STRUCTURE AND STEREOCHEMISTRY OF COMPOUNDS FROM CASSIPOUREA SPECIES

by

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of the requirements for the degree of

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Department of Chemistry
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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: .....

C. W. TAYLOR

I hereby certify that this statement is correct.

Signed: .....

PROFESSOR S. E. DREWES

SUPERVISOR

Department of Chemistry
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Pietermaritzburg

DECEMBER 1993

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The Foundation for Research Development and the University of Natal for financial assistance.

SUMMARY

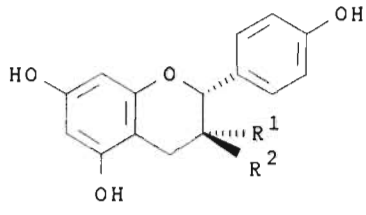
The ethanolic extract from the stem bark of *Cassipourea gerrardii* (RHIZOPHORACEAE) yielded a phenolic fraction, the components of which were extremely intractable.

Using a combination of chromatographic techniques a series of compounds were isolated and include the known compounds afzelechin (**i**), epiafzelechin (**ii**), and epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**iii**). The only previous isolation of the latter compound was from *Ephedra spp.* and this compound is reported to possess hypotensive properties. Three new compounds were also isolated and identified as afzelechin-3-O-α-L-rhamnopyranoside (**iv**), epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**v**) and epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**vi**). The latter compound is the only known example of a trimeric A-type propelargonidin.

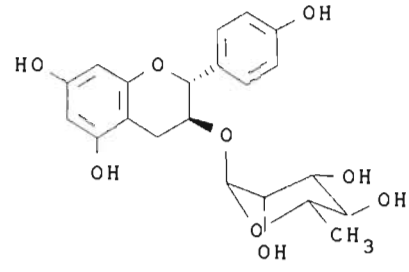
A chemical investigation of the phenolic constituents in the stem bark of *C. gummiflua* has led to the re-isolation of compounds (**i**)-(**v**), the isolation of the biogenetically closely related flavonol, kaempferol-3-O-α-L-rhamnopyranoside (**vii**) and two new A-type proanthocyanidins identified as 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**viii**) and 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**ix**). The latter two compounds are the only examples of naturally occurring O-methylated A-type proanthocyanidins.

A similar investigation into the phenolic constituents in the stem bark of *C. mossambicensis* has led to the isolation of a rarely occurring O-methylated flavan-3-ol identified as 4'-OMe-epigallocatechin (**x**). The isolation of this compound has hitherto been restricted to species in the Celastracea and is accompanied in the stem bark of *C. mossambicensis* by compounds (**i**)-(**vi**).

The structures have been established on the basis of spectroscopic and chemical evidence.



(i) R^1=H; R^2=OH
(ii) R^1=OH; R^2=H



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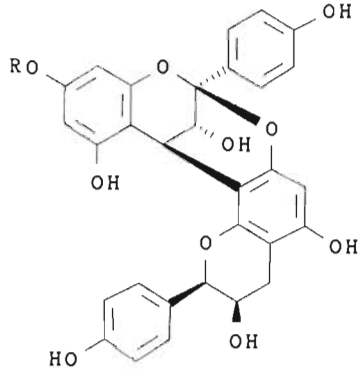
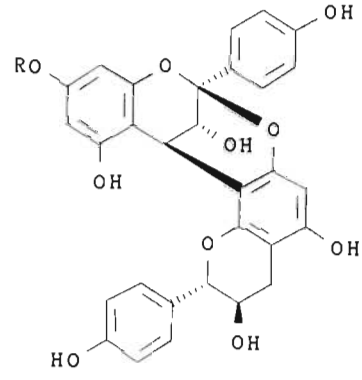
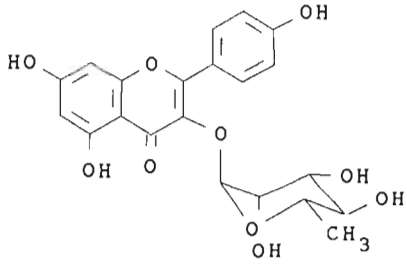


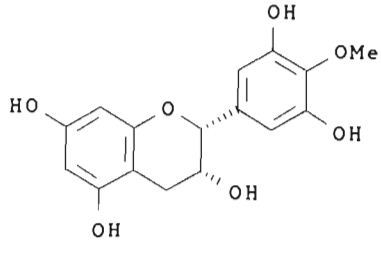
(iii) R=H
(ix) R=OMe



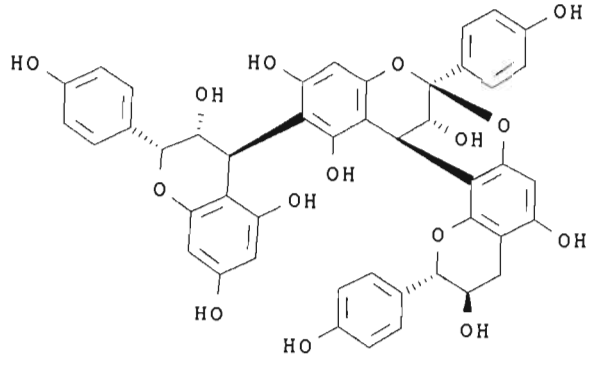
(v) R=H
(viii) R=OMe



(vii)



(x)



(vi)

LIST OF PUBLICATIONS

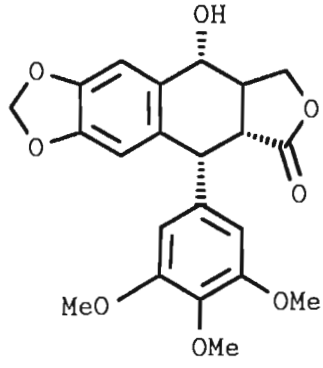
(i) Drewes, S.E., Taylor, C.W. and Balfour-Cunningham, A., (1992) (+)-Afzelechin 3-rhamnoside from *Cassipourea gerrardii*. *Phytochemistry* **31**, 1073.

(ii) Drewes, S.E., Taylor, C.W., Balfour-Cunningham, A., Ferreira, D., Steenkamp. J.A. and Mouton, C.H.L. (1992) Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin from *Cassipourea gerrardii*. *Phytochemistry* **31**, 2491.

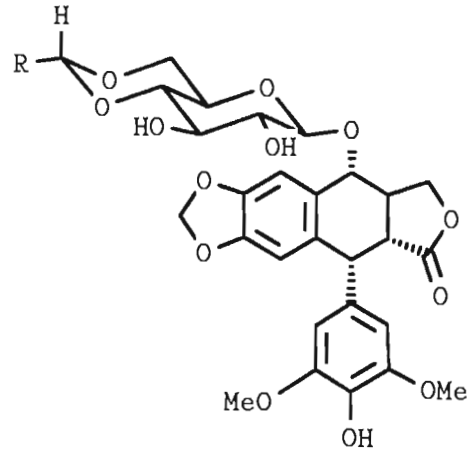
1. INTRODUCTION

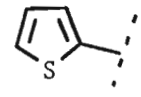
1.1. BACKGROUND

The bewildering variety of chemical compounds produced by nature has awakened the chemists' enthusiasm and curiosity, particularly in his search for biologically-active compounds. A large number of these natural products has emerged from the scientific study of remedies traditionally employed by various cultures. Atropine, quinine, digoxin, colchicine and khellin are a few well known examples.



(1)

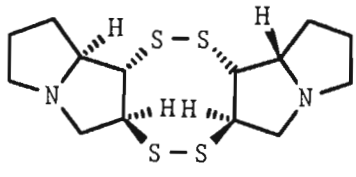


	R
(2)	Me
(3)	

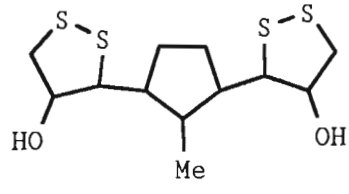
In cases where the biological activity is too low, or the toxicity too high to be of practical use, such compounds may serve as prototype molecules or templates for the development of new bio-active compounds. A good example of this strategy is provided by the aryltetralin lignan podophyllotoxin (**1**) extracted from the rhizomes of *Podophyllum peltatum* and *P. emodii*[1]. Although this compound showed anti-tumour activity in rodents, severe non-specific side effects and no significant response was observed in humans[2]. Systematic

chemical modification of the aryltetralin yielded a series of compounds with promising activity *in vitro* and *in vivo*. Two of these, etoposide (**2**) and teniposide (**3**) have been developed as anticancer drugs and are at present in clinical use.

Cassipourea gerrardii (Rhizophoraceae), commonly referred to as the bastard onion wood on account of its smell, is a small indigenous canopy tree[3]. The bark from this species is extensively sold in herbal medicine shops where it is known as "memzi obomvu" because of its orange-red colour. Previous work on the extractives of *Cassipourea* species by Warren and his associates[4,5] resulted in the isolation of the novel sulphur-containing alkaloids, cassipourine (**4**) from *C. gummiflua*[4] and gerrardine (**5**) from *C. gerrardii*[5].



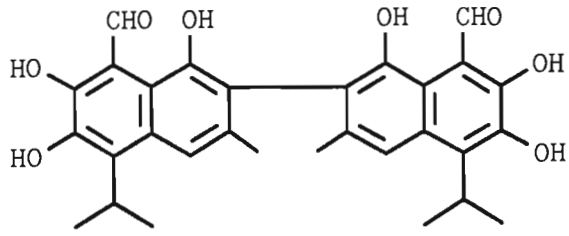
(4)



(5)

The present study was undertaken as part of an ongoing program within this Department aimed at isolating biologically-active compounds from indigenous medicinal plants. The initial objectives were to re-investigate the chemical constituents in the stem bark of *C. gerrardii*, with emphasis on the isolation of phenolic components.

As ubiquitous plant constituents, phenolic compounds are present in many foods, drinks, spices and natural plant drugs. Although their biological activity is less pronounced and less specific than those of the alkaloids, nearly all phenolic compounds, including phenol itself, as well as the polyphenolic tannins possess some biological activity.



(6)

Gossypol (**6**), a polyphenolic dimeric sesquiterpene derived primarily from *Gossypium* species serves as a classic example to illustrate the potential biological activity of phenolic compounds. The contraceptive effect of gossypol was discovered through the observation of subnormal fertility in rural communities in China where crude cottonseed oil was used for preparing food. In extensive clinical tests in China on more than 8800 men, (±)-gossypol was reported to be 99.89% effective as a male contraceptive. Gossypol, apart from being a typical phenolic compound, also shows atropisomerism as a result of restricted rotation about the biaryl axis. In many ways gossypol serves as an ideal model for the polyphenols isolated in this work. Functional group reactivity, in this case phenolic, coupled with preferred conformation as a result of restricted rotation, yield compounds with unique biological properties. Thus gossypol, as the (-)-enantiomer only, possesses pronounced antifertility activity[6]. In addition, the biological activity of phenolic compounds appear, at least in part, to be derived from their ability to bind to proteins and therefore to enzymes.

From the stem bark of *C. gerrardii* a series of phenolic compounds belonging to the proanthocyanidin class of flavonoids was isolated. The combination of relatively uncommon structural features such as the substitution patterns, stereochemistry, as well as the positions of the inter-flavonoid linkages, yielded a series of compounds which were either rarely occurring or novel. From a merely academic viewpoint, these compounds provided a challenge with regard

to their isolation, and in particular, the stereochemical aspects of their structure determination.

In order to provide a better understanding of the more complex compounds discussed in this work, a brief survey of one of the largest and most important classes of phenolic compounds, the flavonoids, is given below.

1.2. THE FLAVONOIDS

The flavonoids constitute one of the most numerous and widespread groups of compounds in the plant kingdom. The chemical structures are based on a C_6-C_3-C_6 skeleton with a chroman ring bearing a second aromatic ring in position 2, 3 or 4 (**Fig. 1.**). In a few cases, the six membered heterocyclic ring occurs in an isomeric open form or is replaced by a five membered ring.

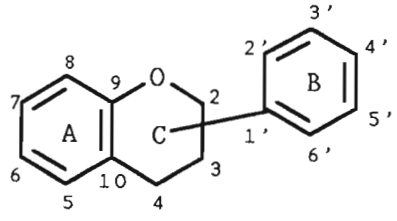
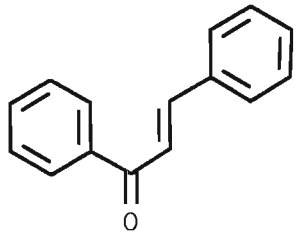
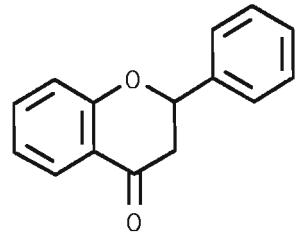
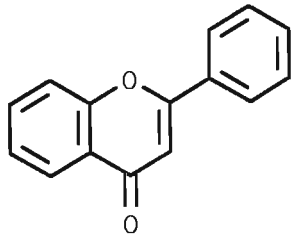


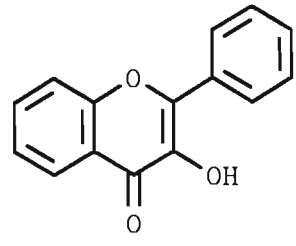
Fig. 1. Basic structure of most flavonoids

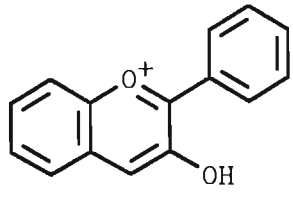
The flavonoids are divided into a number of classes based on the degree of oxidation of the C-ring and the position of the B-ring. Some of these are shown below:

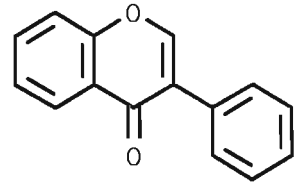
Chalcone 

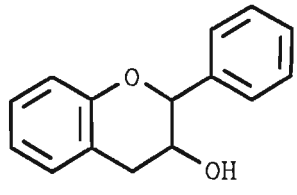
 Flavanone

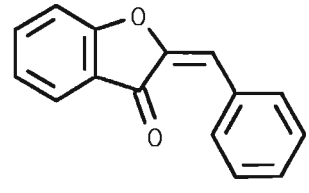
Flavone 

 Flavonol

Anthocyanidin 

 Isoflavone

Flavan-3-ol 

 Aurone

Variations in substitution patterns, stereochemistry and in the nature of the substituents, result in an extensive range of compounds within each class of flavonoid. A comprehensive list of all known flavonoids by Harborne[7] totalling over 4000 structures bears testament to the diversity of this unique class of compounds.

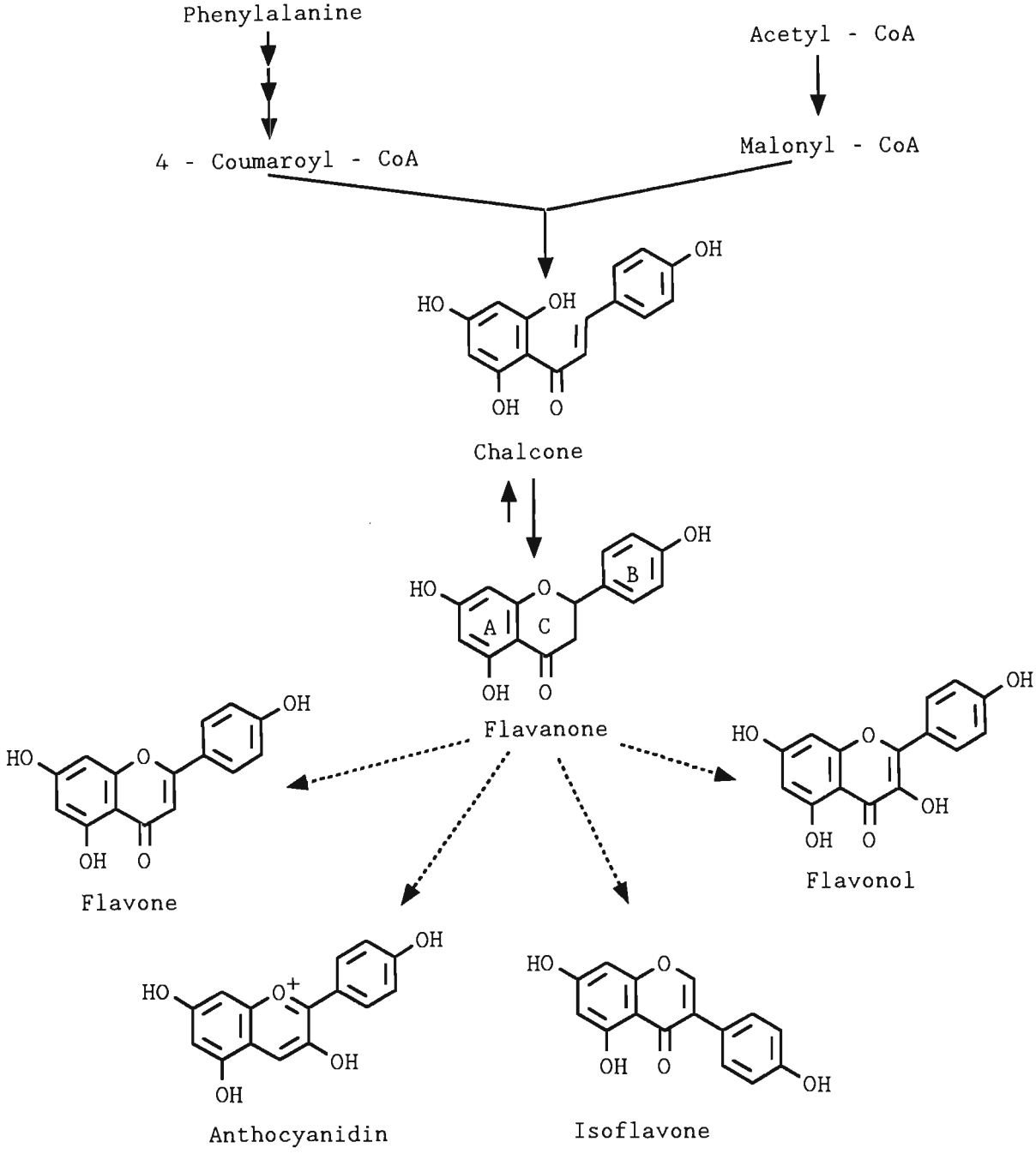
1.2.1. Flavonoid biosynthesis

Earlier work based on results from radioactive tracer studies, and more recently work at the enzyme level has resulted in considerable progress in the elucidation of the biosynthetic pathways of the flavonoids. This subject has been the topic of a number of reviews in recent years[8,9,10]. Thus, the aim of this section is merely to provide a broad overview of the biosynthesis of the flavonoids, to show the relationship between the various flavonoid classes and to highlight some recent developments.

In 1962 Grisebach[11] extended the earlier ideas of Birch[12] and proposed that the first specific reaction in flavonoid biosynthesis was the enzyme mediated condensation of an activated cinnamic acid with three molecules of malonyl-CoA to yield a chalcone or flavanone[12]. Earlier experiments with radioactively labelled precursors served to substantiate this proposal. The biosynthetic relationship of the flavonoids as concluded from these experiments (**Scheme 1.**) may be summarised as follows:

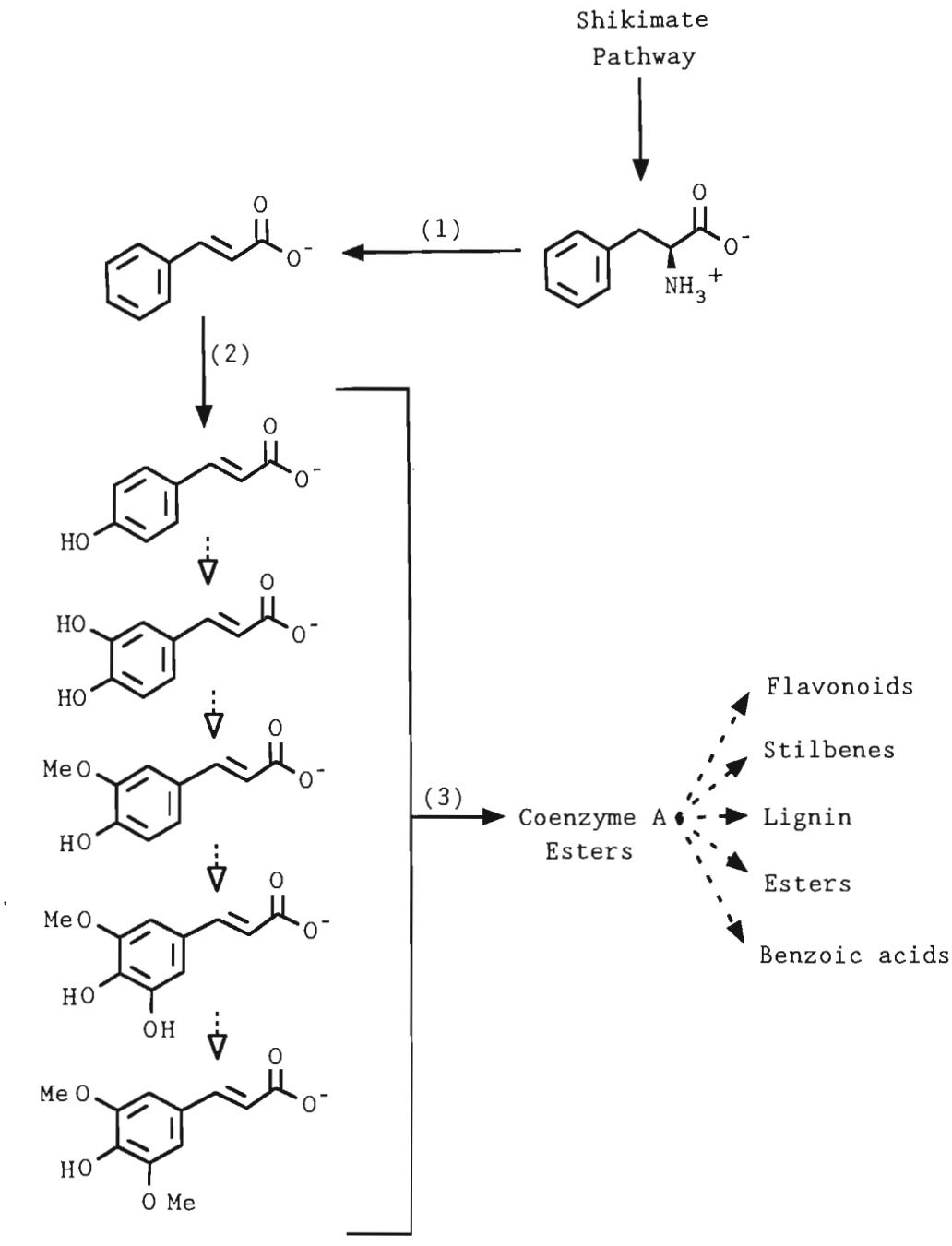
i) Ring A is derived from the head-to-tail condensation of three acetate units (*via* malonate). Ring B and the heterocyclic C-ring carbons at position 2, 3 and 4 are derived from phenylalanine.

ii) All flavonoid classes are biosynthetically closely related with a chalcone being the first common intermediate.



Scheme 1. The biosynthetic relationships of the flavonoids as concluded from *in vivo* labelling experiments.

1.2.1.1. Pathways to precursors of flavonoid formation



Scheme 2. General phenyl propanoid metabolism. Key enzymes: (1) Phenylalanine ammonia-lyase, (2) cinnamate 4-hydroxylase, (3) hydroxycinnamate:CoA ligase.

Malonyl-CoA, one of two substrates for chalcone synthase, is derived from acetyl-CoA *via* the action of a carboxylase enzyme. Although a typical enzyme of fatty acid biosynthesis,

this enzyme is also an integral part of flavonoid biosynthesis.

The pathway leading to the second substrate, usually 4-coumaroyl-CoA is shown (**Scheme 2.**). In this sequence of reactions phenylalanine, derived from the shikimate pathway, is converted to a series of substituted cinnamic acid derivatives. These derivatives are subsequently activated to yield their corresponding CoA esters. Besides the flavonoids, these activated cinnamic acid derivatives serve as precursors for various other classes of phenylpropanoids such as lignan, stilbenes and benzoic acids. For this reason this sequence of reactions was termed "general phenylpropanoid metabolism"[13]. The enzymes catalysing the individual steps are phenylalanine ammonia-lyase, cinnamate 4-hydroxylase and hydroxy-cinnamate:CoA ligase.

Phenylalanine ammonia-lyase is one of the best studied enzymes in phenolic metabolism. It catalyses the elimination of NH_3 from L-phenylalanine to give *trans*-cinnamate. The enzyme from many sources, notably grasses, also acts on L-tyrosine to yield *trans-p*-coumarate. Phenylalanine ammonia-lyase has attracted considerable attention because it provides the link between primary metabolism and the phenylpropanoid pathways. The properties as well as the role of this enzyme have been reviewed by Hanson and Havir[14].

The second enzyme of general phenylpropanoid metabolism, cinnamate 4-hydroxylase, catalyses the 4-hydroxylation of *trans*-cinnamate to yield *trans*-4-coumarate. This enzyme, described as a "microsomal cytochromic P-450-dependent mono-oxygenase that utilises NADPH as a hydrogen donor", has been reviewed together with other oxygenases by Butt and Lamb[15]. This enzyme, first discovered in pea seedlings[16], is the best characterised of plant mono-oxygenases. Of particular interest is the effect of physiological stimuli on the activity of this enzyme in plants, cell suspensions and

excised organs. For example, the accumulation of flavonoids and hydroxycinnamic esters in buckwheat seedlings[17] and of flavonoids in parsley cell cultures[18] is triggered by illumination and correlated with increases in cinnamate 4-hydroxylase activity.

The enzymes involved in the conversion of *trans*-4-coumarate to caffeic acid and its derivatives, such as the partially methylated ferulic acid in which the *p*-hydroxy group is free, are not well understood. Phenolase preparations have been found to catalyse the hydroxylation of *p*-coumaric acid to caffeic acid, usually in the presence of an additional reductant, such as ascorbate[19] or dimethyltetrahydropteridine[20]. Nevertheless, in terms of flavonoid biosynthesis these conversions are of minor importance since evidence suggests that 4-coumarate is the main, if not exclusive precursor for chalcone formation. Finally, activation of the cinnamic acid derivatives *via* the corresponding CoA esters is catalysed by hydroxy cinnamate:CoA ligase and which is often called 4-coumarate:CoA ligase, referring to its preferred substrate.

1.2.1.2. Flavonoid pathways

The condensation of 4-coumaroyl-CoA with three successive molecules of malonyl-CoA to yield naringenin-chalcone is the first step of the true flavonoid pathway. The chalcone is the central intermediate for the formation of all flavonoid classes, and thus chalcone synthase, the enzyme that catalyses this step, may be regarded as the key enzyme of flavonoid biosynthesis. Chalcone synthase has been isolated from several different plant sources. In all cases where this enzyme has been tested, 4-coumaroyl-CoA has proven to be the best substrate, although caffeoyl-CoA and even feruloyl-CoA may be accepted as substrates. That 4-coumaroyl-CoA is the

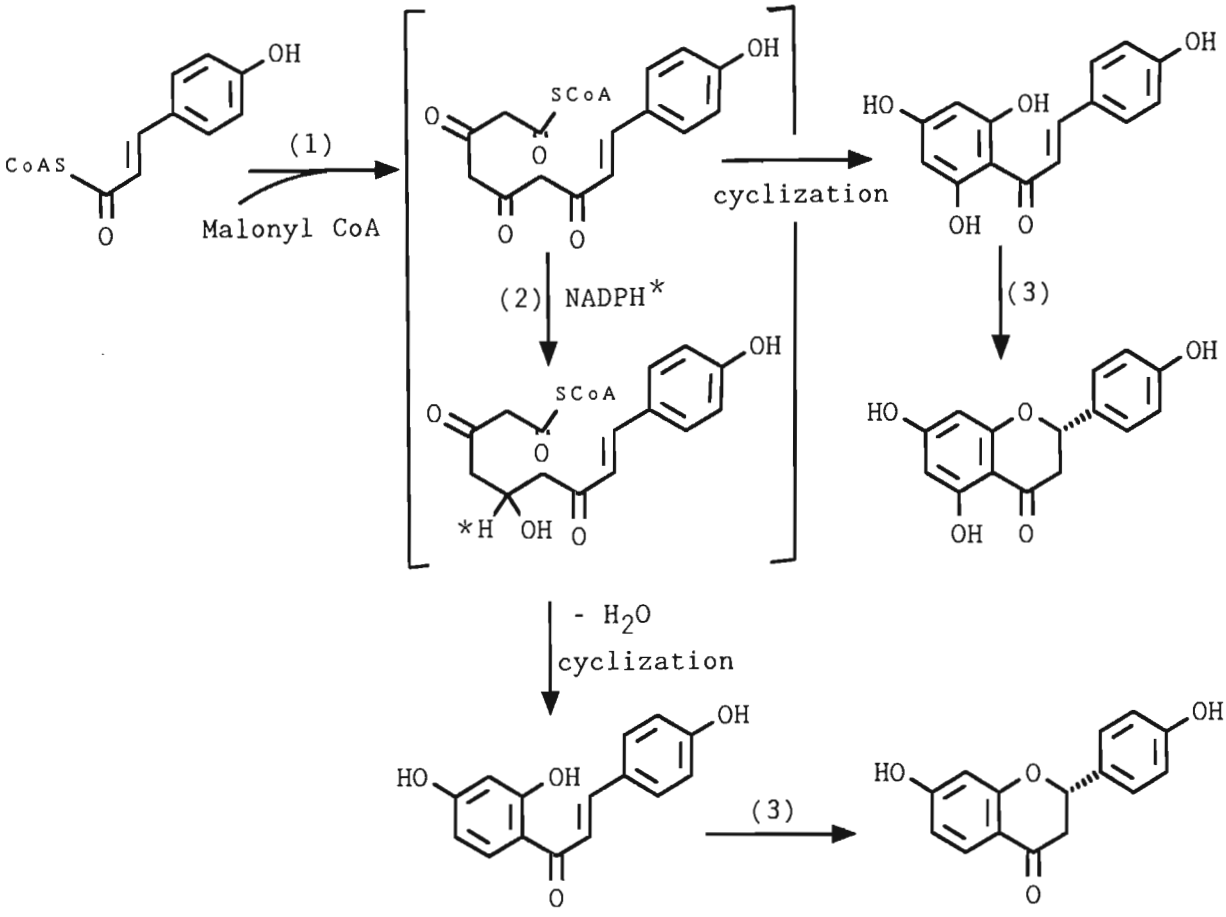
main physiological substrate for chalcone synthase is based mainly on the following evidence:

i) *In vivo* use of caffeoyl-CoA in addition to 4-coumaroyl-CoA has only been demonstrated in *Verbena*[21] and tulip[22,23].

ii) Flavonoid-3'- and -3',5'-hydroxylases as well as various methyltransferases have been demonstrated in a wide variety of plant species.

The second enzyme, chalcone isomerase, is the second key enzyme of flavonoid biosynthesis and catalyses the stereospecific conversion of chalcones to their corresponding (2S)-flavanones. Comparative studies of chalcone isomerases from cell suspension cultures of bean (*Phaseolus vulgaris*), soybean (*Glycine max*) and lucerne (*Medicago sativa*) have shown that the enzyme activity is induced by fungal elicitors, and that they catalyse isomerisation of both naringenin-chalcone and liquiritigenin[24]. The enzyme from *Petunia hybrida*, however, was non-inducible and specific for naningenin-chalcone, suggesting differences and anomalies between the properties of chalcone isomerases from different sources. Rate constants and stereoselectivity of highly purified chalcone isomerase from soybean have been determined[25]. Interestingly, this enzyme catalysed cyclisation of isoliquiritigenin to (2S)-liquiritigenin with a rate constant 36 x 10^6-fold greater than that for spontaneous isomerisation.

In many plants, the occurrence of flavonoids having resorcinol rather than phloroglucinol substitution patterns in the acetate derived aromatic ring has prompted speculation about the possible existence of a 6'-deoxychalcone synthase enzyme. Recently, a reductase enzyme acting concomitantly with chalcone synthase has been detected. The results from these studies have been reviewed by Dewick[26,27] and may be outlined briefly as follows:



Scheme 3. Enzymes: (1) chalcone synthase, (2) reductase, (3) chalcone isomerase.

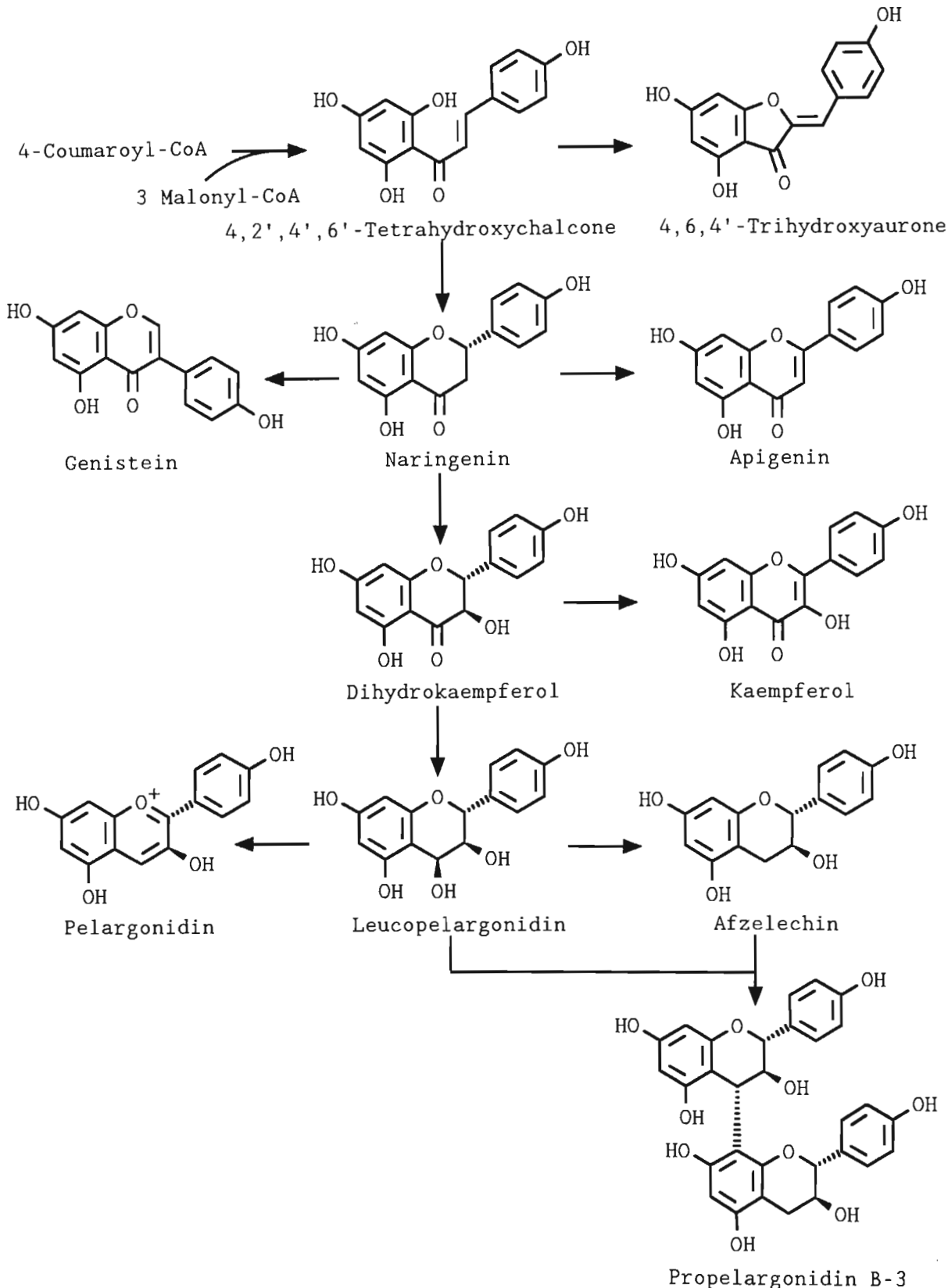
i) The reductase, which requires NADPH as a co-factor has been purified and shown to be a single polypeptide.

ii) This enzyme catalyses transfer of the *pro*-R(A) hydrogen of NADPH to the substrate, either 4-coumaroyl-CoA or caffeoyl-CoA.

iii) Reduction occurs at the polyketide stage since the reductase gives no products when incubated with naringenin-chalcone/naringenin and NADPH.

iv) The sequence of **Scheme 3** is proposed, although it is unknown whether the dehydration step occurs before or after cyclisation, or whether it is spontaneous or enzyme catalysed.

With the exception of the anthocyanins, where a few reactions remain unclear, the key steps leading to the major flavonoid classes have been established (**Scheme 4.**).


4-Coumaroyl-CoA
3 Malonyl-CoA
4,2',4',6'-Tetrahydroxychalcone
4,6,4'-Trihydroxyaurone
Genistein
Naringenin
Apigenin
Dihydrokaempferol
Kaempferol
Pelargonidin
Leucopelargonidin
Afzelechin
Propelargonidin B-3

Scheme 4.

1.2.2. The biological function of flavonoids

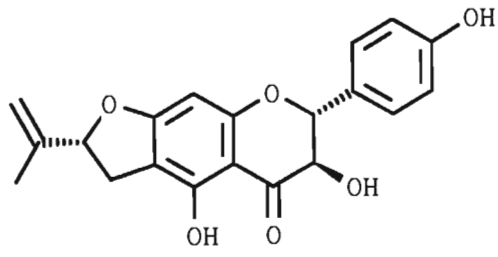
The reason why plants produce these secondary metabolites in such vast quantities, and with such structural variation, has been the focus of a great deal of attention in recent years. It is clear that the role of the flavonoids is multi-functional. They exhibit a wide variety of biochemical, physiological and ecological activities which enable plants to survive the various challenges of their environment.

A major function of the flavonoids is in imparting colour to flowers and fruit in order to attract animals (mainly insects and bees), for the purpose of pollination and seed dispersal. Flower colour has recently been reviewed[28]. Anthocyanins provide most of the pink, orange, red, violet and blue colours to flowers. The chalcones, aurones and yellow flavonols sometimes contribute to yellow flower colour, and these, together with the colourless flavone and flavonol glycosides, are frequently responsible for U.V. patterns which guide insects, especially bees, in their search for nectar.

The flavonoids are conjugated aromatic compounds and as such may act as potent screening agents against harmful U.V. radiation. The U.V. induced activity of a number of enzymes involved in flavonoid biosynthesis has been demonstrated[17,18], and may serve to confirm this theory. The flavanoids are typical phenolic compounds and therefore act as potent antioxidants and metal chelators.

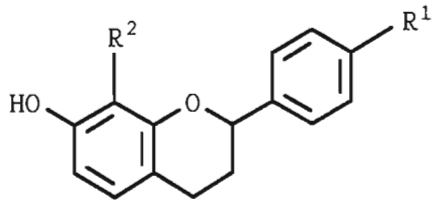
Flavonoids play a role in plant defence against microbial attack. The antimicrobial properties of the proanthocyanidins have been discussed in a recent review[29]. A number of flavonoid phytoalexins have been identified. Phytoalexins are antimicrobial (especially antifungal) compounds produced by plants as a response to fungal or bacterial attack and may be considered as part of a plant's natural defence against

micro-organisms. Most of the flavonoid phytoalexins are isoflavonoids although other classes of flavonoid have been demonstrated. For example, four dihydroflavonols, including (**6**) have been identified as phytoalexins of the legume *Shuteria vestita*[30].



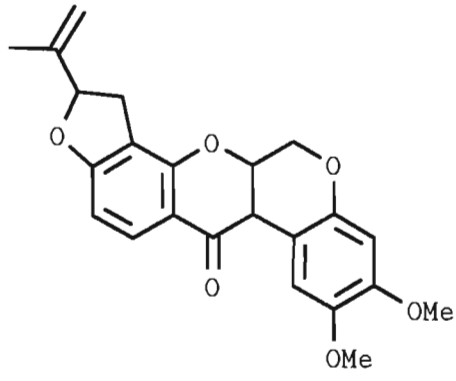
(6)

In another example, three flavans (**7**), (**8**) and (**9**) were isolated as phytoalexins of *Narcissus pseudonarcissus* after inoculation with *Botrytis cinerea*[31].



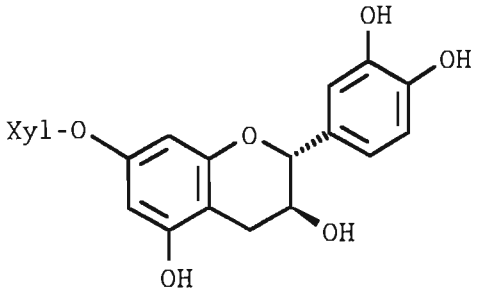
(7) R^1=R^2=H
(8) R^1=OH; R^2=H
(9) R^1=OH; R^2=Me

Plant flavonoids affect insect behaviour, development and growth. The insecticidal activity of the rotenoids, which may be traced to an inhibition of the mitochondrial electron transport system, has been discussed by McClure[32]. Rotenone (**10**) appears to be one of the most active of the naturally occurring rotenoids.



(10)

Besides the insecticidal activity of the rotenoids, the role of flavonoids as feeding stimulants, feeding deterrents and larval growth inhibitors have been demonstrated in a variety of plant species. For example, a flavan-3-ol glycoside (**11**) isolated from *Ulmus americana* is believed to act as a feeding stimulant for the smaller European elm bark beetle *Scolytus multistriatus* (Marsham)[33,34]. The feeding deterrent activity of a number of naturally occurring flavonoids against the aphids *Schizaphis graminum* and *Myzus persicae* have been demonstrated[35]. Many of these, including the dihydrochalcone phlorizin (**12**) and the flavanone eriodictyol (**13**) showed strong deterrency at concentrations well within the range often found in plants. Finally, the cotton leaf anthocyanin, cyanidin 3-glucoside (**14**) has been shown to be particularly inhibitory to the larval growth in the tobacco budworm *Heliothis viriscens*[36].



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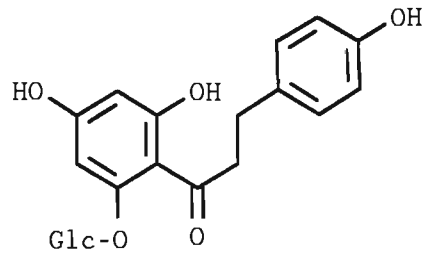

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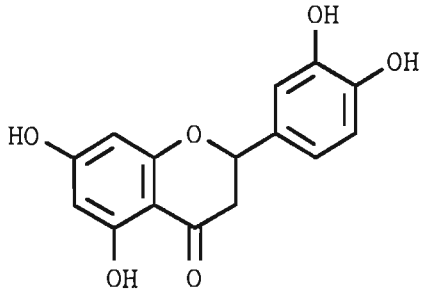

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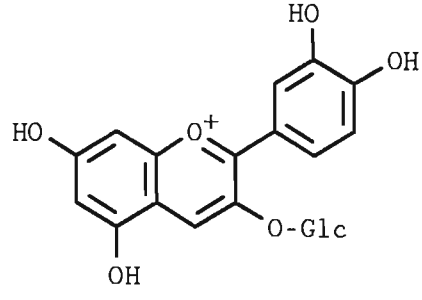
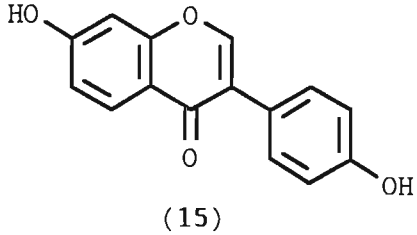


(14)

The oestrogenic activity of simple isoflavones such as daidzein (**15**) and of coumestans such as coumestrol (**16**) have long since been established and extensively discussed by McClure[32]. These compounds are structurally related to the synthetic oestrogen, diethylstilbestrol (**17**).



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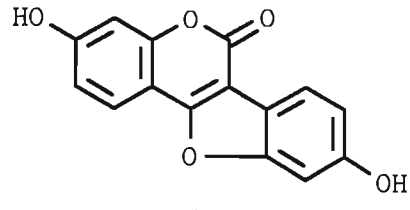

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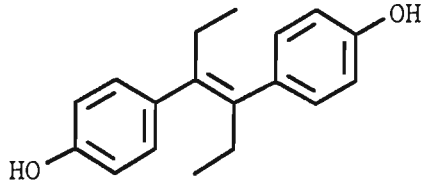


(17)

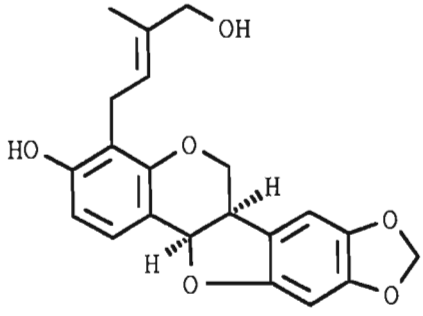
Although not as potent, they nevertheless approach sufficient levels in forage crops such as clovers (*Trifolium spp.*) and lucerne (*Medico sativa*) to exert definite physiological

effects when eaten in quantity[37]. In a review by Smolenski *et al.*[38] these phyto-oestrogens, as well as the proanthocyanidins are featured among the toxic and antinutrient compounds biosynthesised by leguminous plants.

1.2.3. Pharmacology of the flavonoids

In recent years interest in traditional remedies and plant derived drugs have witnessed a spectacular revival which is owed, perhaps in part, to improved methods of biochemistry and clinical pharmacology. The flavonoids in particular, have received renewed interest. Their widespread occurrence in flowering plants, and therefore availability, as well as their overall uniformity in chemical structure, so that structure- activity relationships are easily established, makes this class of compounds ideal for pharmacological screening. It is therefore not surprising that a wide variety of pharmacological activities such as anti-inflammatory, anti-ulcer, anti-anginal, anti-hepatoxic, anti-allergic, antimicrobial, antiviral *etc.* have been attributed to the flavonoids in recent years. A thorough review of the pharmacological activity of the flavonoids has recently been published under the editorship of Cody, Middleton and Harborne[39]. Some of the pharmacological activities displayed by the flavonoids are perhaps best illustrated with a few examples.

An aqueous alcoholic extract of the root of a South American plant called "Cabeca de Negra" is available to plantation workers in the Amazon jungle as an oral antidote against snake and spider venoms. Two pterocarpans, cabenegrin A-1 (**18**) and cabenegrin A-2 (**19**) were isolated as the active principles[40]. Both of these have been shown to possess potent snake anti-venom properties.



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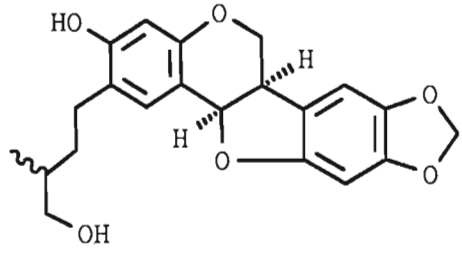
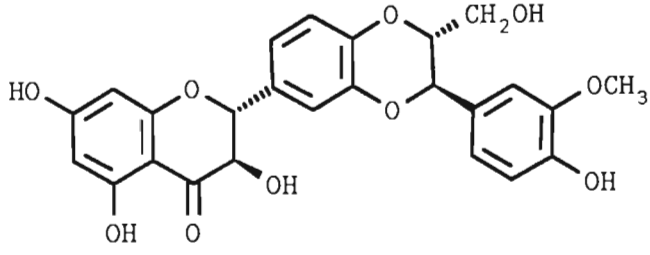


(19)

The anti-hepatoxic activity of the flavonoids has been reviewed by Wagner[41]. Of these, a series of flavanolignans from *Silybum marianum* which include silybin (**20**) and silymonin (**21**) have been shown to possess significant anti-hepatoxic activity[42].



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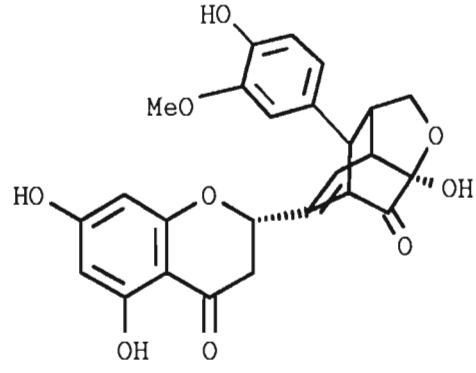
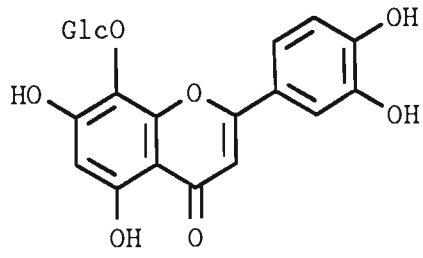


(21)

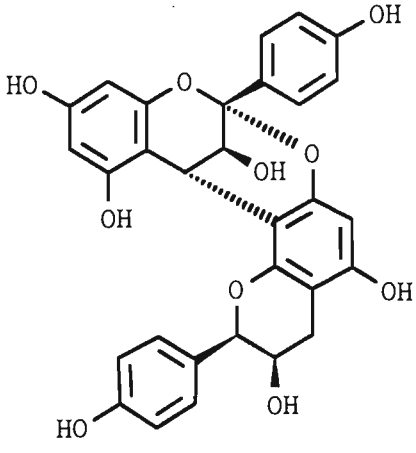
Wagner points out that the discovery of these liver-active flavanolignans was not the result of systematic pharmacological screening but were derived from folk medicine.

A number of flavonoids with anti-inflammatory properties have been isolated in recent years[43]. Of these, hypolaetin-8-glucoside (**22**) isolated[44] from *Sideritis mugronensis* is interesting in that it displays both anti-inflammatory and anti-ulcer properties[45]. The authors suggest that this compound may provide a useful alternative to anti-inflammatory drugs of the aspirin type.



(22)

In the final example, a series of five dimeric, doubly linked flavonoids isolated from *Ephedra spp.*[46-49], including structures (**23**) and (**24**) have been shown to possess significant coronary dilatory, or hypotensive properties.



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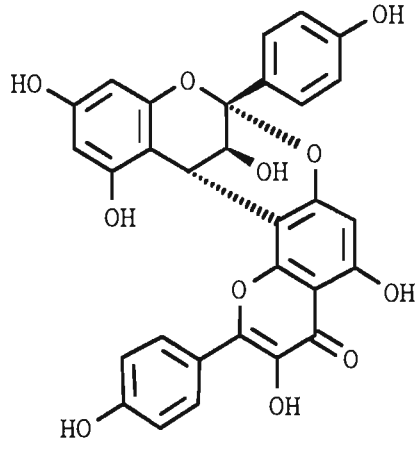


(24)

Recently, (**23**) together with related novel doubly linked proanthocyanidins have been isolated from *Prunus spinosa*[50]. Of particular interest is that this species is well known in regions of Spain, where an infusion prepared from the bark is used in folk medicine for the treatment of hypertension[51].

1.3. THE PROANTHOCYANIDINS

Of the various flavonoid classes the proanthocyanidins (*syn.* condensed tannins) are unique in their ability to exist as complex polymers. Their high molecular weight, and polyphenolic nature imparts to them the ability, amongst others,

to precipitate proteins such as gelatin from solution. This property (often called astringency) is vividly illustrated by their use industrially in the tanning of animal hides to make leather.

From a chemical point of view the proanthocyanidins have, until the last decade or so, remained largely unexplored. Technological advances in the field of chemical separation and identification, coupled with the recognition of the importance of the proanthocyanidins in herbal remedies, nutrition, and agriculture *etc.* has led to an unprecedented interest in this unique class of secondary metabolites.

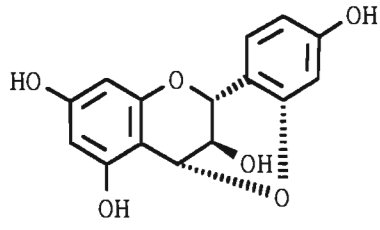
1.3.1. Nomenclature

In light of the recent dramatic increase in both the number and complexity of proanthocyanidins with novel structures, Porter[52], in his review of the flavans and proanthocyanidins has proposed a nomenclature re-appraisal. He points out that Haslam's previous reviews[53,54] of the proanthocyanidins were confined to compounds based on a flavan-3-ol skeleton and recommends that those compounds with an unsubstituted C-ring, the flavans, as well as the naturally occurring flavan-4-ols be included in the proanthocyanidin class of flavonoid.

Following the earlier suggestions of Weinges and Freudenberg, Haslam[54] defined leucoanthocyanidins as monomeric proanthocyanidins, such as the phenolic flavan-3,4-diols, and condensed proanthocyanidins as flavan-3-ol dimers and higher oligomers. Porter[52] suggests that this terminology be modified as follows:

The term leucoanthocyanidin should include all monomeric flavanoids which produce anthocyanidins by cleavage of a C-O bond on heating with mineral acid, including not only the

flavan-3,4-diols, but also flavan-4-ols and unusual metabolites such as cyanomaclurin (**25**). Regarding proanthocyanidins, the term "condensed" should be dispensed and this group should be redefined to include all compounds which produce anthocyanidins by cleavage of a C-C bond.



(25)

A recent system of nomenclature for naming proanthocyanidins was introduced by Hemingway *et al.*[55] and extended for general application by Porter[52], and is described as follows:

i) Proanthocyanidins are named in a similar way to polysaccharides, where C-4 of the flavan monomer unit is (in the nomenclature sense) equivalent to C-1 of the sugar unit in an oligo- or poly-saccharide chain.

ii) The interflavanoid linkage is indicated in the same way as the linkage between sugar units; the bond and it's direction are contained in brackets.

iii) The configuration of the interflavanoid bond at C-4 is indicated by the (α,β) nomenclature[56].

iv) The flavanoid monomer units are defined in terms of the trivial names, for example, catechin, epicatechin, afzelechin, epiafzelechin *etc.*

v) Flavanoid monomer units with the 2S configuration in the case of the flavan-3-ols and 2R configuration in the case of the flavans are distinguished by the *enantio* prefix, thus (+)-epicatechin becomes *ent*-epicatechin.

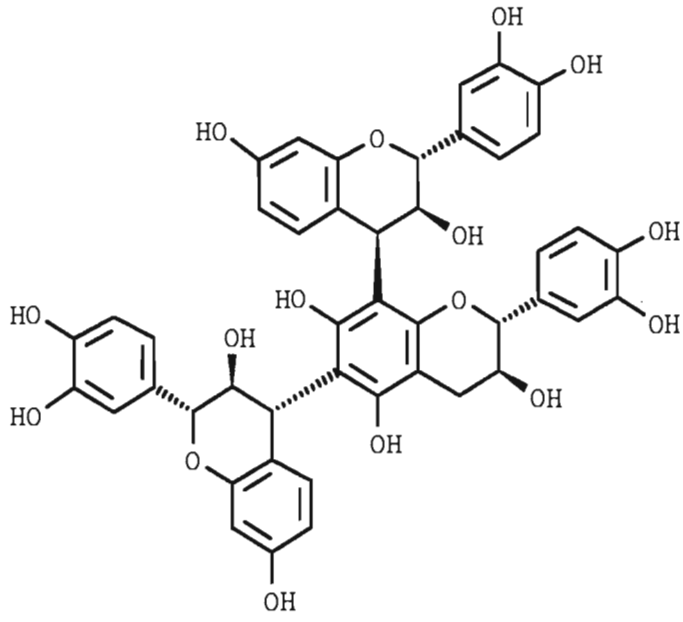
1.3.2. Structure and distribution

A comprehensive register of the naturally occurring compounds in the flavan, flavan-3-ol, leucoanthocyanidin and proanthocyanidin (oligomeric and polymeric) classes, complete to 1987, has been compiled by Porter[52]. The proanthocyanidins which occur widely in plants of a woody habit may be subdivided into two main groups, the singly linked B-type proanthocynidins and those containing a double interflavanoid linkage, the so called A-type proanthocyanidins.

1.3.2.1. B-type proanthocyanidins

Of the B-type proanthocyanidins the procyanidins, and in particular, the (4→8) linked dimers (B-1, B-2, B-3 and B-4) and their corresponding (4→6) linked analogues (B-5, B-6, B-7 and B-8) have a widespread distribution. On the other hand, classes such as the propelargonidins are relatively uncommon in nature.

A number of proanthocyanidins with a 5-deoxy or resorcinol type A-ring, as opposed to the more commonly occurring phloroglucinol type A-ring have been isolated in recent years and include the proguibourtinidins, profisetinidins, prorobinetinidins and promelacacinidins. It is interesting to note that the vast majority of these compounds have been isolated from *Acacia spp.* The 5(A)-deoxy-proanthocyanidins are often associated with structural features that are uncommon in proanthocyanidins with phloroglucinol A-rings, such as the enhanced tendency towards the biosynthesis of angular trimers and the common occurrence of 3,4-*cis* interflavanoid linkages. A typical example of such a compound is provided by the trimeric profisetinidin, fisetinidol-(4β→8)-catechin-(6→4α)-fisetinidol (**26**) isolated from *Acacia mearnsii*[57].

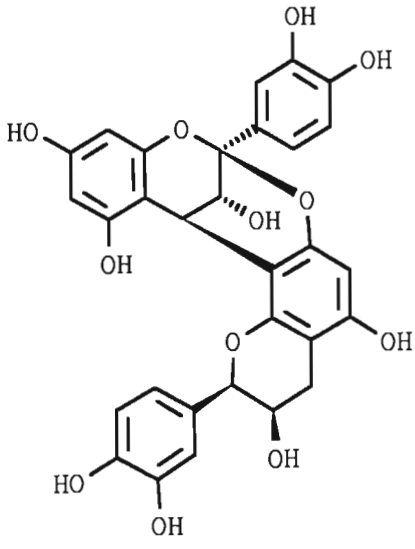


(26)

The highly successful semi-synthetic or biomimetic approach towards the synthesis of oligiomeric flavanoids, adopted by Ferreira and co-workers, has provided great insight into the rationalisation of the condensation sequences observed in these compounds. General principles which have emerged from their studies in this field of chemistry are authoritatively discussed in a recent review[58].

1.3.2.2. A-type proanthocyanidins

Since the first isolation[59] and structure determination[60,61] of proanthocyanidin A-2 (**27**) [epicatechin-(4β→8,2β→O→7)-epicatechin], a variety of analogues possessing the doubly linked unit of either (2β,4β)- (**27**) or (2α,4α)-configuration (**28**) have been reported[46-50,62,63,64]. However, in contrast to the wide distribution of B-type proanthocyanidins those of the A-type are relatively rare and occur in a restricted number of plant species.



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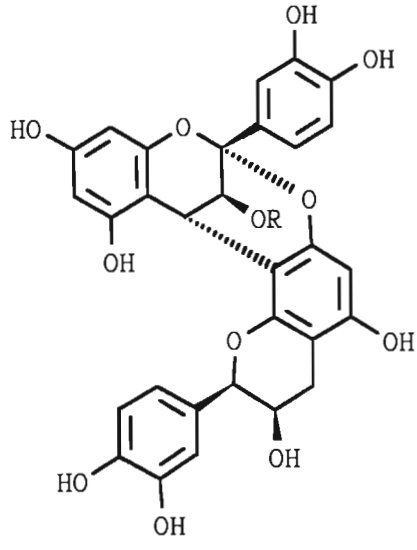
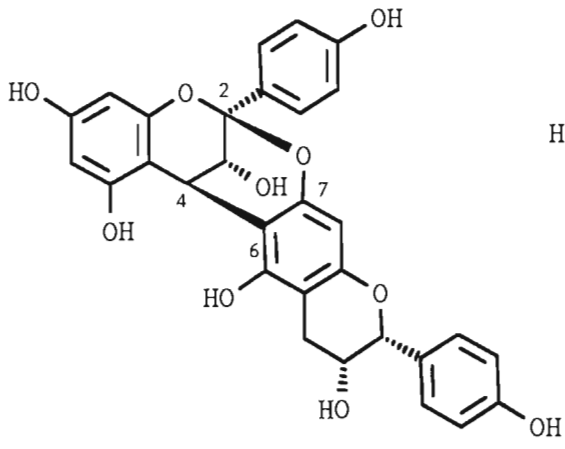


(28) R=H
(29) R=O-β-D-Gal.
(30) R=O-L-Ara.

Although the majority of these fall into the procyanidin class of condensed tannins, constituent units other than those based on the catechins have been isolated, and include a flavonol[46], a flavan[47], epigallocatechin[65] and the afzelechins[48,49,50,66]. Of the three possible linkage isomers *viz.* (4→6,2→O→7), (4→6,2→O→5) and (4→8,2→O→7) the latter mode of linkage is by far the most prevalent. The sole example of a (4→6,2→O→7) linkage is provided by mahuannin C (**31**) [epiafzelechin-(4β→6,2β→O→7)-epiafzelechin] isolated from *Ephedra spp*[49]. Recently epicatechin-(4β→6,2β→O→5)-epicatechin (**32**) has been reported[64] as a new natural product from *Theobroma cacao* and is the only example with the (4→6,2→O→5) mode of linkage.



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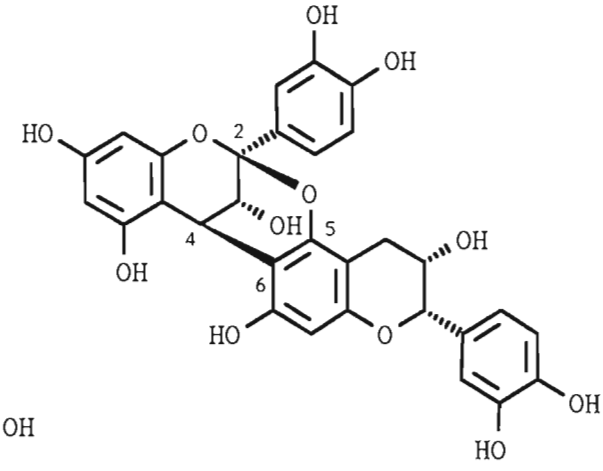
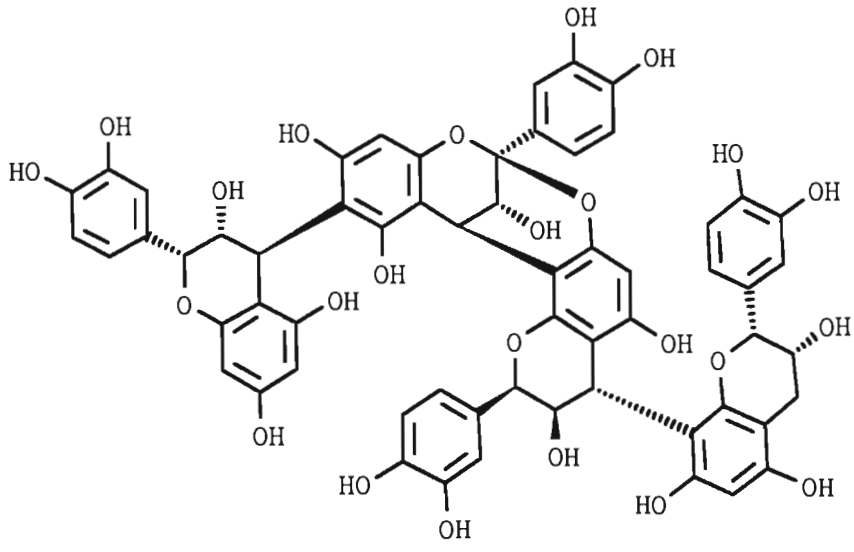


(32)

Interestingly, two A-type proanthocyanidin glycosides, *ent*-epicatechin-3-O-D-galactopyranosyl-(4α→8,2α→O→7)-epicatechin (**29**) and *ent*-epicatechin-3-O-L-arabinopyranosyl-(4α→8,2α→O→7)-epicatechin (**30**) have been isolated from the same source. These, together with epigallocatechin-(4β→8,2β→O→7)-epigallocatechin-3-O-gallate, isolated[65] from commercial oolong tea, are presumably the only known substituted A-type proanthocyanidins. A number of procyanidin oligomers containing both A- and B-type linkages have been isolated[67,68,69]. Of these, a series including a trimer, two tetramers, including (**33**), and a pentamer isolated[69] from *Cinnamomum zeylanicum* are particularly worthy of mention.



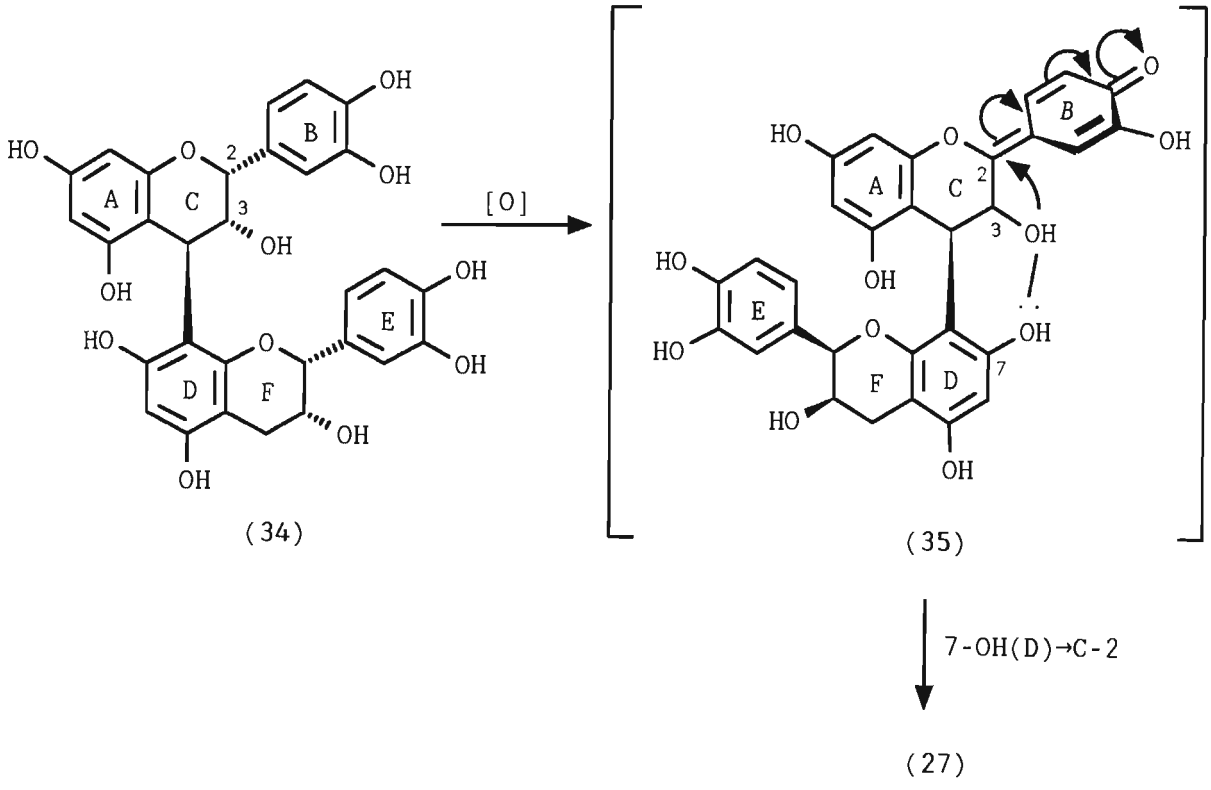
(33)

Structure elucidation was based largely on partial acid-catalysed degradation of these compounds with phenyl-methanethiol, in conjunction with ^{1}H- and ^{13}C-NMR analysis.

