ISOLATION AND IDENTIFICATION OF NOVEL COMPOUNDS FROM INDIGENOUS PLANTS

By

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DECLARATION

I hereby certify that this research is the result of my own investigation, which has not already been accepted in substance for any degree and is not being submitted in candidature for any other degree.

Signed S E Drewes for B M Sehlapelo.

B. M. SEHLAPELO

I certify that this statement is correct.

Signed S E Drewes.

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To my grandmother Nthipi, my mother Onica and
uncle Antipas and all the
Sehlapelo family.

ACKNOWLEDGEMENTS

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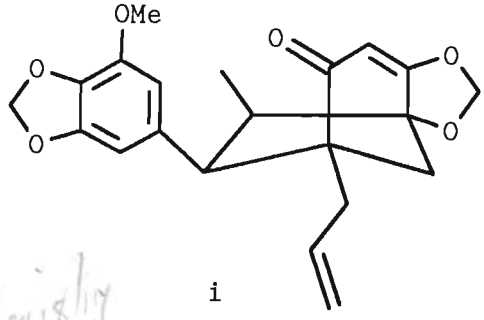
ABBREVIATIONS

Ar	-	aryl
CoA	-	coenzyme A
CI-MS	-	chemical ionization mass spectrometry
E.A.	-	ethyl acetate
IR	-	infra red
HMBC	-	heteronuclear multiple-bond quantum coherence
$LiAlH_4$	-	lithium aluminium hydride
mCPBA	-	metachloroperbenzoic acid
P.E.	-	petroleum ether
pTSOH	-	paratoluenesulfonic acid

SUMMARY

The chemical constituents of four Lauraceae species indigenous to South Africa, namely, *Ocotea bullata*, *Cryptocarya woodii*, *C. latifolia* and *C. myrtifolia*, were investigated. The species yielded eight novel compounds and one known constituent among them.

The three types of compounds isolated include, two neolignans (ocobullenone (**i**) and bullatone (**ii**)), five 6-substituted 5,6-dihydro-α-pyrones, cryptofolione (**iv**) and myrtifolione (**v**) including the known compound goniothalamin (**iii**), and cryptocaryolide A (**vi**) and B (**vii**), and two macrolides foliolide A (**viii**) and B (**ix**).



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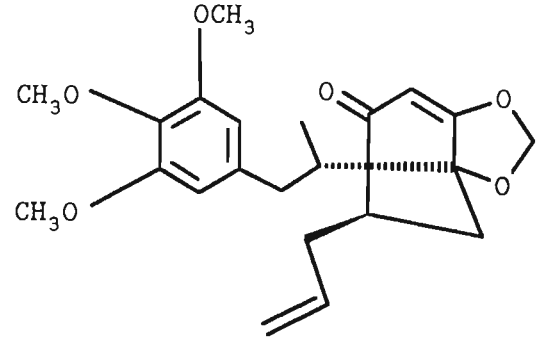

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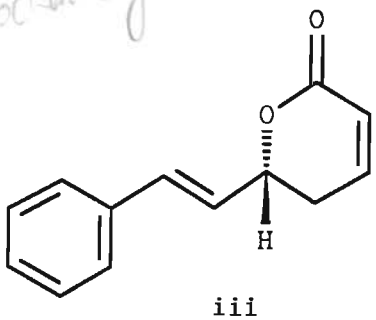

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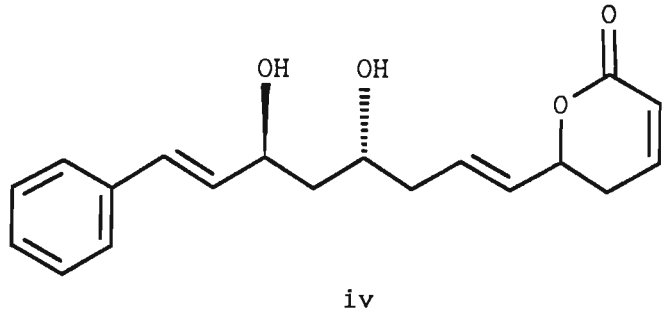

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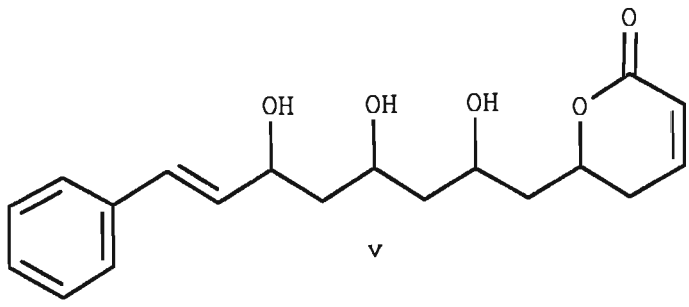

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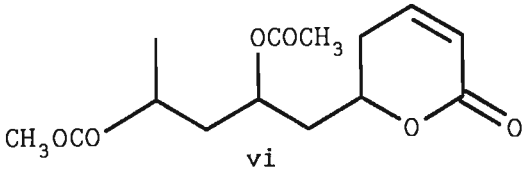

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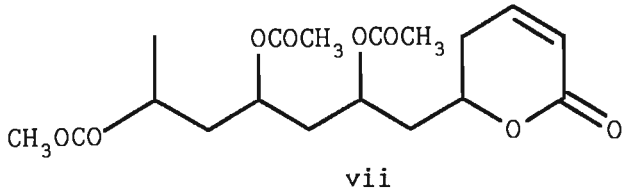

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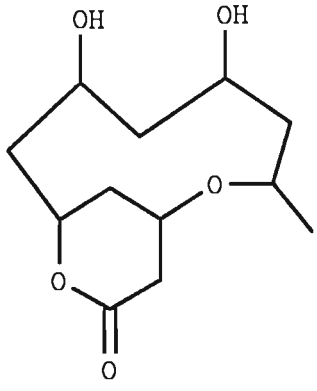

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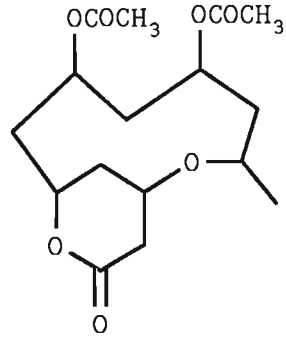

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Flash chromatography technique and the chromatotron were central in the isolation of the compounds. Structural elucidation included the use of techniques such as ^{1}H and ^{13}C NMR spectroscopy, HETCOR, DELAYED or RELAY HETCOR, COSY and DELAYED or RELAY COSY, NOE, ROESY, HMBC, IR and mass spectroscopy. These techniques were instrumental in the satisfactory or complete assignment of the proton and carbon atoms of the compounds isolated. Chemical modifications were useful in determining the relative stereochemistry of some of the compounds.

CONTENTS

The following paper has been published.

Sehlapelo, B.M., Drewes, S.E. and Sandors, P. (1993) *Phytochemistry* **32**, 1352.

CHAPTER ONE

1. INTRODUCTION

This investigation concerns itself with the study of the chemical constituents of four plants belonging to the family lauraceae; one species of *Ocotea* (*Ocotea bullata*) and three species of the genus *Cryptocarya* (*C. woodii*, *C. latifolia* and *C. myrtifolia*).

1.1. DESCRIPTION, DISTRIBUTION AND ETHNOMEDICINAL USES OF THE PLANT SPECIES INVESTIGATED.

1.1.1 *Ocotea bullata* (Burch.) Baill.

O. bullata is a medium to large evergreen tree that grows as high as 8-30m.[1] The bark is brown and scaly when old and pale and attractively coloured in young plants. The leaves are large, dark green, glossy and oblong and of the dimension 5- 10 x 2.5-5 cm. Conspicuous on the leaves are blisters or 'bubbles' on the upper surface in the axils of the veins[1] (**Plate 1**). The blisters or bullae, as they are botanically known, give the plant its specific name[1].

The plant occurs in most of the high forests in South Africa. It is found in the eastern Transvaal and from the north coast of Natal along the coastal areas, down to the western Cape (**Plate 1**).[1]

The plant is known as umNukane in Zulu or Black Stinkwood in

English. Both names are derived from the smell, perceived to be unpleasant, of the freshly cut timber and bark. The timber is commercially used for furniture and panelling and was used in the past by the Afrikaner for wagon construction. The bark is used in folk medicine as a headache remedy either by inhaling the smoke from burning it or by snuffing the powder.[2] The powdered bark is also used as a local application to the bladder in urinary disorders.[3] *Ocotea bullata* has been declared a protected species in South Africa.[1]

1.1.2. *Crptocarya woodii* Engl.

C. woodii is a small to medium sized tree, 5-10m, that occasionally grows up to 15 to 20m in height. The bark is grey and smooth. The leaves are mid-green, silky-textured, ovate and small, 1.5-8 x 1.5-4cm[1] (**Plate 2**). The plant occurs in the woodlands, in forests, in river valleys and on forest margins. The trees are found in the north eastern to south eastern Transvaal and from the southern part of Mozambique along the coast, down to the eastern cape (**Plate 2**).[1] The plant is also known as the Cape laurel in English and umThungwa in Zulu.[2]

1.1.3. *Cryptocarya latifolia* Engl.

C. latifolia which is known as a broad-leaved laurel[1] and umHlangwenya[2] in English and Zulu respectively, is a large tree that can grow up to 20m. The bark is grey-brown to light brown, smooth, and has fine vertical fissures and horizontal ridges occurring occasionally. The leaves are broadly oval and are 5-10 x 2-4cm in size. They are leathery, dark green and finely velvety above, greenish or cinnamon-brown below, and later becoming dull bluish-green. The apex is broadly tapering, rounded or notched and the petiole is short (**Plate 3**).[1]

The tree occurs in evergreen forests from the north to the south coast of Natal (**Plate 3**).[1] The plant is used in Zulu folk medicine, by mixing the ground bark with crocodile fat, as an ointment to treat chest complaints.[1]

1.1.4. *Cryptocarya myrtifolia* Stapf.

C. myrtifolia which is known as umGqabe in Zulu,[2] is a medium to large tree that grows from 10 to 20m. The bark is brown and smooth and the young branchlets are velvety. The leaves are small, 5 x 1.5-2.5cm, green above and bluish below, lanceolate to broadly obovate. The apex is broadly tapering and sometimes abruptly and shortly attenuate, while the base is tapering (**Plate 4**).[1]

The distribution of this *Cryptocarya* species is from the far north coast to the eastern Cape (**Plate 4**). *Cryptocarya myrtifolia* borrows its English name, Camphor laurel, from the fact that the bark, twigs and leaves have a distinct smell of camphor.[1]

Ocotea bullata (Burch.) Baillon
S.A. no: 118 Stinkwood. Stinkhout
Rhod. no: —

PLATE 1

Cryptocarya woodii Engl.
[*C. acuminata* Schinz ex T. R. Sim]
S.A. no: 116 Cape laurel. Kaapse kweper
Rhod. no: —

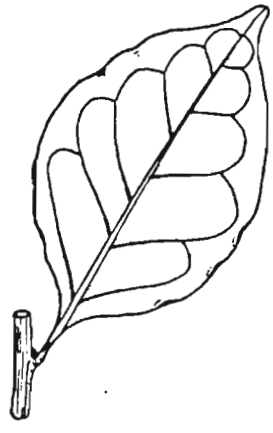


PLATE 2

Cryptocarya latifolia Sonder
S.A. no: 113 Broad-leaved laurel. Breëblaarkweper
Rhod. no: —

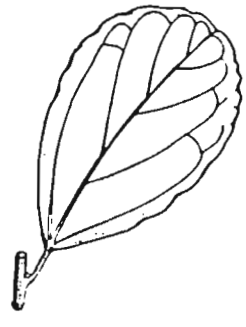
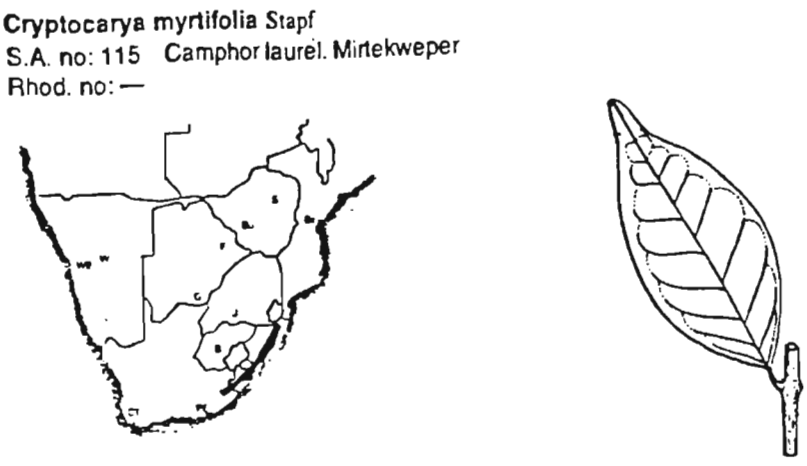


PLATE 3

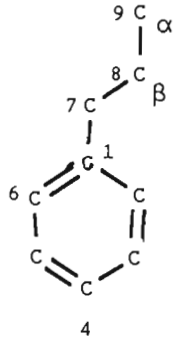


Plates 1-4: The distribution of the four species of plants studied.[1]

1.2. **NEOLIGNANS**

1.2.1. Introduction

Neolignans are a group of secondary plant metabolites that are structurally characterized by the presence of two aryl propanoid ($C_6.C_3$) units.[4] Harworth[5] introduced the term 'lignan' to express the woody tissue from which many examples derive, and implied structures that consist of two units **1a** and **1b** linked 8-8' (β-β'). The name 'neolignan' was conceived



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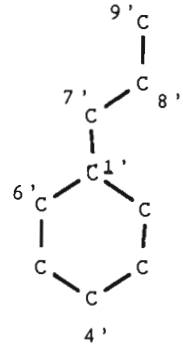
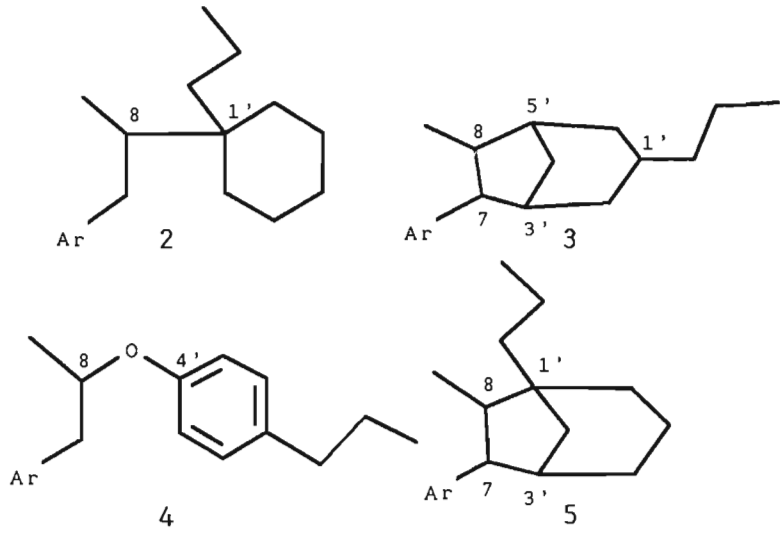


1b

by Gottlieb[6], initially for compounds containing two aryl propanoid fragments that are linked otherwise than β-β'. Neolignans were recently redefined[7] as the products of oxidative coupling of allyl- or propyl phenols.

1.2.2. Nomenclature and numbering

Neolignans show very varied structures, and more than fifteen subgroups have been identified, including the frameworks[7] **2-5** (**Scheme 1**).



Scheme 1

Trivial names proliferate and are still being introduced, even where systematic nomenclature is uncomplicated. In spite of an assortment of skeleta and functions, application of systematic nomenclature to neolignans is still discernible. The $Ar.C_3$ group is written towards the left. Its C-atoms are numbered 1-9, while the carbon atoms of the additional $C_6.C_3$ unit are numbered[8] 1'-9', as in **Scheme 1**. Direct or oxygen links between the units are indicated by identification of the bridgehead positions through the smallest possible numerals, example 7.3' (and not 7.5'), 8.1' in compound **5 Scheme 1**.

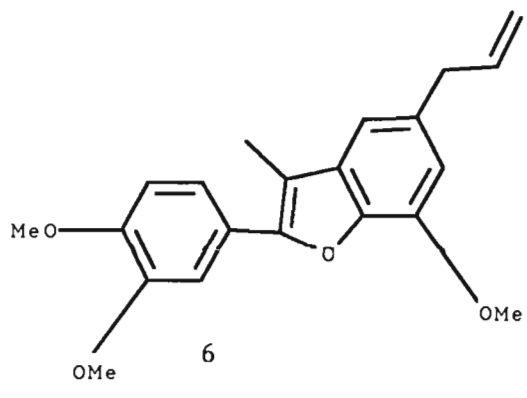
1.2.3. Types of neolignans

Neolignans are classified into subgroups according to the point of linkage of the two $C_6.C_3$ units, example 8.1' (**2**), 8.O.4' (**4**) and 7.3',8.1' (**5**) neolignans. Two types of neolignans are of direct interest in this work; the (8.3')-neolignans and

neolignans with two doubly linked $C_6.C_3$ units.

1.2.3.1. (8.3')-neolignans

These have an oxygen bridge between the two $C_6.C_3$ units, forming benzofurans (e.g. carinatin (**6**)[9] and carinatidin (**7**)[10]) and dihydrobenzofurans (e.g. kadsurenone (**8**)[11] and 2S,3S-dihydrocarinatidin (**9**).[10] Compounds without such a bridge include (-) carinalol (**10**)[9] and lancifolin F (**11**).[12] These compounds vary according to substituents on the C_6 and C_6' rings.



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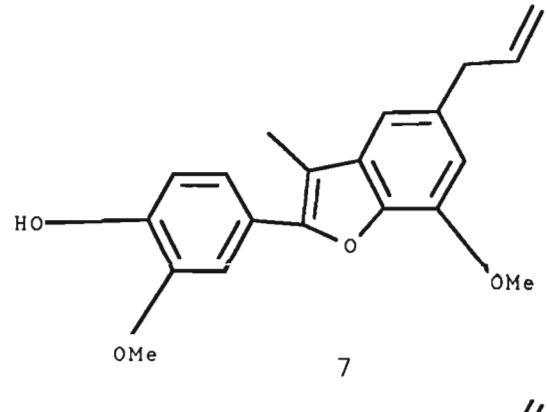

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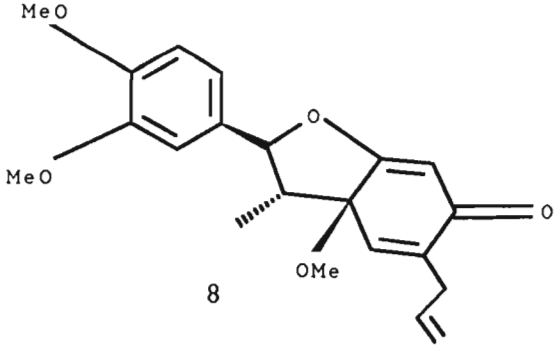

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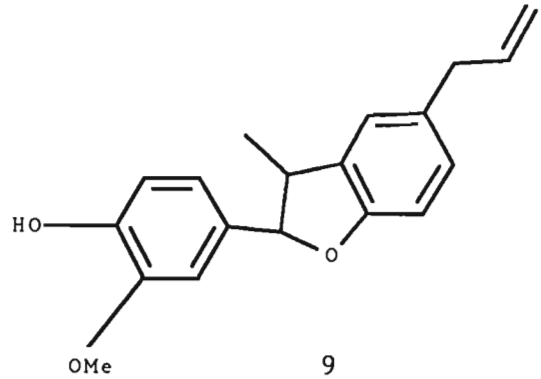

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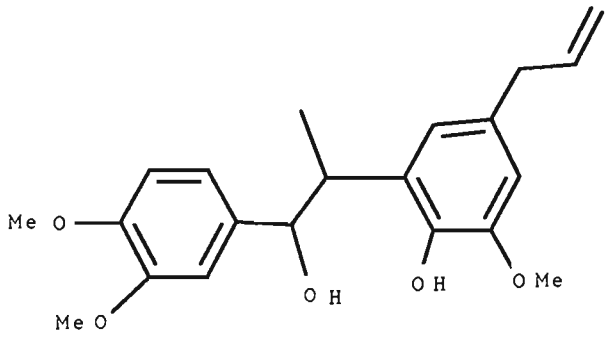

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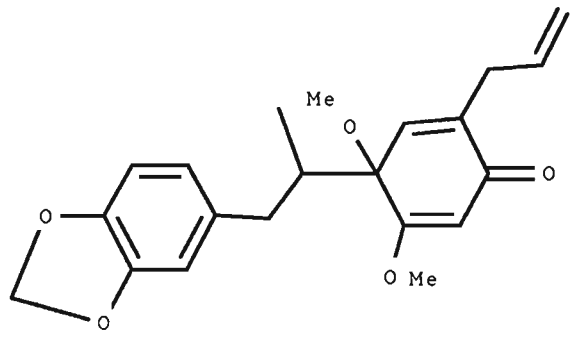

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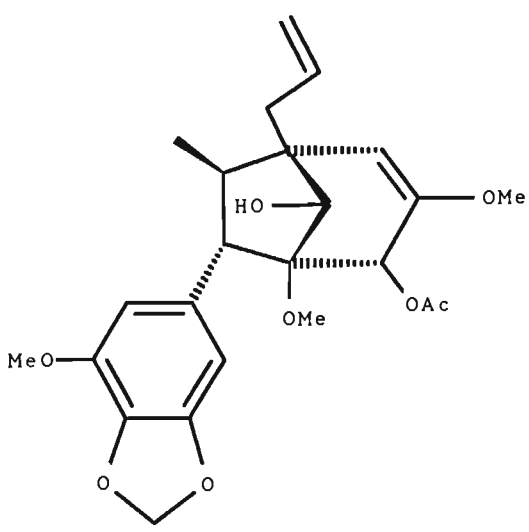
1.2.3.2. Bicyclo [3.2.1] Octanoid neolignans

Bicyclo [3.2.1] octanoid neolignans are a group of neolignans whose $C_6.C_3$ and $C_6'.C_3'$ units are double linked such that the units form a bicyclo [3.2.1] octane system. A survey of Brazilian species of plants by Gottlieb and his group has uncovered an abundance of new examples with the general skeleton 8.1',7.3' and variations of stereochemistry and oxygenation pattern. These include rel-(7S,8R,1'R,2'R,3'S,4S) $\Delta^{8'}$-4'-acetoxy-2'-hydroxy-3,3',5'-trimethoxy-4,5-methylenedioxy-1', 2', 3', 4'-tetrahydro-7.3',8.1'-neolignan (**12**) from a species of *Ocotea*[13], rel-(7S, 8R, 1'R, 2'S, 3'R, 4'R,5'S)-$\Delta^{8'}$-2',4'-dihydroxy-3',5'-dimethoxy-4,5-methylenedioxy -1', 2', 3', 4', 5', 6'-hexahydro-7.3',8.1'- neolignan (**13**), from an Amazonian species of the genus *Aniba*,[14] and canellin-C (**14**) from an *Ocotea catharinensis*.[15]



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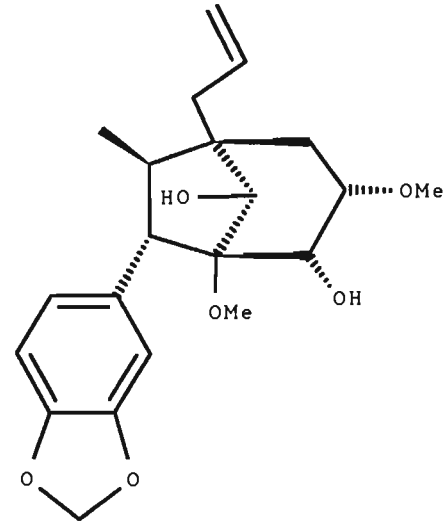

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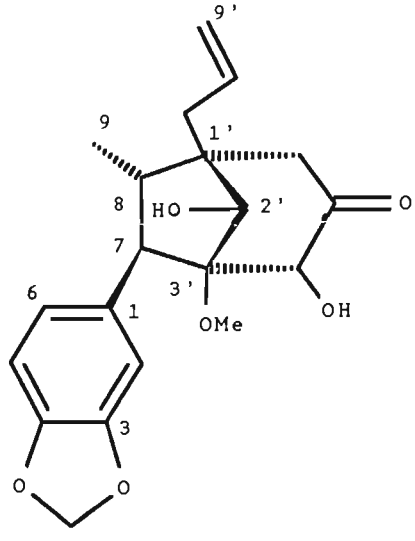

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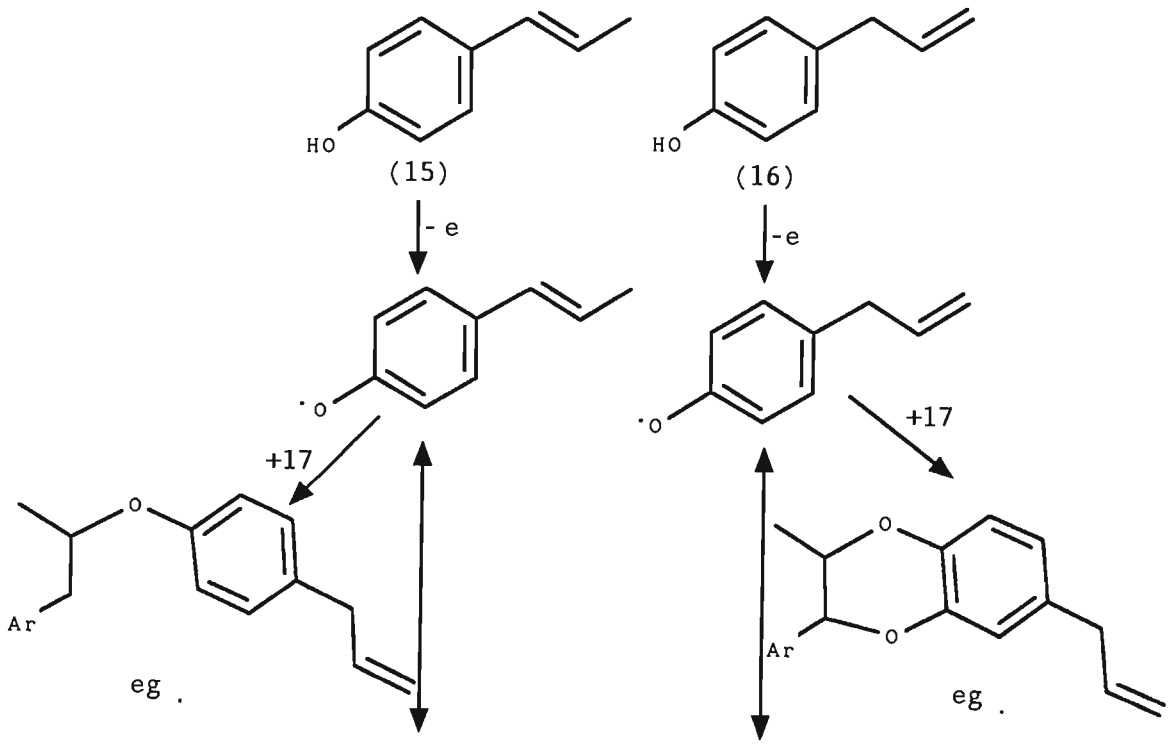
In the variations of bicyclo [3.2.1] octanoid neolignans that have so far been discovered, two features are common. The first is that the neolignan either bears a hydroxy, methoxy, acetoxy or a carbonyl group on the bridgehead carbon (example C-2' in **12, 13** and **14**. The scarcity or absence of a methylenedioxy group on the C_6'.C_3' ring is also a noticeable feature of the bicyclo [3.2.1] octanoid neolignans previously isolated. Secondly, if the allyl group is located at the carbons at the intersection of the two rings forming the bicyclo system, its attachment is such that it results in a

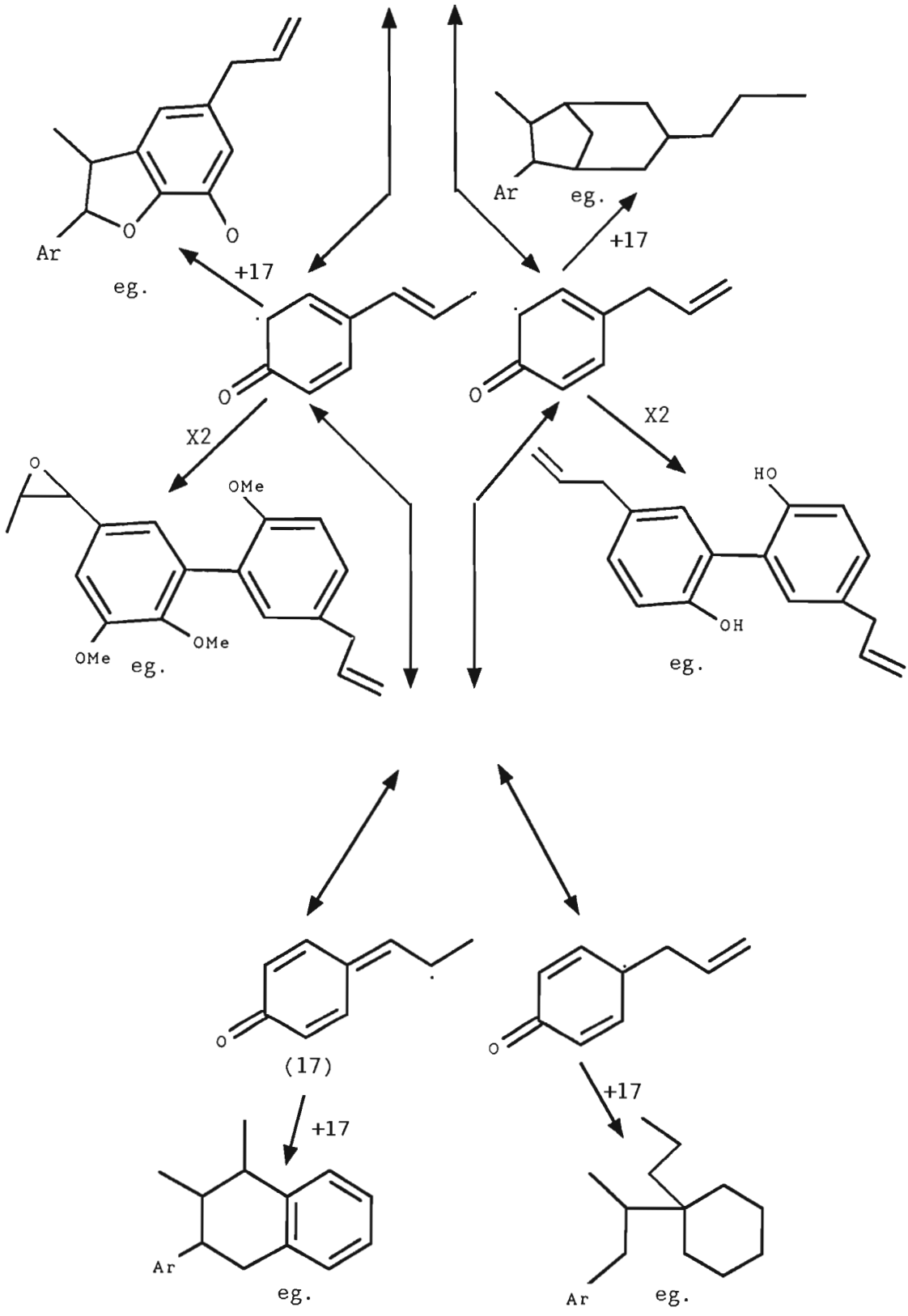
8.1' instead of 8.3' linkage (i.e. bicyclo [3.2.1] octanoid systems with the allyl group adjacent to the aryl group have not been isolated until recently).[16]

1.2.4. Biosynthesis of neolignans

Substantial proof, in the form of studies involving carbon and oxygen-labelled precursors, on the biosynthesis of neolignans has yet to be reported. Gottlieb *et al.*,[8] however, propose that the biogenesis of the majority of neolignans can be rationalized by:

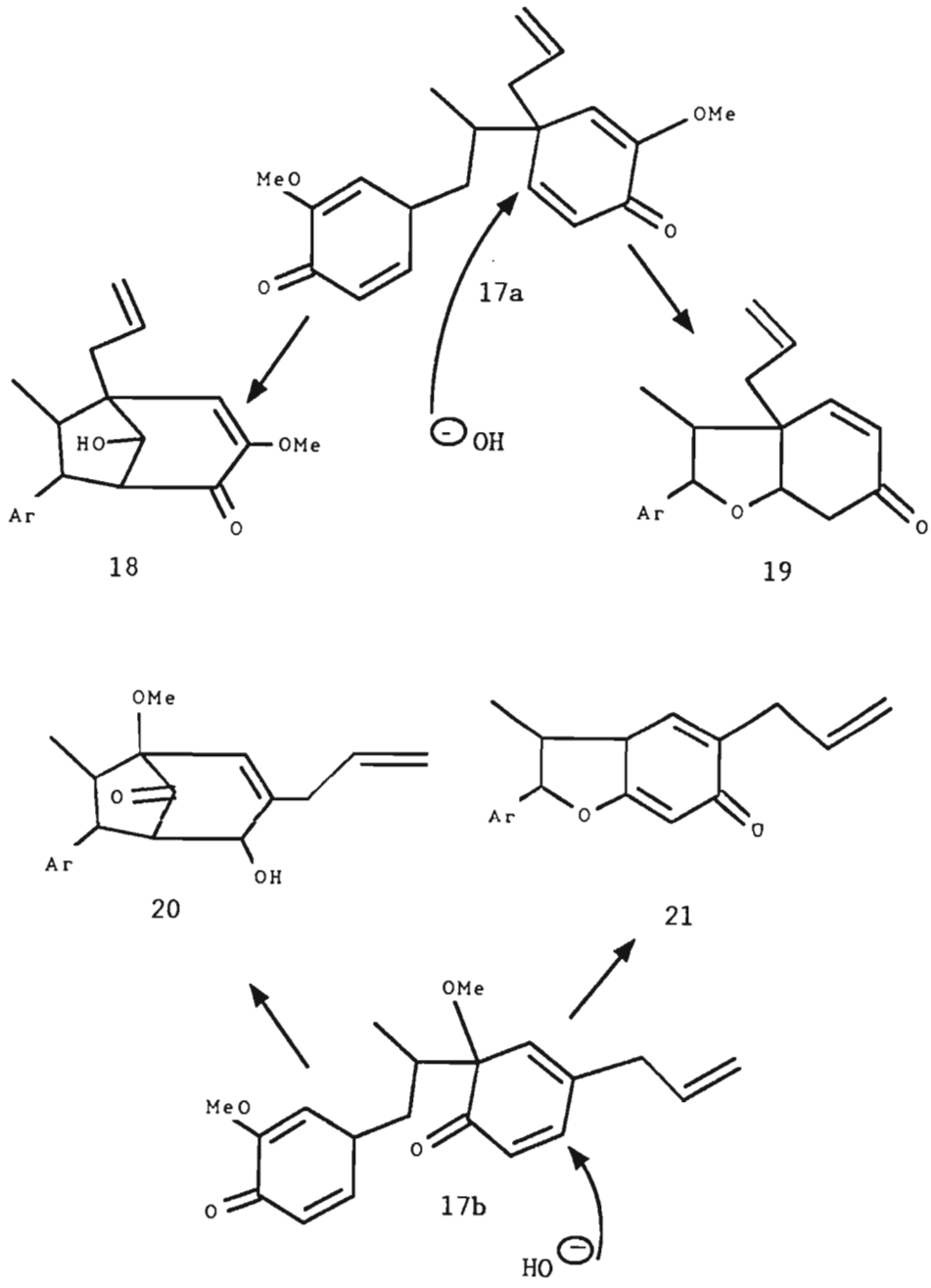
i) the oxidative coupling of a propenylphenol **15** derived starter with either a propenyl or an allylphenol **16** derived termination unit or
ii) coupling of allylphenol derived units (**Scheme 2**).





Scheme 2

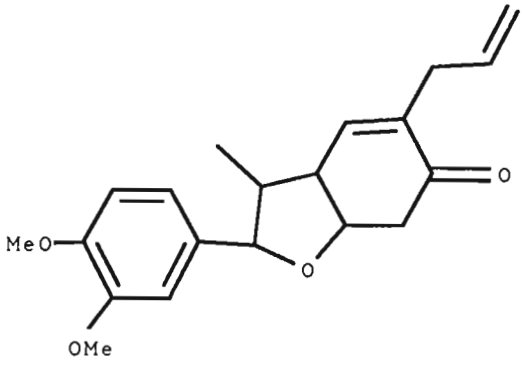
The mechanism of the coupling of two radicals was proposed by Erdtman,[17] who suggested that the coupling step should produce a quinone methide intermediate (example **17a** and **17b**, (**Scheme 3**) which may add water, hydroxyl, hydride or carbanion. This biosynthetic pathway can be postulated to explain the biogenesis of benzofurans and bicyclo [3.2.1] octanoid neolignans as illustrated in **Scheme 3**.



Scheme 3

1.2.5. Biological activity of neolignans

In spite of a phlethora of neolignans that have been isolated from plants, extensive investigation on the pharmacology of neolignans or the effect of neolignans on plants, has not been reported to date. Studies concerning the function of neolignans on plants would seem rewarding in view of the insect antifeeding properties[18] of piperenone (**22**).



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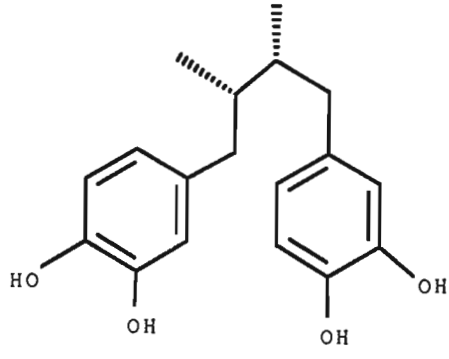

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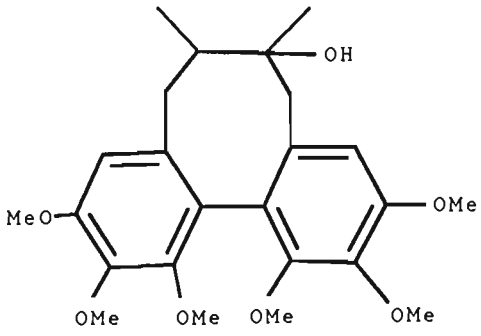
Investigations towards the pharmacologic activity of neolignans should draw inspiration by their structural proximity to those lignans, such as nor-dihydroguaiaretic acid (**23**) which has been implicated in cancer therapy and which acquired such importance.[19] Nor-dihydroguaiaretic acid (**23**) is also used industrially as an antioxidant for food material.[8] Schizandrin (**24**) was found to be one of the active ingredients of *Schizandra chinensis*, a plant used as stimulant in the Orient.[8]



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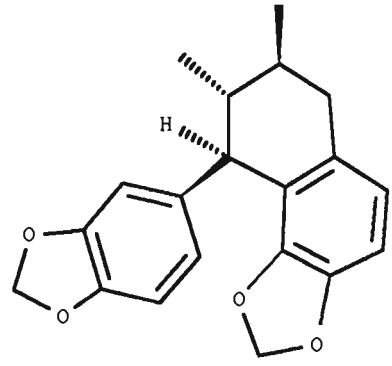

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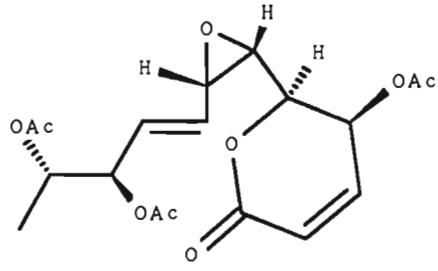
Otobain (**25**) and co-occurring neolignans, were found to be antifungal components of otoba butter, used in Columbia in veterinary practise.[8]

1.3. 6-SUBSTITUTED 5,6-DIHYDRO-α-PYRONES

1.3.1. Introduction

Pyrones constitute an important class of compounds as the pyran-2-one moiety is found in a large number of natural products. Pyrones have been isolated from an extensive range of natural resources, such as plants (example 10-epi-olguine (**26**) from *Hyptis capitata*,[20] a tropical herb), animals (example bufalin (**27**) from *Bufa vulgaris* (toad)),[21] insects (example mossoilactone (**28**) from two species of Australian ants, where it forms part of the ant's defence mechanism),[22] and microbial organisms (example, the polyene-pyrone citreomontanin (**29**), which was isolated from *Penicillium pedemontanum*).[23] However, in order to keep this discussion to an acceptable length, only 6-substituted 5,6-dihydro-α-pyrones will be further deliberated upon here.



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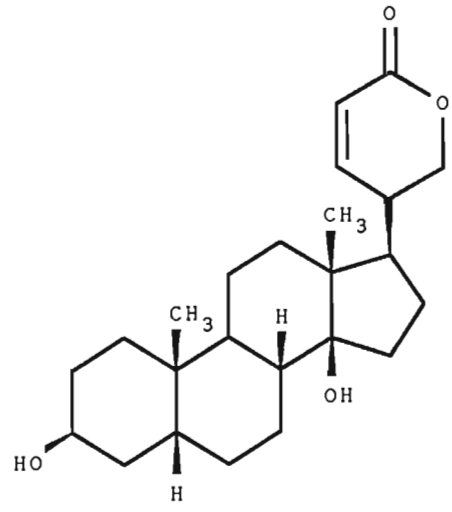

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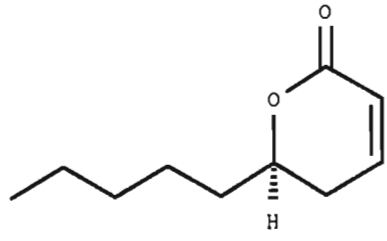

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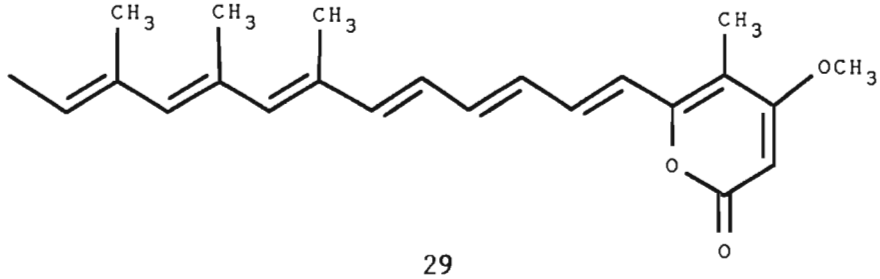

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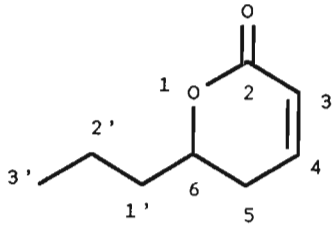
6-Substituted 5,6-dihydro-α-pyrones are widely distributed in fungi and higher plants. They have, however, been frequently found in plants of the families, Lamiaceae, Piperaceae, Annonaceae and Lauraceae. They have been isolated in all parts of plants including fruits, flowers, leaves and bark.[24]

It is interesting to note that regardless of the wide distribution of 5,6-dihydro-α-pyrones in both plants and fungi,

they rarely occur together with α-pyrones[24] and have been found coexisting only in three species.[25,26,27]

1.3.2. Nomenclature and numbering of 6-substituted 5,6-dihydro-α-pyrones

Uniformity within the nomenclature of 6-substituted 5,6-dihydro-α-pyrones is unsatisfactory. Although trivial names are widely used, 5,6-dihydro-α-pyrones can be named and numbered in two ways according to I.U.P.A.C. recommendations.[28] The first recommended nomenclature system identifies the lactone with a corresponding heterocyclic nucleus. Therefore, a substituted α,β-unsaturated-δ- lactone is named as a substituted 5,6-dihydro-2H-pyran-2-one (example 6-propyl-5,6-dihydro-2H-pyran-2-one (**30**).[33] This method is accepted by chemical abstracts and is commonly used. It is admissible to omit the indicated hydrogen and the numerical representation of its position (2H), and also to reduce pyran-2-one to 2-pyrone (or α-pyrone) where no ambiguity exists.



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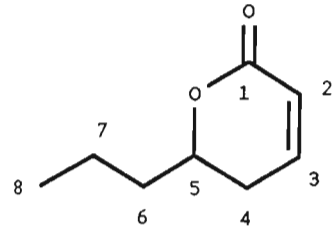

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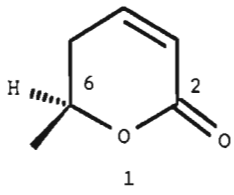
The lactonization of a carboxylic acid hydroxylated at C-5 and replacement of the suffix 'oic' with 'olide' forms the basis of

the second method (example 2-octen-5-olide or 5-propyl -2-penten-5-olide **31**).[28] The numbering sequence illustrated in (**30**), where the lactone ring and side chain positions are differentiated by numbering the latter with prime numbers, will be adopted here. Trivial names will be retained. Where trivial names have not been given, the adopted nomenclature will be employed.

1.3.3. Biosynthesis of 6-substituted 5,6-dihydro-α-pyrones

Biosynthetic studies of only a few 6-substituted 5,6-dihydro -α-pyrones have been reported. These include parasorbic acid[29] (**32**), aspyrone[30] (**33**), psilotin[31] (**34**) and pestalotin[32] (**35**). Most of the studies have been carried out with ^{13}C-labelled acetates using ^{13}C-nmr to determine incorporation.[29,30,32] The biosynthetic pathway of aspyrone (**33**) will be discussed here since it is one of the most extensively investigated 6-substituted 5,6-dihydro-α-pyrone to date.



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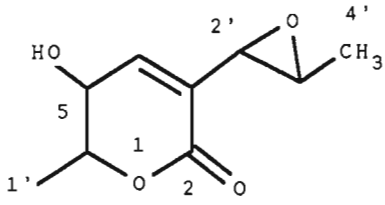

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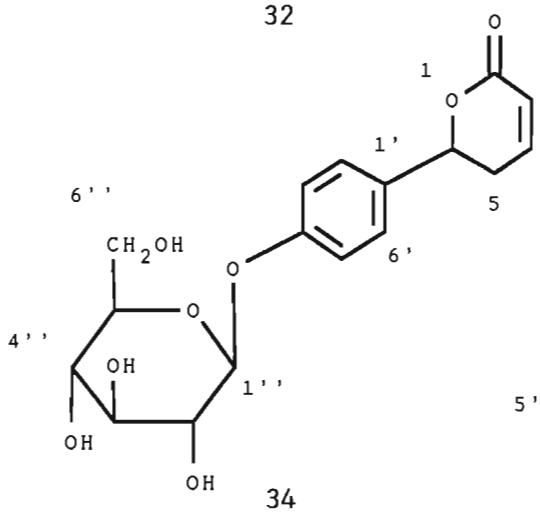

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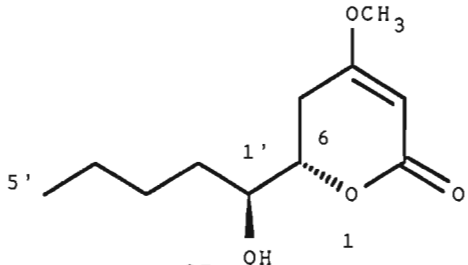

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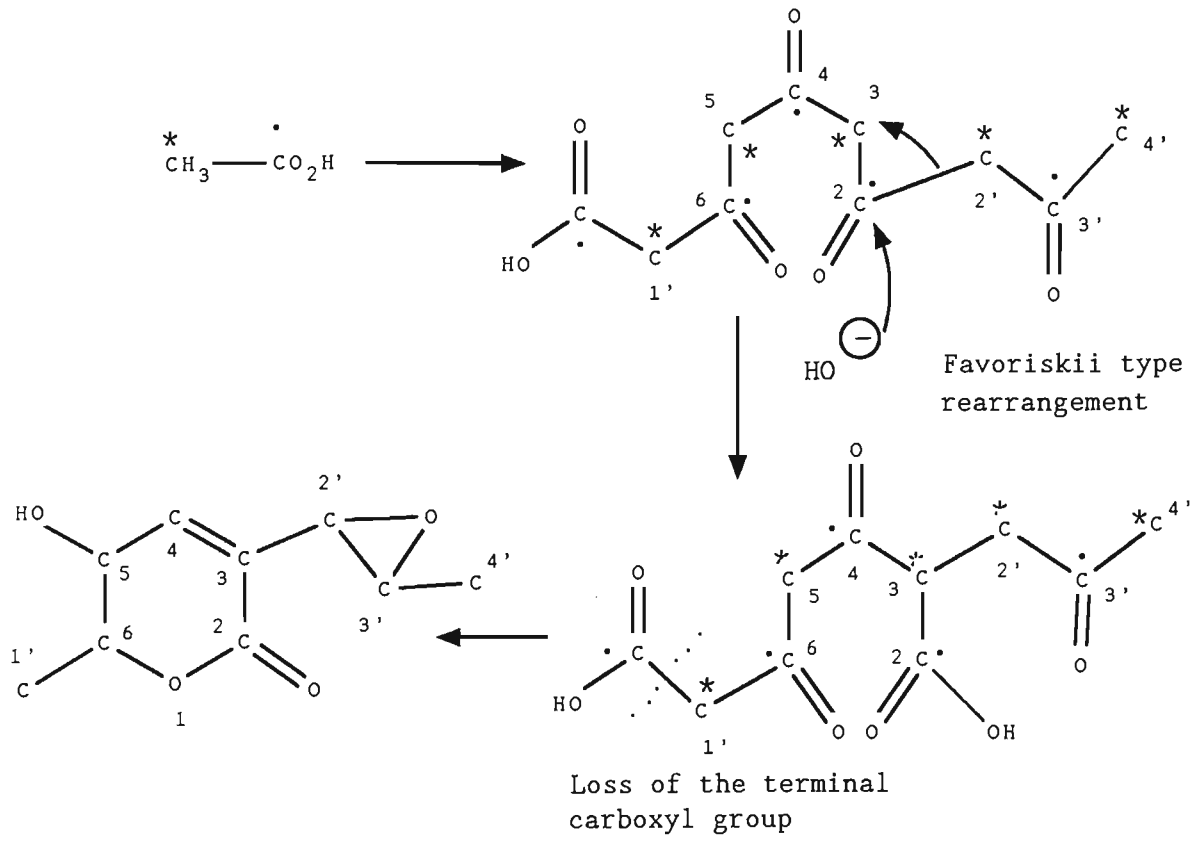
Holker and Simpson[33] and Brereton *et al.*[30] investigated the biosynthesis of aspyrone (**33**) from [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetates (**Scheme 4a**). High enrichment of C-2, C-4, C-6 and C-3' from [1-^{13}C]-acetate and C-3, C-5, C-1', C-2', and C-4' from [2-^{13}C]-acetate was observed. The ^{13}C-nmr spectrum of [1,2-$^{13}C_2$]-acetate enriched aspyrone, showed ^{13}C-^{13}C coupling between the following pairs; C-3 and C-4, C-5 and C-6, C-3' and C-4', thus providing their origin from acetate units which have remained intact throughout the biosynthetic sequence, to give the labelling pattern in **Scheme 4a**.

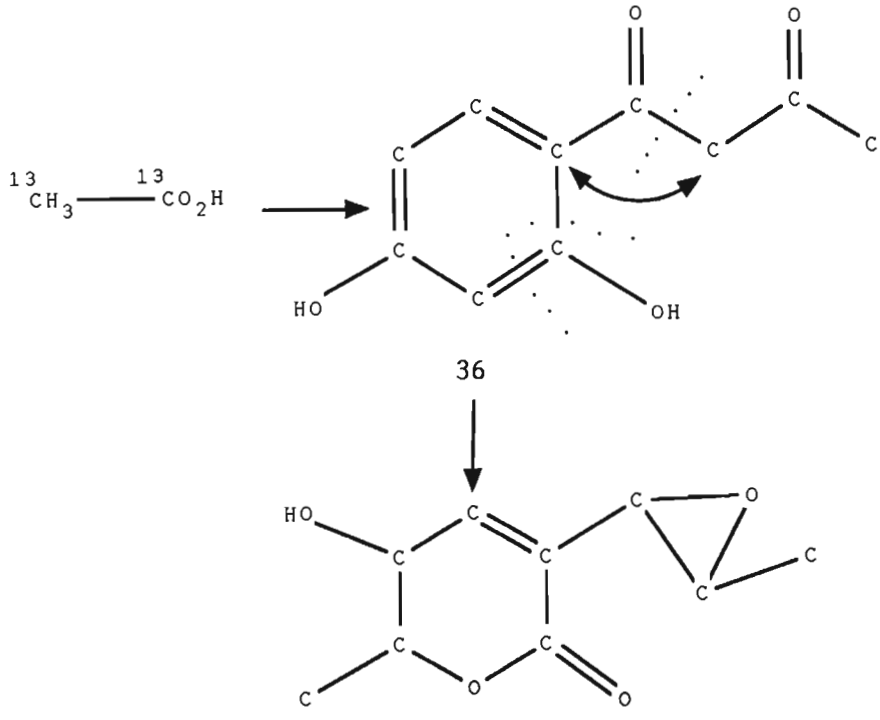


Scheme 4a

Small couplings were observed between C-2 and C-2', indicating that the two carbon atoms must be derived from an originally intact acetate molecule which has undergone an intramolecular rearrangement during the course of the biosynthesis. The above enrichment is consistent with the biosynthetic pathway shown in **Scheme 4a** and indicates that aspyrone (**33**) is formed from a pentaketide precursor via an intramolecular (Favorski type) rearrangement, generating both the observed head-to-head linkage of acetate units, and the 1,3-coupling between C-2 and C-2', followed by the loss of the terminal carboxyl group.

This biosynthetic pathway was confirmed by Copeland[34] during his incorporation studies with ^{14}C- and ^{3}H-labelled acetates when he located labelling sites on the degradation products of aspyrone. The tritium labelling experimental results of

Copeland,[34] however, also accommodate the alternative pathway to the biosynthesis of aspyrone (the intermediacy of **36**), as shown in **Scheme 4b**.



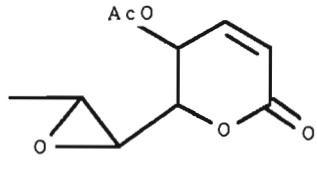
Scheme 4b

1.3.4. Biological activity of 6-substituted 5,6-dihydro-α-pyrones

Naturally occurring 6-subsituted 5,6-dihydro-α-pyrones exhibit a variety of biological activities. A number of them are allelochemicals while others are phytopathogenic, antifungal and bacterial; one has insect antifeeding properties.

Pestalotin (**35**) has a synergistic effect with gibberellic acid

(GA_3) in stimulating the growth of rice-seedlings,[35] while asperline (**37**) displays antibiotic and antifungal activity against a spectrum of microbial organisms.[36] Astepyrone (**38**) was reported to exhibit antiulcerogenic activity in rats.[37]



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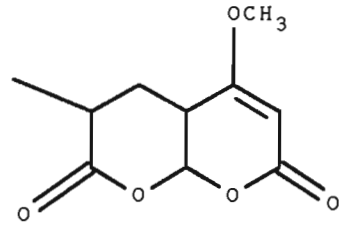

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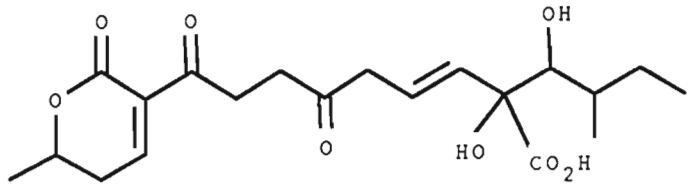
Alternaric acid (**39**) was shown to inhibit spore germination in a number of fungi.[38] Parasorbic acid[39] (**32**) and psilotin[40] (**34**) inhibit seed germination and plant growth, whereas cryptocaryalactone[41] (**40**) forms part of a growth regulatory system. Both phomalactone (**41**) and antibiotic (U-13,1933) (**42**) are active against bacteria.[23] Compounds **39** and **44** have been found to be phytopathogenic.[38,29] Toxin 1 (**43**) is responsible for the brown spot disease on lemons and Rangpur limes while alternaric acid (**39**) causes the collapse of tissues and wilting of plants.[38]



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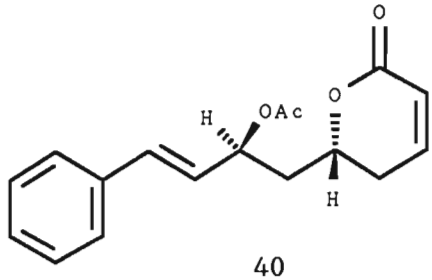

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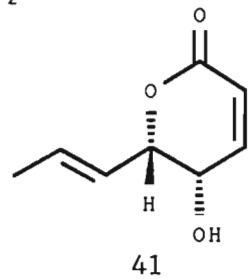

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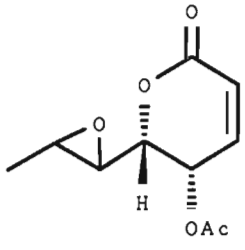

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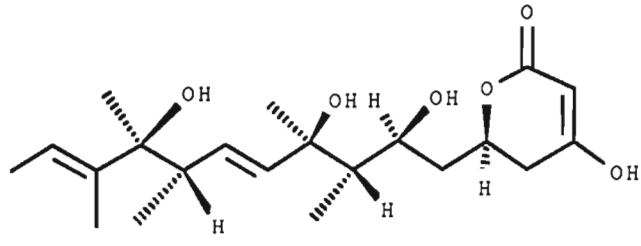
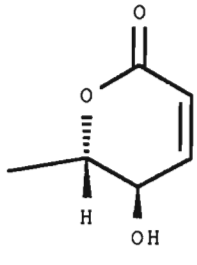


43

Osmundalactone (**44**) inhibits feeding of the larvae of yellow butterfly *Eurema hecabe mandarina* on the fern *Osmunda japonica*; it is said to be the only naturally occurring 6-substituted 5,6-dihydro-α-pyrone known to posses insect antifeeding properties.[24] Goniothalamin (**45**) has been shown to exhibit central nervous system activity.[42]



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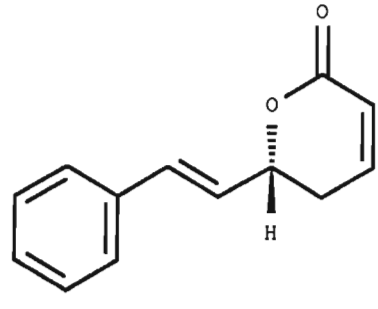

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1.4. MACROLIDES

1.4.1. Introduction

A consistent definition of macrolides is still lacking. The central commonality in the various definitions of macrolides is that they are macrocyclic lactones. Devon and Scott[43] define macrolides as macrocyclic lactones (constructed from linear precursors of acetate or propionate origin) that have a polyene group as one structurally recognizable subset. Manitto[44] describes macrolides as macrocyclic lactones containing a saturated and branched aliphatic chain composed of more than ten carbon atoms. Mazzei *et al.*[45] contend that macrolides are compounds whose chemical structure is characterized by a large lactone ring containing from 12 to 16 atoms to which are attached, via glycoside bonds, one or more sugars.

According to Mazzei *et al.*[45] the lactone ring is substituted by hydroxyl or alkyl groups, one ketone at C-7 in 12-membered macrolides and at C-9 in 14-membered macrolides, and one aldehyde group in 16-membered macrolides. This definition, however, has several shortcomings. For instance macrolidic compounds with more than 16 atoms constituting a ring and without a sugar moiety attached have been isolated and defined as macrolides as shall be shown latter.

1.4.2. Classification of macrolides

Devon and Scott[43] have classified macrolides into three categories:

a) POLYENE MACROLIDES

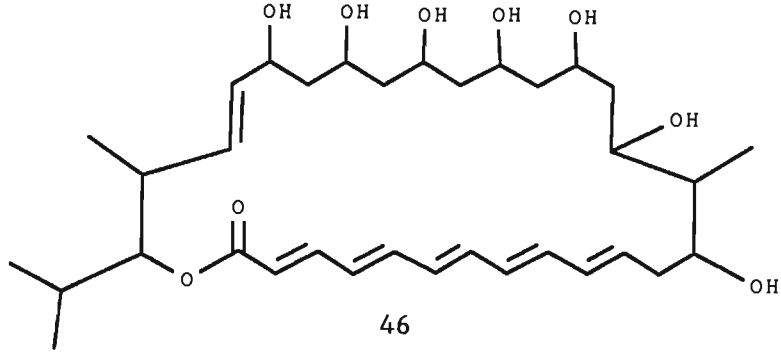
These types of macrolides include polyhydroxy and conjugated compounds such as mycoticin[46] (**46**) and filipin[47] (**47**).

b) NON-POLYENE MACROLIDES

This category encompasses macrolides such as methymycin (**8**) and narbomycin (**49**).[48]

c) MACROLIDIC (MACROLIDE-LIKE) COMPOUNDS

Compounds constituting this group include colletodiol (**50**), a macrocyclic dilactone[49] and zearalactone (**51**).[43]



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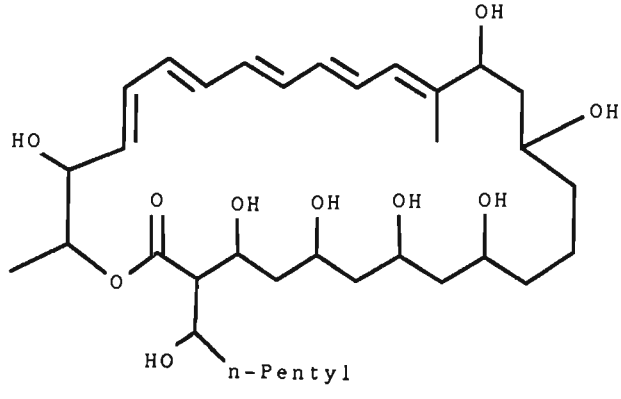

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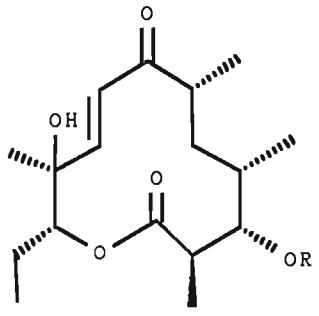

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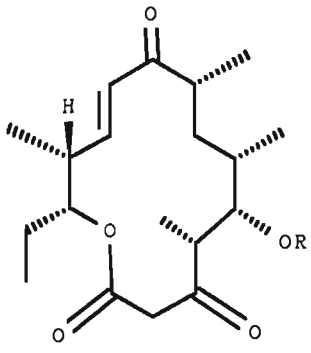

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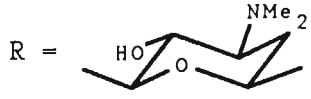

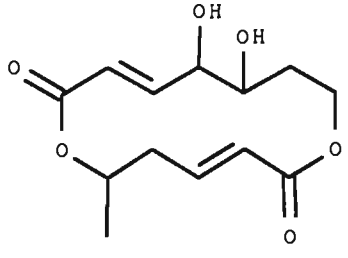

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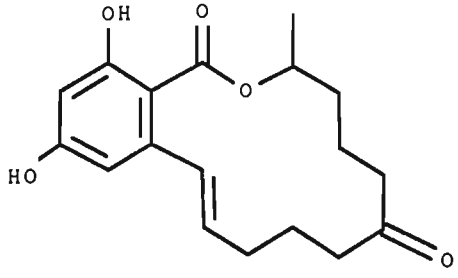

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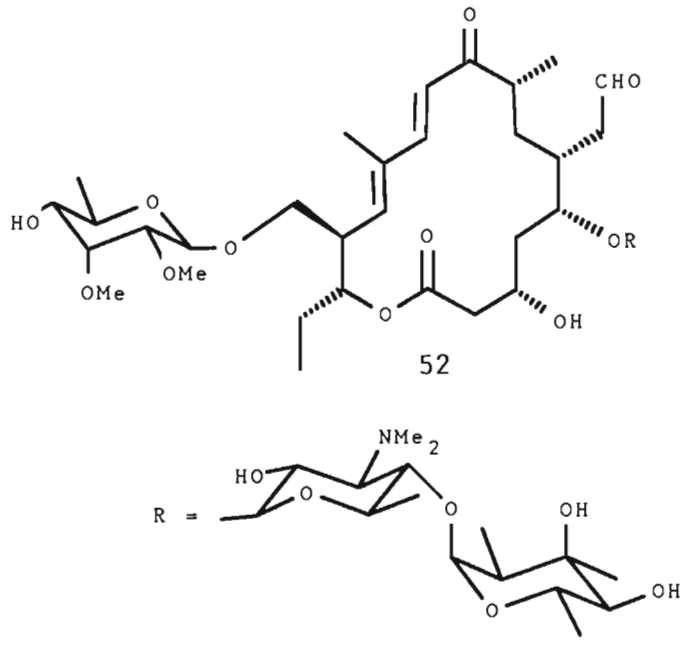
Patterson and Mansuri,[48] however, use the size of the ring to classify macrolides. For example, a 12-membered lactone ring (e.g. methymycin (**48**)), a 14-membered lactone ring (e.g. narbomycin (**49**)) and a 16-membered lactone ring (e.g. tylocin (**52**)).



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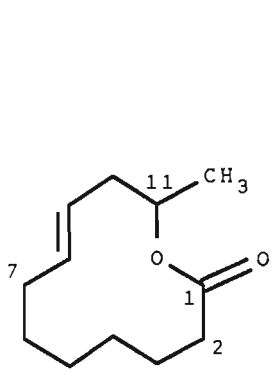
1.4.3. Nomenclature and numbering of macrolides

Structurally simple macrolides such as the lactone of 11-hydroxy-trans-8-dodecenoic acid[50] (**53**) have been assigned names based on the parent hydroxy acid from which they are derived. More complex structures such as mycoticin A (**46**) and tylosin (**52**) are conveniently given trivial names.

Although naming (trivial) of macrolides is arbitrary, the names of macrolides with a sugar moiety attached to them generally bear the suffix 'mycin' (example narbomycin (**49**)), whereas those without a sugar moiety have their names commonly ending with 'olide'[51] (example amphidinolide J (**54**)).



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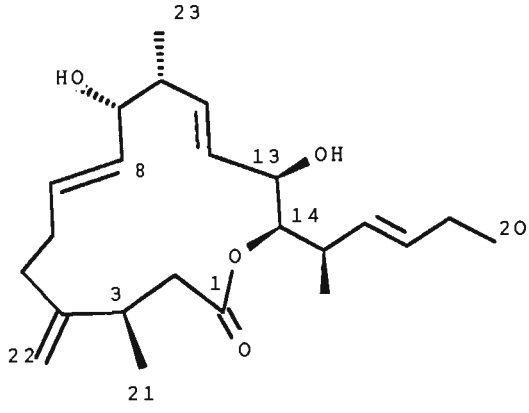

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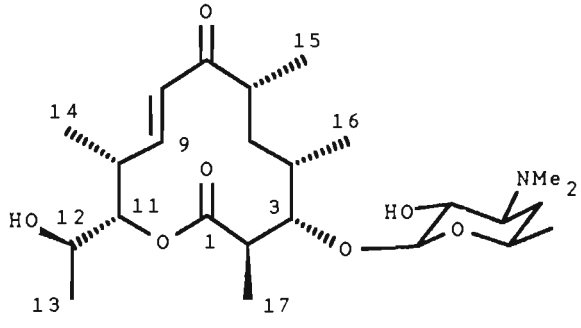

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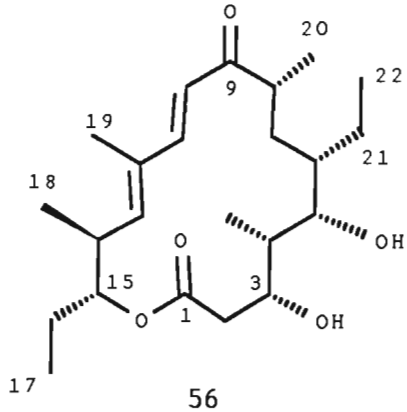
According to the numbering system adopted by Vesonder *et al.*,[50] Kobayashi *et al.*[51] and Inanaga *et al.*[52] in the numbering of **53**, **54** and neomethymycin (**55**) respectively, the carbonyl carbon of the ester moiety is numbered 1. After all the carbons constituting the ring have been numbered, carbons on the longest side chain are then numbered followed by the labelling of the rest of the carbons. This method, however, has been applied only to monolide macrolides.

1.4.4. Biosynthesis of macrolides

Both bacteria and fungi have shown that microorganisms are a rich source of structurally-unique and medicinally-important compounds. Most macrolides discovered to date owe their origin to these two microorganisms. A few macrolides, however, have

been found in insects. Higher plants are seldom, if ever, sources of macrolides. It is with this background that the biosynthesis of macrolides was studied in bacterial cultures.

The biosynthesis of several macrolides including tylactone[53] (**56**), dehydrocurvularin[54] (**57**), nonactin[55] (**58**) and erythromycin A (**59**) and B (**60**),[56,57] have been studied. Erythromycin A and B were among the first macrolides to be discovered and characterized and their biogenesis has been extensively studied.[56,57,59] It is for this reason that the biosynthesis of erythromycin A and B will be discussed here.



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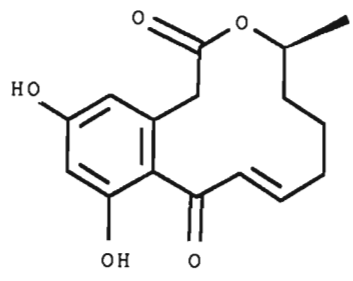

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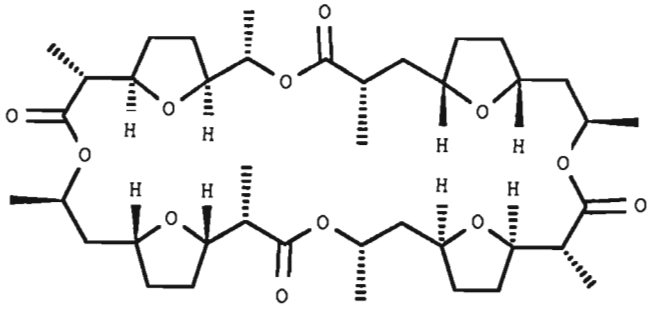

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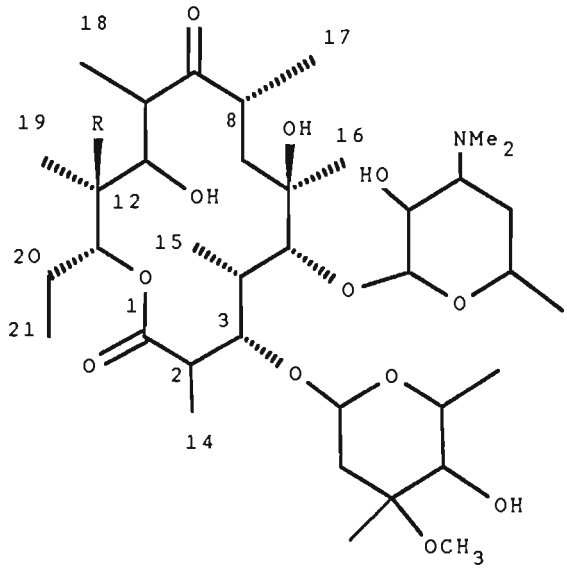
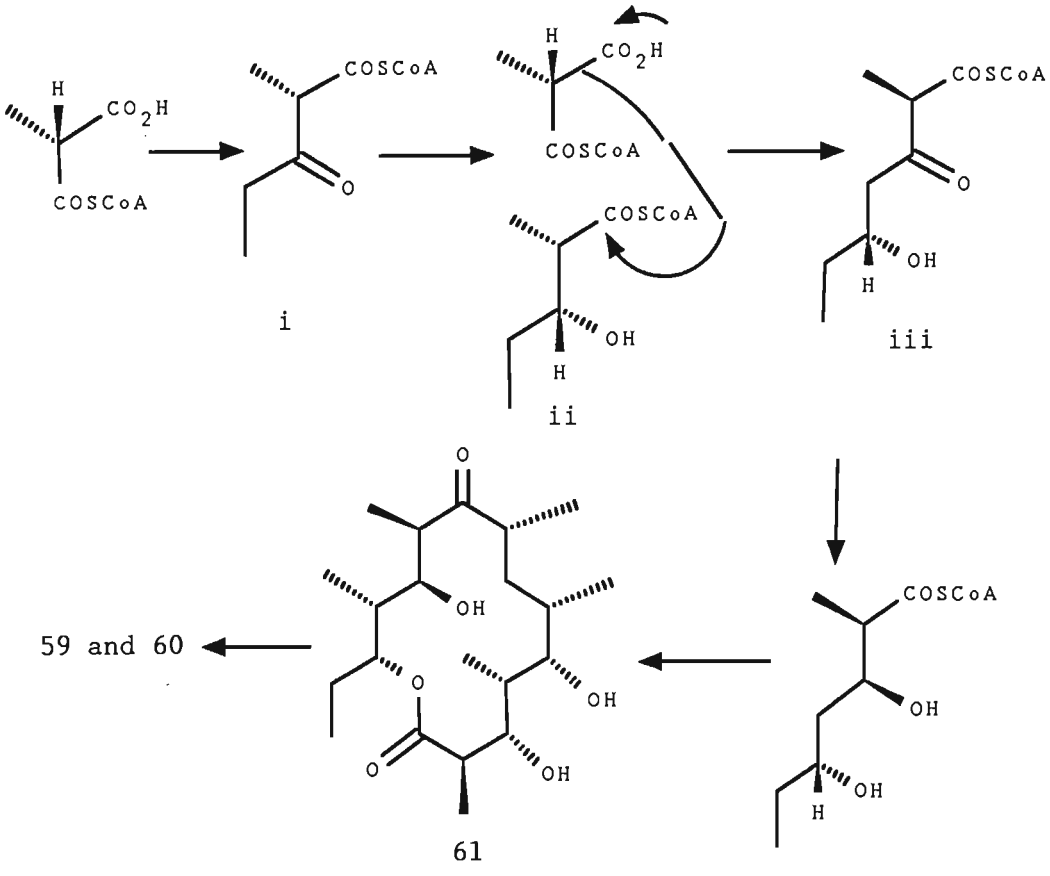


Erythromycin A (**59**), R = OH
Erythromycin B (**60**), R = H

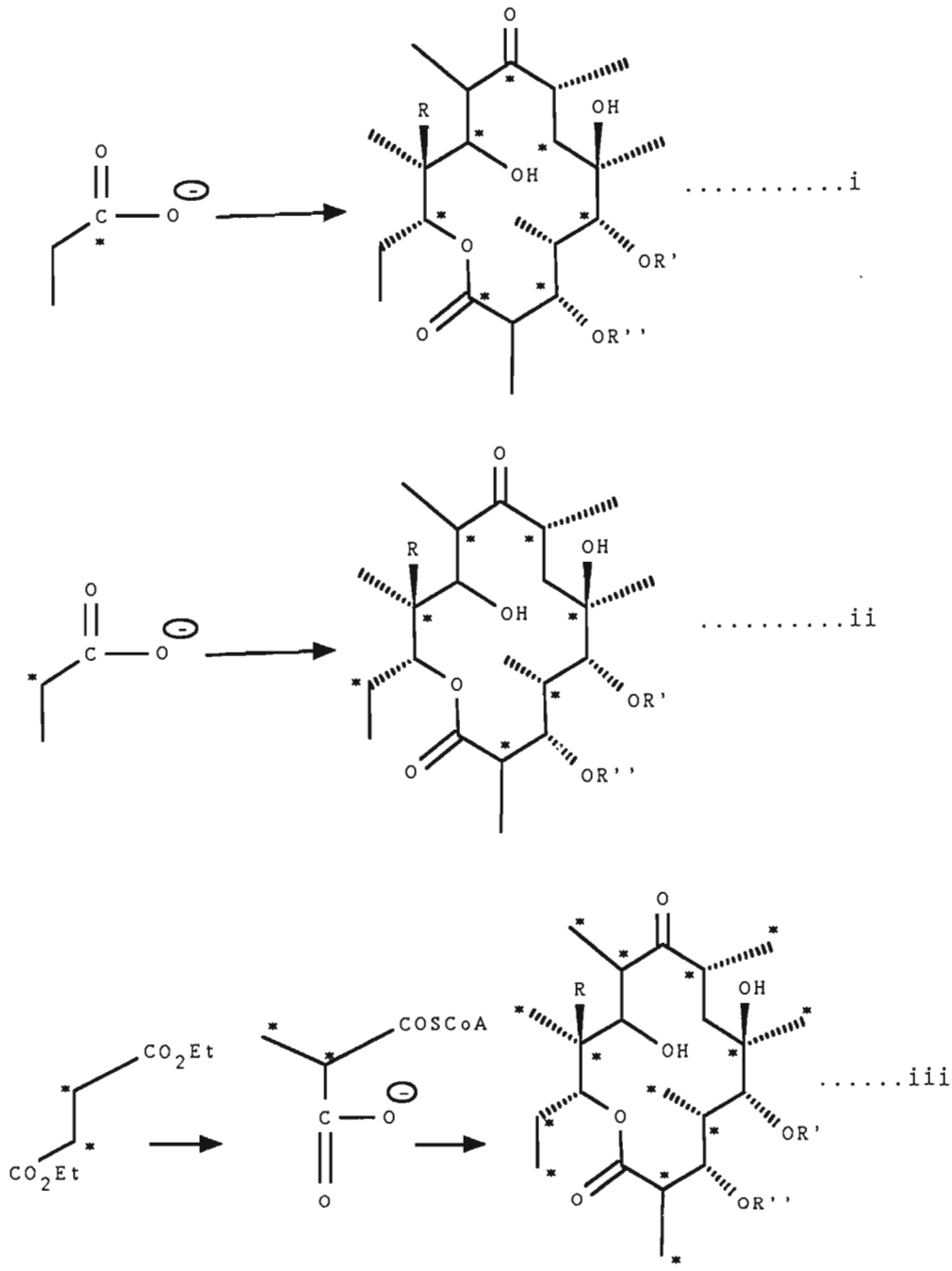
The proposed mechanism for the biosynthesis of erythromycins (**Scheme 5**) is based on feeding of radioactive propionate and methylmalonate to *Streptomyces erythreus*.[56] The resultant macrolides from feeding experiments with [1-^{13}C]-propionate, were converted to the readily crystalline 2'-benzoate esters. The labelled macrolide esters displayed enhanced signals corresponding to the expected positions of labelling; carbons 1, 3, 5, 7, 9, 11 and 13 (**Scheme 6**).

Using the same protocol, samples of erythromycin A and B 2'-benzoates were also generated by incorporation of [2-^{13}C]-propionate. The carbon-13 nmr spectra revealed the expected distribution of label based on the appearance of enhanced signals corresponding to C-2, C-4, C-6, C-8, C-10, C-12 and C-20 (**Scheme 6**)



Scheme 5

The biosynthetic origin of the alkyl substituents, C-14, C-15, C-16, C-17, C-18, C-19 and C-20,21 was established by incorporation experiments with diethyl [2,3-$^{13}C_2$]-succinate. The diethyl [2,3-$^{13}C_2$]-succinate was expected to act as an *in vivo* precursor of [2,2'-$^{13}C_2$]-methylmalonate CoA generated by the action of methylmalonyl CoA mutase, and could therefore be considered as an *in vivo* equivalent of [2,3-$^{13}C_2$]-propionate.[56] The spectra of the resultant labelled erythromycins A and B 2'-benzoates, showed the expected seven pairs of enhanced and coupled doublet for each sample.

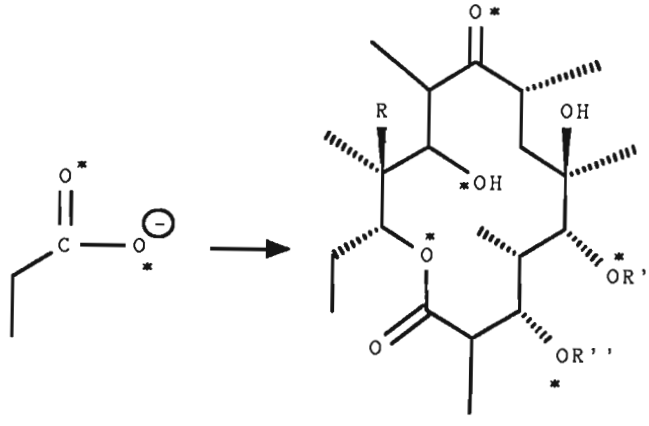


Scheme 6

Labelling of the propionate starter unit (C-20,21) is presumed[56] to be due to decarboxylation of the methylmalonyl CoA. Measurement of ^{13}C-^{13}C coupling constants was used to

assign the critical methine and methyl groups.

Although Corcoran[59] showed that the tertiary-OH groups at C-6 and C-12 are derived from molecular oxygen (O_2), it was Cane and Yang [57] who investigated the biosynthesis of the six remaining oxygen atoms already present in erythromycins. [1-$^{18}O_2$, 1-^{13}C]-propionate was incorporated and the results also analysed using ^{13}C-nmr (**Scheme 7**). The peaks corresponded to C-1, C-3, C-5, C-7, C-9, C-11 and C-13 in both erythromycin A and B benzoates. Each appeared as enhanced pairs of signals



R' = desosaminyl benzoate
R'' = cladinosyl benzoate

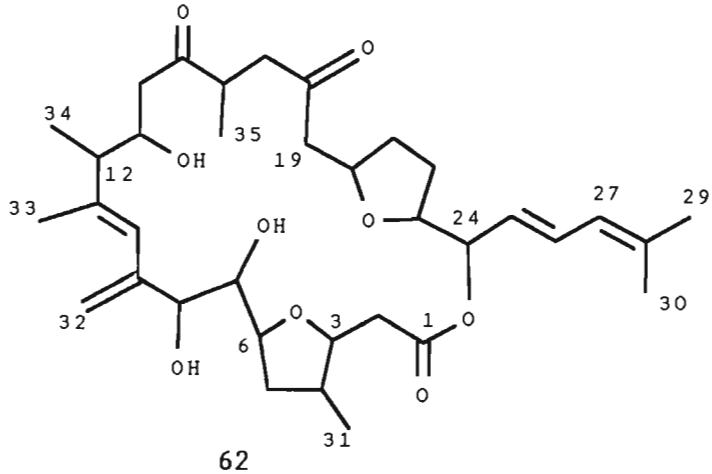
Scheme 7

corresponding to ^{16}O-^{13}C and ^{18}O-^{13}C species, the latter resonance being shifted upfield, according to the type of C-O bond. The results showed that each of the six oxygen atoms present in the first-formed aglycone 6-deoxyerythronolide (**61**), was derived from the propionate precursor. Only the carboxyl oxygen of the lactone originated from the C-1, 2, and 14 propionate unit, the ester oxygen having been derived intact from the corresponding C-13, 20, and 21 propionate moiety.

1.4.5. Biological activity of macrolides

Macrolides are well established as an old class of antibiotics known to humans. They have found application since the early 1950's and are still an important aspect on the chemotherapy of infectious diseases and contribute substantially to the world's oral antibiotic trade.[60] The discovery of new types of macrolides hopes to expand or maintain their role in the control of infectious diseases. New macrolides have been discovered and synthesised in recent years. The biological activity of selected macrolides will be discussed here.

