# Extractives from Seven African Medicinal Plants

by

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## Anyway

People are often unreasonable, illogical and self-centred;

Forgive them anyway.

If you are kind, people may accuse you of selfish, ulterior motives;

Be kind anyway.

If you are successful, you will win some false friends, and have some true enemies;

Succeed anyway.

If you are honest and frank, people may cheat you;

Be honest and frank anyway.

What you spend years building, someone could destroy overnight;

Build anyway.

If you find serenity and happiness, some may be jealous;

Be happy anyway.

The good you do today, people will often forget tomorrow;

Do good anyway.

Give the world the best you have, and it may never be enough;

Give the world your best anyway.

**You see, in the final analysis, it is between you and God.**

**It was never between you and them anyway.**

**Mother Theresa**

**This thesis is dedicated to the loving memory of**

**my grandmother**

## SHIRLEY EMILY SLABBERT

**1936 - 1979**

**and**

**my grandfather**

## CHARLES FELIX MARGOT

**1918 - 1997**

**May their souls and the souls of all the faithfully departed, through the mercy of God, rest in peace.**

**Amen**

# Abstract

This PhD thesis describes a phytochemical investigation of seven medicinal plants, namely, *Ledebouria ovatifolia* (Hyacinthaceae), *Eucomis pole-evansii* (Hyacinthaceae), *Lachenalia rubida* (Hyacinthaceae), *Drimia capitata* (Hyacinthaceae), *Papaver aculeatum* (Papaveraceae), *Spilanthes mauritiana* (Asteraceae) and *Tachiadenus longiflorus* (Gentianaceae).

The southern African Hyacinthaceae is a large, chemically and morphologically diverse group of plants. Plants of the genus *Ledebouria* are used extensively by traditional healers in Kwazulu-Natal, particularly in enemas and as purgatives for both humans and cattle. Investigations of *Ledebouria ovatifolia* led to the isolation of three compounds, a novel norlignan, a class, which has never before been found in this family, and two eucosterol-type compounds. Chemical investigations of *Eucomis pole-evansii* and *Lachenalia rubida* have revealed the presence of two homoisoflavonoids of the 3-benzyl-4-chromanone type, a novel 3-benzylidene–4-chromanone type homoisoflavonoid as well as a novel 3-benzylchromone. Investigations of *Drimia capitata* have yielded a novel bufadienolide and its glycoside

Plants of the family Papaveraceae have been of great interest chemically, as they contain alkaloids such as morphine and codeine. Morphine is an intense analgesic used to treat chronic pain, while codeine is milder and is found in cough syrups and headache remedies. The species *Papaver aculeatum* is thought to be a premature member of the Papaveraceae and it was thought that it might contain precursors to these alkaloids. This plant yielded an alkaloid, (+)-*N*-acetylanonaine.

In South Africa, the African plant *Spilanthes mauritiana* (Asteraceae), is used medicinally by the Zulus as an oral local analgesic for the relief of toothache. Other medicinal usage of this plant includes healing broken limbs, stomach-ache, diarrhoea, bladder complaints and headaches. This plant yielded one known and one novel isobutylamide. The known isobutylamide, spilanthol, has been attributed with larvicidal and other insecticidal properties.

Members of the family Gentianaceae commonly accumulate bitter substances called iridoids. The species *Tachiadenus longiflorus* yielded the known triterpenoid, oleanolic acid; two known coumarins, scopoletin and scoparone; and what appears to be an iridoid derivative. Syntheses of aesculetin, scoparone and isoscopoletin were also performed for comparison purposes.

The final chapter in this thesis is an attempt to synthesise the norlignan isolated from *Ledebouria ovatifolia*. This procedure involves firstly the synthesis of the appropriate chalcone, secondly the formation of the appropriate Grignard reagent and its attachment to the chalcone, thirdly reduction of the vinyl ketone to form the vinyl alcohol and finally dehydration to form the norlignan. This unfortunately did not occur, however a novel cyclisation product was formed and was identified as (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene.

# Preface

The experimental work described in this thesis was carried out in the School of Pure and Applied Chemistry, University of Natal, Durban, from February 2000 to September 2003, under the supervision of Professor D.A. Mulholland.

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

Signed:

**A. Langlois**, BSc., BSc. (Hons.), MSc. (Natal)

I hereby certify that the above statement is correct.

Signed:

**Professor D.A. Mulholland**, PhD. (Natal)

# List of Abbreviations

| | |
|---|---|
| ADEPT | - distortionless enhancement by polarisation transfer |
| ADP | - adenosine diphosphate |
| ATP | - adenosine triphosphate |
| brd | - broad doublet |
| brs | - broad singlet |
| $^{13}C$ NMR | - carbon-13 nuclear magnetic resonance |
| CoA | - coenzyme A |
| COSY | - correlated nuclear magnetic resonance spectroscopy |
| d | - doublet |
| dd | - double doublet |
| dq | - doublet of quartets |
| dt | - doublet of triplets |
| DMAPP | - dimethylallyl diphosphate |
| Glc | - glucose |
| $^{1}H$ NMR | - proton nuclear magnetic resonance |
| HETCOR | - heteronuclear shift correlation |
| HMBC | - heteronuclear multiple bond coherence |
| HMG-CoA | - hydroxymethylgluteryl coenzyme A |
| HSQC | - heteronuclear multiple quantum coherence |
| Hz | - Hertz |
| IPP | - isopentenyl diphosphate |
| IR | - infra-red |
| m | - multiplet |
| NADPH | - nicotinamide adenine dinucleotide phosphate |
| NMR | - nuclear magnetic resonance |
| NOESY | - nuclear Overhauser effect spectroscopy |
| Pi | - inorganic phosphate |
| PP | - diphosphate |
| ppm | - parts per million |
| q | - quartet |
| s | - singlet |
| t | - triplet |
| TLC | - thin layer chromatography |
| UV | - ultraviolet |

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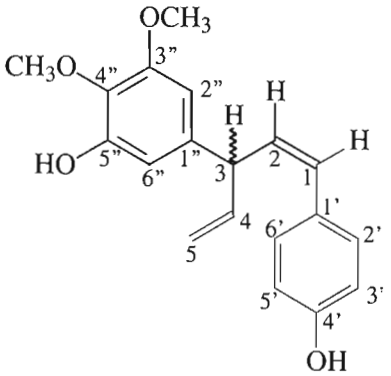
# Structures of Isolated Compounds



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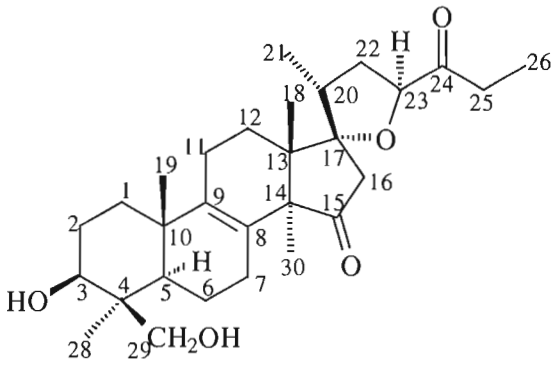

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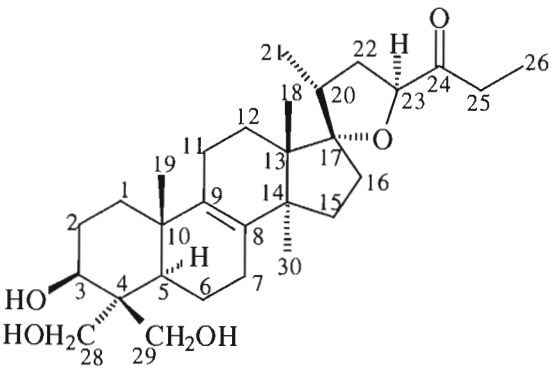

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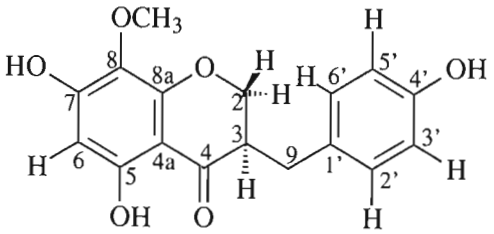

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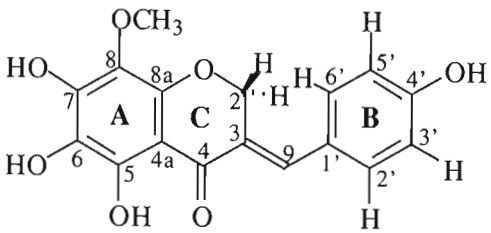

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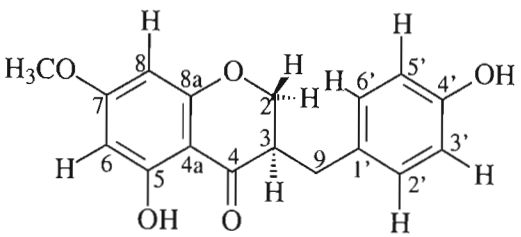

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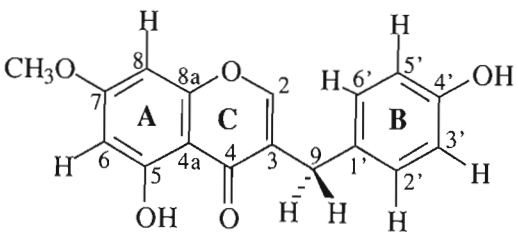

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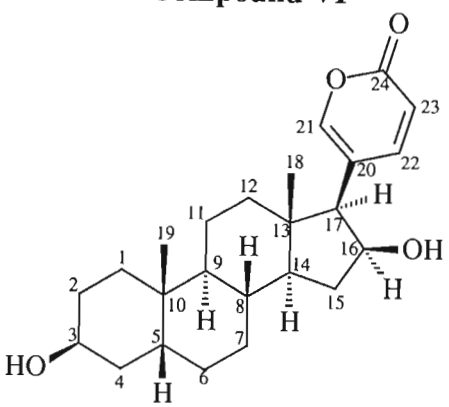

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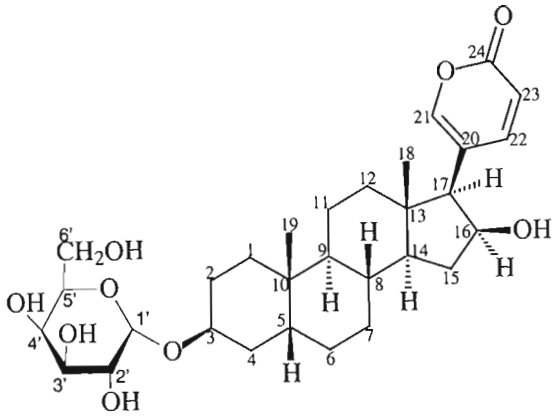

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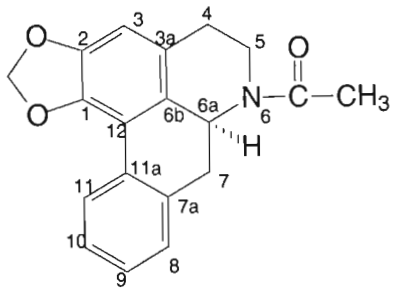

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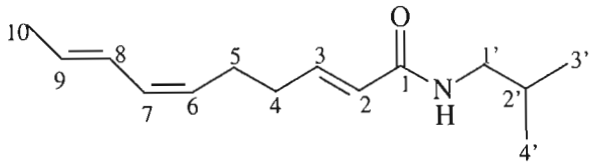

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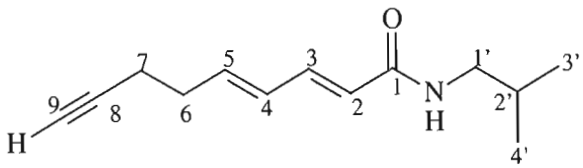

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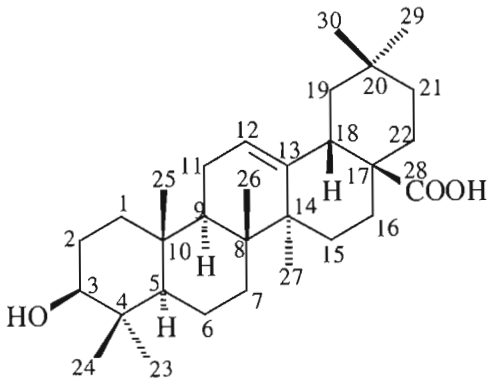

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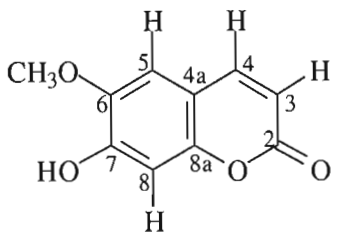

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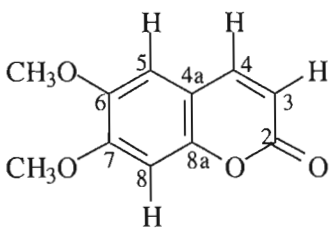

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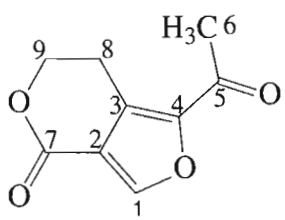
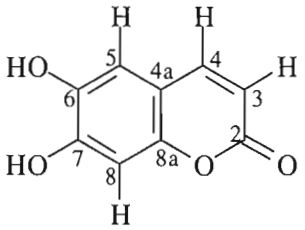
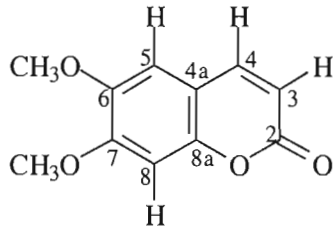


**Compound XVI**

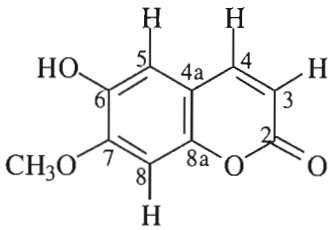
# Structures of Synthesised Compounds



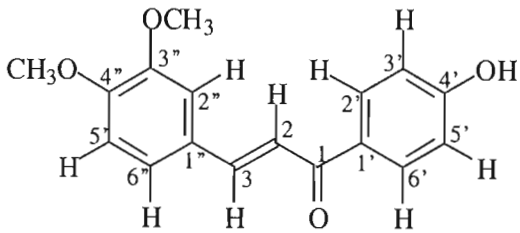
**Compound XVII**



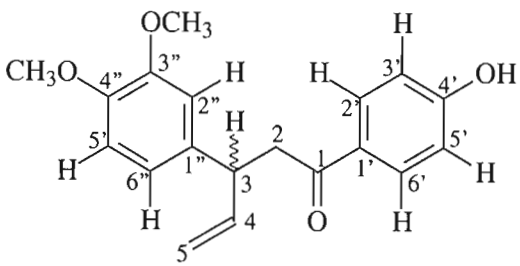
**Compound XVIII**



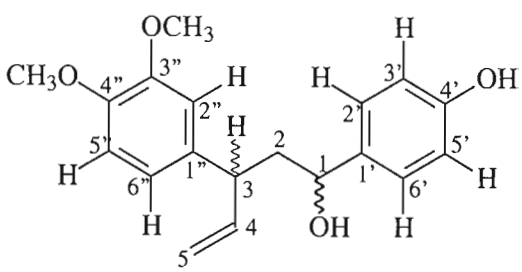
**Compound XIX**



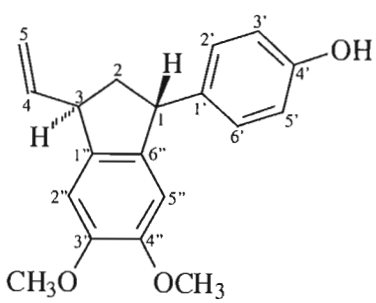
**Compound XX**



**Compound XXI**



**Compound XXII**



**Compound XXIII**

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Lastly I would like to thank the Lord for the many gifts He has given me. I believe in You, I adore You, I trust in You and I love You eternally, my Lord and my God.

**Do not let anyone look down on you because you are young,**
**but be an example for the believers in your speech,**
**your conduct, your love, faith and purity.**
**1 Timothy 4:12**

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# CHAPTER 1

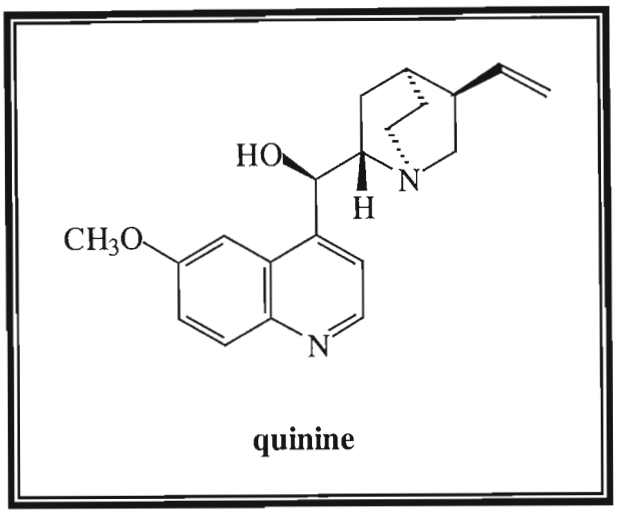
# General Introduction

## 1.1 The Importance of Medicinal Plants

Until relatively recently, the primary source of all the medicines in the world was plants, and they are continuing to provide mankind with new remedies[1]. Plant-based traditional medicine systems play a vital role in health care, and the World Health Organisation (WHO) estimates that approximately eighty percent of the world's inhabitants are dependant on traditional medicines for their primary health care[2]. In the remaining twenty percent of the population, plant products also play an important role in health care, as an analysis of prescribed drugs indicated that approximately twenty-five percent contained plant extracts or active principles derived from higher plants[2]. Currently approximately one hundred and nineteen chemical substances isolated from ninety plant species are considered as important drugs and are used in one or more countries[2]. Seventy-four percent of these one hundred and nineteen drugs were discovered as a result of chemical investigations directed at isolating the active substances from traditional medicinal plants[2].

Natural products and their derivatives are extensively used in clinical drugs with quinine, morphine, codeine, aspirin and reserpine being a few well-known examples[1]. The development of anti-cancer drugs such as paclitaxel, from *Taxus brevifolia*, and vincristine, from *Catharanthus roseus*, has been a significant breakthrough for pharmaceutical companies[1], however new analogues are currently being isolated and synthesised. In South Africa, many of the medicines used are still derived from plant sources, which can be bought informally from traditional healers, or commercially[1]. Many South African plants, including *Aloe ferox* (Cape aloe), *Agathosma betulina* (buchu) and *Harpagophytum procumbens* (devil's claw) are known and used worldwide[1].

The bark of the quinine tree, *Cinchona pubescens* (Rubiaceae), yields the alkaloid, quinine (Figure 1.1) which, before the recent development of more effective synthetic drugs such as quinacrine, chloroquine and primaquine, provided the only treatment for malaria[1,3].




**quinine**

Figure 1.1. The structure of quinine.

However, with the increase in resistant parasites, it was necessary to search for other agents, both synthetic and natural product based[2]. From the extract of a traditional Chinese herbal remedy plant, *Artemisia annua* (Wormwood) (Compositae/ Asteraceae), the active agent artemisinin, a sesquiterpene endoperoxide, was isolated. This plant had been used for centuries in China in the treatment of fevers and as an antimalarial[2,4]. Artemisinin has been shown to be responsible for the antimalarial properties of this plant, as it is an effective blood schizontocide in humans infected with malaria and it shows virtually no toxicity[4]. Artemisinin can be reduced to the lactol (hemiacetal) dihydroartemisinin, which has been used for the semi-synthesis of a range of analogues of which the acetal, artemether, has been shown to be a very promising antimalarial agent[4]. When compared to artemisinin, these analogues show increased activity and also a reduction in the probability of a re-infection[4]. Dihydroartemisinin rapidly clears the blood of malarial parasites, however, it does not have a prophylactic effect[4]. Investigations have also led to the identification of artemether, which is now in widespread use throughout the world as a potent treatment for malaria[2]. Chemically, these analogues are unlike any other class of current antimalarial agent and may become a significant group of drugs in the battle

against this life-threatening disease[4]. The structures of artemisinin, artemether and dihydroartemisinin are shown in figure 1.2.

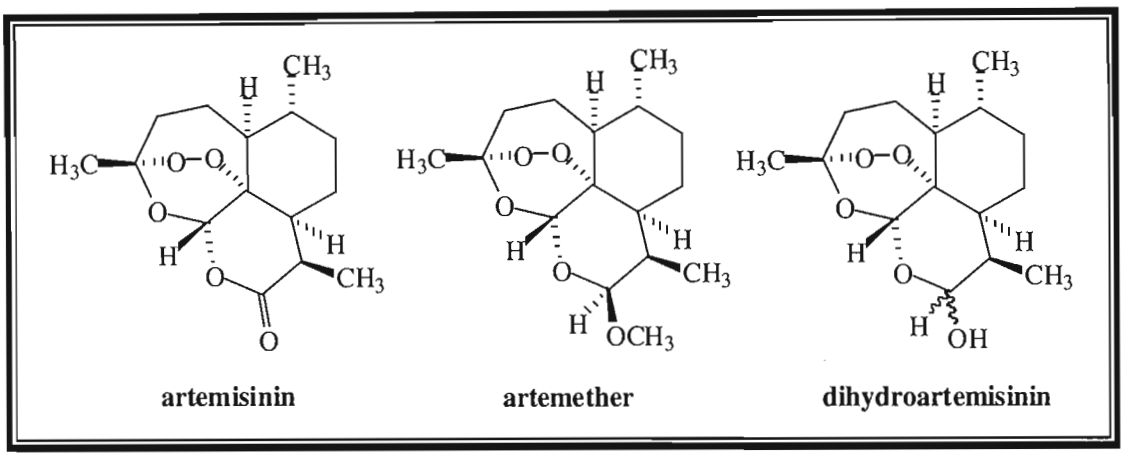



Figure 1.2. The structures of artemisinin, artemether and dihydroartemisinin[2].

Atropine (Figure 1.3), the racemic form of hyoscyamine[5], and other tropane alkaloids are isolated from *Atropa belladonna*[1] (Solanaceae). These compounds are used in eye drops, injected to treat Parkinson's disease and used in skin patches to treat motion sickness[1]. Atropine produces an increased heart rate, dilated pupils, dry skin and anaesthetises the nerve endings in the skin[6].

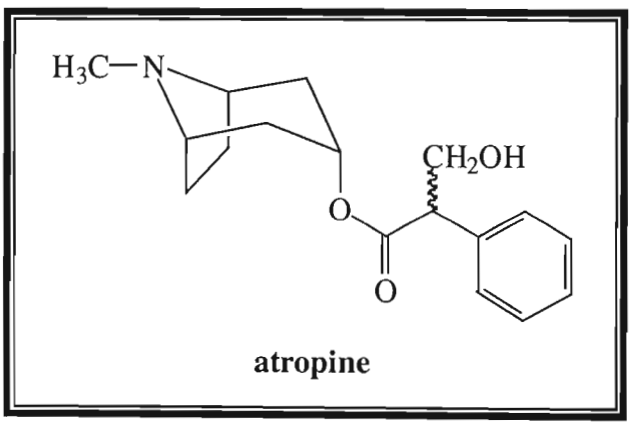



Figure 1.3. The structure of atropine[5].

Morphine and codeine are obtained from the unripe fruit capsules of the opium poppy, *Papaver somniferum* (Papaveraceae). Crude 'opium', the air-dried latex from *P. somniferum*, contains about twenty five percent of its weight as opium alkaloids, with

morphine and codeine being the major components[2]. Morphine is a powerful analgesic and narcotic and continues to be one of the most vital analgesics for the relief of severe pain, usually in terminal patients. It also induces a state of euphoria and mental detachment, together with nausea and vomiting[5]. Codeine, the 3-*O*-methyl ether of morphine, is the opium alkaloid that is most widely used[5]. It is a milder analgesic and is found in cough syrups and headache remedies[1]. Most of the prescribed codeine is manufactured by semi-synthesis from morphine due to the very small amounts of this compound found in opium[5]. From morphine, the addictive drug, heroin, is also produced[7]. Figure 1.4 shows the structures of morphine, codeine and heroin.

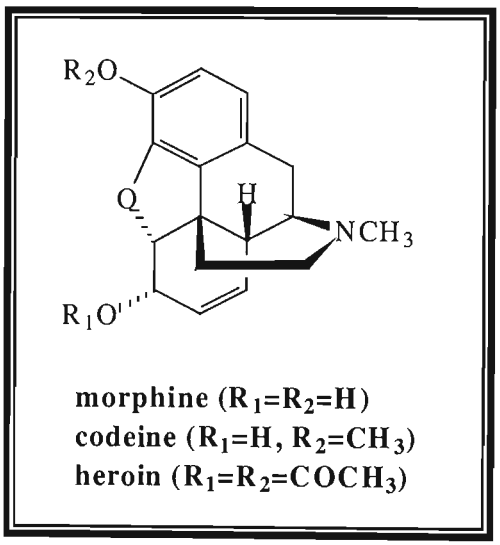



Figure 1.4. The structures of morphine, codeine and heroin.

A very well known and most used drug in pain relief is aspirin (Figure 1.5). It is the acetyl derivative of salicylic acid and is used to lower fever, relieve pain, reduce inflammation and thin the blood[8]. Aspirin was originally derived from salicin (Figure 1.5), the active ingredient in willow bark (*Salix* species, Salicaceae)[8].

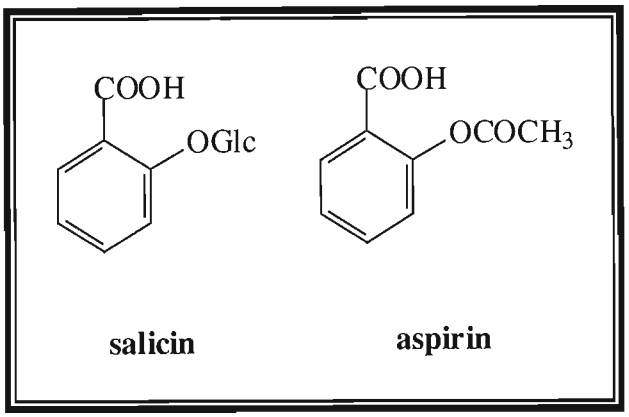



Figure 1.5. The structures of salicin and aspirin.

Reserpine (Figure 1.6) is an alkaloid isolated from the root of the snakeroot plant, *Rauwolfia serpentina* (Apocynaceae), a small evergreen climbing shrub native to the Indian subcontinent[9]. The plant was used for centuries to treat insanity as well as physical illnesses such as fevers and snakebites[9]. Reserpine has been reported to cause many toxic side effects including nightmares, Parkinsonism, gastrointestinal disturbances and severe depression[5,9]. Reserpine has also been suggested to play a role in the promotion of breast cancers, however, it is widely used as an antihypertensive and a mild tranquilizer[5].

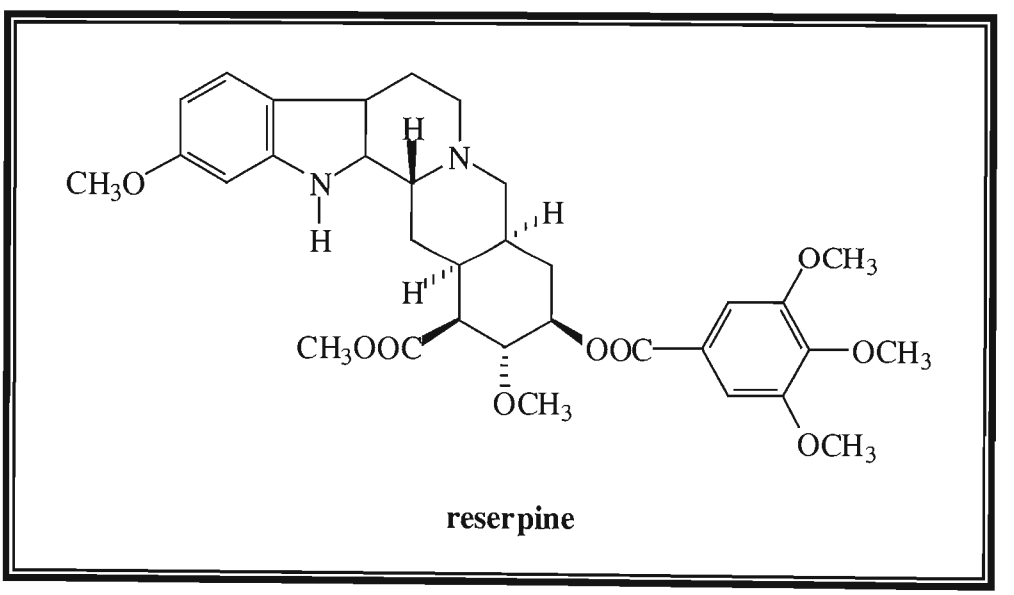



Figure 1.6. The structure of reserpine.

The treatment of cancer is poorly defined in terms of folklore and traditional medicine, thus the claims that plants have a long history of effective use in the treatment of cancer, can be viewed with some scepticism[2]. The Madagascan periwinkle, *Catharanthus roseus* (Apocynaceae), due to its folklore usage as a tea for diabetics, was originally investigated for potential hypoglycaemic activity[2,5]. Although the blood sugar levels of rabbits were not affected by the plant extracts, the test animals became susceptible to bacterial infection due to low white blood cell levels[5]. Thus anticancer activity was suggested, which initiated the exhaustive investigation of the constituents[5]. Chemical investigations yielded a mixture of alkaloids, two of which, vinblastine and vincristine[2,10], shown in figure 1.7, were introduced into cancer chemotherapy and have proved to be vital in the fight against cancer. Vinblastine and vincristine show very little structural difference, however, there is a significant difference in the spectrum of human cancers that respond to these two drugs[5]. Vinblastine is used to treat Hodgkin's disease (a cancer affecting the lymph glands, spleen and liver), advanced breast cancer and advanced testicular carcinoma[5,10]. Vincristine has superior antitumor activity compared to vinblastine but is more neurotoxic[5]. A high rate of remission of childhood leukaemia has been shown in treatment using this drug[5]. Some other cancer conditions, including lymphomas, small cell lung cancer, and cervical and breast cancers also respond positively to this drug[5]. These alkaloid drugs need to be injected, and are both currently used in combination with other drugs in cancer chemotherapy[5].

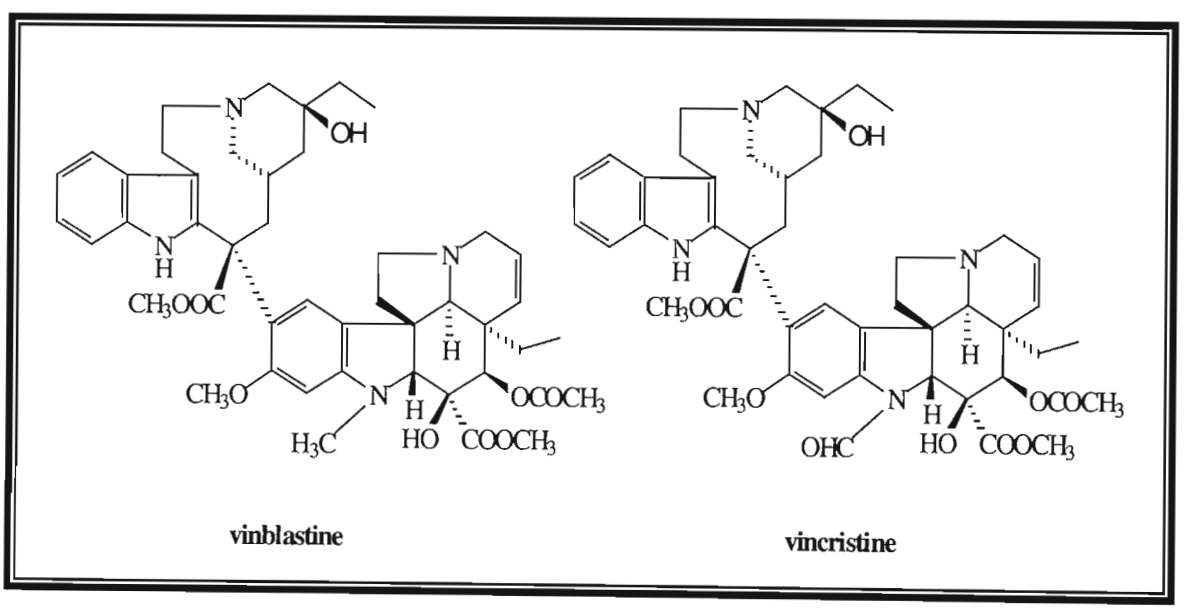



Figure 1.7. The structures of vinblastine and vincristine[2].

Selective chemical modifications of vinblastine and vincristine have led to two semi-synthetic derivatives, vinorelbine and vindesine, shown in figure 1.8, being introduced for the treatment of cancer[2,5,10]. Recently, the use of vinorelbine has been approved in the United States, while vindesine is still undergoing clinical trials[2]. Vindesine has been used in the treatment of acute lymphoid leukaemia in children[5]. Vinorelbine is orally active and has a broader anticancer activity, however, with fewer neurotoxic side-effects than either vinblastine or vincristine[5].

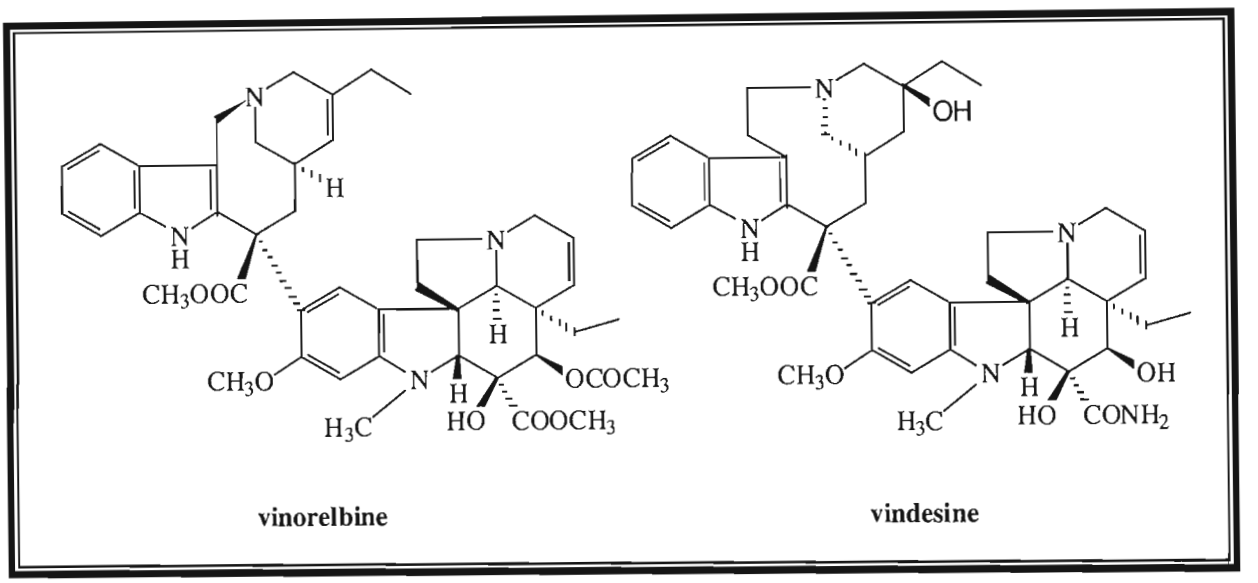



Figure 1.8. The structures of vinorelbine and vindesine[2].

A very effective treatment for breast and ovarian cancer is the drug paclitaxel, which is sold as Taxol[1,5]. It is a diterpenoid derivative found in the bark of *Taxus brevifolia* (Taxaceae), commonly known as the Pacific Yew[1,5,11]. Several Native American tribes have used parts of this plant for non-cancerous treatments[2], while the leaves of *Taxus baccata,* the common yew, are used in the traditional Asiatic Indian medicine system, with a single reported use in the treatment of cancer[2]. The problem with paclitaxel is the difficulty in obtaining the drug. The amount of paclitaxel in the yew bark is very low and the tree is very slow growing, and only yields enough paclitaxel to treat one patient. Although paclitaxel has been produced by total synthesis, this is not commercially viable. Docetaxel (sold as Taxotere), is a side chain analogue of paclitaxel, which has also been produced by semi-synthesis from 10-deacetylbaccatin III[4]. Docetaxel has improved water solubility compared with paclitaxel and is being

used clinically against ovarian and breast cancers[4]. The structures of paclitaxel and docetaxel are shown in figure 1.9.

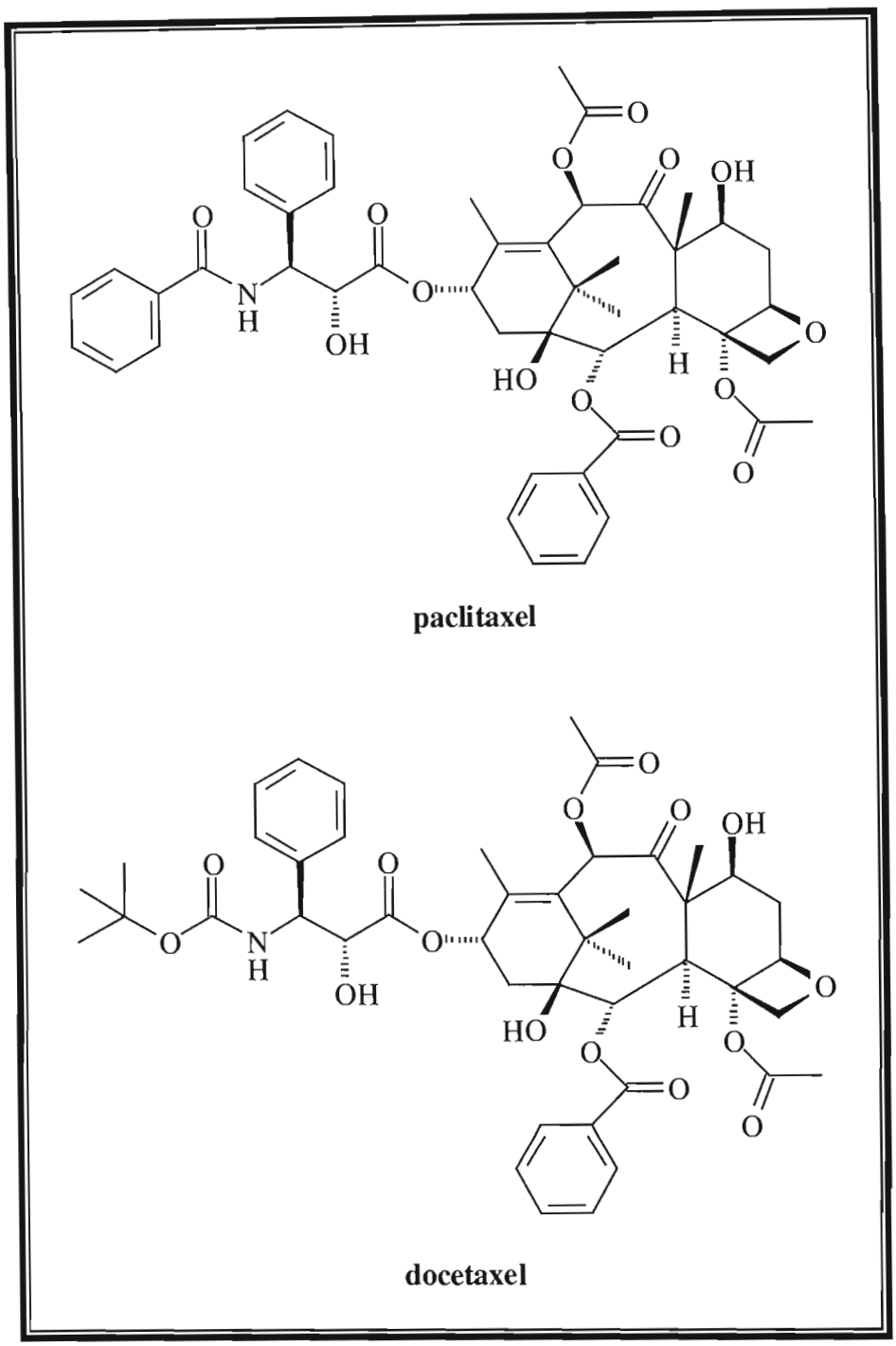



Figure 1.9. The structures of paclitaxel and docetaxel.

Plants provided an important source of raw materials for medicines in the early part of the 20th century; however, later, synthetic analogues were produced to replace many of these plant-derived medicines[12]. Recently, plants have once again become a

significant source of new medicines due to problems with drug resistant micro-organisms, the side effects from modern drugs as well as diseases which emerge where no medicines are available[12]. The vast medicinal knowledge of indigenous people is captivating researchers around the world. The major discoveries listed in this chapter, show the importance of plants in providing new target molecules for drug development.

Southern Africa is an important centre of plant diversity in the world, producing a large number of plant species (approximately thirty thousand higher plants in South Africa and three thousand in Botswana), many of which are endemic to the area[13]. One of the world's richest floral regions boasting over six thousand endemic species is the Cape Floristic Region[14]. A wide variety of plant types, which have adapted to the different habitats, is a result of the great variation in climatic types from the sub-tropical eastern coast to the semi-desert vegetation of much of the region, to the Mediterranean climate of the Western Cape[13]. The indigenous people of southern Africa have a long history of traditional plant usage for medicinal purposes[13]. The southern African medicinal plant trade is a vital part of the economy with over seven hundred plant species reported as being traded in the region[15]. The factors of the large numbers, uniqueness and variety of the plants, commercial and social importance, and ethno-botanical information available make natural products research an important area of research for the region[13].

In this work, the chemical investigation of seven African medicinal plants, namely, *Ledebouria ovatifolia* (Hyacinthaceae), *Eucomis pole-evansii* (Hyacinthaceae), *Lachenalia rubida* (Hyacinthaceae), *Drimia capitata* (Hyacinthaceae), *Papaver aculeatum* (Papaveraceae), *Spilanthes mauritiana* (Asteraceae) and *Tachiadenus longiflorus* (Gentianaceae), has been undertaken to find compounds with biological activity. Three main classes of compounds were isolated, namely, homoisoflavonoids, triterpenoids and coumarins and therefore the biosynthesis of these three classes will be discussed in detail.

## 1.2 Introduction to Homoisoflavonoids

Homoisoflavonoids, also referred to as homoisoflavanones, are a group of naturally occurring compounds isolated predominantly from the Hyacinthoideae subfamily of the Hyacinthaceae (Liliaceae *sensu lato*) family[16-20]. Bohler and Tamm first isolated this class of compounds in 1967 from *Eucomis bicolor* and many others have subsequently been isolated from other species of *Eucomis*[21-24], *Scilla*[25-27], *Veltheimia*[20], *Drimiopsis*[28], *Muscari*[17] and *Ledebouria*[29].

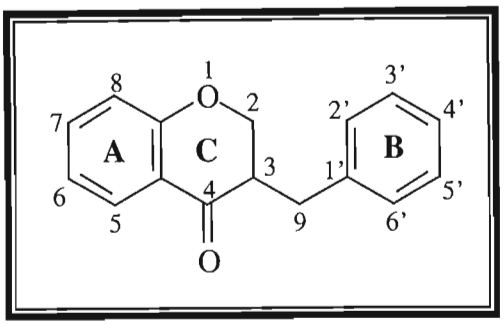



Figure 1.10. The numbering system of a typical homoisoflavonoid.

Homoisoflavonoids (Figure 1.10) belong to a homogeneous group of naturally occurring oxygen heterocycles[30]. However, the term "homoisoflavonoid" is a misnomer since these compounds are not biogenetically related to isoflavonoids and do not undergo the 2,3-aryl rearrangement of the $C_6$-$C_3$-$C_6$ group that typically occurs in the biosynthesis of isoflavonoids[31,32]. In addition, homoisoflavonoids have a sixteen-carbon skeleton as opposed to the fifteen-carbon atoms characteristic of the isoflavonoid skeleton. Thus, although this term is often used when making reference to these compounds, the systematic name 3-benzyl-4-chromanones is more correct. Characteristically, homoisoflavonoids have an additional benzylic carbon atom situated between the B and C-rings, leading to a sixteen-carbon skeleton. This skeleton bears either a chromane, chromone or chromanone group, which has a benzyl or benzylidene group situated at the 3-position[19]. Homoisoflavonoids vary from one another by the substitution patterns on the A and B-rings. Common substituents are hydroxy, methoxy, acetoxy and in some cases even methyl and aldehyde groups.

Homoisoflavonoids are generally classified into three basic types[30]; 3-benzyl-4-chromanones (Figure 1.11), 3-benzyl-3-hydroxy-4-chromanones (Figure 1.12) and 3-benzylidene-4-chromanones (Figure 1.13). The latter are characterised by the presence of a 3,9-double bond, which can be in the (*Z*) or (*E*) configuration[30].

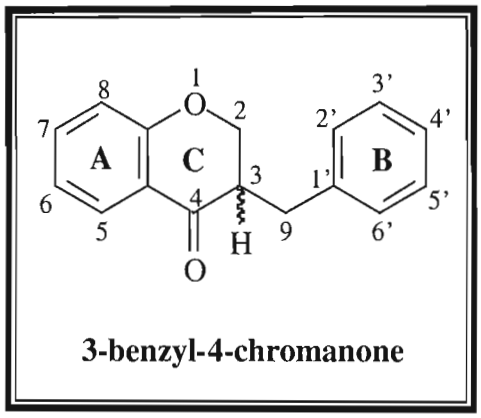



Figure 1.11. The structure of a 3-benzyl-4-chromanone homoisoflavonoid.

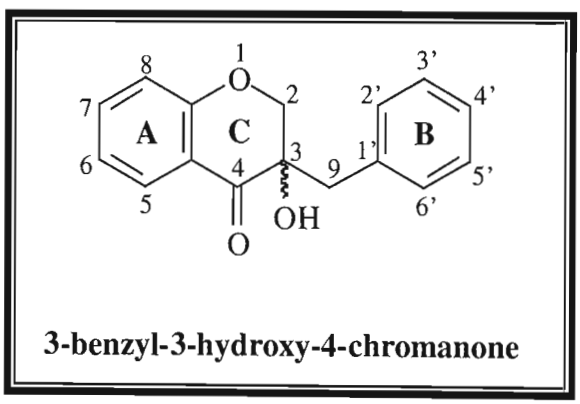



Figure 1.12. The structure of a 3-benzyl-3-hydroxy-4-chromanone homoisoflavonoid.

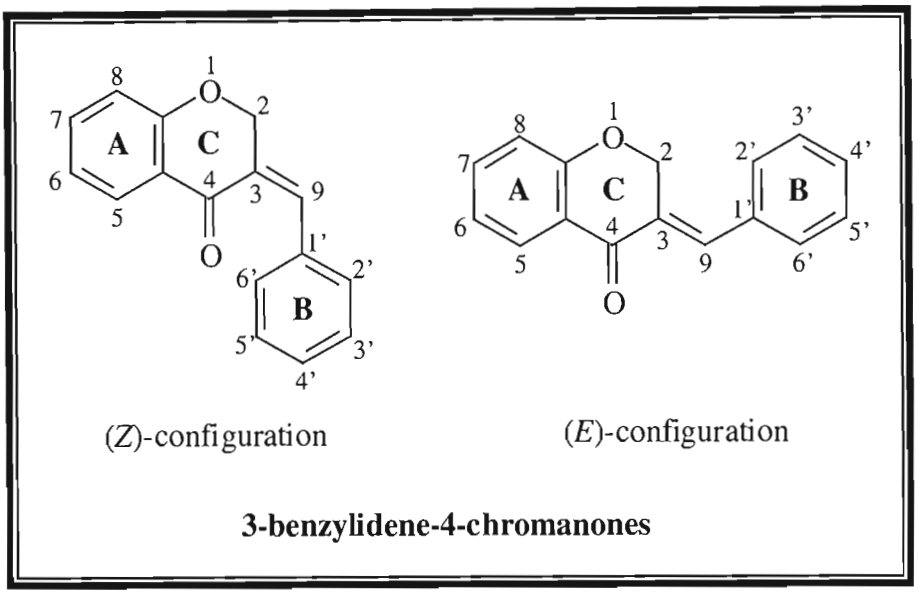



Figure 1.13. The structure of 3-benzylidene-4-chromanone homoisoflavonoids.

In addition to these three basic structural types, numerous homoisoflavonoids containing a fourth ring have been isolated[19,33]. These unusual compounds include scillascillin, which has a unique 3-*spiro*-cyclobutene system, brazilin and dracaenone (Figure 1.14). Brazilin and dracaenone have been isolated from the non-Hyacinthaceae species, *Caesalpinia spp* (Caesalpinaceae) and *Dracaena loureiri* (Dracaenaceae) respectively. The C-4 carbonyl group is lost in brazilin and rather a cyclopentene ring is observed, while in dracaenone, a cyclohexene ring is observed.

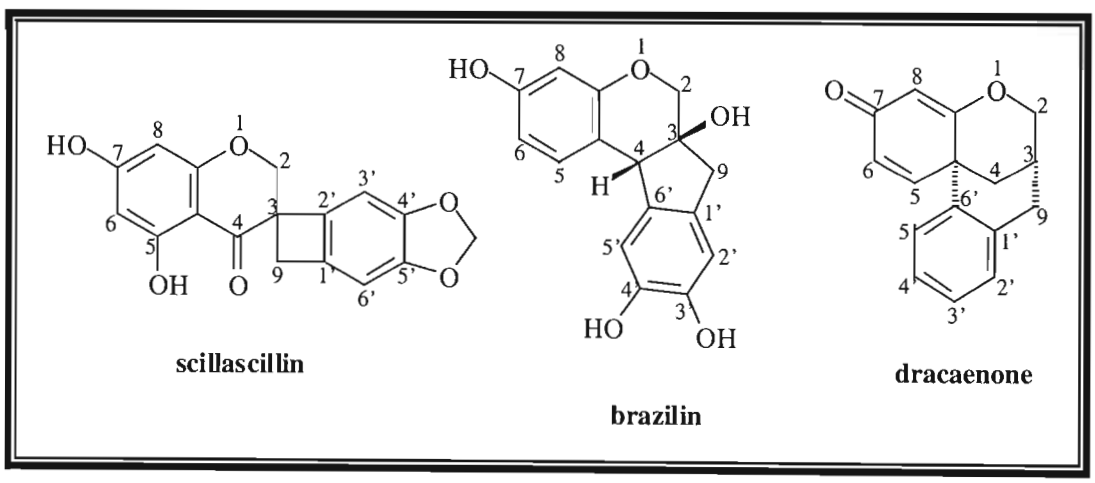



Figure 1.14. The structures of scillascillin, brazilin and dracaenone.

### 1.2.1 The Biosynthesis of Homoisoflavonoids

Aromatic compounds derived from plants can be formed by one of two biosynthetic pathways, namely, the shikimate pathway (originating from carbohydrates) or the polyketide pathway (originating from acetyl and malonyl coenzyme A units)[34-36]. Homoisoflavonoids are of mixed origin and the biosynthesis involves both of the pathways, with ring B being shikimate-derived and ring A polyketide-derived[32]. This is shown in Figure 1.15.

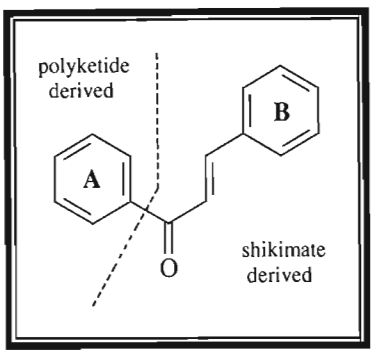
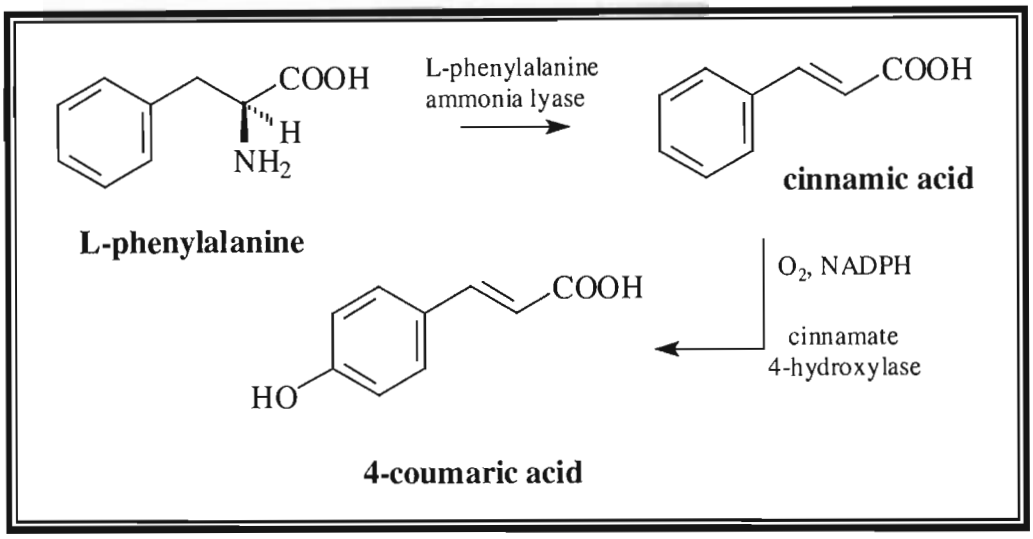


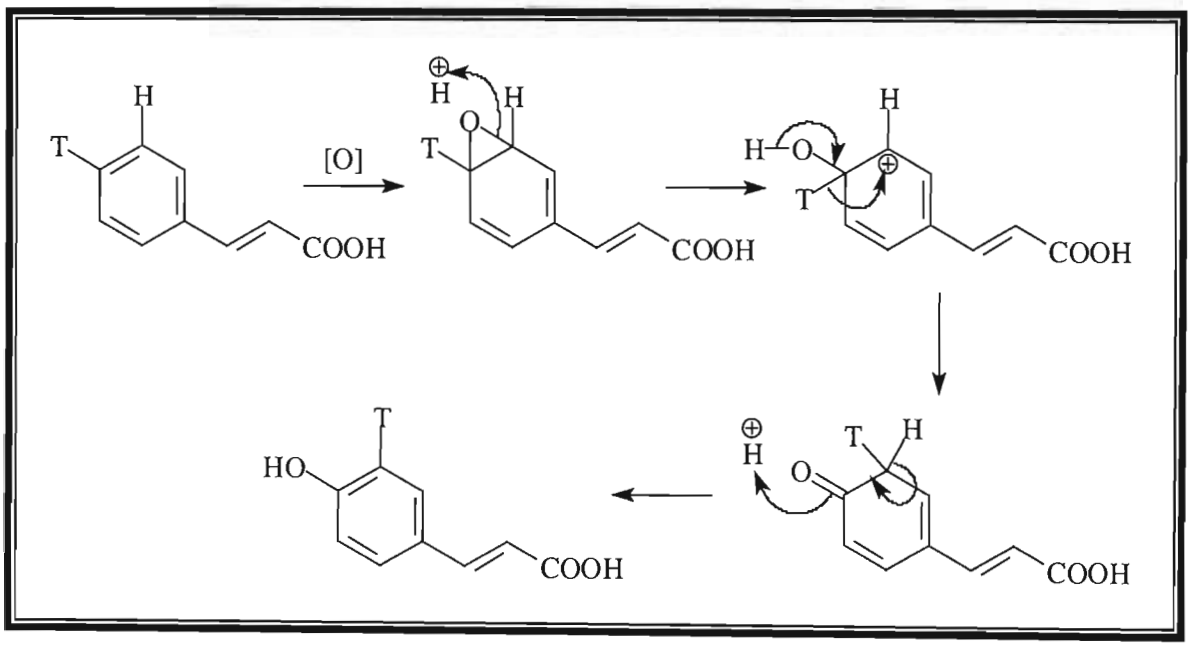

Figure 1.15. The origin of the A and B rings.

An important stage in the biosynthesis of homoisoflavonoids is the formation of a chalcone as chalcones are known to be the direct precursors of homoisoflavonoids. This is achieved in the following way: shikimate derived L-phenylalanine is converted to cinnamic acid in a deamination reaction catalysed by the enzyme L-phenylalanine ammonia lyase[37]. This involves the elimination of ammonia in an *anti*-periplanar fashion to yield *trans*-cinnamic acid. Hydroxylation of the cinnamic acid then occurs, mediated by the mono-oxygenase enzyme, cinnamate-4-hydroxylase, which results in the formation of 4-coumaric acid (Scheme 1.1).



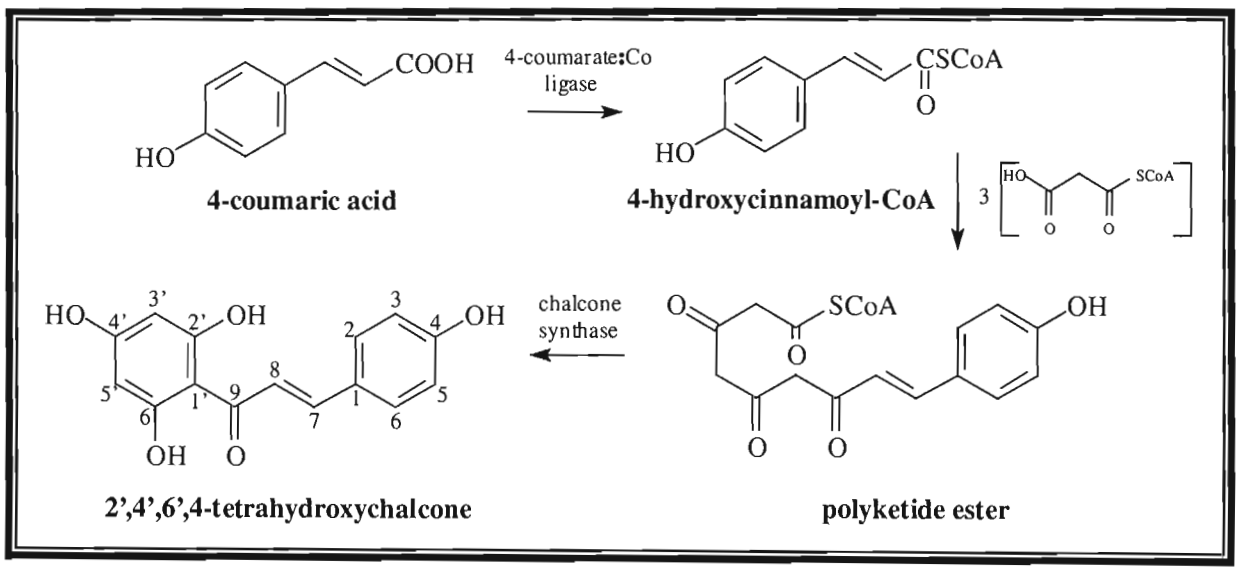

Scheme 1.1. The formation of 4-coumaric acid[38].

This reaction mechanism involves a hydrogen shift called the NIH shift (Scheme 1.2). This shift was first discovered at the National Institute of Health in Bethesda, hence the phenomenon's name, and was established using tritium labelling experiments[36]. This mechanism involves the replacement of the proton in the *para* position with a hydroxy group and the proton's subsequent migration to the *meta* position.



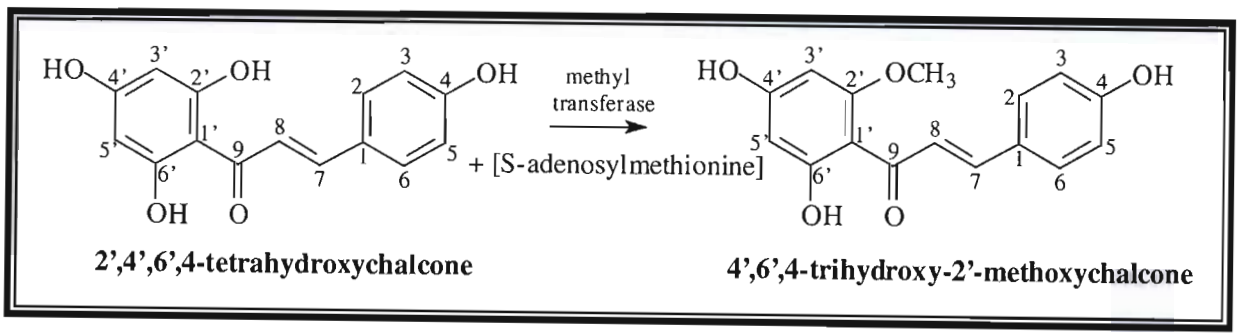

Scheme 1.2. The mechanism of the NIH shift[39].

The 4-coumaric acid formed is converted to the CoA ester, 4-hydroxycinnamoyl-CoA, by the enzyme 4-coumarate:CoA ligase. The 4-hydroxycinnamoyl-CoA molecule then combines with three malonyl CoA derived acetate units to give the polyketide ester. This resultant ester then cyclises *via* a Claisen type condensation to yield the tetrahydroxychalcone. This reaction is catalysed by the enzyme chalcone synthase[36-38] (Scheme 1.3).



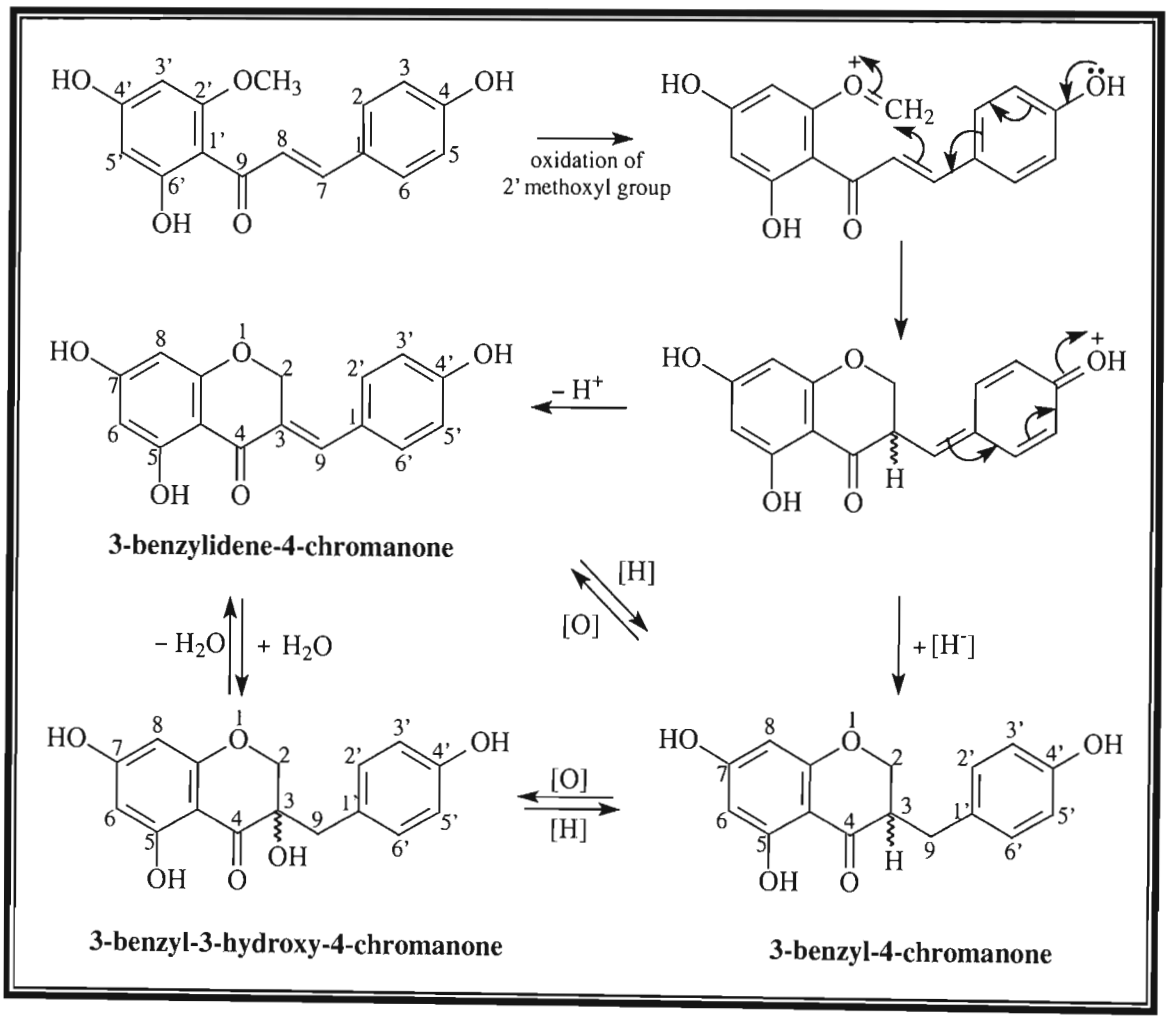

Scheme 1.3. The formation of the tetrahydroxychalcone[38].

A common methylating agent in living systems is methionine, probably as S-adenosylmethionine, which provides the tetrahydroxychalcone with an additional methyl group forming 4,4',6'-trihydroxy-2'-methoxychalcone (Scheme 1.4). This reaction is mediated by a methyltransferase[32,37].




Scheme 1.4. The formation of 2'-methoxy-4',6',4-trihydroxychalcone.

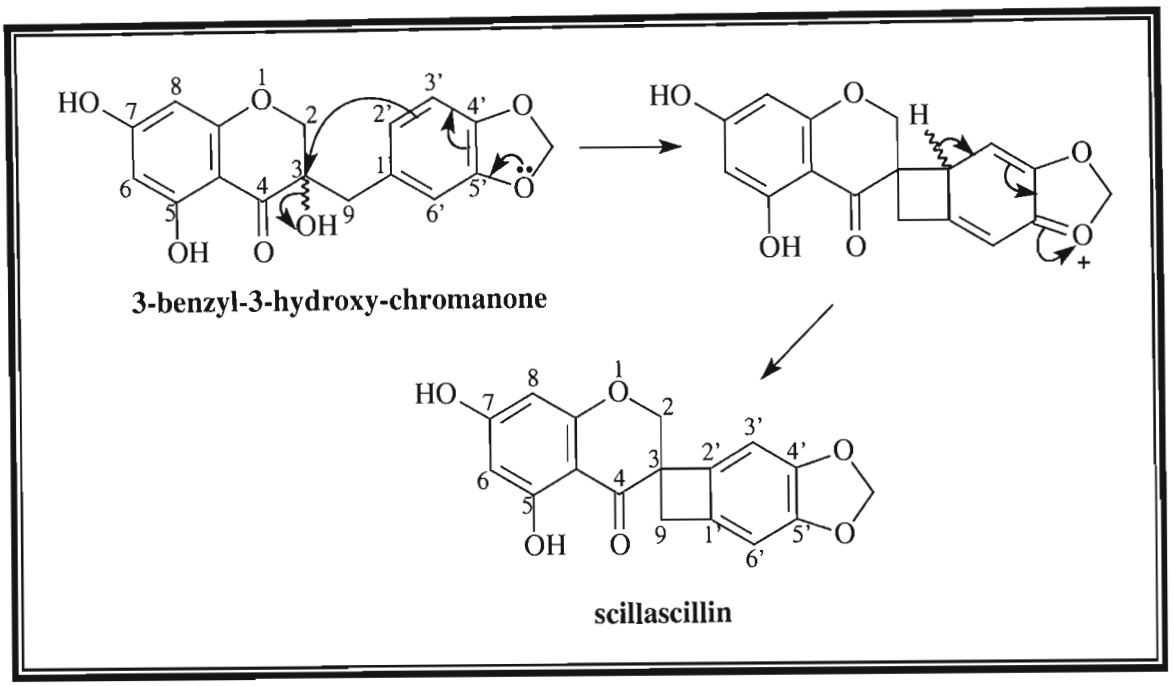
Dewick, who proposed the biosynthetic scheme for the conversion of chalcones to homoisoflavonoids[32], suggests that the methoxy group, derived from L-methionine, in the precursor 2'-methoxychalcone is oxidised, and subsequent cyclisation results in the three basic types of homoisoflavonoids (Scheme 1.5). The 3-benzyl-4-chromanone types are produced by the addition of a hydride ion, while the loss of a proton leads to the formation of the 3-benzylidene-4-chromanone types. 3-Benzyl-3-hydroxy-4-chromanones are either formed by the addition of water to a 3-benzylidene-4-chromanone type homoisoflavonoid or by the oxidation of the C-3 position of a 3-benzyl-4-chromanone type homoisoflavonoid.



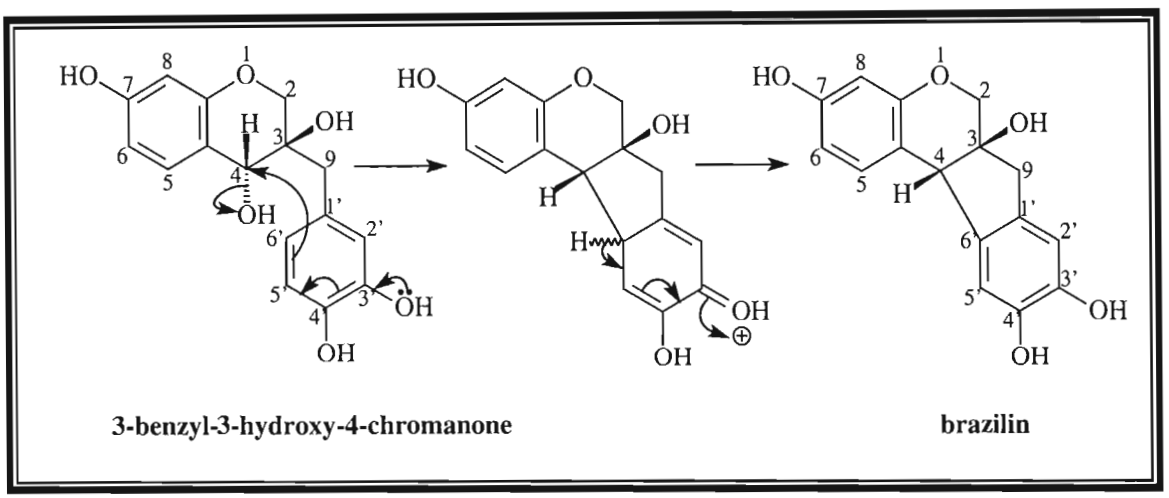

Scheme 1.5. The proposed biosynthetic routes to the homoisoflavonoids[32].

The formation of scillascillin, brazilin and dracaenone-type compounds can be explained by more complex mechanisms. Dewick[32], Bhandari[37] and co-workers have proposed some of these mechanisms. Scillascillin and brazilin are thought to form

from activated 3-benzyl-3-hydroxy-4-chromanone precursors, which cyclise to form a cyclobutene ring in scillascillin[32] (Scheme 1.6) and a cyclopentene ring in the case of brazilin[32] (Scheme 1.7).

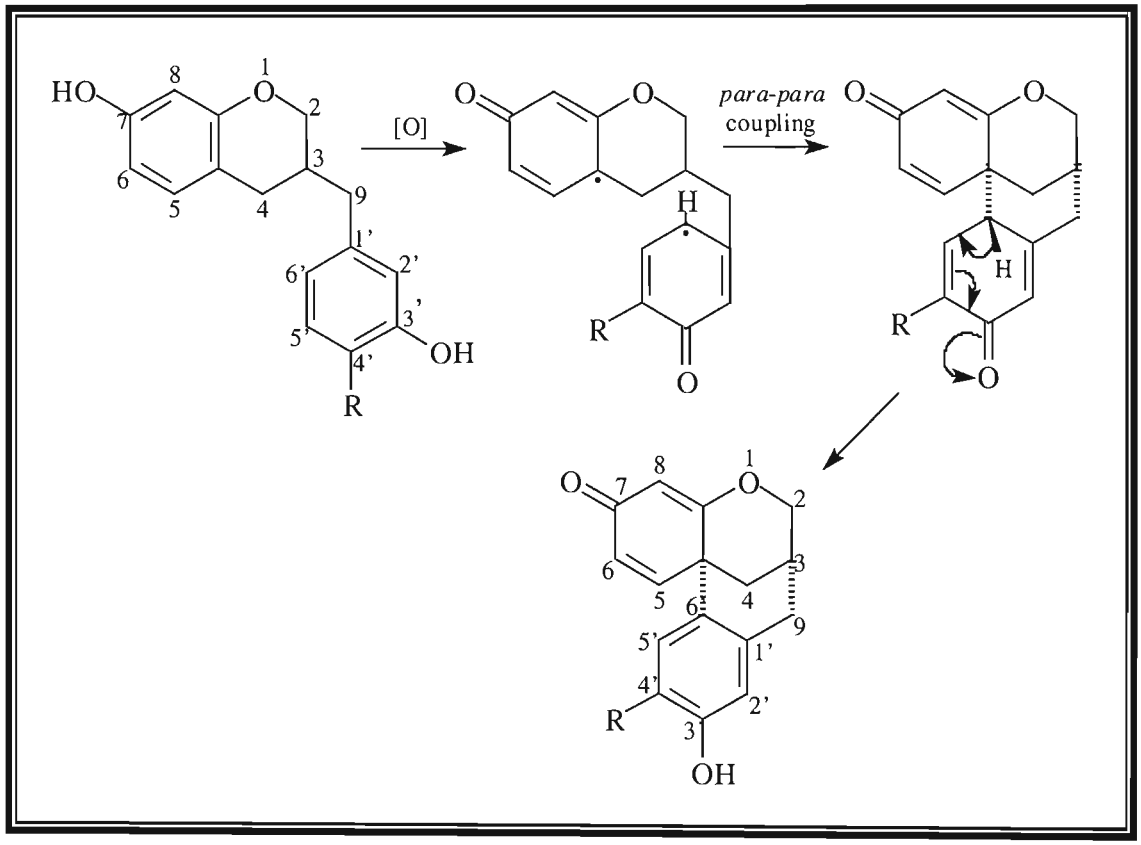



Scheme 1.6. The proposed formation of scillascillin[32].




Scheme 1.7. The proposed formation of brazilin[32].

Dracaenone-type compounds, on the other hand, are thought to be derived from 3-benzylchroman-type compounds, which cyclise *via para-para* phenolic oxidative coupling to yield a tetracyclic intermediate[40]. Dienone-phenol rearrangement of this intermediate affords the dracaenone skeleton, as shown in Scheme 1.8.




Scheme 1.8. The biosynthesis of dracaenone-type compounds[40].

### 1.2.2 The Biological Activities of Homoisoflavonoids

Homoisoflavonoids have many diverse biological activities and considerable research is still necessary in order to appreciate them fully. Within the plant, these compounds are concentrated in the waxy layers between the storage leaves of the bulbs[33] and this specific distribution has prompted the investigation of their biological significance. From previous studies these compound have been shown to possess anti-mutagenic, anti-inflammatory, anti-bacterial and analgesic properties[33,40-42], which could explain their widespread ethnobotanical usage by traditional healers.

An investigation of the roots of *Hoffmanseggia intricata*[41] (Fabaceae) showed two homoisoflavonoids which had antimutagenic and antitoxic properties. These compounds, identified as intricatin (8-hydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone) and intricatinol (7,8-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone), both shown in figure 1.16, displayed varying toxicity inhibition of 2-amino-anthracene, acetylaminofluorine and ethylmethanesulphonate towards *Salmonella typhimurium*[41]. Intricatinol, the dihydroxy analogue, was found to be broadly active and it was postulated that the presence of a dihydroxy group in ring A could lead to increased potency of the homoisoflavonoid[41]. Investigations of a crude extract from the bulbs of *Muscari comosum*[42] (Hyacinthaceae), revealed extensive anti-inflammatory properties with the inhibition of croton oil-induced dermatitis in the mouse ear. The inhibitory effect of this administration was comparable to the potent anti-inflammatory drug, indomethacin[42]. In general, phenolic compounds are known for their antibiotic properties.

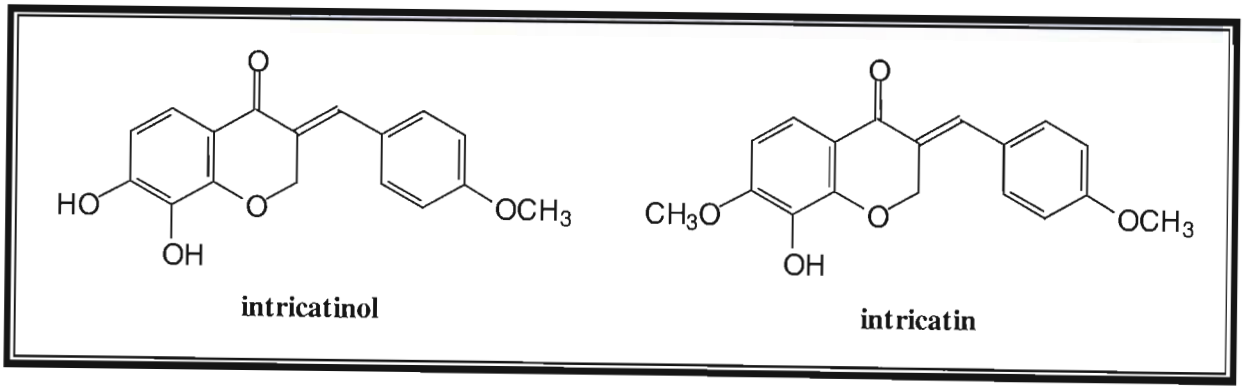



Figure 1.16. The structures of intricatin and intricatinol.

## 1.3 Introduction to Triterpenoids

The terpenes are amongst the most widespread groups of natural products and are derived from a common biosynthetic pathway based on mevalonate as parent. Terpenes are typically found in higher plants, lower animals (coelenterates, molluscs and arthropods), fungi, algae and lichens[4]. Members of this class also form the basis of mammalian sex hormones, pheromones and plant hormones.

Despite being structurally diverse, terpenoids are composed of a single unifying feature, the $C_5$ isoprene unit. In 1953 Ruzicka[43] proposed the "Biogenetic Isoprene Rule" which stated that all terpenoids are formed by the head-to-tail linkage of isoprene units. This rule was later improved and extended to include different types of terpenoids derived from a single parent compound unique to that class i.e. geraniol ($C_{10}$), farnesol ($C_{15}$), geranylgeraniol ($C_{20}$), and squalene ($C_{30}$) (Figure 1.17), involving tail-to-tail or head-to-tail cyclisation and/or rearrangement[4].

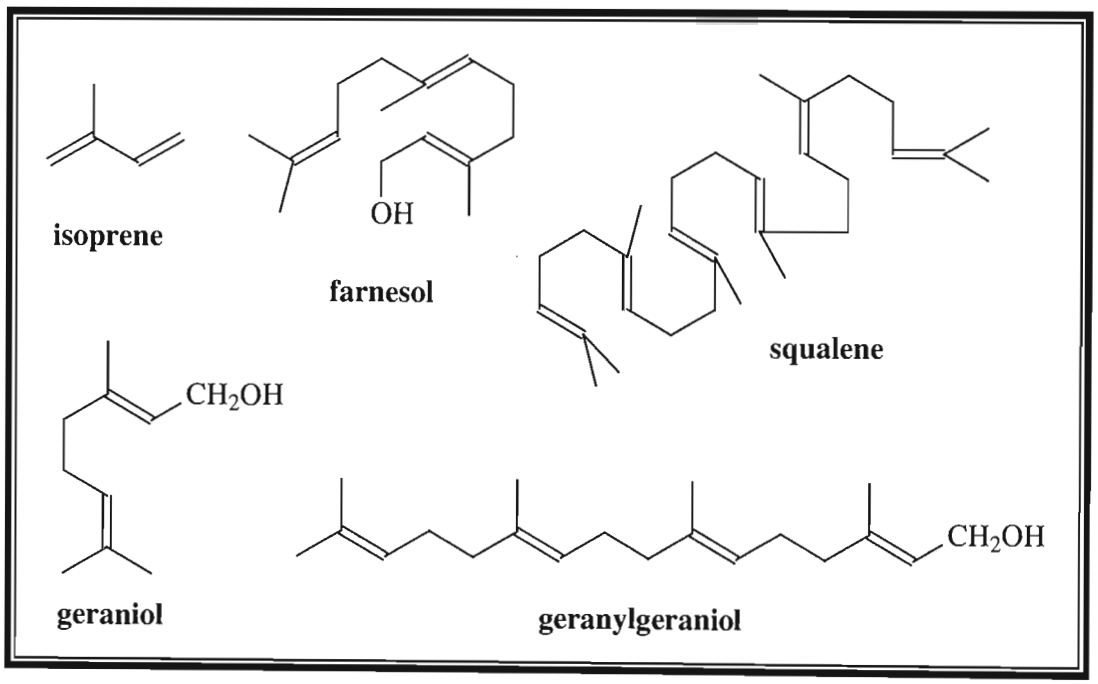
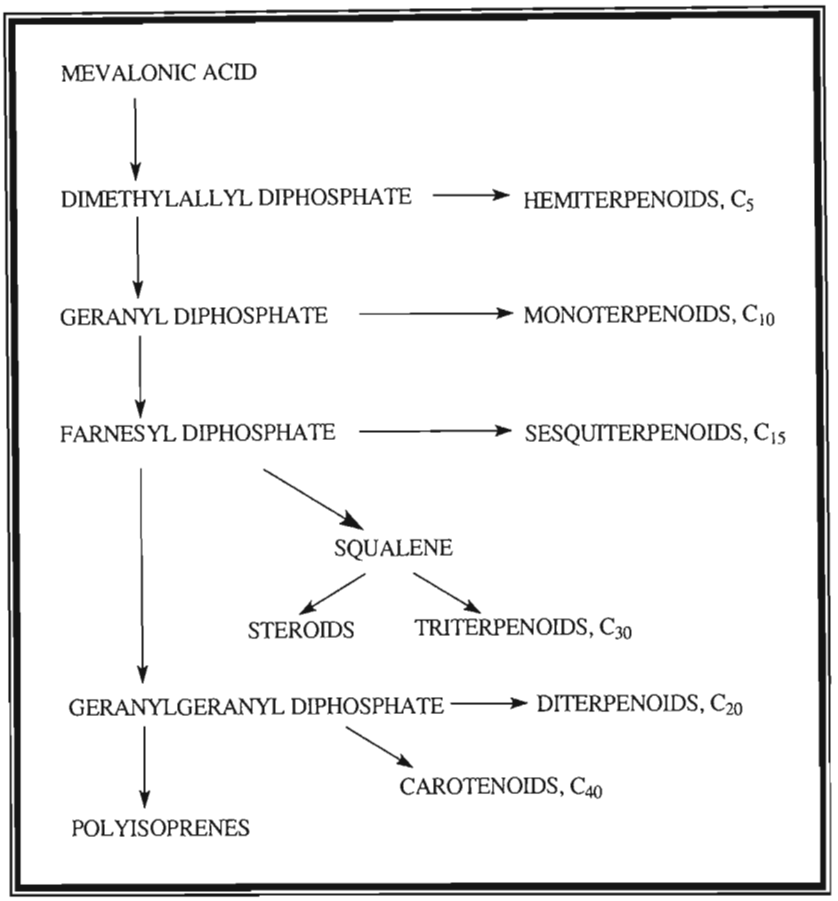



Figure 1.17. Some examples of the structures of terpenoids.

Terpenoids are grouped according to the number of isoprene units they contain i.e. hemiterpenoids, $C_5$; monoterpenoids, $C_{10}$; sesquiterpenoids, $C_{15}$; diterpenoids, $C_{20}$; triterpenoids, $C_{30}$; and carotenoids, $C_{40}$ (Scheme 1.9).




Scheme 1.9. Terpenoid groups derived from mevalonic acid[44].

However, isoprene itself is not the biogenetic precursor of terpenoids and is only rarely found in nature[45]. The biochemically active isoprene units are the diphosphate esters, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP)[4] (Figure 1.18)

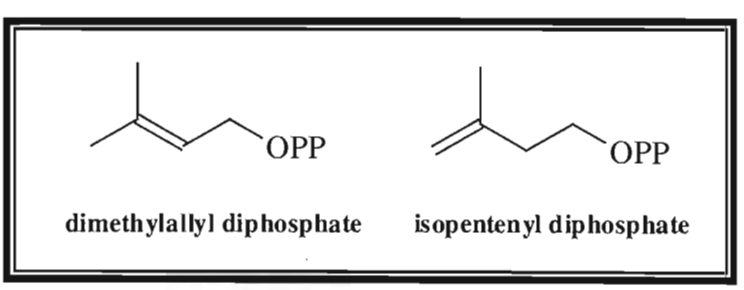
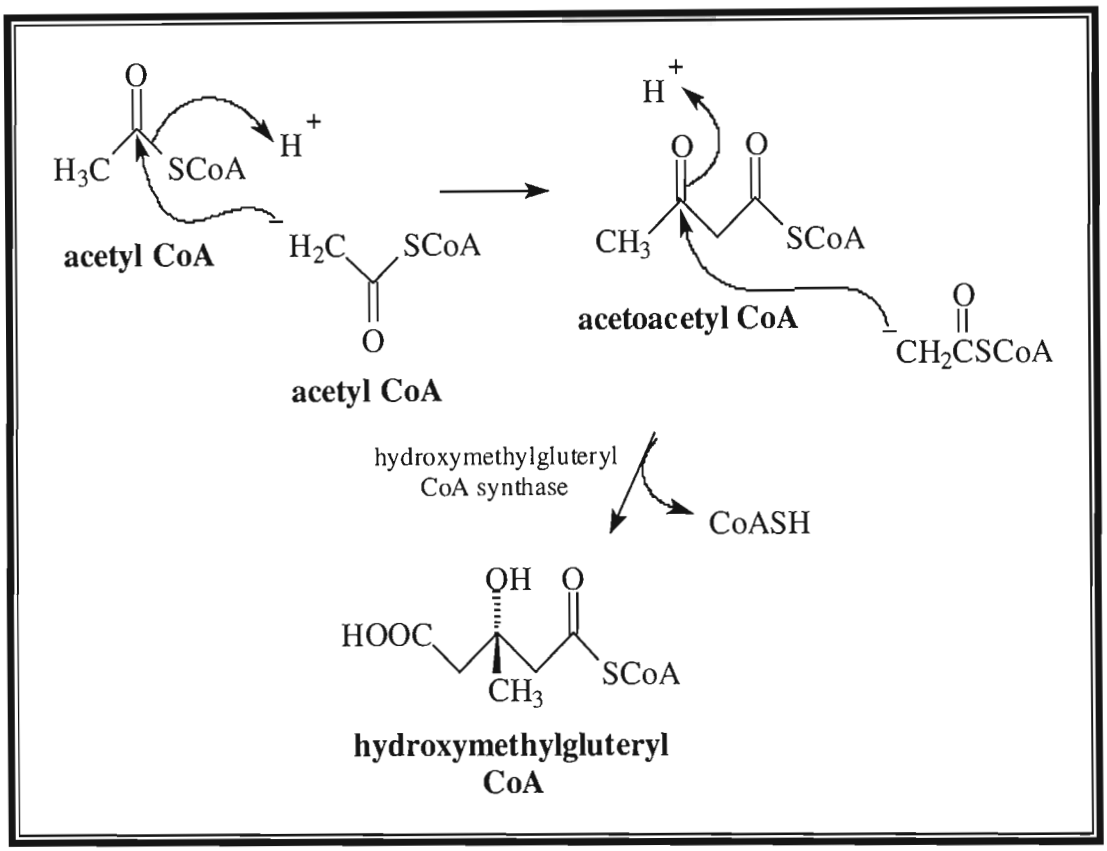



Figure 1.18. The biochemically active isoprene units.

### 1.3.1 The Biosynthesis of Triterpenoids

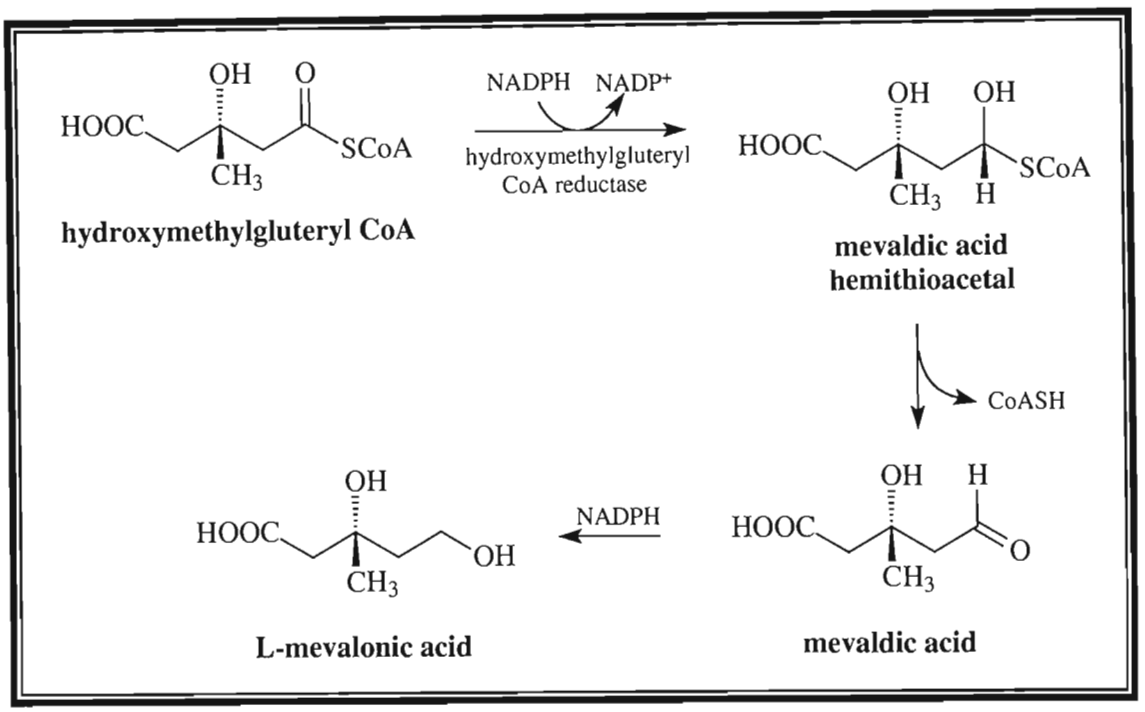
The triterpenoids form a very diverse group of naturally occurring compounds, which are widely distributed throughout the Plant Kingdom. The starting material for the synthesis of triterpenoids is acetyl CoA (Scheme 1.10).




Scheme 1.10. The formation of hydroxymethylgluteryl CoA[4].

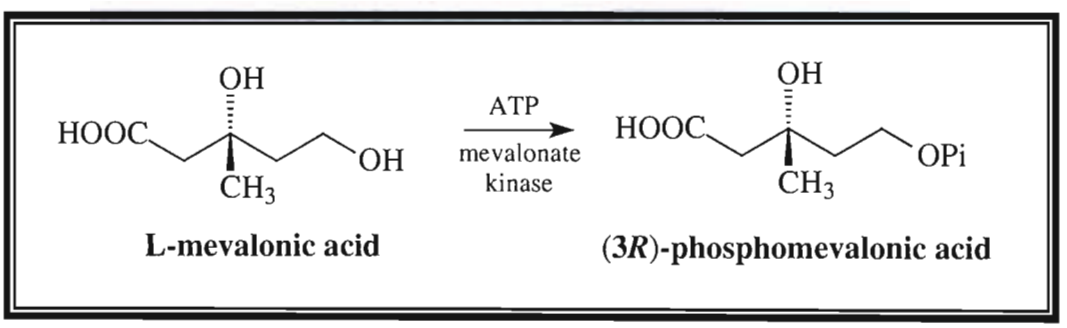
Two molecules of acetyl CoA combine in a Claisen condensation to form acetoacetyl CoA. A third molecule of acetyl CoA is incorporated *via* a stereospecific aldol addition[4] to form (3*S*)-3-hydroxy-3-methylgluteryl CoA (HMG-CoA), these reactions being catalysed by acetyl CoA acetyltransferase and HMG-CoA synthase, respectively. Acetoacetyl CoA is more acidic than the third acetyl CoA molecule and thus serves as the nucleophile. NADPH then reduces the thio ester, HMG-CoA *via* the hemithioacetal to the aldehyde, (3*R*)-mevaldic acid, which is in turn reduced by NADPH to (3*R*)-mevalonic acid in higher plants (Scheme 1.11), the enzyme catalysing this reaction being hydroxymethylgluteryl CoA reductase[46]. The

conversion of HMG-CoA into mevalonic acid is irreversible, and hence, mevalonic acid has no metabolic future except in terpene formation[45].




Scheme 1.11. The formation of L-mevalonic acid[4,46].

Mevalonic acid is the primary precursor of all the terpenoids biosynthesised by plants. The catalytic phosphorylation of mevalonate to form (3*R*)-phosphomevalonic acid (Scheme 1.12) occurs *via* the enzyme mevalonate kinase and is ATP dependent. Only the *R* form is utilised by organisms for producing terpenes, as the *S* form is metabolically inert[43].




Scheme 1.12. The phosphorylation of mevalonic acid[4,46].

This phosphorylation is a primary point at which control of terpenoid biosynthesis operates, as mevalonic acid kinase activity has been found to be inhibited by such products of the acetate-mevalonate pathway as geranyl, farnesyl, geranylgeranyl and phytyl diphosphates[44] (Figure 1.19).

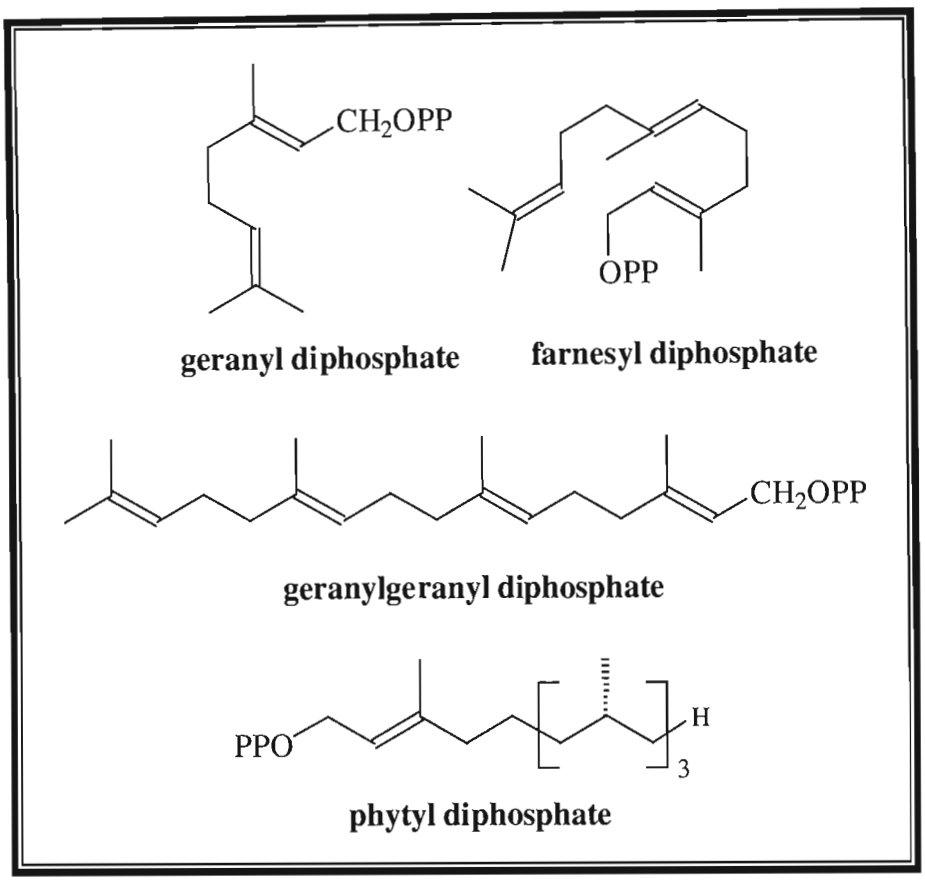
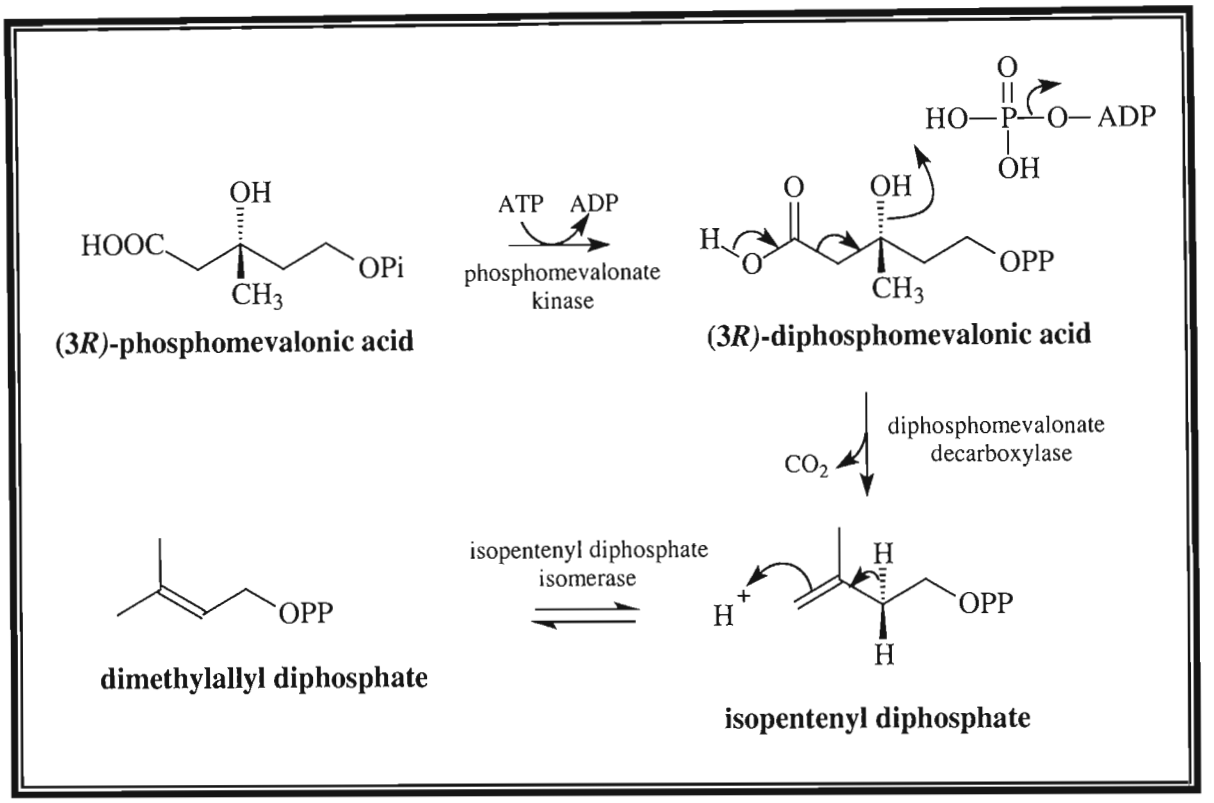



Figure 1.19. The inhibiting products of the acetate mevalonate pathway.

The conversion of (3*R*)-phosphomevalonic acid into (3*R*)-diphosphomevalonic acid (Scheme 1.13) is catalysed by phosphomevalonate kinase. A concerted decarboxylation-dehydration of (3*R*)-diphosphomevalonic acid gives isopentenyl diphosphate, the biogenic isoprene unit, and is catalysed by the enzyme diphosphomevalonate decarboxylase, a highly stereospecific enzyme involved in the *trans* elimination of the carboxyl and hydroxyl groups[44].

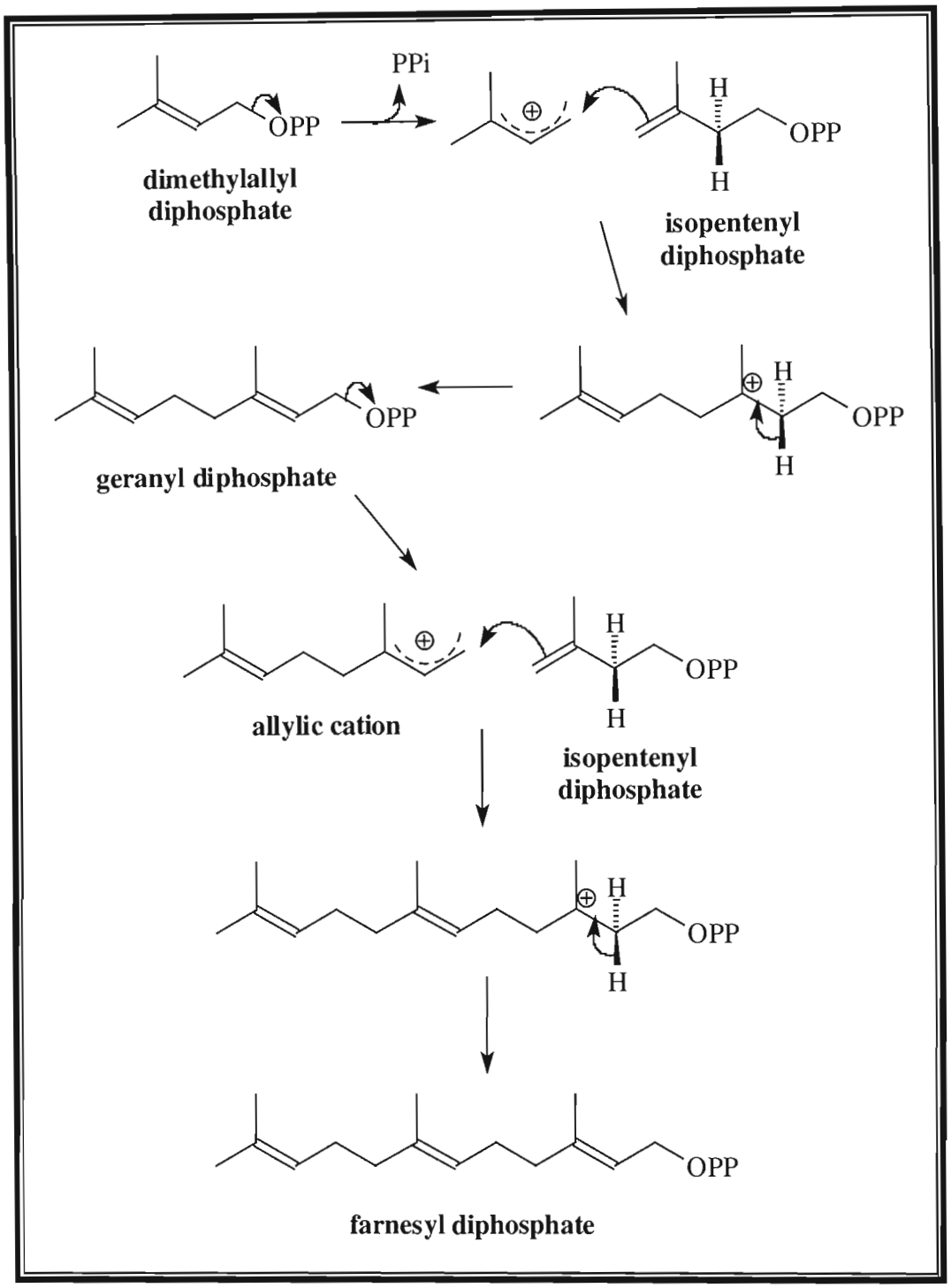
The interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) is an equilibrium reaction catalysed by the enzyme isopentenyl-diphosphate-Δ-isomerase. Although the isomerisation is reversible, the equilibrium

lies heavily on the side of DMAPP[4]. This generates a reactive electrophilic DMAPP and a nucleophilic IPP, due to the terminal double bond.



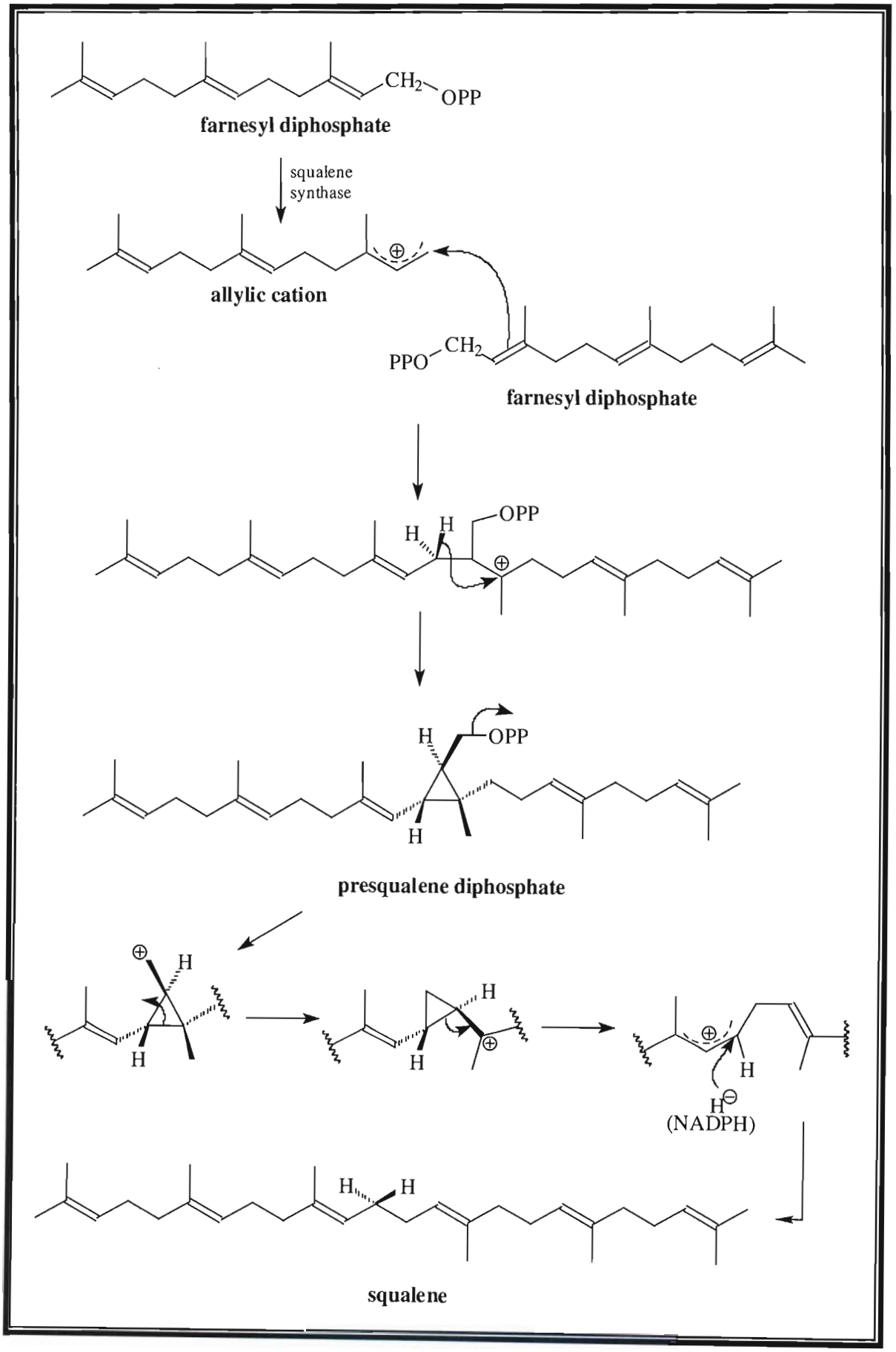

Scheme 1.13. The formation and isomerisation of isopentenyl diphosphate[4,46].

Isopentenyl diphosphate condenses with dimethylallyl diphosphate, *via* the enzyme prenyl transferase to yield geranyl diphosphate, which, in turn, condenses with isopentenyl diphosphate to form farnesyl diphosphate (Scheme 1.14). Up till this point the cytosol has been the site of all the reactions, with the exception of those involving HMG-CoA reductase[46].



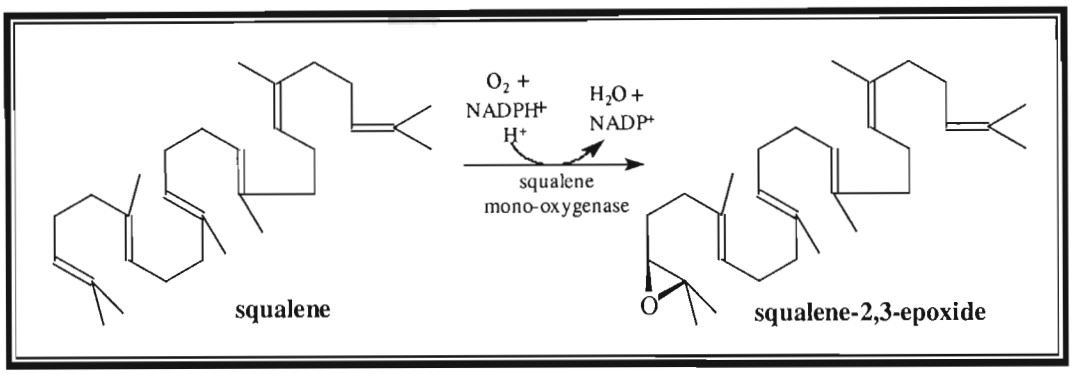

Scheme 1.14. The formation of farnesyl diphosphate[46].

The NADPH-dependant enzyme farnesyl transferase, bound to membranes of the endoplasmic reticulum, joins two molecules of farnesyl diphosphate, tail-to-tail, to give presqualene diphosphate (Scheme 1.15), which then undergoes diphosphate elimination and rearrangement to yield squalene[46]. This reaction is catalysed by squalene synthase and is NADPH dependent[44].



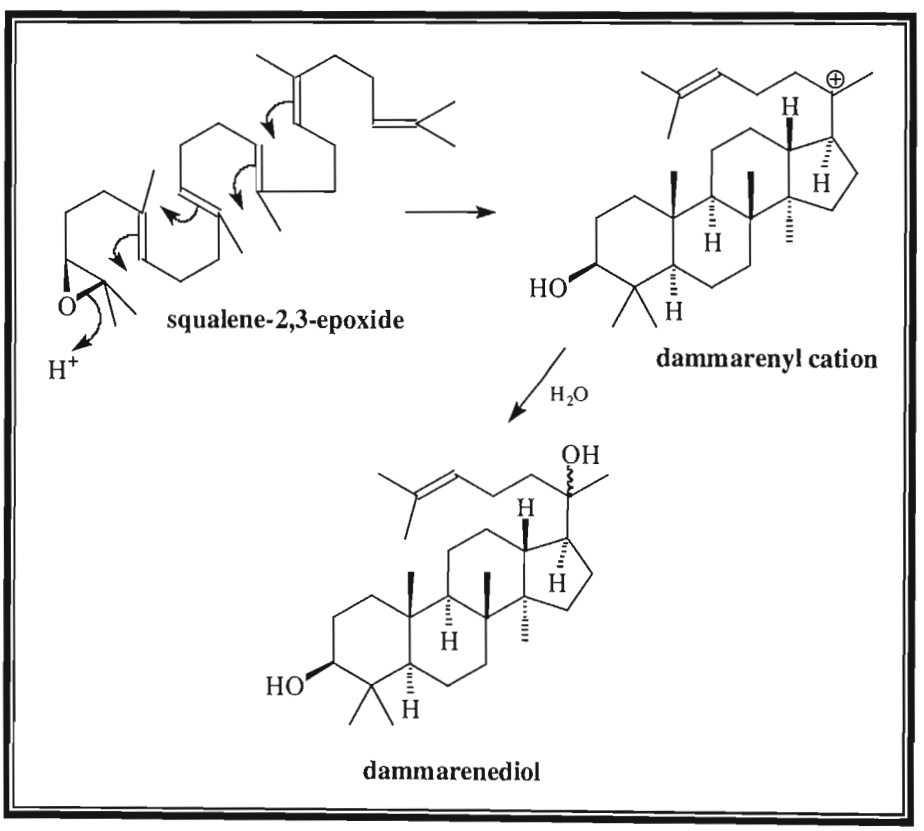

Scheme 1.15. The formation of squalene[4].

Cyclisation of squalene occurs *via* the intermediate squalene-2,3-oxide, catalysed by squalene mono-oxygenase, which forms from a reaction involving $O_2$ and NADPH cofactors[4,44,47] (Scheme 1.16).



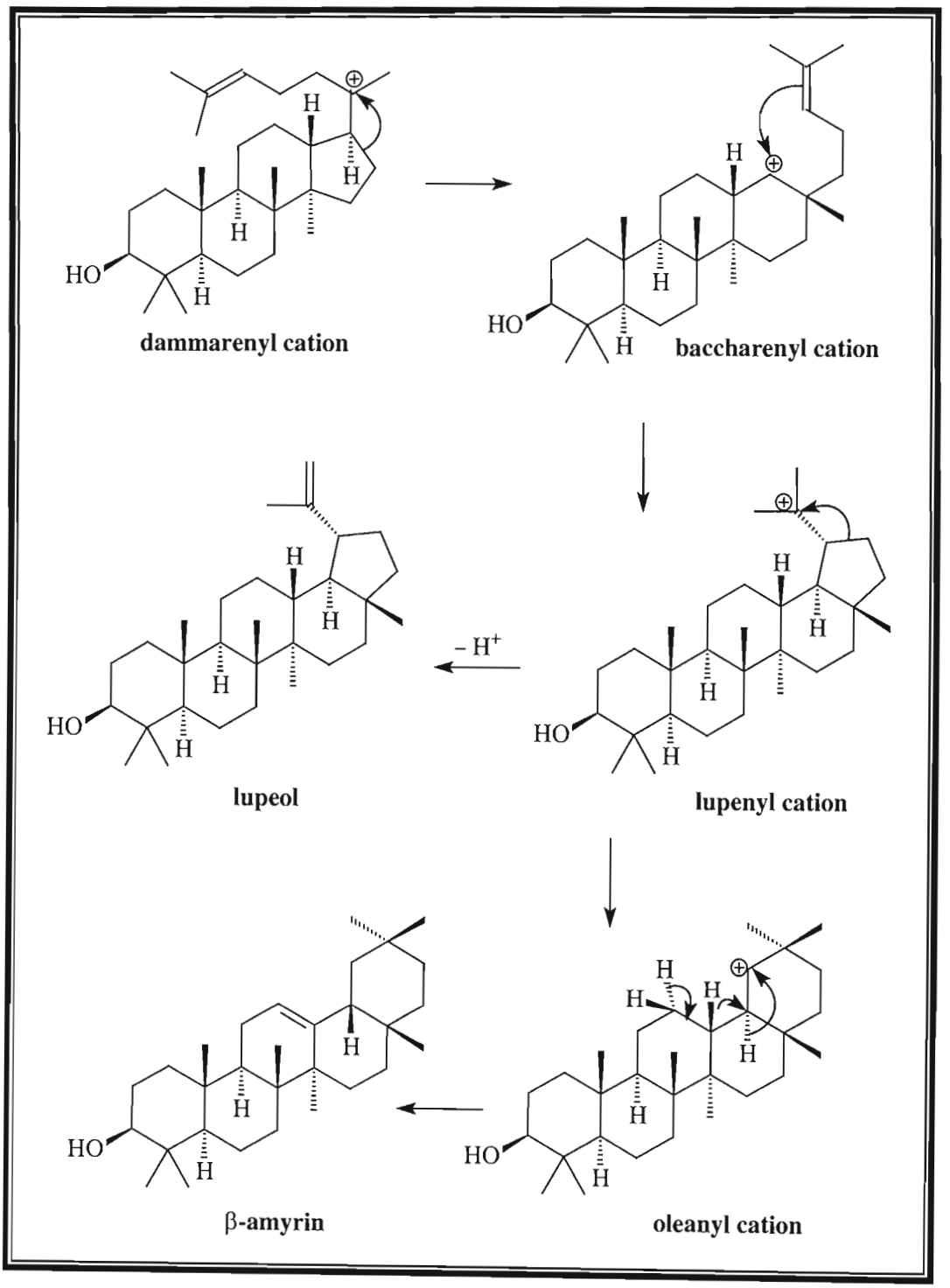

Scheme 1.16. The formation of the squalene-2,3-epoxide intermediate[46].

One of the simplest cyclisations of squalene-2,3-epoxide, that forming the dammarene derivative, dammarenediol, *via* the dammarenyl cation, is shown in scheme 1.17. Protonation of the epoxide group initiates a series of 1,2-shifts of methyl groups and hydride ions.



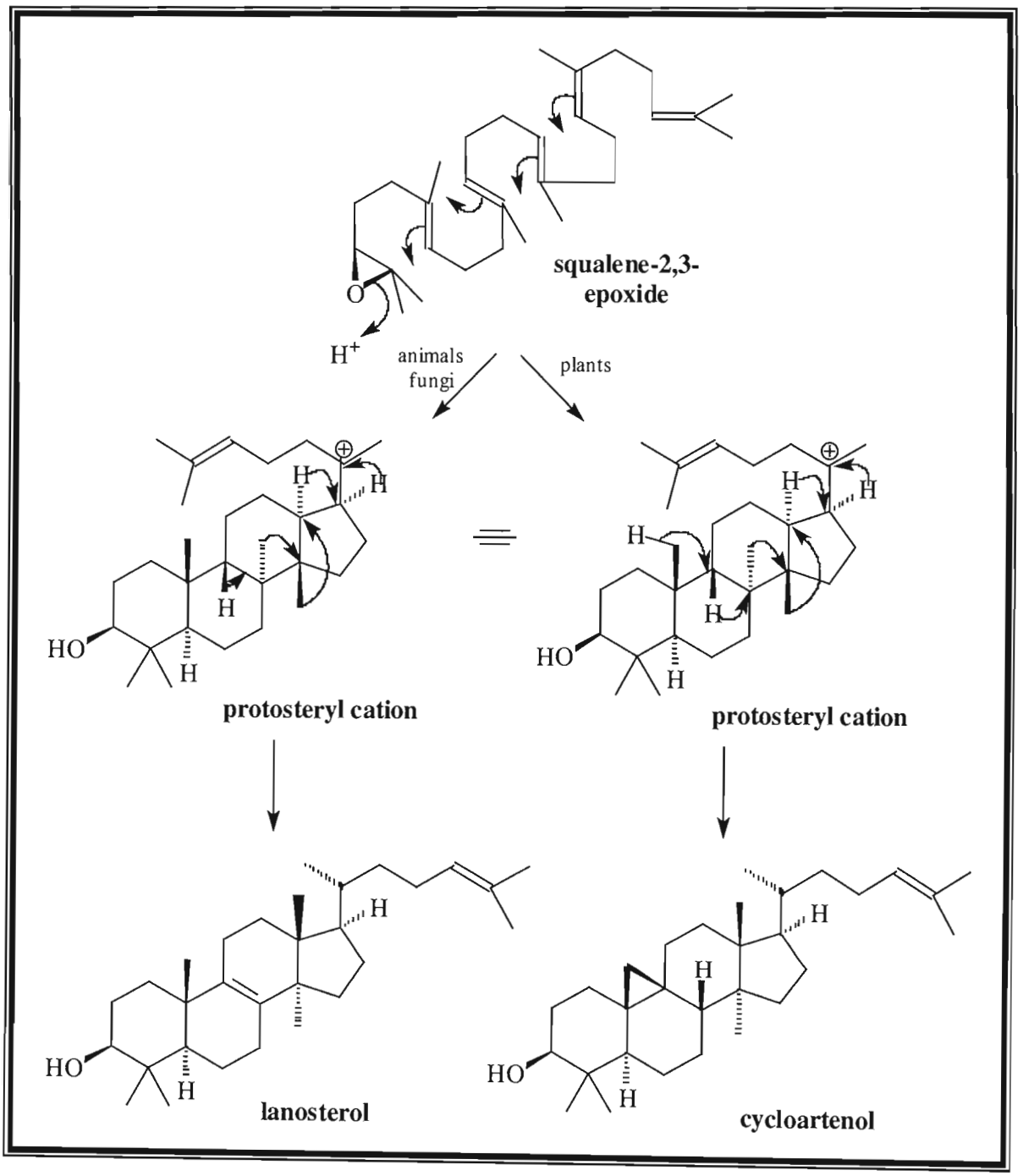

Scheme 1.17. The cyclisation of squalene-2,3-epoxide[4].

The pentacyclic triterpenoids, such as lupeol and β-amyrin are biosynthesised from a tetracyclic precursor of the dammarene type by a rearrangement resulting in the expansion of ring D and the formation of ring E[4] (Scheme 1.18).




Scheme 1.18. The rearrangement of the dammarenyl cation[4].

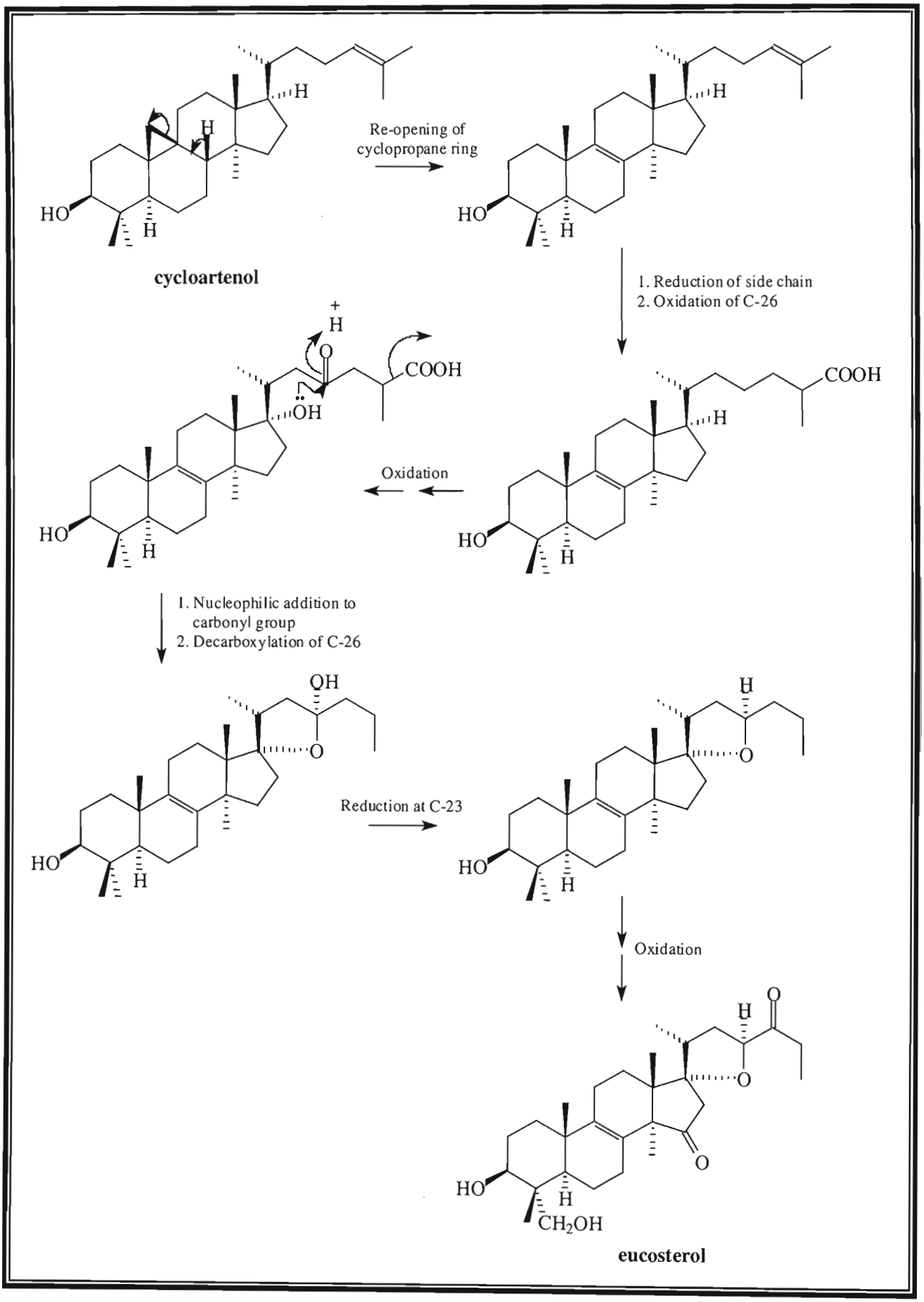
In animals, squalene-2,3-epoxide, *via* the protosteryl cation intermediate, can cyclise to form the lanosterol family of tetracyclic triterpenoids (Scheme 1.19). Lanosterol is a typical triterpenoid found in animals and is the precursor for cholesterol biosynthesis.




Scheme 1.19. The formation of lanosterol and cycloartenol[4].

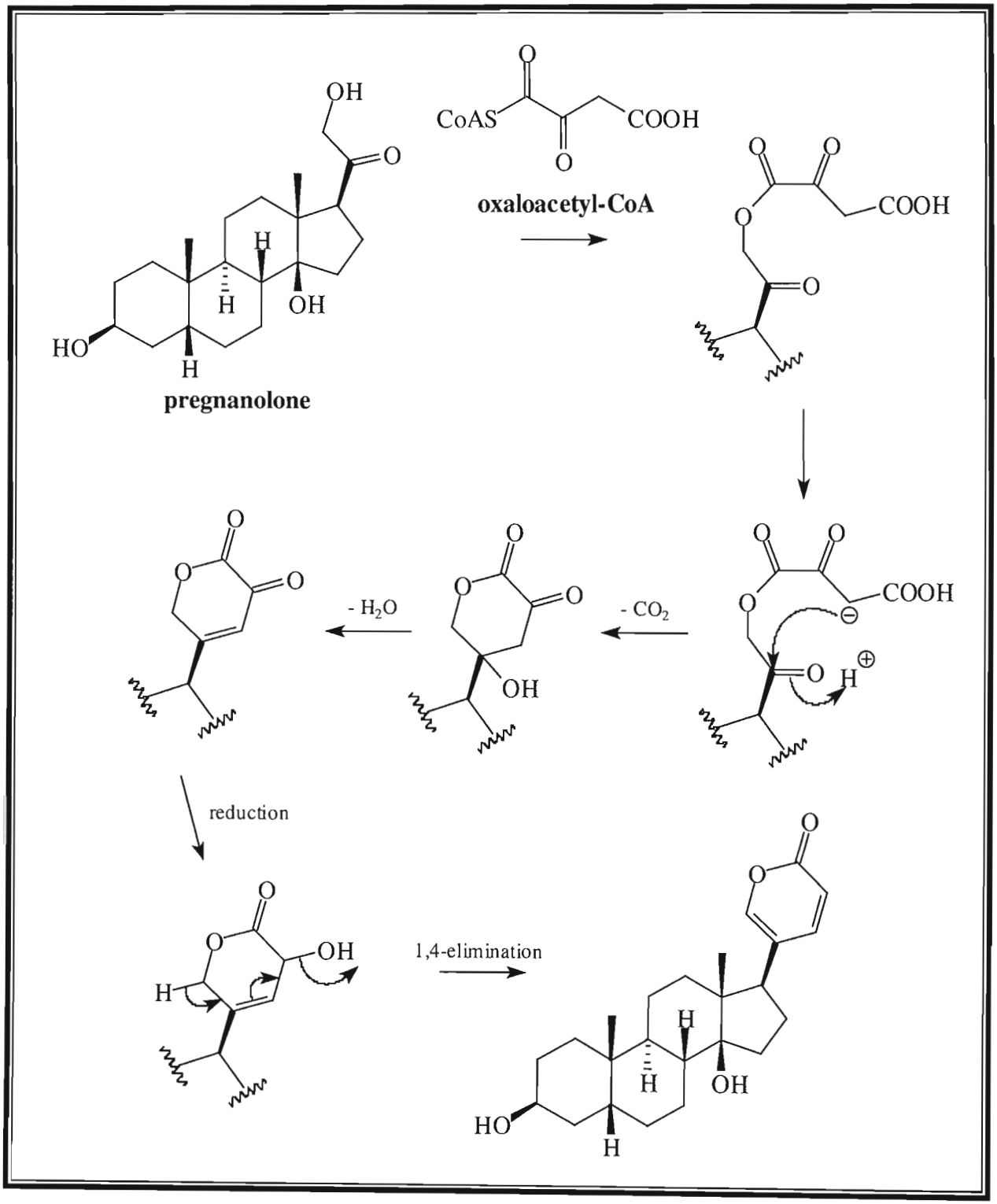
Cycloartenol, a cyclisation product of squalene in plants, is similarly formed *via* squalene-epoxide[4,44,45] (Scheme 1.19). Cycloartenol contains a cyclopropane ring generated by inclusion of the methyl group at C-10. The hydrogen at C-9 migrates to C-8 and the carbocation so formed is quenched by cyclopropane formation and loss of one of the methyl protons.

Through its conversion to 24-methylenecycloartenol or cholesterol, cycloartenol is the precursor of a wide range of compounds collectively termed the phytosterols, which contain varying numbers of carbon atoms. Lanosterol, however, is not implicated in the biogenesis of phytosterols[47]. However for many of the plant sterols the cyclopropane ring of cycloartane has to be re-opened. Manipulation of the cycloartane skeleton results in the formation of the eucosterol class of compounds (Scheme 1.20). Eucosterol is a spirocyclic nortriterpenoid, which has a basic lanosterol triterpernoid skeleton where the side chain has been modified to produce a hemiketal[4]. This hemiketal is converted to an ether by reduction. Variations in these compounds are due to different degrees of oxygenation in the aglycone and differing combinations of sugars in the glycosides.




Scheme 1.20. The proposed formation of eucosterol[48].

Bufadienolides are cardiac glycosides which are characterised by a steroidal aglycone coupled to a sugar group. They are characterised by having a β-substituted, doubly unsaturated, six-membered lactone ring (pentadienolide) at C-17[4]. Bufadienolides are derived from the addition of an oxaloacetyl-CoA molecule to a pregnanolone[4], such as 5β-pregnan-3β,14β,21-triol-20-one as shown in scheme 1.21.




Scheme 1.21. The biosynthesis of a bufadienolide from a pregnanolone precursor[4].

The large number of bufadienolides that have been isolated is primarily due to the extensive variation in the oxygenation pattern of the aglycone, and the variation in the sugars attached at the C-3 position. Generally, tertiary methyl groups are present at C-10 and C-13, however, occasionally an aldehyde group replaces the methyl group at C-10[4]. In most cases a β-hydroxyl group is present at C-14, while a $\Delta^4$-double bond is also common[4].

Most natural triterpenoids and steroids are oxygenated at C-3, the original epoxide oxygen from squalene-2,3-oxide, usually as alcohols but some as ketones. Triterpenoids are distinguished from each other by unsaturations, additional hydroxyl groups, and frequently carbonyl groups[49].

### 1.3.2 The Biological Activities of Triterpenoids

In plants, the pentacyclic triterpenoids often exist as glycosides, the resulting compounds are termed saponins. Saponins, even at low concentrations, produce frothing in aqueous solutions because they have soap-like properties. Saponins are known to be highly toxic to cold-blooded animals and are lethal if injected as they haemolyse the red blood corpuscles. Medicinally these compounds are used as anti-inflammatory agents, antibiotics, fungicides, and, most importantly, molluscicides[44].

A large range of cholestane glycosides have been isolated from poisonous *Ornithogalum thyrsoides* and *O. saundersiae* species[50]. These steroidal compounds have been reported to exhibit potent cytostatic activities both *in vitro* (animal and human cell lines) and *in vivo* (mouse leukaemia)[51-53]. Many species of *Urginea* have been identified as toxic causing cardio activity and gastro-intestinal irritation, and are of agricultural concern[54]. Links have been established between hyacinthaceous plants and deaths caused by homicidal agents and traditional medicine preparations.

The mechanism of action of bufadienolides and the cumulative toxicity of plants containing them have been extensively reviewed by Kellerman *et al.*[55]. *Digitalis*-like compounds have been detected in low quantities in mammalian tissue[4]. The compound 19-norbufalin is found in the human eye lenses and at higher concentrations if a cataract is present. The bufadienolides are believed to regulate ATPase activity[4].

Previously, plants containing cardiotonic glycosides were used as arrowhead poisons. Nowadays, they are used as heart drugs[4]. Although these drugs strengthen a weak heart, the toxic dose is extremely close to the therapeutic dose, so the optimal dosage control must be exercised[4]. Extracts of *Digitalis* are used in the successful treatment of dropsy, which is an excessive accumulation of fluid in the body tissues[4]. The extract was found to alleviate this condition by improving the blood supply to the kidneys, thereby removing the excess fluid[4].

## 1.4 Introduction to Coumarins

Coumarins are one of the most important biologically active classes of compounds of plant origin. They are made up of a benzene ring fused with a pyrone ring in a linear manner and are δ-lactones, or more specifically α-benzopyrones[56].

Coumarins can be placed into one of five groups, namely, group A (coumarins with substitution mainly on the benzene ring), group B (coumarins showing substitution only on the pyrone ring), group C (furanocoumarins), group D (3,4-benzocoumarins) and group E (pyranocoumarins)[56].

### 1.4.1 Group A Coumarins

This category consists of coumarin itself as well as umbelliferone. Other members of this group include coumarin methyl ethers or isoprenoid substituted benzene rings, especially at the 8-position[56]. An example of a coumarin belonging to group A is aurapten, a constituent of grapefruit peel and of the root barks of *Feronia elephantum* and *Aegle marmelos*. Some examples of group A coumarins are given in figure 1.20.

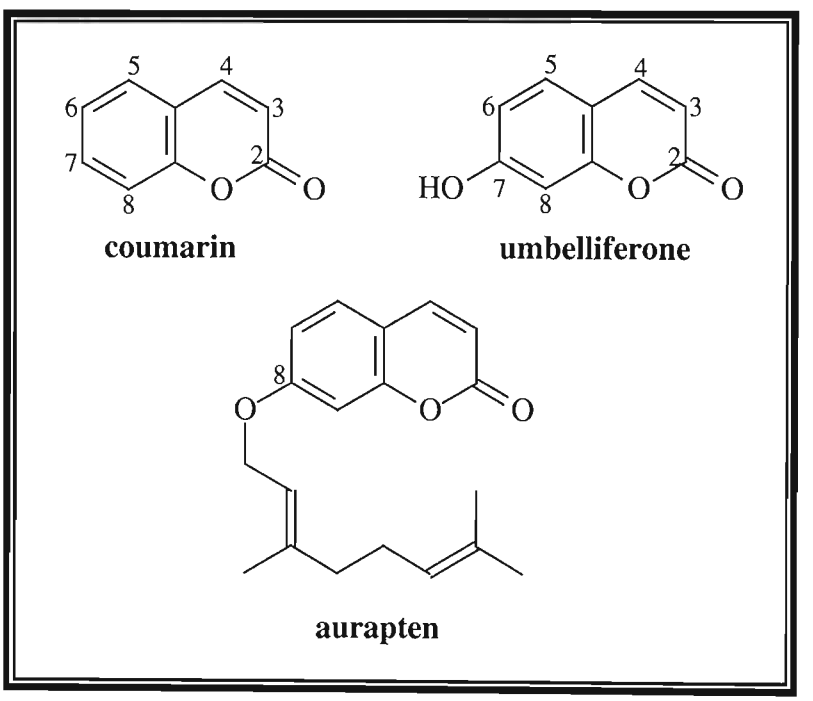



Figure 1.20. The structures of coumarin, umbelliferone and aurapten[56].

### 1.4.2 Group B Coumarins

The first phenylcoumarin to be isolated from a natural source was dalbergin, isolated from the heartwood of *Dalbergia sissoo*, which also occurs with its methyl ether[56]. This coumarin has the phenyl group attached at the 4-position. The structure of dalbergin is shown in figure 1.21.

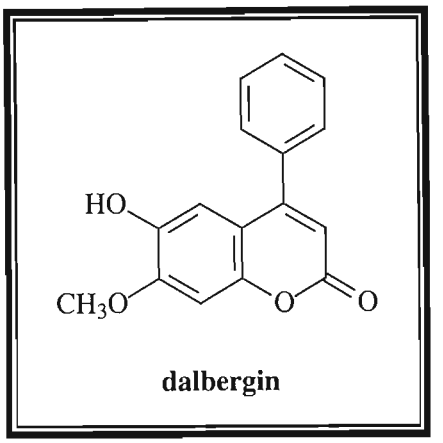



Figure 1.21. The structure of dalbergin[56].

### 1.4.3 Group C Coumarins

In furocoumarins, it is more common for rings to fuse in a linear fashion than in one of the angular modes, however, both linear and angular fusion occurs in some plants[56]. Furocoumarins have isoprenoid derived residues attached to either oxygen or carbon. The isopropyldihydrofuran ring possibly arises from the cyclisation of isoprenoid residues onto neighbouring hydroxyl groups in substituted coumarins[56]. Angelicin, which is a constituent of *Angelica archangelica* and *Psoralea corylifolia*, is an example of a group C coumarin where the rings are fused in an angular fashion[56]. Psoralen has a linear type of structure and was first isolated from the seeds of *Psoralea corylifolia*[56]. Figure 1.22 shows the structures of angelicin and psoralen.

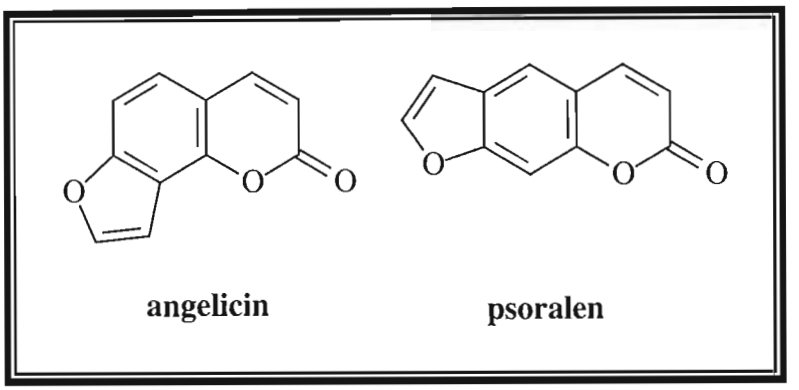



Figure 1.22. The structures of angelicin and psoralen[56].

### 1.4.4 Group D Coumarins

In the group D coumarins, a benzene ring is fused at the 3,4-position. The fungus *Alternaria tenuis*[56] produces the only known fungal 3,4-benzocoumarin, alternariol, as well as its methyl ether. This fungus is weakly parasitic on certain plants but is mainly a saprophyte. The structure of alternariol is shown in figure 1.23.

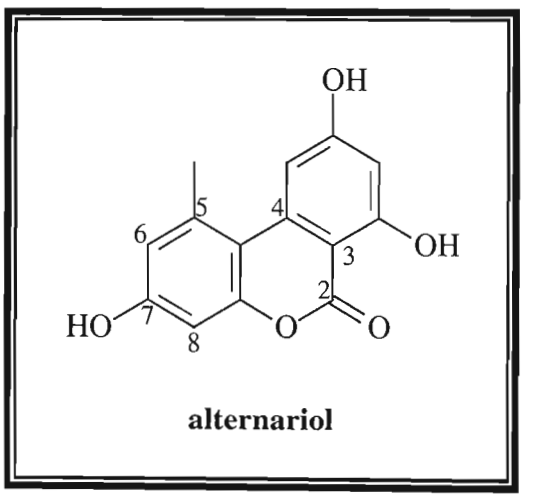
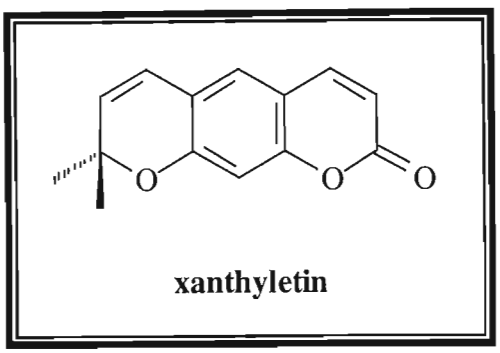



Figure 1.23. The structure of alternariol[56].

### 1.4.5 Group E Coumarins

The pyran ring system, found in pyranocoumarins, is formed when an isoprenoid unit is attached to a carbon and oxygen atom on the coumarin nucleus[57]. This group of coumarins can be either linear or angular[57]. An example of this type of coumarin is xanthyletin (Figure 1.24), which is extracted from the bark of *Xanthoxylum americanum*, the fruits of *Luvanga scandens*, the bark of *Citrus acida* and the heartwood of *Chloroxylon swietenia*[57].



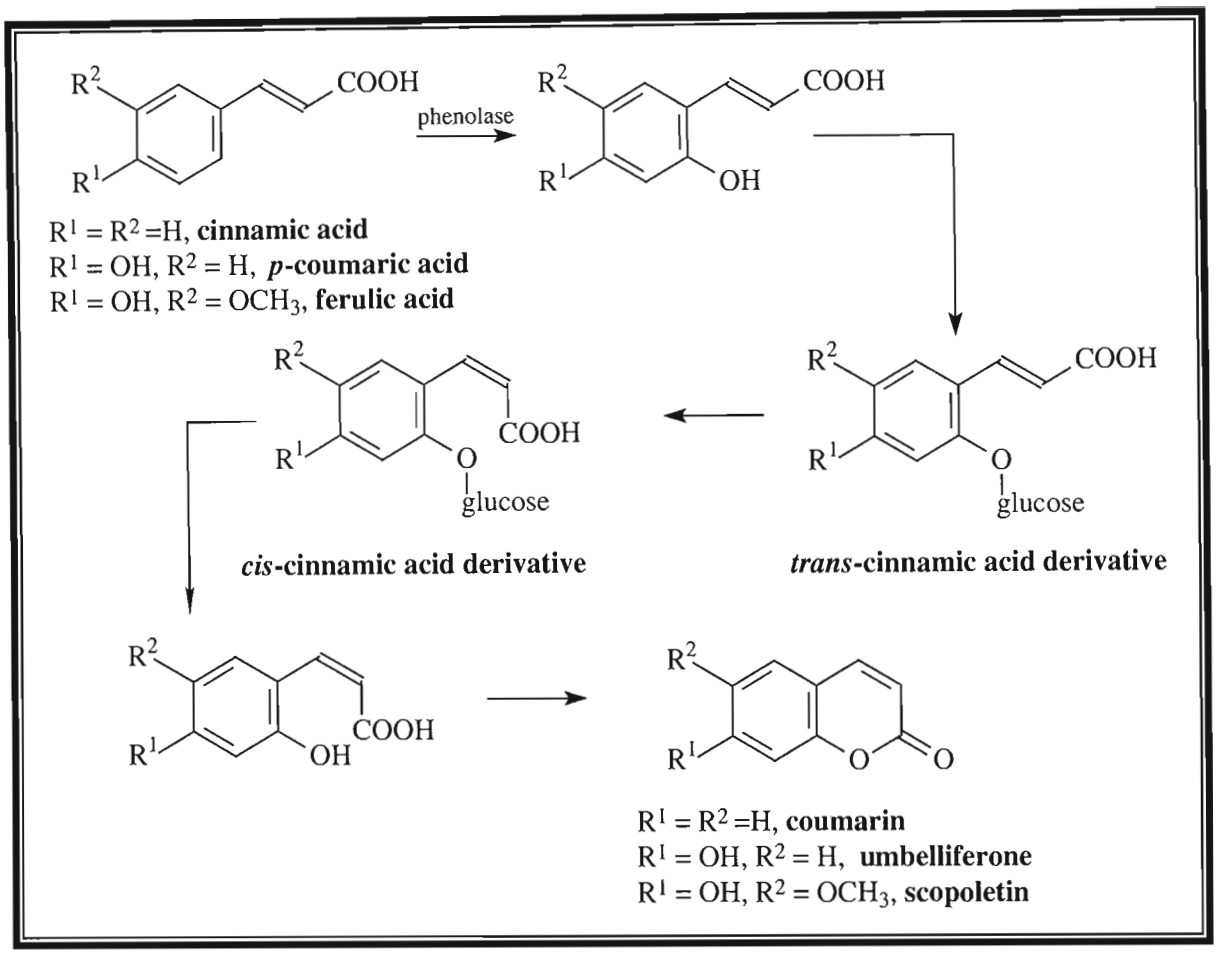

**xanthyletin**

Figure 1.24. The structure of xanthyletin.

### 1.4.6 The Biosynthesis of Coumarins

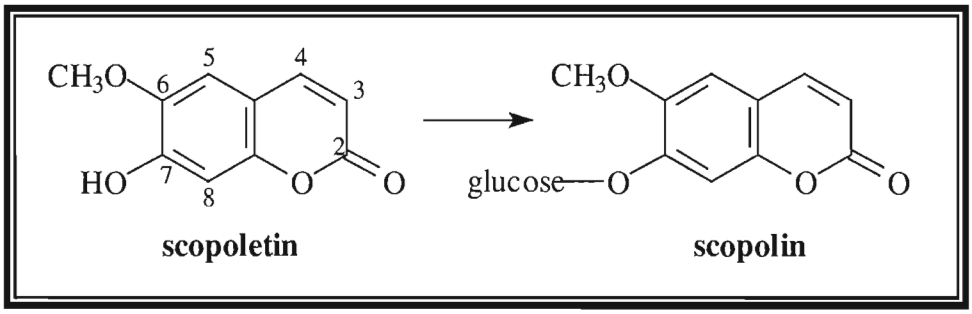
The biosynthesis of coumarins (Scheme 1.22) involves the hydroxylation of cinnamic acid *ortho* to the side-chain, a reaction catalysed by phenolase[38,58,59].

The *ortho*-hydroxy group undergoes glucosylation resulting in isomerisation of *trans*-cinnamic acid derivatives to the less stable *cis*-form. In the case of a single isolated double bond, *cis/trans* isomerisation would be unfavourable, however, cinnamic acid has a fully conjugated double bond system, which allows this process to occur quite readily[38]. Subsequent hydrolysis of the sugar group, *via* enzymatic cleavage of the glycoside, leads to spontaneous cyclisation and lactone formation, resulting in the final coumarin product. Both *cis/trans* isomerisation and lactonisation are enzyme mediated and light is not necessary for coumarin biosynthesis[38].




Scheme 1.22. The biosynthesis of coumarins[58].

Most naturally occurring coumarins contain an oxygen substituent at the 7-position, introduced by *para*-hydroxylation followed by *ortho*-hydroxylation of cinnamic acid. Umbelliferone is regarded as the parent compound of the 7-oxycoumarins. Prior to isomerisation and ring closure, several hydroxy groups may be introduced and methylated, such as ferulic acid giving scopoletin. Ferulic acid in tobacco tissue cultures converts to scopoletin while the subsequent formation of a glucoside is absent. However, in the intact plant, scopoletin accumulates as the glucoside scopolin[58] (Scheme 1.23).




Scheme 1.23. The formation of scopolin[38].

Daphnin in *Daphne odora* of the family Thymelaeaceae and cichoriin in chicory (*Cichorium intybus,* Compositae) are biosynthesised from *p*-coumaric acid and not caffeic acid (Figure 1.25), the second hydroxy group being introduced after ring closure[58], as shown in Scheme 1.24.

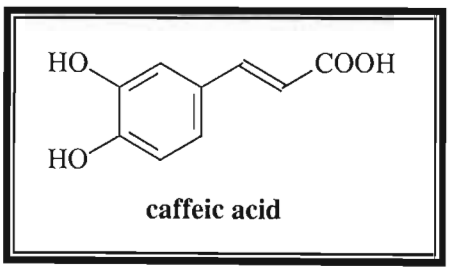
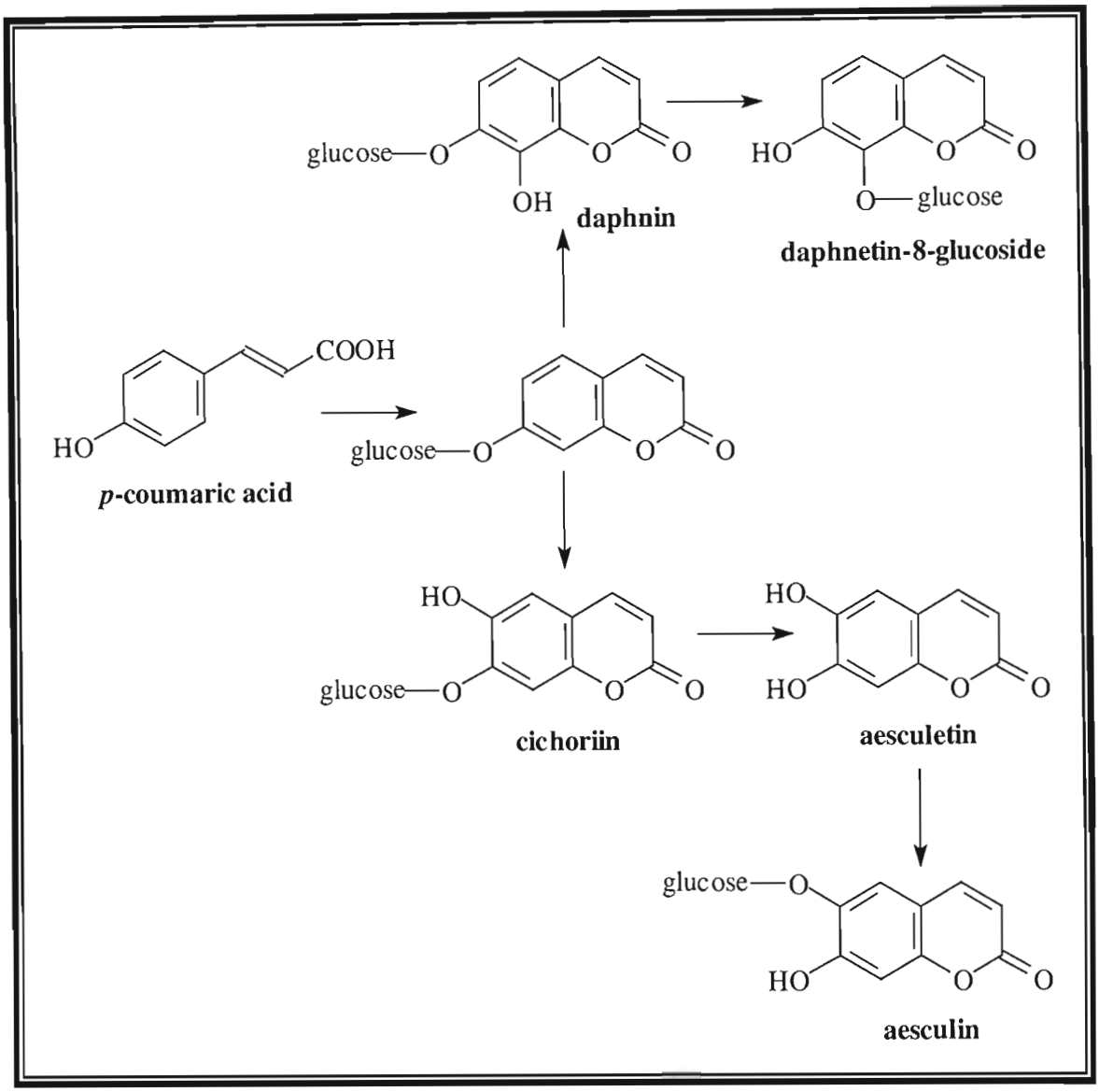


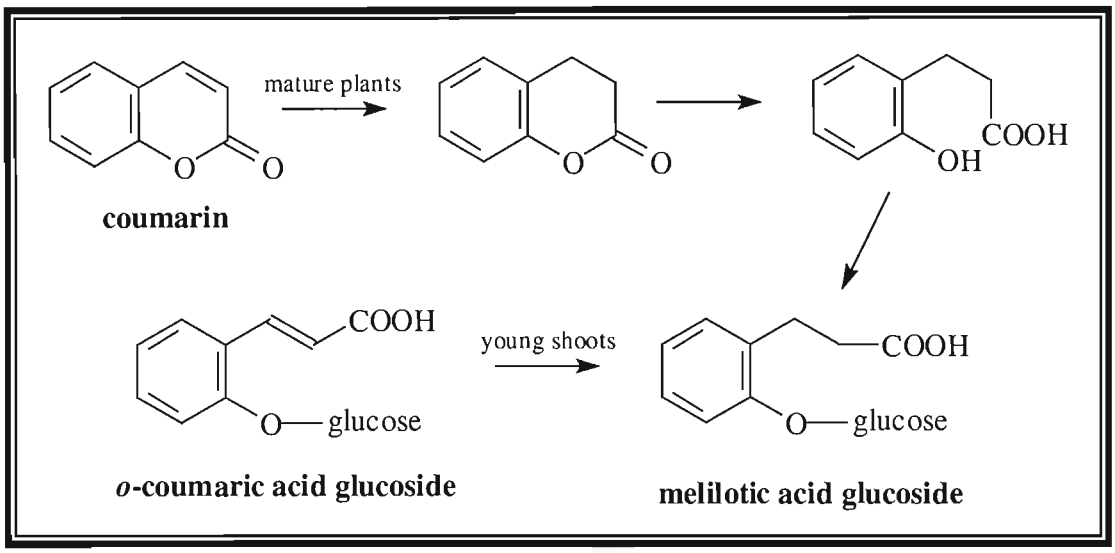

Figure 1.25. The structure of caffeic acid.

Chicory contains an enzyme that catalyses the transglucosylation of cichoriin to aesculin, with aesculetin being an intermediate. A similar enzyme in *Daphne* catalyses the transglucosylation of daphnin to daphnetin-8-glucoside. In both cases, the reactions are irreversible and the enzymes responsible are highly specific.



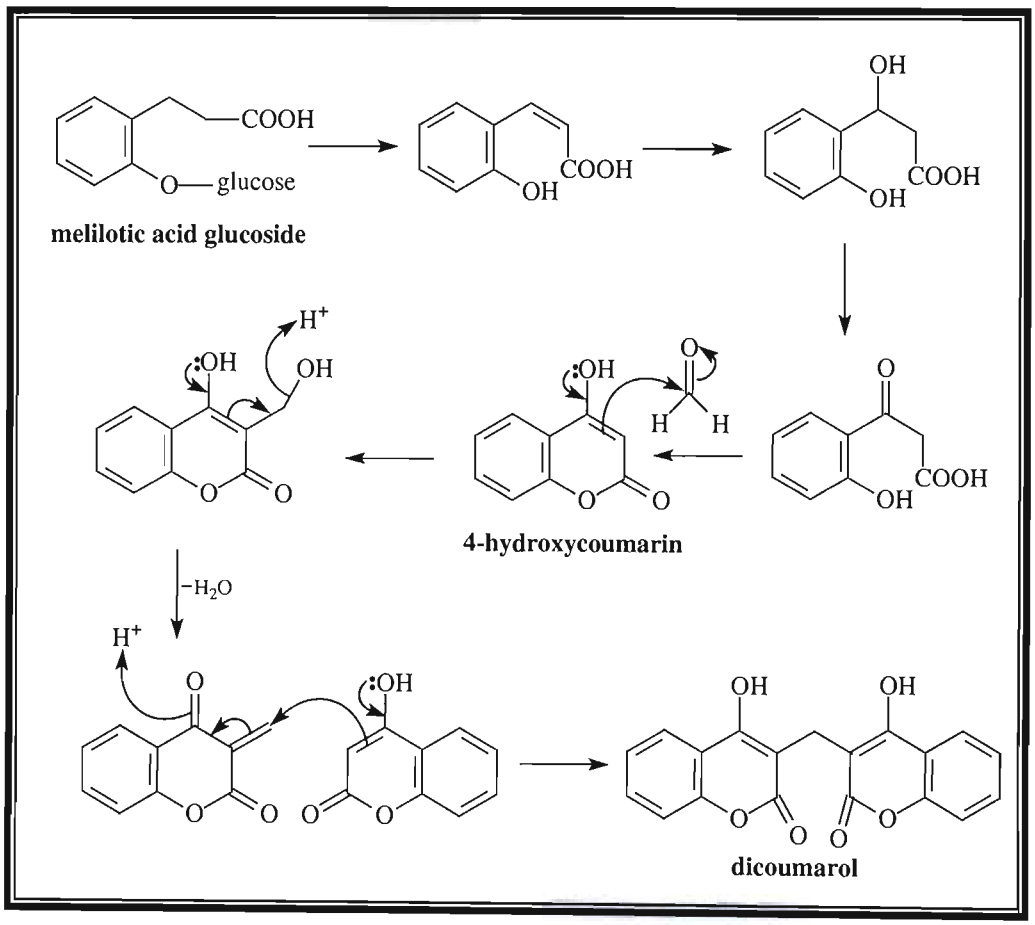

Scheme 1.24. The biosynthesis of daphnin, cichoriin, aesculetin and aesculin[58].

Coumarin itself was isolated initially from tonka beans (*Coumaraouna odorata*) and then from various melilot species, e.g. *Melilotus officinalis* and from *Asperula odorata*. It has a pleasant odour and is likened to that of newly mown hay. Evidence shows that plants contain the glucosides of *trans*- and *cis*-*o*-coumaric acid, and that coumarin is only produced through damage during the harvesting process resulting in enzymatic hydrolysis and lactonisation[38]. Melilotic acid glucoside is the bound form of coumarin in sweet clover. In mature plants, biosynthesis of the glucoside occurs *via* reduction of coumarin (Scheme 1.25), followed by ring cleavage, and finally glucosylation. In shoots, however, melilotic acid glucoside is formed directly from *o*-coumaric acid glucoside.




Scheme 1.25. The biosynthesis of melilotic acid glucoside in sweet clover[58].

Fermenting sweet clover produces 4-hydroxycoumarin, which can react with formaldehyde from microbial degradation reactions to give dicoumarol (Scheme 1.26).

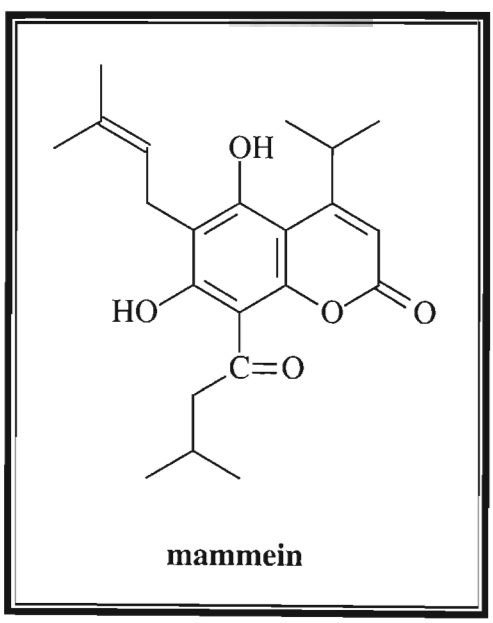



Scheme 1.26. The proposed formation of dicoumarol in spoilt hay[38,58].

Characteristically, coumarins fluoresce (usually blue) in ultraviolet light. Those derived from umbelliferone fluoresce with a blue colour when irradiated with visible light, while, if the coumarin has a free 7-hydroxy group, a green fluorescence is seen in alkaline solution[56].

### 1.4.7 The Biological Activities of Coumarins

Coumarins have been shown to have an extensive range of biological activities, and coumarin itself is highly toxic to mammals. Certain umbelliferone derivatives act as sunburn preventatives[56]. This they achieve by being able to absorb a wide range of ultraviolet frequencies, dissipate the energy as fluorescence, and finally to modify the erythermal response to ultraviolet light[56]. Umbelliferone (page 36) has been found active against undulant fever, the infection by *Brucella malitensis* and *B. abortus*[56]. Mammein (Figure 1.26) is of special interest as it shows insecticidal properties[56].




**mammein**

Figure 1.26. The structure of mammein.

Dicoumarol possesses blood anticoagulant properties, which causes intestinal bleeding in livestock[4,56], resulting in death. However, dicoumarol and synthetic analogues can be used therapeutically as anticoagulants, by inhibiting the synthesis of certain proteins concerned with the blood-clotting mechanisms. Hence, these

compounds have found clinical use in the prevention of thrombosis and to aid the dissolution of blood clots[56]. Vitamin K, an example of which is shown in figure 1.27, is a blood-clotting promoter, and dicoumarol, being an analogue of the vitamin, acts as a competitive inhibitor of the enzymes concerned in the synthesis of blood-clotting proteins[58]. Novobiocin (Figure 1.27), a metabolite of *Streptomyces niveus*, has been found to exhibit antibiotic properties[56], and chartreusin (Figure 1.27), found in *Streptomyces* species, is a glycosidic antibiotic[56].

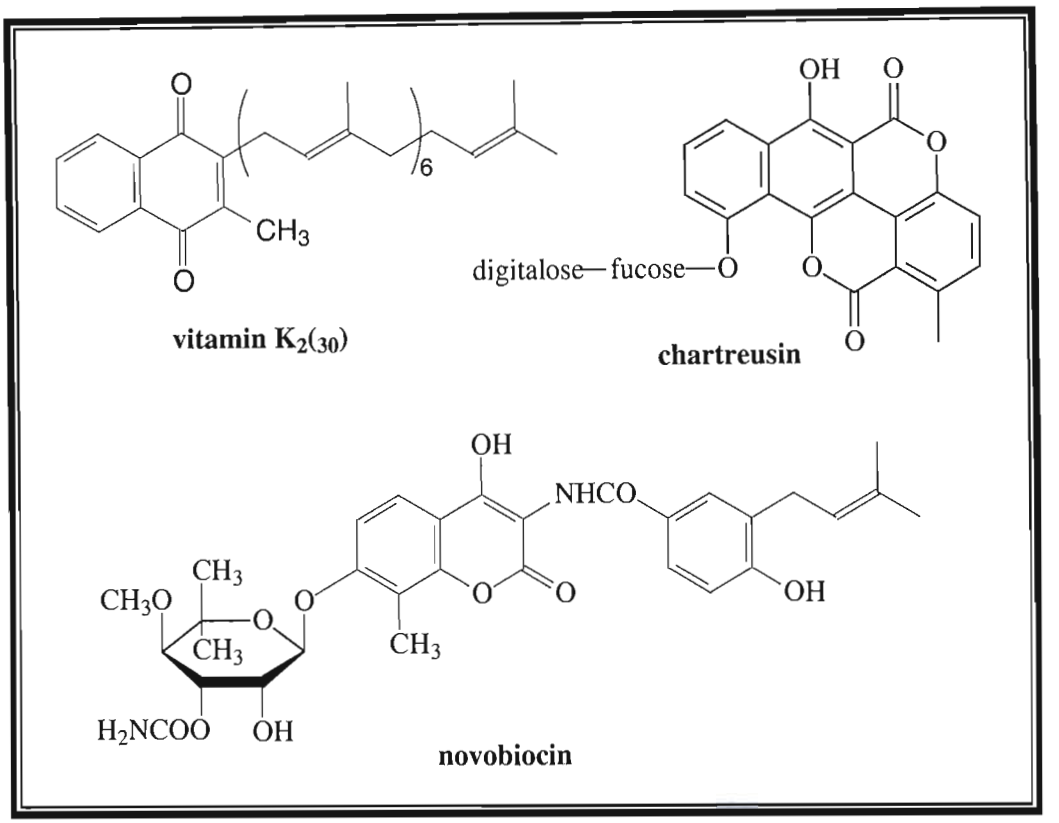



Figure 1.27. The structures of vitamin $K_{2(30)}$, chartreusin and novobiocin.

Furocoumarins are highly toxic to fish, but show little toxicity towards other animals[56]. For this reason plant extracts have been added to streams to paralyse the fish and hence they can be easily caught without their edibility being affected[56]. Generally, furocoumarins appear to photosensitise the skin and produce a poisonous character in plants[56]. Psoralen (Page 38) has been used for the treatment of alopecia[56], while the seeds of *Psoralea corylifolia* are used in the Hindu Ayurvedic system of medicine as laxatives and diuretics as well as in the treatment of leucoderma and leprosy[56]. Psoralen and its derivatives show photodynamic activity and hence may be taken orally to promote sun tanning of the skin[58].

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# CHAPTER 2

# Extractives from the Hyacinthaceae

## 2.1 Introduction to the Hyacinthaceae

The species *Ledebouria ovatifolia, Eucomis pole-evansii, Lachenalia rubida* and *Drimia capitata* all belong to the family Hyacinthaceae (Liliaceaea *sensu lato*). Based on chemotaxonomical, morphological, cytological and molecular data, members of the Hyacinthaceae can be divided up into five sub-families. The Hyacinthoideae, Urgineoideae and Ornithogaloideae occur in southern Africa[1], while the Clorogaloideae and Oziroeoideae are restricted to Northern America and Andean South America respectively[2]. *Ledebouria ovatifolia*, *Eucomis pole-evansii* and *Lachenalia rubida* belong to the sub-family Hyacinthoideae, while *Drimia capitata* belongs to the Urgineoideae sub-family.

Since early times, the generic names, *Hyacinthus, Ornithogalum* and *Scilla* have been used and represent the most important genera of the Hyacinthaceae. In his Genera Planatarum (1754), Linné based his characterisations of these three genera mainly on their floral characteristics[3]. Consequently, some species belonging to the Hyacinthaceae were incorrectly classified and distributed into these three genera resulting in highly unnatural classifications[3].

