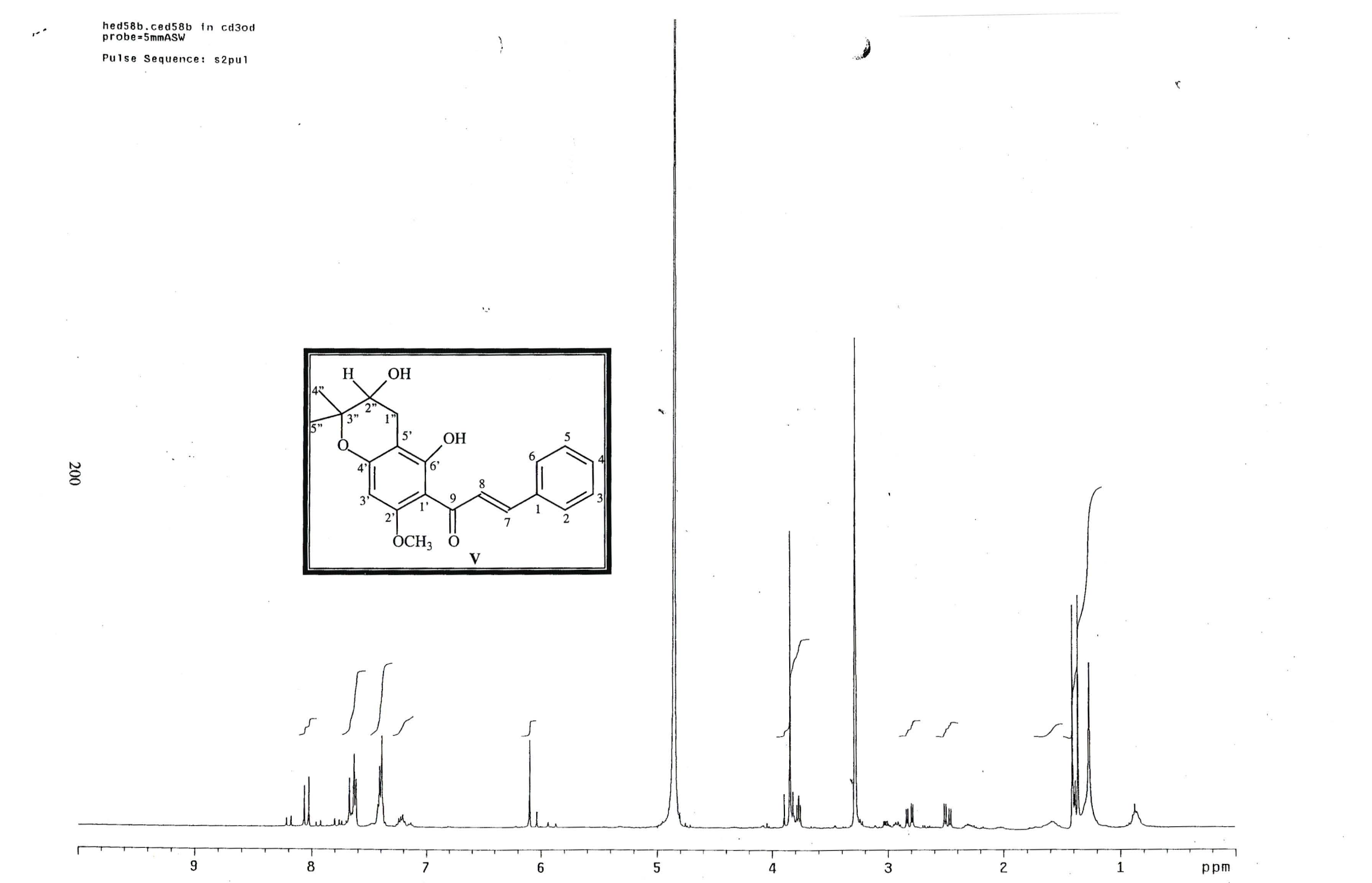
Ultra violet spectrum of compound **IV**, flavokawin B



Infrared spectrum of compound **IV**, flavokawin B



Mass spectrum of compound **IV**, flavokawin B

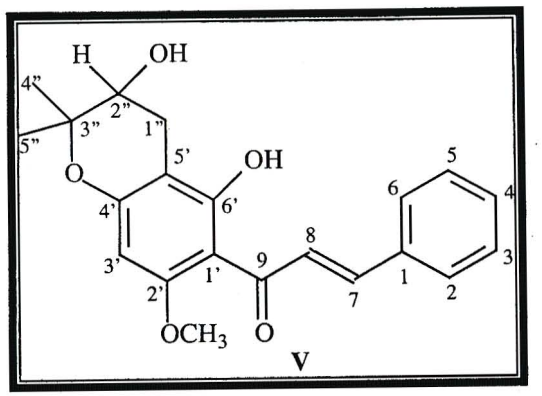


^{1}H NMR spectrum of compound **V**, 2'-methoxyhelikrausichalcone

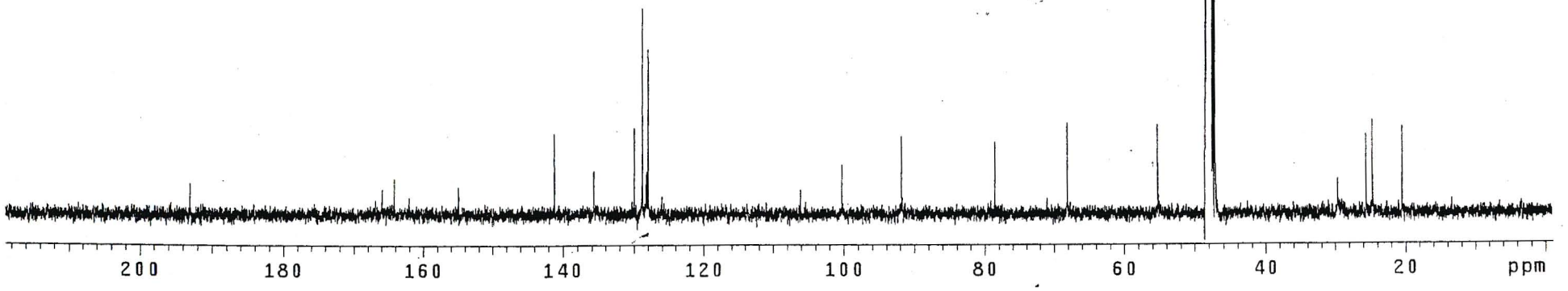
ced58b.ced58b in cd3od
probe=5mmASW

Pulse Sequence: s2pul

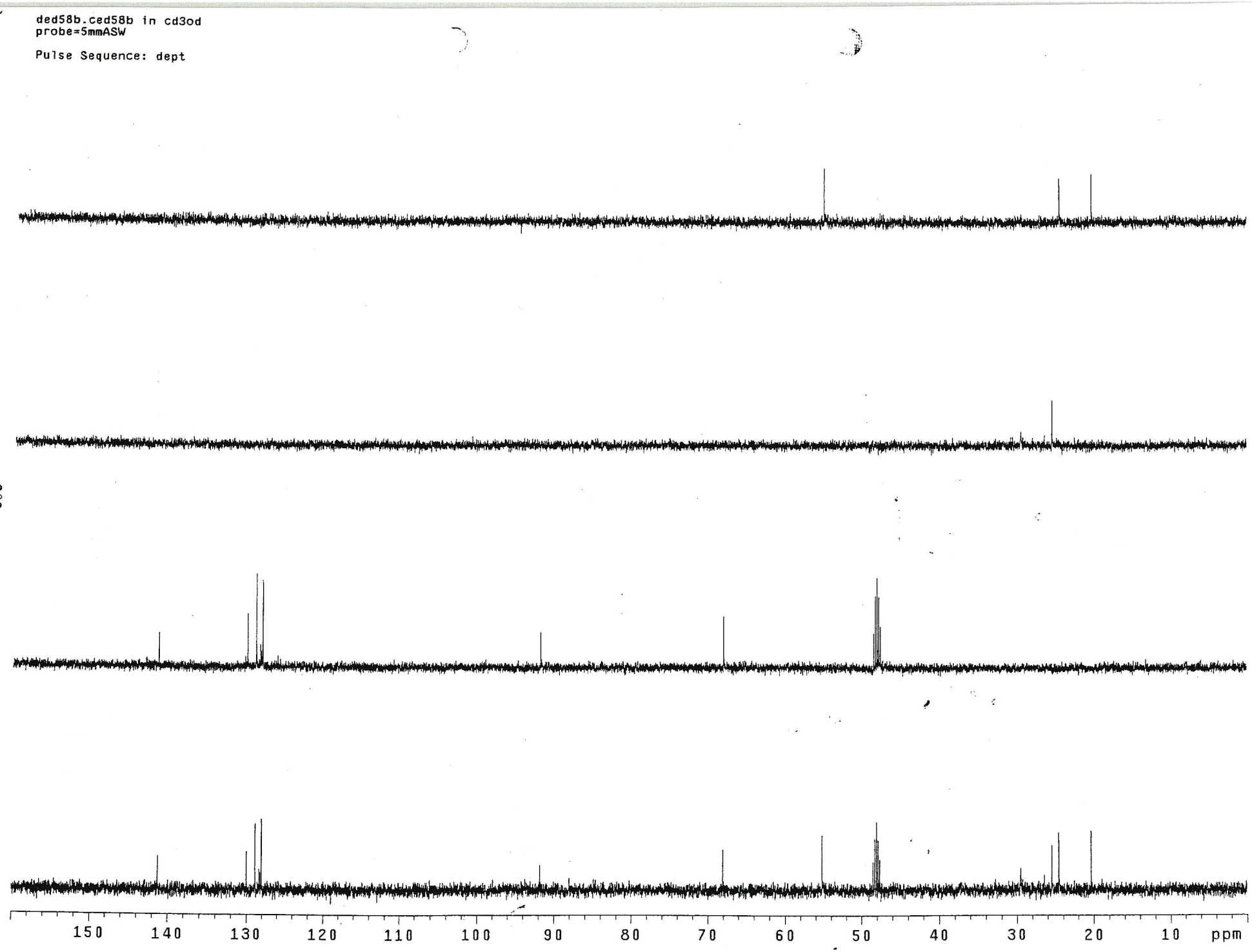
INDEX	FREQUENCY	PPM	HEIGHT
1	19413.	193.044	5.1
2	16688.604	165.945	4.3
3	16514.709	164.215	5.9
4	15586.946	154.990	4.5
5	14199.117	141.190	13.1
6	13647.495	135.705	7.1
7	13068.406	129.947	14.0
8	12953.962	128.809	33.3
9	12900.554	128.278	6.5
10	12889.873	128.171	7.0
11	12873.851	128.012	15.2
12	12870.799	127.982	26.8
13	10668.888	106.087	4.0
14	10085.222	100.283	8.0
15	9233.755	91.816	12.6
16	7903.148	78.585	11.7
17	6849.497	68.108	14.7
18	5556.275	55.249	14.4
19	4887.920	48.603	-4.4
20	4878.001	48.505	560.8
21	4863.505	48.361	30.5
22	4856.638	48.292	1613.3
23	4842.142	48.148	112.0
24	4835.275	48.080	3224.9
25	4813.912	47.867	4000.0
26	4792.549	47.655	3781.5
27	4771.186	47.443	1993.5
28	4749.823	47.230	608.7
29	2981.276	29.645	5.6
30	2573.854	25.593	12.8
31	2481.535	24.675	15.1
32	2058.854	20.472	14.0



V



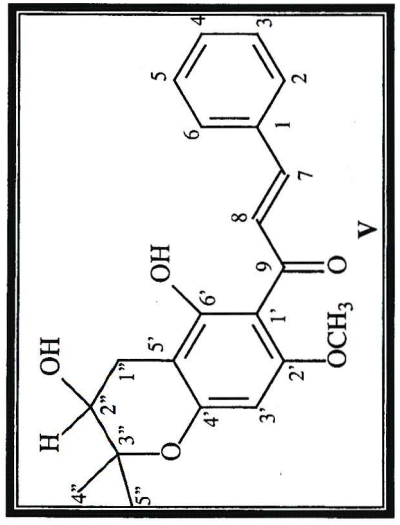
^{13}C NMR spectrum of compound **V**, 2'-methoxyhelikrausichalcone

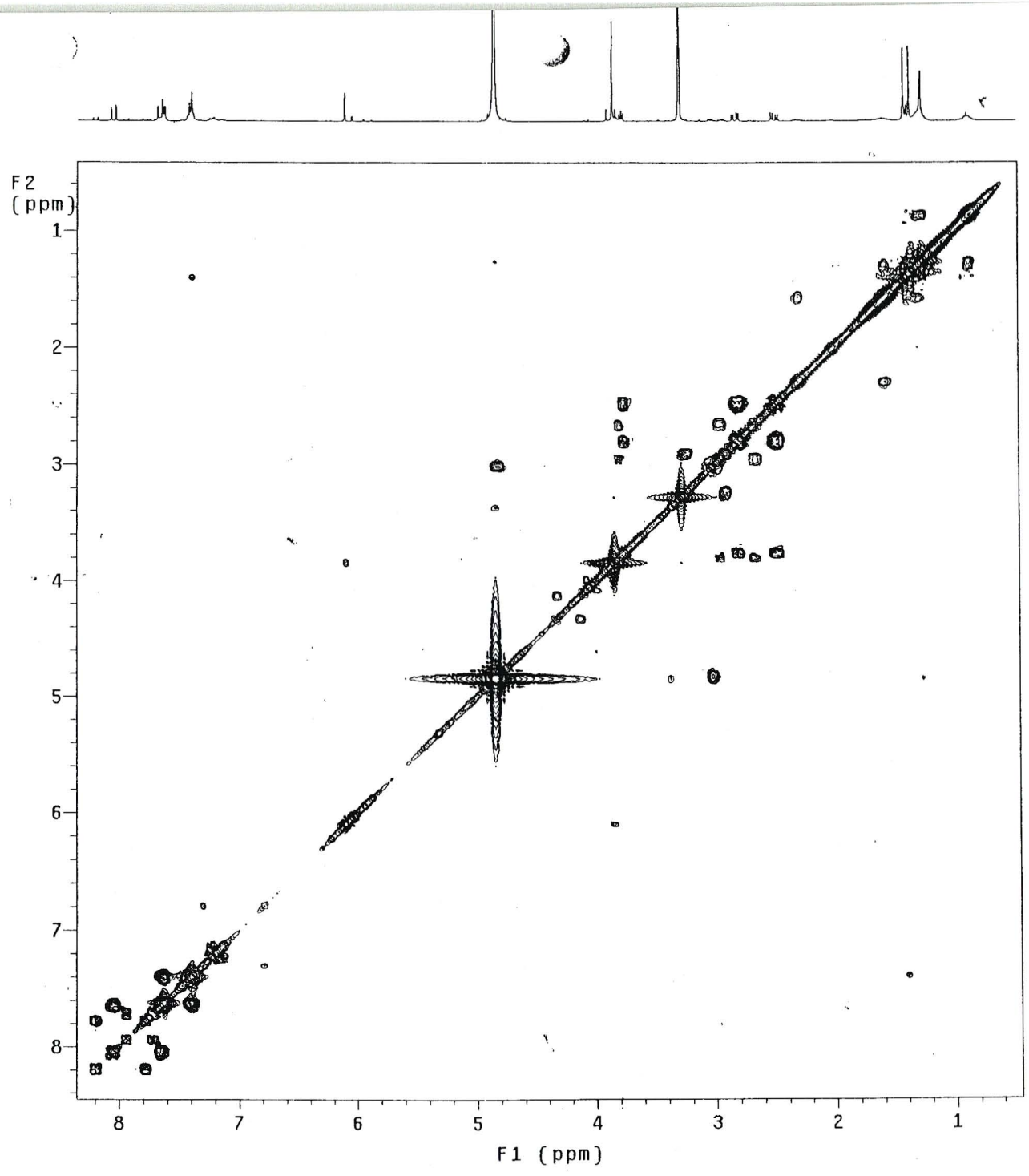


ADEPT spectrum of compound **V**, 2'-methoxyhelikrausichalcone

cyed58b.ced58b in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

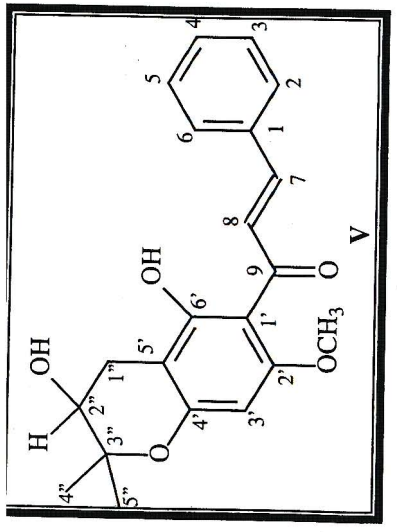


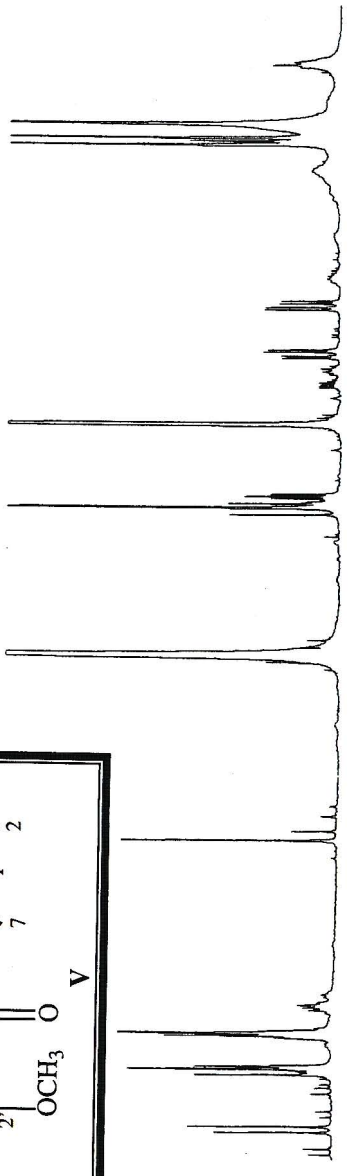


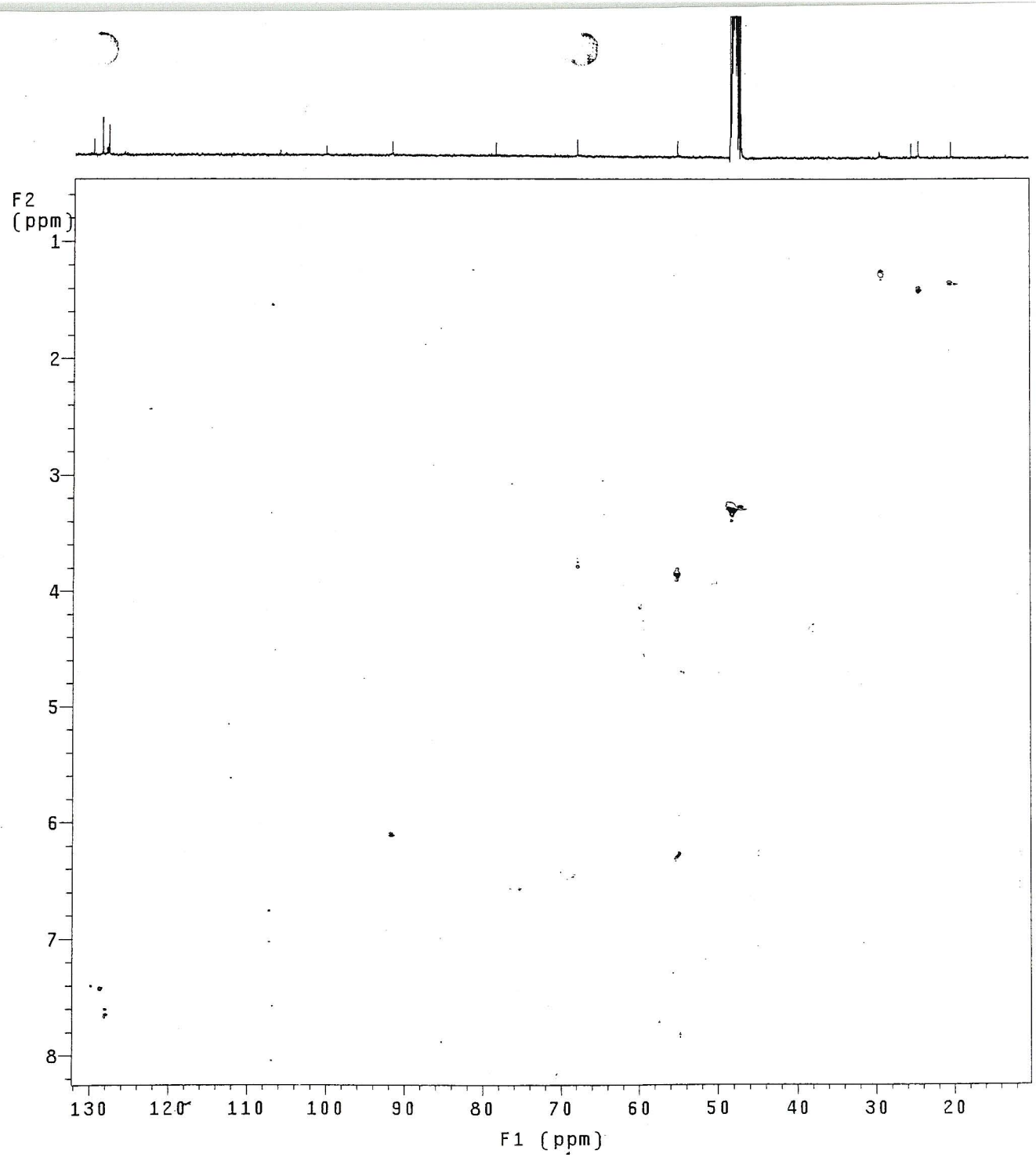
COSY spectrum of compound **V**, 2'-methoxyhelikrausichalcone

HQed58b.ced58b in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

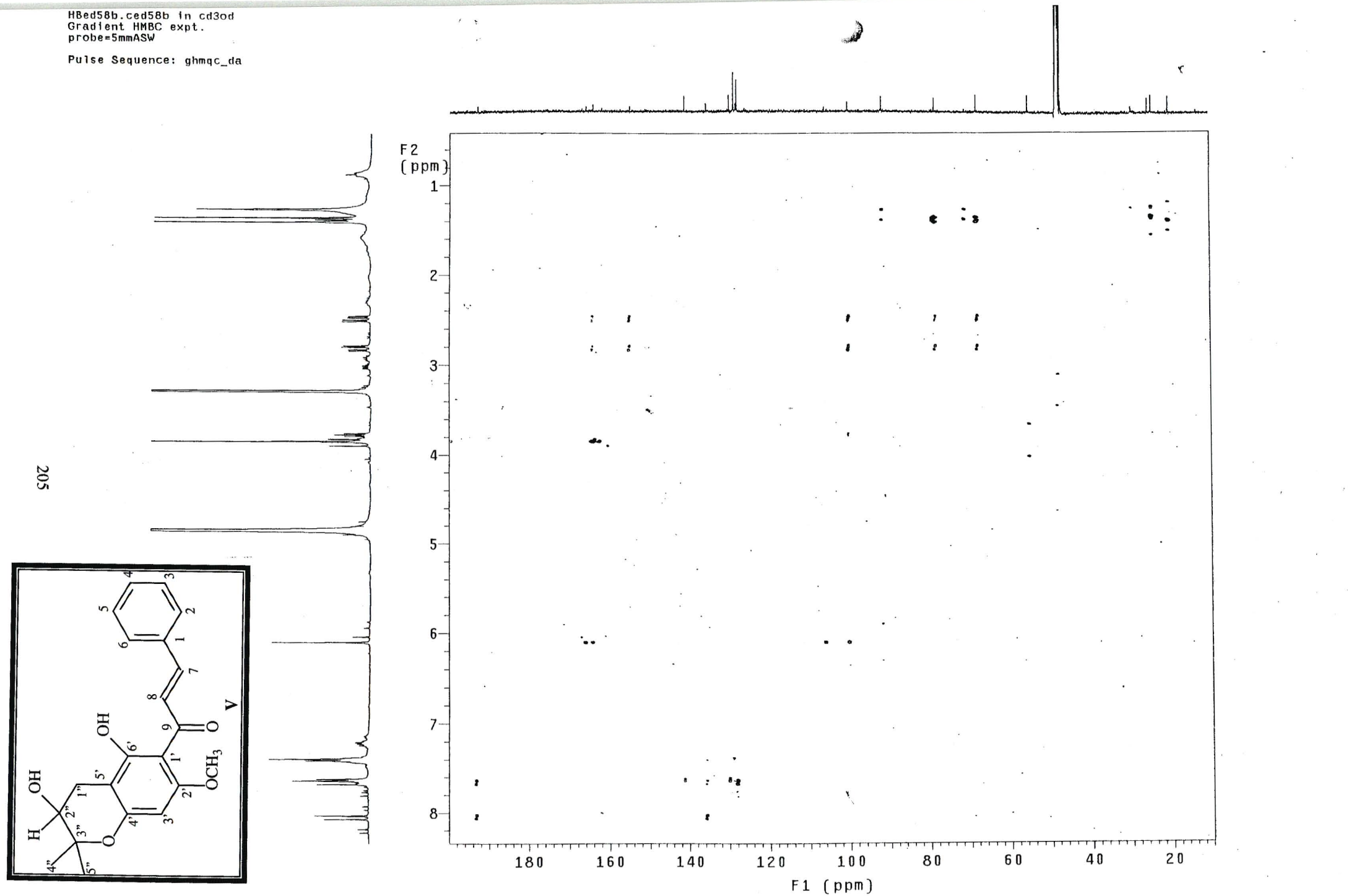
Pulse Sequence: ghsqc_da



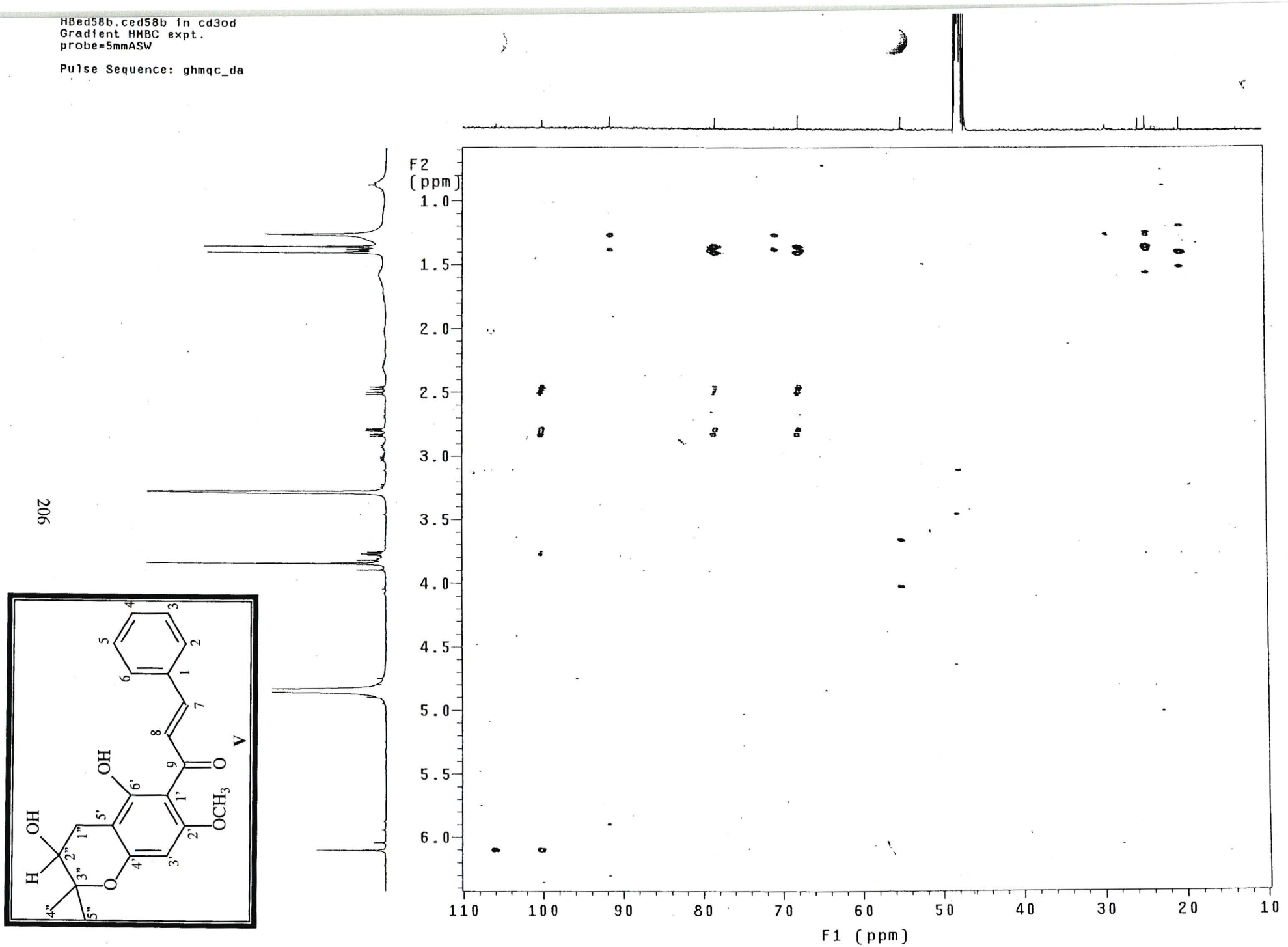




HSQC spectrum of compound **V**, 2'-methoxyhelikrausichalcone



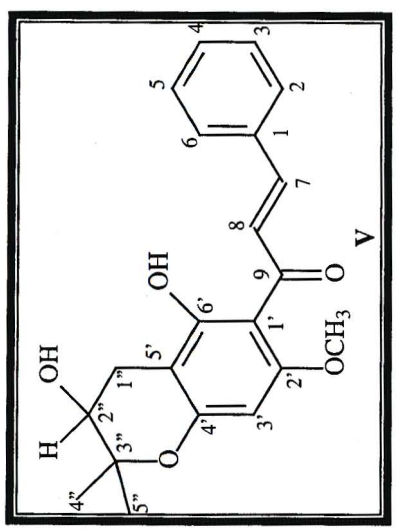
HMBC spectrum of compound **V**, 2'-methoxyhelikrausichalcone



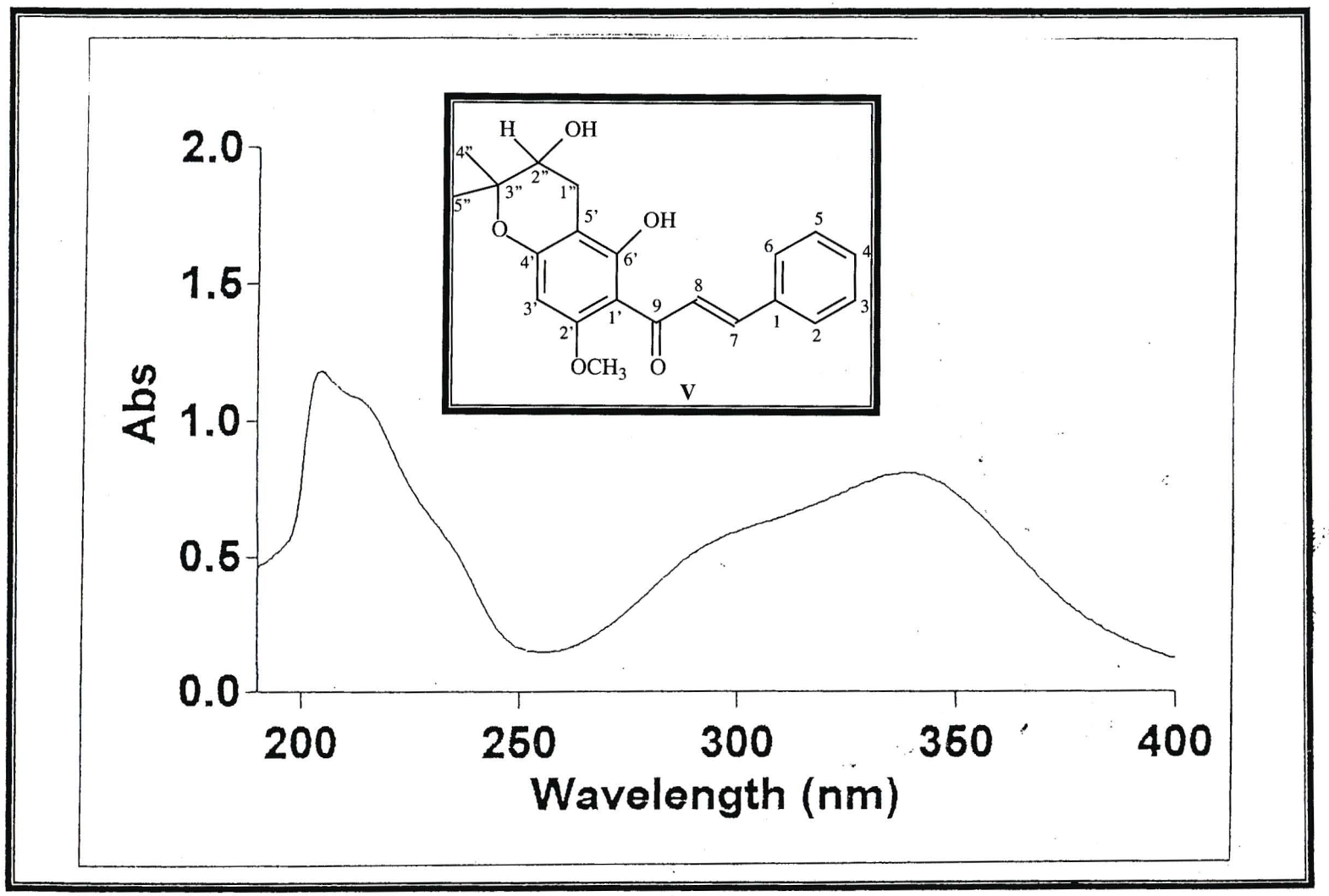
Expanded HMBC spectrum of compound **V**, 2'-methoxyhelikrausichalcone

NOed58b.ced58b in cd3od
Gradient NOESY expt.
using presat-h2o
mix=1sec
probe=5mmASW

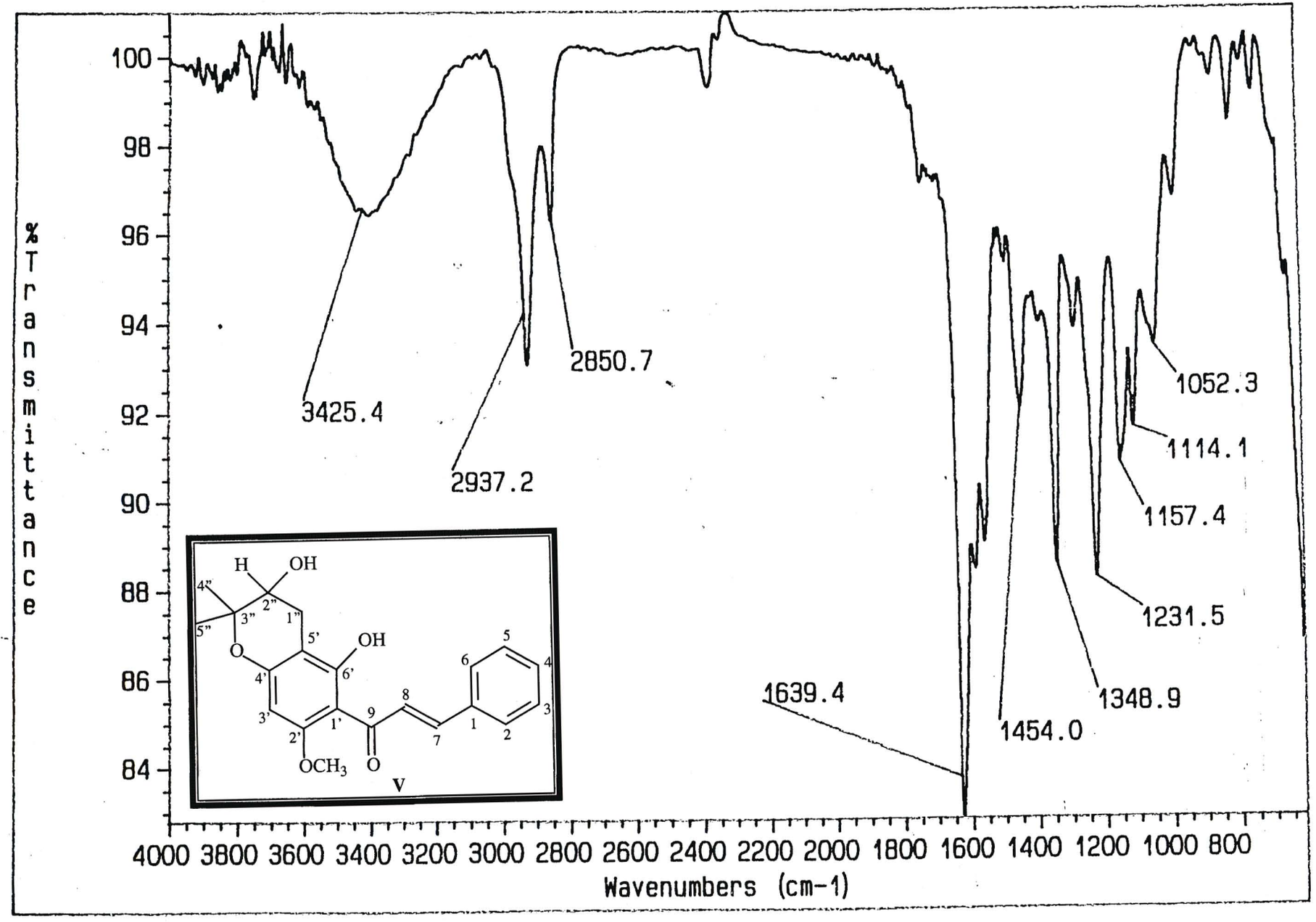
Pulse Sequence: noesy_da



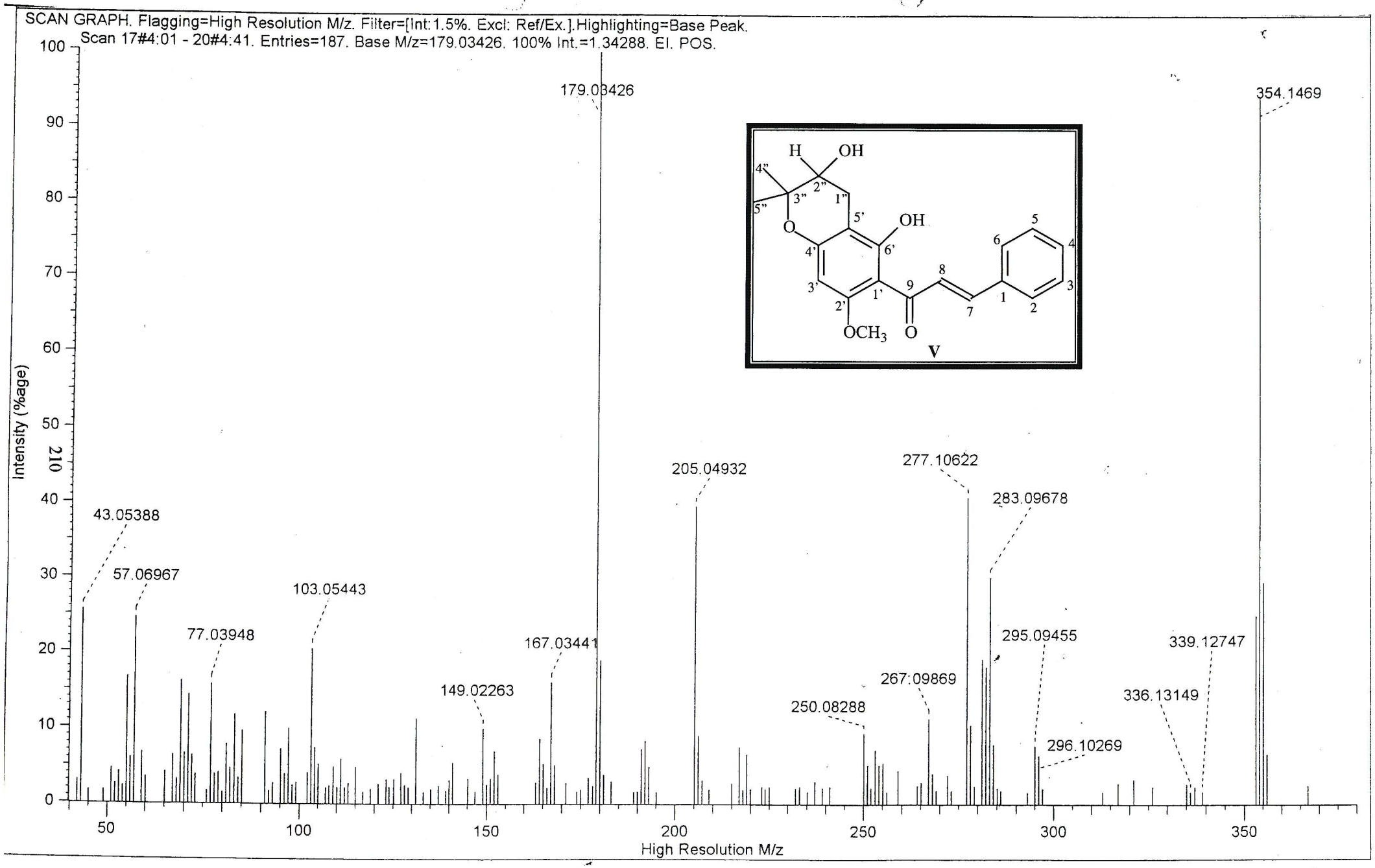
NOESY spectrum of compound **V**, 2'-methoxyhelikrausichalcone



Ultra violet spectrum of compound **V**, 2'-methoxyhelikrausichalcone



Infrared spectrum of compound **V**, 2'-methoxyhelikrausichalcone

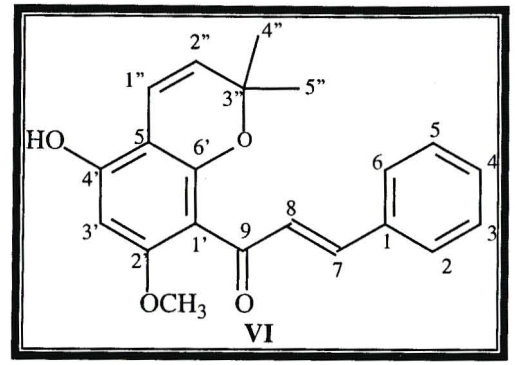


Mass spectrum of compound **V**, 2'-methoxyhelikrausichalcone

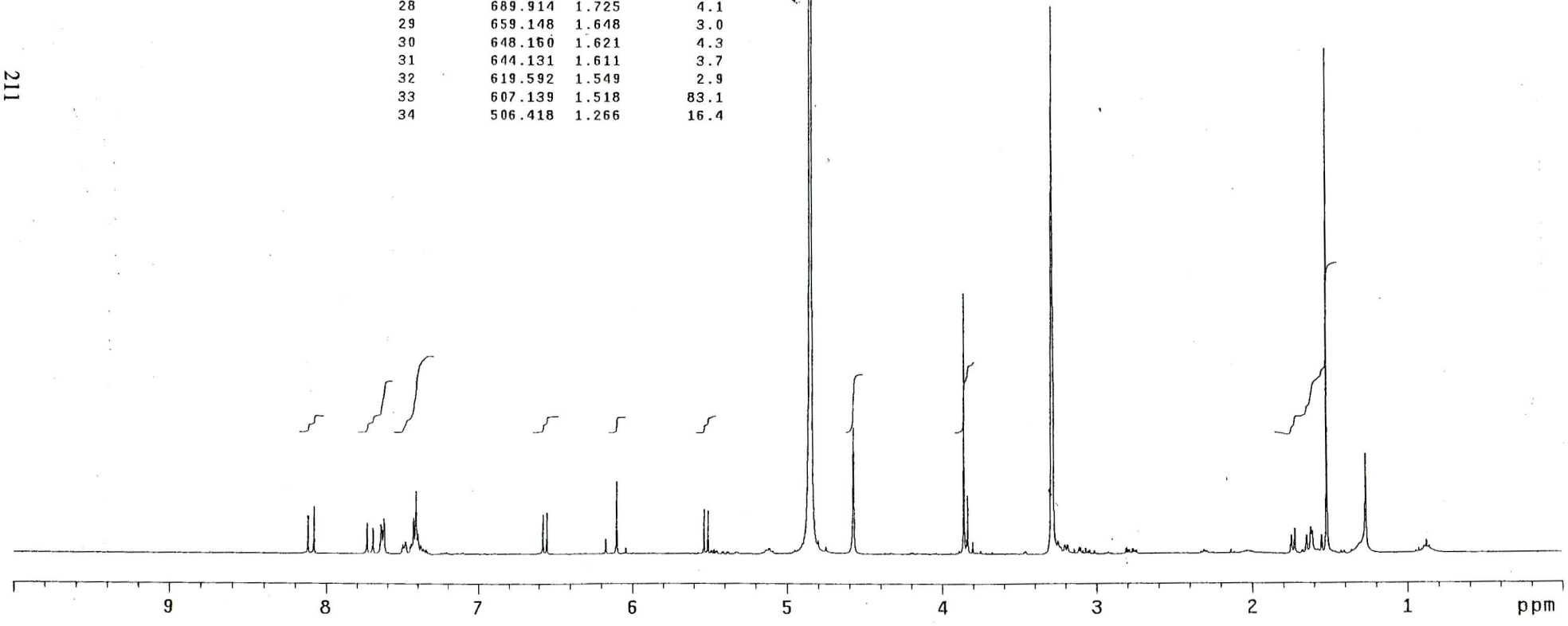
hed18.ced18 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3246.56	8.117	6.2
2	3230.83	8.078	7.7
3	3092.937	7.733	5.1
4	3077.188	7.694	4.2
5	3056.678	7.643	4.8
6	3054.663	7.638	4.5
7	3048.986	7.623	5.8
8	3047.338	7.619	5.1
9	2970.790	7.428	5.9
10	2963.648	7.410	10.3
11	2958.886	7.398	3.3
12	2957.238	7.394	3.0
13	2629.254	6.574	6.5
14	2619.365	6.549	6.8
15	2440.630	6.102	12.0
16	2212.817	5.533	7.3
17	2202.928	5.508	7.1
18	1940.504	4.852	1200.0
19	1829.894	4.575	20.9
20	1543.663	3.860	42.9
21	1533.957	3.835	9.7
22	1318.047	3.296	35.8
23	1316.399	3.291	65.9
24	1314.934	3.288	90.1
25	1313.286	3.284	66.3
26	1311.821	3.280	35.5
27	699.070	1.748	3.0
28	689.914	1.725	4.1
29	659.148	1.648	3.0
30	648.160	1.621	4.3
31	644.131	1.611	3.7
32	619.592	1.549	2.9
33	607.139	1.518	83.1
34	506.418	1.266	16.4



VI

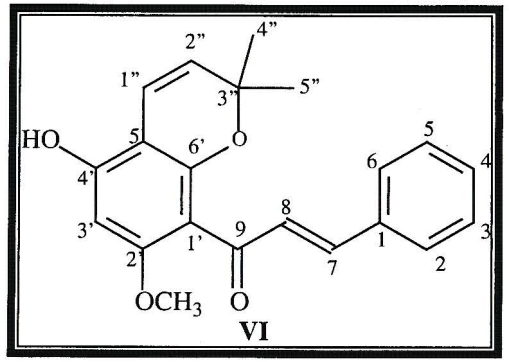


^{1}H NMR spectrum of compound **VI**, cedreprenone

ced18.ced18 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

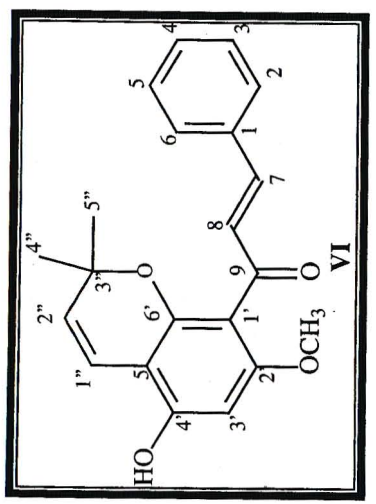
INDEX	FREQUENCY	PPM	HEIGHT
1	19397.9[illegible]	92.885	3.6
2	16779.457	166.848	4.0
3	16239.280	161.476	4.6
4	15645.695	155.574	5.0
5	14282.280	142.017	9.9
6	13633.762	135.568	5.7
7	13089.006	130.151	12.3
8	12961.592	128.884	30.2
9	12918.103	128.452	5.5
10	12910.473	128.376	5.6
11	12873.851	128.012	30.5
12	12809.762	127.375	12.3
13	12677.007	126.055	4.6
14	12549.592	124.788	11.6
15	12309.259	122.398	3.8
16	11695.836	116.298	11.1
17	10660.496	106.003	4.0
18	10388.881	103.303	4.0
19	9284.111	92.317	9.6
20	7852.030	78.077	8.6
21	5564.668	55.333	15.8
22	4877.238	48.497	776.3
23	4862.742	48.353	73.8
24	4855.876	48.285	2379.2
25	4847.483	48.201	72.1
26	4834.513	48.072	4944.8
27	4813.150	47.860	6000.0
28	4791.787	47.647	5192.0
29	4770.424	47.435	2561.6
30	4749.061	47.223	830.3
31	2982.802	29.660	4.5
32	2717.291	27.020	28.5
33	2496.795	24.827	3.8

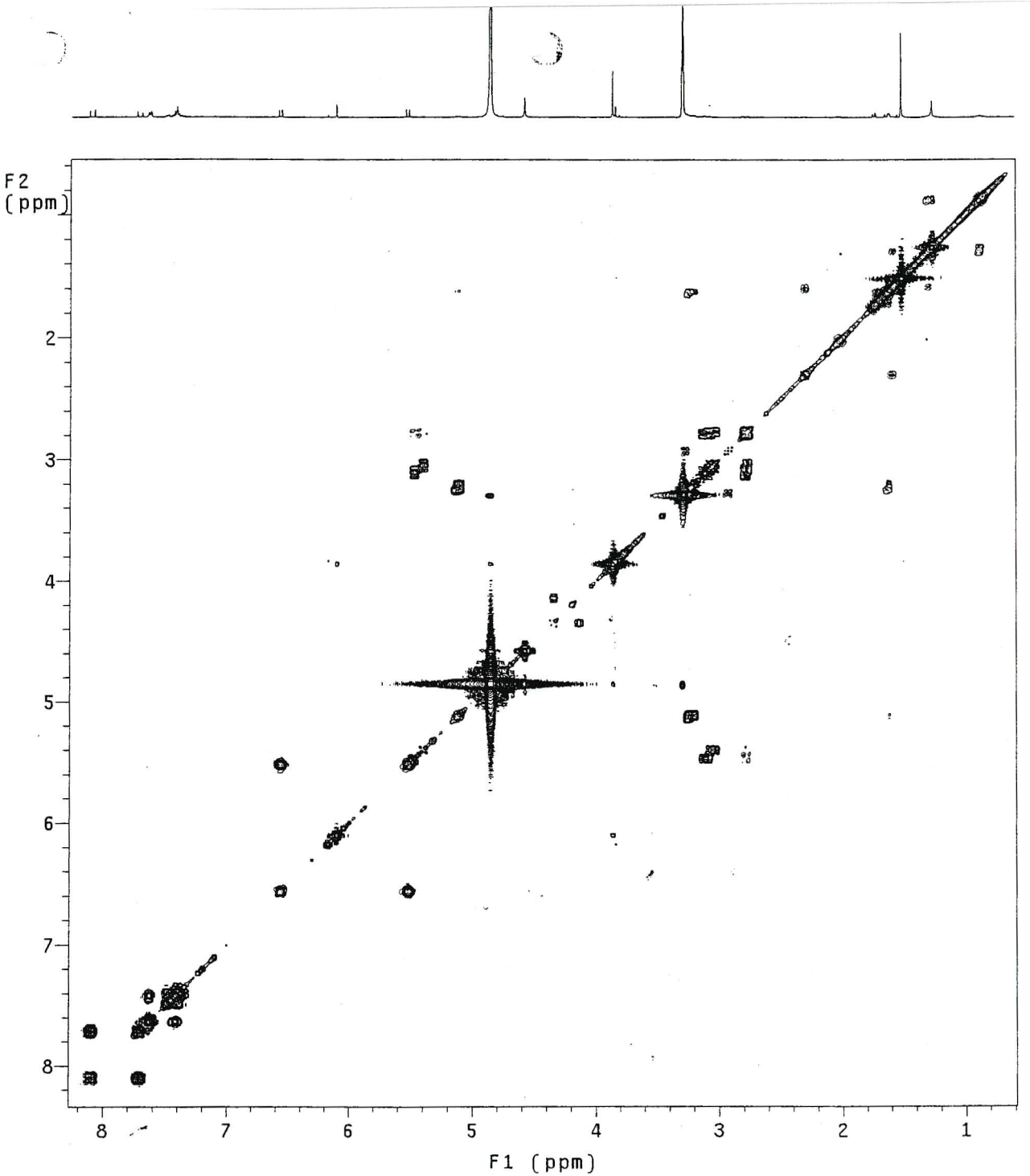


^{13}C NMR spectrum of compound **VI**, cedreprenone

cyed18.ced18 in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

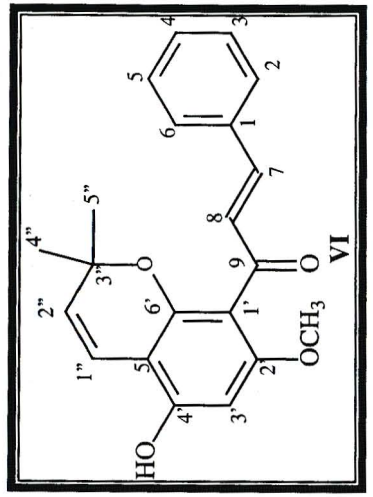


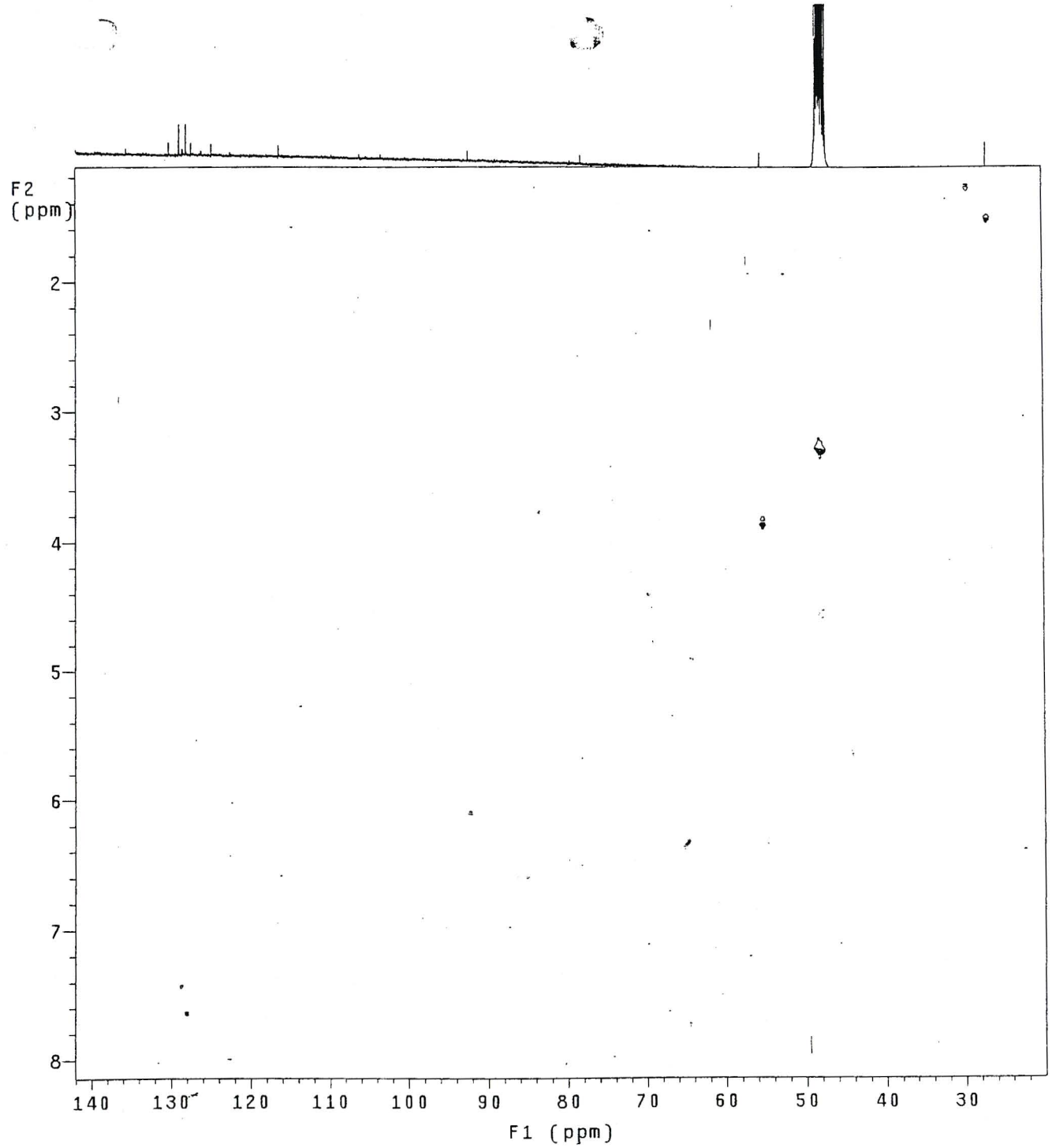


COSY spectrum of compound **VI**, cedreprenone

HQed18.ced18 in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

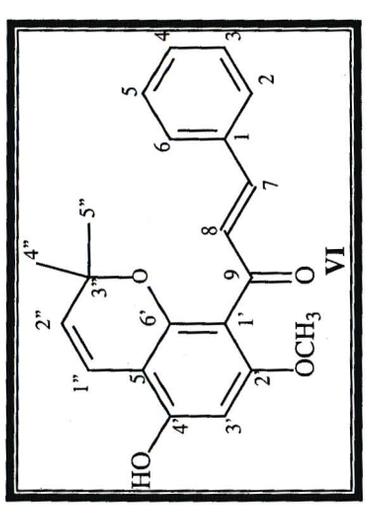


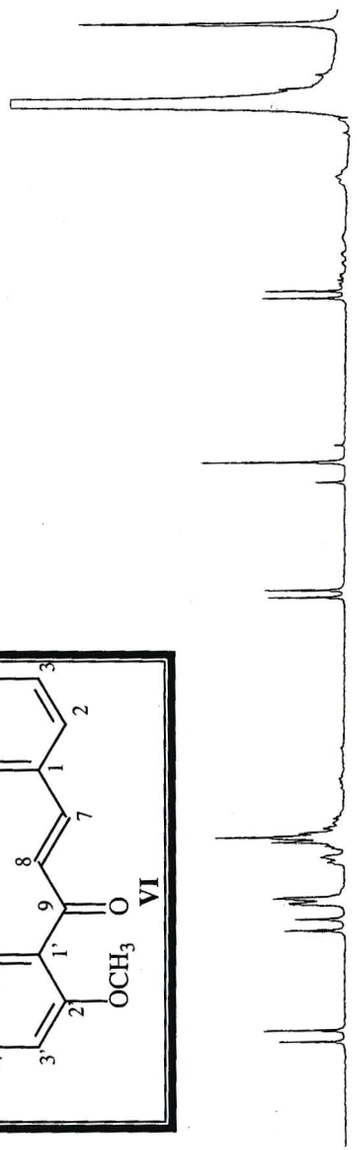


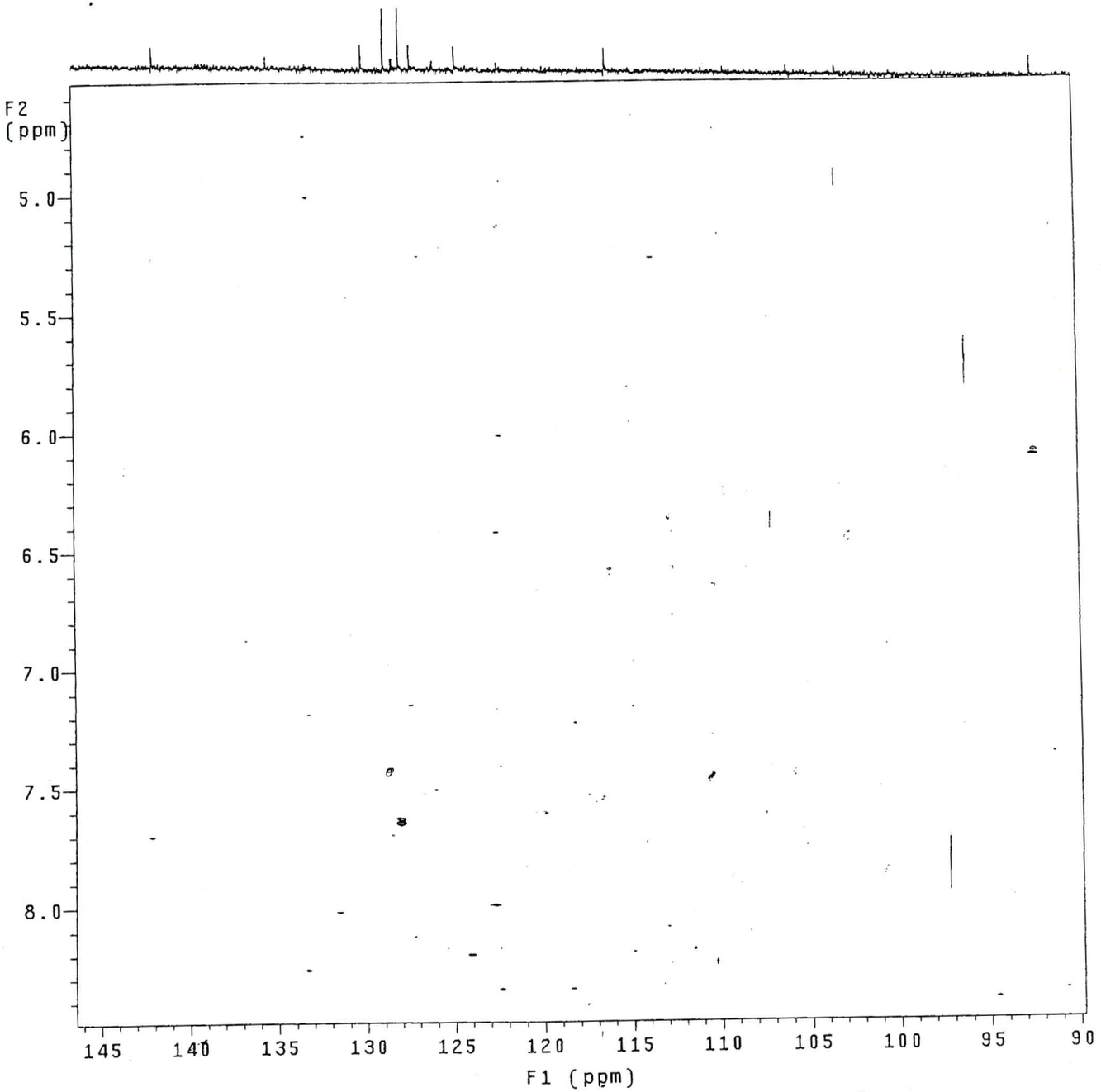
HSQC spectrum of compound **VI**, cedreprenone