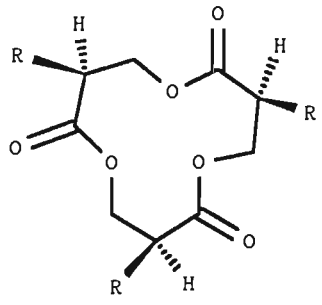
Amphidinolide F (**62**) showed some activity against murine lymphoma L1210 cells and human epidermoid carcinoma Kb cells.[61]



62

Amphidinolide F

Amphidinolide J (**54**) was also found to be cytotoxic.[51] Some macrolides (example enterobactin (**63**), which transport iron from the environment into bacterial cells (e.g. *Escherichia. coli*) by forming a macro-bridged hexacoordinate trianion **64**) were found to stimulate bacterial growth.[62]



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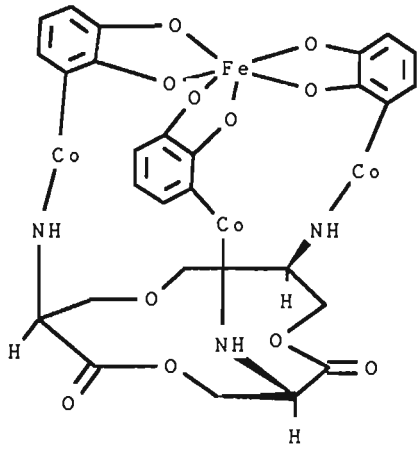

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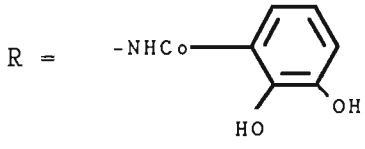
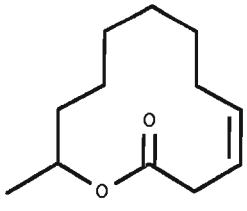


Other macrolides (e.g. **65** and **66**) are used by beetles of the genera *Oryzaephilus* and *Cryptolestes* in their pheromone system.[63] Grahamimycin A_1 (**67**), a macrodiolide, exhibits antibacterial activity against species such as *Staphylococcus* and *Pseudomonas*.[64]



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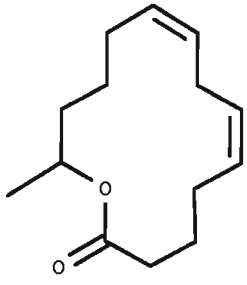

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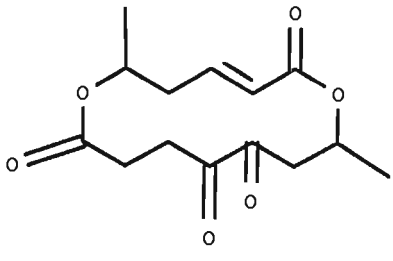

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CHAPTER TWO

2. **DISCUSSION**

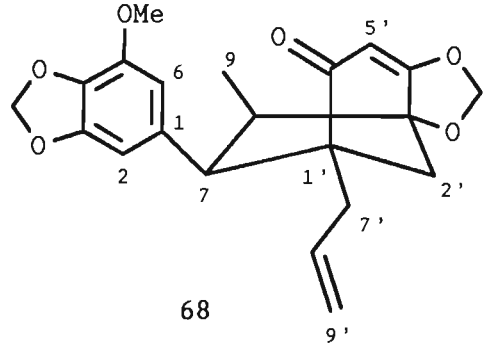
The four Lauraceae species studied yielded eight novel compounds and one known constituent. Structural elucidation of the compounds included techniques such as ^{1}H and ^{13}C NMR spectroscopy, HETCOR, DELAYED or RELAY HETCOR, COSY and DELAYED or RELAY COSY, NOE, ROESY, IR spectroscopy, HMBC techniques, mass spectroscopy and chemical modifications of the parent compounds isolated. These techniques enabled the satisfactory or complete assignment of the protons and carbon atoms of the compounds and the relative stereochemistry of some compounds. Compounds will be discussed in the same manner as they were discussed in the introduction (i.e. according to the class to which they belong).

2.1. **NEOLIGNANS**

Two novel compounds that belong to this class were isolated from *Ocotea bullata*. Ocobullenone (**74**) was isolated from the chloroform extract (**Experimental**, 3.3.1.1.) whereas bullatone (**74**) was isolated from the ethyl acetate extract (**Experimental**, 3.3.1.2.)). The chemical profile of the chloroform extract of the bark of *Ocotea bullata* revealed a number of other compounds that were close (on TLC plate) to the two compounds isolated from this plant. Most of the compounds were found to be oils with very close Rf values and were difficult to isolate with the techniques employed.

2.1.1. Ocobullenone (**68**)

The literature review indicated that plants belonging to the family Lauraceae, especially *Ocotea* species, abound with neolignans and more so with bicyclo [3.2.1] octanoid neolignans.[65,66,15,67] Scrutiny of the spectroscopic data of ocobullenone (**68**) showed that it has certain structural features common to neolignans of the bicyclo [3.2.1] octanoid type. However, it was discovered that **68** was the first known bicyclo [3.2.1] neolignan to be fully deoxygenated at the carbon (C-2') between the bridgeheads.[16] Bicyclo [3.2.1] octanoid neolignans usually either have a hydroxy,[68,69] an ester[70,71] or a carbonyl moiety[72,13] at C-2'. Another unique



68

feature is the methylenedioxy group at the C-3',4' position on the cyclohexenone ring. The ^{13}C NMR showed 19 resolved peaks, comprising (from DEPT) two methyl, five methylene, six methine and eight quaternary carbons. Signals due to the methylenedioxy groups, O_2CH_2-3',4' (101.3ppm) and O_2CH_2-3,4 (101.5ppm), were almost superimposing whereas peaks due to C-8' and C-4 (134.4ppm) were on top of each other. An infrared peak at 1641 cm^{-1} revealed the presence of an α,β-unsaturated carbonyl group. Ocobullenone (**68**) gave a satisfactory C, H and

N analysis and the ^{1}H (**Appendix 68a**) and ^{13}C (**Appendix 68c**) NMR data were in accord with the proposed structure. COSY, DELAYED COSY, HETCOR and DELAYED HETCOR (**Appendices 68d, 68e, 68f,** and **68g** respectively) NMR techniques were employed to prove connectivities in the molecule **68**.

Where ambiguities still existed, these were resolved using the ^{1}H HMBC technique (**Table 1**). These experiments were particularly useful in establishing the position of attachment of the benzene ring to the cyclopentane moiety and to locate C-5' in the cyclic system. Thus, long range coupling of H-9 (methyl protons) to C-3', C-7 and C-8 together with coupling of H-7' to C-7 and C-6' (**Table 1**) established the position of the linkage in **68** as 7.1',8.3' and pinpointed the position of the methyl group relative to the allyl side chain. As indicated in the Introduction, the 7.1',8.3' linkage in ocobullenone is unique in that previously isolated bicyclo [3.2.1] octanoid neolignans have the common 7.3',8.1' linkage.[73,74]

Table 1: Significant HMBC correlations for ocobullenone **68** (2,3- and 4-bond couplings)

Proton Irradiated	Carbon Observed
H-8	C-4', C-1', C-1, C-3'
H-7'a	C-6', C-7, C-2'
H-2'a	C-6', C-4', C-7, C-8, C-7'
H-7'b	C-6', C-7, C-2'
H-2'b	C-6', C-4', C-7, C-8, C-7', C-9
H-2	C-7
H-6	C-7
O_2CH_2-3,4	C-3, C-4
H-8'	C-1'
O_2CH_2-3',4'	C-3', C-4'
H-5'	C-3', C-1'
OCH_3	C-5
H-7	C-6', C-1', C-2, C-6, C-7'

The relative stereochemistry of **68** was established using ROESY technique (**Table 2a**). Coupling of both H-2 and H-6 with the methyl group (H-9), and with H-8, proved the aryl and methyl

Table 2a: Selected ROESY experimental results for **68**

Proton Irradiated	Observed Enhancement
H-9	OCH_2O-3',4', H-2, H-6
H-2'a	H-8, H-7
H-8	H-2'a, H-7
H-7	H-2'a, H-8
H-2 and H-6	H-7, H-9

group to be *cis* to each other. This feature is also unique in that most bicyclo [3.2.1] octanoid neolignans have the aryl and methyl groups *trans* to one another.[75,76,77] Since both H-7 and H-8 also couple with one of the H-2' protons, these protons must also be *cis* to one another. NOE experiment (**Table 2b**) on **69** confirmed the above allocation, and, in particular that the

Table 2b: Selected NOE experimental results for **69**

Proton Irradiated	Observed Enhancement
H-6'	OCH_3, H-2'b, H-5'
H-9	H-2, H-6
H-8	H-7, H-9, H-2'a
H-7	H-8, H-2'a, H-7'a

allylic side chain is on the same side as H-7, that H-6' assumes an endo orientation, and that the protons on the aliphatic methylenedioxy group are geminally coupled.

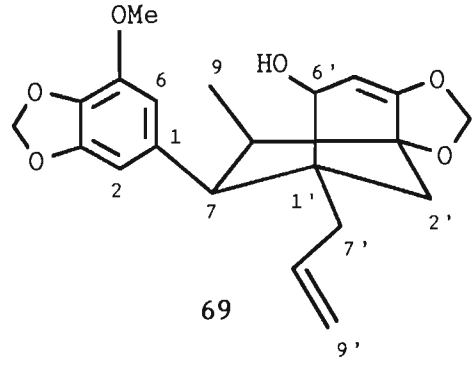
2.1.1.1. Reactions on ocobullenone (**68**)

In an attempt to confirm the structure of ocobullenone (**68**), several reactions were carried out. These included: reduction with $LiAlH_4$, hydrogenation, reaction with ethylene glycol using para -toluene sulphonic acid (pTSOH) as a catalyst, and reaction with dilute hydrochloric acid.

2.1.1.1.1. Reduction of **68** with lithium aluminium hydride

Reduction with $LiAlH_4$ (**Experimental**, 3.3.1.1a.) afforded the alcohol **69**, $C_{21}H_{24}O_6$. This proved to be a very useful

derivative since it confirmed the presence of an α,β-unsaturated carbonyl system. In the ^{1}H NMR spectrum (**Appendix 69a**) of **69**, clear coupling between the protons H-5' and H-6' (J=2.7Hz) is observed. The reduction resulted in the chemical shifts of the signals representing carbons on the



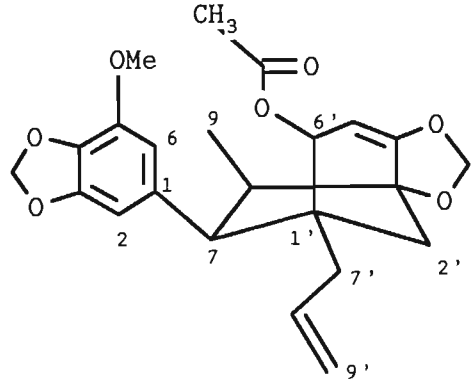
69

α,β-unsaturated carbonyl system, especially C-6'and C-4', shifting upfield to 77.5ppm and 154.7ppm respectively (**Appendix 69c**). Mass spectroscopy showed that **69** had M^{+} 372. Connectivities between the various carbons were deduced from HETCOR (**Appendix 69f**), DELAYED HETCOR (**Appendix 69g**), COSY (**Appendix 69d**), and DELAYED COSY (**Appendix 69e**) spectra. The ^{13}C NMR showed 21 resolved signals representing (from DEPT), three methyl, five methylene, seven methine and six quaternary carbons. The IR spectrum peak (3560 cm^{-1}) revealed the presence of a hydroxyl group which appear as a doublet (J=11.9Hz) at δ1.56 in the ^{1}H NMR spectrum due to coupling with H-6'.

2.1.1.1.2. <u>Acetylation of **69**</u>

The alcohol **69** was acetylated with acetic anhydride in pyridine to afford the acetate **69a** (**Experimental**, 3.3.1.1b.). Mass

spectroscopy showed the acetate had molecular ion of 414.

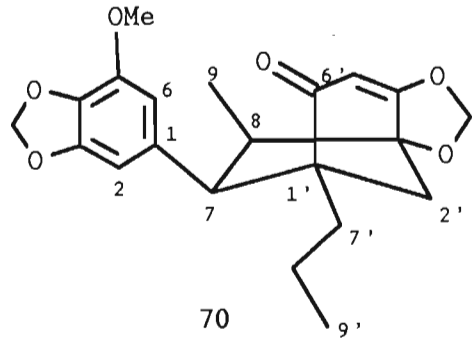


69a

The ^{13}C NMR spectrum (**Appendix 69ac**) showed 23 resolved peaks comprising of, three methyl, five methylene, seven methine and eight quaternary carbons (DEPT analysis). The appearance of carbons at 170.4ppm (ester carbon) and 20.6ppm (methyl carbon) point to **69a** being an ester. The disappearance of the hydroxyl band (3560 cm^{-1}) and the appearance of an ester carbonyl band (1692 cm^{-1}) in the IR spectrum confirmed this transformation. Connectivities in **69a** were arrived at by comparison with the spectral data of **68** and **69** and the interpretation of the HETCOR (**Appendix 69af**), DELAYED HETCOR (**Appendix 69ag**), COSY (**Appendix 69ad**) and DELAYED COSY (**Appendix 69ae**) spectra.

2.1.1.1.3. Hydrogenation of **68**

Ocobullenone (**68**) was hydrogenated using palladium on carbon (**Experimental**, 3.3.1.1c.) in an attempt to prove the position of the double bond at C-4' and C-5'. However, hydrogenation of the allylic side chain was observed giving compound **70**.

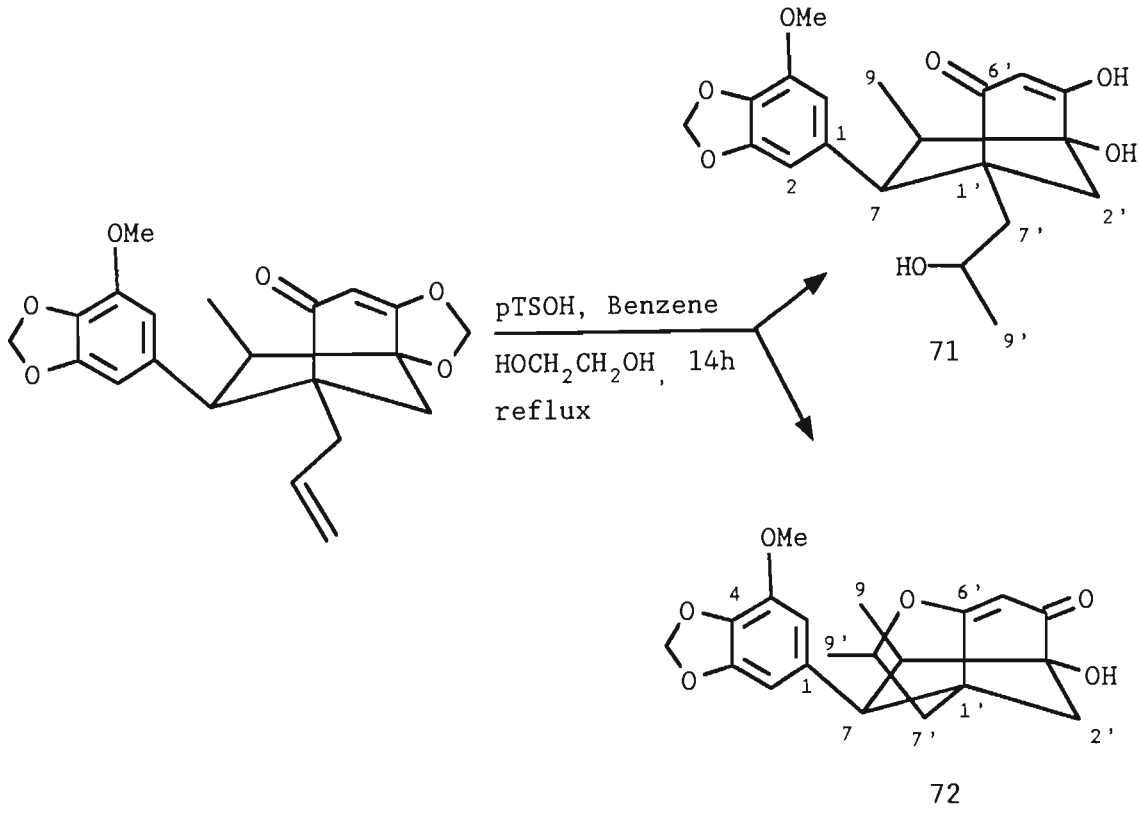


70

Proof for the formation of this product was the appearance of extra methyl and methylene groups in both the ^{1}H (**Appendix 70a**) and ^{13}C (**Appendix 70c**) NMR spectra.

2.1.1.1.4. Acid-catalyzed hydrolysis of **68** with para-toluene sulfonic acid (pTSOH)

Acetals readily form under acid catalyzed conditions.[73,74] When ocobullenone (**68**) in benzene was reacted with ethylene glycol using pTSOH as a catalyst (**Experimental**, 3.3.1.1d.) two products were isolated, **71** and **72** (**Scheme 8**). These were not the expected products. It was clear from the nature of the products that **68** had undergone an acid-mediated hydrolysis. The

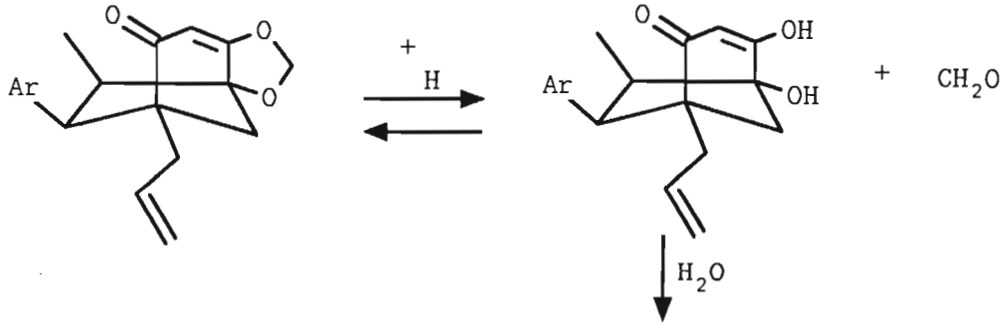


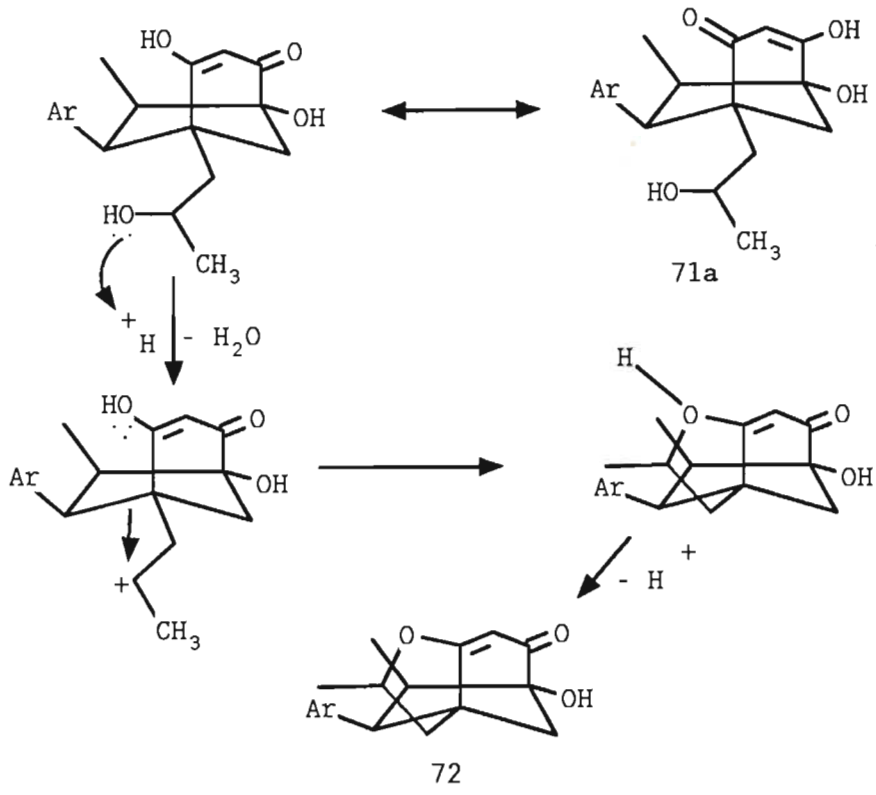
Scheme 8

two products were crystalline. The proposed structure of the major product **72** was arrived at by comparing the spectral data of **72** with those of **68**. Mass spectroscopy showed that **72** had M^+ 358. The ^{13}C NMR spectrum (**Appendix 72c**) showed 18 resolved signals. The other two carbons (methine groups, C-2 and C-6) were missing from both the ^{13}C spectrum and DEPT. However, evidence for the presence of these methine groups was provided by the presence of aromatic protons at δ6.44 (**Appendix 72a**). Only one hydroxyl group (OH-3', δ4.54) was observed. The proton at δ3.80 (H-8') and the quaternary carbon (C-6') at 187.69 ppm could only be that far downfield if they were both attached to an oxygen atom, indicating that C-6' and C-8' must be linked through an oxygen atom.

The ^1H NMR spectrum of compound **71** (**Appendix 71a**) was also compared to that of **68**. Mass spectroscopy indicated that **71** had M^+ 376. The ^1H NMR spectrum showed the appearance of three hydroxyl groups; two hydroxyls (OH-3'and OH-8') at δ1.63 and one hydroxyl group on a vinyl moiety (OH-4') at δ4.54. Both the protons at δ5.06 (H-9') and δ5.75 (H-8') and the methylenedioxy moiety (O_2CH_2-3',4') at δ5.65 that were visible on the ^1H NMR spectrum of ocobullenone (**68**) had disappeared. A new methyl group at δ0.63 (H-9') which coupled with H-8' (δ4.79) was observed.

A mechanism for this transformation is proposed in **Scheme 9**. This mechanism would suggest that some water was present in the reaction mixture. The fact that the hydration of the double bond on the allylic side chain of tautomer **71a** results in an intermediate that is the same as the minor product **71** lends legitimacy to the proposed mechanism.

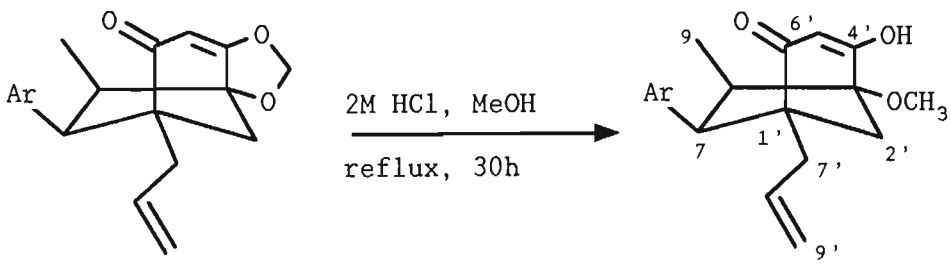




Scheme 9

2.1.1.1.5. Acid-catalyzed hydrolysis of **68** with dilute (HCl)

In view of the hydrolysis of **68** by pTSOH, a reaction was carried out to determine whether a dilute HCl would also effect hydrolysis to give the same products. The reaction was carried out in a polar solvent (methanol) (**Experimental**, 3.3.1.1e.) to facilitate dissolution of the aqueous acid (**Scheme 10**).

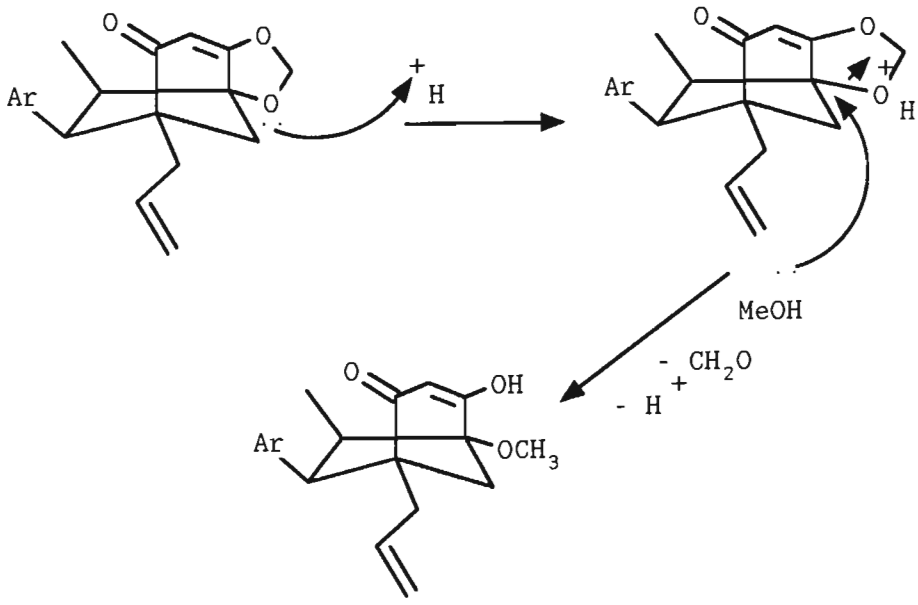


73

Scheme 10

Compound **73** ($C_{21}H_{24}O_6$), an oil, was obtained as the product of hydrolysis. The molecular structure of **73** was deduced by comparison with the spectral data of ocobullenone **68**. The ^{13}C NMR spectrum revealed twenty resolved signals (with the peak due the quaternary carbon, C-4, and the signal due to the methine group C-8' superimposing at 134.47 ppm) (**Appendix 73c**), three methyl, four methylene, six methine and eight quaternary carbons. ^1H NMR (**Appendix 73a**) showed a new OCH_3 group (compared to the ^1H NMR of **68**) at δ3.84 and the disappearance of the O_2CH_2-3',4' at δ5.65). The proton at δ5.48 (H-5') retained its multiplicity (singlet) indicating that no proton was introduced adjacent to it.

The hydrolysis is perceived here to occur via the same mechanism as that in the case where p-TSOH was used as the acid catalyst. However, methanol acts as the nucleophile instead of water (**Scheme 11**).

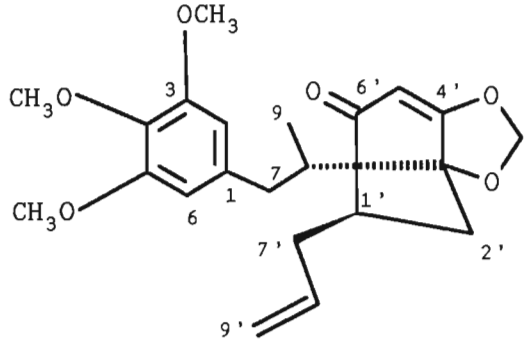


73

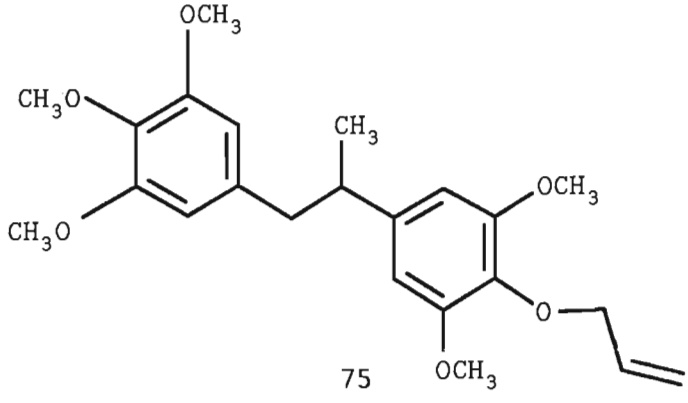
Scheme 11

2.1.2. Bullatone (**74**)

The relationship between bullatone (**74**) and ocobullenone (**68**) is apparent. Bullatone shows the typical 8.3' linkage but the 7.1' bond is severed at the carbon alpha to the carbonyl group. A molecular formula of $C_{22}H_{28}O_6$ was supported by a high resolution mass spectroscopy (HR-MS) peak at 388. The ^{13}C NMR spectrum (**Appendix 74c**) showed 18 resolved carbons comprising (from DEPT) four methyl, five methylene, six methine and seven quaternary carbons. Two pairs of equivalent carbons - two OCH_3 groups (attached to C-3 and C-5 carbons) at 56.23 ppm and two methine groups (C-2 and C-6) at 153.23 ppm - were superimposed. The equivalence of the two methoxy groups is readily explained by the presence of a 3,4,5-trimethoxybenzene moiety on the $C_6.C_3$ unit, a grouping also present in the neolignan aurin[78] (**75**). Spectral data available for isodihydrofutoquinone (**76**),



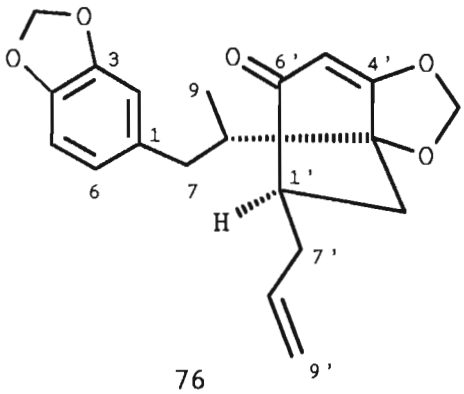
74



75

isolated by Matsui[79] and $\Delta^{8'}$-3',6'-dihydro-3,4,3',4'-bis-methylenedioxy-6'-oxo-8.3'-neolignan (**77**) obtained by Green and Wiener[80], proved very useful for the purposes of comparison. The substituted benzene ring and propyl side chain were identified without difficulty.

The allyl side chain at C-1' was discernible from both the ^{1}H (**Appendix 74a**) and ^{13}C NMR spectra and the chemical shifts were comparable to those found in ocobullenone (**68**). The methylenedioxy group at C-3' and C-4' was characterized by the



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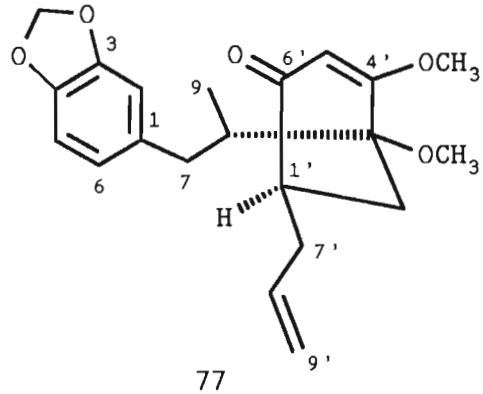


77

typical carbon shift of 99.9 ppm and the methylene hydrogens at δ5.60. The α,β-unsaturated carbonyl moiety had chemical shifts very similar to those found in **68**.

The point of linkage between $C_6.C_3$ and $C_6'C_3'$ units in **74** was established by the HMBC experiment (**Table 3**).

Table 3: Multiple bond connections observed in the HMBC spectra (2,3- and 4-bond couplings) for **74**.

Proton Irradiated	Carbon Observed
H-9 (δ 0.99)	C-3', C-7, C-8
H-2'b (δ 1.79)	C-3', C-1', C-8, C-7'
H-8 (δ 2.09)	C-1, C-3', C-7, C-9
H-7b (δ 2.25)	C-1, C-2, C-9
H-7'b (δ 2.27)	C-6', C-8', C-9', C-1', C-2'
H-1' (δ 2.40)	C-6', C-8', C-7'
H-2'a (δ 2.56)	C-6', C-4', C-3', C-1'
H-7'a (δ 2.69)	C-6', C-8', C-9', C-1', C-2'
H-7a (δ 2.87)	C-3, C-1, C-2, C-3', C-8, C-9
OCH_3-4 (δ 3.80)	C-4
OCH_3-3,5 (δ 3.82)	C-3
H-9' (δ 5.07)	C-8', C-7'
H-5' (δ 5.53)	C-6', C-4', C-3', C-1'
O_2CH_2 (δ 5.60)	C-4', C-3'

The relative stereochemistry at C-1' and C-3' was deduced from a 1D NOE difference experiment (**Table 4**) on **74**. The *trans* relationship between the group attached to C-1' and that connected to C-3' was shown by a large NOE between H-1' and H-8 and between H-9 and H-1'. The planarity constraints of the α,β-unsaturated carbonyl system coupled with the requirements of the methylenedioxy group lend substantial rigidity to the cyclohexenone ring resulting in a half-chair or pseudo-chair

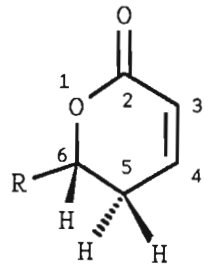
Table 4: 1D NOE Difference Experiment on **74**

Proton Irradiated	Observed correlated proton
H-9(δ0.99)	H-2, H-6, H-8, H-2'a
H-8(δ2.09)	H-2, H-6, H-9, H-1', H-7a, H-2'a
H-1'(δ2.40)	H-8, H-8', H-2'a
H-2'a(δ2.56)	H-2'b, H-1', H-9, H-8'
H-7'a(δ2.69)	H-8', H-1', H-7'b, H-7b
H-7(δ2.87)	H-8, H-7'b, O_2CH_2, H-5, H-2, H-6

conformation. Carbons 1', 6', 5', 4', and 3' exhibit a 5-point coplanarity. A simple Dreiding model made it abundantly clear that the $C_6.C_3$ unit can only be attached to an axial bond from the cyclohexenone ring. This places the allyl side chain on an equatorial bond and *trans* to the $C_6.C_3$ moiety.

2.2. 6-SUBSTITUTED 5,6-DIHYDRO-α-PYRONES

^1H NMR spectroscopy has played a major role in the structural elucidation of most of naturally occurring 6-substituted 5,6-dihydro-α-pyrones. Proton H-3 in **78** is coupled to H-4 (J=9.7-10Hz) and resonates at δ5.9-6.1, indicative of a *cis*



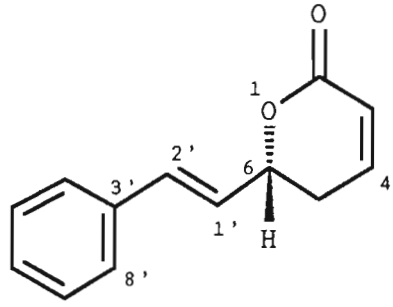
78

olefinic function next to a carbonyl group. Proton H-3 also experiences allylic coupling[82] with the two protons attached to C-5 ($J_{3,5ax}$=1Hz, $J_{3,5eq}$=2-3Hz). The fact that H-4 (δ6.78-7.05) is shielded relative to H-3 is typical of a proton attached to the β-carbon of an α,β-unsaturated carbonyl chromophore. Proton H-4 appears as a triplet of doublets from its coupling to H-3 and also to H-5_{ax} ($J_{4,5ax}$=2-4Hz) and H-5_{eq} ($J_{4,5eq}$=4-6Hz).

The two allylic protons at C-5 show a typical geminal coupling ($J_{5ax,5eq}$=15-19Hz) and a coupling to H-4 and to H-6 ($J_{5ax,6}$=9-12Hz, $J_{5eq,6}$=3-6Hz). These two non-equivalent protons resonate as a complex multiplet with chemical shifts between δ2.3 and δ2.8.

2.2.1. Goniothalamin (**45**)

This styryl-α-pyrone **45** was isolated from *Cryptocarya woodii* (**Experimental**, 3.3.2.1.). It has been isolated before from other plant species[83,42] and both its molecular structure and absolute configuration have been thoroughly investigated. The molecular formula of $C_{13}H_{12}O_2$ was arrived at from the analysis of spectral data. Mass spectroscopy show that goniothalamin (**45**) had M^+ 200.



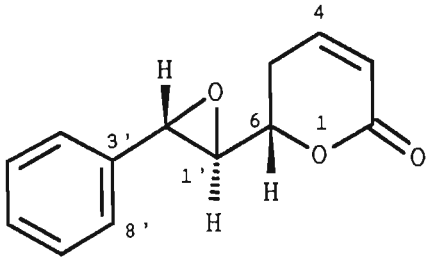
45

The ^{13}C NMR spectrum showed eleven resolved carbon signals (**Appendix 45c**) comprising (from DEPT), one methylene, ten methine and two quaternary carbons. The peaks at 126.67 ppm and 128.67 ppm were almost double the size of other methine groups and this was attributed to the overlapping of signals due to carbons C-4' and C-8' at 126.67 ppm and C-5'and C-7' at 128.67 ppm. Both the melting point and rotation of the isolated **45** were in close agreement to the literature values. As earlier shown,[81,82] proton H-3 in **45** was coupled to H-4 with a coupling constant of about 9.8Hz showing a *cis* relationship between the two protons. Proton H-3 also experienced a long range coupling to C-5 with the expected coupling constants ($J_{3,5ax}$=1.93Hz, $J_{3,5eq}$=3.67Hz). Proton H-4 also coupled to H-5 ($J_{4,5ax}$=3.57Hz, $J_{4,5eq}$=4.16Hz) as envisaged. The splitting pattern due to the two germinal protons at C-5 (**Appendix 45a**) was complex and thus their coupling constants were not determined.

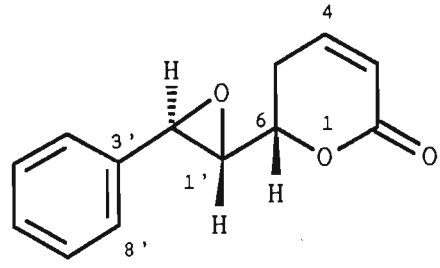
2.2.1.1. Epoxidation of goniothalamin

Goniothalamin was epoxidized using mCPBA[84] (**Experimental**, 3.3.2.1.1.) to give two crystalline products **79** and **80**. The

epoxide **79** was obtained as the major compound. This is in contrast to Sam *et. al.*[83] who obtained **80** as the major product.



79

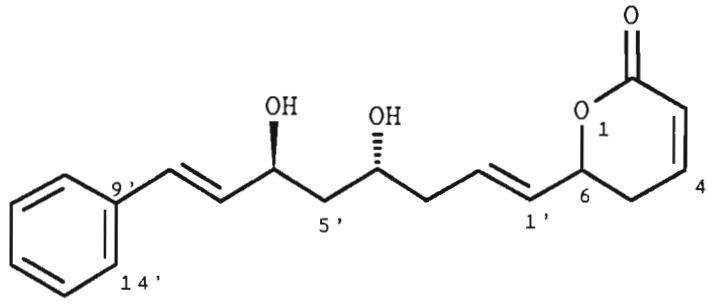


80

The coupling constants between the oxirane ring protons ($J_{1',2'}$=2.01Hz for **80** and $J_{1',2'}$=2.04Hz for **79**) were consistent for *trans*-oxides ($J_{1',2'}$=2.0Hz) and not for *cis*-oxides ($J_{1',2'}$=4.2-4.5Hz).[83,85] Epoxide **80** having a larger $J_{6,1'}$(5.12Hz) and its H-6 proton appearing at a higher field compared to that in **79** ($J_{6,1'}$=3.87Hz) must be the threo-isomer.[86]

2.2.2. Cryptofolione (81)

The benzene extract of the bark of both *Cryptocarya latifolia and Cryptocarya myrtifolia* yielded an oily compound **81** (**Experimental**, 3.3.3.3. and 3.4.) for which the name cryptofolione has been proposed. A molecular formula of $C_{19}H_{22}O_4$ was supported by a HR-MS peak at 314. The ^{13}C NMR spectrum (**Appendix 81c**) revealed 17 resolved carbon signals, consisting of, three methylene, fourteen methine and two quaternary carbons (DEPT analysis). As was the case with



81

goniothalamin (**45**), the two sp^2 methine groups at 126.43 ppm and 128.55 ppm had intensities that were almost double those of other sp^2 methine groups indicating overlapping of two signals each. IR absorption at 700 and 755 cm^{-1} (**Experimental**, 3.3.3.3.). A five-proton multiplet at δ7.23 in the ^1H NMR spectrum (**Appendix 81a**) indicated the presence of a monosubstituted benzene ring. This was further confirmed by ^{13}C resonances at 136.65s, 126.43d (x2), 128.55d (x2) and 127.56d. The presence of the two exocyclic *trans*-disubstituted double bonds was indicated by ^1H NMR which showed two pairs of one-proton multiplets with coupling constants of about 16Hz.

A strong hydroxyl band (3440 cm^{-1}) and an α,β-saturated lactone band (1708 cm^{-1}) were present in the IR spectrum. The presence of an endocyclic double bond was indicated by two sp^2 methine groups which couple to each other in the ^1H-^1H COSY spectrum (**Appendix 81d**) and a coupling constant of 10Hz in the ^1H NMR spectrum showed the *cis* stereochemistry of this double bond.

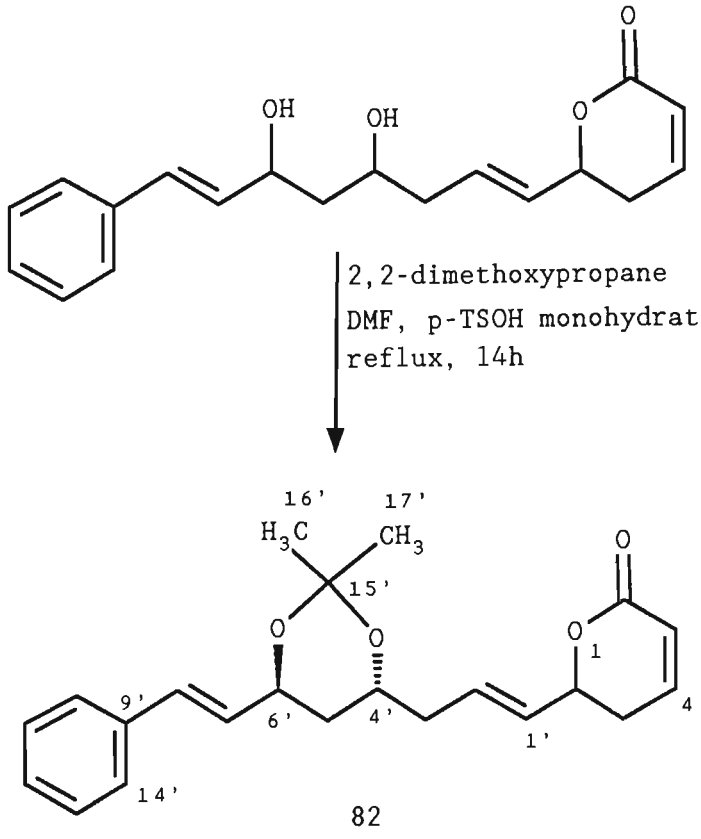
The point of attachment between the α-pyrone moiety and the substituent at C-6 was revealed by the coupling (^1H-^1H COSY spectrum) between H-1' (δ5.63) and H-6 (δ4.88) and coupling between H-2' (δ5.82) and H-6 and between H-1' and H-6 RELAYED COSY spectrum (**Appendix 81e**)). The position of the methylene group C-5' (42,29t ppm) was indicated by ^1H-^1H COSY coupling

between H-4' (δ4.05) and H-5'(δ1.78) between H-6' (δ4.63) and H-5' thus showing that H-5' is sandwiched between the two methine groups. Coupling in the ^{1}H-^{1}H COSY spectrum between H-3' (δ2.28) and H-4' (δ4.05) and between H-3' and H-2' (δ5.82) indicated the position of C-3' as that proposed in **81**.

2.2.2.1. Reactions on cryptofolione (**81**)

2.2.2.1.1. Acetonide derivative of cryptofolione

The relative stereochemistry of cryptofolione (**81**) at C-4' and C-6', as illustrated in structure **81**, was established from the chemical shifts of the methyl groups on the acetonide derivative **82** (**Experimental**, 3.3.3.3.1a.) in **Scheme 12**.



82

Scheme 12

Acetonide formation was discovered by Robinson *et al.*[87] and Tanabe and Bigley[88] as a means of protecting the side chain of some corticosteroids. The method was later used (as far back as 1964) by Brown and MacBride[89] for assignment of relative configuration to flavandiols. Acetonide formation was further and recently exploited by Rychnovsky and Skalitzky[90] and Evans *et al.*[91] for stereochemical assignment of compounds containing a 1,3-diol moiety. According to these authors, acetonides formed from *syn* 1,3-diols exist in a well defined chair conformation with the two methyl group substituents in equatorial positions whereas those from *anti* 1,3-diols exist in a twist conformation in order to avoid the 1,3-diaxial interactions which would be present in a chair conformation.

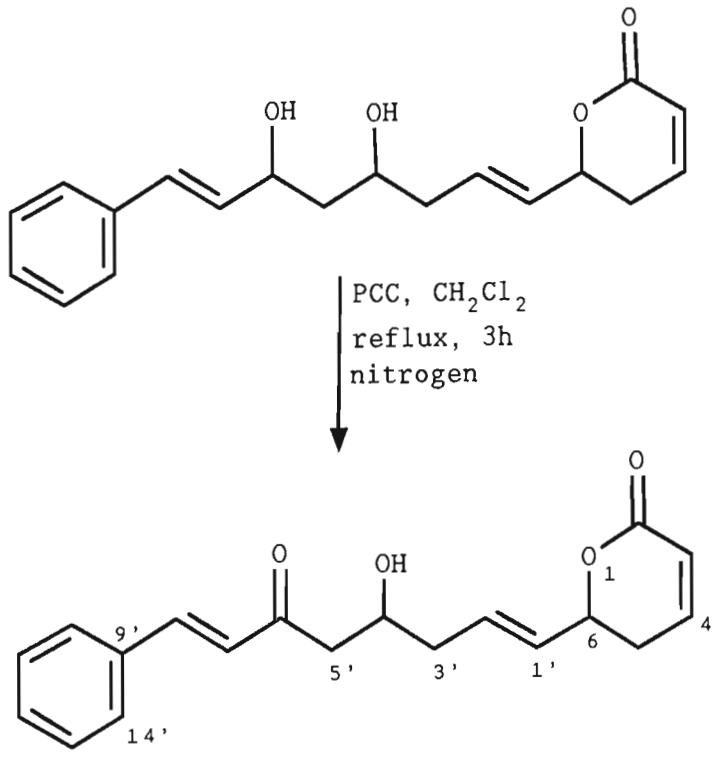
The ^{13}C NMR chemical shifts of the acetonide methyl groups can

be used to distinguish between these two conformations and thus the relative stereochemistry at these centres.[90,91] The ^{13}C NMR spectrum of *syn* 1,3-diol acetonides shows an axial methyl group at 19 ppm and an equatorial methyl group at 30 ppm, whereas that of *anti* 1,3-diol acetonides shows two methyl groups at 25 ppm.

The acetal carbon in acetonides, according to Rychnovsky and Skalitzy[90] and Evans *et al.*,[91] also follow a stereoregular pattern where in *anti* diols it resonates above 100ppm, while in *syn* diols acetonides it appears below 100ppm. Based on the above analysis, the relative stereochemistry of **81** at C-4' and C-6' wherein the acetal carbon C-15' resonates at 100.26ppm and both methyl carbons C-16' and C-17' resonate at approximately 25ppm (**Appendix 82c**), was deduced as *anti* as indicated in structures **81** and **82**.

2.2.2.1.2. Oxidation of Cryptofolione

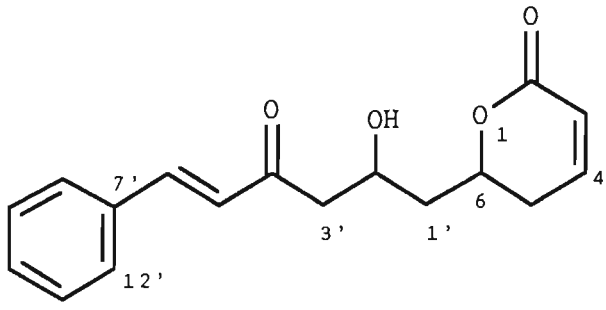
Treatment of cryptofolione (**81**) with pyridinium chlorochromate[92] (**Experimental**, 3.3.3.3.1b.) effected oxidation at the allylic hydroxyl group (C-6') only to give compound **83** (**Scheme 13**). Both the ^{1}H NMR (**Appendix 83a**) and ^{13}C NMR



83

Scheme 13

(**Appendix 83c**) and the IR spectra supported the occurrence of this transformation. A molecular formula of $C_{19}H_{20}O_4$ was supported by a HR-MS peak at 312. A hydroxyl band (3460 cm^{-1}) and a ketone band (1720 cm^{-1}) were present in the IR spectrum. Furthermore, an IR absorption band at 1610 cm^{-1} indicated the presence of an α,β-unsaturated carbonyl system. A carbon signal at 200.46 ppm confirmed the presence of a ketone conjugated to a styryl moiety. The oxidation of the hydroxyl group at C-6' resulted in the shifting of H-7' further downfield (δ7.51) - even more downfield than the benzene protons - and reduced the splitting patterns of both hydrogens (on a double bond, C-7' and C-8') to a doublet each (J=16Hz). This oxidation product **83** is structurally similar to kurzilactone (**84**) which has very recently been isolated from



84

Cryptocarya kurzi by Fu *et al.*.[93] The ^{13}C NMR data of **83** compared with that of kurzilactone (**84**) in **Table 5**. The difference between the two 6-substituted 5,6-dihydro-α,β-unsaturated-α-pyrones is that the double bond at C-1'and C-2' observed in **83** is absent absent in kurzilactone (**84**).

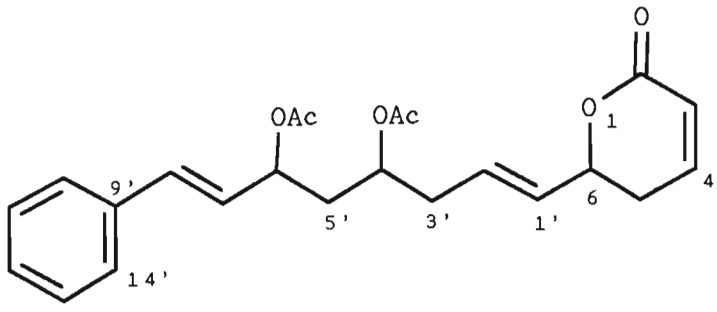
Table 5: ^{13}C NMR ($CDCl_3$) data for compound **83** (50MHz) and **84** (62.5MHz)

Compound 83		Compound 84	
Position	δc	Position	δc
2	164.8s	2	164.2s
3	121.3d	3	121.4d
4	145.0d	4	145.3d
5	29.62d	5	30.0t
6	77.95d	6	74.8d
1'	129.8d		
2'	130.9d		
3'	39.3t	1'	41.8t
4'	67.2d	2'	64.1d
5'	46.2t	3'	47.0t
6'	200.46s	4'	200.3s
7'	126.1d	5'	126.2d
8'	143.7d	6'	143.9d
9'	134.1s	7'	134.2s
10', 14'	128.4d	8', 12'	128.5d
11', 13'	129.0d	9', 11'	129.0d
12'	130.8d	10'	130.9d

Assignment of C-18',12' and C-9',11' may be reversed.

2.2.2.1.3. Acetylation of Cryptofolione

Acetylation of cryptofolione (**81**) using the classical method involving acetic anhydride and pyridine (**Experimental**, 3.3.3.3.1c.) yielded compound **85**. Both hydroxyl groups were acetylated.

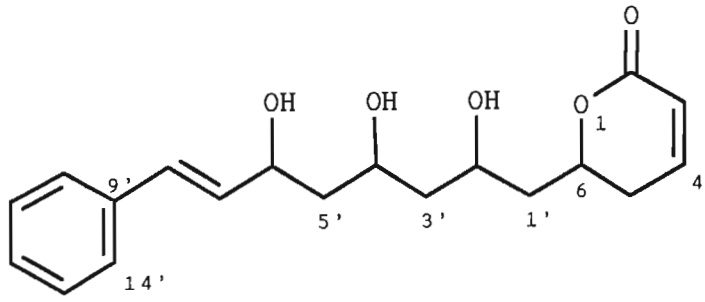


85

The ^{13}C NMR spectrum of **85** (**Appendix 85c**) shows two methyl groups (21.17 ppm and 21.06 ppm) and two ester quaternary carbons at 170.47 ppm and 170.19 ppm. Further evidence of the successful acetylation of **81** was the disappearance of the signal (δ3.46) due to the two hydroxyl groups. The acetyl groups resulted in the downfield shifting of H-4' and H-6' to δ5.05 and δ5.46 respectively (**Appendix 85a**).

2.2.3. Myrtifolione (86)

A crystalline trihydroxy styryl α-pyrone **86** was isolated from the chloroform extract of *Cryptocarya myrtifolia* (**Experimental**, 3.4.1.). The name myrtifolione was proposed for this hygroscopic 6-substituted 5,6-dihydro-α-pyrone. A molecular formula of $C_{19}H_{24}O_5$ was deduced for myrtifolione (**86**) from analysis of both the ^{1}H (**Appendix 86a**) and ^{13}C (**Appendix 86c**) NMR data and comparison with the spectral data of **82**. The ^{13}C NMR spectrum showed 17 resolved signals comprising (from DEPT) four methylene, seven methine and two quaternary carbons. The



86

shortfall of four peaks could be explained from the superimposition of signals due to two pairs of equivalent methine groups on the benzene ring, C-10' and C-14' (127.26ppm) and C-11' and C-13' (129.48ppm) and the closeness of the two peaks due to C-1' (46.83ppm) and C-3' (46.91ppm). The molecular structure of myrtifolione (**86**) was arrived at by comparison of its spectral data with those of cryptofolione (**81**). The absence of the exocyclic double bond at C-1'and C-2' was apparent from both the ^{1}H NMR (peaks at δ5.63 and δ5.82 are missing) and ^{13}C NMR (signals at 121.61 ppm and 131.33 ppm are missing) spectra. The double bond was replaced by a methylene group at C-1' and a methine and a hydroxyl group at C-2'. The methylene group (46.83 ppm) coupled with the proton H-6 in the ^{1}H-^{1}H COSY spectrum (**Appendix 86d**). The fact that no coupling was observed between any two sp^3 methine groups in the ^{1}H-^{1}H COSY spectrum confirms the 1,3-relationship of the hydroxyl groups as presented in the proposed structure.

2.3. MACROLIDES

The four compounds, cryptocaryolide A (**87**) and B (**88**) and foliolide A (**90**) and B (**91**), which were isolated from the three *Cryptocarya* species, *C. woodii, C. latifolia and C. myrtifolia*, will be discussed in turn. To our knowledge this is the first time that macrolides have been isolated from higher plants.

These compounds fall short of the definition of Devon and Scott[43] in that they don't contain a polyene group as a structurally recognizable unit. The absence of one or more sugar units in these compounds and the fact that some of them contain a lactone ring with more than 16 atoms, disqualifies them as macrolides according to the definition of Mazzei *et al.*.[45] Saturated and branched aliphatic chains are not a feature in these compounds hence the definition of Manitto[44] does not fully suit these compounds. (See the Introduction for the definition of macrolides according to respective authors. However, these four compounds **87**, **88**, **90** and **91** fit the definition of macrolides in that they are macrocyclic lactones. All of these compounds were isolated as viscous oils.

Trivial names, which are mainly based on the names of the plant sources, have been proposed for the macrolides isolated. The suggested names bear the suffix 'olide' by virtue of the lack of attachment of a sugar moiety to these molecules.

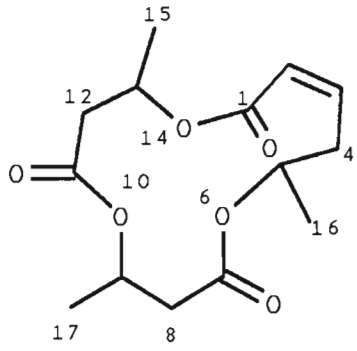
The numbering system of Vesonder *et al.*[50] has been adopted and adapted here. The carbonyl carbon of the ester moiety is numbered 1. In the two macrolides with a double bond adjacent to an ester moiety (**87** and **88**), it is this ester carbon that has been given the first priority in numbering. The rest of the atoms in the ring (including oxygen atoms) are then

numbered, in the direction opposite to the oxygen atom of the ester group, followed by the bridgehead carbon (in compounds **90** and **91**) and the substituents.

2.3.1. CRYPTOCARYOLIDE A (**87**)

Cryptocaryolide A (**87**) was isolated from the benzene extract of *Cryptocarya latifolia* (**Experimental**, 3.3.3.1b). The proposed structure of cryptocaryolide A, a 14-membered macrotriolide (three ester moieties) was based on NMR, IR and mass spectroscopic analysis. A molecular formula of $C_{14}H_{20}O_6$ was supported by a HR-MS peak at 284. The ^{13}C NMR (**Appendix 87c**) showed 13 resolved peaks, comprising three methyl, three methylene, five methine and three quaternary carbons (DEPT analysis). However, an sp^3 methine resonance at 67.62 ppm has intensity almost double that of other methine groups and is considered to represent two superimposed signals. The mass spectrum showed a molecular ion peak at 284. Evidence for the presence of an α,β-unsaturated ester or ester group (1690 cm^{-1}) was present in the IR spectrum.

The part of the ring of cryptocaryolide A that includes O-14, C-1, C-2, C-3, C-4, and C-5 is reminiscent of the α,β-unsaturated-δ-lactone moiety in 6-substituted



87

5,6-dihydro-α-pyrones. Proton H-2 in structure **87** resonates at δ6.00 (**Appendix 87a**) and is coupled to H-3 (J=9.76Hz) indicative of a cis olefinic function adjacent to a carbonyl group. Proton H-2 is also coupled to H-4 by long range coupling to the two protons attached to C-4 ($J_{2,4ax}$=0.96Hz, $J_{2,4eq}$=2.44Hz). The deshielding of H-3 relative to H-2 is typical of a proton attached to the β-carbon of an α,β-unsaturated carbonyl system. The signal for H-3, as in 6-substituted 5,6-dihydro-α-pyrones, appears as a triplet of doublets from its coupling to H-2 and also to $H\text{-}4_{ax}$ ($J_{3,4ax}$=2.27Hz) and $H\text{-}4_{eq}$ ($J_{3,4eq}$=5.80Hz).

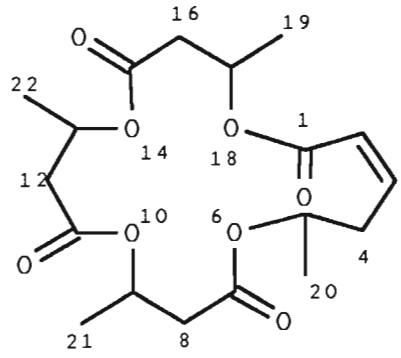
The carbon at C-4 (29.03 ppm) has a typical carbon chemical shift for a methylene carbon adjacent to an α,β-unsaturated ester moiety. Both the $^1H\text{-}^1H$ COSY (**Appendix 87d**) and the $^1H\text{-}^1H$ RELAYED COSY (**Appendix 87e**) show that the methine groups are not coupled to each other, hence no two methine groups are adjacent to each other in the structure. The $^1H\text{-}^1H$ COSY spectrum also show coupling between H-4 to both H-2 and H-3 thus confirming the presence of this moiety in structure **87**. The carbon (67-75ppm) and the proton (δ4.4-5.1) chemical shifts of the three methine groups indicate that these methine groups are each attached to an oxygen atom of an ester group. The methylene carbons at 38.94 ppm and 40.12 ppm have chemical shifts typical of a CH_2 group attached to the carbonyl carbon

of an ester group. The methine group at C-5 is coupled to the methylene group at C-4 (see ^{1}H-^{1}H COSY spectrum) and thus the two groups must be adjacent to each other. The ^{1}H-^{1}H COSY spectrum also shows the methyl group at 19.97 ppm to be attached to one of the methine groups at 67.62 ppm. The ^{1}H-^{13}C DELAYED HETCOR spectrum (**Appendix 87g**) shows this methyl group (19.97 ppm) to also couple to the methylene group at 40.12 ppm. This leaves one methylene group (38.94 ppm) which therefore must be attached to the other methine group at 67.62 ppm. Connectivity of the other methyl groups at 21.01 ppm and 21.14 ppm could not be determined with certainty since their proton signals superimpose with other signals upfield. The methyl group at 21.01 ppm is either connected to the methine group at 67.62 (C-9) or to that at 74.82 ppm (C-5). The same applies to the methyl group at 21.14 ppm.

2.3.2. CRYPTOCARYOLIDE B (**88**)

Cryptocaryolide B (**88**) was isolated from both *Cryptocarya woodii* and *Cryptocarya latifolia*. However, *C. latifolia* was found to be the richer source of this compound (**Experimental**, 3.3.2.2. and 3.3.3.1.).

The molecular structure of cryptocaryolide B (**88**), an 18-membered macrotetrolide (four ester groups), was derived from IR, NMR and Mass Spectroscopic data. In the CI-MS, ions at m/z 371 $[M+H]^{+}$, 399 $[M+C_2H_5]^{+}$ and 412 $[M+C_3H_7]^{+}$ supported the molecular ion at 370 mass units. A molecular formula of $C_{18}H_{26}O_8$ was supported by a HR-MS peak at 370. The ^{13}C NMR spectrum (**Appendix 88c**) showed 17 resolved peaks comprising of (from DEPT), four methyl, three methylene, six methine and four



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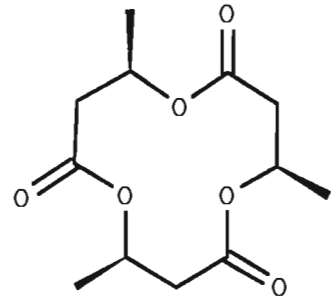


89

quaternary carbons. However, the methylene group at 38.96 ppm was more intense than all other methylene peaks and was considered to represent two superimposed signals. An α,β-unsaturated ester or ester group (1730 cm^{-1}) was evident in the IR spectrum. As was observed in the case of cryptocaryolide A (**87**), proton H-2 in structure **88** resonates at δ6.01 (**Appendix 88a**) and is coupled to H-3 (J=9.76Hz) indicating a *cis* olefinic moiety next to a ester group. The long range coupling of H-2 to the two protons at C-4 ($J_{2,4ax}$=1.16Hz, $J_{2,4eq}$=2.49Hz) was also evident in the spectrum. Proton H-3 was deshielded relative to H-2; this is usual of a proton attached to the β-carbon of an α,β-unsaturated system.

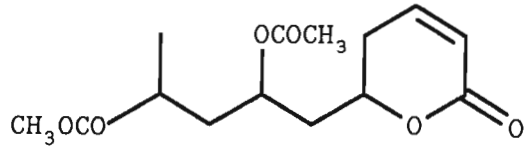
As in **87**, the ^{1}H-^{1}H COSY (**Appendix 88d**) and ^{1}H-^{1}H DELAYED COSY (**Appendix 88e**) spectra of compound **88** show no immediate or long range correlation respectively between any two sp^3 methine protons. A methine proton instead seemed to couple to both a methyl and a methylene proton. On the other hand, coupling between the proton at C-4 and carbons C-2 and C-3 were evident in the ^{1}H-^{13}C DELAYED HETCOR Spectrum (**Appendix 88g**). The ^{1}H-^{13}C DELAYED HETCOR spectrum also showed coupling between protons at C-19 and the carbon at C-16 and one of the methine carbons at about 68 ppm.

The structure of cryptocaryolide B (**88**) compares with that of

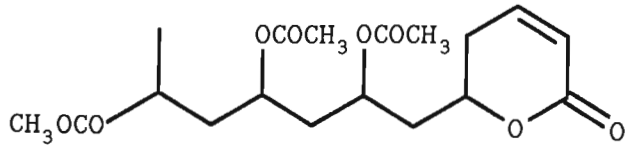
cryptocaryolide A (**87**). Assuming that the basic units in the two structures are a 3-hydroxybutanoic acid and a 2Z-5-hydroxy-2-hexenoic acid, **88** would have four units of 3-hydroxybutanoic acid compared to three units in **87.** The ^{13}C NMR spectra of the 3-hydroxybutanoic acid moieties of the two macrolides compare with that of triolide **89** (R,R,R-4,8,12-trimethyl-1,5,9 -trioxadodeca-2,6,10-trione) synthesized by Seebach *et al.*[94] [^{13}C NMR (100MHz, $CDCl_3$, TMS): δ20.76 (CH_3), 42.15 (CH_2), 68.86 (CH) and 170.02 (CO)].

Revised structures of Cryptocaryolide A (87) and B (88)

Additional spectroscopic data obtained from HMBC experiments (obtained after the submission of this thesis to examiners) necessitates the revision of the structural formulae **87** and **88**. The revised structures are proposed and seem to fit well with NMR data.



87



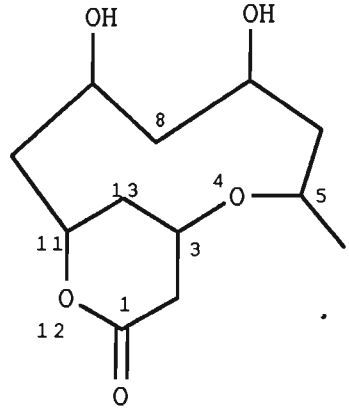
88

Contrary to the initial classification of these two compounds as macrolides, **87** and **88** should be reclassified as 6-substituted 5,6-dihydropyrones.

2.3.3. FOLIOLIDE A (90)

Foliolide A (**90**) was isolated from the chloroform extract of *Cryptocarya latifolia* (**Experimental**, 3.3.3.1.). There was evidence of the presence of this compound in *Cryptocarya myrtifolia* (from a TLC profile) albeit in small amounts.

The planar structure of this bicyclo-macromonolide **90** was deduced by analysis of its ^{1}H and ^{13}C NMR data aided by 2D NMR experiments. The ^{13}C NMR spectrum (**Appendix 90c**) shows 12 resolved peaks comprising, one methyl, five methylene, five methine and one quaternary carbons (DEPT analysis). An ester group (1675 cm^{-1}) was present in the IR spectrum. This was confirmed by a quaternary carbon resonating at 169.56 ppm.



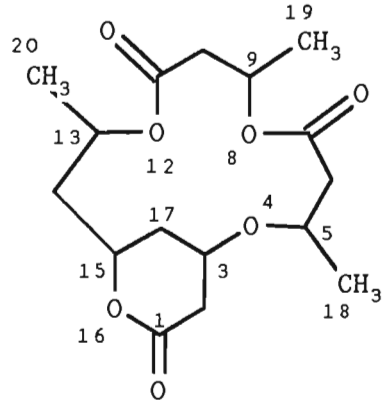
90

It was evident from the ^{1}H-^{1}H COSY (**Appendix 90d**) that no two methine groups coupled with one another (i.e. not attached to each other). The ^{13}C chemical shifts of C-3, C-5, C-7, C-9 and C-11 (65.94-72.62) (**Appendix 90c**) indicate that these five methine carbons are attached to oxygen atoms. The downfield shift of H-11 (δ4.89) in the ^{1}H NMR spectrum (**Appendix 90a**) suggests that C-11 is connected to an ester oxygen. Two of the protons at δ3.67-4.11 show no coupling to carbons in the ^{1}H-^{13}C HETCOR spectrum (**Appendix 90f**) thus indicating the existence of two hydroxyl groups in the molecule. The presence of two hydroxyl groups was further substantiated by running a D_2O NMR experiment (**Appendix 90a**) which resulted in the disappearance of the peaks due to the hydroxyl groups.

Strong coupling of the methyl (C-13) protons in the ^{1}H-^{13}C DELAYED HETCOR (5Hz) spectrum (**Appendix 90g**) to carbons at 44.89 ppm (CH_2, C-6) and 68.13 (CH, C-5) indicate the presence of a propyl moiety (CH_3-CH-CH_2). The downfield shift of H-2 (δ2.86) indicates that the carbon to which these protons are attached bears an ester group. This was also evidenced by a strong coupling of H-2 to C-1 (169.56 ppm) in the ^{1}H-^{13}C DELAYED HETCOR (12Hz) spectrum (**Appendix 90g**). The DELAYED HETCOR (12Hz) spectrum also reveals a moderate coupling of H-3 (δ4.40) and a weak coupling of H-11 (δ4.89) to C-1. The weak H-11 to C-1 coupling is typical of a through-bond coupling interrupted by a heteroatom. The ^{1}H-^{1}H RELAY 2 COSY spectrum (**Appendix 90e**) shows weak coupling between H-3 and H-11 (typical of a through-four-bonds coupling). ^{1}H-^{1}H COSY experiment (**Appendix 90d**) shows coupling of H-12 (δ2.01) to both H-3 (δ4.41) and H-11 (δ4.89). The ^{1}H-^{1}H COSY also shows coupling between H-12 and H-2 (δ2.86). The above experiments indicate that H-13 represents the bridgehead methylene group.

2.3.4. FOLIOLIDE B (91)

The name foliolide B was proposed for the compound **91**, which was isolated from *Cryptocarya latifolia* (**Experimental**, 3.3.3.2.). The molecular structure and formula ($C_{16}H_{24}O_7$) of this bicyclo-macrotriolide is based on the mass, IR, ^1H and ^{13}C NMR spectroscopic data. The mass spectrum showed a molecular ion at m/z 328. A molecular formula of $C_{26}H_{24}O_7$ was supported by a HR-MS peak at 328. The carbon-13 NMR spectrum (**Appendix 91c**) shows 15 resolved signals, with signals at 170.49ppm consisting of two superimposing peaks. The 16 carbon peaks comprise, three methyl, five methylene, five methine and five quaternary carbons (DEPT analysis). Assignment of protons to the respective carbons was done with the aid of the ^1H-^{13}C HETCOR spectrum (**Appendix 91f**).



91

The chemical shifts of three of the quaternary carbons at 169.68-170.49ppm indicate that these must be ester carbons. This was confirmed by an IR band at (1740 cm^{-1}). The chemical shifts of C-3, C-5, C-9, C-13 and C-15 (63.11-72.85ppm) signify that these five methine carbons are attached to oxygens. The

downfield shift, in the ^{1}H NMR spectrum, of H-9, H-13 and H-15 (δ4.88-5.09) especially suggest that C-9, C-13 and C-15 should bind to ester oxygens; therefore H-3 (δ4.33) and H-5 (δ4.96) must be attached to ether oxygens. No coupling was observed between any two methine protons in the ^{1}H-^{1}H COSY spectrum (**Appendix 91d**) indicating that no methine groups immediately connect to each other. The implication of the above analysis is that the two methine group (C-3 and C-5) are connected to each other through an ether linkage since evidence for the presence of a hydroxyl group is lacking.

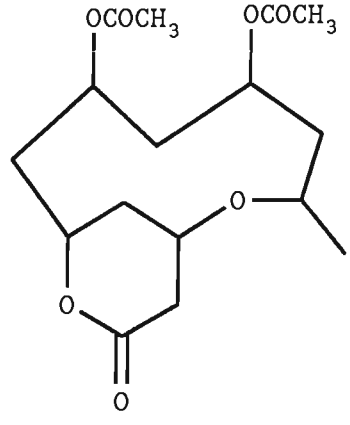
Structural elucidation of the α-pyrone moiety was not difficult. The α-pyrone moiety present in foliolide A (**90**) was found to exist in foliolide B (**91**). This was evidenced by an ester carbon peak at 169.68ppm, two methylene signals at 36.11ppm and 29.50ppm, two methine peaks at 65.79ppm and 72.85ppm in the ^{13}C spectrum of **91**. Both the chemical shifts of H-2 and C-2 and the multiplicity of H-2 in foliolide A (**90**) and foliolide B (**91**) are comparable. The ^{1}H-^{1}H COSY spectrum (**Appendix 91d**) shows coupling of H-3 to both H-2 and H-15. Proton H-2 (δ2.81) also shows correlation in the ^{1}H-^{13}C DELAYED HETCOR (7Hz) spectrum (**Appendix 91g**) to C-2, C-3 and C-17. The ^{1}H-^{1}H COSY spectrum further indicates coupling of H-15 (δ4.88) to both H-14 and H-17. ^{1}H-^{13}C DELAYED HETCOR (12Hz) (**Appendix 91g**) reveals coupling of H-3 to C-1. The above couplings confirm the presence of the α-pyrone moiety and the fact that C-17 is the bridgehead carbon.

Detection of connectivities in the rest of the structure was not easy due to overlapping of peaks representing four methylene and two methyl groups in the lower field of the ^{1}H NMR spectrum. Coupling of H-15 to both H-14 and H-17 was confirmed in the ^{1}H-^{1}H COSY spectrum. It is interesting to note that in the ^{1}H-^{13}C HETCOR spectrum of both foliolide A (**90**) and B (**91**) peaks due to the two protons on C-10 and C-14, respectively, are distributed as three groups of signals. Such distribution of peaks is typical in cyclic structures. The

propyl moiety consisting of C-6, C-5 and C-18 was exhibited by coupling of H-5 (δ4.96) to both H-18 and H-6 in the ^{1}H-^{1}H COSY spectrum, and coupling of H-18 (δ1.23Hz) to both C-6 and C-5. Therefore C-9 (63.11ppm) must be attached to C-10 (39.87ppm) and C-19 (21.23 or 21.34ppm) and C-13 (68.35ppm) must be attached to C-14 (36.87ppm) and C-20 (21.23 or 21.34ppm). Precise assignment of the two methyl groups C-19 and C-20 was difficult.

Revised structure of Foliolide B (**91**)

The revision of the structure of **91** indicates that this macrolide is the diacetate of Foliolide A (**90**) as illustrated in the structural formula below.



91

2.4. Biological activity

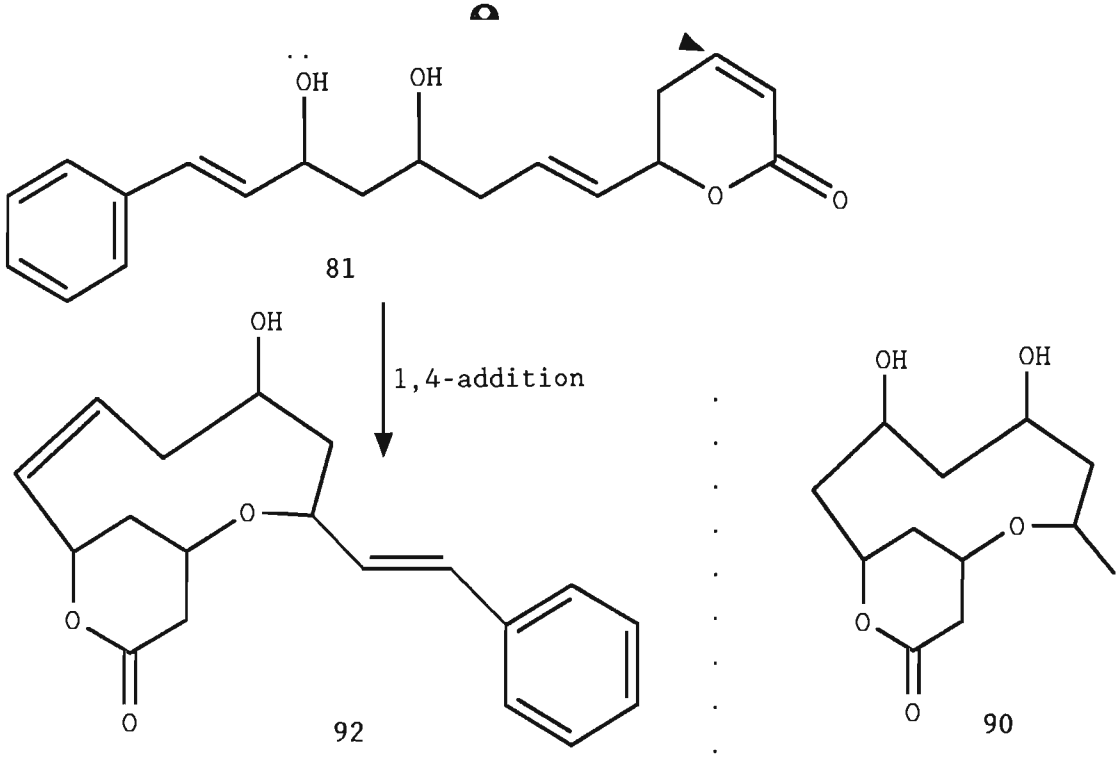
The biological activity of the compounds isolated in this work, except for goniothalamin (**45**) has never been studied before. Previous studies on **45** revealed that this 6-substituted 5,6-dihydro-α-pyrone exhibits central nervous system activity,

antifungal activity[84] and was found to induce foetal abnormalities in mice.[95]

Similarities in the core structure of macrolides such as enterobactin (**63**), which stimulates bacterial growth,[62] and cryptocaryolide A (**87**) and B (**88**) may tempt one to suggest that **87** and **88** to be of biological importance. Indeed plans are underway to study the pharmacology of these structurally interesting compounds.

2.5. Concluding Observations

Given the presence of an α-pyrone moiety in some of the macrolides it is tempting to speculate that a relationship exists between the α-pyrones and the bridged cyclic compounds terminating in an α-pyrone ring. For example, the transformation of compound **81** to the macrocycle **92** via Michael addition of the allylic alcohol group on the α,β-unsaturated system (**Scheme 14**) is an attractive possibility. Although **92** has not been found in the bark of either



Scheme 14

C. latifolia or *C. myrtifolia* its resemblance to **90** is quite striking. For example, both **90** and **92** are 12-membered bicyclic macromonolides with a methylene group at the bridgehead.

CHAPTER THREE

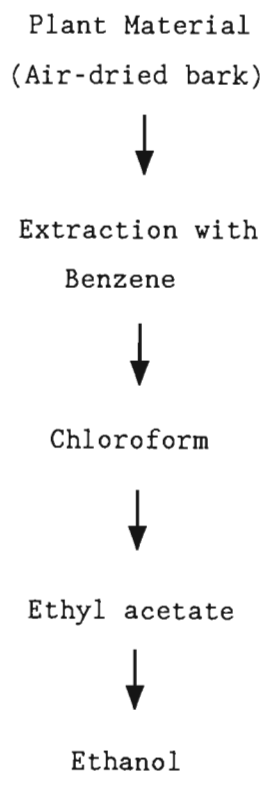
3. **EXPERIMENTAL**

3.1. Instrumentation and Chemicals

Infrared spectra were obtained using a Shimadzu FTIR-4300 spectrophotometer. Optical rotations ([α]) were recorded on a Perkin-Elmer 241 polarimeter. Mass spectra were obtained using Hewlett Parkard gas chromatographic mass spectrometer (HP5988A) and a Varian high resolution mass spectrometer. NMR spectra were recorded on a Varian Gemini 200 and a U400 instruments, using TMS as the internal standard, in chloroform (unless stated otherwise). Melting points (mp) were recorded on a Kofler hot-stage apparatus and are uncorrected. The flash chromatographic technique of Still *et. al.*[95] was employed in running columns on silica gel (Merk 60, 230-400 mesh). The Harison Research (Model 7924T) chromatotron was used to run chromatotron plates on silica gel (Merck 60, PF_{254}, 7749) using UV (254nm) for detection. Separations in both the flash chromatography and the chromatotron were monitored by thin layer chromatography (TLC) using small strips of precoated Kieselgel 60, F_{254}, Merck plastic sheets. Ultraviolet light (254nm) and the anisaldehyde stain[96] were used to detect components on TLC plates. Reactions were monitored with TLC.

3.2. Preparation and Extraction of Plant Material

The plant specimens were identified by Mr. R. Scott-Shaw of the Natal Parks Board. The plant bark were collected from natural specimen, air-dried and milled. Except for *Cryptocarya myrtifolia*, the fine bark was extracted sequentially using a Soxhlet apparatus as illustrated below:



The milled bark of *Cryptocarya myrtifolia* was first extracted with absolute ethanol - this was owing to the long delay in further extractions of the bark. After the solvent (ethanol) was removed, water was added and the extract sequentially extracted with benzene, chloroform and ethyl acetate.

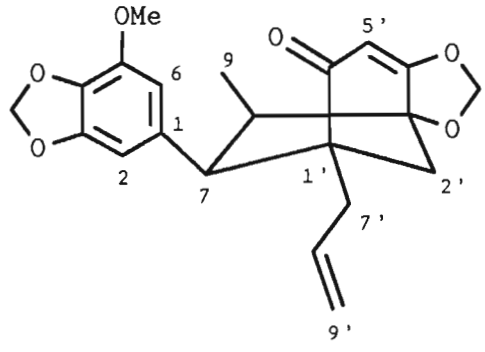
3.3. Fractionation and Isolation of Compounds

Each of the solvents was evaporated to dryness, and the components present investigated using TLC. Thin layer chromatography was employed to develop solvent systems used in the fractionation of extracts on the column or chromatotron. Fractionation of the plant extracts, the isolation of individual compounds and reactions on some of the isolated components are discussed under the plant species below.

3.3.1. *Ocotea bullata*

Bark from a natural specimen of *Ocotea bullata* was collected in the Karkloof range, Natal, South Africa in October 1990. The dried bark (4Kg) was extracted as described in 3.2..

3.3.1.1. Ocobullenone (**68**)



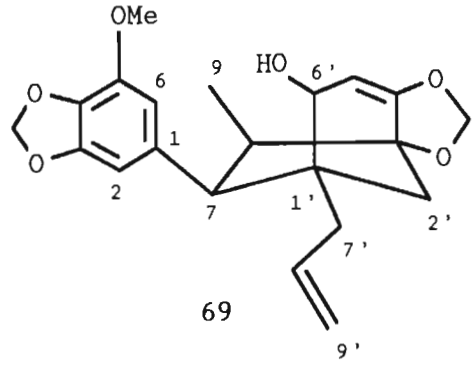
68

$C_{21}H_{22}O_6$
MW=370

The crude chloroform extract (21.3g) was fractionated by column chromatography (cc) using chloroform as eluent. The fractions containing ocobullenone (**68**) were concentrated and further subjected to repeated fractionation by cc using ethyl acetate-hexane (7:2) as eluent to afford a pure crystalline compound (**68**) (1.56g).
Rel-(7R, 8R, 1'R, 3'R)-$\Delta^{8'}$-5-methoxy-bis-3,4;3',4'-methylenedioxy-1',2',3',6'-tetrahydro-6'-oxo-7.1',8.3'-neolignan (**68**). Found: M^+ 370.1424; $C_{21}H_{22}O_6$ requires 370.1415. White crystals, mp 151°C, $[\alpha]_D^{25}$ +204° ($CHCl_3$; c0.16). EI-MS m/s (relative intensity (rel. int.)): 370 $[M]^+$ (15), 192 (11), 178 (100), 137 (29), 91 (27), 69 (46). $IR_{\upsilon max}cm^{-1}$ (KBr disc): 2930, 1641, 1510, 1427, 1363, 1205, 1176, 1049, 929. ^1H NMR: δ0.85 (3H, d, J=7.42Hz, H-9); 2.05 (1H, dd, J=14.18, 8.94Hz, H-7'a); 2.06 and 2.26 (2H, dd, J=10.50Hz, H-2'); 2.56 (1H, dddd, 14.11, 5.75, 1.39, 1.35Hz, H-7'b); 2.87 (1H, dq, J=11.90, 7.40Hz, H-8); 3.37 (1H, d, J=11.90Hz, H-7); 3.83 (3H, s, OMe); 5.06 (2H, m, H-9'); 5.57 (1H, s, H-5'); 5.65 (2H, dd, J=0.33Hz, O_2CH_2-3',4'); 5.75 (1H, m, H-8'), 5.88 (2H, dd, J=1.52Hz, O_2CH_2-3,4); 6.12 (2H, dd,

J=1.70Hz, H-2,6). ^{13}C NMR: δ14.2 (C-9); 37.7 (C-7'); 44.4 (C-8); 46.6 (C-2'); 54.0 (C-7); 56.6 (OMe); 59.8 (C-1'); 91.2 (C-3'); 98.4 (C-5'); 101.3 (O_2CH_2-3',4'); 101.5 (O_2CH_2-3,4); 104.8 (C-6); 111.2 (C-2); 118.5 (C-9'); 129.8 (C-1); 134.4 (C-4, C-8'); 142.7 (C-5); 148.5 (C-3); 177.5 (C-4'); 200.2 (C-6').

3.3.1.1a. Reduction of ocobullenone



69

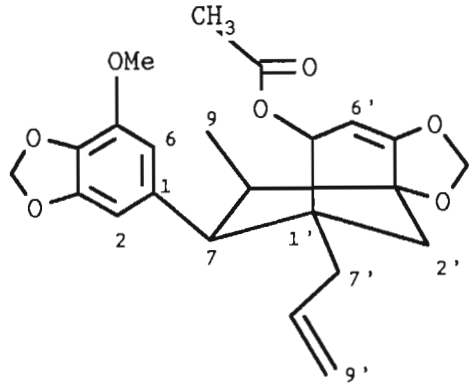
$C_{21}H_{24}O_6$
MW=372

Ocobullenone (**68**) (100mg, $2.702x10^{-4}$ mol) in dry diethyl ether (25ml) was added dropwisely to a stirred suspension of $LiAlH_4$ (16mg, $4.200x10^{-4}$ mol) in dry diethyl ether (25ml) at such a rate as to cause a mild reflux. The reaction mixture was cooled in an ice-bath and water (10ml), NaOH (15%, 10ml), then water (30ml) were added and the mixture stirred for 30min. The aqueous layer was extracted with diethyl ether and the combined organic solvents dried over anhydrous magnesium sulphate. The reaction yielded 98mg of the alcohol, a yellow viscous oil.

$[\alpha]_D^{25}$ + 116° ($CHCl_3$; c0.42). EI-MS m/z (rel. int.): 372 $[M]^+$ (67), 283 (20), 242 (29), 192 (100), 141 (29), 111 (21), 91 (19). $IR_{\nu max}cm^{-1}$ (Nujol): 3560, 2910, 1710, 1640, 1515, 1460, 1328, 1260, 970, 940. ^1H NMR: δ0.97 (3H, d, J=7.40Hz, H-9); 1.56 (1H, d, J=11.90Hz, OH); 1.80, 2.20 (2H, dd, J=10.40, H-2'); 2.25 (1H, dd, J=7.15, 13.78Hz, H-7'a); 2.54 (1H, dd, J=7.15, 13.78Hz, H-7'b); 2.78 (1H, dq, J=7.30, 12.40Hz, H-8); 3.23 (1H, d, J=12.40Hz, H-7); 3.90 (3H, s, OMe); 4.47 (1H, dd, J=2.70, 11.90Hz, H-6'); 4.99 (1H, d, J=2.70Hz, H-5'); 5.16 (2H,

m, H-9'); 5.38 (2H, dd, J=0.8Hz, O_2CH_2-3',4'); 5.78-5.99 (1H, m, H-8'); 5.95 (2H, dd, J=1.4Hz, O_2CH_2-3,4); 6.58 (1H, d, J=1.4Hz, H-2); 6.63 (1H, d, J=1.4Hz, H-6). ^{13}C NMR: δ13.4 (C-9); 40.6 (C-7'); 44.4 (C-2'); 46.8 (C-8); 52.0 (C-7); 52.4 (C-1'); 56.6 (OMe); 77.5 (C-6'); 86.9 (C-3'); 95.0 (C-5'); 98.8 (O_2CH_2-3',4'); 101.5 (O_2CH_2-3,4); 104.7 (C-6); 111.1 (C-2); 118.7 (C-9'); 133.1 (C-1); 134.3 (C-4, C-8'); 142.5 (C-5); 148.9 (C-3); 154.7 (C-4').