The Hyacinthaceae is a diverse family consisting of approximately seventy genera and about one thousand species[3], with large numbers of species in southern Africa (approximately twenty-seven genera and four hundred species) and the Mediterranean[4]. The geographic distribution of the Hyacinthaceae is shown in green in figure 2.1.

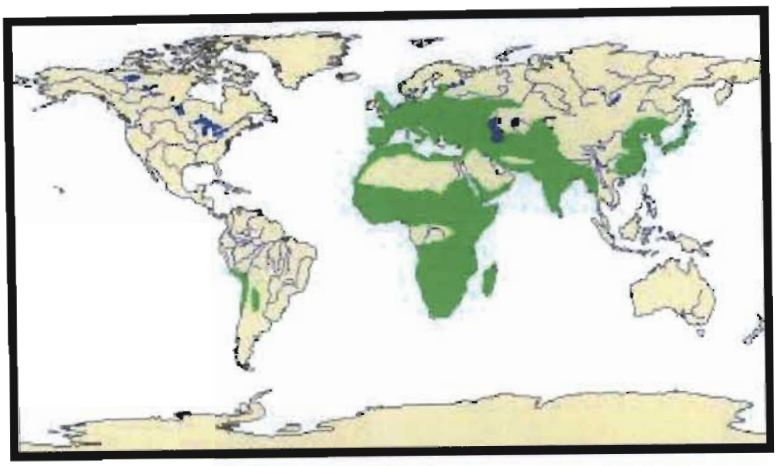


Figure 2.1. The geographic distribution of the Hyacinthaceae[3].

The Hyacinthaceae are mostly perennial bulbous plants with subterraneous or epigeal bulbs[3]. The bulbs grow in grassland or woodlands between trees and bushes. The bulbs themselves have characteristic fleshy scales that produce copious adhesive, elastic threads when separated. The leaves are occasionally grazed but the threads in the bulbs generally prevent them from being eaten by porcupines and Chacma baboons. The inflorescence is a raceme bearing one to hundreds of flowers, which are visited mainly by insects, but in some genera, also birds[3]. Many genera of the Hyacinthaceae are used in the horticultural trade and also in traditional medicine[4].

*Charybdis maritima*, the sea onion, of the subfamily Urgineoideae, has been used medicinally and its use was first described in 1554 BC in the Ebers Papyrus of the Middle Empire of Egypt as a cure for dropsy[3]. Bufadienolides are cardiac active substances isolated from this species as well as from *Urginea indica*[3]. In South Africa, several species of Hyacinthaceae including *Ledebouria cooperi, L. inguinata, L. ovatifolia* and *L. revoluta*, as well as several members of the Urgineoideae are poisonous to grazing animals. The toxic compound, scilliroside (a bufalienolide), found in certain Hyacinthaceae species, is also used to poison rats[3]. Hyacinthaceae are rarely used for human consumption as food. However, in Greece, the bulbs of *Muscari comosum* (Hyacinthoideae) are eaten pickled, and in France, flowers of *Loncomelos pyrenaicus* (Ornithogaloideae) are eaten as a vegetable[3]. In Africa, the bulbs of *Ledebouria apertiflora* and *L. revoluta* of the subfamily Hyacinthoideae, are eaten by the Bushmen[3].

A relatively small portion of the southern African Hyacinthaceae has been investigated phytochemically. The compounds that have been isolated can be classified into four groups, namely, homoisoflavonoids, steroidal type compounds, bufadienolides and miscellaneous compounds.

## 2.2 Extractives from *Ledebouria ovatifolia*

*Ledebouria* (subfamily: Hyacinthoideae, family: Hyacinthaceae) was formerly included in the genus *Scilla*, but has now been independently classified[5]. The three genera that were previously thought to be closely related, *Scilla*, *Schizocarphus* and *Ledebouria*, were grouped according to their morphology, cytology, anatomy and chromatography[6]. Based on their physical characteristics, it was evident that there was a much closer affinity between *Scilla* and *Schizocarphus* than between either of these groups and *Ledebouria*[6]. Based on these findings, Jessop[6] suggested that *Ledebouria* be accepted as a generically distinct genus. *Ledebouria ovatifolia* has been classified previously as *Scilla ovatifolia*, *Scilla lanceaefoli*, *Scilla guttata*, *Scilla climatocarpha*, *Scilla cicatricosa*, *Scilla albomarginata*, *Scilla elevans* and *Scilla collina*[5,6].

Figure 2.2. Photographs of *Ledebouria ovatifolia* (Photographed by Dr. Neil Crouch).

Members of the genus *Ledebouria* are extensively used by traditional healers in Kwazulu-Natal in enemas and as purgatives for both humans and cattle[5,7]. *Ledebouria ovatifolia* bulbs, shown in figure 2.2, are used by the Zulu in enemas for gastro-enteritis and in medicines taken for influenza and backache[5,7]. It has been reported that the plants are rubbed on pubescent female breasts to make them grow[5,7]. In the Transkei, the bulbs are used as purgatives for adults and children over the age of five months[8].

Toxicity levels of *L. ovatifolia* are quite high, as 1.5 kg of fresh bulbs is fatal to sheep within a few hours[9]. Post-mortem findings include, among other effects, general cyanosis, severe hyperaemia and oedema of the lungs, congestion and regressive changes of the liver and kidneys[9]. However, two rabbits recovered after being given 10g of the bulb and 20 g of the leaf and flower of this plant[9]. Tests conducted at Onderstepoort Research Station proved that this species is toxic to both sheep and rabbits at levels of 2 g per kilogram of live mass.

Other species of *Ledebouria* are also used by traditional healers. The Sotho people use *L. revoluta* for the treatment of lumbago and as a charm to drive away lightning[5]. They also used *L. cooperi* as a soothing medication for women in their fourth month of pregnancy and to inebriate boys during circumcision rites[5]. Certain species of *Scilla* and *Eucomis* including *S. natalensis, S. nervosa, E. autumnalis, E. bicolour* and *E. comosa* are extensively used by traditional healers as purgatives and in enemas[5], which makes our chemical investigations interesting as these three genera have been reported to be a rich source of homoisoflavonoids.

Species of *Ledebouria* are perennial, deciduous and usually solitary bulbous herbs. The bulbs are found below the ground and are oblong in shape with fleshy scales[4]. There are approximately forty-six species extending across Africa to the southern tip of India with about thirty-eight species being indigenous to southern Africa[4].

*Ledebouria ovatifolia* has been previously worked on by Pohl *et al.* who isolated 4,4'-dihydroxy-2',6'-dimethoxychalcone and 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone[10]. Further investigations of this plant have also yielded the novel chalcone, ovatifolin[11]. This plant was chemically investigated for a second time as the

plant in this study was the Highveld form which was collected from the Blyde Nature Reserve, while the first investigation was performed on *Ledebouria ovatifolia* that was purchased from the Warwick Triangle market in Durban, Kwazulu-Natal. The structures of the compounds isolated by Pohl *et al.* are shown in figure 2.3.

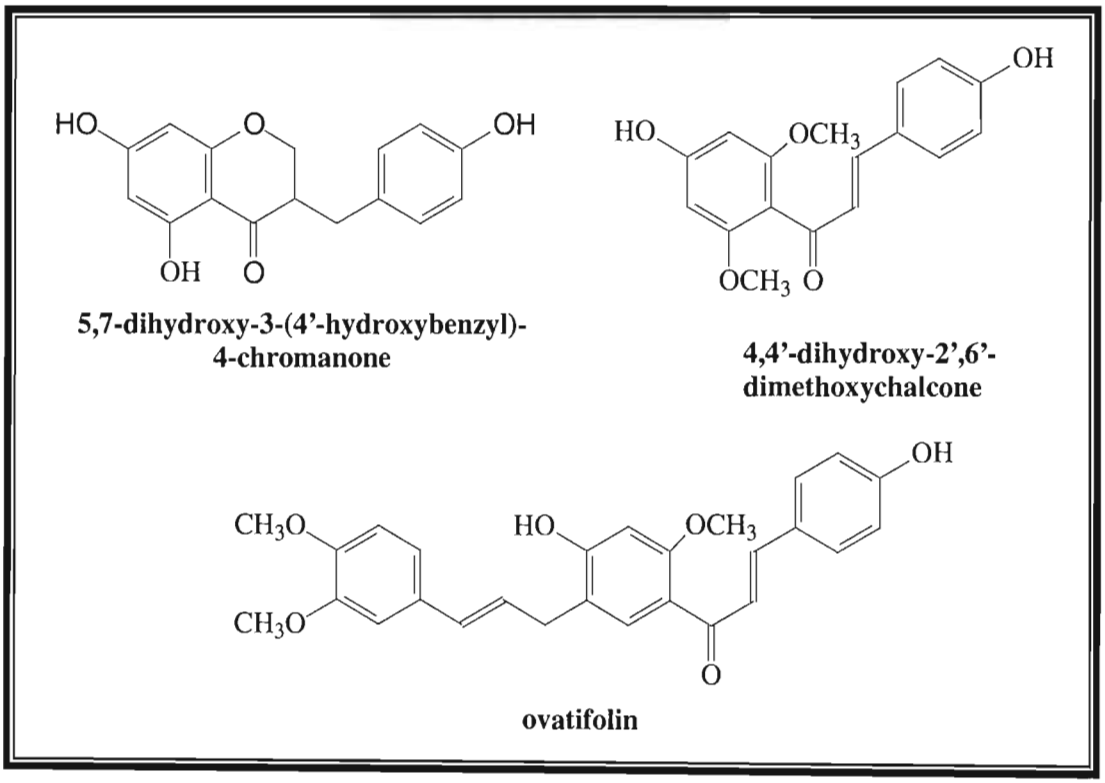



Figure 2.3. Compounds previously isolated from *Ledebouria ovatifolia.*[10, 11]

## 2.2.1 Results and Discussion

The dichloromethane extract of *Ledebouria ovatifolia* (Bak) Jessop yielded three compounds (**compounds I-III**). The extract from the *L. ovatifolia* (Highveld form) collection yielded different compounds compared to the *L. ovatifolia* collected from the Warwick Triangle. This confirms that the environment that a plant grows in plays an important role in the type of compounds that plant produces. No compounds were isolated from the ethyl acetate extract while the methanol extract yielded only sugars.

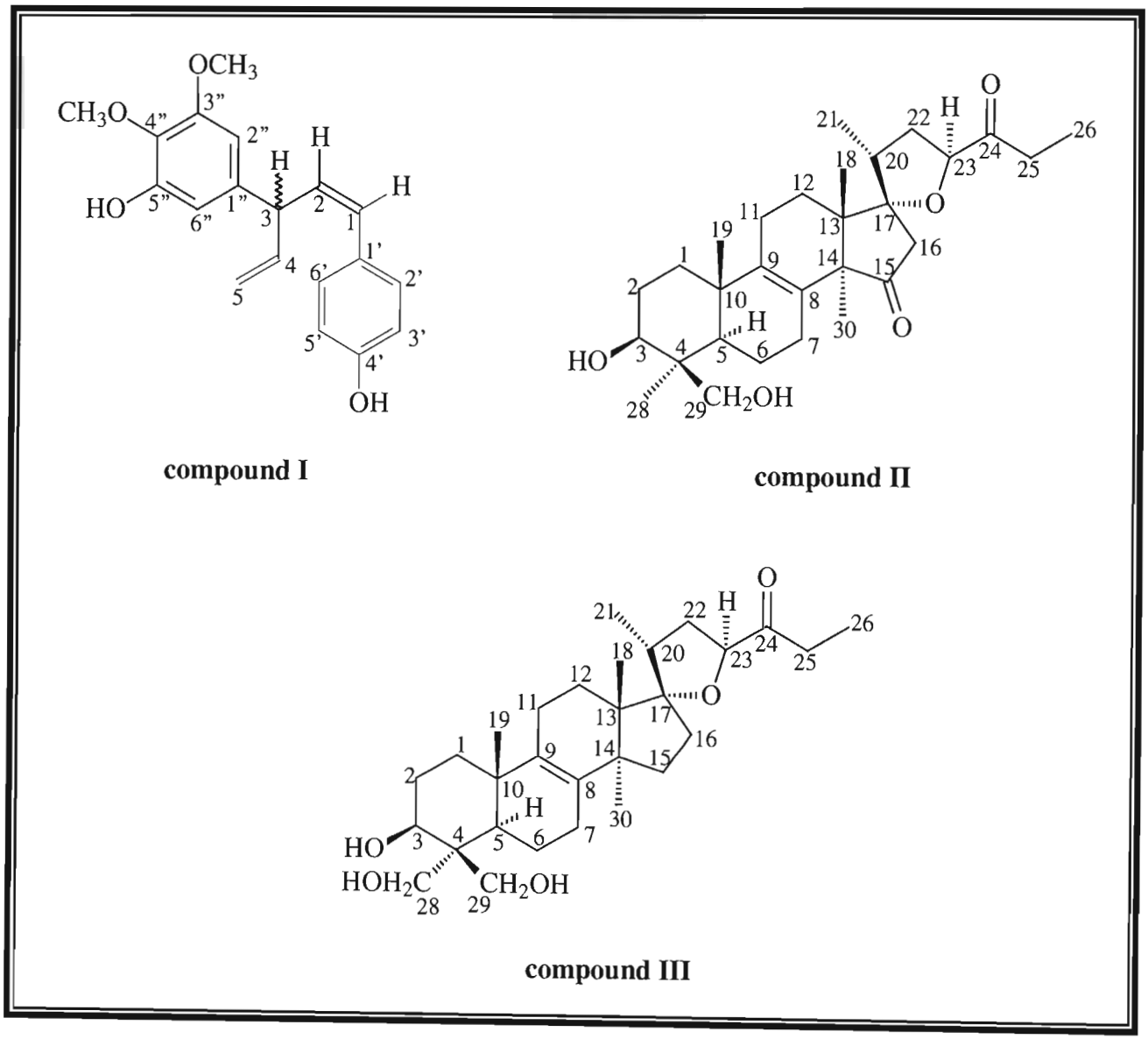


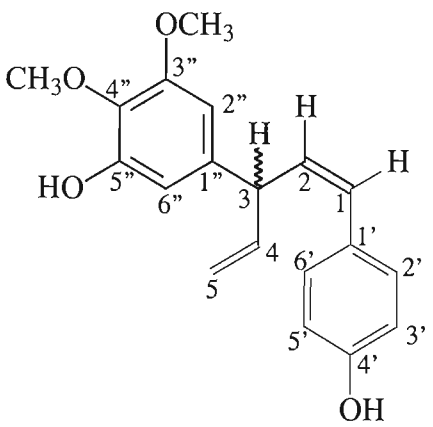

Figure 2.4. The structures of compounds I-III.

### 2.2.1.1 Structural Elucidation of Compound I

### (-)-(*Z*)-1-(4'-hydroxyphenyl)-3ξ-(5"-hydroxy-3",4"-dimethoxyphenyl) -1,4-pentadiene

(Spectra 1a-i, pages 208-216)

The first compound isolated from *Ledebouria ovatifolia* was isolated as a yellow gum and was identified as the novel norlignan, (-)-(*Z*)-1-(4'-hydroxyphenyl)-3ξ-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene (Figure 2.5). This is the first time that a norlignan has been isolated from the Hyacinthaceae family*.




**(-)-(Z)-1-(4'-hydroxyphenyl)-3-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene**

Figure 2.5. The structure of compound I.

The high resolution mass spectrum showed a parent ion peak at *m/z* 312.13690, which was consistent with the molecular formula of $C_{19}H_{20}O_4$ ($C_{19}H_{20}O_4$ requires 312.13616). A double bond equivalence of ten was deduced.

The infra-red spectrum showed a broad band at 3412 $cm^{-1}$ due to the O-H stretching vibrations and two sharp peaks at 2923 $cm^{-1}$ and 2847 $cm^{-1}$ due to the asymmetric and

* A colleague in the group has simultaneously isolated two norlignans, *Z*-1,3-bis(4-hydroxyphenyl)-1,4-pentadiene from *Drimiopsis burkei* and *E*-1,3-bis(4-hydroxyphenyl)-1,4-pentadiene from *Drimiopsis maculata*.

symmetric C-H stretching respectively. The $CH_2$ and $CH_3$ bending vibrations were observed at 1355 $cm^{-1}$, 1230 $cm^{-1}$ and 1105 $cm^{-1}$.

The $^1H$ NMR spectrum showed a pair of two-proton doublets at δ 7.16 and δ 6.78 ($J$ = 8.6 Hz) assigned to H-2'/6' and H-3'/5' respectively, which indicated a *para*-substituted aromatic ring. The HMBC spectrum showed a correlation between the H-3'/5' proton resonance and the fully substituted C-4' carbon resonance at δ 154.6. The methine carbon signal assigned to C-3'/5' was observed at δ 115.1. The methine carbon peak ascribed to C-2'/6'(δ 130.0) showed correlations in the HMBC spectrum with H-1(δ 6.52) and H-3'/5'.

The H-1 one-proton signal (δ 6.52, d, $J$ = 11.7 Hz) in the $^1H$ NMR spectrum occurred as a doublet, with one peak of the doublet being hidden under the H-6" resonance. In the COSY spectrum, the H-1 signal was seen to be coupled to a one-proton double doublet resonance appearing at δ 5.66 ($J$ = 10.1, 11.7 Hz), which was assigned to H-2. A coupling constant of 11.7 Hz between H-1 and H-2 suggests that the substitution pattern is *cis* across the double bond. The *cis* configuration was further proved by the fact that H-1 showed correlations in the NOESY spectrum to H-2'/6' as well as to H-2.

The H-3 proton signal at δ 4.42 (1H, dd, $J$ = 6.2, 10.0 Hz) showed correlations in the NOESY spectrum with H-2'/6', H-2" and H-6". A model showed that these correlations were only possible for the *cis* isomer. The methine carbon resonances at δ 129.0, δ 131.2 and δ 47.6 were ascribed to C-1, C-2 and C-3 respectively. In the COSY spectrum, the H-2 proton resonance was seen to be coupled to the H-1 and H-3 protons, while the one-proton multiplet at δ 5.99, due to H-4, was seen to be coupled to 2H-5 and H-3. The pair of double doublet resonances due to H-5 (δ 5.18 and δ 5.17) only showed coupling in the COSY spectrum with the H-4 signal. The C-4 methine carbon signal occurred at δ 140.2 while the C-5 methylene carbon resonance occurred at δ 115.4.

Two *meta* coupled protons were present on the B ring, and their resonances occurred at δ 6.31 (1H, d, H-2", $J$ = 1.8 Hz) and δ 6.50 (1H, d, H-6", $J$ = 1.8 Hz). Both the H-2" and H-6" resonances showed correlations in the NOESY spectrum with H-3. The

corresponding methine carbon resonances occurred at δ 103.5 and δ 107.2 respectively. In the HMBC spectrum the C-2" signal showed only a correlation with H-6", while the C-6" resonance showed only a correlation in the HMBC spectrum with H-2".

In the $^{1}$H NMR spectrum, two sharp singlets were observed at δ 3.80 and δ 3.85, each integrating to three protons, which indicated the presence of two methoxy groups. The methoxy group, which had a methyl carbon resonance at δ 55.8, was positioned at C-3" (δ 152.3, C) as it showed a correlation in the NOESY spectrum with H-2". The second methoxy group protons showed no correlations in the NOESY spectrum. This methoxy group would have to be positioned on C-4" (δ 134.0, C) as if it were placed at position C-5", we would see a correlation in the NOESY spectrum with H-6" and we do not. Furthermore, if the second methoxy group was placed at C-5", the ring would be symmetrical, which it is not. The two hydroxy groups were attached to the fully substituted carbon whose resonances occurred at δ 149.2 (C-5") and δ 154.6 (C-4').

This compound was found to be a new norlignan identified as (-)-(*Z*)-1-(4'-hydroxyphenyl)-3ξ-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene. When tested for anti-inflammatory activity, compound I, at a concentration of 10 μg/cm$^3$ showed 72.7% inhibition of prostaglandin synthesis using the microsomal cell screen, 42.2% inhibition using the Cox-1 screen and 23.4% inhibition using the Cox-2 screen (page 72).

The NMR data for compound I is tabulated in table 2.1 and the HMBC and NOESY correlations are depicted in figure 2.6 and figure 2.7 respectively.

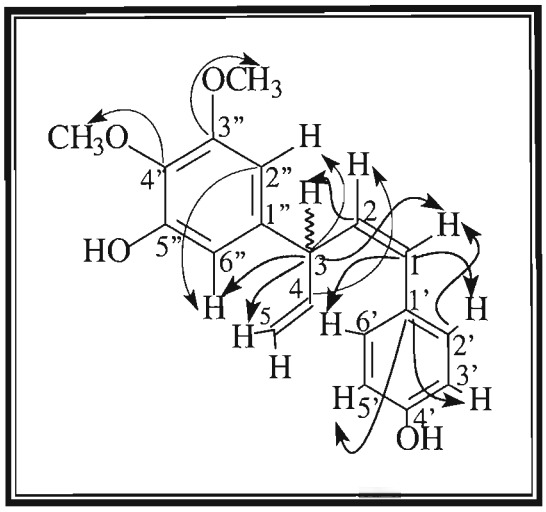



Figure 2.6. The HMBC (C → H) correlations for compound I.

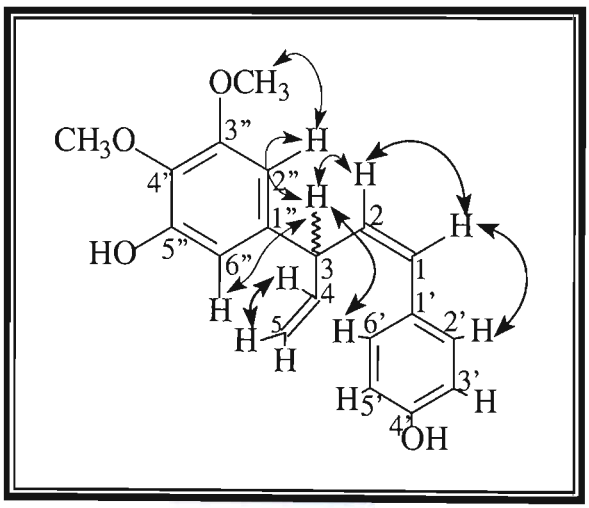



Figure 2.7. The NOESY correlations for compound I.

**Table 2.1. NMR data and correlations for compound I ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1 | 6.52 (1H, d, *J*=11.7 Hz) | 129.0 (CH) | H-2 | H-2'/-6' | H-2, H-2'/6' |
| 2 | 5.66 (1H, dd, *J*=10.1, 11.7 Hz) | 131.2 (CH) | H-1, H-3 | H-3 | H-1, H-3, H-6" |
| 3 | 4.42 (1H, dd, *J*=6.2, 10.0 Hz) | 47.6 (CH) | H-2, H-4 | 2H-5, H-2'', H-6'' | H-2, H-2'/6', H-2'', H-6'' |
| 4 | 5.99 (1H, m) | 140.2 (CH) | H-3, 2H-5 | H-2, H-3 | H-5 |
| 5a | 5.18 (1H, dd, *J*=1.5, 10.3Hz)) | 115.4 ($CH_2$) | H-4 | - | H-4 |
| 5b | 5.17 (1H, dd, *J*=1.5, 17.2Hz) | | H-4 | | H-4 |
| 1' | - | 129.7 (C) | - | H-3'/5' | - |
| 2' | 7.16 (1H, d, *J*=8.6Hz) | 130.0 (CH) | H-3'/5' | H-1, H-3'5', H-2'/6' | H-1, H-3, H-3'/5' |
| 3' | 6.78 (1H, d, *J*=8.6Hz) | 115.1 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| 4' | - | 154.6 (C) | - | H-2'/6', H-3'/5' | - |
| 5' | 6.78 (1H, d, *J*=8.6Hz) | 115.1 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| 6' | 7.16 (1H, d, *J*=8.6Hz) | 130.0 (CH) | H-3'/5' | H-1, H-2'/6'(w), H-3'/5' | H-1, H-3, H-3'/5' |
| 1'' | - | 139.7 (CH) | - | H-2, H-3 | - |
| 2'' | 6.31(1H, d, *J*=1.8Hz) | 103.5 (CH) | H-6'', $OCH_3$ at H-3'' | H-6'' | H-3, $OCH_3$ at C-3" |
| 3'' | - | 152.3 (C) | - | H-2'', $OCH_3$ at C-3" | - |
| 4'' | - | 134.0 (C) | - | H-2'', H-6'', $OCH_3$ at C-4" | - |
| 5'' | - | 149.2 (C) | - | H-6'' | - |
| 6'' | 6.50 (1H, d, *J*=1.8Hz) | 107.2 (CH) | H-2'' | H-2'' | H-2, H-3 |
| $OCH_3$ at C-3'' | 3.80 (3H, s) | 55.8 ($CH_3$) | H-2'' | - | H-2'' |
| $OCH_3$ at C-4'' | 3.85 (3H, s) | 60.9 ($CH_3$) | - | - | - |

### 2.2.1.2 Structural Elucidation of Compound II

### (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-norlanost-8-ene-15,24-dione

(Spectra 2a-p, pages 217-232)

The second compound isolated from *Ledebouria ovatifolia* was isolated as white needle-like crystals and was identified as the known compound, (23*S*)-17α,23-epoxy-3β, 29-dihydroxy-27-norlanost-8-ene-15,24-dione, commonly known as eucosterol (Figure 2.8). Eucosterol has been previously isolated from *Eucomis bicolour*, *E. autumnalis*, *E. punctata* and *E. pole-evansii*[12].

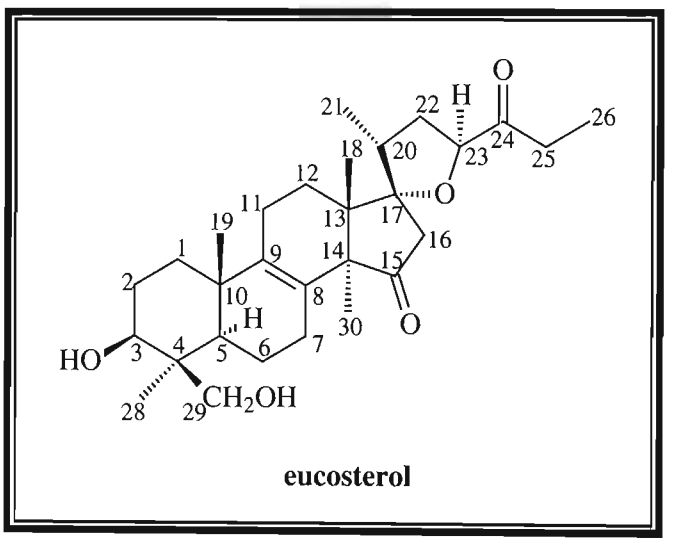



Figure 2.8. The structure of compound II.

The mass spectrum revealed compound II to have a molar mass of 472.3189 g $mol^{-1}$, which corresponded to a molecular formula of $C_{29}H_{44}O_5$ ($C_{29}H_{44}O_5$ requires 472.3189) and a double bond equivalence of eight.

The infra-red spectrum of compound II showed the presence of an absorption at 3488 $cm^{-1}$ which was due to the O-H stretching vibrations. Absorptions were also observed at 2943 $cm^{-1}$ and 2878 $cm^{-1}$, which could be assigned to the C-H symmetric and asymmetric stretchings respectively. The C=O stretching vibrations were evident at 1740 $cm^{-1}$and 1731 $cm^{-1}$, while the $CH_2$ and $CH_3$ bending vibrations could be seen at 1463 $cm^{-1}$ and 1381 $cm^{-1}$. The absorption at 1038 $cm^{-1}$ was due to C-O stretchings.

The $^{1}H$ NMR spectrum showed the presence of four singlet methyl proton resonances at δ 0.89, δ 0.87, δ 1.19 and δ 1.32, each integrating to three protons. By comparison of NMR data for compound II with the literature data[12] for eucosterol, these resonances were ascribed to 3H-18, 3H-19, 3H-28 and 3H-30 respectively and this was subsequently confirmed by means of the HMBC spectrum. A methyl proton doublet integrating to three protons was observed at δ 1.07 ($J$ = 6.8 Hz) and was assigned to 3H-21. This resonance was seen to be coupled in the COSY spectrum to H-20 (δ 2.19, m). The methyl proton resonance at δ 1.01 (t, $J$ = 7.2 Hz), ascribed to 3H-26, was seen to be coupled in the COSY spectrum to 2H-25 (δ 2.44, q, $J$ = 7.2 Hz).

The doublet methyl proton resonance assigned to 3H-21 was seen to be correlated in the NOESY spectrum to a triplet methine proton resonance at δ 4.62. This was assigned to H-23 as it was seen to be coupled in the COSY spectrum to 2H-22 (δ 1.87, m). The corresponding carbon resonances were observed at δ 81.5 (C-23) and δ 36.6 (C-22), using the HSQC spectrum. The C-21 methyl group was assigned an α configuration as this resonance showed a correlation in the NOESY spectrum to 3H-18. A model was constructed and from the model it was evident that if C-20 and 3H-18 were β in orientation, then a correlation would be seen in the NOESY spectrum between 3H-21 and 3H-18, confirming that 3H-21 is α in orientation. Due to the 3H-21 resonance being correlated to H-23 in the NOESY spectrum, it can be concluded that H-23 is also of the α orientation.

In the $^{13}C$ NMR spectrum, twenty-nine carbon resonances were observed, which indicated a nortriterpenoid-type skeleton. The two fully substituted resonances at δ 215.7 and δ 212.6 were due to the two carbonyl group carbons at C-15 and C-24 respectively. These two carbon resonances could be differentiated as C-15 showed an HMBC correlation to 2H-16 (δ 2.71 and δ 2.16) and 3H-30, while H-24 was seen to be correlated in the NOESY spectrum with 2H-22, H-23, 3H-26 and 3H-30. The $^{13}C$ NMR spectrum also showed the presence of two carbon resonances at δ 135.8 (C) and δ 132.6 (C), which indicated the presence of a double bond. This double bond was found to be situated between C-8 and C-9. This was confirmed by the HMBC spectrum, which showed correlations of the peak at δ 132.6 (C-8) with H-6β (δ 1.77),

H-6α (δ 1.32) and 3H-30 (δ 1.32), while the carbon resonance at δ 135.8 (C-9) showed correlations in the HMBC spectrum with H-5 (δ 1.11), H-12β (δ 1.53) and 3H-19 (δ 0.87).

The oxygenated methylene carbon resonance (δ 64.2) was assigned to C-29 as it showed correlations in the HMBC spectrum to H-5 and 3H-28. The methine carbon resonance ascribed to C-3 (δ 80.3) also showed a correlation in the HMBC spectrum to 2H-29 (δ 4.16 and δ 3.29, 2 x d, $J$ = 11.0 Hz). The COSY spectrum showed coupling between the two H-29 resonances, as did the NOESY spectrum. The 3H-28 resonance was assigned the alpha configuration as it was seen to correlated in the NOESY spectrum with H-3. The 2H-29 resonance was thus assigned the beta configuration which was confirmed by the NOESY spectrum as H-29B was seen to be correlated with 3H-19.

The molecular formula of compound II was found to be $C_{29}H_{44}O_5$ and hence a double bond equivalence of eight was deduced. This confirmed the 17,23-ether linkage, as it fitted in with the extra ring needed by the double bond equivalence calculation. This compound was also acetylated in order to confirm the presence of the 17,23-ether. No acetylation at C-17 and C-23 was observed indicating that these two oxygenated carbon atoms did not have hydroxy groups attached but rather formed part of an ether ring system. The only acetylations that did occur were at C-3 and C-29.

The NMR data of compound II was compared to the literature data[12] of eucosterol and the $^{13}C$ NMR values correlated well, but certain values were slightly different due to this compound being run in $CDCl_3$ and the literature data being run in $C_5D_5N$. However, due to the HMBC correlations, it was possible to make conclusive assignments and hence correct the literature data for those assignments that were uncertain to Ziegler *et al.*[12]. The NMR data and correlations for compound II are listed in table 2.2.

**Table 2.2. NMR data and correlations for compound II ($CDCl_3$) and literature data for eucosterol ($C_5D_5N$)[12]**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for eucosterol[12] (22.63 MHz) | $^{13}$C NMR data for acetylated eucosterol (100 MHz) |
|---|---|---|---|---|---|---|---|
| 1α | 1.71 (1H, m) | 35.1 ($CH_2$) | H-1β, H-2β | 3H-19, H-5 | H-1β, H-3, H-6α, H-7α, 3H-28 | 35.8 | 35.3 |
| 1β | 1.16 (1H, m) | | H-1α, H-2α | | H-1α | | |
| 2α | 1.76 (1H, m) | 27.9 ($CH_2$) | H1β, H-3 | - | H-3 | 28.9 ($CH_2$) | 29.7 |
| 2β | 1.76 (1H, m) | | H-1α, H-3 | | - | | |
| 3 | 3.38 (1H, m) | 80.3 (CH) | H-2β, H-2α | 2H-1, 2H-29 | H-1α, H-5, H-2α, 3H-28 | 79.9 (CH) | 80.0 |
| 4 | - | 42.4 (C) | - | 2H-1, H-5, 3H-28, 2H-29 | - | 43.1 (C) | 40.9 |
| 5 | 1.11 (1H, m) | 50.4 (CH) | 2H-6 | 2H-1, 2H-6, 3H-19, 3H-28 | H-3 | 51.1 | 50.9 |
| 6α | 1.32 (1H, m) | 18.0 ($CH_2$) | H-6β, H-7β | H-5 | H-1α, H-6β, H-7α | 20.8† | 18.9 |
| 6β | 1.77 (1H, m) | | H-5, H-6α, H-7α | | H-6α | | |
| 7α | 2.28 (1H, m) | 26.4 ($CH_2$) | 2H-6, H-7β | H-5, 2H-6 | H-1α, H-6α, H-7β, 3H-30 | 32.3 ($CH_2$) | 26.8 |
| 7β | 2.57 (1H, m) | | H-6α, H-7α | | H-7α, 3H-19 | | |
| 8 | - | 132.6 (C) | - | 2H-6, 3H-30 | - | 133.1 (C) | 133.2 |
| 9 | - | 135.8 (C) | - | H-5, H-12β, 3H-19 | - | 136.6 (C) | 135.4 |
| 10 | - | 37.0 (C) | - | H-5, 2H-6, 2H-11, 3H-19 | - | 37.6 | 37.2 |
| 11α | 2.07 (1H, m) | 20.3 ($CH_2$) | H-11β, H-12α | 2H-12 | H-11β | 18.8 ($CH_2$) | 20.4 |
| 11β | 1.99 (1H, m) | | H-11α, H-12β | | H-11α, H-12β, 3H-19 | | |
| 12α | 2.28 (1H, m) | 22.7 ($CH_2$) | H-11α, H-12β | 3H-18 | H-12β | 23.4‡ | 22.7 |
| 12β | 1.53 (1H, m) | | H-11β, H-12α | | H-11β, H-12α, 3H-19, 3H-26 | | |
| 13 | - | 47.3 (C) | - | 2H-12, 2H-16, 2H-18, 3H-30 | - | 47.7 (C) | 47.3 |
| 14 | - | 57.7 (C) | - | 2H-12, 2H-16, 3H-18, 3H-30 | - | 57.9 (C) | 57.7 |
| 15 | - | 215.7 (C) | - | 2H-16, 3H-30 | - | 215.1 (C) | 215.5 |
| 16α | 2.71 (1H, m) | 51.6 ($CH_2$) | H-16β | - | H-20, 2H-22, H-16β, 3H-30 | 51.9 | 51.6 |
| 16β | 2.16 (1H, m) | | H-16α | | H-16α, 3H-19, 2H-22, 3H-26 | | |
| 17 | - | 90.9 (C) | - | H-16α, 3H-18, 3H-21, 2H-22 | - | 91.2 (C) | 90.9 |
| 18 | 0.89 (3H, s) | 19.7 ($CH_3$) | - | 2H-12 | 3H-21 | 19.9 | 19.1 |
| 19 | 0.87 (3H, s) | 20.2 ($CH_3$) | - | H-5 | H-7β, H-11β, H-12β, H-16β, H-29B | 20.4† | 20.3 |
| 20 | 2.19 (1H, m) | 43.2 (CH) | 3H-21, H-22 | H-16β, 3H-21, 2H-22 | H-16α, 2H-22 | 43.4 (CH) | 43.3 |
| 21 | 1.07 (3H, d, *J*=6.8Hz) | 17.0 ($CH_3$) | H-20 | 2H-22 | 3H-18, 2H-22, H-23 | 17.2 | 17.1 |
| 22 | 1.87 (2H, m) | 36.6 ($CH_2$) | H-20, H-23 | 3H-21 | 2H-16, H-20, 3H-21, H-23, 2H-25, 3H-26 | 27.2 | 36.6 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **23** | 4.62 (1H, t) | 81.5 (CH) | 2H-22 | 2H-22 | | 81.8 (CH) | 81.6 |
| **24** | - | 212.6 (C) | - | 2H-22, 2H-25, 3H-36 | - | 211.7 (C) | 212.1 |
| **25** | 2.44 (2H, q, *J*=7.2Hz) | 32.2 ($CH_2$) | 3H-26 | 3H-26 | 2H-22, H-23, 3H-26, 3H-30 | 36.9 | 32.3 |
| **26** | 1.01 (3H, t, *J*=7.2Hz) | 7.2 ($CH_3$) | 2H-25 | 2H-25 | H-12β, H-22, 2H-25 | 7.6 ($CH_3$) | 7.3 |
| **28** | 1.19 (3H, s) | 22.2 ($CH_3$) | - | H-5 | H-1α, H-3, H-29A | 23.3‡ | 22.4 |
| **29A** | 3.29 (1H, d, *J*=11.0Hz) | 64.2 ($CH_2$) | H-29B | H-5, 3H-28 | 3H-28, H-29B | 64.3 ($CH_2$) | 65.3 |
| **29B** | 4.16 (1H, d, *J*=11.0Hz) | | H-29A | | 3H-19, H-29A | | |
| **30** | 1.32 (3H, s) | 23.6 ($CH_3$) | - | - | H-7α, H-16α, 2H-25 | 24.1 | 23.7 |
| **$\underline{C}H_3CO$ at C-29** | - | - | - | - | - | - | 21.2* |
| **$CH_3\underline{C}O$ at C-29** | - | - | - | - | - | - | 171.1** |
| **$\underline{C}H_3CO$ at C-3** | - | - | - | - | - | - | 21.1* |
| **$CH_3\underline{C}O$ at C-3** | - | - | - | - | - | - | 170.6** |

†, ‡ literature values are reported to be interchangeable, however, from the NMR data of compound II the correct assignments could be made.

*,** values are interchangeable

### 2.2.1.3 Structural Elucidation of Compound III

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

(Spectra 3a-i, pages 233-241)

The third compound to be isolated from *Ledebouria ovatifolia* was isolated as an orange gum and was found to be a derivative of the eucosterol-type nortriterpenoids identified as (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one (Figure 2.9). This compound has been isolated previously from *Muscari comosum*[13] (Hyacinthaceae), *Eucomis montana*[14] and *Ledebouria zebrina*[15].

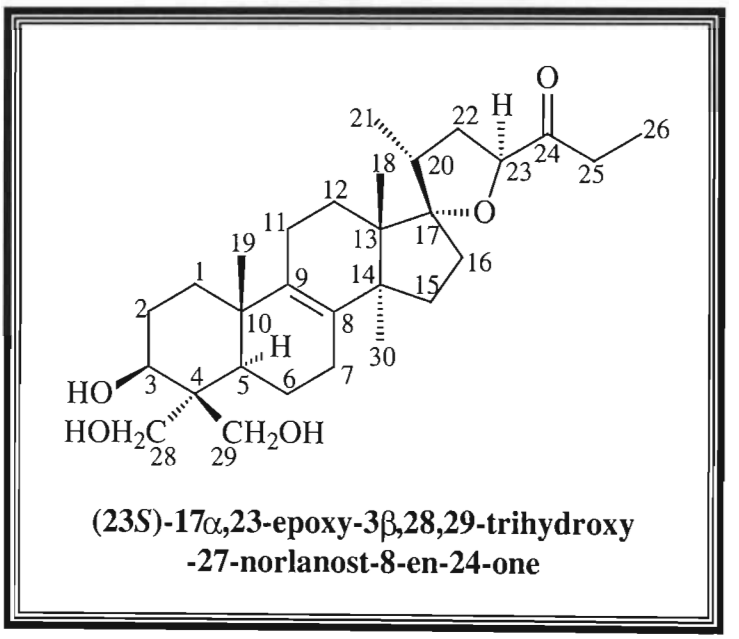



Figure 2.9. The structure of compound III.

The mass spectrum showed a molecular ion peak at *m/z* 474.33398 g $mol^{-1}$, which corresponded to a molecular formula of $C_{29}H_{46}O_5$ ($C_{29}H_{46}O_5$ requires 474.33453). A double bond equivalence of seven was deduced from this molecular formula.

In the infra-red spectrum, peaks were observed at 3437 $cm^{-1}$ (O-H stretching), 2929 $cm^{-1}$ (aliphatic C-H stretching), 2853 $cm^{-1}$ (C-H stretching) and 1731 $cm^{-1}$ (C=O stretching).

The $^1H$ NMR spectrum showed the presence of five methyl group proton resonances, two of which, 3H-21 and 3H-26, were not easily recognised as they were

superimposed at δ 1.04. This was confirmed by the HSQC spectrum which clearly showed the two methyl carbon resonances ascribed to C-21 (δ 17.1) and C-26 (δ 7.4) correlating to the signal at δ 1.04. These methyl group proton resonances were assigned by comparison to eucosterol. In the HMBC spectrum, the resonances due to C-17 (δ 97.0) and C-24 (δ 213.6) showed correlations to the proton signal at δ 1.04, further proving the presence of the 3H-21 and 3H-26 proton resonances at δ 1.04. The C-17 resonance showed a correlation in the HMBC spectrum to the proton singlet at δ 0.86 and this signal was therefore assigned to 3H-18. In the same way, the proton singlet at δ 0.92 was assigned to the resonance at C-19 as it showed a correlation in the HMBC spectrum with C-9. The HMBC spectrum also showed a correlation of the carbon resonance due to C-8 with the remaining three-proton methyl group singlet resonance at δ 1.19. This proton signal was ascribed to the 3H-30 methyl protons. Thus when this compound was compared with eucosterol, it was evident that this compound was one methyl group deficient, which suggested that the methyl groups at C-4 had been modified.

In further comparing this compound with eucosterol, it was observed in the $^{13}C$ NMR spectrum of this compound that only one carbonyl group was present indicating that the C-15 ketone was absent. Furthermore, only five methyl group proton resonances could be seen in the $^{1}H$ NMR spectrum and, in addition, the presence of an additional hydroxy methyl group, whose resonances was observed at δ 3.75 (d, $J$ = 11.2 Hz) and δ 4.13 (d, $J$ = 11.2 Hz) was observed. This group was placed at C-4 because the C-3 (δ 77.6) resonance showed correlations in the HMBC spectrum with the methylene group protons. Thus this resonance was assigned 2H-28. The HMBC spectrum also showed correlation of the methine carbon resonance due to C-3 with the 2H-29 (δ 3.73 and δ 4.33, 2 x d, $J$ = 11.5 Hz) proton signals as in eucosterol.

All the correlations found in the COSY, NOESY and HMBC spectra are tabulated in table 2.3. By comparison with literature[13], this compound was found to be the known compound (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one. Table 2.3 shows the NMR data and correlations for compound III as well as the literature NMR values for this compound.

**Table 2.3. NMR data and correlations for compound III ($CDCl_3$) and literature data for (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one ($CDCl_3$)[13]**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR data for (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one[13] |
|---|---|---|---|---|---|---|
| **1** | 1.72 (2H, m) | 35.2 ($CH_2$) | - | 3H-19 | 3H-19 | 35.3 |
| **2** | 1.76 (2H, m) | 27.3 ($CH_2$) | H-3 | 2H-1 | 3H-19 | 27.5 |
| **3** | 3.72 (1H, m) | 77.6 (CH) | H-2 | 2H-28, 2H-29 | 2H-28 | 77.86 |
| **4** | - | 45.8 (C) | - | H-5 | - | 45.96 |
| **5** | 1.25 (1H, m) | 47.0 (CH) | H-6 | 3H-19, 2H-28, 2H-29 | H-7 | 47.29 |
| **6** | 1.46 (2H, m) | 18.6 ($CH_2$) | H-5 | - | 3H-19 | 18.72 |
| **7** | 1.98 (2H, m) | 26.1 ($CH_2$) | - | - | H-5 | 26.24 |
| **8** | - | 133.9 (C) | - | 3H-30 | - | 134.14 |
| **9** | - | 135.2 (C) | - | 3H-19 | - | 134.48 |
| **10** | - | 36.7 (C) | - | H-5, 3H-19 | - | 36.50 |
| **11α** | 2.12 (1H, m) | 20.7 ($CH_2$) | 2H-12 | - | - | 20.78 |
| **11β** | 1.94 (1H, m) | | 2H-12 | | 2H-12, 3H-19 | |
| **12α** | 1.38 (1H, m) | 24.8 $CH_2$) | 2H-11 | 3H-18 | - | 24.91 |
| **12β** | 2.18 (1H, m) | | 2H-11 | | 3H-18, 3H-21 | |
| **13** | - | 48.5 (C) | - | 3H-18, 3H-30 | - | 48.69 |
| **14** | - | 50.4 (C) | - | 3H-18, 3H-30 | - | 50.55 |
| **15α** | 1.60 (1H, m) | 31.6 ($CH_2$) | 2H-16 | 3H-30 | 3H-30 | 31.70 |
| **15β** | 1.32 (1H, m) | | 2H-16 | | H-16β | |
| **16α** | 2.15 (1H, m) | 39.6 ($CH_2$) | 2H-15 | - | - | 39.70 |
| **16β** | 1.60 (1H, m) | | 2H-15 | | H-15β, 3H-18, H-20, 3H-21 | |
| **17** | - | 97.0 (C) | - | 3H-18, 3H-21, 2H-22 | - | 97.18 |
| **18** | 0.86 (3H, s) | 19.4 ($CH_3$) | - | 3H-21 | H-12β, H-16β, H-20, 3H-21 | 19.51 |
| **19** | 0.92 (3H, s) | 19.1 ($CH_3$) | - | - | 2H-1, 2H-6, 2H-7, H-29B | 19.23 |
| **20** | 2.14 (1H, m) | 43.5 (CH) | 3H-21 | 3H-21 | H-16β, 3H-18 | 43.63 |
| **21** | 1.04 (3H, d) | 17.1 ($CH_3$) | H-20 | 2H-22 | H-12β, 3H-18, H-23 | 17.18 |
| **22** | 1.78 (2H, m) | 36.7 ($CH_2$) | H-23 | 3H-21 | 3H-21, H-23 | 36.77 |
| **23** | 4.64 (1H, dd, *J* =7.3, 10.4 Hz) | 81.4 (CH) | H-22 | - | 3H-21, 2H-22, 2H-25 | 81.52 |
| **24** | - | 213.6 (C) | - | 3H-26 | - | 213.63 |
| **25** | 2.53 (2H, q, *J*=7.33 Hz) | 32.2 ($CH_2$) | 3H-26 | 3H-26 | H-23, 3H-26 | 32.30 |
| **26** | 1.04 (3H, t) | 7.4 ($CH_3$) | 2H-25 | 2H-25 | H-25 | 7.39 |
| **28A** | 3.75 (1H, d, *J*=11.2 Hz) | 70.9 ($CH_2$) | H-28B | H-5, H-29A | H-28B | 71.27 |
| **28B** | 4.13 (1H, d, *J*=11.2 Hz) | | H-28A | | - | |
| **29A** | 3.75 (1H, d, *J*=11.5 Hz) | 63.6 ($CH_2$) | H-29B | H-3, H-5, 3H-28 | 3H-19, H-29B | 63.81 |
| **29B** | 4.33 (1H, d, *J*=11.5 Hz) | | H-29A | | - | |
| **30** | 1.19 (3H, s) | 25.8 ($CH_3$) | - | 3H-18 | H-15α, H-16β | 25.91 |

## 2.2.2 Foreword to Experimental Sections

*Nuclear Magnetic Resonance (NMR) Spectroscopy*

All the NMR spectra were recorded using a 300 MHz Varian Gemini NMR spectrometer or a Varian Unity Inova 400 MHz NMR spectrometer. The spectra were obtained using the solvents deuterated chloroform ($CDCl_3$) and deuterated methanol ($CD_3OD$). For the NMR data, chemical shifts are expressed in $\delta$ (ppm) from tetramethylsilane as an internal standard and coupling constants ($J$) are given in hertz (Hz). The spectra were referenced against the central line of the deuteriochloroform signal at $\delta_C$ 77.0, the $C\underline{H}Cl_3$ singlet at $\delta_H$ 7.24, the deuteriomethanol signal at $\delta_C$ 49.0 or the $C\underline{H}D_2OD$ signal at $\delta_H$ 3.34.

*Infra red (IR) Spectroscopy*

The infrared spectra were recorded using a Nicolet Impact 400 D spectrometer. In all cases the dissolved sample was dropped onto a NaCl disc and the spectrometer was calibrated against air.

*Ultra violet (UV) Spectroscopy*

UV absorption spectra were obtained on a Varian DMS 300 UV-visible spectrophotometer using dichloromethane as a solvent. The NaOAc and $AlCl_3$ used for the bathochromic shift tests were prepared by dissolving 0.5 g of each salt (anhydrous) in 100 $cm^3$ redistilled methanol.

*Mass Spectrometry (MS)*

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

High resolution mass spectra were also run by Mr John Hill at Kent Mass Spectrometry in London on a VG (now Waters) 70-SE magnetic sector by direct insertion probe using an accelerating voltage of 8KV and a mass range of 3000. Electron impact (EI) used an ionising potential of 70 eV, 100 $\mu$A trap current and a

source temperature of 200°C, while Fast Atom Bombardment (FAB) was performed using a Ceasium ion gun, Matrix:MNOBA.

Low resolution mass spectra (LRMS) were recorded by Dr L Fourie at the Potchefstroom University, who performed EI analyses on a Micromass Autospec-TOF. The samples were run in EI mode at 70eV.

***Melting Point Determination***

A Kofler micro-hot stage melting point apparatus was used to record the melting points of all the crystalline compounds isolated in this work. All values are uncorrected.

***Optical Rotation (OR)***

All the optical rotations, except those for compounds VI, VIII, X, XXI and XXII were recorded at room temperature in chloroform on an Optical Activity Ltd AA-5 automatic polarimeter together with a series A2 stainless steel (4 x 200 mm) unjacketed flow tube. The optical rotation for compounds VI, VIII, X, XXI and XXII were recorded at room temperature in chloroform or methanol on a Perkin Elmer polarimeter (Model 341).

***Column Chromatography***

Glass columns ranging from 1 cm to 4 cm in diameter were used in the separation procedure. Merck 9385 silica gel was used as the solid phase and elution was allowed to proceed *via* gravity. Solvents used included hexane, dichloromethane, ethyl acetate and methanol in varying proportions depending on the polarity of compounds being isolated.

***Thin Layer Chromatography (TLC)***

The progress of column chromatography was monitored using thin layer chromatographic plates. Aluminium backed plates (Merck Art 5554) coated with silica gel (0.2 mm thick) and containing a fluorescent indicator (F254) were used. The plates were developed using anisaldehyde spray reagent consisting of anisaldehyde, conc. $H_2SO_4$ and methanol in a ratio of 1:2:97, followed by heating of the plates.

***Anti-inflammatory and anti-bacterial Screening***

Biological activity screening of compounds I, IV, V, XIV, XVII, XVIII, XIX and XX, was performed by Karen du Toit at the University of Natal, Pietermaritzburg. The anti-inflammatory activity screening involved the use of microsomal cells and the enzymes, Cox-1 and Cox-2. Cox-1 and Cox-2 are the cyclooxygenase enzymes that, in the arachidonic acid cascade, are involved in the formation of prostaglandins. The percentage inhibition is measured against indomethacin as a positive control. The indomethacin's activity ranged between 70-80% in the microsomal cells and 60-70% in the Cox-1 assay. The anti-bacterial activity testing was performed on compound IV. This activity is measured as the minimum inhibitory concentration (MIC), which is the lowest concentration at which the bacterium, *Staphylococcus aureus* is inhibited. The concentration of sample used in the anti-inflammatory screening was 10 $\mu g/cm^3$ of solvent, and was the same for all samples submitted.

## 2.2.3 Experimental

The bulbs (2168 g) of *Ledebouria ovatifolia* (Highveld form) were collected from the Blyde Nature Reserve by Dr. Neil Crouch. A voucher specimen (N. Crouch 854) is retained at the Natal Herbarium. The bulbs were chopped into small pieces and extracted with dichloromethane and methanol using continuous agitation for approximately 48 hours. The excess solvents were removed using the rotor evaporator resulting in the dichloromethane (15.81g) and methanol (115.89 g) crude extracts. The methanol extract was partitioned with ethyl acetate resulting in an ethyl acetate crude extract of 5.49 g.

***Isolation of compounds I-III***

The dichloromethane and ethyl acetate extracts were chromatographed using silica gel (Merck 9385) as the stationary phase on a crude column (3 cm in diameter) in order to separate the compounds.

The mobile phase used for the dichloromethane extract was a dichloromethane : methanol step gradient [100 % dichloromethane (fractions 1-20), 1% methanol in dichloromethane (fractions 21-45), 2% methanol in dichloromethane (fractions 46-

60), 5% methanol in dichloromethane (fractions 61-80), 10% methanol in dichloromethane (fractions 81-100), 20% methanol in dichloromethane (fractions 100-120), 100% methanol (fractions 121-130)]. Fractions of 40 $cm^3$ each were collected in each step. Elution using a 5% methanol in 95% dichloromethane solvent system afforded compounds I and II. Compound I was purified using the same mobile phase on a column (2 cm in diameter) with silica gel as the stationary phase, while compound II eluted pure from the crude column. Elution with 10 % methanol in dichloromethane yielded compound III, which was purified using the same mobile phase on a column (1 cm in diameter).

The ethyl acetate extract contained the same compounds isolated from the dichloromethane extract, while the methanol extract contained a large amount of sugar.

***Acetylation of compound II***

Approximately 10 mg of the compound was placed in a stoppered round-bottomed flask with pyridine (1 $cm^3$) and acetic anhydride (1 $cm^3$). The contents of the flask were allowed to react at room temperature for 24 hours. Thereafter, methanol (3 x 10 $cm^3$) was added to remove the excess acetic anhydride, and the pyridine was removed by the addition of toluene (3 x 10 $cm^3$). The final traces of toluene were removed by further addition of methanol (3 x 10 $cm^3$). All solvents were removed on a rotor evaporator.

### 2.2.3.1 Physical data for Compound I

**Name:** (-)-(*Z*)-1-(4'-hydroxyphenyl)-3ξ-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene

**Physical appearance:** yellow gum

**Yield:** 129.1 mg

**Mass:** HRMS [$M^+$] at *m/z* 312.13690, $C_{19}H_{20}O_4$ requires 312.13616

EIMS: *m/z* (rel. int.): 312 (100.00), 297 (13.37), 295 (9.32), 281(17.23), 265 (7.33), 218 (5.14), 205 (19.12), 204 (10.39), 203 (7.88), 181 (6.21), 179 (5.32), 173 (17.16), 167 (9.58), 165 (5.58), 159 (5.60), 158 (18.56), 157 (11.24), 145 (7.58), 133 (5.15), 115 (8.82), 107 (40.96), 91 (6.55), 77 (6.28), 71 (6.54), 69 (5.51), 57 (8.06), 55 (6.11)

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3412, 2923, 2847, 1355, 1230, 1105

**Optical Rotation:** $[\alpha]_D = -161.9°$ (c = 0.278 g/100 $cm^3$; $CHCl_3$)

**$^1$H and $^{13}$C NMR Data**: Refer to table 2.1 (page 61)

### 2.2.3.2 Physical data for Compound II

**Name:** (23*S*)-17α,23-epoxy-3β, 29-dihydroxy-27-norlanost-8-ene-15,24-dione

**Synonyms:** eucosterol

**Physical appearance:** white needle-like crystals

**Yield:** 293.6 mg

**Melting Point:** 234-236°C, Literature = 235-236°C [12]

**Mass:** LRMS [$M^+$] at *m/z* 472.3189, $C_{29}H_{44}O_5$ requires 472.3189

EIMS: *m/z* (rel. int.): 472 (32.15), 454 (10.72), 439 (7.70), 415 (15.07), 414 (21.58), 397 (14.79), 379 (8.68), 343 (11.37), 303 (24.78), 257 (10.88), 239 (11.01), 181 (18.69), 171 (14.91), 159 (15.02), 157 (14.12), 155 (97.53), 137 (16.28), 135 (19.60), 133 (17.18), 129 (14.95), 125 (24.45), 123 (27.48), 121 (24.57), 113 (15.94), 111 (30.90), 109 (45.34), 99 (30.61), 97 (36.28), 83 (70.39), 69 (100.00), 57 (43.94), 55 (70.52), 43 (94.42)

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3488, 2943, 2878, 1740, 1731, 1289, 1038

**Optical Rotation:** $[\alpha]_D$ = +23.0° (c = 0.217 g/ 100$cm^3$; $CHCl_3$),

Literature $[\alpha]_D^{25}$ = +20.4 ± 2° (c = 1.00, $CHCl_3$)[12]

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.2 (pages 65 and 66)

### 2.2.3.3 Physical data for Compound III

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

**Physical appearance:** orange gum

**Yield:** 21.7mg

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

EIMS: *m/z* (rel. int.): 474 (51), 459 (27), 456 (8), 417 (25), 57 (100)

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3437, 2929, 2853, 1731

**Optical Rotation:** $[\alpha]_D$ = -22.7° (c = 0.088 g/100 $cm^3$; $CHCl_3$),

Literature $[\alpha]_D$ = -27.0° (c = 0.5)[13]

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.3 (page 69)

## 2.3 Extractives from *Eucomis pole-evansii*

Plants of the genus *Eucomis* (subfamily: Hyacinthoideae, family: Hyacinthaceae) are perennial bulbous herbs, which can either grow solitary or gregariously. The bulb is large and oblong in shape with the outer scales being dark and membranous[4]. Approximately eleven species are found in the area ranging from South Africa to Zimbabwe and Malawi. *Eucomis* species are widespread but are absent from drier areas, thus they are mainly found in grasslands, forests, swamps and along riverbanks[4]. These plants are used in traditional medicine and also make beautiful garden plants due to their unusual shape. The common name 'pineapple lily' is derived from the raceme, which resembles a pineapple[4].

Figure 2.10. Photograph of *Eucomis pole-evansii* (Photographed by Dr. Neil Crouch).

### 2.3.1 Results and Discussion

The dichloromethane extract of *Eucomis pole-evansii* N.E.Br. yielded two homoisoflavonoids, **compound IV** being a 3-benzyl-4-chromanone type homoisoflavonoid and **compound V** being a 3-benzylidene-4-chromanone type homoisoflavonoid. This extract also yielded the same nortriterpenoid, eucosterol, that was previously isolated from *Ledebouria ovatifolia*. The methanol extract was investigated, however it contained the same compounds that were found in the dichloromethane extract as well as a large amount of sugar.

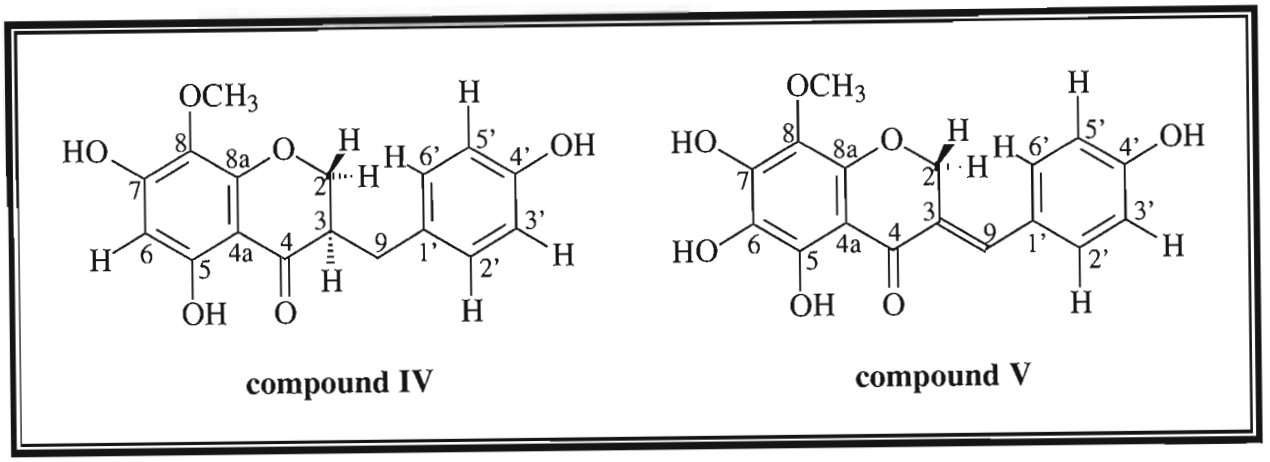



Figure 2.11. The structures of compounds IV and V.

### 2.3.1.1 Structural Elucidation of Compound IV

### (-)-[R]-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone

(Spectra 4a-p, pages 242-257)

The first compound to be isolated from *E. pole-evansii* was isolated as yellow crystals and was identified as (-)-[R]-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone, commonly known as 3,9-dihydropunctatin. This compound was previously isolated from *Muscari comosum*[16].

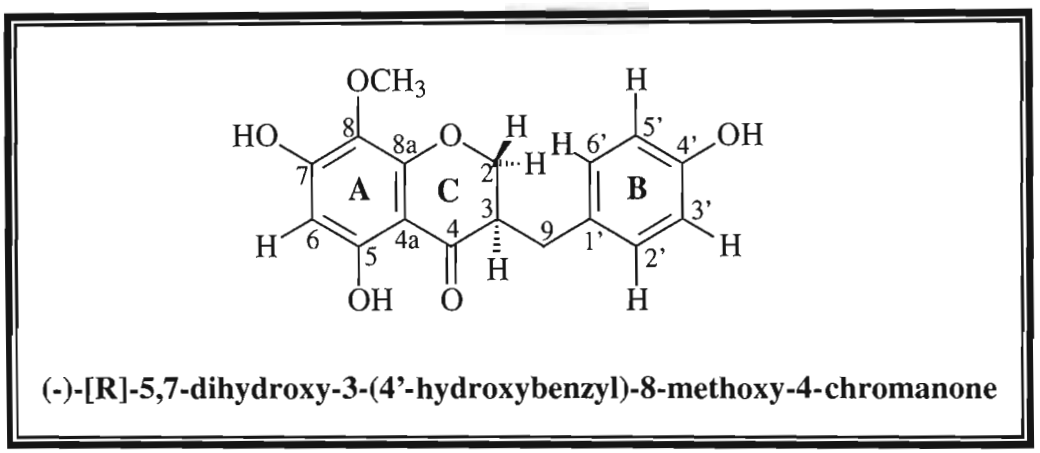
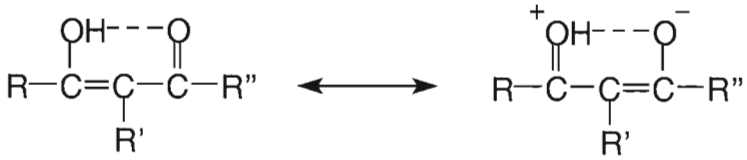



Figure 2.12. The structure of compound IV.

The mass spectrum revealed compound IV to have a molar mass of 316.09469 g $mol^{-1}$, which corresponded to a molecular formula of $C_{17}H_{16}O_6$ ($C_{17}H_{16}O_6$ requires 316.09469) and a double bond equivalence of ten.

The infra-red spectrum of compound IV showed an absorption at 3390 $cm^{-1}$ due to the stretching vibrations of the hydroxy group. Absorptions were also evident at 2918 $cm^{-1}$ (C-H asymmetric stretching) and 2847 $cm^{-1}$ (C-H symmetric stretching). The C=O stretching vibration was observed at 1637 $cm^{-1}$ and is shifted considerably as β-hydroxy ketones have a chelation effect which causes a shift in the carbonyl frequency[17]. 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[17] and explains the low frequency absorption stretch of compound IV.

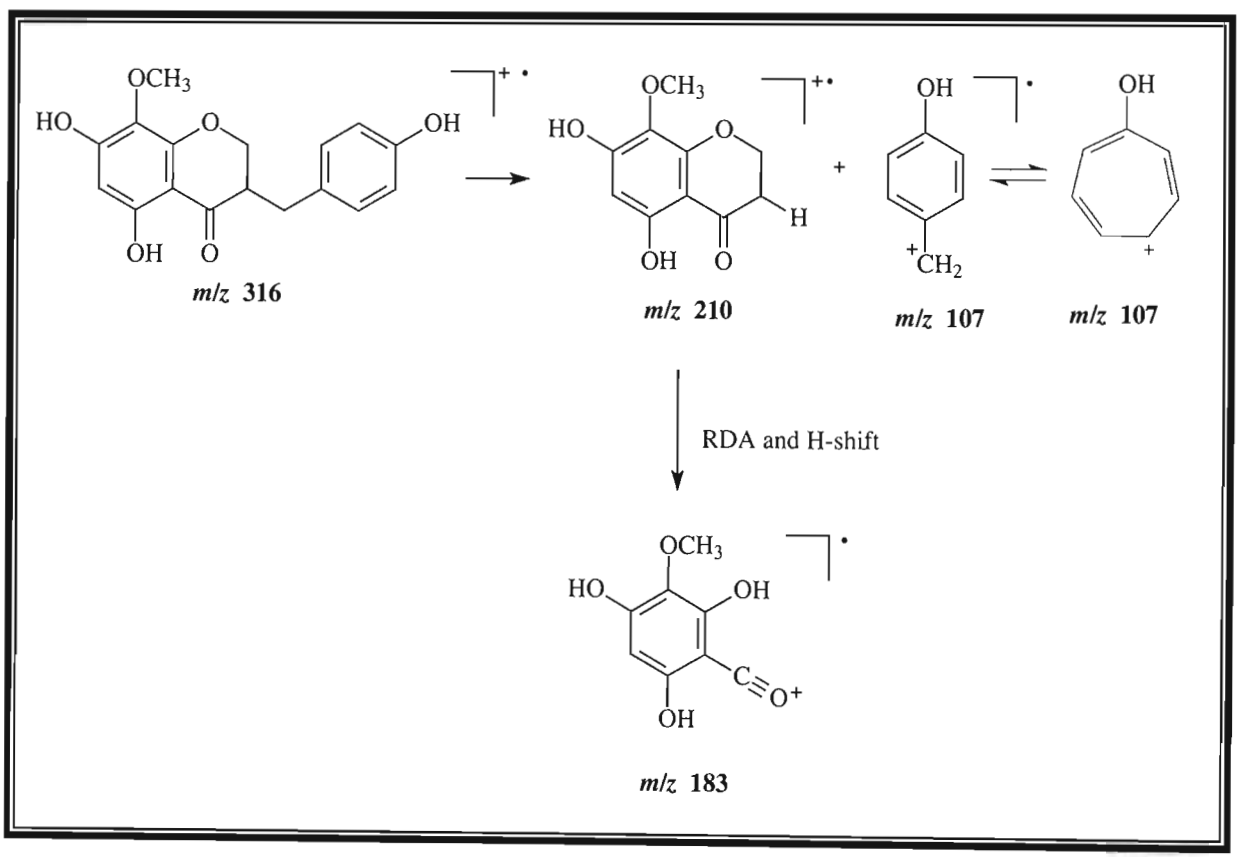
The absorption at 1518 $cm^{-1}$ in the infra-red spectrum was due to the aromatic C=C stretching. The $CH_2$ and $CH_3$ bending vibrations were observed at 1452 $cm^{-1}$, 1268 $cm^{-1}$ and 1165 $cm^{-1}$, while the absorption at 1018 $cm^{-1}$ was due to the C-O stretching.

The NMR data for compound IV indicated that it was a homoisoflavanone of the 3-benzyl-4-chromanone type. The definitive $^1H$ NMR resonances were the two pairs of double doublets at δ 4.35 (1H, $J$ = 4.1, 11.3 Hz), δ 4.18 (1H, $J$ = 7.2, 11.3 Hz) and δ 3.17 (1H, $J$ = 4.1, 13.8), δ 2.69 (1H, $J$ = 10.1, 13.8) and the multiplet at δ 2.82 (1H), which are typical of the two ABX systems for the 2H-2, 2H-9 and H-3 protons respectively[18].

In the $^1H$ NMR spectrum a pair of doublets, each integrating to two protons, was observed at δ 7.08 ($J$ = 8.3 Hz) and δ 6.78 ($J$ = 8.3 Hz). These signals were assigned to the respective H-2'/6' and H-3'/5' protons of the B-ring and the corresponding methine carbons were found at δ 130.3 (C-2'/6') and δ 115.6 (C-3'/5'). In the COSY spectrum, coupling was observed between the H-2'/6' proton resonance and the H-3'/5' proton resonance. A coupling constant of 8.3 Hz is indicative of a *para* substituted aromatic ring, thus the C-4' (δ 154.4) position must be substituted. The

H-2'/6' resonance exhibited correlations in the NOESY spectrum with H-3'/5', 2H-9 and H-3, and its corresponding methine carbon showed a correlation in the HMBC spectrum with 2H-9. The methylene carbon resonance ascribed to C-9 was observed at δ 31.9 and showed correlations in the HMBC spectrum with the 2H-2 and H-2'/6' resonances.

The mass spectrum of homoisoflavonoids is used to identify the substitution pattern on the B ring. In general, an intense peak at *m/z* 121 is indicative of an A-4 and retro Diels Alder (RDA) fragmentation pattern and this corresponds to a methoxybenzyl/methoxytropylium ion[19]. However, a base peak at *m/z* 107 is indicative of a hydroxybenzyl/hydroxytropylium ion[19]. In compound IV a peak at *m/z* 107 is observed indicating that a hydroxy group is positioned at C-4' (Scheme 2.1).




Scheme 2.1. The MS fragmentation pattern for compound IV.

In the $^{13}C$ NMR spectrum of homoisoflavonoids, the C-5, C-6 and C-8 chemical shifts are generally used to confirm the A-ring substitution pattern. The C-5 resonance usually occurs at δ 165.8 when there is a 5,7-dihydroxy substitution pattern[20].

However, the presence of a methoxy substituent at C-6 results in C-5 resonating about 9.0 ppm upfield from the norm[20]. Furthermore when C-6 or C-8 is unsubstituted, these resonances are observed at δ 97.1-97.3 and δ 95.8-96.0 respectively if C-7 carries a hydroxy group. When C-7 carries a methoxy group, these resonances are at a higher field[20].

The only position that was protonated on the A ring was the C-6 position (δ 95.9, CH), which corresponded to the singlet signal in the $^1$H NMR spectrum at δ 6.11. The C-5 and C-4a resonances showed correlations in the HMBC spectrum to H-6 and the hydroxyl group proton at C-5, while the C-8 resonance showed correlations in the HMBC spectrum to H-6, the hydroxyl group proton at C-7 and methoxy group protons at C-8. The C-7 resonance could be assigned as this resonance showed a correlation in the HMBC spectrum to H-6 and the hydroxyl group proton at C-7, while the C-8a resonance was seen to be correlated in the HMBC spectrum with 2H-2.

The sharp singlet at δ 3.38, integrating to three protons, was due to the presence of protons of a methoxy group. The corresponding methyl carbon resonance appeared at δ 61.5. In the HMBC spectrum the 3H-methoxy group protons showed a correlation to C-8, indicating that the methoxy group occurred at C-8.

The ultra violet (UV) spectra are useful in determining the substitution pattern on the A ring of homoisoflavonoids when the compounds are analysed using NaOAc and $AlCl_3$. The original UV spectra usually give absorption maxima between 295-310 nm. However, when NaOAc or $AlCl_3$ solutions are added, this causes a shift in the maxima in certain instances. For example, when a bathochromic shift is observed with NaOAc, this indicates the presence of a hydroxy group at the C-7 position[16, 21], while a bathochromic shift with $AlCl_3$, indicates the presence of a hydroxy group at the C-5 position[16, 21]. The absence of a shift implies a methoxy group is present at the respective positions.

In this compound, a bathochromic shift (+36 nm) was observed with NaOAc confirming that a hydroxy group occurred at C-7. The H-7 hydroxy group proton resonance occurred as a singlet at δ 6.39 and showed a correlation in the HMBC

spectrum to C-6. The $^{1}H$ NMR spectrum showed a singlet resonance at δ 11.95 due to the hydroxy group protons, which indicated the presence of a hydroxy group at C-5. Further evidence of a hydroxy group at C-5 was revealed by a positive bathochromic shift (+19 nm) in the UV spectrum with $AlCl_3$. This was confirmed by the presence of the C-4 carbonyl resonance at δ 197.7. The carbonyl carbon resonance is deshielded due to chelating effects with the hydroxy group and this causes it to be shifted downfield in the $^{13}C$ NMR spectrum to almost δ 200.0[20]. In the event of a methoxy group being present at C-5, the carbonyl carbon would resonate much further upfield of δ 200.0. In the HMBC spectrum the fully substituted C-4 carbon resonance showed correlations with 2H-9, 2H-2 and H-3(w).

Five fully substituted carbon resonances were observed for carbons of the A ring and were assigned to the signals at δ 157.7 (C-5), δ 102.4 (C-4a), δ 127.6 (C-8), δ 160.2 (C-7) and δ 153.0 (C-8a). The absolute stereochemistry at C-3 has been determined by Professor D. Ferreira at the University of Mississippi, USA, for a series of homoisoflavanones of this type from the group using circular dichroism. In all cases the absolute stereochemistry at C-3 was shown to be R, implying that H-3 must be α. It is assumed that the stereochemistry would be the same for compound IV.

By comparison with the literature data[16], compound IV was identified as the known homoisoflavanoid, (-)-[R]-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone. Compound IV, at a concentration of 10 $\mu g/cm^3$, showed a 28.2% inhibition of prostaglandin synthesis using the microsomal cell screen and a 2.5% inhibition using the Cox-2 screen (page 72). This compound also showed an anti-bacterial activity at a MIC value of 0.98mM. This minimum inhibitory concentration (MIC) is the lowest concentration at which the bacterium, *Staphylococcus aureus* is inhibited.

The data for compound IV, as well as the literature data is given in Table 2.4. The $^{13}C$ NMR data for compound IV did not correlate well with that of literature and this may be due to these compounds being run in different solvents. However, using HMBC correlations it was possible to make conclusive assignments for compound IV. The HMBC and NOESY correlations for compound IV are shown in figure 2.13 and

figure 2.14 respectively.

In order to confirm the structure, compound IV was acetylated. In the $^1$H NMR spectrum, three singlet peaks, each integrating to three protons, were observed at δ 2.36, δ 2.32, and δ 2.28 which were assigned to the acetate groups at C-5 (δ 145.6, C), C-7 (δ 148.6, C) and C-4' (δ 149.5, C) respectively. In the NOESY spectrum, the acetate group at C-5 was seen to be correlated with the proton peak ascribed to H-6, the acetate group at C-7 showed a correlation with the proton signal due to the methoxy group at C-8 (δ 138.8, C) and the acetate group at C-4' was seen to be correlated with the proton resonance assigned to H-3'/5'. Thus the positions of the hydroxy groups in compound IV were confirmed. The NMR data and correlations for the acetylated compound IV are shown in table 2.5.

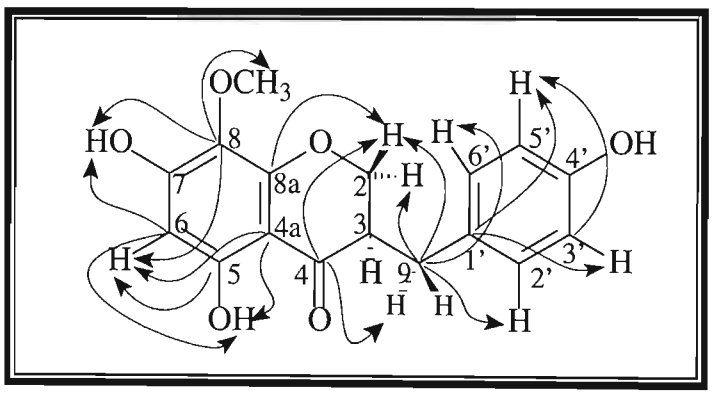



Figure 2.13. The HMBC (C → H) correlations for compound IV.

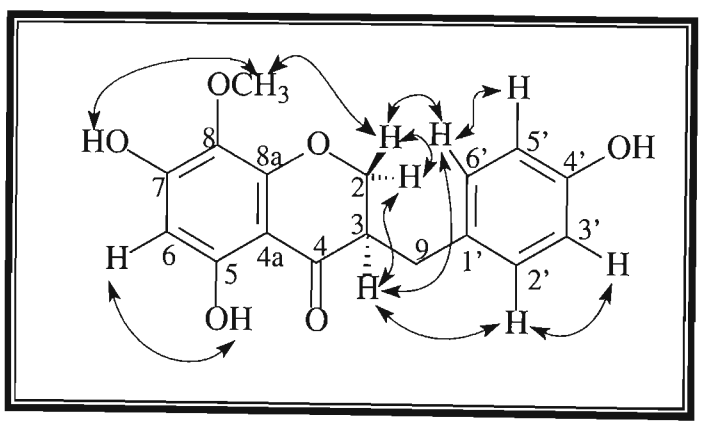
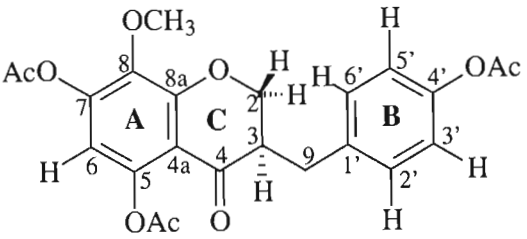



Figure 2.14. The NOESY correlations for compound IV.

**Table 2.4. NMR data and correlations for compound IV ($CDCl_3$) and literature data for 3,9-dihydropunctatin ($CD_3OD$)[16]**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for 3,9-dihydropunctatin[16] (100 MHz) |
|---|---|---|---|---|---|---|
| 2A | 4.35 (1H, dd, *J*=4.1, 11.3 Hz) | 69.4 ($CH_2$) | H-2B, H-3 | 2H-9 | H-2B, H-3 | 70.5 |
| 2B | 4.18 (1H, dd, *J*=7.2, 11.3 Hz) | | H-2A, H-3 | | H-2A, H-3, H-9B, H-2'/6' | |
| 3 | 2.82 (1H, m) | 46.8 (CH) | H-2A, H-2B, H-9A, H-9B | H-2A, 2H-9 | H-2A, H-2B, H-9A, H-9B H-2'/6' | 48.0 |
| 4 | - | 197.7 (C) | - | 2H-2, H-3, H-9B | - | 199.3 |
| 4a | - | 102.4 (C) | - | H-6, OH at C-5 | - | 102.8 |
| 5 | - | 157.7 (C) | - | H-6, OH at C-5 | - | 161.0† |
| 6 | 6.11 (1H, s) | 95.9 (CH) | - | OH at C-5, OH at C-7 | OH at C-5 | 97.2 |
| 7 | - | 160.2 (C) | - | H-6, OH at C-7 | - | 161.6† |
| 8 | - | 127.6 (C) | - | H-6, OH at C-7, $OCH_3$ at C-8 | - | 130.1 I |
| 8a | - | 153.0 (C) | - | 2H-2 | - | 155.6 |
| 9A | 3.17 (1H, dd, *J*=4.1, 13.8 Hz) | 31.9 ($CH_2$) | H-3, H-9B, | 2H-2, H-2'/6' | H-3, H-9B, H-2'/6' | 33.1 |
| 9B | 2.69 (1H, dd, *J*=4.1, 13.8 Hz) | | H-3, H-9A | | H-2B, H-3, H-9A, H-2'/6' | |
| 1' | - | 129.8 (C) | - | H-3, H-3'/5' | - | 129.8 I |
| 2' | 7.08 (1H, d, *J*=8.3 Hz) | 130.3 (CH) | H-3'/5' | 2H-9, H-2'/6' | H-2B, H-3, H-9A, H-9B, H-3'/5' | 131.1 |
| 3' | 6.78 (1H, d, *J*=8.3 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5' | H-2'/6' | 116.4 |
| 4' | - | 154.4 (C) | - | H-2'/6', H-3'/5' | - | 157.1† |
| 5' | 6.78 (1H, d, *J*=8.3 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5 | H-2'/6' | 116.4 |
| 6' | 7.08 (1H, d, *J*=8.3 Hz) | 130.3 (CH) | H-3'/5' | 2H-9, H-2'/6' | H-2B, H-3, H-9A, H-9B, H-3'/5' | 131.1 |
| $OCH_3$ at C-8 | 3.83 (3H, s) | 61.5 ($CH_3$) | - | - | 2H-2(w), OH at C-7(w) | 61.5 |
| OH at C-5 | 11.95 (1H, s) | - | - | - | H-6 | - |
| OH at C-7 | 6.39 (1H, s) | - | - | - | $OCH_3$ at C-8(w) | - |

†, I values given as interchangeable in literature




**[R]-5,7-diacetoxy-8-methoxy-3-(4'-acetoxybenzyl)-4-chromanone**

Figure 2.15. The structure of acetylated compound IV.

**Table 2.5. NMR data and correlations for the acetylated compound IV ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 2A | 4.43 (1H, dd, *J*=4.4, 11.5 Hz) | 69.5 ($CH_2$) | H-2B, H-3 | H-9A, H-9B | H-2B, H-3, H-2'/6'(w) |
| 2B | 4.23 (1H, dd, *J*=8.8, 11.5 Hz) | | H-2A, H-3 | | H-2A, H-3, H-9B, H-2'/6'(w) |
| 3 | 2.84 (1H, m) | 47.8 (CH) | H-2A, H-2B, H-9A, H-9B | - | H-2A, H-2B, H-9A, H-9B, H-2'/6' |
| 4 | - | 190.9 (C) | - | H-2A, H-2B | - |
| 4a | - | 112.5 (C) | - | H-6 | - |
| 5 | - | 145.6† (C) | - | H-6 | - |
| 6 | 6.45 (1H, s) | 110.9 (CH) | - | - | 3H-OAc at C-5 |
| 7 | - | 148.6† (C) | - | H-6 | - |
| 8 | - | 138.8 (C) | - | H-6, $OCH_3$ at C-8 | - |
| 8a | - | 156.4 (C) | - | H-2A, H-2B | - |
| 9A | 3.24 (1H, dd, *J*=4.3, 14.2 Hz) | 31.5 ($CH_2$) | H-3, H-9B | H-2A, H-2'/6' | H-3, H-9B, H-2'/6' |
| 9B | 2.63 (1H, dd, *J*=10.6, 14.1 Hz) | | H-3, H-9A | | H-2B(w), H-3, H-9A, H-2'/6' |
| 1' | - | 135.4 (C) | - | H-9A, H-9B, H-3'/5' | - |
| 2' | 7.19 (1H, d, *J*=8.4 Hz) | 130.0 (CH) | H-3'/5' | H-9A, H-9B, H-2'/6' | 2H-2(w), H-3, H-9A, H-9B, H-3'/5' |
| 3' | 7.02 (1H, d, *J*=8.6 Hz) | 121.9 (CH) | H-2'/6' | H-3'/5' | H-2'/6', 3H-OAc at C-4' |
| 4' | - | 149.5 (C) | - | H-2'/6', H-3'/5' | - |
| 5' | 7.02 (1H, d, *J*=8.6 Hz) | 121.9 (CH) | H-2'/6' | H-3'/5' | H-2'/6', 3H-OAc at C-4' |
| 6' | 7.19 (1H, d, *J*=8.4 Hz) | 130.0 (CH) | H-3'/5' | H-9A, H-9B, H-2'/6' | 2H-2(w), H-3, H-9A, H-9B, H-3'/5' |
| $OCH_3$ at C-8 | 3.81 (s) | 61.0 ($CH_3$) | - | - | 3H-OAc at C-7 |
| $\underline{C}H_3CO_2$ at C-5 | 2.36 (s) | 21.0 ($CH_3$) | - | - | H-6 |
| $\underline{C}H_3CO_2$ at C-7 | 2.32 (s) | 20.7 ($CH_3$) | - | - | $OCH_3$ at C-8 |
| $\underline{C}H_3CO_2$ at C-4' | 2.28 (s) | 21.1 ($CH_3$) | - | - | H-3'/5' |
| $CH_3\underline{C}O_2$ at C-5 | - | 169.5 (C) | - | 3H-OAc at C-5 | - |
| $CH_3\underline{C}O_2$ at C-7 | - | 167.9 (C) | - | 3H-OAc at C-7 | - |
| $CH_3\underline{C}O_2$ at C-4' | - | 169.5 (C) | - | 3H-OAc at C-4' | - |

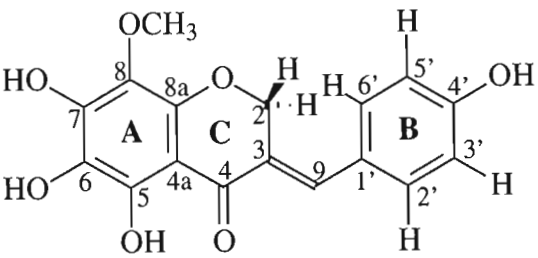
† Values may be interchangable

### 2.3.1.2 Structural Elucidation of Compound V

### (*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone

(Spectra 5a-i, pages 258-266)

The second compound to be isolated from *Eucomis pole-evansii* was isolated as yellow crystals and was identified as the novel compound (*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone (compound V).




**(*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone**

Figure 2.16. The structure of compound V.

The mass spectrum indicated a molar mass of 330 g $mol^{-1}$, which corresponded to a molecular formula of $C_{17}H_{14}O_7$ ($C_{17}H_{14}O_7$ requires 330) and a double bond equivalence of eleven. Further fragments were observed at *m/z* 298 ($M^+$ - $CH_3OH$) and *m/z* 107. The latter indicated the presence of a hydroxytropylium ion.

The infra-red spectrum of compound V showed an absorption at 3381 $cm^{-1}$, which was due to the O-H stretching vibration. Absorption peaks were also observed at 2917 $cm^{-1}$ and 2846 $cm^{-1}$, which were due to the C-H asymmetric and symmetric stretching respectively. The C=O stretching vibration was observed at 1634 $cm^{-1}$, while the aromatic C=C stretching was observed at 1514 $cm^{-1}$. The $CH_2$ and $CH_3$ bending absorptions were observed at 1378 $cm^{-1}$, 1307 $cm^{-1}$ and 1170 $cm^{-1}$, and the absorption peak at 1034 $cm^{-1}$ was due to the C-O stretching.

The appearance of the resonances at δ 5.32 (2H, s, 2H-2) and δ 7.72 (1H, s, H-9) in the $^{1}$H NMR spectrum, as well as the appearance of an α,β-unsaturated carbonyl carbon resonance at δ 185.3 (C-4, C) in the $^{13}$C NMR spectrum, indicated the presence of a 3-benzylidene (i.e. a 3(9)-unsaturated) system[22]. In the COSY spectrum, the 2H-2 resonance was seen to be weakly coupled to H-9.

The $^{1}$H NMR spectrum showed a pair of doublet proton resonances, each integrating to two protons, at δ 7.14 ($J$ = 8.4 Hz) and δ 6.83 ($J$ = 8.4 Hz), which were assigned to H-2'/6' and H-3'/5' respectively. The corresponding methine carbon resonances were observed at δ 132.3 and δ 115.8. The C-2'/6' carbon resonance showed a correlation in the HMBC spectrum to H-9, and C-9 (δ 137.7), in turn, showed a correlation in the HMBC spectrum to 2H-2. The C-4 carbon resonance was seen to be correlated in the HMBC spectrum with 2H-2 and H-9. In the NOESY spectrum the proton resonance assigned to H-2'/6' showed a correlation to H-3'/5', H-9 and 2H-2. The fact that a correlation was not observed in the NOESY spectrum between H-9 and 2H-2, indicated that this compound must be the (*E*)-isomer. Also, the chemical shifts for C-2 (δ 67.7) of this 3-benzylidene-4-chromanone agreed with the value for eucomin[22], thus confirmed the same *E* configuration to be assigned to this derivative[22]. Furthermore, an *E*-orientation of the double bond was indicated by the position of the H-2 and H-9 proton signals at δ 5.32 and δ 7.72 respectively[23].

The A ring substitution pattern was deduced by UV absorption, whereby positive bathochromic shifts were observed with the addition of both NaOAc (+30 nm) and $AlCl_3$ (+50 nm). This indicated that hydroxy groups were present at C-5 and C-7. The $^{1}$H NMR spectrum also showed the presence of a singlet resonance integrating to three protons at δ 3.72, which indicated a methoxy group. This was placed at C-8 (δ 127.8) as it showed a NOESY correlation to 2H-2. A model was built in order to confirm that this NOESY correlation was possible.

From the NMR data this compound was identified as the novel 3-benzylidene-4-chromanone, *E*-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chroman-one. Compound V, at a concentration of 10 μg/cm$^3$, showed a 29.7% inhibition of prostaglandin synthesis using the microsomal cell screen (page 72). The HMBC and

NOESY correlations are depicted in figures 2.17 and 2.18 respectively, while all the NMR data for this compound is given in table 2.6.

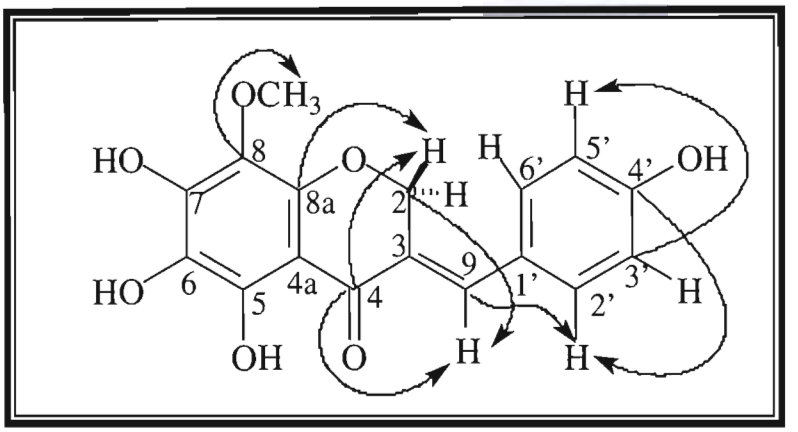



Figure 2.17. The HMBC (C → H) correlations for compound V.

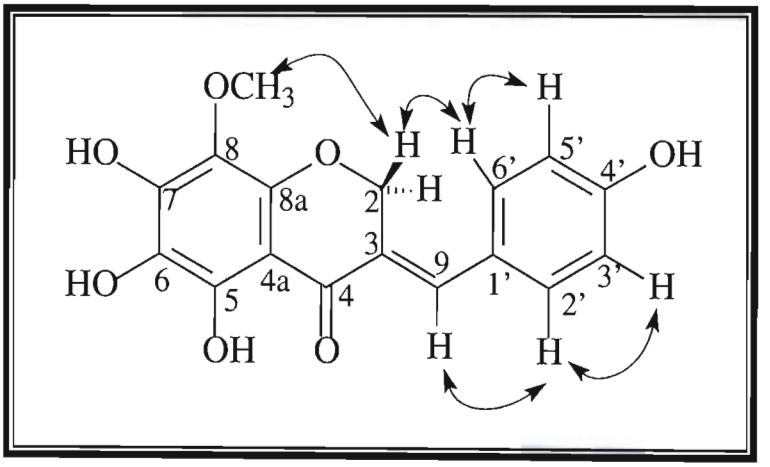



Figure 2.18. The NOESY correlations for compound V.

**Table 2.6. NMR data and correlations for compound V ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 2 | 5.32 (2H, s) | 67.7 ($CH_2$) | H-9 | H-9 | H-2′/6′, $OCH_3$ at C-8 |
| 3 | - | 126.4 (C) | - | 2H-2, H-9 | - |
| 4 | - | 185.3 (C) | - | 2H-2, H-9 | - |
| 4a | - | * | - | * | - |
| 5 | - | * | - | * | - |
| 6 | - | 102.6 (C) | - | OH at C-7 | - |
| 7 | - | * | - | * | - |
| 8 | - | 127.8 (C) | - | OH at C-7, $OCH_3$ at C-8 | - |
| 8a | - | 153.0 (C) | - | 2H-2 | - |
| 9 | 7.72 (1H, s) | 137.7 (CH) | 2H-2 | 2H-2, H-2′/6′ | H-2′/6′ |
| 1′ | - | 125.6 (C) | - | H-3′/5′ | - |
| 2′ | 7.14 (1H, d, *J*=8.4 Hz) | 132.3 (CH) | H-3′/5′ | H-9, H-2′/6′ | 2H-2, H-9, H-3′/5′ |
| 3′ | 6.83 (1H, d, *J*=8.4 Hz) | 115.8 (CH) | H-2′/6′ | H-3′/5′ | H-2′/6′ |
| 4′ | - | 158.8 (C) | - | H-2′/6′, H-3′/5′ | - |
| 5′ | 6.83 (1H, d, *J*=8.4 Hz) | 115.8 (CH) | H-2′/6′ | H-3′/5′ | H-2′/6′ |
| 6′ | 7.14 (1H, d, *J*=8.4 Hz) | 132.3 (CH) | H-3′/5′ | H-9, H-2′/6′ | 2H-2, H-9, H-3′/5′ |
| $OCH_3$ at C-8 | 3.72 (3H, s) | 61.2 ($CH_3$) | - | - | 2H-2 |
| OH at C-7 | 6.01 (1H, s) | - | - | - | - |

* Carbon resonances and HMBC correlations not able to be determined from spectra

## 2.3.2 Experimental

*Eucomis pole-evansii* bulbs (2950 g) were collected from Mac Mac Pools, Mpumalanga by Dr. Neil Crouch. A voucher specimen (N. Crouch 856) is retained at the Natal Herbarium for verification purposes. The bulbs were chopped and extracted with dichloromethane and methanol for approximately 48 hours at room temperature using continuous agitation. The extracts were concentrated using a rotor evaporator resulting in the dichloromethane (34.05 g) and methanol (141.58 g) crude extracts. Column chromatography using silica gel (Merck 9385) was used for separation of the compounds.

*Isolation of compounds IV and V*

The dichloromethane extract was chromatographed using silica gel (Merck 9385) as the stationary phase and a column of 3 cm in diameter.

The mobile phase used for the dichloromethane extract was a dichloromethane : methanol step gradient [100% dichloromethane (fractions 1-40), 1% methanol in dichloromethane (fractions 41-80), 3% methanol in dichloromethane (fractions 81-100), 5% methanol in dichloromethane (fractions 101-120), 10% methanol in dichloromethane (fractions 121-145). 20% methanol in dichloromethane (fractions 146-160), 100% methanol (fractions 161-180)]. Fractions of 40 $cm^3$ each were collected in each step.

Both compounds IV and V were eluted from the column using 2% methanol in 98% dichloromethane and were purified on a second column (1 cm in diameter) using a 100% dichloromethane solvent system. These compounds were distinguished from each other as compound IV exhibited a red coloured spot on thin layer chromatographic plates, while compound V was seen as an orange coloured spot, when the plate was sprayed with anisaldehyde spray reagent.

The methanol extract was investigated, however, it contained the same compounds that were isolated from the dichloromethane extract as well as a large amount of sugar.

*Acetylation of compound IV*

Approximately 10 mg of the compound was placed in a stoppered round-bottomed flask with pyridine (1 $cm^3$) and acetic anhydride (1 $cm^3$). The contents of the flask were allowed to react at room temperature for 24 hours. Thereafter, methanol (3 x 10 $cm^3$) was added to remove the excess acetic anhydride, and the pyridine was removed by the addition of toluene (3 x 10 $cm^3$). The final traces of toluene were removed by further addition of methanol (3 x 10 $cm^3$). All solvents were removed on a rotor evaporator.

### 2.3.2.1 Physical data for Compound IV

**Name:** (-)-[R]-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone

**Synonyms:** 3,9-dihydropunctatin

**Physical appearance:** yellow crystals

**Yield:** 19.8 mg

**Melting Point:** 196-200°C, Literature = 205-206°C ($CHCl_3$-hexane)[16]

**Mass:** LRMS [$M^+$] at *m/z* 316.09469, $C_{17}H_{16}O_6$ requires 316.09469

EIMS: *m/z* (rel. int.): 316 (71.10), 301 (1.39), 299 (2.05), 210 (45.33), 209 (15.82), 195 (21.06), 183 (7.56), 167 (10.40), 139 (7.82), 107 (100.00), 77 (11.86), 69 (8.01), 55 (7.30)

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3390, 2918, 2847, 1637, 1518, 1452, 1268, 1165, 1165, 1018

**Ultra violet:** $\lambda_{max}^{MeOH}$ nm (log ε): 296 (4.20), 343 (3.28)

With NaOAc: 332

With $AlCl_3$ : 315

**Optical Rotation:** $[\alpha]_D$ = -27.8° (c = 0.09 g/100 $cm^3$; $CHCl_3$),

Literature $[\alpha]_D^{24}$ = -37° (c = 0.3, MeOH)[16]

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.4 (page 83)

### 2.3.2.2 Physical data for Compound V

**Name:** (*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone

**Physical appearance:** yellow amorphous

**Yield:** 11.2 mg

**Mass:** LRMS [$M^+$] at *m/z* 330, $C_{17}H_{14}O_7$ requires 330

EIMS: *m/z*: 330, 316, 298, 167, 107, 71, 57, 55, 44, 28

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$: 3381, 2917, 2846, 1634, 1514, 1378, 1307, 1170, 1034

**Ultra violet:** $\lambda_{max}^{MeOH}$ nm (log ε): 354 (3.73)

With NaOAc: 384

With $AlCl_3$ : 404

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.6 (page 88)

## 2.4 Extractives from *Lachenalia rubida*

Plants belonging to the genus *Lachenalia* Jacq., (subfamily: Hyacinthoideae, family: Hyacinthaceae) are perennial bulbous herbs. The bulb varies in size and has either a hard or soft outer covering of dry, membranous scales, which occasionally form a neck[4]. There are approximately one hundred and ten species extending across southern Africa. *Lachenalia* is the largest genus of the Hyacinthaceae in southern Africa with several species only known from a single locality[4]. This work is the first time a plant from this genus is being investigated chemically. Plants of this genus occur in a wide range of habitats and are cultivated as ornamentals. There is no evidence of *Lachenalia* plants being used by traditional healers, however, they also seem to be a rich source of homoisoflavonoids.

Figure 2.19. Photograph of *Lachenalia rubida* (Photographed by Dr. Neil Crouch).

### 2.4.1 Results and Discussion

The dichloromethane extract of *Lachenalia rubida* yielded two compounds, the first (**compound VI**), being a 3-benzyl-4-chromanone type homoisoflavanone and the second (**compound VII**), a novel 3-benzyl-4-chromone type homoisoflavone. The latter is a 3-benzylidene-4-chromanone with the double bond having been shifted from the 3,9-position to the 2,3-position.

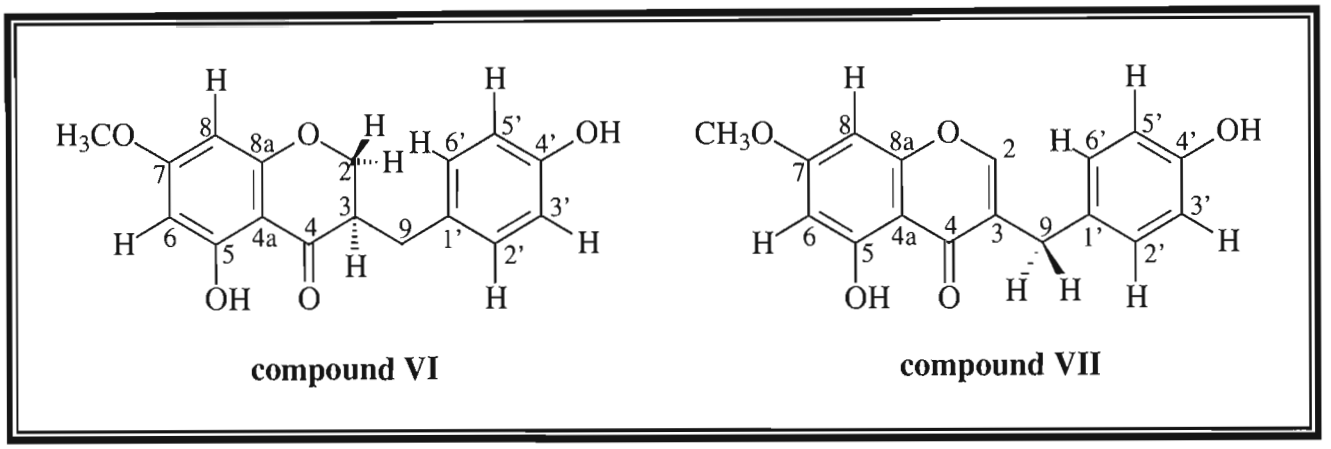



Figure 2.20. The structures of compounds VI and VII.

### 2.4.1.1 Structural Elucidation of Compound VI

### (-)-[R]-5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone

(Spectra 6a-k, pages 267-277)

The first compound isolated from *Lachenalia rubida* was identified as the homoisoflavanone, (-)-[R]-5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone. This compound has been reported by Adinolfi *et al.*[20], however, it is not clear from the paper whether this compound was isolated as a natural compound or if it resulted from a methylation reaction.

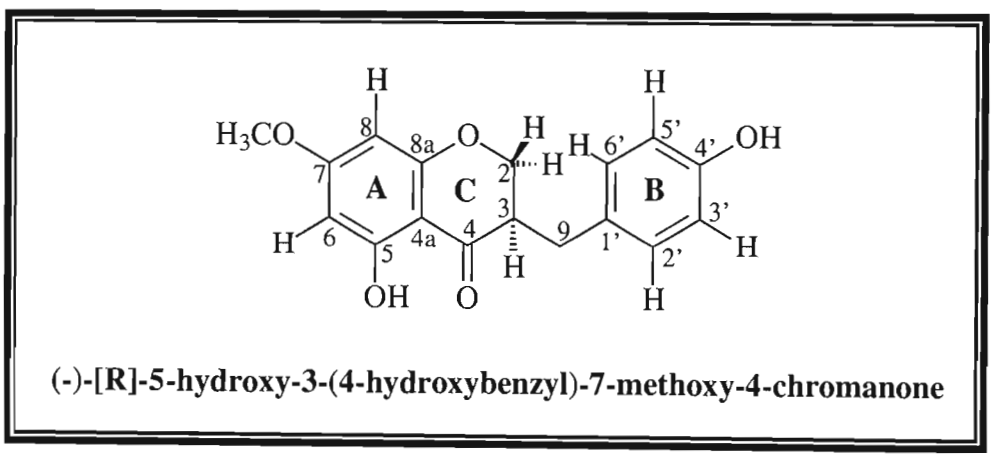



Figure 2.21. The structure of compound VI.

The mass spectrum indicated compound VI to have a molecular mass of 300 g $mol^{-1}$, which corresponded to a molecular formula of $C_{17}H_{16}O_5$ ($C_{17}H_{16}O_5$ requires 300) and

a double bond equivalence of ten. Further fragmentation was seen at *m/z* 193 and *m/z* 107. The latter indicated the presence of a hydroxytropylium ion.

The infra-red spectrum showed absorptions at 3407 $cm^{-1}$ (O-H stretching), 2918 $cm^{-1}$ (C-H asymmetric stretching), 2849 $cm^{-1}$ (C-H symmetric stretching) and 1640 $cm^{-1}$ (C=O stretching). The absorption at 1513 $cm^{-1}$ was due to the aromatic C=C stretching, while the $CH_2$ and $CH_3$ bending absorptions could be seen at 1443 $cm^{-1}$, 1374 $cm^{-1}$ and 1156 $cm^{-1}$.

On first inspection of the $^1H$ NMR spectrum, it was evident that this compound was a 3-benzyl-4-chromanone type homoisoflavonoid, having a hydroxy group attached at position 5 and protonated at the C-6 and C-8 positions. Evidence of the hydroxy group attached to C-5 is given in the $^1H$ NMR spectrum by the singlet peak at δ 12.09 due to the hydrogen bonded hydroxy group proton and in the $^{13}C$ NMR spectrum by the downfield position of the carbonyl resonance at C-4 (δ 197.9). This signal at δ 12.09, showed a correlation in the NOESY spectrum with H-6 (δ 6.04, d, $J$ = 2.4 Hz) and correlations in the HMBC spectrum to C-5 (δ 164.5, C), C-4a (δ 102.6, C) and C-6 (δ 95.0, CH). Further evidence supporting the presence of a hydroxy group at C-5 was revealed by a positive bathochromic shift (+20 nm) in the UV spectrum with $AlCl_3$.

The $^1H$ NMR spectrum showed the presence of one methoxy group proton resonance at δ 3.79 (s), which was seen in the NOESY spectrum to be correlated with both the H-6 and H-8 (δ 5.95, d, $J$ = 2.1 Hz) proton resonances. The methoxy group was therefore placed at C-7 (δ 167.8, C). This was confirmed by the HMBC spectrum, which showed a correlation between C-7 and the methoxy group proton resonance. No bathochromic shift was observed with NaOAc, indicating that a methoxy group occurred at C-7, an observation, which was also corroborated by the appearance of resonances due to H-6 and H-8 downfield of 6.0 ppm[20]. The HMBC spectrum also showed correlations of the proton resonance assigned to H-6 with C-5, C-4a and C-7.

The B ring showed a *para* substitution pattern, indicated by a pair of doublet proton resonances at δ 7.08 ($J$ = 8.5 Hz, H-2'/6') and δ 6.78 ($J$ = 8.3 Hz, H-3'/5'). These doublet resonances are seen to be coupled to each other in both the COSY and

NOESY spectra. The methine carbon signal at δ 130.0, assigned to C-2'/6', showed correlations in the HMBC spectrum with H-3'/5', H-9A (δ 3.15, dd, $J$ = 4.2, 13.6 Hz) and H-9B (δ 2.68, dd, $J$ = 10.5, 13.6 Hz), while the fully substituted carbon resonance at δ 154.4 (C-4') showed correlations in the HMBC spectrum with H-2'/6' and H-3'/5'. In the NOESY spectrum the H-2'/6' peak showed correlations with the proton signals assigned to H-3'/5', H-3 and 2H-9.

In the C ring, the proton resonance due to H-2A (δ 4.26, dd, $J$ = 4.2, 11.4 Hz) was seen to be coupled in the COSY spectrum with H-2B (δ 4.09, dd, $J$ = 7.0, 11.4 Hz) and the H-3 (δ 2.78, m) proton peak. The proton resonance attributed to H-3 showed coupling in the COSY spectrum with the 2H-9 and 2H-2 proton signals and was seen to be coupled in the NOESY spectrum to 2H-2, 2H-9 and H-2'/6'. In the HMBC spectrum the methylene carbon resonance at δ 32.0 (C-9) showed correlations with H-2'/6' and 2H-2.

The absolute stereochemistry at C-3 has been determined by Professor D. Ferreira at the University of Mississippi, USA, for a series of homoisoflavanones of this type from the group using circular dichroism. In all cases the absolute stereochemistry at C-3 was shown to be R, implying that H-3 must be α. It is assumed that the stereochemistry would be the same for compound VI.

From the use of NMR spectroscopy and other spectral data, this compound was found to be the homoisoflavonoid, (-)-[R]-5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone. The NMR data for compound VI is given in table 2.7 and is compared to the literature data[20] for this compound. The values do not correlate exactly as they are run in different solvents. The HMBC and NOESY correlations for this compound are shown in figures 2.22 and 2.23 respectively.

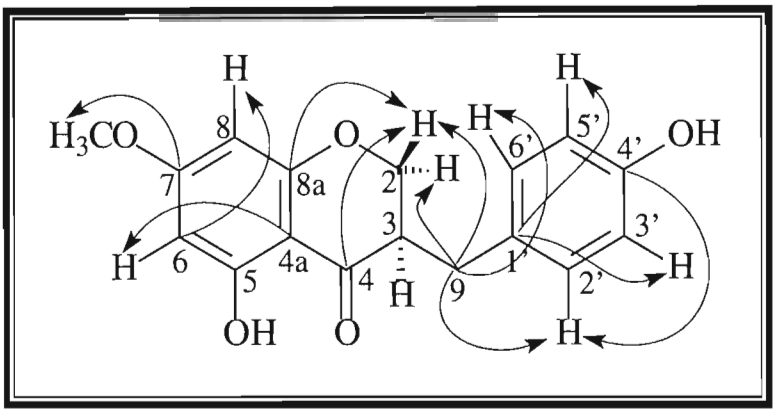



Figure 2.22. The HMBC (C → H) correlations for compound VI.

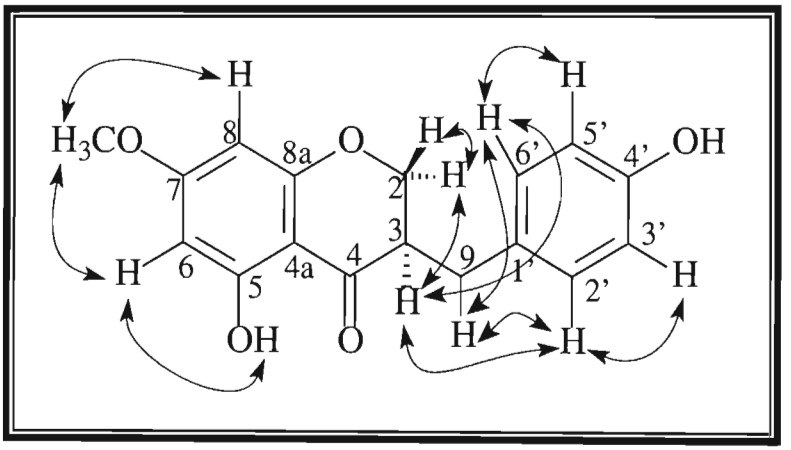



Figure 2.23. The NOESY correlations for compound VI.

**Table 2.7. NMR data and correlations for compound VI ($CDCl_3$) and literature data for 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone ($CD_3OD$)[20]**

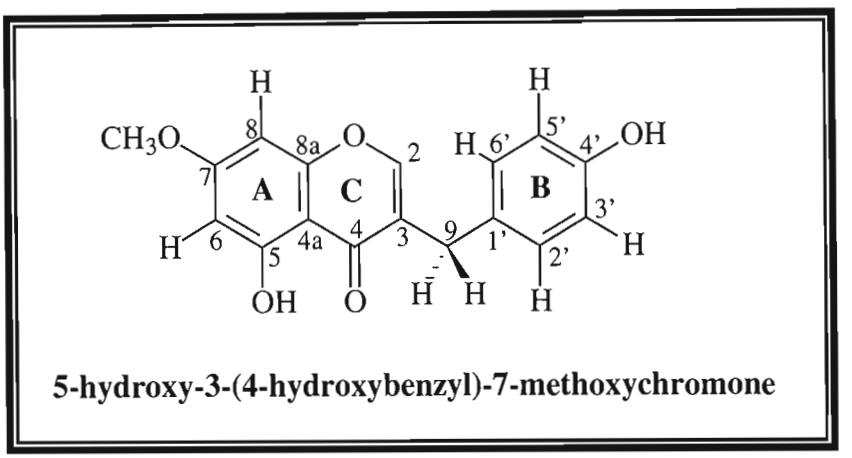
| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone (100MHz)[20] |
|---|---|---|---|---|---|---|
| **2A** | 4.26 (1H, dd, *J*=4.2, 11.4 Hz) | 68.9 ($CH_2$) | H-2B, H-3 | H-9A, H-9B | H-2B, H-3 | 70.4 |
| **2B** | 4.09 (1H, dd, *J*=7.0, 11.4 Hz) | | H-2A, H-3 | | H-2A, H-3 | |
| **3** | 2.78 (1H, m) | 46.8 (CH) | H-2A, H-2B, H-9A, H-9B | H-9A, H-9B | H-2A, H-2B, H-9A, H-9B, H-2'/6' | 48.4 |
| **4** | - | 197.9 (C) | - | H-2A, H-2B, H-9A(w), OH at C-5 | - | 200.2 |
| **4a** | - | 102.6 (C) | - | H-6 | - | 103.0 |
| **5** | - | 164.5 (C) | - | H-6(w), OH at C-5 | - | 165.6 |
| **6** | 6.04 (1H, d, *J*=2.4 Hz) | 95.0 (CH) | H-8 | H-8(w), OH at C-5 | $OCH_3$ at C-7 | 95.8 |
| **7** | - | 167.8 (C) | - | H-6(w), $OCH_3$ at C-7 | - | 169.4 |
| **8** | 5.95 (1H, d, *J*=2.1 Hz) | 93.9 (CH) | H-6 | - | $OCH_3$ at C-7 | 94.6 |
| **8a** | - | 162.8 (C) | - | H-2A, H-2B | - | 164.6 |
| **9A** | 3.15 (1H, dd, *J*=4.2, 13.6 Hz) | 32.0 ($CH_2$) | H-3, H-9B | H-2A, H-2B, H-2'/6' | H-3, H-9B, H-2'/6' | 32.9 |
| **9B** | 2.68 (1H, dd, *J*=10.5, 13.6 Hz) | | H-3, H-9A | | H-3, H-9A, H-2'/6' | |
| **1'** | - | 130.0 (C) | - | H-2'/6', H-3'/5' | - | 130.2 |
| **2'** | 7.08 (1H, d, *J*=8.5 Hz) | 130.3 (CH) | H-3'/5' | H-9A, H-9B, H-3'/5' | H-3, H-9A, H-9B, H-3'/5' | 131.2 |
| **3'** | 6.78 (1H, d, *J*=8.3 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5' | H-2'/6' | 116.5 |
| **4'** | - | 154.4 (C) | - | H-2'/6', H-3'/5' | - | 157.3 |
| **5'** | 6.78 (1H, d, *J*=8.3 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5' | H-2'/6' | 116.5 |
| **6'** | 7.08 (1H, d, *J*=8.5 Hz) | 130.3 (CH) | H-3'/5' | H-9A, H-9B, H-3'/5' | H-3, H-9A, H-9B, H-3'/5' | 131.2 |
| **$OCH_3$ at C-7** | 3.79 (3H, s) | 55.7 ($CH_3$) | - | - | H-6, H-8 | 56.2 |
| **OH at C-5** | 12.09 (1H, s) | - | - | | H-6 | - |

### 2.4.1.2 Structural Elucidation of Compound VII

### 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxychromone

(Spectra 7a-i, pages 278-286)

The second compound to be isolated from *Lachenalia rubida* was identified as the novel homoisoflavone, 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxychromone. These types of homoisoflavones have been reported from *Ophiopogon japonicus*[24] (Liliaceae) as well as from *Dracaena draco*[25] (Dracaenaceae).




**5-hydroxy-3-(4-hydroxybenzyl)-7-methoxychromone**

Figure 2.24. The structure of compound VII.

The mass spectrum revealed compound VII to have a molar mass of 298 g $mol^{-1}$, which corresponded to a molecular formula of $C_{17}H_{14}O_5$ ($C_{17}H_{14}O_5$ requires 298) and a double bond equivalence of eleven. The peak at *m/z* 107 indicated the presence of a hydroxytropylium ion which implied that a hydroxyl group was situated on ring B.

In the infra-red spectrum, the O-H stretching vibration was observed at 3396 $cm^{-1}$. The absorptions at 2914 $cm^{-1}$ and 2845 $cm^{-1}$ were due to the C-H asymmetric and symmetric stretching vibrations respectively. The C=O stretching could be seen at 1651 $cm^{-1}$, while the aromatic C=C stretching vibration was observed at 1512 $cm^{-1}$. The $CH_2$ and $CH_3$ bending vibrations were seen at 1442 $cm^{-1}$, 1303 $cm^{-1}$ and 1164 $cm^{-1}$.

Compound VII was contaminated with a slight impurity, the peaks of which have been cancelled out in the relevant spectra. The $^1H$ NMR spectrum showed the

presence of a pair of doublet resonances at δ 7.12 (2H, $J$ = 8.3 Hz) and δ 6.77 (2H, $J$ = 8.3 Hz), which were indicative of a *para* disubstituted aromatic ring B system and were assigned to H-2'/6' and H-3'/5' respectively. The corresponding methine carbon resonances were observed at δ 130.1 (C-2'/6') and δ 115.5 (C-3'/5') in the $^{13}$C NMR spectrum. The C-2'/6' resonance was seen to correlate in the HMBC spectrum with the singlet two-proton resonance at δ 3.67, ascribed to 2H-9. This 2H-9 peak was seen to be correlated in the NOESY spectrum with H-2'/6', which further confirmed the peak assignment. The C-9 methylene carbon resonance observed at δ 29.7, showed HMBC correlations to the H-2'/6' resonance and the singlet olefinic proton resonance at δ 7.45 (1H). Due to this peak showing correlations in both the COSY and NOESY spectra to 2H-9, this peak was assigned to H-2.

According to Kirkiacharian *et al.*[22], the chemical shift for the C-2 methine carbon resonance in 3-benzylchromones should occur at approximately δ 152.5. Thus, the presence of the 2,3-double bond was confirmed by the resonance at δ 153.3 ascribed to C-2. Further confirmation was given by the fully substituted carbon resonance at δ 123.2, which was assigned to C-3 as it showed HMBC correlations to H-2 and 2H-9. The presence of the *endo* double bond shifts the C-3 absorption upfield by 6.4 ppm compared with their *exo* isomers, the 3-benzylidene-4-chromanones. 3-Benzylidene–4-chromanones can isomerise to 3-benzylchromones by migration of the exocyclic double bond into the pyrone ring. This chemical transformation requires drastic conditions and is irreversible[22].

From the $^{1}$H NMR spectrum, it is evident that the A ring is protonated at the standard C-6 (δ 98.0) and C-8 (δ 92.3) positions, by the presence of two doublet proton resonances, each integrating to one proton, at δ 6.32 (H-6, $J$ = 2.2 Hz) and δ 6.31 (H-8, $J$ = 2.2 Hz). The H-6 and H-8 proton superimposed resonances showed a correlation in the NOESY spectrum to the methoxy group proton resonance at δ 3.82 (3H, s) indicating that a methoxy group is positioned at C-7 (δ 165.4, C). This was confirmed in the UV absorption spectrum where no bathochromic shift was observed with NaOAc, further proving that a methoxy group occurred at C-7. This observation was also corroborated by the appearance of resonances due to H-6 and H-8 downfield of 6.0 ppm[20]. The presence of a hydroxy group at C-5 (δ 162.2, C) was indicated by

the singlet hydroxy group proton resonance at δ 12.66 (1H). Further evidence supporting the presence of a hydroxy group at C-5 was revealed by a positive bathochromic shift (+21 nm) in the UV spectrum with $AlCl_3$.

From the NMR data, this compound was identified as the novel 3-benzylchromone, 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxychromone. The HMBC and NOESY correlations are shown in figures 2.25 and figure 2.26, while all the NMR data and correlations for compound VII are listed in table 2.8.

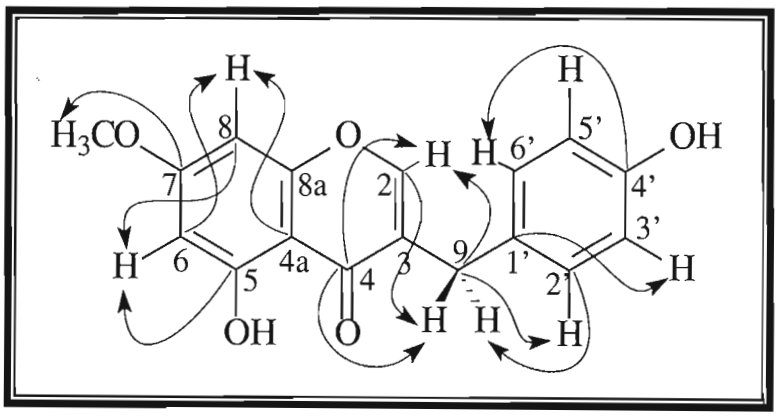



Figure 2.25. The HMBC (C → H) correlations for compound VII.

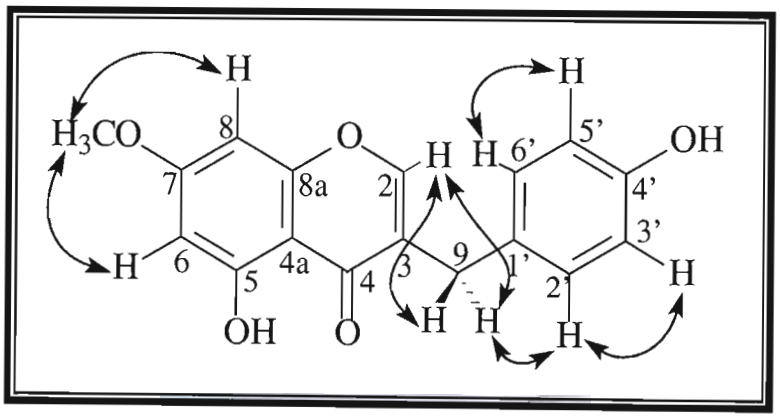



Figure 2.26. The NOESY correlations for compound VII.

## Table 2.8. NMR data and correlations for compound VII ($CDCl_3$)

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| **2** | 7.45 (1H, s) | 153.3 (CH) | 2H-9 | 2H-9 | 2H-9, H-2'/6'(w) |
| **3** | - | 123.2 (C) | - | H-2, 2H-9 | - |
| **4** | - | 181.6 (C) | - | H-2, 2H-9 | - |
| **4a** | - | 106.0 (C) | - | H-6, H-8, OH at C-5 | - |
| **5** | - | 162.2 (C) | - | H-6, OH at C-5 | - |
| **6** | 6.32 (1H, d, *J*=2.2 Hz) | 98.0 (CH) | - | H-8, OH at C-5 | $OCH_3$ at C-7 |
| **7** | - | 165.4 (C) | - | H-6, H-8, $OCH_3$ at C-7 | - |
| **8** | 6.31 (1H, d, *J*=2.2 Hz) | 92.3 (CH) | - | H-6 | $OCH_3$ at C-7 |
| **8a** | - | 158.1 (C) | - | H-8 | - |
| **9** | 3.67 (2H, s) | 29.7 ($CH_2$) | H-2 | H-2, H-2'/6' | H-2, H-2'/6' |
| **1'** | - | 130.4 (C) | - | H-2'/6', H-3'/5' | - |
| **2'** | 7.12 (1H, d, *J*=8.3 Hz) | 130.1 (CH) | H-3'/5' | 2H-9, H-2'/6' | 2H-9, H-3'/5' |
| **3'** | 6.77 (1H, d, *J*=8.3 Hz) | 115.5 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| **4'** | - | 154.3 (C) | - | H-2'/6', H-3'/5' | - |
| **5'** | 6.77 (1H, d, *J*=8.3 Hz) | 115.5 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| **6'** | 7.12 (1H, d, *J*=8.3 Hz) | 130.1 (CH) | H-3'/5' | 2H-9, H-2'/6' | 2H-9, H-3'/5' |
| **$OCH_3$ at C-7** | 3.82 (3H, s) | 55.8 ($CH_3$) | - | - | H-6, H-8 |
| **OH at C-5** | 12.66 (1H, s) | - | - | - | - |

## 2.4.2 Experimental

*Lachenalia rubida* bulbs (1666 g) were collected from Bloubergstrand by Graham Duncan. A voucher specimen is held in the Compton Herbarium and the voucher specimen number is G.D. Duncan 442. The dried bulbs were chopped and extracted with dichloromethane and methanol at room temperature for 48 hours using continuous agitation. The extracts were evaporated down using a rotor evaporator, which afforded the dichloromethane (4.92 g) and methanol (39.55 g) extracts.

***Isolation of compounds VI and VII***

The dichloromethane extract was chromatographed using silica gel (Merck 9385) as the stationary phase in a column of 3 cm in diameter.

The mobile phase used for the dichloromethane extract was a hexane : dichloromethane : methanol step gradient [20% dichloromethane in hexane (fractions 1-15), 40% dichloromethane in hexane (fractions 16-30), 80% dichloromethane in hexane (fractions 31-65), 100% dichloromethane (fractions 66-80), 1% methanol in dichloromethane (fractions 81-99), 5% methanol in dichloromethane (fractions 100-110), 10% methanol in dichloromethane (fractions 111-119), 100% methanol (fractions 120-130)]. Fraction sizes of 40 $cm^3$ were collected in each step.

Both compounds VI and VII eluted from the column using 1% methanol in 99% dichloromethane and were purified firstly on a 1 cm in diameter column and then on a Pasteur pipette column using a 100% dichloromethane solvent system. The compounds were distinguished from one another by the colour of the spot on the thin layer chromatographic plate when sprayed with anisaldehyde spray reagent. Compound VI exhibited a red spot, while compound VII was seen as an orange spot.

The methanol extract was separated using column chromatography, however only sugar was isolated from this column.

### 2.4.2.1 Physical data for Compound VI

**Name:** (-)-5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-4-chromanone

**Physical appearance:** amorphous

**Yield:** 12.3 mg

**Mass:** LRMS [$M^+$] at *m/z* 300, $C_{17}H_{16}O_5$ requires 300

EIMS: *m/z*: 300, 194, 193, 167, 107, 28

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$: 3407, 2918, 2849, 1640, 1513, 1443, 1374, 1156

**Ultra violet:** $\lambda_{max}^{MeOH}$ nm (log ε): 290 (4.04)

With NaOAc : 290

With $AlCl_3$ : 310

**Optical Rotation:** $[\alpha]_D$ = -7.1° (c = 0.028 g/100 $cm^3$; $CHCl_3$),

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.7 (page 96)

### 2.4.2.2 Physical data for Compound VII

**Name:** 5-hydroxy-3-(4-hydroxybenzyl)-7-methoxy-chromone

**Physical appearance:** amorphous

**Yield:** 7.1 mg

**Mass:** LRMS [$M^+$] at *m/z* 298, $C_{17}H_{14}O_5$ requires 298

EIMS: *m/z*: 298, 167, 107, 77, 28

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$: 3396, 2914, 2845, 1651, 1512, 1442, 1303, 1164

**Ultra violet:** $\lambda_{max}^{MeOH}$ nm (log ε): 290 (3.52)

With NaOAc: 290

With $AlCl_3$ : 311

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.8 (page 100)

## 2.5 Extractives from *Drimia capitata*

Plants of the genus *Drimia*, (subfamily: Urgineoideae, family: Hyacinthaceae), are perennial, deciduous bulbous herbs, varying in size and sometimes forming clumps. The bulbs are oval in shape with loose scales that are reddish in colour[4]. There are approximately thirteen species in southern Africa, which enjoy a varied habitat, but they are most frequently found in open grasslands, amongst rocks or occasionally in marshy ground. Plants of the genus *Drimia* are used in traditional medicine and as protective charm mixtures. *Drimia capitata* has not previously been investigated chemically and no uses of this plant are reported in literature.

Figure 2.27. Photograph of *Drimia capitata* (Photographed by Dr. Neil Crouch).

### 2.5.2 Results and Discussion

The methanol extract of *Drimia capitata* (Jacq.) yielded a bufadienolide (**compound VIII**) and the glycoside of compound VIII (**compound IX**). Unfortunately no compounds were isolated from the dichloromethane extract.

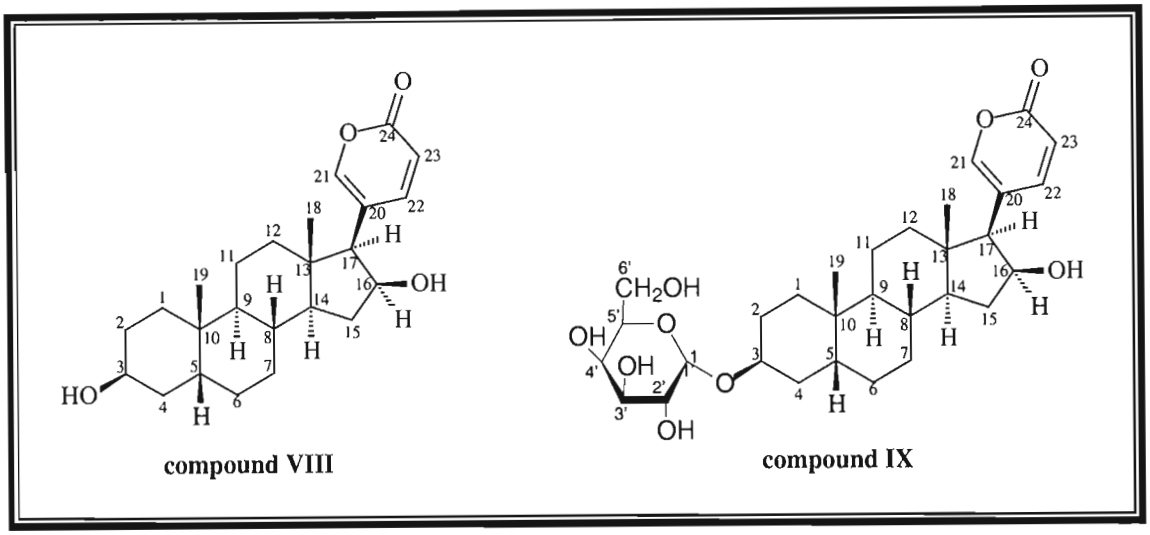



Figure 2.28. The structures of compounds VIII and IX.

### 2.5.1.1 Structural Elucidation of Compound VIII

### 5β-3β,16β-dihydroxybufa-20,22-dienolide

(Spectra 8a-g, pages 287-293)

The first compound isolated from *Drimia capitata* was found to be a bufadienolide, identified as 5β-3β,16β-dihydroxybufa-20,22-dienolide.

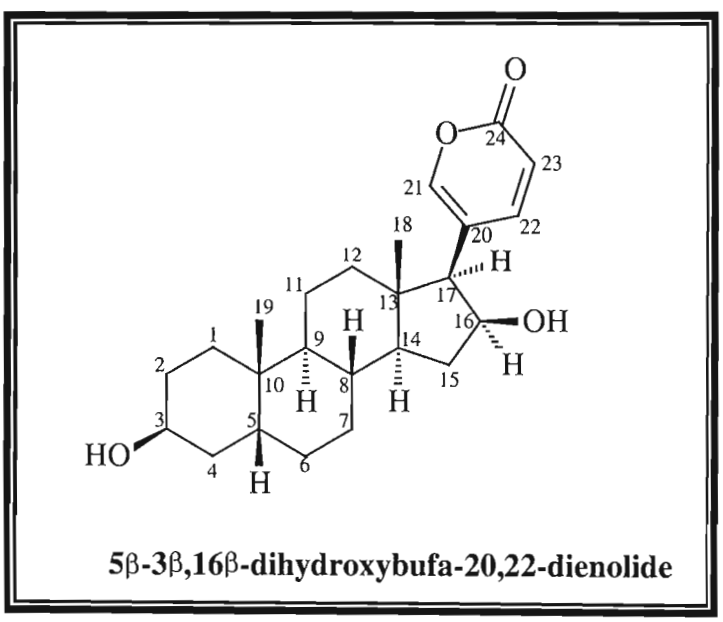



Figure 2.29. The structure of compound VIII.

Compound VIII belongs to the class of compounds known as the bufadienolides. These compounds are often isolated with a sugar attached at C-3, however, this compound was isolated as the aglycone.

The mass spectrum showed a parent ion peak at *m/z* 386, which was consistent with the molecular formula of $C_{24}H_{34}O_4$ ($C_{24}H_{34}O_4$ requires 386). A double bond equivalence of eight was deduced.

The presence of the characteristic lactone ring of the bufadienolide was indicated in the $^1$H NMR spectrum by three double doublet proton resonances at δ 7.59 (1H, $J$ = 1.0, 2.5 Hz), δ 7.77 (1H, $J$ = 2.6, 9.7 Hz) and δ 6.25 (1H, $J$ = 1.0, 9.7 Hz), which are assigned to H-21, H-22 and H-23 respectively. The corresponding methine carbon resonances were observed in the $^{13}$C NMR spectrum at δ 152.3, δ 151.2 and δ 114.4, using the HSQC spectrum. The H-21 and H-23 proton resonances were seen to be coupled in the COSY spectrum with H-22. The fully substituted carbon resonance ascribed to C-24 was observed at δ 164.8 and showed correlations in the HMBC spectrum to H-21, H-22 and H-23.

In the HMBC spectrum the C-20 fully substituted carbon resonance at δ 118.8 showed correlations to H-17 (δ 2.16, 1H, d, $J$ = 7.5 Hz), H-21 and H-23. The H-17 proton resonance was seen to be coupled in the COSY spectrum to H-16 (δ 4.39, 1H, m). The methine carbon resonance, due to C-17, was observed at δ 58.2 and showed correlations in the HMBC spectrum to H-15α (2.32, 1H, m) and the three-proton singlet at δ 0.85 ascribed to 3H-18. The 3H-18 proton resonance, which, for biosynthetic reasons, is known to be in the β orientation, showed no correlation in the NOESY spectrum to H-17 thus confirming that H-17 is in the α orientation. The H-17 proton resonance, however, showed correlations in the NOESY spectrum to H-16, H-21, H-22 and H-14 (δ 1.05) indicating that the lactone ring and H-16 are also α in orientation.

The $^1$H NMR spectrum showed the presence of a second three-proton singlet signal at δ 0.99, integrating to three protons, and this was assigned to 3H-19. This proton resonance was seen to be correlated in the NOESY spectrum to H-5 (δ 1.76, 1H, m),

H-8 (δ 1.47, 1H, m) and 2H-1 (δ 1.27 and δ 1.44). Furthermore, as the 3H-19 proton resonance is in the β orientation this indicates that H-5 and H-8 are also of the β orientation. The H-5 proton resonance was seen to be coupled in the COSY spectrum to 2H-4 (δ 2.00 and δ 1.30), while H-9 (δ 1.57, 1H, m) was seen to be coupled to H-8 (δ 1.47, 1H, m) and H-11α (δ 1.43). The H-9 proton is in the α orientation on biosynthetic grounds, and, this was confirmed by the absence of correlations in the NOESY spectrum to 3H-18, H-5 and H-8. The $^{1}$H NMR spectrum showed a one-proton multiplet resonance at δ 4.03, which was assigned to H-3 as it was seen to be coupled in the COSY spectrum to 2H-4. The H-3 proton resonance was assigned the usual α configuration as it showed no correlation in the NOESY spectrum to H-5β, however the NOESY spectrum did show correlations to the resonances at δ 1.45, δ 2.00 and δ 1.96, assigned to H-2α, H-4α and H-6α respectively. The C-5 (δ 37.9), C-8 (δ 41.4), C-9 (δ 36.8) and C-10 (δ 36.4) carbon resonances showed HMBC correlations to 3H-19.

The presence of the hydroxy group at C-16 was indicated in the $^{13}$C NMR spectrum by a methine carbon resonance at δ 73.9 in the oxygenated carbon region of the spectrum. In the HMBC spectrum this C-16 methine carbon resonance showed correlations to H-17 and 2H-15 (δ 1.29 and δ 2.32), while the C-13 methine carbon resonance at δ 45.0 showed a correlation to H-16, thus confirming that the hydroxy group was situated at C-16. In the COSY spectrum, the H-16 proton resonance was seen to be coupled to the 2H-15 proton resonances.

This compound was identified as the novel bufadienolide, 5β-3β,16β-dihydroxybufa-20,22-dienolide. The HMBC and NOESY correlations are shown in figures 2.30 and 2.31 while all the NMR data and correlations are shown in table 2.9.

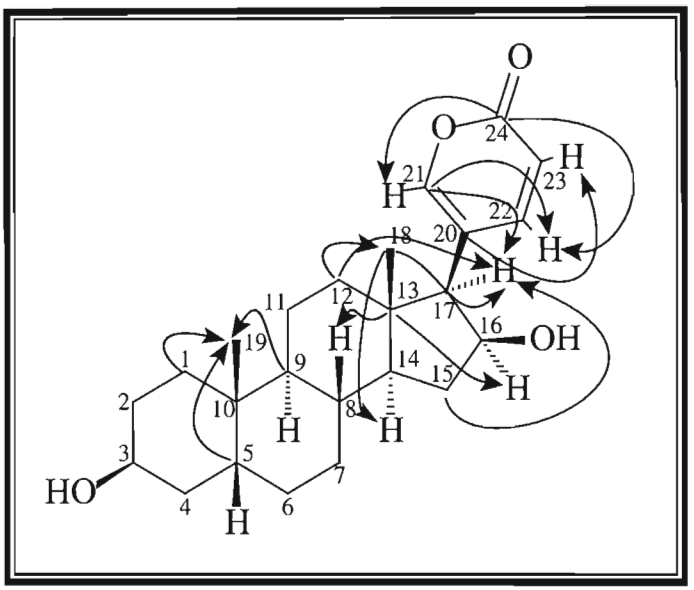



Figure 2.30. The HMBC (C → H) correlations for compound VIII.

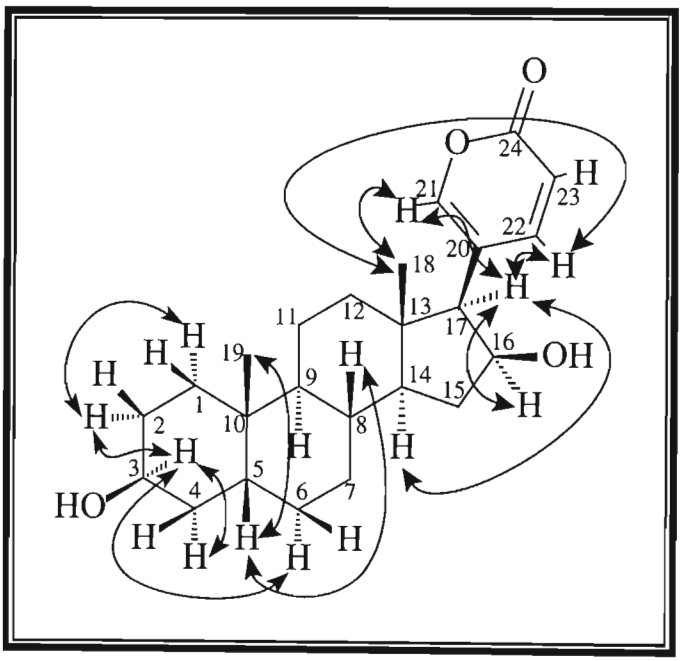



Figure 2.31. The NOESY correlations for compound VIII.

## Table 2.9. NMR data and correlations for compound VIII ($CD_3OD$)

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1α | 1.27 (1H, m) | 31.0 ($CH_2$) | H-1β, 2H-2 | 3H-19 | H-1β, H-2α, 3H-19 |
| 1β | 1.44 (1H, m) | | H-1α | | H-1α, 3H-19 |
| 2α | 1.45 (1H, m) | 28.5 ($CH_2$) | H-1α, H-2β | - | H-1α, H-2β, H-3 |
| 2β | 1.54 (1H, m) | | H-2α | | H-2α |
| 3 | 4.03 (1H, bs) | 67.7 (CH) | 2H-4 | - | H-2α, H-4α, H-6α |
| 4α | 2.00 (1H, m) | 34.3 ($CH_2$) | H-3, H-4β, H-5 | - | H-3 |
| 4β | 1.30 (1H, m) | | H-3, H-4α, H-5 | | 3H-19 |
| 5 | 1.76 (1H, m) | 37.9 (CH) | 2H-4 | 3H-19 | H-8, 3H-19 |
| 6α | 1.96 (1H, m) | 27.6 ($CH_2$) | H-5, H-6β | - | H-3, H-6β, H-7 |
| 6β | 1.20 (1H, m) | | H-6α, 2H-7 | | H-6α |
| 7 | 1.16 (1H, m) | 27.8 ($CH_2$) | 2H-6, H-8 | - | H-8 |
| 8 | 1.47 (1H, m) | 41.4 (CH) | H-9, H-14, 2H-7 | 3H-19(w) | H-5, H-19 |
| 9 | 1.57 (1H, m) | 36.8 (CH) | H-8, H-11α | 3H-19 | - |
| 10 | - | 36.4 (C) | - | 3H-19 | - |
| 11α | 1.43 (1H, m) | 21.5 ($CH_2$) | H-9, H-11β | - | H-12α |
| 11β | 1.26 (1H, m) | | H-11α | | 3H-18, 3H-19 |
| 12α | 1.11 (1H, m) | 39.3 ($CH_2$) | H-12β | H-17, 3H-18 | H-11α, H-17 |
| 12β | 1.50 (1H, m) | | H-12α | | H-15β, 3H-18 |
| 13 | - | 45.0 (C) | - | H-8, H-15α, H-16, H-17 | - |
| 14 | 1.05 (1H, m) | 54.3 (CH) | H-8, 2H-15 | H-15β, 3H-18 | H-17 |
| 15α | 2.32 (1H, m) | 37.8 ($CH_2$) | H-14, H-15β, H-16 | H-17 | H-15β, H-16 |
| 15β | 1.29 (1H, m) | | H-14, H-15α, H-16 | | H-12β, H-15α |
| 16 | 4.39 (1H, m) | 73.9 (CH) | 2H-15, H-17 | H-15β, H-17 | H-15α, H-17 |
| 17 | 2.16 (1H, d, *J*=7.5 Hz) | 58.2 (CH) | H-16 | H-15α, 3H-18 | H-12α, H-14, H-16, H-21, H-22 |
| 18 | 0.85 (3H, s) | 15.2 ($CH_3$) | - | H-14, H-17 | H-12β, H-21, H-22 |
| 19 | 0.99 (3H, s) | 24.4 ($CH_3$) | - | - | 2H-1, 2H-4, H-5, H-8 |
| 20 | - | 118.8 (C) | - | H-17, H-21, H-23 | - |
| 21 | 7.59 (1H, dd, *J*=1.0, 2.5 Hz | 152.3 (CH) | H-22 | H-17, H-22 | H-17, 3H-18 |
| 22 | 7.77 (1H, dd, *J*=2.6, 9.7 Hz) | 151.2 (CH) | H-21, H-23 | H-17, H-21 | H-17, 3H-18, H-23 |
| 23 | 6.25 (1H, dd, *J*=1.0, 9.7 Hz) | 114.4 (CH) | H-22 | - | H-22 |
| 24 | - | 164.8 (C) | - | H-21, H-22, H-23 | - |

### 2.5.1.2 Structural Elucidation of Compound IX

### 5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside

(Spectra 9a-i, pages 294-302)

The second compound isolated from *Drimia capitata* was identified as the novel bufadienolide glycoside, 5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside.

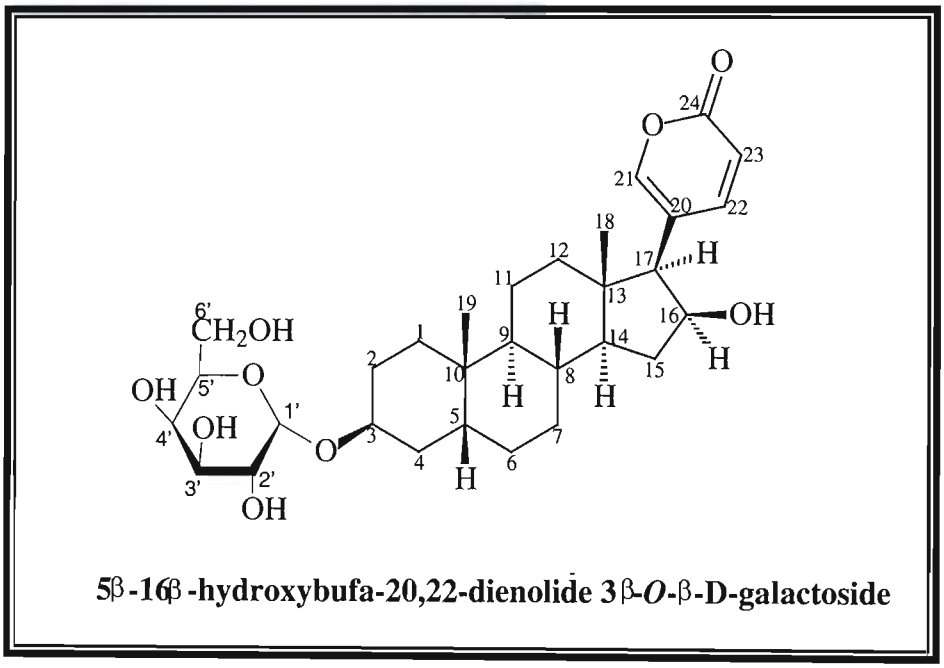



Figure 2.32. The structure of compound IX.

The mass spectrum of compound IX indicated a molar mass of 548 using Fast Atom Bombardment (FAB) mass spectrometry, which corresponded to a molecular formula of $C_{30}H_{44}O_9$ ($C_{30}H_{44}O_9$ requires 548). A double bond equivalence of nine was deduced.

The infra-red spectrum showed bands at 3395 $cm^{-1}$ (O-H stretching), 2928 $cm^{-1}$ (C-H stretching), 1702 $cm^{-1}$ (C=O stretching), 1454 $cm^{-1}$ ($CH_2/CH_3$ bending) and 1028 $cm^{-1}$ (C-O stretching).

By comparison of the NMR data for compound IX with that of compound VIII, it was determined that the aglycone group of compound IX was identical to that of compound VIII. The presence of the sugar group was observed in the $^1H$ NMR

spectrum by the doublet one-proton resonance at δ 4.31($J$ = 7.9 Hz), assigned to the anomeric proton of the sugar, H-1'. This proton resonance showed coupling in the NOESY spectrum to H-3 (δ 4.06, 1H, m), thus confirming the sugar attachment at C-3 (δ 75.5). In the COSY spectrum, the H-1' proton resonance was seen to be coupled to a one-proton multiplet resonance at δ 3.18 which was ascribed to H-2'. The corresponding C-1' methine carbon resonance, was observed in the $^{13}C$ NMR spectrum at δ 102.6 and showed a correlation in the HMBC spectrum to H-2'. The COSY spectrum showed coupling between the H-2' proton resonance and H-1' and a one-proton multiplet resonance at δ 3.32, due to H-3'. In the HMBC spectrum, C-3' (δ 78.2) showed a correlation with H-2'. In the COSY spectrum the H-3' proton resonance was seen to be coupled to H-2' and a one-proton multiplet resonance at δ 3.26, assigned to H-4', which was, in turn, coupled to a one-proton multiplet resonance at δ 3.22, ascribed to H-5'. In the COSY spectrum, the H-3' proton resonance showed a correlation to H-4', which in turn showed a correlation to H-5'.

In the HMBC spectrum the C-4' methine carbon resonance at δ 71.6, showed a correlation to H-3' and a two-proton multiplet resonance at δ 3.64 and δ 3.82. These proton resonances were assigned to H-6'A and H-6'B respectively and both the COSY and NOESY spectra showed correlations between the two H-6 resonances and H-5'. The methylene carbon resonance, C-6', was observed at δ 62.7 in the $^{13}C$ NMR spectrum.

In order to ascertain what the sugar group was, this compound was subjected to enzyme hydrolysis using β-glucosidase at 37°C for one week. The resultant mixture was sent to Forestry and Forest Products, which has a high throughput sugar Gas Chromatography analysis system, run by Professor Andrew Spark, who identified the sugar as galactose by comparing it to a range of standards. The instrument used was a Perkin Elmer HPLC with Dionex Anion exchange column run with pure water. A post column pH adjustment to pH 14 (350mM NaOH) was performed and pulsed amperometric detection was used.

Compound IX was identified as the novel bufadienolide glycoside, 5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside. The NMR data and correlations for compound IX are shown in table 2.10.

**Table 2.10. NMR data and correlations for compound IX ($CD_3OD$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1 | 1.48 (2H, m) | 31.5 ($CH_2$) | H-2 | 3H-19 | H-2 |
| 2 | 1.65 (2H, m) | 27.7 ($CH_2$) | H-1, H-3 | - | H-1 |
| 3 | 4.06 (1H, m) | 75.5 (CH) | H-2, 2H-4 | 2H-11, H-1' | H-4α, H-6α, H-1' |
| 4α | 1.86 (1H, m) | 31.0 ($CH_2$) | H-3, H-4β, H-5 | - | H-3, H-4α |
| 4β | 1.54 (1H, m) | | H-3, H-4α, H-5 | | H-4β |
| 5 | 1.82 (1H, m) | 37.9 (CH) | H-4α | 3H-19 | H-4β, H-8, 3H-19 |
| 6α | 1.65 (1H, m) | 27.4 ($CH_2$) | H-6β | 3H-19(w) | H-3, H-6β |
| 6β | 1.48 (1H, m) | | H-6α | | H-6α |
| 7α | 1.17 (1H, m) | 27.5 ($CH_2$) | H-7β, H-8 | H-5 | H-7β |
| 7β | 1.92 (1H, m) | | H-7α | | H-7α, 3H-19 |
| 8 | 1.46 (1H, m) | 41.6 (CH) | H-7α, H-9, H-14 | 3H-19 | H-5, 3H-18, 3H-19 |
| 9 | 1.56 (1H, m) | 36.8 (CH) | H-7α, H-8, H-11α, H-12α, 3H-19 | H-7α, H-12α, 3H-19 | - |
| 10 | - | 36.1 (C) | - | 3H-19, H-8, H-4β | - |
| 11α | 1.46 (1H, m) | 21 ($CH_2$) | H-9, 2H-12 | - | H-11β, H-12α |
| 11β | 1.26 (1H, m) | | 2H-12 | | H-11α, 3H-19 |
| 12α | 1.12 (1H, m) | 39.3 ($CH_2$) | 2H-11, H-12β | H-17, 3H-18 | H-11α |
| 12β | 1.50 (1H, m) | | 2H-11, H-12α | | 3H-18, 3H-19 |
| 13 | - | 45.0 (C) | - | 3H-18, H-17, H-15x, H-16, H-8(w) | - |
| 14 | 1.06 (1H, m) | 54.3 (CH) | H-8, 2H-15 | 2H-15, H-17, 3H-18 | H-15α, H-17 |
| 15α | 2.33 (1H, m) | 37.8 ($CH_2$) | H-14, H-15β | - | H-14, H-15β, H-16 |
| 15β | 1.30 (1H, m) | | H-14, H-15α | | H-15α, 3H-18 |
| 16 | 4.37 (1H, m) | 73.9 (CH) | H-17, 2H-15 | 2H-15, H-17 | H-15α, H-17 |
| 17 | 2.16 (1H, d, *J*= 7.5 Hz) | 58.2 (CH) | H-16 | H-15α, H-21 | H-14, H-16, H-21, H-22 |
| 18 | 0.85 (3H, s) | 15.2 ($CH_3$) | - | H-17 | H-8, H-12β, H-15β, H-21, H-22 |
| 19 | 0.98 (3H, s) | 24.2 ($CH_3$) | - | - | H-4β, H-5, H-7β, H-11β, H-12β |
| 20 | - | 118.8 (C) | - | H-17, H-21, H-23 | - |
| 21 | 7.59 (1H, dd, *J*=2.5, 1.0 Hz) | 152.2 (CH) | H-22 | H-17, H-22 | H-17, H-18 |
| 22 | 7.77 (1H, dd, *J*=2.5, 9.7 Hz) | 151.2 (CH) | H-21, H-23 | H-17, H-21 | H-17, H-18, H-23 |
| 23 | 6.25 (1H, dd, *J*=1.0, 9.7 Hz) | 114.4 (CH) | H-22 | - | H-22 |
| 24 | - | 164 (C) | - | H-21, H-22, H-23 | - |
| 1' | 4.31 (1H, d, *J*=7.9Hz) | 102.6 (CH) | H-2' | H-2' | H-3, H-2', H-3', H-4', H-5' |
| 2' | 3.18 (1H, m) | 75.1 (CH) | H-1', H-3' | H-1' | H-1' |
| 3' | 3.32 (1H, m) | 78.2 (CH) | H-2', H-4' | H-2' | H-1', H-4' |
| 4' | 3.26 (1H, m) | 71.6 (CH) | H-3', H-5' | H-3', 2H-6' | H-1', H-3' |
| 5' | 3.22 (1H, m) | 77.8 (CH) | H-4', 2H-6' | H-1', H-4', H-6'A | H-1', 2H-6' |
| 6'A | 3.64 (1H, m) | 62.7 ($CH_2$) | H-5', H-6'B | - | H-5', H-6'B |
| 6'B | 3.82 (1H, m) | | H-5', H-6'A | | H-5', H-6'A |

## 2.5.2 Experimental

*Drimia capitata* bulbs were collected from Stutterheim by R. McMaster and a voucher specimen (SN) was retained at the Natal Herbarium. The dried plant material (873 g) was chopped and extracted with dichloromethane and methanol at room temperature for 48 hours using continuous agitation. The excess solvents were removed using a rotor evaporator and yielded the dichloromethane (6.47 g) and methanol (61.31 g) extracts.

*Isolation of compounds VIII and IX*

The dichloromethane and methanol extracts were separated using column chromatography (3 cm in diameter) and silica gel (Merck 9385) as the stationary phase.

The mobile phase used for the dichloromethane extract was a dichloromethane : methanol step gradient [100% dichloromethane (fractions 1-10), 1% methanol in dichloromethane (fractions 11-20), 2% methanol in dichloromethane (fractions 21-30), 5% methanol in dichloromethane (fractions 31-40) 10% methanol in dichloromethane (fractions 41-50)]. Fraction sizes of 100 $cm^3$ were collected in each step. Elution with 5% methanol in dichloromethane afforded compound VIII, which was purified on a pasteur pipette column using the same solvent system.

The methanol extract was separated using a dichloromethane : methanol step gradient [100% dichloromethane (fractions 1-30), 1% methanol in dichloromethane (fractions 31-50), 2% methanol in dichloromethane (fractions (51-90), 3% methanol in dichloromethane (fractions 91-106), 5% methanol in dichloromethane (fractions 107-130), 7% methanol in dichloromethane (fractions 131-200), 10% methanol in dichloromethane (fractions 201-220), 20% methanol in dichloromethane (fractions 221-249), 100% methanol (fractions 250-260)] as the mobile phase. In each step, 100 $cm^{-3}$ fractions were collected. Compound IX eluted from the column using a 5% methanol in dichloromethane solvent system and was purified on a column (2 cm in diameter) using 1% methanol in dichloromethane as the mobile phase.

### 2.5.2.1 Physical Data for Compound VIII

**Name:** 5β-3β,16β-dihydroxybufa-20,22-dienolide

**Physical appearance:** white amorphous

**Yield:** 12.2 mg

**Mass:** $[M^+]$ at *m/z* 386, $C_{24}H_{34}O_4$ requires 386

**Optical Rotation:** $[\alpha]_D = +16.7°$ (c = 0.006 g/100 $cm^3$; MeOH),

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.9 (page 108)

### 2.5.2.2 Physical Data for Compound IX

**Name:** 5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside

**Physical appearance:** cream powdered substance

**Yield:** 55.5 mg

**Mass:** $[M^+]$ at *m/z* 548 FAB, $C_{30}H_{44}O_9$ requires 548

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3395, 2928, 1702, 1454, 1028

**Optical Rotation:** $[\alpha]_D = +66.4°$ (C = 0.226 g/100 $cm^3$; $CHCl_3$)

**$^1$H and $^{13}$C NMR Data:** Refer to table 2.10 (page 111)

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# CHAPTER 3

# Extractives from the Papaveraceae

## 3.1 Introduction to the Papaveraceae

Plants belonging to the family Papaveraceae may be annual, biennial or perennial herbs having hairs or spines[1]. They usually contain white, yellow or orange sticky latex in all parts of the plant except the seeds. There are approximately twenty-three genera with about two hundred and forty species distributed mainly in the temperate regions of the northern hemisphere and extending into central and South America. In southern Africa there are four genera, three of which are exotic, and about eight species, only one of which, *Papaver aculeatum*, is indigenous[1]. Many species of Poppy (*Papaver*) are cultivated as ornamentals and a few species have become locally established.

Plants belonging to the family Papaveraceae are a rich source of opium, which is used medicinally, and in the illegal production of heroin. The latex of some species is used in the treatment of warts, for eye disorders and as a dye source for colouring feathers[2].

## 3.2 Extractives from *Papaver aculeatum*

Plants of the genus *Papaver* are annual, biennial or perennial herbs with the green parts having hairs or bristles, or spines in the case of *P. aculeatum*.

Figure 3.1. Photograph of *Papaver aculeatum* (Photographed by Dr. Neil Crouch).

The sap of *P. aculeatum* is milky or coloured. There are approximately eighty species of *Papaver* widespread in the northern hemisphere. This project was initiated by Professor P. Tetenyi of the Institute of Research into Medicinal Plants in Budapest, Hungary who communicated that the South African species was considered primitive amongst the *Papavers* and could contain simple alkaloid precursors.

## 3.2.1 Results and Discussion

The methanol extract of the leaves of *Papaver aculeatum* Thunb. yielded an alkaloid, *N*-acetylanonaine (**compound X**).

### 3.2.1.1 Structural Elucidation of Compound X

### (+)-*N*-acetylanonaine

(Spectra 10a-n, pages 303-316)

The only compound to be isolated from *Papaver aculeatum* was identified as the alkaloid, (+)-*N*-acetylanonaine. (-)-*N*-acetylanonaine has been previously isolated from *Liriodendron tulipifera*[3] (Magnoliaceae) and the root bark of *Zanthoxylum simulans*[4] (Rutaceae).

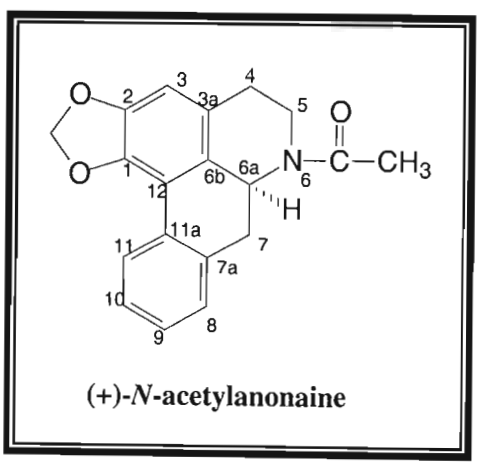
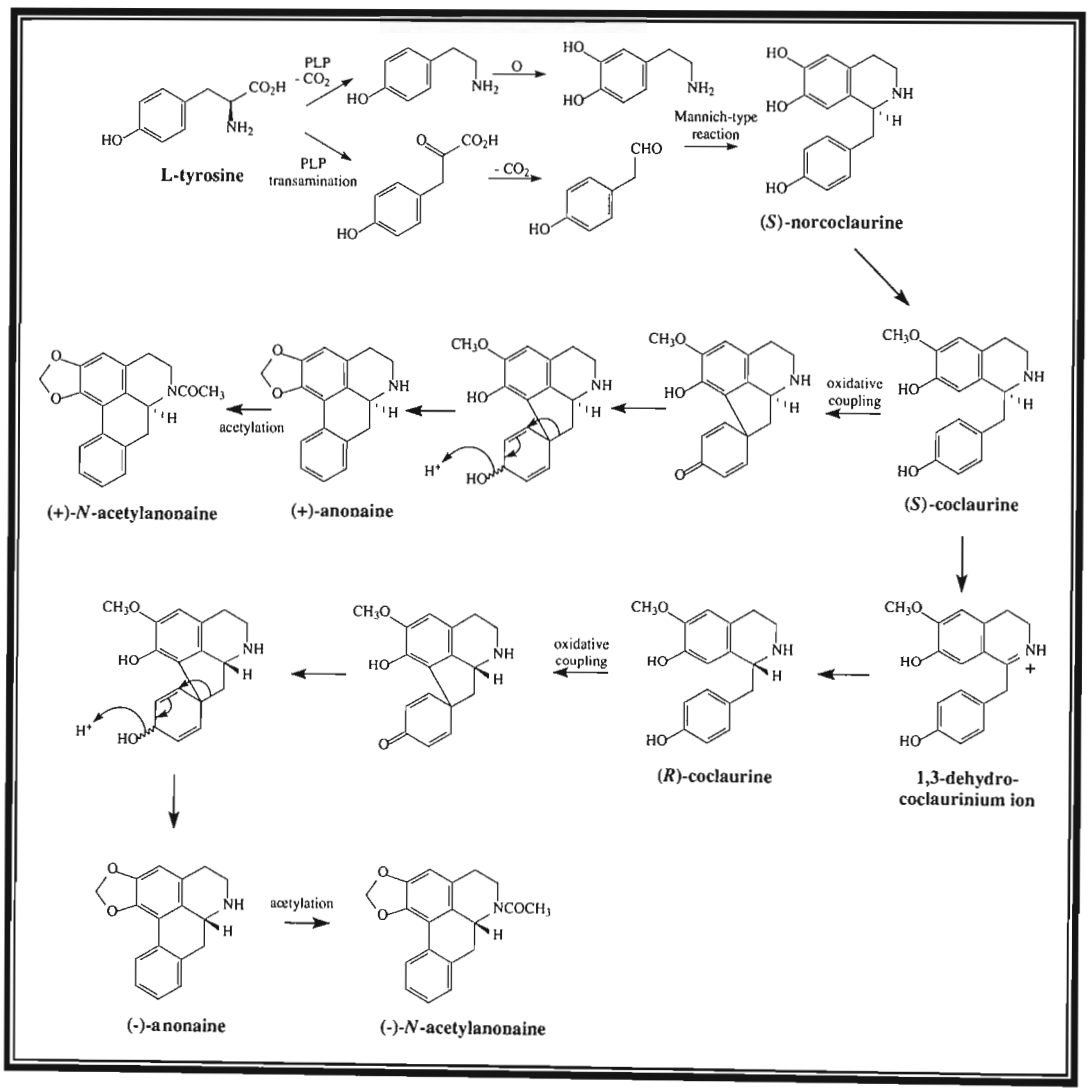



Figure 3.2. The structure of compound X.

The proposed biosynthesis of compound X is shown in scheme 3.1. This involves the combination of two molecules of tyrosine, one forming dopamine and the other forming 4-hydroxyphenylacetaldehyde. These two molecules then undergo a

Mannich-type reaction to form the trihydroxy alkaloid norcoclaurine, formed sterospecifically as the (*S*)-enantiomer[5]. The next step in the biosynthesis is the formation of a dienone, which forms *via* oxidative *ortho-para* coupling to the phenol group. After reduction of the carbonyl group, a rearrangement occurs, restoring the aromatic ring and expelling the hydroxy group[5]. Many biosynthetic pathways of alkaloids feature this type of rearrangement, and it occurs because keto-enol tautomerism is not possible for rearomatisation when coupling involves positions already substituted[5]. The final step in the biosynthesis is the acetylation of anonaine to form (+)-*N*-acetylanonaine. The formation of (-)-*N*-acetylanonaine occurs *via* the same biosynthetic route, however, the change in configuration from the (*S*)-enantiomer to the (*R*)-enantiomer is achieved by an oxidation-reduction process and the formation of an intermediate, (*R*)-coclaurine.




Scheme 3.1. The proposed biosynthesis of (+)-*N*-acetylanonaine and (-)-*N*-acetylanonaine[5].

The high resolution mass spectrum indicated a molar mass of 307.11974 g $mol^{-1}$, which corresponded to a molecular formula of $C_{19}H_{17}NO_3$ ($C_{19}H_{17}NO_3$ requires 307.12084). A double bond equivalence of twelve was deduced.

The infra-red spectrum showed a broad band at 3444 $cm^{-1}$ due to the O-H stretching vibrations and a peak at 2921 $cm^{-1}$ due to the asymmetric C-H stretching. The sharp peak at 1648 $cm^{-1}$ was due to the presence of a carbonyl group. The $CH_2$ and $CH_3$ bending vibrations were observed at 1223 $cm^{-1}$ and 1055 $cm^{-1}$.

The $^1H$ NMR spectrum showed two non-equivalent proton resonances for the non-equivalent protons of the methylene dioxy group at δ 6.08 and δ 5.97. A singlet proton resonance at δ 6.65 was assigned to H-3. The C-1 and C-2 fully substituted carbon resonances at δ 144.2 and δ 148.1 both showed coupling in the HMBC spectrum to the methylene dioxy group protons and H-3. The $^1H$ NMR spectrum showed a double doublet at δ 8.10 ($J$ = 7.9 Hz), which was seen to be coupled in the COSY spectrum to three superimposed proton resonances between δ 7.23 and δ 7.35. The $^1H$ NMR spectrum indicated the presence of a three-proton acetate methyl group proton resonance at δ 2.22. The $^{13}C$ NMR spectrum indicated a further methine carbon resonance at δ 52.2 (C-6a) and three methylene carbon resonances at δ 43.3 (C-5), δ 34.5 (C-7) and δ 31.4 (C-4). The COSY spectrum showed coupling between protons of two of the methylene carbons, C-5 (H-5α and H-5β, δ 3.27 and δ 4.11) and C-4 (H-4, δ 2.81) and coupling between the methine proton (H-6a, δ 5.04, dd, $J$ = 4.5 Hz and 14.0 Hz) and the third methylene group protons (2H-7, δ 3.07 and δ 2.84).