HQed18.ced18 in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



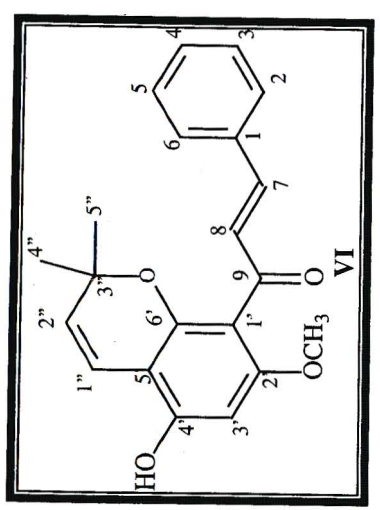


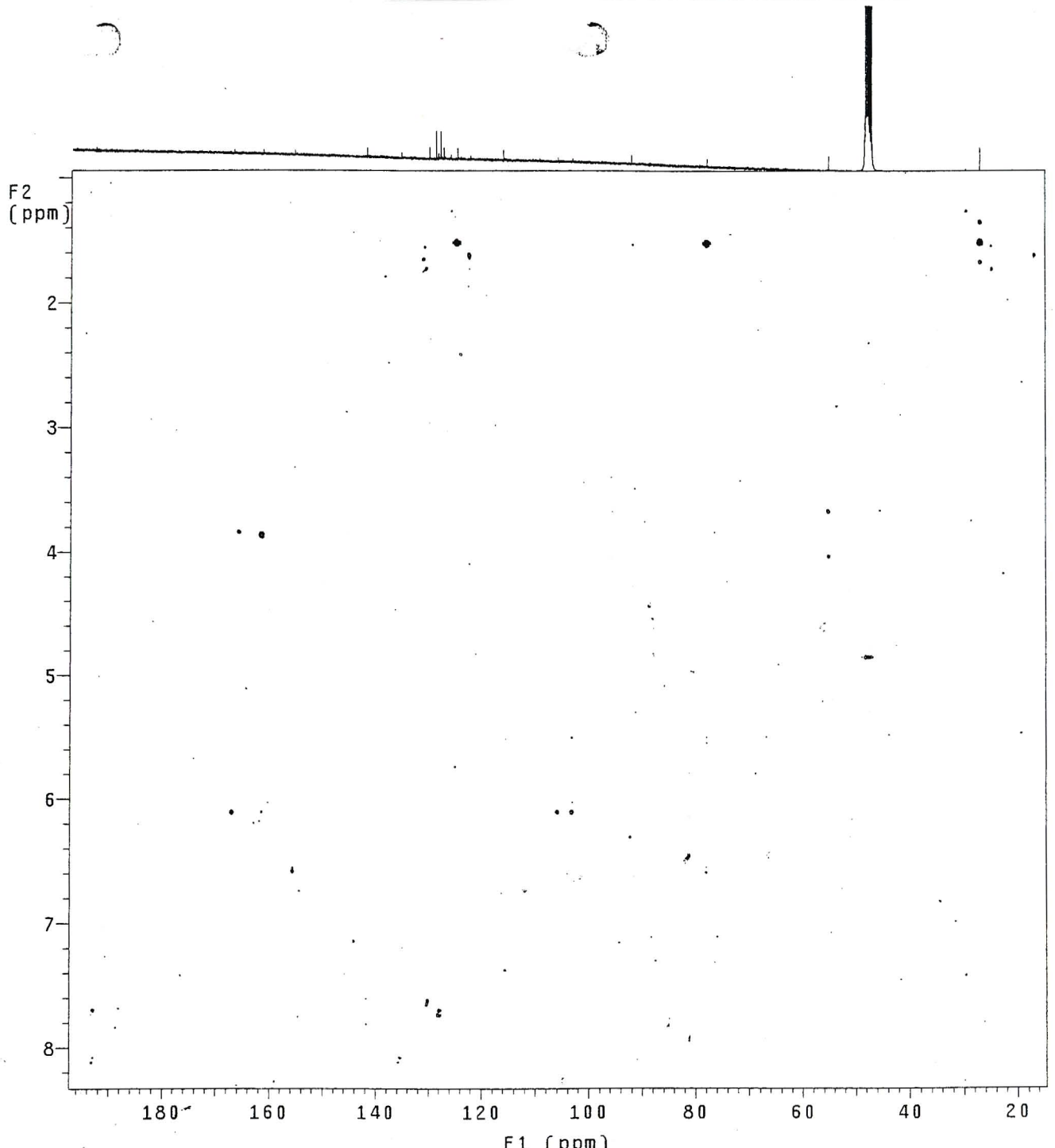


Expanded HSQC spectrum of compound **VI**, cedreprenone

HBed18.ced18 in cd3od
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da

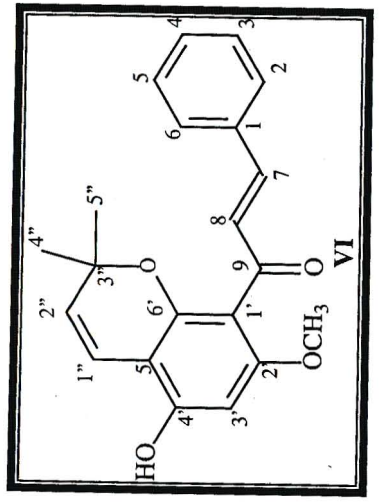


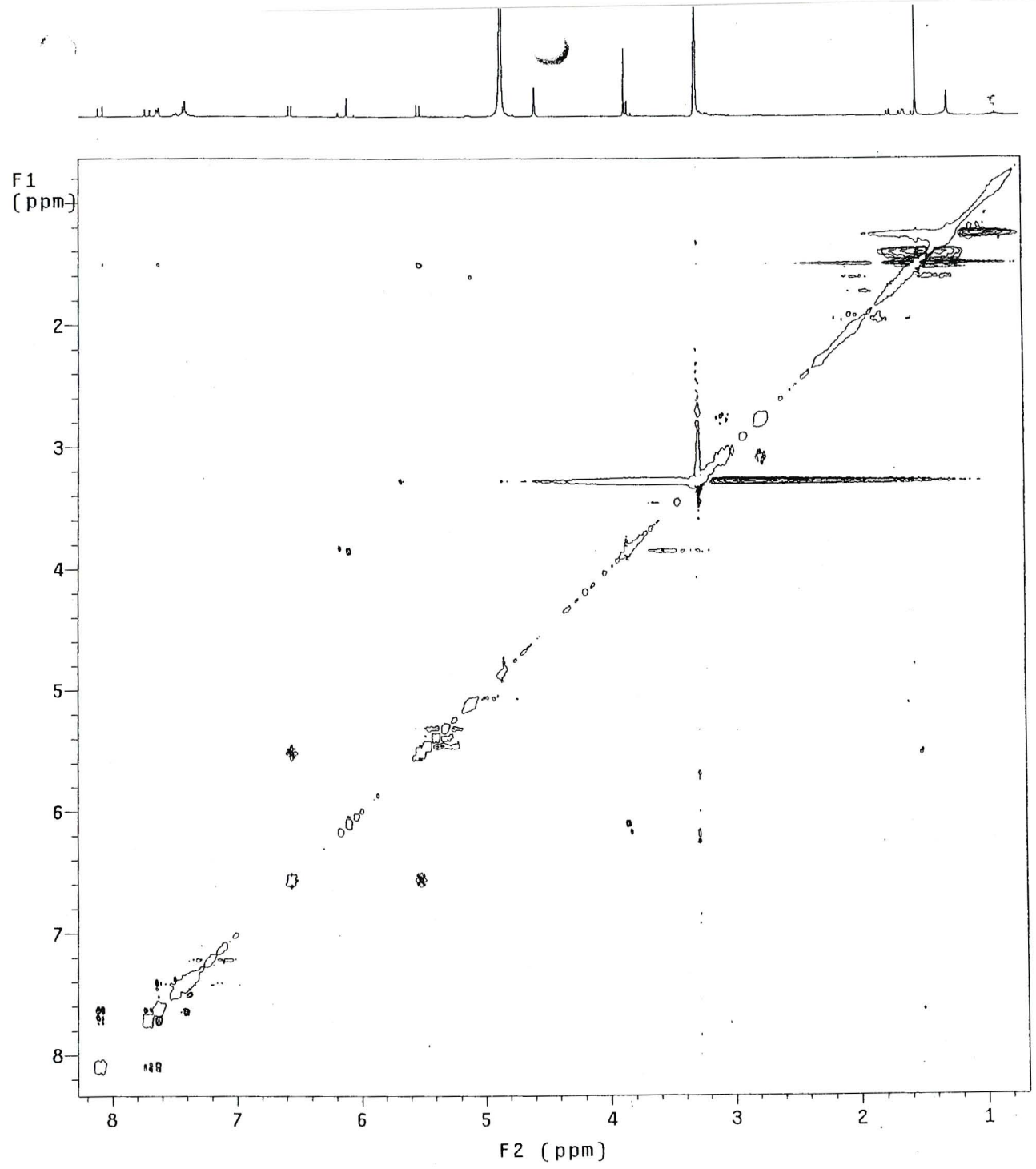


HMBC spectrum of compound **VI**, cedreprenone

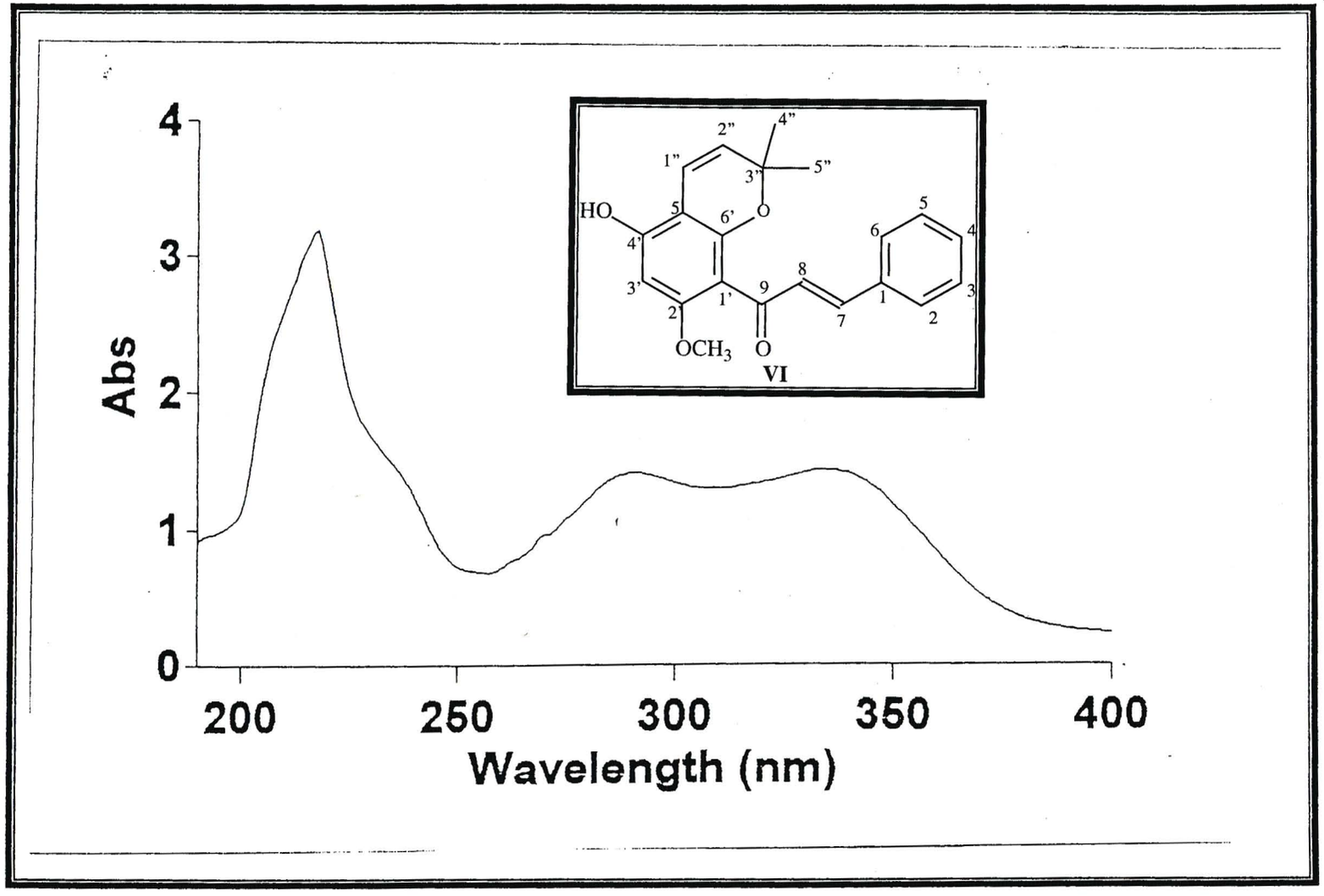
NOed18.ced18 in cd3od
Gradient NOESY expt.
with H2O suppression
probe=5mmASW

Pulse Sequence: noesy_da

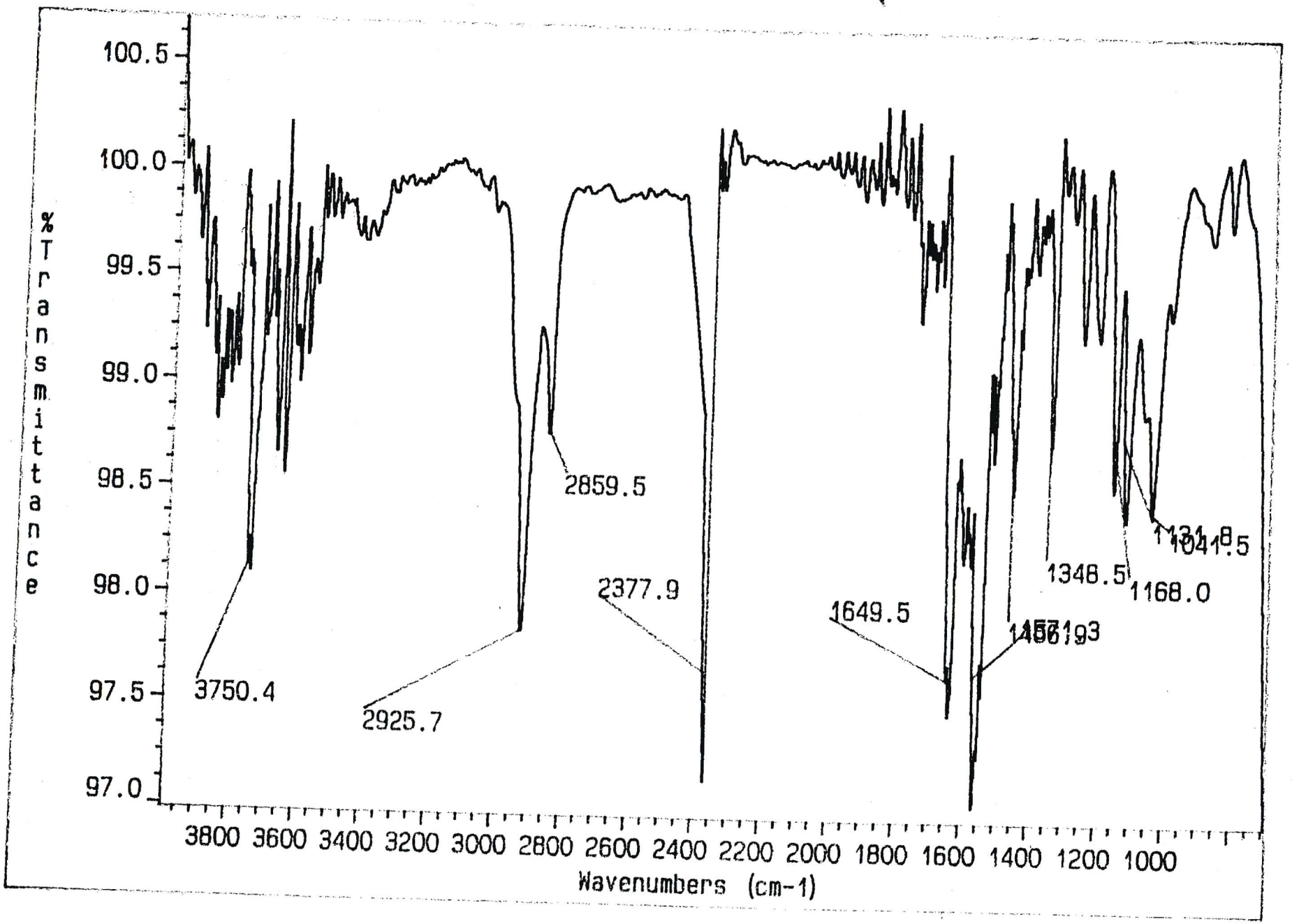




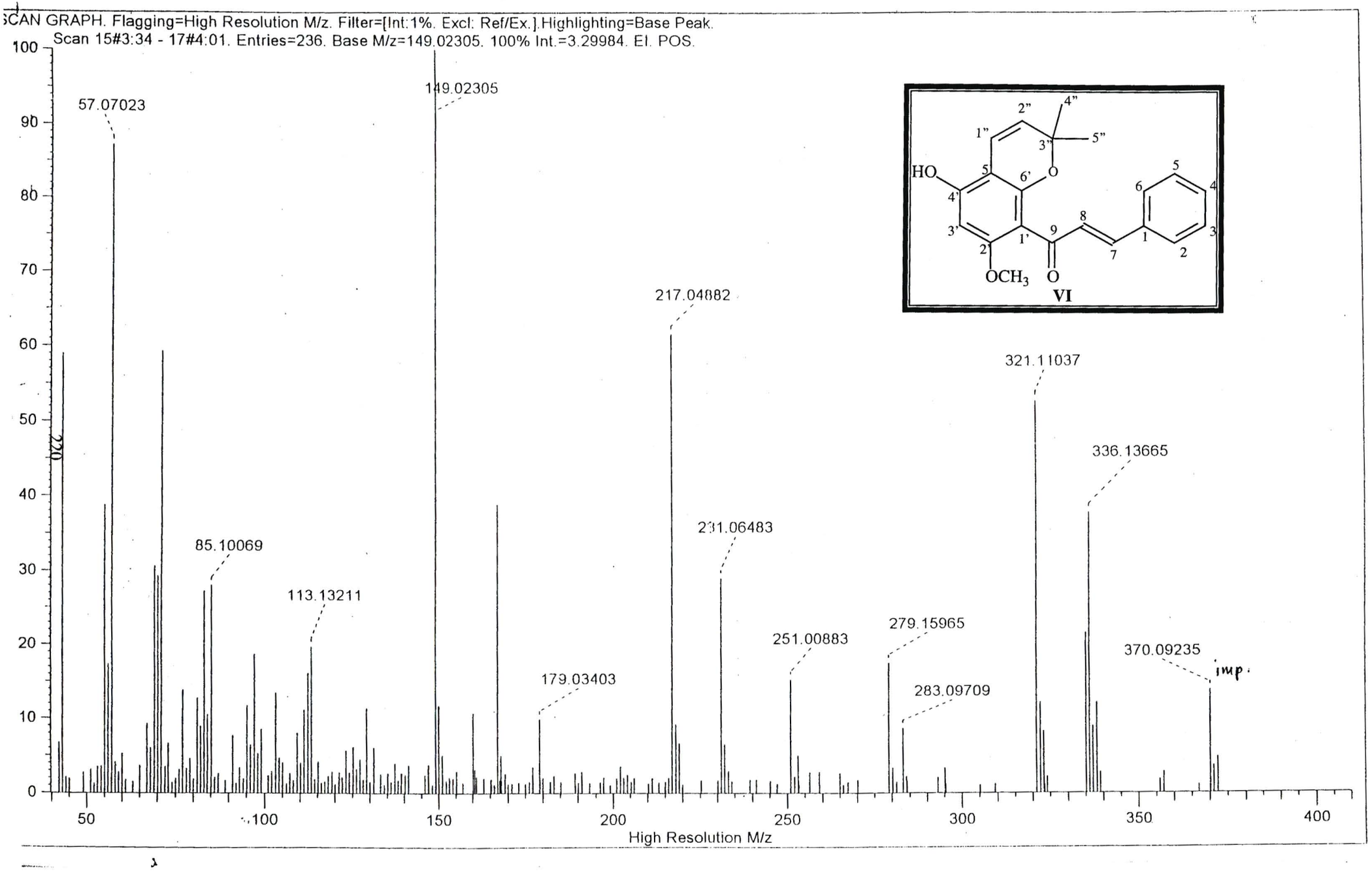
NOESY spectrum of compound **VI**, cedreprenone



Ultra violet spectrum of compound **VI,** cedreprenone



Infrared spectrum of compound **VI,** cedreprenone

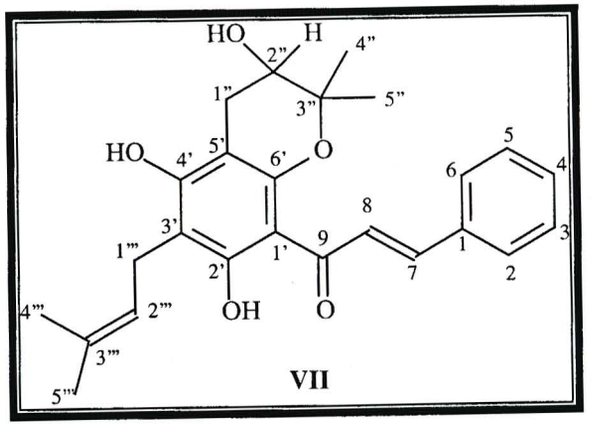


Mass spectrum of compound **VI**, cedreprenone

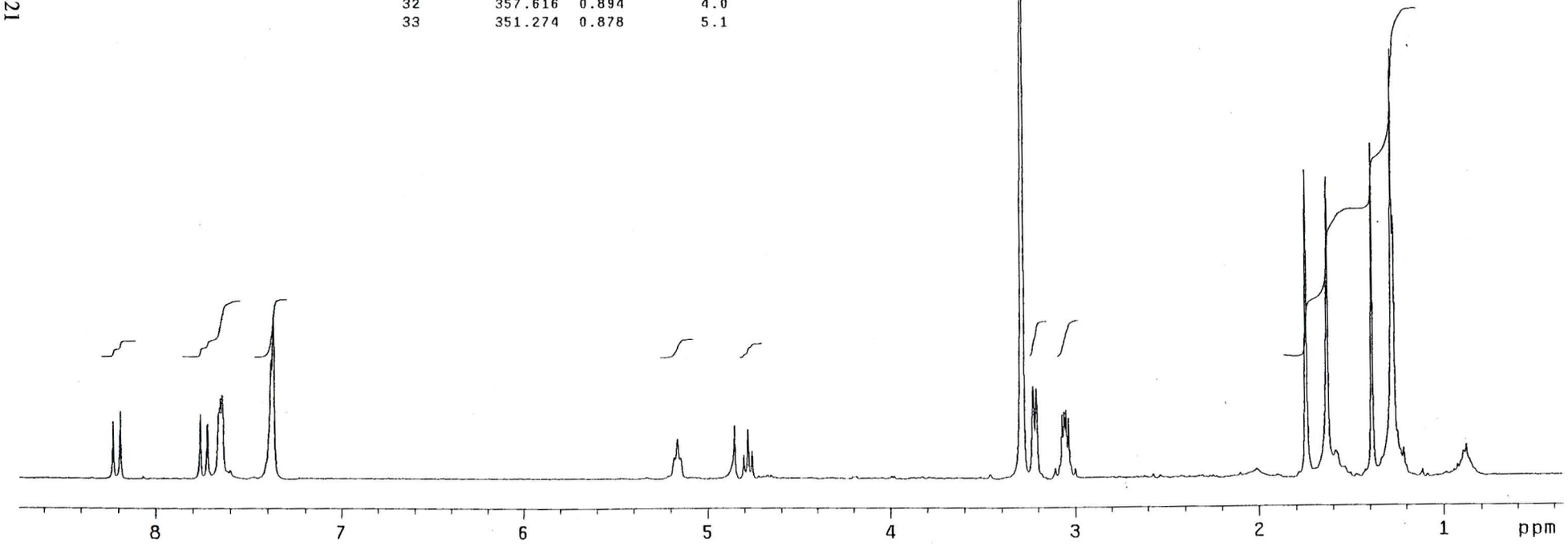
h1ed90c.ced90c in cd3od
using presat-h2o
probe=5mmASW

Pulse Sequence: presat_da

INDEX	FREQUENCY	PPM	HEIGHT
1	3291.8[illegible]	[illegible].231	9.3
2	3276.06[illegible]	8.191	11.0
3	3104.826	7.763	10.5
4	3089.278	7.724	8.9
5	3065.136	7.664	10.9
6	3061.863	7.656	13.1
7	3057.976	7.646	13.7
8	2953.431	7.384	19.6
9	2949.748	7.375	26.5
10	2072.679	5.182	3.5
11	2065.723	5.165	6.5
12	2058.972	5.148	3.5
13	1941.538	4.854	8.8
14	1921.488	4.804	3.8
15	1912.282	4.781	8.1
16	1903.280	4.759	4.5
17	1314.884	3.288	125.0
18	1290.743	3.227	15.1
19	1283.582	3.209	14.7
20	1227.525	3.069	10.4
21	1223.024	3.058	10.9
22	1218.932	3.048	11.1
23	1213.613	3.034	9.8
24	698.256	1.746	50.3
25	652.223	1.631	49.2
26	633.810	1.585	4.2
27	554.225	1.386	54.7
28	511.262	1.278	70.2
29	506.556	1.267	42.7
30	498.373	1.246	7.5
31	485.484	1.214	4.6
32	357.616	0.894	4.0
33	351.274	0.878	5.1



VII

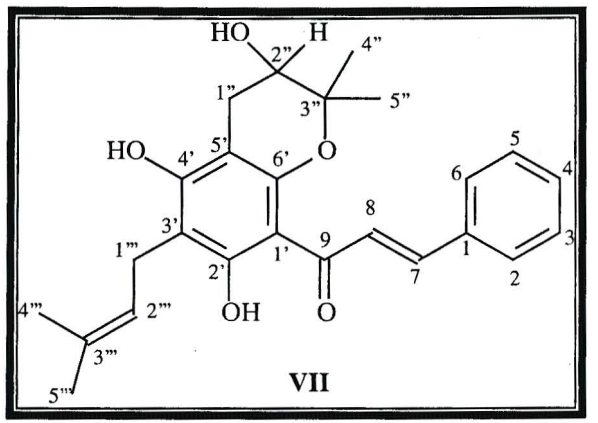


^{1}H NMR spectrum of compound **VII**, cedrediprenone

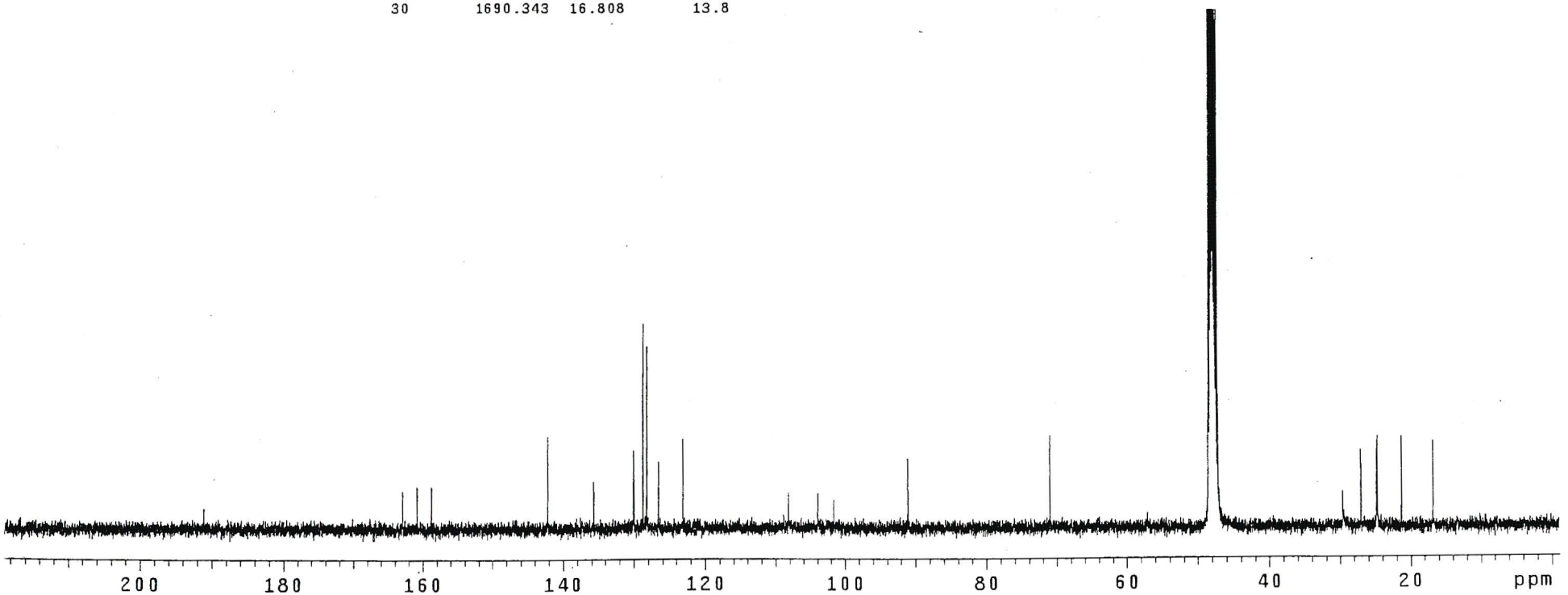
ced90c.ced90c in cd3od
probe=5mmASW

Pulse Sequence: s2pul

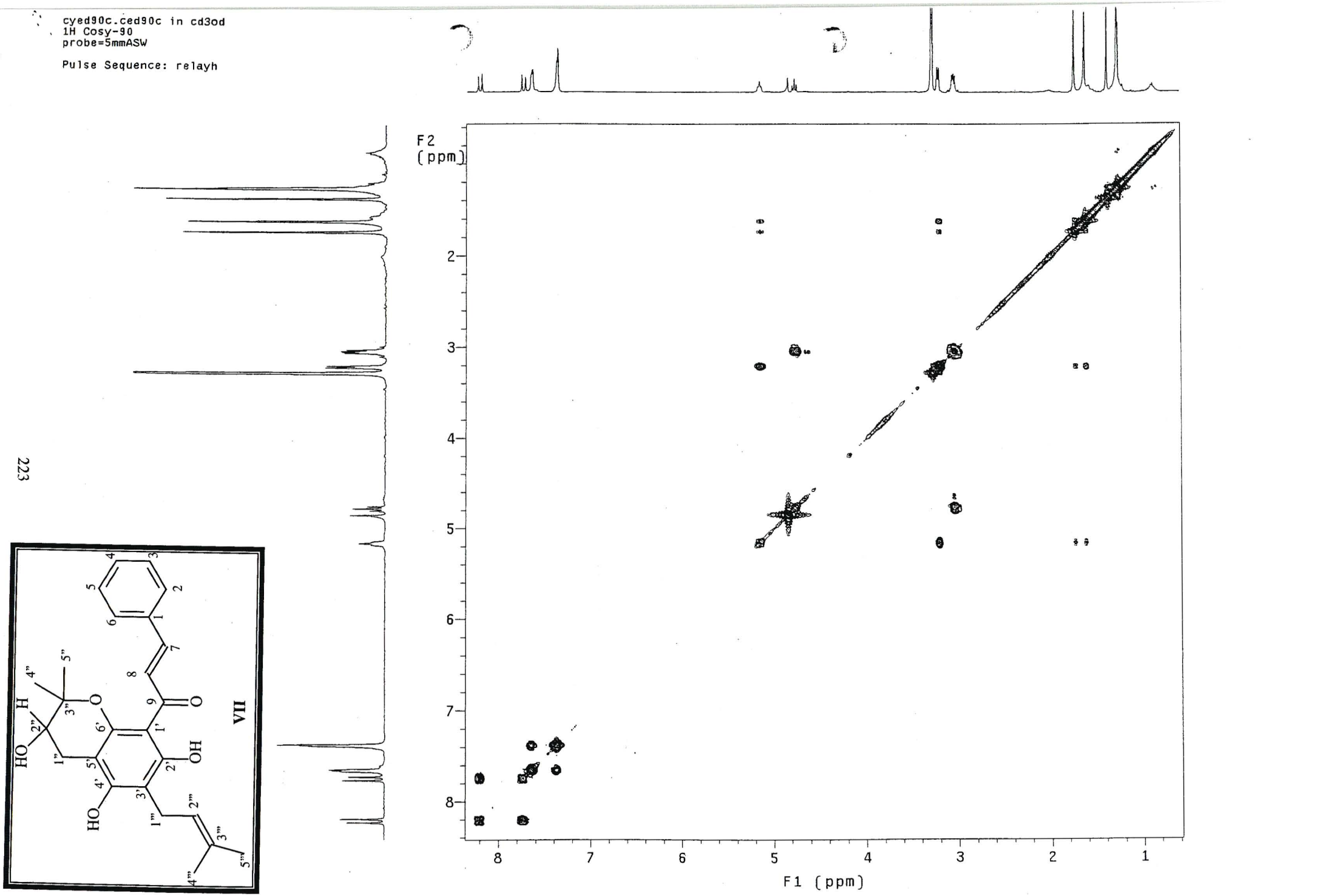
INDEX	FREQUENCY	PPM	HEIGHT
1	19220.9[illegible]	191.125	3.3
2	16386.53[illegible]	162.941	6.2
3	16174.428	160.831	6.9
4	15966.902	158.768	6.9
5	14299.065	142.184	15.0
6	13643.680	135.667	7.7
7	13082.903	130.091	7.7
8	13074.510	130.007	12.7
9	12945.569	128.725	33.1
10	12890.636	128.179	29.5
11	12725.073	126.533	10.8
12	12380.214	123.103	14.6
13	10877.940	108.166	5.8
14	10452.207	103.932	5.7
15	10219.504	101.618	4.6
16	9157.459	91.058	11.3
17	7142.475	71.022	14.9
18	4877.238	48.497	418.9
19	4855.876	48.285	1259.5
20	4834.513	48.072	2533.7
21	4813.150	47.860	3000.0
22	4791.787	47.647	2608.0
23	4770.424	47.435	1321.4
24	4749.061	47.223	441.9
25	2982.039	29.652	5.8
26	2720.343	27.050	12.4
27	2502.135	24.880	12.5
28	2486.876	24.728	14.6
29	2140.491	21.284	14.6
30	1690.343	16.808	13.8



VII



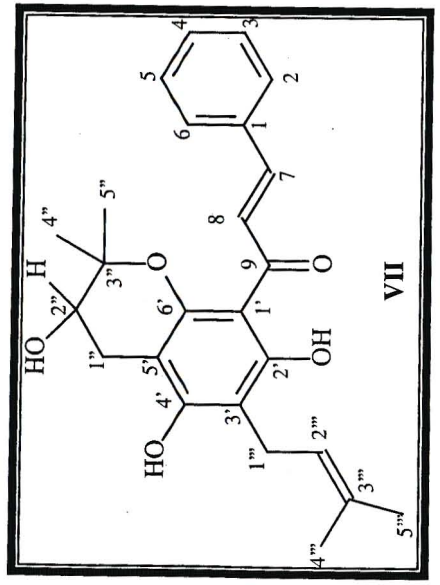
^{13}C NMR spectrum of compound **VII**, cedrediprenone

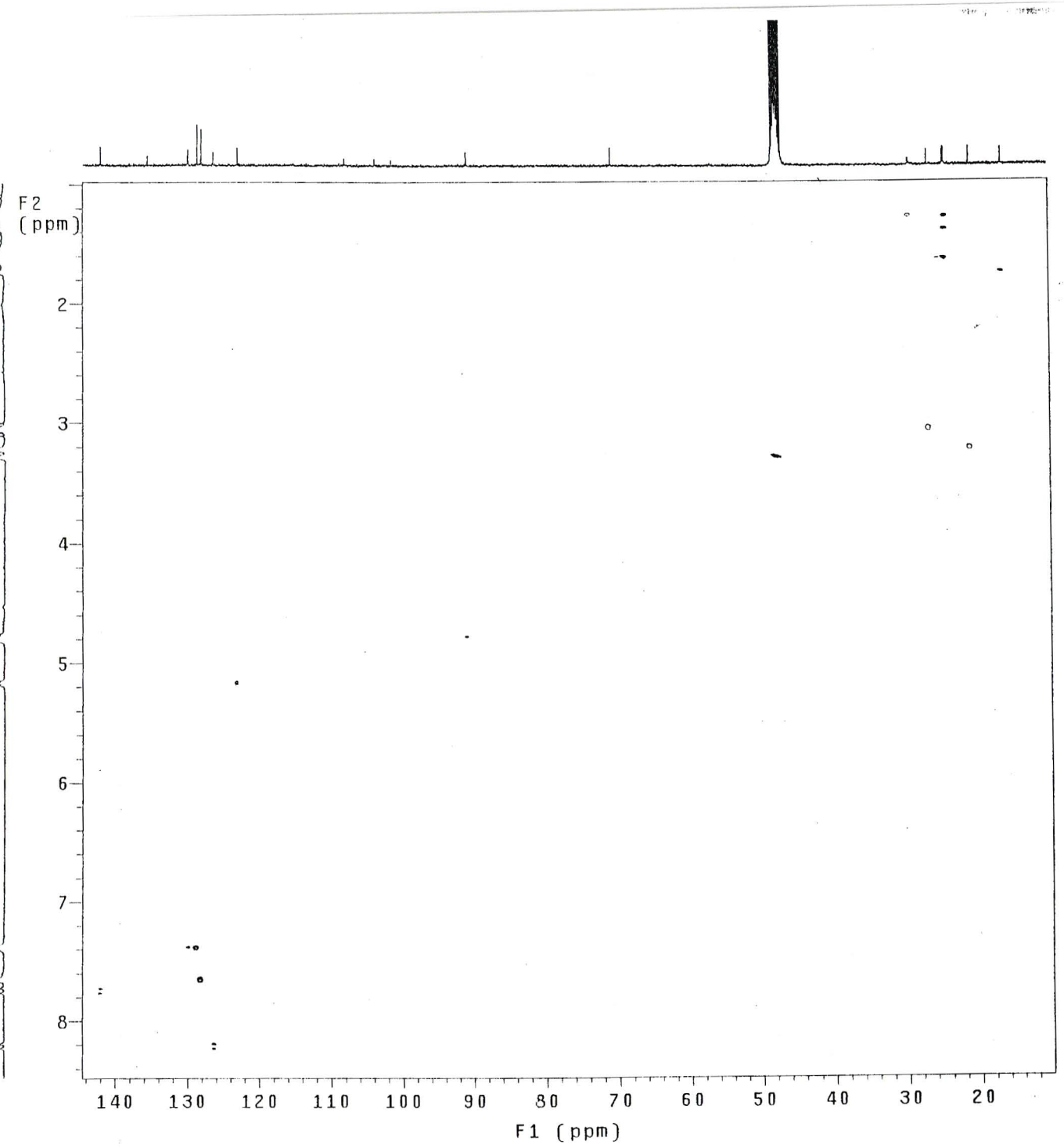


COSY spectrum of compound **VII**, cedrediprenone

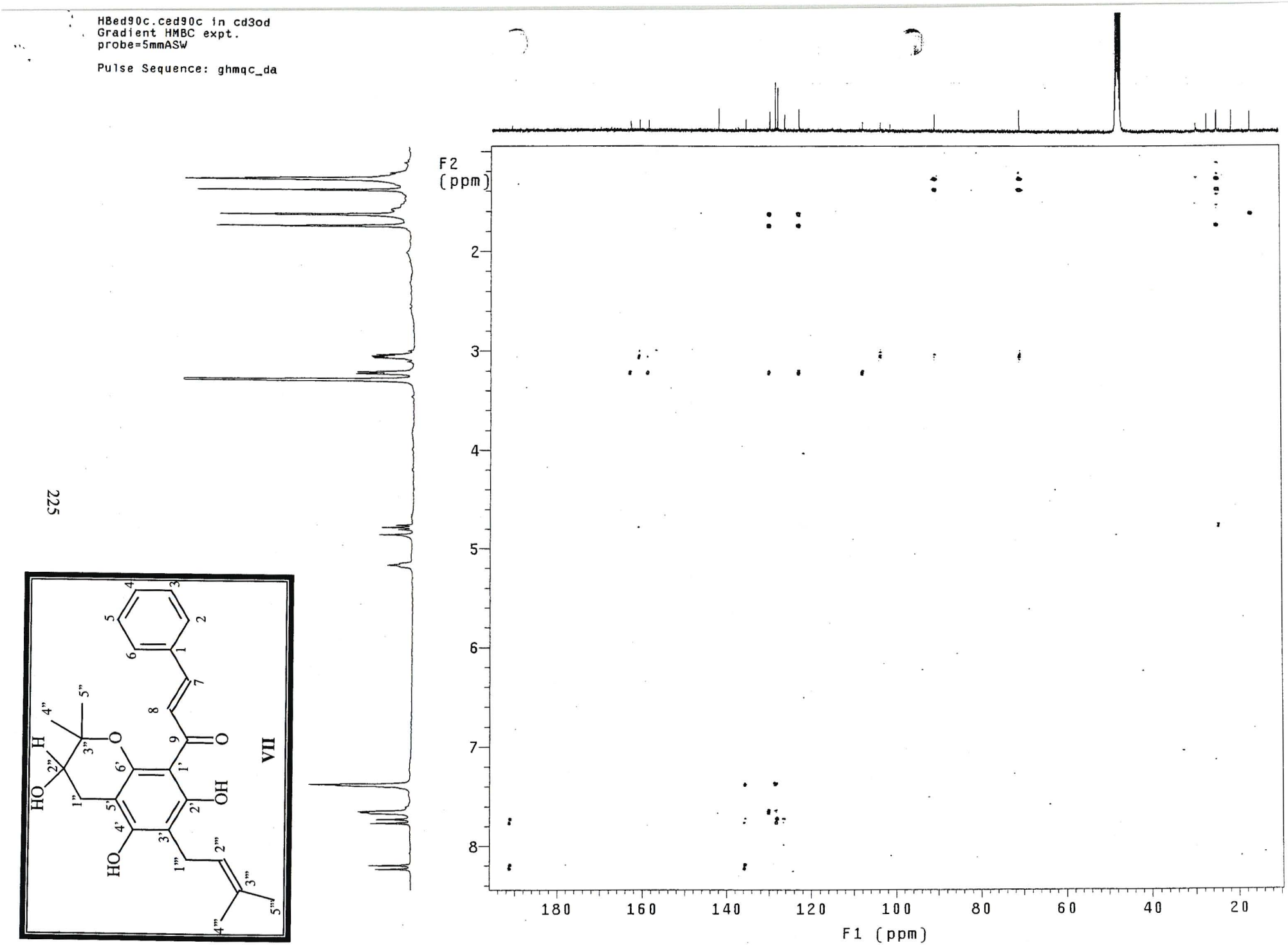
HQed90c.ced90c in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da





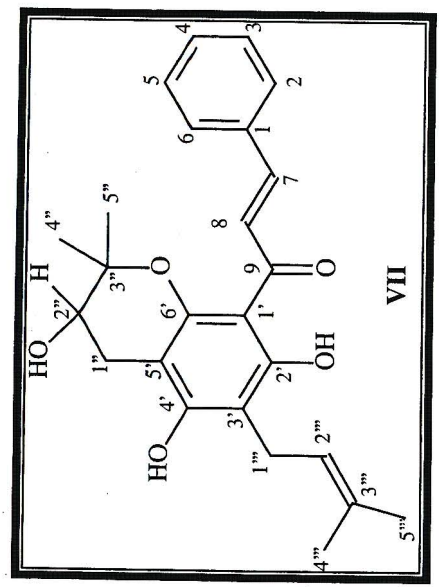
HSQC spectrum of compound **VII**, cedrediprenone

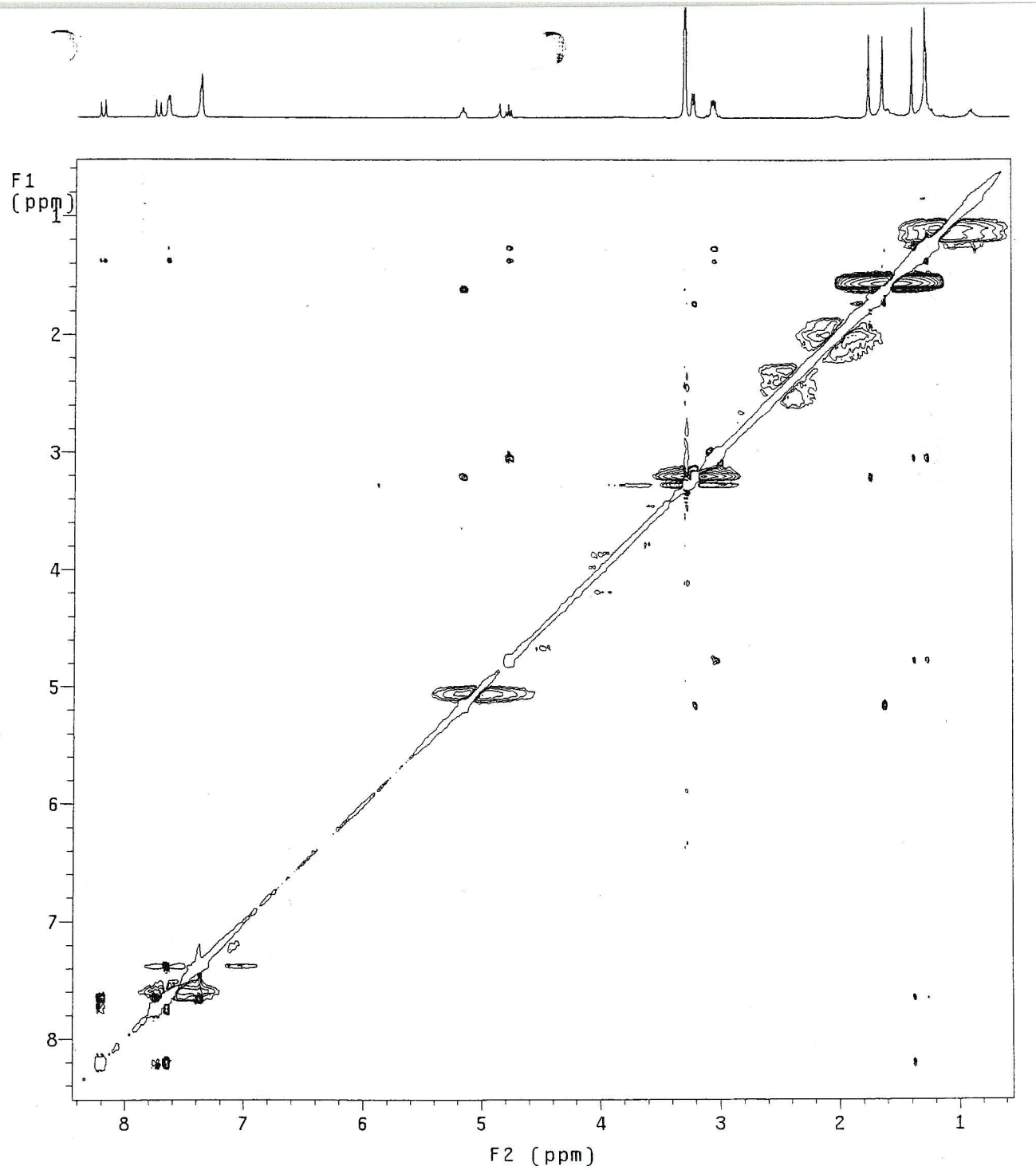


HMBC spectrum of compound **VII,** cedrediprenone

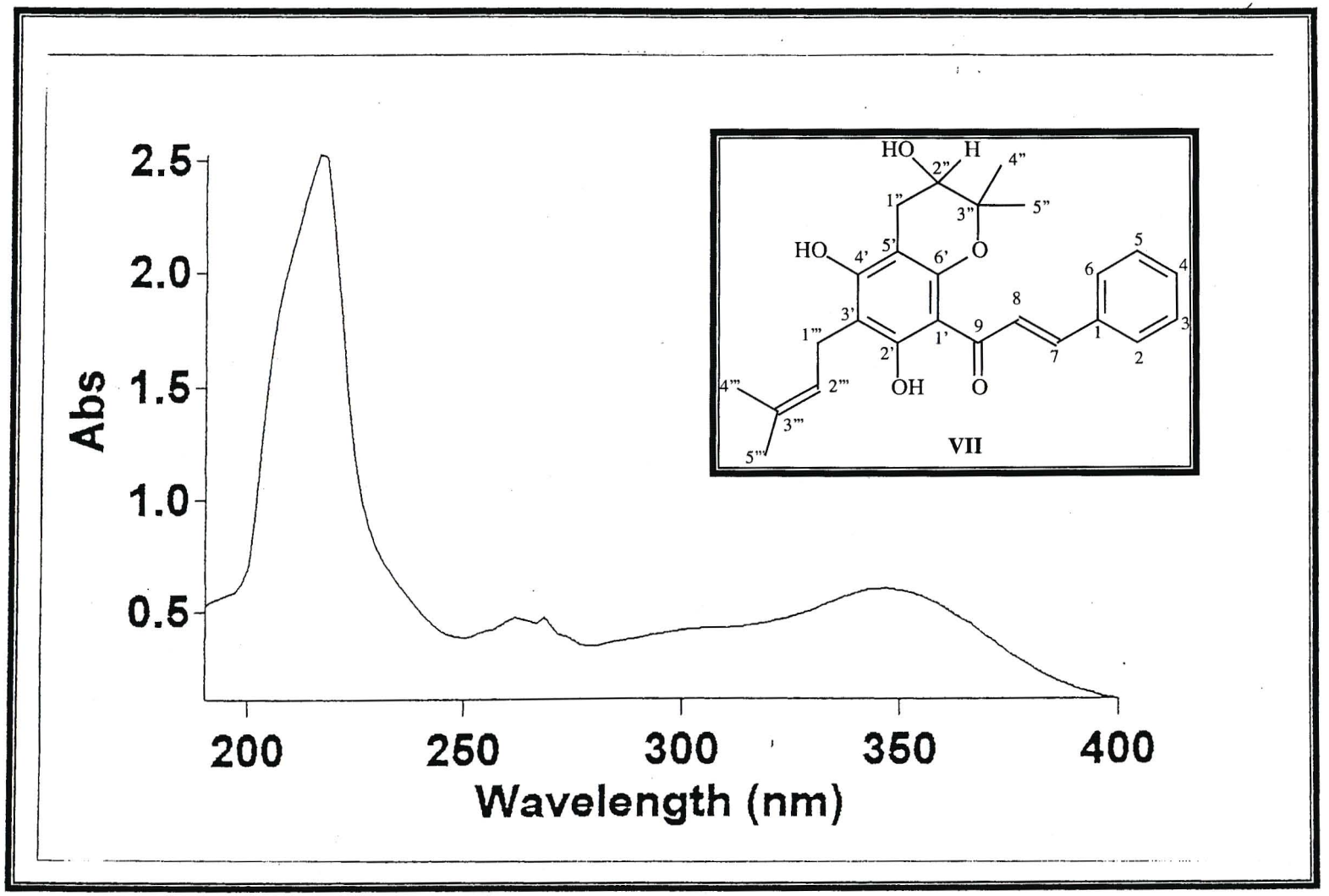
NOed90c.ced90c in cd3od
Gradient NOESY expt.
using presat-h2o
probe=5mmASW

Pulse Sequence: noesy_da



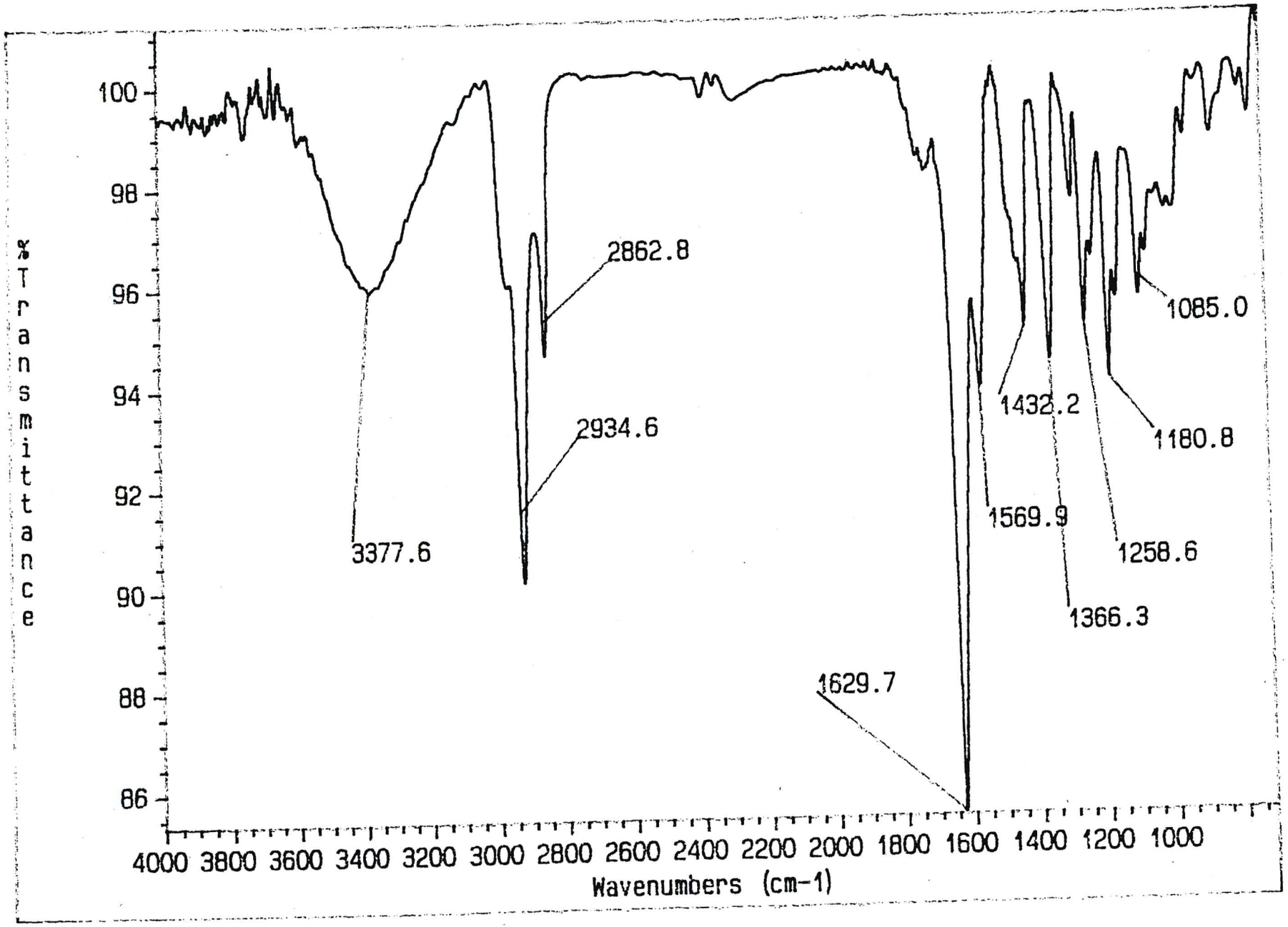


NOESY spectrum of compound **VII**, cedrediprenone

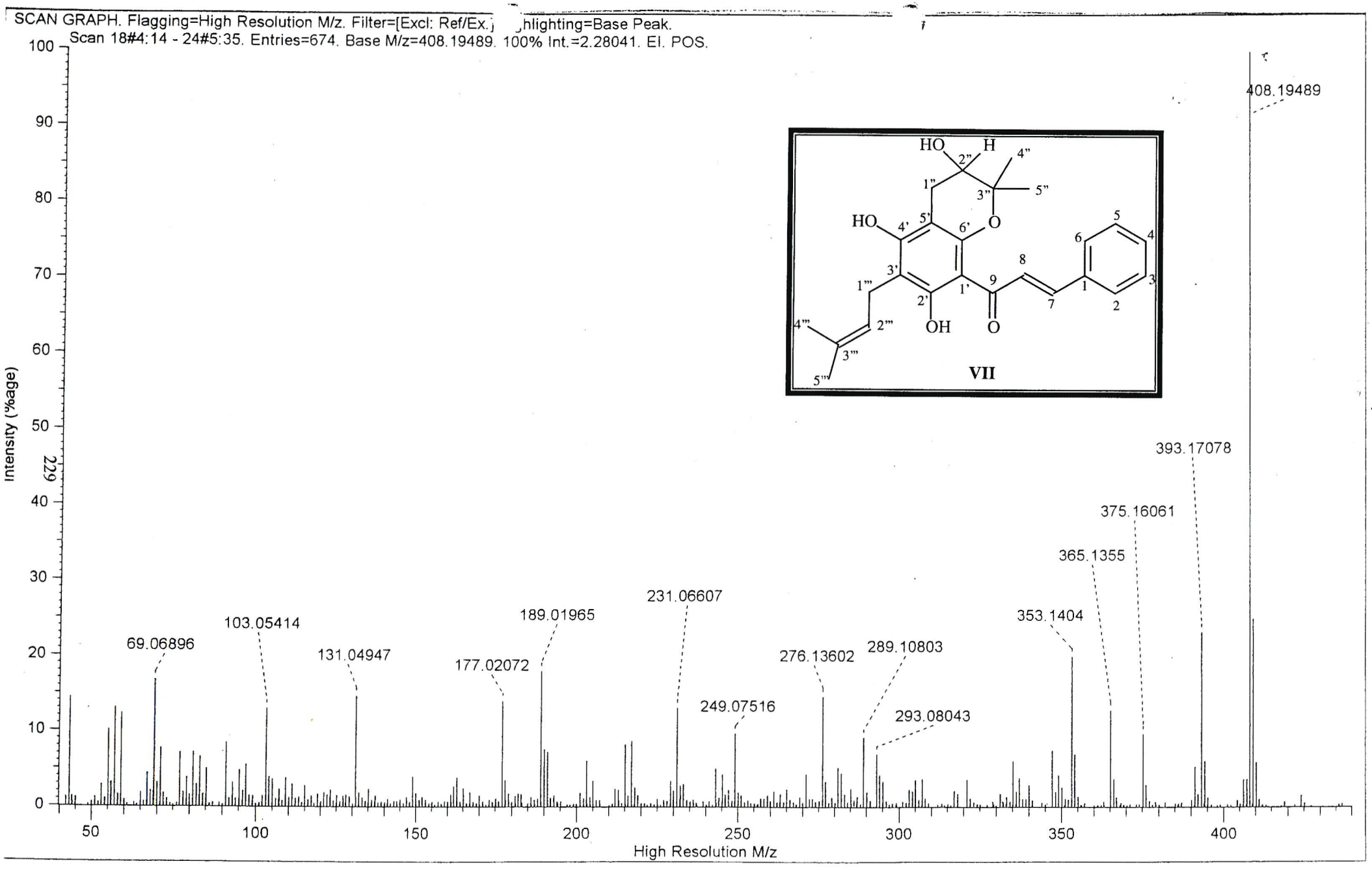


Ultra violet spectrum of compound **VII,** cedrediprenone

CED90C



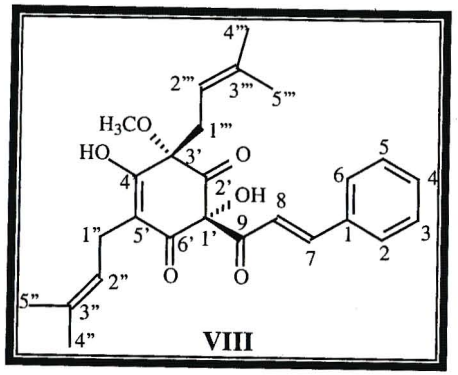
Infrared spectrum of compound **VII**, cedrediprenone



Mass spectrum of compound **VII**, cedrediprenone

Appendix II

NMR, Ultra violet, Infrared and Mass spectra for Chapter 4. Extractives from *Cedrelopsis microfoliata*

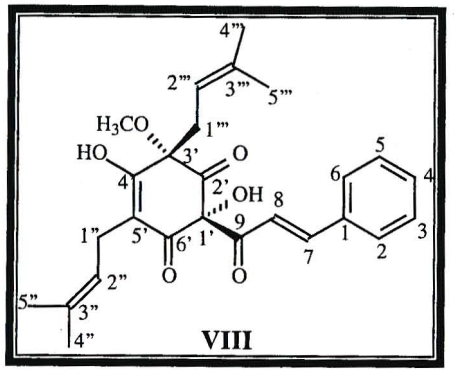


VIII

^{1}H NMR spectrum of compound **VIII,** microfolian in $CDCl_3$

hmic40.mic40 in cd3od
probe=5mmASW

Pulse Sequence: s2pul



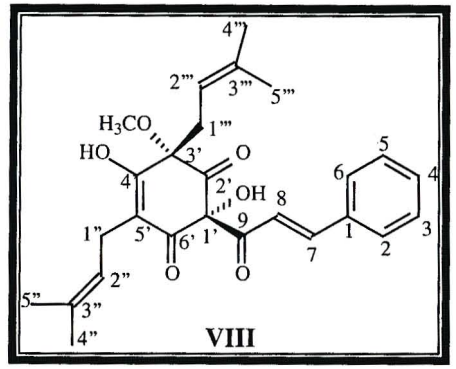
VIII

^{1}H NMR spectrum of compound **VIII,** microfolian in CD_3OD

cmic40.mic40 in cd3od
probe=5mmASW

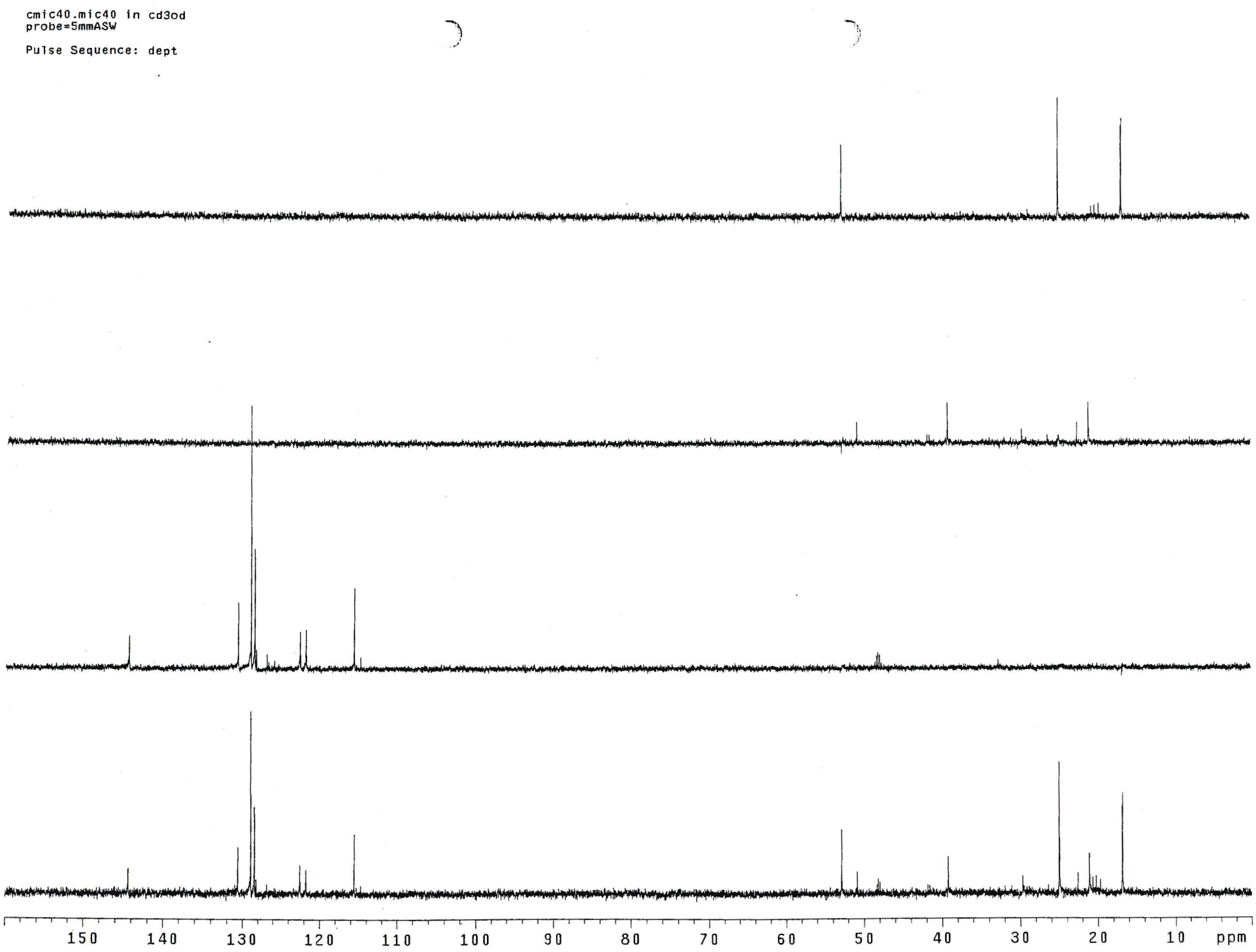
Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	19593.2	194.827	6.2	40	2079.454	20.677	4.2
2	19265.190	191.565	4.5	41	2036.728	20.252	4.7
3	18564.027	184.593	6.9	42	1699.499	16.899	31.7
4	16921.368	168.259	5.5	43	1689.580	16.800	33.3
5	14511.169	144.293	12.3				
6	13781.777	137.040	12.6				
7	13610.110	135.333	20.7				
8	13205.740	131.312	9.2				
9	13130.206	130.561	18.7				
10	12963.881	128.907	74.0				
11	12913.525	128.406	37.2				
12	12897.503	128.247	9.4				
13	12891.399	128.186	8.0				
14	12754.829	126.828	5.0				
15	12322.992	122.534	13.1				
16	12245.933	121.768	12.9				
17	11619.540	115.540	25.3				
18	11536.377	114.713	4.7				
19	11524.170	114.591	7.3				
20	10878.703	108.173	5.3				
21	8491.393	84.435	10.0				
22	5316.705	52.867	19.9				
23	5117.572	50.887	13.6				
24	4880.290	48.527	119.1				
25	4858.927	48.315	341.1				
26	4844.431	48.171	19.2				
27	4837.564	48.103	674.6				
28	4816.201	47.890	800.0				
29	4794.839	47.678	704.3				
30	4773.476	47.465	353.3				
31	4752.113	47.253	111.8				
32	4180.654	41.571	4.7				
33	3945.661	39.234	13.2				
34	3127.765	31.101	4.1				
35	2984.328	29.675	5.3				
36	2513.580	24.994	44.0				
37	2509.002	24.948	35.9				
38	2273.247	22.604	12.9				
39	2123.706	21.117	14.0				