3.3.1.1b. Acetylation of the alcohol **69**



69a

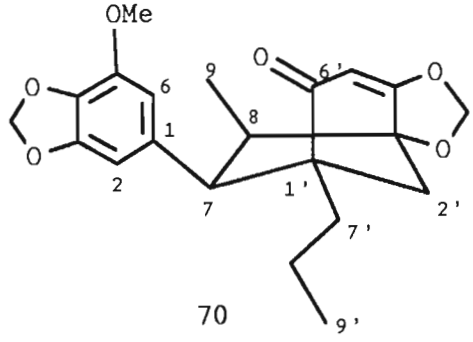
$C_{22}H_{26}O_7$
MW=414

The alcohol (**69**) (50mg, $1.351x10^{-4}$ mol) was dissolved in pyridine (3ml) and acetic acid anhydride (3ml) was added. The reaction mixture was stirred overnight. Water (20ml) was added and the mixture extracted with chloroform (30ml X 3). The organic layer was washed with hydrochloric acid (30ml, 0.5N) and dried over anhydrous $MgSO_4$ and concentrated to yield 45mg of the acetate, **69a**, a yellow oil.

$[\alpha]_D^{25}$ + 109° ($CHCl_3$; c0.42). EI-MS m/z (rel. int.): 414 $[M]^+$ (4), 324 (31), 283 (7), 192 (100), 150 (28), 109 (10), 91 (9). $IR_{\nu max}cm^{-1}$ (Nujol): 2920, 1735, 1692, 1635, 1463, 1379, 1135, 938, 834. ^1H NMR: δ0.95 (3H, d, J=7.33Hz, H-9); 1.62 (3H, s, $COOCH_3$); 1.92 (1H, d, J=10.46Hz, H-2'a); 2.12 (1H, dd, J=7.54, 13.98Hz, H-7'a); 2.29 (1H, d, J=10.46Hz, H-2'b); 2.32 (1H, dd, J=7.38, 13.88Hz, H-7'b); 2.77 (1H, dq, J=12.61, 7.33Hz, H-8); 3.25 (1H, d, J=12.62Hz, H-7); 3.87 (3H, s, OCH_3); 4.97 (1H, d, J=2.85Hz, H-5'); 5.13 (2H, m, H-9'); 5.43 (2H, dd, J=0.92Hz, O_2CH_2-3',4'); 5.53 (1H, d, J=2.84Hz, H-6'); 5.79 (1H, m, H-8');

5.92 (2H, d, J=1.39Hz, O_2CH_2-3,4); 6.50 (2H, dd, J=1.55Hz, H-2, H-6). ^{13}C NMR: δ13.56 (C-9); 20.64 ($COO\underline{C}H_3$); 40.77 (C-7'); 44.69 (C-2'); 46.05 (C-8); 51.32 (C-1'); 53.09 (C-7); 56.54 (OCH_3); 77.92 (C-6'); 87.14 (C-3'); 90.54 (C-5'); 98.98 (O_2CH_2-3',4'); 101.04 (O_2CH_2-3,4); 107.36 (C-6); 112.59 (C-2); 118.97 (C-9'); 132.29 (C-1); 133.48 (C-4); 133.67 (C-8'); 141.66 (C-5); 147.21 (C-3); 156.60 (C-4'); 170.45 ($\underline{C}OOCH_3$).

3.3.1.1c. Hydrogenation of Ocobullenone



70

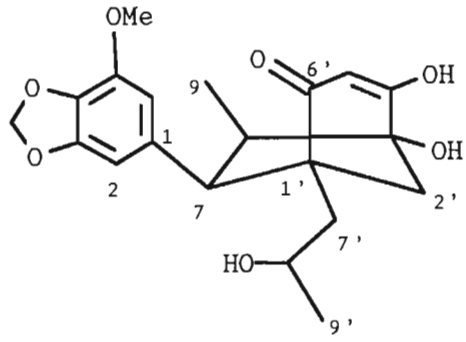
$C_{21}H_{24}O_6$
MW=372

A suspension of **68** (45mg, $1.215x10^{-4}$ mol) and palladium on charcoal was magnetically stirred under hydrogen for 3h to yield the crystalline compound **70** (40mg).

$[\alpha]_D^{23}$ + 152° ($CHCl_3$; c0.243). ^1H NMR: δ0.86 (3H, d, J=7,16Hz, H-9'); 0.88 (3H, d, J=7.41Hz, H-9); 1.09-1.33 (3H, overlapping multiplet, H-7',8'); 1.92 (1H, multiplet, H-7'); 2.22 (2H, dd, J=2.22, 10.44Hz, H-2'); 2.91 (1H, dq, J=11.91, 7.39Hz, H-8); 3.34 (1H, d, J=11.90Hz, H-7); 3.84 (3H, s, OCH_3); 5.59 (1H, s, H-5'); 5.68 (2H, dd, J=0.42, 9.29Hz, O_2CH_2-3',4'); 5.90 (2H, dd, J=1.46, 4.30Hz, O_2CH_2-3,4); 6.17 (2H, dd, J=1.655Hz, H-2,6). ^{13}C NMR: δ14.17 (C-9); 14.71 (C-9'); 18.65 (C-8'); 37.16 (C-7'); 44.16 (C-8); 46.5 (C-2'); 56.31 (C-7); 56.58 (OCH_3-5); 60.42 (C-1'); 91.32 (C-3'); 98.62 (C-5'); 101.29 (O_2CH_2-3,4); 101.47 (O_2CH_2-3',4'); 104.79 (C-6); 110.92 (C-2); 129.99 (C-1); 134.29 (C-4); 142.60 (C-5); 148.32 (C-3); 177.42 (C-4'); 200.41 (C-6').

3.3.1.1d. Acid-catalyzed hydrolysis of **68** with pTSOH

A solution of ocobullenone (200mg, $5.39x10^{-4}$ mol) in dry benzene (10ml) was added into a three-necked round-bottommed flask (100ml) containing ethylene glycol (1ml, 0.179 mol) in dry benzene (50ml). After a Dean-Stark water take-off was in place, a catalytic amount of para-toluene sulphonic acid monohydrate (p-TSOH) was added and the mixture refluxed for 14h. The benzene was evaporated and the products dissolved in chloroform (50ml). The organic layer was washed with potassium bicarbonate (2N, 30mlX3) then with water. The chloroform extract was dried over anhydrous magnesium sulphate. The products were chromatographed on silica gel column using petroleum ether (40-60^{o}C)-ethyl acetate (7:5) as eluent to give the minor product (12mg) **71** and the major product (75mg) **72**.

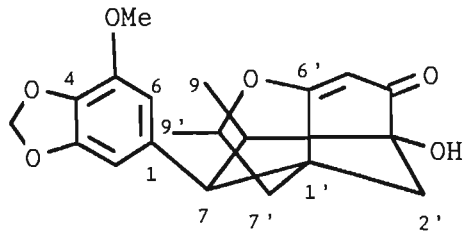


71

$C_{20}H_{24}O_7$

MW=376

Mp 254-255°C. ^{1}H NMR: δ0.63 (3H, d, J=6.50Hz, H-9'); 0.70 (3H, d, J=7.69Hz, H-9); 1.63 (2H, overlapping s, OH-3',8'); 1.79 (1H, dd, J=4.40, 13.36Hz, H-7'a); 2.05 (1H, d, J=10.53Hz, H-2'a); 2.44 (1H, d, J=10.53Hz, H-2'b); 2.48 (1H, dd, 9.87, 13.35Hz, H-7'b); 2.70 (1H, dq, 7.71, 11.81Hz, H-8); 3.45 (1H, d, J=11.81Hz, H-7); 3.85 (3H, s, OCH_3); 4.54 (1H, s, OH-4'); 4.79 (1H, ddq, J=4.40, 6.51, 8.89Hz, H-8'); 5.96 (2H, dd, J=1.42Hz, H-2,6). ^{13}C NMR: δ13.04 (C-9); 20.57 (C-9'); 36.57 (C-7'); 44.83 (C-8); 51.35 (C-2'); 56.83 (C-7, OCH_3); 58.41 (C-1'); 83.59 (C-8'); 84.79 (C-3'); 96.49 (C-5'); 101.47 (O_2CH_2); 200.60 (C-6'). Peaks due to C-4' and carbons on the benzene ring (both doublets and quaternary carbons) were not observed on the ^{13}C spectrum (see **Appendix 71c**).



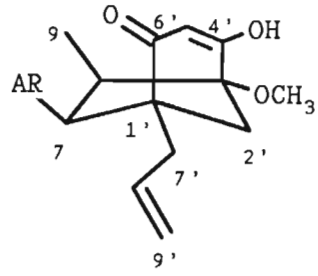
72

$C_{20}H_{24}O_6$
MW=358

Mp 199-200°C. $[\alpha]_D^{23}$ cm^{-1} -263° ($CHCl_3$; c0.314). ^1H NMR: δ0.71 (3H, d, 7.79Hz, H-9); 1.29 (3H, d, J=6.21Hz, H-9'); 1.89 (1H, dd, J=10.62, 12.91Hz, H-7'a); 2.01 (1H, d, J=10.55Hz, H-2'a); 2.22 (1H, dd, J=5.69, 12.83Hz, H-7'b); 2.43 (1H, d, J=10.53Hz, H-2'b); 2.71 (1H, dq, J=7.73, 11.74Hz, H-8); 3.53 (1H, d, J=11.72, H-7); 3.80 (1H, overlapping m, H-8'); 3.87 (3H, s, OCH_3); 4.54 (1H, s, OH-3'); 5.57 (1H, s, H-5'); 5.98 (2H, s, O_2CH_2); 6.44 (2H, s, H-2,6). ^{13}C NMR: δ13.02 (C-9); 20.68 (C-9'); 39.83 (C-2'); 45.27 (C-8); 50.51 (C-7'); 55.31 (C-7); 56.50 (OCH_3); 59.17 (C-1'); 83.49 (C-8'); 84.27 (C-3'); 95.87 (C-5'); 101.48 (O_2CH_2); 131.44 (C-1); 134.67 (C-4); 143.06 (C-5); 148.65 (C-3); 187.69 (C-6'); 200.53 (C-4'). Peaks due to C-2 and C-6 were not observed on the ^{13}C spectrum (**Appendix 72c**).

3.3.1.1e. Acid-catalyzed hydrolysis of **68** with mild HCl

Ocobullenone (**68**) (115mg, $3.10x10^{-4}$ mol) was weighed into a three-necked flask (100ml) and methanol (20ml) was added. Dilute HCl (1.5ml, 0.2M) was added and the mixture refluxed. A further portion of HCl (1ml, 2M) was added and the reaction allowed to continue for 30h. The reaction mixture was evaporated to near-dryness and water (20ml) added. The mixture was extracted with chloroform (30mlx3) and the combined chloroform washings dried over $MgSO_4$. The reaction products were chromatographed on a column using petroleum ether-ethyl acetate (7:10) as eluent to yield a colourless oil (36mg) (**73**).



73

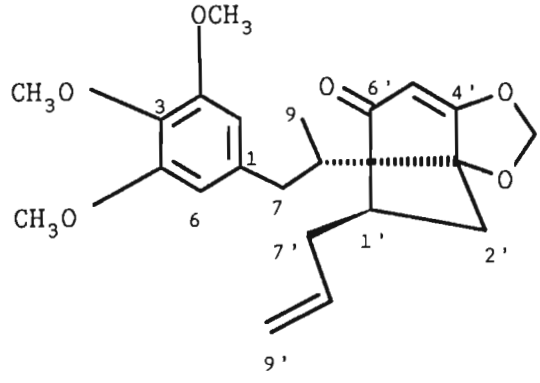
$C_{21}H_{24}O_6$
MW=372

^1H NMR: δ0.73 (1H, d, J=7.58Hz, H-9); 2.10 (3H, overlapping m, H-2', H-7'a); 2.56 (2H, m, H-7'b); 2.74 (1H, dq, J=7.55, 11.70Hz, H-8); 3.47 (1H, d, J=11.90Hz, H-7); 3.84 (6H, overlapping s, OCH_3-3', OCH_3-5); 5.12 (2H, m, H-9'); 5.48 (1H, s, H-5'); 5.73-5.83 (1H, m, H-8'); 5.91 (2H, dd, J=1.49, 4.31Hz, O_2CH_2); 6.15 (2H, dd, J=1.56, H-2,6). ^{13}C NMR: δ12.79 (C-9); 37.07 (C-7'); 47.88 (C-8); 49.11 (C-2'); 53.96 (C-7); 56.53 (OCH_3-3'); 56.61 (OCH_3-5); 61.04 (C-1'); 81.76 (C-3');

101.08 (C-5'); 101.29 (O_2CH_2); 104.37 (C-6); 111.08 (C-2); 118.42 (C-9'); 130.44 (C-1); 134.47 (C-4,8'); 142.62 (C-5); 148.41 (C-3); 179.60 (C-4'); 200.74 (C-6').

3.3.1.2. Bullatone (**74**)

The residue from the ethyl acetate extract of the bark of *Ocotea bullata* (12.5g) was fractionated by cc using chloroform and final purifications by gradient elution with petroleum ether(40-60°C)-ethyl acetate to afford **74** (14mg) as a crystalline compound.



74

$C_{22}H_{28}O_6$

MW=388

High Res.-MS: Found 388.1865; $C_{22}H_{28}O_6$ requires 388.1886. Mp=105°C. $[\alpha]_D^{22}$ + 175° (CH_2Cl_2; c0.08). EI-MS m/z (rel. int.): 388 $[M]^+$ (8), 209 (35), 208 (42), 193 (16), 182 (18), 181 (100), 148 (19), 139 (48), 137 (20), 79 (17), 77 (20), 69 (33), 55 (46), 53 (20). ^{1}H NMR: δ0.99 (3H, d, J=6.72Hz, H-9); 1.79 (1H, dd, J=12.67Hz, H-2'b); 2.09 (1H, ddq, J=2.44, 6.78, 12.59Hz, H-8); 2.25 (1H, dd, J=12.58, 12.66Hz, H-7b); 2.27 (1H, overlapping m, H-7'b); 2.40 (1H, m, H-1'); 2.56 (1H, dd, J=5.19, 12.97Hz, H-2'a); 2.69 (1H, m, H-7'a); 2.87 (1H, dd, J=2.44, 12.67Hz, H-7a); 3.80 (3H, s, OCH_3-4); 3.82 (6H, overlapping s, OCH_3-3,5); 5.07 (2H, m, H-9'); 5.53 (1H, s, H-5'); 5.60 (2H, dd, very small coupling, O_2CH_2); 5.70 (1H, m, H-8'); 6.27 (2H, s, H-2,6). ^{13}C NMR: δ13.34 (C-9); 35.69

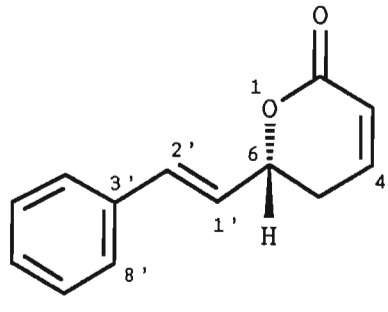
(C-2'); 36.29 (C-7'); 38.84 (C-7); 41.18 (C-8); 42.44 (C-1'); 56.23 (OCH_3-3,5); 60.82 (OCH_3-4); 85.44 (C-3'); 99.85 (C-5'); 99.95 (O_2CH_2); 106.15 (C-2,6); 117.66 (C-9'); 135.18 (C-1); 136.81 (C-4); 152.23 (C-3,5); 175.76 (C-4'); 198.77 (C-6').

3.3.2. *Cryptocarya woodii*

The fresh bark of *Cryptocarya woodii* was collected in the Karkloof nature reserve, Natal, South Africa in July 1992. The dried bark (3.7Kg) was extracted as in 3.2..

3.3.2.1. Goniothalamin (**45**)

The crude benzene extract of *Cryptocarya woodii* was fractionated by cc using petroleum ether (40-60°C)-ethyl acetate (7:2) as eluent. The fractions containing goniothalamin were further fractionated on a column and then on a chromatotron plate (4mm) with the same solvent system to afford a pure crystalline sample of **45** (566mg).



45

$C_{13}H_{12}O_2$

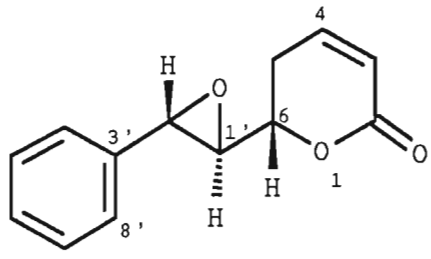
MW=200

Mp 79-81°C (lit. 81-82°C). $[\alpha]_D^{23}$ + 169° ($CHCl_3$; c0.053) (lit. + 178.5°). ^1H NMR: δ2.52 (2H, m, H-5); 5.09 (1H, m, H-6); 6.08 (1H, ddd, J=1.93, 3.67, 9.82Hz, H-3); 6.26 (1H, dd, J=6.32, 15.95Hz, H-1'); 6.72 (1H, dd, J=1.26, 15.95Hz, H-2'); 6.91 (1H, ddd, 3.57, 4.16, 9.88Hz, H-4); 7.33 (5H, m, H-4',5',6',7',8'). ^{13}C NMR: δ29.83 (C-5); 76.44 (C-6); 121.54 (C-3); 125.60 (C-1'); 126.67 (C-4',8'); 128.33 (C-6'); 128.67 (C-5',7'); 133.05 (C-2'); 135.70 (C-3'); 144.78 (C-4); 163.92 (C-2).

3.3.2.1.1. Epoxidation of **45**

Goniothalamin (**45**) (200mg, 9.988×10^{-4} mol) dissolved in dichloromethane (20ml) was added into a stirred solution of mCPBA (516mg, 2.99×10^{-3} mol) in dichloromethane (30ml) over a period of 30min. The reaction mixture was refluxed with stirring in a water bath (40-60°C) overnight, then washed with a solution of 10% sodium bicarbonate (30mlx3), water and dried over anhydrous magnesium sulphate. After the solvent was removed, the products were fractionated on a column using petroleum ether (40-60°C)-dichloromethane-acetone-ethyl acetate (10:2:1:1) as eluent to afford two crystalline products; isogoniothalamin oxide **79** (66mg) and goniothalamin oxide **80** (11mg).

3.3.2.1.1a. Isogoniothalamin oxide (**79**)

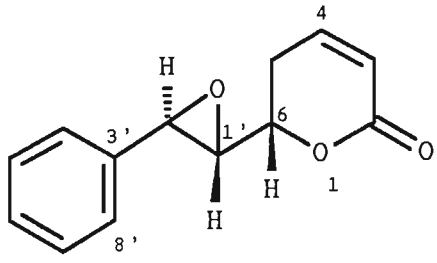


79

$C_{13}H_{12}O_3$
MW=216

Mp 114-117°C (lit. 111-114°C)[83]. $[\alpha]_D^{23}$ -110.1° ($CHCl_3$; c0.53) (lit. -106.0°)[83]. $IR_{\upsilon max}cm^{-1}$ (KBr): 1710, 1375, 1230, 1060, 1015, 800. EI-MS m/z (rel. int.): 216 $[M]^+$ (10), 147 (15), 131 (22), 128 (20), 110 (35), 105 (20), 97 (23), 91 (40), 90 (30), 82 (91), 68 (100), 66 (37). ^1H NMR: δ2.57 (2H, m, H-5); 3.24 (1H, dd, J=2.04, 3.87Hz, H-1'); 4.06 (1H, d, J=2.04Hz, H-2'); 4.46 (1H, dd, J=4.00, 5.59, 9.81Hz, H-6); 6.09 (1H, m, H-3); 6.93 (1H, m, H-4); 7.30 (5H, overlapping m, H-4',5',6',7',8'). ^{13}C NMR: δ26.16 (C-5); 55.02 (C-1'); 62.10 (C-2'); 75.33 (C-6); 121.39 (C-3); 125.70 (C-4',8'); 128.60 (C-5',7'); 135.90 (C-3'); 144.24 (C-4); 162.95 (C-2). Carbon due to C-6' was not observed in the ^{13}C spectrum (See **Appendix 79c**).

3.3.2.1.1b. Goniothalamin oxide (**80**)



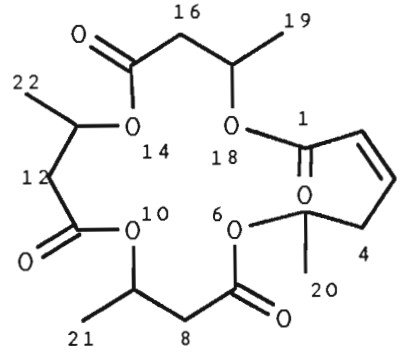
80

$C_{13}H_{12}O_3$
MW=216

Mp 89-91°C (lit. 90-94°C)[83]. $[\alpha]_D^{23}$ + 104.3° ($CHCl_3$; c0.045) (lit. + 100.7°)[83]. EI-MS m/z (rel. int.): 216 $[M]^+$ (2), 105 (19), 97 (28), 91 (66), 90 (41), 89 (46), 82 (100), 81 (30), 69 (29), 68 (47), 66 (40), 65 (26), 63 (23), 51 (22), 41 (32), 39 (49). $IR_{\nu max}cm^{-1}$ (KBr): 1715, 1370, 1236, 1026, 800. ^1H NMR: δ2.30 (2H, m, H-5); 3.26 (1H, dd, J=2.00, 5.12Hz, H-1'); 3.88 (1H, d, J=2.01Hz, H-2'); 4.46 (1H, ddd, J=5.12, 6.48, 10.25Hz, H-6); 6.02 (1H, ddd, J=1.63, 1.99, 9.66Hz, H-3); 6.91 (1H, ddd, J=3.45, 8.53, 9.79Hz, H-4); 7.30 (5H, overlapping m, H-4',5',6',7',8'). ^{13}C NMR: δ25.61 (C-5); 56.86 (C-1'); 61.44 (C-2'); 76.87 (C-6); 121.20 (C-3); 125.68 (C-4',8')*; 128.54 (C-6'); 128.60 (C-5',7')*. (* = Interchangeable carbons).

3.3.2.2. Cryptocaryolide B (**88**)

Fractions (from the benzene extract of *C. woodii*) containing cryptocaryolide B were chromatographed using petroleum ether (30-60°)-ethyl acetate (9:3), then repeatedly chromatographed with petroleum ether (30-60°)-ethyl acetate-chloroform (9:6:1.5). Chromatography on the chromatotron, using the same solvent system, yielded a pure sample of **88** (101mg), a yellow oil.



88

$C_{18}H_{26}O_8$
MW=370

High Res.-MS: Found 370.1570; $C_{18}H_{26}O_8$ requires 370.3960. $[\alpha]_D^{25}$ +43.8° ($CHCl_3$; c0.63). CI-MS (CH_4) m/z (rel. int.): 412 $[M+C_3H_7]^+$ (0.4), 399 $[M+C_2H_5]^+$ (5), 371 $[M+H]^+$ (4), 339 (2), 313 (3), 312 (12), 311 (100), 251 (6), 219 (1), 191 (10), 173 (1). $IR_{\nu max}cm^{-1}$ (Neat): 2950, 1730, 1435, 1370, 1245, 1040, 960, 820. ^1H NMR: δ1.23 (3H, d, J=6.24Hz, H-19); 1.77, 2.15 (2H, overlapping m, H-16); 1.87-2.25 (4H, overlapping m, H-8,12); 2.05 (9H, overlapping d, H-20,21,22); 2.40 (2H, m, H-4); 4.49 (1H, m, H-5); 4.83-5.13 (3H, overlapping m, H-9, 13, 17); 6.01 (1H, ddd, J=1.16, 2.49, 9.76Hz, H-2); 6.89 (1H, ddd, J=2.80, 5.72, 9.78Hz, H-3). ^{13}C NMR: δ19.95 (C-19); 21.13 (C-20)*; 21.16 (C-21)*; 21.30 (C-22)*; 29.13 (C-4); 38.96

(C-8)^{x}; 38.96 (C-12)^{x}; 40.16 (C-16)^{x}; 67.63 (C-9)^{o}; 67.77 (C-13)^{o}; 68.14 (C-17)^{o}; 74.89 (C-5); 121.24 (C-2); 144.93 (C-3); 163.78 (C-1); 170.42 (C-7)$^{+}$; 170.52 (C-11)$^{+}$; 170.58 (C-15)$^{+}$. *, x, o, + = Exchangeable carbons.

3.3.3. *Cryptocarya latifolia*

The bark of the natural specimen of *Cryptocarya latifolia* was collected from Qudeni Forest, Natal, South Africa in March 1993. The dried bark (2.0Kg) was extracted as described in 3.2..

3.3.3.1. Cryptocaryolide B (**88**) and A (**87**)

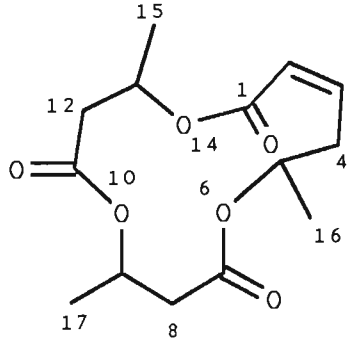
The benzene extract (19.2g) was chromatographed on a column using petroleum ether (30-60^{o}C)-ethyl acetate (9:3) as eluent. Fractions containing **87** and **88** were chromatographed on a column with petroleum ether-ethyl acetate-chloroform (9:6:1.5) as eluent. Fractions containing predominantly cryptocaryolide B (**88**) were further fractionated on a chromatotron (4mm plate) using the same solvent system as above to yield a pure sample of **88** (2.16g) a colourless to light yellow oil.

Fractions containing mainly cryptocaryolide A (**87**) were fractionated on a column using petroleum ether-ethyl acetate-chloroform (P.E.-E.A.-$CHCl_3$) (9:6:2) as eluent. Cryptocaryolide A (**87**) was further purified by fractionating on a chromatotron plate (4mm) using the same solvent system as above to yield a pure sample of **87** (270mg), a light yellow oil.

3.3.3.1a. Cryptocaryolide B (**88**)

See 3.3.2.2. for data.

3.3.3.1b. Cryptocaryolide A (87)



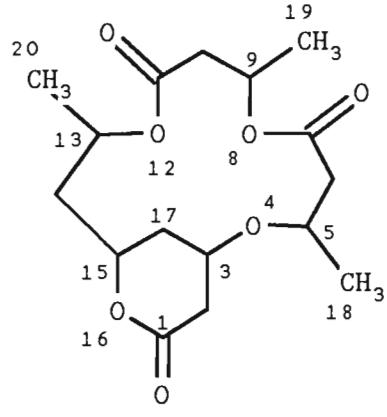
87

$C_{14}H_{20}O_6$
MW=284

High Res.-MS: Found 284.1250; $C_{14}H_{20}O_6$ requires 284.1260. $[\alpha]_D^{22}$ +55.8° ($CHCl_3$; c1.06). CI-MS (CH_4) m/z (rel. int.): 325 (3), 314 (1), 313 (6), 285 $[M+1]^+$ (15), 265 (0.4), 253 (4), 243 (1), 226 (16), 225 (100), 207 (0.5), 193 (4), 183 (2), 182 (0.5), 166 (3), 165 (23), 147 (3), 121 (5). $IR_{\nu max}$ cm^{-1} (Neat): 2950, 1733, 1435, 1375, 1245, 1040, 960, 820. ^1H NMR: δ1.26 (3H, d, J=6.29Hz, H-15); 1.80, 2.06 (2H, m, H-12); 1.96, 2.19 (2H, m, H-8); 2.06 (6H, overlapping d, J=6.96Hz, H-16, 17); 2.42 (2H, m, H-4); 4.52 (1H, ddq, J=4.48, 6.48, 12.00Hz, H-5); 4.98 (1H, m, H-9); 5.13 (1H, m, H-13); 6.00 (1H, ddd, J=0.96, 2.44, 9.76Hz, H-2); 6.91 (1H, ddd, J=2.27, 5.80, 9.67Hz, H-3). ^{13}C NMR: δ19.97 (C-15); 21.01 (C-17)*; 21.14 (C-16)*; 29.03 (C-4); 38.94 (C-8); 40.12 (C-12); 67.62 (C-9,13); 74.82 (C-5); 121.00 (C-2); 144.99 (C-3); 163.69 (C-1); 170.31 (C-7)+; 170.44 (C-11)+. *, + =Interchangeable carbons.

3.3.3.2. Foliolide B (**91**)

Fractions containing foliolide B (**91**) were repeatedly chromatographed on a column then on a chromatotron plate (4mm) with P.E.-E.A. (5:7:3) to yield a pure sample of **91** (111mg), a yellow oil.



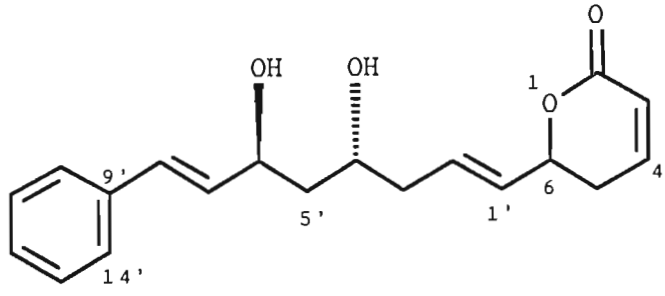
91

$C_{16}H_{24}O_7$
MW=328

High Res.-Ms: Found 328.3078; $C_{16}H_{24}O_7$ requires 328.3590. $[\alpha]_D^{23}$ -145° ($CHCl_3$; c0.27). $IR_{\nu max}$ cm^{-1} (Neat): 2945, 1740, 1430, 1240, 1045, 965, 820. CI-MS (CH_4) m/z (rel. int.): 369 $[M+C_2H_5]^+$ (0.7), 329 $[M+H]^+$ (0.2), 328 $[M]^+$ (0.04), 297 (2), 269 (100), 237 (4), 209 (12), 181 (0.7), 169 (2). ^1H NMR: δ1.23 (3H, d, J=6.30Hz, H-18); 1.50-2.25 (8H, overlapping m, H-6, 10, 14, 17); 2.03 (6H, overlapping d, J=1.88Hz, H-19, 20); 2.81 (2H, m, H-2); 3.87 (1H, m, H-9); 4.33 (1H, m, H-3); 4.88 (1H, m, H-15); 4.96 (1H, m, H-5); 5.09 (1H, m, H-13). ^{13}C NMR: δ20.03 (C-18); 21.23 (C-19)*; 21.34 (C-20)*; 29.50 (C-17); 36.11 (C-2); 36.87 (C-14); 39.71 (C-6); 39.87 (C-10); 63.11 (C-9); 67.89 (C-5); 68.35 (C-13); 72.85 (C-15); 169.68 (C-1); 170.49 (C-7,11). * = Exchangeable carbons.

3.3.3.3. Cryptofolione (81)

Fractions containing **81** were repeatedly chromatographed on a column then on a chromatotron plate (2mm) using E.A.-CH_2Cl_2-P.E.-MeOH (5:2:7:1) as eluent. A pure yellow oil (51mg) was obtained.



81

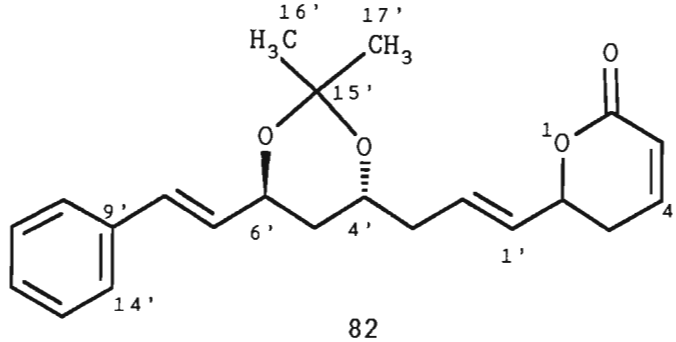
$C_{19}H_{22}O_4$
MW=314

High Res.-Ms: Found 314.1503; $C_{19}H_{22}O_4$ requires 314.1518. $[\alpha]_D^{23}$ +27° ($CHCl_3$; c0.52). $IR_{\nu max}cm^{-1}$ (Neat): 3440, 2942, 1700, 1385, 1250, 1055, 975, 825, 755, 700. ^1H NMR: δ1.78 (2H, m, H-5'); 2.38 (2H, m, H-3'); 2.41 (2H, m, H-5); 3.46 (2H, overlapping m, OH-4',6'); 4.05 (1H, m, H-4'); 4.63 (1H, m, H-6'); 4.88 (1H, m, H-6); 5.63 (1H, dd, J=6.33, 15.66Hz, H-1'); 5.82 (1H, m, H-2'); 6.00 (1H, overlapping m, H-3); 6.23 (1H, dd, J=5.81, 15.99Hz, H-7'); 6.61 (1H, dd, J=1.01, 16.00Hz, H-8'); 6.83 (1H, overlapping m, H-4); 7.23 (5H, overlapping m, H-10', 11',12',13',14'). ^{13}C NMR: δ29.63 (C-5); 40.35 (C-3'); 42.29 (C-5'); 68.04 (C-4'); 70.05 (C-6'); 77.96 (C-6); 121.29 (C-3); 126.43 (C-10',14'); 127.56 (C-12'); 128.55 (C-11',13'); 129.61 (C-8'); 129.70 (C-1'); 131.33 (C-2'); 131.93 (C-7'); 136.65 (C-9'); 145.10 (C-4); 164.27 (C-2).

3.3.3.3.1. Reactions on Cryptofolione (**81**)

3.3.3.3.1a. Acetonide derivative of Cryptofolione

A catalytic amount of pTSOH (22mg) was added to a solution of cryptofolione (**81**) (1.597g, 5.08×10^{-1} mol) in dimethyl formamide (5ml) and 2,2-dimethoxypropane (20ml). The reaction mixture was refluxed for 14h. Sodium bicarbonate (0.5%, 150ml) was added and the mixture extracted with chloroform (3x100ml). The combined chloroform extracts were dried over anhydrous $MgSO_4$ and chromatographed on a column using E.A.-$CHCl_3$-P.E.-MeOH (5:2:20:1) as eluent. The acetonide derivative **82** (481mg) was obtained as a yellow oil.



82

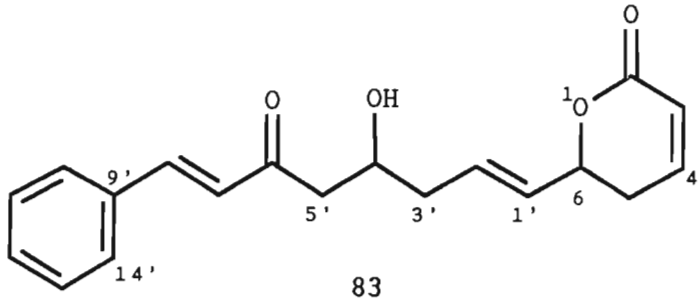
$C_{22}H_{26}O_4$
MW=354

$[\alpha]_D^{23}$ +79° (CH_2Cl_2; c1.36). $IR_{\nu max}cm^{-1}$ (Neat): 2940, 1705, 1380, 1255, 1055, 970, 825, 750. ^1H NMR: δ1.40 (6H, overlapping s, H-16',17'); 1.76 (2H, m, H-5'); 2.30 (4H, overlapping m, H-5, 3'); 3.92 (1H, m, H-4'); 4.48 (1H, m, H-6'); 5.62 (1H, m, H-1'); 5.80 (1H, m, H-2'); 6.01 (1H, ddd, J=1.83, 3.67, 9.82Hz, H-3); 6.20 (1H, dd, J=6.22, 16.01Hz, H-7'); 6.54 (1H, dd, J=0.77, 15.99Hz, H-8'); 6.77 (1H, ddd, J=4.22, 8.45, 9.89Hz, H-4); 7.28 (5H, overlapping m, H-10', 11', 12', 13', 14'). ^{13}C NMR: δ24.59 (C-17')*; 25.59 (C-16')*; 29.60 (C-5); 37.31 (C-5'); 38.44 (C-3'); 65.73 (C-4'); 67.51

(C-6'); 77.79 (C-6); 100.26 (C-15'); 121.14 (C-3); 126.40 (C-10', 14'); 127.58 (C-12'); 128.51 (C-11', 13'); 129.46 (C-1'); 129.87 (C-7'); 130.04 (C-8'); 130.40 (C-2'); 136.46 (C-9'); 145.08 (C-4); 163.81 (C-2). * =Exchangeable carbons.

3.3.3.3.1b. Oxidation of Cryptofolione

81 (1.076g, $3.42x10^{-1}$) in anhydrous CH_2Cl_2 (10ml) was added to the orange suspension of pyridinium chlorochromate (2.21g, $1.026x10^{-4}$) in anhydrous CH_2Cl_2 (25ml) which as a result changed to a brown-black colour. The reaction mixture was refluxed for 3h under nitrogen. After cooling, dry diethyl ether (50ml) was added. The brown-black suspension was decanted leaving a black gum, which was washed with diethyl ether, at the bottom of the flask. The combined organic solvents were filter, concentrated and chromatographed on a column using E.A.-$CHCl_3$-P.E.-MeOH (4:2:7:1) to give compound **83** (98mg), a yellow oil, as a major product.



83

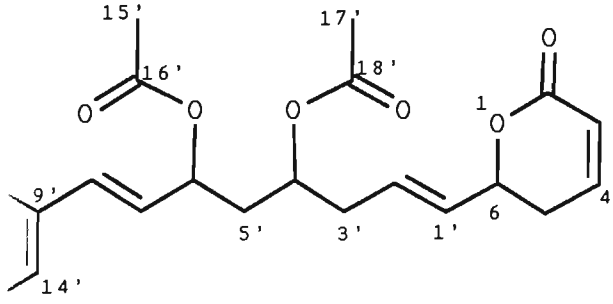
$C_{19}H_{20}O_4$
MW=312

High Rea.-Ms: Found 312.1360; $C_{19}H_{20}O_4$ requires 312.1361. $[\alpha]_D^{23}$ +101° (CH_2Cl_2; c.0.86). DIP CI-MS (CH_4) m/z (rel. int.): 353 (1), 341 (1), 314 (6), 313 $[M+H]^+$ (31), 295 (17), 277 (3), 209 (3), 207 (3), 195 (4), 187 (4), 175 (17), 167 (35), 149 (39), 147 (100), 131 (53), 107 (15). ^1H NMR: δ2.31 (2H, m, H-3'); 2.45 (2H, m, H-5); 2.61 (2H, m, H-5'); 3.55 (1H, broad s, OH-4'); 4.32 (1H, broad m, H-4'); 4.91 (1H, m, H-6); 5.71 (1H, m, H-1'); 5.91 (1H, m, H-2'); 6.03 (1H, m, H-3); 6.74 (1H, d, J=16.20Hz, H-8'); 6.90 (1H, m, H-4); 7.49 (5H, overlapping m, H-10', 11', 12', 13', 14'); 7.52 (1H, d, J=16.16Hz, H-7'). ^{13}C NMR: δ29.62 (C-5); 39.29 (C-3'); 46.19

); 77.95 (C-6); 121.32 (C-3); 126.12 (C-7'); 128.97 (C-11',13'); 129.80 (C-1'); 130.80 -2'); 134.06 (C-9'); 143.71 (C-8'); 145.02 ; 200.46 (C-6').

ation of Cryptofolione

1 (200mg, 6.369×10^{-4} mol) in pyridine (5ml) hydride (5ml). The mixture was refluxed for s added and the mixture extracted with CH_2Cl_2 anic layer was then extracted with HCl (0.5N, ned organic solvent was dried over anhydrous was chromatographed on a column using 2:10) to give a hygroscopic yellow d **85** (107mg).



85

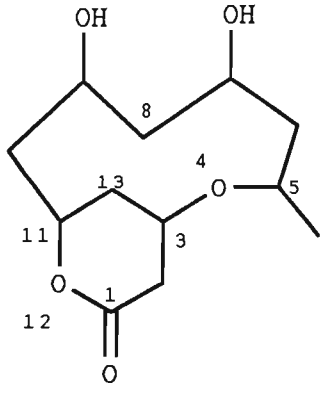
$C_{23}H_{26}O_6$
MW=393

^{23} +53° ($CHCl_3$; c0.053). CI-MS (CH_4) m/z 6), 398 (31), 397 (100), 396 (21), 395 (9), 223 (4), 207 (7). ^{1}H NMR: δ1.93 (2H, m, , H-15'); 2.06 (3H, s, H-17'); 2.28 (4H, , 5); 4.89 (1H, m, H-6); 5.05 (1H, m, H-4'); ; 5.60-5.86 (2H, overlapping m, H-1', 2'); erlapping m, H-3, 7'); 6.62 (1H, dd, J=0.55,

15.99Hz, H-8'); 6.87 (1H, ddd, J=4.21, 8.42, 9.89Hz, H-4); 7.32 (5H, overlapping m, H-10',11',12',13',14'). ^{13}C NMR: δ21.06 (C-15')*; 21.17 (C-17')*; 29.57 (C-5); 37.55 (C-3'); 38.44 (C-5'); 68.66 (C-4'); 70.68 (C-6'); 77.63 (C-6); 121.49 (C-3); 126.57 (C-10', 14'); 126.90 (C-7'); 128.08 (C-12'); 128.57 (C-11', 13'); 129.28 (C-2'); 130.43 (C-1'); 132.76 (C-8'); 135.98 (C-9'); 144.70 (C-4); 163.89 (C-2); 170.19 (C-16')+; 170.47 (18')+. *, + = Exchangeable carbons.

3.3.3.3.4. Foliolide A (90)

The chloroform extract (5.250g) of *Cryptocarya latifolia* was fractionated on a column with E.A.-CH_2Cl_2-P.E.-MeOH (5:2:7:1). Fractions containing foliolide A (**90**) were subsequently chromatographed with E.A.-CH_2Cl_2-P.E.-MeOH (9:2:4:1), (8:2:3:1) then (7:2:4:1) on a column and finally with (9:2:2:1) on a chromatotron to yield a pure sample of **90** (60mg), a yellow oil.



90

$C_{12}H_{20}O_5$
MW=244

$[\alpha]_D^{24}$ -128° ($CHCl_3$; c0.04). $IR_{\nu max}$ cm^{-1} (Nujol): 2940. 1733, 1435, 1045. 960. CI-MS (CH_4) m/z (rel. int.): 245 $[M+H]^+$ (53),

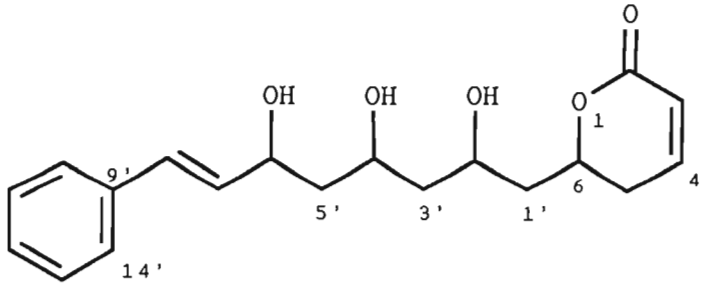
191 (17), 183 (25), 165 (22), 157 (37), 155 (18), 141 (100), 139 (39). ^{1}H NMR: δ1.18 (3H, d, J=6.16Hz, H-13); 1.52 (2H, m, H-6); 1.59, 1.73 and 2.08 (2H, m, H-10); 1.59 (2H, m, H-8); 2.01 (2H, m, H-12); 2.86 (2H, m, H-2), 3.67-4.11 (5H, overlapping m, H-5, 7, 9, OH-7, OH-9); 4.41 (1H, m, H-3); 4.89 (1H, m, H-11). ^{13}C NMR: δ23.64 (CH_3); 29.40 (C-13); 36.35 (C-2), 37.07 (C-10); 42.91 (C-8); 44.89 (C-6); 65.94 (C-3); 66.41 (C-7)*; 68.13 (C-5); 71.91 (C-9)*; 72.62 (C-11); 169.56 (C-1). * = Interchangeable carbons.

3.4. *Cryptocarya myrtifolia*

The benzene extract (42.2g) of *Cryptocarya myrtifolia* was fractionated on a column using P.E.-E.A.-$CHCl_3$ (3:3:1) as eluent. Fractions containing cryptofolione (**81**) were concentrated and chromatographed as in 3.3.3.3. to yield **81** (3.516g).

3.4.1. Myrtifolione (**86**)

The chloroform extract of *C. myrtifolia* (8.78g) was fractionated on a column with CH_2Cl_2-MeOH (15:2) as eluent. Fractions containing myrtifolione (**86**) were further and repeatedly chromatographed on a chromatotron using Hexane-E.A.-CH_2Cl_2-MeOH (7:3:3:1) as eluent. A white, hygroscopic and crystalline compound **86** (98mg) was isolated.



86

$C_{19}H_{24}O_5$
MW=332

Mp 103-105°C (Highly hygroscopic compound). $[\alpha]_D^{23}$ +69° ($CHCl_3$; c0.050). DIP CI-MS m/z (rel. int.): 371 (2), 343 (15), 331 $[M-H]^+$ (2), 315 (62), 213 (16), 211 (31), 193 (28), 185 (100), 167 (68), 155 (51), 141 (71), 131 (94), 117(46). ^1H

NMR: δ1.88 (2H, m, H-3'); 1.98 (2H, m, H-5'); 2.07 (2H, m, H-1'); 2.02 (2H, m, H-5); 4.50 (1H, m, H-2'); 4.76-4.92 (2H, overlapping m, H-4', 6); 5.04 (1H, m, H-6'); 5.62-5.90 (3H, broad m, OH-2',4',6'); 5.94 (1H, ddd, J=1.10, 2.67, 9.83Hz, H-3); 6.59 (1H, m, H-4); 6.65 (1H, dd, J=5.40, 15.93Hz, H-8'); 6.89 (1H, dd, J=1.10, 16.02Hz, H-7'); 7.19-7.38 (5H, overlapping m, H-10', 11',1 2',13', 14'). ^{13}C NMR: δ29.83 (C-5); 44.19 (C-5'); 46.83 (C-1'); 46.91 (C-3'); 65.19 (C-2'); 65.90 (C-4'); 69.90 (C-6'); 76.86 (C-6); 121.78 (C-3); 127.26 (C-10', 14'); 128.02 (C-12'); 128.98 (C-7'); 129.48 (C-11', 13'); 136.31 (C-8'); 138.17 (C-9'); 146.46 (C-4); 164.98 (C-2).

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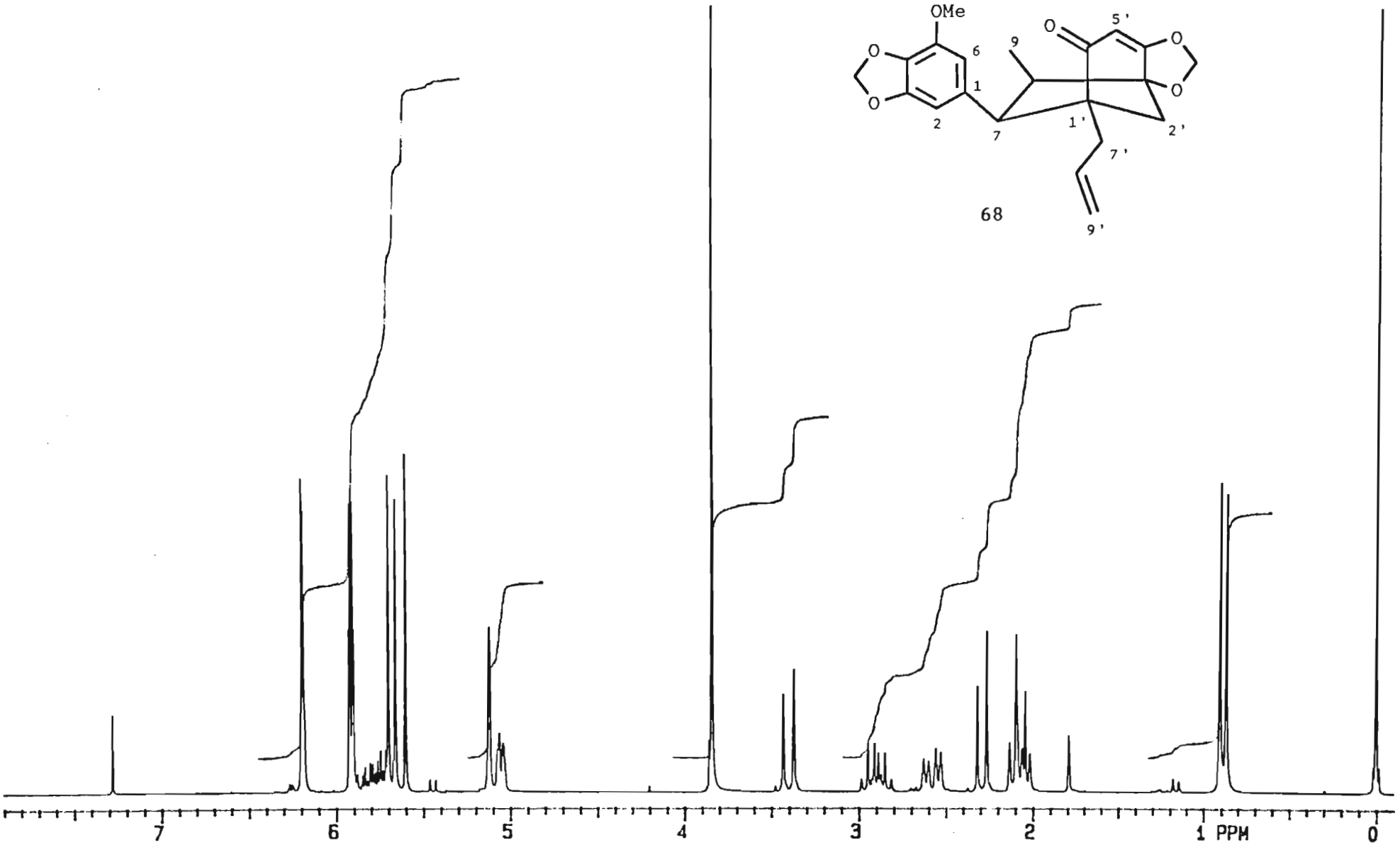
96a. A modified anisaldehyde solution was freshly prepared from ethanol (95%, 465ml), glacial acetic acid (5ml), concentrated sulphuric acid (17ml) and p-anisaldehyde (13ml) (added in this order) and used as a dip. The solution was thoroughly cooled (liquid nitrogen) before suphuric acid and p-anisaldehyde were added.

96b. Dying reagent for Thin Layer and Paper Chromatography, p. 3. Merck, E., Darmstadt, Germany.

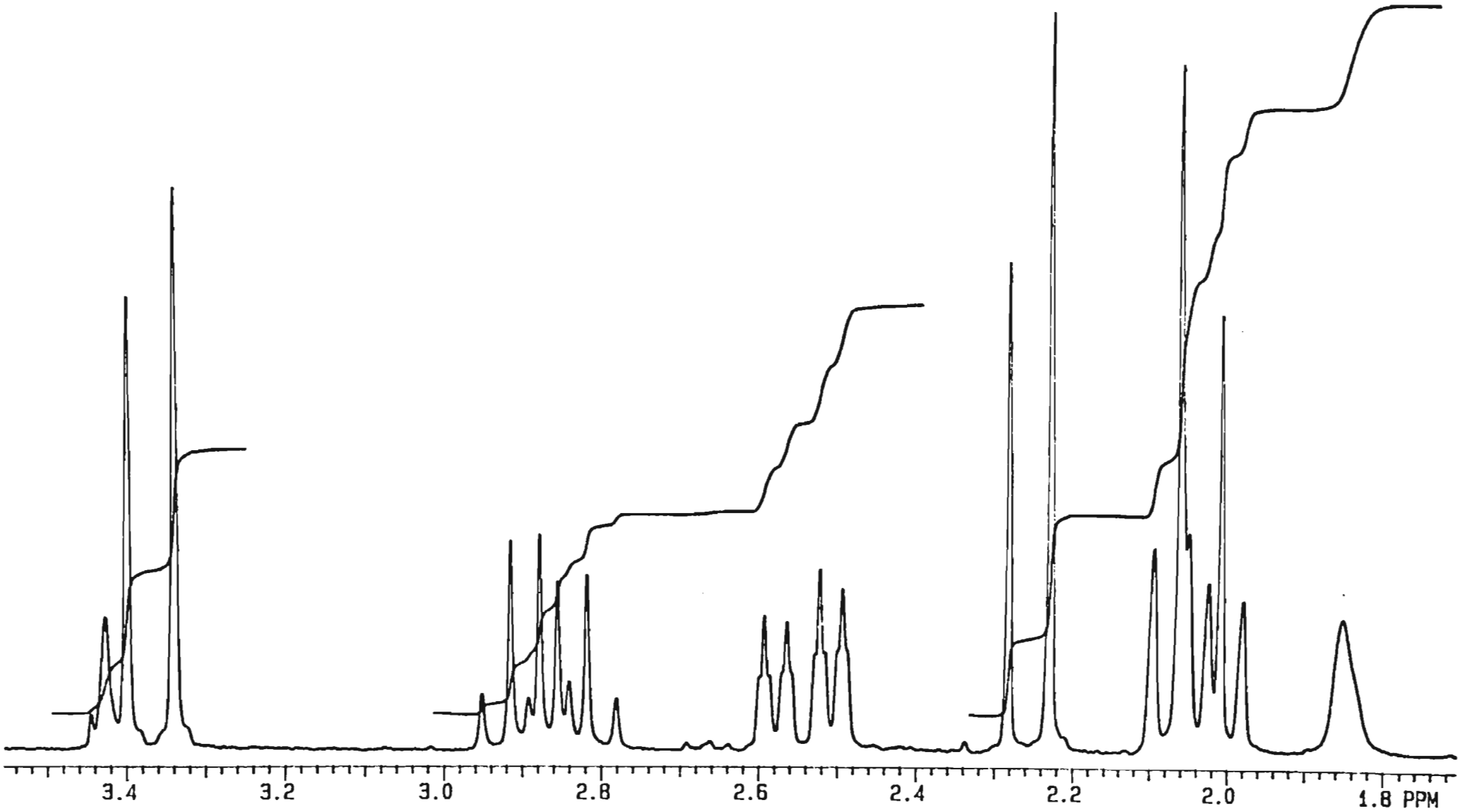
5. APPENDIX

A collection of all the NMR spectra, in numerical order of the compounds as they are numbered in the Introduction and Discussion. Spectra were recorded at 200MHz (^{1}H NMR) and 50MHz (^{13}C NMR) unless labelled otherwise.

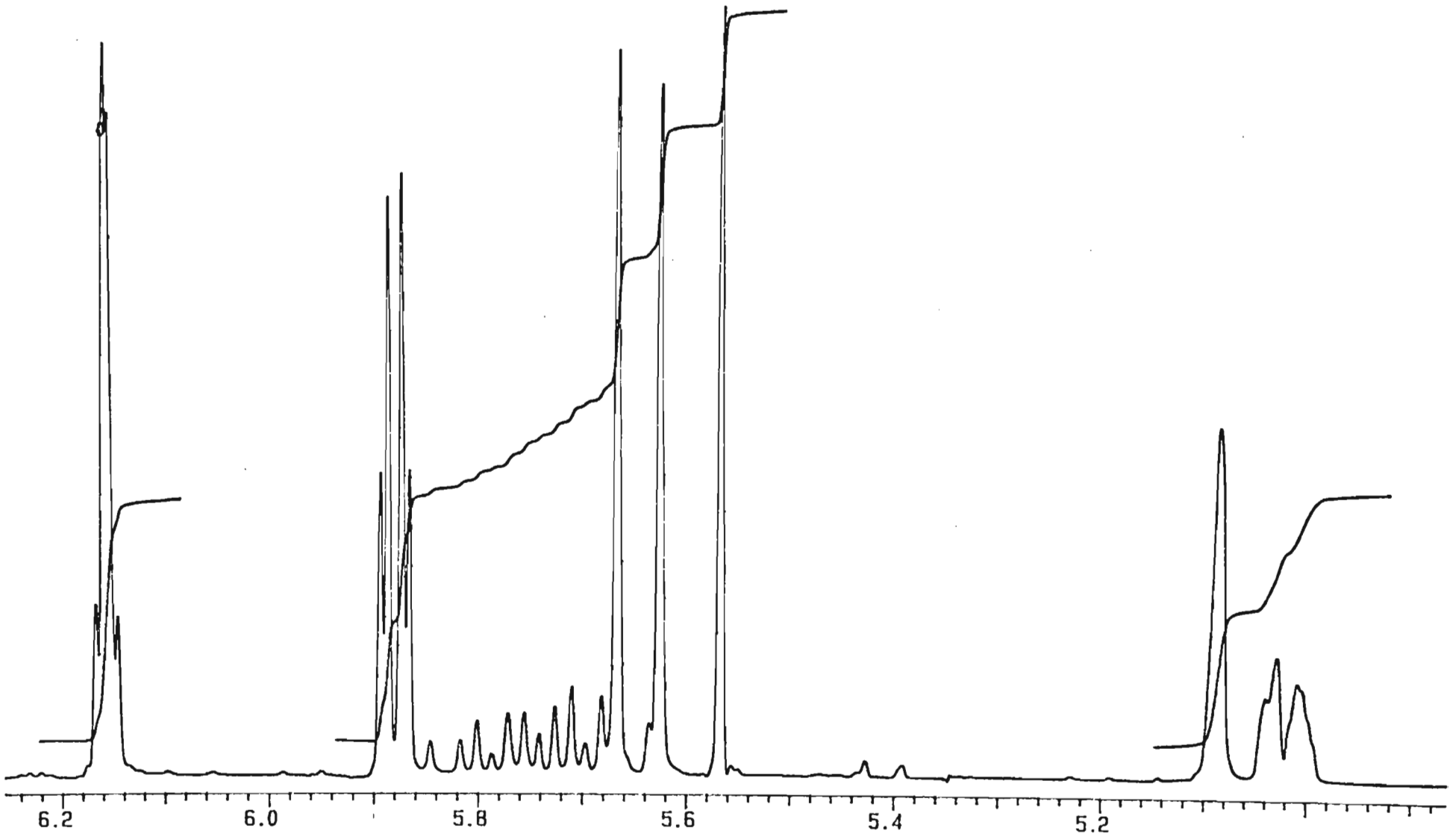
a	=	^{1}H NMR
b	=	expanded ^{1}H NMR
c	=	^{13}C NMR
d	=	^{1}H-^{1}H COSY
e	=	^{1}H-^{1}H DELAYED OR RELAYED COSY
f	=	^{1}H-^{13}C HETCOR
g	=	^{1}H-^{13}C DELAYED HETCOR
s	=	NMR solvent
*	=	impurities



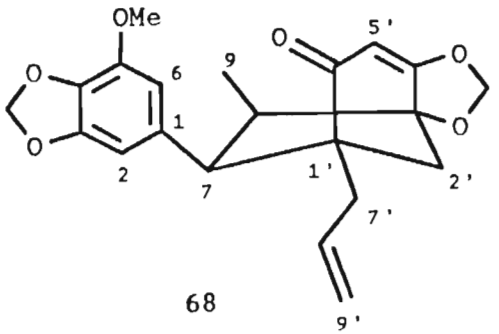
68a ^{1}H NMR spectrum of ocobullenone in $CDCl_3$



68b Expanded ^{1}H NMR spectrum of ocobullenone in $CDCl_3$

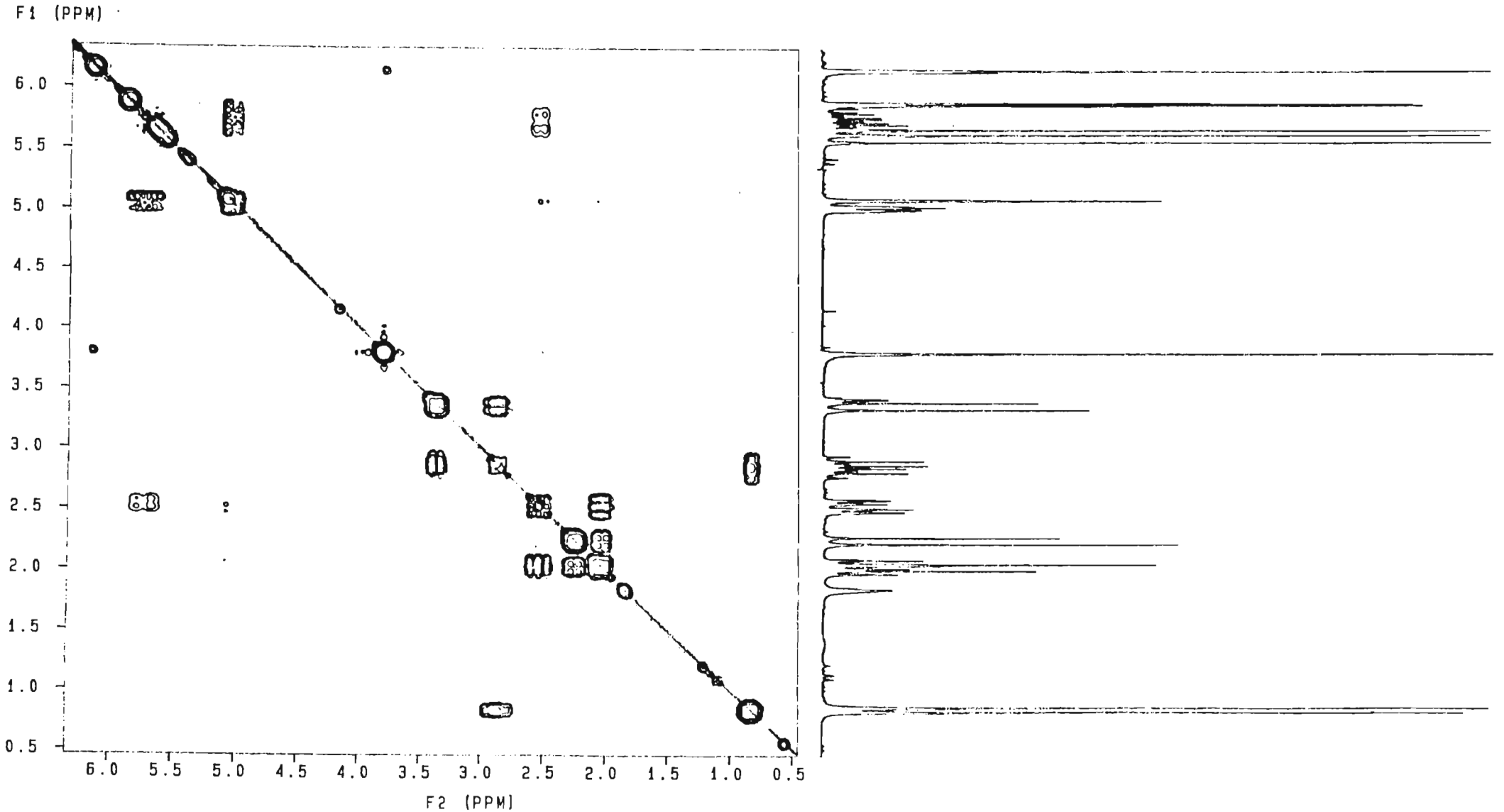


68b Expanded ^{1}H NMR spectrum of ocobullenone in $CDCl_3$

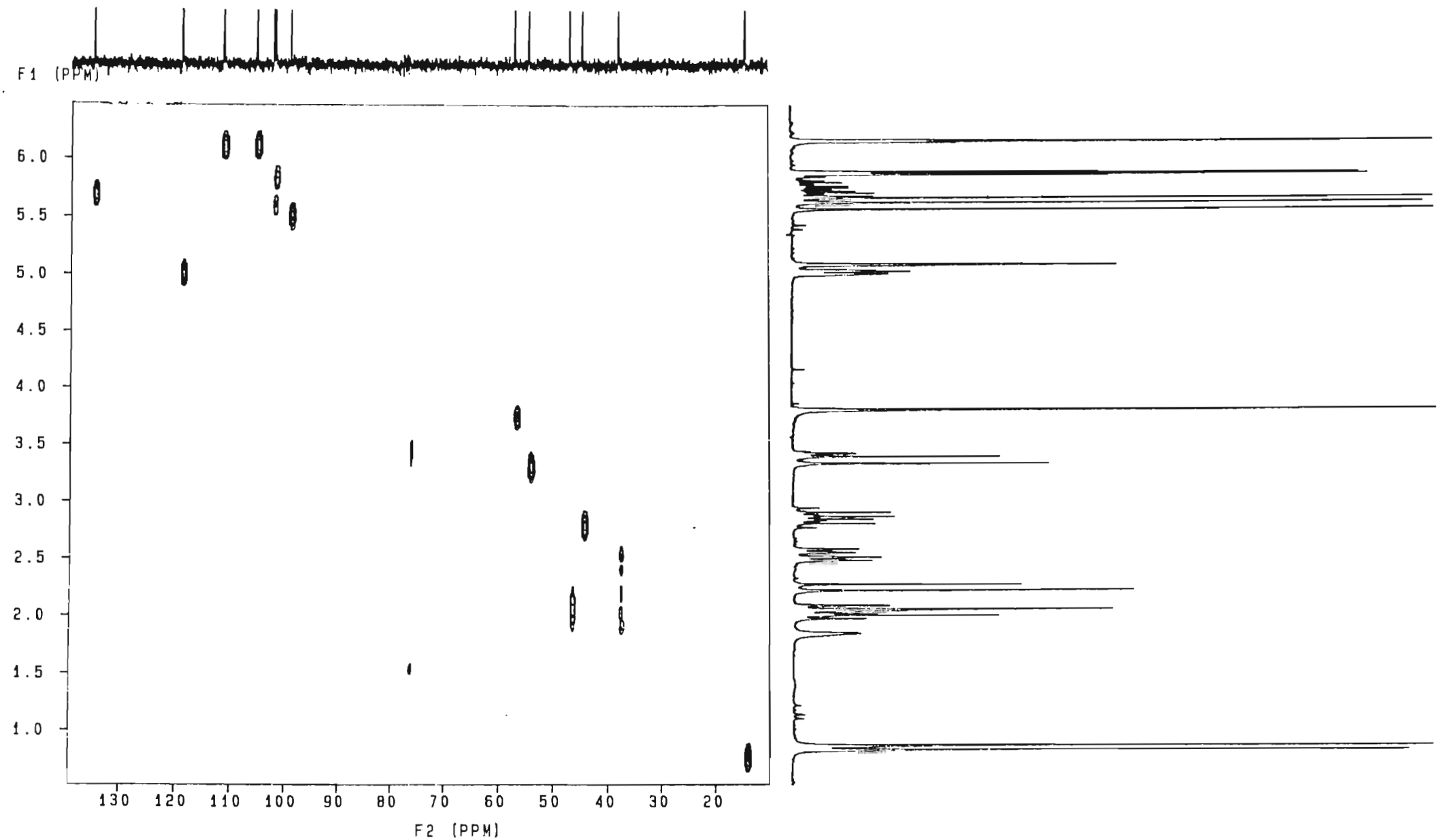


68

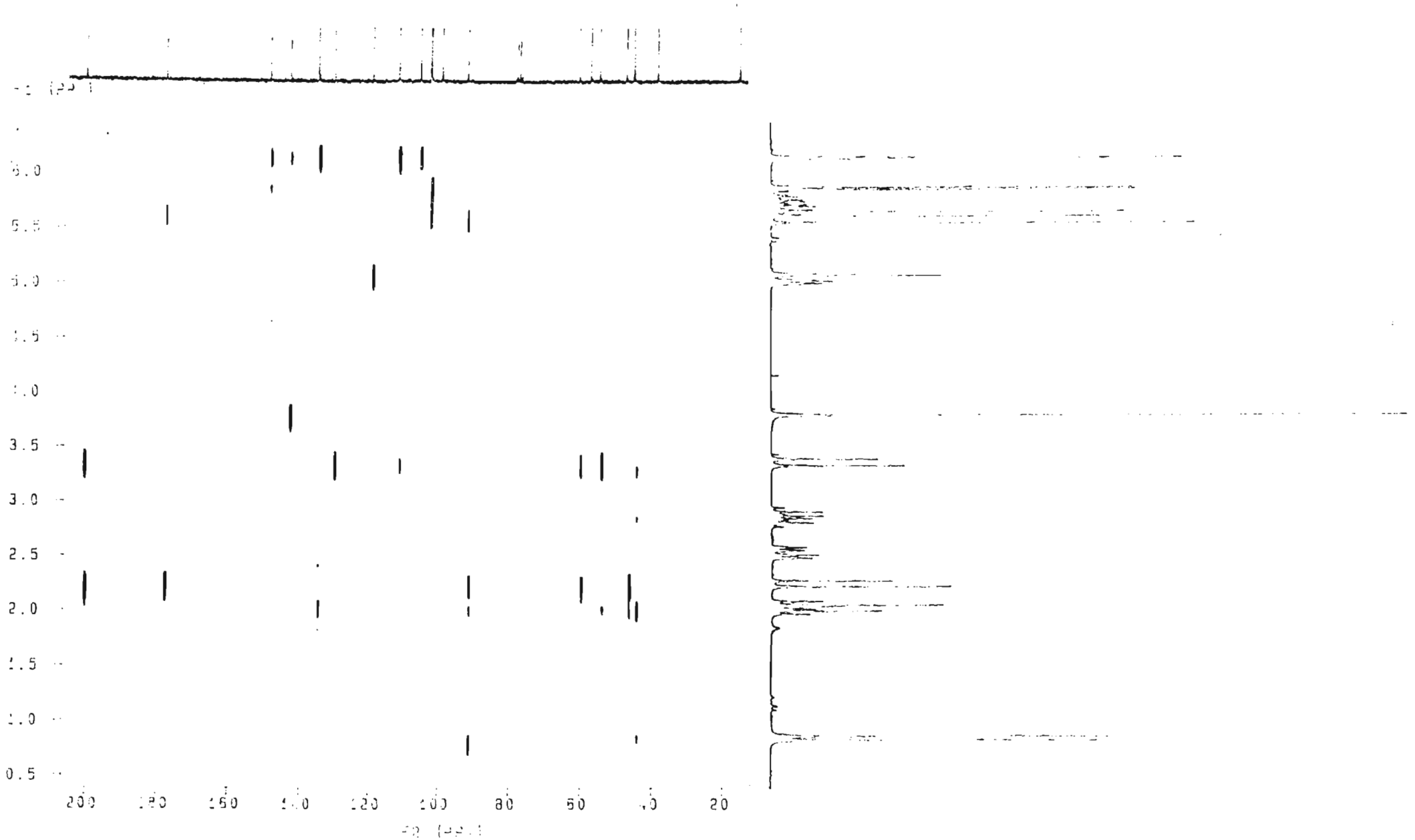
68c ^{13}C NMR spectrum of ocobullenone in $CDCl_3$



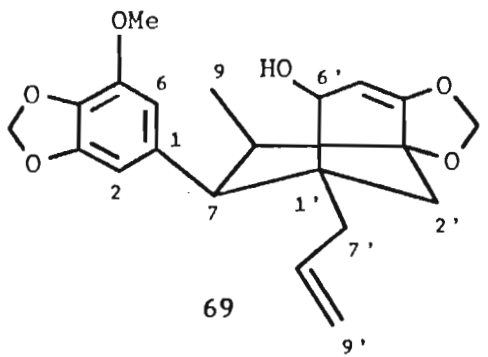
68d ^{1}H-^{1}H COSY spectrum of ocobullenone in $CDCl_3$



68f ^{1}H-^{13}C HETCOR spectrum of ocobullenone in $CDCl_3$

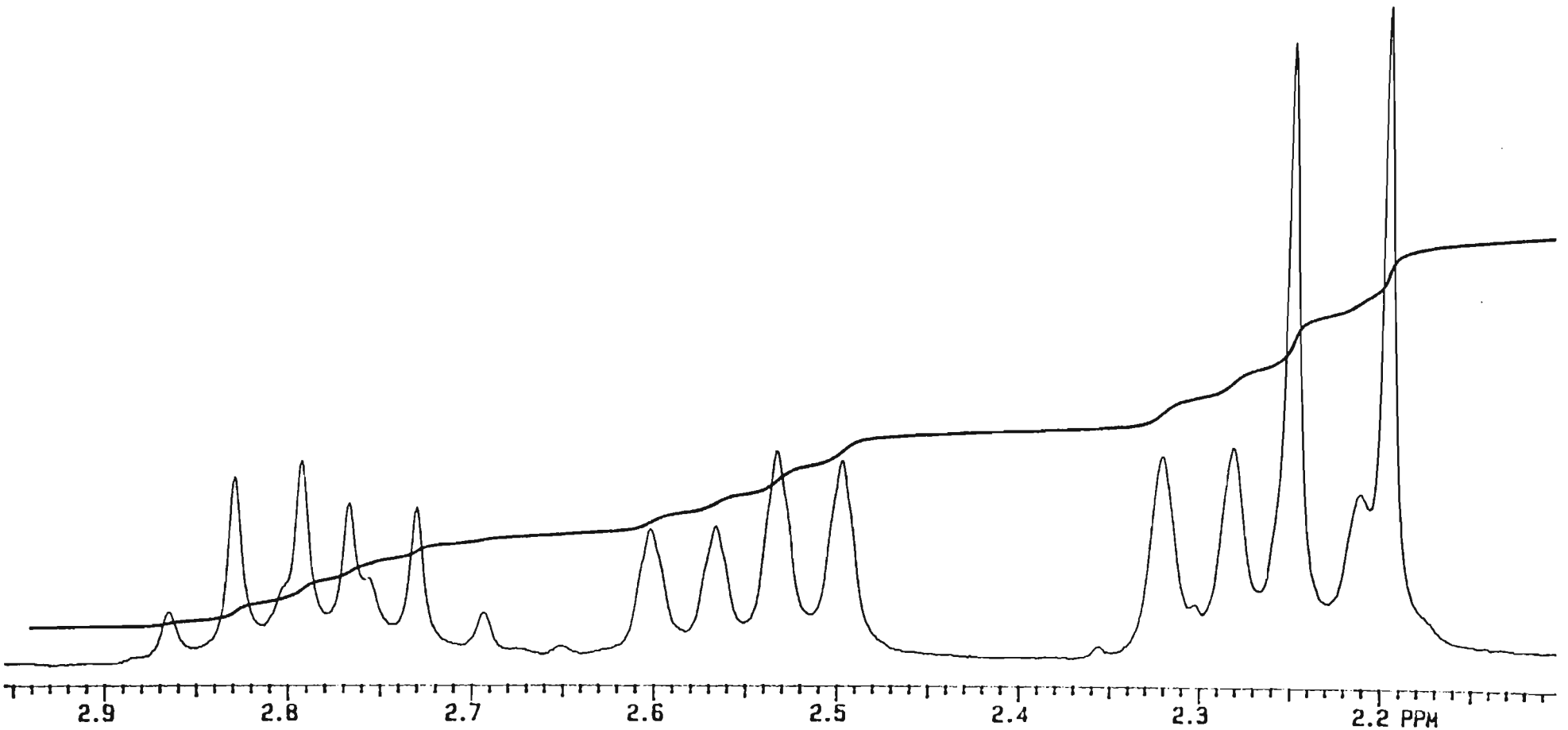


68g ^{1}H-^{13}C DELAYED HETCOR spectrum of ocobullenone in $CDCl_3$

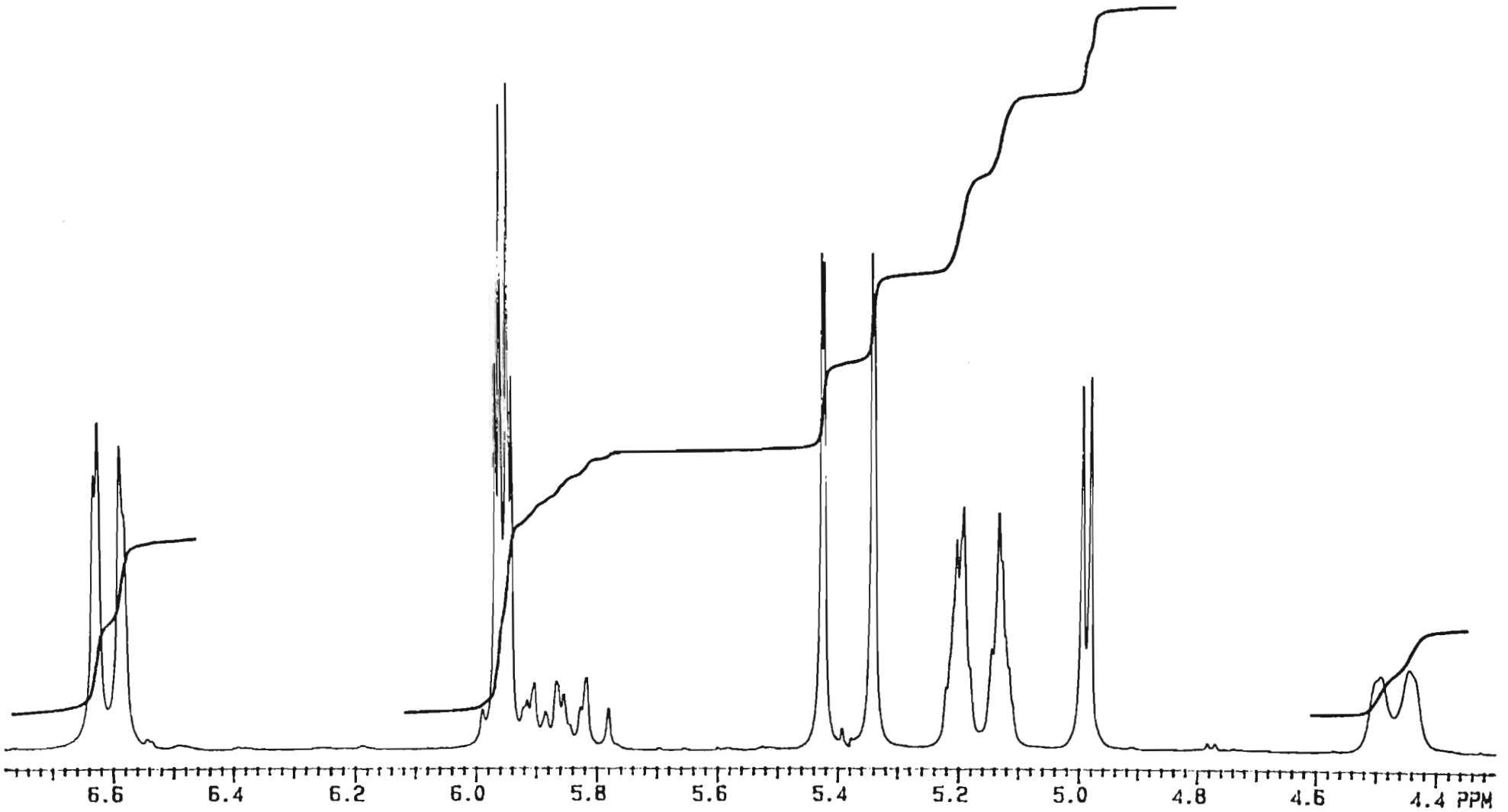


69

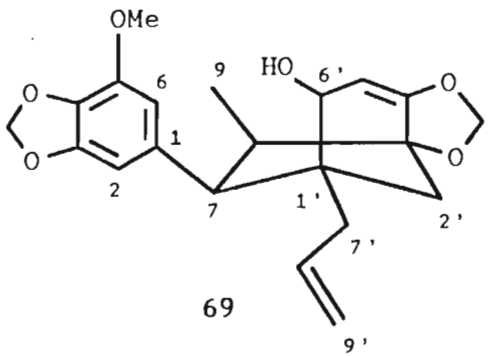
69a ^{1}H NMR spectrum of the alcohol 69 in $CDCl_3$