Use of 1-D NOE spectra and the NOESY spectrum enabled the assignment of the relative stereochemistry of the aliphatic protons. It was initially assumed that H-6a was β as in the literature[3], and the other protons were assigned relative to this. However, when the optical rotation was determined, it became clear that compound X was the enantiomer of the known compound and H-6 was actually α. The $[\alpha]_D$ value obtained for compound X was + 410° while the $[\alpha]_D$ value[3] for (-)-*N*-acetylanonaine is -356° and the $[\alpha]_D$ value[3] for (-)-*N*-acetylasimilobine is -405°.

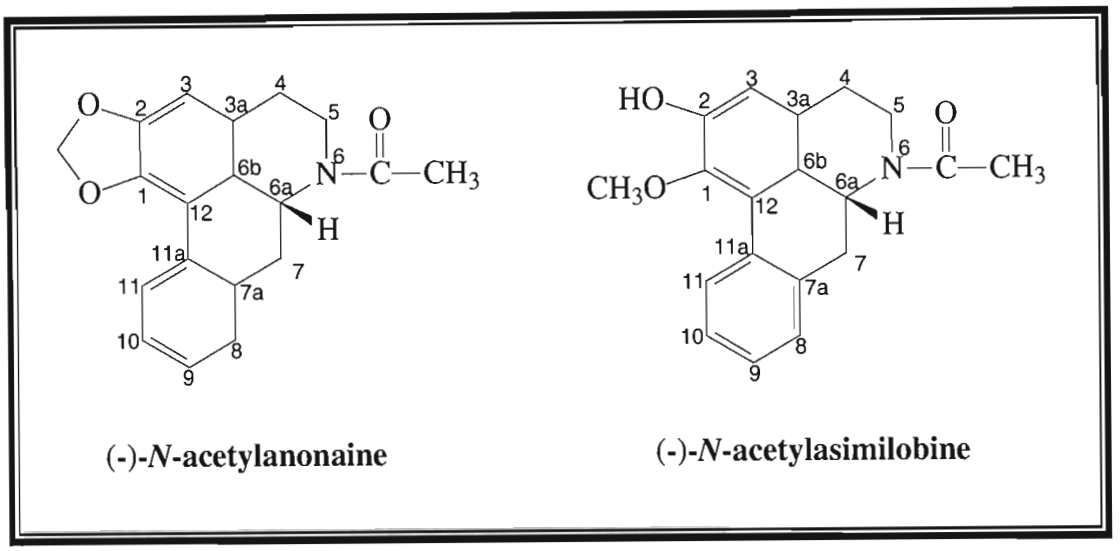



Figure 3.3. The structures of (-)-*N*-acetylanonaine and (-)-*N*-acetylasimilobine[3]

The H-6a proton showed a correlation in the NOESY spectrum to the one H-7 proton resonance at δ 3.07 indicating this proton to be H-7α and the other proton of the methylene group co-occurred with the 2H-4 resonance at δ 2.84 to be H-7β. Irradiation of H-3 showed positive correlations in the NOESY spectrum with both the superimposed H-4 protons, thus they were not distinguished as α or β.

Irradiation of the H-5 resonance at δ 4.11 showed no correlation in the NOESY spectrum with H-6a (which is α in orientation), indicating it must have the β stereochemistry. This resonance showed a correlation with H-5α (δ 3.27), 2H-4 and the acetate methyl group proton resonance. Irradiation of H-11 only gave a NOE correlation with the superimposed aromatic proton resonances.

There was great difficulty in obtaining literature NMR data for comparison purposes as Hufford *et al.*[3] only gives selected $^1$H NMR data and the literature reference[4] only refers to the NMR data for the isolated (-)-*N*-acetylanonaine being compared to an authentic sample.

Compound X was identified as the alkaloid, (+)-*N*-acetylanonaine. The NMR data and correlations are shown in table 3.1.

**Table 3.1. NMR data and correlations for compound X ($CD_3OD$) and literature data for (-)-*N*-acetylanonaine ($CDCl_3$)[3]**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^1$H NMR literature data for (-)-*N*-acetylanonaine[3] |
|---|---|---|---|---|---|---|
| **1** | - | 144.2 (C)$^{\dagger}$ | - | H-3, $OCH_2O$ | - | - |
| **2** | - | 148.1 (C)$^{\dagger}$ | - | H-3, $OCH_2O$ | - | - |
| **3** | 6.65 (1H, s) | 108.5 (CH) | - | - | 2H-4 | 6.87 (1H, s) |
| **3a** | - | 128.1 (C) | - | | - | - |
| **4** | 2.81 (2H, m) | 31.4 ($CH_2$) | 2H-5 | H-3 | H-3, H-5α, H-5β | ± |
| **5α** | 3.27 (1H, m) | 43.3 ($CH_2$) | 2H-4, H-5β | $COCH_3$ | 2H-4, H-5β, $COCH_3$ | |
| **5β** | 4.11 (1H, m) | | 2H-4, H-5α | | 2H-4, H-5α | ± |
| **6a** | 5.04 (1H, dd, *J*=4.5 Hz and 14.0 Hz) | 52.2 (CH) | H-7α, H-7β | H-7α, H-7β | H-7α, $COCH_3$ | ± |
| **6b** | - | 126.9 (C) | - | H-3, H-7α, H-7β | - | - |
| **7α** | 3.07 (1H, m) | 34.5 ($CH_2$) | H-6a, H-7β | - | H-6a, H-7β $COCH_3$ | |
| **7β** | 2.84 (1H, m) | | H-6a, H-7α | | H-7α | ± |
| **7a** | - | 137.0 (C)$^{\ddagger}$ | - | H-7α, H-7β, H-11, X | - | - |
| **8** | 7.30 (1H, X) | 128.8 (CH)$^{\dagger}$ | X | * | X | 7.37 (1H, m) |
| **9** | 7.30 (1H, X) | 128.9 (CH) | X | * | X | 7.37 (1H, m) |
| **10** | 7.30 (1H, X) | 129.6 (CH)$^{\dagger}$ | X | * | X | 7.37 (1H, m) |
| **11** | 8.10 (1H, dd, *J*=7.9 Hz) | 128.4 (CH) | X | | X | 8.18 (1H, dd) |
| **11a** | - | 131.9 (C)$^{\ddagger}$ | - | H-7β, X | - | - |
| **12** | - | 127.9 (C) | - | H-11 | - | - |
| **$OCH_2O$** | 6.08 and 5.97 (1H each, d, *J*=1.0 Hz) | 102.3 ($CH_2$) | - | - | - | 6.20 and 6.08 (1H, each, d, *J*=1.8 Hz) |
| **$\underline{C}OCH_3$** | - | 169.1 (C) | - | $COCH_3$ | - | - |
| **$CO\underline{C}H_3$** | 2.22 (3H, s) | 22.3 ($CH_3$) | - | - | H-5α, H-7α | 2.23 (3H, s) |

X = three superimposed aromatic resonances

$^{\dagger,\ddagger}$ values may be interchangeable, * cannot be assigned due to superimposed peaks

$^{\pm}$ $^1$H NMR data not available in literature[3]

## 3.2.2 Experimental

*Papaver aculeatum* was collected from Hella Hella by Dr. Neil Crouch and a voucher specimen (N. Crouch 848) was retained at the Natal Herbarium. The dried plant material was divided up into seeds, capsules and leaves/stems, and each was extracted with methanol at room temperature for 48 hours using continuous agitation. The excess solvent was removed using a rotor evaporator, which afforded the seed methanol extract (5.01 g), the capsule methanol extract (11.33 g) and the leaves/stem methanol extract (188.52 g). The three extracts were run on a GC-Mass Spectrometer to ascertain the components of the extracts. From this data, the three extracts were considered to be similar and thus due to the greater quantity of the leaves/stem extract, this extract was subjected to column chromatography in order to separate the compounds.

***Isolation of compound X***

All compounds were separated on a crude column (3 cm in diameter) using silica gel (Merck 9385) as the stationary phase and a mobile phase of a dichloromethane : methanol gradient step [100% dichloromethane (fractions 1-30), 1% methanol in dichloromethane (fractions 31-50), 2% methanol in dichloromethane (fractions 51-60), 5% methanol in dichloromethane (fractions 61-90), 10% methanol in dichloromethane (fractions 91-110), 10 dichloromethane : 2 methanol (fractions 111-130), 10 dichloromethane : 3 methanol (fractions 131-150) 100% methanol (fraction 151-160)]. In each step a fraction size of 40 $cm^3$ was collected. Compound X was eluted from the crude column using a 1% methanol in dichloromethane solvent system and was purified on a column (1 cm in diameter) using the same solvent.

Two other compounds were isolated from this extract, however they were isolated in such a small yield that there was too little material to purify further, hence they were not investigated further.

### 3.2.2.1 Physical Data for Compound X

**Name:** (+)-*N*-acetylanonaine

**Synonyms:** 6-acetyl-1,2-methylenedioxynoraporphine

**Physical appearance:** cream needles

**Yield:** 14.4 mg

**Melting Point:** 220-222°C, Literature = 229-230°C[3] for (-)-*N*-acetylanonaine

**Mass:** HRMS [$M^+$] at *m/z* 307.11974, $C_{19}H_{17}NO_3$ requires 307.12084

EIMS: *m/z*: 307, 248, 236, 235, 206, 178, 165, 149, 72, 57, 43

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3444, 2921, 2383, 2288, 1648, 1419, 1223, 1055, 935

**Optical Rotation:** $[\alpha]_D$ = + 410.7° (c = 0.028 g/100 $cm^3$;MeOH),

Literature $[\alpha]_D^{25}$ = - 356° (c = 0.49; $CHCl_3$)[3] for (-)-*N*-acetylanonaine

**$^1$H and $^{13}$C NMR Data:** Refer to table 3.1 (page 121)

## 3.3 References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# CHAPTER 4

# Extractives from the Asteraceae

## 4.1 Introduction to the Asteraceae (Compositae)

Plants belonging to the family Asteraceae may be annual, biennial or perennial herbs, shrubs or trees. These plants are rarely aquatic and are sometimes succulent, typically having resin ducts or latex canals[1]. There are approximately one thousand five hundred and thirty-five genera containing about twenty-five thousand species. These plants usually prefer a cosmopolitan habitat and are absent only from Antarctica[1]. In southern Africa alone, there are two hundred and forty-six genera and two thousand three hundred and five species.

### 4.1.1 Introduction to Alkamides (Isobutylamides)

The alkamides comprise a group of closely related fatty acid amides with characteristic olefinic and acetylenic patterns[2]. Purely olefinic derivatives occur in the Piperaceae, Aristolochiaceae and Rutaceae, while the Asteraceae tribes Heliantheae and Anthemideae additionally contain a great number of acetylenic amides[2]. Some olefinic isobutylamides have been found to possess insecticidal activity[2] and to be effective as molluscicides and cercaricides (cercariae are the haploid forms of schistosomes which penetrate the skin of humans and cause disease) and thus are important for the use against bilharzia[3]. Alkamides have been reported to have a pungent taste and produce local anaesthesia of the mucous membranes accompanied by profuse salivation[2]. Thus plants that contain these compounds have been used medicinally since ancient times as sialogogues, antitussives and analgesics[2].

## 4.2 Extractives from *Spilanthes mauritiana*

The genus *Spilanthes* belongs to the Heliantheae tribe and plants are annual herbs found mainly rooting near rocks in damp places. There are six species, with two species, including *S. mauritiana* (Pers.) DC., being indigenous to southern Africa[1].

Figure 4.1. Photograph of *Spilanthes mauritiana.*(Photographed by Dr. Neil Crouch).

In South Africa, the African plant *Spilanthes mauritiana*, of the family Asteraceae, is used medicinally by the Zulus as an oral local analgesic for the relief of toothache[4]. This is achieved by applying the moistened, powdered leaves to hollow teeth in order to relieve the pain[4]. Plants of this species are given the name *isisinini* by the Zulus, which is derived from the Zulu noun for gums (*insini*). The first recorded use in southern Africa was by Wood[5] in 1897, who, because of its peculiar pungent taste, noted its name as the 'electric' plant.

The Xhosa use the flowers for treating toothache and pyorrhoea[4], while the Chagga use the roots for toothache and sore throats, and unspecified parts of the plants are used as fever, articular rheumatism and snakebite remedies[6]. The Tsonga rub the leaves of this plant on mouth ulcers as an analgesic[7]. Other medicinal usage of *S. mauritiana* includes healing broken limbs, stomachache, diarrhoea, bladder complaints and headaches. The leaves stimulate the flow of saliva and numb the mouth. Antiscorbutic, diuretic and digestive stimulant properties have also been reported[6].

Previous chemical investigations of *Spilanthes mauritiana* have yielded volatile oils, while an acid amide, spilanthol (affinin, *N*-isobutyl-deca-2,6,8-trienamide), a sterol and a nonreducing polysaccharide have been isolated from the flowers[4]. Spilanthol acts as a strong local analgesic and has a distinctive hay-like odour. It has also been attributed with larvicidal and other insecticidal properties. Whole plant extracts have been reported to have extensive activities, including antifungal, antibacterial, antiprotozoal, antiviral and anthelmintic effects[8].

Jondiko *et al.*[9] isolated a potent mosquito larvicide, *N*-isobutyl-2*E*,4*E*,8*E*,10*Z*-dodeca-2,4,8,10-tetraenamide from the methanol extract of the fresh aerial parts of *Spilanthes mauritiana*. This compound gave 100% mortality against 3rd instar larvae of *Aedes aegypti*[9] at 5-10 mg/cm$^3$. This compound was not isolated in my chemical investigation of this plant, however, some related compounds were isolated.

### 4.2.1 Results and Discussion

This plant was initially the project of postdoctoral student Dr. J.J. Nair who investigated this plant in an attempt to isolate the compounds responsible for the numbing action of the plant. Dr. Nair isolated two compounds but he left the department before these compounds could be identified. I was given Dr Nair's work to complete and to determine the structures of the compounds that he had isolated. Dr Nair managed to run some 2-D spectra before leaving, however, even though his compounds were stored in a freezer, they still decomposed. These isobutylamide compounds are highly unstable[10] and several efforts to re-isolate this type of compound proved unsuccessful as they rapidly decomposed. Thus, the spectral data used in elucidating the structures of these compounds were taken from Dr Nair who managed to run only incomplete sets of spectra for analysis.

Two alkamides, one known, spilanthol (**compound XI**), previously isolated from the flowers of the plant, and one novel (**compound XII**) were isolated from the hexane extract of *Spilanthes mauritiana* (Pers) DC.

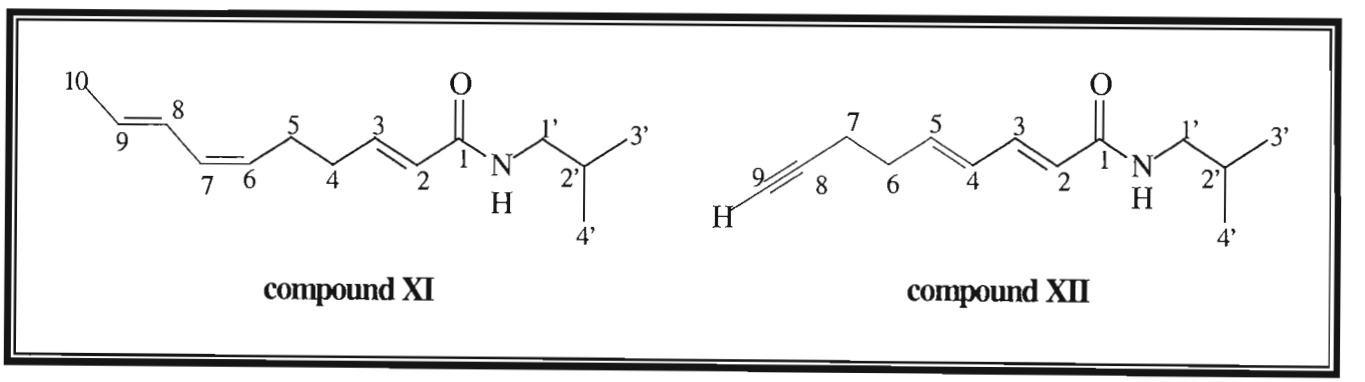


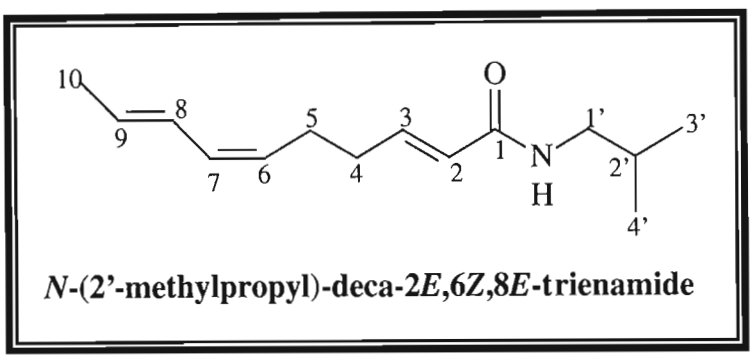

Figure 4.2. The structures of compounds XI and XII.

### 4.2.1.1 Structural Elucidation of Compound XI

### *N*-(2'-methylpropyl)-deca-2*E*,6Z,8*E*-trienamide (Spilanthol)

(Spectra 11a-e, pages 317-321)

The first compound isolated from this plant was a pale yellow viscous oil identified as the known isobutyl amide, *N*-(2'-methylpropyl)-deca-2*E*,6Z,8*E*-trienamide, commonly known as spilanthol. This compound has been isolated previously from the flowers of *Spilanthes mauritiana*[6], and the roots of *Heliopsis longipes*[3] and *Spilanthes oleraceae*[11].




***N*-(2'-methylpropyl)-deca-2*E*,6*Z*,8*E*-trienamide**

Figure 4.3. The structure of compound XI.

Due to these alkamides being so unstable, they decompose almost immediately after isolation. Thus only the $^{1}H$, $^{13}C$, COSY and HETCOR spectra were available to elucidate this structure.

The $^{13}C$ NMR spectrum showed the presence of fourteen carbon resonances. The fully substituted carbon signal at δ 168.8 indicated the presence of a carbonyl group. The COSY spectrum showed coupling between the proton resonance due to H-2' (δ 1.81, tqq, 1H) and 2H-1' (δ 3.09) as well as to the 3H-3'/3H-4' six-proton doublet resonance at δ 0.95. The COSY spectrum showed coupling between the proton peak at δ 5.95 (d, H-2) and H-3 (δ 6.80, dt), which, in turn, was coupled to 2H-4 (δ 2.32, m). The proton signal attributed to 2H-4 was seen to be coupled in the COSY spectrum to 2H-5 (δ 2.32, m); 2H-5 was seen to be coupled to H-6 (δ 5.28, dt). The H-6 resonance was seen to be coupled to H-7 (δ 5.98, dd) and the H-7 resonance was,

in turn, seen to be coupled to H-8 (δ 6.32, brdd); H-8 was seen to be coupled to H-9 (δ 5.71, dq) and this proton resonance was coupled to 3H-10 (δ 1.78, brd).

The coupling constants could not be obtained from the $^1$H NMR spectrum, as the line list for the spectrum had not been stored. Thus, in order to determine whether compound XI was the *trans-cis-trans* isomer or the all *trans* isomer, the spectrum was enlarged using a photocopier and the coupling constants calculated manually. A coupling constant of 11 Hz for $J_{6,7}$ and approximately 16 Hz for $J_{2,3}$ and $J_{8,9}$ indicated that this isolated compound is *N*-(2'-methylpropyl)-deca-2*E*,6Z,8*E*-trienamide. The $^1$H NMR data for compound XI was compared to that of literature[10] and the data correlated well.

Compound XI was found to be the known alkamide identified as spilanthol. Table 4.1 shows the NMR data for compound XI including the $^1$H NMR literature data[10] and $^{13}$C NMR literature data[11] for spilanthol.

**Table 4.1. NMR data and correlations for compound XI ($CD_3OD$) and literature data for spilanthol[10,11] ($CDCl_3$)**

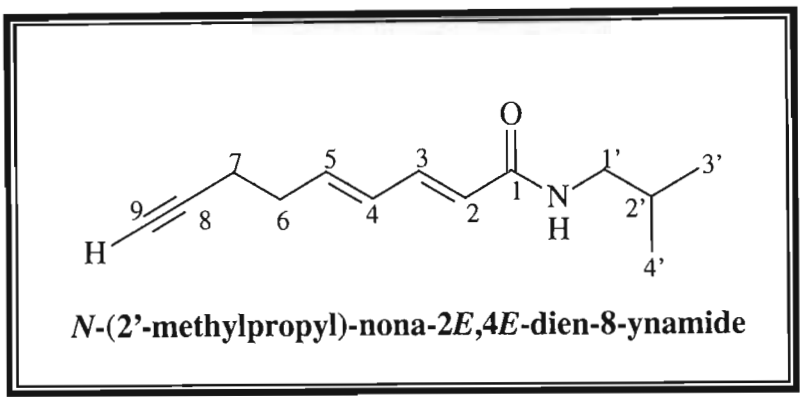
| Carbon Number | $^1$H NMR data (300 MHz) | $^{13}$C NMR data (75 MHz) | COSY correlations | $^1$H NMR literature data for spilanthol[10] (250 MHz) | $^{13}$C NMR literature data for spilanthol[11] (15.1 Hz) |
|---|---|---|---|---|---|
| **1** | - | 168.8 (C) | - | - | 166.3 |
| **2** | 5.95 (1H, d) | 125.2 (CH) | H-3 | 5.83 (1H, dt, *J*=15Hz, 1.5Hz) | 124.5 |
| **3** | 6.80 (1H, dt) | 144.7 (CH) | H-2, 2H-4 | 6.82 (1H, dt, *J*=15Hz, 6.5Hz) | 143.4 |
| **4** | 2.32 (1H, m) | 33.2 ($CH_2$) | H-3, 2H-5 | 2.28 (1H, m) | 32.2 |
| **5** | 2.32 (1H, m) | 27.5 ($CH_2$) | 2H-4, H-6 | 2.28 (1H, m) | 26.5 |
| **6** | 5.28 (1H, dt) | 128.5 (CH) | 2H-5, H-7 | 5.26 (1H, dt, *J*=11Hz, 7Hz) | 127.8 |
| **7** | 5.98 (1H, dd) | 130.7 (CH) | H-6, H-8 | 5.97 (1H, dd, *J*=11Hz, 11Hz) | 129.6 |
| **8** | 6.32 (1H, brdd) | 128.0 (CH) | H-7, H-9 | 6.29 (1H, brdd, *J*=11Hz, 15Hz) | 126.9 |
| **9** | 5.71 (1H, dq) | 130.6 (CH) | H-8, 3H-10 | 5.70 (1H, dq, *J*=15Hz, 7Hz) | 130.0 |
| **10** | 1.78 (3H, brd) | 18.3 ($CH_3$) | H-9 | 1.78 (3H, brd, *J*=7Hz) | 18.3 |
| **1'** | 3.09 (2H, dd) | 48.0 ($CH_2$) | H-2' | 3.14 (2H, dd, *J*=7Hz, 6.5Hz) | 47.0 |
| **2'** | 1.81 (1H, tqq) | 29.7 (CH) | 2H-1', 3H-3', 3H-4' | 1.80 (1H, tqq, *J*=6.5Hz, 7Hz, 7Hz) | 28.7 |
| **3'** | 0.95 (3H, d) | 20.5 ($CH_3$) | H-2' | 0.93 (3H, d, *J*=7Hz) | 20.2 |
| **4'** | 0.95 (3H, d) | 20.5 ($CH_3$) | H-2' | 0.93 (3H, d, *J*=7Hz) | 20.2 |

### 4.2.1.2 Structural Elucidation of Compound XII

### *N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide

(Spectra 12a-e, pages 322-326)

The second compound to be isolated from *Spilanthes mauritiana* was identified as the novel compound, *N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide.




***N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide**

Figure 4.4. The structure of compound XII.

The $^1$H NMR spectrum showed the presence of a doublet resonance, integrating to two protons, at δ 3.10 ($J$ = 6.8 Hz), which was due to 2H-1'. This resonance was shifted downfield due to the deshielding effect of the nitrogen atom. This resonance was seen to be coupled in the COSY spectrum to the proton resonance at δ 1.87, which was assigned to H-2'. The H-2' proton resonance was, in turn, seen to be coupled in the COSY spectrum to two three-proton doublets superimposed at δ 0.96 ($J$=6.8 Hz), which were ascribed to 3H-3' and 3H-4'. The corresponding carbon resonance were observed at δ 20.5 (C-3' and C-4').

The doublet resonance at δ 6.02 ($J$ = 15.1 Hz) was assigned to H-2 and was seen to be coupled in the COSY spectrum to a double doublet proton resonance at δ 7.15 (1H, $J$ = 10.7Hz and 15.1 Hz), which was due to H-3. The large coupling constant indicated the presence of a *trans* double bond between C-2 and C-3. The H-3 proton resonance was also seen to be coupled in the COSY spectrum to a proton resonance at δ 6.30 (H-4, dd, $J$ = 10.7, 15.3 Hz), which, in turn, was seen to be coupled in the COSY spectrum to H-5 (δ 6.16). The coupling constant of 15.3 Hz for H-4 indicated

a *trans* double bond between C-4 and C-5. The COSY spectrum showed coupling of the H-5 proton with the two-proton resonance at δ 2.43, assigned to H-6. The H-7 proton resonance was observed at δ 2.43 and was seen to be coupled in the COSY spectrum to H-6.

In the $^{13}C$ NMR spectrum, the fully substituted carbon resonance at δ 169.4 indicated the presence of a carbonyl group and hence was assigned to C-1. The downfield shifted carbon resonances at δ 124.2 (C-2), δ 141.5 (C-3), δ 131.2 (C-4) and δ 140.6 (C-5) indicated the presence of double bonds between C-2 and C-3 as well as between C-4 and C-5. The presence of the triple bond between C-8 and C-9 was confirmed by the fully substituted carbon resonance at δ 77.5 (C-8) and the methine carbon resonance at δ 66.8 (C-9)[12]. The one-proton singlet resonance at δ 2.51 in the $^{1}H$ NMR spectrum was assigned to H-9.

This compound was found to be the novel alkamide, *N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide. The NMR data for compound XII are shown in table 4.2.

**Table 4.2. NMR data and correlations for compound XII ($CD_3OD$)**

| Carbon Number | $^{1}H$ NMR data (300 MHz) | $^{13}C$ NMR data (75 MHz) | COSY correlations |
|---|---|---|---|
| 1 | - | 169.4 (C) | - |
| 2 | 6.02 (1H, d, *J*=15.1 Hz) | 124.2 (CH) | H-3 |
| 3 | 7.15 (1H, dd, *J*=10.7, 15.1 Hz) | 141.5 (CH) | H-2, H-4 |
| 4 | 6.30 (1H, dd, *J*=10.7, 15.3 Hz) | 131.2 (CH) | H-3, H-5 |
| 5 | 6.16 (1H, m) | 140.6 (CH) | H-4, H-6 |
| 6 | 2.43 (2H, m) | 32.4 ($CH_2$)± | H-5, H-7 |
| 7 | 2.43 (2H, m) | 19.3 ($CH_2$) ± | H-6 |
| 8 | - | 77.5 (C) | - |
| 9 | 2.51 (1H, s) | 66.8 (CH) | - |
| 1' | 3.10 (2H, d, *J*=6.8 Hz) | 48.1 ($CH_2$) | H-2' |
| 2' | 1.87 (1H, m) | 29.8 (CH) | H-1', H-3', H-4' |
| 3' | 0.96 (3H, d, *J*=6.8 Hz) | 20.5 ($CH_3$) | H-2' |
| 4' | 0.96 (3H, d, *J*=6.8 Hz) | 20.5 ($CH_3$) | H-2' |

± Values are uncertain due to insufficient spectra

### 4.2.2 Experimental

Cultivated material of *Spilanthes mauritiana* (611 g) was obtained from Silverglen Medicinal Plant Nursery for Dr. Nair by Dr. Neil Crouch and a voucher specimen (N. Crouch 788) was retained at the Natal Herbarium. The dried leaves were extracted using hexane for 48 hours at room temperature using continuous agitation. The hexane extract (5.10 g) resulted from the removal of excess solvent using a rotor evaporator. Column chromatography using silica gel (Merck 9385) was used to separate the compounds. Compound XI eluted from the column using a 20% ethyl acetate in 80% hexane solvent system, while compound XII eluted using 30% ethyl acetate in 70% hexane solvent system as the mobile phase. Dr. Nair who initially isolated these two isobuylamides recorded the data reported here.

#### 4.2.2.1 Physical Data for Compound XI

**Name:** *N*-(2'-methylpropyl)-deca-2*E*,6Z,8*E*-trienamide
**Synonyms:** spilanthol, affinin, *N*-isobutyl-deca-2*E*,6Z,8*E*-trienamide
**Physical appearance:** pale yellow viscous oil
**Yield:** 5 mg before decomposition
**Melting Point:** No sample, Literature = 23°C[13]
**Mass:** No sample
**Infra-red:** No sample
**$^{1}$H and $^{13}$C NMR Data:** Refer to table 4.1 (page 128)

#### 4.2.2.2 Physical Data for Compound XII

**Name:** *N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide
**Yield:** 4 mg before decomposition
**Melting Point:** No sample
**Mass:** No sample
**Infra-red:** No sample
**$^{1}$H and $^{13}$C NMR Data:** Refer to table 4.2 (page 130)

## 4.3 References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# CHAPTER 5

## Extractives from the Gentianaceae

## 5.1 Introduction to the Gentianaceae

Plants of the family Gentianaceae may be annual, biennial or perennial herbs, which may be erect or straggling. The plants are rarely shrubs or trees[1]. There are approximately eighty-nine genera containing one thousand two hundred species. These plants are mostly found in temperate and subtropical regions. In southern Africa alone, nine genera and seventy species exist[1].

## 5.2 Extractives from *Tachiadenus longiflorus*

The Madagascan plant, *Tachiadenus longiflorus* Griseb. belongs to the family Gentianaceae. This family is a diverse lineage of over one thousand five hundred angiosperm species, including many tropical and temperate trees, shrubs and herbs with a wide range of floral types and colours[2]. Plants belonging to the family Gentianaceae have many economical uses such as providing dyes and flavourings. Many species accumulate bitter iridoid substances, which are used medicinally. The plants are often used as cultivated ornaments[3]. A basic iridoid structure, as well as that of an isolated iridoid derivative known as longiflorone[4], are shown in figure 5.1.

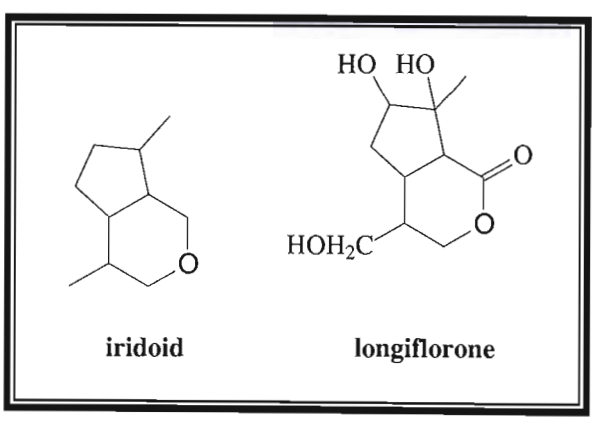



Figure 5.1. The structures of a basic iridoid and longiflorone[4].

Figure 5.2. Photograph of *Tachiadenus longiflorus*
(Photograph provided by Dr M. Randrianarivelojosia).

A decoction of the aerial parts of *T. longiflorus* is drunk to relieve stomach pains and dyspepsia, while a leaf decoction is drunk as a purgative or to alleviate gall bladder ailments[5].

### 5.2.1 Results and Discussion

The hexane extract of *Tachiadenus longiflorus* Griseb. yielded oleanolic acid (**compound XIII**) in large quantities, while the dichloromethane extract yielded two coumarins, scopoletin (**compound XIV**) and scoparone (**compound XV**). An iridoid derivative (**compound XVI**) was isolated from the ethyl actetate extract.

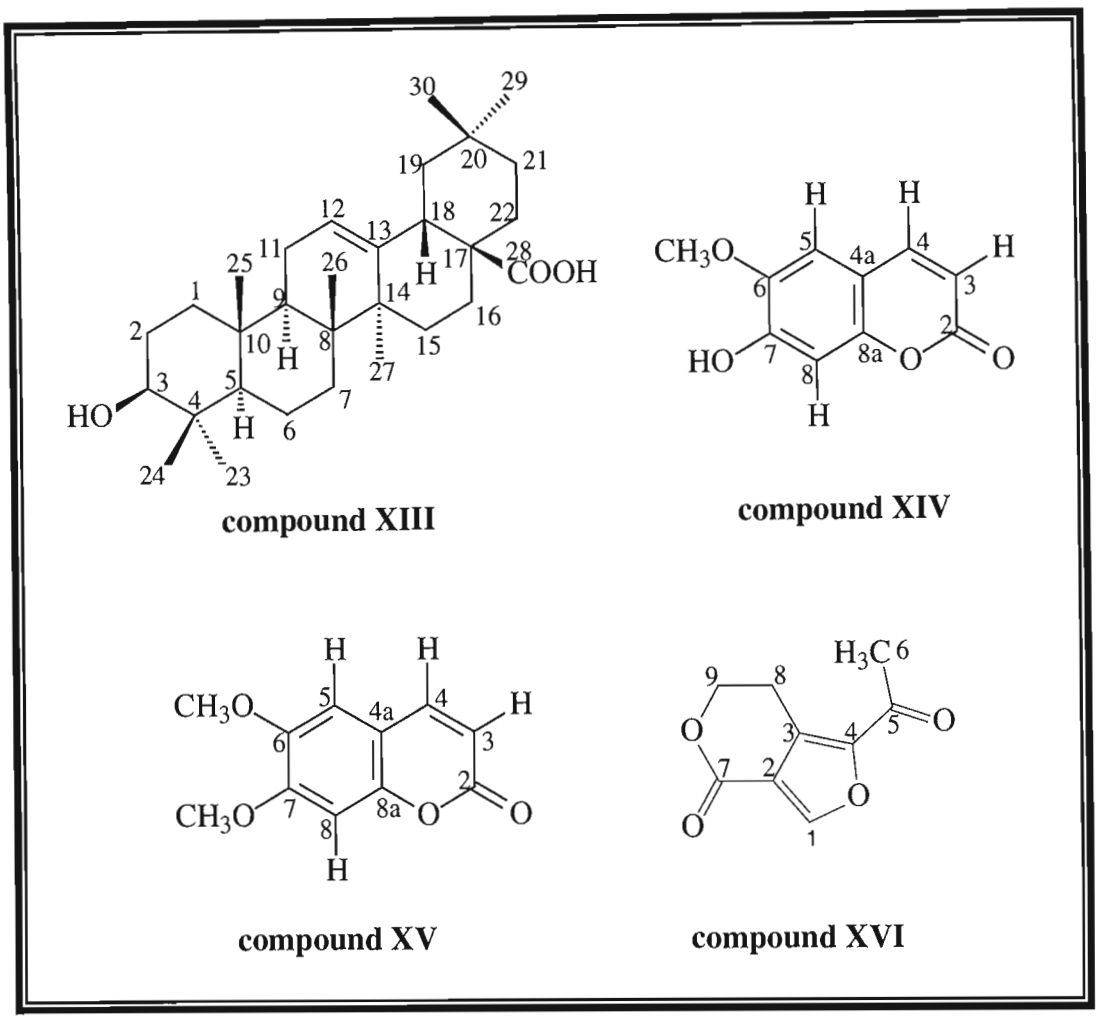



Figure 5.3. The structures of compounds XIII – XVI.

### 5.2.1.1 Structural Elucidation of Compound XIII

### 3β-hydroxy-12-oleanen-28-oic acid

(Spectra 13a-n, pages 327-340)

The first compound to be isolated from *Tachiadenus longiflorus* was a green-brown precipitate, which crystallised out of the hexane extraction in large quantities (approximately 2.5% of the hexane extract). This compound was identified as 3β-hydroxy-12-oleanen-28-oic acid, commonly known as oleanolic acid[6] (compound XIII). Oleanolic acid (Figure 5.3) has been reported to show antiulcer properties, anti-inflammatory activity and is an inhibitor of type 1 allergic reactions[7]. It occurs as glycosides in mistletoe, cloves (*Syzygium aromaticum*), sugar beet (*Beta vulgaris*) and in olive leaves, however it also exists as a widely distributed aglycone.

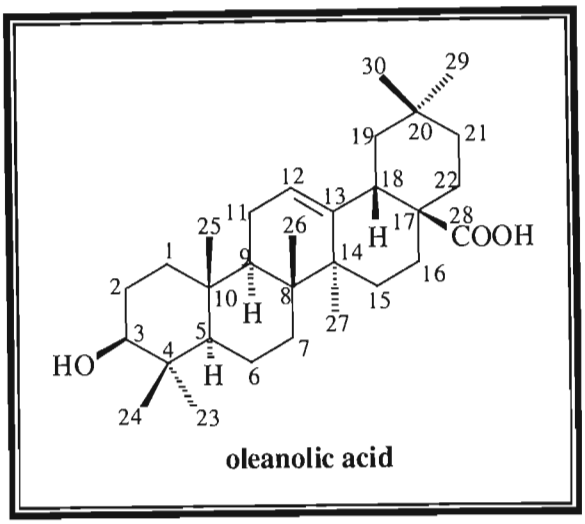



Figure 5.4. The structure of compound XIII.

The mass spectrum showed the presence of a parent ion peak at *m/z* 456.3578, which was consistent with the molecular formula of $C_{30}H_{48}O_3$ ($C_{30}H_{48}O_3$ requires 456.3603). A double bond equivalence of seven was deduced.

The infra-red spectrum showed absorption bands at 3412 $cm^{-1}$ (O-H stretching), 2923 $cm^{-1}$ and 2869 $cm^{-1}$ (asymmetric and symmetric C-H stretchings) and 1692 $cm^{-1}$ (C=O stretching).

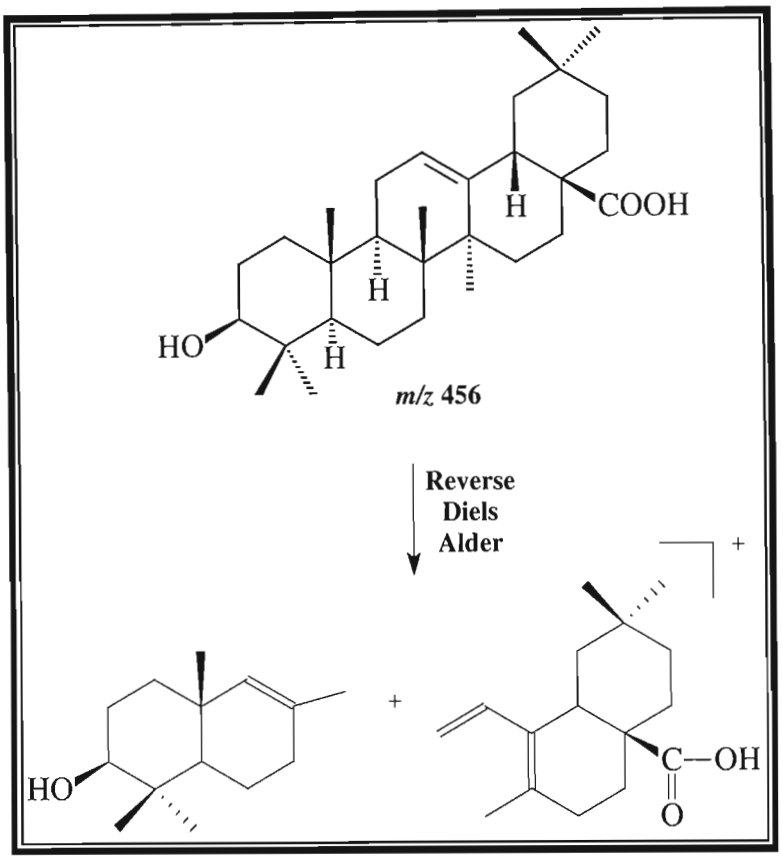
The $^1H$ NMR spectrum showed the presence of seven singlet proton resonances at δ 0.73, δ 0.75, δ 0.88, δ 0.89, δ 0.90, δ 0.96 and δ 1.11, each integrating to three protons. These singlet proton resonances were ascribed to 3H-26, 3H-24, 3H-29, 3H-25, 3H-30, 3H-23 and 3H-27 respectively by comparison of compound XIII to the literature data[6] for oleanolic acid and these were confirmed using the HMBC spectra. The triplet proton resonance at δ 5.25, integrating to one proton, indicated the presence of a double bond. This proton resonance, ascribed to H-12, was seen to be coupled in the COSY spectrum to the proton resonance at δ 1.84 (2H, m), which was due to H-11. The corresponding carbon resonances, obtained from the HSQC spectrum, were observed at δ 122.6 (C-12, CH) and δ 23.4 (C-11, $CH_2$). In the HMBC spectrum the methine carbon resonance due to C-12 showed a correlation to a one-proton resonance at δ 2.80, which was assigned to H-18. The proton resonance ascribed to H-18 was seen to be coupled in the COSY spectrum with 2H-19 (δ 1.15

and δ 1.58), while C-18 (δ 41.0) showed a correlation in the HMBC spectrum to 2H-16 (δ 1.59 and δ 1.92).

The proton resonance at δ 3.20 (1H, m) indicated the presence of a 3β-equatorial hydroxy group ($W_{1/2}$ = 16 Hz). The proton resonance ascribed to H-3 was seen to be coupled in the COSY spectrum to a proton resonance at δ 1.56 (2H), which was due to 2H-2. The methine carbon resonance at δ 79.1, assigned to C-3, showed correlations in the HMBC spectrum to 2H-1 (δ 0.92 and δ 1.58), 3H-23 and 3H-24. The H-5 proton resonance did not show a correlation in the NOESY spectrum to 3H-25, which biosynthetically is β in orientation, thus indicating that H-5 is α in orientation; and since H-5α is correlated in the NOESY spectrum to H-3, we can conclude that H-3 is also α in orientation.

The $^{13}C$ NMR spectrum showed the presence of a fully substituted carbon resonance at δ 183.5, which indicated, in conjunction with the IR spectrum, the presence of a carboxylic acid group. This carbon resonance was positioned at C–17 (δ 46.5, C) as it showed correlations in the HMBC spectrum to 2H-16, H-18 and 2H-22 (δ 1.54 and δ 1.72, each 1H). The two carbon resonances at δ 143.6 (C-13, C) and δ 122.6 (C-12, CH), confirmed the presence of the $\Delta^{12}$ double bond.

The molecular formula of $C_{30}H_{48}O_2$ indicated seven double bond equivalents. However, it was deduced that one alkene double bond and one carboxylic acid group were present, thus indicating the presence of five ring systems. The presence of six quaternary carbon resonances and one methyl group converted to a carboxylic acid group, in the $^{13}C$ NMR spectrum, suggested an oleanane-type compound. The olefinic carbon resonances at δ 122.6 (CH) and δ 143.6 (C), were characteristic of the $\Delta^{12}$ double bond in olean-12-enes[8]. The olean-12-ene structure of compound XIII was further suggested by the presence of a peak at *m/z* 248 ($[C_{16}H_{24}O_2]^+$), in the mass spectrum, due to a reverse Diels Alder fragmentation (Scheme 5.1). The $^{13}C$ NMR data of compound XIII was compared to the literature data[6] of oleanolic acid, and the data correlated well.

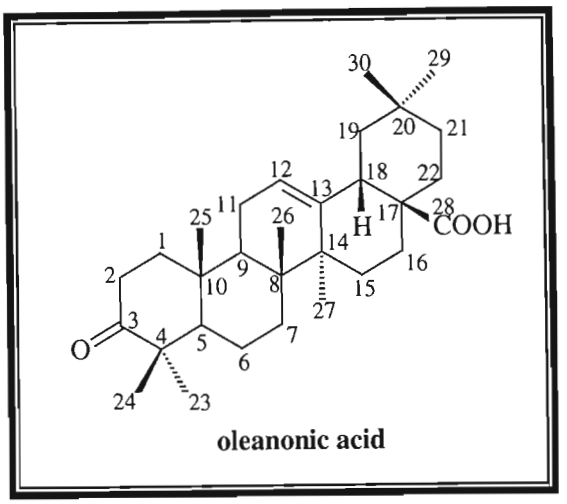



Scheme 5.1 The MS fragmentation pattern for compound XIII.

Compound XIII was oxidised using Jones' reagent, to yield white crystals, identified as oleanonic acid (Figure 5.5). The secondary alcohol at C-3 was oxidised to a ketone group. The NMR data and correlations for compound XIII and the literature $^{13}C$ NMR data for oleanolic acid are reported in table 5.1. The $^{13}C$ NMR data for oleanolic acid, and oleanonic acid are compared in table 5.2.

**Table 5.1. NMR data and correlations for compound XIII ($CDCl_3$) and literature data for oleanolic acid[6] ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for oleanolic acid[6] (50.1 MHz) |
|---|---|---|---|---|---|---|
| 1α | 0.92 (1H, m) | 38.4 ($CH_2$) | H-2 | 3H-25 | H-1β, H-5 | 38.5 |
| 1β | 1.58 (!H, m) | | | | H-1α | |
| 2 | 1.56 (2H, m) | 27.1 ($CH_2$) | 2H-1, H-3 | 2H-1 | H-3 | 27.4 |
| 3 | 3.20 (1H, m) | 79.1 (CH) | H-2 | 2H-1, 3H-23, 3H-24 | H-2, H-5, 3H-23 | 78.7 |
| 4 | - | 38.7 (C) | - | 3H-23, 3H-24 | - | 38.7 |
| 5 | 1.69 (1H, d, *J*=9.5 Hz) | 55.2 (CH) | 2H-6 | 3H-23, 3H-24, 3H-25 | H-1α, H-3, H-6α | 55.2 |
| 6β | 1.30 (1H, m) | 18.3 ($CH_2$) | H-5, 2H-7 | - | H-6α | 18.3 |
| 6α | 1.51 (1H, m) | | | | H-5, H-6β | |
| 7β | 1.26 (1H, m) | 32.6 ($CH_2$) | 2H-6 | H-6(w), 3H-26 | H-7α | 32.6 |
| 7α | 1.38 (1H, m) | | | | H-7β, 3H-27 | |
| 8 | - | 39.2 (C) | - | H-6, H-11, 3H-26, 3H-27 | - | 39.3 |
| 9 | 1.52 (1H, m) | 47.6 (CH) | H-11 | H-5, H-11, 3H-25 | H-11 | 47.6 |
| 10 | - | 37.1 (C) | - | H-2, H-6, H-11, 3H-25 | - | 37.0 |
| 11 | 1.84 (2H, m) | 23.4 ($CH_2$) | H-9, H-12 | 3H-26(w) | H-9, H-12, 3H-25, 3H-26 | 23.1 |
| 12 | 5.25 (1H, t, *J*=3.4 Hz) | 122.6 (CH) | H-11 | H-18 | H-11, H-18, H-19β | 122.1 |
| 13 | - | 143.6 (C) | - | H-11, 2H-15, H-18, 2H-19 | - | 143.4 |
| 14 | - | 41.6 (C) | - | H-9, 3H-26, 3H-27 | - | 41.6 |
| 15α | 1.05 (1H, m) | 27.7 ($CH_2$) | 2H-16 | 2H-16(w), 3H-27 | H-15β | 27.7 |
| 15β | 1.68 (1H, m) | | | | H-15α, 3H-26 | |
| 16A | 1.59 (1H, m) | 22.9 ($CH_2$) | 2H-15 | H-18 | H-16B | 23.4 |
| 16B | 1.92 (1H, m) | | | | H-16A | |
| 17 | - | 46.5 (C) | - | 2H-16, H-22 | - | 46.6 |
| 18 | 2.80 (1H, m) | 41.0 (CH) | 2H-19 | H-12, 2H-16, H-19(w), 3H-29(w) | H-12, H-19β, 3H-30 | 41.3 |
| 19β | 1.15 (1H, m) | 45.9 ($CH_2$) | H-18 | 3H-30, 2H-19(w), H-21(w), H-22, 3H-29 | H-12, H-18, H-19α | 45.8 |
| 19α | 1.58 (1H, m) | | | | H-19β | |
| 20 | - | 30.7 (C) | - | H-19(w), H-21(w), H-22, 3H-29 | - | 30.6 |
| 21α | 1.29 (1H, m) | 33.8 ($CH_2$) | 2H-22 | H-19(w), H-22(w), 3H-29,3H-30 | H-21β, 3H-29 | 33.8 |
| 21β | 1.17 (1H, m) | | | | H-21α, H-22α | |
| 22α | 1.72 (1H, m) | 32.4 ($CH_2$) | 2H-21 | 2H-21(w) | H-21β, H-22β, 3H-29 | 32.3 |
| 22β | 1.54 (1H, m) | | | | H-22α, 3H-30 | |
| 23 | 0.96 (3H, s) | 28.1 ($CH_3$) | - | 3H-24 | H-3, 3H-24 | 28.1 |
| 24 | 0.75 (3H, s) | 15.6 ($CH_3$) | - | H-6(w), 3H-23 | 3H-23 | 15.6 |
| 25 | 0.89 (3H, s) | 15.3 ($CH_3$) | - | 3H-26(w) | H-11, 3H-26 | 15.3 |
| 26 | 0.73 (3H, s) | 17.1 ($CH_3$) | - | 3H-25(w) | H-15β, 3H-25 | 16.8 |
| 27 | 1.11 (3H, s) | 25.9 ($CH_3$) | - | H-7(w) | H-7α | 26.0 |
| 28 | - | 183.5 (C) | - | 2H-16, H-18, 2H-22 | - | 181.0 |
| 29 | 0.88 (3H, s) | 33.1 ($CH_3$) | - | 2H-19, 2H-21, 3H-30 | H-21α, H-22α | 33.1 |
| 30 | 0.90 (3H, s) | 23.6 ($CH_3$) | - | 2H-19(w), 3H-29 | H-22β | 23.6 |



oleanonic acid

Figure 5.5. The structure of oleanonic acid.

**Table 5.2. A comparison of $^{13}C$ NMR data for oleanolic acid ($CDCl_3$) and oleanonic acid ($CDCl_3$)**

| Carbon Number | $^{13}C$ NMR data for oleanolic acid (100 MHz) | $^{13}C$ NMR data for oleanonic acid (100 MHz) |
|---|---|---|
| 1 | 38.4 ($CH_2$) | 39.2 ($CH_2$) |
| 2 | 27.1 ($CH_2$) | 34.1 ($CH_2$) |
| 3 | 79.1 (CH) | 217.8 (C) |
| 4 | 38.7 (C) | 47.4 (C) |
| 5 | 55.2 (CH) | 55.3 (CH) |
| 6 | 18.3 ($CH_2$) | 19.5 ($CH_2$) |
| 7 | 32.6 ($CH_2$) | 32.1 ($CH_2$) |
| 8 | 39.2 (C) | 39.1 (C) |
| 9 | 47.6 (CH) | 46.8 (CH) |
| 10 | 37.1 (C) | 36.8 (C) |
| 11 | 23.4 ($CH_2$) | 22.9 ($CH_2$) |
| 12 | 122.6 (CH) | 122.4 (CH) |
| 13 | 143.6 (C) | 143.6 (C) |
| 14 | 41.6 (C) | 41.7 (C) |
| 15 | 27.7 ($CH_2$) | 27.6 ($CH_2$) |
| 16 | 22.9 ($CH_2$) | 23.5 ($CH_2$) |
| 17 | 46.5 (C) | 46.5 (C) |
| 18 | 41.0 (CH) | 41.1 (CH) |
| 19 | 45.9 ($CH_2$) | 45.8 ($CH_2$) |
| 20 | 30.7 (C) | 30.6 (C) |
| 21 | 33.8 ($CH_2$) | 33.8 ($CH_2$) |
| 22 | 32.4 ($CH_2$) | 32.4 ($CH_2$) |
| 23 | 28.1 ($CH_3$) | 26.4 ($CH_3$) |
| 24 | 15.6 ($CH_3$) | 21.4 ($CH_3$) |
| 25 | 15.3 ($CH_3$) | 15.0 ($CH_3$) |
| 26 | 17.1 ($CH_3$) | 16.9 ($CH_3$) |
| 27 | 25.9 ($CH_3$) | 25.8 ($CH_3$) |
| 28 | 183.5 (C) | 183.4 (C) |
| 29 | 33.1 ($CH_3$) | 33.0 ($CH_3$) |
| 30 | 23.6 ($CH_3$) | 23.5 ($CH_3$) |

### 5.2.1.2 Structural Elucidation of Compound XIV

### 7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one (Scopoletin)

(Spectra 14a-h, pages 341-348)

The second compound to be isolated from *Tachiadenus longiflorus* was a crystalline material identified as 7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one, commonly known as scopoletin[9] (compound XIV). This compound could not be detected on a thin layer chromatographic plate using anisaldehyde spray reagent, instead it fluoresced with a bright blue spot under ultra violet (UV) light at 366 nm. It is possible to make assignments of the structural class from the colour of the fluorescence. Characteristically 7-alkoxycoumarins show a purple fluorescence while 7-hydroxycoumarins and 5,7-dioxygenated coumarins fluoresce with a blue colour[10]. Scopoletin has been reported to be used as an antispasmodic agent[7]. It occurs widely in the plant world including the root of *Gelsemium sempervirens*, *Atropa belladonna* and *Fabiana imbricata* to name just a few. The structure of compound XIV is shown in figure 5.6.

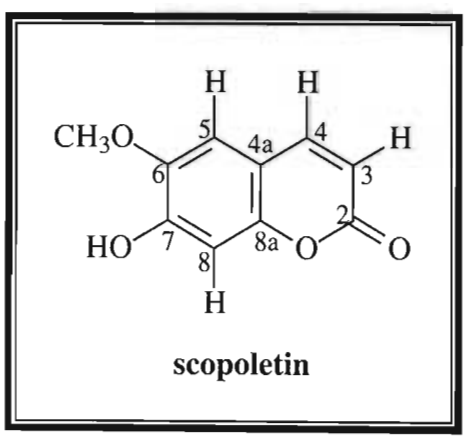



Figure 5.6. The structure of compound XIV.

Mass spectrometry showed a molecular ion peak at *m/z* 192, which indicated a molecular formula of $C_{10}H_8O_4$ ($C_{10}H_8O_4$ requires 192). Characteristic peaks were observed at *m/z* 177 [$M^+$ - 15], *m/z* 149 [$M^+$ - 15 - 18] and *m/z* 121. A double bond equivalence of seven was deduced.

The infra-red spectrum showed an intense band at 3338 $cm^{-1}$ due to the O-H stretching vibration. Less intense bands at 2914 $cm^{-1}$ and 2845 $cm^{-1}$ were due to the asymmetric and symmetric C-H stretching respectively. The carbonyl stretching vibration of the α,β-unsaturated lactone was shown as a strong sharp band at 1716 $cm^{-1}$. The bands at 1297 $cm^{-1}$ and 1139 $cm^{-1}$ were due to the $CH_2$ and $CH_3$ bending vibrations.

In the $^1H$ NMR spectrum the two one-proton doublet signals at δ 6.25 and δ 7.58 were ascribed to the H-3 and H-4 protons respectively. They appear as doublets as they belong to an isolated spin system and are split by each other. The coupling constant of 9.3 Hz is indicative of *cis* coupling (this was also confirmed by a correlation in the NOESY spectrum between H-3 and H-4). Both the H-3 and H-4 proton resonances showed coupling in the HMBC spectrum with the signal due to C-2 (δ 161.5). The two resonances at δ 6.83 and δ 6.90, each integrating to one proton, were assigned to the H-5 and H-8 protons respectively. An expanded spectrum showed that these signals are doublets and the coupling constant of 0.4 Hz indicates *para* coupling to one another. The singlet proton resonance at δ 3.93, integrating to three protons was due to the three equivalent protons of the methoxy group at C-6, which was seen to correlate in the HMBC spectrum to the resonance ascribed to C-6. The hydoxy group proton signal was observed as a broad singlet at δ 6.10 (1H).

The $^{13}C$ NMR spectrum showed the presence of ten carbon resonances. The HSQC spectrum was used to assign the proton resonances. In the HMBC spectrum the carbon resonance ascribed to C-8a showed a correlation with the signal assigned to H-5 and that of H-4, while the C-4a carbon peak showed correlations in the HMBC spectrum with H-3 and H-8. The resonance at δ 56.4 was due to the methoxy group carbon while the methine signals at δ 113.4, δ 143.3, δ 107.4 and δ 103.2 were ascribed to C-3, C-4, C-5 and C-8 respectively using the HSQC spectrum. The fully substituted signal at δ 161.5 was shifted far downfield and was due to the carbonyl group. Four other fully substituted carbon signals were observed at δ 111.5 (C-4a), δ 144.0 (C-6), δ 149.7 (C-7) and δ 150.2 (C-8a).

In the COSY spectrum, coupling is evident between the H-3 and H-4 protons and also between the three equivalent protons of the methoxy group and H-5. The NOESY spectrum showed coupling of the H-4 proton resonance with that of H-3 and H-5, while a correlation was evident in the NOESY spectrum between H-5 and the methoxy group proton resonance at C-6.

A literature search indicated that this compound was scopoletin. Compound XIV, at a concentration of 10 $\mu g/cm^3$, showed a 7.5% inhibition of prostglandin synthesis using the microsomal cell screen and a 4.5% inhibition using the Cox-2 screen (page 72).

The NMR data for compound XIV and the literature data[9] for scopoletin are given in Table 5.3. The C-4a, C-5 and C-8a literature values for scopoletin do not agree with the $^{13}C$ NMR data for compound XIV. This problem has arisen previously with other students in the research group whose $^{13}C$ NMR data also did not match that of the quoted literature values for this compund, thus an attempted synthesis of scopoletin was performed to confirm the structure. This synthesis (reported later in this chapter) proved unsuccessful, although the related coumarins, aesculetin, scoparone and isoscopoletin were synthesised and NMR data acquired for these compounds were used for comparison purposes. The NMR data for compound XIV is tabulated in table 5.3, while the HMBC and NOESY correlations for this compound are shown in figures 5.7 and 5.8 respectively.

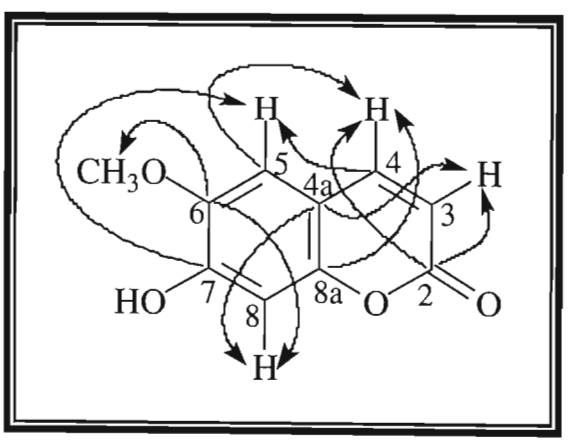



Figure 5.7. The HMBC (C → H) correlations for compound XIV.

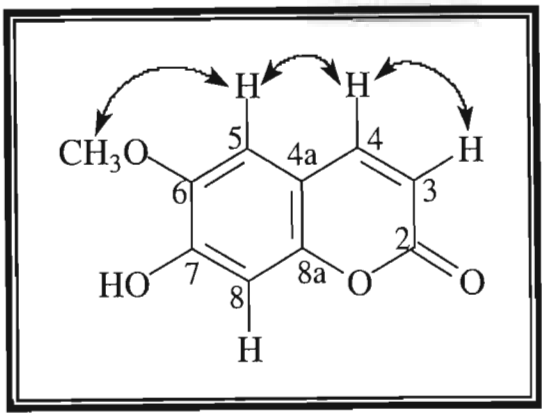



Figure 5.8. The NOESY correlations for compound XIV.

**Table 5.3. NMR data and correlations for compound XIV ($CDCl_3$) and literature data for scopoletin ($CDCl_3$)** [9]

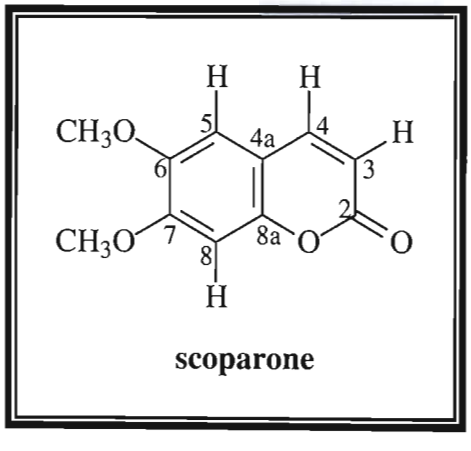
| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data 100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for scopoletin[9] (62.9 MHz) |
|---|---|---|---|---|---|---|
| **2** | - | 161.5 (C) | - | H-3, H-4 | - | 160.3 (C) |
| **3** | 6.25 (1H, d, *J*=9.3 Hz) | 113.4 (CH) | H-4 | - | H-4 | 114.4 (CH) |
| **4** | 7.58 (1H, d, *J*=9.3 Hz) | 143.3 (CH) | H-3 | H-5 | H-3, H-5 | 143.4 (CH) |
| **4a** | - | 111.5 (C) | - | H-3, H-8 | - | 114.4 (C) |
| **5** | 6.83 (1H, d, *J*=0.4 Hz) | 107.4 (CH) | $OCH_3$ at C-6 | H-4 | H-4, $OCH_3$ at C-6 | 113.9 (CH) |
| **6** | - | 144.0 (C) | - | H-5, H-8, $OCH_3$ at C-6 | - | 143.3 (C) |
| **7** | - | 149.7 (C) | - | H-5 | - | 149.8 (C) |
| **8** | 6.90 (1H, d, *J*=0.4Hz) | 103.2 (CH) | - | - | - | 104.2 (CH) |
| **8a** | - | 150.2 (C) | - | H-4, H-5, H-8 | - | 147.2 (C) |
| **$OCH_3$ at C-6** | 3.93 (3H, s) | 56.4 ($CH_3$) | H-5 | - | H-5 | 56.3 ($CH_3$) |

### 5.2.1.3 Structural Elucidation of Compound XV

### 6,7-dimethoxy-2*H*-1-benzopyran-2-one (Scoparone)

(Spectra 15a-h, pages 349-356)

The third compound isolated from this species was identified as the known coumarin, 6,7-dimethoxy-2*H*-1-benzopyran-2-one, commonly known as scoparone[9] (compound XV). It was isolated as a crystalline material from the dichloromethane extract. Compound XV (Figure 5.8) was also not detected using anisaldehyde spray reagent on a thin layer chromatographic plate and rather fluoresced under ultra violet light of 366 nm, as a bright purple spot. Scoparone has been previously isolated from several citrus oils and is also found in *Artemisia scoparia* and *Zanthoxylum setosum*. This compound has an $LD_{50}$ (rat,oral) value of 292 mg/kg[7].




**scoparone**

Figure 5.9. The structure of compound XV.

The mass spectrum indicated a molar mass of 206 g $mol^{-1}$, which corresponded to a molecular formula of $C_{11}H_{10}O_4$ ($C_{11}H_{10}O_4$ requires 206). A double bond equivalence of seven was deduced.

The infra-red spectrum showed peaks at 2914 $cm^{-1}$ and 2849 $cm^{-1}$ which were due to the C-H asymmetric and symmetric stretching respectively. The C=O stretching was observed at 1717 $cm^{-1}$, while the C=C stretching was seen at 1616 $cm^{-1}$. The bands at 1380 $cm^{-1}$, 1279 $cm^{-1}$ and 1138 $cm^{-1}$ indicated the $CH_2$ and $CH_3$ bending vibrations, while the C-O stretching vibration was observed at 1014 $cm^{-1}$.

The $^1$H NMR spectrum of compound XV showed a pair of doublets at δ 6.26 and δ 7.60, both integrating to one proton, which corresponded to H-3 and H-4 respectively. These two protons are *cis* to each other as shown by a coupling constant of 9.3 Hz. The COSY spectrum showed coupling between H-3 and H-4. The methine carbon signal at δ 113.5 was assigned to C-3 while the methine carbon signal at δ 143.3 was assigned to C-4. The H-3 and H-4 proton resonances showed correlations in the NOESY spectrum, which confirmed the *cis* orientation. The C-3 resonance showed no correlations in the HMBC spectrum while that of C-4 showed a correlation in the HMBC spectrum with H-5. The HMBC spectrum also showed a correlation between the C-5 resonance and H-4.

In the $^1$H NMR spectrum, two one-proton doublet peaks are observed at δ 6.83 ($J$ = 0.4 Hz) and δ 6.82 ($J$ = 0.4 Hz). These resonances are due to the H-5 and H-8 protons respectively, which are *para* coupled to one another. The methine carbon resonances for C-5 and C-8 were observed at δ 107.9 (C-5) and δ 100.0 (C-8). The final two singlet peaks, which appeared in the $^1$H NMR spectrum both integrated to three protons and were found to be methoxy group protons. The carbon resonances were observed at δ 3.90 ($OCH_3$ at C-6) and δ 3.93 ($OCH_3$ at C-7). The carbon signals for the methoxy groups occur at δ 56.3 ($OCH_3$ at C-6) and δ 56.4 ($OCH_3$ at C-7). In the NOESY spectrum the H-5 proton resonance showed a correlation with H-4 and the methoxy protons of the methoxy group at C-6, while the H-8 proton signal showed a correlation in the NOESY spectrum with the proton resonance at δ 3.93 (3H, s, $OCH_3$ at C-7). The fully substituted carbon resonance due to C-7 showed HMBC correlations with H-5 and the methyl protons of the methoxy group at C-7, while the fully substituted carbon resonance due to C-6 showed correlations in the HMBC spectrum with H-8 and the methyl protons of the methoxy group at C-6. Thus confirming the attachment of the two methoxy groups at C-6 and C-7.

The fully substituted carbon signals ascribed to C-2, C-4a, C-6, C-7 and C-8a were observed at δ 161.4, δ 111.4, δ 146.3, δ 152.8 and δ 150.0 respectively. The carbon signal assigned to C-2 showed a correlation in the HMBC spectrum with the H-3 and H-4 proton signals. In the HMBC spectrum correlations were also seen between C-4a

and the four proton resonances due to H-3, H-5, H-8 and H-4(w). The H-4 and H-8 proton peaks showed a correlation in the HMBC spectrum with C-8a.

From this data and by comparison with literature data[9], compound XV was found to be the known coumarin, scoparone. This compound, at a concentration of 10 $\mu g/cm^3$, showed a 4.5% inhibition of prostaglandin synthesis using the microsomal cell screen (page 72).

The NMR data for compound XV and scoparone is shown in Table 5.4, while the HMBC and NOESY correlations are illustrated in figures 5.10 and 5.11 respectively. The $^{13}C$ NMR data for compound XV did not correlate well with that of literature[9], and hence the attempted synthesis of scoparone was also performed so as to obtain NMR data that could be correctly assigned. This synthesis, which will also be discussed later in this chapter, proved successful, thereby proving that the assignments for compound XV were correct.

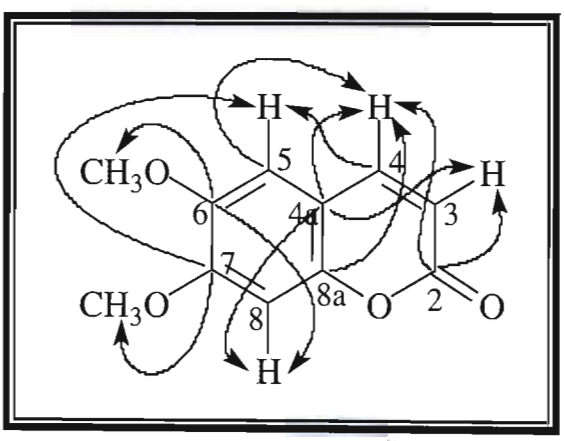



Figure 5.10. The HMBC (C → H) correlations for compound XV.

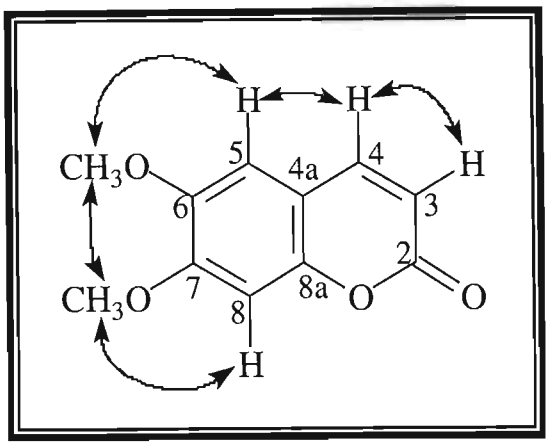



Figure 5.11. The NOESY correlations for compound XV.

**Table 5.4. NMR data and correlations for compound XV ($CDCl_3$) and literature data for scoparone[9] ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations | $^{13}$C NMR literature data for scoparone[9] (62.9 MHz) |
|---|---|---|---|---|---|---|
| **2** | - | 161.4 (C) | - | H-3, H-4 | - | 160.2 (C) |
| **3** | 6.26 (1H, d, *J*=9.3 Hz) | 113.5 (CH) | H-4 | - | H-4 | 114.9 (CH) |
| **4** | 7.60 (1H, d, *J*=9.3 Hz) | 143.3 (CH) | H-3 | H-5 | H-3, H-5 | 143.5 (CH) |
| **4a** | - | 111.4 (C) | - | H-3, H-4, H-5, H-8 | - | 114.9 (C) |
| **5** | 6.83 (1H, d, *J*=0.4Hz) | 107.9 (CH) | | H-4 | H-4, $OCH_3$ at C-6 | 113.6 (CH) |
| **6** | - | 146.3 (C) | - | H-8, $OCH_3$ at C-6 | - | 148.8 (C) |
| **7** | - | 152.8 (C) | - | H-5, $OCH_3$ at C-7 | - | 149.4 (C) |
| **8** | 6.82 (1H, d, *J*=0.4Hz) | 100.0 (CH) | - | - | $OCH_3$ at C-7 | 104.5 (CH) |
| **8a** | - | 150.0 (C) | - | H-4, H-5, H-8 | - | 147.5 (C) |
| **$OCH_3$ at C-6** | 3.90 (3H, s) | 56.3 ($CH_3$) | - | - | H-5, $OCH_3$ at C-7 | 56.3 ($CH_3$) |
| **$OCH_3$ at C-7** | 3.93 (3H, s) | 56.4 ($CH_3$) | - | - | H-8, $OCH_3$ at C-6 | 55.2 ($CH_3$) |

### 5.2.1.4 Structural Elucidation of Compound XVI

## Angelone

(Spectra 16a-h, pages 357-364)

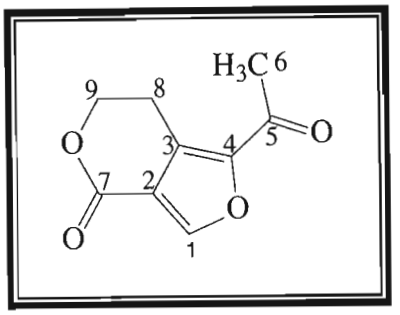
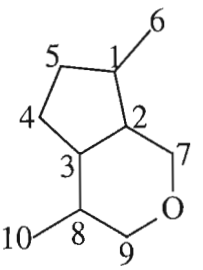
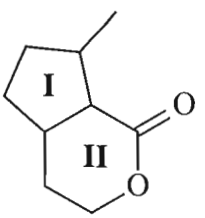



Figure 5.12. The structure of compound XVI*.

The fourth compound isolated from *Tachiadenus longiflorus* provided difficulties in determining the structure, as we were unable to determine the structure based purely on the NMR data. As this compound was isolated from a member of the Gentianaceae family, it was thought that the compound could be an iridoid. Iridoids have the following basic skeleton:




The methyl group at C-10 is often oxidatively lost and iridoids with the basic structure shown below are formed.




From the COSY spectrum it was evident that the ring II structure was present, with protons for two methylene groups at δ 4.50 (H-9, t, $J$ = 6.0 Hz) and δ 3.19 (H-8, t, $J$ = 6.0 Hz) seen to be coupled together in the COSY NMR spectrum. In the HMBC spectrum a correlation was observed between C-7 (160.8) and H-9. The $^1$H NMR

* Numbering of sidechain does not fit in with IUPAC rules due to there being an extra carbon atom.

spectrum showed a singlet at δ 8.12 (H-1, 1H) and a methyl group three-proton resonance at δ 2.50 (H-6, 3H, s).

The high resolution mass spectrum indicated a molecular mass of 180.04220 g $mol^{-1}$, which corresponded to a molecular formula of $C_9H_8O_4$ ($C_9H_8O_4$ requires 180.04226). A double bond equivalence of six was deduced. The mass spectrum appeared to indicate the presence of a methyl ketone with peaks at *m/z* 165 [M - $CH_3$] and *m/z* 137 [M - $CH_3$ - CO].

The infra-red spectrum showed peaks at 2916 $cm^{-1}$ and 2845 $cm^{-1}$ which were due to the C-H asymmetric and symmetric stretching respectively. The peak at 1746 $cm^{-1}$ is indicative of a saturated six-ring lactone, while the peak at 1686 $cm^{-1}$ indicates the presence of an α,β-unsaturated ketone carbonyl group. The C=C stretching was observed at 1610 $cm^{-1}$. The bands at 1408 $cm^{-1}$, 1248 $cm^{-1}$ and 1082 $cm^{-1}$ indicated the $CH_2$ and $CH_3$ bending vibrations, while the C-O stretching vibration was observed at 1019 $cm^{-1}$.

The lack of further correlations in the COSY and NOESY spectra made it difficult to suggest a structure. Hence this compound was sent to Professor Jean-Marc Nuzillard at the Université de Reims, France, who ran the NMR data through the LSD (Logic for Structure Determination) Program[11]. The LSD program produces planar structures according to 2D NMR correlation data and sub-structural information provided by the user. This program was used to elucidate a possible structure for compound XVI and the most likely structure suggested by the program, was that shown in figure 5.13.

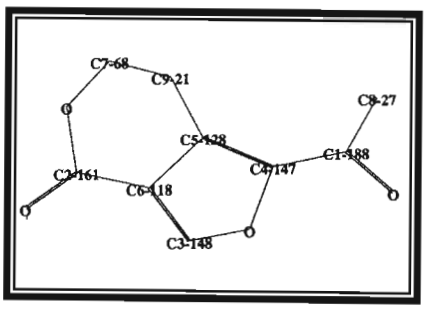



Figure 5.13. The structure of compound XVI as given by the LSD program*.

* The numbering system used here was according to the decending order of carbon data and not according to the IUPAC numbering system.

Then after being given the proposed structure from the LSD analysis, we were able to tentatively confirm this structure using HMBC and NOESY correlations. The structure also agreed with the methyl ketone group suggested by the mass spectrum. In the HMBC spectrum C-5 (δ 188.4) showed correlations with the 3H-6 resonance and the 2H-8 resonance, while C-7 (δ 160.8) showed a correlation in the HMBC spectrum with 2H-9. The C-1 (δ 148.2) methine carbon resonance was seen to be correlated weakly to H-8 in the HMBC spectrum ($J_4$ correlation), while the C-4 (δ 147.4) carbon resonance showed correlations in the HMBC spectrum with H-1, 3H-6, 2H-8 and weakly with the 2H-9 resonances.

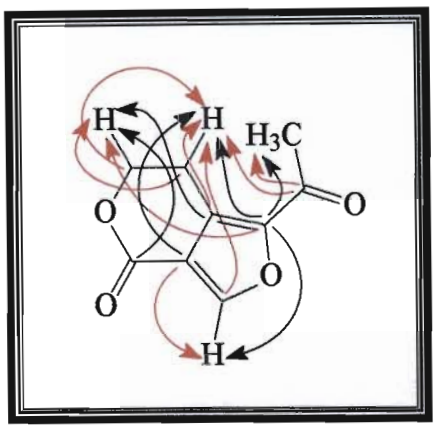



Figure 5.14. The HMBC (C → H) correlations for compound XVI.
($J_3$ in black, $J_2$ and $J_4$ in red)

The fully substituted C-3 carbon resonance at δ 128.3 showed correlations to 2H-9 and 2H-8 in the HMBC spectrum, while C-2 (δ 117.6) was correlated to H-1 and 2H-8. In the COSY spectrum, the 2H-9 resonance was seen to be coupled to the 2H-8 resonance and a correlation in the NOESY spectrum was also evident between these two resonances. The corresponding C-9 methylene carbon resonance at δ 68.5 showed an HMBC correlation to the 2H-8. The 3H-6 singlet proton resonance showed a weak correlation to the 2H-8 resonance in the NOESY spectrum. In the NOESY spectrum, the 2H-8 proton resonance was seen to be correlated to the 2H-9 and weakly to the 3H-6 resonance, while the corresponding methylene carbon resonance at δ 21.3, showed a correlation in the HMBC spectrum with H-9. However, biosynthetically the formation of this compound is difficult to explain.

The sample was sent to Dr. Peter Sandor at the Varian Applications Laboratory in Darmstadt, Germany, for the acquisition of an INADEQUATE spectrum to try and prove the structure but insufficient material was available for the experiment.

We have requested more plant material to be collected by Dr. M. Randrianarivelojosia, however, we were informed that this is the wrong time of year for collecting this plant and so this compound will be investigated further at a later stage.

The HMBC correlations and the NOESY correlations for compound XVI are given in figure 5.14 and figure 5.15 respectively and the NMR data and correlations are tabulated in table 5.5.

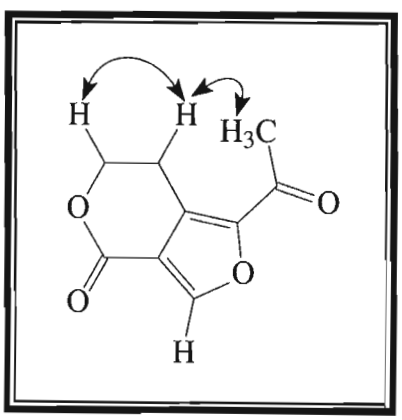



Figure 5.15. The NOESY correlations for compound XVI.

**Table 5.5. NMR data and correlations for compound XVI ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1 | 8.12 (1H, s) | 148.2 (CH) | - | 2H-8(w) | - |
| 2 | - | 117.6 (C) | - | H-1, 2H-8 | - |
| 3 | - | 128.3 (C) | - | 2H-9, 2H-8 | - |
| 4 | - | 147.4 (C) | - | H-1, 2H-9(w), 3H-6, 2H-8 | - |
| 5 | - | 188.4 (C) | - | 3H-6, 2H-8 | - |
| 6 | 2.50 (3H, s) | 26.8 ($CH_3$) | - | - | 2H-8(w) |
| 7 | - | 160.8 (C) | - | 2H-9 | - |
| 8 | 3.19 (2H, t, *J*=6.0 Hz) | 21.3 ($CH_2$) | 2H-9 | 2H-9 | 2H-9, 6H-6(w) |
| 9 | 4.50 (2H, t, *J*=6.0 Hz) | 68.5 ($CH_2$) | 2H-8 | 2H-8 | 2H-8 |

## 5.2.2 Experimental

The stem and bark of *Tachiadenus longiflorus,* was collected from Feverive, Madagascar and identified by Dr Milijaona Randrianarivelojosia. A voucher specimen was retained at the University of Antananarivo, Madagascar (09-99 MJ/DUL). The milled stem and bark (755 g) were collectively extracted using soxhlet extraction for approximately 24 hours using hexane, dichloromethane, ethyl acetate and methanol respectively. The excess solvent was removed using a rotor evaporator, which afforded the hexane (70 g), dichloromethane (54 g), ethyl acetate (27 g) and methanol (92 g) crude extracts. NMR analysis of the crude methanol extract indicated that only sugars were present and hence was not examined further.

***Isolation of compounds XIII - XV***

The hexane, dichloromethane and ethyl acetate extracts were subjected to column chromatography using silica gel (Merck 9385) on a column (3 cm in diameter) in order to separate the compounds.

Compound XIII was the predominant compound in this plant and precipitated out of the hexane extract in large quantities. Column chromatography run on the hexane extract yielded only more compound XIII and large quantities of fatty acids.

The mobile phase used for the dichloromethane extract was a dichloromethane : methanol step gradient [100% dichloromethane (fractions 1-40), 5% methanol in dichloromethane (fractions 41-80), 10% methanol in dichloromethane (fractions 81-120), 100 methanol (fractions 121-130)]. Compounds XIV and XV were eluted using 5% methanol in dichloromethane and were purified on a column (2 cm in diameter) using a 100% dichloromethane solvent system.

The mobile phase used for the ethyl acetate extract was a dichloromethane : methanol gradient step [100% dichloromethane (fractions 1-40), 1% methanol in dichloromethane (fractions 41-80), 2% methanol in dichloromethane (fractions 81-120), 5% methanol in dichloromethane (fractions 121-140), 10% methanol in dichloromethane (fractions 141-160), 100% methanol (fractions 161-170).

Compound XVI was eluted using a 1% methanol in dichloromethane solvent system and was purified on a Pasteur pipette column using 100% dichloromethane.

***Jones' oxidation of compound XIII***

Compound XIII (0.10 g) was dissolved in acetone (10 $cm^3$) and the solution was stirred at room temperature in a flat-bottomed flask. The Jones' reagent was prepared as follows: concentrated $H_2SO_4$ (23 $cm^3$) was diluted with water to a volume of 100 $cm^3$ and chromic trioxide (26.74 g) was dissolved in this solution. The Jones' reagent (0.1 $cm^3$) was then added slowly to the solution in the flat-bottomed flask and was allowed to stir for 5 hours. The solution was diluted with water and then washed with bicarbonate solution (5 $cm^3$). The product was extracted into dichloromethane (2 x 5 $cm^3$) and the solvent was removed using a rotor evaporator.

### 5.2.2.1 Physical Data for Compound XIII

**Name:** 3β-hydroxy-12-oleanen-28-oic acid

**Synonyms:** oleanolic acid, oleanol

**Physical appearance**: yellow-green crystalline precipitate

**Yield:** 39 g

**Melting Point:** 307-309°C, Literature = 306-308°C[7]

**Mass:** $[M]^+$ at *m/z* 456.3578, $C_{30}H_{48}O_3$, requires 456.3603

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$: 3412, 2923, 2852, 1692

**Optical rotation:** $[\alpha]_D$ = +53.6° (c = 0.14 g/100 $cm^3$; $CHCl_3$),
Literature $[\alpha]_D$ = + 79.5° ($CHCl_3$)[7]

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.1 (page 139)

### 5.2.2.2 Physical Data for Compound XIV

**Name:** 7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one

**Synonyms:** scopoletin, 7-hydroxy-6-methoxycoumarin, aesculetin 6-methyl ether

**Physical appearance:** cream coloured needles

**Yield:** 3.6 mg

**Melting Point:** 196-198°C, Literature = 204°C[9]

**Mass:** $[M^+]$ at *m/z* 192, $C_{10}H_8O_4$ requires 192

EIMS: *m/z*: 192, 177, 149, 121

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3338, 2914, 2845, 1716, 1297, 1139

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.3 (page 144)

### 5.2.2.3 Physical Data for Compound XV

**Name:** 6,7-dimethoxy-2*H*-1-benzopyran-2-one

**Synonyms:** scoparone, 6,7-dimethoxycoumarin, scoparin

**Physical appearance:** cream coloured needles

**Yield:** 9.4 mg

**Melting Point:** 141-143°C, Literature = 147°C[9]

**Mass:** $[M^+]$ at *m/z* 206, $C_{11}H_{10}O_4$ requires 206

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 2914, 2849, 1717, 1616, 1380, 1279, 1138, 1014

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.4 (page 148)

### 5.2.2.4 Physical Data for Compound XVI

**Name:** angelone

**Physical appearance:** light brown crystals

**Yield:** 7 mg

**Melting Point:** insufficient sample

**Mass:** HRMS [$M^+$] at *m/z* 180.04220, $C_9H_8O_4$ requires 180.04226

EIMS: *m/z* (rel. int.): 180 (100.00), 165 (51.77), 152 (24.10), 150 (18.91), 135 (22.28), 124 (16.44), 122 (55.29), 109 (21.17), 94 (4.67), 79 (24.68), 71 (5.33), 51 (19.83), 43 (64.09)

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ :3349, 2916, 2845, 1746, 1686, 1610, 1544, 1408, 1248, 1082, 1019

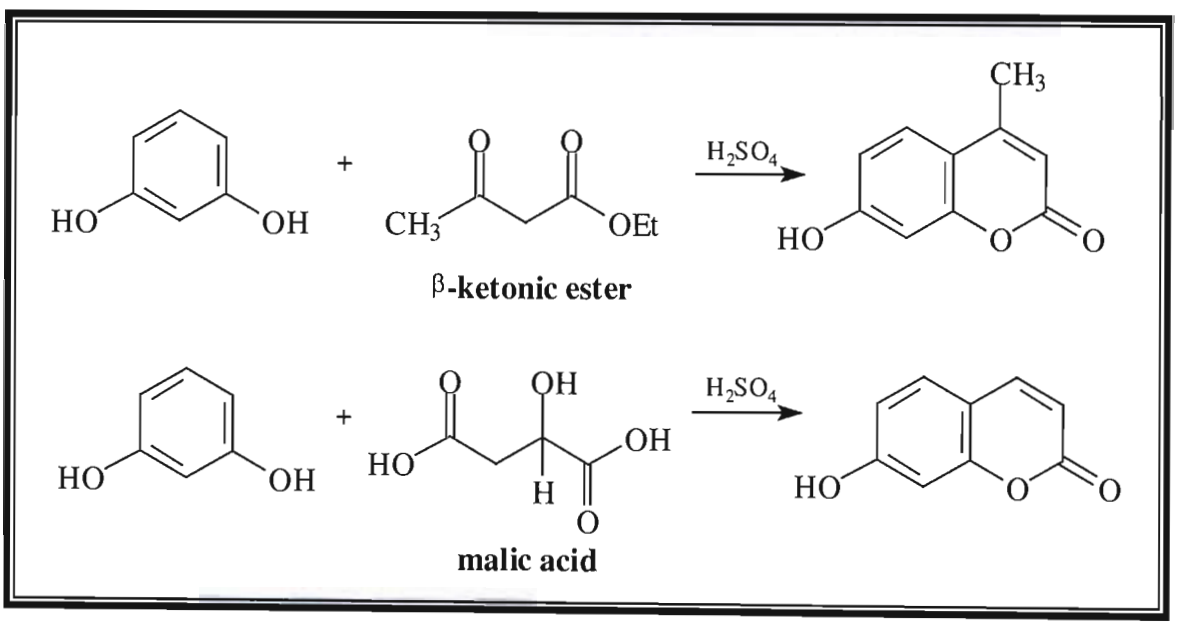
**$^1$H and $^{13}$C NMR Data:** Refer to table 5.5 (page 152)

## 5.3 The Synthesis of Coumarins

The synthesis of coumarins, specifically scopoletin and scoparone, was attempted in order to confirm the NMR data obtained for compounds XIV and XV. The NMR data for these compounds did not correlate well with the relevant literature values[9] and so it was decided to synthesise these compounds so as to confirm the NMR assignments of compounds XIV and XV.

### 5.3.1 Introduction

A reaction by which coumarins may be synthesised is known as the Pechmann condensation and involves condensation of phenols with either malic acid or β-ketonic esters in the presence of Lewis acid catalysts[12].

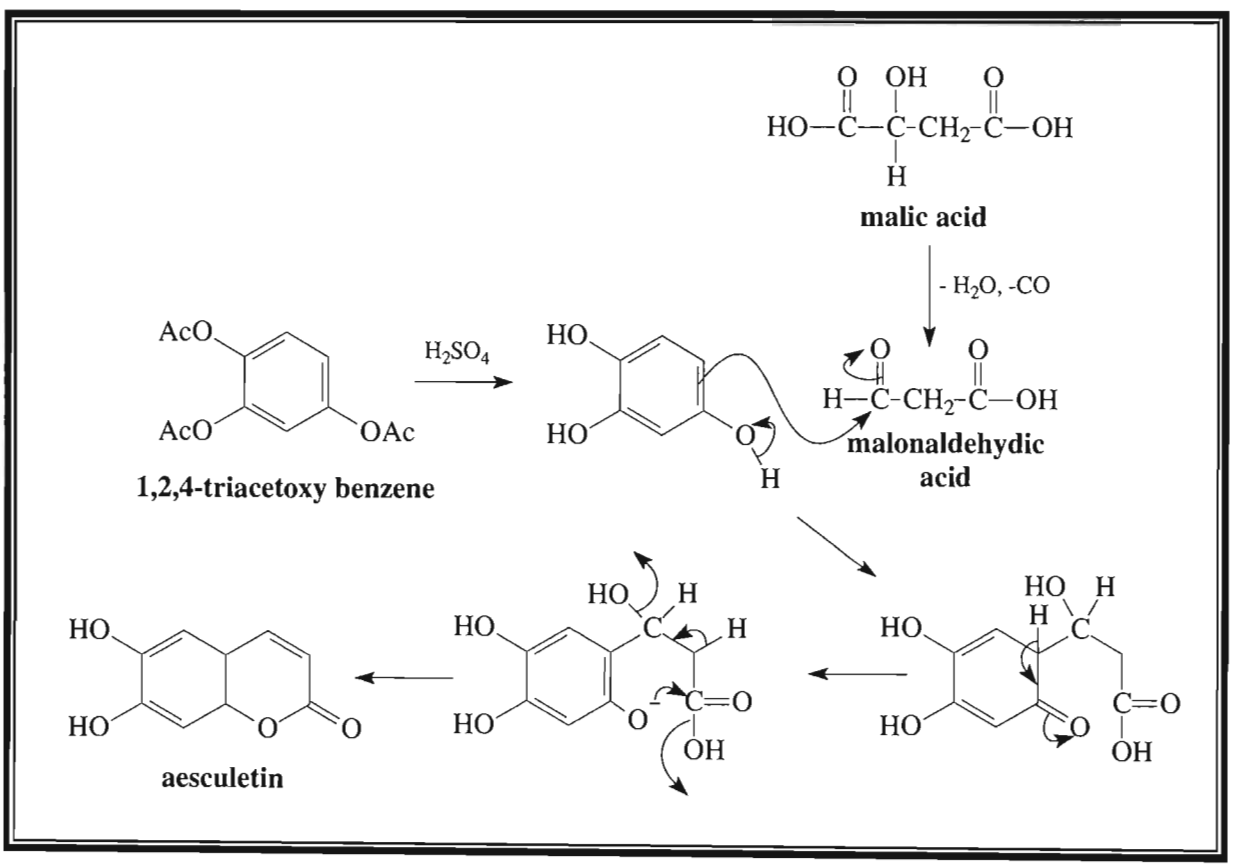



Scheme 5.2. The two types of Pechmann condensation reactions[12].

The product of the Pechmann reaction depends on the nature of the phenol, the nature of the β-ketonic ester and the condensing agent used[12].

## 5.3.2 The Synthesis of Aesculetin

The synthesis of aesculetin is the first step in the synthesis of scoparone, thus this compound had to be synthesised initially. In the synthesis of aesculetin, the three acetate groups of the starting material 1,2,4-triacetoxybenzene are cleaved off under the acidic conditions to give hydroxyquinol. Next, condensation of malic acid with the phenol occurs, which takes place in three stages. Firstly, the malic acid is converted into malonaldehydic acid and formic acid, which is further decomposed into water and carbon monoxide[12]. In the second stage, reaction of the aldehyde with the phenol yields an unstable addition product. Two molecules of water are released in the third step resulting in the formation of the coumarin derivative[12]. This reaction is made possible as malonaldehydic acid contains a β-carbonyl group, which resembles the acetoacetic ester in its reaction with a phenol to yield a coumarin[12].




Scheme 5.3. The proposed mechanism of the Pechmann condensation[12].

### 5.3.2.1 Structural Elucidation of Compound XVII

### 6,7-Dihydroxy-2*H*-1-benzopyran-2-one (Aesculetin)

(Spectra 17a-h, pages 365-372)

This compound was isolated in 59% yield as dark brown crystals.

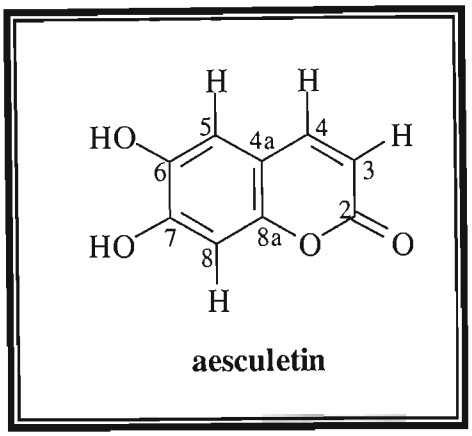



Figure 5.16. The structure of compound XVII.

The mass spectrum indicated a molar mass of 178 g $mol^{-1}$ which indicated a molecular formula of $C_9H_6O_4$ ($C_9H_6O_4$ requires 178) and a double bond equivalence of seven.

The infra-red spectrum showed bands at 3262 $cm^{-1}$ (O-H stretching), 1680 $cm^{-1}$ (C=O stretching), 1569 $cm^{-1}$ (C=C stretching), 1394 $cm^{-1}$, 1293 $cm^{-1}$ and 1149$cm^{-1}$ ($CH_2$ and $CH_3$ bending).

The $^1H$ NMR spectrum showed the presence of a pair of doublet resonances at δ 7.77 (1H, $J$ = 9.3 Hz) and δ 6.17 (1H, $J$ = 9.3 Hz), which were assigned to H-4 and H-3 respectively. These two pairs of doublets were seen to be coupled to each other in the COSY and NOESY spectrums.

The downfield fully substituted carbon resonance at δ 164.3 in the $^{13}C$ NMR spectrum indicated the presence of a carbonyl group and was assigned to C-2 due to this resonance being correlated to H-3 and H-4 in the HMBC spectrum. The fully substituted carbon signal at δ 112.8 was due to H-4a as it was seen to be correlated to

H-3 and H-8 (δ 6.74, 1H, d, $J$ = 0.4 Hz) in the HMBC spectrum. The remaining doublet proton resonance at δ 6.93 ($J$ = 0.4 Hz), integrating to one proton, was assigned to H-5 as it was seen to be correlated to H-4 in the NOESY spectrum and showed a correlation in the HMBC spectrum to C-4 (δ 146.1)

The two fully substituted carbon resonances in the $^{13}C$ NMR spectrum observed at δ 144.6 and δ 152.0 were due to C-6 and C-7 respectively based on a comparison of $^{13}C$ NMR data for these carbon atoms in scopoletin, scoparone and isoscopoletin. Also, stronger HMBC correlations were observed between C-6 and H-8 as well as between C-7 and H-5, while slightly weaker correlations in the HMBC spectrum were observed between C-6 and H-5 as well as between C-7 and H-8. The remaining fully substituted carbon resonance at δ 150.5 was ascribed to H-8a and showed correlations in the HMBC spectrum to H-4, H-5 and H-8. Compound XVII, at a concentration of 10 μg/cm$^3$, showed a 27.7% inhibition of prostaglandin synthesis using the microsomal cell screen and a 2.3% inhibition using the Cox-2 screen (page 72).

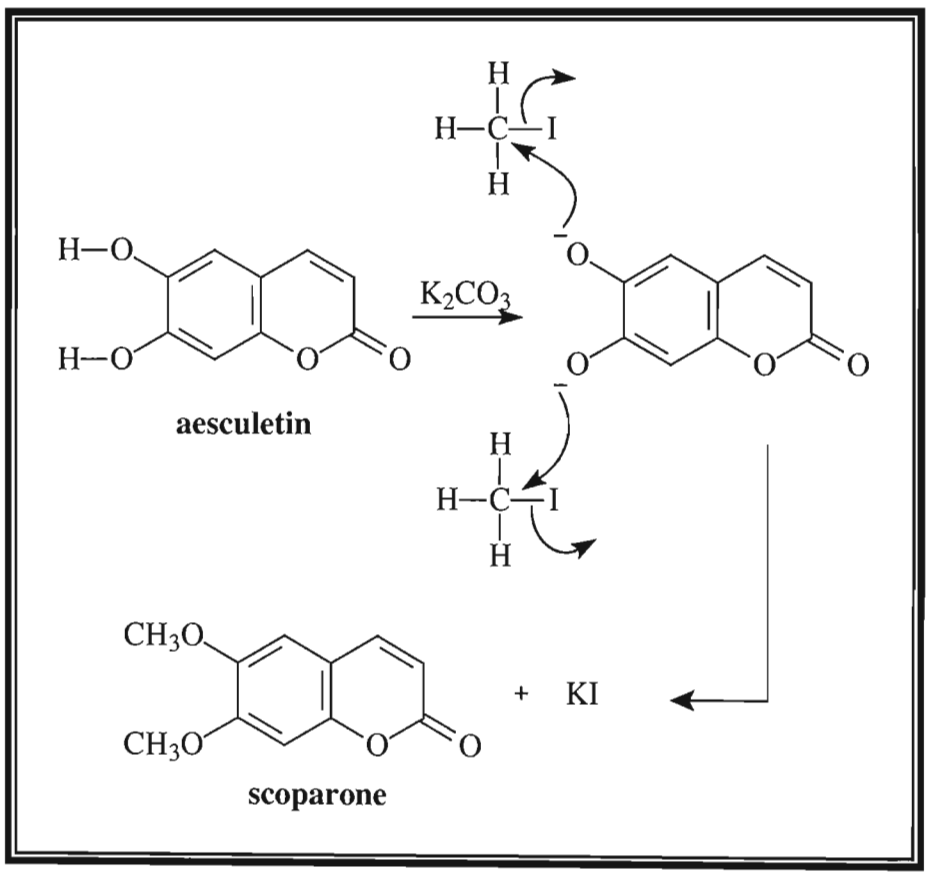
The NMR data and correlations for aesculetin and the literature data[9] for aesculetin are tabulated in table 5.6.

**Table 5.6. NMR data and correlations for compound XVII ($CD_3OD$) and literature data for aesculetin[9] ($CDCl_3$)**

| Carbon Number | $^1H$ NMR data (400 MHz) | $^{13}C$ NMR data (100 MHz) | COSY correlations | HMBC correlations (C→H) | NOESY correlations | $^{13}C$ NMR literature data for aesculetin[9] (62.9 MHz) |
|---|---|---|---|---|---|---|
| 2 | - | 164.3 (C) | - | H-3, H-4 | - | 160.4 |
| 3 | 6.17 (1H, d, $J$=9.3 Hz) | 112.5 (CH) | H-4 | - | H-4 | 112.4 |
| 4 | 7.77 (1H, d, $J$=9.3 Hz) | 146.1 (CH) | H-3 | H-5 | H-3, H-5 | 143.5 |
| 4a | - | 112.8 (C) | - | H-3, H-8 | - | 112.4 |
| 5 | 6.93 (1H, d, $J$=0.4Hz) | 113.0 (CH) | - | H-4 | H-4 | 114.2 |
| 6 | - | 144.6 (C) | - | H-5, H-8 | - | 142.7 |
| 7 | - | 152.0 (C) | - | H-5, H-8 | - | 150.2 |
| 8 | 6.74 (1H, d, $J$=0.4Hz) | 103.6 (CH) | - | - | - | 104.4 |
| 8a | - | 150.5 (C) | - | H-4, H-5, H-8 | - | 148.1 |

### 5.3.3 The Synthesis of Scoparone

The methylation of aesculetin to form scoparone involves reacting one mole of aesculetin with two moles of iodomethane with an excess of potassium carbonate in acetone and refluxing overnight[13]. The potassium carbonate renders the solution basic, thereby abstracting the protons from the hydroxy groups of aesculetin. This creates nucleophilic sites on aesculetin, which bind to the electron deficient carbon atom of iodomethane. Subsequently, the iodide group from iodomethane is released and complexes with the excess potassium ions to form potassium iodide. This proposed reaction mechanism is shown in scheme 5.4.




Scheme 5.4. The proposed mechanism for the methylation of aesculetin to form scoparone[13].

### 5.3.3.1 Structural Elucidation of Compound XVIII

### 6,7-dimethoxy-2*H*-1-benzopyran-2-one (Scoparone)

(Spectra 18a-f, pages 373-378)

This compound was isolated in 82% yield as yellow crystals.

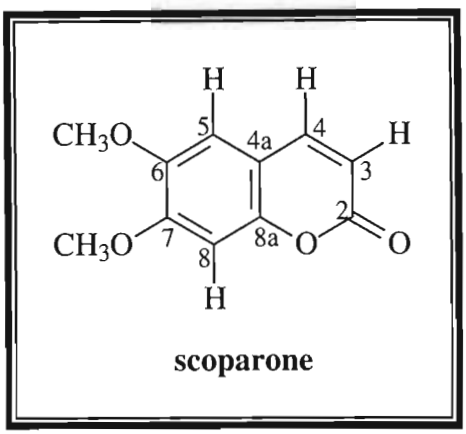



Figure 5.17. The structure of compound XVIII.

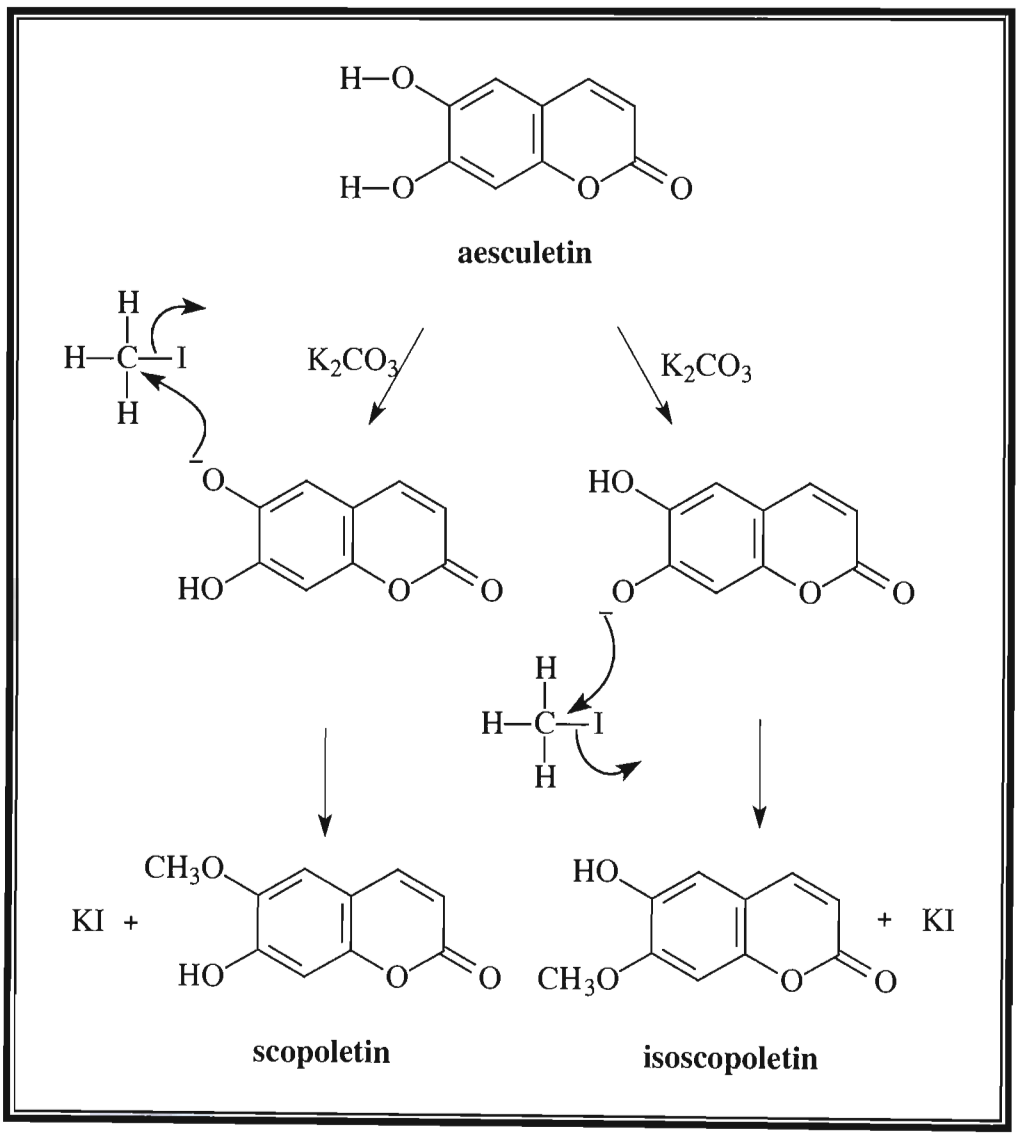
The NMR spectral data for this compound was identical to the isolated scoparone (compound XV) and so will not be discussed again in detail. Table 5.7 lists all the NMR data and correlations for this compound. The NMR data for compound XVIII did not correlate well with literature[9], however it did correlate very well with the NMR data for scoparone (compound XV) isolated from *Tachiadenus longiflorus*.

**Table 5.7. NMR data and correlations for compound XVIII ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations (C→H) | NOESY correlations |
|---|---|---|---|---|---|
| **2** | - | 161.4 (C) | - | H-3, H-4 | - |
| **3** | 6.26 (1H, d, *J*=9.3 Hz) | 113.5 (CH) | H-4 | - | H-4 |
| **4** | 7.59 (1H, d, *J*=9.2 Hz) | 143.3 (CH) | H-3 | H-5 | H-3, H-5 |
| **4a** | - | 111.4 (C) | - | H-3, H-8 | - |
| **5** | 6.83 (1H, s) | 107.9 (CH) | $OCH_3$ at C-6 | H-4 | H-4, $OCH_3$ at C-6 |
| **6** | - | 146.3 (C) | - | H-8, $OCH_3$ at C-6 | - |
| **7** | - | 152.8 (C) | - | C-7, $OCH_3$ at C-7 | - |
| **8** | 6.81 (1H, s) | 100.0 (CH) | $OCH_3$ at C-7 | - | $OCH_3$ at C-7 |
| **8a** | - | 150.0 (C) | - | H-4, H-5 | - |
| **$OCH_3$ at C-6** | 3.92 (3H, s) | 56.3 ($CH_3$) | - | - | H-5 |
| **$OCH_3$ at C-7** | 3.89 (3H, s) | 56.3 ($CH_3$) | - | - | H-8 |

### 5.3.4 The Synthesis of Isoscopoletin

The methylation reaction was repeated in the same way as that for scoparone[13], however, a mono-methoxy compound was required and thus only one mole of iodomethane was reacted with one mole of aesculetin. However a large amount of scoparone was still formed, thus separation of the compounds by column chromatography was necessary. One of the compounds isolated from this reaction was isoscopoletin. This reaction was initially performed so as to obtain scopoletin, as we wanted to check the NMR data of compound XIV, however, scopoletin was not isolated from this reaction.




Scheme 5.5. The proposed formation of scopoletin and isoscopoletin[13].

### 5.3.4.1 Structural Elucidation of Compound XIX

### 6-Hydroxy-7-methoxy-2*H*-1-benzopyran-2-one (Isoscopoletin)

(Spectra 19a-h, pages 379-386)

This compound was isolated, after column chromatography, in 1.0% yield as a pale yellow substance.

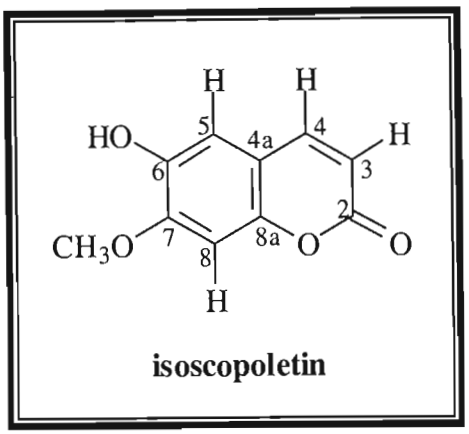



Figure 5.18. The structure of compound XIX.

The mass spectrum of compound XIX indicated a molecular mass of 192 g $mol^{-1}$, which indicated a molecular formula of $C_{10}H_8O_4$ ($C_{10}H_8O_4$ requires 192). A double bond equivalence of seven was deduced.

The infra-red spectrum showed a broad band at 3415 $cm^{-1}$ due to the O-H stretching vibration. The carbonyl stretching vibrations of the α,β-unsaturated lactone was shown as a sharp peak at 1695 $cm^{-1}$. The peaks at 1301 $cm^{-1}$ and 1149 $cm^{-1}$ were due to the $CH_2$ and $CH_3$ bending vibrations.

The $^1H$ NMR spectrum showed the presence of a pair of doublets at δ 7.58 (1H, $J$ = 9.4 Hz) and δ 6.26 (1H, $J$ = 9.4 Hz), which were assigned to H-4 and H-3 respectively. The corresponding methine carbons, identified from the HSQC spectrum, were observed at δ 143.4 (C-4) and δ 113.9 (C-3). This region of the $^{13}C$ NMR spectrum indicates the presence of a double bond. The H-4 and H-3 doublet proton resonances were seen to be coupled to each other in the COSY spectrum.

The $^{13}C$ NMR spectrum showed a fully substituted carbon resonance at δ 161.5 indicating the presence of a carbonyl group. This carbon resonance was ascribed to C-2 as it showed correlations in the HMBC spectrum to both H-3 and H-4. The doublet proton resonance at δ 6.95 ($J$ = 0.4 Hz), integrating to one proton, was assigned to H-5 as it showed correlations in both the HMBC and NOESY spectra to H-4. The resonance at δ 6.81 (1H, d, J=0.4 Hz) was therefore assigned to H-8. The doublet proton resonance due to H-4 was seen to be correlated in the NOESY spectrum to H-3, H-5 and H-8 (δ 6.81, 1H, d, $J$ = 0.4 Hz).

The $^{1}H$ NMR spectrum showed the presence of one methoxy group at δ 3.95 (3H, s), which could be positioned at C-6 or C-7. The NOESY spectrum showed a correlation between this methoxy group and H-8, thus positioning the methoxy group at C-7 (δ 150.1, C). This was further proved by the HMBC spectrum, which showed correlations between C-7 and the methoxy at C-7, as well as to H-5 and H-8. A hydroxy group was placed at the remaining, C-6 (δ 142.7, C), position. Compound XIX, at a concentration of 10 μg/cm$^3$, showed a 21.6% inhibition of prostaglandin synthesis using the microsomal cell screen (page 72).

The literature melting point of scopoletin (204°C) is very similar to that of isoscopoletin (207.5 - 209°C) and thus the formation of isoscopoletin could not be confirmed based on the recorded melting point of compound XIX. The NMR data and correlations for compound XIX and the literature data[9] for isoscopoletin are given in table 5.8.

**Table 5.8. NMR data and correlations for compound XIX ($CDCl_3$) and literature data for isoscopoletin ($CDCl_3$)[9]**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations (C→H) | NOESY correlations | $^{13}$C NMR literature data for isoscopoletin[9] (62.9 MHz) |
|---|---|---|---|---|---|---|
| **2** | - | 161.5 (C) | - | H-3, H-4 | - | 160.3 |
| **3** | 6.26 (1H, d, *J*=9.4 Hz) | 113.9 (CH) | H-4 | - | H-4 | 113.2 |
| **4** | 7.58 (1H, d, *J*=9.4 Hz) | 143.4 (CH) | H-3 | H-5 | H-3, H-5, H-8 | 143.3 |
| **4a** | - | 112.2 (C) | - | H-3, H-8 | - | 113.2 |
| **5** | 6.95 (1H, d, *J*=0.4Hz) | 111.0 (CH) | - | H-4 | H-4 | 114.1 |
| **6** | - | 142.7 (C) | - | H-8 | - | 144.5 |
| **7** | - | 150.1 (C) | - | H-5, H-8, $OCH_3$ at C-7 | - | 150.3 |
| **8** | 6.81 (1H, d, *J*=0.4Hz) | 99.3 (CH) | - | - | H-4, $OCH_3$ at C-7 | 104.2 |
| **8a** | - | 149.2 (C) | - | H-4 | - | 149.4 |
| **$OCH_3$ at C-7** | 3.95 (3H, s) | 56.4 ($CH_3$) | - | - | H-8 | 55.4 |

## 5.3.5 A Comparison of the $^{13}$C NMR Data of Selected Coumarins

A table comparing the carbon NMR data of these synthetic and naturally occurring compounds could be compiled (Table 5.9). From this data it is evident that whether the coumarins have two hydroxy groups or two methoxy groups, or even one methoxy and one hydroxy group attached at C-6 and C-7, the carbon values of the coumarins are very similar.

**Table 5.9. $^{13}C$ NMR data for compound XVII ($CD_3OD$), compound XVIII ($CDCl_3$), compound XIV ($CDCl_3$) and compound XIX ($CDCl_3$)**

| Carbon Number | $^{13}C$ NMR data for synthesised aesculetin, compound XVII (100MHz) | $^{13}C$ NMR data for synthesised scoparone, compound XVIII (100 MHz) | $^{13}C$ NMR data for naturally occurring scopoletin, compound XIV (100 MHz) | $^{13}C$ NMR data for synthesised isoscopoletin, compound XIX (100 MHz) |
|---|---|---|---|---|
| 2 | 164.3 (C) | 161.4 (C) | 161.5 (C) | 161.5 (C) |
| 3 | 112.5 (CH) | 113.5 (CH) | 113.4 (CH) | 113.9 (CH) |
| 4 | 146.1 (CH) | 143.3 (CH) | 143.3 (CH) | 143.4 (CH) |
| 4a | 112.8 (C) | 111.4 (C) | 111.5 (C) | 112.2 (C) |
| 5 | 113.0 (CH) | 107.9 (CH) | 107.4 (CH) | 111.0 (CH) |
| 6 | 144.6 (C) | 146.3 (C) | 144.0 (C) | 142.7 (C) |
| 7 | 152.0 (C) | 152.8 (C) | 149.7 (C) | 150.1 (C) |
| 8 | 103.6 (CH) | 100.0 (CH) | 103.2 (CH) | 99.3 (CH) |
| 8a | 150.5 (C) | 150.0 (C) | 150.2 (C) | 149.2 (C) |
| $OCH_3$ at C-6 | - | 56.3 ($CH_3$) | 56.4 ($CH_3$) | - |
| $OCH_3$ at C-7 | - | 56.3 ($CH_3$) | - | 56.4 ($CH_3$) |

## 5.3.6 Conclusion

The purpose of synthesising scopoletin and scoparone was so that we could prove that the $^{1}H$ and $^{13}C$ NMR data of these two coumarins isolated from *Tachiadenus longiflorus* were accurate and the data in the literature were incorrect.

Unfortunately, scopoletin was not synthesised so the NMR data of naturally occurring scopoletin could not be compared to that of synthesised scopoletin. However, the $^{1}H$ and $^{13}C$ NMR data of the synthesised scoparone, was identical to that of the naturally occurring scoparone which confirmed that our NMR data was correct. It can therefore be concluded that the NMR data obtained from the naturally occurring coumarins, scopoletin and scoparone were correct, thus confirming that the literature data for these compounds were inaccurate.

### 5.3.7 Experimental

***Synthesis of aesculetin***

1,2,4-Triacetoxybenzene (20.0 g, 79.4 mmol) and (+)-malic acid (11.7 g, 83.3 mmol) were powdered in a mortar. The mixture was added to concentrated sulphuric acid (32 $cm^3$) in one addition in a large beaker. The mixture was stirred for five minutes using a mechanical stirrer and then heated on a steam bath (100°C) with continuous stirring for one hour. Heating and stirring were continued for a further ninety minutes, after which the mixture was left to cool, poured onto crushed ice (250 g) and then left in the refrigerator for forty-eight hours. The brown precipitate formed was filtered, washed with copious amounts of water, and dried to provide aesculetin (8.32 g, 59 %).

***Synthesis of scoparone***

Aesculetin (1.0 g, 5.6 mmol), potassium carbonate (3.88 g, 28.1 mmol) and iodomethane (1.4 $cm^3$, 22.5 mmol) were refluxed for twenty-four hours in acetone (25 $cm^3$). The mixture was filtered, and the acetone solution was concentrated *in vacuo* to give pure scoparone (949.6 mg, 82%).

***Synthesis of isoscopoletin***

Aesculetin (1.0 g, 5.6 mmol), potassium carbonate (3.01 g, 30.4 mmol) and iodomethane (0.30 $cm^3$, 4.8 mmol) were refluxed for twenty-four hours in acetone (25 $cm^3$). The mixture was filtered, and the acetone solution was concentrated *in vacuo* to give a mixture of coumarins. The mixture was separated using column chromatography (Merck 9385) and a 100% dichloromethane solvent system. A large portion of the extract consisted of unreacted aesculetin as well as a large amount of the dimethoxy product, scoparone. Scoparone eluted first off the column, while pure isoscopoletin was collected in subsequent fractions (10 mg, 1.0 %).

### 5.3.7.1 Physical data for compound XVII

**Name:** aesculetin

**Synonyms:** 6,7-dihydroxy-2*H*-1-benzopyran-2-one

**Physical appearance:** dark brown crystals

**Melting point:** 260-264°C, Literature = 268-270°C[9]

**Yield:** 8.32 g

**Mass:** $[M^+]$ at *m/z* 178, $C_9H_6O_4$ requires 178

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3262, 1680, 1569, 1394, 1293, 1149

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.6 (page 160)

### 5.3.7.2 Physical data for compound XVIII

**Name:** scoparone

**Synonyms:** 6,7-dimethoxy-2*H*-1-benzopyran-2-one

**Physical appearance:** yellow crystals

**Melting point:** 141-143°C, Literature = 147°C[9]

**Yield:** 949.6 mg

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.7 (page 162)

### 5.3.7.3 Physical data for compound XIX

**Name:** isoscopoletin

**Synonyms:** 6-hydroxy-7-methoxy-2*H*-1-benzopyran-2-one

**Physical appearance:** pale yellow substance

**Melting point:** insufficient sample, Literature = 207.5-209°C[9]

**Yield:** 10 mg

**Mass:** $[M^+]$ at *m/z* 192, $C_{10}H_8O_4$ requires 192

EIMS: *m/z*: 192, 177, 149, 121

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3415, 1695, 1633, 1571, 1526, 1447, 1385, 1301, 1149

**$^1$H and $^{13}$C NMR Data:** Refer to table 5.8 (page 166)

## 5.4 References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# CHAPTER 6

# The Attempted Synthesis of (*E*)-1-(4'-hydroxyphenyl)-3-(5''-hydroxy-3'',4''-dimethoxyphenyl)-1,4-pentadiene

## 6.1 Introduction

Although the norligan that was isolated from *Ledebouria ovatifolia* was found to be (*Z*)-1-(4'-hydroxyphenyl)-3-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene, it was initially thought that compound I was isolated as the *trans* isomer due to confusion in the literature discussed below. However, only after completing the synthesis of compound XXIII, was it determined that compound I was the *cis* isomer, based on NMR correlations and the coupling constant ($J$ = 11.7 Hz) of H-1 and H-2.

There is confusion in the literature regarding *cis/trans* isomers of norlignans and the structure of the known norlignan, hinokiresinol (Figure 6.1), has presented researchers with difficulties in the past.

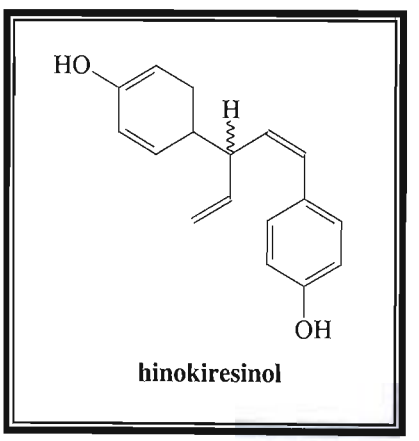


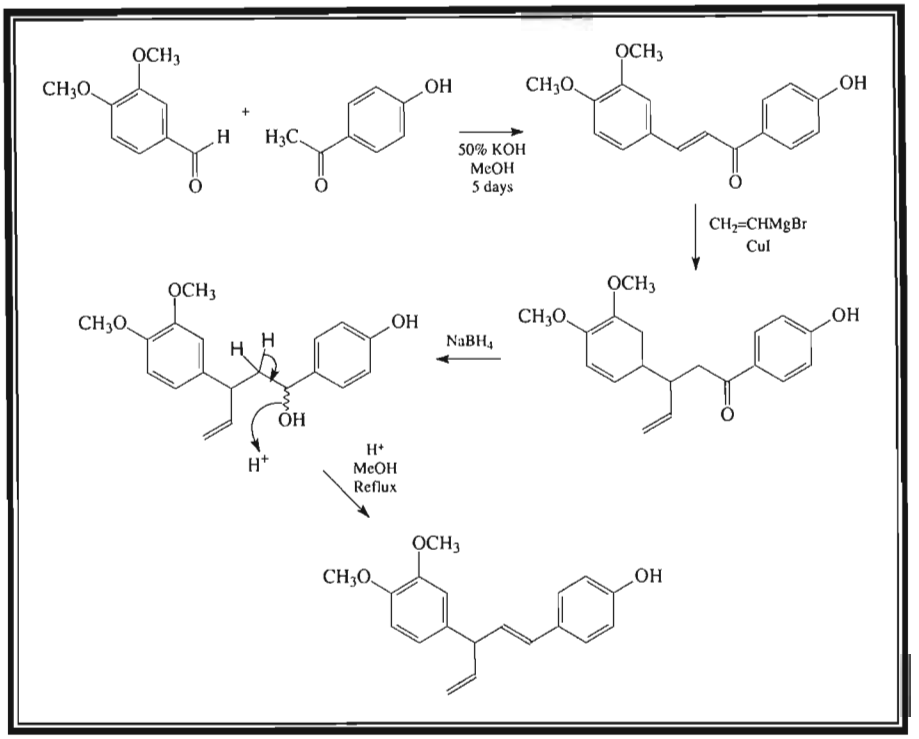

Figure 6.1. The structure of hinokiresinol.

In 1965 Hirose *et al.*[1], who isolated hinokiresinol from the wood of *Chamaecyparis obtuse*, concluded that the $\Delta^{1,2}$ double bond was *trans* based on an infra-red absorption at 967 $cm^{-1}$ and no chemical shifts or coupling constants for H-1 and H-2 were given. In 1967, Enzell *et al.*[2] assumed the *trans* stereochemistry, based on the work of Hirose *et al.*, to be correct and investigated the absolute stereochemistry at C-3 through a series of chemical transformations.

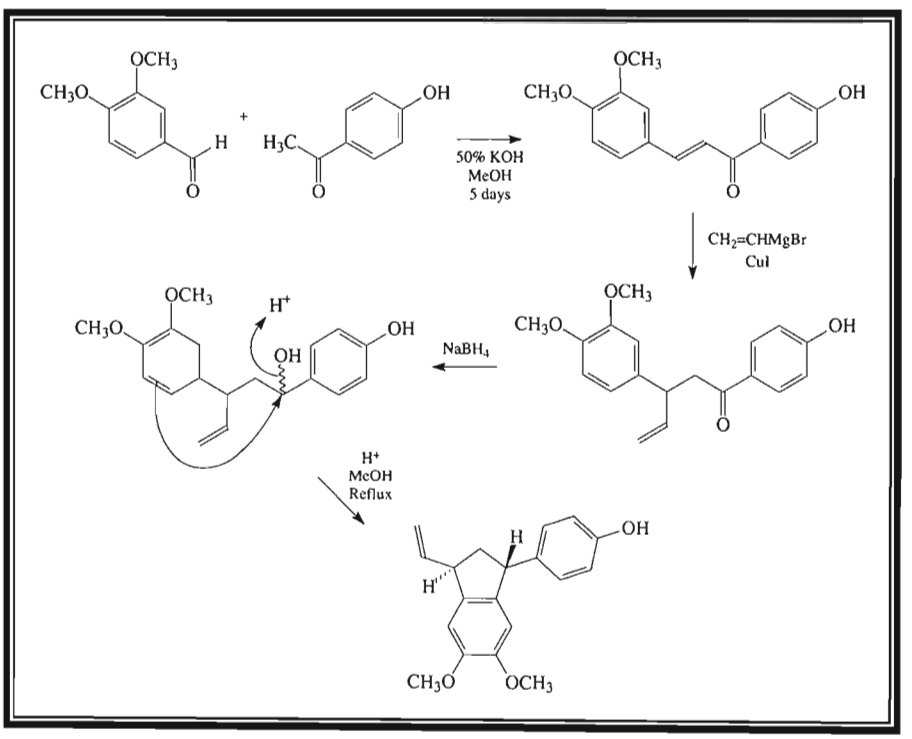
In 1967, Beracierta and Whiting[3] identified their synthetic (±)-dimethylether product as *trans,* based on a $J_{1,2}$ coupling constant of 12 Hz and an infra-red absorption of 967 $cm^{-1}$. They also commented that the value of 12 Hz was at the "low end of the range", lower than the value of 16 Hz found in the *trans* intermediate and higher than the value of 9 Hz found in the *cis* intermediate.

In 1988, Ameer *et al.*[4] synthesised both the *cis* and *trans* isomers and obtained $J_{1,2}$ coupling constants of 11.3 Hz and 15.9 Hz respectively. This indicated that the compound hinokiresinol reported previously in the literature to have a *trans* configuration, actually had the *cis* configuration. In 1996, Tsui and Brown[5] isolated (+)-nyasol from *Asparagus cochinchinensis*, and from the NOESY spectrum, they showed that their compound had the *cis* stereochemistry and reported a $J_{1,2}$ coupling constant of 11.2 Hz. Thus the attempted synthesis in this chapter is of the *trans* isomer and not of the isolated *cis* isomer.

The starting material for the norlignan, (*E*)-1-(4'-hydroxyphenyl)-3-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene i.e. 5-hydroxy-3,4-dimethoxy-benzaldehyde was not readily available, and due to time constraints, the derivative 3,4-dimethoxy-benzaldehyde, which was available, was used instead. It was thought that the synthesis could be performed using the latter starting material to see if the reaction was successful, and if it was, then the starting material could be obtained to make the required substituted benzaldehyde. Thus the synthesis of the norlignan (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxy)prop-2-en-1-one was attempted based on the procedure reported by Muraoka and co-workers[6]. Scheme 6.1 shows the proposed overall reaction for what product should have been formed, while scheme 6.2 shows the proposed overall reaction for the product actually formed in the reaction.




Scheme 6.1. The proposed overall reaction for the expected product[6].

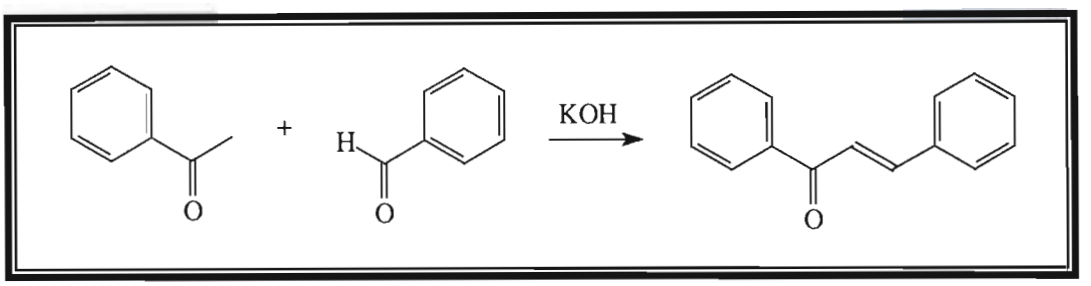



Scheme 6.2. The proposed overall reaction for the obtained product.

The key synthetic intermediate in the formation of the norlignan is the chalcone, (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one, which was prepared according to the reported method of Martin *et al.*[7].

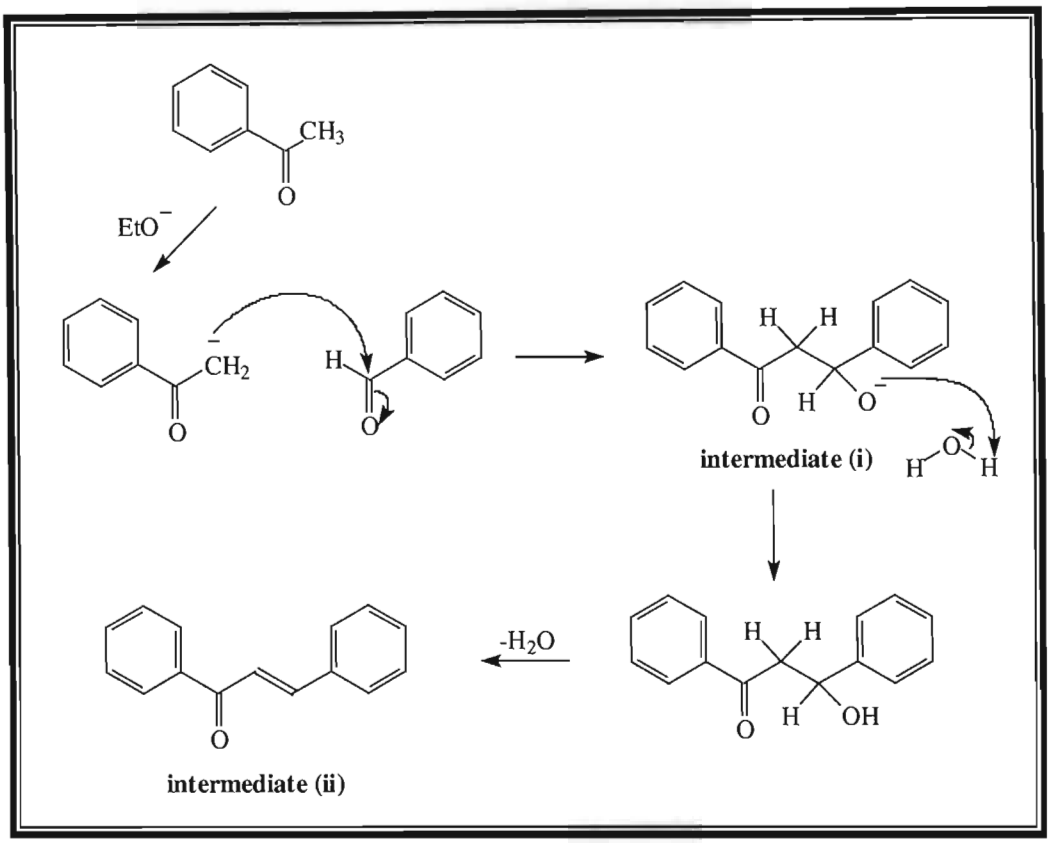
### 6.1.1 Chalcone Synthesis

Chalcones can be synthesised using various methods, but the simplest involves the Claisen-Schmidt condensation. This approach involves reacting equimolar quantities of acetophenone with benzaldehyde in the presence of potassium hydroxide resulting in the formation of an α,β-unsaturated ketone (Scheme 6.3). Substituted chalcones can be synthesised in the same manner using appropriately substituted acetophenones and benzaldehydes.



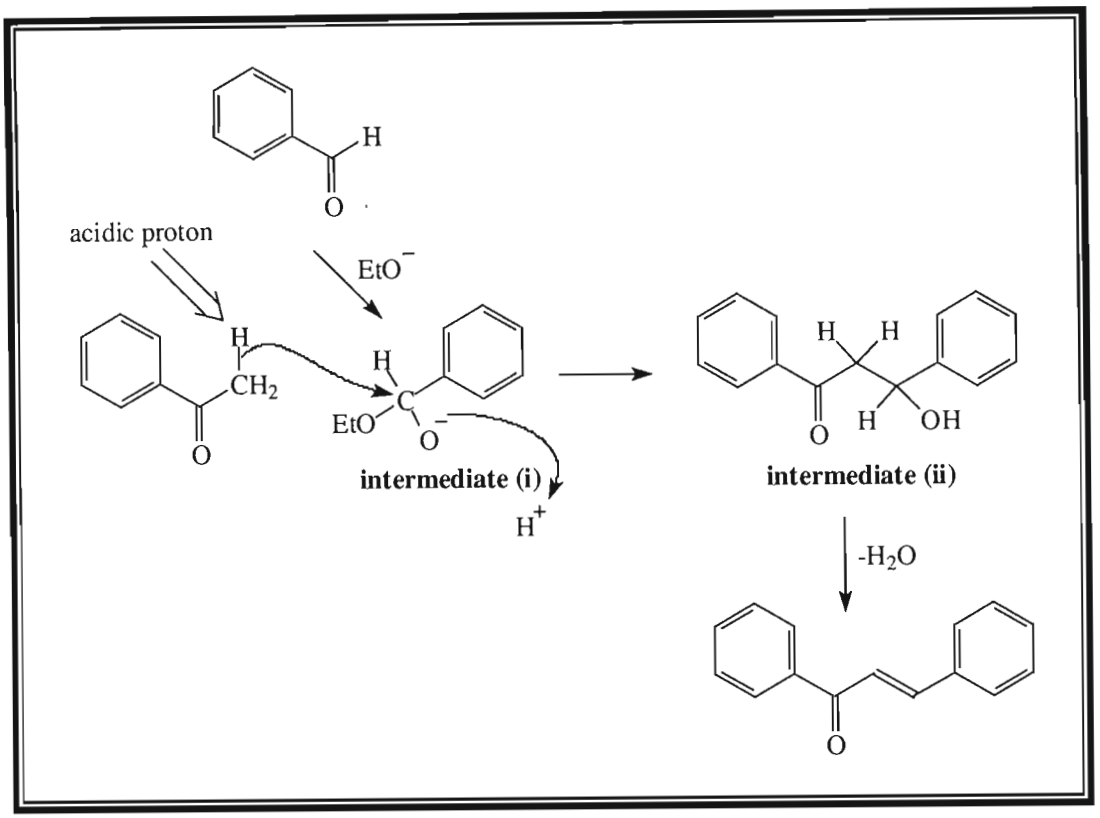

Scheme 6.3. Chalcone formation by the Claisen-Schmidt condensation.

The base catalysed formation of chalcones[8-10] and their derivatives[9, 10] was subjected to kinetic studies, and two mechanisms have been proposed for the reaction of benzaldehyde and acetophenone in the presence of a base catalyst. The first mechanism involves base abstraction of a proton from the methyl group of acetophenone, which creates a nucleophile, and attacks the electrophilic carbon atom of benzaldehyde to form the first intermediate (i), which then accepts a proton from water to form the second intermediate (ii). The latter undergoes dehydration to form the chalcone. This mechanism is currently the most accepted one (Scheme 6.4).




Scheme 6.4. Mechanism one of chalcone formation[8].

The second proposed mechanism involves the ethoxide anion, which acts as a nucleophile and attacks the electrophilic carbon atom of benzaldehyde, forming the first intermediate (i). The nucleophilic acetophenone then attacks the electrophilic centre in intermediate (i). The negative oxygen ion simultaneously accepts a proton from the solution to form the second intermediate (ii), which then undergoes dehydration to form the chalcone (Scheme 6.5).



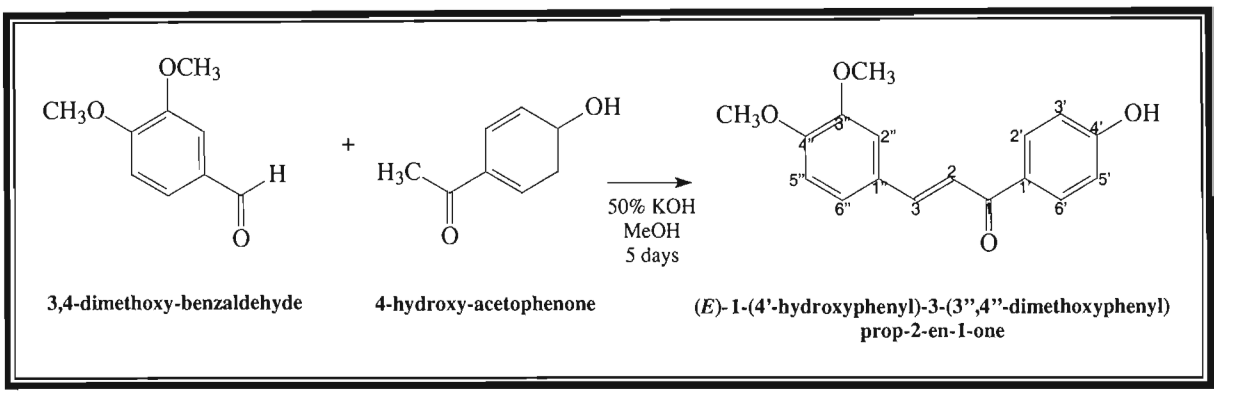

Scheme 6.5. Mechanism two of chalcone formation[8].

The alkali concentration in the Claisen-Schmidt reaction usually ranges between 10 and 60%. The reaction can be carried out at 50°C for twelve to fifteen hours or at room temperature for five to seven days[7]. However, under these conditions, the Cannizzaro reaction also takes place, thus decreasing the yield of the desired chalcone. The Cannizzaro reaction occurs when an aldehyde with no α-hydrogen atom reacts with the concentrated aqueous alkali, which results in half the aldehyde being converted to a carboxylic acid, and the other half to an alcohol.

A variety of condensing agents other than aqueous alkali have also been used to form chalcones. These include alkali metal alkoxides[11,12], magnesium *tert*-butoxide[13], hydrogen chloride[14, 15], aluminium chloride[16] and boron trifluoride[16].

### 6.1.2 Synthesis of (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxy-phenyl)prop-2-en-1-one

The synthesis of the (*E*)-4'-hydroxy-3",4"-dimethoxy-chalcone was attempted using the Claisen-Schmidt condensation reaction and proved successful (Scheme 6.6). The product was obtained in 56% yield.




Scheme 6.6. The formation of (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)-prop-2-en-1-one.

#### 6.1.2.1 Confirmation of the Structure of Compound XX (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one

(Spectra 20a-h, pages 387-394)

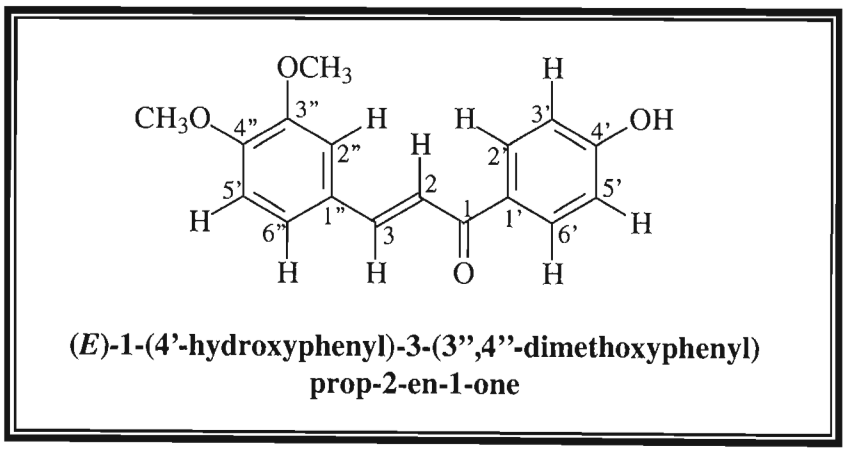



Figure 6.2. The structure of compound XX.

The high resolution mass spectrum showed a parent ion peak at *m/z* 284.10485, which was consistent with the molecular formula of $C_{17}H_{16}O_4$ ($C_{17}H_{16}O_4$ requires 284.10486). A double bond equivalence of ten was deduced.

The infra-red spectrum showed a broad band at 3273 $cm^{-1}$ due to the O-H stretching of the hydroxy group. The sharp peak at 1608 $cm^{-1}$ was assigned to the C=O stretching vibrations, while the peak at 1517 $cm^{-1}$ was due to the C=C stretchings. The $CH_2$ and $CH_3$ bending vibrations were observed at 1442 $cm^{-1}$, 1271 $cm^{-1}$ and 1159 $cm^{-1}$, while the C-O stretching was seen at 1025 $cm^{-1}$.

The $^1H$ NMR spectrum showed the presence of a pair of doublets at δ 7.37 and δ 7.31, both integrating to one proton and having a coupling constant of 15.6 Hz, which were ascribed to H-2 and H-3 respectively. In the COSY spectrum, coupling can be seen between these two proton signals. The NOESY spectrum also shows coupling of H-2 with the proton resonances assigned to H-2'/6' (δ 7.93, 1H, $J$ = 8.2 Hz), H-2'' (δ 7.12, 1H, d, $J$ = 2.0 Hz) and H-6'' (δ 7.19, 1H, dd, $J$=2.0, 8.2 Hz), while H-3 showed a correlation in the NOESY spectrum to H-2, H-2'' and H-6''.

The fully substituted carbon resonance at δ 189.5 (C-1) indicated that a carbonyl group was positioned at C-1, and this carbon resonance showed correlations in the HMBC spectrum to H-2, H-3 and H-2'/6'. In the HMBC spectrum the fully substituted carbon resonance due to C-4' (δ 151.4) showed correlations to H-2'/6' and H-3'/5' (δ 6.88, 1H, $J$ = 8.2 Hz).

The two methoxy groups at C-3'' (δ 149.4) and C-4'' (δ 151.4) were observed in the $^1H$ NMR spectrum as two singlets, each integrating to three protons, at δ 3.91 ($OCH_3$ at C-3'') and δ 3.89 ($OCH_3$ at C-4''). The methine carbon ascribed to C-6'' (δ 123.2) showed correlations in the HMBC spectrum to H-3 and H-2'', while H-6'' was seen to be coupled in the NOESY spectrum to H-2, H-3 and H-5''. The fully substituted carbon resonance assigned to C-4'' showed correlations in the HMBC spectrum with H-2'', H-5'', H-6'' and the protons of the methoxy group attached at C-4''. In the $^{13}C$ NMR spectrum the resonances assigned to the carbons of the methoxy groups at C-3'' and at C-4'' were observed at δ 56.2.

From the NMR data this synthesised compound was confirmed to be the chalcone, (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one. Compound XX, at a concentration of 10 μg/cm$^3$, showed a 22.8% inhibition of prostaglandin synthesis using the microsomal cell screen (page 72).

The NMR data and correlations for this compound are listed in table 6.1, while the HMBC and NOESY correlations are shown in figure 6.3 and figure 6.4 respectively.

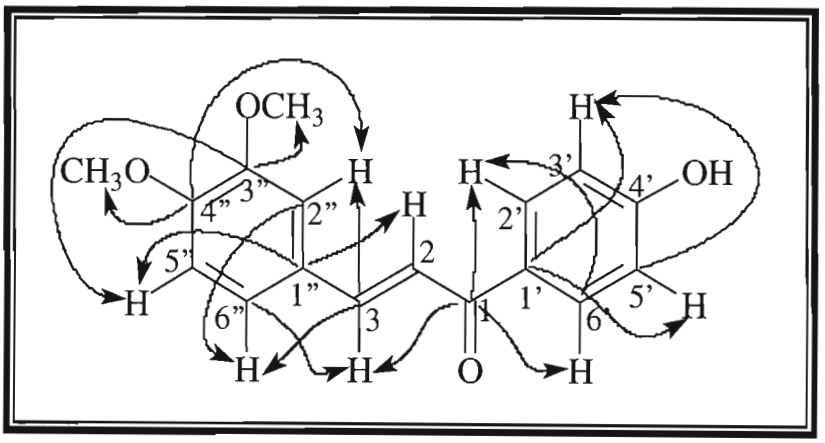



Figure 6.3. The HMBC (C → H) correlations for compound XX.

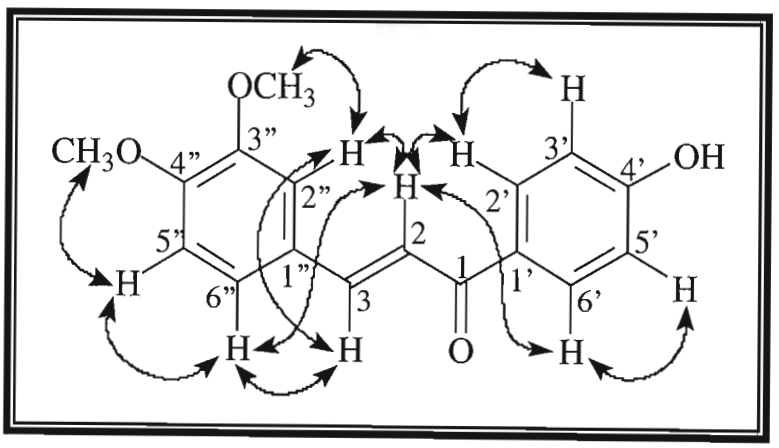


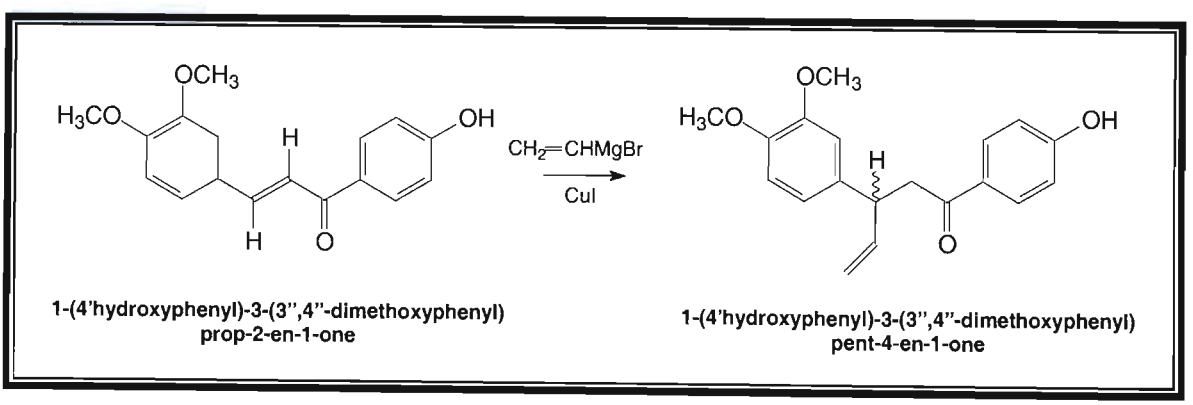

Figure 6.4. The NOESY correlations for compound XX.

**Table 6.1. NMR data and correlations for compound XX ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1 | - | 189.5 (C) | - | H-2, H-3, H-2'/6' | - |
| 2 | 7.37 (1H, d, *J*=15.6 Hz) | 120.1 (CH) | H-3 | - | H-3, H-2'/6', H-2'', H-6'' |
| 3 | 7.71 (1H, d, *J*=15.6 Hz) | 144.4 (CH) | H-2 | H-2'', H-6'' | H-2, H-2'', H-6'' |
| 1' | - | 130.6 (C) | - | H-3'/5' | - |
| 2' | 7.93 (1H, d, *J*=8.2 Hz) | 131.3 (CH) | H-3'/5' | H-2'/6' | H-2, H-3'/5' |
| 3' | 6.88 (1H, d, *J*=8.2 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| 4' | - | 161.6 (C) | - | H-2'/6', H-3'/5' | - |
| 5' | 6.88 (1H, d, *J*=8.2 Hz) | 115.6 (CH) | H-2'/6' | H-3'/5' | H-2'/6' |
| 6' | 7.93 (1H, d, *J*=8.2 Hz) | 131.3 (CH) | H-3'/5' | H-2'/6' | H-2, H-3'/5' |
| 1'' | - | 128.3 (C) | - | H-2, H-3(w), H-5'' | - |
| 2'' | 7.12 (1H, d, *J*=2.0 Hz) | 110.3 (CH) | - | H-3, H-6'' | H-2, H-3, $OCH_3$ at C-3'' |
| 3'' | - | 149.4 (C) | - | H-2'', H-5'', $OCH_3$ at C-3'' | - |
| 4'' | - | 151.4 (C) | - | H-2'', H-5'', H-6'', $OCH_3$ at C-4'' | - |
| 5'' | 6.86 (1H, d, *J*=8.2 Hz) | 111.3 (CH) | H-6'' | - | H-6'', $OCH_3$ at C-4'' |
| 6'' | 7.19 (1H, dd, *J*=2.0, 8.2 Hz) | 123.2 (CH) | H-5'' | H-3, H-2'' | H-2, H-3, H-5'' |
| $OCH_3$ at C-3' | 3.91 (3H, s) | 56.2 ($CH_3$) | - | - | H-2'' |
| $OCH_3$ at C-4' | 3.89 (3H, s) | 56.2 ($CH_3$) | - | - | H-5'' |

## 6.1.3 Synthesis of 1-(4'-hydroxyphenyl)-3-(3'',4''-dimethoxyphenyl)-pent-4-en-1-one

Firstly the Grignard reagent, vinylmagnesium bromide, had to be synthesised according to the method reported in Vogel[17]. Once synthesised, the Grignard reagent was reacted with the chalcone in the presence of cuprous iodide to give the adduct 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one (Scheme 6.7).




Scheme 6.7. The proposed attachment of the Grignard reagent to the chalcone.

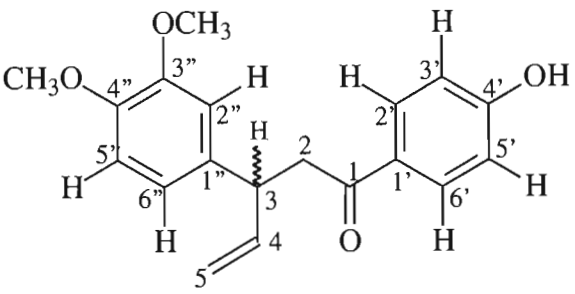
In this reaction step, a Michael-type addition reaction is favoured and hence the vinyl group attacks C-3 instead of the carbonyl group carbon.

### 6.1.3.1 Confirmation of the Structure of Compound XXI

### 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one

(Spectra 21a-g, pages 395-401)

This compound was isolated in a 20% yield as a red amorphous compound.




**1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one**

Figure 6.5. The structure of compound XXI.

The high resolution mass spectrum showed a parent ion peak at *m/z* 312.13615, which was consistent with the molecular formula of $C_{19}H_{20}O_4$ ($C_{19}H_{20}O_4$ requires 312.13616). A double bond equivalence of ten was deduced.

In the $^1$H NMR spectrum, the H-2'/6' proton resonance was observed at δ 7.83 (2H, d, $J$ = 8.4 Hz) and this showed a correlation in the NOESY spectrum to a triplet proton resonance at δ 3.32 (2H) in the NOESY spectrum. This resonance was assigned to 2H-2 as it was seen in the COSY spectrum to be coupled to a proton resonance at δ 4.03, ascribed to H-3. The 2H-2 proton resonance showed correlations in the NOESY spectrum to H-3, H-2'/6', H-2" (δ 6.74), H-6" (δ 6.76) and a vinyl methylene proton resonance at δ 5.04 (H-5A, dd, $J$ = 1.5, 10.0 Hz) and δ 5.00 (H-5B, dd, $J$ = 1.5, 16.8 Hz), assigned to 2H-5.

The COSY spectrum showed coupling between the 2H-5 proton resonance and a resonance at δ 6.00, ascribed to H-4, which was, in turn, seen to be coupled in the COSY spectrum to H-3. In the HMBC spectrum the C-2 methylene carbon resonance at δ 43.6 showed correlations to H-3 and H-4, while the C-3 methine carbon resonance at δ 44.5 showed coupling in the HMBC spectrum with 2H-2, H-4, 2H-5, H-2'' and 2H-6''.

The $^{1}H$ NMR spectrum showed the presence of two singlet proton resonances at δ 3.78 (3H) and δ 3.79 (3H), which were assigned to the methoxy groups at C-3" and C-4" respectively. The carbonyl group at C-1 was indicated by the fully substituted carbon resonance at δ 198.5 (C-1) and showed correlations in the HMBC spectrum to 2H-2, H-3 and H-2'/6'.

This synthesised compound was confirmed to be the novel vinyl ketone, 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one. The NMR correlations are tabulated in table 6.2, while the HMBC and NOESY correlations are shown in figure 6.6 and figure 6.7 respectively.

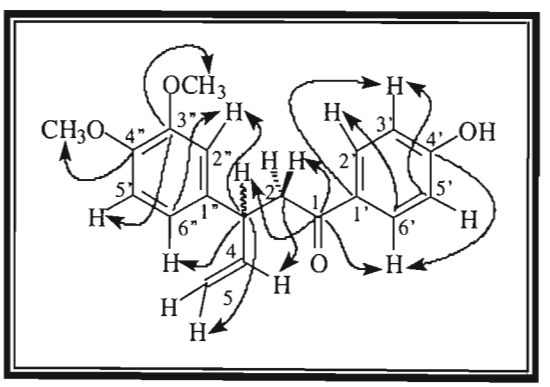



Figure 6.6. The HMBC (C → H) correlations for compound XXI.

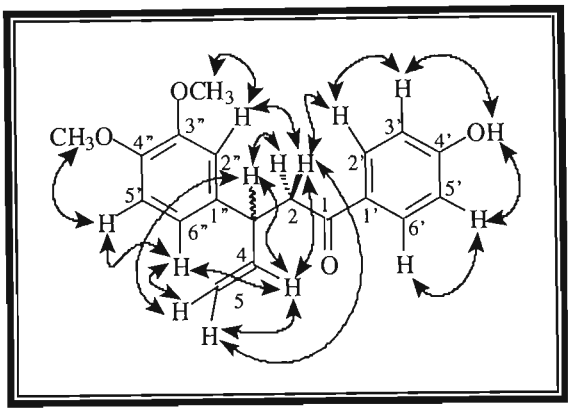


Figure 6.7. The NOESY correlations for compound XXI

**Table 6.2. NMR data and correlations for compound XXI ($CDCl_3$)**

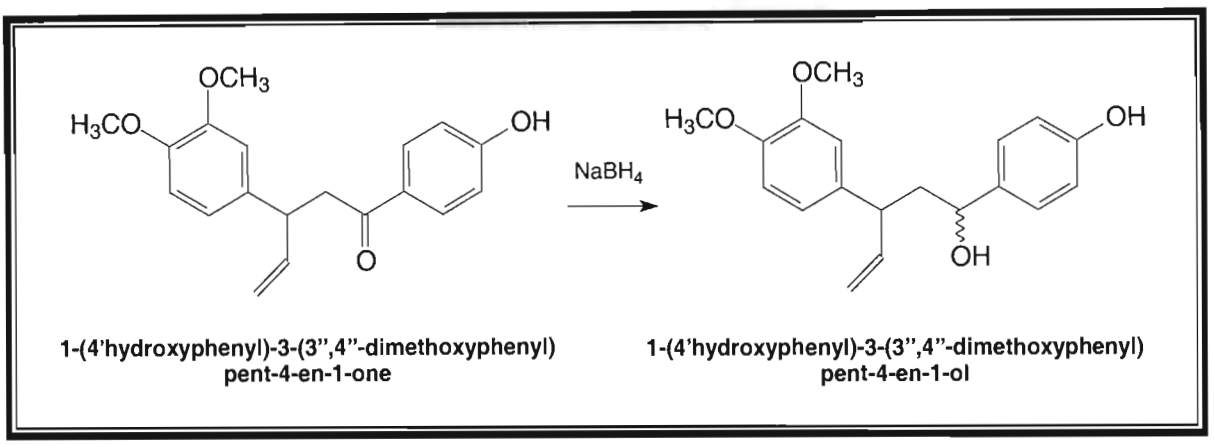
| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| **1** | - | 198.5 (C) | - | 2H-2, H-3, H-2'/6' | - |
| **2** | 3.32 (2H, m) | 43.6 ($CH_2$) | H-3 | H-3, H-4 | H-3, 2H-5, H-2'/6', H-2", H-6" |
| **3** | 4.03 (1H, m) | 44.5 (CH) | 2H-2, H-4 | 2H-2, H-4, 2H-5, H-2'', H-6'' | 2H-2, 2H-5, H-4, H-2'/6', H-2", H-6" |
| **4** | 6.00 (1H, dt) | 140.6 (CH) | H-3, 2H-5 | 2H-2, H-3, 2H-5 | 2H-2, H-3, 2H-5, H-2", H-6" |
| **5A** | 5.04 (1H,dd, *J*=1.5, 10.0Hz) | 114.4 ($CH_2$) | H-4 | H-3 | 2H-2, H-3, H-4, H-2", H-6" |
| **5B** | 5.00 (1H,dd, *J*=1.5, 16.8Hz) | | H-4 | | 2H-2, H-3, H-4, H-2", H-6" |
| **1'** | - | 129.2 (C) | - | H-3'/5' | - |
| **2'** | 7.83 (1H, d, *J*=8.4 Hz) | 130.8 (CH) | H-3'/5' | H-2'/6' | 2H-2, H-3'/5' |
| **3'** | 6.86 (1H, d, *J*=8.4 Hz) | 115.4 (CH) | H-2'/6' | H-3'/5' | H-2'/6', OH at C-4' |
| **4'** | - | 161.4 (C) | - | H-2'/ 6', H-3'/5' | - |
| **5'** | 6.86 (1H, d, *J*=8.4 Hz) | 115.4 (CH) | H-2'/6' | H-3'/5' | H-2'/6', OH at C-4' |
| **6'** | 7.83 (1H, d, *J*=8.4 Hz) | 130.8 (CH) | H-3'/5' | H-2'/6' | 2H-2, H-3'/5' |
| **1''** | - | 135.6 (C) | - | 2H-2, H-3, H-4, H-5 | - |
| **2''** | 6.74 (1H,)[§] | 111.1 (CH) | - | H-3, H-6" | $OCH_3$ at C-3'' |
| **3''** | - | 148.6 (C)[†] | - | H-5", $OCH_3$ at C-3" | - |
| **4''** | - | 147.3 (C)[†] | - | H-2", H-6", $OCH_3$ at C-4" | - |
| **5''** | 6.76 (1H,)[§] | 111.3 (CH) | H-6" | - | $OCH_3$ at C-4'', H-6'' |
| **6''** | 6.76 (1H)[§] | 119.4 (CH) | H-5" | H-3, H-2" | H-5'' |
| **$OCH_3$ at C-3''** | 3.78 (3H, s) | 55.7 ($CH_3$)[‡] | - | - | H-2'' |
| **$OCH_3$ at C-4''** | 3.79 (3H, s) | 55.6 ($CH_3$)[‡] | - | - | H-5'' |
| **OH at C-4'** | 8.28 (brs) | - | - | - | H-3'/5' |

[†, ‡] values may be interchangeable

[§] superimposed resonances (*J* could not be determined)

### 6.1.4 Attempted Synthesis of 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol

According to Muraoka and co-workers[6], the reduction of the 1,4-adduct with sodium borohydride (Scheme 6.8) should yield a diastereoisomeric mixture of the vinyl alcohol, 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol.



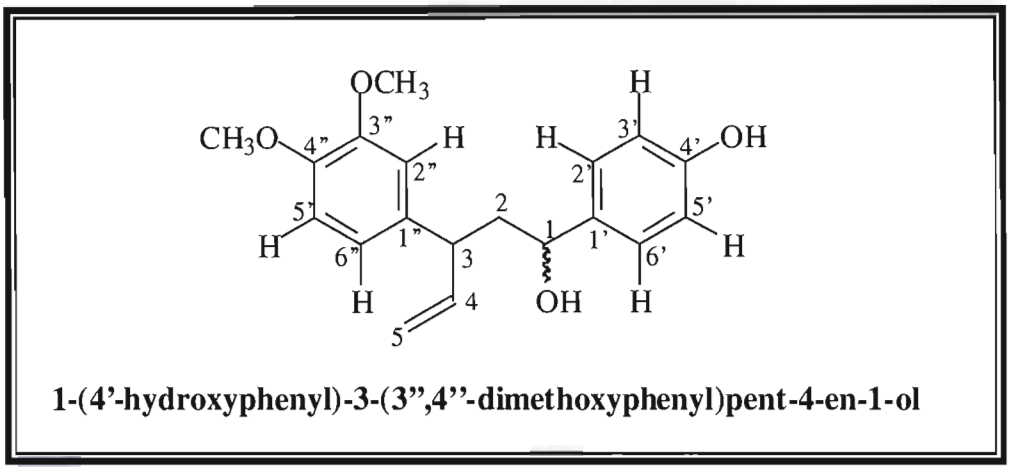

Scheme 6.8. The proposed reduction of the 1,4-adduct.

This synthesis proved successful, however, only $^{1}H$ NMR and $^{13}C$ NMR spectra were obtained as this stage and indicated a mixture of isomers that were not separated and were used as the starting material for the next synthetic step.

#### 6.1.4.1 Confirmation of the Structure of Compound XXII 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol

(Spectra 22a-b, pages 402-403)

This compound (Figure 6.8) was isolated in a 23.3% yield as a brown oil.



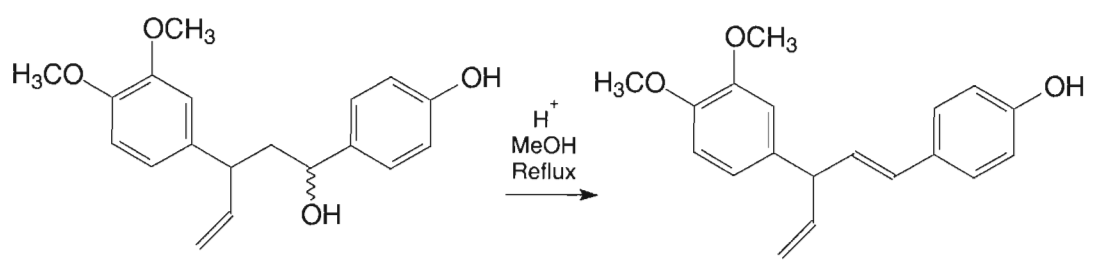

**1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol**

Figure 6.8. The structure of compound XXII.

Due to this being a mixture of isomers, only the $^{1}H$ and $^{13}C$ NMR spectra were run. The $^{13}C$ NMR spectrum showed traces of the vinyl ketone, which have been marked in the spectrum. The presence of the hydroxy group at C-1 was confirmed by the methine carbon signal at δ 79.0 in the $^{13}C$ NMR spectrum. In the $^{1}H$ NMR spectrum, a multiplet occurred at approximately δ 1.9, shifted from δ 3.32 in the $^{1}H$ NMR spectrum for compound XXI, and was assigned to 2H-2. This indicated that C-1 was no longer fully substituted, indicating the formation of the alcohol.

## 6.1.5 Attempted Synthesis of 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)-1,4-pentadiene

The acid catalysed dehydration of the vinyl alcohol should have yielded the required norlignan[6] (Scheme 6.9).

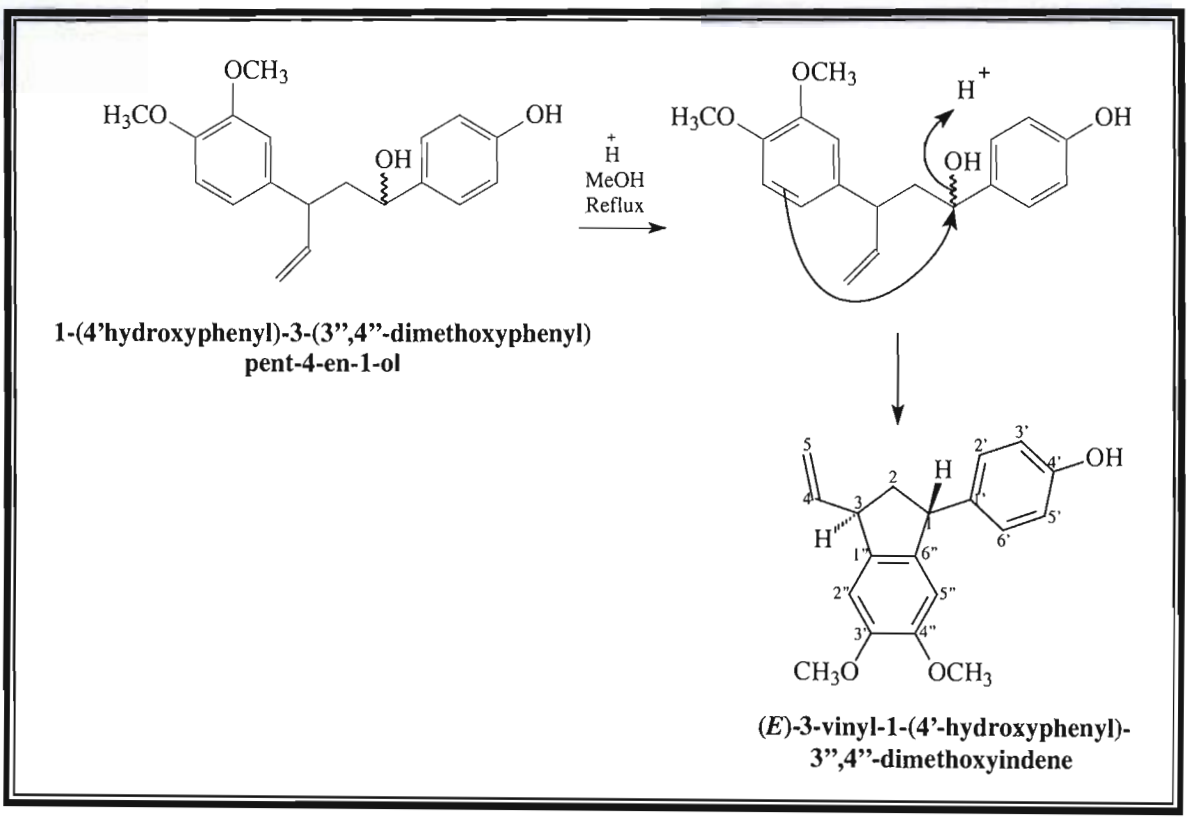



**1-(4'hydroxyphenyl)-3-(3",4"-dimethoxyphenyl) pent-4-en-1-ol**

**1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl) -1,4-pentadiene**

Scheme 6.9. The proposed formation of the norlignan.