Owing to the close structural relationship between proantho-cyanidins of the B- and A-type, Porter[52] has proposed that the A-type proanthocyanidins are formed from their corresponding B-type proanthocyanidins *via* an enzyme mediated hydroxylation at C-2(C), in much the same way as the auto-oxidation at C-2(C) in the chemical conversion of procyanidins to cyanidins[70].

The conversion of B- to A-type proanthocyanidins under mildly basic conditions has recently been demonstrated by Ferreira and co-workers[71,72]. These authors propose that the transformation of, for example, procyanidin B-2 (**34**), into the corresponding A-type proanthocyanidin (**27**) involves the oxidative removal of a hydride ion at C-2(C) as the initial step. They suggest that although trace amounts of oxygen may effect the transformation of (**34**) to (**35**), it seems more reasonable that the prevailing conditions induce oxidation of the *o*-dihydroxy functionality of the pyrocatechol B- or

E-rings to an o-quinone which subsequently serves as oxidant for the conversion of (**35**) to (**27**) shown below.



(34)

(35)

7-OH(D)→C-2

(27)

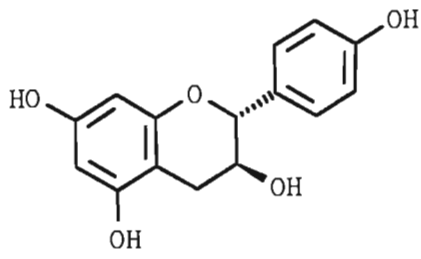
2. DISCUSSION

2.1. OVERVIEW

The initial objective of this study was to isolate and characterise biologically active compounds from *Cassipourea gerrardii* (RHIZOPHORACEAE). The bark from this indigenous South African species is well known in the local herbal medicine trade, where it is known as "memezi obomvu". The ground bark is used, mainly by Zulu women, in the form of a face pack - in this fashion it is reported to have skin whitening properties. Demand for this product, which is estimated at over eight tonnes per annum, has resulted in a serious decline in the wild population of this species[73].

In light of the increasing vulnerability of this species and since previous chemical studies[5] were confined to the alkaloid constituents, a re-investigation was warranted in order to gain a more comprehensive knowledge of the chemical constituents of this species.

TLC analysis of the extractives of the stem bark from *C. gerrardii* revealed, upon staining with diazotised benzidine reagent, a phenolic rich fraction. Using a combination of chromatographic techniques, a series of compounds belonging to the proanthocyanidin class of flavonoids were isolated. These include the known compounds afzelechin (**36**), epiafzelechin (**37**) and epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**38**) as well as three novel compounds identified as afzelechin-3-O-α-L-rhamnopyranoside[74] (**39**), epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin[75] (**40**) and epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**41**). In contrast to the naturally occurring catechin and gallocatechin derivatives afzelechin derivatives are relatively rare and occur in a restricted number of plant species.



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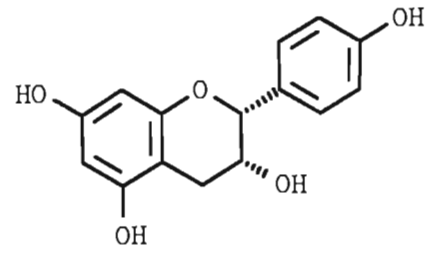
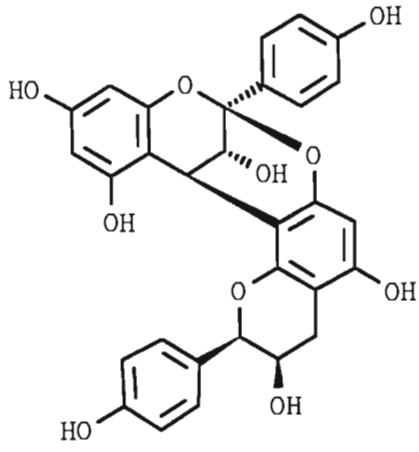


(37)



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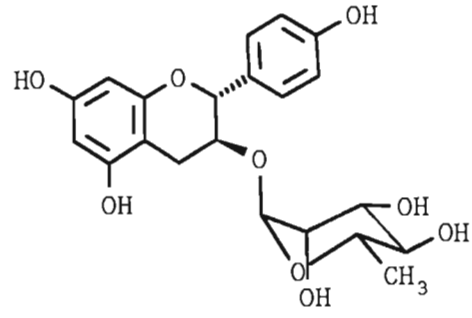

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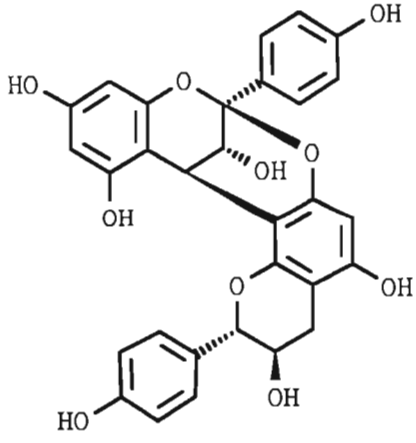

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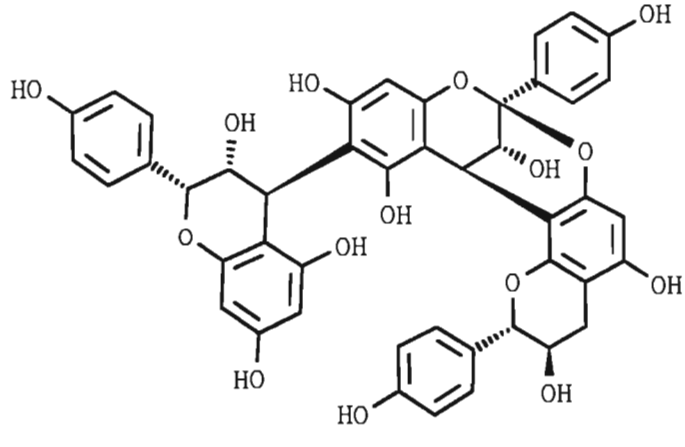
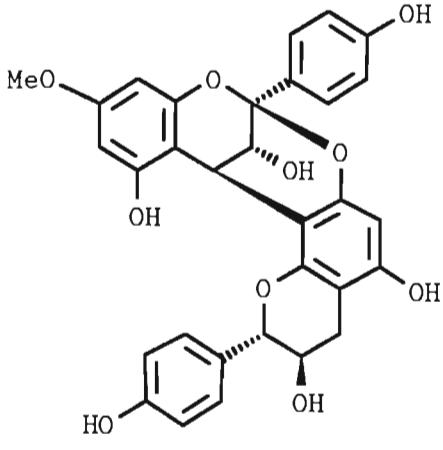


(41)

As a result of these findings this work was extended to include a similar study of two related species *viz. C. gummiflua* and *C. mossambicensis*.

As was the case with *C. gerrardii*, previous chemical studies on *C. gummiflua* were restricted to the isolation of alkaloid constituents[4]. Using a similar strategy to that developed for the phenolic constituents in *C. gerrardii*, eight compounds were isolated. Of these (**36**), (**37**), (**38**), (**39**), and (**40**) were found to occur in *C. gerrardii*. Two new compounds were also isolated and identified as 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**42**) and 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**43**). A flavonol glycoside was isolated and identified as the known compound kaempferol-3-O-α-L-rhamnopyranoside (**44**).



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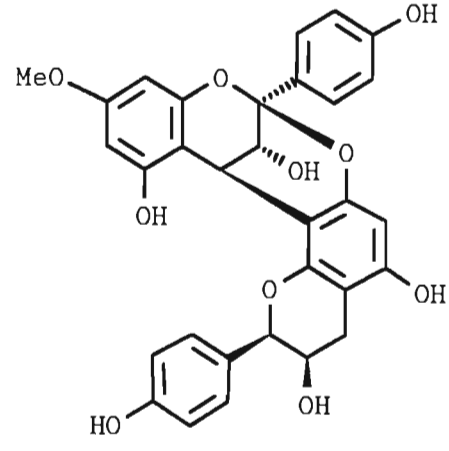
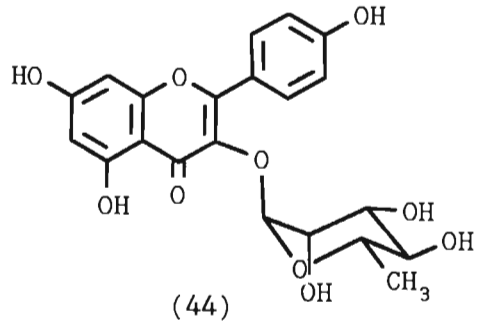
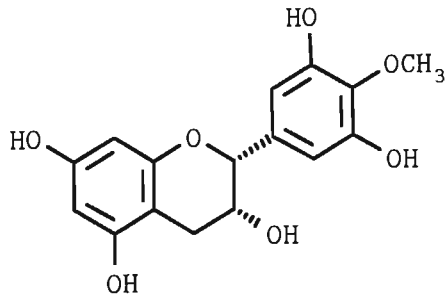


(43)



(44)

Stem bark from *C. mossambicensis*, a hitherto unstudied species, yielded besides (**36**), (**37**), (**38**), (**39**), (**40**) and (**41**) a rarely occurring flavan-3-ol identified as 4'-OMe-epigallocatechin (**45**).



(45)

The distribution of compounds isolated from *Cassipourea* species during the course of this work is summarised in **Table 1.**.

Table 1. Distribution of compounds isolated from *Cassipourea spp.*

Compound	*C. gerrardii*	*C. gummiflua*	*C.mossambicensis*
(36)	x	x	x
(37)	x	x	x
(38)	x	x	x
(39)	x	x	x
(40)	x	x	x
(41)	x	-	x
(42)	-	x	-
(43)	-	x	-
(44)	-	x	-
(45)	-	-	x

A concise botanical description of these species, including their distribution in Southern Africa has been published[3].It is interesting to note that members of the Rhizophoraceae family have, for many years, been used in the tanning industry[76]. The present isolation of "tannins", or more specifically, proanthocyanidins from *Cassipourea* species is therefore not surprising.

2.2. EXTRACTION AND ISOLATION

Owing to the close similarity of the phenolic profiles encountered in the *Cassipourea* species studied, the extraction and isolation strategies developed for the isolation of compounds from *C. gerrardii* were easily extended to both *C. gummiflua* and *C. mossambicensis*. These strategies are discussed in this section. A characteristic feature of the phenolic extractives of these species was the occurrence of a large number of diastereomeric pairs. The separation of these diastereomers proved to be particularly challenging, although reasonable success was achieved with HPLC and HSCCC methods. HSCCC is a relatively recent development in the field of chromatography and thus the practical merits of this technique will be discussed.

The solvents most commonly employed for the extraction of phenolic compounds are EtOH (95%), MeOH and aqueous acetone (60-80%). Preliminary extractions on small quantities of bark revealed that acetone (60%) was the most effective solvent, but was judged unsuitable owing to the difficulty associated with reducing the aqueous fraction to workable volumes after extraction. EtOH (95%) was chosen as a suitable alternative.

Following exhaustive extraction (Soxhlet) of the air dried and powdered stem bark, the extractives were concentrated under reduced pressure, suspended in water, and in the case of *C. gerrardii* this was followed by successive extractions with hexane, $CHCl_3$ and EtOAc. A similar procedure was adopted

with the extractives from *C. gummiflua* and *C. mossambicensis* except that the step involving extraction with hexane was omitted.

TLC analysis of these fractions revealed upon staining with suitable reagents (diazotised benzidine and anisaldehyde reagent) that the phenolic compounds were restricted exclusively to the EtOAc fractions. TLC analysis of the EtOAc extractives from *C. gerrardii*, *C. gummiflua* and *C. mossambicensis* are shown (**Plate 1.**).

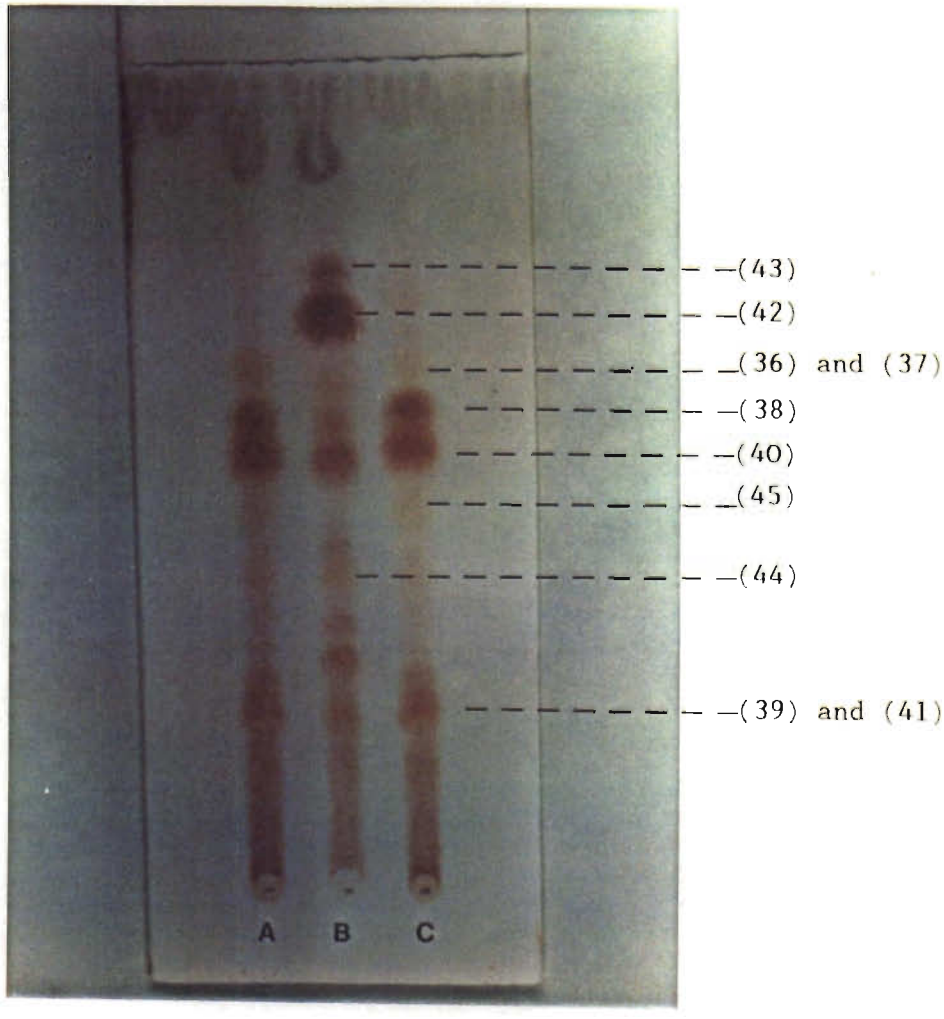


Plate 1. (X1) TLC chromatogram of the EtOAc extractives from *C. gerrardii* (A), *C. gummiflua* (B) and *C. mossambicensis* (C). Compound numbers shown in parenthesis. Solvent: acetone-CH_2Cl_2 -MeOH (39:60:1)

For silica gel based separations ($CHCl_3$ or CH_2Cl_2)-acetone-MeOH proved to be a useful solvent system. By varying the solvent ratios between (70:30:0) and (39:60:1) suitable Rf values, and separations devoid of tailing could be obtained for any of the desired phenolic compounds. The solvent system was extremely useful in that gradient elutions could easily be performed simply by increasing the ratio of acetone in the system in stepwise increments. At higher solvent polarity (acetone content > 50%) the addition of MeOH served to prevent tailing. A direct attempt to purify the crude EtOAc extract from *C. gerrardii* on silica gel by column chromatography, however, proved to be unsuitable due to the similar Rf values of many of these compounds.

A solution to this problem proved to be reverse phase column chromatography over Sephadex LH-20. Trial separations using EtOH (95%) as eluent demonstrated that monomeric flavan-3-ols and their glycosides were eluted first, followed by the dimers and trimers. A surprising feature of these separations was the high degree of plant material irreversibly adsorbed to the column and which resulted in rapid deterioration of the Sephadex LH-20. It was found that an initial crude fractionation of the phenolic plant material on silica gel by column chromatography using $CHCl_3$-acetone (2:3) as eluent prevented this occurrence and also served to remove less polar non-phenolic contaminants. This procedure was also adopted with the phenolic fractions from *C. gummiflua* and *C. mossambicensis* except for the following modifications:

i) Better fractionation was achieved on the silica gel "pre-columm" using a gradient elution with the solvent system previously described.

ii) The solvent system for the Sephadex LH-20 separations was changed. Using aqueous MeOH and increasing the MeOH-H_2O ratios from (50:50) to (100:0) in stepwise increments, better control of the separations was achieved. This resulted in complete separation between the monomeric

and dimeric compounds.

Subsequent purification of these fractions over silica gel (Flash and centrifugal thin layer chromatography) afforded the phenolic compounds either in pure form, or in the case of the diastereomers, as diastereomeric mixtures.

The question arose as to whether these diastereomeric pairs were produced *in vivo*, or whether one of the diastereomers within each pair was an artifact, produced *via* epimerization either during the period that the bark was drying or during the extraction process. The logical solution was to rapidly extract a small piece of fresh bark, under mild conditions, followed by immediate TLC analysis. In general TLC analysis of crude extractives seldom provides good results. Resolution is generally poor presumably due to the large number and vastly differing polarities of compounds usually encountered in these extracts. The concentration of individual components within such mixtures is usually low, hence the problem associated with the visualization of these compounds on the chromatogram. The following simple method was devised which solved some of these problems and may serve as a generally useful method for the rapid screening of plant phenolics:

A small quantity of fresh bark (ca. 1g) was macerated and vigorously shaken for a few secs in a test tube half filled with aqueous acetone (60%). Saturation of the mixture with NaCl resulted in a phase separation. The upper layer (acetone) was immediately subjected to TLC analysis. Upon staining with diazotised benzidine reagent, the diastereomeric pairs [(**38**) and (**40**)] and [(**42**) and (**43**)] were clearly discernible, thus verifying their biosynthetic origin.

The separation of the diastereomers within each diastereomeric pair proved to be particularly challenging. Although these separations could be detected on TLC, it is

surprising that they could not be effected on a preparative scale with silica gel, either by centrifugal or preparative TLC. In both methods the diastereomers within each pair travelled as unresolved bands, even at low sample concentrations. Partial success was achieved with reverse phase column chromatography over Toyopearl HW-40F. A mixture of (**38**) and (**40**) from *C. gerrardii* was subjected to repeated chromatography over Toyopearl HW-40F with EtOH (80%) as eluent. In this way pure fractions of the major diastereomer (**40**) were obtained. The minor diastereomer (**38**) could only be obtained in an enriched form due to tailing of (**40**). An attempted final purification of the trimer (**41**) resulted in extremely poor recovery, presumably due to decomposition on the column. Owing to the apparent harshness of the columm conditions, further separations using this method were avoided.

HPLC proved to be a useful tool for the separation of diastereomers. Small quantities of (**36**) and (**37**) were separated on a semi-preparative C-18 reverse phase columm with solvent programming. Complete separation of (**38**) and (**40**) was also achieved by HPLC using a C-18 preparative columm with H_2O-MeOH (60:40) as eluent. The methylated dimers (**42**) and (**43**) could not, however, be separated on the C-18 columm largely due to the insolubility of these compounds in aqueous solvent systems.

Countercurrent chromatography (CCC) designates a family of liquid-liquid partition chromatographic methods which do not employ a solid supporting matrix. As a result, these methods benefit from a number of advantages over liquid chromatography: there is total recovery of the introduced sample and no irreversible adsorption on a solid matrix, tailing is minimised, denaturation of sample is minimal and solvent consumption is low.

High-speed countercurrent chromatography (HSCCC) is a relatively recent development in this field. Unlike droplet countercurrent chromatography (DCCC) which relies on gravity for the retention of stationary phase, HSCCC relies on centrifugal force to retain the stationary phase. Consequently, the mobile phase can be pumped at much higher flow rates, thus allowing much faster separation times. Both DCCC and centrifugal partition chromatography (CPC) have been discussed in a recent review[77]. Separation of the diastereomers (**38**) and (**40**) as well as the diastereomers (**42**) and (**43**) was achieved by HSCCC with a P.C. Inc. Multilayer Coil Planet CCC instrument.

Briefly, the separation column consists of a Teflon tube (i.d. 2.6mm volume ca. 400ml) wrapped as a coil around a spool. The coil is balanced by a counter-weight and describes a planetary motion about a central axis[78] (**Fig. 2.**).

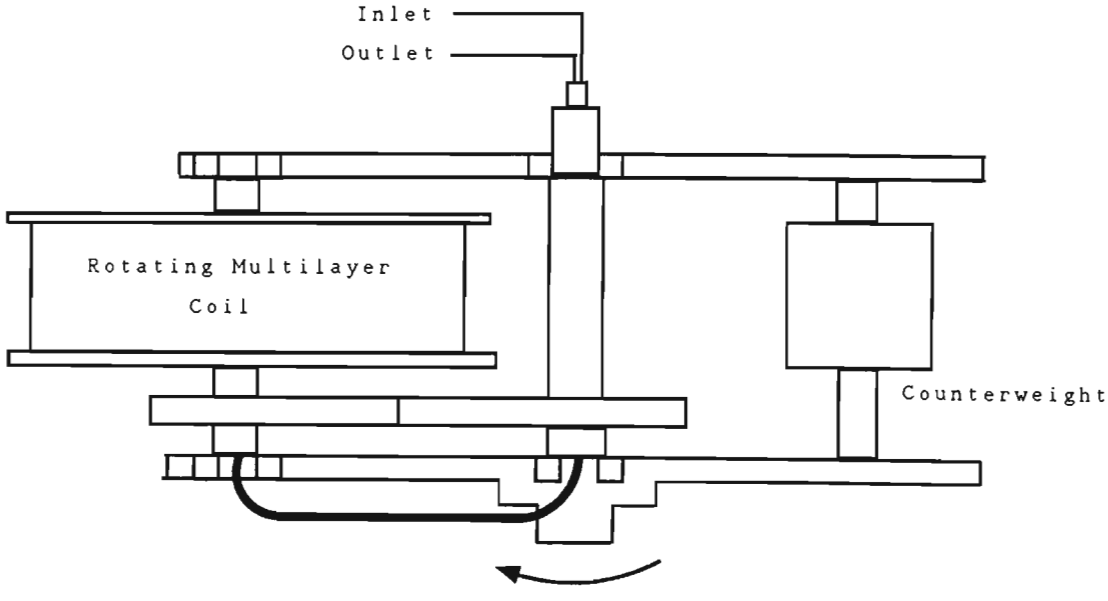


Fig. 2. Schematic diagram of the MLCCC instrument.

Rotation speeds for the separations are generally of the order of 700-800rpm. Concerning the principle of the

technique, the motion of the coil generates a train of dynamic mixing zones. This repetitive mixing and settling process, ideal for solute partitioning, occurs at over 13 times per second[79].

The procedure for loading the instrument[80] consists first of pumping stationary phase into the stationary coil and then introducing mobile phase, after commencing rotation. When the necessary stationary phase has been displaced and mobile phase alone leaves the coil, the sample is introduced *via* a loop. Sample volumes between 10 and 30ml are recommended. Furthermore, since the sample may be introduced in either the mobile or stationary phase or a mixture of the two phases sample solubility seldom presents a problem.

For successful separations the choice of the two-phase solvent system is critical. Unlike DCCC which is limited to solvents which form droplets the centrifugal apparatus can accommodate a broader variety of solvent systems, ranging in polarity from hexane-H_2O, EtOAc-H_2O, 1-butanol-H_2O and 2-butanol-H_2O. The solute partitioning coefficient can be systematically shifted by varying the content of a third or fourth component. Either phase may be chosen as the mobile phase. For good retention of the stationary phase rapid partitioning of the phases is a pre-requisite.

EtOAc-H_2O-MeOH-hexane proved to be a useful solvent system for the separation of the diastereomers (**38**) and (**40**) as well as the diastereomers (**42**) and (**43**). Partitioning of solutes into the organic layer is usually favoured by increasing the EtOAc content, while transfer to the aqueous phase is promoted by adding MeOH. In this way suitable partition coefficients[81] (ca. K = 1) may be obtained for compounds over a wide polarity range. For each diastereomeric pair, solvent ratios were varied until a relatively even distribution of these compounds was observed in the upper and lower solvent phases as judged by TLC. Separation of these diastereomers

proved to be extremely sensitive to the solvent composition. Good separations with the upper phase as the mobile phase were only achieved after a fair degree of "trial and error".

A far better method involved the employment of a gradient elution for the separation of these compounds. This method allowed greater flexibility without the need for the relatively precise mixing ratios required when using an isocratic elution system. Using the solvent system H_2O-EtOAc-hexane-MeOH (30:48:24:21), with the upper phase as the mobile phase, and increasing the polarity of this phase in a linear fashion with water saturated EtOAc (**Fig. 3.**) separation of all four compounds was readily achieved.

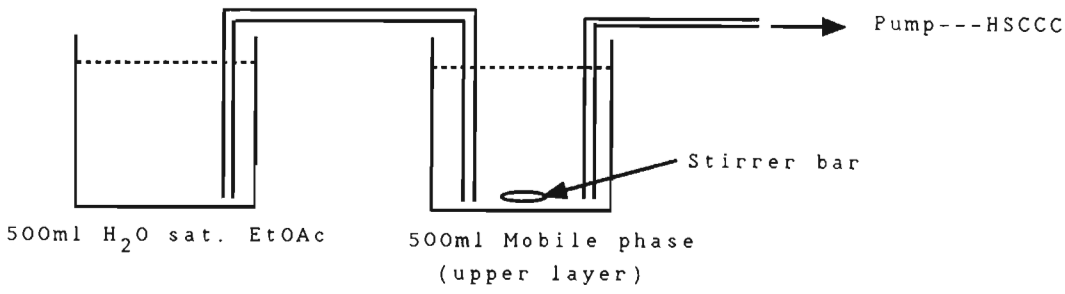


Fig. 3. Gradient elution system.

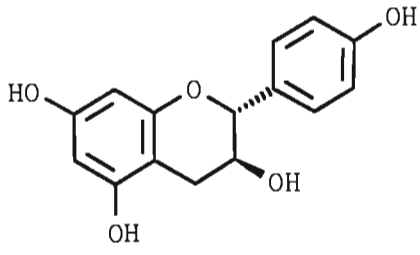
A solvent flow rate of 60ml/h and sample loads of between 350 and 450mg was achieved. Increasing any of these parameters resulted in poor retention of the stationary phase. Separation times were relatively slow, varying between 8 hours for compounds (**42**) and (**43**) and 12.5 hours for compounds (**38**) and (**40**).

Solvent flow rate and sample loading is dependent to a large degree on the composition of the solvent system. It is however worth noting that solvent flow rates of 240ml/h and sample loads of up to 2g are possible[77]. Good resolution, simple operation and low solvent consumption makes HSCCC a versatile technique for the separation of natural products.

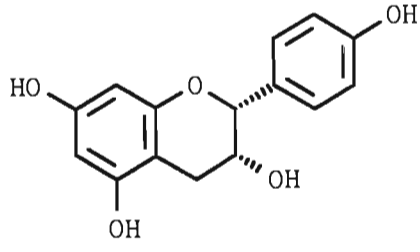
2.3. STRUCTURAL ELUCIDATION

2.3.1. Afzelechin and epiafzelechin

Compounds (**36**) and (**37**) were both isolated as pale yellow crystalline solids and identified as afzelechin[82] (**36**) and epiafzelechin[83] (**37**) respectively, by comparison of their spectroscopic and physical data with those described in the literature.



(36)



(37)

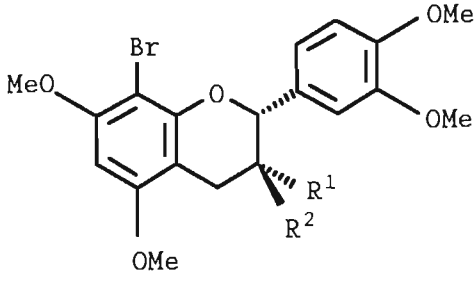
Both afzelechin and epiafzelechin were isolated from the stem bark of *C. gerrardii* (0.0016% and 0.0016% respectively), *C. gummiflua* [0.006% and (0.001% detected *via* ^{1}HNMR, but not isolated) respectively] and *C. mossambicensis* (0.003% and 0.008% respectively).

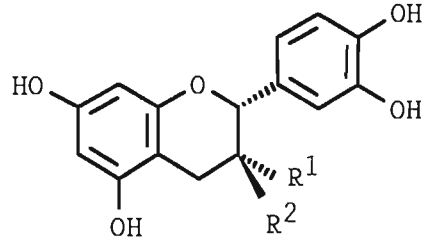
Most of the compounds isolated during the course of this work are based on a 5,7,4'-flavan-3-ol nucleus, thus (**36**) and (**37**) serve as useful model compounds. It is therefore appropriate to discuss the conformation of the flavan heterocycle in these type of compounds, and to compare, briefly, the ^{13}C- and ^{1}H-NMR spectra of (**36**) and (**37**).

2.3.1.1. Conformation of the flavan heterocycle

The preferred conformation of flavan derivatives in which the heterocyclic ring approximates a half chair conformation,

with the B-ring in a quasi-equatorial position has been discussed by Clarke-Lewis[84]. More recently, definitive evidence of the shape of the flavan-3-ol ring system has come from X-ray analysis of 8-bromo-5,7,3',4'-tetra-OMe-catechin[85] (**46**), 8-bromo-5,7,3',4'-tetra-OMe-epicatechin[54] (**47**) and epicatechin[86] (**48**).

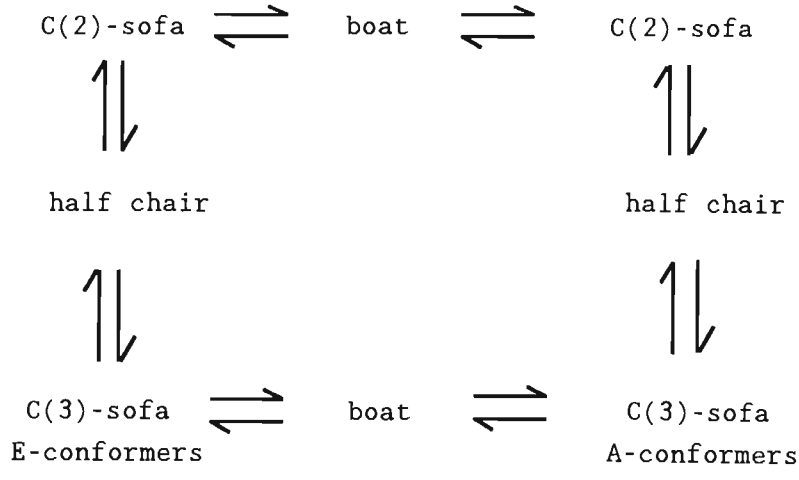




(46) R^1=H; R^2=OH
(47) R^1=OH; R^2=H

(48) R^1=OH; R^2=H
(49) R^1=H; R^2=OH

Based on combined X-ray, crystallographic, molecular mechanical (MM2) and variable temperature ^{1}HNMR studies of (**48**) and (**49**) and their derivatives Porter, Mattice and co-workers[87] have shown that the C-ring conformation may be described in terms of the C-ring equilibrium:



where E- and A-conformers are those with the B-ring equatorial or axial respectively. **Fig. 4.** depicts the ground-state energy conformations that may be adopted by the flavan heterocycle. The conformations are viewed along the plane indicated by the arrow.

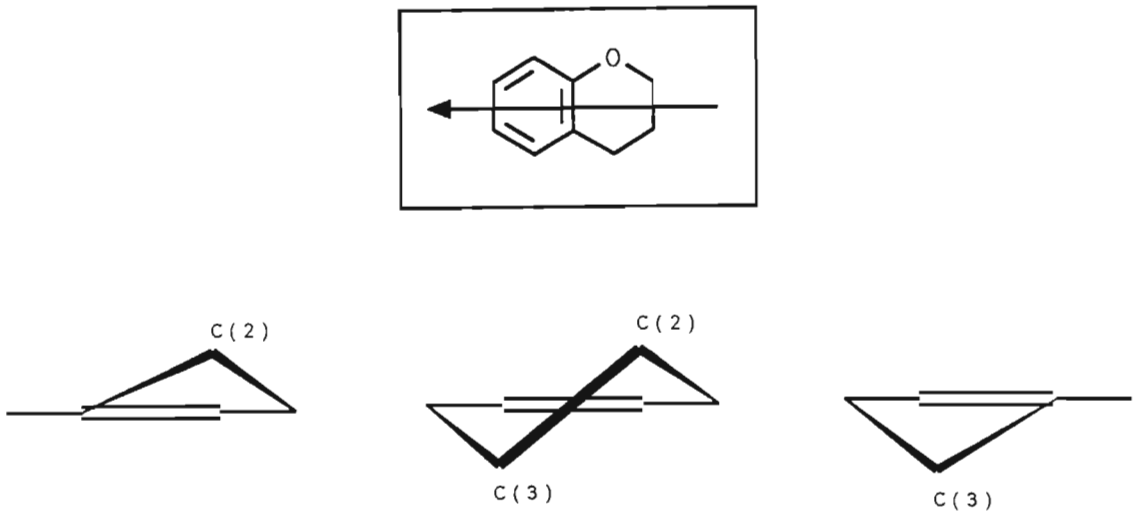


Fig. 4. Ground-state energy conformations adopted by the flavan heterocycle.

The conversion of E- to A-conformers, or *vice versa* proceeds *via* the boat conformation which is the high energy transition state. An unequal conformational energy for the E- and A-conformers is manifested by an unequal population of the two states, the one with the lower energy being populated to a greater extent.

Using low temperature ^{1}HNMR spectroscopy, conformational exchange was demonstrated in both catechin (**49**) and epicatechin (**48**) with E:A ratios of 62:38 and 86:14 respectively[87]. Acetylation of the 3-hydroxy group of catechin altered the ratio to 48:52, thus favouring the A-conformation.

In both (**48**) and (**49**) substitution at C(4) by a hydroxy or aryl substituent strongly favoured the E-conformation. This was explained by a balance between strain energies arising from 1,2-repulsions, 1,3-diaxial interactions, and the *pseudo*-allylic effect[88]. It was concluded that the preferred conformers of catechin-4 and epicatechin-4 units would adopt the C(2)-sofa and the half-chair ground state energy conformations respectively.

2.3.1.2. NMR spectra of afzelechin and epiafzelechin

The diastereomers afzelechin and epiafzelechin differ in their stereochemistry at the C(3) stereocenter. As expected the ^{1}HNMR spectra of (**36**) and (**37**) are similar, differing mainly in the coupling constants of the C-ring proton resonances (**Table 2**.).

Table 2. ^{1}HNMR (200MHz) spectral data for compounds (36) and (37) in $[(CD_3)_2CO]$.

H	36	37
2(C)	4.59 *d* (7.69)	4.92 *br s*
3(C)	4.04 *m*	4.193 *m*
$4(C)_{ax}$	2.94 *dd* (5.27, 16.02)	2.88 *dd* (4.33, 16.74)
$4(C)_{eq}$	2.53 *dd* (8.48, 16.02)	2.73 *dd* (3.11, 16.74)
6(A)	5.87 *d* (2.31)	5.91 *d* (2.31)
8(A)	6.02 *d* (2.31)	6.01 *d* (2.31)
2',6'(B)	7.25 *d* (8.70)	7.35 *d* (8.73)
3',5'(B)	6.83 *d* (8.70)	6.80 *d* (8.73)

In the ^{1}HNMR spectra of flavan-3-ols and the polymeric B-type proanthocyanidins the signal due to the proton at position 2 on the heterocycle is of particular diagnostic value. A large

coupling constant ($J_{2,3}$ ca. 7-9Hz) indicates a 2,3-*trans* stereochemistry, while a small coupling constant ($J_{2,3}$ ca. 1Hz) indicates a 2,3-*cis* stereochemistry.

An interesting observation may be made concerning the ^{1}HNMR spectra of (**36**) and (**37**). Besides the obvious difference in the signals arising from the C-ring protons, the H-2',6'(B) proton signals of (**36**) are upfield (δ-0.10) relative to the same signals in (**37**). This relative upfield shift suggest greater steric interaction between these protons and the 3(C)-OH group. This observation is confirmed by Dreiding models and may serve as a method for determining the 2,3-relative stereochemistry of the upper unit in A-type proanthocyanidins (see later).

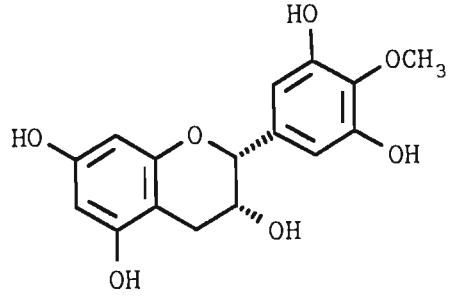
2.3.1.3. Relative *vs.* absolute stereochemistry

A number of flavan-3-ols with the 2S or so called *ent*-configuration have been isolated. This enantiomerism is not restricted to the flavan-3-ols. A number of proanthocyanidins of the B-type, and in particular those of the A-type, have been isolated containing one or more units with the *ent*-configuration. It is therefore clear that, although the coupling constants provide useful information concerning the relative stereochemistry, they are no longer acceptable as being indicative of the absolute stereochemistry in these compounds.

The proposal by Ellis[89], that proanthocyanidins with the 2S stereochemistry arise from their 2R counterparts by the action of a C-2 epimerase enzyme seems plausible, particularly if one considers that in cases where proanthocyanidins with the 2S configuration have been isolated, the corresponding 2R isomers generally co-occur.

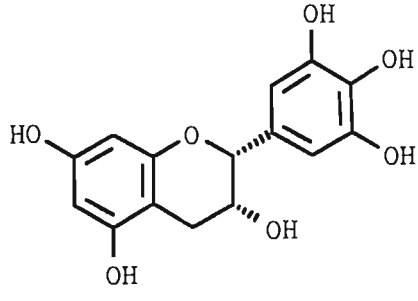
2.3.2. 4'-OMe-epigallocatechin

Compound (**45**) was isolated from the stem bark of *C. mossambicensis* (0.0125%) and identified as 4'-OMe-epigallocatechin.



(45)

The ^{13}C- and ^{1}H-NMR spectra of (**45**) were similar to those recorded in the literature for epigallocatechin[90] (**50**), the significant differences being the inclusion of a 3H singlet at δ3.821 in the ^{1}HNMR spectrum and an additional ^{13}CNMR resonance at δ61.077.



(50)

The HETCOR spectrum of (**45**) showed strong coupling between these signals. The chemical shift of these signals are characteristic of a methoxy group and it was thus logically inferred that (**45**) was an O-methylated epigallocatechin. These observations are supported by the positive FAB mass spectrum which displayed a $[M+H]^+$ ion at m/z 321. The methoxy group was established to be located at position 4'(B) based

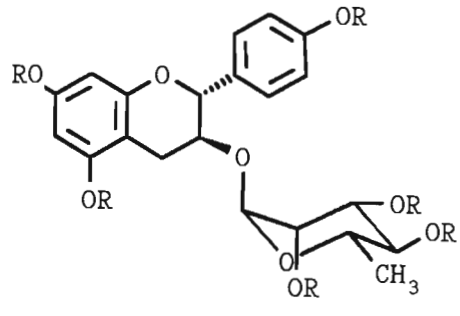
on the downfield shift of C-4'(B) (δ+3.18) in (**45**) relative to the same signal in (**50**). This was confirmed by the DELAYED HETCOR (10Hz) spectrum, which displayed weak, but distinct coupling between the methoxy protons and C-4'.

4'-OMe-epigallocatechin was origionally isolated from the root of *Ouratea sp.*[91] and given the trivial name Ourateacatechin. This compound has subsequently been isolated from the root bark of further species in the Celastracea[92,93,94].

Comparison of physical and spectral data of (**45**) with that recorded in the literature serves to confirm the proposed structure. The isolation of (**45**) from *C. mossambicensis* is significant in that its occurrence has hitherto been restricted to species in the Celastracea.

2.3.3. Afzelechin-3-O-α-L-rhamnopyranoside

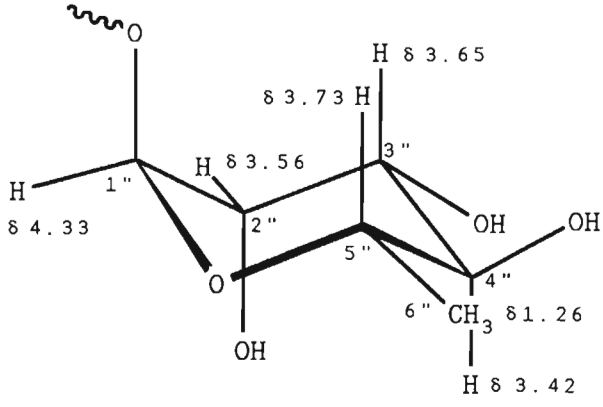
The novel flavan-3-ol glycoside (**39**) was isolated as a semi-crystalline hygroscopic solid from *C. gummiflua* (0.023%), *C. gerrardii* (0.0007%) and *C. mossambicensis* (0.030%). Its structure has been established from spectroscopic and hydrolytic studies as afzelechin-3-O-α-L-rhamnopyranoside[74].



(**39**) R = H

(**39a**) R = Ac

The ^{1}H-NMR and ^{13}C-NMR spectra of (**39**) recorded in acetone-d_6 clearly revealed the presence of a 5,7,4′-trihydroxy flavan-3-ol nucleus. Furthermore, the proton coupling constant of H-2(C) (δ4.74, *d*, *J*=7.85) was consistent with a 2,3-*trans* relative stereochemistry. The remaining ^{13}C- and ^{1}H-NMR signals, together with the positive FAB mass spectrum which displayed an $[M+H]^+$ ion at *m/z* 421, suggested the presence of a carbohydrate moiety of the methyl pentose type. Analysis of the ^{1}HNMR spectrum following D_2O exchange, in conjunction with the COSY spectrum enabled ^{1}H-^{1}H correlations and coupling constants of the remaining proton signals to be established as follows:



$J_{1",2"}$ =1.57Hz

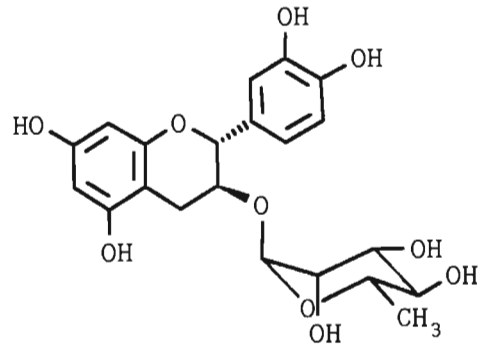
$J_{2",3"}$ =3.35Hz

$J_{3",4"}$ =9.44Hz

$J_{4",5"}$ =9.44Hz

$J_{5",6"}$ =6.20Hz

Comparison of these results, together with the chemical shift positions of the remaining carbons with those recorded in the literature for the structurally closely related compound, catechin-3-O-α-L-rhamnopyranoside (**51**) isolated from *Quercus miyagii*[95] confirms the rhamnose moiety.



(51)

It has been reported that the configuration at the anomeric carbon centres of rhamnose may be assigned on the basis of the $J_{H-1"}$ coupling constant[95]. Based on an examination of the Dreiding models for both the α- and β- anomer it would appear that the dihedral angle between H-1" and H-2" in both anomers is very similar (ca. 55°) and should thus lead to almost identical coupling constants for these protons. The α-configuration was thus assigned to the glycosidic linkage based on the chemical shift of C-3" and C-5" of rhamnose, in which the signals due to the α-anomer (δ72.5 and 69.4 respectively) resonate at higher field than those in the β-anomer (δ75.4 and 73.5)[96,97].

The location of the rhamnose moiety was judged to be at C-3 based on the downfield shift of this resonance (δ+6.58) relative to the same signal in afzelechin (**36**). Enzymic hydrolysis with hesperidinase afforded the aglycone which was confirmed to be afzelechin by comparison with an authentic sample.

Acetylation of (**39**) gave a hexa-acetate (**39a**) which was also fully characterised and which serves to confirm the structure of (**39**) as afzelechin-3-O-α-L-rhamnopyranoside. Although flavan-3-ols are widely distributed in nature their glycosides are rarely found. It has been suggested[52] that

their apparent scarcity may be due to incorrect techniques used for their isolation. Although (**39**) gave a positive colour reaction with both anisaldehyde and benzidine reagents, it did not stain as intensely as either the flavan-3-ol monomers (**36**) and (**37**), or the flavonol glycoside (**44**) and consequently was almost overlooked. If this relative insensitivity towards these commonly used stain reagents is displayed by the flavan-3-ol glycosides in general, it might further explain their apparent scarcity.

The following interesting observations could be made from a study of the delayed ^{1}H-^{1}H COSY spectrum of (**39**) using a coupling constant of 5Hz:

i) Weak, but quite distinct coupling between the phenolic proton signals on C-5 and C-7 while that from C-4′ shows no coupling. This allows accurate "labelling" of the position of the phenolic protons.

ii) Distinct coupling between the signals from the C-4′ hydroxyl group and the signals from 2′,6′ (but not 3′,5′) protons and between C-7 hydroxyl and H-6 and H-8.

iii) Very strong couplings between the signals from H-2′,6′ and H-2 and slightly weaker couplings between H-3′,5′ and H-2.

iv) Strong coupling between the signals from H-8 to H-4 and the corresponding coupling between H-6 and H-4.

These long range couplings served to confirm the proposed structure as well as providing a generally applicable method for establishing the relationship between protons separated by more than one bond.

2.3.4. Kaempferol-3-O-α-L-rhamnopyranoside

The flavonol glycoside (**44**) was isolated from the stem bark of *C. gummiflua* (0.026%) as a pale yellow amorphous powder and identified as kaempferol-3-O-α-L-rhamnopyranoside.

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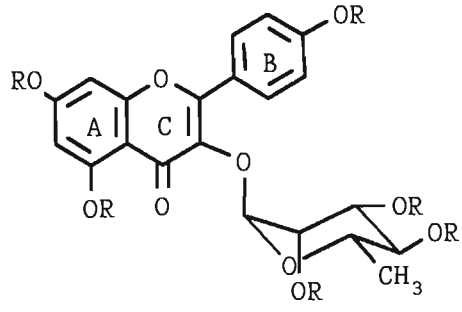
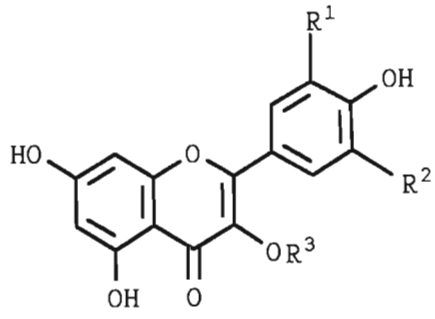


(44) R = H

(44a) R = Ac

In the phenolic region of the ^{1}HNMR spectra of (**44**), the 5,7,4'-trihydroxy substitution pattern is clearly discernible. The remaining proton signals, including the characteristic anomeric proton resonance at (δ5,41, *d*, *J*=1.74Hz) and the methyl resonance at (δ0.96, *d*, *J*=5.79Hz) clearly suggests the presence of a methyl pentose glycoside. From the ^{13}CNMR spectrum of (**44**), and based on the presence of a carbonyl resonance at δ179.45 together with the olefinic resonances at δ159.13 and δ136.13 it was logically inferred that the aglycone was a flavonol. The positive FAB mass spectrum of (**44**) showed a $[M+H]^{+}$ peak at *m/z* 434 consistent with a molecular formula $C_{21}H_{20}O_{10}$. Comparison of the ^{13}CNMR data of (**44**) with that published[98] for kaempferol (**52**), kaempferol-3-O-β-D-glucoside (**53**), myricetin-3-O-α-L-rhamnopyranoside (**54**) and quercetin-3-O-α-L-rhamnopyranoside (**55**) served to establish the structure of (**44**) as kaempferol-3-O-α-L-rhamnopyranoside.



(52) $R^1=R^2=R^3=H$

(53) $R^1=R^2=H$; R^3=glucose

(54) $R^1=R^2=OH$; R^3=rhamnose

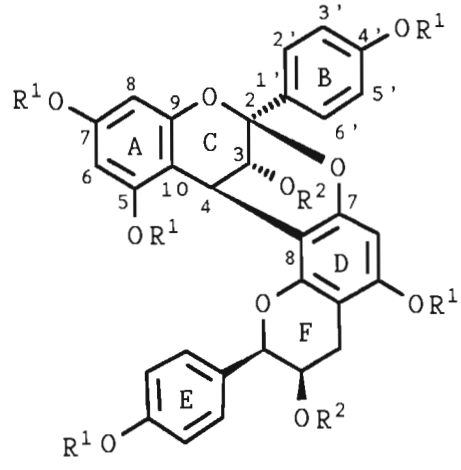
(55) $R^1=OH$; $R^2=H$; R^3=rhamnose

The ^{13}CNMR shifts of the carbons on the sugar moiety are in close agreement with the corresponding signals of the flavonol rhamnosides (**54**) and (**55**). Comparison with both (**52**)and (**53**) leaves no doubt that the aglycone is kaempferol. Furthermore, the downfield shift of C-2 (δ +12.33) in (**44**) relative to the same signal in (**52**) is consistent with glycosidation at C-3.

Although biogenetically closely related to the flavan-3-ol glycosides, flavonol glycosides, unlike the flavan-3-ol glycosides have a widespread distribution in the plant kingdom. The isolation of kaempferol-3-O-α-L-rhamnopyranoside has been reported from a number of plant sources and comparison of physical and spectral data of (**44**) and the hexa-acetate derivative (**44a**) to that recorded in the literature[99] serves to confirm the proposed structure.

2.3.5. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin

The A-type proanthocyanidin dimer (**38**) was isolated from the stem bark of *C. gerrardii* (0,036%), *C. gummiflua* (0.045%) and *C. mossambicensis* (0.109%) and identified as epiafzelechin-(4β→8,2β→O→7)-epiafzelechin.



(38) $R^1=R^2=H$

(38a) $R^1=Me$; $R^2=H$

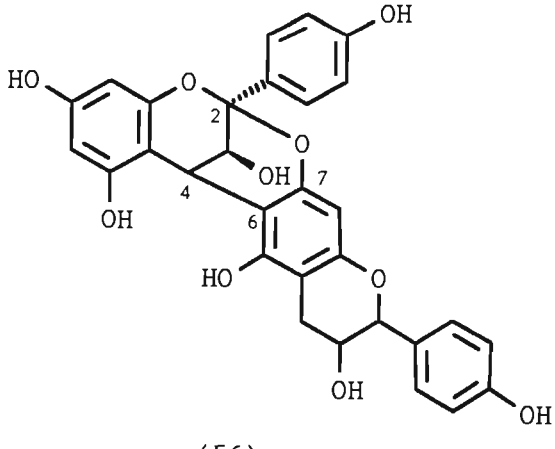
(38b) $R^1=Me$; $R^2=Ac$

The positive FAB mass spectrum of compound (**38**) displayed an $[M+H]^+$ ion at *m/z* 545. Upon methylation with ethereal diazomethane compound (**38**) yielded a pentamethyl ether (**38a**) which was acetylated with acetic anhydride in pyridine to afford the pentamethyl ether di-acetate (**38b**). It was thus inferred that (**38**) had, as part of it's structure, five phenolic and two alcoholic hydroxyl groups.

Analysis of the ^{1}HNMR spectrum of (**38**) revealed, at lowest field in the aromatic region of the spectrum, a pair of A_2B_2 spin systems. The chemical shifts and coupling constants of these signals were similar to those for the B-ring proton signals of epiafzelechin (**37**). The chemical shifts and coupling constants of several other signals in the ^{1}HNMR

spectrum were also similar to those recorded for epiafzelechin. Of these, a pair of *meta*-coupled doublets (δ6.05 and 6.11, both *J*=2.6Hz) were similar to the A-ring signals for epiafzelechin and three signals in the aliphatic region of the spectrum at δ5.0, 4.1 and 2.9 closely matched the C-ring signals of epiafzelechin. A 1H singlet at δ6.13 suggested the presence of a penta-substituted benzene with oxygen functions at the 1,3,5-positions. These observations implied that (**38**) comprised of two 5,7,4'-trihydroxy-flavan-3-ol units. The remaining proton signals, present as a pair of 1H doublets (*J*= 3.5Hz) at δ4.10 and δ4.46 together with the ^{13}CNMR resonances at δ29.25 and ketal resonance at δ100.26 indicate that the flavan-3-ol units are joined *via* a C-4(C) carbon-carbon linkage and a C-2(C) ether linkage. The characteristic coupling constants and chemical shifts of these signals are generally regarded as being of diagnostic value for establishing the presence of A-type linkages in these type of compounds[50,67,71].

Three types of linkage isomers are possible *viz.* (4→6,2→O→7) (**56**), (4→6,2→O→5) (**57**) and (4→8,2→O→7) (**38**).



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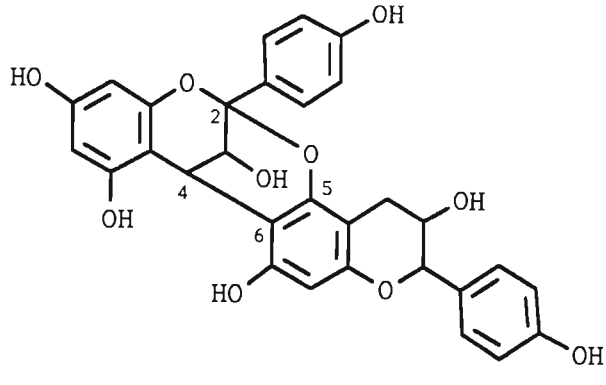


(57)

Selection of the mode of linkage could not be determined with any certainty from a study of the ^{13}C- and ^{1}H-NMR spectra of (**38**). The ^{1}HNMR spectrum of the pentamethyl ether derivative (**38a**) showed, together with four methoxy signals between δ3.72 and 3.82, one upfield methoxy signal at δ3.49. The ^{1}H-^{1}H COSY spectrum of (**38a**) displayed weak, but distinct coupling between the high field methoxy proton signal and H-6(A). These results established the methoxy group to be located at position 5(A). This unusual upfield shift may be interpreted in terms of a through space interaction with the lower structural unit and is only possible in the (4→8,2→O→7) mode of linkage represented by (**38**).

The absolute stereochemistry at C-4(C) for (**38**) was established from the sign of the Cotton effect at ca. 200-220nm on the basis of the report[100], that such compounds having 4R configurations exhibit a positive couplet and those with 4S configurations a negative couplet in this wavelength region. Circular dichroism measurements in EtOH revealed a negative Cotton effect at 271nm ($[\theta]=-16.6 \times 10^3$) and a positive Cotton effect at 222nm ($[\theta]=71.9 \times 10^3$), thus indicating a 4R configuration. The configuration at C-2(C) is thus automatically assigned. The 3R configuration was tentatively assigned to C-3(C) based on the observation that the proton resonance for H-2',6'(B) (δ7.53, J=8.8Hz) matched closely with the corresponding signal for epiafzelechin (**37**) (δ7.4, J=8.7Hz).

Following a literature search on A-type proanthocyanidins compound (**38**) was found to be identical to mahuannin B, previously isolated from the roots of *Ephedra sp.*[48]. These workers determined the absolute stereochemistry of mahuannin B by application of NMR and CD spectroscopy. They also confirmed the absolute stereochemistry at the C-3 positions by esterification of the pentamethyl ether derivative (**38a**) with (±)-2-phenylbutanoic acid by application of the Horeau

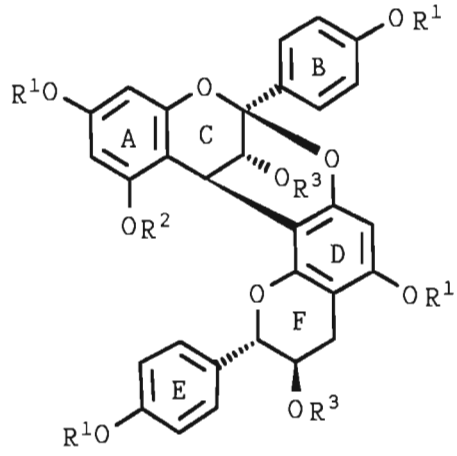
Brooks method.

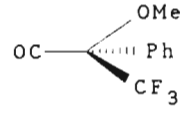
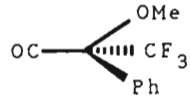
No data for the derivative (**38a**) and only partial data for the derivative (**38b**) was reported[48]. Both these derivatives have now been fully characterised. It is interesting to note that the signal due to H-3(C) in the ^{1}HNMR spectrum of the pentamethyl ether derivative (**38a**) is unusual. Instead of appearing as a "characteristic" doublet (J=3.5-4.0Hz), additional coupling is observed (δ4.717, *dd*, J=3.44, 5.94Hz). The ^{1}H-^{1}H COSY spectrum clearly reveals that this additional coupling is due to OH-3(C) (δ1.941, *d*, J=5.94Hz).

In general, the ^{1}H-^{1}H COSY spectra, often in conjunction with the DELAYED ^{1}H-^{1}H COSY spectra, proved extremely useful for the accurate assignment of the ^{1}HNMR signals in the spectra of the A-type dimers and their derivatives.

2.3.6. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin

The novel A-type proanthocyanidin[75] (**40**) was isolated as a pale yellow amorphous powder from the stem bark *C. gummiflua* (0.105%), *C. gerrardii* (0.09%) and *C. mossambicensis* (0.218%). Its structure has been established from spectroscopic studies as epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin and is the major compound in the EtOAc soluble extracts from both *C. gerrardii* and *C. mossambicensis*. The absolute stereochemistry at the C-3 stereocenters was determined by application of a novel modified Mosher's method (Mouton, C.H.L., Steenkamp, J.A., and Ferreira, D. unpublished results).



	R^1	R^2	R^3	
(40)	H	H	H	
(40a)	Me	H	H	
(40b)	H			(R)
(40c)	H			(S)
(40d)	Me	Me	Me	

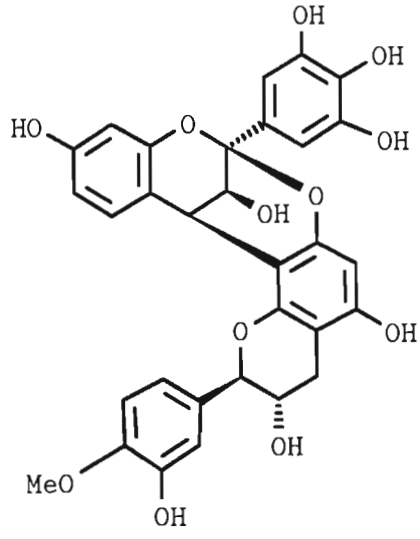
The positive FAB mass spectrum of (**40**) was identical to that recorded for epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**38**) (mahuannin B). The ^{13}C- and ^{1}H-NMR spectra of (**40**) were similar to those recorded for (**38**), the significant differences being only the chemical shifts and ^{1}H coupling constants of the F-ring protons and carbons. The presence of a 1H doublet (δ4.83, J=7.95Hz) in the ^{1}HNMR spectrum of (**40**) attributed to H-2(F) clearly indicates a 2,3-*trans* configuration of the lower F-ring substituents. Furthermore, the presence of a pair of 1H doublets (J=3.56Hz) at δ4.12 and δ4.13 together with ^{13}CNMR resonances at δ29.5 and δ100.37 indicate that the flavan-3-ol units are joined *via* a C-4(C) carbon-carbon linkage and a C-2(C) ether linkage. Methylation of (**40**) with ethereal diazomethane yielded the tetramethyl ether (**40a**) and not a pentamethyl ether as expected. The ^{1}HNMR spectrum of (**40a**) revealed the presence of a sharp 1H

singlet at δ6.67. Based on the HETCOR spectrum of (**40a**) it was concluded that the proton was phenolic. The DELAYED HETCOR (7Hz) spectrum displayed strong coupling between the phenolic proton and the adjacent C-5(A) and slightly weaker coupling to the C-6(A) and C-10(A) carbons. These results establish that the phenolic hydroxyl is located at position 5(A). The unusual high field shift and uncharacteristic sharpness of this phenolic proton resonance, together with the definite non-reactivity towards diazomethane, suggests a through-space interaction with the lower structural unit. Of the three possible linkage isomers *viz.* (4→6,2→O→7), (4→6,2→O→5) and (4→8,2→O→7) stereomodels suggest that it is only in the latter mode of linkage that such steric interactions could exist. Confirmation of the mode of linkage was established by applying the powerful ^{1}HNOE technique to the tetramethyl ether (**40a**). Besides the stereochemically insignificant but structurally important NOE association of 5(A)-OH (δ6.67) with H-4(C) (δ4.31, 19.1%) and H-6(A) (δ6.01, 3.4%) this proton exhibited selective associations with both H-2',6'(E) and H-2(F) (δ7.34, *d*, *J*=6.4Hz; δ4.83, *d*, *J*=7.95Hz; 8.0% and 0.73% respectively). These selective NOE associations thus establish the (4→8,2→O→7) mode of linkage represented by (**40**). The absolute stereochemistry at C-4(C) for (**40**) was established from the sign of the Cotton effect near 230nm[101]. Circular dichroism measurements in MeOH revealed a negative Cotton effect at 258nm ([θ] =-5.8 X 10^3) and a positive Cotton effect at 232nm ([θ] =1.7 x 10^3). This indicates a 4R configuration. The configuration at C-2(C) is thus automatically assigned.

2.3.6.1. <u>Configuration at the C-3(C) and C-3(F) stereocenters</u>

The characteristic C-ring proton signals for H-3(C) and H-4(C) in the A-type proanthocyanidins invariably display coupling constants ($J_{3,4}$=3.5-4.0Hz) for these signals. By reference to X-ray data for proanthocyanidin A-2 (**27**) and ^{13}CNMR comparisons, these coupling constants have

consequently been accepted as being indicative of a 3,4-*trans* configuration in these type of compounds. Dreiding models of the A-type proanthocyanidins show very similar dihedral angles (ca.60°) between H-3(C) and H-4(C) in both 3,4-*trans* and 3,4-*cis* analogues which should lead to almost identical coupling constants for these protons. These observations were confirmed by Ferreira *et al.*[71] following their unambiguous synthesis of robinetinidol-(4β→8,2β→O→7)-4'-OMe-catechin (**58**) which possesses the 3,4-*cis* configuration of the heterocyclic C-ring.



(58)

Furthermore, these workers demonstrated that A-type analogues with the 3,4-*cis* configuration or 3,4-*trans* configuration could be differentiated on the basis of ^{1}HNOE techniques, which may be summarised as follows:

Selective NOE associations were observed between H-3(C) and H-8(A) in A-type proanthocyanidins with the 3,4-*cis* configuration but not in those analogues with the 3,4-*trans* configuration. In the acetate derivatives, selective NOE associations were only observed between H-3(C) and H-6(D) in those analogues with the 3,4-*trans* configuration.

Clearly, in order to complete the structure determination of (**40**), assessment of the absolute stereochemistry of not only the lower unit but also at the C-3(C) stereocenter is required.

It has been established that ^{1}HNMR analysis of R-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of different sets of enantiomeric flavan-3-ols and 4-arylflavan-3-ols, respectively, permits assignment of the absolute configuration at C-3 in these compounds[104]. By comparing the ^{1}HNMR data in both the R-(+)- and S-(-)-MTPA esters, this methodology has been extended to assess the absolute configuration in these compounds when only one isomer is available (Mouton, C.H.L., Steenkamp, J.A. and Ferreira, D., unpublished results). A structural appraisal of the A-type proanthocyanidins revealed that this method would be well suited for assigning the absolute stereochemistry at C-3(C) and C-3(F) in these compounds. The principle of the technique is outlined as follows:

Mosher proposed[102,103] that, in solution, the conformation of the R-(+)- and S-(-)-MTPA esters is such that the carbinyl proton, ester carbonyl and trifluoromethyl groups of the MTPA moiety lie in the same plane (**Fig. 5.**). When the MTPA group is in the hypothesized conformation, Mosher pointed out that the ^{1}HNMR signal of L^2 of the R-(+)-MTPA ester will appear upfield relative to that of the S-(-)-MTPA ester due to the diamagnetic effect of the benzene ring.

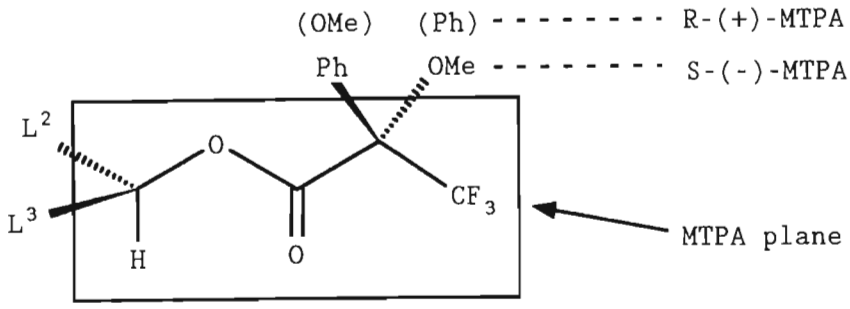


Fig. 5. Configurational correlation model for the R-(-)- and S(-)-MTPA derivatives proposed by Mosher.

Ferreira *et al*[104] have shown that the MTPA esters of flavan-3-ols and 4-arylflavan-3-ols adopt conformations compatible with those proposed by Mosher (**Fig. 5.**), in which the α-trifluoromethyl group, carbonyl and carbinyl hydrogen are in the same plane and are approximately eclipsed. Thus, in these compounds with a 3R configuration shielding, and hence an upfield shift of the B-ring proton resonances, occurs in the S-(-)-MTPA esters relative to the corresponding R-(+)-MTPA esters. Conversely, the B-ring proton resonances are upfield in the R-(+)-MTPA esters relative to those in the corresponding S-(-)-MTPA esters for those compounds with the 3S configuration.