VIII

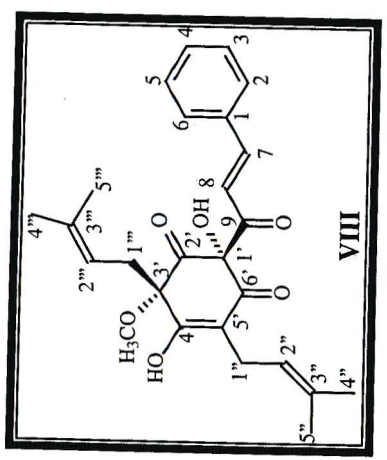
^{13}C NMR spectrum of compound **VIII**, microfolian in CD_3OD

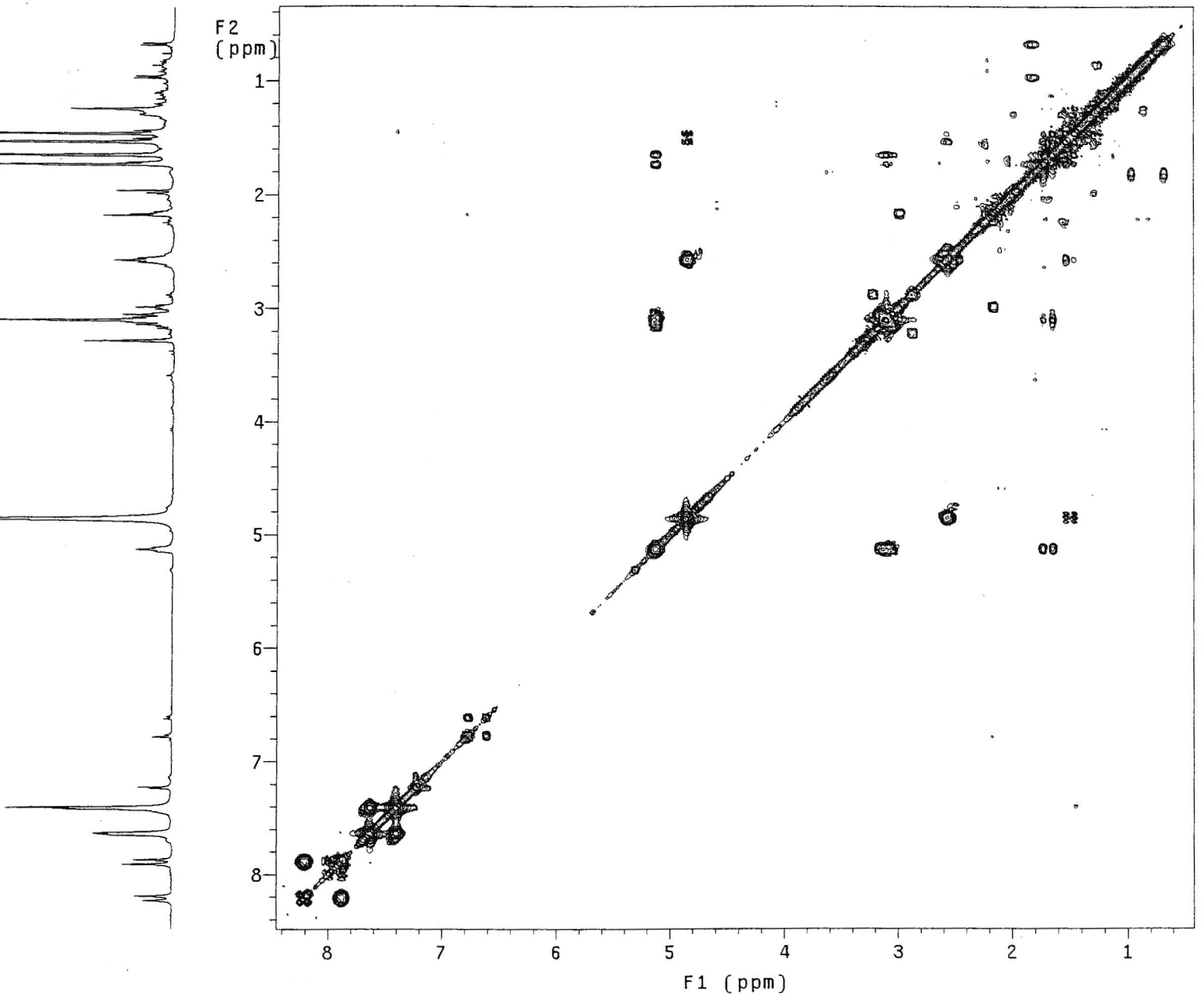


ADEPT spectrum of compound **VIII**, microfolian

cymic40.mic40 in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

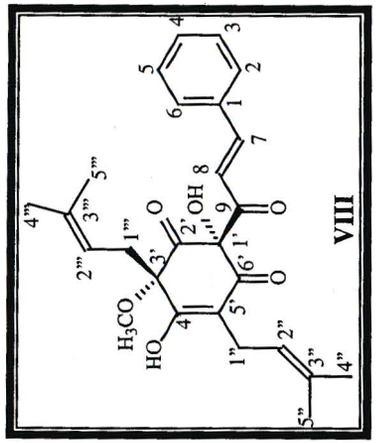


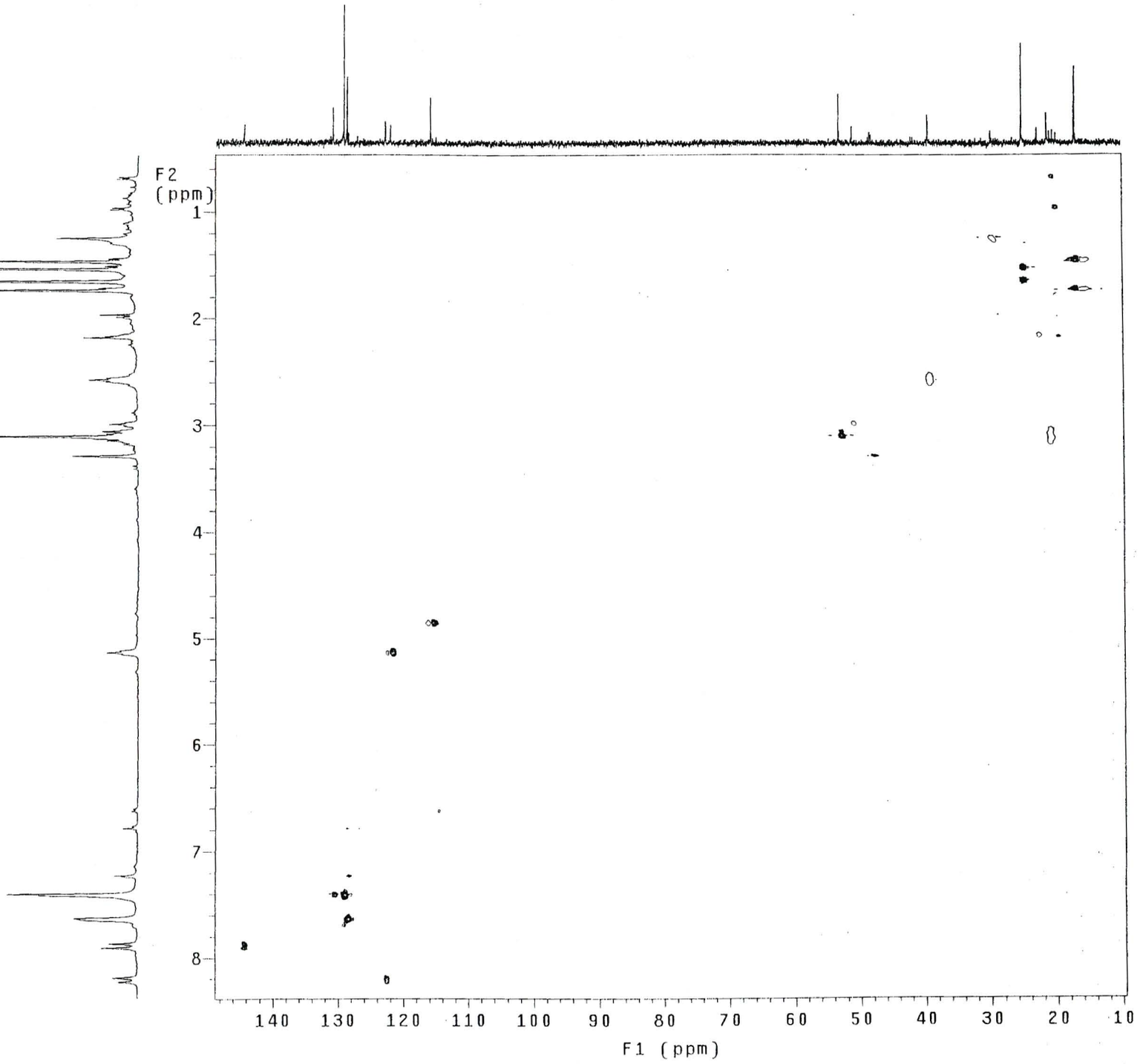


COSY spectrum of compound **VIII,** microfolian

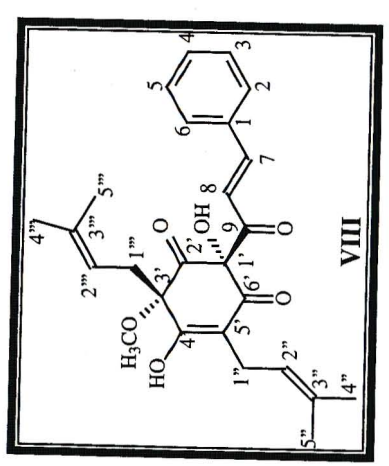
HQmic40.mic40 in cd3od
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da





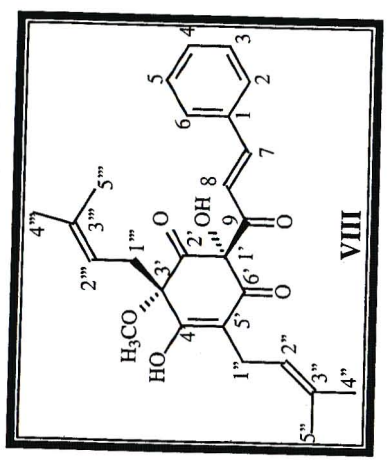
HSQC spectrum of compound **VIII**, microfolian

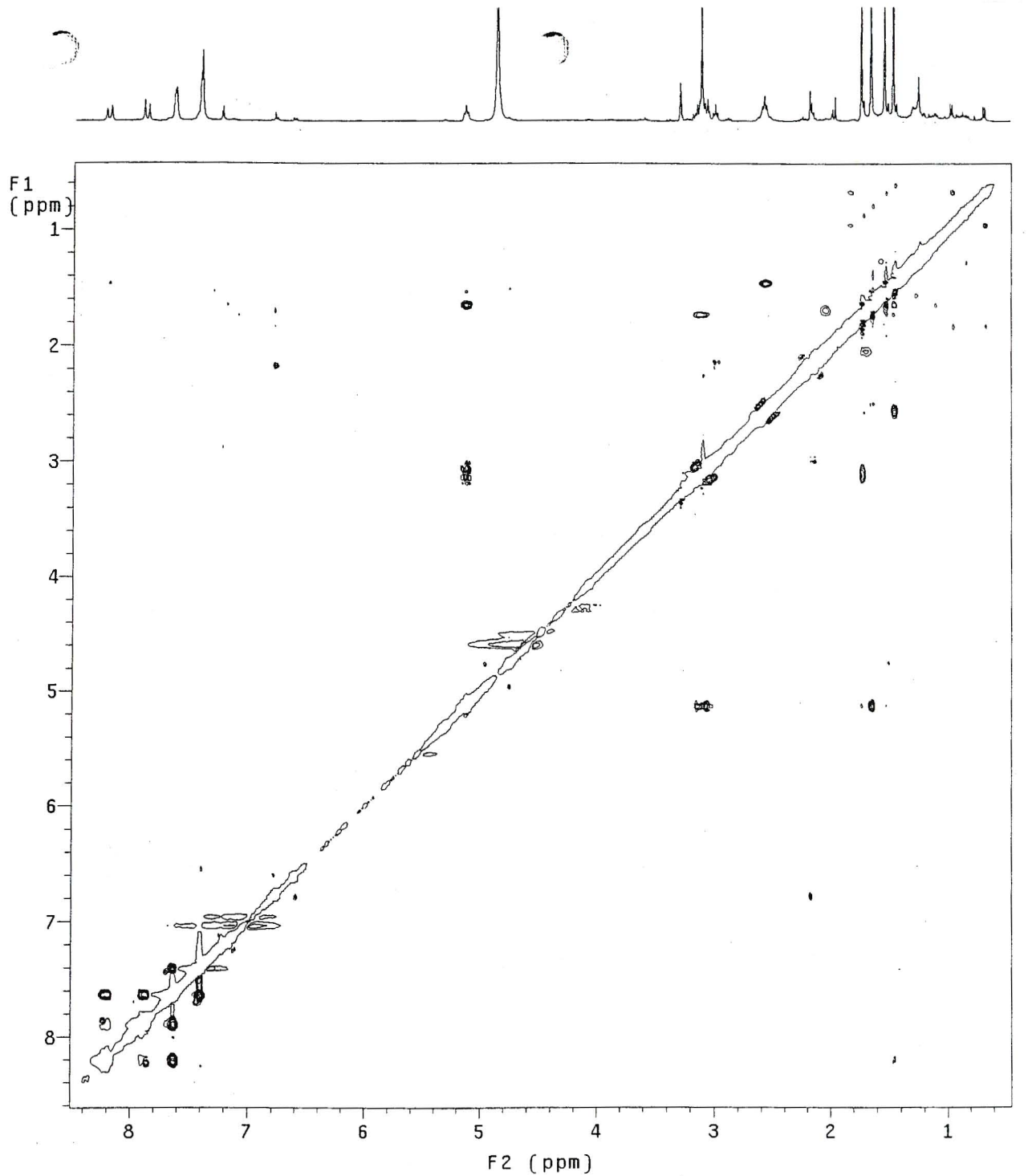


HMBC spectrum of compound **VIII**, microfolian

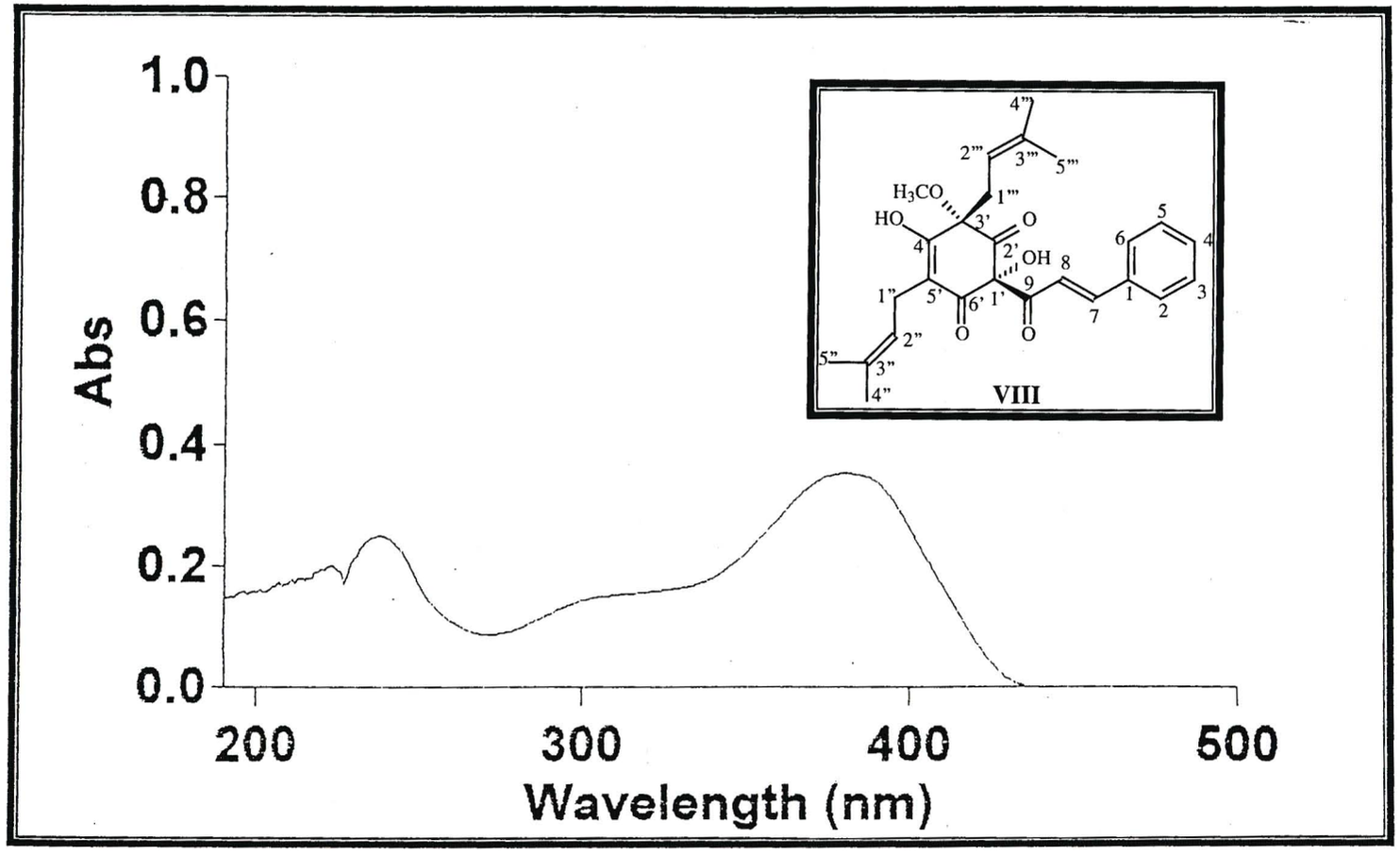
NOmic40.mic40 in cd3od
Gradient NOESY expt.
using presat h2o
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

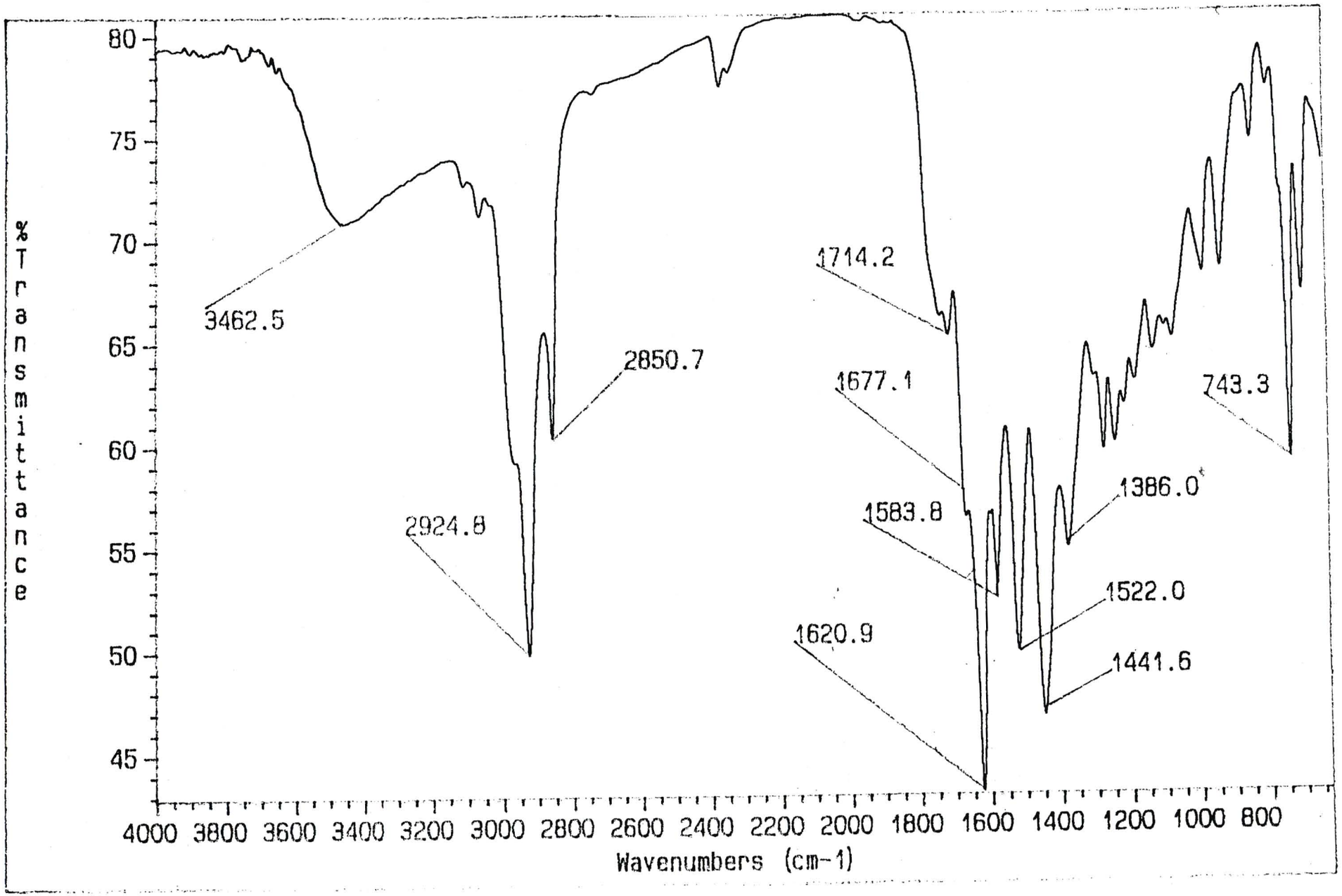




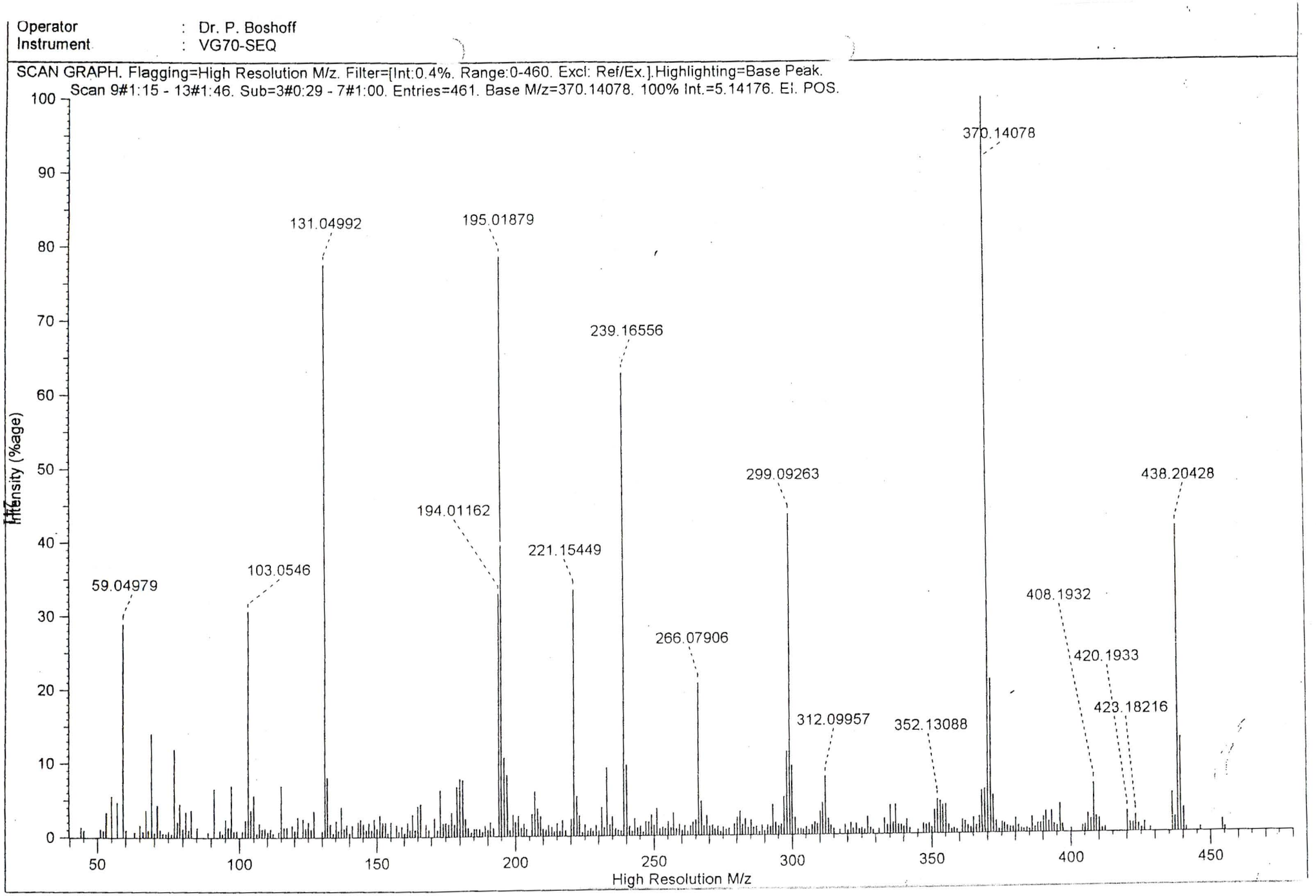
NOESY spectrum of compound **VIII,** microfolian



Ultra violet spectrum of compound **VIII,** microfolian



Infrared spectrum of compound **VIII,** microfolian

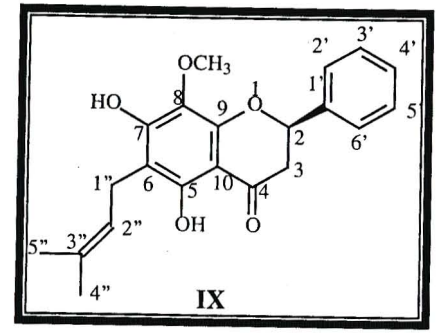


Mass spectrum of compound **VIII**, microfolian

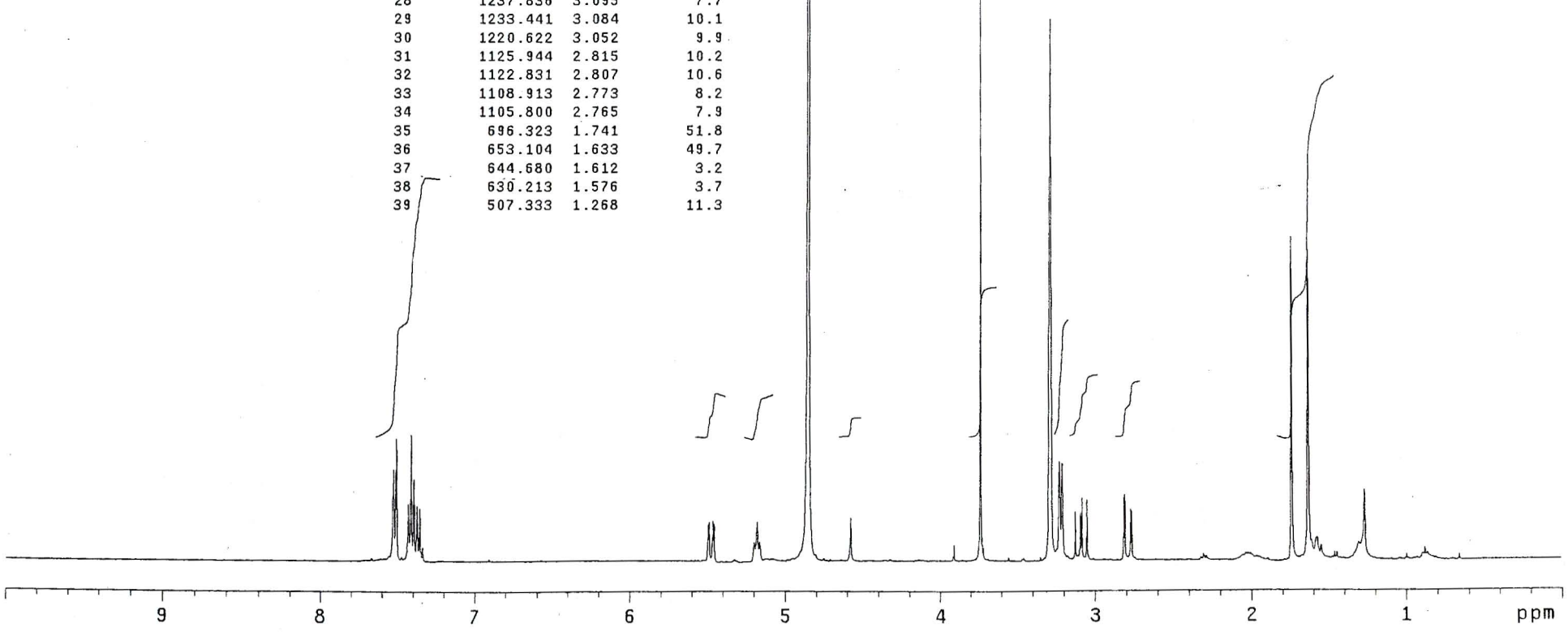
hmi19a.mic19a in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	3009.9	7.526	14.8				
2	3002.85	7.508	19.7				
3	2972.804	7.433	6.9				
4	2971.522	7.430	9.2				
5	2969.691	7.425	4.5				
6	2964.563	7.412	20.3				
7	2957.055	7.394	13.2				
8	2948.814	7.373	8.9				
9	2944.053	7.361	3.2				
10	2941.672	7.355	8.5				
11	2197.984	5.496	5.9				
12	2194.870	5.488	6.3				
13	2185.164	5.464	6.4				
14	2182.234	5.456	5.9				
15	2073.089	5.183	5.1				
16	2071.807	5.180	6.2				
17	2070.526	5.177	4.9				
18	2064.482	5.162	3.0				
19	1941.419	4.854	600.0				
20	1831.542	4.579	6.9				
21	1494.767	3.737	113.8				
22	1316.399	3.291	66.5				
23	1314.934	3.288	87.1				
24	1313.469	3.284	64.7				
25	1291.310	3.229	16.0				
26	1284.168	3.211	15.7				
27	1250.655	3.127	7.9				
28	1237.836	3.095	7.7				
29	1233.441	3.084	10.1				
30	1220.622	3.052	9.9				
31	1125.944	2.815	10.2				
32	1122.831	2.807	10.6				
33	1108.913	2.773	8.2				
34	1105.800	2.765	7.9				
35	696.323	1.741	51.8				
36	653.104	1.633	49.7				
37	644.680	1.612	3.2				
38	630.213	1.576	3.7				
39	507.333	1.268	11.3				



IX

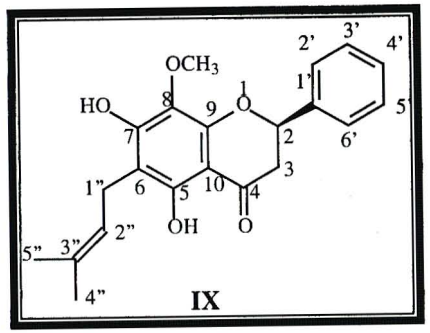


^{1}H NMR spectrum of compound **IX,** microfolione .

cmi19a.mic19a in cd3od
probe=5mmASW

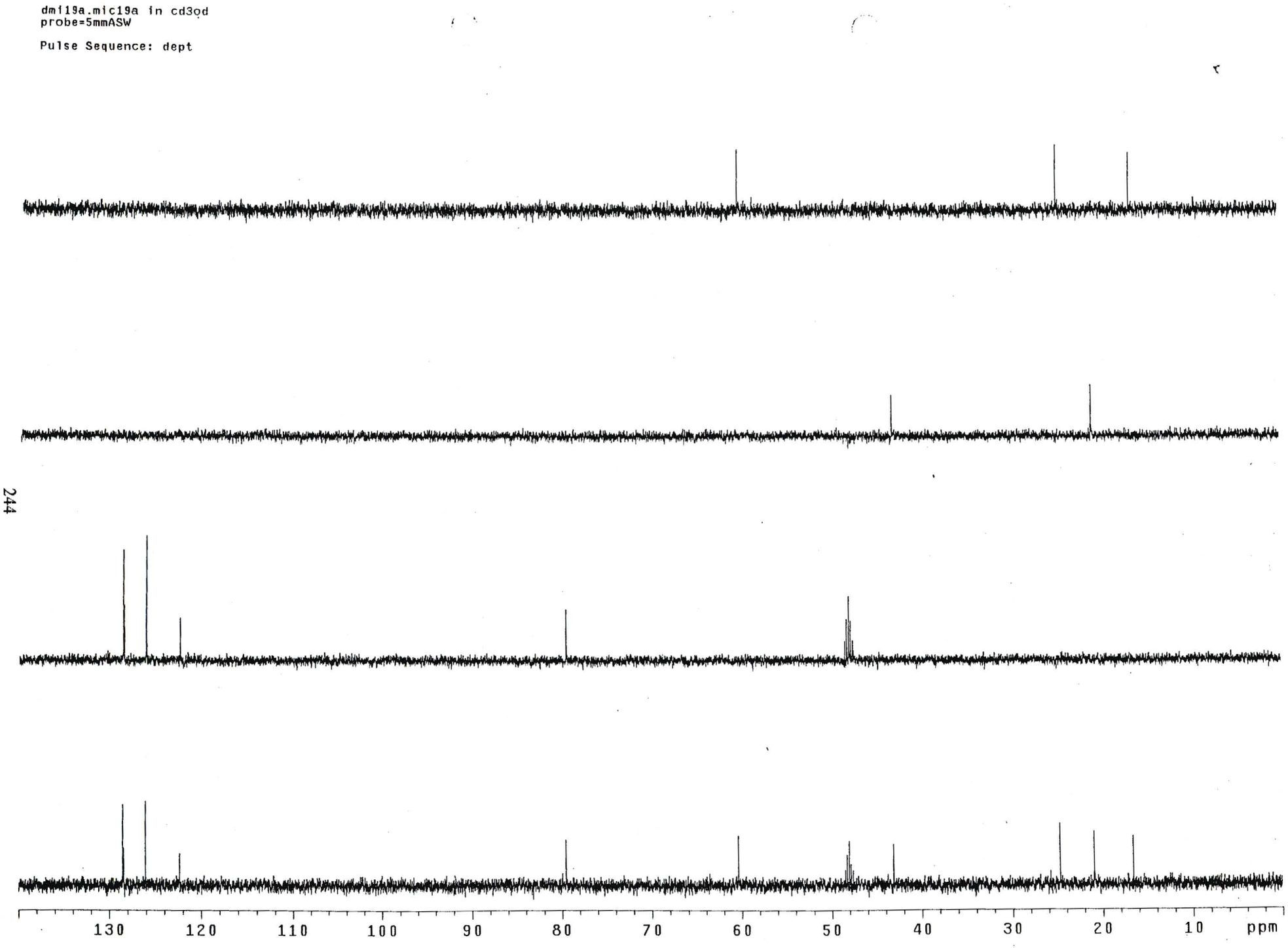
Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	19732.88	196.215	6.9
2	15858.561	157.691	4.7
3	15776.924	156.879	5.1
4	15265.739	151.796	5.8
5	14003.036	139.240	7.4
6	13134.021	130.599	7.6
7	12922.681	128.498	37.3
8	12913.525	128.406	13.0
9	12901.318	128.285	4.1
10	12675.481	126.039	28.9
11	12304.681	122.352	13.3
12	10906.170	108.446	7.4
13	10234.763	101.770	4.9
14	8003.860	79.587	13.7
15	6081.194	60.469	14.0
16	4877.238	48.497	268.9
17	4868.846	48.414	15.3
18	4855.876	48.285	811.0
19	4847.483	48.201	30.1
20	4834.513	48.072	1655.5
21	4826.120	47.989	34.8
22	4813.150	47.860	2000.0
23	4804.757	47.776	29.1
24	4791.787	47.647	1760.5
25	4770.424	47.435	892.7
26	4749.061	47.223	294.5
27	4348.505	43.240	12.1
28	2499.084	24.850	13.4
29	2119.891	21.079	11.6
30	1685.765	16.763	11.4



IX

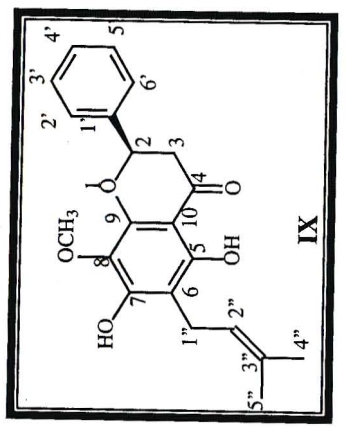
^{13}C NMR spectrum of compound **IX**, microfolione

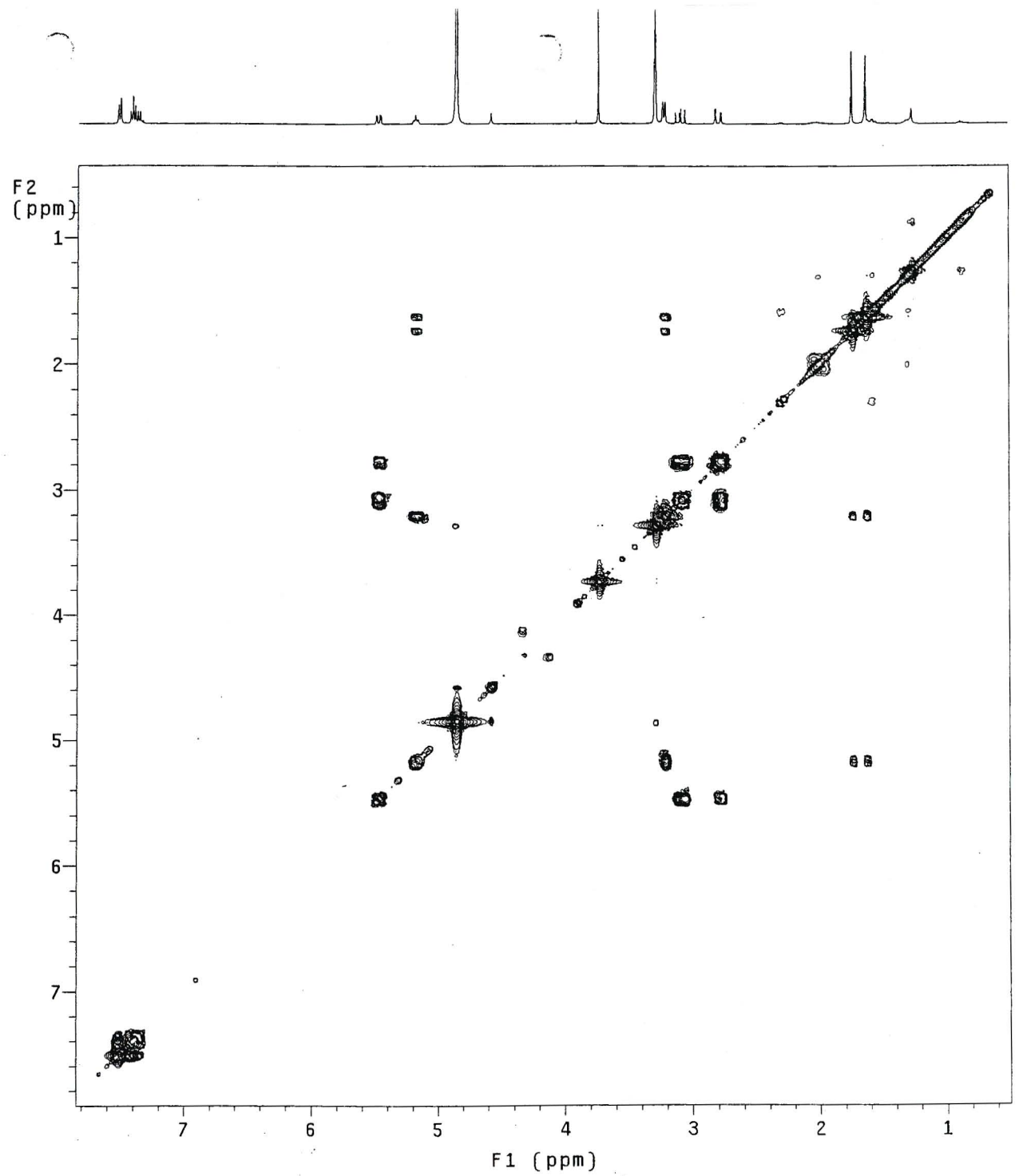


ADEPT spectrum of compound **IX**, microfolione

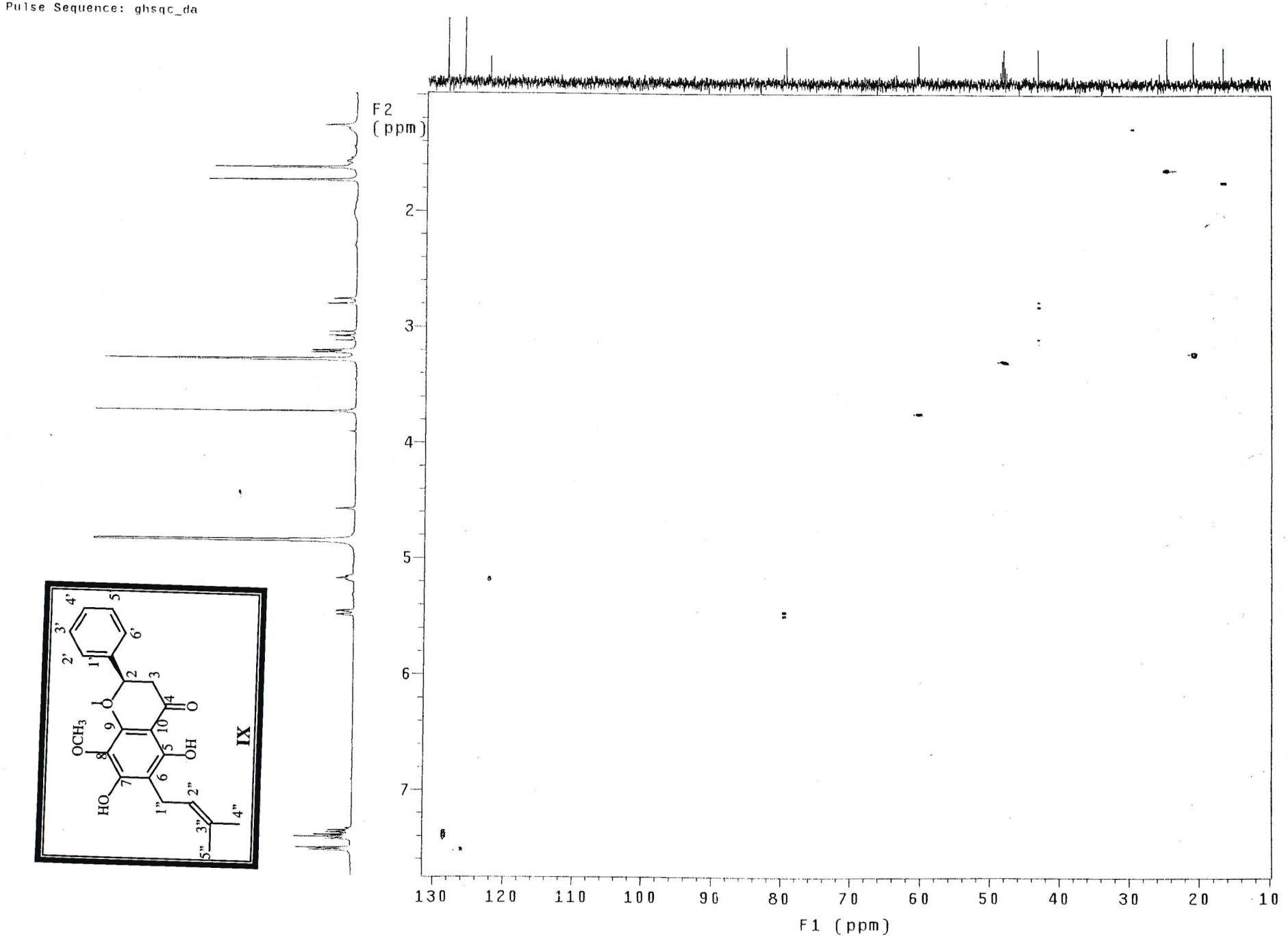
cymi19a.mic19a in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

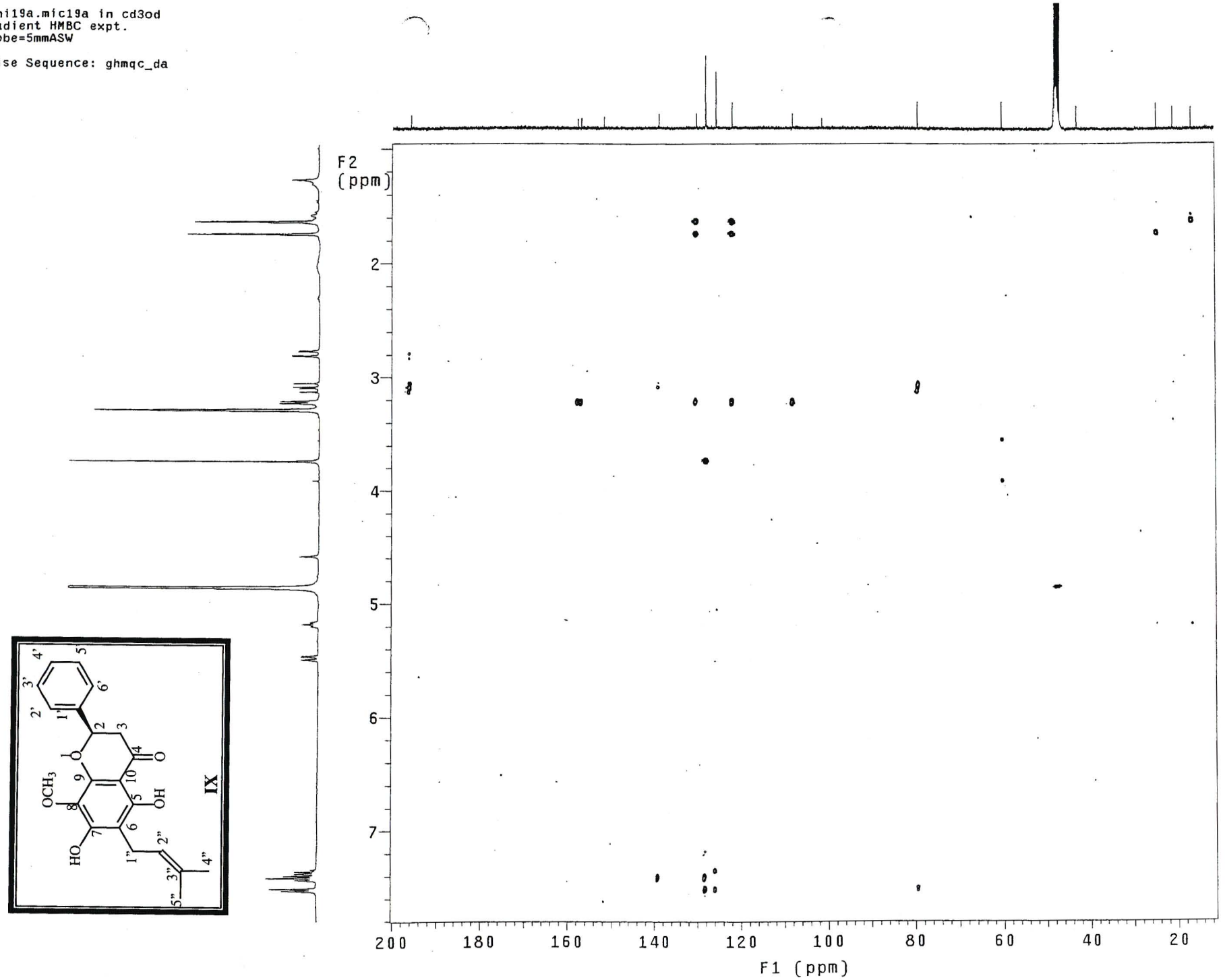




COSY spectrum of compound **IX**, microfolione



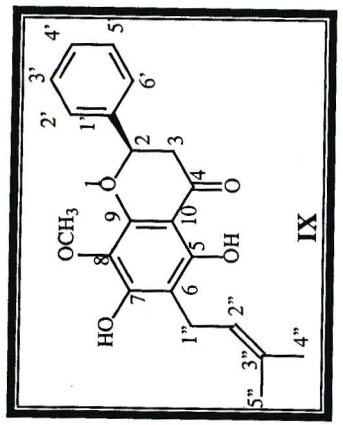
HSQC spectrum of compound **IX**, microfolione

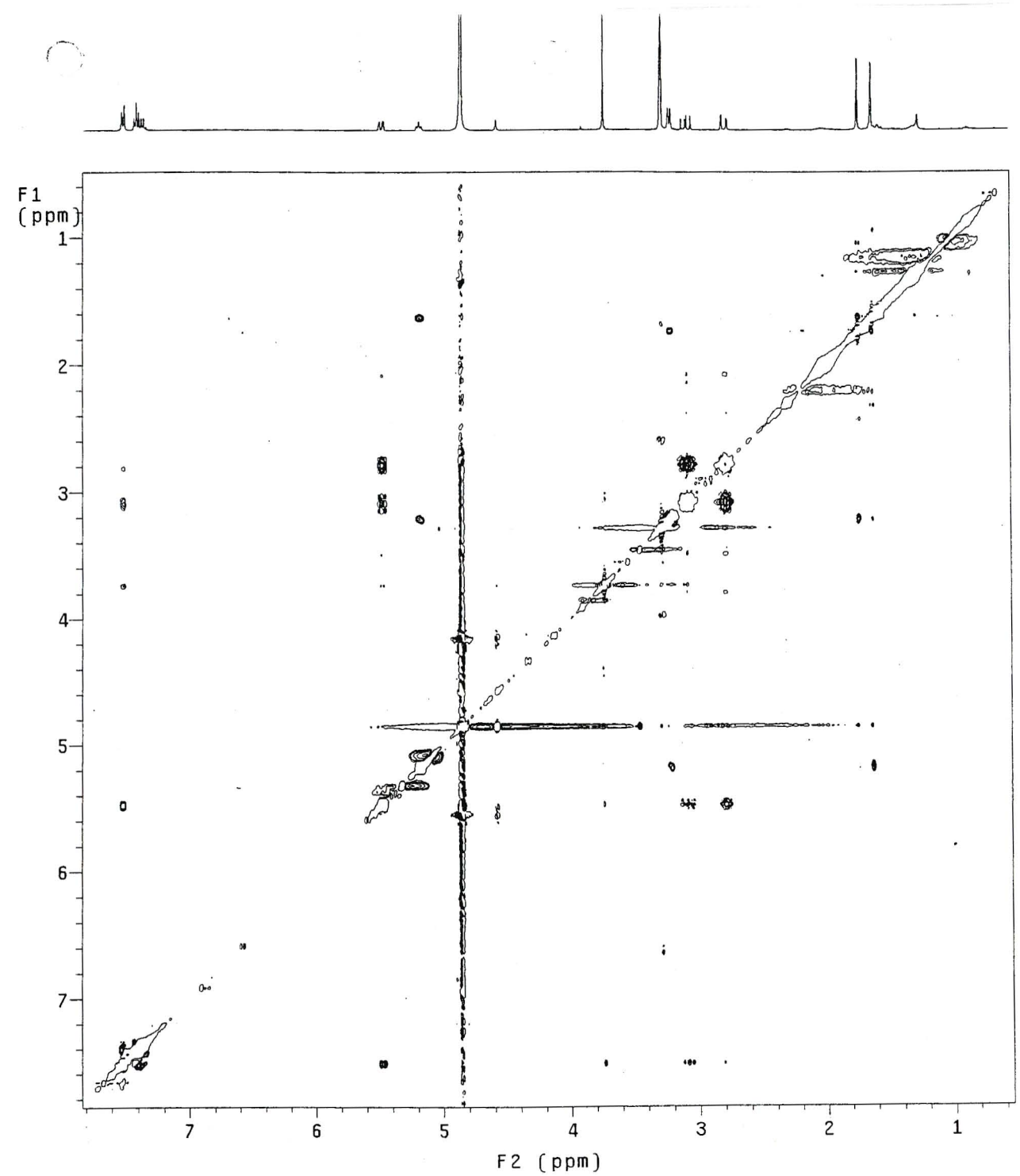


HMBC spectrum of compound **IX**, microfolione

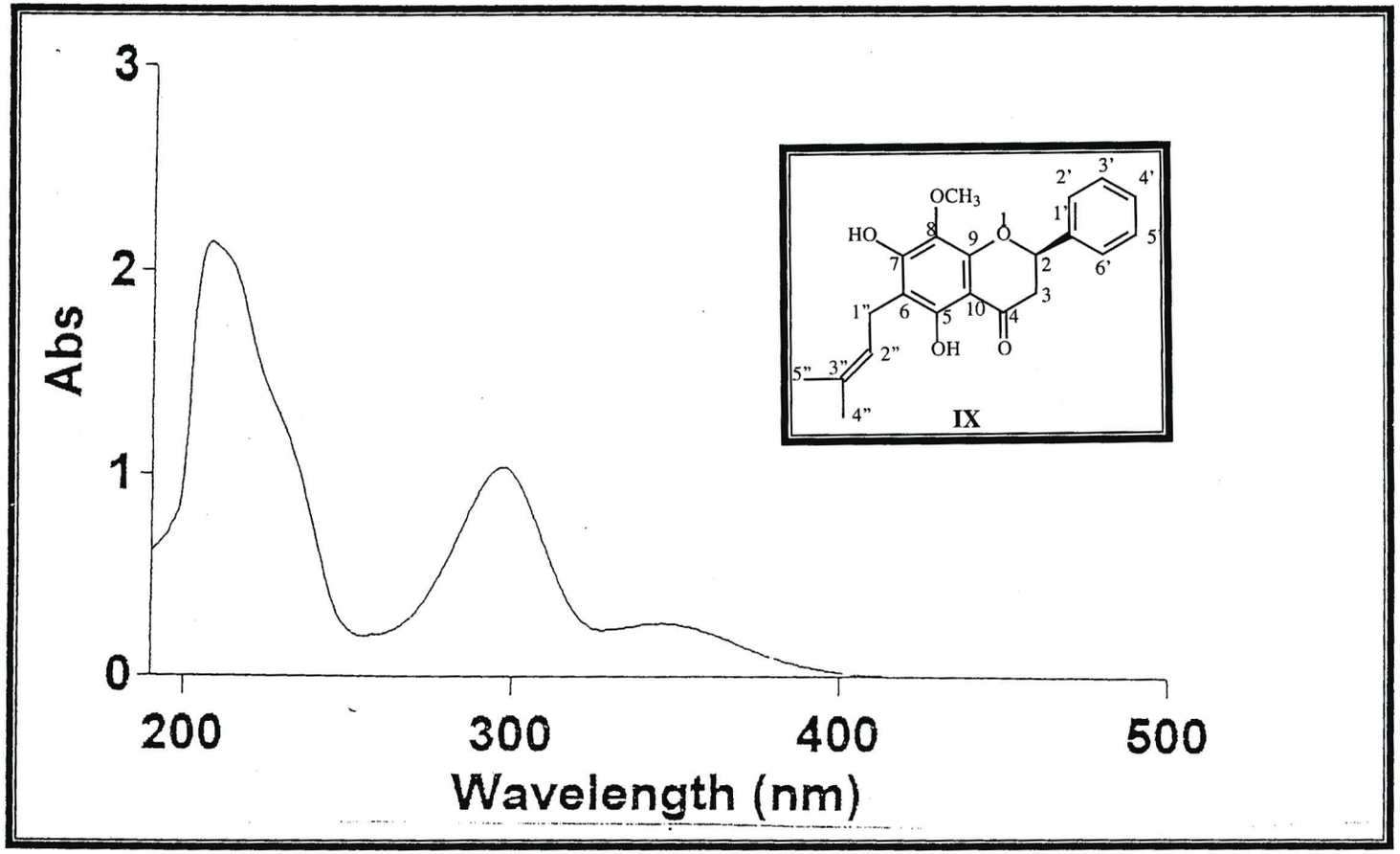
NOmi19a.mic19a in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



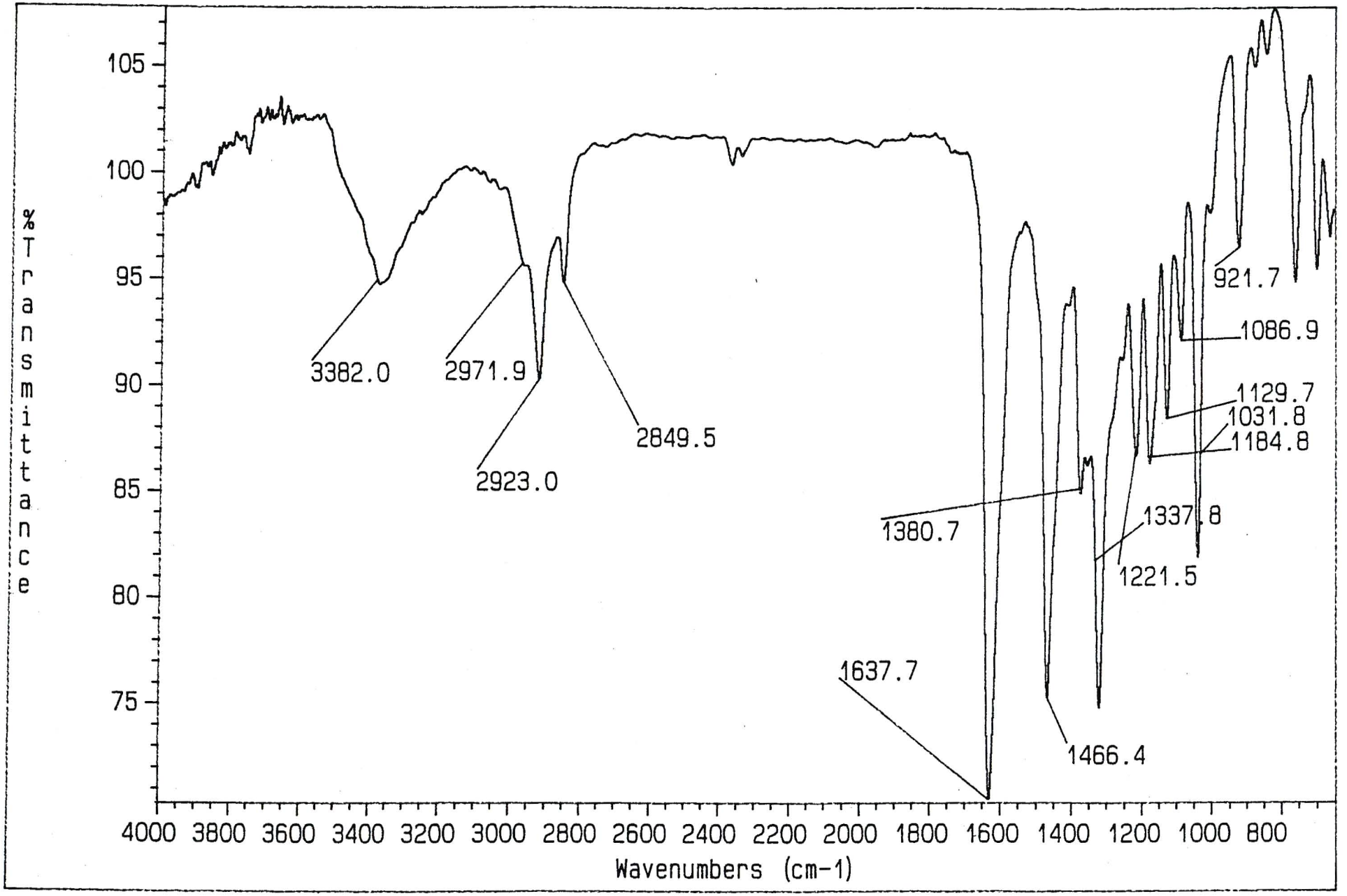


NOESY spectrum of compound **IX**, microfolione



Ultra violet spectrum of compound **IX,** microfolione

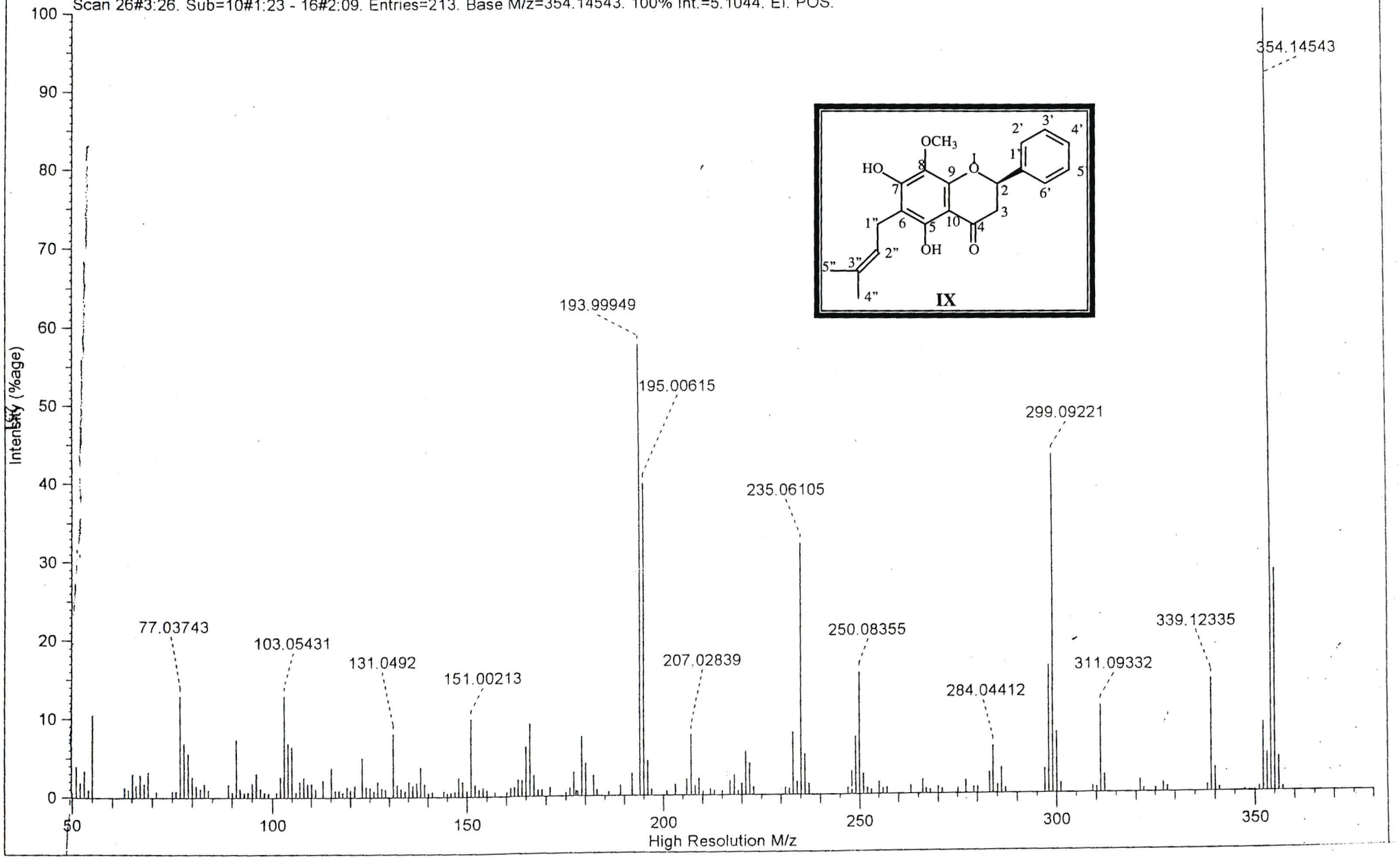
MIC19A



Infrared spectrum of compound **IX**, microfolione

File Title :
Operator : Dr. P. Boshoff
Instrument : VG70-SEQ MIC19A

SCAN GRAPH. Flagging=High Resolution M/z. Filter=[Int:0.4%. Range:0-360. Excl: Ref/Ex.].Highlighting=Base Peak.
Scan 26#3:26. Sub=10#1:23 - 16#2:09. Entries=213. Base M/z=354.14543. 100% Int.=5.1044. EI. POS.