However, the dehydration of the hydroxy group to form a double bond between C-1 and C-2, using 7% $H_2SO_4$, was not successful and rather the compound cyclised to form the novel indene-type compound (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene (Scheme 6.10). This occurred *via* a nucleophilic attack on the alcohol by the aromatic ring.




Scheme 6.10. The proposed formation of (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene.

### 6.1.5.1 Structural Elucidation of Compound XXIII

### (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene

(Spectra 23a-h, pages 404-411)

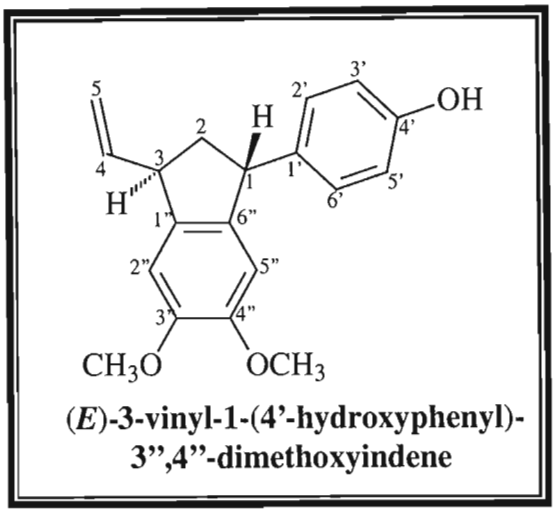



Figure 6.9. The structure of compound XXIII.

The mass spectrum showed a parent ion peak at *m/z* 296, which was consistent with the molecular formula of $C_{19}H_{20}O_3$ ($C_{19}H_{20}O_3$ requires 296). A double bond equivalence of ten was deduced.

The $^1$H NMR spectrum showed the presence of a pair of doublets at δ 6.95 (2H, $J$ = 8.6 Hz) and δ 6.73 (2H, $J$ = 8.6 Hz) which are ascribed to the H-2'/6' and H-3'/5' protons respectively. These two proton resonances are seen to be coupled to one another in the COSY spectrum. The H-3'/5' proton signal showed a correlation in the NOESY spectrum to H-2'/6', while the C-3'/5' methine carbon resonance at δ 115.3 was correlated to H-2'/6' in the HMBC spectrum. In the HMBC spectrum the C-2'/6' methine carbon resonance at δ 128.8 showed a correlation to a one-proton superimposed resonance at δ 4.31 which was assigned to H-1. The corresponding carbon resonance at δ 49.0 (C-1) was correlated in the HMBC spectrum to 2H-2 (δ 2.24 and δ 2.36) and a one-proton singlet proton resonance at δ 6.53 which was assigned to H-5''. This H-5'' proton resonance also showed a correlation in the NOESY spectrum to H-1.

The H-2'' proton resonance showed a correlation in the NOESY spectrum to a one-proton resonance at δ 3.85 which was ascribed to H-3 as it also showed a correlation in the NOESY spectrum to H-2A and 2H-5 (δ 5.06 and δ 5.03) and was seen to be coupled in the COSY spectrum to 2H-2. Due to the fact that no correlation could be seen in the NOESY spectrum between H-1 and H-3, the relative stereochemistry could be determined, and these protons were observed to be *trans* to one another. In the HMBC spectrum the C-3 methine carbon resonance at δ 48.3 showed a correlation to H-2'', 2H-2 and a two-proton resonance at δ 5.05 which was due to the vinyl methylene protons, 2H-5. In the COSY spectrum the 2H-5 proton resonance was seen to be coupled with a one-proton multiplet at δ 5.86, which was assigned to H-4 as the corresponding methine carbon resonance at δ 141.3 showed correlations in the HMBC spectrum to 2H-2 and H-3. The C-5 methylene carbon resonance at δ 114.4 also showed a correlation in the HMBC spectrum to H-3.

In the $^{1}$H NMR spectrum two singlet proton resonances, both ascribed to three protons, were observed at δ 3.86 and δ 3.75 and were assigned to the methoxy group attached to C-3'' and the methoxy group attached to C-4'' respectively. In the NOESY spectrum the methoxy group at C–3'' showed a correlation to H-2'', while the methoxy group at C-4'' showed a correlation to H-5''. The C-3'' fully substituted carbon resonance was thus assigned to the resonance at δ 148.6 as it was correlated in the HMBC spectrum to the methoxy group at C-3'' proton resonance, similarly, the C-4'' fully substituted carbon resonance was due to the resonance at δ 148.5. Four additional fully substituted carbon resonance were observed in the $^{13}$C NMR spectrum at δ 154.0, 138.0, 137.4 and 129.4 and were ascribed to C-4', C-1', C-6'' and C-1'' respectively.

This compound was found to be the novel indene-type compound identified as (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene. The NMR data for compound XXIII is tabulated in table 6.3 and the HMBC and NOESY correlations (both showing the relative stereochemistry) are shown in figure 6.10 and figure 6.11 respectively.

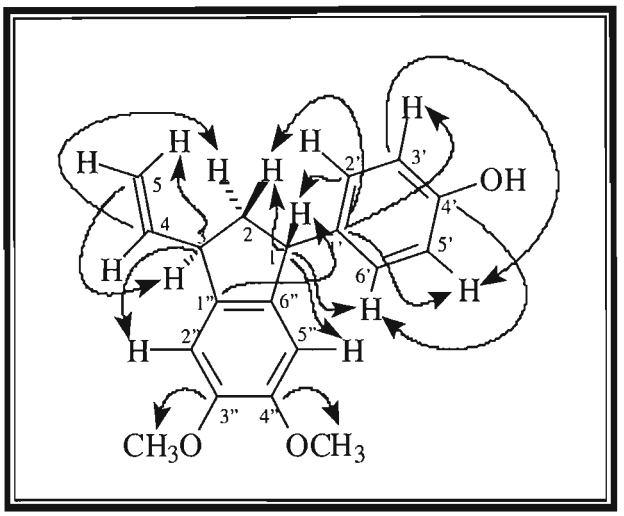



Figure 6.10. The HMBC (C → H) correlations for compound XXIII.

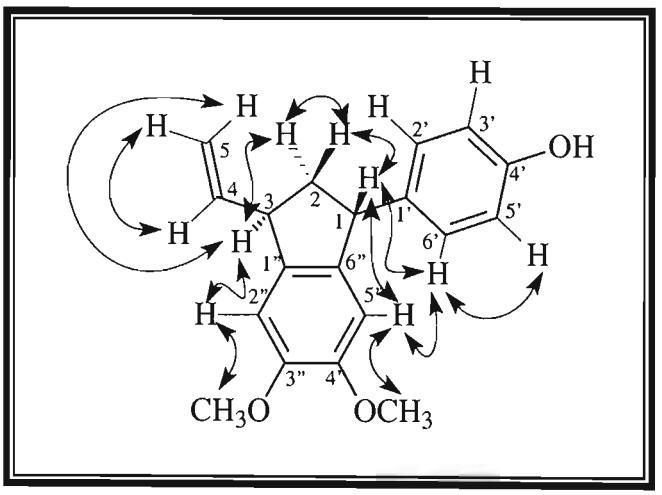



Figure 6.11. The NOESY correlations for compound XXIII.

**Table 6.3. NMR data and correlations for compound XXIII ($CDCl_3$)**

| Carbon Number | $^1$H NMR data (400 MHz) | $^{13}$C NMR data (100 MHz) | COSY correlations | HMBC correlations C → H | NOESY correlations |
|---|---|---|---|---|---|
| 1 | 4.31 (1H, dd, *J*=5.5, 8.1Hz) | 49.0 (CH) | 2H-2 | 2H-2, H-2'/6', H-5'' | 2H-2, H-2'/6', H-5'' |
| 2A | 2.24 (1H, m) | 43.8 ($CH_2$) | H-1, H-2B, H-3 | H-1(w) | H-1, H-2B, H-3, H-2'/6' |
| 2B | 2.36 (1H, m) | | H-1, H-2A, H-3 | | H-1, H-2A |
| 3 | 3.85 (1H, m) | 48.3 (CH) | 2H-2 | 2H-2, H-4(w), 2H-5, H-2'' | H-2A, 2H-5, H-2'' |
| 4 | 5.86 (1H, m) | 141.3 (CH) | 2H-5 | 2H-2, H-3 | 2H-5 |
| 5A | 5.06 (1H, dd, *J*=1.8, 17.2Hz) | 114.4 ($CH_2$) | H-4 | H-3 | H-3. H-4 |
| 5B | 5.03 (1H,dd, *J*=10.6Hz) | | H-4 | | H-3, H-4 |
| 1' | - | 138.0 (C) | - | H-1, 2H-2, H-3'/5' | - |
| 2' | 6.95 (2H, d, *J* =8.6 Hz) | 128.8 (CH) | H-3'/5' | H-1, H-2'/6' | H-1, H-2A, H-3'/5', H-5'' |
| 3' | 6.73 (2H, d, *J*=8.6 Hz) | 115.3 (CH) | H-2'/6' | H-2'/6', H-3'/5' | H-2'/6' |
| 4' | - | 154.0 (C) | - | H-2'/6', H-3'/5'(w) | - |
| 5' | 6.73 (2H, d, *J*=8.6 Hz) | 115.3 (CH) | H-2'/6' | H-2'/6', H-3'/5' | H-2'/6' |
| 6' | 6.95 (2H, d, *J* =8.6 Hz) | 128.8 (CH) | H-3'/5' | H-1, H-2'/6' | H-1, H-2A, H-3'/5', H-5'' |
| 1'' | - | 129.4 (C) | - | H-1 | - |
| 2'' | 6.71 (1H, s) | 107.3 (CH) | - | - | H-3, $OCH_3$ at C-3'' |
| 3'' | - | 148.6 (C) | - | H-2'', $OCH_3$ at C-3'' | - |
| 4'' | - | 148.5 (C) | - | H-5'', $OCH_3$ at C-4'' | - |
| 5'' | 6.53 (1H, s) | 107.9 (CH) | - | - | H-1, H-2'/6', $OCH_3$ at C-4'' |
| 6'' | - | 137.4 (C) | - | H-5'', $OCH_3$ at C-4''(w) | - |
| $OCH_3$ at C-3' | 3.86 (3H, s) | 56.0 ($CH_3$) | - | - | H-2'' |
| $OCH_3$ at C-4' | 3.75 (3H, s) | 56.0 ($CH_3$) | - | - | H-5'' |

## 6.1.6 Experimental

***Synthesis of 1-(4'-hydroxyphenyl)-3-(3'',4''-dimethoxyphenyl)prop-2-en-1-one***

Potassium hydroxide pellets (90 g) were dissolved in water (90 $cm^3$) in a round-bottomed flask. The 4'-hydroxyacetophenone (20 g in 220 $cm^3$ methanol; 0.1469 moles) was added to the flask followed by the 3,4-dimethoxybenzaldehyde (24.41g in 220 $cm^3$ methanol; 0.1469 moles) and the flask was stoppered. The contents of the flask were left to stir for 5 days. The solution turned from yellow to orange in colour. The resultant solution was diluted with 500 $cm^3$ of deionised water and then acidified

with 500 $cm^3$ of 4M hydrochloric acid. The reaction solution was then extracted with dichloromethane (5 x 500 $cm^3$ portions). The chalcone (23.3 g, 56%) crystallised out as bright yellow crystals from the dichloromethane extract.

***Formation of the Grignard reagent***

A dry three-necked round bottom flask, under nitrogen, was equipped with a magnetic stirrer bar, a dry-ice condenser and an addition funnel. Magnesium (1.5 g, 0.06 mol) was placed inside the flask with 13 ml dry tetrahydrofuran (THF). In the addition funnel was placed vinyl bromide (3.8 $cm^3$, 54.5 mmol) in dry THF (37 $cm^3$). Approximately 2 $cm^3$ of this solution was added to the flask, with very rapid stirring, to initiate the reaction. After initiation, the remaining vinyl bromide solution was added slowly to maintain steady reflux. Once all the magnesium had been consumed the reaction flask was allowed to cool.

***Synthesis of (+)-1-(4'-hydroxyphenyl)-3-(3'',4''-dimethoxyphenyl)pent-4-en-1-one***

Under nitrogen, a solution of vinylmagnesium bromide in tetrahydrofuran (THF, 50 $cm^3$, 54.5 mmol) was added to a suspension of cuprous iodide (520 mg, 2.7 mmol) in THF (50 $cm^3$) at 0°C, and the mixture was stirred at 0°C for 15 minutes. To the mixture was added a solution of the chalcone (7.3 g, 24.3 mmol) in THF (20 $cm^3$) at room temperature for 24 hours. The reaction was quenched by the addition of 10% hydrochloric acid to the mixture, which was then extracted with diethyl ether. The extract was washed successively with aqueous sodium hydrogen carbonate and brine and then evaporated to give a red amorphous substance, identified by NMR as the vinyl ketone (1478 mg, 20%).

***Synthesis of 1-(4'-hydroxyphenyl)-3-(3'',4''-dimethoxyphenyl)pent-4-en-1-ol***

A mixture of the vinyl ketone (1421 mg, 4.5 mmol), sodium borohydride (0.33 g, 30.9 mmol) and ethanol (20 $cm^3$) was stirred at room temperature for three days and then concentrated using a rotor evaporator. The residue was diluted with 50 $cm^3$ brine, acidified with 10% hydrochloric acid solution and extracted with benzene. The extract was washed successively with aqueous sodium hydrogen carbonate and brine and then evaporated to give a brown oil (1316.7 mg, 93%). This is a diastereoisomeric mixture of the vinyl alcohol

***Synthesis of (E)-3-vinyl-1-(4'-hydroxyphenyl)-3'',4''-dimethoxyindene***

A mixture of the vinyl alcohol (1284.5 mg, 4.3 mmol), 7% hydrochloric acid (20 $cm^3$) and methanol (33 $cm^3$) was refluxed for 24 hours using steam. Once cool, the reaction mixture was poured into aqueous sodium hydrogen carbonate and extracted with chloroform. The extract was washed with brine and evaporated to give a brown oil (532 mg). This was subjected to column chromatography (2 $cm^3$ in diameter) using 100% dichloromethane as the mobile phase and silica gel (Merck 9385) as the stationary phase to yield the final product (149 mg, 11.7%).

### 6.1.6.1 Physical data for Compound XX

**Name:** (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one

**Physical appearance:** yellow crystals

**Melting point:** 84-86°C

**Yield:** 56%

**Mass:** HRMS [$M^+$] at *m/z* 284.10485, $C_{17}H_{16}O_4$ requires 284.10486

EIMS: *m/z*: 284, 269, 253, 241, 226, 210, 191, 181, 163, 147, 138, 121, 105, 93, 77, 65

**Infra-red:** $\nu_{max}^{NaCl}$ $cm^{-1}$ : 3273, 1608, 1517, 1442, 1271, 1159, 1025

**$^1$H and $^{13}$C NMR Data:** Refer to table 6.1 (page 180)

### 6.1.6.2 Physical data for Compound XXI

**Name:** 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one

**Physical appearance:** red amorphous

**Yield:** 20%

**Mass:** HRMS [$M^+$] at *m/z* 312.13615, $C_{19}H_{20}O_4$ requires 312.13616

EIMS: *m/z*: 312, 284, 253, 229, 191, 177, 146, 121, 93, 77, 65, 51

**Optical Rotation:** $[\alpha]_D$ = +0.11° (c = 1.932 g/100 $cm^3$; $CHCl_3$)

**$^1$H and $^{13}$C NMR Data:** Refer to table 6.2 (page 183)

### 6.1.6.3 Physical data for Compound XXIII

**Name:** (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene

**Physical appearance:** brown amorphous

**Yield:** 11.7%

**Mass:** [$M^+$] at *m/z* 296, $C_{19}H_{20}O_3$ requires 296

**$^1$H and $^{13}$C NMR Data:** Refer to table 6.3 (page 190)

## 6.2 References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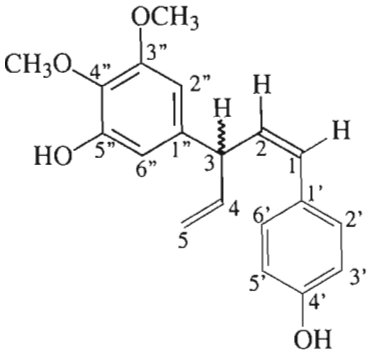
# Chapter 7

# Conclusion

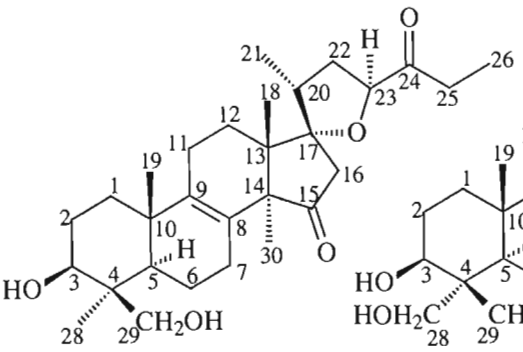
This thesis involved the chemical investigation of seven African medicinal plants as well as the synthesis, or partial synthesis, of certain isolated compounds.

The first plant family investigated in this work was the Hyacinthaceae, and in this chemical investigation, four plants were investigated, namely *Ledebouria ovatifolia, Eucomis pole-evansii, Lachenalia rubida* and *Drimia capitata.* A diverse range of compounds were isolated from this plant family including a norlignan, homoisoflavonoids, nortriterpenoids and bufadienolides.

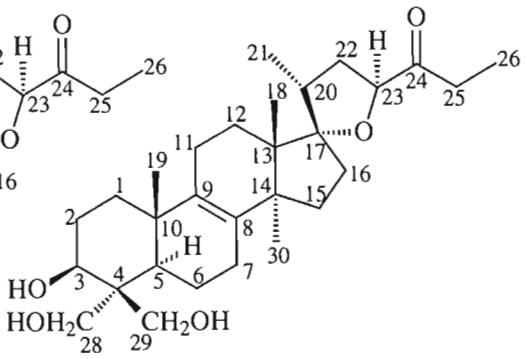
*Ledebouria ovatifolia* yielded a novel norlignan, (-)-(*Z*)-1-(4'-hydroxyphenyl)-3-(5"-hydroxy-3",4"-dimethoxyphenyl)-1,4-pentadiene (**compound I**) and two nortriterpenoids, (23*S*)-17α,23-epoxy-3β,29-dihydroxy-27-norlanost-8-ene-15,24-dione (**compound II**) and (23*S*)-17α,23-epoxy-3β,28,29-trihydroxy-27-norlanost-8-en-24-one (**compound III**). This was the first time that a norlignan had been isolated from the Hyacinthaceae family. *Ledebouria ovatifolia* had previously been worked on by Pohl *et al.* who isolated 4,4'-dihydroxy-2',6'-dimethoxychalcone and 5,7-dihydroxy-3-(4'-hydroxybenzyl)-4-chromanone[1]. Further investigations of this plant have also yielded the novel chalcone, ovatifolin[2]. This plant was chemically investigated for a second time as the plant in this study was the Highveld form which was collected from the Blyde Nature Reserve, while the first investigation was performed on *Ledebouria ovatifolia* that was purchased from the Warwick Triangle market in Durban, Kwazulu-Natal. This confirmed that the environment that a plant grows in plays an important role in the type of compounds that plant produces.



**compound I**

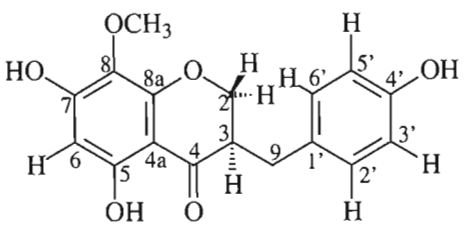


**compound II**

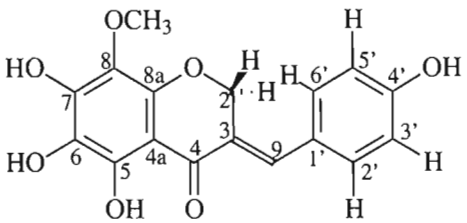


**compound III**

*Eucomis pole-evansii* yielded a 3-benzyl-4-chromanone type homoisoflavonoid, (-)-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone (**compound IV**) and a novel 3-benzylidene-4-chromanone type homoisoflavonoid, (*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone (**compound V**).

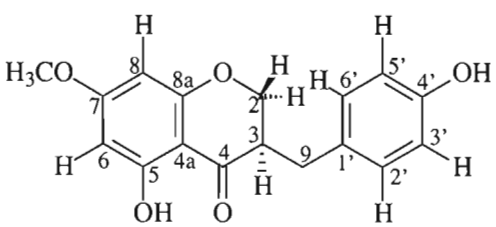


**compound IV**

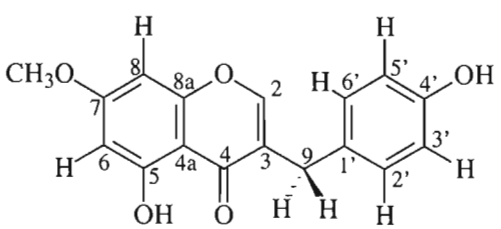


**compound V**

*Lachenalia rubida* yielded a 3-benzyl-4-chromanone type homoisoflavanone, (-)-5-hydroxy-3-(4'-hydroxybenzyl)-7-methoxy-4-chromanone (**compound VI**) and a novel 3-benzyl-4-chromone type homoisoflavone, 5-hydroxy-3-(4'-hydroxybenzyl)-7-methoxychromone (**compound VII**). Compound VI has been reported by Adinolfi *et al.*[3], however, it was not clear from the paper whether this compound was isolated as a natural compound or if it resulted from a methylation reaction. Compound VII is an unusual type of homoisoflavonoid that has not been widely isolated.

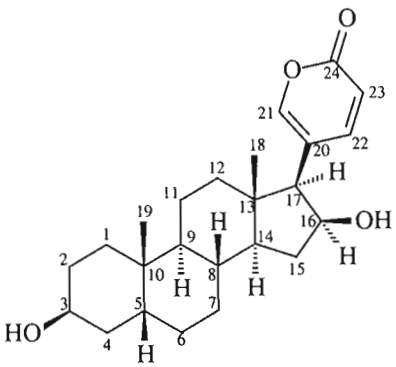


**compound VI**

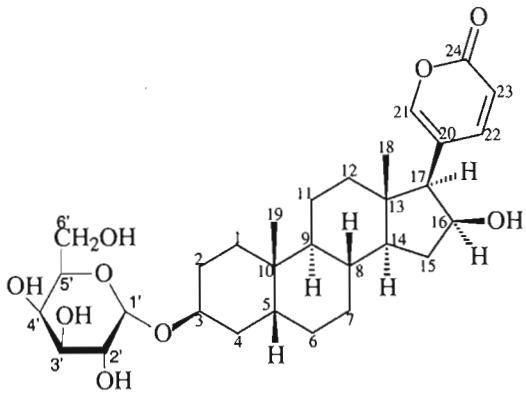


**compound VII**

*Drimia capitata* yielded a novel bufadienolide 5β-3β,16β-dihydroxybufa-20,22-dienolide (**compound VIII**) and the novel glycoside of compound VIII, 5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside (**compound IX**). The identity of the sugar group was unsure, thus a hydrolysis of compound IX with β-glucosidase was performed in order to cleave off the sugar from the aglycone. The sugar was identified as galactose.

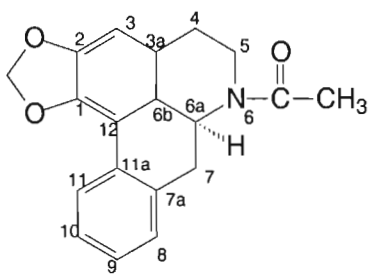


**compound VIII**



**compound IX**

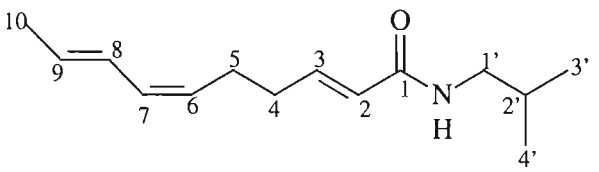
The fifth plant to be investigated was *Papaver aculeatum* of the family, Papaveraceae. This project was initiated by Professor P. Tetenyi of the Institute of Research into Medicinal Plants in Budapest, Hungary who communicated that the South African species was considered primitive amongst the *Papavers* and could contain simple alkaloid precursors. This plant yielded an alkaloid, (+)-*N*-acetylanonaine (**compound X**).



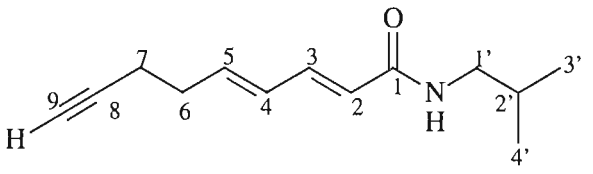
**compound X**

The Asteraceae was the third plant family to undergo chemical investigation and *Spilanthes mauritiana,* yielded one known isobutyl amide, *N*-(2'-methylpropyl)-deca-2*E*,6*Z*,8*E*-trienamide (**compound XI**) and a novel isobutyl amide, *N*-(2'-methylpropyl)-nona-2*E*,4*E*-dien-8-ynamide (**compound XII**). This work was initially the project of postdoctoral student Dr. J.J. Nair who investigated this plant in an

attempt to isolate the compounds responsible for the numbing action of the plant. Dr. Nair isolated these two compounds but he left the department before they could be identified. Dr Nair managed to run some 2-D spectra before leaving, however, even though his compounds were stored in a freezer, they still decomposed. These isobutylamide compounds are highly unstable[4] and several efforts to re-isolate this type of compound proved unsuccessful as they rapidly decomposed.

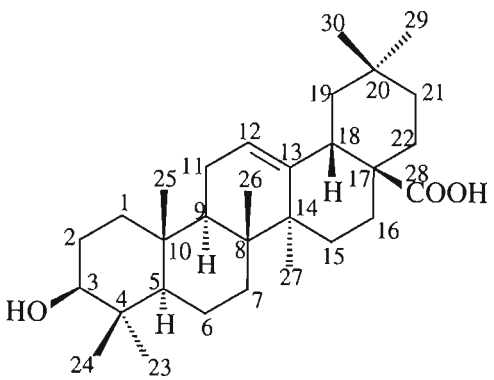


**compound XI**

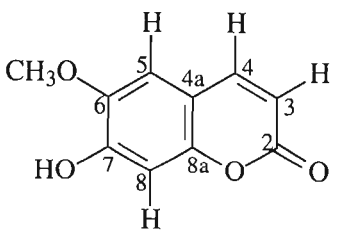


**compound XII**

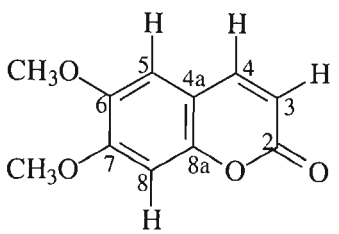
The seventh and final plant investigated was *Tachiadenua longiflorus* from the family, Gentianaceae. This plant yielded a wide variety of compounds including an oleane triterpenoid, oleanolic acid (**compound XIII**), which precipitated out of the hot hexane extract in a large quantity. Other compounds isolated included two coumarins, scopoletin (**compound XIV**) and scoparone (**compound XV**), as well as an iridoid derivative, angelone (**compound XVI**).



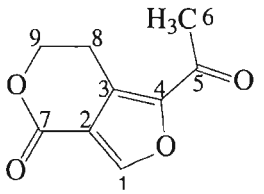
**compound XIII**



**compound XIV**

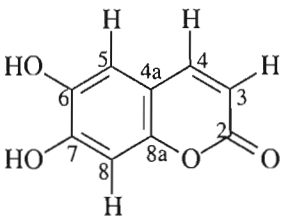


**compound XV**

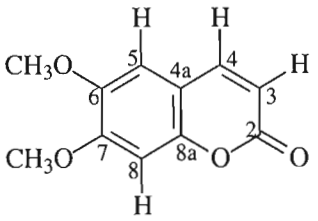


**compound XVI**

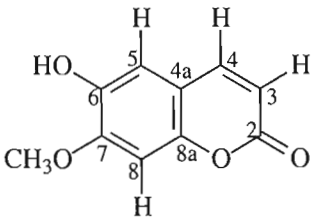
The isolation of the two coumarins prompted their syntheses, as the NMR data for these compounds did not correlate well with literature. The synthesis of aesculetin (**compound XVII**) and scoparone (**compound XVIII**) proved successful, however scopoletin was unable to be synthesised. However, isoscopoletin (**compound XIX**) was successfully synthesised, but in a low yield.



**compound XVII**

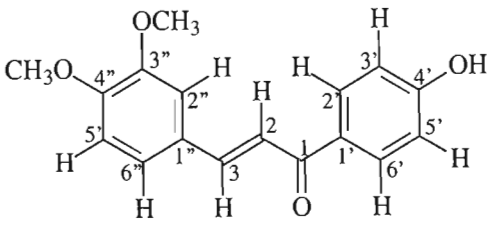


**compound XVIII**

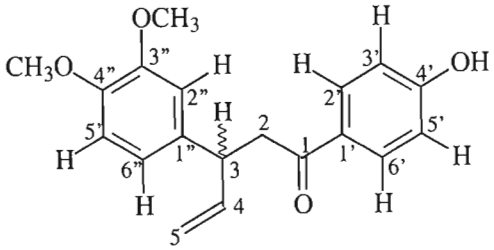


**compound XIX**

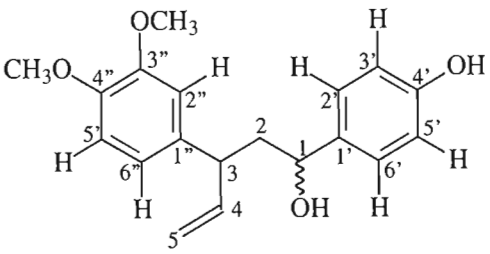
The isolation of the norlignan from *Ledebouria ovatifolia* prompted its attempted synthesis. However, due to confusion in the literature, it was initially thought that compound I was of the *trans* orientation and thus the synthesis was performed according to the method of Muraoka and co-workers[5]. The starting material, 5-hydroxy-3,4-dimethoxy-benzaldehyde, was not readily available, and due to time constraints, the derivative 3,4-dimethoxy-benzaldehyde, which was readily available was used instead. It was presumed that if the reaction was successful with this starting material, then compound I could be synthesized using the same procedure, with the appropriate starting material. The initial step in the reaction resulted in the formation of the chalcone, (*E*)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one (**compound XX**). The formation of the appropriate Grignard reagent was required and this was reacted with the chalcone to yield the vinyl ketone, (+)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one (**compound XXI**). Reduction of the ketone to form the vinyl alcohol, 1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol (**compound XXII**) resulted in a mixture of isomers which were then dehydrated to give the final product, (*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene (**compound XXIII**). Thus the formation of the norlignan was unsuccessful. However, the synthesis of the indene-type compound was interesting and this work will be investigated further by a student in the research group.



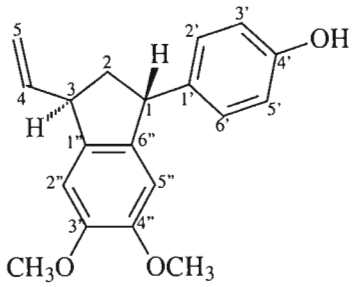
**compound XX**



**compound XXI**



**compound XXII**



**compound XXIII**

1-D and 2-D NMR spectroscopy were used to elucidate all the structures isolated in this work, while mass spectrometry, infra-red analysis and ultra-violet spectroscopy were used to confirm the structures. Seven novel compounds resulted from the sixteen natural products isolated. Compounds I, IV, V, XIV, XVII, XVIII, XIX and XX were screened for biological activity and these provided promising results (Table 7.1). The anti-inflammatory activity given is measured as a percentage inhibition of prostaglandin synthesis using indomethacin as a positive control.

**Table 7.1. Table summarising biological activity screening results**

| | Anti-inflammatory activity | | | Anti-bacterial activity |
|---|---|---|---|---|
| **Compound** | **Microsomal cellscreen** | **COX-1 screen** | **COX-2 screen** | |
| **I** | 72.7 | 42.2 | 23.4 | - |
| **IV** | 28.2 | - | 2.5 | - |
| **V** | 29.7 | - | - | MIC 0.98 mM |
| **XIV** | 7.5 | - | 4.5 | - |
| **XVII** | 27.7 | - | 2.3 | - |
| **XVIII** | 4.5 | - | - | - |
| **XIX** | 21.6 | - | - | - |
| **XX** | 22.8 | - | - | - |

In earlier times, all drugs and medicinal agents were derived from natural products in higher plants. Today many drugs are synthesised to compete with the growing demand for medicinal agents. However, the study of natural products has its advantages over synthetic drugs as it primarily leads to compounds having new structural features and novel biological activities[6]. Higher plants serve as an important source of new drugs and novel compounds isolated from them are extremely useful as lead structures for synthetic modification and optimisation of bioactivity[6]. The future of higher plants as sources of medicinal compounds for use in investigation, prevention and treatment of diseases is very promising[6].

## 7.1 References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6) http://chemistry.uah.edu/Faculty/setzer/natprod.html

# APPENDIX

# List of Spectra


Page

**(-)-(Z)-1-(4'-hydroxyphenyl)-3-(5''-hydroxy-3'',4''-dimethoxyphenyl)-1,4-pentadiene**

Spectrum 1a: $^{1}H$ NMR spectrum of compound I ($CDCl_3$) (400 MHz) 208
Spectrum 1b: $^{13}C$ NMR spectrum of compound I ($CDCl_3$) (100 MHz) 209
Spectrum 1c: ADEPT spectrum of compound I 210
Spectrum 1d: HSQC spectrum of compound I 211
Spectrum 1e: COSY spectrum of compound I 212
Spectrum 1f: HMBC spectrum of compound I 213
Spectrum 1g: NOESY spectrum of compound I 214
Spectrum 1h: Mass spectrum of compound I 215
Spectrum 1i: Infra-red spectrum of compound I 216

**(23*S*)-17α,23-epoxy-3β, 29-dihydroxy-27-norlanost-8-ene-15,24-dione**

Spectrum 2a: $^{1}H$ NMR spectrum of compound II ($CDCl_3$) (400 MHz) 217
Spectrum 2b: $^{13}C$ NMR spectrum of compound II ($CDCl_3$) (100 MHz) 218
Spectrum 2c: ADEPT spectrum of compound II 219
Spectrum 2d: HSQC spectrum of compound II 220
Spectrum 2e: COSY spectrum of compound II 221
Spectrum 2f: HMBC spectrum of compound II 222
Spectrum 2g: NOESY spectrum of compound II 223
Spectrum 2h: Mass spectrum of compound II 224
Spectrum 2i: Infra-red spectrum of compound II 225
Spectrum 2j: $^{1}H$ NMR spectrum of acetylated compound II ($CDCl_3$) (400 MHz) 226
Spectrum 2k: $^{13}C$ NMR spectrum of acetylated compound II ($CDCl_3$) (100 MHz) 227
Spectrum 2l: ADEPT spectrum of acetylated compound II 228
Spectrum 2m: HSQC spectrum of acetylated compound II 229
Spectrum 2n: COSY spectrum of acetylated compound II 230
Spectrum 2o: HMBC spectrum of acetylated compound II 231
Spectrum 2p: NOESY spectrum of acetylated compound II 232

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

Spectrum 3a: $^{1}H$ NMR spectrum of compound III ($CDCl_3$) (400 MHz) 233
Spectrum 3b: $^{13}C$ NMR spectrum of compound III ($CDCl_3$) (100 MHz) 234
Spectrum 3c: ADEPT spectrum of compound III 235
Spectrum 3d: HSQC spectrum of compound III 236
Spectrum 3e: COSY spectrum of compound III 237
Spectrum 3f: HMBC spectrum of compound III 238
Spectrum 3g: NOESY spectrum of compound III 239
Spectrum 3h: Mass spectrum of compound III 240
Spectrum 3i: Infra-red spectrum of compound III 241

**(-)-5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methoxy-4-chromanone**

Spectrum 4a: $^{1}H$ NMR spectrum of compound IV ($CDCl_3$) (400 MHz) 242
Spectrum 4b: $^{13}C$ NMR spectrum of compound IV ($CDCl_3$) (100 MHz) 243
Spectrum 4c: HSQC spectrum of compound IV 244
Spectrum 4d: COSY spectrum of compound IV 245
Spectrum 4e: HMBC spectrum of compound IV 246
Spectrum 4f: NOESY spectrum of compound IV 247
Spectrum 4g: Mass spectrum of compound IV 248
Spectrum 4h: Ultra-violet spectrum of compound IV 249
Spectrum 4i: Infra-red spectrum of compound IV 250
Spectrum 4j: $^{1}H$ NMR spectrum of acetylated compound IV ($CDCl_3$) (400 MHz) 251
Spectrum 4k: $^{13}C$ NMR spectrum of acetylated compound IV ($CDCl_3$) (100 MHz) 252
Spectrum 4l: ADEPT spectrum of acetylated compound IV 253
Spectrum 4m: HSQC spectrum of acetylated compound IV 254
Spectrum 4n: COSY spectrum of acetylated compound IV 255
Spectrum 4o: HMBC spectrum of acetylated compound IV 256
Spectrum 4p: NOESY spectrum of acetylated compound IV 257

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**(*E*)-5,6,7-trihydroxy-3-(4'-hydroxybenzylidene)-8-methoxy-4-chromanone**

Spectrum 5a: $^{1}H$ NMR spectrum of compound V ($CDCl_3$) (400 MHz) 258
Spectrum 5b: $^{13}C$ NMR spectrum of compound V ($CDCl_3$) (100 MHz) 259
Spectrum 5c: HSQC spectrum of compound V 260
Spectrum 5d: COSY spectrum of compound V 261
Spectrum 5e: HMBC spectrum of compound V 262
Spectrum 5f: NOESY spectrum of compound V 263
Spectrum 5g: Mass spectrum of compound V 264
Spectrum 5h: Ultra-violet spectrum of compound V 265
Spectrum 5i: Infra-red spectrum of compound V 266

**(-)-5-hydroxy-3-(4'-hydroxybenzyl)-7-methoxy-4-chromanone**

Spectrum 6a: $^{1}H$ NMR spectrum of compound VI ($CDCl_3$) (400 MHz) 267
Spectrum 6b: $^{13}C$ NMR spectrum of compound VI ($CDCl_3$) (100 MHz) 268
Spectrum 6c: ADEPT spectrum of compound VI 269
Spectrum 6d: HSQC spectrum of compound VI 270
Spectrum 6e: COSY spectrum of compound VI 271
Spectrum 6f: HMBC spectrum of compound VI 272
Spectrum 6g: NOESY spectrum of compound VI 273
Spectrum 6h: NOESY spectrum of compound VI 274
Spectrum 6i: Mass spectrum of compound VI 275
Spectrum 6j: Ultra-violet spectrum of compound VI 276
Spectrum 6k: Infra-red spectrum of compound VI 277

**5-hydroxy-3-(4'-hydroxybenzyl)-7-methoxy-chromone**

Spectrum 7a: $^{1}H$ NMR spectrum of compound VII ($CDCl_3$) (400 MHz) 278
Spectrum 7b: $^{13}C$ NMR spectrum of compound VII ($CDCl_3$) (100 MHz) 279
Spectrum 7c: HSQC spectrum of compound VII 280
Spectrum 7d: COSY spectrum of compound VII 281
Spectrum 7e: HMBC spectrum of compound VII 282
Spectrum 7f: NOESY spectrum of compound VII 283
Spectrum 7g: Mass spectrum of compound VII 284
Spectrum 7h: Ultra-violet spectrum of compound VII 285
Spectrum 7i: Infra-red spectrum of compound VII 286

**5β-3β,16β-dihydroxybufa-20,22-dienolide**

Spectrum 8a: $^{1}H$ NMR spectrum of compound VIII ($CD_3OD$) (400 MHz) 287
Spectrum 8b: $^{13}C$ NMR spectrum of compound VIII ($CD_3OD$) (100 MHz) 288
Spectrum 8c: HSQC spectrum of compound VIII 289
Spectrum 8d: COSY spectrum of compound VIII 290
Spectrum 8e: HMBC spectrum of compound VIII 291
Spectrum 8f: NOESY spectrum of compound VIII 292
Spectrum 8g: Mass spectrum of compound VIII 293

**5β-16β-hydroxybufa-20,22-dienolide 3β-*O*-β-D-galactoside**

Spectrum 9a: $^{1}H$ NMR spectrum of compound IX ($CD_3OD$) (400 MHz) 294
Spectrum 9b: $^{13}C$ NMR spectrum of compound IX ($CD_3OD$) (100 MHz) 295
Spectrum 9c: ADEPT spectrum of compound IX 296
Spectrum 9d: HSQC spectrum of compound IX 297
Spectrum 9e: COSY spectrum of compound IX 298
Spectrum 9f: HMBC spectrum of compound IX 299
Spectrum 9g: NOESY spectrum of compound IX 300
Spectrum 9h: Mass spectrum of compound IX 301
Spectrum 9i: Infra-red spectrum of compound IX 302

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**(+)-*N*-acetylanonaine**

Spectrum 10a: $^{1}$H NMR spectrum of compound X ($CD_3OD$) (400 MHz) 303
Spectrum 10b: $^{13}$C NMR spectrum of compound X ($CD_3OD$) (100 MHz) 304
Spectrum 10c: HSQC spectrum of compound X 305
Spectrum 10d: HSQC spectrum of compound X 306
Spectrum 10e: COSY spectrum of compound X 307
Spectrum 10f: HMBC spectrum of compound X 308
Spectrum 10g: NOESY spectrum of compound X 309
Spectrum 10h: NOE spectrum of compound X showing irradiation of H-5β 310
Spectrum 10i: NOE spectrum of compound X showing irradiation of H-3 311
Spectrum 10j: NOE spectrum of compound X showing irradiation of H-6a 312
Spectrum 10k: NOE spectrum of compound X showing irradiation of H-11 313
Spectrum 10l: NOE spectrum of compound X showing irradiation of $OCH_2O$ 314
Spectrum 10m: Mass spectrum of compound X 315
Spectrum 10n: Infra-red spectrum of compound X 316

***N*-(2'-methylpropyl)-deca-2*E*,6*Z*,8*E*-trienamide**

Spectrum 11a: $^{1}$H NMR spectrum of compound XI ($CD_3OD$) (300 MHz) 317
Spectrum 11b: $^{13}$C NMR spectrum of compound XI ($CD_3OD$) (75 MHz) 318
Spectrum 11c: ADEPT spectrum of compound XI 319
Spectrum 11d: HETCOR spectrum of compound XI 320
Spectrum 11e: COSY spectrum of compound XI 321

***N*-(2'-methylprpoyl)-nona-2*E*,4*E*-dien-8-ynamide**

Spectrum 12a: $^{1}$H NMR spectrum of compound XII ($CD_3OD$) (300 MHz) 322
Spectrum 12b: $^{13}$C NMR spectrum of compound XII ($CD_3OD$) (75 MHz) 323
Spectrum 12c: ADEPT spectrum of compound XII 324
Spectrum 12d: HETCOR spectrum of compound XII 325
Spectrum 12e: COSY spectrum of compound XII 326

**3β-hydroxy-12-oleanen-28-oic acid**

Spectrum 13a: $^{1}$H NMR spectrum of compound XIII ($CDCl_3$) (400 MHz) 327
Spectrum 13b: $^{13}$C NMR spectrum of compound XIII ($CDCl_3$) (100 MHz) 328
Spectrum 13c: HSQC spectrum of compound XIII 329
Spectrum 13d: COSY spectrum of compound XIII 330
Spectrum 13e: HMBC spectrum of compound XIII 331
Spectrum 13f: NOESY spectrum of compound XIII 332
Spectrum 13g: Mass spectrum of compound XIII 333
Spectrum 13h: Infra-red spectrum of compound XIII 334
Spectrum 13i: $^{1}$H NMR spectrum of oxidised compound XIII ($CDCl_3$) (400 MHz) 335
Spectrum 13j: $^{13}$C NMR spectrum of oxidised compound XIII ($CDCl_3$) (100 MHz) 336
Spectrum 13k: HSQC spectrum of oxidised compound XIII 337
Spectrum 13l: COSY spectrum of oxidised compound XIII 338
Spectrum 13m: HMBC spectrum of oxidised compound XIII 339
Spectrum 13n: NOESY spectrum of oxidised compound XIII 340

**7-hydroxy-6-methoxy-2*H*-1-benzopyran-2-one**

Spectrum 14a: $^{1}$H NMR spectrum of compound XIV ($CDCl_3$) (400 MHz) 341
Spectrum 14b: $^{13}$C NMR spectrum of compound XIV ($CDCl_3$) (100 MHz) 342
Spectrum 14c: HSQC spectrum of compound XIV 343
Spectrum 14d: COSY spectrum of compound XIV 344
Spectrum 14e: HMBC spectrum of compound XIV 345
Spectrum 14f: NOESY spectrum of compound XIV 346
Spectrum 14g: Mass spectrum of compound XIV 347
Spectrum 14h: Infra-red spectrum of compound XVI 348

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**6,7-dimethoxy-2*H*-1-benzopyran-2-one**

Spectrum 15a: $^{1}$H NMR spectrum of compound XV ($CDCl_3$) (400 MHz) 349
Spectrum 15b: $^{13}$C NMR spectrum of compound XV ($CDCl_3$) (100 MHz) 350
Spectrum 15c: HSQC spectrum of compound XV 351
Spectrum 15d: COSY spectrum of compound XV 352
Spectrum 15e: HMBC spectrum of compound XV 353
Spectrum 15f: NOESY spectrum of compound XV 354
Spectrum 15g: Mass spectrum of compound XV 355
Spectrum 15h: Infra-red spectrum of compound XV 356

**angelone**

Spectrum 16a: $^{1}$H NMR spectrum of compound XVI ($CDCl_3$) (400 MHz) 357
Spectrum 16b: $^{13}$C NMR spectrum of compound XVI ($CDCl_3$) (100 MHz) 358
Spectrum 16c: HSQC spectrum of compound XVI 359
Spectrum 16d: COSY spectrum of compound XVI 360
Spectrum 16e: HMBC spectrum of compound XVI 361
Spectrum 16f: NOESY spectrum of compound XVI 362
Spectrum 16g: Mass spectrum of compound XVI 363
Spectrum 16h: Infra-red spectrum of compound XVI 364

**6,7-dihydroxy-2*H*-1-benzopyran-2-one**

Spectrum 17a: $^{1}$H NMR spectrum of compound XVII ($CD_3OD$) (400 MHz) 365
Spectrum 17b: $^{13}$C NMR spectrum of compound XVII ($CD_3OD$) (100 MHz) 366
Spectrum 17c: HSQC spectrum of compound XVII 367
Spectrum 17d: COSY spectrum of compound XVII 368
Spectrum 17e: HMBC spectrum of compound XVII 369
Spectrum 17f: NOESY spectrum of compound XVII 370
Spectrum 17g: Mass spectrum of compound XVII 371
Spectrum 17h: Infra-red spectrum of compound XVII 372

**6,7-dimethoxy-2*H*-1-benzopyran-2-one**

Spectrum 18a: $^{1}$H NMR spectrum of compound XVIII ($CDCl_3$) (400 MHz) 373
Spectrum 18b: $^{13}$C NMR spectrum of compound XVIII ($CDCl_3$) (100 MHz) 374
Spectrum 18c: HSQC spectrum of compound XVIII 375
Spectrum 18d: COSY spectrum of compound XVIII 376
Spectrum 18e: HMBC spectrum of compound XVIII 377
Spectrum 18f: NOESY spectrum of compound XVIII 378

**6-hydroxy-7-methoxy-2*H*-1-benzopyran-2-one**

Spectrum 19a: $^{1}$H NMR spectrum of compound XIX ($CDCl_3$) (400 MHz) 379
Spectrum 19b: $^{13}$C NMR spectrum of compound XIX ($CDCl_3$) (100 MHz) 380
Spectrum 19c: HSQC spectrum of compound XIX 381
Spectrum 19d: COSY spectrum of compound XIX 382
Spectrum 19e: HMBC spectrum of compound XIX 383
Spectrum 19f: NOESY spectrum of compound XIX 384
Spectrum 19g: Mass spectrum of compound XIX 385
Spectrum 19h: Infra-red spectrum of compound XIX 386

**1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)prop-2-en-1-one**

Spectrum 20a: $^{1}$H NMR spectrum of compound XX ($CDCl_3$) (400 MHz) 387
Spectrum 20b: $^{13}$C NMR spectrum of compound XX ($CDCl_3$) (100 MHz) 388
Spectrum 20c: HSQC spectrum of compound XX 389
Spectrum 20d: COSY spectrum of compound XX 390
Spectrum 20e: HMBC spectrum of compound XX 391
Spectrum 20f: NOESY spectrum of compound XX 392
Spectrum 20g: Mass spectrum of compound XX 393
Spectrum 20h: Infra-red spectrum of compound XX 394

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**(+)-1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-one**

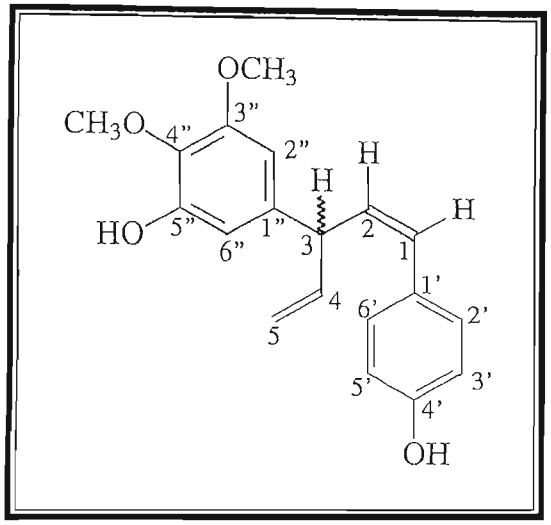
Spectrum 21a: $^{1}H$ NMR spectrum of compound XXI ($CDCl_3$) (400 MHz) 395
Spectrum 21b: $^{13}C$ NMR spectrum of compound XXI ($CDCl_3$) (100 MHz) 396
Spectrum 21c: HSQC spectrum of compound XXI 397
Spectrum 21d: COSY spectrum of compound XXI 398
Spectrum 21e: HMBC spectrum of compound XXI 399
Spectrum 21f: NOESY spectrum of compound XXI 400
Spectrum 21g: Mass spectrum of compound XXI 401

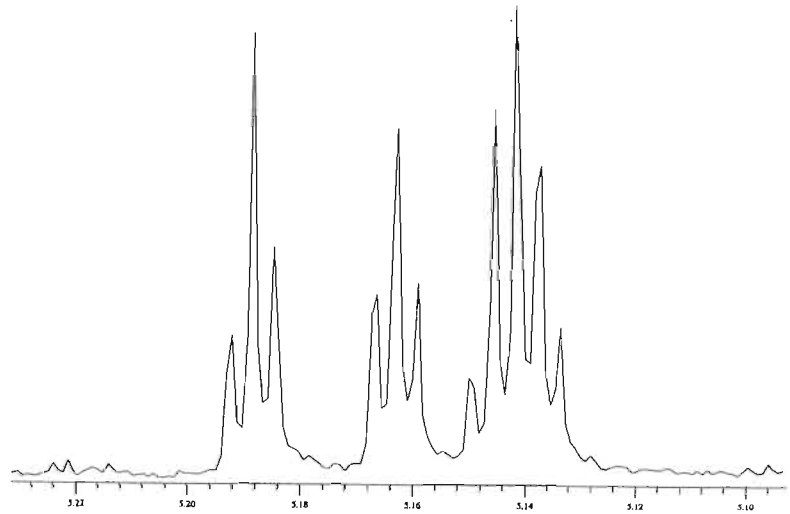
**1-(4'-hydroxyphenyl)-3-(3",4"-dimethoxyphenyl)pent-4-en-1-ol**

Spectrum 22a: $^{1}H$ NMR spectrum of compound XXII ($CDCl_3$) (400 MHz) 402
Spectrum 22b: $^{13}C$ NMR spectrum of compound XXII ($CDCl_3$) (100 MHz) 403

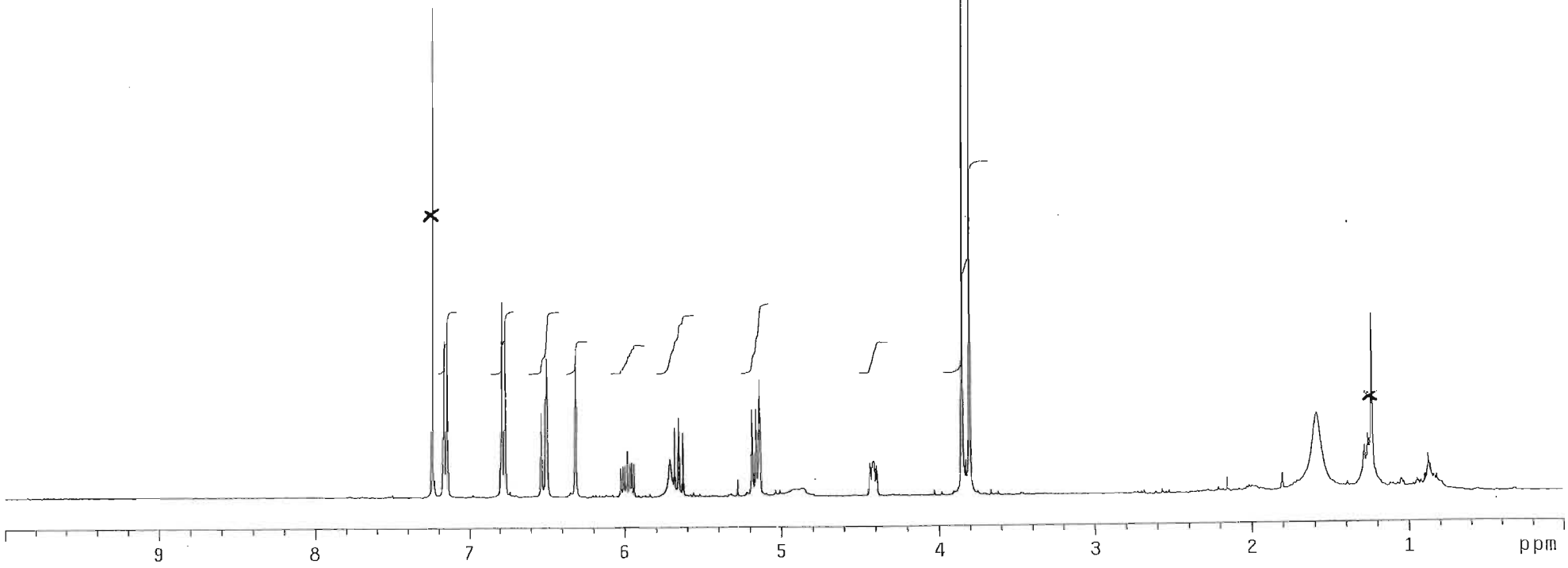
**(*E*)-3-vinyl-1-(4'-hydroxyphenyl)-3",4"-dimethoxyindene**

Spectrum 23a: $^{1}H$ NMR spectrum of compound XXIII ($CDCl_3$) (400 MHz) 404
Spectrum 23b: $^{13}C$ NMR spectrum of compound XXIII ($CDCl_3$) (100 MHz) 405
Spectrum 23c: ADEPT spectrum of compound XXIII 406
Spectrum 23d: HSQC spectrum of compound XXIII 407
Spectrum 23e: COSY spectrum of compound XXIII 408
Spectrum 23f: HMBC spectrum of compound XXIII 409
Spectrum 23g: NOESY spectrum of compound XXIII 410
Spectrum 23h: Mass spectrum of compound XXIII 411

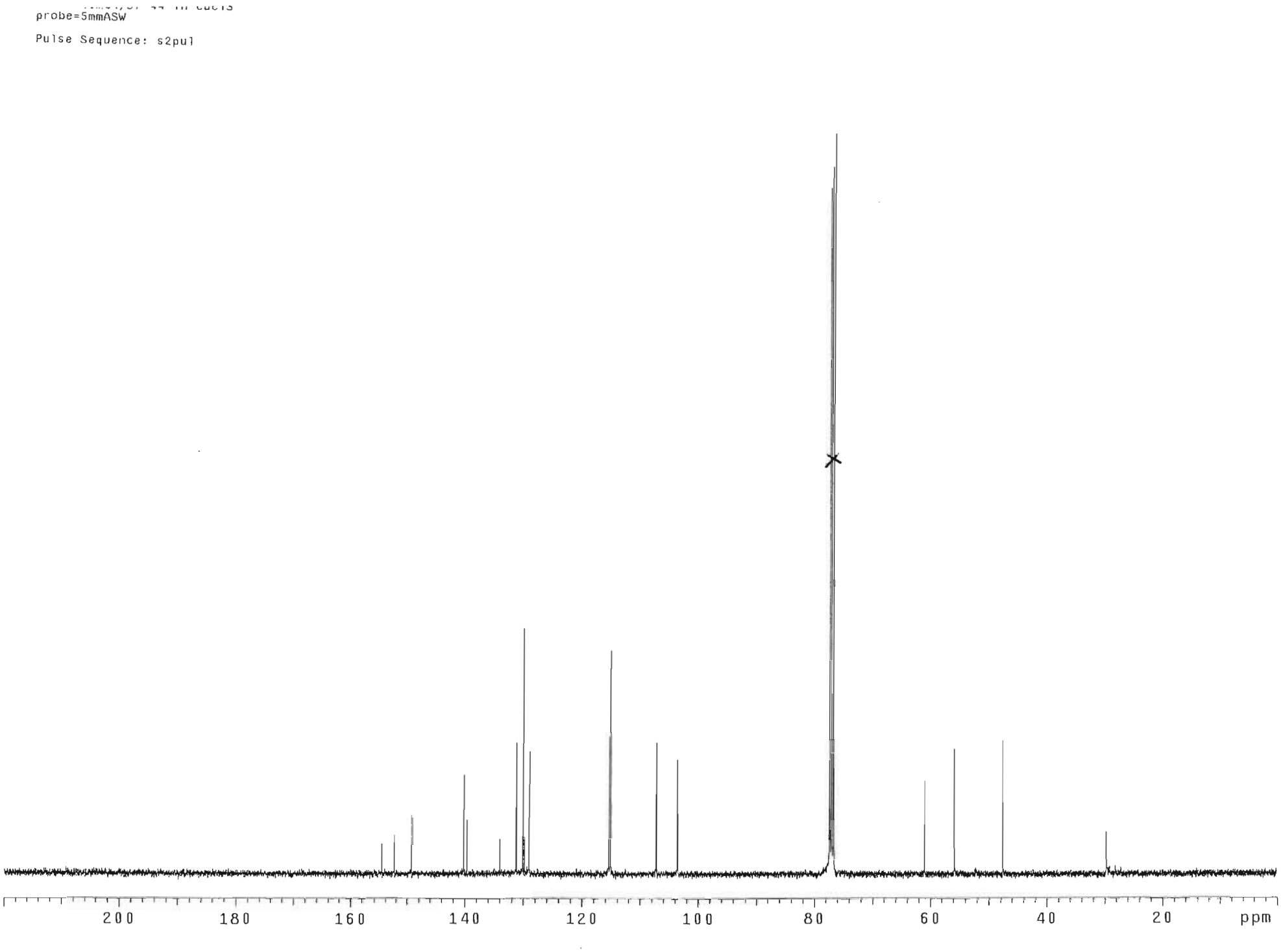


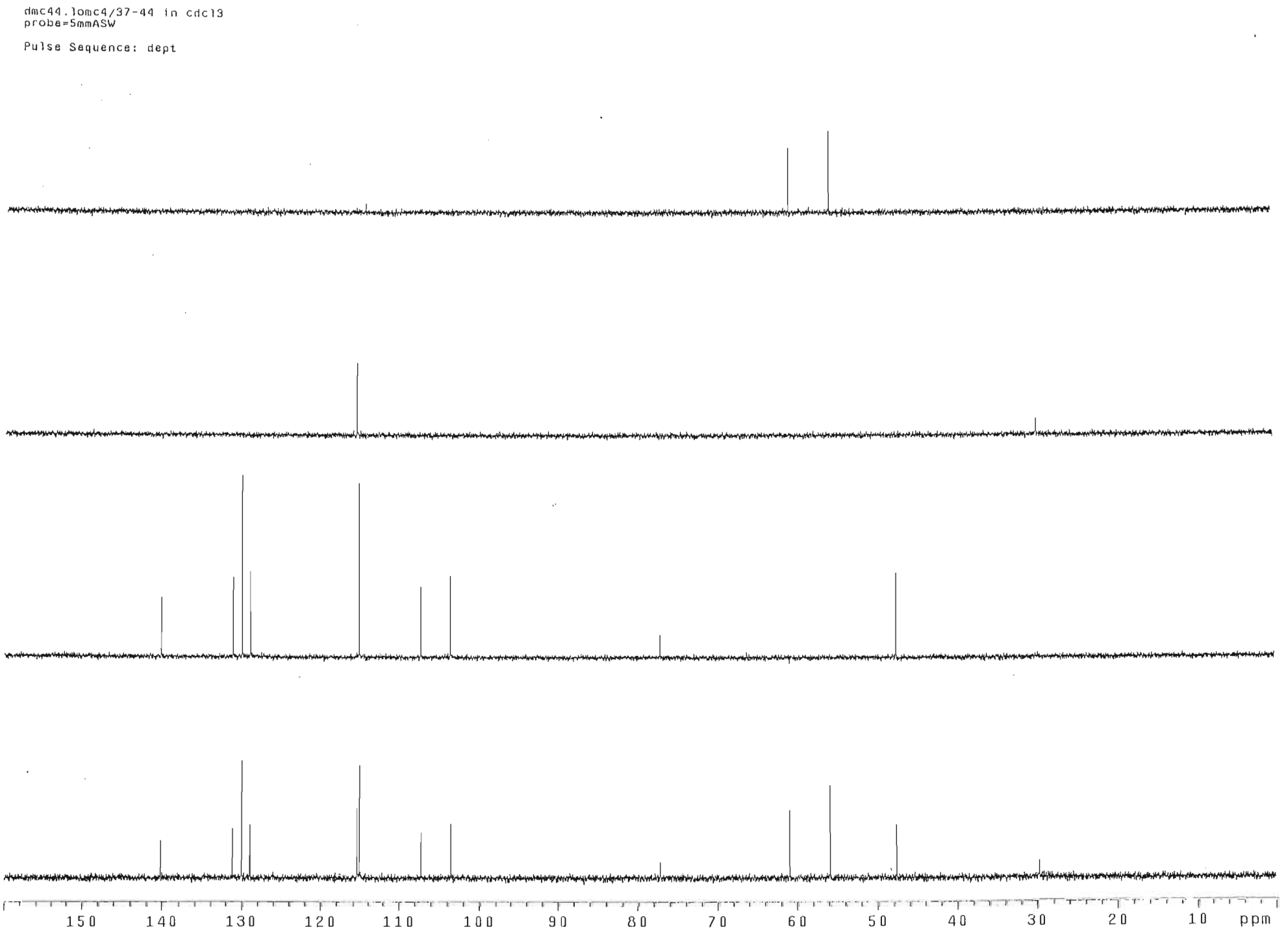




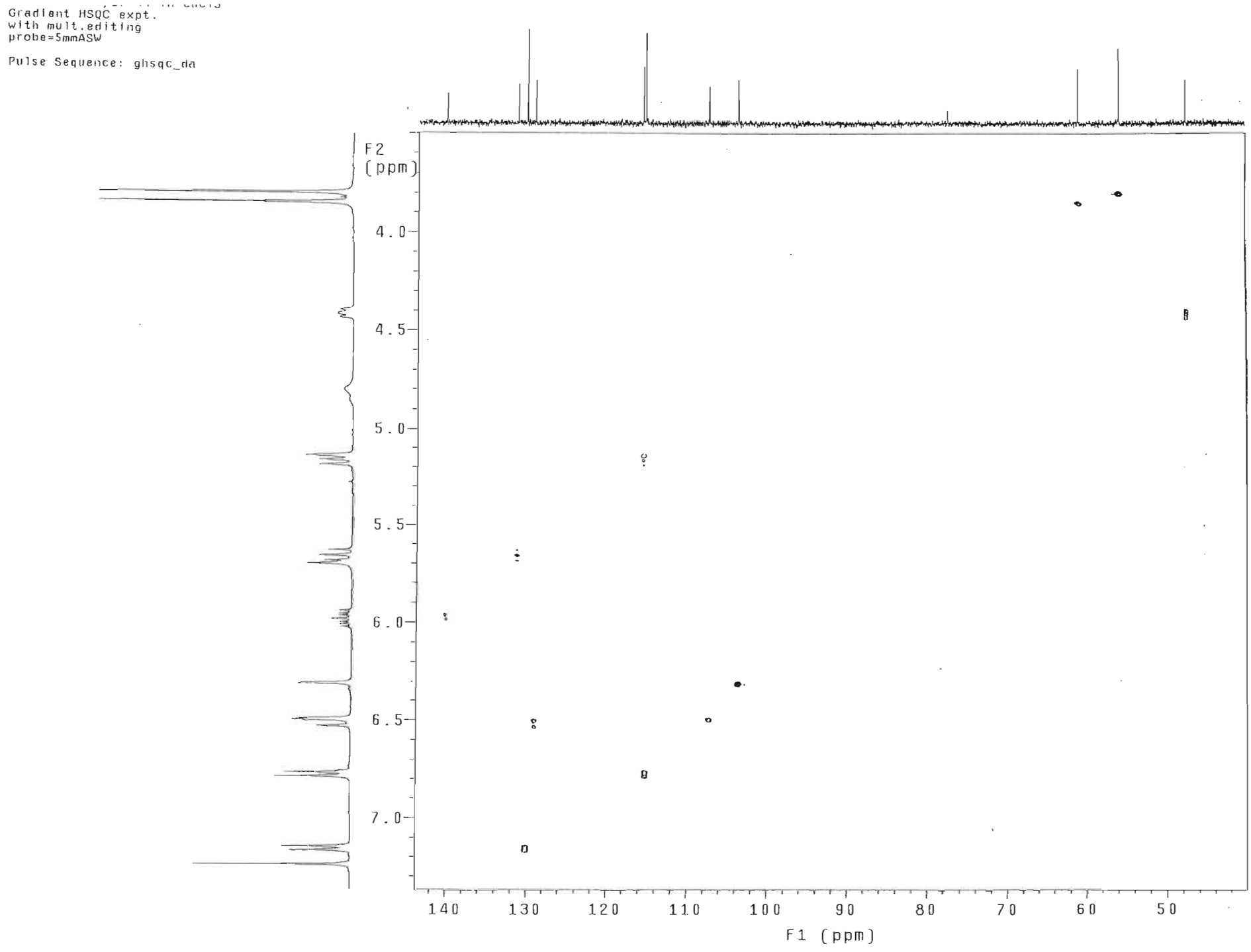

**Spectrum 1a: $^{1}$H NMR spectrum of compound I ($CDCl_3$) (400 MHz)**



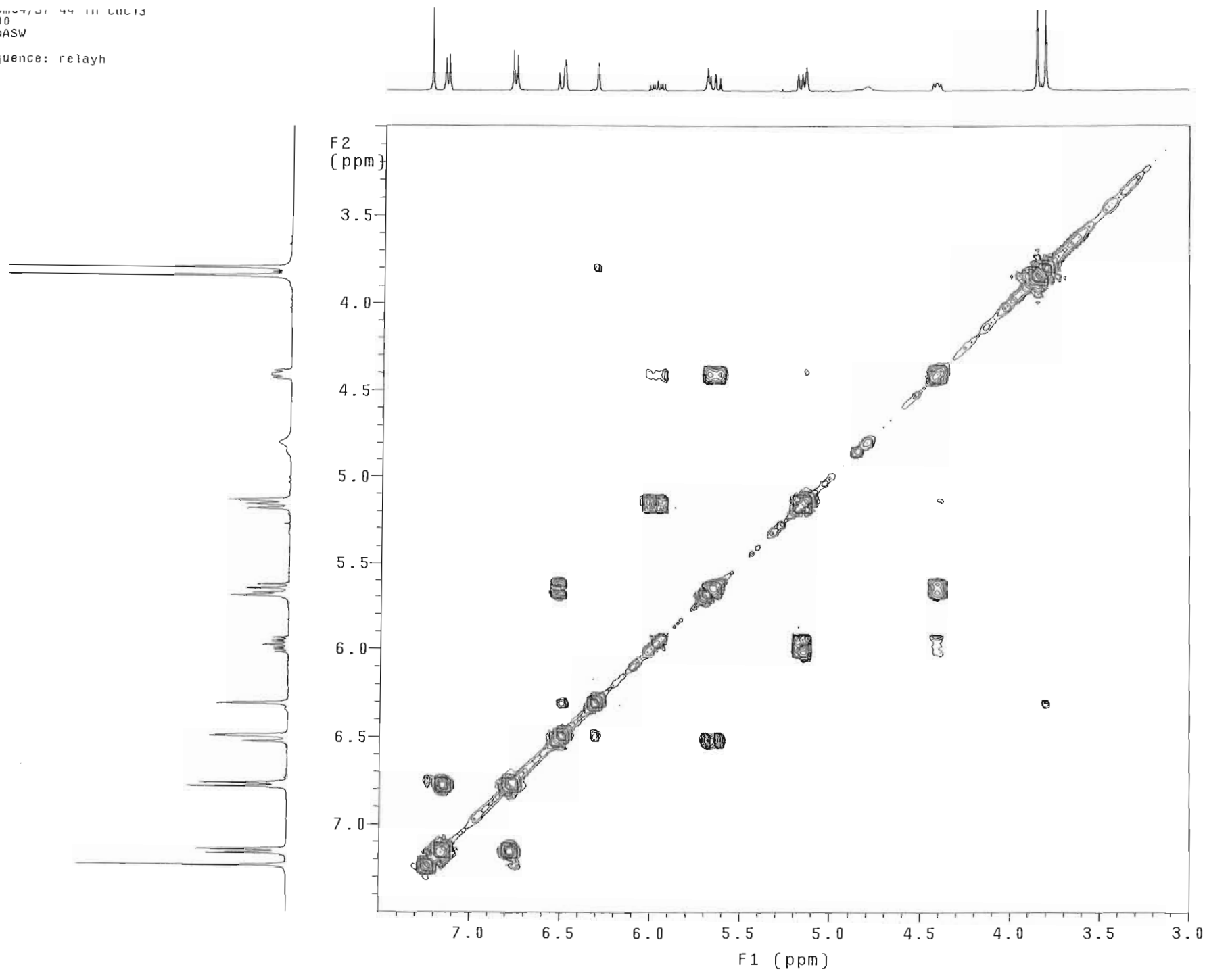

**Spectrum 1b: $^{13}C$ NMR spectrum of compound I ($CDCl_3$)(100 MHz)**



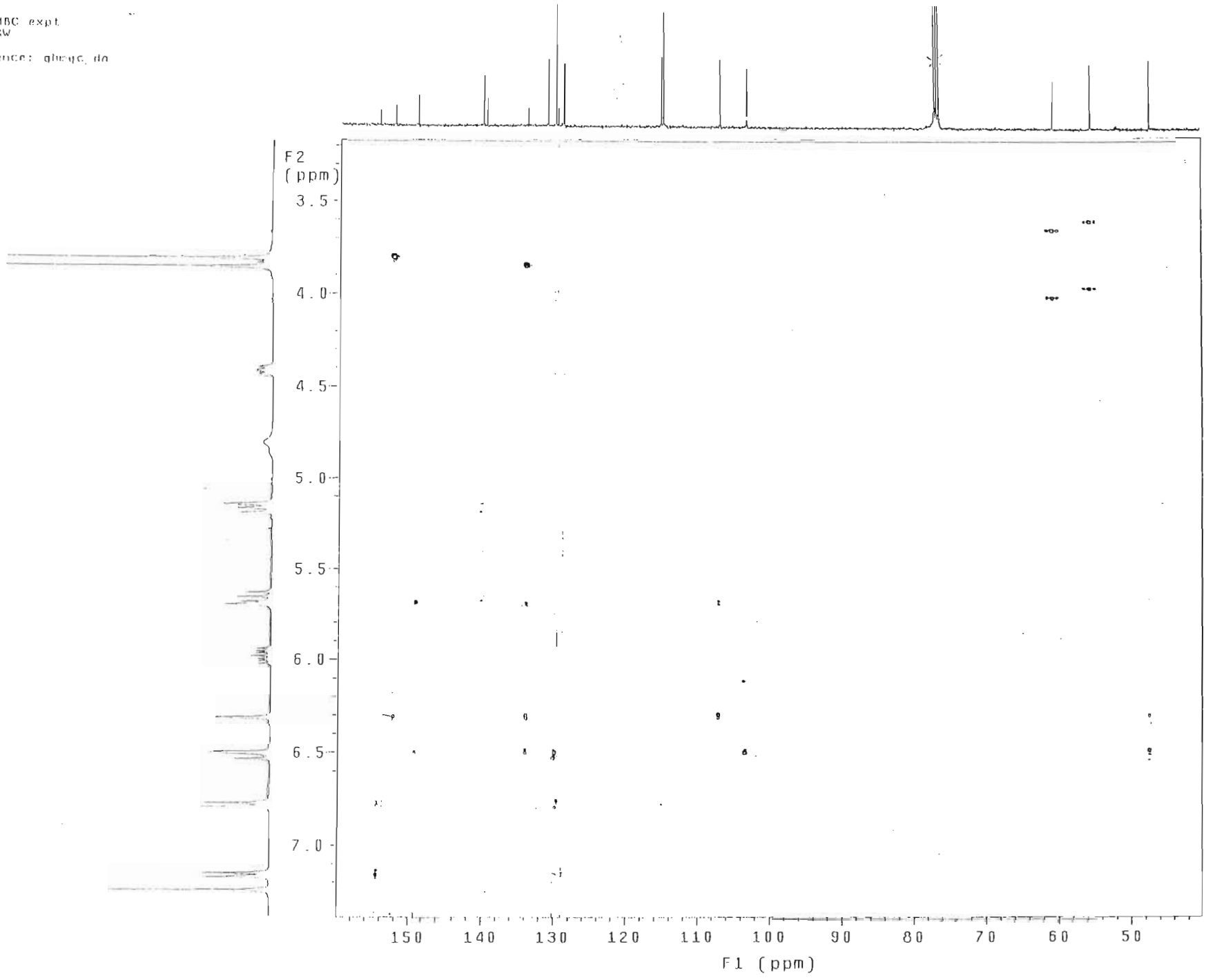

**Spectrum 1c: ADEPT spectrum of compound I**



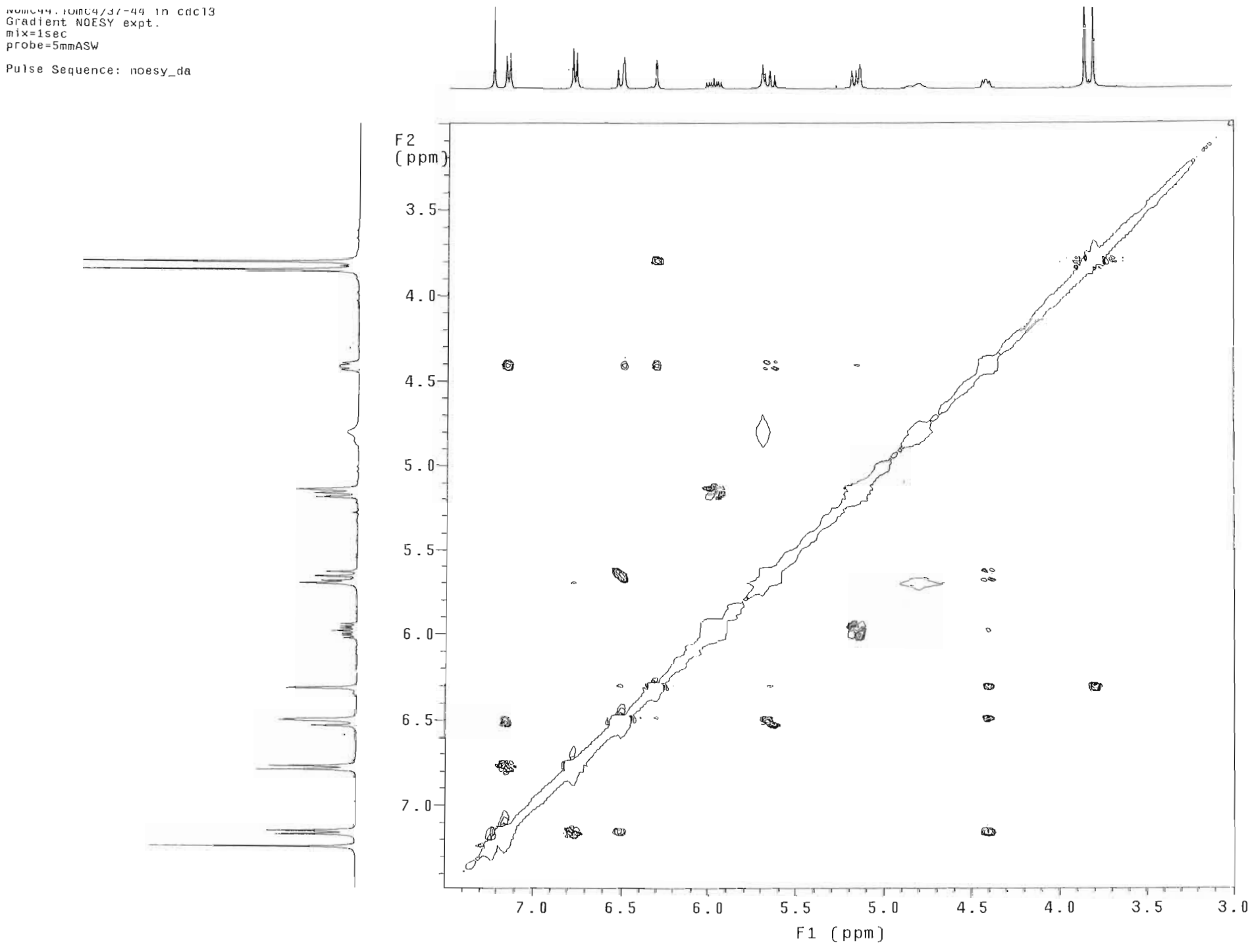

**Spectrum 1d: HSQC spectrum of compound I**



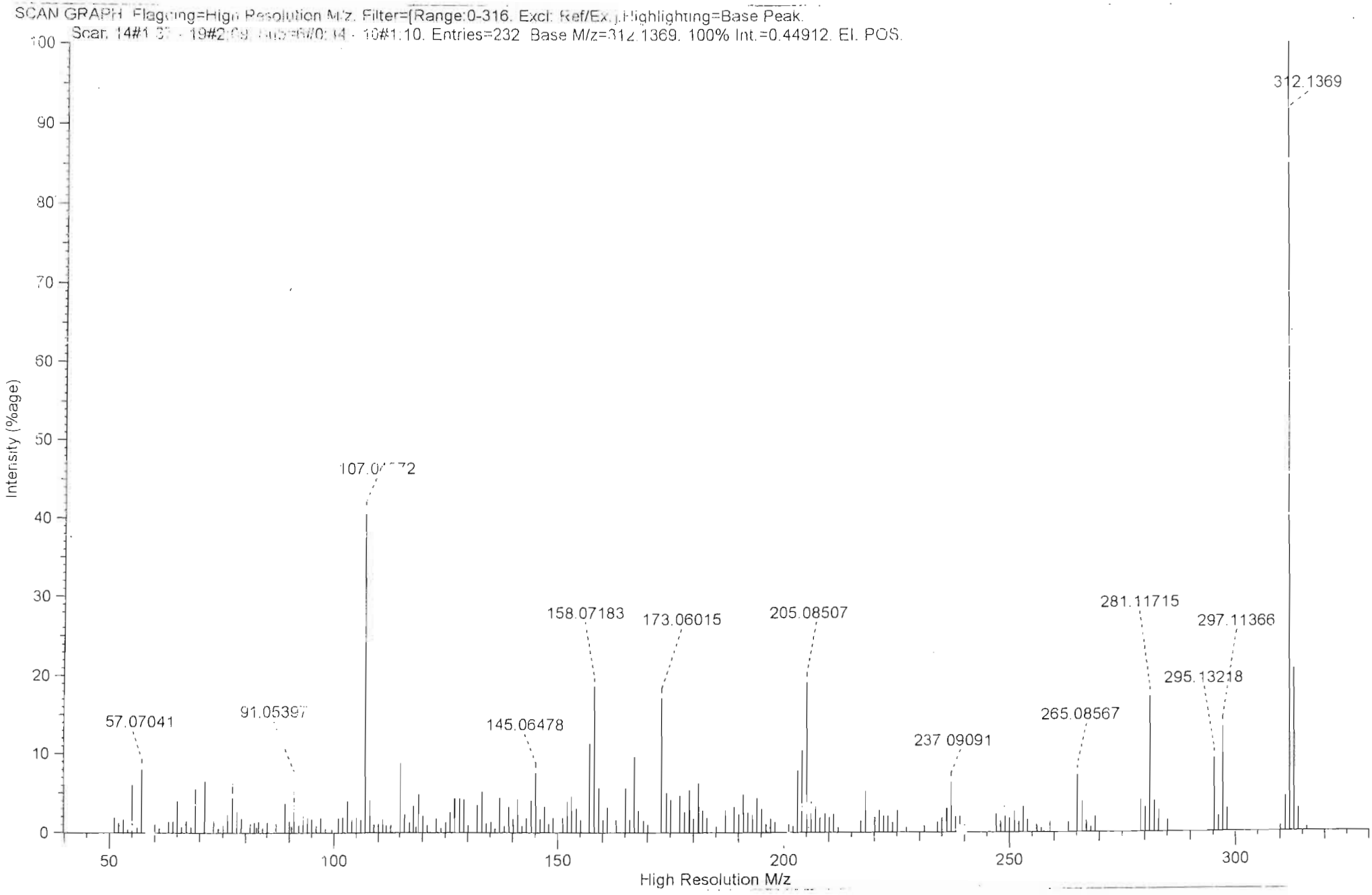

**Spectrum 1e: COSY spectrum of compound I**



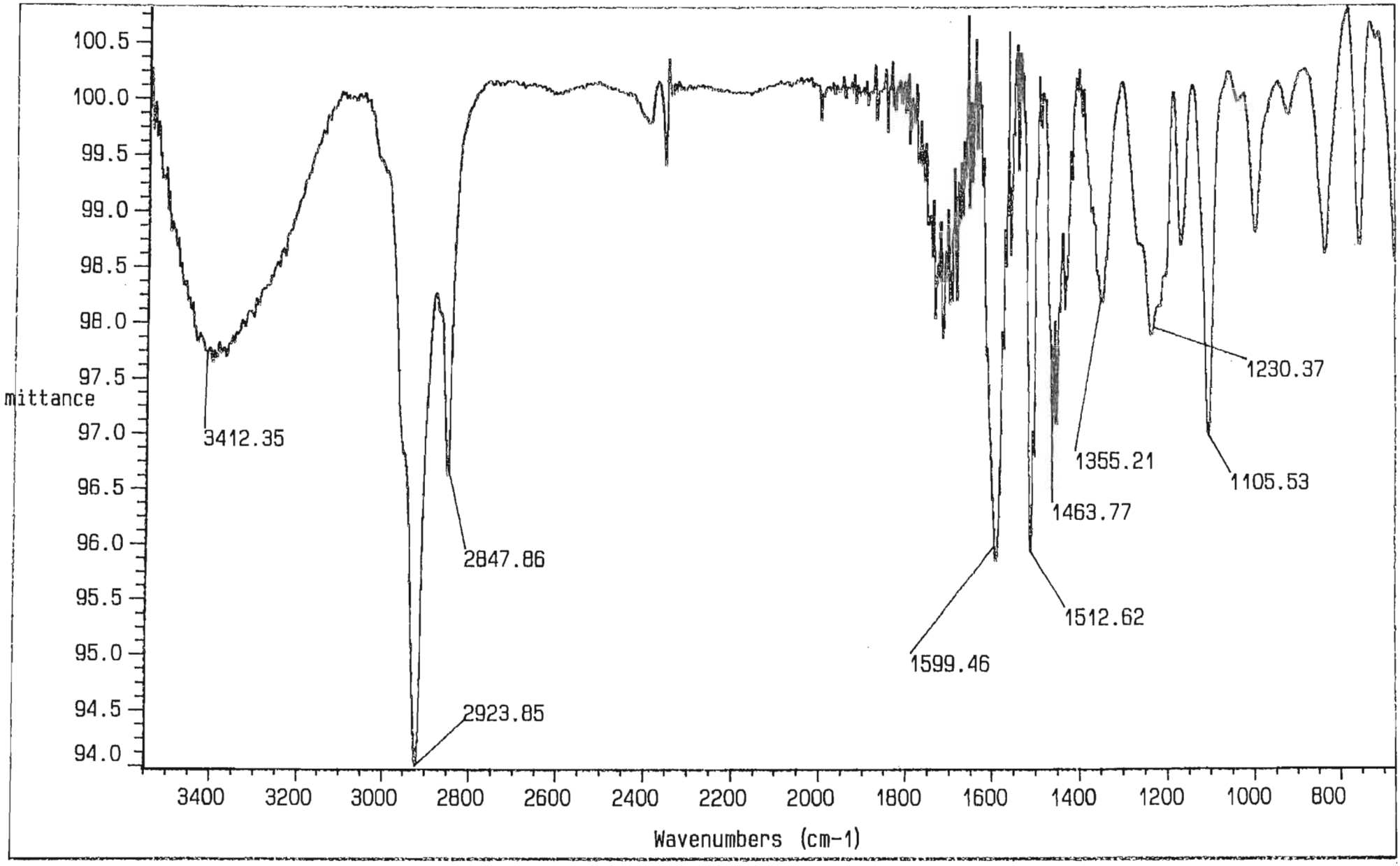

**Spectrum 1f: HMBC spectrum of compound I**




**Spectrum 1g: NOESY spectrum of compound I**




**Spectrum 1h: Mass spectrum of compound I**

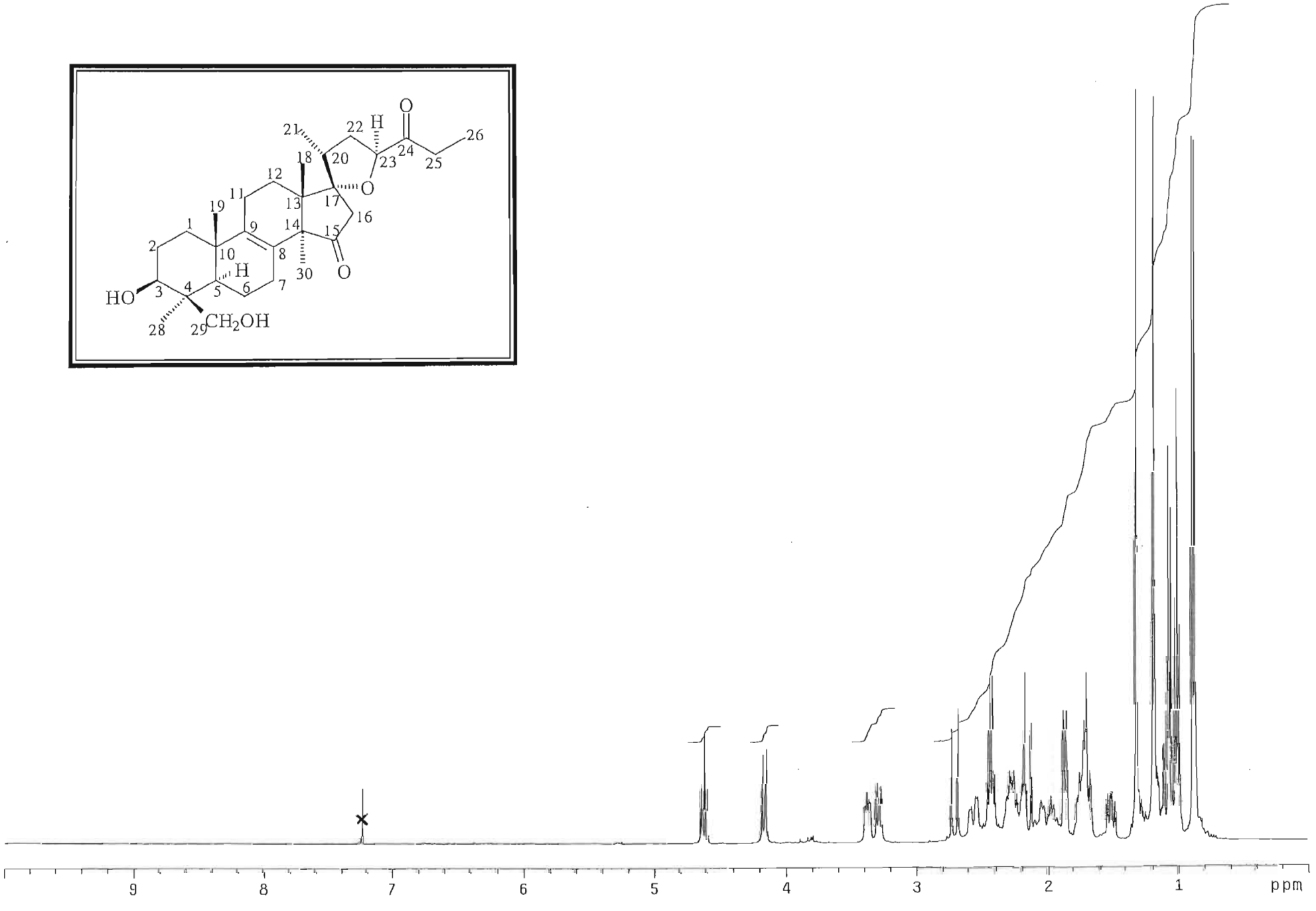



**Spectrum 1i: Infra-red spectrum of compound I**

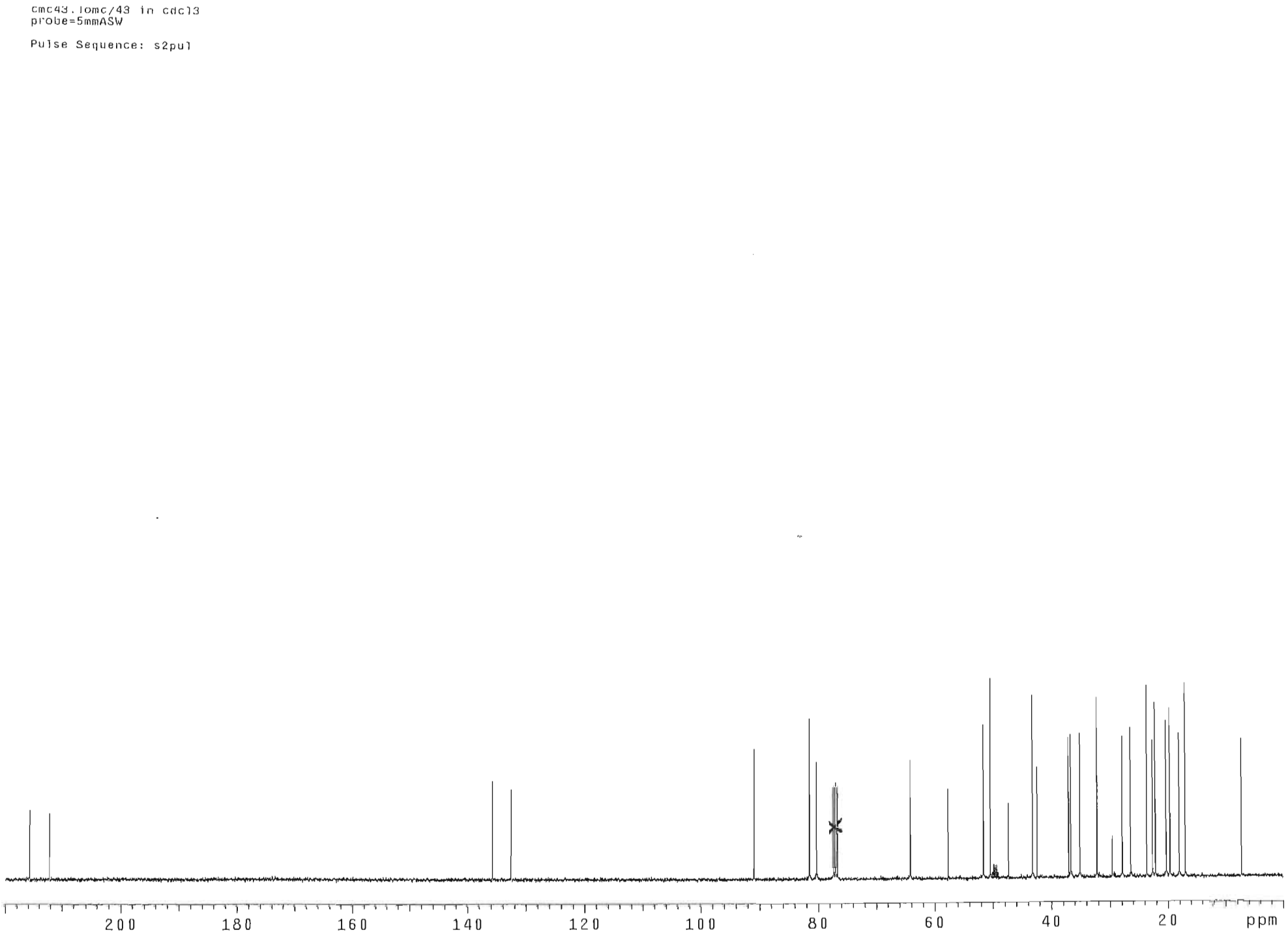
Pulse Sequence: s2pul



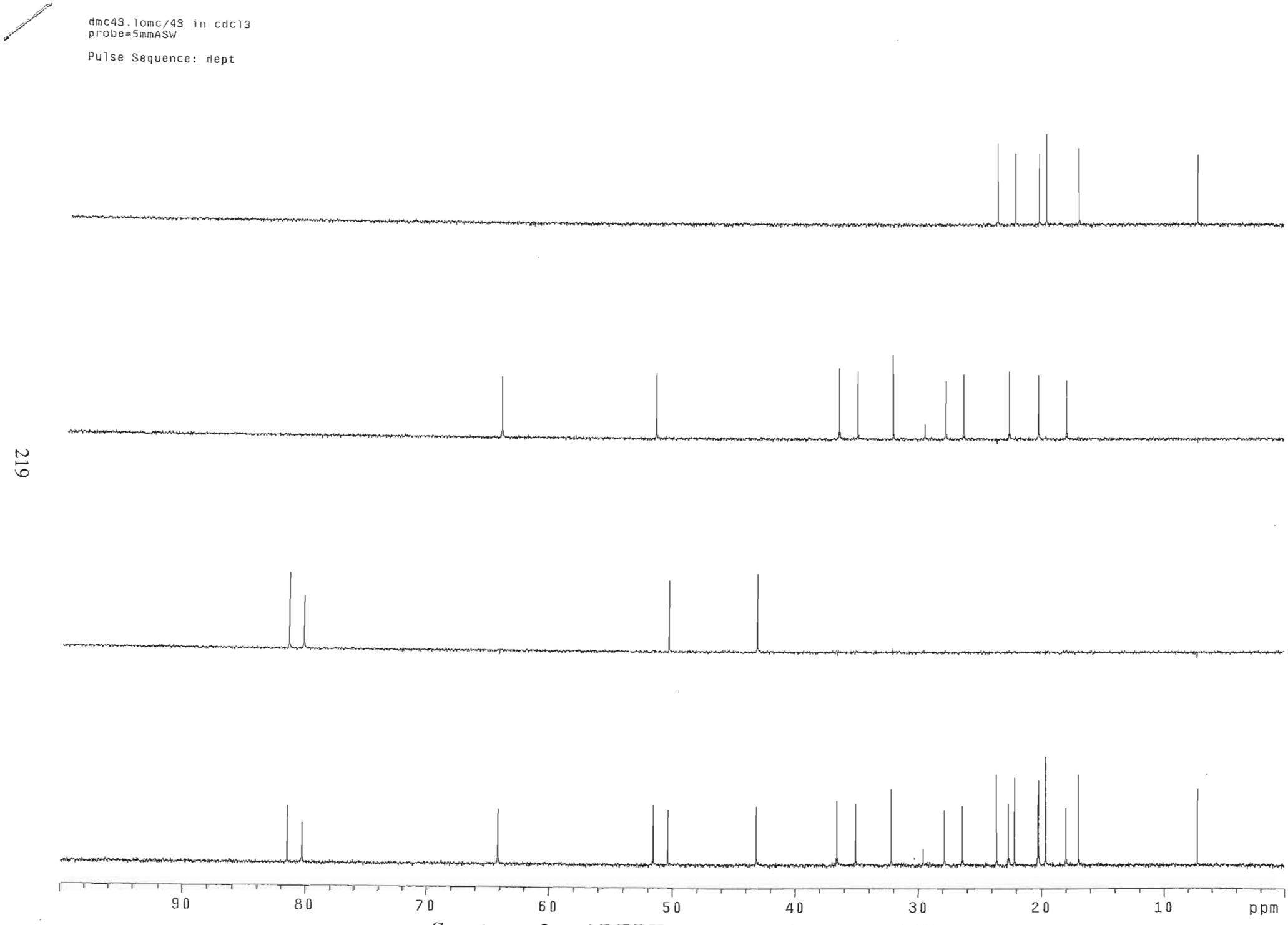


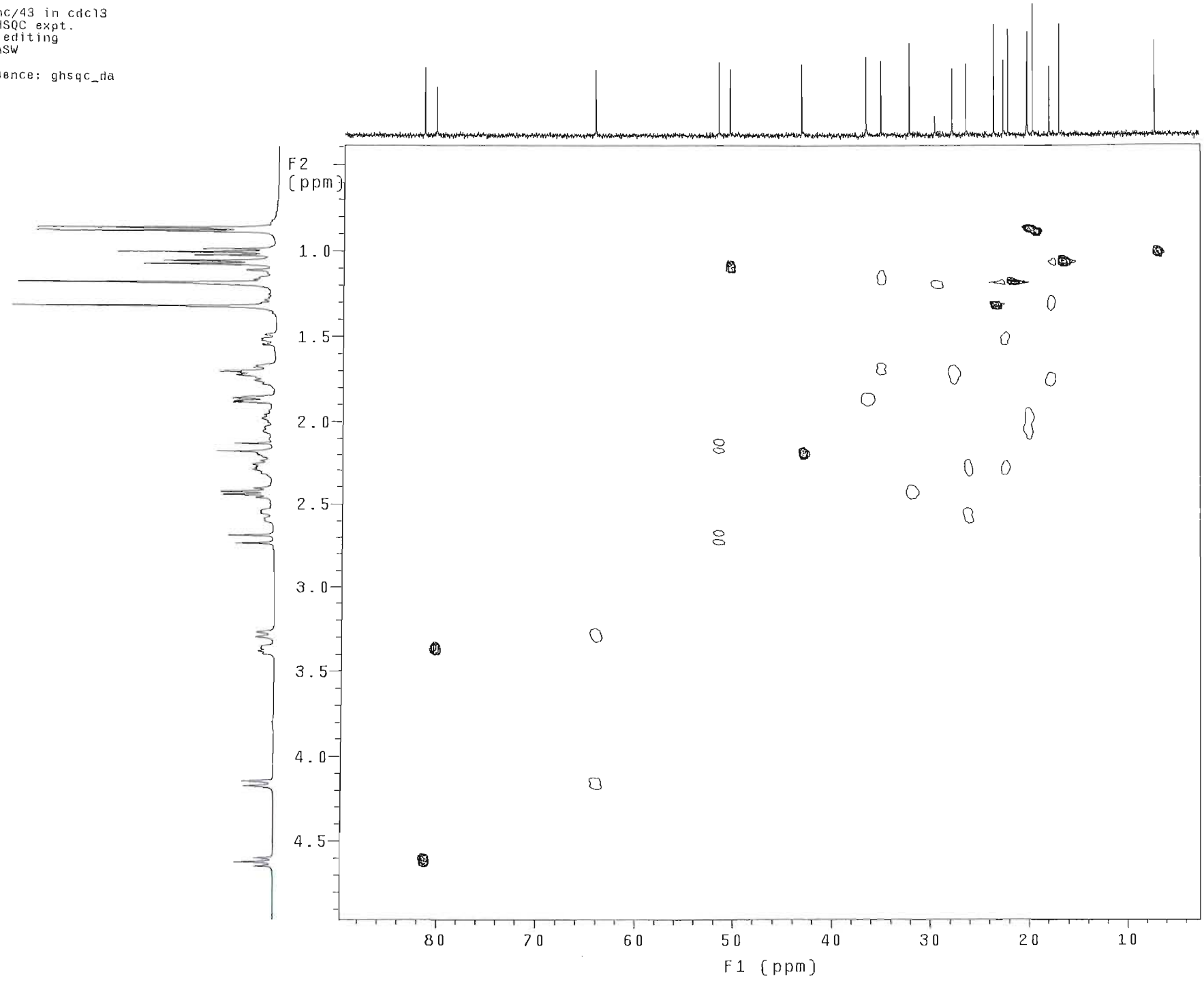

**Spectrum 2a: $^{1}H$ NMR spectrum of compound II ($CDCl_3$) (400 MHz)**



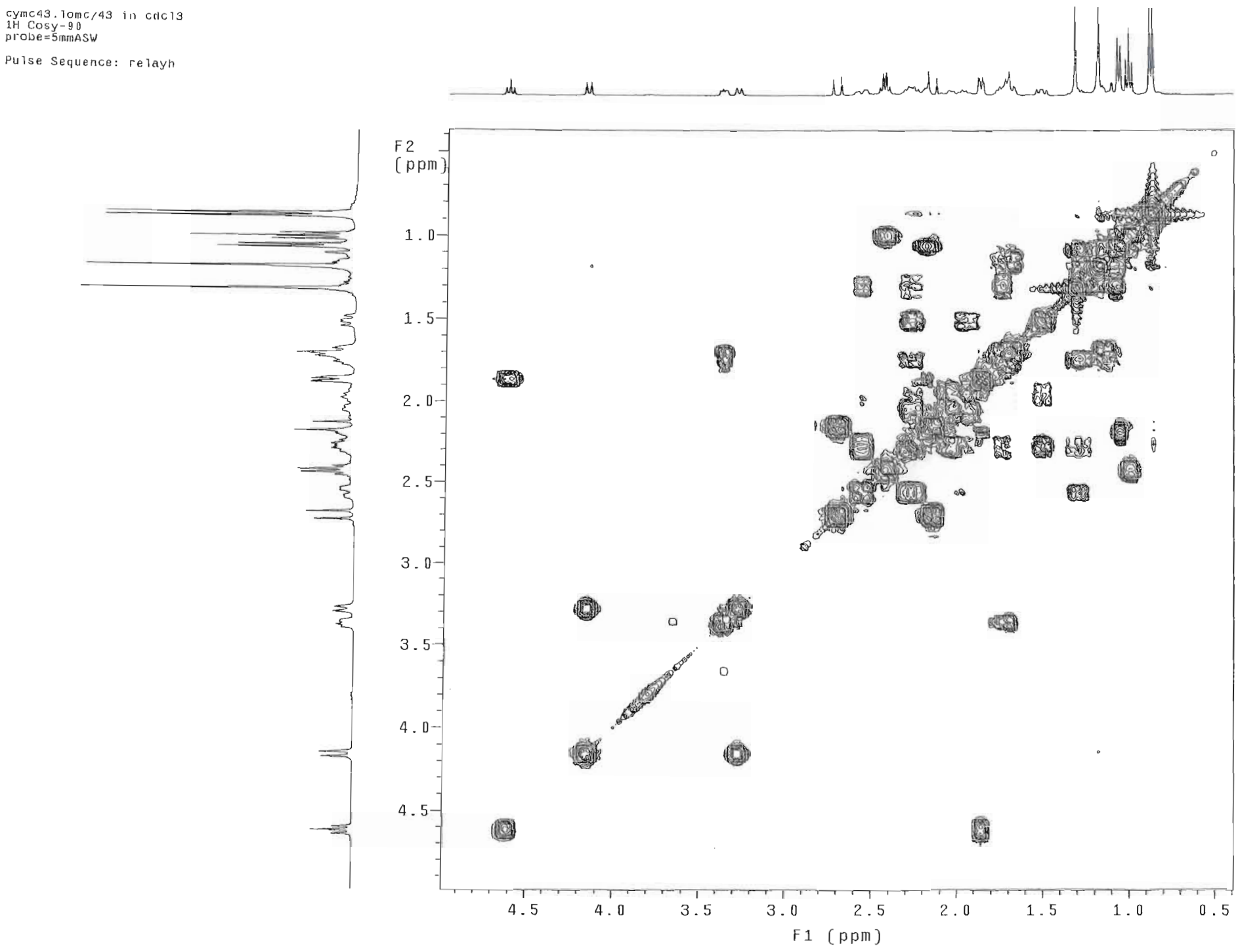

**Spectrum 2b: $^{13}C$ NMR spectrum of compound II ($CDCl_3$) (100 MHz)**



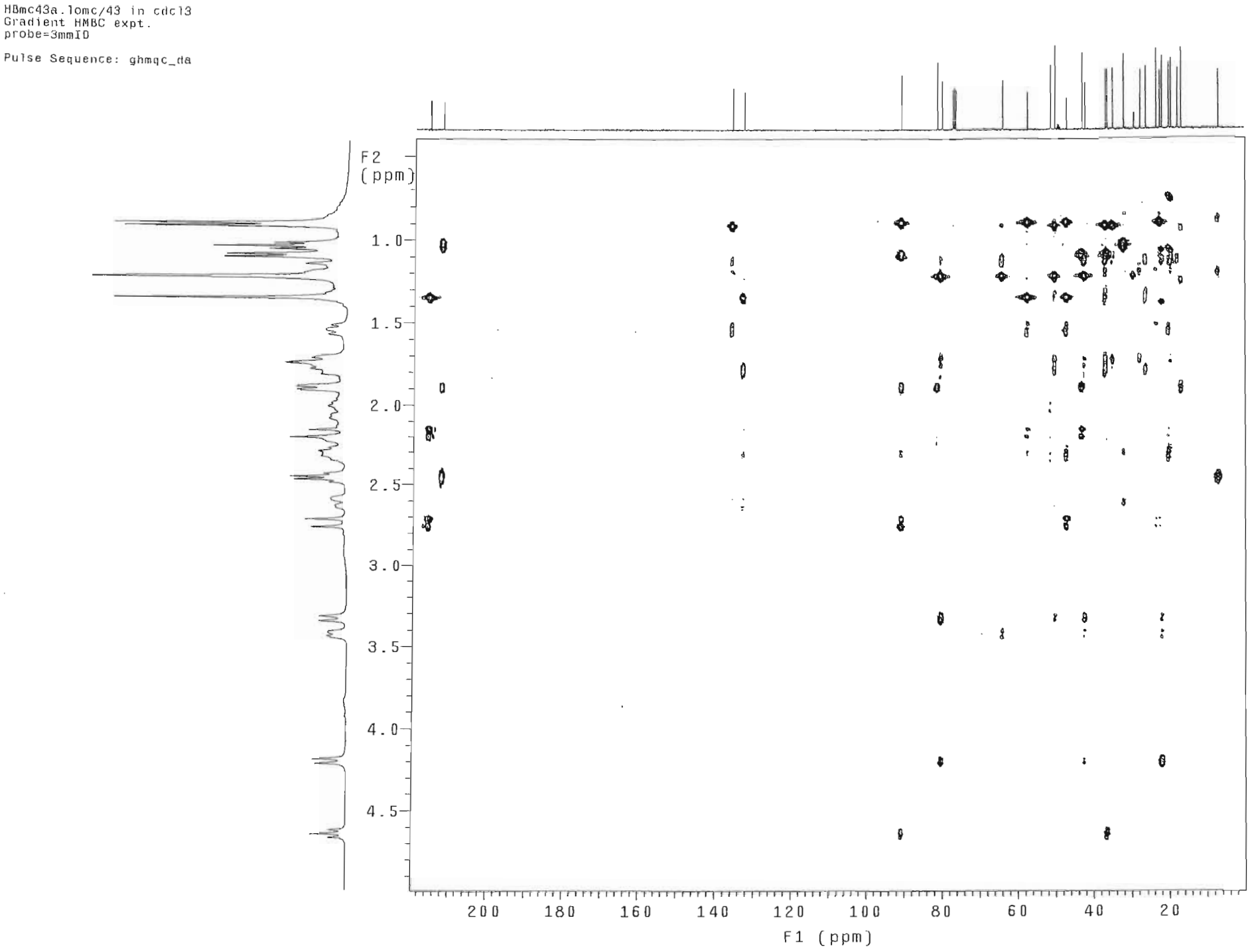

**Spectrum 2c: ADEPT spectrum of compound II**



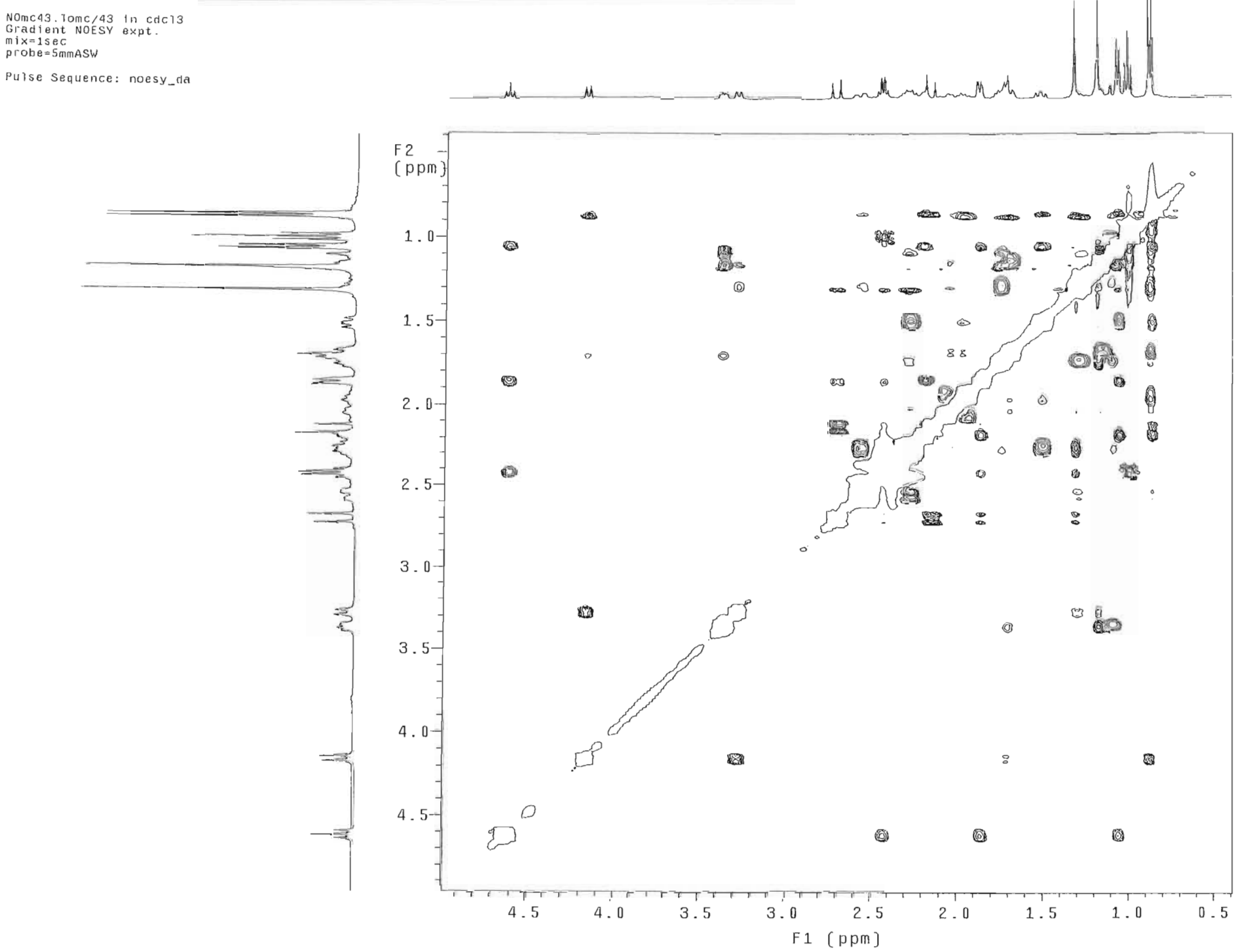

**Spectrum 2d: HSQC spectrum of compound II**



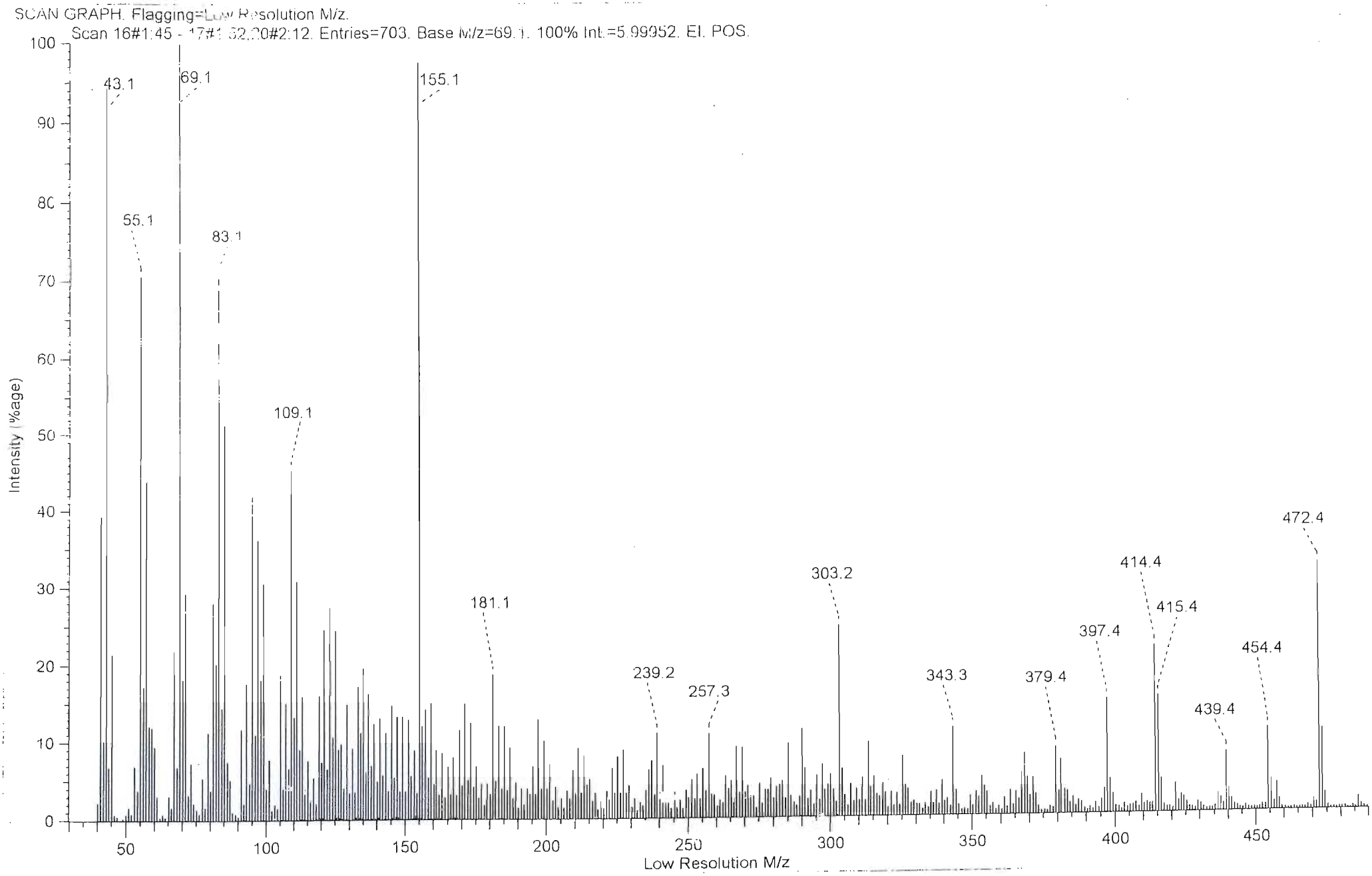

**Spectrum 2e: COSY spectrum of compound II**



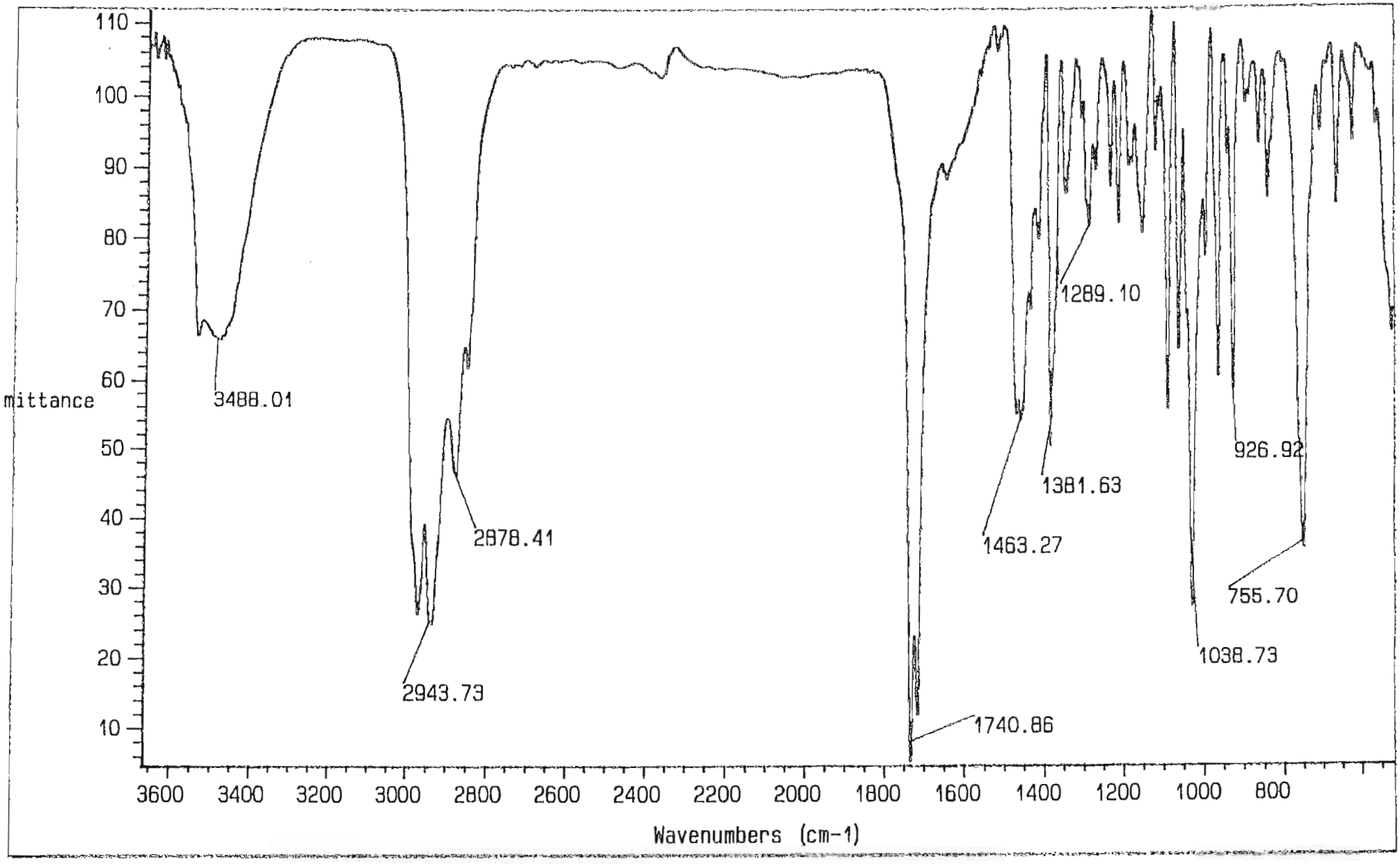

**Spectrum 2f: HMBC spectrum of compound II**




**Spectrum 2g: NOESY spectrum of compound II**

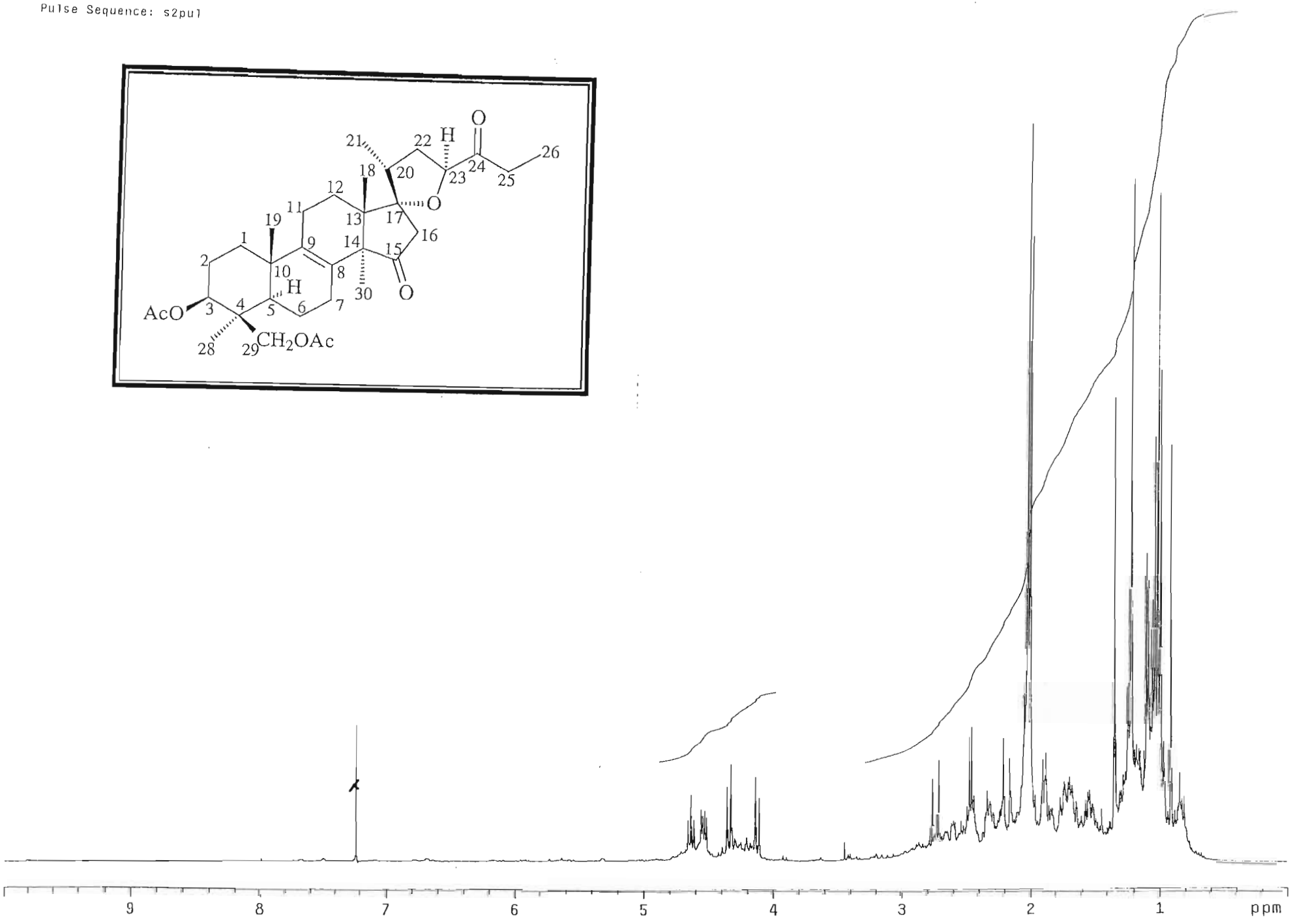



**Spectrum 2h: Mass spectrum of compound II**

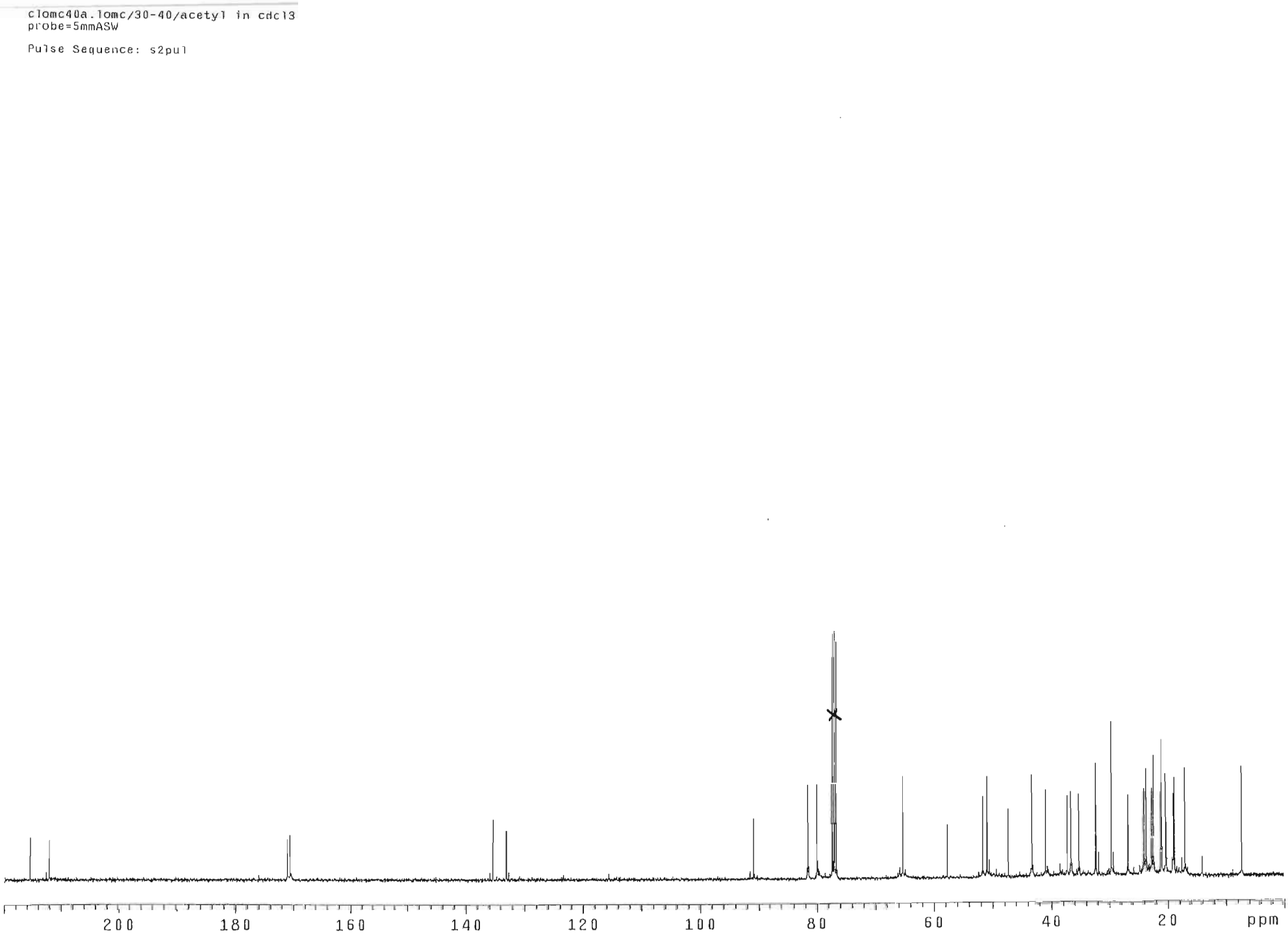



**Spectrum 2i: Infra-red spectrum of compound II**

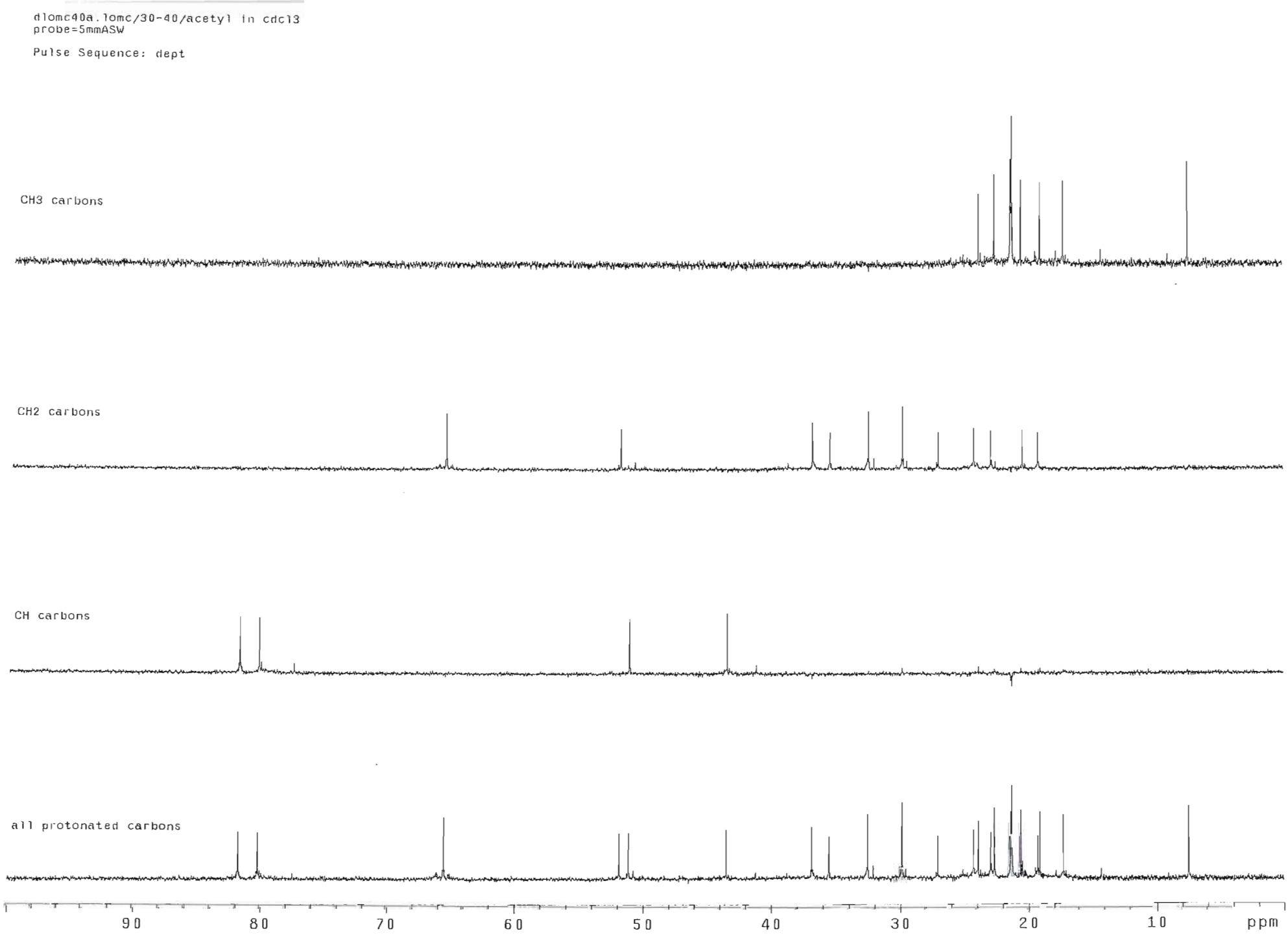
Pulse Sequence: s2pul




**Spectrum 2j: $^{1}H$ NMR spectrum of acetylated compound II ($CDCl_3$) (400 MHz)**



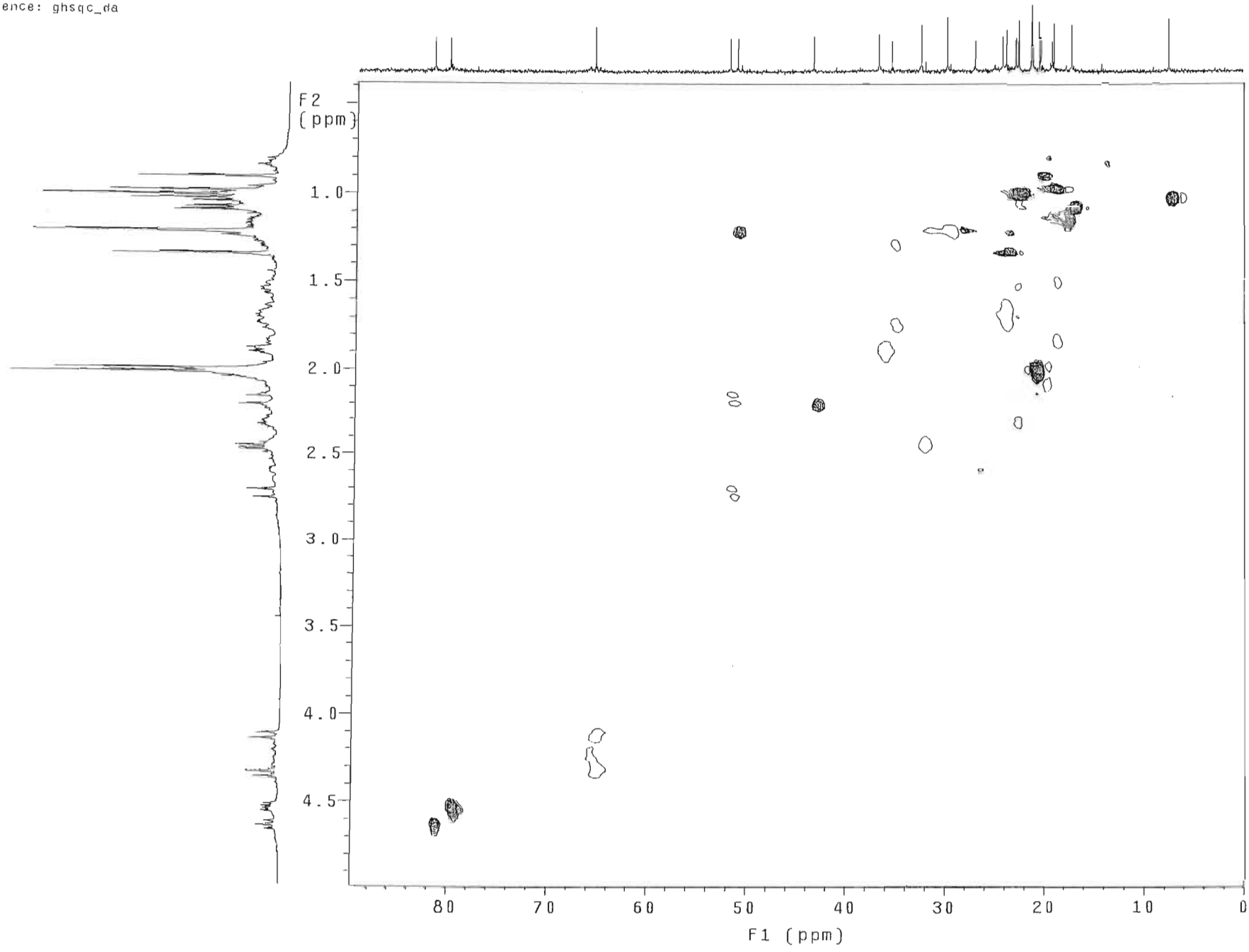

**Spectrum 2k: $^{13}C$ NMR spectrum of acetylated compound II ($CDCl_3$) (100 MHz)**



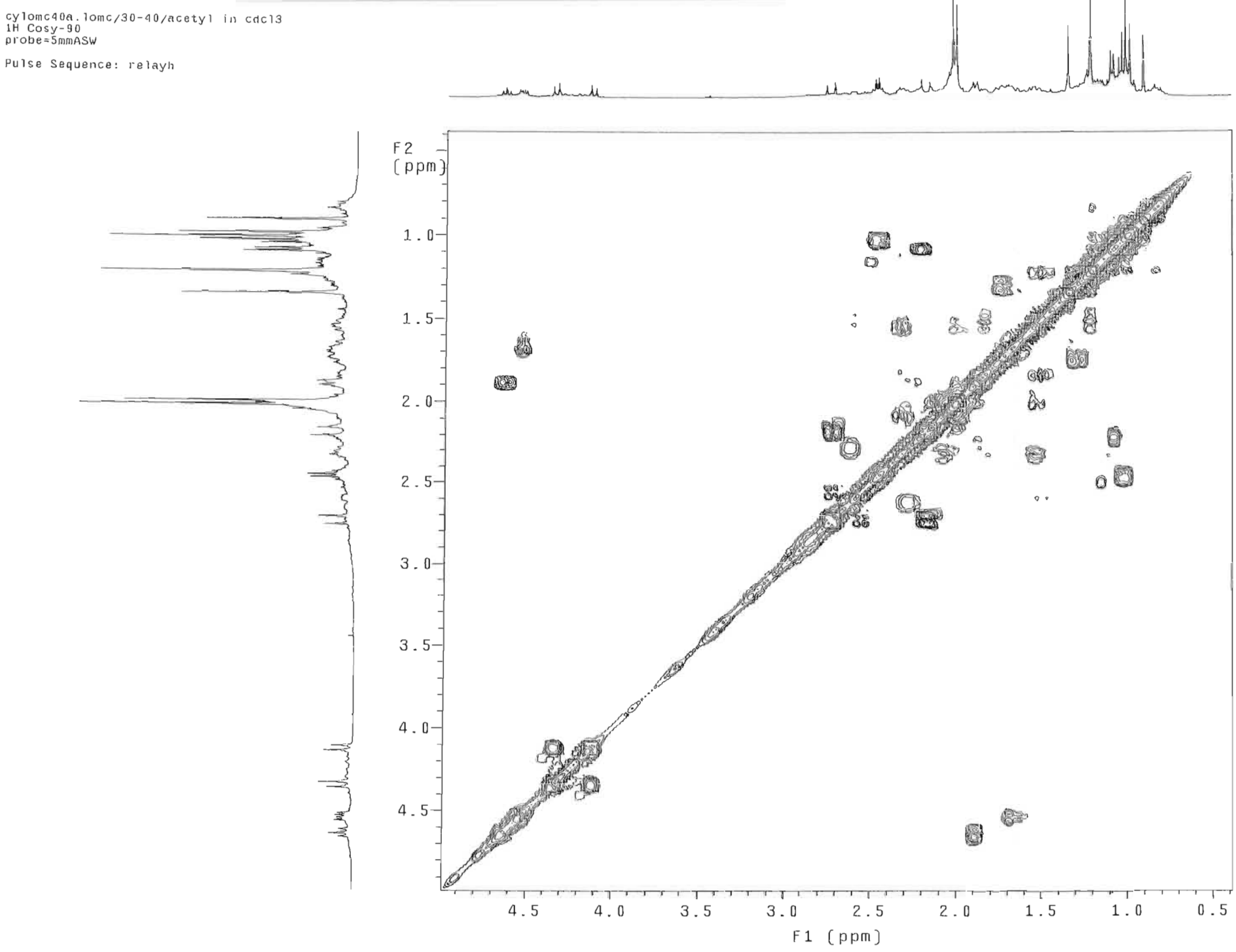

**Spectrum 2l: ADEPT spectrum of acetylated compound II**

HQ1omc40a.1omc/30-40/acetyl in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