69b Expanded ^{1}H NMR spectrum of the alcohol 69 in $CDCl_3$

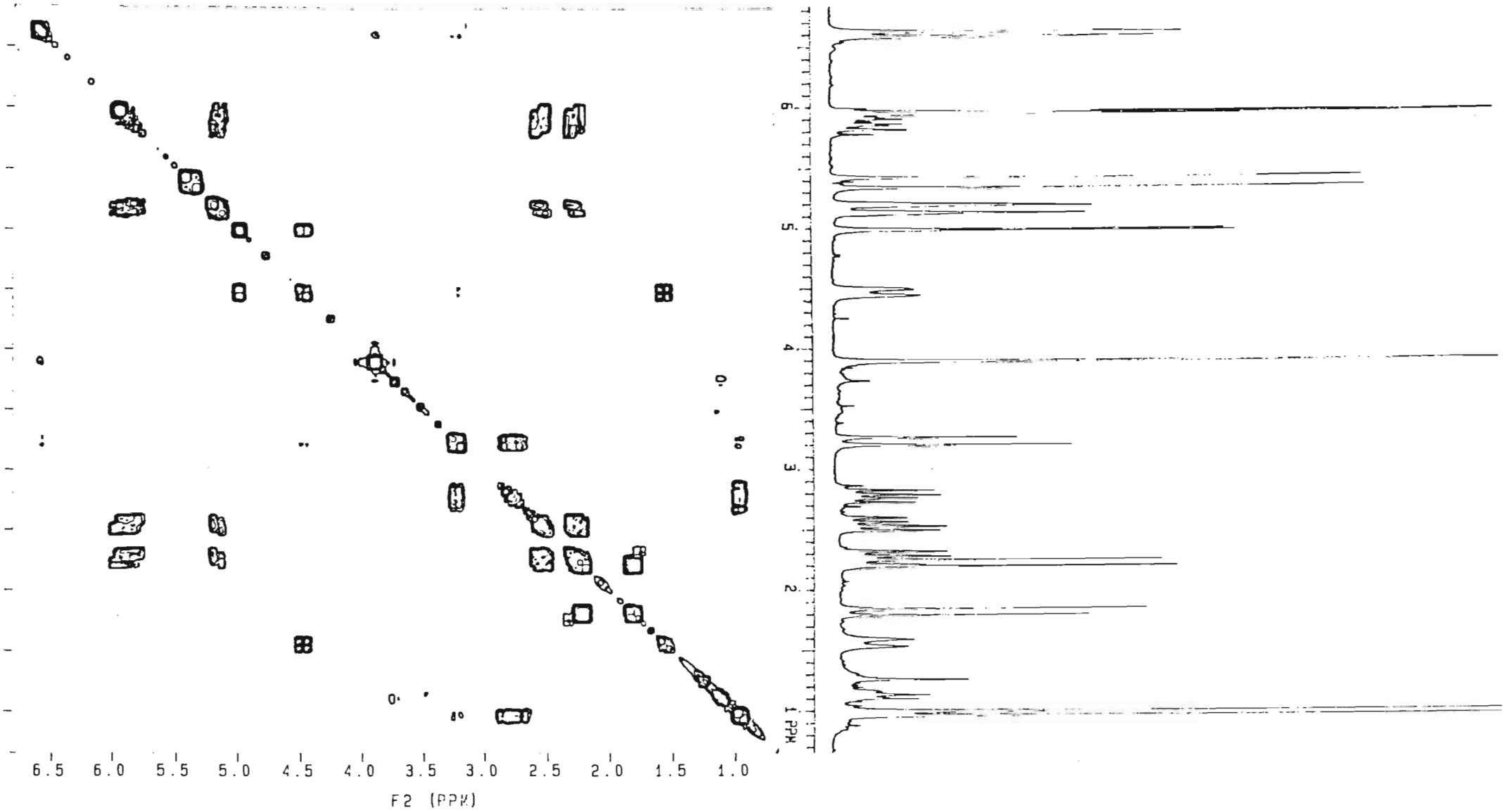


69b Expanded ^{1}H NMR spectrum of the alcohol 69 in $CDCl_3$

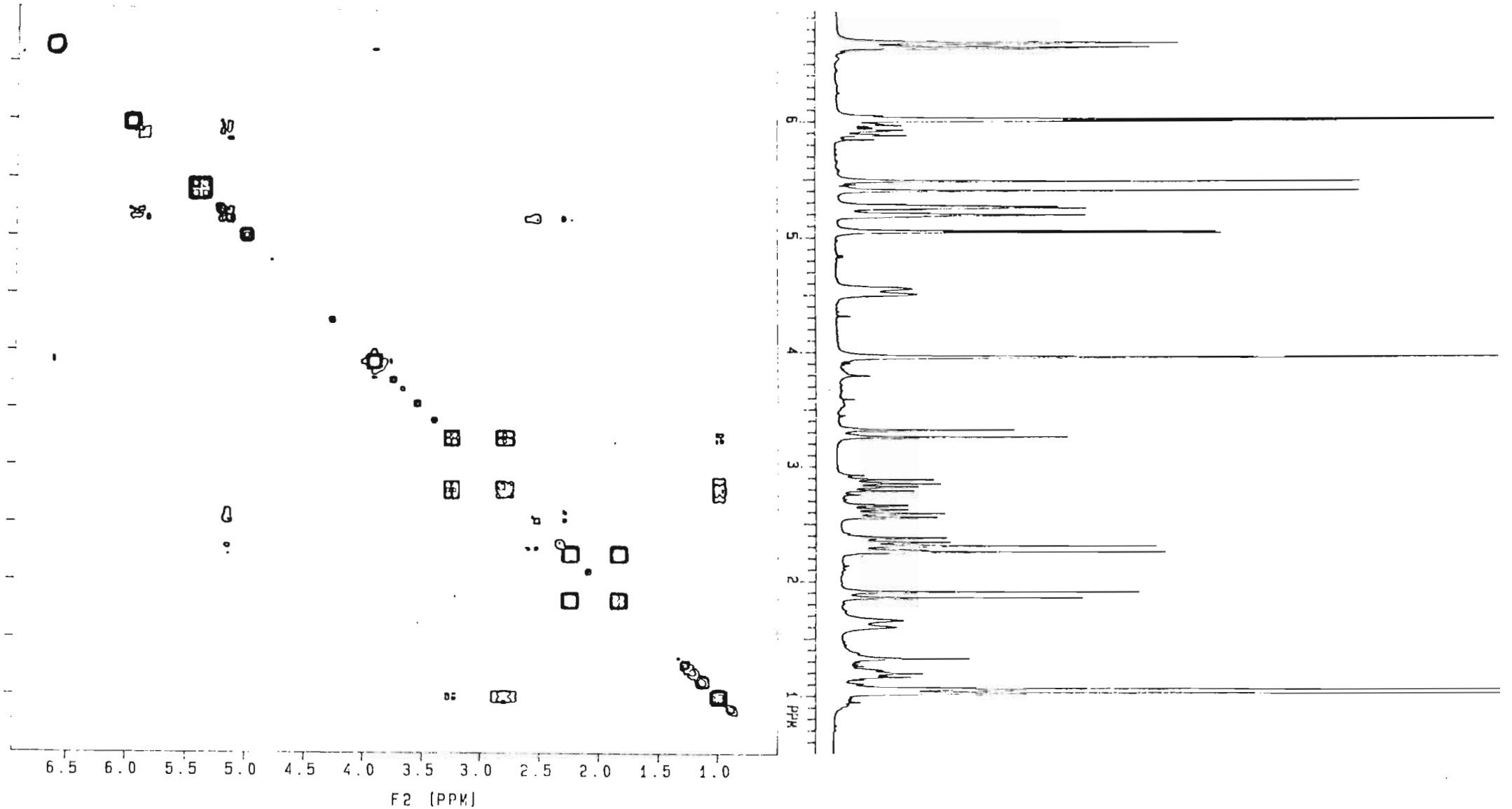


69

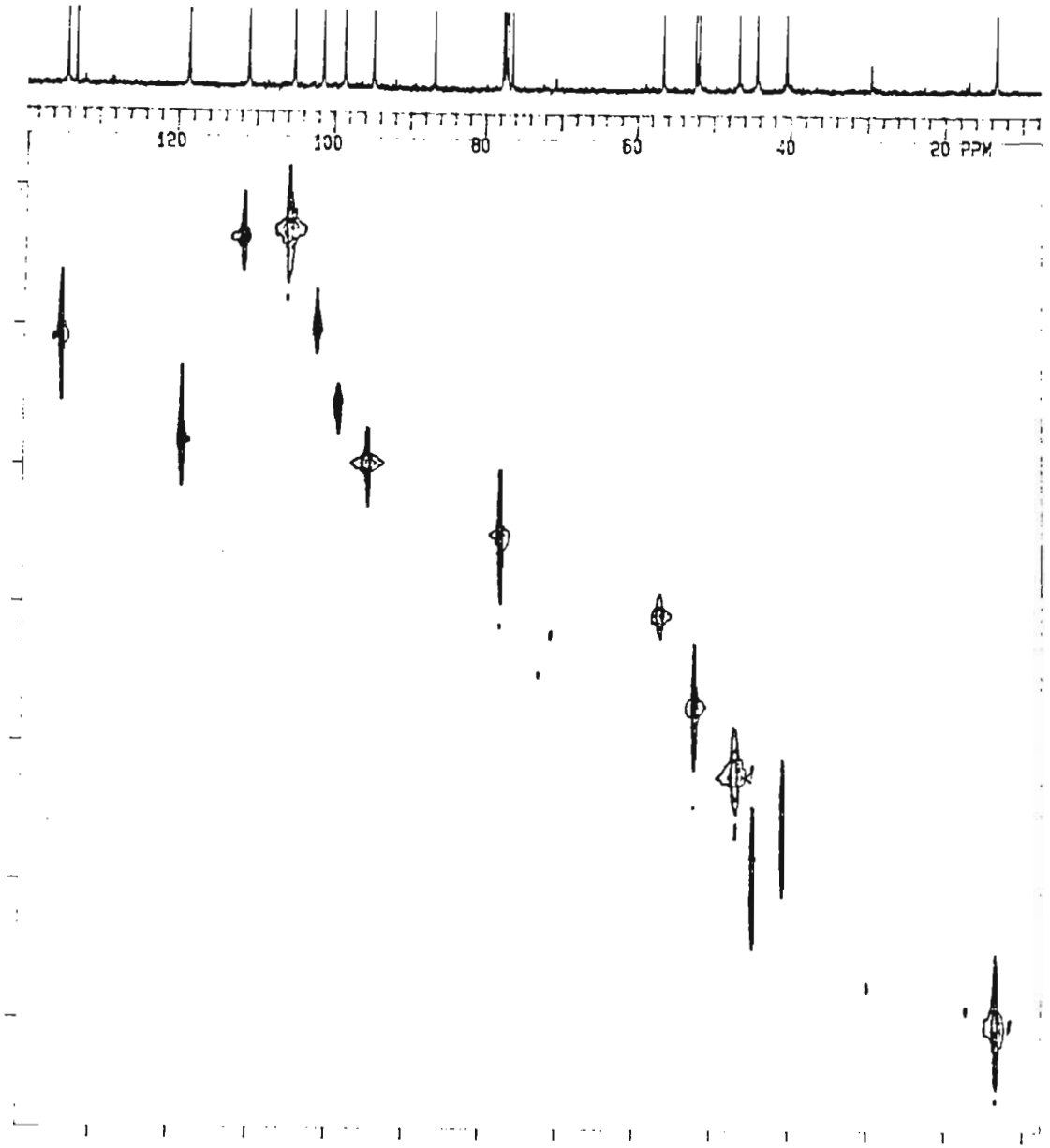
69c ^{13}C NMR spectrum of the alcohol 69 in $CDCl_3$

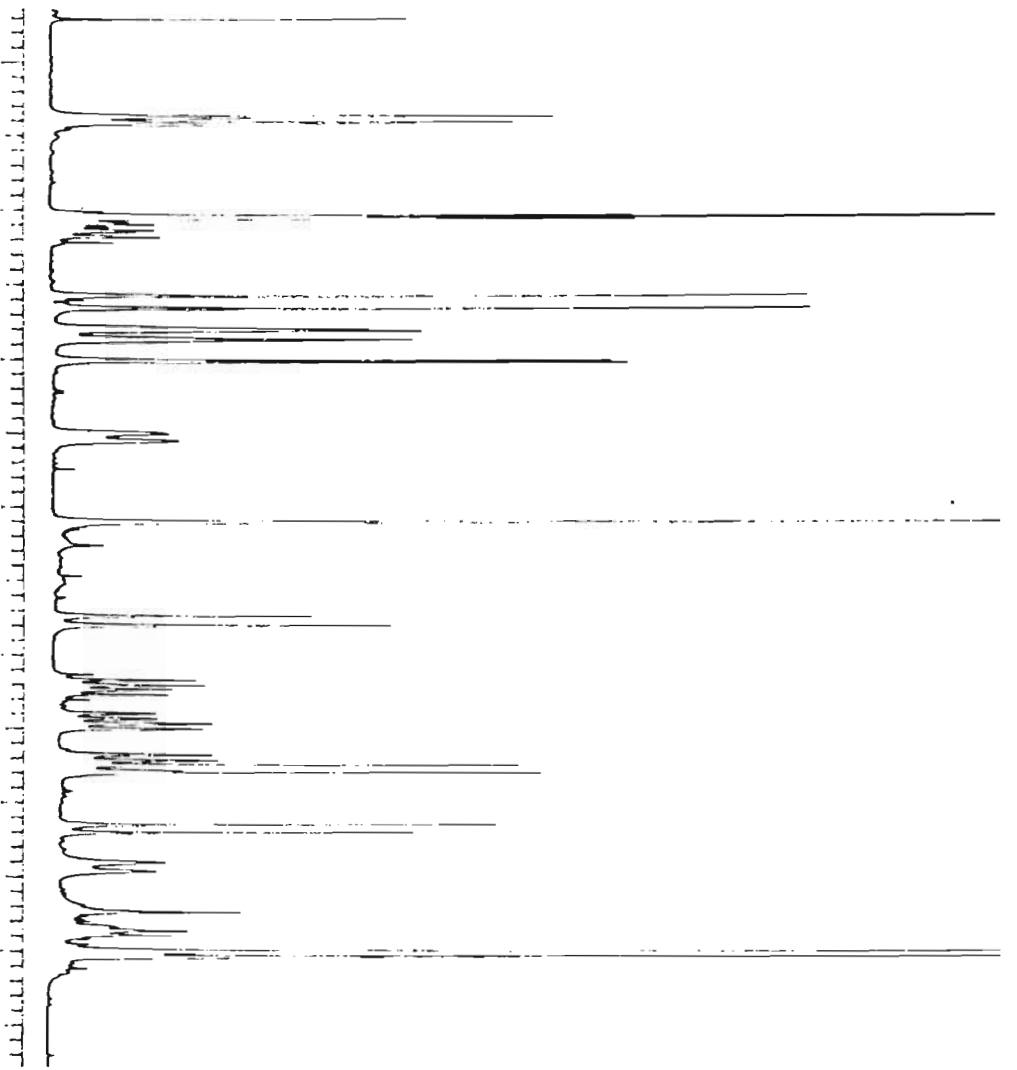


69d ^{1}H-^{1}H COSY spectrum of the alcohol **69** in $CDCl_3$

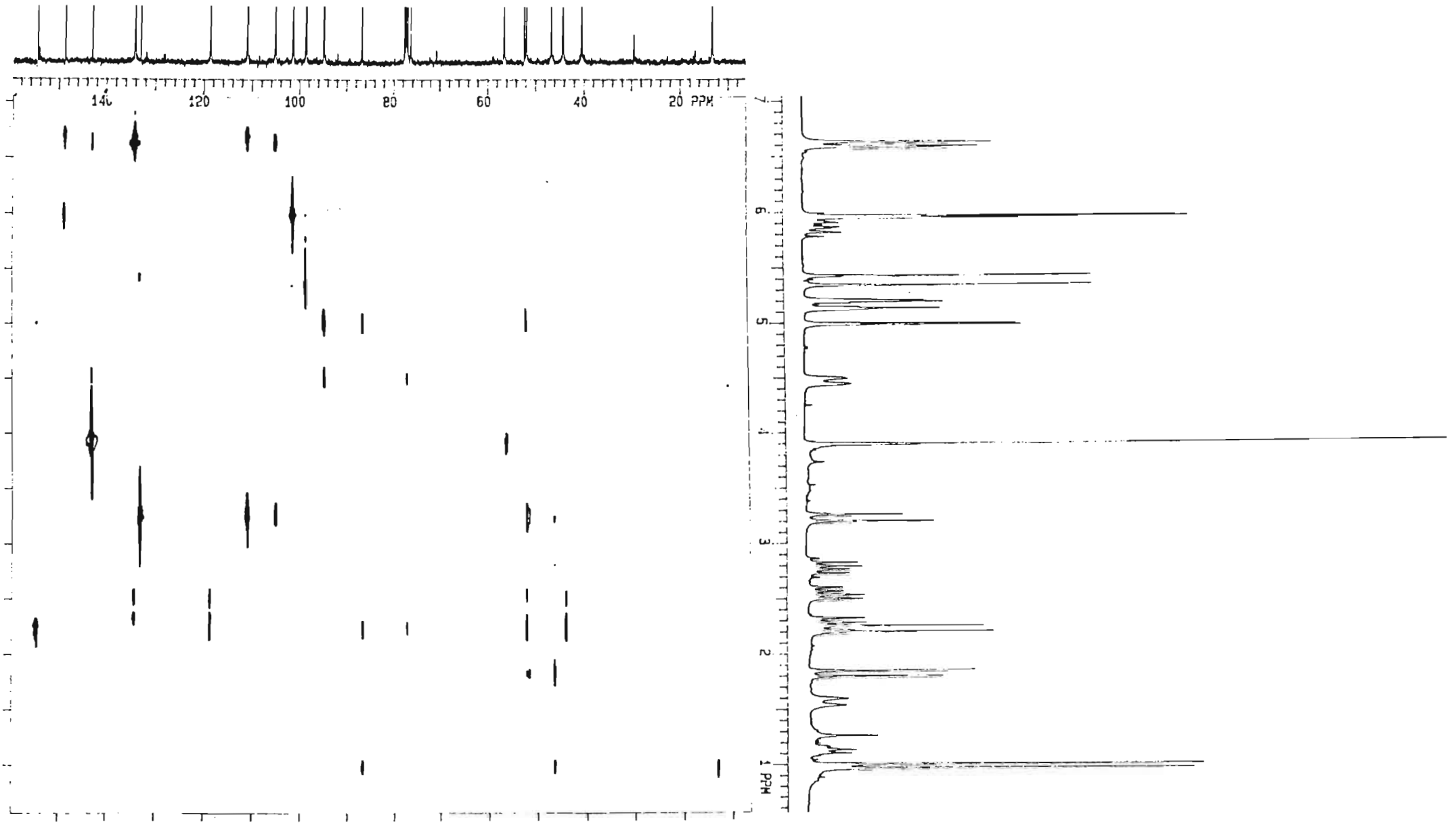


69e ^{1}H-^{1}H DELAYED COSY spectrum of the alcohol 69 in $CDCl_3$

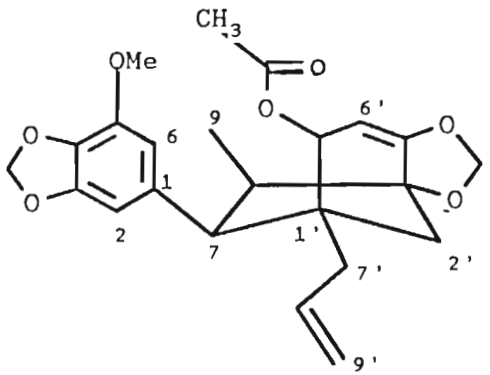




69f ^{1}H-^{13}C HETCOR spectrum of the alcohol **69** in $CDCl_3$

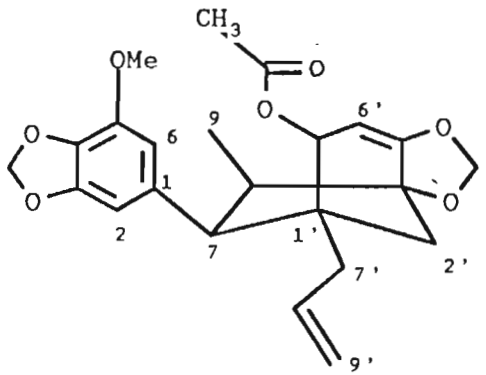


69q ^{1}H-^{13}C DELAYED HETCOR spectrum of the alcohol 69 in $CDCl_3$



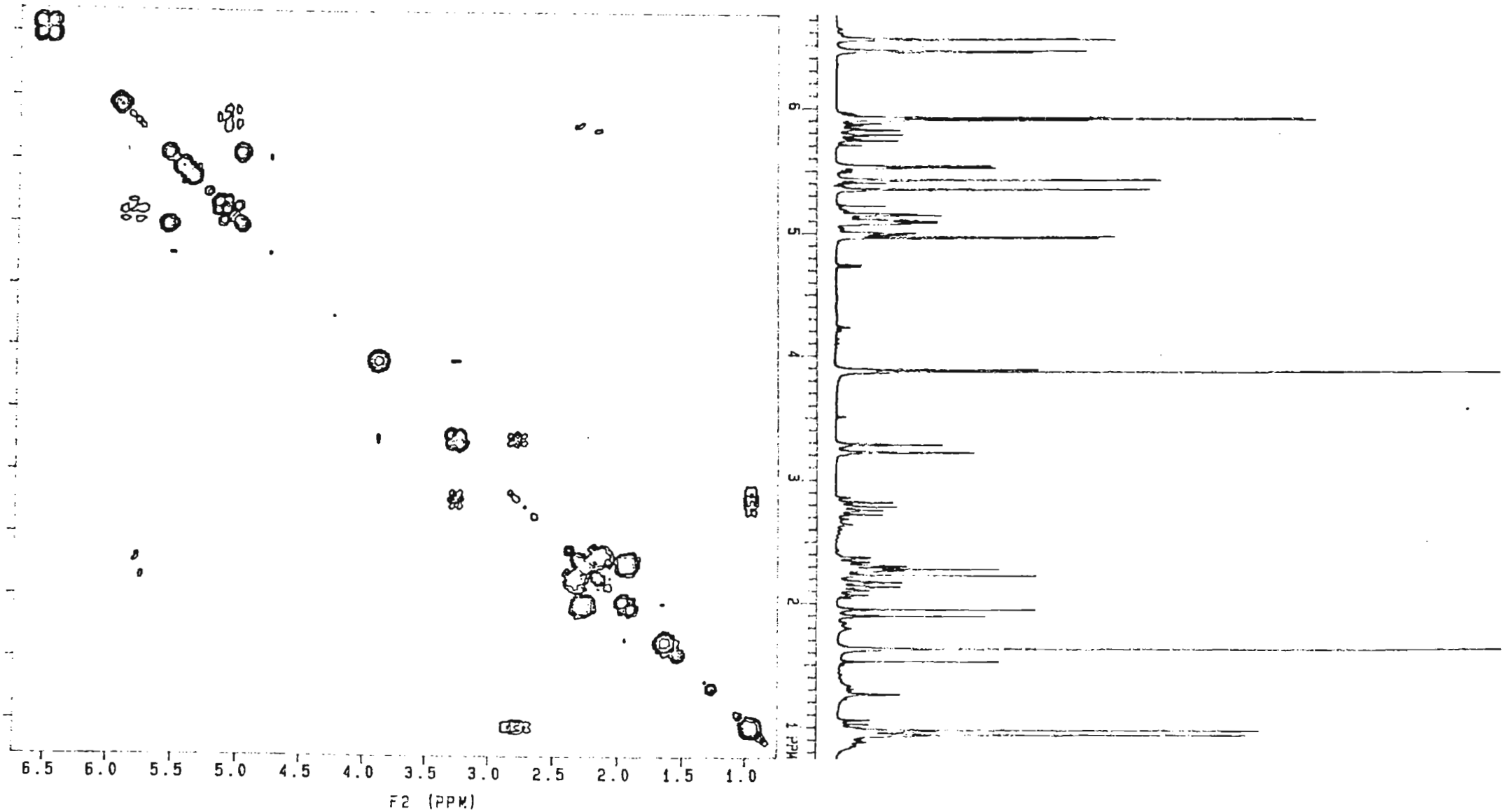
69a

69aa ^{1}H NMR spectrum of the acetate **69a** in $CDCl_3$

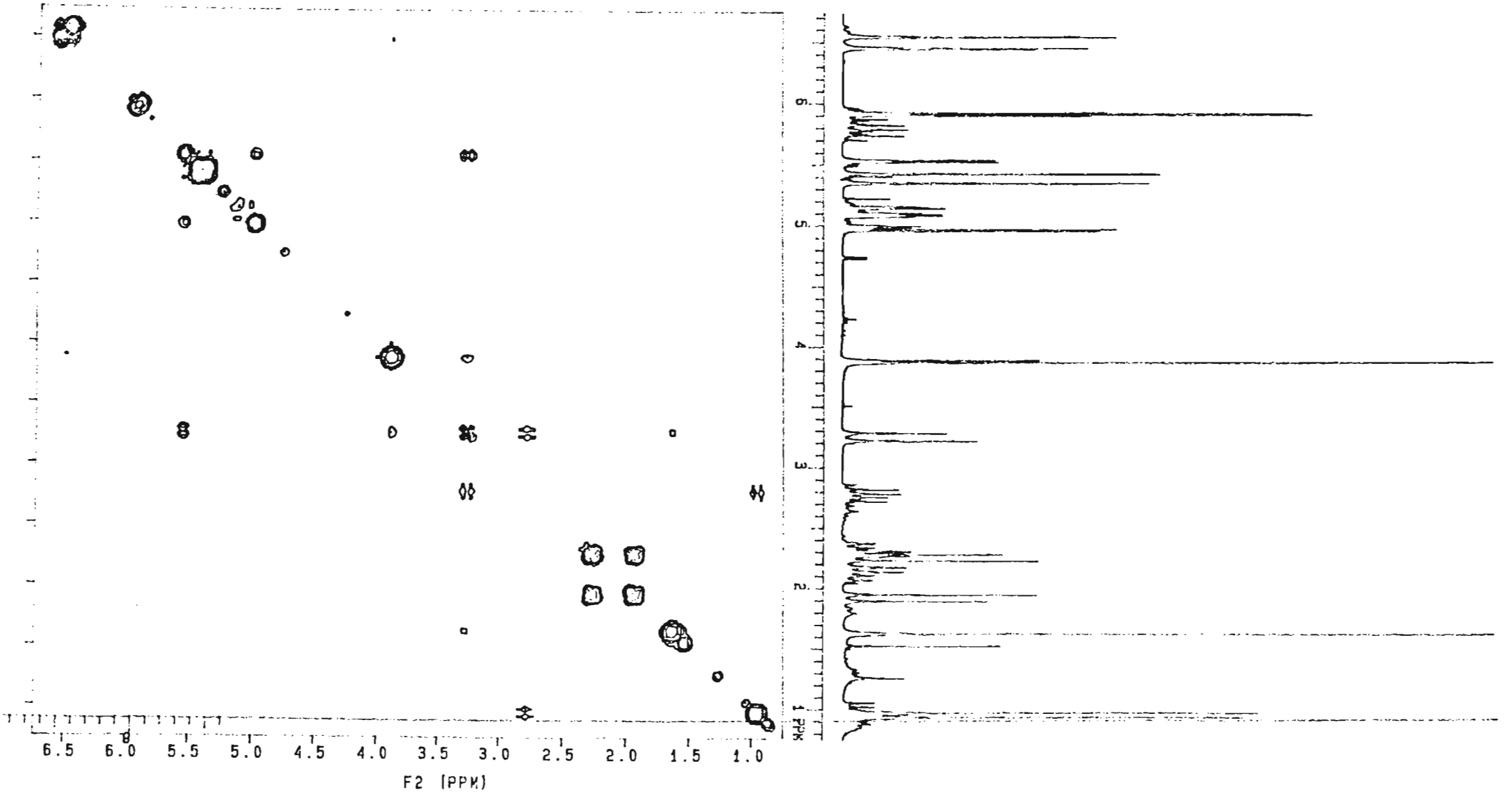


69a

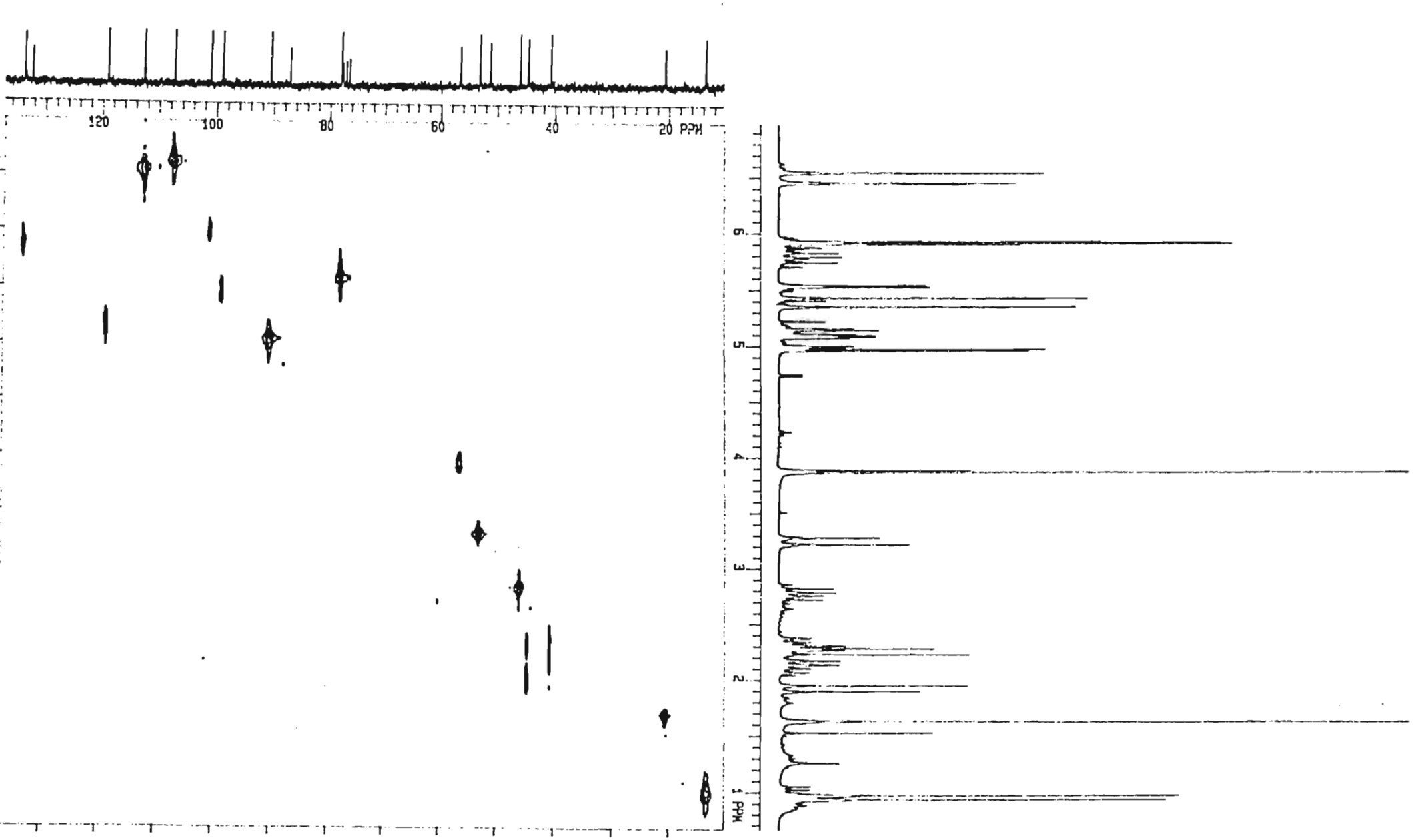
69ac ^{13}C NMR spectrum of the acetate **69a** in $CDCl_3$



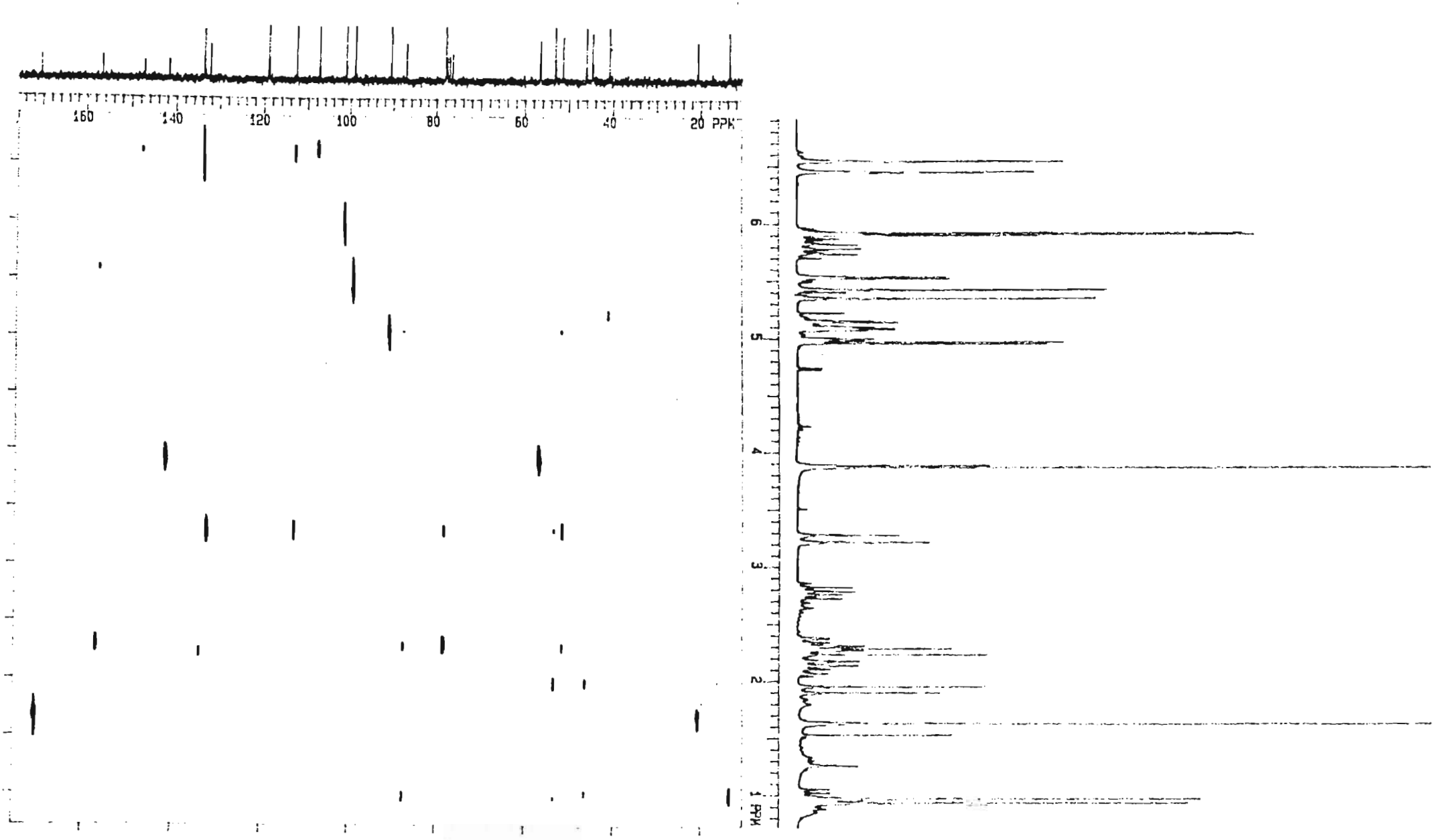
69ad ^{1}H-^{1}H COSY spectrum of the acetate 69a in $CDCl_3$



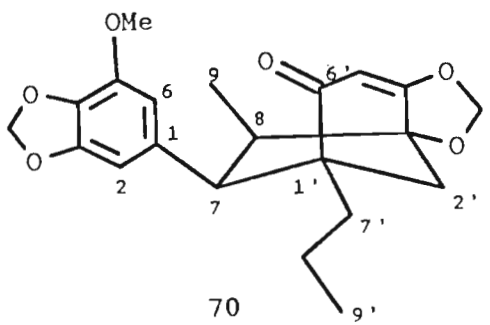
69ae ^{1}H-^{1}H DELAYED COSY spectrum of the acetate 69a in $CDCl_3$



69af ^{1}H-^{13}C HETCOR spectrum of the acetate **69a** in $CDCl_3$



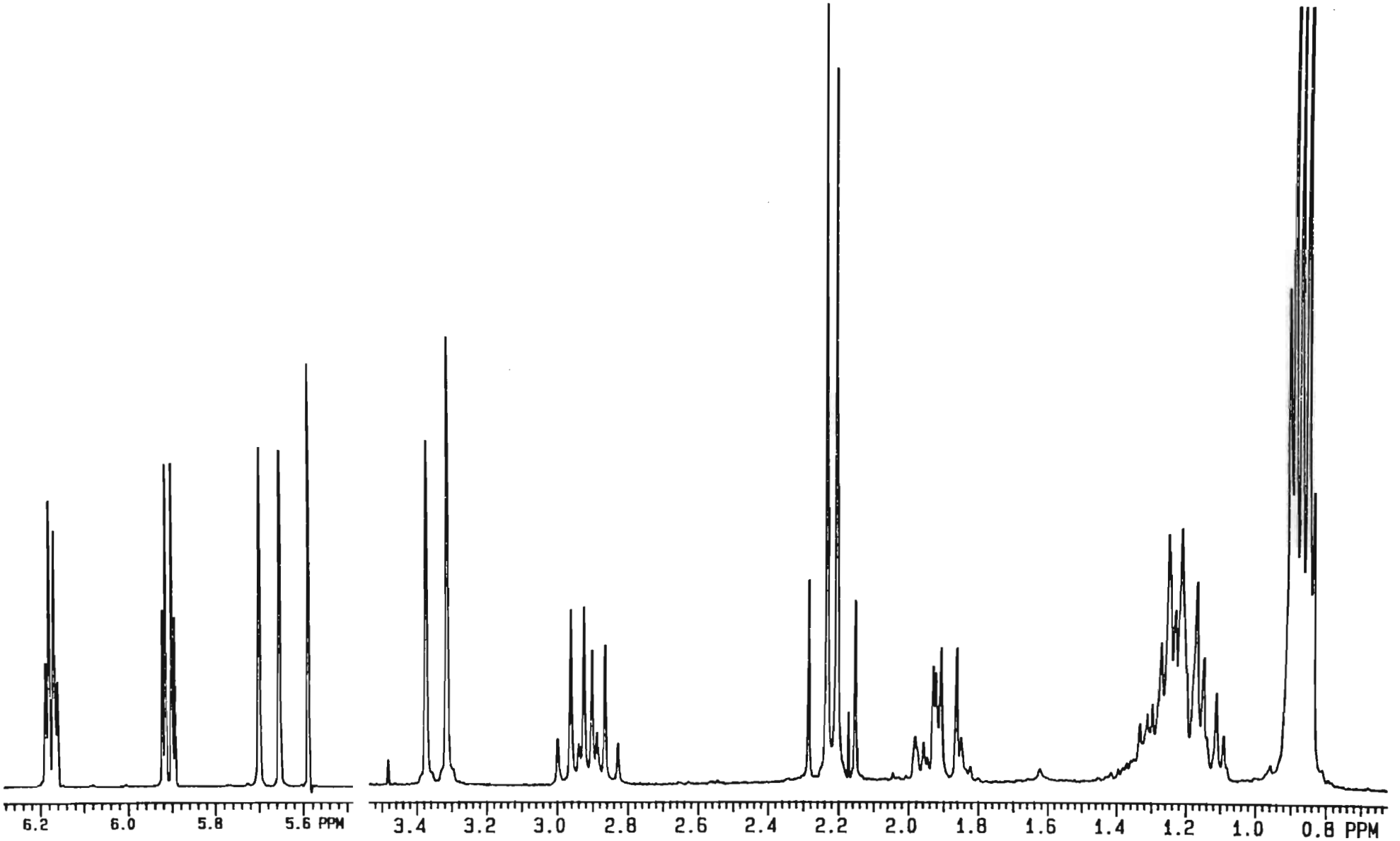
69aq ^{1}H-^{13}C DELAYED HETCOR spectrum of the acetate 69a in $CDCl_3$



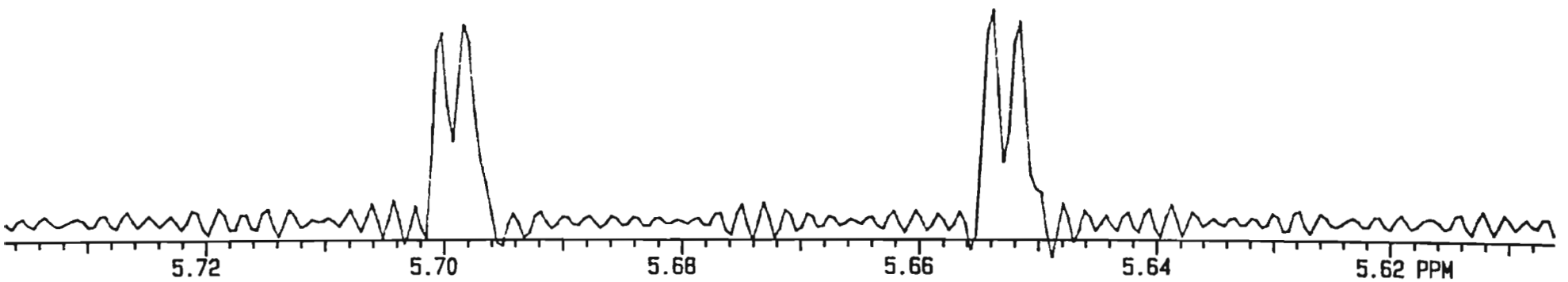
70

9 8 7 6 5 4 3 2 1 PPM

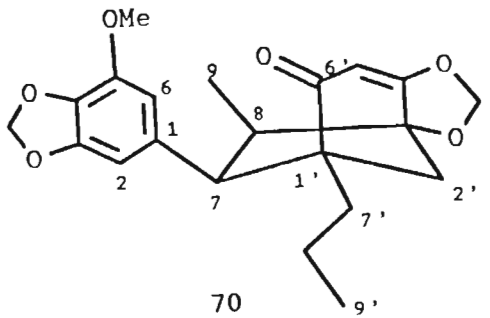
70a ^{1}H NMR spectrum of **70** in $CDCl_3$



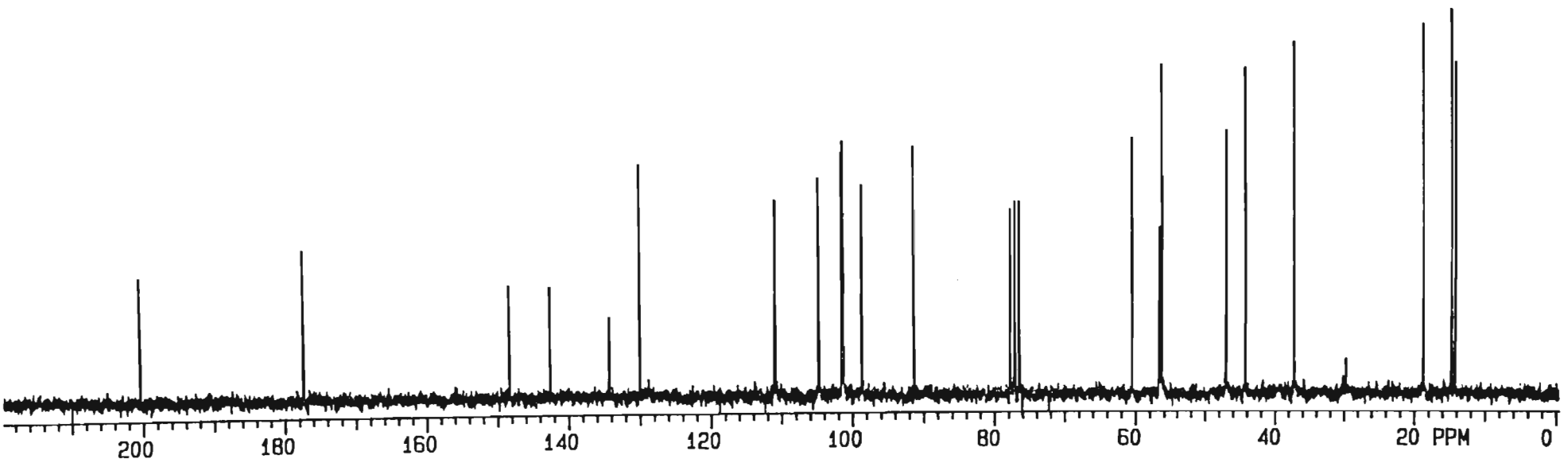
70b Expanded ^{1}H NMR spectrum of **70** in $CDCl_3$



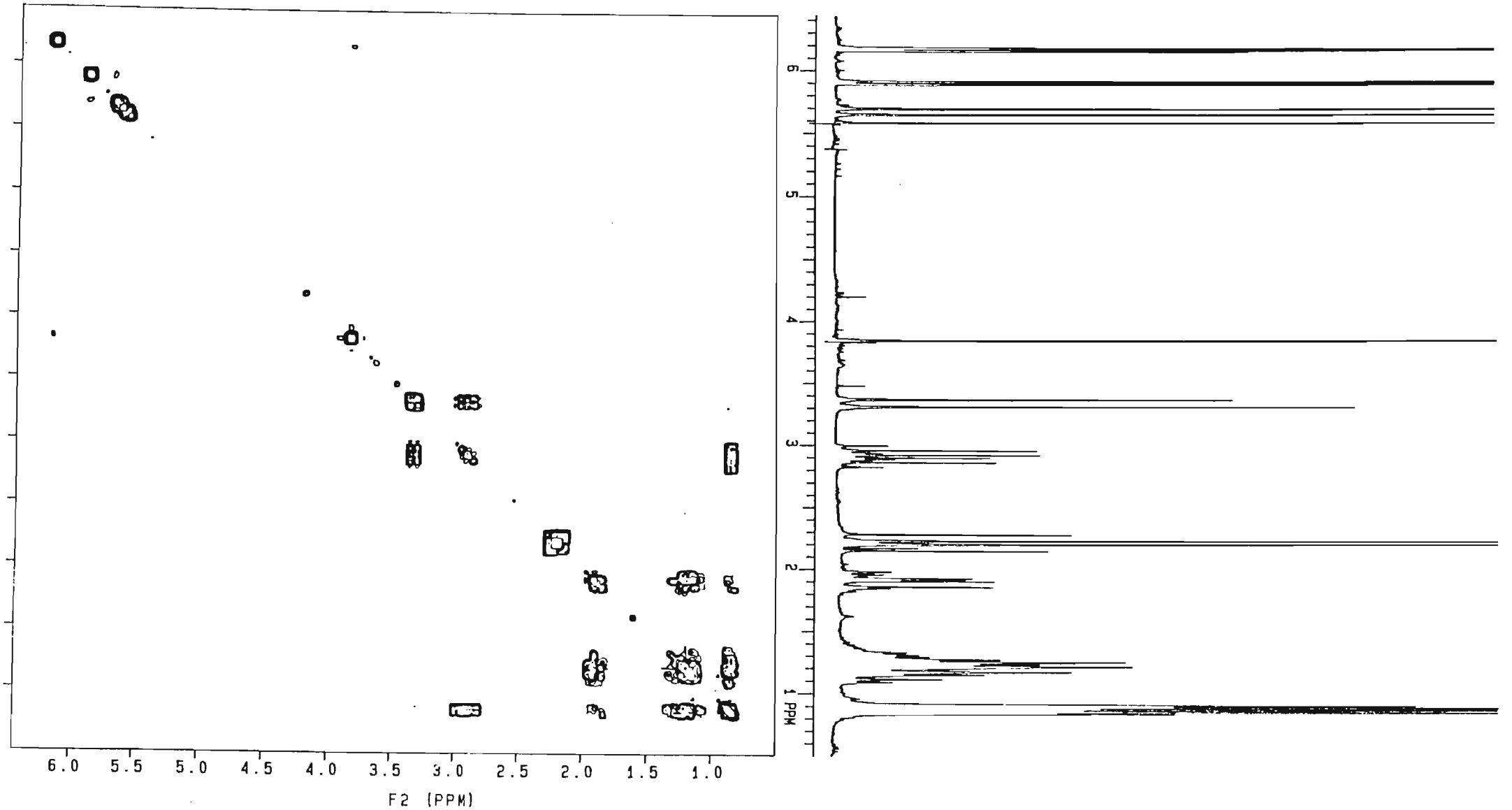
70b Expanded ^{1}H NMR spectrum of 70 in $CDCl_3$



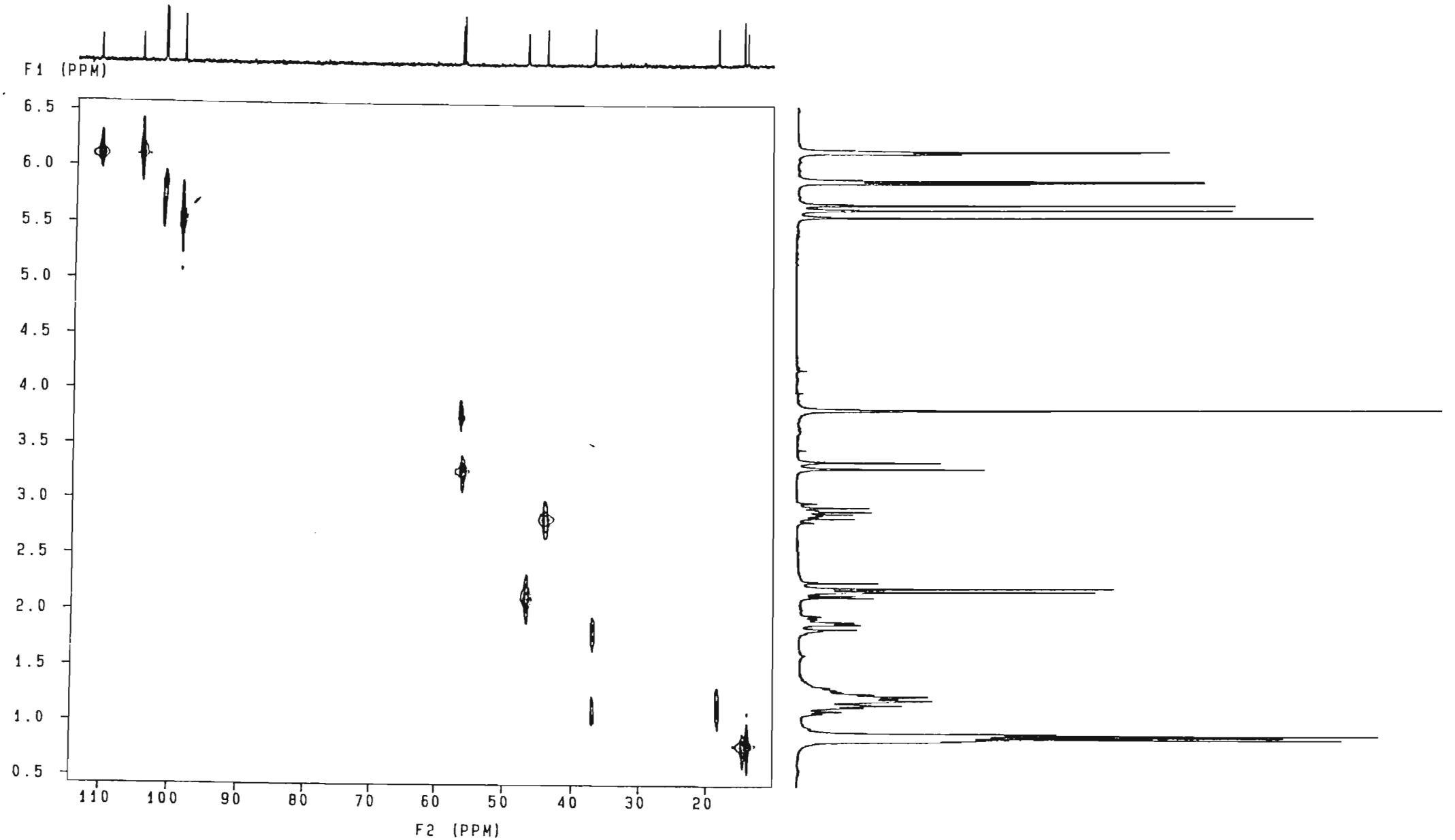
70



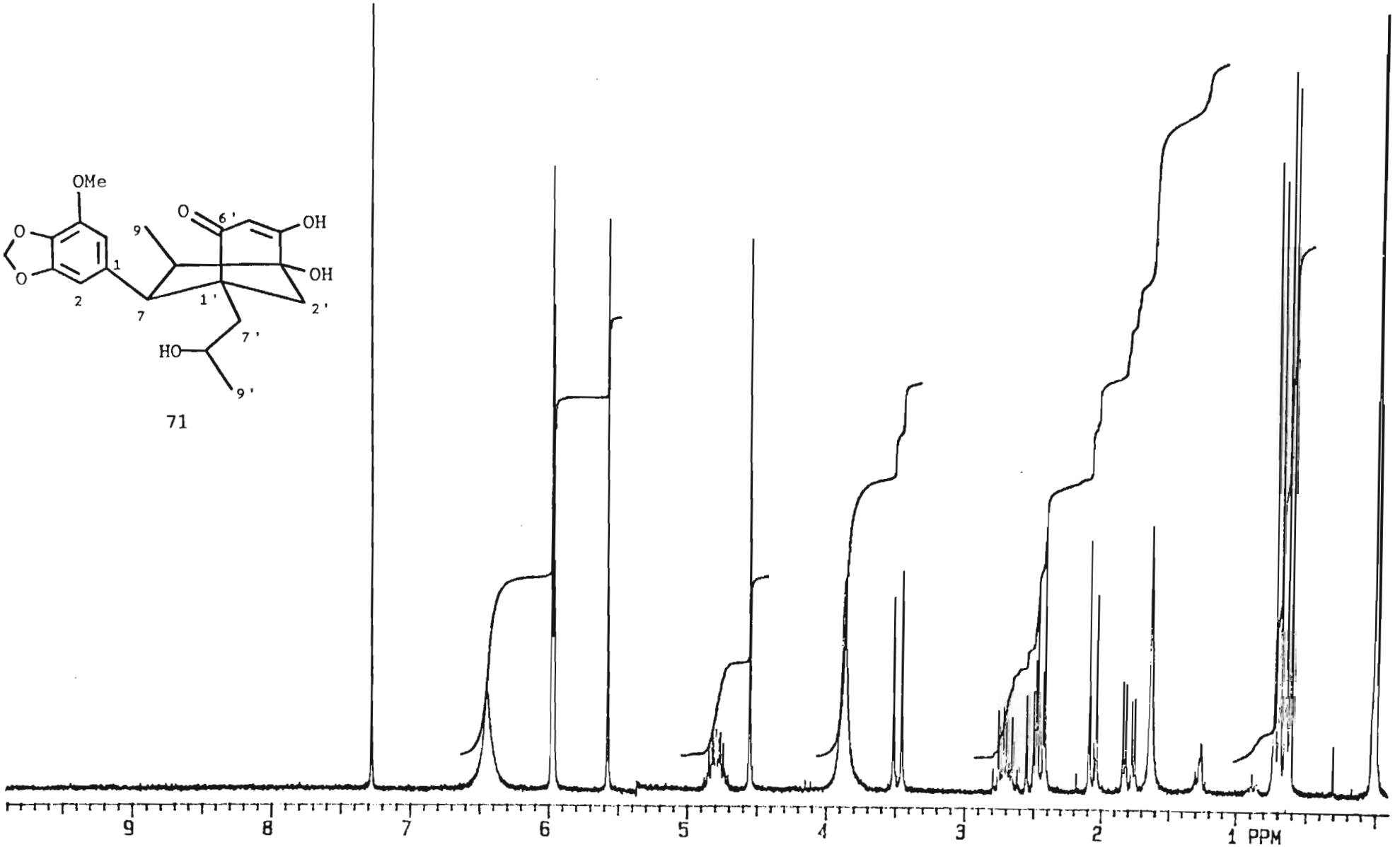
70c ^{13}C NMR spectrum of **70** in $CDCl_3$



70d ^{1}H-^{1}H COSY spectrum of **70** in $CDCl_3$

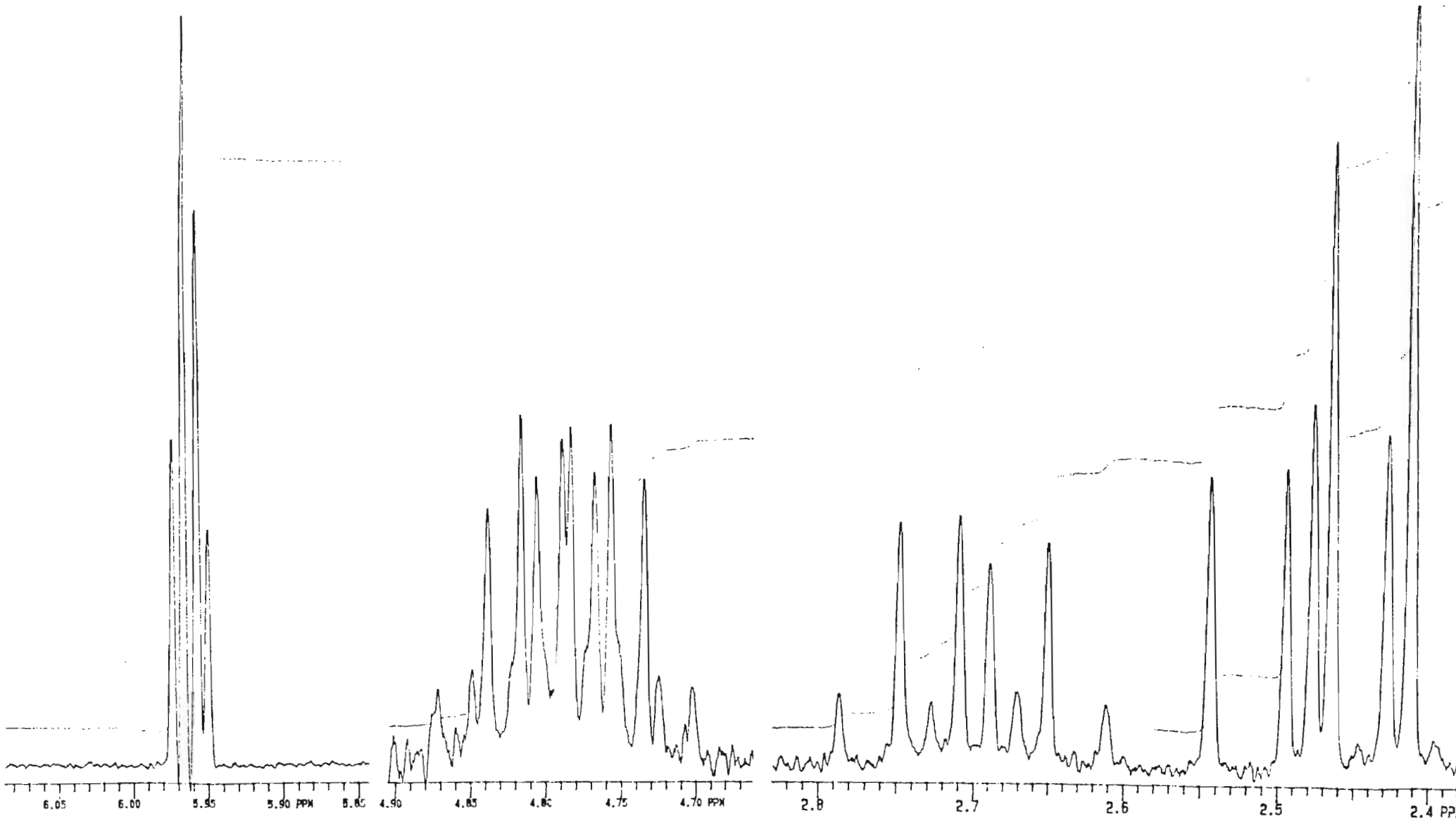


70f ^{1}H-^{13}C HETCOR spectrum of **70** in $CDCl_3$

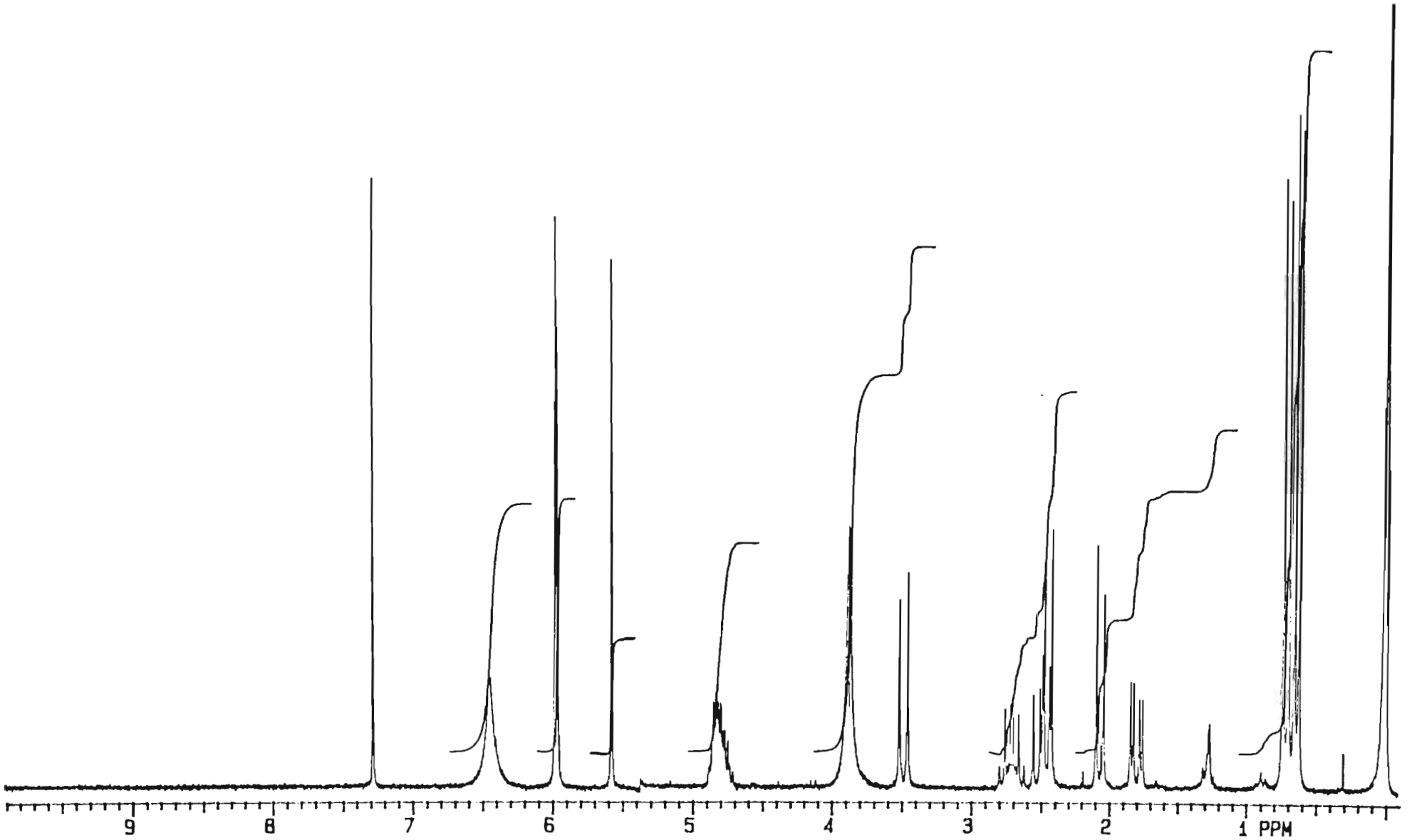


71

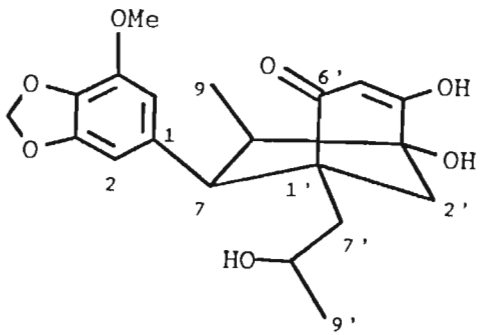
71a ^{1}H NMR spectrum of **71** in $CDCl_3$



71b Expanded ^{1}H NMR spectrum of **71** in $CDCl_3$

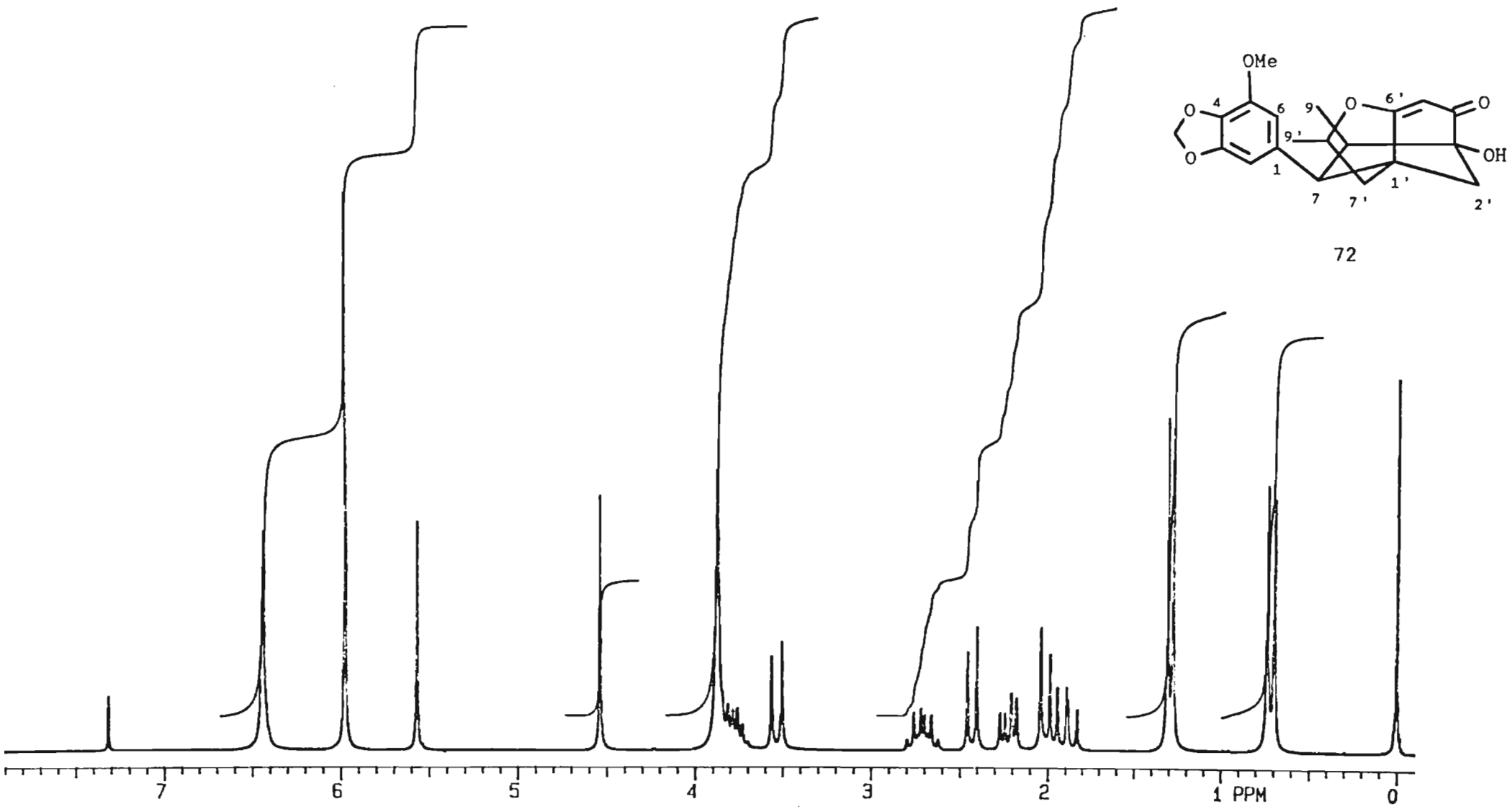


71a ^{1}H NMR spectrum of **71** in $D_2O/CDCl_3$



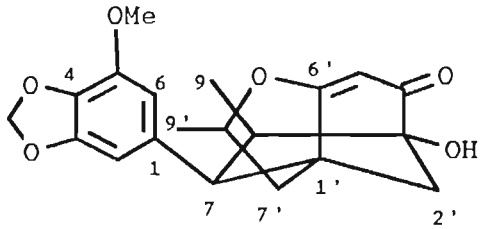
71

71c ^{13}C NMR spectrum of **71** in $CDCl_3$



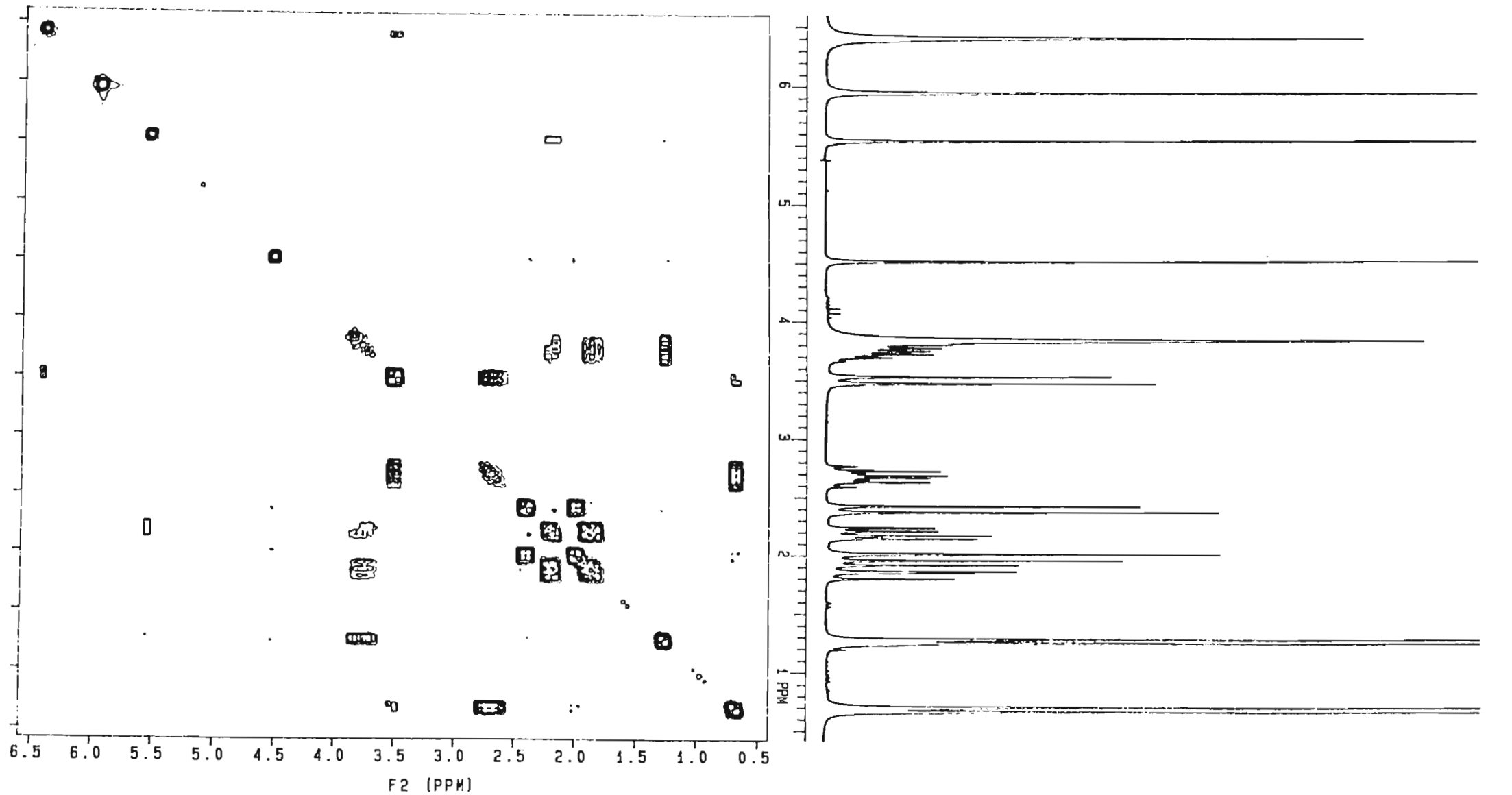
72

72a ^{1}H NMR spectrum of 72 in $CDCl_3$

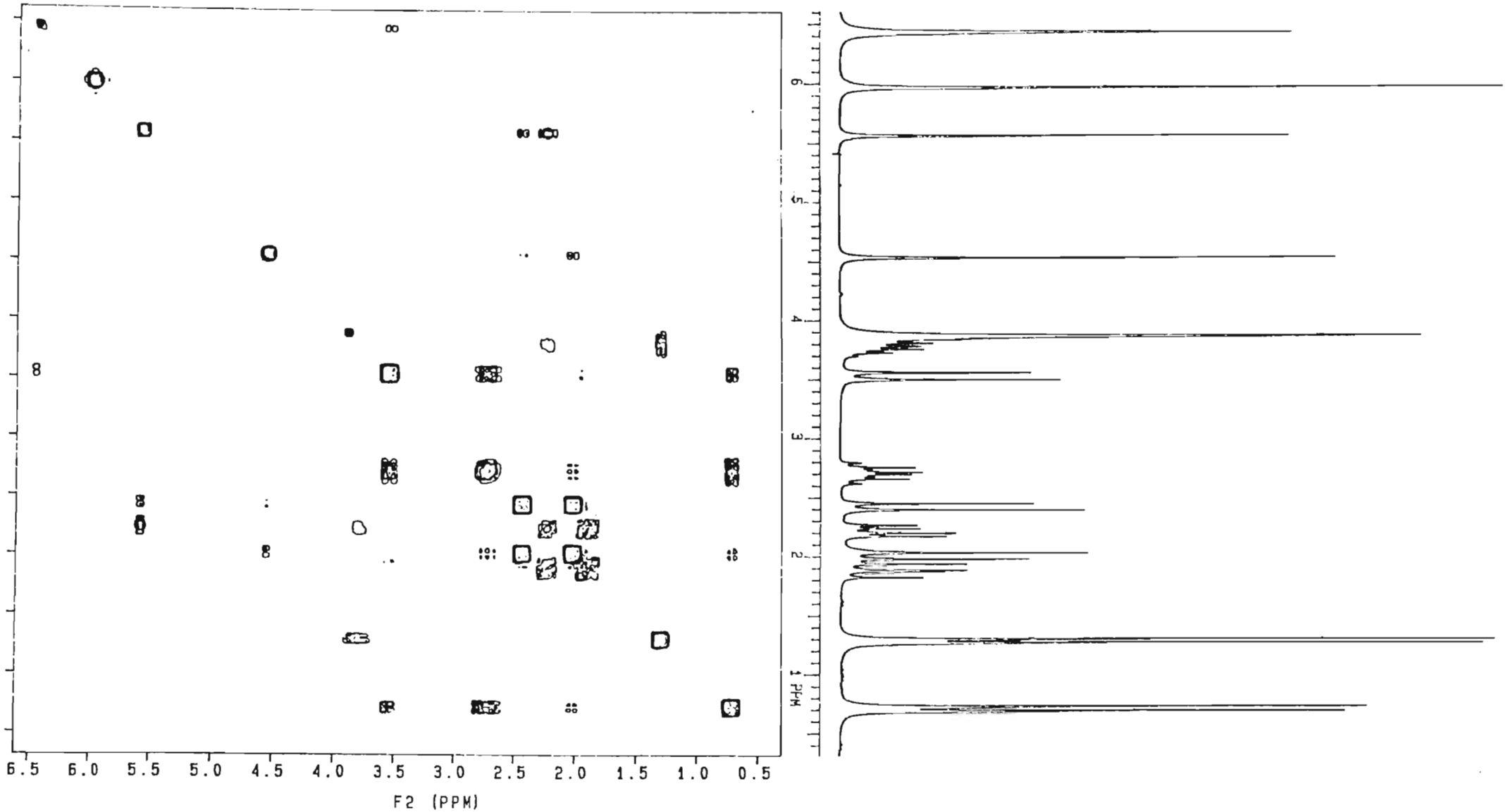


72

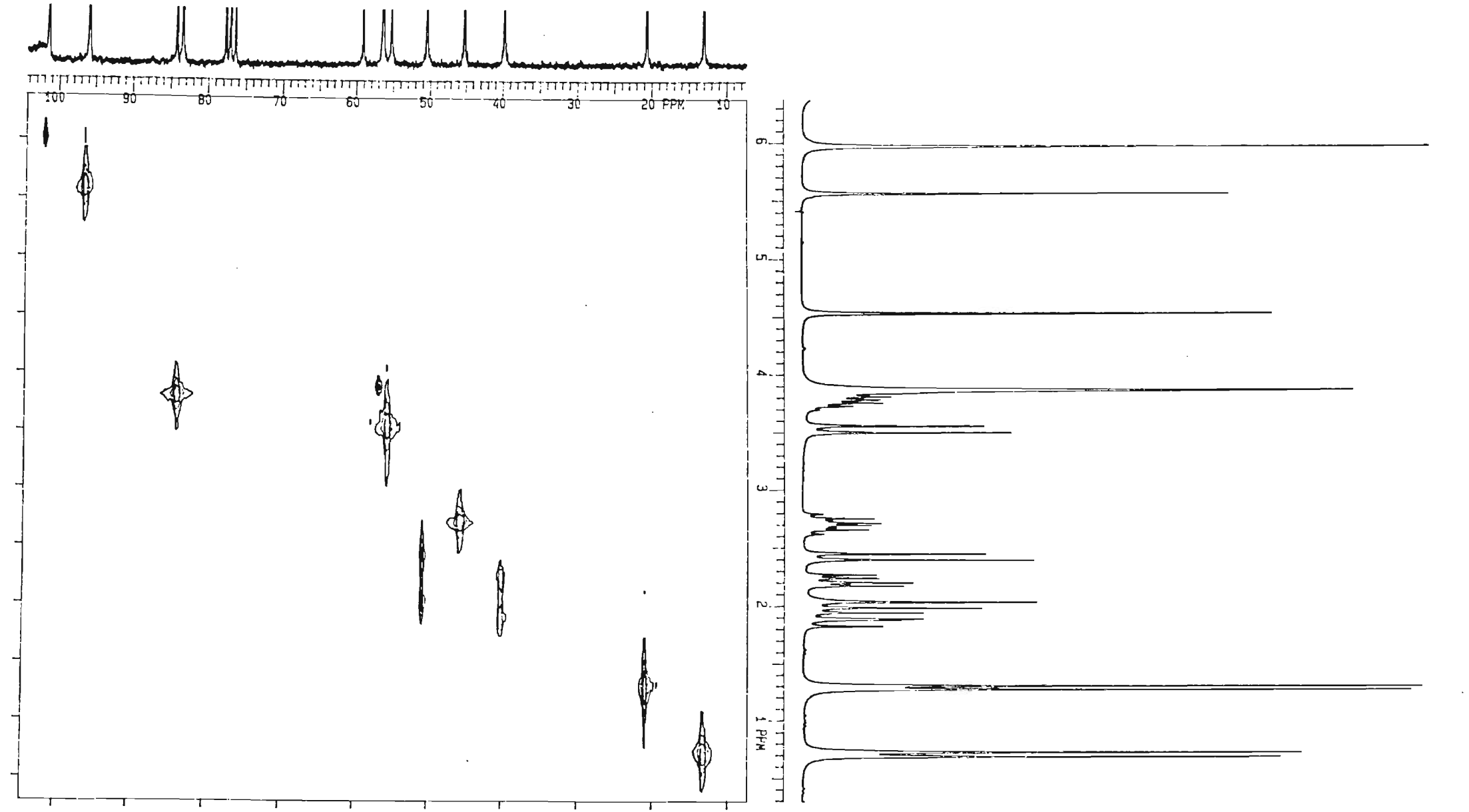
72c ^{13}C NMR spectrum of **72** in $CDCl_3$



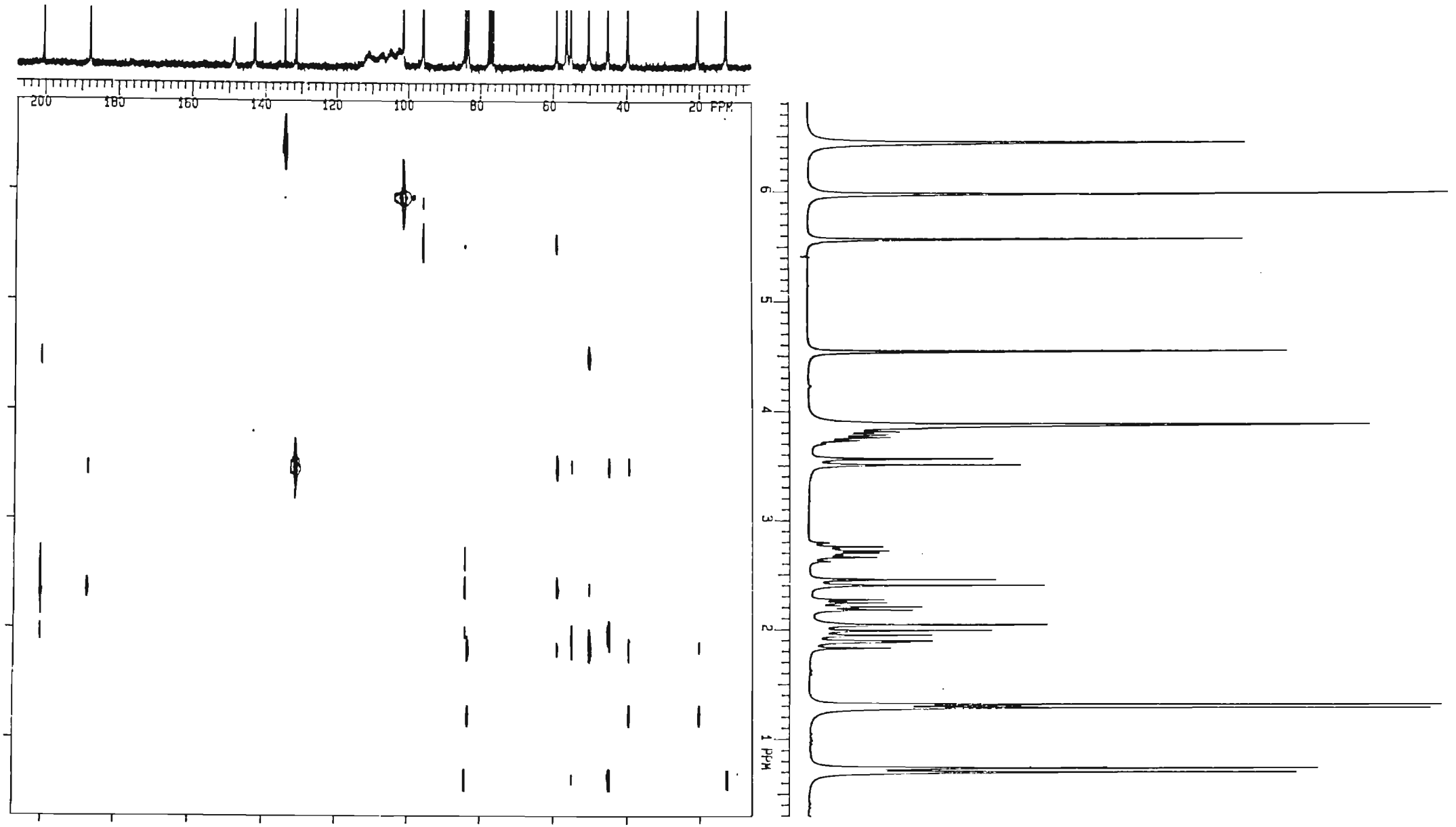
72d ^{1}H-^{1}H COSY spectrum of **72** in $CDCl_3$



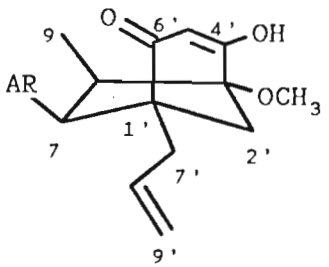
72e ^{1}H-^{1}H DELAYED COSY spectrum of **72** in $CDCl_3$



72f ^{1}H-^{13}C HETCOR spectrum of **72** in $CDCl_3$

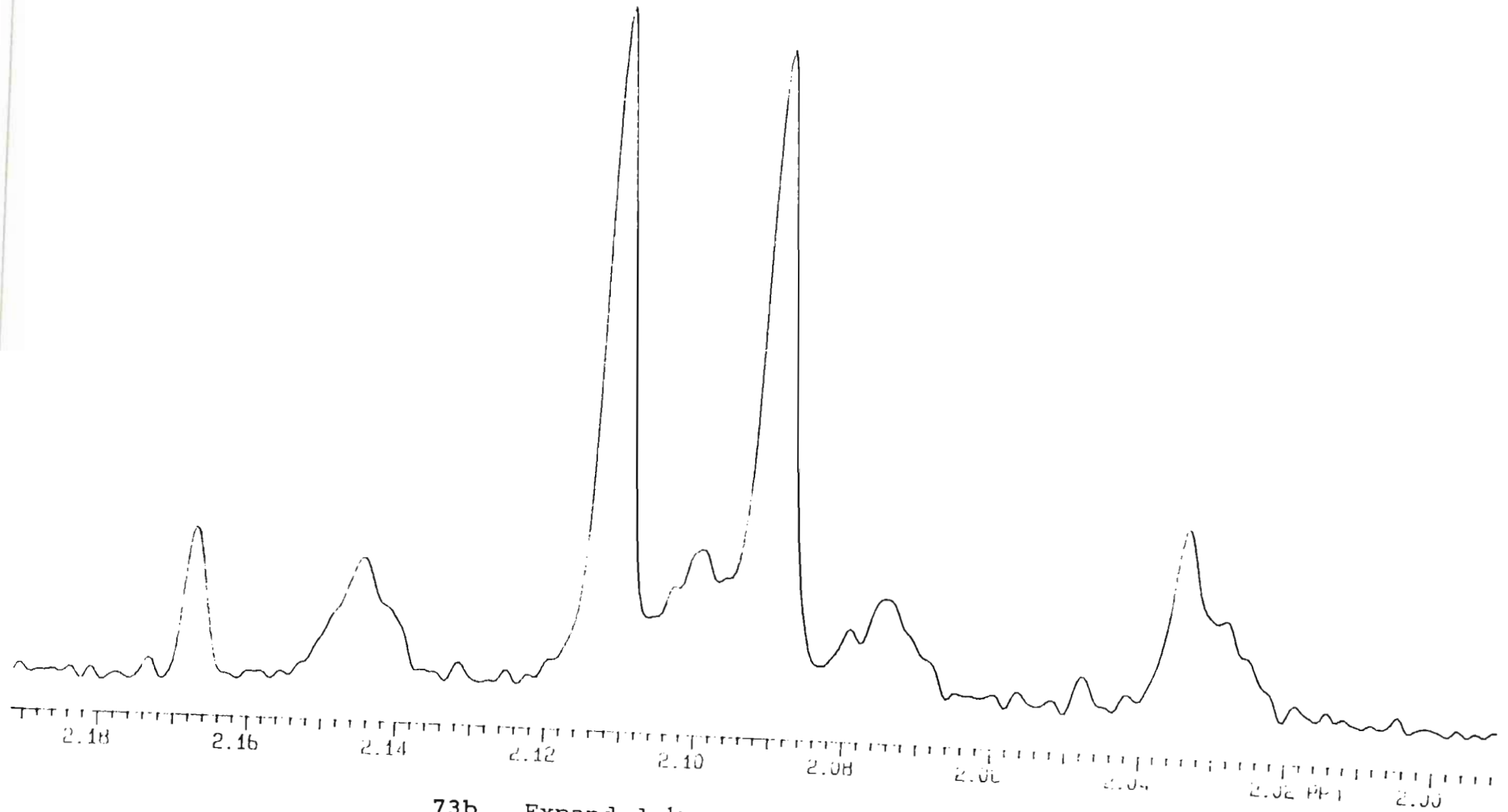


72a ^{1}H-^{13}C DELAYED HETCOR spectrum of 72 in $CDCl_3$

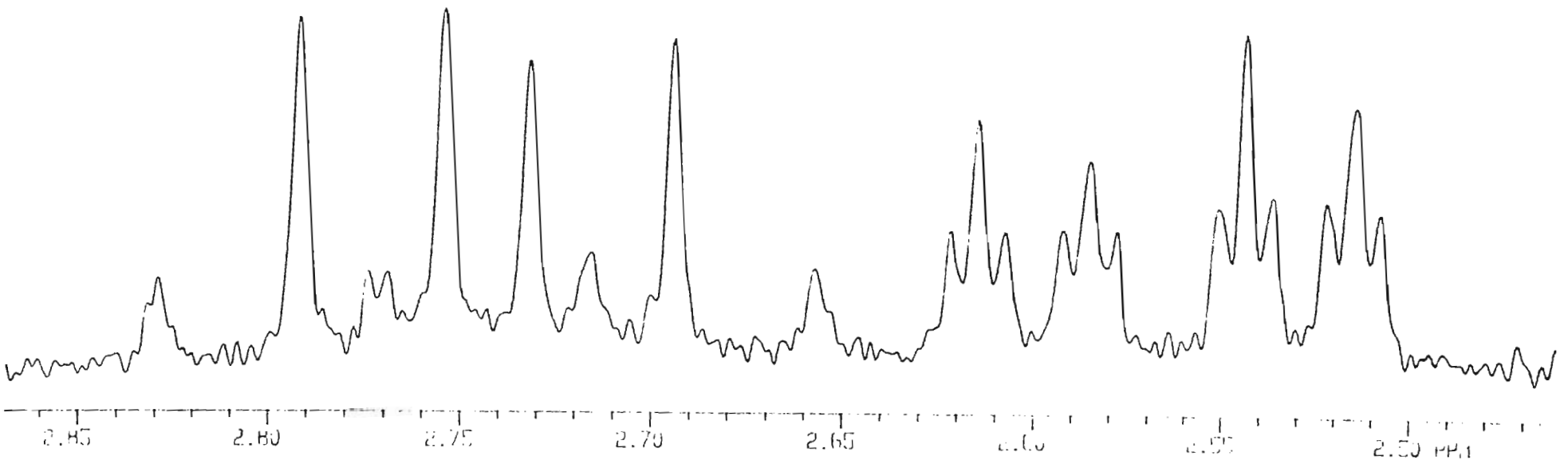


73

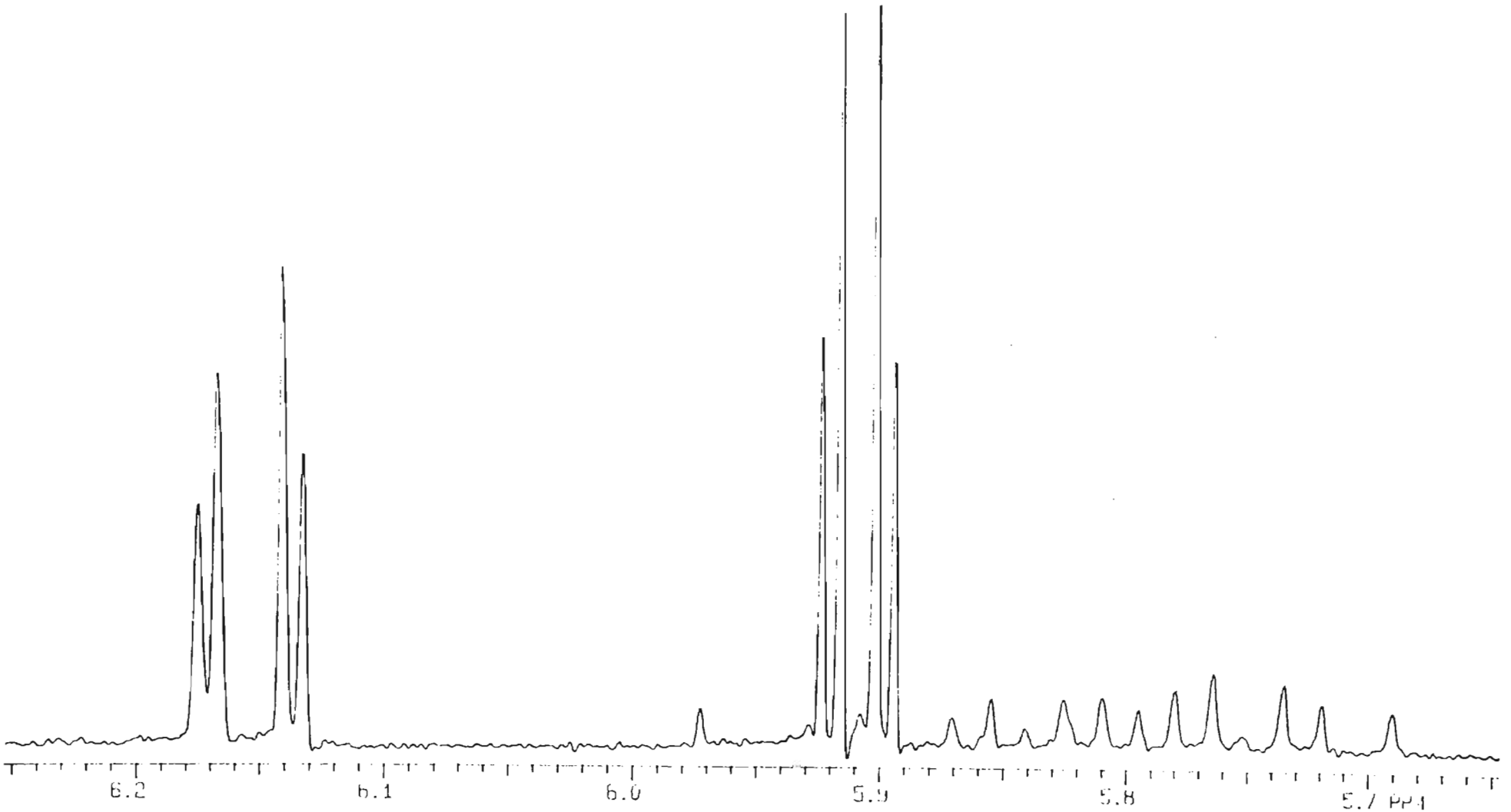
73a ^{1}H NMR spectrum of 73 in $CDCl_3$