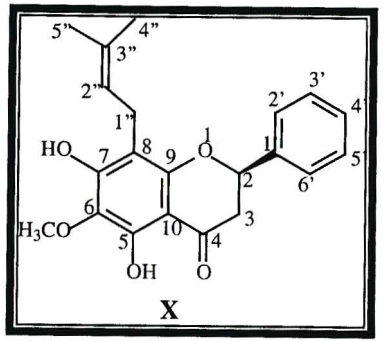


Mass spectrum of compound **IX**, microfolione

hmi30.mic30 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	2995.6	7.490	18.9
2	2988.555	7.472	27.2
3	2965.662	7.415	9.3
4	2964.380	7.412	12.2
5	2957.421	7.394	26.7
6	2949.913	7.376	16.8
7	2942.405	7.357	11.8
8	2937.827	7.345	4.6
9	2935.446	7.340	11.0
10	2166.669	5.417	8.2
11	2163.556	5.410	8.8
12	2154.033	5.386	8.9
13	2150.920	5.378	8.3
14	2064.666	5.162	4.4
15	2057.524	5.144	8.7
16	2050.199	5.126	4.4
17	1941.603	4.855	550.0
18	1511.798	3.780	137.0
19	1318.047	3.296	24.1
20	1316.582	3.292	41.4
21	1314.934	3.288	54.4
22	1313.286	3.284	39.8
23	1311.821	3.280	21.8
24	1289.113	3.223	11.9
25	1285.450	3.214	13.5
26	1282.520	3.207	13.0
27	1278.675	3.197	11.4
28	1235.273	3.089	9.0
29	1222.637	3.057	9.2
30	1218.242	3.046	12.2
31	1205.606	3.014	11.7
32	1120.634	2.802	13.0
33	1117.521	2.794	13.1
34	1103.420	2.759	9.9
35	1100.307	2.751	9.4
36	639.919	1.600	67.5
37	627.650	1.569	71.6
38	505.502	1.264	7.7



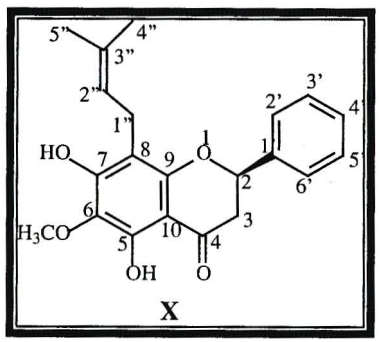
X

^{1}H NMR spectrum of compound **X**, (+)-agrandol

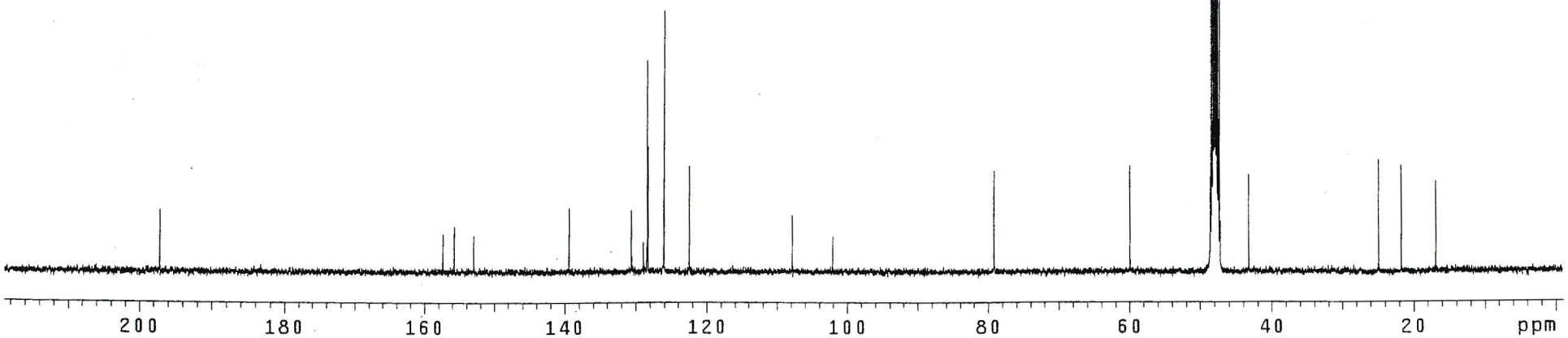
cmi30.mic30 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

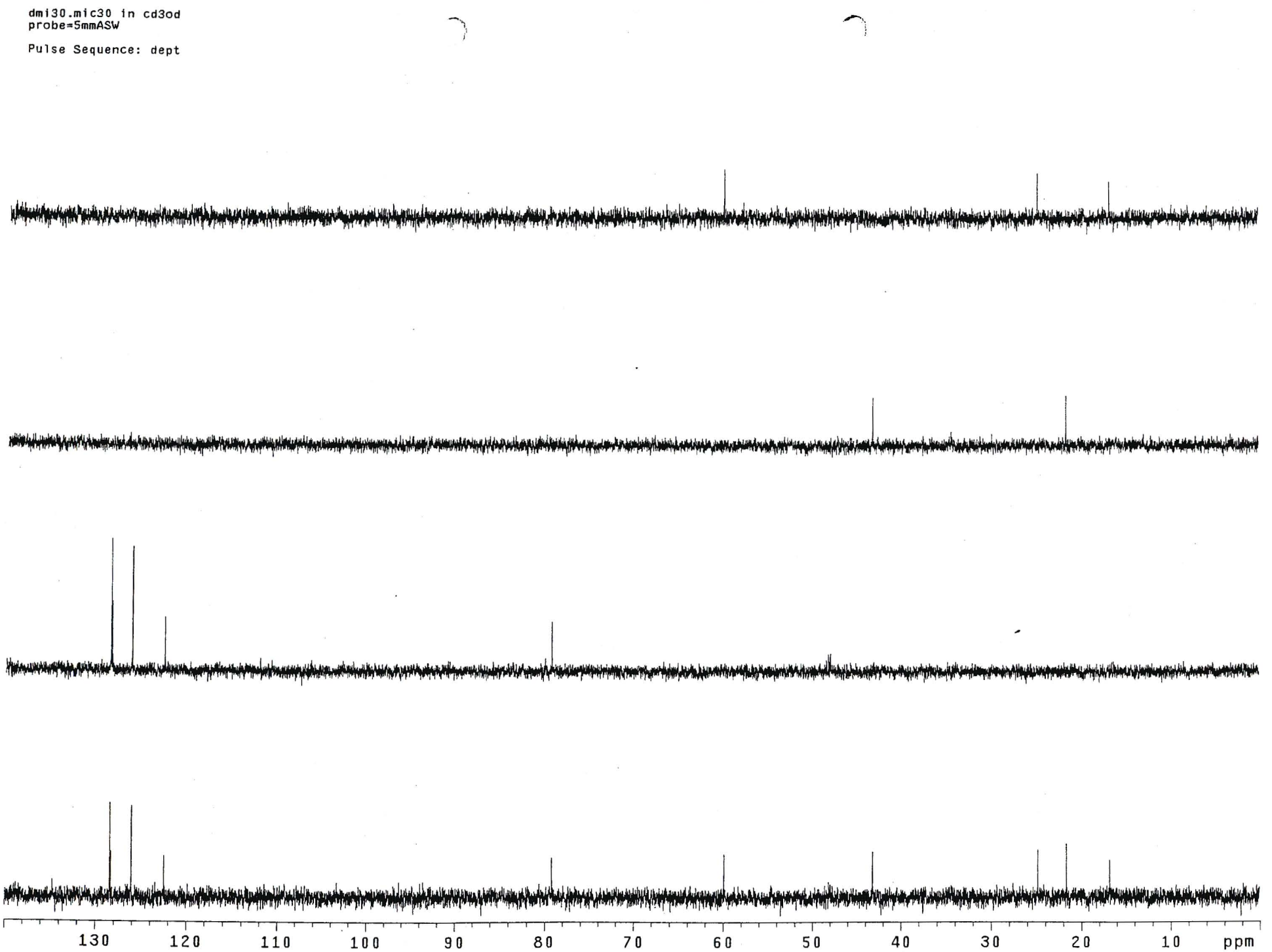
INDEX	FREQUENCY	PPM	HEIGHT
1	19826.7	197.148	10.0
2	15824.95	157.357	6.2
3	15661.717	155.733	7.4
4	15377.895	152.911	5.9
5	14018.295	139.392	10.4
6	13134.784	130.607	10.0
7	12912.762	128.399	34.0
8	12900.555	128.278	20.2
9	12674.718	126.032	42.0
10	12313.836	122.443	17.2
11	10839.792	107.786	9.2
12	10261.467	102.036	5.8
13	7963.423	79.185	16.3
14	6023.971	59.900	17.2
15	4878.001	48.505	145.1
16	4856.638	48.292	432.5
17	4835.276	48.080	857.5
18	4813.913	47.867	1000.0
19	4792.550	47.655	859.3
20	4771.187	47.443	434.4
21	4749.824	47.230	145.2
22	4343.165	43.187	15.8
23	2499.084	24.850	18.1
24	2178.639	21.663	17.1
25	1690.343	16.808	14.6



X



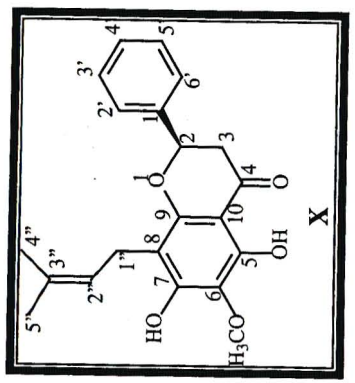
^{13}C NMR spectrum of compound **X**, (+)-agrandol



ADEPT spectrum of compound **X**, (+)-agrandol

cymi30.mic30 in cd3od
1H Cosy-90
probe=5mmASW

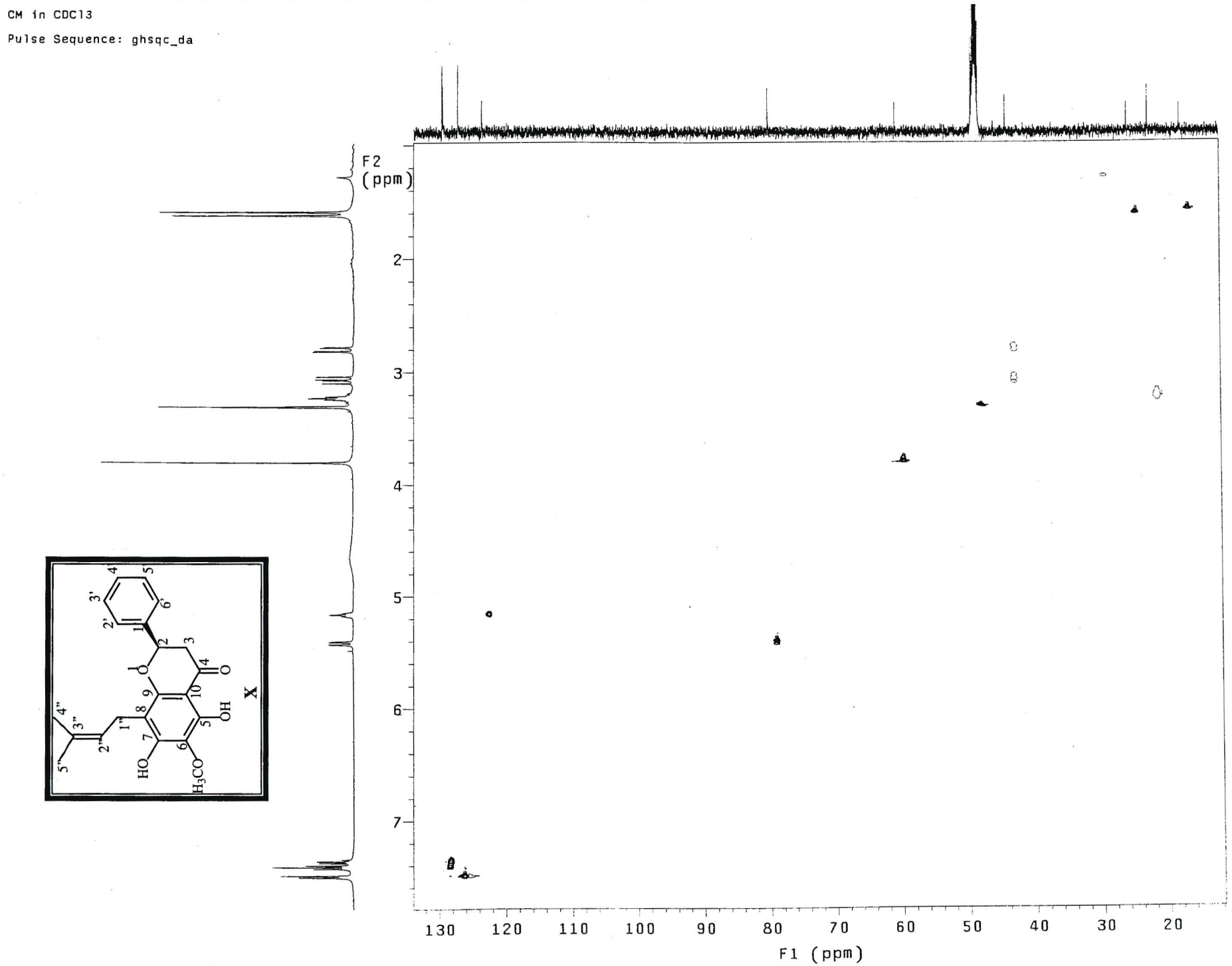
Pulse Sequence: relayh



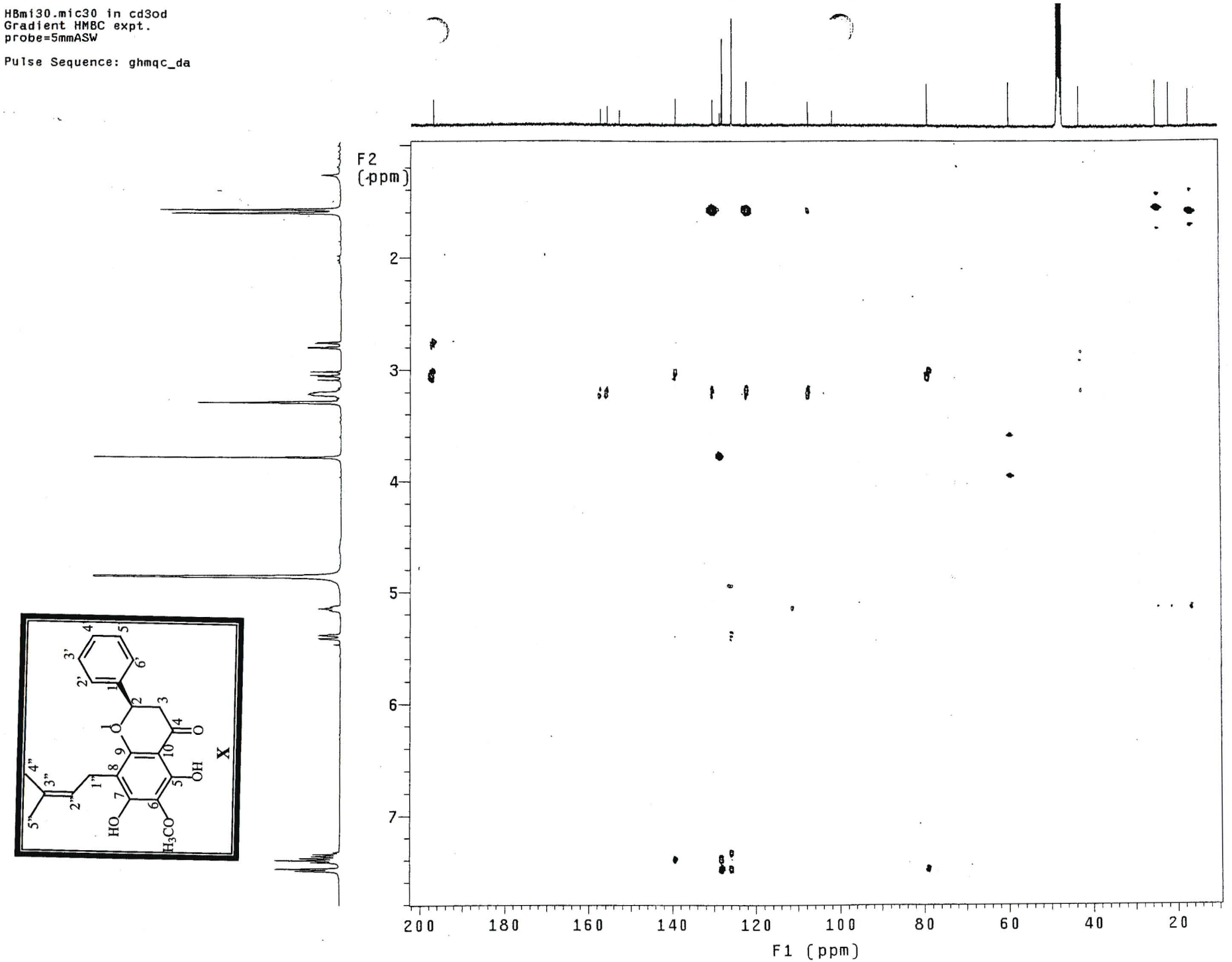
F2 (ppm)

F1 (ppm)

COSY spectrum of compound **X**, (+)-agrandol



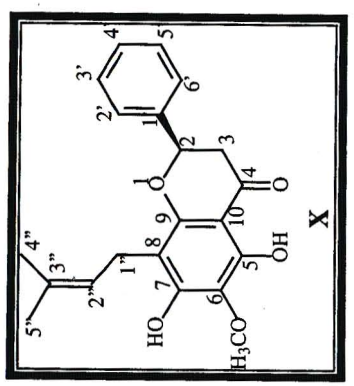
HSQC spectrum of compound **X**, (+)-agrandol

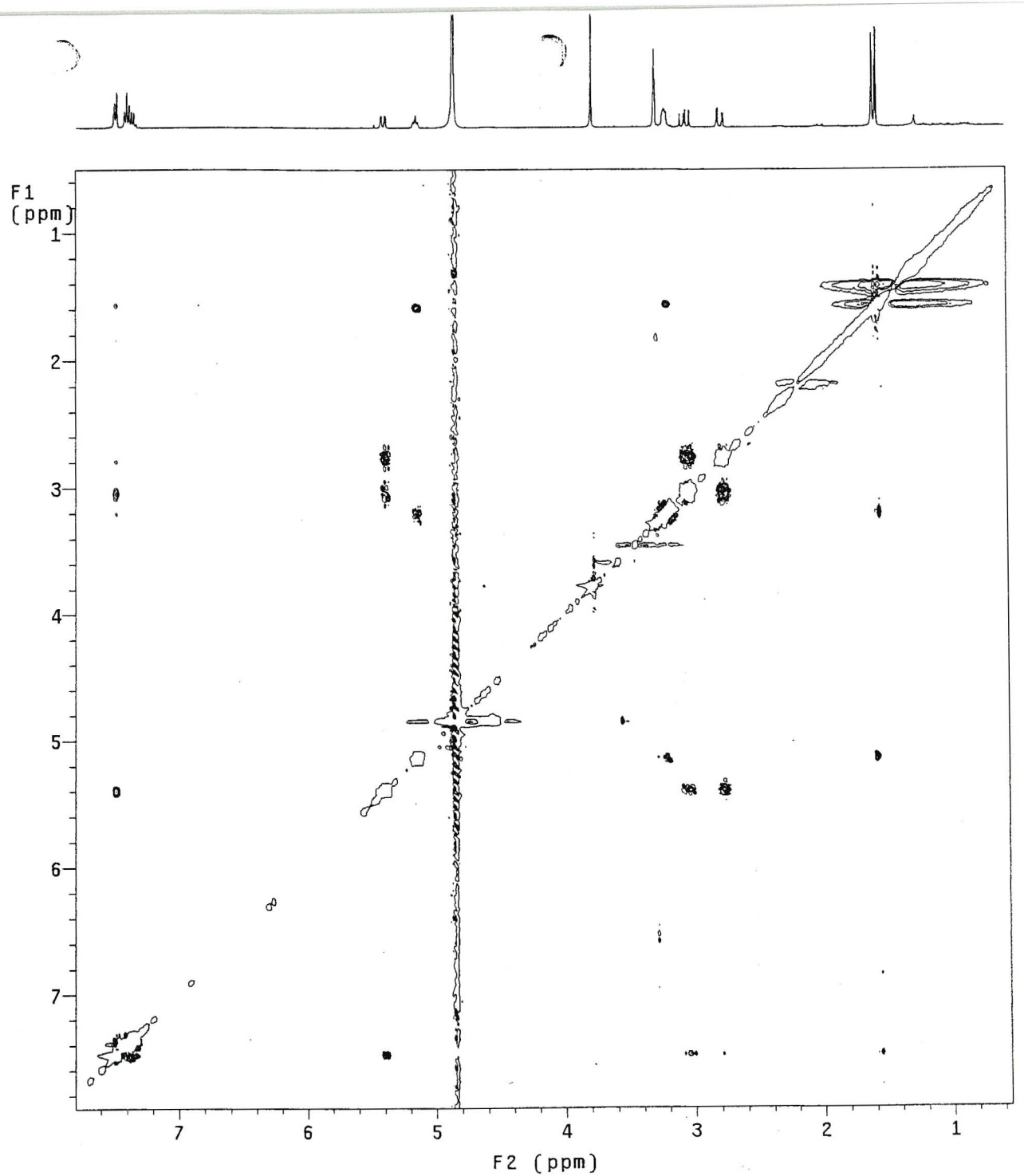


HMBC spectrum of compound **X**, (+)-agrandol

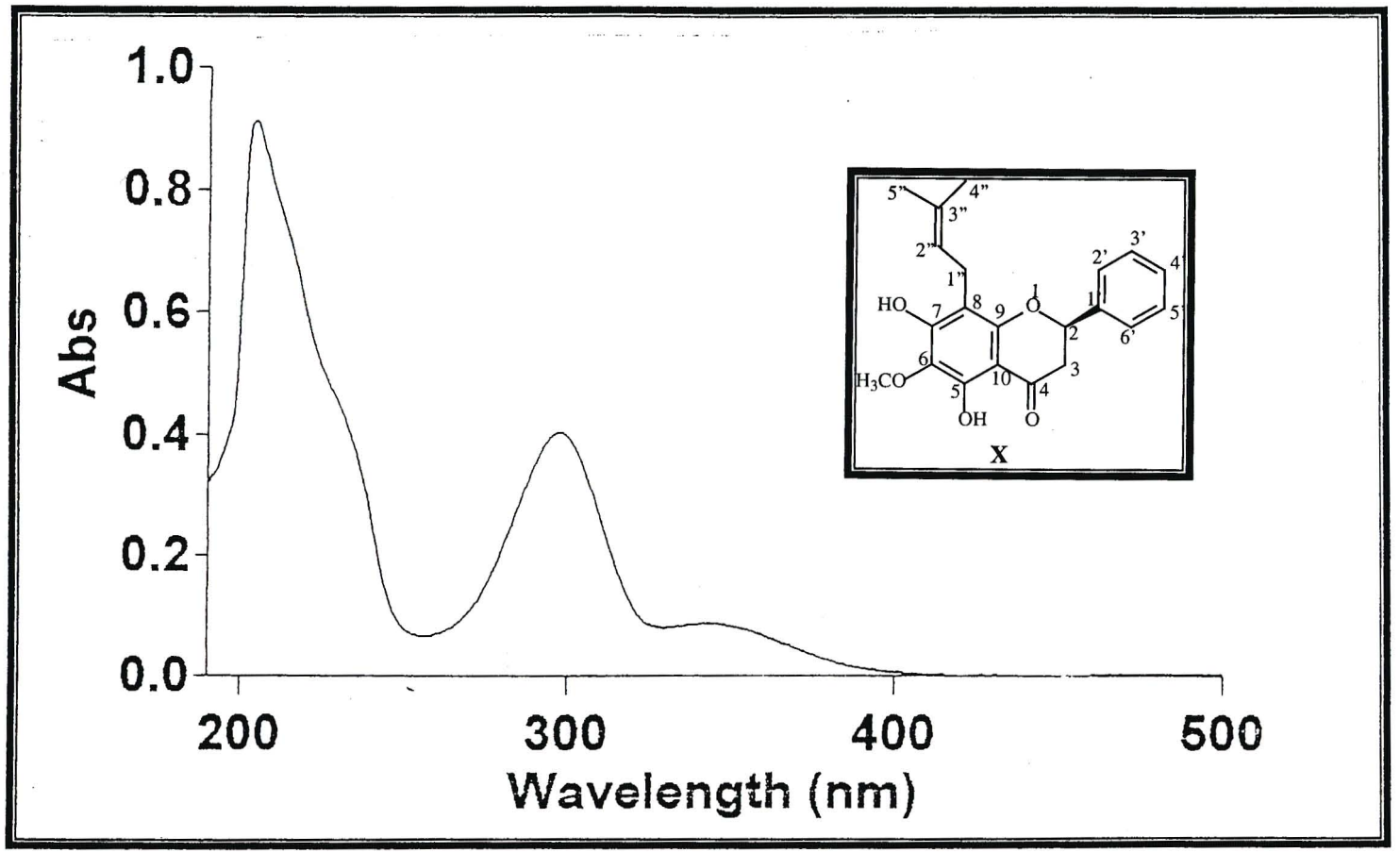
NOmi30.mic30 in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



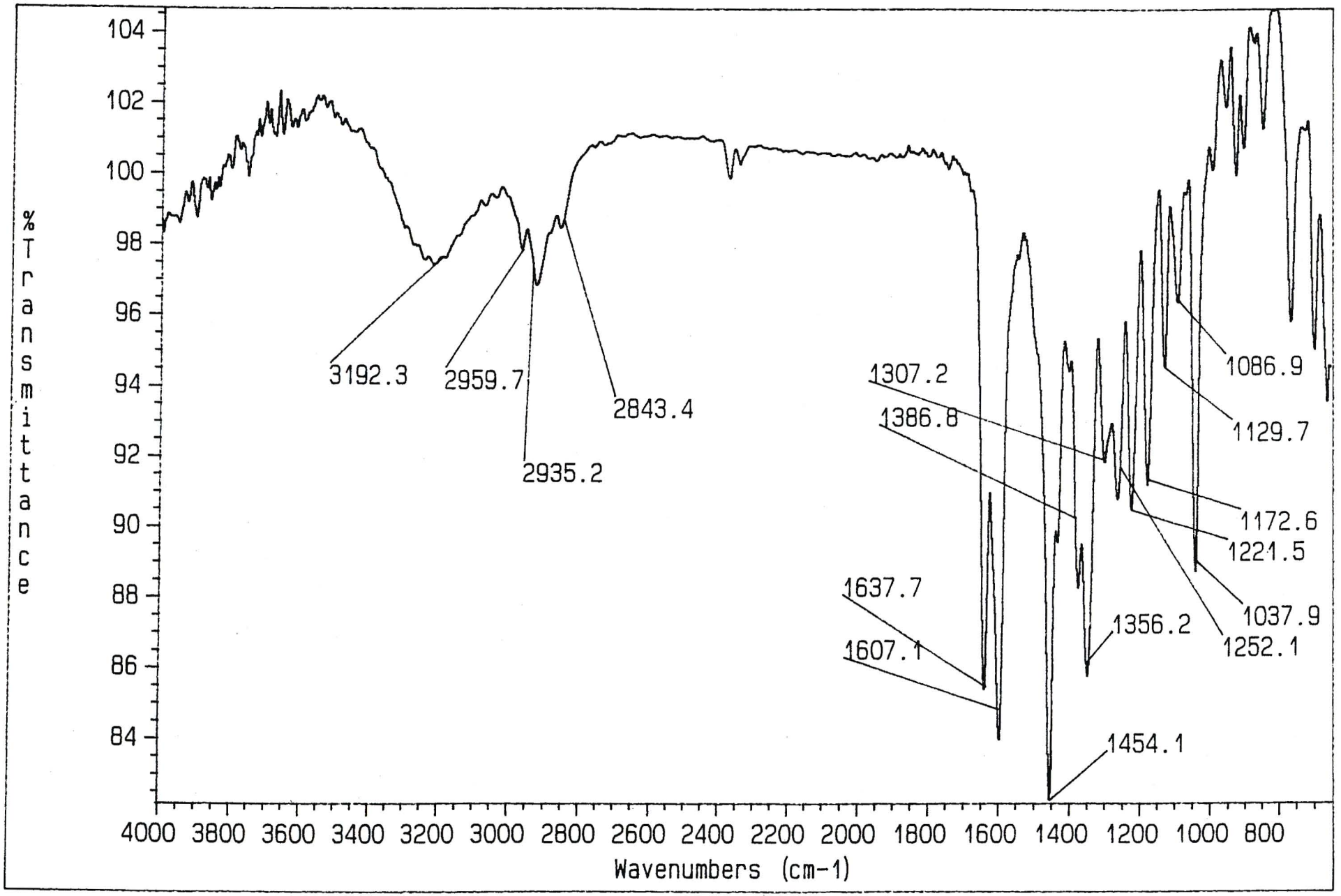


NOESY spectrum of compound **X**, (+)-agrandol



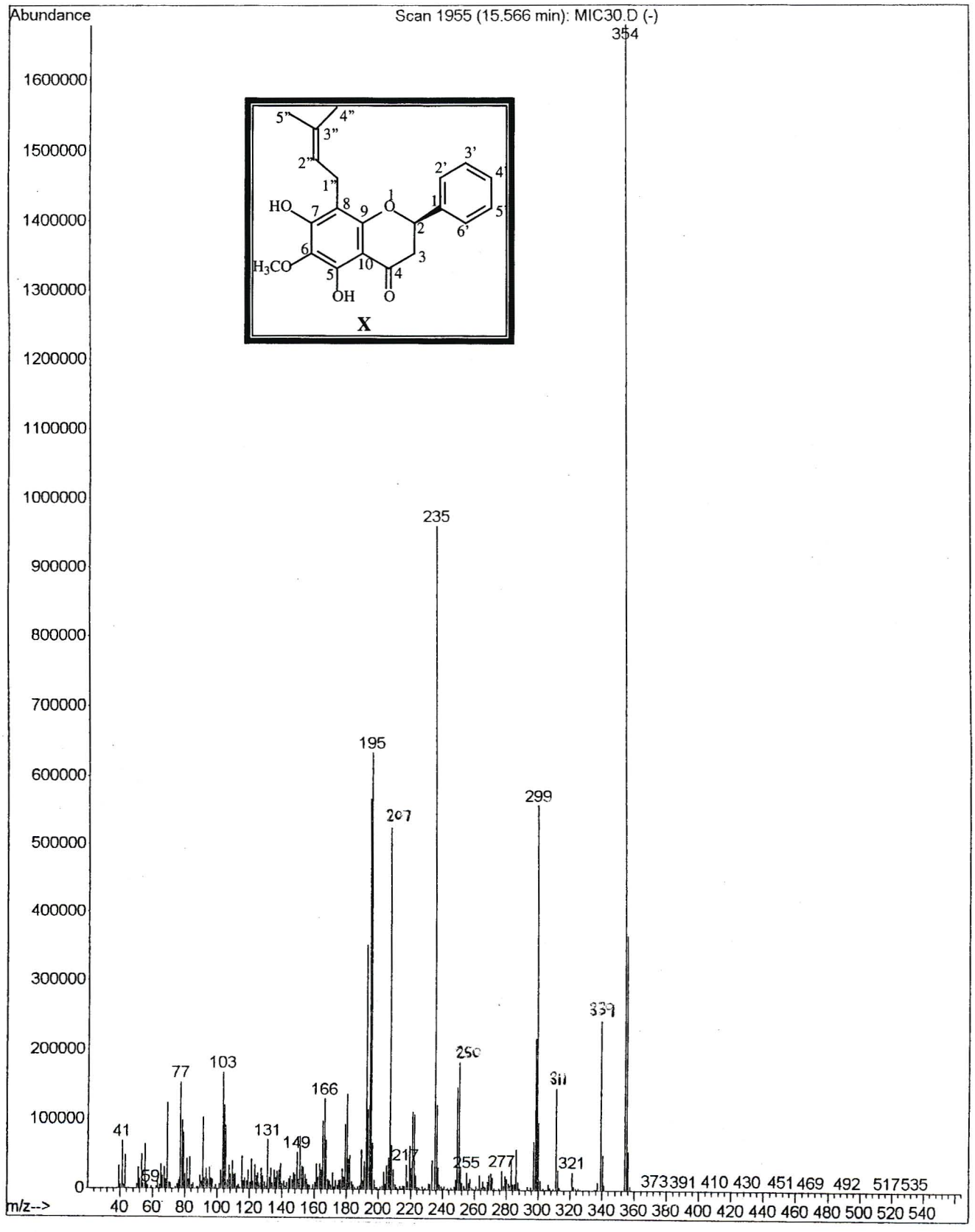
Ultra violet spectrum of compound **X,** (+)-agrandol

MIC30



Infrared spectrum of compound **X**, (+)-agrandol

File : D:\NEIL\MIC30.D
Operator : Bret
Acquired : 20 Dec 2000 12:50 using AcqMethod NEW
Instrument : Instrumen
Sample Name: MIC30
Misc Info : 1ul inject, MeCl2, splitless
Vial Number: 8

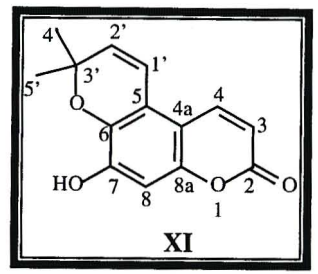


Mass spectrum of compound **X**, (+)-agrandol

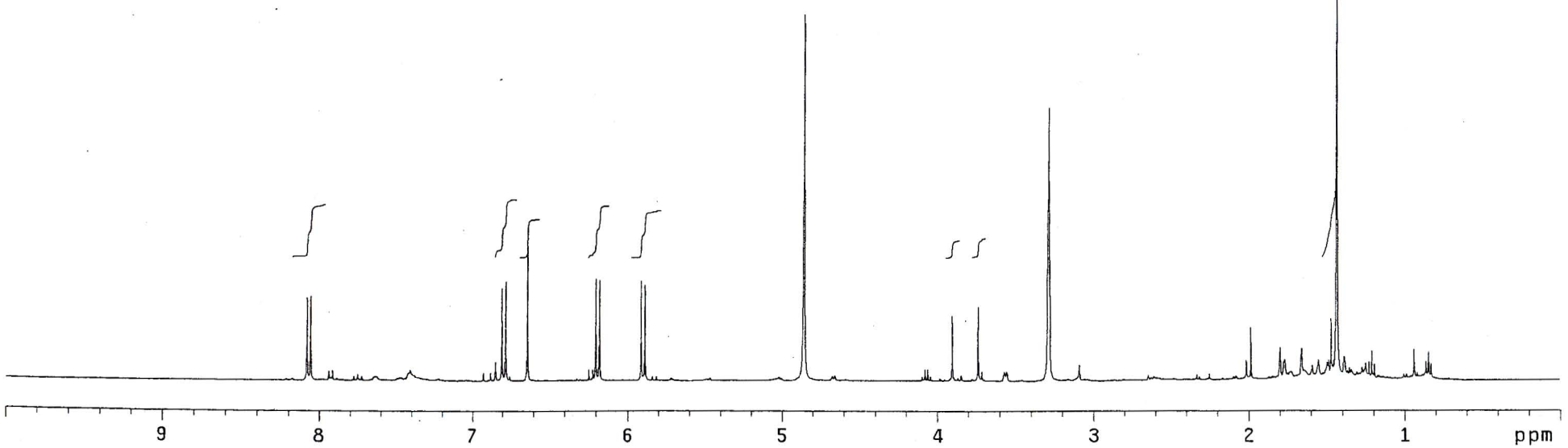
hmlc23a.m*cm23a in cd3od
probe*5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3231.7	8.080	13.4
2	3222.045	8.056	13.7
3	2720.818	6.803	15.0
4	2710.746	6.778	16.1
5	2655.075	6.639	21.9
6	2480.919	6.203	16.6
7	2471.396	6.179	16.3
8	2365.730	5.915	16.3
9	2355.658	5.890	15.5
10	1943.251	4.859	59.2
11	1562.159	3.906	10.7
12	1495.317	3.739	12.0
13	1318.047	3.296	18.3
14	1316.399	3.291	32.9
15	1314.934	3.288	44.2
16	1313.286	3.284	32.3
17	1311.638	3.280	17.2
18	794.664	1.987	8.7
19	588.460	1.471	10.1
20	573.810	1.435	200.0



XI

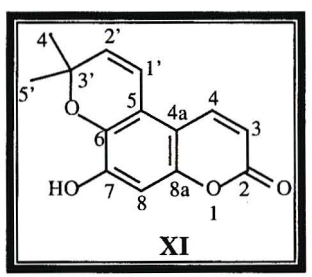


^{1}H NMR spectrum of compound **XI,** cedrecoumarin A

cmic23a.micm23a in cd3od
probe=5mmASW

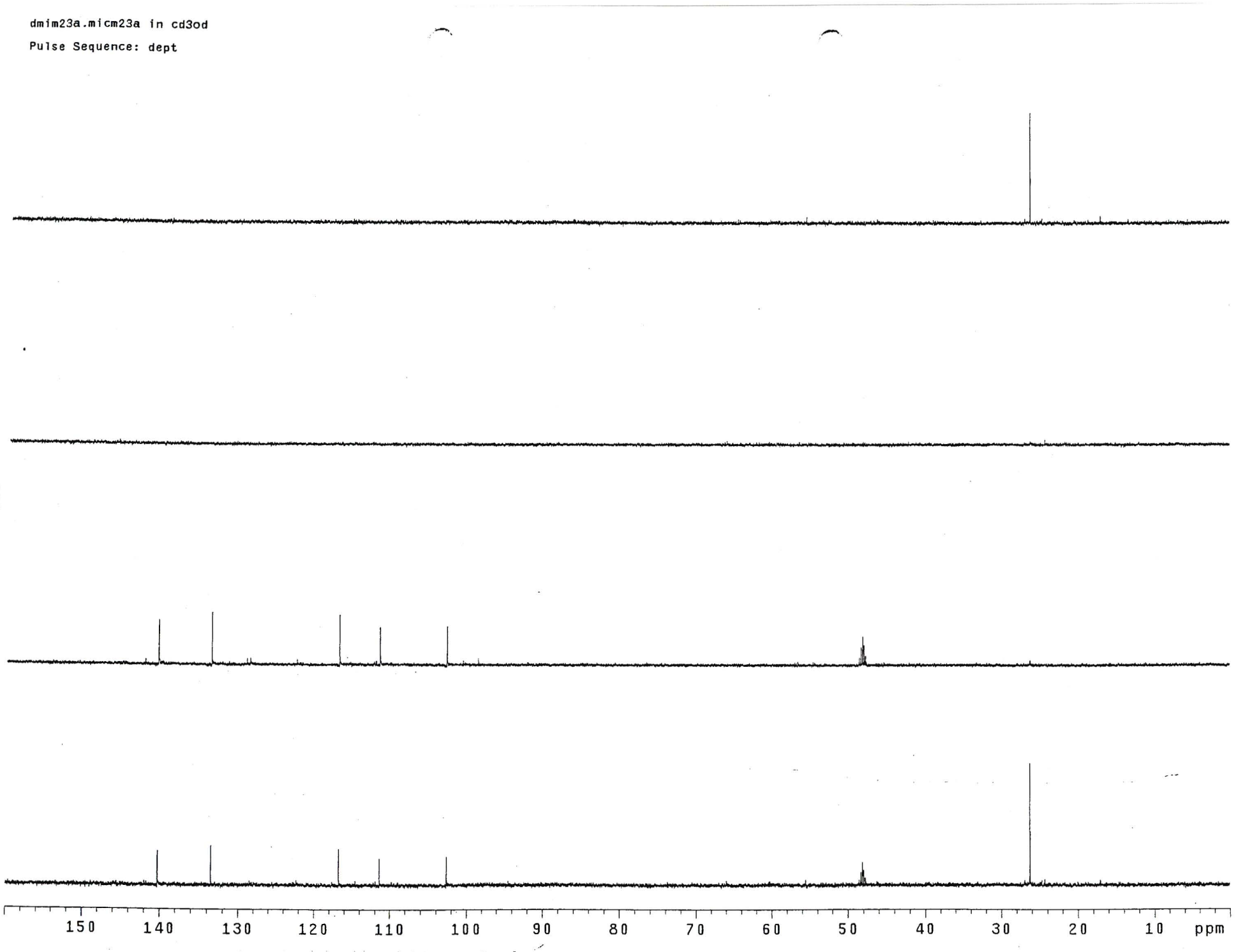
Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	16343.0	162.508	6.0
2	15129.169	150.438	5.3
3	15084.917	149.998	6.0
4	14108.325	140.287	12.7
5	13862.651	137.844	6.0
6	13425.473	133.497	17.4
7	11831.644	117.649	6.6
8	11738.562	116.723	14.0
9	11203.726	111.405	15.7
10	10792.489	107.316	6.6
11	10321.741	102.635	15.2
12	7693.334	76.499	7.8
13	4879.527	48.520	48.5
14	4858.164	48.307	137.5
15	4843.668	48.163	10.8
16	4836.801	48.095	260.1
17	4815.439	47.883	300.0
18	4794.076	47.670	260.5
19	4772.713	47.458	134.3
20	4751.350	47.245	45.4
21	2640.232	26.253	35.8



XI

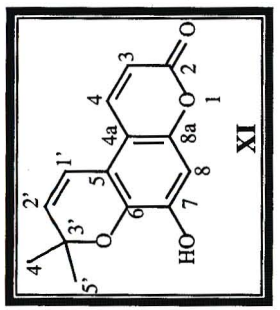
^{13}C NMR spectrum of compound **XI**, cedrecoumarin A



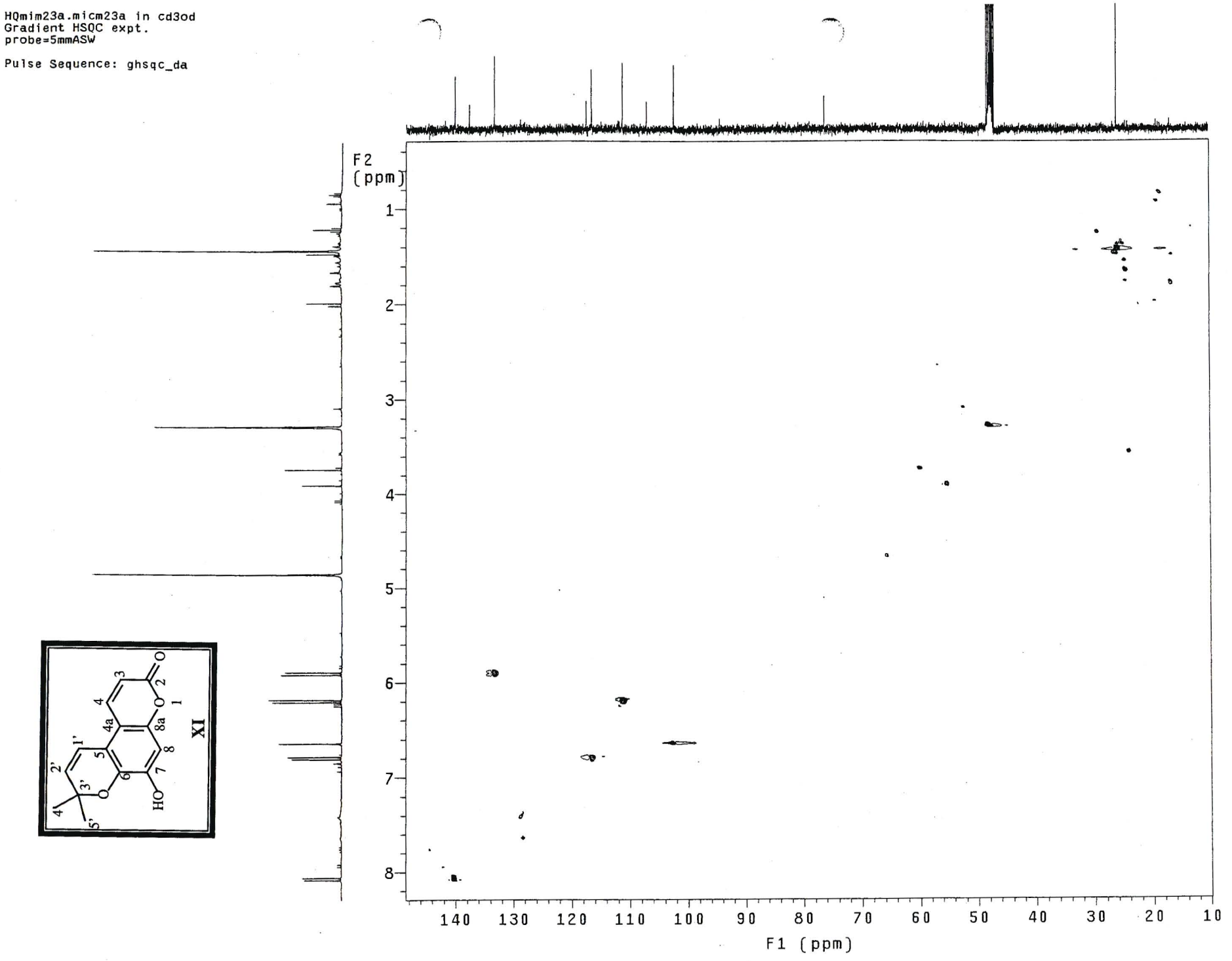
ADEPT spectrum of compound **XI**, cedrecoumarin A

cymim23a.micm23a in cd3od
1H Cosy-90
probe=5mmASW

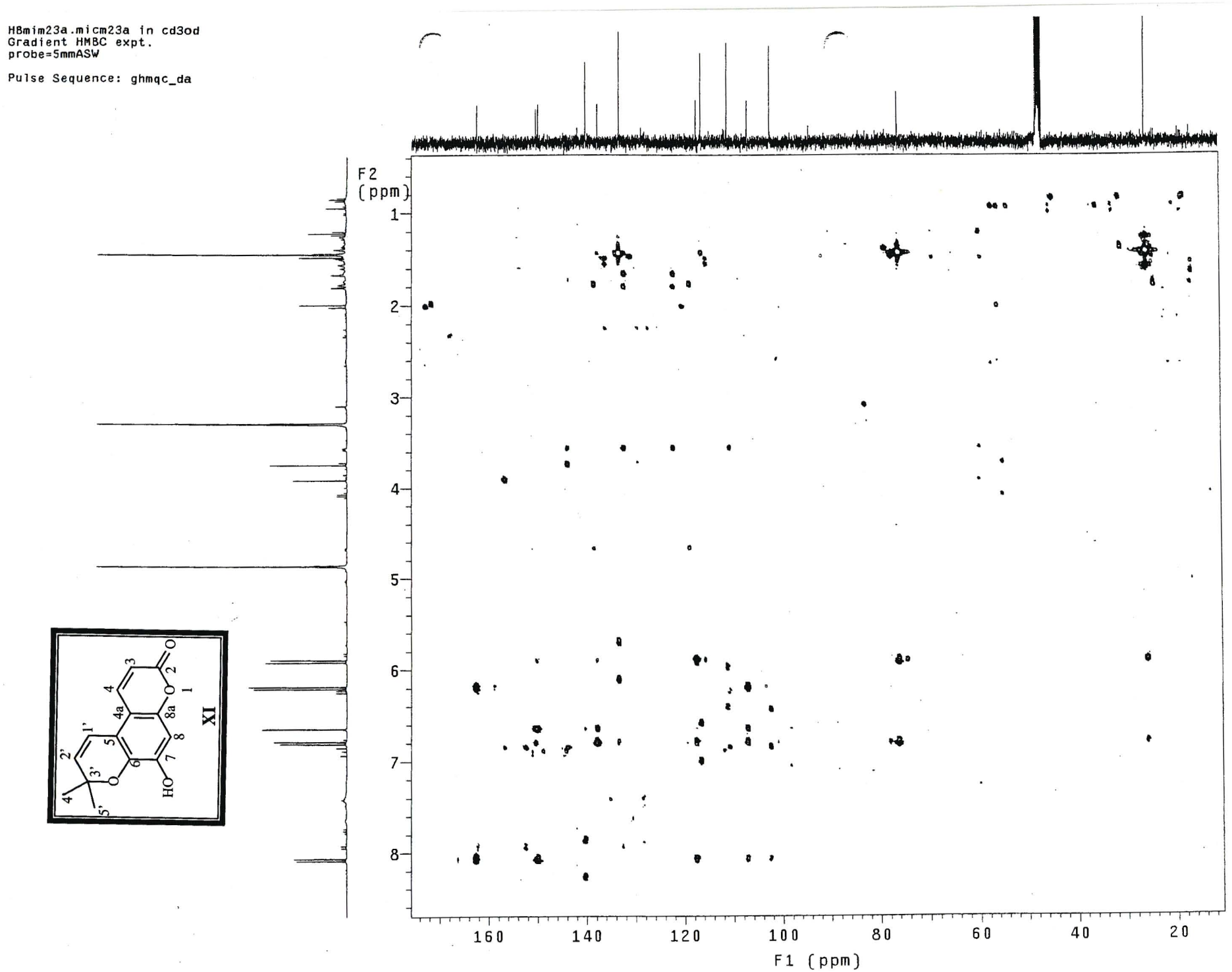
Pulse Sequence: relayh



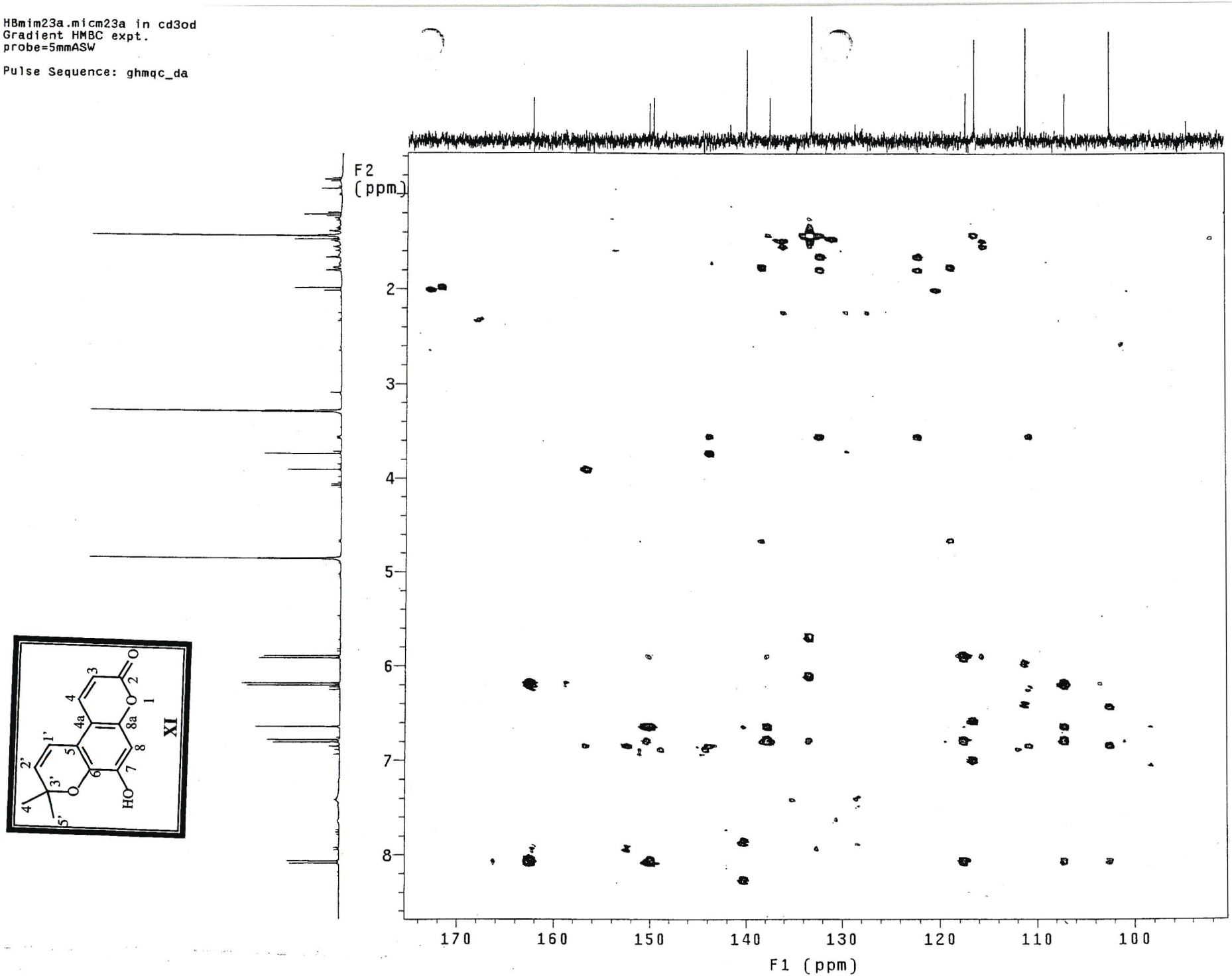
COSY spectrum of compound **XI**; cedrecoumarin A



HSQC spectrum of compound **XI**, cedrecoumarin A



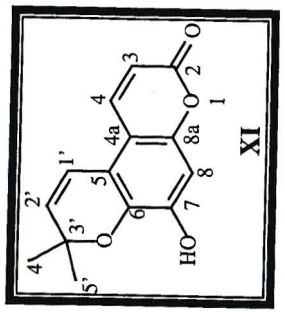
HMBC spectrum of compound **XI,** cedrecoumarin A

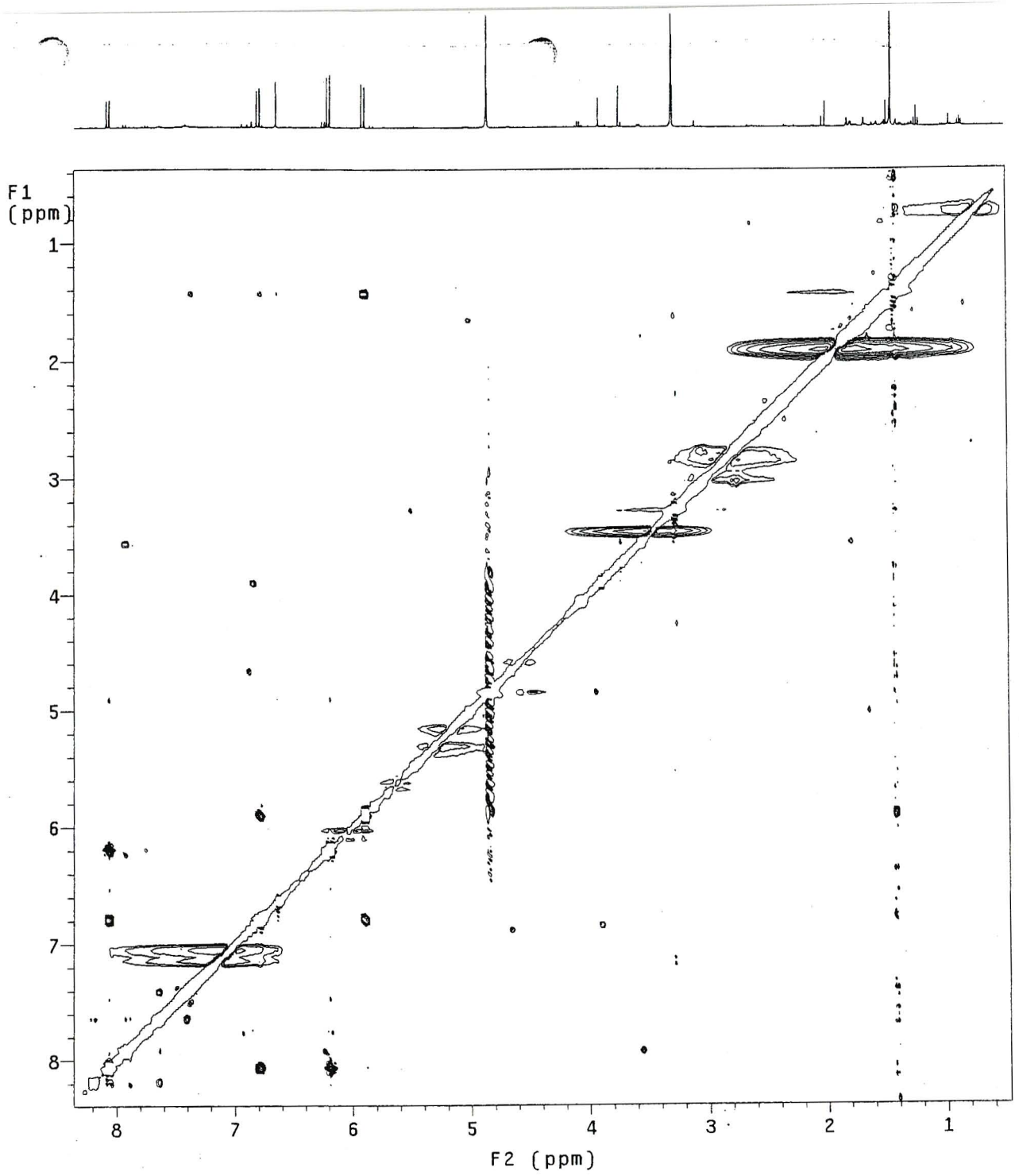


Expanded HMBC spectrum of compound **XI**, cedrecoumarin A

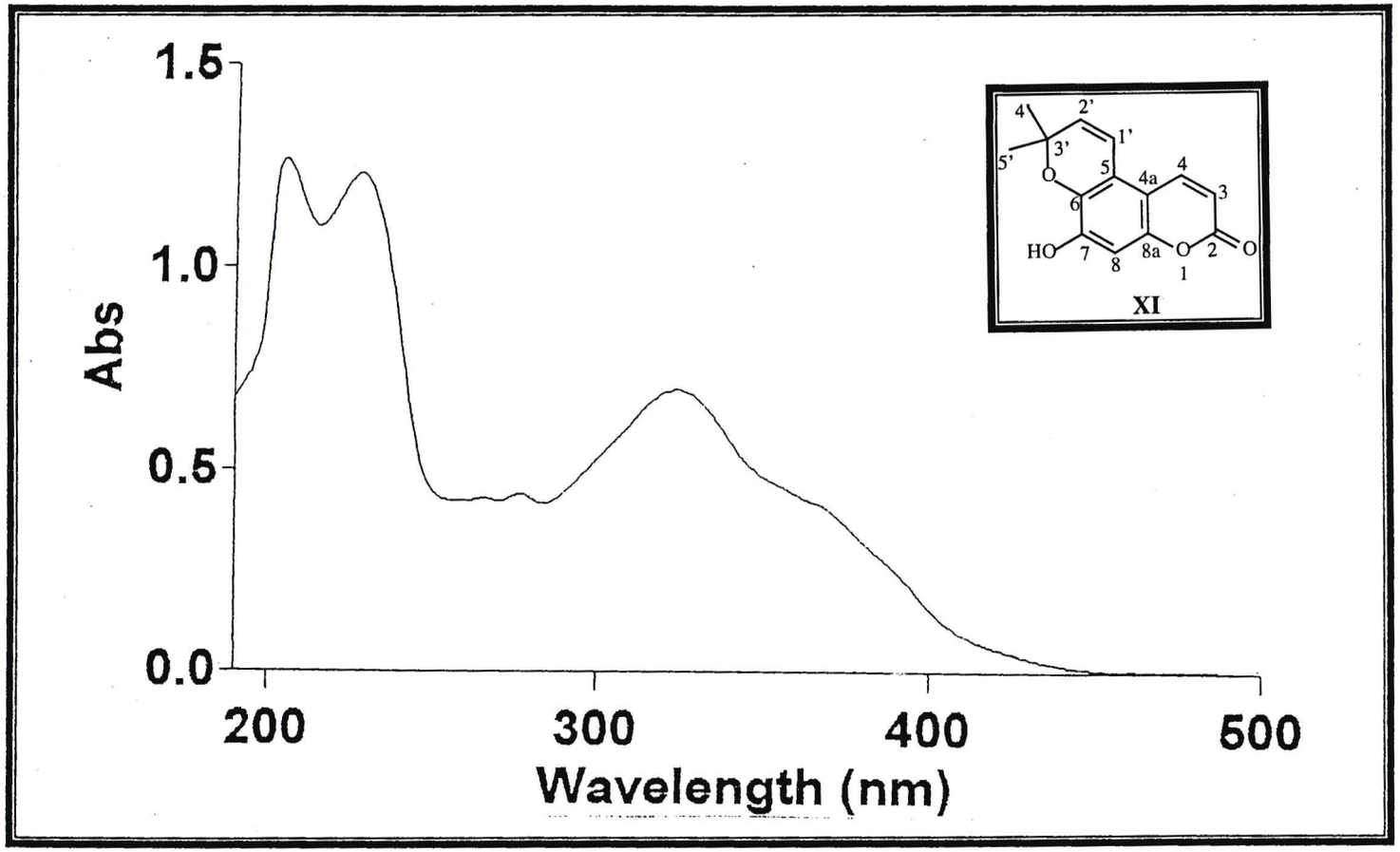
NOmim23a.micm23a in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

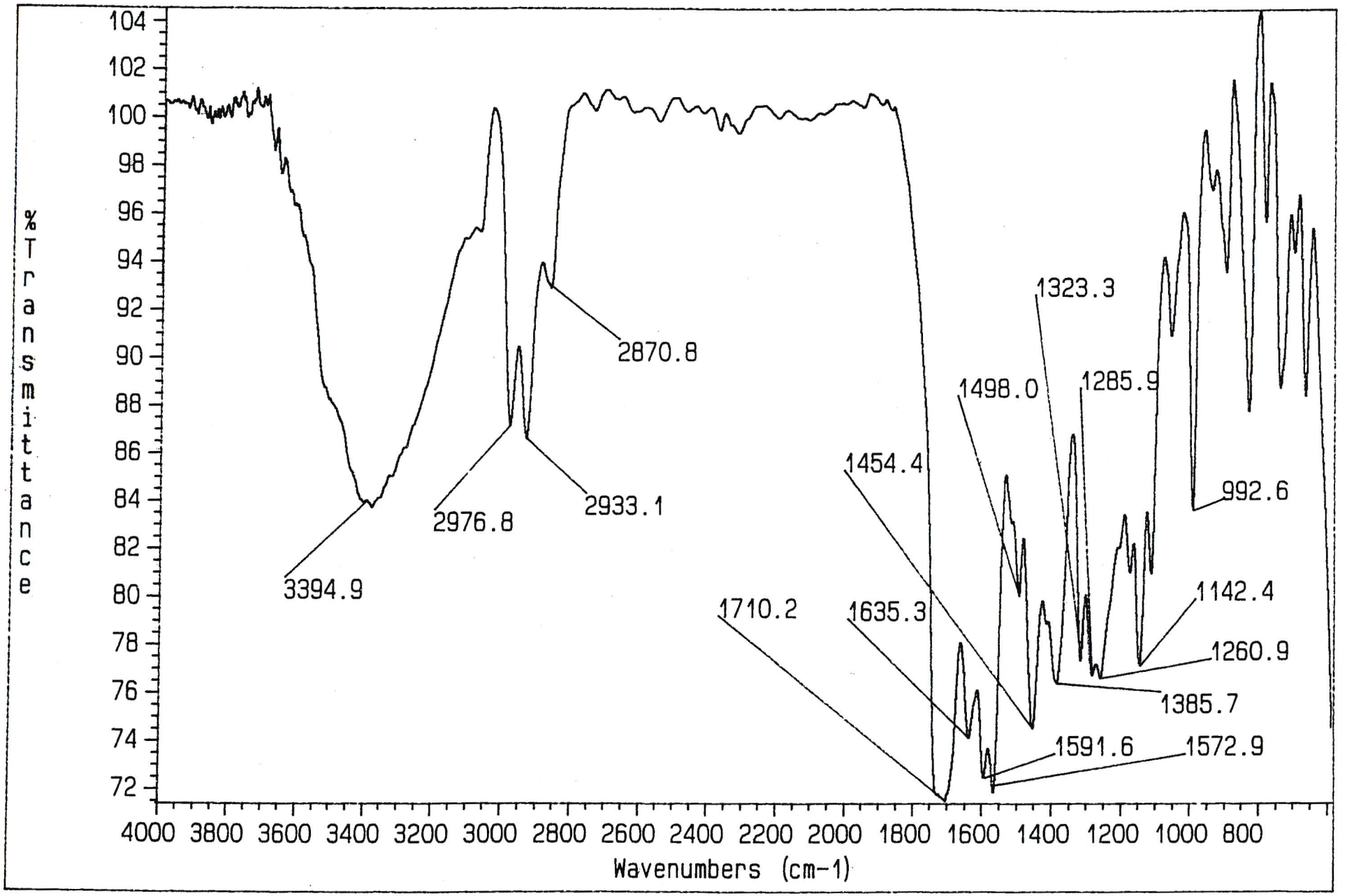




NOESY spectrum of compound **XI,** cedrecoumarin A

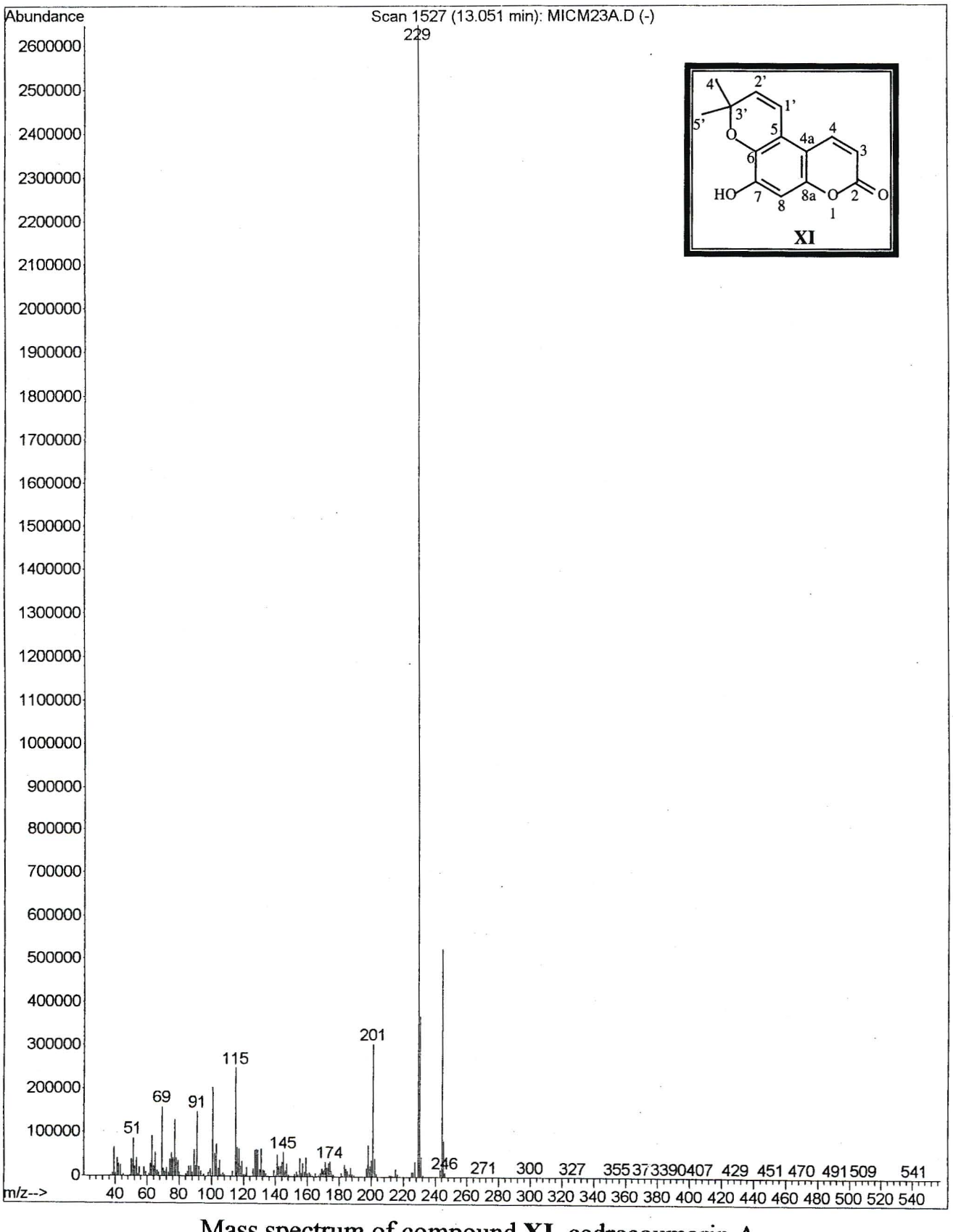


Ultra violet spectrum of compound **XI,** cedrecoumarin A



Infrared spectrum of compound **XI,** cedrecoumarin A

File : D:\NEIL\MICM23A.D
Operator : Bret
Acquired : 20 Dec 2000 11:00 using AcqMethod NEW
Instrument : Instrumen
Sample Name: MICM23A
Misc Info : 1ul inject, MeCl2, 75:1 split
Vial Number: 10