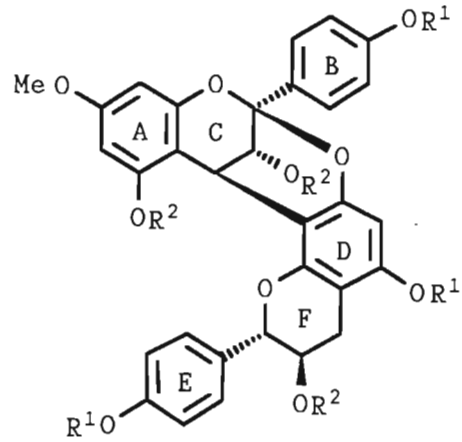
The R-(+)- and S-(-)-MTPA esters (**40b**) and (**40c**) respectively, were prepared from the tetramethyl ether (**40a**) *via* standard literature procedures[102]. ^{1}HNOE association of H-3(C) and H-3(F), H-2(F) with H-2',6'(B) and H-2',6'(E), respectively, permitted accurate labelling of these aromatic protons in both the R-(+)- and S-(-)-MTPA esters. ^{1}HNMR spectra of the R-(+)- and S-(-)-MTPA esters revealed that shielding, and therefore an upfield shift of both H-2',6'(B) and H-2',6'(E) (δ-0.17 and δ-0.18, respectively) occurs in the S-(-)-MTPA ester relative to the R-(+)-MTPA ester. Thus, the R configuration may be assigned to both C-3(C) and C-3(F). Finally, since the relative stereochemistry between H-2(F) and H-3(F) is known, C-2(F) may be assigned the S

configuration. The heptamethyl ether derivative (**40d**) was also fully characterised and serves to confirm the proposed structure of (**40**) as epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin.

An interesting observation may be made concerning the optical rotations that have been recorded for A-type proanthocyanidins[52,62,68]. Without exception, a positive rotation is observed in those compounds with the β-configuration of the interflavanoid bonds, while in those compounds with the α-configuration, a negative optical rotation is observed.

2.3.7. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin

The novel A-type proanthocyanidin (**42**) was isolated from the stem bark of *C. gummiflua* (0.5%) as a white crystalline compound and identified as 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin. It is also the major compound in the EtOAc soluble fraction from *C. gummiflua*.



(42) $R^1=R^2=H$

(42a) $R^1=R^2=Ac$

(40a) $R^1=Me$; $R^2=H$

The ^{13}C- and ^{1}H-NMR spectra of (**42**) were very similar to those recorded for epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**40**), except for the inclusion of a 3H singlet at δ3.677 in the ^{1}HNMR spectrum and a carbon resonance at δ55.63. The HETCOR spectrum of (**42**) displayed strong coupling between these signals. These results, together with the positive FAB mass spectrum which showed an $[M+H]^+$ ion at m/z 559 are consistent with an O-methylated A-type proanthocyanidin dimer. The DELAYED ^{1}H-^{1}H COSY spectrum of (**42**) displayed strong coupling between the methoxy signal and the H-6(A) and H-8(A) signals, thus establishing the methoxy group to be located at position 7(A). Additional evidence for the location of the methoxy group at position 7(A) was obtained from the selective ^{1}HNOE association of this group with both H-6(A) and H-8(A) (17% and 14% respectively).

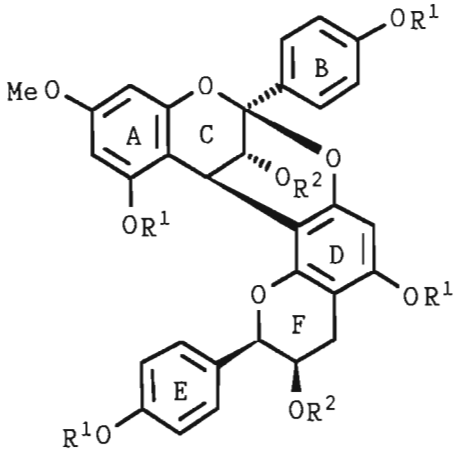
Besides the methoxy resonance and the chemical shifts of H-6(A) and H-8(A) which are downfield (δ+0.1 and δ+0.12 respectively) relative to the corresponding signals in (**40**), the chemical shifts and coupling constants of the remaining proton resonances in the ^{1}HNMR spectrum of (**42**) are virtually coincident with the corresponding signals in the spectrum of (**40**). Similarly, comparisons of the ^{13}CNMR spectra of (**42**) and (**40**) reveal that besides the obvious inclusion of the methoxy resonance, the spectra are almost identical, with only slight differences being observed in the chemical shift positions of the A-ring carbons. These differences are in close agreement with the predicted chemical shift differences between an OH- and a OMe-substituted benzene as calculated from ^{13}CNMR correlation tables[105]. These observation suggest that except for the methoxy group at position 7(A), (**42**) is identical to (**40**).

Methylation of (**42**) with ethereal diazomethane yielded a tetramethyl ether identical to (**40a**), thus confirming the structure of (**42**) as 7-OMe- epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin.

Acetylation of (**42**) with acetic anhydride in pyridine yielded the hexa-acetate derivative (**42a**) which was also fully characterised and which serves to confirm the proposed structure.

2.3.8. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin

The novel A-type dimer (**43**) was isolated from the stem bark of *C. gummiflua* (0.125%) as a white crystalline solid and identified as 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin.



(**43**) $R^1=R^2=H$

(**43a**) $R^1=R^2=Ac$

(**38a**) $R^1=Me$; $R^2=H$

The spectral data for (**43**) was very similar to those recorded for 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**42**). The positive FAB mass spectrum of (**43**) yielded an $[M+H]^+$ ion at m/z 559 identical to that obtained for (**42**). The ^{13}C- and ^1H-NMR spectra for (**43**) were similar to those recorded for (**42**), the significant differences being only the chemical shifts and ^1H coupling constants of the F-ring protons and carbons. The presence of a 1H singlet at δ4.988 attributed to H-2(F), together with the characteristic chemical shifts and ^1H coupling constants of the remaining F-ring protons clearly

indicates a 2,3-*cis* configuration of the lower F-ring substituents.

As with compound (**42**) selective ^{1}HNOE associations were observed between the methoxy resonance (δ4.98) and both H-6(A) and H-8(A) (δ6.128, *d*, *J*=1.85; δ6.223, *d*, *J*=1.85Hz; 11.2% and 9.2% respectively), thus establishing the location of the methoxy group at position 7(A).

Comparison of the ^{13}C- and ^{1}H-NMR spectra of (**43**) to those recorded for epiafzelechin-(4β→8,2β→O→7)-epiafzelechin (**38**) revealed that besides the inclusion of the methoxy resonances the spectra were almost identical, with only minor differences in the chemical shifts of the A-ring protons and carbons.

Methylation of (**43**) yielded a pentamethyl ether derivative (**38a**) identical to that obtained from the methylation of (**38**). These results thus establish the structure of (**43**) as 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin. Acetylation of (**43**) afforded the hexa-acetate derivative (**43a**) which was fully characterised and which serves to confirm the proposed structure.

Two A-type proanthocyanidin glycosides[64] and a gallate ester[65] are presumably the only known substituted A-type proanthocyanidins. The isolation of compound (**42**) and (**43**) is thus significant and represents the first isolation of O-methylated A-type proanthocyanidins.

2.3.8.1. Selective reactivity of the OH-5(A) group

The apparently selective restricted access of diazomethane to OH-5(A) in A-type analogues has previously been reported[74,50]. The non-reactivity of the OH-5(A) group in compounds (**42**) and (**40**) towards diazomethane may conveniently be explained in terms of steric effects derived from the

close proximity of the A- and E-rings. These steric effects are clearly reflected in the ^{1}HNMR spectrum of (**42a**) in which the OAc-5(A) signal resonates at unusually high field (δ2.044). That methylation of the OH-5(A) group proceeds smoothly in the epimers (**43**) and (**38**) under identical reaction conditions, is not as easily explained. Logic suggests that in these compounds, the OH-5(A) group is sterically less hindered than the corresponding epimers (**42**) and (**40**) thus permitting access to diazomethane. This then should be reflected by a downfield shift of the OAc-5(A) signal in the ^{1}HNMR spectrum of (**43a**) relative to the same signal in (**42a**). This is not the case and the OAc-5(A) signal in (**43a**) resonates upfield (δ-0.1) relative to the same signal in (**42a**). Dreiding models suggest that the distances between OH-5(A) and the lower unit in these compounds are not significantly different. Clearly then, a more plausible explanation must exist for the non-reactivity of OH-5(A) in compounds (**42**) and (**40**).

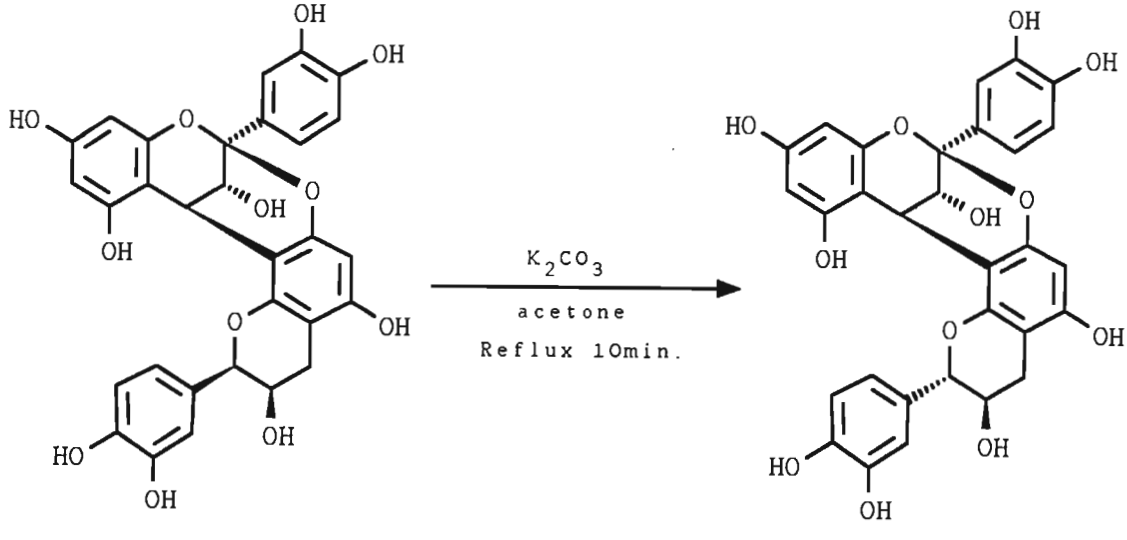
Based on Dreiding models hydrogen bonding between OH-5(A) and the F-ring heteroatom seems possible. The OH-5(A) resonance in the ^{1}HNMR spectrum of the tetramethyl ether derivative (**40a**) is uncharacteristically well resolved, suggesting that it may indeed be involved in such bonding. A closer inspection of the Dreiding models of these compounds reveals that if hydrogen bonding were to occur, the conformation adopted by the F-ring in compounds (**42**) and (**40**) places one of the lone pairs on the F-ring heteroatom in an optimal position for such bonding to occur. In compounds (**43**) and (**38**) the conformation of the F-ring is such that neither of the lone pairs on the heteroatom are optimally orientated for hydrogen bonding.

In summary, these observations suggest that hydrogen bonding will only occur in those A-type dimers with a 2(F)S-configuration combined with the (4β→8,2β→O→7) mode of linkage and in their corresponding enantiomers *viz.* those

compounds with a 2(F)R-configuration combined with the (4α→8,2α→O→7) mode of linkage. The combination of hydrogen bonding, low reaction temperatures (ca.-15°C) and steric hindrance could well explain the apparently selective restricted access of diazomethane to the OH-5(A) group in these compounds.

2.3.8.2. Base catalysed rearrangement of the 7-OMe-dimers

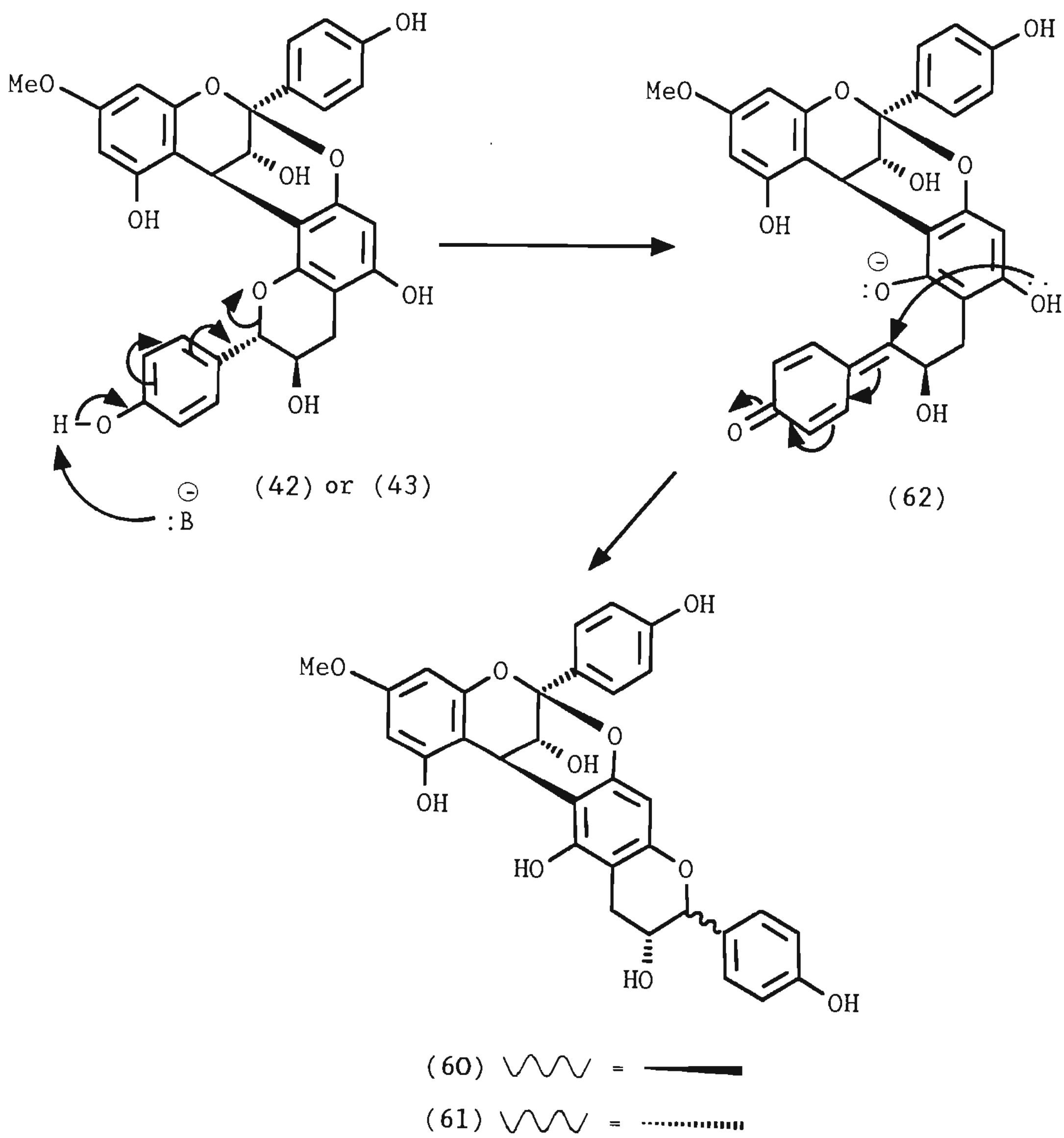
The 7(A)-OMe-dimers (**42**) and (**43**), differ only in their stereochemistry at position 2(F). Thus, a method for the conversion of (**42**) to (**43**), or *vice versa*, by epimerization of the C-2(F) stereocenter would not only sere to confirm the structures of these compounds, but also of their "parent" compounds (**38**) and (**40**). Exactly such a reaction, the base catalysed epimerization of epicatechin-(4β→8,2β→O→7)-epicatechin (**27**) has been reported[62] (**Scheme 5.**). In this report[62], treatment of (**27**) with K_2CO_3 in acetone under reflux afforded the product epicatechin-(4β→8,2β→O→7)-*ent*-catechin (**59**) in high yield (75%) after ten minutes.



(27) (59)

Scheme 5.

Thus, using this method the epimerization of compound (**42**) at position C-2(F) was attempted. Surprisingly, under these reaction conditions (**42**) yielded two new compounds identified as 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin (**60**) and 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin (**61**) in moderate yields (40% and 20% respectively) after 8.5 hours. The reaction was monitored by TLC, and at no time was the epimer (**43**) or any other intermediate detected.



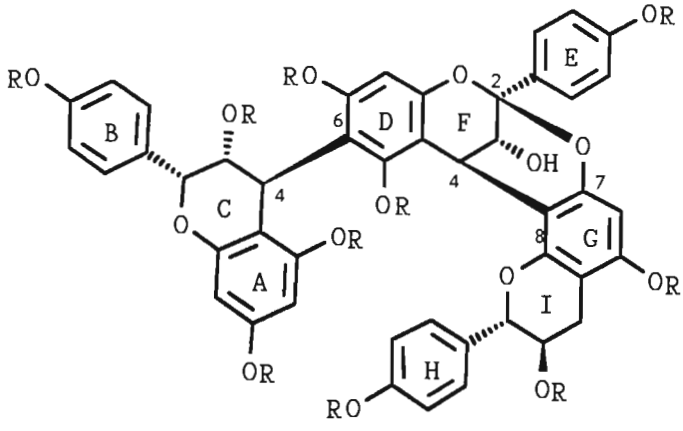
Scheme 6. Proposed route to the formation of (60) and (61).

The ^{13}C- and ^{1}H-NMR spectra of compounds (**60**) and (**61**) were almost identical to those recorded for (**42**) and (**43**) respectively, the significant difference being the $^{1}HNMR$ shift position of H-2',6'(E) and H-2(F) which suggest that the lower unit is no longer under the steric or electronic influence of the upper unit. The mechanism for the formation of these products (**Scheme 5.**) presumably occurs *via* an E-ring quinone-methide (**62**), followed by rotation about the $C_{(3)}$-$C_{(4)}$ bond and subsequent recyclization.

Perhaps of more significance, the base catalysed conversion of (**43**) under these conditions afforded identical products to those obtained from the conversion of (**42**). These results thu serve to confirm the structures of both (**42**) and (**43**).

2.3.9. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent* afzelechin

The novel A-type proanthocyanidin (**41**) was isolated from the stem bark of *C. gerrardii* (0.014%) as a brown amorphous solid and from *C.mosambicensis* (ca. 0.02%) as the peracetate derivative.



(41) R=H

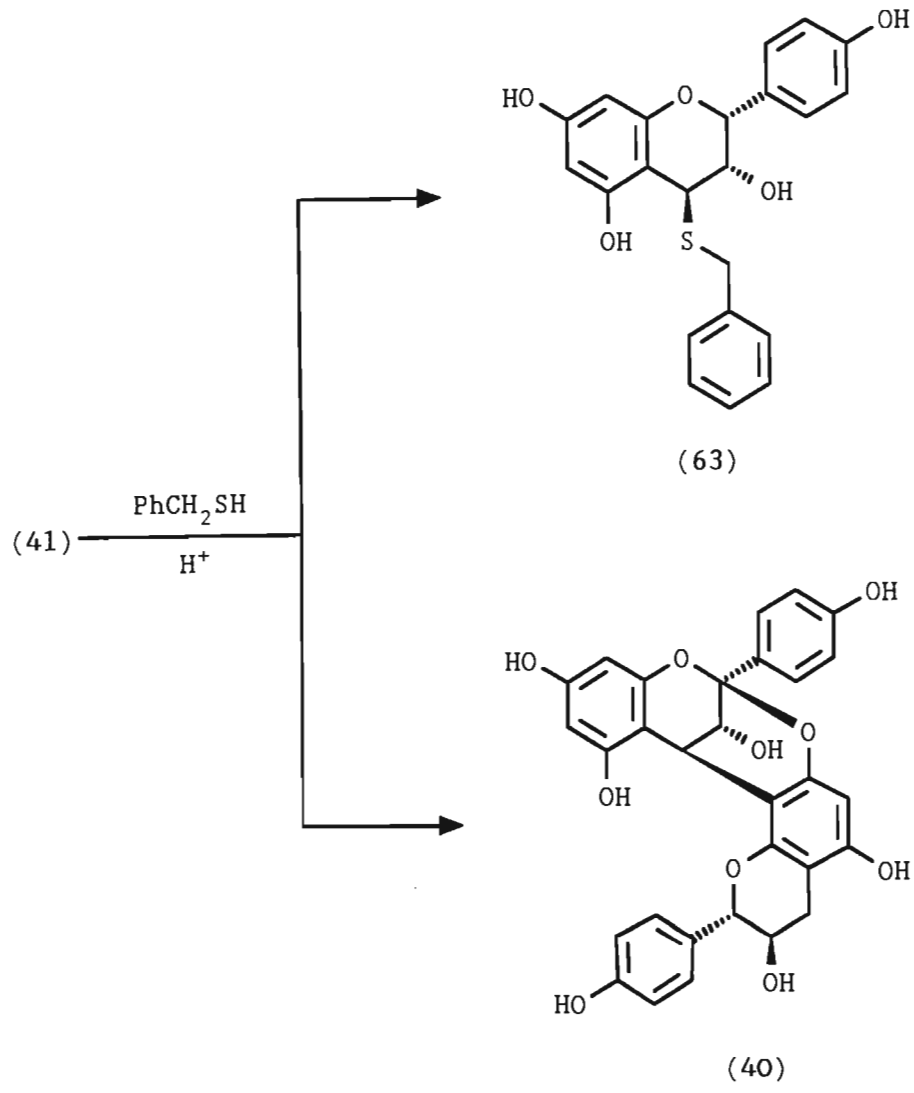
(41a) R=OAc

The positive FAB mass spectrum displayed an $[M]^+$ ion at m/z 816 which suggested a triflavanoid moiety. The ^{1}HNMR spectrum of (**41**) revealed a complicated spin pattern due to rotational isomerism[106,107,108], rendering its interpretation impossible. In contrast, the ^{1}HNMR spectrum of the peracetate (**41a**) was surprisingly free of these effects. Analysis of this spectrum revealed, at lowest field in the aromatic region, three A_2B_2 spin systems (δ7.66, 7.14, *d*, *J*=8.84Hz; δ7.40, 7.02, *d*, *J*=8.61Hz; δ7.34, 7.09 *d*, *J*=8.57Hz) suggesting the presence of three *para*-substituted aromatic rings, a pair of *meta*-coupled doublets (δ6.76, δ6.72, *d*, *J*=2.29Hz) and two residual 1H singlets at δ6.50 and δ6.51.

Analysis of the aliphatic region of the spectrum was facilitated by a study of the ^{1}H-^{1}H COSY spectrum of (**41a**) which displayed three separate spin systems. Of these, a pair of 1H doublets (*J*=3.77Hz) at δ5.56 and δ4.65 closely matched those of the C-ring signals in the acetylated derivatives (**42a**) and (**43a**). These signals, together with the ^{13}CNMR resonances at δ27.379 and ketal resonance at δ98.021 clearly indicate the presence of an A-type linkage. Furthermore, the presence of a strongly shielded acetoxy resonance at δ2.03 in the ^{1}HNMR spectrum establishes the (4→8,2→O→7) mode of linkage. Three proton resonances, including a 1H broad singlet at δ5.25, a 1H doublet of doublets (δ5.513, *J*=2.44, 1.78Hz) and a 1H doublet (δ4.796, *J*=2.44Hz) were in close agreement with the heterocyclic ring signals in peracetylated proanthocyanidins containing a B-type linkage[109]. The coupling constants were also consistent with a 2,3-*cis*-3,4-*trans* stereochemistry. The remaining spin system, including a 2H multiplet at δ2.68 and two proton signals (δ5.3-5.4, overlapping signals) together with ^{13}CNMR resonances at δ22.99, 68.276 and 78.57 closely matched the signals of the lower heterocyclic ring in compound (**42a**).

In order to establish the structures and the stereochemistry of the component units in (**41**), cleavage of the B-type

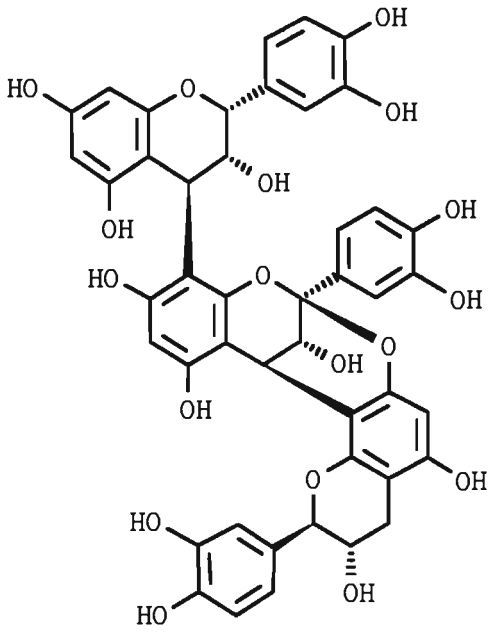
linkage was attempted[108]. Treatment of (**41**) with phenylmethanethiol and HOAc afforded two products, epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**40**) and a thioether identified as epiafzelechin-4β-benzylthioether (**63**) by comparison of its physical and spectral data with that recorded in the literature[110] (**Scheme 7**.).



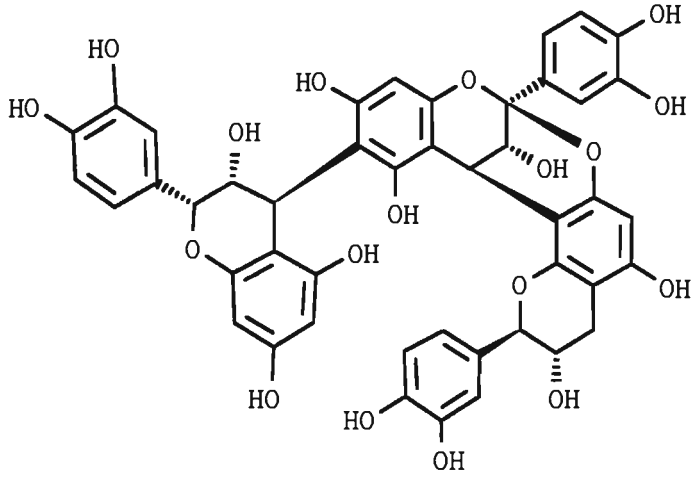
Scheme 7. Thiolytic degradation of (41).

Clearly, two possible modes of linkage exist between the "upper" monomeric unit and the "lower" A-type unit *viz*. (4β→6) and (4β→8). Selection of the mode of linkage was based on a comparison of the ^{13}C- and ^{1}H-NMR spectral properties of (**41**) with those of two related compounds (**64**) and (**65**)

isolated from *Vaccinium vitis-idaeda*[68].

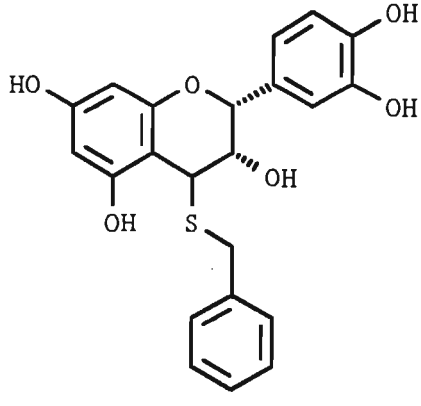


(64)

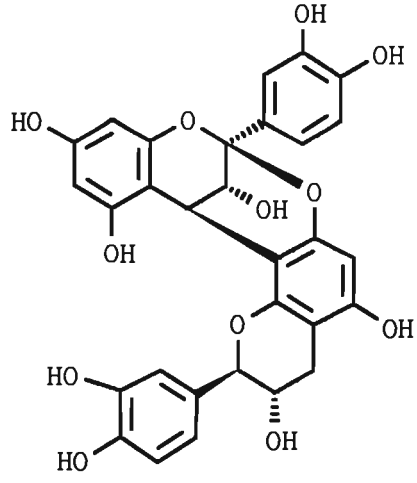


(65)

According to this report[68] both compounds yielded identical mass spectra. Upon thiolytic degradation with phenylmethanethiol both (**64**) and (**65**) yielded identical products *viz.* epicatechin-4β-benzylthioether (**66**) and epicatechin-(4β→8,2β→O→7)-catechin (**67**).



(66)



(67)

While the ^{13}C- and ^{1}H-NMR spectra for (**64**) were first order those for (**65**) were complicated owing to the existance of

several conformers. This conformational isomerism was thought to be probably caused by steric hindrance to free rotation about the interflavanoid linkages. Dreiding models revealed that in the (4→6) linked isomer the close proximity of the B- an H-rings could give rise to these effects, while in the case of the (4→8) linked isomer such steric hindrance was not observed.

Based on these results, the (4→6) mode of linkage was assigned to (**41**) thus establishing its structure as epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin.

That essentially only one conformer is evident in the ^{13}C- and ^{1}H-NMR spectra of the acetate derivative (**41a**) is surprising[109] and can only be explained in terms of the increased energy barriers to rotation introduced by the bulky acetate groups. These increased rotational energy barriers, together with the steric effects between the B- and H-rings presumably restrict free rotation to such extent that the molecule is "frozen" into one conformation only.

3. EXPERIMENTAL

3.1. GENERAL PROCEDURES AND INSTRUMENTATION

Melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were determined using Perkin-Elmer 240B and 2400 elemental analysers. NMR spectra were recorded on a Gemini 200 instrument, using tetramethylsilane as the internal standard, in the specified solvents. Optical rotations were determined on a Perkin-Elmer 241 digital polarimeter. Infra-red spectra were recorded on a Shimadzu FTIR-4300 spectrophotometer.

3.1.1. Chromatography

HSCCC was performed on a P.C. Inc. Multilayer Coil Planet CCC apparatus: Coil rotation speed, 700rpm; Solvent system, H_2O-EtOAc-hexane-MeOH (30:48:24:21), with the upper phase (500ml) as the mobile phase, and increasing the polarity of this phase in a linear fashion with water saturated EtOAc (500ml); Solvent flow rate, 60ml/hr; Maximum sample load, 450mg. Reverse phase column chromatography was carried out with Toyopearl HW-40F (30-60μm; TosoHaas) and Sephadex LH-20 (25-100μm; Pharmacia Fine Chemical Co., Ltd.) with the eluents specified. Preparative column chromatography with silica gel (230-400 mesh; Merck 60) was performed using the technique of Still *et al*[111]. Centrifugal TLC (Chromatotron, Model 7924T) was used for the separation of derivatives and for the final purification of minor compounds. All separations and reactions were monitored by TLC using precoated Kieselgel 60 F_{254} plastic sheets (0.2mm; Merck). Spots were detected with benzidine or anisaldehyde-H_2SO_4 reagents. For silica gel based separations CH_2Cl_2-Me_2CO-MeOH with solvent ratios between (70:30:0) and (39:60:1) was used as the mobile phase for natural products and CH_2Cl_2-Me_2CO with solvent ratios between (100:0) and (95:5) for all derivatives, unless otherwise stated. Reverse phase HPLC was

conducted on a Waters system. Semi-preparative HPLC, column: Spheri-10 RP 18 (25 x 0.46cm); Sample load, ca. 5-10mg; The elution conditions were as follows: flow rate 1.5ml min^{-1}; solvent A, 0.6% HOAc in aqueous MeOH (10%) buffered to pH 3.4 with triethylamine; solvent B, MeOH; elution starting with 5% B with a linear gradient from 5 to 20% B in 10 min and from 20 to 60% B in 20 min. Preparative HPLC, column: Bondapak C18 (15-20μm) (RCM 10 x 2.5cm); Sample load, ca. 50mg; The elution conditions were as follows: flow rate 7.0ml min^{-1}; solvent, aqueous MeOH (46%); isocratic elution.

3.1.2. Chemical methods

3.1.2.1. Acetylation

The dry compound was dissolved in a 1:1 $^{v}/v$ mixture of pyridine/acetic anhydride (ca. 0.1ml/mg sample) and the reaction allowed to proceed for ca. 8 hours at room temperature. The reaction was quenched with water and the product extracted with ether.

3.1.2.2. Methylation with diazomethane

Phenolic material (ca. 100mg) was dissolved in MeOH (ca. 10ml) and cooled to -15°C. Diazomethane was generated by hydrolysis of N-methyl-N-nitrosotoluene-*p*-sulphonamide (ca. 20g) with 40% KOH (aq) (ca. 50ml) and collected as an ethereal solution (ca. 200ml) at -15°C. The ether layer was decanted, dried over KOH pellets (3x) and added to the MeOH solution. The reaction was allowed to proceed for 24 hours at -15°C, after which excess diazomethane was allowed to evaporate at room temperature.

3.2. EXTRACTION AND ISOLATION

Air dried and powdered stem bark of *C. gerrardii* (2.48kg) was exhaustively extracted (Soxhlet) with EtOH (95%) and the

combined extracts concentrated to dryness under reduced pressure. The residue was suspended in water and successively extracted with hexane, $CHCl_3$ and EtOAc. Evaporation of the EtOAc fraction afforded a residue (10g) which was fractionated on silica gel by column chromatography using $CHCl_3$-Me_2CO (2:3) as eluent. This solvent removed less polar, non-phenolic contaminants. The residue (8.0g) was applied to a Sephadex LH-20 column and eluted with EtOH (95%), collecting 6ml fractions: tubes 38-58 afforded a mixture of compounds (**36**),(**37**) and (**39**); tubes 60-100 contained compounds (**38**), (**40**) and (**41**). Further silica gel based separations of these fractions afforded (**36**) and (**37**) as a diastereomeric mixture (80mg), (**39**) (170mg), (**38**) and (**40**) as a diastereomeric mixture (3.0g) and (**41**) (340mg).

Air dried and powdered stem bark of *C. gummiflua* (2.93kg) was exhaustivly extracted (Soxhlet) with EtOH (95%) and the combined extracts concentrated to dryness under reduced pressure. The residue was suspended in water and successively extracted with $CHCl_3$ and EtOAc. Evaporation of the EtOAc fraction afforded a residue (51.0g) of which a portion (10g) was fractionated on silica gel using CH_2Cl_2-acetone (70:30 - 25:65, in step-wise increments) to yield compounds (**42**) and (**43**) as a diastereomeric mixture (2.8g) and a phenolic rich fraction (4.3g). The phenolic fraction was subjected to column chromatography on Sephadex LH-20 and eluted with increasing amounts of MeOH in water in step-wise increments (50:50-80:20) to afford (**39**) (133mg), (**36**) (170mg), (**44**) (151mg) and an oligomeric fraction (3.3g). Further silica gel based separations of this fraction afforded (**38**) and (**40**) (0.86g) and further quantities of (**42**) and (**43**) (0.8g) as diastereomeric mixtures.

Air dried and powdered stem bark of *C. mossambicensis* (1.6kg) was exhaustively extracted (Soxhlet) with EtOH (95%) and the combined extracts concentrated to dryness under reduced pressure. The residue was suspended in water, defatted with

$CHCl_3$ and extracted with EtOAc. Evaporation of the EtOAc fraction afforded a residue (21.0g) of which a portion (10g) was fractionated on silica gel using CH_2Cl_2-acetone (75:25 - 30:70, in step-wise increments) to give three fractions: fr. 1 (3.7g), fr. 2 (777mg) and fr. 3 (1.1g). Each fraction was subjected to chromatography over Sephadex LH-20 and eluted with increasing amounts of MeOH in water in step-wise increments (60:40-100:0). Following further silica gel based separations: fr. 1 afforded compounds (**45**) (50mg), (**36**) and (**37**) as a diastereomeric mixture (74mg) and (**38**) and (**40**) as a diastereomeric mixture (2.0g); Fr. 2 yielded two compounds as an inseparable mixture (450mg). Acetylation of this mixture with acetic anhydride in pyridine and subsequent separation on silica gel afforded (**41a**) (180mg) and a compound of unresolved structure (ca.80mg); Fr. 3 afforded compound (**39**) (224mg).

3.2.1. Separation of diastereomers

Compounds (**36**) and (**37**) were separated by semi-preparative HPLC (R_t13.4 and 15.4min respectively). Compounds (**42**) and (**43**) were separated using HSCCC (R_t8.0 and 7.3hr respectively). Separation of compounds (**38**) and (**40**) was achieved using reparative HPLC (R_t15.7 and 11.4min respectively) and by HSCCC (R_t11.5 and 12.1hr respectively).

3.3. SPECTRAL DATA

Afzelechin (**36**). A pale yellow crystalline solid, mp 176°C, R_f 0.46 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 275 $[M+H]^+$; $[\alpha]_D^{24}$ +19.32° (MeOH; *c* 0.176), $[\alpha]_{546}^{24}$ +22.16° (MeOH; *c* 0.176); ^{1}HNMR (Me_2CO-d_6): δ2.53 [1H, *dd*, *J*= 8.5, 16.0Hz, H-4(C)$_{eq}$], 2.95 [1H, *dd*, *J*=5.3, 16.0Hz, H-4(C)$_{ax}$], 4.04 [1H, *m*, H-3(C)], 4.59 [1H, *d*, *J*=7.7Hz, H-2(C)], 5.87 [1H, *d*, *J*=2.3Hz, H-6(A)], 6.02 [1H, *d*, *J*=2.3Hz, H-8(A)], 6.81 [2H, *d*, *J*=8.7Hz, H-3',5'(B)], 7.25 [2H, *d*, *J*=8.7Hz, H-2',6'(B)]; 8.08, 8.27 and 8,40 [each 1H, *s*, 3 X OH]; ^{13}CNMR (Me_2CO-d_6): δ29.08

[C-4(C)], 68.24 [C-3(C)], 82.57 [C-2(C)], 95.34 [C-8(A)], 96.03 [C-6(A)], 100.60 [C-10(A)], 115.67 [C-3',5'(B)], 129.55 [C-2',6'(B)], 131.24 [C-1'(B)], 156.85, 157.09, 157.62 and 157.92 [C-4'(B), C-5(A), C-7(A), and C-9(A)].

Epiafzelechin (**37**). A pale yellow crystalline solid, mp 241°C, R_f 0.46 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 275 $[M+H]^+$; $[\alpha]_D^{23}$ -51.52° (MeOH; *c* 0.066), $[\alpha]_{546}^{23}$ -62.12° (MeOH; *c* 0.066); ^{1}HNMR (Me_2CO-d_6): δ2.73 [1H, *dd*, *J*= 3.1, 16.7Hz, H-4(C)$_{eq}$], 2.89 [1H, *dd*, *J*=4.3, 16.7Hz, H-4(C)$_{ax}$], 3.66 [1H, *d*, *J*=5.86, OH-3(C)], 4.19 [1H, *m*, H-3(C)], 4.92 [1H, *brs*, H-2(C)], 5.91 [1H, *d*, *J*=2.3Hz, H-6(A)], 6.01 [1H, *d*, *J*=2.3Hz, H-8(A)], 6.80 [2H, *d*, *J*=8.7Hz, H-3',5'(B)], 7.35 [2H, *d*, *J*=8.7Hz, H-2',6'(B)], 7.99, 8.16 and 8.29 [each 1H, *s*, 3 X OH]; ^{13}CNMR (Me_2CO-d_6): δ29.10 [C-4(C)], 66.80 [C-3(C)], 79.43 [C-2(C)], 95.66 [C-8(A)], 96.13 [C-6(A)], 99.69 [C-10(A)], 115.41 [C-3',5'(B)], 129.10 [C-2',6'(B)], 131.46 [C-1'(B)], 157.15, 157.53, and 2 X 157.59 [C-4'(B), C-5(A), C-7(A), and C-9(A)].

Epiafzelechin-(4β→8,2β→O→7)-*epiafzelechin* (**38**). Isolated as a pale yellow amorphous powder, mp 220-223°C, R_f 0.34 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 545 $[M+H]^+$; $[\alpha]_D^{25}$ +54.21° (MeOH; *c* 0.107), $[\alpha]_{365}^{25}$ +199.07° (MeOH; *c* 0.107); CD $[\theta]_{212}$0.0, $[\theta]_{222}$71.9 X 10^3, $[\theta]_{256}$0.0, $[\theta]_{271}$-16.6 X 10^3; ^{1}HNMR (CD_3OD): δ2.79 [1H, *dd*, *J*=1.95, 17.15Hz, H-4(F)$_{eq}$], 3.00 [1H, *dd*, *J*=4.95, 17.15Hz, H-4(F)$_{ax}$], 4.10 [1H, *d*, *J*=3.45Hz, H-3(C)], 4.258 [1H, *m*, H-3(F)], 4.46 [1H, *d*, *J*=3.45Hz, H-4(C)], 5.00 [1H, *brs*, H-2(F)], 6.05 [1H, *d*, *J*=2.57Hz, H-6(A)], 6.11 [1H, *d*, *J*=2.57Hz, H-8(A)], 6.13 [1H, *s*, H-6(D)], 6.86 [2H, *d*, *J*=8.81Hz, H-3',5'(B)], 6.87 [2H, *d*, *J*=8.81Hz, H-3',5'(E)], 7.54 [4H, *d*, *J*=8.81Hz, H-2',6'(E), H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.25 [C-4(C)], 29.94 [C-4(F)], 67.02 [C-3(F)], 68.02 [C-3(C)], 81.64 [C-2(F)], 96.49 and 96.54 [C-6(D), C-8(A)], 98.22 [C-6(A)], 100.26 [C-2(C)], 102.37, 104.14 and 107.20 [C-10(A), C-8(D) and C-10(D)],

115.49 [C-3',5'(B)], 115.9 [C-3',5'(E)], 129.45 [C-2',6'(E)], 129.94 [C-2',6'(B)], 130.60 [C-1'(E)], 131.82 [C-1'(B)], 152.19, 152.26, 154.24, 156.57, 157.05, 158.07, 158.27 and 158.83 [C-5(A), C-5(D), C-7(A), C-7(D), C-9(A), C-9(D), C-4'(B) and C-4'(E)].

Epiafzelechin-(4β→8,2β→O→7)-*epiafzelechin-pentamethyl ether* (**38a**). Methylation of (**38**) with ethereal diazomethane, afforded (**38a**) as an amorphous powder, mp 135°C, R_f 0.27 [CH_2Cl_2-acetone (97:3)]; $[\alpha]_D^{23}$ +61.36° ($CHCl_3$; *c* 0.176) $[\alpha]_{546}^{23}$ +73.86° ($CHCl_3$; *c* 0.176); IR $\nu_{max}cm^{-1}$: 1612, 1515, 1250; ^{1}HNMR ($CDCl_3$): δ1.88 [1H, *brs*, OH-3(F)], 1.94 [1H, *d*, *J*=5.94Hz, OH-3(C)], 2.84 [2H, *m*, H-4(C)], 3.49 [3H, *s*, OMe-5(A)], 3.72 and 3.74 [each 3H, *s*, OMe-7(A) and OMe-5(D)], 3.81 and 3.82 [each 3H, *s*, OMe-4'(B) and OMe-4'(E)], 4.17 [1H, *dd*, *J*=3.44, 5.94Hz, H-3(C)], 4.41 [1H, *m*, H-3(F)], 4.93 [1H, *d*, *J*=3.44Hz, H-4(C)], 4.96 [1H, *brs*, H-2(F)], 6.06 [1H, *d*, *J*=2.33Hz, H-6(A)], 6.20 [1H, *s*, H-6(D)], 6.28 [1H, *d*, *J*=2.33Hz, H-8(A)], 6.957 [4H, *d*, *J*=8.89Hz, H-3',5'(B) and H-3',5'(E)], 7.60 [2H, *d*, *J*=8.89Hz, H-2',6'(E)], 7.66 [2H, *d*, *J*=8.89Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ27.58 [C-4(C)], 28.86 [C-4(F)], 55.34, 55.30 and 55.47 [5 X OMe], 65.52 [C-3(F)], 67.42 [C-3(C)], 78.24 [C-2(F)], 92.06 [C-6(D)], 83.10 [C-8(A)], 93.30 [C-6(A)], 98.88 [C-2(C)], 103.64 [C-10(A)], 106.69 [C-8(D)], 113.62 and 113.69 [C-3',5'(E) and C-3',5'(B)], 127.98 [C-2',6'(E)], 128.10 [C-2',6'(B)], 130.09 [C-1'(E)], 130.60 [C-1'(B)], 151.23 [C-9(D)], 151.61 [C-7(D)], 152.94 [C-9(A)], 157.63 [C-5(D)], 158.98 [C-5(A)], 159.24 [C-4'(E)], 159.87 [C-7(A)], 160.13 [C-4'(B)].

Epiafzelechin-(4β→8,2β→O→7)-*epiafzelechin-pentamethyl ether diacetate* (**38b**). Acetylation of (**38a**) afforded (**38b**) as an amorphous powder, mp 145°C, R_f 0.33 [CH_2Cl_2]; $[\alpha]_D^{21}$ +4.33° ($CHCl_3$; *c* 0.462), $[\alpha]_{546}^{21}$ +6.49° ($CHCl_3$; *c* 0.462); IR$\nu_{max}cm^{-1}$:1742, 1614, 1516, 1123; ^{1}HNMR ($CDCl_3$): δ1.79 and

1.88 [each 3H, *s*, OAc-3(C), OAc-3(F)], 2.77 [1H, *dd*, *J*=2.50, 17.95Hz, H-4(F)$_{eq}$], 3.02 [1H, *dd*, *J*=5.13, 17.95Hz, H-4(F)$_{ax}$], 3.56 [3H, *s*, OAc-5(A)], 3.82, 3.73, 3.78 and 3.82 [each 3H, *s*, OAc-4'(E), OAc-5(D), OAc-7(A) and OAc-4'(B)], 4.91 [1H, *d*, *J*=3.33Hz, H-4(C)], 5.11 [1H, *brs*, H-2(F)], 5.45 [1H, *d*, *J*=3.33Hz, H-3(C)], 5.60 [1H, *m*, H-3(F)], 6.10 [1H, *d*, *J*=2.30Hz, H-6(A)], 6.21 [1H, *s*, H-6(D)], 6.31 [1H, *d*, *J*=2.30Hz, H-8(A)], 6.92 [4H, *d*, *J*=8.78Hz, H-3',5'(B) and H-3',5'(E)], 7.49 [2H, *d*, *J*=8.78Hz, H-2',6'(E)], 7.63 [2H, *d*, *J*=8.78Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ20.77 and 20.98 [OCO$\underline{C}$H$_3$-3(C) and -3(F)], 25.76 [C-4(C)], 25.87 [C-4(F)], 55.23, 55.34 and 55.45 [5 X OMe], 67.39 [C-3(F)], 67.86 [C-3(C)], 76.66 [C-2(F)], 91.78 [C-6(D)], 93.02 [C-6(A)], 93.42 [C-8(A)], 97.46 [C-2(C)], 101.48 [C-10(D)], 104.33 [C-10(A)], 106.25 [C-8(D)], 113.33 and 113.66 [C-3',5'(B), and C-3',5'(E)], 127.64 [C-2',6'(E)], 128.03 [C-2',6'(B)], 129.83 [C-1'(E)], 130.35 [C-1'(B)], 151.27 [C-9(D)], 151.78 [C-7(D)], 153.15 [C-9(A)], 157.34 [C-5(D)], 158.69 [C-5(A)], 159.15 [C-4'(E)], 159.70 [C-7(A)], 160.10 [C-4'(B)], 169.39 and 170.36 [O$\underline{C}$OCH$_3$-3(C) and -3(F)].

Afzelechin-3-O-α-L-rhamnopyranoside (**39**). This is a semi-crystalline hygroscopic powder, mp 110-115°C, R_f 0.23 [CH_2Cl_2-acetone (2:3)]; FAB-MS *m/z* 421 $[M+H]^+$; $[\alpha]_D^{28}$ -14.95° (Me_2CO; *c* 0.107), $[\alpha]_{365}^{28}$ -37.38° (Me_2CO; *c* 0.107); UV λ_{max}^{MeOH} nm: 213, 224, 279; IR ν_{max}cm^{-1}: 3384, 1614, 1317, 1141; ^{1}HNMR (Me_2CO-d_6): δ1.26 [3H, *d*, *J*=6.20Hz, H-6"], 2.67 [1H, *dd*, *J*=16.27, 8.18Hz, H-4(C)$_{eq}$], 2.92 [1H, *dd*, *J*=16.27, 5.71Hz, H-4(C)$_{ax}$], 3.42 [1H, *dd*, *J*=9.44, 9.44Hz, H-4"], 3.56 [1H, *dd*, *J*=3.35, 1.57Hz, H-2"], 3.65 [1H, *dd*, *J*=9.43, 3.35Hz, H-3"], 3.73 [1H, *dq*, *J* =9.44, 6.20Hz, H-5"], 4.02 [1H, *ddd*, *J*=7.85, 5.71, 8,18Hz, H-3(C)], 4.33 [1H, *d*, *J*=1.57Hz, H-1"], 4.74 [1H, *d*, *J*=7.85Hz, H-2(C)], 5.94 [1H, *d*, *J*=2.29Hz, H-6(A)], 6.09 [1H, *d*, *J*=2.29Hz, H-8(A)], 6.88 [2H, *d*, *J*=8.57Hz, H-3',5'(B)], 7.28 [2H, *d*, *J*=8.57Hz, H-2',6'(B)], 8.2 [1H, *s*, OH-5(A)], 8.5 [1H, *s*, OH-7(A)], 8.6 [1H, *s*, OH-4'(B)]; ^{1}HNMR

(CD_3OD): δ1.28 [3H, *d*, *J*=6.22Hz, H-6"], 2.68 [1H, *dd*, *J*=16.08, 8.57Hz, H-4(C)$_{eq}$], 2.95 [1H, *dd*, *J*=16.08, 5.72Hz, H-4(C)$_{ax}$], 3.33 [1H, *dd*, *J*=9.43, 9.43Hz, H-4"], 3.50 [1H, *dd*, *J*=3.30, 1.66Hz, H-2"], 3.61 [1H, *dd*, *J*=9.43, 3.30Hz, H-3"], 3.72 [1H, *dq*, *J* =9.43, 6.22Hz, H-5"], 3.97 [1H, *ddd*, *J*=8.00, 5.57, 8,57Hz, H-3(C)], 4.29 [1H, *d*, *J*=1.66Hz, H-1"], 4.69 [1H, *d*, *J*=8.00Hz, H-2(C)], 5.89 [1H, *d*, *J*=2.30Hz, H-6(A)], 5.98 [1H, *d*, *J*=2.30Hz, H-8(A)], 6.82 [2H, *d*, *J*=8.53Hz, H-3',5'(B)], 7.25 [2H,*d*,*J*=8.53Hz,H-2',6'(B)]; ^{13}CNMR (Me_2CO-d_6): δ17.94 [Me-6"], 27.89 [C-4(C)], 69.67 [C-5"], 71.51 [C-2"], 72.29 [C-3"], 73.66 [C-4"], 74.82 [C-3(C)], 80.45 [C-2(C)], 95.52 [C-6(A)], 96.41 [C-8(A)], 100.35 [C-10(A)], 101.49 [C-1"], 115.99 [C-3',5'(B)], 129.38 [C-2',6'(B)], 131.09 [C-1'(B)], 156.91, 157.38,158.04 and 158.32 [C-5(A), C-7(A), C-9(A) and C-4'(B)].

Enzymic hydrolysis of compound (**39**). The glycoside (45mg) was dissolved in an acetate buffer (0.02M, 10ml, pH 3.8) and incubated (40°) with hesperidinase (20mg) for 12hr. Extraction with EtOAc afforded the crude aglycone, which, after separation over silica gel was identified as afzelechin (**36**) by comparison with an authentic sample. The sugar residue was confirmed to be rhamnose by co-TLC with an authentic sample.

Afzelechin-3-O-α-L-rhamnopyranoside-hexa-acetate (**39a**). Amorphous powder, mp 69°C; (Found: C, 58.52; H, 5.62. requires: C, 58.92; H 5.4%); $[\alpha]_D^{28}$ +23.89° (Me_2CO; *c* 0.180), $[\alpha]_{365}^{28}$ +90.0° (Me_2CO; *c* 0.180); UV λ_{max}^{MeOH} nm: 210; IR ν_{max}cm^{-1}: 3430, 1753, 1620, 1130; ^{1}HNMR ($CDCL_3$): δ1.17 [3H, *d*, *J*=6.28Hz, H-6"], 1.96, 2,04 and 2.05 [each 3H, *s*, OAc-2", -3" and -4"] 2.27, 2.28 and 2.33 [each 3H, *s*, OAc-5(A), -7(A) and -4'(B)], 2.78 [1H, *dd*, *J*=16.36, 9.09Hz, H-4(C)$_{eq}$], 2.90 [1H, *dd*, *J*=16,36, 6.13Hz, H-4(C)$_{ax}$], 3.85 [1H, *dq*, *J*=9.82, 6.28Hz, H-5"], 3.97 [1H, *m*, H-3(C)], 3.99 [1H, *d*, *J*=1.61Hz, H-1"], 4.87 [1H, *d*, *J* =8.46Hz, H-2(C)], 4.95 [1H, *dd*, *J*=1.61,

3.43Hz, H-2"], 4.97 [1H, *dd*, *J*=9.86, 10.03Hz, H-4"], 5.21 [1H, *dd*, *J*=10.03, 3.43Hz, H-3"], 6.58 [1H, *d*, *J*=2.21Hz, H-6(A)], 6.63 [1H, *d*, *J*=2.21Hz, H-8(A)], 7.09 [2H, *d*, *J*=8.52Hz, H-3',5'(B)], 7.43 [2H, *d*, *J*=8.52Hz, H-2',6'(B)]; ^{13}CNMR ($CDCL_3$): δ17.28 [Me-6"], 20.67, 20.79 and 21.03 [6 X $OCO\underline{C}H_3$], 27.54 [C-4(C)], 67.09 [C-5"], 68.81 and 68.85 [C-3", C-2"], 70.82 [C-4"], 74.37 [C-3(C)], 79.48 [C-2(C)], 97.98 [C-1"], 107.82 [C-8(A)], 108.73 [C-6(A)], 111.03 [C-10(A)], 122.02 [C-3',5'(B)], 127.84 [C-2',6'(B)], 134.97 [C-1'(B)], 149.29, 149.80, and 150.86 [C-5(A), C-7(A) and C-4'(B)], 155.03 [C-9(A)], 168.34, 168.94, 169.02, 169.58, 169.85 and 169.99 [6 X $O\underline{C}OCH_3$].

Epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin* (**40**). This is an amorphous, hygroscopic powder, mp 238-245°C, R_f 0.31 [CH_2Cl_2-acetone (50:50)]; FAB-MS *m/z* 545 $[M+H]^+$; $[\alpha]_D^{25}$ +64.29° (MeOH; *c* 0.126), $[\alpha]_{365}^{25}$ +199.21° (MeOH; *c* 0.126); UV λ_{max}^{MeOH} nm 237.0, 237.2; IR ν_{max} cm^{-1}: 3492, 1614, 1516, 1140, 832; CD $[\theta]_{200}$+0.73 X 10^3, $[\theta]_{232}$+1.7 X 10^3, $[\theta]_{241}$0.0, $[\theta]_{258}$-5.8 X 10^3, $[\theta]_{285}$0.0; ^{1}HNMR (CD_3OD): δ2.63 [1H, *dd*, *J*=8.39, 16.41Hz, H-4(F)$_{eq}$], 2.99 [1H, *dd*, *J*=5.60, 16.41Hz, H-4(F)$_{ax}$], 4.12 [1H, *d*, *J*=3.56Hz, H-3(C)], 4.21 [1H, *ddd*, *J*=8.39, 5.60, 7.95Hz, H-3(F)], 4.31 [1H, *d*, *J*=3.56Hz, H-4(C)], 4.83 [1H, *d*, *J*=7.95Hz, H-2(F)], 6.01 [1H, *d*, *J*=2.22Hz, H-6(A)], 6.13 [1H, *d*, *J*=2.22Hz, H-8(A)], 6.15 [1H, *s*, H-6(D)], 6.85 [2H, *d*, *J*=8.89Hz, H-3',5'(B)], 6.88 [2H, *d*, *J*=8.57Hz, H-3',5'(E)], 7.34 [2H, *d*, *J*=8.57Hz, H-2',6'(E)], 7.55 [2H, *d*, *J*=8.89Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.15 [C-4(C) and C-4(F)], 67.66 [C-3(C)], 68.04 [C-3(F)], 84.14 [C-2(F)], 96.48 and 96.53 [C-6(D), C-8(A)], 98.06 [C-6(A)], 100.37 [C-2(C)], 103.1 [C-10(D)], 103.93 [C-10(A)], 106.74 [C-8(D)], 115.48 [C-3',5'(B)], 116.26 [C-3',5'(E)], 129.46 [C-2',6'(B)], 129.80 [C-1'(E)], 130.10 [C-2',6'(E)], 131.57 [C-1'(B)], 151.40 [C-9(D)], 152.09 [C-7(D)], 154.20 [C-9(A)], 156.03 [C-5(D)], 156.69 [C-5(A)], 158.01 [C-7(A)], 158.66 [C-4'(B)], 158.76 [C-4'(E)].

Epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin-tetramethyl ether*. (**40a**). Methylation of (**40**) with ethereal diazomethane afforded (**40a**) as an amorphous powder, mp 152-154°C, R_f 0.13 [CH_2Cl_2-acetone (95:5)]; $[\alpha]_D^{25}$ +40.00° ($CHCl_3$; *c* 0.095), $[\alpha]_{365}^{25}$ +104.21° ($CHCl_3$; *c* 0.095); IRν_{max}cm^{-1}: 3445, 1603, 1516, 1252, 1036, 831; ^{1}HNMR ($CDCl_3$): δ2.00 and 2.03 [each 1H, *s*, OH-3(C) and OH-3(F)], 2.57 [1H, *dd*, *J*=16.58, 9.02Hz, H-4(F)$_{eq}$], 3.06 [1H, *dd*, *J*=16.58, 5.94Hz, H-4(F)$_{ax}$], 3.81, 3.74, 3.81 and 3.70 [each 3H, *s*, OMe-4'(E), -5(D), -4'(B) and -7(A)], 4.12 [1H, *d*, *J*=3.65Hz, H-3(C)], 4.13 [1H, *m*, H-3(F)], 4.27 [1H, *d*, *J*=3.65Hz, H-4(C)], 4.73 [1H, *d*, *J*=8.63Hz, H-2(F)], 6.13 [1H, *d*, *J*=2.47Hz, H-6(A)], 6.25 [1H, *s*, H-6(D)], 6.27 [1H, *d*, *J*=2.47Hz, H-8(A)], 6.67 [1H, *s*, OH-5(A)], 6.96 [2H, *d*, *J*=8.92Hz, H-3',5'(B)], 6.98 [2H, *d*, *J*=8.70Hz, H-3',5'(E)], 7.38 [2H, *d*, *J*=8.70Hz, H-2',6'(E)], 7.64 [2H, *d*, *J*=8.92Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ27.40 [C-4(C)], 28.01 [C-4(F)], 55.30-55.64 [OMe-7(A), -5(D), -4'(E), -4'(B)], 66.52 [C-3(C)], 67.34 [C-3(F)], 83.37 [C-2(F)], 93.00 [C-6(D)], 94.72 [C-8(A)], 96.90 [C-6(A)], 99.07 [C-2(C)], 102.51 [C-10(A)], 103.28 [C-10(D)], 105.61 [C-8(D)], 113.65 [C-3',5'(B)], 114.56 [C-3',5'(E)], 127.74 [C-1'(B)], 128.22 [C-2',6'(B)], 129.3 [C-2',6'(E)], 130.12 [C-1'(E)], 149.67 [C-9(D)], 150.96 and 152.36 [C-9(A) and C-7(D)], 155.25 [C-5(A)], 157.30 [C-5(D)], 159.94 [C-7(A)], 160.21 [C-4'(B)], 160.45 [C-4'(E)].

Epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin-heptamethyl ether*. (**40d**). NaH (3 eq.) was added to a stirred solution of (**40**) (70mg) in anhydrous THF (30ml) at 0°. The mixture was stirred at room temperature for 30 min, treated with MeI (3 eq.) and stirred for a further 6 hr. The reaction was quenched with water and extracted with ether. Purification by flash chromatography (CH_2Cl_2) afforded the heptamethyl ether (42mg) as a pale yellow amorphous powder, mp 134°C, $[\alpha]_D^{25}$ -78.0°

($CHCl_3$; *c* 0.100), $[\alpha]_{365}^{25}$ -332.0° ($CHCl_3$; *c* 0.100); IRν_{max}cm^{-1}: 1615, 1518, 1250, 1115, 1040; MS *m/z* (rel. int.): 642.2397 $[M]^+$ (30) (calc. for $C_{37}H_{38}O_{10}$, 642.2465), 611 (52), 447 (41), 164 (64), 149 (100), 121 (57), 91 (69); ^{1}HNMR ($CDCl_3$): δ2.44 [1H, *dd*, *J*=16.95, 4.53Hz, H-4(F)$_{eq}$], 2.73 [1H, *dd*, *J*=16.95, 3.85Hz, H-4(F)$_{ax}$], 2.97 [3H, *s*, OMe-3(C)], 3.24 [3H, *s*, OMe-5(A)], 3.35 [3H, *s*, OMe-3(F)], 3.65 [3H, *s*, OMe-5(D)], 3.69 [1H, *m*, H-3(F)], 3.70, 3.71 and 3.77 [each 3H, *s*, OMe-4'(E), -4'(B) and -7(A)], 3.79 [1H, *d*, *J*=3.59Hz, H-3(C)], 4.99 [1H, *d*, *J*=3.59Hz, H-4(C)], 5.21 [1H, *d*, *J*=7.23Hz, H-2(F)], 5.94 [1H, *d*, *J*=2.32Hz, H-6(A)], 6.13 [1H, *s*, H-6(D)], 6.24 [1H, *d*, *J*=2.32Hz, H-8(A)], 6.74 [2H, *d*, *J*=8.72Hz, H-3',5'(E)], 6.90 [2H, *d*, *J*=8.91Hz, H-3',5'(B)], 7.10 [2H, *d*, *J*=8.72Hz, H-2',6'(E)], 7.61 [2H, *d*, *J*=8.91Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ21.46 [C-4(F)], 24.47 [C-4(C)], 54.88-57.39 [7 X OMe], 75.67 [C-3(C)], 76.01 [C-3(F)], 78.23 [C-2(F)], 91.61 [C-6(D)], 92.31 [C-6(A)], 92.80 [C-8(A)], 98.42 [C-2(C)], 101.29 [C-10(D)], 104.30 [C-10(A)], 105.72 [C-8(D)], 113.15 [C-3',5'(B)], 113.54 [C-3',5'(E)], 127.38 [C-2',6'(E)], 128.30 [C-2',6'(B)], 131.37 [C-1'(E)], 131.40 [C-1'(B)], 151.04 [C-9(D)], 151.40 [C-7(D)], 153.72 [C-9(A)], 157.09 [C-5(D)], 158.19 [C-5(A)], 158.96 [C-4'(E)], 159.51 and 159.80 [C-4'(B) and C-7(A)].

Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin* (**41**). Brown amorphous solid; R_f 0.14 [CH_2Cl_2-acetone-MeOH (50:49:1)]; FAB-MS *m/z* 816 $[M]^+$.

Acid-catalysed thiolytic degradation of (**41**). A mixture of (**41**) (200mg), benzylmercaptan (1.5ml) and HOAc in MeOH (10ml) was refluxed under N_2 for 8hr. The reaction mixture was concentrated under reduced pressure and the residue chromatographed over silica gel to afford two products, epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin (**40**) (50mg) and epiafzelechin-4β-benzylthioether (**63**) (18mg).

Epiafzelechin-4β-benzylthioether (**63**). Amorphous solid, mp

106-108°C, (Found: C, 64.01; H, 5.09. $C_{22}H_{20}O_5 \cdot 1H_2O$ requires C, 63.75; H, 5.35%); $[\alpha]_D^{23}$ -17.02° (MeOH; *c* 0.094); ^{1}HNMR (CD_3OD): δ 3.87 [1H, *dd*, *J*=2.37, 0.97Hz, H-3(C)], 3.99 [2H, *s*, CH_2S-()], 4.11 [1H, *d*, *J*=2.37Hz, H-4(C)], 5.32 [1H, *brs*, H-2(C)], 5.95 [1H, *d*, *J*=2.33Hz, H-6(A)], 6.01 [1H, *d*, *J*=2.33Hz, H-8(A)], 6.80 [2H, *d*, *J*=8.46Hz, H-3',5(B)], 7.23 [2H, *d*, *J*=8.46Hz, H-2',6'(B)], 7.25-7.47 [5H, *m* (toluene-thiolyl ring protons)]; ^{13}CNMR (CD_3OD): δ38.35 [CH_2S], 44.42 [C-4(C)], 71.87 [C-3(C)], 75.88 [C-2(C)], 96.09 [C-8(A)], 97.01 [C-6(A)], 100.46 [C-10(A)], 116.12 [C-3',5'(B)], 128.25 [C-4(toluene-thiolyl ring)], 129.34, 129.87 and 130.38 [C-2',6'(B), C-3,5 and C-2,6(toluene-thiolyl ring)], 131.67 [C-1'(B)], 140.97 [C-1(toluene-thiolyl ring)], 157.57, 158.11, 159.17 and 159.33 [C-5(A), C-7(A), C-9(A) and C-4'(B)].

Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin peracetate* (**41a**). Acetylation of (**41**) afforded the peracetate (**41a**) as an amorphous solid. ^{1}HNMR ($CDCl_3$): δ1.74-2.35 [each 3H, *s*, 11 X OAc], 2.86 [2H, *m*, H-4(I)], 4.65 [1H, *d*, *J*=3.77Hz, H-4(F)], 4.80 1[H, *d*, *J*=2.44Hz, H-4(C)], 5.25 [1H, *brs*, H-2(C)], 5.3-5.4 [2H, overlapping signals, H-2(I) and H-3(I)], 5.51 [1H, *dd*, *J*=2.44, 1.78Hz, H-3(C)], 5.56 [1H, *d*, *J*=3.77Hz, H-3(F)], 6.51 and 6.52 [each 1H, *s*, H-8(D) and H-6(G)], 6.72 [1H, *d*, *J*=2.29Hz, H-6(A)], 6.76 [1H, *d*, *J*=2.29Hz, H-8(A)], 7.02 [2H, *d*, *J*=8.61Hz, H-3',5'(B)], 7.09 [2H, *d*, *J*=8.57Hz, H-3',5'(H)], 7.14 [2H, *d*, *J*=8.84Hz, H-3',5'(E)], 7.34 [2H, *d*, *J*=8.87Hz, H-2',6'(H)], 7.40 [2H, *d*, *J*=8.61Hz, H-2',6'(B)], 7.66 [2H, *d*, *J*=8.84Hz, H-2',6'(E)]; ^{13}CNMR ($CDCl_3$): δ 19.95-21.14 [11 X OCO$\underline{C}H_3$], 22.99 [C-4(I)], 27.38 [C-4(F)], 34.71 [C-4(C)], 66.55 [C-3(F)], 68.28 [C-3(I)], 70.29 [C-3(C)], 74.87 [C-2(C)], 78.86 [C-2(I)], 98.02 [C-2(F)], 103.85 [C-6(G)], 106.17 [C-10(G)], 107.72 [C-8(D)], 109.20 [C-8(G)], 108.92 [C-8(A)], 111.14 [C-10(A)], 111.35 [C-6(A)], 113.43 [C-10(D)], 116.41 [C-6(D)], 121.31, 121.39 and 121.82 [C-3',5'(B), C-3',5'(E) and C-3',5'(H)],127.71, 127.81 and 127.90 [C-2',6'(B), C-2',6'(E)

and C-2',6'(H)], 134.26, 134.37 and 134.54 [C-1'(B), C-1'(E) and C-1'(H)], 147.89, 148.33, 149.04, 149.67, 149.74, 150.39, 150.54, 150.69, 150.77, 150.83, 151.36 and 154.99 [C-5(A), -5(D), -5(G), -7(A), -7(D),- 7(G), -9(A), -9(D), -9(G), -4'(B), -4'(E) and -4'(H)], 167.34-170.37 [11 X $O\underline{C}OCH_3$].