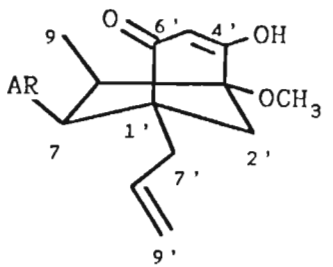
73b Expanded ^{1}H NMR spectrum of **73** in $CDCl_3$



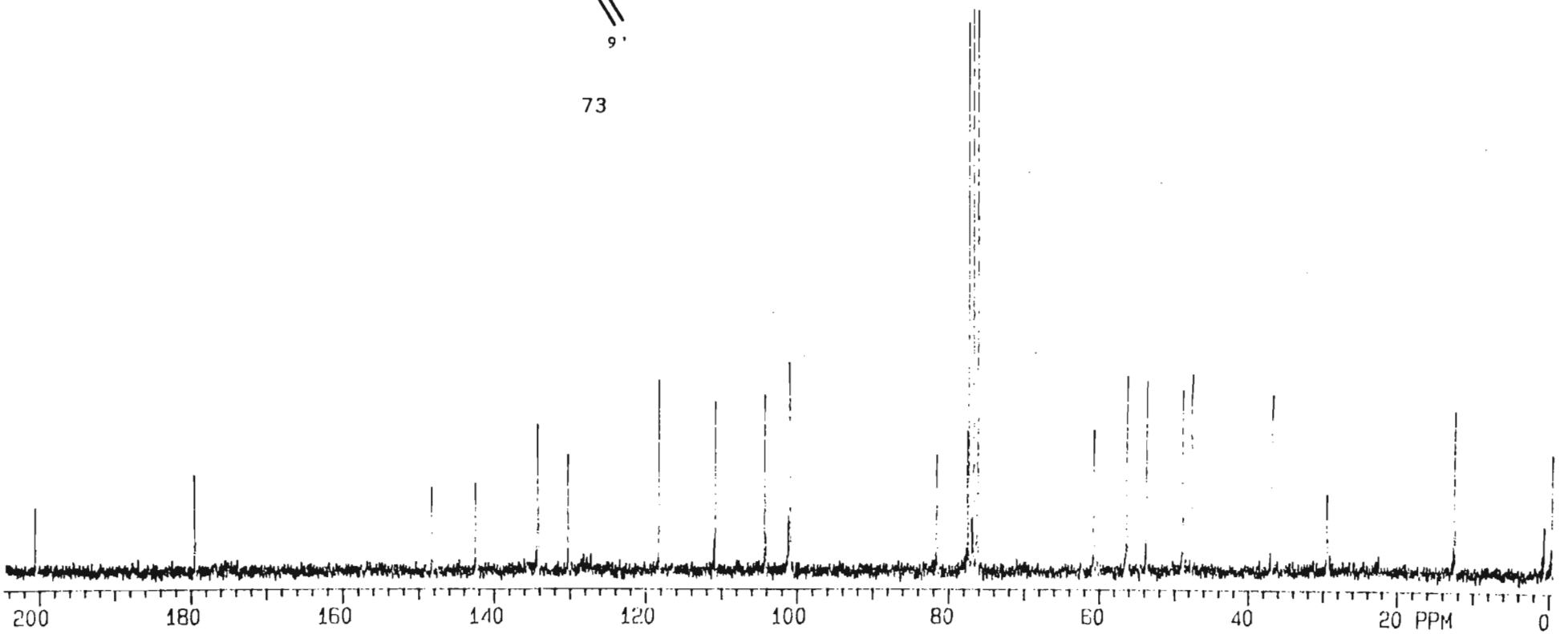
73b Expanded ^{1}H NMR spectrum of **73** in $CDCl_3$



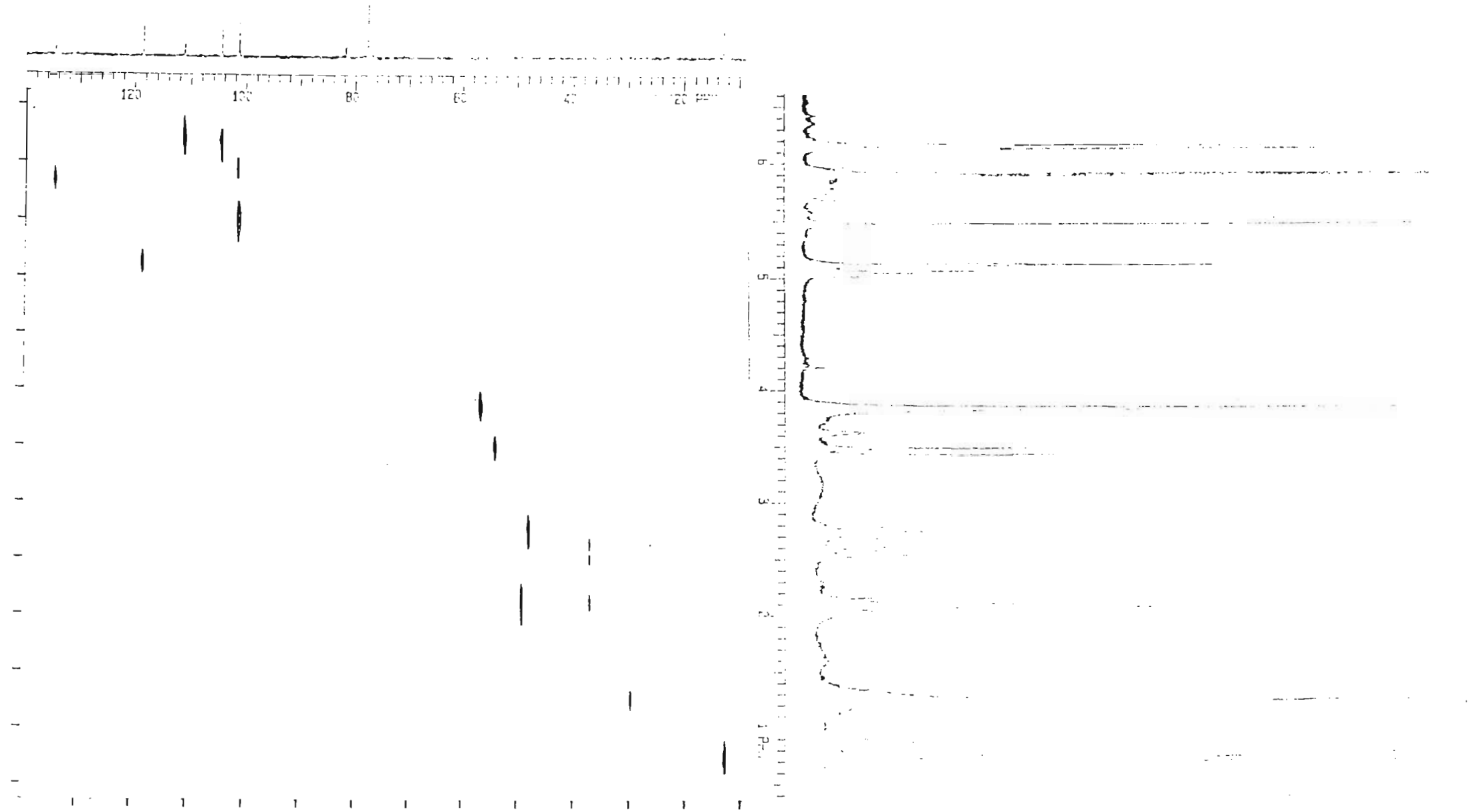
73b Expanded ^{1}H NMR spectrum of **73** in $CDCl_3$



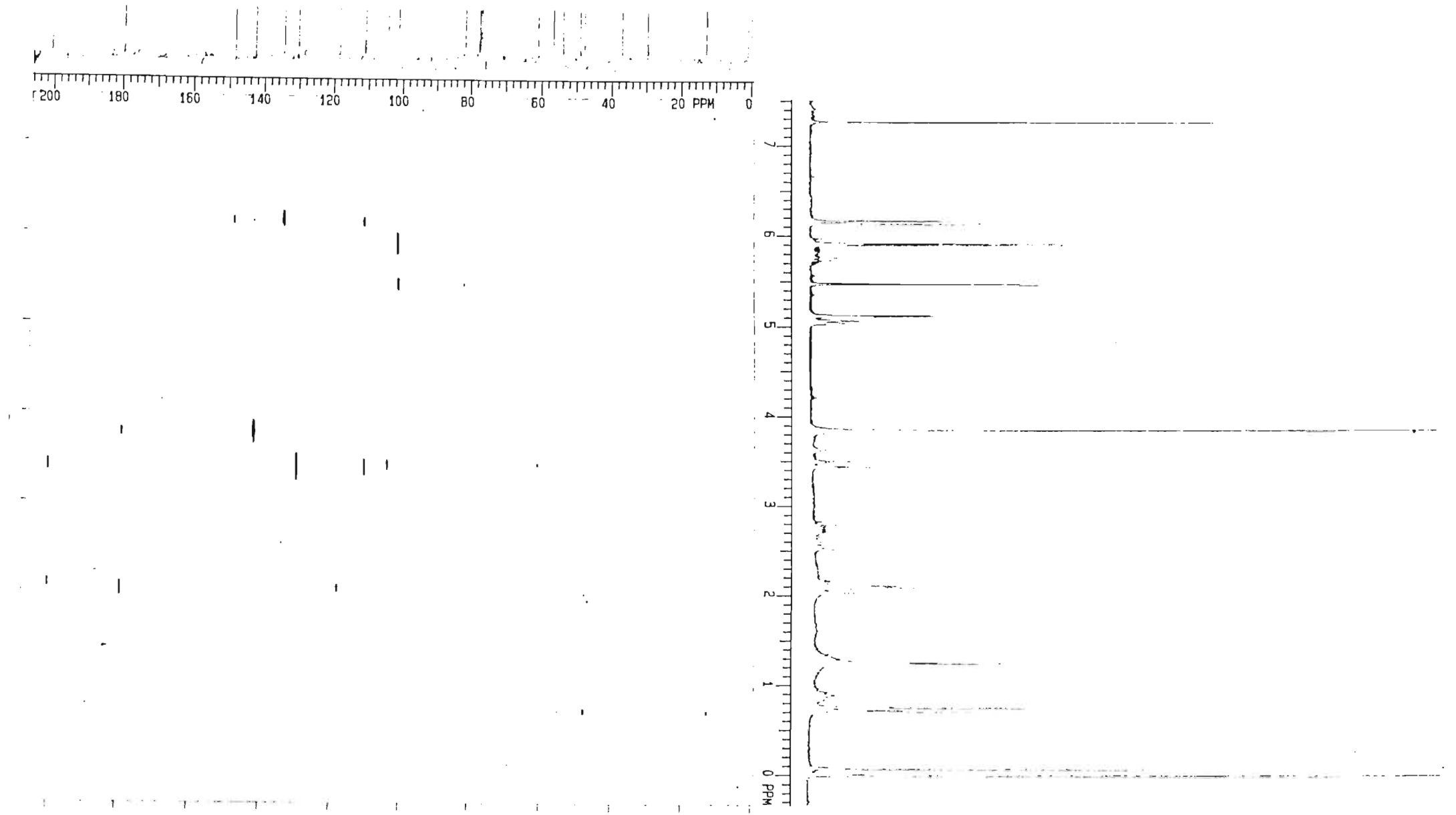
73



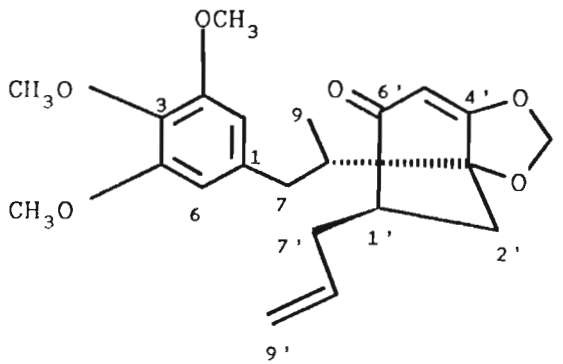
73c ^{13}C NMR spectrum of **73** in $CDCl_3$



73f ^{1}H-^{13}C HETCOR spectrum of **73** in $CDCl_3$

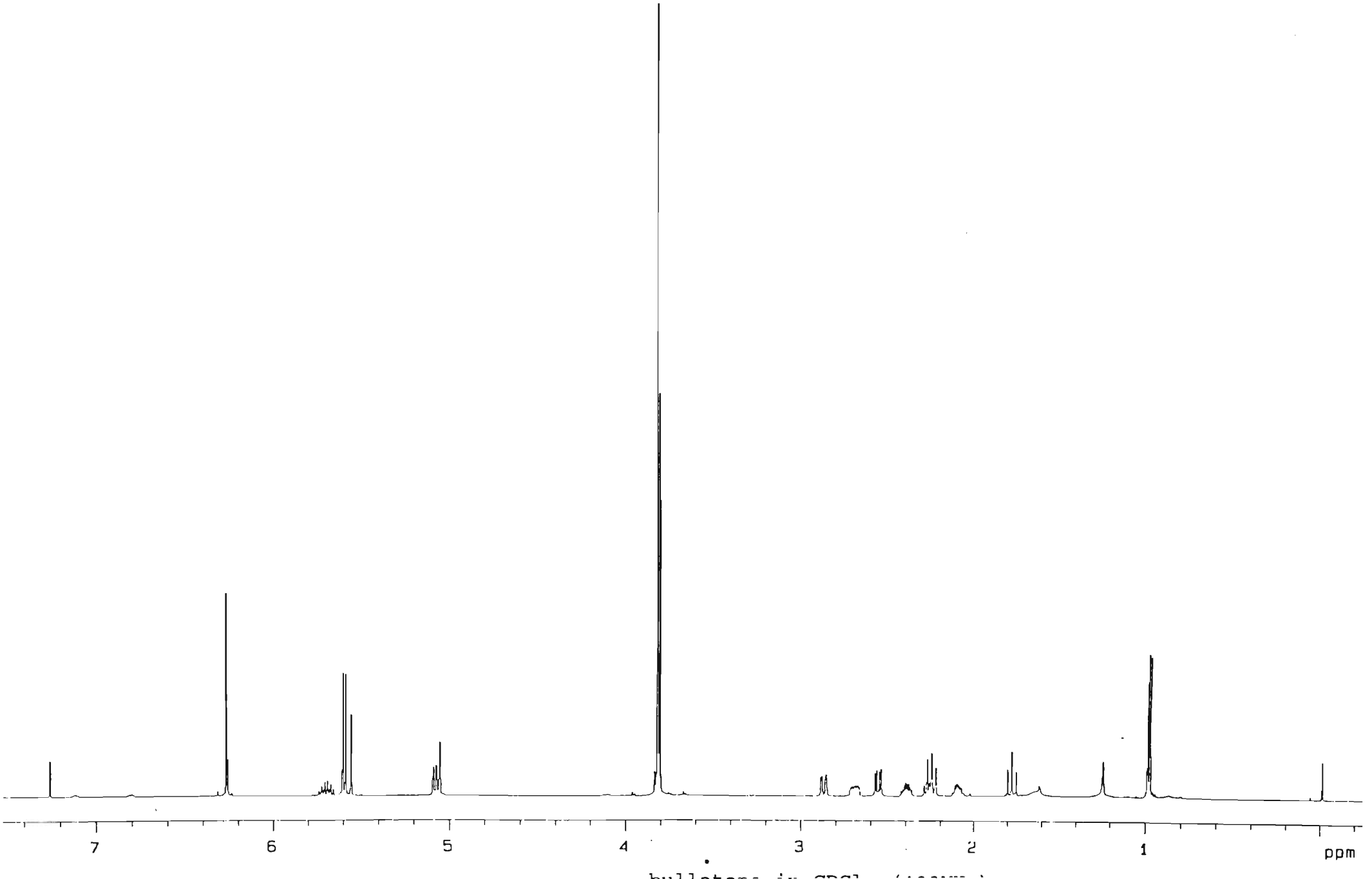


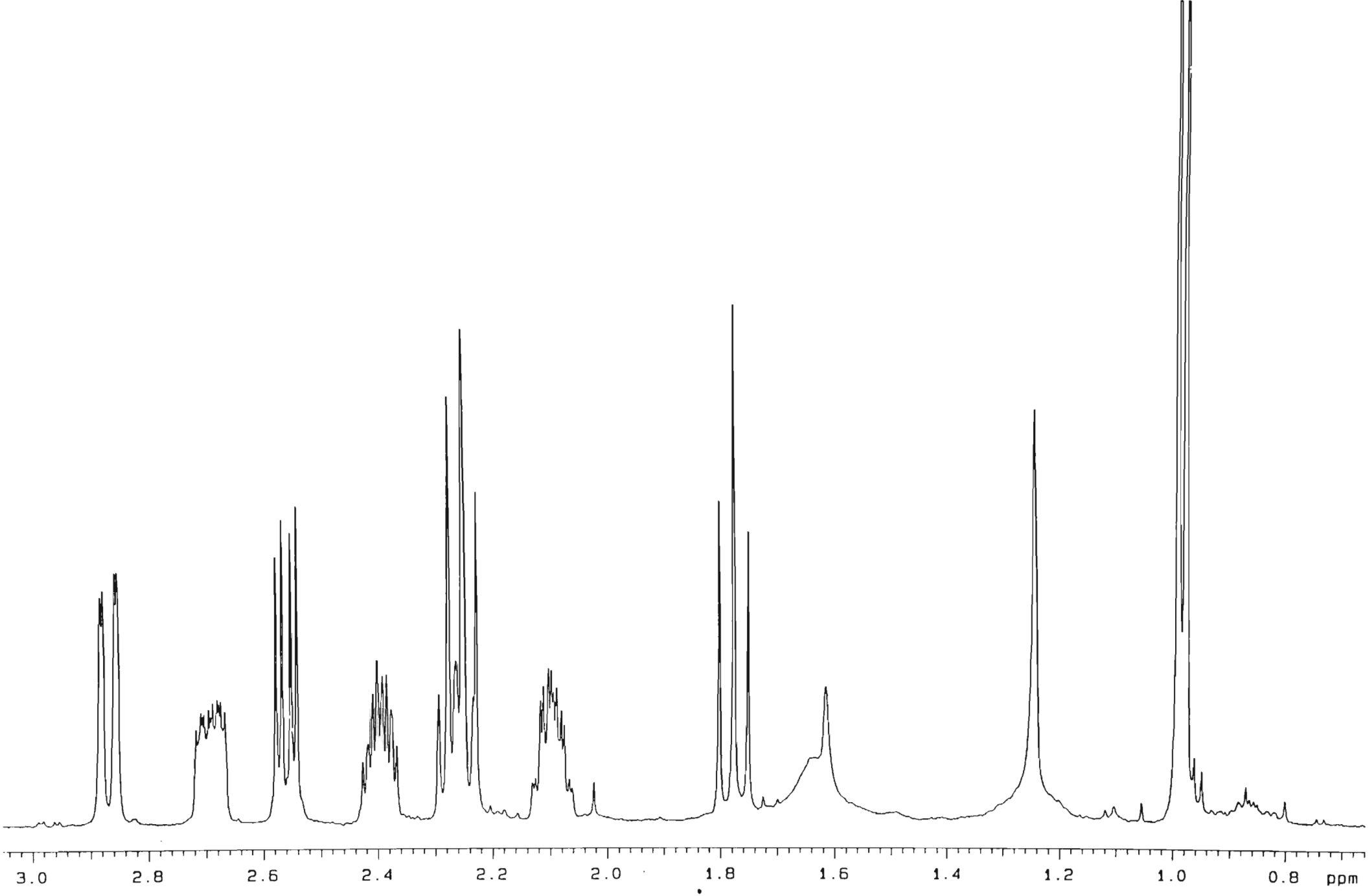
73g ^{1}H-^{13}C DELAYED HETCOR spectrum of 73 in $CDCl_3$



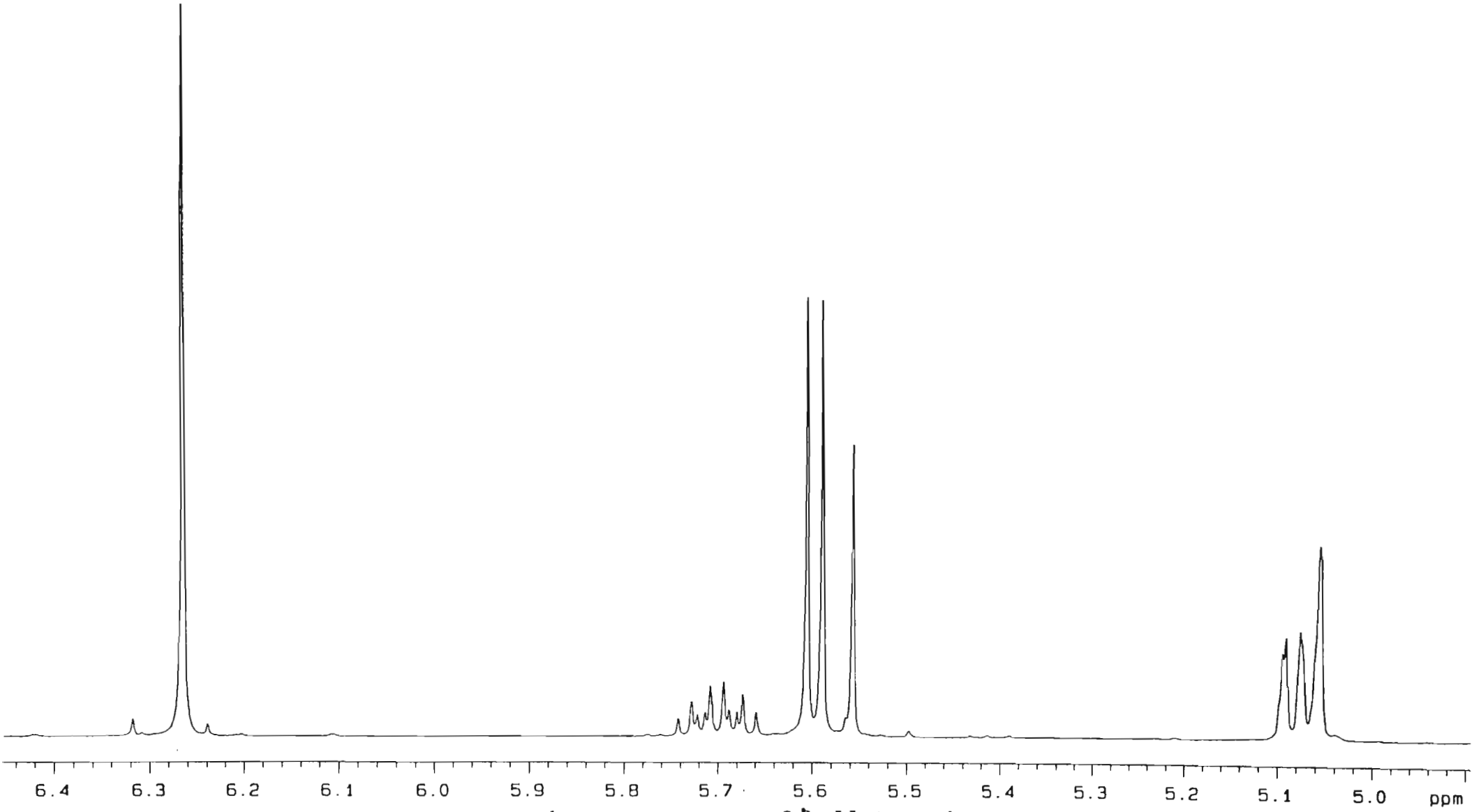
74

74a ^{1}H NMR spectrum of bullatone in $CDCl_3$

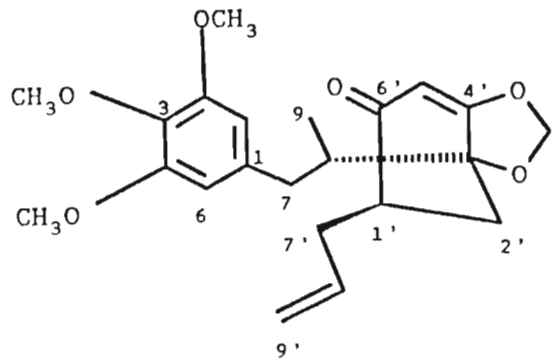

7
6
5
4
3
2
1
ppm



74b Expanded ^{1}H NMR spectrum of bullatone in $CDCl_3$ (400MHz)

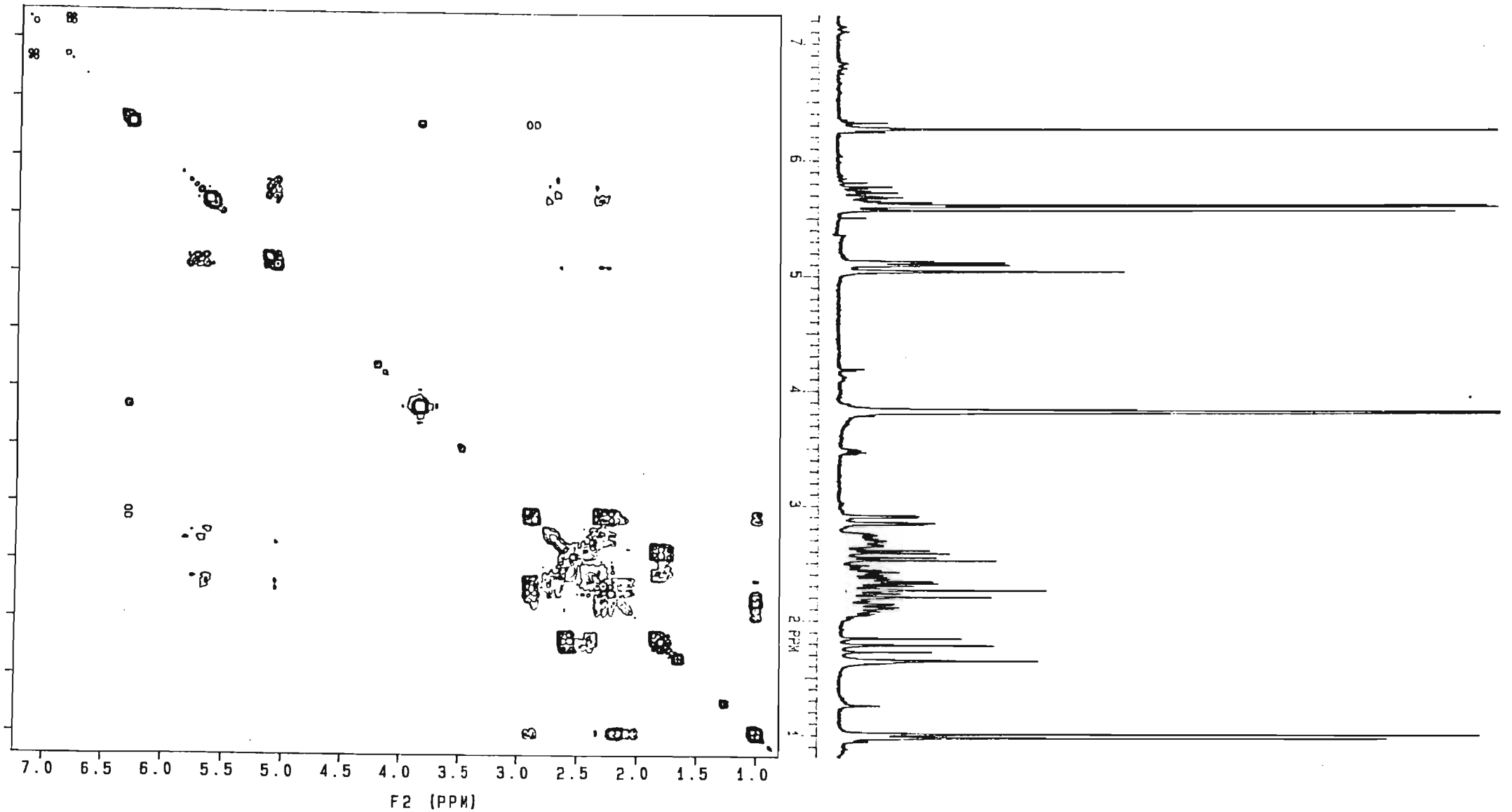


74b Expanded ^{1}H NMR spectrum of bullatone in $CDCl_3$ (400MHz)



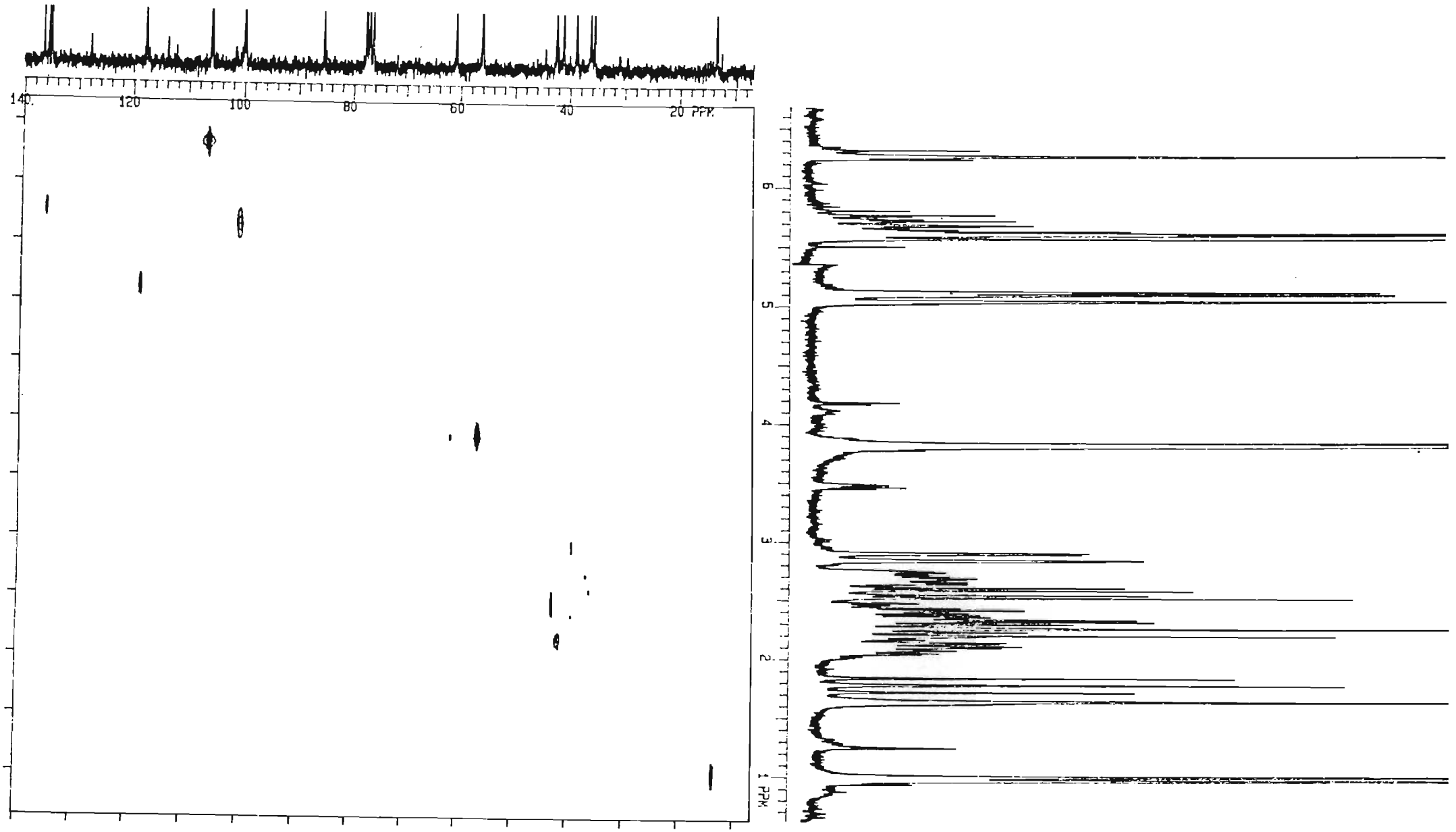
74

74c ^{13}C NMR spectrum of bullatone in $CDCl_3$

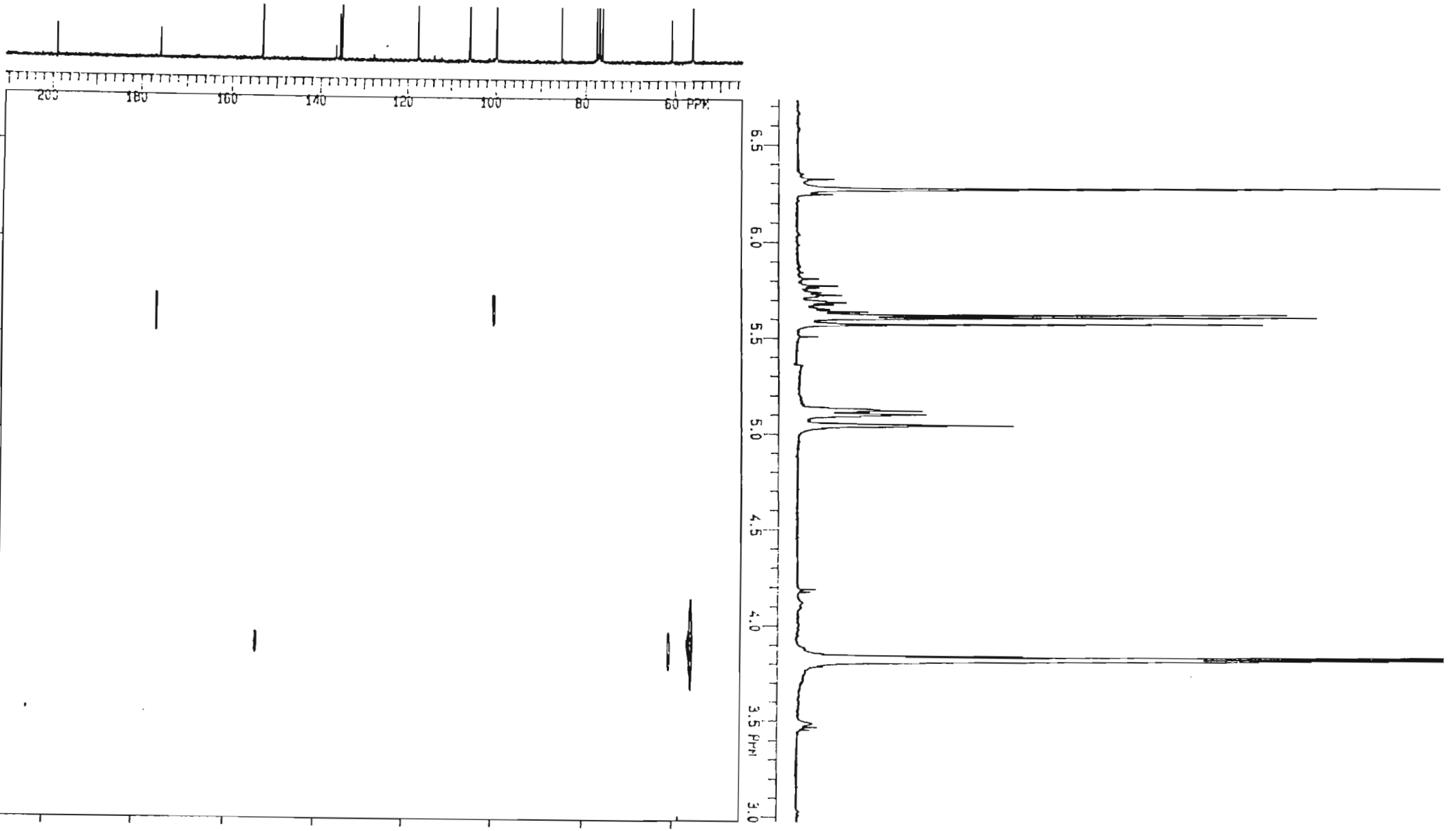


74d ^{1}H-^{1}H COSY spectrum of bullatone in $CDCl_3$

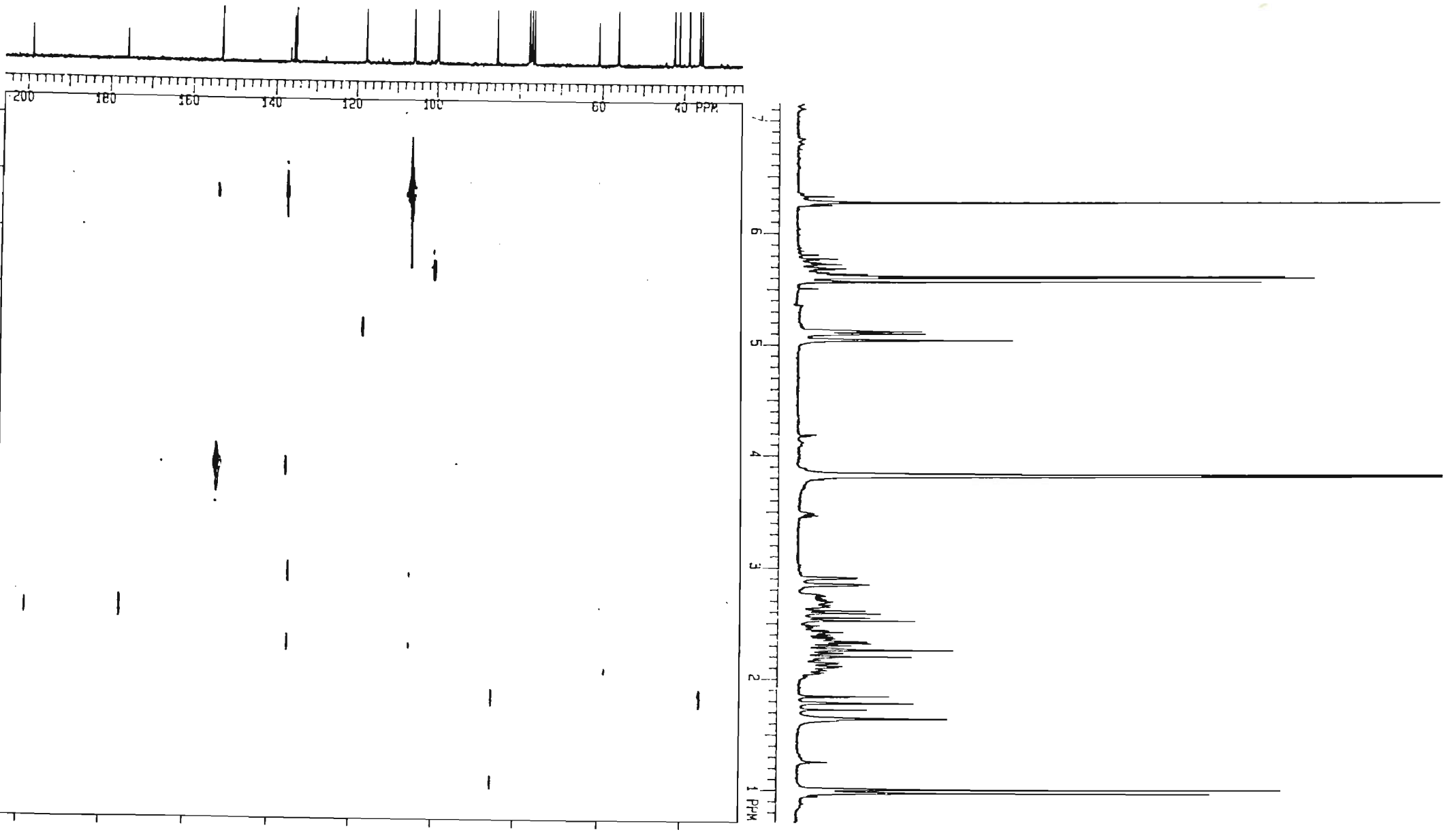
74d ^1H-^1H COSY spectrum of bullatone in $CDCl_3$ (400MHz)



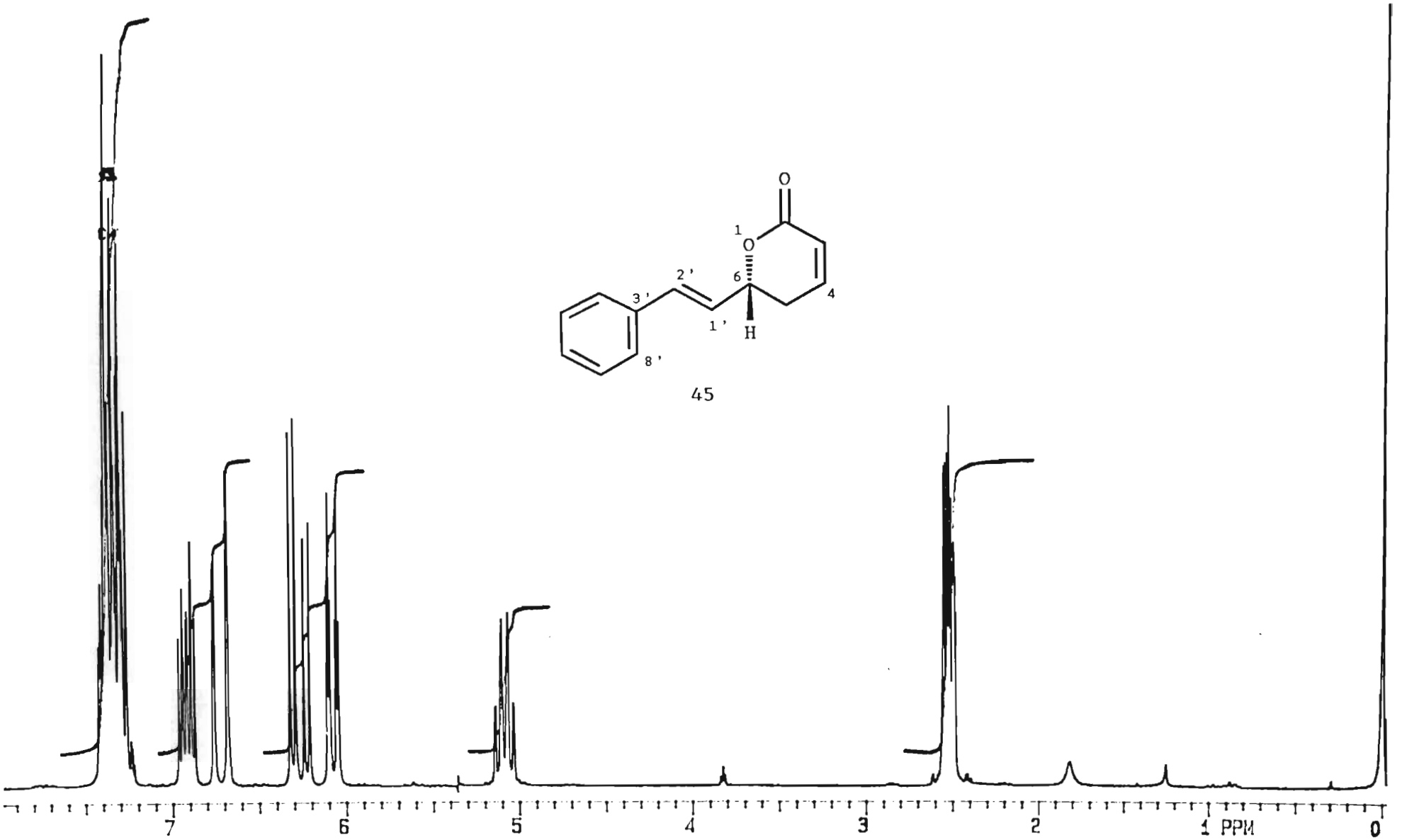
74f ^{1}H-^{13}C HETCOR spectrum of bullatone in $CDCl_3$



74g ^{1}H-^{13}C HETCOR spectrum of bullatone in $CDCl_3$ (3.5Hz)

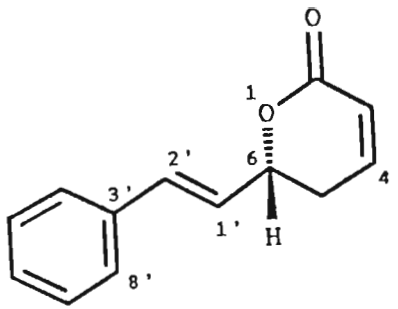


74g ^{1}H-^{13}C HETCOR spectrum of bullatone in $CDCl_3$ (12Hz)



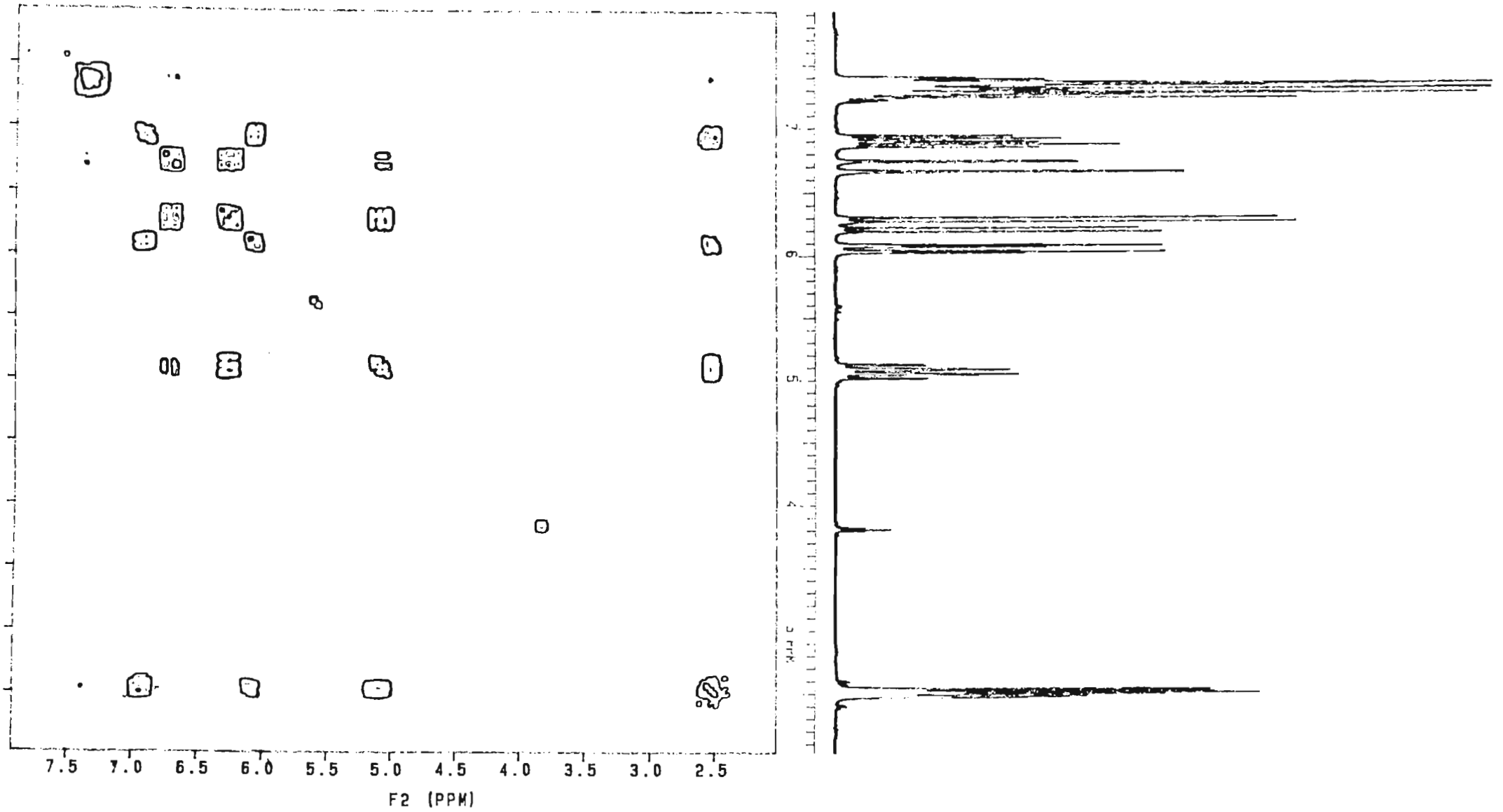
45

45a ^{1}H NMR spectrum of goniothalamin in $CDCl_3$

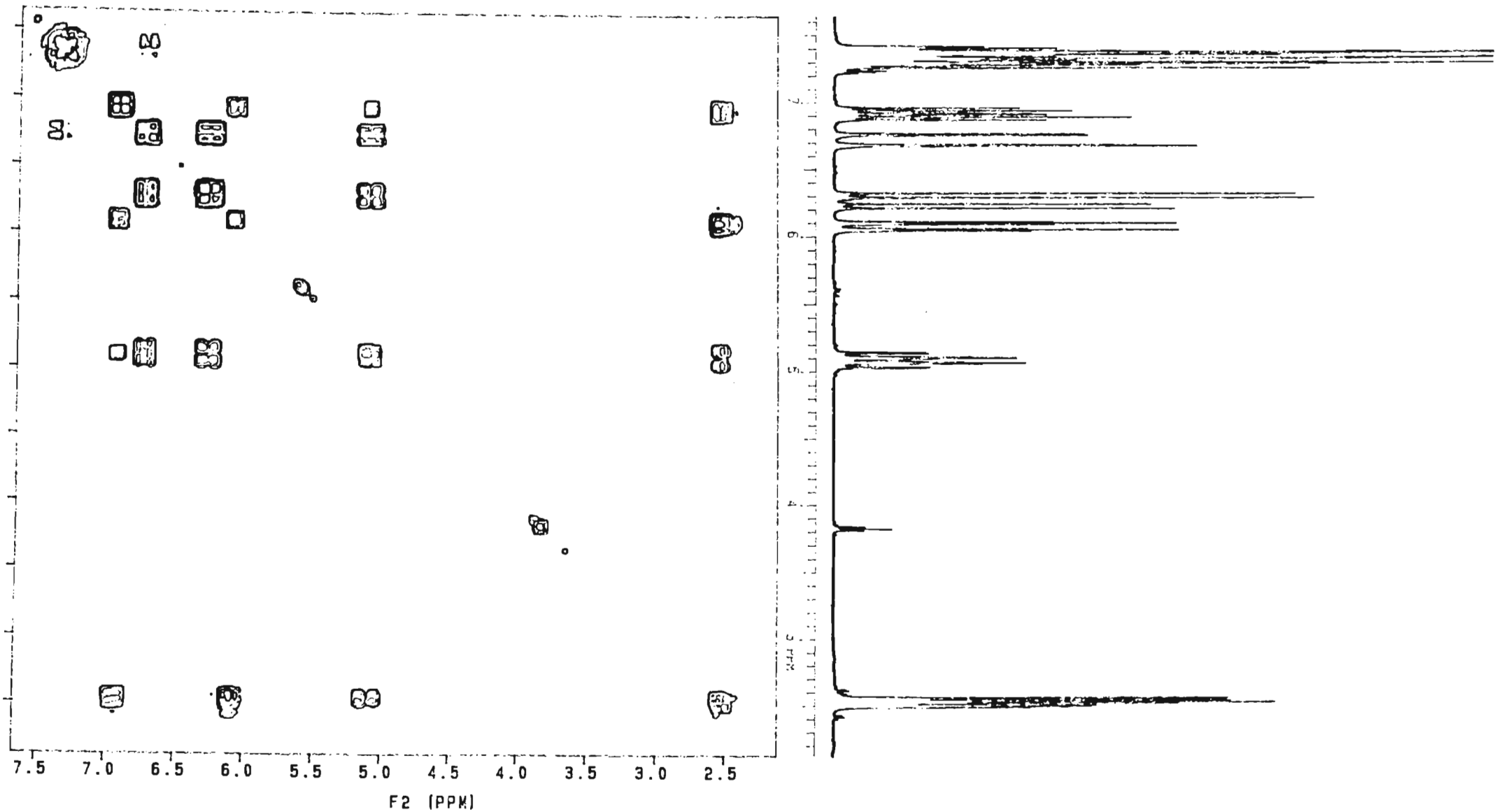


45

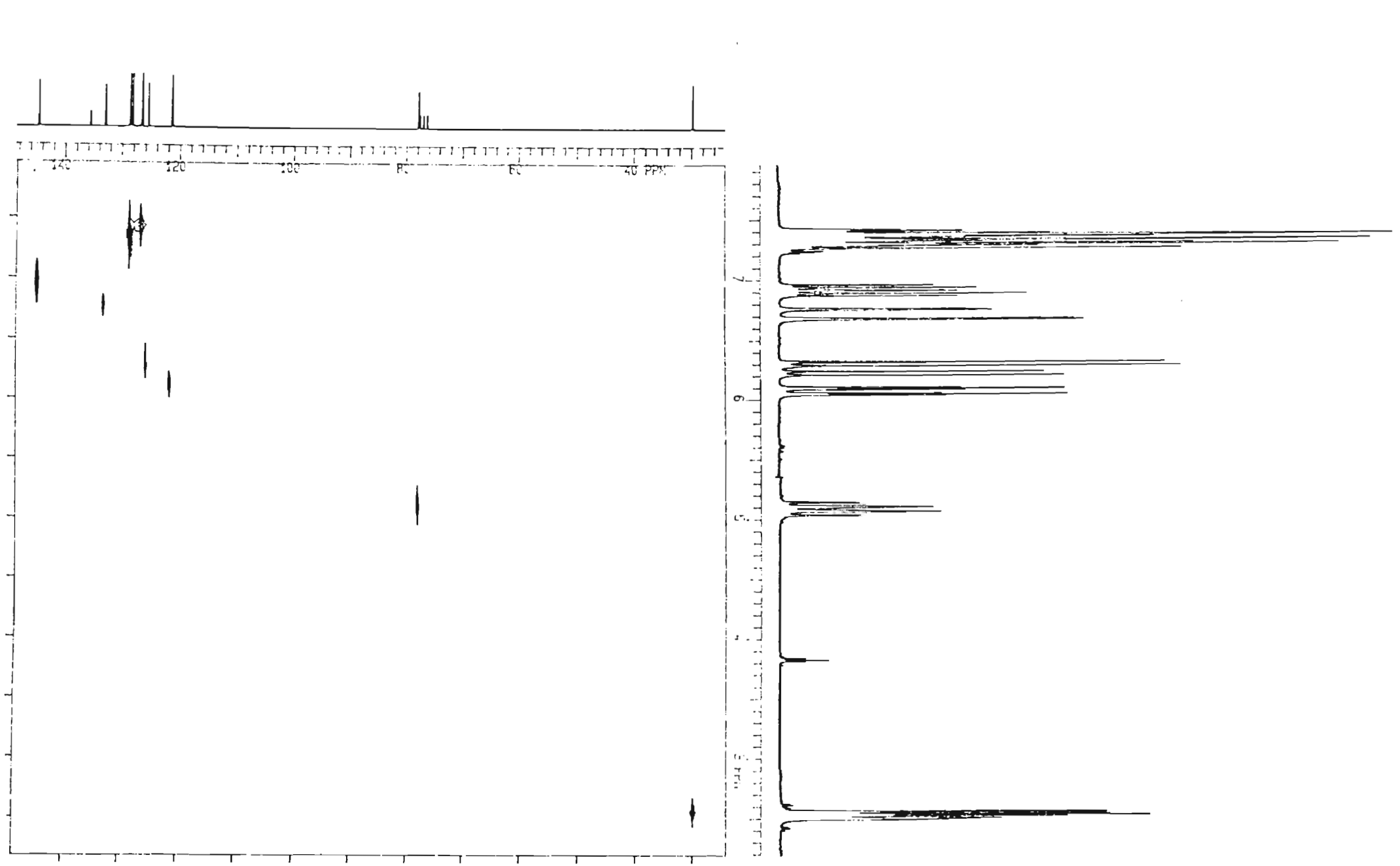
45c ^{13}C NMR spectrum of goniothalamin in $CDCl_3$



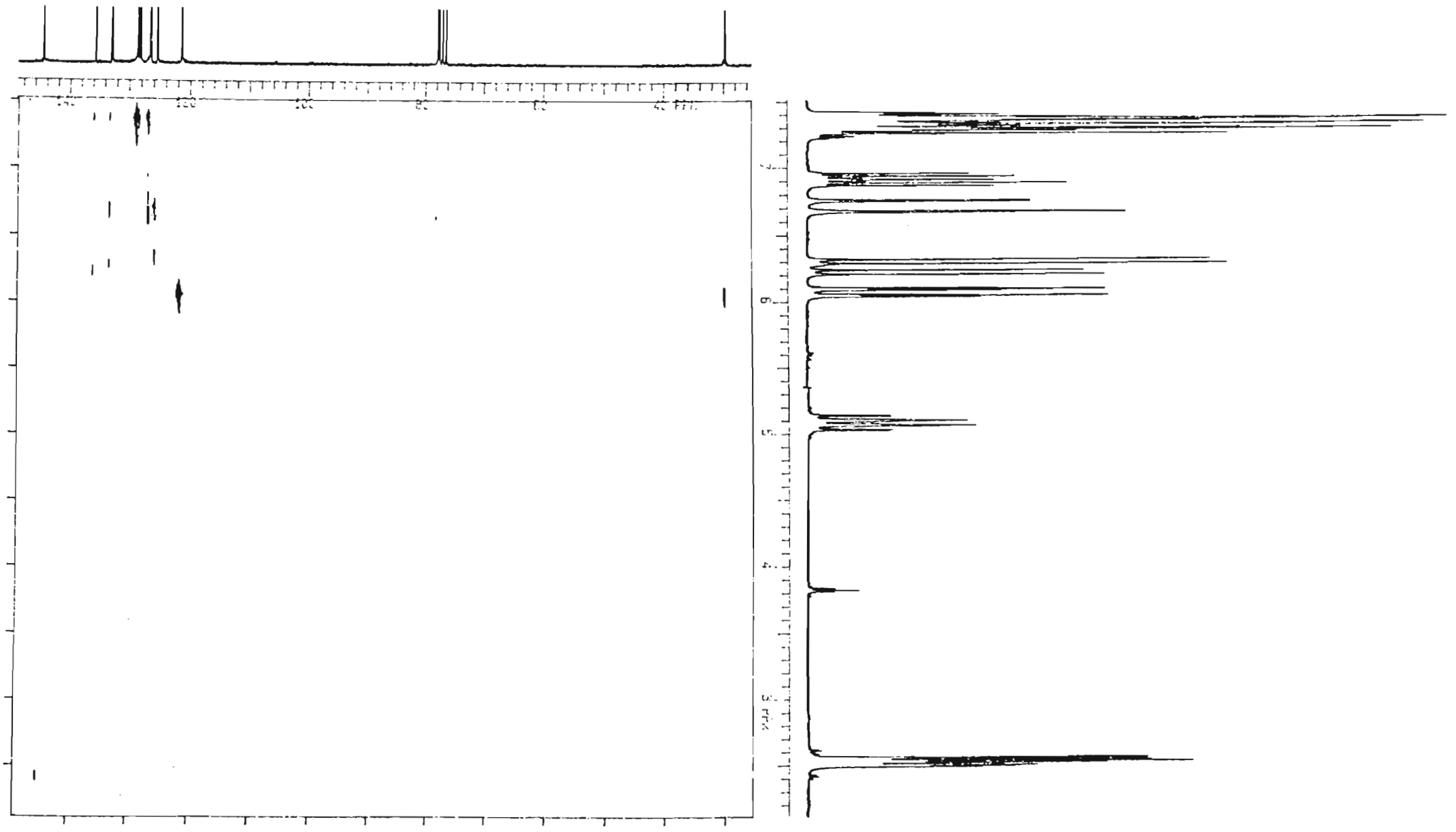
45d ^{1}H-^{1}H COSY spectrum of goniothalamin in $CDCl_3$



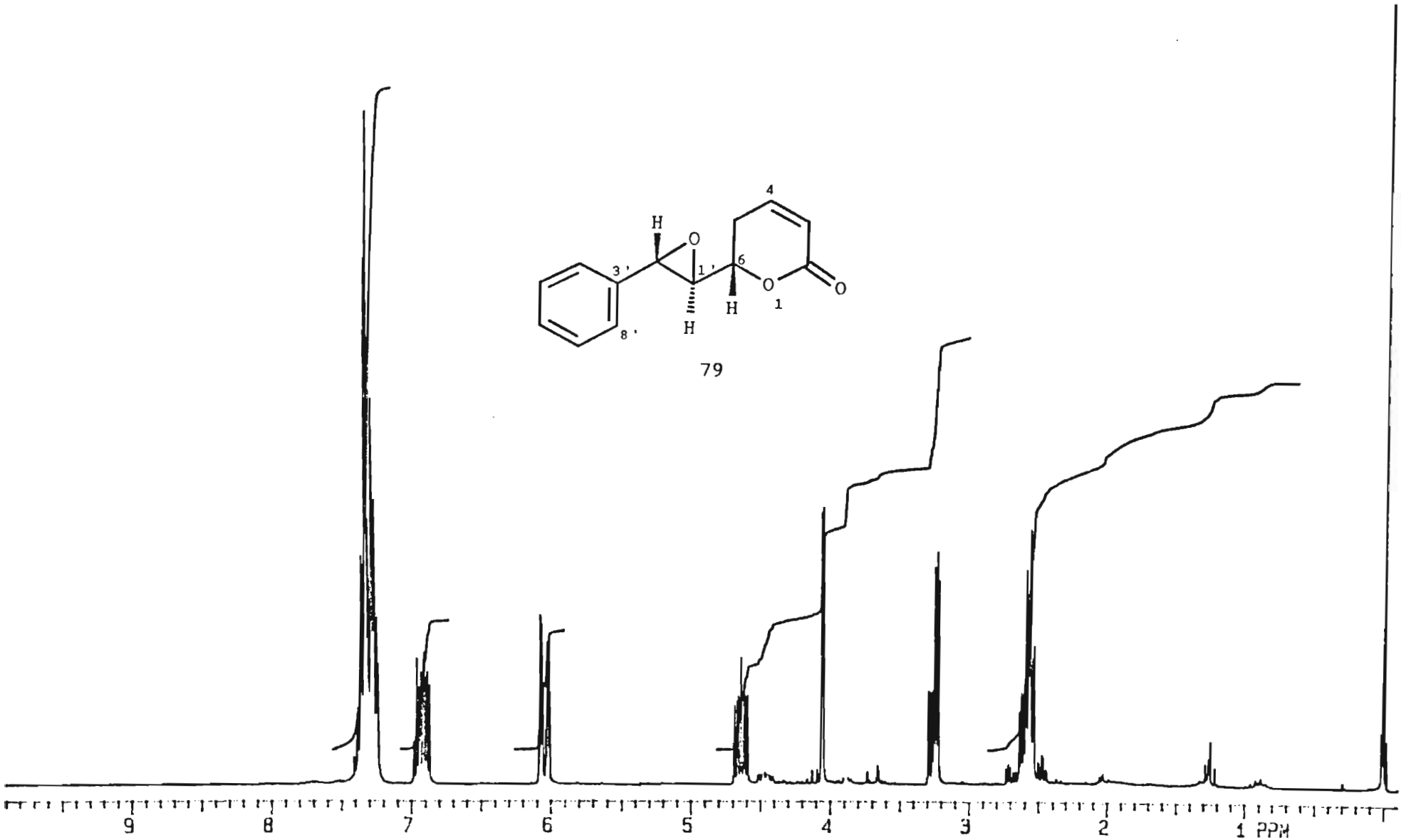
5e ^{1}H-^{1}H DELAYED COSY spectrum of goniothalamin in $CDCl_3$



45f ^{1}H-^{13}C HETCOR spectrum of goniothalamin in $CDCl_3$

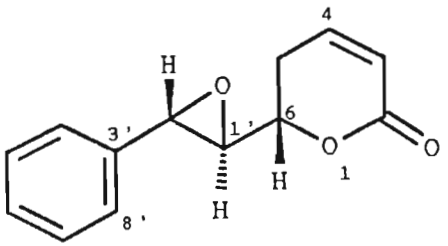


^{1}H-^{13}C DELAYED HETCOR spectrum of goniothalamin in $CDCl_3$



79

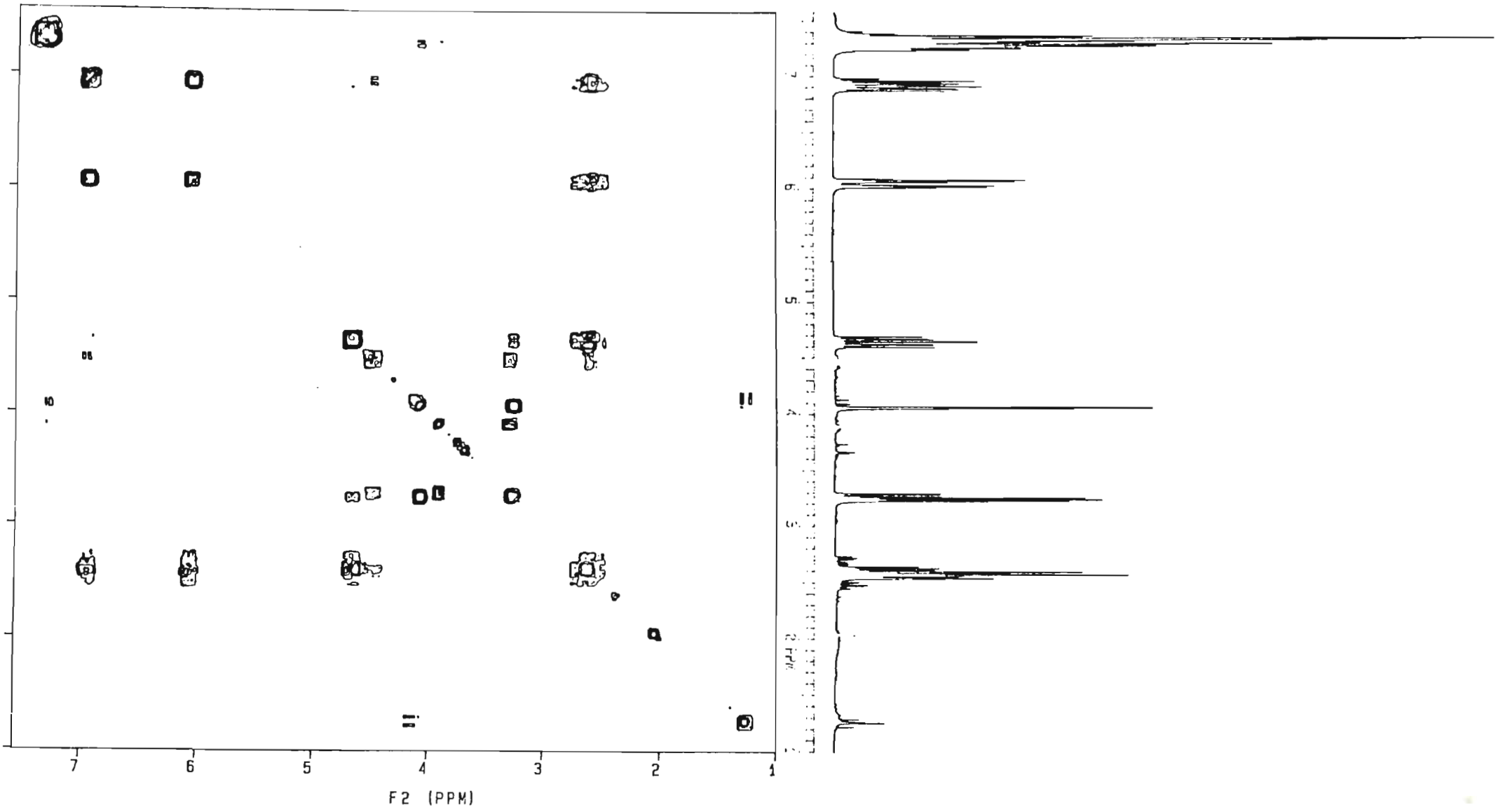
79a ^{1}H NMR spectrum isogoniothalamin of oxide in $CDCl_3$



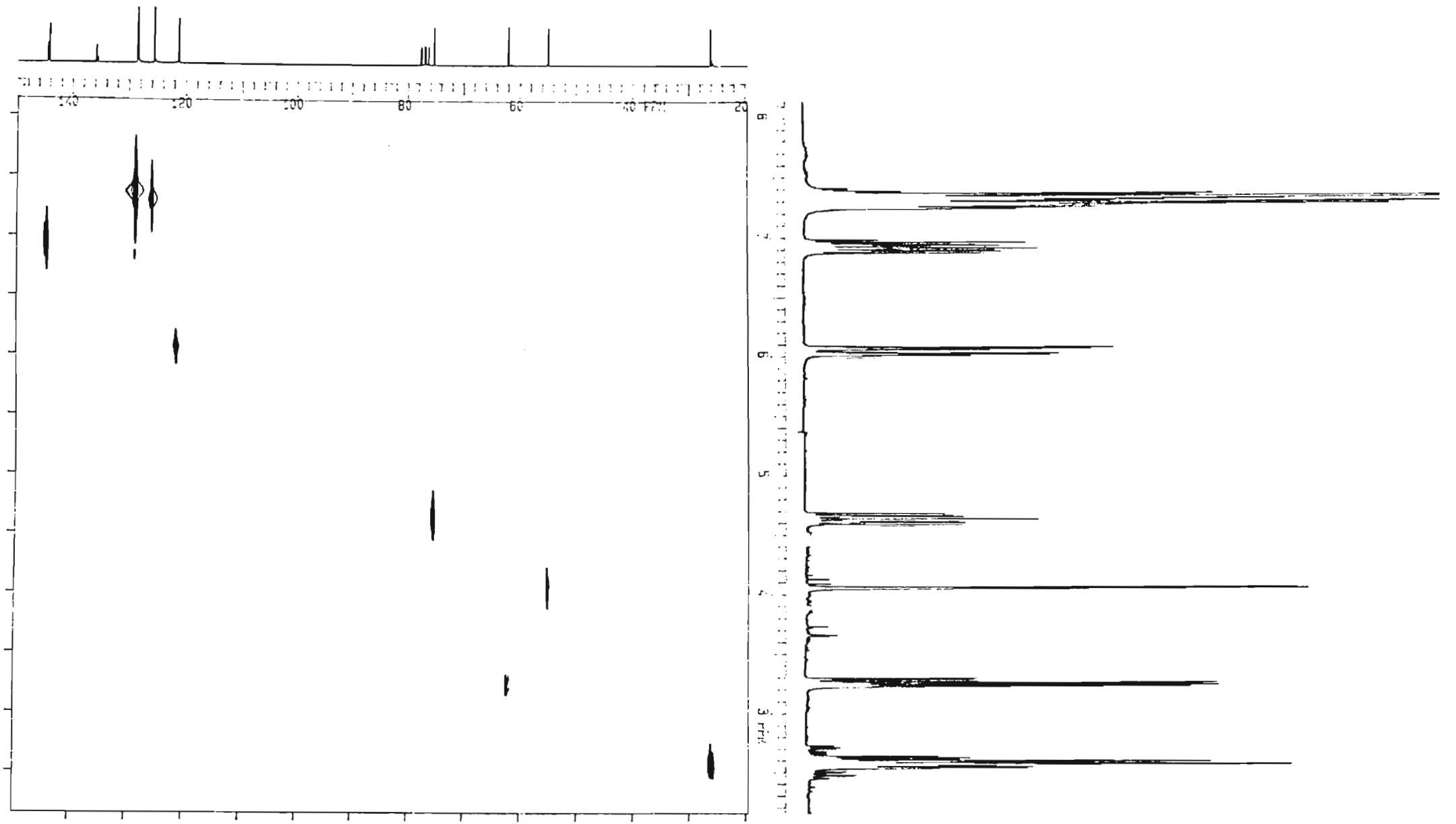
79

79c ^{13}C NMR spectrum of isogoniothalamin oxide in $CDCl_3$

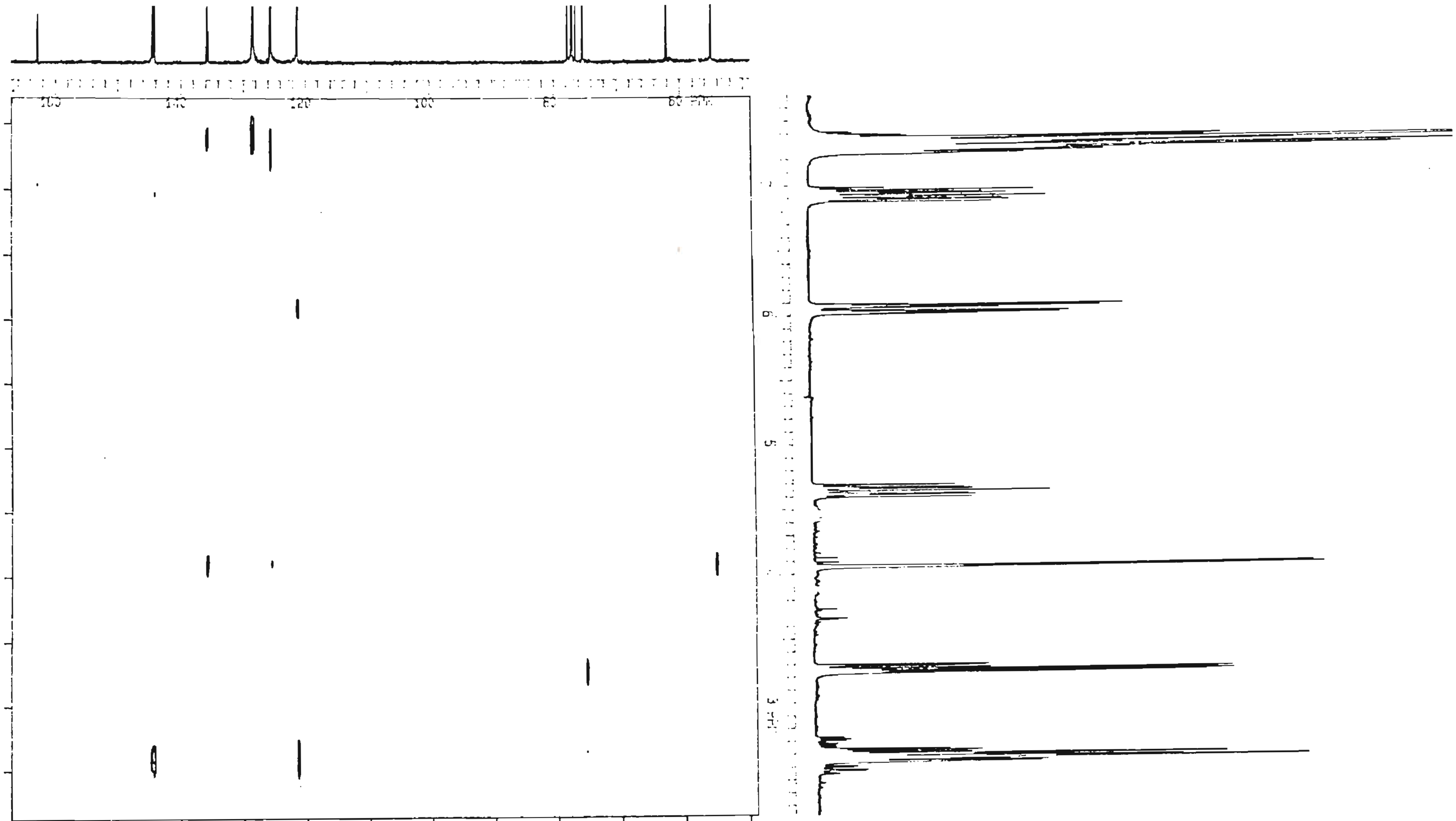
79d ^1H-^1H COSY spectrum of isogoniothalamin oxide in $CDCl_3$



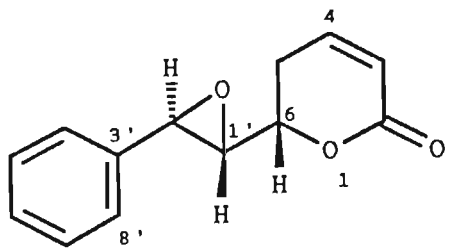
e ^{1}H-^{1}H DELAYED COSY spectrum of isogoniothalamin oxide in



'9f ^{1}H-^{13}C HETCOR spectrum of isogoniothalamin oxide in

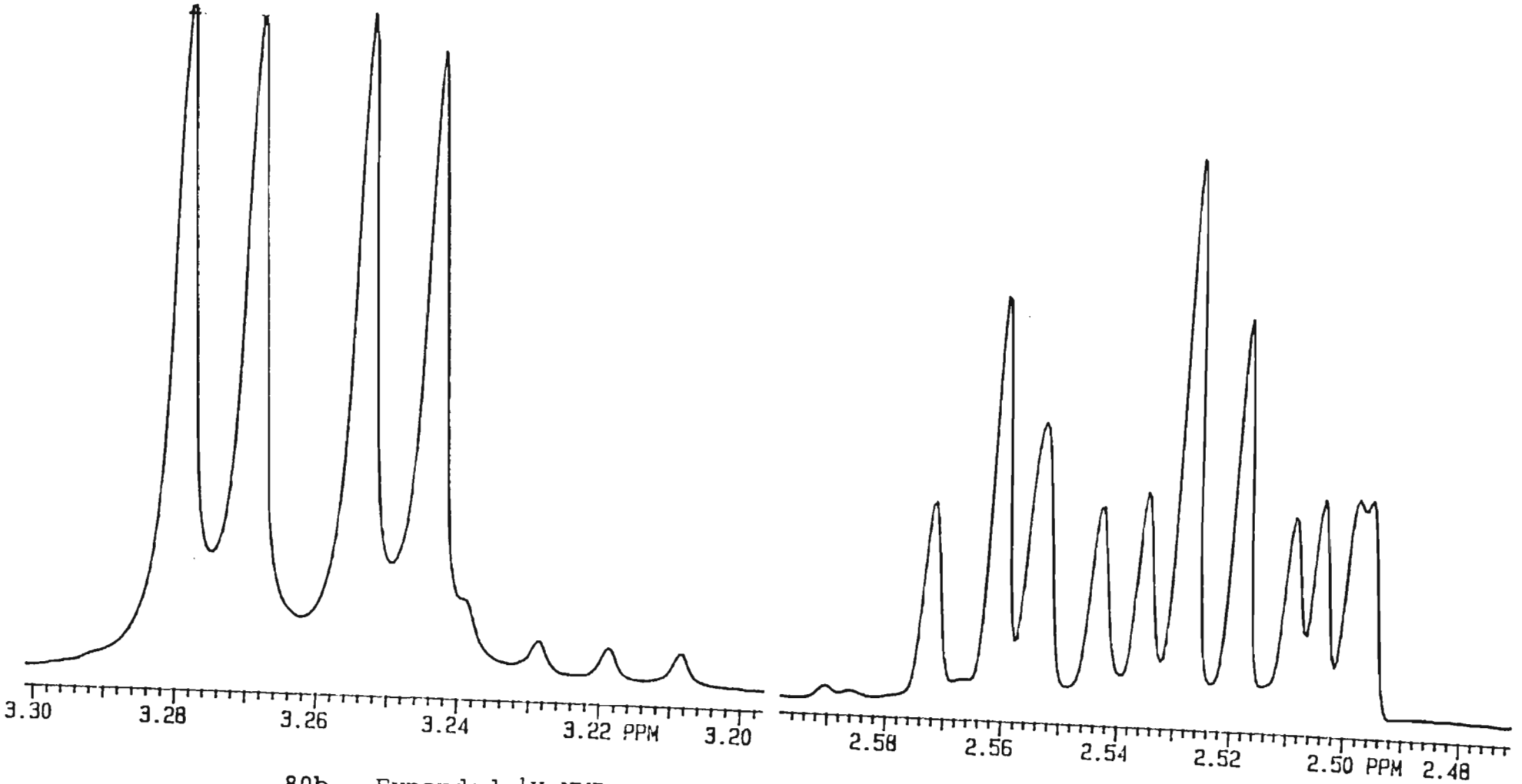


…niothalamin oxide in

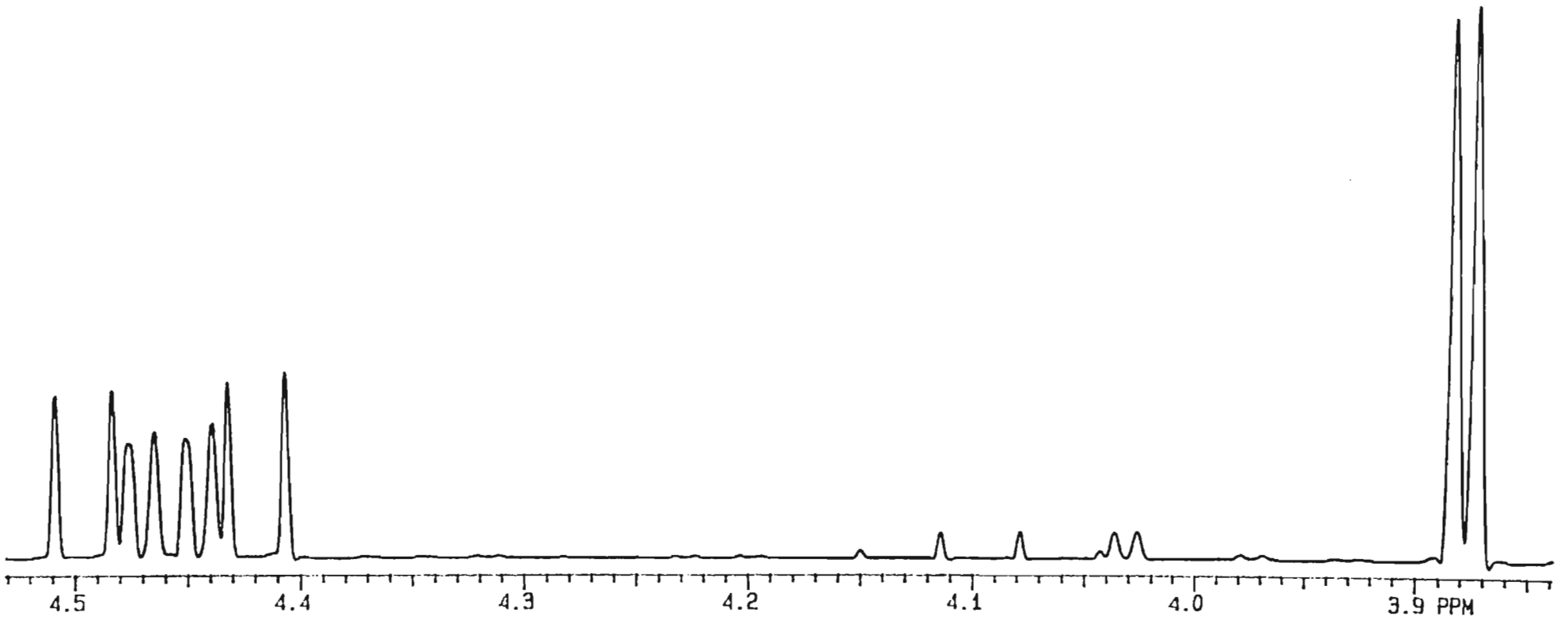


80

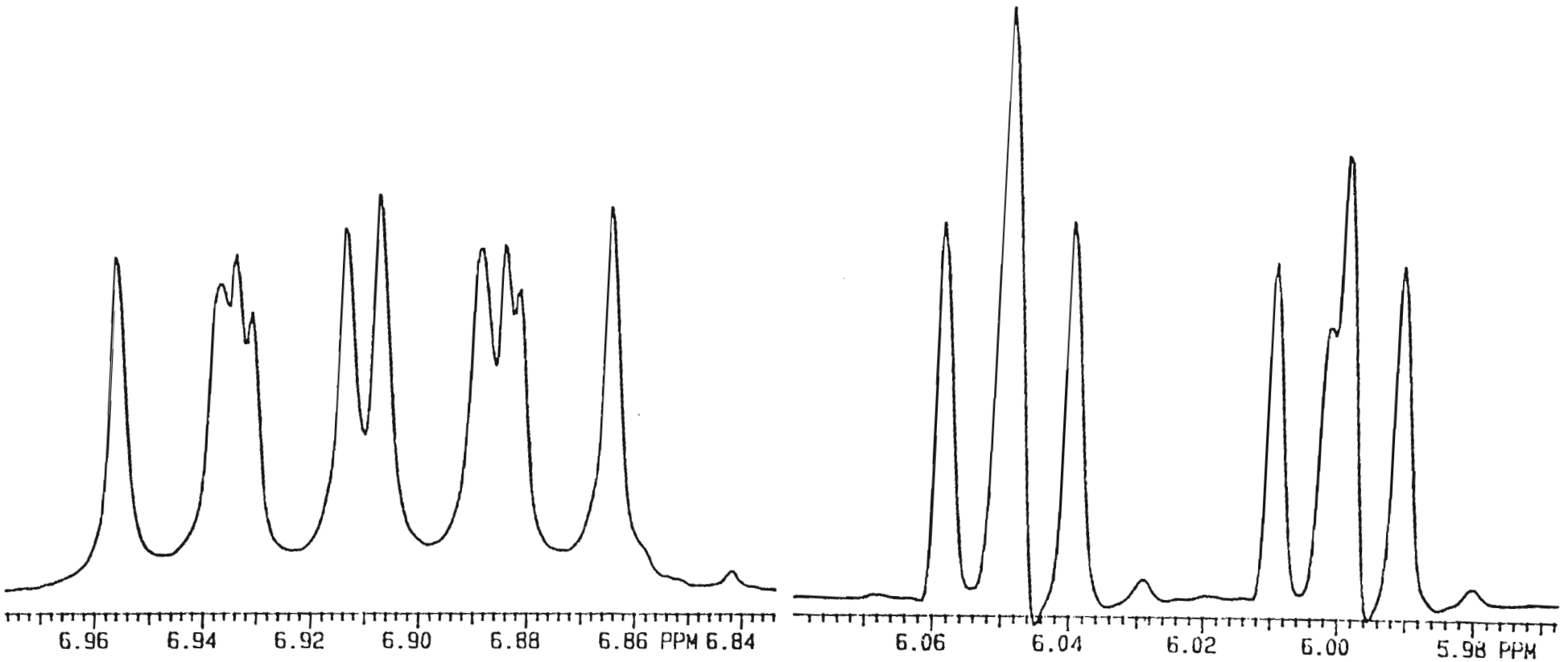
80a ^{1}H NMR spectrum of goniothalamin oxide in $CDCl_3$