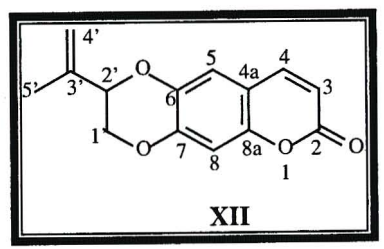


Mass spectrum of compound **XI**, cedrecoumarin A

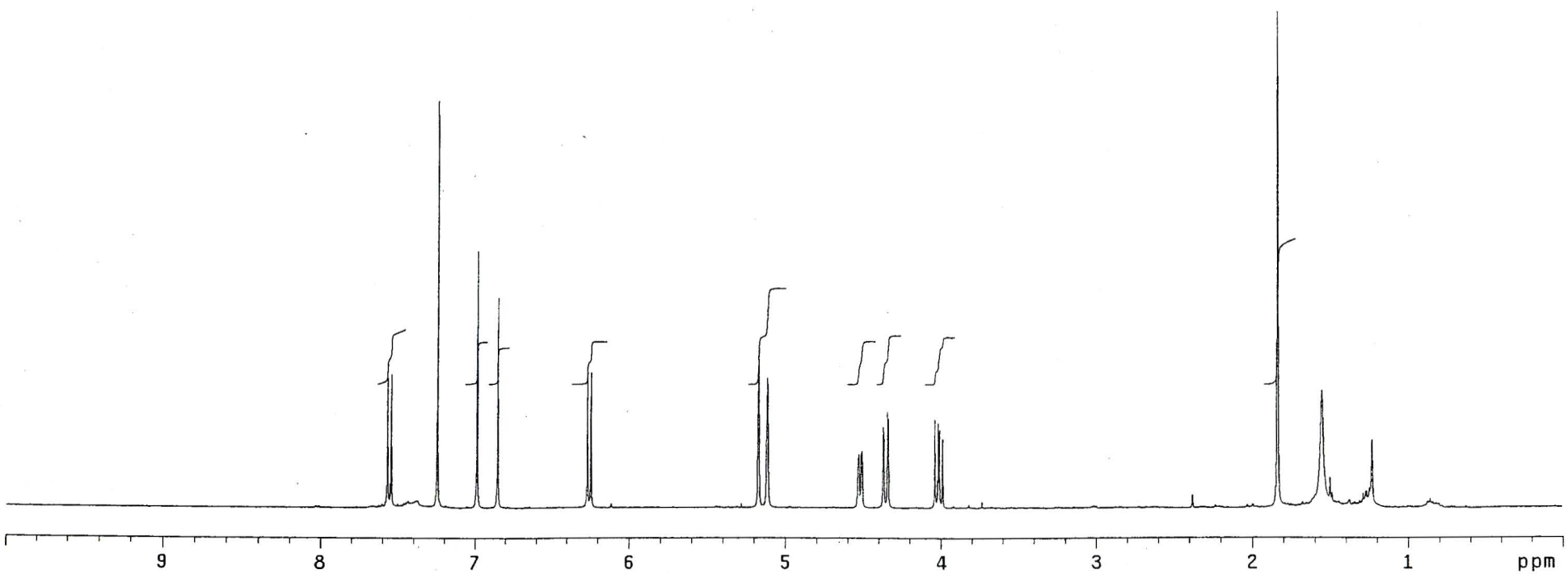
hmim12.micm12(micm blue1) in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUEN	PPM	HEIGHT
1	3026.4	7.567	20.9
2	3016.9	7.543	21.6
3	2896.798	7.243	65.4
4	2794.429	6.987	41.3
5	2738.941	6.848	33.8
6	2507.099	6.269	22.7
7	2497.576	6.245	21.9
8	2068.321	5.171	24.7
9	2045.430	5.114	21.0
10	1811.757	4.530	8.6
11	1803.699	4.510	9.2
12	1748.943	4.373	13.1
13	1746.746	4.367	12.3
14	1737.589	4.345	15.5
15	1735.209	4.339	14.4
16	1615.442	4.039	14.3
17	1607.201	4.019	13.7
18	1604.088	4.011	12.6
19	1595.664	3.990	11.2
20	735.505	1.839	80.0
21	622.148	1.556	19.0
22	493.225	1.233	11.1



XII

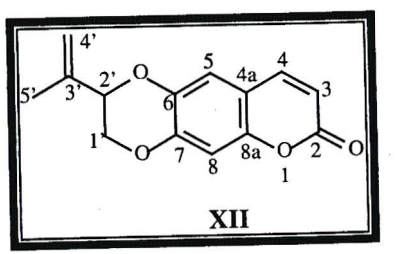


^{1}H NMR spectrum of compound **XII,** obliquin

cmim12.micm12(micm blue1) in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	16229.3	161.379	3.2
2	15029.254	149.445	4.7
3	14763.743	146.805	5.4
4	14400.573	143.194	12.1
5	14164.817	140.849	4.4
6	14013.751	139.347	7.3
7	11569.218	115.040	11.9
8	11537.936	114.729	9.7
9	11502.840	114.380	11.0
10	11385.344	113.211	5.9
11	10568.211	105.086	11.5
12	7800.945	77.570	250.0
13	7789.501	77.456	11.2
14	7768.901	77.251	247.1
15	7737.619	76.940	236.5
16	7637.671	75.946	11.7
17	6821.301	67.828	13.6
18	1926.895	19.160	12.3



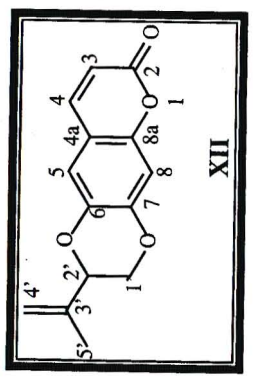
XII

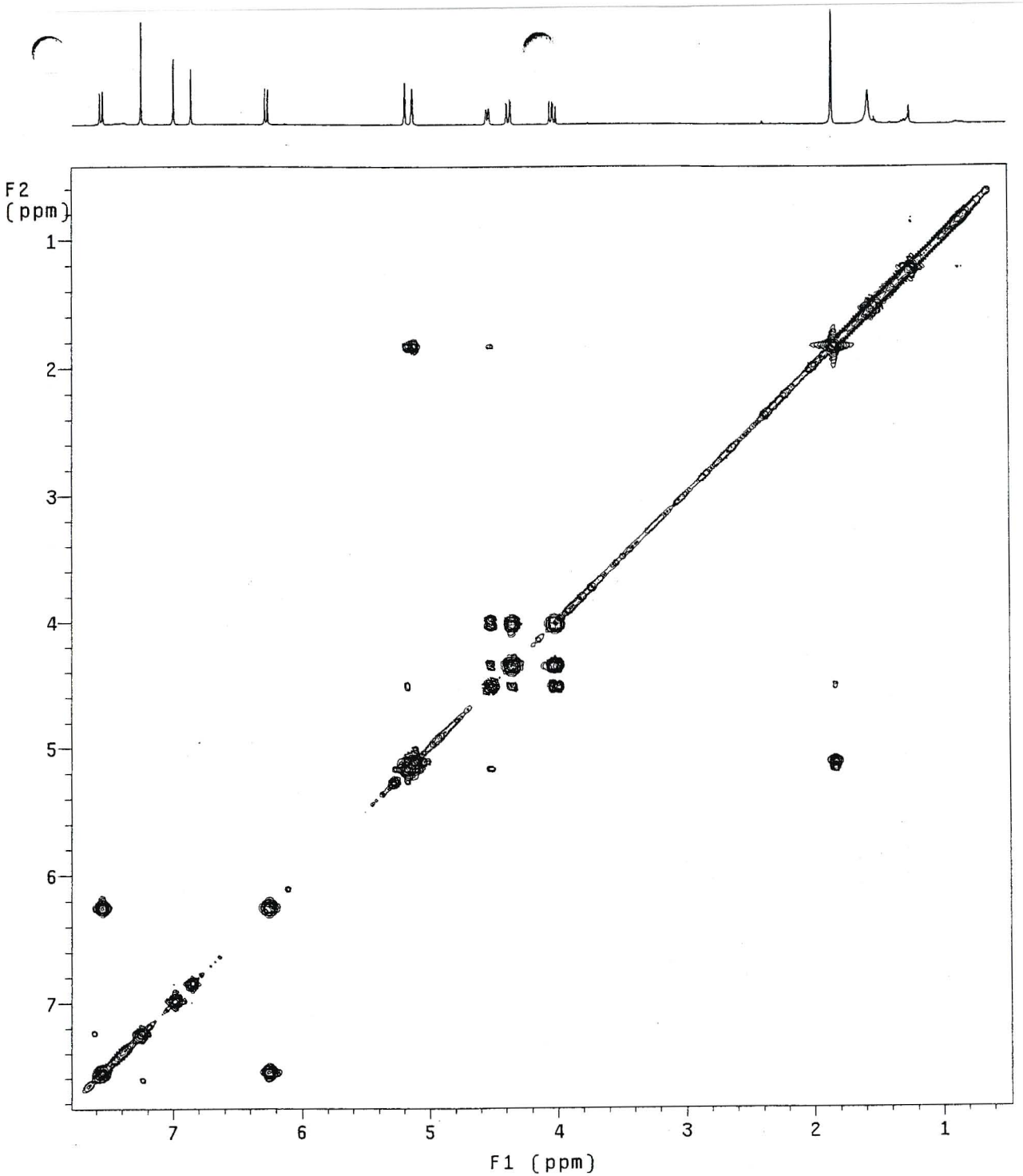
200 180 160 140 120 100 80 60 40 20 ppm

^{13}C NMR spectrum of compound **XII,** obliquin

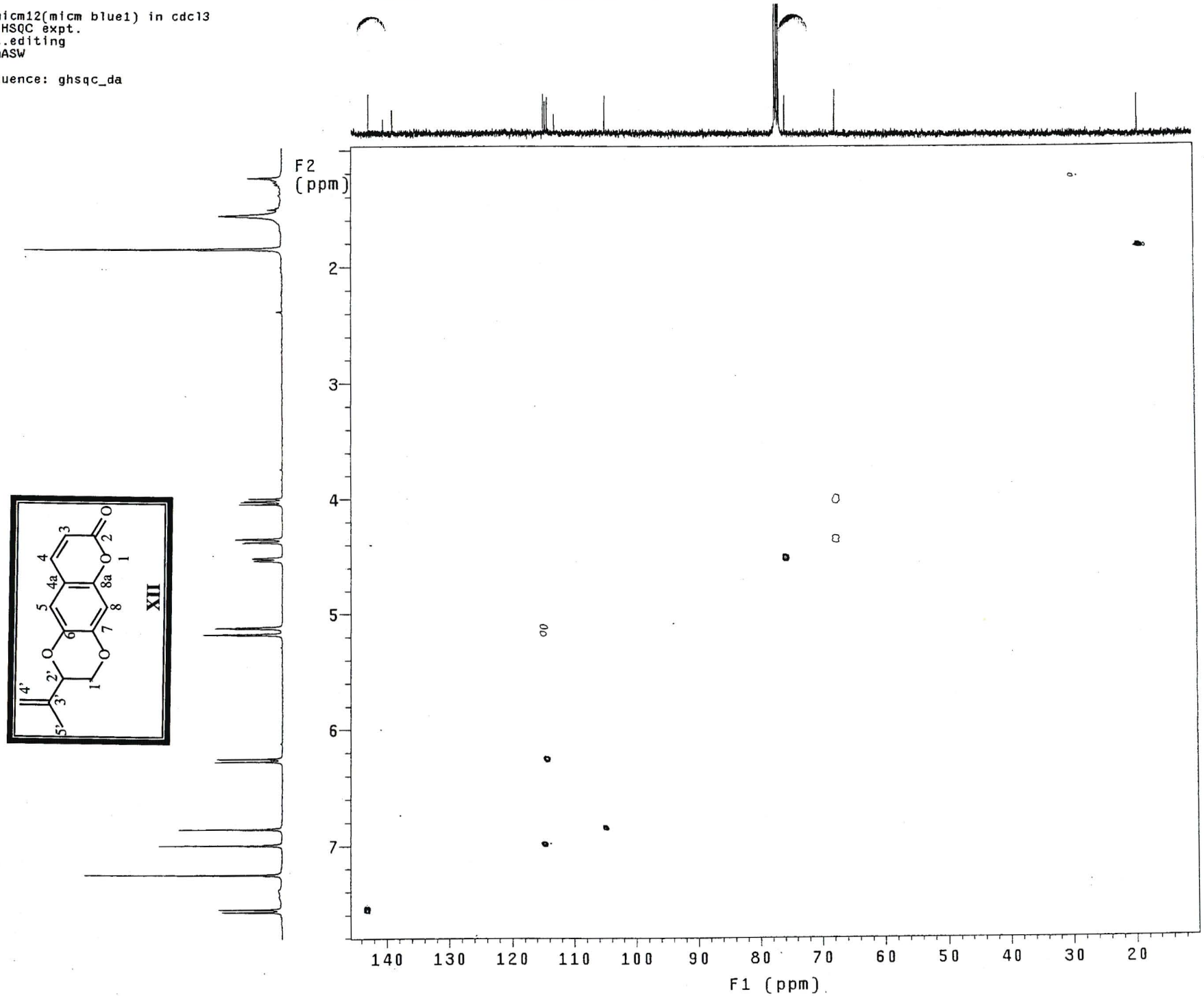
cymim12.micm12(micm blue1) in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

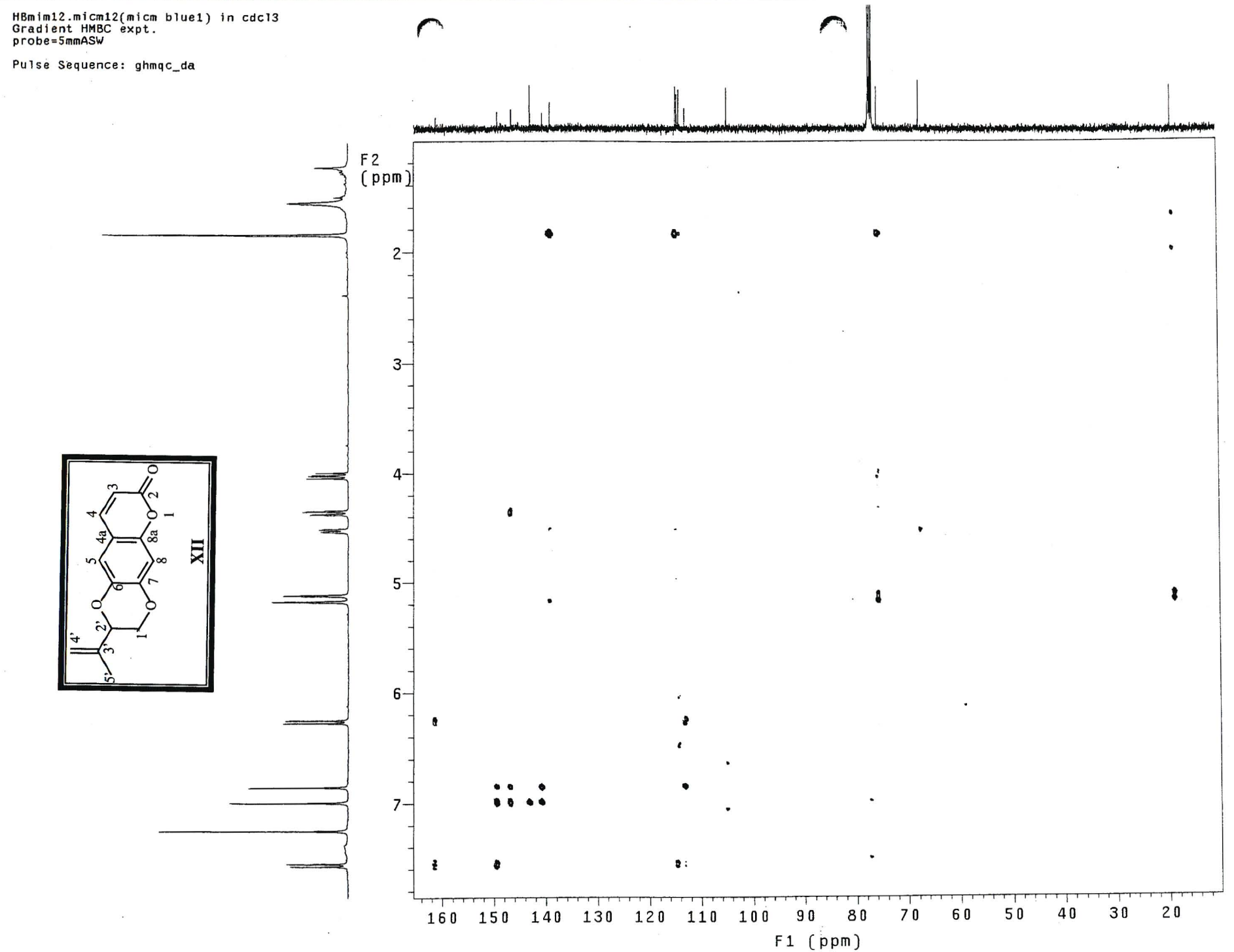




COSY spectrum of compound **XII**, obliquin



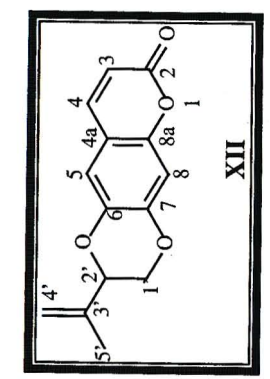
HSQC spectrum of compound **XII**, obliquin

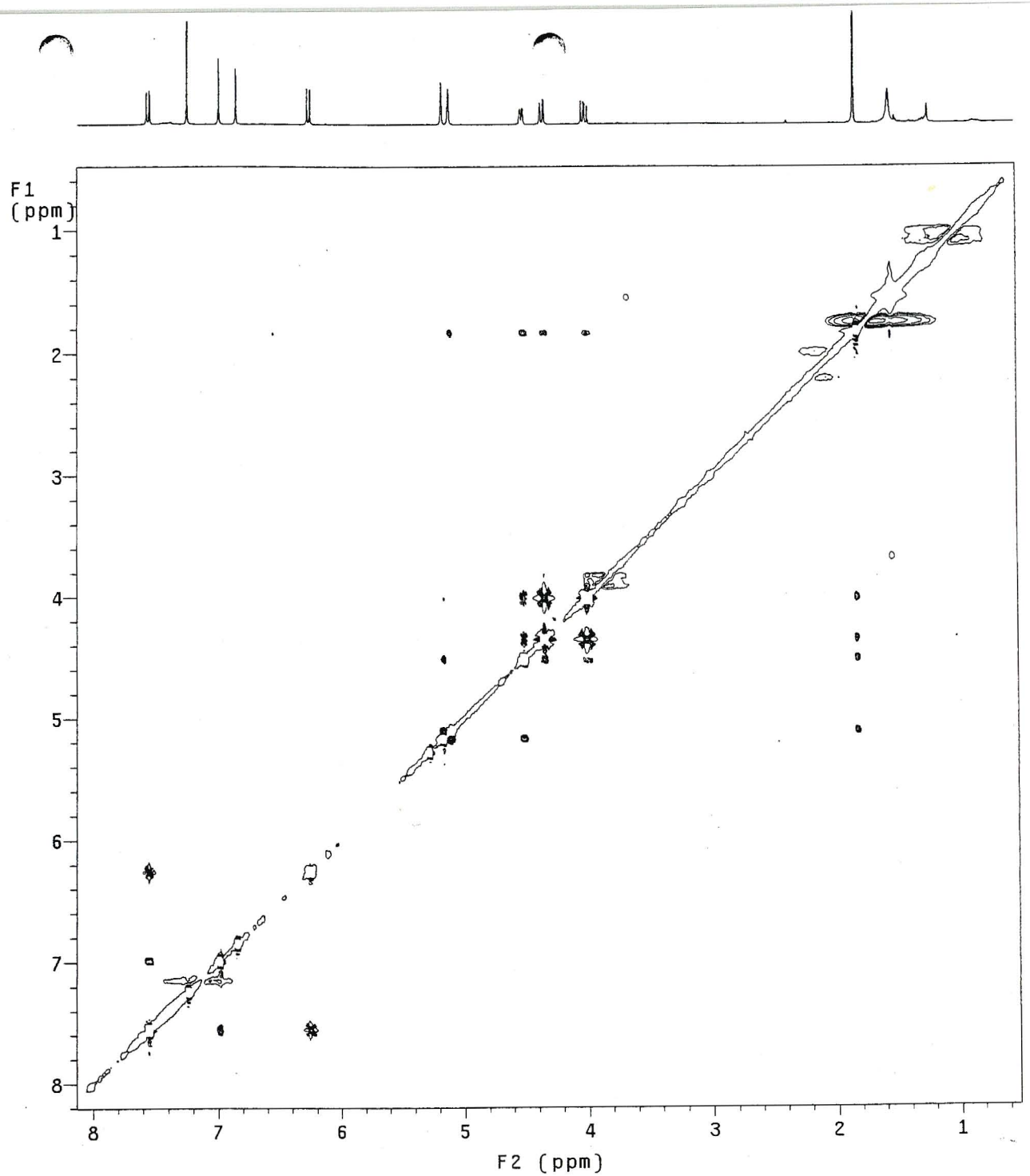


HMBC spectrum of compound **XII,** obliquin

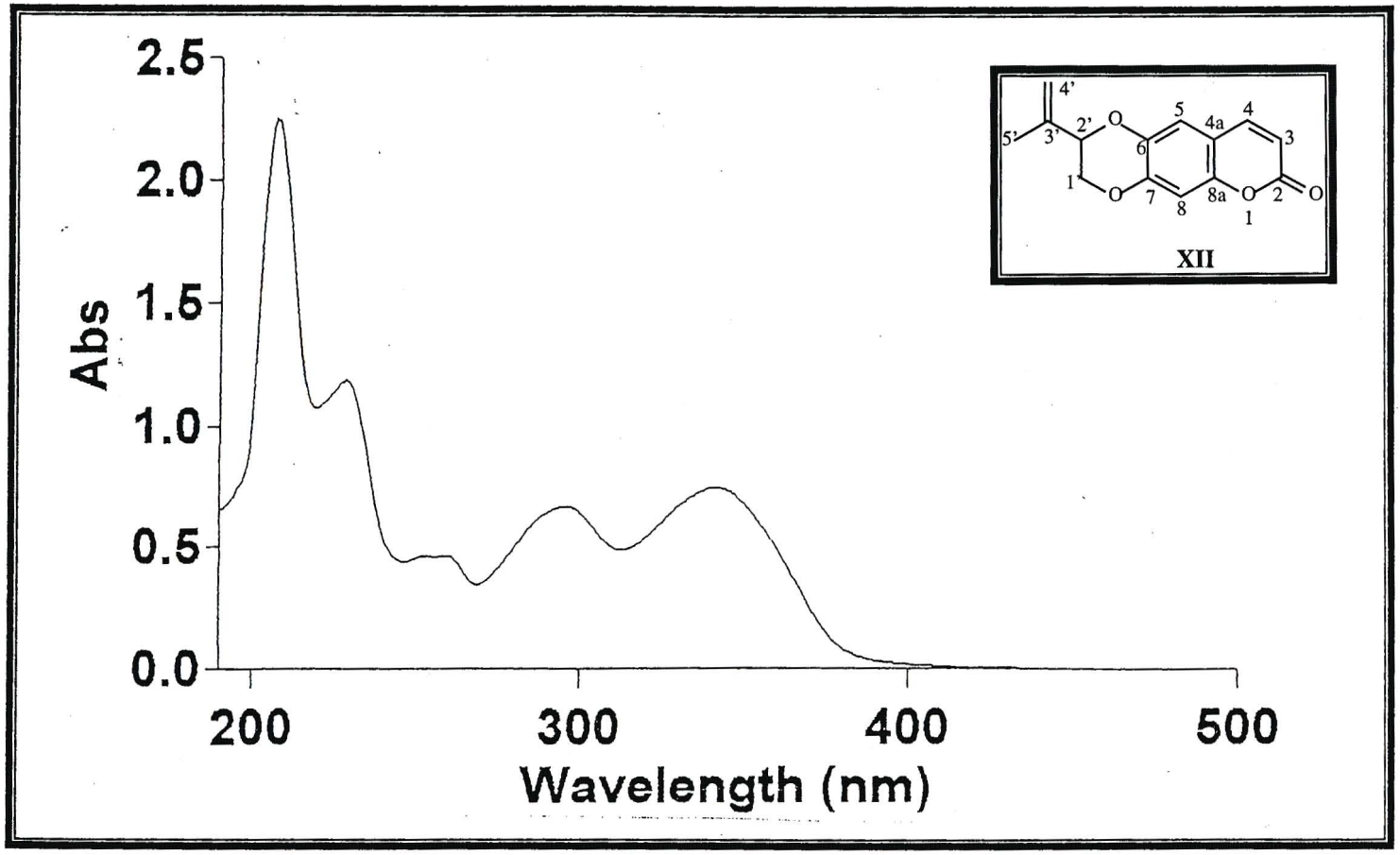
NOmim12.micm12(micm blue1) in cdcl3
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

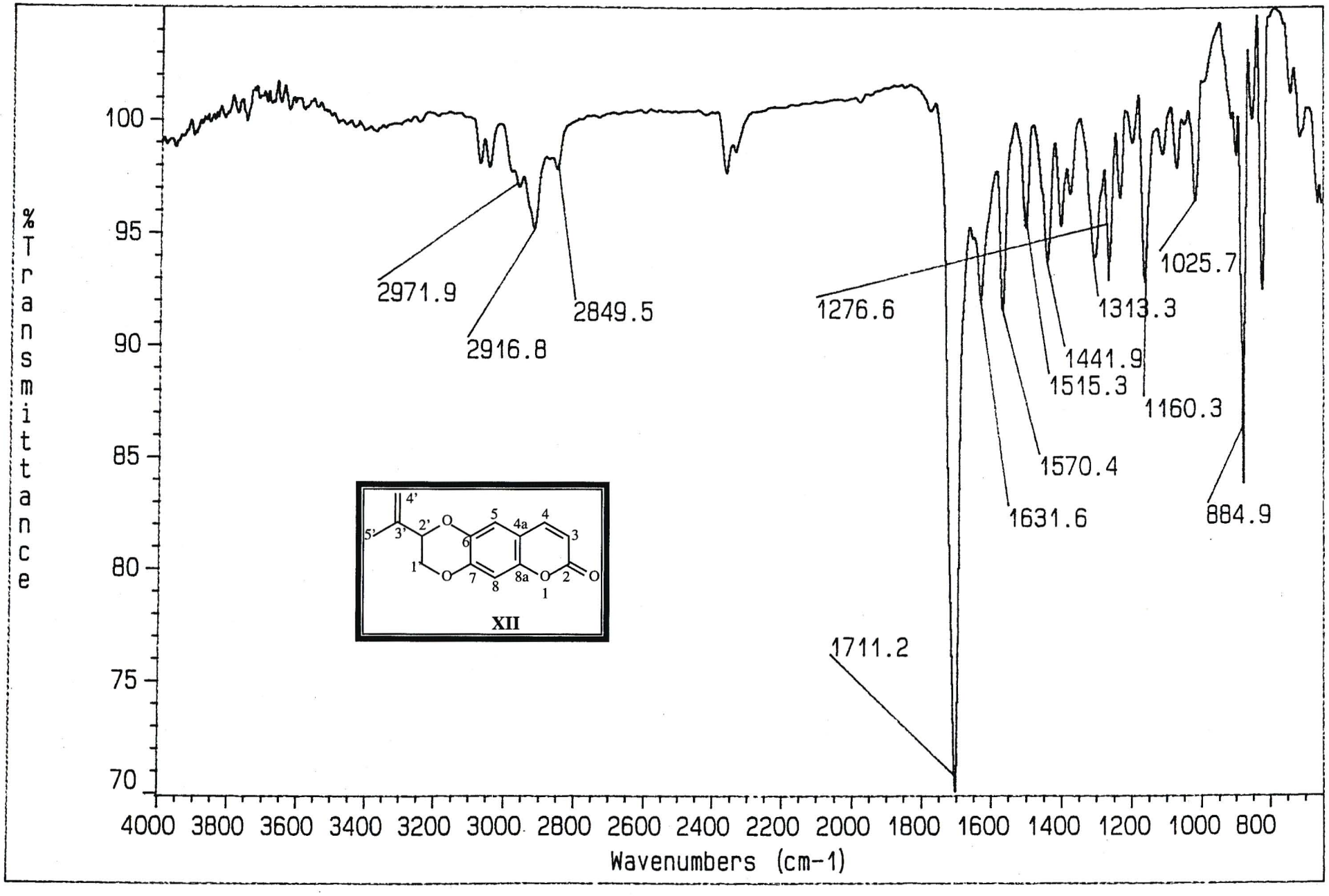




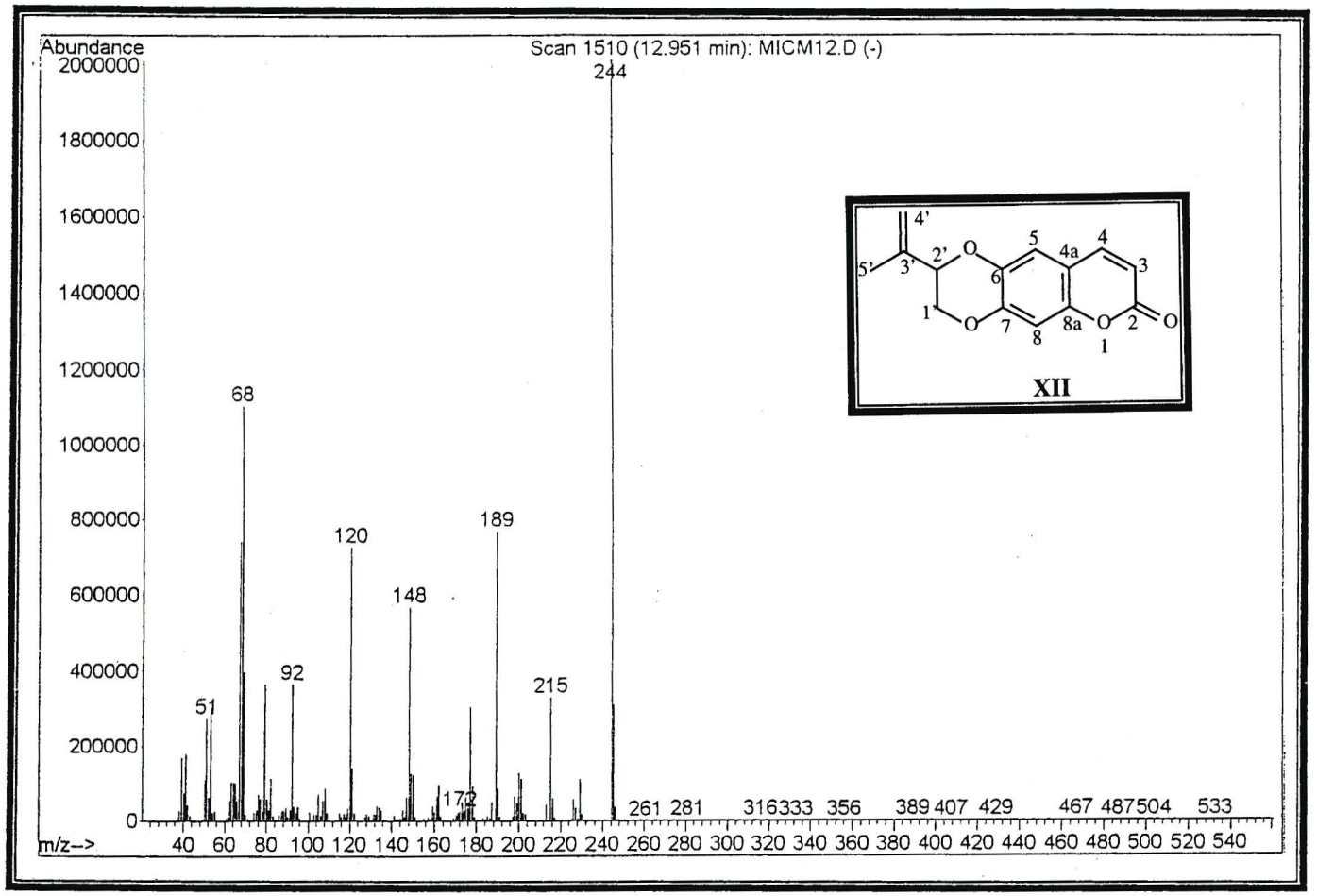
NOESY spectrum of compound **XII**, obliquin



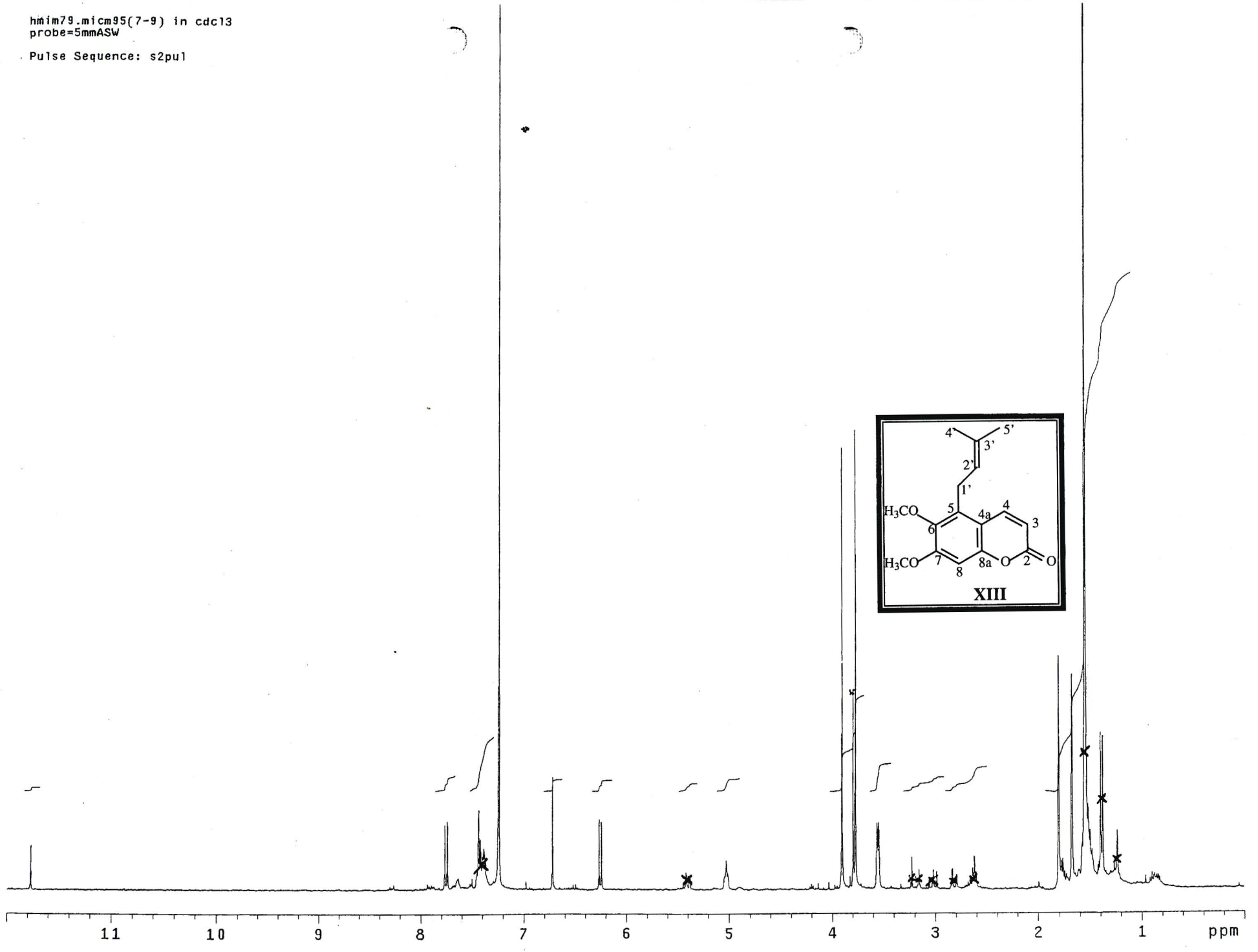
Ultra violet spectrum of compound **XII,** obliquin



Infrared spectrum of compound **XII**, obliquin



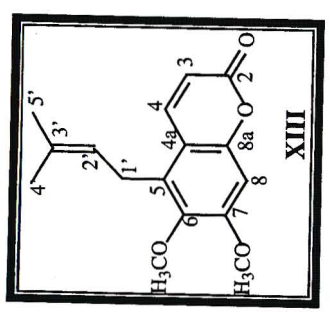
Mass spectrum of compound **XII**, obliquin



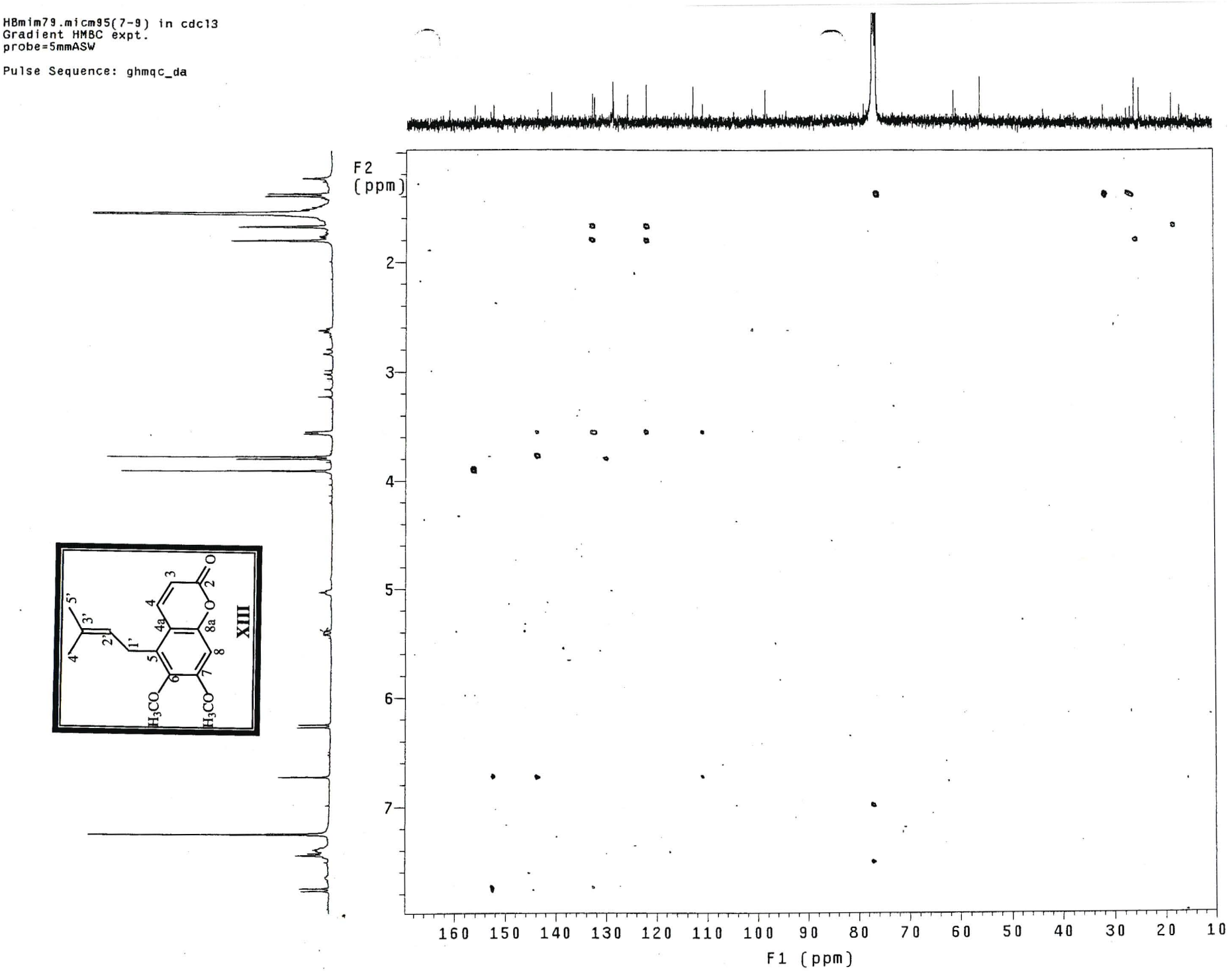
^{1}H NMR spectrum of compound **XIII**, microfolicoumarin

HQmim79.micm95(7-9) in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



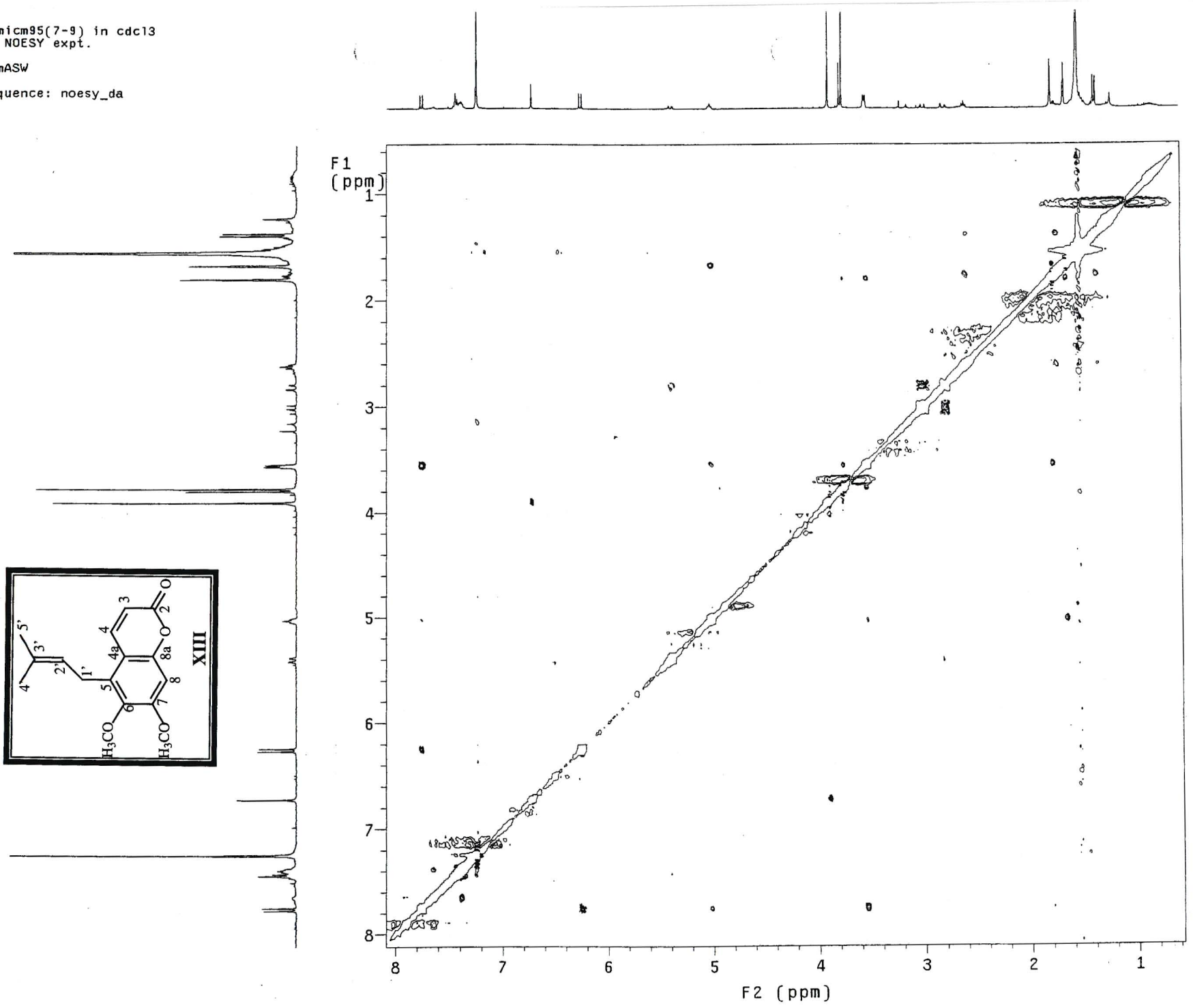
HSQC spectrum of compound **XIII,** microfolicoumarin



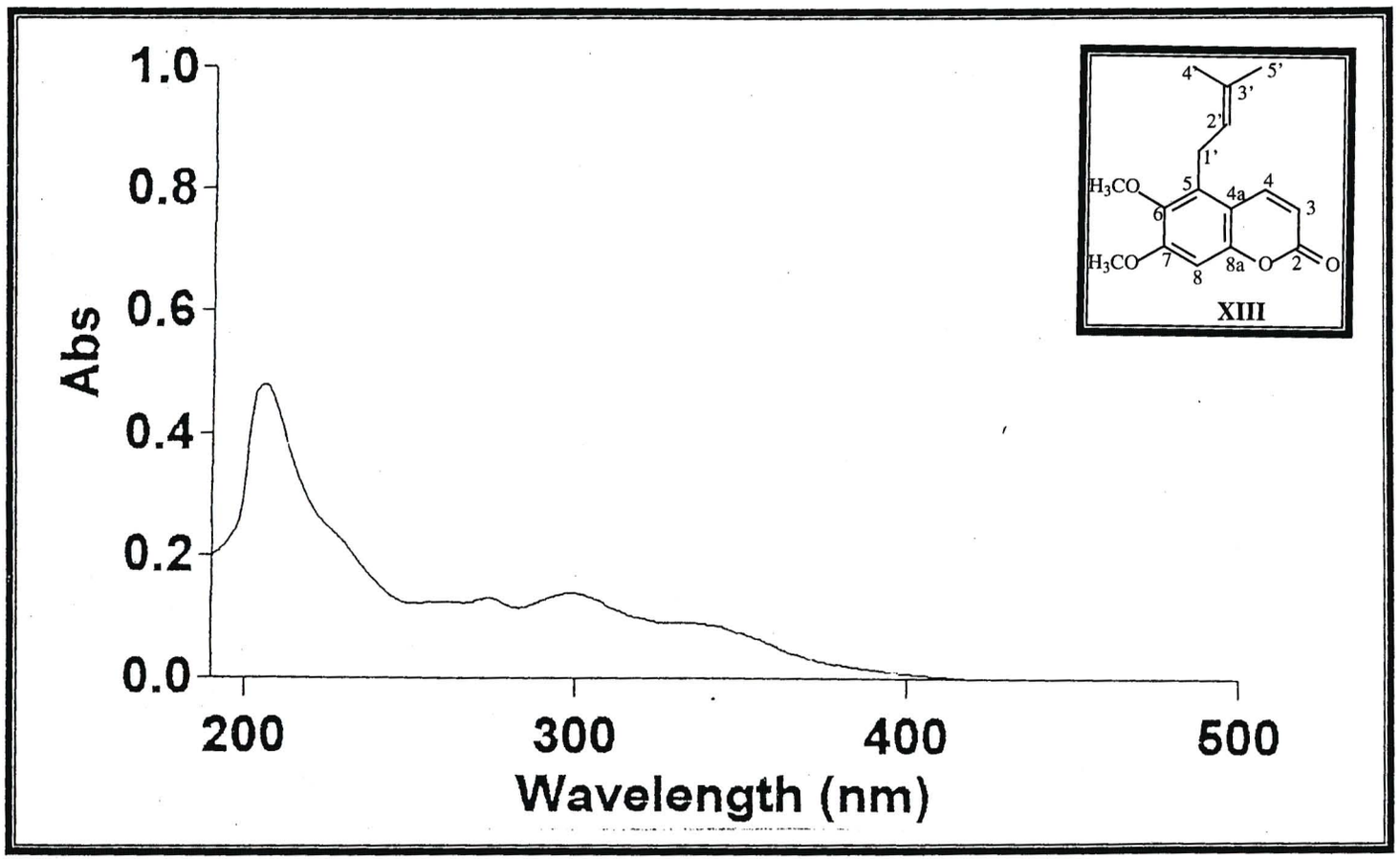
HMBC spectrum of compound **XIII,** microfolicoumarin

NOmim79.micm95(7-9) in cdcl3
Gradient NOESY expt.
mix=1sec
probe=5mmASW

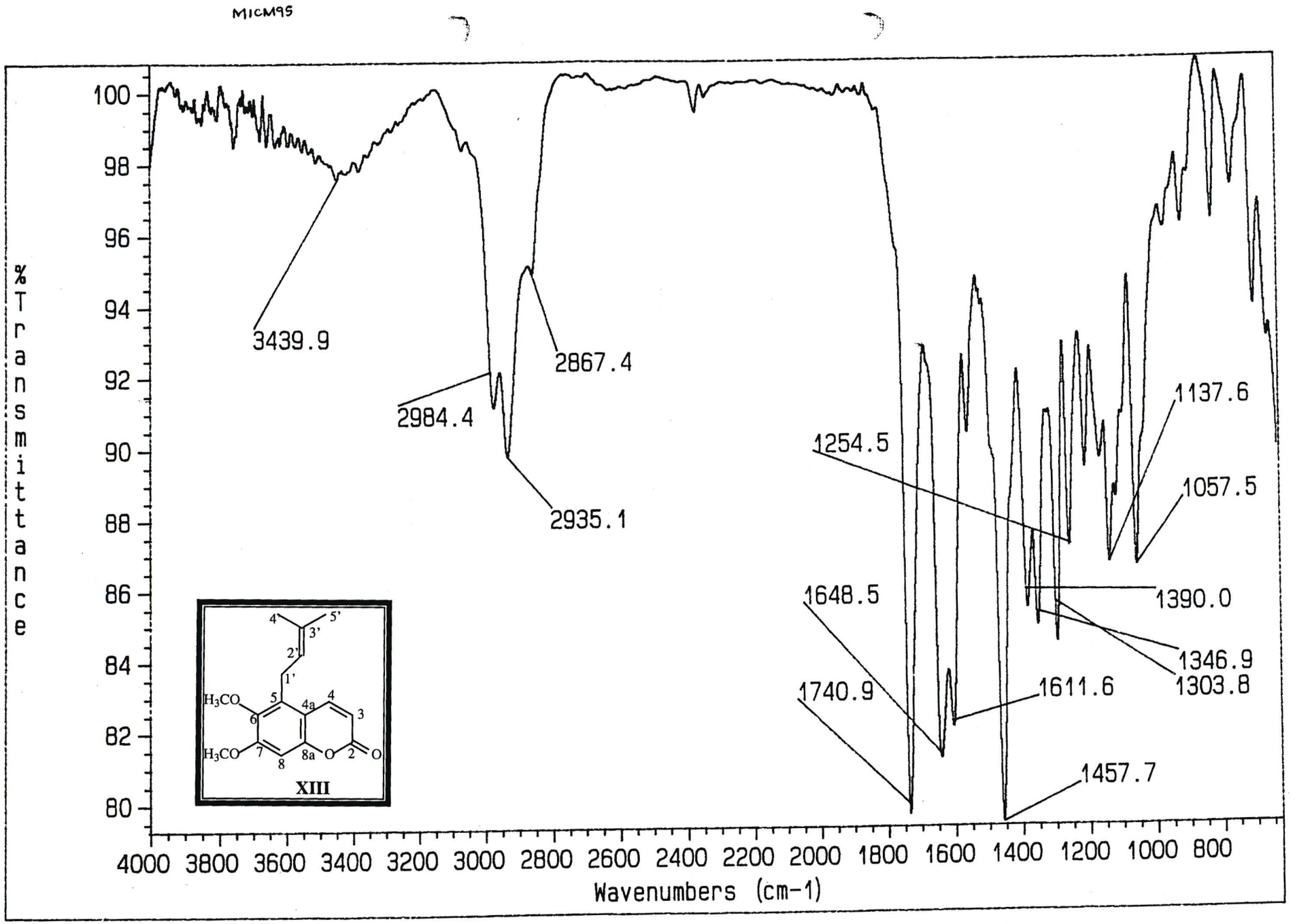
Pulse Sequence: noesy_da



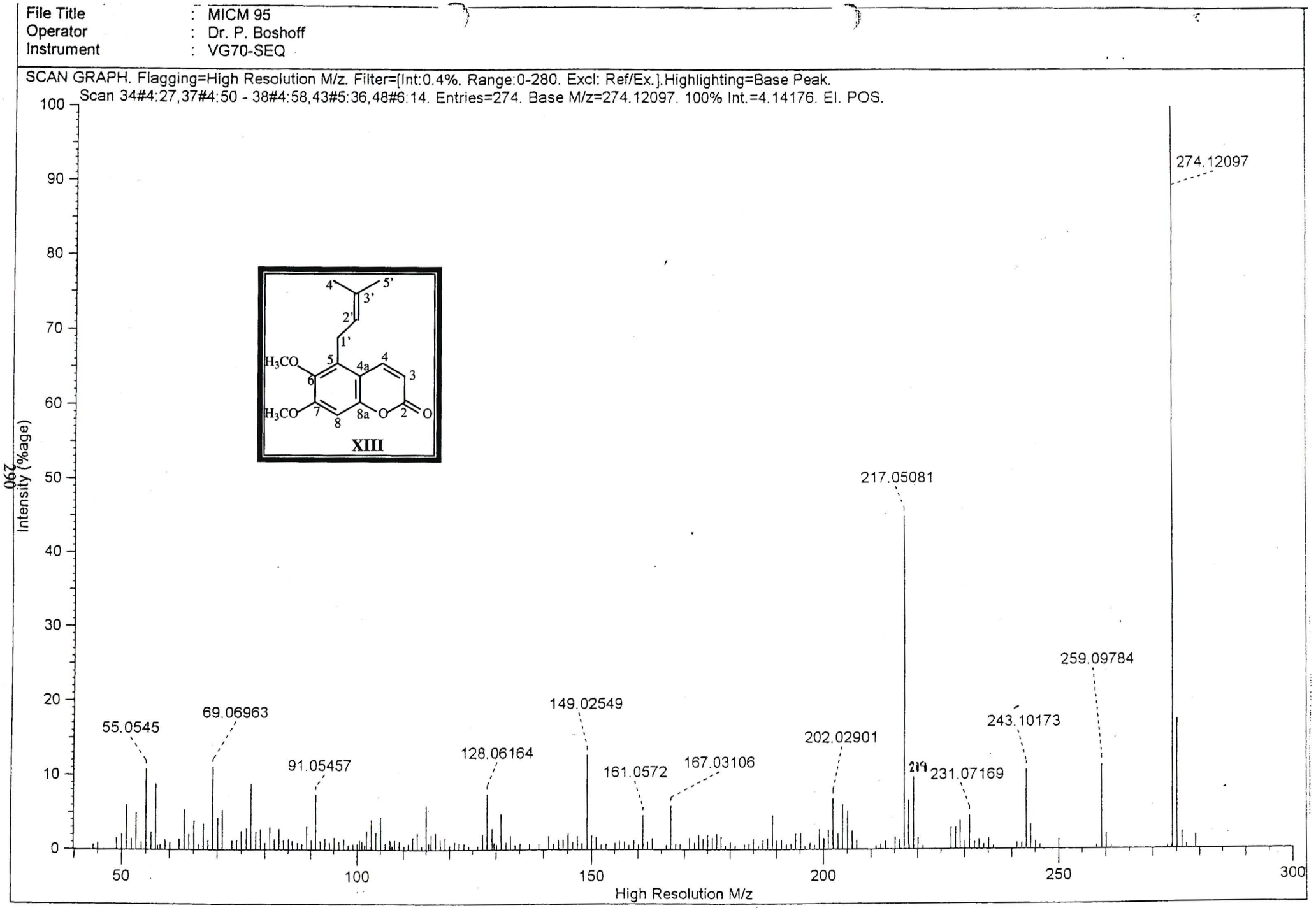
NOESY spectrum of compound **XIII**, microfolicoumarin



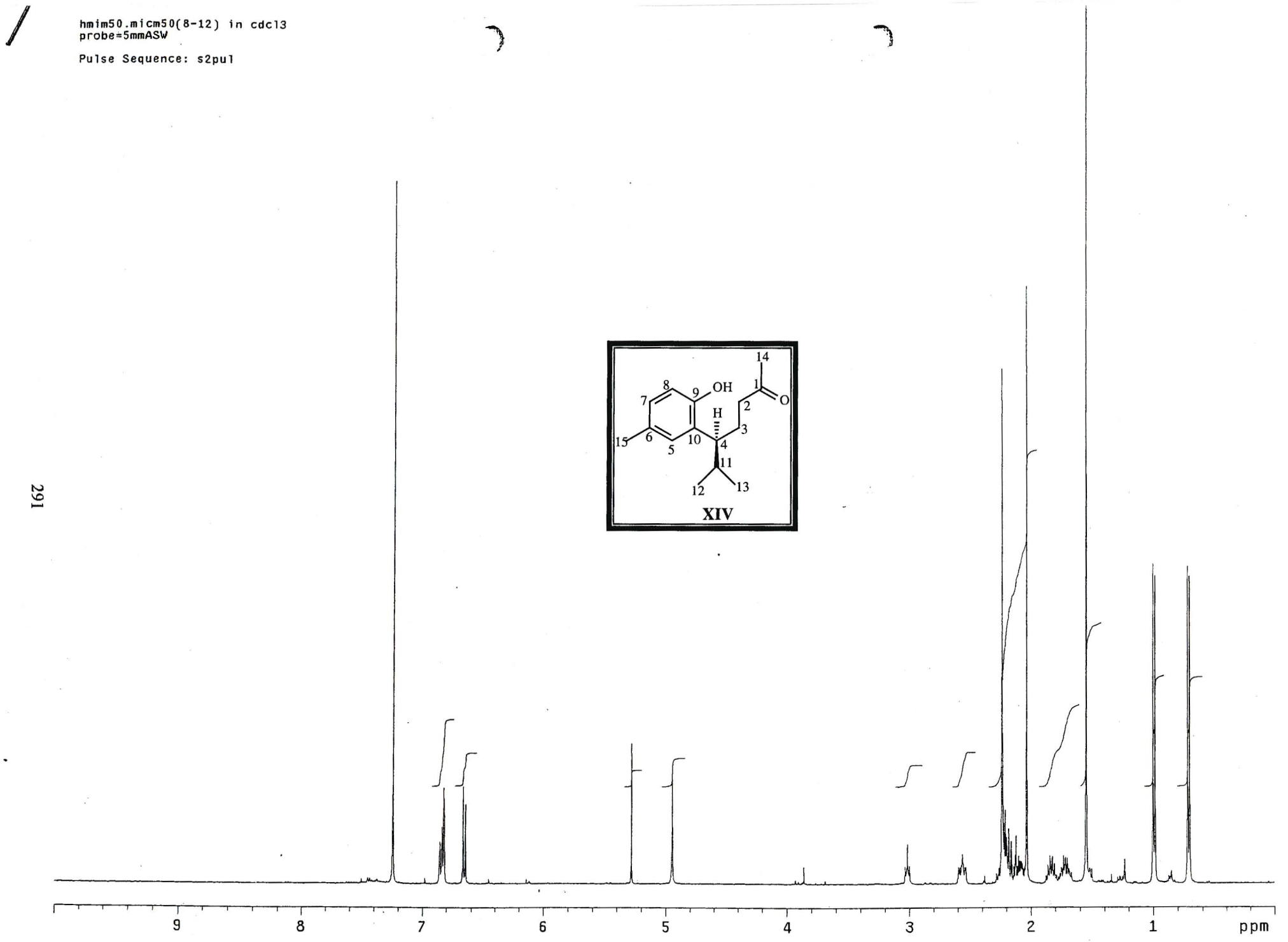
Ultra violet spectrum of compound **XIII,** microfolicoumarin



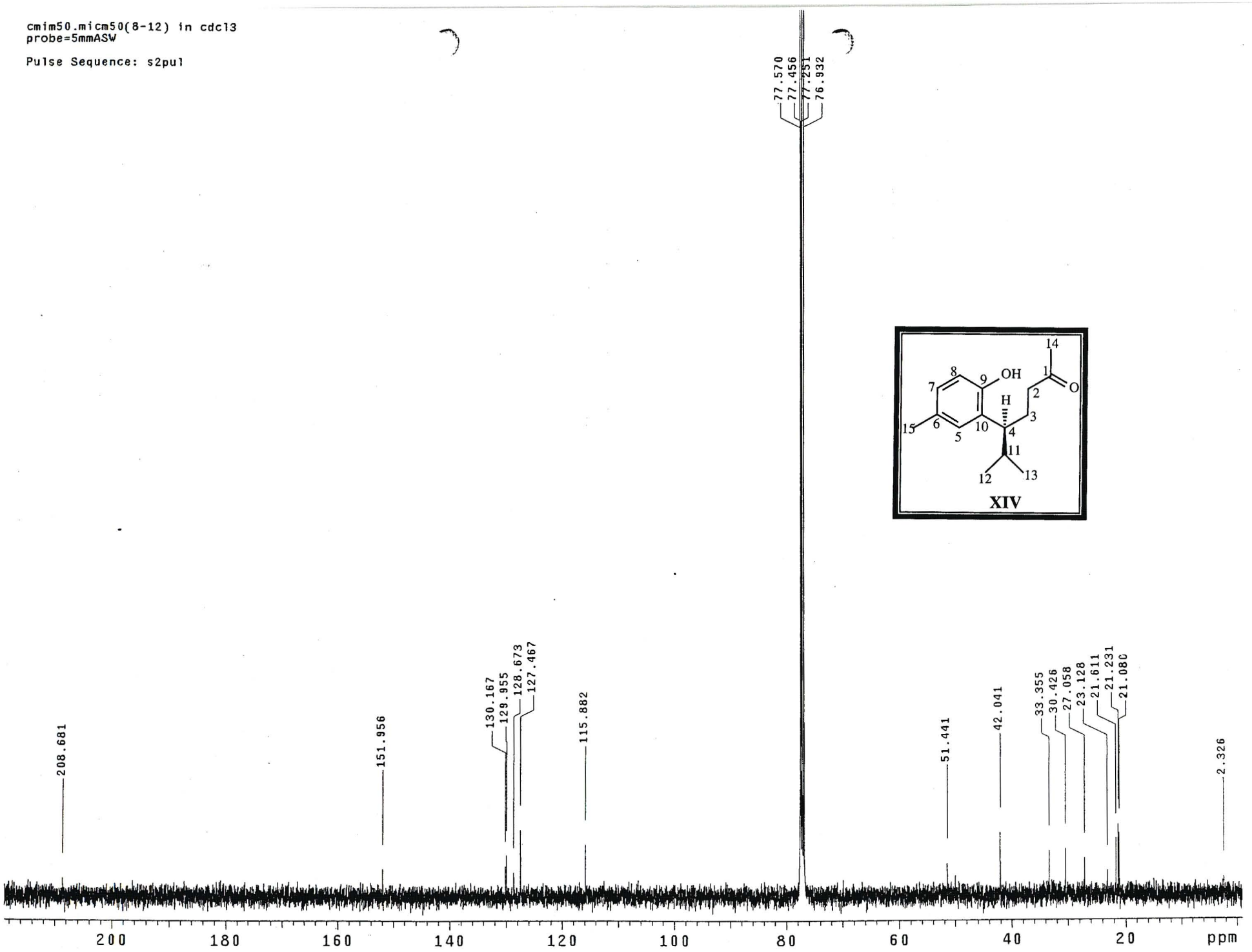
Infrared spectrum of compound **XIII,** microfolicoumarin