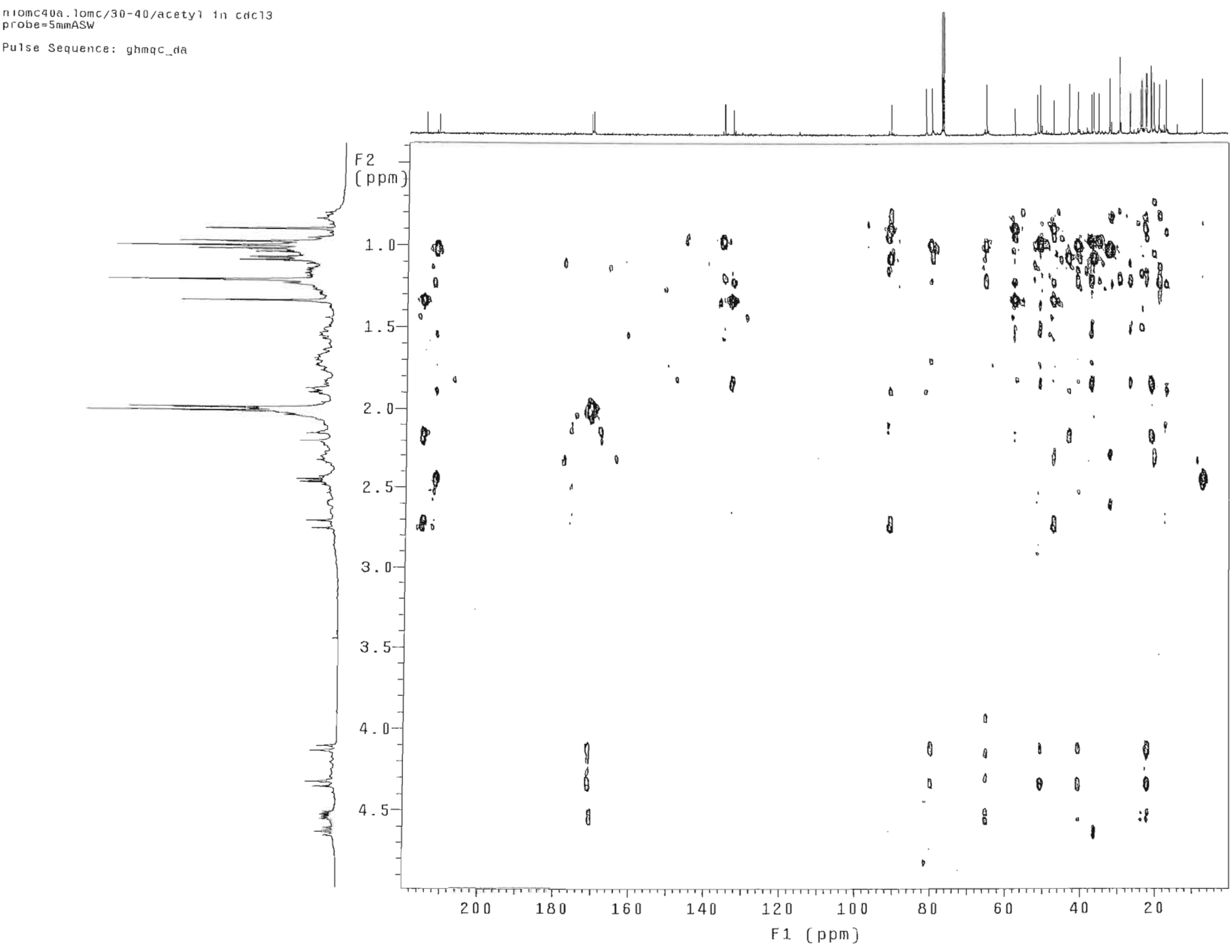
Pulse Sequence: ghsqc_da



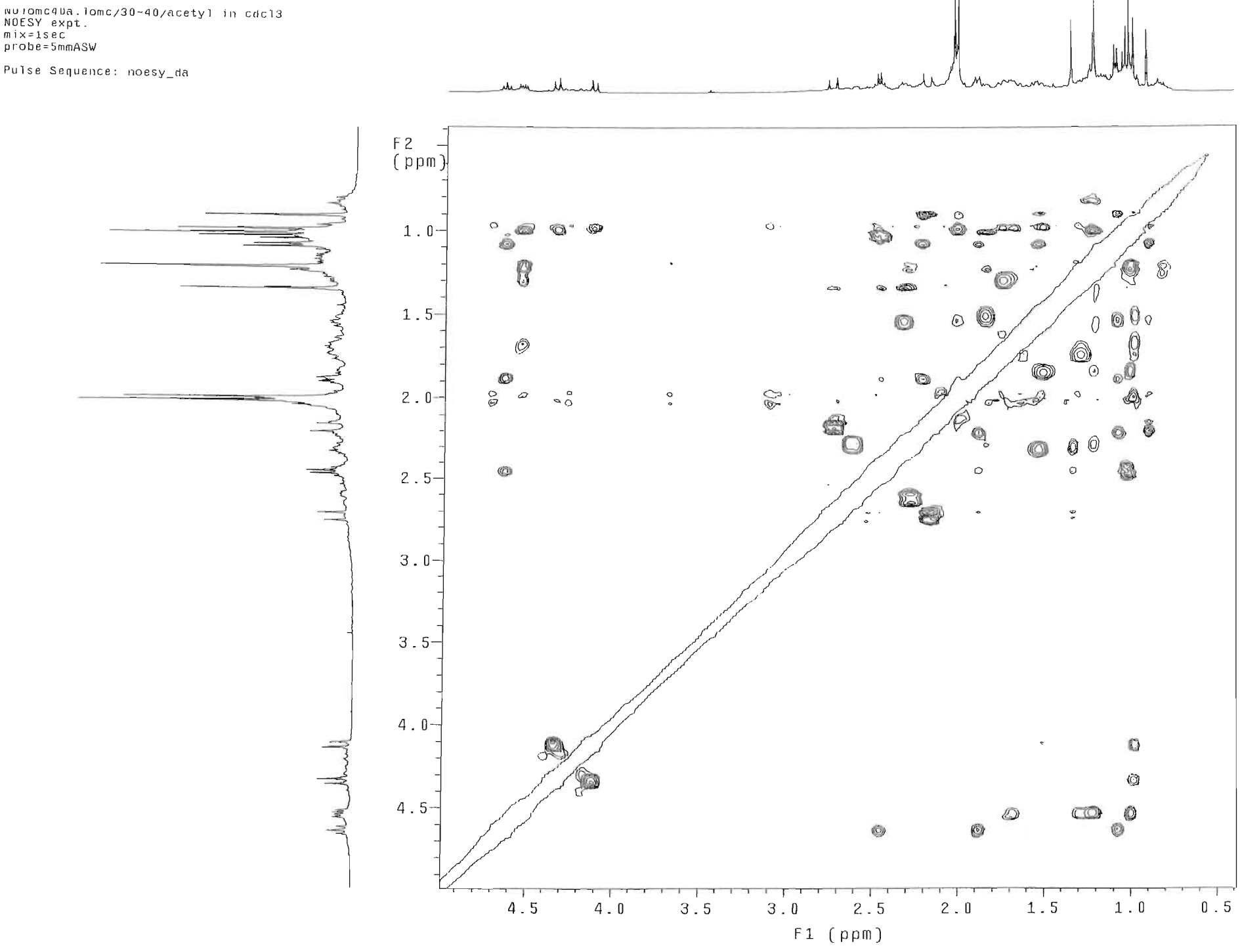

**Spectrum 2m: HSQC spectrum of acetylated compound II**



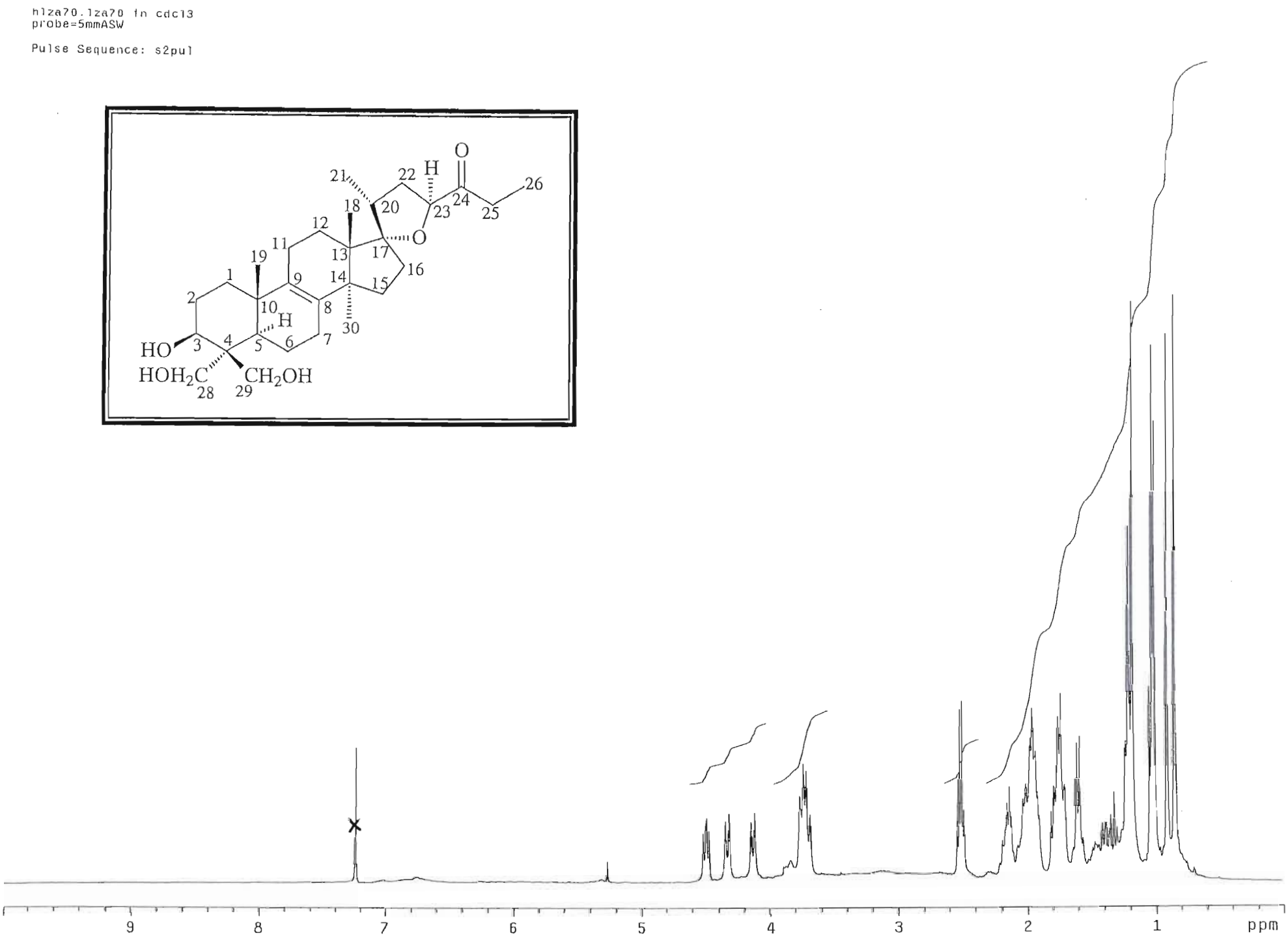

**Spectrum 2n: COSY spectrum of acetylated compound II**



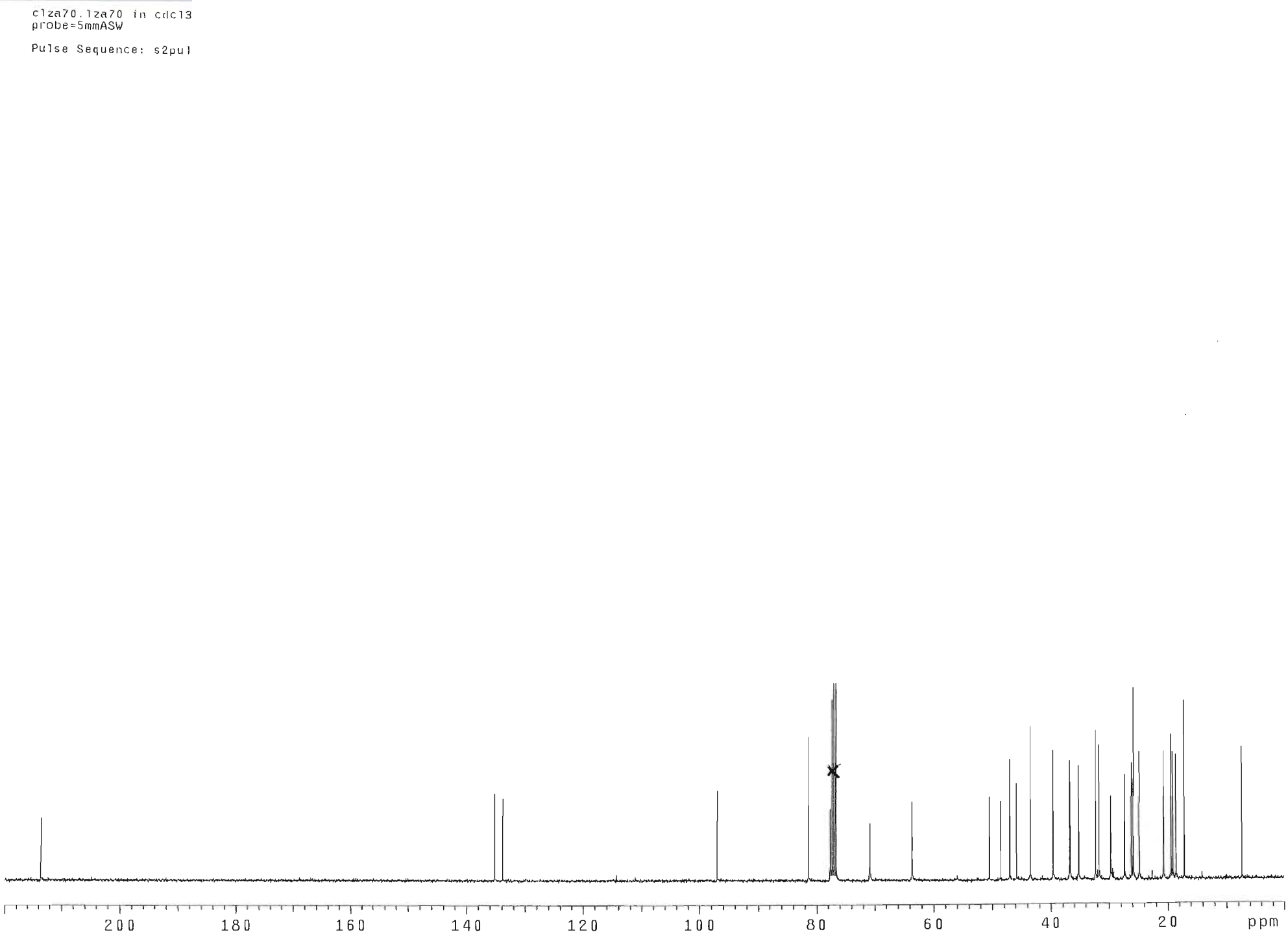

**Spectrum 2o: HMBC spectrum of acetylated compound II**



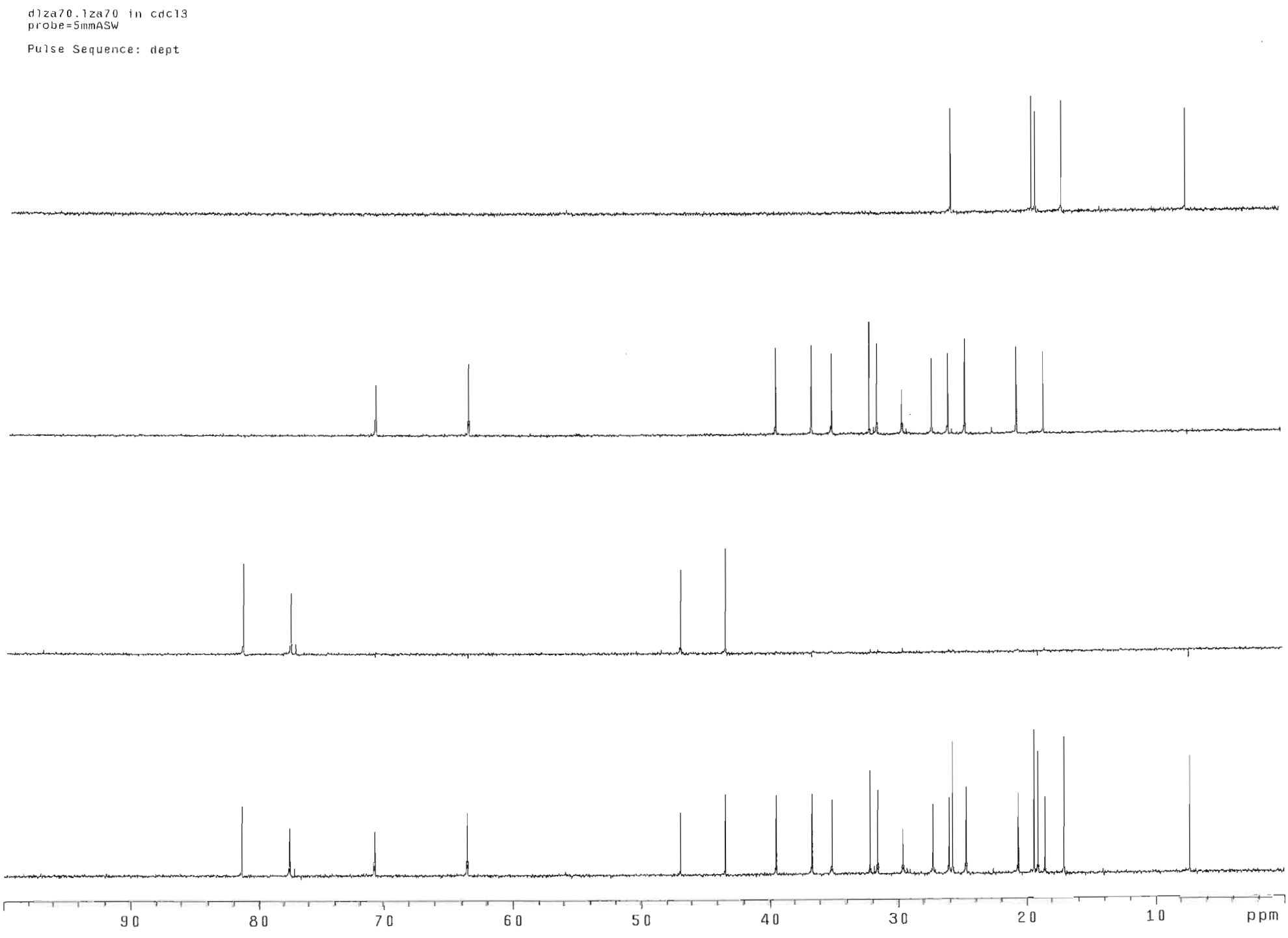

**Spectrum 2p: NOESY spectrum of acetylated compound II**



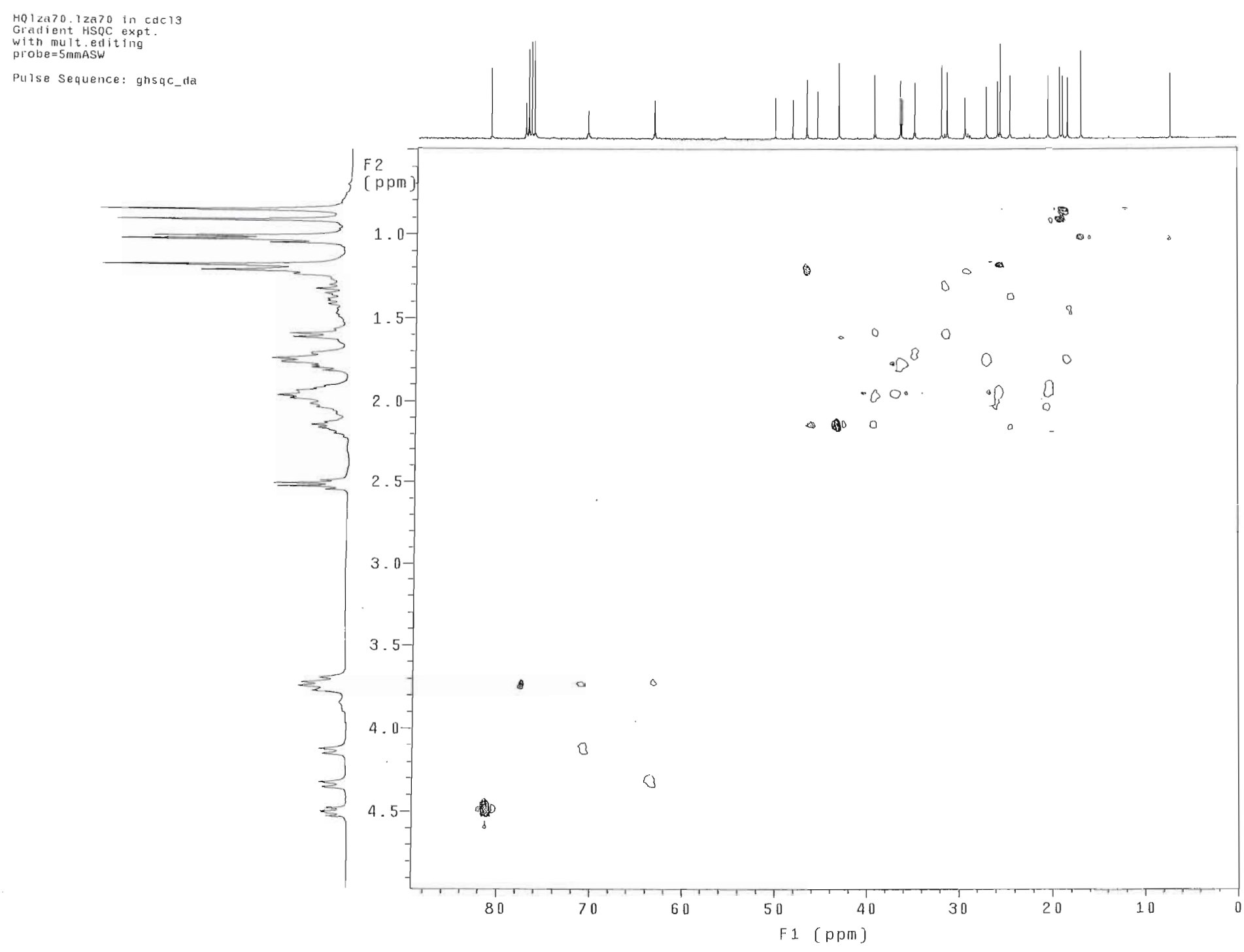

**Spectrum 3a: $^{1}H$ NMR spectrum of compound III ($CDCl_3$) (400 MHz)**



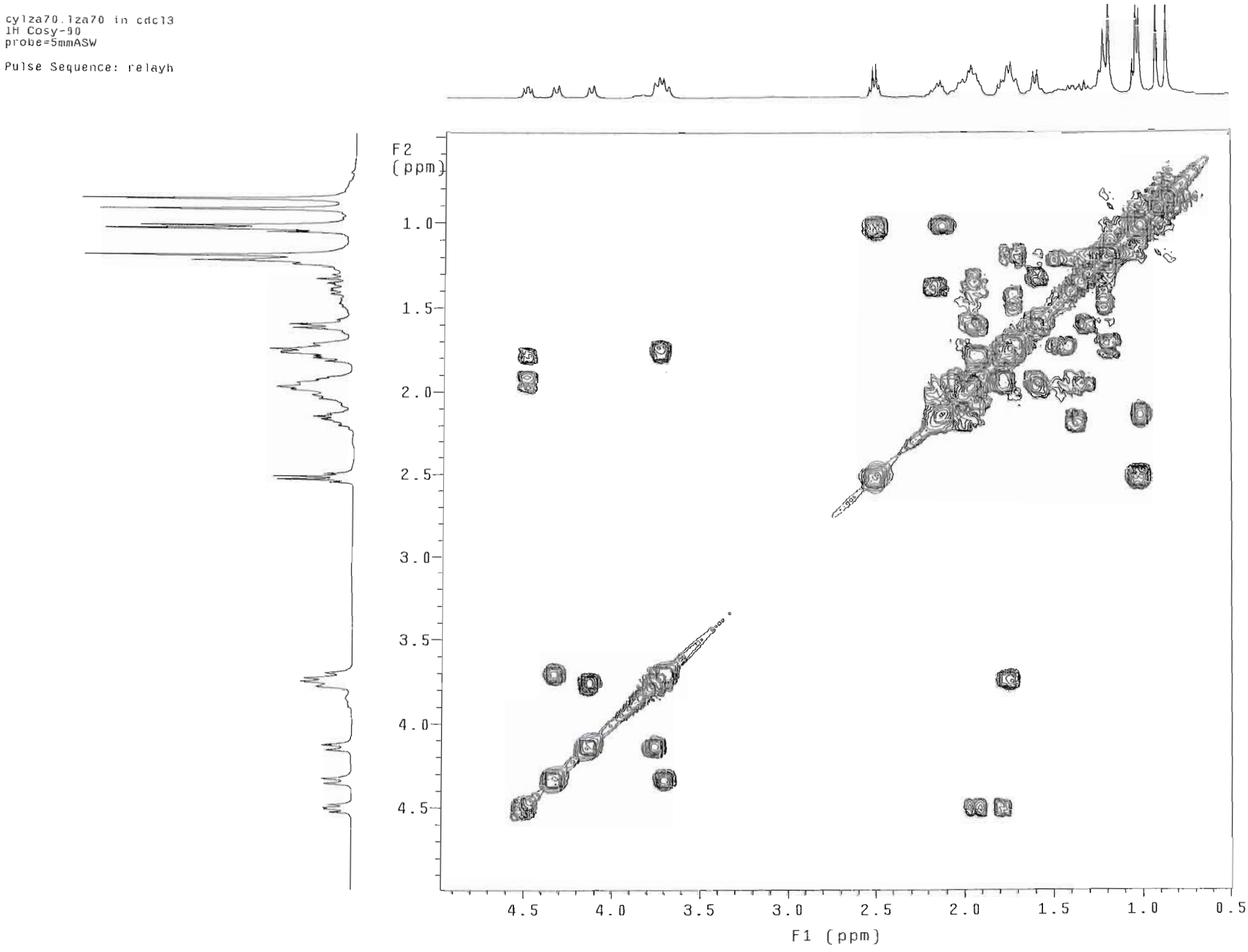

**Spectrum 3b: $^{13}C$ NMR spectrum of compound III ($CDCl_3$) (100 MHz)**



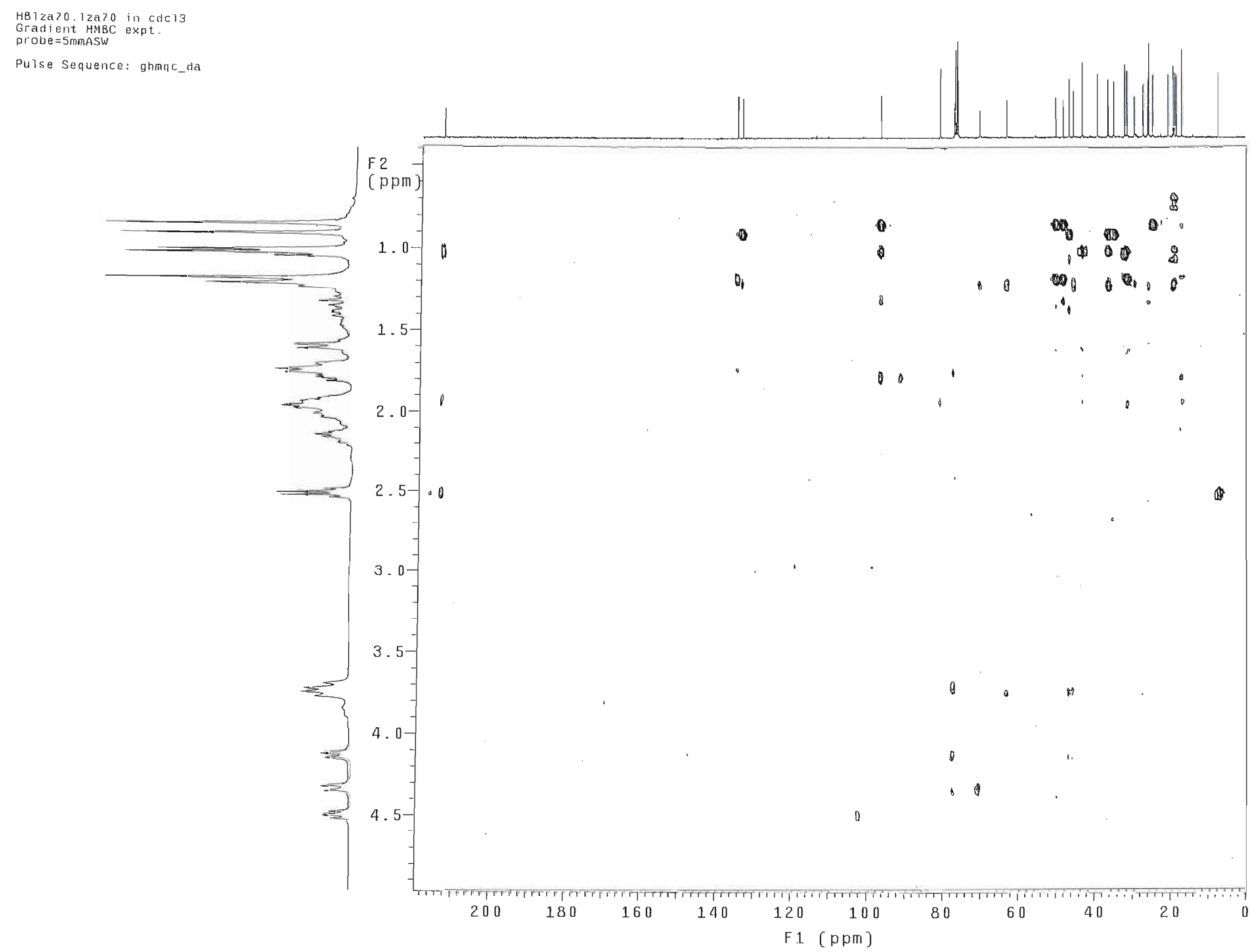

**Spectrum 3c: ADEPT spectrum of compound III**



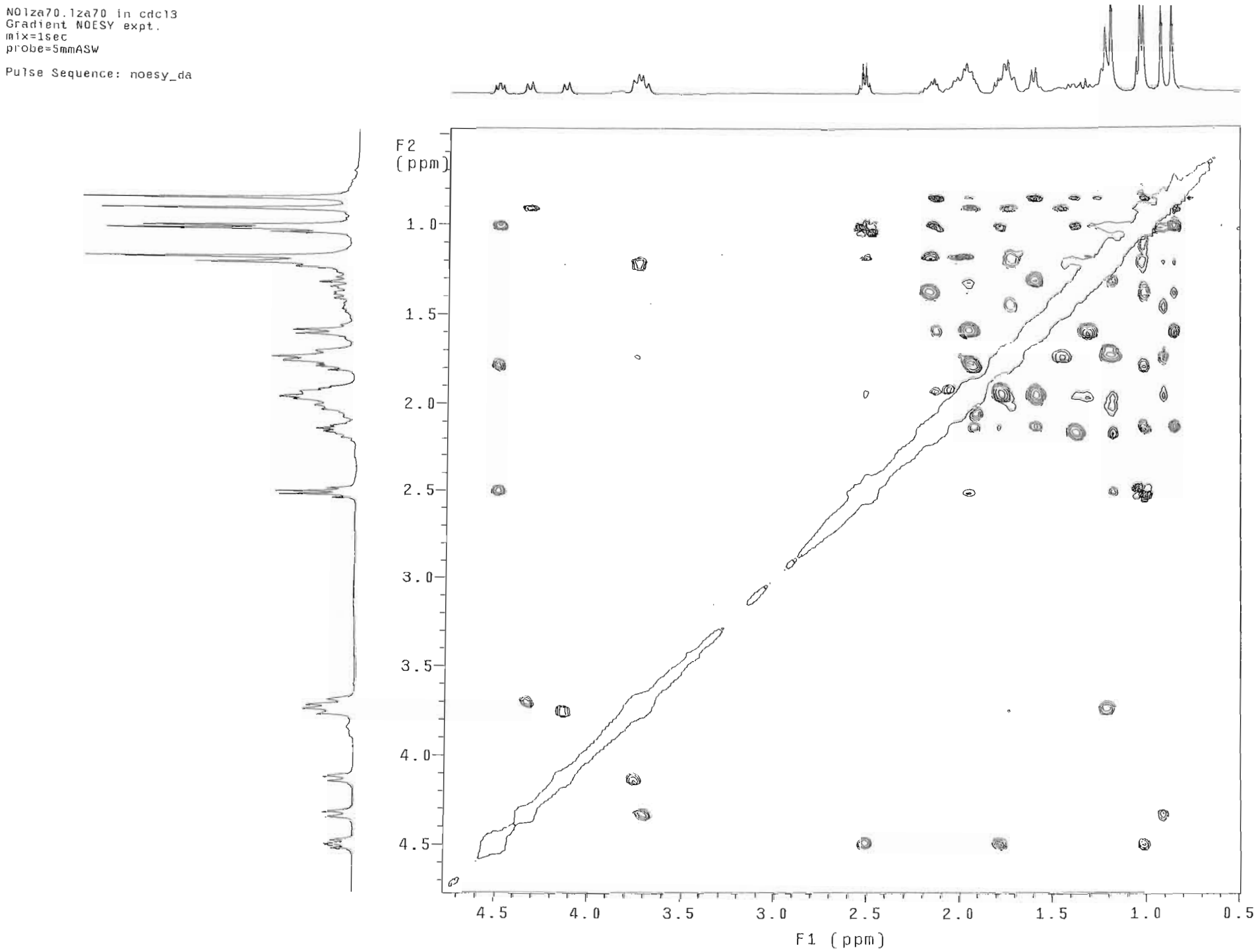

**Spectrum 3d: HSQC spectrum of compound III**



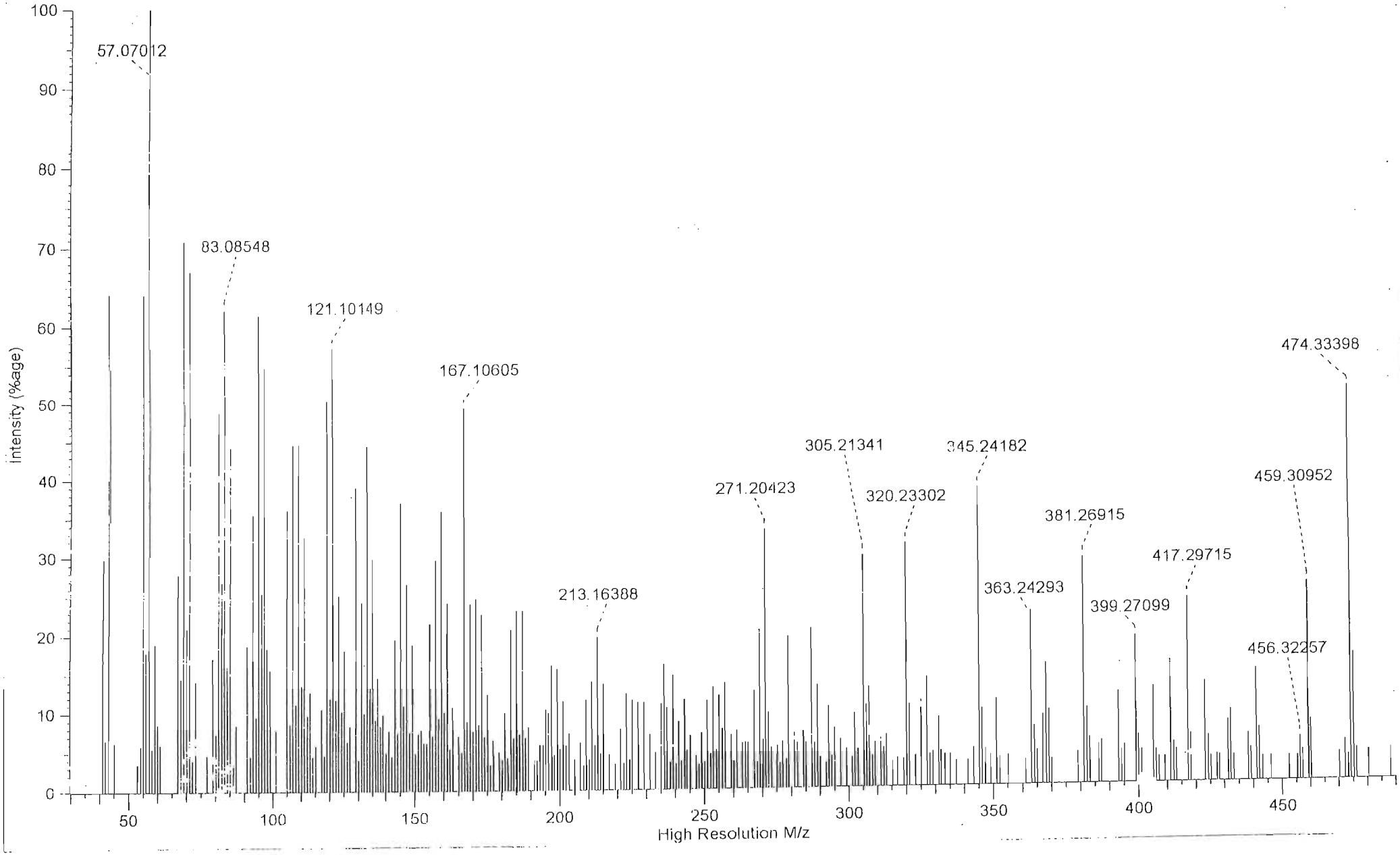

**Spectrum 3e: COSY spectrum of compound III**



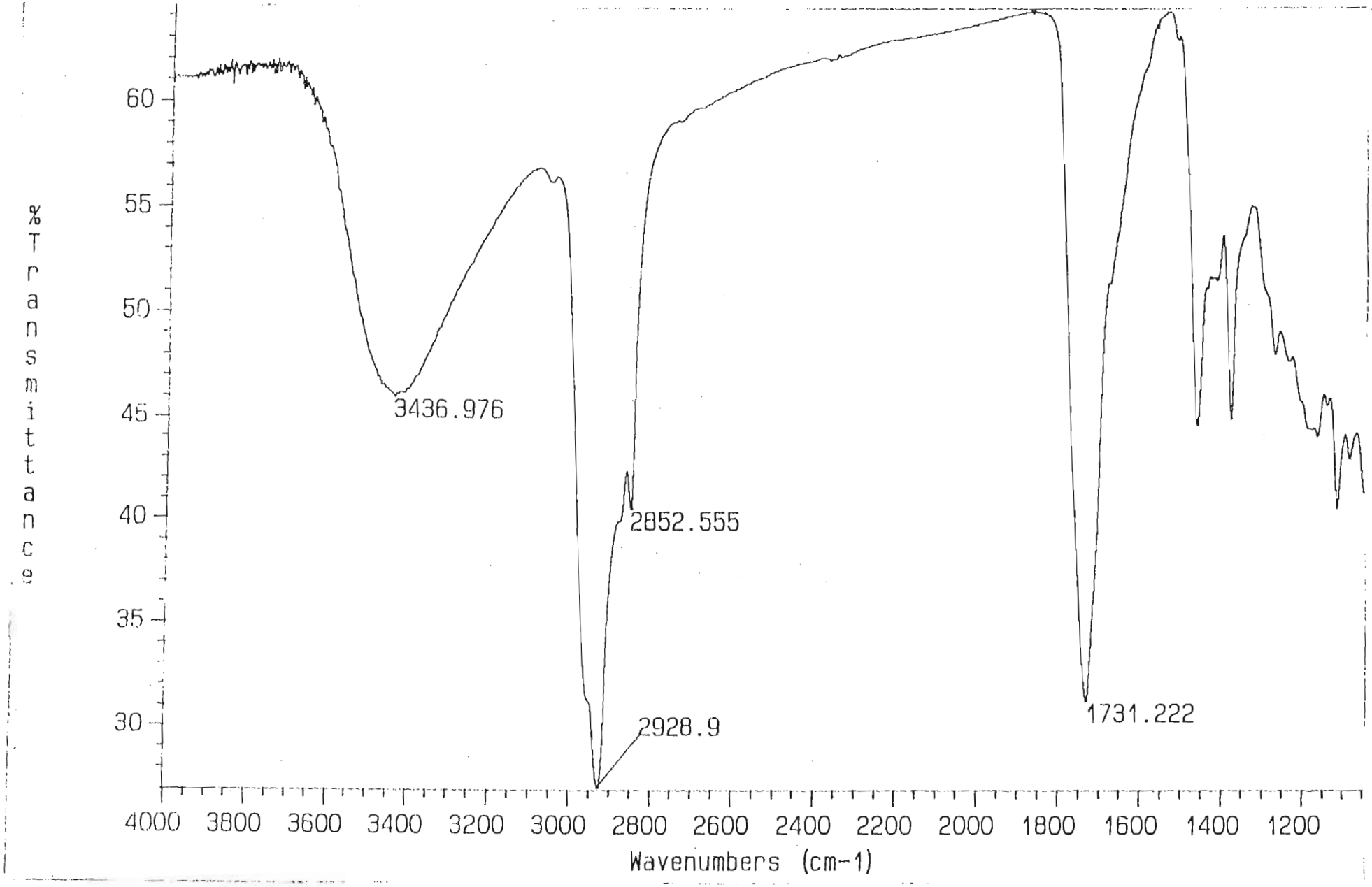

**Spectrum 3f: HMBC spectrum of compound III**



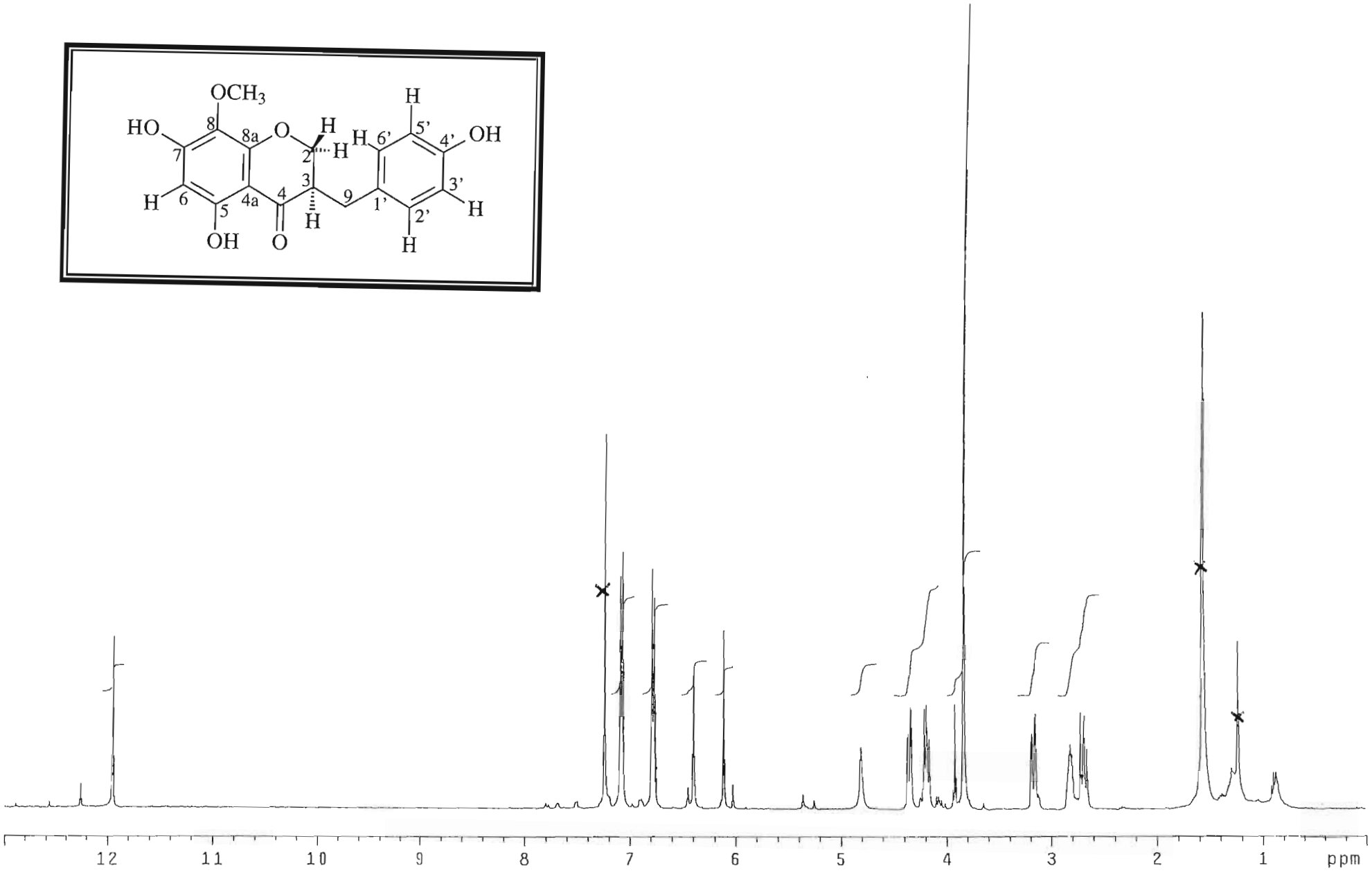

**Spectrum 3g: NOESY spectrum of compound III**



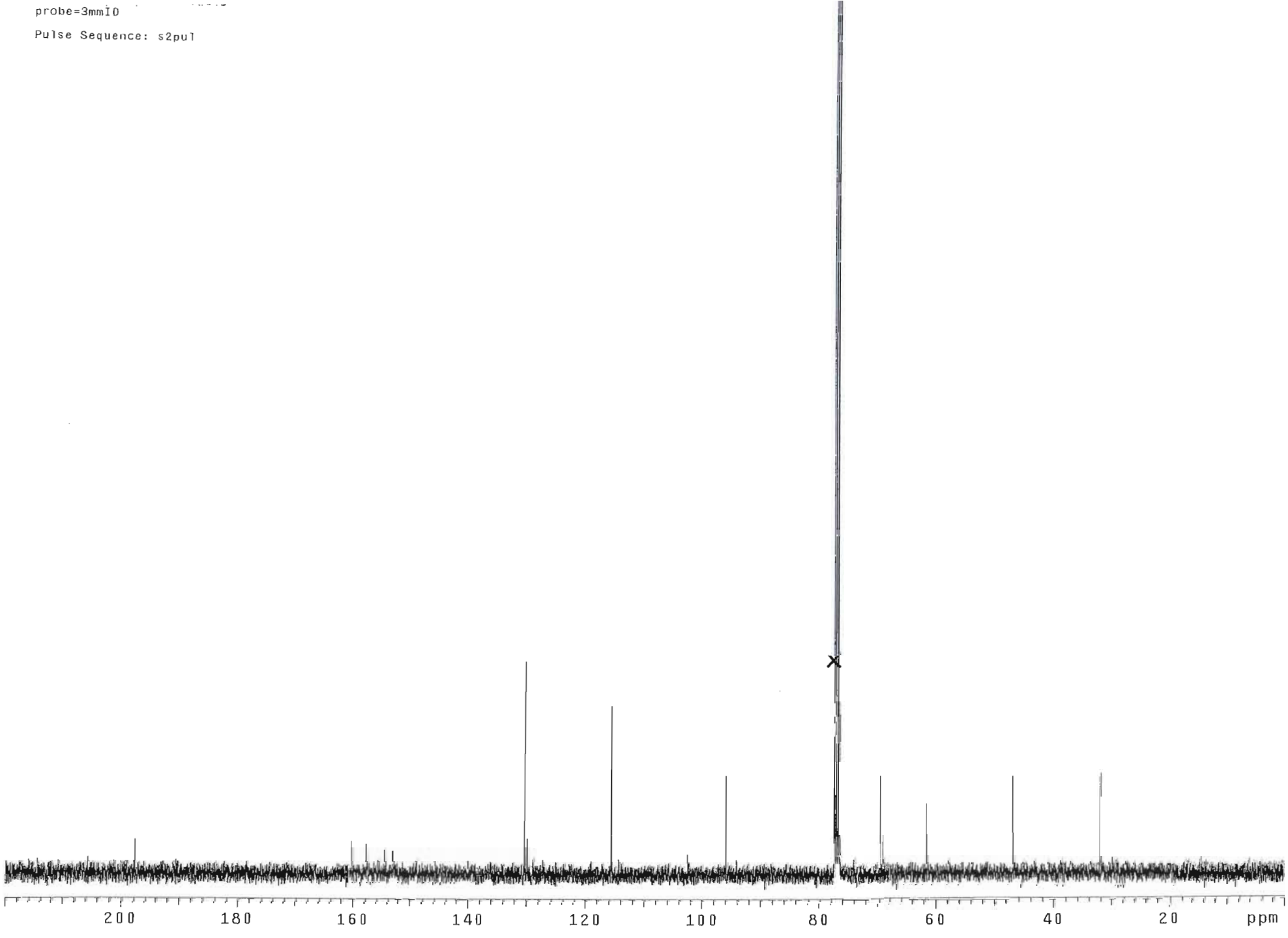

**Spectrum 3h: Mass spectrum of compound III**



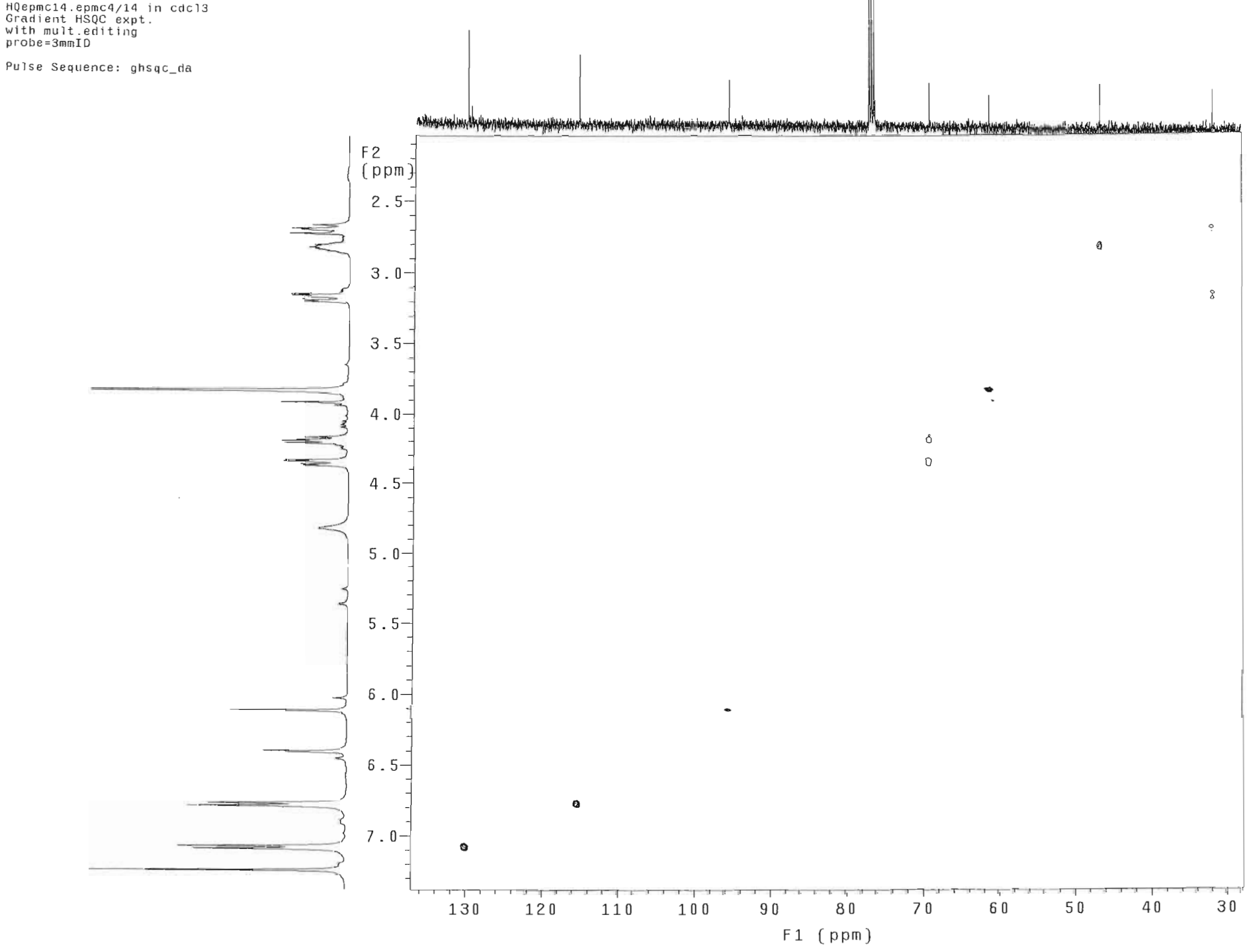

**Spectrum 3i: Infra-red spectrum of compound III**



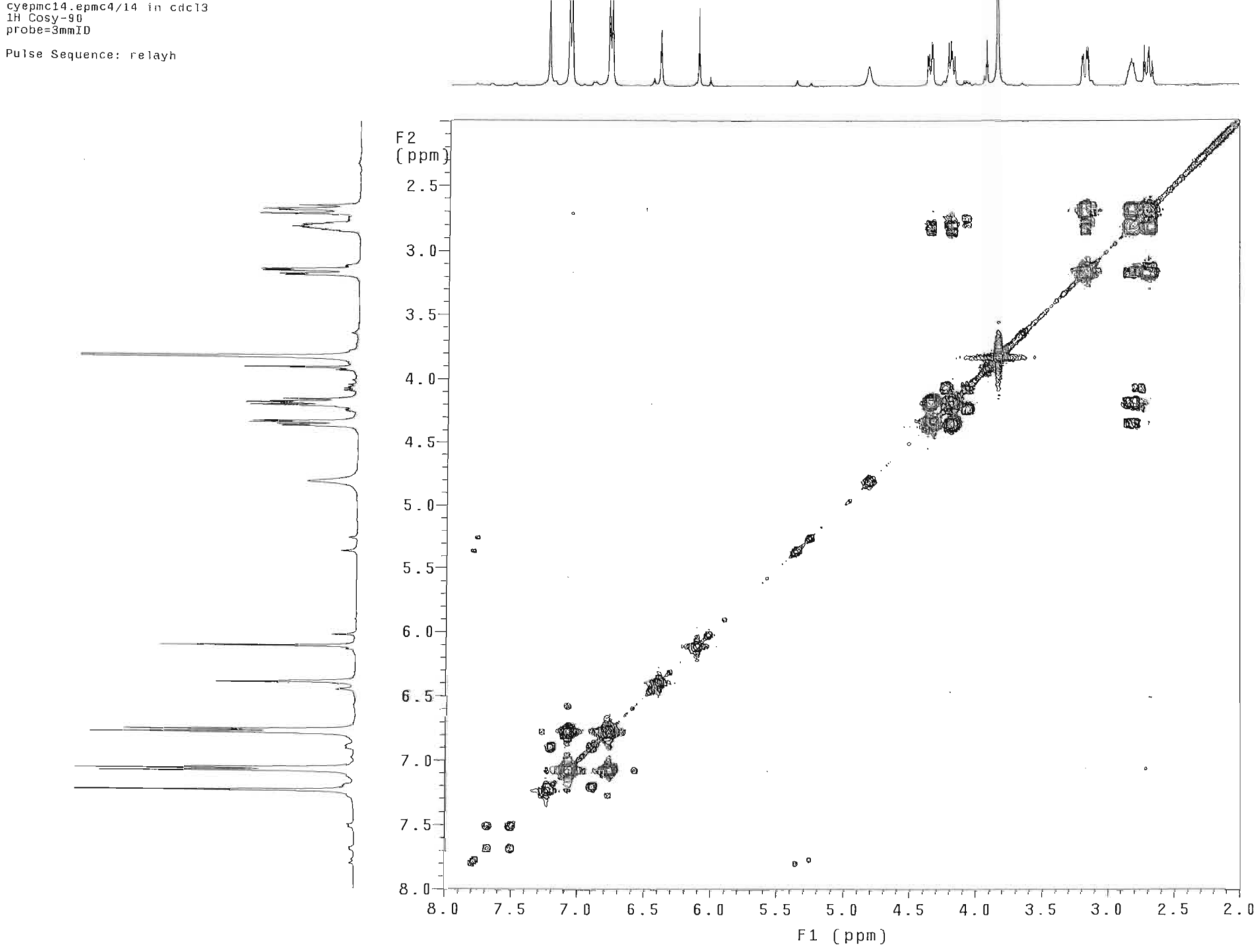

**Spectrum 4a: $^{1}H$ NMR spectrum of compound IV ($CDCl_3$) (400 MHz)**



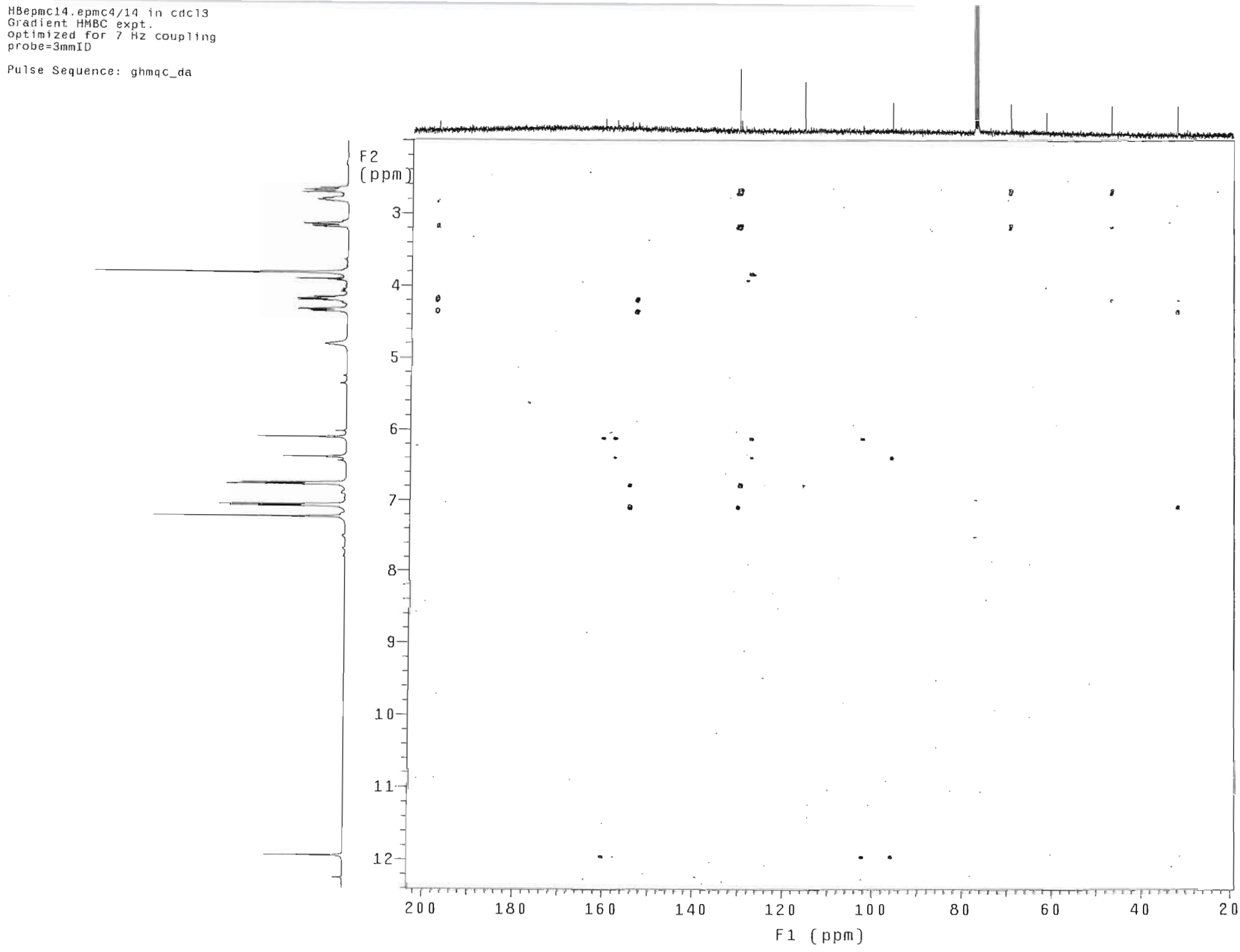

**Spectrum 4b: $^{13}C$ NMR spectrum of compound IV ($CDCl_3$) (100 MHz)**



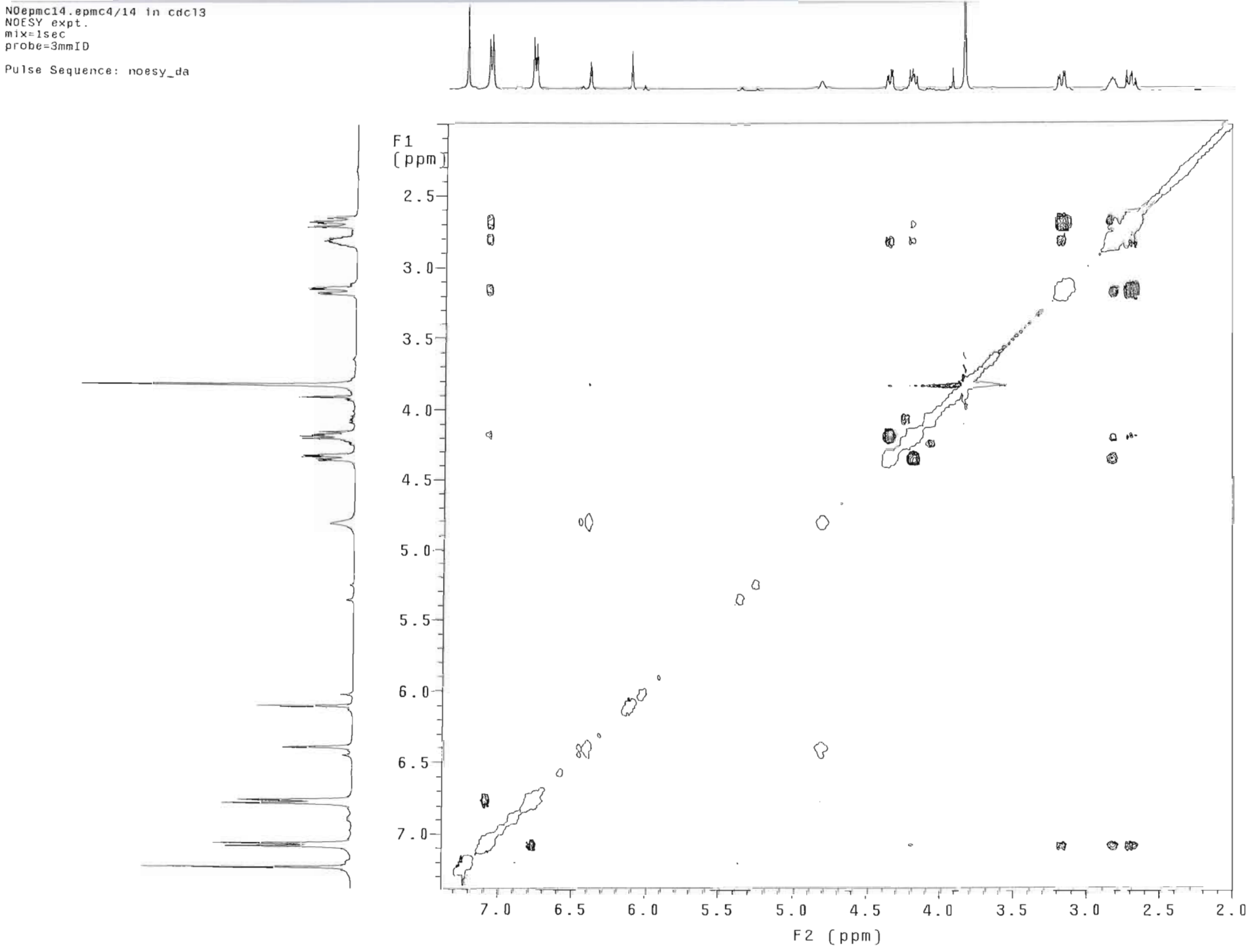

**Spectrum 4c: HSQC spectrum of compound IV**



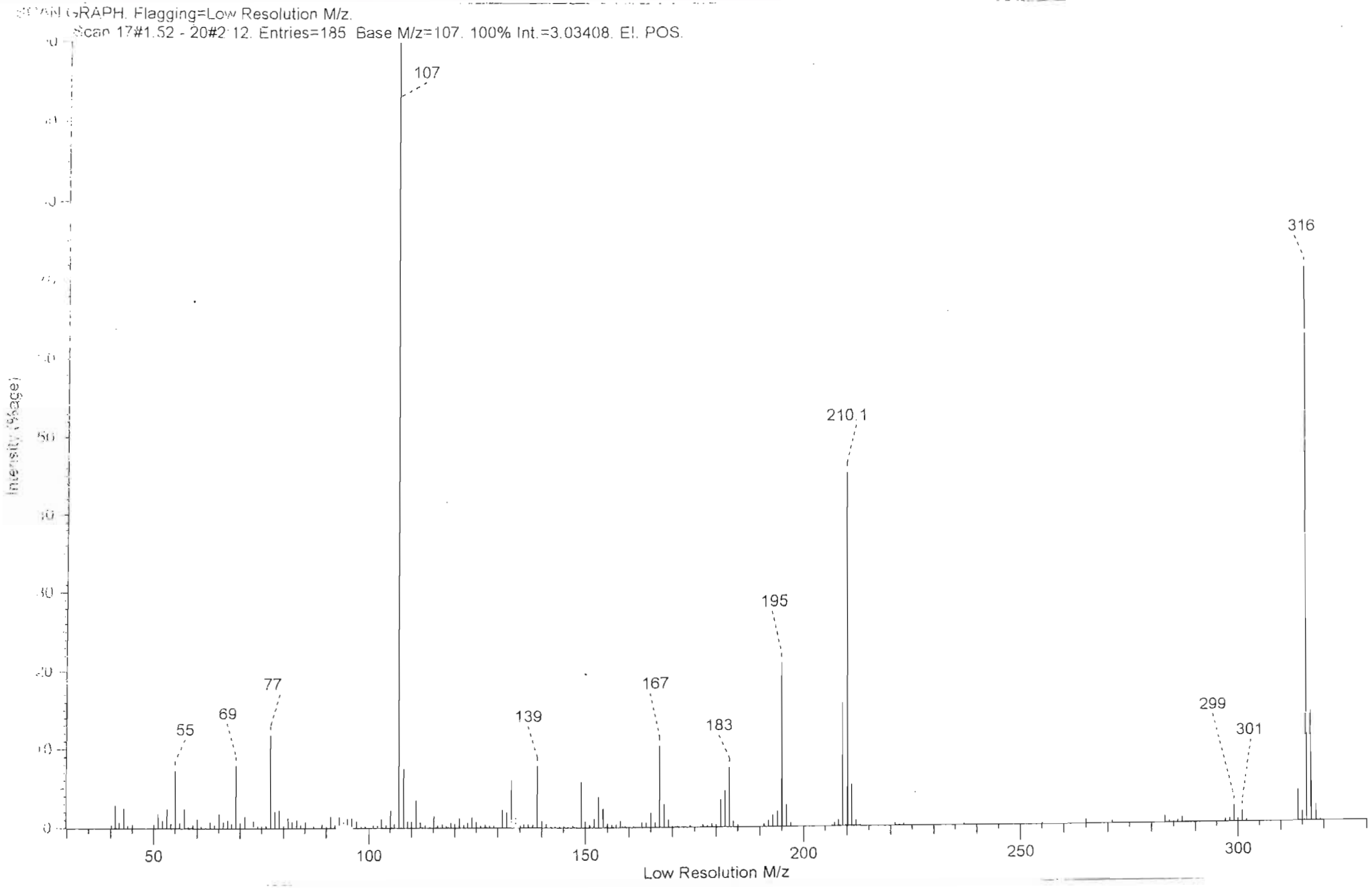

**Spectrum 4d: COSY spectrum of compound IV**



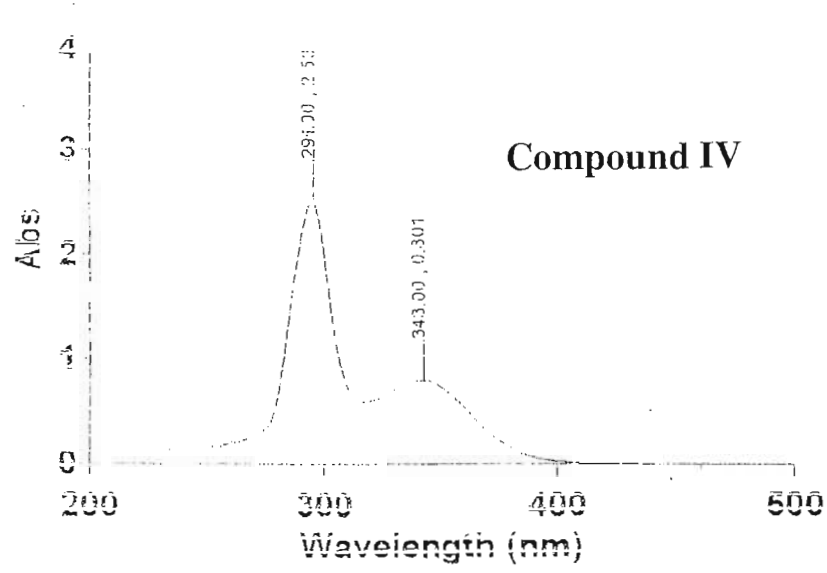

**Spectrum 4e: HMBC spectrum of compound IV**

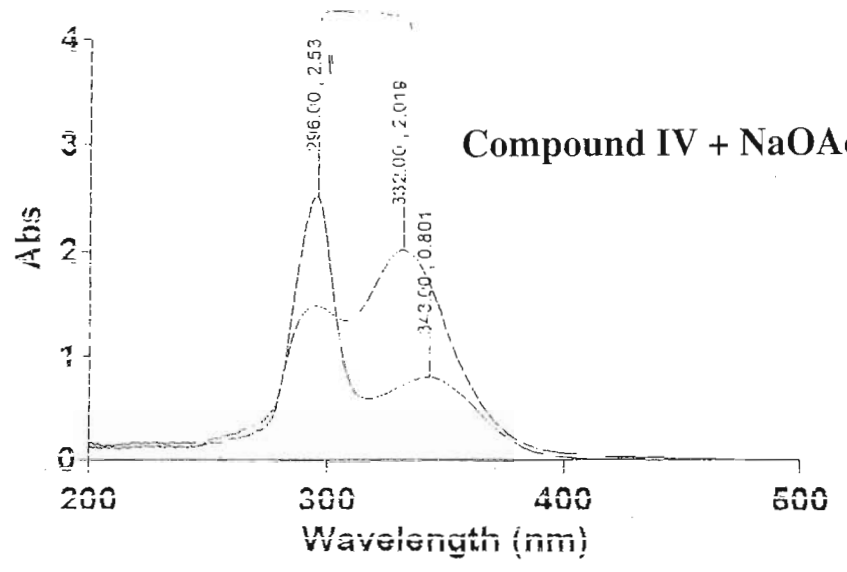


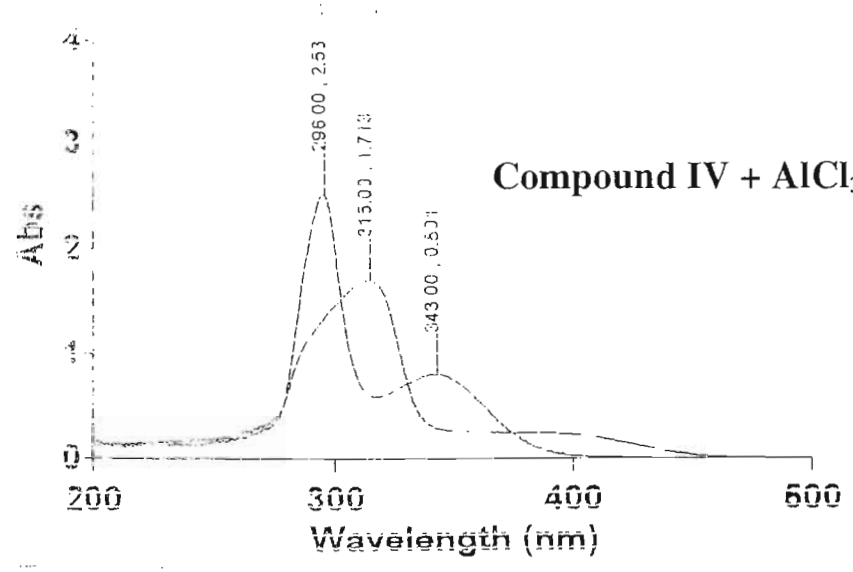

**Spectrum 4f: NOESY spectrum of compound IV**



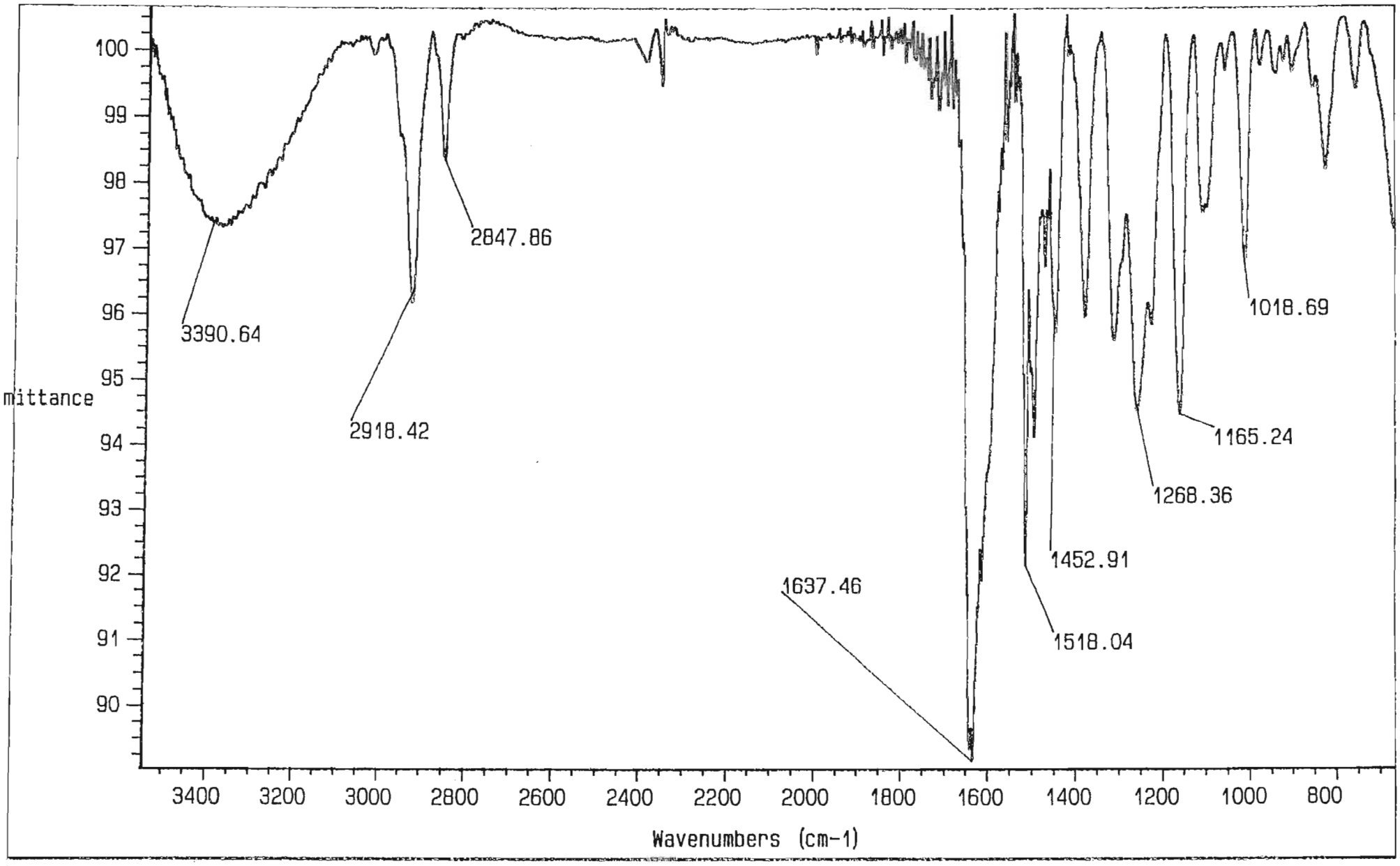

**Spectrum 4g: Mass spectrum of compound IV**



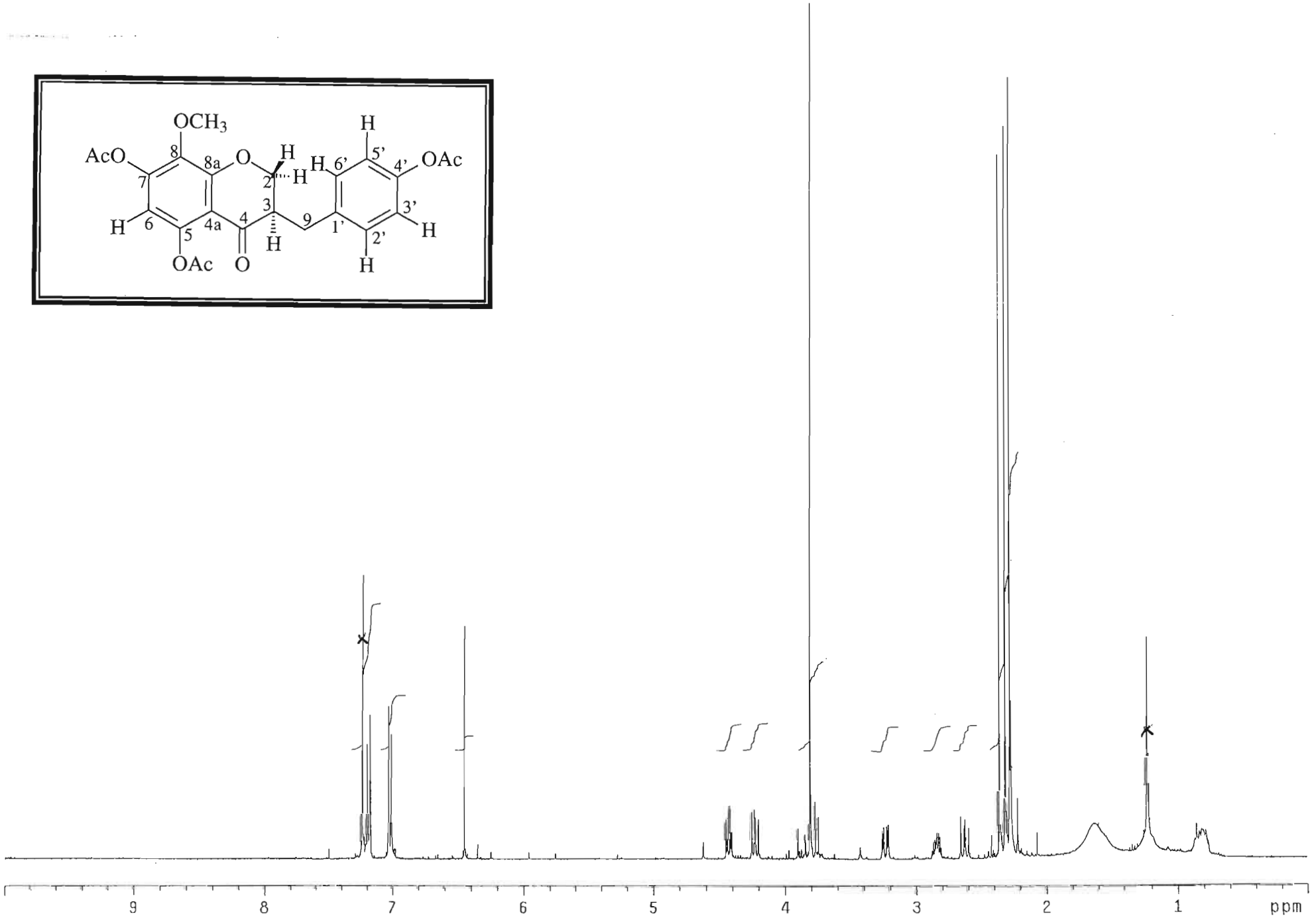




**Spectrum 4h: Ultra-violet spectrum of compound IV**

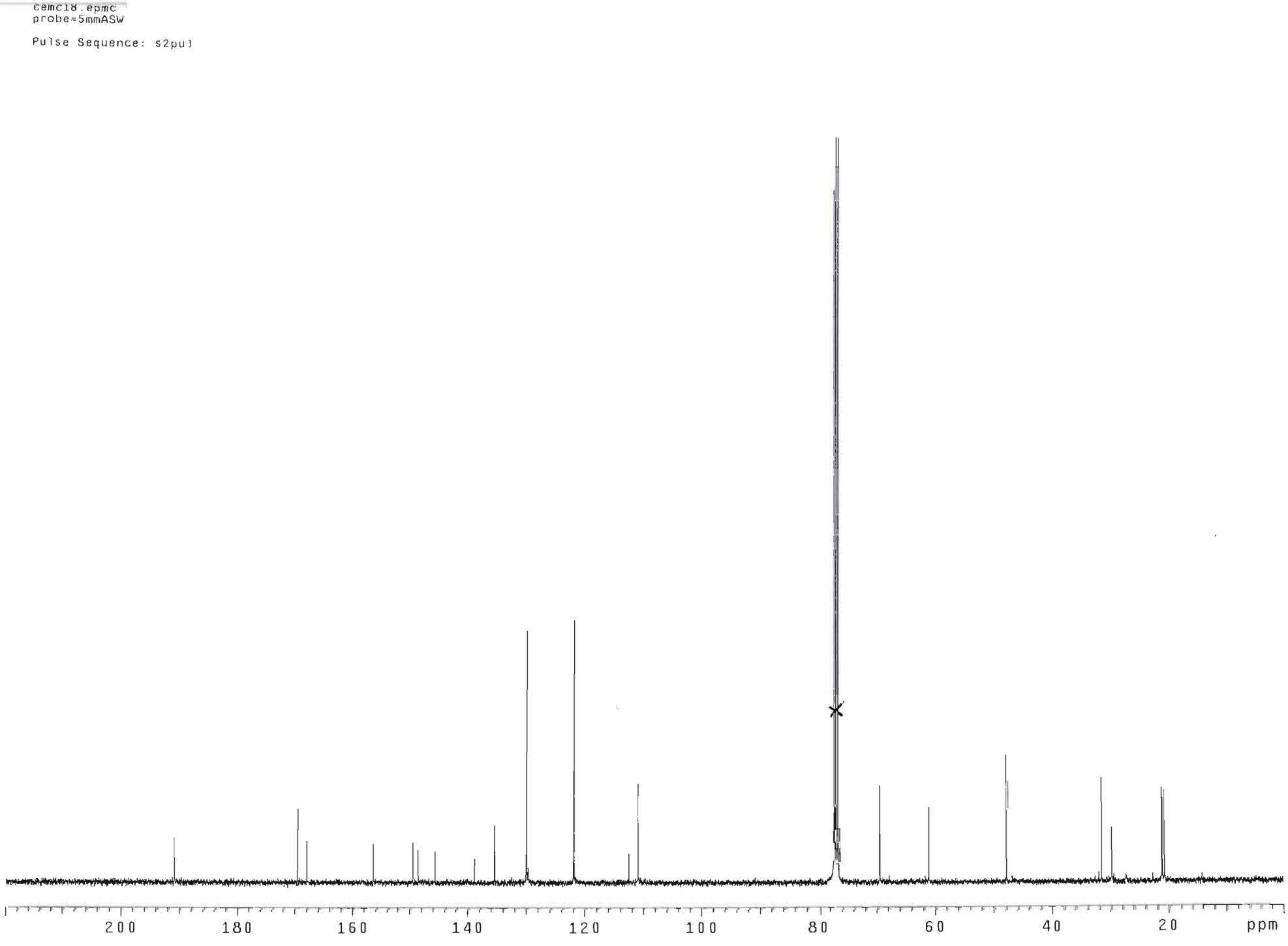



**Spectrum 4i: Infra-red spectrum of compound IV**

Pulse Sequence: s2pul



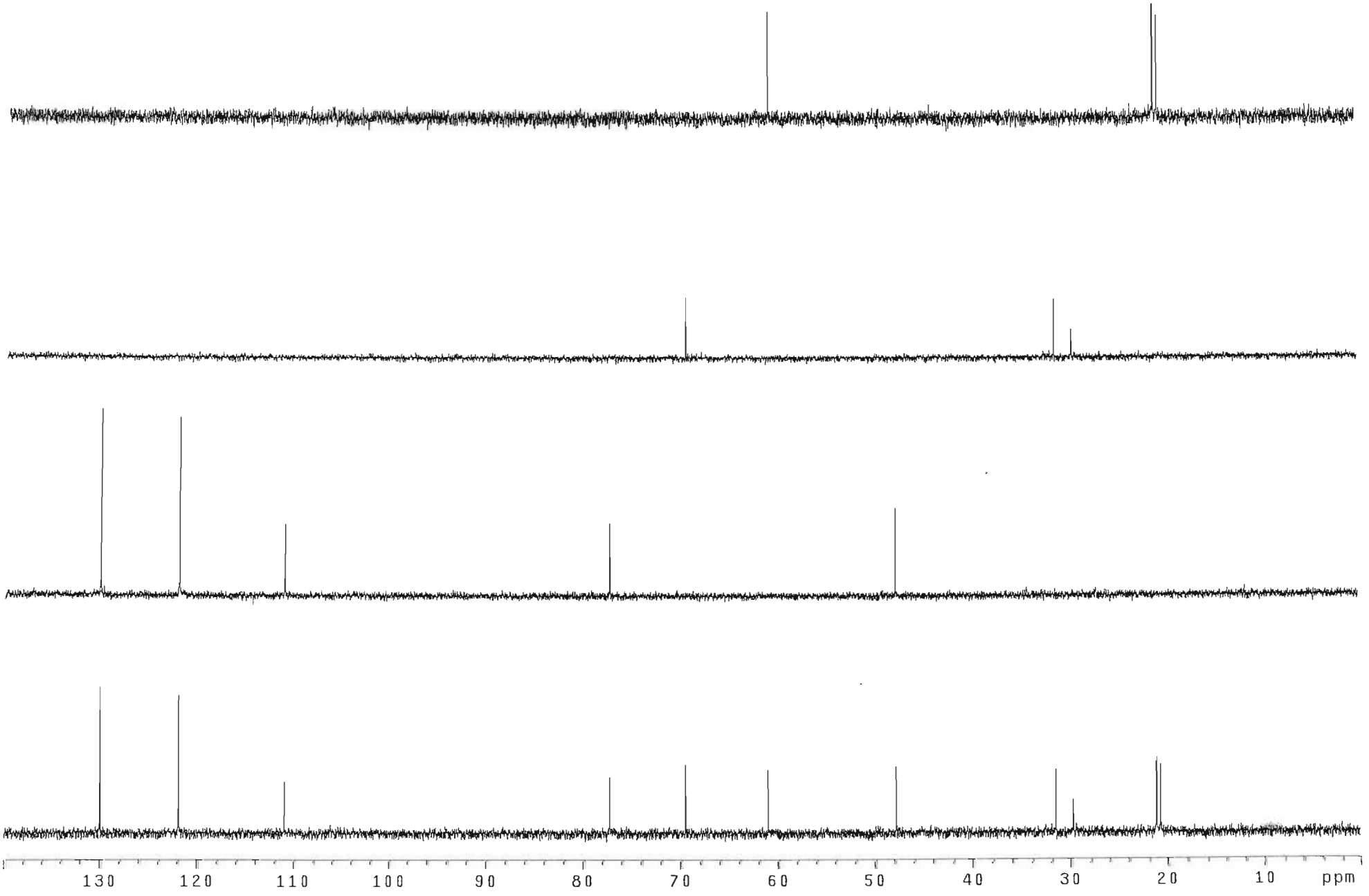

**Spectrum 4j: $^{1}H$ NMR spectrum of acetylated compound IV ($CDCl_3$) (400 MHz)**




**Spectrum 4k: $^{13}C$ NMR spectrum of acetylated compound IV ($CDCl_3$) (100 MHz)**

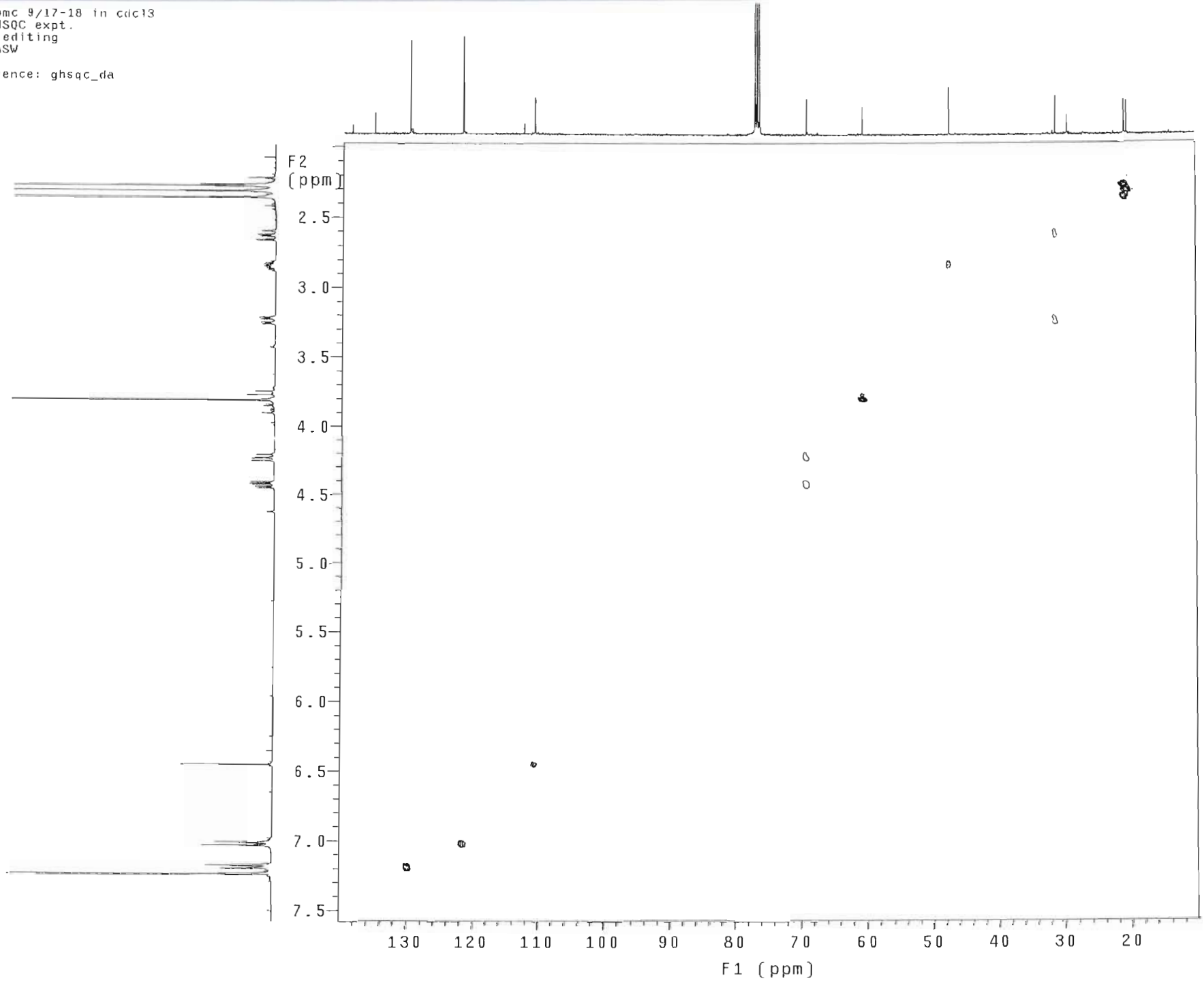
demc18.epmc 9/17-18 in cdcl3
probe=5mmASW