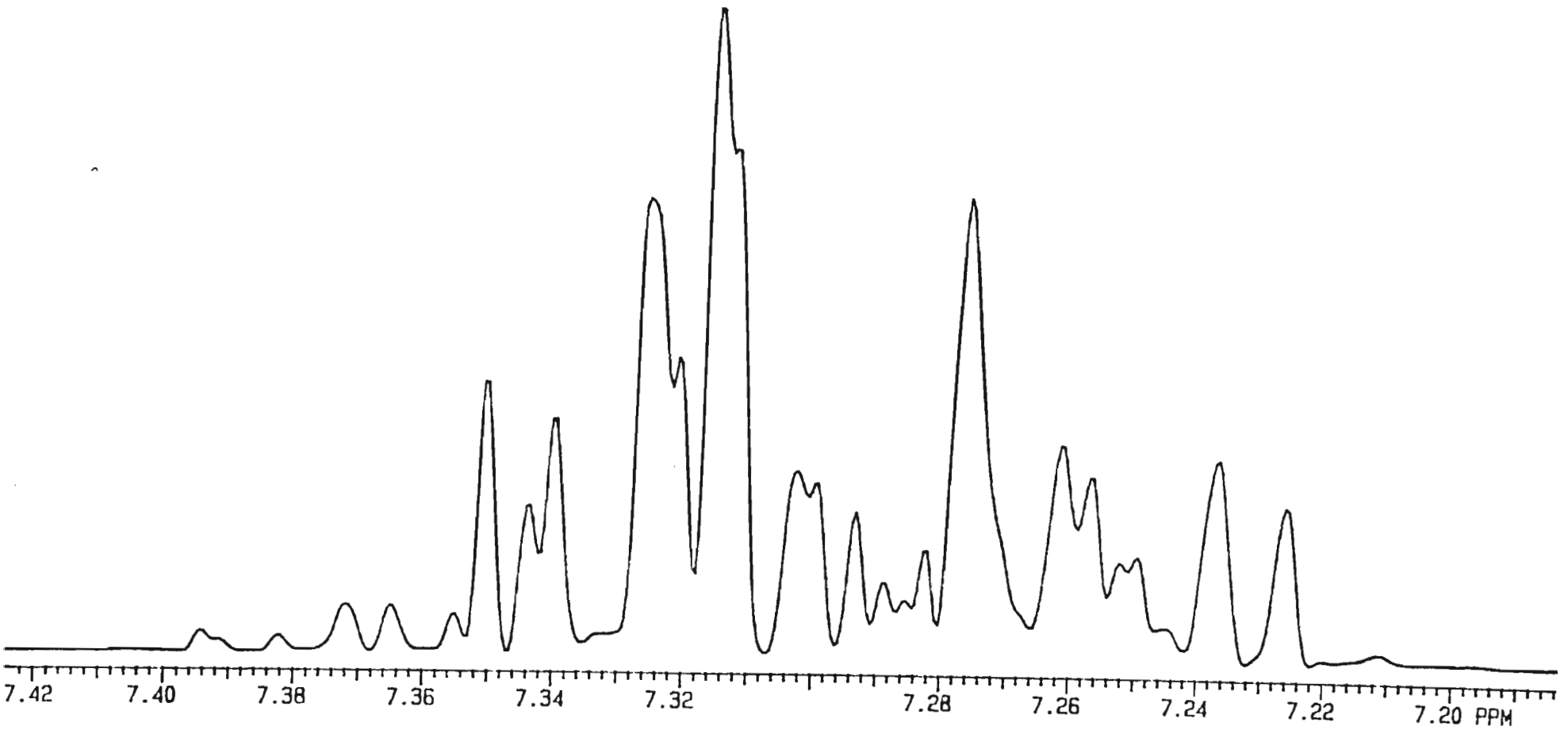
80b Expanded ^{1}H NMR spectrum of goniothalamin oxide in $CDCl_3$



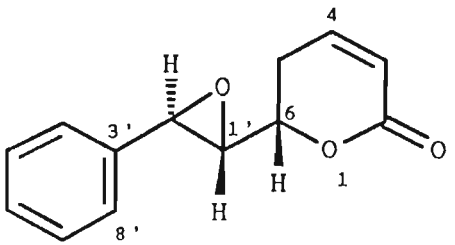
80b Expanded ^{1}H NMR spectrum of goniothalamin oxide in $CDCl_3$



80b Expanded ^{1}H NMR spectrum of goniothalamin oxide in $CDCl_3$



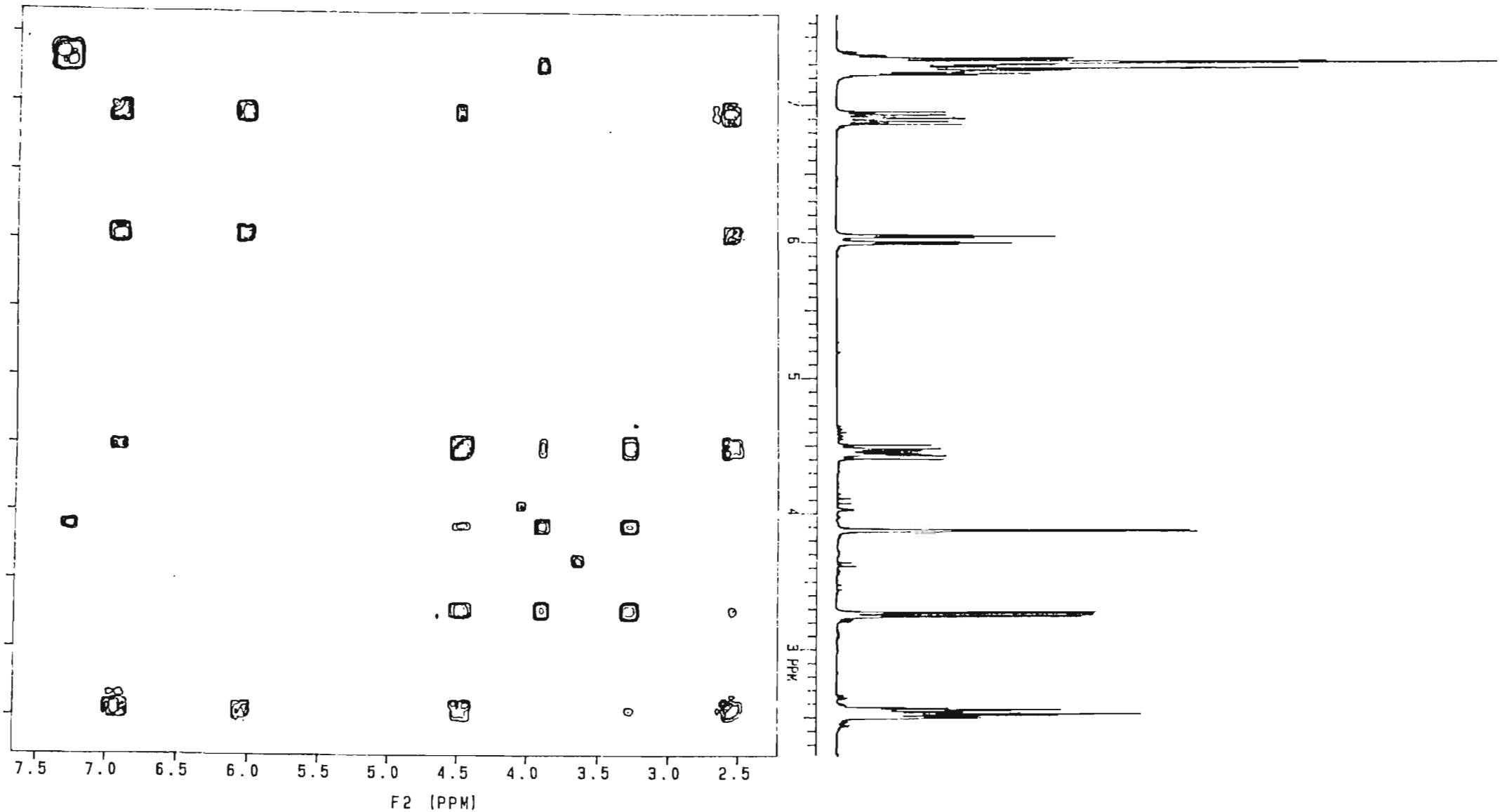
80b Expanded ^1H NMR spectrum of goniothalamin oxide in $CDCl_3$



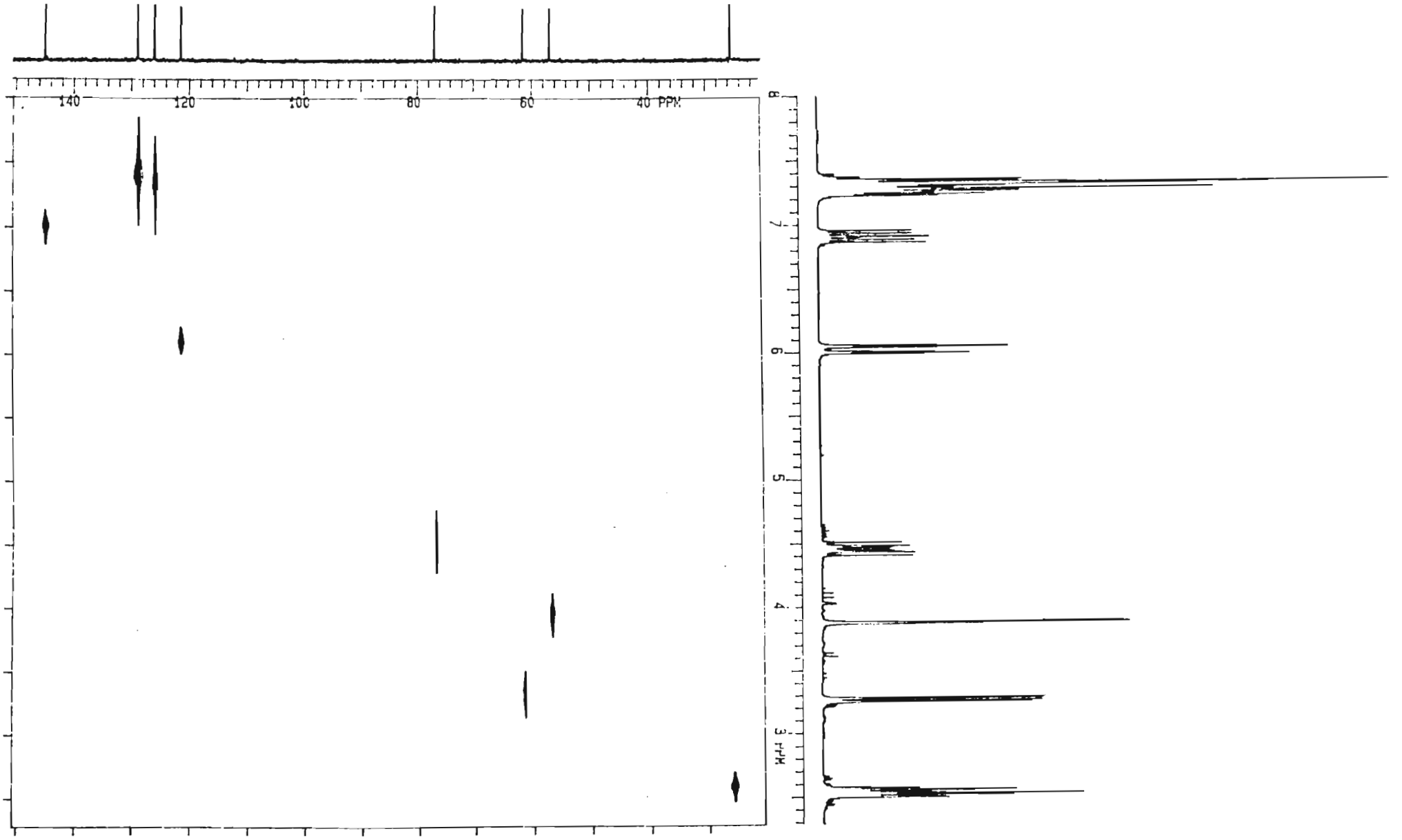
80

80c ^{13}C NMR spectrum of goniothalamin oxide in $CDCl_3$

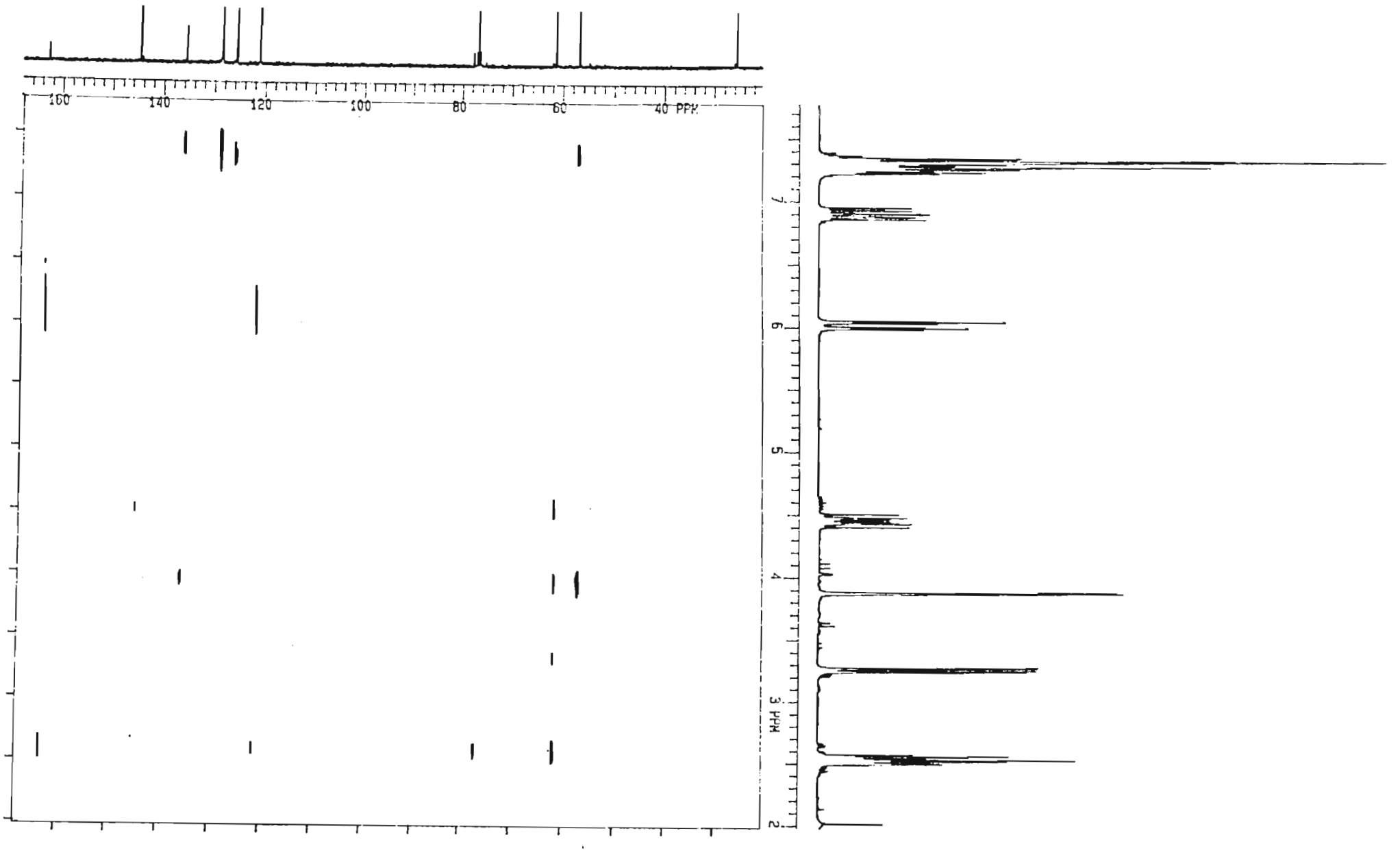
Abb. 10d ^1H-^1H COSY spectrum of goniothalamin oxide in $CDCl_3$



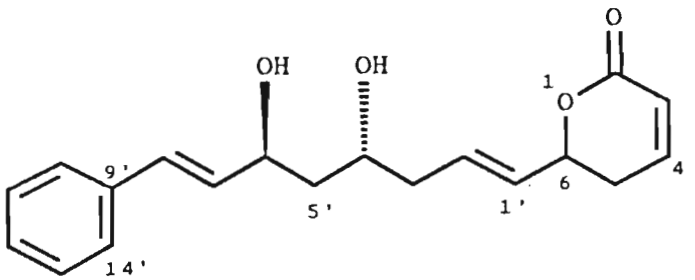
0e ^{1}H-^{1}H DELAYED COSY spectrum of goniothalamin oxide in



f. ^{1}H-^{13}C HETCOR spectrum of goniothalamin oxide in $CDCl_3$

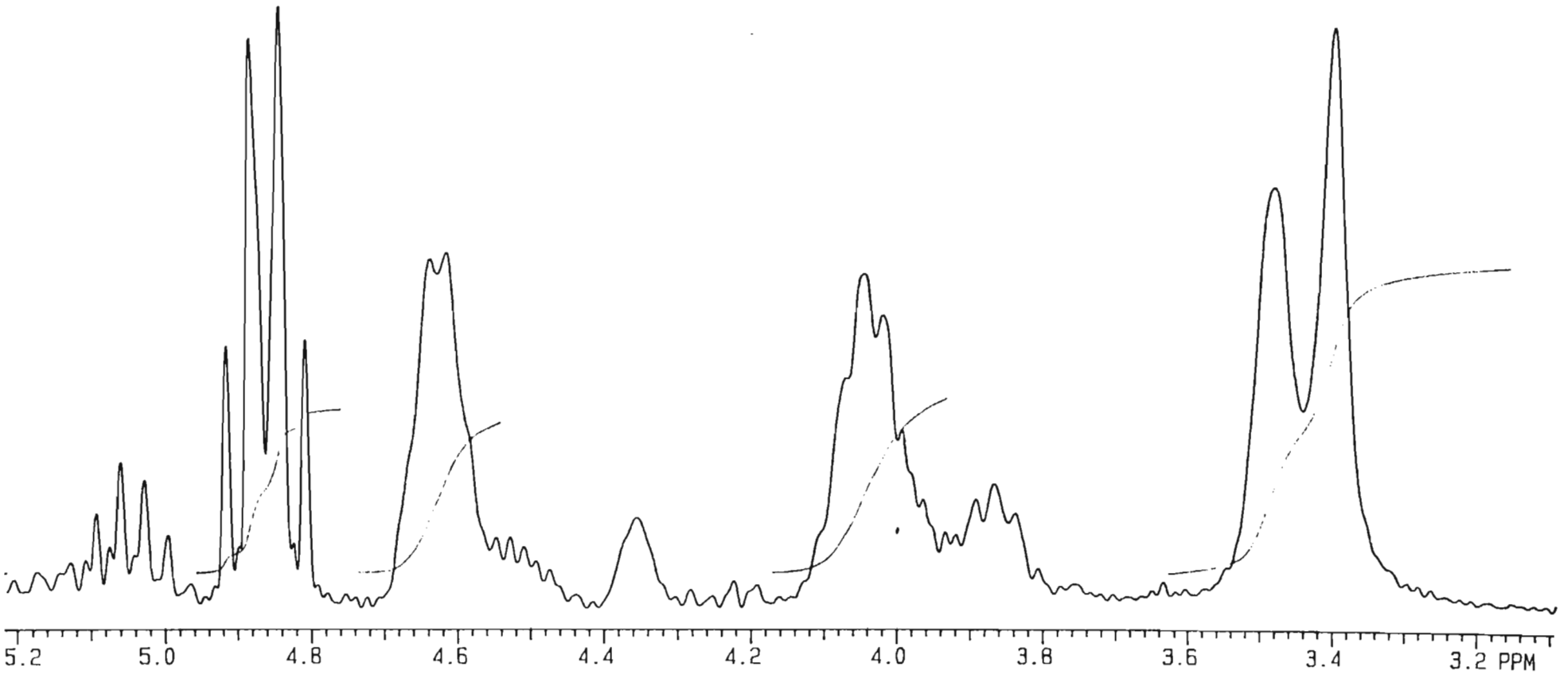


^{1}H-^{13}C DELAYED HETCOR spectrum of goniothalamin oxide in $CDCl_3$

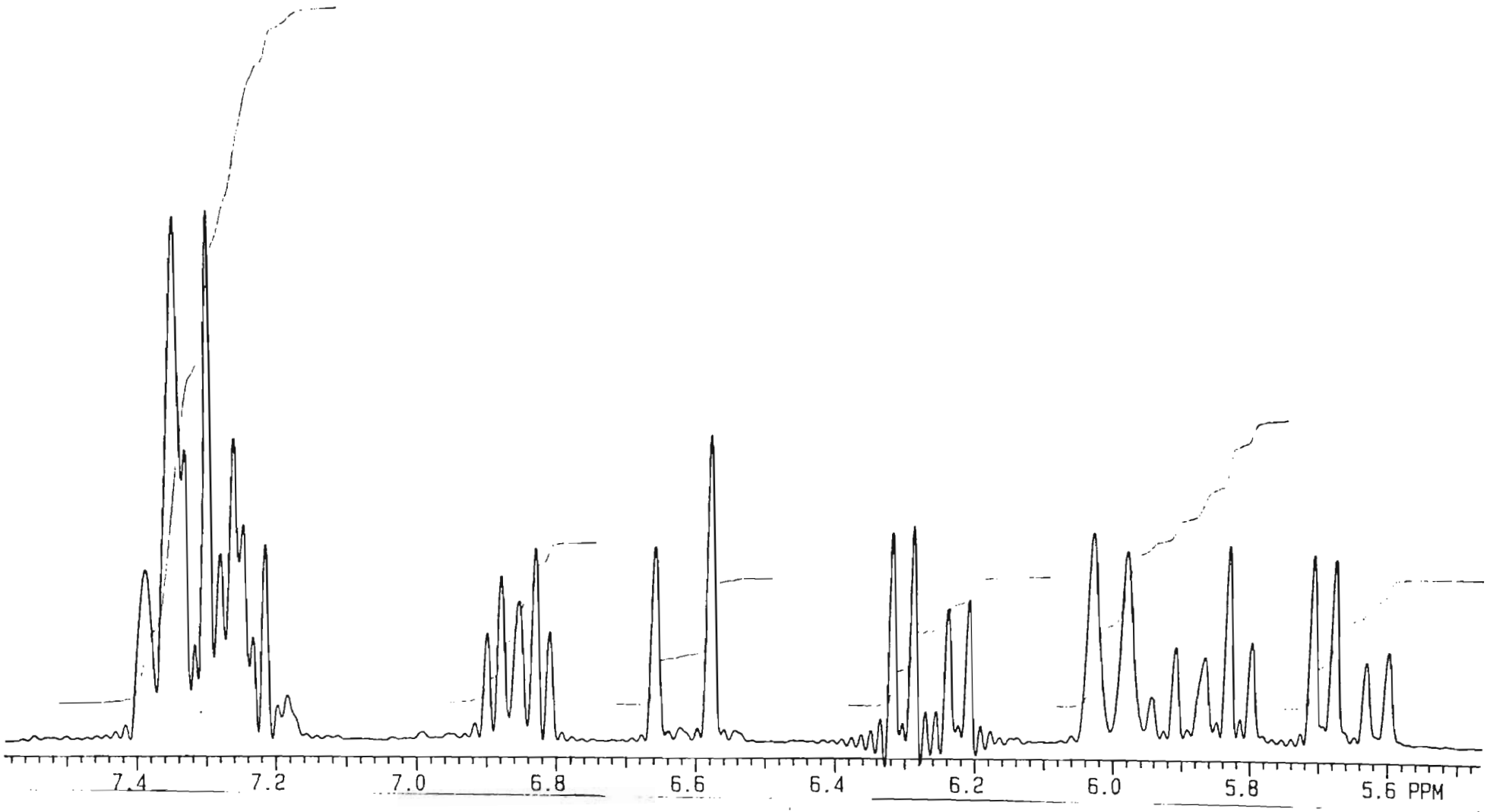


81

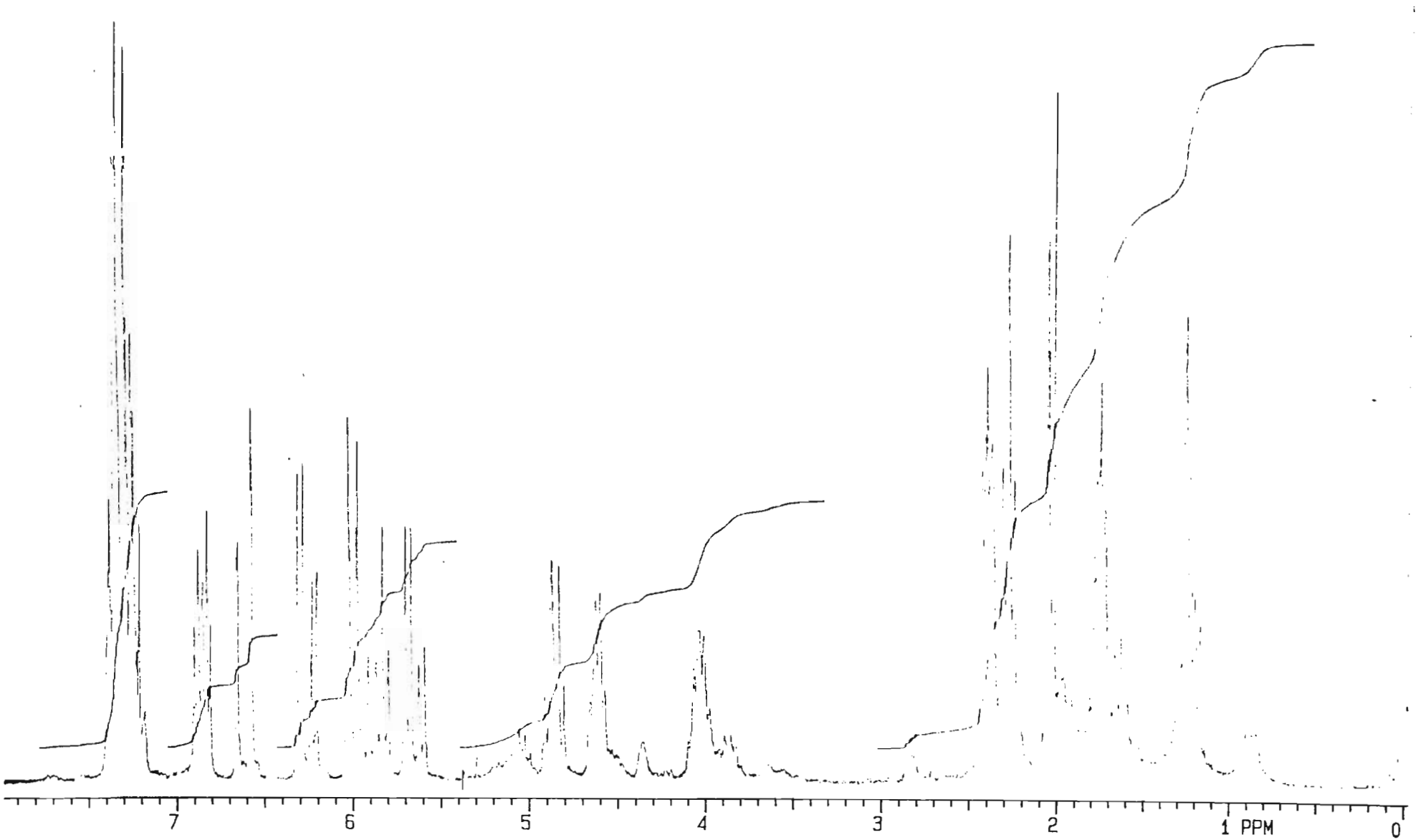
81a ^{1}H NMR spectrum of cryptofolione in $CDCl_3$



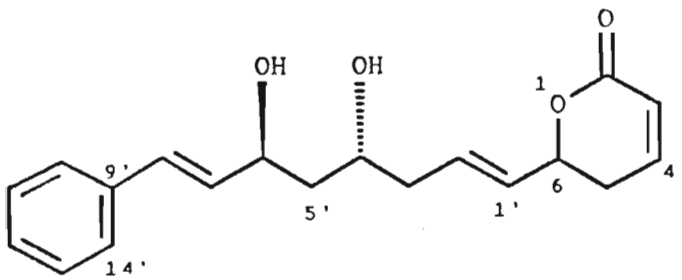
81b Expanded ^{1}H NMR spectrum of cryptofolione in $CDCl_3$



81b Expanded ^{1}H NMR spectrum of cryptofolione in $CDCl_3$

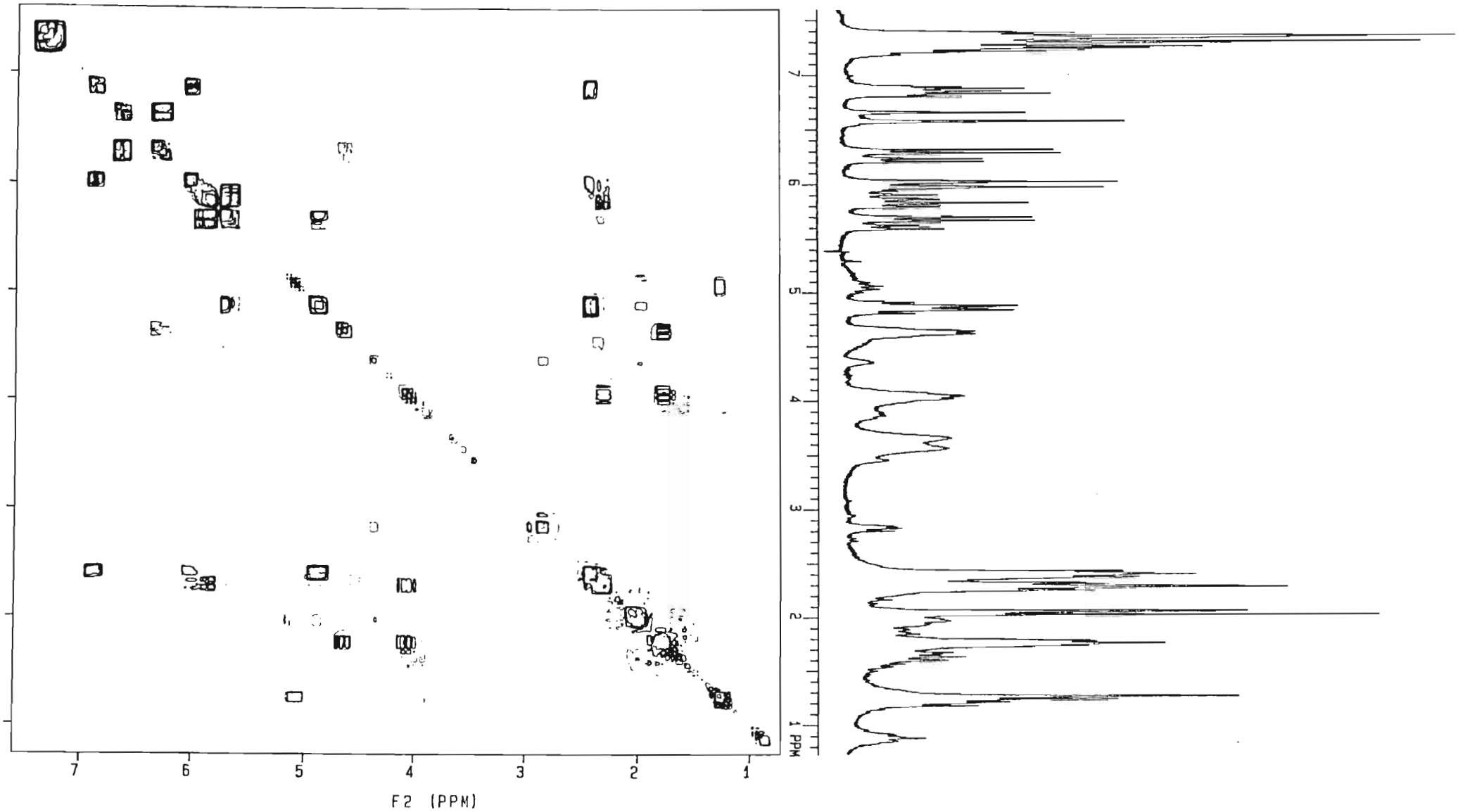


81a ^{1}H NMR spectrum of cryptofolione in D_2O $CDCl_3$

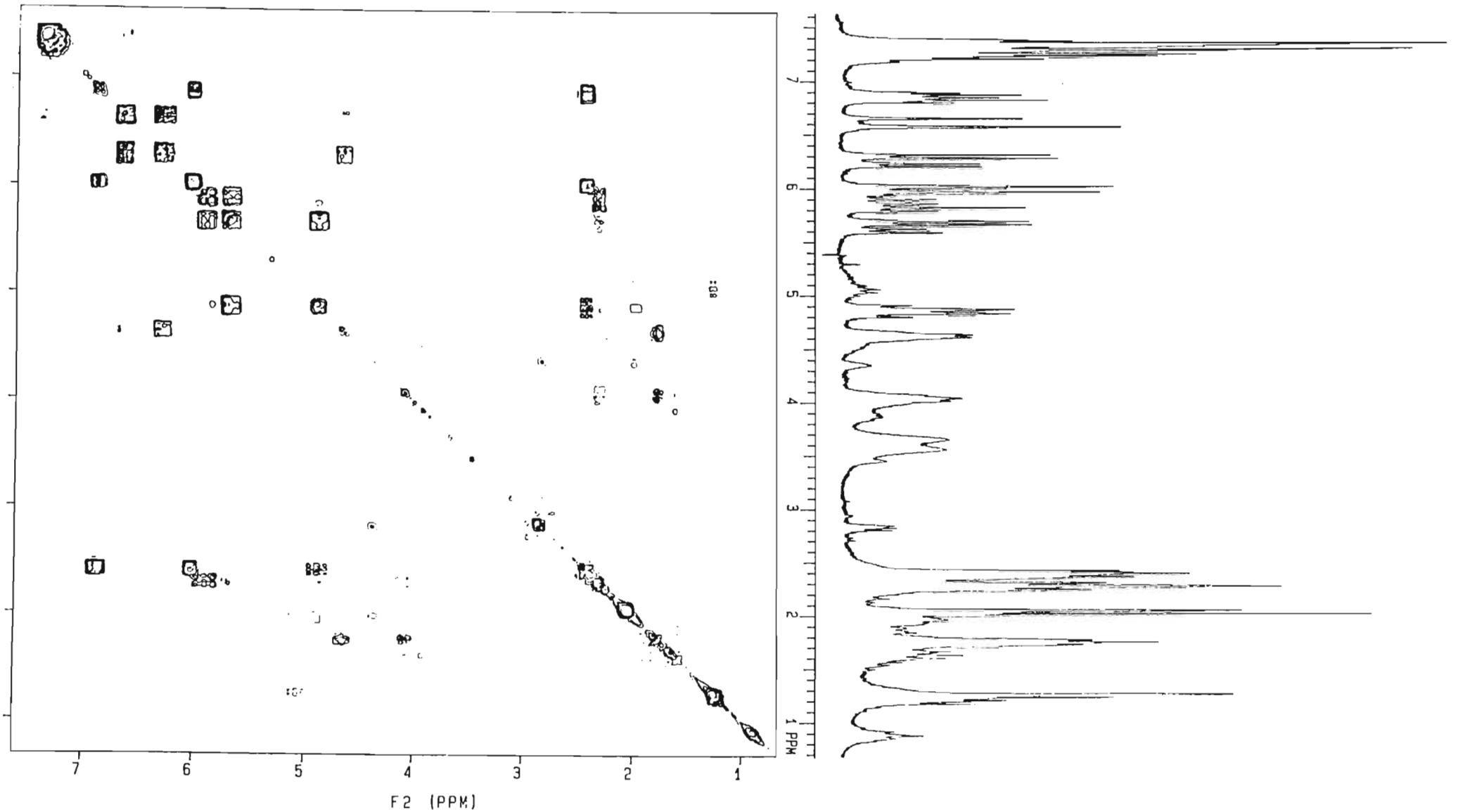


81

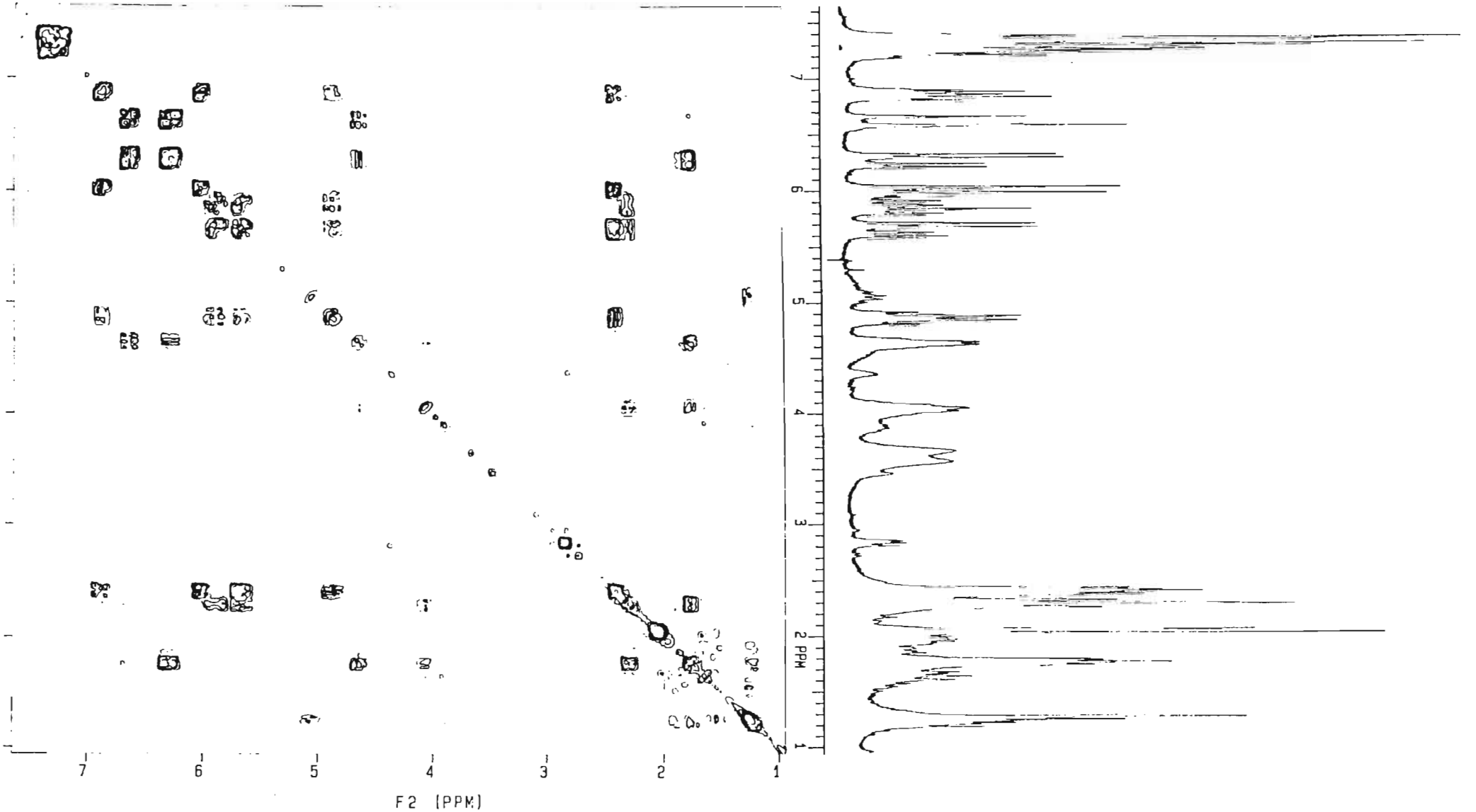
81c ^{13}C NMR spectrum of cryptofolione in $CDCl_3$



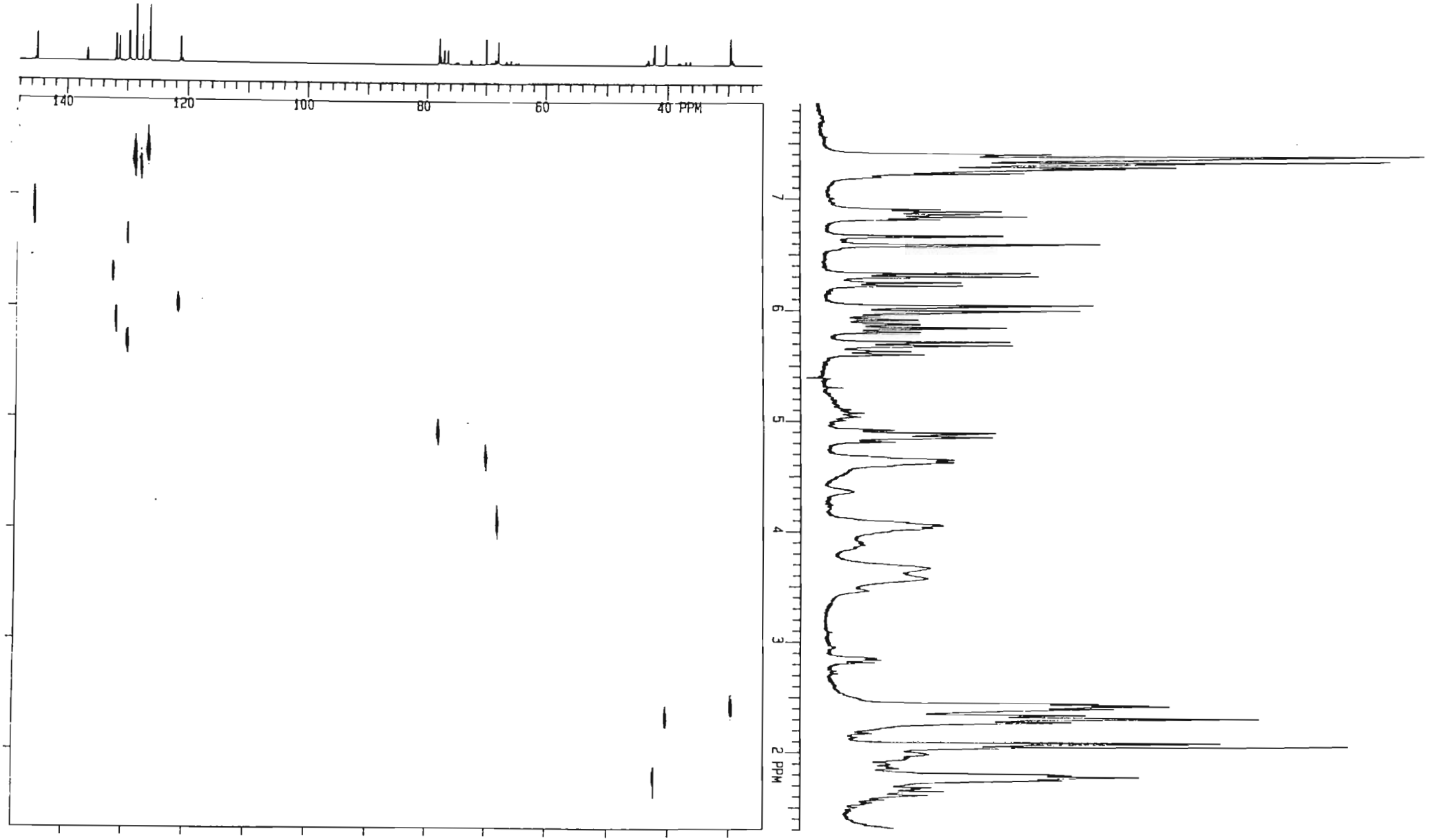
81d ^{1}H-^{1}H COSY spectrum of cryptofolione in $CDCl_3$



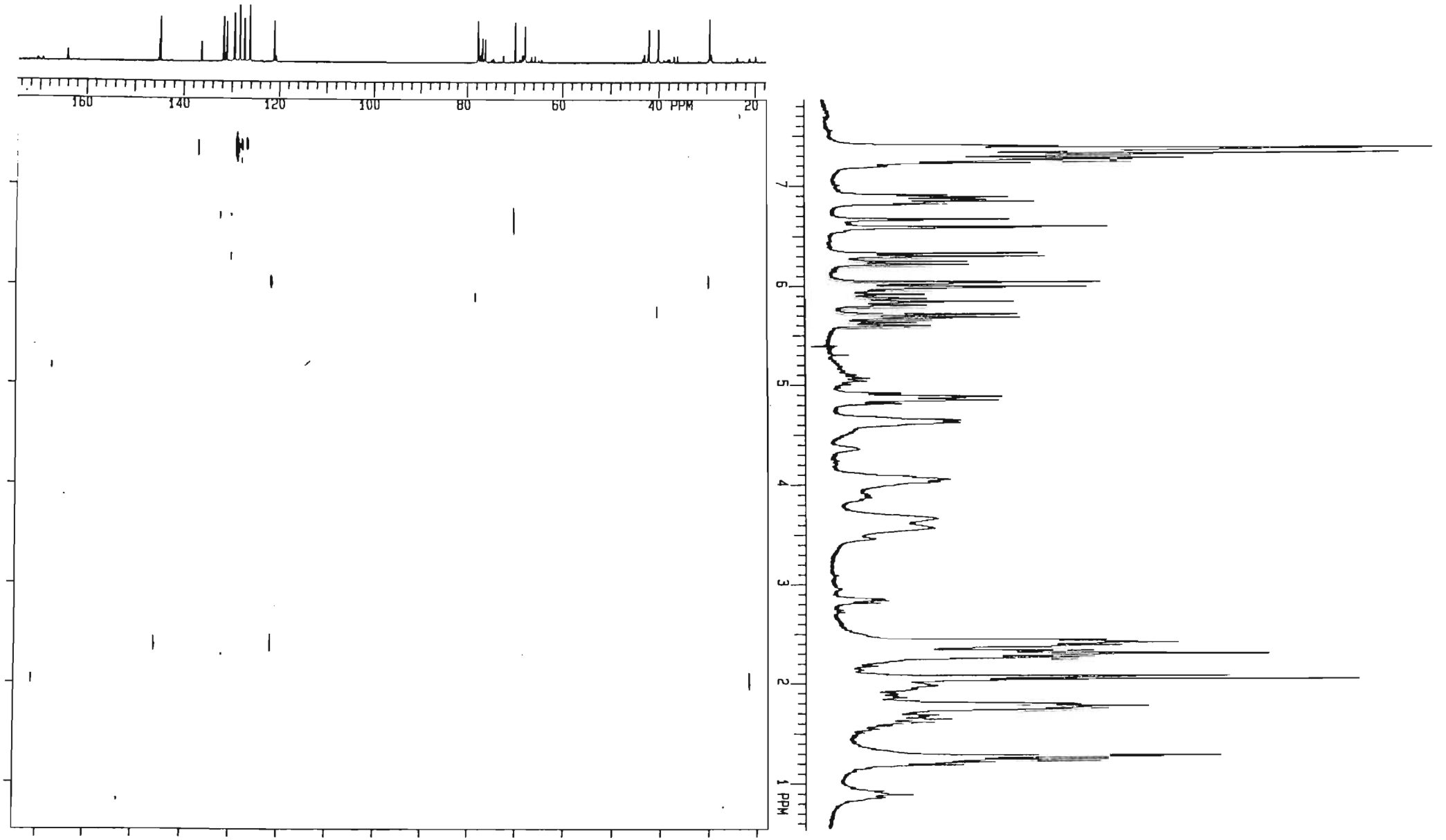
81e ^{1}H-^{1}H DELAYED COSY spectrum of cryptofolione in $CDCl_3$



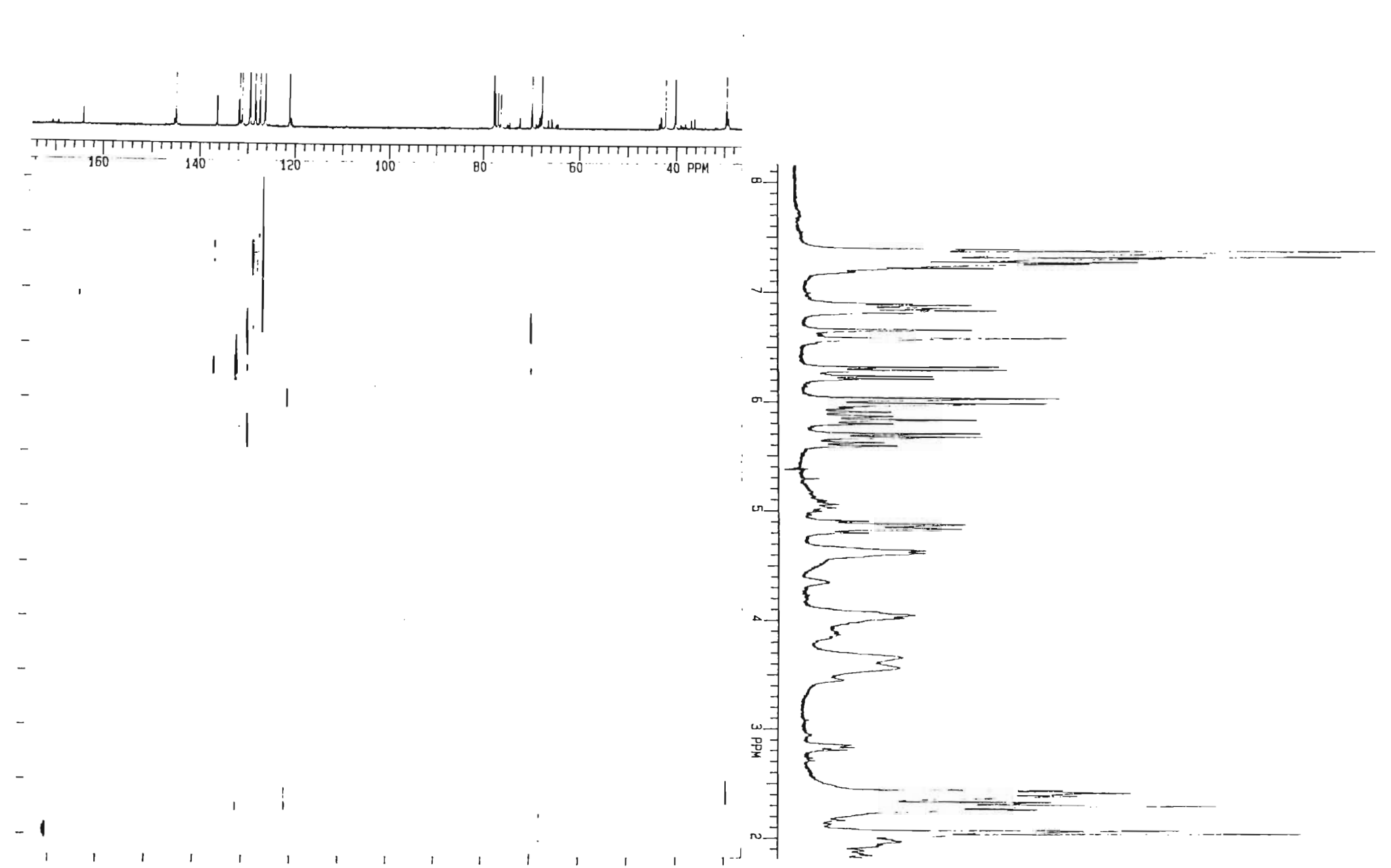
81e ^{1}H-^{1}H RELAYED COSY spectrum of cryptofolione in $CDCl_3$



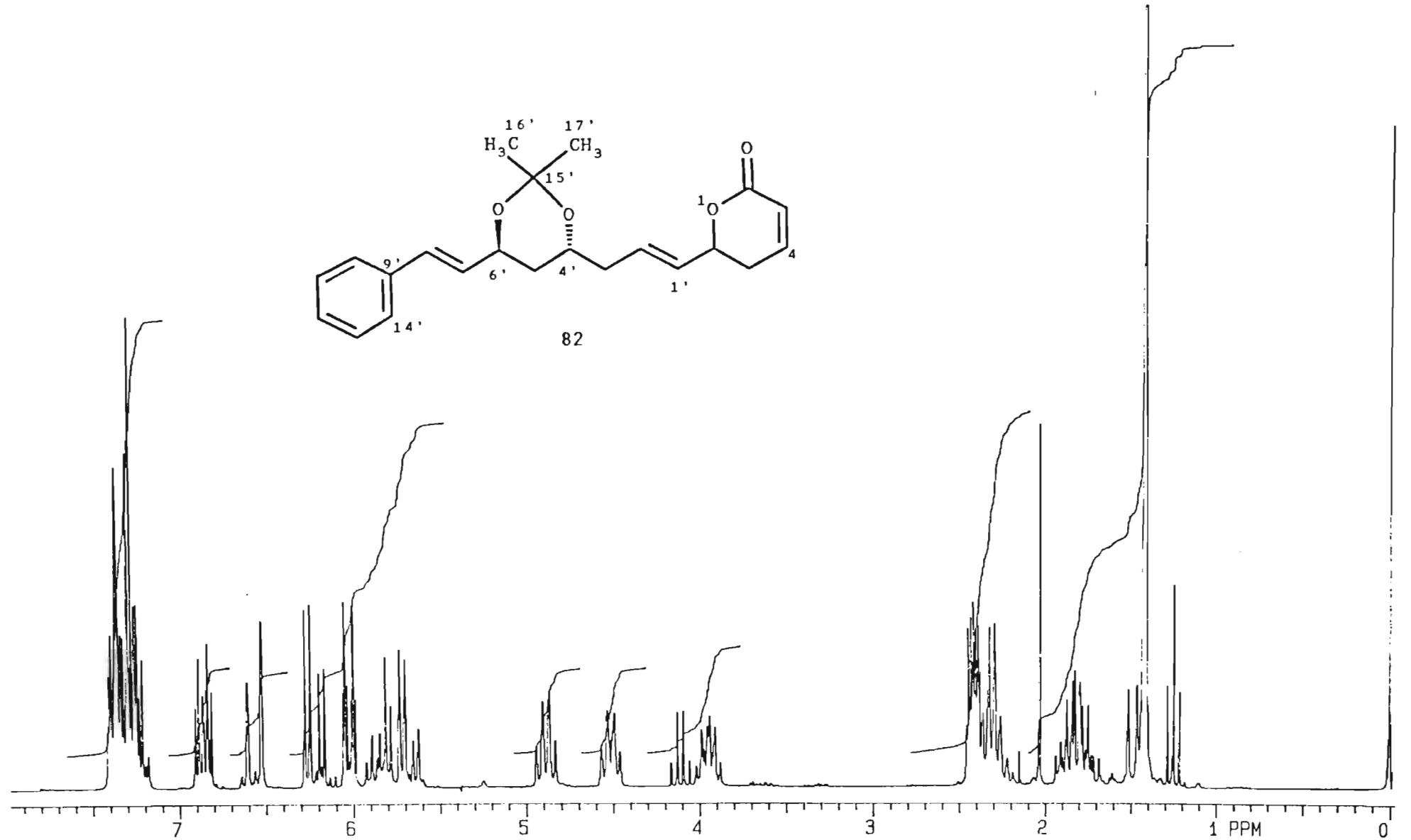
81f ^{1}H-^{13}C HETCOR spectrum of cryptofolione in $CDCl_3$



^{1}H-^{13}C DELAYED HETCOR spectrum of cryptofolione in $CDCl_3$

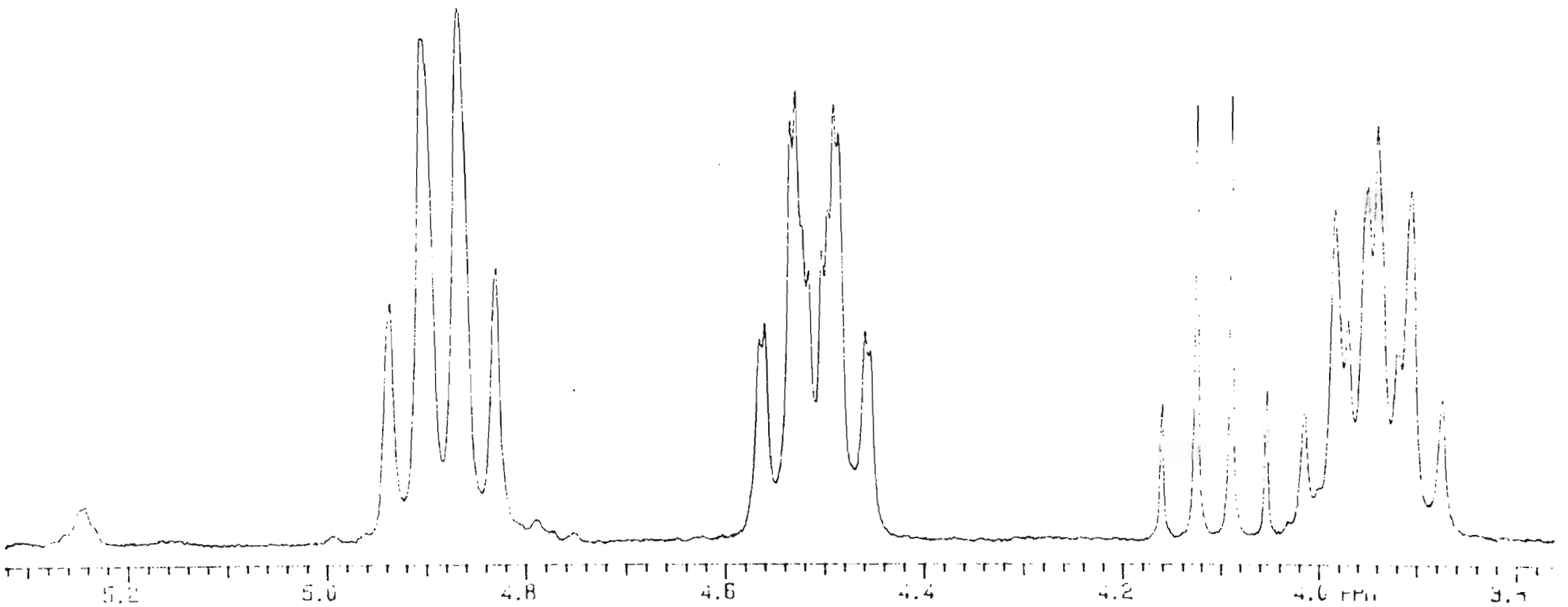


^{1}H-^{13}C DELAYED HETCOR spectrum of cryptofolione in $CDCl_3$

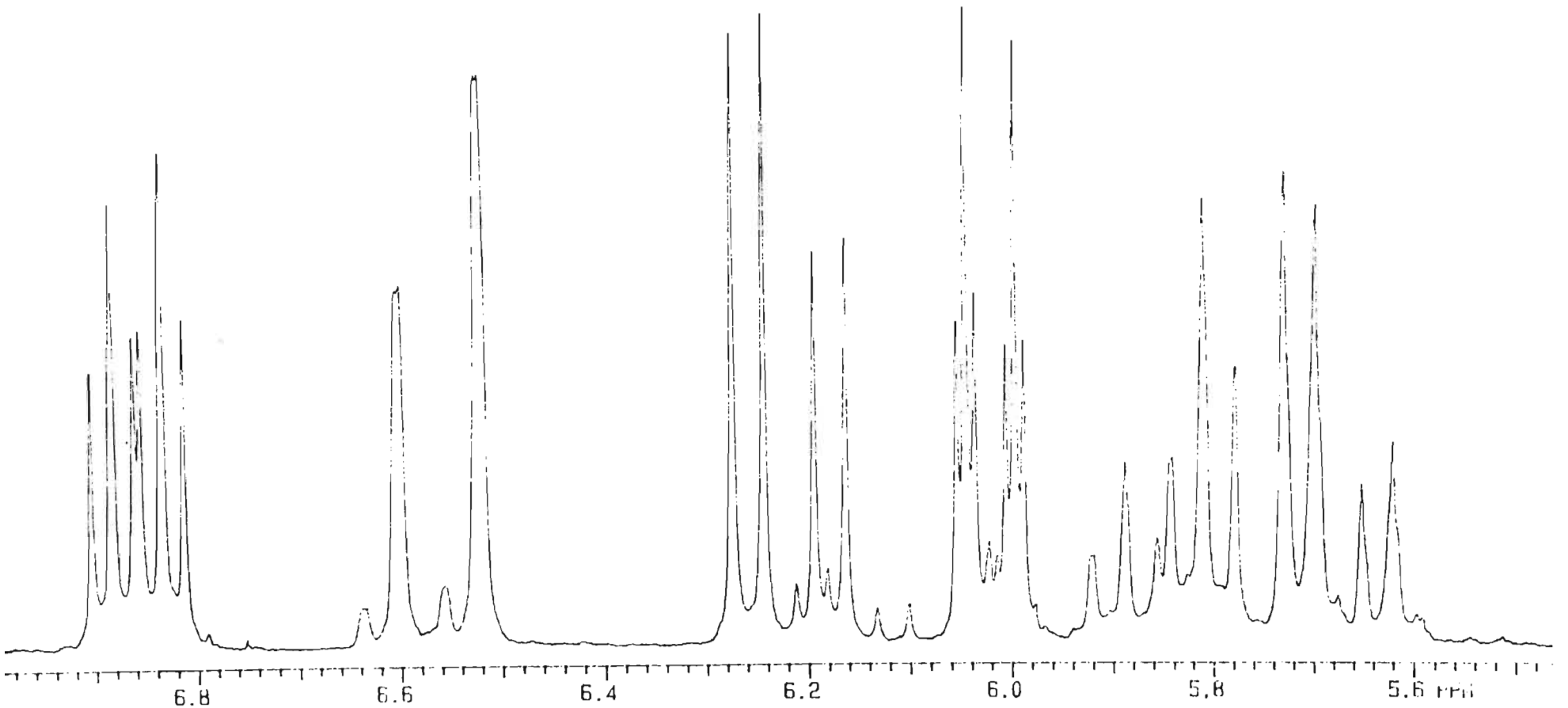


82

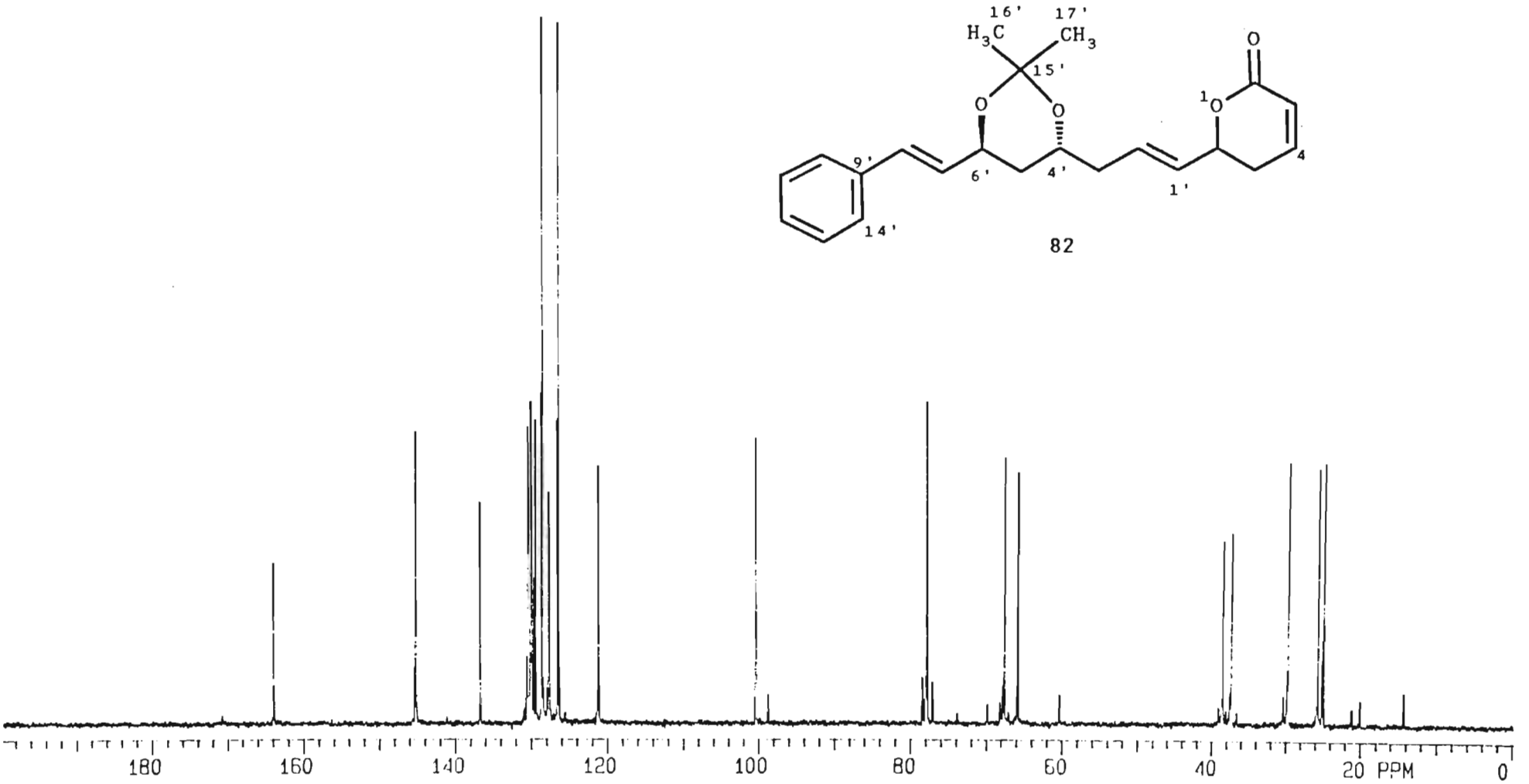
82a ^{1}H NMR spectrum of **82** in $CDCl_3$



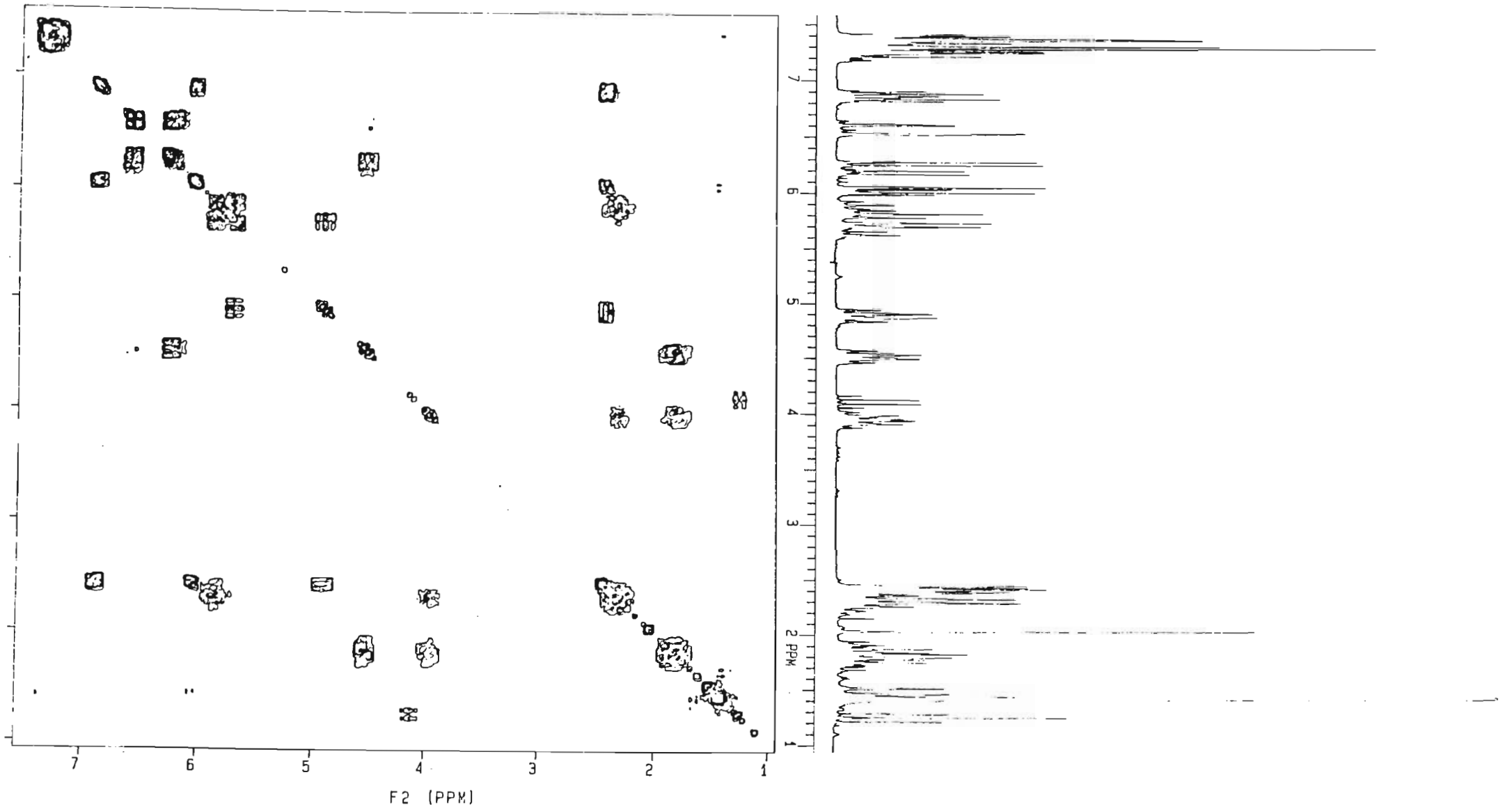
82b Expanded ^1H NMR spectrum of **82** in $CDCl_3$



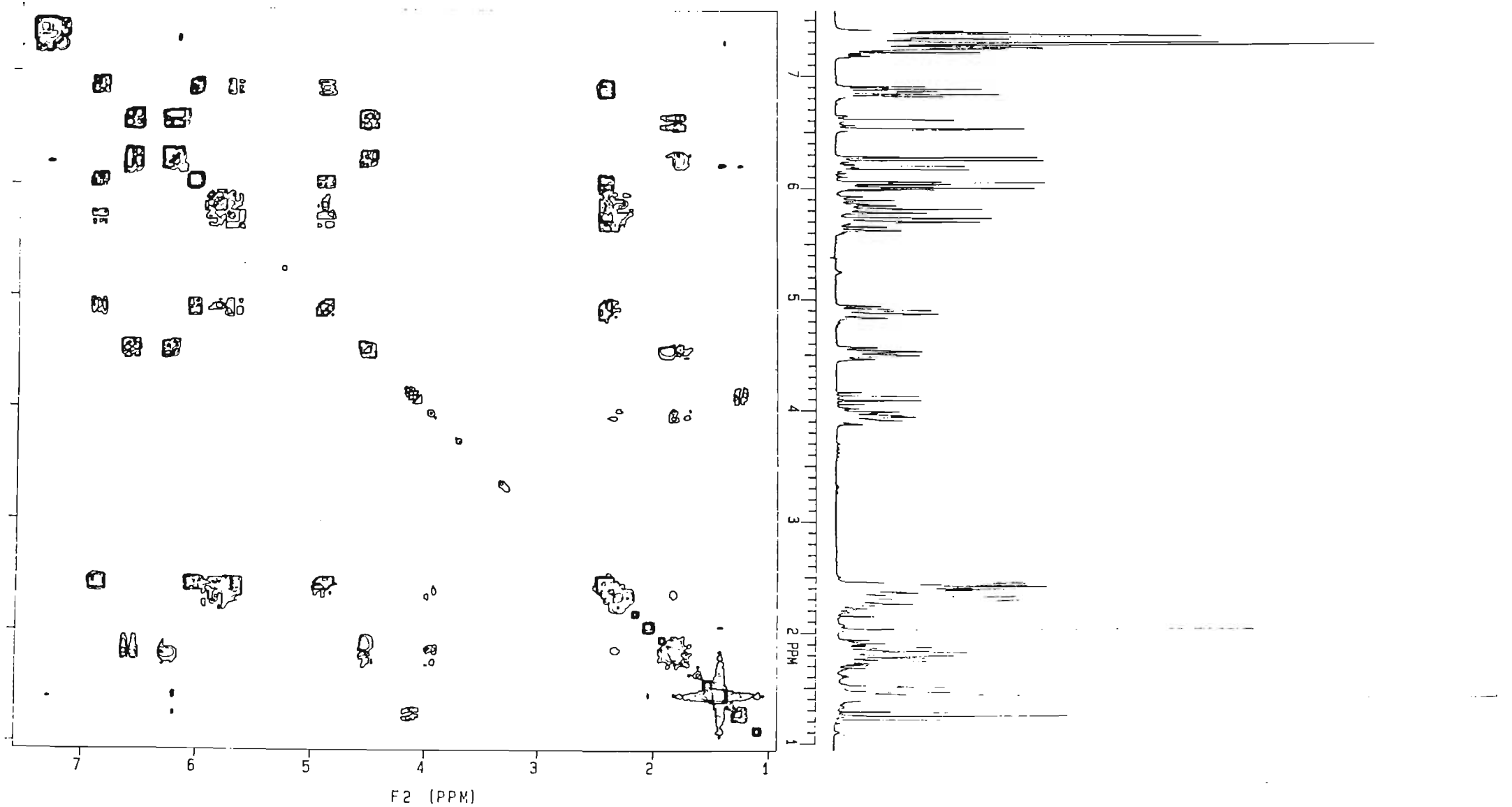
82b Expanded ^{1}H NMR spectrum of **82** in $CDCl_3$



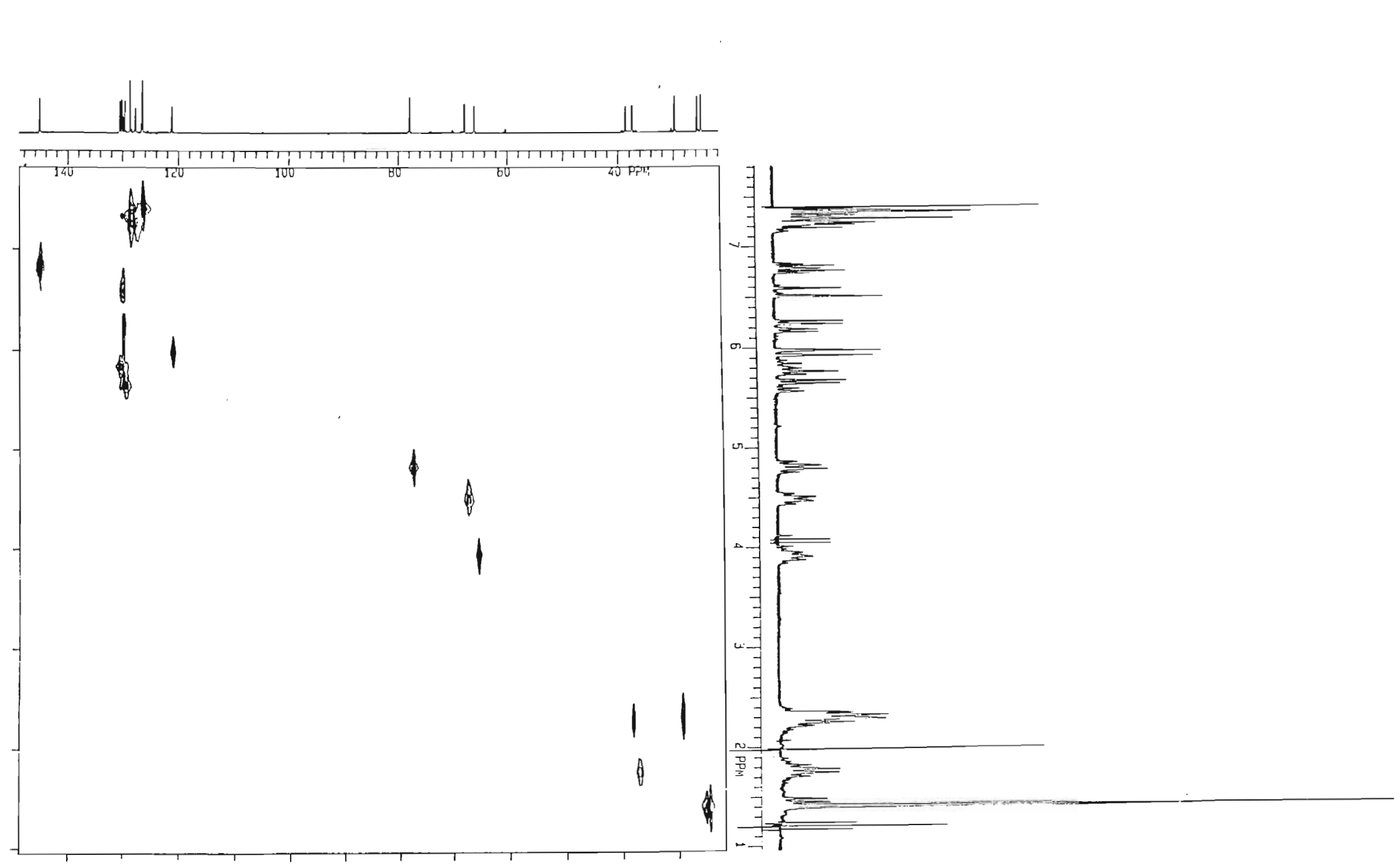
82c ^{13}C NMR spectrum of **82** in $CDCl_3$



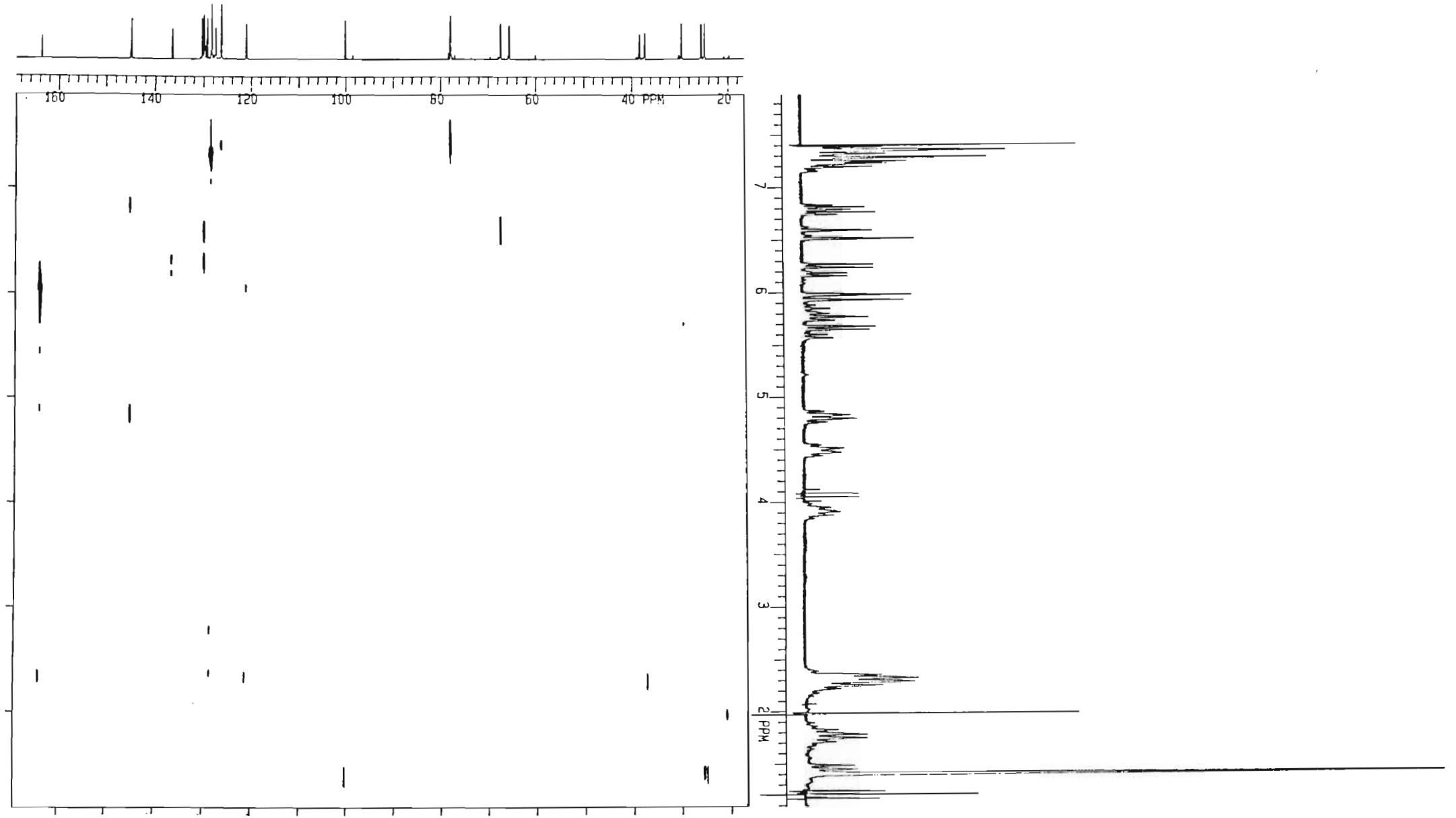
82d ^{1}H-^{1}H COSY spectrum of **82** in $CDCl_3$