Mass spectrum of compound **XIII,** microfolicoumarin



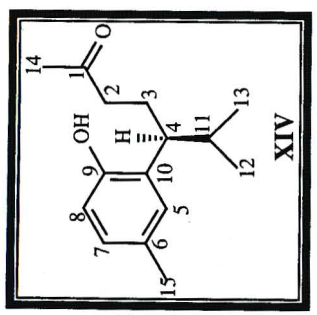
^{1}H NMR spectrum of compound **XIV**, sesquichamaenol

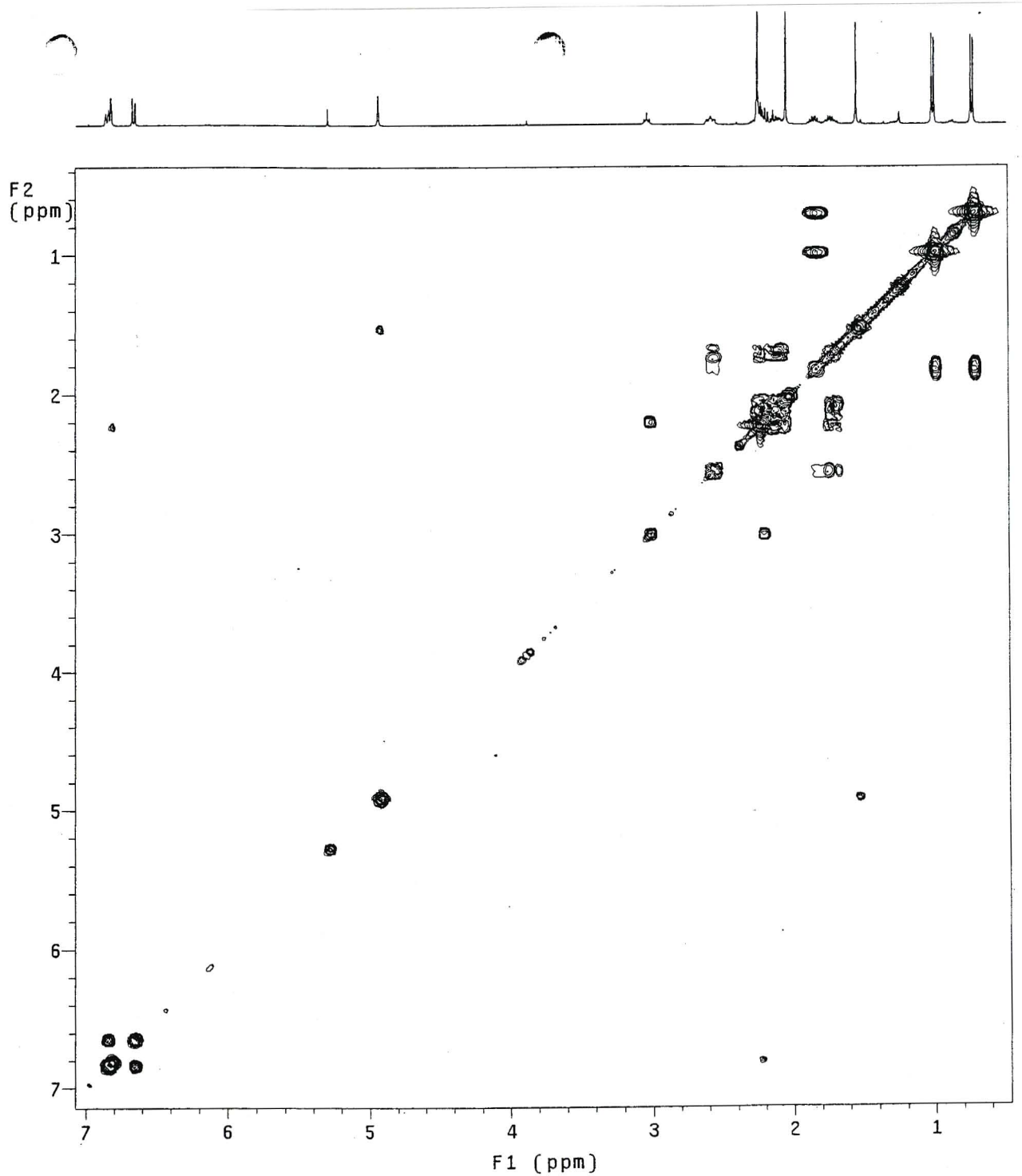


^{13}C NMR spectrum of compound **XIV**, sesquichamaenol

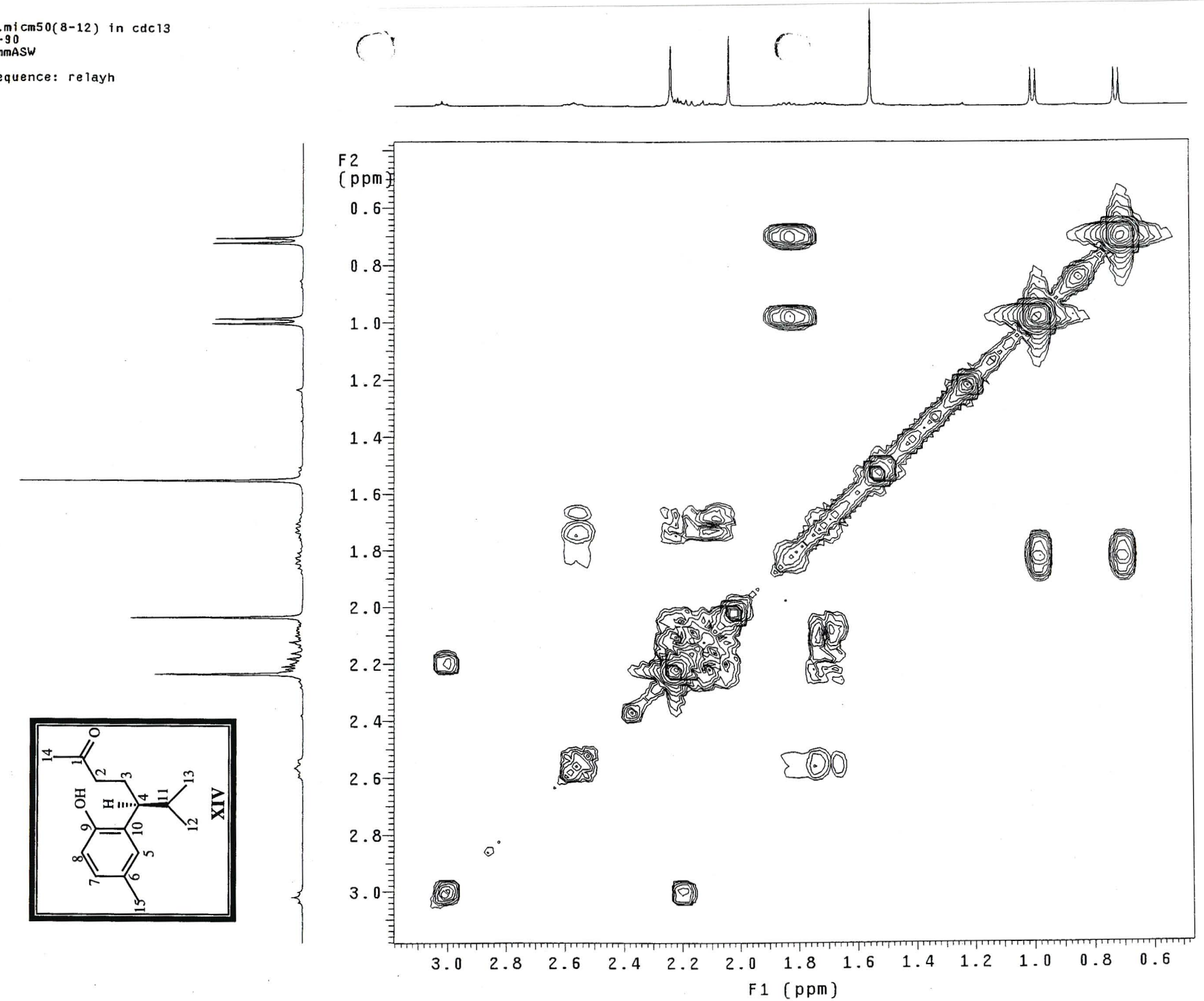
cymim50.micm50(8-12) in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

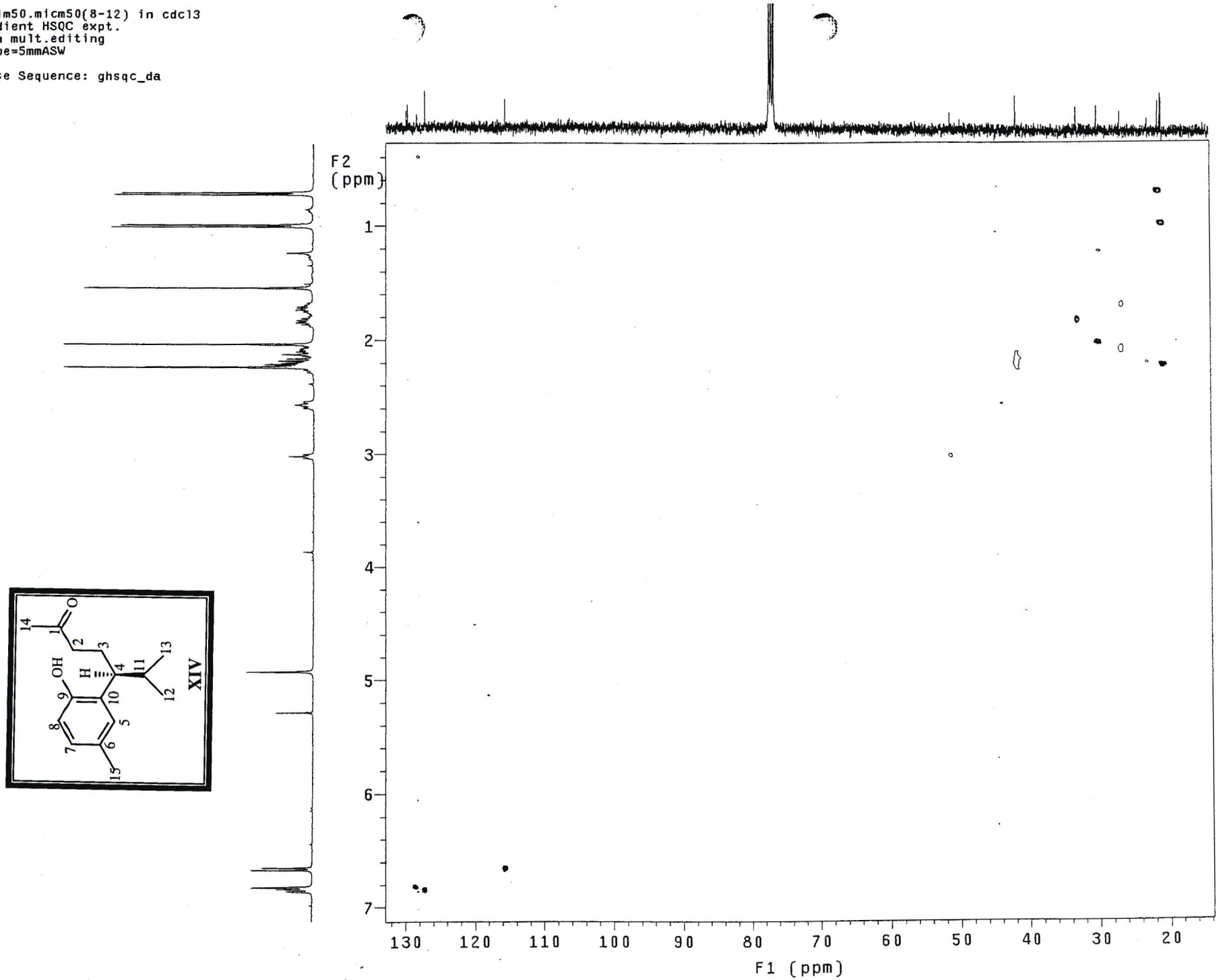




COSY spectrum of compound **XIV**, sesquichamaenol



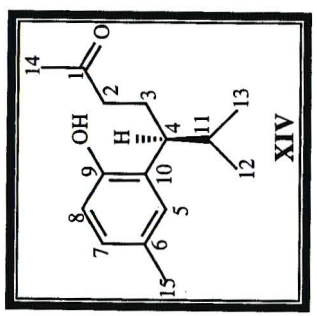
Expanded COSY spectrum of compound **XIV**, sesquichamaenol

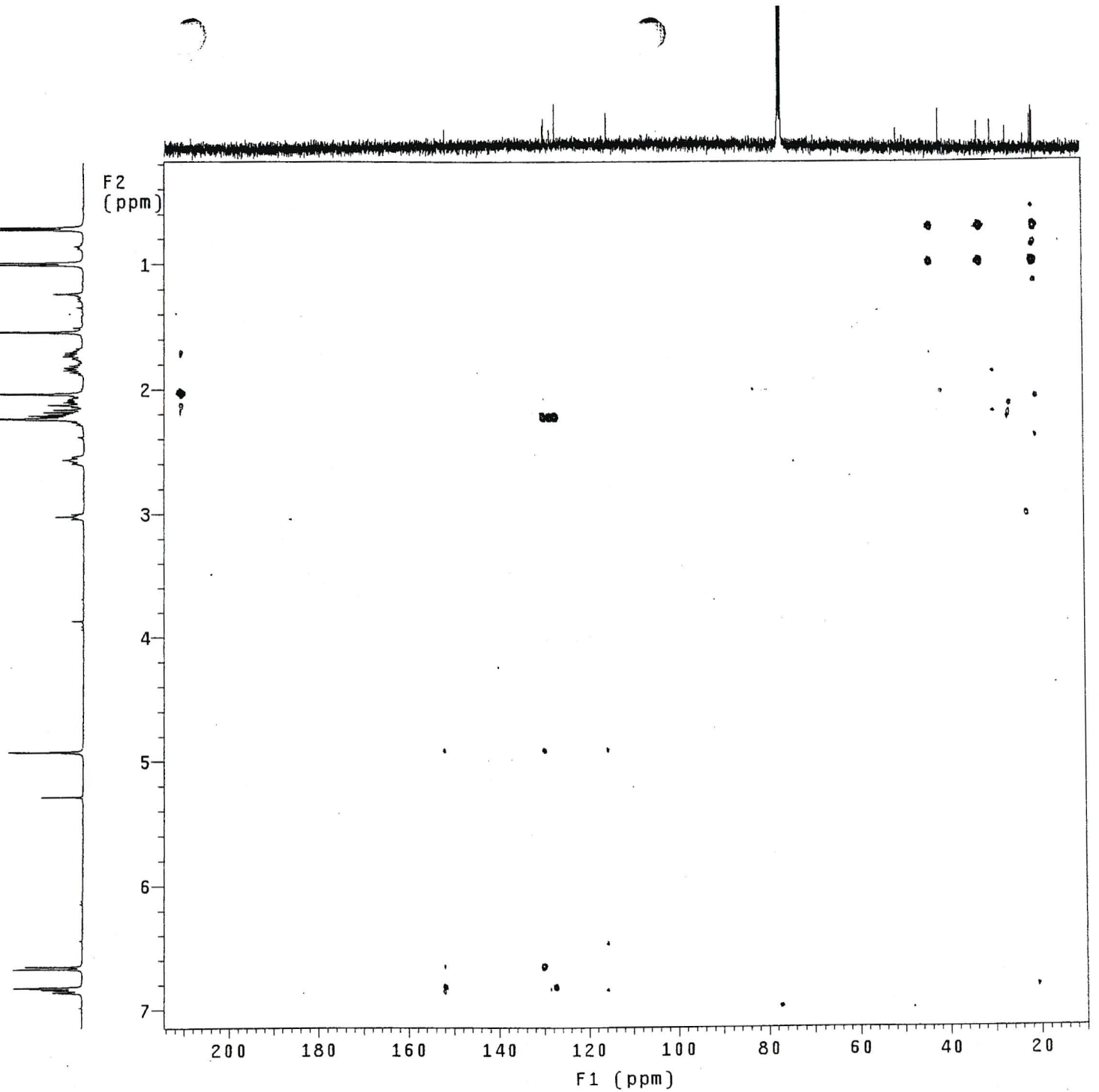


HSQC spectrum of compound **XIV**, sesquichamaenol

HBmim50.micm50(8-12) in cdcl3
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da

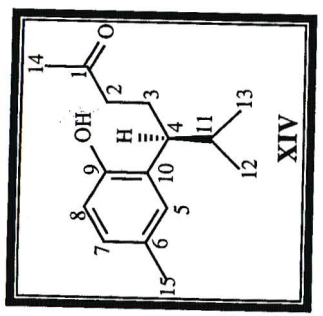




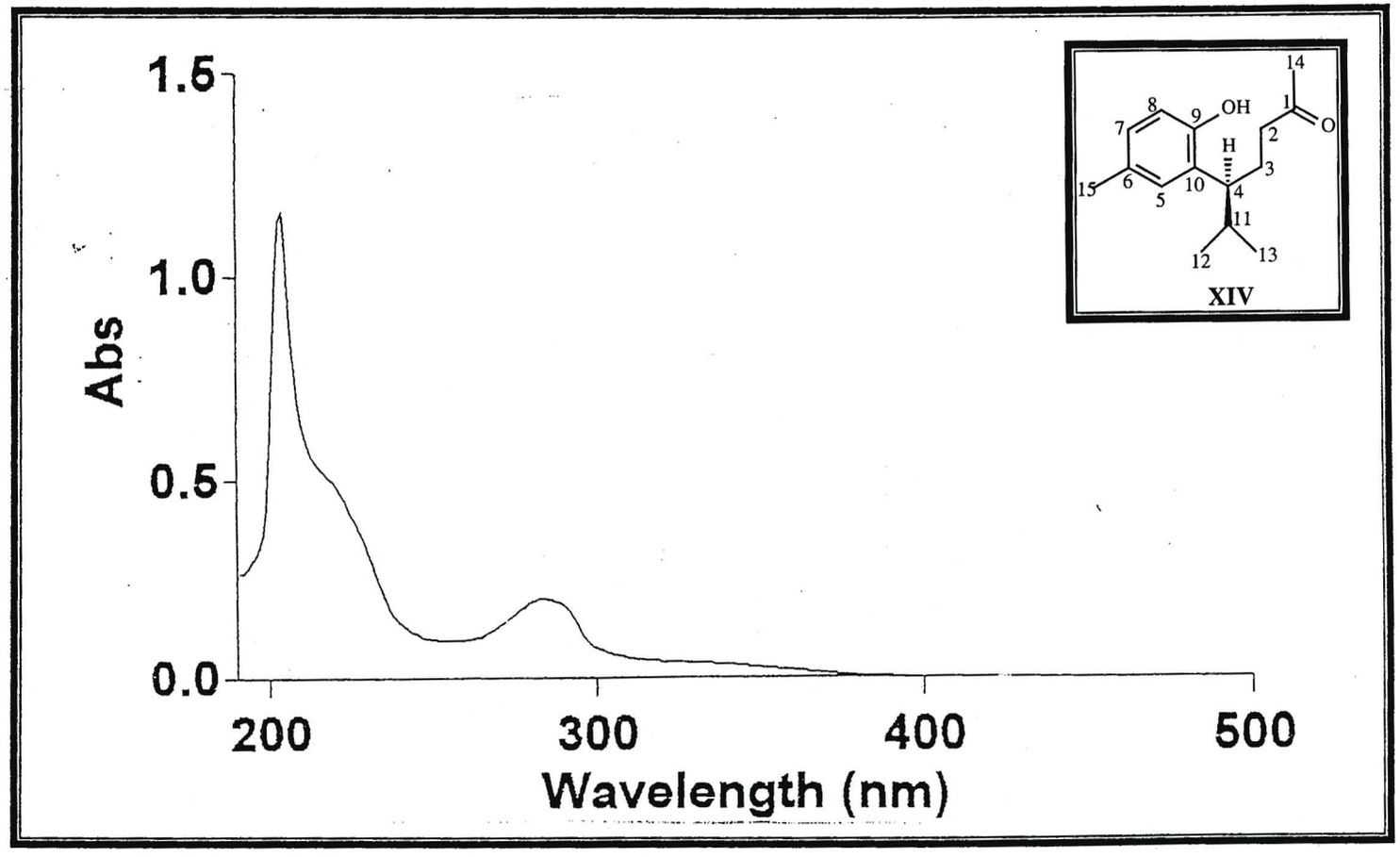
HMBC spectrum of compound **XIV**, sesquichamaenol

NOmim50.micm50(8-12) in cdcl3
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

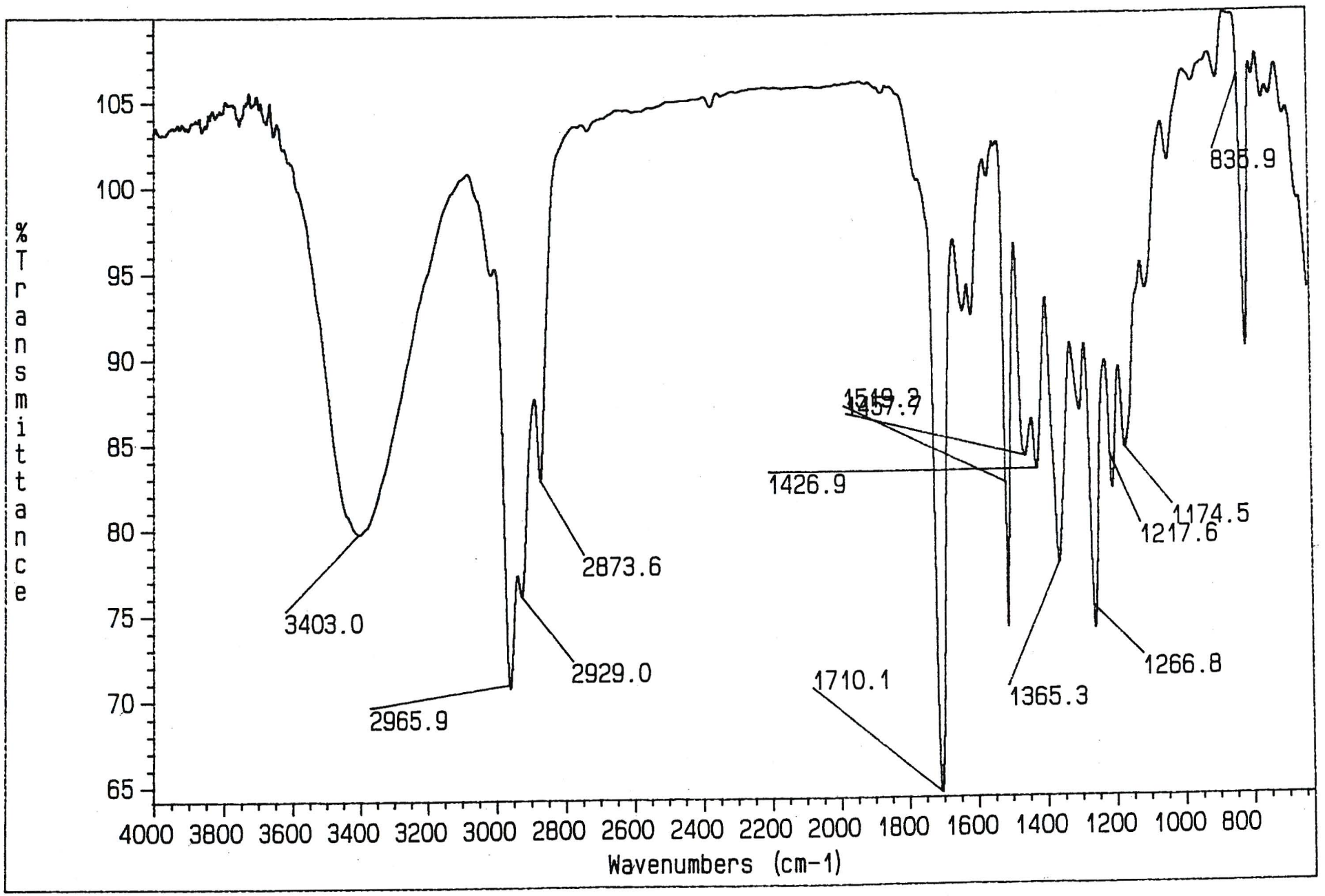


NOESY spectrum of compound **XIV**, sesquichamaenol



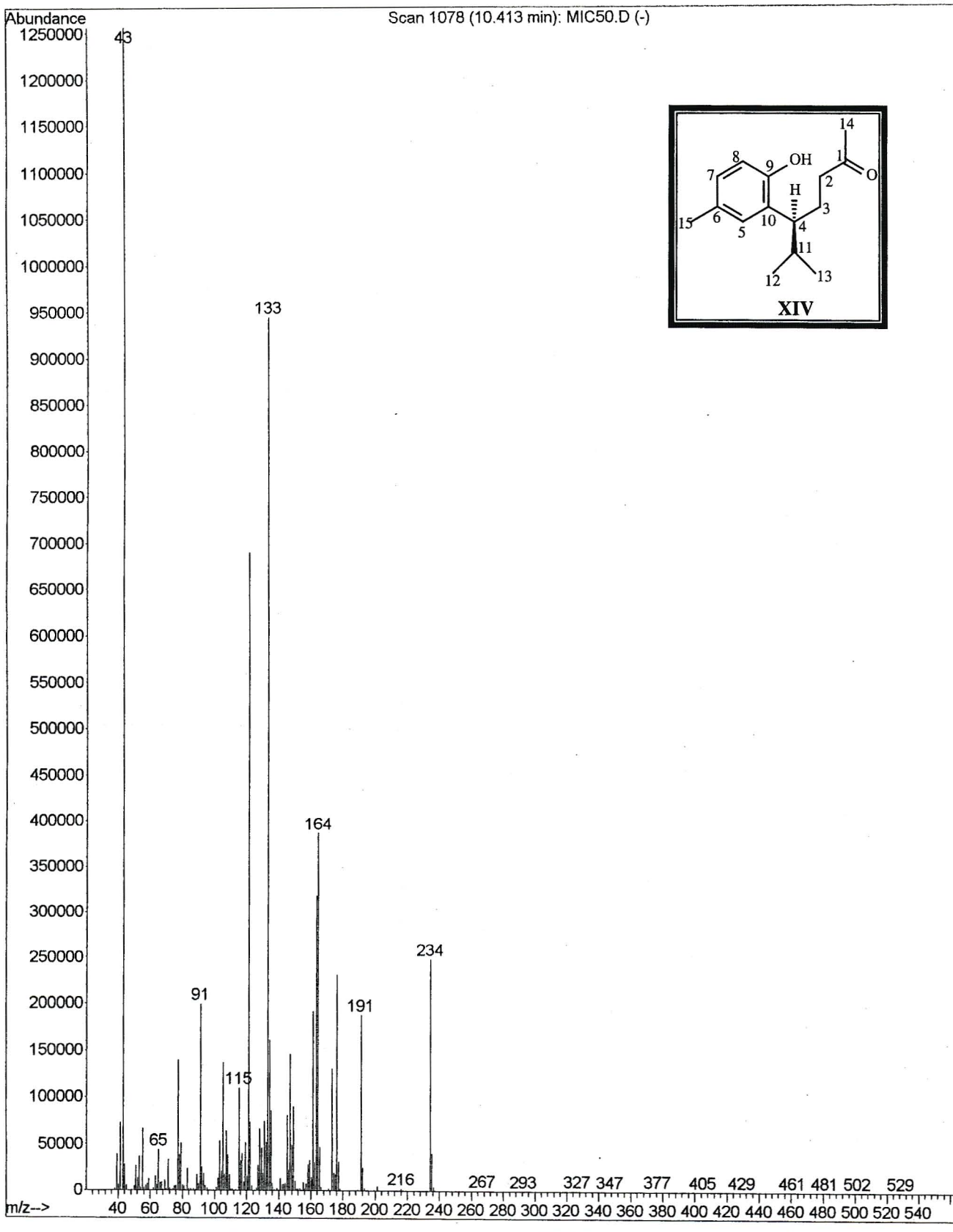
Ultra violet spectrum of compound **XIV**, sesquichamaenol

MICMSC



Infrared spectrum of compound **XIV**, sesquichamaenol

File : D:\NEIL\MIC50.D
Operator : Bret
Acquired : 20 Dec 2000 13:33 using AcqMethod NEW
Instrument : Instrumen
Sample Name: MIC50 (8-12)
Misc Info : 1ul inject, MeCl2, splitless
Vial Number: 7



Mass spectrum of compound **XIV**, sesquichamaenol

Appendix III

NMR, Ultra violet, Infrared and Mass spectra

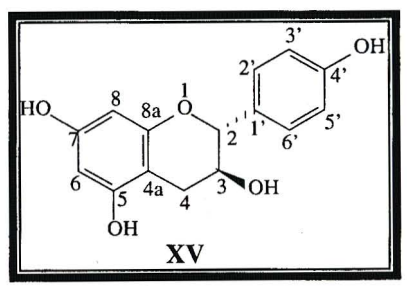
for

Chapter 5. Extractives from *Khadia alticola*

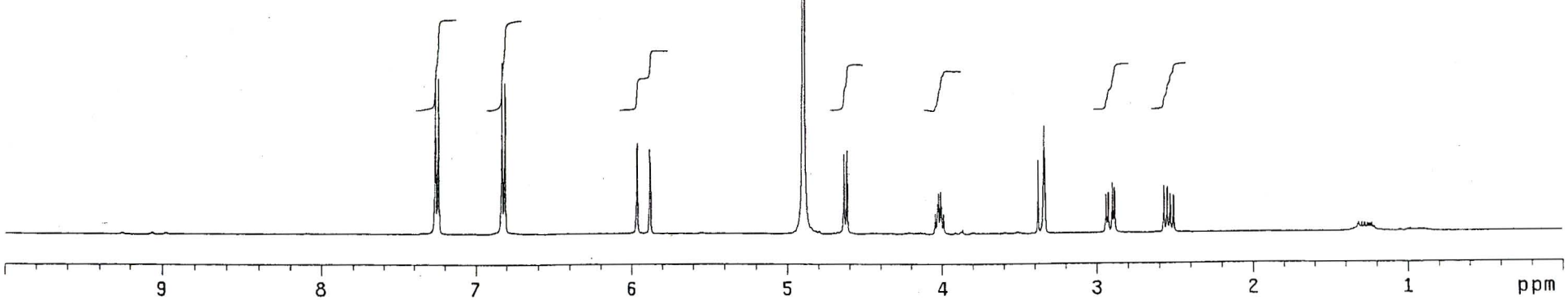
hkad23.khad22-23 in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	2904.1[illegible]	7.261	24.0
2	2902.486	7.257	8.6
3	2897.541	7.245	11.6
4	2895.710	7.240	25.1
5	2734.373	6.837	6.4
6	2731.809	6.830	27.6
7	2729.795	6.825	9.1
8	2725.217	6.814	10.8
9	2723.202	6.809	24.3
10	2387.160	5.969	13.9
11	2384.779	5.963	14.7
12	2352.915	5.883	13.7
13	2350.717	5.878	11.6
14	1958.088	4.896	200.0
15	1854.253	4.636	12.9
16	1846.562	4.617	13.6
17	1611.424	4.029	4.9
18	1608.860	4.023	6.6
19	1603.366	4.009	6.8
20	1600.802	4.003	4.3
21	1351.380	3.379	11.9
22	1338.927	3.348	7.9
23	1337.462	3.344	13.5
24	1335.814	3.340	17.4
25	1334.166	3.336	12.2
26	1332.701	3.332	6.4
27	1175.759	2.940	6.2
28	1170.265	2.926	6.5
29	1159.644	2.900	8.2
30	1154.150	2.886	7.3
31	1027.974	2.570	7.6
32	1019.550	2.549	7.4
33	1011.858	2.530	6.3
34	1003.434	2.509	6.1



XV

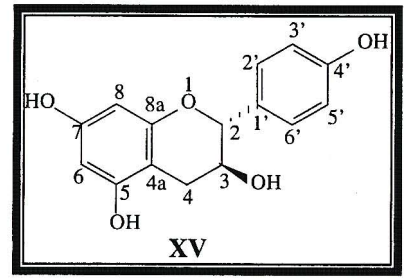


^{1}H NMR spectrum of compound **XV**, (+)-afzelechin

ckad23.khad22-23 in cd3od
probe=5mmASW

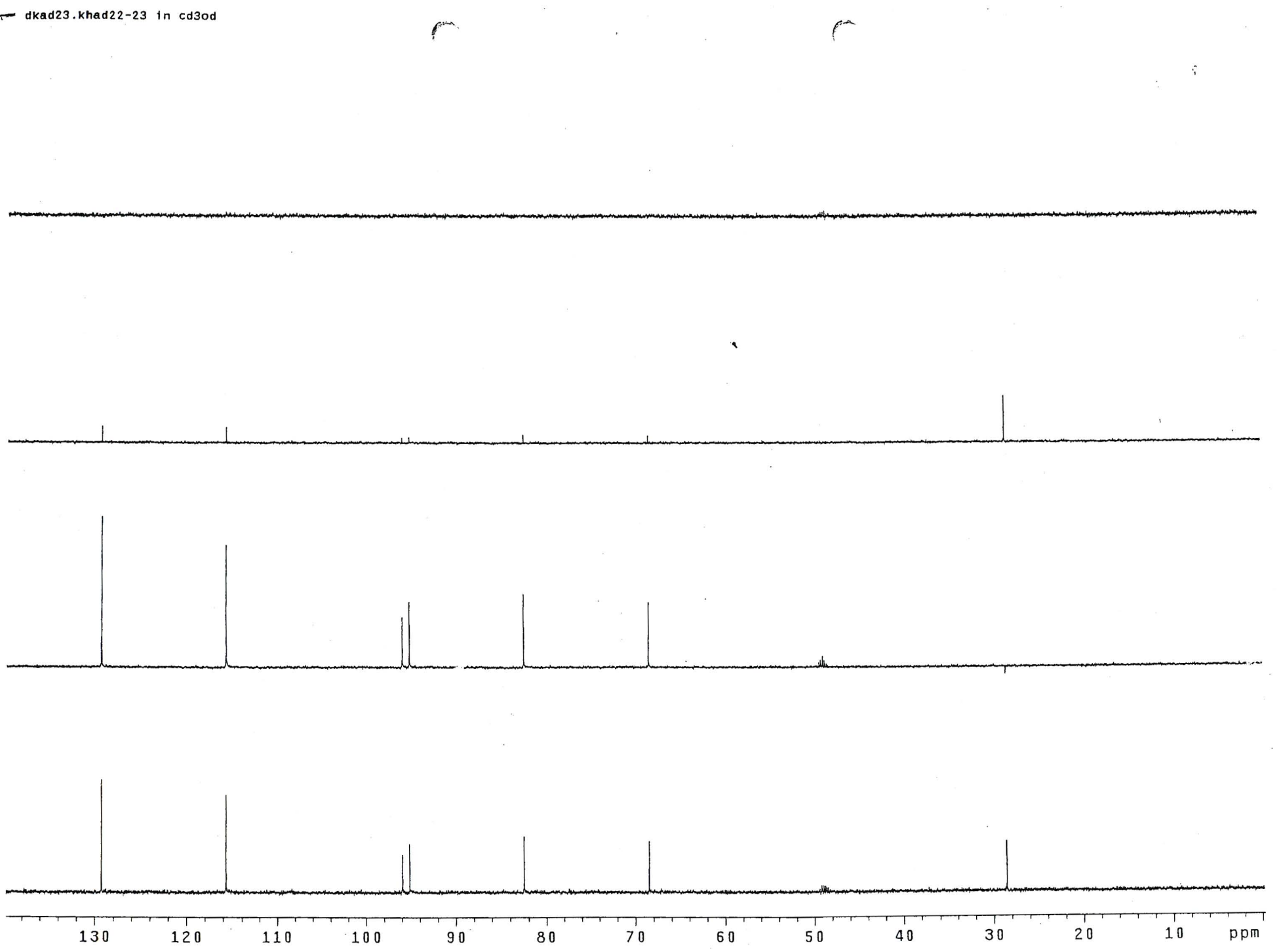
Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	15925.8	158.362	10.4
2	15871.669	157.823	7.9
3	15844.203	157.550	8.0
4	15786.217	156.974	9.0
5	13221.900	131.475	15.4
6	13034.974	129.616	57.1
7	11669.270	116.036	44.4
8	10147.160	100.901	10.0
9	9684.804	96.303	16.7
10	9602.404	95.484	19.4
11	8331.308	82.844	25.6
12	6921.353	68.824	21.1
13	4991.821	49.637	21.5
14	4970.458	49.425	64.4
15	4949.095	49.212	128.3
16	4927.732	49.000	150.0
17	4906.369	48.788	128.3
18	4885.006	48.575	63.7
19	4863.643	48.363	21.1
20	2904.354	28.880	18.9



XV

^{13}C NMR spectrum of compound **XV**, (+)-afzelechin

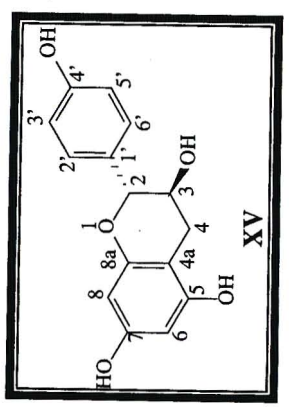


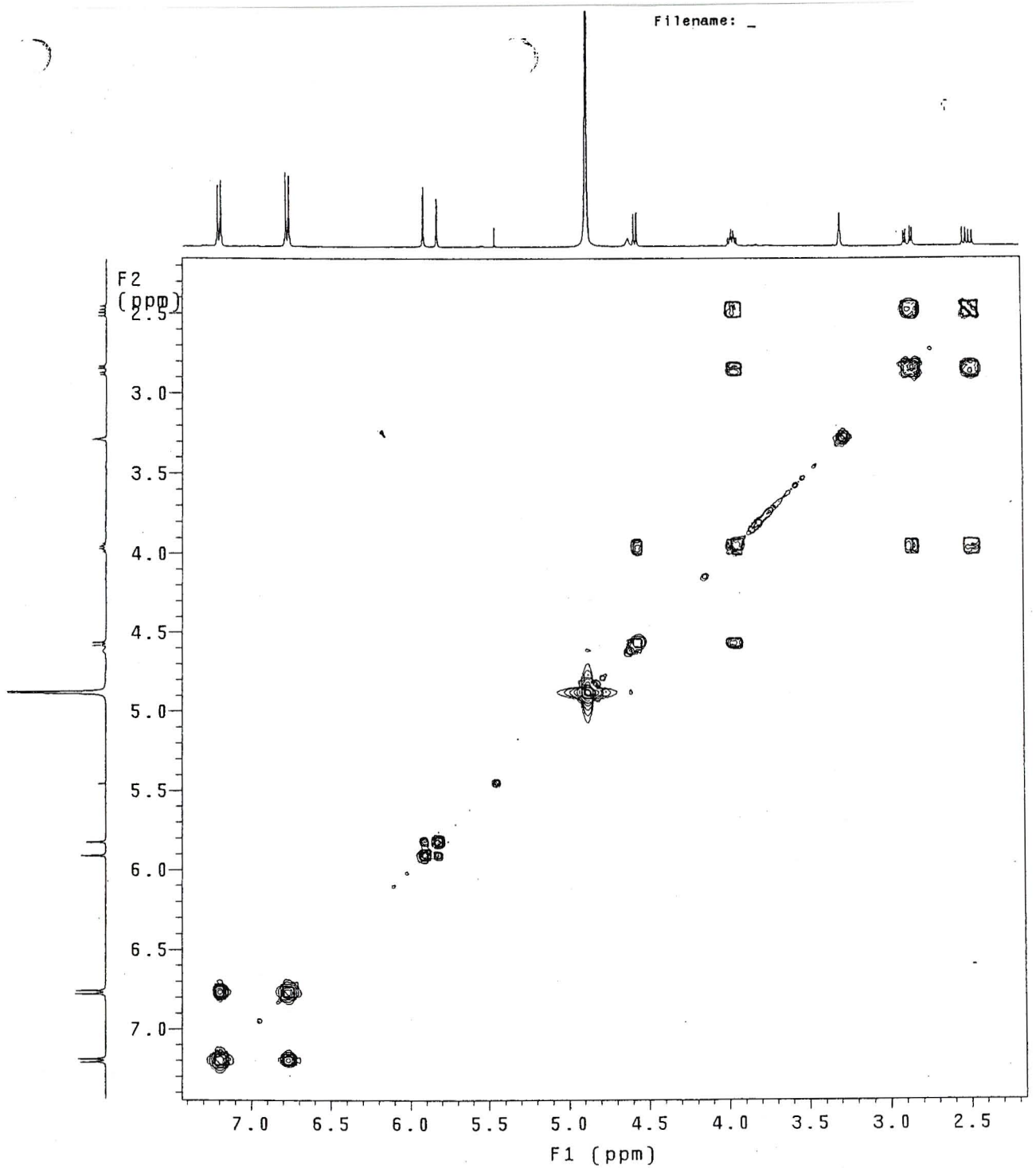
ADEPT spectrum of compound **XV**, (+)-afzelechin

khad22-23

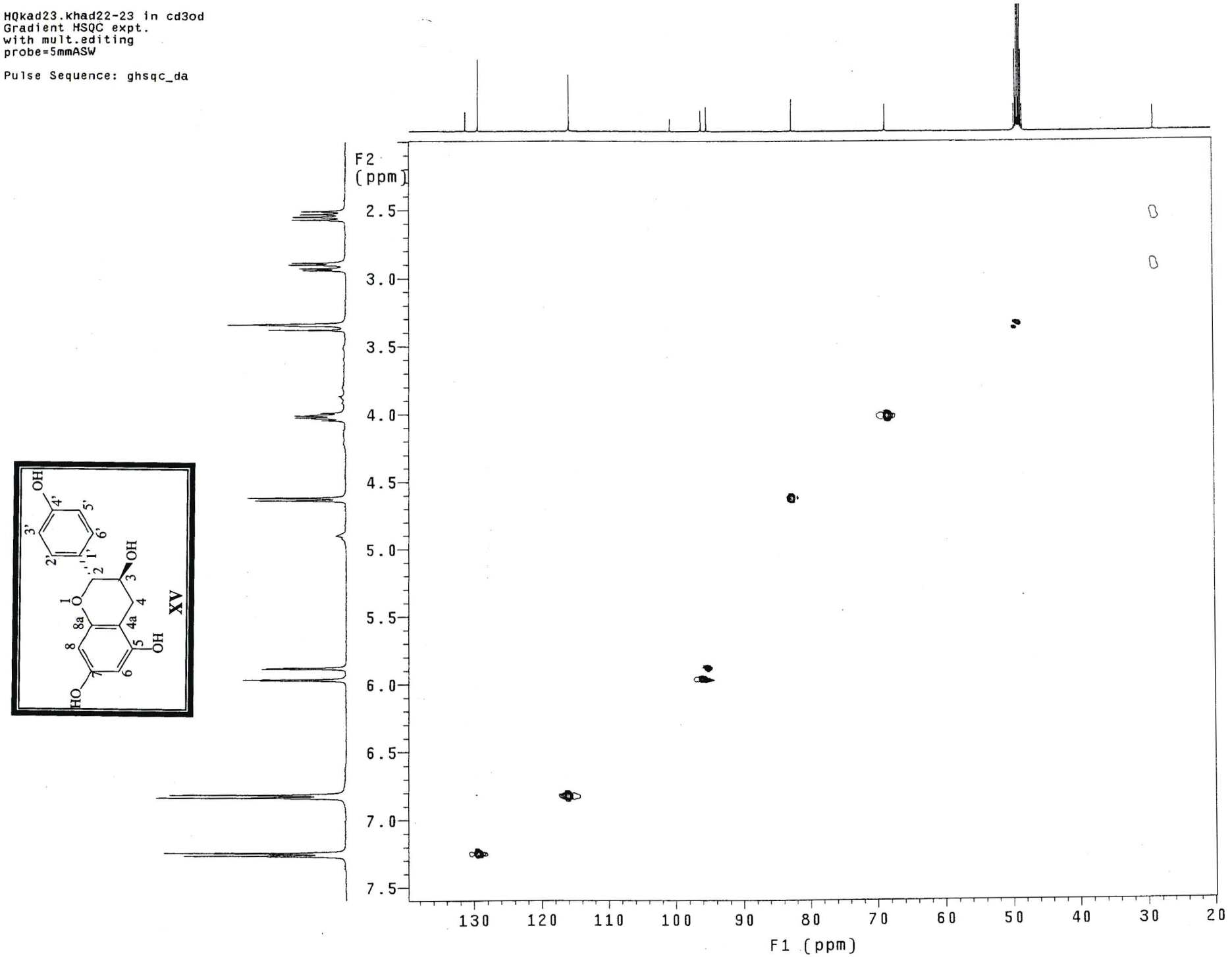
Solvent: cd3od
Ambient temperature
INOVA-400 "undnmr400"

PULSE SEQUENCE: relayh
Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.163 sec
Width 3132.1 Hz
2D Width 3132.1 Hz
16 repetitions
256 increments
OBSERVE H1, 399.9502544 MHz
DATA PROCESSING
Sine bell 0.082 sec
F1 DATA PROCESSING
Sine bell 0.041 sec
FT size 1024 x 1024
Total time 82 minutes

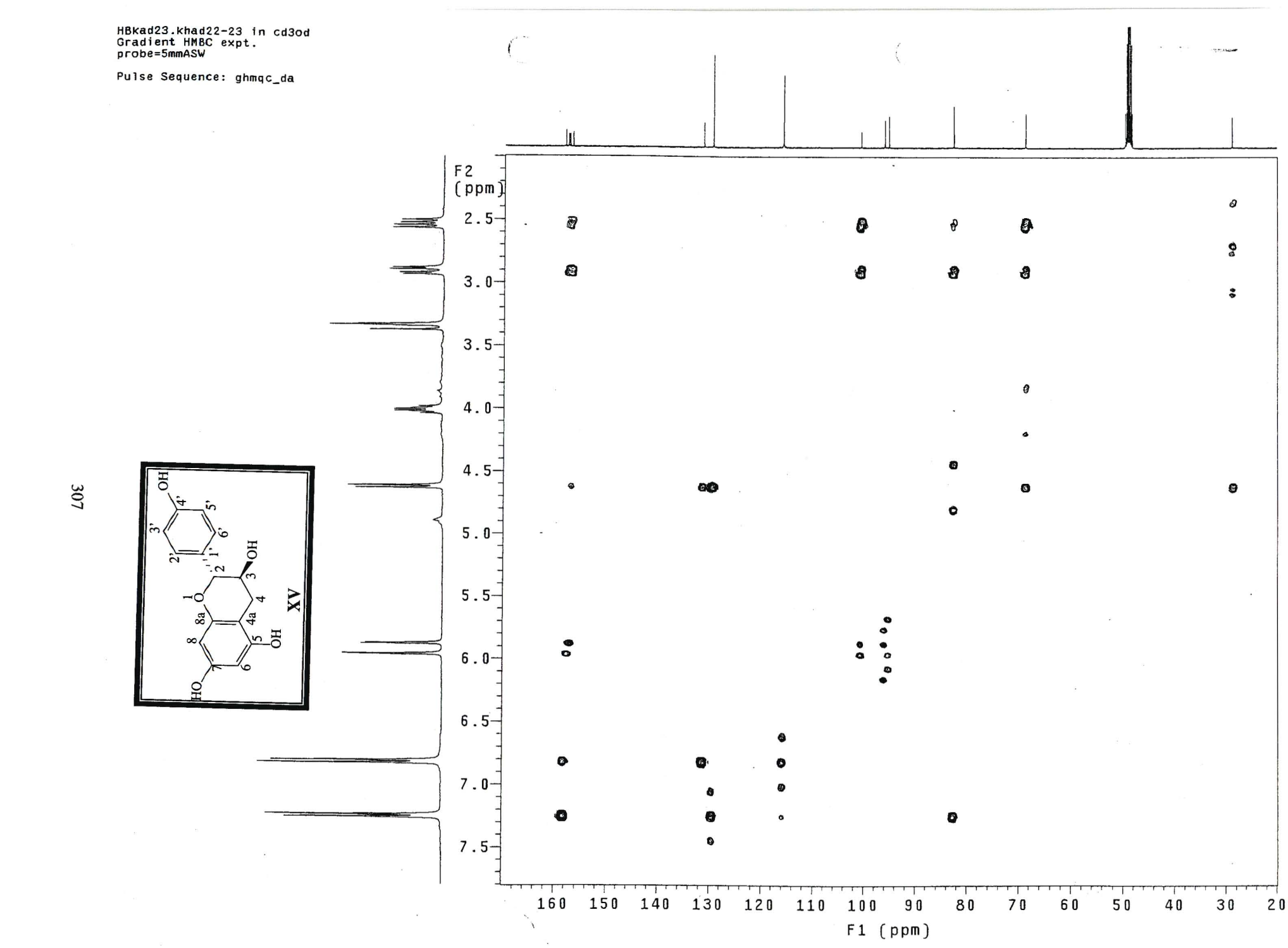




COSY spectrum of compound **XV**, (+)-afzelechin



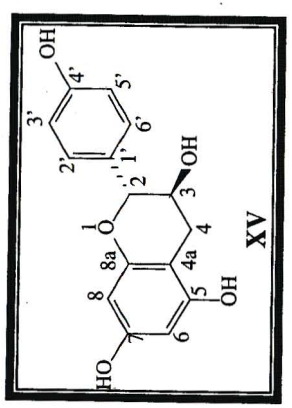
HSQC spectrum of compound **XV**, (+)-afzelechin



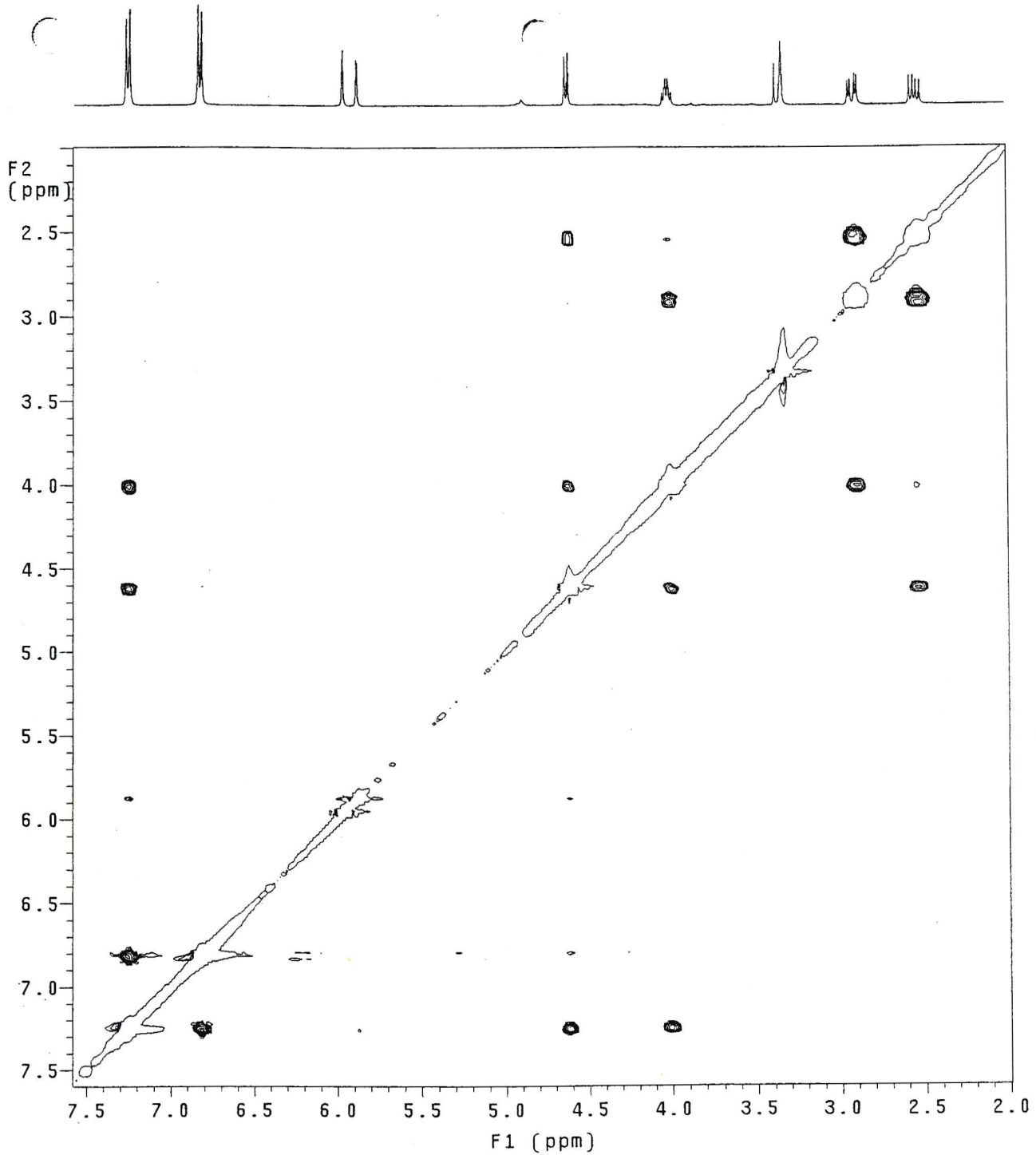
HMBC spectrum of compound **XV**, (+)-afzelechin

NOkad23.khad22-23 in cd3od
Gradient NOESY expt.
using presat_h2o
mix=1sec
probe=5mmASW

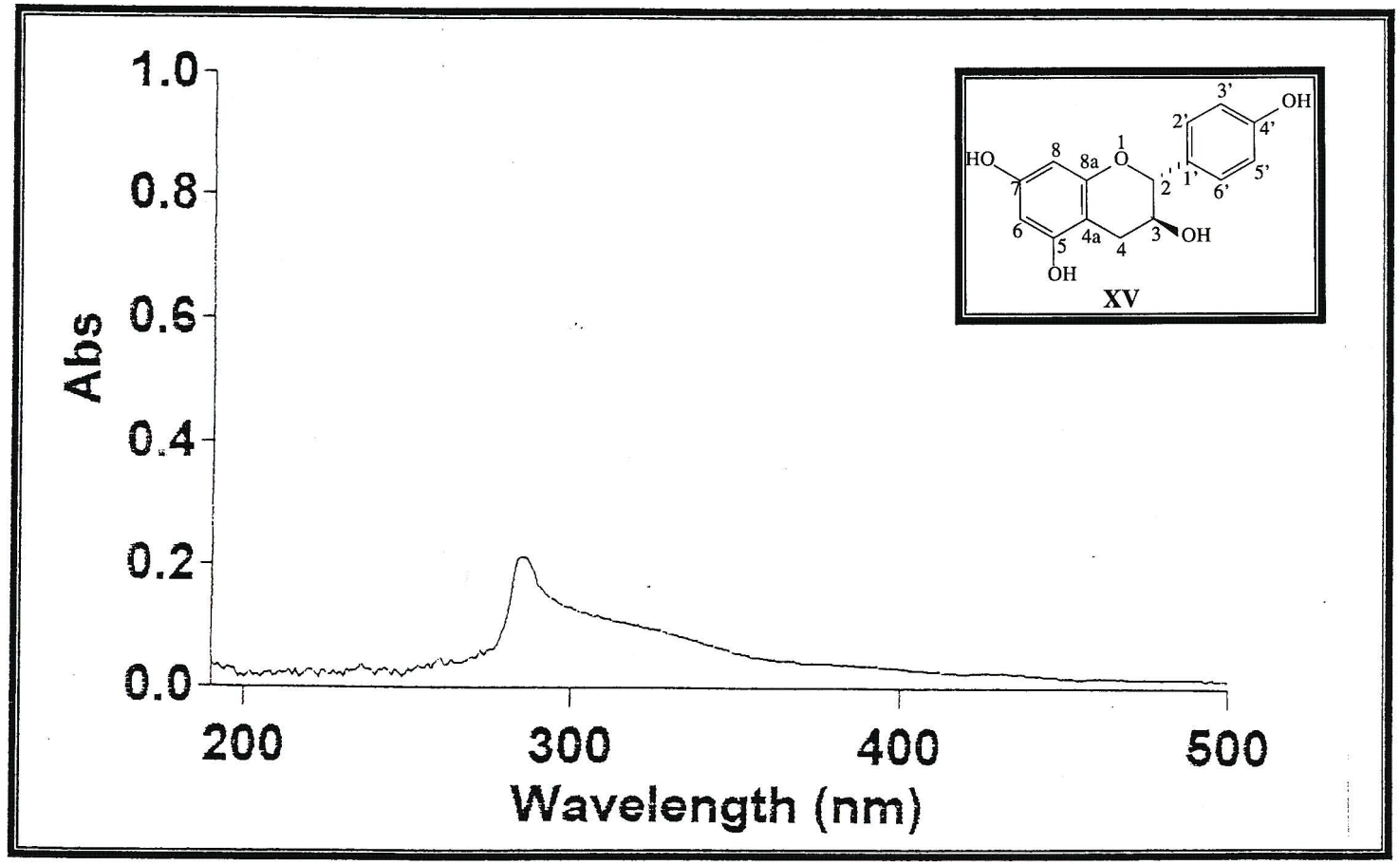
Pulse Sequence: noesy_da



XV

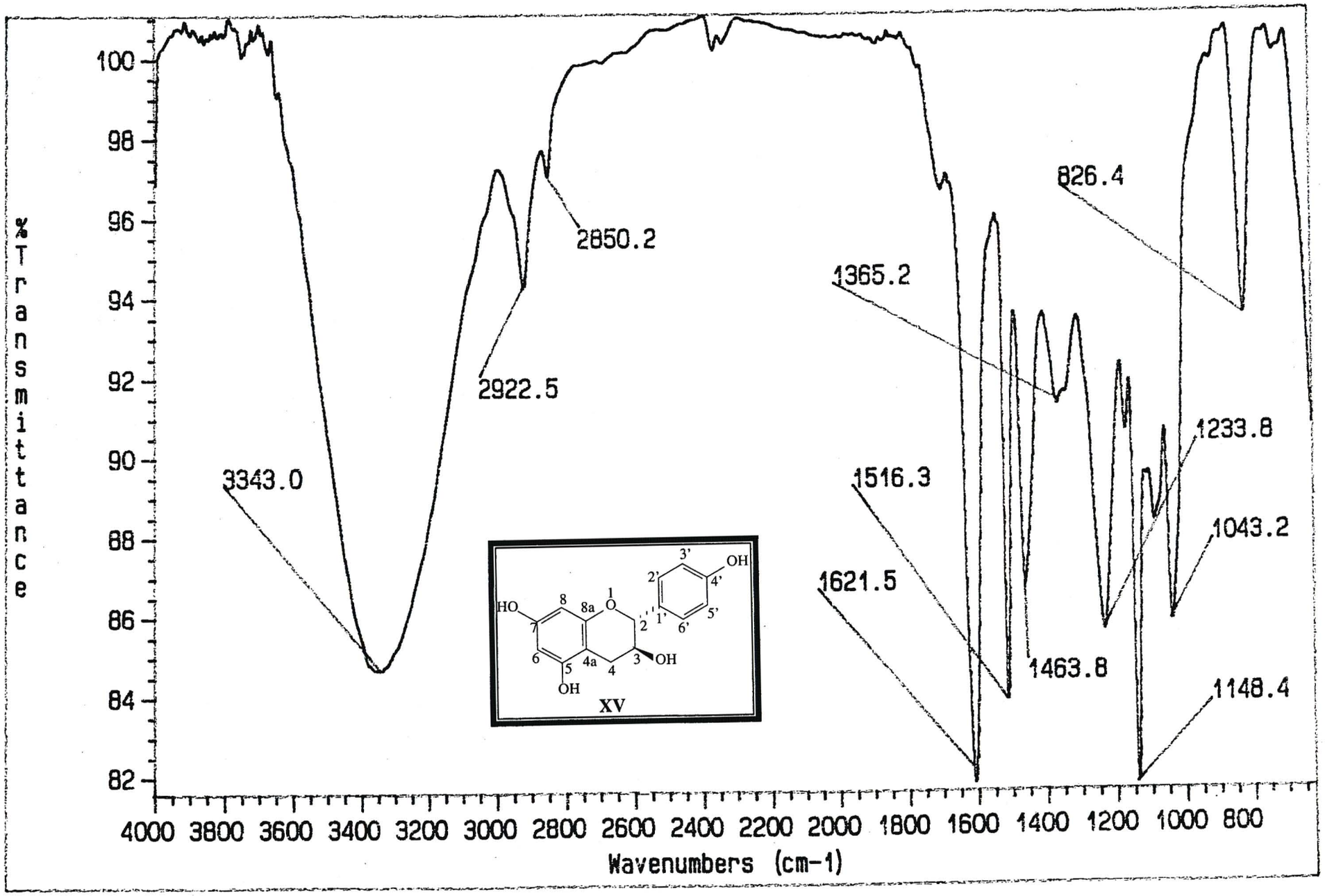


NOESY spectrum of compound **XV,** (+)-afzelechin

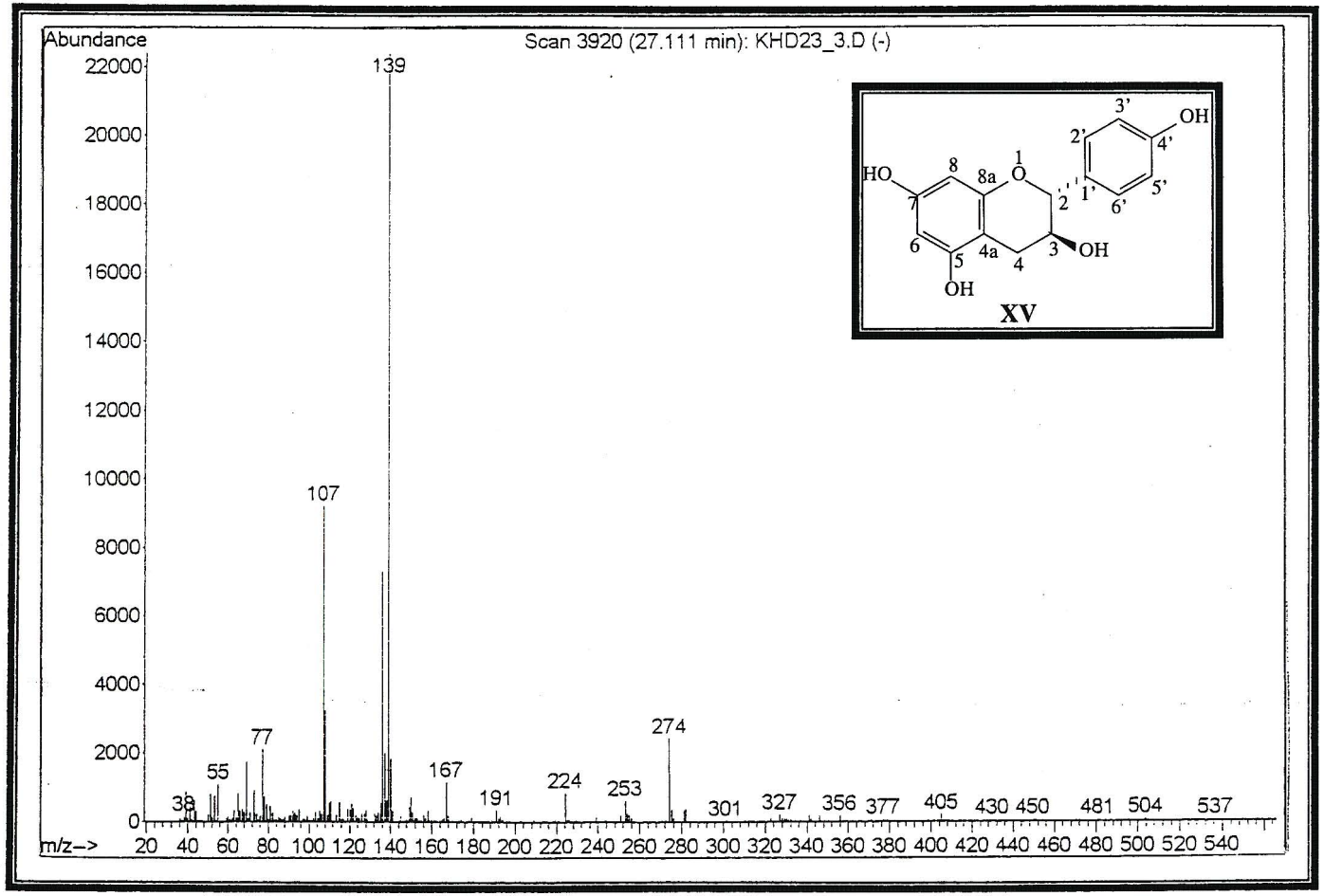


Ultra violet spectrum of compound **XV**, (+)-afzelechin

KHAD21-22



Infrared spectrum of compound **XV**, (+)-afzelechin



Mass spectrum of compound **XV**, (+)-afzelechin

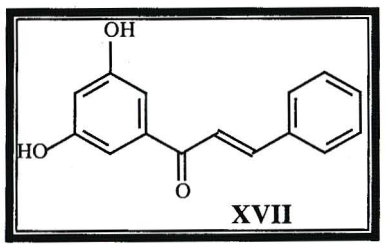
Appendix IV

NMR, Ultra violet, Infrared and Mass spectra for Chapter 6. Investigations into the synthesis of chalcones and prenylated chalcones

h3oh2.3,5-oh2 chalcone in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3116.015	7.791	15.6
2	3100.266	7.752	43.7
3	3096.786	7.743	26.0
4	3092.757	7.733	24.5
5	3090.926	7.728	21.7
6	3048.623	7.623	29.9
7	3033.057	7.584	19.4
8	2985.810	7.466	32.5
9	2982.330	7.457	43.8
10	2980.316	7.452	41.9
11	2793.524	6.985	51.9
12	2791.326	6.979	53.8
13	2624.129	6.561	13.3
14	2622.115	6.556	22.4
15	2620.100	6.551	13.6
16	1965.413	4.914	200.0
17	1351.197	3.378	12.9
18	1335.814	3.340	24.7