7-OMe-epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin* (**42**).This is a white crystalline solid, mp 245-247°C, R_f 0.24 [CH_2Cl_2-acetone (70:30)]; FAB-MS *m/z* 559 $[M+H]^+$; (Found: C, 63.69; H, 5.07. $C_{31}H_{26}O_{10}\cdot H_2O$ requires C, 63.59; H, 4.59%); IR $\nu_{max}cm^{-1}$: 3522, 3302, 1628, 1144; $[\alpha]_D^{23}$ +67.65° (MeOH; *c* 0.136), $[\alpha]_{578}^{23}$ +70.95° (MeOH; *c* 0.136); ^{1}HNMR (CD_3OD): δ2.65 [1H, *dd*, *J*=8.34, 16.37Hz, H-4(F)$_{eq}$], 3.00 [1H, *dd*, *J*=5.47, 16.37Hz, H-4(F)$_{ax}$], 3.68 [3H, *s*, OMe-7(A)], 4.15 [1H, *d*, *J*=3.52Hz, H-3(C)], 4.21 [1H, *ddd*, *J*=8.34, 5.49, 7.82Hz, H-3(F)], 4.36 [1H, *d*, *J*=3.52Hz, H-4(C)], 4.84 [1H, *d*, *J*=7.82Hz, H-2(F)], 6.11 [1H, *d*, *J*=2.42Hz, H-6(A)], 6.18 [1H, *s*, H-6(D)], 6.25 [1H, *d*, *J*=2.42Hz, H-8(A)], 6.88 [2H, *d*, *J*=8.79Hz, H-3',5'(B)], 6.88 [2H, *d*, *J*=8.56Hz, H-3',5'(E)], 7.35 [2H, *d*, *J*=8.56Hz, H-2',6'(E)], 7.58 [2H, *d*, *J*=8.79Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.15 [C-4(C) and C-4(F)], 55.63 [OMe-7(A)], 67.49 [C-3(C)], 67.97 [C-3(F)], 84.06 [C-2(F)], 96.02 [C-8(A)], 96.59 [C-6(D)], 96.95 [C-6(A)], 100.42 [C-2(C)], 103.15 [C-10(D)], 104.96 [C-10(A)], 106.53 [C-8(D)], 115.52 [C-3',5'(B)], 116.26 [C-3',5'(E)], 129.46 [C-2',6'(B)], 129.74 [C-1'(E)], 130.12 [C-2',6'(E)], 131.42 [C-1'(B)], 151.40 [C-9(D)], 152.03 [C-7(D)], 154.21 [C-9(A)], 155.98 [C-5(D)], 156.60 [C-5(A)], 158.56 [C-4'(E)], 158.68 [C-4'(B)], 160.84 [C-7(A)].

7-OMe-epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin-hexa-acetate* (**42a**). Acetylation of (**42**) afforded the hexa-acetate as an amorphous solid, mp 145°C, R_f 0.37 [CH_2Cl_2-acetone (97:3)]; $[\alpha]_D^{20}$ -101.34° ($CHCl_3$; *c* 0.372) $[\alpha]_{546}^{20}$ -121.23° ($CHCl_3$; *c* 0.372); IR $\nu_{max}cm^{-1}$: 1760, 1626, 1371, 1200 1063, 849; HNMR ($CDCl_3$): δ1.78 and 1.76 [each 3H, *s*, OAc-3(C) and -3(F)],

2.04 [3H, *s*, OAc-5(A)], 2.24, 2.28 and 2.31 [each 3H, *s*, OAc-4'(B), -4'(E) and -5(D)], 2.60 [2H, *m*, 4(F)], 3.78 [3H, *s*, OMe-7(A)], 4.60 [1H, *d*, *J*=3.95Hz, H-4(C)], 5.28-5.35 [3H, overlapping signals, H-2(F), -3(F) and -3(C)], 6.12 [1H, *d*, *J*=2.43Hz, H-6(A)], 6.47 [1H, *s*, H-6(D)], 6.60 [1H, *d*, *J*=2.43Hz, H-8(A)], 7.02 [2H, *d*, *J*=8.81Hz, H-3',5'(E)], 7.16 [2H, *d*, *J*=8.87Hz, H-3',5'(B)], 7.24 [2H, *d*, *J*=8.81Hz, H-2',6'(E)], 7.72 [2H, *d*, *J*=8.87Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ20.15,-21.14 [6 X OCO$\underline{C}H_3$], 22.36 [C-4(F)], 27.03 [C-4(C)], 55.20 [OMe-7(A)], 67.25 [C-3(C)], 68.28 [C-3(F)], 78.46 [C-2(F)], 97.86 [C-2(C)], 99.62 [C-8(A)], 102.54 [C-6(A)], 103.43 [C-6(D)], 105.68 [C-10(D)], 108.16 [C-10(A)], 109.51 [C-8(D)], 121.11 [C-3',5'(B)], 121.64 [C-3',5'(E)], 127.45 [C-2',6'(E)], 128.24 [C-2',6'(B)], 134.75 and 134.88 [C-1'(B) and C-1'(E)], 148.82 [C-5(D)], 149.18 [C-5(A)], 150.43, 150.65 and 150.69 [C-7(D), C-9(D) and C-4'(E)], 151.38 [C-4'(B)], 153.94 [C-9(A)], 159.49 [C-7(A)], 168.57-170.46 [6 X O$\underline{C}OCH_3$].

Base catalysed conversion of 7-OMe-epiafzelechin-(4β→8,2β→O→7)-ent-*afzelechin* (**42**).A mixture of (**42**) (200mg) and K_2CO_3 (ca. 300mg) was refluxed in acetone (ca. 4ml) for 8,5 hours. After removal of potassium carbonate by filtration, the filtrate was concentrated to dryness. The residue was subject to chromatography over silica gel to afford two new compounds (**60**) (80mg) and (**61**) (40mg).

7-OMe-epiafzelechin-(4β→6,2β→O→7)-ent-*afzelechin* (**60**).This is a white amorphous powder, mp 230-234°C, R_f 0.22 [CH_2Cl_2-acetone (70:30)]; IRν_{max}cm^{-1}: 3474, 1603, 1517, 1144; $[\alpha]_D^{20}$ +31.71° (MeOH; *c* 0.432), $[\alpha]_{578}^{20}$ +33.57° (MeOH; *c* 0.432); ^{1}HNMR (CD_3OD): δ2.79 [2H, *ddd*, H-4(F)], 3.73 [3H, *s*, OMe-7(A)], 4.10 [1H, *m*, H-3(F)], 4.20 [1H, *d*, *J*=3.62Hz, H-3(C)], 4.37 [1H, *d*, *J*=3.62Hz, H-4(C)], 6.12 [1H, *s*, H-8(D)], 6.14 [1H, *d*, *J*=2.26Hz, H-6(A)], 6.25 [1H, *d*, *J*=2.26Hz, H-8(A)], 6.81 [2H, *d*, *J*=8.60Hz, H-3',5'(E)], 6.88 [2H, *d*, *J*=8.84Hz, H-3',5'(B)], 7.23 [2H, *d*, *J*=8.60Hz,

H-2',6'(E)], 7.59 [2H, *d*, *J*=8.84Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ28.40 [C-4(F)], 29.72 [C-4(C)], 55.75 [OMe-7(A)], 67.38 [C-3(C)], 68.43 [C-3(F)], 82.54 [C-2(F)], 94.89 [C-8(A)], 95.99 [C-8(D)], 96.66 [C-6(A)], 100.75 [C-2(C)], 103.66 [C-10(D)], 105.27 [C-10(A)], 108.53 [C-6(D)], 116.03 [C-3',5'(B)], 116.06 [C-3',5'(E)], 129.29 [C-2',6'(E)], 129.64 [C-2',6'(B)], 131.30 [C-1'(E)], 131.43 [C-1'(B)], 151.92 [C-9(D)], 152.43, 152.45 and 155.21 [C-5(A), C-7(D) and C-5(D)], 154.52 [C-9(A)], 158.26 [C-4'(E)], 158.89 [C-4'(B)], 160.96 [C-7(A)].

7-OMe-epiafzelechin-(4β→6,2β→O→7)-*epiafzelechin* (**61**).This is a white amorphous powder, mp 241-244°C, R_f 0.32 [CH_2Cl_2-acetone (70:30)]; IRν_{max}cm^{-1}: 3474, 1603, 1517, 1144; $[\alpha]_D^{20}$+49.17° (MeOH; *c* 0.120), $[\alpha]_{578}^{20}$+52.50° (MeOH; *c* 0.120); ^{1}HNMR (CD_3OD): δ2.93 [2H, *m*, H-4(F)], 3.74 [3H, *s*, OMe-7(A)], 4.19 [1H, *m*, H-3(F)], 4.20 [1H, *d*, *J*=3.58Hz, H-3(C)], 4.38 [1H, *d*, *J*=3.58Hz, H-4(C)], 4.88 [1H, *brs*, H-2(F)], 6.12 [1H, *d*, *J*=2.21Hz, H-6(A)], 6.17 [1H, *s*, H-8(D)], 6.24 [1H, *d*, *J*=2.21Hz, H-8(A)], 6.88 [2H, *d*, *J*=8.60Hz, H-3',5'(E)], 6.88 [2H, *d*, *J*=8.88Hz, H-3',5'(B)], 7.33 [2H, *d*, *J*=8.60Hz, H-2',6'(E)], 7.58 [2H, *d*, *J*=8.88Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.78 [C-4(C) and C-4(F)], 55.76 [OMe-7(A)], 66.99 [C-3(F)], 67.41 [C-3(C)], 79.88 [C-2(F)], 94.89 [C-8(A)], 95.95 [C-6(A)], 96.98 [C-8(D)], 100.72 [C-2(C)], 102.74 [C-10(D)], 105.33 [C-10(A)], 108.60 [C-6(D)], 115.53 [C-3',5'(B)], 115.72 [C-3',5'(E)], 129.10 [C-2',6'(E)], 129.64 [C-2',6'(B)], 131.39 [C-1'(E)], 131.49 [C-1'(B)], 151.90 [C-9(D)], 152.93 [C-5(D)], 154.54 [C-9(A)], 155.20 [C-5(A)], 155.83 [C-7(D)], 157.88 [C-4'(E)], 158.92 [C-4'(B)], 160.99 [C-7(A)].

7-OMe-epiafzelechin-(4β→8,2β→O→7)-*epiafzelechin* (**43**).This is a white crystalline solid, mp 235-237°C, R_f 0.56 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 559 $[M+H]^+$; (Found: C, 62.56; H, 5.41. $C_{31}H_{26}O_{10}\cdot 2H_2O$ requires C, 62.62; H, 5.085%);

IRν_{max}cm^{-1}: 3373, 1626, 1516, 1142; $[\alpha]_D^{23}$ +57.81° (MeOH; *c* 0.192), $[\alpha]_{578}^{23}$ +60.42° (MeOH; *c* 0.192); ^{1}HNMR (CD_3OD): δ2.79 [1H, *dd*, *J*=2.45, 17.40Hz, H-4(F)$_{eq}$], 2.99 [1H, *dd*, *J*=4.59, 17.40Hz, H-4(F)$_{ax}$], 3.73 [3H, *s*, OMe-7(A)], 4.12 [1H, *d*, *J*=3.34Hz, H-3(C)], 4.25 [1H, *m*, H-3(F)], 4.49 [1H, *d*, *J*=3.34Hz, H-4(C)], 4.99 [1H, *s*, H-2(F)], 6.13 [1H, *d*, *J*=1.85Hz, H-6(A)], 6.14 [1H, *s*, H-6(D)], 6.22 [1H, *d*, *J*=1.85Hz, H-8(A)], 6.86 [2H, *d*, *J*=8.84Hz, H-3',5'(B)], 6.87 [2H, *d*, *J*=8.66Hz, H-3',5'(E)], 7.54 [2H, *d*, *J*=8.66Hz, H-2',6'(E)], 7.55 [2H, *d*, *J*=8.84Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.63 [C-4(C)], 30.27 [C-4(F)], 56.04 [OMe-7(A)], 67.36 [C-3(F)], 68.29 [C-3(C)], 81.94 [C-2(F)], 96.48 [C-8(A)], 96.83 [C-6(D)], 96.93 [C-6(A)], 100.68 [C-2(C)], 102.75 [C-10(D)], 105.59 [C-10(A)], 107.36 [C-8(D)], 115.87 [C-3',5'(B)], 116.25 [C-3',5'(E)], 129.81 [C-2',6'(B)], 130.29 [C-2',6'(E)], 130.96 [C-1'(E)], 132.08 [C-1'(B)], 152.56 [C-9(D)], 154.60, 156.90, 156.94 and 157.361 [C-5(A), C-5(D), C-7(A) and C-7(D)], 158.59 [C-4'(E)], 159.19 [C-4'(B)], 161.20 [C-7(A)].

7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate (**43a**). Acetylation of (**43**) afforded the hexa-acetate as an amorphous solid, mp 125-127°C, R_f 0.26 [CH_2Cl_2-acetone (97:3)]; $[\alpha]_D^{20}$ -80.10° ($CHCl_3$; *c* 0.206) $[\alpha]_{546}^{20}$ -95.15° ($CHCl_3$; *c* 0.206); HNMR ($CDCl_3$): δ1.46 and 1.73 [each 3H, *s*, OAc-3(C) and -3(F)], 1.95 [3H, *s*, OAc-5(A)], 2.27, 2.30 and 2.31 [each 3H, *s*, OAc-4'(B), -4'(E) and -5(D)], 2.88 [2H, *m*, 4(F)], 3.80 [3H, *s*, OMe-7(A)], 4.54 [1H, *d*, *J*=4.00Hz, H-4(C)], 5.22-5.31 [3H, overlapping signals, H-2(F), -3(F) and -3(C)], 6.26 [1H, *d*, *J*=2.47Hz, H-6(A)], 6.49 [1H, *s*, H-6(D)], 6.60 [1H, *d*, *J*=2.47Hz, H-8(A)], 7.12 [2H, *d*, *J*=8.66Hz, H-3',5'(E)], 7.16 [2H, *d*, *J*=8.88Hz, H-3',5'(B)], 7.49 [2H, *d*, *J*=8.66Hz, H-2',6'(E)], 7.70 [2H, *d*, *J*=8.88Hz, H-2',6'(B)]; ^{13}CNMR ($CDCl_3$): δ19.72,-21.17 [6 X OCO$\underline{C}H_3$], 25.47 [C-4(F)], 27.11 [C-4(C)], 55.52 [OMe-7(A)], 66.62 [C-3(F)], 67.79 [C-3(C)], 77.82 [C-2(F)], 98.09 [C-2(C)], 99.30 [C-8(A)], 102.78

[C-6(A)], 103.68 [C-6(D)], 105.59 [C-10(D)], 108.15 [C-10(A)], 109.69 [C-8(D)], 121.11 [C-3',5'(B)], 121.32 [C-3',5'(E)], 128.26 [C-2',6'(B)], 129.22 [C-2',6'(E)], 134.17 [C-1'(E)] 134.59 [C-1'(B)] , 149.20 and 150.25 [C-5(D) and C-7(D)], 149.37 [C-5(A)], 150.96 [C-4'(E)], 151.37 [C-4'(B)], 152.00 [C-9(D)], 154.15 [C-9(A)], 168.73-170.31 [6 X O$\underline{C}$OCH$_3$].

Kaempferol-3-O-α-L-rhamnopyranoside (**44**). This is a pale yellow amorphous powder, mp 167-170°C, R_f 0.15 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 434 $[M+H]^+$; $[\alpha]_D^{24}$ -140.6° (MeOH; *c* 0.348); IR $\nu_{max}cm^{-1}$: 1610, 1362, 1176, 973; ^{1}HNMR (CD_3OD): δ0.96 [1H, *d*, *J*=5.79Hz, H-6"], 3.35-3.40 [each 1H, H-4", H-5"], 3.77 [1H, *dd*, *J*=3.39, 9.32Hz, H-3"], 4.28 [1H, *dd*, *J*=3.39, 1.74Hz, H-2"], 5.41 [1H, *d*, *J*=1.74Hz, H-1"], 6.19 [1H, *d*, *J*=2.1Hz, H-6(A)], 6.35 [1H, *d*, *J*=2.1Hz, H-8(A)], 6.95 [2H, *d*, *J*=8.90Hz, H-3',5'(B)], 7.77 [2H, *d*, *J*=8.90Hz, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ17.63 [C-6"], 71.86, 71.94, 72.05 and 73.15 [C-2", C-3", C-4" and C-5"], 94.73 [C-8(A)], 99.78 [C-6(A)], 103.39 [C-1"], 105.85 [C-10(A)], 116.43 [C-3',5'(B)], 122.55 [C-1'(B)], 131.86 [C-2',6'(B)], 136.13 [C-3(C)], 158.36 [C-9(A)], 159.13 [C-2(C)], 161.40 [C-4'(B)], 163.03 [C-5(A)], 165.67 [C-7(A)], 179.45 [C-4(C)].

Kaempferol-3-O-α-L-rhamnopyranoside-hexa-acetate (**44a**). Acetylation of (**44**) according to standard procedures yielded (**44a**) as an amorphous powder, mp 99°C, R_f 0.20 [CH_2Cl_2-acetone (97:3)]; $[\alpha]_D^{23}$ -154.55° ($CHCl_3$; *c* 0.220) $[\alpha]_{546}^{23}$ -197.27° ($CHCl_3$; *c* 0.220); IR $\nu_{max}cm^{-1}$: 1753, 1629, 1371, 1215; ^{1}HNMR ($CDCl_3$): δ0.872 [3H, *d*, *J*=6.30Hz, H-6"], 1.985, 1.991, 2.13, 2.33, 2.34 and 2.44 [each 3H, *s*, 6 X OAc], 3.26 [1H, *dq*, *J*=9.87, 6.30Hz, H-5"], 4.92 [1H, *dd*, *J*=1.00, 9.87Hz, H-4"], 5.25 [1H, *dd*, *J*=3.38, 1.00Hz, H-3"], 5.58 [1H, *d*, *J*=1.78Hz, H-1"], 5.65 [1H, *dd*, *J*=1.78, 3.38Hz, H-2"], 6.85 [1H, *d*, *J*=2.24Hz, H-6(A)], 7.29 [1H, *d*, *J*=2.24Hz, H-8(A)], 7.29 [2H, *d*, *J*=8.97Hz, H-3',5'(B)"] 7.92 [2H, *d*, *J*=8.97Hz, H-2',6'(B)"]; ^{13}CNMR ($CDCl_3$): δ17.00 [C-6"], 20.66,

20.73, 20.90, 21.08 21.10 and 21.17 [6 X $OCO\underline{C}H_3$], 68.42 [C-5"], 68.82 and 69.07 [C-2" and C-3"], 70.25 [C-4"], 98.04 [C-1"], 108.98 [C-8(A)], 113.60 [C-6(A)], 115.14 [C-10(A)], 122.21 [C-3',5'(B)], 127.45 [C-1'(B)], 130.17 [C-2',6'(B)], 136.96 [C-3(C)], 150.35, 152.74, 153.94, 155.30 and 156.67 [C-4'(B), C-9(A), C-7(A), C-5(A) and C-2(C)], 168.01, 168.70, 169.41, 169.65, 169.96 and 170.03 [6 X $O\underline{C}OCH_3$], 172.25 [C-4(C)].

4'-OMe-epigallocatechin (**45**) this is a reddish brown crystalline solid, mp 144°C, R_f 0.3 [CH_2Cl_2-acetone (55:45)]; FAB-MS *m/z* 321 $[M+H]^+$; $[\alpha]_D^{23}$ -60.00° (EtOH; *c* 0.12), $[\alpha]_{546}^{23}$ -76.67° (EtOH; *c* 0.12); ^{1}HNMR (CD_3OD): δ2.75 [1H, *dd*, *J*= 2.9, 16.8Hz, H-4(C)$_{eq}$], 2.91 [1H, *dd*, *J*=4.3, 16.8Hz, H-4(C)$_{ax}$], 3.82 [1H, *s*, OMe-4'(B)], 4.21 [1H, *m*, H-3(C)], 4.80 [1H, *brs*, H-2(C)], 5.96 [1H, *d*, *J*=2.2Hz, H-6(A)], 5.98 [1H, *d*, *J*=2.2Hz, H-8(A)], 6.56 [2H, *s*, H-2',6'(B)]; ^{13}CNMR (CD_3OD): δ29.49 [C-4(C)], 61.08 [OMe-4'(B)], 67.70 [C-3(C)], 79.96 [C-2(C)], 96.18 [C-6(A)], 96.72 [C-8(A)], 100.35 [C-10(A)], 107.46 [C-2',6'(B)], 136.37 [C-4'(B)], 136.92 [C-1'(B)],151.67 [C-3',5'(B)], 157.46, 157.97, and 158.28 [C-5(A), C-7(A), and C-9(A)].

REFERENCES

1. Kelly, M.G. and Hartwell, J.L. (1954) *J. Natl. Cancer Inst.* **14**, 967.
2. Jardine, J. (1980) in *Anticancer Agents based on Natural Product Models* (Cassidy, J.M. and Douros, J.D., eds.), p. 319. Academic Press, New York.
3. Palmer, E. and Pitman, N. (1972) *Trees of Southern Africa*, Vol. 3, p. 1613. A.A. Balkema, Cape Town.
4. Warren, F.L., Cooks, R.G. and Williams, D.H. (1967) *J. Chem. Soc. (C)* 286.
5. Wright, W. and Warren, F.L. (1967) *J. Chem. Soc. (C)* 283.
6. Wang, Y., Luo, Y. and Tang, X. (1979) *Acta Pharm. Sinica* **11**, 622.
7. Harborne, J.B. (ed.) (1988) *The Flavonoids-Advances in Research since 1980*, Chapman and Hall, London and New York.
8. Ebel, J. and Hahlbrock, K. (1982) in *The Flavonoids-Advances in Research* (Harborne, J.B. and Mabry, T.J., eds.), p. 641. Chapman and Hall, London.
9. Hahlbrock, K. (1981) in *The Biochemistry of plants* (Conn, E.E.and Stumpf, P.K., eds.), Vol. 7, p. 425. Academic Press, New York.
10. Heller, W. and Forkmann, G. (1988) in *The Flavonoids-Advances in Research since 1980* (Harborne, J.B., ed.), p. 399. Chapman and Hall, London and New York.
11. Birch, A.J. and Donovan, F.W. (1953) *Aust. J. Chem.* **6**, 360.
12. Grisebach, H. (1962) *Planta medica.* **10**, 385.
13. Ebel, J., Schaller-Hekeler, B., Knobloch, K.H., Wellmann, E., Grisebach, H. and Hahlbrock, K. (1974) *Biochem. Biophys. Acta.* **362**, 417.
14. Hanson, K.R. and Havir, E.A. (1981) in *The Biochemistry of Plants* (Stumpf, P.K. and Conn, E.E., eds.), Vol. 7, p. 577. Academic Press, New York.

15. Butt, V.S. and Lamb, C.J. (1981) in *The Biochemistry of Plants* (Stumpf, P.K. and Conn, E.E., eds.), Vol. 7, p. 627. Academic Press, New York.
16. Russell, D.W. and Conn, E.E. (1967) *Arch. Biochem. Biophys.* **122**, 256.
17. Amrhein, N. and Zenk, M.H. (1970) *Naturwissenschaften* **57**, 312.
18. Hahlbrock, K., Ebel, J., Ortmann, R., Sutter, A., Wellmann, E. and Grisebach, H. (1971) *Biochem. Biophys. Acta.* **244**, 7.
19. Satô, M. (1969) *Phytochemistry* **8**, 353.
20. Vaughan, P.F.T. and Butt, V.S. (1972) *J. Biochem.* **127**, 641.
21. Stotz, G., Spribille, R. and Forkmann, G. (1984) *J. Plant. Physiol.* **116**, 173.
22. Sütfeld, R., Kehrel, B. and Wiermann, R. (1978) *Z. Natürforsch.* **33c**, 841.
23. Sütfeld, R. and Wiermann, R. (1981) *Z. Naturforsch.* **36c**, 30.
24. Dixon, R.A., Blyden, E.R., Robbins, M.P., van Tunen, A.J. and Mol, J.N. (1988) *Phytochemistry* **27**, 2801.
25. Bednar, R.A. and Hadcock, J.R. (1988) *J. Biol. Chem.* **263**, 9582.
26. Dewick, P.M. (1990) *Natural Product Reports* **7**, 165.
27. Dewick, P.M. (1991) *Natural Product Reports* **8**, 149.
28. Brouillard, R. (1988) in *The Flavonoids-Advances in Research since 1980* (Harborne, J.B., ed.), p. 399. Chapman and Hall, London and New York.
29. Scalbert, A. (1991), *Phytochemistry* **30**, 3875.
30. Ingham, J.L., Tahara, S. and Dziedzic, S.Z. (1986) *J. Nat. Prod.* **49**, 631.
31. Coxon, D.T., O'Neill, T.M., Mansfield, J.W. and Porter, E.A. (1980) *Phytochemistry* **19**, 889.
32. McClure, J.W. (1975) in *The Flavonoids* (Harborne, J.B., Mabry, T.J. and Mabry, H., eds.), p. 970. Chapman and Hall, London.

33. Doskotch, R.W., Chatterji, S.K. and Peacock, J.W. (1970) *Science* **167**, 380.
34. Doskotch, R.W., Mikhail, A.A. and Chatterji, S.K. (1973) *Phytochemistry* **12**, 1153.
35. Dreyer, D.L. and Jones, K.C. (1981) *Phytochemistry* **20**, 2489.
36. Hedin, P.A., Jenkins, J.N., Collum, D.H., White, W.H. and Parrott, W.L. (1983) in *Plant Resistance to Insects* (Hedin, P.A., ed.), p. 347. American Chemical Society, Washington D.C..
37. Bickoff, E.M., Livingstone, A.L., Hendrickson, A.P. and Booth, A.N. (1962) *J. Agric. Food Chem.* **10**, 410.
38. Smolenski, S.J., Kinghorn, A.D. and Balandrin, M.F. (1981) *Economic Botany* **35**, 321.
39. Cody, V., Middleton, E. and Harborne, J.B.,(eds.) (1986) *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships*, p. 87. A.R. Liss Inc., New York.
40. Nakagawa, M., Nakanishi, K., Darko, L. and Vick, J.A. (1982) *Tetrahedron Lett.* **23**, 3855.
41. Wagner, H. (1986) in *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships* (Cody, V., Middleton, E. and Harborne, J.B., eds.), p. 545. A.R. Liss Inc., New York.
42. Hikino, H., Kiso, Y., Wagner, H. and Fiebig, M. (1984) *Plant. med.* 248.
43. Gabor, M. (1986) in *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships* (Cody, V., Middleton, E. and Harborne, J.B., eds.), p. 471. A.R. Liss Inc., New York.
44. Villar, A., Gasco, M.A., Manez, S. and Cortes, D. (1985) *Plant. Med.* **45**, 70.
45. Villar, A., Gasco, M.A. and Alcaraz, M.J. (1984) *J. Pharm. Pharmacol.* **36**, 820.

46. Hikino, H., Takahashi, M. and Konno, C. (1982) *Tetrahedron Lett.* **23**, 673.
47. Kasahara, Y. and Hikino, H. (1983) *Heterocycles* **20**, 1953.
48. Hikino, H., Shimoyama, N., Kasahara, Y., Takahashi, M. and Konno, C. (1982) *Heterocycles* **19**, 1381.
49. Kasahara, Y., Shimoyama, H., Konno, C. and Hikino, H. (1983) *Heterocycles* **20**, 1741.
50. Kolodziej, H., Sakar, M.K., Burger, J.F.W., Engelshowe, R. and Ferreira, D. (1991) *Phytochemistry* **30**, 2041.
51. González, A.G., Irizar, C.I., Ravelo, A.G. and Fernádez, M.F. (1992) *Phytochemistry* **31**, 1432.
52. Porter, L.J. (1988) in *The Flavonoids-Advances in Research since 1980* (Harborne, J.B., ed.), p. 21. Chapman and Hall, London and New York.
53. Haslam, E. (1975) in *The Flavonoids* (Harborne, J.B. and Mabry, T.J.,eds.), p. 505. Chapman and Hall, London.
54. Haslam, E. (1982) in *The Flavonoids-Advances in Research* (Harborne, J.B. and Mabry, T.J. eds.), p. 417. Chapman and Hall, London.
55. Hemingway, R.W., Foo, L.Y. and Porter, L.J. (1982) *J. Chem. Soc., Perkin Trans 1* 1209.
56. IUPAC (1979) *Nomenclature of Organic Chemistry*, Pergamon, Oxford.
57. Botha, J.J., Viviers, P.M., Young, D.A., du Preez, I.C., Ferreira, D., Roux, D.G. and Hull, W.E. (1982) *J. Chem. Soc., Perkin Trans 1* 527.
58. Ferreira, D., Steynberg, J.P., Roux, D.G. and Brandt, E.V. (1992) *Tetrahedron* **48**, 1743.
59. Mayer, W., Goll, L. Arndt, E.V. and Mannschreck, A. (1966) *Tetrahedron Lett.* 429.
60. Jacques, D., Haslam, E., Bedford, G.R. and Greatbanks, D. (1974) *J. Chem. Soc., Perkin Trans 1* 2663.
61. Otsuka, H., Fujioka, S., Komiya, T., Mizuta, E. and Takemoto, M. (1982) *Yakugaku Zasshi* **102**, 162.
62. Nonaka, G., Morimoto, S., Kinjo, J., Nohara, T. and Nishioka, I. (1987) *Chem. Pharm. Bull.* **35**, 149.

63. Baldé, A.M., Pieters, L.A., Gergely, A., Kolodziej, H., Claeys, M. and Vlietinck, A.J. (1991) *Phytochemistry* **30**, 337.
64. Porter, L.J., Ma, Z. and Chan, B.G. (1991) *Phytochemistry* **30**, 1657.
65. Hashimoto, F., Nonaka, G. and Nishioka, I. (1989) *Chem. Pharm. Bull.* **37**, 3255.
66. Ohigashi, H., Minami, S., Fukui, H., Koshimizu, K.,Mizutani, F., Sugiura, A. and Tomana, T. (1982) *Agric. Biol. Chem.* **46**, 2555.
67. Baldé, A.M., Pieters, L.A., Wray, V., Kolodziej, H., van den Berghe, D.A., Claeys, M. and Vlietinck, A.J. (1991) *Phytochemistry* **30**, 4129.
68. Morimoto, S., Nonaka, G. and Nishioka, I. (1988) *Chem. Pharm. Bull.* **36**, 33.
69. Nonaka, G., Morimoto, S. and Nishioka, I. (1983) *J. Chem. Soc., Perkin Trans 1* 2139.
70. Porter, L.J., Hrstich, L.N. and Chan, B.G. (1986) *Phytochemistry* **25**, 223.
71. Cronjé, A., Burger, J.F.W., Brandt, E.V., Kolodziej, H. and Ferreira, D. (1990) *Tetrahedron Lett.* **31**, 3789.
72. Burger, J.F.W., Kolodziej, H., Hemingway, R.W., Steynberg, J.P., Young, D.A. and Ferreira, D. (1990) *Tetrahedron* **46**, 5733.
73. Cunningham, A. (1988) in *An Investigation of the herbal medicine trade in Natal/Kwazulu* (INR Investigational Report No. 29).
74. Drewes, S.E., Taylor, C.W. and Balfour-Cunningham, A. (1992) *Phytochemistry* **31**, 1073.
75. Drewes, S.E., Taylor, C.W., Balfour-Cunningham, A., Ferreira, D., Steenkamp. J.A. and Mouton, C.H.L. (1992) *Phytochemistry* **31**, 2491.
76. Howes, F.N. (1953) in *Vegetable Tanning Materials* Butterworth Scientific Publications, London.
77. Hostettmann, K. and Marston, A. (1990) *Analytica Chimica Acta* **236**, 63.
78. Ito, Y. (1986) *Anal. Chem.* **17**, 65.

79. Ito, Y. (1984) *J. Chromatogr.* **301**, 377.
80. Slacanin, I., Marston, A. and Hostettmann, K. (1989) *J. Chromatogr.* **482**, 234.
81. Conway, W.D. (1991) *J. Chromatogr.* **538**, 27.
82. Hills, W.E. and Carle, A. (1960) *Aust. J. Chem.* 390.
83. Waterman, P.G. and Faulkner, D.F. (1979) *Planta Medica* **37**, 178.
84. Clark-Lewis, J.L. (1968) *Aust. J. Chem.* **21**, 2059, 2032.
85. Roux, D.G., Engel, D.W., Hattingh, M. and Hundt, H.K.L. (1978) *J. Chem. Soc., Chem. Commun.* 695.
86. Fronczek, F.R., Gannuch, G., Mattice, W.L., Tobiason, F.L., Broeker, J.L. and Hemingway, R.W. (1984) *J. Chem. Soc., Perkin Trans II* 1611.
87. Porter, L.J., Wong, R.Y., Benson, M., Chan, B.G., Vishwanadhan, V.N., Gandour, R.D. and Mattice, W.L. (1986) *J. Chem. Research* (S) 86.
88. Johnson, F. (1968) *Chem. Rev.* **68**, 375.
89. Ellis, C.J., Foo, L.Y. and Porter, L.J. (1983) *Phytochemistry* **22**, 483.
90. Cai, Y., Evans, F.J., Roberts, M.F., Phillipson, J.D., Zenk, M.H. and Gleba, Y.Y. (1991) *Phytochemistry* **30**, 2033.
91. Delle Monache, F., D'Albuqueque, I.L., Ferrari, F. and Marini-Bettolo, G.B. (1967) *Tetrahedron Lett.* 4211.
92. Delle Monache, F., Pomponi, M., Marini-Bettolo, G.B., D'Albuqueque, I.L. and de Lima, O.G. (1976) *Phytochemistry* **15**, 573.
93. Kamal, G.M., Gunaherath, B., Gunatilaka, A.A.L., Sultanbawa, M.U.S. and Balasubramanian, S. (1982) *J. Nat. Prod.* **45**, 140.
94. Weeralinga, G., Bohlin, L., Verpoorte, R., and Kumar, V. (1985) *Phytochemistry* **24**, 2093.
95. Ishimaru, K., Nonaka, G. and Nishioka, I. (1987) *Phytochemistry* **26**, 1167.
96. Rasoanaivo, P., Ratsimamanga-Urverg, S., Messana, I., de Vicente, Y. and Geleffi, C. (1990) *Phytochemistry* **29**, 2040.

97. Bock, K.,Pederson, C. and Pederson, H. (1984) *Adv. Carbohydr. Chem. Biochem.* **42**, 193.
98. Markham, K.R. and Chari, V.M. (1982) in *The Flavonoids-Advances in Research* (Harborne, J.B. and Mabry, T.J., eds.), p. 19. Chapman and Hall, London.
99. Masuda, T., Jitoe, A., Kato, S. and Makatani, N. (1991) *Phytochemistry* **30**, 2391.
100. Barrett, M.W., Klyne, W., Scopes, P.M., Fletcher, A.L., Porter, L.J. and Haslam, E. (1979) *J. Chem. Soc., Perkin Trans I* 2375.
101. Botha, J.J., Young, D.A., Ferreira, D. and Roux, D.G. (1981) *J. Chem. Soc., Perkin Trans I* 1213.
102. Dale, J.A. and Mosher, H.S. (1973) *J. Am. Chem. Soc.*, **95**, 512.
103. Sullivan, G.R., Dale, J.A. and Mosher, H.S. (1973) *J. Org. Chem.* **38**, 2143.
104. Hundt, A.F., Burger, J.F.W., Steynberg, J.P., Steenkamp, J.A. and Ferreira, D. (1990) *Tetrahedron Lett.* **31**, 5073.
105. Brown, D.W. (1985) *J. Chem. Ed.* **62**, 209.
106. Weinges, K., Marx, H.D. and Göritz, K. (1970) *Chem. Ber.* **103**, 2336.
107. Du Preez, I.C., Rowan, A.C., Roux, D.G. and Feeney, J. (1971) *J. Chem. Soc., Chem. Commun.* 315.
108. Thompson, R.S., Jacques, D., Haslam, E. and Tanner, R.J.W. (1972) *J. Chem. Soc., Perkin Trans I* 1387.
109. Kolodziej, H. (1986) *Phytochemistry* **25**, 1209.
110. Scholz, E. and Rimpler H. (1989) *Plant. Med.* **55**, 379.
111. Still, W.C., Kahn, M. and Mitra, A. (1978) *J. Org. Chem.* **43**, 2923.

APPENDIX-NMR SPECTRA

1.1. Afzelechin, ^{1}HNMR spectrum (acetone-d_6)

1.2. Afzelechin, ^{13}CNMR spectrum (acetone-d_6)

2.1. Epiafzelechin, ^{1}HNMR spectrum (acetone-d_6)

2.2. Epiafzelechin, ^{13}CNMR spectrum (acetone-d_6)

3.1. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, ^{1}HNMR spectrum (CD_3OD)

3.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, ^{13}CNMR spectrum (CD_3OD)

3.3 Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, HETCOR spectrum (CD_3OD)

4.1. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether, ^{1}HNMR spectrum ($CDCl_3$)

4.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether, ^{13}CNMR spectrum ($CDCl_3$)

4.3. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether, ^{1}H-^{1}H COSY spectrum ($CDCl_3$)

4.4. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether, HETCOR spectrum ($CDCl_3$)

4.5. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether, DELAYED HETCOR spectrum (7.5Hz) ($CDCl_3$)

5.1. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate, ^{1}HNMR spectrum ($CDCl_3$)

5.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate, ^{13}CNMR spectrum ($CDCl_3$)

5.3. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate, ^{1}H-^{1}H DELAYED COSY spectrum (0.1sec) ($CDCl_3$)

5.4. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate, HETCOR spectrum ($CDCl_3$)

5.5. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate, DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

6.1. Afzelechin-3-O-α-L-rhamnopyranoside, ^{1}HNMR spectrum (acetone-d_6 + D_2O)

6.2. Afzelechin-3-O-α-L-rhamnopyranoside, ^{13}CNMR spectrum (acetone-d_6)

6.3. Afzelechin-3-O-α-L-rhamnopyranoside, ^{1}H-^{1}H DELAYED COSY spectrum (5Hz) (acetone-d_6)

6.4. Afzelechin-3-O-α-L-rhamnopyranoside, HETCOR spectrum (5Hz) (acetone-d_6)

7.1. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate, ^{1}HNMR spectrum ($CDCl_3$)

7.2. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate, ^{13}CNMR spectrum ($CDCl_3$)

7.3. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate, HETCOR spectrum ($CDCl_3$)

8.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, ^{1}HNMR spectrum (CD_3OD)

8.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, ^{13}CNMR spectrum (CD_3OD)

8.3. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, HETCOR spectrum (CD_3OD)

8.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, DELAYED HETCOR spectrum (7Hz) (CD_3OD)

9.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, ^{1}HNMR spectrum ($CDCl_3$)

9.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, ^{13}CNMR spectrum ($CDCl_3$)

9.3. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, ^{1}H-^{1}H COSY spectrum ($CDCl_3$)

9.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, HETCOR spectrum ($CDCl_3$)

9.5. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

10.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether, ^{1}HNMR spectrum ($CDCl_3$)

10.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether, ^{13}CNMR spectrum ($CDCl_3$)

10.3. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether, ^{1}H-^{1}H COSY spectrum ($CDCl_3$)

10.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether, HETCOR spectrum ($CDCl_3$)

10.5. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether, DELAYED HETCOR spectrum (6Hz) ($CDCl_3$)

11.1. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{1}HNMR spectrum ($CDCl_3$)

11.2. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{1}HNMR spectrum δ6.4-7.8 ($CDCl_3$)

11.3. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{13}CNMR spectrum ($CDCl_3$)

11.4. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{1}H-^{1}H COSY spectrum ($CDCl_3$)

11.5. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{1}H-^{1}H COSY spectrum δ2.5-5.8 ($CDCl_3$)

11.6. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate, ^{1}H-^{1}H DELAYED COSY spectrum ($CDCl_3$)

12.1. Epiafzelechin-4β-benzylthioether, ^{1}HNMR spectrum (CD_3OD)

12.2. Epiafzelechin-4β-benzylthioether, ^{13}CNMR spectrum (CD_3OD)

13.1. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, ^{1}HNMR spectrum (CD_3OD)

13.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, ^{13}CNMR spectrum (CD_3OD)

13.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, ^{1}H-^{1}H DELAYED COSY spectrum (2.5Hz) (CD_3OD)

13.4. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin, DELAYED HETCOR spectrum (7Hz) (CD_3OD)

14.1. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate, ^{1}HNMR spectrum ($CDCl_3$)

14.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate, ^{13}CNMR spectrum ($CDCl_3$)

14.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate, HETCOR spectrum ($CDCl_3$)

14.4. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate, DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

15.1. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin, ^{1}HNMR spectrum (CD_3OD)

15.2. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin, ^{13}CNMR spectrum (CD_3OD)

15.3. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin, HETCOR spectrum (CD_3OD)

15.4. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin, DELAYED HETCOR spectrum (7.5Hz) (CD_3OD)

16.1. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin, ^{1}HNMR spectrum (CD_3OD)

16.2. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin, ^{13}CNMR spectrum (CD_3OD)

16.3. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin, HETCOR spectrum (CD_3OD)

16.4. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin, DELAYED HETCOR spectrum (7.5Hz) (CD_3OD)

17.1. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, ^{1}HNMR spectrum (CD_3OD)

17.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, ^{13}CNMR spectrum (CD_3OD)

17.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin, DELAYED HETCOR spectrum (7Hz) (CD_3OD)

18.1. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate, ^{1}HNMR spectrum ($CDCl_3$)

18.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate, ^{13}CNMR spectrum ($CDCl_3$)

18.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate, HETCOR spectrum ($CDCl_3$)

18.4. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate, DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

19.1. Kaempferol-3-O-α-L-rhamnopyranoside, ^{1}HNMR spectrum (CD_3OD)

19.2. Kaempferol-3-O-α-L-rhamnopyranoside, ^{13}CNMR spectrum (CD_3OD)

19.3. Kaempferol-3-O-α-L-rhamnopyranoside, ^{1}H-^{1}H COSY spectrum (CD_3OD)

19.4. Kaempferol-3-O-α-L-rhamnopyranoside, HETCOR spectrum (CD_3OD)

20.1. Kaempferol-3-O-α-L-rhamnopyranoside-hexa-acetate, ^{1}HNMR spectrum ($CDCl_3$)

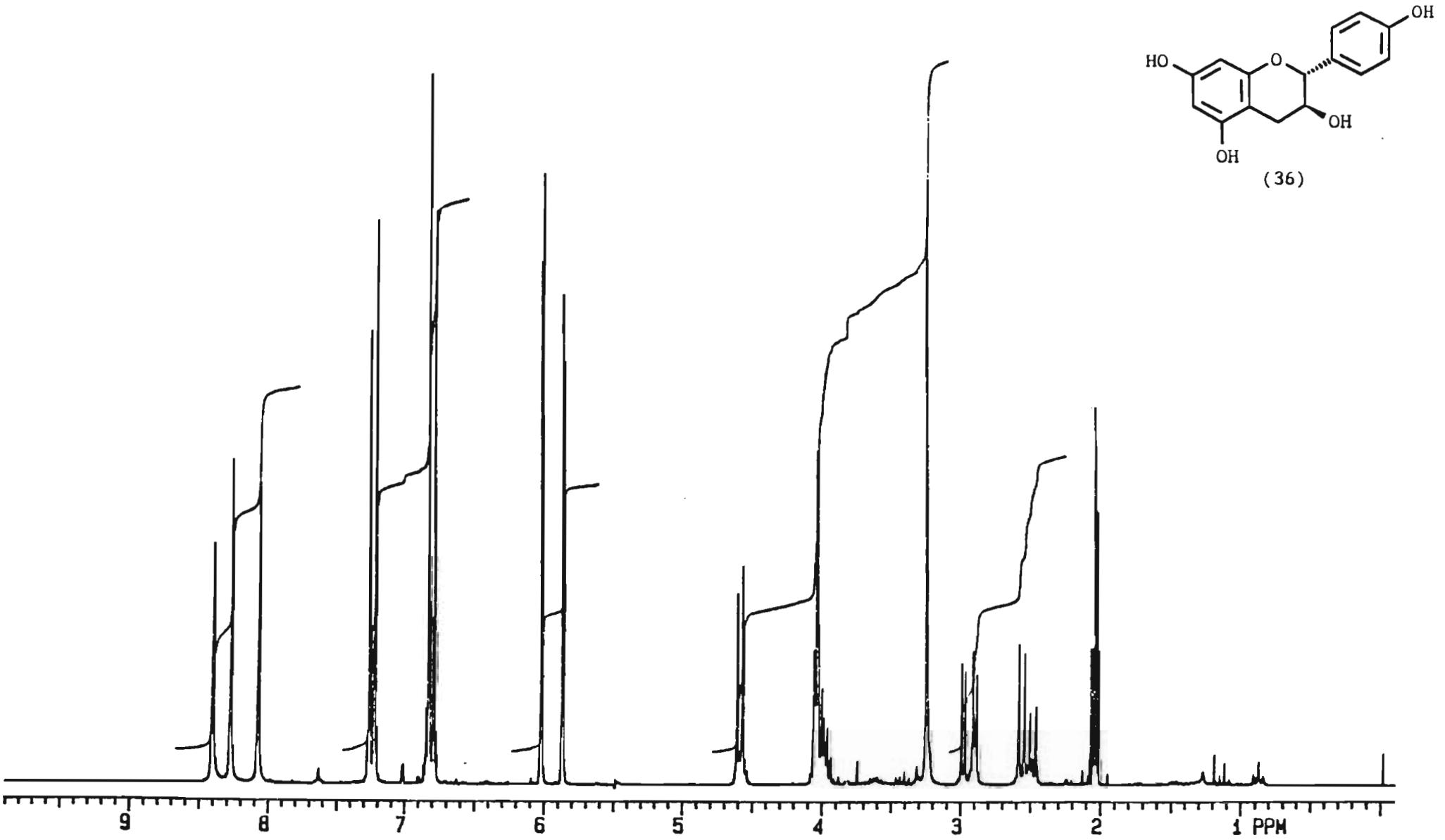
20.2. Kaempferol-3-O-α-L-rhamnopyranoside-hexa-acetate, ^{13}CNMR spectrum ($CDCl_3$)

21.1. 4'-OMe-epigallocatechin, ^{1}HNMR spectrum (CD_3OD)

21.2. 4'-OMe-epigallocatechin, ^{13}CNMR spectrum (CD_3OD)

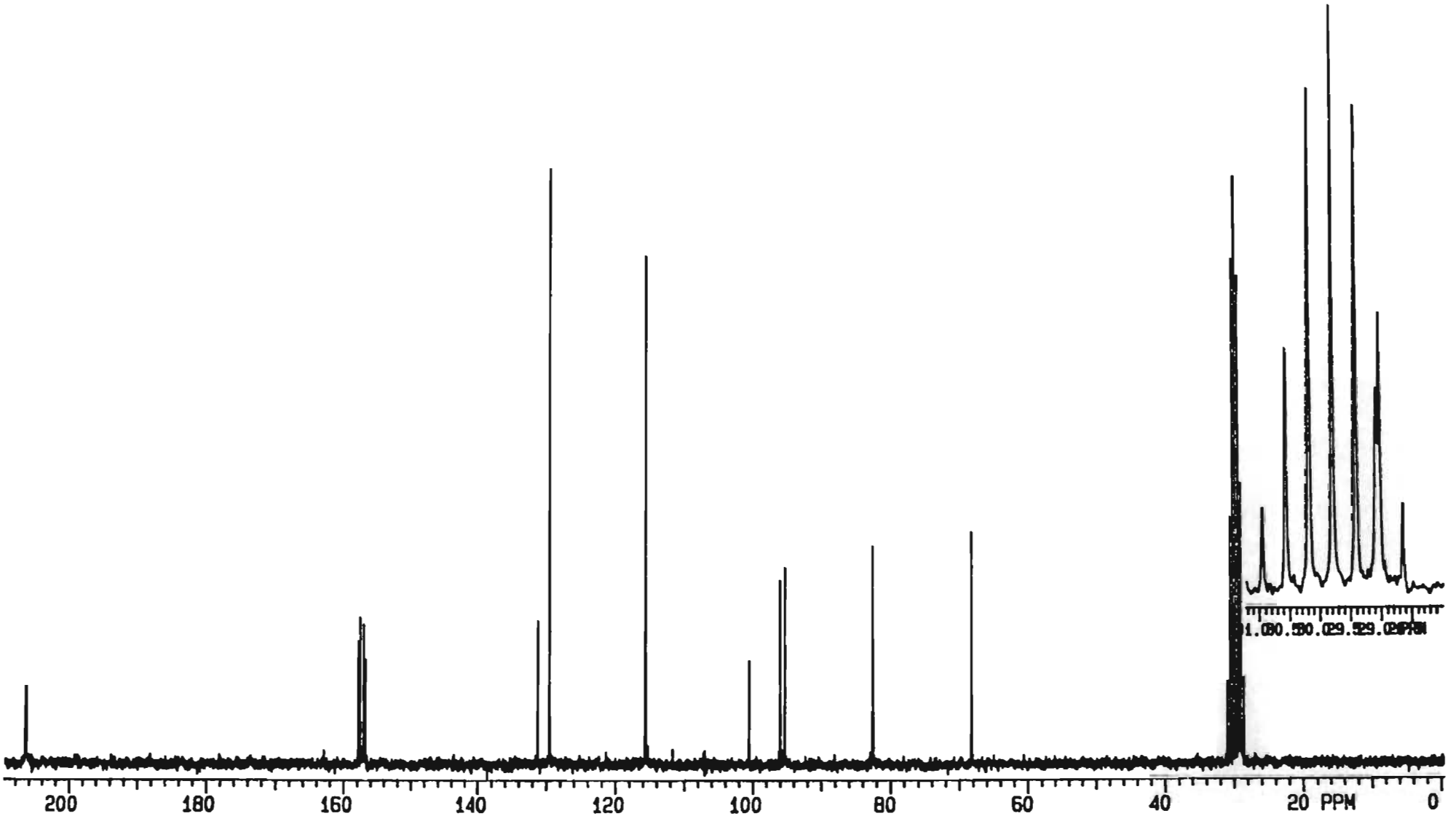
21.3. 4'-OMe-epigallocatechin, HETCOR spectrum (CD_3OD)

21.4. 4'-OMe-epigallocatechin, DELAYED HETCOR spectrum (7Hz) (CD_3OD)

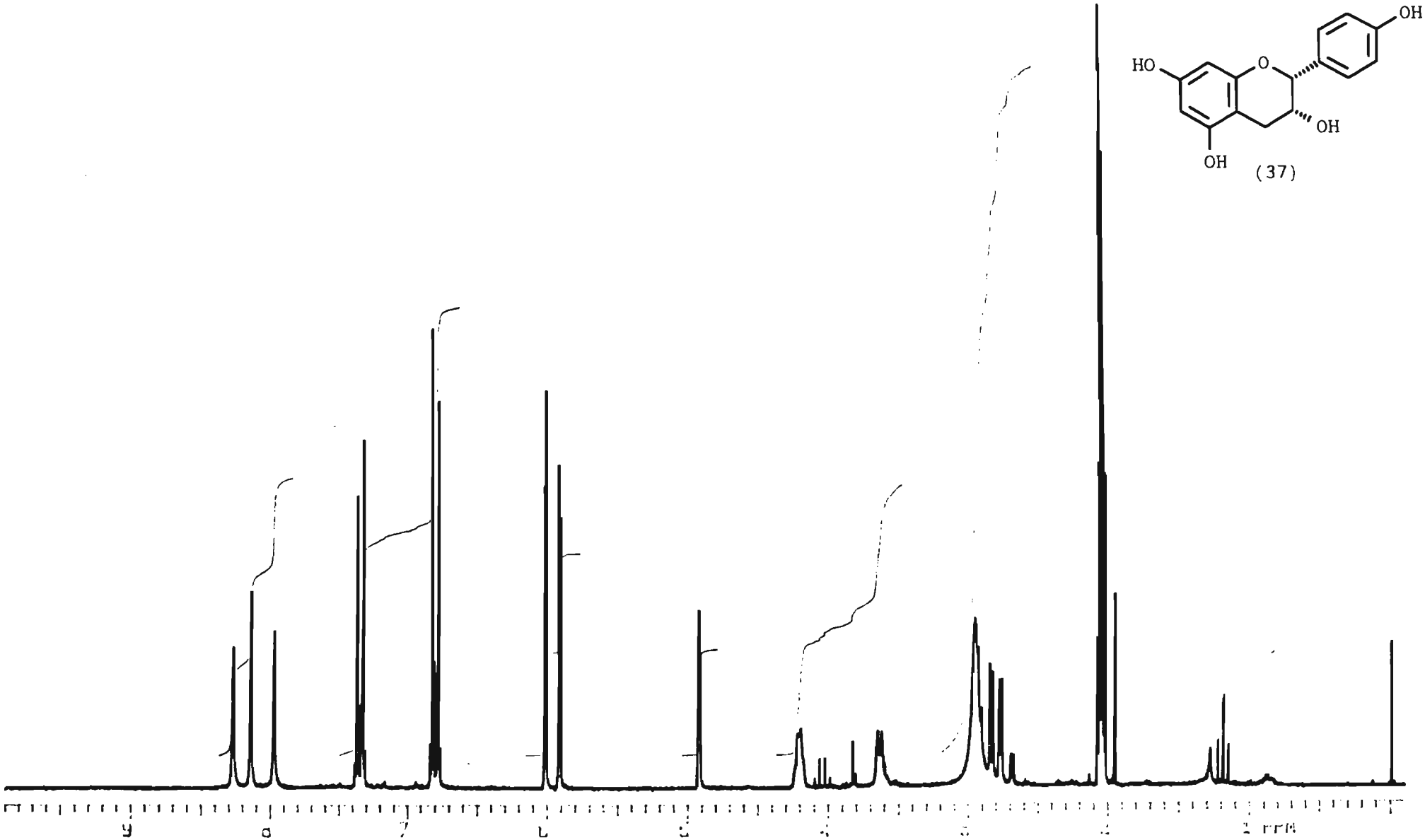


(36)

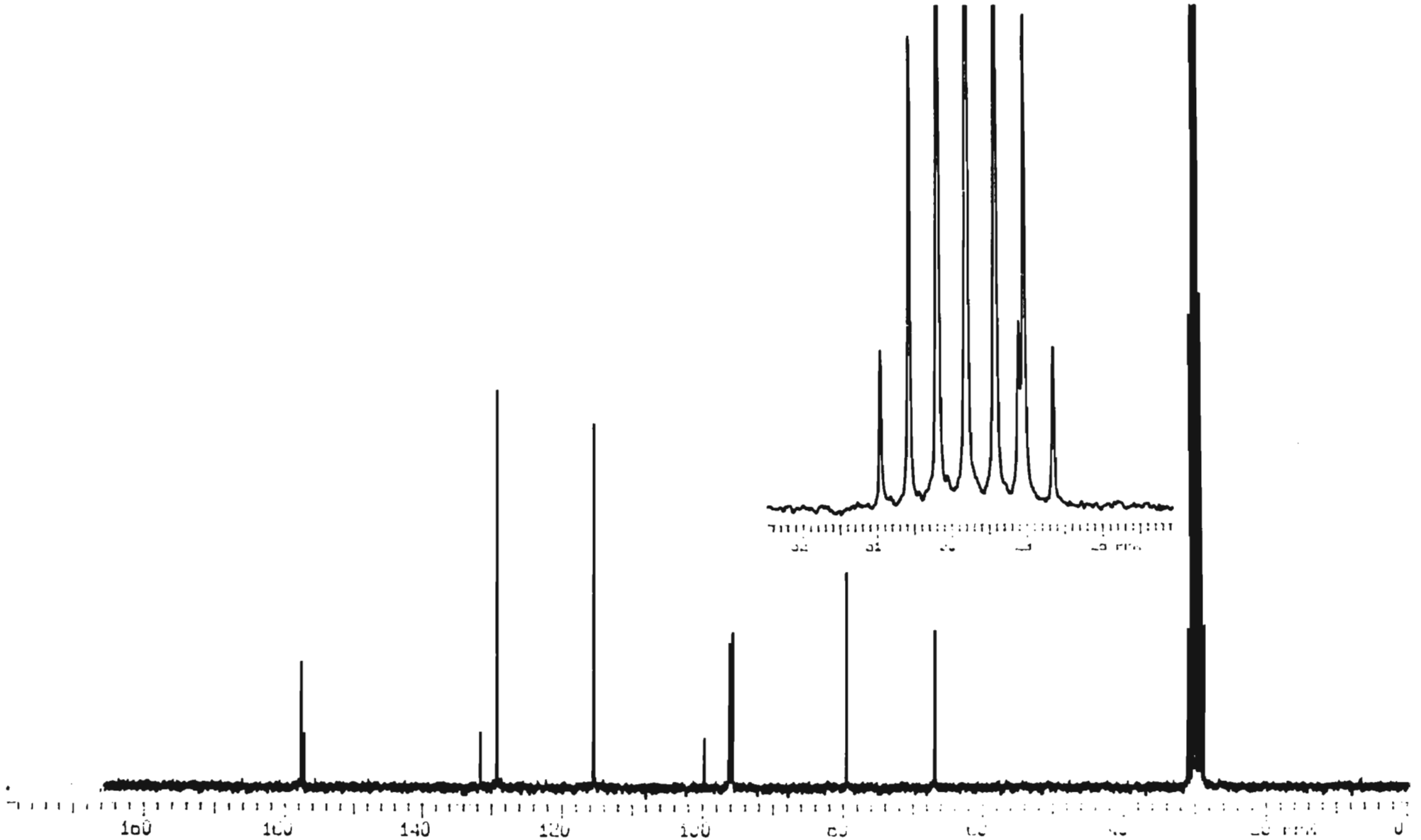
1.1. Afzelechin ^{1}HNMR spectrum (acetone-d_6)



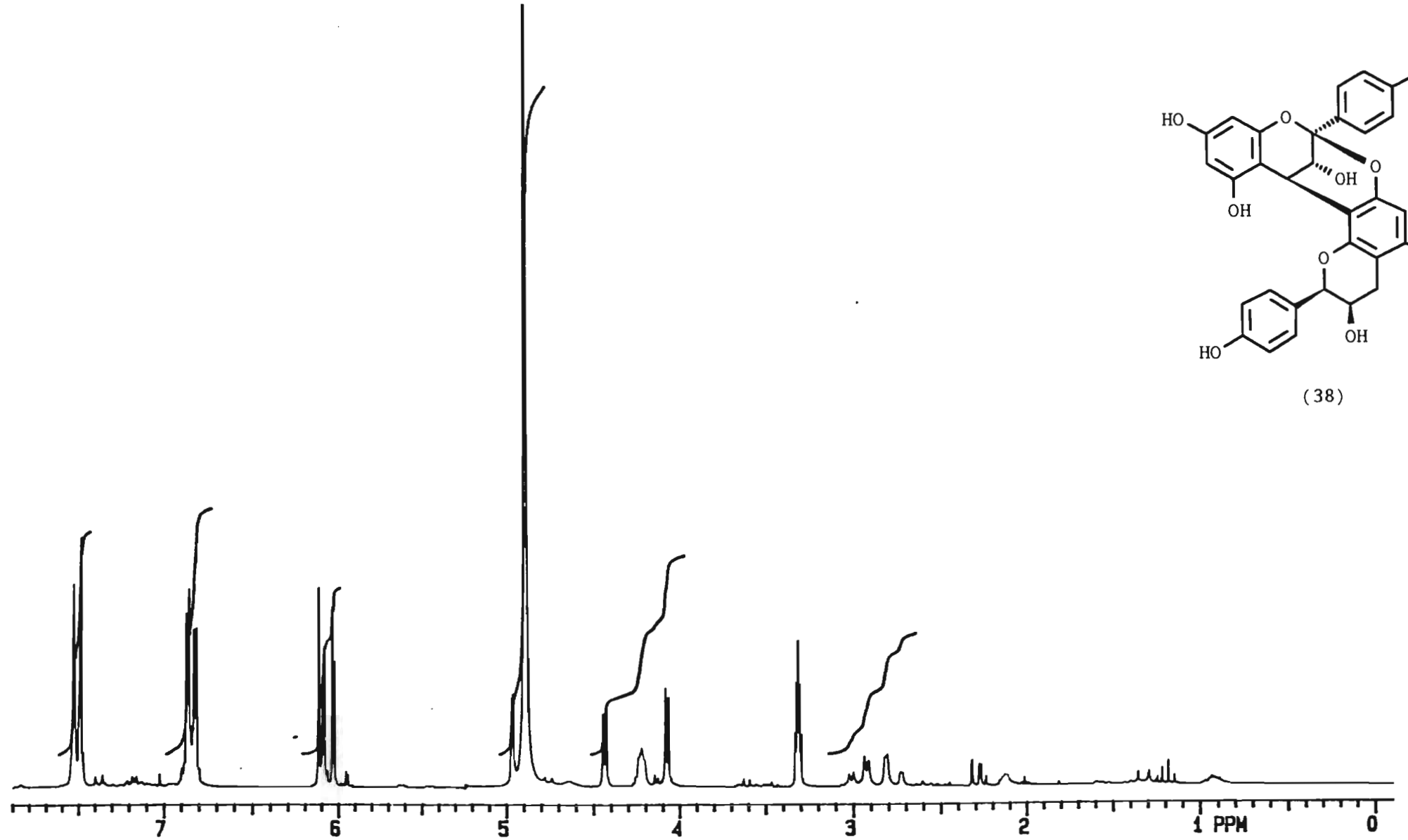
1.2. Afzelechin ^{13}CNMR spectrum (acetone-d_6)