Pulse Sequence: dept

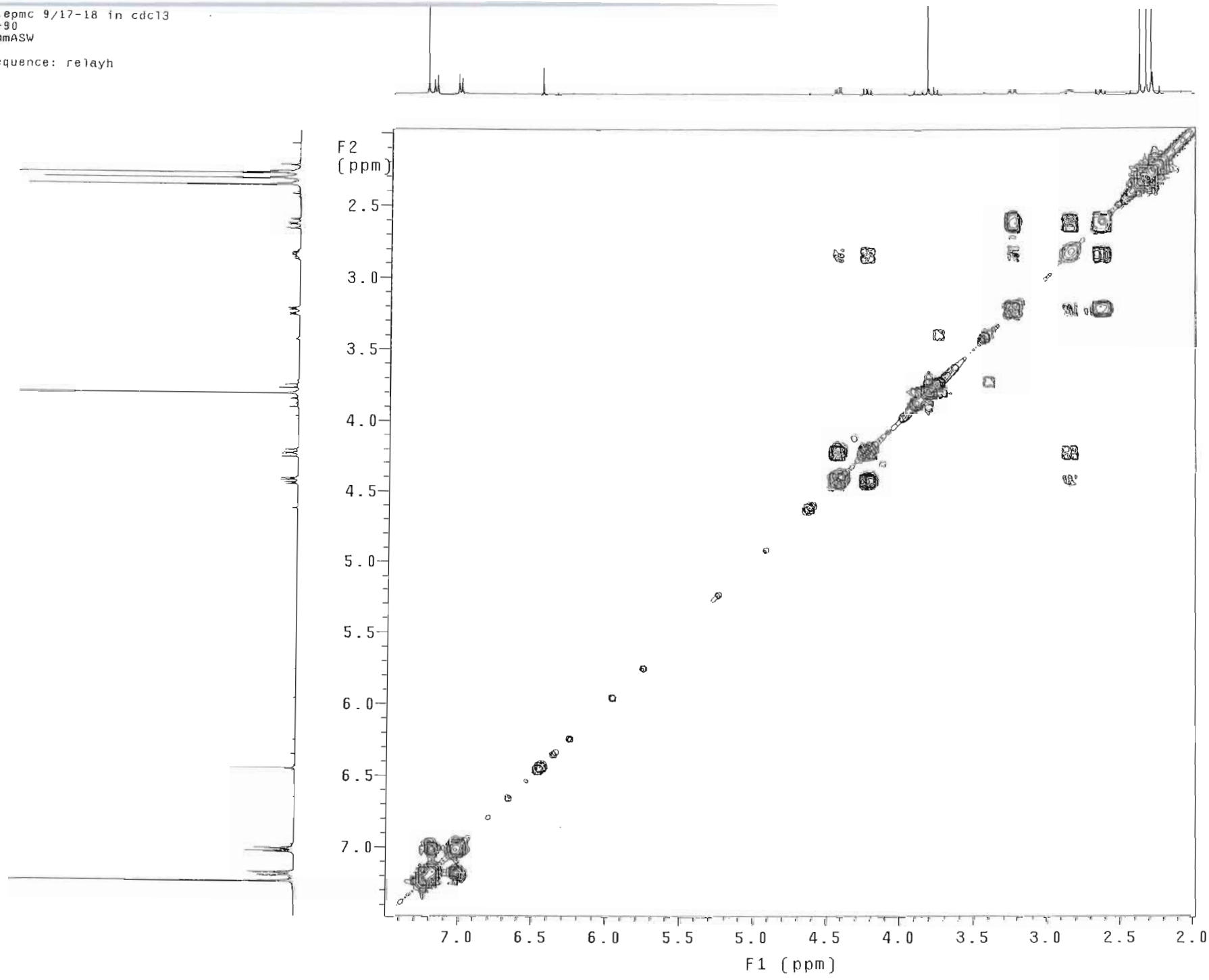




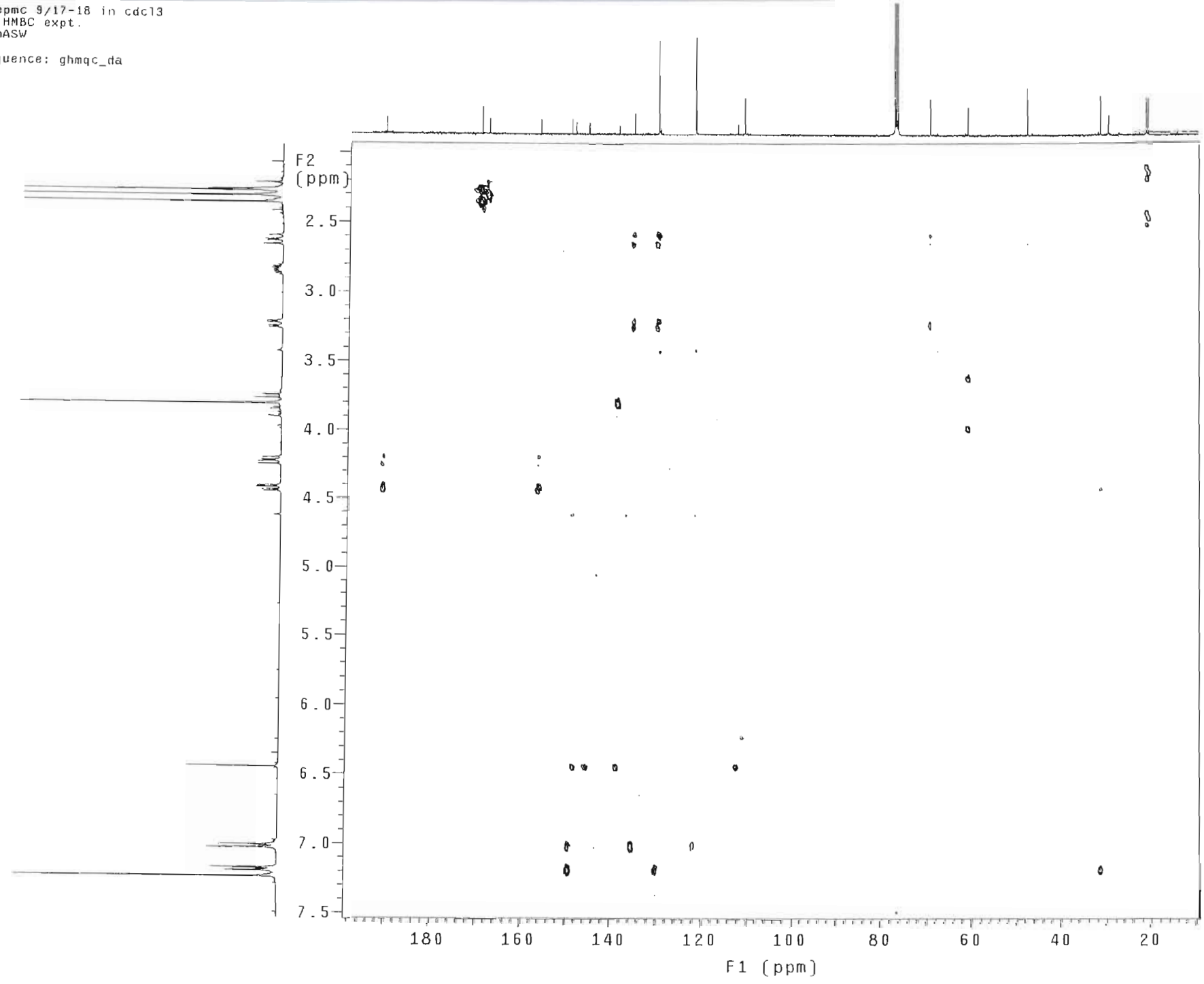

**Spectrum 4l: ADEPT spectrum of acetylated compound IV**



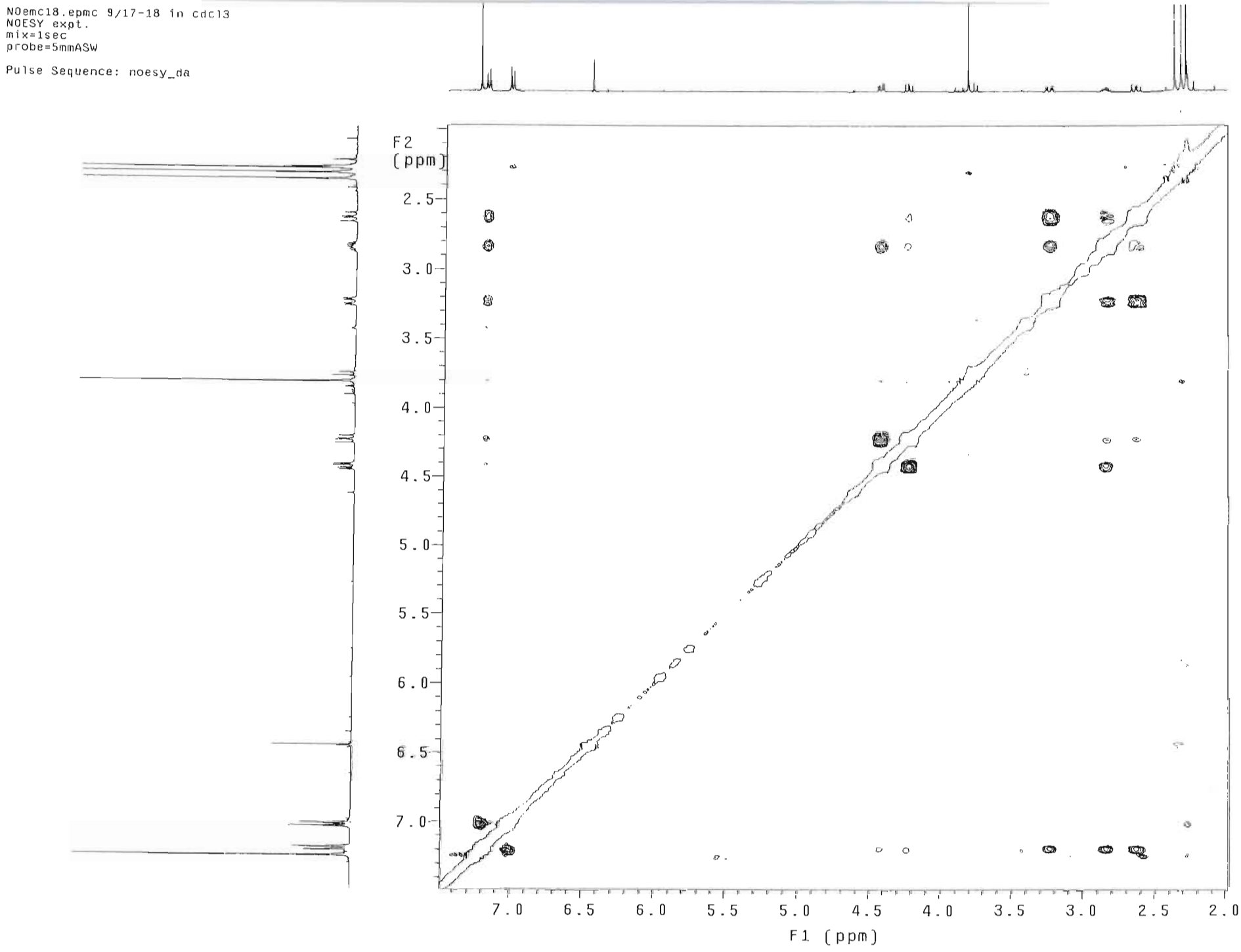

**Spectrum 4m: HSQC spectrum of acetylated compound IV**




**Spectrum 4n: COSY spectrum of acetylated compound IV**




**Spectrum 4o: HMBC spectrum of acetylated compound IV**

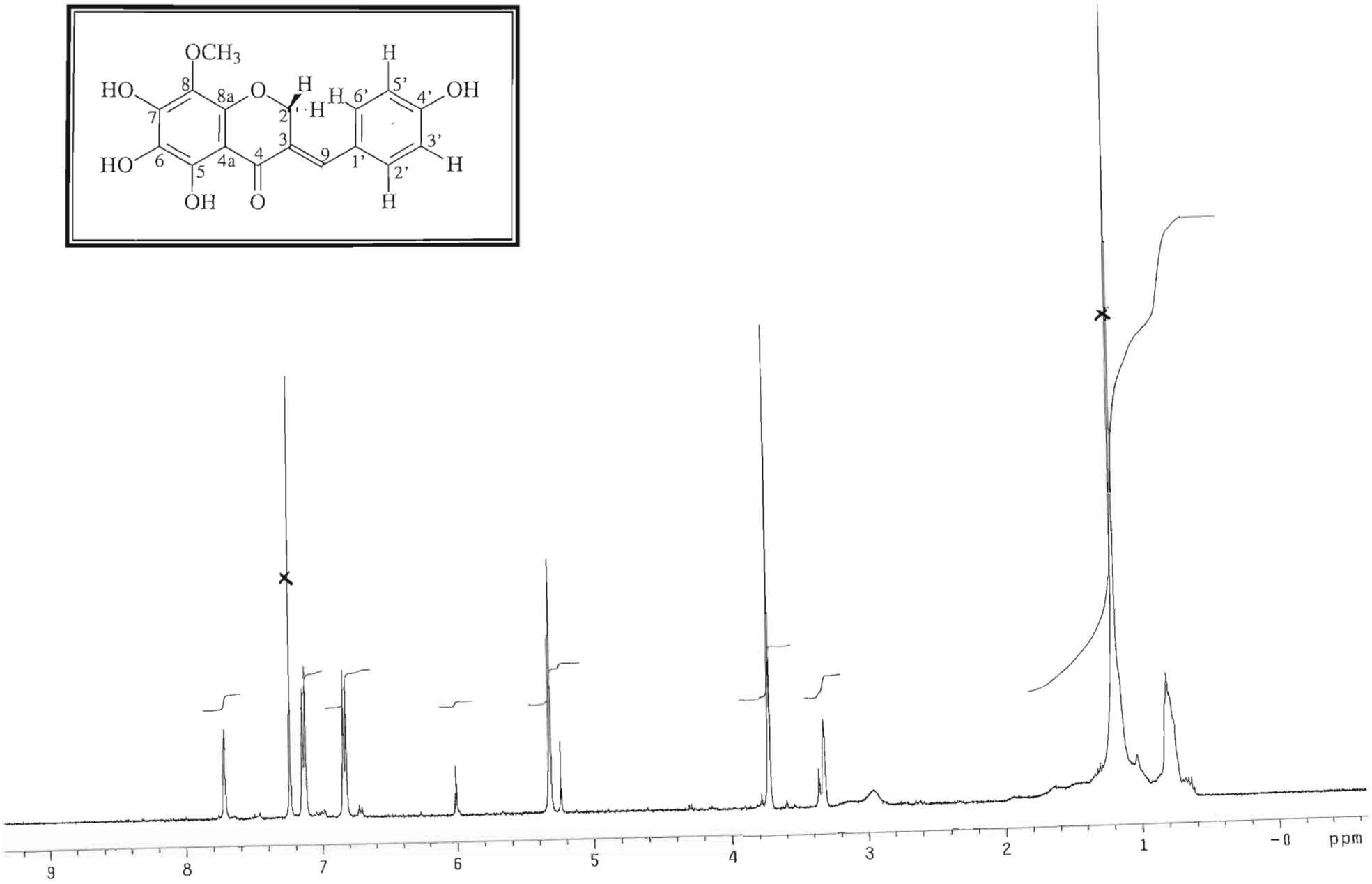



**Spectrum 4p: NOESY spectrum of acetylated compound IV**

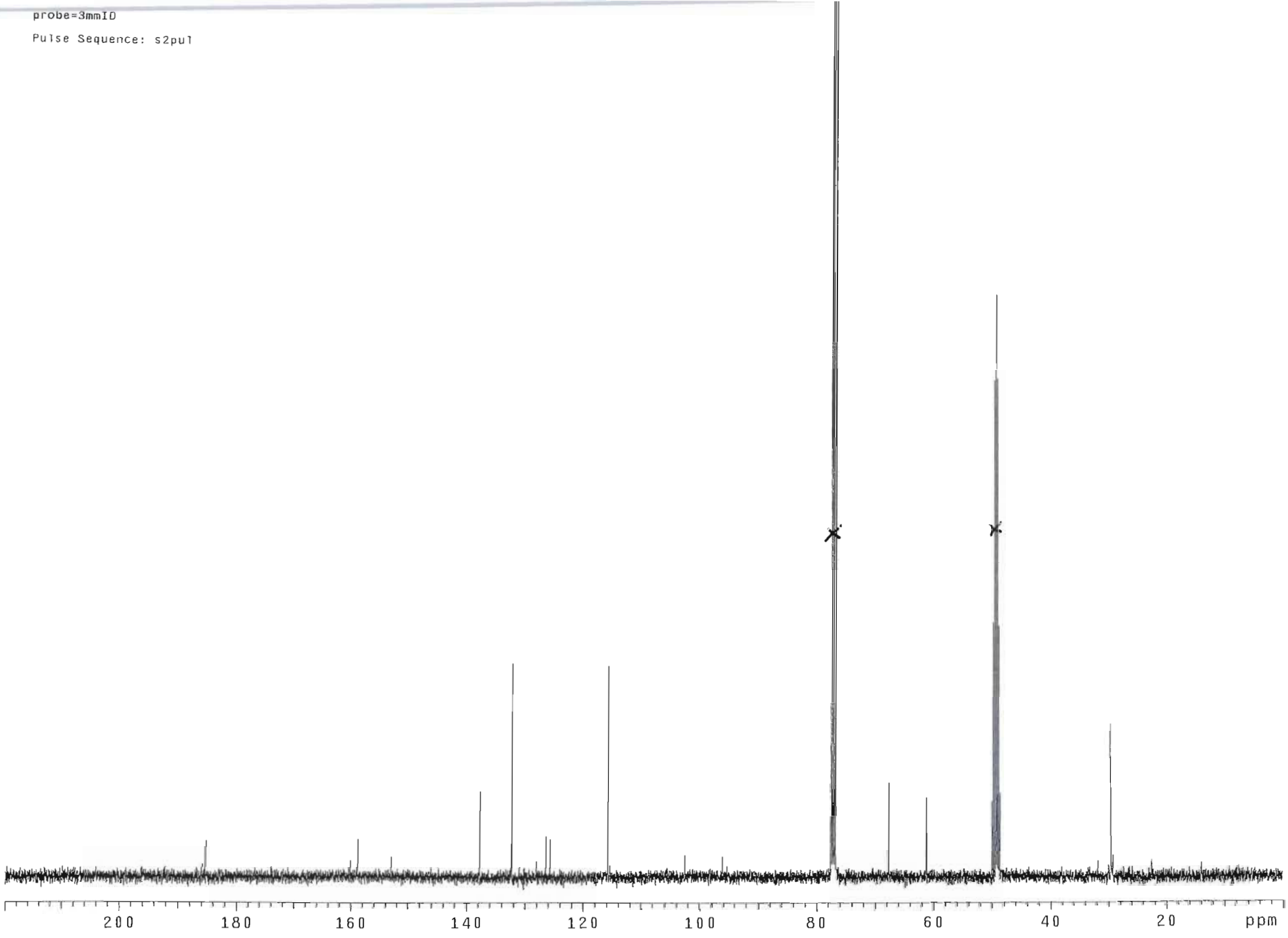
using presat_h2o
satpwr=-14
probe=3mmID

Pulse Sequence: presat_da

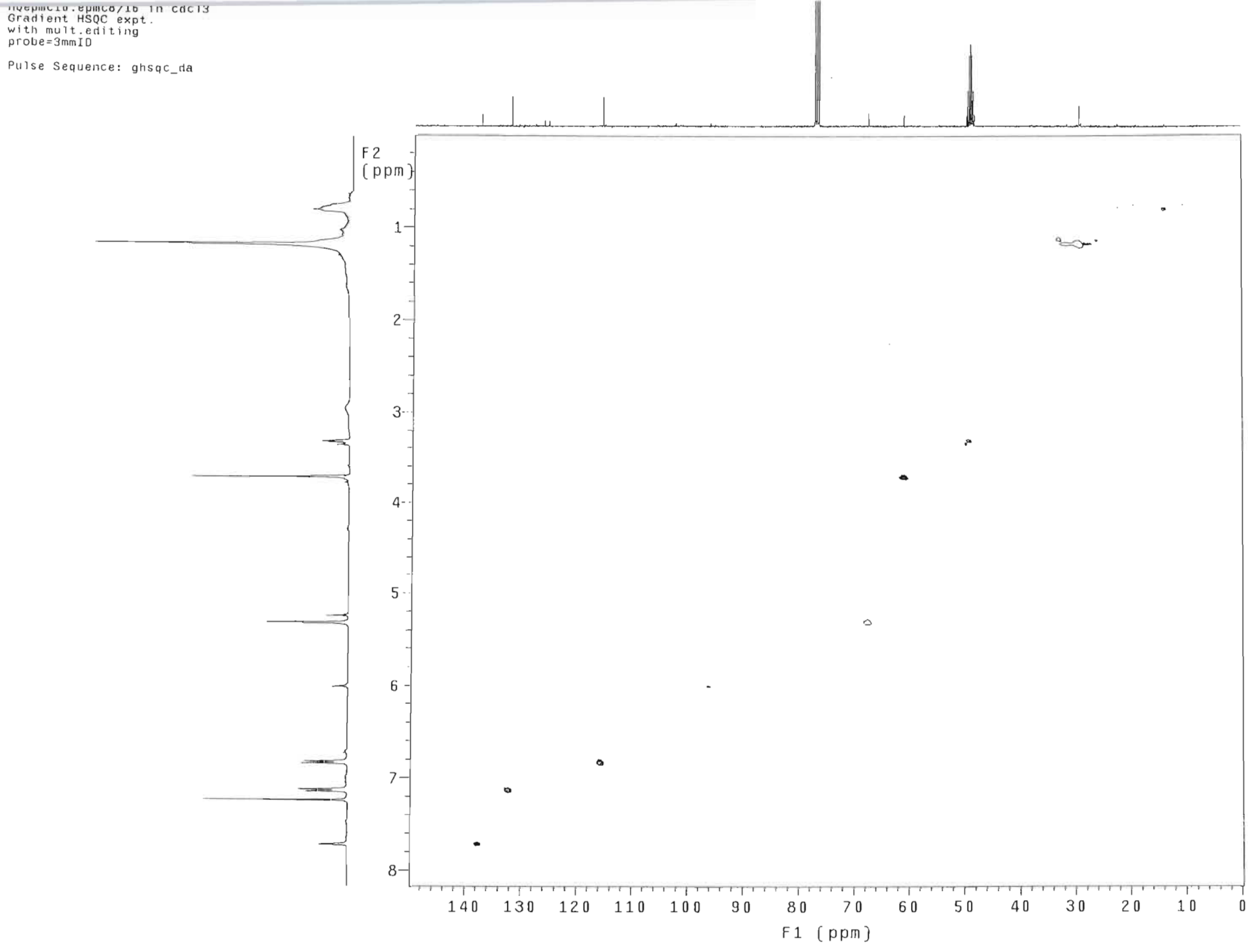


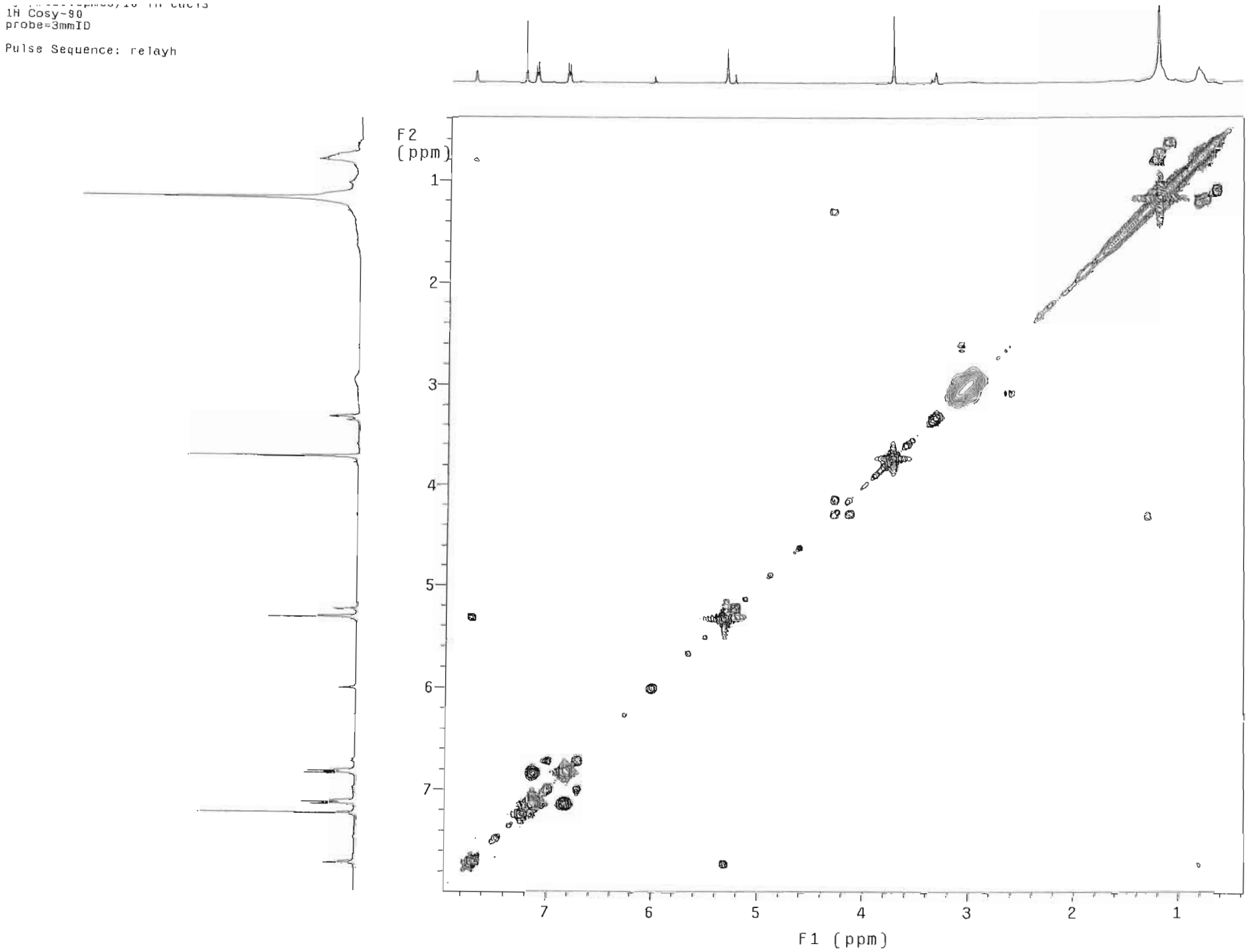

**Spectrum 5a: $^{1}$H NMR spectrum of compound V ($CDCl_3$) (400 MHz)**



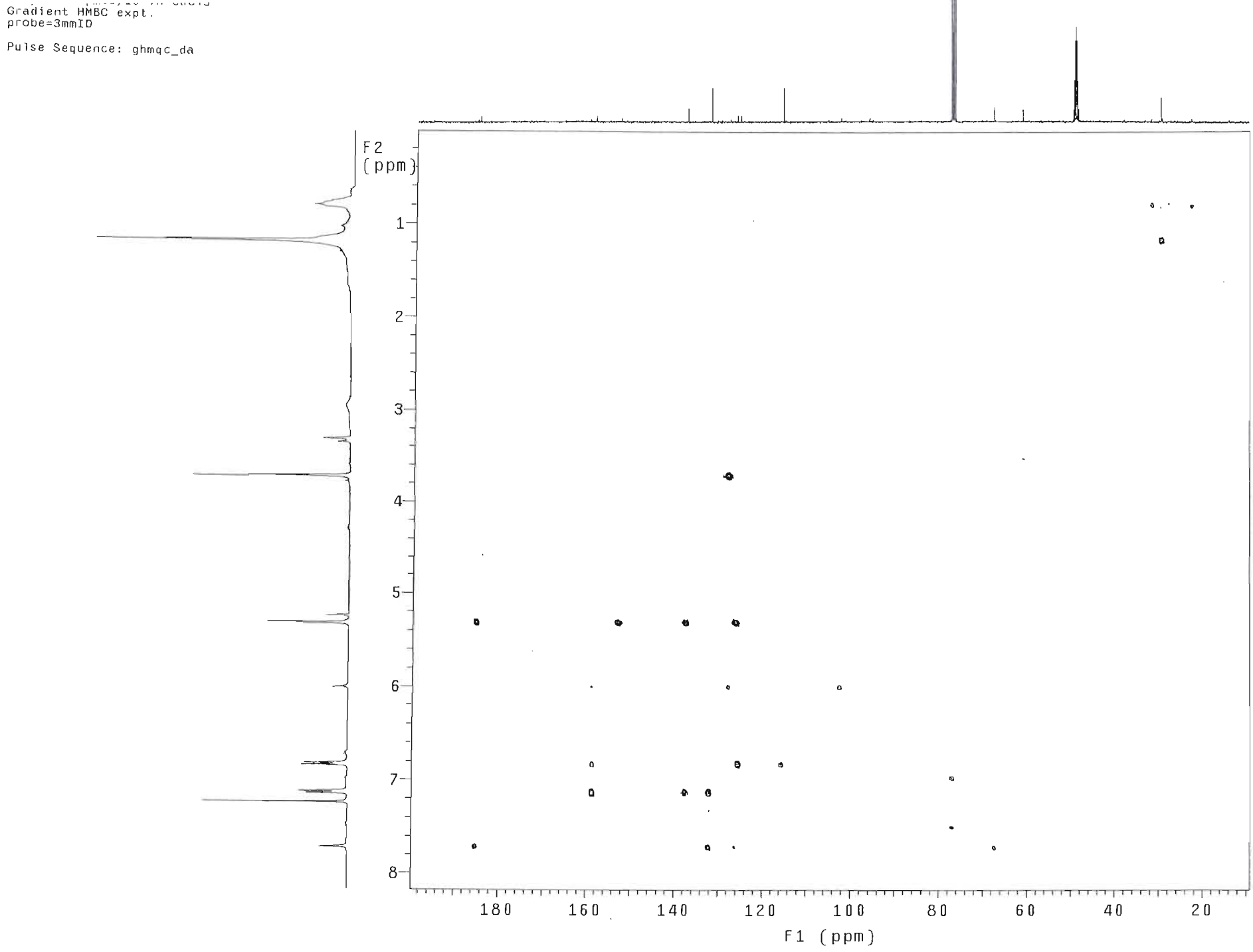

**Spectrum 5b: $^{13}C$ NMR spectrum of compound V ($CDCl_3$) (100 MHz)**



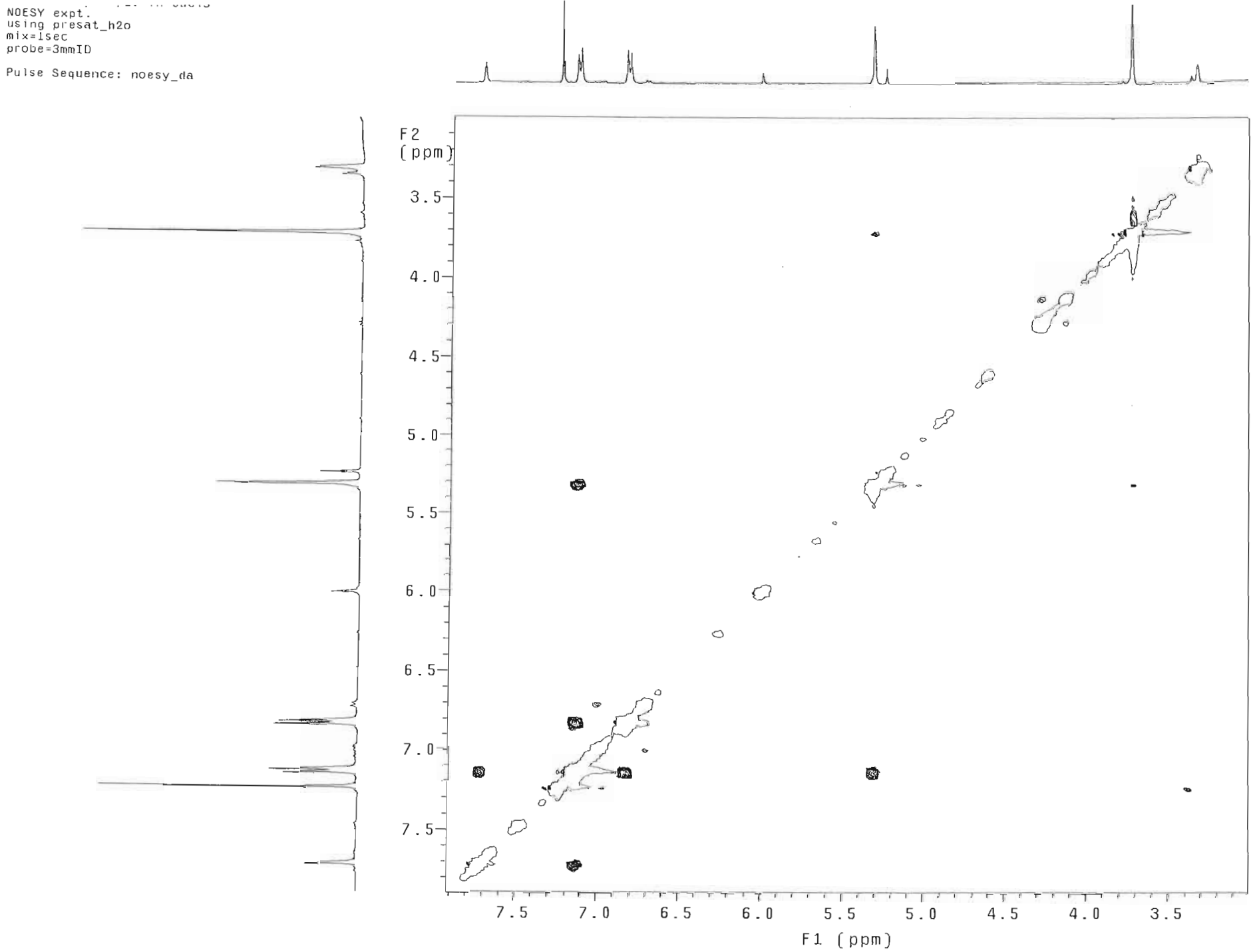

**Spectrum 5c: HSQC spectrum of compound V**



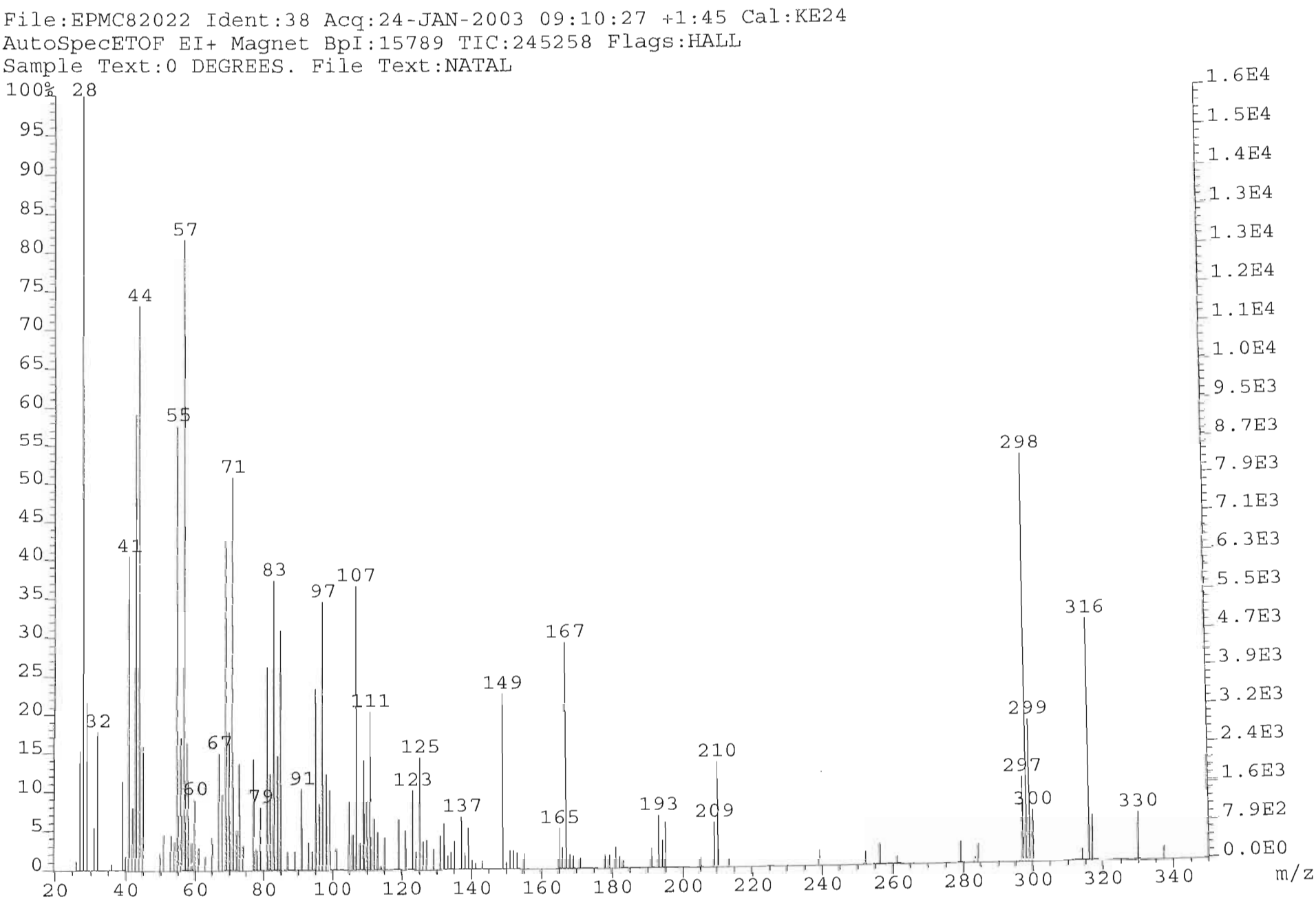

**Spectrum 5d: COSY spectrum of compound V**



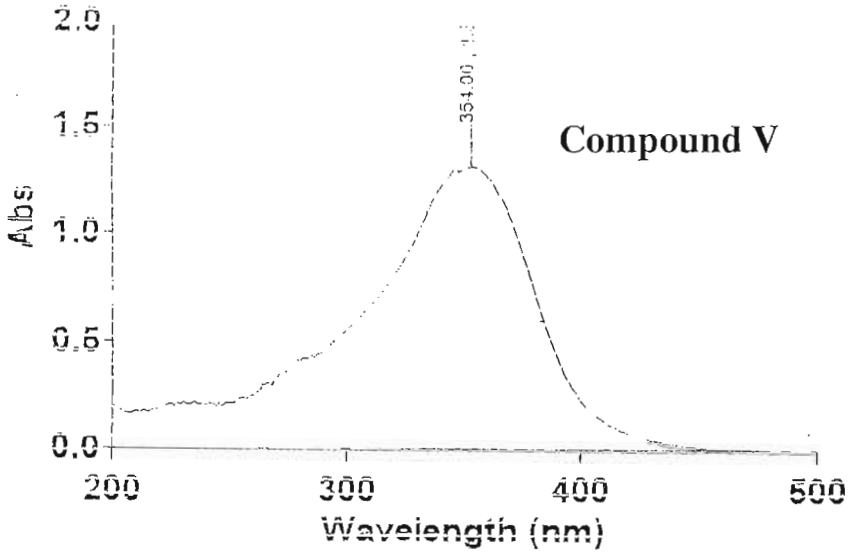

**Spectrum 5e: HMBC spectrum of compound V**

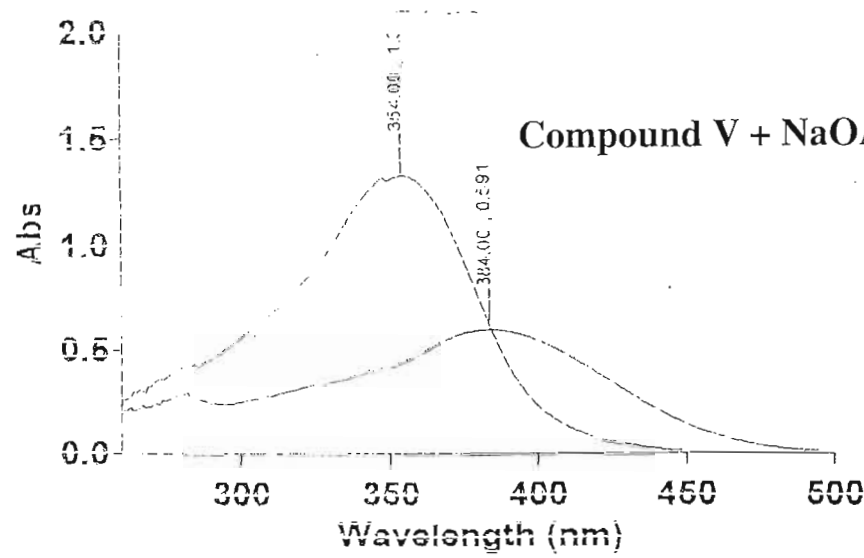


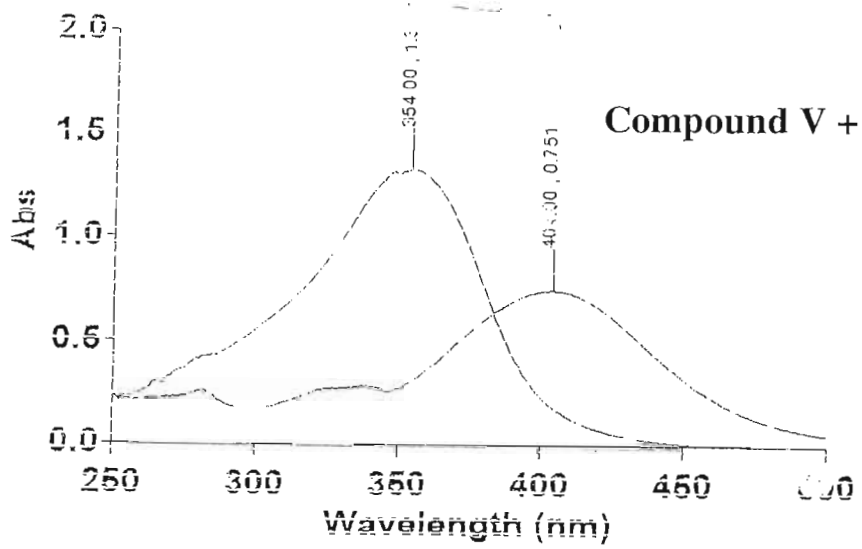

**Spectrum 5f: NOESY spectrum of compound V**



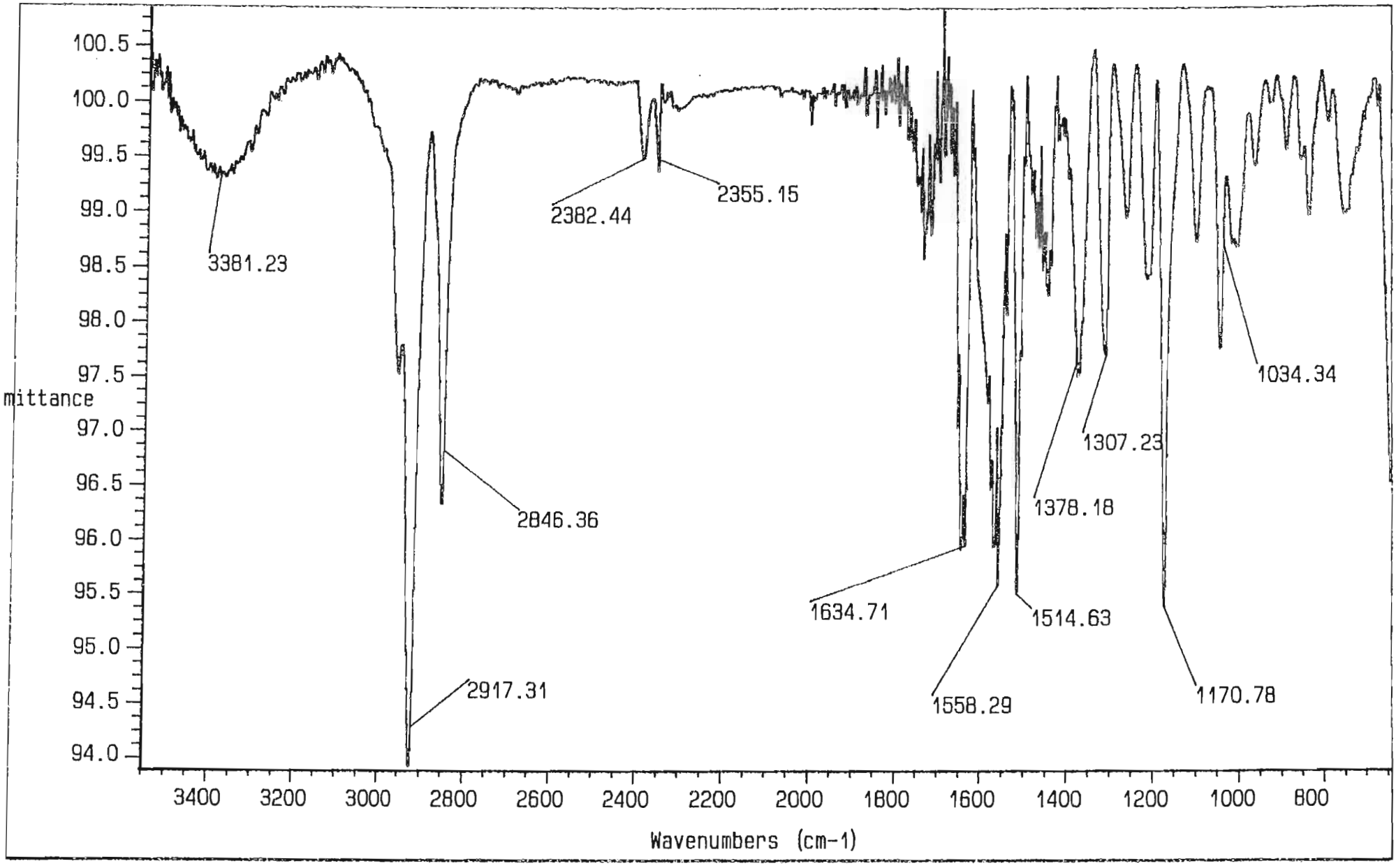

**Spectrum 5g: Mass spectrum of compound V**



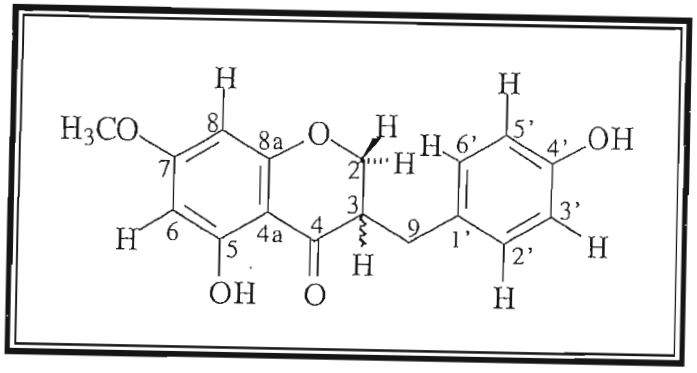




**Spectrum 5h: Ultra-violet spectrum of compound V**

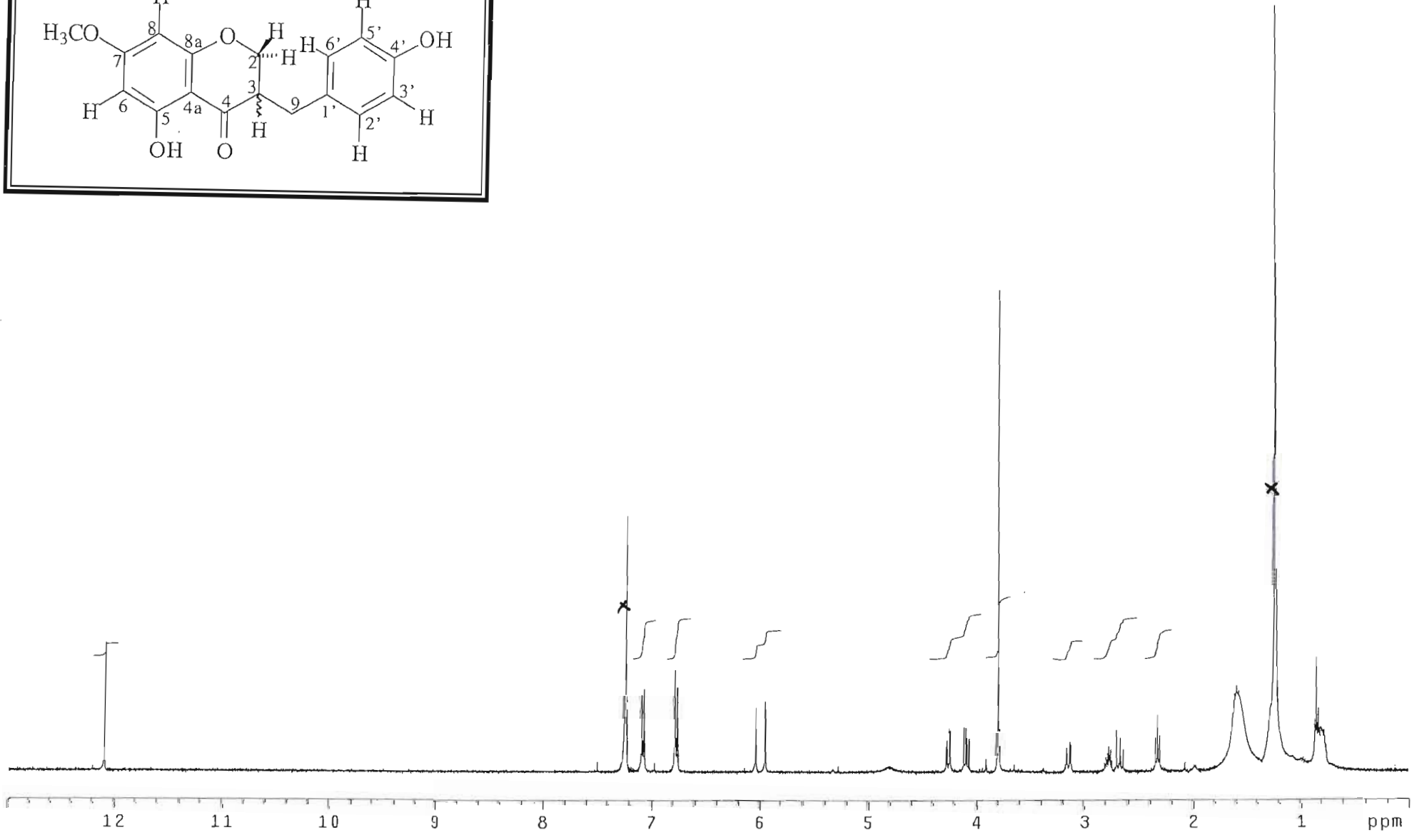



**Spectrum 5i: Infra-red spectrum of compound V**

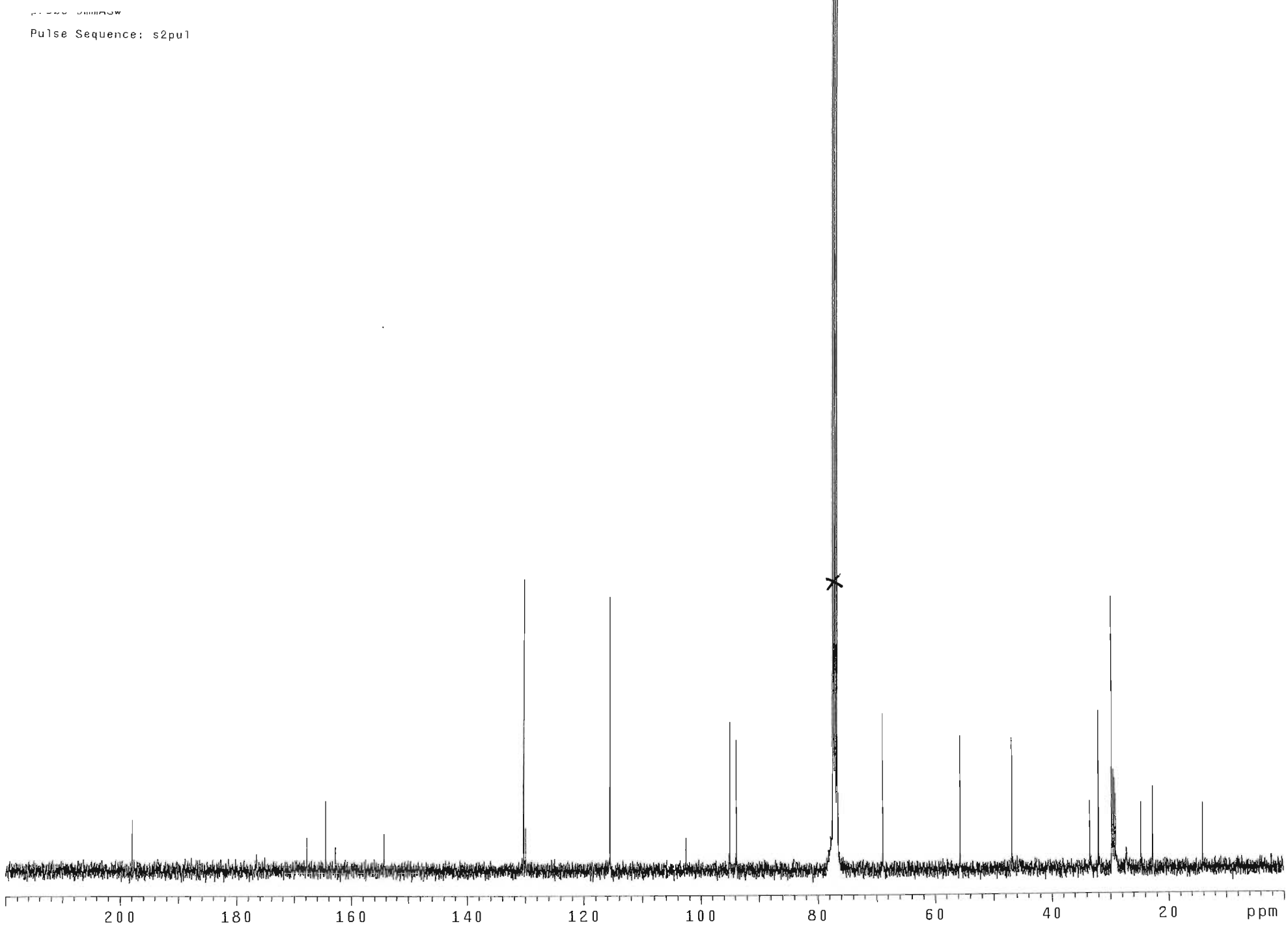
Pulse Sequence: s2pul



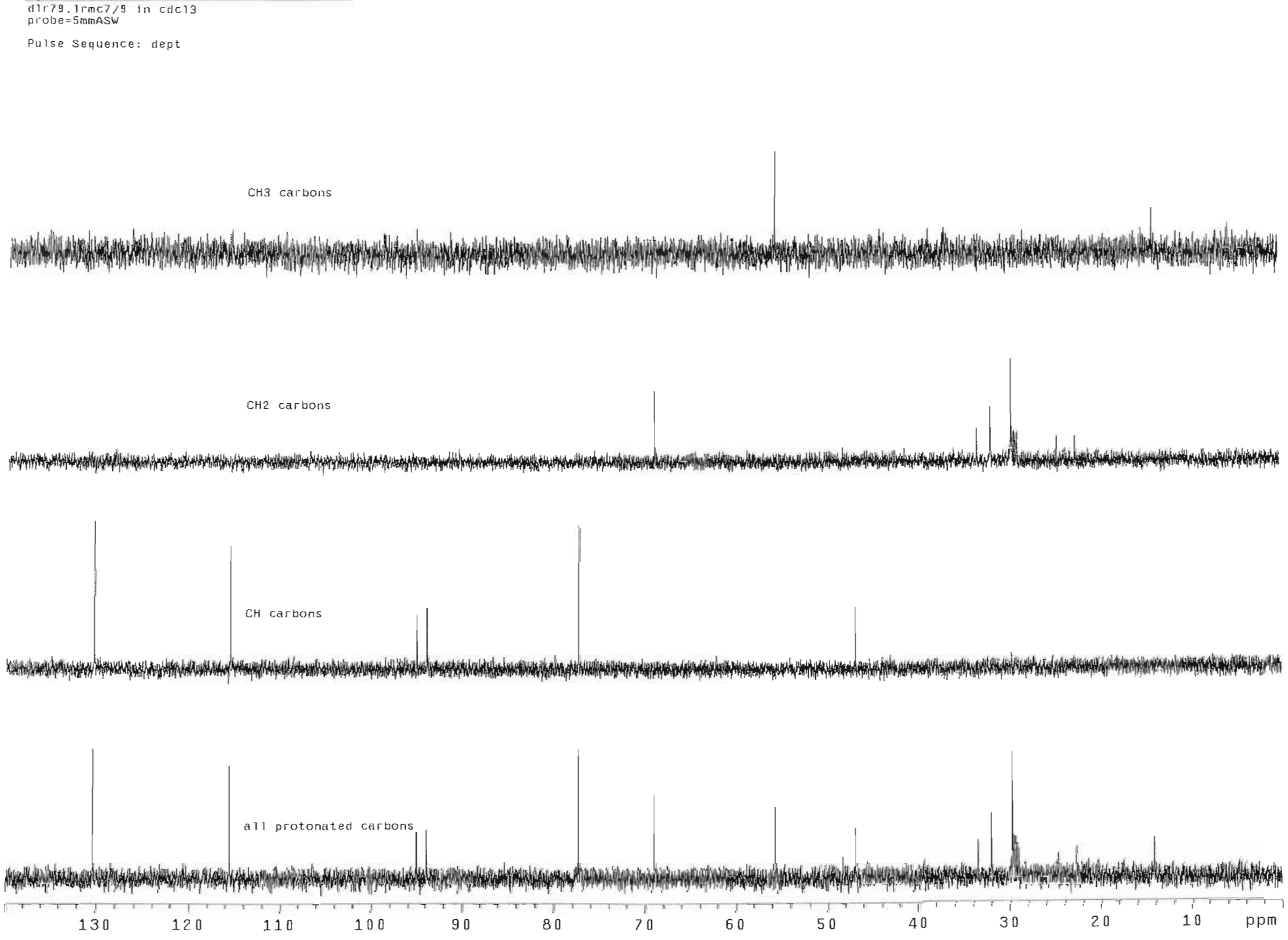


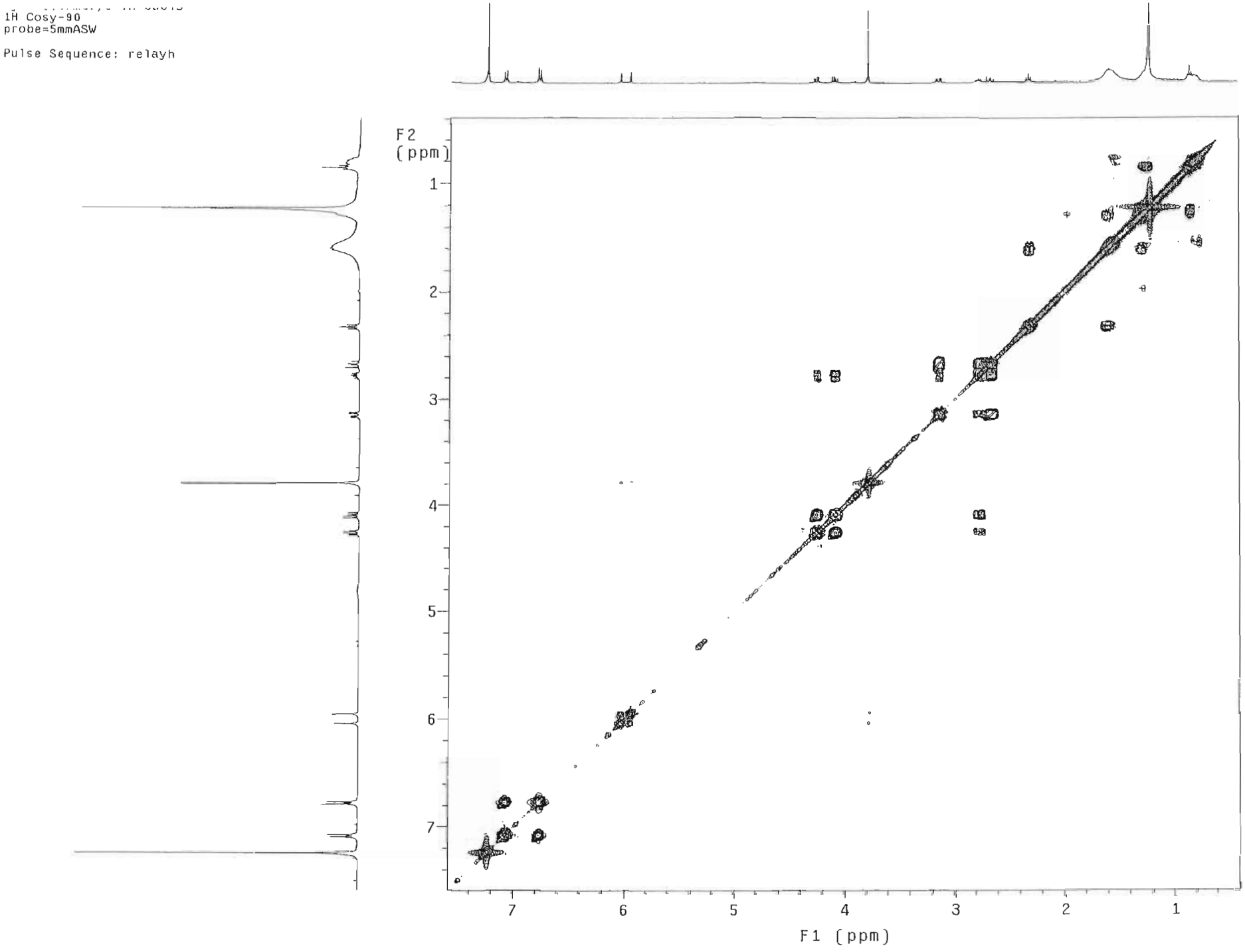

**Spectrum 6a: $^1$H NMR spectrum of compound VI ($CDCl_3$) (400 MHz)**



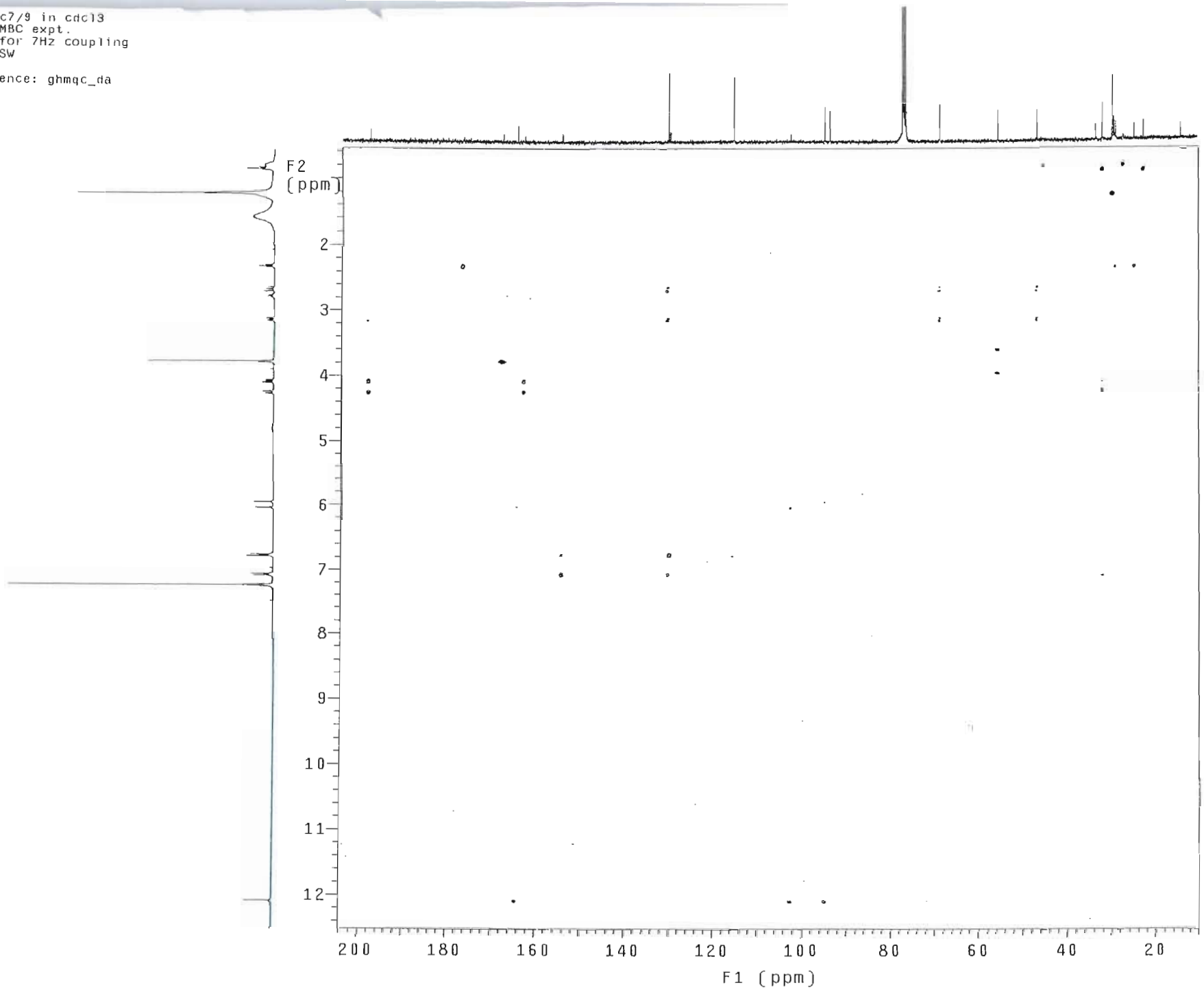

**Spectrum 6b: $^{13}C$ NMR spectrum of compound VI ($CDCl_3$) (100 MHz)**



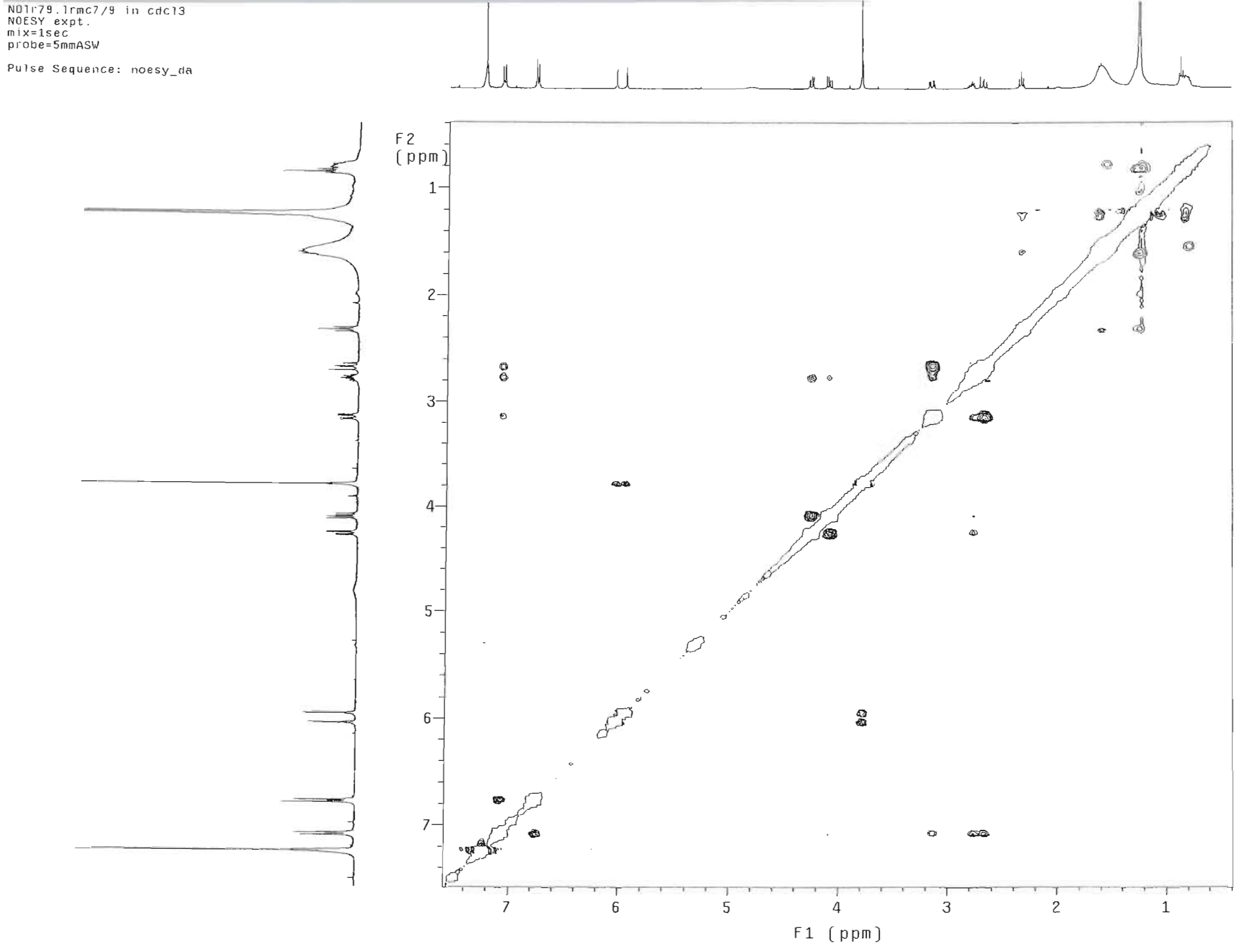

**Spectrum 6c: ADEPT spectrum of compound VI**



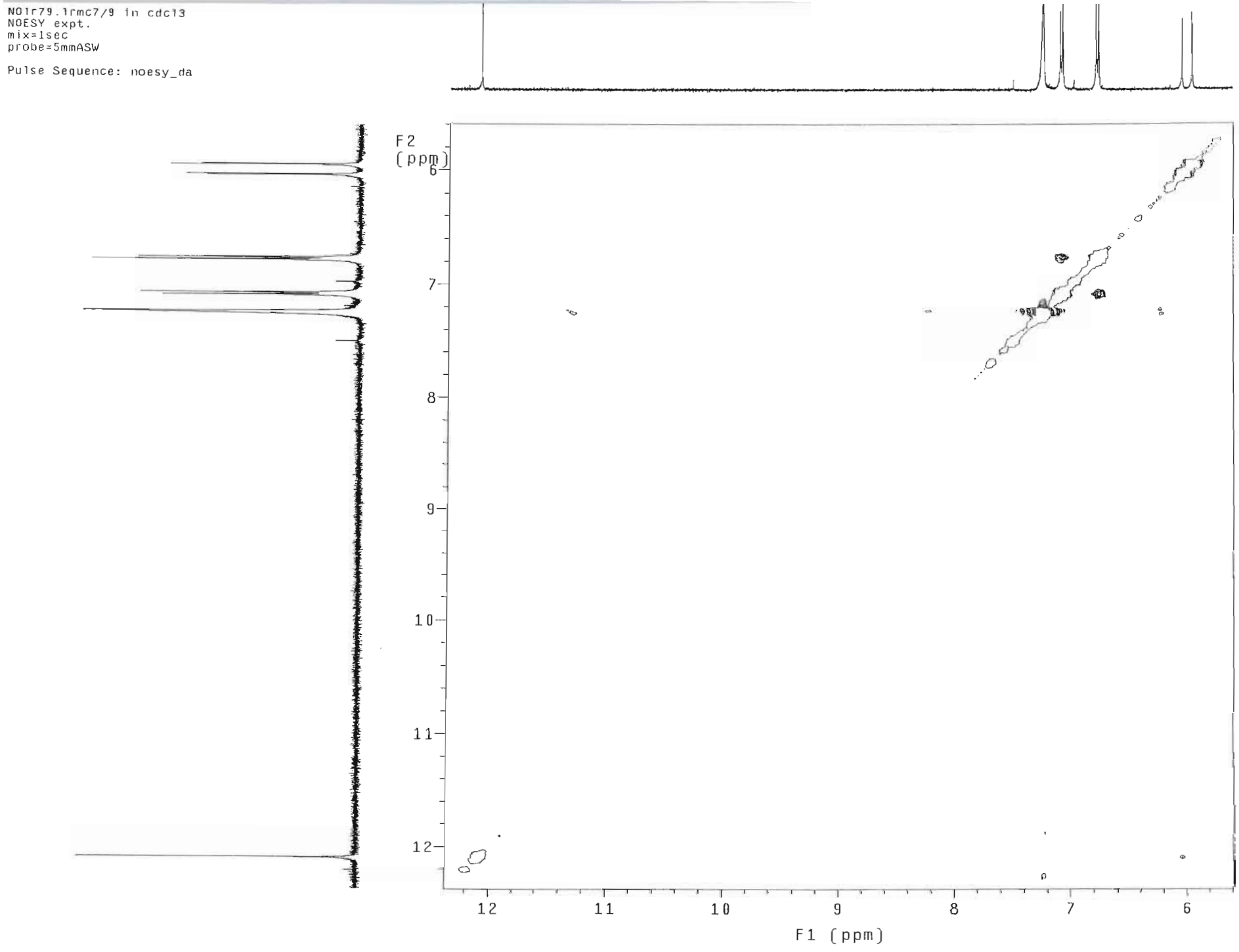

**Spectrum 6e: COSY spectrum of compound VI**



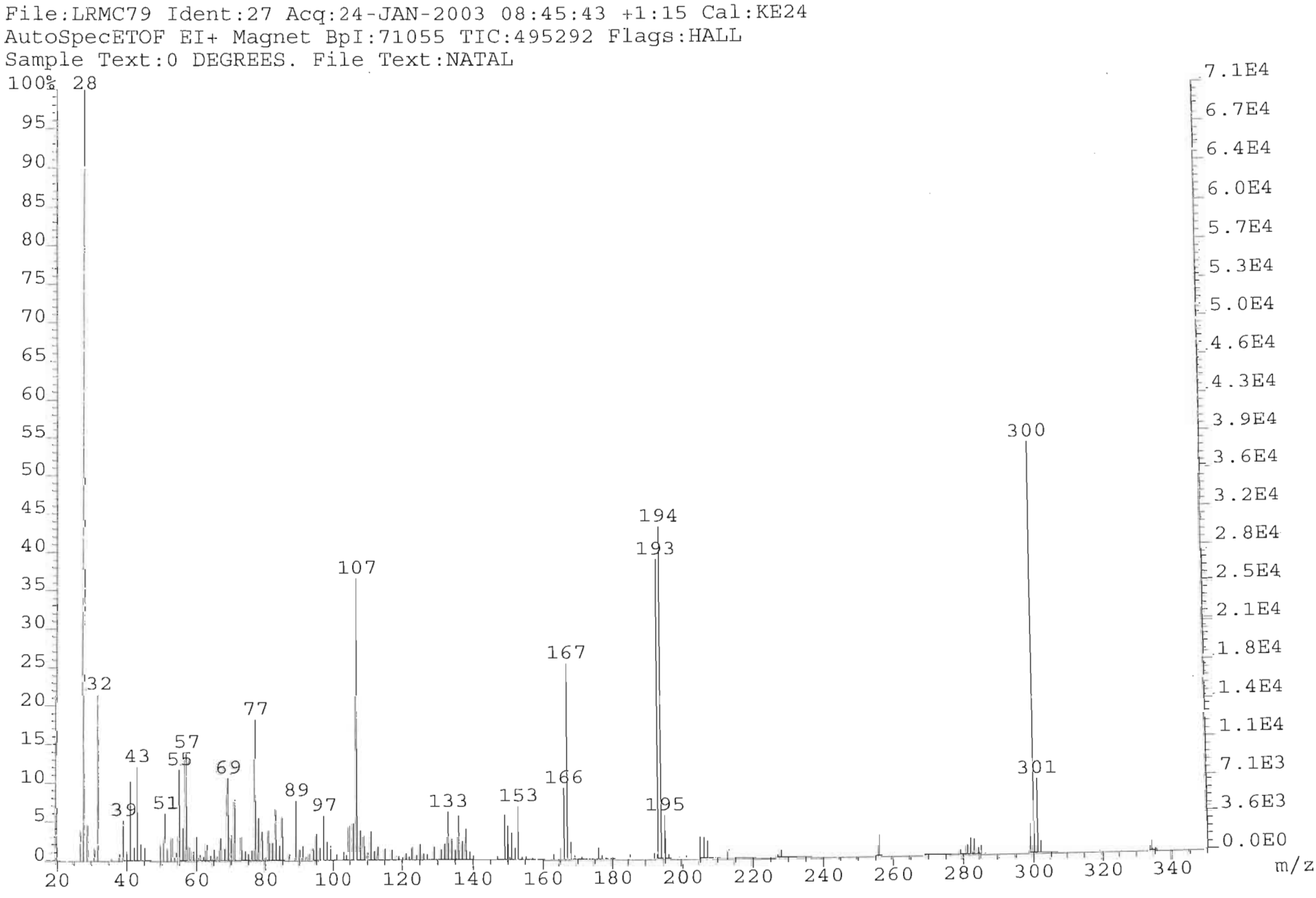

**Spectrum 6f: HMBC spectrum of compound VI**



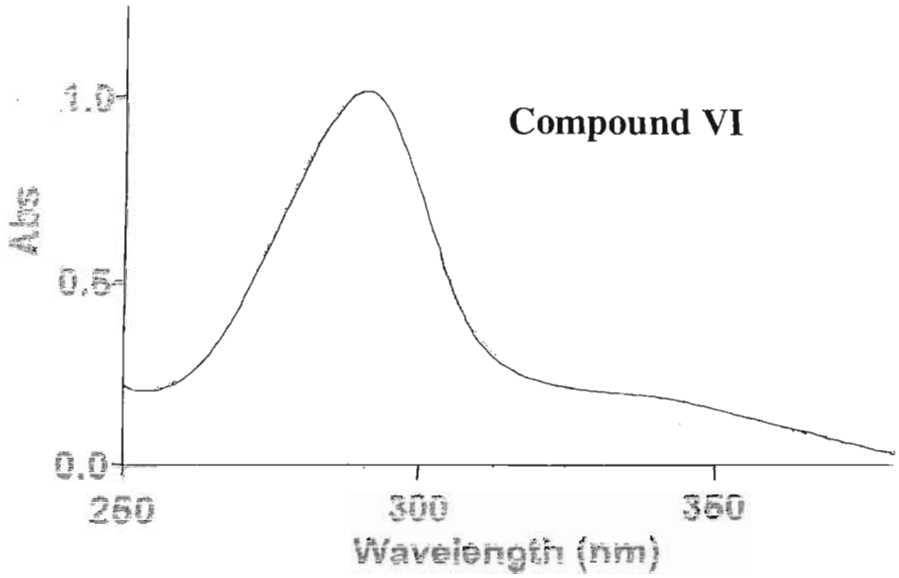

**Spectrum 6g: NOESY spectrum of compound VI**

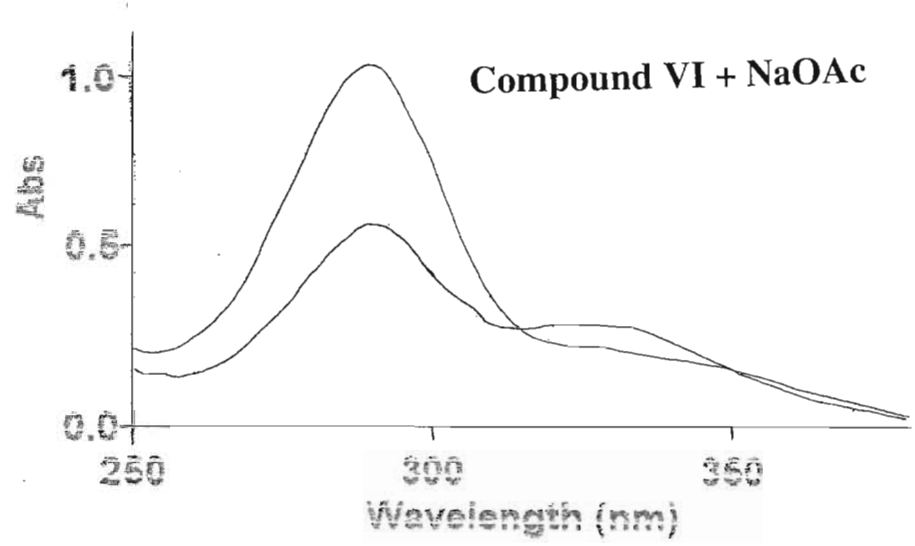


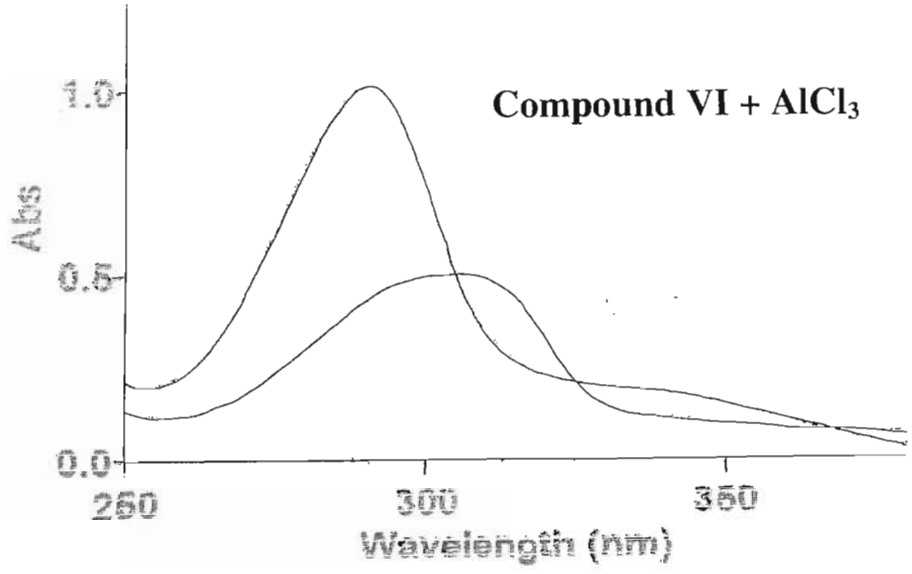

**Spectrum 6h: NOESY spectrum of compound VI**



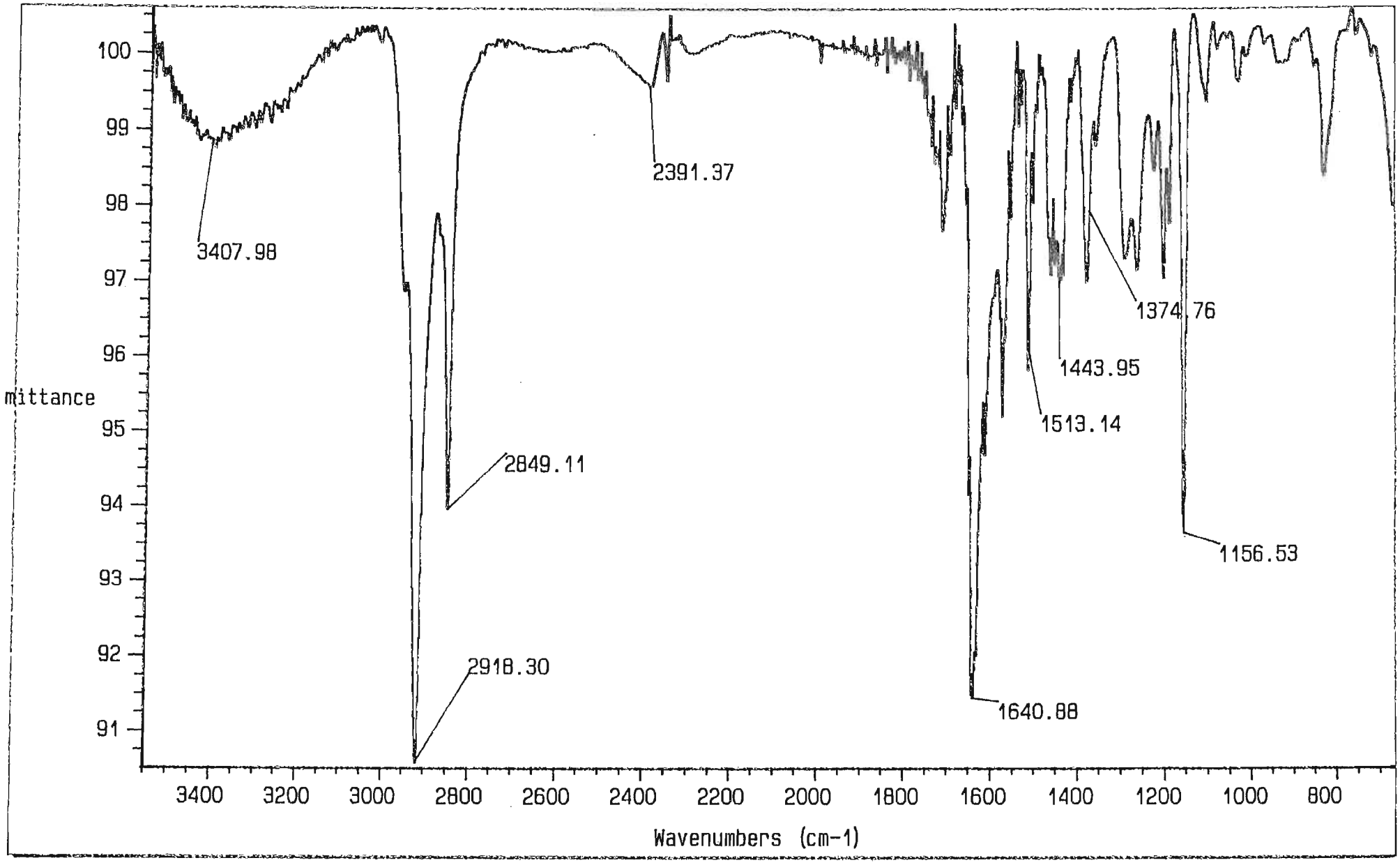

**Spectrum 6i: Mass spectrum of compound VI**



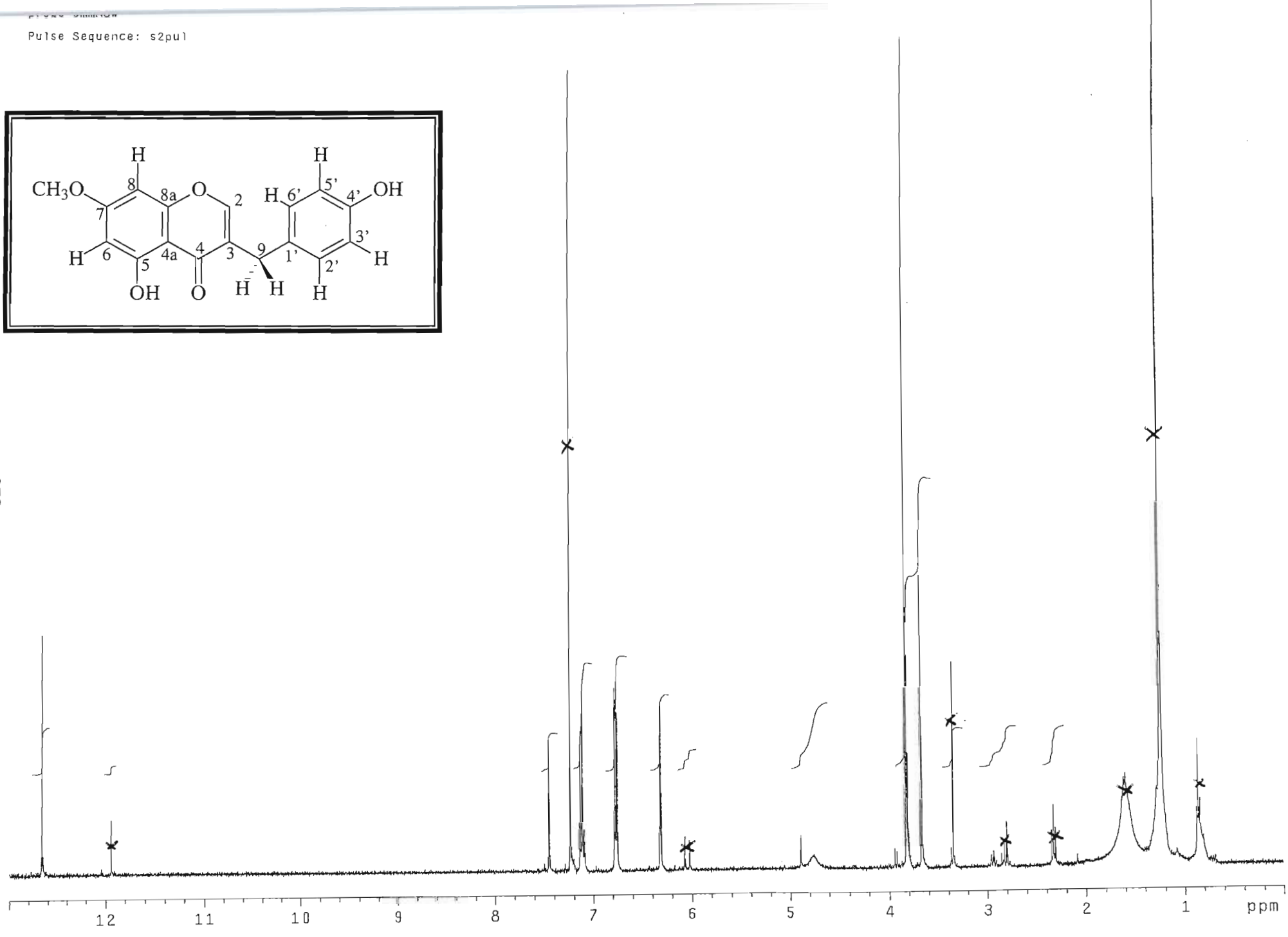

**Spectrum 6j: Ultra-violet spectrum of compound VI**



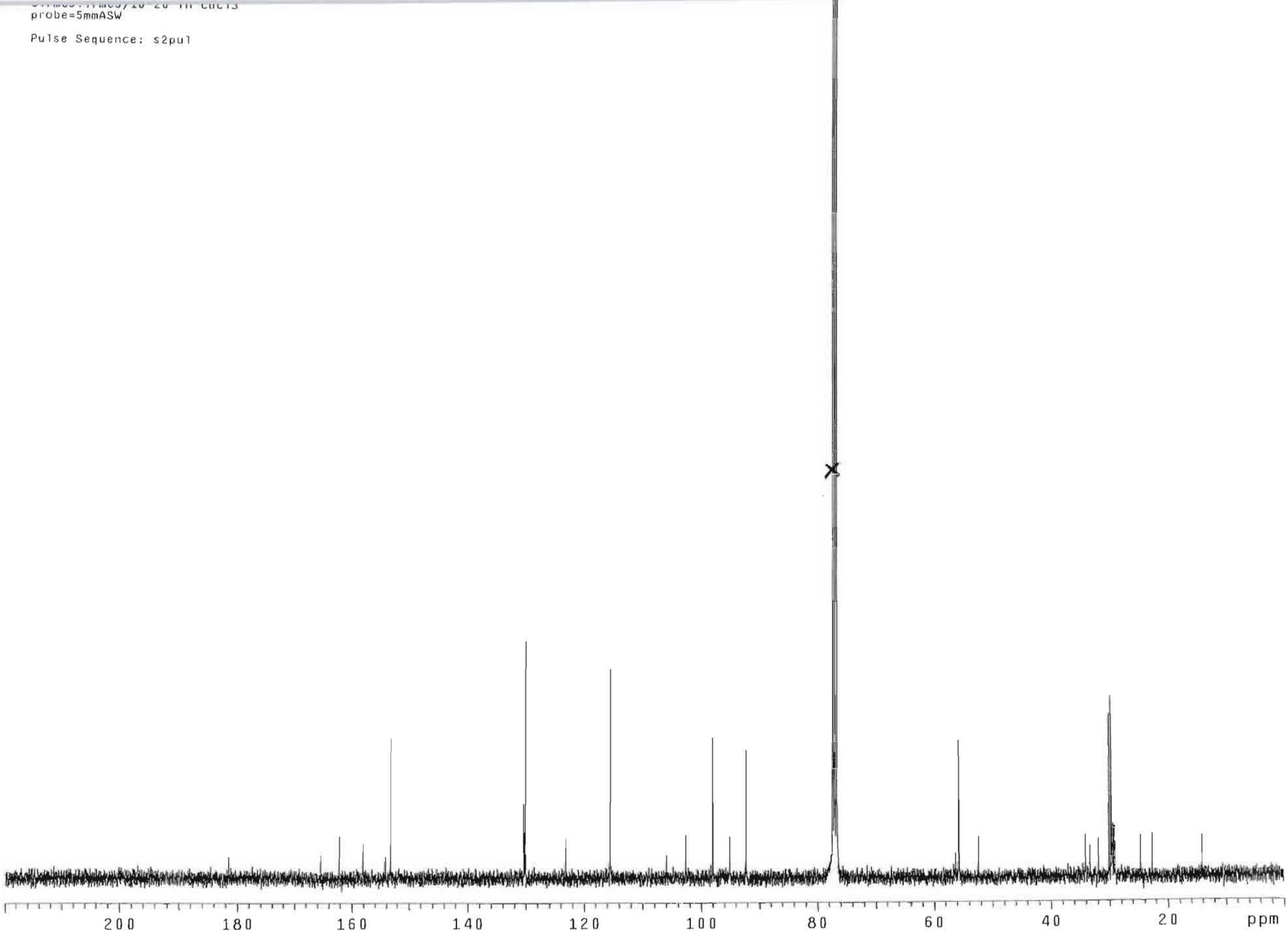

**Spectrum 6k: Infra-red spectrum of compound VI**

Pulse Sequence: s2pul

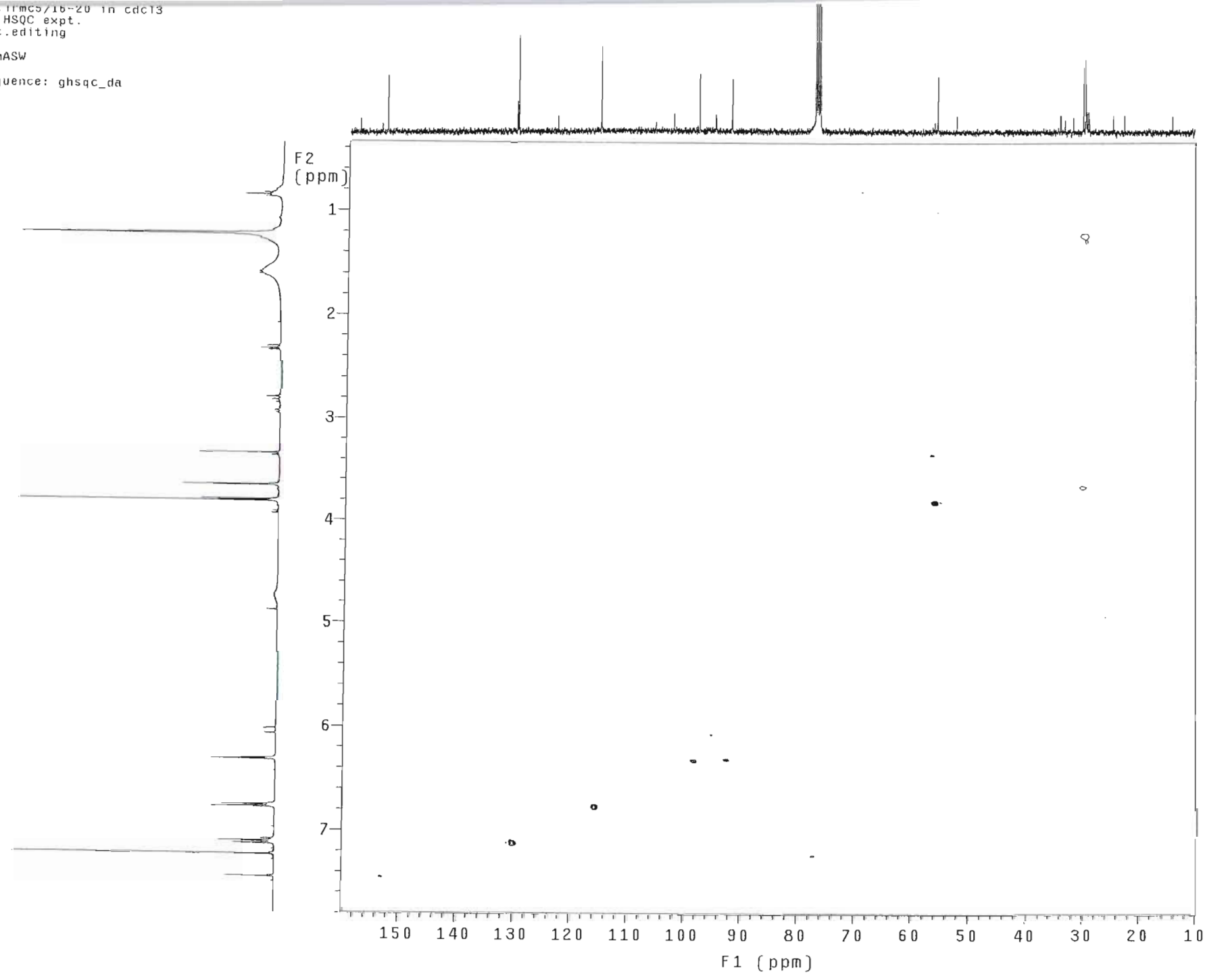



**Spectrum 7a: $^1$H NMR spectrum of compound VII ($CDCl_3$) (400 MHz)**



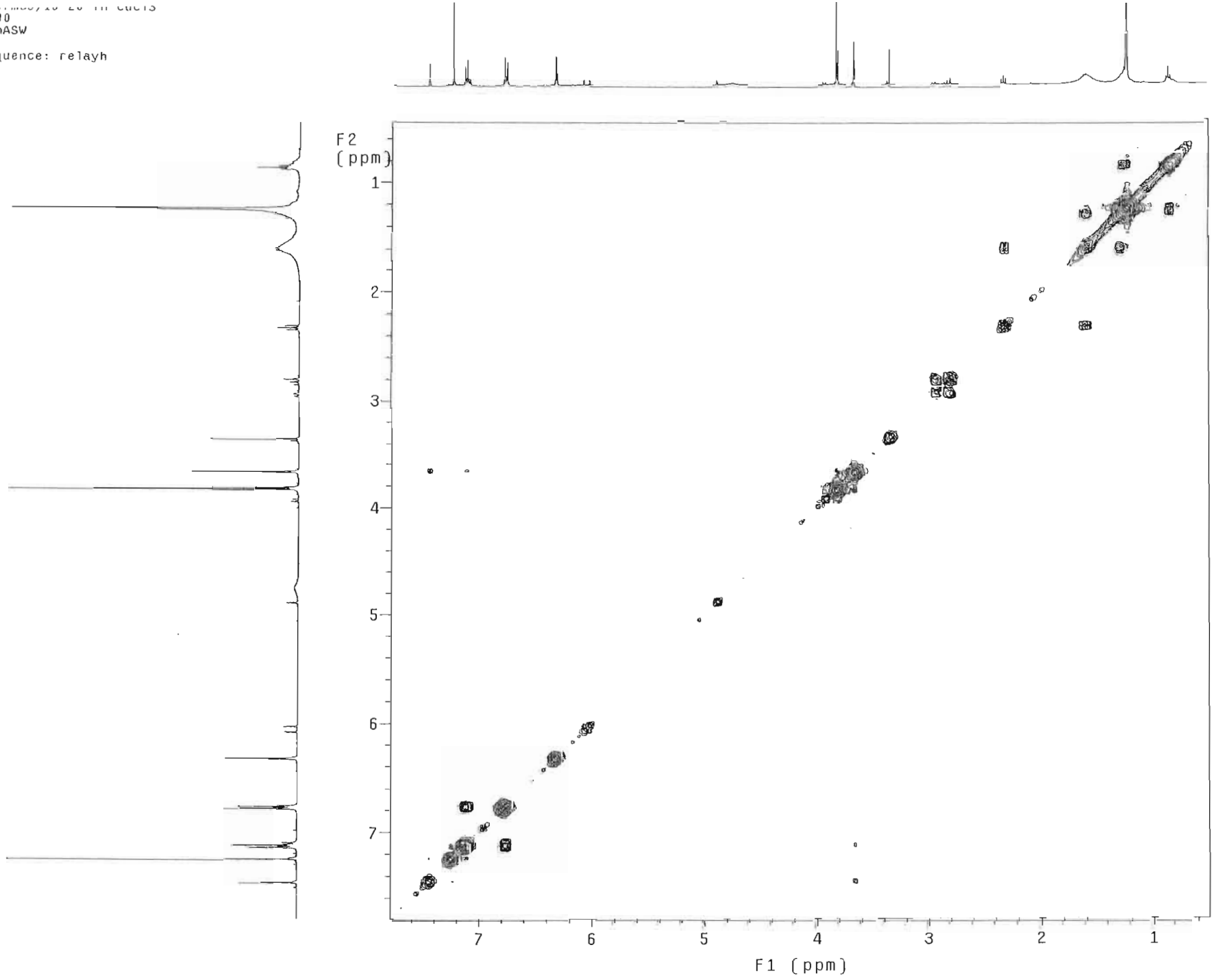

**Spectrum 7b: $^{13}C$ NMR spectrum of compound VII ($CDCl_3$) (100 MHz)**



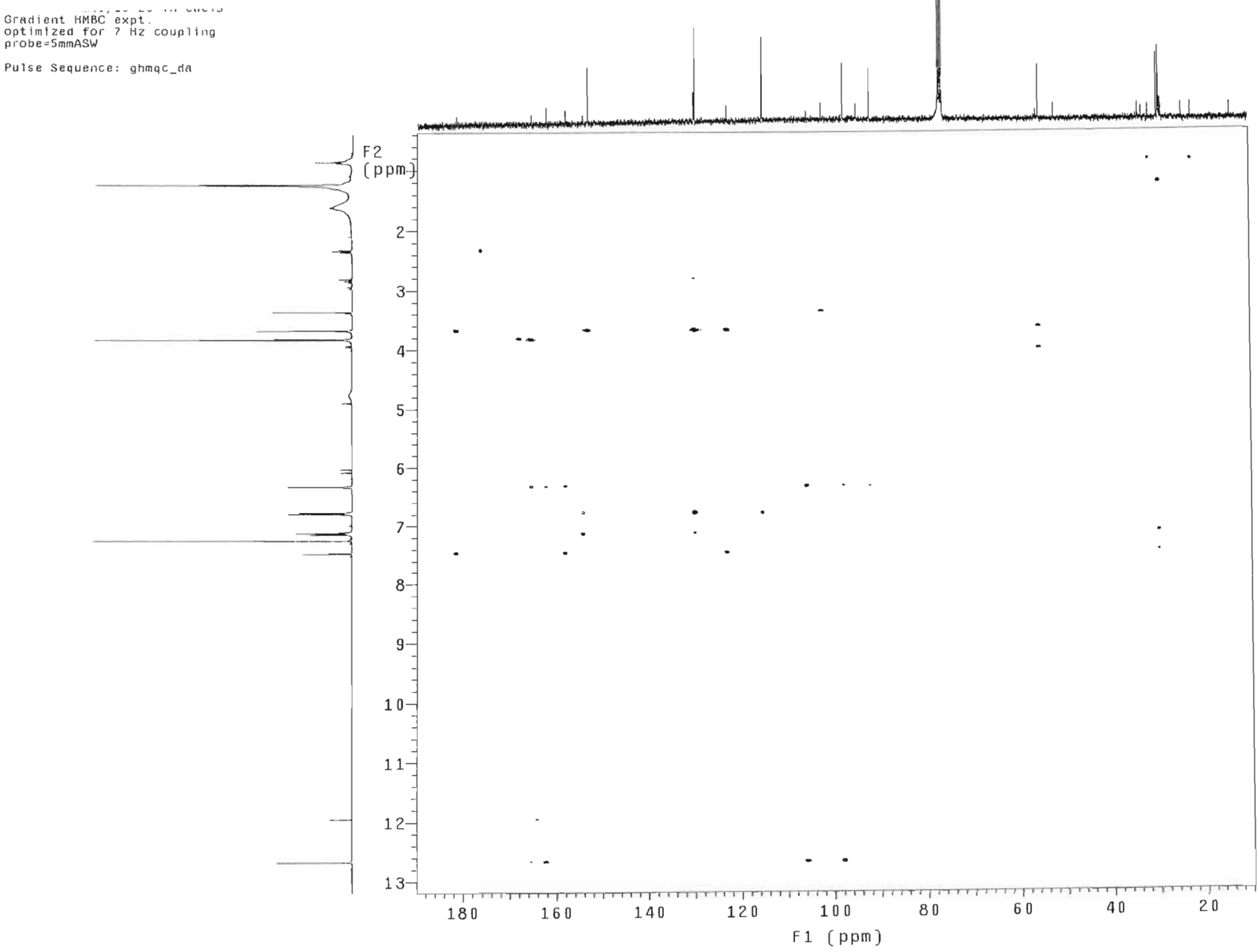

**Spectrum 7c: HSQC spectrum of compound VII**

1H Cosy-90
probe=5mmASW

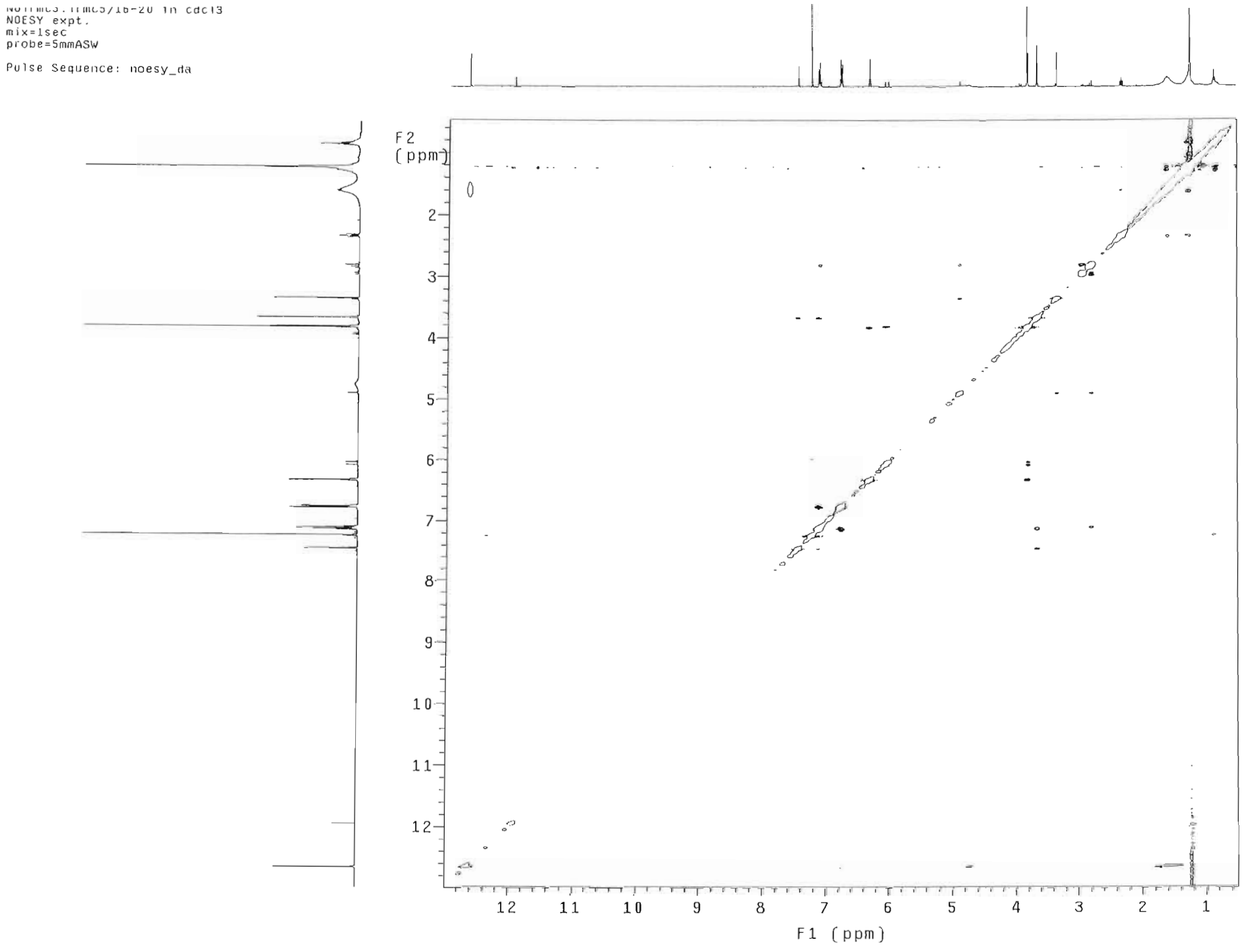
Pulse Sequence: relayh



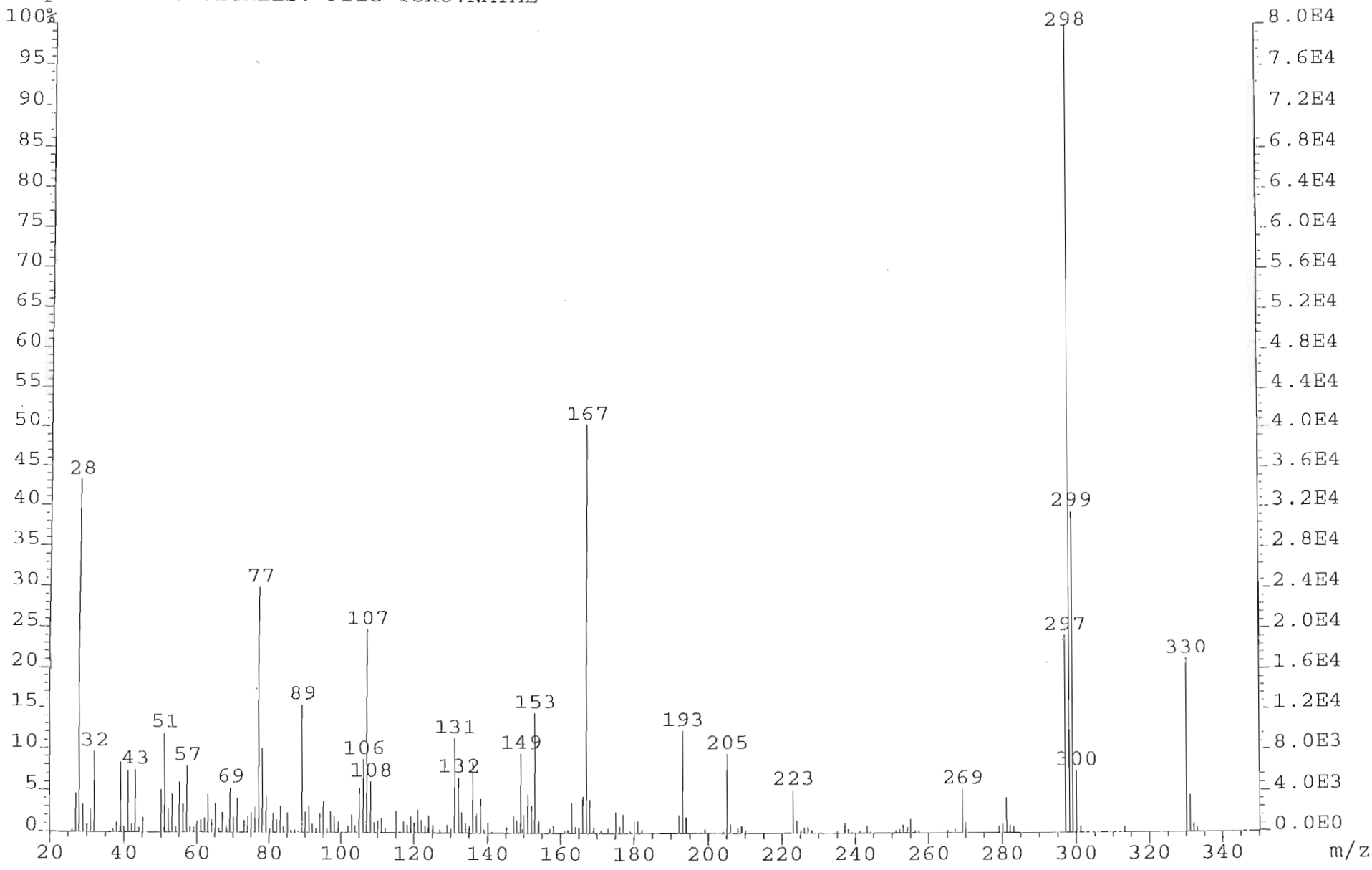

**Spectrum 7d: COSY spectrum of compound VII**



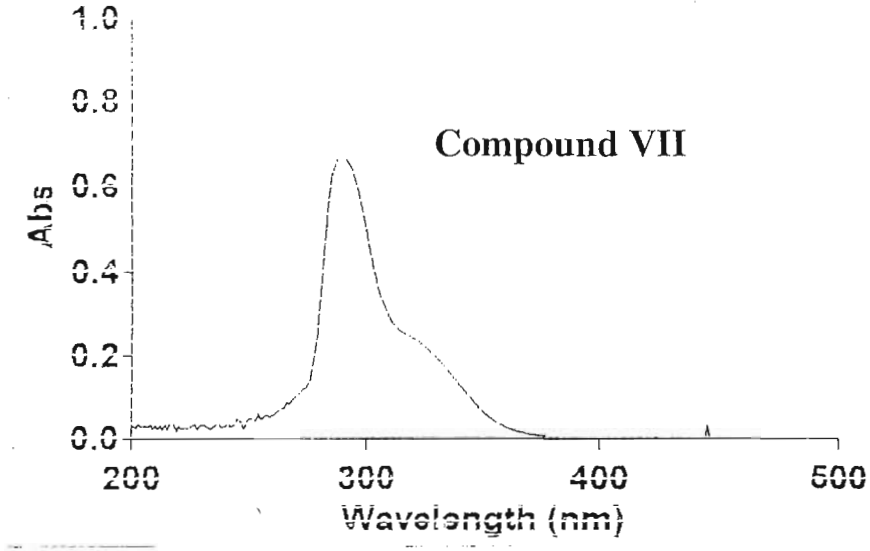

**Spectrum 7e: HMBC spectrum of compound VII**

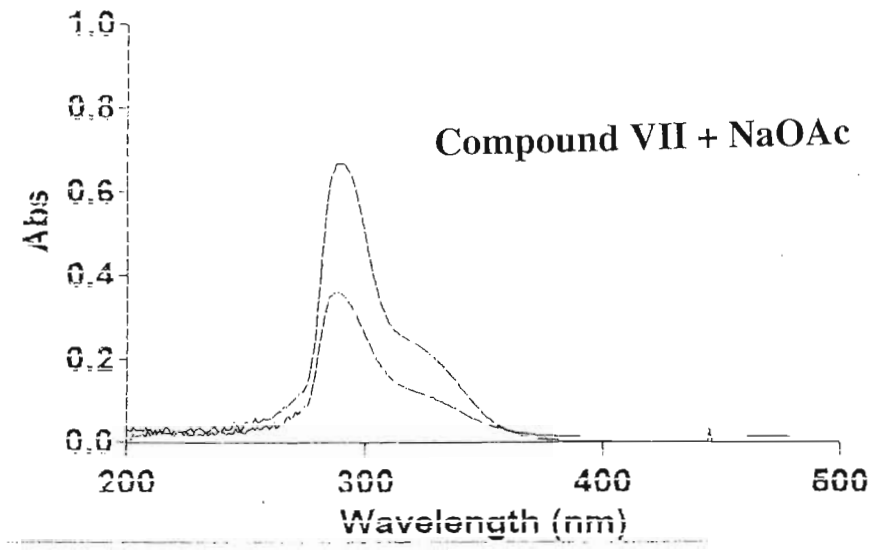


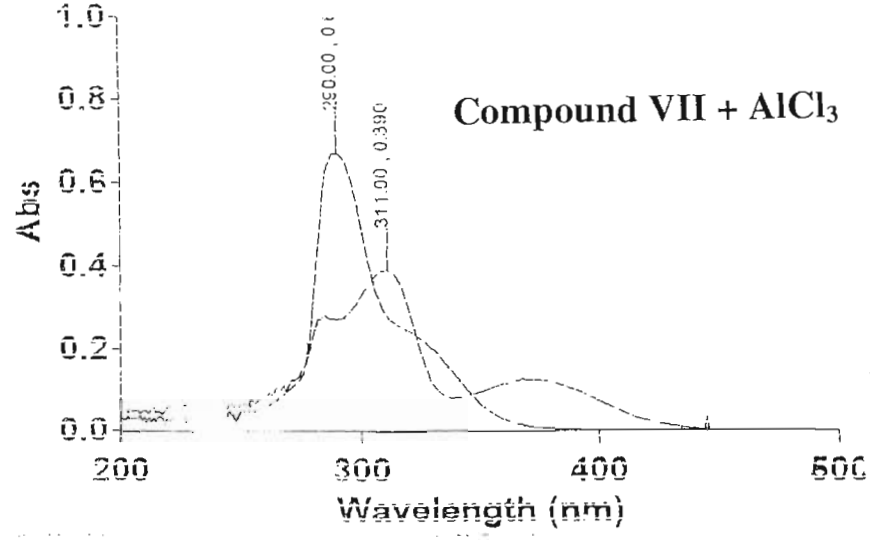

**Spectrum 7f: NOESY spectrum of compound VII**



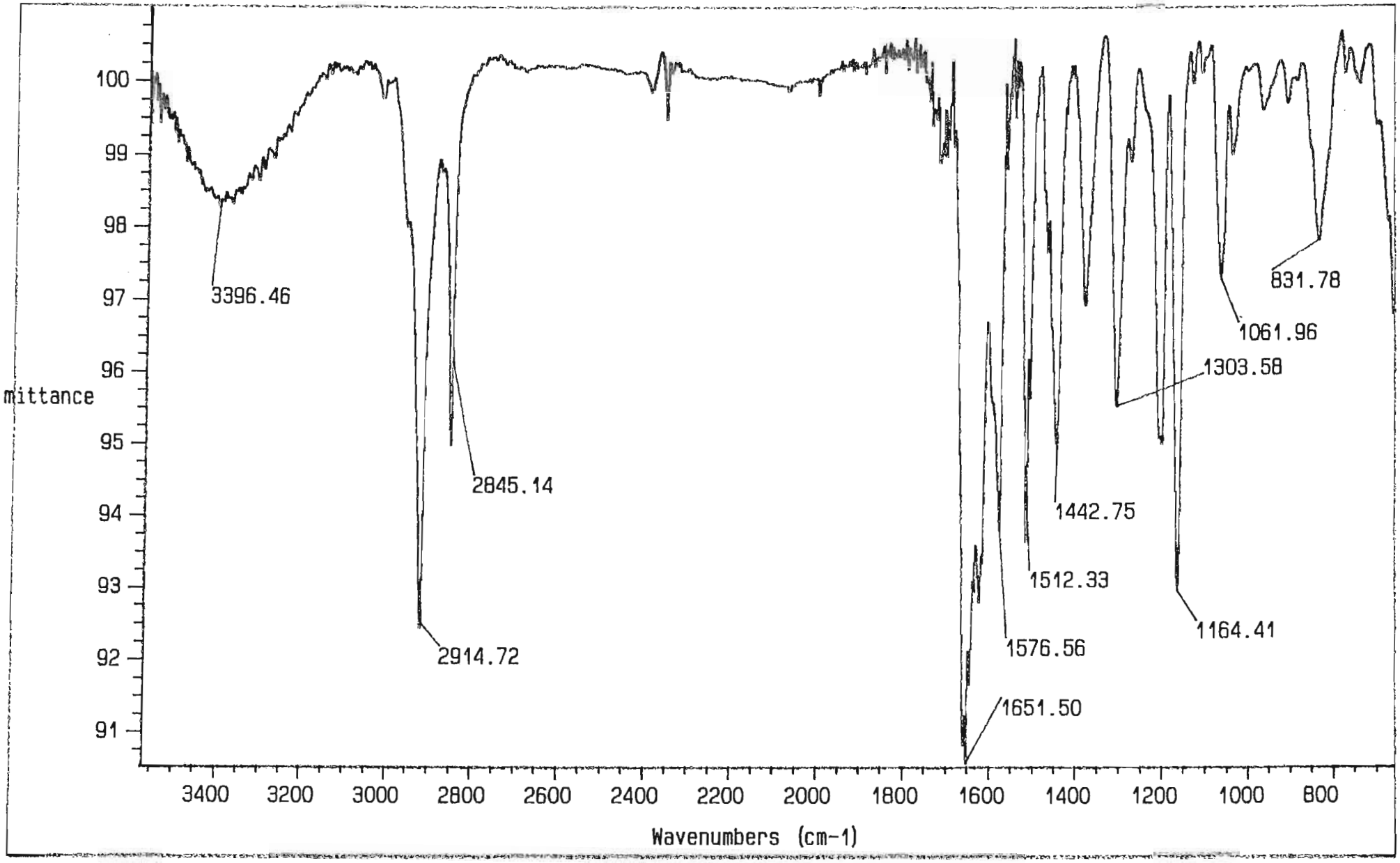

**Spectrum 7g: Mass spectrum of compound VII**




**Spectrum 7h: Ultra-violet spectrum of compound VII**

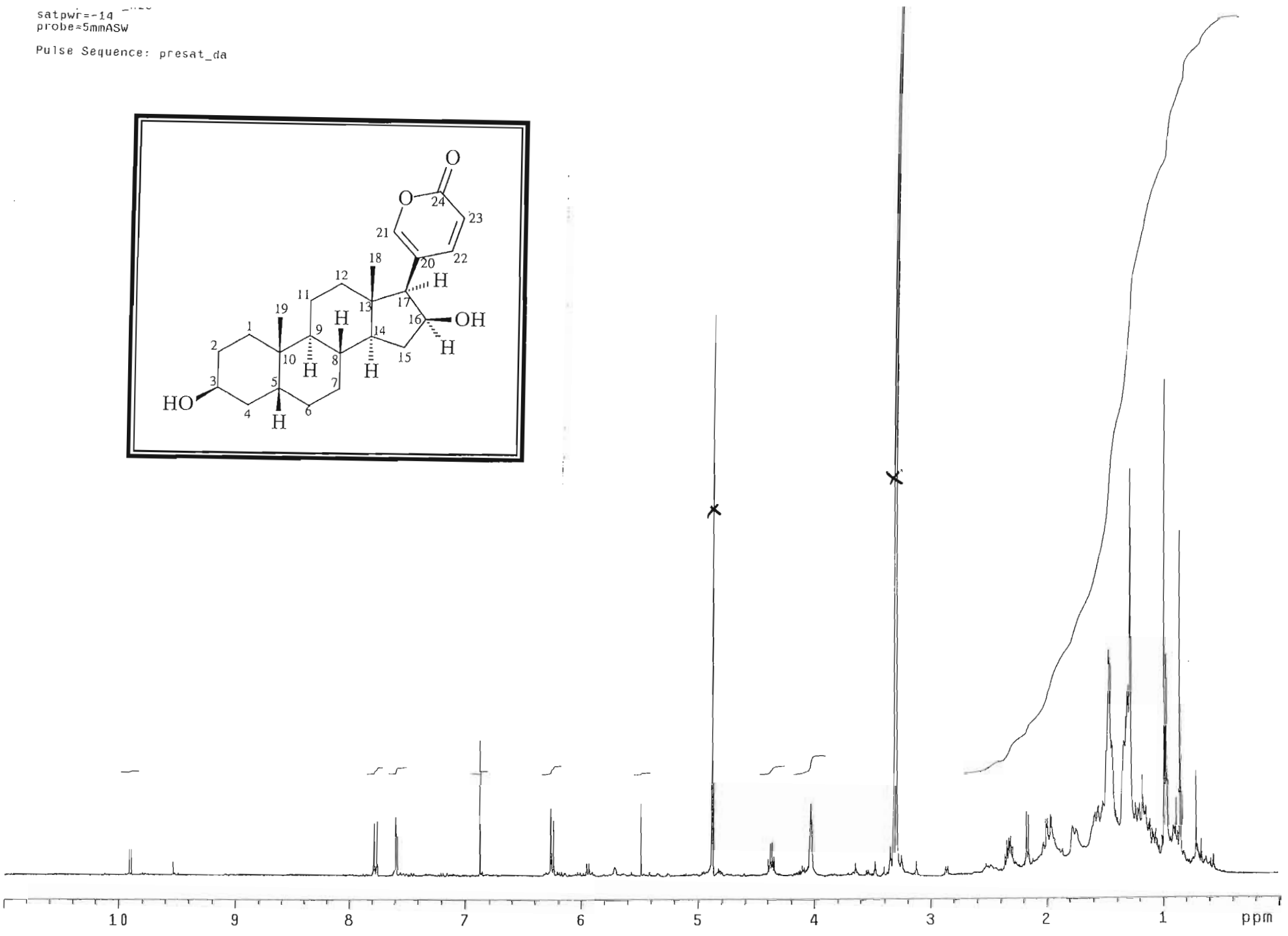



**Spectrum 7i: Infra-red spectrum of compound VII**

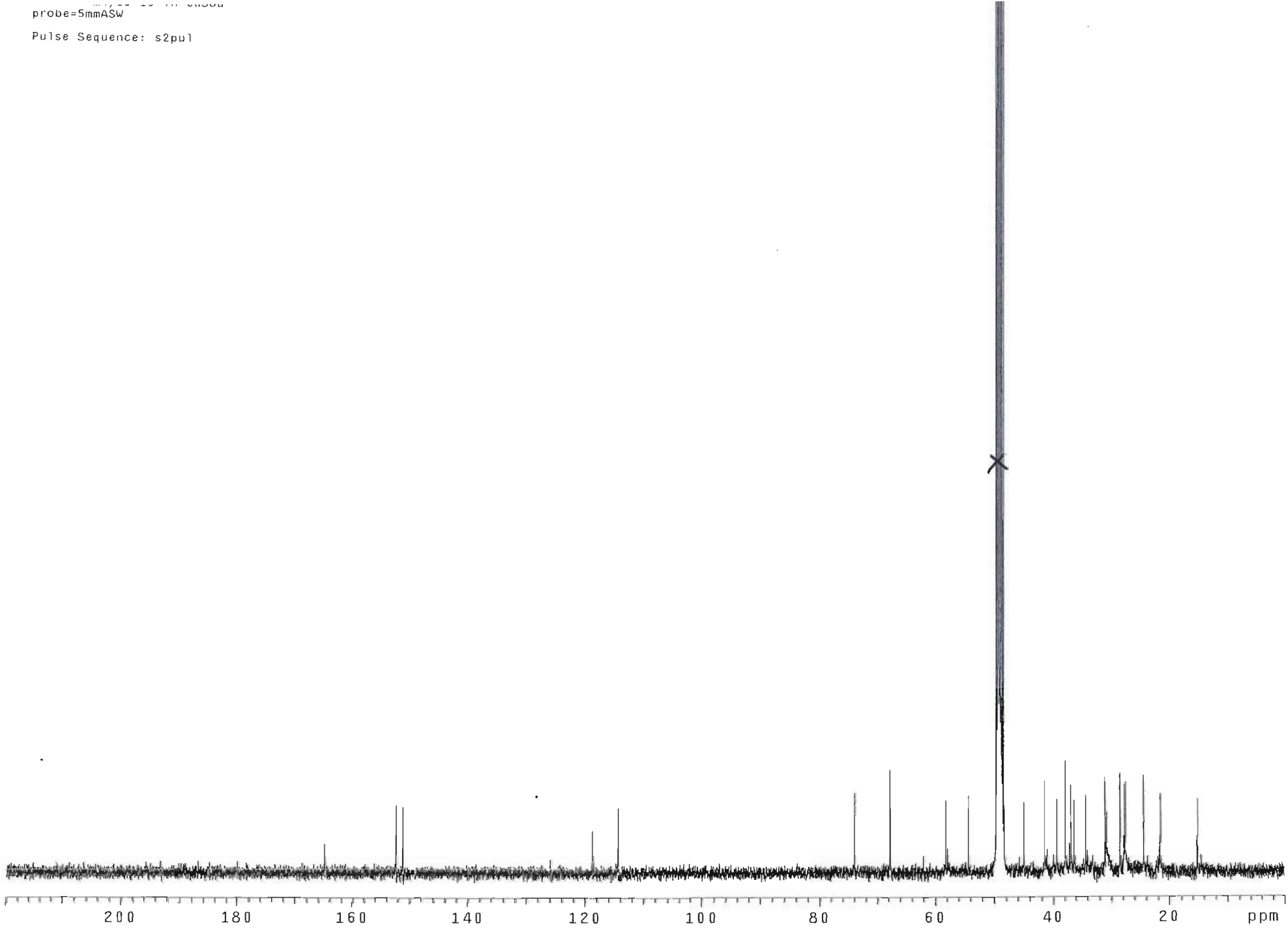
satpwr=-14
probe=5mmASW

Pulse Sequence: presat_da

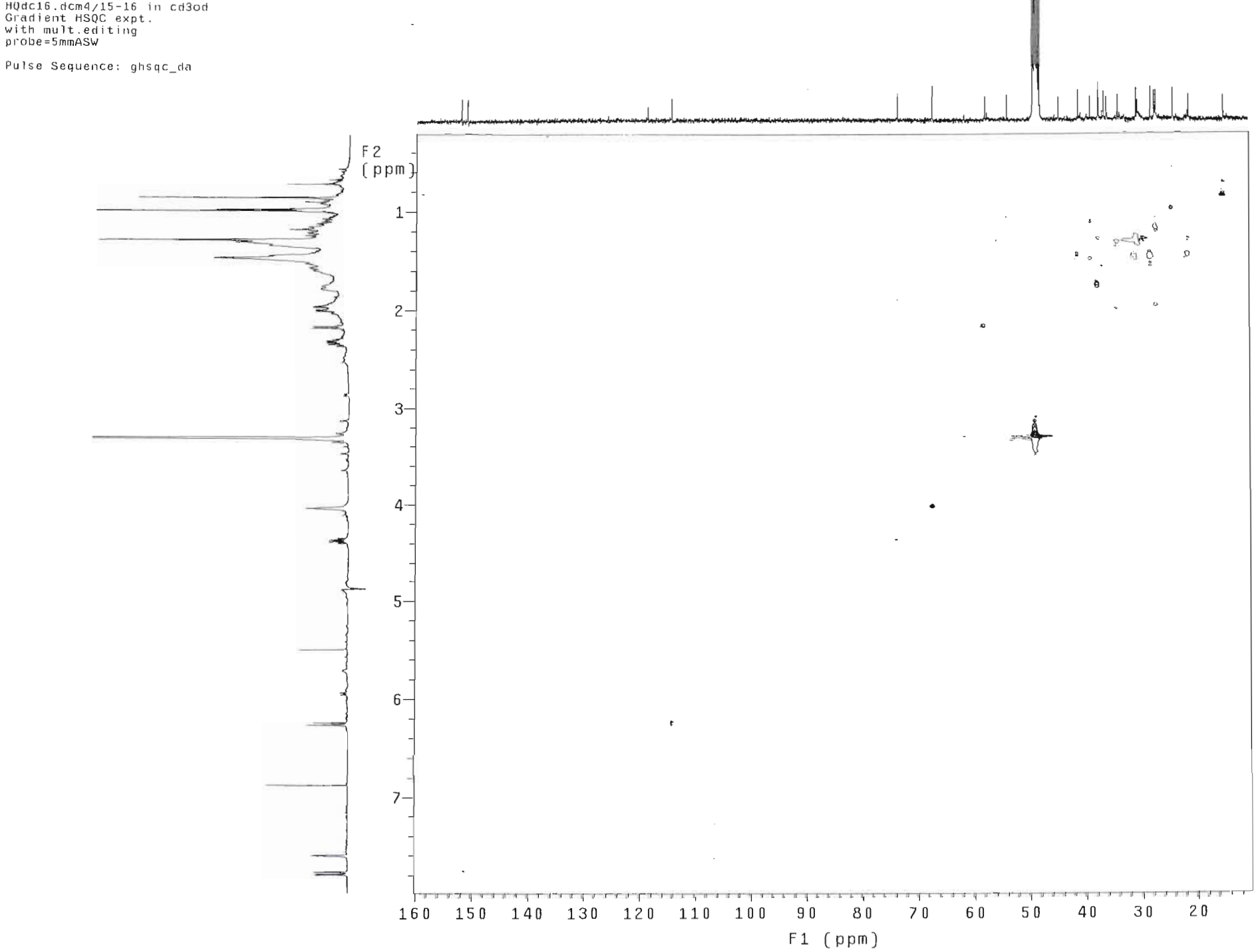


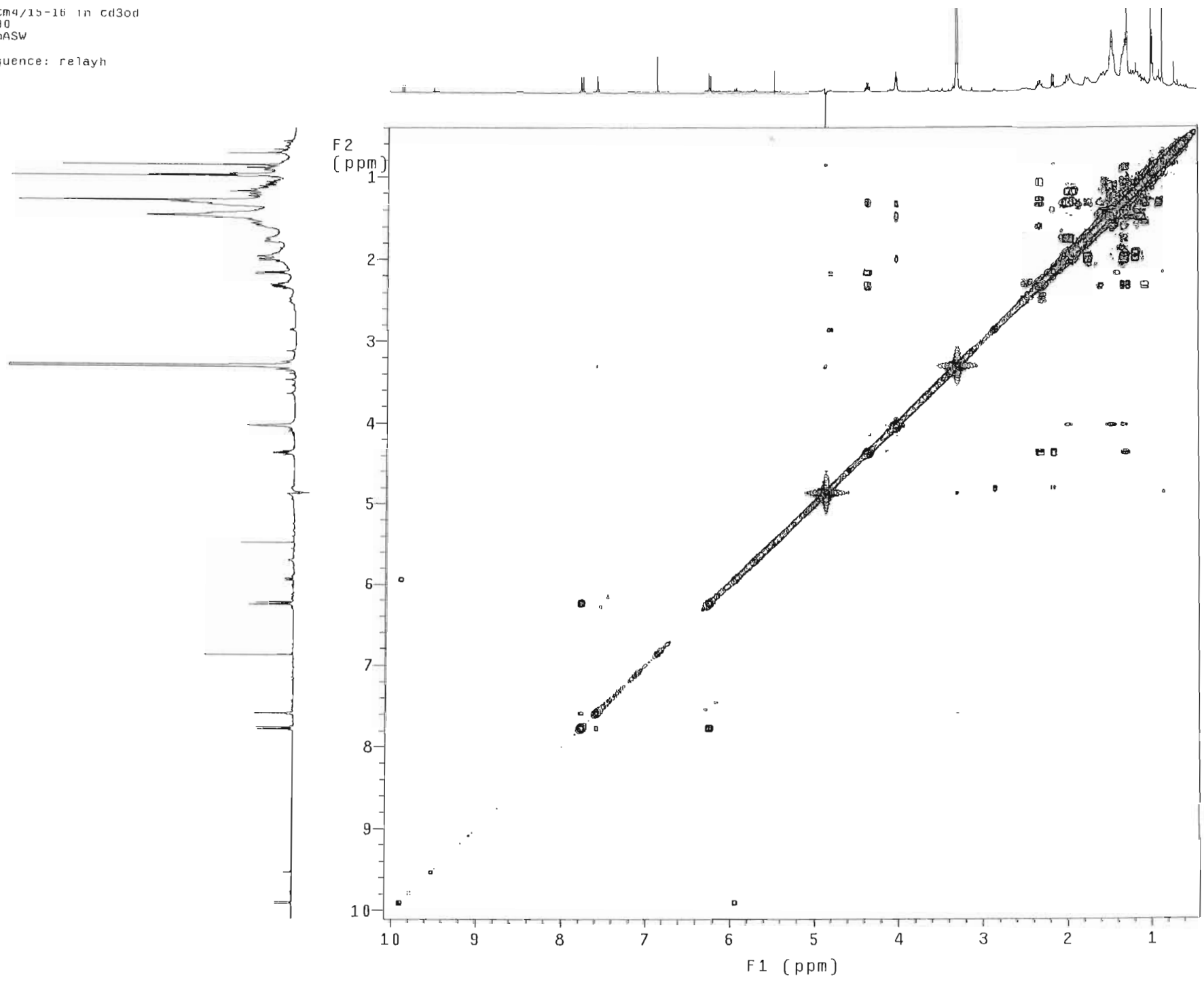

**Spectrum 8a: $^{1}$H NMR spectrum of compound VIII ($CD_3OD$) (400 MHz)**



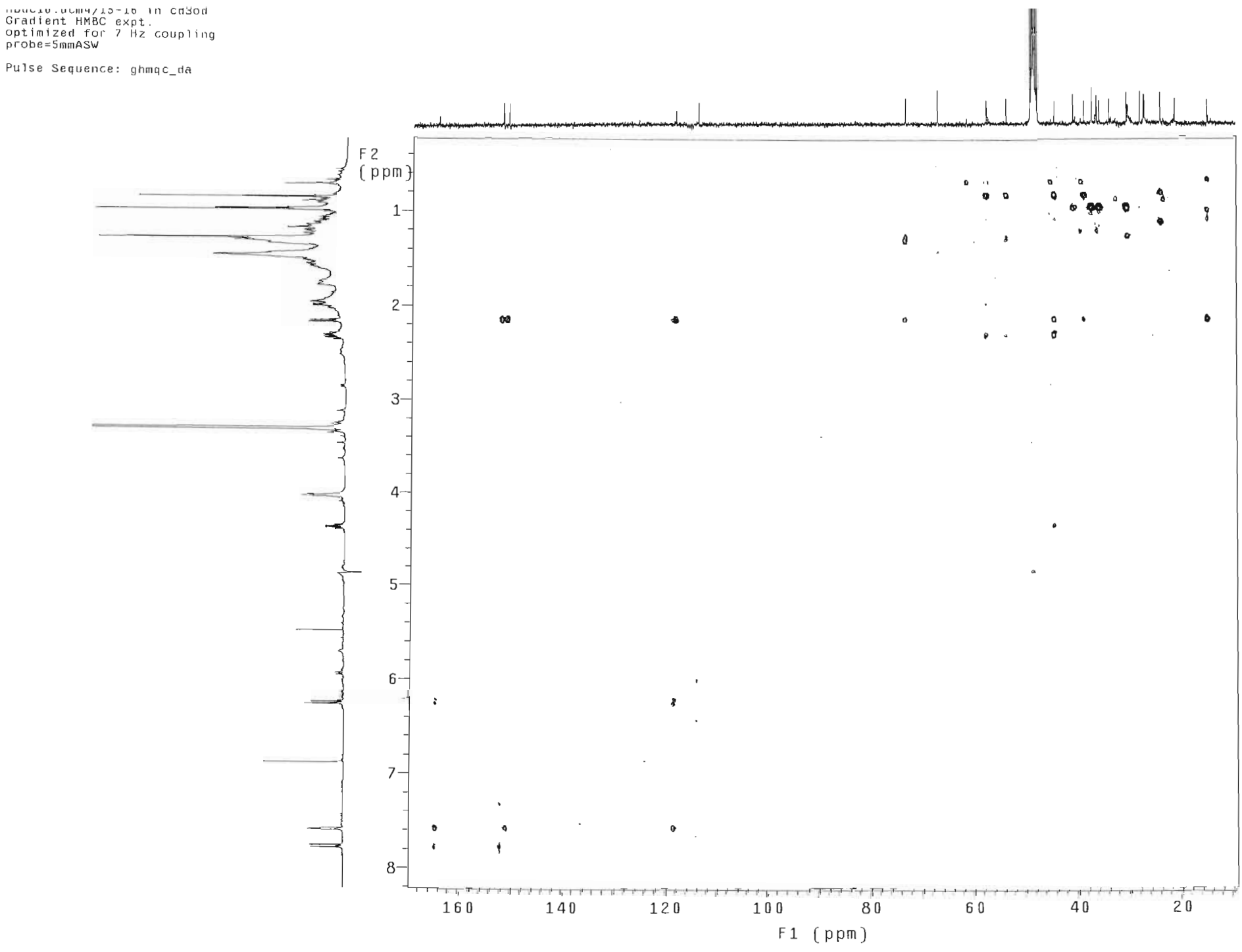

**Spectrum 8b: $^{13}C$ NMR spectrum of compound VIII ($CD_3OD$) (100 MHz)**




**Spectrum 8c: HSQC spectrum of compound VIII**



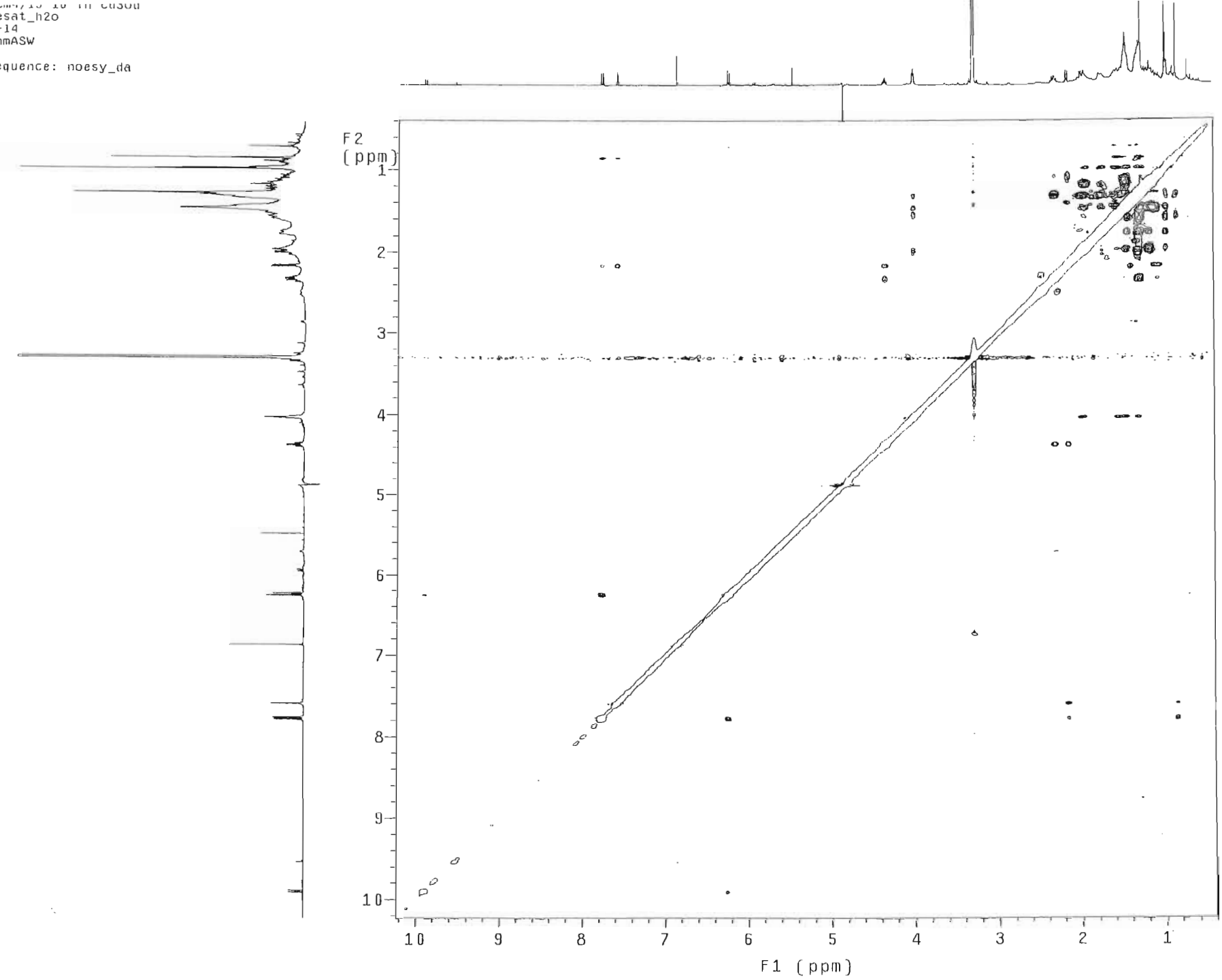

**Spectrum 8d: COSY spectrum of compound VIII**



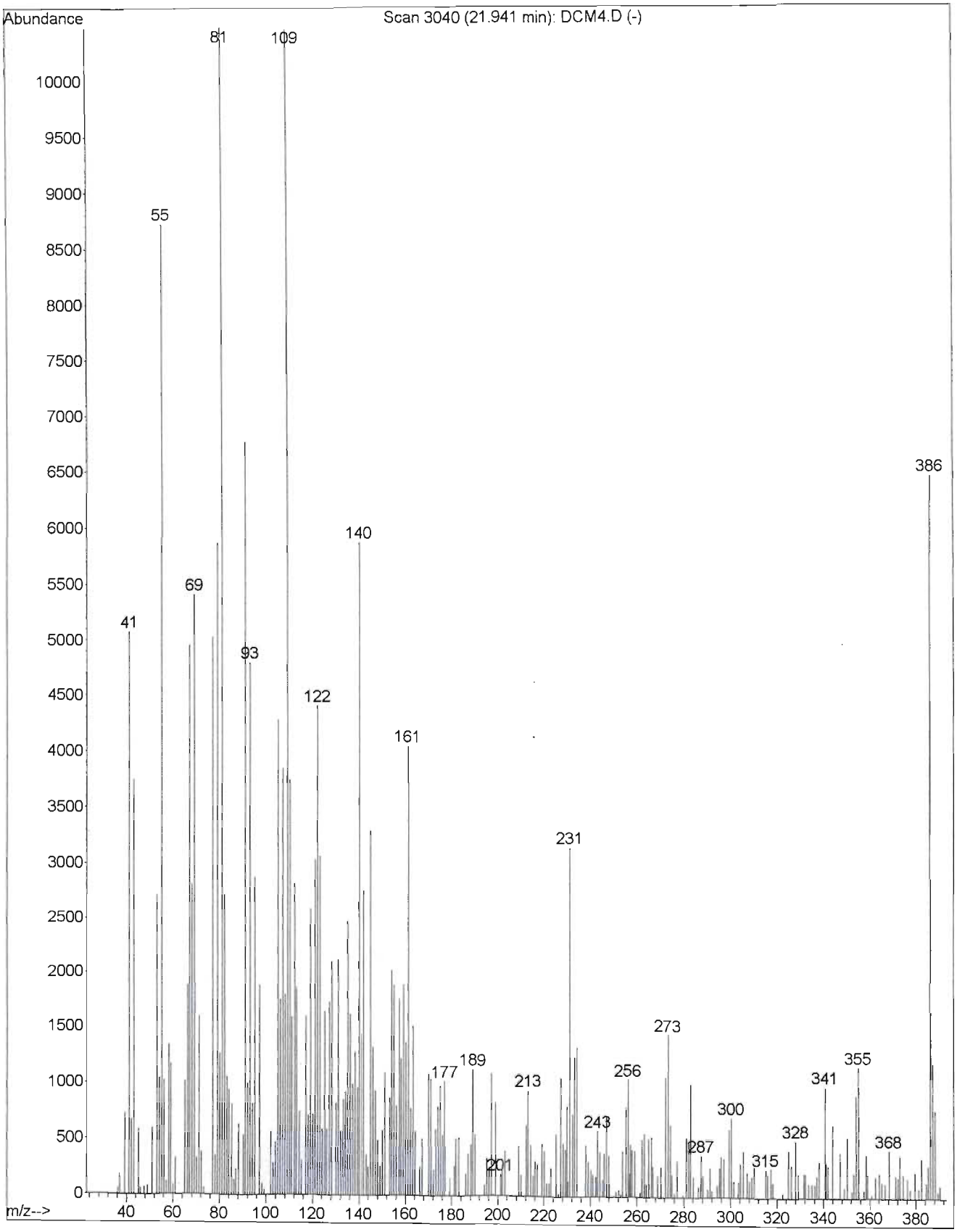

**Spectrum 8e: HMBC spectrum of compound VIII**

with presat_h2o
satpwr=-14
probe=5mmASW

Pulse Sequence: noesy_da




**Spectrum 8f: NOESY spectrum of compound VIII**

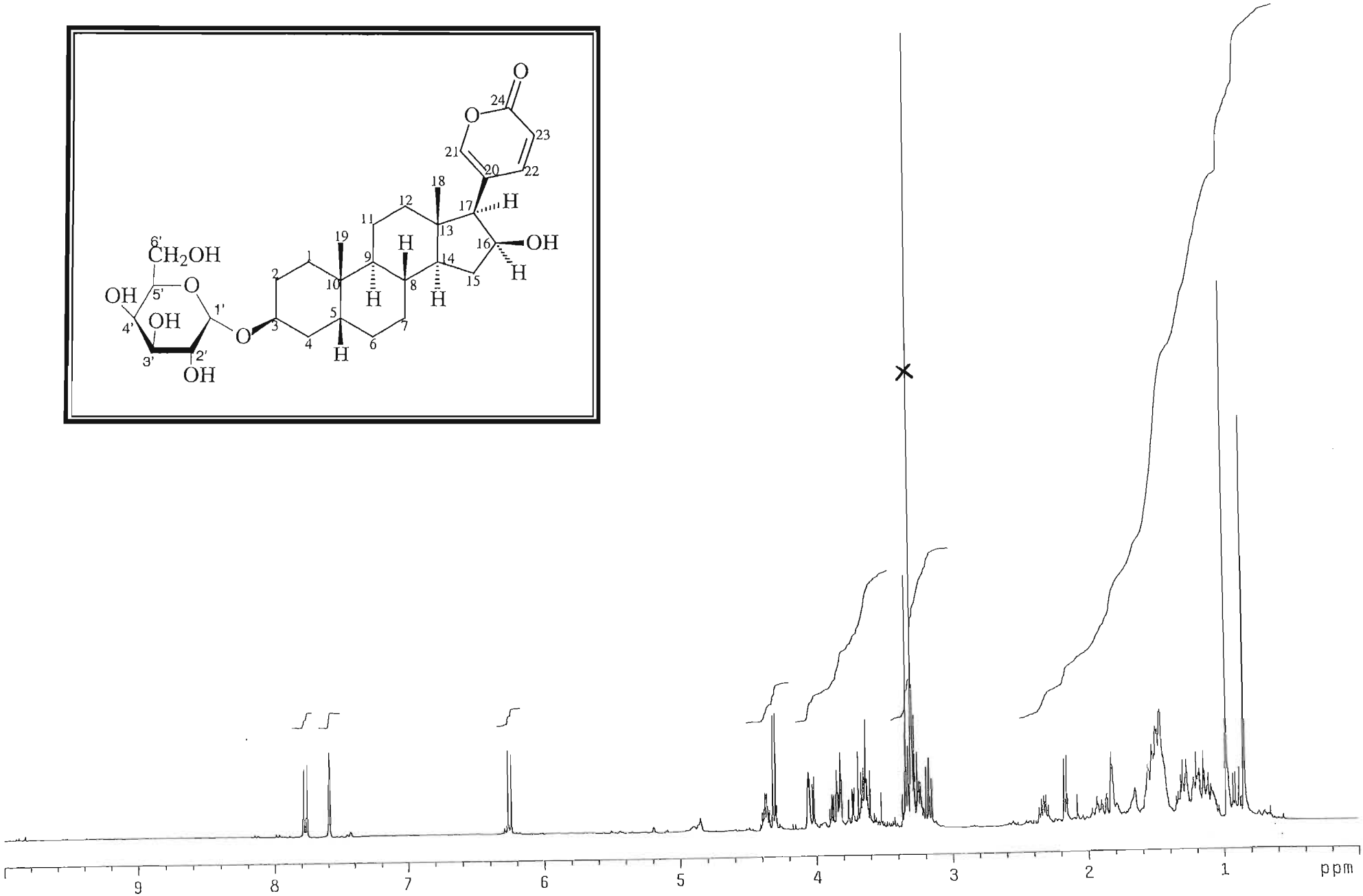



**Spectrum 8g: Mass spectrum of compound VIII**

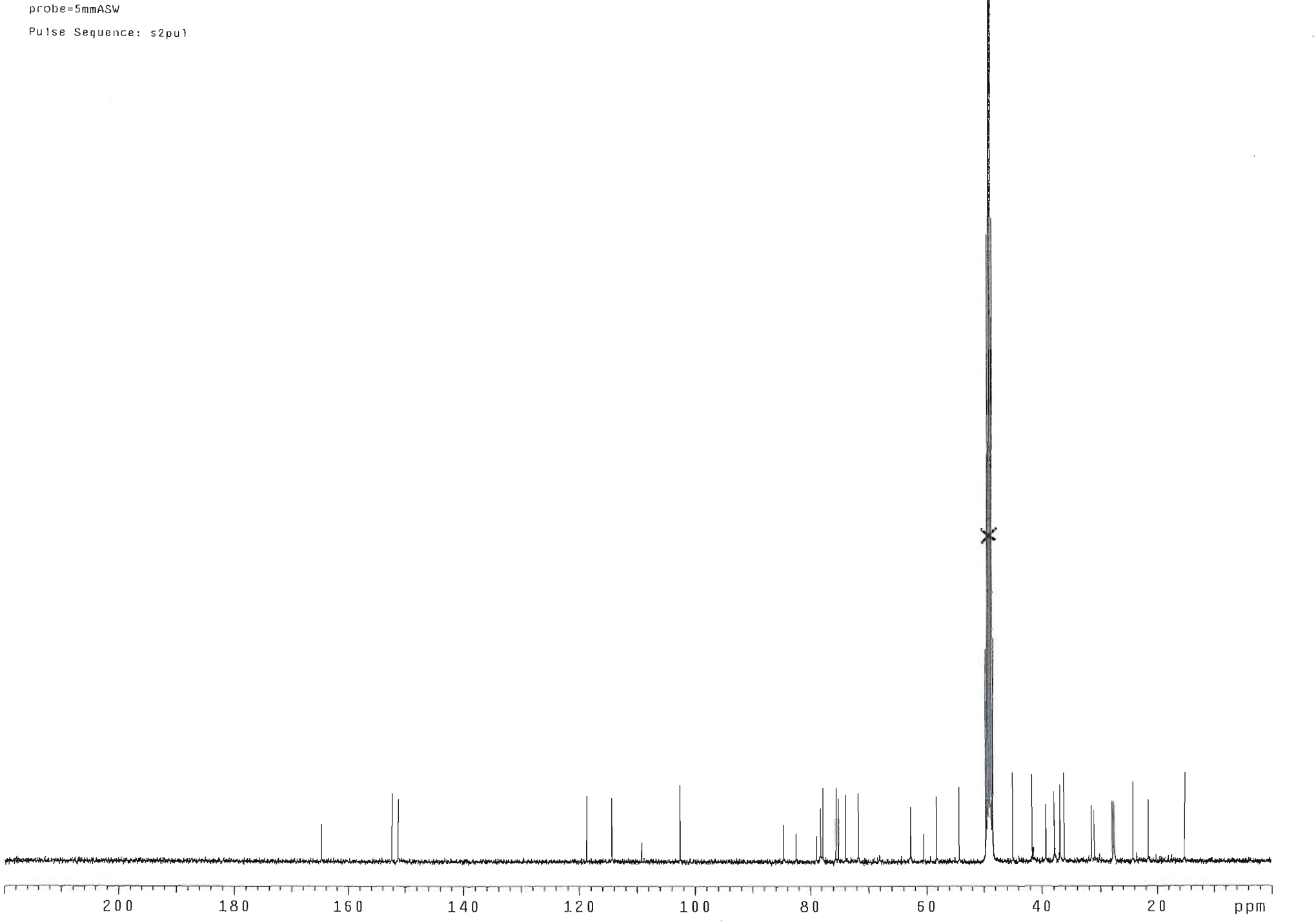
satpwr=-12
probe=5mmASW

Pulse Sequence: presat_da

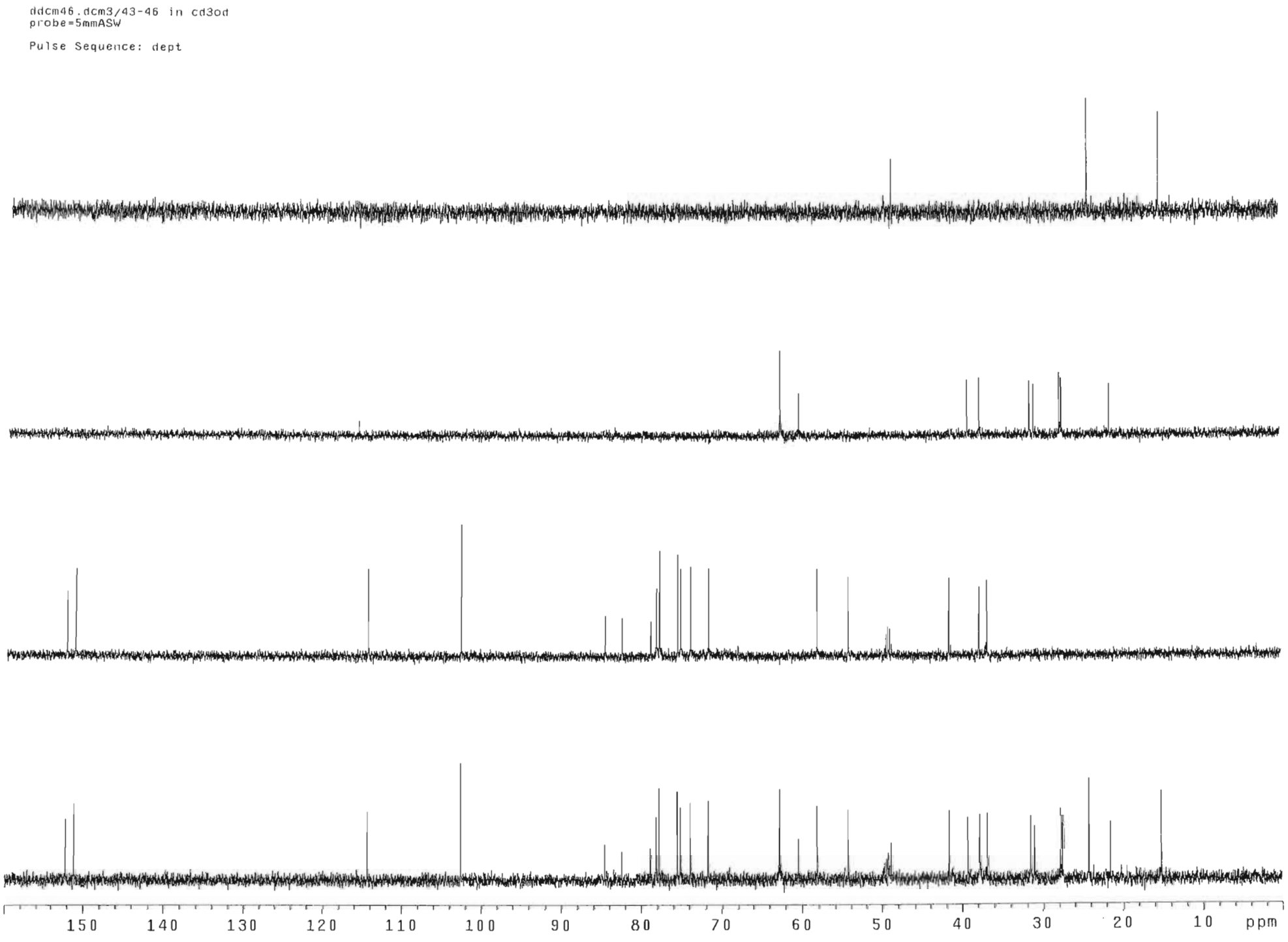



**Spectrum 9a: $^{1}$H NMR spectrum of compound IX ($CD_3OD$) (400 MHz)**

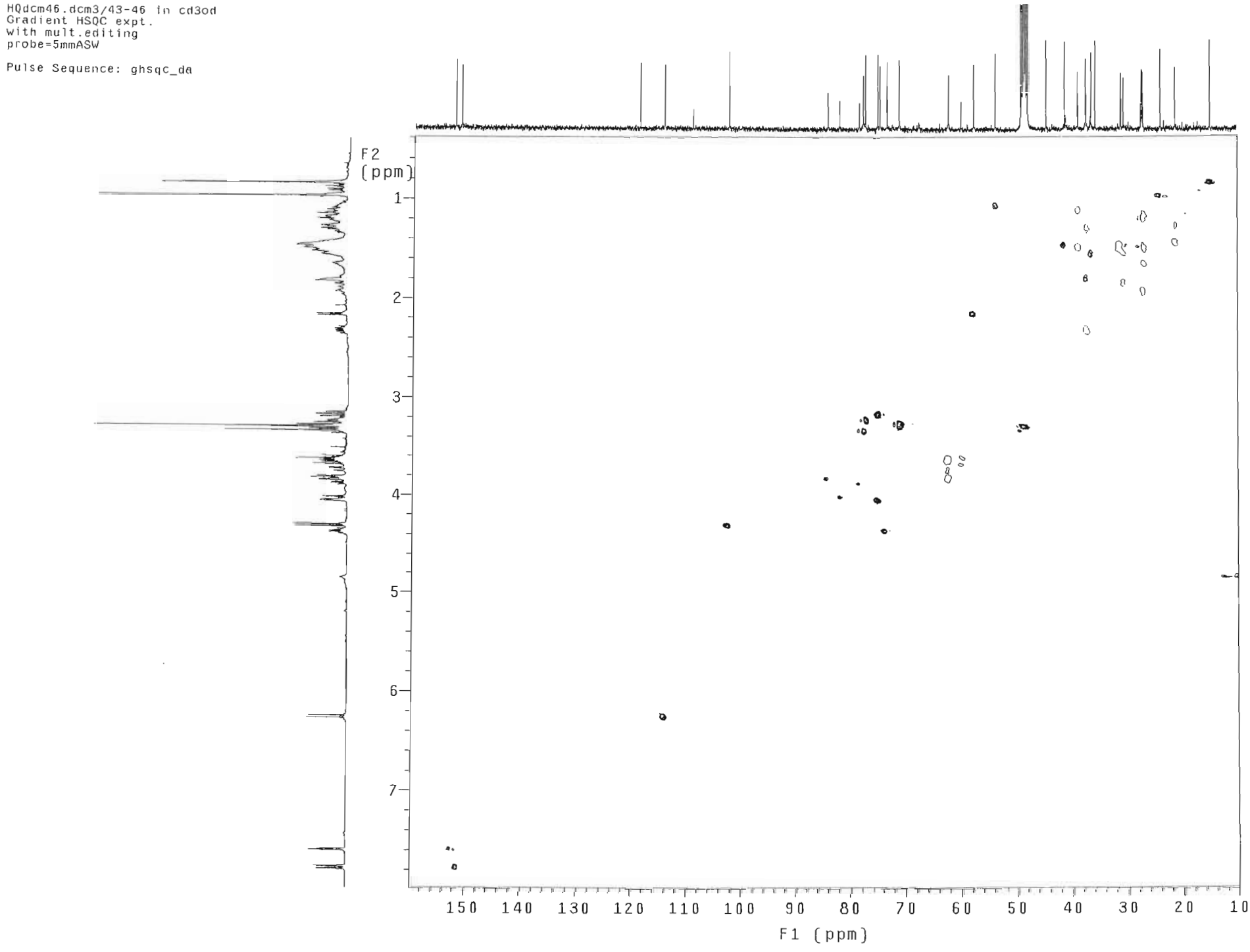



**Spectrum 9b: $^{13}C$ NMR spectrum of compound IX ($CD_3OD$) (100 MHz)**

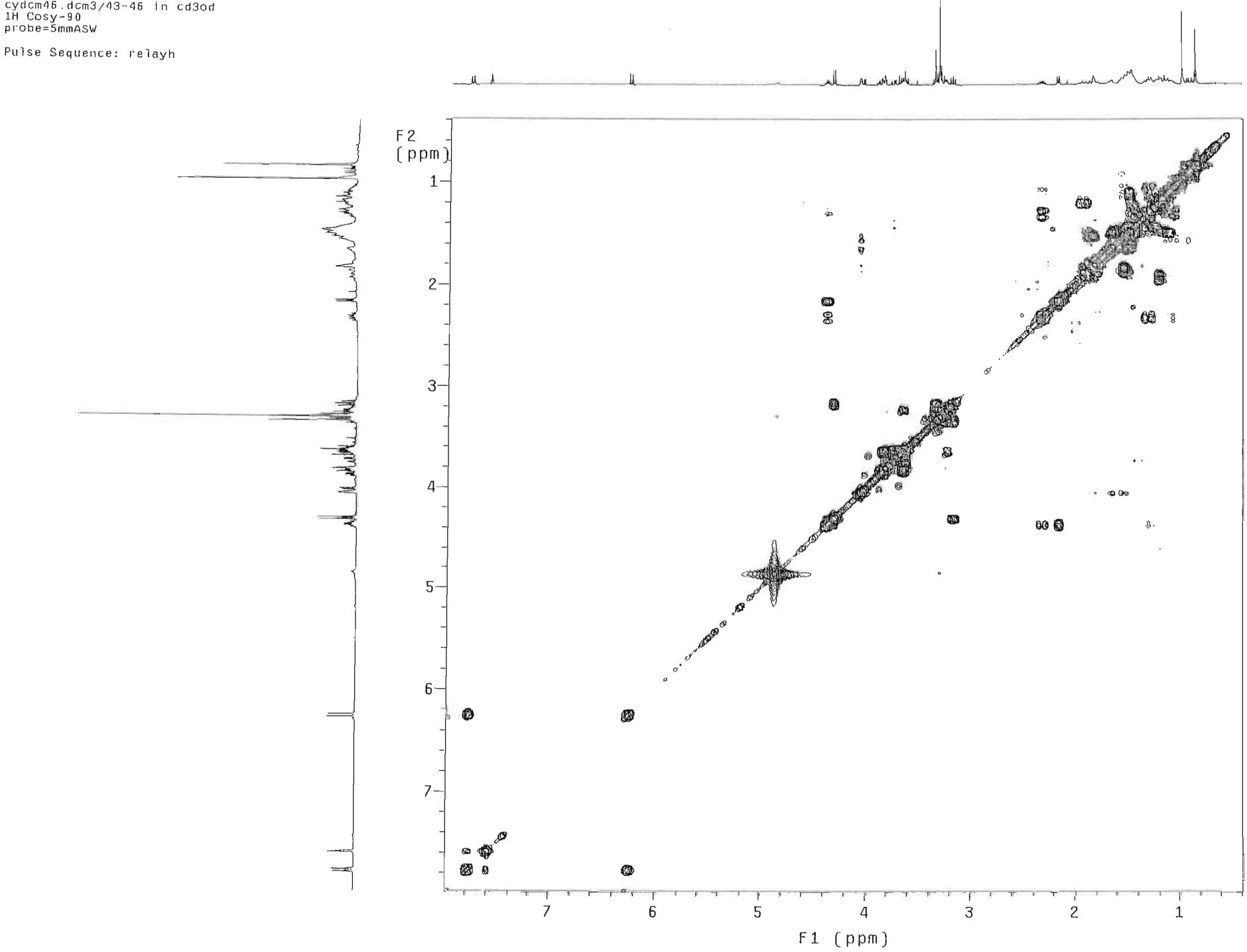




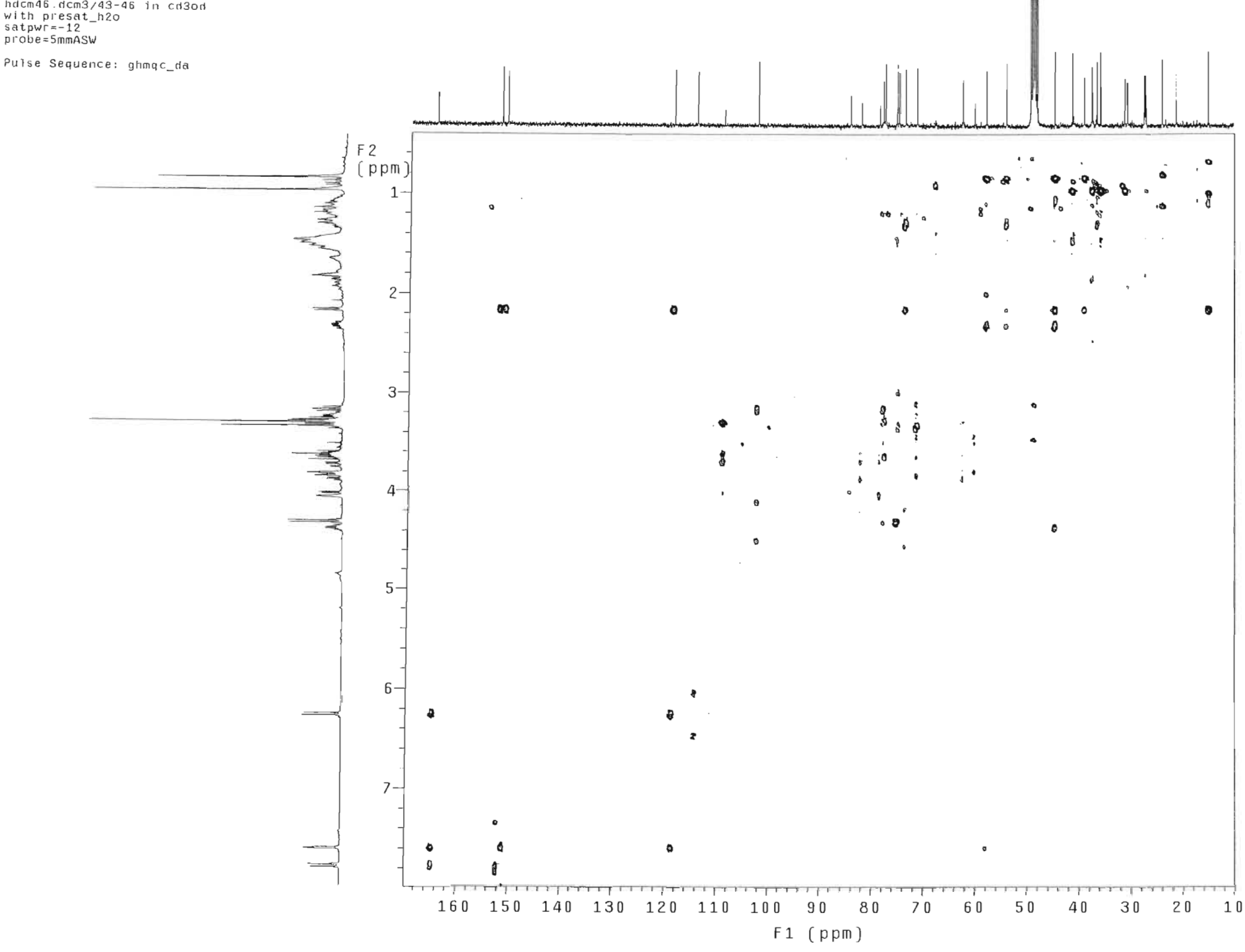

**Spectrum 9c: ADEPT spectrum of compound IX**



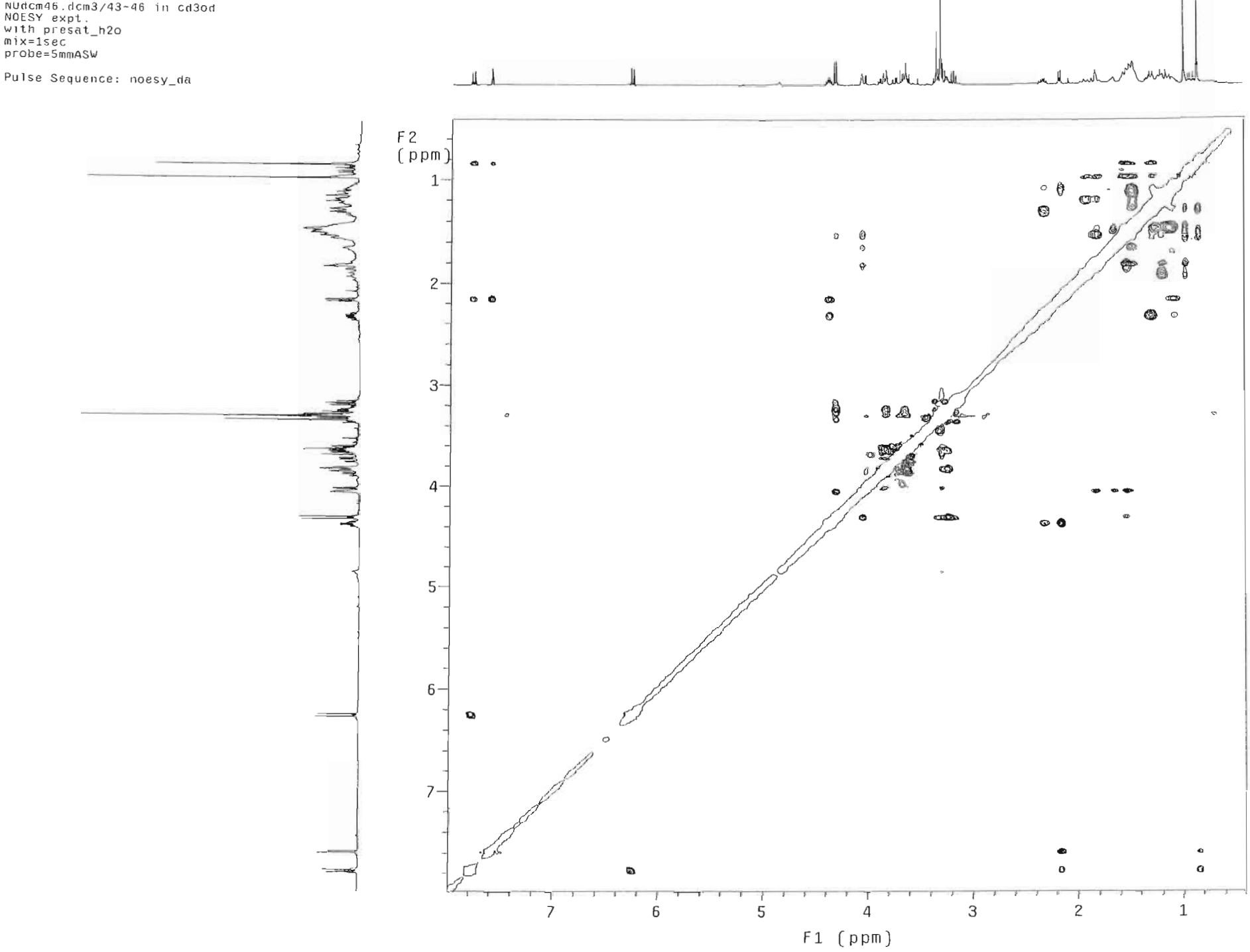

**Spectrum 9d: HSQC spectrum of compound IX**



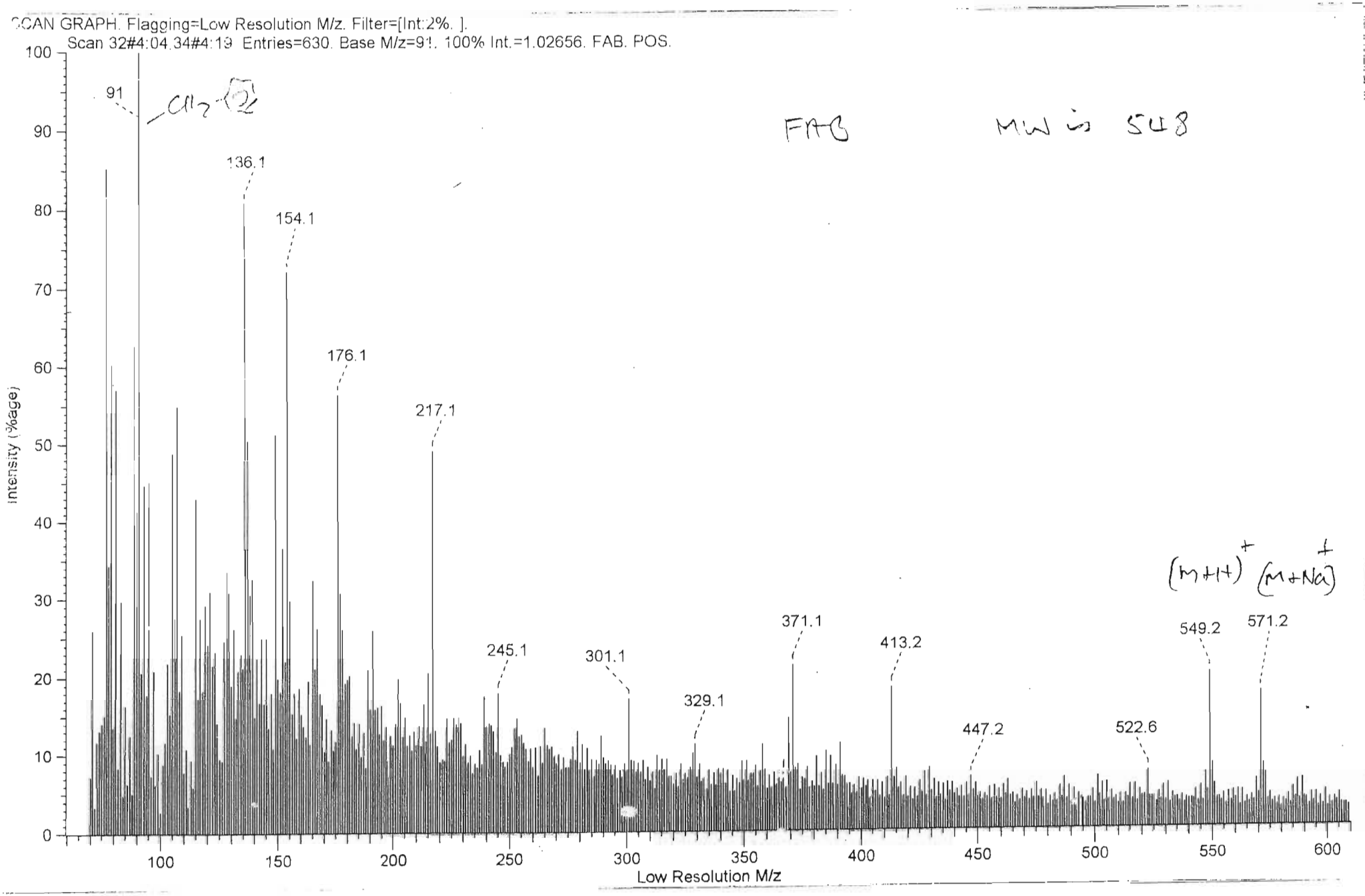

**Spectrum 9e: COSY spectrum of compound IX**



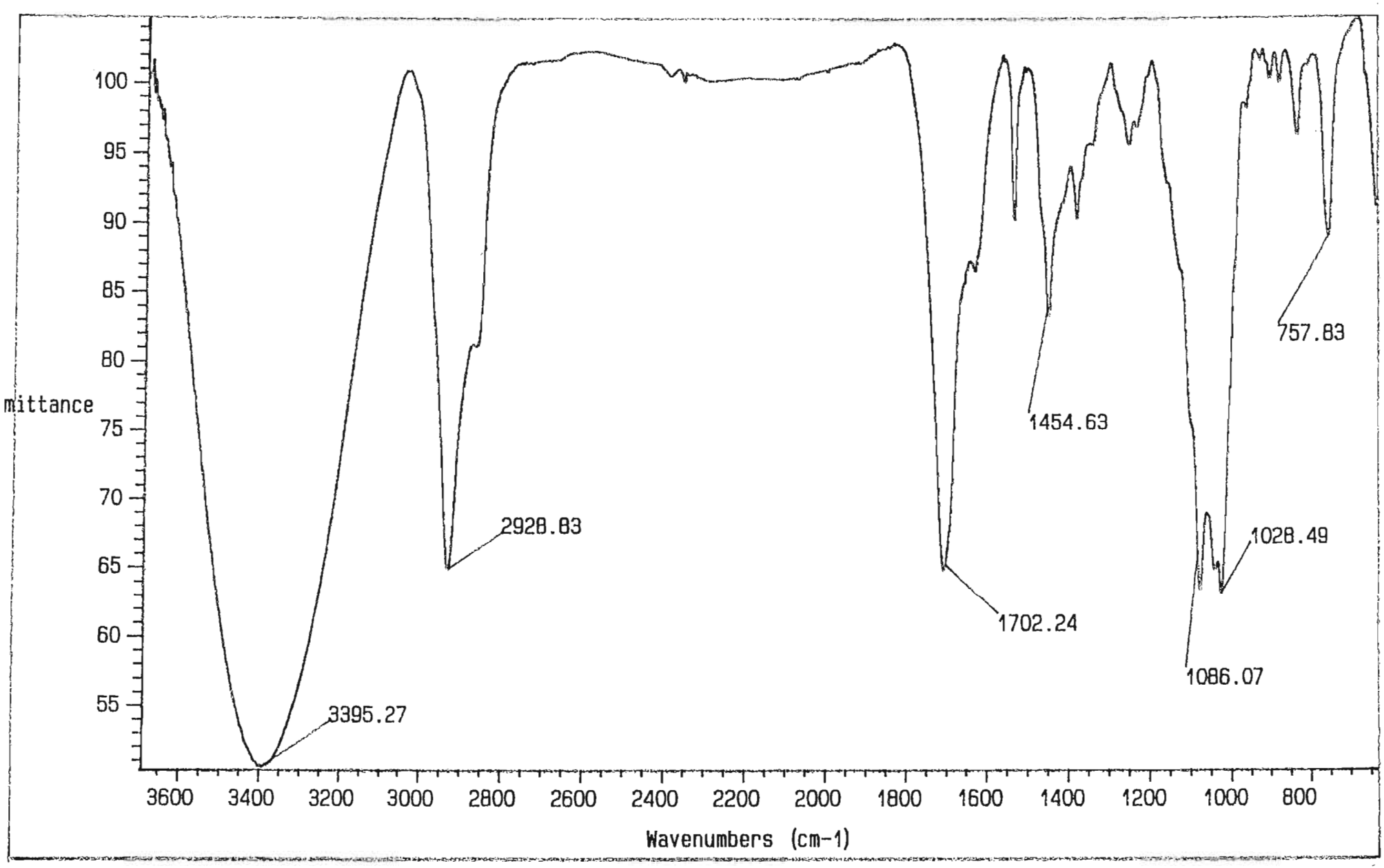

**Spectrum 9f: HMBC spectrum of compound IX**




**Spectrum 9g: NOESY spectrum of compound IX**




**Spectrum 9h: Mass spectrum of compound IX**

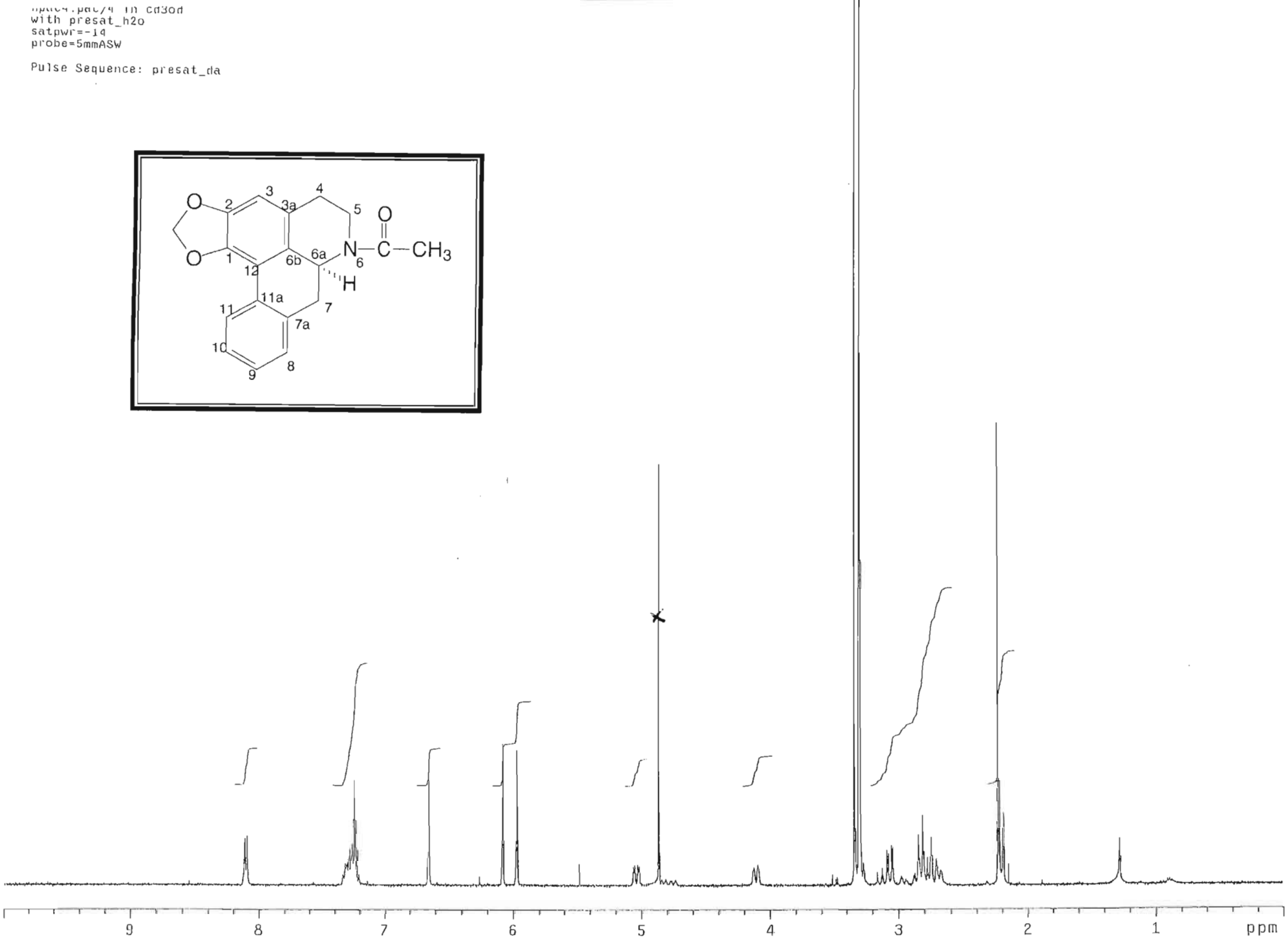



**Spectrum 9i: Infra-red spectrum of compound IX**

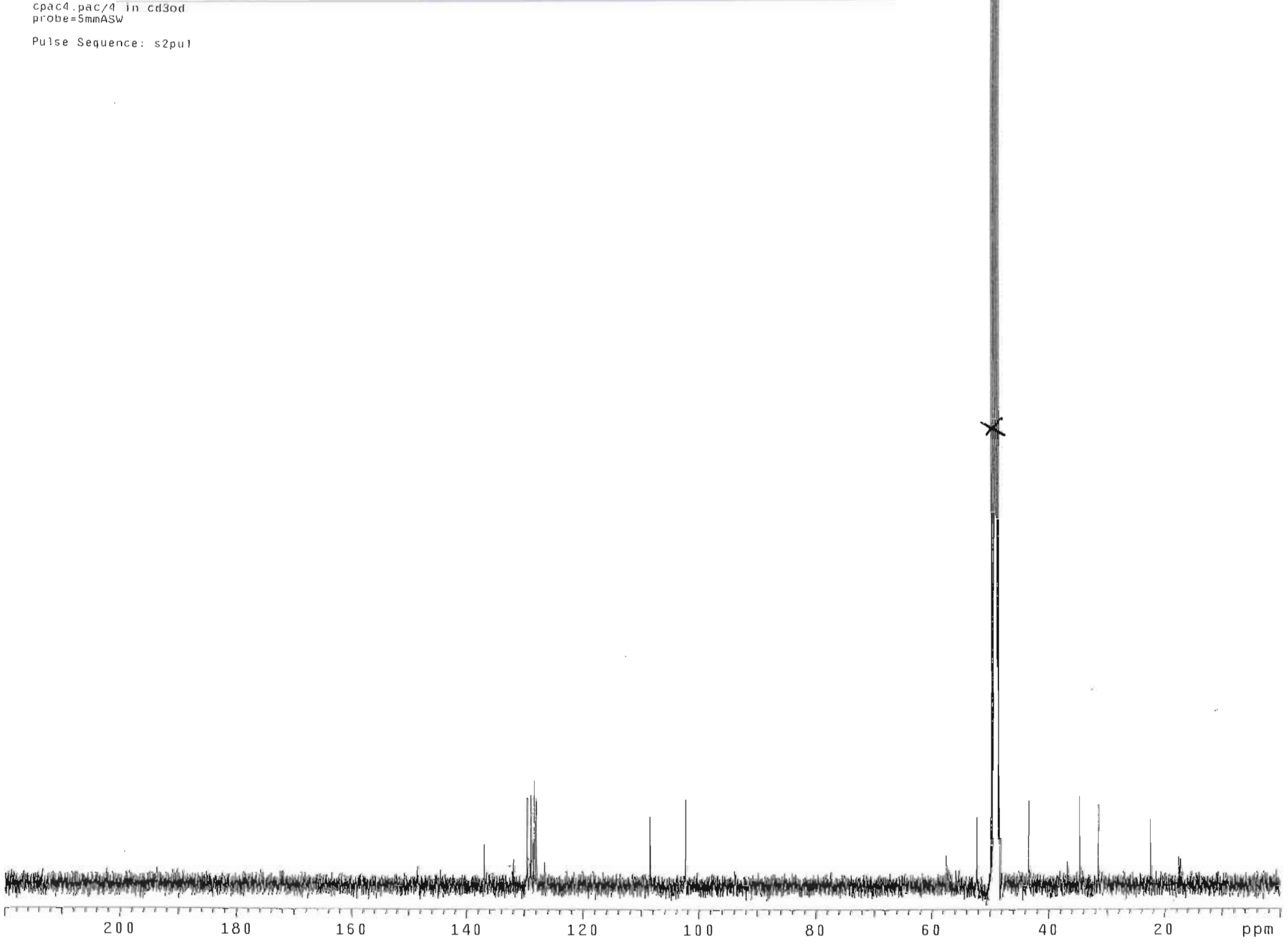
with presat_h2o
satpwr=-14
probe=5mmASW

Pulse Sequence: presat_da

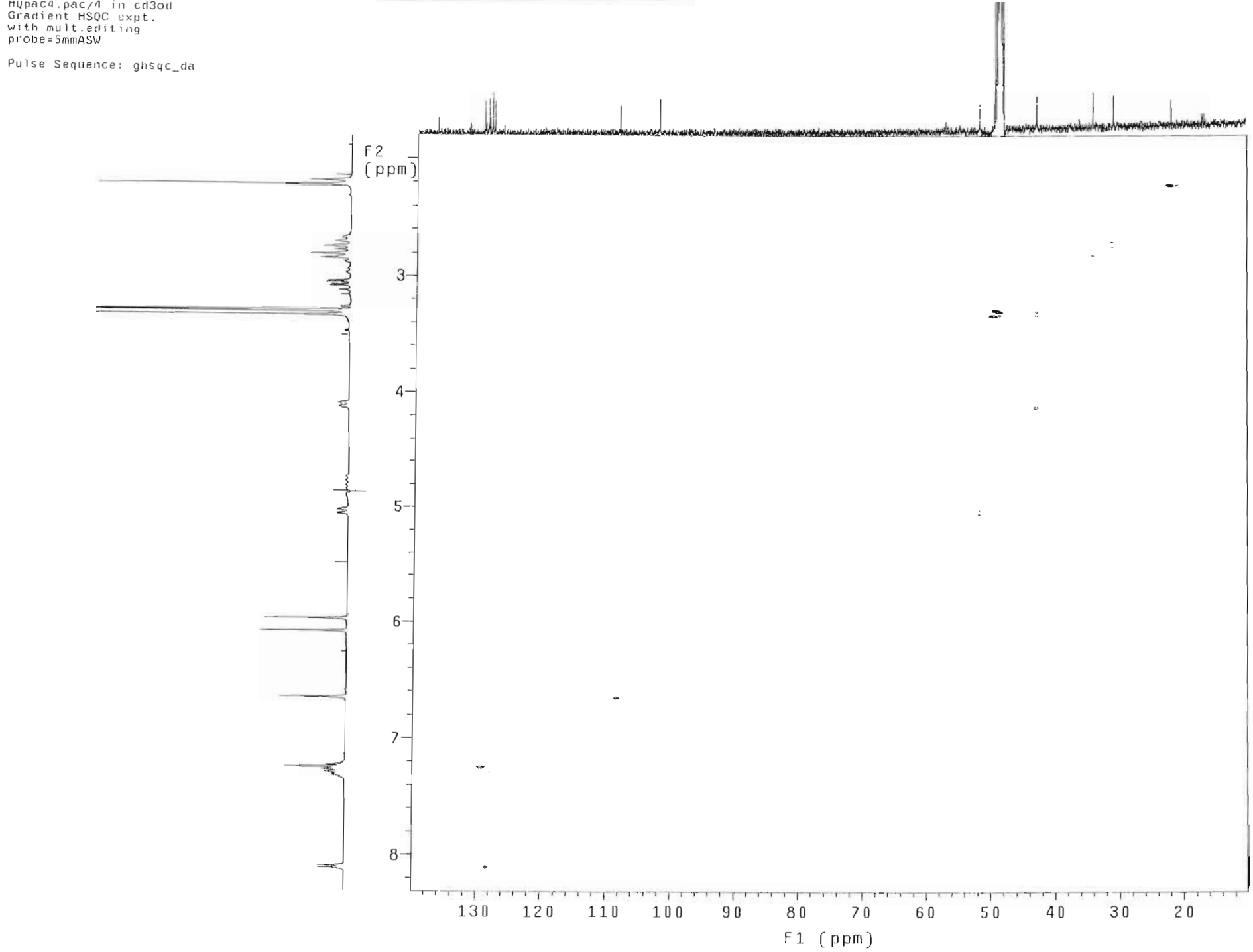


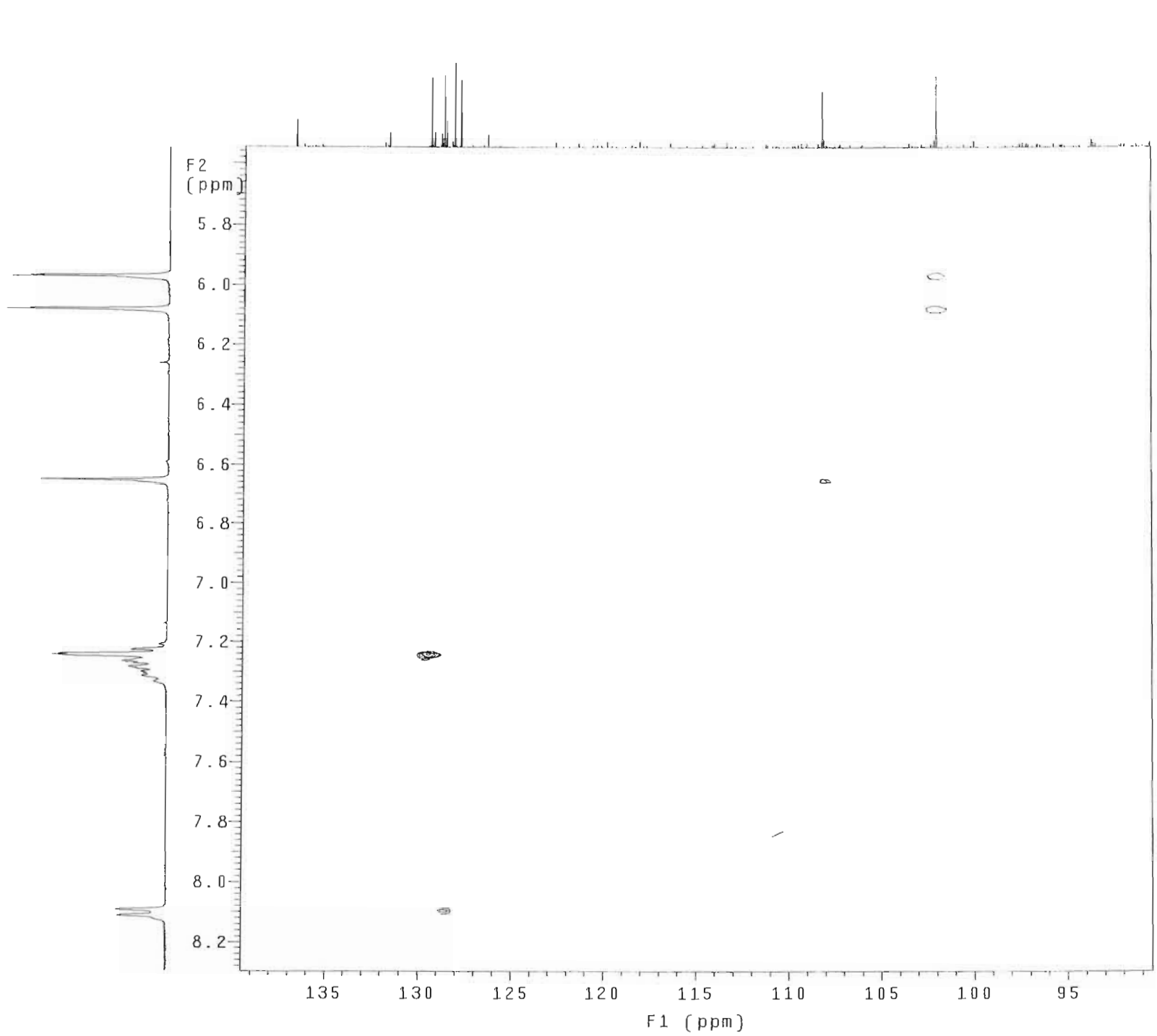

**Spectrum 10a: $^{1}H$ NMR spectrum of compound X ($CD_3OD$) (400 MHz)**



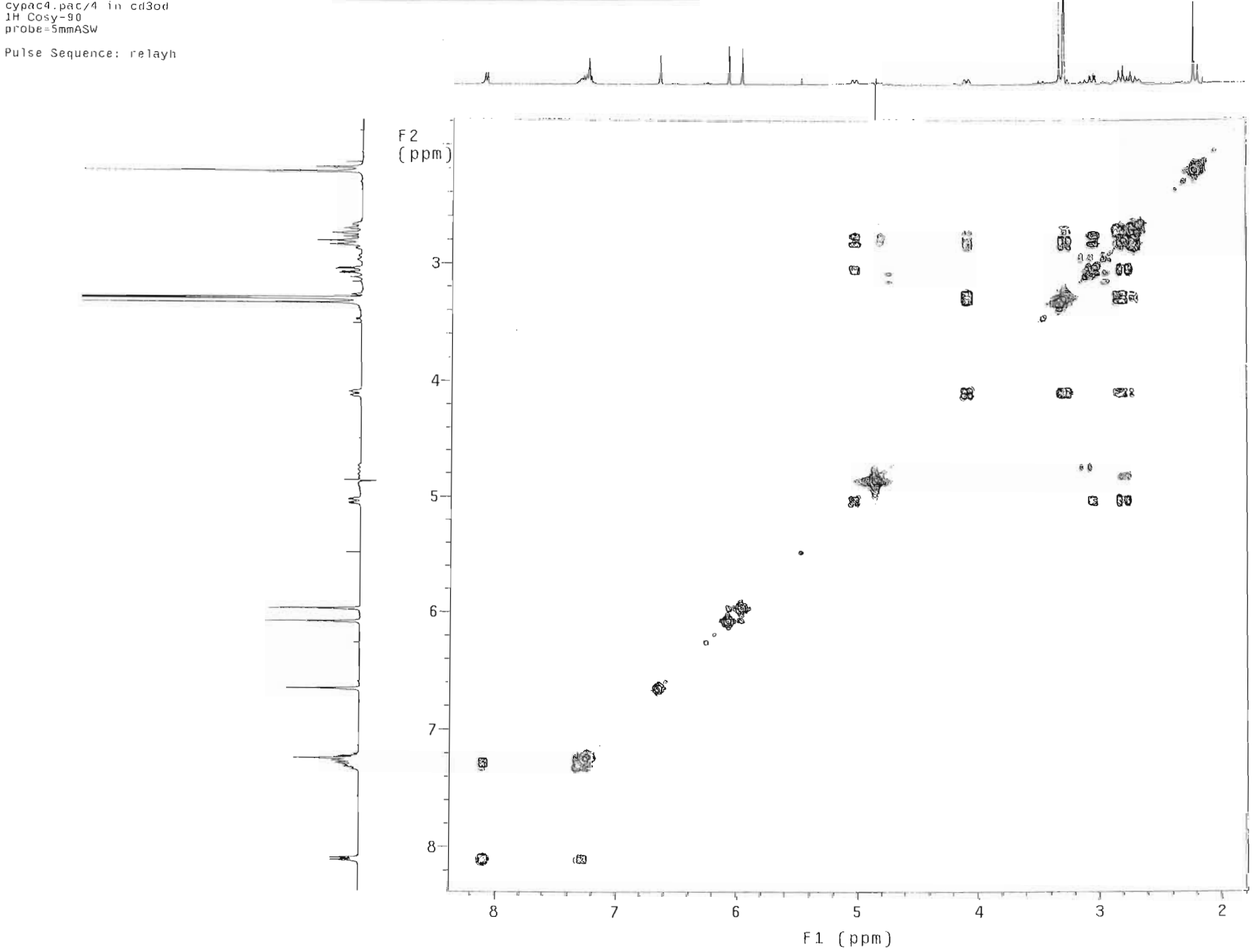

**Spectrum 10b: $^{13}C$ NMR spectrum of compound X ($CD_3OD$) (100 MHz)**



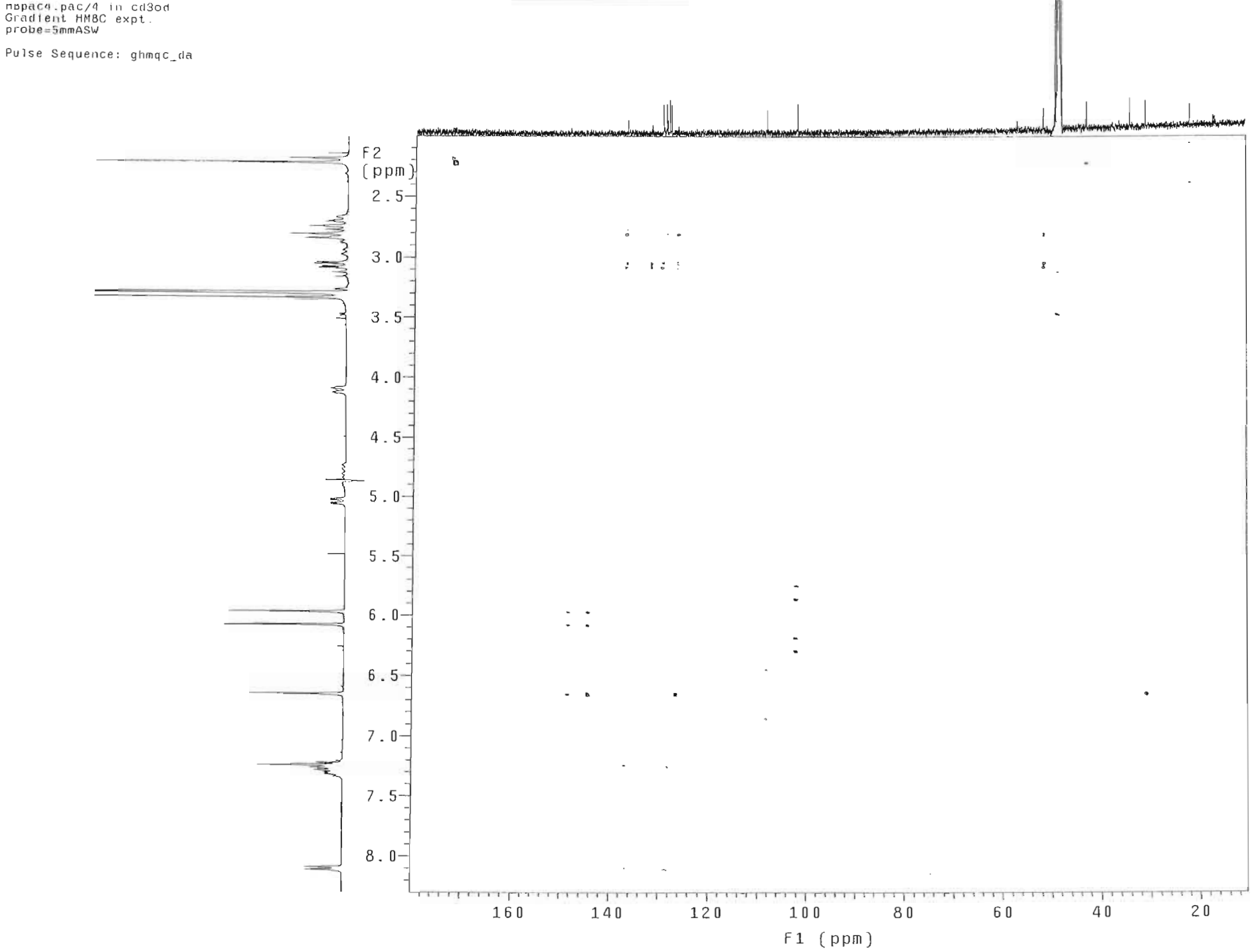

**Spectrum 10c: HSQC spectrum of compound X**



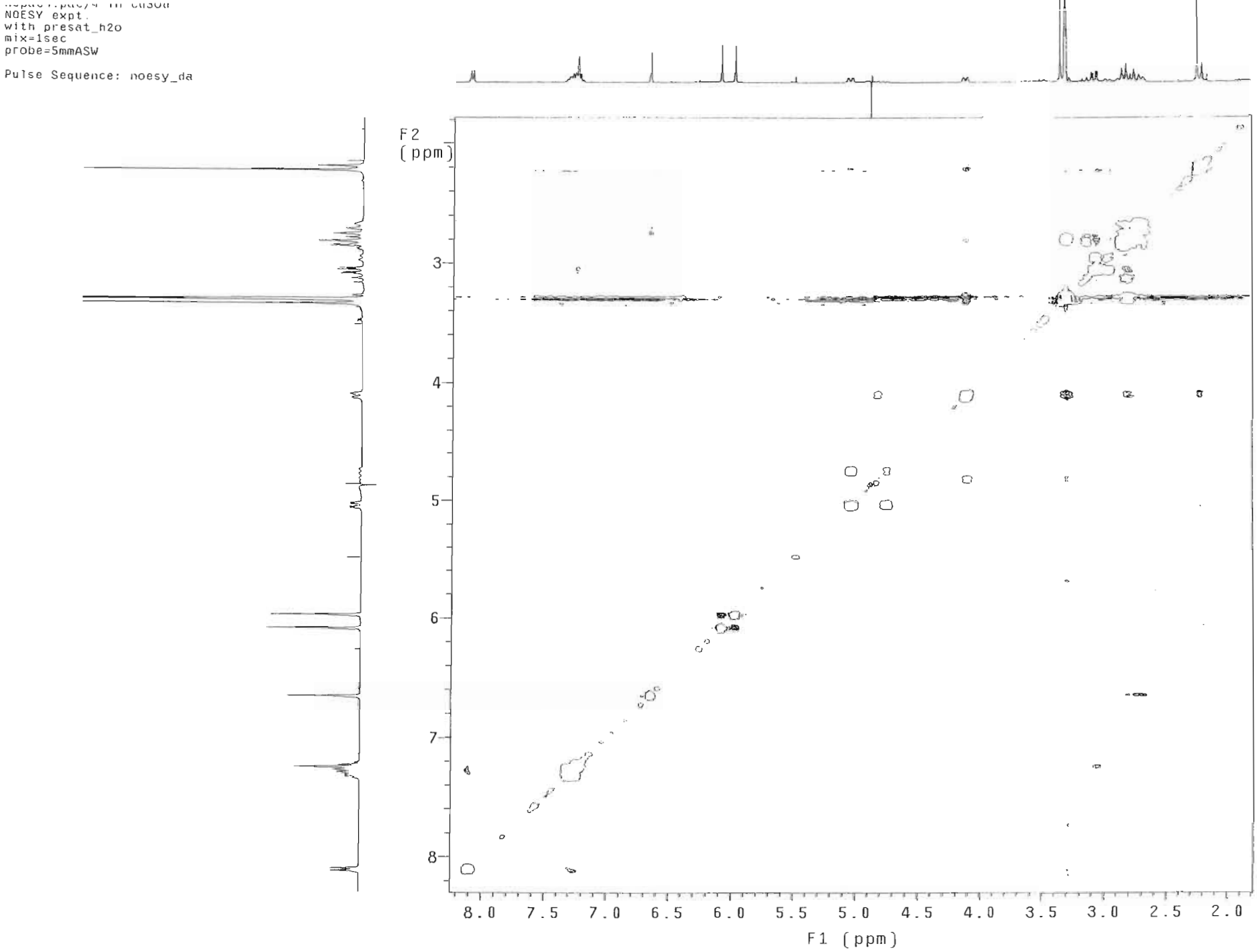

**Spectrum 10d: HSQC spectrum of compound X**



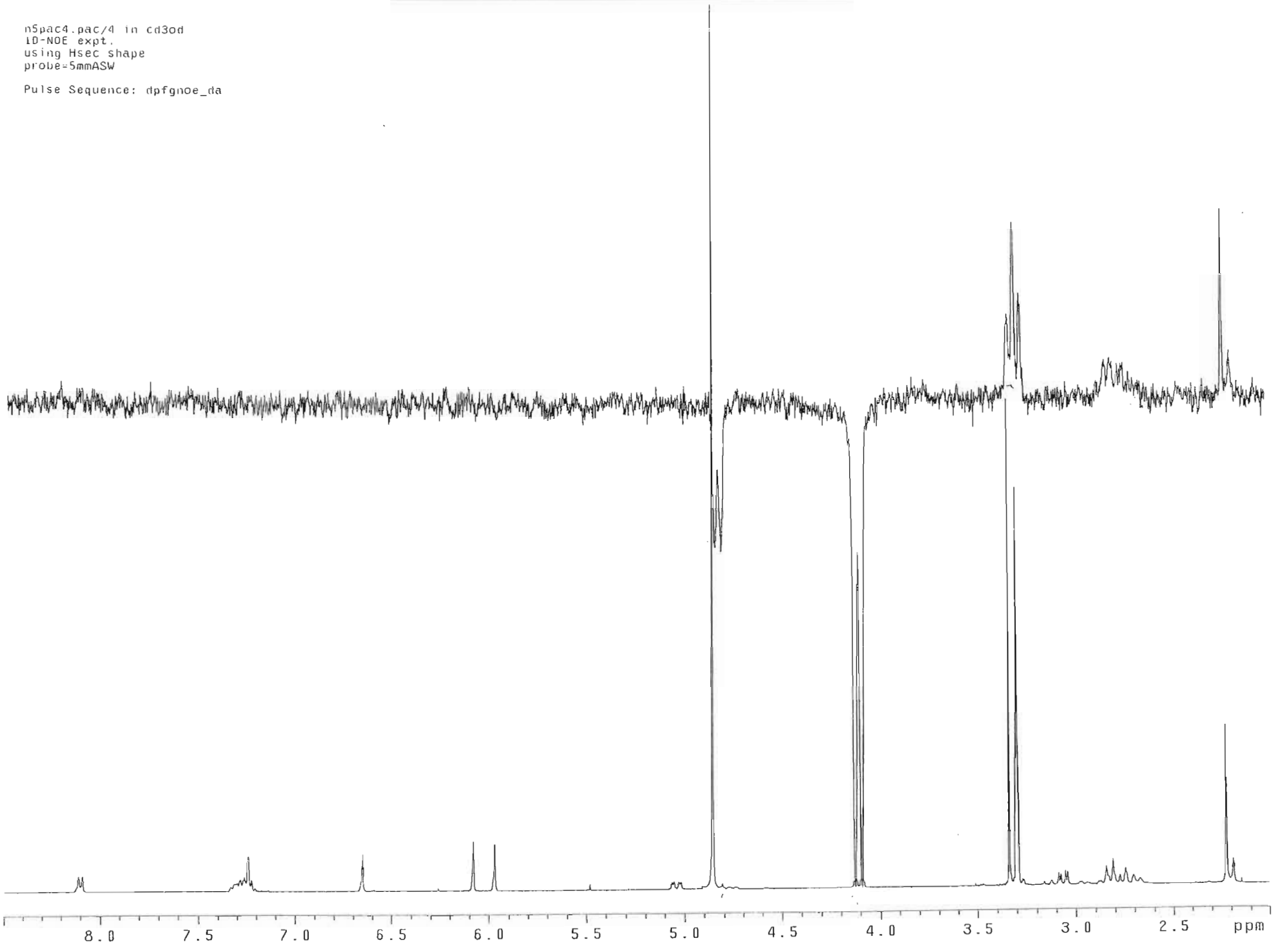

**Spectrum 10e: COSY spectrum of compound X**



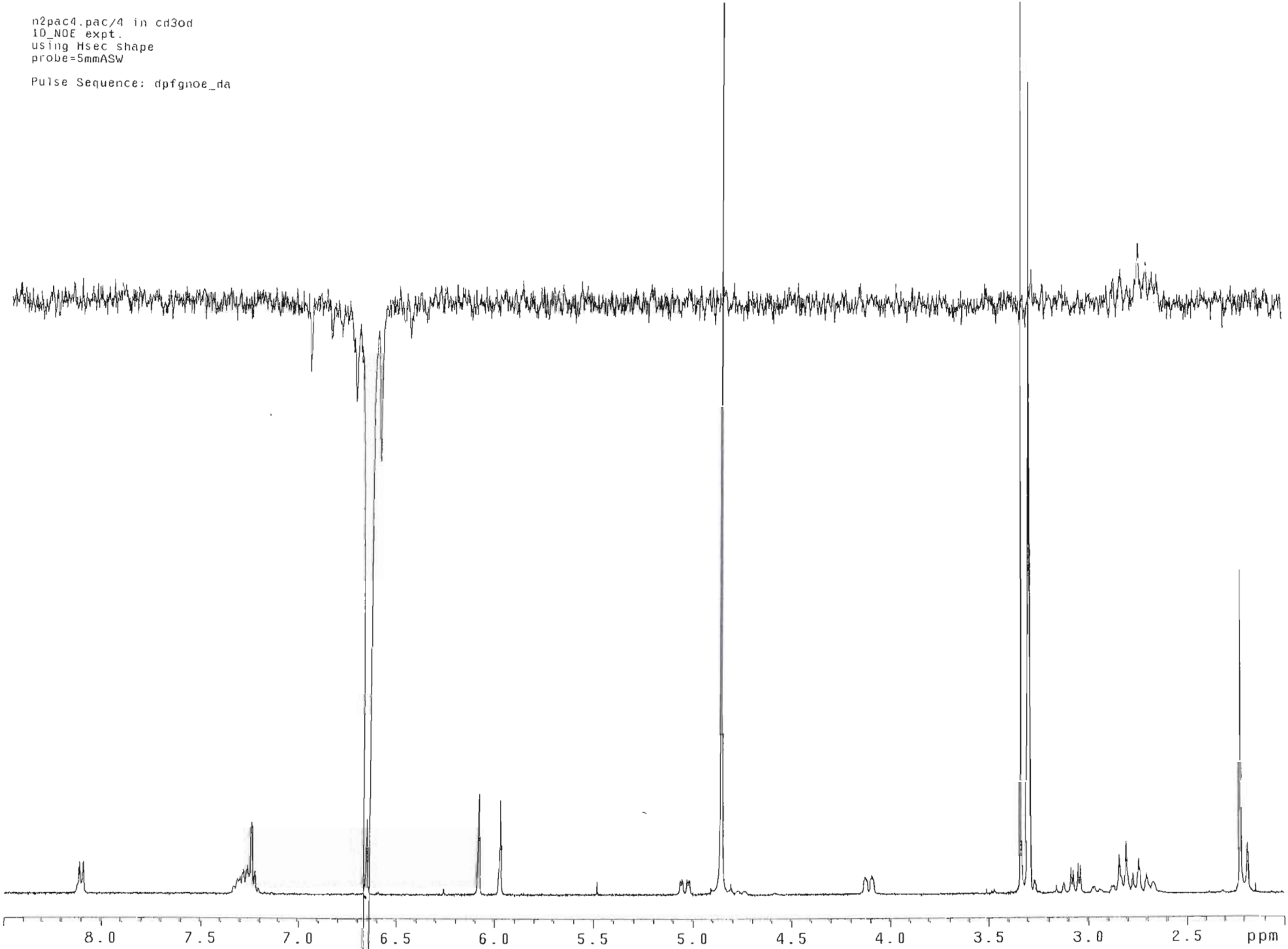

**Spectrum 10f: HMBC spectrum of compound X**



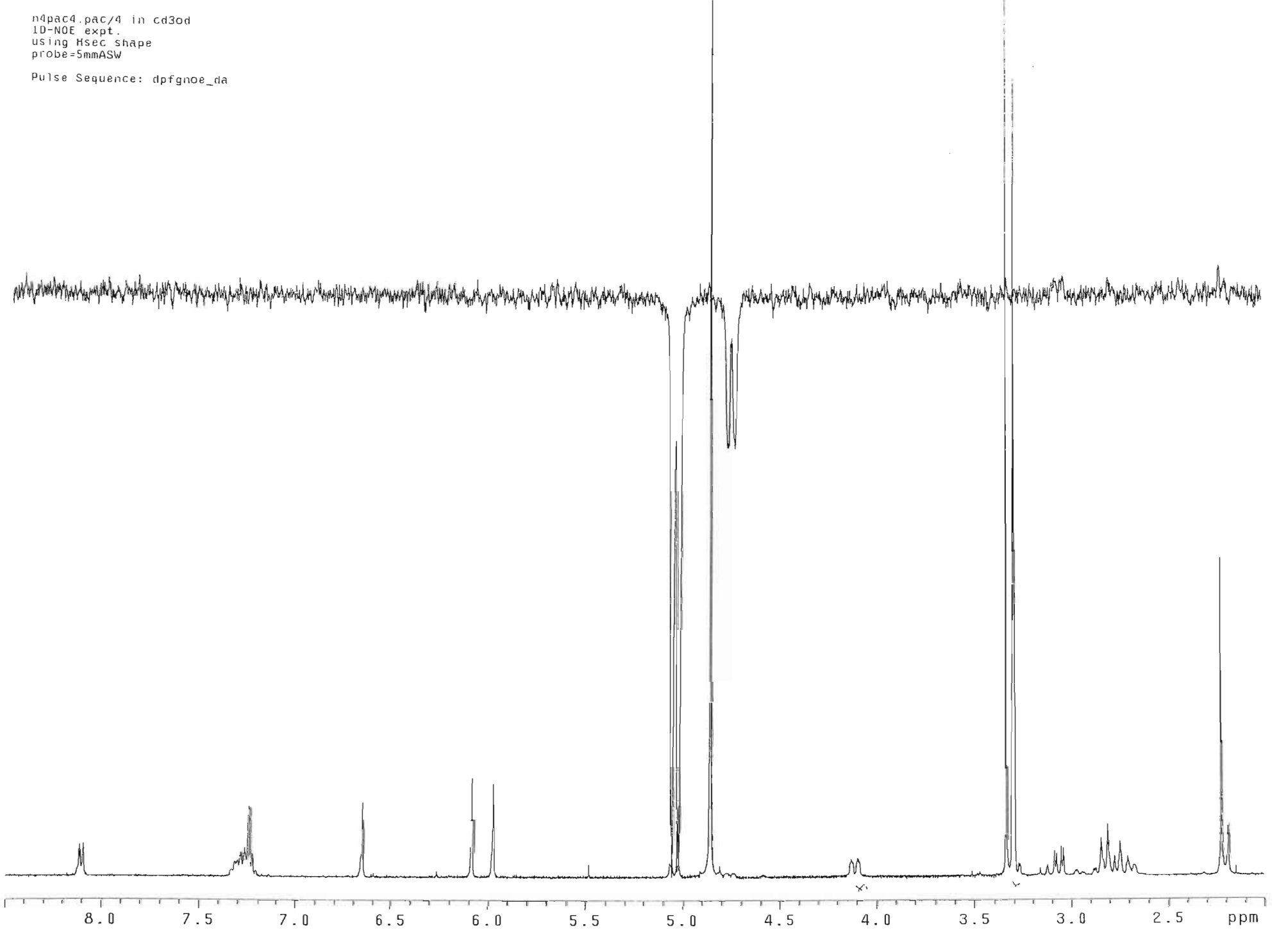

**Spectrum 10g: NOESY spectrum of compound X**



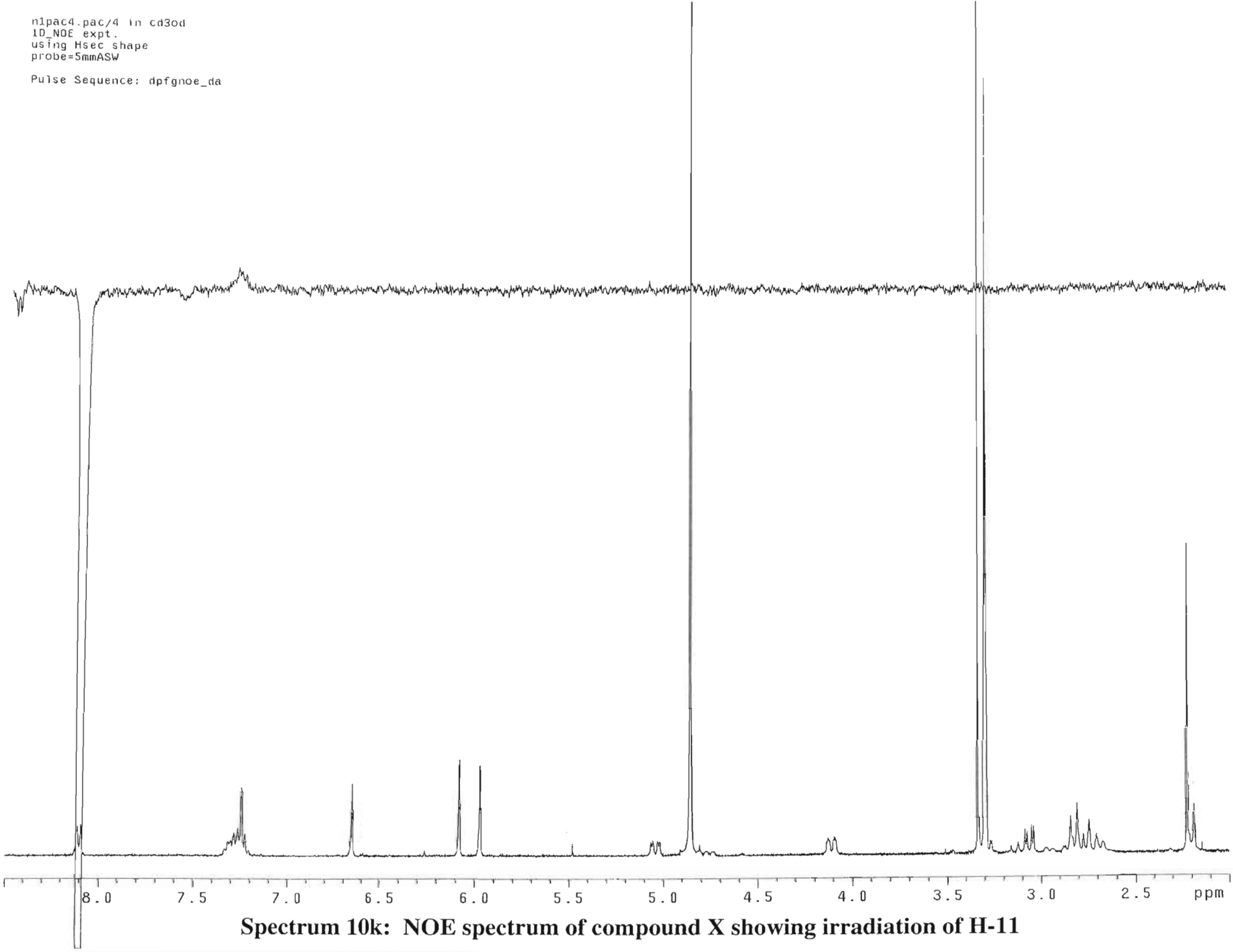

**Spectrum 10h: NOE spectrum of compound X showing irradiation of H-5β**



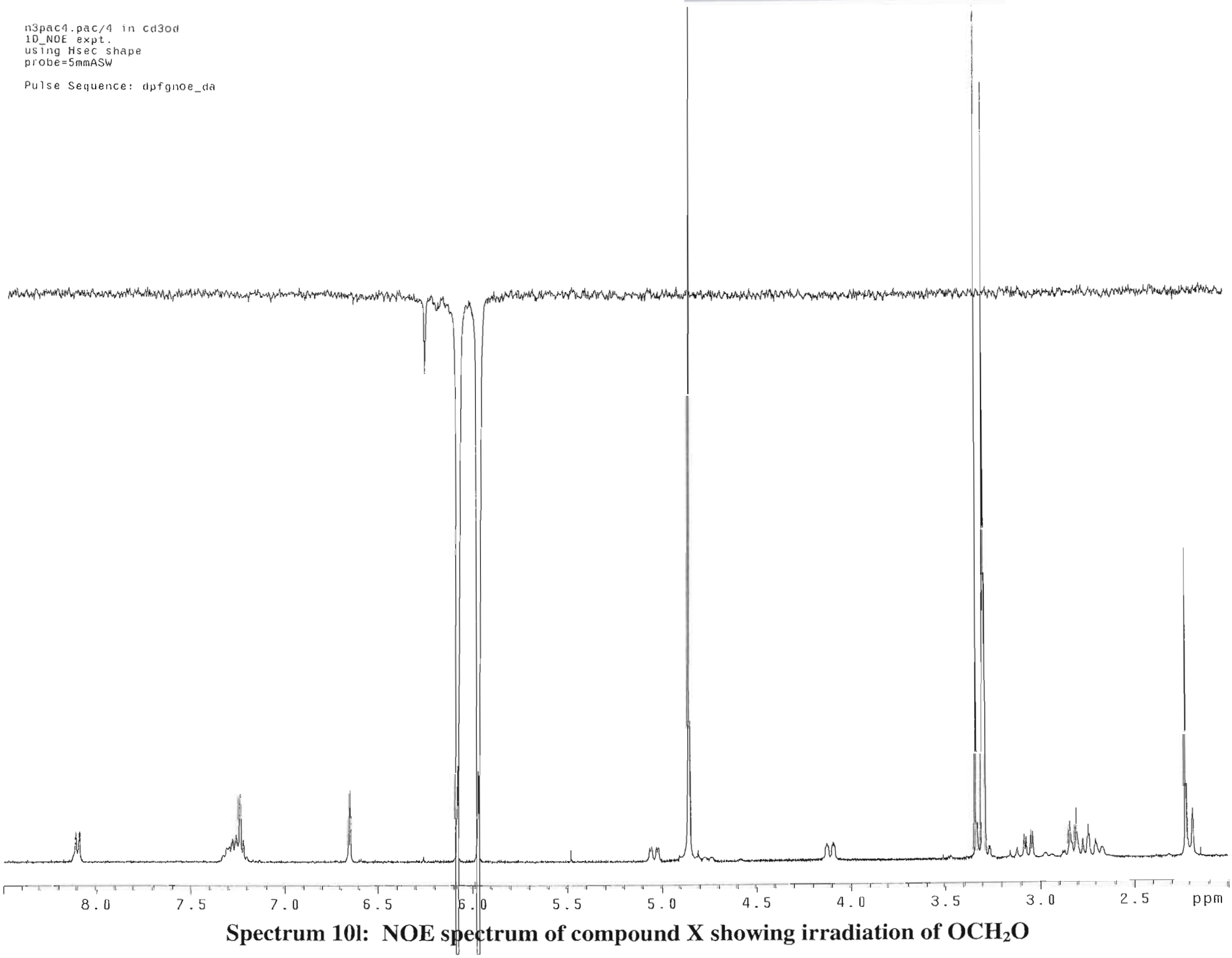

**Spectrum 10i: NOE spectrum of compound X showing irradiation of H-3**



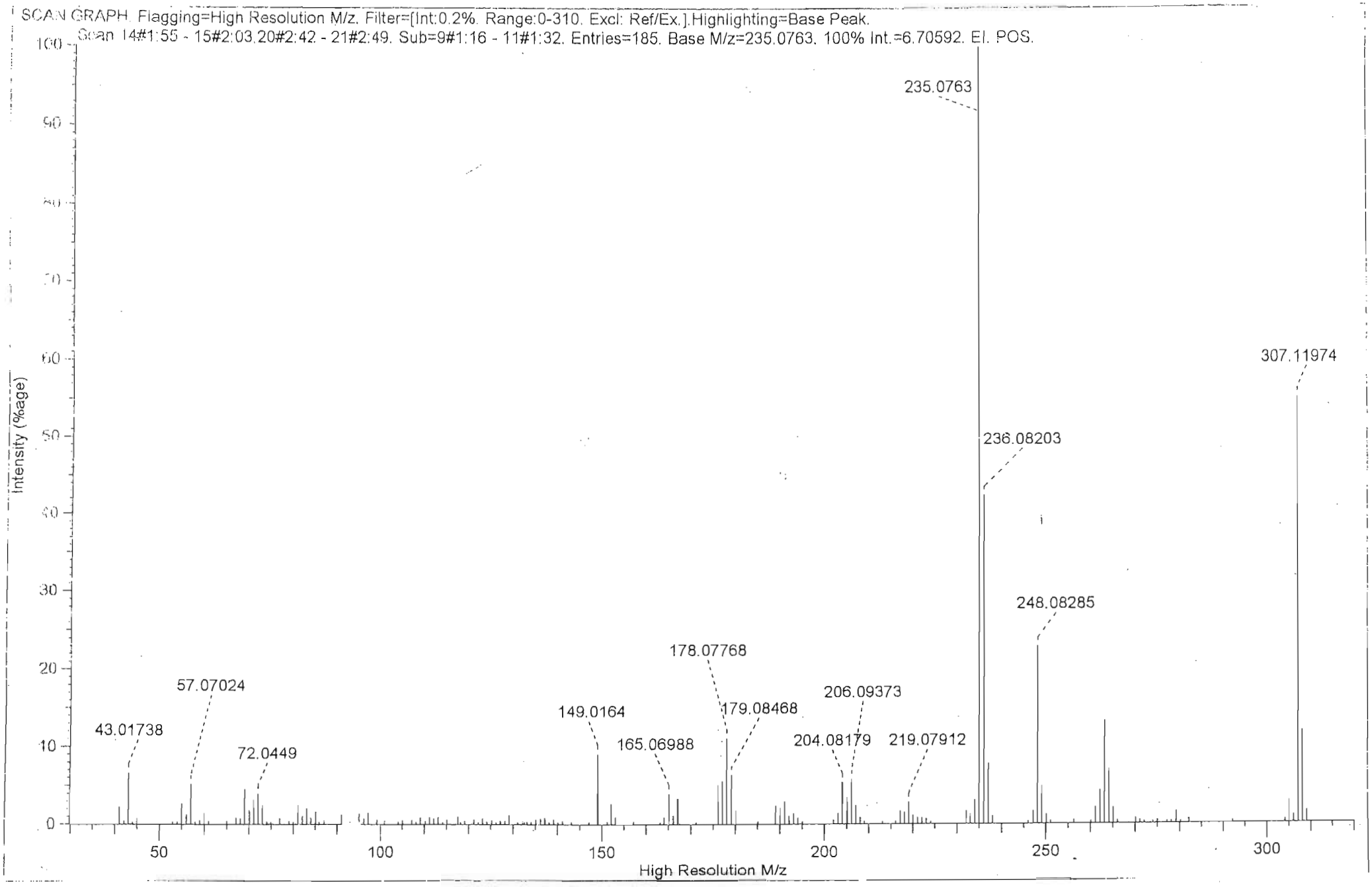