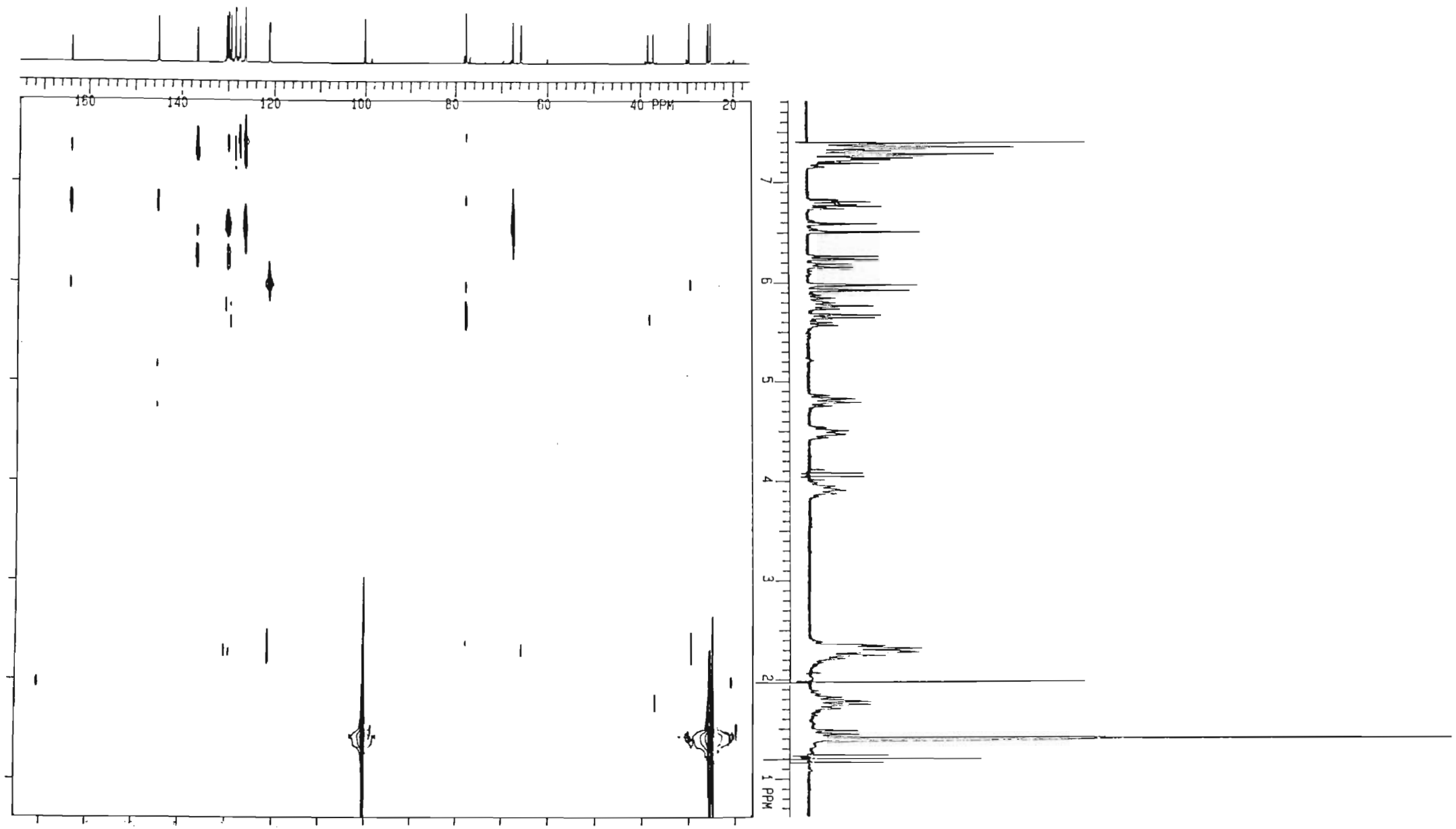
82e ^{1}H-^{1}H RELAYED COSY spectrum of **82** in $CDCl_3$



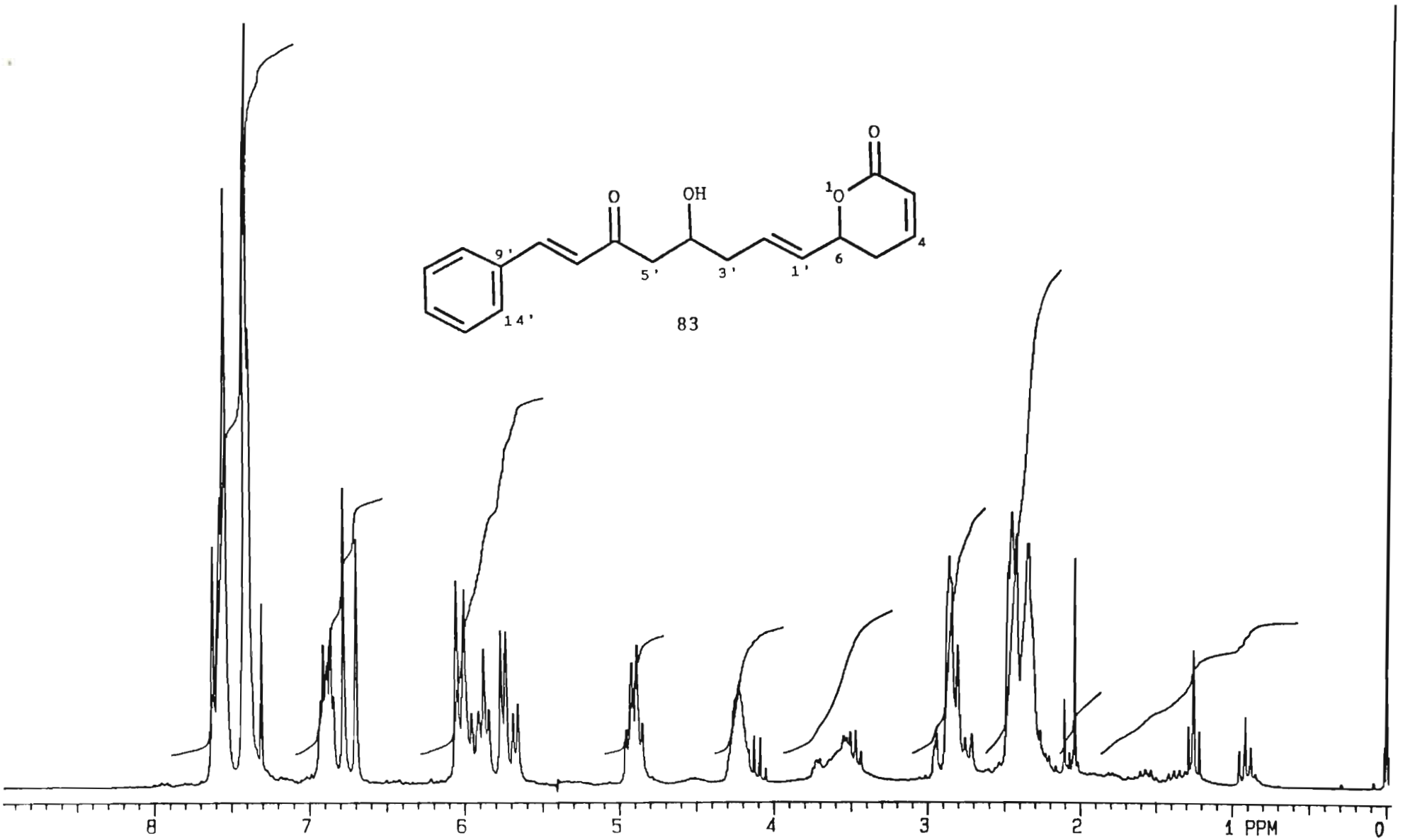
82f ^{1}H-^{13}C HETCOR spectrum of **82** in $CDCl_3$



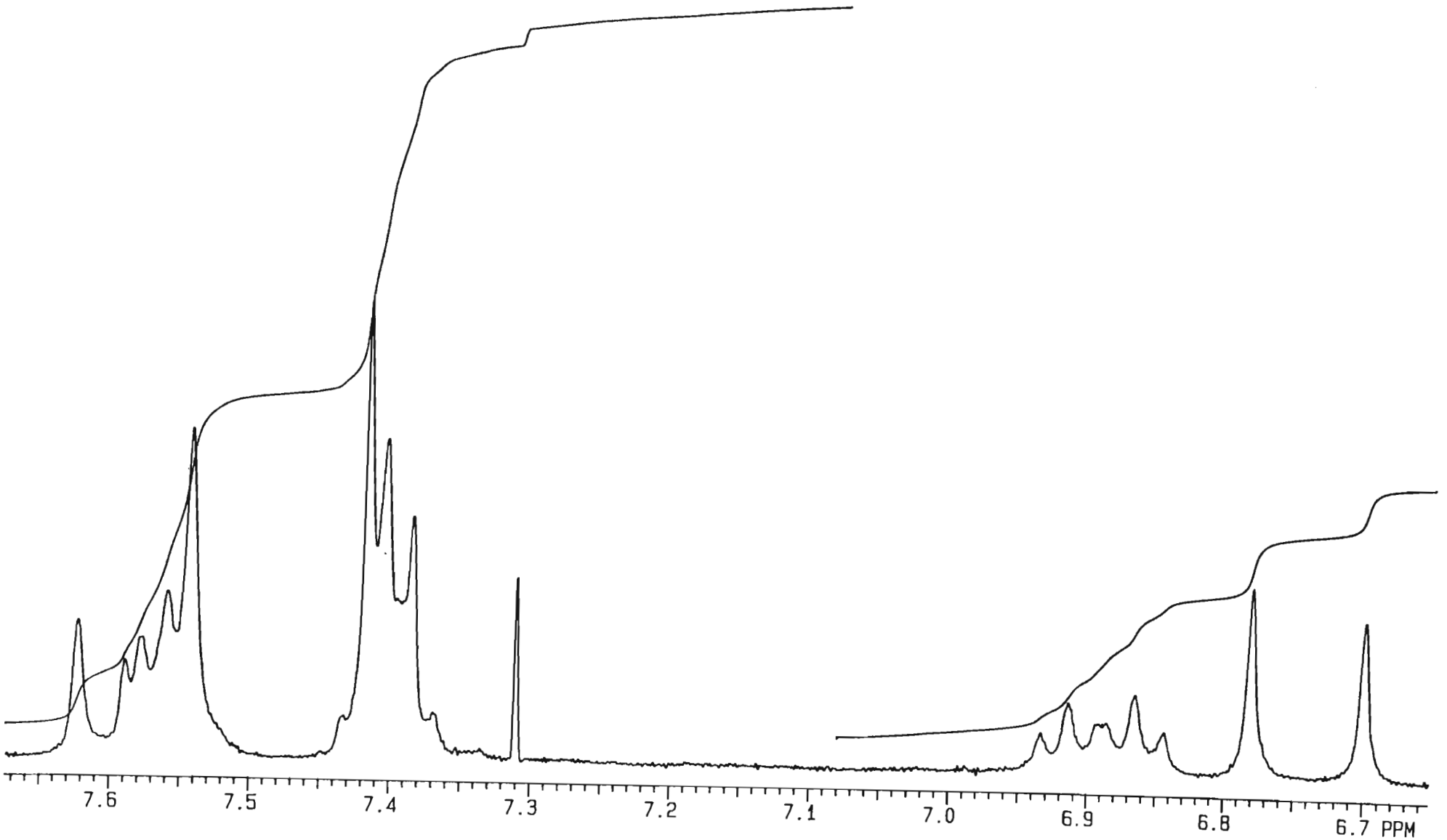
82 — ^{1}H-^{13}C DELAYED HETCOR spectrum of **82** in $CDCl_3$ (4Hz)



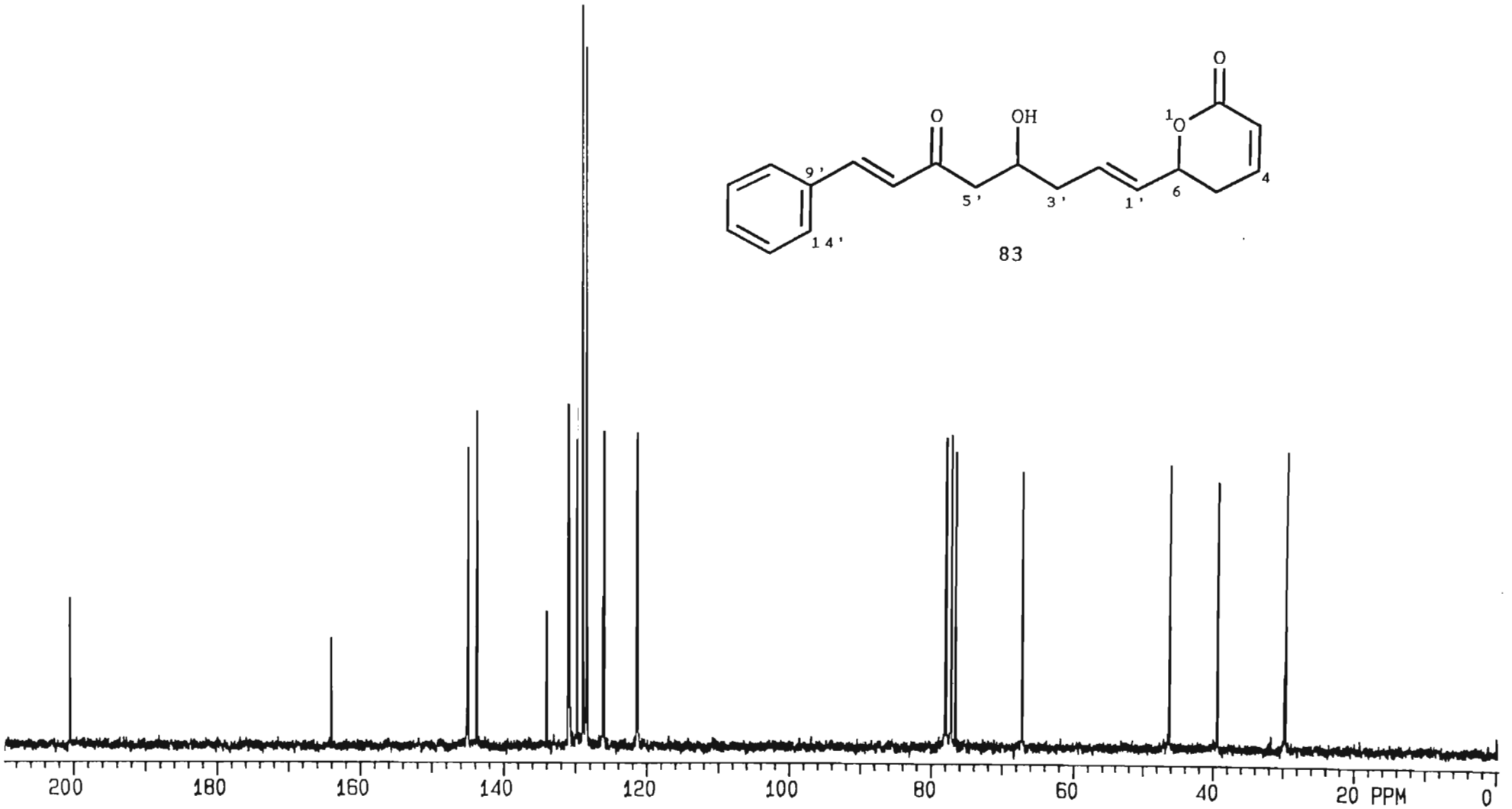
2g ^{1}H-^{13}C DELAYED HETCOR spectrum of **82** in $CDCl_3$ (10Hz)



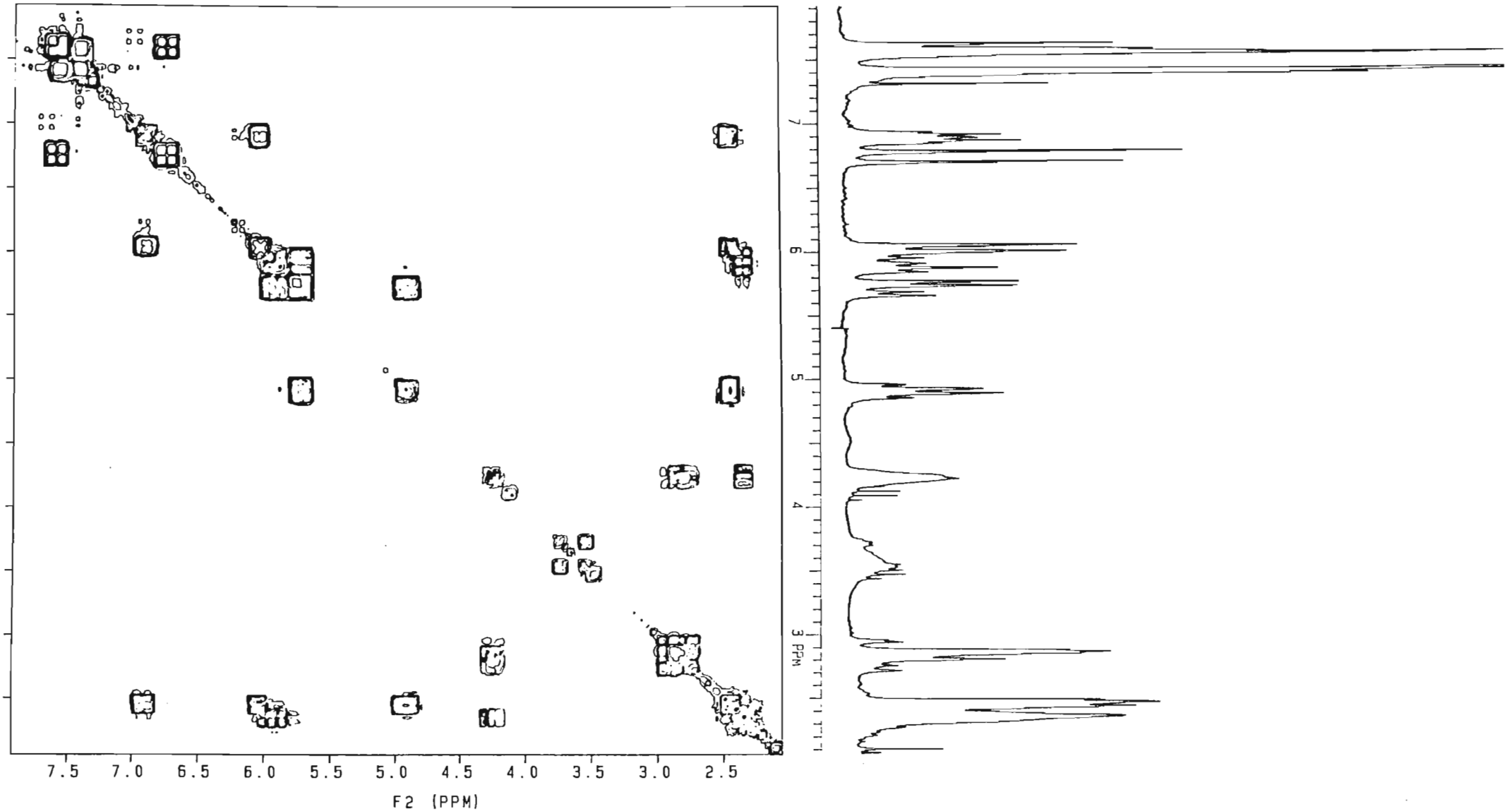
83a ^{1}H NMR spectrum of cryptofolione in $CDCl_3$



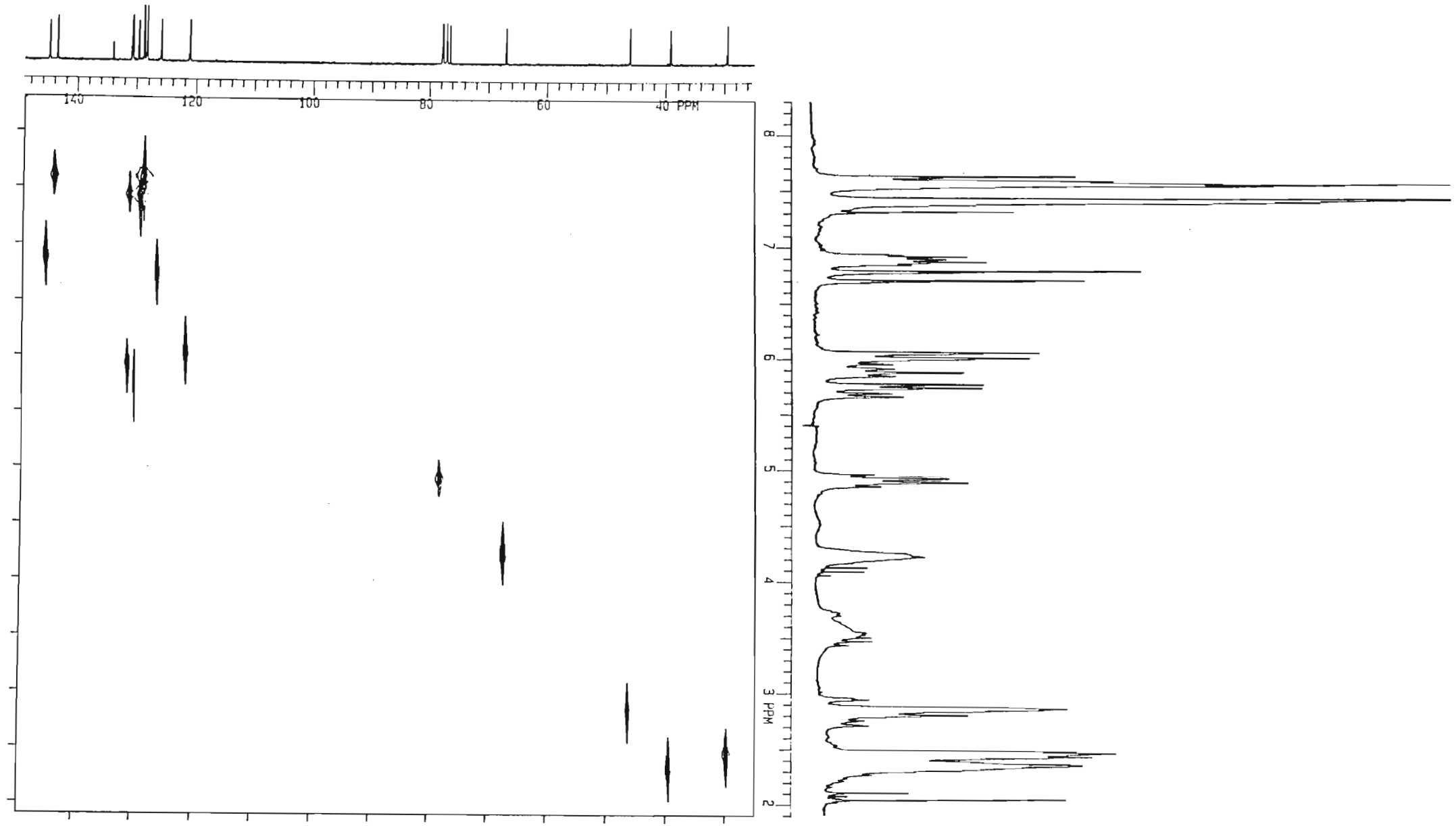
83b Expanded ^{1}H NMR spectrum of cryptofolione in $CDCl_3$



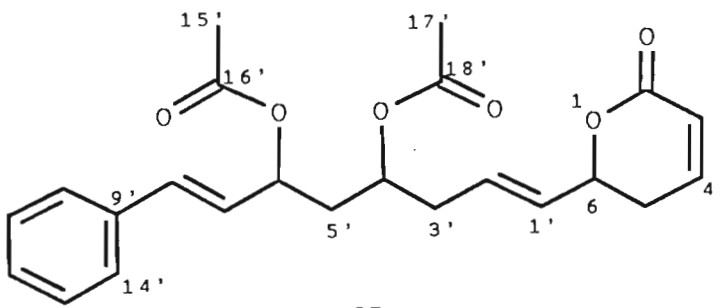
83c ^{13}C NMR spectrum of cryptofolione in $CDCl_3$



83d ^{1}H-^{1}H COSY spectrum of cryptofolione in $CDCl_3$

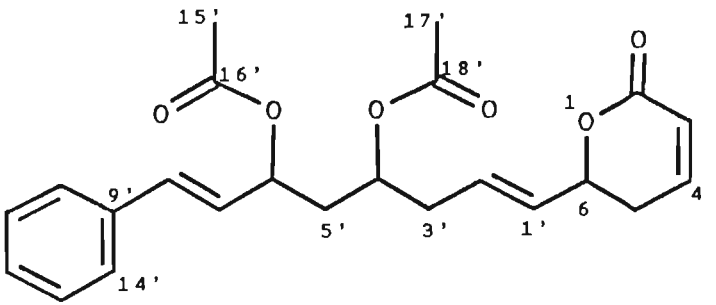


83f ^{1}H-^{13}C HETCOR spectrum of cryptofolione in $CDCl_3$

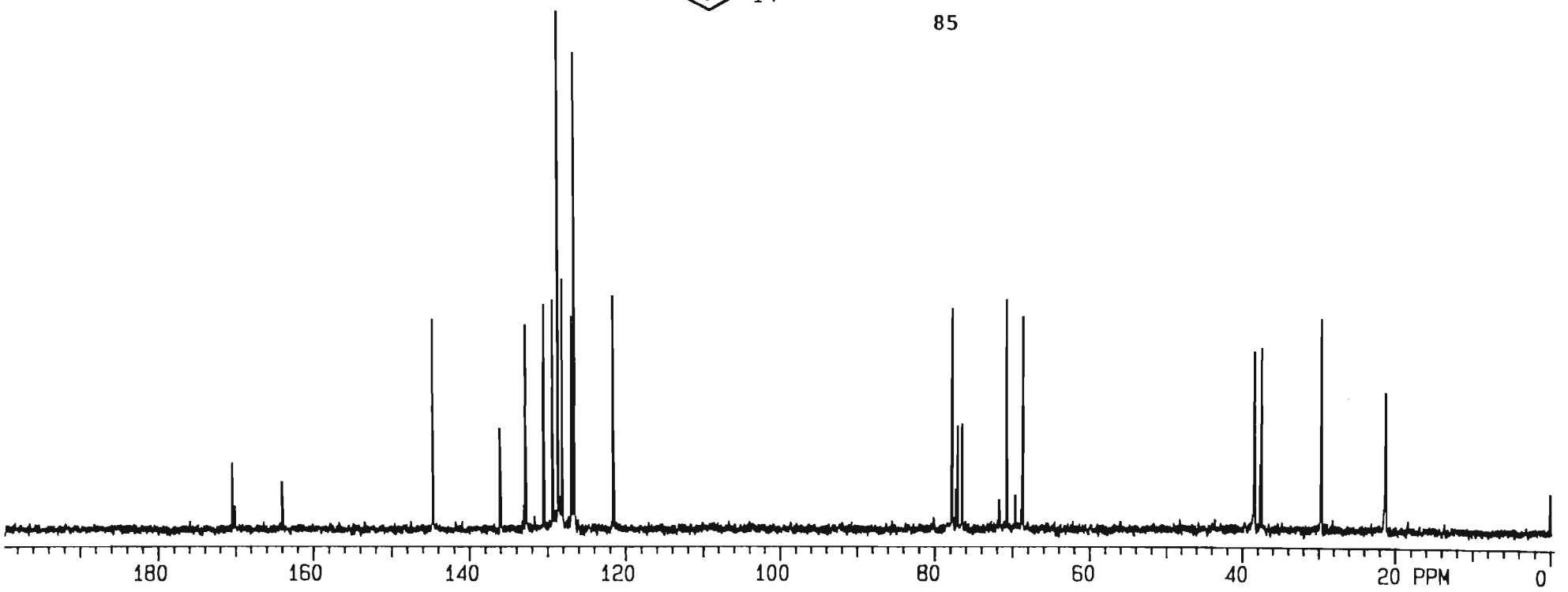


85

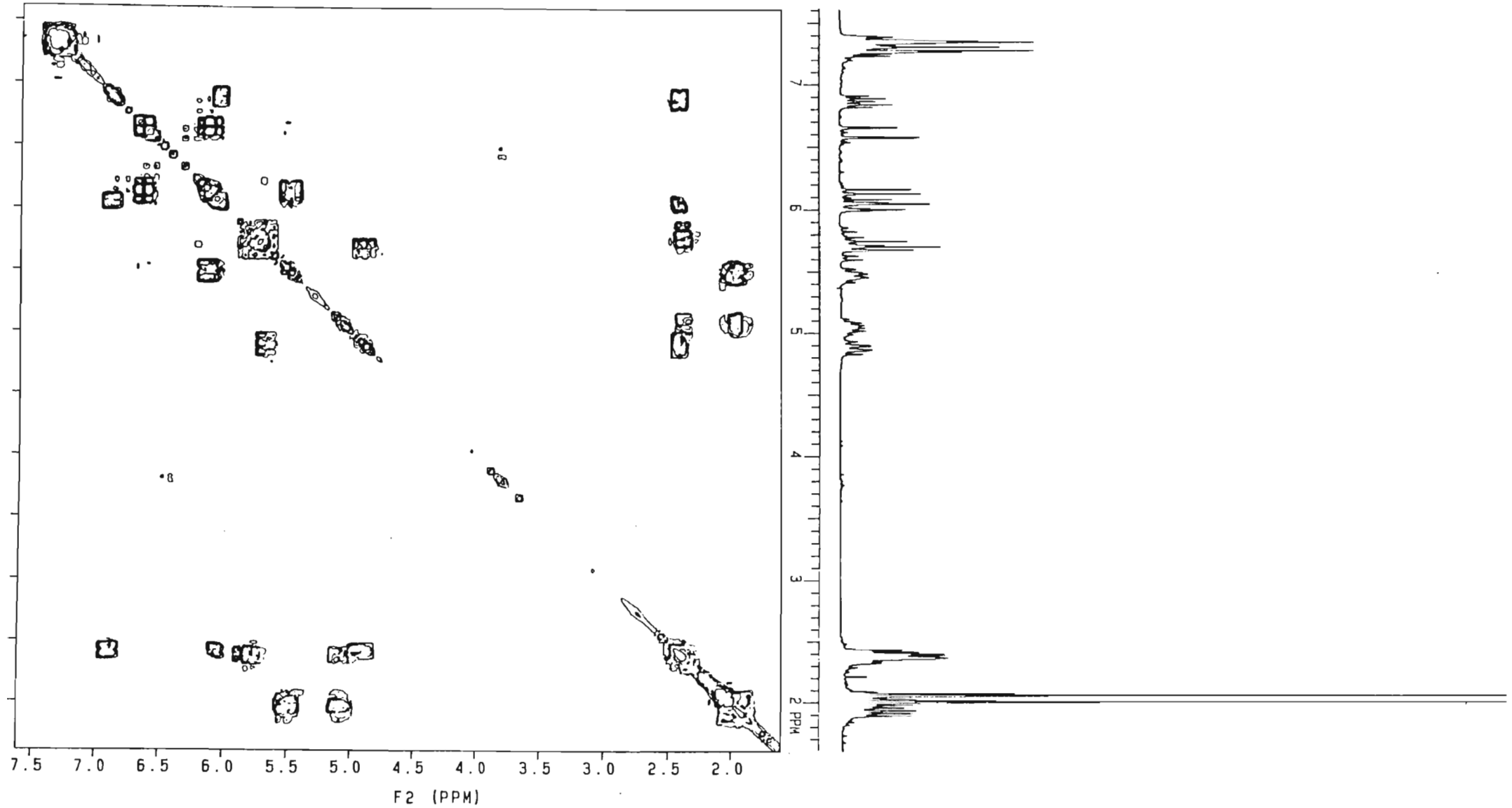
85a ^{1}H NMR spectrum of **85** in $CDCl_3$



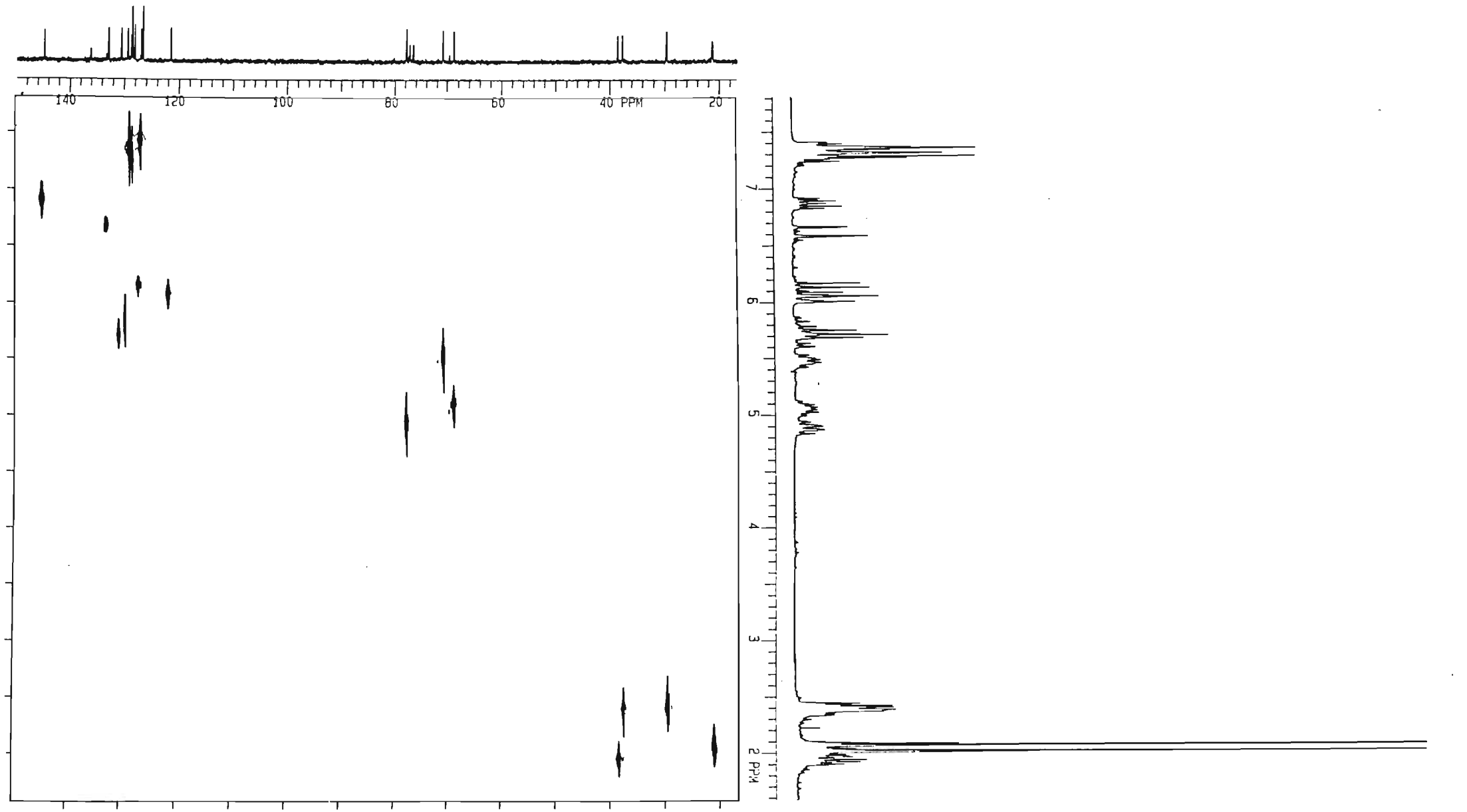
85



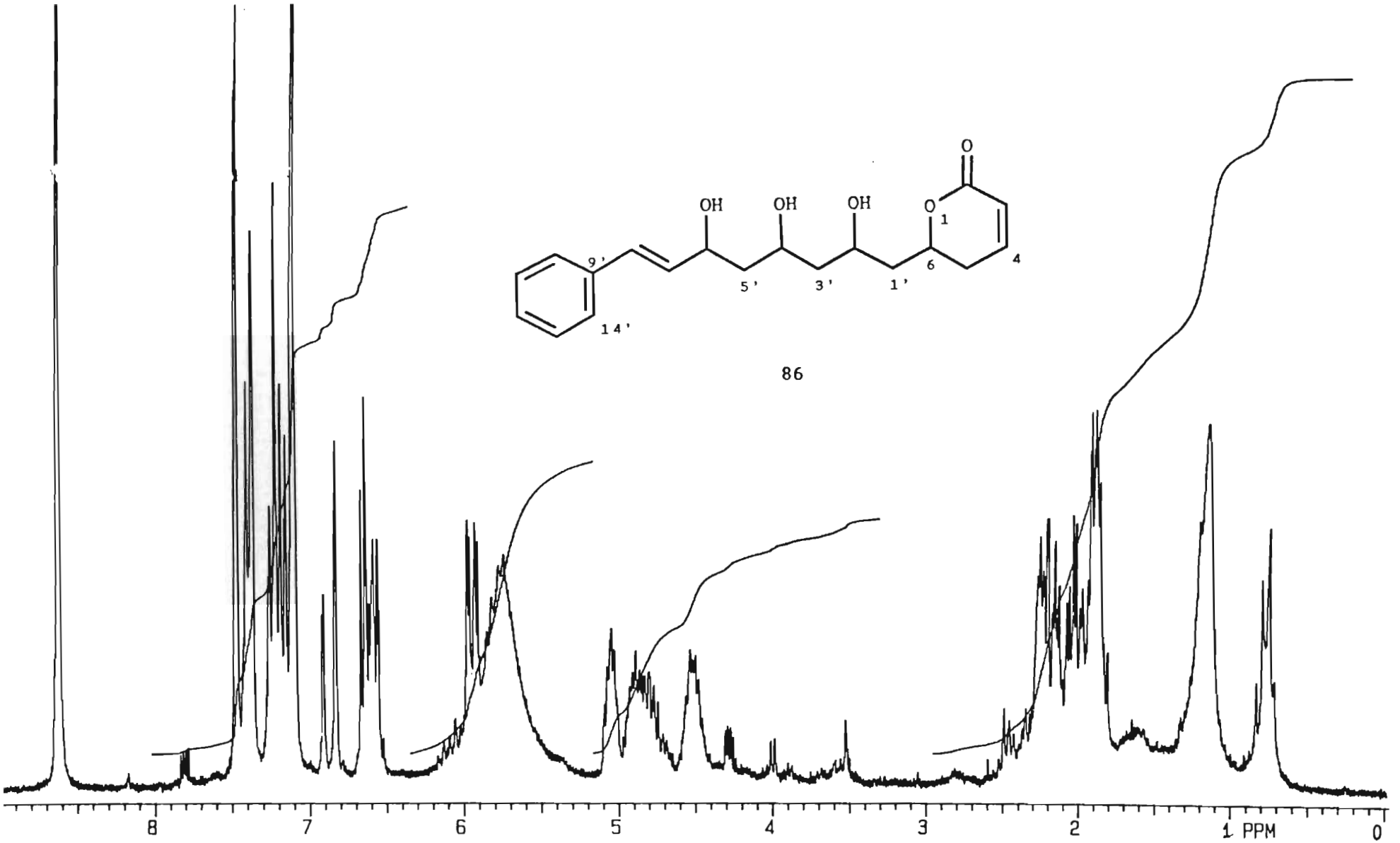
85c ^{13}C NMR spectrum of **85** in $CDCl_3$



85d ^{1}H-^{1}H COSY spectrum of **85** in $CDCl_3$

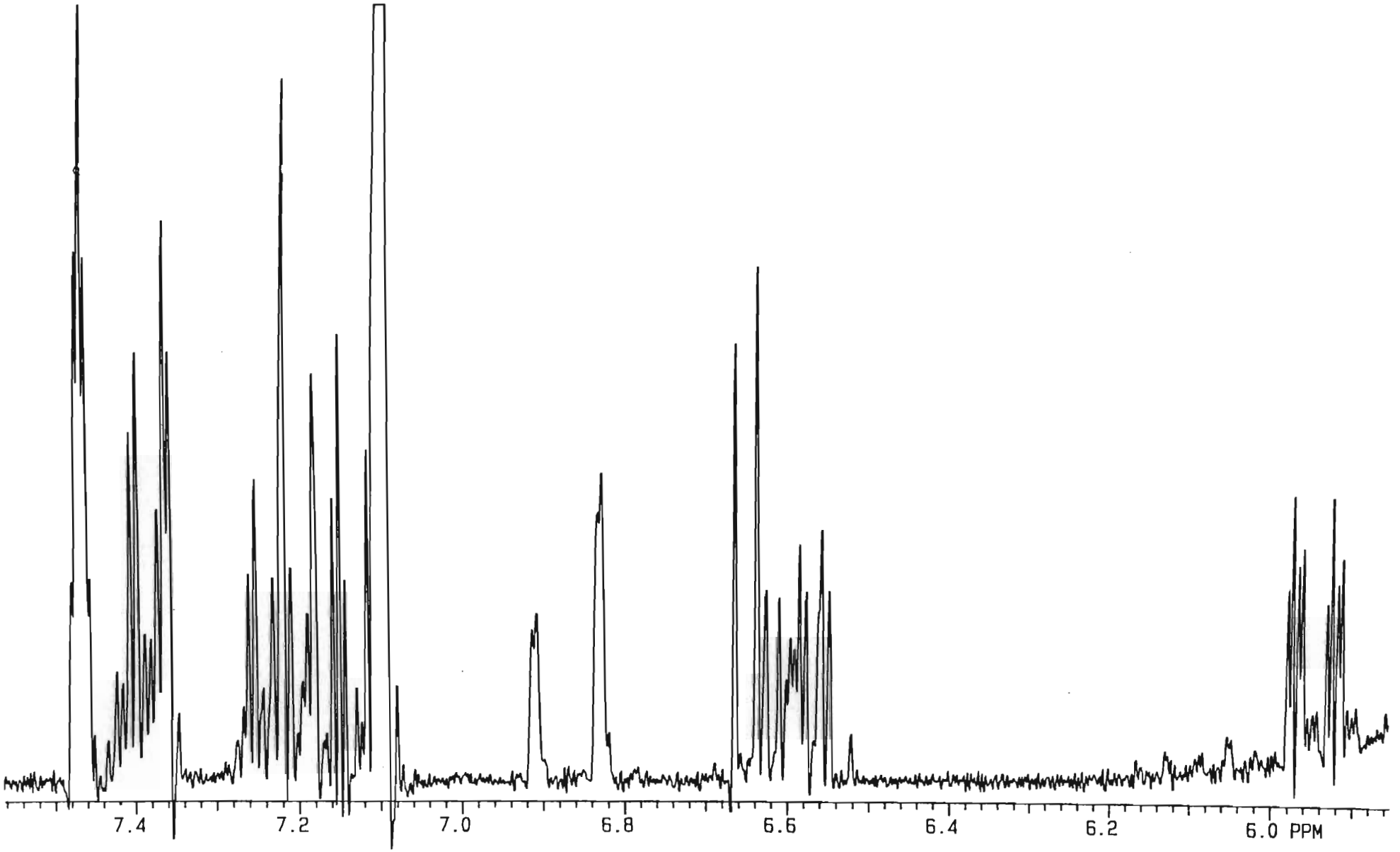


85f ^{1}H-^{13}C HETCOR spectrum of 85 in $CDCl_3$

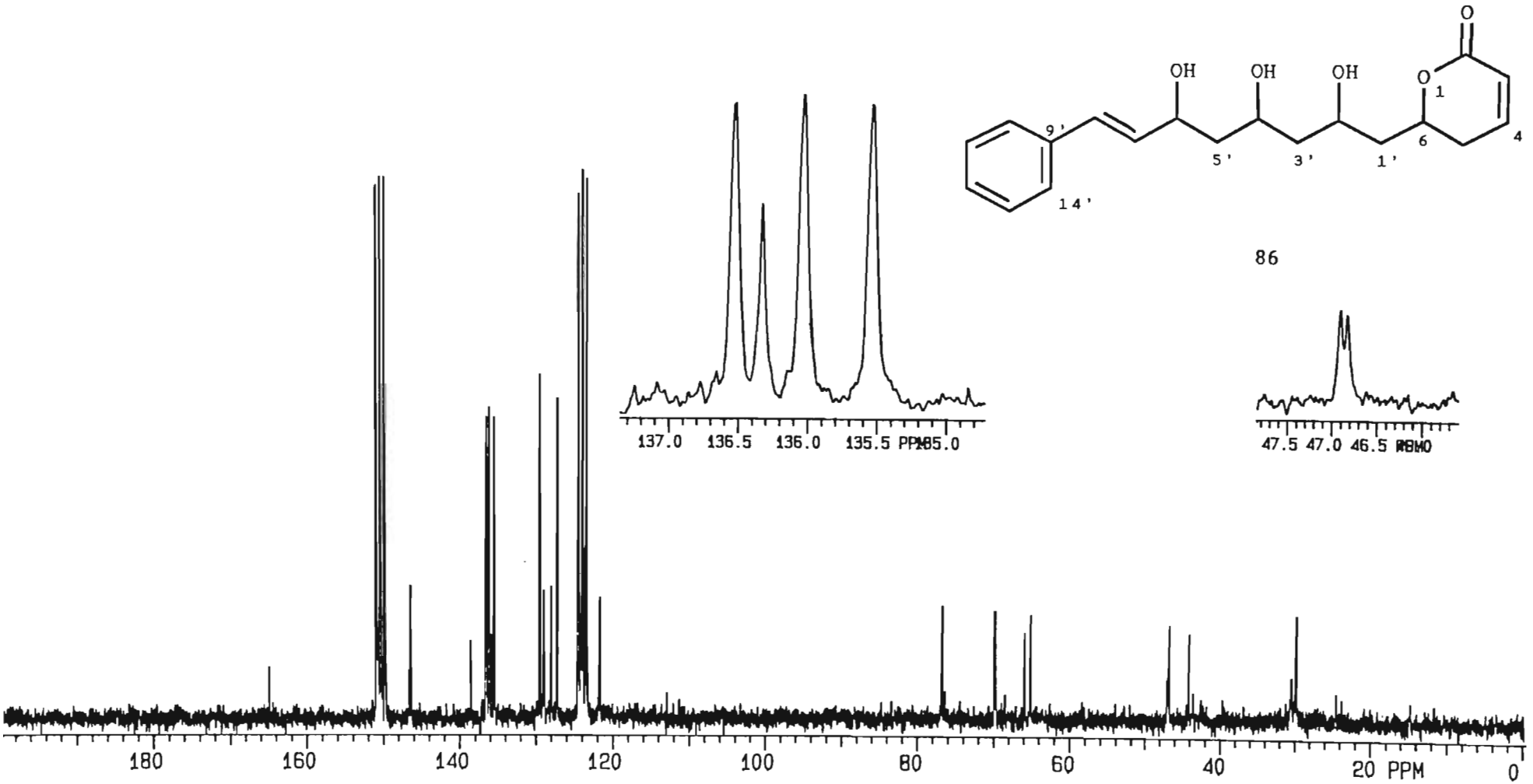


86

86a ^{1}H NMR spectrum of myrtifolione in pyridine-d_5



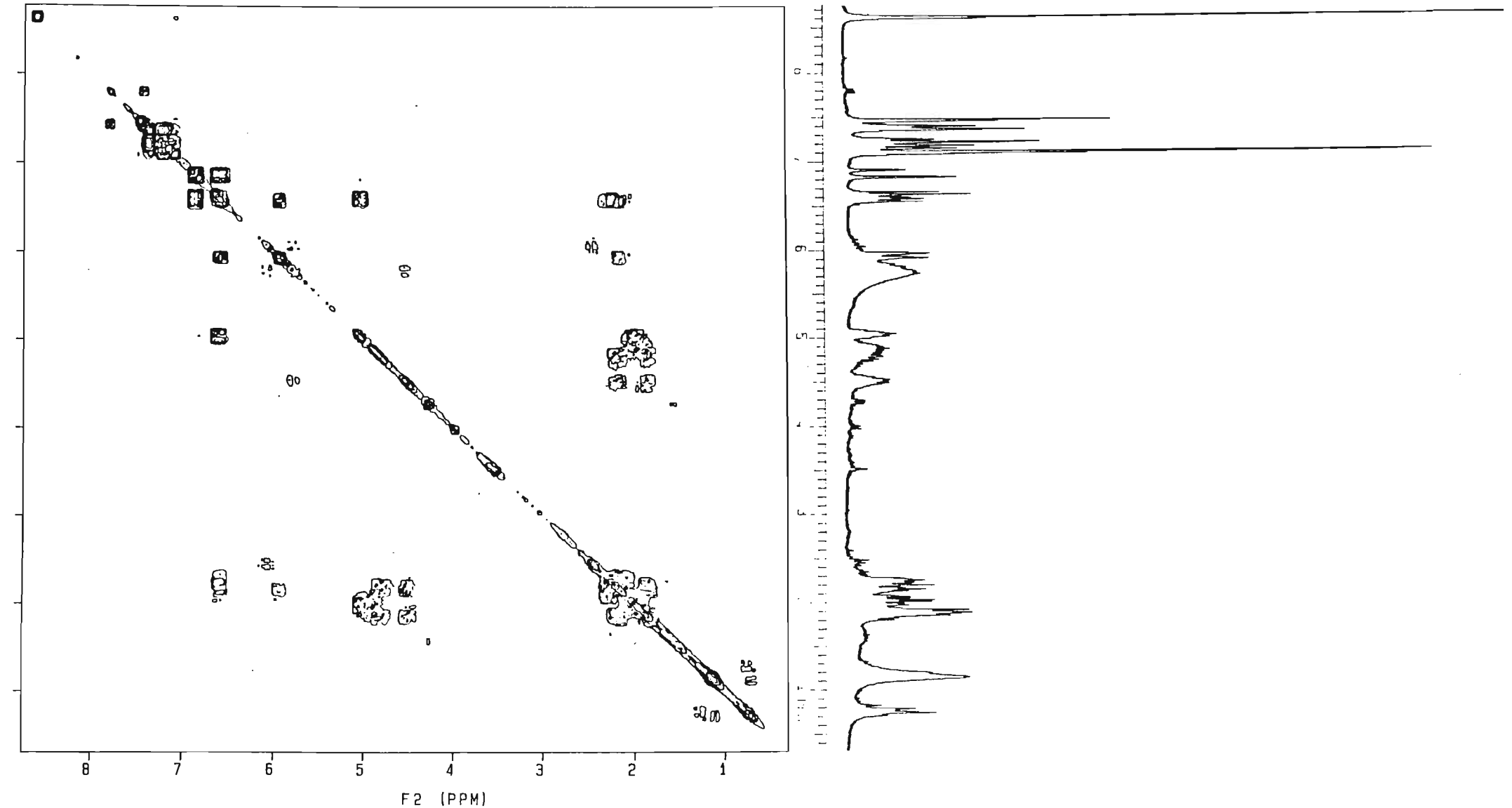
86b Expanded ^{1}H NMR spectrum of myrtifolione in pyridine-d_5



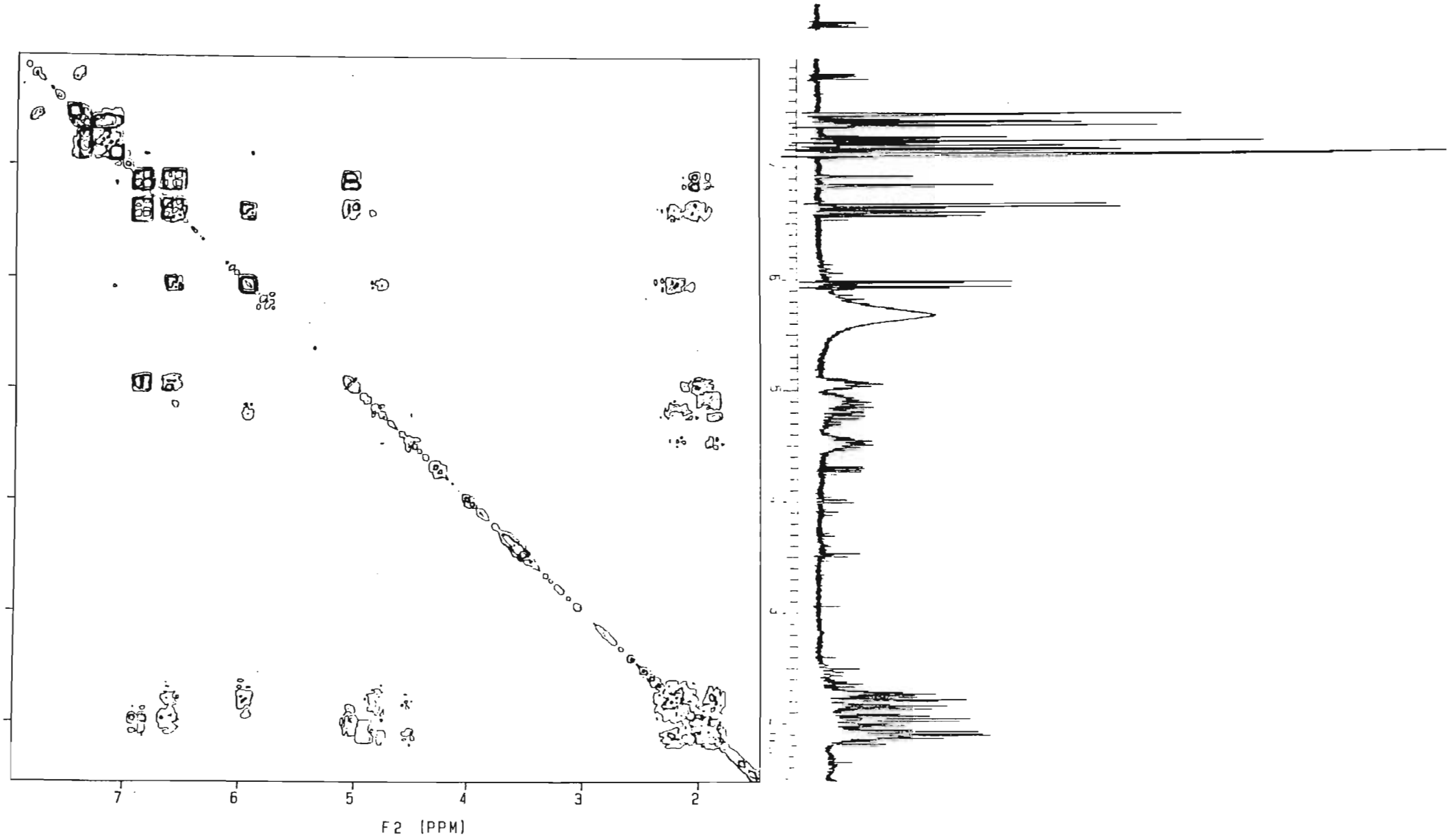
86

180 160 140 120 100 80 60 40 20 PPM 0

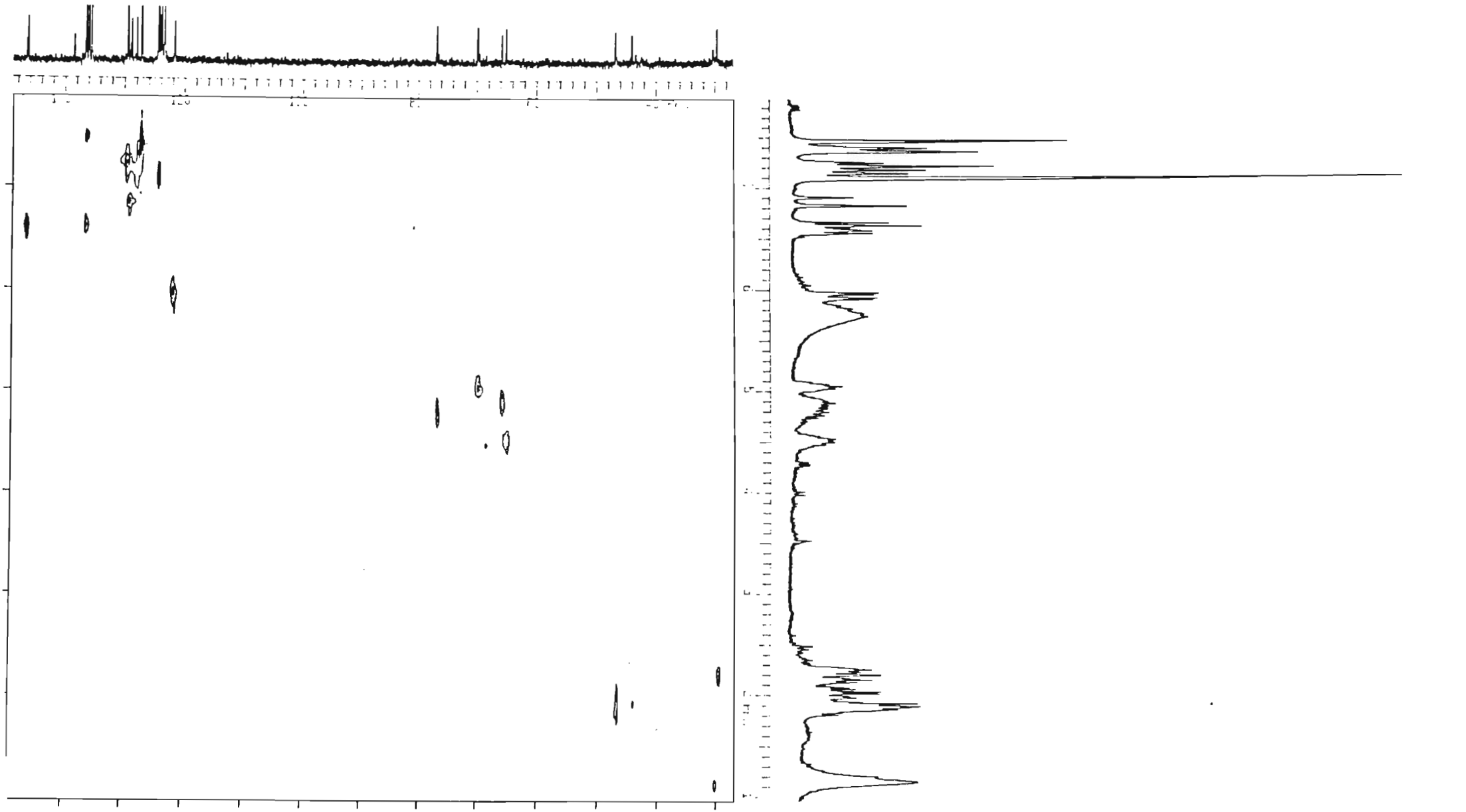
86c ^{13}C NMR spectrum of myrtifolione in pyridine-d_5



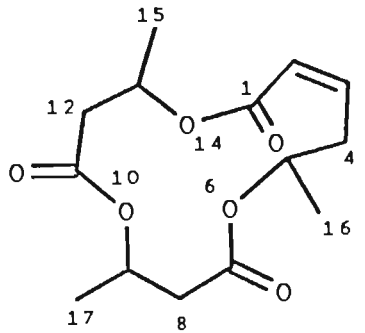
86d ^{1}H-^{1}H COSY spectrum of myrtifolione in pyridine-d_5



86e ^{1}H-^{1}H RELAYED COSY spectrum of myrtifolione in

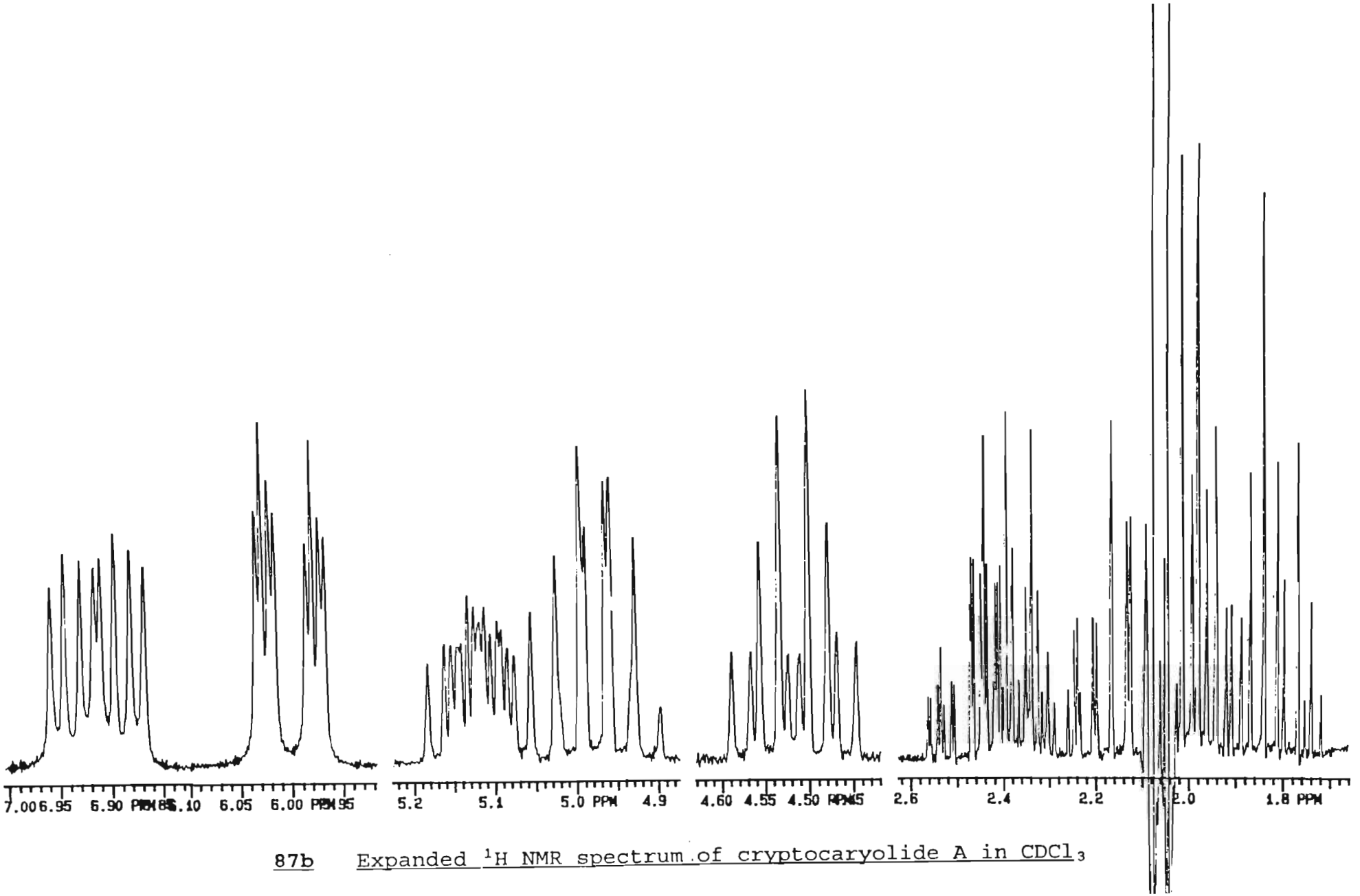


^{1}H-^{13}C HETCOR spectrum of myrtifolione in pyridine-d_5

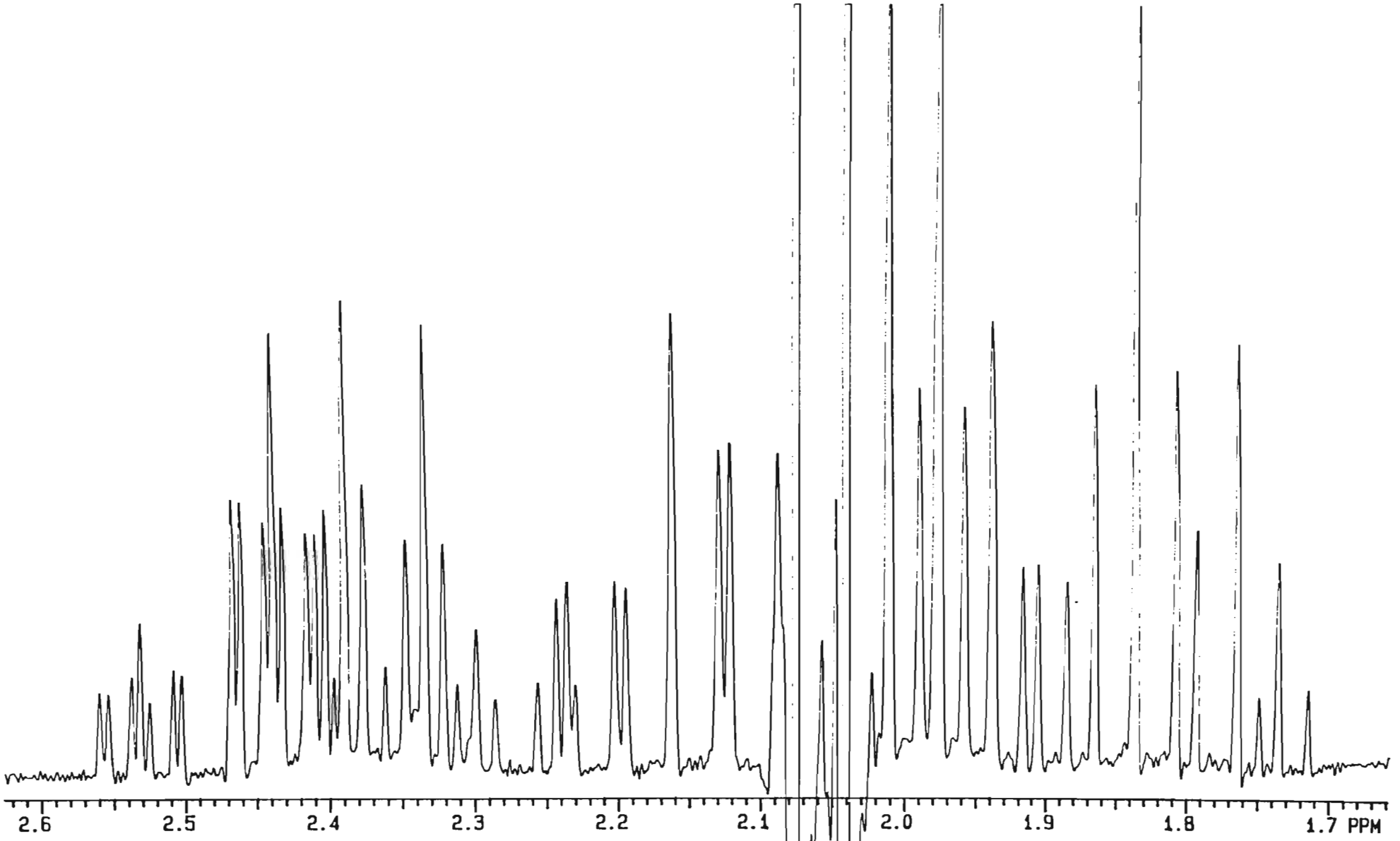


87

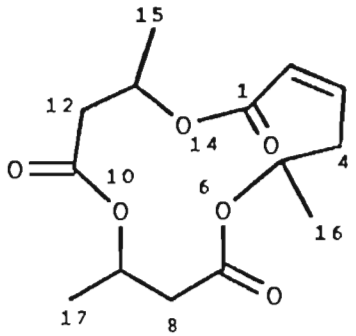
87a ^{1}H NMR spectrum of cryptocaryolide A in $CDCl_3$



87b Expanded ^1H NMR spectrum of cryptocaryolide A in $CDCl_3$



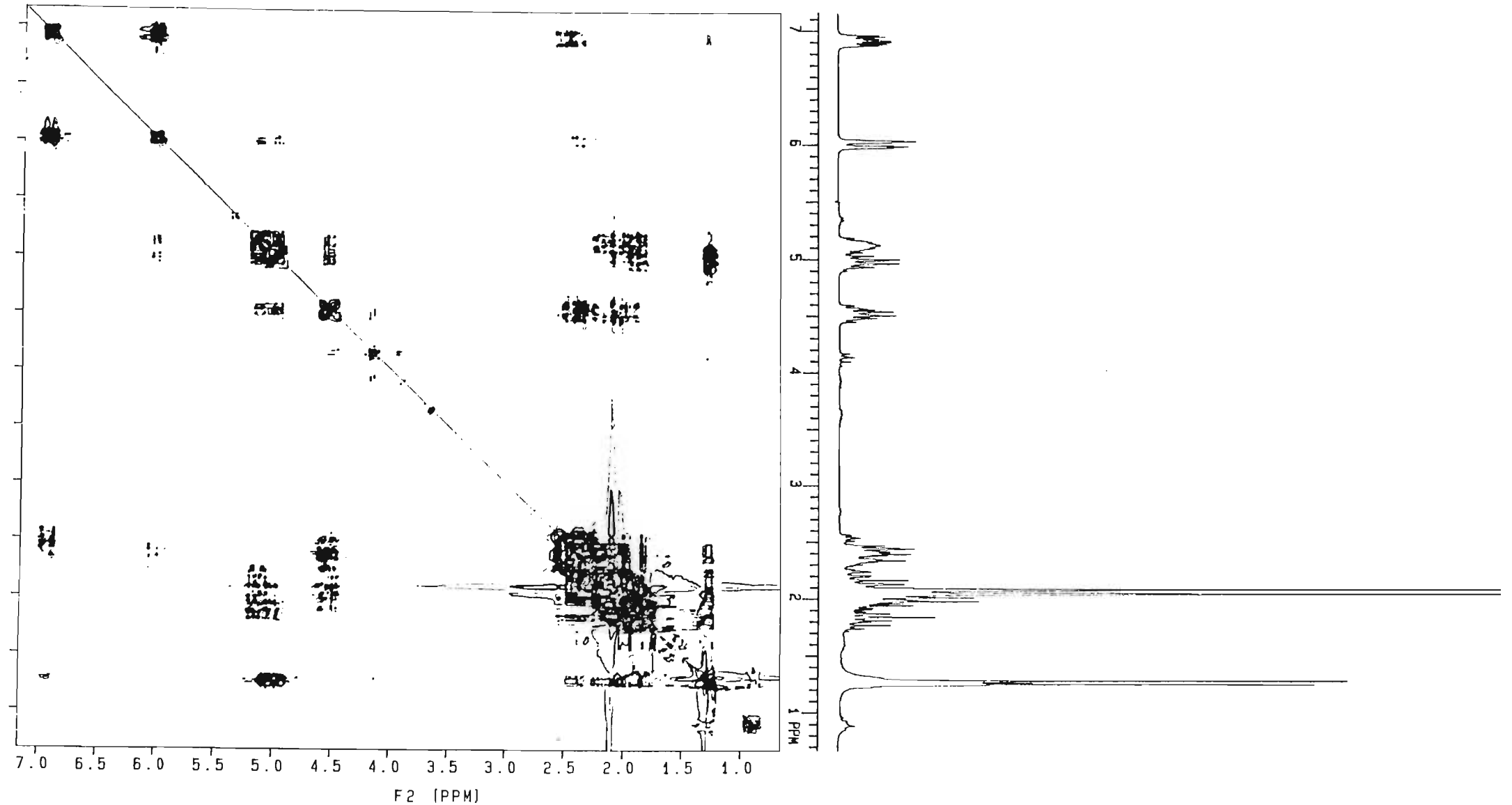
87b Expanded ^{1}H NMR spectrum of cryptocaryolide A in $CDCl_3$



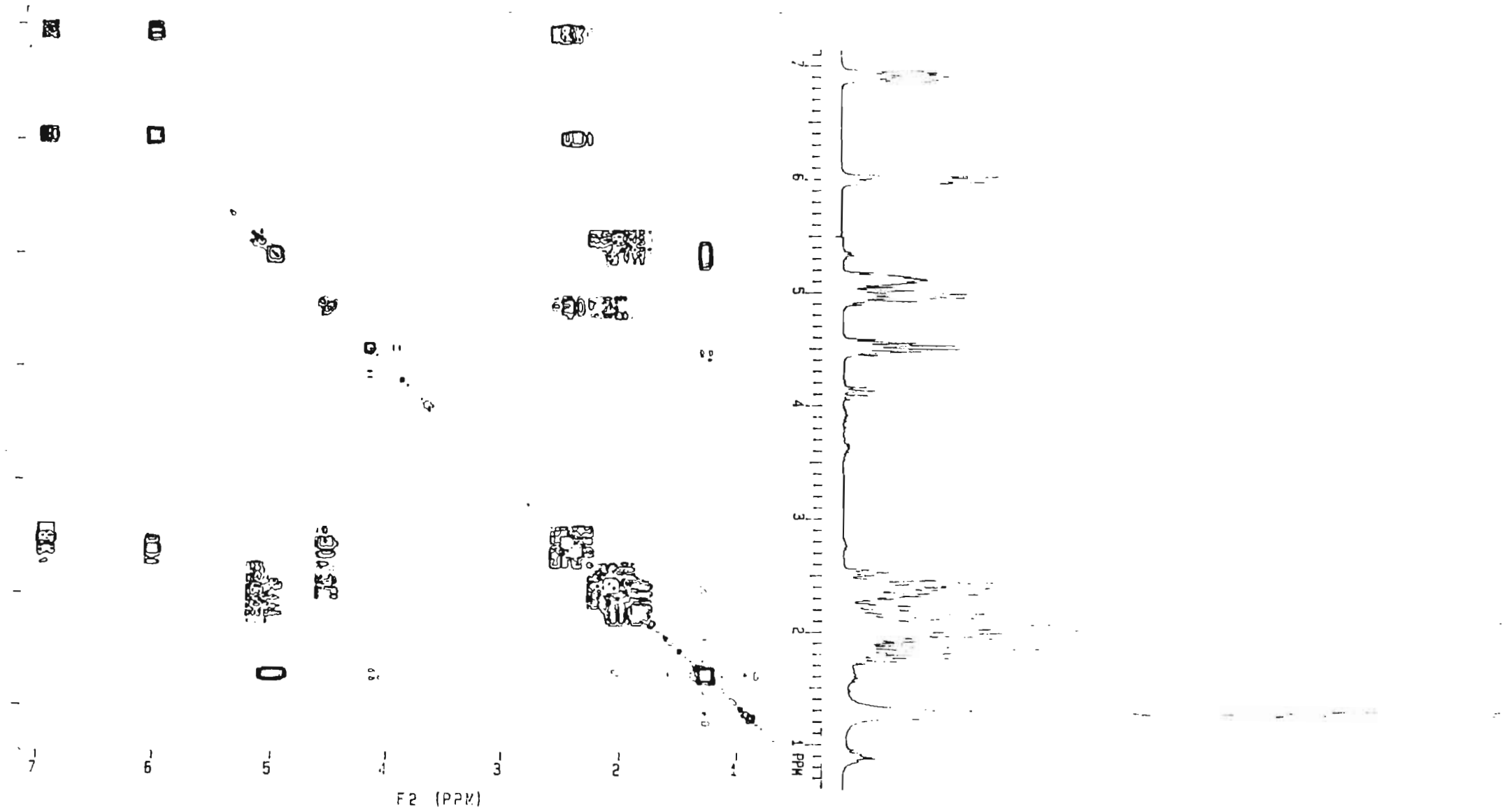
87

180 160 140 120 100 80 60 40 20 PPM 0

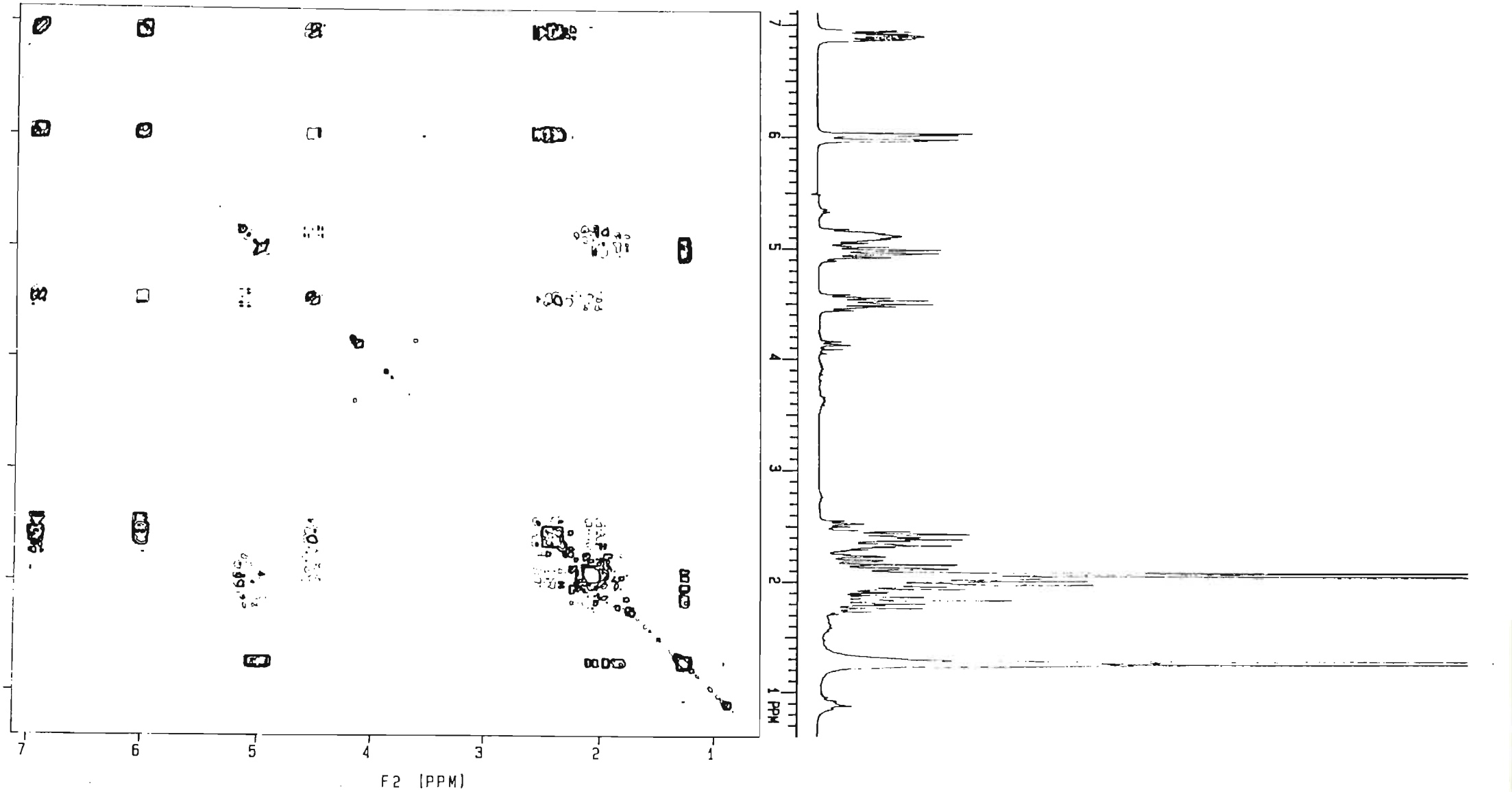
87c ^{13}C NMR spectrum of cryptocaryolide A in $CDCl_3$



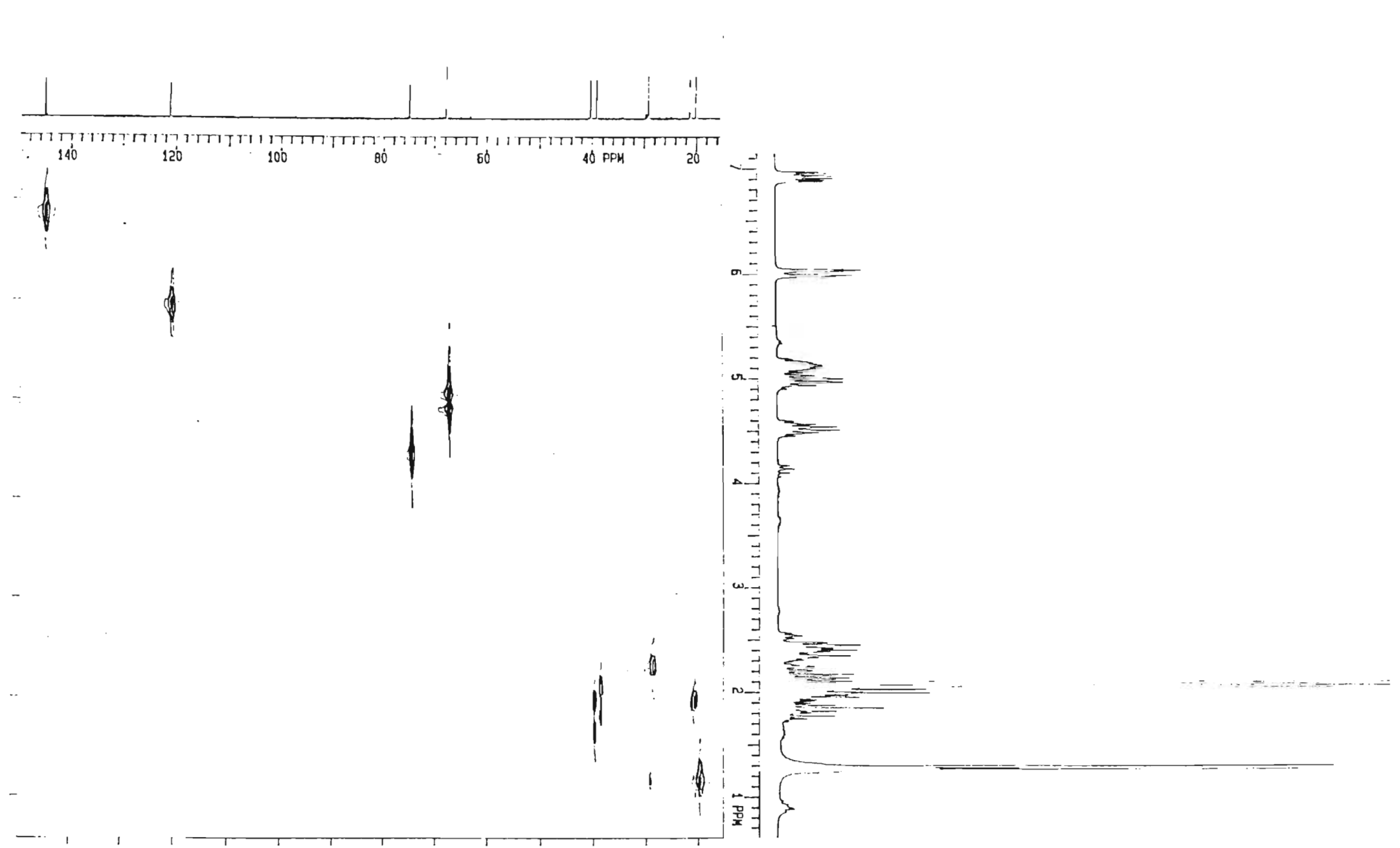
^{1}H-^{1}H PHASE SENSITIVE COSY spectrum of cryptocaryolide A in $CDCl_3$