2.1. Epiafzelechin ^{1}HNMR spectrum (acetone-d_6)

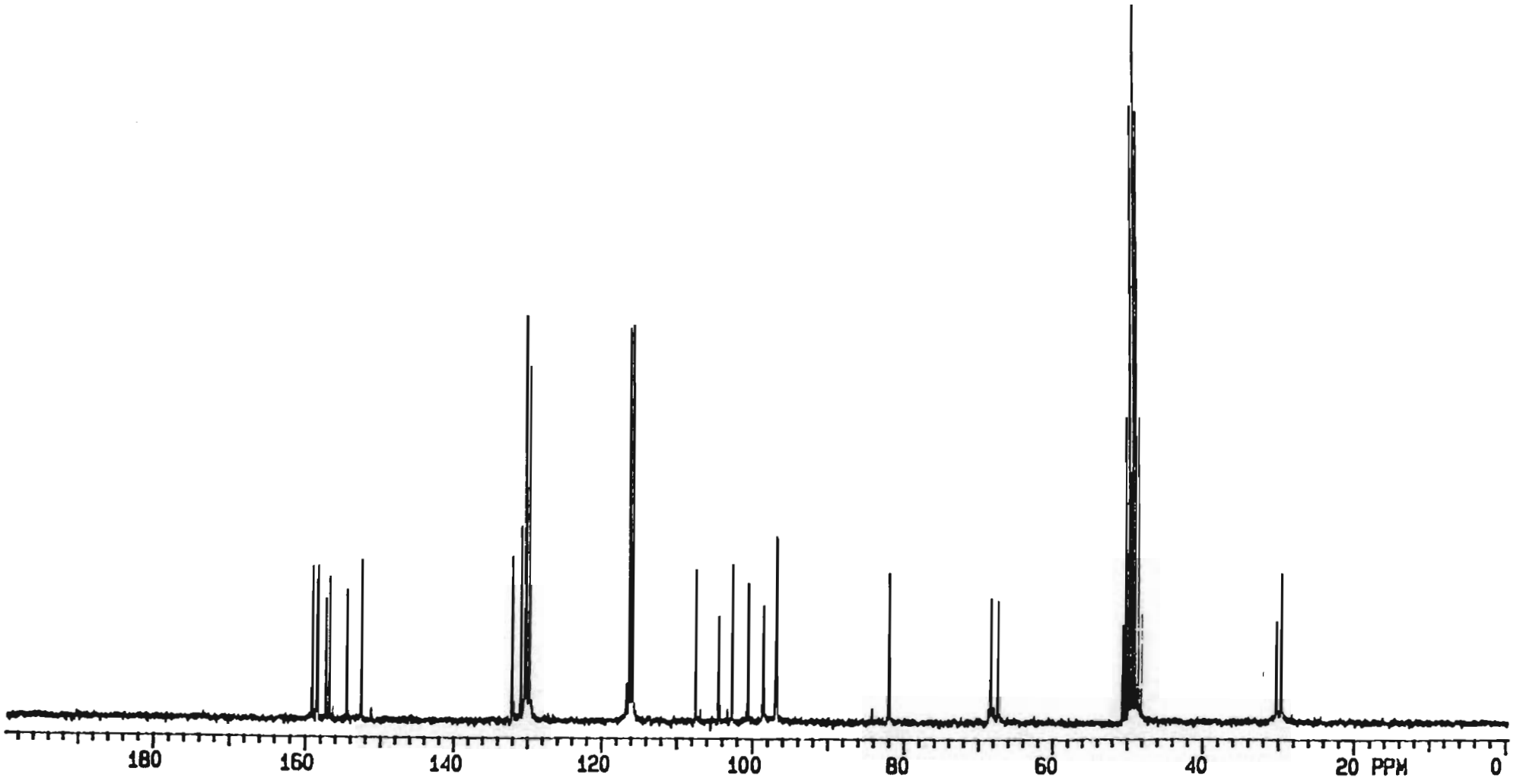


2.2. Epiafzelechin ^{13}CNMR spectrum (acetone-d_6)

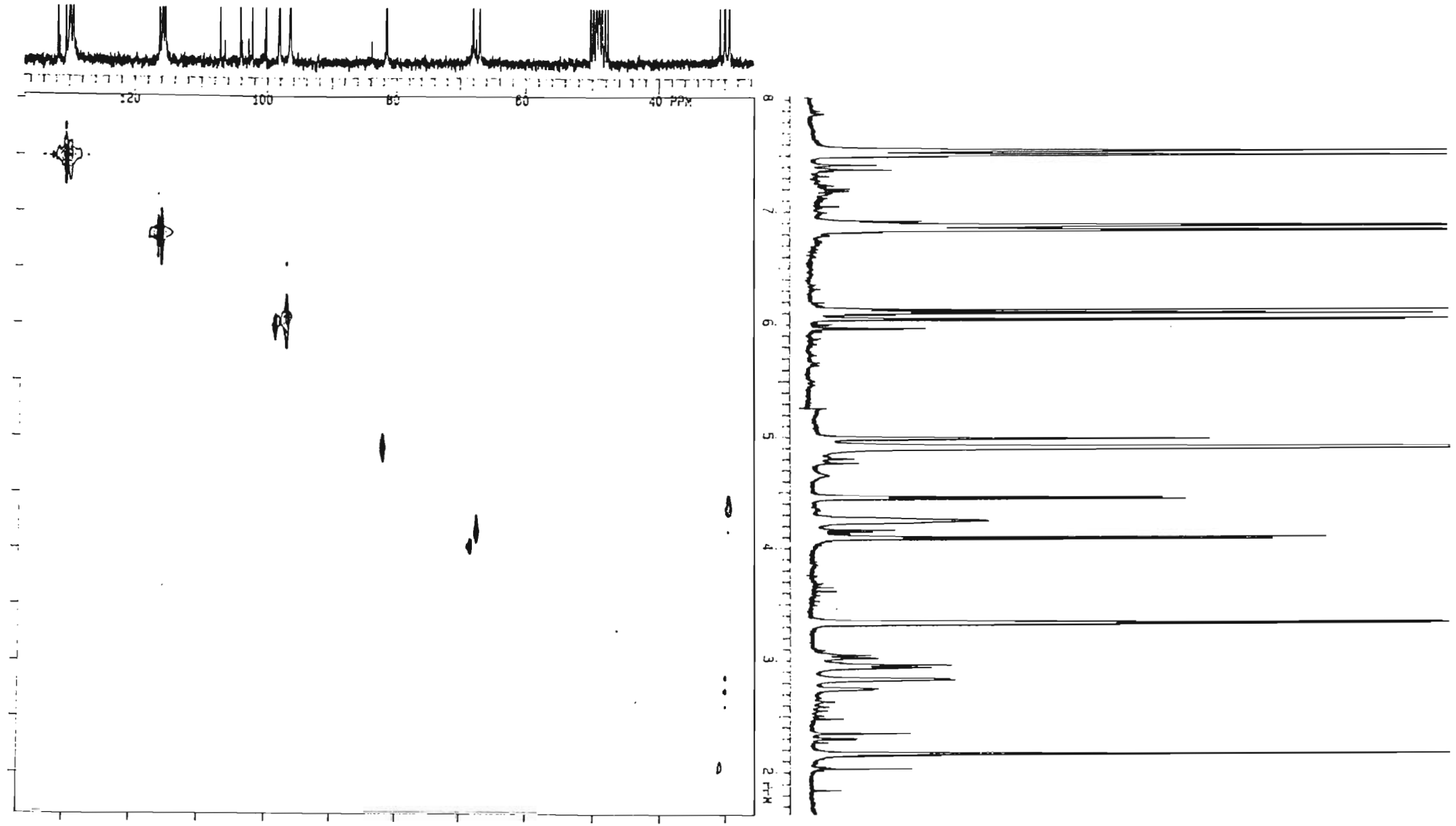


(38)

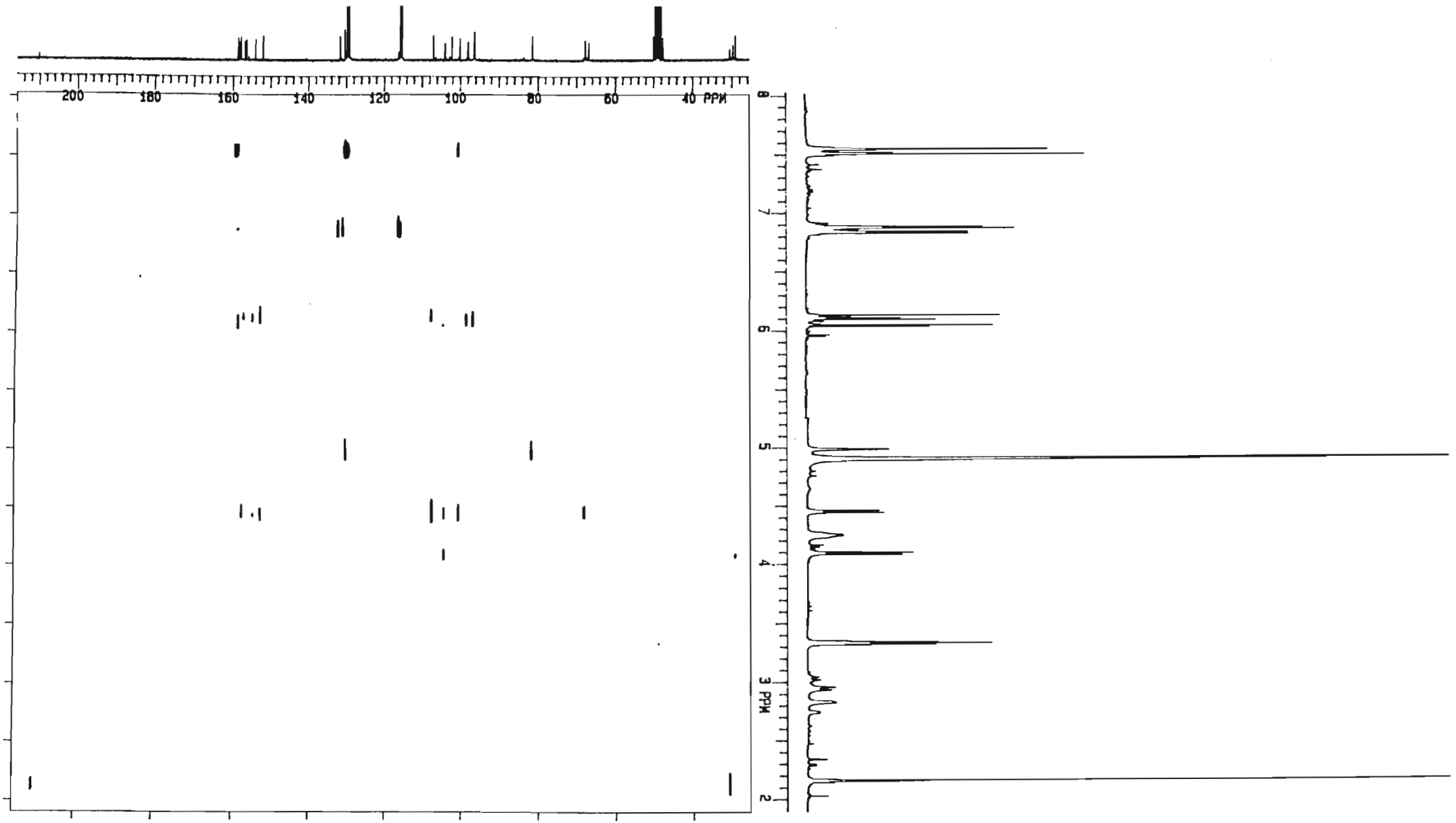
3.1. Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin ^{1}HNMR spectrum (CD_3OD)



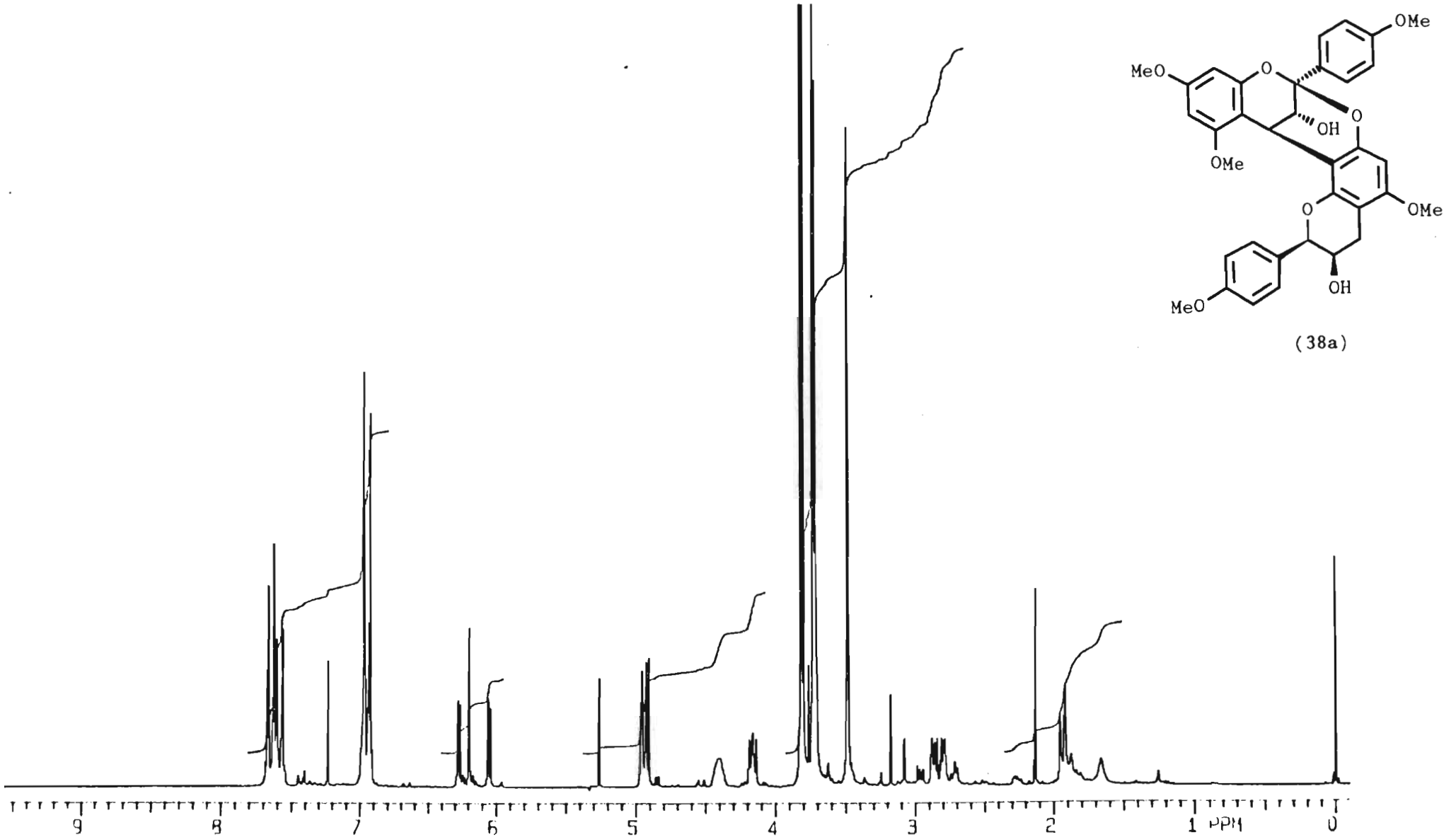
3.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin ^{13}CNMR spectrum (CD_3OD)



3.3. Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin HETCOR spectrum (CD_3OD)

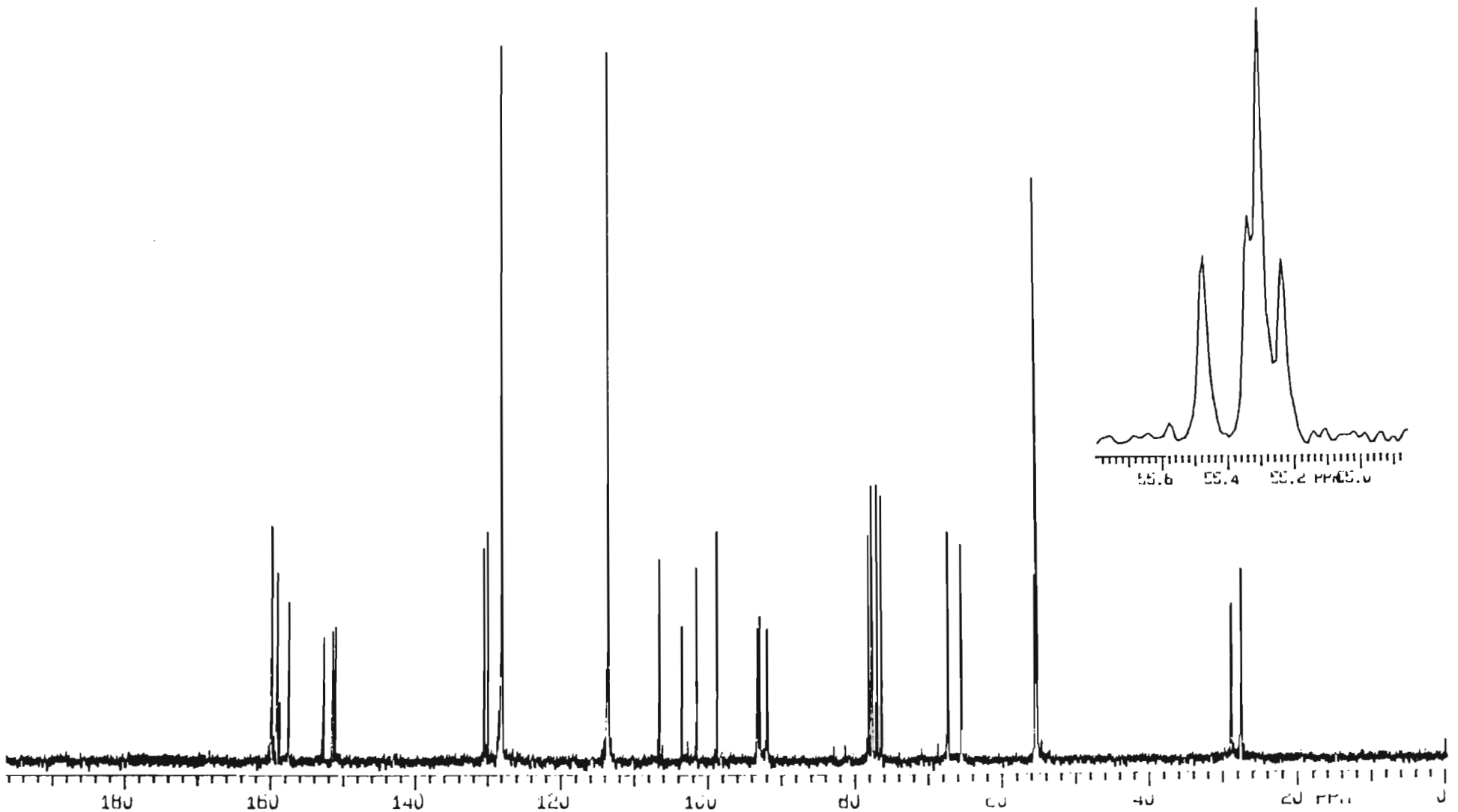


3.4. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin DELAYED HETCOR spectrum (7.5Hz) ($CDCl_3$)

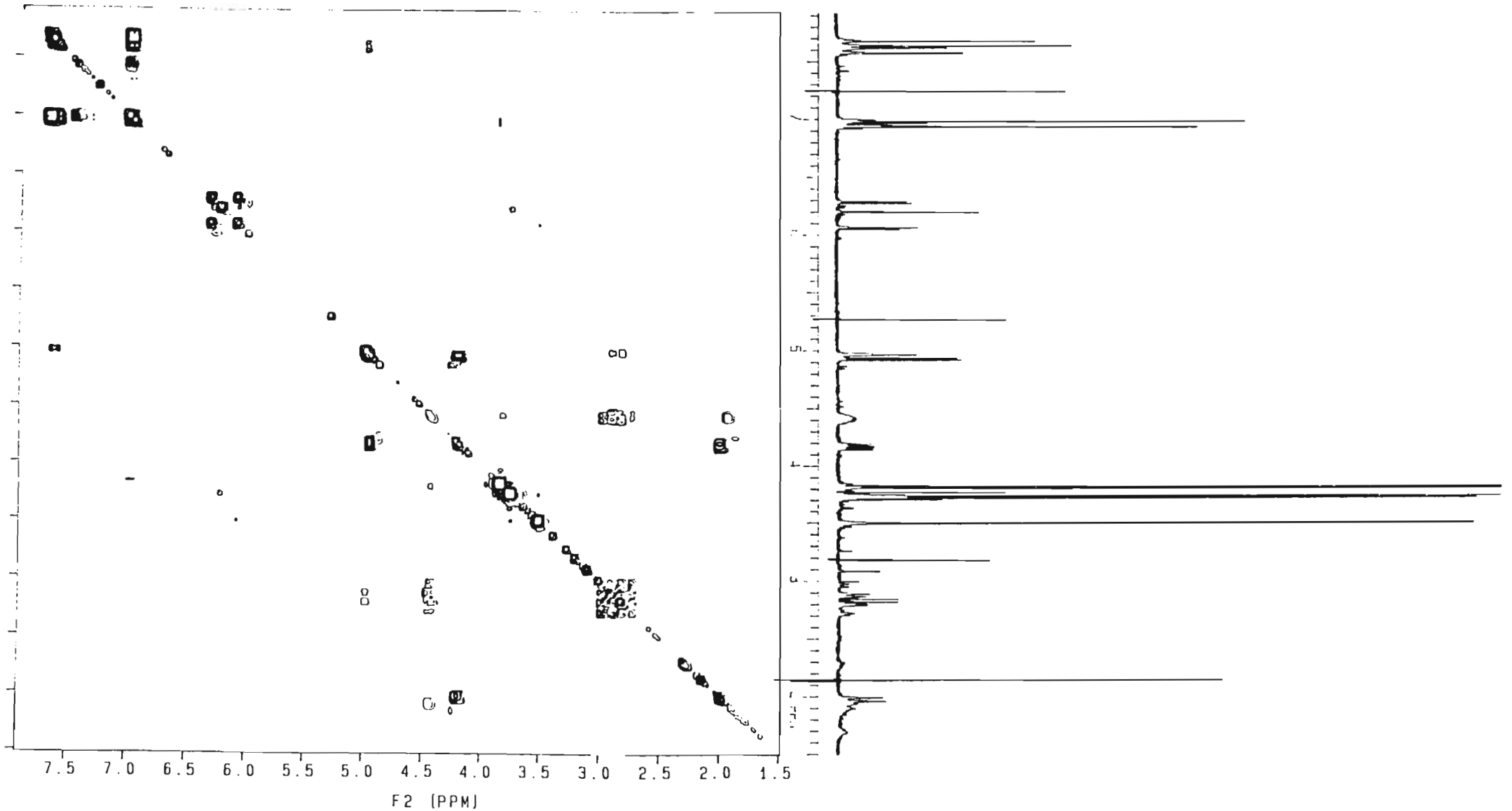


(38a)

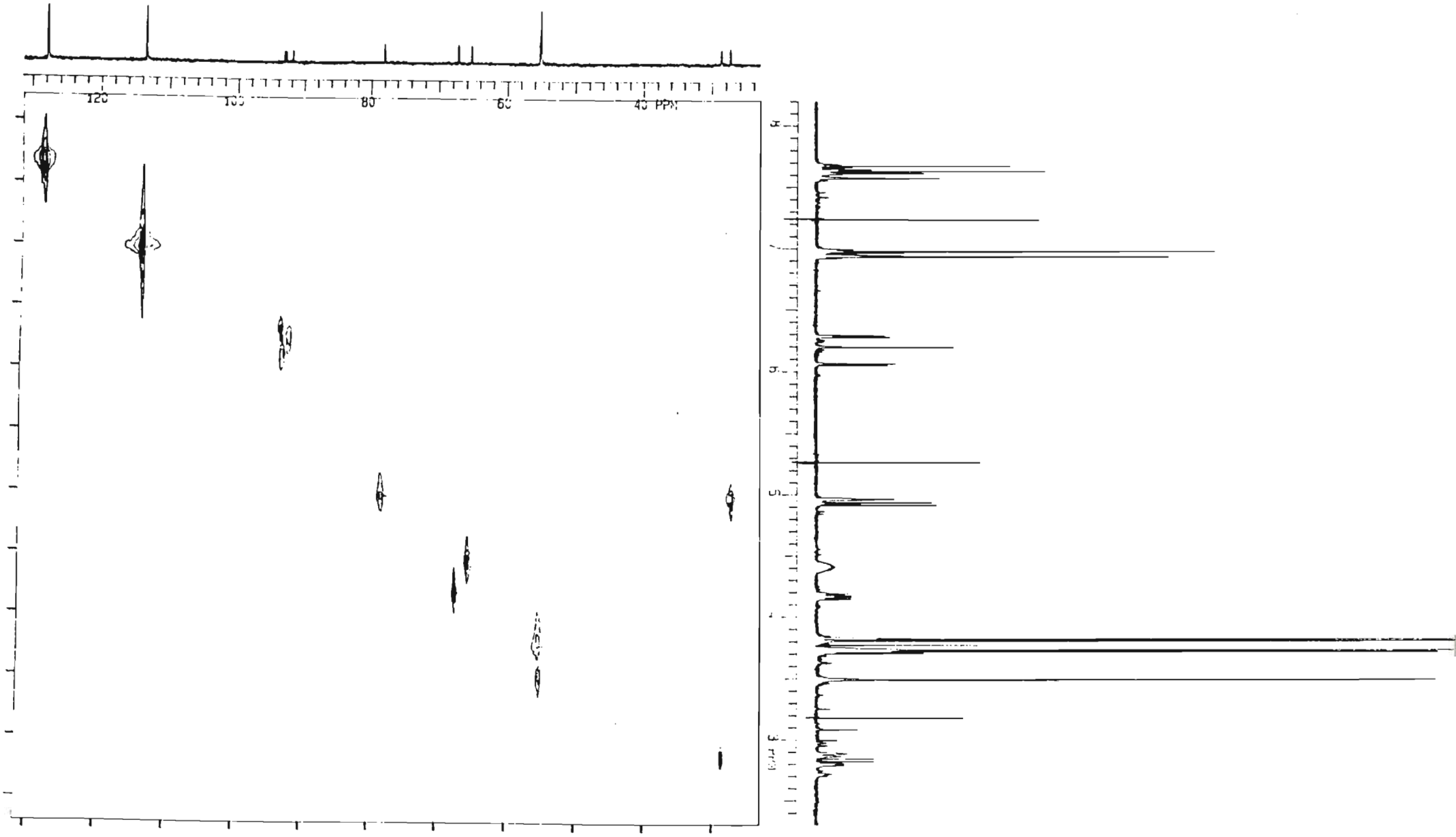
4.1. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether ^{1}HNMR spectrum ($CDCl_3$)



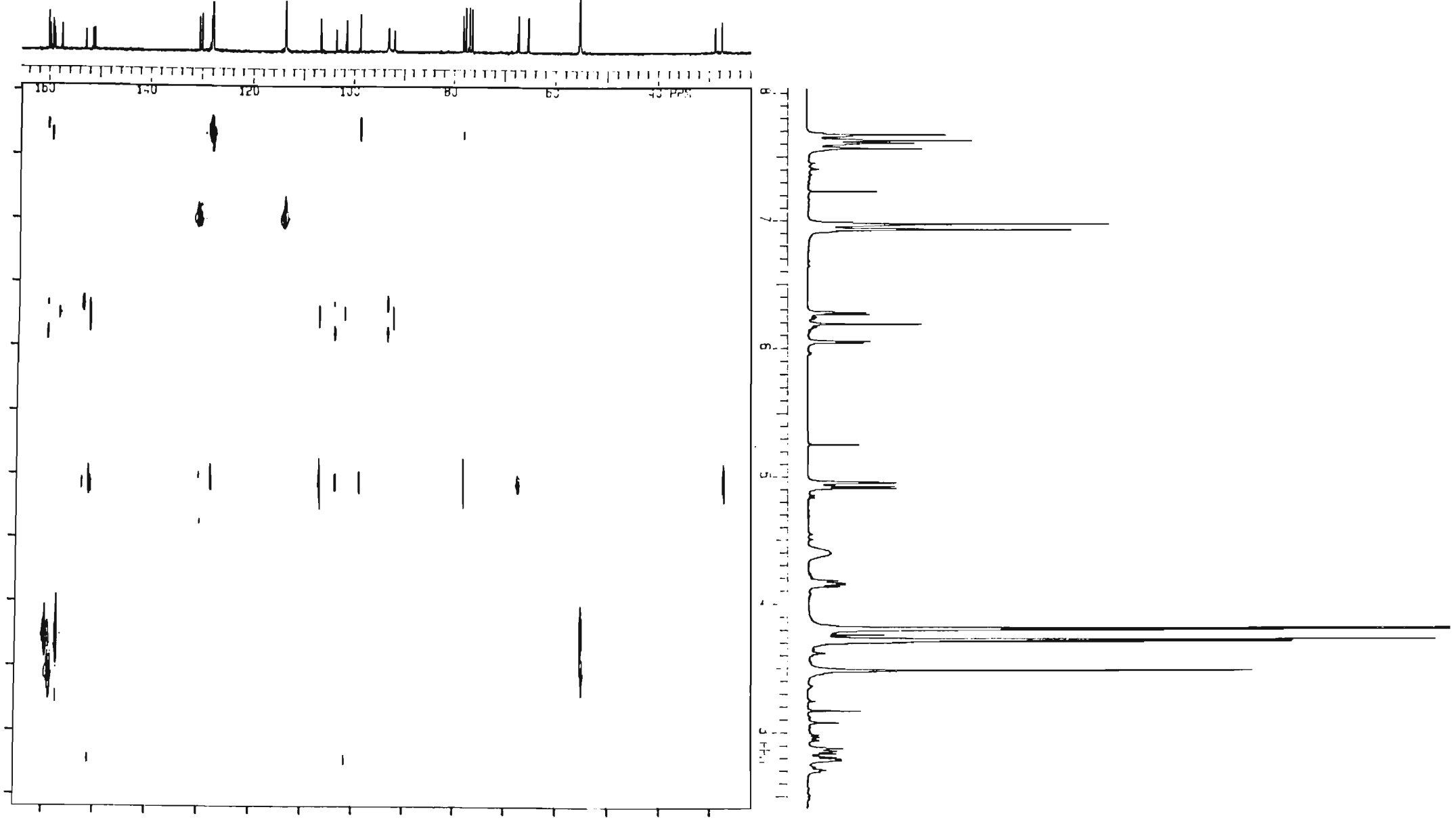
4.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether ^{13}CNMR spectrum ($CDCl_3$)



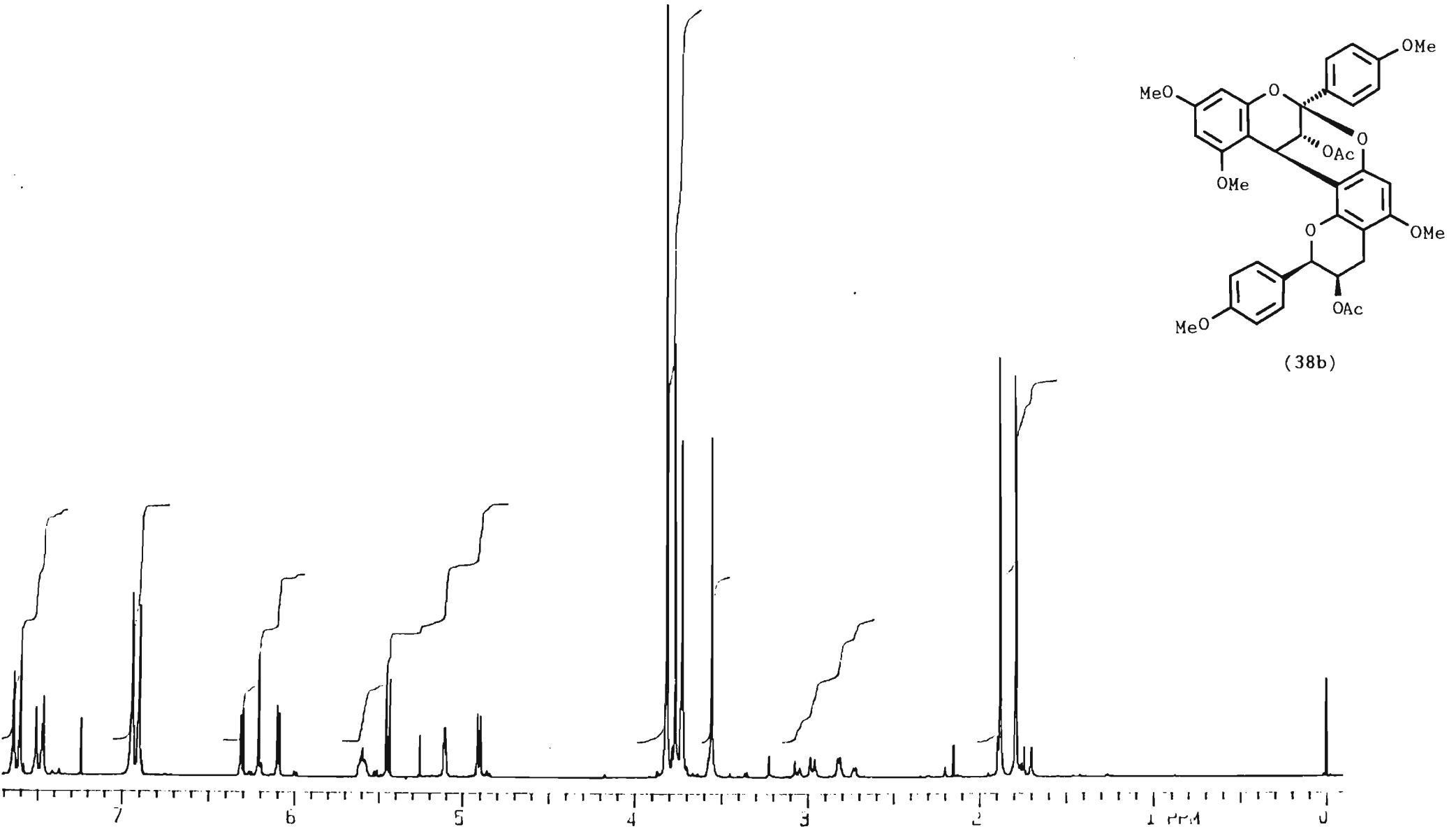
4.3. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether ^{1}H-^{1}H COSY spectrum ($CDCl_3$)



4.4. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether HETCOR spectrum ($CDCl_3$)

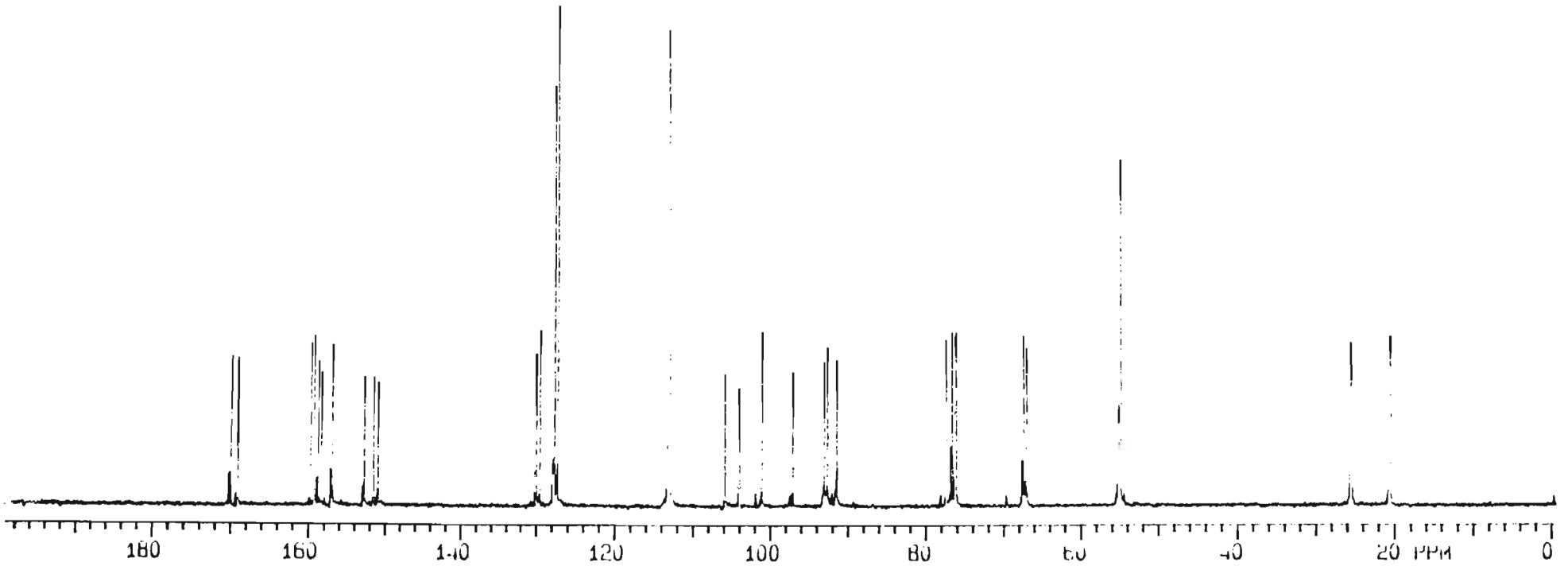


4.5. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether DELAYED HETCOR spectrum (7.5Hz) ($CDCl_3$)

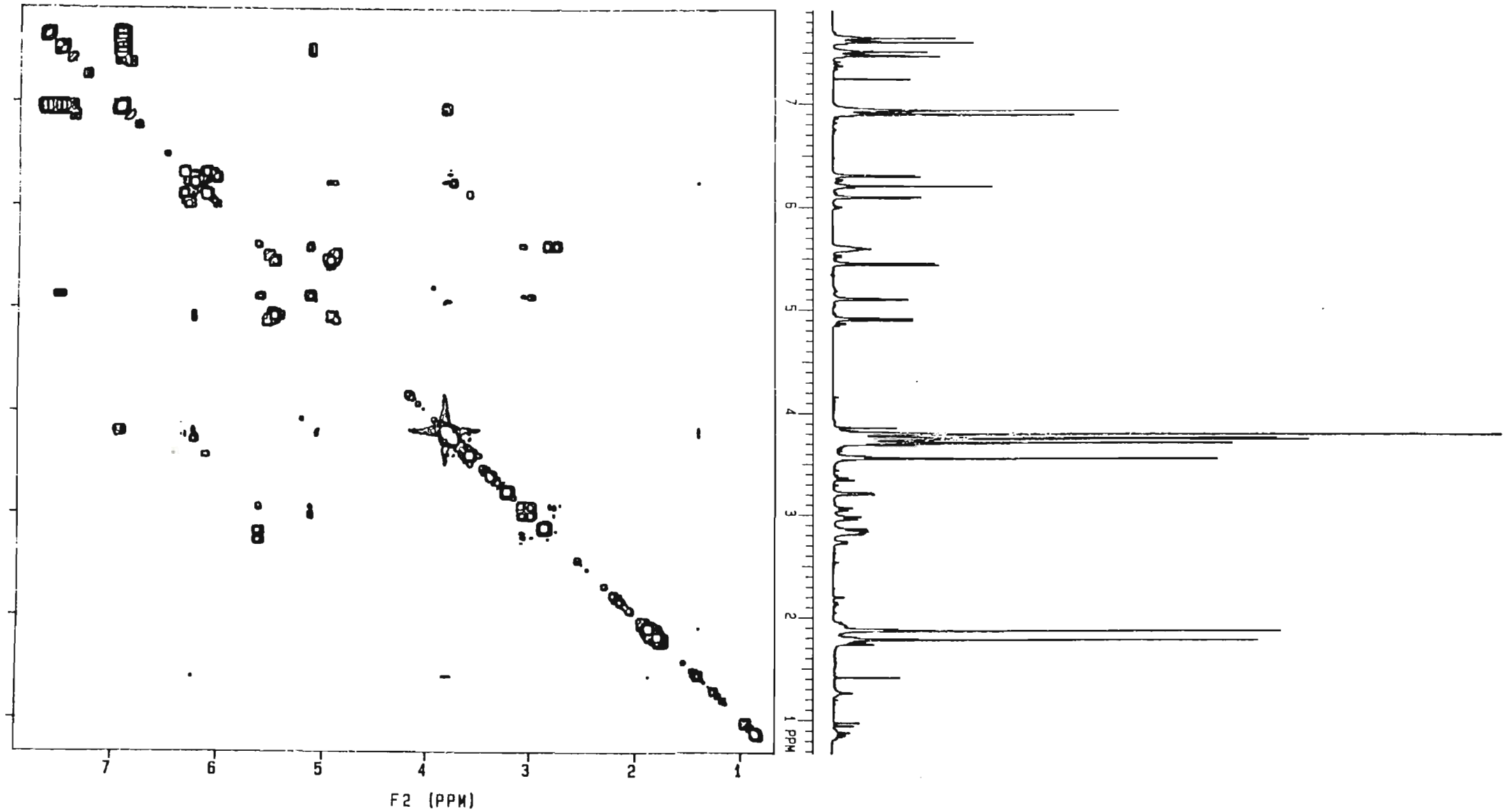


(38b)

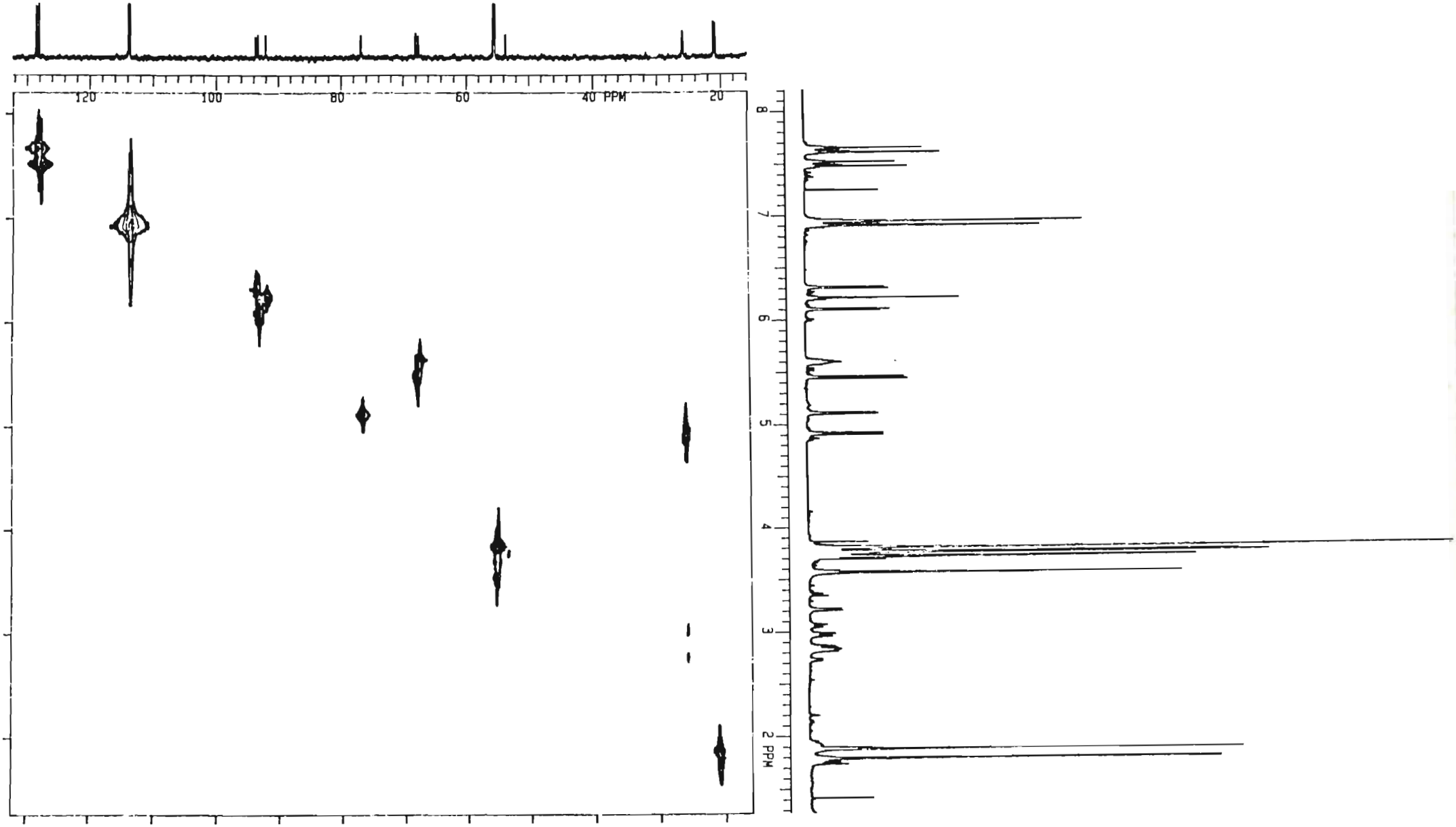
5.1. Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-pentamethyl ether diacetate ^{1}HNMR spectrum ($CDCl_3$)



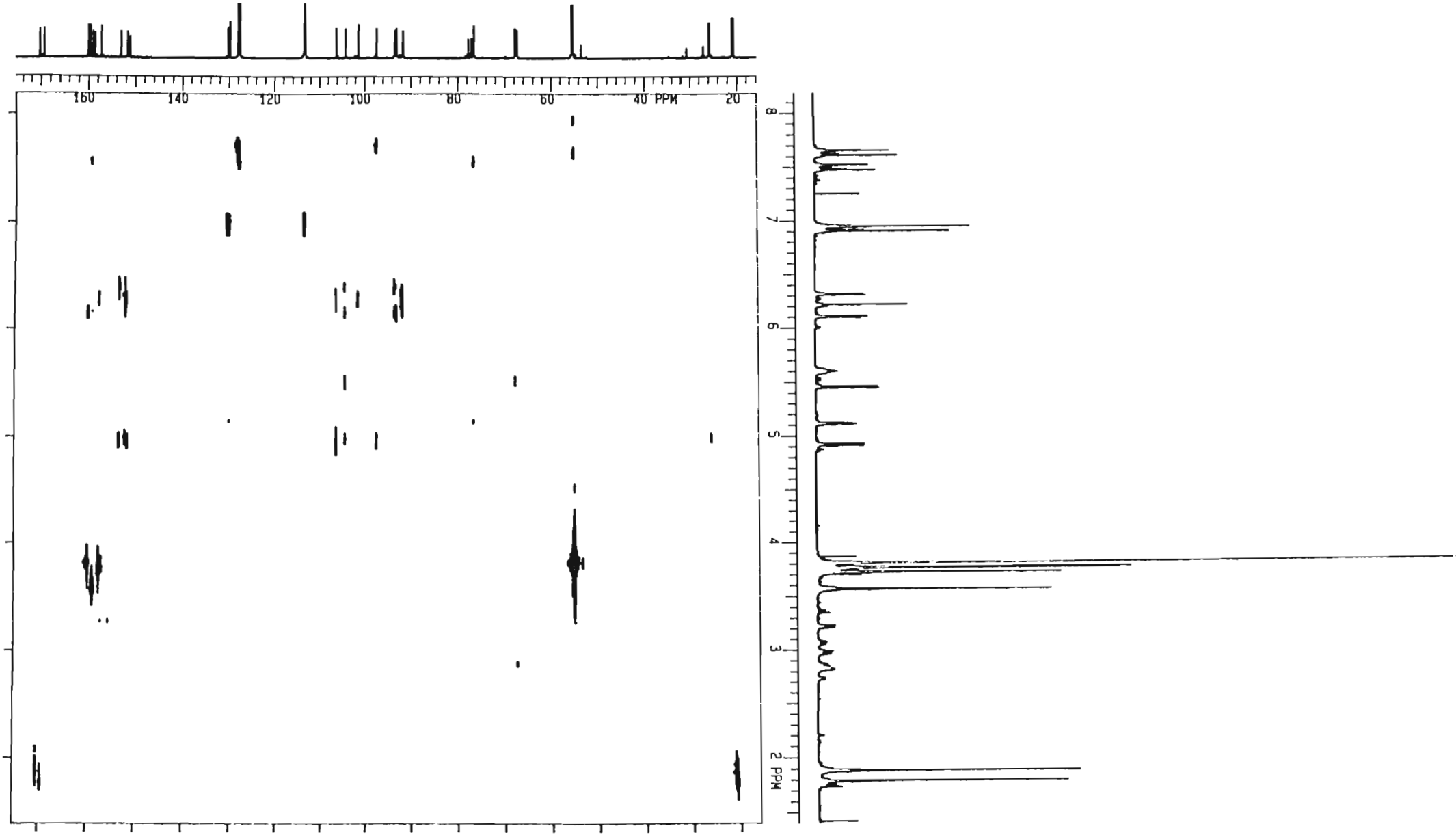
5.2. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate ^{13}CNMR spectrum ($CDCl_3$)



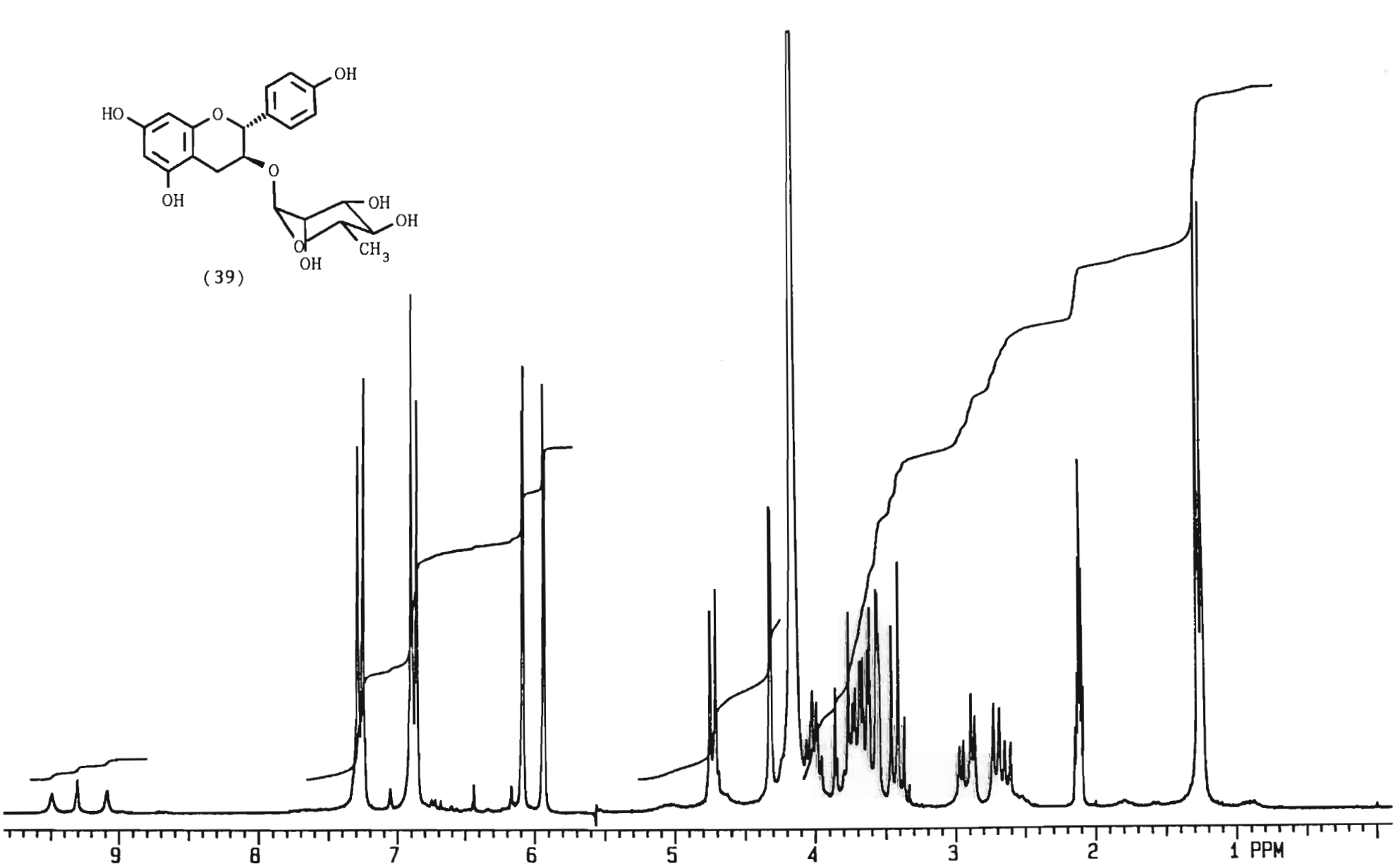
5.3. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate ^{1}H-^{1}H DELAYED COSY spectrum (0.1sec) ($CDCl_3$)



5.4. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate HETCOR spectrum ($CDCl_3$)

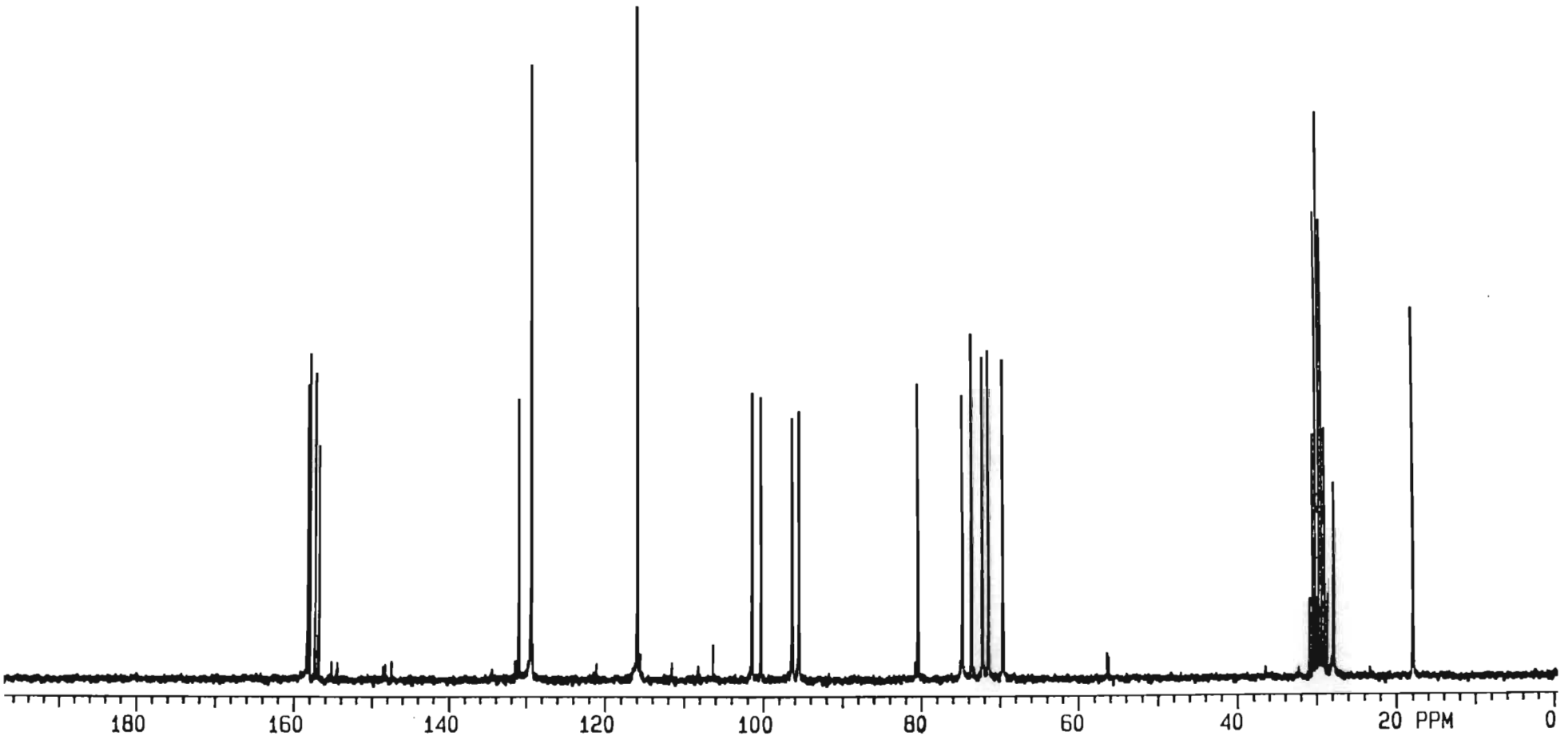


5.5. Epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-pentamethyl ether diacetate DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

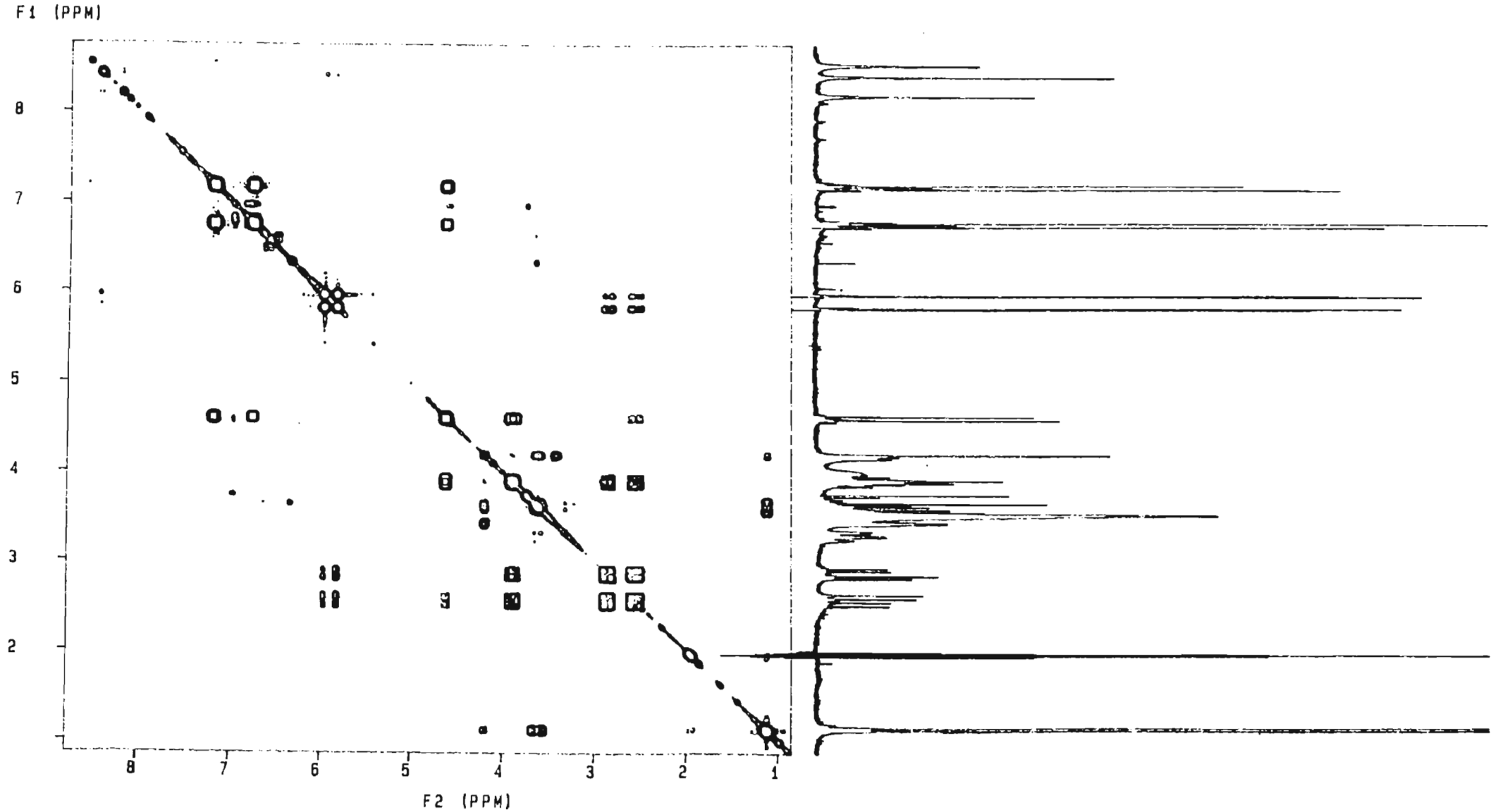


(39)

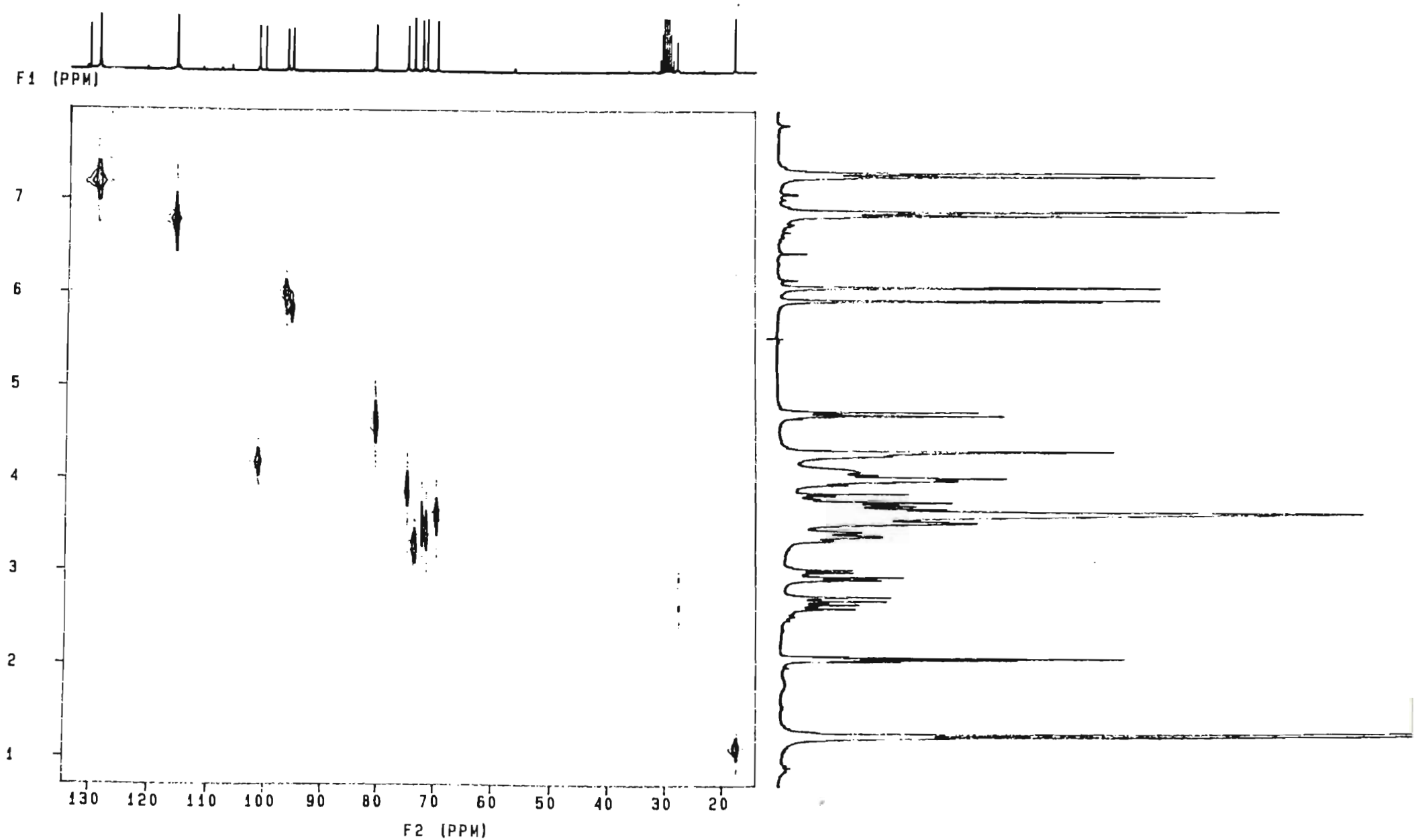
6.1. Afzelechin-3-O-α-L-rhamnopyranoside ^{1}HNMR spectrum (acetone-d_6 + D_2O)



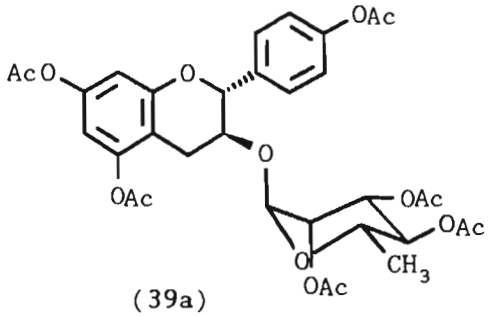
6.2. Afzelechin-3-O-α-L-rhamnopyranoside ^{13}CNMR spectrum (acetone-d_6)



6.3. Afzelechin-3-O-α-L-rhamnopyranoside ^{1}H-^{1}H DELAYED COSY spectrum (5Hz) (acetone-d_6 + D_2O)

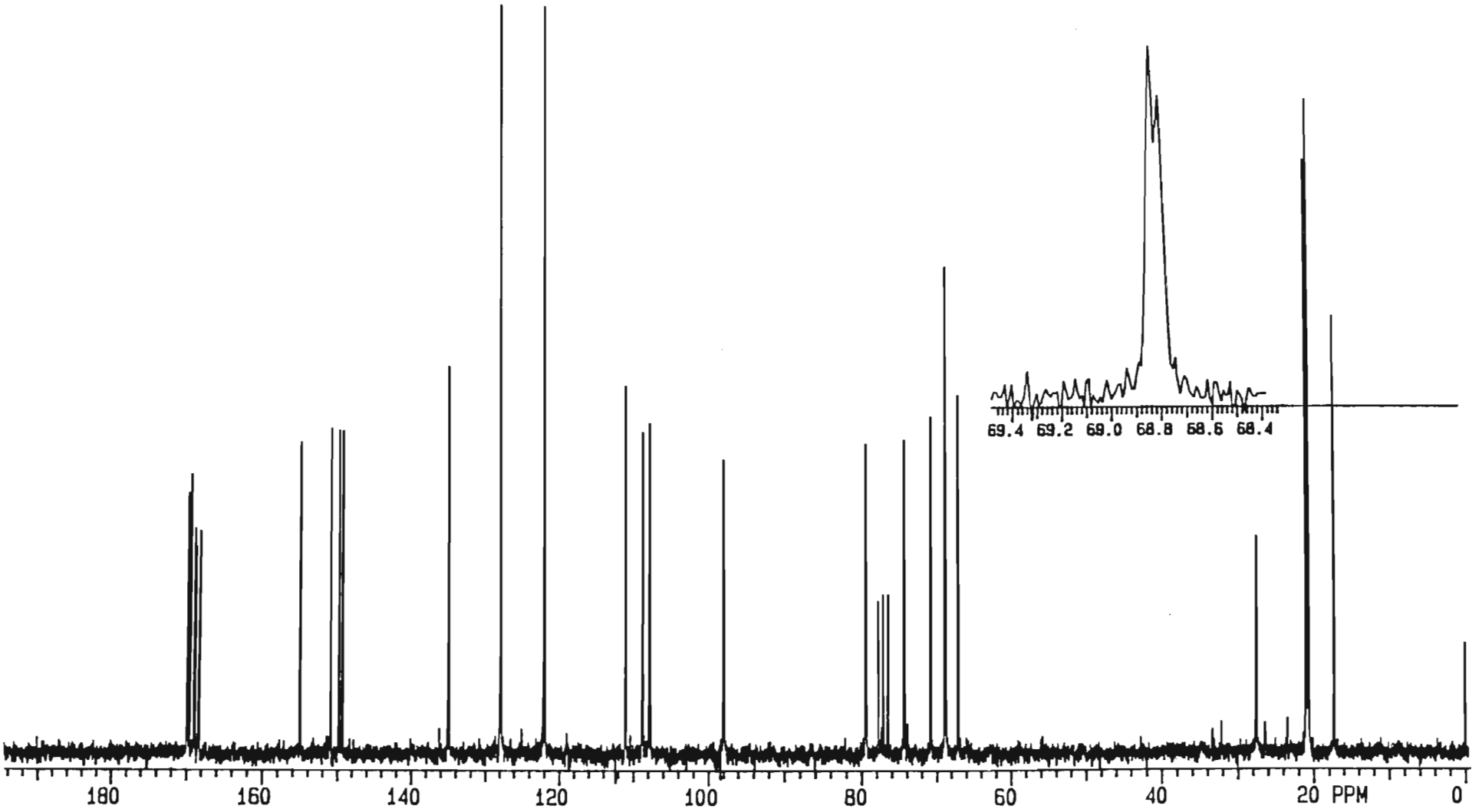


6.4. Afzelechin-3-O-α-L-rhamnopyranoside HETCOR spectrum (acetone-d_6)

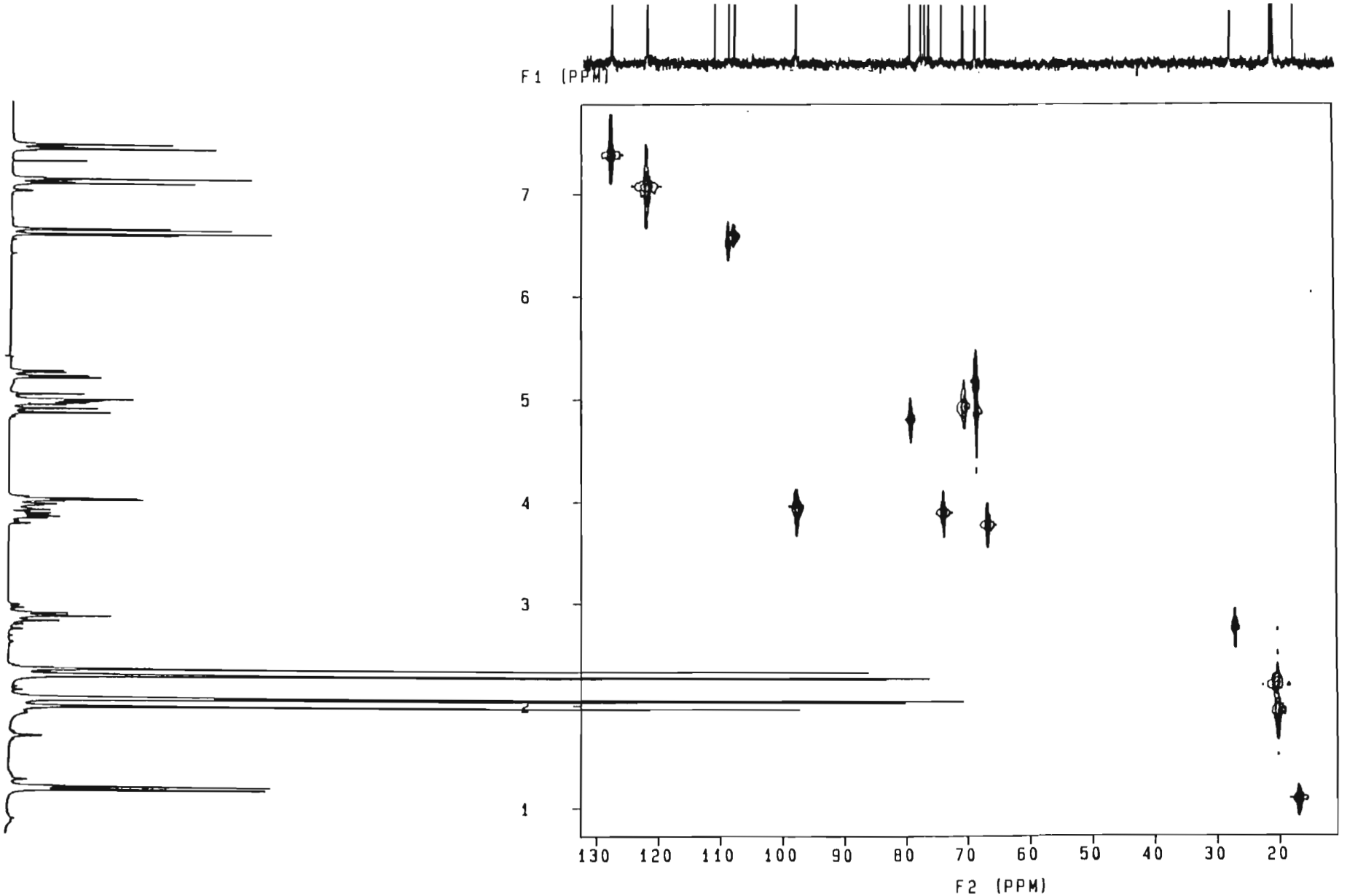


(39a)