**Spectrum 10j: NOE spectrum of compound X showing irradiation of H-6a**



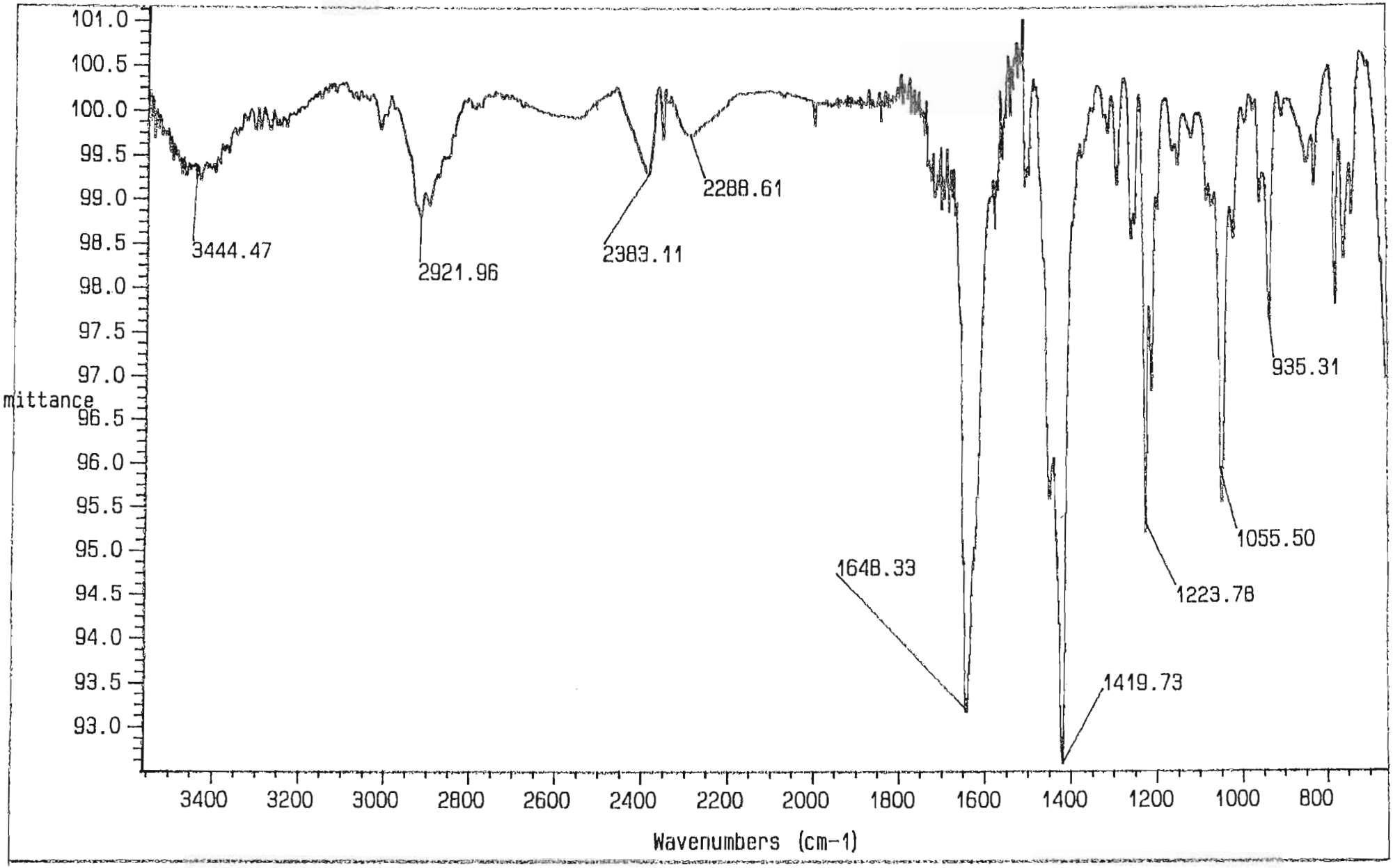

**Spectrum 10k: NOE spectrum of compound X showing irradiation of H-11**



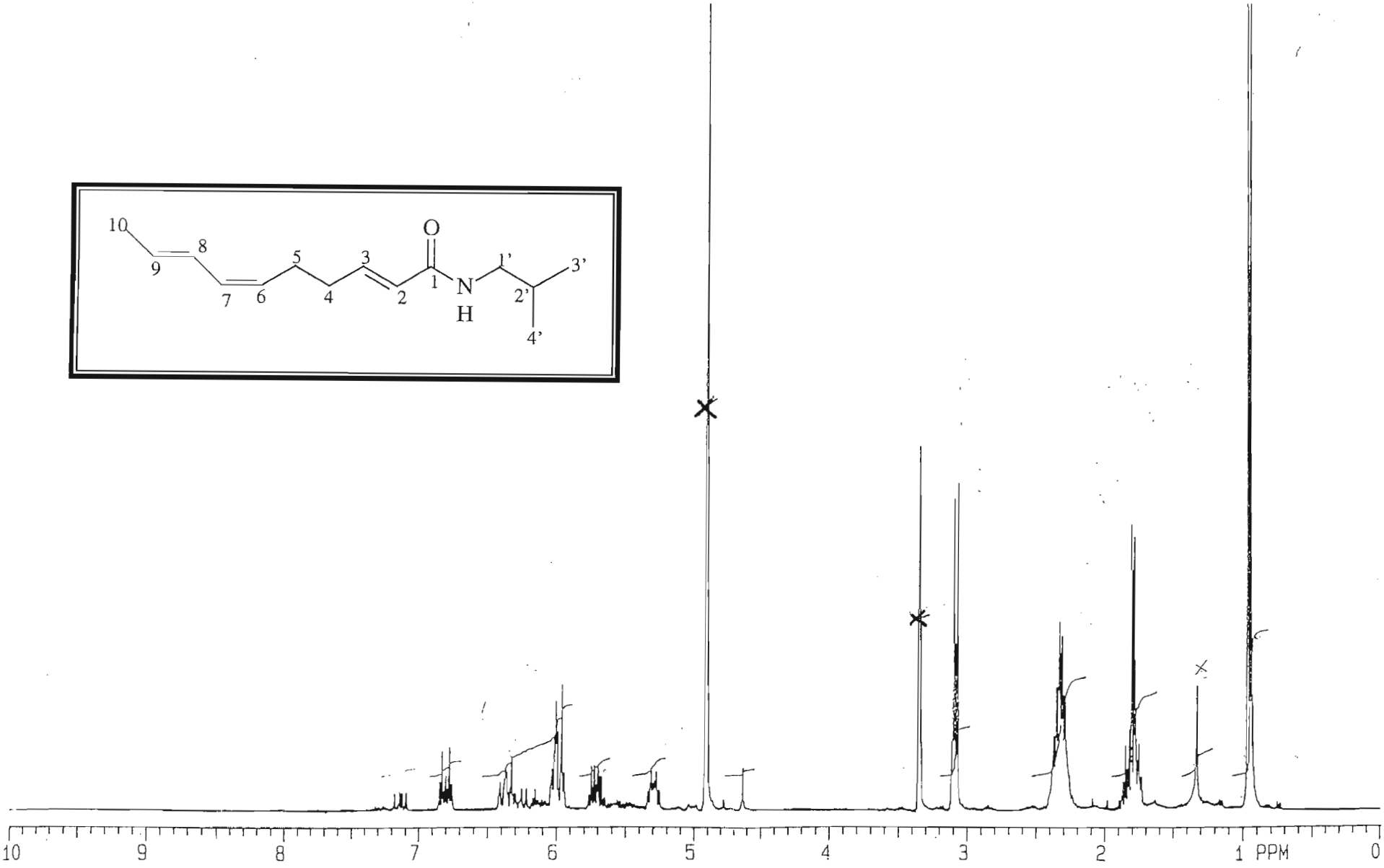

**Spectrum 10l: NOE spectrum of compound X showing irradiation of $OCH_2O$**



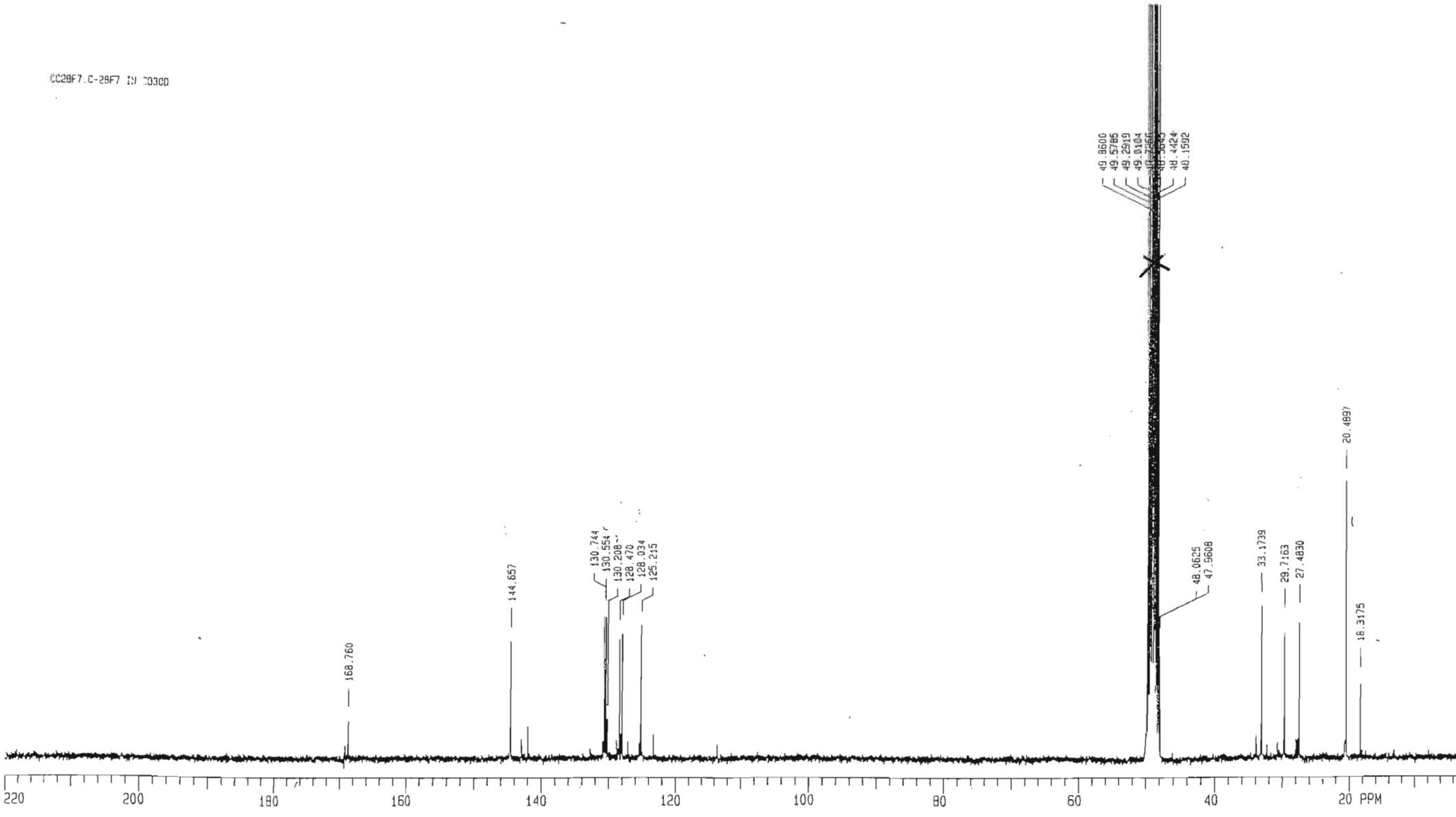

**Spectrum 10m: Mass spectrum of compound X**



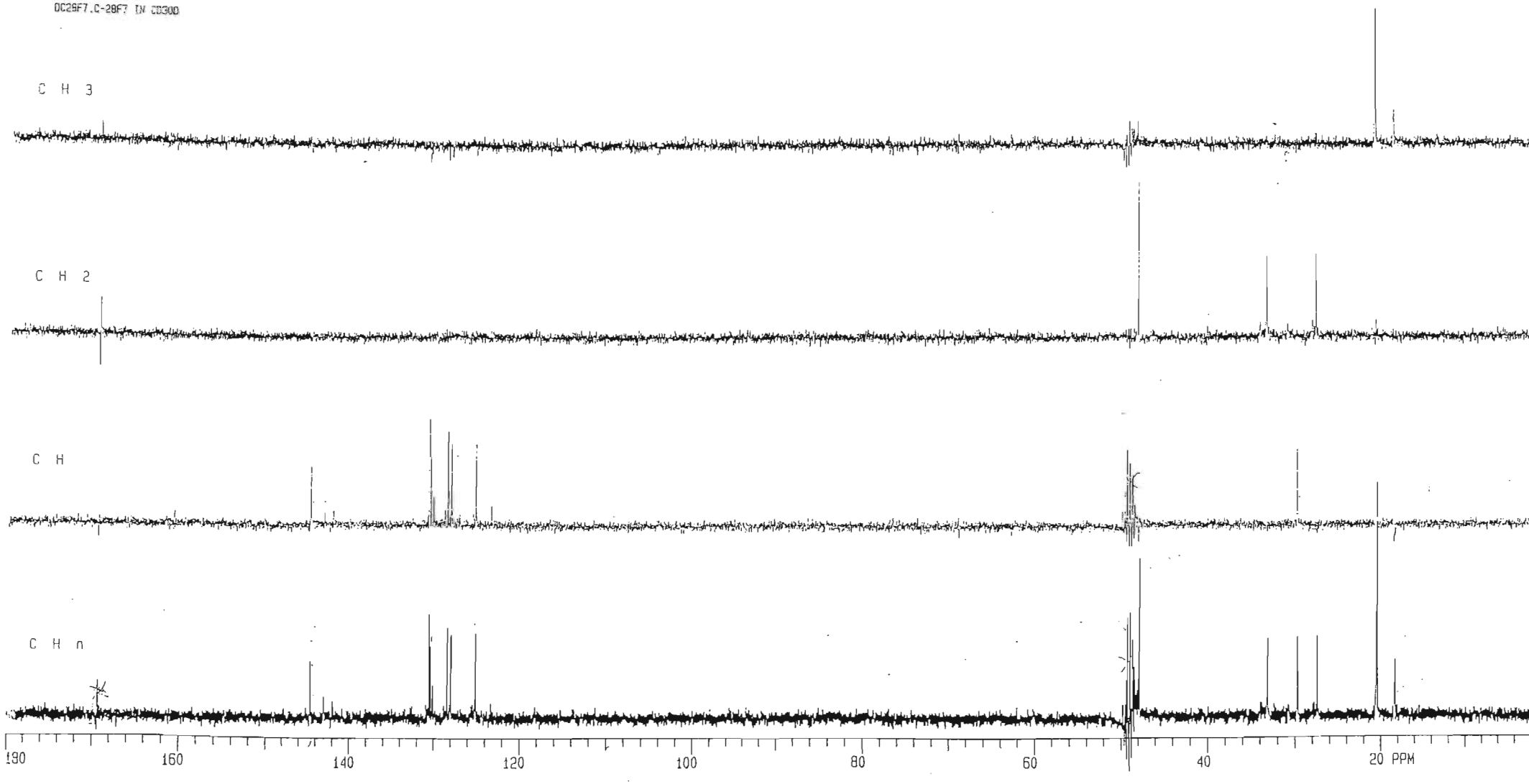

**Spectrum 10n: Infra-red spectrum of compound X**



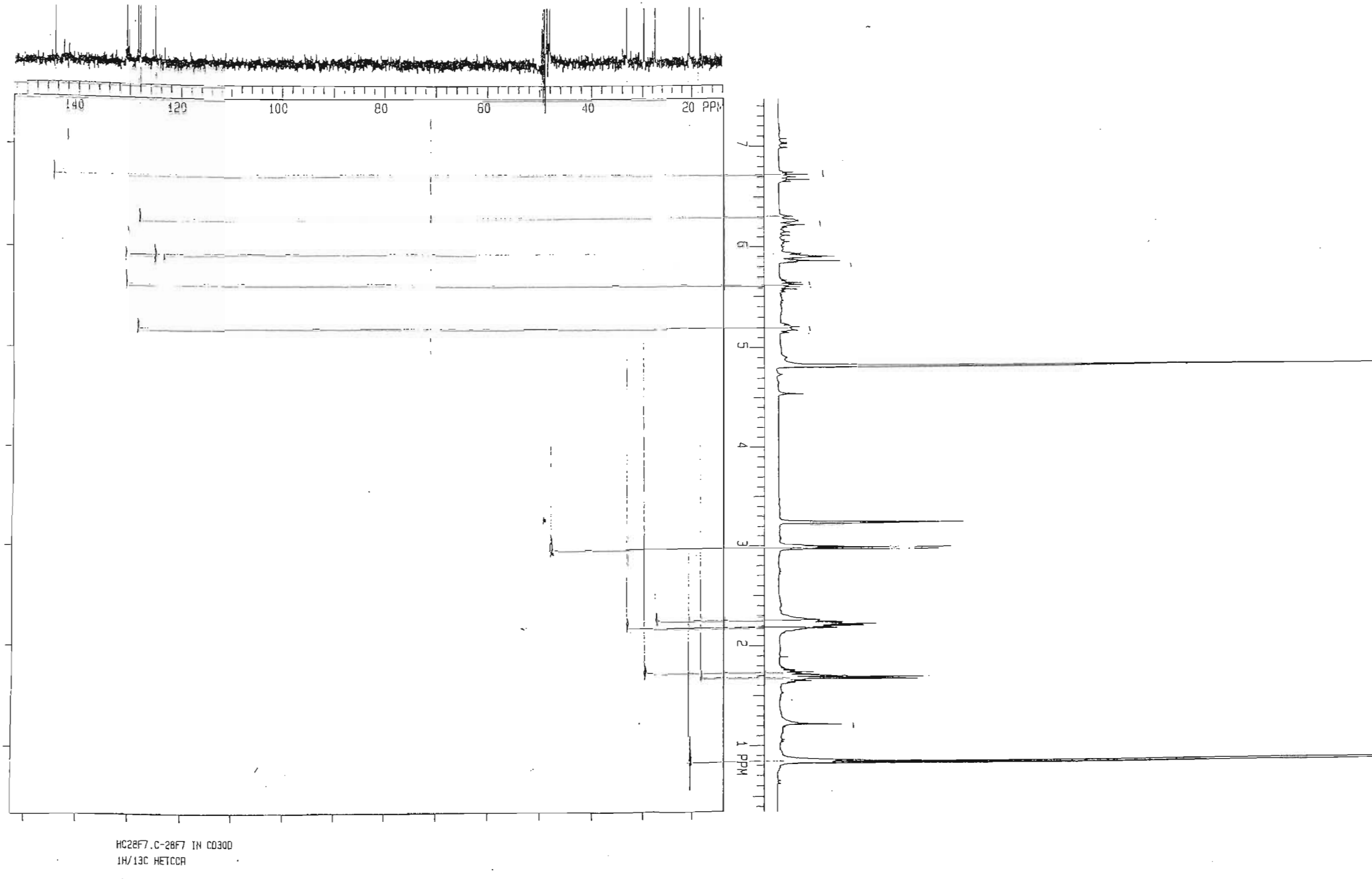

**Spectrum 11a: $^1$H NMR spectrum of compound XI ($CD_3OD$) (300 MHz)**



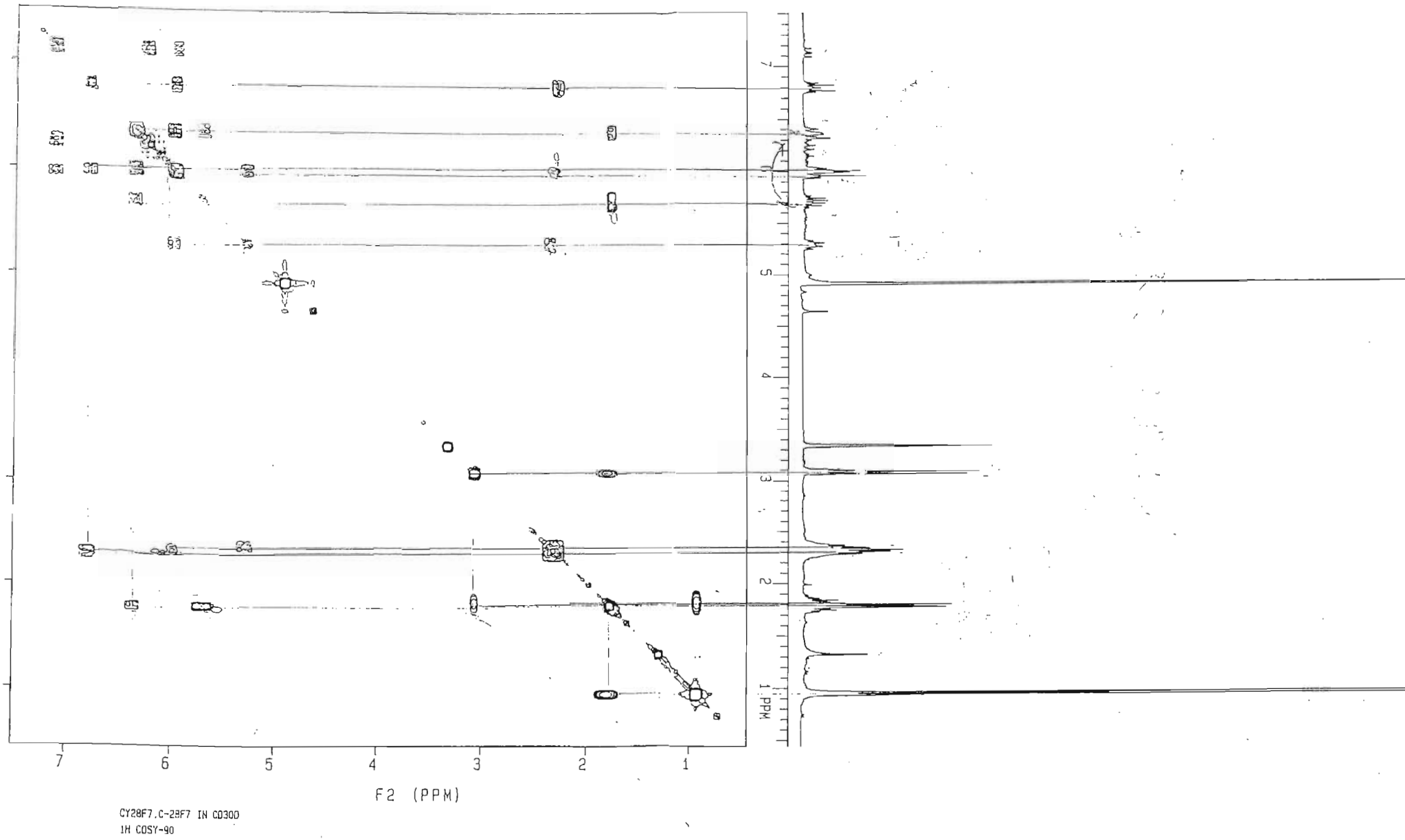

**Spectrum 11b: $^{13}C$ NMR spectrum of compound XI ($CD_3OD$) (75 MHz)**



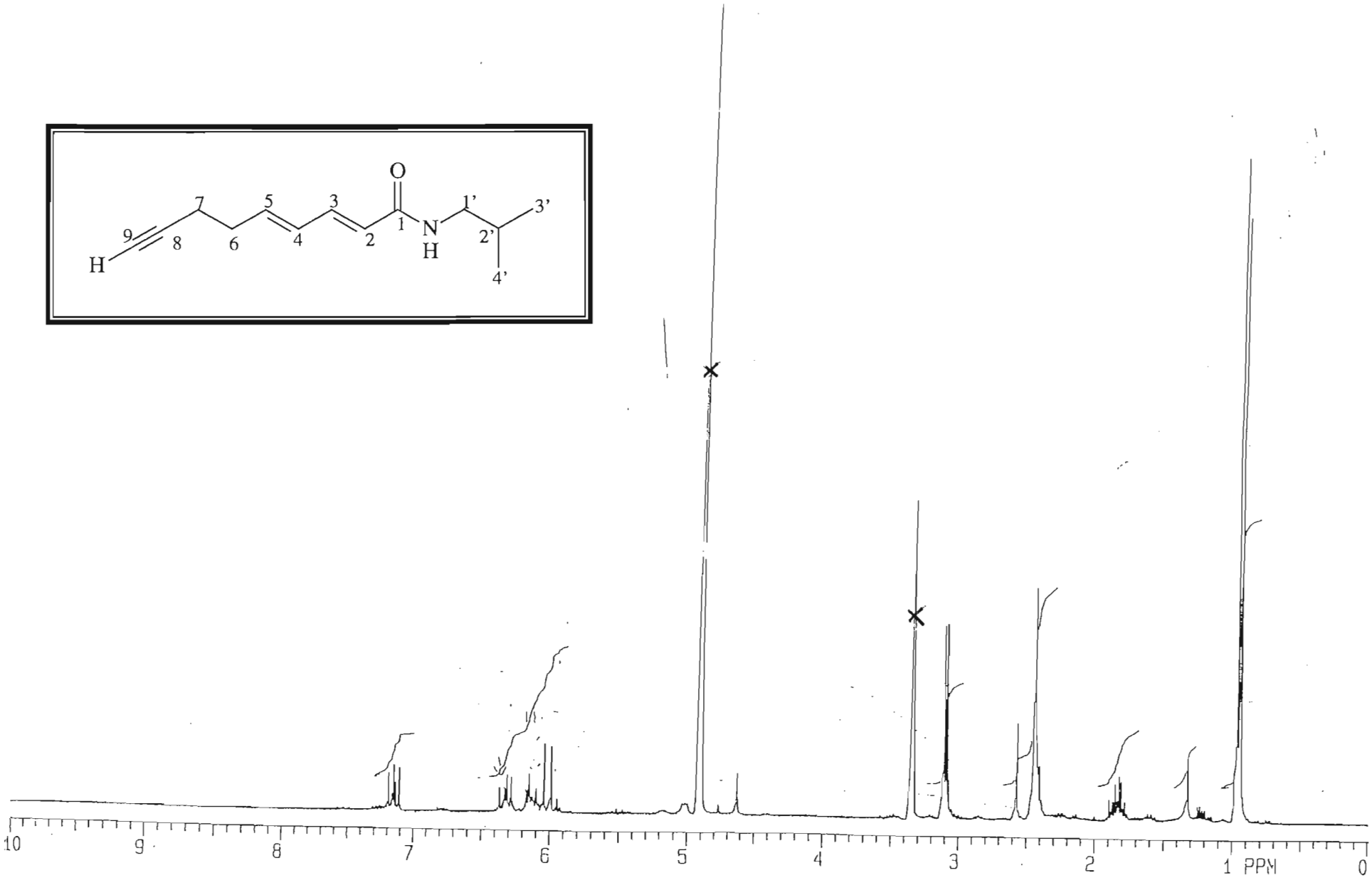

**Spectrum 11c: ADEPT spectrum of compound XI**



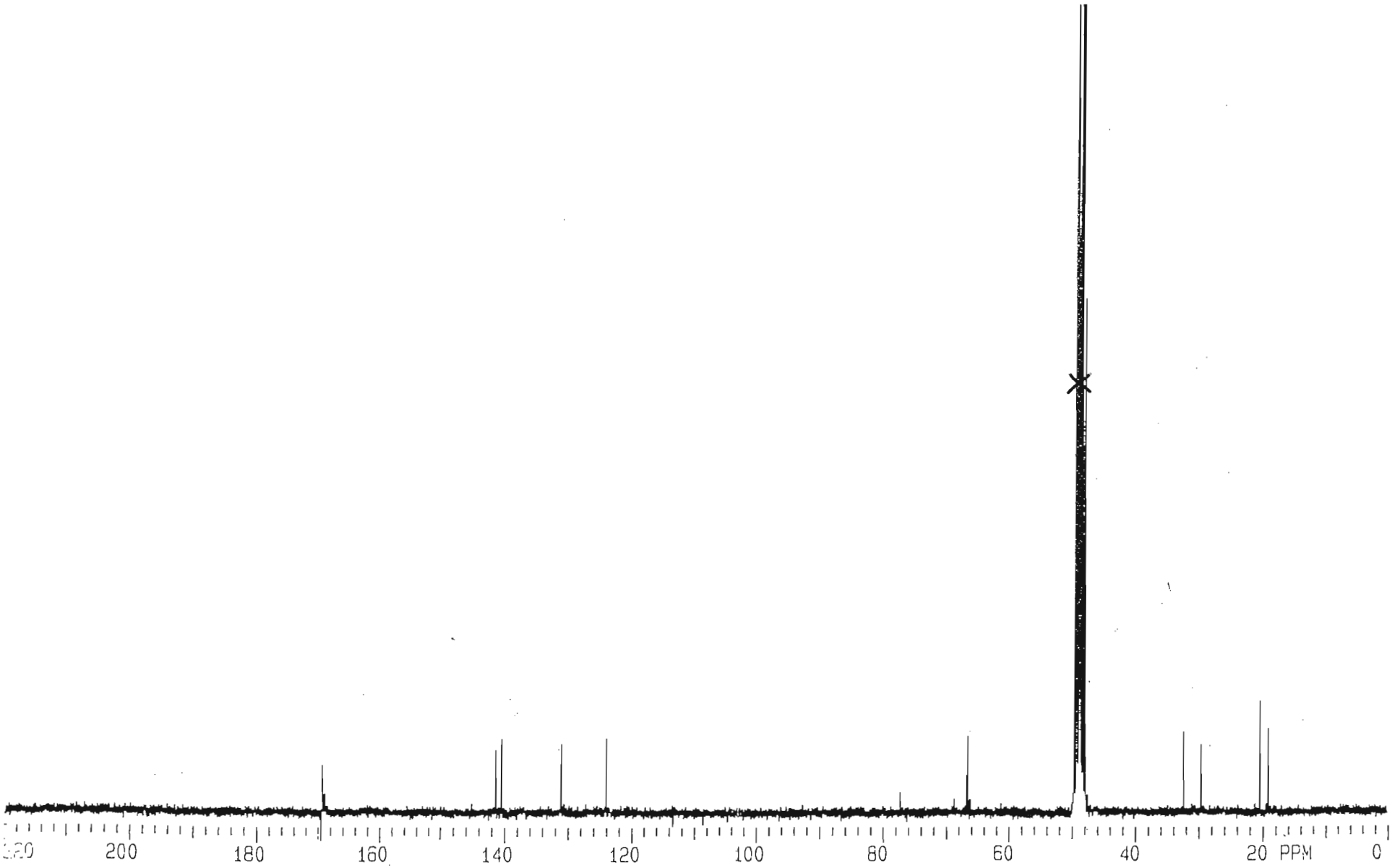

**Spectrum 11d: HETCOR spectrum of compound XI**



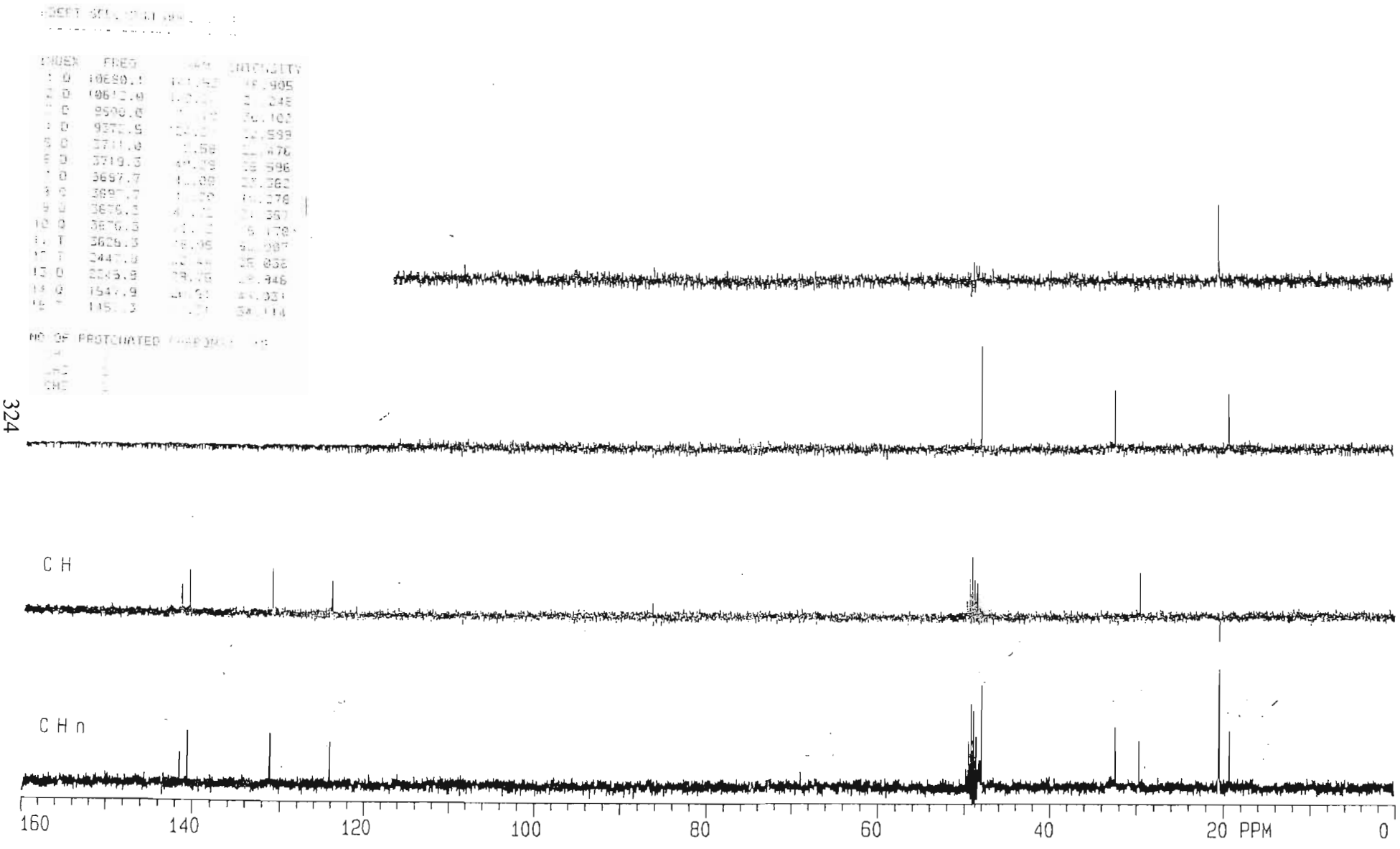

**Spectrum 11e: COSY spectrum of compound XI**



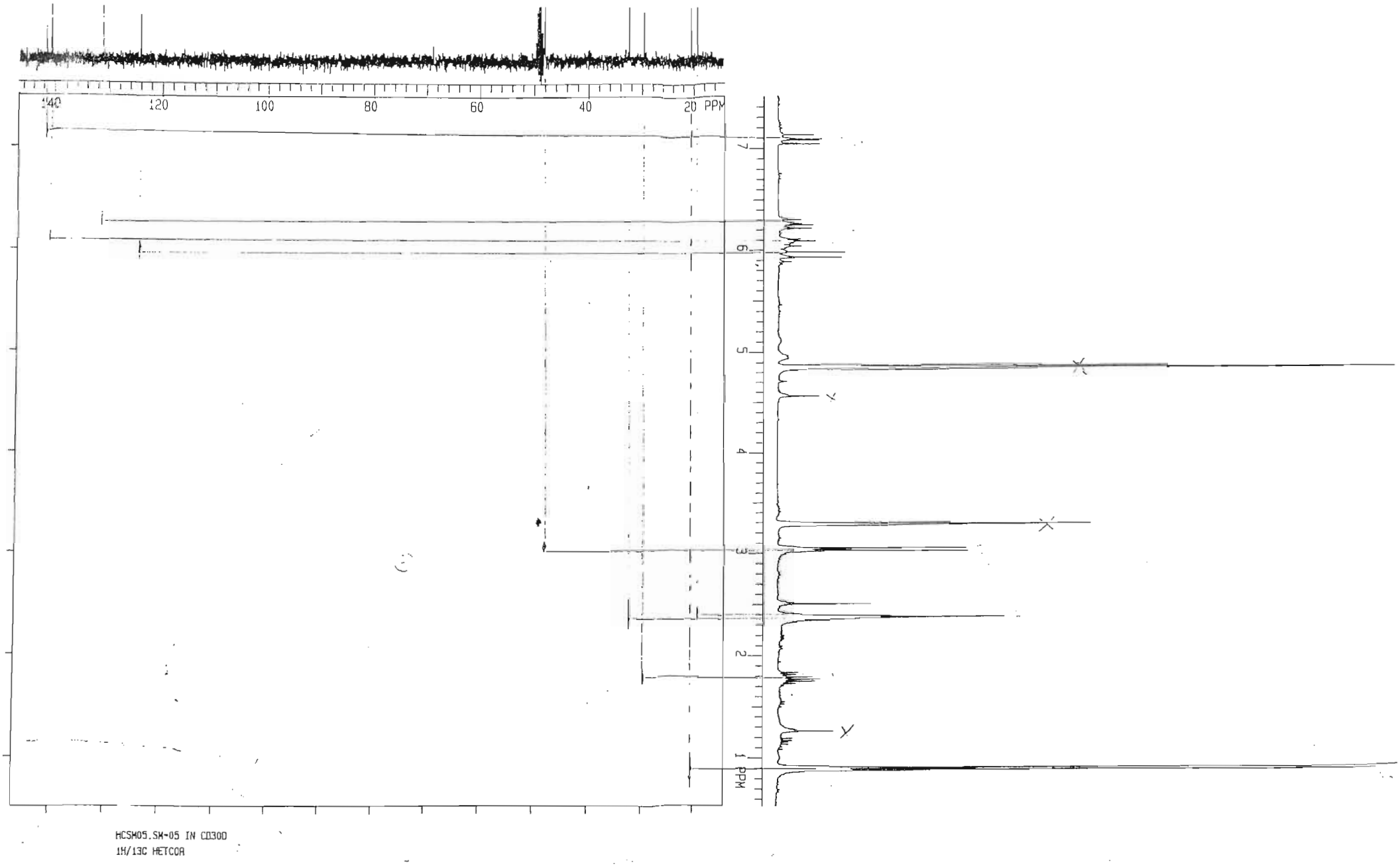

**Spectrum 12a: $^{1}$H NMR spectrum of compound XII ($CD_3OD$) (300 MHz)**



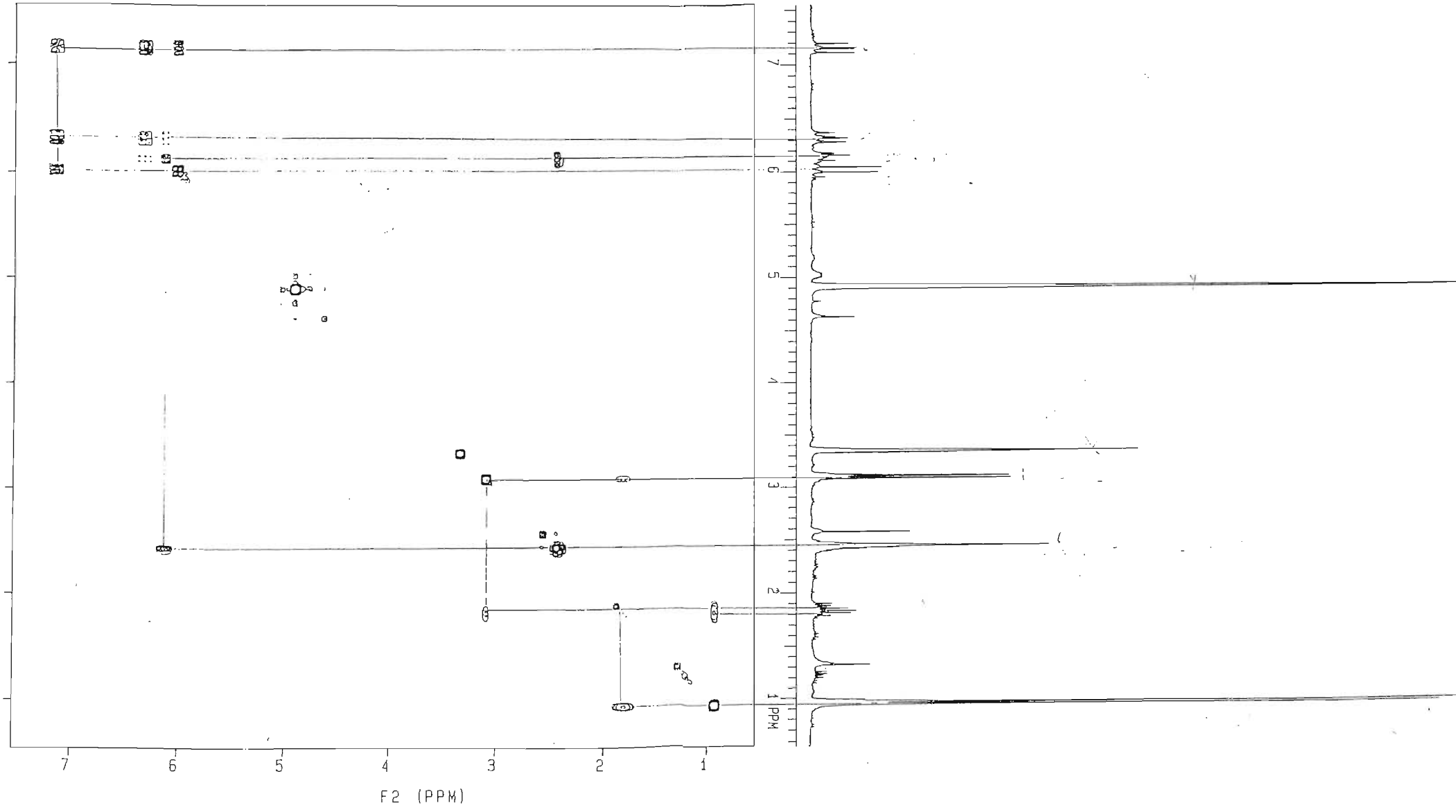

**Spectrum 12b: $^{13}C$ NMR spectrum of compound XII ($CD_3OD$) (75 MHz)**




**Spectrum 12c: ADEPT spectrum of compound XII**

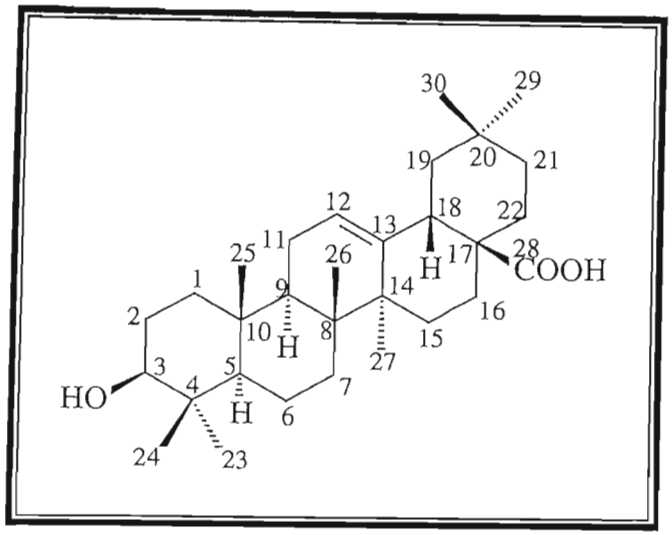


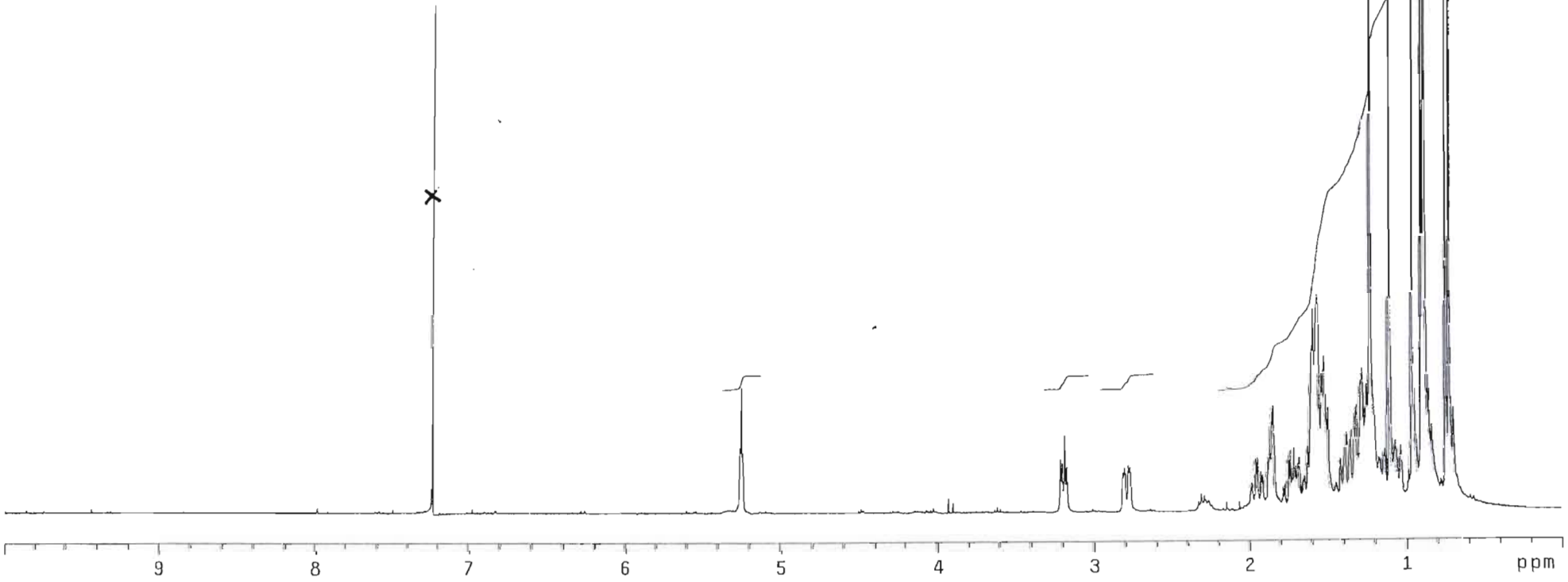

**Spectrum 12d: HETCOR spectrum of compound XII**



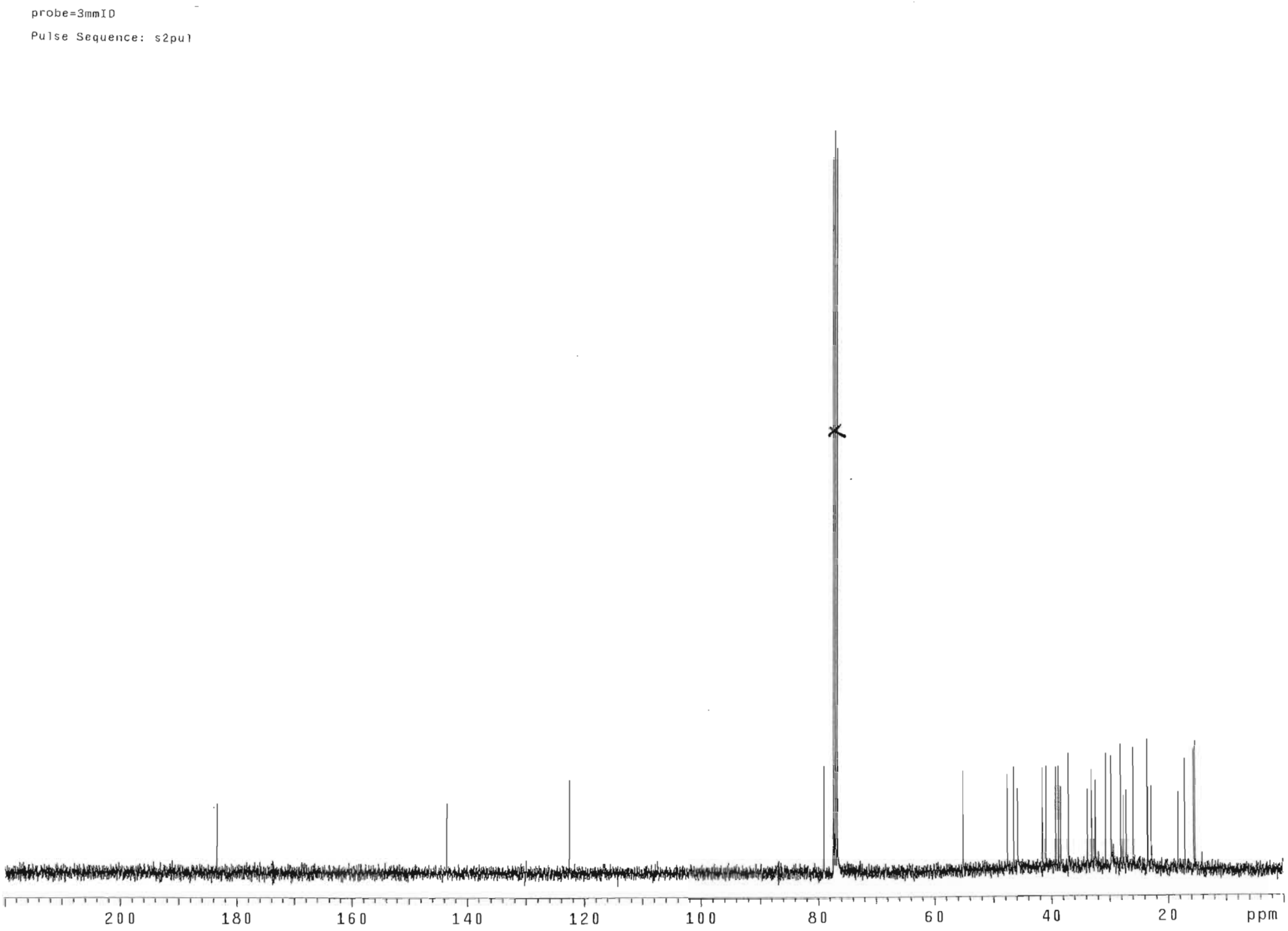

**Spectrum 12e: COSY spectrum of compound XII**

Pulse Sequence: s2pul

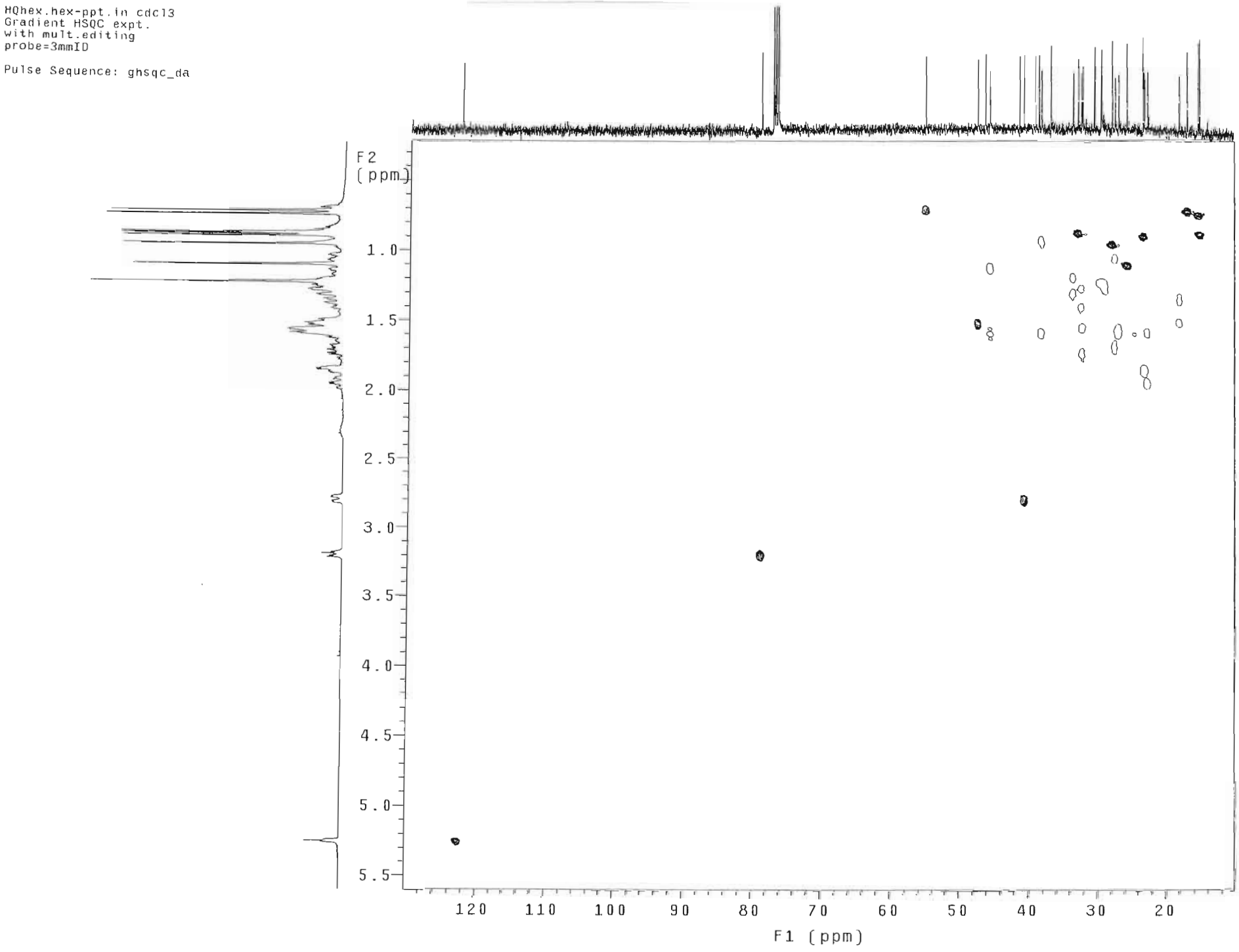


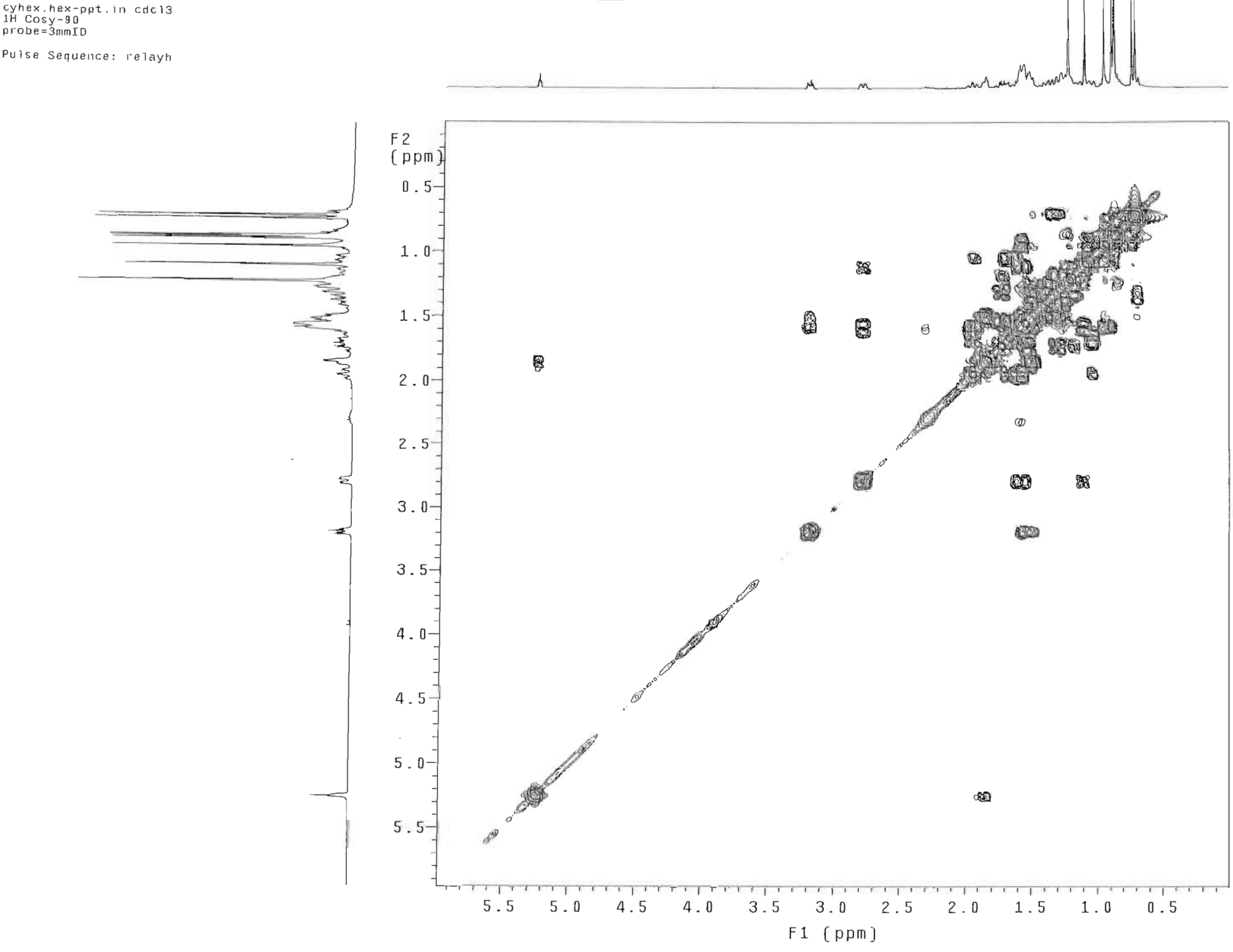

**Spectrum 13a: $^{1}H$ NMR spectrum of compound XIII ($CDCl_3$) (400 MHz)**



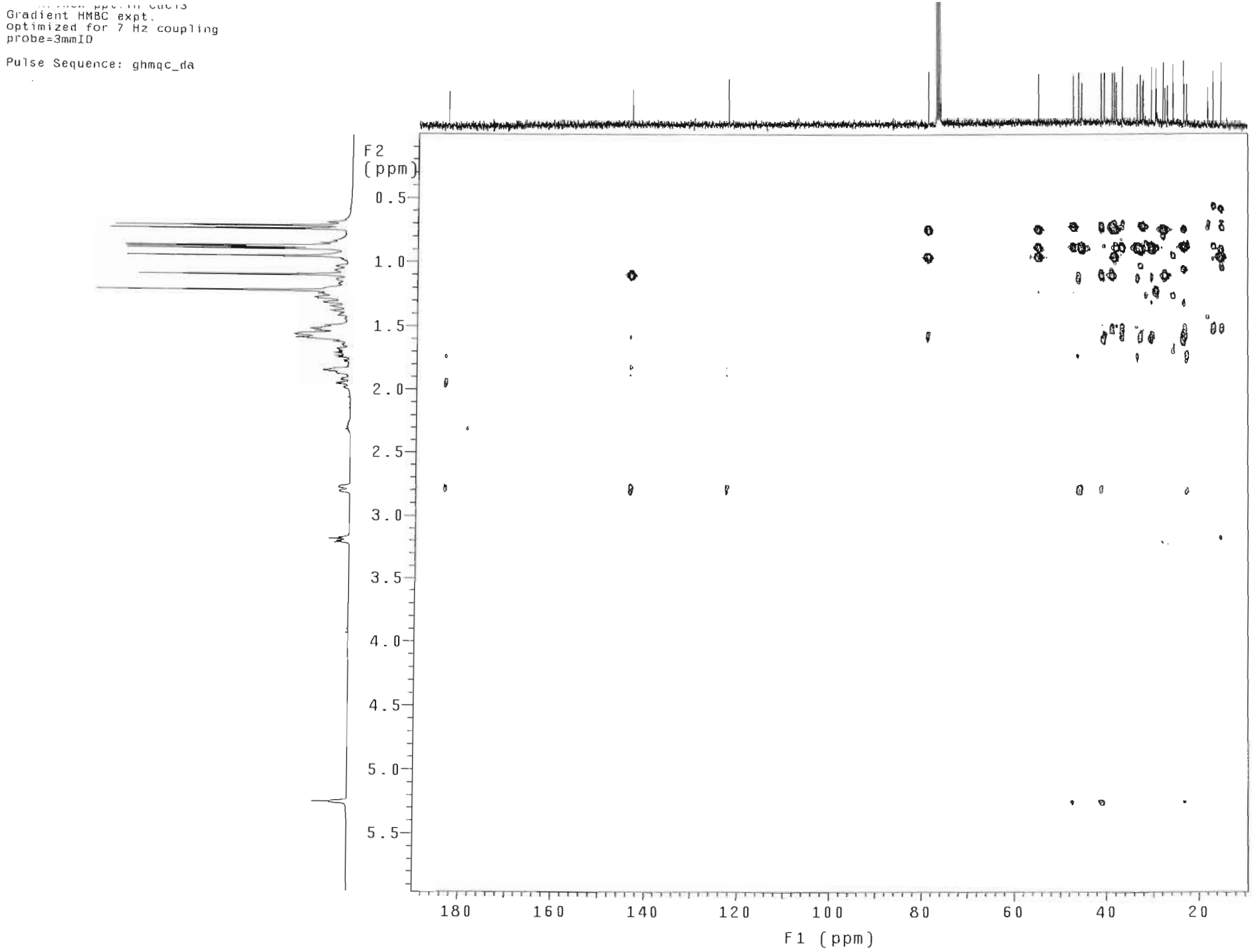

**Spectrum 13b: $^{13}C$ NMR spectrum of compound XIII ($CDCl_3$) (100 MHz)**



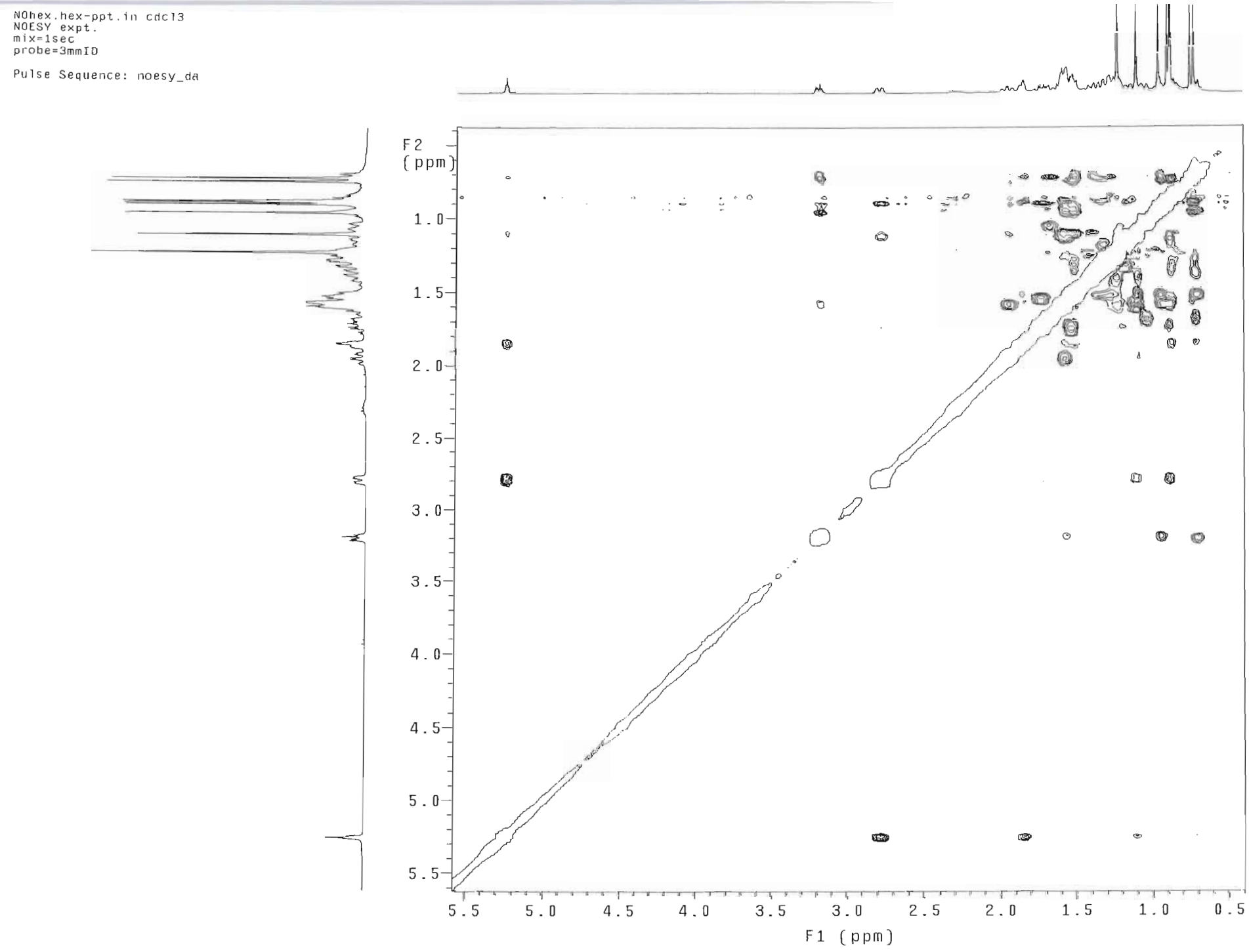

**Spectrum 13c: HSQC spectrum of compound XIII**



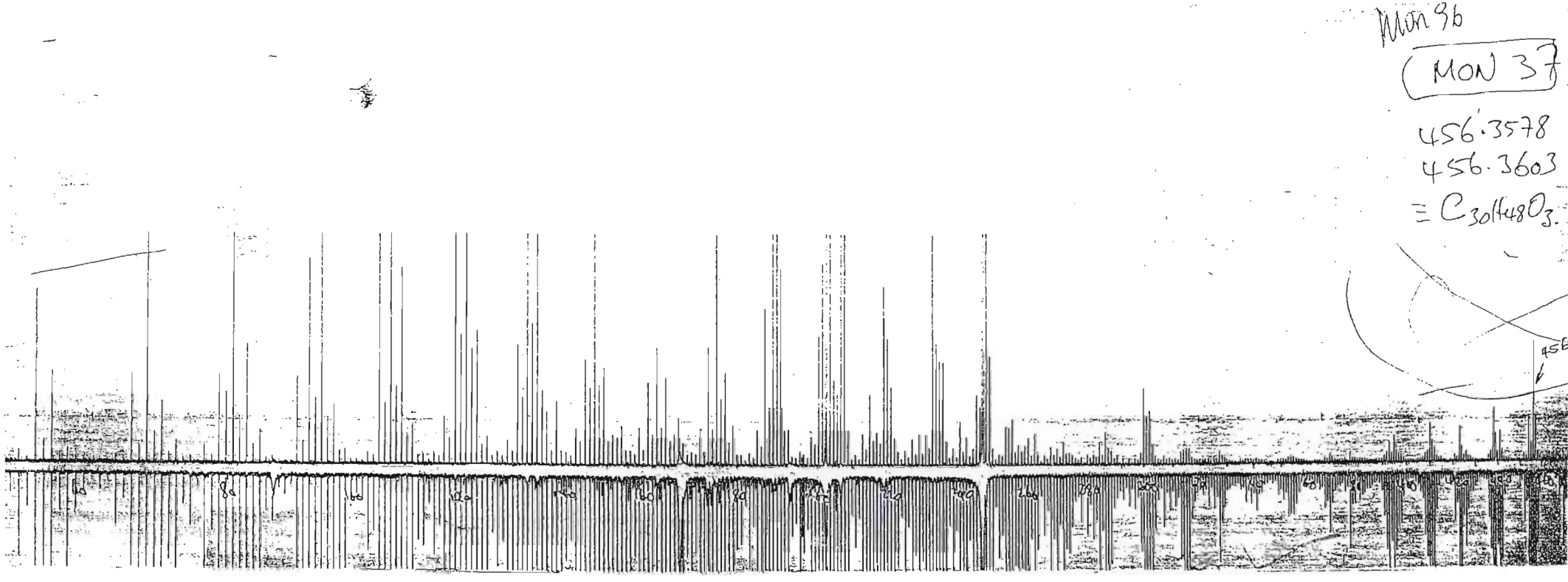

**Spectrum 13d: COSY spectrum of compound XIII**



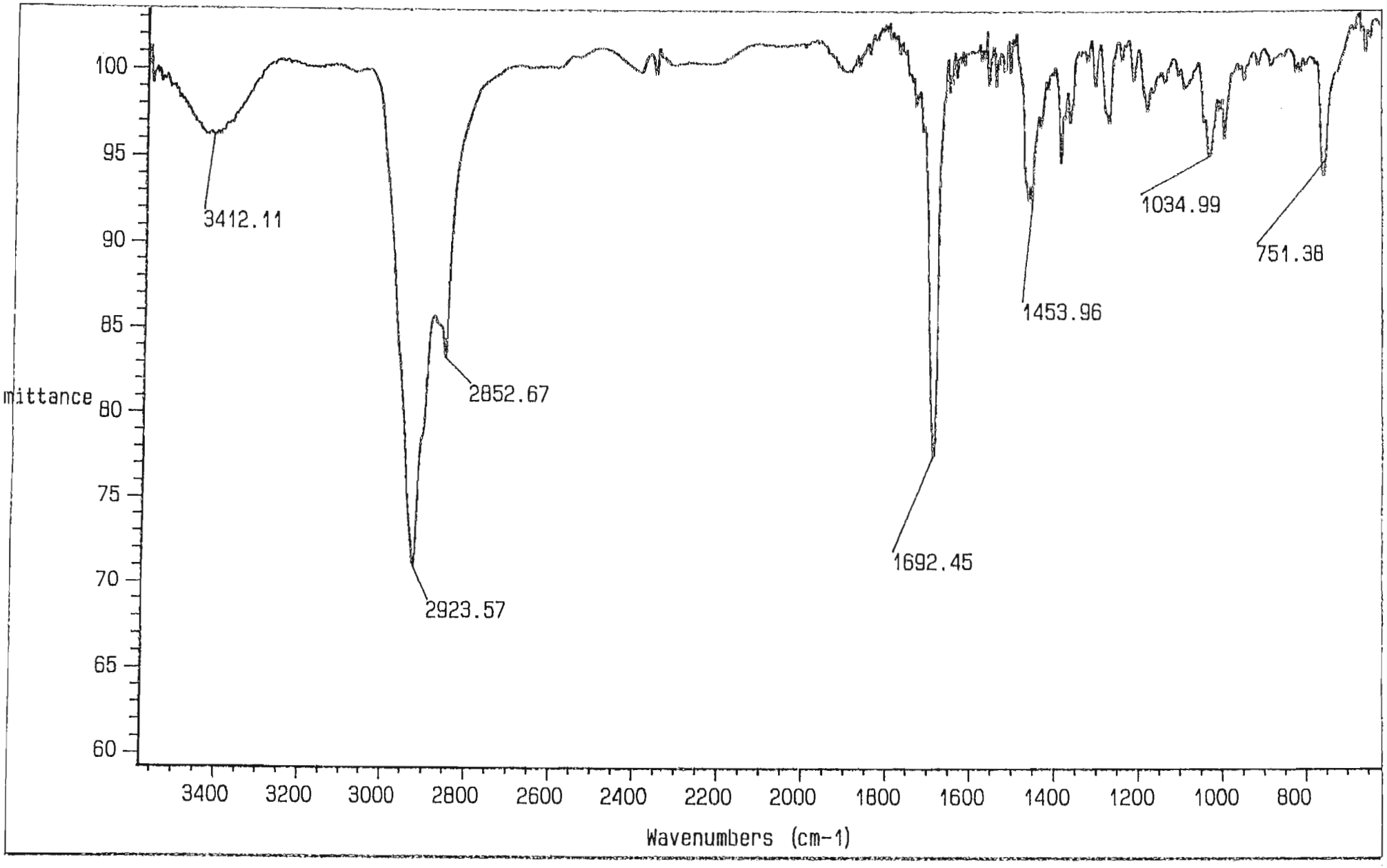

**Spectrum 13e: HMBC spectrum of compound XIII**



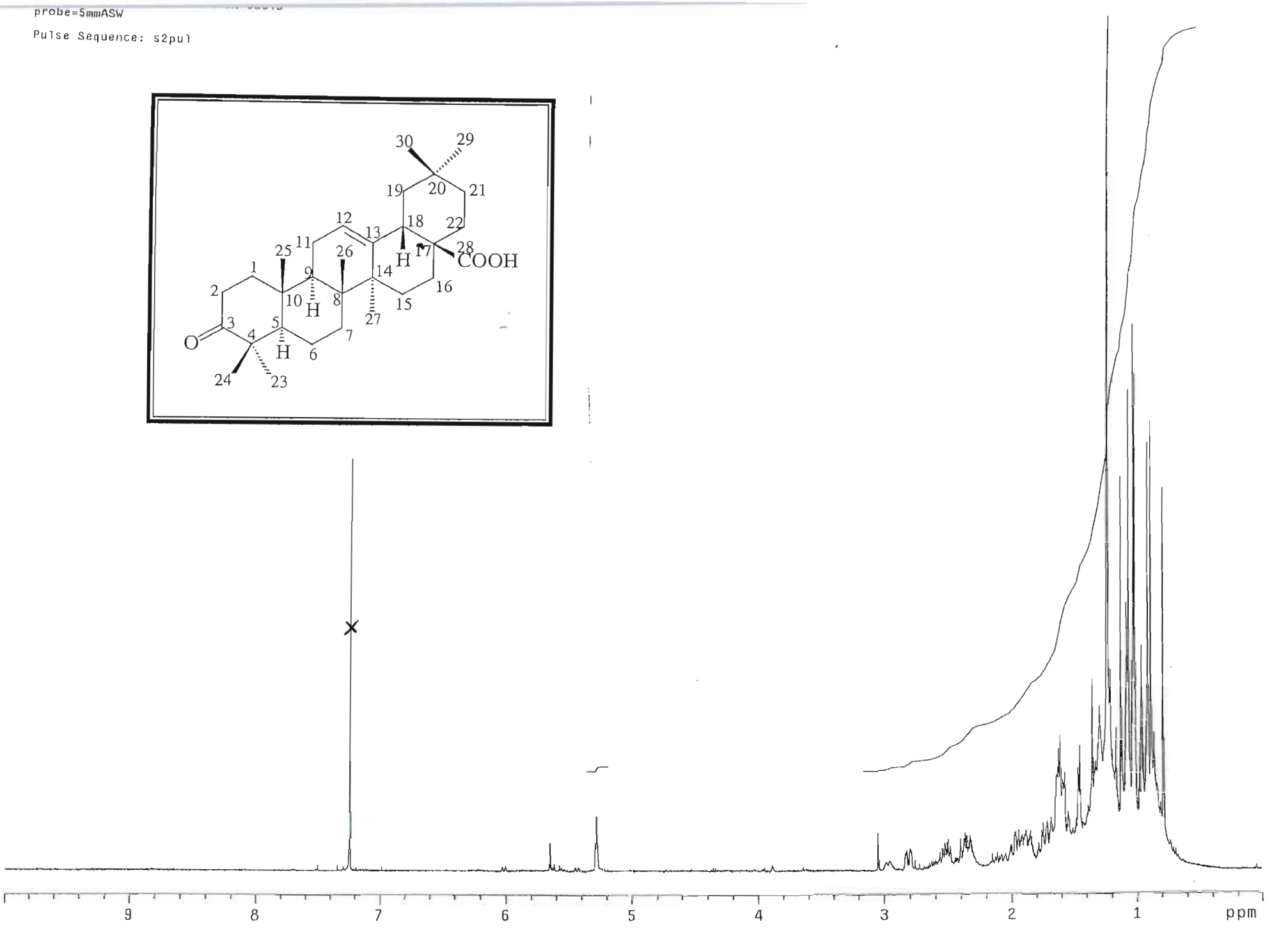

**Spectrum 13f: NOESY spectrum of compound XIII**



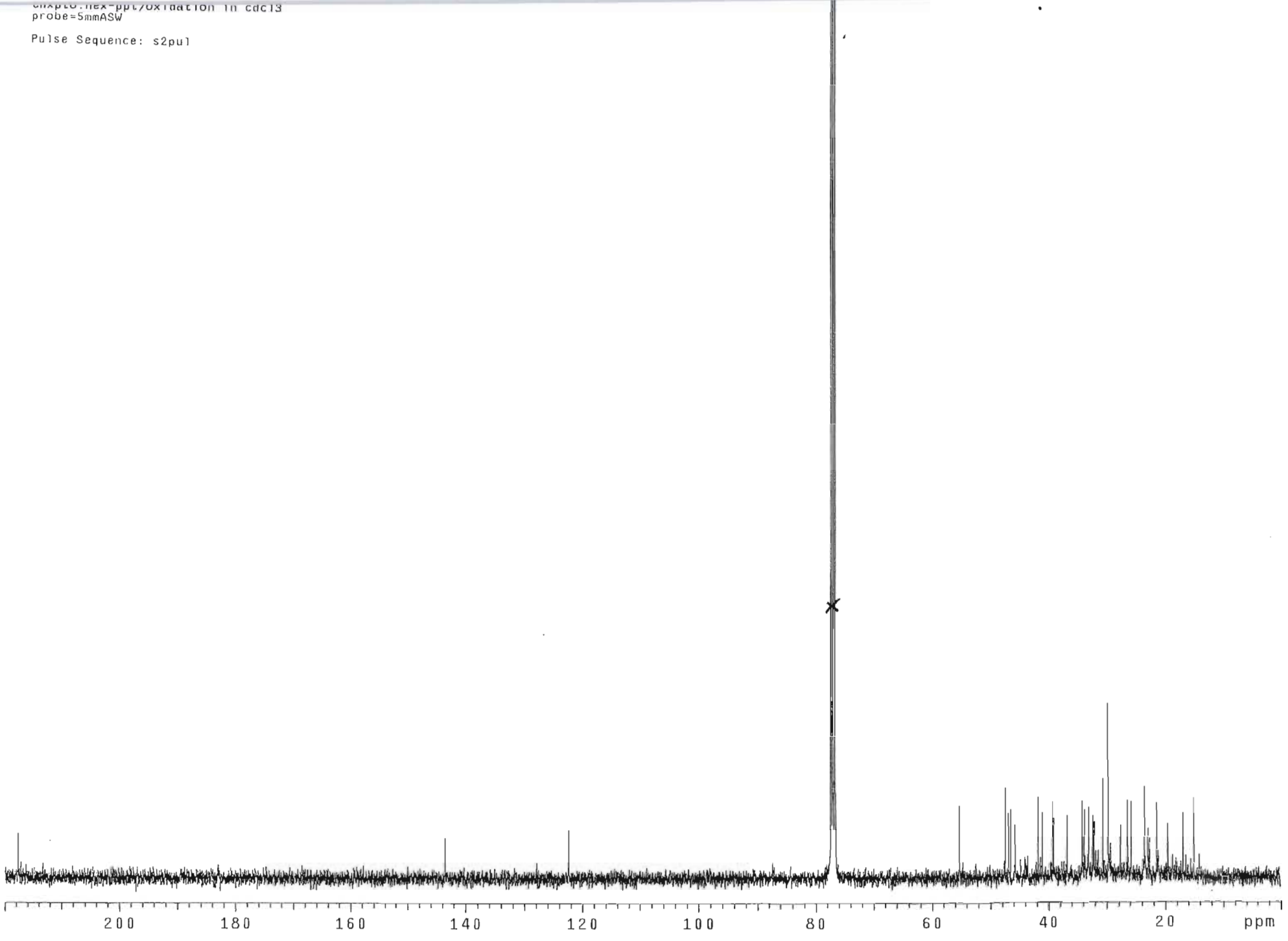

**Spectrum 13g: Mass spectrum of compound XIII**



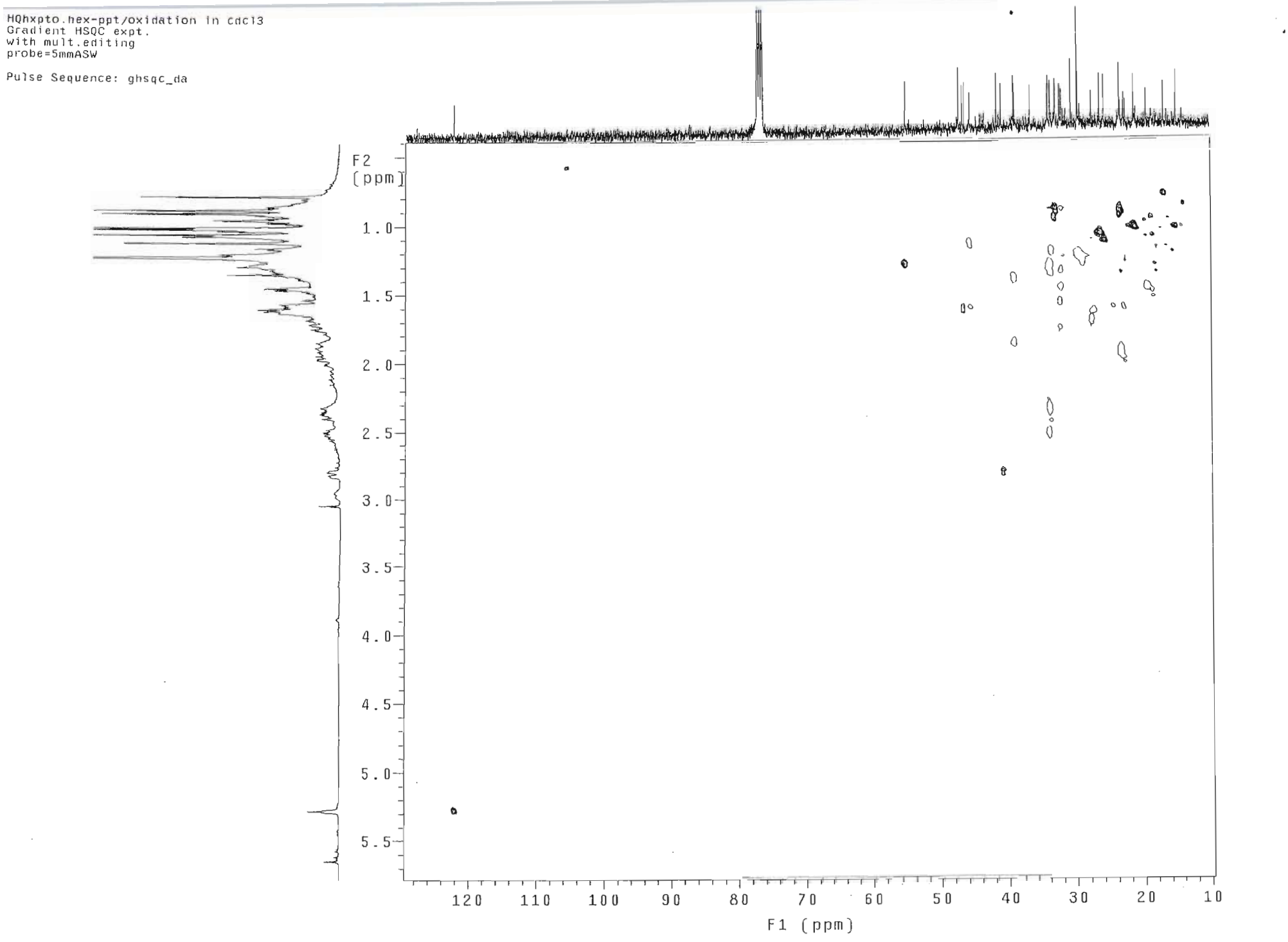

**Spectrum 13h: Infra-red spectrum of compound XIII**



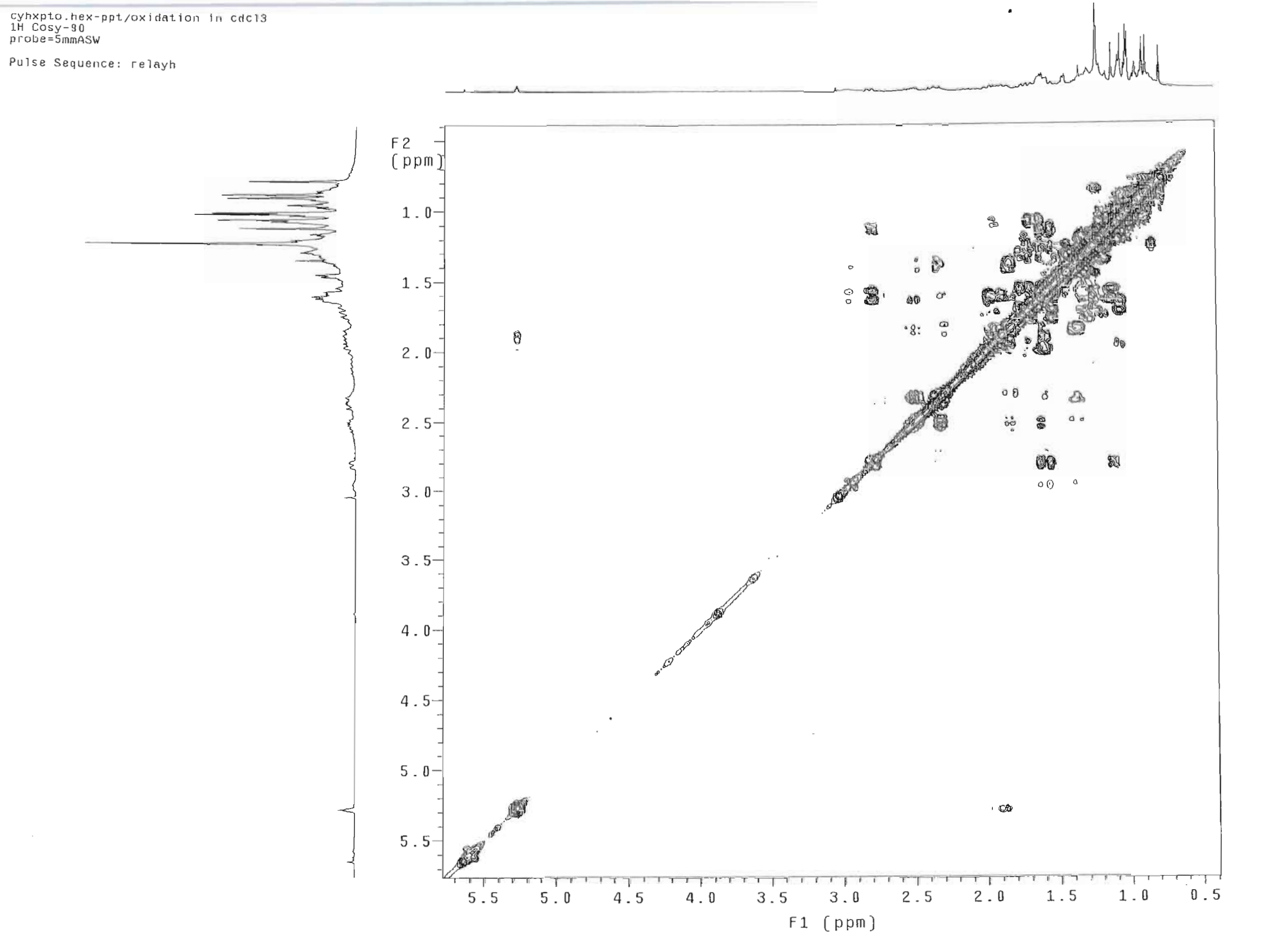

**Spectrum 13i: $^{1}$H NMR spectrum of oxidised compound XIII ($CDCl_3$) (400 MHz)**



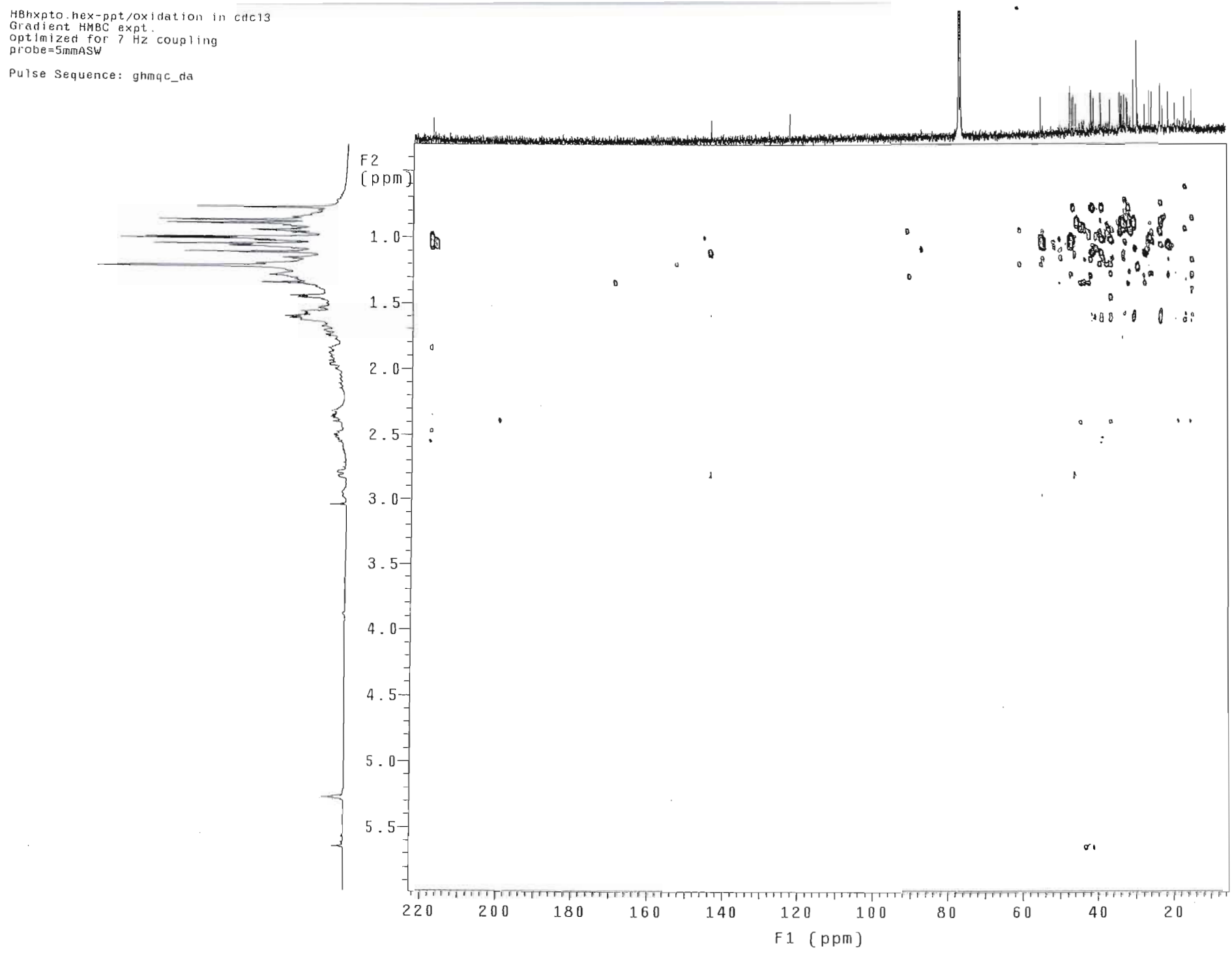

**Spectrum 13j: $^{13}C$ NMR spectrum of oxidised compound XIII ($CDCl_3$) (100 MHz)**



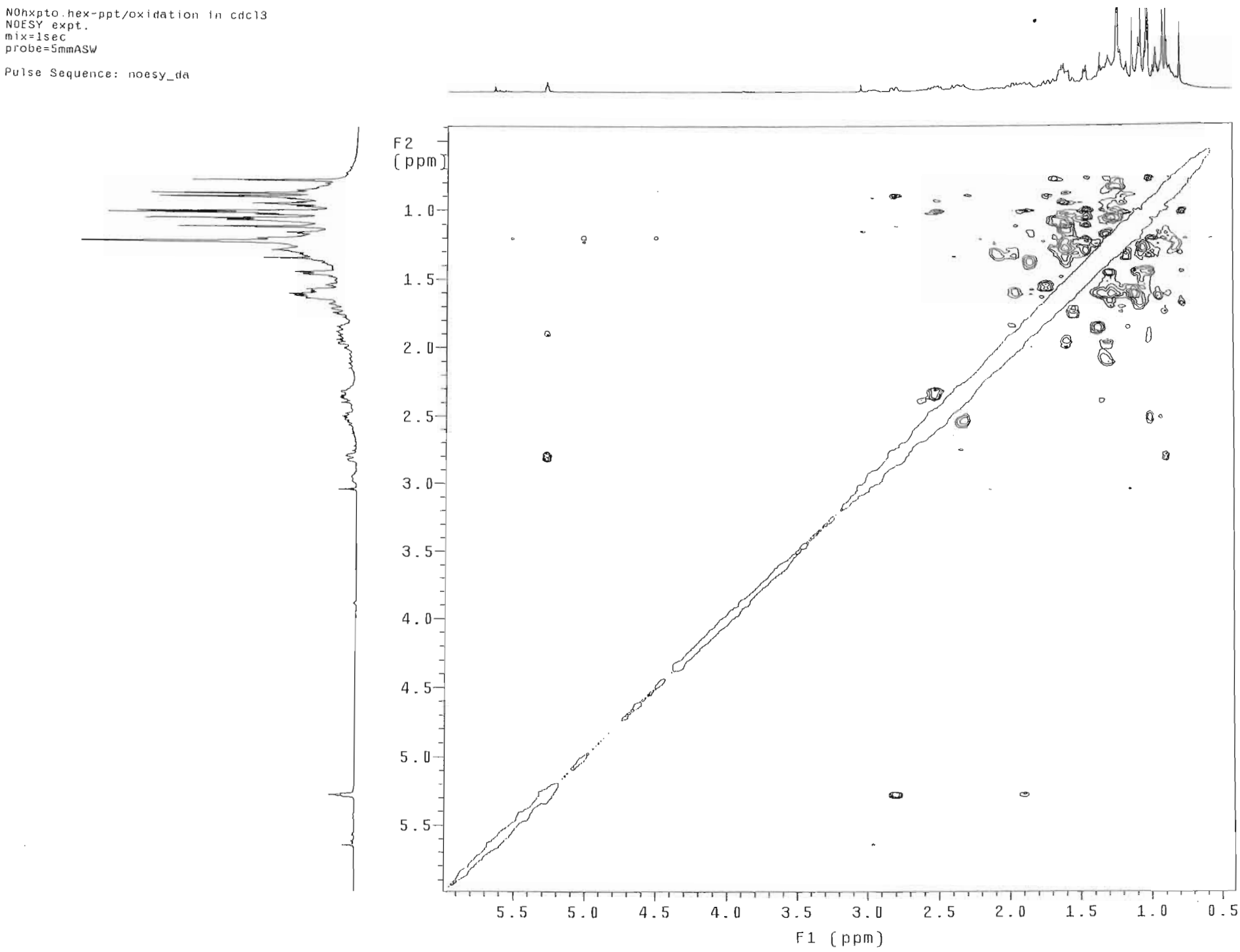

**Spectrum 13k: HSQC spectrum of oxidised compound XIII**




**Spectrum 13l: COSY spectrum of oxidised compound XIII**



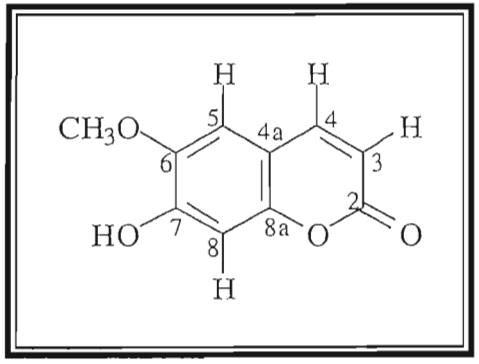

**Spectrum 13m: HMBC spectrum of oxidised compound XIII**




**Spectrum 13n: NOESY spectrum of oxidised compound XIII**

probe=3mmID

Pulse Sequence: s2pul

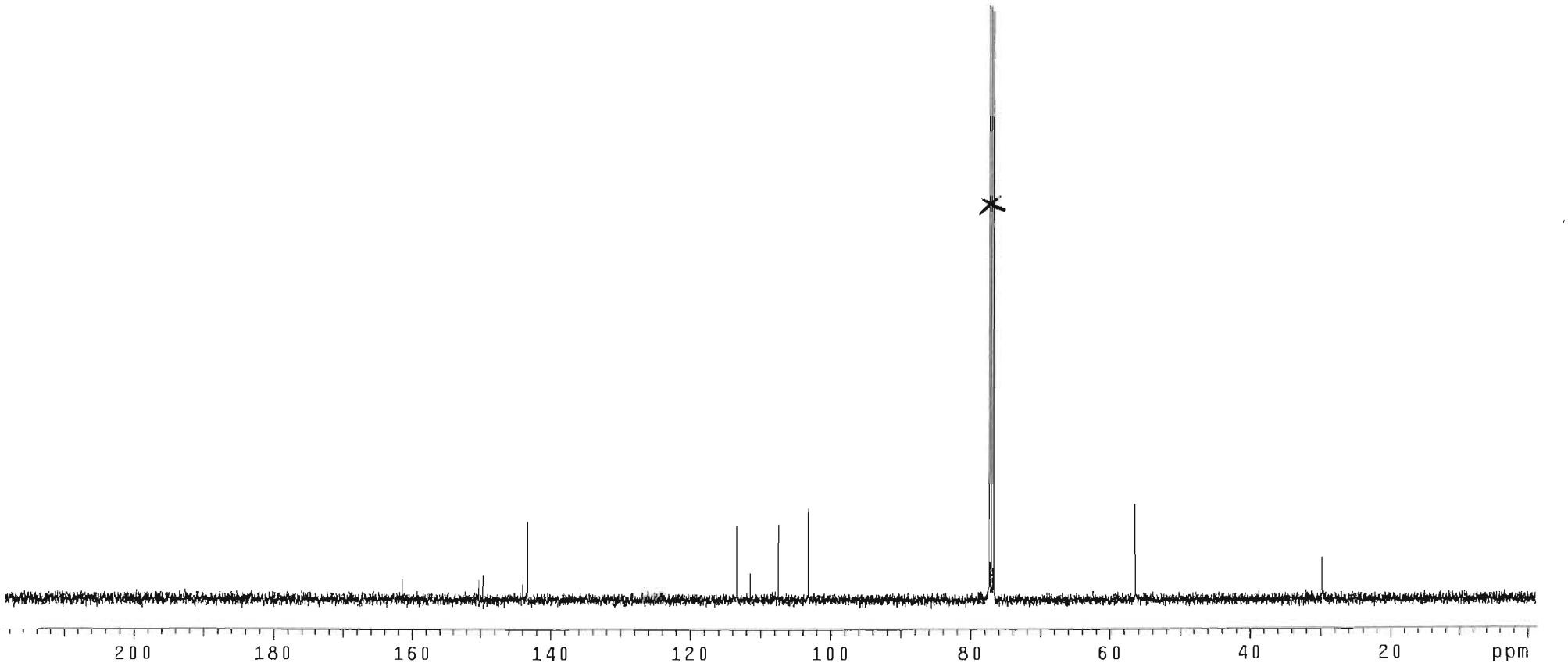




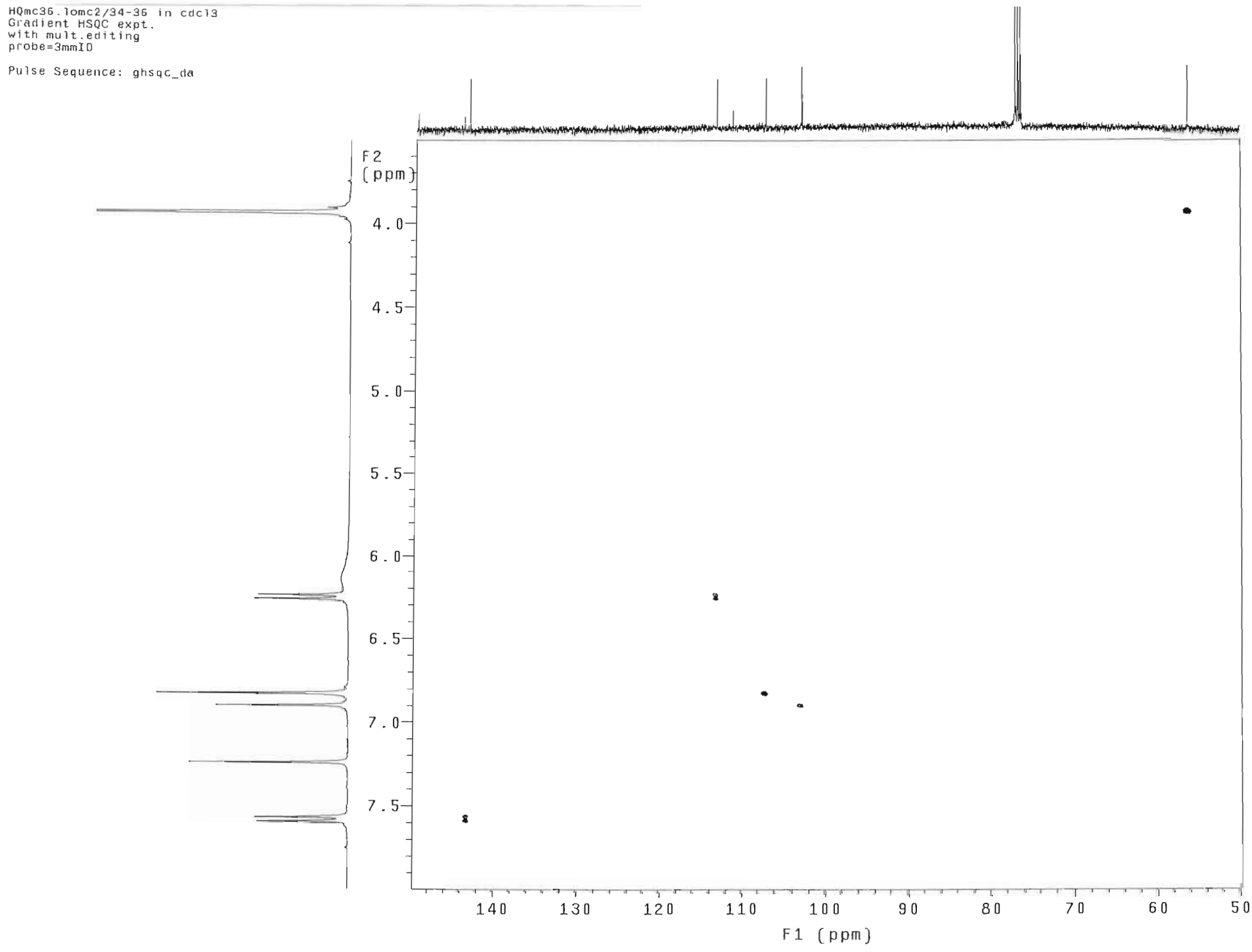

**Spectrum 14a: $^{1}H$ NMR spectrum of compound XIV ($CDCl_3$) (400 MHz)**

probe=5mmID

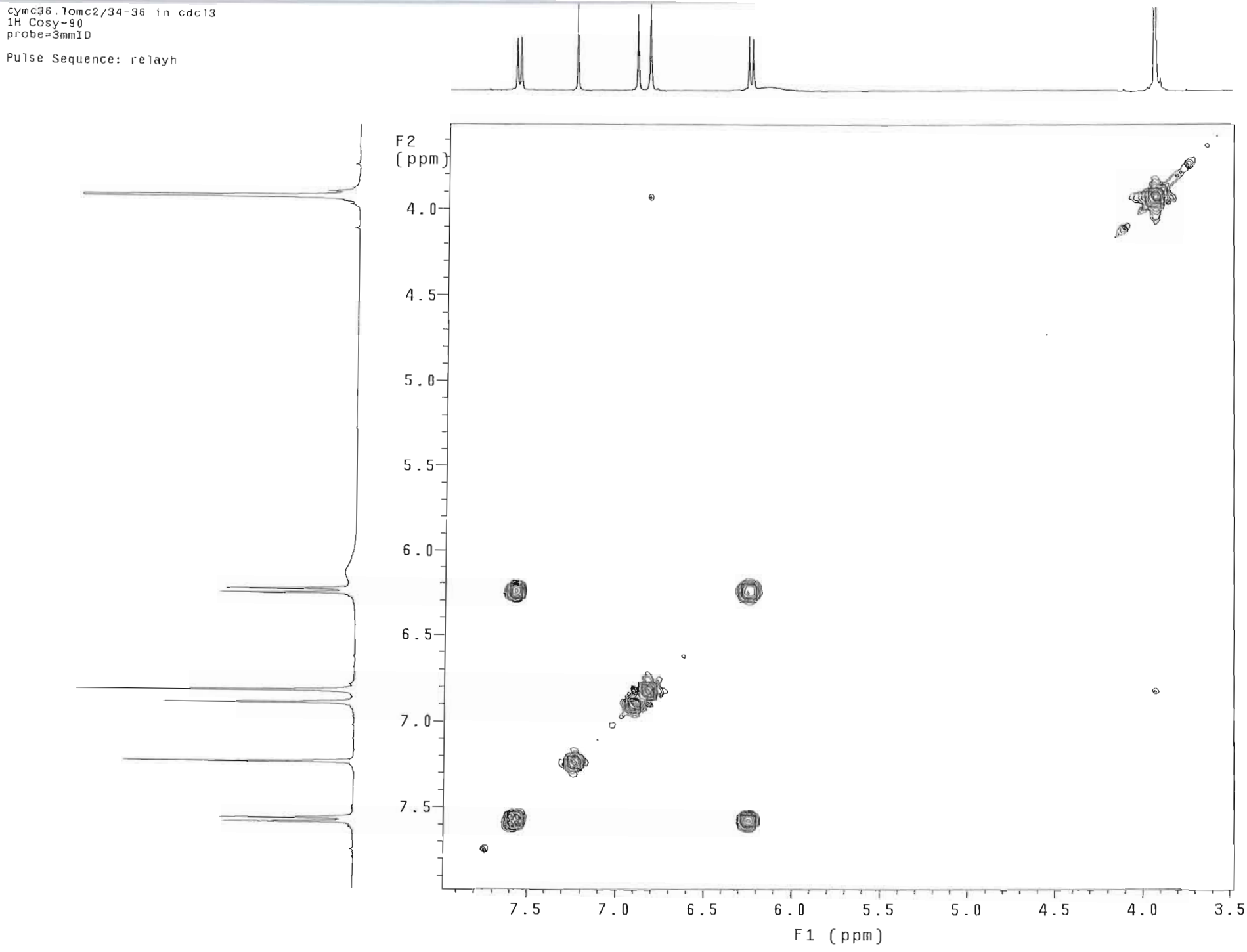
Pulse Sequence: s2pul



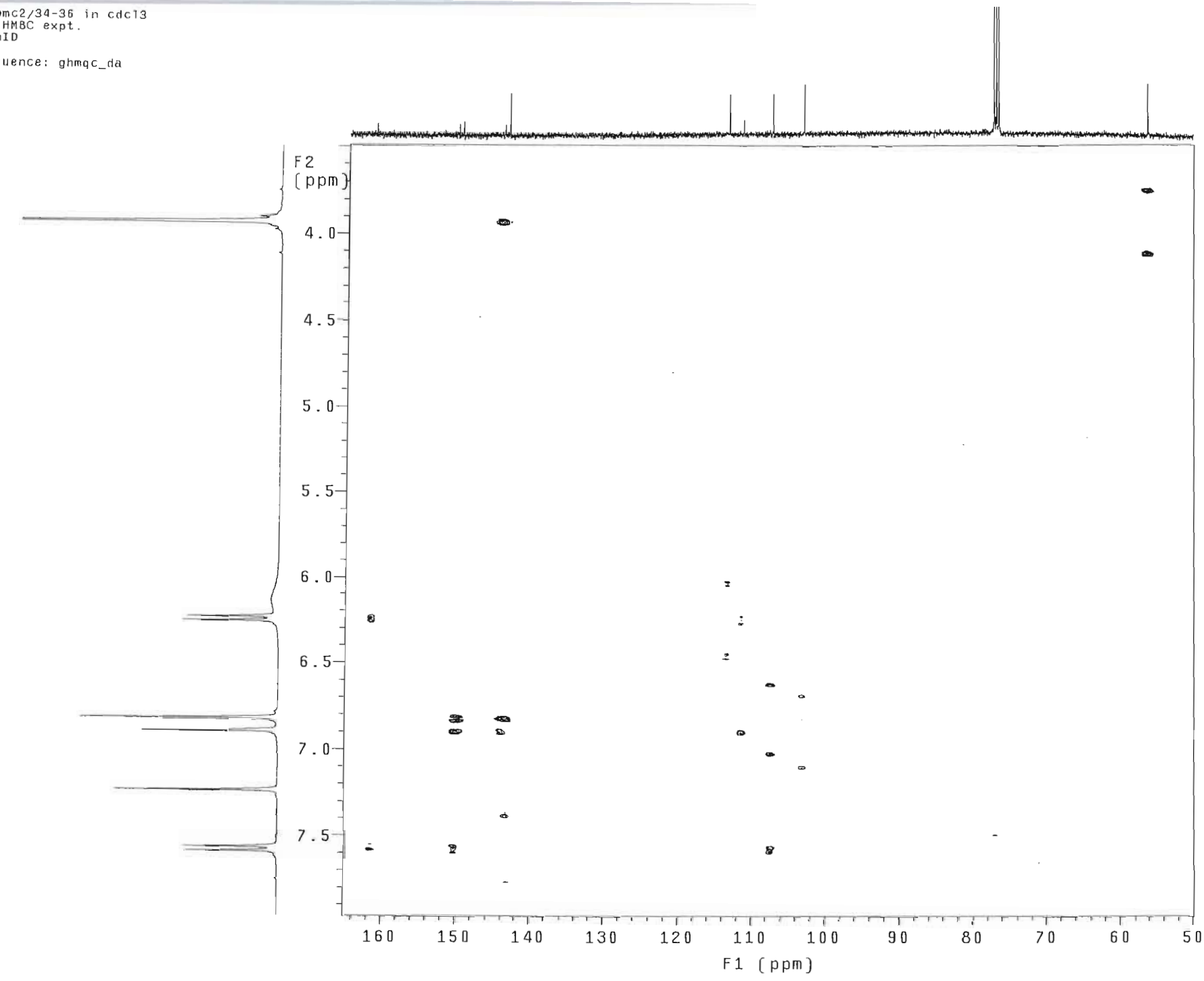

**Spectrum 14b: $^{13}C$ NMR spectrum of compound XIV ($CDCl_3$) (100 MHz)**



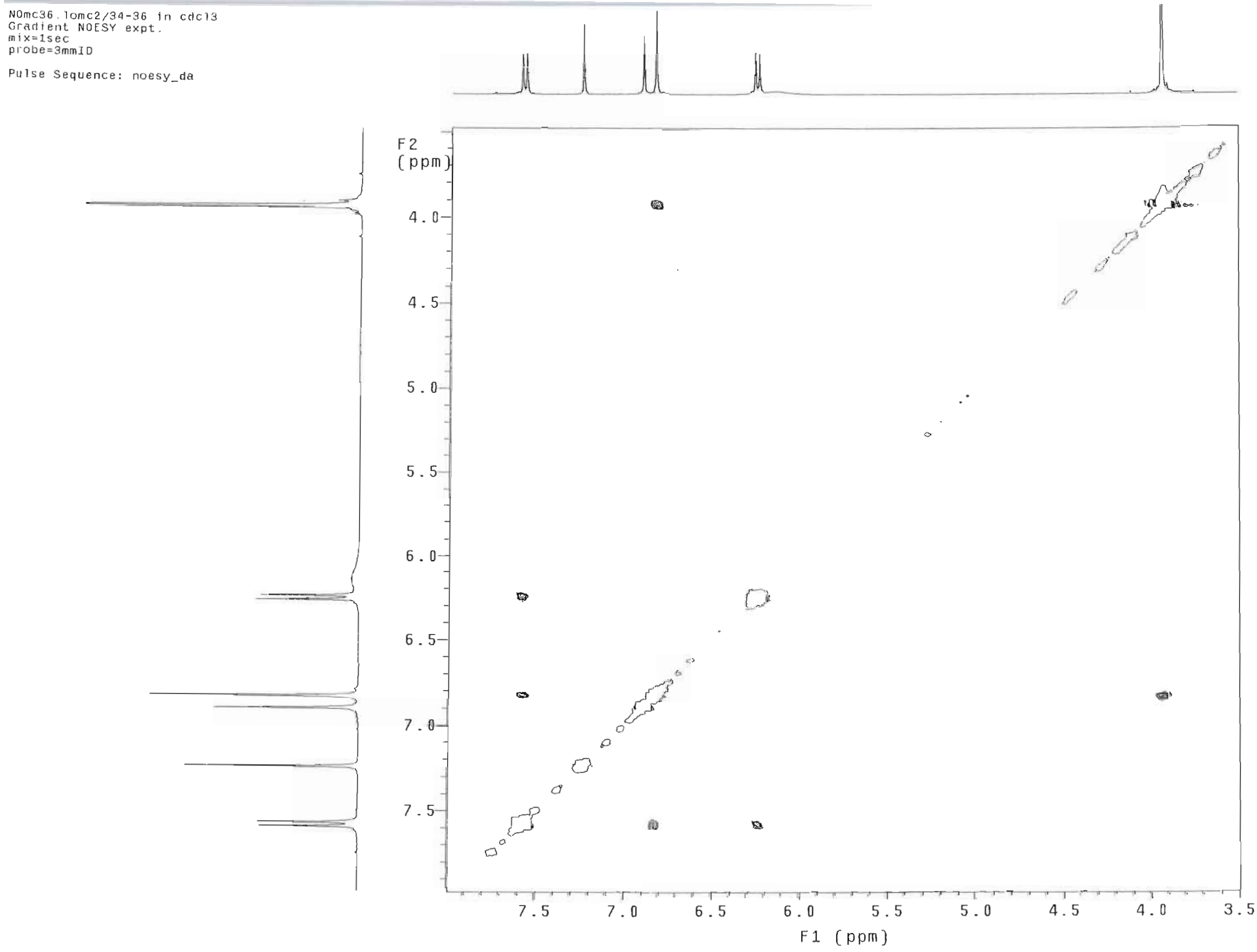

**Spectrum 14c: HSQC spectrum of compound XIV**



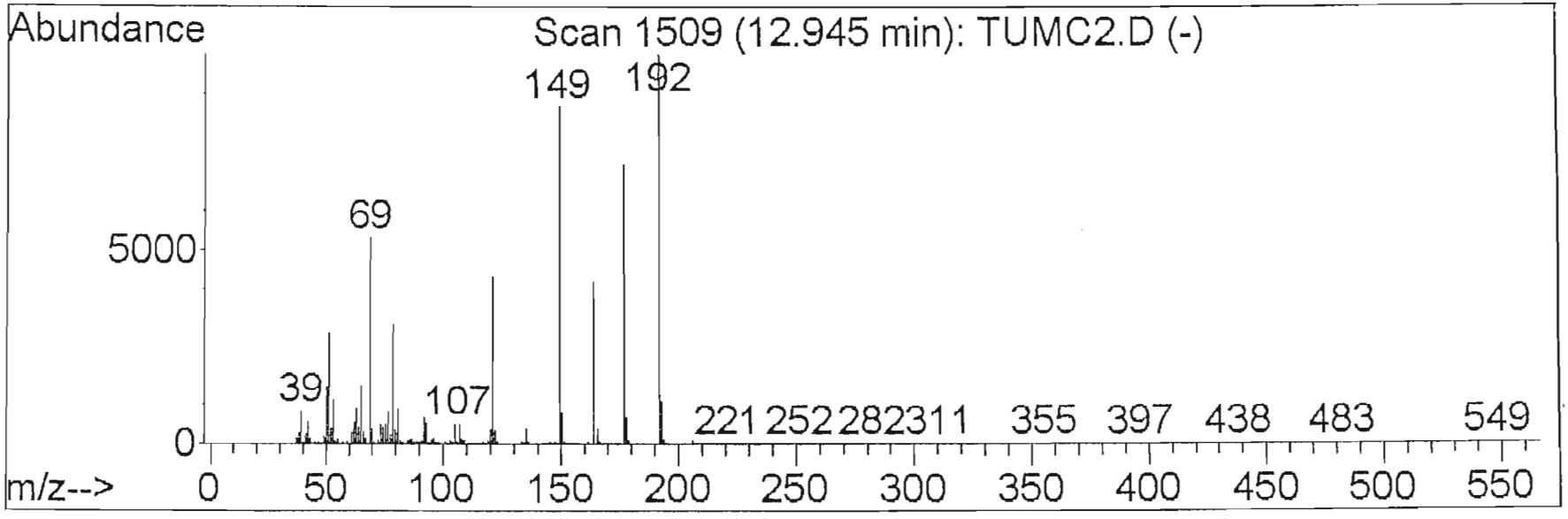

**Spectrum 14d: COSY spectrum of compound XIV**



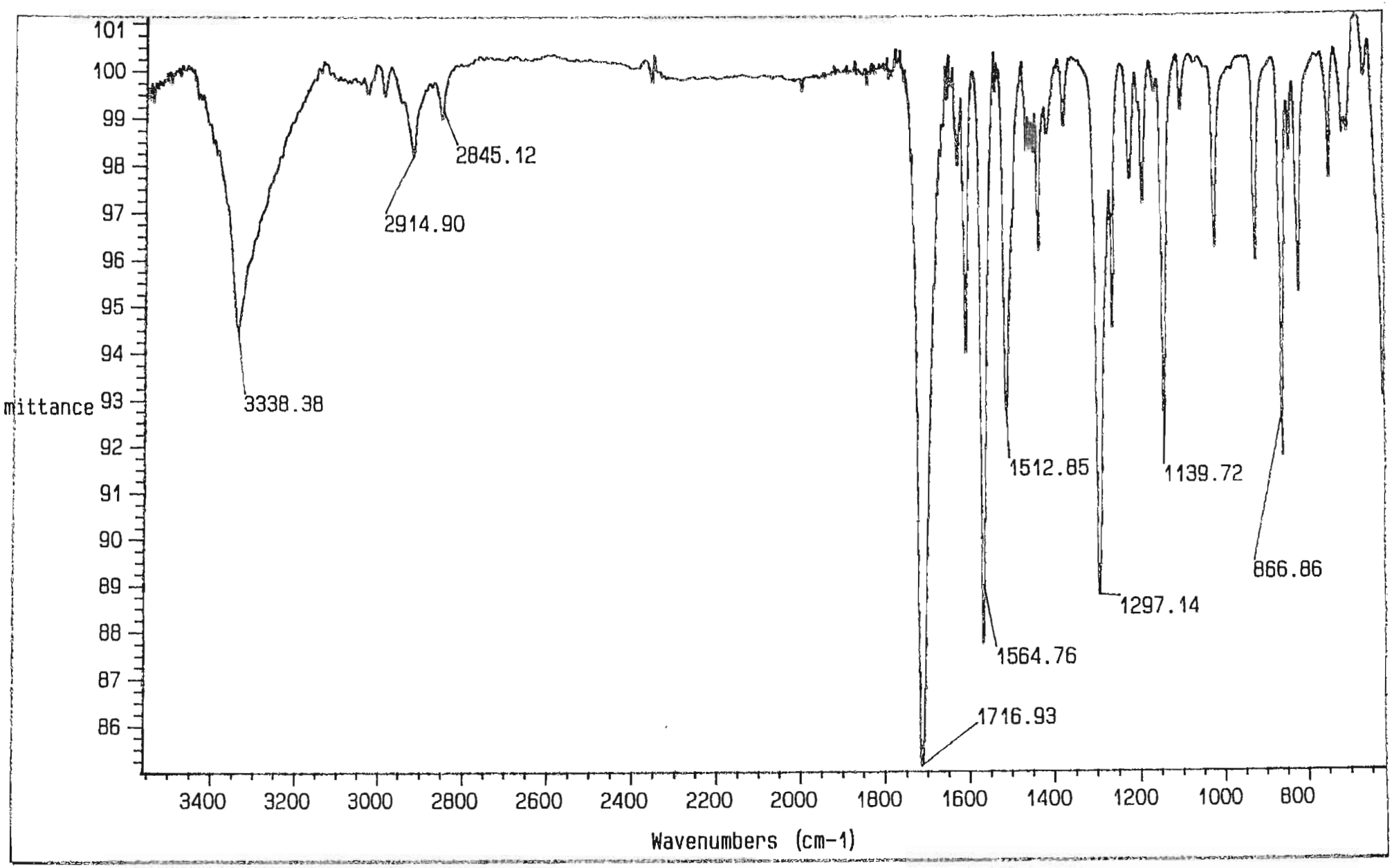

**Spectrum 14e: HMBC spectrum of compound XIV**



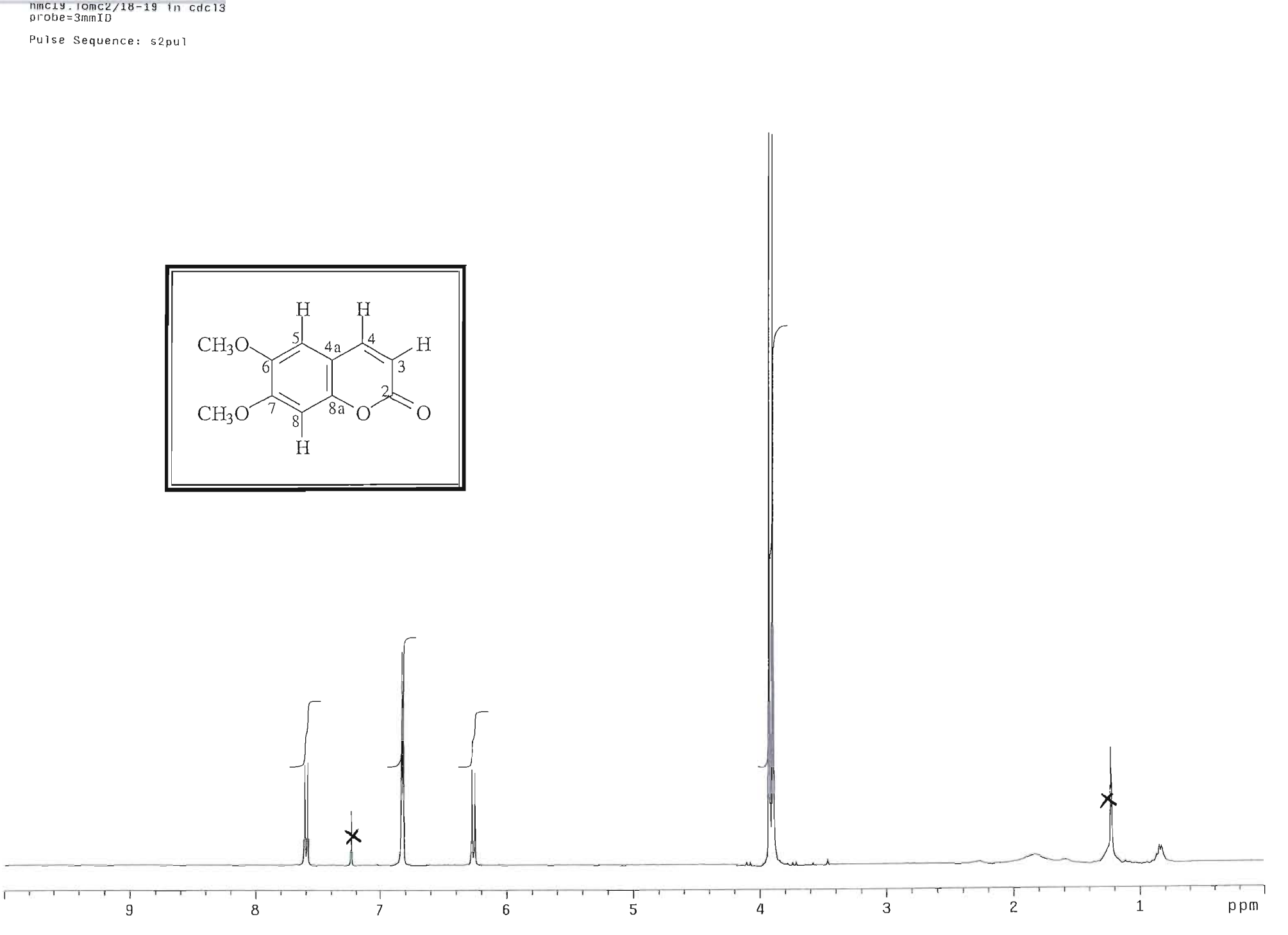

**Spectrum 14f: NOESY spectrum of compound XIV**




**Spectrum 14g: Mass spectrum of compound XIV**



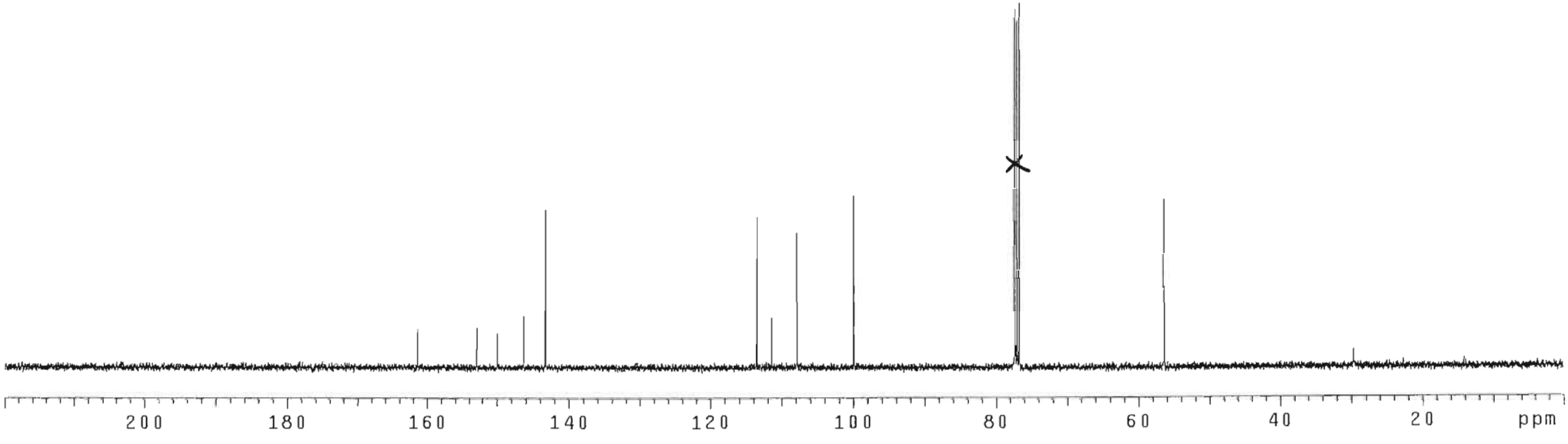

**Spectrum 14h: Infra-red spectrum of compound XIV**



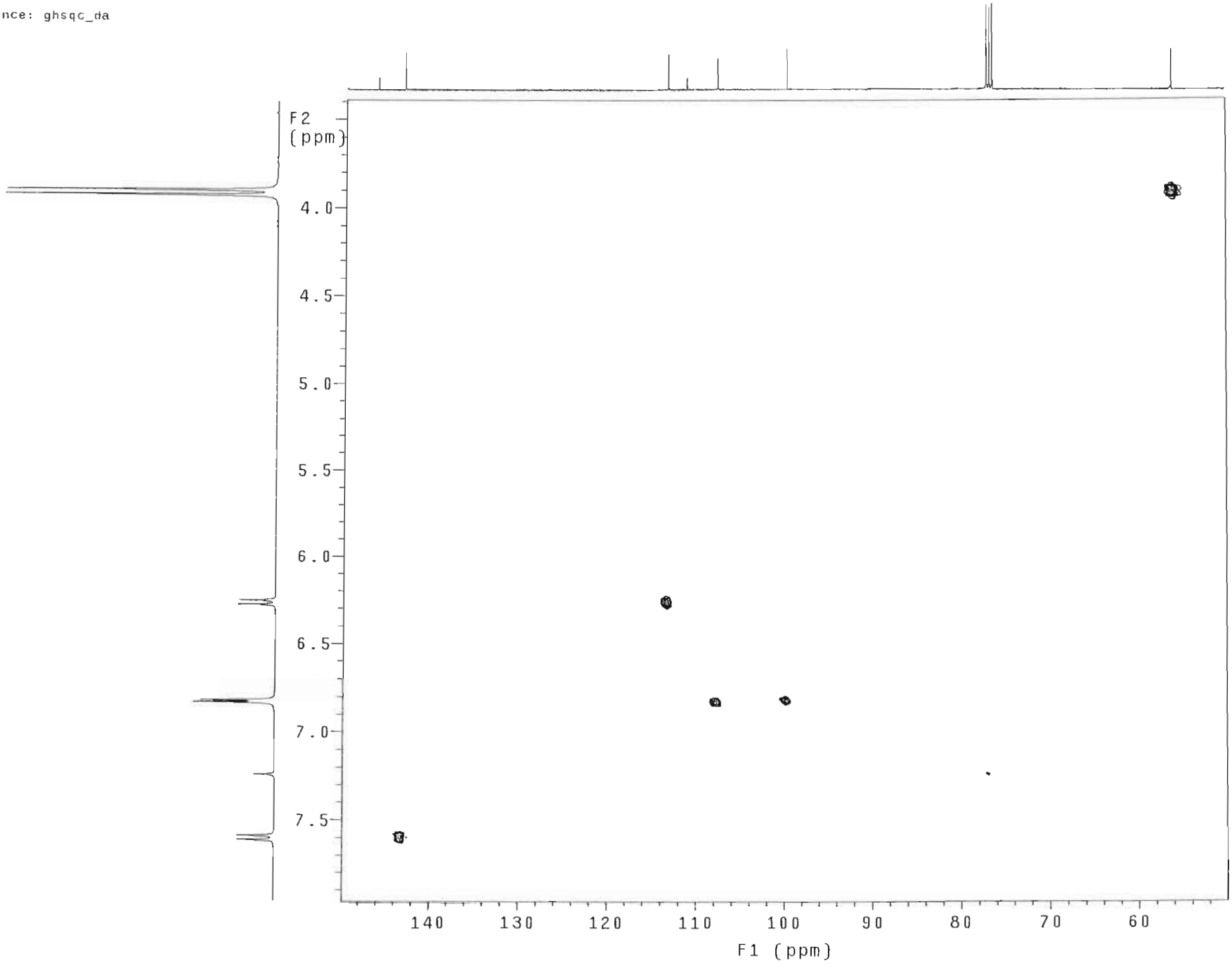

**Spectrum 15a: $^{1}H$ NMR spectrum of compound XV ($CDCl_3$) (400 MHz)**

cmc19.1omc2/18-19 in cdcl3
probe=3mmID

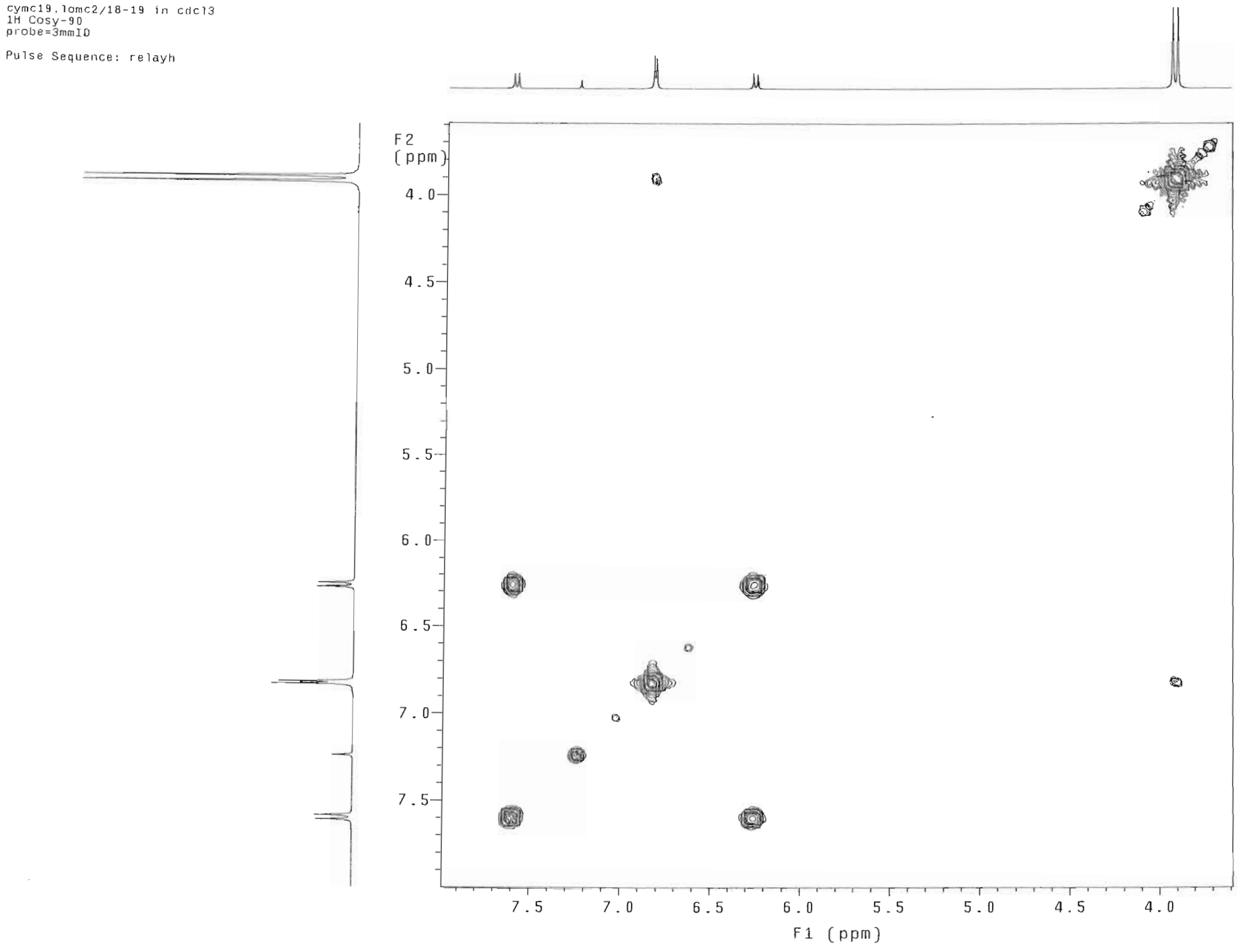
Pulse Sequence: s2pul



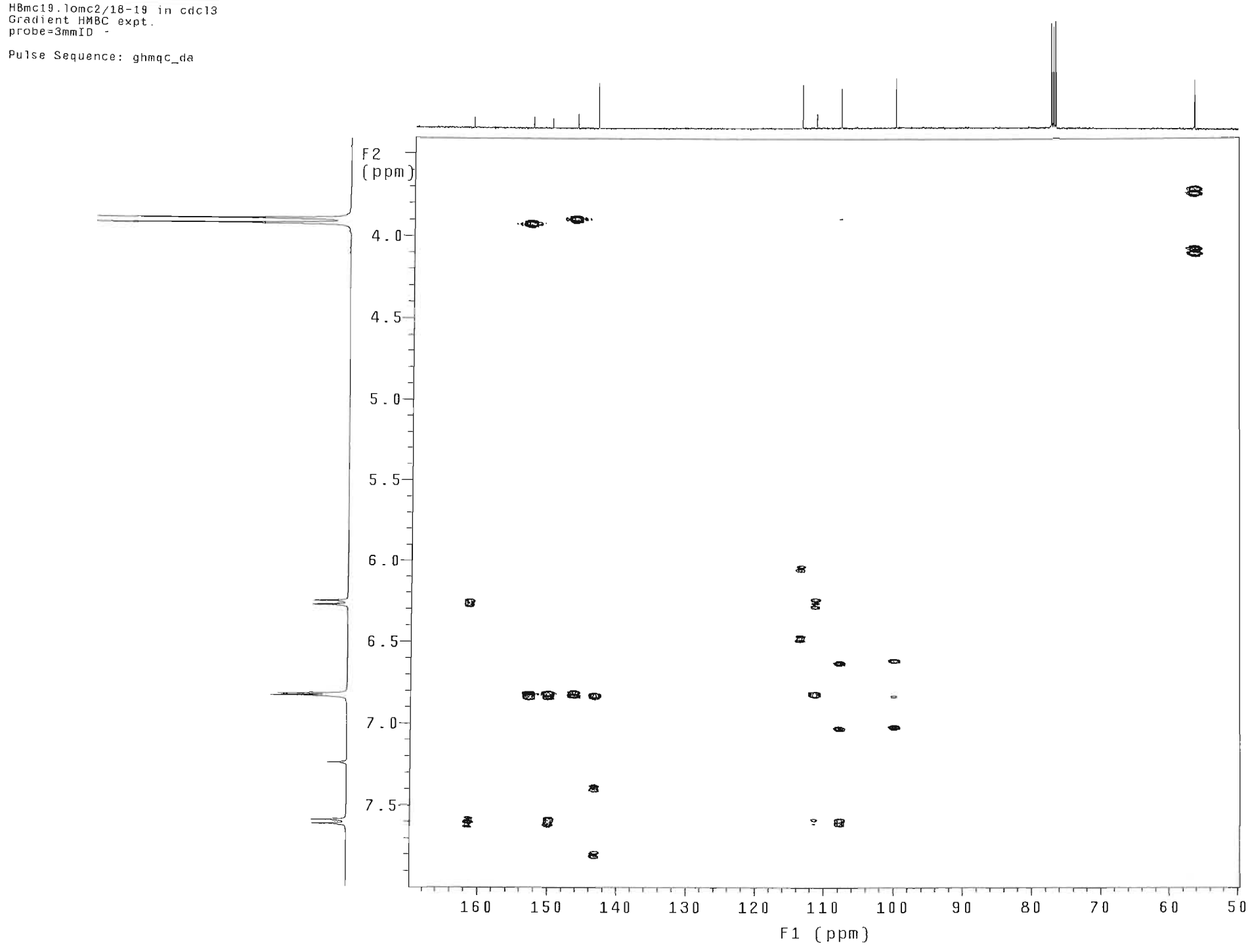

**Spectrum 15b: $^{13}C$ NMR spectrum of compound XV ($CDCl_3$) (100 MHz)**



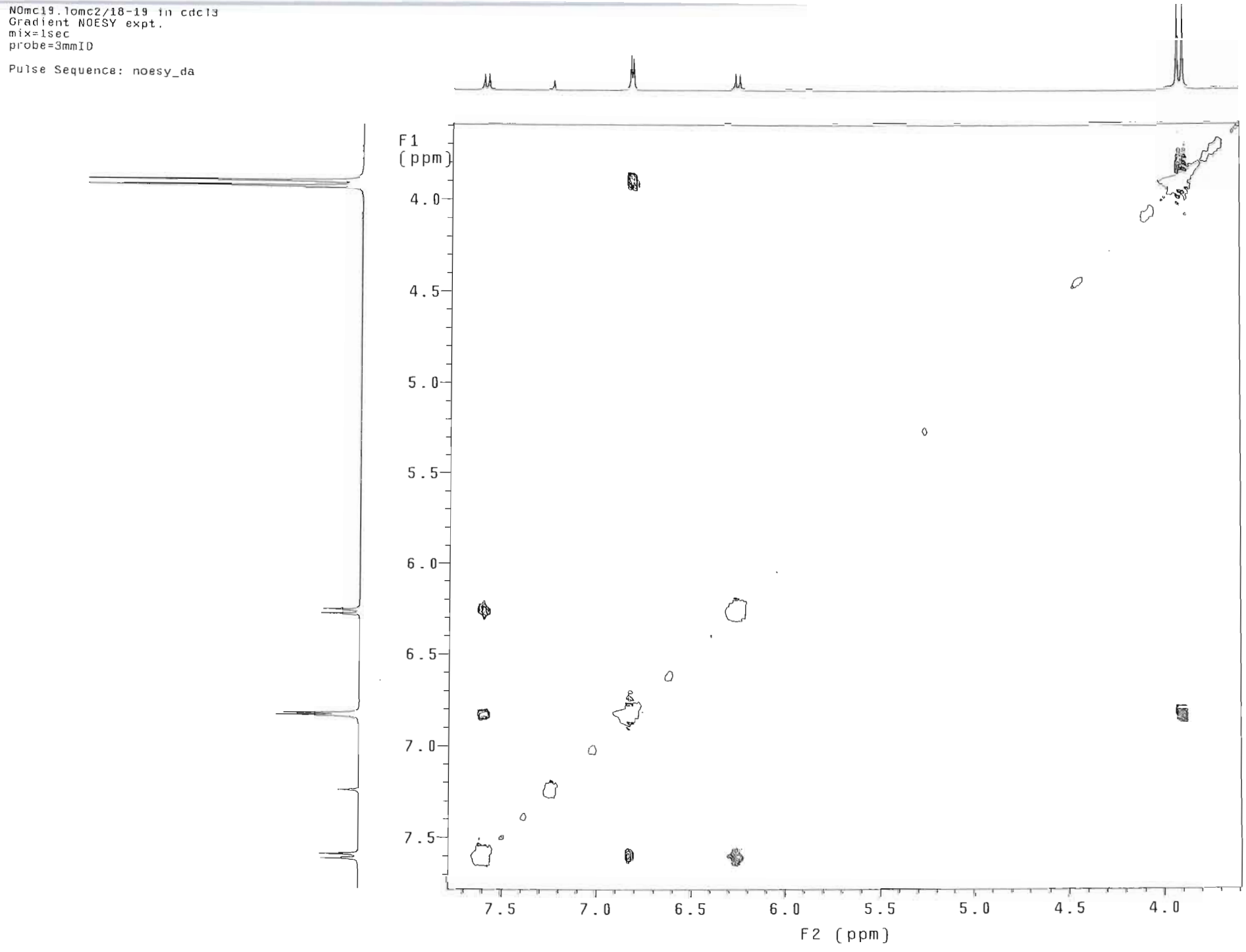

**Spectrum 15c: HSQC spectrum of compound XV**



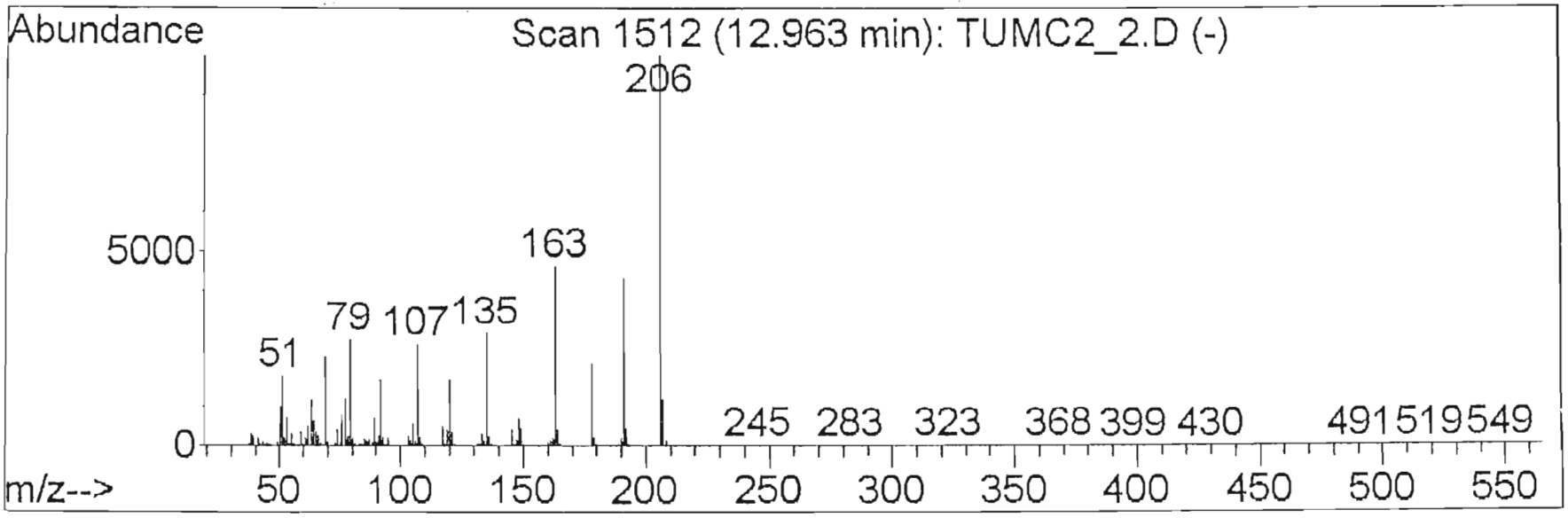

**Spectrum 15d: COSY spectrum of compound XV**



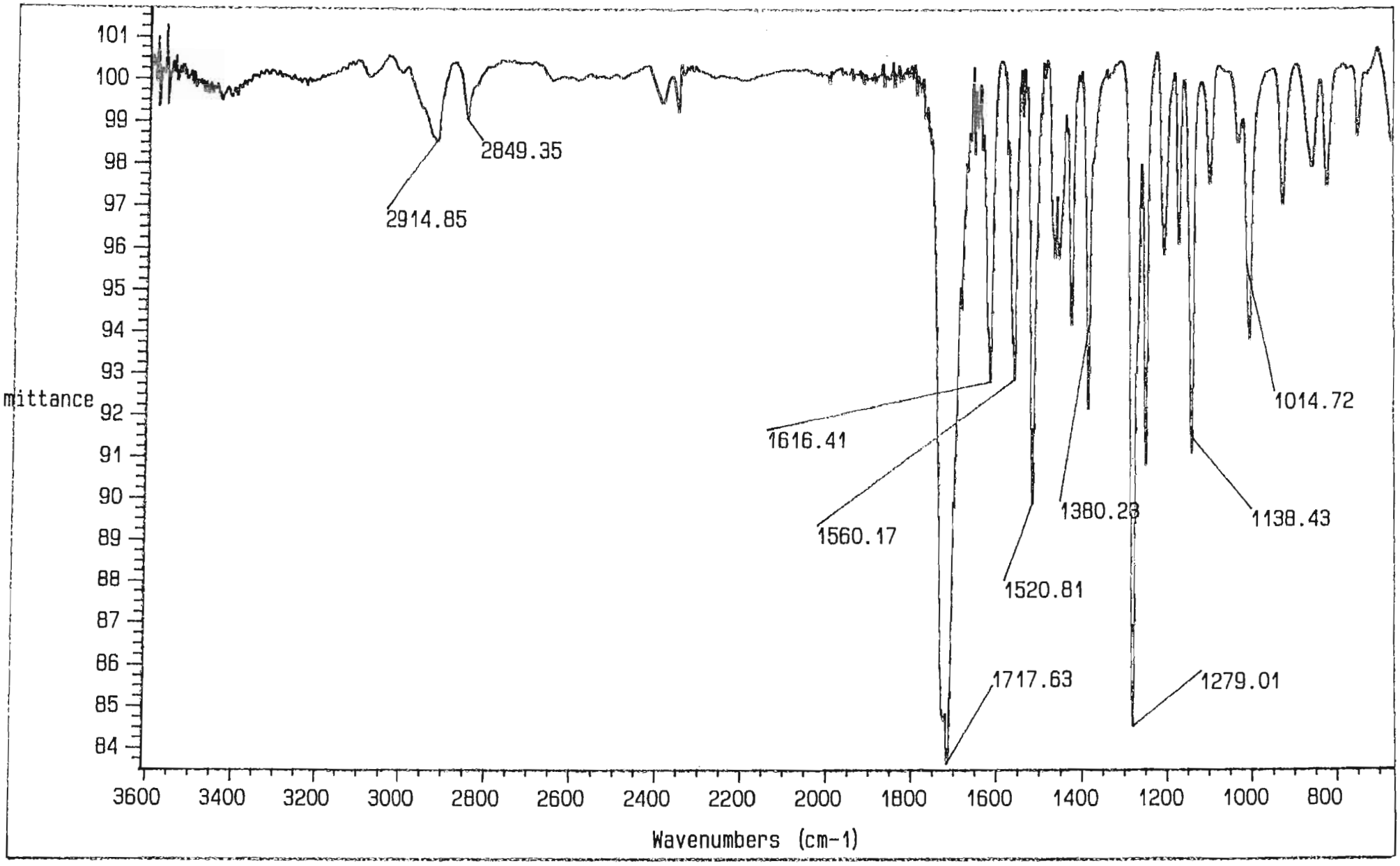

**Spectrum 15e: HMBC spectrum of compound XV**




**Spectrum 15f: NOESY spectrum of compound XV**




**Spectrum 15g: Mass spectrum of compound XV**

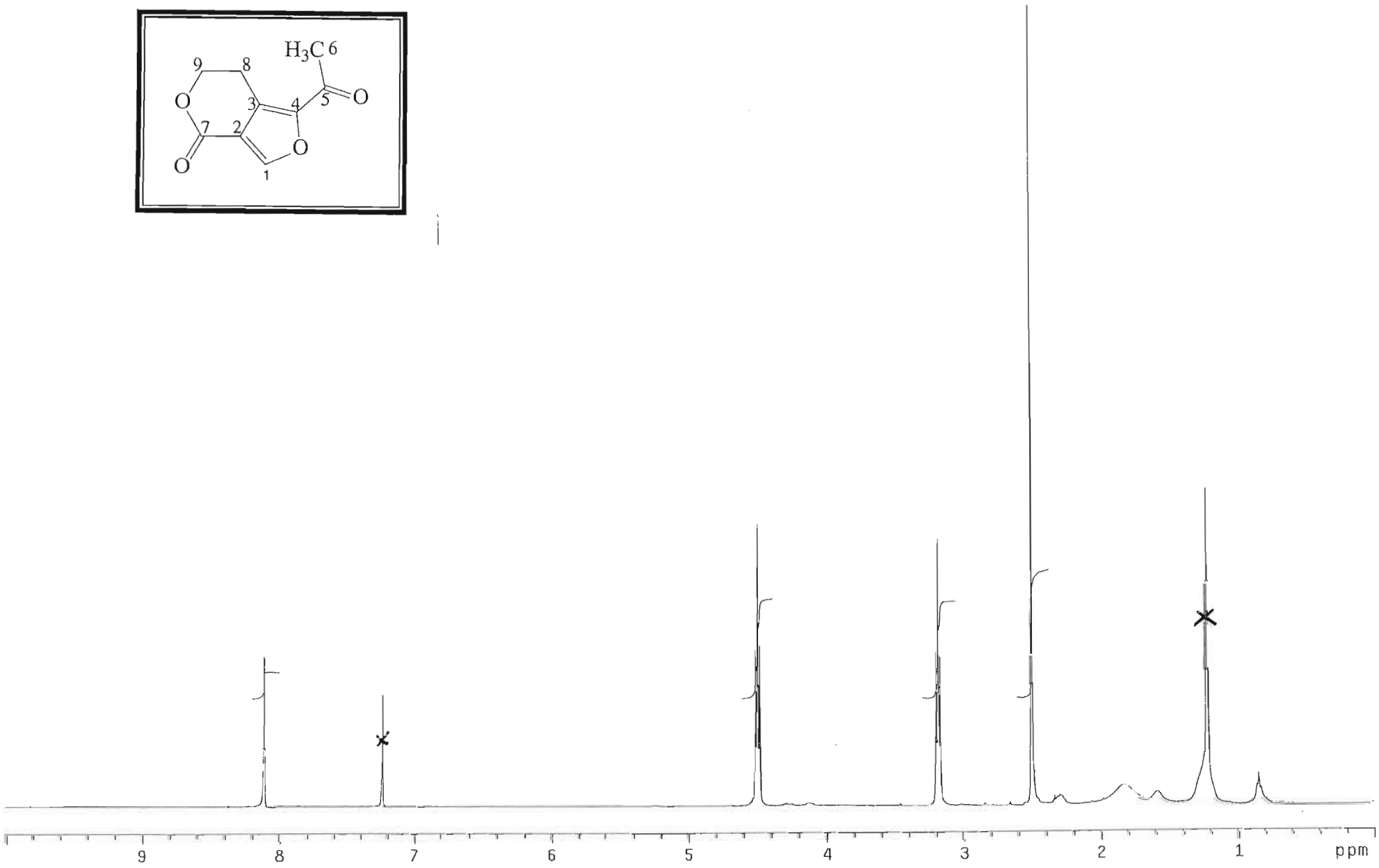



**Spectrum 15h: Infra-red spectrum of compound XV**

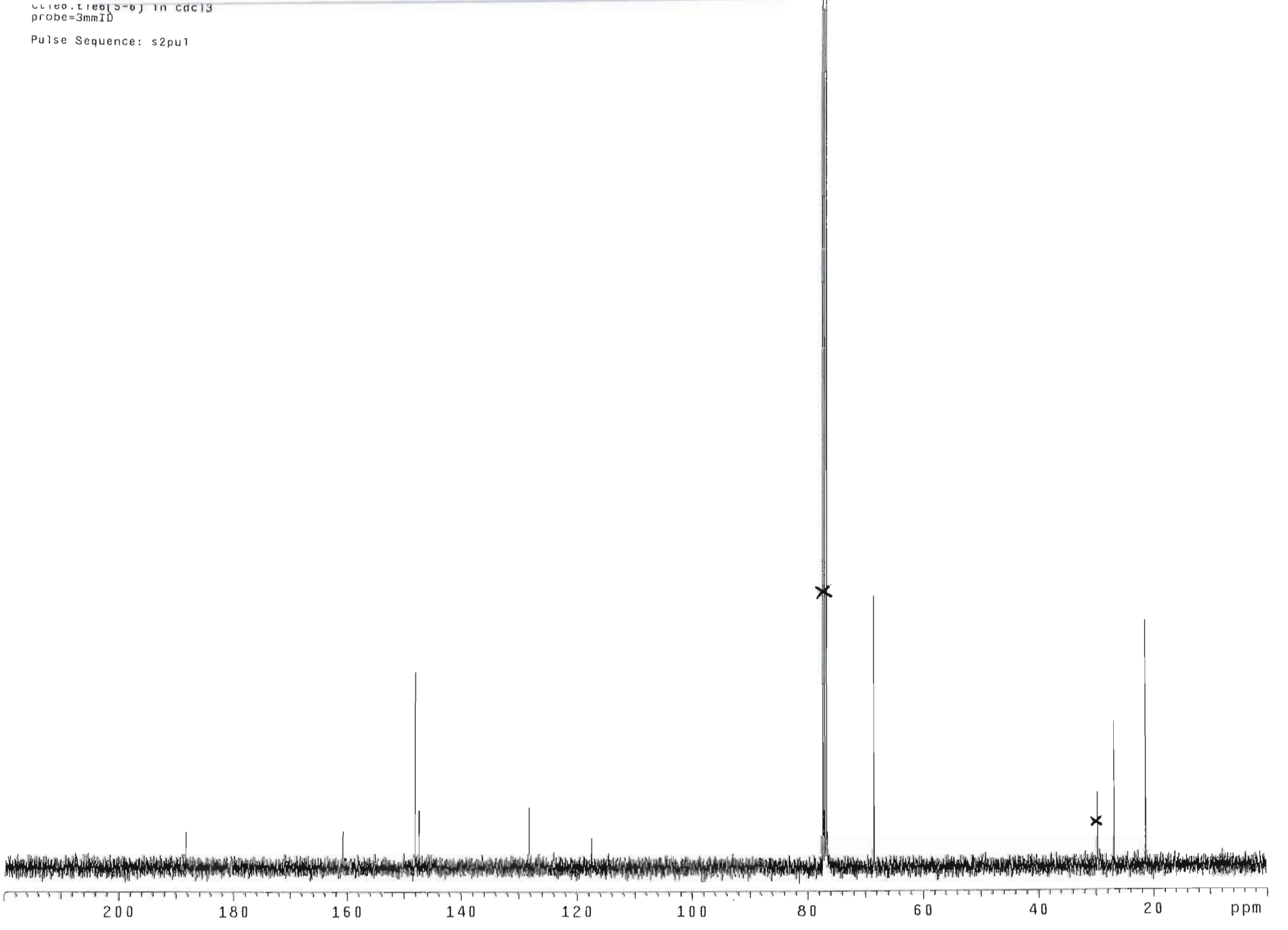
nt1e6.t1e6(5-6) in cdcl3
probe=3mmID

Pulse Sequence: s2pul

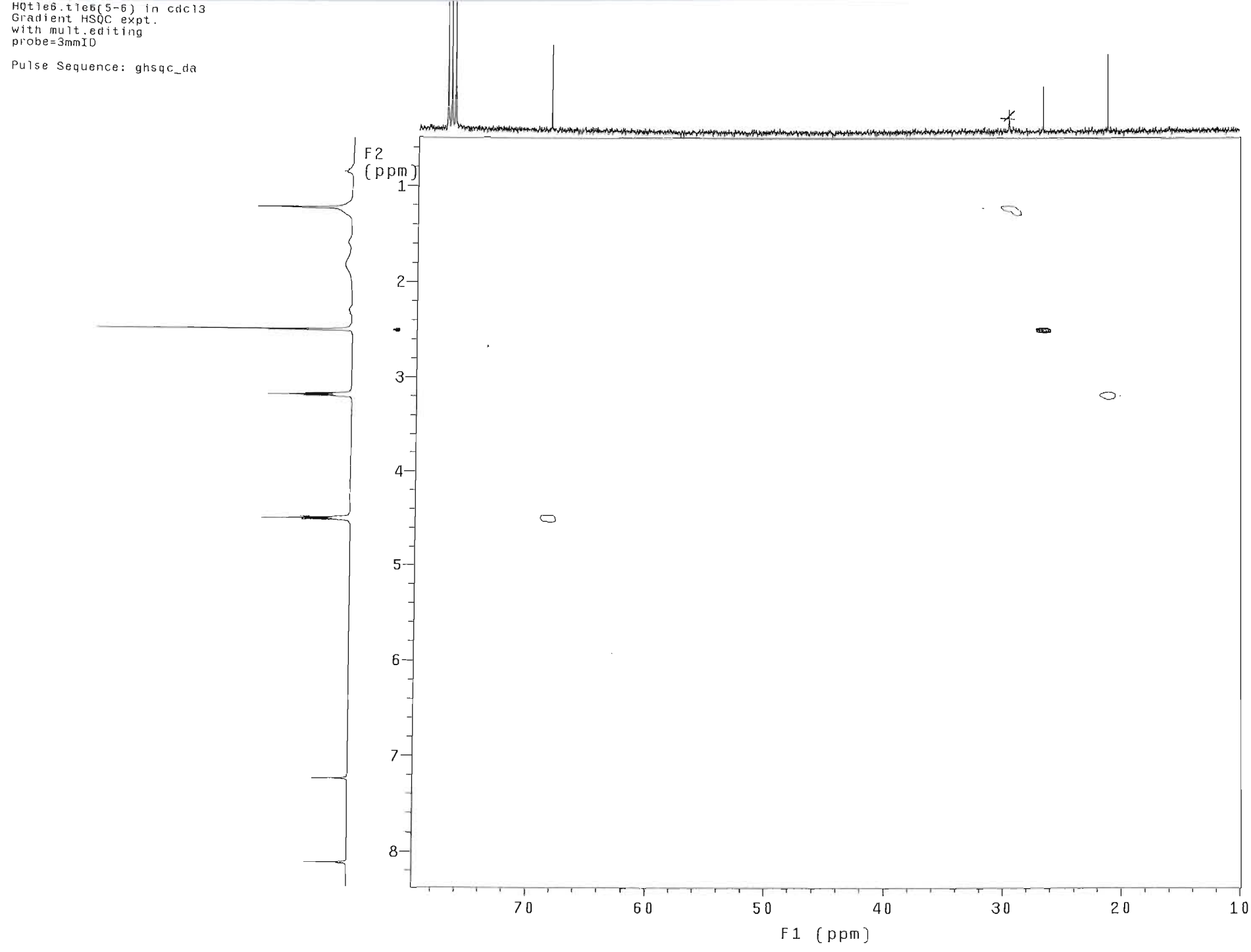


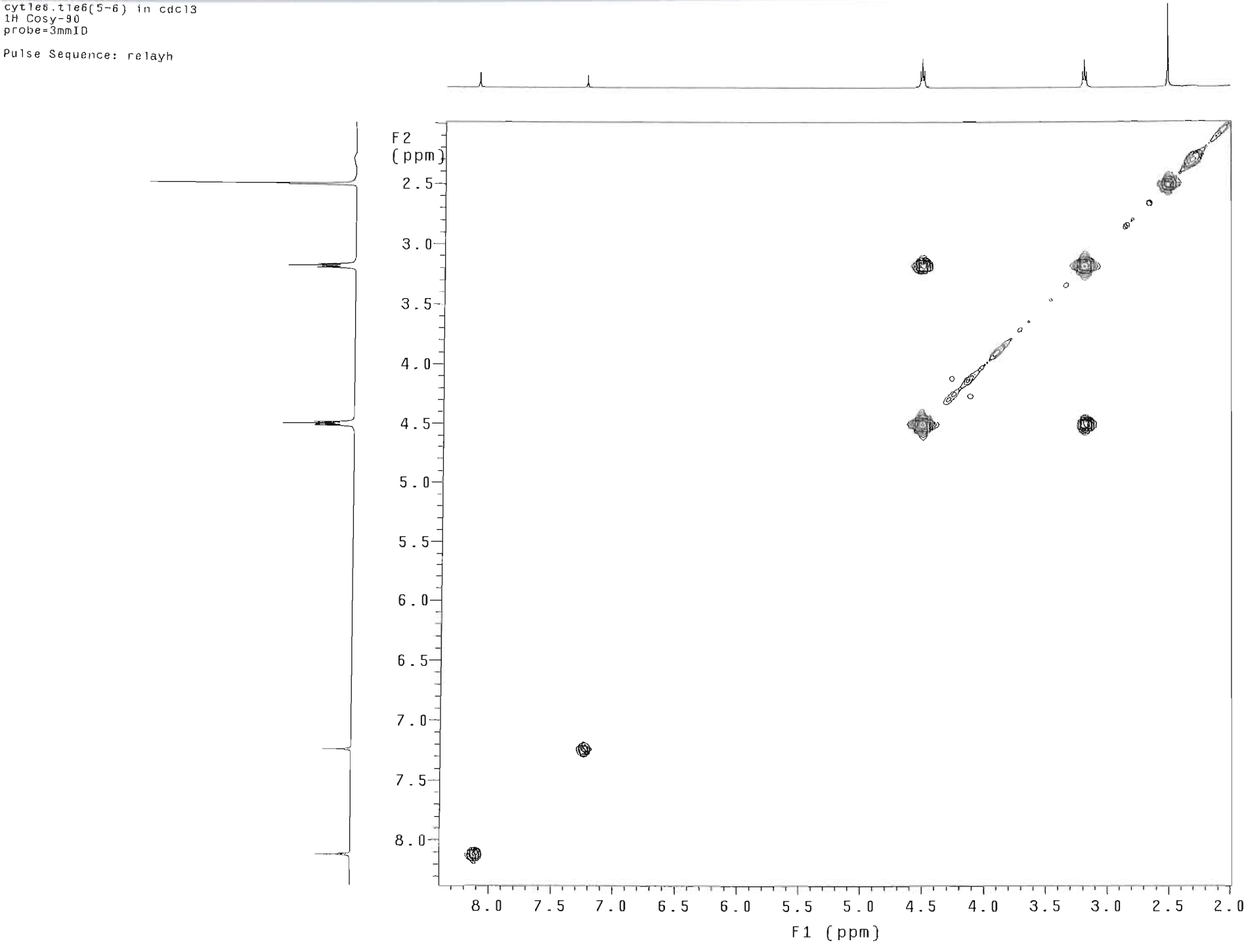

**Spectrum 16a: $^{1}$H NMR spectrum of compound XVI ($CDCl_3$) (400 MHz)**



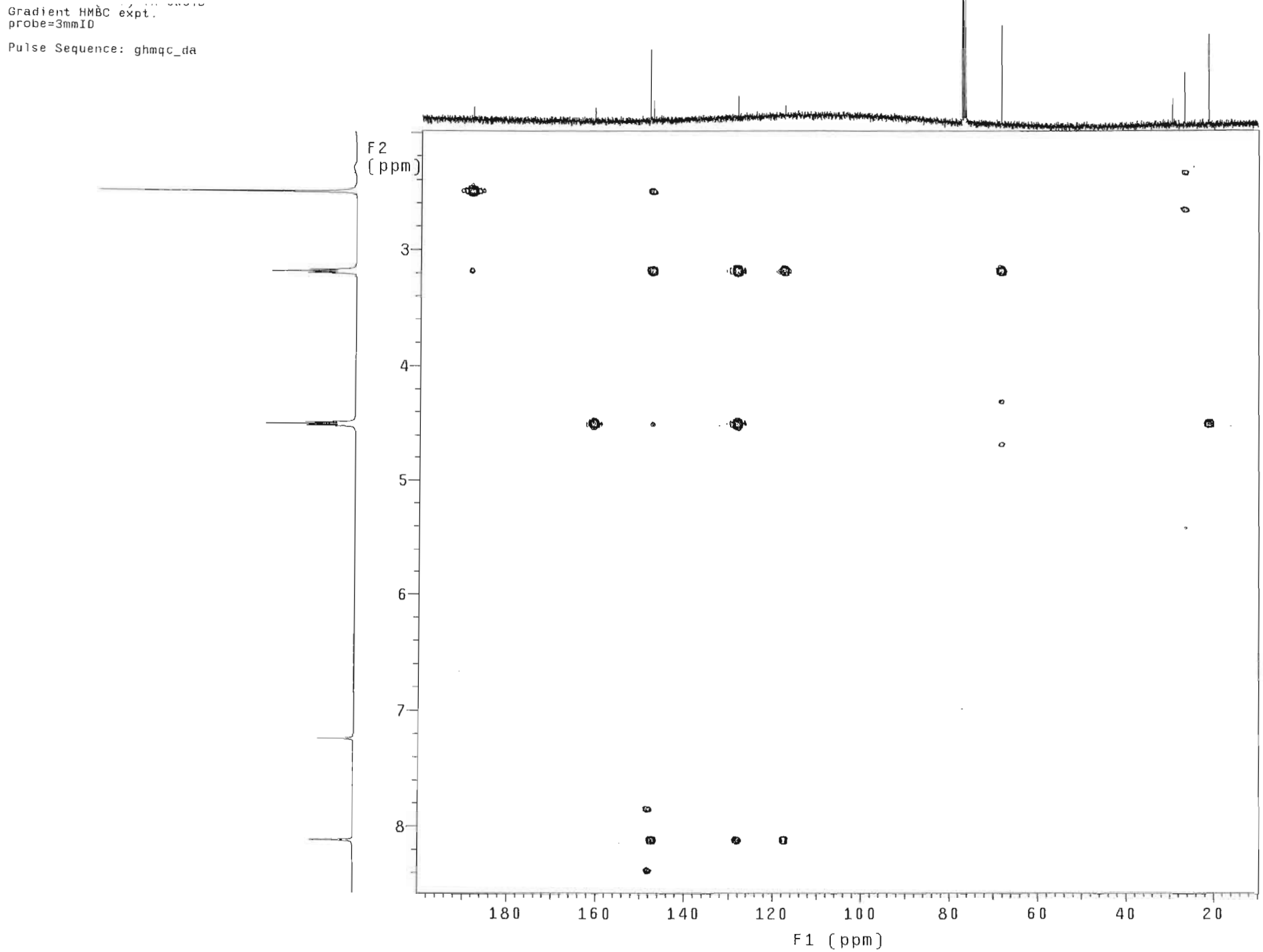

**Spectrum 16b: $^{13}C$ NMR spectrum of compound XVI ($CDCl_3$) (100 MHz)**



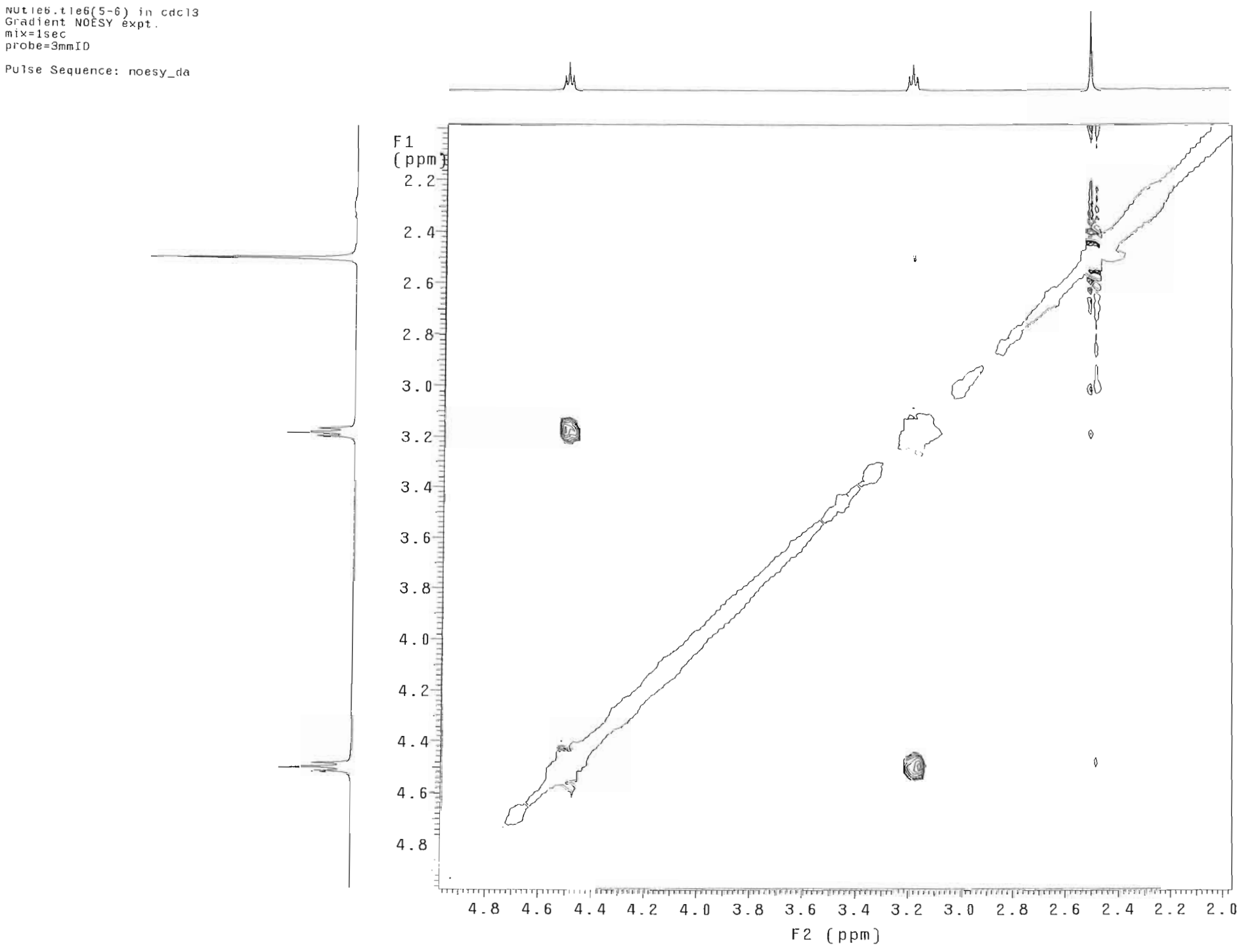

**Spectrum 16c: HSQC spectrum of compound XVI**



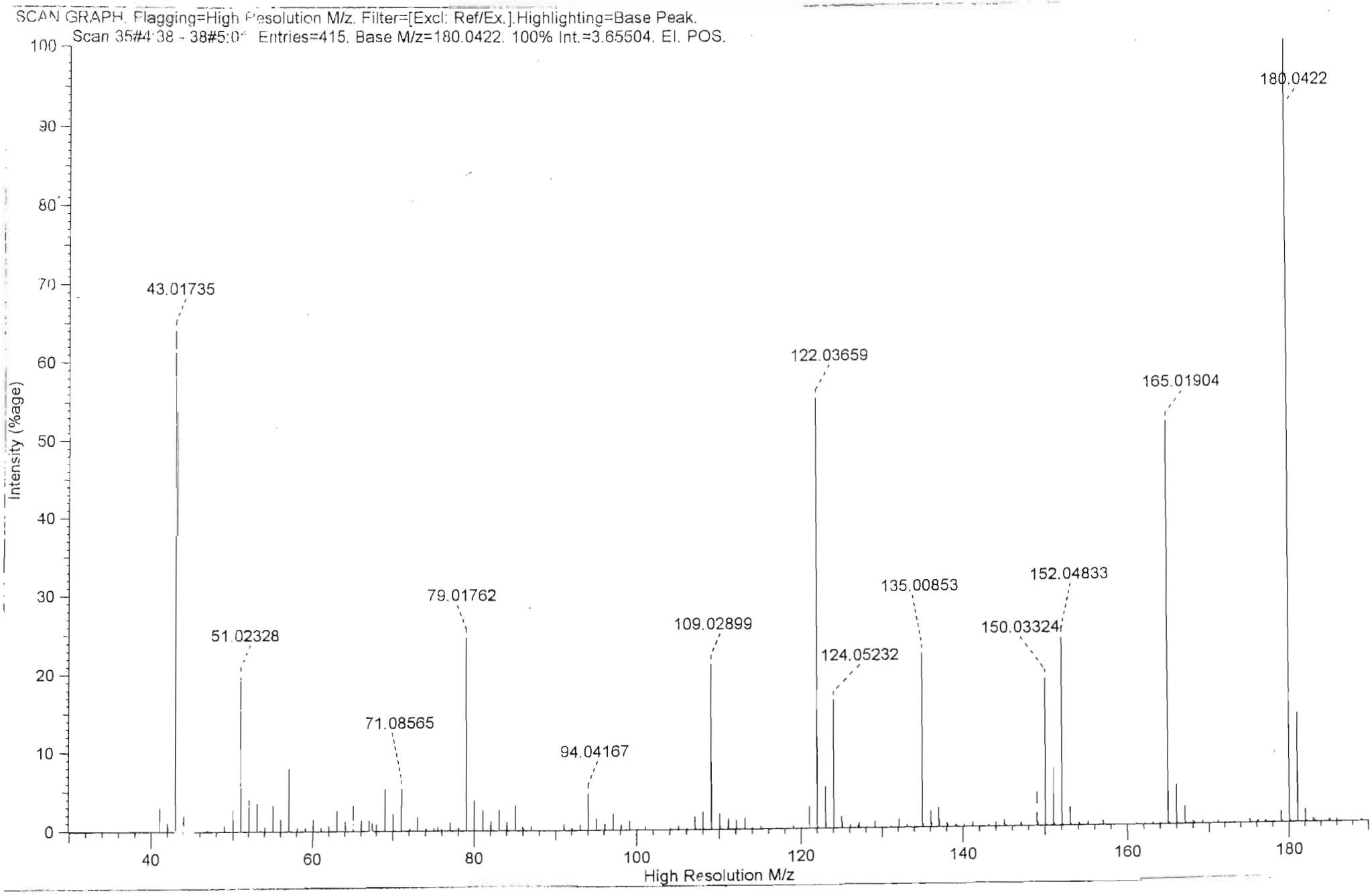

**Spectrum 16d: COSY spectrum of compound XVI**



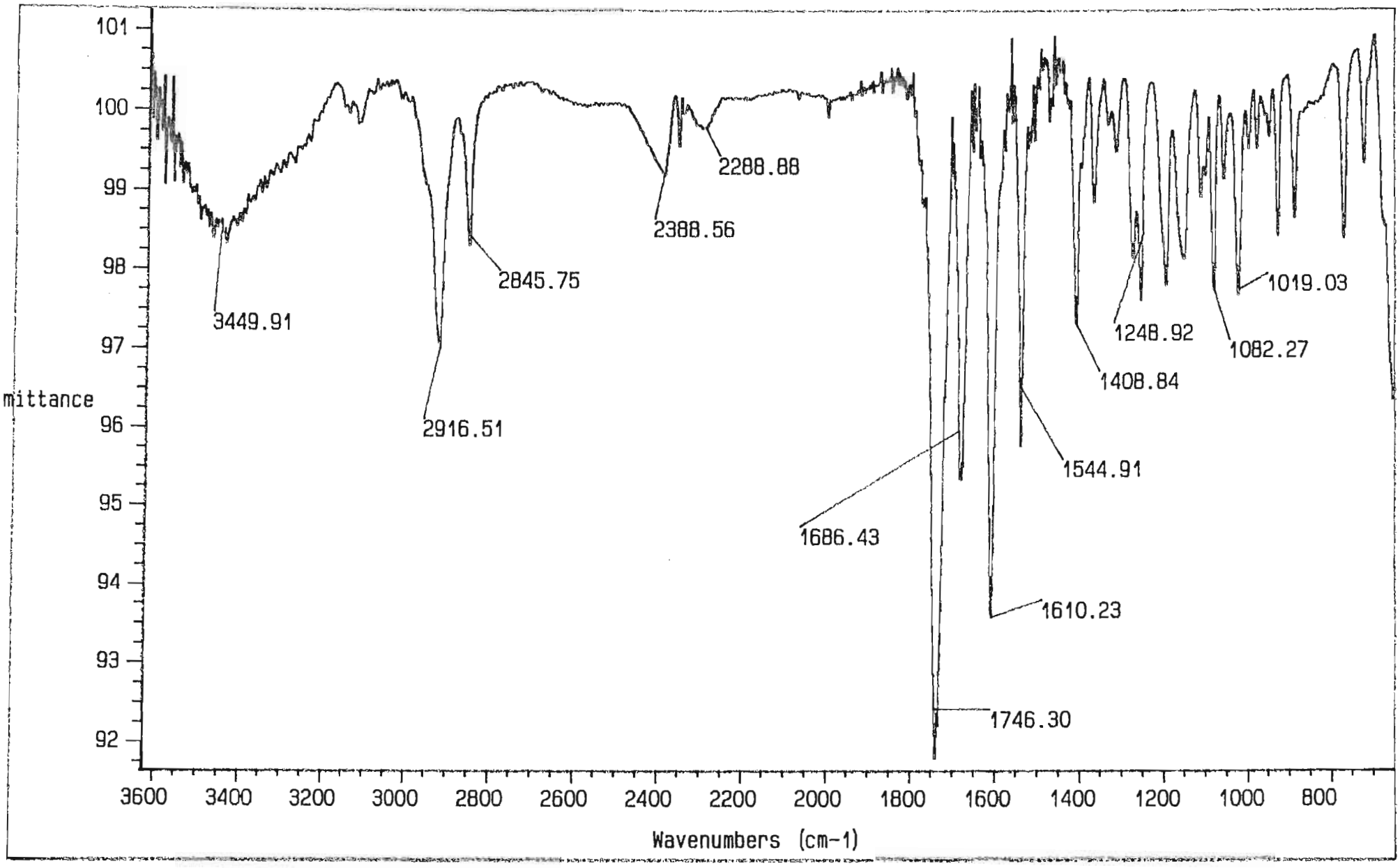

**Spectrum 16e: HMBC spectrum of compound XVI**




**Spectrum 16f: NOESY spectrum of compound XVI**




**Spectrum 16g: Mass spectrum of compound XVI**

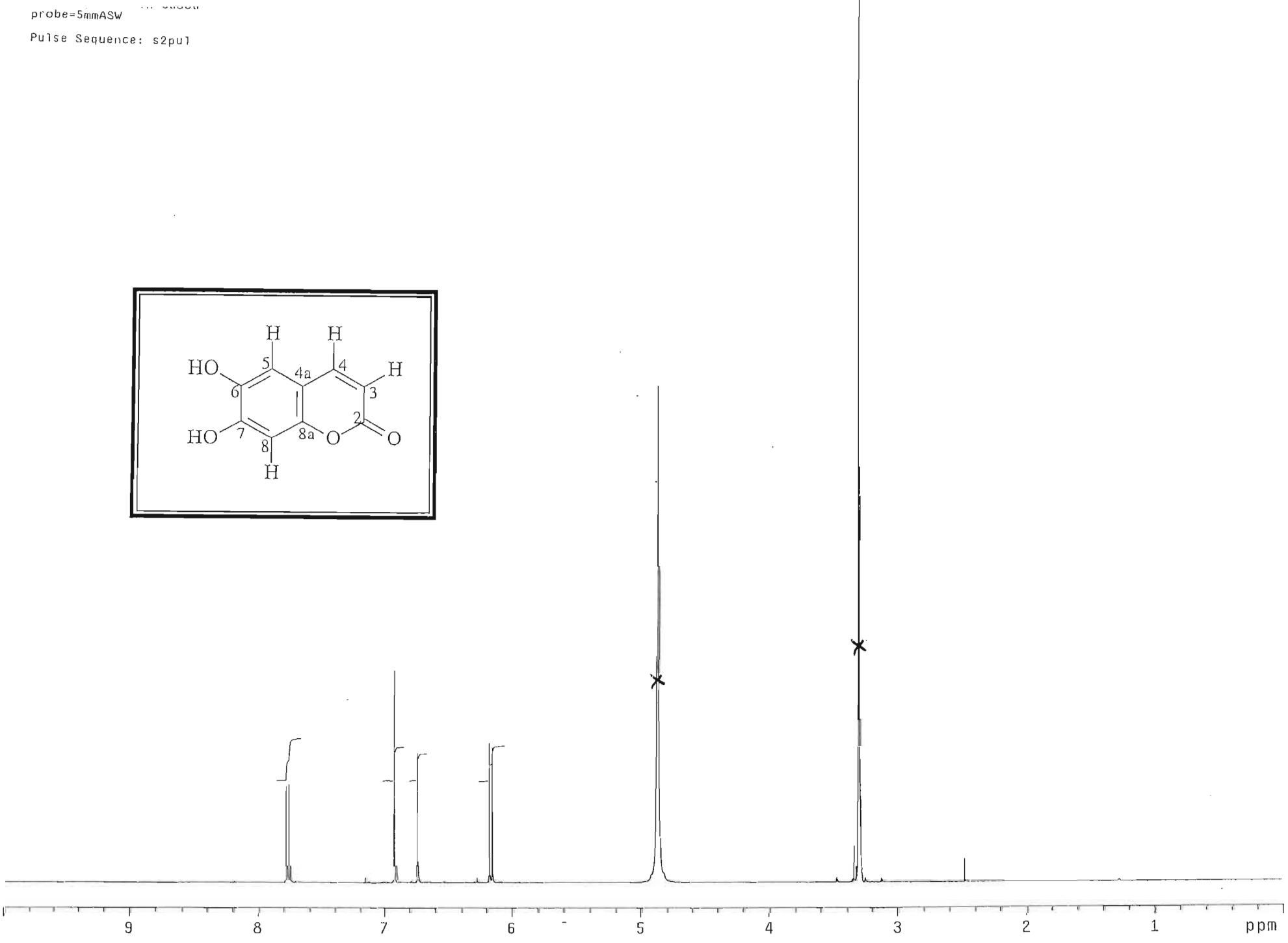



**Spectrum 16h: Infra-red spectrum of compound XVI**

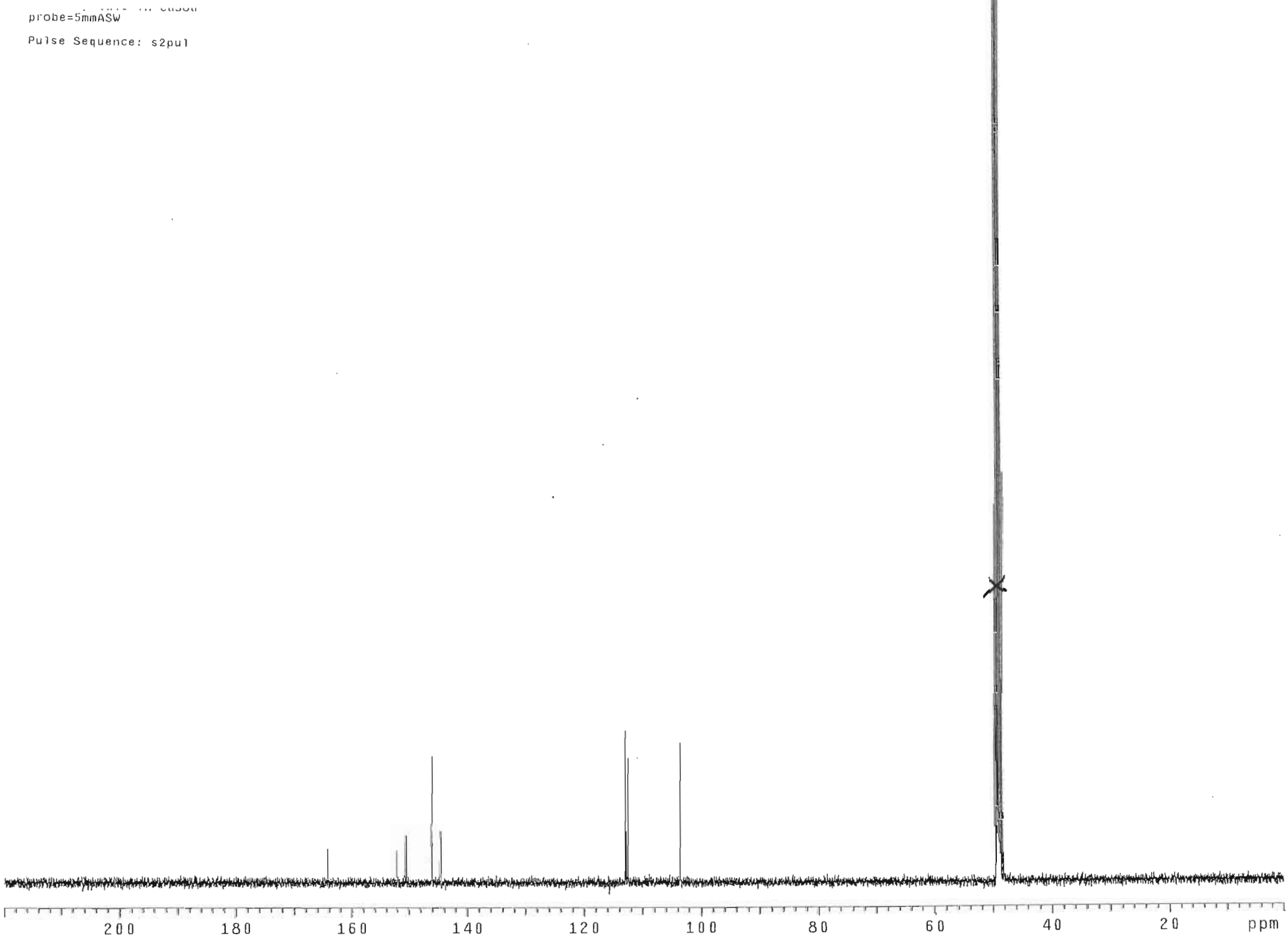
probe=5mmASW

Pulse Sequence: s2pul

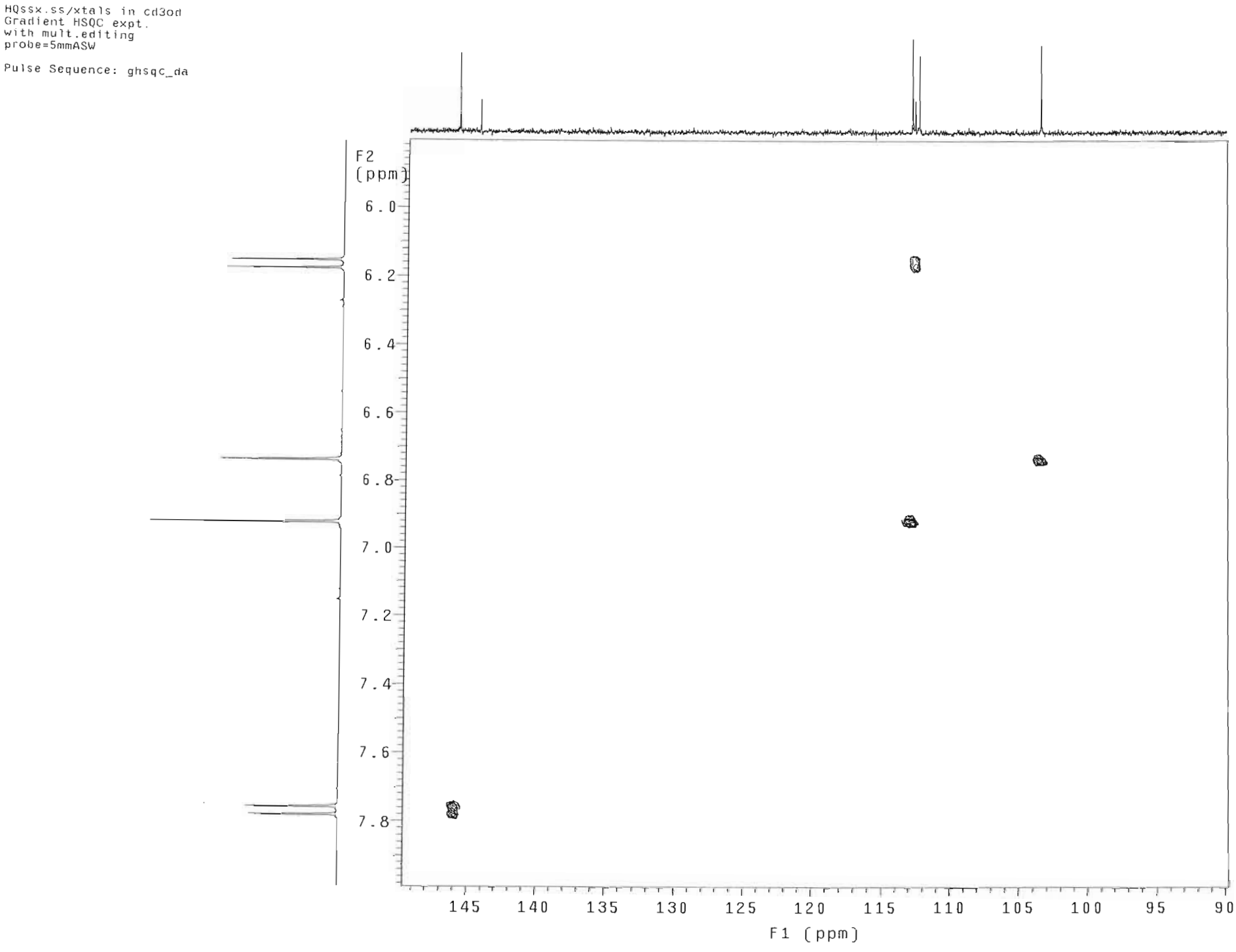


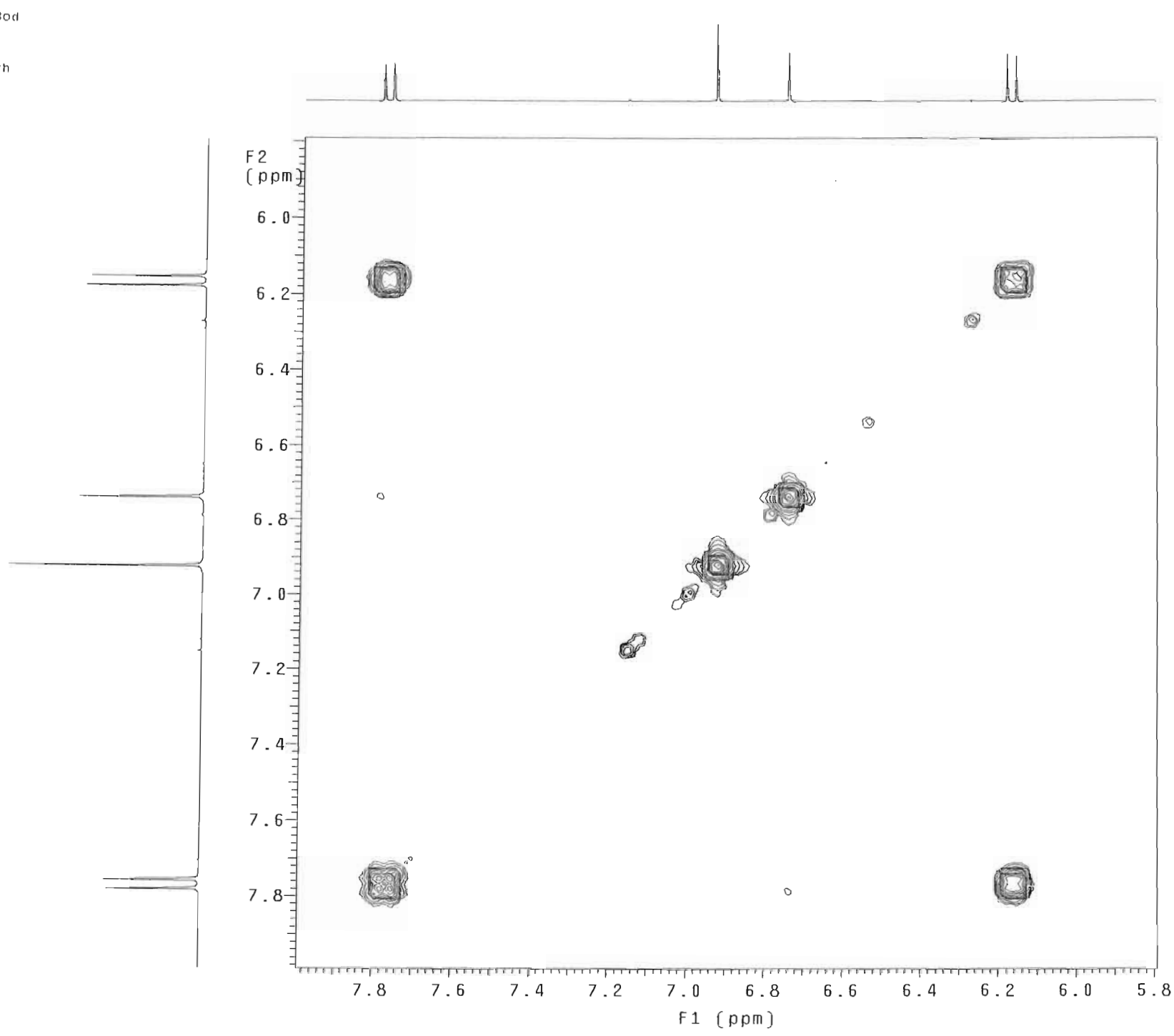

**Spectrum 17a: $^{1}$H NMR spectrum of compound XVII ($CD_3OD$) (400 MHz)**



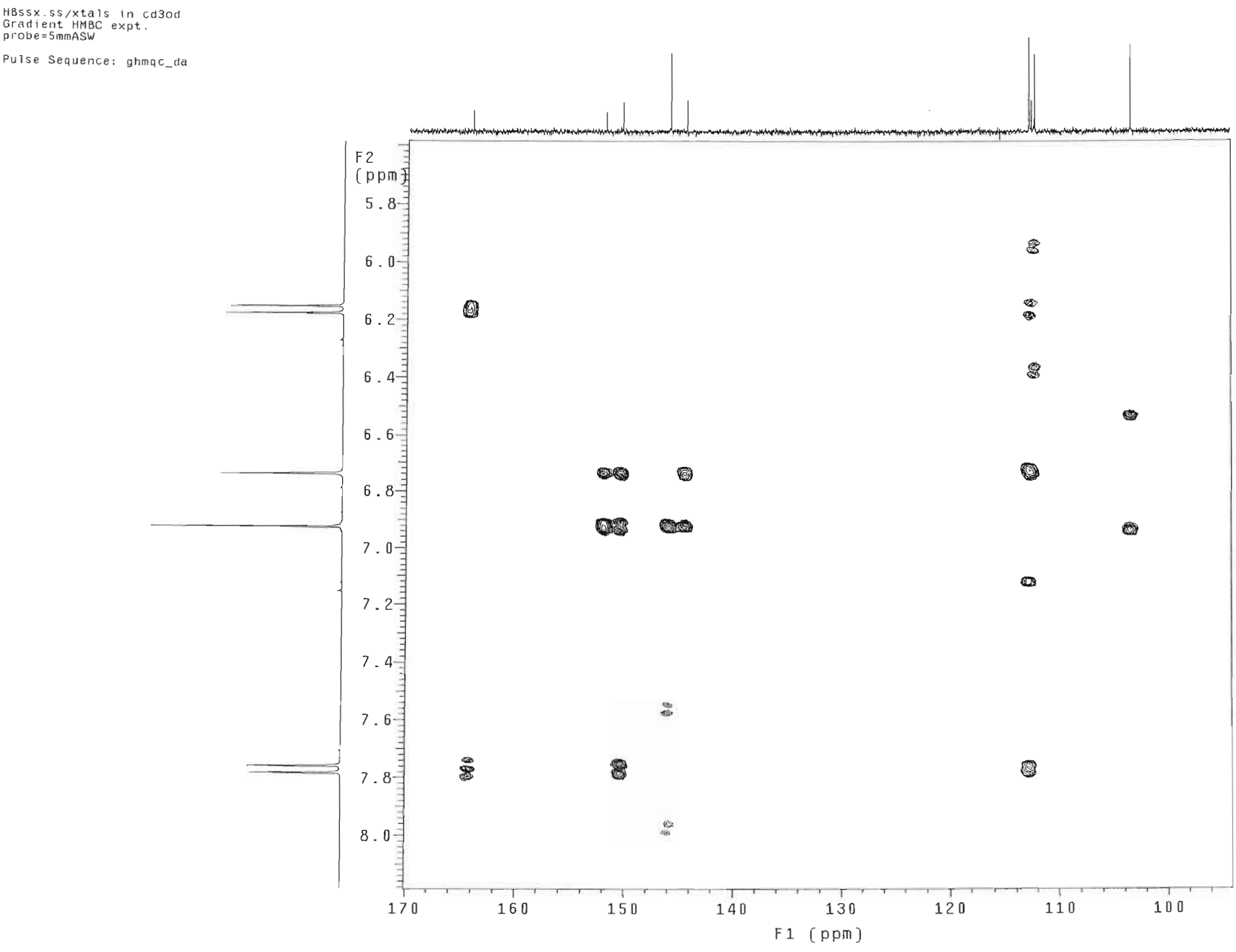

**Spectrum 17b: $^{13}C$ NMR spectrum of compound XVII ($CD_3OD$) (400 MHz)**



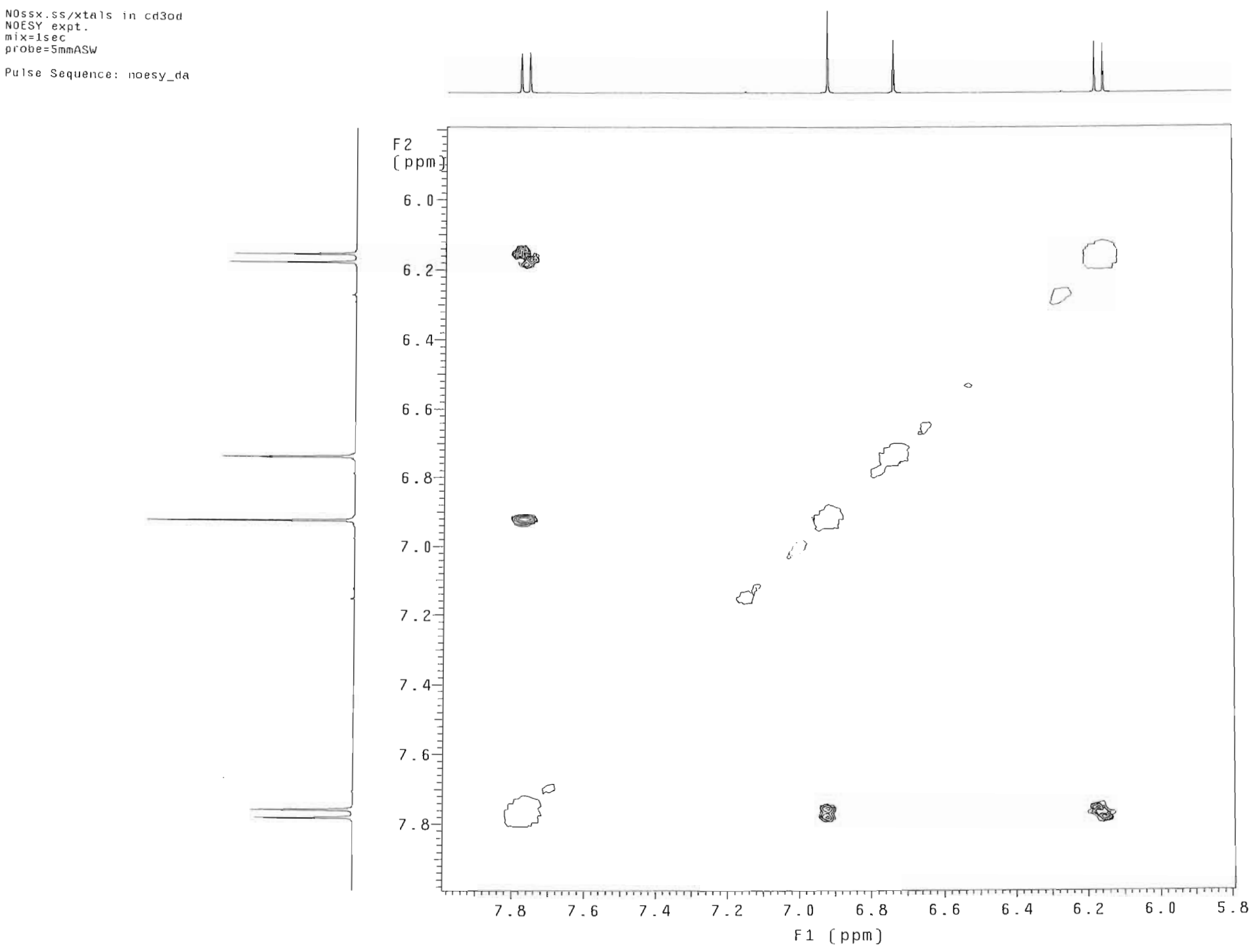

**Spectrum 17c: HSQC spectrum of compound XVII**



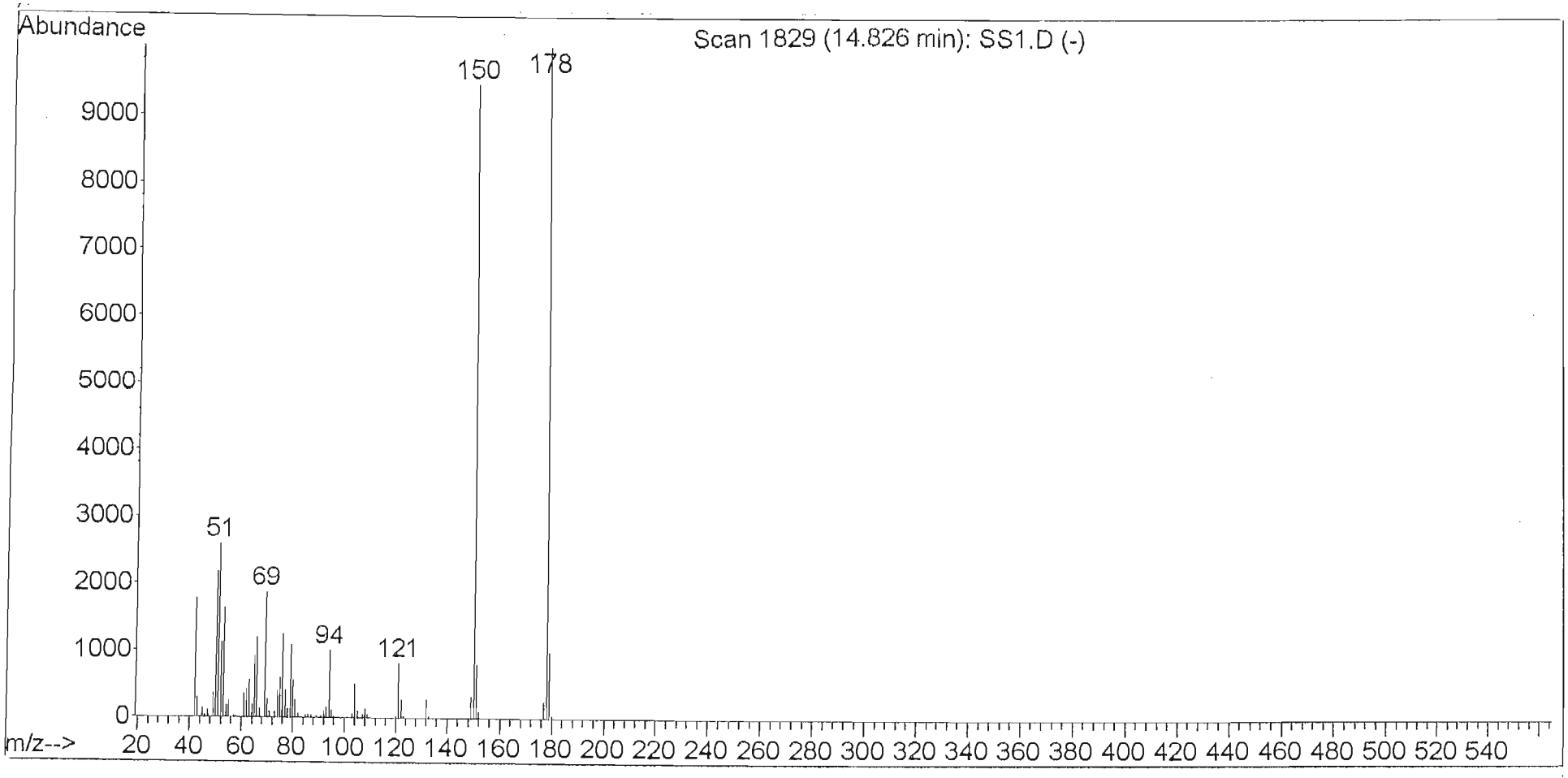

**Spectrum 17d: COSY spectrum of compound XVII**



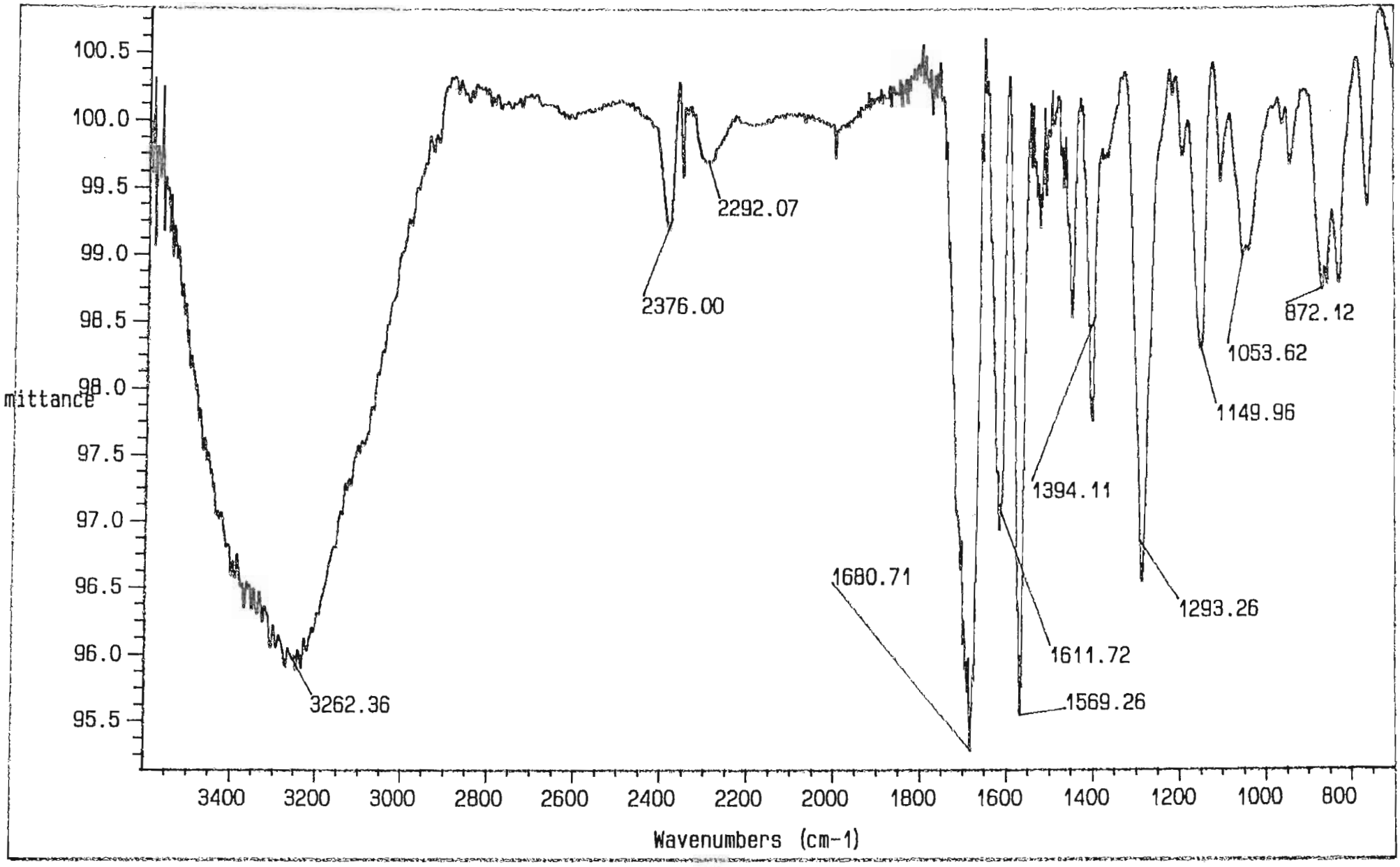

**Spectrum 17e: HMBC spectrum of compound XVII**




**Spectrum 17f: NOESY spectrum of compound XVII**




**Spectrum 17g: Mass spectrum of compound XVII**

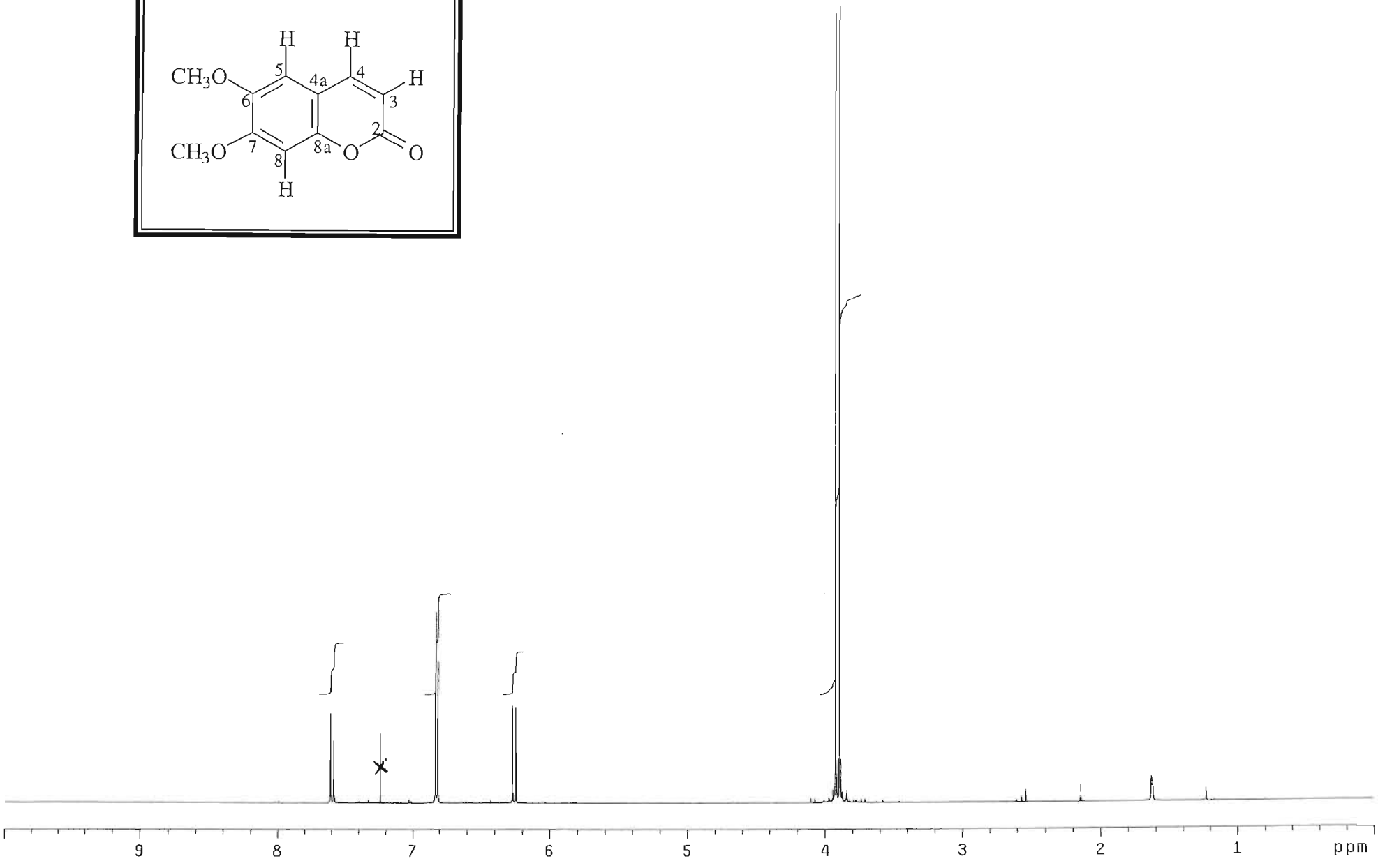



**Spectrum 17h: Infra-red spectrum of compound XVII**

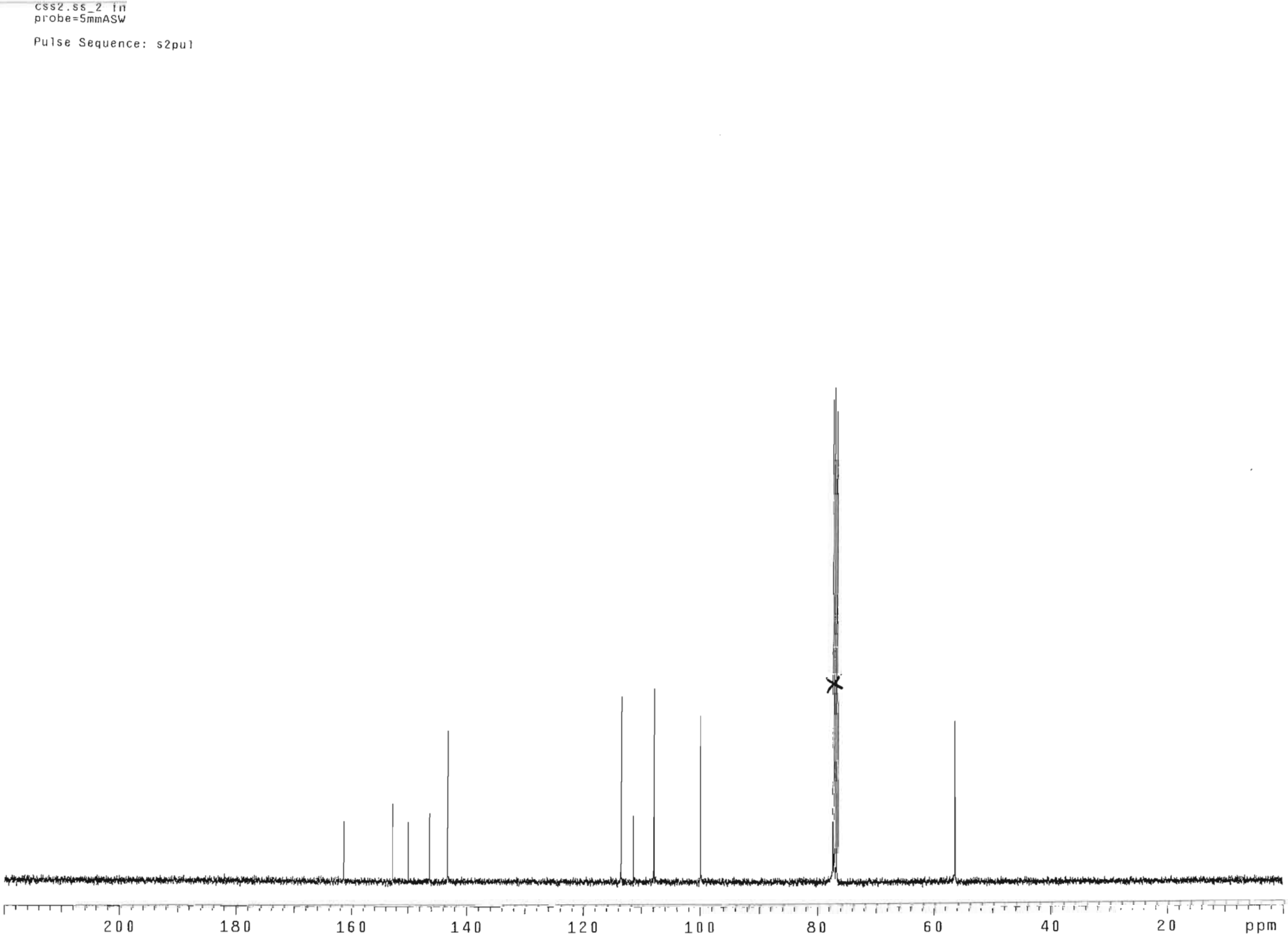
nss2.ss_2 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