XVII

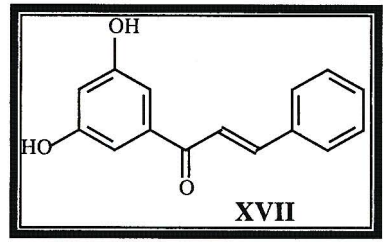
9 8 7 6 5 4 3 2 1 ppm

^{1}H NMR spectrum of compound **XVII,** 3',5'-dihydroxychalcone

c3oh2.3,5-oh2 chalcone in cd3od
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	19359.9[illegible]	192.510	8.1
2	16102.084	160.115	24.6
3	14687.551	146.049	25.1
4	14215.277	141.353	10.5
5	13700.277	136.232	10.5
6	13248.603	131.740	23.4
7	13083.040	130.094	51.7
8	13035.737	129.624	55.0
9	12400.952	123.312	24.9
10	10895.626	108.343	22.1
11	10852.137	107.911	48.7
12	4991.821	49.637	31.6
13	4970.458	49.425	92.3
14	4949.095	49.212	176.7
15	4927.732	49.000	200.0
16	4905.606	48.780	166.1
17	4884.243	48.568	89.5
18	4862.880	48.355	31.7

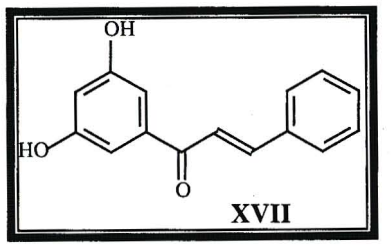


XVII

^{13}C NMR spectrum of compound **XVII**, 3',5'-dihydroxychalcone

c3oh2.3,5-oh2 chalcone in cd3od
probe=5mmASW

Pulse Sequence: dept



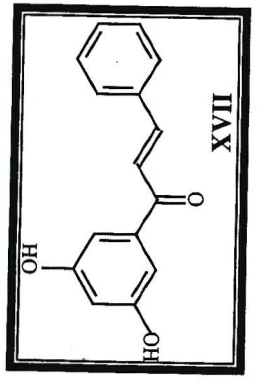
XVII

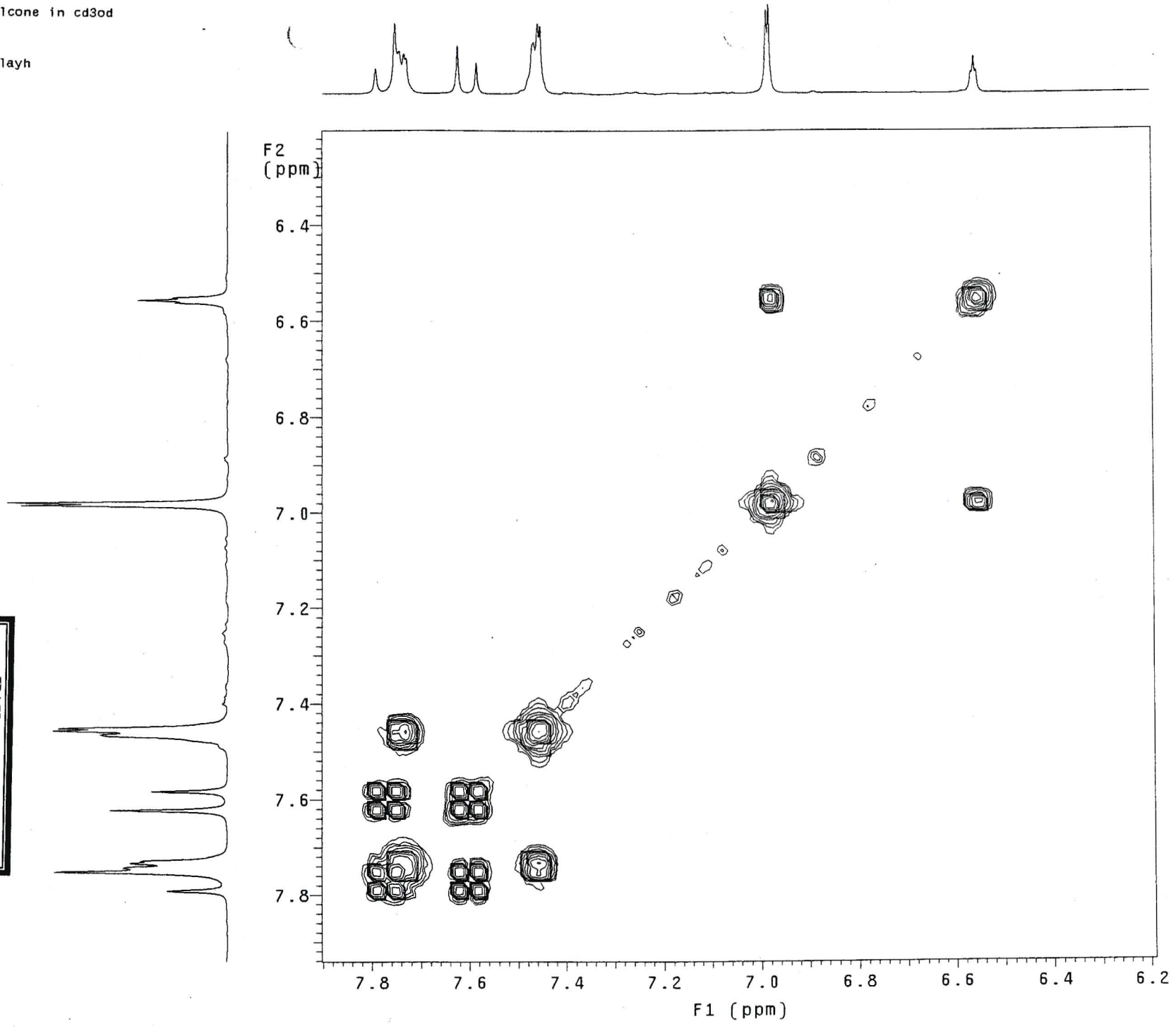
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

ADEPT spectrum of compound **XVII,** 3',5'-dihydroxychalcone

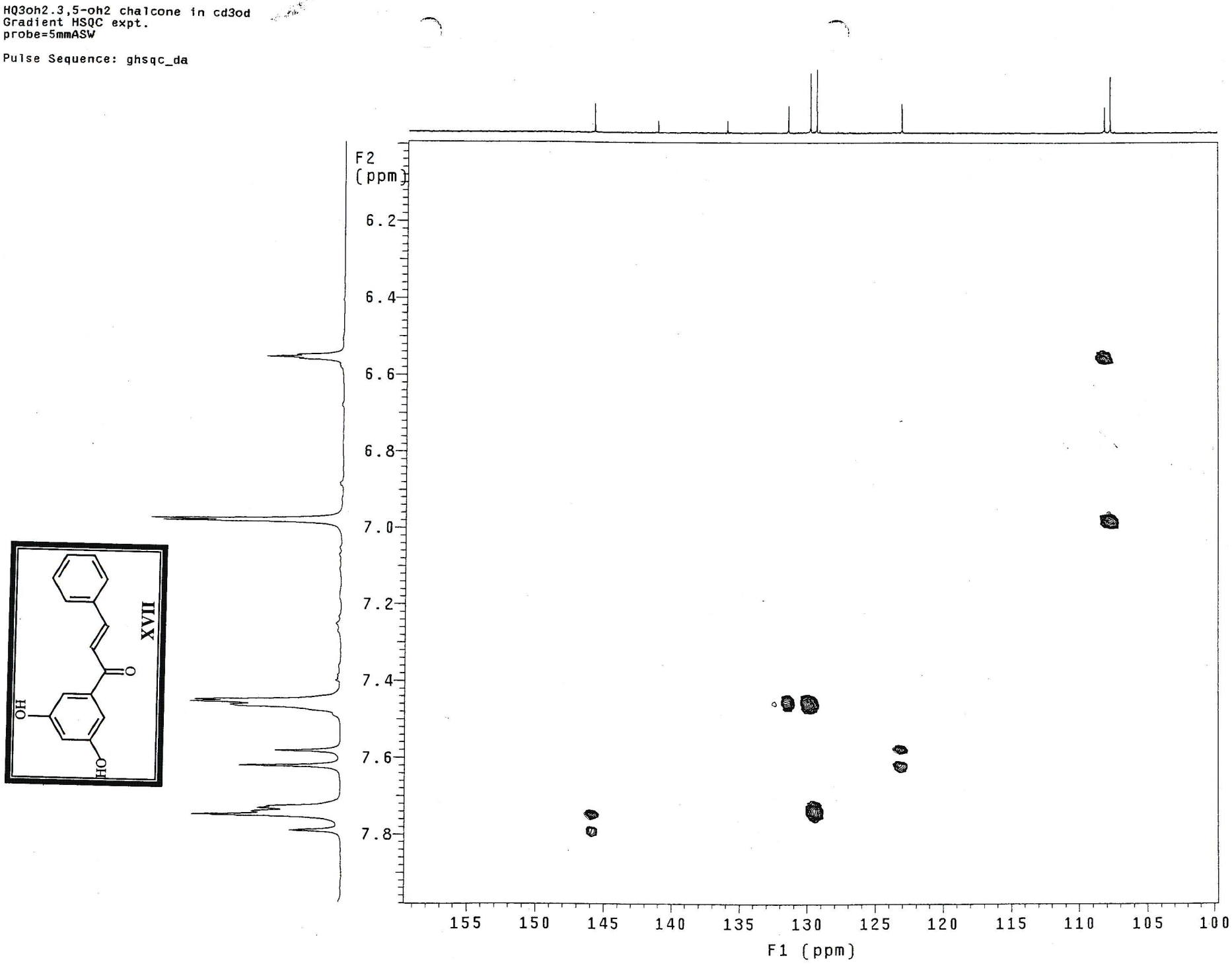
cy3oh2.3,5-oh2 chalcone in cd3od
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

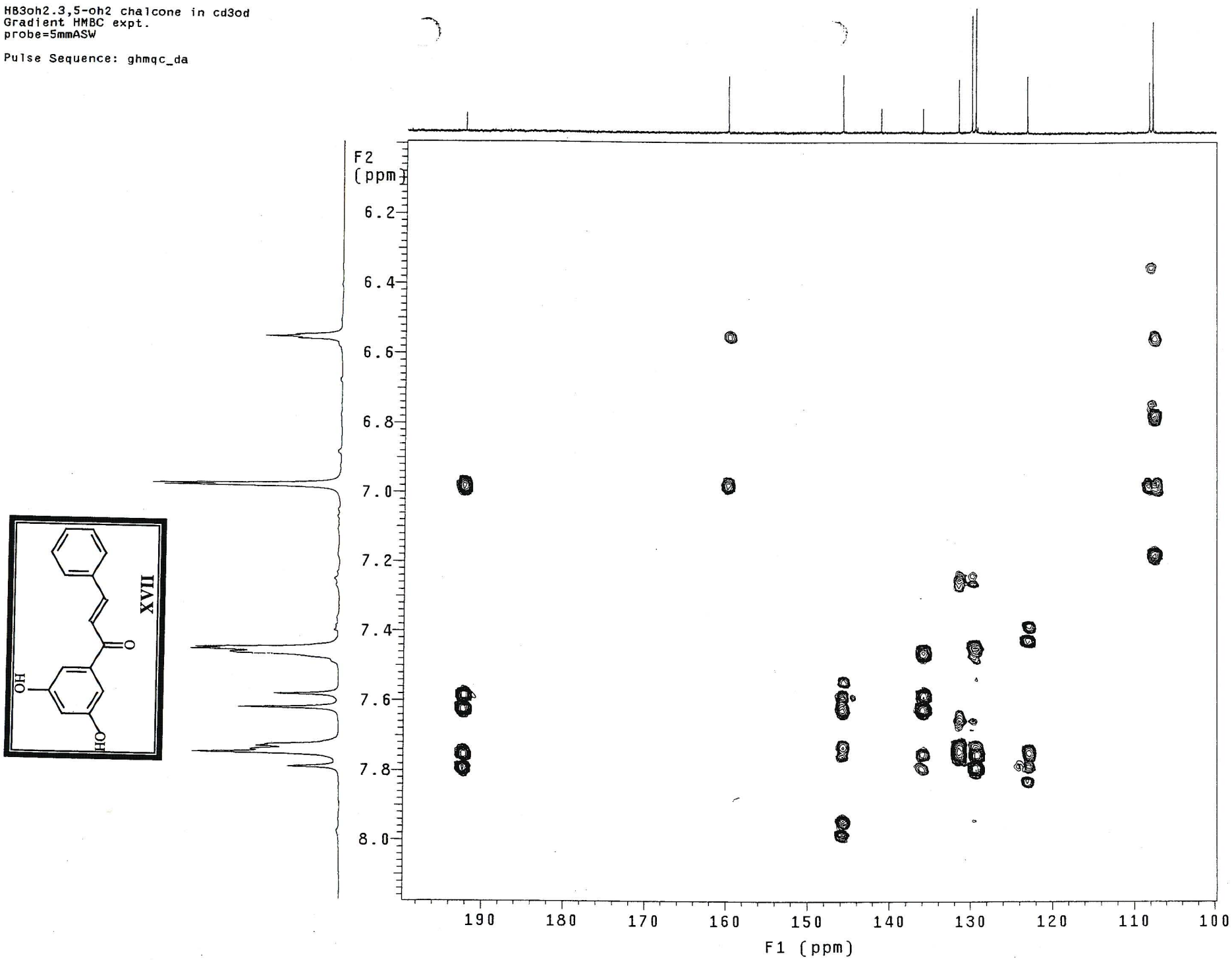




COSY spectrum of compound **XVII,** 3',5'-dihydroxychalcone



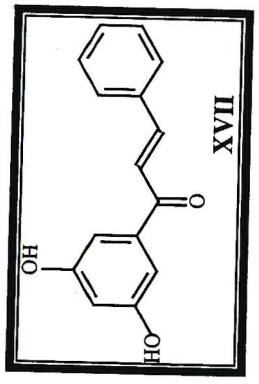
HSQC spectrum of compound **XVII,** 3',5'-dihydroxychalcone

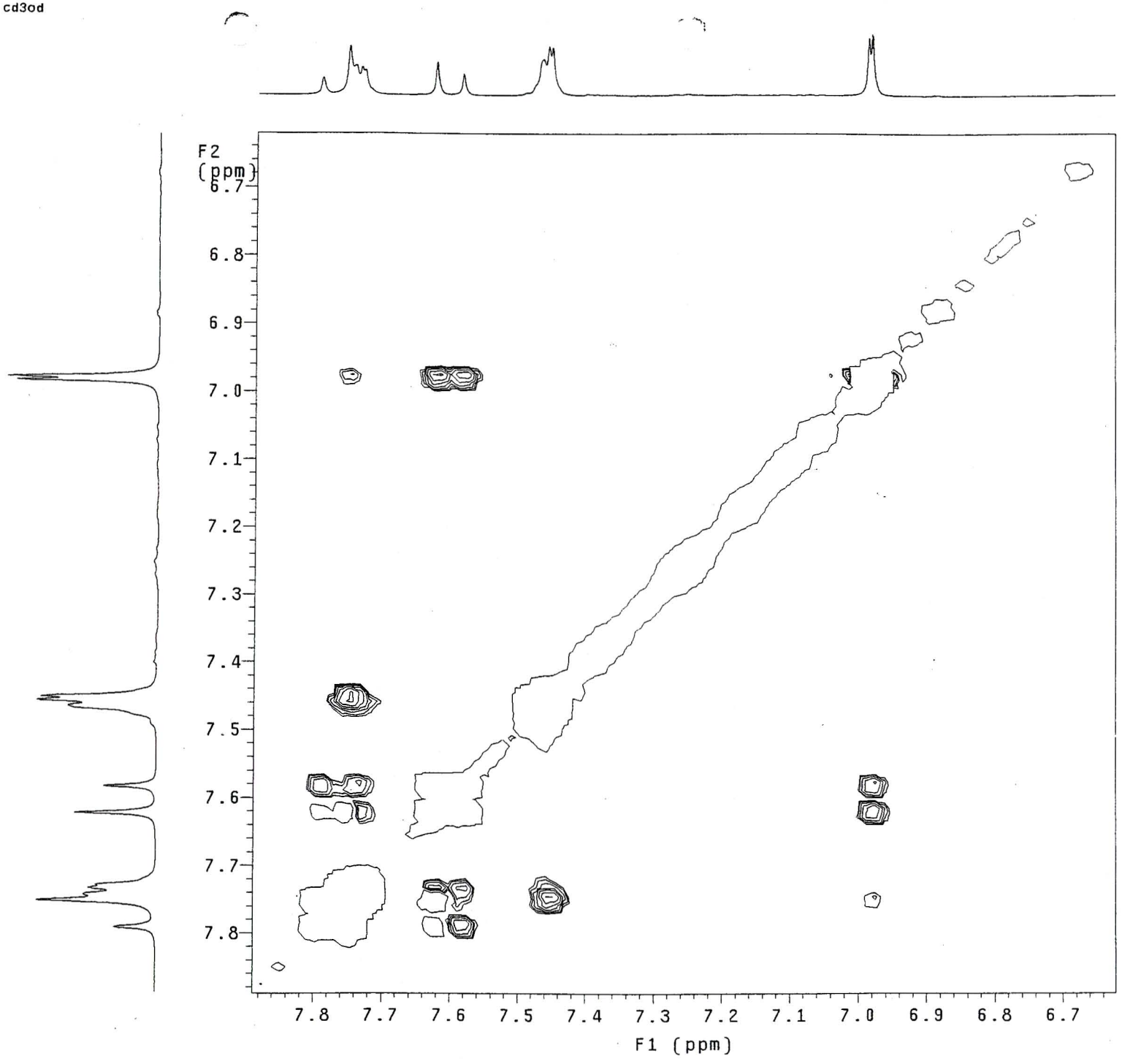


HMBC spectrum of compound **XVII**, 3',5'-dihydroxychalcone

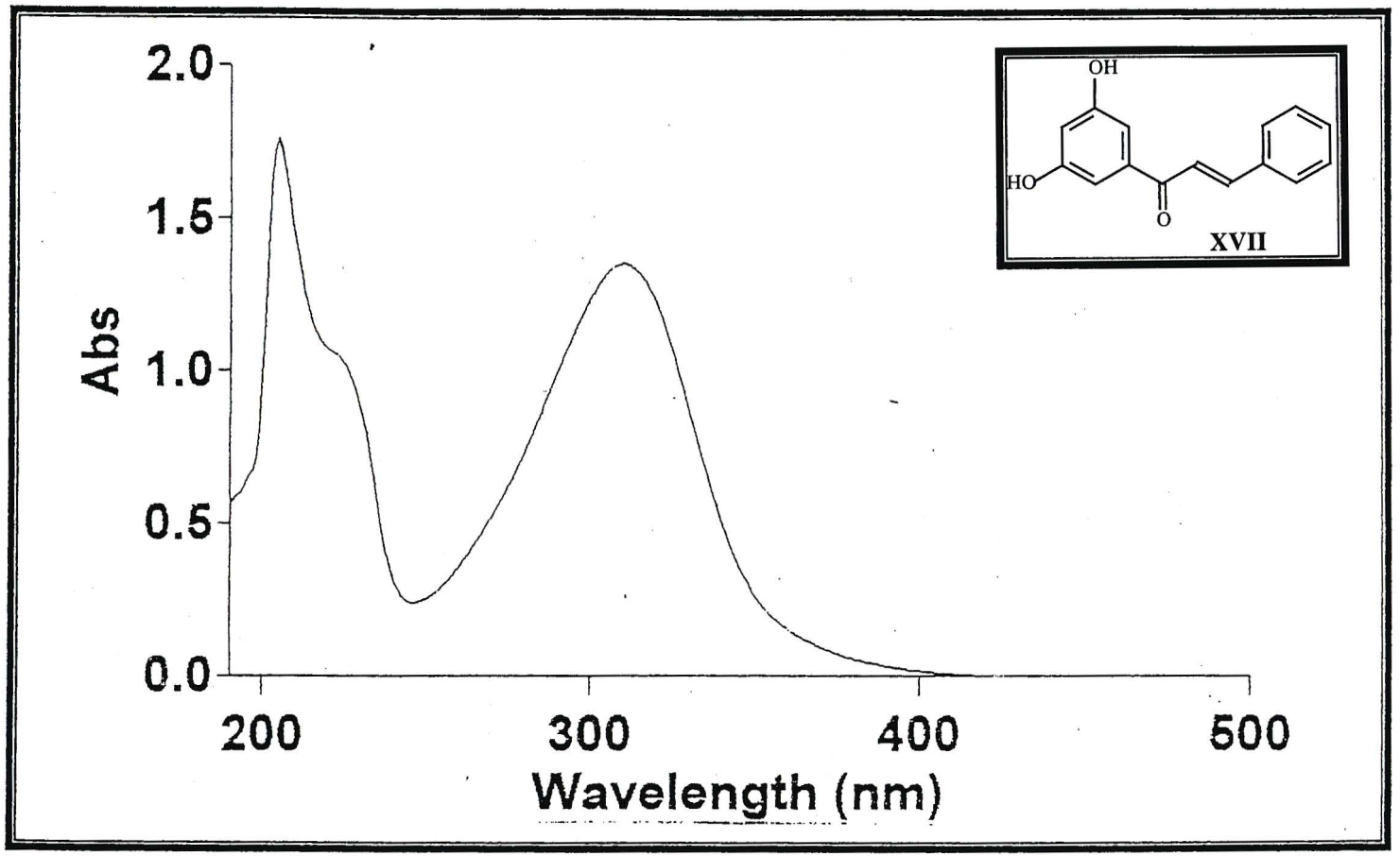
NO3oh2.3,5-oh2 chalcone in cd3od
Gradient NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da



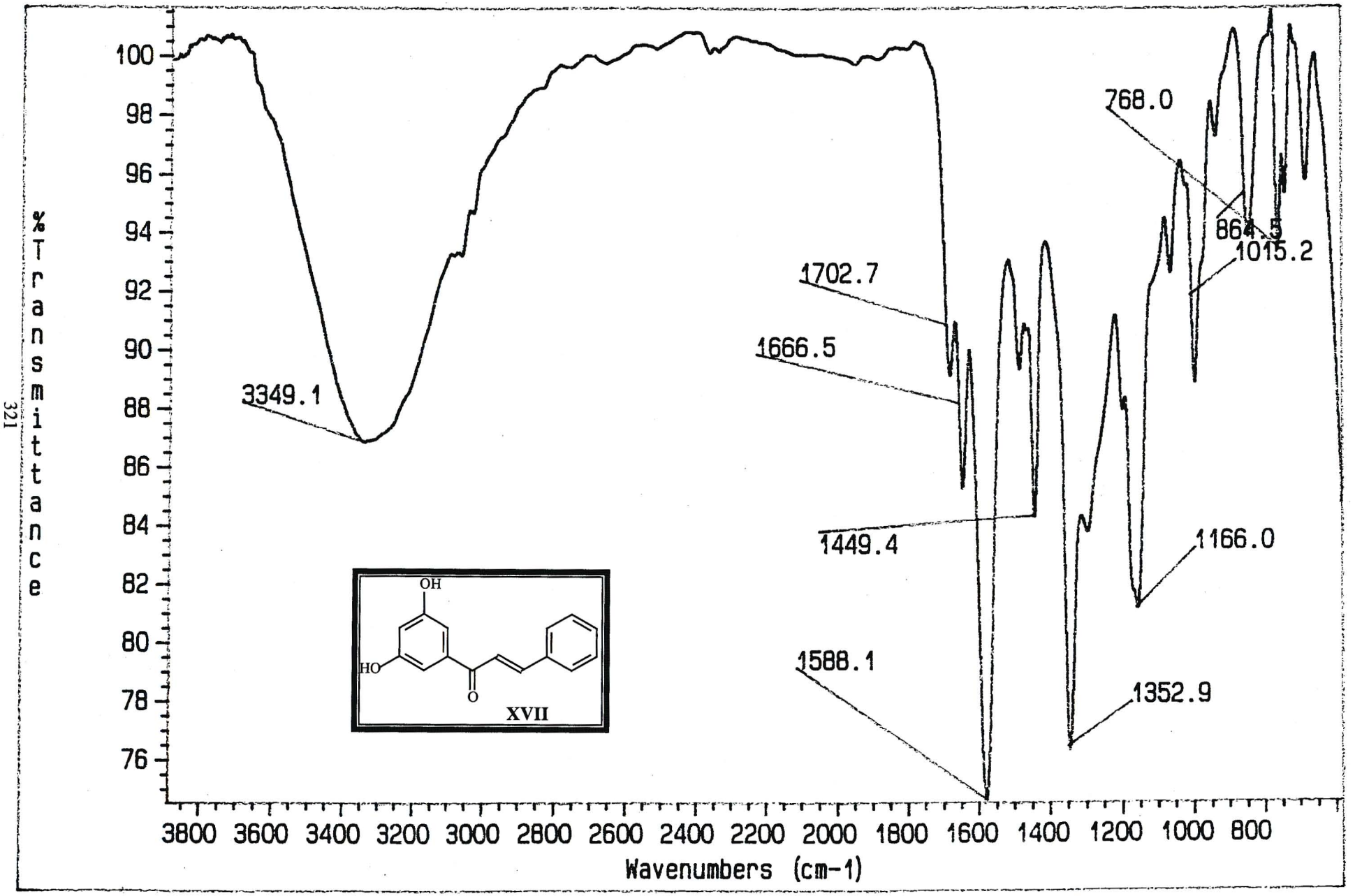


NOESY spectrum of compound **XVII**, 3',5'-dihydroxychalcone



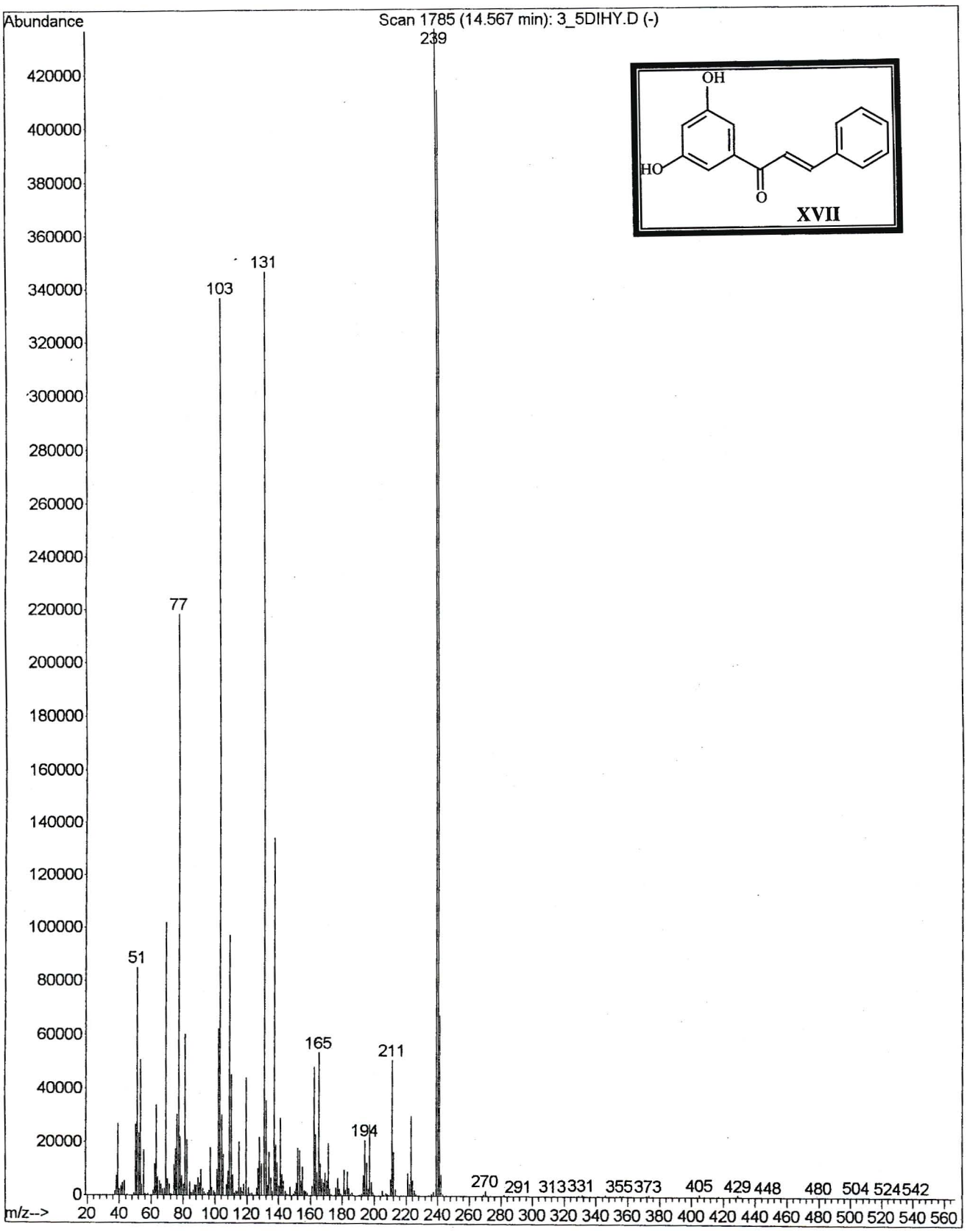
Ultra violet spectrum of compound **XVII,** 3',5'-dihydroxychalcone

3',5'-dihydroxychalcone (synthetic)



Infrared spectrum of compound **XVII,** 3',5'-dihydroxychalcone

File : D:\NEIL\3_5DIHY.D
Operator : Bret
Acquired : 10 Apr 2001 9:09 using AcqMethod NEW
Instrument : Instrumen
Sample Name: 3',5'-dihydroxychalcone synthetic
Misc Info : 1ul inject, 1:75 split, MeOH, 20dpm
Vial Number: 56

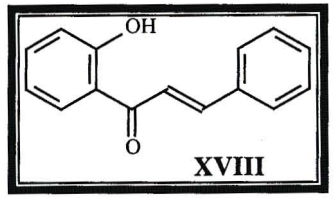


Mass spectrum of compound **XVII**, 3',5'-dihydroxychalcone

h2oh.2oh chalcone in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	3168.632	7.923	43.9	40	2772.157	6.931	41.2
2	3166.434	7.917	37.1	41	2771.058	6.929	26.5
3	3164.969	7.914	35.5	42	2765.015	6.914	22.4
4	3158.377	7.897	35.9	43	2763.733	6.910	20.2
5	3156.728	7.893	36.8				
6	3153.066	7.884	57.5				
7	3062.234	7.657	100.0				
8	3059.670	7.650	34.2				
9	3058.937	7.648	38.3				
10	3058.205	7.647	34.0				
11	3057.472	7.645	36.4				
12	3056.007	7.641	40.0				
13	3054.542	7.637	40.5				
14	3053.077	7.634	28.7				
15	3052.162	7.631	45.4				
16	3046.851	7.618	65.1				
17	3000.519	7.502	18.2				
18	2999.054	7.499	18.9				
19	2993.377	7.485	22.5				
20	2992.095	7.481	34.8				
21	2990.630	7.478	23.4				
22	2984.953	7.463	22.0				
23	2983.305	7.459	21.9				
24	2974.332	7.437	11.6				
25	2971.585	7.430	30.7				
26	2970.486	7.427	98.8				
27	2968.655	7.423	55.8				
28	2967.190	7.419	97.6				
29	2966.091	7.416	52.6				
30	2964.076	7.411	85.2				
31	2962.245	7.407	21.7				
32	2895.586	7.240	10.3				
33	2811.896	7.031	34.0				
34	2810.797	7.028	39.7				
35	2803.655	7.010	31.4				
36	2802.373	7.007	35.0				
37	2780.214	6.952	25.0				
38	2779.116	6.949	22.8				
39	2773.072	6.934	28.2				



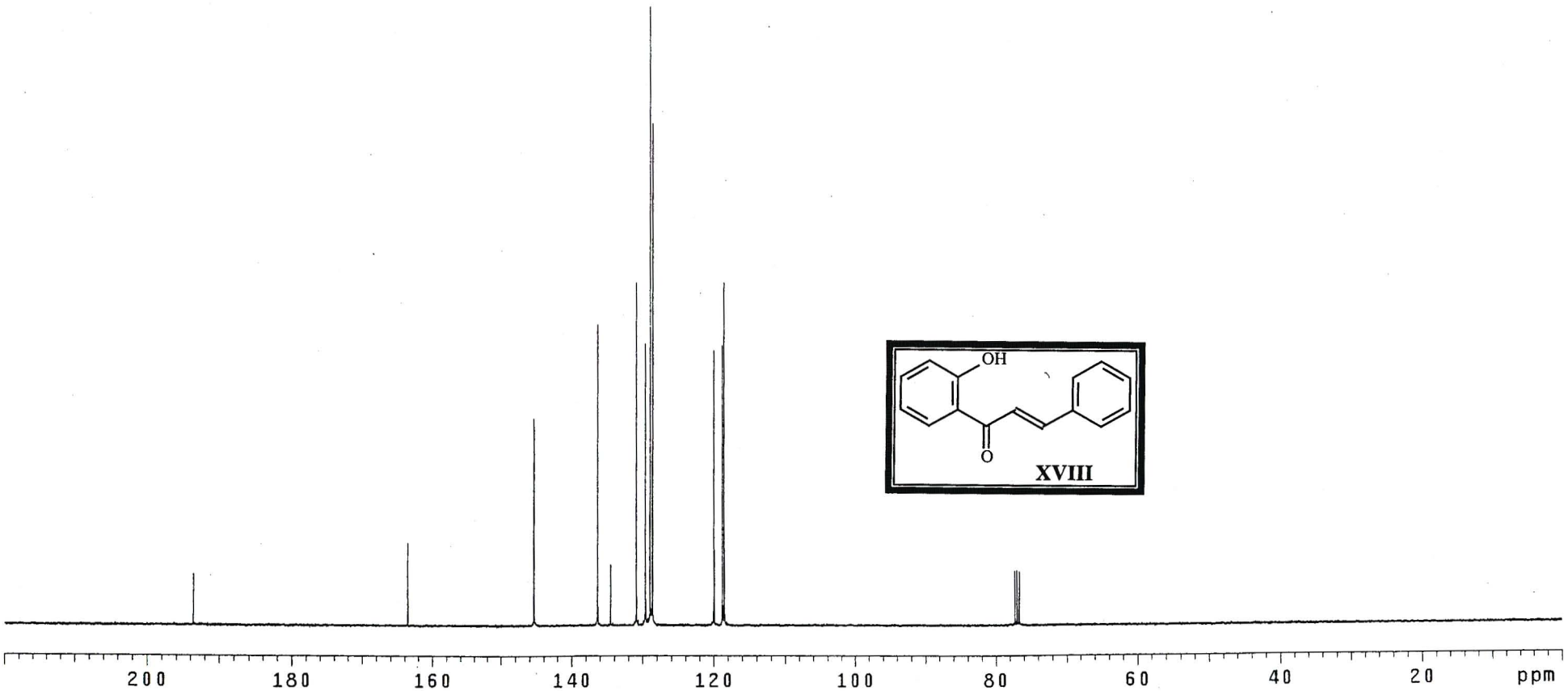
XVIII

^{1}H NMR spectrum of compound **XVIII**, 2'-hydroxychalcone

c2oh.2oh chalcone in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

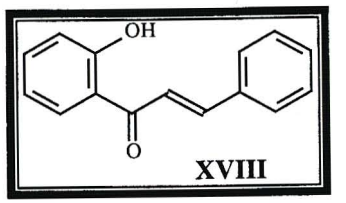
INDEX	FREQUENCY	PPM	HEIGHT
1	19473.8	193.643	8.3
2	16444.888	163.524	13.5
3	14621.399	145.392	33.5
4	13710.891	136.338	48.6
5	13525.493	134.494	9.9
6	13160.466	130.864	55.4
7	13032.748	129.594	45.6
8	12969.300	128.963	100.0
9	12933.045	128.603	81.1
10	12067.032	119.992	44.4
11	12060.440	119.926	12.4
12	11946.730	118.795	45.3
13	11922.010	118.549	55.3
14	7774.871	77.311	8.8
15	7742.735	76.992	9.0
16	7710.600	76.672	8.7



^{13}C NMR spectrum of compound **XVIII**, 2'-hydroxychalcone

d2oh.2oh chalcone in cdcl3
probe=5mmASW

Pulse Sequence: dept



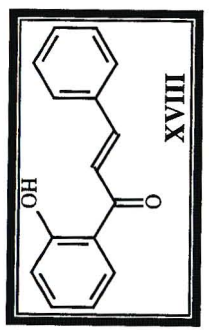
XVIII

150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

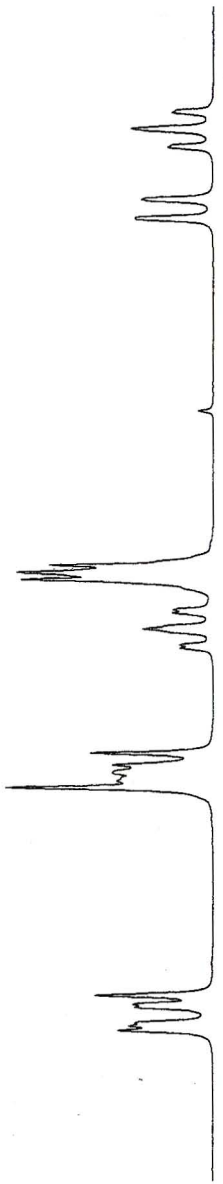
ADEPT spectrum of compound **XVIII,** 2'-hydroxychalcone

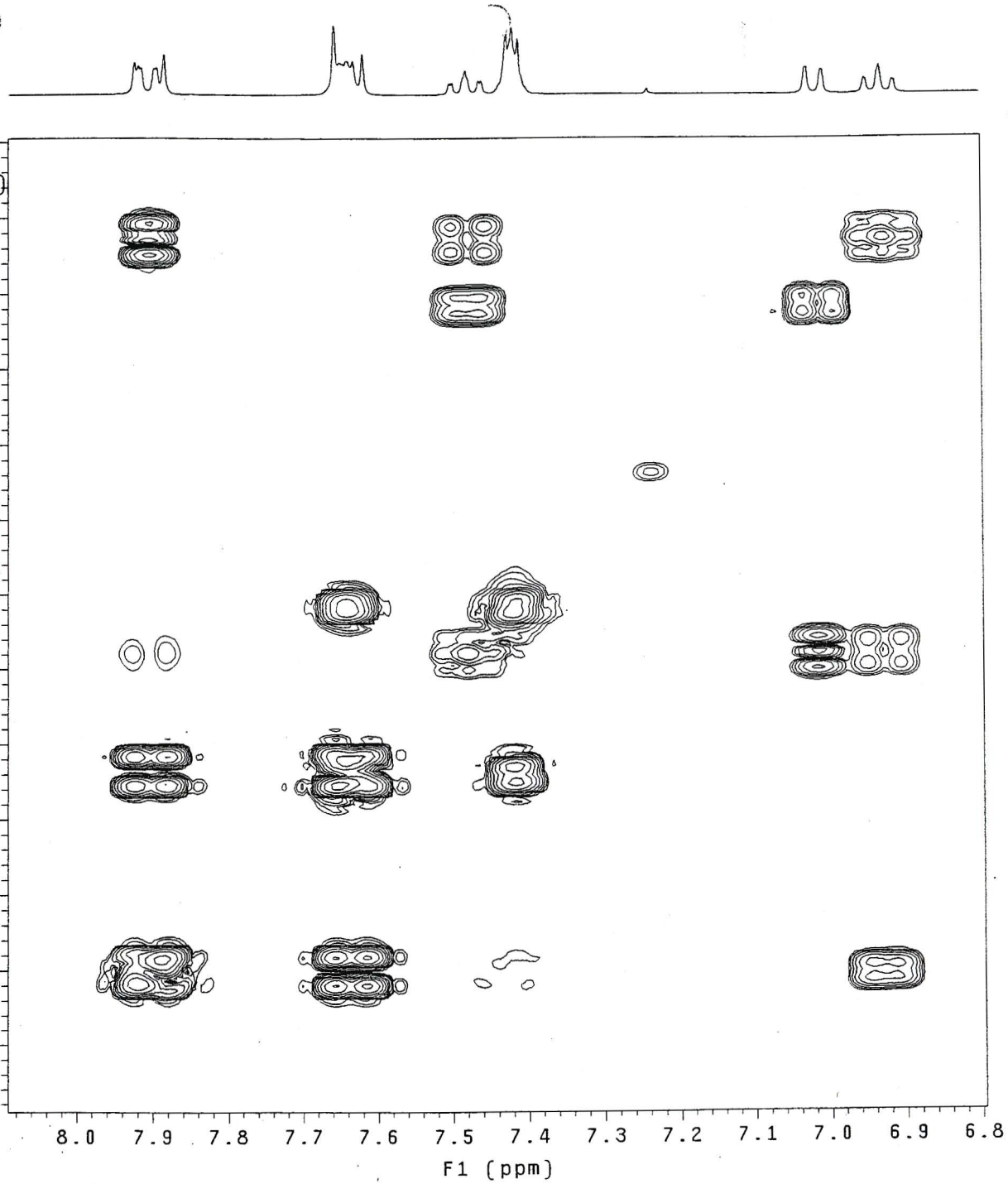
cy2oh.2oh chalcone in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh



XVIII

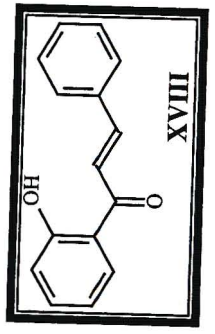




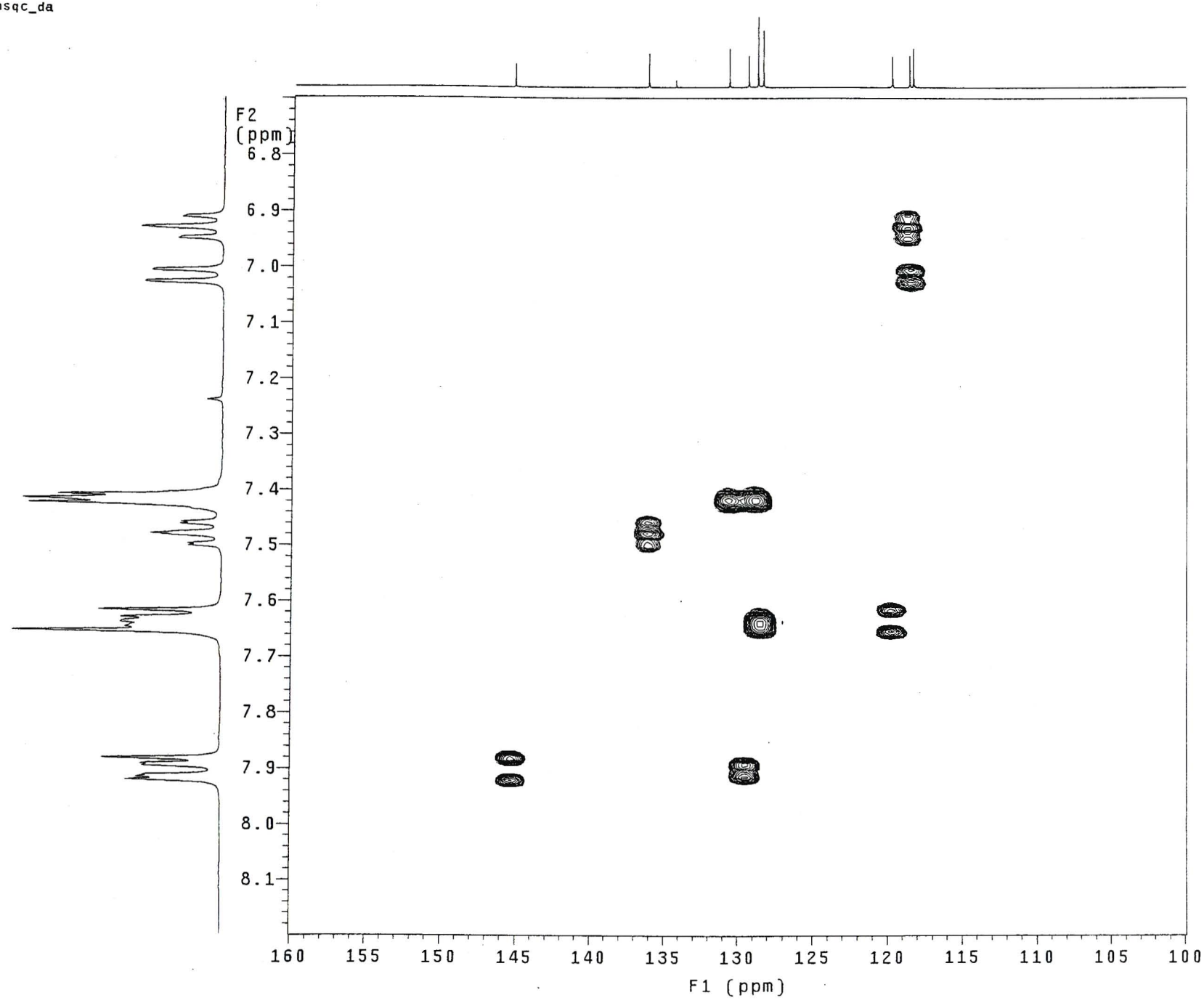
COSY spectrum of compound **XVIII,** 2'-hydroxychalcone

HQ2oh.2oh chalcone in cdcl3
Gradient HSQC expt.
probe=5mmASW

Pulse Sequence: ghsqc_da



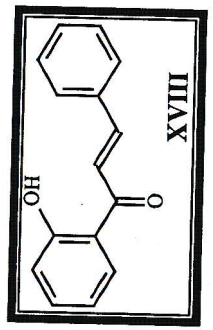
XVIII

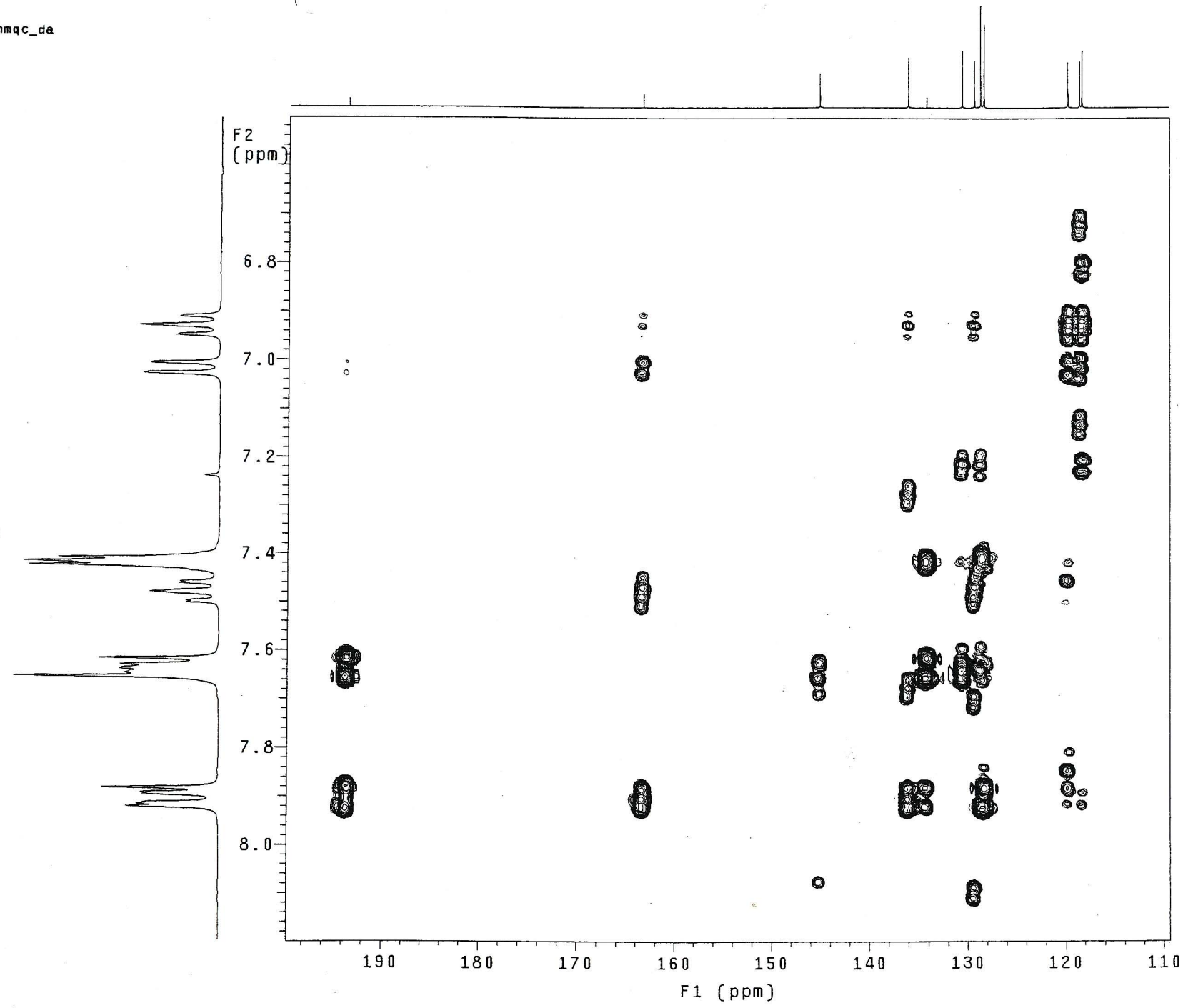


HSQC spectrum of compound **XVIII,** 2'-hydroxychalcone

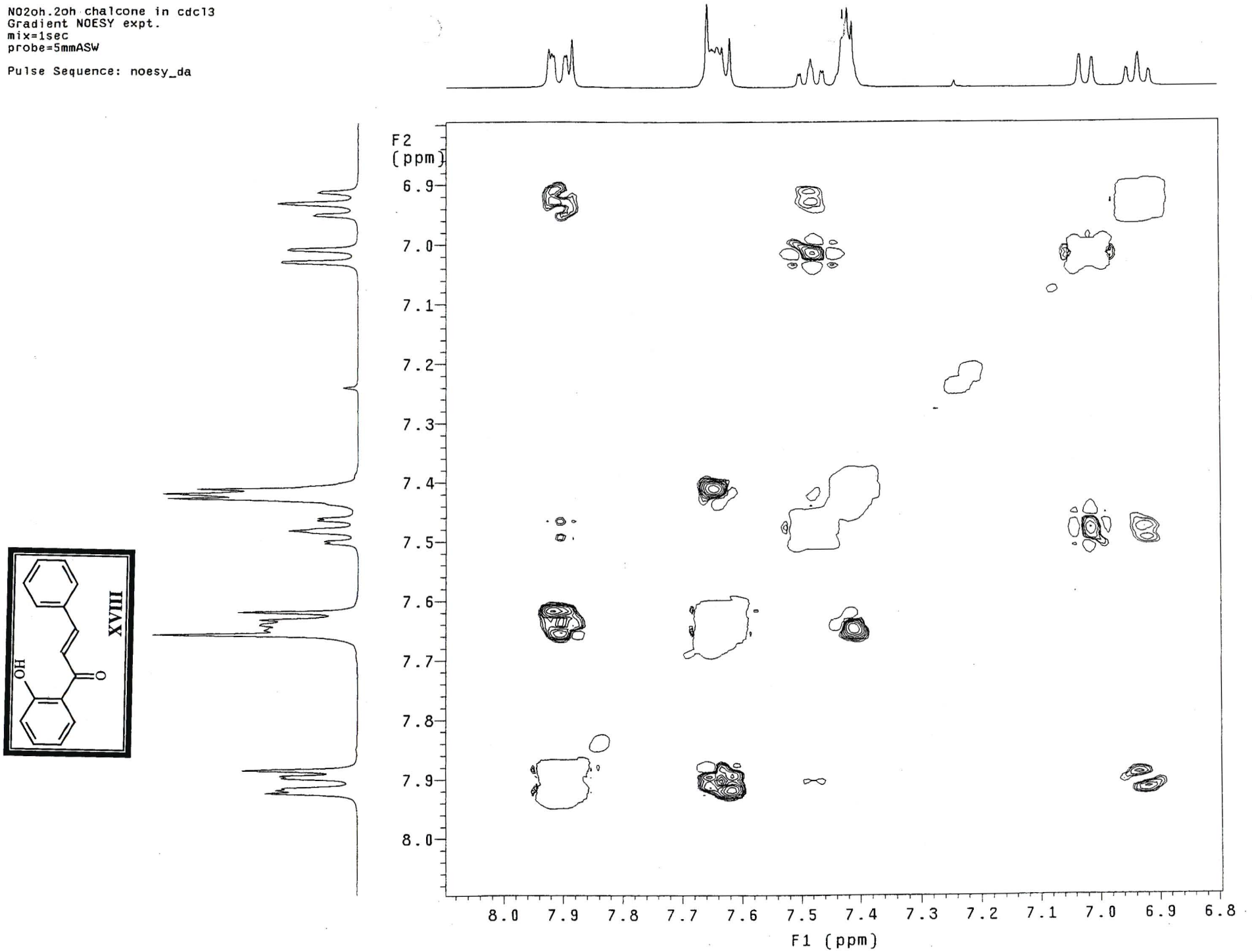
HB2oh.2oh chalcone in cdcl3
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da

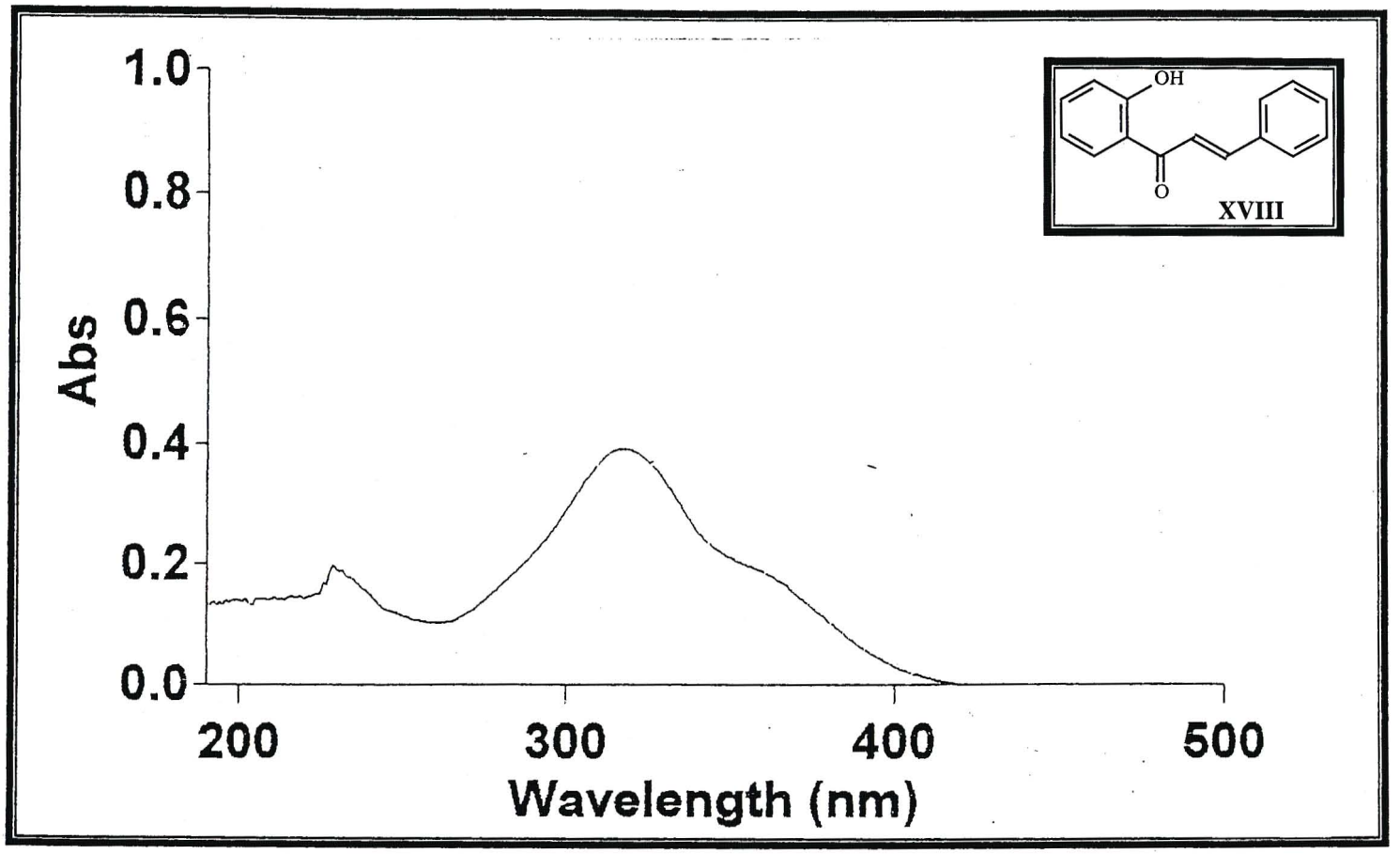




HMBC spectrum of compound **XVIII,** 2'-hydroxychalcone

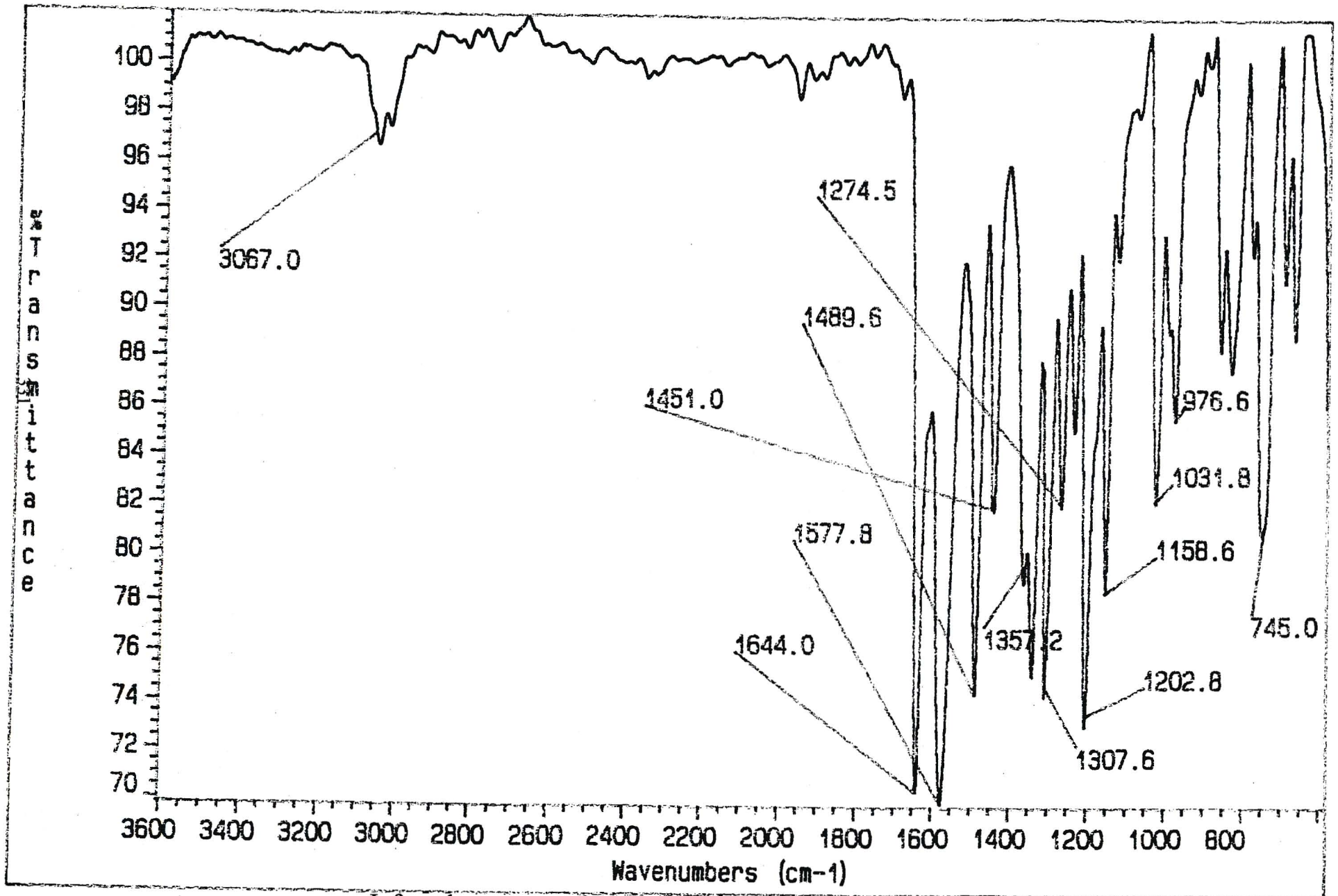


NOESY spectrum of compound **XVIII,** 2'-hydroxychalcone



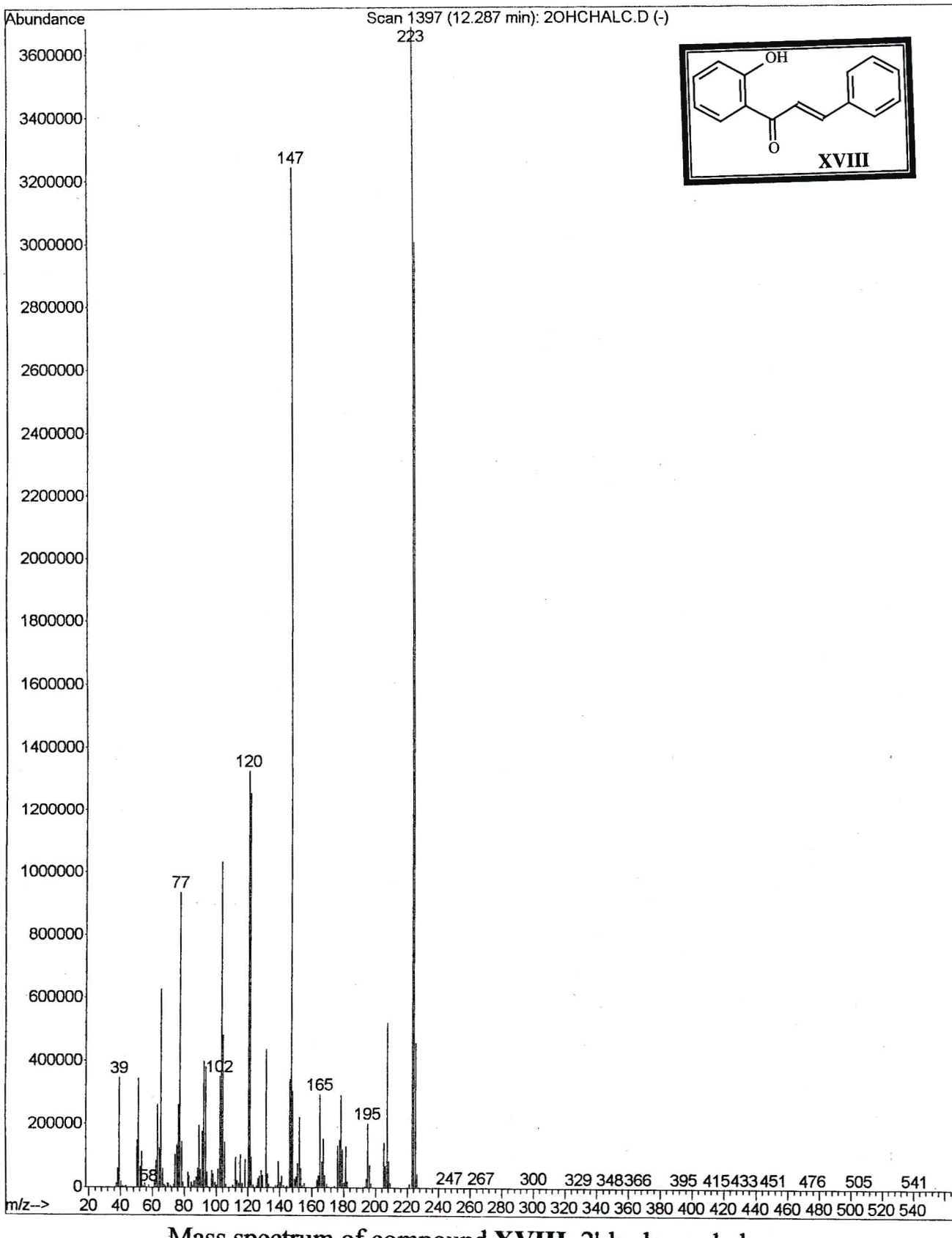
Ultra violet spectrum of compound **XVIII,** 2'-hydroxychalcone