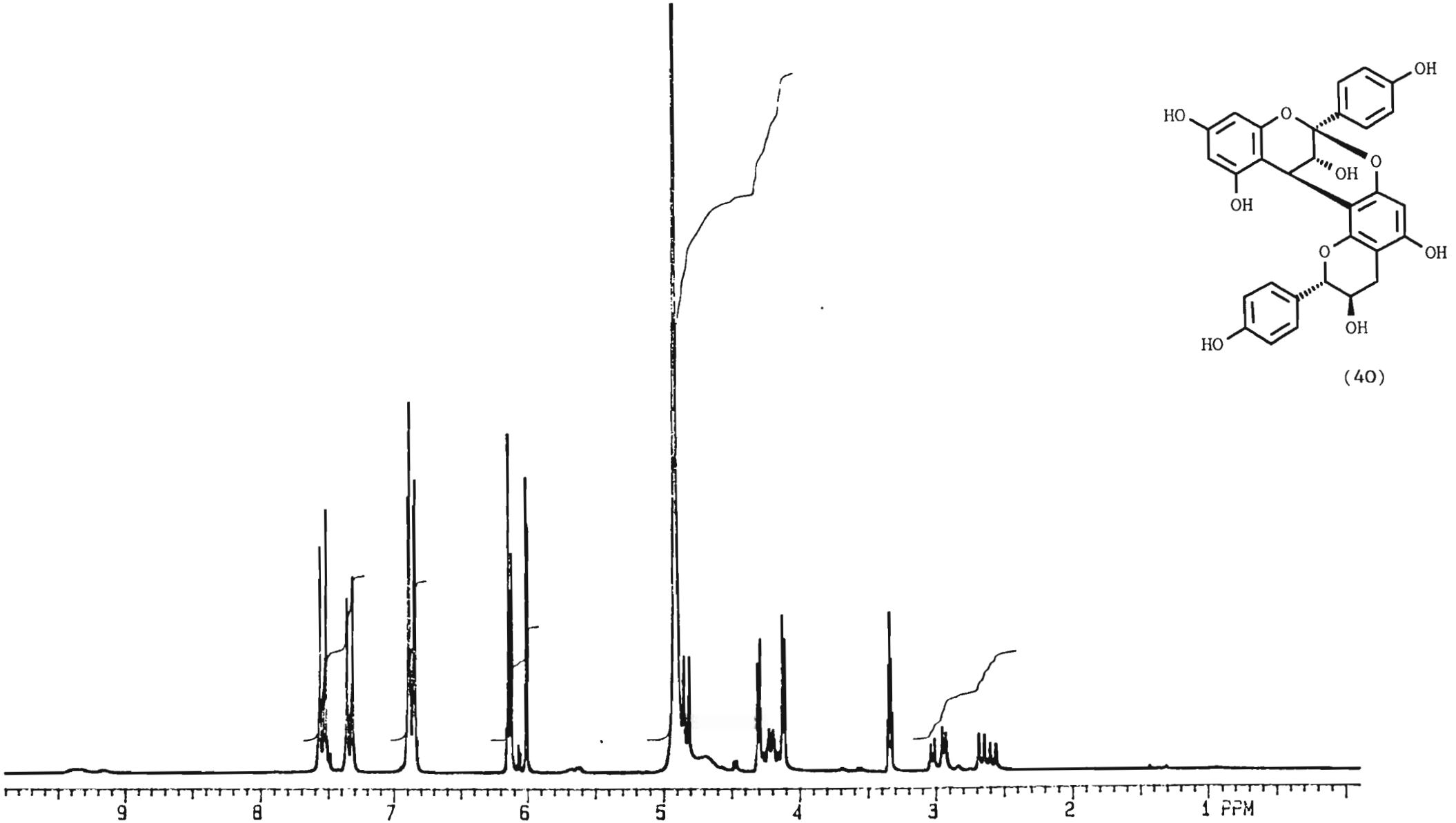
7.1. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate ^{1}HNMR spectrum ($CDCl_3$)



7.2. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate ^{13}CNMR spectrum ($CDCl_3$)

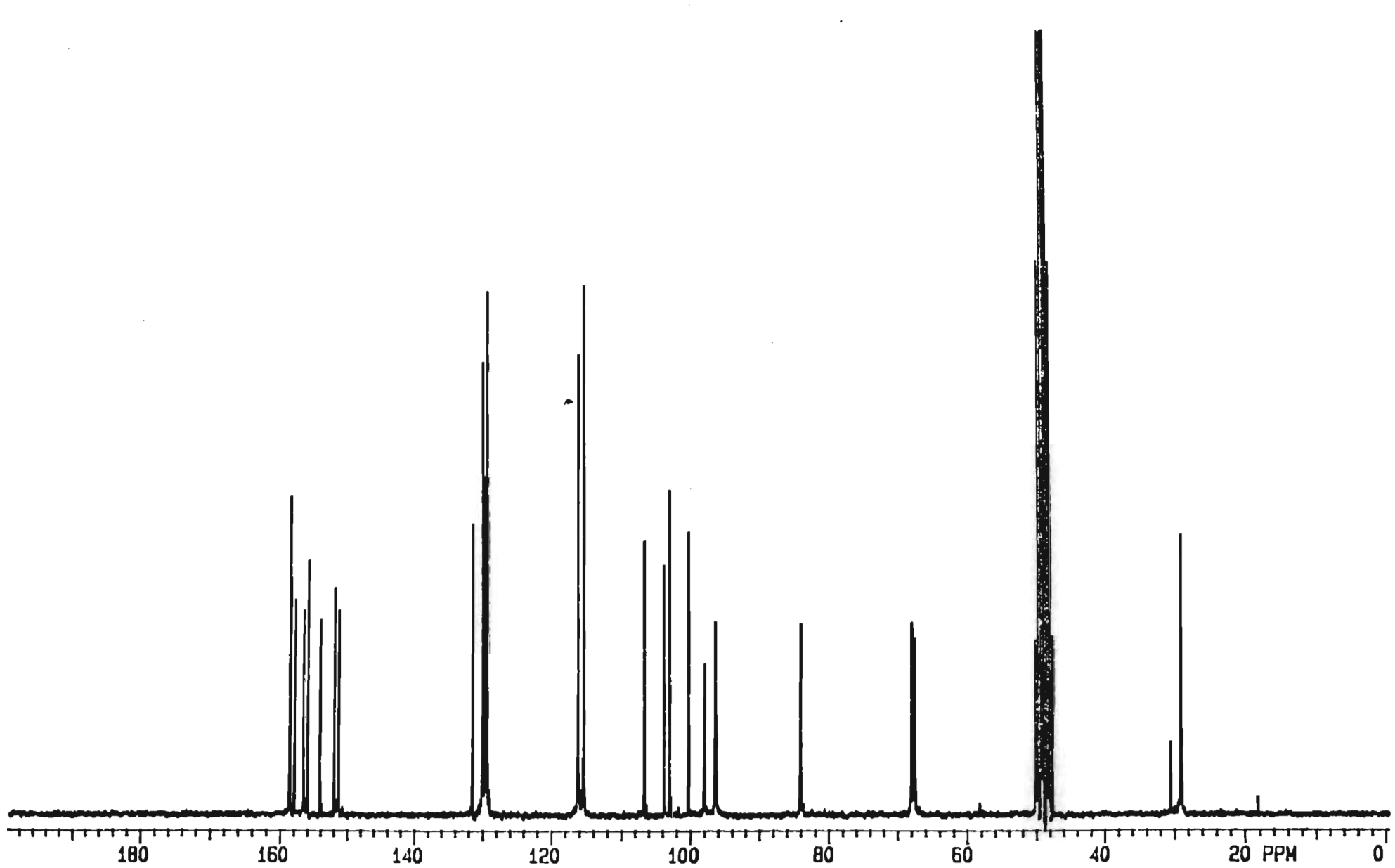


7.3. Afzelechin-3-O-α-L-rhamnopyranoside hexa-acetate HETCOR spectrum ($CDCl_3$)

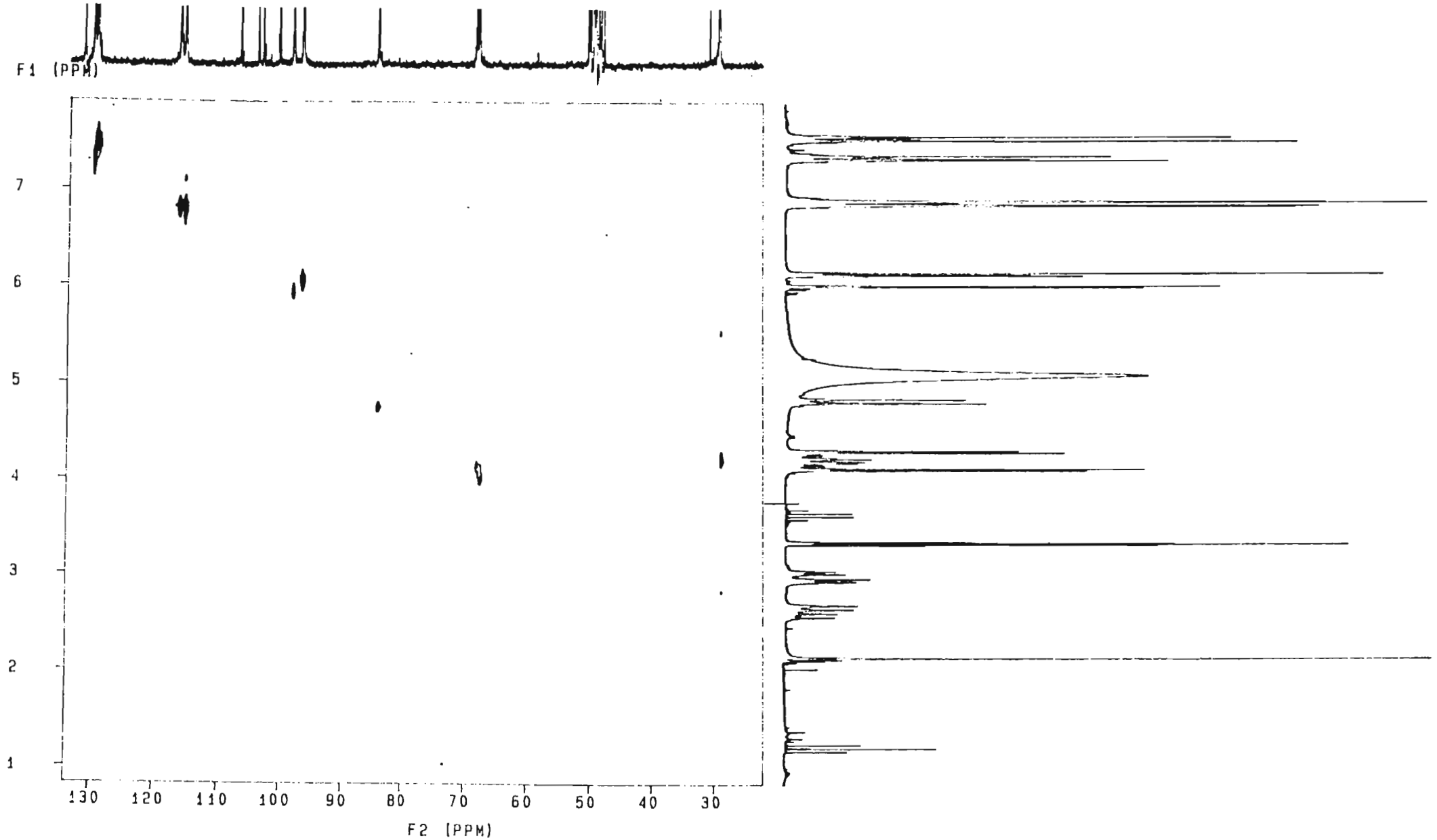


(40)

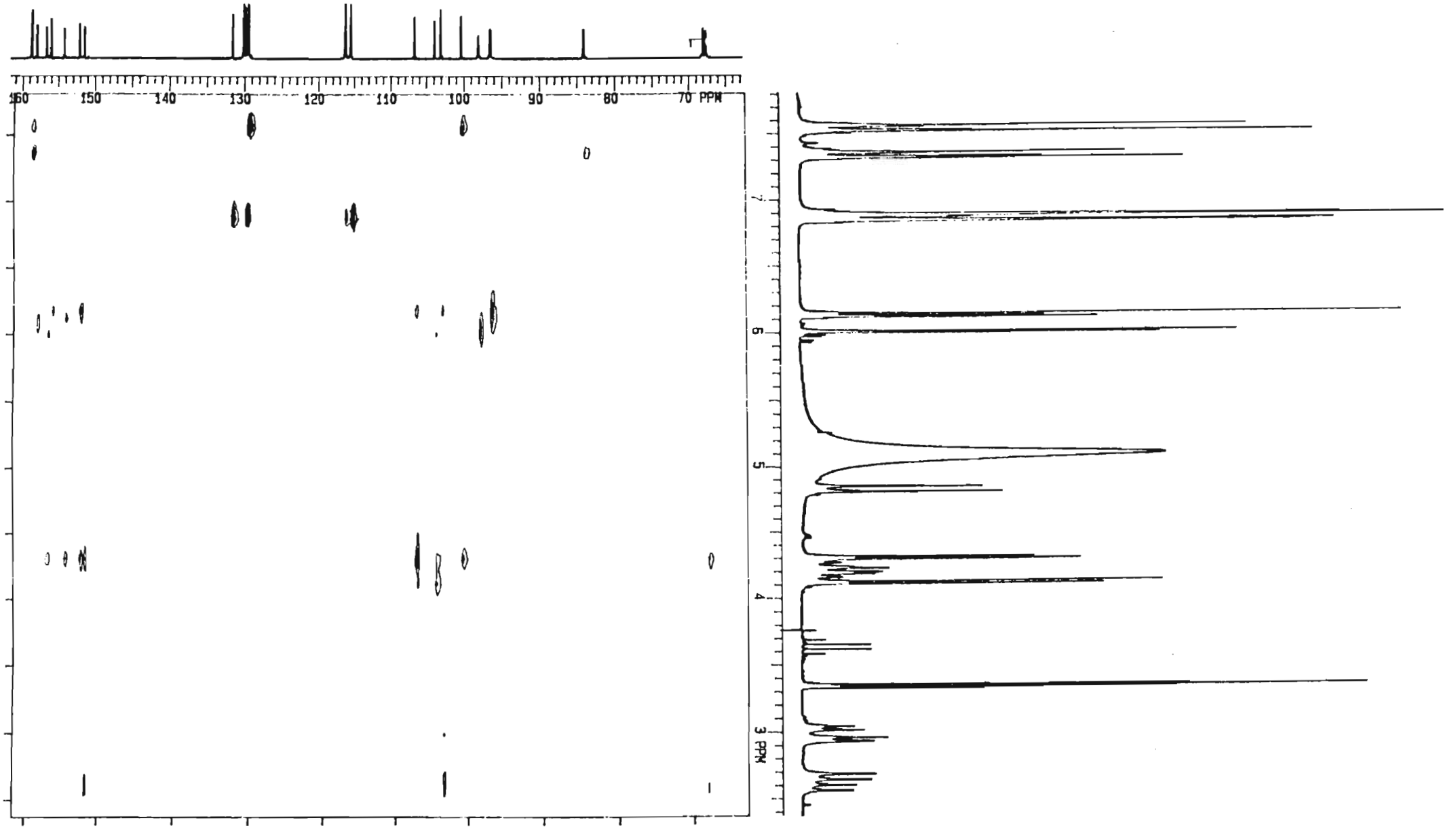
8.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin ^{1}HNMR spectrum (CD_3OD)



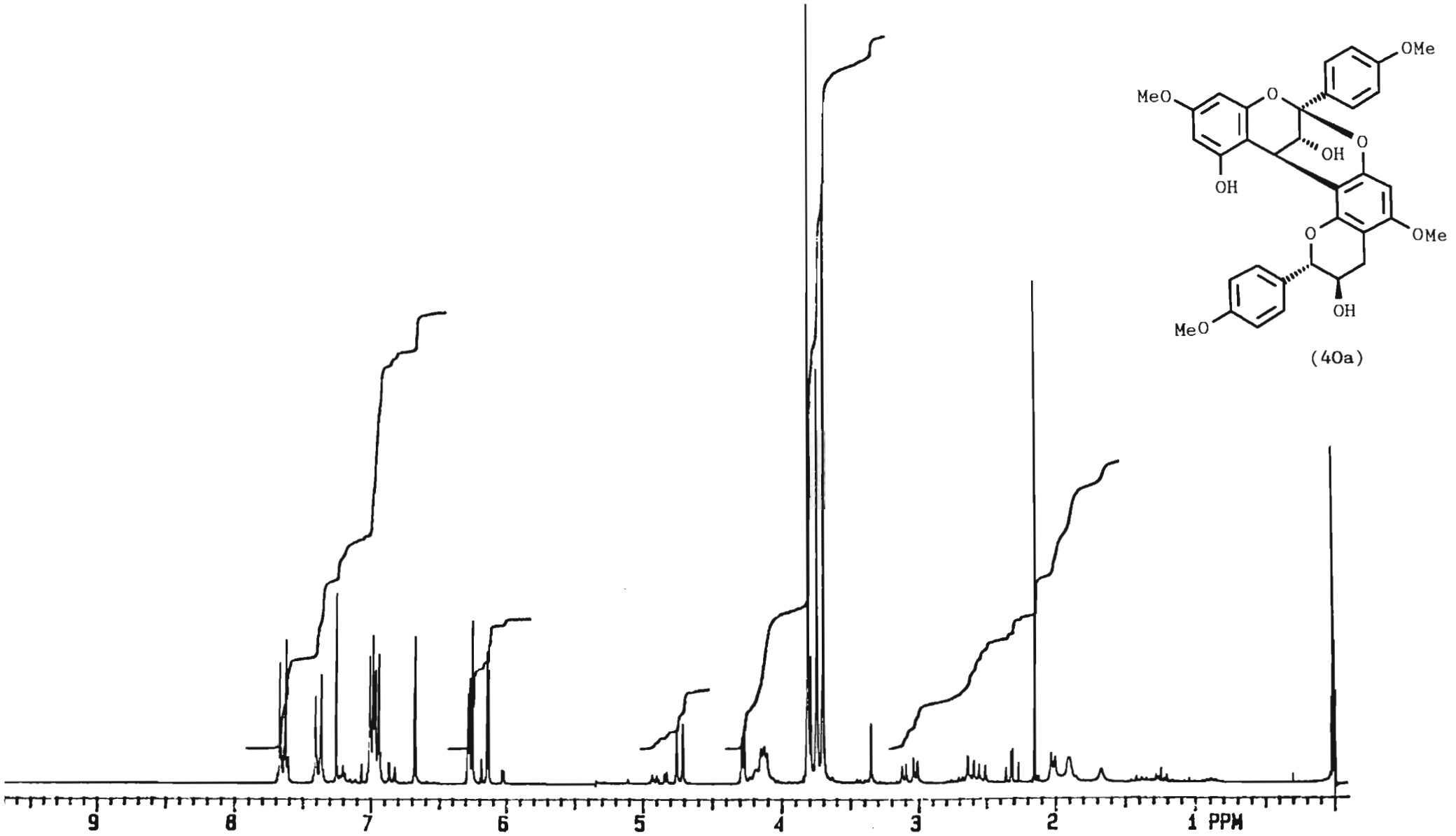
8.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin ^{1,3}CNMR spectrum (CD_3OD)



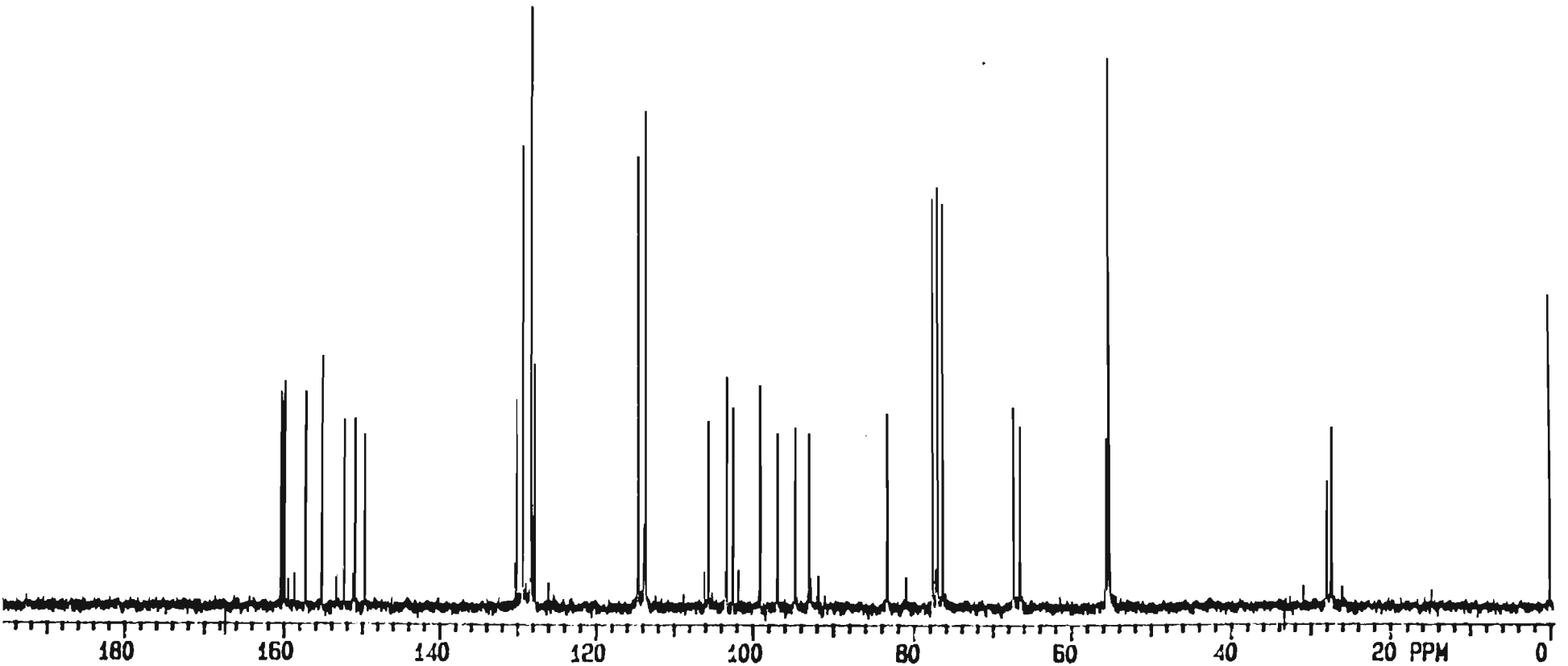
8.3. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin HETCOR spectrum (CD_3OD)



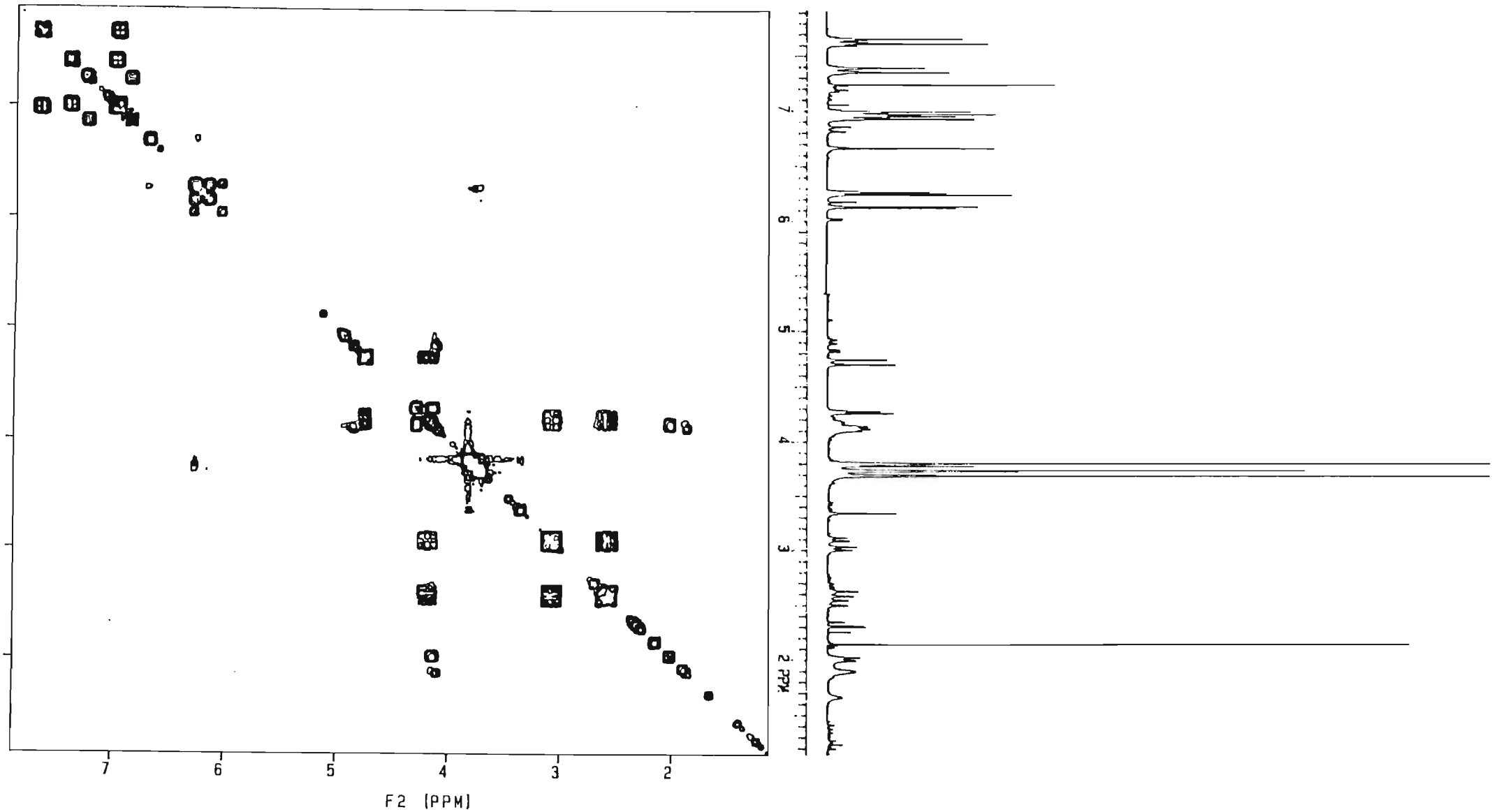
8.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin DELAYED HETCOR spectrum (7Hz) (CD_3OD)



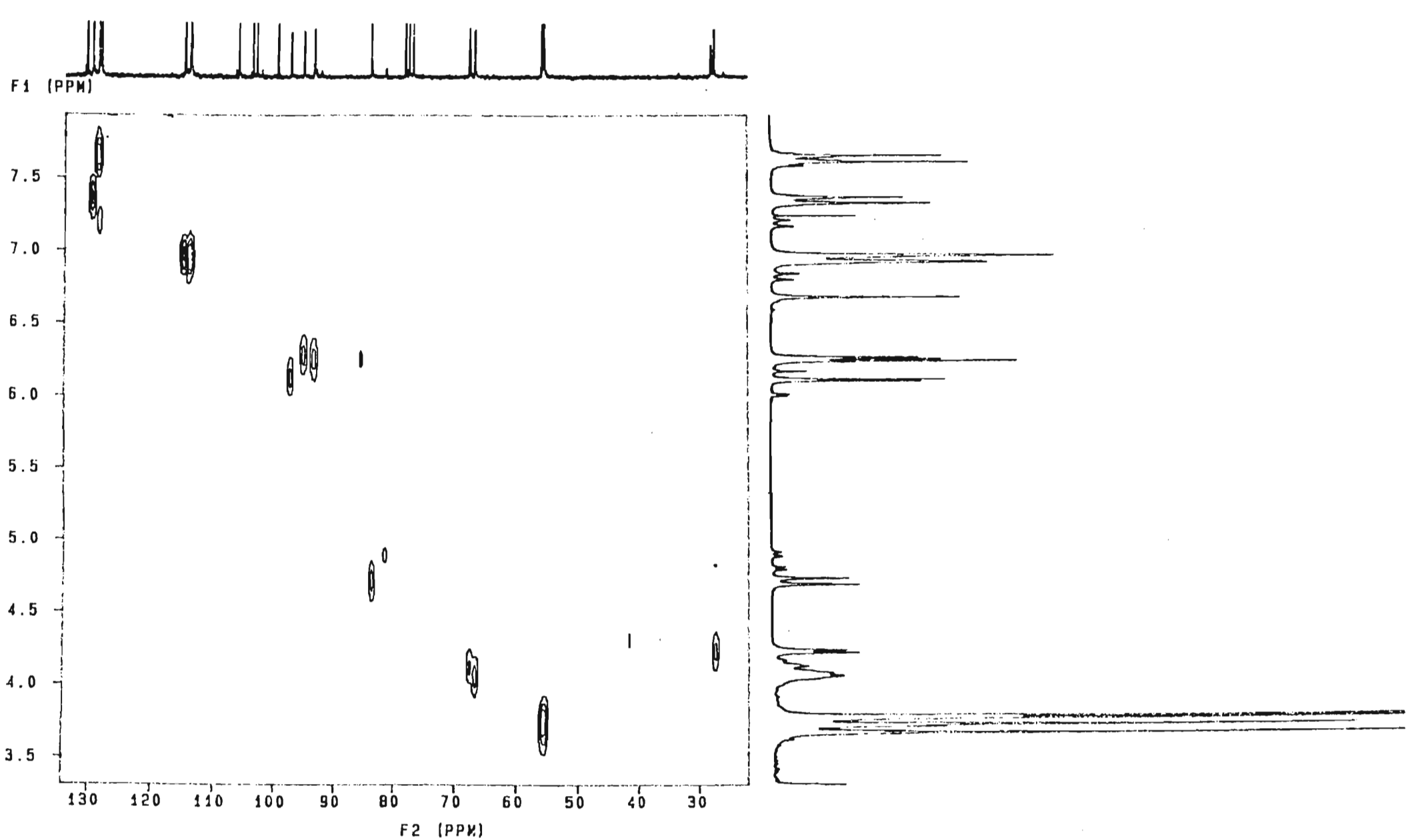
9.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether ^{1}HNMR spectrum ($CDCl_3$)



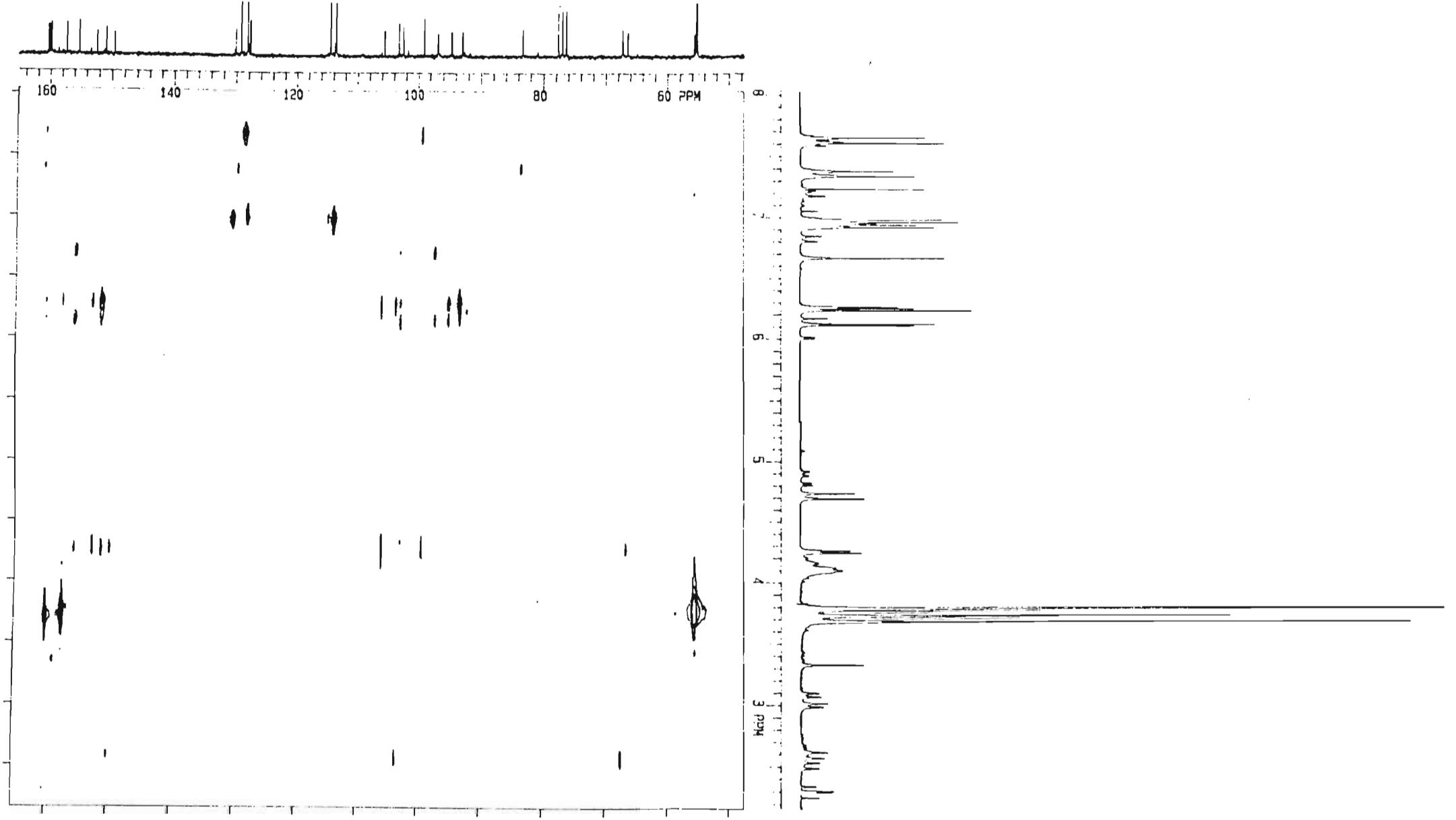
9.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether ^{13}CNMR spectrum ($CDCl_3$)



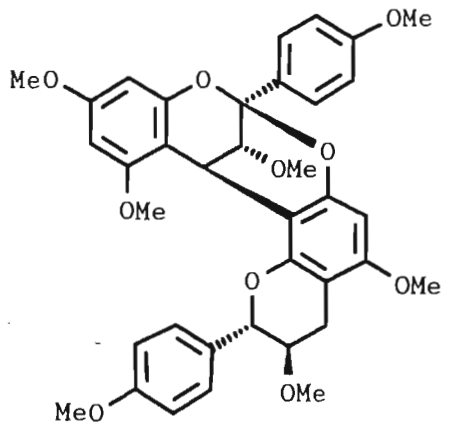
9.3. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether ^{1}H-^{1}H COSY spectrum ($CDCl_3$)



9.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether HETCOR spectrum ($CDCl_3$)

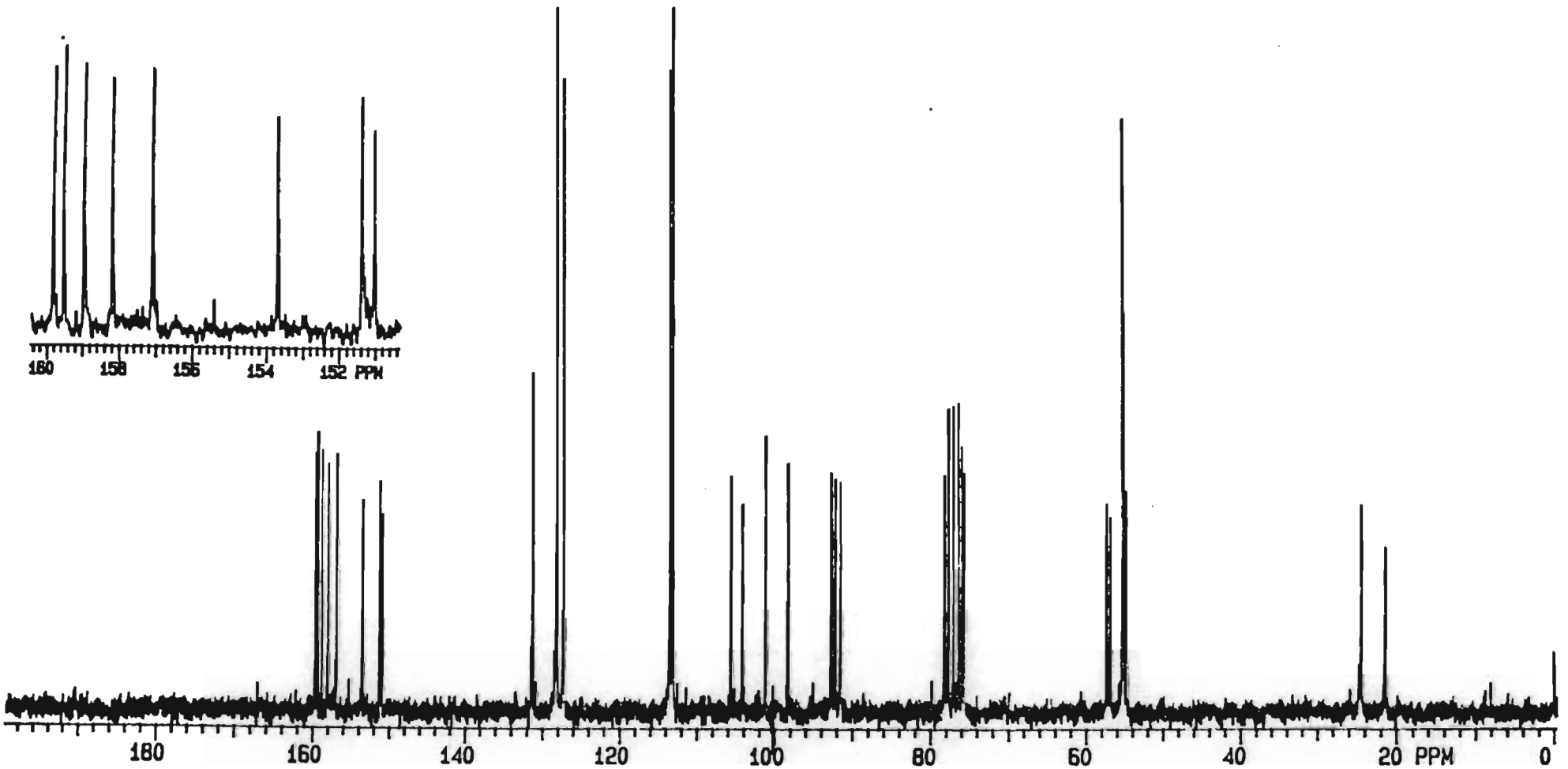


9.5. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-tetramethyl ether DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

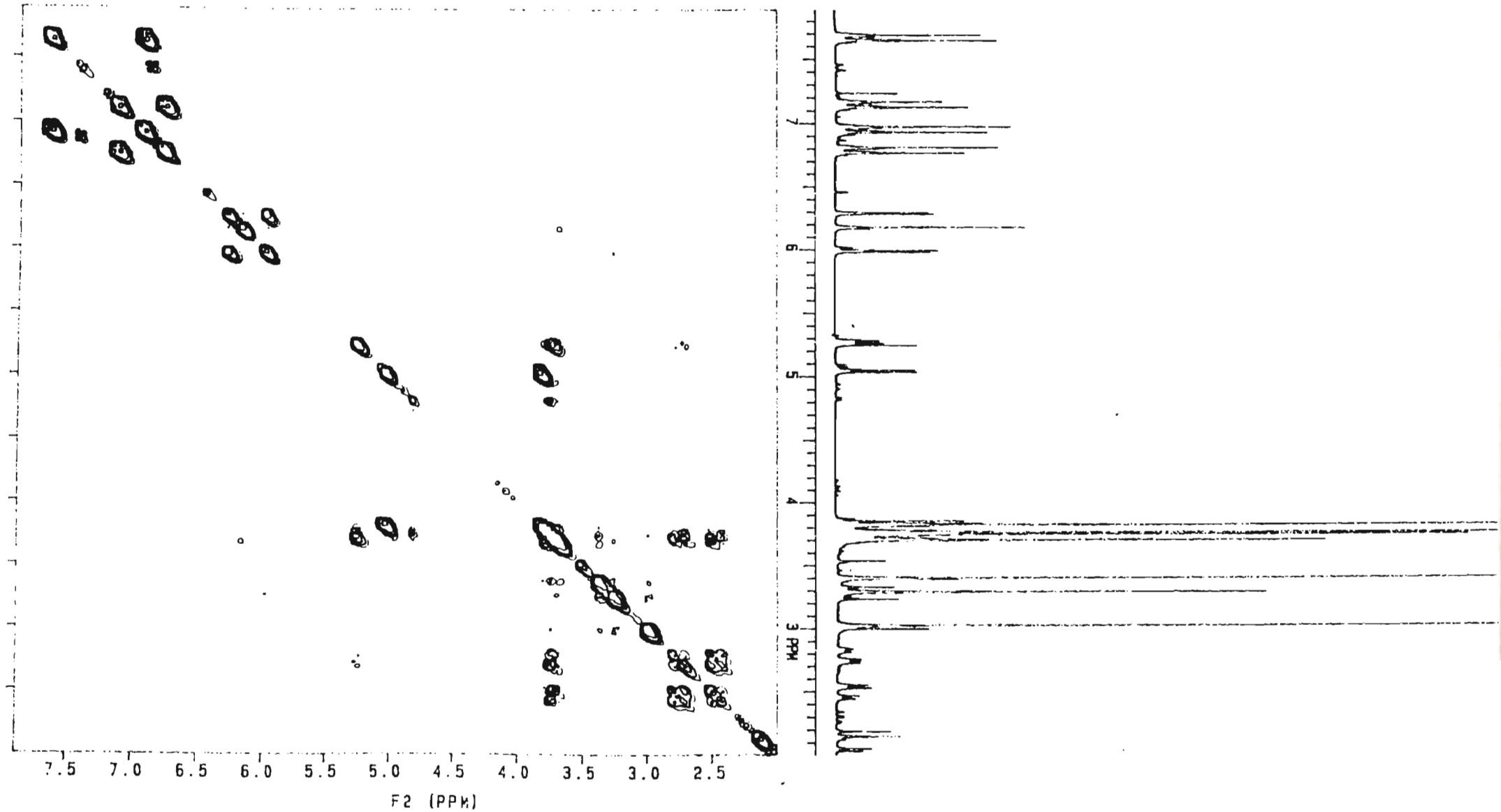


(40d)

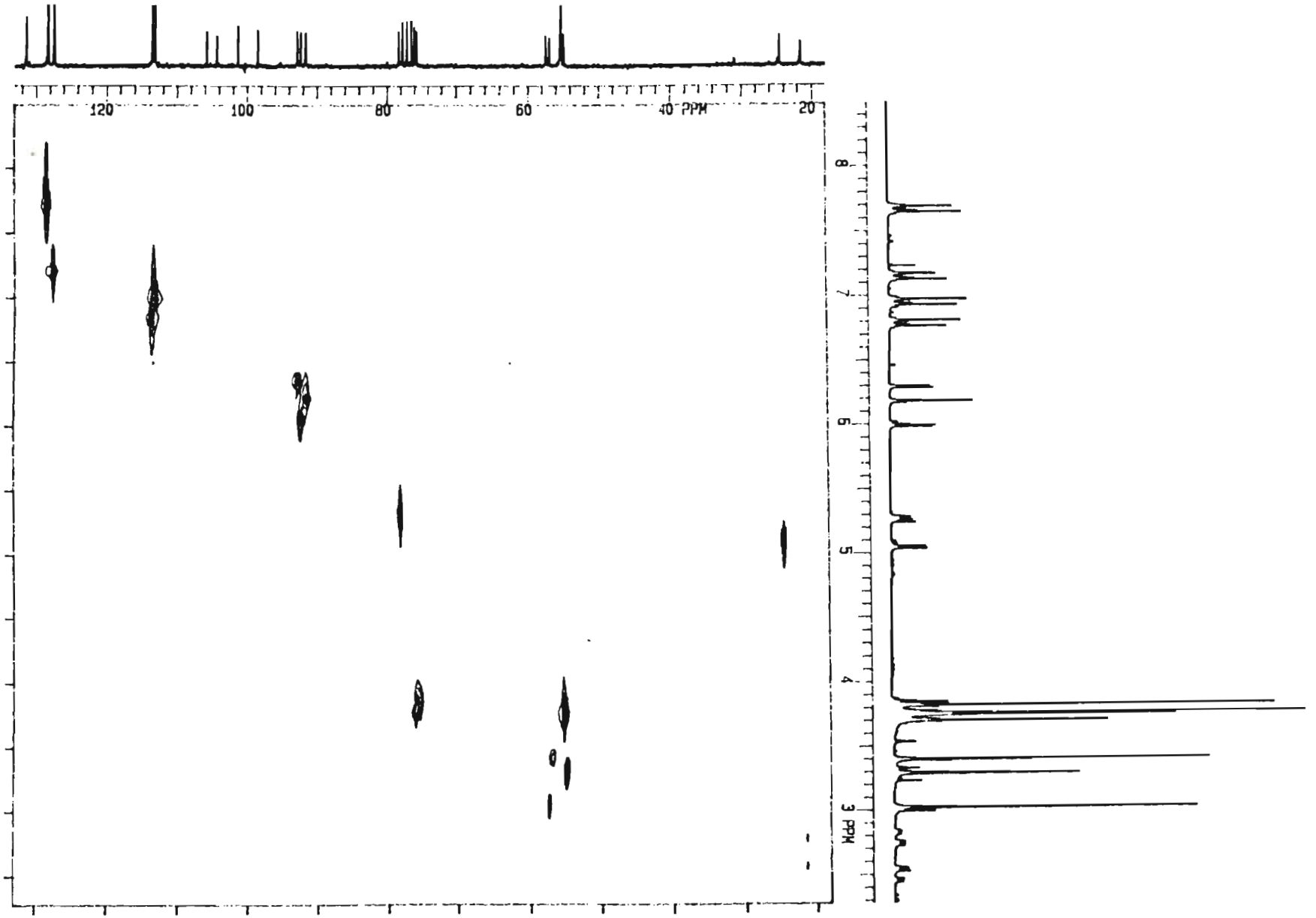
10.1. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether ^{1}HNMR spectrum ($CDCl_3$)



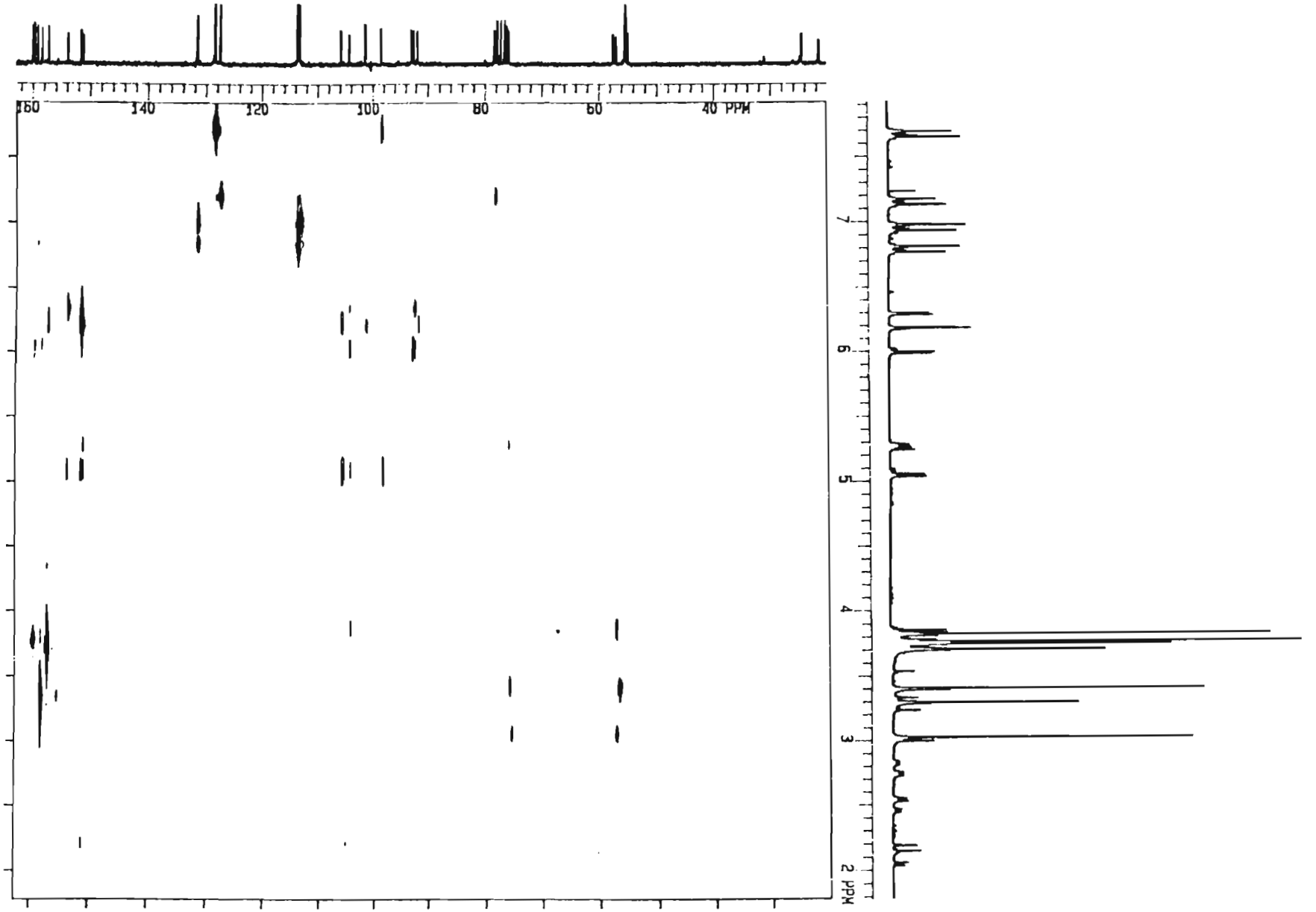
10.2. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether ^{13}CNMR spectrum ($CDCl_3$)



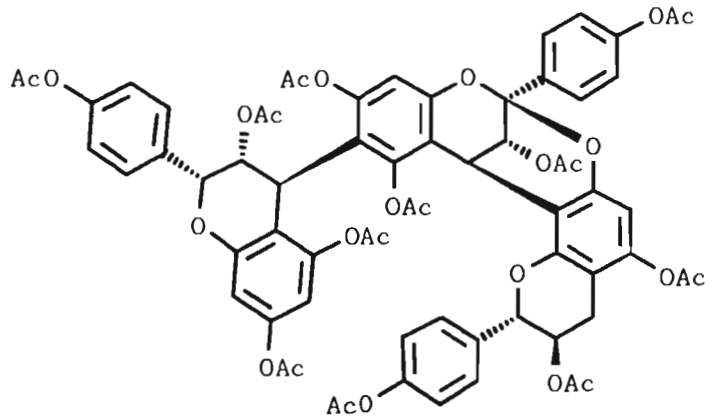
10.3. Epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin-heptamethyl ether ^{1}H-^{1}H COSY spectrum ($CDCl_3$)



10.4. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether HETCOR spectrum ($CDCl_3$)

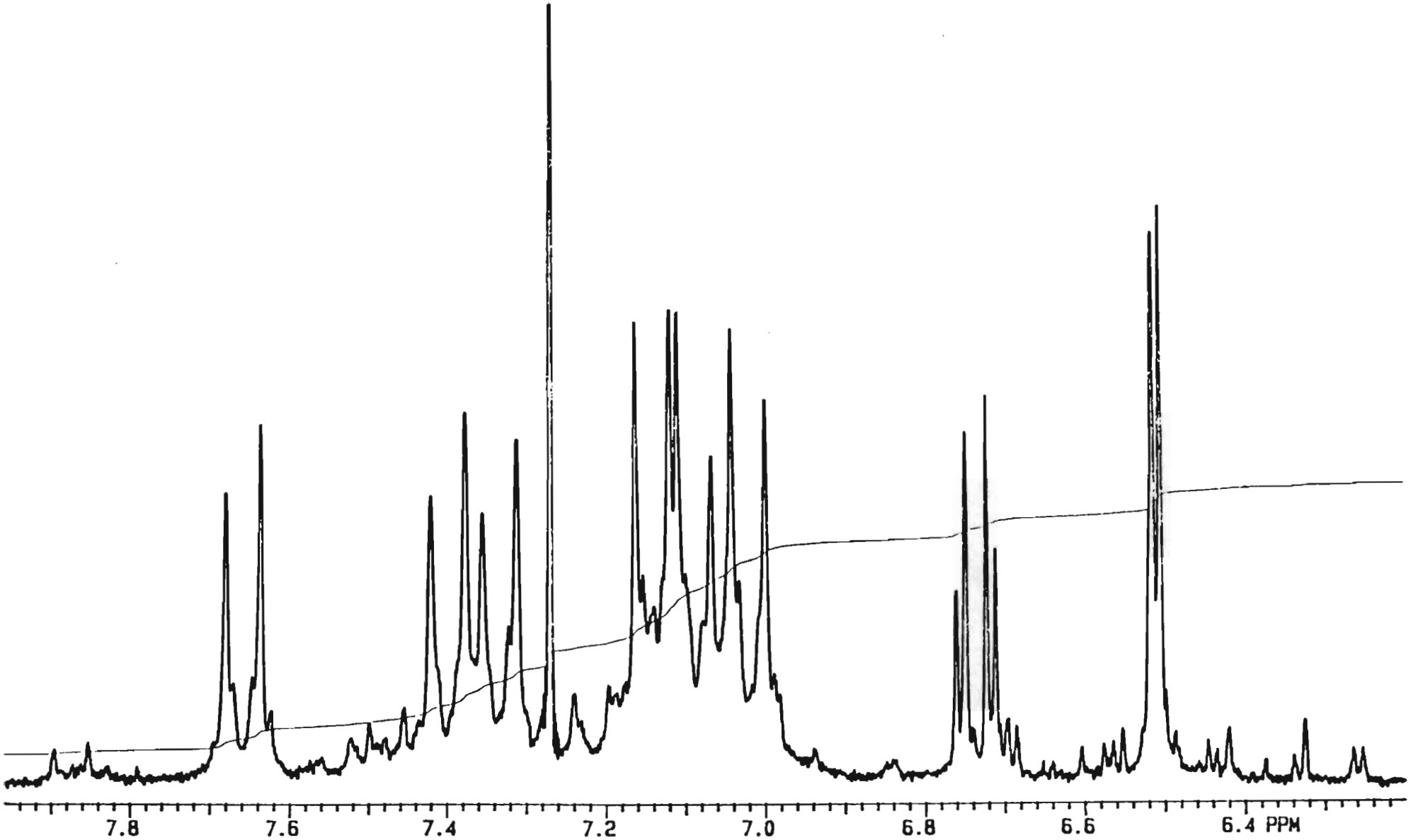


10.5. Epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-heptamethyl ether DELAYED HETCOR spectrum (6Hz) ($CDCl_3$)

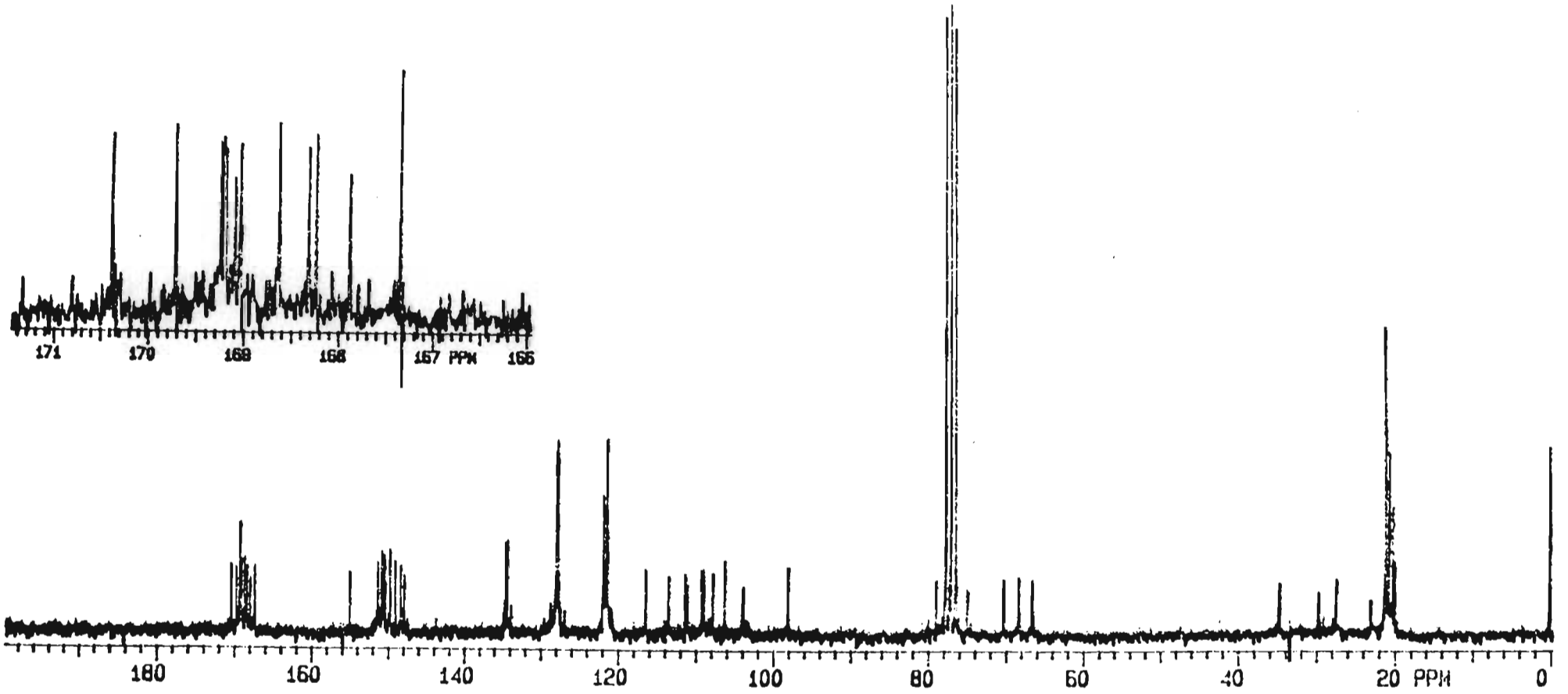


(41a)

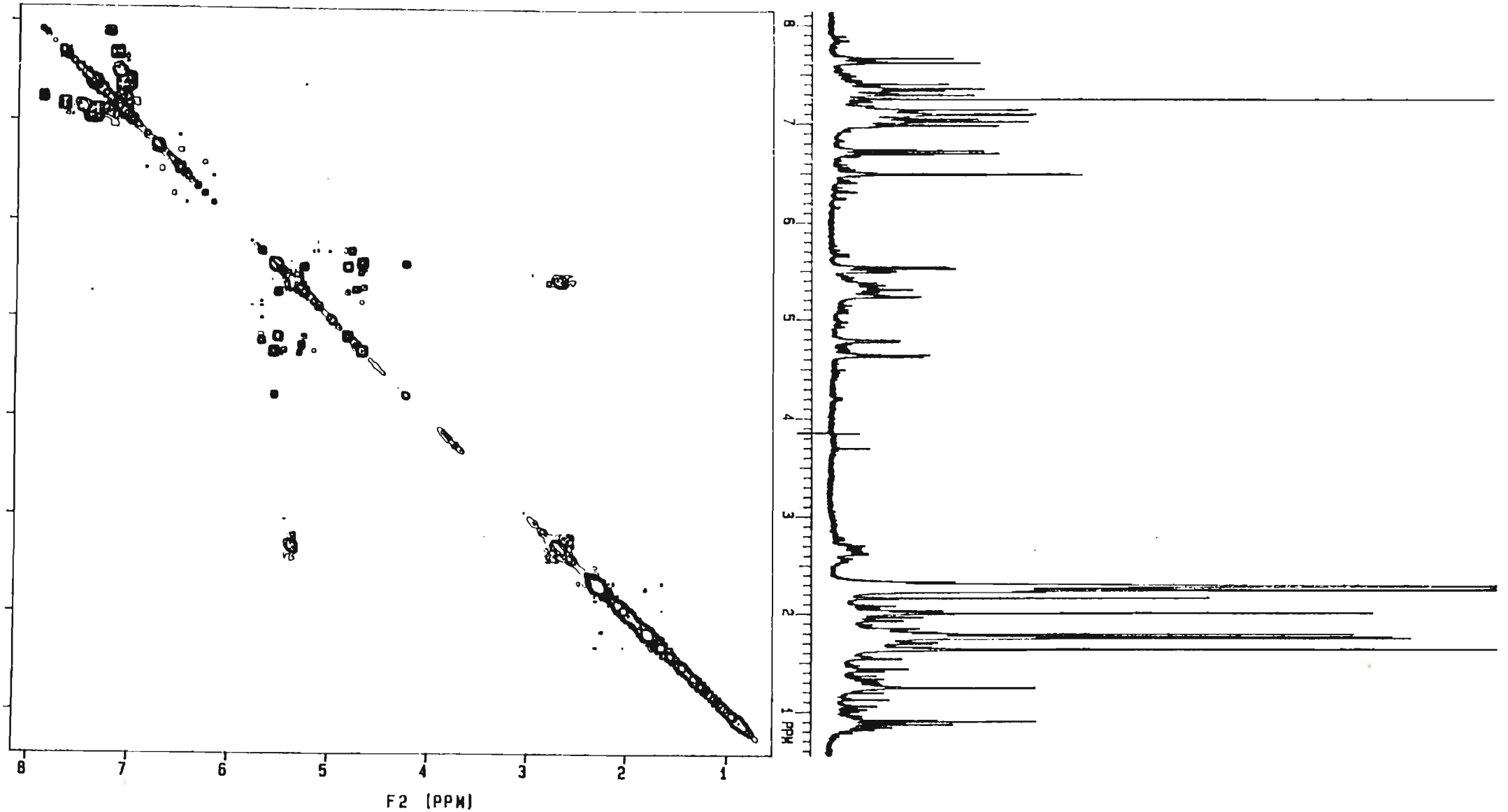
11.1. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
^{1}HNMR spectrum ($CDCl_3$)



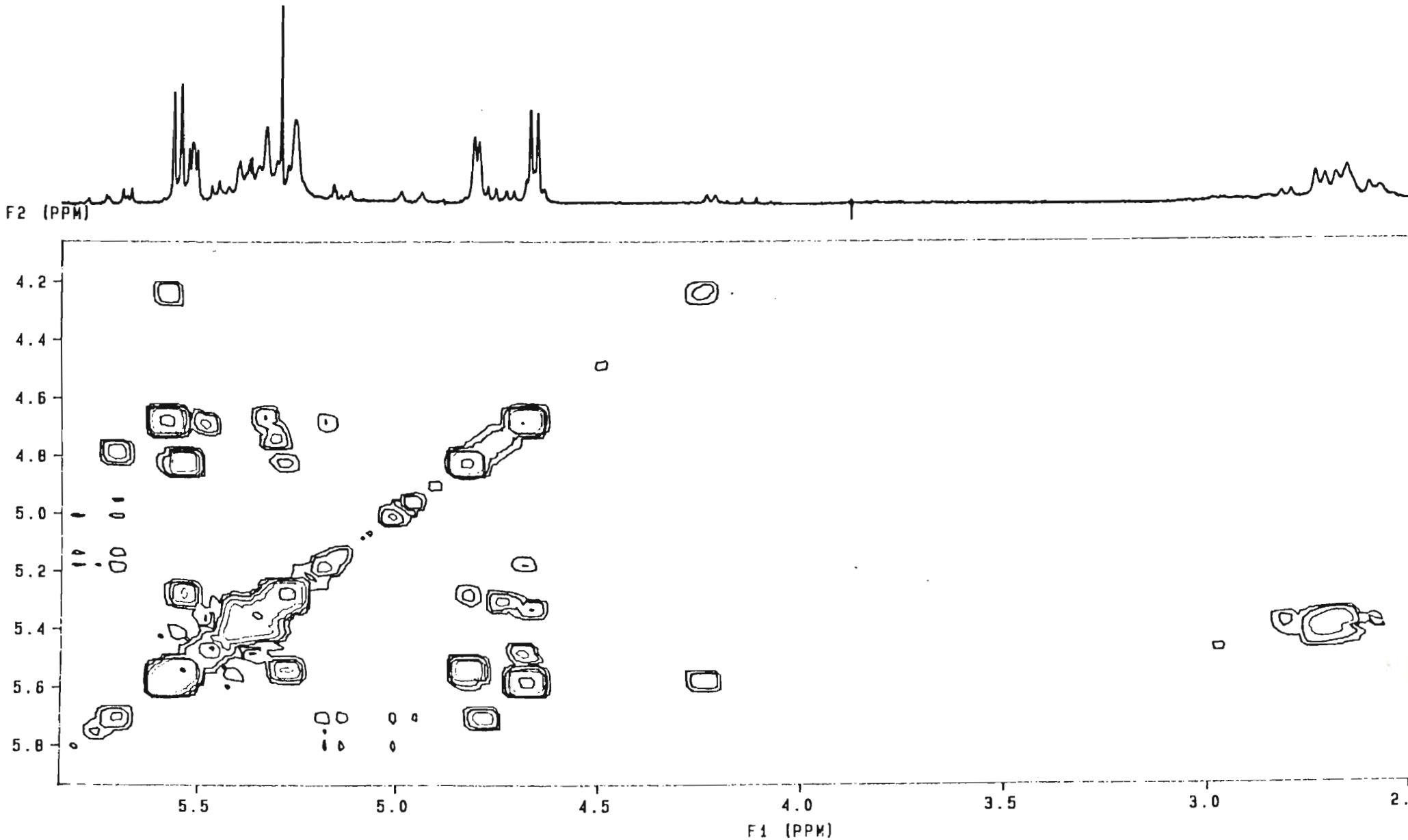
11.2. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
^{1}HNMR spectrum δ6.4-7.8 ($CDCl_3$)