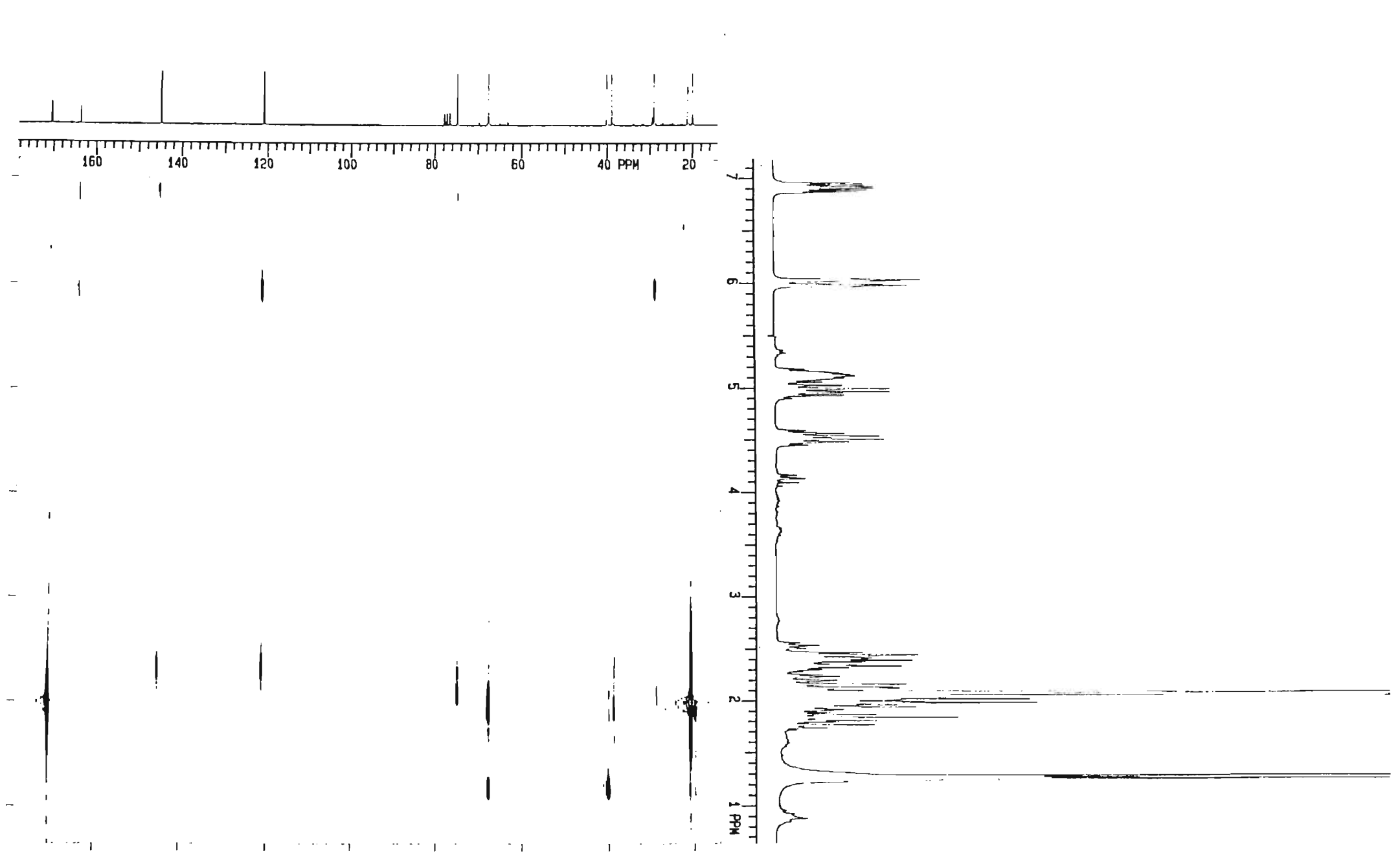
87d ^{1}H-^{1}H COSY spectrum of cryptocaryolide A in $CDCl_3$



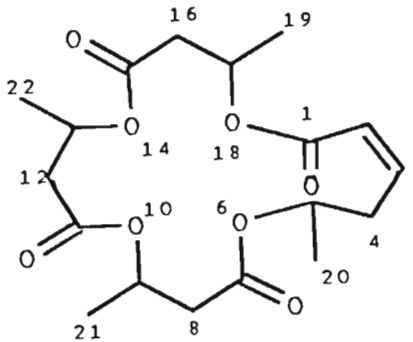
^{1}H-^{1}H RELAYED COSY spectrum of cryptocaryolide A in $CDCl_3$



7f ^{1}H-^{13}C HETCOR spectrum of cryptocaryolide A in $CDCl_3$

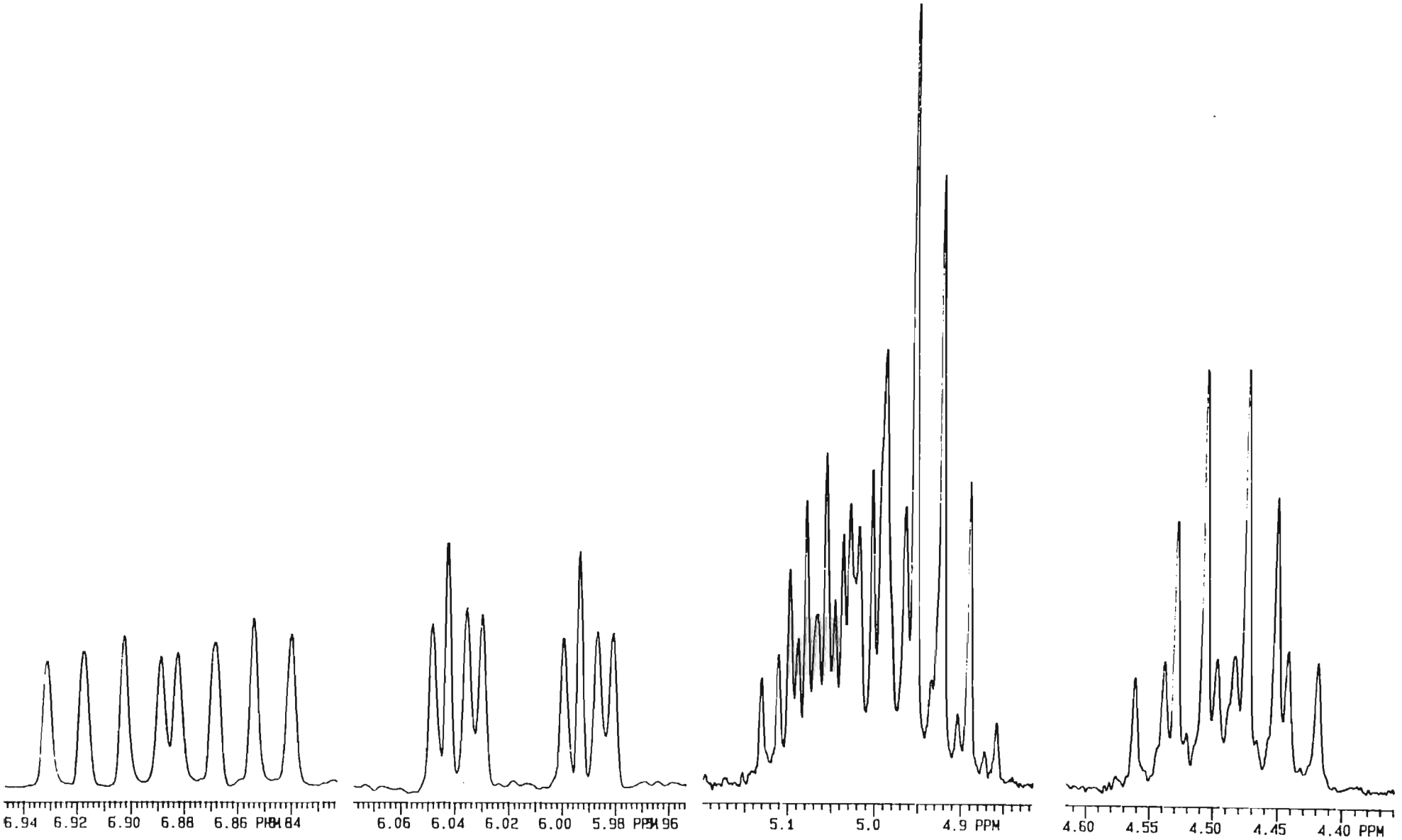


7g ^{1}H-^{13}C HETCOR spectrum of cryptocaryolide A in $CDCl_3$

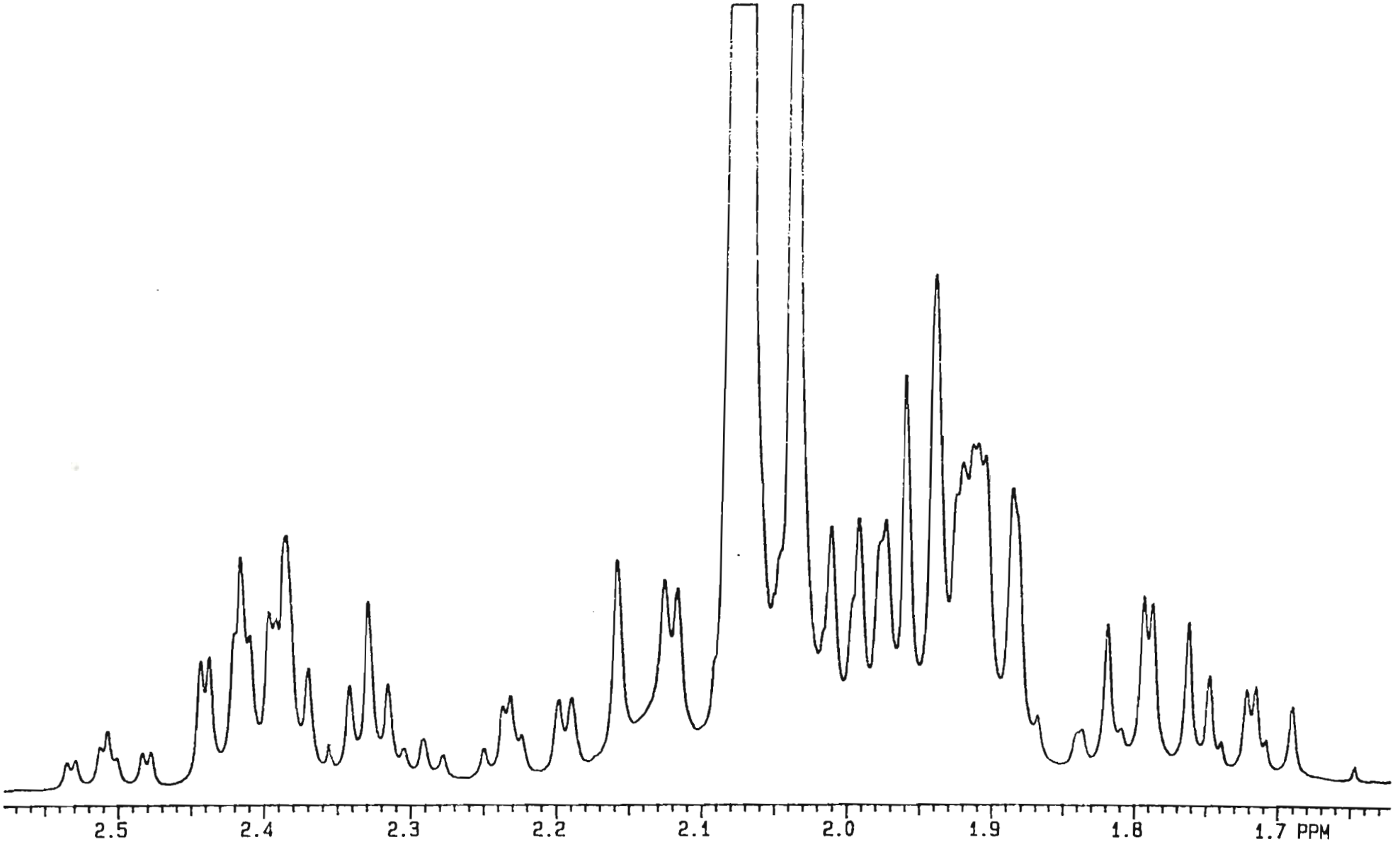


88

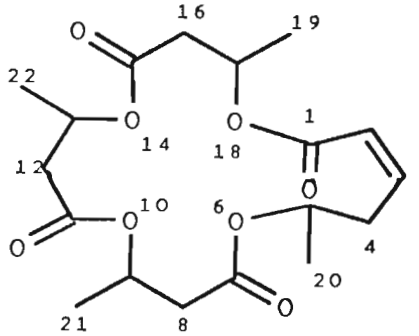
88a ^{1}H NMR spectrum of cryptocaryolide B in $CDCl_3$



88b Expanded ^{1}H NMR spectrum of cryptocaryolide B in $CDCl_3$



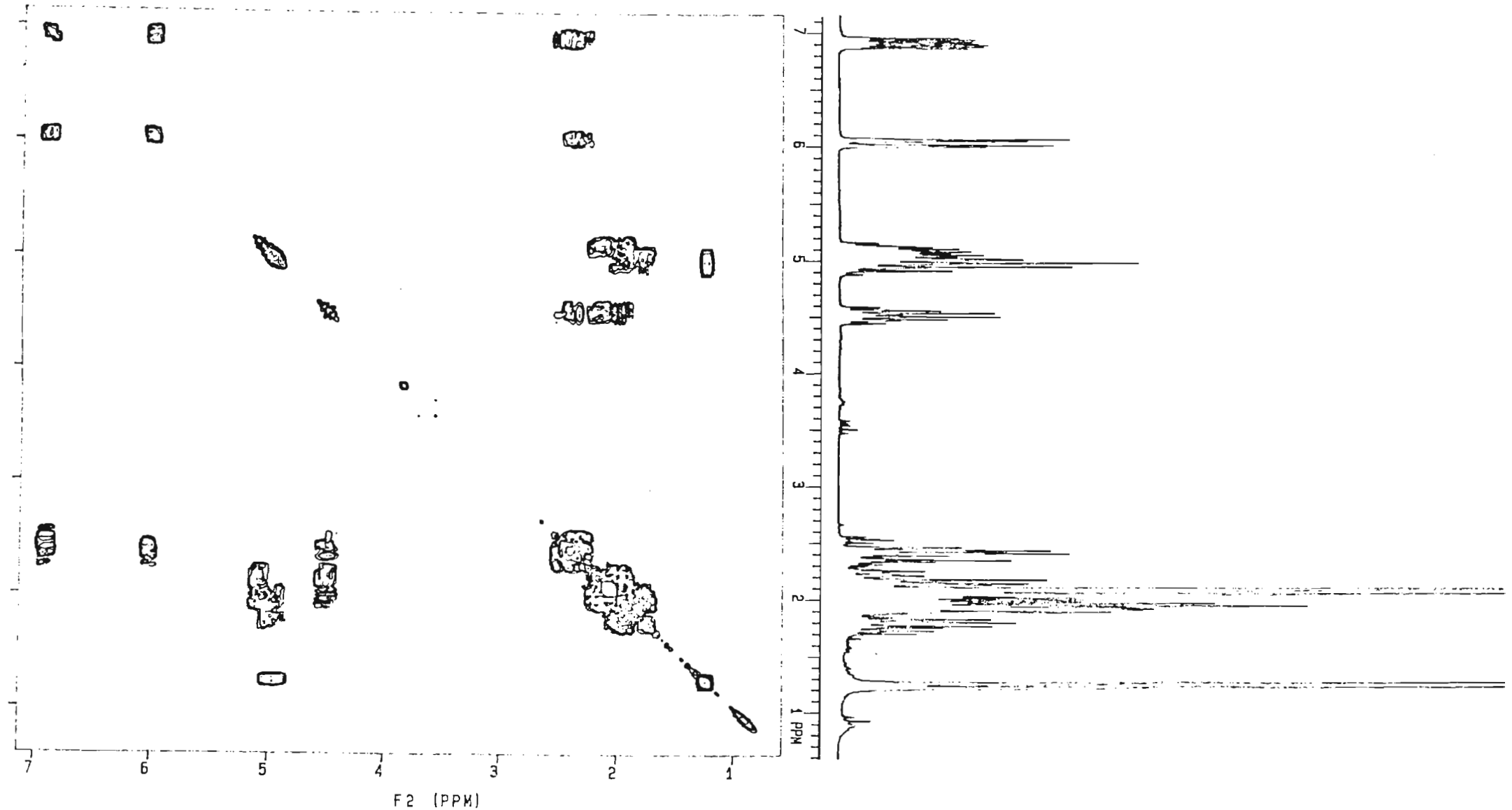
88b Expanded ^{1}H NMR spectrum of cryptocaryolide B in $CDCl_3$



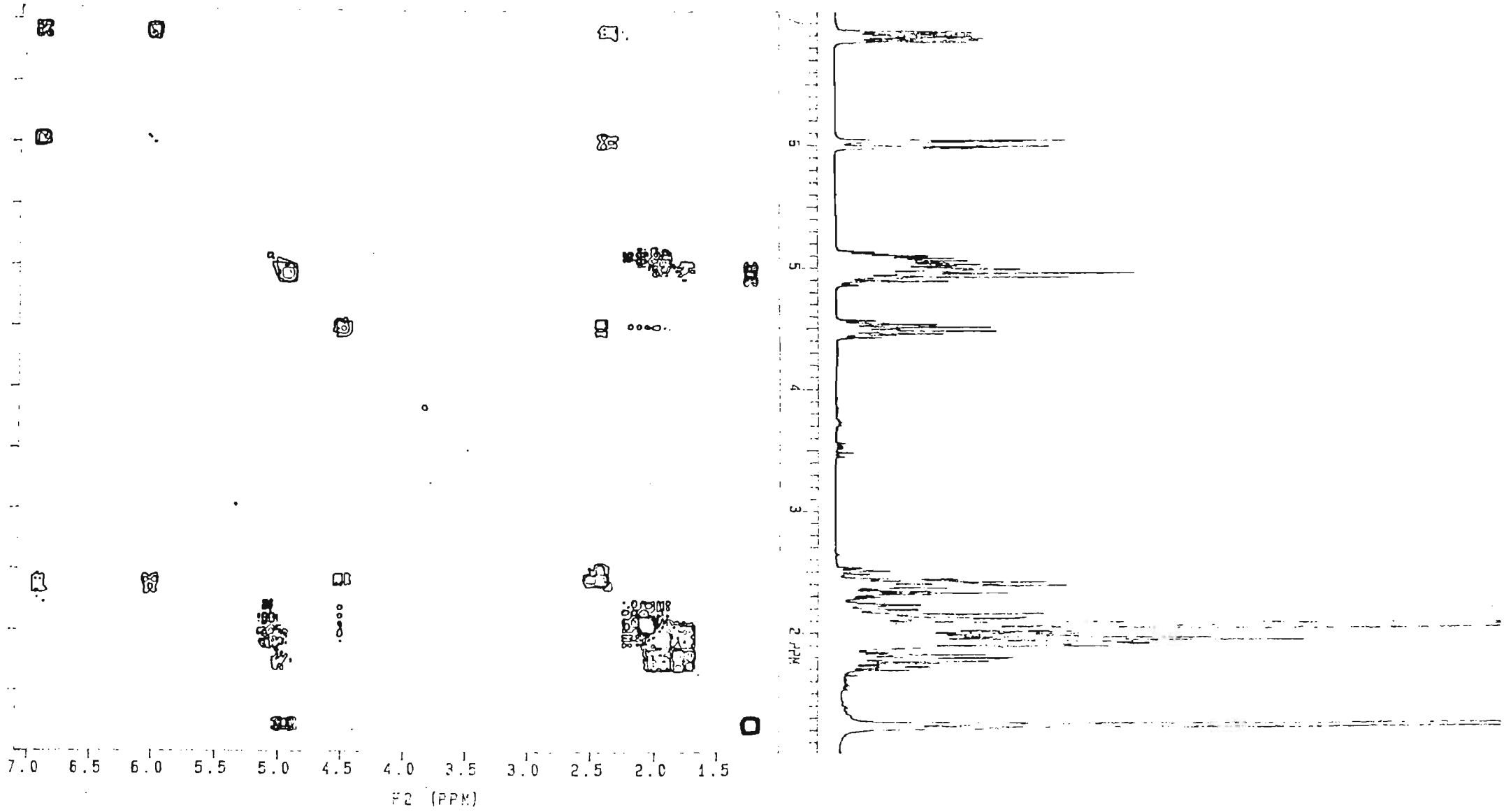
88

180 160 140 120 100 80 60 40 20 PPM 0

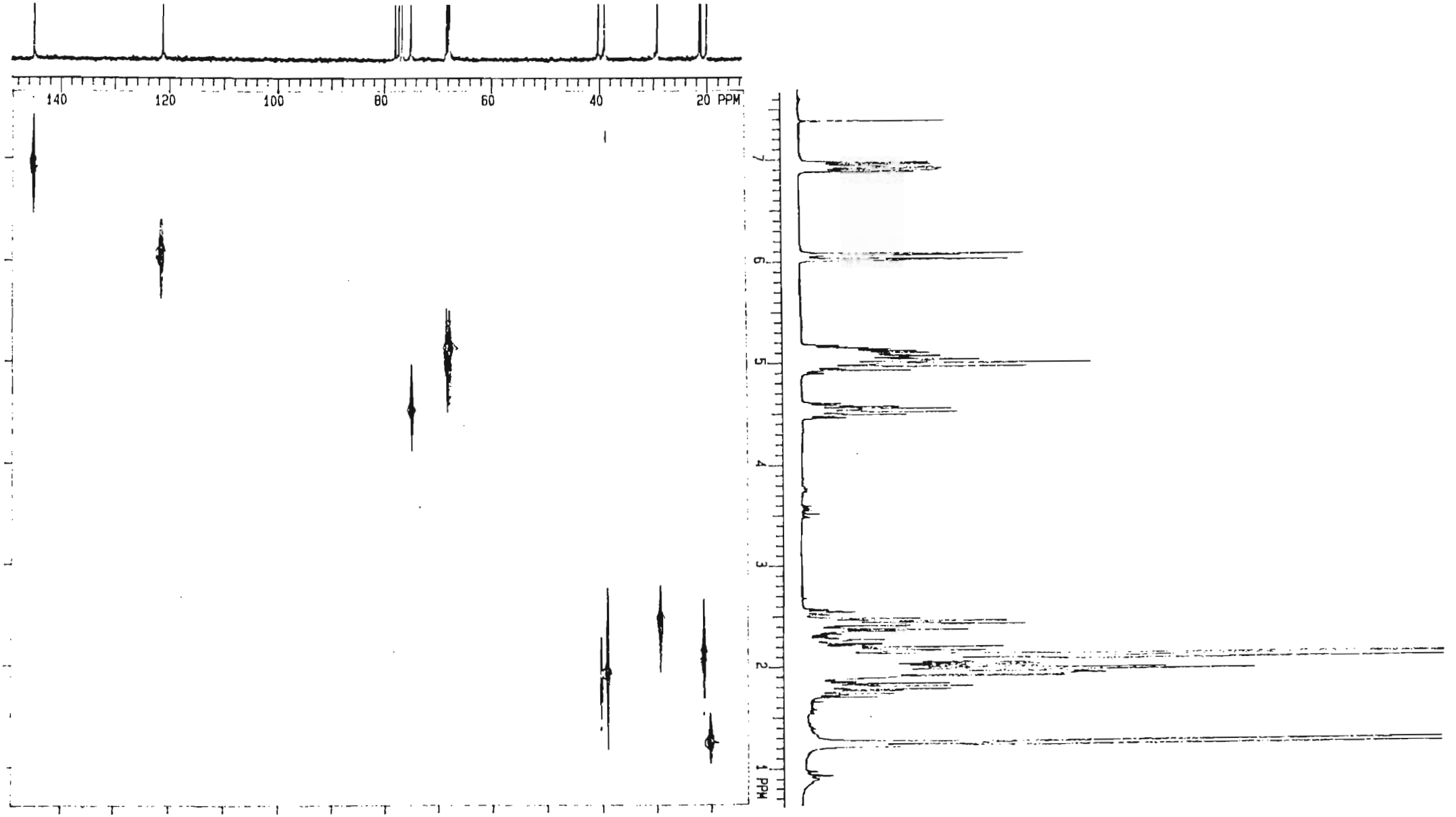
88c ^{13}C NMR spectrum of cryptocaryolide B in $CDCl_3$



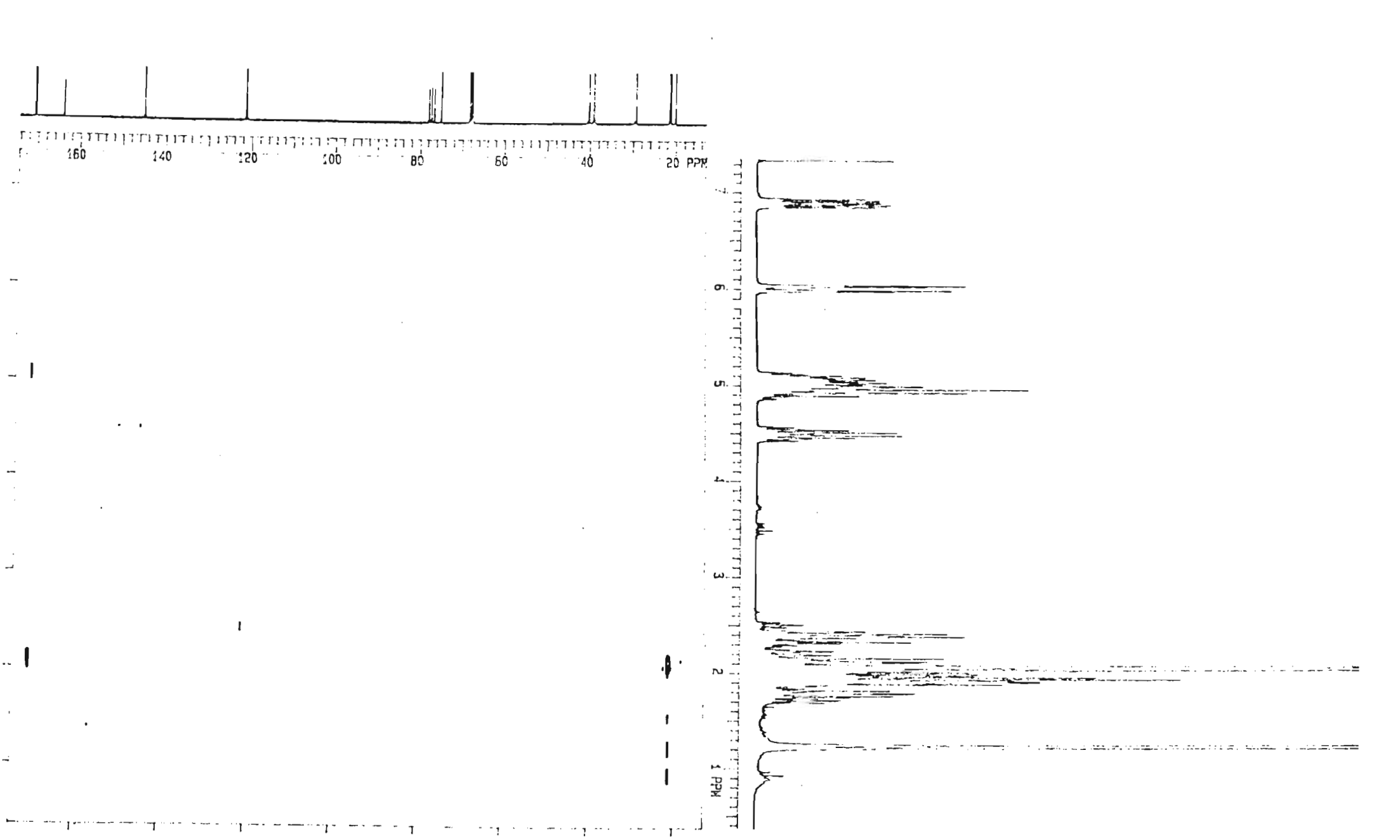
88d ^{1}H-^{1}H COSY spectrum of cryptocaryolide B in $CDCl_3$



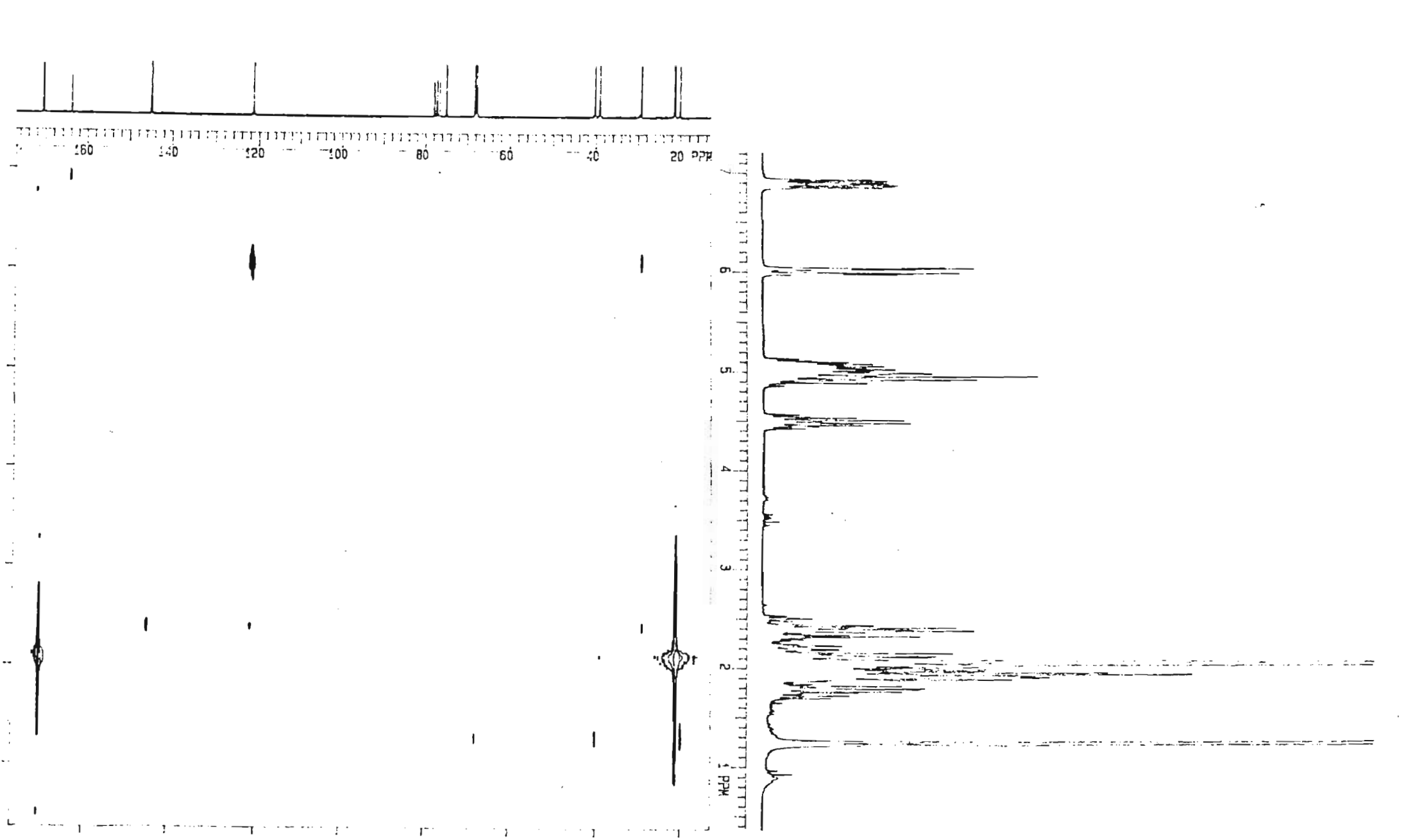
^{1}H-^{1}H DELAYED COSY spectrum of cryptocaryolide B in $CDCl_3$



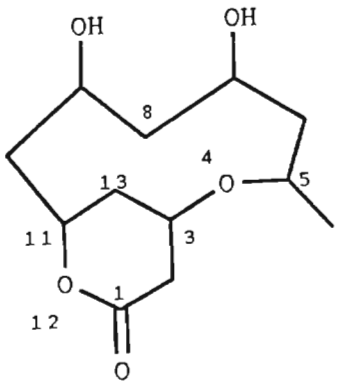
^{13}C HETCOR spectrum of cryptocaryolide B in $CDCl_3$



^{1}H-^{13}C DELAYED HETCOR spectrum of cryptocaryolide B in

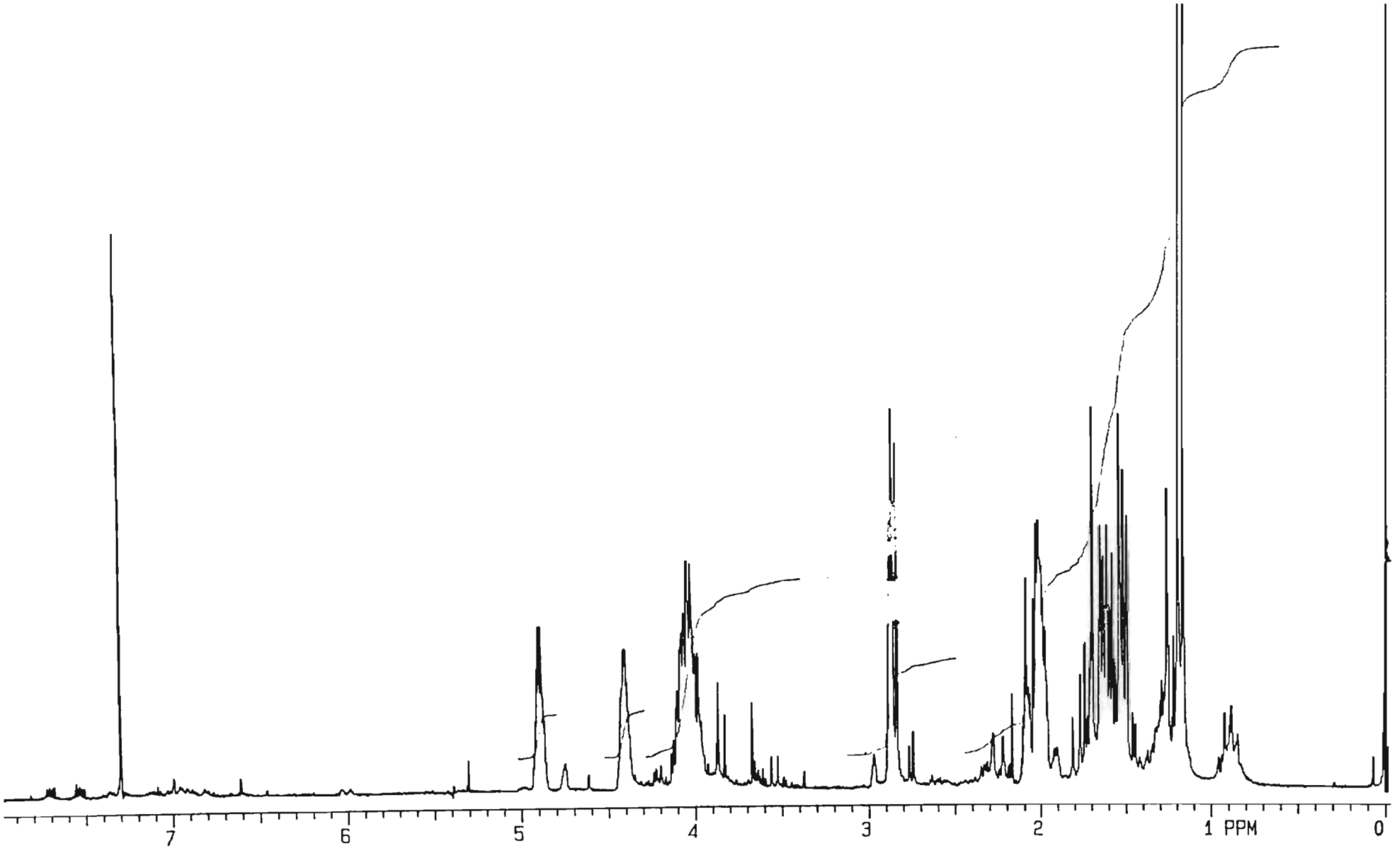


^{1}H-^{13}C DELAYED HETCOR spectrum of cryptocaryolide B in $CDCl_3$ (7Hz)

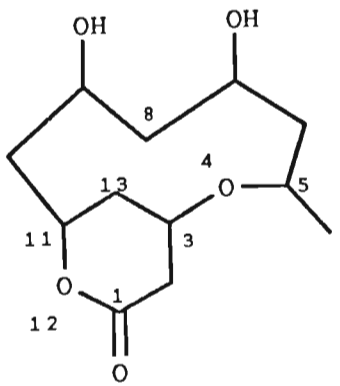


90

90a ^{1}H NMR spectrum of foliolide A in $CDCl_3$

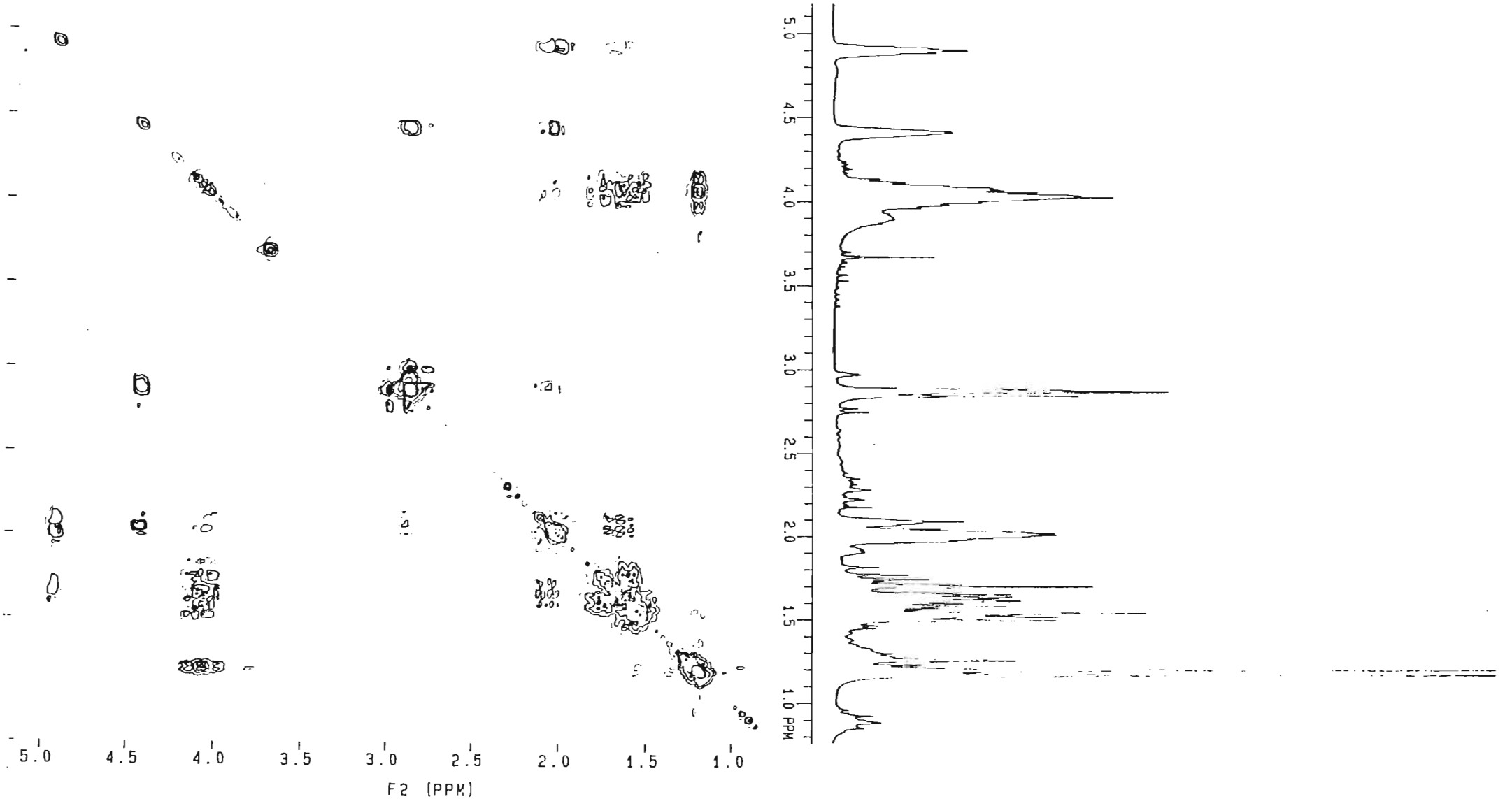


90a ^{1}H NMR spectrum of foliolide A in $D_2O/CDCl_3$

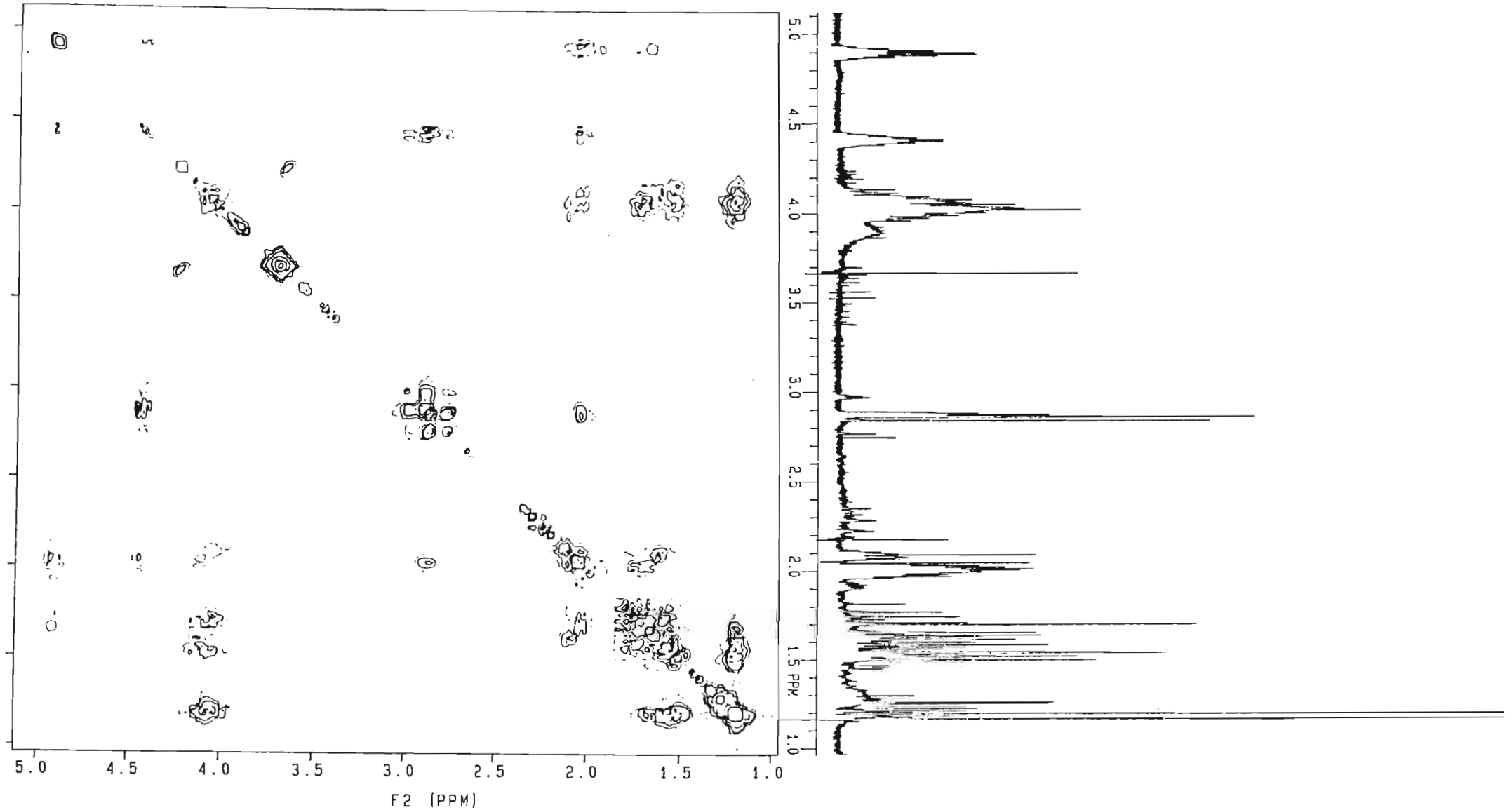


90

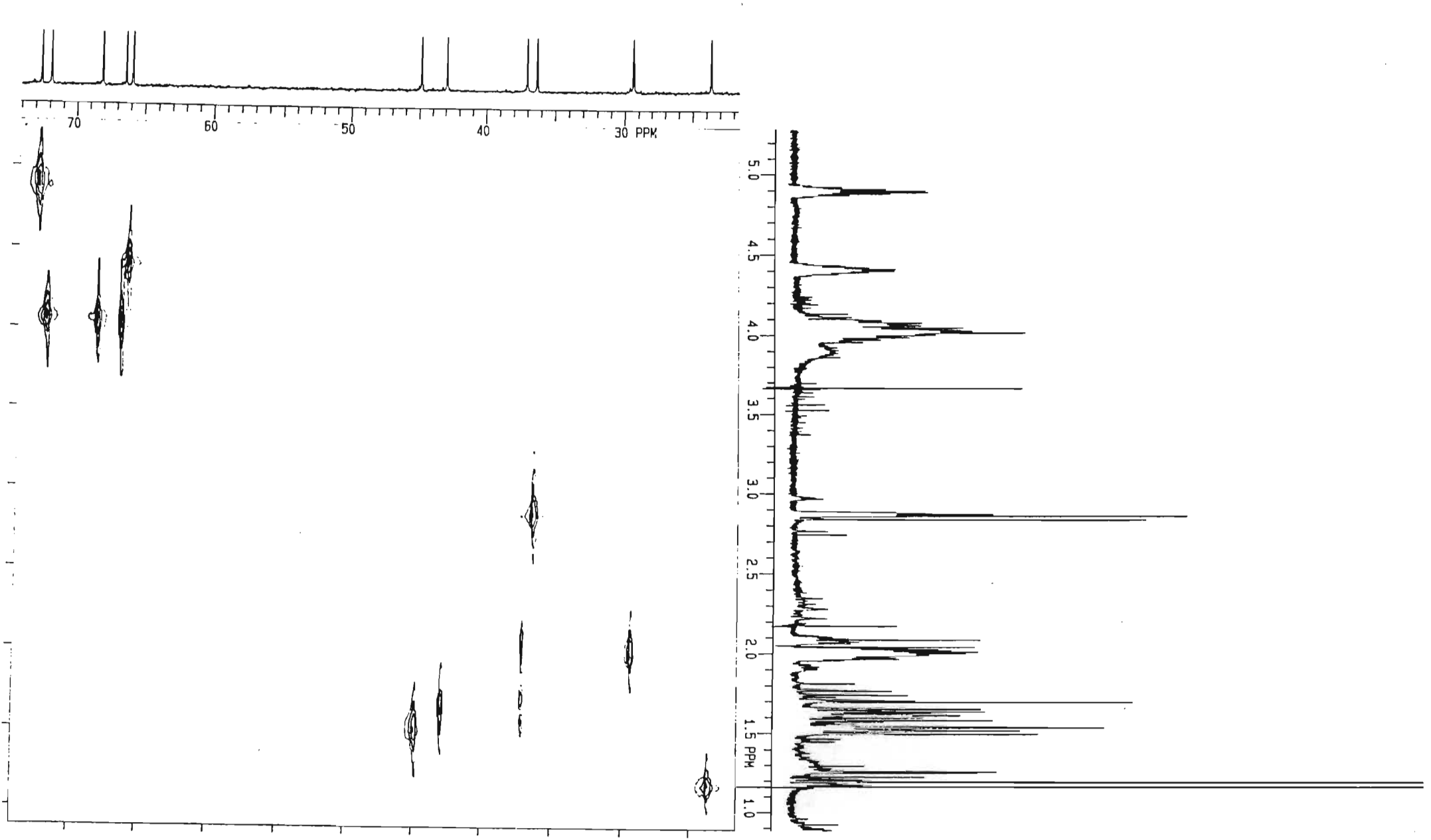
90c ^{13}C NMR spectrum of foliolide A in $CDCl_3$



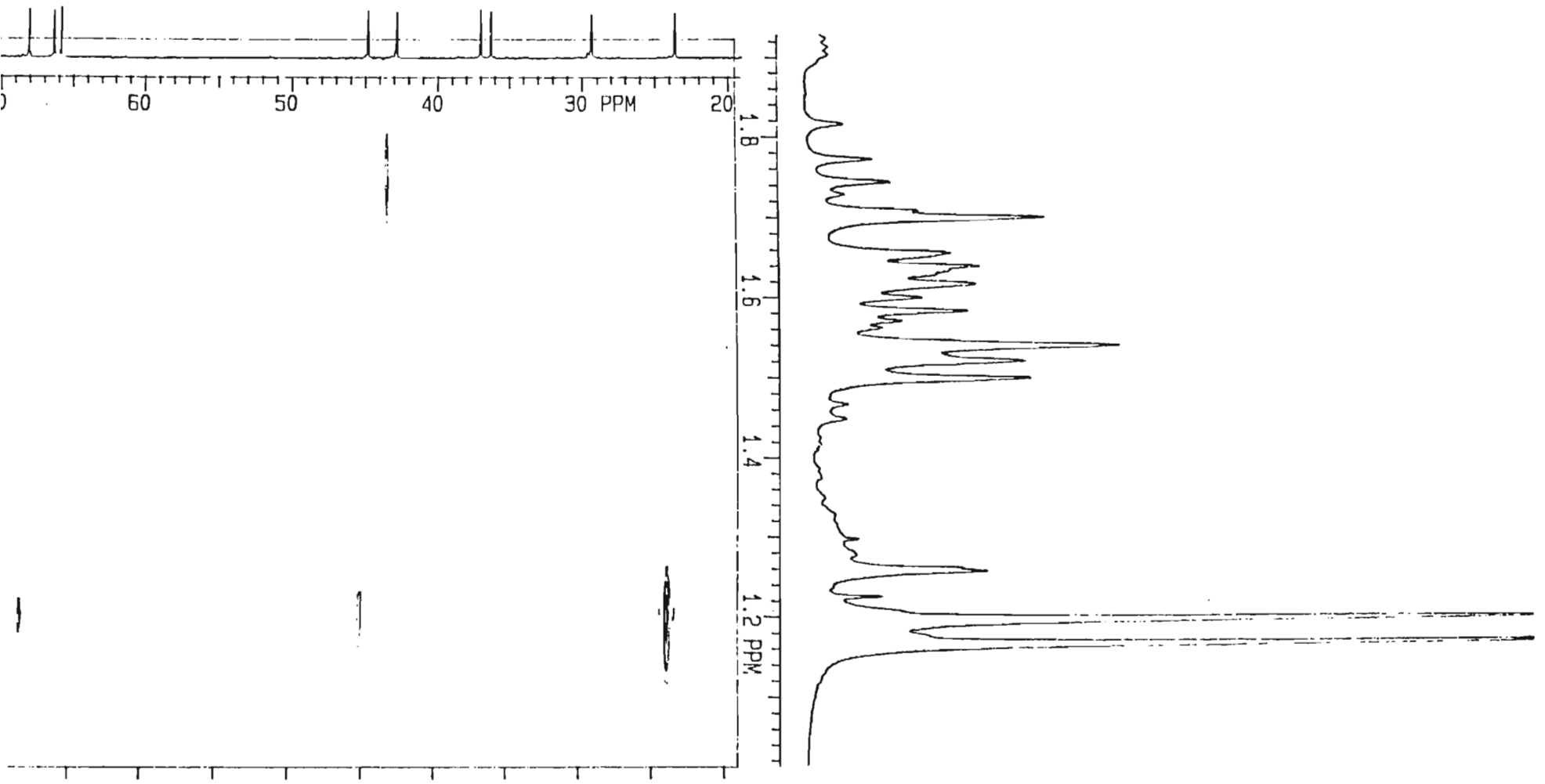
90d ^{1}H-^{1}H COSY spectrum of foliolide A in $CDCl_3$



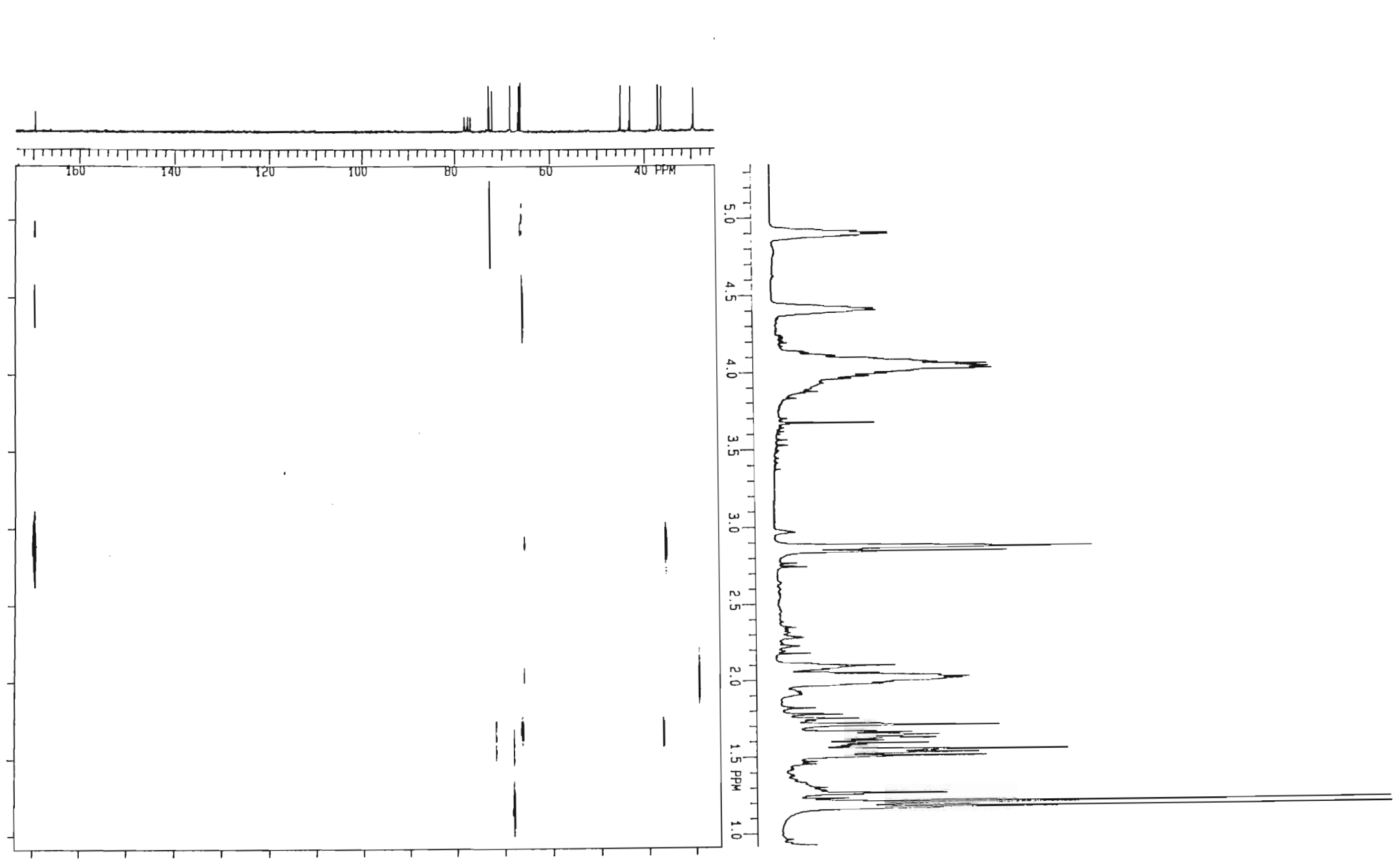
90e ^{1}H-^{1}H RELAYED COSY spectrum of foliolide A in $CDCl_3$



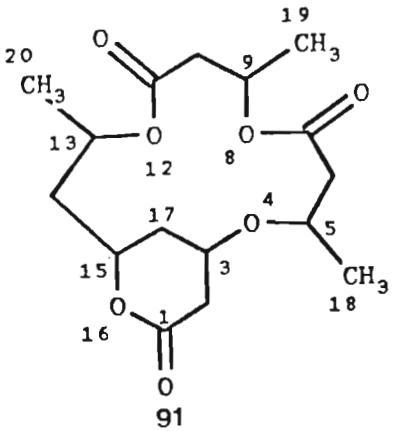
90f ^{1}H-^{13}C HETCOR spectrum of foliolide A in $CDCl_3$



0g ^{1}H-^{13}C DELAYED HETCOR spectrum of foliolide A in $CDCl_3$ (5Hz)

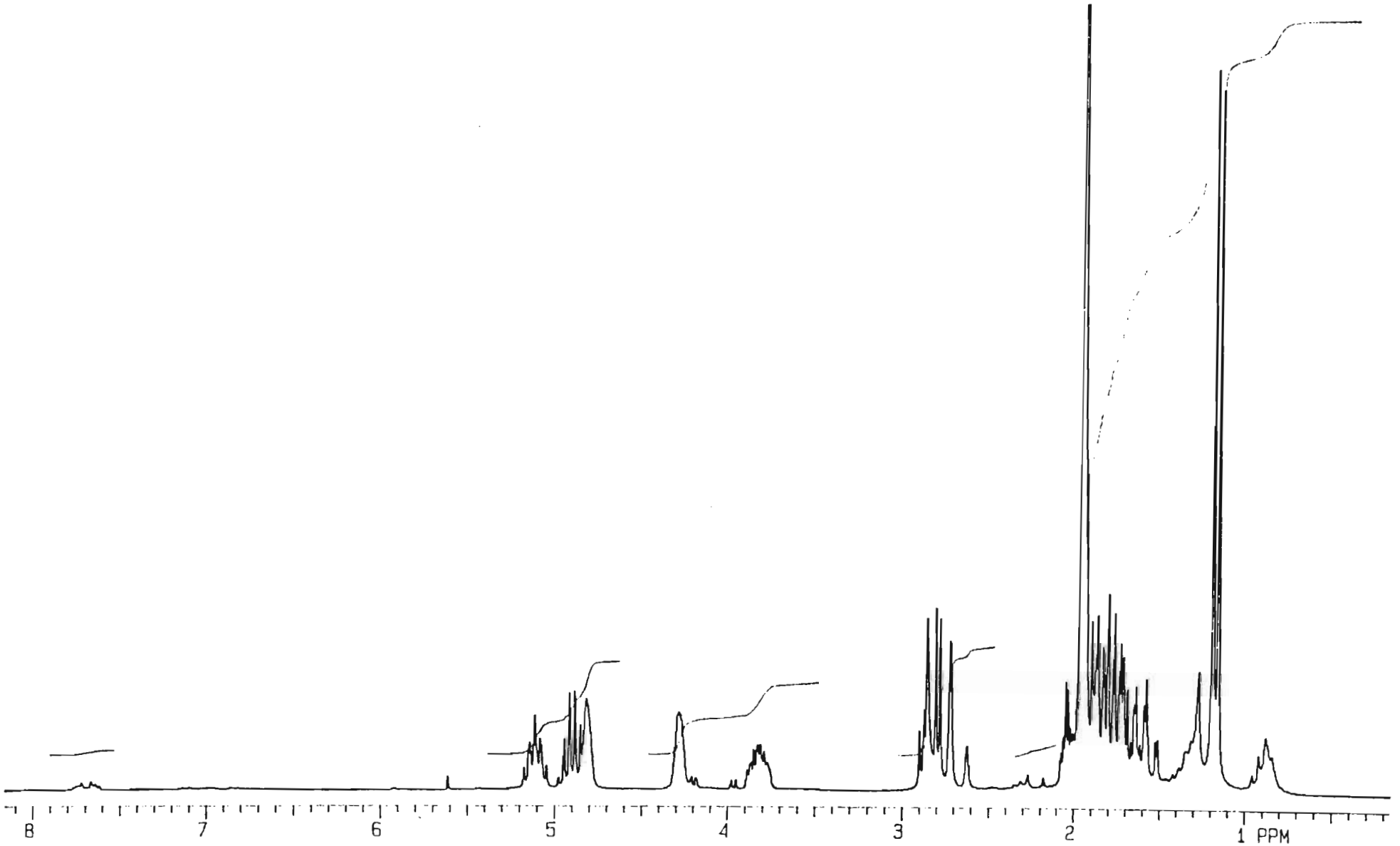


90q ^{1}H-^{13}C DELAYED HETCOR spectrum of foliolide A in $CDCl_3$ (12Hz)

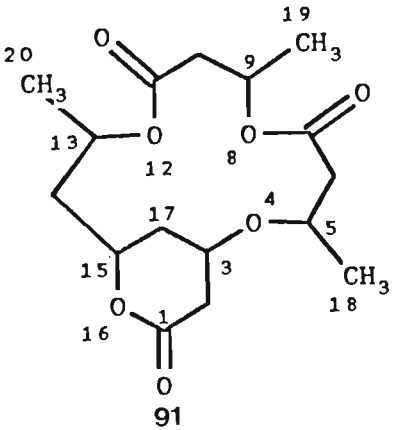


91

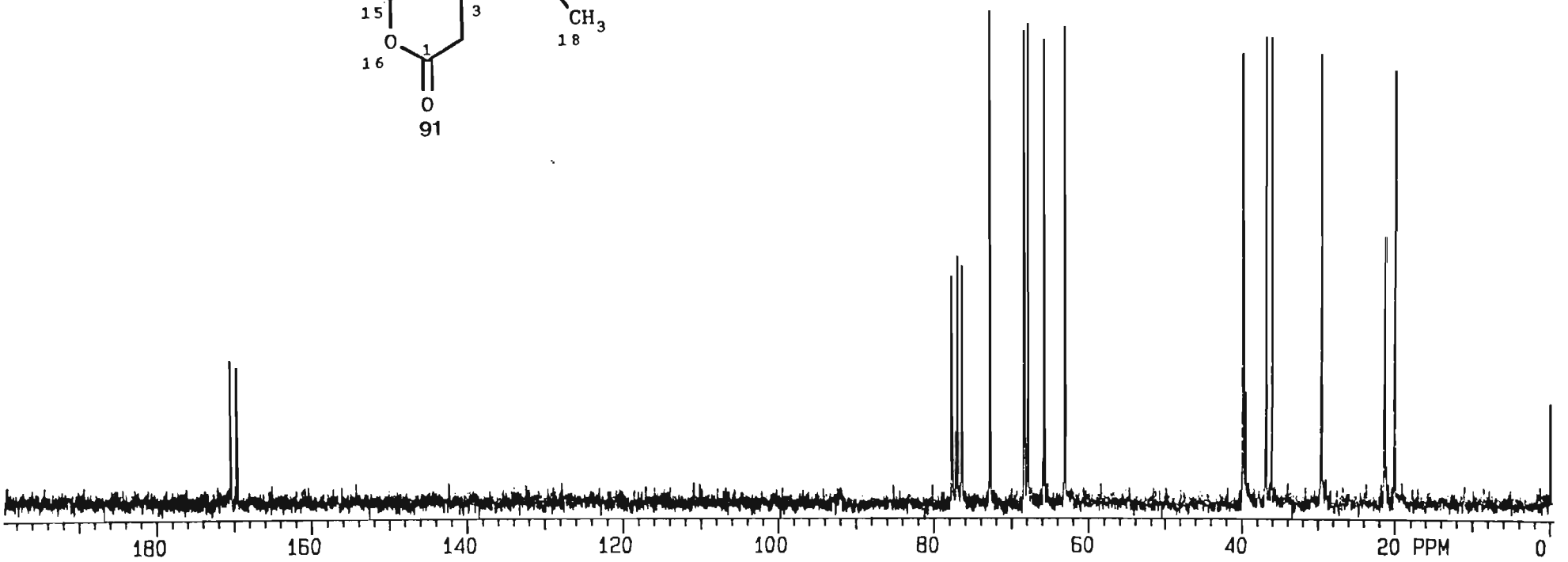
91a ^{1}H NMR spectrum of foliolide B in $CDCl_3$



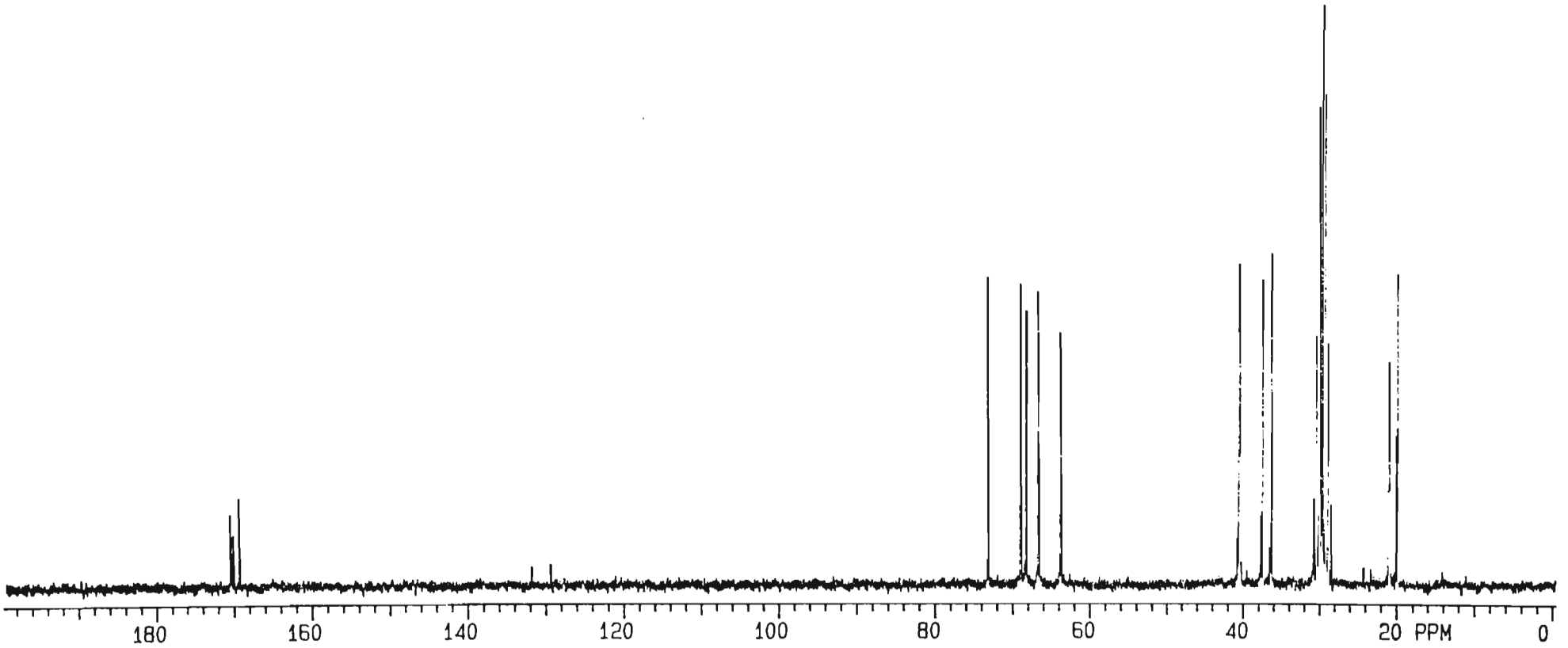
91a ^{1}H NMR spectrum of foliolide B in Acetone-d_6



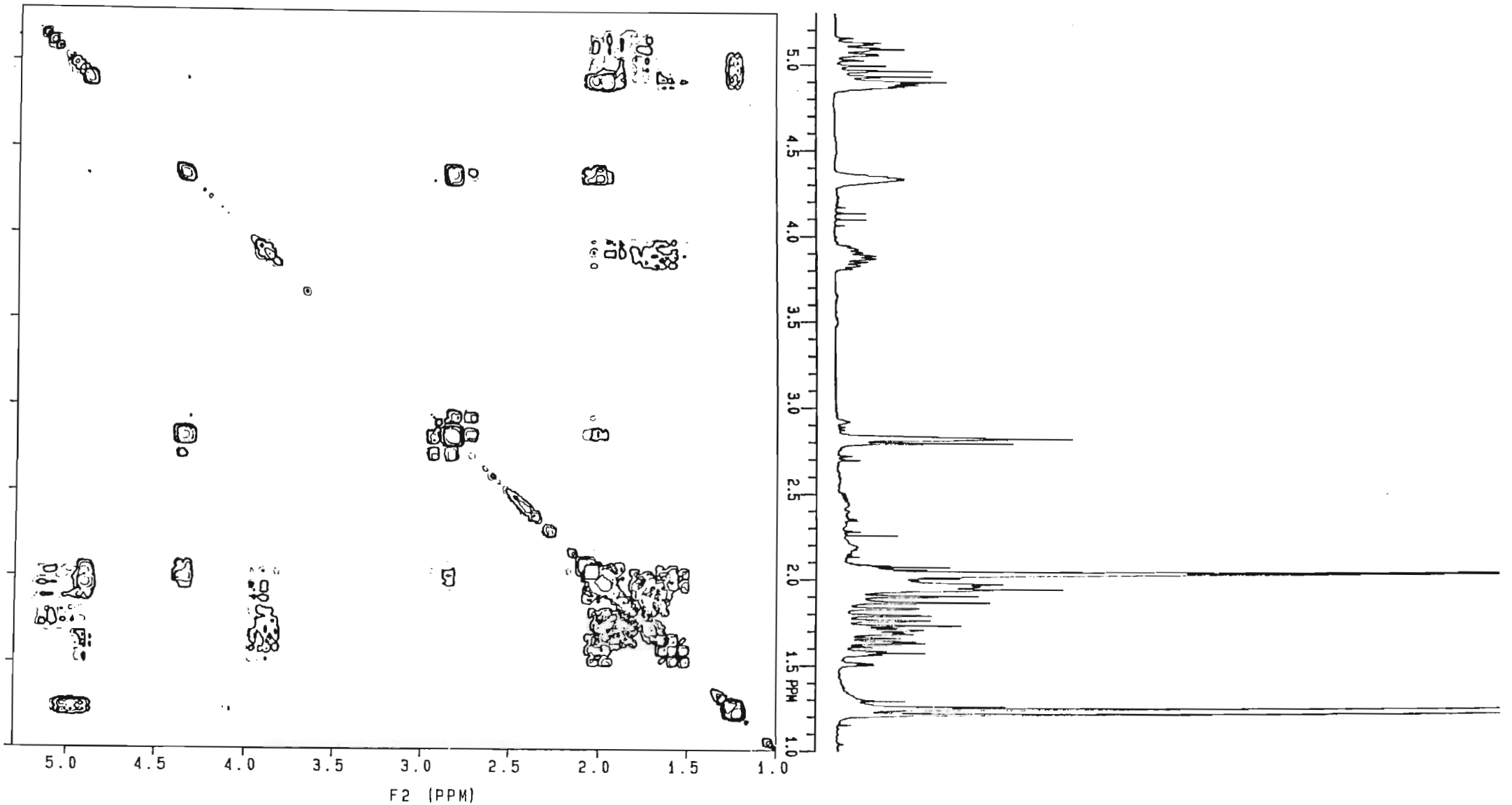
91



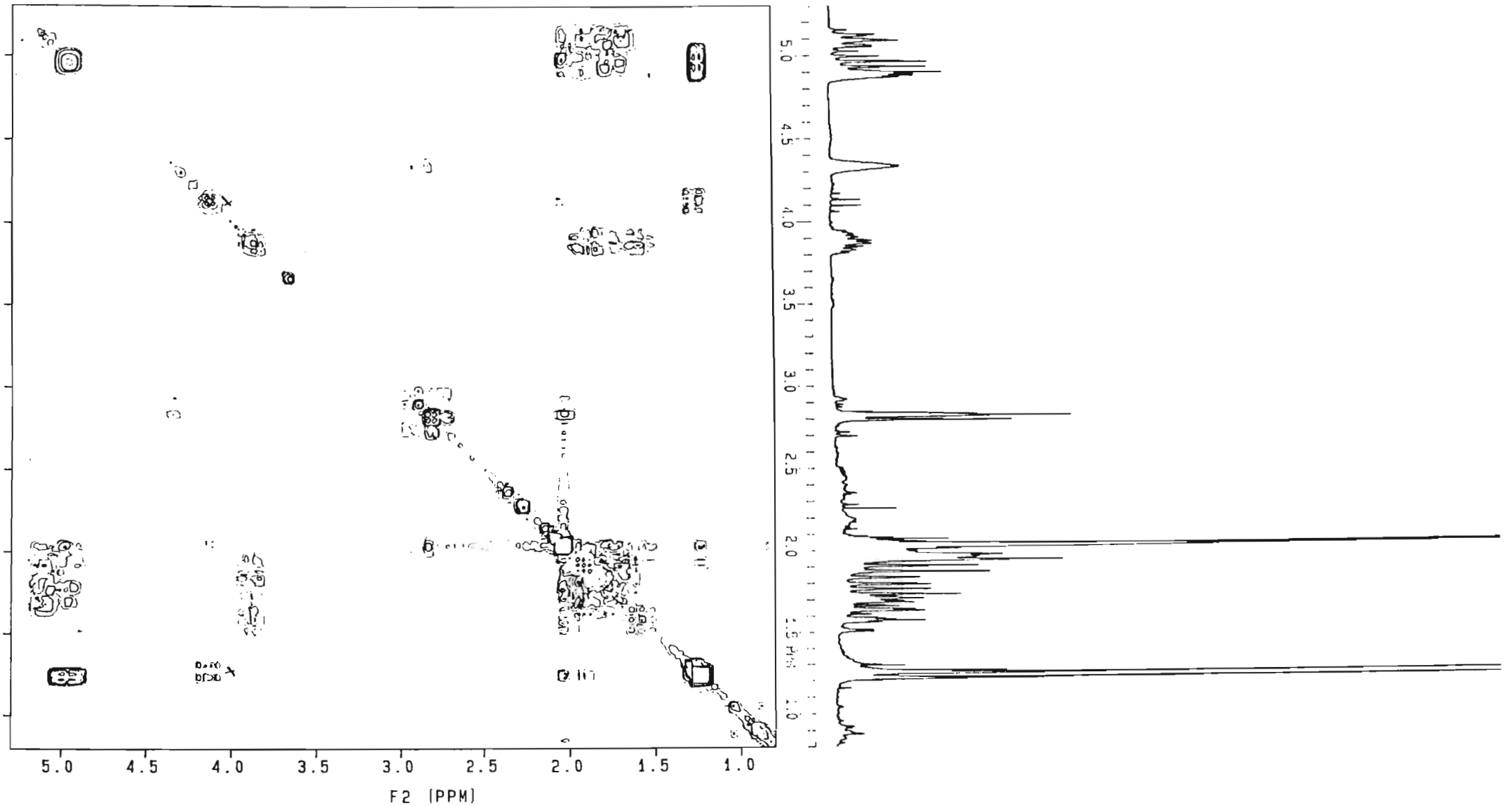
91c ^{13}C NMR spectrum of foliolide B in $CDCl_3$



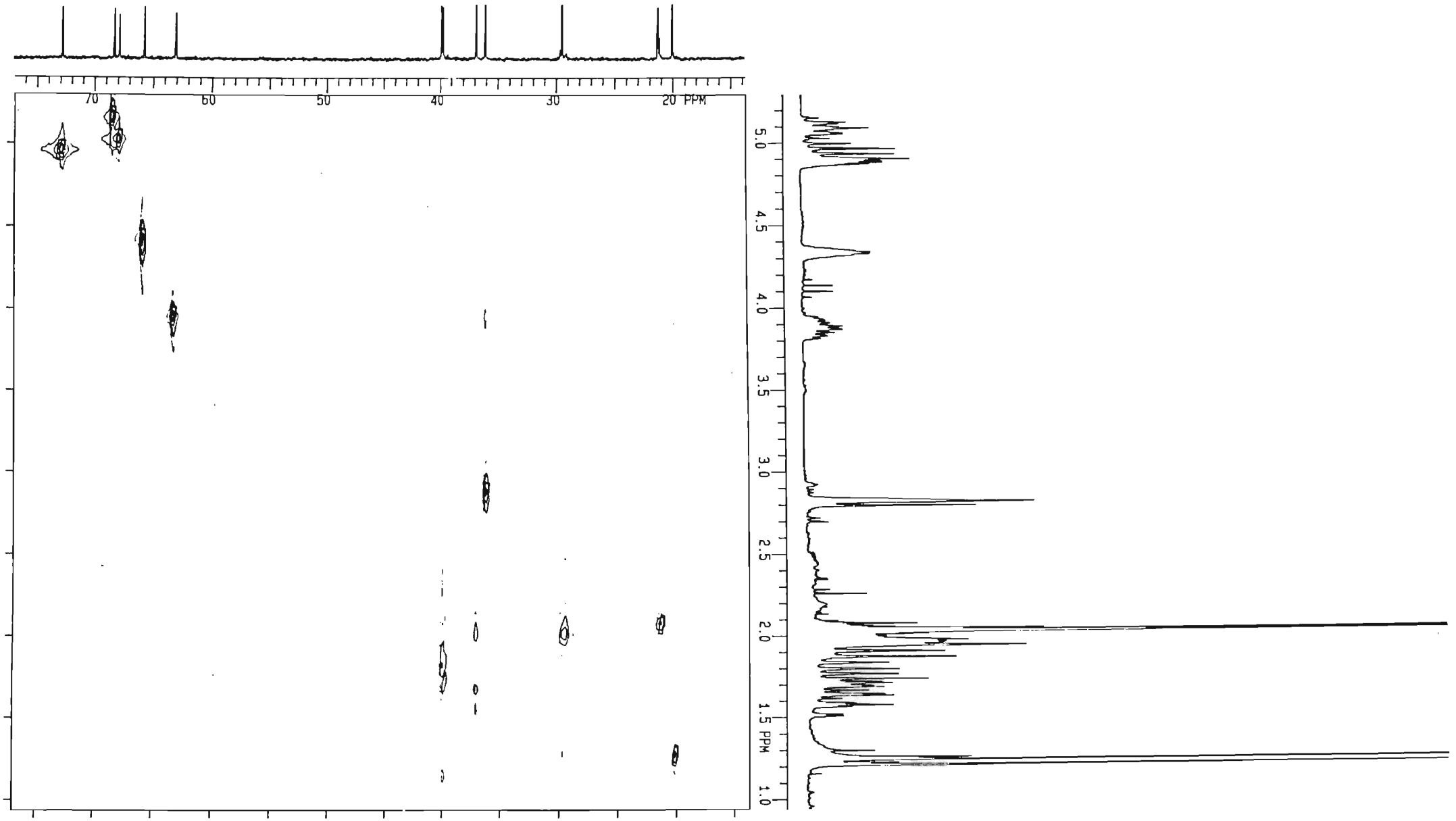
91c ^{13}C NMR spectrum of foliolide B in Acetone-d_6



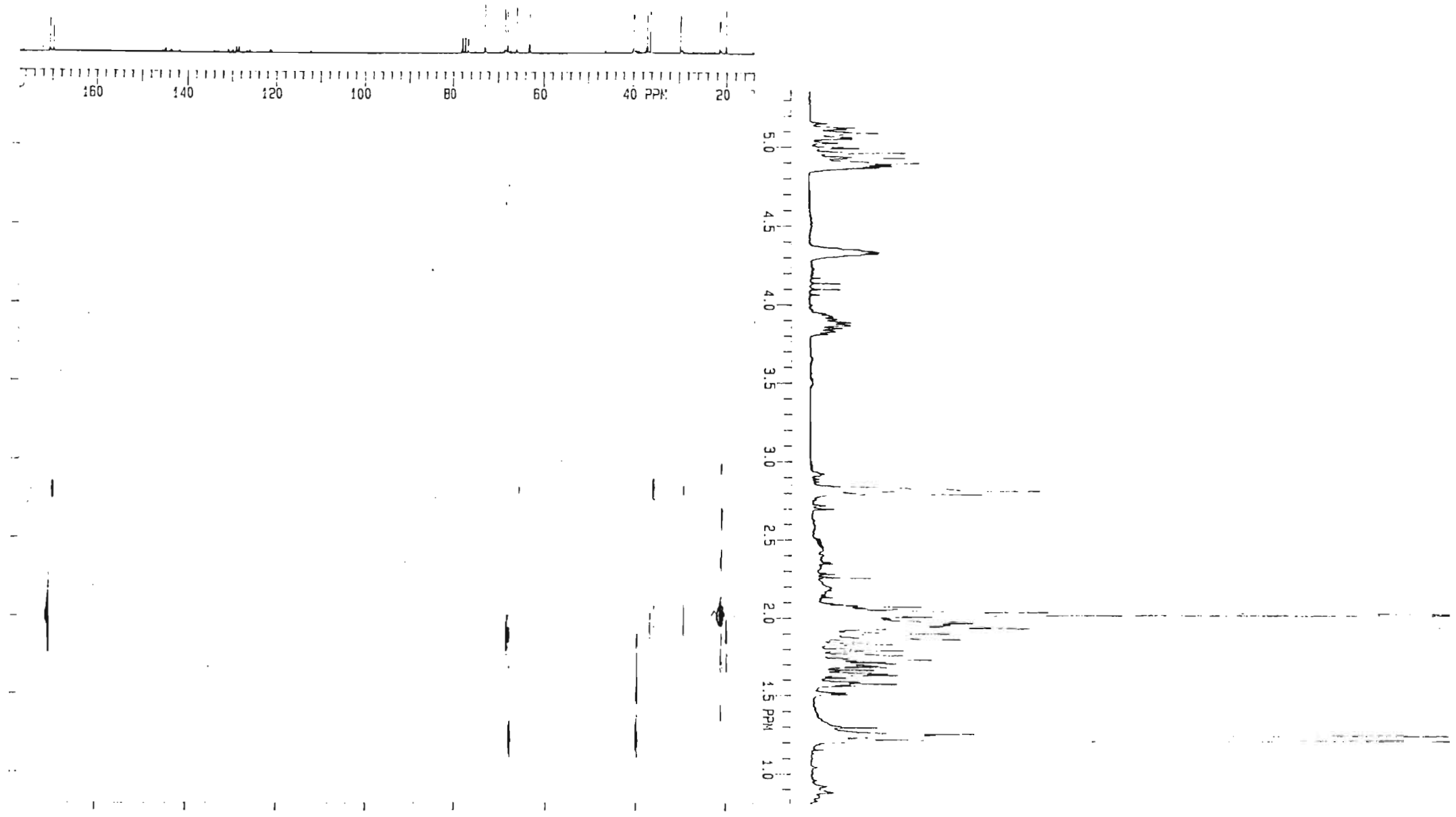
91d ^{1}H-^{1}H COSY spectrum of foliolide B in $CDCl_3$



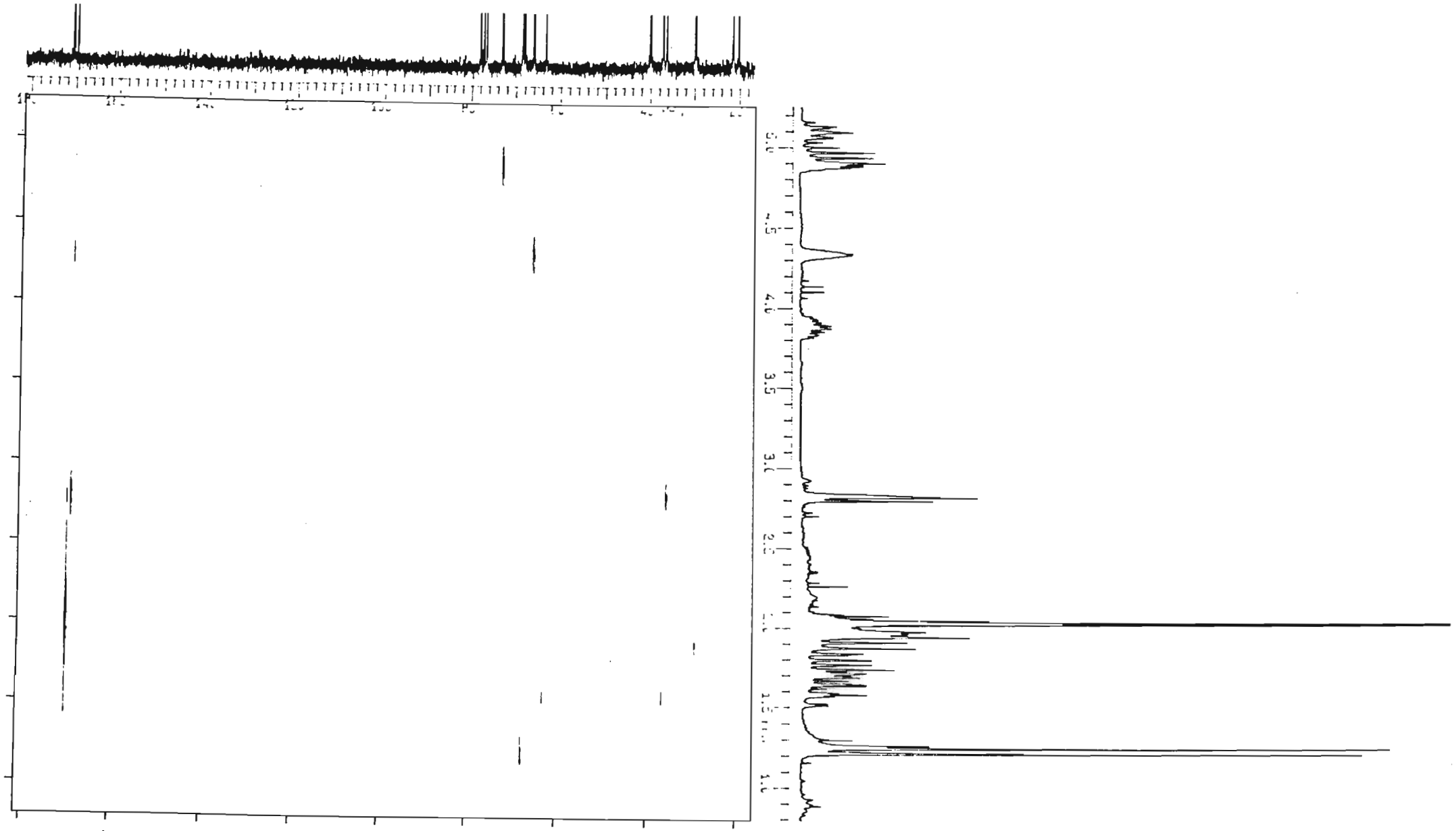
91e ^{1}H-^{1}H DELAYED COSY spectrum of foliolide B in $CDCl_3$



91f ^{1}H-^{13}C HETCOR spectrum of foliolide B in $CDCl_3$



91g ^{1}H-^{13}C DELAYED HETCOR spectrum of foliolide B in $CDCl_3$ (7Hz)



91g ^{1}H-^{13}C DELAYED HETCOR spectrum of foliolide B in $CDCl_3$ (12Hz)