2'-hydroxychalcone (synthetic)



Infrared spectrum of compound **XVIII**, 2'-hydroxychalcone

File : D:\NEIL\2OHCHALC.D
Operator : Bret
Acquired : 6 Apr 2001 15:52 using AcqMethod NEW
Instrument : Instrumen
Sample Name: 2' OH chalcone
Misc Info : 1ul inject, 1:75 split, MeCl2, 20dpm
Vial Number: 57

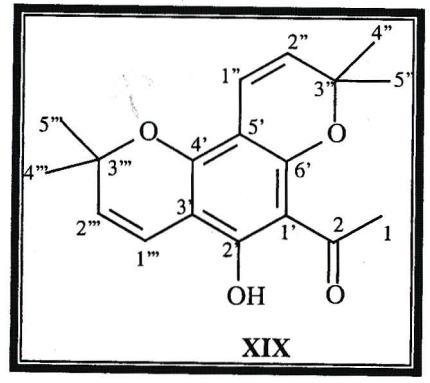


Mass spectrum of compound **XVIII**, 2'-hydroxychalcone

cchrom.chromene reaction in cdcl3
probe=5mmASW

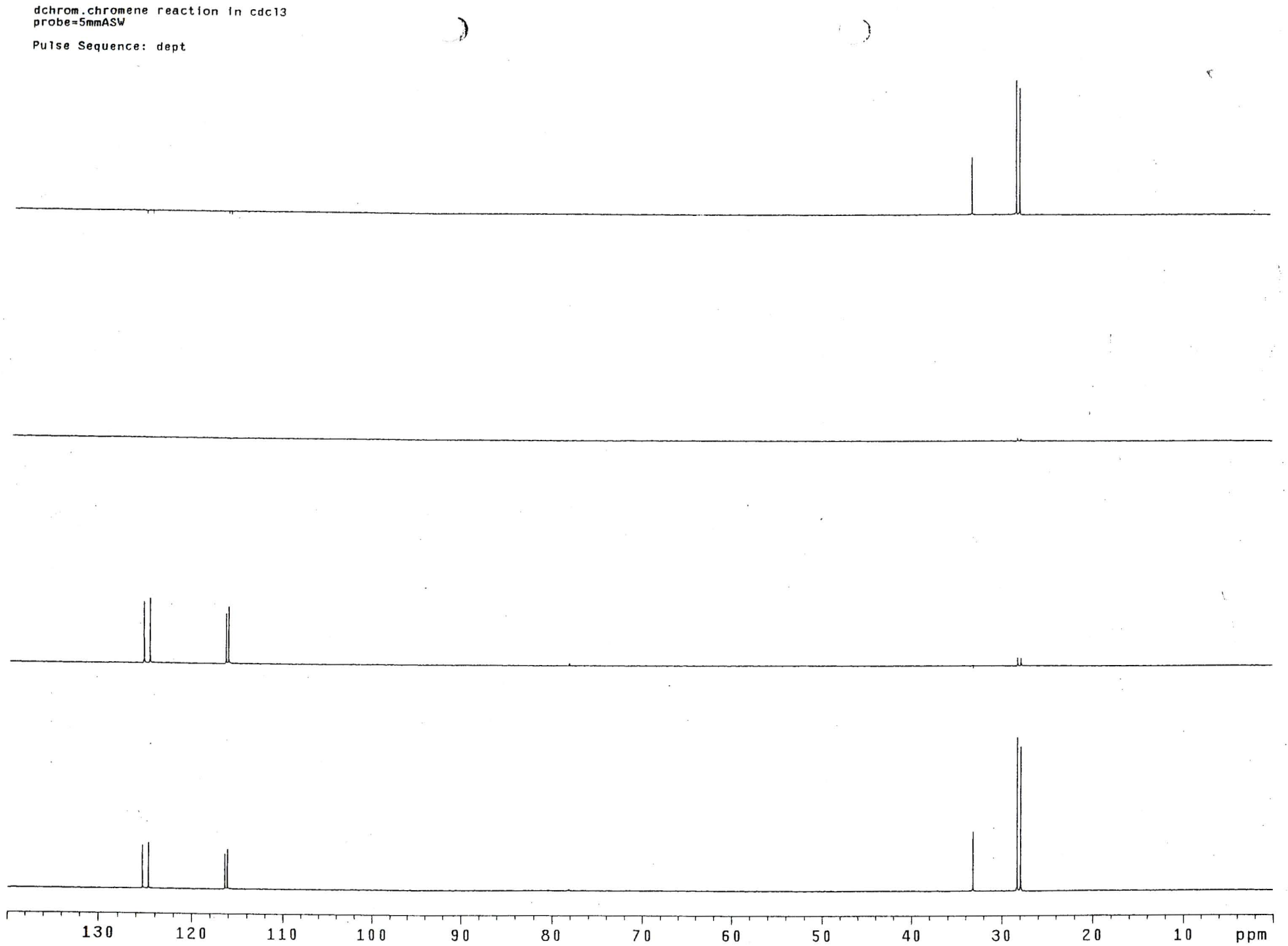
Pulse Sequence: s2pul

INDEX	FREQUE"	PPM	HEIGHT
1	20442.	203.271	9.5
2	16141.659	160.509	8.2
3	15753.560	156.649	11.8
4	15583.819	154.962	6.6
5	12603.449	125.326	42.3
6	12538.354	124.678	40.1
7	11704.476	116.386	35.7
8	11676.461	116.108	35.6
9	10606.922	105.473	10.1
10	10271.559	102.138	7.3
11	7865.509	78.213	27.7
12	7855.621	78.114	15.0
13	7775.695	77.320	9.3
14	7743.559	77.000	9.6
15	7711.423	76.680	9.0
16	3336.039	33.173	39.4
17	2846.590	28.306	100.0
18	2809.510	27.937	90.9



XIX

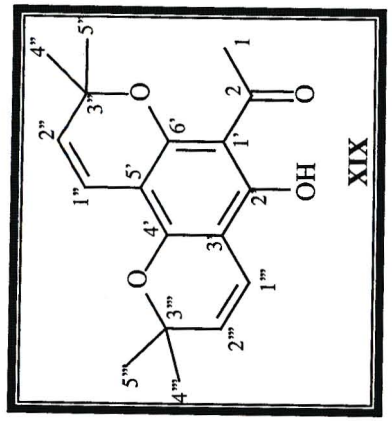
^{13}C NMR spectrum of compound **XIX**, chromene intermediate

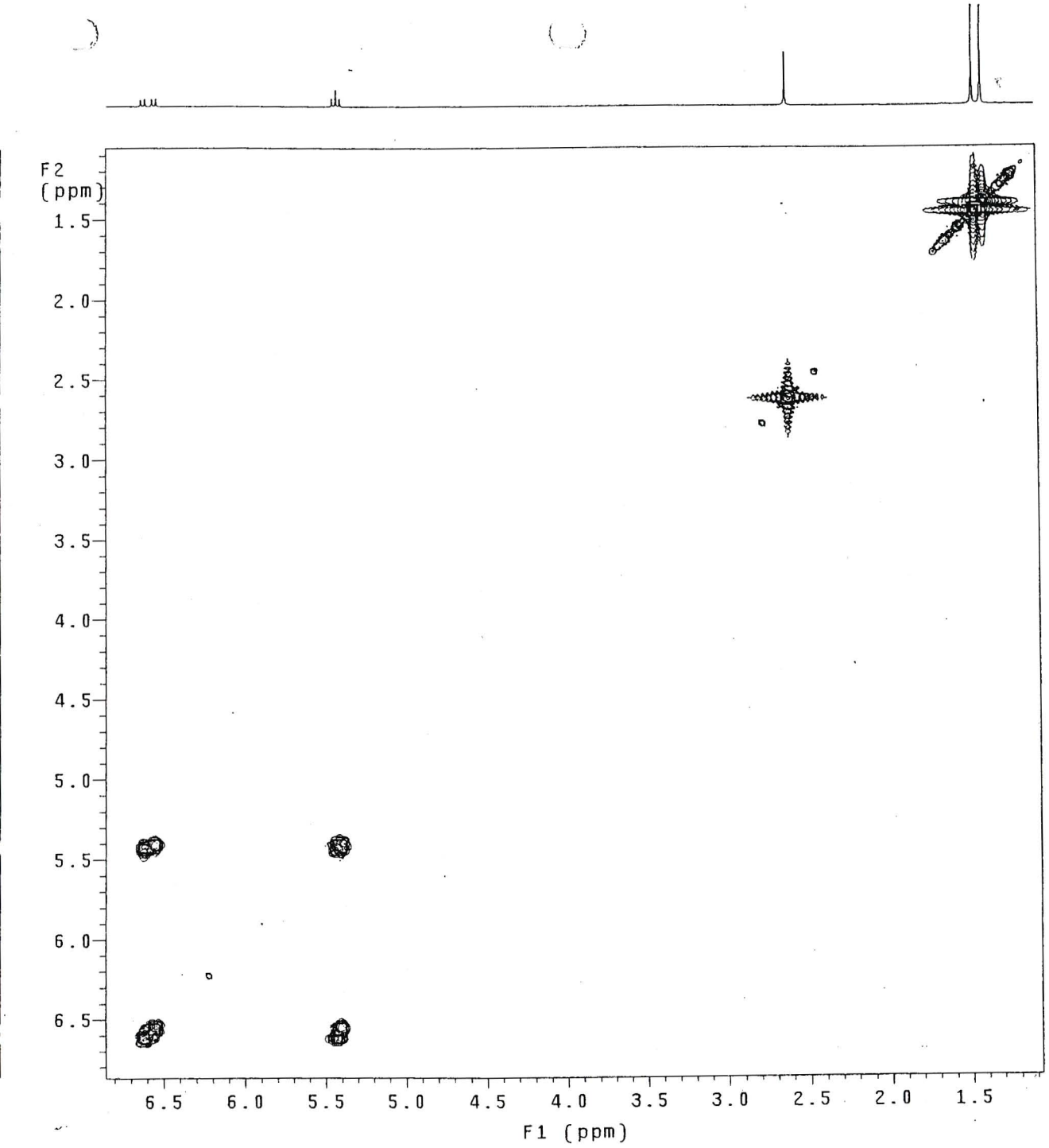


ADEPT spectrum of compound **XIX**, chromene intermediate

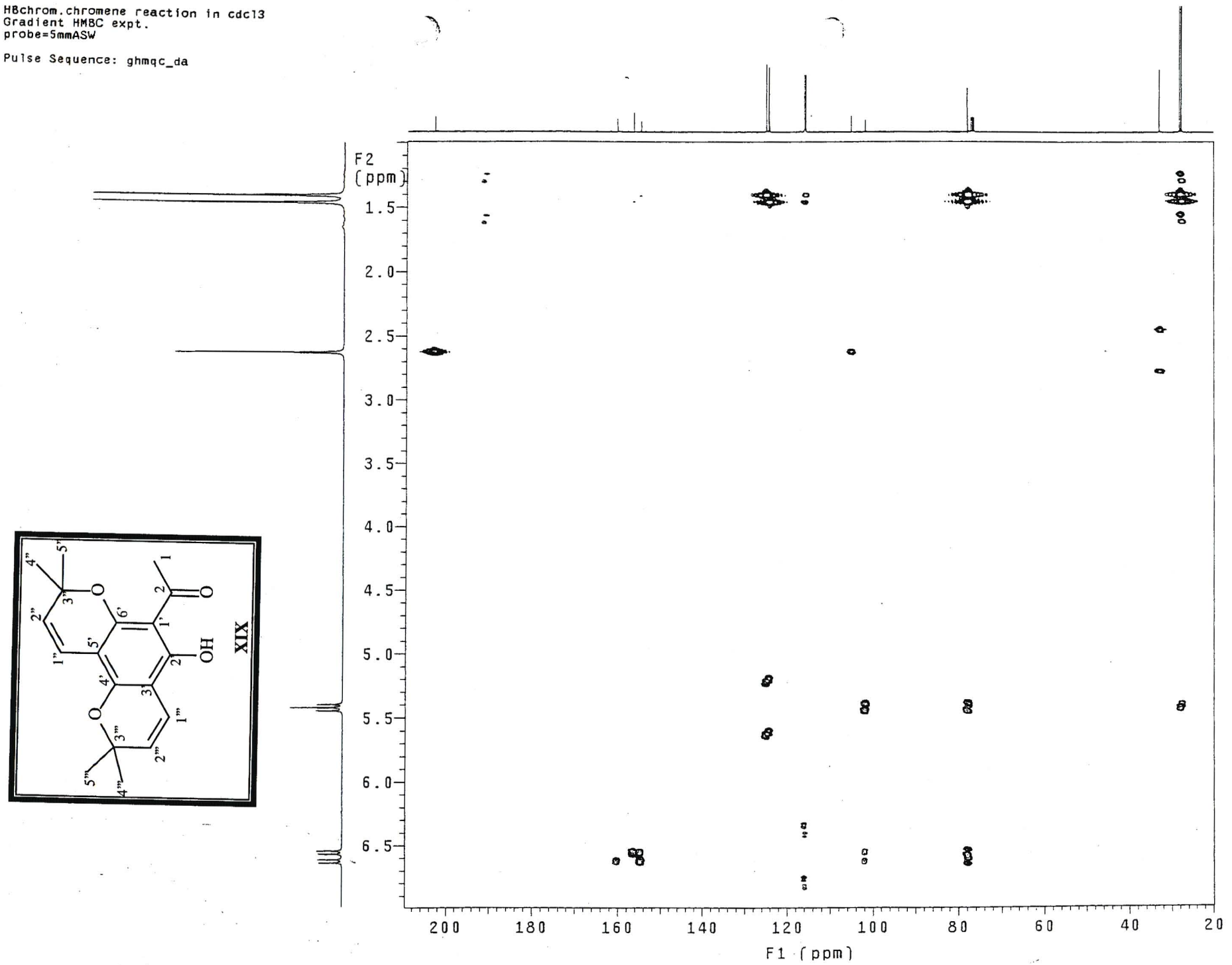
cychrom.chromene reaction in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh





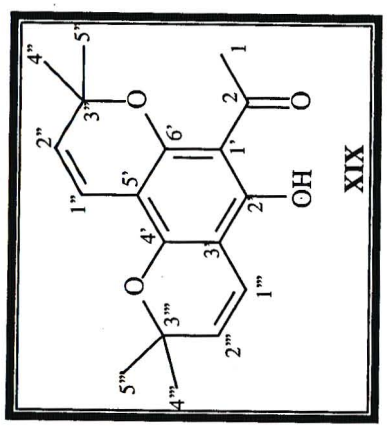
COSY spectrum of compound **XIX,** chromene intermediate



HMBC spectrum of compound **XIX**, chromene intermediate

HQchrom.chromene reaction in cdcl3
Gradient HSQC expt.
probe=5mmASW

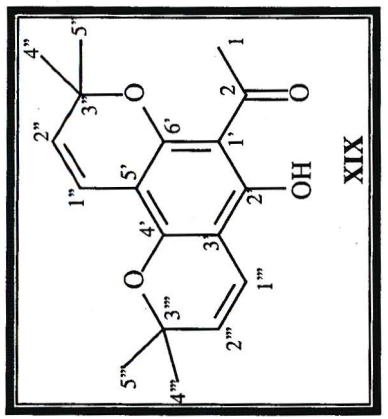
Pulse Sequence: ghsqc_da



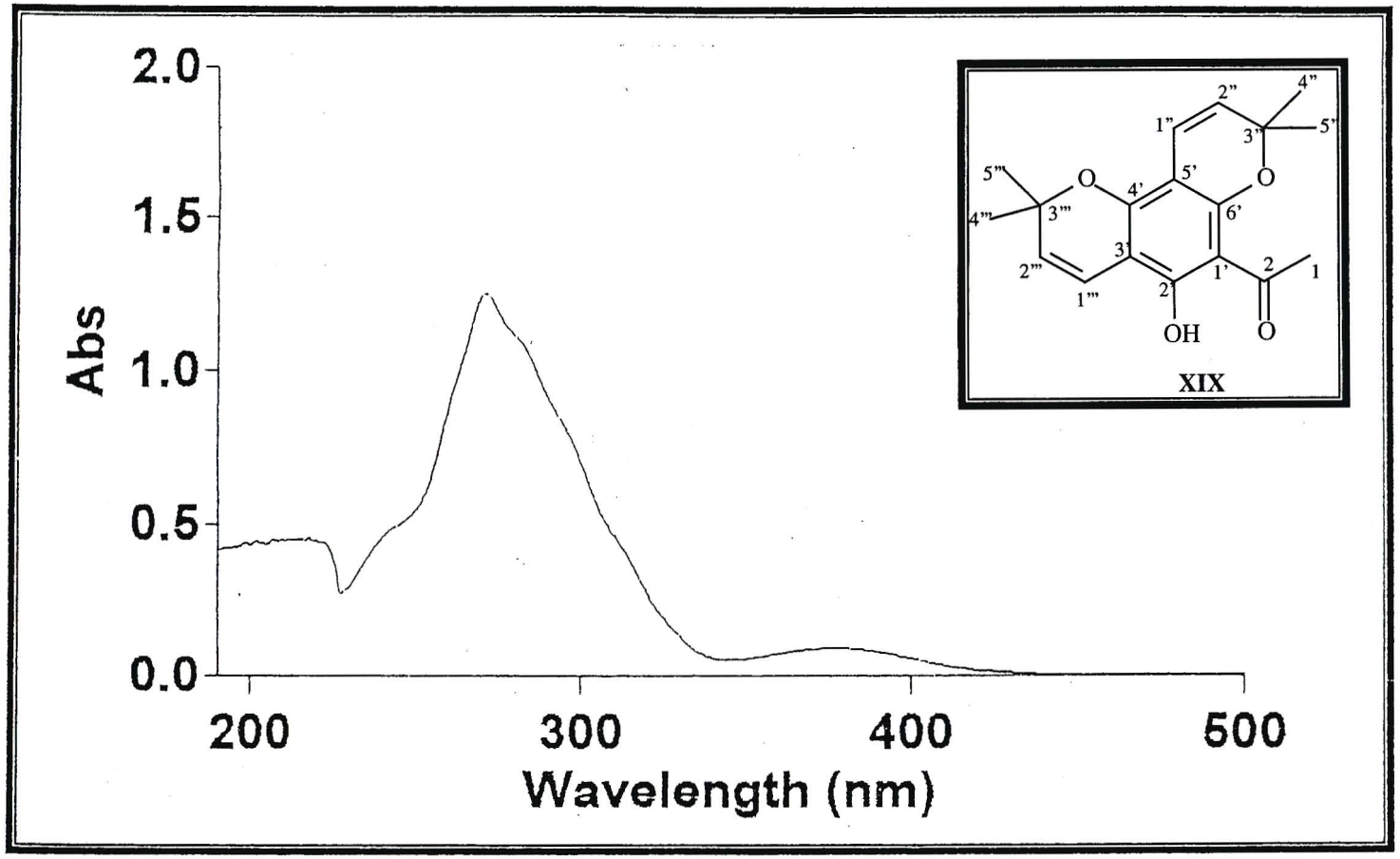
HSQC spectrum of compound **XIX**, chromene intermediate

NOchrom.chromene reaction in cdcl3
Gradient NOESY expt.
mix=1sec
probe=5mmASW

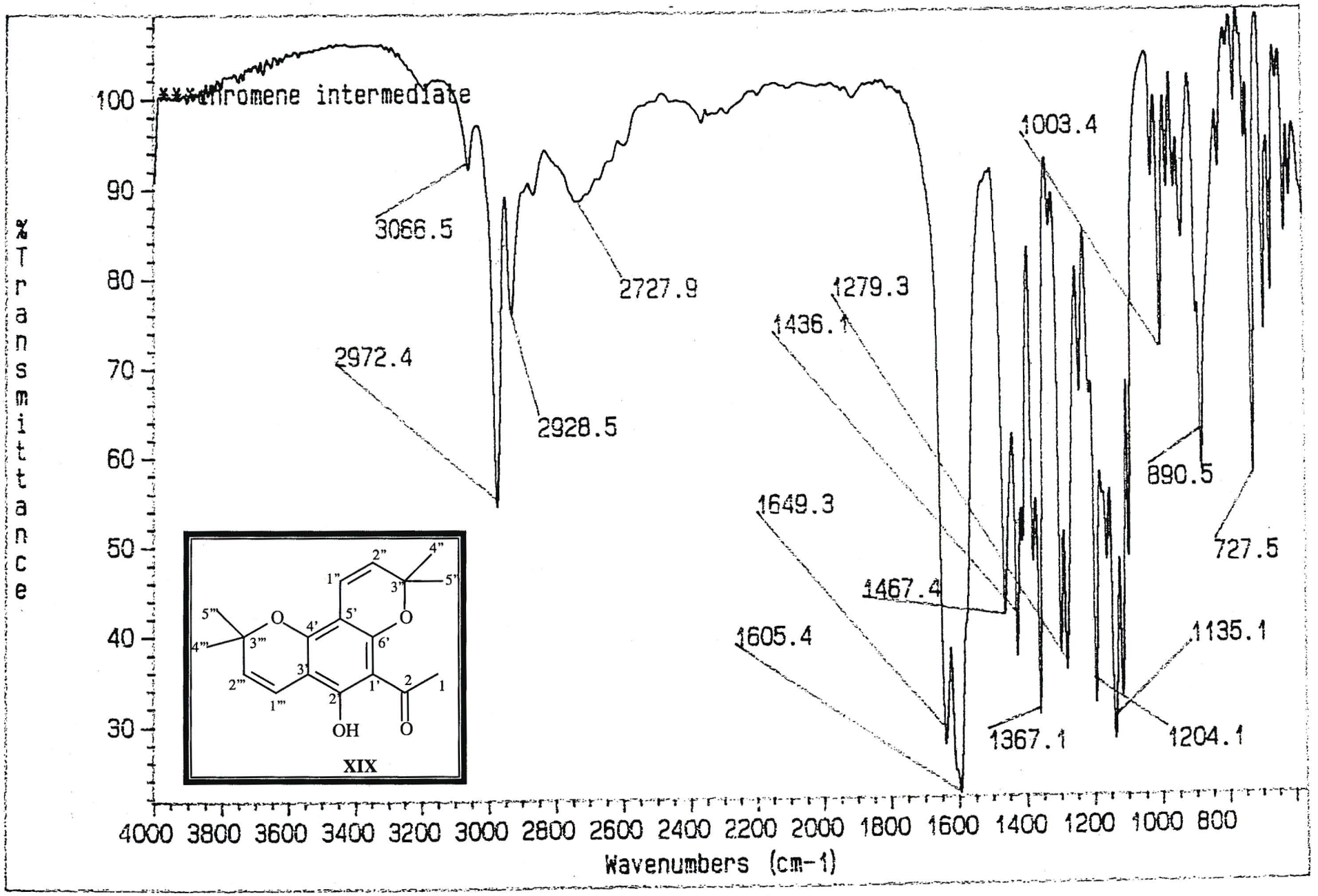
Pulse Sequence: noesy_da



NOESY spectrum of compound **XIX,** chromene intermediate

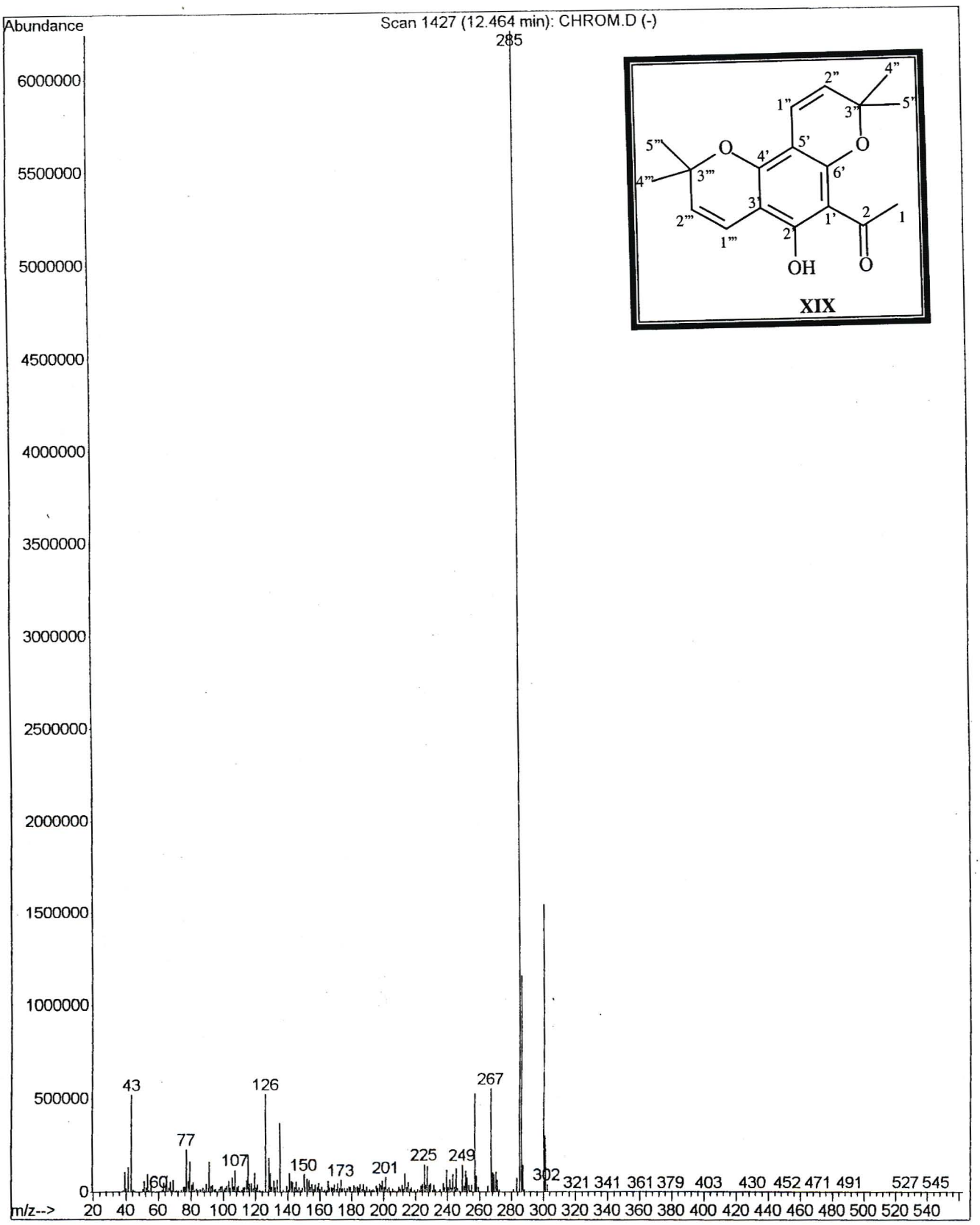


Ultra violet spectrum of compound **XIX**, chromene intermediate



Infrared spectrum of compound **XIX**, chromene intermediate

File : D:\NEIL\CHROM.D
Operator : Bret
Acquired : 6 Apr 2001 15:04 using AcqMethod NEW
Instrument : Instrumen
Sample Name: Chrom
Misc Info : 1ul inject, 1:75 split, MeCl2, 20dpm
Vial Number: 58

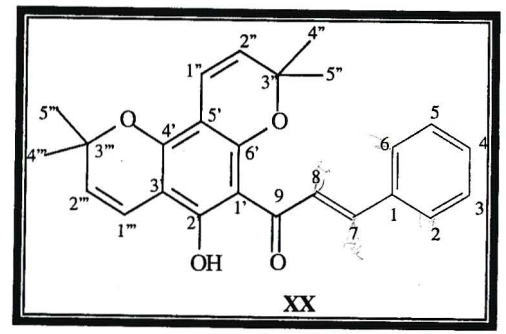


Mass spectrum of compound **XIX**, chromene intermediate

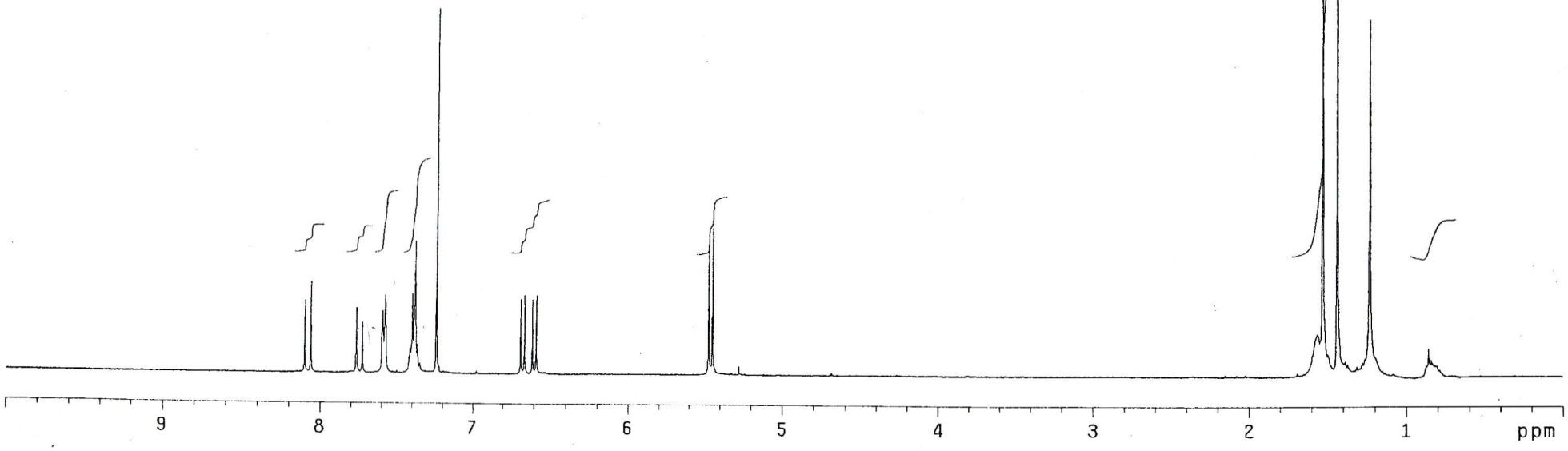
hdcrne.dichromenochalcone in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	3239.5	8.100	12.0
2	3223.7	8.061	15.0
3	3105.452	7.765	10.8
4	3089.886	7.726	8.4
5	3038.427	7.597	9.9
6	3036.596	7.593	10.4
7	3030.552	7.577	12.9
8	3028.904	7.573	11.6
9	2959.132	7.399	13.2
10	2951.624	7.380	21.9
11	2895.586	7.240	59.8
12	2673.633	6.685	12.4
13	2663.561	6.660	13.1
14	2643.417	6.609	12.4
15	2633.528	6.585	13.0
16	2189.805	5.475	23.4
17	2179.733	5.450	24.2
18	626.247	1.566	7.1
19	612.695	1.532	150.0
20	575.337	1.439	148.0
21	492.745	1.232	58.8



XX

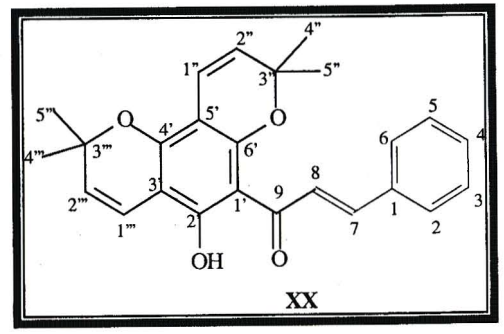


^{1}H NMR spectrum of compound **XX**, dichromenochalcone

cdcrne.dichromenochalcone in cdcl3
probe=5mmASW

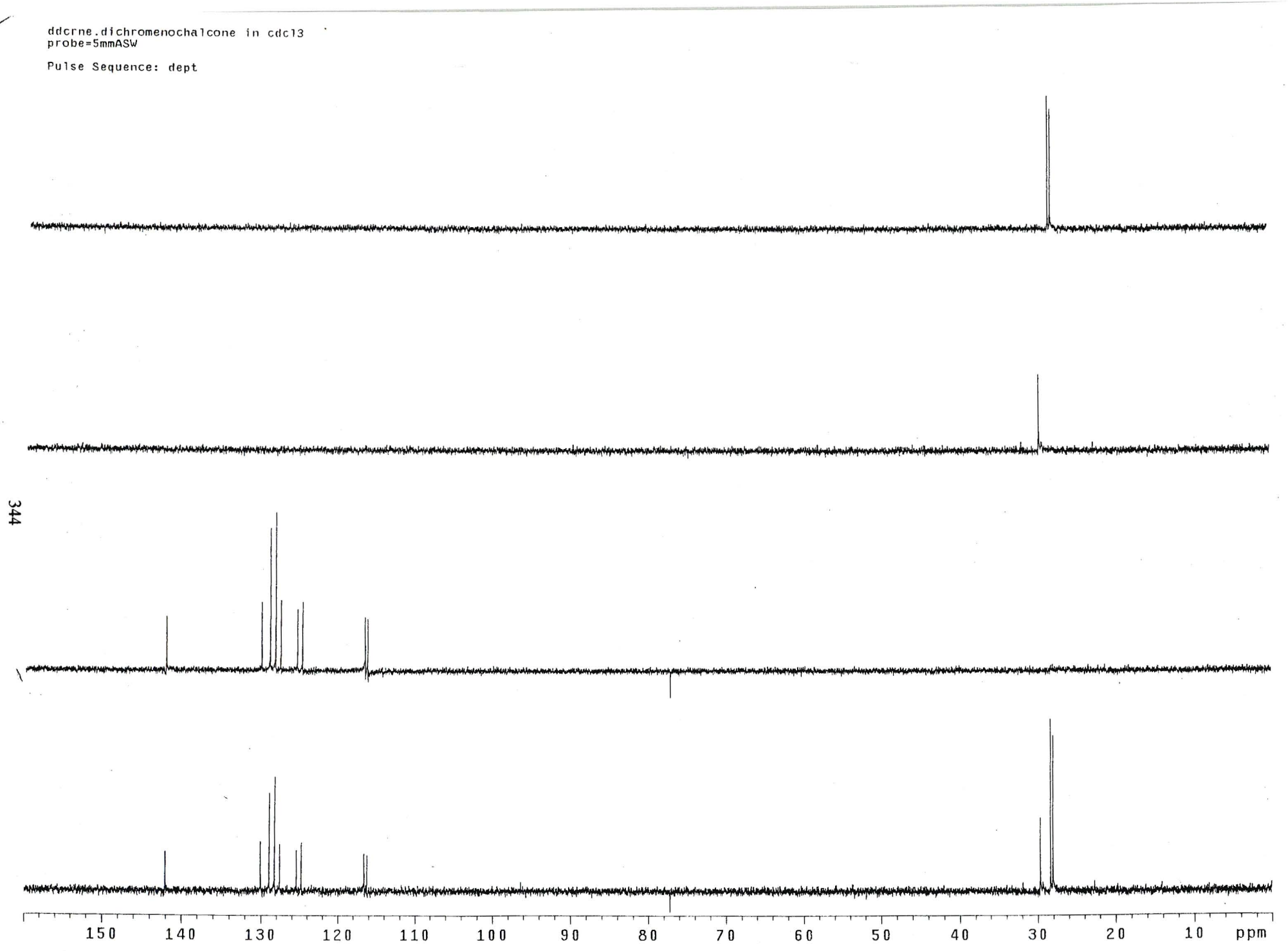
Pulse Sequence: s2pul

INDEX	FREQUENCY	PPM	HEIGHT
1	19401.3	192.922	8.2
2	16236.416	161.451	7.2
3	15709.065	156.207	4.9
4	15619.250	155.314	5.5
5	14287.683	142.073	24.5
6	13640.027	135.633	7.6
7	13078.066	130.045	28.5
8	12967.652	128.947	62.3
9	12896.789	128.242	54.2
10	12830.046	127.579	24.3
11	12611.688	125.407	22.6
12	12547.417	124.768	22.3
13	11724.252	116.583	23.2
14	11687.996	116.222	22.9
15	10654.713	105.948	4.5
16	10319.350	102.613	5.2
17	10311.110	102.531	4.5
18	7875.397	78.311	12.6
19	7872.101	78.278	13.5
20	7775.694	77.320	118.5
21	7764.158	77.205	9.0
22	7743.559	77.000	120.0
23	7722.135	76.787	5.7
24	7712.247	76.689	120.0
25	2985.844	29.690	33.1
26	2855.653	28.396	56.0
27	2824.342	28.085	50.1



XX

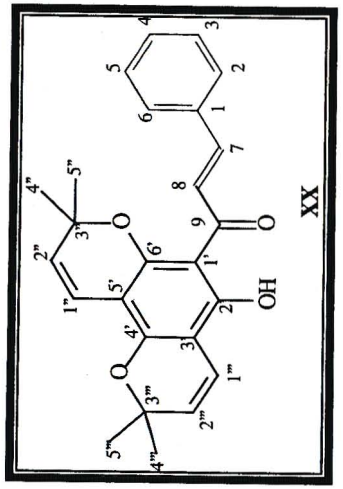
^{13}C NMR spectrum of compound **XX**, dichromenochalcone

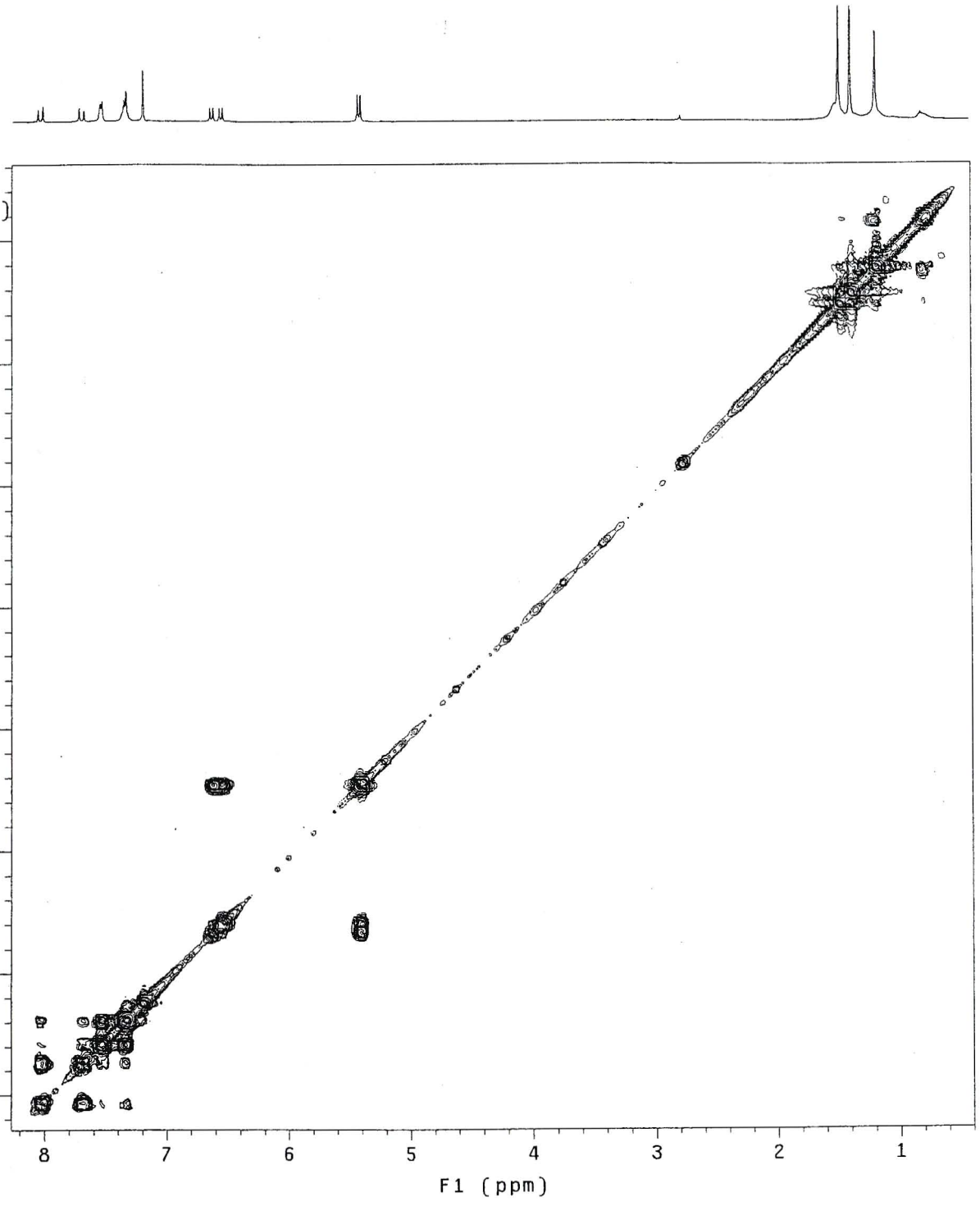


ADEPT spectrum of compound **XX**, dichromenochalcone

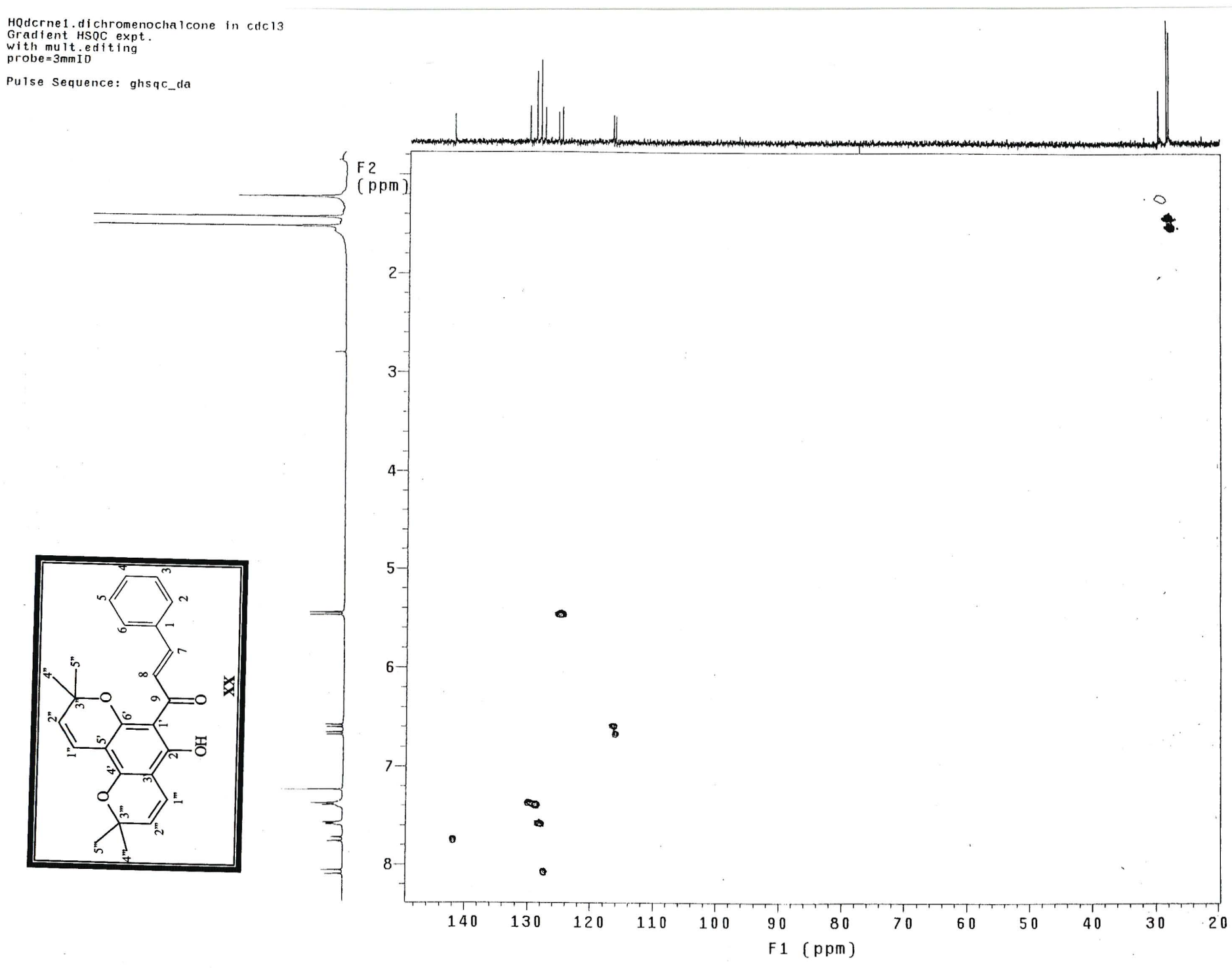
cydcrne.dichromenochalcone in cdcl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

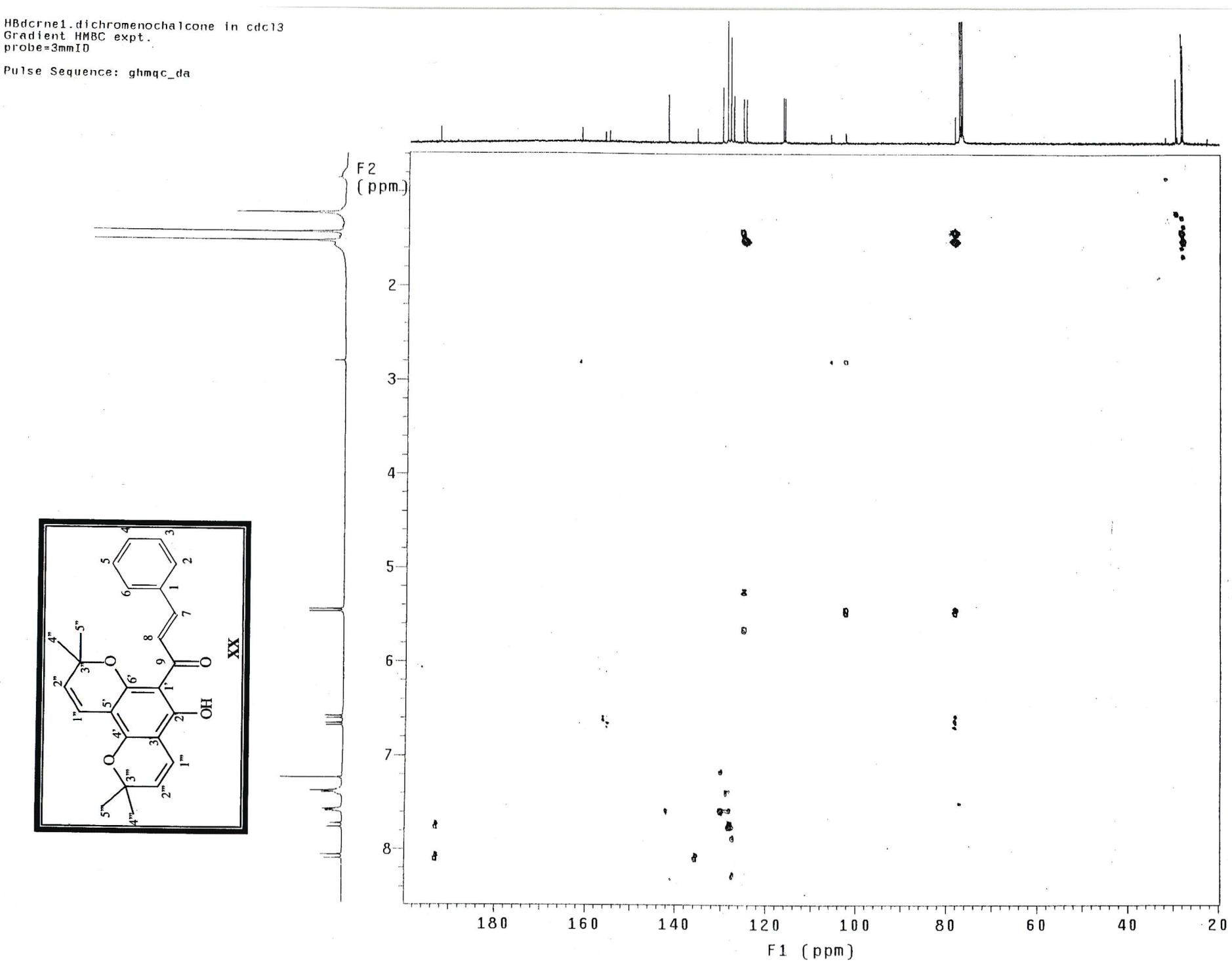




COSY spectrum of compound **XX**, dichromenochalcone



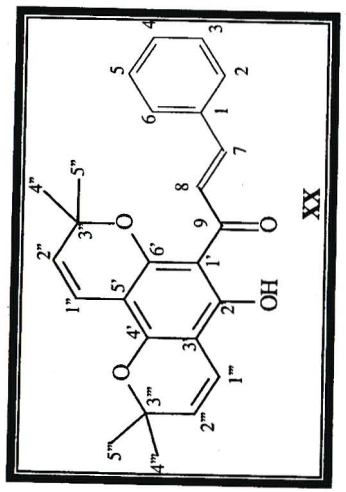
HSQC spectrum of compound **XX**, dichromenochalcone

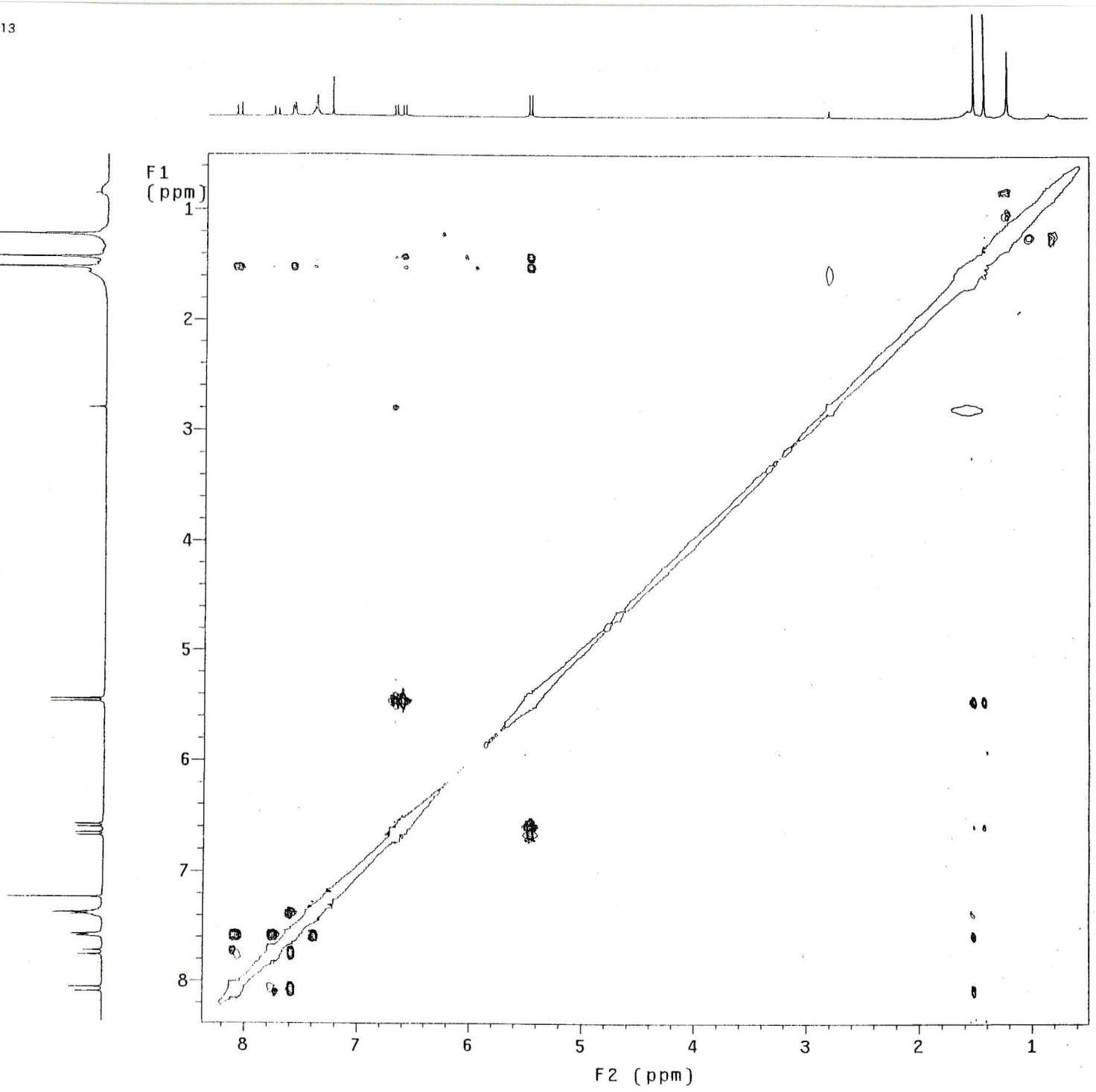


HMBC spectrum of compound **XX**, dichromenochalcone

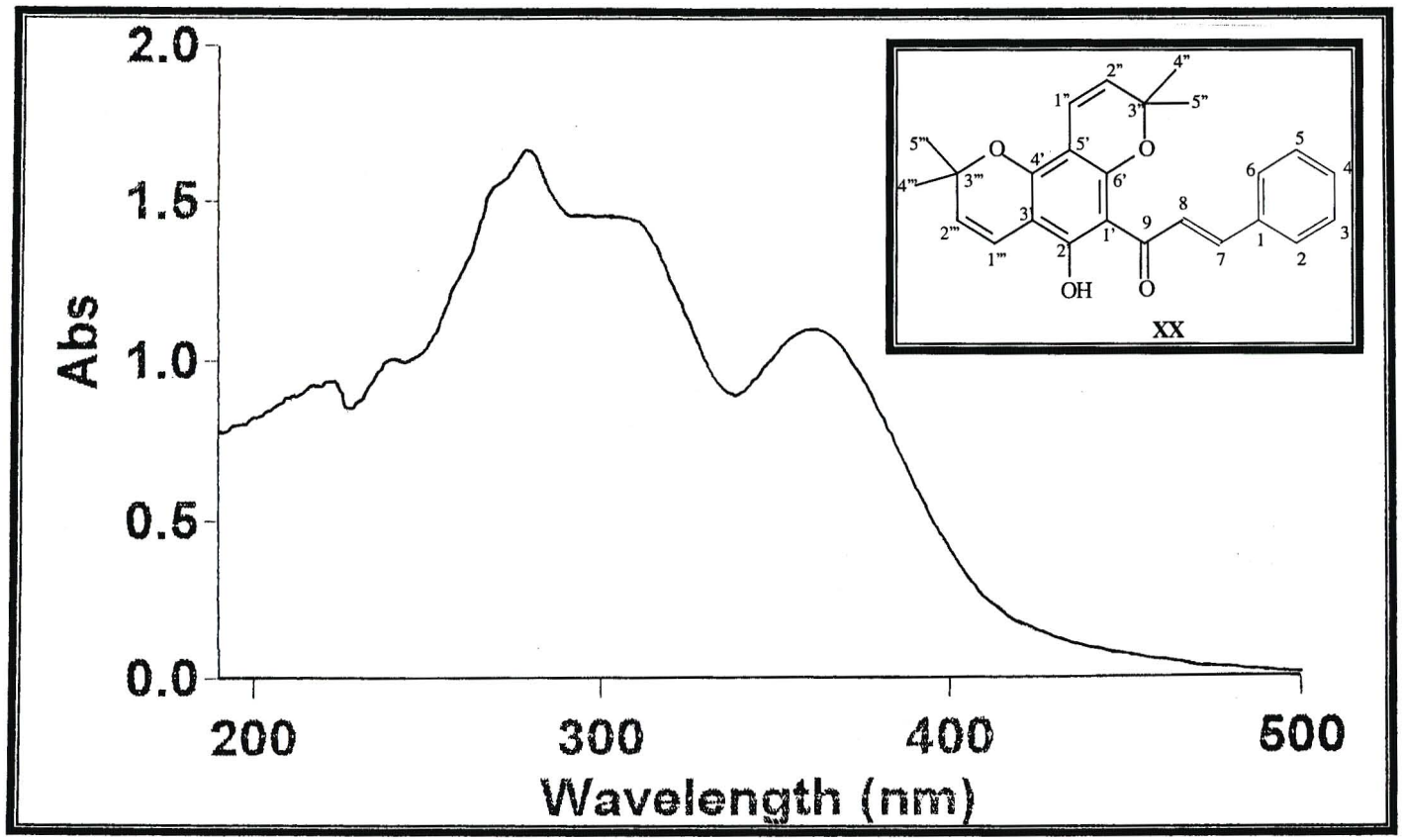
hdcrne1.dichromenochalcone in cdcl3
probe=3mmid

Pulse Sequence: noesy_da

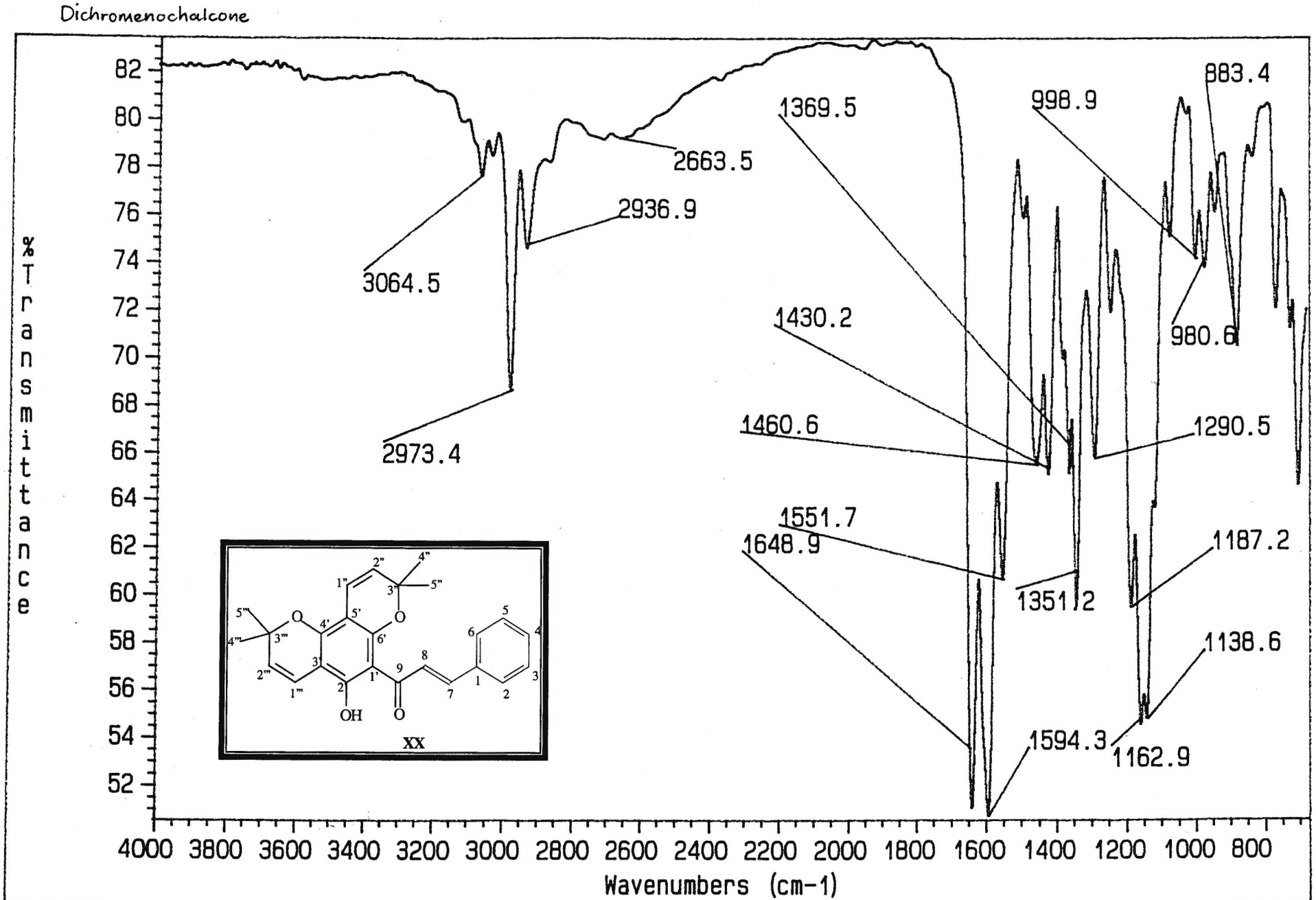




NOESY spectrum of compound **XX**, dichromenochalcone

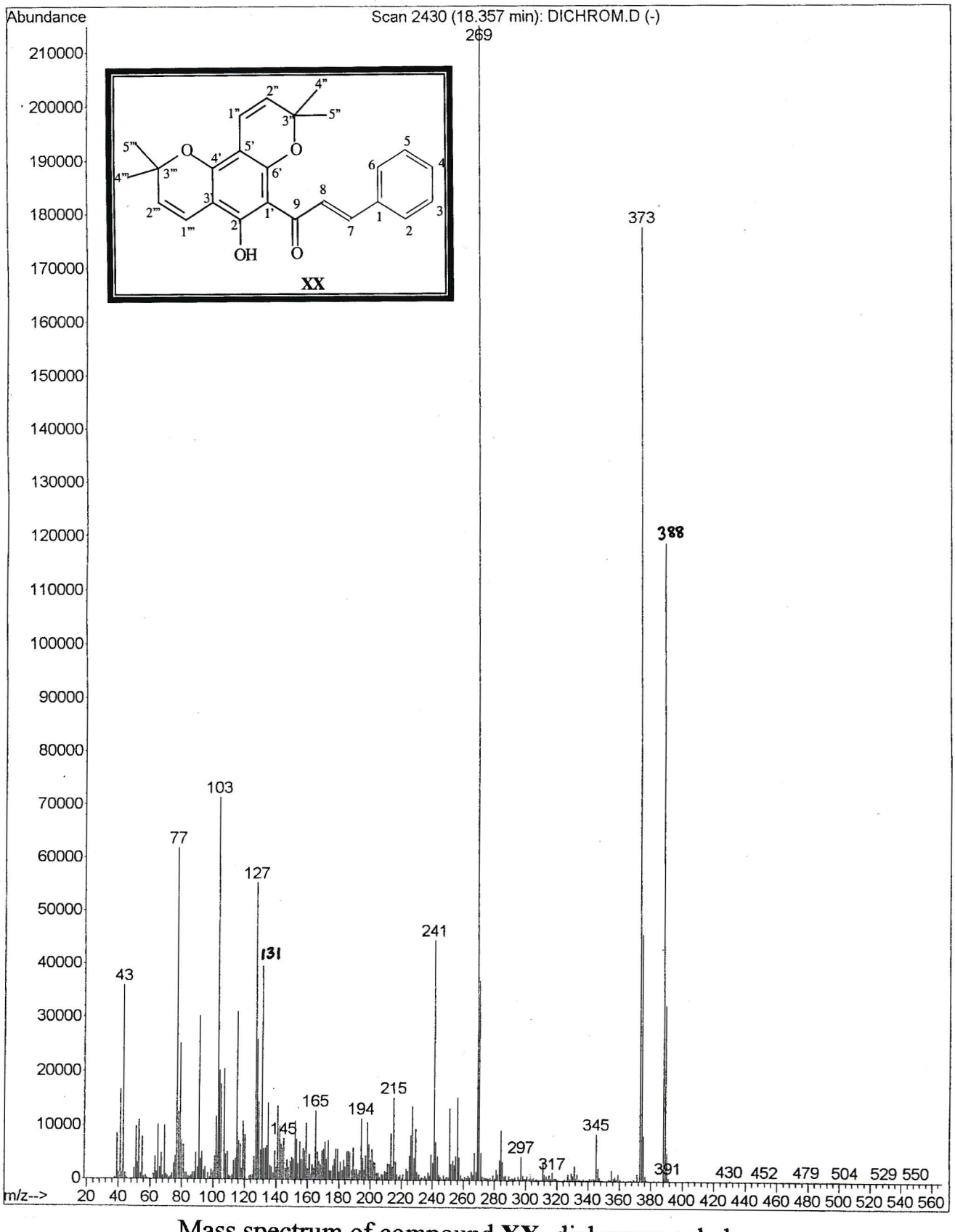


Ultra violet spectrum of compound **XX**, dichromenochalcone



Infrared spectrum of compound **XX**, dichromenochalcone

File : D:\NEIL\DICHROM.D
Operator : Bret
Acquired : 20 Jun 2001 10:45 using AcqMethod NEW
Instrument : Instrumen
Sample Name: Dichromenochalcone
Misc Info : 1ul inject, 1:75 split, MeCl2, 20dpm
Vial Number: 50



Mass spectrum of compound **XX**, dichromenochalcone