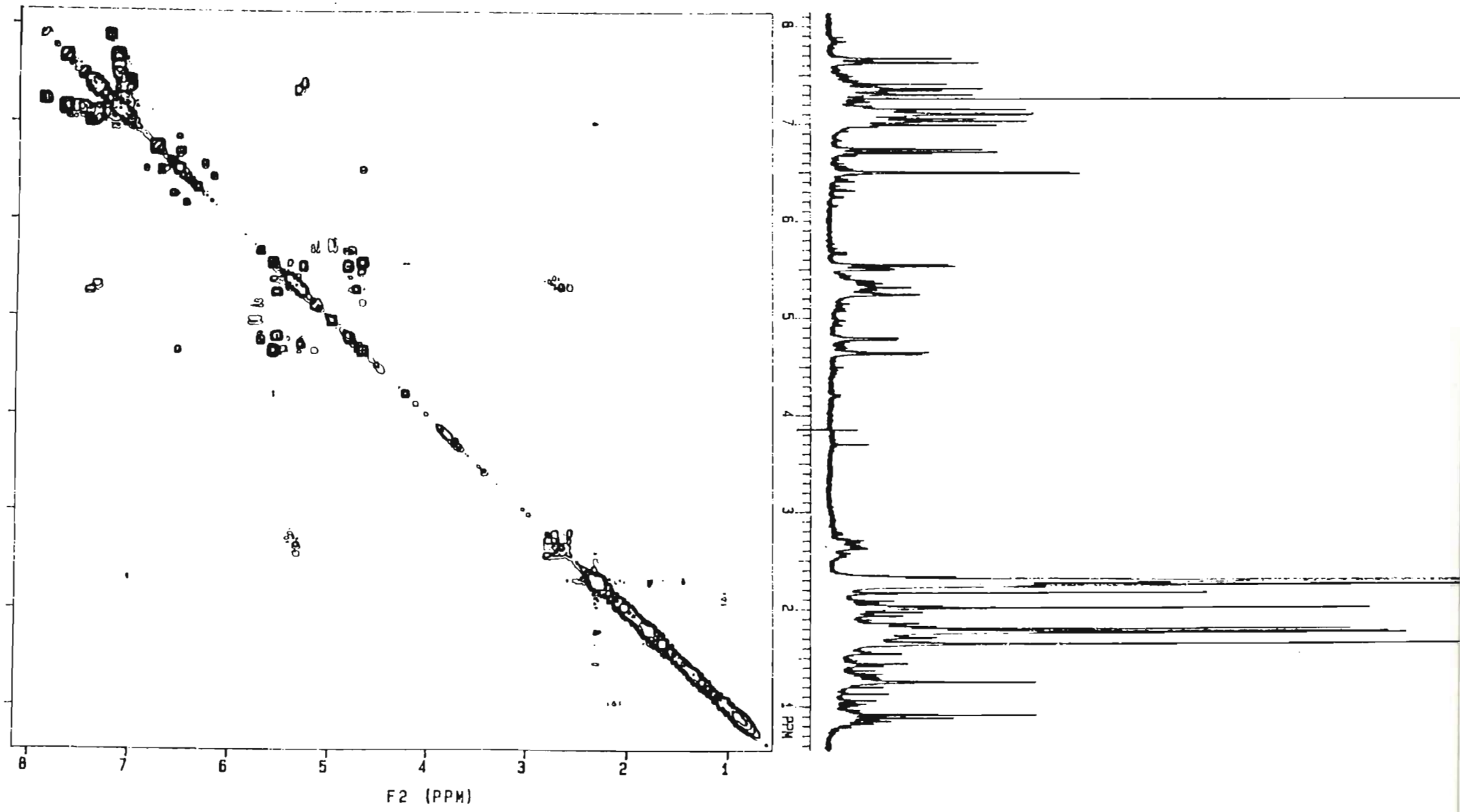
11.3. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
^{13}CNMR spectrum ($CDCl_3$)



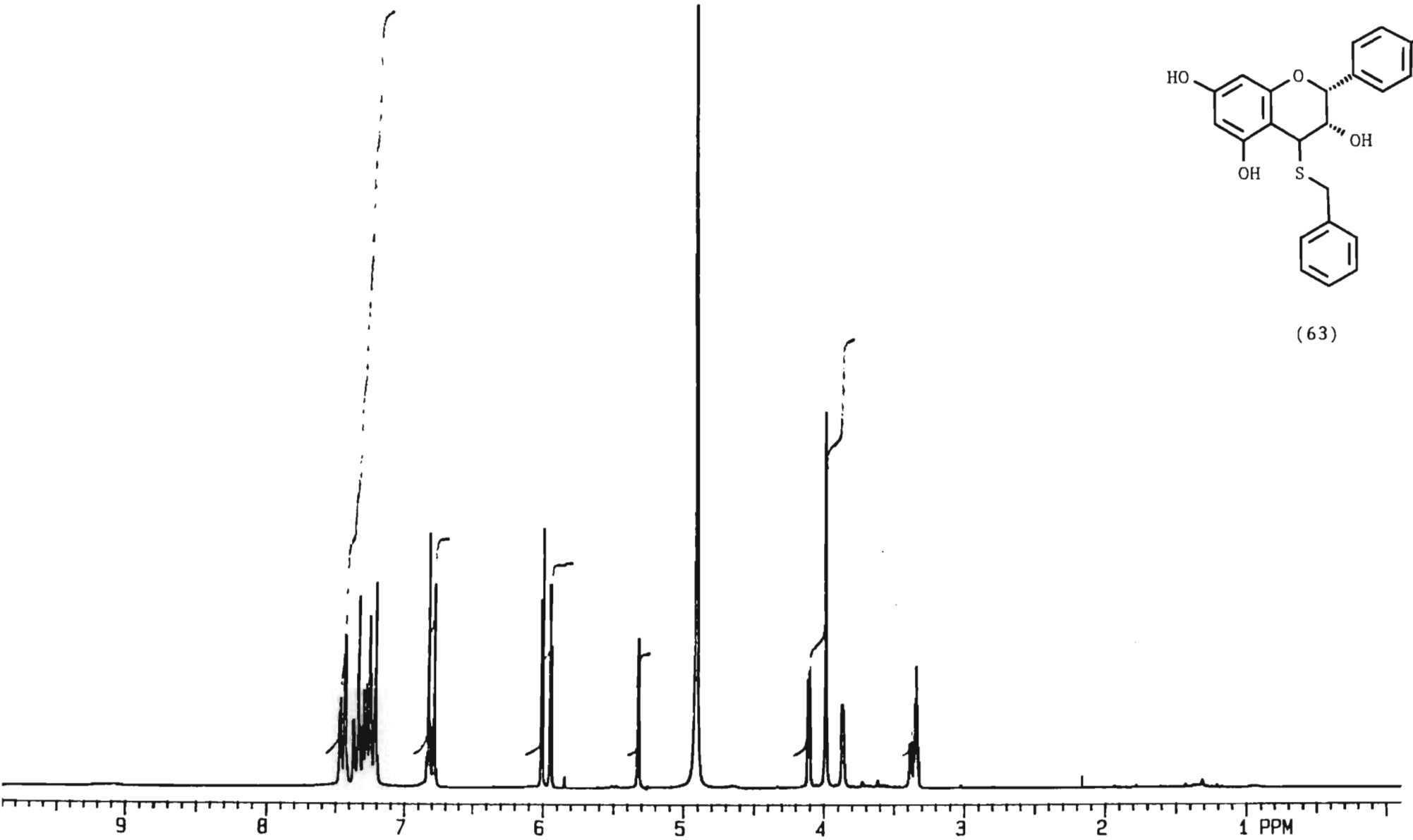
11.4. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
^{1}H-^{1}H COSY spectrum ($CDCl_3$)



11.5. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
^{1}H-^{1}H COSY spectrum δ2.5-5.8 ($CDCl_3$)

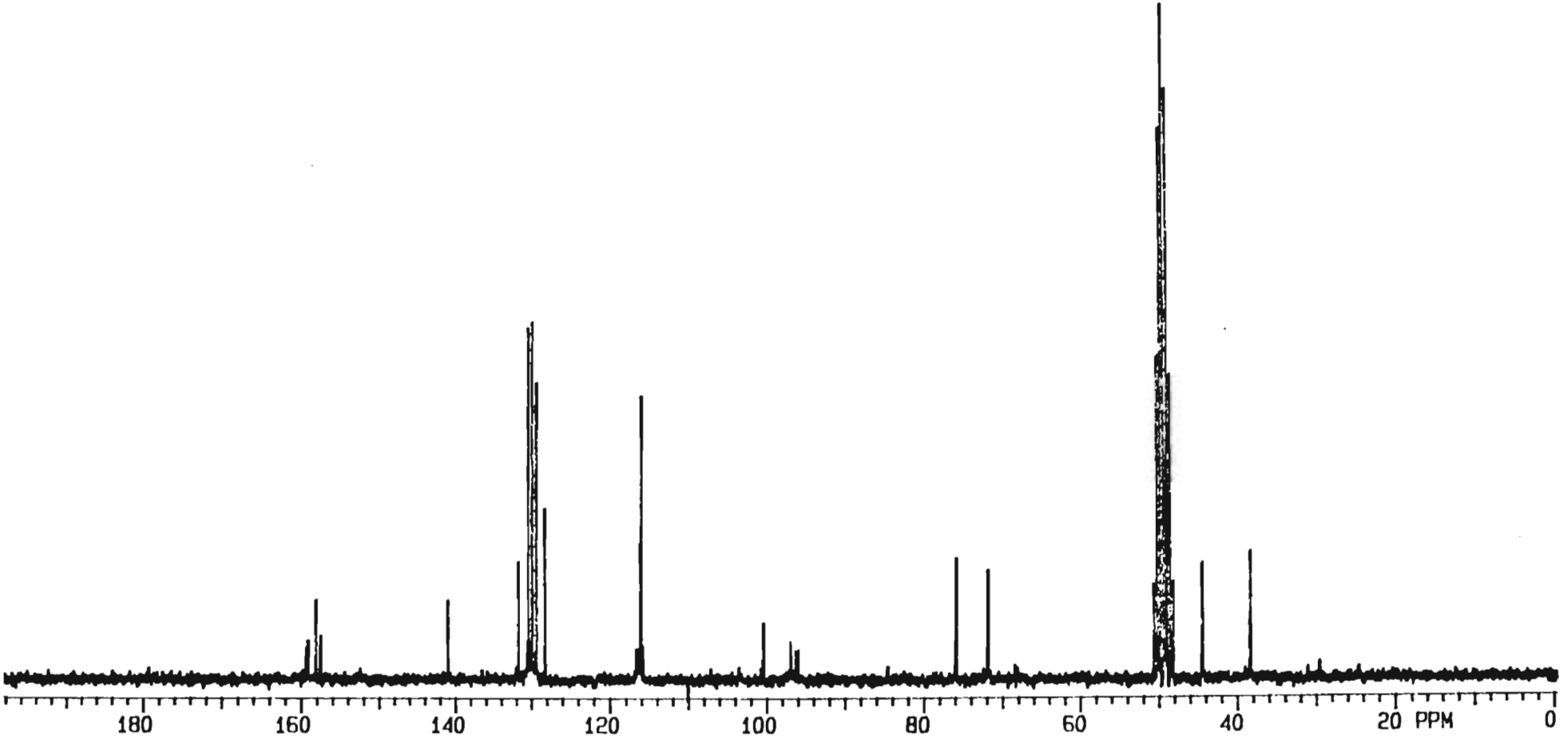


11.6. Epiafzelechin-(4β→6)-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin peracetate
DELAYED ^{1}H-^{1}H COSY spectrum ($CDCl_3$)

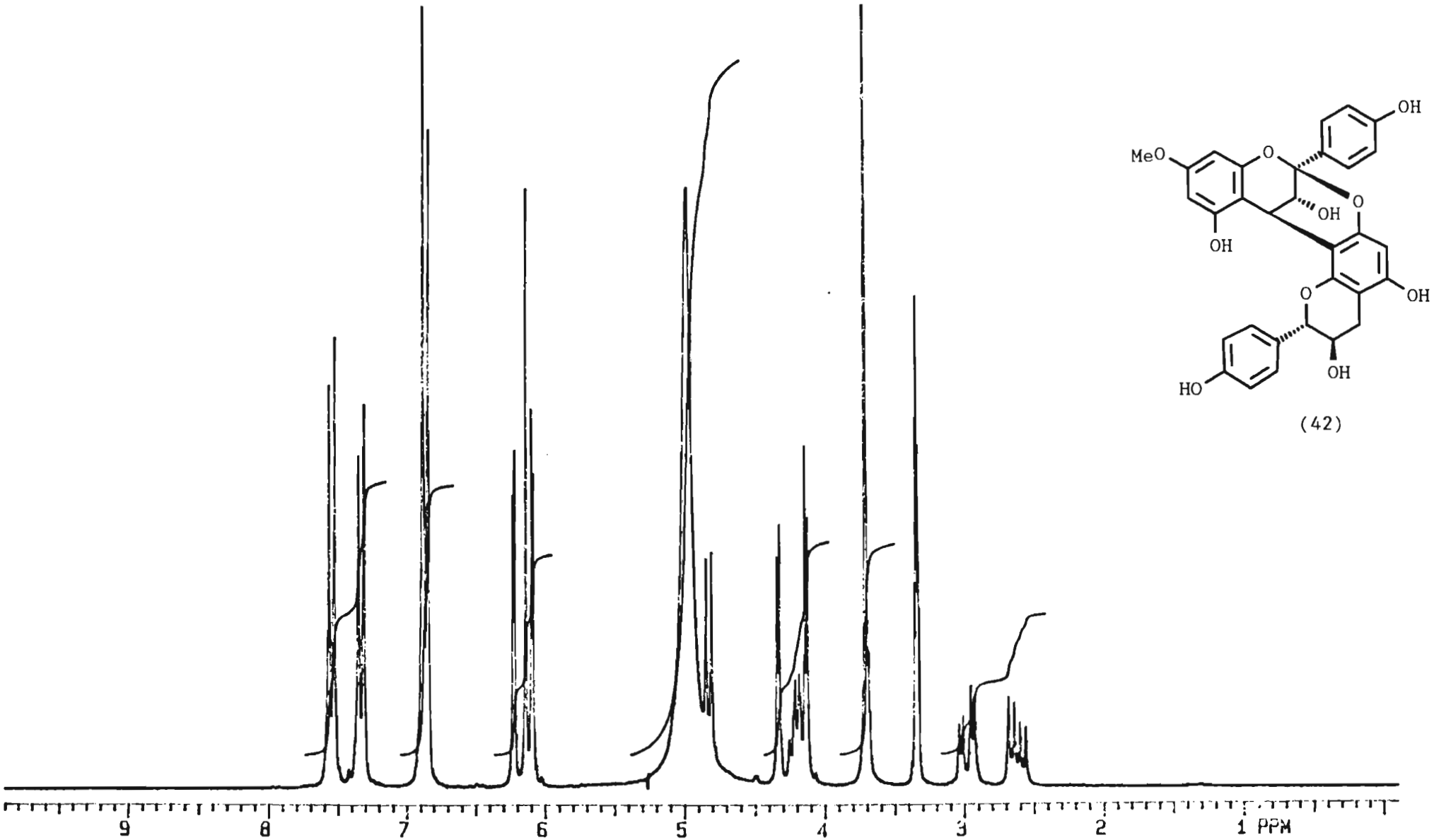


(63)

12.1. Epiafzelechin-4β-benzylthioether ^{1}HNMR spectrum (CD_3OD)

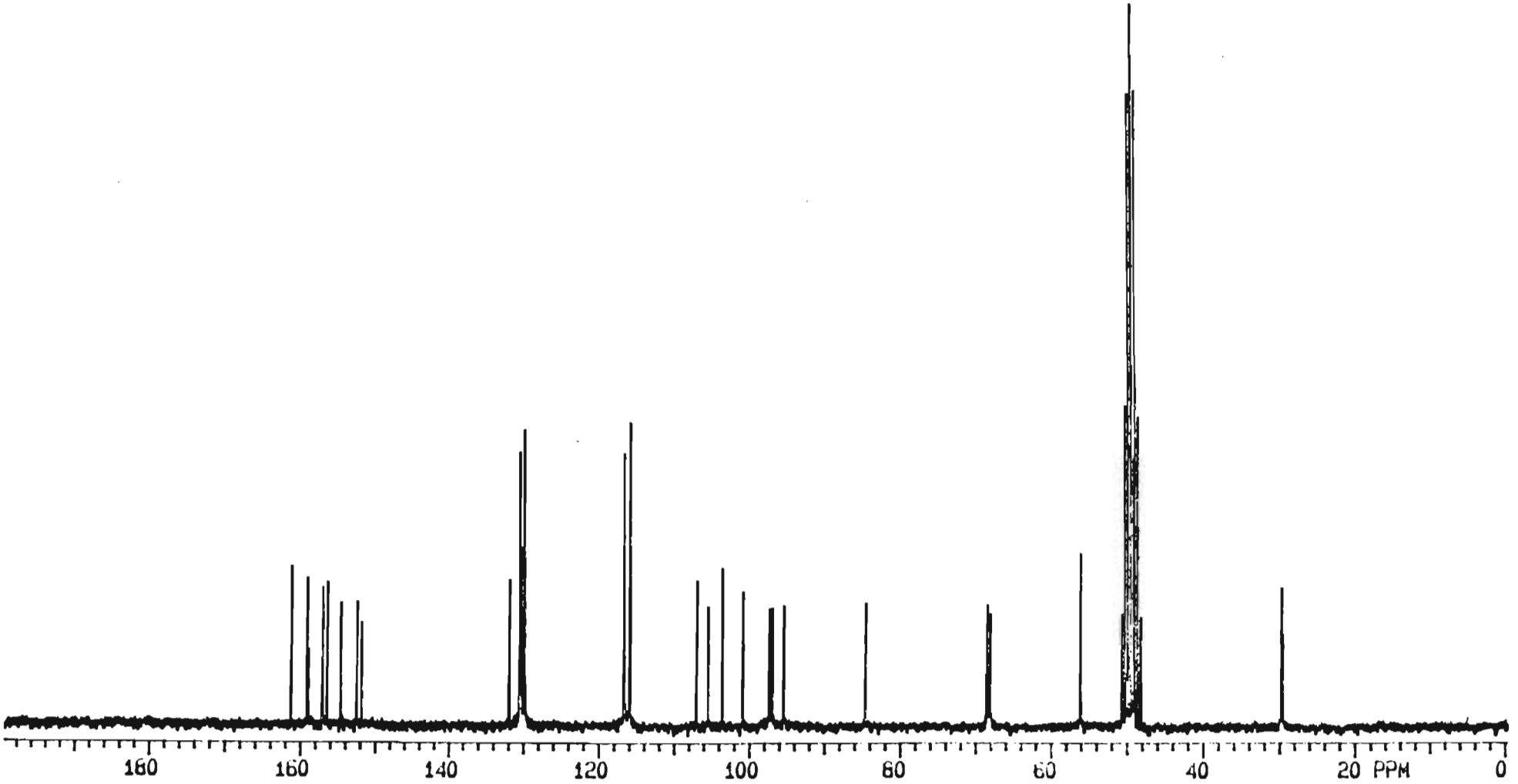


12.2. Epiafzelechin-4β-benzylthioether ^{13}CNMR spectrum (CD_3OD)

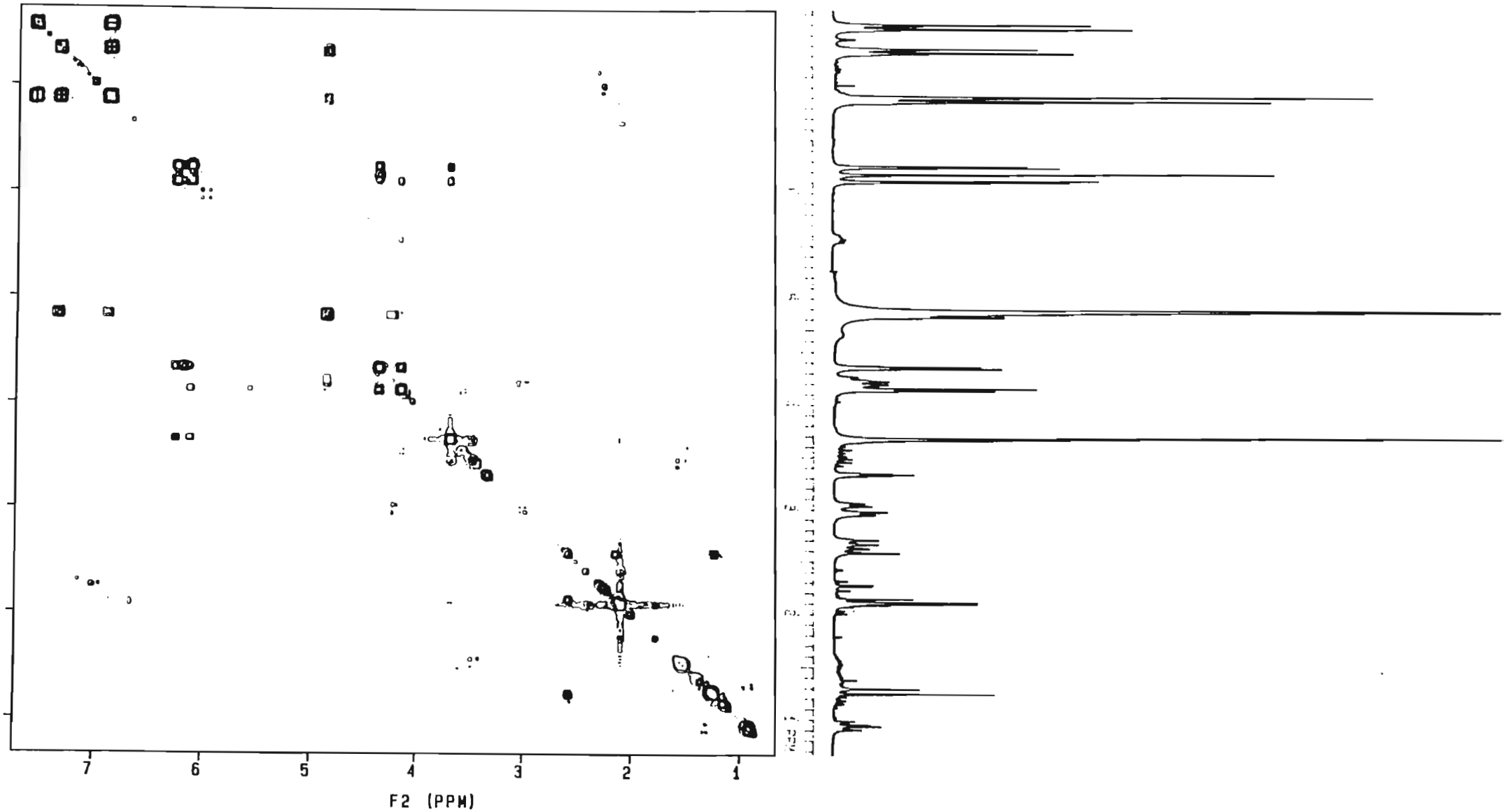


(42)

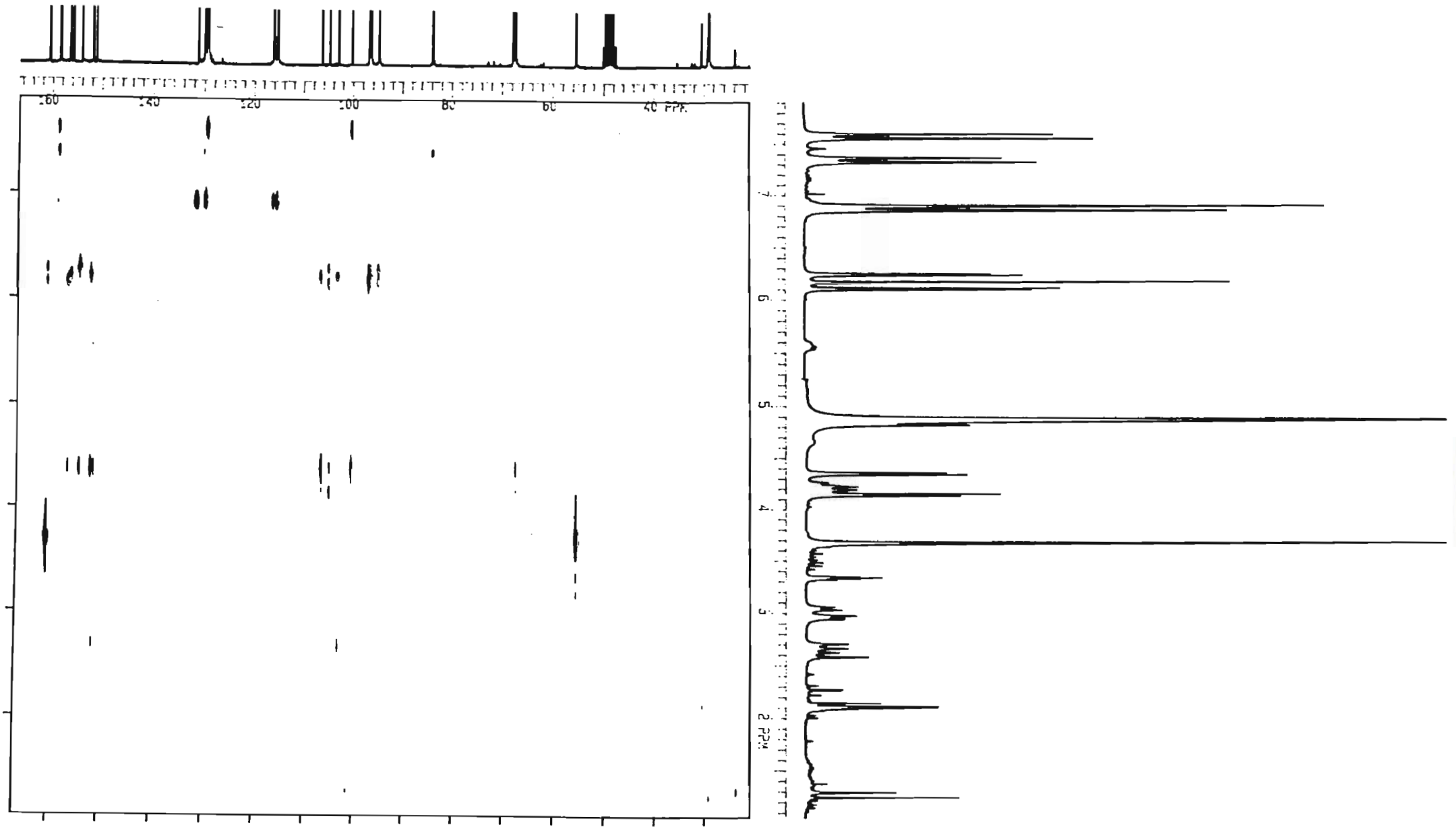
13.1. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin ^{1}HNMR spectrum (CD_3OD)



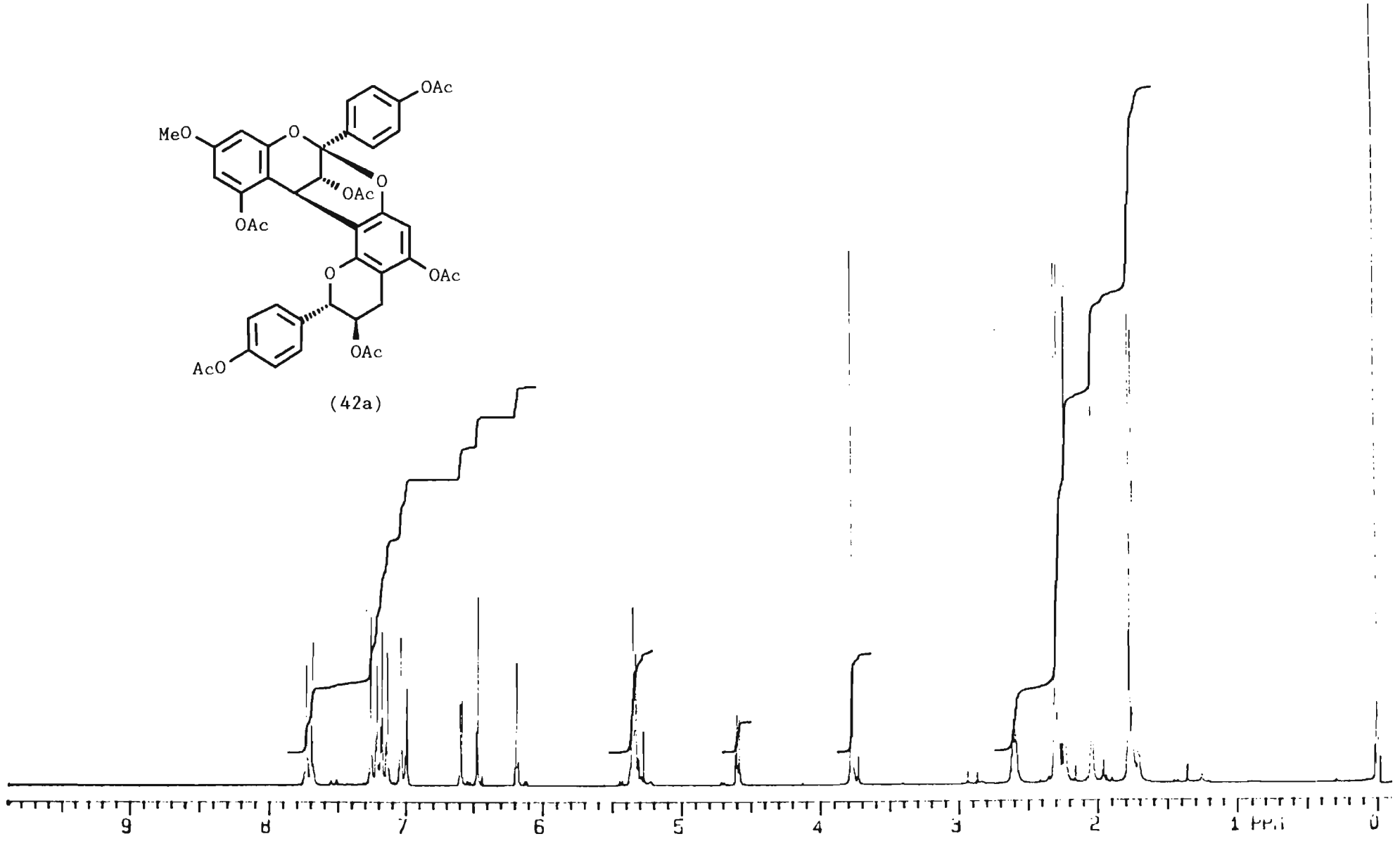
13.2. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin ^{13}CNMR spectrum (CD_3OD)



13.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin DELAYED COSY spectrum (2.5Hz) (CD_3OD)

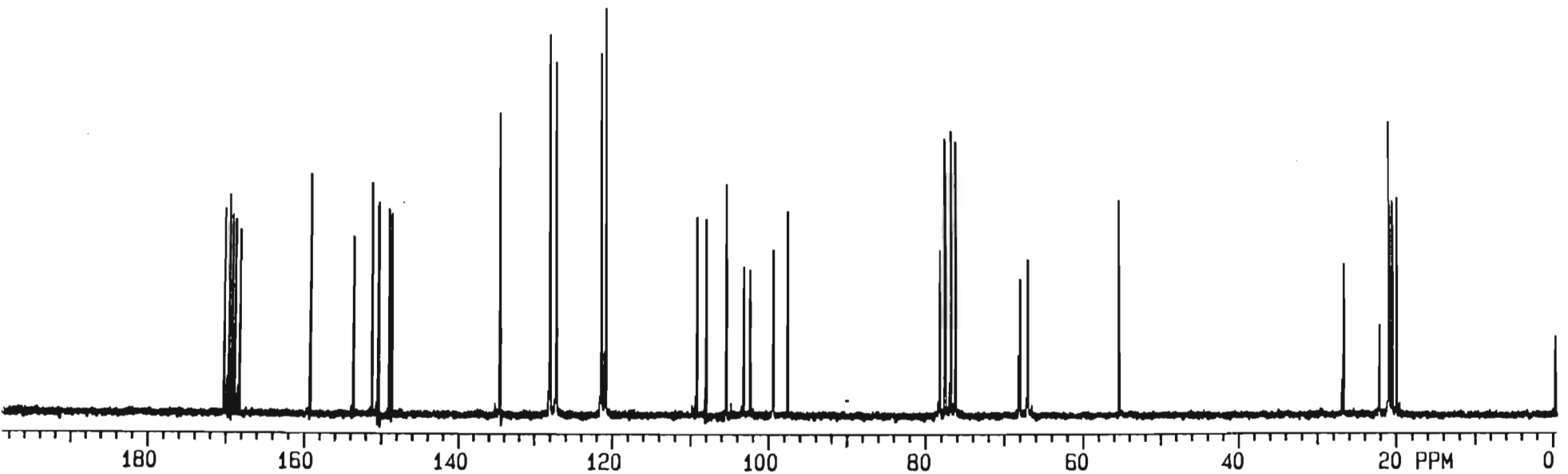


13.4. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin DELAYED HETCOR spectrum (7Hz) (CD_3OD)

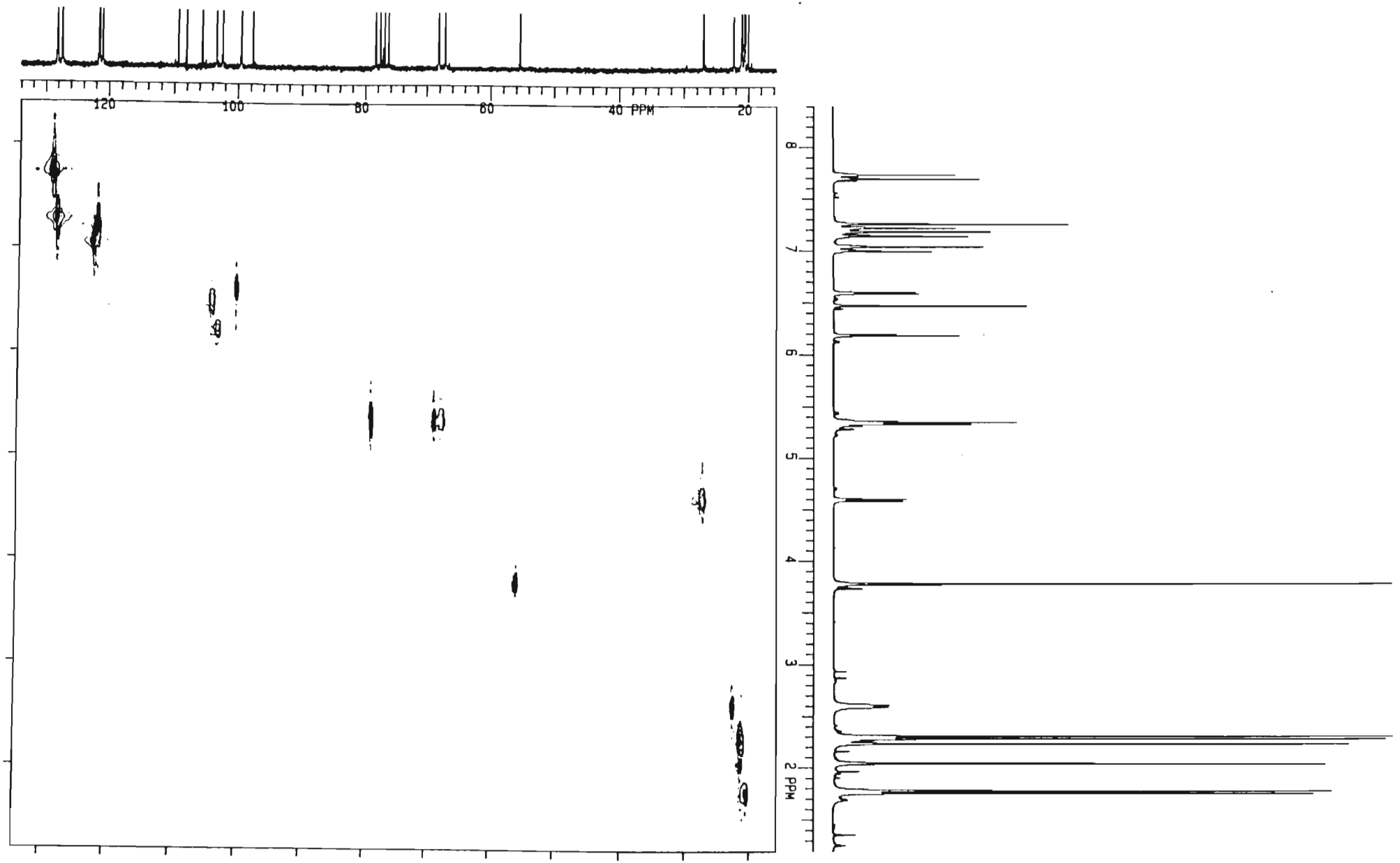


(42a)

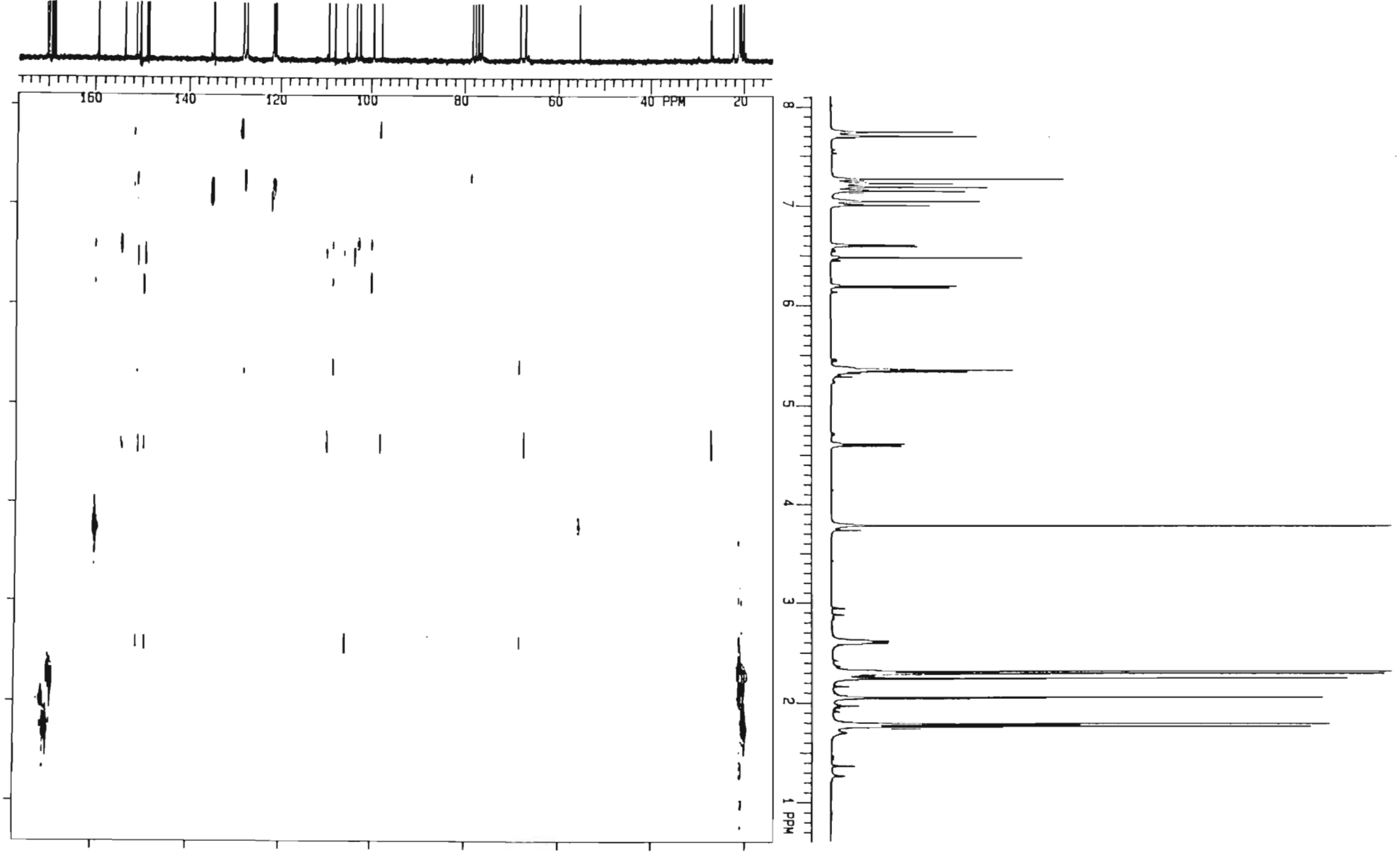
14.1. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate ^{1}HNMR spectrum ($CDCl_3$)



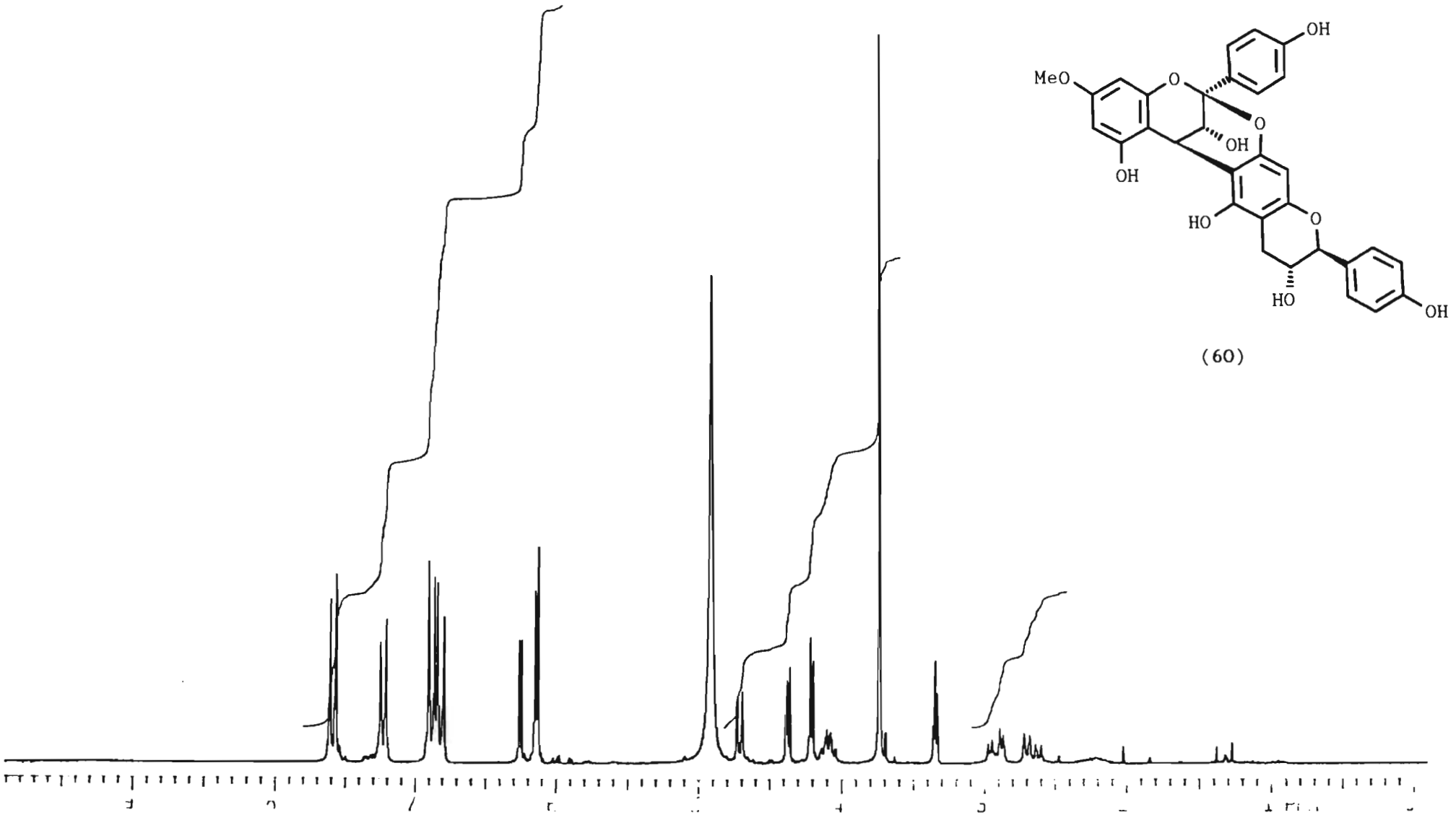
14.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-*ent*-afzelechin-hexa-acetate ^{13}CNMR spectrum ($CDCl_3$)



14.3. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin-hexa-acetate HETCOR spectrum ($CDCl_3$)

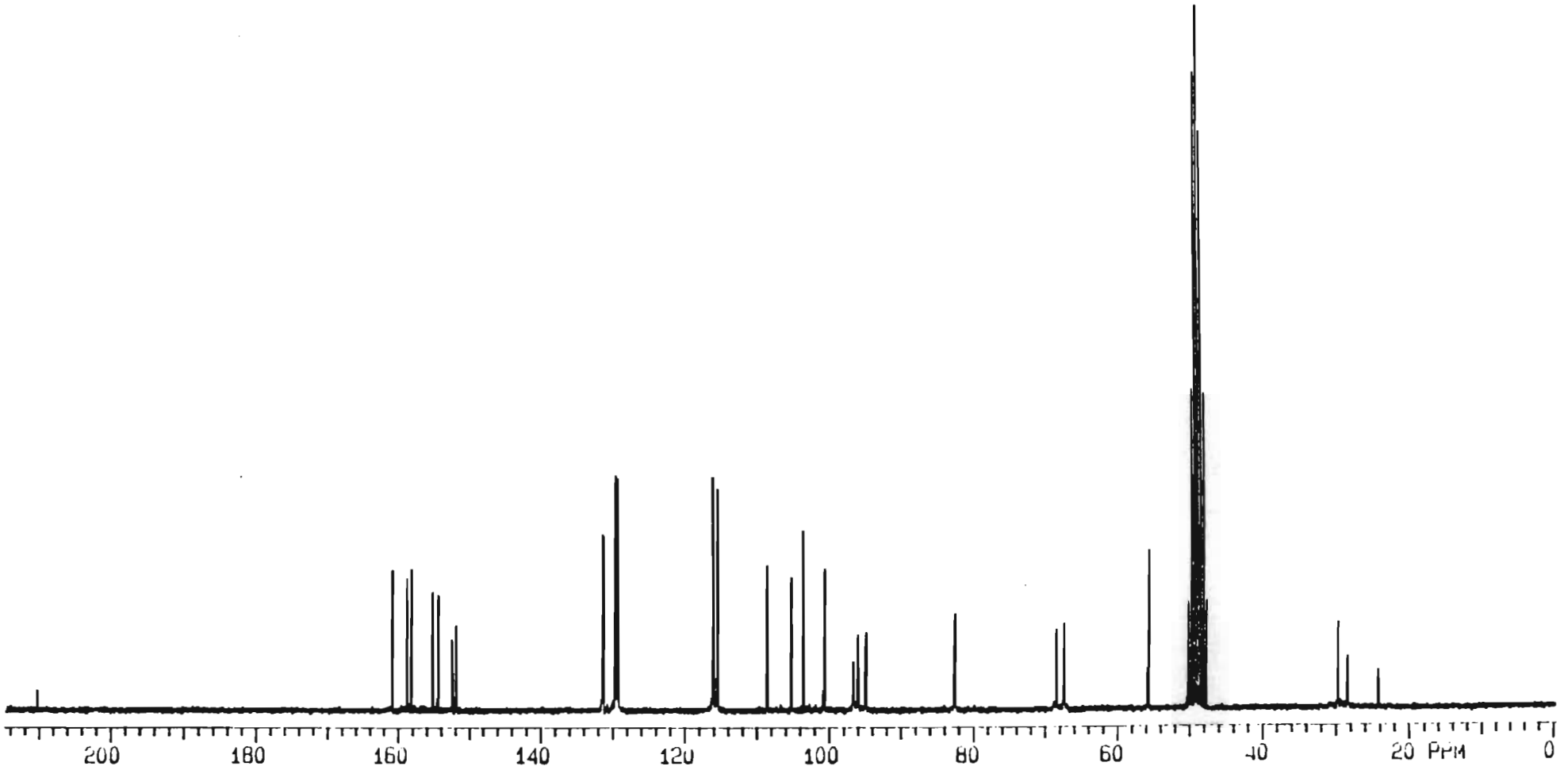


14.4. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-*ent*-afzelechin-hexa-acetate DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

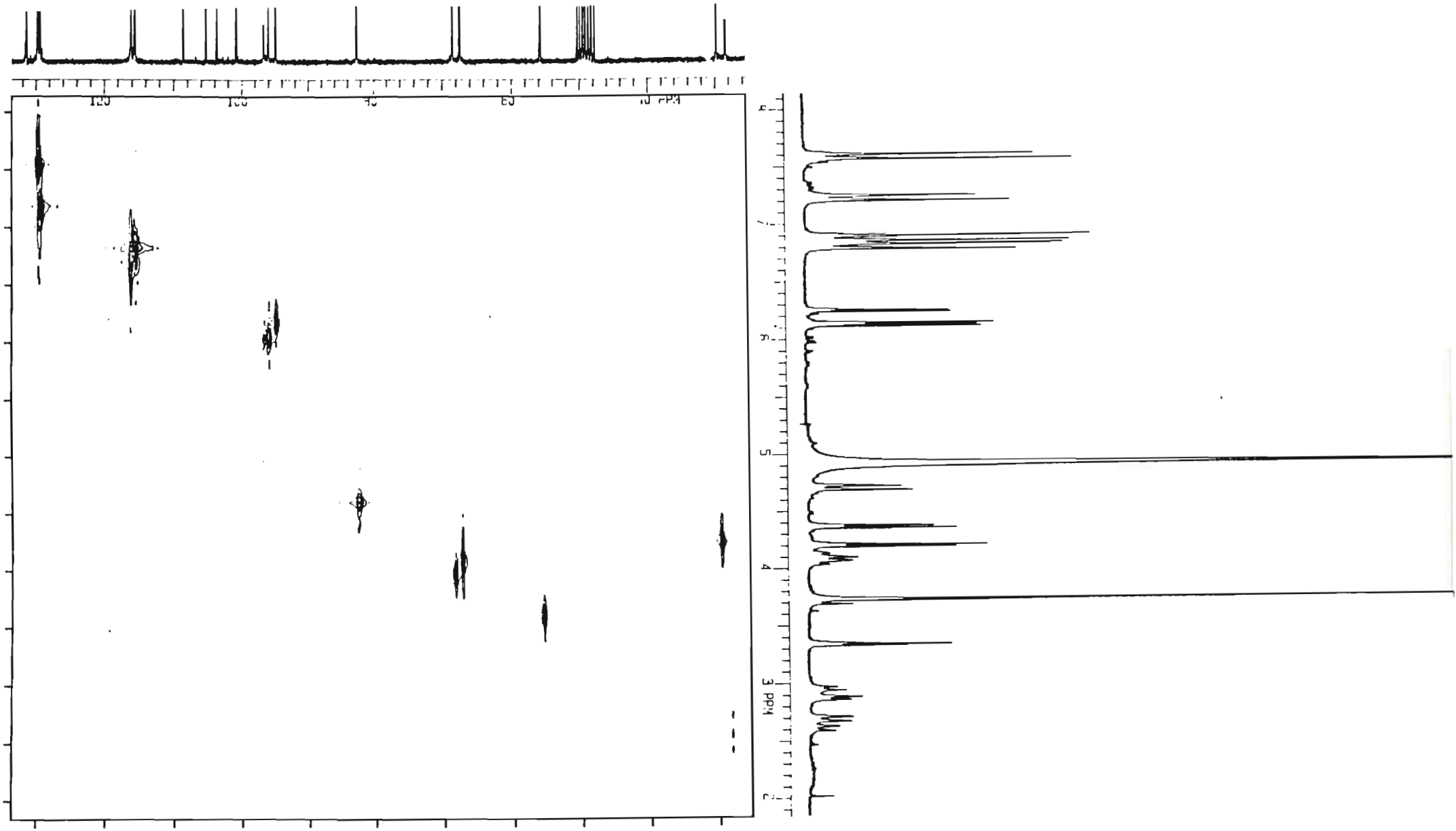


(60)

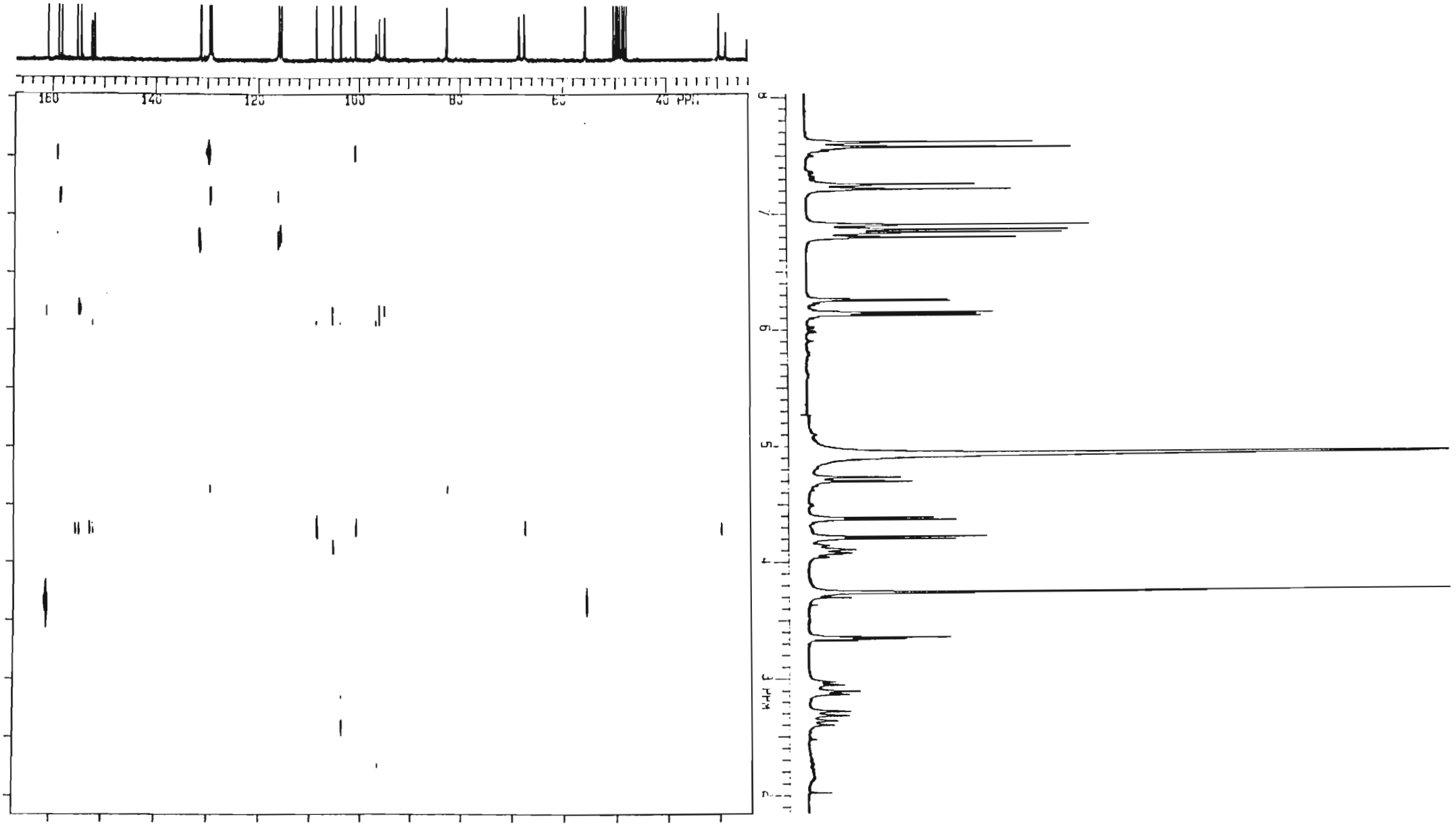
15.1. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin ^{1}HNMR spectrum (CD_3OD)



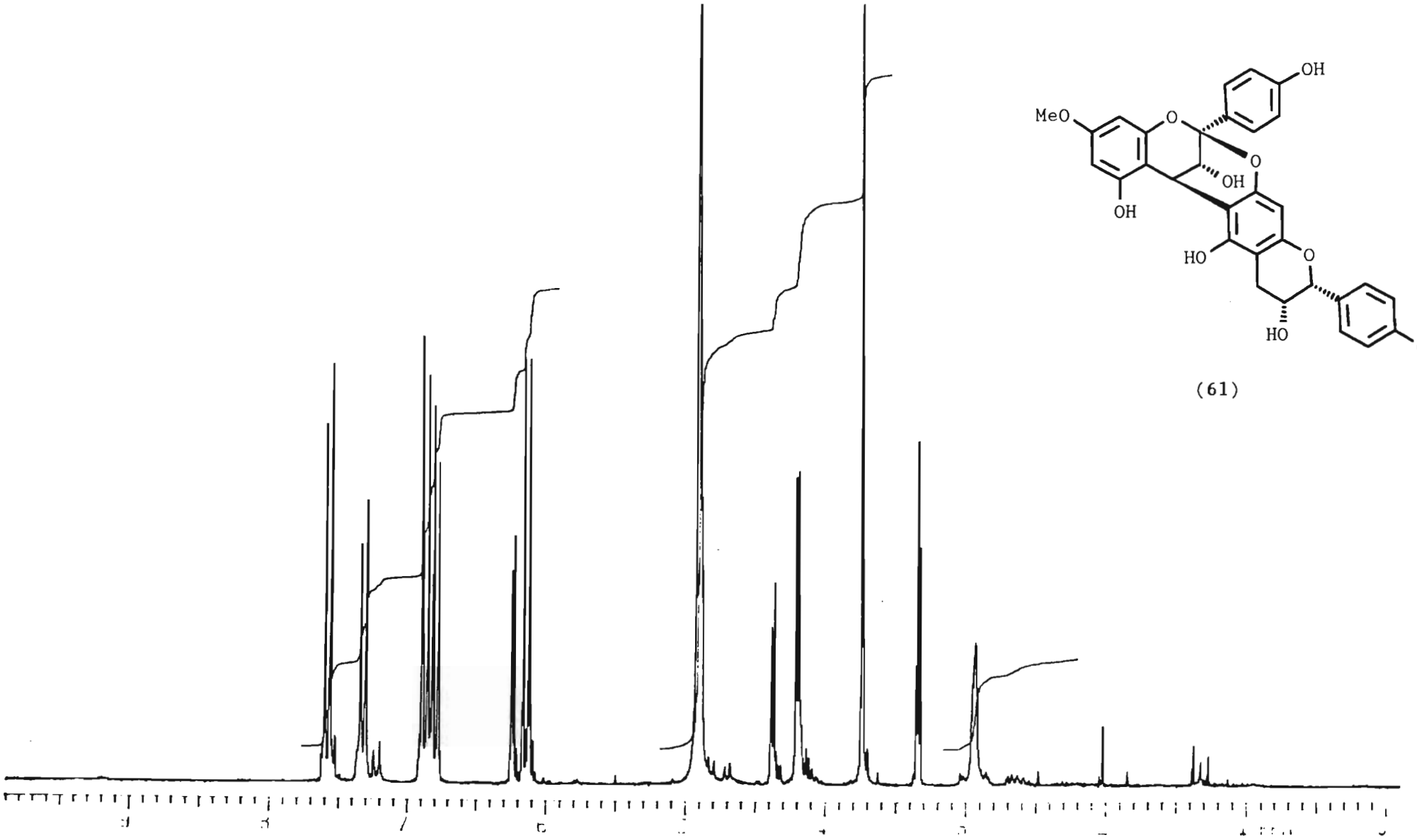
15.2. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin ^{13}CNMR spectrum (CD_3OD)



15.3. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin HETCOR spectrum (CD_3OD)

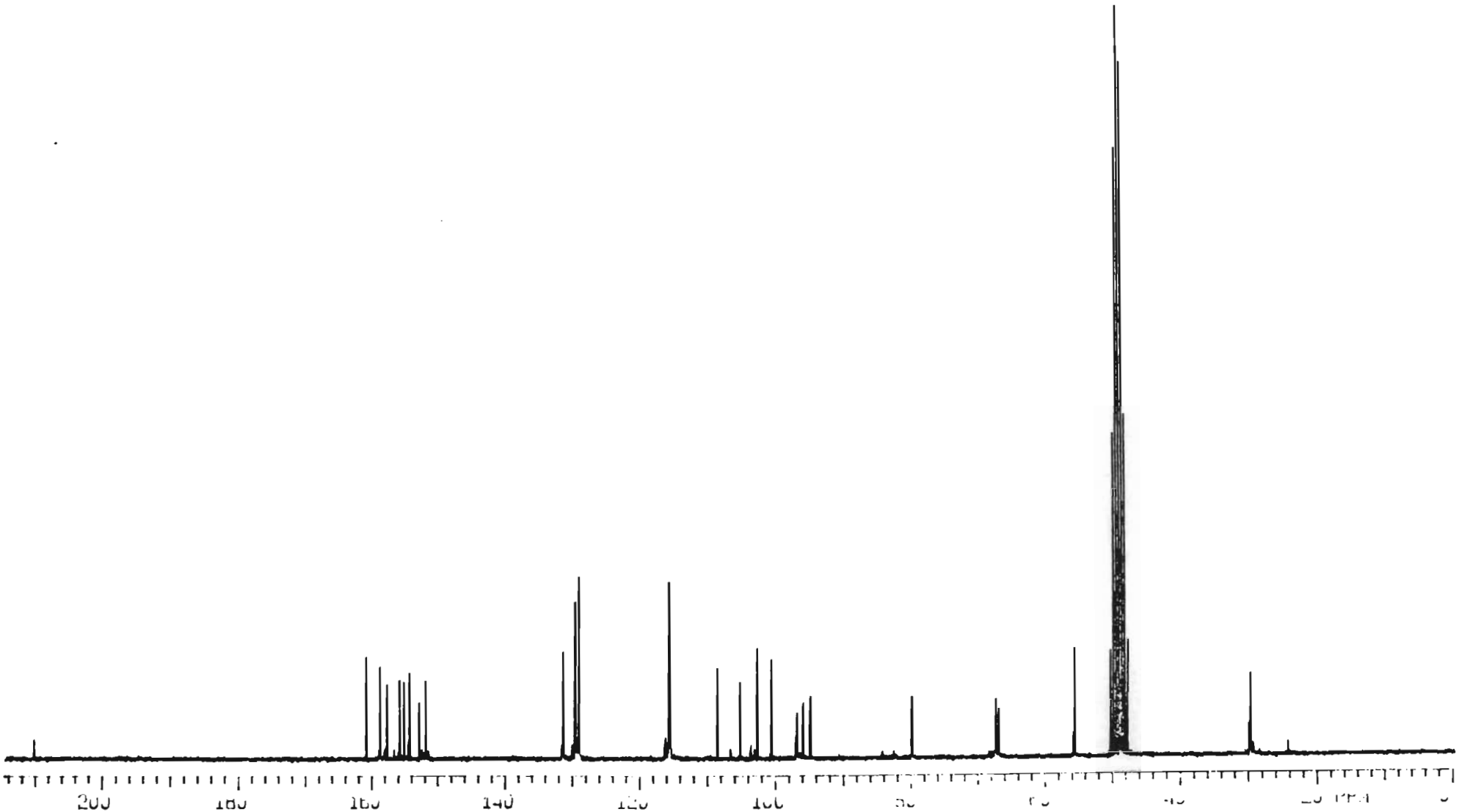


15.4. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-*ent*-afzelechin DELAYED HETCOR spectrum (7.5Hz) (CD_3OD)