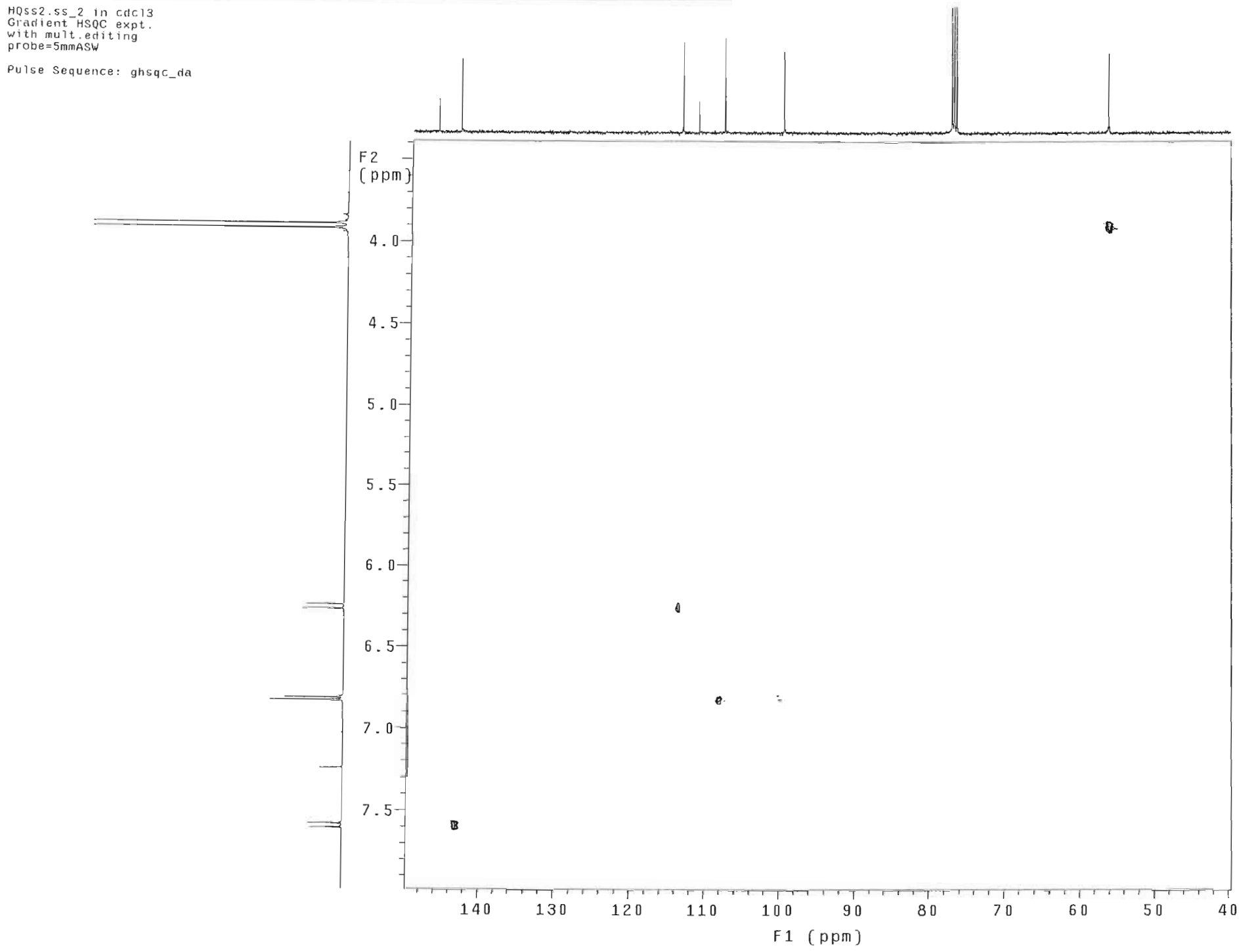


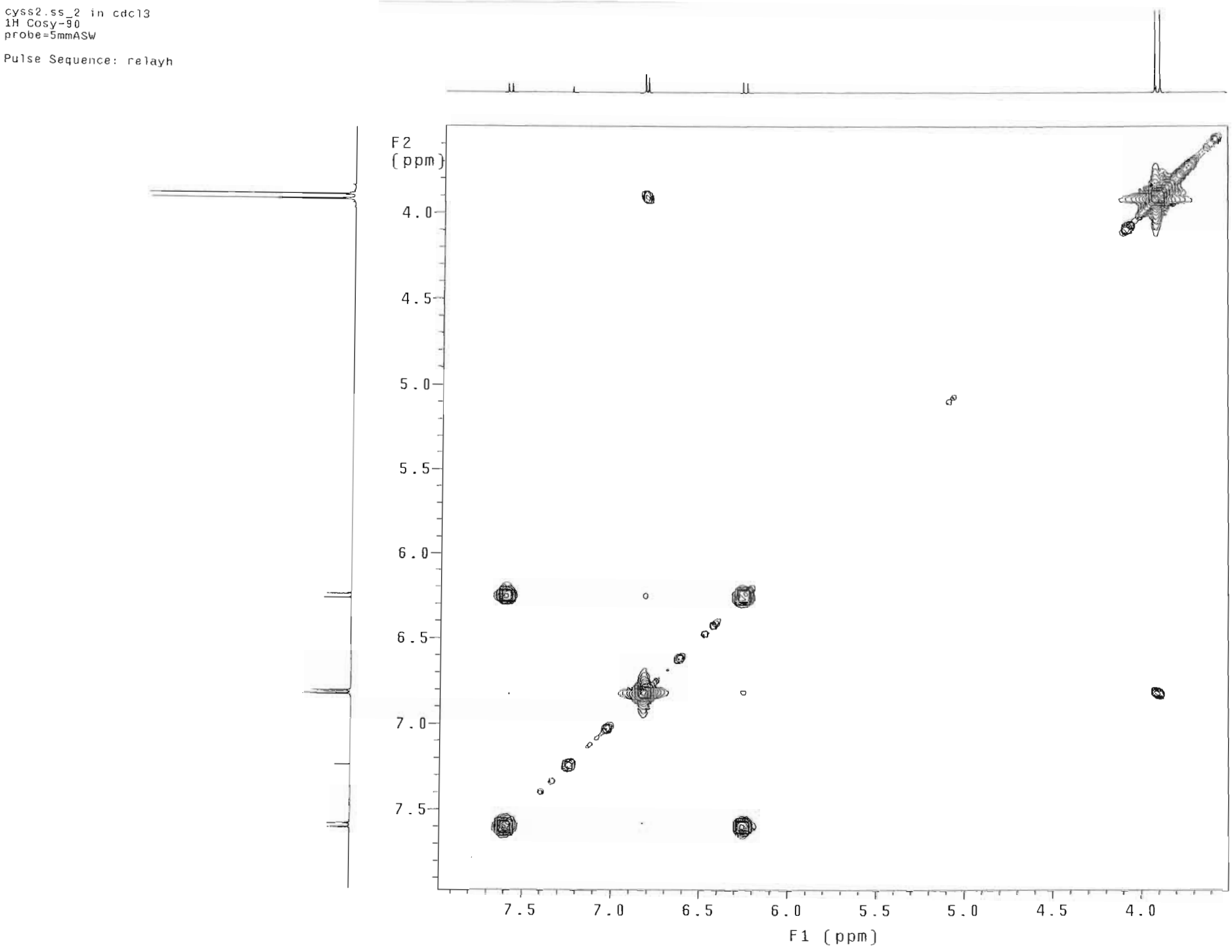

**Spectrum 18a: $^1$H NMR spectrum of compound XVIII ($CDCl_3$) (400 MHz)**



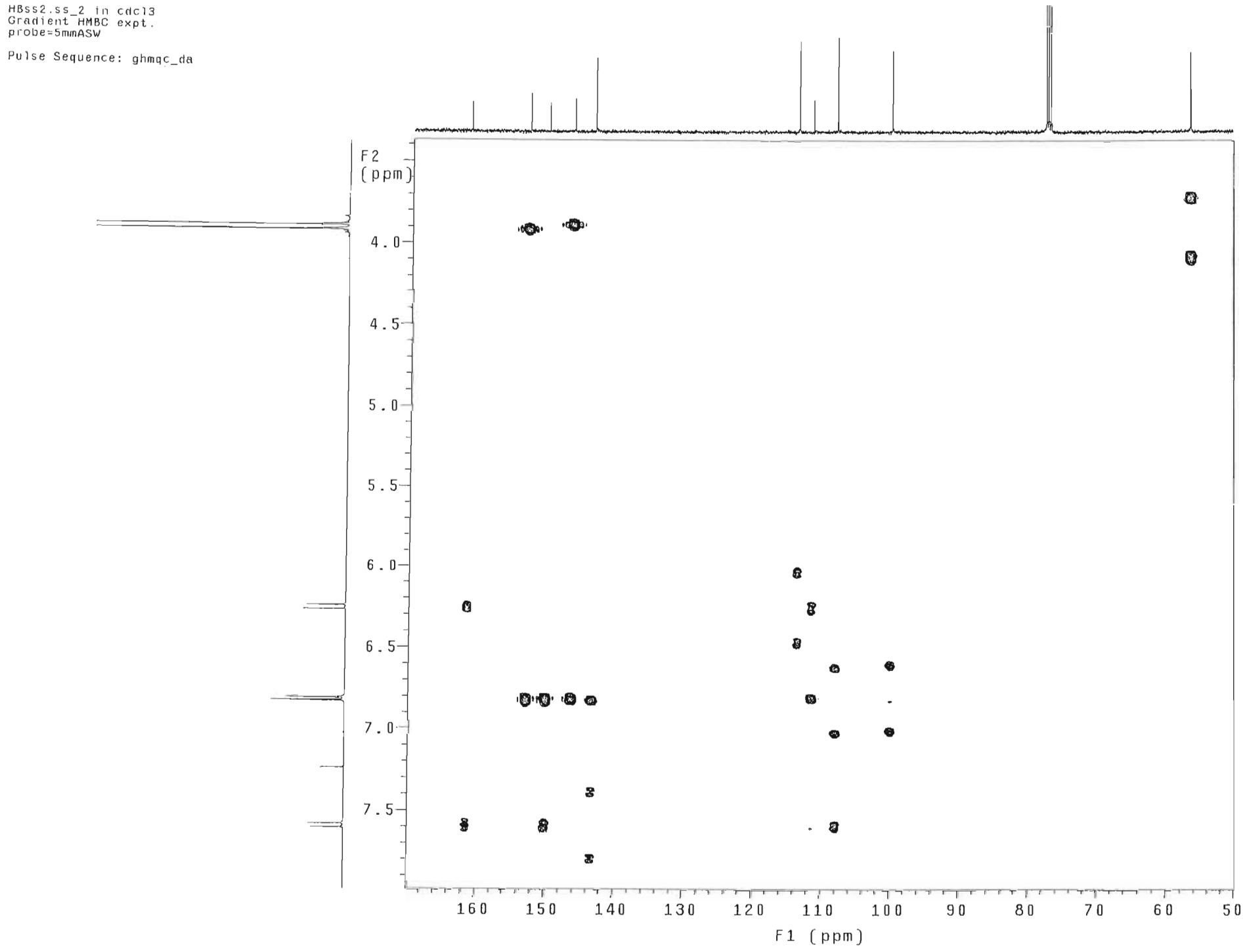

**Spectrum 18b: $^{13}C$ NMR spectrum of compound XVIII ($CDCl_3$) (100 MHz)**



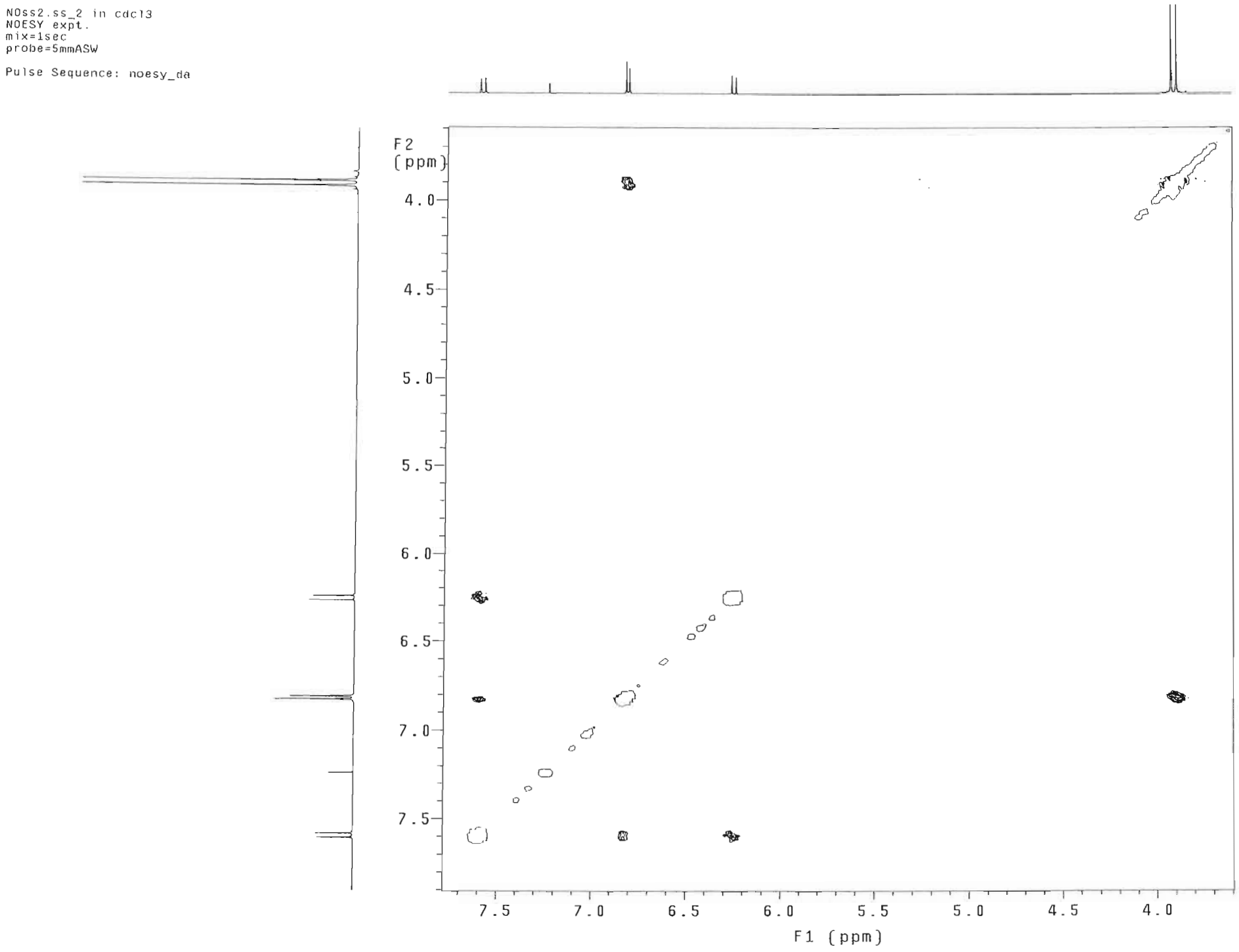

**Spectrum 18c: HSQC spectrum of compound XVIII**




**Spectrum 18d: COSY spectrum of compound XVIII**




**Spectrum 18e: HMBC spectrum of compound XVIII**

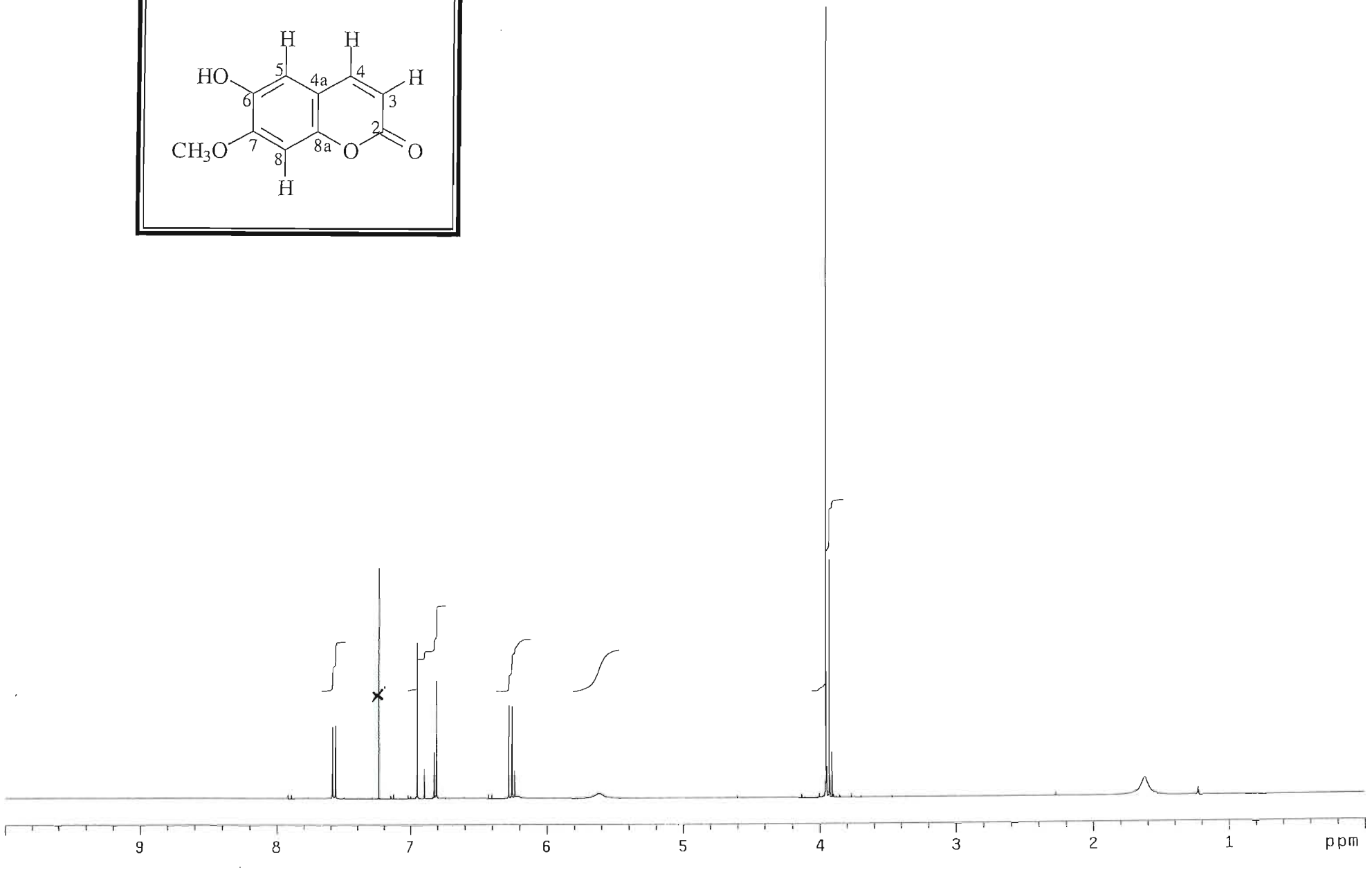



**Spectrum 18f: NOESY spectrum of compound XVIII**

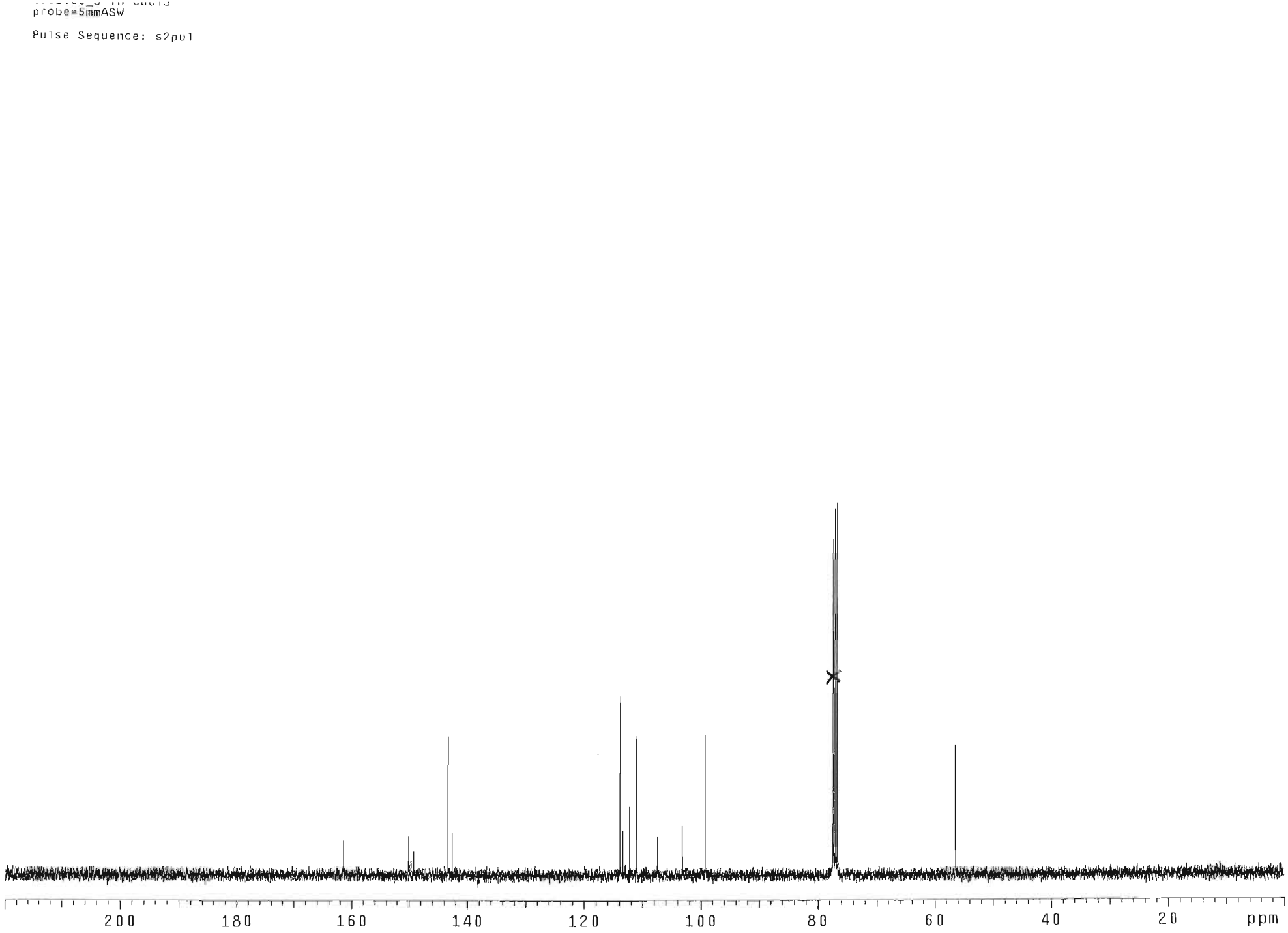
hss3.ss_3 in cdcl3
probe=5mmASW

Pulse Sequence: s2pul

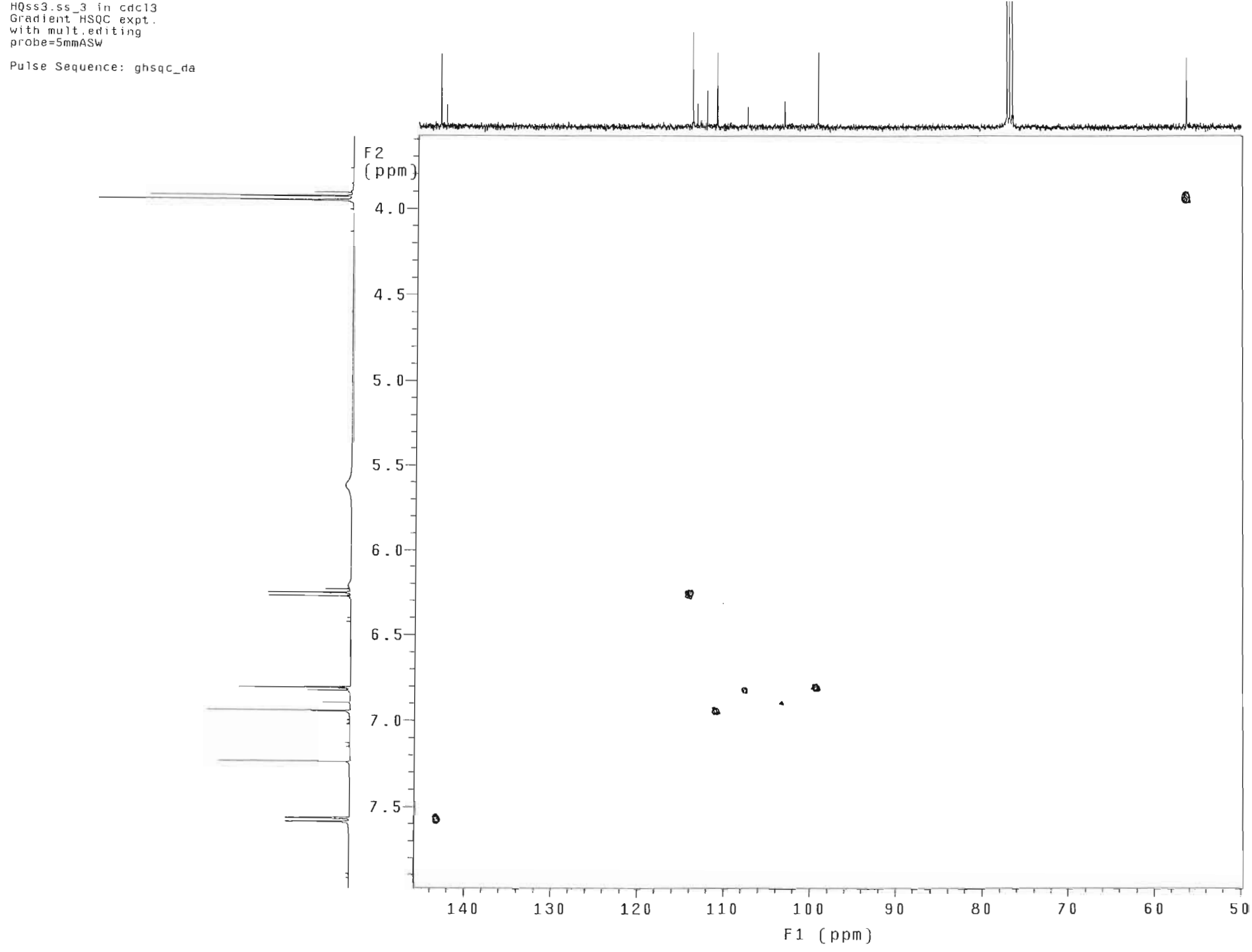


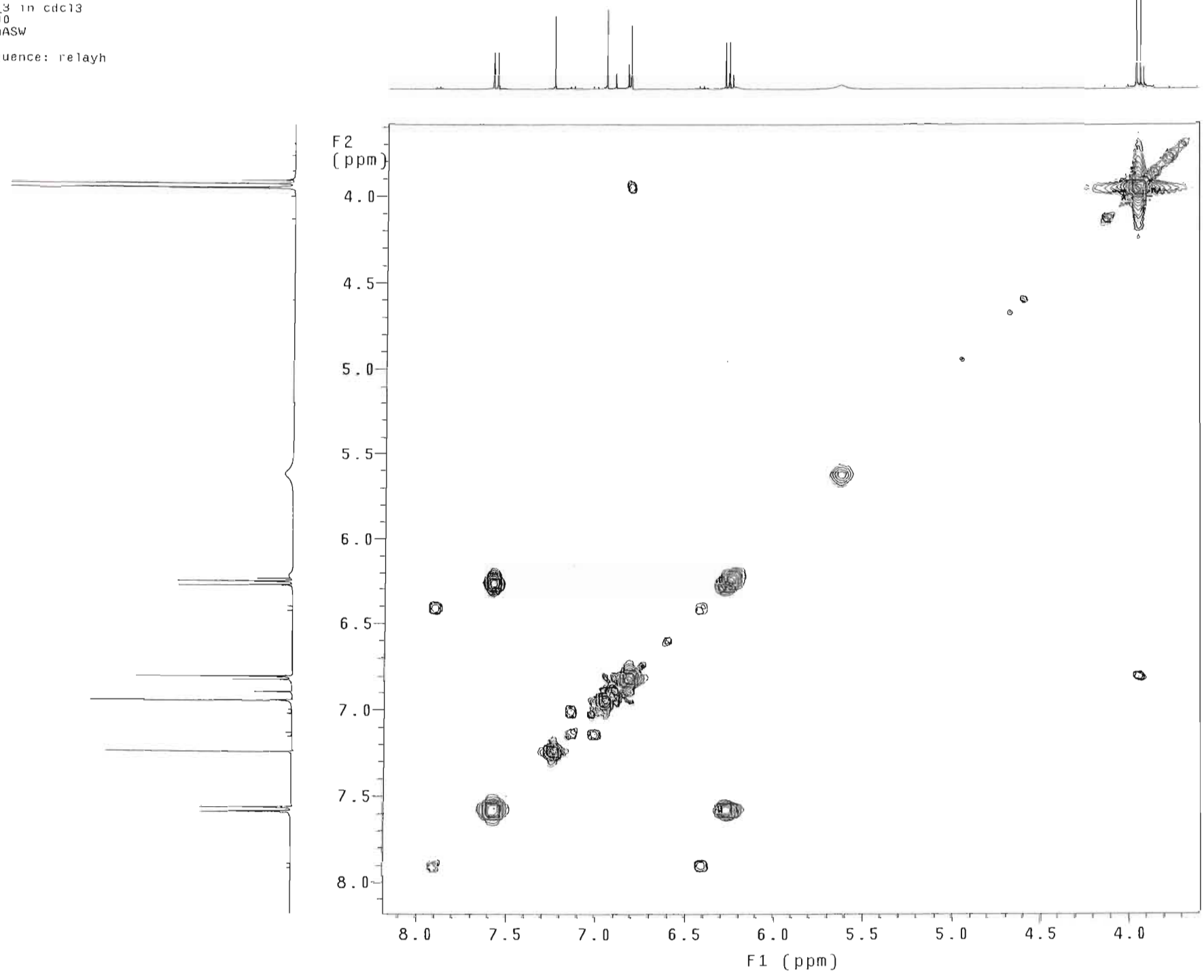

**Spectrum 19a: $^1$H NMR spectrum of compound XIX ($CDCl_3$) (400 MHz)**



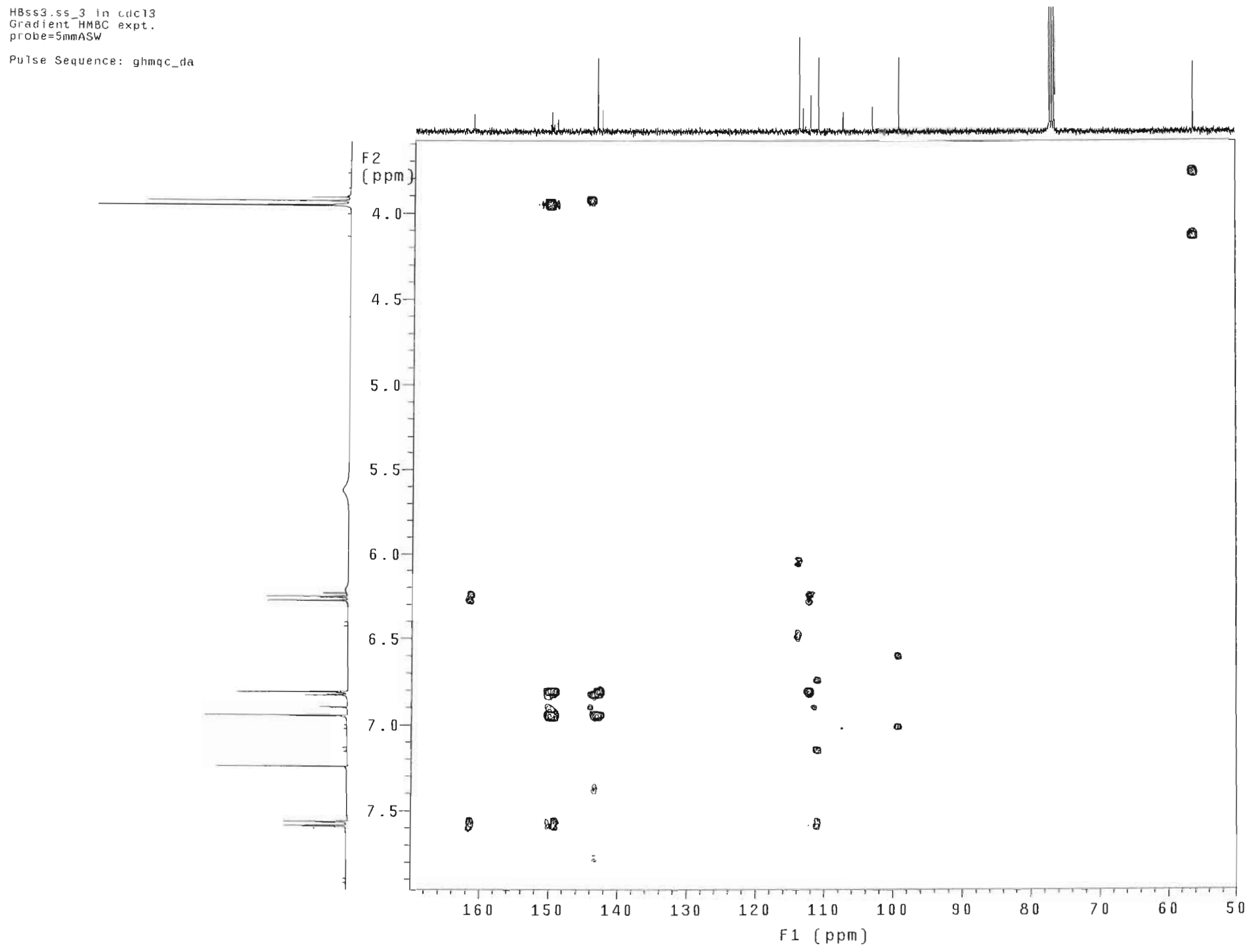

**Spectrum 19b: $^{13}C$ NMR spectrum of compound XIX ($CDCl_3$) (100 MHz)**



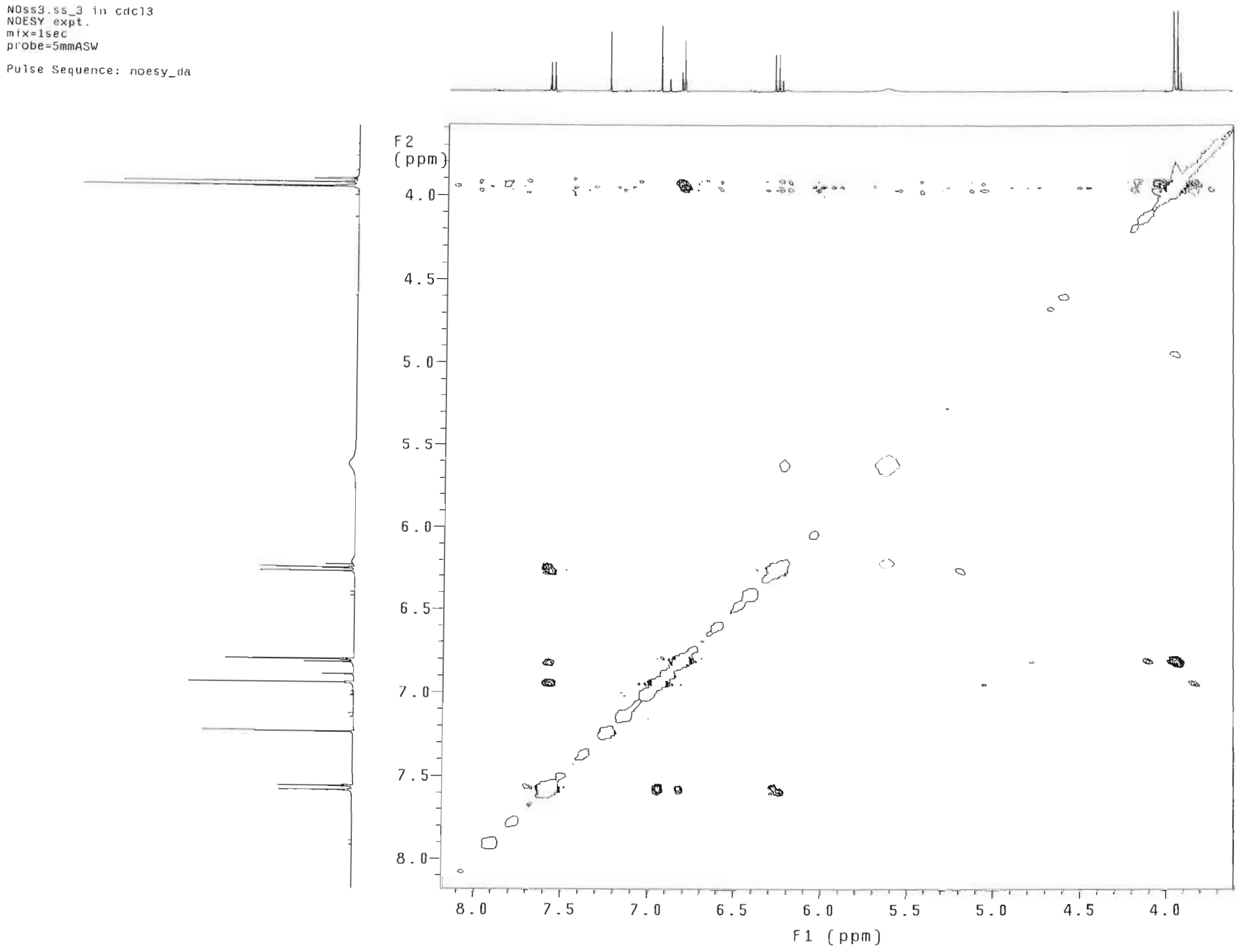

**Spectrum 19c: HSQC spectrum of compound XIX**



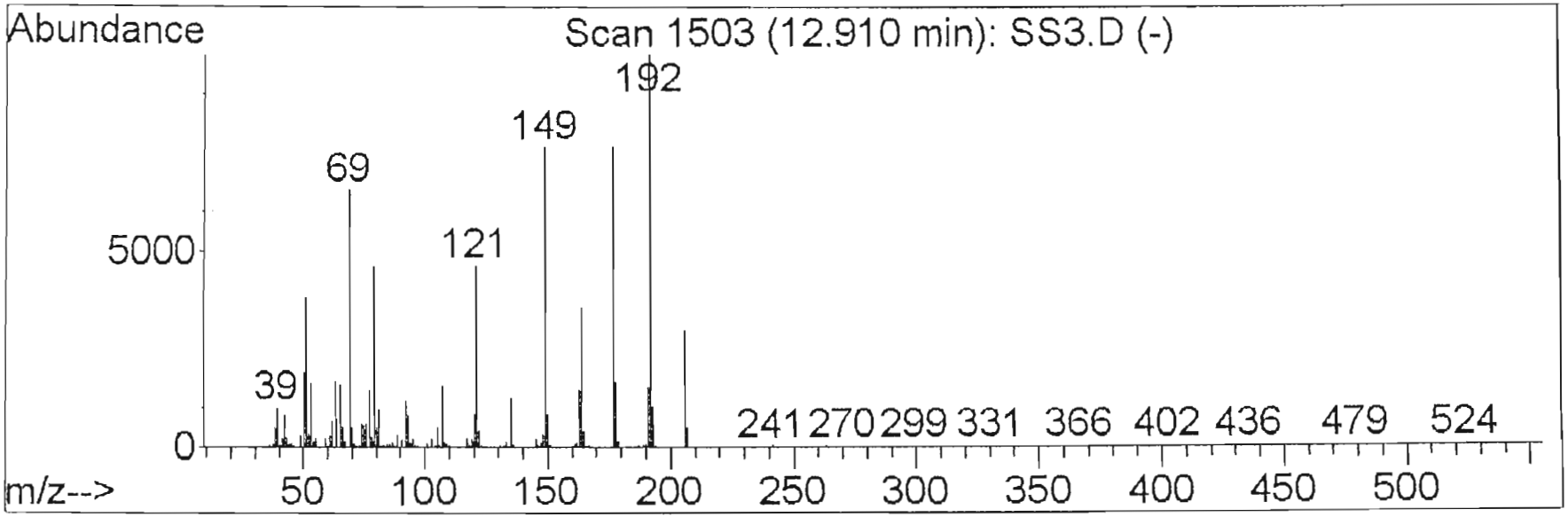

**Spectrum 19d: COSY spectrum of compound XIX**



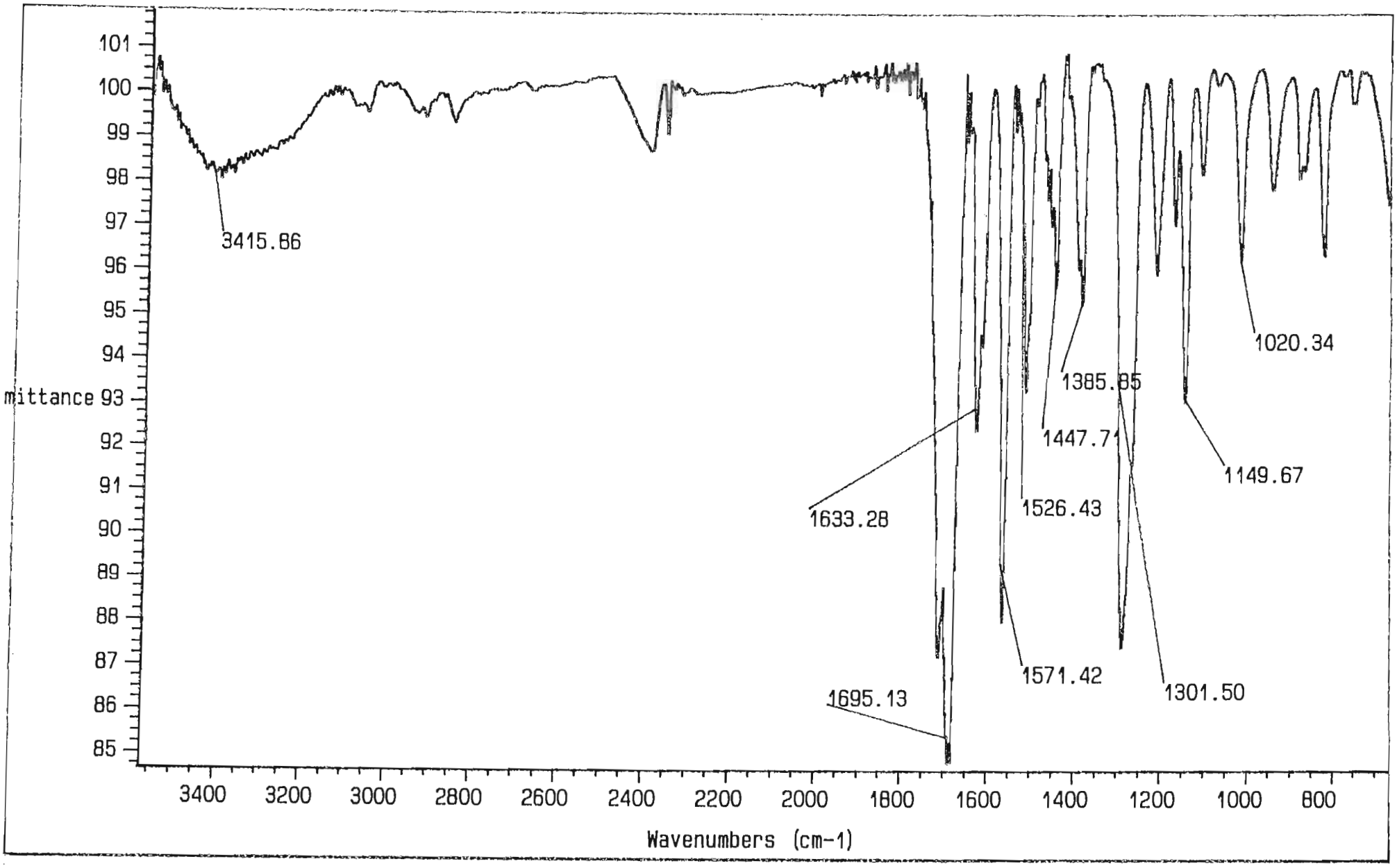

**Spectrum 19e: HMBC spectrum of compound XIX**




**Spectrum 19f: NOESY spectrum of compound XIX**



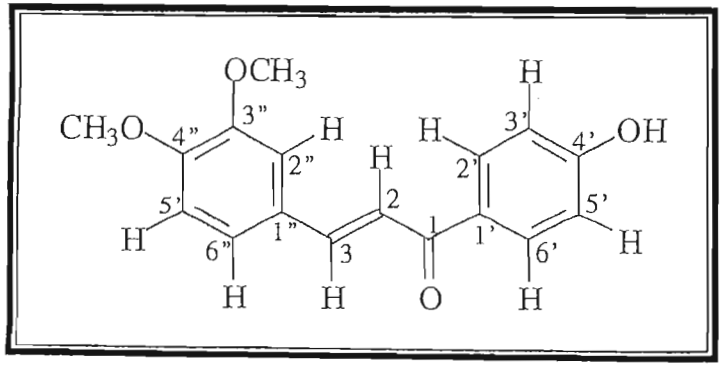

**Spectrum 19g: Mass spectrum of compound XIX**

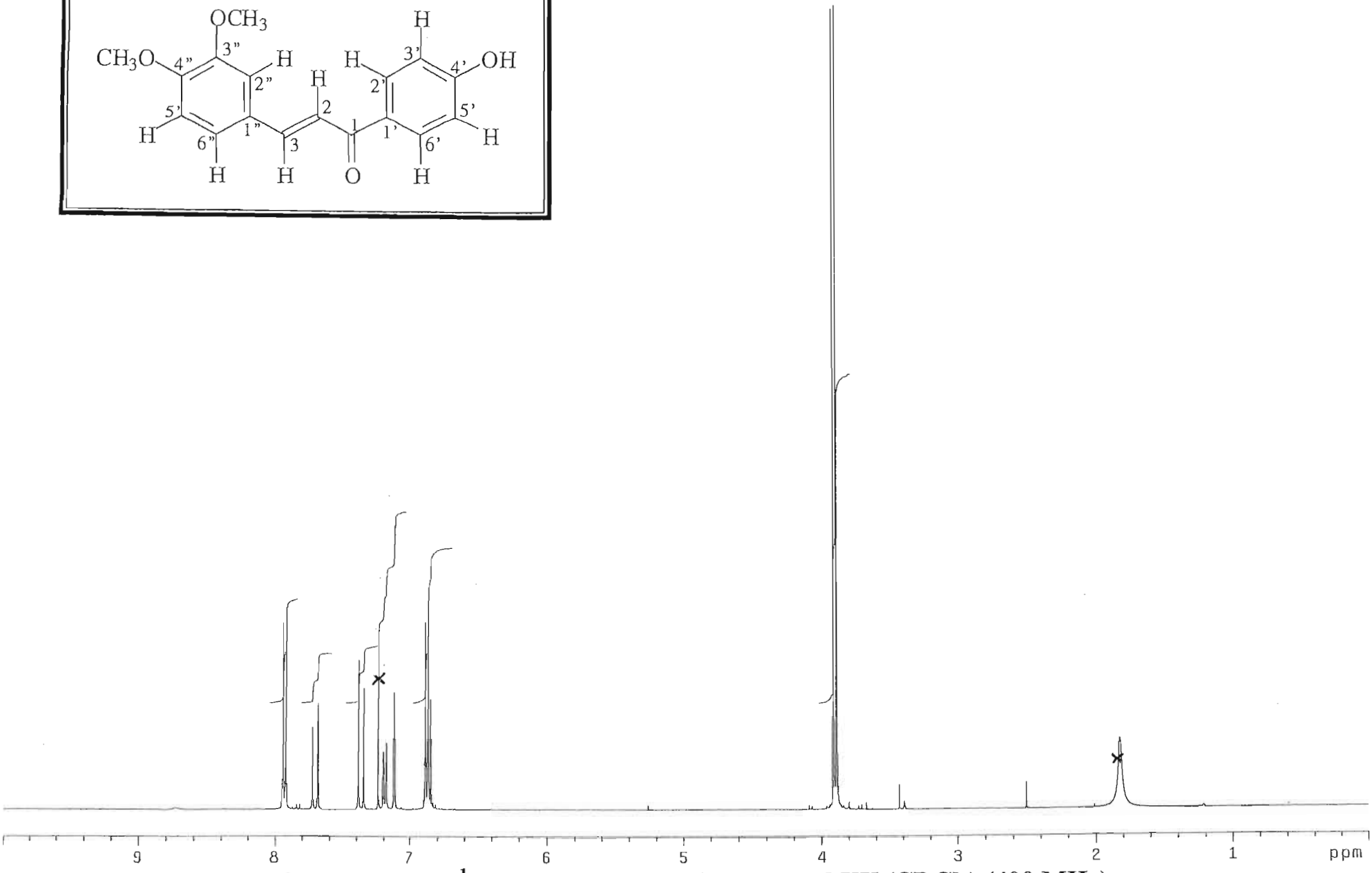



**Spectrum 19h: Infra-red spectrum of compound XIX**

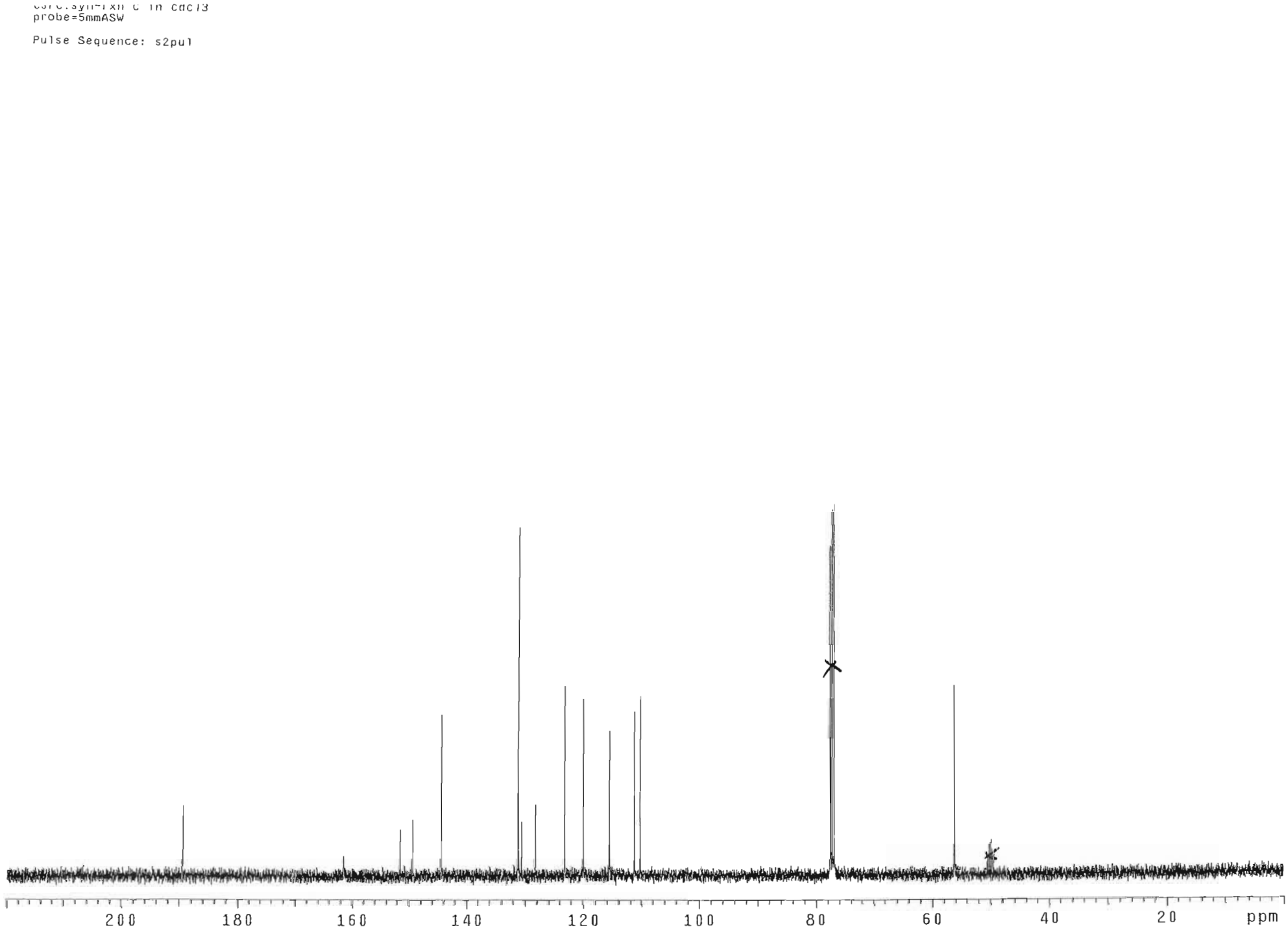
syn-rxn c

Pulse Sequence: s2pul

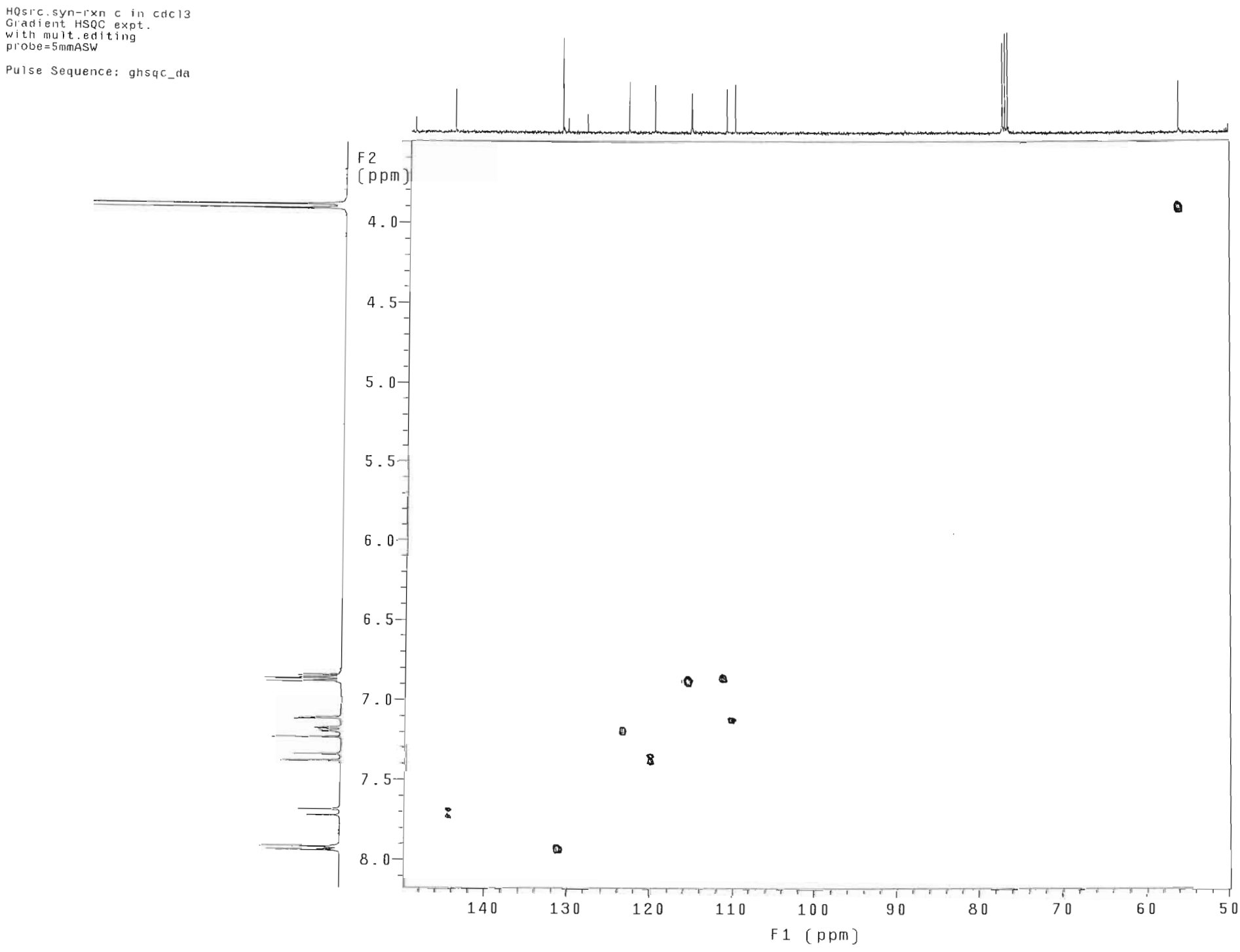


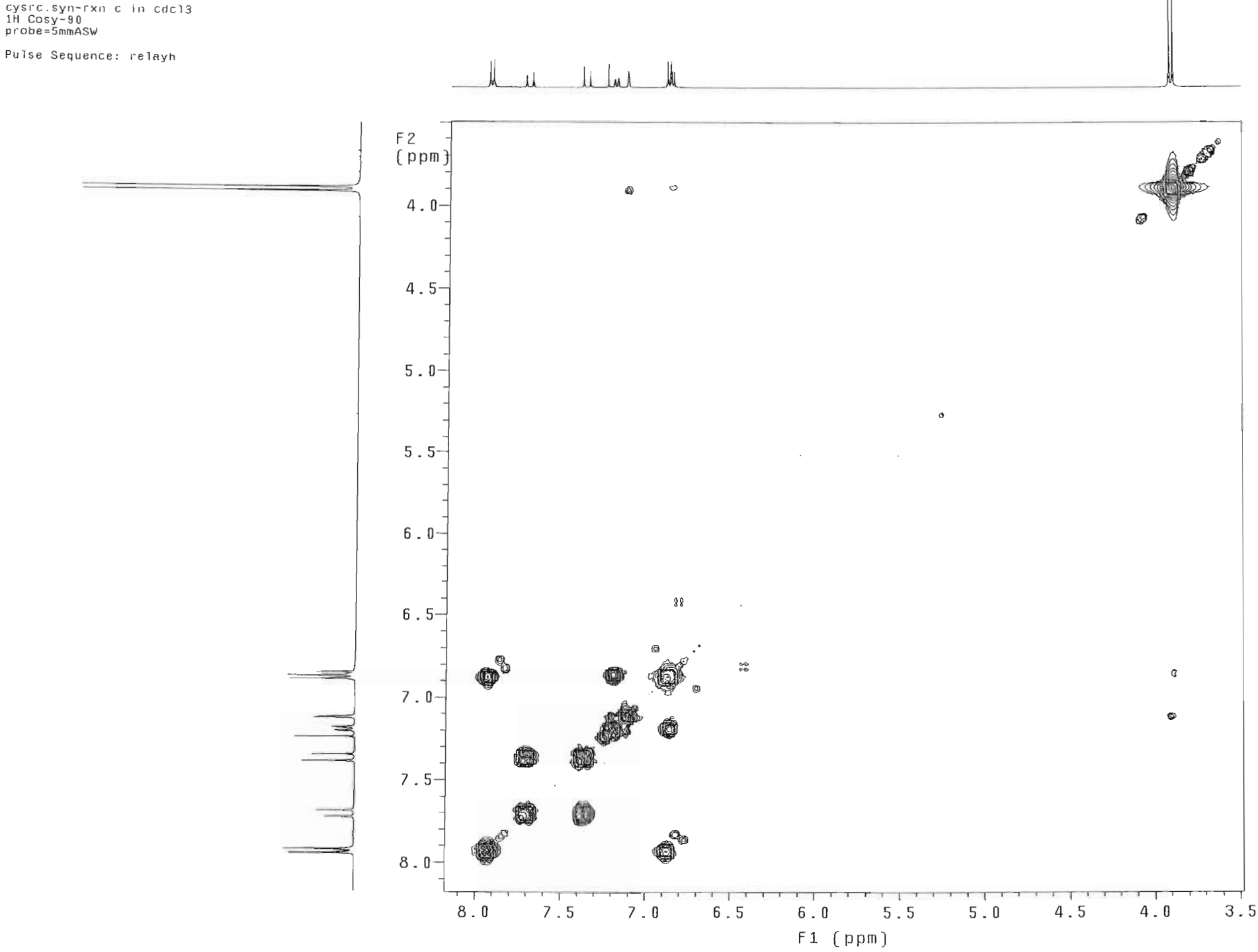

**Spectrum 20a: $^1$H NMR spectrum of compound XX ($CDCl_3$) (400 MHz)**



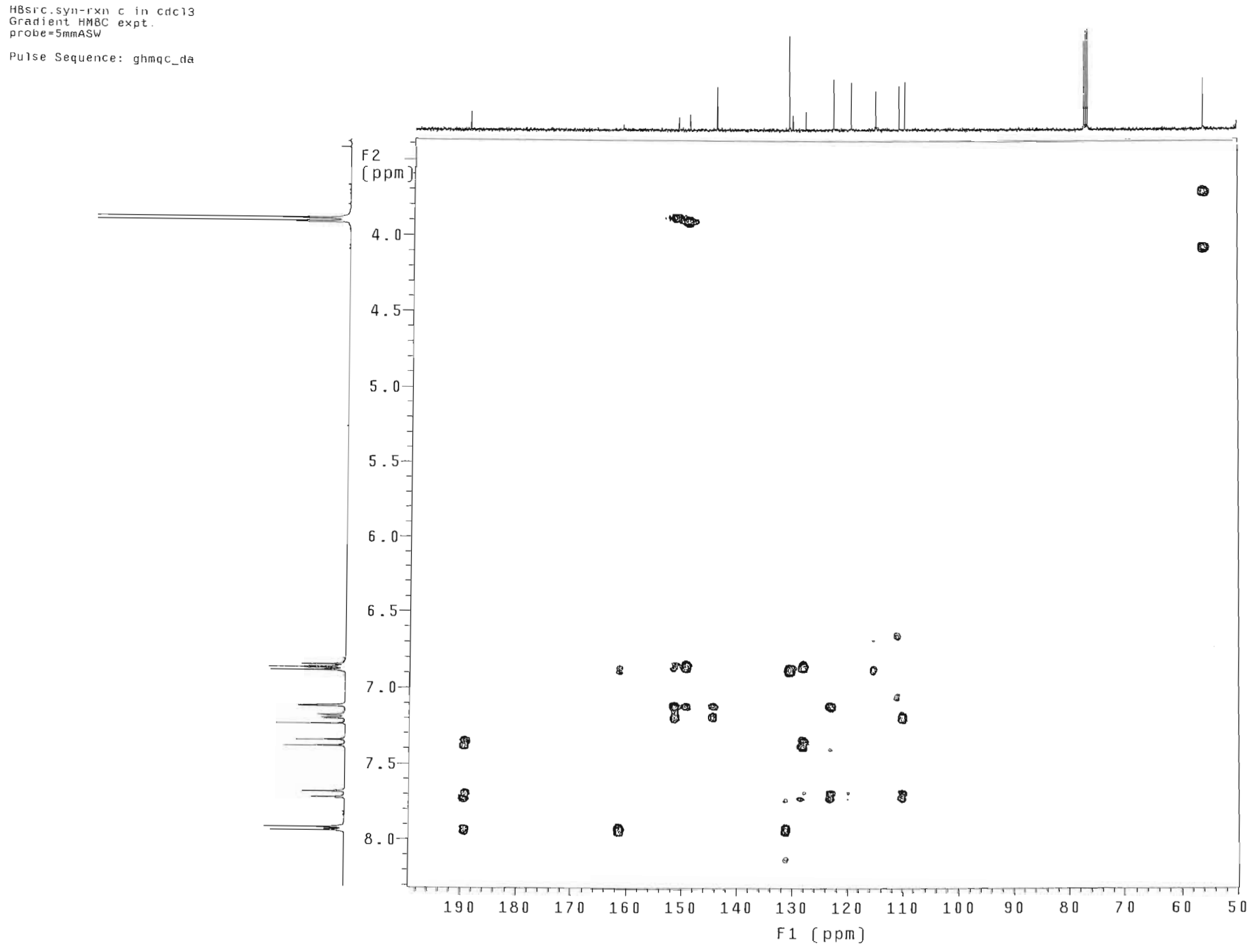

**Spectrum 20b: $^{13}C$ NMR spectrum of compound XX ($CDCl_3$) (100 MHz)**



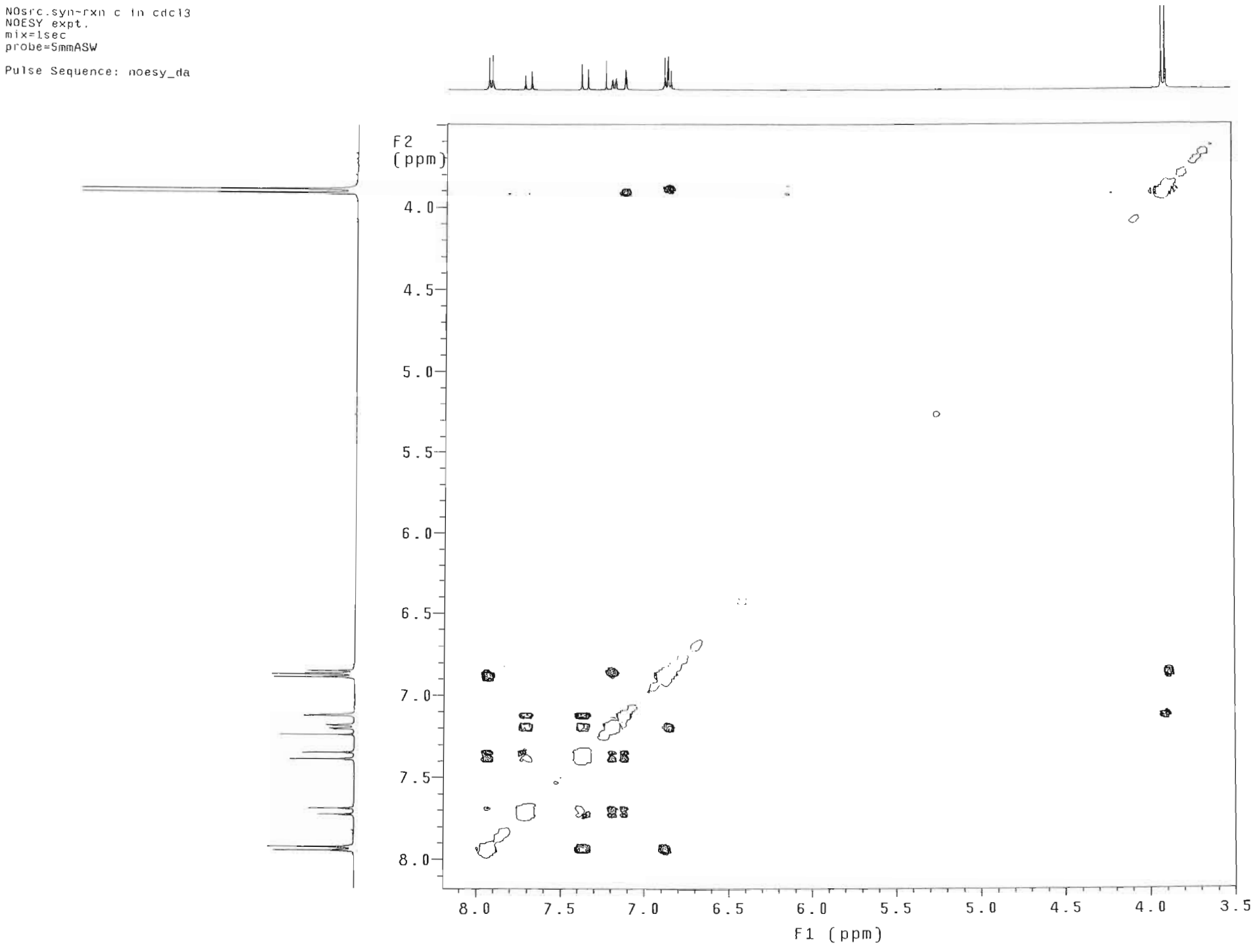

**Spectrum 20c: HSQC spectrum of compound XX**



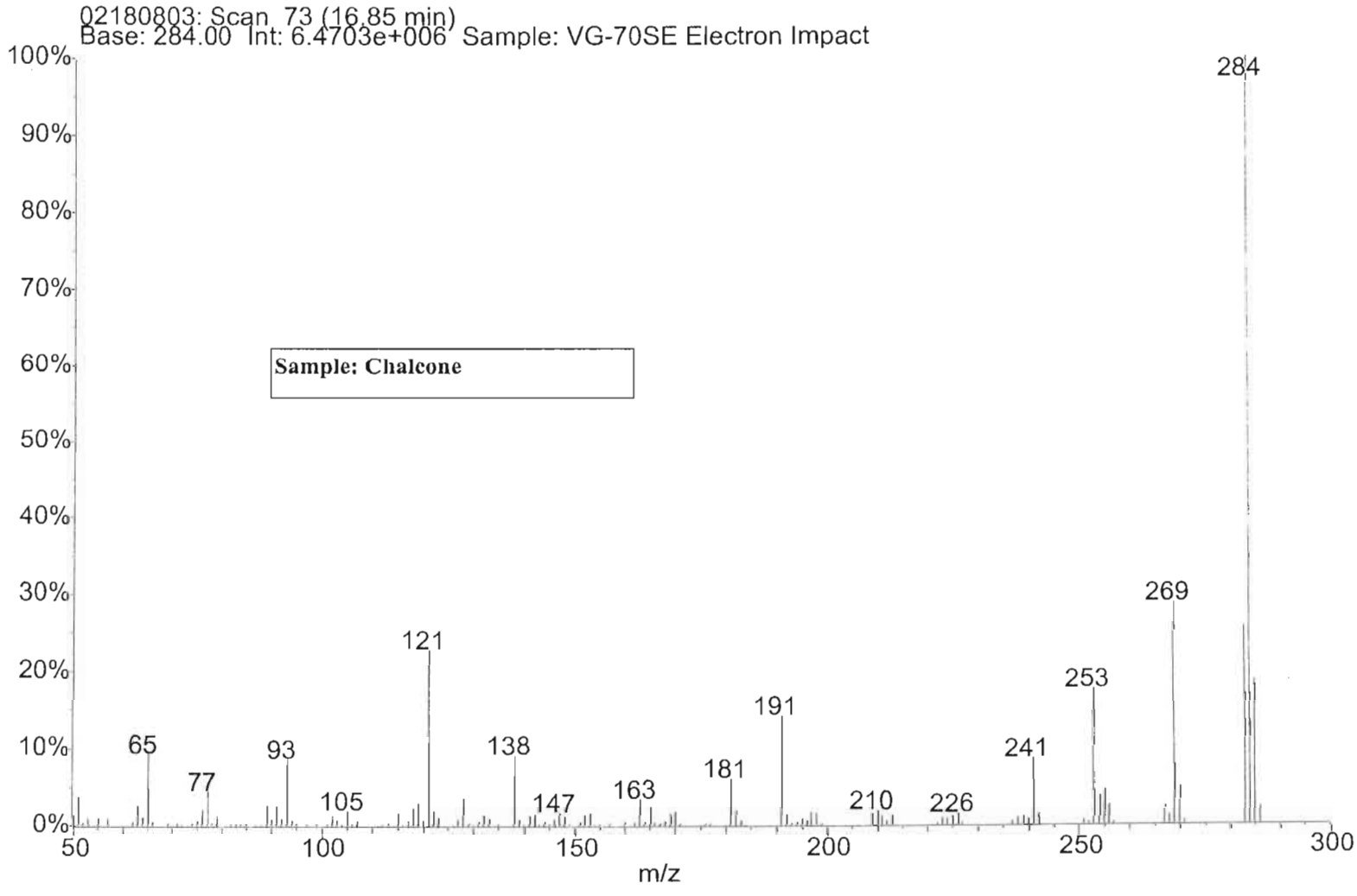

**Spectrum 20d: COSY spectrum of compound XX**



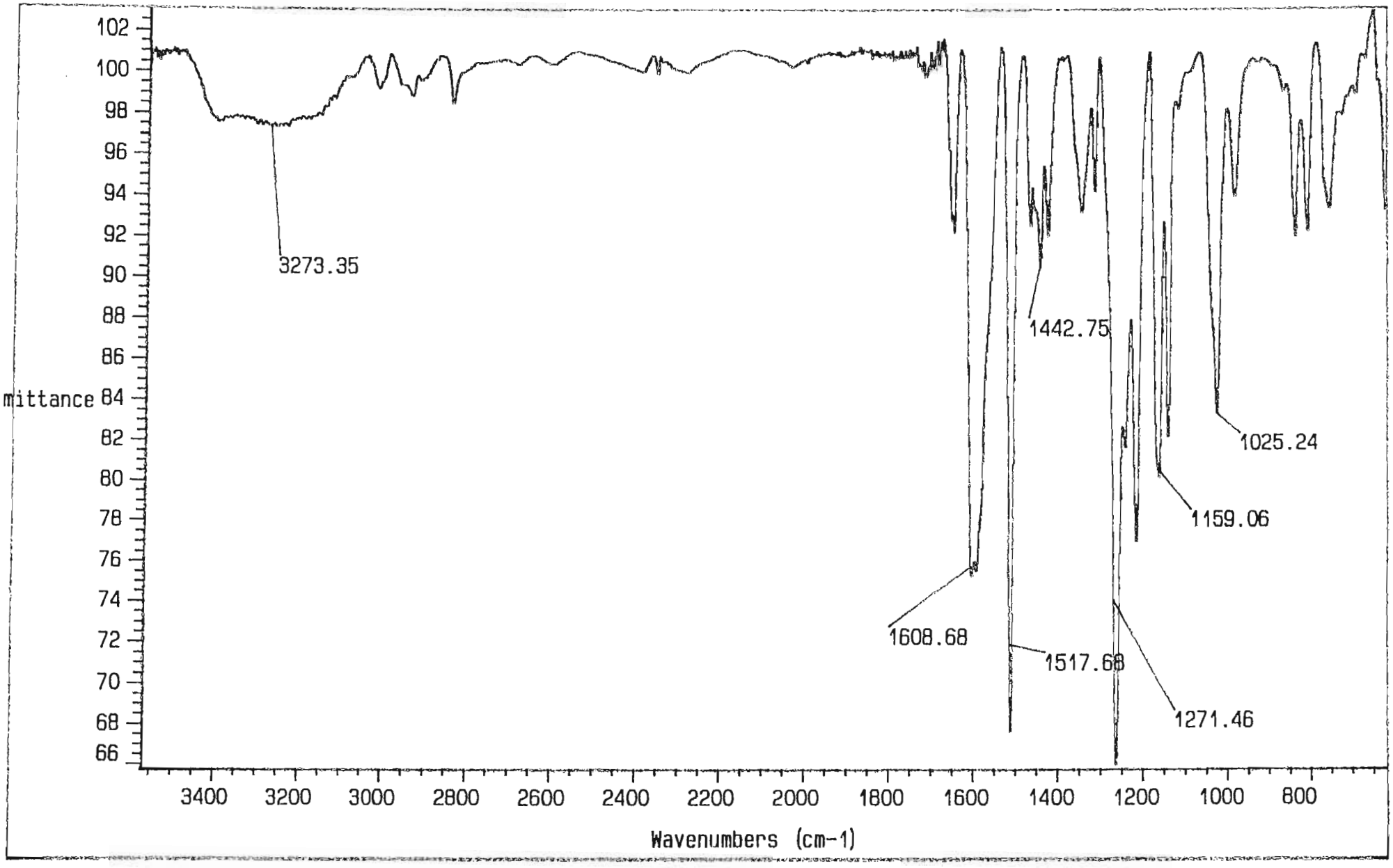

**Spectrum 20e: HMBC spectrum of compound XX**



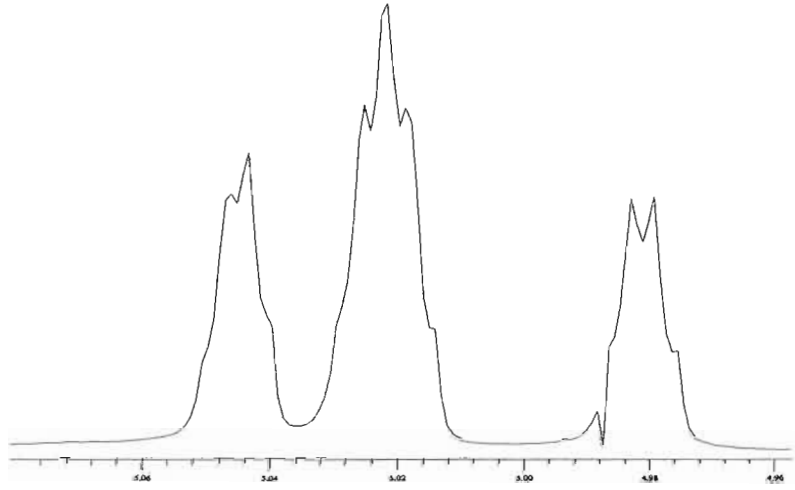

**Spectrum 20f: NOESY spectrum of compound XX**

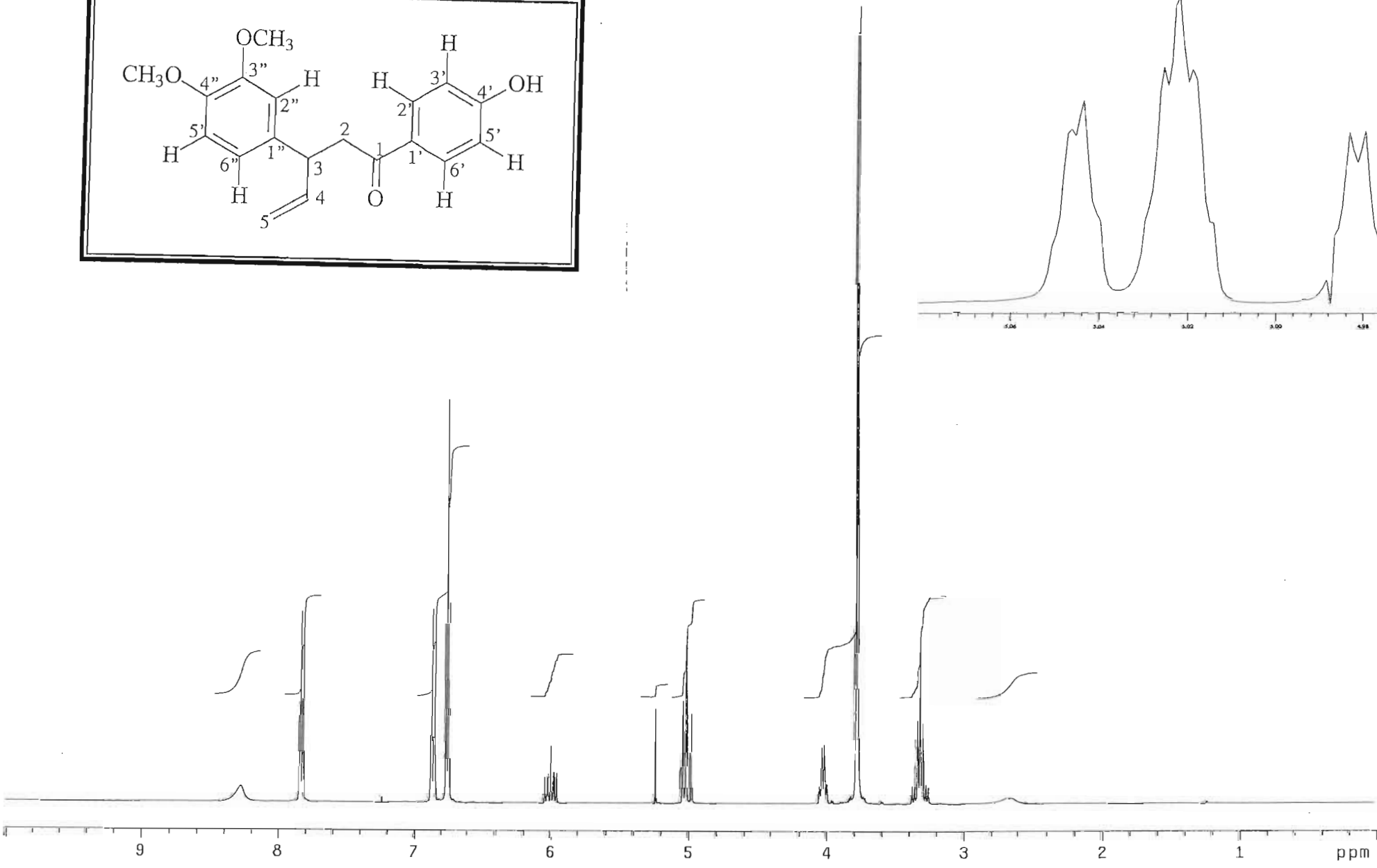



**Spectrum 20g: Mass spectrum of compound XX**

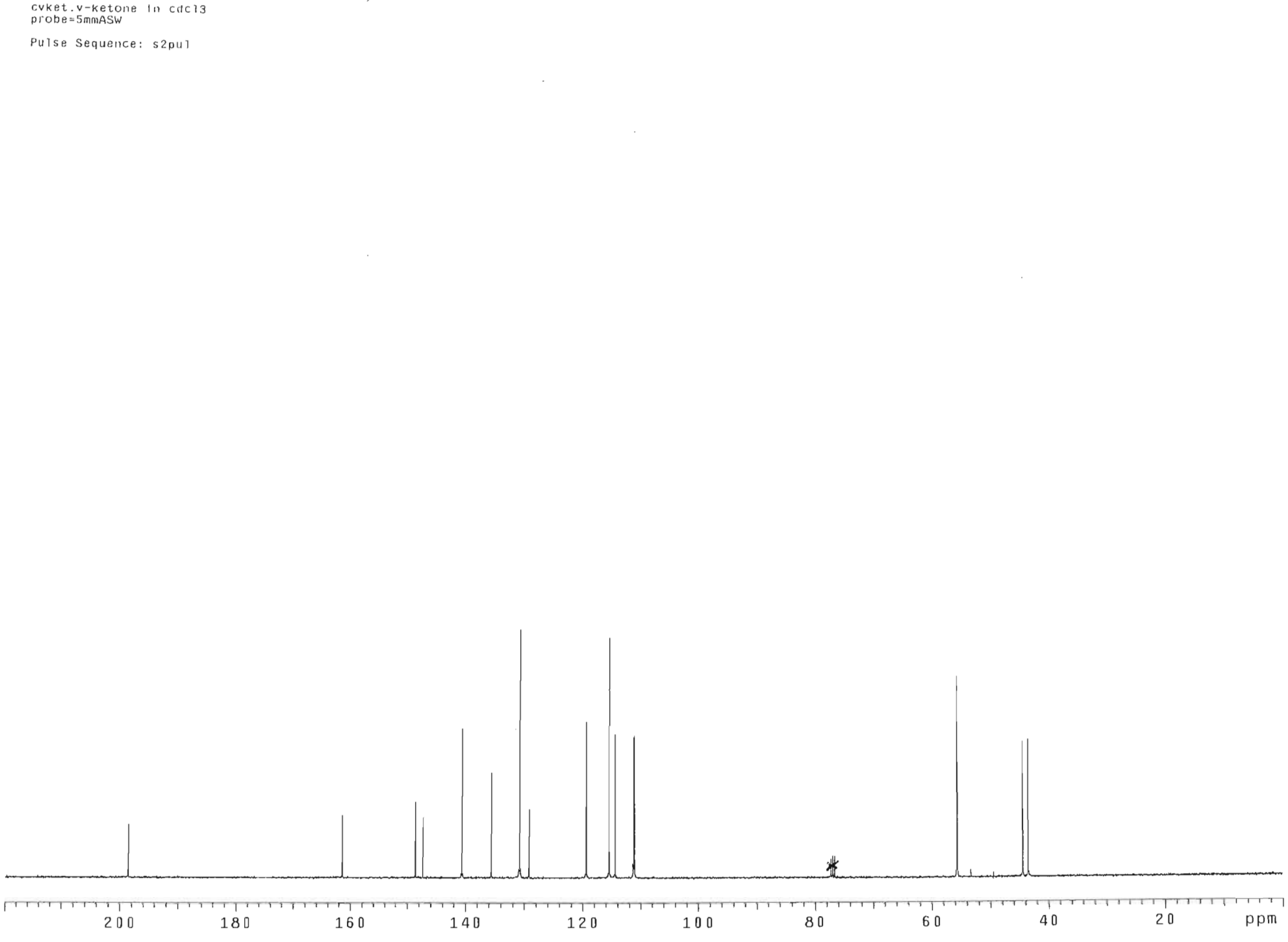



**Spectrum 20h: Infra-red spectrum of compound XX**

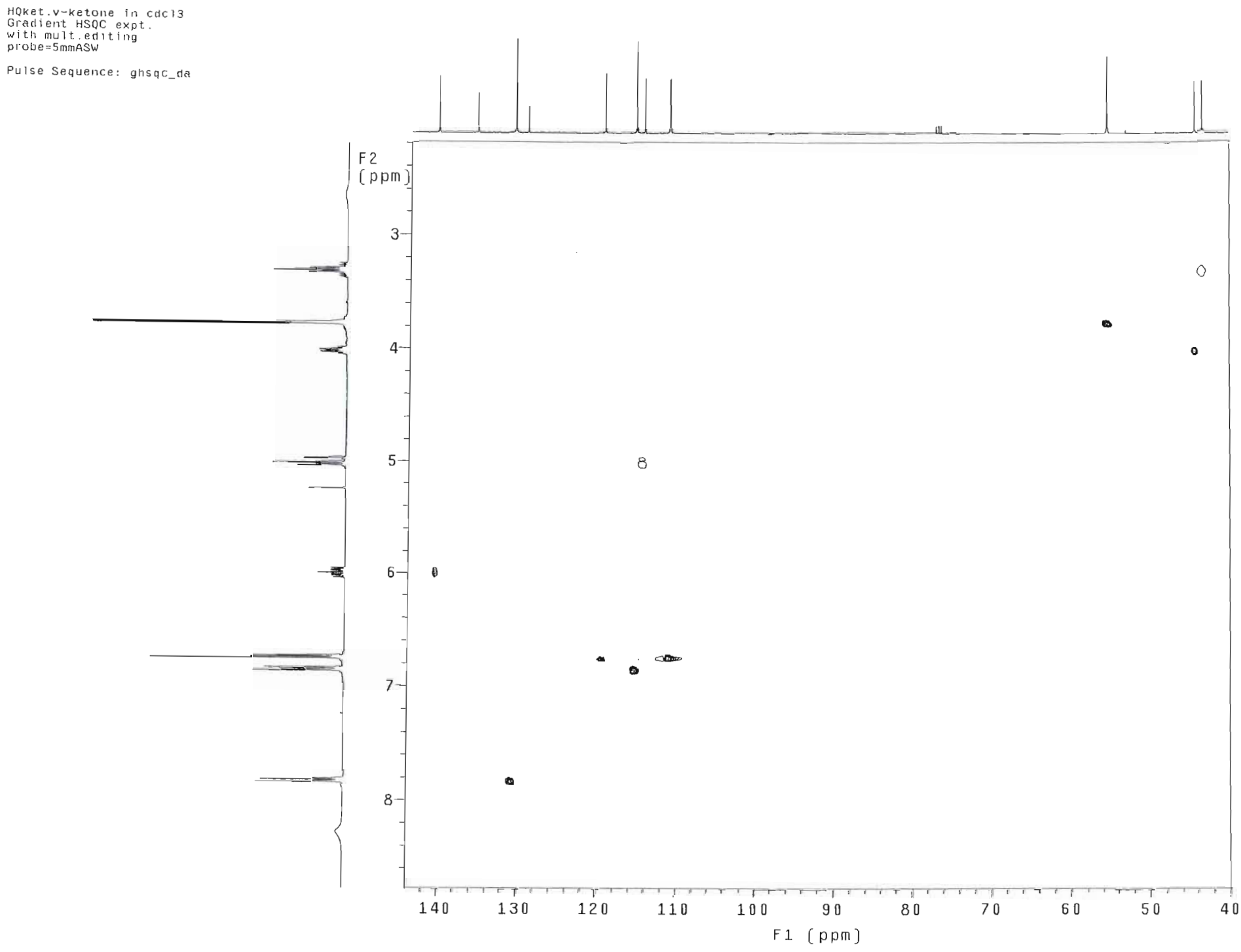




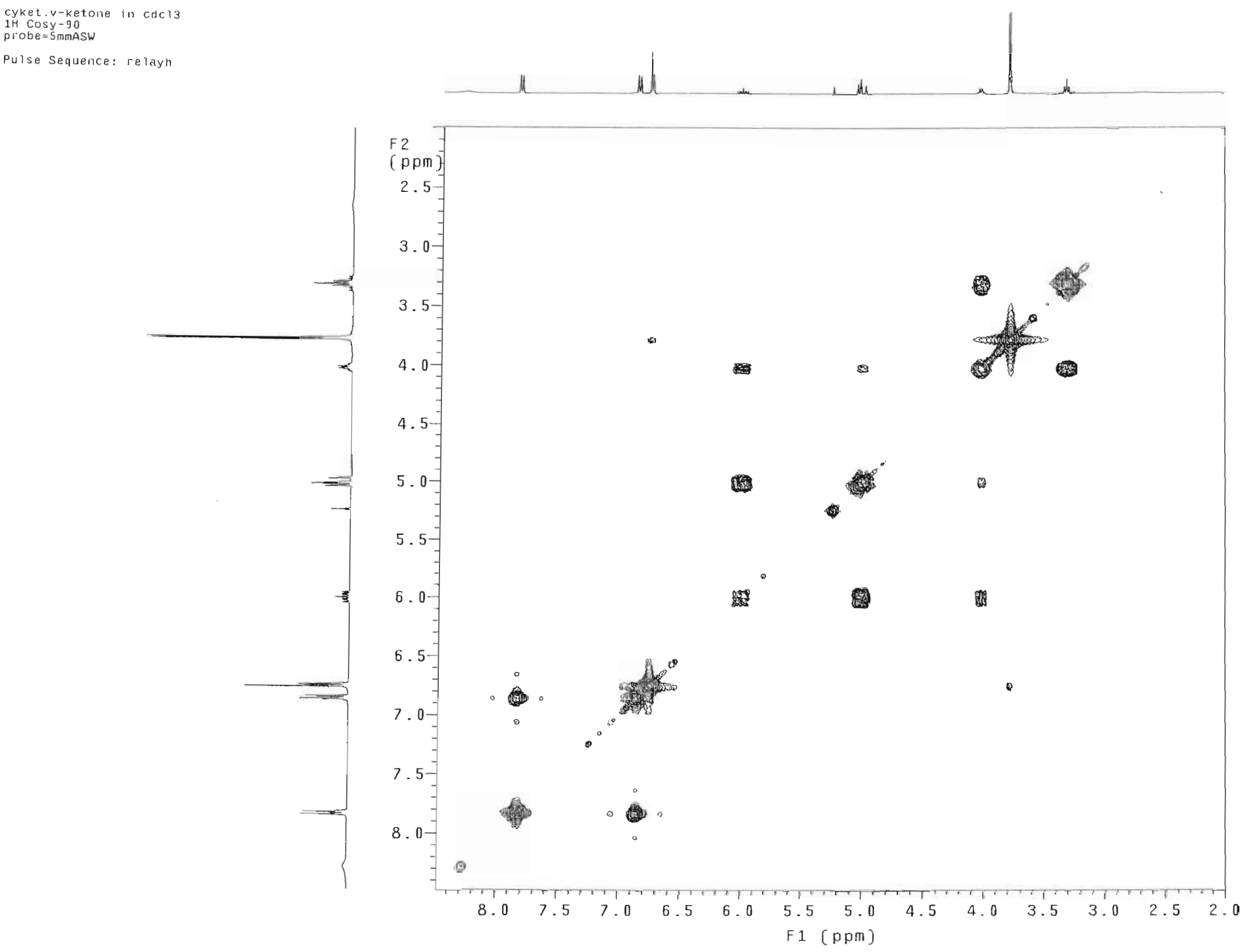

**Spectrum 21a: $^{1}$H NMR spectrum of compound XXI ($CDCl_3$) (400 MHz)**



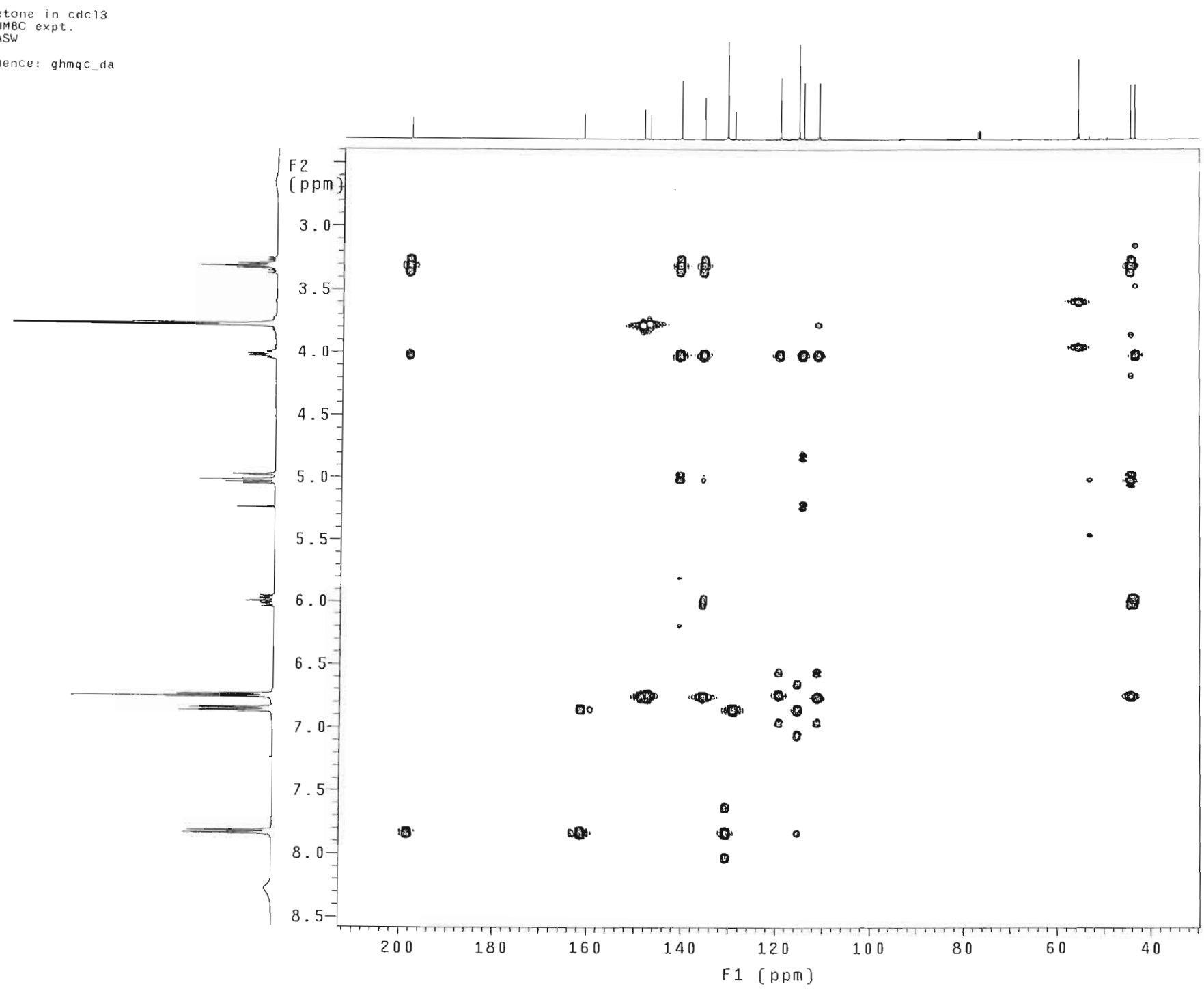

**Spectrum 21b: $^{13}C$ NMR spectrum of compound XXI ($CDCl_3$) (100 MHz)**



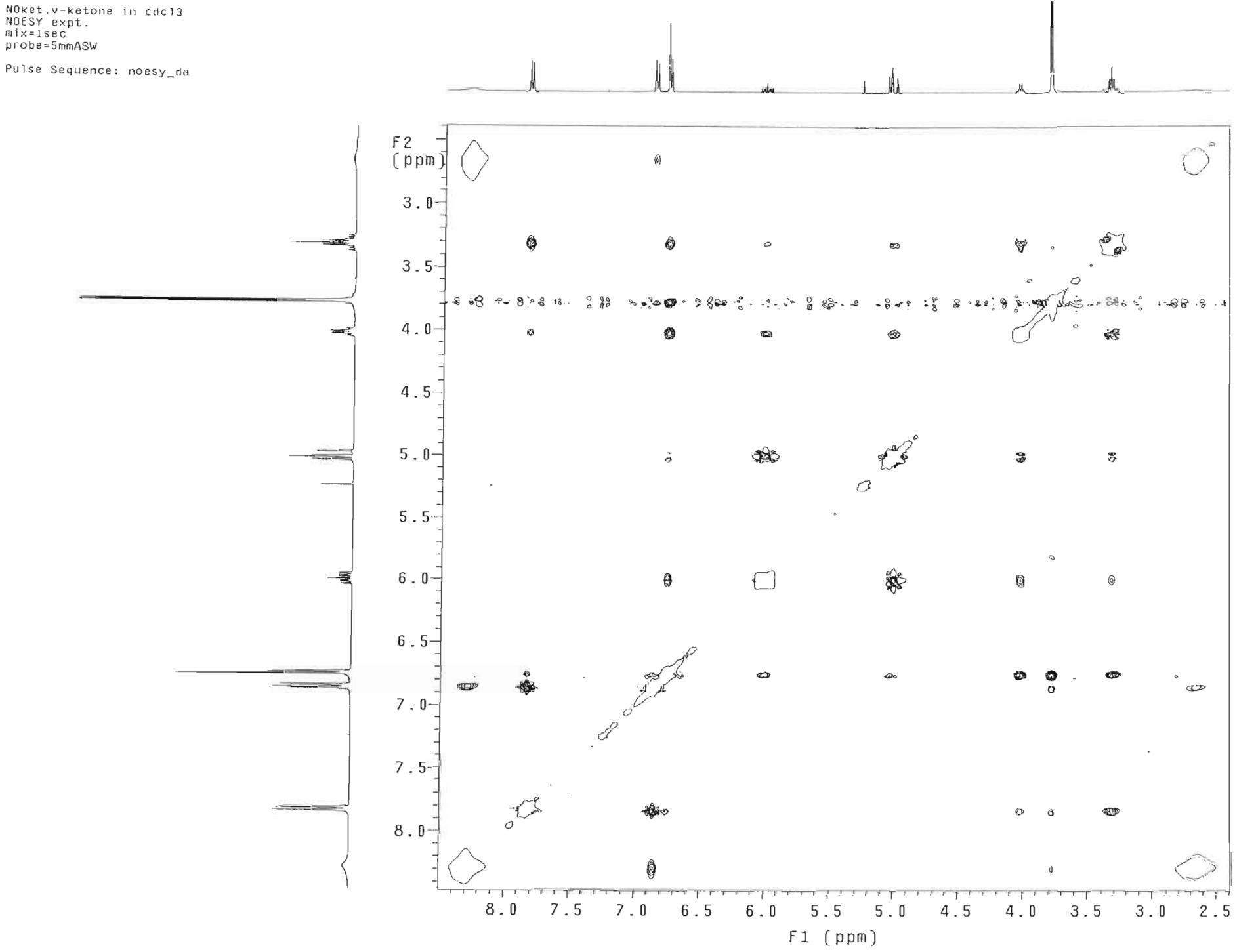

**Spectrum 21c: HSQC spectrum of compound XXI**



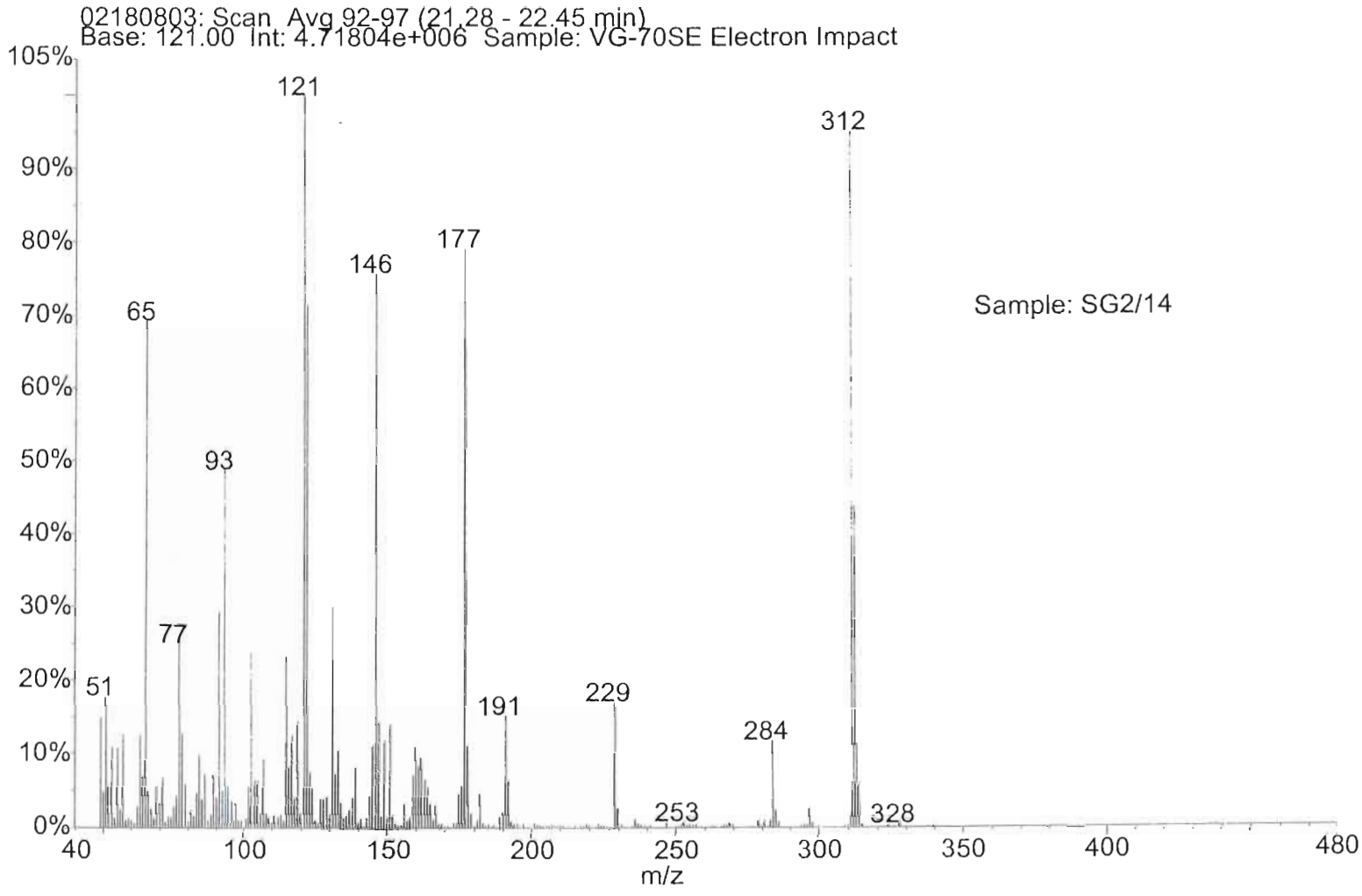

**Spectrum 21d: COSY spectrum of compound XXI**




**Spectrum 21e: HMBC spectrum of compound XXI**




**Spectrum 21f: NOESY spectrum of compound XXI**



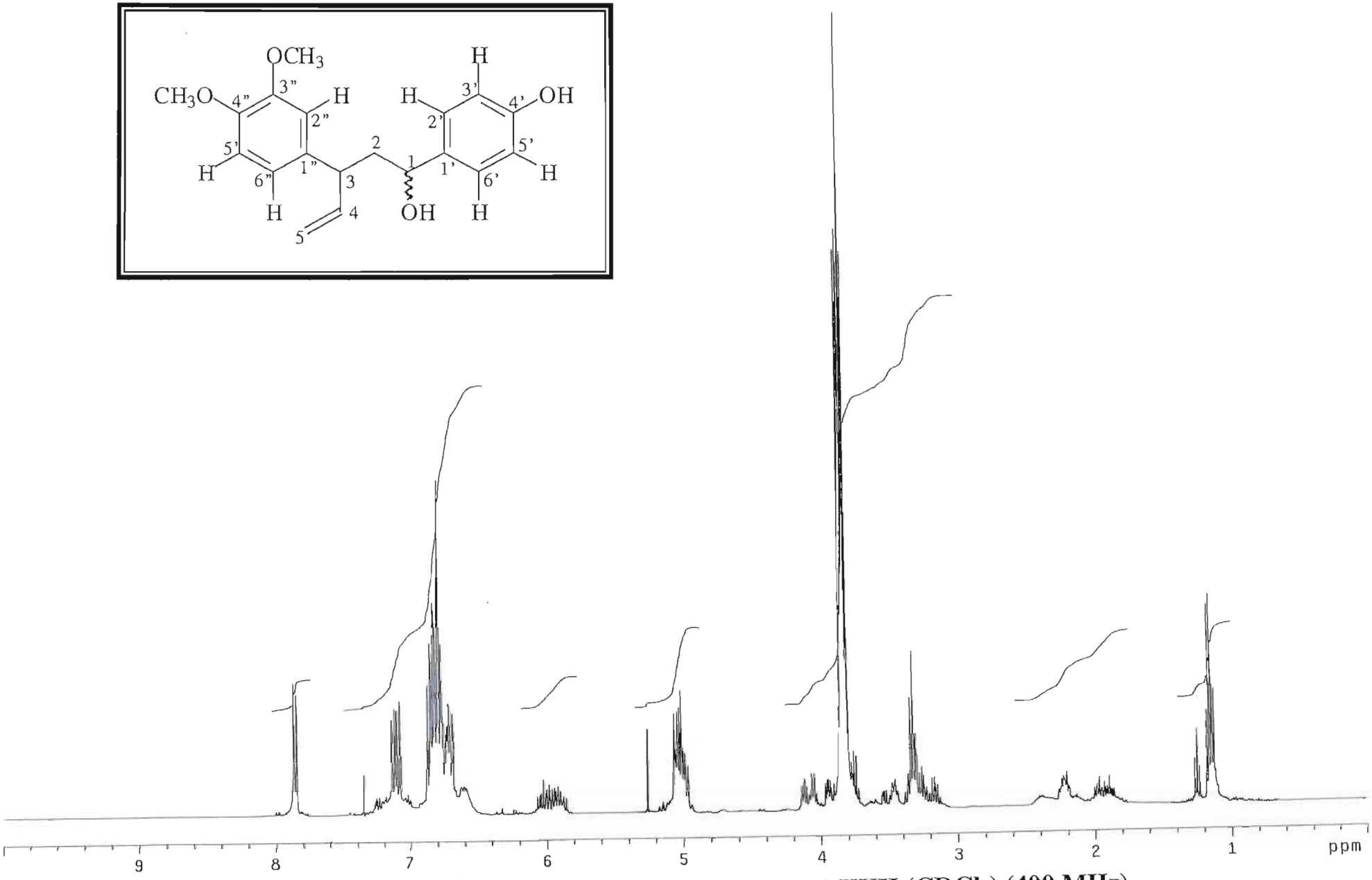

**Spectrum 21g: Mass spectrum of compound XXI**

hvalc.v-alcohol in cdcl3
probe=5mmASW

Pulse Sequence: s2pul





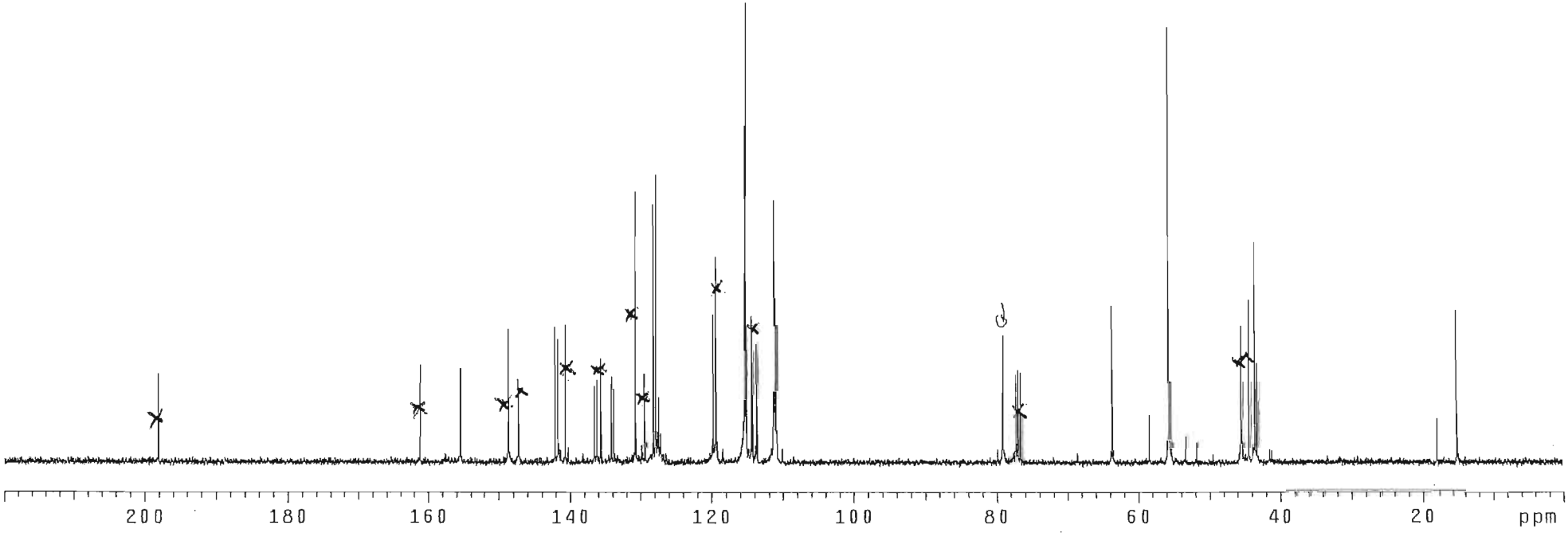




**Spectrum 22a: $^1$H NMR spectrum of compound XXII ($CDCl_3$) (400 MHz)**

probe=5mmASW

Pulse Sequence: s2pul

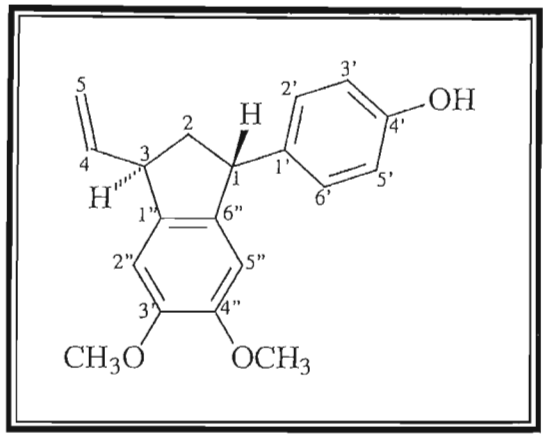
**x = vinyl ketone impurity**

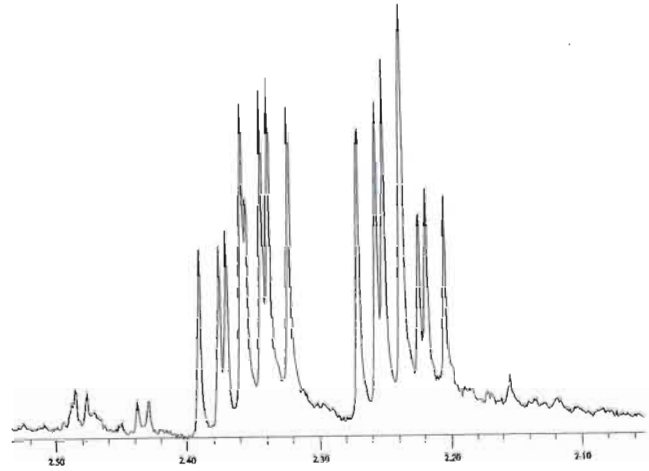


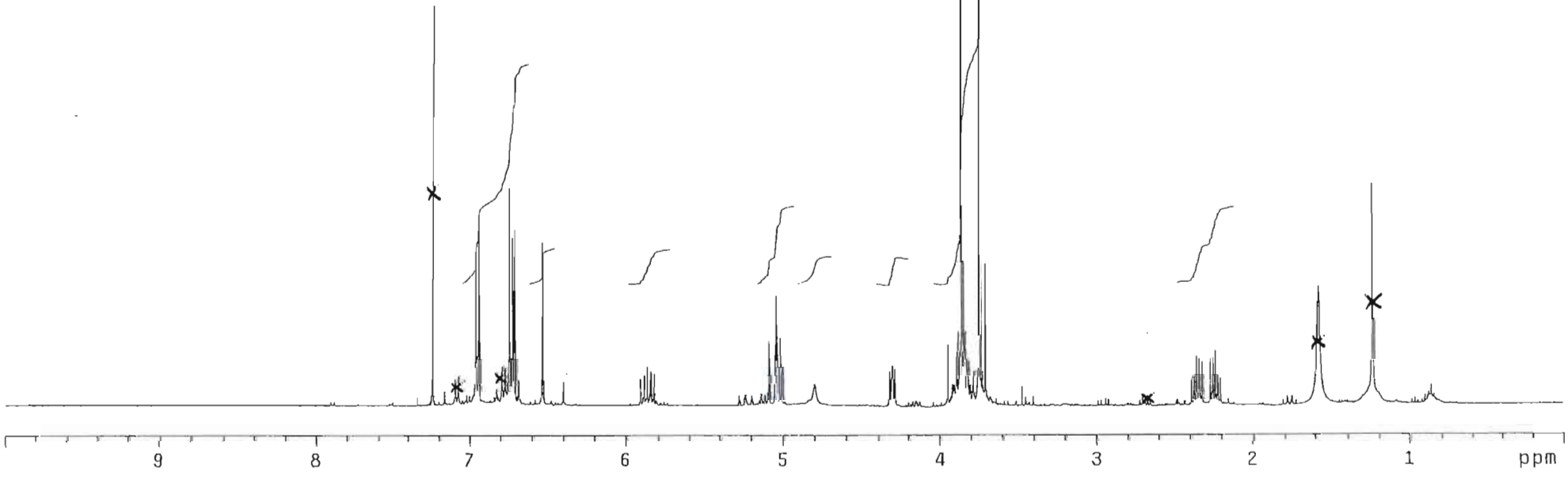

**Spectrum 22b: $^{13}C$ NMR spectrum of compound XXII ($CDCl_3$) (100 MHz)**

probe=5mmASW

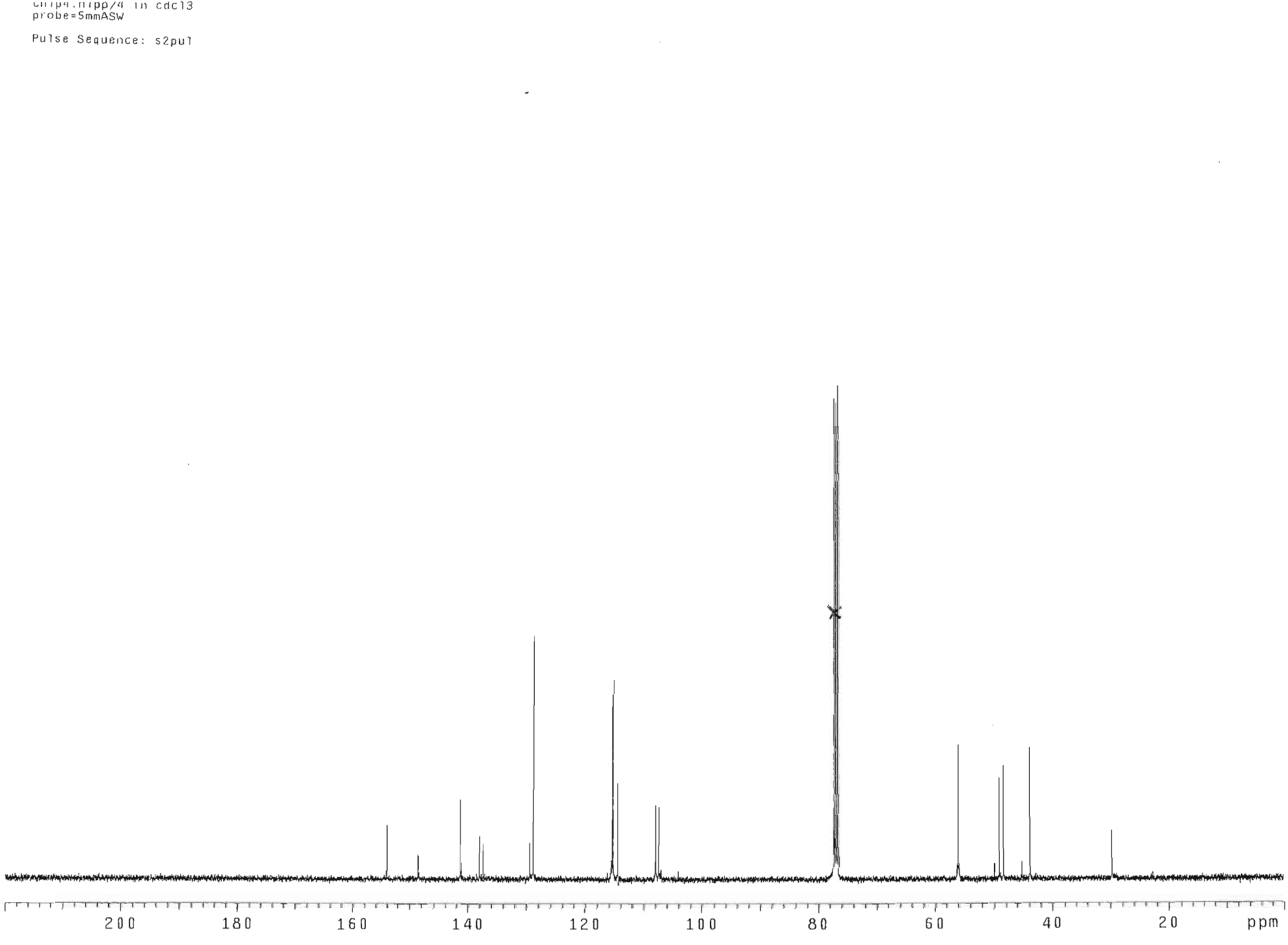
Pulse Sequence: s2pul



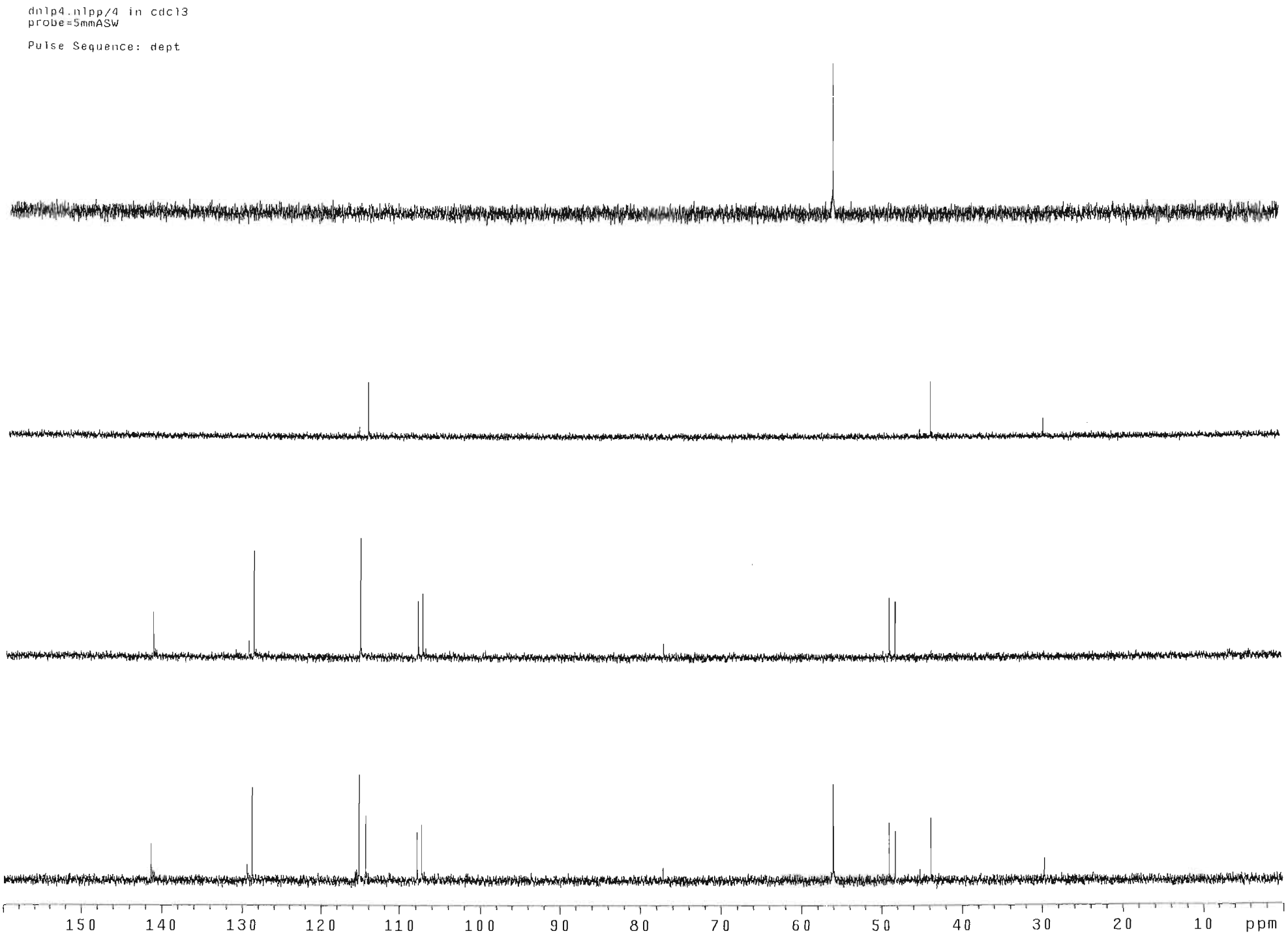





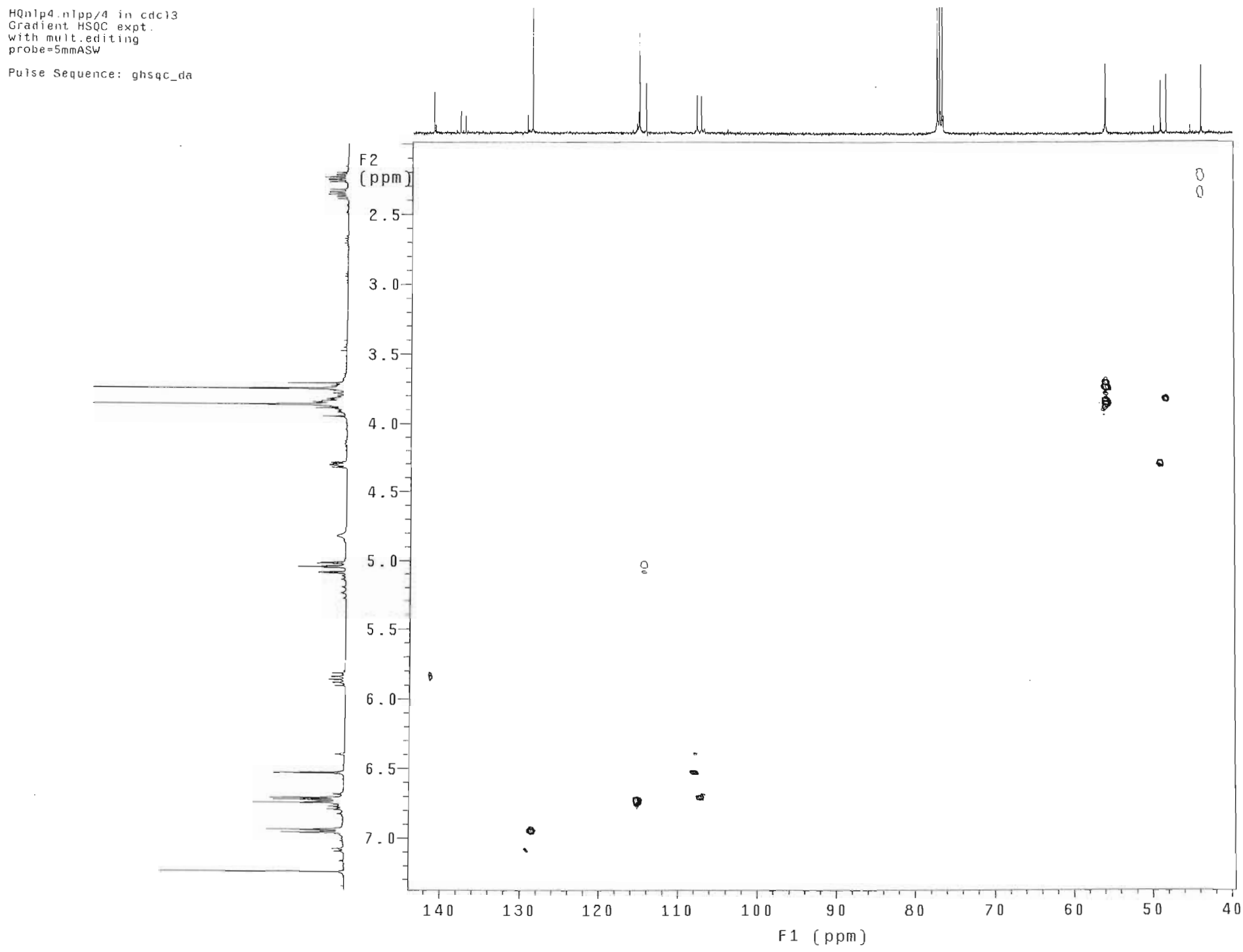

**Spectrum 23a: $^{1}H$ NMR spectrum of compound XXIII ($CDCl_3$) (400 MHz)**



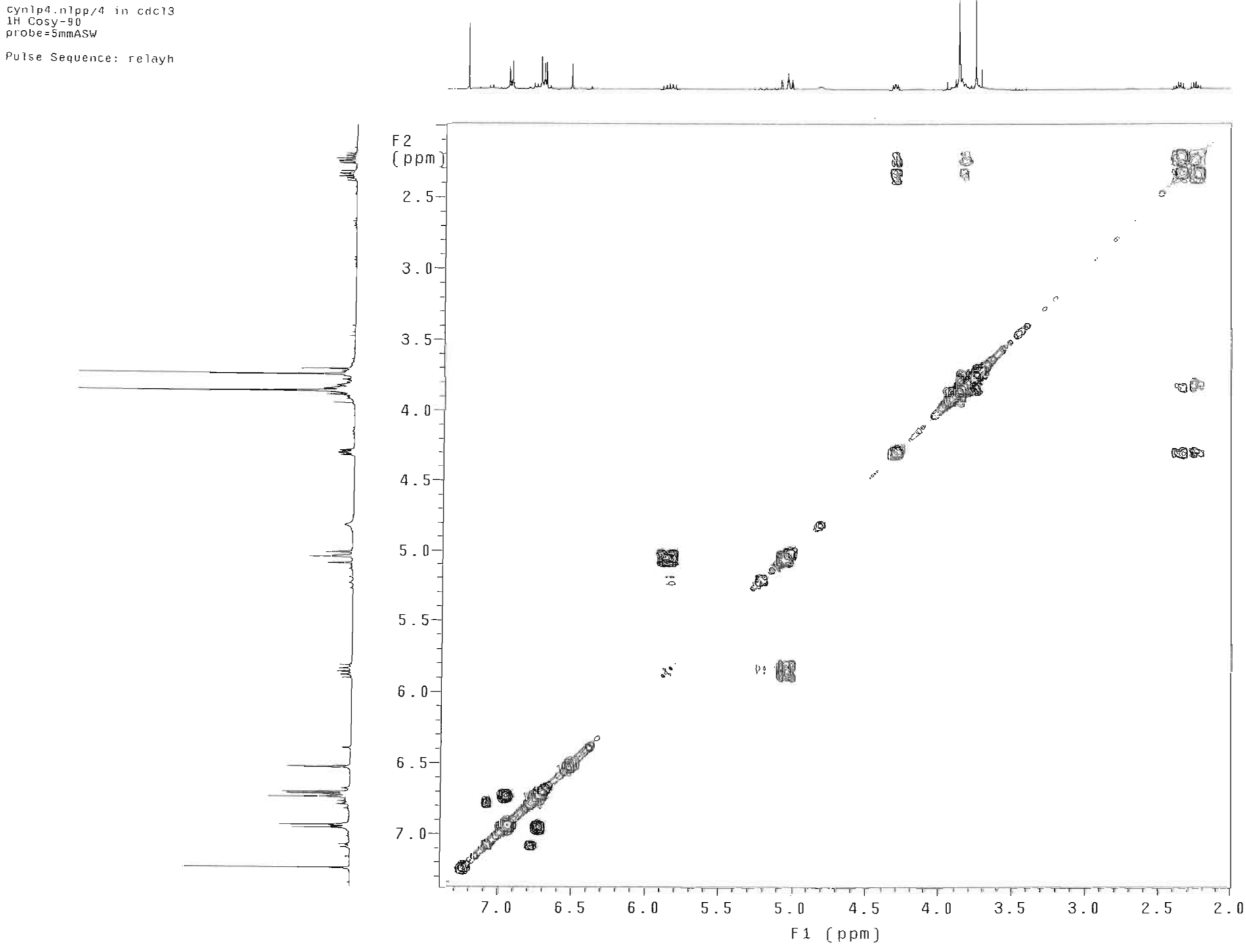

**Spectrum 23b: $^{13}C$ NMR spectrum of compound XXIII ($CDCl_3$) (100 MHz)**



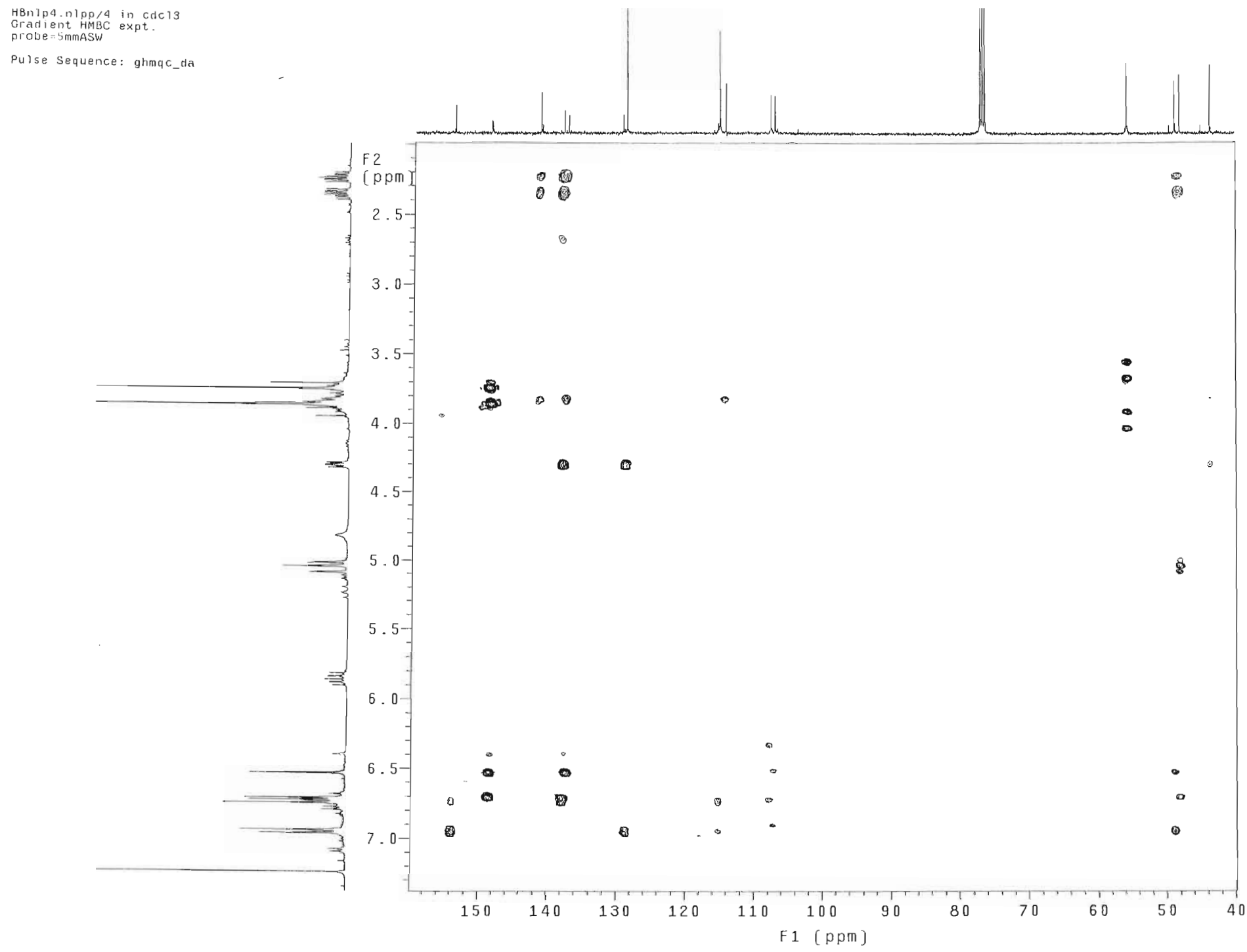

**Spectrum 23c: ADEPT spectrum of compound XXIII**



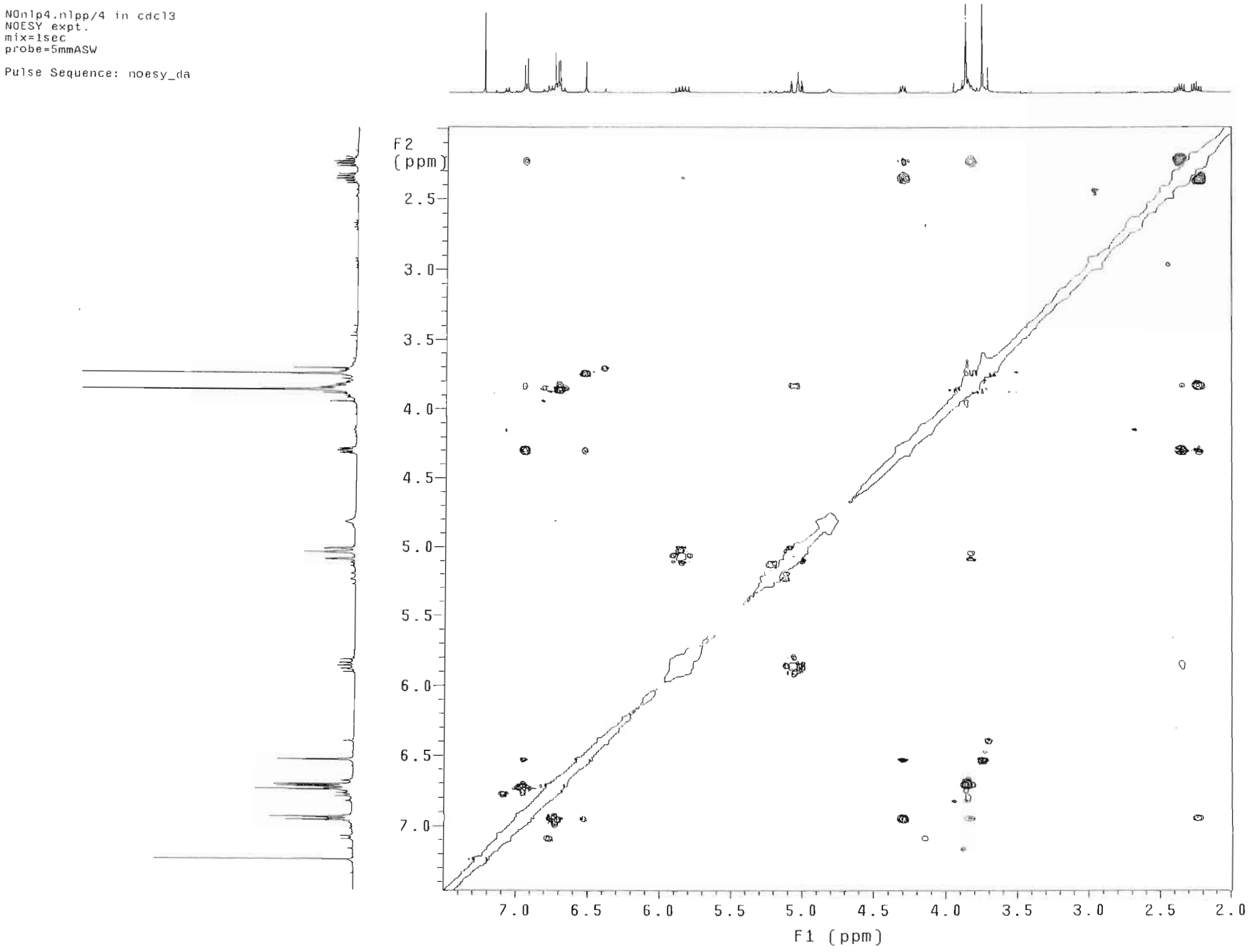

**Spectrum 23d: HSQC spectrum of compound XXIII**



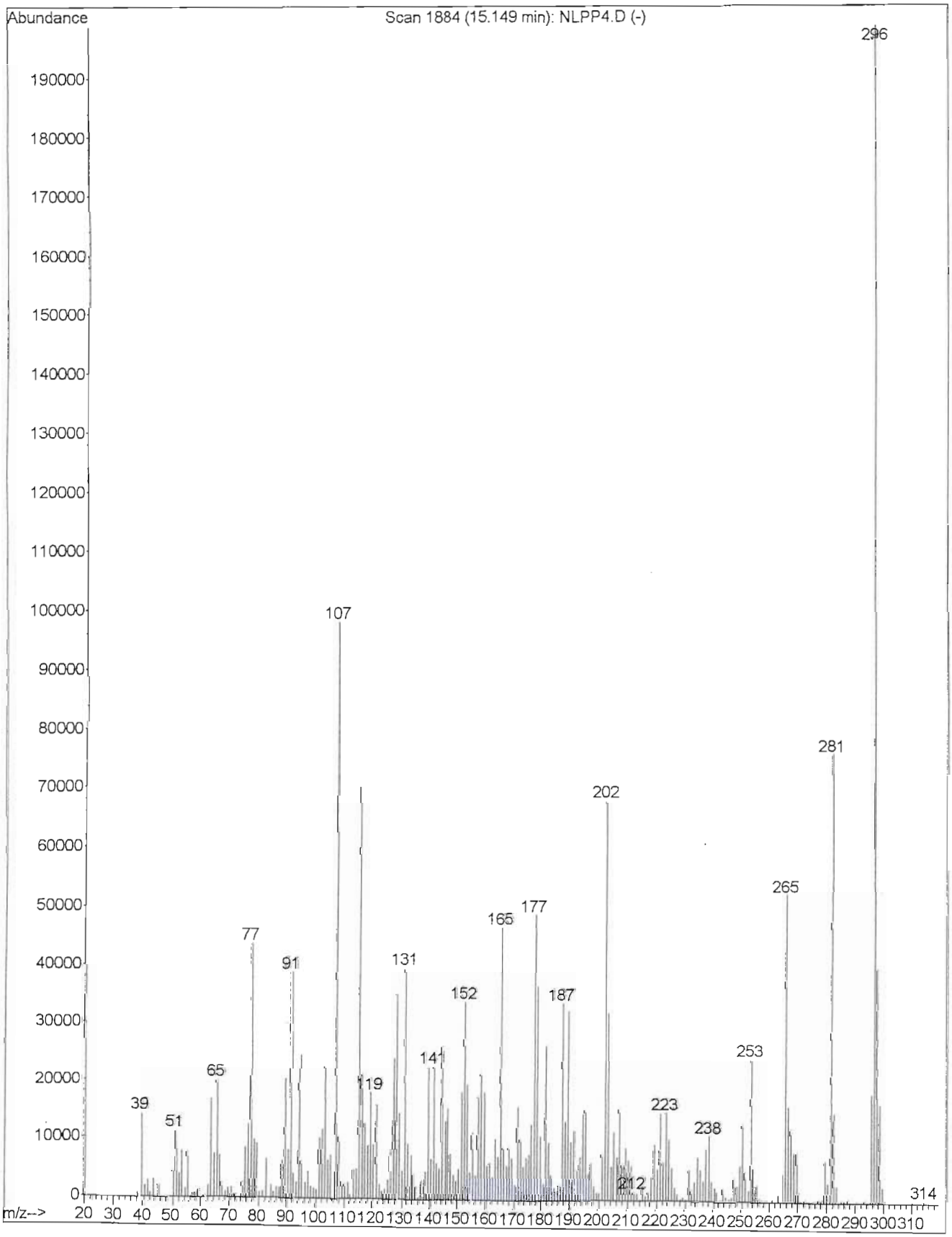

**Spectrum 23e: COSY spectrum of compound XXIII**




**Spectrum 23f: HMBC spectrum of compound XXIII**




**Spectrum 23g: NOESY spectrum of compound XXIII**




**Spectrum 23h: Mass spectrum of compound XXIII**