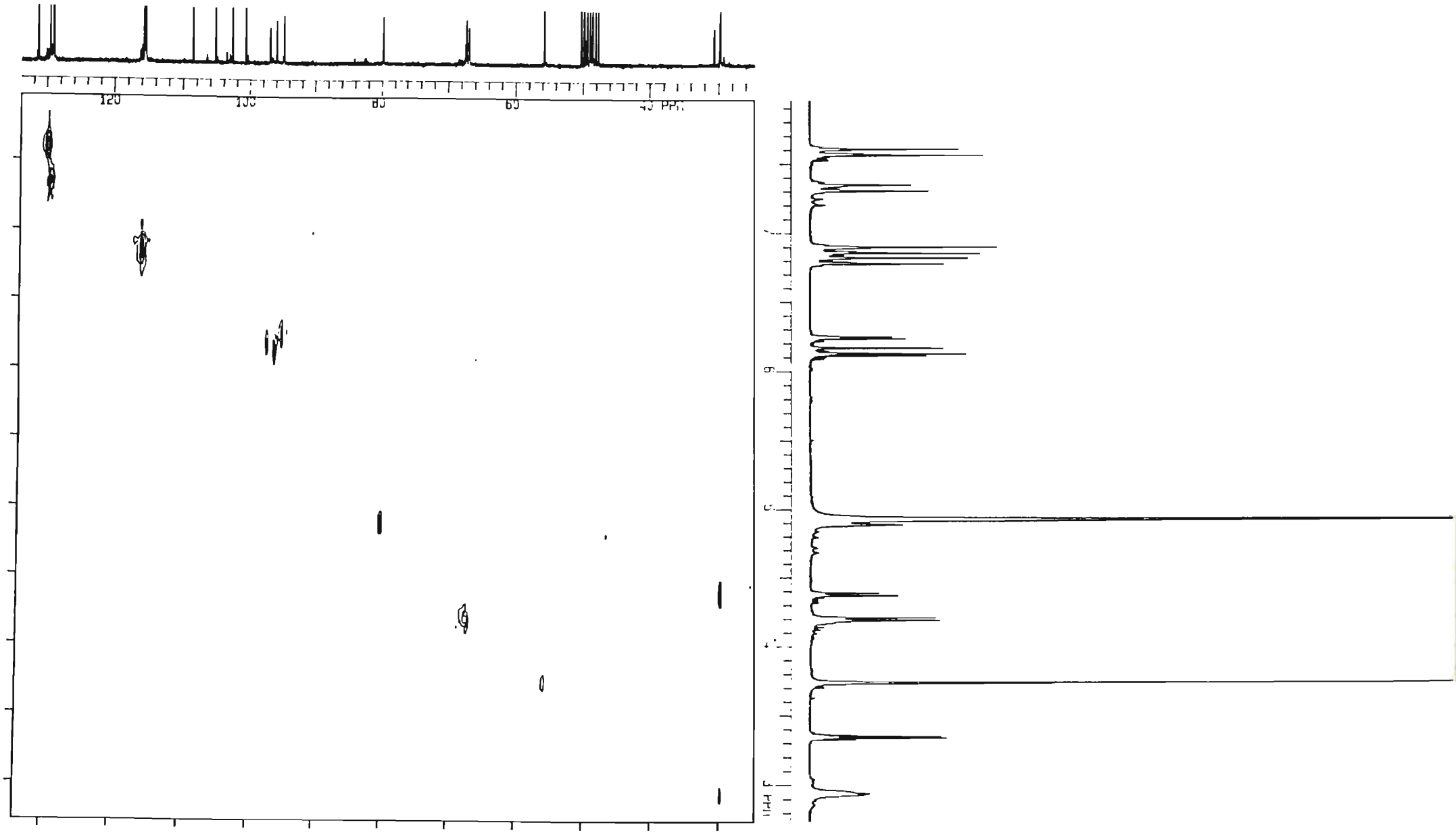


(61)

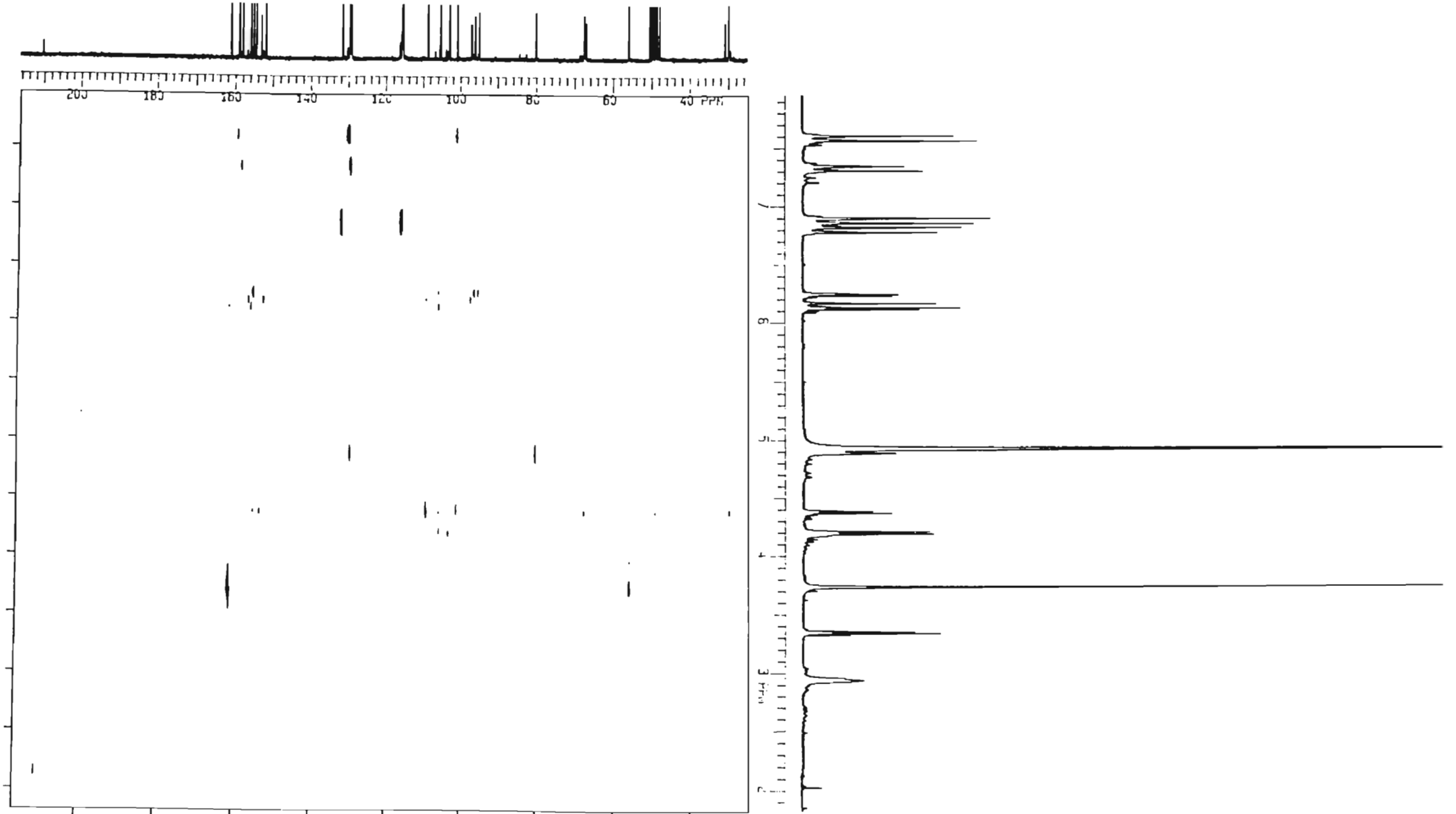
16.1. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin ^{1}HNMR spectrum (CD_3OD)



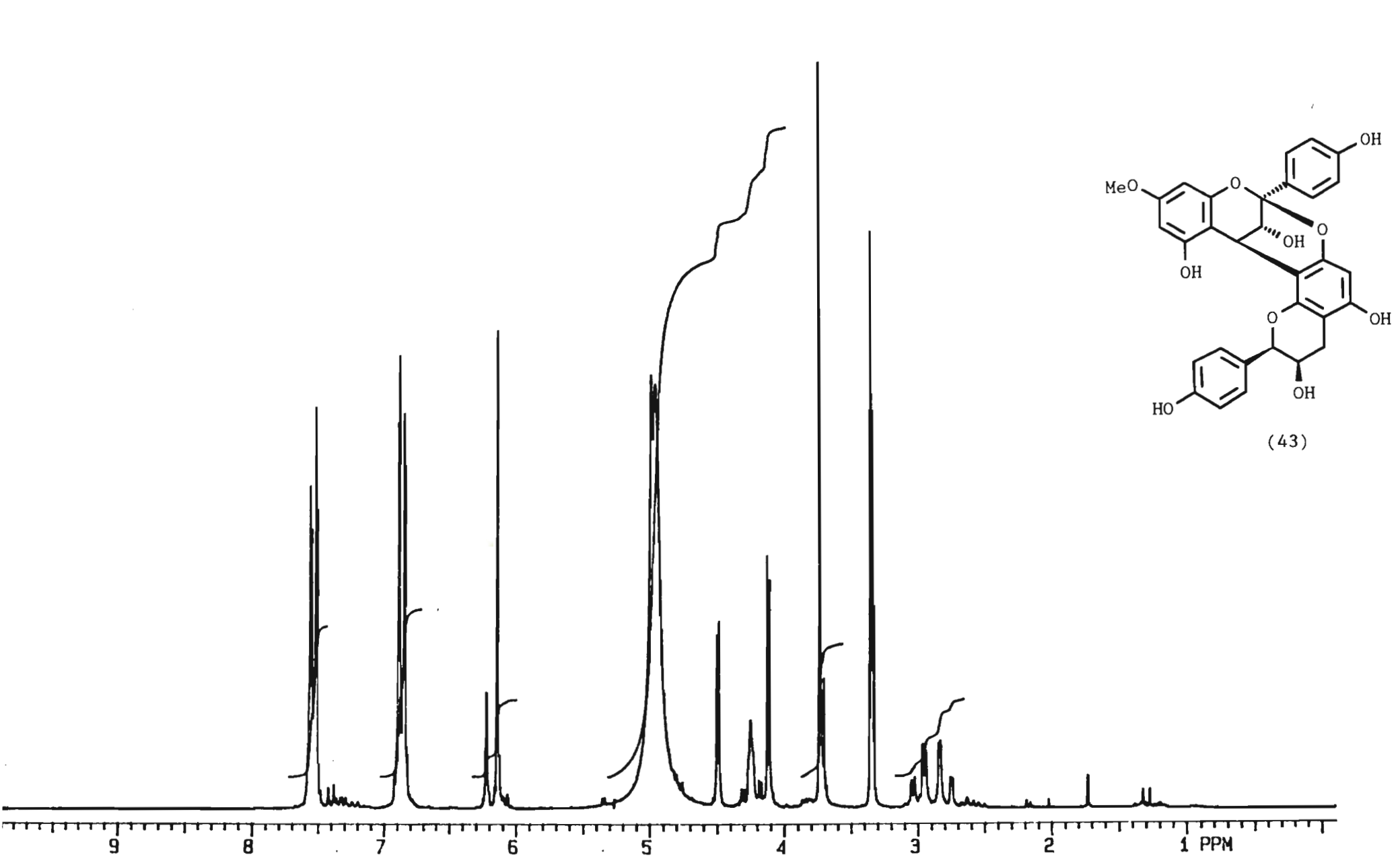
16.2. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin ^{13}CNMR spectrum (CD_3OD)



16.3. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin HETCOR spectrum (CD_3OD)

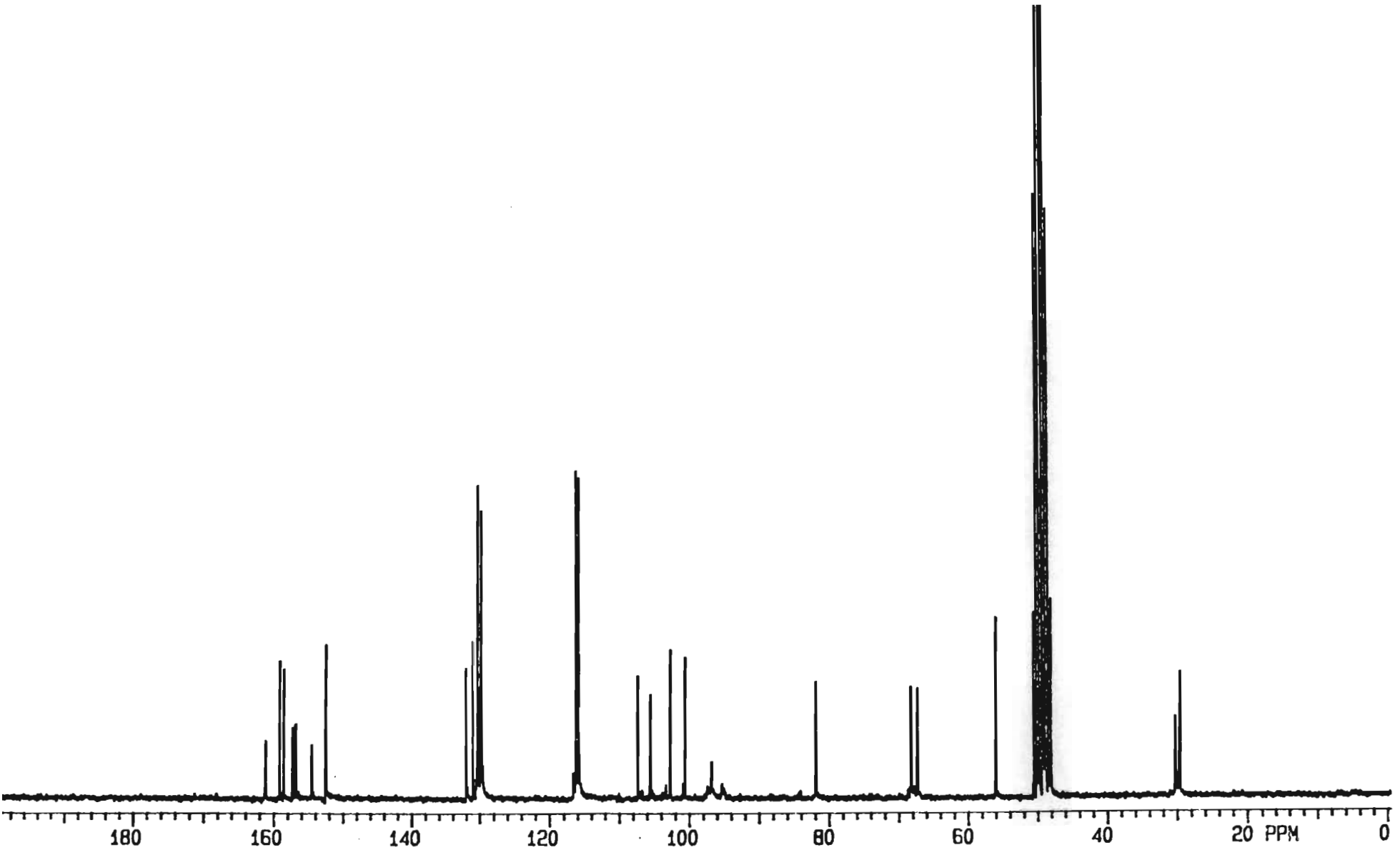


16.4. 7-OMe-epiafzelechin-(4β→6,2β→O→7)-epiafzelechin DELAYED HETCOR spectrum (7.5Hz) (CD_3OD)

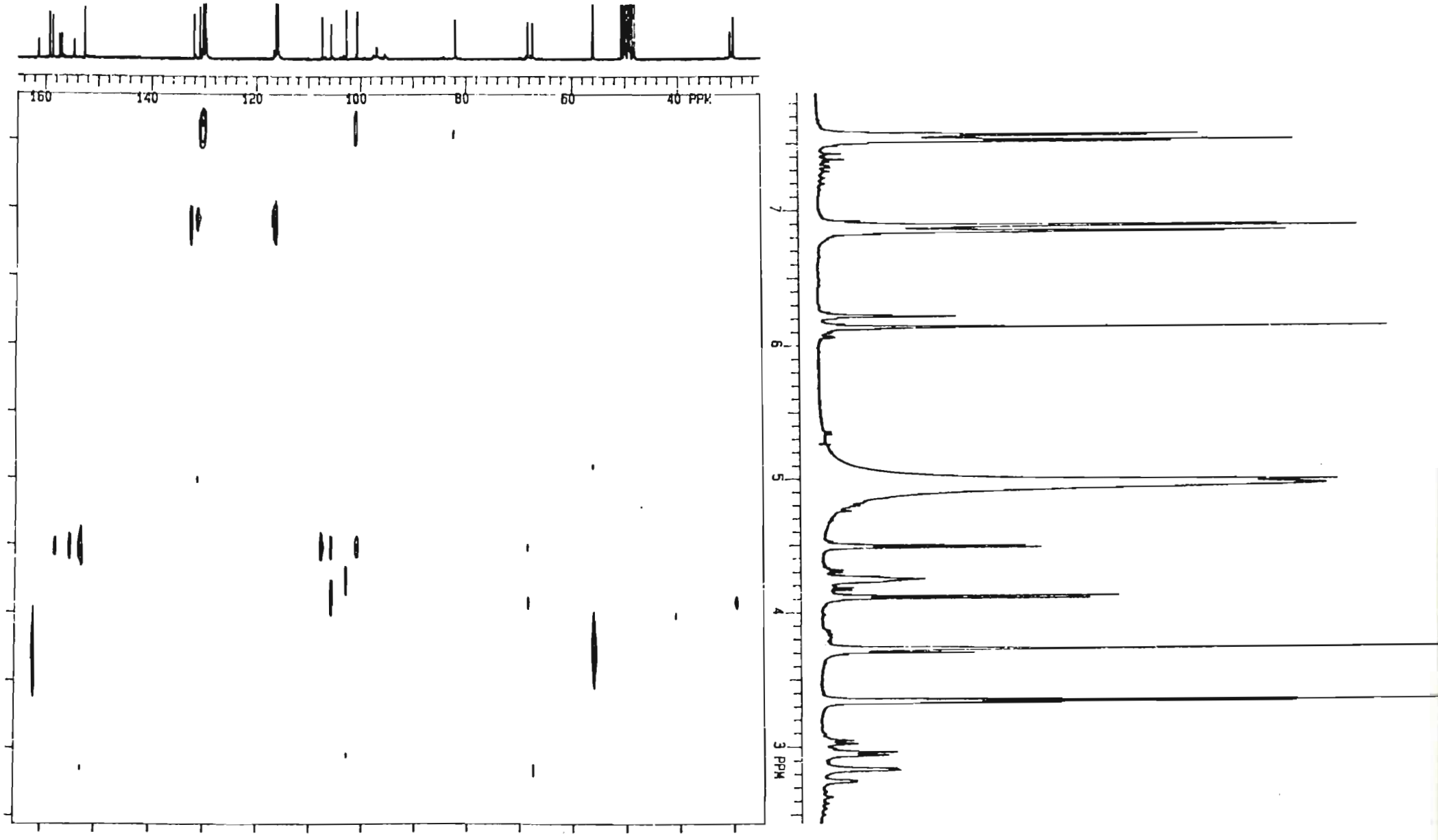


(43)

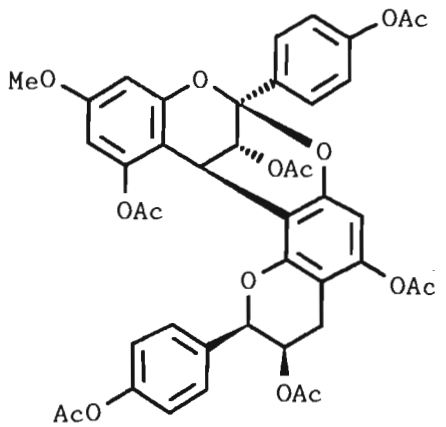
17.1. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin ^{1}HNMR spectrum (CD_3OD)



17.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin ^{13}CNMR spectrum (CD_3OD)

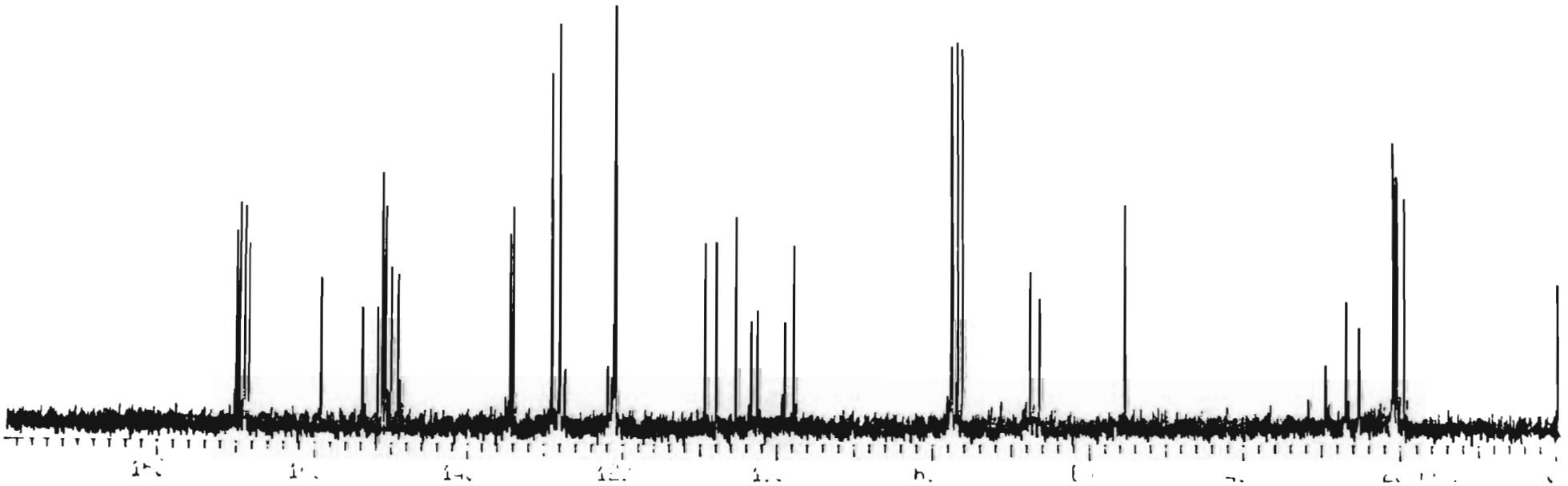


17.3. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin DELAYED HETCOR spectrum (7Hz) (CD_3OD)

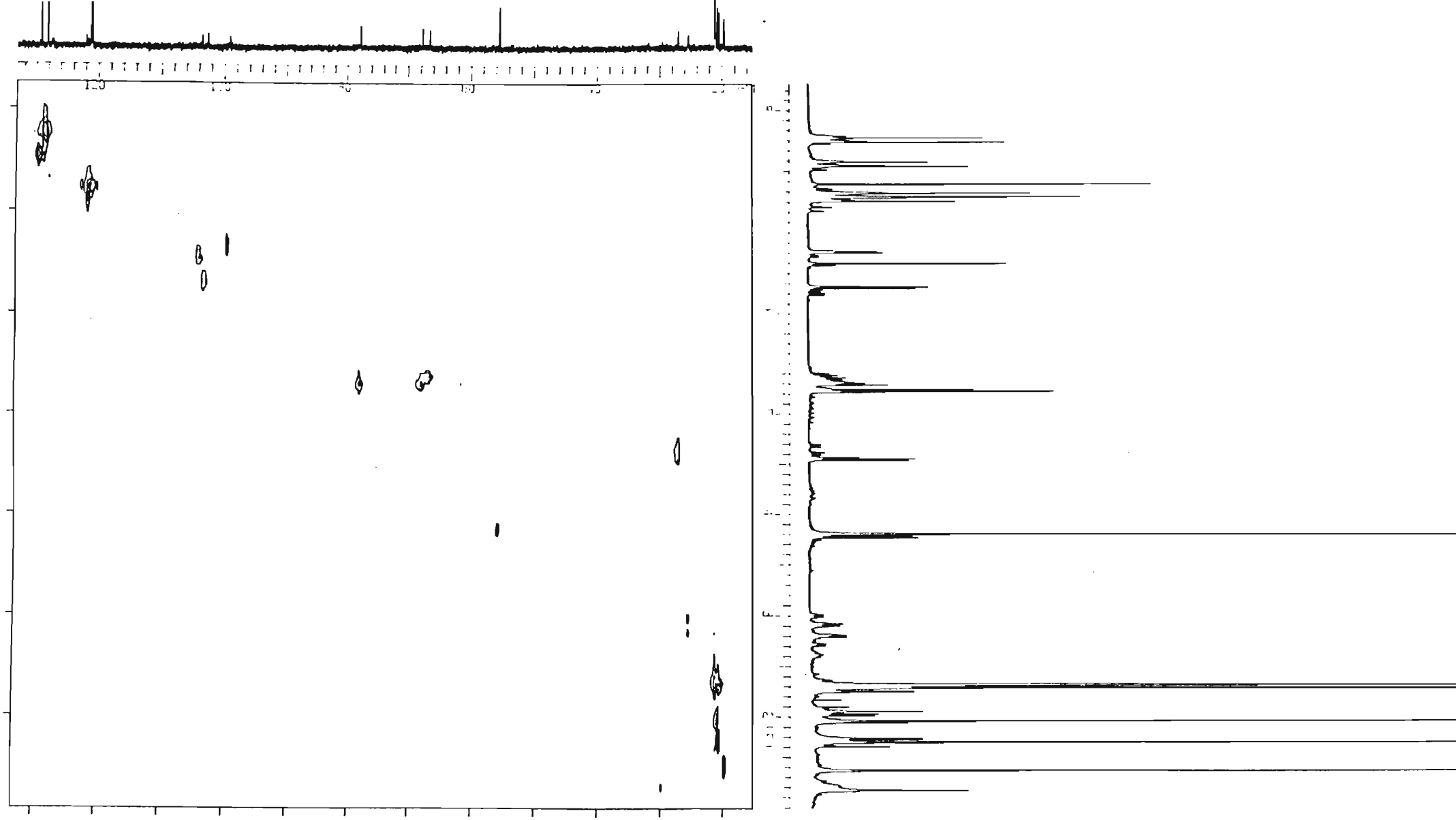


(43a)

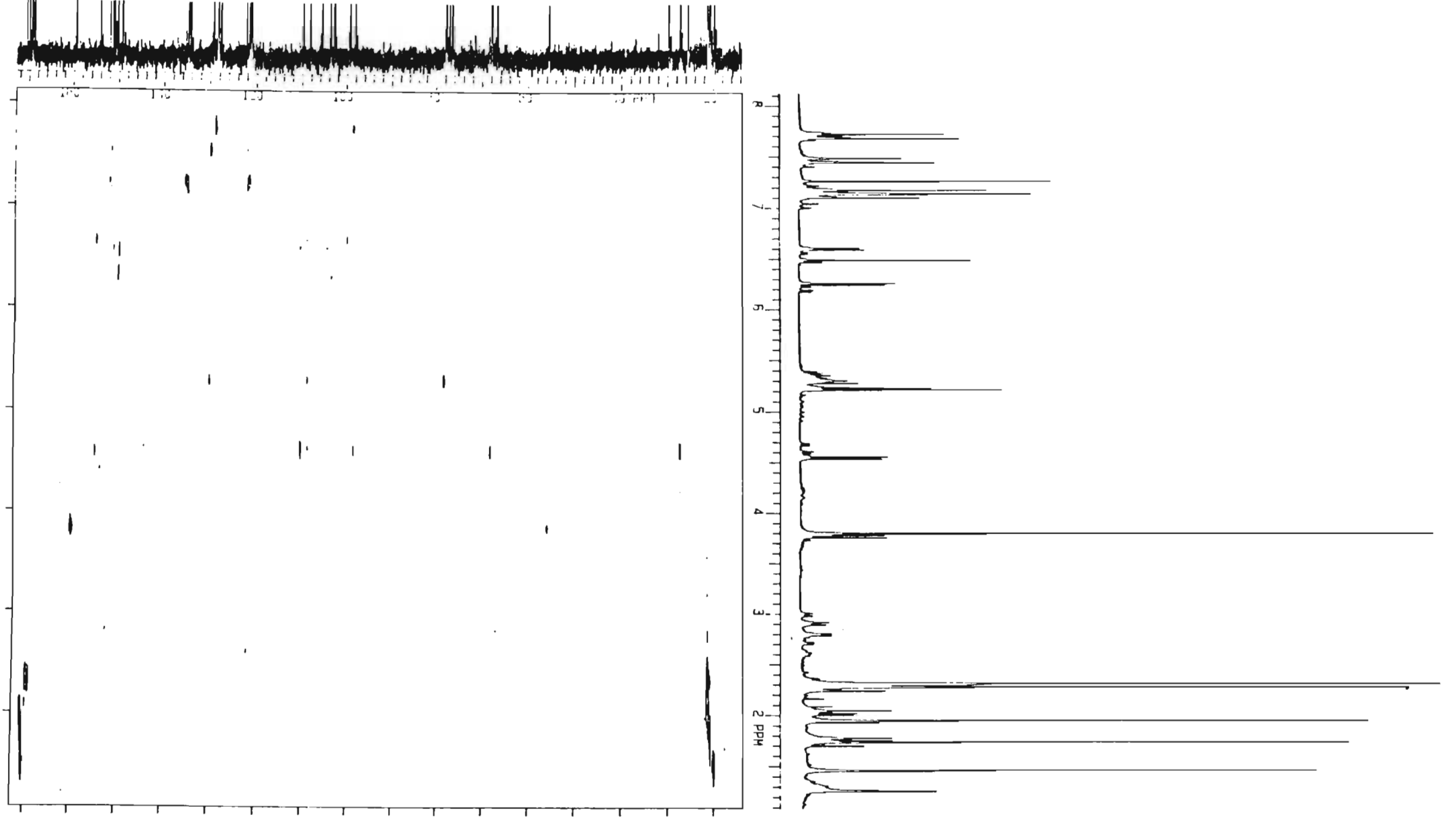
18.1. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-hexa-acetate ^{1}HNMR spectrum ($CDCl_3$)



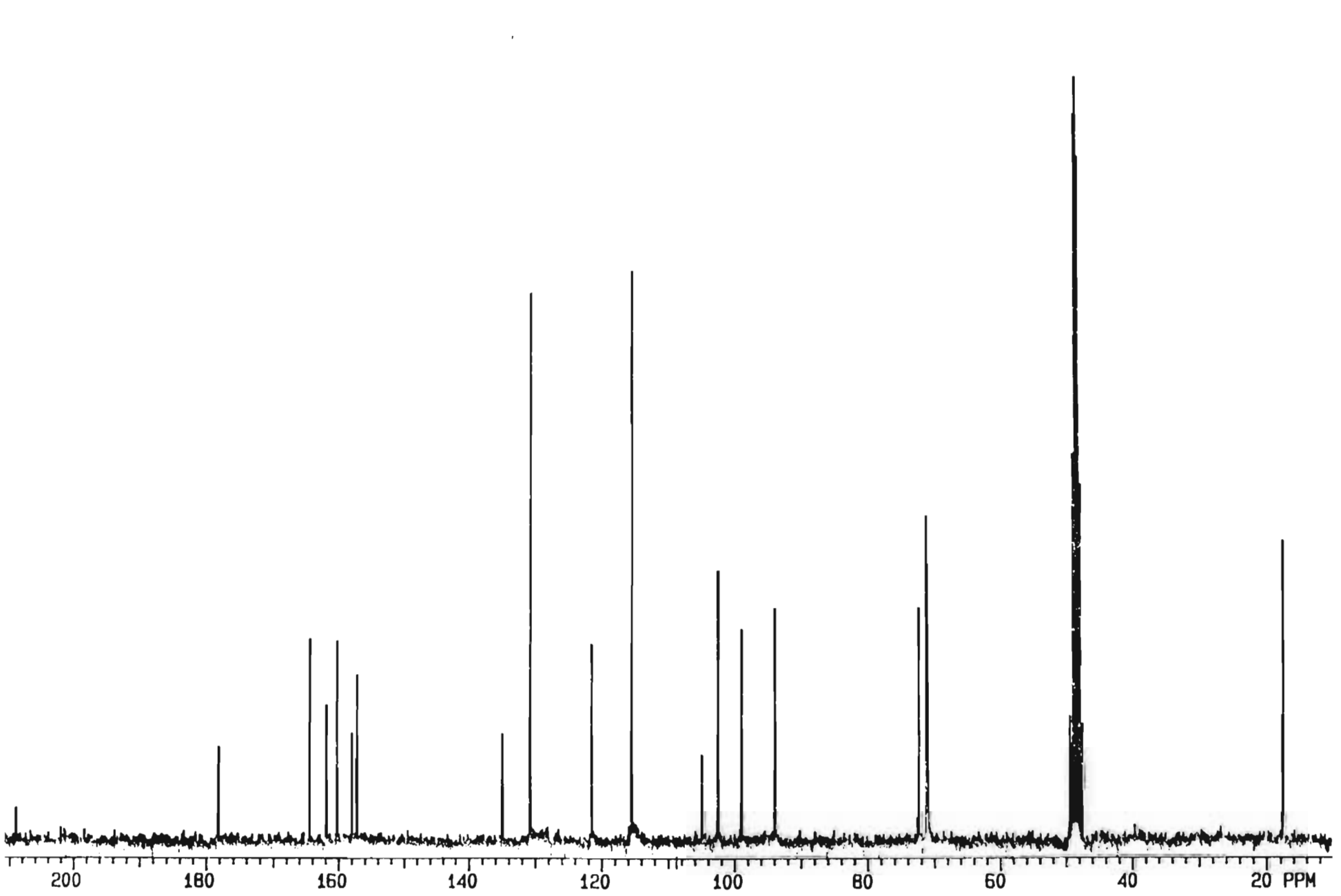
18.2. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate ^{13}CNMR spectrum ($CDCl_3$)



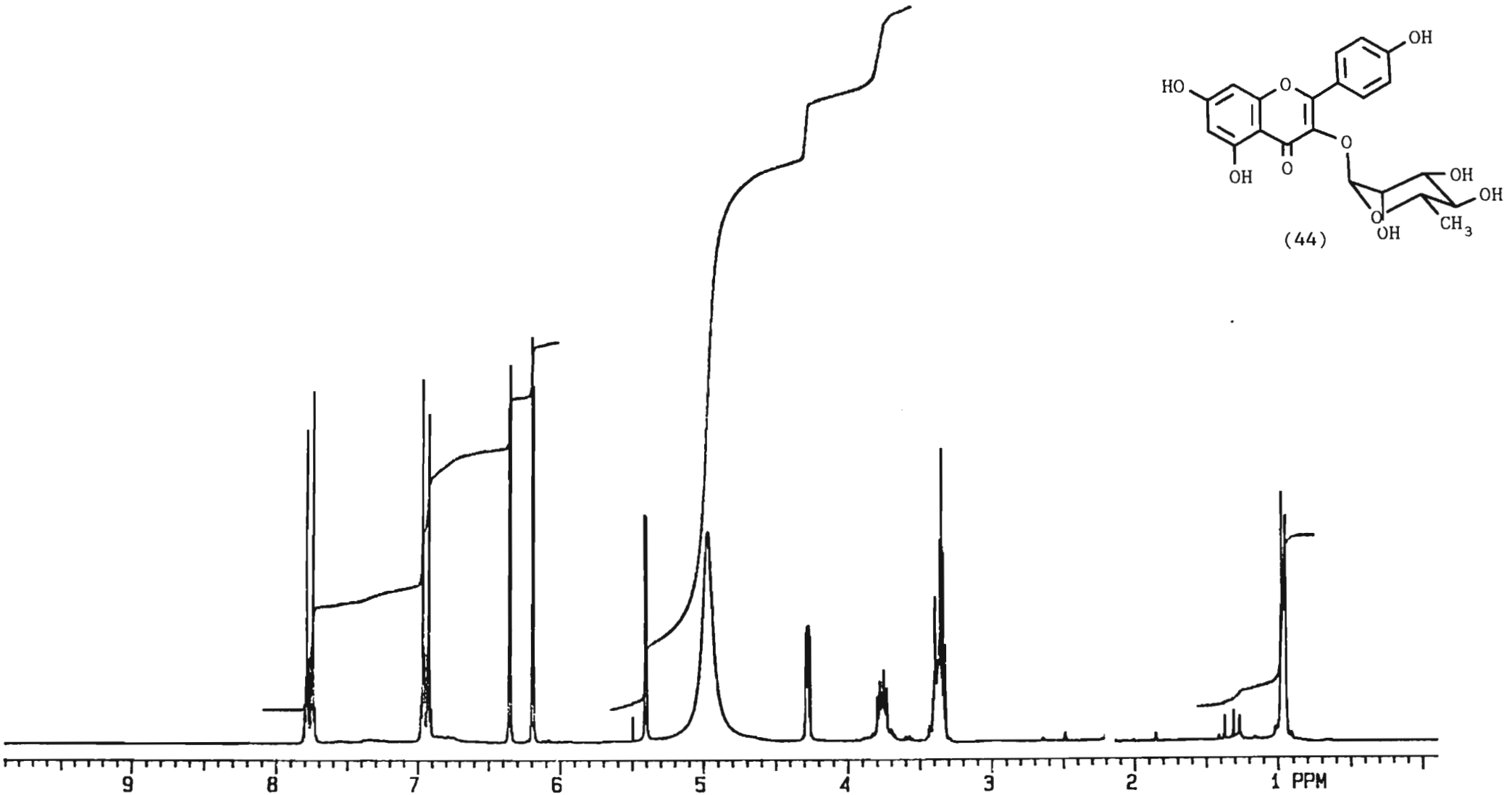
18.3. 7-OMe-epiafzelechin-(4β→8,2β→O→7)-epiafzelechin-hexa-acetate HETCOR spectrum ($CDCl_3$)



18.4. 7-OMe-epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-hexa-acetate DELAYED HETCOR spectrum (7Hz) ($CDCl_3$)

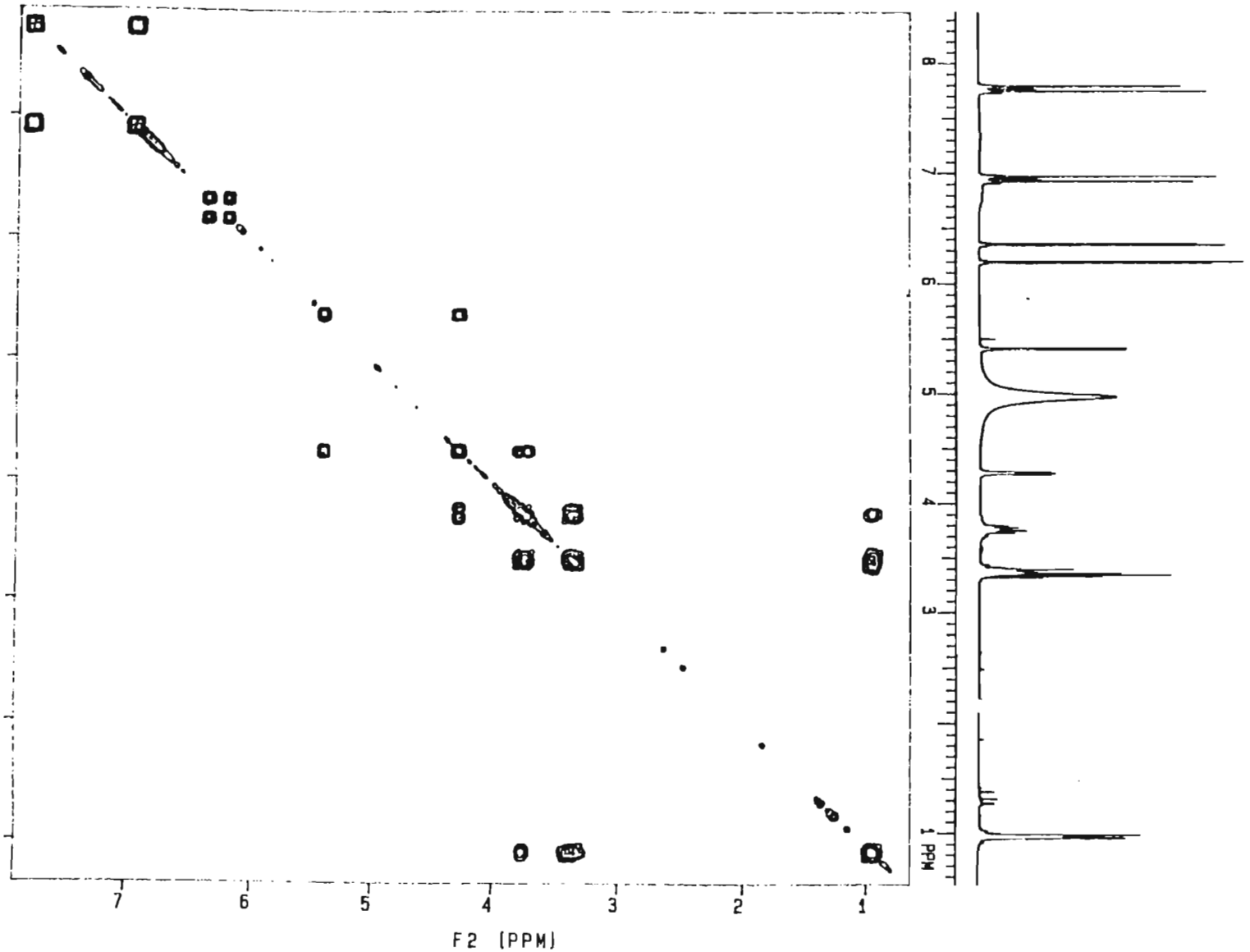


19.2. Kaempferol-3-O-α-L-rhamnopyranoside ^{13}CNMR spectrum (CD_3OD)

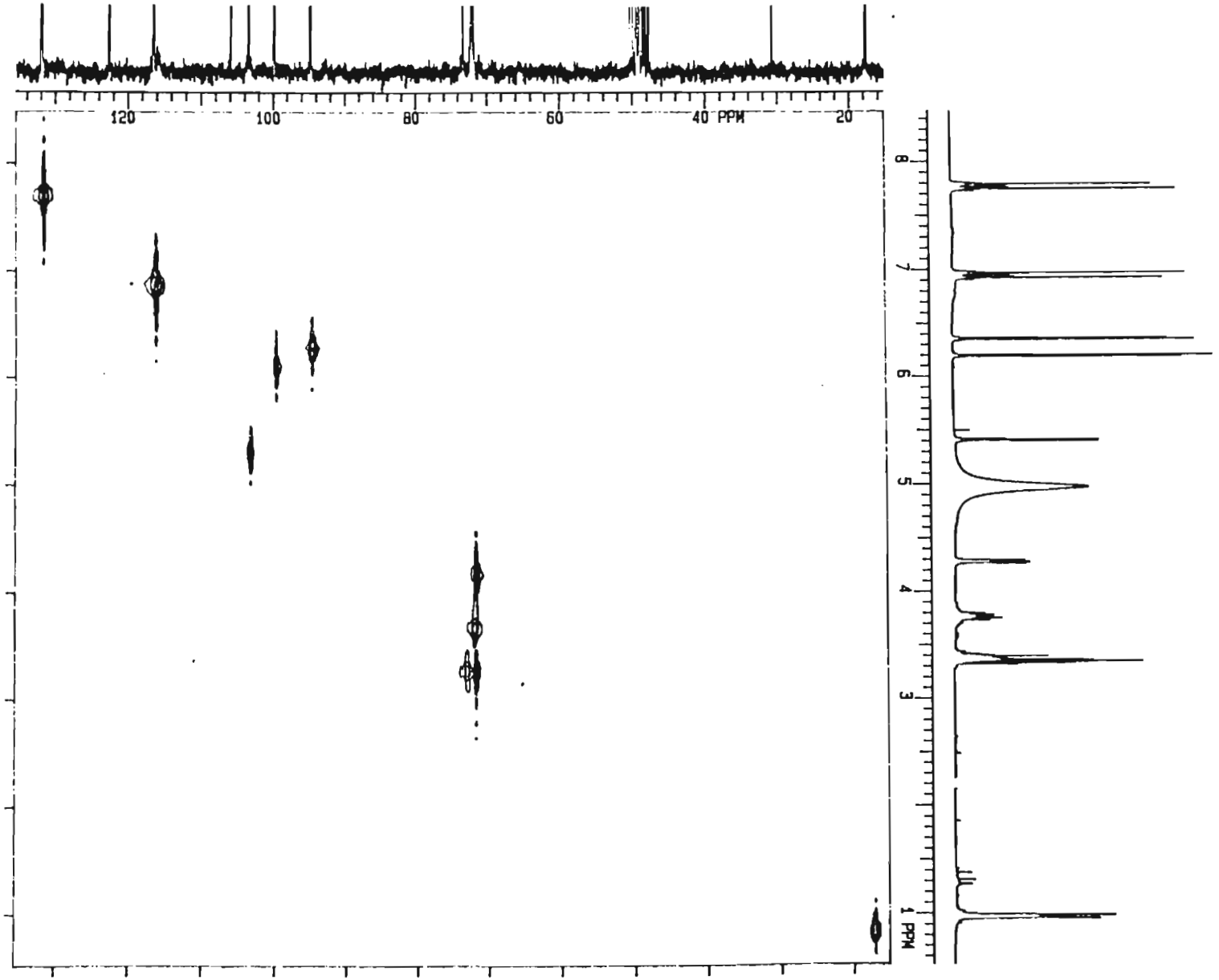


(44)

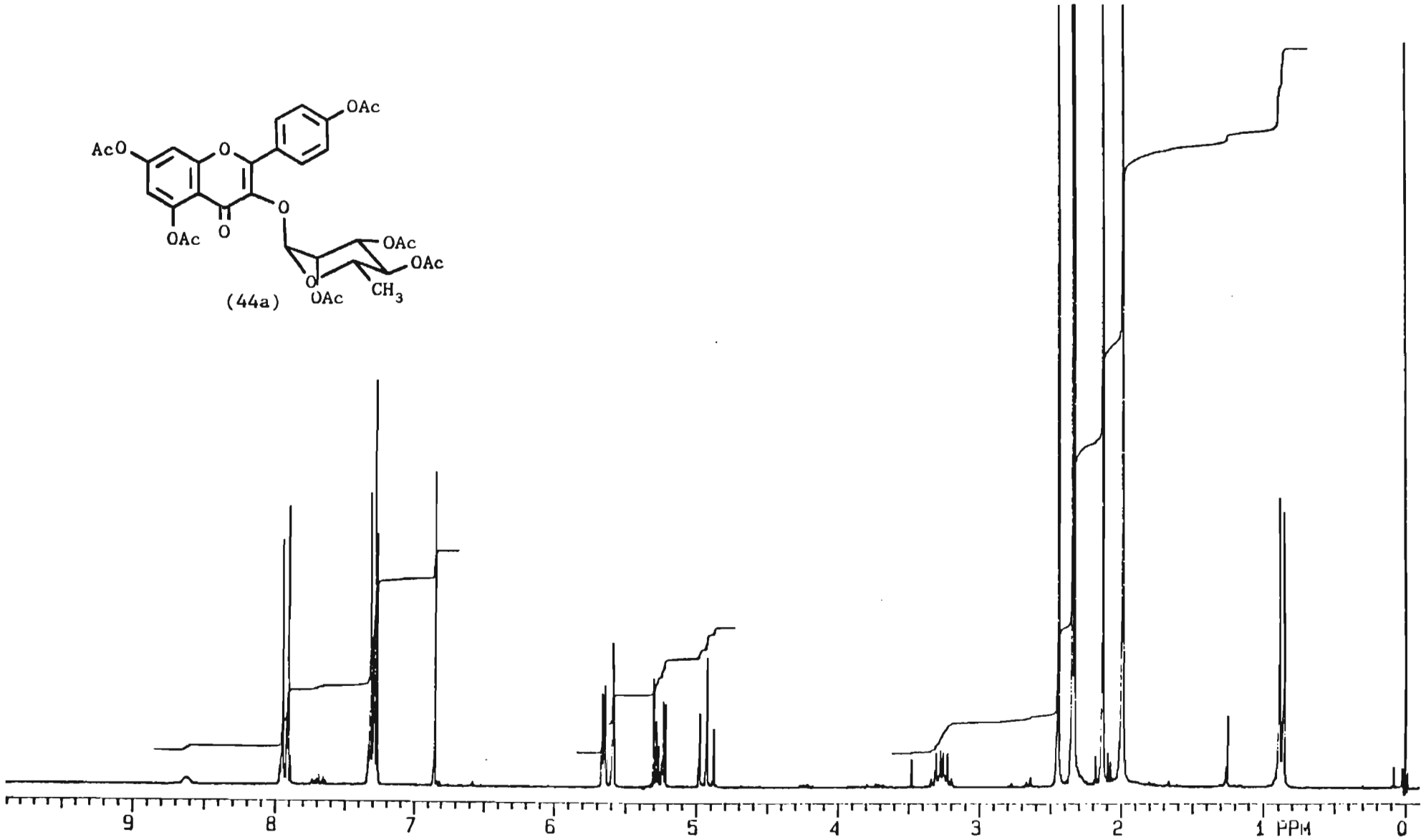
19.1. Kaempferol-3-O-α-L-rhamnopyranoside ^{1}HNMR spectrum (CD_3OD)



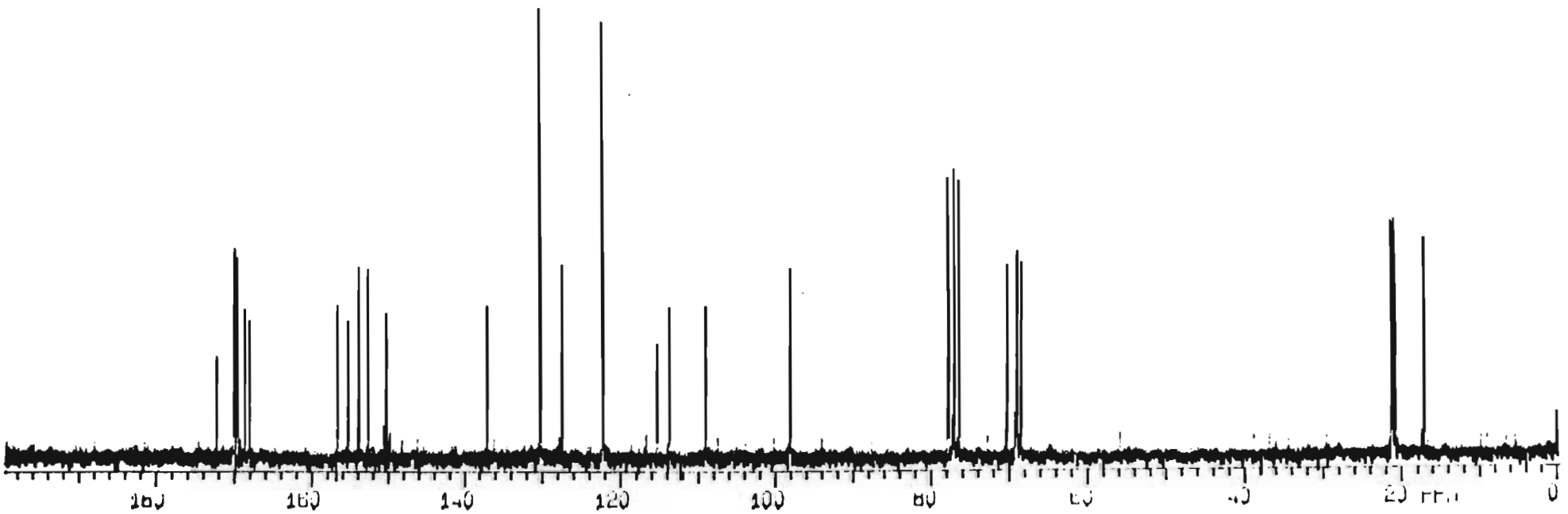
19.3. Kaempferol-3-O-α-L-rhamnopyranoside ^{1}H-^{1}H COSY spectrum (CD_3OD)



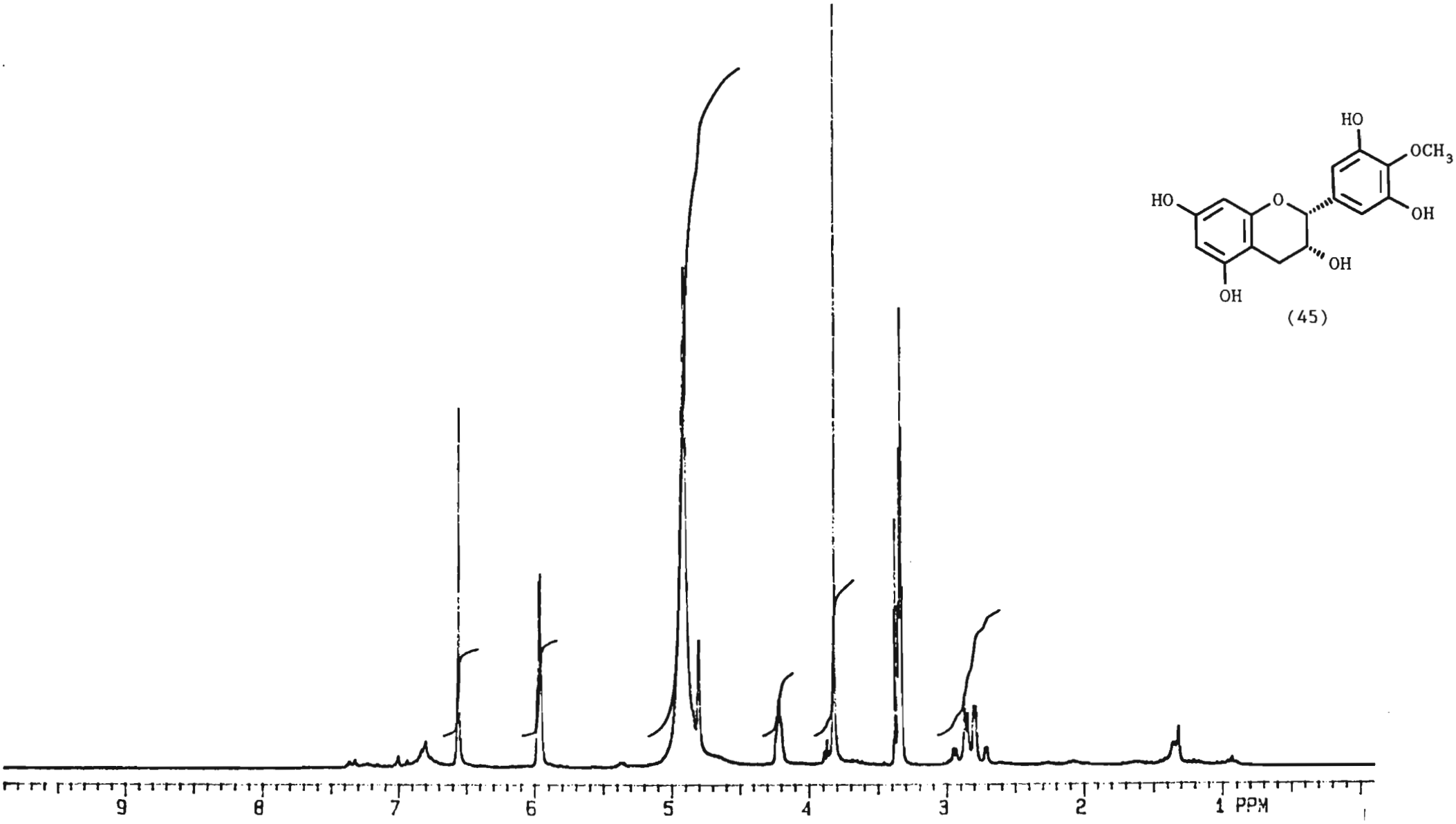
19.4. Kaempferol-3-O-α-L-rhamnopyranoside HETCOR spectrum (CD_3OD)



20.1. Kaempferol-3-O-α-L-rhamnopyranoside-hexa-acetate ^{1}HNMR spectrum ($CDCl_3$)

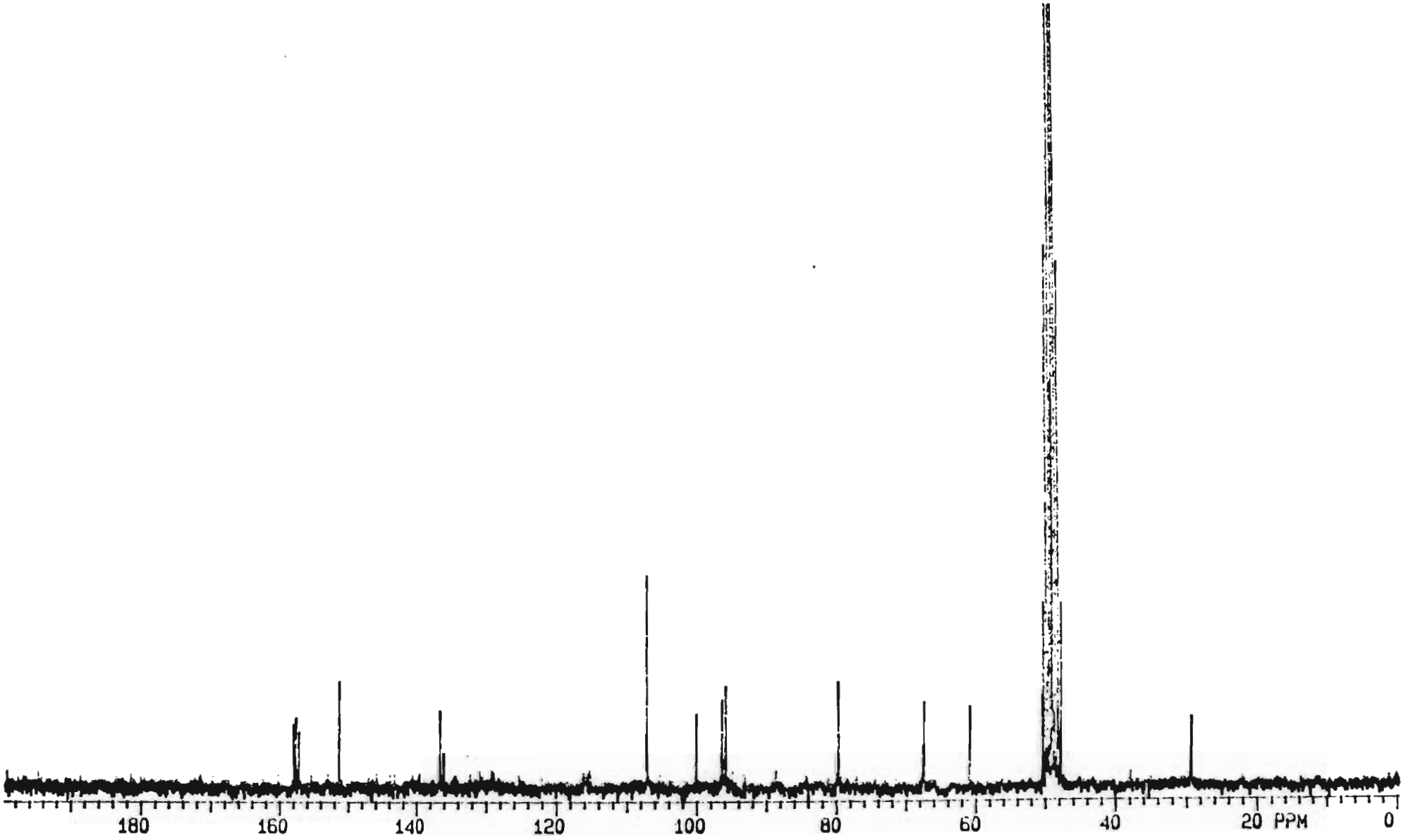


20.2. Kaempferol-3-O-α-L-rhamnopyranoside-hexa-acetate ^{13}CNMR spectrum ($CDCl_3$)

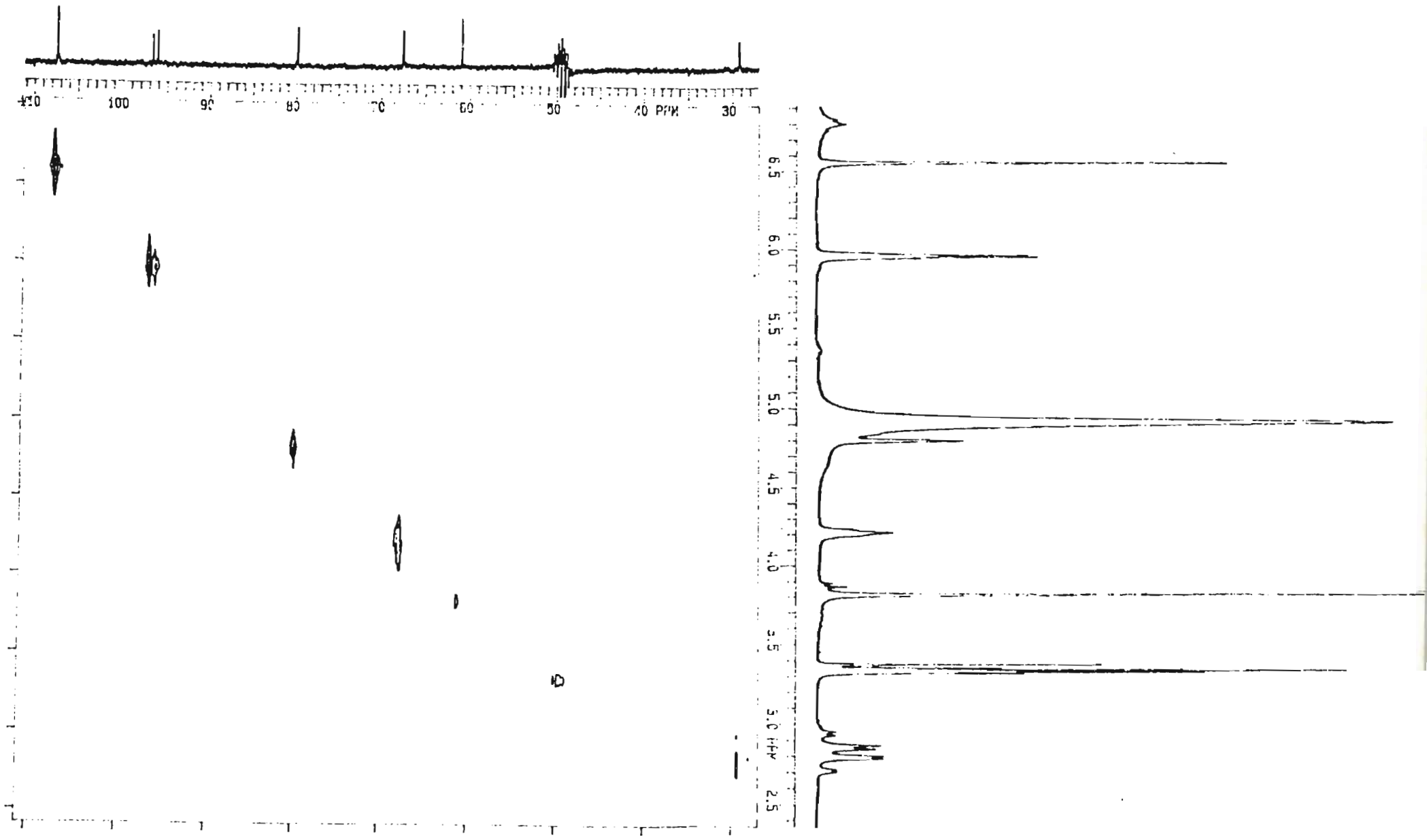


(45)

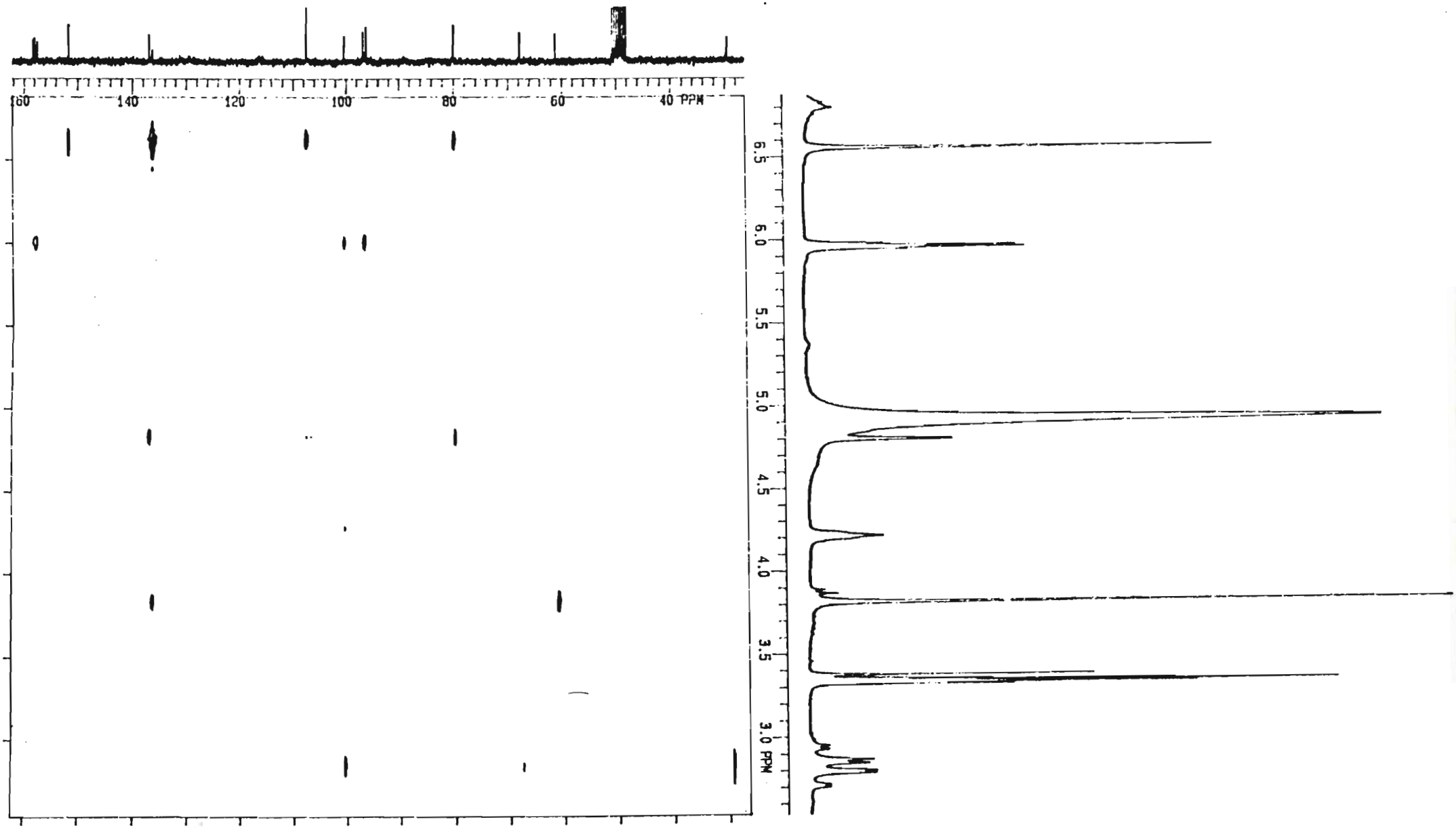
21.1 4'-OMe-epigallocatechin ^{1}HNMR spectrum (CD$_3$OD)



21.2. 4'-OMe-epigallocatechin ^{13}CNMR spectrum (CD_3OD)



21.3. 4'-OMe-epigallocatechin HETCOR spectrum (CD_3OD)



21.4. 4'-OMe-epigallocatechin DELAYED HETCOR spectrum (10Hz) (CD_3OD)